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ALFRED WERNER FUND, MASTER'S STUDENT SCHOLARSHIPS



The Alfred Werner Fund of the SCS Foundation, established in 2014, continues the initiatives and projects of the former foundation 'Stiftung für Stipendien auf dem Gebiete der Chemie', also known as the 'Alfred Werner Stiftung'. The SCS Foundation offers this scholarship program in collaboration with the Swiss chemical and pharmaceutical industry (see box with supporting companies).

The program supports Master degree studies for excellent students from foreign countries in Chemistry or Biochemistry at a Swiss University or at a Federal Institute of Technology. The Foundation offers up to ten scholarships of CHF 25'000 each year for students selected by the partner universities (see box). The goal of the scholarship program is to bring in young talent to Swiss Universities or the Federal Institutes of Technology.

See <https://scs-foundation.ch/> for information about the Alfred Werner alumni.

Partner Universities / Federal Institutes of Technology

The program is supported by




Summary of the Master Theses from Students of the Term 2018–2020



Michelle Gaspard

Nationality: Canada

Bachelor at:

Polytechnique Montreal, Canada

Master at: *EPF Lausanne*

Master thesis supervisor:

Prof. Horst Pick

Surface Plasmon Resonance (SPR) Signal Analysis for Characterizing Heterogeneous Analyte Solutions

Glycosylation is one major types of post-translational modification (PTM) in protein biosynthesis and has been deemed to be a critical quality attribute during the production process of therapeutic monoclonal antibodies (mAbs).^[1] In fact, several studies have shown that different states of glycosylation may have a significant impact on the effectiveness of a therapeutic mAbs. Different glycoforms can affect pharmacokinetic parameters such as serum half-life, immunogenicity, safety, efficacy or even stability.^[2] Because process parameters such as pH and temperature can affect the PTMs and result in production batches with highly heterogeneous glycosylation patterns, it would be preferable if the glycosylation of antibodies was monitored throughout the course of their production. Our group decided to investigate Surface Plasmon Resonance (SPR)-based biosensors to see if it could be a potential answer to this issue as it would characterize cell cultures faster than current methods and could be integrated in a continuous production line, if developed well.

The overall objective of our group was to develop a reliable and robust detection system for on-line quality assessment of recombinant proteins present in complex culture media when produced in a bioreactor. With this system, we would be able to estimate accurately the molar composition of any protein solution according to their glycoforms. To do so, we had to understand the correlation of the interaction kinetic data between the product of interest and the biosensor surface with the critical quality attributes of the product.

To characterize the interactions of the analytes with the biosensors, an algorithm had to be created using MATLAB. For this purpose, we concluded that the analytes followed a simple Langmuir 1:1 interaction model.^[3] This kinetic relation was used to create the algorithm and be able to determine the composition of a heterogeneous solution.

The algorithm was also useful to demonstrate the contribution of each analyte to the SPR response. Unfortunately, not all glycoforms have the same affinity to the ligand on the sensor chip. For example, antibodies onto whom were attached *N*-glycans containing a fucose sugar inherently have lower affinities to the sensor chip ligand than others. They usually contribute less to the response obtained during an SPR assay.

With that information, we were able to determine that some analytes could be combined when analyzing a multi-analyte SPR

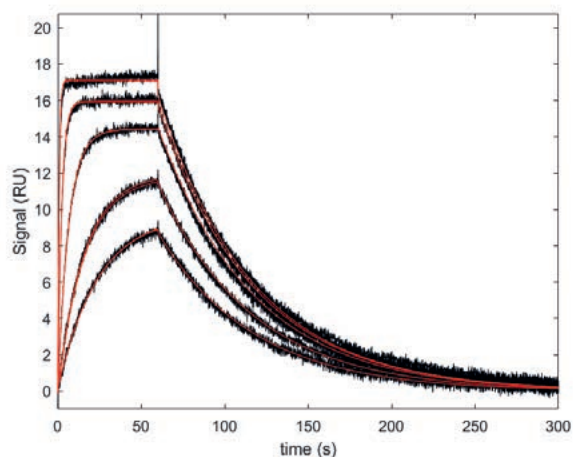


Fig. 1. Kinetic analysis of the SPR response of a solution containing four analytes. The black dots correspond to the SPR sensorgram obtained from the experimental data. The red line represents the fit obtained by the MATLAB model.

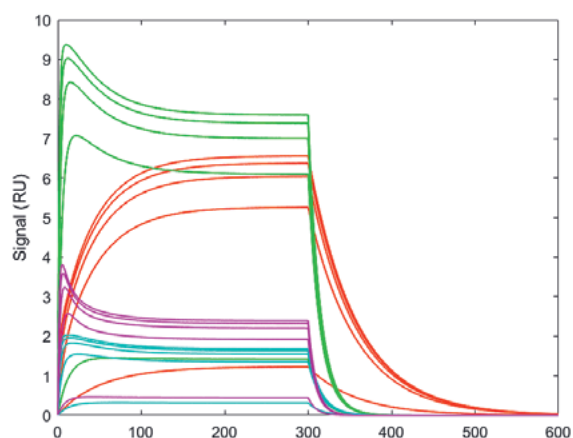


Fig. 2. Kinetic analysis of the contributions of each analyte to the SPR response of each mixture at different total concentrations. The solution contains four analytes (four colors). The sum of the contribution of each analyte gives the expected SPR sensorgram of the solution.

sensorgram. Multiple case studies showed that the analytes could be pooled together when they had lower affinity to the sensor and similar kinetics. This result was important because it enabled us to confirm that it could be possible to have a broad sense of the glycosylation profile of a batch of mAbs throughout the production process. It will also enable to continuously assess the quality of them and adjust the parameters if the profile deviated from the desired final product.

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Future Plans

I was in Canada visiting family when COVID-19 restrictions were applied. Thus, I had to stay in the country and perform my

research from home. Now, I am using a previous training as a long-term care aid to work as an essential worker in Canada for a while and help in any way I can. After this period, I am considering pursuing PhD at the National Research Council of Canada in environmental biotechnology. However, if it is possible, I am also considering coming back to Switzerland for studies or for work. I wish to thank my advisor for his support in this exceptional situation.



Oleh Hordiichuk

Nationality: *Ukraine*
 Bachelor at: *Ivan Franko National University of Lviv, Ukraine*
 Master at: *ETH Zurich*
 Master thesis supervisor: *Prof. Maksym Kovalenko*

Hybrid Luminescent Materials by Incorporating Metal Halides into Metal–Organic and Covalent Organic Frameworks

Metal–organic and covalent organic frameworks (MOFs and COFs) are employed as molecular templates for crystallization of main group metal halide (MH) compounds – materials which are known to exhibit exceptional luminescence and other optoelectronic properties.

Constraining spatial dimensionality of electronically extended systems by tailoring crystal structure, size and shape of inorganic compounds at the nanoscale often endows the resulting materials with extraordinary optical, electronic, magnetic and catalytic properties. A recent prominence of inorganic and hybrid – organic–inorganic – lead halide perovskites as exceptional materials for optoelectronic applications has stimulated studies of other main group MH compounds leading to discoveries of materials with diverse and novel functionalities.^[1–4] We envisioned an extension of the structural space accessible for MHs, in terms of atomic connectivity and electronic dimensionality, when using atomically-defined porous templates as hosts for MH crystallization. Atomically defined MH clusters and their properties can thus be engineered well beyond those few structural motives known from the conventional free-space crystallization.^[2–4]

Several 2D and 3D COFs and MOFs were studied for the possibility of confining main group MHs. In the case of 2D COF, its interaction with some amine group MHs in solution and in melt led to the formation of photoluminescent products (Fig. 1). When reacted in melt, the system exhibited temperature-dependent photoluminescence behavior. However, the chemical and structural nature of luminescent species could not be unambiguously resolved, since no structure determination was within reach due to the microcrystalline (with a very low degree of crystallinity) nature of the employed COFs. With one of the MOFs attempted, we succeeded to obtain an atomically defined (from single-crystal X-ray diffraction) MH cluster within the MOF pores (Fig. 1).

The example of the MH cluster confined in the MOF emphasizes how the geometry and functionality of the pores and the linkers dictate *via* directing the structure of the resulting MH units, forcing the latter to acquire the geometry necessary to fit into the cavity and which may not otherwise form in the free-space crystallization from the solvents. We thus anticipate that such molecular templating of the MH crystallization will evolve into a useful materials design strategy. Future work shall focus

on the explorations of other frameworks as hosts and other MHs as guests, offering vast compositional and structural space to dive in.

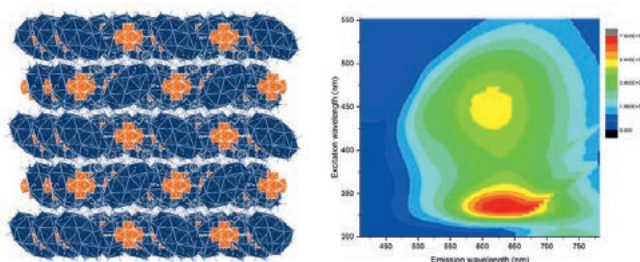


Fig. 1. A novel materials design strategy employs metal–organic and covalent organic frameworks as molecular templates for crystallization of main group metal halide compounds – materials which exhibit exceptional luminescence and other optoelectronic properties. Crystal structure determination with atomic resolution (left) facilitates establishing of structure-properties relationships (e.g. photoluminescent properties, right).

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Future Plans

I have started a doctorate in Prof. Kovalenko's Functional Inorganic Materials Group at ETH Zurich. I continue exploration of hybrid metal halide materials.



Artem Kononenko

Nationality: *Ukraine*
 Bachelor at: *Institute of Organic Chemistry and Biochemistry, Prague*
 Master at: *University of Geneva*
 Master thesis supervisor:
Prof. Nicolas Winssinger

Suprastaple: Delivering Structural Organization and Molecular Diversity

A novel peptide stapling strategy based on PNA-mediated assembly was developed, leading to a new peptide screening platform. The technology was named 'Suprastaple', in reference to the fact that stapling makes use of supramolecular interactions. Suprastaple was shown to enhance peptide's resistance to proteolysis and not to disrupt its binding affinity to the target protein. In addition, such a PNA tag allowed to create a library of α -helical peptides through combining and subsequent ligation of N- and C-terminal libraries.

Protein–protein interactions (PPIs) govern myriads of intracellular and extracellular processes of exceptional pharmacological interest. However, targeting PPIs by means of small molecule drugs has proven to be a challenging task mostly because of geometrical reasons. The interfaces between proteins are predominantly flat, lacking grooves and pockets that are commonly implicated in binding with the small molecules.^[1]

As over 60% of all multiprotein complexes feature an α -helix at the interface,^[2] mimicry of α -helical folding pattern appears to be a promising strategy for targeting PPIs. But native helical peptides have major shortcomings as therapeutic agents, resulting from their low conformational stability. One of the strategies to enforce a constrained alpha-helical conformation in short peptides is peptide stapling, which implies peptide's side chain macrocyclization. Being a promising strategy, a route from a peptide sequence at the interface to high affinity stapled peptide is challenging and requires laborious experimental optimization. The integration of peptide stapling with high-throughput screening (HTS) methods, particularly phage-display^[3] and mRNA-display^[4] was shown to be an effective solution to this problem. However, both phage and mRNA display technologies are limited in the possibility of sequence diversification using unnatural amino acids. In this work we developed a new approach for short peptides screening, which allows to diversify α -helical peptides using unnatural amino acids and can be compatible with DNA-encoded technologies.

In the first part of the project, several designs of Suprastaple were tested by measuring their influence on the binding affinity of model peptide sequence and its resistance to proteolysis. The importance of the stapling position was demonstrated by performing affinity measurements using fluorescence polarimetry (Fig. 1A) and surface plasmon resonance. A significant improvement of the proteolytic stability of suprapstapled peptide comparing to single PNA control was shown using serum stability assay (Fig. 1B). Finally, a test library of suprapstapled peptides was screened against a specific target protein and several hits were identified that are strongly in line with data obtained by Pentelute and co-workers^[5] (Fig. 1C).

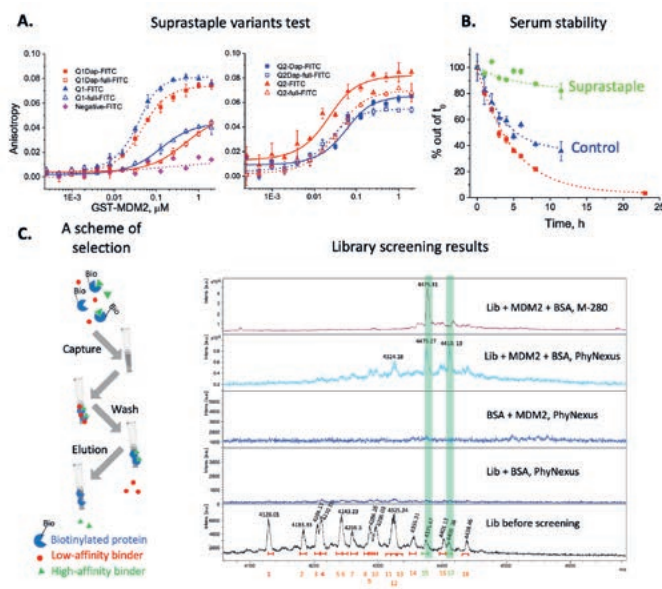


Fig. 1. A) Binding data of model peptide stapled with different variants of Suprastaple. B) Serum stability assay of suprapstapled peptide versus control. C) Selection scheme and MS reads from test library selections. Binding hits are highlighted in green.

All in all, an incorporation of suprapstaple into a highly optimized α -helical peptide sequence was shown to have any detrimental impact on binding affinity while substantially enhancing proteolytic stability of the suprapstapled peptide relative to a control. Based on this data, a new screening strategy for α -helical peptides discovery that utilizes PNA-mediated assembly was developed.

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Future Plans

I am excited about HTS technologies and bioengineering, so after my graduation I have joined the group of Prof. Maartje Bastings at EPFL to work on the development of HTS technology based on DNA-mediated assemblies.



Jane Marsden

Nationality: Irish

Bachelor at:

University of Limerick

Master at:

University of Geneva and EPFL

Master thesis supervisor:

Prof. Bruno Correia

Experimental Evaluation of *de novo* Designed Protein Binders to Create Site-Specific Interactions

Protein–protein interactions (PPIs) are essential to the proper functioning of many biological processes in the cell. Being able to predict PPIs based solely on protein structure and design novel interactions remains an unsolved problem in computational biology. This project experimentally tested the ability of a novel computational approach developed in the lab – Molecular Surface Interaction Fingerprinting (MaSIF)^[4] to identify protein binding interfaces based upon the protein's molecular surface and find a complementary binding motif for that specific site. Combining high-throughput screening with yeast surface display, sequencing, protein expression and characterisation, and interface analysis, we were able to show the experimental testing of computationally designed protein complexes and create a high-affinity site-specific protein binder to PD-L1.

expressed, purified and characterised to examine the specificity of the interaction and accuracy of the prediction by MaSIF. In the case of PD-L1, after three selection rounds followed by further interface optimisation, a site-specific *de novo* protein binder with a double-digit nanomolar affinity to PD-L1 was obtained. The binder was shown to be monomeric, α -helical and had a molecular weight of 12.5 kDa. Mutagenesis data from the design revealed the importance of an amino acids' side chain properties in the formation of protein complexes at specific sites. Altogether, this project illustrates the importance of using experimental methods to test, guide and feedback computational design efforts when creating novel PPIs. It also supports the potential of the MaSIF framework for the structure-based prediction and design of site-specific PPIs.

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Future Plans

After graduating in January 2020, I worked briefly on an early-stage development project within the laboratory of Prof. Christian Heinis at the EPFL. After, I began my professional career in industry with Vifor Pharma at their site in Villars-sur-Glâne, working within their quality department.



Daria Morina

Nationality: Ukraine

Bachelor at:

V. N. Karazin Kharkiv National University, Ukraine

Master at:

ETH Zurich

Master thesis supervisor:

Prof. Dr. Bill Morandi

Synthesis of Macrocycles and Molecular Cages via Catalytic C–S/C–S Metathesis

In this work, a number of building blocks containing arylthioether moieties were synthesized and subsequently employed in a Pd-catalyzed C–S/C–S metathesis for the formation of cages and macrocycles. Different reaction conditions and the influence of templating effects were investigated. The products obtained were characterized by mass spectroscopy. Additionally, gel permeation chromatography was tested as an alternative method of characterization.

The nowadays popular synthesis of macrocycles and molecular cages can presuppose a number of challenges that include the wide distribution of homologous products in resulting reaction mixture, kinetic issues, and undesired formation of polymeric structures.^[1] To overcome the possible obstacles, different synthetic tools can be used. Dynamic covalent chemistry (DCC) is one of the strategies for supramolecules' synthesis.^[2] According to the DCC concept, the core reaction to form supramolecules has to meet a number of requirements: mild conditions, reversibility, and relatively high speed. The C–S metathesis reaction reported by Prof. Morandi's group in 2017 meets all these conditions and could thus potentially be used for the synthesis of macrocycles and molecular cages.^[3]

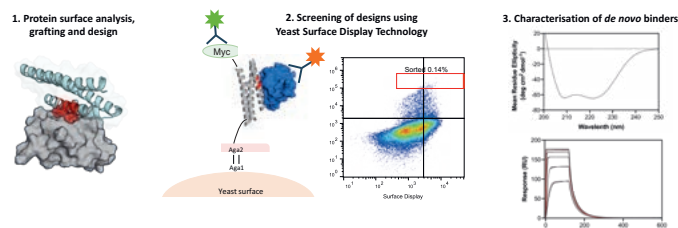
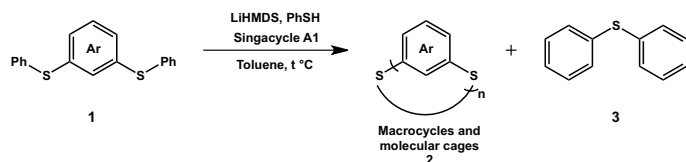


Fig. 1. Overview of the different steps involved in the project to develop a *de novo* protein binder targeting a specific site on PD-L1.

Yeast surface display technology^[2] was the chosen screening platform to test individual designed binders as well as libraries based on a binder to the target protein PD-L1. Fluorescence-activated cell sorting (FACS)^[3] was used to isolate high-affinity designs from a library of binders. This directed evolution approach enabled the selection of improved binder sequences which were

Therefore, in the first part of the research, after the synthesis of the building blocks, the C–S bond metathesis reaction was tested in the synthesis of molecular cages and macrocycles under different screening conditions. Concentrations of starting material, base, catalyst, co-catalyst and temperature were evaluated as variables (Scheme 1).



Scheme 1. Generalized scheme of macrocycles and molecular cages synthesis via C–S/C–S metathesis reaction.

The analysis of the reaction mixtures showed the presence of macrocycles with different numbers of units in case of the macrocycles' formation. On the other hand, the more challenging desired molecular cages were not observed in the reaction mixtures. However, products with a structure close to the cages were detected.

The presence of sulfur atoms and aromatic rings on the building blocks opens the door for a more efficient method for supramolecules' synthesis: templated synthesis. Thus, to improve the results, the most successful reaction conditions were tuned with the addition of a templating agent (cations and aromatic molecules). However, it was discovered that in most cases the templating agents cause an inhibition of the metathesis reaction.

Future research will be focused on increasing the yields of supramolecules, identifying purification methods and adjusting conditions for better product selectivity.

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Future Plans

After completion of my Masters at ETH Zurich, I will start my doctoral studies as a member of Prof. Dr. Karl Gademann group. The research will be focused on the synthesis and modification of natural products.



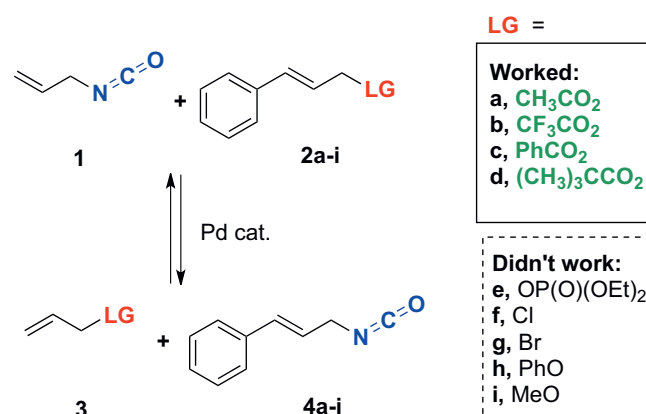
Olivera Živojinović
Nationality: Serbia
Bachelor at: *University of Belgrade*
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Master thesis supervisor:
Prof. Bill Morandi

Palladium-catalyzed Metathesis of Allylic Carboxylates and Isocyanates

Isocyanates ($R-N=C=O$) are interesting compounds due to their dual reactive nature. Upon a nucleophilic attack onto the electrophilic carbon, the nitrogen's latent nucleophilic nature is revealed^[1] Diisocyanates have found tremendous application

in the polymer industry as starting materials for polyurethanes, which are applied in many industry spheres and can be found everywhere from construction sites and automobiles to kitchens and wardrobes. The density and rigidity of polyurethanes depend on the production technology and their chemical composition^[2] which is why new monomer structures are attractive synthetic goals.

This master thesis aims to develop a Pd-catalyzed exchange between carboxylate and isocyanate. Trading a leaving group such as carboxylate with isocyanate is safer than other methods such as using poisonous phosgene or acyl azides. Using the model reaction (Scheme 1), isocyanate transfer from an allyl donor to a cinnamyl substrate has been developed.

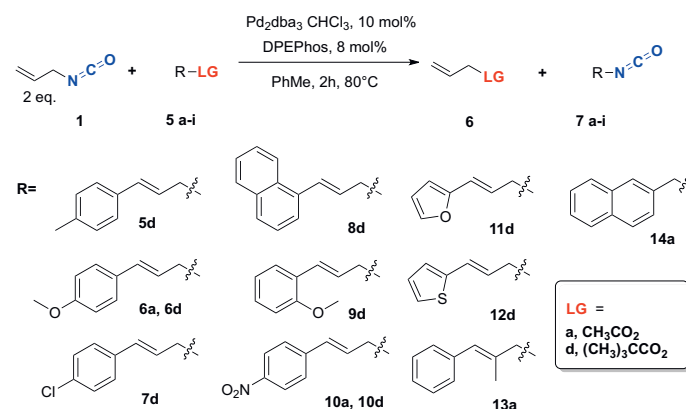


Scheme 1. Examination of leaving groups in the model reaction.

In a closed system, it was not feasible to push the reaction towards 100% yield of cinnamyl isocyanate, and it has been proven that this transfer reaction attains an equilibrium^[3] with the equilibrium constant lying on the side of reactants:

$$\left(K_{OAc} = \frac{[3][4a]}{[1][2a]} = 0.25 \pm 0.05\right) \quad (1)$$

However, already by adding 2 equivalents of allyl isocyanate **1**, the ratio of compounds **4a:2a** reaches 1:1. After further optimization, optimal reaction conditions have been found, and the substrate scope has been explored with regard to the leaving group (Scheme 1) as well as the main scaffold (Scheme 2). Only carboxylates proved useful as substrates.



Scheme 2. Substrate scope of cinnamyl carboxylates.

All substituted cinnamyl examples resulted in a starting material to product ratio ranging from 1:1 to 2:1, except for **8d** and **10a,d**, which gave complex mixtures. Attempts to develop an exchange reaction between naphthyl acetate (**14a**) and allyl isocyanate **1** was also investigated. This transformation was far less successful than cinnamyl examples, and only ~5% product formation was observed.

³¹P NMR studies and DFT calculations have been performed to help rationalize the mechanism of this transformation. Our proposed mechanism involves allyl isocyanate undergoing oxidative addition in the same manner as allyl carboxylates, forming palladium-η³-allyl isocyanate ion pair with a possibility of ion exchange for acetate^[4] which is contrary to the previously proposed mechanism for such transformation of isocyanates^[3] Literature has been thoroughly reviewed for examples of reductive elimination and oxidative addition of isocyanates. In addition, a mechanistic pathway has been calculated for Buchwald's aryl isocyanation, one of the rare published reactions proposed to involve a C-NCO bond formation through reductive elimination^[5]

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Future Plans and Acknowledgement

I am very grateful to Alfred Werner Foundation for this scholarship, which allowed me to study at prestigious ETH. During my master's studies, I also got an opportunity to spend a

year in Roche medicinal chemistry department as an intern. After defending my thesis, I plan to start my PhD studies in the group of Prof. Morandi.

Alfred Werner Scholars 2020–2022

The following students were awarded a scholarship by the Selection Committee of the Alfred Werner Fund:

Nika Asatiani, University of Geneva
BSc from San Diego State University, San Diego, USA (native of Georgia)

Patricia Brandl, EPFL Lausanne
BSc from Technical University of Vienna, Vienna, Austria

Chiara Compagnoni, University of Zurich
BSc from University of Milano-Bicocca, Milan, Italy

Chung Sum Leung, ETH Zurich
BSc from Hong Kong University of Science and Technology, Hong Kong, China

I-Hsuan Lin, ETH Zurich
BSc from University of Tokyo, Tokyo, Japan (native of Taiwan)

Gudlaugur Bjarki Ludviksson, ETH Zurich
BSc from University of Iceland, Reykjavik, Iceland

Dieu Khanh An Nguyen, University of Geneva
BSc from University of Strasbourg, Strasbourg, France (native of Vietnam)

Andrii Suponytskyi, ETH Zurich
BSc from Warsaw University of Technology, Warsaw, Poland (native of the Ukraine)