BP 26: Focus Session mRNA Physics

Time: Thursday 15:00–17:30 Location: TOE 317

Invited Talk BP 26.1 Thu 15:00 TOE 317
Decoding Molecular Plasticity in the Dark Proteome of the Nuclear Transport Machinery — •Edward Lemke — JGU & IMB Mainz, GE

The mechanisms by which intrinsically disordered proteins (IDPs) engage in rapid and highly selective binding is a subject of considerable interest and represents a central paradigm to nuclear pore complex (NPC) function. Nuclear transport receptors (NTRs) can move small proteins and mRNA through the central channel of the NPC which is filled with hundreds of phenylalanine-glycine-rich nucleoporins (FG-Nups) reaching millimolar concentrations with elusive conformational plasticity. We have now developed a semi-synthetic strategy to equip the living cell with up to three genetic codes and label FG-Nups inside functional NPCs site-specifically with small FRET probes. This allowed us to develop an experimental approach and use fluorescent lifetime imaging microscopy (FLIM) to directly decipher the plasticity of FG-Nups. Our study enabled a conformational look on the densely packed IDPs in the sub-resolution (roughly (50 nm)^3 small cavity) cavity of the NPC. We extracted the scaling exponent, which directly describes the conformations of FG-Nups at their functional status as well as the solvent quality in the cellular and even inner NPC environment. Pairing our data with coarse grained simulations enabled us to complement the missing half of protein mass that due to its dynamics are not present in even the most recent electron tomograms of the NPC.

BP 26.2 Thu 15:30 TOE 317
Decomposition of ensemble fluorescence signals from translation experiments and simulations — •Nadin Haase, Simon Christ, and Sophia Rudorf — Institute of Cell Biology and Biophysics, Leibniz University Hannover, Germany

Proteins are major components of cells and perform all kinds of tasks essential for survival. Those parts of the DNA that contain blueprints for the synthesis of proteins are transcribed to messenger RNA (mRNA). Complex biomolecular machines called ribosomes translate the mRNA by assembling the corresponding building blocks of proteins, the amino acids. This process can be studied by in-vitro ensemble experiments through monitoring time-dependent output signals such as fluorescence time traces, where the ensemble signal is a superposition of the individual signals from all molecules in the reaction. However, translation is a stochastic process and thus even for initially synchronized reactions the ensemble signal may not reflect the features in the individual signals, especially on longer time scales. Here we present an approach to decompose the ensemble signal to reveal hidden information about the individual steps of the translation process. We explore the limits of this method and study the conditions under which a meaningful solution can be gathered.

BP 26.3 Thu 15:45 TOE 317
Creating an evolution machine with 2-3-cyclic RNA — •Dieter Braun — Systems Biophysics, Ludwig Maximilian University Munich

For life to start, a simple physical non-equlibrium mechanism combined with a most robust chemistry of a few molecules had to reach the regime of Darwinian molecular evolution. We found RNA oligomerization and templated ligation from very mildly activated RNA with 2-3-cyclic phosphates [1], with wet-dry cycling at heated air-water interfaces [2]. The oligomerization operated at elevated pH 9-10 without added salts at temperatures between 4-40°C and created oligomers of all four bases with 15 percent yield. It operated in a water-poor 'dry' state. Replication was possible with templated ligation and showed with only 1mM MgCl2 a strongly base-selective templated ligation with 25 percent yield. If catalytic conditions to recycle the hydrolytically opened 2-3-cyclic phosphate from linear 2 prime or 3 prime endings wcould be found, the reaction would operate indefinitely without feeding in a thermal gradient setting. We will show preliminary experiments for local feeding, vesiculation and protein expression in the same setting. The experiments suggest a most simple scenario for the emergence of life from only two nucleotide molecules, implemented in an early Earth volcanic setting under a CO2 atmosphere. It showcases how physics and chemistry could have acted together in a geologically abundant microfluidic setting to create Darwinian evolution.

[1] ChemSystemsChem doi.org/10.1002/syst.202200026 (2022)
[2] Nature Physics doi.org/10.1038/s41567-022-01516-z (2022)

BP 26.4 Thu 16:00 TOE 317
RNA G-quadruplex folding is a multi-pathway process with a variety of short-lived intermediate states — •Marijana Ugrina[1], Ines Burkhart[2], Diana Müller[2], Harald Schwalbe[2], and Nadine Schwierz[1] — [1]University of Augsburg, Augsburg, Germany — [2]Goethe University, Frankfurt am Main, Germany

The folding kinetics of regulatory RNAs is crucial for their function. Here, we provide molecular insights into the folding pathways of a G-quadruplex from telomeric repeat-containing RNA by combining all-atom molecular dynamics and coarse-grained simulations with circular dichroism experiments. The ion atmosphere surrounding the highly charged quadruplex plays a crucial role in folding. To correctly capture the electric double-layer in implicit solvent coarse-grained simulations, we develop a matching procedure based on all-atom simulations in explicit water. This procedure allows us to provide quantitative agreement between the experiments and simulations as judged by the number of native contacts at different salt concentrations and temperatures. Folding of the quadruplex is on the timescale of minutes and the coarse-grained simulations using the three-interactions site model are therefore ideal to resolve the folding pathways and intermediate states. The results reveal that the folding is sequential with each pathway passing through two transient, on-pathway intermediates: A hairpin and a triplex or double hairpin state. Since these intermediates are degenerate with at two to four alternative conformations per state, quadruplex folding is a multi-pathway process with high conformational entropy.

15 min. break

BP 26.5 Thu 16:30 TOE 317
Codon position-specific engineering of translation kinetics — Judith Müller[1], Gerlinde Schwake[1], Anita Reiser[1], Daniel Woschée[1], Zahra Alirezaeizanjani[3], Joachim Rädler[1], and •Sophia Rudorf[2] — [1]Faculty of Physics, Ludwig Maximilian University of Munich — [2]Faculty of Natural Sciences, Leibniz University Hannover — [3]independent researcher

Recently, we introduced Live Imaging on Single Cell Arrays (LISCA), which combines time-lapse microscopy of single cells on microstructured surfaces with automated image analysis. LISCA enables us to observe and analyze the time-evolution of protein expression in hundreds of individual cells in parallel. Here, we combine LISCA with our software OCTOPOS, which simulates ribosome movement on the ORF and is used to generate reporter genes with varying ribosome speeds and densities. We predict and monitor the translation kinetics of synonymous variants of eGFP to determine mRNA functional lifetimes and translation rates with high accuracy in single lung tissue cells. Our approach allows us to study how provoked ribosome jams on specific positions within the ORF influence mRNA stability, thus linking ribosome dynamics and mRNA biophysics.

BP 26.6 Thu 16:45 TOE 317
The pH dependent phase transition in lipid nanoparticle cores leads to changes of protein expression in single cells — •Julian Philipp and Joachim Rädler — LMU, Munich, Germany

Lipid nanoparticles developed into the most powerful delivery platform for mRNA-based vaccination and therapies. In general, LNPs are particles exhibiting PEG-lipid and DSPC at the surface and ionizable lipid, cholesterol and mRNA in the core. However, the pH dependent changes induced by ionizable lipids in the context of endosomal release are little understood. In particular the ionizable lipids MC3, KC2 and DLin are known to exhibit remarkably different efficacy despite similar pK values. Here, we study the structural response of ionizable lipids with cholesterol as a function of pH using synchrotron X-ray scattering. All three core phases exhibit a sequence of ordered mesophases in the range of pH 7 to 4, beginning with an isotropic swollen phase above their pK value. Lowering the pH reveals transitions to inverse micellar $P6_3/mmc$ / $Fd3m$, inverse hexagonal H_{II} and bicontinous cubic $Pn3m$ phases. If polyA, as mRNA surrogate, is added to the core phases, coexistence of pure lipid phases and condensed nucleic acid

lipid H_{II}^{c} phase occurs. We show that the observed core structures are consistent with the SAXS scattering of mRNA containing core phases and full LNP. The difference in structural features of DLin versus MC3 and KC2 phases is also consistent with the delayed onset and reduced level of GFP expression observed in single cell time courses after transfection with DLin LNPs compared to MC3/KC2. We conclude that pH dependent core phase transitions trigger endosomal release.

BP 26.7 Thu 17:00 TOE 317

pH-dependent behavior of ionizable cationic lipids in mRNA-carrying lipoplexes investigated by molecular dynamics simulations — •Giovanni Settanni[1,2], Wolfgang Brill[3], Heinirich Haas[3], and Friederike Schmid[1] — [1]Department of Physics, Johannes Gutenberg University Mainz, Germany — [2]Faculty of Physics and Astronomy, Ruhr University Bochum, Germany — [3]BioNTech SE, Mainz, Germany

Lipid-based nanoparticles and lipoplexes are successful nanocarriers for mRNA-based therapies. The molecular structure of these assemblies is still not fully understood. Lipoplexes including the ionizable lipid 2-dioleyloxy-N,N-dimethyl-3-aminopropane (DODMA), under specific conditions, have a pH-dependent lamellar structure, where lipid bilayers are separated by mRNA-rich layers. Here, the structure and dynamics of these lipoplexes are investigated at varying pH and mRNA-concentration using multiscale molecular dynamics simulations[1]. It is observed that the interaction between DODMA and RNA is slightly attractive only at low pH levels. This results into a pH-dependent relocation of the RNA inside the multilayers, from bilayer's surface at low pH to a more uniform distribution inside the hydrophilic slabs at high pH. Also, at high pH, DODMA lipids shift toward the hydrophobic part of the bilayer, thus increasing their leaflet-flipping rate, a phenomenon which may ultimately affect the fusion process of the lipoplex with the endosomal membrane.

[1] Settanni, G., Brill, W., Haas, H. and Schmid, F. (2022), Macromol. Rapid Commun. 43:2100683. https://doi.org/10.1002/marc.202100683

BP 26.8 Thu 17:15 TOE 317

Charge and structural properties of transfection lipid layers adsorbing mRNA — •Miriam Grava[1], Ibrahim Mohd[2,3], Julio Pusterla[1], Julian Philipp[4], Joachim Rädler[4], Olaf Soltwedel[1], Nadine Schwierz[2,3], Heinrich Haas[5], and Emanuel Schneck[1] — [1]Technische Universität Darmstadt, Germany — [2]University of Augsburg, Germany — [3]Max-Planck-Institute of Biophysics, Frankfurt am Main, Germany — [4]Ludwig-Maximilians-Universität München, Germany — [5]BioNTech Corporation, Mainz, Germany

Some of the most effective COVID-19 vaccines are based on cationic lipid-based delivery systems for messenger RNA (mRNA), a promising technology for a broader use in biomedical applications. Its efficiency depends on pH variations and ionic conditions of the bulk phase. We combine x-ray scattering and x-ray fluorescence on monolayers of positively chargeable transfection lipid mixtures with atomistic molecular dynamics (MD) simulations in order to determine their pH-dependent structural properties and the protonation degree. While the experiments yield electron density profiles and surface charge densities, the MD simulations yield the area per molecule, the conformation of different lipid species, and the counter-ion distributions. The analysis of experimental and simulation data provides detailed information on the transfection lipid layer characteristics. We applied the same experimental techniques to transfection lipid layers before and after mRNA adsorption, which yields insights into the structure of the adsorbed layers and the interfacial electrostatic balance.