
Session 25: From Experimental Data to in Silico Simulations and Way Back

Lectures

L25.1

Cell penetration and membrane fusion: two sides of the same coin

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Cell penetrating peptides have a unique potential for targeted drug delivery, therefore, mechanistic understanding of their membrane action has been sought since their discovery over 20 years ago. While ATP-driven endocytosis is known to play a major role in their internalization, there has been also ample evidence for the importance of passive translocation for which the direct mechanism, where the peptide is thought to directly pass through the membrane via a temporary pore, has been widely advocated. In this talk, I will question this view and demonstrate that arginine-rich cell penetrating peptides can instead enter vesicles and cells by inducing multilamellarity and fusion, analogously to the action of calcium ions. The molecular picture of this penetration mode, which differs qualitatively from the previously proposed direct mechanism, is provided by molecular dynamics simulations. In addition, the kinetics of vesicle agglomeration and fusion by non-arginine, non-alanine, and calcium ions are documented in real time by fluorescence techniques and the induction of multilamellar phases in vesicles and cells is revealed both via electron microscopy and fluorescence spectroscopy. We thus show that the newly identified passive cell penetration mechanism is analogous to vesicle fusion induced by calcium ions, demonstrating that the two processes are of a common mechanistic origin.

L25.2

Modeling of protein structure and dynamics with the UNRES force field assisted by limited experimental data and knowledge-based information

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Theoretical methods have become a common means to predict protein structure and to simulate the dynamics of proteins. We developed a hybrid approach by combining knowledge-based information with the physics-based UNRES force field. UNRES uses a highly simplified representation of proteins, in which each amino-acid is modeled by only two bodies, yet the force field is rigorously physics-based. A simple representation enables us to carry out simulations about 1000 faster than with the all-atom representation. Our hybrid approach can use ancillary experimental information such as small angle X-ray scattering (SAXS) [1]. Restraints derived from knowledge-based models and distance distribution from SAXS are used in multiplexed replica exchange molecular dynamics simulations. SAXS restraints are incorporated into the target function as a maximum-likelihood term that guides the shape of the simulated structures towards that defined by SAXS. We tested our hybrid approach by participating in the CASP12 as the KIAS-Gdansk group [2]. Recently, we have implemented a web server for coarse-grained simulations using UNRES force field. Local energy minimization, canonical molecular dynamics simulations, replica exchange and multiplexed replica exchange molecular dynamics simulations can be run with the current UNRES server [3].

References:

- Karczyńska A *et al.* (2017) *Proteins* **86**: 228-239.
- Karczyńska A *et al.* (2018) *J Mol Graph Model* **83**: 92-99.
- Czaplewski C *et al.* (2018) *Nucleic Acids Res* gky328.

Oral presentations

O25.1

Protein hydraulics: water driven substrate binding cooperativity to protein kinase A

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Catalytic subunits of protein kinases share a common fold centered around several conserved, precisely arranged functional motifs. Crystal structures representing diverse kinases reveal a number of buried water molecules whose positions are equally well preserved as types of most functionally important amino acids. It is unknown whether they play any important role, and whether their removal – disturbing local interaction patterns to no smaller degree than amino acid mutations – can affect kinase stability and function.

By conducting molecular dynamics simulations we demonstrate that communication between the ATP and protein substrate binding sites in protein kinase A involves an isolated water molecule embedded between the DFG and YRD regions. Modification of its hydrogen bonding pattern induced by ATP binding affects the configuration of the DFG+1 residue. This in turn shifts the conformational equilibrium of the peptide substrate positioning loop, promoting more frequent occurrence of binding competent state. Furthermore, we show that conformational flexibility of the peptide binding segment is lubricated by buried, disordered water cluster not fully resolved in X-ray structures. The above findings complement well NMR-based data on changes in local kinase mobility upon substrate binding, and provide mechanistic explanation for experimentally observed binding cooperativity of the two substrates.

Reference:

Setny P, Wisniewska MD (2018) *PNAS* published ahead of print.

O25.2

DNA binding to DnaA replication initiator protein – implications on the structure of the replication initiation complex

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DnaA protein is a highly conserved multifunctional protein, a key component of bacterial DNA replication machinery. DnaA protein binds to specific sequences (DNA-boxes) on double stranded DNA (dsDNA) and also is able to bind to single stranded DNA (ssDNA) when the replication complex is formed. The functionality of DnaA protein is regulated by ATP (ADP) binding. Many structural data are available related to the mechanism of binding of DnaA to dsDNA, and recently also a structure of DnaA molecules bound to ssDNA were determined by x-ray crystallography [1]. Binding to ssDNA required formation of tight DnaA oligomers, on another hand binding of DnaA to dsDNA could be accomplished by the monomeric form of protein. Due to dynamics of the replication initiation complex, none of experimental methods is able to present reliable data to provide answers to two key questions: if single DnaA molecule is able to simultaneously bind ssDNA and dsDNA and how (if at all) DnaA molecules bound to dsDNA and ssDNA interact with one another. Using theoretical tools such as sequence comparison, molecular modeling, structure prediction, and molecular dynamics simulations, we proposed possible answers for the questions raised above.

Reference:

Richardson TT, Harran O, Murray H (2016) *Nature* **534**: 412.

Posters

P25.1

A novel approach for the *in situ* visualization of individual gene location within the nucleus

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Fluorescent *in situ* hybridization (FISH) is one of the main methods used in cytogenetic studies. The conventional FISH relies on hybridization of several hundred nucleotide long DNA probes and is not well suited for detection of short sequences of chromosomal DNA. One of the solutions to overcome this problem is applying DNA padlock oligonucleotides, which specifically bind to short (20-30 nt) DNA sequences. However, this technique may be challenging to use due to the necessity of making chromatin accessible for hybridization. Therefore, the DNA padlock technique requires an application of a complex set of oligonucleotides that enhance padlock interaction with the sequence of interest and minimize the cross-interactions between oligonucleotides.

Here, we present a new approach for DNA padlock experiments, in which we applied short “opener” oligomers, composed of 2'-O-methyl-RNA and LNA nucleotides. These oligomers hybridize firmly to one strand of chromosomal DNA making the other available for binding with the padlock oligonucleotide. We also developed user-friendly software for designing whole sets of oligonucleotides needed to perform DNA padlock-based experiments. By using our software one can assure the specificity and selectivity of the used oligonucleotides. Finally, we present the results of *in situ* visualization of the selected genes in mouse brain cells.

P25.2

NANOS1 and NANOS3 paralogs repress distinct somatic pathways in human germ cells

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Nanos proteins are evolutionary conserved RNA-binding proteins (RBPs) acting as post-transcriptional repressors. They are crucial for germ cell development and maintenance throughout the animal kingdom. Each of three mammalian Nanos paralogs are essential for fertility. However, their roles in germ cells are not equal. The molecular basis of functional differences of Nanos paralogs has not been established yet. We hypothesize that distinct Nanos paralogs regulate different mRNA pools by binding different protein cofactors. Thus we aimed to identify mRNA pools regulated by NANOS1 and NANOS3 as well as proteins binding each paralog in human germ cell line (TCam-2). Using NANOS1 or NANOS3 overexpression followed by RNA-sequencing, as well as NANOS1 and NANOS3 Co-IP followed by mass spectrometry, we identified mostly distinct mRNA pools regulated by NANOS paralogs and mostly different groups of NANOS1 and NANOS3 interacting proteins. Furthermore, using systems biology tools we built distinct biological interaction networks of NANOS1 and NANOS3 regulated mRNAs each consisting of protein-protein and protein-DNA interactions. Gene ontology analysis on identified clusters showed that NANOS1 and NANOS3 repress distinct somatic pathways in human germ cells.

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P25.3

Understanding bedaquiline: atomistic insights into the inhibition of ATP synthase Fo subunit

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Tuberculosis is one of the world's leading causes of death, harbouring over 1.4 million deaths in 2011 alone. With a 4-week incubation of sputum cultures being the only reliable method of diagnosis even identifying the disease poses a challenge. *Mycobacterium tuberculosis*, the species responsible for the disease, is a slow-growing bacteria with high mutation rate. Every year, this battle proves more difficult due to the appearance of new multi-drug resistant strains (MDR). Current first-line treatment regime, which typically includes four chemicals: isoniazid, rifampicin, pyrazinamide and ethambutol, lacks the potential to successfully and permanently eliminate the bacteria. Relatively recently, the diarylquinoline drug bedaquiline has been included in the second-line treatment regime, introduced if the first-line therapy fails due to presence of MDR strains.^[1]

The precise mechanism of action of bedaquiline is not currently known and the molecular basis for selectivity also remains to be identified. To address these questions, molecular dynamics simulations of dissociation and association of the drug to Fo ATP synthase complex, supposed molecular target, have been performed. To investigate its selectivity, we docked the drug to the Fo main component – c-ring, derived from various organisms. Here, we present the results of these simulations and a cheminformatical analysis of the docking outcomes. Furthermore, we report on the results of the first Bayesian Cryo-EM density guided refinement of the atomistic model of the Fo complex.^[2]

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References:

1. Zumla A *et al.* (2013) *Nature Reviews Drug Discovery* **12**: 388-404.
2. Guo H *et al.* (2017) *Science* **358**: 936-940.