Friday, March 25, Session III

L12

SUPERNOVA: A DEOXYRIBOZYME THAT CATALYZES A CHEMILUMINESCENT REACTION

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Functional DNA molecules are useful components in nanotechnology and synthetic biology. To expand the toolkit of functional DNA parts, in this study we used artificial evolution to identify a glowing deoxyribozyme called Supernova [1]. This deoxyribozyme transfers a phosphate from the 1,2-dioxetane substrate CDP-Star to its 5' hydroxyl group, which triggers a chemiluminescent reaction and a flash of blue light. Comparative sequence analysis of 135,000 variants of Supernova obtained using in vitro selection and high-throughput sequencing indicated that the catalytic core of the deoxyribozyme is made up of 38 conserved nucleotides and 46 total positions. It also revealed the unusual secondary structure of Supernova, which consists of a purine motif triple helix and two purine-rich elements. An engineered version of Supernova can be programmed to only produce light in the presence of an oligonucleotide complementary to its 3' end, demonstrating that catalytic activity can be coupled to ligand binding. We anticipate that Supernova will be useful in a wide variety of applications, including as a signaling component in allosterically regulated sensors and in logic gates of molecular computers.

1. K. Svehlova, O. Lukšan, M. Jakubec, E. A. Curtis, *Angew. Chem. Int. Ed. Engl.*, **61**, (2022), e202109347.

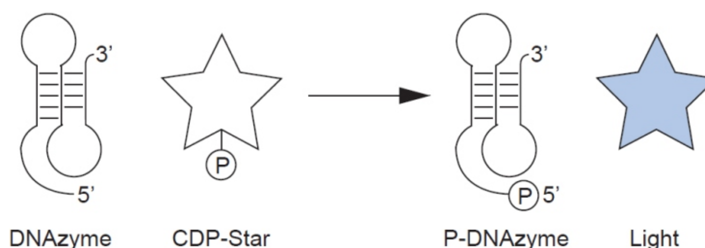

Figure 1. Production of light using the Supernova deoxyribozome.

L13

NEW CLASS OF STRUCTURALLY ROBUST NON-ANTIBODY PROTEIN SCAFFOLDS FOR DIRECTED EVOLUTION

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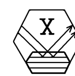
Engineered small non-antibody protein scaffolds are a promising alternative to antibodies and are especially attractive for use in protein therapeutics and diagnostics. The advantages include smaller size and a more robust, single-domain structural framework with a defined binding surface amenable to mutation. This calls for a more systematic approach in designing new scaffolds suitable for use in one or more methods of directed evolution. We hereby describe a process based on an analysis of protein structures from the Protein Data Bank and their experimental examination. The candidate protein scaffolds were subjected to a thorough screening including computational evaluation of the mutability, and experimental determination of their expression yield in *E. coli*, solubility, and thermostability. In the next step, we examined several variants of the candidate scaffolds including their wild types and alanine mutants. We proved the applicability of this systematic procedure by selecting a monomeric single-domain human protein with a fold different from previously known scaffolds. The newly developed scaffold contains two independently mutable surface patches. We demonstrated its functionality by training it as a binder against human interleukin-10, a medically important cytokine. The procedure yielded scaffold-related variants with nanomolar affinity.



L14

ATOMIC FORCE MICROSCOPY IN STRUCTURAL BIOLOGY

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Atomic force microscopy (AFM) can generate images within ranges of resolution that are of particular interest in biology [1]. Although atomic resolution may not be possible with biological samples, a great deal of information can still be obtained from images that provide structures at a slightly lower level of resolution.

Our laboratory is performing research in the field of imaging of biomolecules [2], mapping the elastic properties of cells and their clusters [3], and characterization of contractile properties of cardiomyocytes and their clusters [4].

The laboratory's flagship is the large AFM microscope JPK NanoWizard 4XP installed on a Leica DMi8 optical microscope with a fluorescence module. Both microscopes can operate simultaneously in the so-called directoverlay mode, thus combining AFM and optical microscopy abilities. Moreover, this microscope is not only an imaging tool, however helps to map elastic properties of various samples with nanometer resolution. One of the main advantages is the ability to work in semi-physiological conditions.

Combining the AFM with microfluidic, so-called FluidFM enables the possibility to aspirate and/or deliver extremely low volumes. This feature can be used when injecting or removing small volumes from individual cells. Using stiffer cantilevers, the system can investigate cell adhesion on new types of implant materials.

Keeping on the cutting-edge current AFM technology, the new generation of the MultiMode AFM microscope, version 8HR, was built for imaging with the maximum resolution that current commercial setups allow. This AFM setup will help the structural biologist image the biomolecules (DNA, proteins, molecular complexes) on a single molecular level.

Moreover, the multielectrode array (MEA) can be simultaneously connected with an AFM microscope, thus studying mechanoelectrical feedback of cardiac cells, tightly connected with some heart pathologies, such as catecholaminergic polymorphic ventricular tachycardia (CPVT).

1. Czajkowsky, D. M., Iwamoto, H., & Shao, Z.: *J. Electron Microscopy* **49**(**3**) (2000) 395–406.
2. Horňáková, V., Přibyl, J. & Skládal, P: *Monatsh Chem* **147** (2016) 865–871.
3. Raudenska M., Kratochvilova M., et al.: *Scientific Reports* **9** (2019) 1660, 1-11.
4. Caluori G, Pribyl J, et al.: *Biosens Bioelectron.* **124-125** (2019) 129-135.

CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2018127 and European Regional Development Fund-Project "UP CIISB "(No. CZ.02.1.01/0.0/0.0/18_046/0015974), is gratefully acknowledged for the financial support of the measurements at the CF Nanobiotechnology.

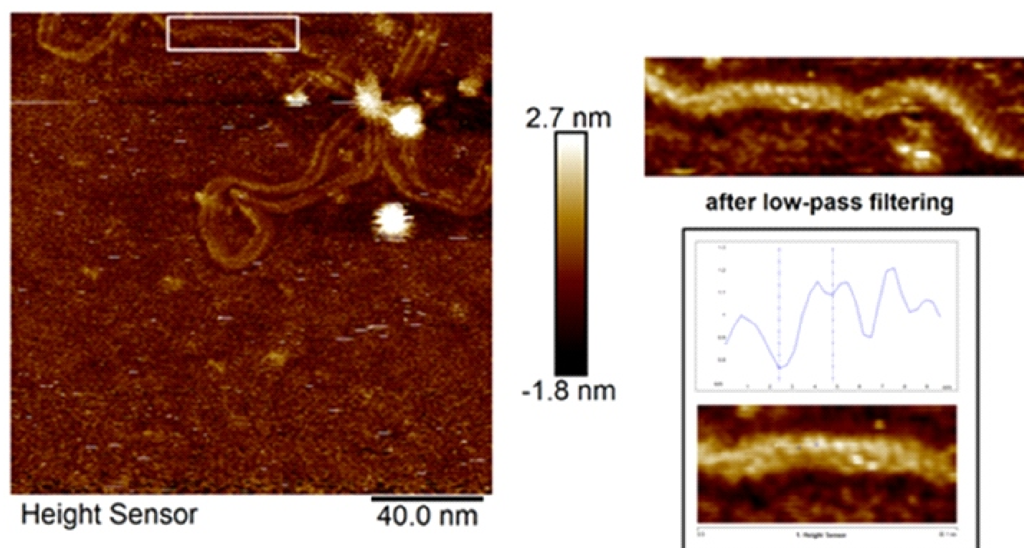

Figure 1. DNA structure studied by the AFM.

L15

STRUCTURAL MASS SPECTROMETRY – AN ADVANCED TOOL IN PROTEIN STRUCTURE ANALYSIS

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Structural mass spectrometry is a fast-growing field on analytical chemistry representing a new approach for protein structural studies.

The Structural Mass Spectrometry facility offers access to an ultra-high resolution 15T-Solarix XR FT-ICR (Bruker Daltonics), timsToF Pro (Bruker Daltonics) and to a MALDI-TOF Bruker Autoflex Speed (Bruker Daltonics) mass spectrometers.

This instrumentation allows determination of precise mass of biological macromolecules, characterization of posttranslational modifications, peptide mass fingerprinting, detection of small molecule/metabolites and monitoring of protein structural changes/protein-protein interactions under physiological conditions by hydrogen-deuterium exchange, chemical cross-linking and native mass spectrometry.

In the Centre of Molecular Structure, the tools of structural mass spectrometry are well established and allow to look beyond the edge of traditional structural techniques.

L16

STRUCTURAL CHANGES AND THEIR CONSEQUENCES FOR AZURIN OXIDATION IN VACUUM AND ON GOLD INTERFACES

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Charge transfer processes facilitated by metalloproteins play an essential role in biology and technological applications, including novel vacuum-based nanobioelectronic devices. Detail understanding of the charge-transport mechanism and its dependence on the environment of a protein is thus very desirable. In an aqueous solution, electrons flow through biomatter by sequential, thermally-activated hopping between the available redox sites. However, the interaction of the proteins with metal surfaces has profound consequences on the electronic structure, as suggested by the temperature-independent current-voltage response measured in vacuum junctions [1]. Such behavior could be, in principle, explained either by coherent tunneling mechanism or by substantial lowering of the so-called

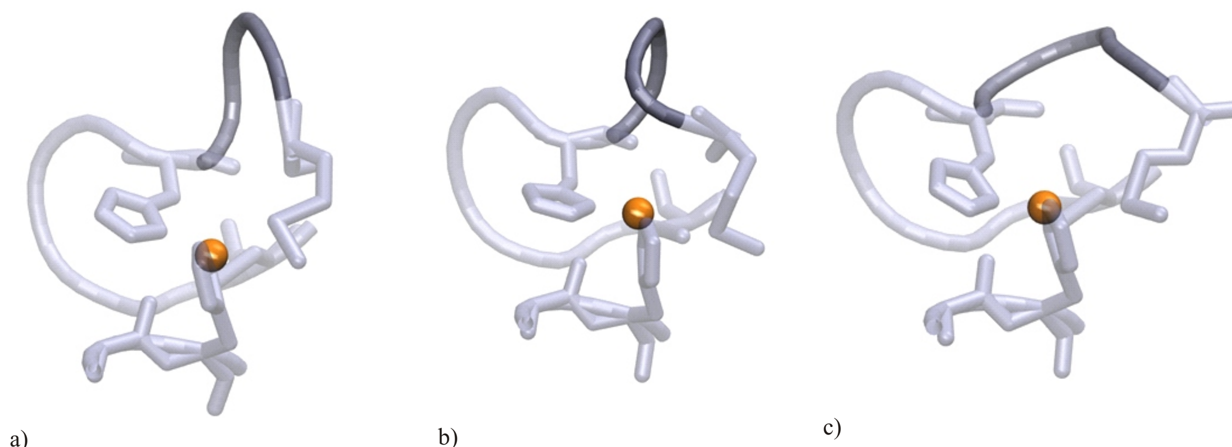
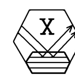


Figure 1. Observed loop twists between His117 and Met121 (dark grey) next to the redox Cu site (orange).



reorganization free energy controlling the barrier heights in the hopping mechanism.

We investigated such environmental effects on structure and oxidation reorganization free energies of the Azurin protein, as extracted to vacuum and consequently adsorbed to clean gold surfaces. For that, we employed molecular dynamics (MD) computational techniques and QM/MM sampling [2] within the framework of density-functional theory (DFT). We reproduced the experimental value of the reorganization free energy in the solution (~0.7 eV). However, the energy is not reduced upon the extraction of Azurin to vacuum due to its increased flexibility near the redox Cu site (c.f. Fig. 1). On the gold surfaces, the reorganization energy varies between 0.6 and 0.9 eV, depending on the particular adsorption structure. The structural flexibility is balanced there with the metal adsorption and polarization effects. In either case, the reorganization free energy is kept relatively high, which does not support the hopping mechanism of electron transport on vacuum biometallic interfaces.

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2. Futera, Z. et al.: Coherent Electron Transport across a 3 nm Bioelectronic Junction Made of Multi-Heme Proteins. *J. Phys. Chem. Lett.* **11**, 9766-9774, 2020.

L17

MICROFLUIDIC MODULATION SPECTROSCOPY (MMS): A NOVEL IR-BASED TECHNIQUE PROVIDING AUTOMATED, HIGHLY-SENSITIVE PROTEIN SECONDARY STRUCTURE CHARACTERISATION IN IN SITU CONDITIONS

Patrick King

RedShiftBio Inc.

Infra-Red (IR) analysis has been long accepted as a powerful tool in protein characterization, particularly in the Amide I band (~1600 – 1700 cm^{-1}), which gives detailed secondary-structural information that can be critical in determining protein structure-activity relationships, stability, batch-to-batch comparisons and in formulation studies as a few examples. Technologies traditionally used for secondary structure analysis, such as benchtop Fourier Transform IR (FTIR) or Circular Dichroism (CD), suffer from a number of issues that have prevented their routine use in this area, preventing this application from reaching its full potential. These include concentration and buffer restrictions, incompatibility with a range of excipients, a lack of automation, low spectral reproducibility and for FTIR, water subtraction problems.

Microfluidic Modulation Spectroscopy (MMS) is a new key technology that was brought to market in 2019 by RedShift Bioanalytics. It focuses on the IR Amide I region to produce exceptionally high data quality and reproducibility that aim to solve the aforementioned issues encountered with traditional technologies. It is fully automated, running samples from 24- and 96-well plates, compatible with a very broad concentration range (0.1 to >200 mg/ml), and is also compatible with a wide range of complex buffer systems and excipients, including those that absorb in the amide I region, surfactants and organic solvents. The platform includes a powerful software package that facilitates data analysis, and can be included in the automation procedure. This presentation highlights the technical benefits of MMS and its application in the protein structural workflow, giving relevant application examples.