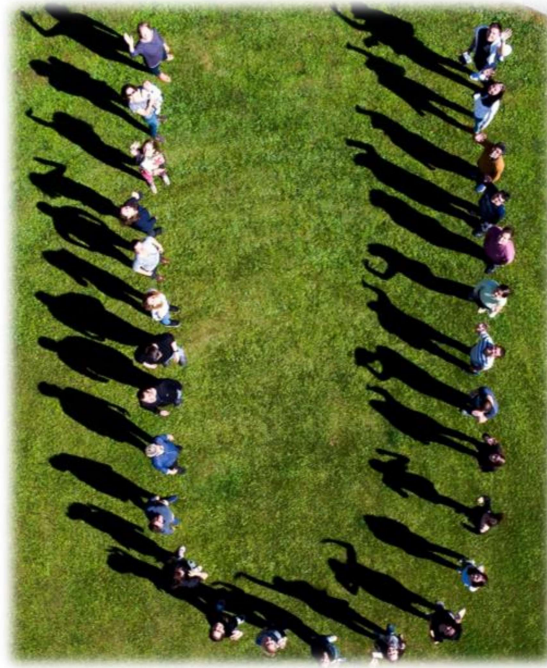


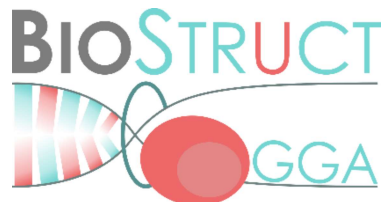
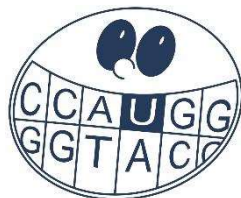
ANNUAL MEETING 2023

**GENOME METABOLISM RESEARCH GROUP
INSTITUTE OF MOLECULAR LIFE SCIENCES**



Mottó: "Csak akkor születtek nagy dolgok,
Ha bátrak voltak, akik mertek"

Motto: "Great things are only done when there are bold
Men that are venturesome, come to the fore"



Budapest

18-20 December 2023

STATEMENT OF MISSION

Similarly to the previous years, we decided to come together for these 3 days in order to synthesize our recent work and to tell other lab members what we achieved during the last year. This occasion provides an excellent opportunity to acquire a deeper understanding of the issues we are working on and to put them into a wider scientific context. Discussions after each presentation are expected to initiate fresh ideas and novel routes of successful project realization. The intensive 3-days training is also intended to enhance teamwork and knowledge transfer between lab members.

PRACTICAL INFORMATION

Presentations will cover the work performed during the last year in the lab.

Chairs are kindly requested to observe the time allowed for presentations (15 min) and lead the discussion sessions (10-15 min).

Everybody is fully expected to take active part in the discussions.

Venue: HUN-REN TTK Research Centre for Natural Sciences, Budapest 1117, Magyar tudósok krt 2.

PARTICIPANTS

Angéla Békési

András Benedek

Nikolett Emődi

Márk Hegedüs

Eszter Holub

Richard Izrael

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Dániel Molnár

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András Telek

Judit Tóth

Otília Tóth

Zoé Tóth

Rita Váradiné Hirmondó

Beáta G. Vértessy

PROGRAMME

Monday, 18 December, 2023

9:00 Morning coffee

Session 1: Potential role of mycobacterial proteins

Chair: Gergely Nándor Nagy

9:30 **Hirmondó Váradiné Rita:** Do Mycobacteria really have no actin?
Characterization of a potential Mbl protein

10:00 **Dániel Molnár:** The AdnA-dUTPase story: results and perspectives

10:30 **Dániel Molnár:** *In situ* captured antimicrobial action of membrane-incising peptide lamelle

11:00 Coffee break

Session 2: Application of enzymes in biocatalysis

Chair: Beáta G. Vértessy

11:30 **András Telek:** Baby steps towards discovering and engineering industrial biocatalysts

12:00 **András Benedek:** An enzyme library for a sustainable pharmaceutical chemistry

12:30-14:00 Lunch break

Session 3: Moonlighting function of dUTPase in eukaryotic cells

Chair: Rita Váradiné Hirmondó

14:00 **Otília Tóth:** Investigation the role of the dUTPase enzyme during mitosis

14:30 **Nikolett Nagy:** Investigation of the physiological role of the dUTPase enzyme

Tuesday, 19 December, 2023

9:00 Morning coffee

Session 4: Investigation of protein interactions

Chair: Angéla Békési

9:30 **Zoé Tóth:** Mycobacteria-specific loop shields *M. tuberculosis* dUTPase from being efficiently inhibited by tailored form of inhibitor protein

10:00 **Nikolett Emődi:** Investigating the activity and interactions of oncogenic KRas proteins

10:30 **Gergely Nándor Nagy:** Understanding the sulfation specificity of heparan sulfate glycosaminoglycans: NDST3 characterization project overview

11:00 Coffee break

Session 5: Role of genomic uracil in *Plasmodium falciparum*

Chair: András Benedek

11:30 **Richard Izrael:** Role of uracil in the antigenic variation and genetic diversity of the malaria parasite, *Plasmodium falciparum*

12:00 **Márk Hegedüs:** Investigation of the presence and formation of genomic uracil in the malaria pathogen, *Plasmodium falciparum* strain

12:30-14:00 Lunch break

Wednesday, 20 December, 2023

9:00 Morning coffee

Session 6: Effects of drug-induced uracilation and the role of uracil in embryonic development

Chair: Judit Tóth

9:30 **Angéla Békési:** Pathways affected by thymidylate synthase inhibitors in HCT116 cells: genomic uracilation, transcription changes and post-transcriptional regulation by TS

10:00 **Eszter Holub:** Potential roles of APOBEC3 DNA cytosine deaminases in drug induced mutagenesis and splicing

10:30 **Viktória Perey-Simon:** Role and effect of uracil metabolism on zebrafish embryonic development

11:00 Coffee break

Session 7: Amylase project and closing remarks

11:30 **Askar Kholikov:** Cloning and expression of thermostable alpha-Amylase gene

12:00 **Beáta G. Vértessy:** Closing remarks

12:30-14:00 Lunch break

14:00 **Laboratory Christmas celebration**

Do Mycobacteria really have no actin?

Characterization of a potential Mbl protein

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In rod shaped bacteria, MreB family proteins were shown to form helical actin-like cables that could comprise cytoskeletal elements with a direct role in cell shape determination ¹. Interestingly, the rod shaped mycobacteria have no MreB system identified, and therefore, the exact mechanism of controlling cell shape is not yet known. In contrast to most rod-shaped bacteria, the deposition of newly synthesised peptidoglycan in mycobacteria seems to be restricted to the poles ^{2,3}. Interestingly, we found that the cell wall metabolism enzyme RmlA, acting to crosslink of peptidoglycane and arabinogalactane layers of the cell wall localizes in a helical pattern at the cylindrical part of the cell as well as at the poles ⁴. Other proteins involved in PG synthesis (CwsA, MurG, GlfT2, Pks13, e.g.) are also not just found at the poles, but distributed along the lateral cell body ^{5,6}. These data indicate that crosslinking the nascent layers or even cell wall biosynthesis may not be restricted exclusively to the cell poles. However, it is thought so far that the laterally localized synthetic enzymes are not active in the absence of a multiprotein complex, recruited by a cytoskeletal scaffold, such as FtsZ or MreB ⁷. A possible explanation how mycobacteria grow and what governs cell shape, is that the functionally inert lateral walls guides the width of the bacterium. However, it is contradictory to this passive cell-shape-maintenance theory, that spheroplasts formed in *M. smegmatis* through degradation of PG are able to regenerate wild-type rod-shaped cells without guidance from a preexisting cylindrical cell wall ⁸. Recent evidence suggests that FtsZ also forms dynamic helices in bacteria besides Z-ring formation ⁹. Alternatively, there may be actin-like proteins encoded by mycobacteria that have not yet been described, due to a lack of sequence homology ⁷.

Therefore, to establish these concerns and how mycobacteria retain their cell shape, we planned several different approaches. With extensive homology searching for already known bacterial cytoskeletal elements (mainly actin homologs, like MreB and Mbl), we found two homologs of *B. subtilis* Mbl. We would like to investigate the localization, the effect of KO and *in vitro* polymerization capacity of these candidates. We will also follow a more indirect line of investigating cell shape maintenance in *Mycobacteria* by inhibiting and staining bacterial actins.

- 1 Jones, L. J. F. *et al. Cell* 104, 913–922 (2001)
- 2 Daniel, R. A. *et al. Cell* 113, 767–776 (2003)
- 3 Thanky, N. R. *et al. Tuberculosis* 87, 231–236 (2007)
- 4 Hirmondó, R. *et al. PLoS One* 17, (2022)
- 5 Plocinski, P. *et al. J. Bacteriol.* 194, 6398–6409 (2012)
- 6 Meniche, X. *et al. Proc. Natl. Acad. Sci. U. S. A.* 111, (2014)
- 7 Hett, E. C. *et al. Microbiol. Mol. Biol. Rev.* 72, 126–156 (2008)
- 8 Melzer, E. S. *et al. Cytoskeleton* 75, 498–507 (2018)
- 9 Thanedar, S. *et al. Curr. Biol.* 14, 1167–1173 (2004)

The AdnA-dUTPase story: results and perspectives

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Tuberculosis is still one of the top 10 causes of death worldwide in spite of century-long efforts to combat it. In addition, multidrug-resistant tuberculosis also denotes a public health crisis. In the host, as an intracellular pathogen, TB bacilli faces harsh conditions and various types of drugs during treatment. Therefore, *Mycobacteria* has a specific set of DNA repair enzymes to avoid DNA damage. In *Mycobacteria*, homologous recombination driven repair processes have particularly important role in genome integrity maintenance. End resection of DNA double strand breaks (DSBs) as the starting step of homologous recombination is one of the most essential DNA repair mechanisms in every organism. This motor driven process is usually provided in prokaryotes either by an *E. coli*-type RecBCD, or by a *Bacillus*-type AddA helicase nuclease complex. However *Mycobacteria* have RecBCD encoded in their genome, it is suggested to be involved rather in single strand annealing double strand break repair pathway, than homologous recombination. As an alternative pathway for end resection, a novel mycobacterial heterodimeric helicase nuclease complex, AdnAB (ATP dependent nuclease) was implicated in homologous recombination. AdnAB is a heterodimeric DSB end resecting machine with distinctive properties compared to RecBCD or AddAB. Each AdnA and AdnB subunits contain an N-terminal UvrD like helicase domain, and a C-terminal nuclease domain, but functionally the two domains differ. AdnAB has an ATP dependent, and independent nuclease activity, and the capability to act as a molecular ruler.

Interestingly, our preliminary results indicates that dUTPase, an enzyme with nucleotide pool sanitizing function, may interact with AdnA, the main helicase subunit, participating in HR.

To reveal the moonlighting function of the dUTPase enzyme in *Mycobacteria*, we investigated this interaction by several aspects. We established an in vivo system comprising specific CRISPR cleavage on a fluorescent reporter. By analyzing fluorescence changes (signal intensity, wavelength) we can deduce the mode of DNA repair and its effectiveness in different mutant strains.

To confirm the interaction in vivo, we used mycobacterial protein fragment complementation assays. We also successfully managed to express and purify the AdnAB complex, and now we are performing experiments to validate and further investigate the dUTPase interactions using a multitude of methods.

1. K. M. Sinha, M.-C. Unciuleac, M. S. Glickman, S. Shuman, *Genes & Development*, **23**, (2009), 1423.

2. N. Jia, M. C. Unciuleac, C. Xue, E. C. Greene, D. J. Patel, S. Shuman, *PNAS*, **116**, (2019), 24507.

3. M. C. Unciuleac, A. Meir, C. Xue, G. M. Warren, G. C. Greene, S. Shuman, *PNAS*, **118** (2021), e2023955118.

***In situ* captured antimicrobial action of membrane-incising peptide lamelle**

K. el Battioui¹, S. Chakraborty¹, A. Wacha¹, D. Molnár^{2, 3*}, M. Quemé-Peña¹, I. Cs. Szigyártó¹, Cs. L. Szabó¹, A. Bodor¹, K. Horváti¹, G. Gyulai¹, Sz. Bősze¹, J. Mihály¹, B. Jezsó¹, L. Románszki¹, J. Tóth², Z. Varga¹, I. Mándity¹, T. Juhász¹ T. Beke- Somfaia¹

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New compounds with unique mechanisms of action are needed to combat the growing issue of antimicrobial resistance. Supramolecular assemblies, which combine the complex membrane attacking mechanisms of natural host defense peptides with the improved biostability of non-natural compounds offer a promising alternative to current small molecule antibiotics. However, for such membrane-targeting compounds the direct visual insight on the toxic agents in bacteria is still lacking. To this end, we employed a design strategy focusing on an inducible assembly mechanism and utilized electron microscopy (EM) to follow the formation of supramolecular peptide structures triggered by bacterial cell surface lipopolysaccharides (LPS). Inspired by the alternating chirality backbone pattern of some effective peptide antimicrobials, we designed lysine-rich heterochiral β 3-peptides, termed lamellin-2K and lamellin-3K, with optimal residual spacing for enhanced coordination on the phosphate groups of LPS. Combined molecular dynamics simulations (MD), EM and bacterial assays confirmed that the phosphate-induced conformational change of these lamellins led to the formation of thin, striped lamellar layers, where each stripe represents double arrays of H-bonded peptide molecules that are interconnected by phosphate ions. EM micrographs of Gram-negative bacteria show that the lamellae incised the cell envelope, while leakage and antibacterial activity assays prove that growth inhibition starts already at submicromolar concentrations. Detailed image analysis demonstrated that the lamellae penetrating deep into the bacterial cell have a rather uniform size distribution and, surprisingly, only a few of these supramolecules are sufficient to cause major cell wall damage making them efficient in destroying target cells. Our findings also provide a missing mechanistic link for membrane-targeting agents, connecting how the antibiotic mechanism is built up from individual molecules through on-site formation of the active supramolecules that lead to bactericidal activity.

I. G. N. Tew R. W. Scott, M.L. Klein, W. F. DeGrado, *Acc Chem. Res.*, **43**, (2010), 30.

Baby steps towards discovering and engineering industrial biocatalysts

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Biocatalysis has become a key technology in the chemical industry by enabling green processes yielding high-value compounds[1]. The development of bioinformatics and molecular biology tools in the past decades enables rapid discovery and engineering of potent biocatalysts. However, the implementation of biocatalysis in the industry requires specialized knowledge and a highly interdisciplinary mindset to reach its full potential. In this presentation I highlight how an academic-industrial collaboration can be beneficial for such an implementation process. Servier set up collaboration with our group to widen its biocatalysis portfolio towards novel enzymes and enzyme classes. This project focuses on enzymatic reductive aminations that is an emerging target reaction for biocatalysis especially in the pharmaceutical industry[2]. The research goes to two main directions: discovery and engineering.

On the discovery side, a further collaboration with a bioinformatics group resulted in the characterization of 6 novel opine dehydrogenases (ODHs)[3] from hot spring metagenomes. These enzymes have unprecedented substrate scope and high thermostability that makes them a valuable addition to the reductive amination toolbox. Moreover, key positions determining amino acid substrate specificity has been discovered by structural analysis and site-directed mutagenesis[4]. This result sets ground for future engineering of these enzymes.

For the industrial application natural enzymes often have to be engineered to achieve sufficient activity or to be able to withstand harsh process conditions. In this project we develop a cell-free workflow for mutagenesis and enzyme expression to allow fast production and screening of small variant libraries even in a chemistry lab. The first results including mutant design and parallel mutagenesis is presented on the example of a ketimine reductase enzyme (KIREN) which could be engineered for the biocatalytic synthesis of a key intermediate of several ACE2 inhibitor drugs.

The results of this collaborative project exemplify the potential of academic-industrial collaborations to bring together and build up essential know-how for the implementation of new enabling technologies for a greener chemical industry.

1. Buller R, Lutz S, Kazlauskas RJ, et al (2023) From nature to industry: Harnessing enzymes for biocatalysis. *Science* (80-) 382:eadh8615. <https://doi.org/10.1126/science.adh8615>
2. Gilio AK, Thorpe TW, Turner N, Grogan G (2022) Reductive aminations by imine reductases: from milligrams to tons. *Chem Sci* 13:4697–4713. <https://doi.org/10.1039/D2SC00124A>
3. Telek A, Molnár Z, Vértessy BG, Tasnádi G (2023) Opine dehydrogenases, an underexplored enzyme family for the enzymatic synthesis of chiral amines. *Biotechnol Bioeng* 120:2793–2808. <https://doi.org/10.1002/bit.28469>
4. Telek A, Molnár Z, Takács K, et al Discovery and biocatalytic characterization of opine dehydrogenases by metagenome mining. *Appl Microbiol Biotechnol* In Press:

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An enzyme library for a sustainable pharmaceutical chemistry

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Amide bond formation is the most heavily utilized transformation in the pharmaceutical sector [1]. Given so it is rather surprising that the widely used amide bond synthesis methods suffer from low efficiency and poor sustainability, especially at larger scales. Although a wide range of coupling reagents have been developed, toxicity, high cost and low atomic efficiency of amide coupling reactions has remained a central problem. Therefore, development of more sustainable catalytic systems would be urgently needed [2].

A promising solution for greener amide bond synthesis would be to utilize enzymes to catalyze the coupling reaction. Amide-bond synthetases (ABSs) are ATP-dependent enzymes being able to accept an amine directly and couple it with acyl-AMP which is an activated form of carboxyl group for the amine attack. These enzymes are able to catalyse amide coupling in water-based solutions under mild reaction conditions (e.g. 30 °C at pH 7.5).

In pharmaceutical synthesis a wide range of carboxylic acid and amine substrates are needed to be coupled. Finding a sole ABS enzyme to couple any of these substrates with considerable efficiency is a real challenge. Our aim is to explore new ABS enzymes from sequence databases and test them for several pharmaceutically important substrates, establishing an enzyme library standing ready to couple the desired amine-acid substrate pairs with regio- and stereoselective specificity. We hope that our results will boost the application of ABSs on a broad range of reactions, thereby significantly reducing the ecological footprint of pharmaceutical industry.

[1] *J. Med. Chem.* **59**, 4385–4402 (2016)

[2] *Nat Catal* **2**, 10–17 (2019)

[3] *Nature* **593**, 391–398 (2021)

Investigation the role of the dUTPase enzyme during mitosis

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The dUTPase enzyme has an important role in nucleotide metabolism by hydrolyzing dUTP into dUMP and pyrophosphate [1]. Through the catalytic activity of the dUTPase enzyme it decreases the dUTP/dTTP ratio in the cells. If the dUTP/dTTP level is high, dUTP can be incorporated into the DNA. Cytosine deamination is also resulted in uracil which leads to mutations. Therefore, uracil bases are excised via the base excision repair (BER) mechanism. As the genomic uracil level increase in the DNA, the overactivation of the BER leads to single- and double-strand breaks and causes cell death. In human cells it has been recently described that dUTPase has four isoforms [2], and the nuclear isoform has the most abundant expression. Knock-out of the *Dut* gene causes embryonic lethality [3]. Since the *Dut* knock-out embryos die in the blastocyst stage during embryonic development and the meiotic-mitotic transition occurs in the blastocyst stage [4], this phenomenon suggests that dUTPase may have an important and yet unknown role in mitosis and development. Using yeast two hybrid technique, the interacting proteins of human dUTPase have been identified and the results contain several proteins which participate in mitosis. Furthermore, it has been shown, that the dUTPase enzyme is phosphorylated by Cdk1 and translocates to the cytosol in mitosis [5]. Using α -tubulin and dUTPase immunohistochemical staining, we investigated the localisation of the dUTPase enzyme during the different stages of mitosis in mouse embryonic fibroblast (MEF), human embryonic kidney (HEK293T) and human colorectal carcinoma (HCT116) cell lines. We found that the dUTPase localises around the mitotic spindle in metaphase, while during telophase and cytokinesis it presents in the midbody. Furthermore we optimized a co-immunoprecipitation method for identification of the interaction partners of the dUTPase. The localisation pattern and the previous results all suggest that the dUTPase enzyme may has a potential moonlighting role in cell division.

1. Vértessy BG, Tóth J., *Acc. Chem. Res.*, 2009, doi: 10.1021/ar800114w
2. Rácz GA, Nagy N, Vértessy BG. et al., *Sci. Rep.*, 2023, doi: 10.1038/s41598-023-32970-1
3. Pálkás HL, Rácz GA, Vértessy BG. et al, *Biomolecules*, 2019, doi: 10.3390/biom9040136
4. Aurélien C, Melina S, Jan E, Takashi H, *J. Cell. Biol.*, 2012, doi: 10.1083/jcb.201202135
5. Róna G, Vértessy BG. et al., *Acta Cryst. D. Biol. Crystallogr.*, 2013, doi: 10.1107/S0907444913023354

Investigation of the physiological role of the dUTPase enzyme

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Maintaining the genomic integrity is of utmost importance therefore preventive repair is essential. Accordingly, dUTPase enzyme has a prominent role by the hydrolysis of dUTP to dUMP [1]. The dUTP nucleotide can be incorporated into the DNA if the dUTP/dTTP ratio is high in the cells. Uracil bases are excised from the DNA by the UDG enzyme family members UNG and SMUG1 glycosylases which are part of the base excision repair mechanism (BER). As the amount of the excised uracil bases grows in the DNA, it generates a recurring cycle causing single- and double-stranded DNA breaks and can lead cell death. Interestingly, the *Ung* and *Smug1* knockout mice are viable and fertile [2]. However, knockout of the dUTPase with CRISPR/Cas9 in mice leads to early embryonic lethality [3].

To consider the importance of dUTPase in cellular physiology, it is paramount to address the essential function of the enzyme, therefore our aim is to use the CRISPR base editing technology to create a catalytically inactive enzyme in mouse embryonic fibroblast (MEF) cell lines and mice as well. The inactive mutant is unable to coordinate the catalytic water for the hydrolysis of the dUTP because of the change of the conserved aspartic acid to an asparagine on the amino acid level (D100N) [4]. To date, we have successfully created heterozygous and inactive cell lines using *Ung* *-/-* *Smug1* *-/-* MEF cells. We determined the genomic uracil level of the inactive mutant cell lines and found that the uracil level highly increased in both cases, however the 200 μ M thymidine supplementation decreased the genomic uracil level in the heterozygous cells but not in the inactive cells. Additionally, we observed that the proliferation rate is decreased in the inactive dUTPase mutant cells. We also measured the dUTPase and thymidylate synthase protein expression with western blot and found that in the *Ung* *-/-* *Smug1* *-/-* MEF cells both dUTPase and thymidylate synthase expression is elevated, while in the inactive mutants dUTPase expression is decreased. Moreover, we characterised the localisation of dUTPase with immunocytochemistry. Normally, dUTPase localises to the nucleus, but in the inactive mutant cells the nuclear localisation is altered. As the inactive dUTPase mutant cells are viable with high genomic uracil content, our results suggest that the catalytic activity is not the essential function of the dUTPase enzyme, but it may have a moonlighting function.

1. Ladner, R.D. and S.J. Caradonna, *The Journal of Biological Chemistry* (1997)

2. Alsøe, L., Nilsen H., et al., *Sci Rep* (2017)

3. Palinkas, H.L., et al., *Biomolecules* (2019)

4. Barabás, O., et al., *The Journal of Biological Chemistry* (2004)

Mycobacteria-specific loop shields *M. tuberculosis* dUTPase from being efficiently inhibited by tailored form of inhibitor protein

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Maintaining adequate intracellular dNTP levels is pivotal for preserving genomic stability (1). One of the enzymes responsible for regulating dUTP/dTTP levels is dUTPase, which hydrolyses dUTP, preventing uracil from being incorporated into DNA instead of thymine. Diminished activity of this enzyme can disrupt the balance of dUTP/dTTP levels, which may indirectly lead to the hyperactivation of the base excision repair mechanism, DNA double-strand breaks, and cell death (2). The design of species-specific dUTPase inhibitor could help in defence against human pathogens, like *Mycobacterium tuberculosis*, as the emergence of multidrug-resistant strains causes an increasing problem in the treatment of tuberculosis (3). The enzyme dUTPase has been proved to be essential in *M. smegmatis*, a laboratory model organism of *M. tuberculosis*. The mycobacterial dUTPase has a short mycobacteria-specific surface loop region, the presence of which is still essential for bacterial survival (4). A known dUTPase inhibitor, the staphylococcal protein Stl strongly inhibits the *Mycobacterium tuberculosis* dUTPase (MtDUT) (5, 6). The understanding of their interaction (7) may help in the design of efficient MtDUT inhibitors.

For this reason, we have crystallized the complex of MtDUT and a truncated Stl protein variant, Stl¹⁻¹⁵⁹ and the loop-lacking enzyme variant MtDUT^{ΔA133-S137}. Bio-layer interferometry (BLI) measurements were performed in favour of the analysis of the protein-protein binding kinetics. In order to examine whether shorter, truncated version of Stl possesses a more effective inhibitory effect on MtDUT activity than the full-length protein, we tested the effect of Stl¹⁻¹⁵⁹ by steady-state activity measurements. Furthermore, the formation of protein-protein complexes was studied by mass photometry, mass spectrometry, and hydrogen-deuterium exchange mass spectrometry methods.

According to the BLI and the steady-state activity measurement results the full-length Stl is a high affinity inhibitor of MtDUT, and it inhibits enzyme activity more effectively than the Stl¹⁻¹⁵⁹. However, the inhibition of MtDUT by the full-length Stl is still not complete, which is presumably due to the formation of a 3:2 complex in addition to the 3:3 binding stoichiometry during MtDUT-Stl complex formation. Structural analysis of MtDUT^{ΔA133-S137} revealed that the loop is likely to play a role in the fixation of the C-terminal arm of the enzyme. In the absence of the loop, the enzyme is similarly inhibited by the full-length and truncated Stl proteins. Thus, the C-terminal arm of the wild-type enzyme is less inhibited by the short Stl protein due to its greater stability.

1. B. A. Kunz, S. E. Kohalmi, T. A. Kunkel, C. K. Mathews, E. M. McIntosh, J. A. Reidy, *Mutat. Res.* **318**, 1–64 (1994).
2. B. G. Vértessy, J. Tóth, *Acc. Chem. Res.* **42**, 97–106 (2009).
3. J. Heyckendorf, C. Lange, J. Martensen, in *Emerging Infectious Diseases* (Elsevier, 2014), pp. 239–253.
4. I. Pecsí, R. Hirmondo, A. C. Brown, A. Lopata, T. Parish, B. G. Vértessy, J. Tóth, *PLoS ONE*. **7**, e37461 (2012).
5. E. Maiques, N. Quiles-Puchalt, J. Donderis, J. R. Ciges-Tomas, C. Alite, J. Z. Bowring, S. Humphrey, J. R. Penadés, A. Marina, *Nucleic Acids Res.* **44**, 5457–5469 (2016).
6. R. Hirmondó, J. E. Szabó, K. Nyíri, S. Tarjányi, P. Dobrotka, J. Tóth, B. G. Vértessy, *DNA Repair (Amst)*. **30**, 21–27 (2015).
7. C. Sanz-Frasquet, J. R. Ciges-Tomas, C. Alite, J. R. Penadés, A. Marina, T, *Microbiol. Spectr.* **11**, e0323222 (2023).

Investigating the activity and interactions of oncogenic KRas proteins

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Mutations in the Ras protein family are responsible for one of the highest prevalence in cancer [1]. Most commonly affecting the pancreas, rectum, colon and lung, these lesions remain a challenge for medical science today.

The KRas protein, encoded by the Ras gene, plays a central role in regulating signaling pathways. This enzyme can control signaling as a binary switch through its GTPase activity, thereby affecting cell proliferation and division. The function of the KRas protein is facilitated by GAP (GTPase activating protein) proteins, which can increase the rate of GTP hydrolysis by several times. However, this finely tuned system can easily be disrupted by mutations in the active site of the KRas protein. Just one change in an amino acid weakens the interaction between KRas-GAP proteins, therefore the rate of hydrolysis is significantly reduced, resulting that the protein being unable to fulfil its role in the signaling network. This inhibits the proper functioning of cellular processes, leading to uncontrolled cell division and to the development of tumors.

Several approaches have been developed to restore the signaling, the most common one is drug treatment with inhibitor molecules. However, the restoration of the correct interaction between mutant KRas and GAP proteins holds promise too.

Despite being an important target, the mechanism of GAP-catalysed hydrolysis has not been precisely defined yet. To understand this, our collaborators have set up a model to systematically design GAP mutants that can activate the KRas G12D mutant, which is the most abundant KRas mutant in the human body [2]. In addition, researchers have recently discovered a GAP-like protein, RGS3, which can also cause a catalytic effect on mutant KRas proteins [3].

My aim is to test the GAP proteins in practice. For this, I investigated the interaction between the mutant KRas and GAP variants by using biolayer interferometry. Also, I measured the helper proteins' ability to activate KRas mutants by using MESG-PNP enzyme activity assay. These studies may help to understand the interaction of these proteins and thus improve the development of tumor therapy methods.

- 1 A. R. Moore, S. C. Rosenberg, F. McCormick, S. Malek RAS-targeted therapies: is the undruggable drugged? *Nat Rev Drug Discov.* 2020 Aug;19(8):533-552.
- 2 D. Berta, S. Gehrke, K. Nyíri, B. G. Vértessy, E. Rosta Mechanism-Based Redesign of GAP to Activate Oncogenic Ras *J. Am. Chem. Soc.* **2023**, 145, 37, 20302–20310
- 3 C. Li, A. Vides, D. Kim, J. Y. Xue, Y. Zhao, P. Lito The G protein signaling regulator RGS3 enhances the GTPase activity of KRAS *Science* **2021** Oct 8;374(6564):197-201

Understanding the sulfation specificity of heparan sulfate glycosaminoglycans: NDST3 characterization project overview

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Heparan sulfate (HS) glycosaminoglycans (GAGs) are major components of the extracellular matrix (EM) with diverse biological functions, including cell signalling and communication, connective tissue integrity, and host-pathogen interactions[1]. Heparin, a form of HS, is an essential anticoagulant medicine. HS biosynthesis enzymes work together in ways yet poorly understood to assemble and decorate GAG polysaccharide chains leading to great structural and functional diversity[2]. The bifunctional N-deacetylase/N-sulfotransferase (NDST) enzymes catalyze the first steps toward the formation of HS N-sulfated domains. Preliminary results from our collaborators (Rebecca Miller, Henrik Clausen) indicate that the variability of the HS sulfation patterns is grounded in the various specificities of the NDST isoenzymes. However, the mechanism of how these specificities are brought about remains elusive. Our project intends to fill this gap in knowledge with a multidisciplinary approach to study the modification mechanism of HS GAG polysaccharide substrates by NDST3. Our project has three overarching objectives: 1) to address the functional role and cooperation of NDST3 enzyme domains; 2) to decipher how the molecular structure defines the specificity of NDST3 and 3) to dissect how NDST3-induced HS modifications modulate the overall HS epitope diversity.

NDST3 is a large, ca. 100 kDa sized glycoprotein that we could not produce by recombinant expression in *E. coli*. Therefore, we adopted a divide and conquer approach for enzyme investigations and started to engineer individual domains of this multi-domain enzyme. We managed to produce the NDST3 sulfotransferase domain in a maltose binding protein (MBP) fusion construct. Construct design and/or purification protocol needs further refinement to improve protein solubility after removal of MBP.

Building on our initial efforts we will pursue the following research directions in 2024.

For NDST3 enzyme activity and specificity characterization, we will produce separate sulfotransferase and deacetylase enzyme forms in *E. coli*, and assess their Michaelis-Menten enzyme kinetics using colorimetric assays. These constructs will be also shipped to our collaborators in Copenhagen for analysis of the patterns of enzyme modifications along heparan sulfate chains using state-of-the-art glycomics techniques [3,4].

Towards structural studies we will optimize NDST3 constructs for stability and protein crystallization and follow-up on promising results. Alternatively, as a rescue strategy we will use homology modelling to test residues and segment with an expected prominent role in enzyme specificity.

1. Esko, J. D. & Lindahl, U. *J Clin Invest* **108** (2001) 169-173, (2001).
2. Annaval T, Wild R, Créton Y, Sadir R, Vivès RR, Lortat-Jacob H. *Molecules*. (2020) Sep 14;25(18):4215.
3. Karlsson, R., Chopra, P., Joshi, A., Yang, Z., Vakhrushev, S.Y., Clausen, T.M., Painter, C.D., Szekeres, G.P., Chen, Y.H., Sandoval, D.R., et al. Dissecting structure-function of 3-O-sulfated heparin and engineered heparan sulfates. *Sci Adv* 7,(2021) eabl6026.
4. Miller, R.L., Guimond, S.E., Schworer, R., Zubkova, O.V., Tyler, P.C., Xu, Y., Liu, J., Chopra, P., Boons, G.J., Grabarics, M., et al. *Nat Commun* **11** (2020) 1481L.

Role of uracil in the antigenic variation and genetic diversity of the malaria parasite, *Plasmodium falciparum*

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Malarial infections threaten hundreds of millions of people yearly despite the immense effort to eradicate the disease in the past decades. The main underlying reason is the unparalleled genetic diversity of the malaria parasite *Plasmodium falciparum*, the main causative agent of lethal malarial infections. The quick emergence of resistance against different antimalarial drugs and the antigenic variation to evade the immune system makes it challenging pathogen to eliminate.

Here, we focus on understanding the role of the non-canonical base uracil in the genomic integrity of *P. falciparum*. Previously, we have demonstrated elevated levels of genomic uracil¹, which begs the question of how and why it is present in the genome despite a working DNA repair machinery. To elucidate its role, we have established a CRISPR-Cas9-based conditional knock-out system² to examine the role of *Pf*UNG and base excision repair on the genetic variability. We characterized the impact of *Pf*UNG knock-out on the cell physiology of *P. falciparum* parasites. Additionally, to investigate the genomic distribution of uracil, we utilized a DIP-Seq-like method that has been developed by our laboratory³. The method noted as U-DNA-Seq exploits a catalytically inactive uracil DNA glycosylase (UNG), the primary protein in base excision repair that recognizes uracil in the genome. Using this sensor to pull down uracilated DNA fragments, we generated next-generation sequencing data to determine uracil-enrichment and to explore its genomic distribution.

Our results indicate an interesting role of uracil in antigenic variation. The genomic data showed an unusually high and structured enrichment in the main variable antigenic families of *var*, *rif* and *stevor* genes alike. In this study, we dissected the different aspects how and why uracil is incorporated in such a distinct manner, during which we identified a putative cytidine deaminase complex that can have a prominent role in the observed uracil enrichment.

1. Molnár, P., Marton, L., Izrael, R., Pálincás, H. L. & Vértessy, B. G. Uracil moieties in *Plasmodium falciparum* genomic DNA. *FEBS Open Bio* **8**, 1763–1772 (2018).
2. Knuepfer, E., Napiorkowska, M., van Ooij, C. & Holder, A. A. Generating conditional gene knockouts in *Plasmodium* – a toolkit to produce stable DiCre recombinase-expressing parasite lines using CRISPR/Cas9. *Sci Rep* **7**, 3881 (2017).
3. Pálincás, H. L. *et al.* Genome-wide alterations of uracil distribution patterns in human DNA upon chemotherapeutic treatments. *Elife* **9**, 1–37 (2020).

Investigation of the presence and formation of genomic uracil in the malaria pathogen, *Plasmodium falciparum* strain

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Plasmodium falciparum is one of the most dangerous pathogens causing malaria, resulting in the death of hundreds of thousands of people worldwide each year. This unicellular parasite possesses a complex life cycle typical of *Apicomplexa* protist species. Additionally, the parasite's genome exhibits unique characteristics. One of its most defining features is an extremely high AT content, making it one of the most distinct base pair compositions among eukaryotes¹. Due to these genomic traits, researchers have concluded that the high AT content could be the result of an evolutionary mutational bias, potentially providing the parasite an adaptive evolutionary advantage, which could significantly contribute to the genetic diversity of the parasite.

To elaborate the mechanism of the AT mutation bias, we focused on the major contributor of such base pair mutations, the presence of uracil. The accumulation of uracil moieties in the genome has been previously identified via dot-blot based genomic uracil quantification². We investigated the distribution of uracil using the U-DNA sequencing method³, revealing that *var* genes in the parasite contain significantly higher levels of uracil, which genes play a crucial role in immune evasion. The incorporation of uracil into the genome can occur through multiple pathways in eukaryotes. In the present work, we have focused on investigating the oxidative deamination of cytosine, with an emphasis on a putative cytosine deaminase enzyme complex. Firstly, we have identified two enzymes with potential cytosine deaminase activity through homology search and AlphaFold modelling (PF3D7_1349400; PF3D7_1204600). Based on the modelling results, the mentioned proteins form a complex and exhibit a high degree of similarity to other enzymes described in the literature as adenosine deaminases acting on tRNA (ADAT). Therefore, we named the identified proteins as *PfADAT2* and *PfADAT3* respectively and the complex as *PfADAT2/3*. Previously ADAT enzymes have been shown to bear a cytosine deaminase activity not only on tRNA, but in the context of DNA as well⁴. In our experiments, we examined whether the *PfADAT2/3* complex identified *in silico* exhibited similar activity *in vitro*, potentially explaining the uracilation in the malaria parasite. Furthermore, we tried to characterize the DNA substrate preference of the enzyme complex to further understand what drives uracilation into the *var* gene loci.

1. Hamilton, W. L. *et al.* Extreme mutation bias and high AT content in *Plasmodium falciparum*. *Nucleic Acids Res* **45**, 1889 (2017).
2. Molnár, P., Marton, L., Izrael, R., Pálkás, H. L. & Vértessy, B. G. Uracil moieties in *Plasmodium falciparum* genomic DNA. *FEBS Open Bio* **8**, 1763 (2018).
3. Pálkás, H. L. *et al.* Genome-wide alterations of uracil distribution patterns in human DNA upon chemotherapeutic treatments. *Elife* **9**, 1–37 (2020).
4. Gao, Z. *et al.* Amphioxus adenosine-to-inosine tRNA-editing enzyme that can perform C-to-U and A-to-I deamination of DNA. *Commun Biol* **6**, (2023).

Pathways affected by thymidylate synthase inhibitors in HCT116 cells: genomic uracilation, transcription changes and post-transcriptional regulation by TS

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The colon cancer HCT116 derived cell lines with altered DNA repair capacities (MMR proficient and deficient as well as UNG inhibited versions) have been used as a model system to study genomic uracil incorporation upon thymidylate synthase (TS) inhibitory drug treatments [1]. Despite the same target gene, the two TS inhibitors, the antifolate RTX and the nucleotide analogue 5FdUR act differently in terms of cytotoxicity, induced genomic uracil patterns and induced mutagenicity (manuscript is under preparation).

Whole transcriptome sequencing also revealed essential differences upon the two drug treatments that are also influenced by the cellular DNA repair capacity. In addition to this, by immunoprecipitation coupled RNA sequencing, we demonstrated that the RNA-binding ability of TS is also differently affected by the two drugs. This prompts us to address the post-transcriptional aspects of the cell responses also.

Moreover, the U-DNA-Seq approach has been further developed preserving the single stranded DNA component of the genomic DNA isolates that previously might have been lost or underrepresented. The genomic uracil profiles generated by ssU-DNA-Seq were similar to the corresponding original profiles with minor differences only. However, the base substitution profiles calculated from the input data by variant calling (GATK mutect2 tool) were markedly altered that might be due to the increased vulnerability of the ssDNA. This might imply lessons regarding the detection of DNA damages/mutations in NGS data in general.

We are working on interpretation of the detected differences in U-DNA profiles (RTX vs 5FdUR, dsDNA vs ssDNA, MMR proficient vs deficient, UNG inhibited vs UNG active). Moreover, we started a data integration (U-score, relative transcription levels, transcriptional changes, replication timing, variability, TS-binding etc.) to be able to unravel the impact of genomic uracil on the transcriptional regulation that will be our major focus in the coming year.