CDK16's activity. [4] Here, we focus on how 14-3-3 proteins control CDK16's activity.

1. P. Mikolcevic, R. Sigl, V. Rauch, M. W. Hess, K. Pfaller, M. Barisic, L. J. Pelliniemi, M. Boesl, S. Geley, *Mol Cell Biol*,, **32**, (2012), 868.
2. X. Wang, R. Liu, S. Li, W. Xia, H. Guo, W. Yao, X. Liang, Y. Lu, H. Zhang, *Biomed Pharmacother.,* **164**, (2023), 114929.
3. S. N. Shehata, M. Deak, N. A. Morrice, E. Ohta, R. W. Hunter, V. L. Kalscheuer, K. Sakamoto, *Biochem J.*, **469**, (2015), 409.
4. S. R. Graeser, J. Gannon, R. Y. C. Poon, T. Dubois, A. Aitken, T. Hunt, *J Cell Sci*, **115**, (2002), 3479.

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L14

FROM MOLECULE TO MECHANISM: INTEGRATED INSTRUMENTATION FOR STRUCTURAL BIOLOGY

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SPECION, s.r.o. , Květnového vítězství 332/31, Praha 4

Modern structural biology depends on combining complementary technologies to understand the structure and dynamics of proteins, DNA, and RNA. This presentation will showcase biophysical analysis using Applied Photophysics (circular dichroism and stopped-flow kinetics) for studying protein folding, conformational stability, and biomolecular interactions. We will also introduce Refeyn's mass photometry for rapid, label-free measurement of molecular mass, oligomerization, and sample heterogeneity in solution. Finally, advanced imaging solutions from Leica Microsystems enable high-resolution visualization of molecular organization and protein localization within cells, linking molecular structure to biological function.

Friday, March 20, Session IV

L15

EXPLORING THE SEQUENCE SPACE OF A FLUORESCENT DEOXYRIBOZYME USING STRUCTURED LIBRARIES, SELECTION AND MACHINE LEARNING

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Finding ways to more comprehensively explore the sequence space of complex functional motifs is an important and unresolved question in nucleic acid engineering. Standard approaches use libraries in which a single variant of a motif is randomly mutagenized at a low level. This provides comprehensive coverage of sequence space over short mutational distances, but only limited information about more distant variants. Here we describe a new approach that uses libraries made up of sequences consistent with the multiple constraints of a desired target motif. Functional variants are rapidly identified in a single round of selection followed by high-throughput sequencing, and rules relating sequence to function elucidated using machine learning. This method was tested using a fluorescent deoxyribozyme recently discovered in our group called Aurora. Single-step selections showed that a secondary structure library based on Aurora contained approximately 40-fold more unique catalytic sequences than one generated by random mutagenesis. Furthermore, models developed by machine learning could quantitatively predict read numbers and identify the most active variants using small subsets of sequences as training sets. By combining secondary structure libraries, selection and machine learning in this way, sequence space can be explored far more quickly and efficiently than in standard approaches.

L16

COMPUTATIONAL APPROACHES FOR BIOMOLECULAR ELECTRONICS FROM MOLECULAR BRIDGES TO PROTEIN JUNCTIONS

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The conductive properties of molecular systems are determined by several factors, including structural motifs, specific chemical groups and bonding, presence of redox sites and their potentials, interaction with the surrounding environment, and thermal motions. In contact with a metal, the relative positions of electronic states in a (bio)molecule and in the metal (the so-called band alignment) also play a crucial role, affecting electronic transport. Therefore, the mechanism and efficiency of charge transport through a particular molecular system might change drastically in different environments. For example, redox proteins are known to transfer electrons by a hopping mechanism in their native biological environments; however, they could facilitate electron tunneling in junctions between two metallic contacts, as utilized in molecular nanoelectronics [1, 2]. Development in this field has been accompanied by advances in computational techniques that help to interpret experimental measurements and bring insightful details.

The non-equilibrium Green's function (NEGF) methods, combined with many-body electronic-structure treatments such as density functional theory (DFT), have become the standard framework for calculating tunneling currents and conductance in open systems, where metallic electrodes couple to discrete molecular states. Despite their success, NEGF approaches remain limited to small systems due to their high computational cost. For investigating biomolecular junctions based on peptides or proteins, and other real applications, approximate yet accurate techniques are needed. The approximation can be done at several levels, from semi-empirical or tight-binding treatments of the electronic structure, post-SCF band-alignment correction schemes, state-projection-based estimation of self-energies, to neglecting collective effects or coupling variations, and treating external-field effects in the linear-response regime [3]. On the other hand, the soft-matter character of these systems, where thermal fluctuations are unavoidable, requires dynamical approaches to electron transport that also take into account non-adiabatic effects and nuclear-electronic couplings. In the presentations, the accuracy of these techniques will be demonstrated on model biomolecular systems, in the context of molecular nanoelectronics.

1. Futera, Z., Wu, X., Blumberger, J.: Tunneling-to-Hopping Transition in Multiheme Cytochrome Bioelectronic Junctions. *J. Phys. Chem. Lett.* 14, 445 (2023).
2. Jonnalagadda, G. N., Wu, X., Hronek, L., Futera, Z.: Structural, Solvent, and Temperature Effects on Protein Junction Conductance. *J. Phys. Chem. Lett.* 15, 11608 (2024).
3. Hronek, L., Jonnalagadda, G. N., Futera, Z.: Computational Aspects of the Transport Calculations on (Bio)molecular Junctions, *J. Chem. Theory Comput.* (submitted).

Computational resources were supplied by the project "e-Infrastruktura CZ" (e-INFRA LM2018140) provided within the program Projects of Large Research, Development and Innovations Infrastructure.

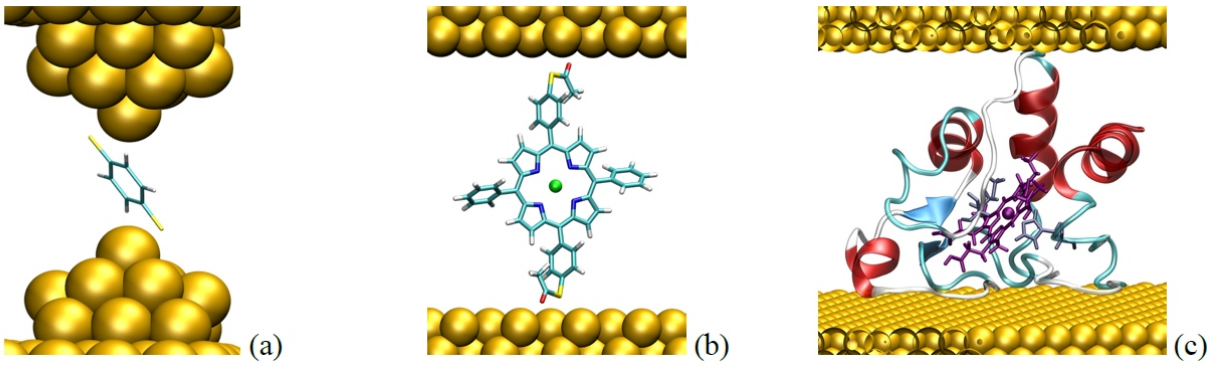

Figure 1. Atomistic models of (a) benzene-1,4-dithiolate bridge (BDT), (b) Zn-porphyrin junction (ZnTPP), and (c) cytochrome c between two flat gold contacts used for electron transport calculations.

L17

ANNOTATION, VALIDATION, REFINEMENT, AND MODELING OF NUCLEIC ACID STRUCTURES

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Recent advancements of the DNATCO web server [1] (https://dnatco.datmos.org) significantly enhance intuitive annotation, validation, modeling, and refinement of nucleic acid structures (Fig. 1). DNATCO leverages a universal structural alphabet to comprehensively describe DNA and RNA backbone conformations [2]. To improve accessibility and interoperability, the underlying computational pipeline has been re-implemented as a C++ library, which now powers the DNATCO web server and enables standalone tools, including integration into widely-used structural biology software such as CCP4 software suite. Additionally, the latest developments from the Nucleic Acid Valence Geometry Working Group [3] will be presented. This initiative attempts to establish and standardize nucleic acid valence geometry parameters, facilitating consistent usage across modeling, refinement, and validation platforms.

1. Černý et al., Nucleic Acids Research 2026, https://doi.org/10.1093/nar/gkaf1491.
2. Černý et al., Nucleic Acids Research 2020, http://dx.doi.org/10.1093/nar/gkaa383
3. Černý et al., Nucleic Acids Research 2026, https://doi.org/10.1093/nar/gkaf1335

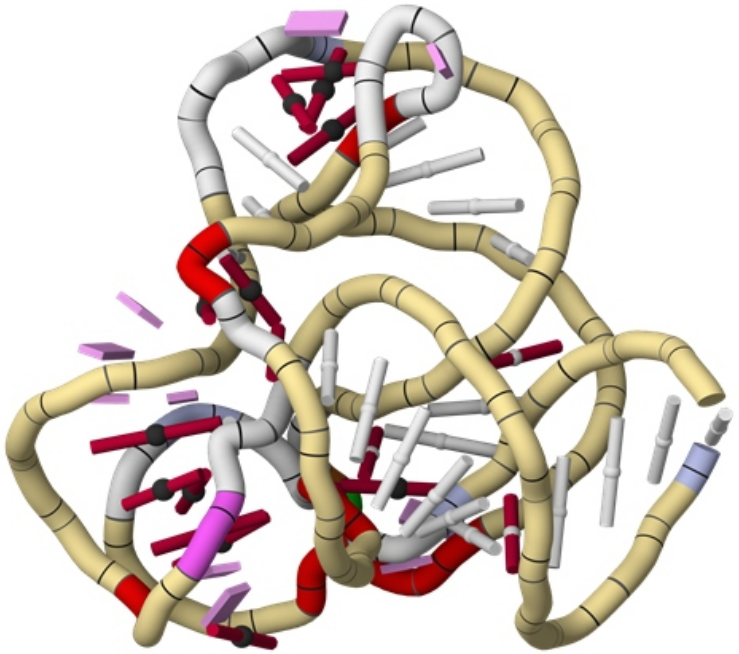

Figure 1. Structural visualization in DNATCO v5.0. An 80-nt fragment of 23S rRNA (PDB ID: 4qvi) is shown, with backbone conformations represented by the color-coded NtC tube and base pairing depicted as a ladder.

L18

MICROSCOPIC SCOOPING FOR UNBIASED, SUBCELLULAR, SPATIAL OPTOPROTEOMIC DISCOVERY

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Microscopy-guided proteomics at organelle-level resolution has the power to reveal previously unknown proteins in disease- or function-specific regions.

We present a breakthrough method for spatial protein purification using in situ subcellular photo-biotinylation, enabling precise labeling of proteins within user-defined regions and fully automated replication across thousands of fields of view.

As a compelling example, stress granules have historically been difficult to characterize due to their dynamic and membrane-less nature. Using optoproteomics, two-photon illumination was directed to G3BP1-positive stress granules to trigger localized photo-biotinylation, enabling selective purification and subsequent mass spectrometric analysis. This automated workflow revealed previously unrecognized high-confidence interactors and achieved 96% specificity upon validation.

Syncell's Microscoop enables hypothesis-free, high-resolution mapping of subcellular proteomes within precisely defined regions of interest, advancing our understanding of dynamic cellular structures such as stress granules.

Friday, March 20, Session V

L19

C-TERMINAL DOMAIN OF THE FILAMENTOUS HEMAGGLUTININ FhaB IS CRUCIAL FOR INTERACTION OF *BORDETELLA PERTUSSIS* WITH CILIATED EPITHELIAL CELLS

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Bordetella pertussis, the etiological agent of whooping cough (pertussis), produces a ~370 kDa filamentous hemagglutinin (FhaB) that functions as a key adhesin required for colonization of the respiratory tract. FhaB is secreted via a two-partner secretion (TPS) pathwayas an extended hairpin and, under *in vitro* conditions, it is proteolytically processed to release the ~230 kDa 'mature' FHA antigen used in acellular pertussis vaccines. Here, we show that FhaB remains largely unprocessed in *B. pertussis* adhering to ciliated airway epithelial cells and that its C-terminal domain (CT) is essential for host engagement. Using solution NMR spectroscopy, we determined the structure of the CT and found that it adopts a previously unrecognized, compact protein fold. Genetic ablation of the CT does not impair FhaB folding, secretion, or surface display, but abolishes bacterial adhesion to primary human nasal ciliated epithelial cells and prevents nasal colonization, shedding, and transmission in a murine catarrhal infection model. These findings establish the CT as a critical determinant of upper airway colonization and identify full-length FhaB, rather than processed FHA, as the biologically relevant adhesin during infection. Our results reframe the model of FhaB biogenesis and uncover a unique virulence mechanism with direct implications for pertussis vaccine design.