Annual Report
2020 and
2020-2021
Implementantation period: 01/2020 – 02/2022

Presented by the Austrian Board of Trustees
of the Austrian Member Universities

Chair: Prof. Dr. Gabriele Kotsis
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Preface by the National Coordinator Austria

Prof. Dr. Gabriele Kotsis,
Johannes Kepler University Linz

Contributions to Sustainability

In 2017, the ASEA-UNINET Plenary Meeting was held in Graz, Austria, with a special focus on the topic of “Sustainability”. Constituting as a pre-event and for the first time ever ASEA-UNINET invited sustainability experts and specialists, across different scientific fields within the network, to participate in a Sustainability Workshop. The program aimed to foster exchange of ideas, collective analysis and matchmaking of expertise and interests to generate ideas for project collaborations within the framework of sustainability. Due to those efforts, several projects have been started contributing to sustainability from various perspectives and disciplines. The ASEA-UNINET Sustainability News have been launched as a digital magazine reporting on latest projects, research, initiatives and personalities regarding all scientific disciplines working on sustainable development within and without the network of ASEA-UNINET.

To further strengthen the activities of the network in that direction, Prof. Dr. A Min Tjoa (Vienna University of Technology, Austria) and Assoc.Prof. Dr. Ngo Chi Trung (Hanoi University of Science and Technology, Vietnam) have been elected as coordinators for technology, innovation and sustainability projects for Europe respectively for South-East Asia at the following ASEA-UNINET Plenary meeting in Danang, Vietnam, in 2019.

At this meeting, I was honored by being elected as ASEA-UNINET President. One of my first actions as President was to invite the network to the next ASEA-UNINET Plenary to be held in July 2020 in Linz, Austria. At that time nobody would have imagined how our world would change under the dominance of Coronavirus disease 2019! Covid-19 was challenging our personal lives as well as our professional contacts. This is especially true for a network like ASEA-UNINET, which defines its existence through cooperation, exchange of ideas, mobility of researchers, etc.

As you can see in this report, ASEA-UNINET has found ways to cope with those challenges. Our network is alive and active. We had to change the ways and means for cooperation, but we continue to work together, to develop innovative project ideas, to learn from each other and to benefit from the diversity that our network offers in many dimensions. This is a significant step towards our vision of being as a role model for cooperation and quality research across different regions worldwide. We have not only produced significant contributions to sustainability research but proven to be sustainable as a network! I would like to thank all of you for your continuing efforts in keeping ASEA-UNINET alive!

Gabriele Kotsis
Scholarships provided by or with the participation of ASEA-UNINET in 2020 and in 2021

The table below lists the scholarships by category to show the source of funding.

A) Ernst Mach Grant ASEA-UNINET

The Ernst Mach Grant ASEA-UNINET within the framework of the ASEAN-European Academic University Network (ASEA-UNINET) enables student and researcher stays in Austria of three months or more. The target groups are PhD students, Postdocs, Postgraduates and (Under-)Graduates in research fields and in fields of music practice, typically coming from ASEAN member institutions of ASEA-UNINET.

Ernst Mach Grant ASEA-UNINET Mobilities 2020

<table>
<thead>
<tr>
<th>Country</th>
<th>PhD</th>
<th>Postdoc</th>
<th>Sandwich</th>
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<tr>
<td>The Philippines</td>
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<tr>
<td>Thailand</td>
<td>4</td>
<td>5</td>
<td>1 Music</td>
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<tr>
<td>Vietnam</td>
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</table>

Ernst Mach Grant ASEA-UNINET Mobilities 2021

| Country     | PhD  | Postdoc | Sandwich | Music |
|-------------|------|---------|----------|
| Indonesia   | 15   | 2       | 1 Sandwich | 1 Music |
| The Philippines | 2  |       | 1 Music    |        |
| Thailand    | 11   | 4       | 2 Sandwich | 4 Music |
| Vietnam     | 8    | 2       | 1 Music    |        |

B) Ernst Mach ASEA-UNINET Short-Term Research Grant (NEW!)

The Ernst Mach ASEA-UNINET Short-Term Research Grant within the framework of the ASEAN-European Academic University Network (ASEA-UNINET) enables Postdocs at the ASEAN member institutions of ASEA-UNINET a research stay in Austria of one or two months.

Ernst Mach ASEA-UNINET Short-Term Research Grant Mobilities 2021

<table>
<thead>
<tr>
<th>Country</th>
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<tr>
<td>Indonesia</td>
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C) Grants with the participation of ASEA-UNINET

**Overseas Scholarships for PhD (Pakistan)**

Overseas Scholarships for PhD in Selected Phase II and III (OSSII and III) are scholarship programmes funded by the Higher Education Commission Pakistan (HEC) and administered by the OeAD. The selection committee is composed of HEC, Pakistani professors and professors of ASEA-UNINET.

OSS II 2020: 17 Mobilities  
OSS II 2021: 3 Mobilities  
OSS III 2021: 1 Mobility

**Indonesia-Austria Scholarship Programme (IASP)**

Indonesia-Austria Scholarship Programme (IASP) is a scholarship programme for Indonesian Postgraduates, who are qualified for a Doctoral/PhD programme in Austria. The selection of candidates is conducted in cooperation with ASEA-UNINET. IASP is financed by the Ministry of Education and Culture (KEMENDIKBUD) and the OeAD.

IASP 2020: 15 Mobilities  
IASP 2021: 23 Mobilities
Reports

of the

ASEA-UNINET

Austrian Member Universities
Researchers from the University of Innsbruck had applied for projects in both the 2020 call and the 2020-2021 call. Because of the travel restrictions due to the COVID-19 pandemic, unfortunately none of these projects could be carried out.
Molecularly Imprinted Polymer Matrices for Diagnostic Applications: Synthesis and Characterization of Molecular Imprinted Polymer Nanoparticles (Nano-MIP) Against Equine Spleen Ferritin

Project Report “ASEA 2020 / UNI WIEN / 4”

Project Team

Univ.-Prof. Dr. Peter A. Lieberzeit, University of Vienna, Department of Physical Chemistry (Project Leader): Full professor since 2011, specialized in the design of novel “artificial antibodies” based on self-organization and the design of mass-sensitive, optical and electrochemical sensors. Currently 134 papers in Web of Science and roughly 200 conference contributions. Holder of the Fritz Feigl Award of the Austrian Society of Analytical Chemistry and the ISOEN Wolfgang Göpel Award. Member of the Editorial Board of “Sensors and Actuators B: Chemical” (IF=7.0). Organizer and General Chair of the “17th International Meeting on Chemical Sensors – IMCS2018” and Chairman of the International Steering Committee of IMCS conferences. Collaborations in Southeast Asia since 2009.

Prof. Dr. Kiattawee Choowongkomon, Kasetsart University Bangkok, Faculty of Science, Department of Biochemistry: BSc from Chulalongkorn University, Bangkok; MSc in Biochemistry from Lehigh University, PA, USA; PhD in Cell Physiology from Case Western Reserve University, OH, USA. Currently Associate Professor at Kasetsart University, Bangkok. He is mainly interested in Protein Purification, Protein Structure, Protein NMR, Protein Crystallography, Cloning and Expression Protein, Protein Simulation, Biosensors. Currently 96 papers in Web of Science. Broad interest in both fundamental and applied sciences.

Supaporn Klangprapan, PhD Kasetsart University, Bangkok.
Figure 1 summarizes the synthesis procedure: nNano-MIPs were synthesized from polymerization of mix monomers and crosslinker; N-tertbutylacrylamide (TBAm), N-isopropylacrylamide (NIPAm), acrylic acid (AAC), N-(3-aminopropyl) methacrylamide hydrochloride (APMA) and N,N'-methylenebisacrylamide (BIS) in presence of ferritin on solid phase. After 3 h polymerization at 39°C, the resulting “MIP nanobodies” were eluted from the solid support. Ideally, they retain recognition cavities that are complementary to ferritin in terms of size, shape and functionality. The morphology and structure of the nano-MIP were characterized using scanning electron microscopy (SEM). The interaction of nano-MIP and ferritin was characterized by a direct assay with a quartz crystal microbalance (QCM).

![Figure 1. Schematic of synthesis PCV2 Ferritin nanoparticles](image)

1) Characterization of nanoparticles

The nanoparticles were first eluted with milli-Q water 39°C and then filtrated to remove large particles. Figure 2 shows SEM images revealing some of the resulting nano-MIP: they are spherical with diameters of around 120-150 nm. Some of them are visible as individual nanoparticles, whereas others agglomerate on the silicon wafers (Figure 2A). Overall, nano-NIP (NIP: non-imprinted polymer) are spherical and smaller than nano-MIP with various sizes 65-82 nm in diameter (Figure 2B).
2) Immobilization of Ferritin on QCM surfaces

In a first step it was necessary to modify QCM gold electrode with cysteamine to generate -NH₂ functionalities on the surface. Those serve as anchor groups for glutaraldehyde that in turn helps immobilizing Ferritin on the device surface. Figure 3A shows SEM images of ferritin with spherical shape and 17-20 nm in diameter distributed on the QCM surface. This confirms that ferritin is attached to the sensor and ready for direct binding assay between the protein and the synthesized particles. For that purpose, we exposed the modified QCM to nano-MIP for 30 min and washed with water to remove unbound nano-MIP. As one can see, some nano-MIPs (yellow arrow) are present on the corresponding surface (Figure 3B). This shows that nano-MIP actually bind to ferritin, though a large number of immobilized ferritin molecules remains on the surface.

Figure 3. SEM images of A) ferritin attached to the QCM gold electrode B) surface of sensor after exposing it to 100 µg/mL of nano-MIP for 30 min. Red arrows denote ferritin, yellow arrows nano-MIP.
3) QCM binding studies of ferritin and nanoparticles

Figure 4A shows the sensor responses toward nano-MIP at concentrations of 12.5, 25, 50, 75, 100 and 150 µg/mL leading to Δf = 29, 43, 65, 96, 130 and 150 Hz, respectively. Evidently, the frequency shift increases when increasing concentrations of nano-MIPs. Furthermore, nano-MIP show negligible no interaction on channel 2 (red line), which contains only cysteamine on the gold surface. This result demonstrates that nano-MIP actually bind to ferritin, while nano-NIPs shows Δf=10 Hz at concentrations of 75 and 100 µg/m, which is basically negligible.

![Figure 4A: QCM sensitivity test of PCV2 and reference to A) nano-MIP nanoparticles and B) nano-NIP. Responses of channels marked in blue result from Ferritin, those in red from nonspecific interactions with cysteamine.](image)

4) Selectivity

We assessed selectivity of nano-MIP against proteins that are present in the blood system, such as human serum albumin (HSA) and γ-globulin. For that purpose, we modified the two electrodes of a QCM with ferritin on one electrode (channel 1, blue line) and HSA or γ-globulin on the other (channel 2, red line), respectively. Figure 5 shows the outcome when exposing such devices to solutions containing 150 µg/mL nano-MIP: on average, the signal responses of the ferritin-coated channel are 3 times higher than HSA channel with resulting frequency shifts Δf = -150 Hz and -50 Hz, respectively (Figure 5A). While ferritin and γ-globulin coated channels show signal responses around -50 Hz (Figure 5B). This preliminary result clearly shows selectivity towards HSA, but the need to further optimize the system with regard to γ-globulin.
Figure 5. Selectivity tests of nano-MIP. A) Ferritin vs HSA sensor surfaces towards nano-MIP. B) Ferritin vs γ-Globulin sensor surfaces towards nano-MIP.

Currently, there is one manuscript in preparation.
Theoretical Methods in Drug Design

Project leader:
A.Univ.Prof. Dr. Peter Wolschann, University of Vienna, Institute of Theoretical Chemistry

Cooperation members:
Univ.Prof. Dr. Supot Hannongbua, Chulalongkorn University, Faculty of Science
Assoc.Prof. Dr. Thanyada Rungrotmongkol, Chulalongkorn University, Department of Biochemistry

Invited persons (November 2021):
Dr. Khanittha Kerdpol, Chulalongkorn University, Department of Chemistry
Dr. Sarinya Hadsadee, Chulalongkorn University, Department of Biochemistry
Mr. Napat Kongtaworn, MSc, Chulalongkorn University, Program in Bioinformatics and Computational Biology

Introduction

Drug design is very important for pharmacy and medicinal chemistry. Beyond experimental techniques, like X-ray crystallography, NMR spectroscopy and cryo-electron microscopy, theoretical methods are of essential interest. In particular, molecular dynamics simulations as well as protein folding are methods which are under development using increasing computer facilities and they are frequently used for modeling of drug targets. A rather recent break-through method – Alphafold – will tremendously increase the possibilities for the prediction of protein structures. The drug candidates interacting with various targets can be characterized by the complex geometries and, rather important, by the drug-target interaction energies, based on several methods, like molecular mechanics generalized Born surface area (MM-GBSA), molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) methods or the solvated interaction energy (SIE) procedure. As new methods are continuously und development they have to be tested by application on many biological systems.

The main topic of the visit of the three scientists from Chulalongkorn University was the training of theoretical methods (AMBER20 and Gaussian19) at large computer systems (VSC3 and VSC4).

Project details: The project of Dr. Kanittha Kerdpol concerns studies of the inclusion complexation of emodin with various β-cyclodextrin derivatives in order to enhance the water solubility of this poorly soluble drug. Emodin (6-methyl-1,3,8-trihydroxyanthraquinone) stands out for anti-cancer activities toward various cancer cell lines via DNA intercalation, cell cycle arrest and induction of apoptosis. However, its usage in pharmaceutical applications has been restricted due to its poor aqueous solubility. To address this issue, a series of complexation of emodin with βCD and its derivatives: hydroxypropyl-β-cyclodextrin (HPβCD), 2,6-di-O-methyl-β-cyclodextrin (DMβCD), and sulfobutylether-β-cyclodextrin (SBEβCD), were performed to identify the most promising drug carrier
for emodin with regards to water solubility enhancement and augmented biological properties through host-guest complexation. The best stability constant was observed in ED/DMβCD complex at 25°C. The resulting negative ΔG_{bind, exp} values indicates that the formation of inclusion complexes was spontaneous and energetically favorable. The potent cytotoxicity of ED and all studied complexes were investigated using cholangiocarcinoma cell lines. Molecular dynamics simulations will be performed to explain the host-guest interaction at molecular level.

A manuscript entitled *In vitro* studies on inclusion complexation of emodin with various β-cyclodextrin derivatives is already on the way to be submitted for publication.

The project of Dr. Sarinya Hadsadee concerns theoretical considerations on the substrate binding mechanism of organophosphate pesticides on methyl parathion. The draft version of a publication is enclosed.

Mr. Napat Kongtaworn has been working on N501Y mutation in receptor binding domain of SARS-CoV-2 spike protein strengthens its binding to human angiotensin-converting enzyme 2 receptor. Evidently, this topic is of actual interest and the research has to be processed rather rapidly. The abstract of a provided summary is given here: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been indicated as a world pandemic. The SARS-CoV-2 has spread to over 85 million confirmed cases and over 1.8 deaths in January 2021 by the World Health Organization (WHO) (WHO, 2021). Although most The recent N501Y mutation in the receptor binding domains (RBD) of SARS-CoV-2 spike protein has been reported to increase its binding efficiency to the human angiotensin-converting enzyme 2 (ACE2) receptor, enhancing viral infection and reducing vaccine effectiveness. In this work, the structural effect of N501Y RBD on the binding to ACE2 was investigated using all-atom MD simulations and free energy calculations based on solvated interaction energy method. The obtained results revealed that the binding affinity toward ACE2 of N501Y RBD (-14.19 ± 0.74 kcal/mol) was higher than that of WT RBD (-13.71 ± 0.71 kcal/mol), consistent well with the lower water accessibility at the protein – protein interface and the higher compactness of N501Y RBD/ACE2 complex, driven by a formation of π-π interaction (Y501-Y41). Moreover, the increased susceptibility of hot-spot residues of N501Y RBD, including F486, N487, P490, P492, and Q493, especially Y501, resulted in promoting the formation of H-bonds and contacting atoms. These structural and energetic information could be useful for the design of novel vaccines or inhibitors against the newly emerging coronavirus strains.

Preliminary draft version of the manuscript of Dr. Sarinya Hadsadee

Substrate binding mechanism of methyl parathion hydrolase towards organophosphate pesticides

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²Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
³Department of Theoretical Chemistry, University of Vienna, Währinger Strasse 17, Vienna 1090, Austria
⁴Center of Excellence in Hazardous Substance Management, Chulalongkorn University, Bangkok 10330, Thailand
⁵Program in Bioinformatics and Computational Biology, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand
1. Introduction

Organophosphates (OP) have been used for the major application in chemical warfare agents and pesticides. OP pesticides accounts for total of 38% of global pesticide consumption. OP compounds are the main components of herbicides, pesticides, and insecticides. OP compounds are also the main components of nerve gas. Moreover, OP pesticides residue has found in agriculture leading to the environmental pollution.

Besides, these OP pesticides are also significant adverse effects on animals and non-target species including humans. Due to their acetylcholinesterase inhibitory activity have a profound effect on the nervous system. OP pesticides are classified based on chemical structure. For example, phosphotriester consists of phosphate center with three $O$-linked groups such as methyl-paraoxon (MPO) and dichlorvos (DDVP). Whereas, thiophosphotriesters have the sulfur replaced the phosphoryl oxygen such as profenofos (PF).

To overcome this environmental hazard, “green and clean” is required to degrade these pesticides. The enzymatic bioremediation is known to contribute the hydrolysis of OP pesticides. Substantial enzymes show the identify to promote the degradation of OP pesticides including phosphotriesterase (PTE), organophosphorus hydrolases (OPHs), serum paraoxonase (PONs), methyl parathion hydrolases (MPHs), diisopropylfluorophosphate fluorohydrolase (DFPase), organophosphate acid anhydrolases (OPAAs), and Phosphotriesterase-Like-Lactonases (PLLs). The OP-degrading enzyme was first discovered in 1946, which hydrolyzed diisopropylfluorophosphate (DFP). Later in 1973, the researcher found that the OP diazinon was hydrolyzed from the soil bacterium *Flavobacterium sp.* (strain ATCC 27551). *Agrobacterium radiobacter, Enterobacter aerogenes,* and *Pseudomonas diminuta* were later observed to have enzymes that degrade OPs.

Specifically, the methyl paraoxon hydrolases (MPHs) are encoded by *mpd* (methyl parathion degradation) genes. The *mpd* genes were first identified in *Plesiomonas* sp. strain M6. Subsequently, *mpd* genes are discovered in various bacterial species such as *Achromobacter, Ochrobactrum, Stenotrophomonas* and *Pseudomonas*. However, the *mpd* genes are found that they do not share sequence homology with other OP-degrading genes. In addition, MPH is a one catalytic activity enzyme for hydrolysis of OP, and MPH has ability to degrade OP pesticides. The MPH enzyme is a dimeric protein as shown in Figure 1, and each subunit contains a mixed hybrid binuclear zinc center.
Figure 1. The 3D structure of homodimer MPH, in which chain A and B are shaded by deep blue and light blue colors, respectively. The close-up regions for active site; Zn metal ions with its coordinating amino acids

The monomer structure is composed of αβ/βα sandwich typical of the metallo-hydrolase/oxidoreductase fold. Two internal mixed β-sheets are flanked either side by three solvent-exposed α-helices. Each subunit is composed of a β-lactamase-like domain, which includes the binuclear metal center. The binuclear metal site is located between the two β-sheets and is surrounded by two αβ-loops. The residues W179, F196 and F119 are three residues that create an aromatic cluster at the entrance of the catalytic center. It is seen that Zn$^{2+}$ is the native metal ion for this enzyme. However, the metallo-β-lactamase (MBL) super family enzymes can be constituted with other metal ions such as Fe$^{2+}$, Mn$^{2+}$, Co$^{2+}$ and Ni$^{2+}$.

Computationally studies are carried out to understand the mechanism of this enzyme. First, nucleophilic attack terminal hydroxide ion. Next, the bulky substrate using the virtue of the large active site volume of MPH binds in-line for nucleophilic attack on the organophosphate. The nucleophilic attacks the aryl ester, which requires very different binding modes for the substrates, resulting also in transition state stabilization from different active site residues.

Here in this study, we present the substrate binding mechanism of MPH enzyme towards different classes of OP pesticides such as phosphotriesters, thiophosphotriesters. The substrate binding mechanism of dichlorvos (phosphotriester) and profenofos (thiophosphotriesters) is compared with natural substrate methyl paraxon (phosphotriester). The molecular structure is as depicted in in Table 1. The promiscuity of the metal ion with respect to cobalt and zinc is also analyzed with respect to binding of these substrates. Since recently this enzyme is known to be evolved for its the ability to hydrolyze a wide range of man-made organophosphates. The binding mode of the substrate for this is key to enzyme catalysis. There have been very less computational studies carried out on this enzyme that elucidates the mechanism of substrate binding. The information presented in here could provide detailed insights of OPs binding to the enzyme and how the active site modifies itself to fit in various classes of OP pesticides.
2. Computational Methods

2.1 System preparation and Molecular Docking

The three-dimensional structures of MPH from *Ochrobactrum* sp. has not been resolved yet. The sequence alignment between the MPH from *Ochrobactrum* sp. and *Pseudomonas* sp. was performed by BLAST. Since the similarity was 99.7% (One residue mismatch in signal peptide sequence) the Zn(II) bound MPH from *Pseudomonas* sp. WBC-3 with a 2.4 Å resolution (accession code 1P9E) was used as initial native structure. Initial native structure, Co and Zn metals were contained in structure of MPH. To create our initial structure, the Zn(II) metal was replaced with Co(II) in discovery studio. The protonation states of all ionizable amino acids (D, E, K, R and H) were assigned at pH 8.0 using PROPKA3.1. Their environments were also visually explored by considering the possibility of hydrogen bonding with the surrounding residues. The charges were calculated by using Gaussian. The AMBER ff14SB force field was applied for the protein. Methyl paraoxon, dichlorvos and profenofos were built by using the Gaussview6.0 program. Substrate parameterization for theses pesticides were generated by antechamber module implemented in AMBER16 with using general AMBER force field (GAFF) by parmchk program. Partial charges were set to fit the electrostatic potential generated at the HF/6-31G(d) level by the RESP model. Seven OP pesticides were docked with 100 independent runs into the active site of the MPH using CDOCKER module in the Accelrys Discovery Studio 2.5Accelrys Inc for MD simulations. The amino acid residues surrounding the metal ions such as H147, H149, H234, D255 and H302 were chosen to define the binding site for the docking process. The lowest distance between the phosphodiester bond and the metal ion were selected to start for MD simulations. Additionally, we also checked the orientation of the pesticide that nucleophilic attack in-line. The suitable docked conformers for the MPH and pesticide molecule were selected for subsequent molecular dynamics (MD) simulation. Afterward, the missing hydrogen atoms were added by using the LeaP module of

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<td>Dimethyl</td>
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AMBER16. The system of AMBER16 LEAP module was immersed in a pre-equilibrated truncated cubic box of TIP3P water molecules with an internal offset distance of 10 Å. The box dimension was $115 \times 115 \times 115$ Å$^3$, with approximately 20300 water molecules. All systems were neutralized with explicit Cl- counterions. Calculations were performed with ff14SB build in Amber force field and TIP3P water model. Geometry optimization was carried out in two steps. Firstly, the solvent and counter ions were initially minimized by 1500 steps of steepest descent (SD) and 3000 steps of conjugate gradient (CG) methods, while the other molecules were restrained by using a force constant of 500 kcal/mol·Å$^2$. The second step, unrestrained minimization of all the atoms in the simulation cell was carried out. The protein was minimized by SD (1000 steps) and CG (2500 steps) methods with a constrained solvent. Finally, the SD (1000 steps) and CG (2500 steps) minimizations were fully applied to the whole system. Moreover, the systems were heated under constant volume and periodic boundary conditions from 0 to 310 K.

2.2 Molecular dynamics (MD) simulations

MD simulations were simulated based on the standard procedure using isothermal–isobaric ensemble (NPT) at a constant pressure of 1 atm equilibrated at 310 K for 500 ns. The calculated simulation time step was set to 2 fs under periodic boundary condition. Hydrogen bonds restrain in structure for their equilibrium lengths were set by the SHAKE algorithm. The particle-mesh of Ewald’s summation method was used for non-bonded interactions, a cut off at 10 Å was performed. The constant temperature and pressure were controlled by using a Langevin dynamics and Berendsen barostat, respectively. MD simulations of the complexes were performed by the AMBER16 software package coupled with the PMEMD module. The trajectories were collected for every 10 ps. For the RMSD calculations, the CPPTRAJ package was provided in Amber tools that was used to generate trajectories for each residue and at an amino acid resolution. The individual chain of MPH (chain A and chain B) and the pesticide was plotted along simulation time as shown in Supplementary Figure 1. The RMSD results of the MPH–pesticide complexes were found that the simulations were fairly stable. Finally, the last 50 ns (450–500 ns) of MD simulations were considered. Trajectory analysis was carried out using cpptraj in AMBER16 package. Binding free energy and per residue energy contribution were calculated by MM/PBSA method.

3. Results and discussion

3.1 Substrate binding

Methyl parathion hydrolase enzyme was found in soil dwelling bacteria that uses methyl parathion as sole source of carbon and nitrogen. In this study, the substrate binding of Ochrobactrum MPH against with three different OPs pesticides including methyl paraxon, dichlorvos and profenofos were determined and compared. Additionally, the MPH enzymes were performed by using cobalt and zinc. In this work, we docked three pesticides in the active site of zinc bound and cobalt bound MPH in cDocker embedded in discovery studio. 100 poses were obtained for each system and the pose with lowest distance between the pesticide and the metal ion. The lowest interaction energy was considered as initial structure. The active site is composed of His and Asp residues surrounding the metal ions. The hydrophobic pocket is help to accommodate the substrate, and the substrate is fitted in the pocket. Figure 2 depicts the selected docked structures. It must be noted that the water molecule close to the two metal ions. Therefore, form of the crystal structure was retained the role in catalysis. Six system interactions between enzymes and substrate were investigated. The results show that all the six systems exhibited
good interaction energy (Table 2) in range of -32 to -36 kcal/mol. Thus, six systems were used as initial structures for molecular dynamics simulations.

Molecular dynamics simulations were carried out in triplicates for all the 6 systems for 500 ns using AMBER16. The system stability was analyzed by RMSD calculations, and it was found that all the systems were stable throughout the simulations (supplementary figure 1). The MPH enzyme contains 2 metal ions in its active site. As shown in Figure 2, the active sites of two enzymes are very similar, but both enzymes have different divalent cation metals, namely Co(II) and Zn(II). The bond distance of two metals ion were investigated.

The distance and position of two metal ions from one and another can be calculated to deduce the stability of both the metal ions and hence the probability of higher enzyme activity. In structure of Co(II) metal, the distance between the two metal ions in the crystal structure was found to 3.5 Å. For the structure of Zn(II) metal, the stimulations the distance between the Zn metal ions was found be stable between 3.0 - 3.25 Å. Whereas the cobalt metal ions had a distance in the range of 3.25 - 3.6 Å. The difference in the distance was attributed to the radius of the metal ion. The distance between the metal ions and the nature of metal ions was found to very important specifically when nonspecific substrates were catalyzed. Obviously, cobalt-MPH systems show that Co-Co distance were obtained slightly higher in dichlorvos comparison to the methyl paraxon and profenofos. Besides, the best results of the three replicates is considered.

![Figure 2. Active site of initial structures for MD simulations of all the systems](image_url)
Figure 3. Distance between α and β ions located in the MPH active site in complex with organophosphate pesticides

The active structure of the enzyme consists of a bi-nuclear metal ion coordinated to aspartate and histidine residues. The alpha metal ion is embedded deep inside with D151, D255, H152 and H302. In contrast, the beta metal ion is coordinated to D255, H147, H149 and H234. Both the metal ions are also bridged together by a water molecule which plays a role in hydrolysis of the substrate. We calculated the bond distance between the metal ions and residues. It was observed that none of the pesticides coordinated with alpha metal ion throughout the simulations. The coordination number of the alpha metal ion varied for the different pesticides. We can hypothesize that this change in the coordination number is due to subtle changes in the geometry which occurs to accommodate the side chains of the substrate. Further, the coordination of the beta metal ion to the amino acids changed completely to accommodate the pesticides. The metal ion lost the coordination with the histidine residues. The coordination distance between the beta metal ions and the pesticides were in the range of 1.90 – 1.98 Å. These results show that the geometric constraints at the beta metal ion could be attributed to the in-line nucleophilic attack on the organophosphate pesticide. These results correspond to the hypothesized mechanism of the enzyme earlier. However the changes in the coordination number in different pesticide systems can probe that they have different binding strategy.
Table 2. Distance (Å) of the α and β ions with surrounding amino acids, pesticide, and water.

<table>
<thead>
<tr>
<th>ALPHA</th>
<th>Metal ion</th>
<th>D151</th>
<th>H152</th>
<th>D255</th>
<th>H302</th>
<th>ROH (Crystal water)</th>
<th>Pesticide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal ion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl paraxon</td>
<td>Cobalt</td>
<td>1.83 ± 0.04</td>
<td>2.72 ± 0.38</td>
<td>1.87 ± 0.06</td>
<td>2.21 ± 0.06</td>
<td>1.80 ± 0.12</td>
<td>3.84 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>1.79 ± 0.04</td>
<td>3.53 ± 0.33</td>
<td>1.79 ± 0.04</td>
<td>4.04 ± 0.83</td>
<td>1.79 ± 0.09</td>
<td>5.11 ± 0.06</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>Cobalt</td>
<td>1.87 ± 0.05</td>
<td>2.30 ± 0.21</td>
<td>4.17 ± 0.20</td>
<td>2.10 ± 0.12</td>
<td>1.77 ± 0.04</td>
<td>3.98 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>1.75 ± 0.03</td>
<td>2.01 ± 0.11</td>
<td>1.74 ± 0.03</td>
<td>2.04 ± 0.11</td>
<td>1.79 ± 0.05</td>
<td>4.50 ± 0.04</td>
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<tr>
<td>Profenofos</td>
<td>Cobalt</td>
<td>1.85 ± 0.05</td>
<td>2.07 ± 0.08</td>
<td>4.88 ± 0.58</td>
<td>1.83 ± 0.05</td>
<td>2.18 ± 0.18</td>
<td>1.75 ± 0.04</td>
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<tr>
<td></td>
<td>Zinc</td>
<td>1.84 ± 0.05</td>
<td>4.88 ± 0.58</td>
<td>1.83 ± 0.05</td>
<td>2.18 ± 0.18</td>
<td>1.75 ± 0.04</td>
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<tr>
<th>BETA</th>
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<th>H147</th>
<th>H149</th>
<th>H234</th>
<th>D255</th>
<th>ROH (Crystal water)</th>
<th>Pesticide</th>
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<td></td>
<td></td>
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<tr>
<td>Methyl paraxon</td>
<td>Cobalt</td>
<td>5.36 ± 0.27</td>
<td>4.55 ± 0.42</td>
<td>6.06 ± 0.21</td>
<td>1.89 ± 0.06</td>
<td>1.80 ± 0.04</td>
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<tr>
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<td>Zinc</td>
<td>5.05 ± 0.55</td>
<td>4.22 ± 0.52</td>
<td>6.84 ± 0.68</td>
<td>1.81 ± 0.04</td>
<td>1.80 ± 0.04</td>
<td>1.90 ± 0.06</td>
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<tr>
<td>Dichlorvos</td>
<td>Cobalt</td>
<td>4.69 ± 1.28</td>
<td>5.53 ± 0.66</td>
<td>5.29 ± 0.76</td>
<td>2.74 ± 0.76</td>
<td>1.78 ± 0.04</td>
<td>1.91 ± 0.10</td>
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<tr>
<td></td>
<td>Zinc</td>
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<td>2.33 ± 0.16</td>
<td>8.12 ± 0.83</td>
<td>1.80 ± 0.05</td>
<td>1.78 ± 0.04</td>
<td>1.90 ± 0.04</td>
</tr>
<tr>
<td>Profenofos</td>
<td>Cobalt</td>
<td>4.90 ± 0.33</td>
<td>3.65 ± 0.83</td>
<td>6.36 ± 0.30</td>
<td>1.86 ± 0.05</td>
<td>1.81 ± 0.04</td>
<td>1.98 ± 0.08</td>
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<tr>
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<td>Zinc</td>
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<td>2.02 ± 0.08</td>
<td>1.99 ± 0.07</td>
<td>1.76 ± 0.04</td>
<td>1.73 ± 0.03</td>
<td>1.96 ± 0.55</td>
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</table>

3.2 Binding affinity and key binding residues

The binding affinities of the three pesticides in presence of zinc and metal ion in the active site was calculated by MM/PBSA method. The stable MD trajectories (450-500 ns) was used for energy calculations. A total of 500 snapshots were taken from the last 50 trajectories to analyze the binding energy. The counterions and water molecules were stripped. The final binding free energy was determined as the average of all the snapshots, and the standard errors were also reported (Table 3). The metal ion dependent binding changes was observed from the results. The results showed that the
binding free energy of cobalt-MPH was higher than zinc-MPH systems about 3-5 times. However, in the case of profenofos the zinc-MPH showed better binding in energy than the cobalt MPH. These results indicated that the different classes of organophosphates could have different mode of substrate binding.

Table 3.

<table>
<thead>
<tr>
<th></th>
<th>ΔG_{bind} (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cobalt</td>
</tr>
<tr>
<td>Methyl paraxon</td>
<td>-11.32 ± 6.28</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>-14.03 ± 2.06</td>
</tr>
<tr>
<td>Profenofos</td>
<td>-20.27 ± 2.34</td>
</tr>
</tbody>
</table>

To understand the role of amino acid, the contribution of substrate binding and per-residue binding free energy were calculated. Figure 5 depicted the contribution of individual amino acid residues in the chain A towards substrate binding. The residue V65, L67, F85, F196, L273 played important role in substrate binding. It must be noted that F85 and F196 were residues present in substrate binding pocket. These residue form π-π or π-alkyl interactions with the substrate. From these results, it could be deduced that all three pesticides bound to MPH enzyme with cobalt or zinc metal ion, and could subsequently undergo hydrolysis via nucleophilic attack.

Figure 5. Per-residue decomposition free energy (ΔG_{bind,residue}) of all the systems of the MPH
Conclusion

The metallohydrolase enzymes used for bioremediation must have two intrinsic properties. i.e. good stability of the metal ions and wide range of substrate specificity. In this research, we aimed to study the stability of metal ion and understand the substrate binding mechanism of nonspecific substrates in presence of two different metal ions. Both zinc and cobalt metal ion showed good stability throughout the simulations and aided in substrate binding. All the organophosphate pesticides could bind well in the active site and in the confirmation that allows successful the substrate catalysis. The difference in metal ion activity could be attributed by electrostatic properties of the metals themselves. From the analysis of our simulations, it is evident that subtle changes occur in the coordination geometry to accommodate the substrates for subsequent catalysis. These results could pave way for successfully using the MPH enzyme as an excellent bioremediator.

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Theoretical Studies of Functional RNA Structures in Flaviviruses Project

(ASEA 2020-2021/UniWien/1)

Project Report

March 2022

The project is part of a long-standing collaboration on theoretical aspects of virus-host interactions between Dr. Michael Wolfinger and Assoc. Prof. Dr. Thanyada Rungrotmongkol.

**Dr. Michael Wolfinger** ([michael.wolfinger@univie.ac.at](mailto:michael.wolfinger@univie.ac.at)) is a theoretical chemist at the research group Bioinformatics and Computational Biology, Faculty of Informatics, and the Department of Theoretical Chemistry, Faculty of Chemistry, University of Vienna. His main research field is computational prediction of RNA structure. He is particularly interested in viruses and has been working in virus bioinformatics for more than seven years. To date, he has authored more than 40 international publications with h-index of 21.

**Assoc. Prof. Dr. Thanyada Rungrotmongkol** ([t.rungrotmongkol@gmail.com](mailto:t.rungrotmongkol@gmail.com)) is a lecturer in Department of Biochemistry, Faculty of Science, Chulalongkorn University since 2011. Her research career has been devoted to the potential of uniquely detailed, atomic-level insight into biological processes of molecular recognition, structural and dynamics properties of proteins by computational simulations. To date, she has contributed to more than 150 international publications with h-index of 28.

**Nitchakan Darai, MSc** is a PhD student of Prof. Rungrotmongkol at Chulalongkorn University, Bangkok.

**Background**

RNA viruses are responsible for a wide range of diseases in humans, that may result in clinical manifestations such as arthralgia, respiratory syndromes, neurologic disorders and life-threatening haemorrhagic fevers. Despite enormous efforts of unraveling and elucidating characteristic traits of many RNA viruses over the last decades, our understanding of the biology and biochemistry of this important class of pathogens is far from being comprehensive. There are currently more than 220 recognized species of RNA viruses, many of which are zoonotic viruses with ancestral transmission cycles in wildlife. Flaviviruses, including emerging and re-emerging pathogens like Dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV) and Zika virus (ZIKV), cause millions of infections every year, thus representing a major health threat. Importantly, many Flaviviruses are neurotropic. Relatively little is known about the biochemical principles of flavivirus neurotropism, however, recent experimental evidence suggested an active role of the Musashi family of proteins, a group of host RNA-binding proteins, in promoting ZIKV replication, neurotropism, and pathology [1].
Musashi is a highly conserved family of proteins that typically act as translational regulator of target messenger RNA (mRNA) and is involved in cell proliferation and differentiation. While the two Musashi paralogs in mammals, Musashi-1 (Msi1) and Musashi-2 (Msi2), are expressed in stem cells and overexpressed in tumors and leukemias, they are absent in differentiated tissue. Musashi proteins have two RNA recognition motif (RRM) domains, which are also referred to as RNA-binding domains 1 and 2 (RBD1 and RBD2), that have been shown to bind short RNA motifs in a single-stranded structural context, preferentially within the 3’UTR of mRNA. The trinucleotide sequence UAG has been identified as core Musashi binding element (MBE), and its thermodynamic binding specificity was determined by fluorescence polarization assays.

**Objective**

In this project, we set out to assess the three-dimensional structure of the MSI1 RBDs in complex with RNA. To this end, we studied association complexes of MSI1 RNA-binding domains 1 and 2 with different RNA motifs, employing molecular dynamics (MD) approaches to gain more insight into the molecular traits of this type of RNA-protein binding.

**Results**

Our studies are based on published MSI1-RNA complexes, i.e. PDB IDs 2RS2 and 5X3Z, which contain the two RBD domains in complex with the RNA motif GUAGU. We set out to mutate individual RNA nucleotides to study the energetics of MSI1 binding to alternative RNA motifs. To this end, we selected three additional pentamers, i.e., GUUGU, GGAGU, and GAUGU, whose central trinucleotides exhibited high, medium, and low affinities, respectively, in a recent study that assessed the RNA opening energies within the thermodynamic ensemble of ZIKV 3’UTRs.

Analysis of the protein structures without RNA from the PDB with Alphafold2-predicted geometries confirmed that protein structures of the binding domains are highly similar, suggesting that no conformational changes on the protein occur upon binding of the RNA.

Our data show that i) the central trinucleotides of the RNA pentamers are more rigid than the flanking nucleotides, and ii) the flanking nucleotides lack interaction with MSI1 RBDs, suggesting that MSI1 RBD1 and RBD2 require the central trinucleotides for recognition. This is in agreement with earlier studies. Furthermore, our MD simulations show that the central trinucleotides of the RNA motifs exhibit a significantly lower distance to the MSI1 RBDs than the enclosing nucleotides, suggesting that they play an important role in the interaction of MSI1-RBD1 and RBD2 with RNA. Moreover, we identified key residues of the Musashi RBDs that are involved in RNA recognition.

In addition, we assessed the binding free energies, as well as decomposition energies of the MSI-RNA complexes with different methods, highlighting the contributions of different RNA nucleotides, and protein residues to the MSI-RNA complexes.

In summary, our study is a showcase for the feasibility of this kind of theoretical RNA-protein investigations and highlights the selectivity of MSI-RNA interactions. A manuscript entitled “Musashi 1 RNA–binding protein in complex with RNA: A theoretical study”, authored by Nitchakan Darai,
Panupong Mahalapbutr, Peter Wolschann, Vannajan Sanghiran Lee, Michael T. Wolfinger, and Thanyada Rungrotmongkol has been submitted to Scientific Reports [7].

References


Musashi 1 RNA–binding protein in complex with RNA: A theoretical study

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Abstract

The Musashi (MSI) family of RNA–binding proteins, comprising the two homologs Musashi–1 (MSI1) and Musashi–2 (MSI2), typically regulates translation and is involved in cell proliferation and tumorigenesis. MSI proteins contain two ribonucleoprotein–like RNA–binding domains, RBD1 and RBD2, that bind single–stranded RNA motifs with a central UAG trinucleotide with high affinity and specificity. The finding that MSI also promotes the replication of Zika virus, a neurotropic Flavivirus, has triggered further investigations of the biochemical principles behind MSI–RNA interactions. However, a detailed molecular understanding of the specificity of MSI RBD1/2 interaction with RNA is still missing. Here, we performed computational studies of MSI1–RNA association complexes, investigating different RNA pentamer motifs using molecular dynamics simulations with binding free energy calculations based on the solvated interaction energy method. Simulations with Alphafold2 suggest that predicted MSI protein structures are highly similar to experimentally determined structures. The binding free energies show that two out of four RNA pentamers exhibit a considerably higher binding affinity to MSI1 RBD1 and RBD2, respectively. The obtained structural information of MSI1 RBD1 and RBD2 will be useful for a detailed functional and mechanistic understanding of this type of RNA–protein interactions.

Keywords: Musashi proteins, RNA–binding domains, RNA–binding proteins, Alphafold2, MD simulation
Introduction

RNA–binding proteins (RBPs) are key regulators of numerous cellular processes, mediating different aspects of co– and posttranscriptional gene expression. They contain well–defined RNA–binding domains (RBDs) that confer sequence– and/or structure–specificity for endogenous target RNAs\(^1\). Examples of evolutionary conserved RBDs are the RNA recognition motif (RRM), the heterogeneous ribonucleoprotein (hnRNP) K–homology (KH) domain, and the C3H1 zinc–finger (ZF) domain. These bind to a relatively restricted set of the primary RNA sequence space, often utilizing additional contextual traits such as RNA secondary structure or base compositional context for additional specificity\(^2\).

The Musashi (MSI) protein family comprises a group of RBPs that act as translational regulators and are involved in the maintenance and self–renewal of neuronal progenitor and stem cells\(^3\). They have been initially identified in the central nervous system, where they are involved in the regulation of Notch signaling by binding to the mRNA of its antagonist Numb\(^4\). While MSI proteins are typically expressed in stem cells\(^5\), they are absent in differentiated tissue. Being evolutionarily conserved among invertebrates\(^6,7\) and vertebrates\(^8\), there has been emerging evidence that MSI proteins mediate biological processes that regulate the initiation and progression of various cancer cells, including colorectal, breast, lung, and pancreatic cancers, as well as leukemias and glioblastoma\(^9\).

The MSI gene has been duplicated in vertebrates, resulting in the two paralogs Musashi–1 (MSI1) and Musashi–2 (MSI2), each containing two ribonucleoprotein (RNP)–type RNA recognition motifs (RRMs) in their N–terminal regions, followed by a poly(A)–binding protein region. While the structures of the mouse MSI1 and MSI2 RRMs have been solved\(^10–12\) the sequence identity of the regions containing the two RRMs in mouse MSI1 and MSI2 is remarkably high at 86%\(^13\), suggesting a common RNA target motif. For MSI1, this has been determined as (G/A)U\(_n\)AGU (n=1–3) by an in vitro selection approach (SELEX)\(^4\). NMR titration experiments with a series of RNA oligomers revealed that MSI1 RBD1 and RBD2 bind to GUAG and UAG motifs with high affinity\(^13,14\). These data are in line with cross–linking and immunoprecipitation (iCLIP) studies, which revealed the trinucleotide sequence UAG in a single–stranded structural context, predominantly in the 3'UTRs of mRNAs, as a core Musashi binding element (MBE)\(^15,16\). Likewise, quantitative fluorescence anisotropy assays confirmed the binding specificity of the UAG trinucleotide, while nucleotides outside this core MBE have limited contribution to the overall binding free energy\(^17\).

A different aspect of MSI pathobiology has been recently elucidated, i.e., the role of MSI proteins as host factors in viral infections, specifically their capacity to promote Zika virus (ZIKV) replication\(^18\). ZIKV is a mosquito–borne Flavivirus (MBFV) that has been circulating for decades in Africa and Asia, often being misdiagnosed as dengue. During a ZIKV outbreak in the Americas 2015–2017, an unexpectedly high number of congenital malformations coupled with intrauterine growth restrictions, placental damage, and microcephaly has been associated with ZIKV infections\(^19\). While MBFVs are typically horizontally transmitted between arthropod vectors and vertebrate hosts, the capacity for transplacental passage aligns ZIKV with a handful of other MBFVs, including West Nile virus (WNV) and Powassan virus (POWV), that have been shown to cause placental infection and fetal neuropathology\(^20\). The presence of UAG–containing MBEs in the 3'UTRs of Flavivirus genomes, together with in vivo data revealing that MSI not only interacts with ZIKV RNA but also enhances viral replication, has led to the understanding that MSI is involved in ZIKV–induced neurotropism\(^18\). It has
been hypothesized that MSI might stabilize viral RNA, thereby maintaining a sufficient RNA level that is not translated but subjected to purposeful exoribonuclease degradation\textsuperscript{21}. The latter results in the production of short flavivirus RNA (sfRNA), which modulates cellular mRNA decay\textsuperscript{22} and antiviral interferon response\textsuperscript{23,24}. While these findings highlight the instrumental role of MSI in virus–associated cytopathicity, the biochemical foundations and mechanisms of the MSI–mediated congenital neuropathology remain elusive.

Computational prediction of the structural accessibility of RNA binding motifs is a promising approach for the characterization of RNA–protein binding sites. This idea has been applied to several eukaryotic RBPs, resulting in the observation that target site accessibility almost always increases the ability to predict sequence–specific RBP–RNA binding\textsuperscript{25}. We have recently addressed the question as to whether other Flaviviruses have a similar MSI–mediated neurotropic potential to ZIKV by analyzing the affinity of Musashi binding elements (MBEs) in 3'UTR regions to appear in a single–stranded structural context, which is a requirement for efficient MSI–RNA interaction\textsuperscript{21}. To this end, we have shown that the structural accessibility of MBEs along viral RNA molecules can be used as a proxy for predicting MSI–RNA interactions, thereby assessing the neurotropic potential of viruses. By employing a thermodynamic model of RNA folding based on the ViennaRNA package\textsuperscript{26}, we computed the average opening energy that is necessary to keep specific MBEs in an unpaired structural context, rendering them accessible for MSI RRM–RNA interaction. Our data highlighted that MBEs in the 3’ untranslated region (3’UTR) of ZIKV are highly accessible for MSI binding, thereby corroborating earlier studies that addressed the neurotropic potential of flaviviruses and alphaviruses\textsuperscript{20}.

Here we follow up on this idea and model the 3D structure of MSI RBDS with Alphafold2. Subsequently, we investigate MSI–RNA association complexes, employing molecular dynamics (MD) approaches to gain more insight into the molecular traits of this type of RNP binding. Specifically, we focus on the published MSI1 RDB1–RNA complex and MSI1 RDB2–RNA complex (PDB IDs 2RS2 and 5X3Z), as shown in Figures 1A and 1B, which were derived from NMR spectroscopy\textsuperscript{13}. Superimposition of MSI1 RBD1 and MSI1 RBD2 NMR structures and their sequence alignment are shown in Supplementary, Figures S1A and S1B. The RNA component of the complex comprises a canonical MBE with the pentamer sequence GUAGU. We set out to mutate individual RNA nucleotides to study the energetics of MSI1 binding to alternative RNA motifs. To this end, we selected three additional pentamers, i.e., GUUGU, GGAGU, and GAUGU, whose central trinucleotides exhibited high, medium, and low affinities, respectively, within the thermodynamic ensemble of ZIKV 3’UTRs\textsuperscript{21}.

![Figure 1](image-url)  
**Figure 1:** (A) Superimposition of the 20 NMR structures of MSI1 RBD1 (PDB ID: 2RS2) and (B) MSI1 RBD2 (PDB ID: 5X3Z) with the RNA pentamer GUAGU bound.
Materials and Methods

Protein structure prediction with AlphaFold2

AlphaFold2\textsuperscript{27} is an artificial intelligence (AI) approach for highly accurate protein structure prediction. In combination with MMseqs2\textsuperscript{28}, a program for protein sequence search within large databases and generation of high quality protein sequence alignments, AlphaFold2 is capable of simulating high accuracy structures for a wide range of proteins, for which structural data are unavailable. Here, we performed predictions for MSI1 RBD1 and MSI1 RBD2 using ColabFold\textsuperscript{29}, which couples MMseqs2 and AlphaFold2 in publicly available notebooks that can be executed on the Google Cloud infrastructure. We were specifically interested in determining the protein structures in the apo form and comparing these to structures available through PDB. The sequences of MSI1 RBD1 and MSI1 RBD2 were retrieved from PDB IDs 2RS2 and 5X3Z. The first candidate structure (model 1) of both RBDs from ColabFold was selected as the initial conformation to assess GUAGU binding to both RBDs by MD simulations.

Molecular dynamics simulations

The NMR structures of MSI1 RBD1/2:GUAGU complexes were retrieved from PDB IDs 2RS2 and 5X3Z. The LEaP module of AMBER16\textsuperscript{30} was used to construct complexes with three alternative RNA pentamers (GUUGU, GGAGU, and GAUGU) by modifying the central trinucleotides. The protonation states of RNA–protein complexes were computed using the PDB2PQR server at pH 7.4. The AMBER ff14SB and chiOL3 (OL3) force fields were employed for protein and RNA, respectively. According to standard procedures, the missing hydrogen atoms of each system were added by the LeaP module. The added hydrogen atoms were then minimized for 1000 steps by steepest descents (SD) and subsequently by 3000 steps of conjugated gradient (CG). Subsequently, solvation of each system was performed by TIP3P water molecules of approximately 6,800 atoms for RBD1 and 7,300 atoms for RBD2 in a periodic box at a distance of 12 Å apart from the protein surface, resulting in a box dimension of $63 \times 70 \times 62$ Å\textsuperscript{3}, and $70 \times 66 \times 63$ Å\textsuperscript{3}, respectively. The systems were neutralized using Na\textsuperscript{+} counter ions. Periodic boundary condition with isothermal–isobaric ensemble (NPT) ensemble and a step–size of 2 fs for the simulation time were applied. The water molecules and ions were then minimized with 1000 steps of the steepest descent (SD) and continued with 3000 steps of the conjugate gradient (CG) method. The entire system was fully minimized in the last step by the same minimization process. All bonds with hydrogen atoms were constrained using the SHAKE algorithm\textsuperscript{31}. MD simulations under periodic boundary conditions were performed five times for all systems using the AMBER16 software package.

The MD simulation started by heating up the system from 10 to 310 K. Next, the system was equilibrated at a constant temperature of 310 K. 100 ns MD simulation was performed under NPT conditions at 1 atm and 310 K. The last 20 ns MD trajectories were taken for structural and energetics analyses. Root–mean–square displacement (RMSD) and distance between the centers of mass of protein and RNA were calculated by the cpptraj module of AmberTools16\textsuperscript{32}. The interactions between protein and RNA were visualized and analyzed using Discovery Studio Visualizer. Additionally, the solvated interaction energy (SIE)\textsuperscript{33} method was applied to estimate the binding affinities of MSI1 RBD1/2 RNA complexes, and to determine the binding contribution of each nucleotide. SIE is an end–point physics–based scoring function that approximates the binding free energy from the force–field non–bonded interaction terms, continuum solvation, and configurational
entropy linear compensation. For each individual simulation, the SIE binding free energy of the complex was calculated over 200 snapshots from the last 20 ns (1,000 snapshots in total) using the equation:

\[
\Delta G_{\text{bind}} = \alpha \times \left[ E_c(D_{\text{in}}) + \Delta G^R + \Delta E_{\text{vdW}} + \gamma \cdot \Delta MSA(\rho) \right] + C
\]

The binding affinity prediction was estimated by summation of Coulomb interactions (\(\Delta E_c\)) and van der Waals interactions (\(\Delta E_{\text{vdW}}\)), the electrostatic solvation contribution (\(\Delta G^R\)), reaction field energy, and nonpolar desolvation energy. Coulomb and van der Waals interactions of the bound state were calculated with AMBER ff14SB and OL3 molecular mechanics force fields. The electrostatic solvation contribution was carried out using the continuum dielectric model with a solute interior dielectric constant and a solvent dielectric constant. The reaction field energies were considered by the Poisson equation with the boundary element method program. The nonpolar desolvation was estimated by a linear proportionality with the change in the solute molecular surface area. Note that the global proportionality coefficient associated with the loss of conformational entropy upon binding (\(\alpha\)) is 0.104758, while the solute interior dielectric constant \((D_{\text{in}})\) is 2.25. The molecular surface area coefficient (\(\gamma\)) is 0.012894 kcal/mol\(^{-1}\)Å\(^{-2}\), \(\Delta MSA(\rho)\) is the difference in molecular surface area between the bound and free state of the protein and constant \((C)\) is \(-2.89\) kcal/mol\(^{-1}\). These parameters were optimized by fitting to the absolute binding free energy. The binding affinity values of the canonical RNA motif (GUAGU) and three modified RNA motifs (GUUGU, GGAGU, and GAUGU) with MSI1 RBD1 and MSI1 RBD2 from the SIE method were taken from the 200 snapshots of the last 20 ns of the five models of each system (1,000 snapshots in total). For the amino acids involved in each nucleotide binding of the four RNAs, \(\Delta G_{\text{bind, res}}\) calculations based on the MM/PBSA method were performed on the same series of 1,000 snapshots.

Results

Structure prediction of MSI1 RBD1/2:GUAGU

For the five predicted structures of MSI1 RBD1/2 from Alphafold2, the number of sequences per position and the per-residue confidence metric (pLDDT) are used to determine the validity of the Alphafold2 results (Figure S2). For MSI1 RBD1, the core structure is covered by approximately 600 sequences at each position, while there are only approximately 100 sequences in the C terminal region (Figure S2A). Likewise, the model confidence at each position increases up to 90% and drops to 70% and 40%, respectively, at the flexible loops and C-terminal. Interestingly, all predicted structures of the five models of MSI1 RBD1 exhibit a similar structure, except in the C terminal region. A similar situation is found for the MSI1 RBD2 models, except that the model confidence at the two terminals is lower to some extent (Figure S2B). The predicted models of MSI1 RBD1/2, excluding RNA, are comparable to the experimentally solved structures (Figure 2). This leads us to conclude that the MSI RBD1/2 protein conformation is not changed upon the complexation of the RNA.
For further investigations, MD simulations of the protein–RNA association complex were performed. To this end, 100 ns MD simulations were applied on the complex between model 1 of the AlphaFold2 simulations, and the canonical RNA pentamer (GUAGU), which has been extracted from the corresponding NMR structure. The root–mean–square displacement (RMSD) during the simulation was evaluated from the geometric coordinates of all atoms of the complex, as well as from the RBD site with respect to those of the initial structures. As shown in Figure S3A, the RMSD values of the predicted MSI1–RBD1:GUAGU increase up to ~5.0 Å during the first 20 ns, then decrease to ~3.1 Å with a fluctuation of approximately 0.5 Å until the end of the simulation. For MSI1–RBD2 (Figure S3B), RMSD increase is found within the first 20 ns and maintained at around 6.0 Å with a fluctuation at 1.0 Å up to 100 ns. The RBD site exhibits a much lower RMSD of ~1.0 − 1.7 and ~2.0 − 2.3 Å in both systems, respectively. This implies high fluctuation at the protein terminals, especially at the C terminal end, as well as flexible loops, and the 3’ end of the GUAGU pentamer (Figure S3).

To estimate the canonical RNA binding affinity, the SIE method was employed on 200 snapshots taken from the last 20 ns. The $\Delta G_{\text{bind}}$ results of MSI1–RBD1 ($-16.77 \pm 0.66$ kcal/mol) and MSI1–RBD2 ($-16.54 \pm 0.99$ kcal/mol) are comparable, and the Coulomb interaction plays a significant role in RNA binding, approximately 2–3 times higher than the vdW interaction (Table. 1). The energy contributions of the residues for RNA recognition (Figure S4) show that the 5’−G of GUAGU RBD1 interacts with Trp29 in (black), while RBD2 connects with Asp143. Likewise, U2 interacts with Phe23, Gly26, Phe63 and Lys93 in RBD1, while in RBD2, stabilization is detected by Phe112 and Gly115. The remaining nucleotides of the core MBE, i.e. A3, and G4, are stabilized by a larger number of residues: Phe23, Phe63, Ala95, Phe96, and Arg98 interact with A3 in RBD1, while Phe112, Phe152, Ala184, Gln185, Met190, Pro192, and Thr193 interact with A3 in RBD2. Binding of the three phenylalanines Phe23, Phe63, and Phe65 (RBD1), and Phe112, Phe152, and Phe154 (RBD2) is supported by the experimentally reported NMR structures. The fourth nucleotide, G, is associated with Lys21, Met52, Arg61, Phe65, Phe96, and Arg99 in RBD1, and Lys110, Phe152, Phe154, Gln185, Lys187, Pro192, and Arg199 in RBD2. Finally, a large contribution of the 3’−terminal U is due to the C−
terminal residues Pro97, Arg98, Arg99, Gln101, and Pro102 in RBD1, while the terminal U nucleotide flips up and interacts with Met141 and Lys144 in RBD2.

Figure 3: Superimposition between the last MD snapshots taken from five individual simulations of (A) MSI1–RBD1 and (B) MSI1–RBD2 in complex with the four RNA pentamers GUAGU, GUUGU, GGAGU, and GAUGU.

Figure 4: Distances between the centers of mass of each nucleotide and protein in (A) MSI1–RBD1 and (B) MSI1–RBD2, bound with the four RNAs. Data were taken from the last 20 ns of all five simulations (1,000 snapshots in total). Grey boxes cover the area between the 25th and 75th percentiles, while whiskers determine the 5th, and 95th percentiles, respectively. Upward and downward triangles represent maximum and minimum values, respectively. Mean values are indicated by a cross, and outliers are depicted by bullets.

Molecular dynamics study of MSI1–RBD1/2 with alternative RNA motifs

In addition to studying the MSI1–RBD1/2 in complex with GUAGU, which has been obtained from NMR structures, we set out to explore three alternative RNA pentamers, i.e., GUUGU, GGAGU,
and GAUGU, by MD simulations. To this end, nucleotides of the pentamer triples cores were adjusted using the NMR structure to obtain starting geometries for MD. The last snapshots from all simulations were superimposed and are depicted in Figure 3. MSI1–RBD1/2 in complex with the canonical RNA GUAGU show the highest stability among all complexes, i.e., the pentanucleotide is well accommodated within the RBD site. The most considerable difference in pentanucleotide conformation is found for the GAUGU system. As shown in Figure 4, the central trinucleotides of all models are placed significantly closer to the protein center (distance distribution of ~10–12 Å) than the flanking nucleotides (~14–18 Å). The structural fluctuation of the C-terminal (Figure 3) is related to the high mobility of the RNA 3’ end, as seen by large interquartile ranges in Figure 4. A change from A to U at position 3 (GUUGU) moves the C-terminal closer to the 3’ end in RBD1, leading to a better stabilization. For GGAGU, the substitution from U to G at the second position results in increased distances of this nucleotide in both RBDs as well as at the 5’ end in RBD1 and the two ends in RBD2. Interestingly, changing two nucleotides of the trinucleotide core, leading to GAUGU, results in significantly lengthened distances. Remarkably, the range of the distance distributions is substantially wider in the case of GAUGU compared to the original GUAGU pentamer. By considering the distance plot, the structural fluctuation of RNAs within the RBD1/2 site is ranked in the order of GUUGU < GUAGU < GGAGU << GAUGU in RBD1; and GUAGU < GGAGU < GUUGU << GAUGU in RBD2. In other words, RNA motifs with less structural fluctuation show a higher affinity for MSI1–RBD1/2.

The SIE method was applied for ΔG_{bind} calculations to predict the pentanucleotide binding strength to MSI1–RBD1/2. From Figure 5A and Table S1, the ΔG_{bind} values of GUAGU, GUUGU, GGAGU, and GAUGU in complex with MSI1–RBD1 are −15.86 ± 1.22, −16.27 ± 0.93, −14.95 ± 1.46, and −14.39 ± 2.23 kcal/mol, respectively. The overall binding affinity is relatively lower in the case of MSI1–RBD2, i.e., they are −14.92 ± 0.91, −13.53 ± 1.07, −14.62 ± 1.42, and −11.97 ± 1.13 kcal/mol. The energy components of MSI1–RBD1:GUAGU are comparable to the predicted model (Table 1), while the decreased Coulomb interaction (~2-fold) in MSI1–RBD2 is compensated by the reduction in the change of the reaction energy upon binding (2-fold). Although the resulting ΔG_{bind} follows the same trend of the structural data above, RNA–protein interactions must be taken into consideration for RNA recognition by a specific protein. From this perspective, the binding of each nucleotide was evaluated by using the SIE binding free energy and MM/PBSA per–residue decomposition free energy calculations.

The highest binding affinity of GUUGU to RBD1 in Figure 5A can be explained by a strong binding of the central trinucleotide UUG of −5.22 ± 0.18, −6.09 ± 0.35, and −6.56 ± 0.42 kcal/mol (Figure 5B and Table S2). The trinucleotide binding is slightly weaker in GUAGU. The binding free energies of the remaining pentanucleotides GGAGU and GAUGU are significantly weaker, as can also be seen from the individual nucleotide contributions. In the case of RBD2, the GUAGU pentamer has the lowest binding free energy, whose trinucleotide binding free energies are −5.24 ± 0.40, −5.71± 0.65, and −6.11 ± 0.48 kcal/mol, respectively. According to the total energy contributions (Figure 5A), the other pentanucleotides exhibit a substantially weaker binding.

Figure 6 shows the residue contributions for each nucleotide binding MSI1–RBD1 and RBD2. Negative and positive ΔG_{bind,res} values represent the nucleotide stabilization and destabilization, respectively. Most RNA–interacting residues are found on the beta–sheet face; However, certain residues in the flexible loop regions also interact with RNA. For RBD1 (Figure 6A), the G at the 5’ end interacts with Trp29 in all models (black). The U at position 2 of the GUAGU and GUUGU pentamers has interactions with Phe23, Gly26, Phe63, and Lys93, while the G2 of GGAGU binds with Gly26,
Asp91, and Arg99. The situation is different for GAUGU. Although the A is stabilized by Gly26 and Phe63, it is destabilized by Asp91, which is in agreement with our binding free energy data (Figure 5B). The central nucleotide (position 3) of GUAGU, GUUGU and GGAGU interacts with Ala95 and the three phenylalanines Phe23, Phe63, and Phe96. The energy contribution of Ala95 is reduced for the central nucleotide of GAUGU. Among all RNAs, the positively charged residues Arg98 and Arg99 provide the highest stabilization to G4 of GUUGU, relating to its highest binding affinity (Figure 5B). Additionally, Lys21, Met52, and Phe65 are also important for the binding of this nucleotide. Their contributions are lowered in the GAUGU model. At the 3’ end, we observe stabilization from positively charged residues at the C-terminus: Arg98 and Arg99 in GUAGU; Arg61 and Arg98 in GUUGU; and Arg61, Arg98 and Arg99 in GAUGU. These contributions are substantially lower in GGAGU.

For RBD2 the 5’ G of the GUAGU pentanucleotide interacts with Val118, which is located at a structurally similar position as Trp29 in RBD1. The second nucleotide of all pentamers has a weak interaction with Gly115 and Phe152, while the third nucleotide of all pentamers interacts with Phe112 and Phe152, which correspond to residues as Phe23 and Phe63 in RBD1. The fourth nucleotide of all pentamers interacts with Lys110, Phe154, Gln185, and Lys187. For GAUGU, we observed a repulsive interaction between the 3’-terminal G and Lys177, and A2 and Glu180, thus highlighting the poor interaction of GAUGU with MSI1–RBD2 (Figure 6B).

The 2D interaction diagram of MSI1–RBD1 and RBD2 complexes are shown in Figures S5A and S5B. In MSI1–RBD1, Lys21, Gly26, Asp91, Lys93, Val94, Phe96, Pro97, Arg98, and Arg99 are predicted to have conventional H-bonding with RNAs. Moreover, salt bridge interaction was found between A3 of the RBD1–GUAGU model with Arg98. The RBD1–GUUGU model revealed an attractive Coulomb interaction between U3 and Arg98, and Arg99. In the RBD1–GGAGU complex, an attractive electrostatic interaction is observed between A3 and Arg98, Arg99, and G4 with Lys88. For all models, Pi–pi stacking interactions were discovered at Phe23 and Phe65 residues. Interestingly, pi–alkyl interactions were found between A3 and Ala95, as well as G4 with Leu50 in the GUAGU and GUUGU pentamers, respectively (Figure S5A).

In the MSI1–RBD2 model, Lys110, Gly115, Arg150, Glu180, Lys182, Lys183, Gln185, Lys187, Ser191, and Pro192 were predicted to have conventional H-bonding with RNA. Moreover, an attractive charge was found between A3 with Lys182 and Lys187 in the GUAGU pentamer. Likewise, an attractive electrostatic interaction is found between U3 and Lys187 for the GAUGU pentamer. Interestingly, Pi–pi stacked interactions are found at Phe112 and Phe154 for all pentamers. In addition, we found pi–alkyl interactions between A3 with Ala184 in the GUAGU model, G4 with Lys187 in the GUUGU pen–tamer, and U3 with Met190 in the GAUGU pentamer. There is one pi–sigma stacked interaction between A3 with Met190 in the GGAGU pentamer. Two pi–sulfur interactions are found between the A3 and Met190 in GUAGU, as well as between the G2 with Met139 in the GGAGU pentamer. One repulsive interaction by negative charges is found between the A2 and Pro192 in the GAUGU pentamer (Figure S5B).
Figure 5: Binding free energies ($\Delta G_{\text{bind}}$) of pentanucleotide (A) and individual nucleotide (B) binding to MSI1–RBD1/2, calculated by the solvated interaction energy method. Data are taken from the last 20 ns of all five simulations (1,000 snapshots in total). Grey boxes cover the area between the 25th and 75th percentiles, with crosses indicating the mean value. Whiskers determine 5th, and 95th percentiles, respectively. Upward and downward triangles represent maximum and minimum values.
Figure 6: Per-residue binding free energy contribution (ΔG_{residue}^{bind}) for the five nucleotides (nt1–nt5) of (A) MSI1–RBD1:RNAs and (B) MSI1–RBD2:RNAs, derived from the average of 1,000 snapshots of the last 20 ns of GUAGU, GUUGU, GGAGU and GAUGU, respectively. Residues with ΔG_{residue}^{bind} ≤ −0.90 kcal/mol and ≥ 0.60 kcal/mol are labeled. Residues that interact with two nucleotides are underlined.

Table 1: Binding free energy (kcal/mol) of MSI1 RBD1/2:GUAGU complexes calculated by the solvated interaction energy method (n=200, SD=standard deviation).

<table>
<thead>
<tr>
<th>Energy Component (kcal/mol)</th>
<th>RBD1 (±SD)</th>
<th>RBD2 (±SD)</th>
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<tr>
<td>ΔE_{vdW}</td>
<td>−99.44 ± 5.50</td>
<td>−119.58 ± 6.49</td>
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<td>ΔE_{c}</td>
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</tr>
<tr>
<td>ΔG_{bind}</td>
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<td>α</td>
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</tr>
<tr>
<td>ΔG_{bind}</td>
<td>−16.77 ± 0.66</td>
<td>−16.54 ± 0.99</td>
</tr>
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</table>
Discussion

Musashi genes have attracted considerable interest as regulators of stem and progenitor cell characteristics. In the present study, we evaluated the three-dimensional structures of the MSI1 in complex with RNA. To this end, we studied the association of MSI1 RNA-binding domains 1 and 2 (RBD1 and RBD2) with different RNA motifs. We investigated the canonical RNA motif GUAGU, as well as the alternative motifs GUUGU (good binding affinity), GGAGU (weaker binding affinity), and GAUGU (unfavorable binding affinity). We compared the protein structures without RNA from the PDB with Alphafold2-predicted geometries, and found that protein structures of the binding domains are highly similar, therefore no conformational changes on the protein occur upon binding of the RNA. In addition, our results corroborate earlier findings that MSI1 RBD1 and RBD2 structures are remarkably similar, despite variation in the underlying primary sequence. To investigate the properties of the RNA–protein association complexes, we performed molecular dynamics simulations and computed the interaction energies by the SIE method.

In agreement with earlier results\textsuperscript{17}, the central trinucleotides of the RNA pentamers (Musashi binding element, MBE) are more rigid than the flanking nucleotides. Moreover, the flanking nucleotides lack interaction with MSI1 RBDs\textsuperscript{13}, suggesting that MSI1–RBD1 and RBD2 require the central trinucleotides for recognition. Our MD simulations show that the central trinucleotides of the RNA motifs exhibit a significantly lower distance to the MSI1 RBDs than the enclosing nucleotides. Thus, the central trinucleotides play an important role in the interaction of MSI1–RBD1 and RBD2 with RNA.

We identified key residues for MSI1–RBD1 binding, specifically Phe23, Trp29, Phe63, Phe65, Phe96, Arg98 and Arg99 are interacting with nucleotides. Our MD simulations are consistent with the fact that Phe23, Phe63 and Phe65 are conserved among all models and interact with A3 and G4 of the pentanucleotides. For MSI1–RBD2, Lys110, Phe112 Gly115, Phe152, Phe154, Gln185, and Lys187 are in contact with the nucleotides. Stacking interactions between evolutionarily conserved phenylalanine (Phe23:RBD1 and Phe112:RBD2, Phe63:RBD1 and Phe152:RBD2, Phe65:RBD1 and Phe154:RBD2) and non-conserved residue tryptophan (Trp29:RBD1 and Val118:RBD2), phenylalanine (Phe96:RBD1 and Gln185:RBD2) of MSI1 and the aromatic bases and ribose rings of the RNA contribute to target recognition within MSI1.

The SIE calculations lead to the following conclusions: Assessment of the contributions to the overall binding free energy of individual nucleotides of the GUAGU and GGAGU motifs shows that the central core nucleotides have the largest interaction energies, with A3 and G4 nucleotides exhibiting the most pronounced contribution. The flanking nucleotides contribute significantly less. Our calculations show that for RBD1, the GUAGU motif possesses the largest binding free energy, followed by GUAGU. While this appears counterintuitive, it is in line with earlier data that assessed opening energy z scores at the level of RNA secondary structures. RBD2 on the other side has overall smaller interaction energy, with the GUAGU motif showing the highest binding affinity for all pentamers.

Calculated decomposition energies clearly show the contributions of individual amino acids to the complexation of the RNA. For RBD1, we should highlight Phe23, Phe63 and Phe65 because of their substantial interaction with the core motif. In analogy, Phe112, Phe152 and Phe154 of RBD2 show a strong interaction with the core trinucleotides.
In summary, we show here the feasibility of MD and SIE calculations to investigate the selectivity of RNA–protein interaction complexation. Further studies are warranted, such as the binding of a longer RNA chain that includes both binding motifs of the two RBDs of the Musashi proteins.

**Data Availability**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Acknowledgements**

N.D. thanks the Overseas Presentations of Graduate Level Academic Thesis from Graduate School of CU. The Vienna Scientific Cluster (VSC) is acknowledged computing resources.

**Author contributions**


**Funding**

This research project was funded by the Second Century Fund (C2F), Chulalongkorn University, the National Research Council of Thailand (NRCT), NRCT5–RGJ63001–009, and the 90th anniversary of CU Fund (Ratchadaphiseksomphot Endowment Fund). Research reported in this publication was supported by the ASEAN–European Academic University Network (ASEA–UNINET). Open access funding provided by University of Vienna.

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Supplementary File

Musashi 1 RNA binding protein in complex with RNA: A theoretical study

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Table S1: Binding free energies (kcal/mol), including standard deviations, of MSI1–RBD1/2 and RNA pentanucleotides, calculated by the solvated interaction energy (SIE) method (n= 1,000, SD=standard deviation).

<table>
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<th>Energy Component (kcal/mol)</th>
<th>RBD1</th>
<th>RBD2</th>
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<td></td>
<td>GUAGU (±SD)</td>
<td>GUUGU (±SD)</td>
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<td>ΔE_{vdw}</td>
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<td>ΔE_c</td>
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<td>-321.63 ± 28.72</td>
</tr>
<tr>
<td>γΔMSA</td>
<td>-14.51 ± 1.10</td>
<td>-14.52 ± 0.96</td>
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<tr>
<td>ΔG_R</td>
<td>307.32 ± 20.79</td>
<td>308.20 ± 25.86</td>
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<td>C</td>
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<tr>
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<tr>
<td>(^{a})ΔG_{bind}</td>
<td>-15.86 ± 1.22</td>
<td>-16.27 ± 0.93</td>
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The binding free energy (ΔG_{bind}) computed by ΔE_{vdw} and ΔE_c are the van der Waals interaction and Coulomb interaction, respectively. γΔMSA relates to the change of the molecular surface area are induced by RNA binding. ΔG^R indicates the change of the reaction energy upon binding and is calculated by solving the Poisson equation with the boundary element method.

Table S2: The binding free energy (kcal/mol) of pentanucleotide and each nucleotide binding to MSI1–RBD1/2 calculated with the solvated interaction energy method (n=1,000, SD=standard deviation).

<table>
<thead>
<tr>
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<th>nt2 (±SD)</th>
<th>nt3 (±SD)</th>
<th>nt4 (±SD)</th>
<th>nt5 (±SD)</th>
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</thead>
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<tr>
<td>RBD1</td>
<td>GUAGU</td>
<td>-5.23 ± 0.26</td>
<td>-5.17 ± 0.21</td>
<td>-5.92 ± 0.36</td>
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<td>GUUGU</td>
<td>-5.09 ± 0.31</td>
<td>-5.22 ± 0.18</td>
<td>-6.09 ± 0.35</td>
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<tr>
<td></td>
<td>GGAGU</td>
<td>-4.35 ± 1.17</td>
<td>-1.83 ± 0.68</td>
<td>-1.13 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>GAUGU</td>
<td>GUAGU</td>
<td>GUUGU</td>
<td>GGAGU</td>
</tr>
<tr>
<td>------</td>
<td>-----------</td>
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</tr>
<tr>
<td>RBD2</td>
<td>-4.95 ± 0.65</td>
<td>-3.85 ± 1.47</td>
<td>-5.35 ± 0.80</td>
<td>-5.97 ± 0.93</td>
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<tr>
<td>GUAGU</td>
<td>-4.69 ± 0.49</td>
<td>-5.24 ± 0.40</td>
<td>-5.71 ± 0.65</td>
<td>-6.11 ± 0.48</td>
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<tr>
<td>GUUGU</td>
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<td>-5.10 ± 0.30</td>
<td>-5.36 ± 0.54</td>
<td>-5.69 ± 0.66</td>
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<tr>
<td>GGAGU</td>
<td>-4.19 ± 0.76</td>
<td>-5.17 ± 0.27</td>
<td>-5.55 ± 0.78</td>
<td>-6.26 ± 0.46</td>
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<td>GAUGU</td>
<td>-3.98 ± 0.62</td>
<td>-4.79 ± 0.35</td>
<td>-5.20 ± 0.94</td>
<td>-5.71 ± 0.66</td>
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</table>

**Figure S1:** (A) Superimposed NMR structures of MSI1 RBD1 (orange) and MSI1 RBD2 (purple). (B) Clustal Omega (1.2.4) multiple sequence alignment and comparison of MSI1 RBD1 (2RS2) and MSI1 RBD2 (5X3Z). Orange and purple circles highlight residues that interact with RNA in MSI1–RBD1 and MSI1–RBD2.
Figure S2: (A) The number of sequences per position, AlphaFold2 confidence measures (pLDDT) and all five AlphaFold2 models of MSI1–RBD1. (B) The number of sequences per position, AlphaFold2 confidence measures (pLDDT) and all five AlphaFold2 models of MSI1–RBD2.
Figure S3: All–atom RMSD plots for the predicted model1/GUAGU complexes: (A) MSI1–RBD1 and (B) MSI1–RBD2. The residues in RBD site Lys21, Phe23, Leu27, Trp29, Arg61, Gly62, Phe65, Asp91, Lys93, Val94 and Phe96 for RBD1; and Lys110, Phe112, Gly115, Thr146, His149, Arg150, Phe154, Glu180, Lys182, and Gln185 for RBD2 are also shown.

Figure S4: Per–residue binding free energy contribution ($\Delta G_{\text{bind\ residue}}$) for the five nucleotides (nt1–nt5) of (A) MSI1–RBD1:GUAGU and (B) MSI1–RBD2:GUAGU derived from the last 20 ns. Residues with $\Delta G_{\text{bind\ residue}} \leq -0.9$ kcal/mol and $\geq 0.6$ kcal/mol are labeled. Residues interacting with particular nucleotides are color–coded, as given in the figure. Protein residues that interact with two nucleotides are underlined.
Figure S5: Summary of the two-dimensional interaction analysis for GUAGU, GUUGU, GGAGU and GAUGU pentamers with MSI1–RBD1 (A) and MSI1–RBD2 (B). Dotted lines indicate predicted interactions.
Researchers from the University of Graz had applied for projects in both the 2020 call and the 2020-2021 call. Because of the travel restrictions due to the COVID-19 pandemic, unfortunately none of these projects could be carried out.
Researchers from the University of Continuing Education Krems had applied for projects in the 2020-2021 call. Because of the travel restrictions due to the COVID-19 pandemic, unfortunately none of these projects could be carried out.
Researchers from the University of Linz had applied for projects in both the 2020 call and the 2020-2021 call. Because of the travel restrictions due to the COVID-19 pandemic, unfortunately none of these projects could be carried out.
Researchers from the University of Salzburg had applied for projects in the 2020-2021 call. Because of the travel restrictions due to the COVID-19 pandemic, unfortunately none of these projects could be carried out.
Researchers from the University of Klagenfurt had applied for projects in the 2020-2021 call. Because of the travel restrictions due to the COVID-19 pandemic, unfortunately none of these projects could be carried out.
Researchers from the Vienna University of Technology had applied for projects in both the 2020 call and the 2020-2021 call. Because of the travel restrictions due to the COVID-19 pandemic, unfortunately none of these projects could be carried out.
Report of the ASEA-UNINET Project: ASEA 2020-2021 /TU Graz/1

from

Graz University of Technology, Graz, Austria

Computational Lung Sound Analysis for Medical Diagnosis Support

Persons involved:

Univ.-Prof. DI Dr. Franz Pernkopf
Technische Universität Graz
pernkopf@tugraz.at

Franz Pernkopf (Senior Member, IEEE) received the Ph.D. degree from the University of Leoben, Leoben, Austria, in 2002. In 2002 he was awarded the Erwin Schrödinger Fellowship. From 2004 to 2006, he was a Research Associate at the Department of Electrical Engineering, University of Washington, Seattle, WA, USA. From 2010-2019 he was Associate Professor at Graz University of Technology, Graz, Austria. Since 1999, he is Professor for Intelligent Systems at the Signal Processing and Speech Communication Laboratory at Graz University of Technology, Graz, Austria. His research is focused on pattern recognition, machine learning, and computational data analysis with applications in signal and speech processing. Particular interests are probabilistic graphical models, discriminative and hybrid learning paradigms, deep neural networks, and sequence modeling.

Dr. Truc Nguyen
Technische Universität Graz + Danang University of Technology, Vietnam
t.k.nguyen@tugraz.at

Truc Nguyen received the M.Sc. degree in 2013 in computer engineering from University of Ulsan, Ulsan, South Korea. In 2022, she received her PhD degree at Graz University of Technology, Graz, Austria. Her research interests include applied machine learning and pattern recognition with a focus on acoustic scene classification and computational lung sound analysis.
Summary of the project:

The aim of the project was (amongst others) to fund a clinical trial in Vietnam to collect lung auscultation data with the multi-channel recording device developed at Graz University of Technology. As all the costs for material expenses was not funded, the project was limited to computational methods for lung sound analysis using existing data. This means that actually due to the budget cut no personal visits with the University of Medicine & Pharmacy of Ho Chi Minh City, Department of Internal Medicine have been performed.

The project was concentrating on computational methods for lung sound analysis, which are beneficial for computer-aided diagnosis support, storage and monitoring in critical care. The main aim was to exploit deep learning techniques for classification for adventitious lung sounds and respiratory diseases on a large public lung sound dataset – ICBHI 2017 Challenge dataset -- and our multi-channel lung sound dataset.

In particular, we improved the generalization ability and model performance for these tasks by exploiting different transfer learning approaches, in which the pre-trained ResNet models of the ImageNet classification task are used as backbone architectures. We compare the following approaches:

✓ We fine-tune the pre-trained model on a target domain and update all top (i.e. feature representation) layers and bottom (i.e. task-specific) layers. We call this vanilla fine-tuning.
✓ We apply co-tuning to fully transfer the knowledge of the pre-trained model [1] in which representation layers and task-specific layers of both source domain and target domain are collaboratively exploited. Co-tuning learns a relationship between source and target categories. Both, the target labels and the probabilistic source labels determined by the category relationship are used for fine-tuning the model for the target domain [1].

✓ We replace Batch Normalization (BN) layers, which suffer from poor performance in case of a data distribution shift between training and test data. We introduce stochastic normalization (StochNorm) [2] in each residual block of the pre-trained backbone architecture. StochNorm is a parallel structure normalizing the activation of each channel by either mini-batch statistics or moving statistics to avoid influence of sample statistics during training. Thus, it is considered as a regularization method. Furthermore, fine-tuning inherits further prior knowledge of moving statistics of the pre-trained networks compared to vanilla fine-tuning. Both properties help to avoid over-fitting on small datasets such as the ICBHI and our lung sound dataset.

✓ We combine co-tuning and stochastic normalization techniques to take advantages of both techniques.

Furthermore, we apply data augmentation in both time domain and time-frequency domain to account for the class imbalance in the datasets. In addition, we use spectrum correction [3] of the lung sounds to compensate the recording device variations in the ICBHI dataset. This improves the generalization ability by accounting for the recording device differences.

The comparison and evaluation of the performance of the different proposed algorithms is based on experimental data. During the funded three months, we completed more than 500 simulations of the proposed algorithms on two lung sound datasets. We also reviewed recent conference and journal papers with the same setting and datasets. Finally, we analysed the results and included them in an article [4] we submitted to IEEE Transaction on Biomedical Engineering (https://ieeexplore.ieee.org/document/9729496)

References


Protein surface display in lactic acid bacteria - applications in biotechnology

Lactic acid bacteria

Lactic acid bacteria (LAB) are Gram-positive, microaerophilic, non-spore forming bacteria with a complex taxonomic relationship and very variable phenotypic traits. The best-studied model organism for LAB is *Lactococcus lactis*; while most species with biotechnological relevance are found with the Lactobacillaceae, namely the genus *Pediococcus* as well as the largest genus, *Lactobacillus*, with over 100 species. LAB are commercially highly relevant in the feed, food and beverages industries as fermentation starter cultures for the manufacturing of a wide range of fermented food products with improved shelf-life, taste and nutritional properties. As such, many LAB species are safe to be used in food and feed applications, and carry the QPS (qualified presumption of safety) status. They are implied to have health-promoting effects as so-called probiotics, beneficial microbes that form transient or stable populations in animals and humans, mostly in the gastrointestinal tract, by a number of interactions with the epithelial surface and the immune system, beneficial enzymatic activities etc. Due to their nature as part of the human microbiome and their capability of establishing themselves as transient or permanent colonizers of the gastrointestinal tract lactobacilli have attracted increased interest for applications as delivery vehicles for therapeutic proteins as well as oral and mucosal vaccinations. Previously recognized weaknesses of *Lactobacillus*-based cell factories, namely limited production of biomass and lower maximum yields particularly for produced proteins, do not play a major role for such applications due to the direct, local and often long-term application. *Lactobacillus* spp. that are capable of persistent colonization would enable a long term-release of the vaccine or therapeutic protein. To this end, a larger and more flexible “toolbox” than is currently available has to be established. The major requirements are custom strains for antibiotics-free plasmid selection conforming to highest biosafety standards, suitable small and flexibly usable plasmids and sets of constitutive promoters, signal peptide sequences and anchoring/cell display signals that can be interchanged easily. It is one of the main purposes of this project to standardize these tools, to establish new ones as required, and to test these together with our Asian partners for two different applications, as an oral vaccine, LAB cells displaying the viral protein antigen VP28 for application in animal / shrimp feed as well as cells displaying suitable enzymes (amylases, mannanases, chitinases) for biocatalytic applications, where these enzyme-displaying cells are used to hydrolyse oligosaccharides abundantly available in SE Asia (Mannan from copra, Chitin from shrimp and crab shells) into potentially prebiotic oligosaccharides.
Protein display in LAB

One of the most attractive features of cell-surface display is that proteins of interest, which are fused to anchoring motifs, are simultaneously synthesized and self-immobilized on the bacterial cell surface. In addition, heterologous proteins anchored onto the bacterial surface likely have higher tolerance or stability under harsh conditions rather than free proteins, especially when proteins are embedded in the bacterial cell wall. The success of protein display depends on a number of factors, including target protein, host strain, promoter and signal sequence used, as well as the type of anchor selected, and currently cannot be predicted rationally even though we currently start to understand its complexity better. In principle, there are two different ways of anchoring a secreted protein to the bacterial surface: via covalent attachment to the cell membrane or the cell wall, or non-covalently via a protein domain that interacts strongly with components of the cell wall or the membrane (Figure 1). Both systems have been used in LAB, primarily in *L. lactis* and different lactobacilli. The group at BOKU worked extensively in the past to study various expression systems in LAB, protein secretion in LAB as well as protein display using LAB (Intaratrakul et al., 2017; Nguyen et al, 2016; Nguyen et al., 2019; Peterbauer et al., 2011; Suphatpahirapol et al., 2019), and our colleagues from HUST and Da Nang University worked on LAB-based expression systems during their PhD in our group in the frame of an Ernst-Mach ASEA Uninet grant and since being back in Vietnam applied this system for antigen display, amongst others using VP28 (Nguyen et al., 2011; Pham et al., 2018; Pham et al., 2020), or enzyme display (Nguyen et al., 2016, Nguyen et al., 2019).

![Figure 1. Methods for protein display in lactobacilli. A schematic view of the most commonly used anchoring methods that are based on covalent or non-covalent interactions with components of the cell membrane or the cell wall. Dark red shows anchor domains/motifs, which are coupled to the to-be displayed protein. Note that the LysM domains may various positions relative to the to-be expressed protein.](image-url)
Construction of oral vaccines via antigen display

White spot syndrome virus (WSSV) is currently the most serious viral pathogen of crustaceans and especially of shrimp, both worldwide as well as in Vietnam. The first outbreak of WSSV was in Taiwan in 1993, and resulted in an approximately 70% reduction in shrimp production in China. In Vietnam, shrimp cultivation areas have been significantly infected by WSSV since 1999, for instance ~423 ha of infected cultivation areas were recorded in 2011 (data from MARD Vietnam, 2011), causing tremendous economic loss. Until now this virus has remained a major concern for shrimp aquaculture. WSSV causes the formation of white spots on the exoskeleton of shrimp and destroys several organs, thus leading to almost 100% mortality within a very short period of 2-5 days. The WSSV belongs to the genus Whispovirus under the new virus family Nimavirida. WSSV is an enveloped double-stranded DNA virus with a rod-like shape of approximately 275 nm x 120 nm in size. In spite of its discovery relatively long ago and the severe influence on aquaculture, no adequate treatment is available to date or at least has not been demonstrated. Even though several approaches related to feeding, the aqueous environment, etc. can be used to prevent or reduce WSSV infections, vaccination has been shown as the best and most promising approach. It is noted that shrimp lack a true adaptive immune system, but it was found that certain memory responses could be induced by using inactivated pathogens or recombinant proteins against WSSV. Different kinds of “vaccine”, mainly derived from the envelop proteins of WSSV, have been extensively investigated. The genome of WSSV contains 184 open reading frames (ORF), among them 39 ORFs encoding for structural proteins including 22 envelop proteins in WSSV. The envelop protein VP28 is currently best demonstrated as related to viral activity of WSSV in its host. Several studies have shown VP28 as a potential candidate for a vaccine against WSSV.

Joint activities. Construction of the LAB cells displaying the selected viral proteins includes (i) the selection of a suitable promoter, (ii) selection of an efficient signal sequence that drives passage of the protein through the cell membrane to the cell wall, and (iii) selection of a suitable anchor.

Promoter. We tested the strong constitutive promoters, Pgm from L. acidophilus NCFM and SlpA from L. acidophilus ATCC4356 to drive mannanase gene expression. Since results obtained with these when driving expression of different genes were not unambiguous, we are currently studying additional promoters for their applicability in the envisaged systems.

Signal sequences. Our group has a library of signal sequences available, some of which were successfully applied in the past (Nguyen et al., 2016; Suphatpahirapol et al., 2019). We now tested three Sec-type signal peptides derived from L. plantarum WCFS1; Lp_2145, Lp_3050, and Lp_0373 for the secretion of a model protein, α-amylase together with the native signal peptides of the α-amylases from L. plantarum S21 (SP_AmyL) and Lactobacillus amylovorus NRRL B-4549 (SP_AmyA). Among the tested signal peptides, Lp_2145 appears to be optimal giving the highest total and extracellular enzymatic activities of α-amylase AmyL from L. plantarum S21.

Anchoring motif. An important element of a cell-display system is the anchoring motif since this can significantly affect stability and accessibility of the displayed protein / antigen. Here we compared the
cell wall-anchoring LPxTG motif, the lysine motif (LysM), and the lipobox motif. With LPxTG, the anchored heterologous protein is more exposed to the environment, which will be advantageous for accessibility (and hence interaction with the host or polymeric substrates) but this might also decrease stability. With the lipobox motif the protein of interest is more embedded in the cell wall and thus better protected from the harsh conditions in the digestive system, but access to the antigens might be hampered. When comparing these three different systems we fused them accordingly to a chitosanase gene from Bacillus, and expressed the hybrid genes in L. plantarum applying a lactobacillal food-grade expression system derived from the pSIP expression vectors using the alr (alanine racemase) gene as the selection marker. Based on this first comparison we found that all three systems can be successfully applied, and that the LPxTG-based cell wall anchor resulted in the highest enzyme activities displayed. To investigate these different approaches and motifs further we studied expression and cell-surface display of the chitosanase in L. plantarum using two truncated forms of the LPxTG anchor. CsnA, a chitosanase from Bacillus subtilis 168 (ATCC23857), was fused to two different truncated forms (short-S and long-L anchors) of the LPxTG anchor derived from Lp_1229, a key-protein for mannose-specific adhesion in L. plantarum WCFS1. The highest enzymatic activities of CsnA-displaying cells were obtained from the strain carrying the alr-based expression plasmid with the short cell wall anchor S. However, the attachment of chitosanase on L. plantarum cells via the long anchor L was shown to be more stable compared with the short anchor after several repeated reaction cycles. CsnA displayed on L. plantarum cells was thus shown to be catalytically active and stable, converting chitosan into chito-oligosaccharides.

Transfer of materials. These different, newly studied genetic elements that are essential for constructing surface-displaying LAB cells were transferred to our partners as part of the project, where they are foreseen to be used in various applications. Restrictions on lab work as a result of the pandemic and various lockdowns, however, significantly hampered this work, with future contacts and work planned for the coming time. This should also result in further jointly published manuscripts.

References


Pham, ML; Tran, AM; Mathiesen, G; Nguyen, HM; Nguyen, TH. (2020): Cell Wall Anchoring of a Bacterial Chitosanase in *Lactobacillus plantarum* Using a Food-Grade Expression System and Two Versions of an LP x TG Anchor. INT J MOL SCI. 2020; 21(11), 3773

Researchers from the Vienna University of Economics and Business had applied for one project in the 2020 call. Because of the travel restrictions due to the COVID-19 pandemic, unfortunately this project could not be carried out.
The Montanuniversität Leoben did not submit an application in either the ASEA-UNINET project 2020 call or in the 2020-2021 call.
Clinical traineeship of students

Project Number: ASEA 2020 / Vet Med Uni / 1

For the 4-week mandatory internship, we applied for two of the four veterinary university hospitals of Kasetsart University. The institutions where we completed our clinical training were KU VTH Bangkok and KU VTH Hua Hin.

<table>
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ER = Emergency room  
CCU = critical care unit  
OPD = outpatient department

Organizationally, we (in addition to the Office for International Relations of Vetmed Vienna) were in contact with Ms. Supreeyanan Sowapark (Khai) of Kasetsart University via e-mail from the very beginning. She took care of the forwarding of our applications, the letter of acceptance, the reservation of our accommodation and the transfer from Bangkok to Hua Hin and was then also our first contact person in Bangkok for any organizational matters. Furthermore, we were looked after in Bangkok by Mrs. Kataria, who also led us through the veterinary clinic on our first day and handed us our work clothes. In Hua Hin we were picked up by Dr. Aris after the hassle-free transfer by bus and also supported in every way during our time on site. Although the organization proved to be rather difficult due to the COVID-19 situation in advance, we were supported by Ms. Supreeyanan Sowapark (Khai) in the best possible way. She was extremely flexible, always helpful and tried to keep us up to date with the entry requirements for Thailand.
In both Bangkok and Hua Hin, we were assigned to a veterinarian on the respective internship days. Depending on the time resources, the medical history of the current cases was then briefly or extensively described to us. We were able to follow owner talks (which understandably took place mostly in Thai), be present at various examinations (eye examination, neurological examination, laser therapy...) and, if necessary, also take blood ourselves or set venous catheters. In Bangkok, the station of the exotics was particularly exciting for us, as we had never had contact with various animal species such as the short-headed glider or the civet cat before. The entire team of the Exotic Pet Station was very open-minded and was very eager to show us as much as possible. In Hua Hin, we were able to develop our practical skills, especially in the surgical department. We were allowed to intubate under supervision, take blood samples, assist with operations and, if possible, set skin sutures in them. At our request, we were also allowed to accompany the two veterinarians of the large animal department in Hua Hin for a day in the driving practice. As a result, we were able to gain at least a small insight into the Thai sheep and cattle herd care.

During our time in Thailand, we stumbled upon some veterinary and cultural differences. The fact that dogs and cats are dressed in Bangkok and transported in strollers was rather bizarre for us. The topic of euthanasia is also handled significantly differently in Thailand than we know it from Austria. When it comes to dealing with imaging diagnostics (e.g. MRI) and innovative operations in the field of the spine, Thailand seems to us to be a few years ahead of us and a lot of experience. These various differences often inspired us to think and discuss. We often questioned the conditions in our home country more critically. We also learned many things, such as appreciating the availability of medicines, which is almost a matter of course in Austria.

All in all, it was definitely a very varied and rewarding internship. We were able to consolidate or refresh our theoretical, practical and English knowledge and expand our personal horizons a bit.

Sandra PFISTER
Johanna SCHACHERMAIR
Determination of chloramphenicol and nitrofuran residues in shrimp products from Thailand

ASEA-UNINET Cooperation project 01.01.- 30.9.2020
Project Number: ASEA 2020 / Vet Med / 2

Project Leader:

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Department of Farm Animals and Veterinary Public Health
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Cooperation Partner in Thailand:

Dr. Piyada Songsermsakul (Dr rer. nat. from faculty of chemistry, University of Vienna in 2006)
Current position: Assistant professor at department of toxicology, Faculty of Pharmaceutical Sciences, Khon Kaen University, 40002 Thailand
Email: spiyad@kku.ac.th

Project description:

Chloramphenicol and nitrofuran are broad spectrum antibiotics. They are used for treatment and prophylaxis in aquaculture. The presence of these antibiotics in aquaculture products is a worldwide problem. Chloramphenicol and nitrofuran have been detected in shrimp imported into Europe. The use of these antibiotics in aquaculture has been reported in some countries such as the Southeast Asian countries of Bangladesh, India and China. The impact of chloramphenicol and nitrofuran used in the aquaculture industry was shown on both environment and human health. Antibiotic residues in food can lead to the development of drug resistance, hypersensitivity and aplastic anemia. The European Agency for the Medicinal Products concluded that acceptable daily intake (ADI) for chloramphenicol cannot be established. Therefore, the maximum residue limit (MRL) cannot be calculated either. This means zero tolerance for chloramphenicol and nitrofuran residues in food and they have been banned from food production in the EU.
The aims of the study:

1. To develop a method for determination of chloramphenicol and nitrofuran in shrimp based on LC-MS/MS (triple quadrupole)
2. To develop a sample preparation method by solid phase extraction of chloramphenicol and nitrofuran in shrimp products
3. To monitor the contamination of chloramphenicol and nitrofuran in shrimp products from Thailand and the imported products in Austria.

Methods used:

Sampling and sample pre-treatment process will be carried out in Thailand. Sample measurement will be carried out in Vienna at the VetmedUni. For sample preparation liquid-liquid extraction in combination with solid phase extraction (SPE) will be used. The extracts will be analyzed using LC-MS/MS (triple quadrupole mass spectrometer). At the VetmedUni, there is an LC-MS/MS instrument, which will be used for the analysis of antibiotics.

Work plan:

<table>
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<th>Second week</th>
<th>Third week</th>
<th>Fourth week</th>
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<td>Experience on software in LC-MS/MS instrument</td>
<td>Optimization of the MS condition Development of sample preparation SPE</td>
<td>Measurement of samples</td>
<td>Method validation and discussion</td>
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</table>

Results and expected results:

Due to COVID-19, the visits from Drs. Songsermsakul and Sungthong have not been carried out in 2020 and were not possible in 2021 either. The optimization of MS condition for analysis of chloramphenicol has been completed. The standard calibration curve of chloramphenicol has been set up with satisfactory parameters such as sensitivity and linearity, but the work on nitrofuran has yet to be carried out. Due to a combination of COVID-19 and instrument problems, this work has stalled, but with the instrument now up and running, it will be carried out shortly by VetmedUni staff, followed by measurement of the shrimp samples from Thailand.

Intended publication:

The method development and results for chloramphenicol and nitrofuran residues in shrimp samples purchased in Thailand and analysed in Austria are expected to result in a publication in a scientific journal. The journal title to publish the data will be discussed when the laboratory work is completed. Additionally, it is expected that the results will be presented at scientific meetings in Thailand and Austria. The publication will be spearheaded by Dr. Piyada Songsermsakul and done in collaboration with Ao.Univ.Prof.Dr. Ebrahim Razzazi-Fazeli and Dr. Kathrine Holmggaard Bak.

Unfortunately, due to COVID 19 pandemic it was not possible for Dr. Piyada Songsermsakul to visit the Vetmeduni Vienna and perform incoming part of the project. We plan to submit a follow-up project for 2022-2023 in order to analyse samples we purchased in Thailand and Vienna and prepare the publication.
Short CV – Dr. Bak:

2019- Post doc in Meat Science, Meat Technology, and Food Chemistry at the Unit of Food Hygiene and Technology, Institute of Food Safety and Veterinary Public Health, University of Veterinary Medicine Vienna

2018-2019 Assistant Professor in Meat Science, Department of Food Science, University of Copenhagen

2016-2018 Post doc in Meat Science, Department of Food Science, University of Copenhagen

2013-2014 Post doc in Meat Science, Meat Science and Muscle Biology Laboratory, University of Wisconsin-Madison, USA

2012 Ph.D. in Meat Science, University of Copenhagen, Denmark

2008 MSc in Food Science and Technology, University of Copenhagen, Denmark

2006 BSc and Engineer in Food Science and Technology, The Royal Veterinary and Agricultural University, Denmark

Areas of expertise:
Meat science
Meat technology
Food chemistry

Short CV – Dr. Songsermsakul:

1996- Employed at Khon Kaen University, Thailand, currently as Assistant Professor, Department of Pharmacognosy and Toxicology, Faculty of Pharmaceutical Science

2006 Ph.D., Dr.rer.nat. in Analytical Chemistry, University of Vienna, Austria

2001 MSc in Pharmacology, Mahidol University, Thailand

1996 BSc in Pharmaceutical Sciences, Khon Kaen University, Thailand

Areas of expertise:
Toxicology
Food contamination
Food safety
Mycotoxins
LC-MS/MS

* Editors note: Due to the COVID-19 pandemic, no mobilities took place and therefore no funding was paid out.
Identification of candidate receptors for ToxA and/or ToxB from Acute Hepatopancreatic Necrosis Disease in Shrimp

ASEA-UNINET Cooperation project 01.01-30.09.2021
Project Number: ASEA 2020-2021 / Vet Med Uni / 1

Project leader: Prof. Dr. Ebrahim Razzazi-Fazeli

Email: ebrahim.razzazi@vetmeduni.ac.at

2007: Head of Proteomics Unit at the VetCore Facility for Research
2004: University of Veterinary Medicine Vienna, Habilitation
1990 – 1994: Medical University of Vienna, PhD, Institute of Medical and Chemical Laboratory Diagnostic, Department of Bioanalysis and Toxicology
1983 – 1990: University of Natural Resources and Applied Life Sciences, Vienna, Master of Sciences (Food and Biotechnology), Institute of Applied Microbiology
1997 – 2004: University Assistent and Lecturer, University of Veterinary Medicine Vienna, Institute of Nutrition
1994 – 1997: Group leader Quality Control, Boehringer Ingelheim Austria

Areas of expertise:
Bioanalysis / Instrumental Analysis
Proteomics / Protein Analysis
Mass Spectrometry / HPLC-MS

Cooperation Partner: Prof. Dr. Hieu TRAN-VAN (Mr), PhD (Dr. rer. nat.)

Email: tvhieunt@yahoo.com

Position: Associate Dean / University lecturer / Associate Professor in Biotechnology
Institution: Department of Molecular and Environmental Biotechnology
Faculty of Biology and Biotechnology,
University of Science, VNU-HCM
Hochiminh City, Vietnam
Professional Experience:

2018 - present: Vice Dean of Faculty
2016 - present: Associate Professor in Biotechnology
2011 - 2018: Deputy Head of Department
2005 - present: Lecturer of Department
2003 - 2004: Teacher assistant

Education:

2011 PhD. University of Wuerzburg, Germany
2006 MSc. University of Science, VNU-HCM, Vietnam
2003 BSc. University of Science, VNU-HCM, Vietnam

Areas of expertise:

Vaccine development against ETEC associated diarrhea in swine:
Mucosal vaccines: M-cell targeting.
Exploiting GRAS organisms for vaccine delivery
Recombinant glycoprotein production in yeasts

Project Description

Shrimp production plays an important role in the sustainable development of Vietnamese aquaculture. However, along with the expansion of farming scales, the endemic/epidemic that takes place on shrimp farms has affected the production as well as the quality of commercial shrimp. Among them is to mention Acute Hepatopancreatic Necrosis Disease (AHPND), which is one of the common diseases on shrimp, has a mortality rate of up to 70 - 100% of the pond area. The disease is mainly caused by the toxins called ToxA and ToxB produced from strains of *Vibrio parahaemolyticus*, or more recently from *Vibrio* Spp. Outbreaks of AHPND in shrimp causing *V. parahaemolyticus* has caused significant economic losses to the aquaculture industry. Hence, many methods utilized to prevent the spread of this epidemic has discovered, including using antibiotics as standard operating practices for farmed shrimp based on many it's positive benefits. Enforcement, though, maybe lax or nonexistent, allowing indiscriminate use to flourish that leads to the development of antimicrobial resistance or AMR for which there may be no medical treatments available. Besides, the shrimp harvested can be detectable residues from the abuse of these antibiotics in the consumer-ready product that results in banning imported shrimp.

Moreover, the feeding of antibiotics as a treatment as well as a preventative measure in farming can be ineffective because of the rapid death in the infected shrimp population. Alternative treatment without using antibiotics, hence, has become an ideal approach to prevent the outbreak of AHPND along with ensuring the safety of animal-derived food and environment. Therefore, the method to inactivate or prevent the exposure of pathogenic factors is considered an extremely potent strategy in preventing and minimizing the damage caused by this disease.
Especially, the identification and analysis of biomarkers and proteins in the field of veterinary science is very challenging due to the broad spectrum of species analyzed starting from working and companion animals as well as their pathogenic bacteria, viruses and parasites. Performing proteomic approaches on such a variety of organisms the major difficulty is missing database information about protein sequences of species with hitherto not sequenced genomes.

**Planned activities:**

**Sample collection and preparation (Vietnam)**

The samples were collected in laboratory of Molecular Biotechnology at University of Science (Vietnam) and proteins were extracted by suspending in lysis buffer. The lysates were clarified by centrifugation and the supernatants were sent to Vienna for protein analysis using high resolution mass spectrometry.

**Protein Identification using nano HPLC Tandem Mass spectrometry (Austria)**

The protein identification by mass spectrometry was carried out at VetCore Facility / Proteomics Unit / Veterinary Medicine University in Vienna. SDS-PAGE was used in advance as an alternative approaches to identify differentially expressed proteins in *E.coli* BL21. In this case an In-Gel-Digestion protocol was used before performing peptide separation on nanoLC-MS/MS.

Due to the COVID-19 pandemic only some selected samples could be measured in Vienna

**Objectives of Project:**

1. Identification of candidate receptors for ToxA and/or ToxB from Acute Hepatopancreatic Necrosis Disease (AHPND) in shrimp using mass spectrometry
2. Prokaryotic expression of the candidate in *E. coli*
3. To establish an academic cooperation between the two universities in the field of biotechnology and proteomics
4. To set up educational collaboration activities between the two universities Vietnam and Austria in the field of proteomic research and creating a network.
5. To train Vietnamese expert to be able to perform state of the art proteomics at his institute

Unfortunatelly, due to the COVID-19 pandemic, it was not possible to perform the points 3-4

**Incoming Activities:** Due to COVID-19 pandemie no incoming activity could be carried out.

**Outgoing Activities:** Due to COVID-19 pandemie no outgoing activity could be carried out.
The incubator supported by ASEA-UNINET was purchased in Vietnam and has been using in this project as well as for several projects related to recombinant protein expression in laboratory at Department of Molecular and Environmental Biotechnology / Faculty of Biology and Biotechnology in Vietnam.

Based on activities of current project, we plan to submit an ASEA-UNINET project for 2022.
The Effect of DDT Contamination on the Proteome of
Hooded Oyster *Saccostrea cucullata*

**Project Number:** ASEA 2020-2021 / Vet Med Uni / 2

**Project Leader:** a.o.Prof.Dr. Ebrahim Razzazi-Fazeli
E-mail: ebrahim.razzazi@vetmeduni.ac.at

**Current position:** Head of Proteomics Unit at the VetCore Facility for Research

**2004:** Habilitation, University of Veterinary Medicine Vienna, Institute of Nutrition

**1997 – 2007:** Assistant and Lecturer, University of Veterinary Medicine Vienna University, Institute of Nutrition

**1994 – 1997:** Group Leader Quality Control, Boehringer Ingelheim Austria

**1990 – 1994:** PhD, Medical University of Vienna, Institute of Medical and Chemical Laboratory Diagnostic, Department of Bioanalysis and Toxicology

**1983 – 1990:** Master Degree of Sciences (Food and Biotechnology), University of Natural Resources and Applied Life Sciences, Vienna, Institute of Applied Microbiology

**Cooperation Partner:** Ass. Prof. Dr. Sutin Kingtong
E-mail: sutin@go.buu.ac.th

**Current Position:** University lecturer, Department of Biology, Faculty of Science, Burapha University, Chonburi, 20131, Thailand

**2019/2021:** Visiting researcher fellowship at University of Veterinarian Medicine, Vienna, Austria (ASEA-UNINET, Austria)

**2012-2013:** Post-doctorate fellowship at University of Caen, France (Reproseed Project, EU)

**2008-2009:** Proteomic training at the Ludwig Institute for Cancer Research, Australia (the RGJ Ph.D. Programme, TRF, Thailand)

**2001-2004:** Scholarship of Enhancement of Science and Math Specialist Programme (The IPST, Thailand)
Project description

One of the major concerns in marine pollution is the contamination with organochlorine pesticides (OCs). These have been extensively used in the past for agriculture and pest control. DDT (dichloro-diphenyl-tri-chloroethane) is one of the most well known organochlorine pesticides. It was developed as the first of the modern synthetic insecticides in the 1940s with great effect to control malaria, typhus, and the other insect-borne human diseases. Later, it was also effectively applied for insect control in agriculture (U.S. EPA, 1975). According to its effectiveness as a pesticide, DDT was used worldwide.

In Thailand, DDT was first introduced for malaria control trial in 1949 but also in agriculture for insect pest control (Pollution Control Department. 2004). Accumulation of DDT and its derivatives has been reported in Thailand (Kumblad et al., 2001; Siriwong et al., 2009; Boonyatumanond et al., 2002). Although DDT was banned, its residues are still circulating and magnified in food web system (Siriwong et al., 2009). The results indicate generally low DDT concentrations which do not pose any threat to the human population of the area. Nevertheless, environmental impacts should not be disregarded, especially since no studies have been conducted to deny their presence. Further research aiming at an overall assessment of the environmental pollution status including more information on the ecological effects of DDT contamination, would thus provide important information for managing in the future. Therefore, health risks of DDT to animals in wildlife should be investigated and monitored.

Proteome analysis and its application in marine environmental research

In recent years, proteomics has been applied in toxicology to investigate response of organisms exposed to certain environmental chemicals. This robust and unbiased technology enables to understand the whole map of proteins expressed in cells or tissues at certain conditions. The applications of the proteomic approach are numerous in many areas of biology, biochemistry and biomedicine. In context of marine environmental toxicology, proteome response of organisms after experimental exposure of pollutants allows us to reveal and explain molecular mechanisms of such pollutant toxicity and explore potential biomarkers of exposure.

Objectives of the project

The aim of this project was to analyze the proteome of the hooded oyster S. cucullata by using a proteomic approach and generate a reference map of the mantle proteome for this species. Additionally, the effect of DDT on the proteome response was investigated in order to explain molecular mechanisms of DDT toxicity in oyster. Proteome analysis was used to screen for potential biomarkers of DDT exposure in this species aiming to use these as an alternative method for DDT monitoring. Therefore the results obtained are essential for further toxicological studies of marine pollution research.

Oyster collection (Thailand)

Adult hooded oysters S. cucullata were collected from an oyster farm in Chonburi Province, Thailand. Oysters were transported to Marine Aquaculture Laboratory, Faculty of Science, Burapha University and acclimatized.
Toxicity test of DDT to oyster (Thailand)
Exposure experiments were carried out in Thailand. Adult oysters were exposed to various concentrations of DDT (0-2,000 µg/L) for 96 hours. Toxicity of DDT was investigated under laboratory conditions. Seawater with different DDT concentrations was prepared in 40 L glass tanks filled with 20 L seawater. Three replicates were performed for each DDT concentration. During the exposure, animals were fed daily as in acclimatization period. Seawater was changed every 24 hours and dead animals were removed, if present. After 96 hours of exposure, three oysters from each treatment were collected from each tank for histological study and another three oysters were collected for proteomic analysis.

Our preliminary results showed that LC-50 of DDT at 96 hours in oyster is about 900 µg/L.

Histopathological effect of DDT to oyster (Thailand)
Soft part tissues of oyster were dissected and fixed in Bouain’s fixative for 24 hours. Fixed tissues were washed in ethanol 70% until yellow color of the fixative was clear and then gradually dehydrated by passing the tissues through increasing concentrations of 80%, 90% and absolute ethanol. Tissues were then placed in dioxane overnight and embedded in paraffin. Embedded tissues were sectioned with a microtome to ensure the thickness of 6 µm and stained with hematoxylin and eosin (H&E). After staining, tissues were observed under a light microscope.

We have carried out this experiment to observe the histopathological effect of DDT. Results showed that DDT produced histopathological alteration in oyster tissue in dose-dependent manner. For example, mucous cells were increased in size and number after exposed to DDT. Connective tissue cells were also affected.

Proteome analysis of oyster (Austria)
Protein samples were prepared according to Kington et al. (2007) and Kington et al. (2013) Proteins of mantle tissue of oysters exposed to ~ 1% and 10% of the LC-50, which were 10 µg/L and 100 µg/L, respectively and were analyzed after 96 hours of exposure. Mantle proteins were extracted, separated in a 2D-gel and stained with Coomassie blue. Protein spots were detected and analyzed by an image analysis software (Delta2D) in Thailand. Results showed that DDT altered the proteome profiles of exposed oysters comparing to the control.

Gel samples were brought to Vienna and the proteome of the hooded oyster S. cucullata was analysed using LC-MS. The purpose of the proteome analysis in this project was to generate a reference map of all mantle proteins of this species and to investigate the effect of DDT on the proteome response of the hooded oyster in experimental exposure.

Protein spots of interest have now been identified. This will be advantageous for the development of an alternative method to detect DDT in the coastal area. However, validation of biomarkers is further required.
Achieved Project Goals:

1. We could investigate the effect of DDT on proteome response of the hooded oyster *Saccostrea cucullata* in experimental exposure in Thailand.
2. Proteome analysis of the hooded oyster *S. cucullata* was performed by using proteomic approach and producing a reference map of mantle proteins for this species (VetCore Facility, Vienna).
3. Thai-Austria proteomic research network has now been established.

The outcome of this project has revealed some aspects of biochemical pathway to explain molecular mechanism of aqua-toxicity. Moreover, the results of this project were published in a scientific journal by mentioning ASEA-UNINET in acknowledgments.

Toxicity of DDT to the hooded oyster *Saccostrea cucullata*: Mortality, histopathology and molecular mechanisms as revealed by a proteomic approach

Supatta Chueycham\(^a\), Chantragan Srisomsap\(^b\), Daranee Chokchaichamnankit\(^b\), Jisunso Svasti\(^c\), Karin Hummel\(^c\), Katharina Nöbauer\(^c\), Omid Hekmat\(^c\), Ebrahim Razzazi-Fazeli\(^c\), Sutin Kingtong\(^d\).

\(^a\) Environmental Science Program, Faculty of Science, Burapha University, Chonburi 20131, Thailand
\(^b\) Laboratory of Biochemistry, Chulabhorn Research Institute, Vibhavadi-Rangsit Highway, Bangkok 10210, Thailand
\(^c\) VetCore Facility for Research, University of Veterinary Medicine, Veterinärplatz 1, Vienna 1210, Austria
\(^d\) Department of Biology, Faculty of Science, Burapha University, 169 Long Road Bangsera Road, Chonburi 20131, Thailand

Incoming Activities: Dr. Sutin was trained on nanoHPLC-MS/MS analysis of proteins and mass spectrometric analysis of samples in Vienna for one month. Furthermore, he was taught how to use protein databases for identification of proteins and how to deposit proteomics data. A peer reviewed publication was prepared in addition.

Outgoing Activities: Due to COVID-19 pandemic no outgoing activities could be carried out.

Material costs: in the amount of 3000€ were used for buying the chemicals in Thailand for this project.

Based on activities and great outcome of this project an ERNST MACH project has been submitted for 2022. Additionally, we plan to submit an ASEA-UNINET project for 2022.
Masterclasses for Violin and Chamber Music
Project number: ASEA 2020 / mdw / 1

Project Leader
Prof. Mag. Priv.-Doz. Peter Schuhmayer
University of Music and Performing Arts Vienna (mdw)
Joseph Haydn Institut, schuhmayer@mdw.ac.at

Since 1996 faculty of mdw, teaching chamber music and violin. Founding member and first violinist of Artis Quartet Vienna. Concerts in the most important musical centers worldwide, 40 CDs/many international awards, visiting professor at important Univ. in Europe, USA and Asia. Jury member at international competitions.

Responsible person at the partner institution
Prof. Marcin Szawelski, Head of Strings
College of Music, Mahidol University Bangkok
mszawelski@hotmail.com

As a cello and chamber music instructor at the College of Music, Mahidol University he serves as well as head of the String and Chamber Music Dept. He is a member of Thailand Philharmonic Orchestra (TPO) and Mahidol University Baroque Orchestra. Mr. Szawelski is a founding member of Salaya International Chamber Music Society (SICMS)

Report:

After leaving Vienna on February 5th, I arrived at Bangkok airport on February 6th and experienced what was going to be the reality for months and will be as well in the near future-wearing masks at the airport and consequently at the planes.

I was picked up at the airport in Bangkok and started my coaching the next day.

Both, violin students as well as chamber music groups were prepared by their teachers to present their interpretations of various pieces of the main repertoire.
Selective Chamber Music pieces like Mozart Clarinet Quintet or Schubert Arpeggione Sonata were performed by the students and since the semester was about to start shortly before I started my visit, the number of instrumental solo performances were dominating the program of the following days.

The coaching was focusing on interpretation issues like phrasing, understanding of the musical context or the similarities of music and language.

To give some of the groups the impression of how to open up and use high level of energy, as well as to develop communicating qualities within a group I explained but even played with the students for a short while.

Technical issues for improvement of the sound like the use of the bow including speed, pressure and contact point were dominating the coaching of the violinists and viola players.
Substantial representatives of the violin repertoire like the concertos by Mozart, Mendelssohn, Lalo, Sibelius and Bruch were presented by the more advanced students while less known repertoire like Stamitz duo for two violas was on the list of some students of the preparatory school.

I had met many of the students before and worked with during my earlier stays at Mahidol and one of them, Patis Intaramaha was about to start his exchange year in Vienna in my class as part of the partnership program between mdw and Mahidol University shortly after my visit in February.

He is one of the most talented violinists at the School of Music and ready to widen his musical horizon in visiting institutions abroad.
Prof. Marcin Szawelski, the head of the string and chamber music institute was my host and responsible for the coordination of my schedule. It was him who asked me during my stay if would be willing to participate in a new project happening during two days of my stay.

For the first time they organized the Mahidol’s COM String Camp. This project was set up for two days, with the background of inviting amateurs and young kids who already play an instrument to visit the campus, getting individual coaching and finally play together in a string orchestra on the second day.

This idea sounded very attractive and promising to me and I agreed in assisting their teachers during the final day in preparing the kids for the concert. Finally, it turned out that I was supposed to conduct the string orchestra at the performance for family members and students in the afternoon.
After roughly two hours of rehearsals, we performed two arrangements of pieces by Dvorak and “Ode an die Freude” from Beethovens 9th symphony.

The enthusiasm of the kids and elder amateurs participating in this project showed how important it is to present the music and the work which is constantly done in our music institutions to the “outside world”.

Projects like this not just mean to invite young kids to learn an instrument and study it at a later stage in higher music institutions but as well to form new audiences to guarantee concert a life as we know for future generations.

The success of the concerts of the Thailand Philharmonic Orchestra which is in residence at the impressive Prince Mahidol Hall strongly relies on the acceptance of the orchestra from the society in general. It seems that the general strategy of the School of Music takes care about this aspect in many ways.

Credits of photos: Macin Szawelski / Mahidol University
Cultural Heritage Conservation in Southeast Asia
Study Programme and Joint Efforts of Angewandte and Silpakorn
ASEA 2020 / Angewandte / 1

Project Participants

o.Univ.-Prof.Mag.Dr. Gabriela Krist, project leader, Institute of Conservation, University of Applied Arts Vienna, gabriela.krist@uni-ak.ac.at;

Gabriela Krist has been university professor at the University of Applied Arts Vienna since 1999 and is head of the Institute of Conservation. She studied conservation at the Academy of Fine Arts Vienna, as well as art history and archaeology in Vienna and Salzburg. For many years she worked for ICCROM in Rome and at the Austrian Federal Office for the Care of Monuments (Bundesdenkmalamt). She leads education cooperation programmes, conservation campaigns and workshops in India, Nepal, Mongolia and Thailand.

(Comment: In the project application 3 mobilities were foreseen and beginning of 2022 also a request for name change was granted. Because of the extension of quarantine days in Thailand in February, it was decided together with the Thai partner to postpone two mobilities to spring 2022.)

Project Report

In view that conservation training is widely missing in Asia, and here particularly in South East Asia, and that trained conservators to preserve cultural heritage and sites are desperately needed, the Institute of Conservation, University of Applied Arts Vienna and the Silpakorn University International College (SUIC) Bangkok collaborate since 2017 with the aim of counteracting this shortage of skilled professionals. Amongst the major achievements so far are the implementation of a number of further capacity building measures, the establishment of a conservation centre in the premises of SUIC and the development of a Joint Master study programme in Cultural Heritage Conservation and Management. The generous support from ASEA UNINET from the very beginning has been an important base for these undertakings.

In the recent two years, the pandemic made personal meetings and on-site training workshops largely impossible. Nevertheless, both partners aimed to meet and exchange regularly online and also to include each other in online activities, which enabled to keep the cooperation alive. The international
summer schools and lecture series of the Institute of Conservation (International Summer School 2020 Remote – Understanding Pigments, June 2020;

International Summer School 2020 Remote – Sustaining Cultural Heritage through Preventive Conservation and Collection Care, 14-18 September 2020; International Summer School 2021 Remote – Tangible Cultural Heritage – Intangible Cultural Heritage – Conservation, 23-24 September 2021) provided platforms for mutual exchange and learning from and with each other. Topics and themes ranged from materials' science for conservators, preventive conservation and collection care to intangible cultural heritage and conservation. Staff and students of Silpakorn University not only participated in the events but also delivered lectures, thus providing a Thai perspective to the discourse.

Some of the students of the Joint Master Programme Cultural Heritage Conservation and Management together with their teacher in the conservation centre in Bangkok, © SUIC

In addition, the Institute held an international conference on archaeology and conservation along the Silk Road in November 2021 with Thai colleagues in the audience. Also, the Silpakorn University organized two international conferences in hybrid format in 2020 and 2021: the first one entitled “A Revival of Creativity in Art and Design” (18.12.2020) was dedicated to the impact of the pandemic on the production and teaching of art and design.
Amongst the lecturers were Angewandte Vice rector Bernhard Kernegger and Martina Haselberger, who talked about how the university has dealt with the lockdown in spring, what has been gained from the experience and how it has shaped the institution’s approach to teaching during the ongoing pandemic.

The second conference entitled “New Challenges for Art, Design, and Business Management” (27.11.2021) focused on global challenges in the fields of art, design, and business management. At this conference Meral Hietz, staff of the Institute, presented the challenges in the conservation of an outdoor contemporary steel sculpture.

Similar to many planned activities, also the start of the jointly organized study programme had to be postponed because of the ongoing pandemic. The first batch of international students was finally accepted in 2021, six at SUIC and seven at Angewandte, and the summer semester 2022 marked the launch of the programme. The students enrolled at SUIC already started the programme some month earlier with a preparatory foundation phase.

With its unique curriculum set-up, the programme offers students the opportunity to take their competences to a global level and look into conservation and cultural heritage in an international context. They will develop competences in international project work, project and site management,
refine their communication and presentation skills and expand their knowledge of Asian and World Heritage. The programme follows an innovative, transcultural and interdisciplinary approach. Students will study both at the University of Applied Arts Vienna in Vienna and at Silpakorn University in Bangkok, and thereby benefit from both institutions’ individual expertise.

As hands-on and site visits will be an essential part of the study programme, Prof. Gabriela Krist, Dr. Sompid Kattiyapikul and Dr. Sudawadee Chanpiwat met in January in Bangkok to identify partner institutions and training sites in Bangkok. Sites, exhibitions and storages, i.e. the Silpakorn Art Collection, were visited. Collections and areas that could serve as topics and fields of action for practical training of the students were identified. It was also discussed to include traditional painting techniques as a topic for student research projects within the programme.

Prof. Krist also met with the students enrolled at SUIC and discussed their prior experience in conservation. Accordingly, the practical courses of the programme delivered in two phases were planned in detail. The first phase (Practicum in Conservation, 3 AC) is currently carried out by Mag. Eliška Miklovičová, senior conservator employed at Silpakorn University based on discussions and directions given by Gabriela Krist during her January mission. The second phase will take place in April 2022 (Conservation Practice I, 5AC) for which Mag. Franziska Marinovic and Dr. Tanushree Gupta, both staff members of the Institute of Conservation, University of Applied Arts Vienna, will travel to Bangkok. The January visit in Bangkok was further used for strategic planning of the practical training of all students in July and August 2022 in the premises of the Institute of Conservation in Vienna.

SUIC’s conservation centre, which was established with the support of the Institute, is already fully operating and serves as working area for the joint master students. The Institute is further assisting in the selection and purchase of conservation materials and is providing contacts of conservation material companies. In addition, Silpakorn University got approval for building a new conservation laboratory. Various options on best construction and best utilization of space have been discussed on site and back in Vienna with staff member Manfred Trummer, who is experienced to build up restoration workshops and labs.

**Outcome and Forecast**

With the support of ASEA, the long-term collaboration between the Austrian University of Applied Arts Vienna and the Thai Silpakorn University International College could be continued and intensified despite the restrictions and limitations due to the ongoing pandemic.

A major step towards further strengthening of the cooperation was the start of the unique international Joint Master Programme between the two institutions. A group of 13 committed students from Thailand, Vietnam, India, Bangladesh, Iran, Turkey, Germany and Austria passed the entrance exam and was admitted to the study in 2021. The programme should on not only act as a central contact
point for academic training and education in conservation in South East Asia, which is still lacking in the region, but also provide advanced students from all over the world the possibility to enhance their skills and competences in conservation and management.

In the following years regular staff exchange and mutual visits will be important for the success of the study programme.

Mag. Miklovičová, students of the Joint Master Programme Cultural Heritage Conservation and Management and Prof. Krist in Bangkok, © Institute of Conservation, University of Applied Arts Vienna

The next mobilities are planned in April 2022, two senior conservators and teachers at the Institute of Conservation, Dr. Gupta and Mag. Marinovic, will travel to Bangkok to continue the practical courses on site. Further important steps are to continue building up a network of supporting institutions and partners in Thailand for the programme and identify cultural heritage sites, art objects and collections, which could be accessed in the framework of the study programme. The conservation centre and conservation laboratory will be further equipped and expanded.
The Mozarteum University Salzburg did not submit an application in either the ASEA-UNINET project 2020 call or in the 2020-2021 call.
Researchers from the University of Music and Performing Arts Graz had applied for one project in the 2020-2021 call. Because of the travel restrictions due to the COVID-19 pandemic, unfortunately this project could not be carried out.
Researchers from the Medical University Graz had applied for one project in both the 2020 call and in the 2020-2021 call. Because of the travel restrictions due to the COVID-19 pandemic, unfortunately these projects could not be carried out.
Researchers from the Medical University of Innsbruck had applied for projects in both the 2020 call and the 2020-2021 call. Because of the travel restrictions due to the COVID-19 pandemic, unfortunately none of these projects could be carried out. One project took place online.
17th Teaching week of Prof. Schwarz at SUT, Thailand, for ASEA UNINET
Nov. 8 – 11, 2021 (held online*)

Prof. Siegfried Schwarz
Prof. (ret.) of Pathophysiology, Medical University Innsbruck

The teaching cooperation between MUI (Medical University Innsbruck) and SUT (Suranaree University of Technology Medical School in Nakhon Ratchasima, Thailand) was initiated in 2006, just 1 year after the Medical Studies Programme at SUT was implemented and SUT was in search for special teaching input from abroad. Since 2007, a – what we called – an „Integrated Endocrinology Teaching Week” was organized at SUT by Dr. Sanong Suksaweang and Dr. Siegfried Schwarz of MUI. Travel expenses were covered by ASEA UNINET. This cooperation was formalized by a Memorandum of Understanding (MOU) in 2011 and renewed in 2017 between these 2 universities. In 2017, a similar program and cooperation was initiated by Dr. Schwarz with Mae Fah Luang University (MFU) in Chiang Rai, Thailand.

„Integrated“ reflected the idea that students should receive a holistic knowledge of a certain endocrinological disease. This was achieved by

1. a **POL** (problem-based learning) case with a genetic disease called CAH (congenital adrenal hyperplasia) in which symptoms, diagnostic procedures and (life-long) therapeutic approaches are discussed.

2. a **venipuncture practicum** in which students learn how to perform practically the drawing of a blood sample from patients in which hormones have to be measured.

3. a **laboratory practicum** in which students learn how to perform practically an EIA (enzyme immuno assay) for measurement of the hormone cortisol (which is pathologically low in the blood of CAH patients and therefore a diagnostic marker)

4. a **molecular modelling practicum** in which students learn how to use a molecular modelling software with which the 3D structure of a protein that is involved in a particular disease can be generated on the computer and into which the mutated amino acid can be mapped in order to anticipate visually what consequences a „wrong“ amino acid would have for the (loss of) functioning of this protein.

At the end of this course, students were encouraged to perform a **homework** for 30 other (mutated) proteins and associated diseases which they will present a few days later in front of the class and the teachers.

5. On the 4th day of this teaching week Prof. Schwarz gave a 2 hrs lecture on further examples of „**molecular diseases**“, i.e. explaining the mutated structure of a protein and deriving from it the clinical consequences. Also the structural details of the **SARS-2 spike protein** in its interaction with the ACE2 as well as with neutralizing antibodies against various epitopes were presenteed.

6. In former years, the 5th day was reserved for an „academic visitation“ of Dr. Suksaweang and Dr. Schwarz at one of the SUT-affiliated provincial hospitals in Buriram or Surin or Chaiyaphum in which SUT medical students have to perform their clinical studies. Students freely described their experiences which Dr. Suksaweang reported to the Dean, for further and iterative improvement. This visitation was, of course, omitted in 2020 and 2021 as well.
In some years, this teaching week was held twice (the second one called Integrated Hematology Week dealing with a genetic disease called Idiopathic thrombocytopenic purpura).

In 2021, this Teaching week was held for the 17th time, however – due to the SARS CoV-2 pandemic - not in real presence of Dr. Schwarz. Instead, a Zoom connection was built up between Dr. Schwarz and the Medical students at SUT or – more correctly – the SUT students in their respective home „offices“. Since months, students are not allowed to enter the SUT campus premises and buildings.

The Zoom connection worked very well, around 140 students were enrolled, interaction was possible through the „chat“ function. All lectures were recorded by the SUT IT service. However, teaching in real presence is certainly preferable: questions can be answered more easily, more frequently, interaction and feedback is better, etc. It is strongly hoped, that in 2022 the Teaching week can be held in the normal presence format like in previous years.

At the end of the lecture on Nov. 11, a students „speaker“ expressed her thanks for the continuation of this teaching cooperation, also via Zoom. Dr. Sanong then „handed out“ each of the students a document “Certificate of participation”.

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<td>Lect. 2 а.п.в.с. сб.вв &amp; Prof. Schwarz</td>
<td>Clinical specimens and their collection</td>
<td>SDL</td>
<td>Lab 1 а.п.в.с. сб.вв</td>
<td>Lab 2</td>
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<td>Lect. 3 а.п.в.с. сб.вв</td>
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<td>SDL</td>
<td>Lab 3 а.п.в.с. сб.вв</td>
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Screenshot from the opening lecture on Nov. 8, 2021, Prof. Schwarz first row 2nd picture, Dr. Sanong 4th picture f.l.t.r.
Project leaders

Prof. Siegfried Schwarz, MUI

Born 1950, promotion Dr. med.univ. 1975, University of Innsbruck Medical School
Since then Assistant, later „Dozent”, later Professor at the Institute of Experimental Pathophysiology & Immunology, Biocenter of MUI),
Habilitation 1983,
Visiting Associate at the National Institutes of Health, Bethesda, USA, 1986-1988
Retired 2015,
Dr. h.c. sci. (SUT) 2016.
https://siegfriedschwarz.wordpress.com/2016/01/14/siegfried-schwarz/

Dr. Sanong Suksaweang, SUT

Education:
Doctoral: 2005 Ph.D., Pathobiology, Keck School of Medicine at the University of Southern California, Los Angeles, CA, U.S.A.
Master: 2000 M.S., Experimental and Molecular Pathology Keck School of Medicine
Bachelor: 1992 B.Sc., Medical Technology, with second class honor from Khon Kaen University

Working experiences:
2011 – Present International Clinical Elective Coordinator
2009 – Present Head of SUT-Medical Technology Clinics for Influenza Research Laboratory
2007 – Present Design and organization with Prof. Siegfried Schwarz of the annual SUT Integrated Hematology/ Endocrinology Teaching week
2005 – Present Medical Educator at School of Pathology and Laboratory Medicine, Institute of Medicine, SUT
2005 – 2006 Secretary for Medical Mega-Project of the SUT
1995 – 1997 Medical Technologist/Medical Laboratory Scientist, CDC/HIV-AIDS Collaboration Research Unit, Ministry of Public Health, Nonthaburi
1992 – 1995 Medical Technologist/Medical Laboratory Scientist, Bumrungrad International Hospital Laboratory, Bangkok

Awards and credits:
2014 Best Alumni Award for Faculty of Associated Medical Sciences of Khon Kaen University of 2014.

*Editor's note: For this reason, Prof. Schwarz received no funding from ASEA-UNINET.
Establishing a human placental explant model to investigate the effect of Traditional Thai Medicine on human placental cell function

Report for „ASEA 2020/MedUni Wien/2“by Isabella Ellinger, MedUni Vienna

Involved persons

Applicant in Austria

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Medical University of Vienna
Rectorate -IO
Tel.: +43 1 40160 21023
E-Mail: human.salemi@meduniwien.ac.at

Project leader Austria

Ao. Univ. Prof. Dr. Isabella Ellinger
Medical University of Vienna; Center for Pathophysiology, Infectiology and Immunology; Institute for Pathophysiology and Allergy Research
3Q, General Hospital of Vienna; Waehringerguertel 18-20
1090 Vienna, Austria
Tel.: +43 1 40400 51310
E-mail: Isabella.ellinger@meduniwien.ac.at

Short Biography:

Isabella Ellinger got habilitated in Cell Biology and is principal investigator at the Institute for Pathophysiology and Allergy Research, MedUni Vienna. She heads the research group “Pathophysiology of the Placenta” and is involved in teaching and research at the MedUni Vienna (https://www.meduniwien.ac.at/hp/ipa/forschung/forschungsgruppen/ellinger-isabella-pathophysiology-of-the-placenta/home/). Currently, her research focusses on (1) studying placental functions under physiologic and pathologic conditions and (2) advancing automated microscopy.

Host Professor in South-East Asia

Ass. Prof. Dr. Waranya Chatuphonprasert
Mahasarakham University
Pre-clinics, Faculty of Medicine
79/99 Nakorn Sawaan Road, Muang, Maha Sarakham 44000, Thailand
Tel.: +66 850137300
E-mail: Waranya.C@msu.ac.th
Short Biography:

Waranya Chatuphonprasert is involved in teaching and research at the Faculty of Medicine, Mahasarakham University, as well as the Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology (PANPB), Khon Kaen University. Currently, her research focuses on drug metabolizing enzymes, drug transporters, and pharmacological and toxicological activities of Thai herbal plants.

Report

During pregnancy, the placenta is an upstream organ of the fetus that takes over the functions of the lungs, liver, gut, kidneys, and glands while the fetal organs have not yet reached full functionality. Despite its short lifespan of nine months, proper function of the placenta is important and has far-reaching consequences. Dysfunction of the placenta increases the risk of complications for both mother and child during pregnancy and delivery and can lead to fetal programming of diseases in the adults according to the “developmental origins of health and disease” hypothesis by Barker. This theory implies that adult cardiovascular or metabolic diseases can be based on a variety of perinatal insults including maternal nutrition, environmental exposure and maternal lifestyle (Ref. 1) that (among other consequences) may result in changes in placental gene expression patterns which consequently cause short- and long-term adaptations of the developing fetal organs.

To ensure proper placental transport and processing of endogenous maternal nutrients as well as protection of the developing fetus from xenobiotic substances, adequate function of the cytochrome P450 (CYPs) family of metabolizing enzymes and transporters of the ABC or SLC22 family is required. Exposure of the pregnant woman and thus the placenta to substances such as Thai medicinal plants may affect expression levels of CYPs as well as ABC and other transporters.

In their previous collaborative research, Isabella Ellinger (IE) and Waranya Chatuphonprasert (WC) demonstrated that traditional Thai medicine plants such as Pueraria candollei var. mirifica (Ref. 2) and Plumbago indica (Ref.3) indeed alter the expression levels of selected CYPs and ABC transporters, suggesting an adverse effect on placental function. These previous studies were performed in the BeWo choriocarcinoma cell line, which is an appropriate and well-established in vitro model (model 1 in Fig.1) to study placental trophoblast cell function (Ref. 4). However, as also shown in Fig.1, the functional units of the human placenta - the chorionic villi - contain not only trophoblast cells that are in direct contact with maternal blood but also other cell types such as macrophages, endothelial cells, and others. These different cells are known to interact with each other in vivo. Tissue integrity is maintained in placental explant cultures (model 2 in Fig. 1), and various protocols for culturing of these explants have been published previously (e.g. Ref. 5 and 6). In the future, to fully understand how traditional Thai medicinal plants affect placental function, IE and WC want to confirm any significant effect of traditional Thai medicinal plants observed in BeWo cells in this advanced model system (model 2 in Fig. 1).
Fig. 1. The structure and cell type composition of the placental chorionic villi and model systems to study the function of individual chorionic cell types (model 1) or the entire chorionic villi (model 2).

Furthermore, during the ASEA-UNINET-funded stay of IE in Thailand it was discussed among IE, WC and Prof. Kanokwan Jarukamjorn from Khon Kaen University (Fig. 2 and 3) that putative alterations of CYPs and transporter expression patterns due to exposure to aromatic aryl hydrocarbons or hypoxia of placental cell lines (model 1) and placental explants (model 2) should be additional topics of future collaborations between our groups.

Fig. 2. Visiting lab infrastructure of partner WC at the Mahasarakham University, Faculty of Medicine, Pre-clinics, and meeting of IE and WC with Assoc. Prof. Dr. Sirinart Tongsiri (Vice-Dean for Quality Assurance and Research) and Asst. Prof. Teabpaluck Sirithanawutichai (Dean of Faculty of Medicine). Photocredits: W. Chatuphonprasert and Mrs. Jutamas Philachai

Fig. 3. Discussing research projects with Prof. Dr. Kanokwan Jarukamjorn (Head of the Doctor of Philosophy Program in Research). Visiting lab infrastructure of Prof. Dr. Kanokwan Jarukamjorn at Faculty of Pharmaceutical Sciences at Khon Kaen University. Meeting with Ass. Prof. Dr. Waranya Chatuphonprasert, Associate Professor Dr. Tipaporn Kanjararach (Associate Dean for Strategic and International Affairs), Associate Professor Dr. Paiboon Daosodsai. (Dean of Faculty of Pharmaceutical Sciences) and Prof. Dr. Kanokwan Jarukamjorn (Head of the Doctor of Philosophy Program in Research). Photocredits: W. Chatuphonprasert and Mrs. Jutamas Philachai
The following requirements for the set-up of the model system 2 (placental explant culture) were identified:

I. Identification of the most relevant traditional Thai medical plants/herbal medicinal products consumed during pregnancy in Thailand.

II. Investigating their impact on relevant CYPs and ABC/SLC22 transporters in placental cell culture models (model 1). Only those extracts/formulations that result in significant changes of enzymes and transporters in model 1 will be further investigated in the explant model (in reference 2 and 3 we have already identified target genes that are influenced by certain Thai medical plants)

III. Access to term placental tissue immediately after birth. This is given, as both, IE and WC work in close proximity to hospitals; all studies will require approval of the respective ethic committees.

IV. Appropriate cultivation protocols of placental explants. It was decided to follow a recently published protocol that changed previous static explant culture to a flow culture protocol. The experimental set-up of the system is described in detail in the respective publication (6). Installing the appropriate hardware components of the cultivation chamber in both (IE and WC) labs is planned for the next year. Apart from that, the assays to measure morphological and functional intactness of the explant tissue are already established in the labs.

V. Of crucial importance for the investigations are methods to address any alteration in enzyme and transporter gene expression due to placental explant treatments with traditional Thai medical plants in comparison to the control group. Here, two types of read outs are important:

   a. Determination of mRNA and protein expression levels of molecules-of-interest (CYPs, ABC-transporters) in total tissue lysates by RT-qPCR, western blotting, ELISA methods. These methods are established in labs of IE and WC.

   b. Determination of cell-type specific (i.e. trophoblast-specific, macrophage-specific,….) expression levels of protein-of-interests (CYPs, ABC-transporters) found to be altered not only in model 1 (I) but also at total protein level in model 2 (Va). For these analyses, a novel approach has been established in the previous months in IE’s lab and has been introduced to and discussed with WC during the research stay (Fig. 4). It is based on a multiplex immunofluorescence staining algorithm of the placental tissue, automated image acquisition and computer-mediated image segmentation with subsequent cell-type specific analysis of expression levels of protein-of-interests. The method was applied in a pilot study in IE’s research group and the related manuscript is currently prepared for submission. Due to the required facilities (automated microscope, image analysis software), the method can currently only be applied in IE’s group.

VI. To come up with the required financial resources for hardware components of the explant model and research material, WC and IE agreed to write and submit individual (and, if possible, also common) research projects in the near future.
Fig. 4. Online presentation for Khon Kaen and Mahasarakhan University researchers given by I. Ellinger and moderated by W. Chatuphonprasert: “Impact of Gestational Diabetes Mellitus (GDM) on M2-phenotype and number of human placental macrophages assessed in situ by multiplex labelling and automated image analysis”.

Photocredits: Mrs. Jutamas Philachai

References


Reports

on

SP 24 Research Mobilities 2019

in 2020

(Selection)
Biogenic raw materials, Resource Efficiency and in the Mekong Delta

Visit of Prof. Gerhart Braunegg to Vietnam National University HoChiMinh City
Duration of the Stay: 2/1/2020 – 24/1/2020

Project Partners

i) Gerhart Braunegg, Prof. Dr. techn.
PhD in Chemical Engineering, retired University Professor for Bioengineering and Applied Microbiology at TU Graz;

ii) Hai Le Than; Director of Institute of Environment and Resources, Vietnam National University, Dr. techn. TU Wien

iii) Assoc. Prof. Dr. Ho Quoc Bang
2005: Msc degree in Environmental Sciences
2010: Dr. degree in Environmental Sciences
Current position: Head of Department of Air pollution and Climate Change

iv Dr. Tung Travan
Institute of Environment and Resources, Vietnam National University,

Report about the visit of Prof. Gerhart Braunegg to Vietnam National University HoChiMinh City

Professor Braunegg was warmly welcomed by Prof. Hai Le Than and the Institute. The focus of the initial days of the visit was to work on the publication of MSc Nguyễn Thị Thu Thảo, “Analysis of energy efficiency in an integrated agro-ecosystem in an acid soil region: research approach for sustainable livelihood in South-West Vietnam”. The publication will be submitted in February 2020 to Journal of Energy, Sustainability and Society, Elsevier, the collaboration of the universities under the ASEA-UNINET program is mentioned in the acknowledgement.

A further publication entitled Waste treatment and soil cultivation in a zero emission integrated system: a case study at a catfish farming system in Mekong delta, Vietnam, (lead author Tra Van Tung) has also been edited, and the publication is still planned for February 2020, after Prof. Braunegg and Prof. Schnitzer, who has always participated in the collaborative projects so far, have carried out final editing.

Prof. Braunegg sees it as one of the most important tasks to place publications of the IER in international journals with a high impact factor in order to consolidate the scientific development of the institute.

Prof. Braunegg lectured at the university in HCMC on

- A critical introduction to the use and disposal of plastic materials, worldwide and in Vietnam
- Possible replacement of fossil plastics in the packaging sector by plastics made from biogenic raw materials and waste materials.

The audience consisted of staff of the IER, as the students were already in their Tet holidays at that time. (Chinese New Year, which is also celebrated in Vietnam).

Further lectures around similar topics, but also stressing the importance of implementing sustainability in
everyday life, were held at An Giang University in the Mekong Delta. His lecture was introduced by Mr. Nguyen Tran Thien Khanh, Head Management and Postgraduate Studies.

The audience were academic teachers and 2nd year students. The discussions at An Giang University with Ass. Prof. Bao-Son Trinh might result in a further cooperation, as Mr. Bao-Son has a close-connection with PRO-Vietnam (https://www.packaging-gateway.com/news/pro-vietnam-packaging-recycling/) which is an organization of the top 9-biggest packaging companies in Vietnam (Coca-Cola Vietnam, Friesland Campina, La Vie, Nestlé Vietnam, NutiFood, Suntory PepsiCo Vietnam, Tetra Pak Vietnam, TH Group and URC Vietnam)

Prof. Braunegg will attempt to actively participate in this cooperation and subsequently submit a project under ASEA-UNINET. The Vietnamese colleagues have been informed that ASEA-UNINET will no longer award Incoming Grants in the future, but this would not hinder future cooperation, the Vietnamese colleagues assure.

Furthermore, Prof. Braunegg was asked about his expertise when a shrimp farm was visited which had asked the IER for scientific advice. According to Prof. Braunegg, the problem of the breeding facility was the insufficient oxygen supply, which caused the shrimps to prefer to stay at the edge of the tank. Improved ventilation could result in an increased yield.

The director of IER, Professor Hai Le Than, stresses that it is the strong desire to continue the longstanding collaboration between TU Graz and IER, which amounts to now 15 years.

Publications

submitted during the stay of Prof. Braunegg in HCMC

Journal: Journal of Cleaner Production

Waste treatment and soil cultivation in a zero emission integrated system for catfish farming in Mekong delta, Vietnam
Corresponding Author: Hai Le Thanh
Co-Authors: Tung Tra Van, Dr; Thao Nguyen Thi Phuong, MSc; Vi Le Quoc, MSc; Hieu Tran Thi, MSc; Son Le Thanh, MSc student; Gerhart Braunegg, Prof. Dr.; Hans Schnitzer, Prof. Dr.; Sibylle Braunegg

Analysis of energy efficiency in an integrated agro-ecosystem in an acid soil region: research approach for sustainable livelihood in South-West Vietnam. to be submitted

Waste treatment and soil cultivation in a zero emission integrated system: a case study at a catfish farming system in Mekong delta, Vietnam, to be submitted

At least 2 more papers for 2020 in international journals, under collaboration with Austrian scientists Prof. Gerhart Braunegg and Prof. Hans Schnitzer.

It is planned to continue the cooperation in 2020. Prof. Braunegg and his colleagues from the IER will submit a project on biogenic raw materials as packaging plastics in the next call.
Photos:

[Images of various scenes and people, all credited to © Gerhart Braunegg]
ASEA-UNINET SCHOLARSHIP REPORT

Visit period: 13th January 2020 to 2nd February 2020 (3 weeks)

Incoming researcher: Dr. Mohd Almie Alias (Universiti Kebangsaan Malaysia, Malaysia)

Host Professor: Prof. John Dunlop (University of Salzburg, Austria)

Title of project: Theoretical modelling of the role of geometry on biological tissue growth in curved substrates

CV:

Name: Mohd Almie Alias
email: mohdalmie@ukm.edu.my

Dr. Almie obtained his BSc and MSc in mathematics from Universiti Kebangsaan Malaysia. He then completed a PhD in Applied and Computational Mathematics from Monash University, Australia in 2018. His research mainly focuses on moving interface problems for example in biological tissues and their associated analytical and numerical methods. His research interest also spans other topics for example the Eikonal-type equations, triangulation of irregular domains and finite element method, and discrete models for collective migration of cells. Almie believes that a mastery of theoretical mathematics knowledge, ability to perform computer programs, and communications with experimentalists are important elements to produce mathematical models that are reliable and usable. He is currently working as a lecturer in Universiti Kebangsaan Malaysia.

REPORT:

This report comprises information about the activities that have been done during the visit, the post-visit follow-up activities that could be done and conclusions.

Activities:

On the first day, I had a brief introduction to the faculty (various labs, group members, pantry, coffee machine and other facilities). I was given a room specially designated for visitor, with access to internet connection and library portal including access to online journals.

I met Prof. John Dunlop almost everyday (or at least once in two days if there were not a lot of things to be discussed). We normally discussed about my understanding/opinions of his research and how they compare to my research, if there were any related studies in the literature which consequently had brought me to encounter various useful papers, and possible extended projects that take into account both of our research. I also had discussions with the other group members: PhD students and postdoctoral researcher. I also attended a talk presented by a visiting Professor on the ‘4D
shape-morphing polymeric biomaterials’. I spent my other time reading papers and doing simple computer codes.

**Follow-up activities:**

Upon my departure from Austria, we will continue communicating through emails and skype. It is hoped that all the ideas can be implemented and studied thoroughly that finally will produce publications and other research output. We could also plan for more collaborations if there is similar funding from OeAD for the year 2021.

**Conclusions:**

Coming from mathematics department, I am blessed to learnt a lot of things from this research visit, particularly the potentials and limitations of theoretical modelling of tissue growth when compared to lab experiments. I am thankful to the OeAD for supporting the visit which has paved the way for me to go deeper into research. I really appreciate the kindness of Prof. John Dunlop, his research group and University of Salzburg for hosting me during the visit, for suggesting various research topics and reading materials, and for many tips on exploring and surviving in Salzburg. Finally, many thanks to Universiti Kebangsaan Malaysia for endorsing this visit and my study leave.
Title of the project:
Surface display of a bacterial chitosanase in *Lactobacillus plantarum*

Duration of the stay:
01/02/2020 – 29/02/2020

Contact detail:

1. Dr. Hoang-Minh Nguyen, Department of Biotechnology, The University of Da Nang - University of Science and Technology, 54 Nguyen Luong Bang, Da Nang, Viet Nam

2. Priv.-Doz. Dr. Thu-Ha Nguyen, Food Biotechnology Laboratory, Department of Food Science and Technology, University of Natural Resources and Life Sciences. Vienna, Muthgass 18, A-1190 Vienna, Austria.

3. Dr. Mai-Lan Pham, Food Biotechnology Laboratory, Department of Food Science and Technology, University of Natural Resources and Life Sciences. Vienna, Muthgass 18, A-1190 Vienna, Austria.

Background:
Chitosanases (EC 3.2.1.132) release chito-oligosaccharides (CHOS) from chitosan, which are of great interest for many food, feed and biomedical applications due to their nontoxic and high solubility properties. CHOS are used as feed additive to provide positive antimicrobial, anti-oxidative, immunoregulatory, and blood cholesterol limiting effects to pigs. It is expected to successfully anchor a bacterial chitosanase onto the cell surface of *L. plantarum* using non-antibiotic lactobacillal expression system and two truncated forms of the LPxTG cell wall anchor for the development of whole-cell biocatalysts for the production of CHOS.

Result:
During this 4-week-visit at BOKU, I performed flow cytometry and immunofluorescent microscopy to confirm the localization of chitosanase on the cell surface of *L. plantarum*. The fluorescent signals in all recombinant strains carrying the plasmids were detected, inferring the successful display of protein on the bacterial surface. These results complete the data needed for this project and we are now preparing a joint-publication.
Final Report for ASEA-UNINET Scholarship Programme

Name: DR LIM SENG JOE

Scholarship: Stipendien aus Mitteln des ASEA-Uninet, Projektstipendien SP 24

Title: In-depth Structural Characterisation of Functional Polysaccharides from Malaysian Brown Seaweeds

Duration: 1 month (1st – 29th February 2020)

Contact details:
Department of Food Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, MALAYSIA
+60175585339 (Mobile) +60389214273 (office) Email: joe@ukm.edu.my

Short biography of persons involved: (Position, topic and work conducted)

- **Dr Lim Seng Joe**: Senior Lecturer of Department of Food Sciences, The National University of Malaysia. In-depth Structural Characterisation of Functional Polysaccharides from Malaysian Brown Seaweeds. Conducted the monosaccharide profiling and recovery of guluronic acid and mannuronic acid from hydrolysis of sodium alginate.

- **Dr Stefan Böhmdorfer**: Deputy Head of Institute of Chemistry of Renewable Resources, BOKU. Monosaccharide profiling of seaweed polysaccharides; Recovery of guluronic acid and mannuronic acid from hydrolysis of sodium alginate. Advised and demonstrated the use GC-MS/FID for monosaccharide profiling; and thin layer chromatography and subsequently flash chromatography for the separation of the uronic acids.

- **Dr Sonja Schiehser**: Coordinator of the laboratory in Muthgasse 18, BOKU, Vienna. Monosaccharide profiling of the polysaccharide from Malaysian brown seaweeds. Advised the methanolysis, derivitisation, and separation using GC-FID, as well as interpretation of the GC-FID data.

- **Dr Markus Bacher**: Senior Scientist of Institute of Chemistry of Renewable Resources, BOKU. Solid state and liquid state NMR spectra of seaweed polysaccharides. Advised and performing the NMR tests necessary. Also performed NMR analysis on recovered mannuronic acid and guluronic acid standards to determine which is which.

- **Dr Josua Oberlerchner**: Post-doc of Institute of Chemistry of Renewable Resources, BOKU. Hydrolysis and separation of sodium alginate into guluronic acid and mannuronic acid. Advised on the hydrolysis process of sodium alginate (sulphuric acid method and trifluoroacetic acid) and separation of guluronic acid and mannuronic acid through TLC methods (suitable TLC plates, eluents, etc).

- **Dr Irina Sulaeva**: Post-doc of Institute of Chemistry of Renewable Resources, BOKU. Molecular weight of polysaccharides isolated from Malaysian brown seaweeds. Performing the gel permeation chromatography (GPC) on the polysaccharide samples.

- **Dr Julien Jaxel**: Post-doc of Institute of Chemistry of Renewable Resources, BOKU. Separation of mannuronic acid and guluronic acid standards through flash
chromatography. Advising and performing the separation and recovery of the uronic acids using the flash chromatography instrument.

- **Prof Dr Thomas Rosenau**: Professor and Head of Institute of Chemistry of Renewable Resources, BOKU. Structural characteristics of functional polysaccharides from Malaysian brown seaweeds. Advised on interpretation of NMR data and its corresponding structural characteristics.

**Work conducted:**

<table>
<thead>
<tr>
<th>Date</th>
<th>Work performed</th>
<th>Location &amp; person involved</th>
</tr>
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<tbody>
<tr>
<td>3-7 February 2020</td>
<td>Monosaccharide profiling (methanolysis, derivatisation and separation using GC-FID) of brown seaweed polysaccharides (fucoidan, laminaran and alginate).</td>
<td>BOKU, Muthgasse 18, Vienna. Dr Stefan Böhmdorfer Dr Sonja Schiehsen</td>
</tr>
<tr>
<td>10 February 2020</td>
<td>Discussion on NMR analysis with Dr Markus Bacher. Deuterated polysaccharide samples passed to Dr Markus for NMR analysis (Solid state and Liquid state)</td>
<td>BOKU, UFT, Tulln. Dr Markus Bacher</td>
</tr>
<tr>
<td>11-21 February 2020</td>
<td>Producing guluronic acid and manuronic acid standards from sodium alginate hydrolysis. Sodium alginites were hydrolysed using 2 methods, i.e. concentrated sulphuric acid and trifluoroacetic acid (TFA). The efficiency of hydrolysis were tested using thin layer chromatography (TLC) with different eluents. We successfully separated the uronic acid standards using TFA hydrolysis (2M TFA, 100°C, 5 hours, nitrogen gas evaporation, lyophilisation) through TLC.</td>
<td>BOKU, UFT, Tulln. Dr Stefan Böhmdorfer Dr Josua Oberlerchner,</td>
</tr>
<tr>
<td>21 February 2020</td>
<td>Discussion with Dr Irina Sulaeva on molecular weight determination of the polysaccharides using gel permeation chromatography (GPC). Samples were passed to Dr Irina Sulaeva.</td>
<td>BOKU, UFT, Tulln. Dr Irina Sulaeva</td>
</tr>
<tr>
<td>24-25 February 2020</td>
<td>Separation of the guluronic acid and mannuronic acid obtained from TFA hydrolysis of sodium alginate using Flash chromatography. The separated standards were then analysed using NMR to determine which is which.</td>
<td>BOKU, UFT, Tulln. Dr Stefan Böhmdorfer Dr Julien Jaxel</td>
</tr>
</tbody>
</table>
26 February 2020 | Discussion and interpretation of NMR results with Prof Dr Thomas Rosenau and Dr Markus Bacher. | BOKU, UFT, Tulln. Prof Dr Thomas Rosenau Dr Markus Bacher
---|---|---
26-28 February 2020 (planned) | Methanolysis procedures on the guluronic acid and mannuronic acid to determine its presence in the polysaccharide samples. | BOKU, Muthgasse 18, Vienna. Dr Sonja Schiehser

**Results:**

- Monosaccharide profiling has established that the polysaccharide samples showed different profile, where fucoidan contained mainly of fucose; laminaran contains mainly of glucose and mannitol; while alginates are expected to contain mainly of guluronic acid and mannuronic acid (the guluronic acid and mannuronic acid standards have yet to be analysed at the time of writing).

- The guluronic acid and mannuronic acid standards were successfully recovered using Flash chromatography, and thus will be applied to methanolysis procedures to obtain their response factors for alginate monosaccharide profile analysis.

- NMR results are still in interpretation stage, where interpretation of the NMR data will be performed when I am back in Malaysia. Molecular weight determination has yet to be performed, but will be performed by Dr Irina Sulaeva next month. I will be in contact with Dr Irina Sulaeva on the results.

**Intended Publications:**

- I intend to prepare a manuscript for the work conducted here in Austria for submission to scientific journal. Possible journal: Food Chemistry

Prepared by: DR LIM SENG JOE Checked by: DR STEFAN BÖHMDORFER Verified by: PROF DR THOMAS ROSENAU

Scholarship Awardee Supervisor Host Professor
Final report for ASEA-UNINET SP24 – 1-month research stay (2020)

(4th - 31st March 2020, University of Vienna, Austria)
Project: “NLP for Disaster Management”

Contact person: Asst. Prof. Dr. Hathairat Ketmaneechairat
Email: hathairat.k@cit.kmutnb.ac.th

Asst. Prof. Dr. Hathairat Ketmaneechairat is currently a lecturer at the Faculty of College of Industrial Technology, King Mongkut’s University of Technology North Bangkok (CIT-KMUTNB). Her main teaching for Bachelor in the curriculums of Information and Production Technology Management. She interested in the areas of Data Mining, Machine Learning and Natural Language Processing.

Description of scientific topic

According to the important of disaster management, we try to find a method that can be used to support the process of management system in terms of social media information extraction. In the past we published a paper related to Natural Language Processing in order to extract information from twitter into a class of object (Name Entity Recognition-NER) and also a class of massages such as announcement, request and support using Condition Random Field-CFR.

The analysis of natural disaster-related multimedia content got great attention in recent years. Being one of the most important sources of information, social media have been crawled over the years to collect and analyze disaster related multimedia content. Satellite imagery has also been widely explored for disasters analysis. However, we discussed how to create the verification engine for cross check the disaster occurs at any time from the social media and satellites. We also plan to write a paper for the conference indexed by scopus, IEEE etc. under the title of “Verification of Disaster Alert in Twitter Through Satellite Images”. We are now working on the literature reviews and also collecting data from twitter and satellite image in order to create the verification engine of disaster.

Univ-Prof. Dipl.-Ing.Dr. Dr. Prof. Gerlad Quirschmayr,
Asst.Prof.Dr.Hathairat Ketmaneechairat
Final report for ASEA-UNINET SP24 – 1-month research stay (2020)
(4\textsuperscript{th} - 31\textsuperscript{st} March 2020, University of Vienna, Austria)
Project: “NLP for Disaster Management”

Contact person: Asst.Prof.Dr. Maleerat Maliyaem
Email: maleerat.s@it.kmutnb.ac.th

Asst.Prof.Dr. Maleerat Maliyaem is currently a lecturer at the Faculty of Information Technology and Digital Innovation, King Mongkut’s University of Technology North Bangkok (IT-KMUTNB). Her main teaching for Master and PhD in the curriculums of Information Technology and Data Science. She interested in the areas of Natural Language Processing, Information Retrieval, Machine Learning and Artificial Intelligence.

Description of scientific topic

According to the important of disaster management, we try to find a method that can be used to support the process of management system in terms of social media information extraction. In the past we published a paper related to Natural Language Processing in order to extract information from twitter into a class of object (Name Entity Recognition-NER) and also a class of massages such as announcement, request and support using Condition Random Field-CFR.

Text centroids is a method that has been inspired from the center of mass in Physics. It refers to a specific point as one mass of the system gathered, it is balanced around the center. The average of the weighted coordinates of the distributed mass determines the coordinates and position of the object. To reduce the complexity of calculations, normally this will be replaced with a single mass at the position or the center of mass. However, we discussed how to apply centroid text representative to get the term that can be match to the disaster situation on library holding official guideline. We also plan to write a paper for the conference indexed by scopus, IEEE etc. under the title of “\textbf{Centroid-based approach mapping to disaster situation on library holding official guideline}”. We are now working on the review literature and also collecting data from twitter in order to create co-occurrence graph and calculate centroid-based text representative. Then try to do mapping with the library holding official guideline in order to prove the concept of term matching using centroid text representative concept.

(Univ-Prof. Dipl.-Ing.Dr. Dr. Prof. Gerald Quirchmayr)
(Asst.Prof.Dr. Maleerat Maliyaem)
Historical and Cultural Indonesian Research: Wayang Beber

01st February to 21st February 2020, University for Continuing Education Krems, Austria

Wiwik Sri Wulandari, Prima Dona Hapsari, Indiria Maharsi, Warsono

Email address: dona.hapsari@gmail.com

The lecturers of Institut Seni Indonesia Yogyakarta (ISI Yogyakarta) received the grant of SP 24 by the ASEA UNINET to conduct a joint-research program and an art conservation training program at Danube University for Continuing Education, Krems, Austria on February 1st – 21st, 2020. There were four lecturers who joint this program, and it was facilitated and supervised by habil. Mag. Dr. Patricia Engel. The team of ISI Yogyakarta were Wiwik Sri Wulandari, Prima Dona Hapsari, Indiria Maharsi, Warsono. The report is presented individually for the OEAD.

Prima Dona Hapsari is a lecturer at Musicology Study Program, Faculty of Performing Arts, Institut Seni Indonesia Yogyakarta. The topic of conservation is a new insight for her as a lecturer as her background study is language education. By doing the training program at Danube University Krems, Austria under the supervision of Dr Patricia Engel, she received some first steps in understanding and valuable knowledge toward conservation on cultural heritage by using a Wayang Beber for developing a conservation concept. Dr.habil Patricia Engel gave her lectures on several basic understanding of conservation of cultural heritage items, particularly for Wayang Beber.

The training program conducted by Prima Dona Hapsari was done into three categories, i.e. (1) preparation of conservator’s documentation and material understanding, (2) environmental standard and proper storage of the artworks, and (3) comparative study of material Wayang Beber. By carrying out the training program, she had more valuable insight and workload focusing on the scientific aspects. There were important lecturers and information facilitated by Dr Patricia Engel as follows:

a. Visiting Program and Learning the storing and archiving method of the book collection at the Monastery Zwettl Abbey, February 4-5th, 2020

On Tuesday, 4th February 2020 – Wednesday, 5th February 2020, the team of ISI Yogyakarta lecturers learned and understood the idea of the world heritage, the book archiving storage, and the environmental standard of the monastery building, and visited the Zwettl Abbey Monastery, in the north of Austria. They met Dr. Andreas Gamerith as the art historian who worked for the monastery. There they found step-by-step procedure for conservators to understand. It was very important for the team in which they could learn how to do the book archiving and understand the environmental
circumstance which supported the storage room for the manuscripts at the Monastery Zwettl Abbey. The idea of this first step was, that the principles in how to observe a collection of items, books or any other items, the method to make a documentation of the artefacts and their condition and the way how to evaluate a room and to decide whether it can be used for a storage is the same, not matter if we have a collection of archival material, books, textiles, paintings or any other. Along with these absolutely basic tasks, the objective was to introduce the first knowledge about paper, parchment and leather, how to distinguish the sorts and how to identify damage.

The followings are some important points for the team of conservation in regards to book archiving:

(1) **To understand the environment appropriate for the book archives and manuscripts at the Zwettl Monastery**

Zwettl Abbey Monastery is a Sisterißen order monastery located 3 km northeast of the city of Zwettl in Austria. This monastery was built by Hadmar I of Kuenring in 1137 as a monastery under the Heiligenkreuz monastery. This monastery is very strong with Romanesque and Baroque architectural styles. Baroque elements were added in the 18th century (source: [https://en.wikipedia.org/wiki/Zwettl_Abbey#History](https://en.wikipedia.org/wiki/Zwettl_Abbey#History), accessed on March 2nd, 2020)

To understand in details about how the conditions of an ideal document and how to do the document archiving was by viewing and observing the manuscripts and book collection of Zwettl Abbey Monastery which were storage in the archive rooms with the following condition:

- They measure approximately 12 x 10 square meters, with a height of between 4-5 meters.
- They are located on the ground floor.
- The outer walls of the archives are very thick and have been confirmed to be far from moisture or water utilities. The strength of the building construction on the ground floor and wall thickness also has a function to provide security guarantees in the event of an earthquake, extreme weather or a threatening fire hazard.
- The floor of the archive room is made of materials that are not prone to moisture. Previously, the floor of the archive room was built using natural stone floors, but they absorbed water from the pores of the stone so that the floor was renovated using concrete which did not absorb water from the ground.
- Archive storage racks use the Roll O Pact Mobile model, which is sized according to the area of the archive room.
- The condition of the archive room environment at Zwettl Abbey Monastery is good in which there is no threat of termites or humidity due to fungus.
Figure 01. The team were working at the storage room of Zwettl Abbey on 4-5th February, 2020 (Doc. By Patricia Engel, 2020)

(2) Understanding on the Temperature and Humidity
• hygrometer is used as a humidity level controller for the archive space.
• The archive room is in good condition of air circulation.
• The archive room complies with temperature and humidity standards

(3) Understanding on the Light and Lighting
Light and lighting are not blinding, shaded and very contrasting.
In the archive room, there is a window facing the outside of the building, where direct sunlight does not hit the archive.

(4) Understanding on Wind
• Building foundations are designed to be strong to support strong walls so that they can withstand strong winds, heavy rain, extreme weather or even earthquakes.
• Windows and doors are made with strong, high-quality materials to prevent heavy rain and water exposure.

(5) Understanding on the Rack for storing the archives or books
• The archive rack uses a Roll O Pact Mobile model that is robust and can be moved according to the archive to be searched.
• File racks are made of metal that do not rust easily.
• Archive material is free from termites or pests
(6) Archive Security

- There are fire extinguishers in the archive room
- Fire alarm system and fire fight system
- Fire extinguisher / smoke detection
- Hydrants inside and outside the building

(7) To identify and diagnose the decay of the books and paper as the basic aspect of understanding conservation

One important thing that needs to be known to further the world of art conservation is understanding the material to be conserved. Likewise, in our art conservation training agenda at Zwettl Abbey Monastery, we were studying book and paper material contained in the monastery's archive room, especially how to identify the condition of the book and the conditions of its damage. Each of us was assigned to identify the condition of the book, including: what materials are made for the volume of the book cover (wood, cardboard, etc.), book binding (vegetable leather, half leather, a quarter binding, alum leather, partchment, paper, etc.), writing material of the book (carboon, iron gal ink), analysis on the paper made (machine or handmade paper), and the details of damage causes (ink corotion, insect investation, microorganism, and clasp missing or damaged).

Then, we also analyzed or examined in more detail the damages, such as whether it has ink corrosion, folded paper, mold, or damaged media binding conditions, etc. It was very useful for a conservator before conducting material analysis using laboratory tools. As Dr. Patricia Engel once said, a conservator uses his entire senses to understand the environment of the material under his/her body as far as possible, then recognizes when it is sensitive to
the symptoms that cause damage to the material. The survey with well trained and experienced senses is a valuable tool for a conservator and allows him/her to bring forward a hypothesis for an instrumental analyse if this is needed.

To make a documentation of a collection with a large quantity of items a table is of great help:

Table 01. Identify and diagnose the decay of the books and paper at the book archive at Monastery Zwettl Abbey by Dona and Wiwik

(8) Observation on the Art Collection of the Abbey and the Church of Monastery Zwettl Abbey with Dr. Andreas Gamerith

After we carried out the project to identify and diagnose the damage to books and papers in the archive room, we were given an excellent opportunity by Dr. Gamerith as Art Historian to see the works of the Zwettl monastery and the Catholic church collection.
b. One Day conservation exercise at the graphic collection of the Abbey of Heiligenkreuz, Austria, on 10th February 2020

Here is the publication can be search on this website: http://www.stift-heiligenkreuz-sammlungen.at/
Introduction

This summary report describes the activities undertaken by four members of the academic staff of Institut Seni Indonesia Yogyakarta (ISI Yogyakarta): Prima Dona Hapsari, Indira Maharsi, Wiwik Sri Wulandari and Warsono being at the graphic collection of the Abbey of Heiligenkreuz. Under the supervision of Dr. Patricia Engel, the Indonesian scholars who were on internship with Donau-Universität Krems held a practical exercise in surveying a graphic art collection, in this case, the collection of the Abbey of Heiligenkreuz, Austria. The event was organized by three institutions, Institut Seni Indonesia Yogyakarta, Indonesia (ISI Yogyakarta) and Danube University Krems, Austria and the Abbey of Heiligenkreuz under the program of SP24 sponsored by ASEA UNINET.

The work was performed on 10th February 2020 in Heiligenkreuz.

The task

ISI Yogyakarta lecturers were tasked with
(a) Developing a concept of survey of a certain amount of works of graphic art
(b) Developing categories of items to be used in performing the next task: that of developing a comprehensive conservation concept of a more general level
(c) Developing a cataloguing system, to encompass description of the material and the condition of prints and drawings

While in Zwettl everything was so to say prepared for going in medias res, in Heiligenkreuz the situation is one step “earlier”, which gave the scholars the opportunity to apply now themselves what they had learned in Zwettl in terms of how to approach a collection and how to find a way through unsorted heaps of Cultural heritage items, how to systematize and categorize and how to cluster damage.

The method

The survey was conducted in visible (direct, raking and transmitted) light, without magnification.

The categorization was based on the material of the artefacts as well as techniques used, such as

1. pencil drawing,
2. aquarelle painting,
3. wood-cut and lino-cut (Hochdruck) technique,
4. intaglio (Tiefdruck) technique,
5. flat printing (Flachdruck) technique,
6. photography,
7. copy technique.
In cases where a number of techniques used by one artist were identified, the corresponding works were kept together and not sorted according to technique or material.

The reason for choosing this categorizing approach is because the different works of art need certain conservation approach: drawings are most sensitive to abrasion, aquarelles are extremely vulnerable to light exposure, intaglio has fine surface relief, wood-cut and lino-cut also have sensitive surface, while the flat printing, as a more recent technique, involves using contemporary paper which is more sensitive to mould attack and tearing. Photographs need specific storage conditions.

The approach carried out was to observe the art, to perform the separation of the works based on the techniques used, group them by artists and dispose of folders and wrappers which are harmful for the articles.

We selected this method because each category needs different treatment in all aspects of conservation.

**Observation results:**

1. We observed all art works which were on top of the drawer closed and known now how many individual pieces representing each artistic techniques exist in this bundle.
2. We found there are famous artists’ works in the collection, such as prints after Albrecht Duerer, Paul Rubens, Tizian, Mark Chagal, and others.
3. We prepared the next steps by developing the method and system, which now can be applied to the entire collection.

**Next steps:**

1. We suggest that the whole collection be classified according to the system suggested.
2. We recommend developing a catalogue and documentation system or model.
3. We recommend preparing a description of the types of decay present in the collection.

The report was given to the monastery. This way a win win situation was created.
Figure 05. Indonesian lecturers were in frame with the Priest of the Abbey of Heiligenkreuz and Dr. Patricia Engel. (Doc. Abbey of Heiligenkreuz 2020)

Figure 06. Indonesian lecturers analyzed the condition of the graphic arts collection of the Abbey of Heiligenkreuz with Dr. Patricia Engel. (Doc. Abbey of Heiligenkreuz 2020)
Figure 07. Indonesian lecturers observed the graphic arts collection of the Abbey of Heiligenkreuz with Dr. Patricia Engel. (Doc. Abbey of Heiligenkreuz 2020)

Figure 08. Dr. Patricia Engel showed the Indonesian lecturers how to record the data for each collection. (Doc. Abbey of Heiligenkreuz 2020)
c. The understanding of graphic art conservation at Saint Florian, Linz, Austria on 11-12th February, 2020

There are also important things we learned when doing the work at Saint Florian Monastery, Linz, Austria. In order to understand the graphic art, the conservator should also be aware of the condition of each collection, particularly when they are the old collection of the Abbey and Monastery which are very valuable and must be conserved.

The overarching idea to the visit in St. Florian was: after the first step in Zwettl, where everything was new and had to be introduced from scratch, and the application of the new information at the comparatively small Graphic Collection of Heiligenkreuz, where the fact that the same principles of conservation can be applied to archival material and graphic art, not in St. Florian, the team did concrete conservation work in a huge and important collection of art. Finally there was the opportunity to make a conservation concept for 2 prints and a charter made of parchment as well as a copy. This was the end of the practical session and gave the opportunity to get feedback on what the team had learned.

The followings are the step in doing the graphic art conservation:

1. Observing and analysing the condition.
2. Unframing the art works to clean.
3. Cleaning the molds and ensure the environment of the Graphic Art Collections are safe by using the special sponge made of latex.
4. Cleaning the frame and cover glass properly with special cloth and glass cleaner.
5. Pulling out the broken nails and change the new ones.
6. Framing the artworks.
7. Besides that, the team was shown the special collection of Albrecht Duerer’s Graphic Art Works to analyze and the details of his collection to observe.

Figure 09. One of the lecturers of ISI Yogyakarta was framing the collection after cleaning (Doc. By Wiwik Sri Wulandari, 2020)
Amongst the lectures in Krems was the one together with Prof. Dr. Harno Dwi Pranowo in which a documentation of a manuscript was demonstrated and the involvement of material analysis was discussed. Furthermore, the lecturers of ISI Yogyakarta received valuable information of how to handle the old manuscript, figure out the damage.
e. **Microscope Understanding**

Besides receiving information on material analysis, the lecturers were shown how to use the microscope for fibre analysis.
f. Comparative material study of Wayang Beber collection at Volkenkunde Museum, Leiden, the Netherland

The original of Wayang Beber is also found in Volkenkunde Museum, Leiden. There are six scrolls of Wayang Beber Leiden. Based on the Carbon Analysis from Tokyo University Museum, the Wayang Beber of Leiden was made estimated around 1516 – 1596. Mostly, they are all made of Daluang/Daluang with the particular condition that a conservator had conserved them by using the non-daluang paper to restore the damage found on the surface of the scrolls. The damage of each scroll is different. Furthermore, the scrolls of Wayang Beber Leiden do not have the storage box or a wooden box to keep like what is found for the Wayang Beber Wonosari and Wayang Beber Pacitan.

An interview with a conservator of Wayang Beber Leiden in Volkenkunde Museum, Ms. Irina Tsjerovenova on February 17th, 2020 was carried out by the authors to find the information that the Wayang Beber Leiden had already been conserved before. It was confirmed by finding out the paper glued on each scroll which makes it looked thicker. Ms. Irene does the conservation method regularly, such as cleaning the dust regularly, scrolling each scroll by inserting the acid-free paper to maintain the coloured surface free from the more damage and any continuing acidification. Moreover, to maintain the stable condition in storing them, there is a cotton rope on each edge of the scroll to tie it up. To keep them all in a proper storage box, the conservator uses the excellent quality of cardboard.

Based on the information given by Ms. Tsjerovenova, Prof. Sakamoto from the Tokyo University Museum had carried out the carbon fibre analysis for the six scrolls of the Wayang Beber Leiden. However, there was not any analysis for the colour pigment of the Wayang Beber Leiden by Prof. Sakamoto.

There are some hypotheses found from the observation. The authors found some important things as follow:

a. The damage on each scroll, such as discolouration, bend, stein, torn, gold-coloured is peeling off, there are some painted parts on the scroll to cover the hole.

b. According to the story of Wayang Beber Leiden, it has a similar story as Wayang Beber Wonosari, that is 'Remeng Mangunjaya', but the visual depiction of the scenes is different.

c. The drawing style is similar to Wayang Beber Wonosari with few ornaments found in the background.

d. The colour of each scroll still looks bright and clear, particularly the red colour; however, the yellow colour is derived from the gold which has already been peeled off.

e. The coloring technique looks similar to the Wayang Beber Wonosari which is seen from the sketching, inking, and coloring.

f. Each scroll consists of four panels or in Javanese term it is ‘pejagong’.
g. Visiting Program at the Welt Museum Wien

On February 20th, 2020 a meeting with experts in the area of conservation, preservation, and restoration in Weltmuseum was undertaken. The visiting program was very valuable and fruitful to foster the understanding of bark cloth materials of cultural heritage and how to do the preservation on it. Daluwang, the carrier of the Wayang beber is a bark cloth material. The Weltmuseum has a rich collection of this material from all over the world.

The visit was to meet Mag. Roswitha Zobl, an expert on bark cloth at Weltmuseum Wien. Mrs. Zobl showed various bark cloth pieces, discussed the various sorts and how to distinguish them. She furthermore explained what would be the focus on bark cloth
conservation and what tools are used for doing the conservation on the bark cloth as well as how the tools are used properly.

Besides having a look at some examples of old bark cloth, Mrs. Zobl also showed the collection of Indonesian heritage kept at the Museum since the beginning of 20th century. They came in Austria as the gifts and private collections of the Austrian emperors.

Figure 16: Indonesian Researchers were shown one of the bark clothes from Indonesia by Mag. Roswitha Zobl at Welt Museum Wien.(Doc. By Wiwik Sri Wulandari, 2020)

Figure 17. The Indonesian team was in frame with Mag. Roswitha Zobl and the French student at Welt Museum Wien.(Doc. By Wiwik Sri Wulandari, 2020)
Scientific Report

Predictive maintenance using machine learning and SPC methods to optimize end-mill utilization

Name: Mr. Md. Nizam Bin ABD RAHMAN
Country of origin: MALAYSIA
Dates of scholarship: 16.02.2020 - 07.03.2020
Host professor: Univ.Prof. Andreas RAUBER / TU Wien

Executive Summary of Research Proposal

Implementation of IR 4.0 in manufacturing environment will enable the collection and digitization of the in-situ equipment data in cyber-space. These data are readily available to be utilized for the main benefit of IR 4.0 implementation which are the improvement of the productivity and efficiency of the manufacturing system. Peripheral benefits can and should be realized from the readily available digitized data. One of the potential benefits that can be harnessed from such environment is the Predictive Maintenance (PdM) system which can predict when the maintenance should be performed based on in-situ data acquisition. PdM approach is superior than routine or time-based Preventive Maintenance (PM) with regards to cost saving and optimization of part utilization. In general, acquisition of sensory data and machine learning approach is adopted to predict equipment component health, such as bearing and tool wear, without conducting physical inspection on the component. The predicted data will then be used to predict condition of the component. The current approach does not take into consideration on the inherent variability of the sensory data which affect the predicted component condition accuracy. The aim of this research project is to propose a new framework on the implementation of PdM for milling cutting tool using hybrid approach of machine learning and SPC in components health determination. At the end of this research project, the proposed predictive maintenance framework should be able to define the parameters that need to be collected to provide early fault detection, fault detection, and time to failure prediction. The framework will be evaluated on the prediction of cutting tool condition during machining.

Problem Statement:

In large, the current practice in cutting tool replacement is based on cutting time or cutting length. Such practice is aligned with the conventional PM concept. The setback of PM is the possibility of not maximizing the useful life of the replaced parts. On the other hand, the risk of not performing the PM is the possibility of inconformity of the machined surface quality. There are some published works on the modeling of cutting tool wear monitoring system using artificial intelligent (AI) approaches. The modelling of cutting tool wear, predict the wear on cutting tool based on the monitored variables such as vibration, power consumption, etc. However, such modelling lack of guidance on the influence of process variabilities that are necessary to determine the trigger limit to initiate the maintenance. Preventive maintenance based on SPC has been reported as an approach for tool wear management system. Such PdM using SPC requires the measurement of tool wear which will interrupt the production. PdM using SPC approach is lacking in the intelligent to forecast the tool wear based on other process variables. Wholistic PdM framework using both AI and SPC is lacking.
Objectives of the Research:

I. To propose new predictive maintenance framework that combines the machine learning and SPC

II. To evaluate the proposed new predictive maintenance framework in predict the wear of milling cutting tool.

III. To validate the accuracy of the framework.

Methodology:

Phase 1: Experimental Design and Data Collection

- The experiment is going to be conducted using CNC milling machine
- The sensory process data to be collected are vibration, temperature, cutting force, power and acoustic. These data can be collected in time series while the process is running. The generated data collected will be tool wear and surface roughness measurement. The process needs to be interrupted during this data collection. 100 experimental runs will be conducted base on run to failure condition.
- The cutting tool and workpiece materials are tungsten carbide and D2 steel respectively.

Phase 2: Development of Prediction Model and SPC

- The initial part of the development of prediction model is the preprocessing of data, using R programming, where the raw data need to be cleaned in terms of removing duplicates, correct errors, deal with missing values, normalization, data type conversions, randomization, etc. The data then is split into training and evaluation sets.
- Appropriate algorithm will be selected to train the model based on the behavior of the collected data using Pyhton programming. The model is then trained to make prediction correctly as often as possible. For example, for linear regression model, the algorithm needs to learn values for intercept and slope. The trained model will be evaluated against the collected evaluation data sets.
- The sensory data variabilities will be analyzed. Variance of the sensory data variability will be used to predict the variability of the generated data (tool wear and surface roughness) using the developed predictive model. Trigger limit will be recommended based on these analyses using SPC approach.

Phase 3: Validation

- Validation run to test the predictive model and the trigger control limit will be conducted. Error percentage will be calculated to ascertain the validity of the predictive model and approaches.

The proposal is based on discussion with:

- Ao.Univ.Prof. Dr. Andreas Rauber, Head of Research Unit, TU Wien, Institute of Information System Engineering
- Alexander Schindler, Scientist, Information Management, Center for Digital Safety and Security, Austrian Institute of Technology
- Gerhard Reisinger, Institute of Management Science
- Thomas Trautner, Research Associate, IFT, TU Wien
FINAL REPORT

Evaluation of defects in Mg-doped AlGaN layers by electron-beam-induced current technique.

Duration:

22 days (4\textsuperscript{th} Feb until 25\textsuperscript{th} Feb 2020)

Contact details:

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Mobile number: +60125925485

Details of report:

My program at TU Graz has focused on investigating defects characteristics in Mg-doped Al\textsubscript{x}Ga\textsubscript{1-x}N samples using electron beam induced current (EBIC). The samples were grown at Universiti Sains Malaysia (USM) beforehand. This is my first experience of conducting this measurement as the tool is not available in my country.

The measurement started with a series of green LED samples that consists of Mg-doped GaN layer. One of the LED samples has the Mg-doped GaN layer which was grown at 980 \textdegree C, while for the rest of the samples, the GaN layer was grown at 1020 \textdegree C. From optical-electrical measurements which were conducted at USM, the LED sample with 980 \textdegree C grown Mg-doped GaN shows a better performance than its counterparts. The reason is that growing the Mg-doped GaN layer at high temperature of 1020 \textdegree C will destroy the multiquantum wells (MQWs) active region of the LED, thereby leading to degradation of the LED performance. In contrast, the EBIC current was found to be higher for the LEDs with the 1020 \textdegree C grown Mg-doped GaN layer than the one with 980 \textdegree C grown Mg-doped GaN layer. At higher growth temperature, high quality Mg-doped GaN layer can be obtained. Hence, the formation of defects can be suppressed, resulting more EBIC current.

Subsequently, another set of semipolar and non-polar LED samples with Mg-doped GaN layer were measured. As opposed to conventional growth direction; that is in c-plane direction, growing LEDs in semipolar or non-polar direction will eliminate the polarization and spontaneous effects. Such effects always degrade the performance of LEDs. Unfortunately, the samples measured in this work do not show a good diode behavior. This is in turn giving weak signals in the EBIC measurement. The result indicates that the Mg-doped GaN layer in the semipolar and non-polar direction was grown under non-optimized conditions.
The last set is DUV LED samples with Mg-doped AlGaN layer. Due to poor conductivity of p-type and n-type, diode characteristic is hardly seen and therefore, EBIC current is very low. The progress of developing the LEDs still at early stage and many works need to be done for improvement. Recently, we have successfully grown a working DUV LED, which gives a good diode characteristic. Unfortunately, the LED was grown after I arrived in Graz. We plan to send this LED to Graz for the EBIC measurement soon.

Overall, I found that EBIC measurement is an interesting tool to further understand the properties of the Mg-doped Al$_{x}$Ga$_{1-x}$N layers. Such understanding would be helpful for us to take further step towards increasing the performance of our LEDs. Apart from improving the material quality of the samples, we should improve the electrical contact in order to generate more EBIC current and subsequently, leading to more accurate data. To do this, we will fabricate the samples through a series of lithography, etching and deposition process. We will start this work after our fabrication facilities is ready.

Other than conducting the EBIC measurement, I had a chance to give a talk related to my work in Malaysia to researchers at TU Graz in the second week of my program. In the talk, I introduced and shared some scientific results of my research works at USM and our future plan for sustaining nitrides research in Malaysia. I also took an opportunity to visit CVD lab which is under supervision of Dr. Anna Maria Coclite. Her team has setup a home-made atomic layer deposition (ALD) system for depositing monomers layer on silicon substrate. I was excited to learn that she also has deposited AlN films using the system.

After this program, I intend to share my experience on conducting EBIC measurement to researchers at USM, including introducing EBIC measurement to our students. Through my communication with Prof. Peter Hadley and his colleagues alongside posters hanging around the department, I am aware of other research works that are running at TU Graz. Interestingly, my colleagues in Malaysia are also doing similar works. I expect that my visit would initiate more collaborations between USM and TU Graz in future.

**Short biography of participating persons:**

Prof. Peter Hadley is a professor at Institute of Solid-State Physics, TU Graz. His current research interest is to study defects in semiconductor materials and thermal behavior of OLEDs. He is the person who establish EBIC setup at TU Graz.

Dr Anna Maria Coclite is an associate professor at Institute of Solid-State Physics, TU Graz. She is an expert in growing materials using chemical vapor deposition (CVD) technique. Due to her outstanding research work, she has received awards from various organizations.

Confirmed by,

Norzaini Zainal, PhD
Universiti Sains Malaysia

Confirmed by,

Prof. Peter Hadley
Institute of Solid-State Physics, TU Graz
Title of Project:
Expressionism in Austrian and Indonesian Poetry: A Case Study of the Poems of Georg Trakl and Chairil Anwar

Duration of Stay:
from 01.02.2020 to 26.02.2020

Biography of all relevant participating:
(1) Prof. Dr. Achim Hermann Holter, M.A., Department Comparative Literature, University of Vienna, Sensengasse 3a, Phone 43-699-12288725, Email: komparistik@univie.ac.at, Head of Department of Comparative Literature and Supervisor; (2) Dr. Mag. Stefan Albert Kutzenberger, Department Comparative Literature, University of Vienna, Sensengasse 3a, Email: stefan.kutzenberger@univie.ac.at, as partner of research in Austria.

Expressionism is an artist’s tendency to distort reality with emotional effects. Expressionism can be found in literary works, films, paintings, music, and architecture. Expressionism emerged as part of the reaction to impressionism and classical academic art which had reached an established artistic peak which was considered too rigid because it only imitated the nature. Expressionism was very much inspired by the flow of symbolism in 19th century art.

This research aims to compare the style of expressionism in poetry in Austria and Indonesia by taking samples of poem by Georg Trakl from Austria and Chairil Anwar from Indonesia. The two poets are alike in expressionism. Both poets are equally famous in their respective countries. In their work they talk about silence or loneliness, anxiety, and death. Georg Trakl and Chairil Anwar’s poetry about seems to be a prediction of their death. They both died young but with different cause. This research uses a comparative literature approach. In this case, the comparative literature focuses on the study of literature from different cultures, context, and nations.

The results showed similarity and difference between Georg Trakl’s poetry and Chairil Anwar. Theme raised about death, anxiety, solitude, and despair. The theme like that is found in Trakl’s poetry “Nahe des Todes”, “Amin”, while in Anwar’s poetry this theme is found in poetry entitled “Nocturno”, “Nisan”, “Kapada Kawan” (to Friends). Both of these poems feel close to the death because Trakl is a cocaine addict and has psychiatric problems so he always feels lonely, hopeless, and suffering. Anwar also felt close to death and hopeless because of the threat of various diseases he suffered. Because the themes raised are related to the gloom in the poetry, the dominant atmosphere is night, black, and dark. In addition, the two poets also pointed out that the poem was addressed to certain individual. However the individuals mention in Trakl’s poetry are fictitious, like Helian figure
whereas in Anwar’s poetry the individual actually do exist, for example “Sajak Buat Basuki Resobowo” (Poetry for Basuki Resobowo).

In his poetry Trakl very rarely uses character “Me” because he more often appears implicitly through the characters in his poetry, such was Helian, Holderlin, the Grandchild, the daughter, and so on. Trakl hid himself behind the characters. Anwar tends to use characters “Me” in his poetry because character ‘Me” here is used as a strategy for his poetry to escape the Japanese invaders censorship which prohibits the circulation of poetry that contain propaganda.

In Trakl’s poetry, the feeling of pessimism is very much felt. Nevertheless, the pessimism and gloom in Trakl’s poetry is beautiful conveyed. In this case, there is an esthetikation of pessimism and gloom. On the contrary, in the Anwar’s poetry in the silence and gloom that is depicted there is great optimism, as in poetry “Siap dan Sedia” (Ready), “Saya Kembali Ada” (I’m Back), “Jangan Berhenti di Sini” (Don’t Stop Here). The optimism behind the expression of silence and gloom is related to the use of metaphors in the form of colours that dominate Anwar’s poetry, namely black and white. Trakl also uses a more various color, such as blue, yellow, orange, but the dominant on remained black so that the gloom and pessimism seemed clearer.

Researcher
Dr. Novi Siti Kussuji
Title: Assessment of microbial indicators and water quality of the river basin system in the Eastern Economic Corridor

Duration (3 weeks): 4th-24th January 2020

Participants Biographies (see short CV): Dr. Chantima Piyapong, Ph.D in Biology, Staff of the Department of Biology, Faculty of Science Burapha University, Thailand. 8 Scientific Articles.

Prof. Dr. Ruben Sommaruga, Ph.D in Limnology, Director of the Department of Ecology, University of Innsbruck. 134 ISI Publications.

The stay started by visiting on Monday 6th the Deputy Dean for Research and Strategic Affairs of the Faculty of Science, Burapha University, Dr. Songklod Sarapusit to talk about future cooperation activities within the project “Assessment of microbial indicators and water quality of the river basin system in the Eastern Economic Corridor” and beyond. In this meeting, also other international advisers to this project such as Dr. Emanuel Paradis (Univ. of Montpellier, France) and Prof. Marco Celli (Univ. of Trento, Italy) were present including the Dean of Faculty of Science (Photo 1). The project with Dr. Piyapong as Principal Investigator is the largest one of the Burapha University regarding budget in the fiscal year 2019.

Then, a second meeting on the same day was done to discuss methodological problems in obtaining environmental DNA from river samples. Problems at the stage of sample concentration were detected and new filters and filtration sets were recommended to buy. This meeting took place with Dr. Nitcha Chamroensaksri, Dr. Piyapong’s co-investigator, from National Center for Genetic Engineering and Biotechnology (BIOTEC) responsible for DNA extraction and the molecular analysis of those samples. At a later stage, Dr. Chamroensaksri discussed with me about the results of the improved measures to collect and extract environmental DNA. She also plans to apply for ASEA-UNINET mobility program to work about the metagenome analysis with me and my colleague in Austria after finishing all the fieldwork and the laboratory of the above project.

On 7th January, I got in contact with students working at the Dept. of Biology and involved in the project to discuss related scientific publications.

From 8th to 9th January, a small workshop was organized to discuss problems of young scientists to write scientific articles and publish them.

From 10th to 23th January, field trips took place first to the upstream (Kao Yai National Park, World Heritage), then to the middle stream and finally to the mouth of Bangpakong River which is the main river in the Eastern Region of Thailand and is the heart of the above project (Photo 2). In all cases, I supervised Dr. Piyapong on how to plan the sampling, on what criteria to use to select the sampling points and also on how to collect water samples properly. On each occasion and after the field trip, work started at the Laboratory in Burapha. At the laboratory, I revised the protocols to filter the water samples (type of filter, type of filtration unit) in order to improve filtration efficiency and thus, obtain enough environmental DNA.
Finally and together with Dr. Chamroensaksri, we tried a new method to extract DNA from microbial communities for all six stations along the river in order that the above project can be accomplished. The changes made resulted in the first successful recovery of DNA from this river after one year of different trials.

24th January: departure to India

Figure 1. Meeting with the Dean of Faculty of Science, Burapha University
Credit: Assist. Prof. Dr. Songklod Sarapusit, Associate Dean for Research and Strategy, Faculty of Science, Burapha University, Thailand.

From left to right: From Left to Right:
Assist Prof. Pachoenchoke Jintasaeranee, Department of Aquatic Sciences, Faculty of Science, Burapha University, Thailand
Dr. Clara Tattoni, University of Florence, Italy
Prof. Dr. Marco Ciolli, Università di Trento, Italy
Dr. Chantima Piyapong, Department of Biology, Faculty of Science, Burapha University, Thailand
Assist. Prof. Dr. Ekarath Srisook, Dean of Faculty of Science, Burapha University, Thailand
Dr. Emmanuel Paradis, Research Director from French National Research Institute for Sustainable Development (IRD), France
Prof. Dr. Ruben Sommaruga, Department of Ecology, University of Innsbruck, Austria
Figure 2. Fieldtrip to the river mouth of Bangpakong River
Credit: Assist Prof. Pachoenchoke Jintasaeranee

Dr. Chantima Piyapong, Department of Biology, Faculty of Science,
Burapha University, Thailand

Prof. Ruben Sommaruga, Department of Ecology,
University of Innsbruck, Austria
SHORT CV
Ruben SOMMARUGA

Department of Ecology, University of Innsbruck
ruben.sommaruga@uibk.ac.at
https://www.uibk.ac.at/ ecology/staff/persons/sommaruga.html.en
ResearcherID: E-5335-2011

Personal data
Citizenship: Austrian
DOB: 27.4.62
Affiliation Address: Technikerstr. 25, 6020 Innsbruck, Austria

Academic qualifications and employment history

2012-2011-present
Director of the Department of Ecology, University of Innsbruck
Full Professor of Limnology, Department of Ecology, Univ. of Innsbruck.

1999-2011
Associate Professor (with Tenure), Institute of Ecology (formerly Institute of Zoology and Limnology), University of Innsbruck, Innsbruck, Austria.

1999
Habilitation in Limnology at the University of Innsbruck. Austria.
External Reviewer Panel: Prof. Dr. Robert G. Wetzel† (USA), Prof. Dr. Otto Siebeck (Germany), and Dr. Gerhard J. Herndl (The Netherlands).

1998
Assistant Professor, Institute of Zoology and Limnology, University of Innsbruck, Innsbruck, Austria.

1994-1996
Postdoctoral researcher: Spain (University of Barcelona, Mountain Research Center), Italy (Institute of Hydrobiology at Pallanza).

Mar-Jun 1994
Visiting researcher, Scripps Institution of Oceanography, UCSD, La Jolla, California. USA.

1993
Doctor in Natural Sciences (Suma cum laude), University of Innsbruck. Major subjects: Limnology and Plankton Ecology.

1990
M.Sc. in Zoology, University of Innsbruck, Austria.

1988
International Postgraduate Training Course in Limnology. Austria

Honors & Awards (partial)

- Scientific Award from the Principality of Liechtenstein, 2007
- Elected Fellow of the Association for the Sciences of Limnology and Oceanography, 2018
- Swarovski Prize, University of Innsbruck, 1998.
- Appointed member of Faculty of 1000, Biology, 2010
- Elected Board Member of the Austrian Science Foundation (Environmental Sciences, FWF), September 2011 to present.
- Tonolli Award of the International Association of Theoretical and Applied Limnology (SIL), 1992.

Publications (September, 2019)

- Total number of publications: 134
- h-index (ISI Web of Science/Google Scholar): 41/49
- Citations: (ISI Web of Science/Google Scholar): 5069/7649
Curriculum Vitae:

Name: Chantima Piyapong  Nationality: Thai

Current contact information:
Department of Biology,  Tel: +66 (0) 87333-7915
Faculty of Science, Burapha University  Fax: +66 (0) 3839- 3489
Chonburi, 20131, Thailand  Email: chantimap@gobuu.ac.th

Education:

IV. Sukhothai Thammathirat Open University, Thailand (Certificate in English for Specific Careers (Teaching)), 2011
V. University of Leeds, Leeds, United Kingdom (PhD in Biology), 2008
VI. Chulalongkorn University, Bangkok, Thailand (MSc in Zoology), 2000
VII. Chulalongkorn University, Bangkok, Thailand (BSc in Zoology), 1996

Working experience:

• Lecturer, Department of Biology, Faculty of Science, Burapha University, Thailand (March 2012-present)

Recent Research grants:

• Analysis, monitoring and prediction of water quality for sustainable land use of the river basin system in Eastern Economic Corridor (EEC): assessment of microbial indicator system and water quality of the river basin system in Eastern Economic Corridor based on metagenomics analysis (Year 2019) funded by National Research Council of Thailand (NCRT) (PI)

• Dynamics of microbial community profiles corresponding to metagenomics analysis and water quality of the river basin system in Eastern Economic Corridor (Year 2019) funded by National Research Council of Thailand (NCRT) (PI)

• Analysis of ecosystem services in organic rice paddy fields in Nakhon Nayok province (Year 2018) funded by National Research Council of Thailand (NCRT) (PI)

Recent Awards:

• ASEA-UNINET (Ernst Mach Grant-ASEA-UNINET: Post-doc Grant) at the Univeristy of Innsbruck (March-June 2017)

• Newton Fund for Professional Development Mid-career Researchers (training in Bangkok (Thailand) and London (United Kingdom)) in 2015-2016 (10 days)
Scientific Report

<table>
<thead>
<tr>
<th>Study project</th>
<th>Research</th>
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</thead>
<tbody>
<tr>
<td>Name</td>
<td>Ms. Thi Ngoc Lien TRAN</td>
</tr>
<tr>
<td>Country of origin</td>
<td>VIETNAM</td>
</tr>
<tr>
<td>Dates of scholarship</td>
<td>From: 06.02.2020 - To: 27.02.2020</td>
</tr>
</tbody>
</table>

1. **Title of the project:**
   Higher Education Program Management: Experience from Austria

2. **Host institution:**
   Program Management and Teaching and Learning Support, Vienna University of Economics and Business (WU)
   Address: LC Building, Level 5, Welthandelsplatz 1, 1020 Wien, Austria

3. **Contact details:**

   - **Dr. Oliver Vettori (main contact)**
     Dean, Accreditations & Quality Management
     Director, Program Management and Teaching & Learning Support
     Email: oliver.vettori@wu.ac.at
     Phone: +43 1 31336 - 5503
     Dr. Oliver Vettori is currently in charge of the Program Management and Teaching & Learning Support. He has strong expertise in higher education research and management, as well as organization culture and theory.

   - **Dipl.-Kffr. Katrin Althammer**
     Accreditations
     Email: katrin.althammer@wu.ac.at
     Phone: +43 1 31336 - 6181

   - **Dipl.-Ing. Laura Bauer**
     Teaching and Learning Development
     Email: laura.bauer@wu.ac.at
     Phone: +43 1 31336 - 6146

   - **Judith Ivancsits, MA**
     Program Development and Policy
     Email: judith.ivancsits@wu.ac.at
     Phone: +43 1 31336 - 5407
During my stay at WU, I was working with Dr. Oliver Vettori and his staff on the following topics:

- Digital Teaching Services
- Teaching and Learning Development; Learning Ergonomics
- Accreditations
- Evaluation and Quality Enhancement
- Program Management and Policy
- Teaching Coordination

With greatest support from Dr. Oliver Vettori and his staff during my visit, I have gained very deep insights about the functions of the host institution, how the programmes are managed and how the teaching and learning activities are implemented and supported within the university. We discussed a lot about the similarities and the differences regarding the program management, teaching and learning development, accreditations, etc. between the two universities.

The visit was extremely fruitful which has been resulting in a few proposals together. In which, a project application in E-learning will be certainly submitted in the autumn of 2020. We hope there will be more collaboration between the two universities in the very near future.

Finally, I would like to thank OeAD (Austrian Agency for International Cooperation in Education and Research) for providing us with this excellent opportunity through this grant. Particularly, I would like to send a big thanks to Dr. Oliver Vettori and his staff for their utmost assistance during my stay at WU. The visit was actually more successful than expected.

Hue, 01.03.2020

(Thi Ngoc Lien TRAN)
Characterization and application of bacterial fucosyl transferases, enzymes for the production of health-related oligosaccharides

Duration: February 1 – February 29, 2020

Contact detail:

1. Univ. Prof. Dipl. -Ing. Dr.techn. Dietmar Haltrich. Department of Food Science and Technology. University of Natural Resources and Life Science, Vienna. Email: dietmar.haltrich@boku.ac.at

2. Priv.- Doz. Dr. Thu – Ha Nguyen. Department of Food Science and Technology. University of Natural Resources and Life Science, Vienna. Email: thu-ha.nguyen@boku.ac.at

3. Le Vu Khanh Trang. Faculty of Biology-Environmental Science – The University of Danang, University of Science and Education, Vietnam. Email: levukhanhtrang@gmail.com

Background: Human milk is special since it is the only food an infant may take in during the first months of its life, and it contains all the essential nutrients needed by the infant to develop and grow. Moreover, it contains ingredients that go beyond traditional nutrients in that they provide certain health benefits to the infant. Galacto-oligosaccharides (GOS) are recognized as one of the most viral prebiotic oligosaccharides and of the special interest to human nutrition based on the presence of structurally related to oligosaccharides together with different complex structures in human breast milk. (V. Sangwan, S. K. Tomar, R. R. B. Singh, A. K. Singh, and B. Ali, J. Food Sci., 76, R103-R111 (2011)). In order to increase the functionality of these oligosaccharides, the project has been carried out with the aim at attaching fucosyl residues onto these oligosaccharide structures. Currently, we are expressing genes coding for fucosyl transferases recombinantly, and characterize these enzymes biochemically.

Results: During a month joining this project, I accumulated a lot of knowledges and experiences related to enzyme technology including:

- Cloning and expression of fucosyl transferases encoding genes - Purification fucosyl transferases by IMAC
Title of the project
Spatial Assessment of Muslim Cemeteries in Vienna: Negotiating Between Sacred Space and Hybrid Function.

Duration of the stay
From 2nd February to 29th February 2020

Host professor
Dr. Roland Tusch

Participating persons
Participating persons will be decided later during the preparation of the manuscript for the publication. Identified persons will be interviews through offline mode pertaining to the inquiry of burial practices at Islamic sections.

A description of the scientific topics
The research is examining the position of cemeteries within the urban context. Especially in today’s context where cities population are growing rapidly at a faster rate. This study will be exploring the potential of the Islamic cemetery as part of the city's green infrastructures. The purpose of this project is to argue whether Islamic cemeteries should remain exclusively for burials rather than be part of Vienna's urban fabrics. This is because urban cemeteries could offer an extra role to serve as a part of public spaces in the city. Another reason is to examine the possible role of Islamic cemetery as a space that could stimulate integration between Viennese Muslims and predominantly Western societies in comparison to its counterpart which is the Islamic Cemetery Altach in Vorarlberg. The recent development observed that newly open Islamic cemeteries have been built outside the Central Cemetery within Vienna’s suburbia particularly in Inzersdorf. This study is also expected to reveal the motives for local Muslim communities choose to have a segregated and dedicated burial space rather than being placed among other faiths within the same ground which has been practiced in the Central Cemetery.

Work conducted during the research stay
During the research stay, I have conducted a site visit to several sites around Vienna. Observation was recorded by taking photos and videos recording. I have also visited the library of TUW and University of Vienna to get asses into their archives.

Expected Results
The expected outcomes from this study are such as follows:

Pre-visit
1. To demonstrate ways of integrating Muslim's sacred space as part of Vienna's civic space through landscape design.
2. To promote the integration of Muslim migrants into predominantly Western culture through a better understanding of their local identity and cultural space.
3. The findings may be replicated in other European cities which serves as a basis and approach to incorporate Islamic cemetery as part of the city's green infrastructures.
Post-visit
1. To identify the gaps in integrating Islamic cemeteries as part of Vienna's civic space through a better understanding of their burial practices and cultural identity.

2. To examine the feasibility and implementation of hybrid function at Islamic cemeteries as a space that can stimulate cultural tolerance between the local Muslims and Viennese society. Muslim sections at the Central Cemetery was chosen as an example in comparison to its counterpart, which are Islamic cemeteries in Inzersdorf, Vienna and in Altach, Vorarlberg.

3. To draw a comparison between Muslim cemeteries in Vienna and Kuala Lumpur, and how this can contribute further understanding towards striving for sustainability through the inclusion of desirable features and characteristics in the development of urban cemeteries.

Results
This aim of this study is to evaluate the feasibility and flexibility of Islamic cemeteries as an integrated public space by taking the example of Vienna. The case studies of Vienna have offered me a different perspective in understanding the other side of Islamic cemeteries within the geographical and social background of Austria. The findings of this research are expected to contribute to knowledge exchanges through research collaboration by drawing the comparison from the case studies between Kuala Lumpur and Vienna.

Here are some of the things that can be learned by Kuala Lumpur based on the site observation that has been conducted around Vienna. The old cemetery can be integrated into a new development of a neighbourhood park. In the case of Vienna, people are aware of the existence of these old cemeteries within the park. However, users don't seem to be mind because it has been gated from public access and remain untouched. The protected area is rich in terms of its biodiversity and natural greeneries which helps to concealed it naturally from the public's eyes.

My research visit is also beneficial in providing input to the ongoing research which is currently pending for the data collection due to Covid-19 lockdown in Malaysia. The title is Establishing the Requirements for Urban Cemeteries as Public Open Spaces in the Metropolitan Area of Kuala Lumpur.

Intended publication
The manuscript will be submitted to be considered for publication in the Journal of City and Environment Interactions, under Elsevier.

The manuscript is still in the writing process and I expect to submit by September 2020.
Final Report

Stipendien aus Mitteln des ASEA-Uninet, Projektstipendien SP 24

Reference number: ICM-2019-16105

Title of the project: Quaternary (CZTS) alloy nanostructure grown on different substrates: Analysis and characterisation

Duration of the stay: 08.02.2020 - 29.02.2020

Academic supervisor: Univ.Prof.Dr. Günther RUPPRECHTER
Place of study: Vienna University of Technology, Institut für Materialwissenschaften und Technologie Karlsplatz 13, 1040 Vienna

Awardee Scientist: Prof. Dr. Yarub Al-Douri
Nanotechnology and Catalysis Research Center, University of Malaya, Malaysia
Yarub Al-Douri is Professor in University of Malaya. He has initiated Nanotechnology Engineering MSc Program and Nano Computing Laboratory, the first in Malaysia. He has received numerous accolades. Al-Douri is Editor-in-Chief of Experimental and Theoretical NANO TECHNOLOGY, Editor-in-Chief of World Journal of Nano Science and Engineering and Associate Editor of Nano-Micro Letters (Q1).

Researcher: Univ. As. Dipl. -Ing. Dr. Techn. Nevzat Yigit is a post-doc researcher at Institut für Materialwissenschaften und Technologie and working in RUPPRECHTER’s group. He has 12 publications.

Description:

<table>
<thead>
<tr>
<th>Date</th>
<th>Person</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturday 8/2/2020</td>
<td>Univ.Prof.Dr. Günther</td>
<td>Arrival to Vienne at mid-day</td>
</tr>
<tr>
<td></td>
<td>RUPPRECHTER</td>
<td>I have met afternoon Univ.Prof.Dr. Günther RUPPRECHTER afternoon, he has welcomed me and did short-visit to the laboratories available in Institut für Materialwissenschaften und Technologie with Scientific discussions</td>
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<td>Sunday 9/2/2020</td>
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<td>Weed-end</td>
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<tr>
<td>Monday 10/2/2020</td>
<td>Dr. Nevzat Yigit</td>
<td>I have resumed my work at TU Wien including receive magnetic card and my office. Additionally, visit to the laboratories available</td>
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<tr>
<td>Date</td>
<td>Activity</td>
<td>Description</td>
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<tr>
<td>Tuesday 11/2/2020</td>
<td>OeAD Regional &amp; Housing Offices Vienna</td>
<td>I have visited both offices for receiving my scholarship and registration</td>
</tr>
<tr>
<td>Wednesday 12/2/2020</td>
<td>OeaD Housing Officer</td>
<td>I have registered my residence at Wipplingerstrasse 8, Ground floor, Kundenservicecenter</td>
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<tr>
<td>Thursday 13/2/2020</td>
<td>Dr. Nevzat Yigit</td>
<td>Scientific discussions to prepare a paper for publishing</td>
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<td>Friday 14/2/2020</td>
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<td>Saturday 15/2/2020</td>
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<td>Monday 17/2/2020</td>
<td>Univ.Prof.Dr. Günther RUPPRECHTER</td>
<td>Scientific discussions</td>
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<td>Tuesday 18/2/2020</td>
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<td>Seminar 1: Colloidal metals oxides nanoparticles</td>
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<td>Wednesday 19/2/2020</td>
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<td>Scientific discussions</td>
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<td>Thursday 20/2/2020</td>
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<td>Seminar 2: Optical studies of Quantum dots</td>
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<td>Friday 21/2/2020</td>
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<td>Scientific discussions</td>
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<tr>
<td>Saturday 22/2/2020</td>
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<td>Weed-end</td>
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<tr>
<td>Sunday 23/2/2020</td>
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<tr>
<td>Monday 24/2/2020</td>
<td>Univ.Prof.Dr. Günther RUPPRECHTER</td>
<td>Scientific discussions, meetings with scientists, researchers, students. In addition to prepare a paper for publishing</td>
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<td>Tuesday 25/2/2020</td>
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<tr>
<td>Wednesday 26/2/2020</td>
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<tr>
<td>Thursday 27/2/2020</td>
<td>OeaD Housing Officer</td>
<td>I have de-registered my residence at Wipplingerstrasse 8, Ground floor, Kundenservicecenter</td>
</tr>
<tr>
<td>Friday 28/2/2020</td>
<td>Univ.Prof.Dr. Günther RUPPRECHTER</td>
<td>Last meeting and farewell</td>
</tr>
<tr>
<td>Saturday 29/2/2020</td>
<td></td>
<td>Departure to Kuala Lumpur</td>
</tr>
</tbody>
</table>

Prof. Dr. Yarub Al-Douri
Awardee Scientist
Reports on Bernd Rode Award 2019
Mobilities in 2020
The project: Radical induced cationic frontal polymerization for vinyl ether monomers

Duration of the stay: 01.04.2021-07.06.2021

Participating persons:

Dr. techn. Anh Dung Tran
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He received his Master of Science in 2016 at Hanoi University of Science and Technology, Vietnam. From April to September 2017, he worked as a lecturer at the School of Chemical Engineering-Hanoi University of Science and Technology. In October 2017, he joined the group of Univ. Prof. Robert Liska (TU Wien) for his PhD program. After received his PhD degree, he continued working at the same group. His research is focused on photopolymerization, frontal polymerization, and composite materials. He has published 8 papers in peer-reviewed journals.

Univ. Prof. Robert Liska
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He is the leader of the research group-Polymer Chemistry and Technology, Institute of Applied Synthetic Chemistry, TU Wien. He is an expert in the fields of photoinitiator and photopolymerization. With over 20 years of research experience, he has published more than 200 papers in peer-reviewed journals, written more than 10 book-sections, and filed more than 30 patents.

Dr. techn. Patrick Knaack
Institute of Applied Synthetic Chemistry, TU Wien
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He studied at TU Wien, where he received his Master of Science in 2010 and his PhD in 2015. Since 2016, he has worked as a senior scientist at the Institute of Applied Synthetic Chemistry-TU Wien. His research mainly focuses on new initiation technologies for ionic and radical polymerization, the development of low toxic monomers for sustainable coatings, and 3D-printing of Biomaterials. He has published more than 20 papers in peer-reviewed journals and filed 4 patents.

Description of the project

Radical induced cationic frontal polymerization (RICFP) is a fast, energy-efficient, and self-sustaining curing technique for cationic monomers. A formulation for RICFP containing three main components which are a cationic monomer, a photoacid generator, and a radical thermal initiator. In my PhD project, I found out that the frontal polymerization was successful for a formulation based on a vinyl ether monomer (1,4-Cyclohexanediethanol...
divinylether, BVC) even without the presence of radical thermal initiator. These results encourage to investigate further the frontal polymerization for BVC and other vinyl ether monomers.

In this study, 1,4-Cyclohexanediol divinylether (BVC) and 1,4-Butadioldivinylether (DVE), Tri(ethylene glycol) divinyl ether (TEDE) were used as monomers. For each monomer, formulations were prepared with different photoacid generator concentrations (aluminum-based iodonium salt, I-Al).

Reactivity of these formulations was investigated using photo-differential scanning calorimetry (photo-DSC) and simultaneous thermal analysis (STA). The investigation revealed that at 25 °C the reactivity of DVE is higher than those of BVC and TEDE. However, due to the low boiling points of DVE and TEDE, at temperatures higher 80 °C BVC is the most reactive monomer.

The frontal polymerization experiment was also performed for all the above formulations using UV light initiation for a short time (less than 20 s). Due to low boiling points, DVE evaporated during the reaction, which resulted in heat loss and, therefore, unsuccessful frontal polymerization. TEDE was not successfully polymerized by the frontal polymerization due to its low reactivity. A fast frontal polymerization process was observed for all formulations based on BVC, even with a low I-Al concentration of 0.005 mol%.

The successful polymerization BVC is contributed by the high reactivity of the monomer and interaction of radicals formed from BVC and the photoacid generator. On the one hand, the heat released from the high reactive reaction is high enough to decompose the photoacid generator and form initiating species (super acids). On the other hand, the initiating species might also be formed via a redox reaction of BVC-based radicals and the photoacid generator.

The study also indicated that impurities (in BVC monomer) play a role in increasing the reactivity of the frontal formulation. With the presence of impurities, the onset temperature of the frontal formulation decreased from 80 °C to 60 °C, while the heat of the polymerization was higher, compared to the formulation based on distilled BVC. Impurities can be residual catalysts and raw materials from the synthesis reaction, which can interact with the photoacid generator. Further investigations need to be carried out to find out the impurities and all of their effects. These results can be applied to increase reactivity of low reactive systems.

Summary:

In three typical vinyl ether monomers, only 1,4-Cyclohexanediol divinylether (BVC) was successfully polymerized by the frontal polymerization. The mechanism of the process is proposed to be due to the high reactivity of BVC.

Some impurities can be used to increase the reactivity of cationic polymerizable systems. Further experiments need to be carried out to confirm these impurities surely.
**Development of a disposable electrochemical biosensor for detection of cancer biomarker**

Bernd Rode Award Laureates 2019

**Duration of the stay:** 20th February – 6th March 2022

**Participating persons:**

1. Ao.Uni.Prof. Dr. Kurt Kalcher  
   Institute of Chemistry-Analytical Chemistry, Karl-Franzens University,  
   A-8010 Graz, **AUSTRIA**

2. Assoc.Prof. Dr. Anchalee Samphao  
   Department of Chemistry, Faculty of Science, UbonRatchathani University,  
   Ubon Ratchathani, 34190 **THAILAND**

**Especially a description of the scientific topics and work conducted during the research stay:**

- Sum-up Results for the project “Development of a disposable electrochemical biosensor for detection of cancer biomarker”
- Revision and manuscript “An electrochemical sensor for the voltammetric determination of artemisinin based on carbon materials and cobalt phthalocyanine”

**Results:**

In order to investigate the applicability of the electrochemical biosensor for CEA detection, a recovery test compared with an available electrochemiluminescence (ECL) immunoassay were conducted. The serum samples were diluted to 10 and 100 times with phosphate buffer solution (pH 7.4) and different amounts of CEA (0, 1, 10, and 50 ng/mL) were subsequently fortified into each sample dilution. The concentrations of CEA in the prepared samples were analyzed by LSV and EIS methods and continuously calculated by substituting the signal values into the above calibration curve (Figure 1). The electrochemical biosensor presented the recovery in a range from 90.4 to 109.8% with the RSD varying in a range of 1.7% to 9.5%. Furthermore, the CEA content in the diluted human serum samples were also tested by the ECL immunoassay in which the results were obtained from the National Cancer Institute, Thailand. The relative error between the two methods was less than 12%, indicating that the designed biosensor is well fitted to the ECL method. Thus, it can be concluded that the SPCE/GNP-MnO$_2$/Fe$_3$O$_4$@Au/anti-CEA has a satisfactory potential for detection of CEA in real serum samples.
Figure 1 The calibration plot; (A) EIS response and (B) its calibration plot for CEA detection using 5 mM Fe(CN)$_6^{3-/4-}$.

Intended publications (if any) etc.

[1] P. Butmee, G. Tumcharern, G. Thouand, K. Kalcher, A. Samphao, An ultrasensitive immunosensor based on manganese dioxide-graphene nanoplatelets and core shell Fe$_3$O$_4$@Au nanoparticles for label-free detection of carcinoembryonic antigen, Bioelectrochemistry, 2020, 132, 107452. (Q1, IF = 4.722)
Scientific and/or societal education results and publications arising from the last project:


A portable selective electrochemical sensor amplified with Fe3O4@Au-cysteamine-thymine acetic acid as conductive mediator for determination of mercuric ion

Preeyanut Butmee 1, Jittra Mala 1, Chulalak Damphathik 2, Kanjana Kunpatee 3, Gamolwan Tumcharern 1, Margaret Kerr 4, Eda Mehmeti 5, Georg Raber 6, Kurt Kalcher 6, Anchalee Samphao 7,8

1 Department of Chemistry, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani, 34190, Thailand
2 National Nanotechnology, National Science and Technology Development Agency, Pathum Thani, 12120, Thailand
3 Department of Chemistry, Worcester State University, 411 Chandler Street, Worcester, MA, 01608, United States
4 Institute of Chemistry, Analytical Chemistry, University of Giza, A-4010, Giza, Asanta
5 Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani, 34190, Thailand


Enzymatic electrochemical biosensor for glyphosate detection based on acid phosphatase inhibition

Preeyanut Butmee 1, Gamolwan Tumcharern 1, Chompunuch Songsiririthigul 2,3, Marie José Durand 4, Gerald Thouand 4, Margaret Kerr 5, Kurt Kalcher 6, Anchalee Samphao 7,8

Received: 17 June 2021 / Revised: 16 July 2021 / Accepted: 19 July 2021 / Published online: 27 July 2021
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Abstract
A novel enzymatic electrochemical biosensor was fabricated for the indirect detection of glyphosate-based acid phosphatase inhibition. The biosensor was constructed on a screen-printed carbon electrode modified with silver nanoparticles, decorated with electrochemically reduced graphene oxide, and chemically immobilized with acid phosphatase via glutardialdehyde cross-linking. We measured the oxidation current by chronamperometry. The current arose from the enzymatic reaction of acid phosphatase and the enzyme-substrate disodium phenyl phosphate. The biosensing response is a decrease in signal resulting from inhibition of acid phosphatase in the presence of glyphosate inhibitor. The inhibition of acid phosphatase by glyphosate was investigated as a reversible competitive-type reaction based on the Lineweaver-Burk equation. Computational docking confirmed that glyphosate was the inhibitor bound in the substrate-binding pocket of acid phosphatase and that it was able to inhibit the enzyme efficiently. Additionally, the established method was applied to the selective analysis of glyphosate in actual samples with satisfactory results following a standard method.
Research Report for Bernd Rode Award

“LC-MS of extracts from medicinal plants”

1 – 25 February 2022

By

Associate Professor Dr. Piyanuch Rojsanga

And

Associate Professor Dr. Pongtip Sithisarn

Faculty of Pharmacy, Mahidol University

Supported by

Professor Dr. Hermann Stuppner

University of Innsbruck,

Institute of Pharmacy/Pharmacognosy

Center for Chemistry and Biomedicine

And

ASEA-UNINET scholarship
Biography

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Interesting Research Topics or Specialties
1. Analytical method development for pharmaceutical product and herbal medicine
2. Quality control of pharmaceutical product and herbal medicine

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Interesting Research Topics or Specialties
1. Separation of active compounds from the medicinal plants
2. Biological testing of the medicinal plants
3. Quality control of the medicinal plant extract
4. Development of the medicinal plant products

Contact person in University of Innsbruck

Prof. Dr. Hermann Stuppner  
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Institute of Pharmacy/Pharmacognosy, Center for Chemistry and Biomedicine, University of Innsbruck, Innrain 80-82, 6020 Innsbruck, Austria
1. Introduction

Oroxylum indicum (L.) Vent. is a medium-size, deciduous tree in Bignoniaceae. It is a medium size deciduous tree, the leaves are very large pinnate compound leaves. The outside petals are reddish purple with the pale yellow inside. Fruits are flat capsules, broad and sword shaped [1]. The seeds are numerous, flat like papery wings [2]. Mature fruit is acrid and sweet, which promotes anti-helminthic and stomachic effects [3]. The seeds have been used as purgative while the seed paste is applied to the throat for quick relief of tonsil pain [1,2]. Furthermore in Thailand, the young fruits and young flowers of this plant are popularly consumed as vegetable in the North of the country. Some phytochemicals were reported from different part of Oroxylum indicum such as flavonoids, anthraquinones, alkaloids, saponins and fatty acids [4-7].

2. Materials and Methods

2.1 Plant extracts and fractions

Oroxylum indicum extracts and fractions used in the experiment are including young fruit, green fruit, mature fruit coat and seed extracts prepared by maceration with ethanol and yellow precipitate and orange-red crystals obtained from the seed extracts.

2.2 LC-MS analysis

LC-MS analysis of all extracts and fractions from Oroxylum indicum was conducted using the method applied from Sithisarn et al. [8] by Agilent Tech 1260 Infinity II with 1260 DAD and 1260 vial sampler.

2.3 HPLC analysis

High performance liquid chromatography (HPLC) analysis of all extracts and fractions from Oroxylum indicum was conducted using Shimadzu HPLC instrument with LC-20AD XR pump, SIL-20AC XR autosampler, CTO-20AC column oven, SPD-M20A PDA detector and DGU-20A degasser with the method applied from Sithisarn et al. [8].

3. Results and Discussion

3.1 LC-MS analysis

LC-MS was used to analyze all extracts and fractions from Oroxylum indicum. Three compounds, oroxin A, orixin B and chrysin-7-O-glucuronide were additionally found in the
extracts and fraction. Oroxin B, oroxin A and chrysin-7-O-glucuronide were found at the retention time 6.67, 8.71 and 14.46 minutes, respectively (Figure 1). The mass spectra of oroxin B, oroxin A and chrysin-7-O-glucuronide showed the molecular mass ion at m/z [M⁺]/[M⁻] 595.2/593.0, 433.2/431.0 and 431.2/428.8 suggesting the molecular weights of 594, 432 and 430, respectively. The retention times and mass spectra of these three compounds were matched with the data of the reference compounds. Other major compounds found in the extracts and fractions were baicalin, baicalein and chrysin. The retention time, maximum wavelength from UV spectra and molecular mass ion from mass spectra of 6 major compounds in *O. indicum* samples are shown in Table 1. The chemical structures of oroxin B, oroxin A and chrysin-7-O-glucuronide are shown in Figure 2.

![HPLC chromatogram of green fruit extract of *Oroxylum indicum* showing the peaks of oroxin B (1) and oroxin A(2) and chrysin-7-O-glucuronide (4) at the retention times of 6.689, 8.709 and 14.460 minutes, respectively.](image)

**Figure 1** HPLC chromatogram of green fruit extract of *Oroxylum indicum* showing the peaks of oroxin B (1) and oroxin A(2) and chrysin-7-O-glucuronide (4) at the retention times of 6.689, 8.709 and 14.460 minutes, respectively.

**Table 1** Retention time (RT), maximum wavelength (λ<sub>max</sub>) and molecular mass ion ([M⁺]/[M⁻]) of 6 major compounds in *O. indicum* samples

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Compound</th>
<th>RT (min.)</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; (nm)</th>
<th>[M⁺]/[M⁻] (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oroxin B</td>
<td>6.67</td>
<td>278, 316</td>
<td>595.2/593.0</td>
</tr>
<tr>
<td>2</td>
<td>Oroxin A</td>
<td>8.71</td>
<td>278, 316</td>
<td>433.2/431.0</td>
</tr>
<tr>
<td>3</td>
<td>Baicalin</td>
<td>10.65</td>
<td>276, 316</td>
<td>447.2/445.0</td>
</tr>
<tr>
<td>4</td>
<td>Chrysin-7-O-glucuronide</td>
<td>14.46</td>
<td>268, 306</td>
<td>431.2/428.8</td>
</tr>
<tr>
<td>5</td>
<td>Baicalein</td>
<td>17.70</td>
<td>272, 292sh, 318</td>
<td>271.2/268.8</td>
</tr>
<tr>
<td>6</td>
<td>Chrysin</td>
<td>21.57</td>
<td>268, 314</td>
<td>255.0/252.8</td>
</tr>
</tbody>
</table>
Figure 2 Chemical structures of oroxin B (A), oroxin A (B) and chrysin-7-O-glucuronide (C)

3.2 HPLC analysis

For quantitative analysis of 6 major compounds in *Oroxylum indicum* extracts and fractions, the HPLC analysis was developed. Several parameters were optimized and developed including gradient system, column length, column type, column temperature, flow rate and mobile phase. The optimized condition for HPLC analysis of 6 major compounds in *Oroxylum indicum* extracts and fractions are shown in Table 2 and the gradient program is shown in Table 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td></td>
</tr>
<tr>
<td>Column</td>
<td>YMC pack Pro C18, 3 µm (250 x 4 mm), 8 nm (porous)</td>
</tr>
<tr>
<td>Temperature</td>
<td>40 °C</td>
</tr>
<tr>
<td>Mobile phase (gradient system)</td>
<td>Solvent A: 0.1% formic acid in water</td>
</tr>
<tr>
<td></td>
<td>Solvent B: 10% methanol in acetonitrile</td>
</tr>
<tr>
<td>Injection volume</td>
<td>5 µl</td>
</tr>
<tr>
<td>Sample concentration</td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>Detector</td>
<td>UV 285 nm</td>
</tr>
</tbody>
</table>
Table 3 Gradient Condition for HPLC analysis of 6 major compounds in *Oroxylum indicum* extracts and fractions

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% solvent B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>15</td>
<td>33</td>
</tr>
<tr>
<td>22</td>
<td>56</td>
</tr>
<tr>
<td>23</td>
<td>90</td>
</tr>
<tr>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>38</td>
<td>30</td>
</tr>
</tbody>
</table>

Using the optimized condition, the HPLC method was validated using the specific parameter according to ICH guideline 2002 composing of linearity, accuracy and precision (9). Six compounds composed of orixin B, baicalin, oroxin A, chrysin-7-O-glucuronide, baicalein and chrysin were used as reference standard.

The linearity of the method was assessed by analyzing the series of standard mixture in methanol. Three calibration curves including 5 – 6 concentration levels were used. The standard curves were achieved by plotting between the concentration (x-axis) and peak areas of each standard (y-axis). A regression equation of the calibration curve was calculated and correlation coefficient was obtained from data analysis ($r \geq 0.99$).

The accuracy of method was examined by recovery of known amounts of standard mixture added to the sample solution. Standard mixture solutions (0.5, 1, 2 ml) were added into a 5 ml volumetric flask containing 1 ml of sample solution and then adjusted with methanol. The percentage recovery of each standard compound was calculated using the following equation.

$$\% \text{ Recovery} = \frac{C_{\text{spiked}} - C_{\text{non-spiked}}}{C_{\text{added}}} \times 100$$

For precision, repeatability and intermediate precision were obtained by analyzing of sample solution on a same day ($n = 6$) and three different days ($n = 18$), respectively. The relative standard deviation (RSD) was calculated using the following equation.

$$\% \text{RSD} = \frac{SD}{\bar{X}} \times 100$$

When $SD$ = Standard deviation of the recovery (%) of sample

$\bar{X}$ = The average of the recovery (%) of sample
The validation parameters for HPLC method of quantitative analysis of 6 compounds in *O. indicum* extracts were found to be acceptable according to AOAC guideline 2002 [9]. The standard curves of each reference standard are shown in Figure 3

### Table 4 Validation parameters for HPLC method of quantitative analysis of 6 compounds in *O. indicum* extracts

<table>
<thead>
<tr>
<th>Validation parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation of determination</td>
<td>&gt; 0.990</td>
</tr>
<tr>
<td>Linear range, μg/mL</td>
<td>0.65 - 1000</td>
</tr>
<tr>
<td>Repeatability (% RSD, n=6)</td>
<td>&lt; 0.90</td>
</tr>
<tr>
<td>Intermediate precision (% RSD, n=18)</td>
<td>&lt; 1.47</td>
</tr>
<tr>
<td>% recovery</td>
<td>95 - 105</td>
</tr>
</tbody>
</table>
**Figure 3** Standard curves of each reference compound for quantitative analysis of *O. indicum* extracts; A = oroxin B, B = baicalin, C = oroxin A, D = chrysin-7-O-glucuronide, E = baicalein, F = chrysin

The system suitability of the HPLC method was evaluated by analyzing 3 parameters including resolutions, tailing factors and %RSD of the peak area. It was found that all parameters are acceptable as shown in Table 5.

**Table 5** System suitability parameters of HPLC conditions for quantitative analysis of 6 compounds in *O. indicum* extracts

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>&gt; 1.6</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>&lt; 1.4</td>
</tr>
<tr>
<td>%RSD of the peak area</td>
<td>&lt; 0.5</td>
</tr>
</tbody>
</table>

After system suitability was evaluated, the HPLC method was used to analyze the amounts of 6 compounds in the extracts of *O. indicum*. Most of the compounds were found in very low amounts in the young fruit extract while the compounds seem to increase when the fruits grow up. In mature green fruit, it contains some amount of baicalin but other 5 compounds are still available in low amounts. The seeds from both provinces, showed the same patterns of the ratio, they contain high amounts of baicalin, some amounts of oroxin B, chrysin-7-O-glucuronide, chrysin and baicalein and low amount of oroxin A. The old pod coat also contains baicalin as major compound with small amounts of others. The amounts of 6 compounds including oroxin B, baicalin, oroxin A, chrysin-7-O-glucuronide, baicalein and chrysin analyzed by the validated HPLC method are shown in Table 6.

**Table 6** Quantitative analysis of 7 compounds in *O. indicum* extracts by HPLC method

<table>
<thead>
<tr>
<th>Extract</th>
<th>OB (Content (%w/w in the extract))</th>
<th>BL</th>
<th>OA</th>
<th>C7</th>
<th>BE</th>
<th>C</th>
<th>OXA</th>
</tr>
</thead>
<tbody>
<tr>
<td>YF_L</td>
<td>n.d.</td>
<td>0.41 ± 0.00</td>
<td>0.08 ± 0.00</td>
<td>n.d.</td>
<td>0.53 ± 0.00</td>
<td>0.96 ± 0.00</td>
<td>n.d.</td>
</tr>
<tr>
<td>GF_C</td>
<td>0.54 ± 0.00</td>
<td>2.77 ± 0.00</td>
<td>0.72 ± 0.00</td>
<td>0.69 ± 0.00</td>
<td>0.79 ± 0.00</td>
<td>1.11 ± 0.00</td>
<td>1.35 ± 0.00</td>
</tr>
<tr>
<td>OP_C</td>
<td>n.d.</td>
<td>1.44 ± 0.00</td>
<td>0.06 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td>0.09 ± 0.00</td>
<td>0.66 ± 0.00</td>
<td>1.20 ± 0.00</td>
</tr>
<tr>
<td>S_C</td>
<td>7.63 ± 0.00</td>
<td>9.01 ± 0.01</td>
<td>0.34 ± 0.00</td>
<td>0.74 ± 0.00</td>
<td>1.82 ± 0.01</td>
<td>1.90 ± 0.00</td>
<td>n.d.</td>
</tr>
<tr>
<td>S_L</td>
<td>2.90 ± 0.01</td>
<td>9.12 ± 0.01</td>
<td>0.14 ± 0.00</td>
<td>2.47 ± 0.00</td>
<td>0.27 ± 0.00</td>
<td>1.03 ± 0.00</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
3.3 Quantitative Analysis of Multi-components by Single marker (QAMS) method for simultaneous determination of flavonoids contents in *O. indicum* extracts

When single reference is used to determine other compounds in samples, the concentration of each compound (Ci) should be calculated by the ratio between the peak area of the compound in sample solution (Ai) and the peak area of chosen reference compound in a standard solution in a unit concentration (Ak/Ck), and then calibrated by relative conversion factor (RCF)

\[
Ci = \frac{Ai}{Ak/Ck} \times RCF
\]

(1)

The relative conversion factor (RCF) for each flavonoid was calculated as the ratio of peak areas in a unit concentration between standard substance (Ak/Ck) and analyte (Ai/Ci):

\[
RCF = \frac{Ak/Ck}{Ai/Ci}
\]

(2)

To calculate the conversion factors, firstly three series of standard mixture solutions were prepared and analyzed by developed HPLC method; then the ratio at each concentration level of three standard mixture solutions were calculated as Eq. (2); finally, the relative conversion factor of each flavonoid was obtained as the mean values calculated from the triplet of five concentrations.

For the comparison of a new QAMS method with external standard method, standard method difference (SMD) was computed according to the following equation:

\[
SMD = \frac{C_{ES} - C_{QAMS}}{C_{ES}} \times 100
\]

where *C*<sub>ES</sub> and *C*<sub>QAMS</sub> represent the concentrations of flavonoid assayed in *O. indicum* extracts by External Standard method and QAMS method, respectively.
Relative conversion factors (RCF) for all the flavonoid standards at 285 nm are listed in Table 7. It can be seen the response factors at 285 nm for each flavonoid were in range from 0.21 to 3.01. To select the suitable single marker for QAMS method, standard method difference (SMD) between a QAMS method and external standard method of each compound, was calculated as Eq. (3); As shown in Table 8, Baicalin was selected as single marker in QAMS method since the % SMD of other compounds were acceptable as not more than 6.8. Under the described HPLC conditions, the conversion factors for other flavonoids are sequenced as Oroxin B > Chrysin-7-O-glucuronide > Chrysin > Baicalein > Oroxin A at 285 nm.

Table 7 Relative conversion factors (RCF) and SD values of flavonoids of *O. indicum* extracts.

<table>
<thead>
<tr>
<th>Reference compound</th>
<th>Oroxin B</th>
<th>Baicalin</th>
<th>Oroxin A</th>
<th>Chrysin-7-O-glucuronide</th>
<th>Baicalein</th>
<th>Chrysin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other compounds</td>
<td>CF</td>
<td>SD</td>
<td>CF</td>
<td>SD</td>
<td>CF</td>
<td>SD</td>
</tr>
<tr>
<td>Oroxin B</td>
<td>-</td>
<td>0.65</td>
<td>0.05</td>
<td>2.98</td>
<td>0.19</td>
<td>0.08</td>
</tr>
<tr>
<td>Baicalin</td>
<td>1.54</td>
<td>0.11</td>
<td>-</td>
<td>4.56</td>
<td>0.31</td>
<td>1.65</td>
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<tr>
<td>Oroxin A</td>
<td>0.33</td>
<td>0.02</td>
<td>0.21</td>
<td>0.02</td>
<td>-</td>
<td>0.35</td>
</tr>
<tr>
<td>Chrysin-7-O-glucuronide</td>
<td>0.93</td>
<td>0.07</td>
<td>0.61</td>
<td>0.05</td>
<td>2.78</td>
<td>0.20</td>
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<tr>
<td>Baicalein</td>
<td>0.52</td>
<td>0.06</td>
<td>0.34</td>
<td>0.04</td>
<td>1.53</td>
<td>0.18</td>
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<tr>
<td>Chrysin</td>
<td>0.56</td>
<td>0.04</td>
<td>0.37</td>
<td>0.03</td>
<td>1.67</td>
<td>0.11</td>
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Table 8 Comparison for the contents (%w/w) of markers measured by EMS and QAMS in young pod, green pod, and seed of *O. indicum* extracts
### Table 1: Compounds Content, % and SMD, % in Young pod, Green pod and Seed

<table>
<thead>
<tr>
<th>Sample</th>
<th>Young pod</th>
<th>Green pod</th>
<th>Seed</th>
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<td></td>
<td>Content, %</td>
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<td>n.d.</td>
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<tr>
<td>Baicalin</td>
<td>0.41</td>
<td>-</td>
<td>2.77</td>
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<tr>
<td>Oroxin A</td>
<td>0.08</td>
<td>0.08</td>
<td>3.80</td>
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<tr>
<td>Chrysin-7-O-glucuronide</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>Baicalein</td>
<td>0.53</td>
<td>0.57</td>
<td>6.8</td>
</tr>
<tr>
<td>Chrysin</td>
<td>0.92</td>
<td>0.96</td>
<td>5</td>
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*a Baicalin was used as single marker in QAMS method.*

### 3.4. Isolation and structure elucidation of compound OI1

From HPLC analysis, it was found that the pod coat and the mature green extracts contained the unknown peak at the retention time around 27 minutes, therefore, the isolation and identification of this compound was conducted by liquid-liquid extraction, column chromatography and Nuclear Magnetic Resonance (NMR) spectroscopic technique. Figure 4 shows the HPLC chromatogram of pod coat extract containing unknown peak.

![HPLC chromatogram of pod coat extract showing the unknown peak (black arrow).](image)

**Figure 4** HPLC chromatogram of pod coat extract showing the unknown peak (black arrow).
The pod coat extract of *Oroxylum indicum* (10.3 g) was suspended in distilled water (100 ml) and was extracted using continuous liquid-liquid chromatography with petroleum ether (200 ml) for 1 h, then the petroleum ether part was collected and the process was repeated, then the petroleum ether parts were combined. The remaining aqueous part was then extracted with ethyl acetate, and *n*-butanol, respectively using the same procedure. After that, all fractions composed of petroleum ether, ethyl acetate, *n*-butanol and aqueous fractions were obtained. They were dried using rotary evaporator and the yield of each fraction is shown in Table 9. All fractions were then monitored using thin layer chromatography (TLC) using silica gel GF\(_{254}\) precoated plate and ethyl acetate:toluene:formic acid 2.5:2.5:0.75 %v/v as solvent system. TLC analysis of all fraction was monitored under UV 254, UV 366 and NP spray reagent under UV 366 nm. The TLC chromatogram of all fractions from the liquid-liquid chromatography of pod coat extract of *O. indicum* are shown in Figure 5. From the results, petroleum ether fraction showed the major spot at Rf value 0.64 that appeared as dark quenching spot under UV 254 nm. The petroleum ether fraction (100 mg) was dissolved in 3 ml methanol, filtered then the solution was submitted to column chromatography using Sephadex LH20 as stationary phase and dichloromethane:acetone 85:12 v/v as mobile phase. Thirty-seven fractions (2 ml) were collected and monitored by TLC using the analytical conditions as mentioned above, fractions with the similar TLC patterns were combined. Six combined fractions were obtained and showed the TLC chromatogram as in Figure 6 and the yields are shown in Table 10. Fraction 5 showed the major quenching spot under UV 254 nm at the Rf value of 0.68. This spot appeared as dark spots under UV 366 and NP spray reagent under UV 366 nm. Fraction 5 were then analyzed by HPLC using the HPLC condition as mentioned before. Fraction 5 showed the main peak at the retention time 27.82 detected by UV detector at the wavelength of 285 nm (Figure 7). This fraction (compound OI1) appeared as yellow solid, it was then structurally analyzed by NMR spectroscopic technique.

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<tr>
<th>Fraction</th>
<th>Yield in extract (%w/w)</th>
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<td>petroleum ether</td>
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<td>ethyl acetate</td>
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<td>2.57</td>
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<td>aqueous</td>
<td>2.89</td>
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Table 9 Yield (%w/w) of the fractions obtained from liquid-liquid extraction of pod coat extract of *O. indicum*. 
Figure 5 TLC chromatograms of *O. indicum* pod coat extract and fractions; adsorbent: silica gel GF254, solvent system: ethyl acetate-toluene-formic acid 25-25-7.5 v/v, 1 = pod coat extract, 2 = petroleum ether fraction, ethyl acetate fraction, *n*-butanol fraction, aqueous fraction, A = white light, B = UV 254, C = UV366, D = NP + UV366

Table 10 Yield (%w/w) of the combined fractions obtained from column chromatography of petroleum ether fraction from pod coat extract of *O. indicum*.

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<th>Combined fraction</th>
<th>Yield in extract (%w/w)</th>
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<td>Combined fraction 5 (compound OI1)</td>
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<tr>
<td>Combined fraction 6</td>
<td>0.19</td>
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</table>

Figure 6 TLC chromatograms of *O. indicum* pod coat extract and combined fractions; adsorbent: silica gel GF254, solvent system: ethyl acetate-toluene-formic acid 25-25-7.5 v/v, 37 = pod coat extract, X = standard mixture, 1 = combined fraction 1, 2 = combined fraction 2, 3 = combined fraction 3, 4 = combined fraction 4, 5 = combined fraction 5, 6 = combined fraction 6, A = white light, B = UV 254, C = UV366, D = NP + UV366
The $^1$H-NMR data (400.13 MHz, DMSO-$d_6$) of compound OI1 were assigned on the basis of the chemical shifts, splitting patterns and integration values. The signals at $\delta$ 8.07 (1H, m), 7.58 (1H, m), 7.61 (1H, m), 7.58 (1H, m) and 8.07 (1H, m) ppm were assigned to H-2’, H-3’, H-4’, H-5’ and H-6’ of ring B, respectively. The proton signal at $\delta$ 6.64 (1H, s) ppm was assigned to H-8 of ring A while the signal at $\delta$ 6.97 (1H, s) was assigned to H-3 of ring C. The signals at $\delta$ 12.93 (1H, s) and 11.78 (1H, s) ppm indicating proton of OH groups at C-5 and C-7 of ring A while methoxy protons of methoxy substitute group at C-6 of ring A was found at $\delta$ 3.76 (3H, s). The proton signals corresponded to the signals of oroxylin A in reported literature [10].

The $^{13}$C NMR (100.61 MHz, DMSO-$d_6$) of compound OI1 showed 16 signals ascribed to: one methoxy carbon (OCH$_3$); 59.95 (CH$_3$ at C-6); seven methine carbons (CH); 94.41 (C-8), 104.67 (C-3), 126.40 (C-2’), 129.14 (C-3’), 132.01 (C-4’), 129.14 (C-5’) and 126.40 (C-6’); and eight quaternary carbons (C); 163.21 (C-2), 182.27 (C-4), 152.75 (C-5), 131.48 (C-6), 157.69 (C-7), 152.58 (C-9), 104.34 (C-10) and 130.76 (C-1’). Nuclear Overhauser Effect Spectroscopy (NOESY) showed signal indicated the relationship between proton of OH group at C-3 of ring C and proton at C-6’ of ring B while correlation spectroscopy (COSY) suggesting the signals indicated the relationship between proton at C-3’ to the protons at C-2’ and C-4’. The heteronuclear single-quantum correlation spectroscopy (HSQC) and heteronuclear multiple-bond correlation spectroscopy (HMBC) showed the signals indicated the relationship between protons and carbons including methoxy protons (OCH$_3$) at C-6 to C-6, hydroxy (OH) at C-5 to C-6 and C-10, proton at C-8 to C-6, C-7, C-9 and C-4, proton at C-3 to C-4, C-9, C-10 and C-1’, proton at C-2’ to C-2 and proton at C-6’ to C-2.
According to the 1H-NMR and $^{13}$C-NMR data, compound OI1 was identified as oroxylin A. The chemical structure of oroxylin A are shown in Figure 8.

![Chemical structure of oroxylin A](image)

**Figure 8** Chemical structure of oroxylin A

The amounts of oroxylin A in pod coat and green mature fruits extracts were analyzed using HPLC condition as mentioned before (Table 7). It was found that pod coat and green mature fruits extracts contained around 1% w/w of oroxylin A. This compound could be used as a marker for indicating the maturity of the fruit of *O. indicum*.

4. Conclusion

From the experiment, three compounds; oroxin B, oroxin A and chrysin-7-O-glucuronide were additionally identified in *Oroxylum indicum* extracts and fractions by LC-MS technique with 3 previously identified compounds; baicalin, baicalein and chrysin. The optimized HPLC condition was developed and used for quantitative analysis of these 6 major compounds in *Oroxylum indicum* extracts. Young fruit extract contained low amount of all flavone while the seeds extracts were found to be the enrich sources of flavones. The green mature fruit and old pod coat extracts contained the same patterns of flavone with some amount of baicalin and low amount of other 5 compound. Using QAMS technique, baicalin was found to be the suitable single marker for calculation of relative conversion factors (RCF) of other flavones in *O. indicum* extracts. Oroxylin A was isolated and identified in the pod coat extract of *O. indicum*.

5. Acknowledgement

Author would like to thank Professor Dr. Hermann Stuppner, Institute of Pharmacy/Pharmacognosy, Center for Chemistry and Biomedicine, University of Innsbruck
for his support. Author would like to thank Dr. Stefan Schwaiger, Institute of Pharmacy/Pharmacognosy, Center for Chemistry and Biomedicine, University of Innsbruck for his guidance and help about LC-MS and HPLC analysis. Finally, author would like to thank ASEAN-European Academic University Network (ASEA-UNINET) scholarship (Ernst Mach Grant) for financial support.

References

Appendix
## Approved ASEA-UNINET Projects Proposals 2020 and 2020/2021
Classified by the Austrian member universities

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<thead>
<tr>
<th>Applying department</th>
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Callout:
- TH Thailand
- PK Pakistan
- VN Vietnam
- RI Indonesia
- MAL Malaysia
- MMR Myanmar
- RP Philippines

* Project approved by ASEA-UNINET, but funding agreement NOT countersigned by university (reason: travel restrictions due to COVID-19)
| Department of Theoretical Chemistry, Research Group Bioinformatics and Computational Biology | Theoretical Studies of Functional RNA Structures in Flaviviruses | Chulalongkorn University, Department of Biochemistry | x |
| Institute of Theoretical Chemistry | Theoretical Methods of Drug Design | Chulalongkorn University, Faculty of Science Department of Chemistry | x |
| | | Chulalongkorn University, Faculty of Science, Department of Biochemistry | x |
| Center for Teacher Education | IN-CRIS: Strengthening inclusive practices in times of crisis PART 1 OUTGOING | Srinakharinwirot University, Special Education Development Center | x |
| Center for Teacher Education | IN-CRIS: Strengthening inclusive practices in times of crisis PART 2 INCOMING | Srinakharinwirot University, Special Education Development Center | x |
| Department of Evolutionary Anthropology | Immunogenetic diversity of the Maniq people, primary hunter-gatherer in Thailand | Chulalongkorn University, Centre for European Studies | x |
| Department of Physical Chemistry | Synthesis and Characterization of Novel Hydrogel for Wound Dressing Application | Chiang Mai University, Faculty of Science, Department of Chemistry | x |
| Department of Social and Cultural Anthropology | The effects of the Covid-19 crisis on tourism on Mt. Merapi in central Java, Indonesia | Universitas Gadjah Mada, Department of Anthropology | x |
| Department of Evolutionary Anthropology | Evolutionary genetic history and genetic adaption of hunter-gatherers: The case of the Forest Tobelo (Indonesia) | Diponegoro University, Department of Anthropology | x |
| Department of Physical Chemistry | Polymer-based rapid test formats for applications in diagnosis and environment | Kasetsart University, Faculty of Science, Department of Biocemistry | x |
| | | Ubon Ratchathani University, Faculty of Science, Department of Chemistry | x |
| Department of Physical Chemistry | Diagnostic assays: feasibility of Molecularly Imprinted Polymers - the way to assays? | Prince-of-Songkla University, Department of Medical Technology | x |

| 15 projects |
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### Vienna University of Technology (TU Wien)

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<td>Integrated Approaches for the Conservation and Transformation of Cultural Heritage Landscapes in Indonesia CaTCH-I</td>
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5 projects

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Hanoi University of Science and Technology, School of Biotechnology and Food Technology  
Universiti Putra Malaysia, Department of Cell and Molecular Biology |
| Institute of Soil Research | Soil amendment effects on crop yields and heavy metal concentrations in soil and crops on ex-tin mining area on Bangka Island - Sumatra | Universitas Gadjah Mada, Faculty of Agricultural Technology, Department of Agricultural and Biosystem Engineering |
| Institute of Agronomy | Soil Health Training on Bangka-Island | Universitas Gadjah Mada, Faculty of Agricultural Technology, Department of Agricultural and Biosystem Engineering |
| Institute of Landscape Development, Recreation and Conservation Planning (ILEN) | A review on differences in recreation use of urban green spaces in Malaysia and Austria | Universiti Putra Malaysia, Faculty of Forestry, Department of Recreation and Ecotourism |
| Institute of Food Technology | Protein surface display in lactic acid bacteria for the development of oral vaccines | Vietnam National University HCMC, University of Science, Department of Molecular and Environmental Biotechnology  
Hanoi University of Science and Technology, School of Biotechnology and Food Technology |
| Institute for Development Research | Preparing Sustainability Transition Schools - a global initiative, implemented locally | Universitas Gadjah Mada, Faculty of Agricultural Technology, Department of Food and Agriculture Product Technology |

6 projects

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number of participations of each ASEAN partner country

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| number of participations of each ASEAN partner country | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
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<td>The Department of Vocal Studies and Music Theatre</td>
<td>Macbeth in word and action. Workshop for acting and directing students *</td>
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2 projects  

| number of participations of each ASEAN partner country | 1 0 1 0 0 0 0 |

### University of Applied Arts Vienna

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2 projects  

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### Montanuniversität Leoben

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<td>Institute of Chemistry</td>
<td>Development of New Electrochemical Sensors Based on Nanoparticles with Chulalongkorn University</td>
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<tr>
<td>Institute of Chemistry</td>
<td>Development of New Electrochemical Sensors Based on Nanoparticles with Ubon Ratchathani University</td>
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<td>Institute of Systems Sciences, Innovation &amp; Sustainability Research</td>
<td>Low-carbon transitions: Strategies to mitigate adverse effects of ambitious climate policy</td>
<td>Prince-of-Songkla University, Faculty of Environmental Management</td>
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<tr>
<td>Institute of Earth Sciences</td>
<td>Petrology and Geochemistry of Mantle Xenoliths from Denchai area, Phrae Province, Northern Thailand (project in cooperation with the University of Innsbruck) *</td>
<td>Chulalongkorn University, Institute of Geology, Kasetsart University, Institute of Geology</td>
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<td>Institute of Earth Sciences</td>
<td>Granitoids and their surrounding contact aureole from NW-Thailand and SE Myanmar *</td>
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5 projects

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<td>Institute of Applied Mathematics</td>
<td>Space time methods for inverse problems</td>
<td>Hanoi University of Science and Technology (HUST), School of Applied Mathematics and Informatics</td>
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<td>Signal Processing and Speech Communication Laboratory</td>
<td>Location-aware wireless networks</td>
<td>Hanoi University of Science and Technology (HUST), School of electronics and telecommunications</td>
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ASEA-UNINET - Annual Report Austrian Board of Trustees 2020 and 2020-2021 - Page 187
| Institute of Inorganic Chemistry | Structure and dynamic studies of amphiphilic glycolipid liquid crystalline phases | Universiti Malaya, Department of Chemistry  
Universiti Teknologi Mara, Faculty of Chemical Engineering | x | x |
| Institute of Applied Mathematics | Complex methods for partial differential equations | University of Transport and Communications Hanoi, Institute of Analysis  
Hanoi University of Science and Technology, Department of Applied Mathematics | x | x |
| Signal Processing and Speech Communication Laboratory | Computational Lung Sound Analysis for Medical Diagnosis Support | University of Medicine & Pharmacy HCMC, Department of Internal Medicine  
University of Danang, Electrical Engineering Faculty, Division of Automation | x | x |

6 projects  
number of participations of each ASEAN partner country 0 0 7 0 2 0 0

Medical University of Graz

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| Vice-Rector for Teaching and Studies | Clinical Rotations in South East Asia (Thailand, Vietnam, Indonesia) | Chiang Mai University, Faculty of Medicine  
Chulalongkorn University, Faculty of Medicine  
Khon Kaen University, Faculty of Medicine  
Mahidol University, Faculty of Medicine  
Universitas Gadjah Mada, Faculty of Medicine  
University of Medicine and Pharmacy HCMC, International Relations |

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### Clinical Rotations in South East Asia (Thailand, Vietnam, Indonesia)

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2 projects

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<td>Institute 2 - Piano</td>
<td>Language and performance practice of the recitative - The rhetorical power of the recitatives in the operas from Monteverdi to the 19th century</td>
<td>Mahidol University, College of Music, Voice and Musical Theatre Department</td>
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<td>European Research Centre for Book and paper Conservation-Restoration</td>
<td>Glass slides and other archival material about the conservation of UNESCO world heritage Temple Borobudur</td>
<td>Institut Seni Indonesia Yogyakarta, Institute for Fine Arts</td>
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<td>Low-Tech Prototype of a Tradition Based Museum Building for Java</td>
<td>Universitas Gadjah Mada, Institute for Architecture, Institut Seni Indonesia Yogyakarta, Institute for Fine Arts</td>
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| Department of Telecooperation | Monitoring, Managing and Mitigating Environmental Impact for Sustainable Development | Universitas Indonesia, Faculty of Computer Science, Hanoi University of Science and Technology, School of Information and Communication Technology, Universiti Malaya, Department of Information System |   |   |   | x |   |   | x |
| Vice rector for Medicine | ASEA-UNINET Clinical Elective Exchange Program * | Chiang Mai University, Faculty of Medicine, Chulalongkorn University, Faculty of Medicine, Khon Kaen University, Faculty of Medicine, Mahidol University, Faculty of Medicine, Gadjah Mada University, Faculty of Medicine | x |   |   |   |   |   | x |
### University of Salzburg

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<td>Department of Geography and Geology</td>
<td>Urban Nature and Urban Green Spaces, Educational Module</td>
<td>Chulalongkorn University, Social Research Institute (CUSRI) Kasetsart University, Division of Urban and Environmental Planning, Faculty of Architecture King Mongkut's University of Technology Thonburi, School of Liberal Arts</td>
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<td>Migration Industry Brokering Retirees' Mobility: Japan-Thailand and Austria-Thailand in Comparison</td>
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<td>Department of Banking and Finance</td>
<td>Bringing Behavioral and Experimental Finance Expertise to Thailand</td>
<td>Chulalongkorn University, Business School (CBS)</td>
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<td>The Making of Transient Urban Spaces in the Bangkok Metropolitan Region (BMR)</td>
<td>Mahidol University, Institute for Population and Social Research</td>
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<td>Department of General and Inorganic and Theoretical Chemistry</td>
<td>QM / MM simulations employing novel neural network strategies</td>
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<td>Institute of Mineralogy and Petrology</td>
<td>Petrology and Geochemistry of Mantle Xenoliths from the Denchai area, Phrae Province, Northern Thailand (project in cooperation with the University of Graz)</td>
<td>Chulalongkorn University, Department of Geology, Chulalongkorn University, Department of Chemistry, Kasetsart University, Institute of Geology</td>
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7 projects

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<td>Chiang Mai University, Faculty of Medicine</td>
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<th>4 projects</th>
<th>number of participations of each ASEAN partner country</th>
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Sum of approved projects for the implementation period 2020 and 2020-2021: 70
Total number of participations of each ASEAN partner country: 117
### SP24 Mobilities 2019

**including 1-Month Staff Mobilities (Thailand)**

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<tr>
<th>Land</th>
<th>Universität Österreich</th>
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Number 17 and number 27: postponed scholarships
### SP24 Mobilities 2020
#### 1-Month Staff Mobilities (Thailand)

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Number 3, 5, 7 and 10: postponed scholarships

\* cancelled because of COVID-19
## Bernd Rode Award (BRA) 2019 – Laureates
(Implementation period was extended due to COVID-19 till 28/02/2022)

### Junior Category

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<tbody>
<tr>
<td>Dr. Bao Quoc Tang</td>
<td>University of Graz, Austria</td>
<td>€ 2.500,−*</td>
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<tr>
<td>Apinun Kanpiengjai, PhD.</td>
<td>Chiang Mai University, Thailand</td>
<td>€ 2.500,−*</td>
</tr>
<tr>
<td>Anh-Dung Tran, MsC</td>
<td>Technical University of Vienna, AT</td>
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### Senior Category

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<tr>
<td>Pongtip Sithisarn, PhD.</td>
<td>Mahidol University, Bangkok</td>
<td>€ 2.500,−*</td>
</tr>
<tr>
<td>Weena Gera, PhD.</td>
<td>University of the Philippines Cebu</td>
<td>€ 2.500,−*</td>
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<tr>
<td>Manuel Joseph C. Loquias, Dr. Math.</td>
<td>University of the Philippines Diliman</td>
<td>€ 2.500,−*</td>
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### Project Excellence

<table>
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<th>University</th>
<th>Award</th>
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<tbody>
<tr>
<td>Dr. Anchalee Samphao</td>
<td>Ubon Ratchathani University, Thailand</td>
<td>€ 3.000,−*</td>
</tr>
<tr>
<td>Assoc. Prof. Tran Thi Dinh</td>
<td>Vietnam National University of Agriculture</td>
<td>€ 3.000,−*</td>
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<tr>
<td>Univ.-Prof. Dr. Annette Ostendorf</td>
<td>University of Innsbruck, Austria</td>
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</tbody>
</table>

* Financial Award must be reinvested into ASEA-UNINET projects
### Bernd Rode Award (BRA) 2020/2021 – Laureates

(Implementation period till 31/12/2022)

#### Junior Category

<table>
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<tbody>
<tr>
<td>Suphat Phongthai, Ph.D.</td>
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<td>Siska Aditya, Ph.D.</td>
<td>University of Veterinary Medicine Vienna, Austria</td>
<td>€ 2.800,-*</td>
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<tr>
<td>Christian Obermayr, Ph.D.</td>
<td>University of Innsbruck, Austria</td>
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#### Senior Category

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<tr>
<td>Prof. Kanokwan Jarukamjorn, Ph.D.</td>
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#### Project-based Category

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<td>Prof. Mag. Art. Anna Maria Krassnigg</td>
<td>University for Music and Performing Arts Vienna, Austria</td>
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<tr>
<td>Prof. Dr. Peter A. Lieberzeit</td>
<td>University of Vienna, Austria</td>
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* Financial Award must be reinvested into ASEA-UNINET projects