CHEM4710

Project in Chemistry or Biochemistry

2023/2024 Project Presentations

March 23, 2024

University of Manitoba
205 Armes Building

University of Manitoba

Program:

9:00 am	**Introduction**	Dr. Mario Bieringer (course coordinator)
	Opening Remarks	Dr. Georg Schreckenbach (Head of Department of Chemistry)

Time	Presenter	Title	Page	Supervisor
	Chair: Dr. Mario Bieringer			
Session 1				
9:10 am	Lexie Desjarlais	*Characterization of the Newly Found Non-Coding RNA EB120*	3	Dr. S. McKenna
9:30 am	Jadon Khouv	*Revival of the Ružička Reaction*	4	Dr. J. Walsh
9:50 am	Asad Nasir	*Understanding the Role of Near-Cognate Binding in Ribosome Assembly by H/ACA snoRNPs*	5	Dr. U. Kothe
10:10 am	Ocean White	*Investigating the Enthalpic and Entropic Contribution of TMAO to the Folding of Paratox, an intrinsic disordered protein*	6	Dr. M. Khajehpour
10:30 am		**Coffee Break**		
Session 2	**Chair: Dr. Joey Lussier**			
11:00 am	Hoang Duc Luong	*Migration of PAHs from food storage container to Foods: An Estimation of Human Exposure*	7	Dr. G. Tomy
11:20 am	Nadia Hossain	*Investigating the DusA residues involved in binding tRNA*	8	Dr. U. Kothe
11:40 am	Brady Jason	*Electrochemical Detection of Chloramphenicol Resistance in Yeast Cells*	9	Dr. S. Kuss
12:00 pm	Ahmed Zohni	*Molecular Interactions of the Extracellular Matrix Proteins Connective Tissue Growth Factor and Bone-Morphogenic Protein-2*	10	Dr. J Stetefeld
12:20 pm	Mercy Arowolo	*Exploring Depsidone Synthesis: Insights into Grayanic Acid and Its Core Structure*	11	Dr. J. Sorensen
12:40 am	**Closing Remarks**	Dr. Mario Bieringer (course coordinator)		
12:50 pm		**Group Photograph and Pizza Lunch in 539 Parker Building**		

Territory Acknowledgement

The University of Manitoba campuses are located on original lands of Anishinaabeg, Cree, Anisininew, Dakota and Dene peoples, and on the homeland of the Métis Nation.

We respect the Treaties that were made on these territories, we acknowledge the harms and mistakes of the past, and we dedicate ourselves to move forward in partnership with Indigenous communities in a spirit of reconciliation and collaboration.

Characterization of the Newly Found Non-Coding RNA EB120

Lexie Desjarlais *(Dr. Sean McKenna Group)*

Non-coding RNAs are interesting as they carry out diverse functions in the body directly. A family known as Alu-RNAs pose unique properties and are associated with human disease states[1], making them an interesting target for research. A well-known Alu-RNA named BC200 has an interesting, but unknown role in cancer[2]. Transcription of BC200 is known to be upregulated by a protein heterodimer known as SRP9/14[3]. Following transcription, BC200 gets processed down into BC120. Recently, following a sequencing experiment, a new RNA molecule was found at lower, but recognizable levels compared to BC200 which was named EB120. For my research, I synthesized the molecules BC120, EB120, and SRP9/14 to test if SRP9/14 also interacted with EB120 using BC120 as a reference. Biophysical experiments were performed to test the interaction of this complex. Preliminary data suggests complex formation, but further experimentation is required to confirm this result. Discovering another Alu-RNA will create a reference for comparison to BC200 to help contribute to determining the function of BC200.

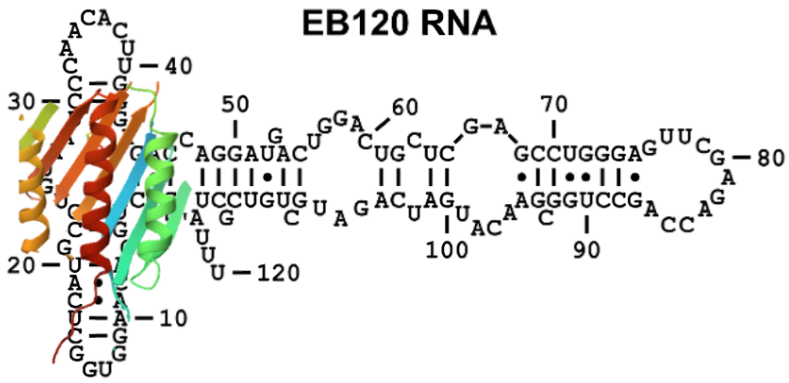


Figure 1. Hypothesized interaction between the signal recognition particle heterodimer SRP9/14 with the non-coding RNA EB120. PDB: 1914.

References

(1) Gussakovsky, D.; McKenna, S. A. Alu RNA and their roles in human disease states. *RNA Biol.* **2021**, *18,* 574-585. DOI: 10.1080/15476286.2021.1989201

(2) Booy, E. P.; McRae, E. K. S.; Koul, A.; Lin, F.; McKenna, S. A. The long non-coding RNA BC200 (BCYRN1) is critical for cancer cell survival and proliferation. *Mol. Cancer.* **2017**, *16* (109). https://doi-org.uml.idm.oclc.org/10.1186/s12943-017-0679-7

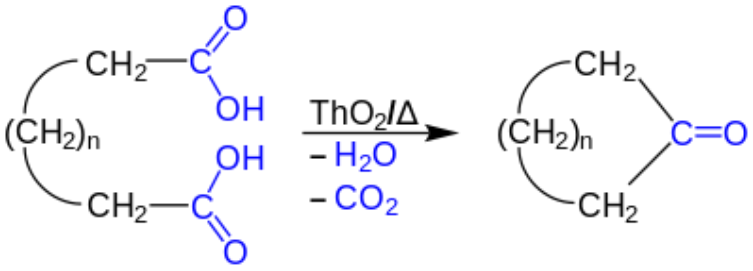
(3) Gussakovsky, D.; Booy, E. P.; Brown, M. J. F.; McKenna, S. A. Nuclear SRP9/SRP14 heterodimer transcriptionally regulates 7SL and BC200 RNA expression. *RNA.* **2023,** *29,* 1185-1200. doi:10.1261/rna.079649.123

(4) Birse, D. E.; Kapp, U.; Cusack, S.; Aberg, A. The crystal structure of the signal recognition particle Alu RNA binding heterodimer, SRP9/14. *EMBO J.* **1997**, *16,* 3757-3766. **DOI:** https://doi.org/10.1093/emboj/16.13.3757, PDB:1914.

Revival of the Ružička Reaction

Jadon Khouv *(Dr. Joshua Walsh Group)*

Molecular strain is a fundamental concept emphasized throughout chemistry, but certain compounds can exist in highly strained conformations. Cyclophanes come to mind as they possess highly strained geometry, leading to unique interactions that few compounds can accomplish. Unfortunately, the synthesis of these cyclophanes prove increasingly difficult. Clues to synthesizing these compounds may come from 1926, when Leopold Ružička brought together two carboxylic acids to form a cyclic ketone through decarboxylative ketonization. During this period, no safety restrictions were put in place; thus, the Ružička method utilized radioactive materials like thorium oxide under high temperature conditions, which is a no-go for modern-day chemists. For this reason, safer alternatives must be investigated to successfully make use of this simple and effective reaction. Once the modernized Ruzicka reaction is fully optimized, these highly strained cyclophanes become synthesized with ease, and the creation of novel cyclophanes are envisioned.



Decarboxylative ketonization performed by Ružička

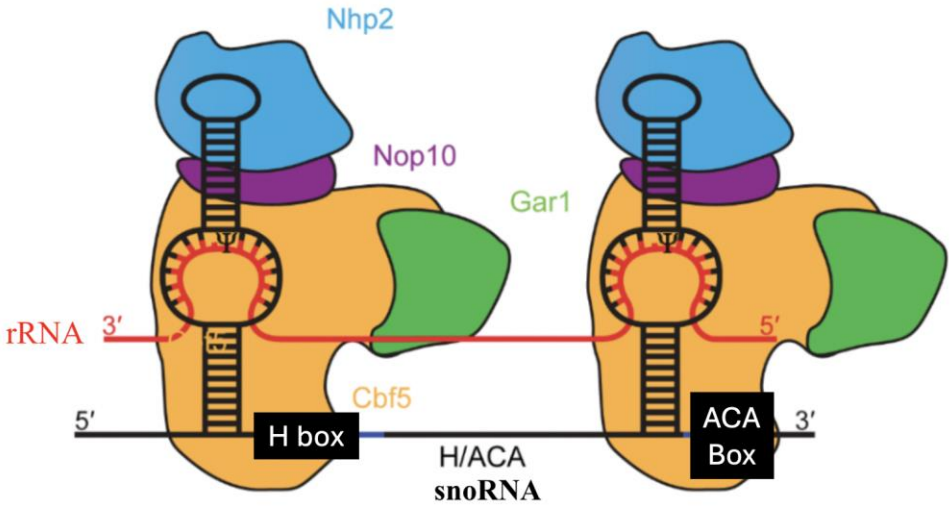
References

(1) L. Ruzicka; M. Stoll; H. Schinz, "Zur Kenntnis des Kohlenstoffringes II. Synthese der carbocyclischen Ketone vom Zehner- bis zum Achtzehnerring". *Helvetica Chimica Acta*. **1926**, 9, 249–264. doi:10.1002/hlca.19260090130.

Understanding the Role of Near-Cognate Binding in Ribosome Assembly by H/ACA snoRNPs

Asad Nasir *(Dr. Ute Kothe Group)*

One of the aspects of protein synthesis that remains understudied is ribosome biogenesis. The H/ACA class of snoRNPs in humans and yeast is known to modify uridine residues in ribosomal RNA (rRNA) to pseudouridine, which are essential rRNA stability and for efficiency of protein synthesis. Sites where the snoRNP binds and modifies the rRNA are known as cognate binding sites. However, it has recently been found that there are sites where the snoRNP will bind but not modify the rRNA, termed near-cognate binding sites. These near-cognate sites can compete for binding sites with their cognate counterparts, and thus decrease levels of pseudouridylation, but they may also assist in promoting correct rRNA folding. Currently, the function of near-cognate binding and its overarching role in ribosome biogenesis remains unclear. My project aims to characterize the differences in cognate and near-cognate binding with the hopes of possibly identifying novel therapeutics for targeting cancer and disease.



References

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Investigating the Enthalpic and Entropic Contribution of TMAO to the Folding of Paratox, an intrinsic disordered protein.

Ocean White *(Dr. Mazdak Khajehpour Group)*

Nature is a phenomenal process that thrives in threatening conditions. In stressful conditions (e.g., high denaturant concentration, changes in osmotic pressure), plants, animals and microorganisms often accumulate high concentration of crowders within their cells to enhance protein stability.[1] One such crowder found in cells of marine animals is trimethylamine N-oxide (TMAO) that allows sea creatures to increase their depth into the ocean without loss of protein functions; in other words, TMAO is found in high concentration to counteract osmotic stress found at low depths.[2,3]

The aim of this project is to study the effects of TMAO addition on the thermal stability of proteins, using paratox as our model system. Paratox was expressed in *Escherichia coli* and purified by his-tag affinity and size exclusion chromatography. Paratox was thermally denatured in various concentrations of TMAO and the secondary structure was studied using circular dichroism spectropolarimetry (CD). The significant contributions that TMAO has on protein folding will be deduced to an enthalpic or entropic effect.

References

1) Fonin, A. V.; Uversky, V. N.; Kuznetsova, I. M.; and Turoverov, K. K. Protein folding and stability in the presence of osmolytes. Molecular Biophysics. 2016, 61, 185-192. https://link.springer.com/article/10.1134/S0006350916020056

2) Velasquez, M. T.; Ramezani, A.; Manal, A.; and Raj, D. S. Trimethylamine N-Oxide: The Good, the Bad and the Unknown. Toxins [Online] 2016, 11, 326. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5127123/#B1-toxins-08-00326 (accessed March 16, 2024).

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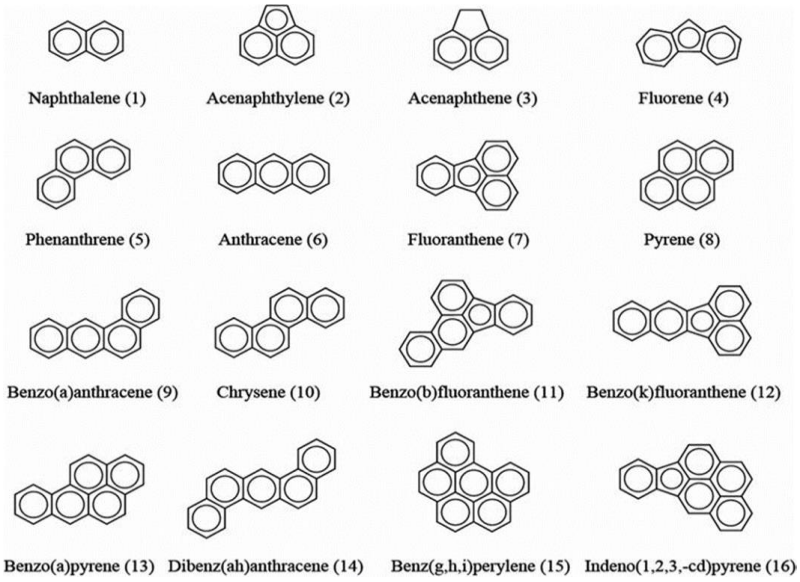
Migration of PAHs from food storage container to Foods: An Estimation of Human Exposure

Hoang Duc Luong *(Dr. Gregg Tomy Group)*

In the area of plastic overconsumption, investigating the potential risks posed by substances leaching from plastic containers, such as polycyclic aromatic hydrocarbons (PAHs), is crucial. This study aims to identify and quantify 16 priority PAHs that migrate from two types of plastics, namely Polycarbonate (PC) and Polyethylene Terephthalate Glycol (PETG), into food. Adhering to guidelines set by the United States Food and Drug Administration (US-FDA), a migration test was designed to simulate the release of PAHs into fatty food simulants under microwave heating conditions. Gas chromatography mass spectrometry (GC-MS) and a validated analytical method were employed to process the data and quantify various PAHs from the list of 16 prioritized ones. The resulting PAH levels were then utilized to estimate the daily human exposure through dietary intake.



References

(1) Zelinkova. Z & Wenzi. T (2015). The Occurrence of 16 EPA PAHs in food-A review.

(2) United State Food and Drug Administration (2007) Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances

Investigating the DusA residues involved in binding tRNA.

Nadia Hossain *(Dr. Ute Kothe Group)*

RNA molecules are essential molecules that are used in many different ways within living organisms. For example, tRNA molecules allow for the construction of proteins that are necessary in everyday biological function. RNA has over 150 different types of modifications that have been discovered, with tRNA molecules carrying the most modifications.[1] Dihydrouridine modifications involves the hydration of a double bond between the 5th and 6th carbon of uracil.[2] Lately, there has been an increase in significance placed on research related to dihydrouridine because of its link to cancer. It has been observed that there are elevated levels of dihydrouridine modifications in human tumour cells located in the lungs, liver, kidneys, prostate, and oral cavity when compared to healthy cells.[3] Following this observation, one of the enzymes in humans that is involved in creating the dihydrouridine modifications, hDus2, is also found to be overexpressing in cancer cells when compared to healthy cells.[2] Determining which residues are important in binding hDus2 to tRNA will allow us to determine how we could inhibit hDus2. In my research, I investigated DusA in *Escherichia coli* because it is a simpler model that is homologous to the hDus2 enzyme in humans. I focused on two separate DusA variants C114A and K153A, wherein the cysteine at position 114 and the lysine at position 153 is changed to an alanine. To be able to examine the binding between DusA and tRNA, I was first required to synthesize tRNA, which was done by performing an in vitro transcription. I also synthesized the enzymes ThiI and IscS which allowed us to thiolate the tRNA. After, I was able to fluorescently label the thiolated tRNA using the dye 5-IAF. Then, using a stopped flow assay, I determined that these residues in DusA are important for binding to tRNA. This result indicates that these residues in the homologous hDus2 may also be important in binding tRNA and may be good targets to use in order to inhibit hDus2 in cancer cells.

Figure 1: Depicts the general outline of preparation that occurred in order to get to stopped-flow assay experiments, starting with using the enzymes ThiI and IscS to add a thiol to the tRNA, then using the fluorescent dye 5-IAF to label the tRNA.

References

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Electrochemical Detection of Chloramphenicol Resistance in Yeast Cells

Brady Jason *(Dr. Sabine Kuss Group)*

Antimicrobial resistance has become one of the most dominant health challenges of our time. More than 1.2 million people died in 2019 as a direct result of antibiotic-resistant bacterial infections, according to the most comprehensive estimate to date of the global impact of antimicrobial resistance. If no effective action is taken, drug-resistant diseases are expected to cause 10 million deaths each year by 2050. *Saccharomyces cerevisiae* (*S. cerevisiae*) is a unicellular fungus vastly studied as a model for eukaryotic cells[1,2]. Some strains of *S. cerevisiae* can present a higher tolerance against chloramphenicol (CAP), however, the resistance mechanism presented by these cells against CAP remains unclear. The objective of the present work is to evaluate the resistance of these cells against CAP electrochemically. Voltametric studies were conducted to evaluate the retention of CAP in resistant and susceptible strains of *S. cerevisiae*. To detect CAP, its electrochemical properties were investigated by cyclic voltametric at a carbon glassy electrode in a three-electrode system.

References

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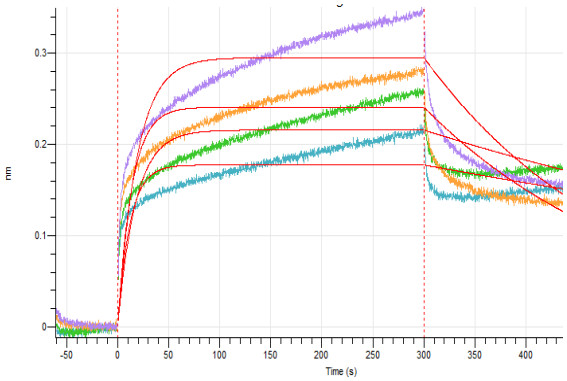
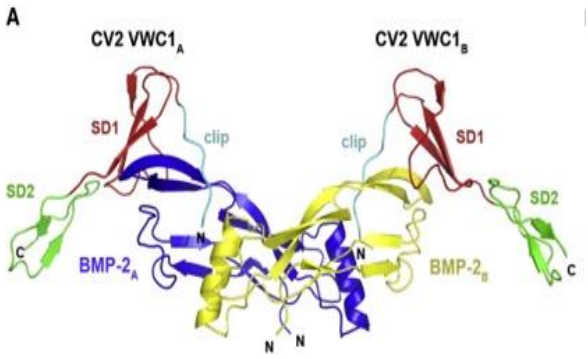
(2) Parapouli, M., Vasileiadis, A., Afendra, A. S. & Hatziloukas, E. *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiology*, 6, 1–31 (2020)

Molecular Interactions of the Extracellular Matrix Proteins Connective Tissue Growth Factor and Bone-Morphogenic Protein-2

Ahmed Zohni *(Dr. Jörg Stetefeld Group)*

Connective Tissue Growth Factor (CTGF) is a secreted protein involved in cellular proliferation, cellular migration and apoptosis. This protein, along with its numerous binding partners, mediate important physiological processes such as tissue repair and tumor suppression. Dysregulation of CTGF has been implicated in various fibrotic disorders and cancers. The protein is overexpressed in pancreatic cancer cell lines and its role in the pathogenesis of pulmonary fibrosis is well established. For those reasons, CTGF has been the target of therapeutic agents. One approach has been to use monoclonal antibodies to block the site through which CTGF binds one of its protein partners, bone morphogenic protein-2 (BMP-2). Following clinical trials, these antibodies were shown to be ineffective in treating cancerous cell lines. The following presentation discusses potential reasons behind the inability of the monoclonal antibody to prevent CTGF binding to BMP-2. The aim is to provide future direction for much needed development of therapeutic agents.





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Exploring Depsidone Synthesis: Insights into Grayanic Acid and Its Core Structure

Mercy Arowolo *(Dr. John Sorensen group)*

Depsidones are crucial compounds that possess antioxidant and antimicrobial properties[1]. The biosynthesis mechanisms of depsidones remain largely unknown[2]. In this presentation, I will focus on the exploration of novel synthesis routes for Grayanic acid, a representative depsidone, focusing on its core structure. This core structure serves as a key intermediate, enabling us to explore synthesis strategies like the Ullman reaction. This presentation will highlight the strategies employed, the hurdles faced, and the key findings that contribute to advancing our understanding of depsidone synthesis pathways.

References

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(2) Kealey, J. T.; Craig, J. P.; Barr, P. J. Identification of a Lichen Depside Polyketide Synthase Gene by Heterologous Expression in Saccharomyces Cerevisiae. *Metabolic Engineering Communications* **2021**, *13*, e00172. https://doi.org/10.1016/j.mec.2021.e00172.