Saturday, March 25, Session VI

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ILLUMINATING THE MECHANISM OF NanoLuc LUCIFERASE ACTION

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NanoLuc, a superior -barrel fold luciferase, was engineered 10 years ago but the nature of its catalysis remains puzzling. Here experimental and computational structural techniques were combined, revealing that imidazopyrazinone luciferins bind to an intra-barrel catalytic site but also to an allosteric site shaped on the enzyme surface [1]. Binding to the allosteric site prevents simultaneous binding to the catalytic site, and vice versa, through concerted conformational changes. We demonstrate that restructuration of the allosteric site can boost dramatically the luminescent reaction in the remote active site. Mechanistically, an intra-barrel arginine coordinates the imidazopyrazinone component of luciferin to attack O_2 via a radical charge-transfer mechanism, while it protonates the excited amide product to secure high emission intensity. Concomitantly, an aspartate, supported by two tyrosines, fine-tune the electronic state of the amide product, promoting the formation of the blue colored emission. Thus, we show that NanoLuc, despite its structural dissimilarity, employs analogous tricks to secure a blue light-emitting phenolate anion, as we recently revealed for *Renilla*-type luciferase [2]. Such information should be critical to engineer the next-generation of light-producing biosystems.

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TIMING OF ICSI WITH RESPECT TO MEIOTIC SPINDLE STATUS

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The aim of this study was to evaluate the efficiency of using meiotic spindle (MS) visibility and relative position to the polar body (PB), as indicators of oocyte maturation, to optimize intracytoplasmic sperm injection (ICSI) timing. This was a cohort study of patients younger than 40 years with planned ICSI, the timing of which was determined by MS status, compared with those without MS evaluation. The angle between PB and MS, and MS visibil-

ity were evaluated by optical microscope with polarizing filter. Oocytes with MS evaluation were fertilized according to MS status either 5-6 hours after ovum pick-up (OPU) or 7-8 hours after OPU. Oocytes without MS evaluation were all fertilized 5-6 hours after OPU. For patients over 35 years visualization of MS influenced pregnancy rate (PR): 182 patients with MS visualization had 32% PR (58/182); while 195 patients without MS visualization had 24% PR (47/195). For patients under 35 years, visualization of MS did not influence PR: 140 patients with MS visualization had 41% PR (58/140), while 162 patients without MS visualization had 41% PR (66/162). Visualization of MS therefore appears to be a useful parameter for assessment of oocyte maturity and ICSI timing for patients older than 35.

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EXPLORING SEQUENCE SPACE USING SECONDARY STRUCTURE LIBRARIES AND SINGLE-STEP SELECTIONS

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Once thought to act primarily as a storage molecule for genetic information, it is now known that DNA and RNA can have many other functions [1]. Our group is particularly interested in the ability of nucleic acids to act as catalysts. It is not yet possible to design nucleic acid sequences with catalytic activity. However, using powerful methods of artificial evolution, rare molecules that catalyze a desired reaction can be isolated from random sequence libraries of 10^{15} (or more) sequences. We are inspired by the power of artificial evolution, and are using this technique to learn more about the interesting and useful things that nucleic acids can do. In this presentation I will describe methods recently developed in the group to explore sequence space using secondary structure libraries (libraries enriched for a secondary structure of interest) and single-step selections (selections that can be performed in a single round rather than the ten or more that are often required). In one example of this approach, RNA-cleaving deoxyribozymes were isolated from a structured library containing a randomized region of only 12 nucleotides (corresponding to 10^7 different sequences) in a single round of selection followed by high-throughput sequencing [2]. In another example, a novel synthetic method [3] was used to construct a library enriched for sequences with the potential to form the secondary structure of Supernova [4], a light-producing deoxyribozyme recently discovered in our group. Active variants were isolated from this library using single-step selections, including some with improved catalytic efficiencies. These examples highlight how structured libraries can be used in combination with single-step selections to rapidly obtain information about functional nucleic acid motifs.

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ARBRE – ASSOCIATION OF RESOURCES FOR BIOPHYSICAL RESEARCH IN EUROPE

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Molecular-scale biophysics is a dynamic interdisciplinary field that aims to study biological macromolecules and assemblies as a whole, at an intermediate level between atomic-resolution structural descriptions and cellular-level observations with significant applications in biomedicine and drug discovery. There has been established numerous biophysical core facilities and other laboratories enabling users of various background to use the advanced instrumentation. Since the development of science is enormous over last decades, the collaboration and sharing of know how between such facilities is necessary in order to keep and develop the state of the art technologies.

In 2014, the ARBRE-MOBIEU network was initiated, aiming to seed a large-scale pan-European interdisciplinary clustering, allowing to ally and synergize the power of spectroscopic, hydrodynamic, real-time microfluidic, thermodynamic and single-molecule approaches [1]. In its early years, the network was supported by a European COST action, resulting in involvement of several dozens of laboratories throughout Europe. In 2021, based on the established contacts and collaborations, the initiative has been turned into a scientific society ARBRE (Association of Resources for Biophysical Research in Europe).

The main objectives of the society are to: i) create an optimal environment for the development of innovative integrative biophysical approaches; ii) disseminate knowledge, e.g. through the organization of workshops and training schools; iii) facilitate the transnational access to instrumentation and expertise for a wide user community; iv) provide a platform for scientists to establish early contacts with instrument developers. The users can already benefit from several outcomes, such as development of standards for interaction techniques [2], establishing of standard operating procedures (SOP's) [3] or formulating recommendations for protein quality control [4] and stability assessment [5].

Those interested in the association activities can visit the web pages www.arbre-biophysics.eu for more information or contact its representatives directly.

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