Exploring polymorphism in oligomeric, macromolecular machines using integrative and interactive approaches — Study system: homologous recombination

Day 1 : Polymorphism of protein oligomers

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Helical Polymers: Conservation, Plasticity and Chaos

Most protein (by mass) in bacterial, archaeal and eukaryotic cells is frequently found in the form of helical polymers. Cryo-EM has emerged as the method of choice to study these assemblies, as reaching a near-atomic resolution is now the rule rather than the exception. Our studies on a large range of such polymers have illuminated a number of points. While tertiary structure can be highly conserved despite large degrees of sequence divergence (and examples will be shown from archaeal viruses), quaternary structure can be very labile, and small changes in sequence can lead to large changes in quaternary structure. This can be an evolutionary mechanism for the amplification of small sequence changes. We have powerful tools for predicting tertiary structure, but due to the lability of quaternary structure, no such reliable tools exist for quaternary structure. Using peptides, we can show that this problem is an example of deterministic chaos. One of the interesting aspects of helical symmetry is that different symmetries may still allow local interactions to be relatively conserved. Examples will be shown from bacterial mating pili and inflammasomes. The concept of relatively conserved (but not identical) local interactions led to the theory of quasi-equivalence in the 1960s to explain icosahedral viruses. We can now show how quasi-equivalent interactions have led archaeal rod-like viruses to expand into spindle-shaped viruses.

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Spontaneous and induced assemblies of antimicrobial peptidic compounds

Basic amphipathic membrane-active peptides target bacteria via various mechanisms, the key factor in their action involves attraction to the negatively charged bacterial membrane surface accompanied by peptide disorder-to-order structural transition often coupled with assembly formation. We have recently reported that similar peptide sequestration and conformational change occurred in the presence of small molecules of synthetic and natural origin with several model peptides. Extending the investigation to various membrane-active peptidic compounds that either contain, or consist entirely of non-natural amino acids, we and others have found that similar oligomerization and fibril formation can occur as in case of natural filament forming processes, such as for amyloid systems. In the current presentation I aim to present some of our molecular understanding on supramolecular scaffolds of peptide sequences, from initial one- or two-component systems, to identifying complex multicomponent assemblies. As interesting model systems, AMP interactions and assemblies with heme, quorum sensing molecules, synthetic drugs, food colors, or components of extracellular vesicles will be described. We hope that in the future we will gain insight at the molecular level to these assembling phenomena, which are hoped to help development of future design processes for therapeutic purposes, based on and exploiting supramolecular scaffolds of peptides.

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Atomistic simulations of nucleic acid-processing motors

Motor proteins such as helicases play essential roles in genome replication and transcription. A central question is how the chemical energy from ATP hydrolysis is used by these molecular machines to unwind and translocate nucleic acid strands. Molecular dynamics (MD) simulations of such systems are limited owing to the long timescale of their conformational changes. Here we present our effort in studying motor action at biologically relevant timescale by combing MD simulations with enhanced sampling techniques. We first show simulations of an exemplary ring-shaped hexameric helicase (Rho), in which the six subunits via highly coordinated relative motion translocate a single-stranded RNA substrate. An essential allosteric control pathway that links substrate dynamics with the ATP hydrolysis cycle is identified. Next we present our recent results in direct characterization of how a four-domain helicase switches from unwinding DNA to rezipping DNA after changing its shape. Our work illustrates the molecular basis for the stepwise translocation of helicases and provides insights into the structure-function relationships of these machines.

Day 2 : Recombination filaments: plasticity and function

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Showing how to make a RAD51 filaments

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Boundaries Play Crucial Roles in RecA Mediated Homologous Recombination

RecA nucleoprotein filaments form on single-stranded DNA (ssDNA). The filaments search double stranded DNA (dsDNA) for a sequence region that is homologous to the invading ssDNA bound to the primary binding site in RecA (site I). Homology is tested by attempting strand exchange. Strand exchange forms a heteroduplex product that pairs the invading strand in site I with one of the strands in the dsDNA. Homology testing is preceded by initial homology independent contacts in which the dsDNA is bound to flexible C-terminal domains in the ssDNA-RecA filament[1, 2]. The sequence independent binding to the flexible C-terminal domains allows homology testing to incorporate one dimensional diffusion[1, 2], hopping between nearby sequence regions,[1, 2] and intersegmental transfers.[3] After binding to C-terminal domains, favorable interactions between a negatively charged DNA backbone and positive residues in the secondary RecA binding site (site II) extend the dsDNA and position it to attempt strand exchange. The number of base pairs that can initially bind to site II is limited by the tradeoff between the favorable electrostatic energy, which depends linearly on the number of base pairs bound to site II, and the non-linear unfavorable mechanical energy due to the mechanical stress [2] Atomistic molecular dynamics simulations indicate that if the dsDNA is homologous with the

invading strand, then 3 successive base duplets strand exchange and form heteroduplex products bound to site I. Boundaries between the heteroduplex and the flaking homoduplex tails play important roles. For example, in the simulations strand exchange is not observed if the site II bound dsDNA is not flanked by homoduplex tails. [2] Furthermore, the homoduplex tails promote homology stringency by driving reversal of exchange products formed by regions of accidental homology. [4] The boundaries between RecA monomers also play important roles. For example, nucleoprotein filaments in which some interfaces between RecA monomers have bound ADP and other have bound ATP have more unstable heteroduplex products than filaments with only bound ATP.[4, 5] The presence of mixed interfaces also drives the unbinding of dsDNA from site I, whether that dsDNA is formed by strand exchange [4], direct binding of RecA to dsDNA [6], or annealing to ssDNA in site I. Importantly, applying an external force > 30 pN to the ends of dsDNA bound to site I prevents ATP hydrolysis driven unbinding of dsDNA from site I.[6]

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FRET-based real-time analysis provides new insights into Rad51-driven DNA strand exchange reaction

Rad51 forms a nucleoprotein filament on single-stranded DNA (ssDNA) named to promote DNA strand exchange during homologous recombination. This filament named presynaptic filament binds to double-stranded DNA (dsDNA), searches for homology, and promotes transfer of the complementary strand, producing a new heteroduplex. We previously developed a real-time assay for DNA strand exchange reaction by fission yeast Rad51. The result shows that strand exchange by Rad51 proceeds via two distinct three-strand intermediates, C1 and C2. The first complex C1 contains the intact donor duplex. On the other hand, the second complex C2 contains newly formed heteroduplex DNA (ref 1). RecA-family recombinases including Rad51 possess the conserved DNA binding sites, Site I and Site II. Site I contains the conserved motifs, loop 1 (L1) and loop 2 (L2). Our mutant analysis with a mutation of the conserved Arg in L1 suggests that L1 is involved in the C1 complex formation. On the other hand, mutant analysis with a mutation of the conserved Val in L2 suggests that L2 mediates the C1-C2 transition, producing the heteroduplex. In addition, mutant analysis suggests that Site II serves as the DNA entry position for initial Rad51 filament formation, as well as for donor dsDNA incorporation (ref 2).

1. Two three-strand intermediates are processed during Rad51-driven DNA strand exchange. Ito K, Murayama Y, Takahashi M, Iwasaki H. ***Nature Struct Mole Biol*** (2018) **25**: 29–36.
2. Real-time tracking reveals catalytic roles for the two DNA binding sites of Rad51. Ito K, Murayama Y, Kurokawa Y, Kanamaru S, Kokabu Y, Maki T, Argunhan B, Tsubouchi H, Ikeguchi M, Takahashi M, Iwasaki H. ***Nat Commun.*** (2020) 11. 2950.

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Structural changes of RecA protein/DNA complex filament promoted by ATP/Mg^{2+} analyzed using small-angle X-ray scattering and compared with its models

RecA protein promotes DNA strand exchange for homologous recombination. For the reaction, RecA binds at first single-stranded DNA with ATP and forms a filamentous complex. Its structure was modeled from the X-ray crystal structure of RecA oligomer. We like to know if the model fits well with the structure of long filament in solution and how ATP affects the structure. Furthermore, we recently observed Mg2+ , which is required for the activation of RecA, also affects the RecA structure. To elucidate this structural change, we investigate the filament structure by measuring the SAXS pattern and comparing it with the computed scattering profile from the model. The scattering profile of RecA-DNA filament clearly depends upon ATP, which is in good agreement with the theoretical one.

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Exploring RecA filament deformations by *in silico* sampling of RecA/RecA binding geometries

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Protein filaments active in homologous recombination can exist in different forms depending on parameters such as the salt concentration, the presence or absence of bound DNA or the hydrolysis state of the bound cofactor (ATP or ADP), or the binding of accessory proteins. Their response to changes in the environment or to applied mechanical forces is mediated by variations in the subunit-subunit binding geometries, that can vary from small interface changes to large displacements. Relating the interface geometry to the overall filament shape can help decipher how changes in the environment influence the physicochemical and mechanical properties of the recombination filaments. Using docking simulations to sample the possible binding modes between two consecutive subunits, we have shown that the preferred RecA-RecA binding geometries are encoded on the surface of the protein rigid core. Interface sampling via molecular dynamics simulations has also proven useful for the integration of experimental information from SAXS experiments into atomic-level models. Finally, we will discuss the possibility that different RecA-RecA interfaces can coexist within the same filament, and its consequences for the homologous recombination process.

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RAD52 integrates within RAD51 presynaptic filaments and stimulates homology search mechanism in humans

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Homologous recombination (HR) ensures the high-fidelity repair of double-strand breaks and stalled/collapsed replication forks. HR uses a homologous template to accurately repair DNA and maintain genome stability. In the early stages of HR, RAD51 assembles on the newly formed single stranded (ss) DNA in presence of Replication protein A (RPA) and forms the presynaptic filaments responsible for homology search by probing and interaction with double stranded (ds) DNA, resulting in synaptic complexes formation. Once homology is identified, base-pairing and bound proteins stabilize a synaptic complex preceding the strand intertwining that creates a displacement-loop (D-loop). RAD51 is assisted by a number of partners from filament formation and architecture to its strand invasion activity, among them, RAD51 mediators are proteins helping filament assembly and stabilization either by catalyzing RAD51 nucleation on ssDNA or by decelerating its dissociation from ssDNA. Whereas in yeast, Rad52 plays this Rad51 mediator role, in humans, BRCA2 has been shown to nucleate RAD51 filaments onto ssDNA covered by RPA, ensuring the function, on the other hand, the human RAD52 was considered to be a dispensable activity in light of the mild phenotype of the knocked out mice (notably in response to DNA damaging agents). However we have observed that silencing RAD52 led to a 40% decrease in gene conversion, its precise function in HR mechanism remaining enigmatic. To better understand RAD52 molecular mode of action, we have used Electron Microscopy (EM) by studying the effect of RAD52 on RAD51 filament formation, architecture and activity. We showed it does not help RAD51 filament assembly on RPA-covered ssDNA but rather interacts with RPA-ssDNA resulting in its compaction. Interestingly, RAD52 fixation on RPA covered ssDNA decreases the ability of BRCA2 to catalyze RAD51 filaments assembly, then inhibiting BRCA2 mediation activity. In addition, RAD52 can form mixed filaments with RAD51 that are 2 to 3 times more active in homology search and strand invasion processes.

Day 3 : Dynamics of oligomeric filaments

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Deciphering the conformational dynamics of a type IV pilus

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Ftsz monomer interactions and their importance to explain filament properties

FtsZ is a cytoskeletal bacterial tubulin-like protein. In vivo it is attached to the inner membrane of the bacteria by binding other membrane proteins. This protein is able to homopolymerize and form dynamic filaments that can adopt different shapes. These filaments can acquire the form of a contractile ring (called Z-Ring) that helps the bacteria to constrict its membrane during the cell division process. Depending on the experimental conditions like the crowding agents or the attachment over a surface, the FtsZ polymers display a wide variety of properties and characteristics like curvature, torsion, bundling, treadmilling or cooperativity. Here I summarize these properties and theoretical models that aim to relate the shape of the filaments observed experimentally with the monomer flexibility and interactions.

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Conformational dynamics of nuclear-encoded PPR proteins regulating gene expression in organelles