A well-studied model of partition is that of the *E. coli* sex factor, F plasmid. We reconstituted its partition system by introducing fluorescent SopA, SopB and plasmid (encoding *sopC*) into a DNA carpeted flowcell (nucleoid biomimetic) and visualized the system dynamics using TIRFM. We found SopA-ATP dynamically bound the DNA carpet, and the steady-state carpet density was lowered by SopB. When all components were infused onto the DNA carpet with ATP, the plasmids transiently bound the carpet with colocalized SopA and SopB. Over time, the static plasmids released SopA, began to wiggle, and finally popped off when little to no SopA remained. The results suggest that plasmid movement and release are coupled to SopB stimulated ATP-hydrolysis by SopA. The tethered particle motion of some wiggling plasmid clusters produced SopA craters on the DNA carpet, suggesting that plasmid-bound SopB not only releases SopA on the plasmid but also releases nearby SopA on the DNA carpet. We propose SopB communicates with both SopA on the plasmid and nucleoid to control the transport of plasmid via an ATP-driven diffusion-ratchet mechanism.
Study of this unique transport system is vital because surface-mediated patterning as a means for cargo carrying is inherently different from the classical motor protein or the actin/microtubule-mechanisms of transport.

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Dynamic Self-Organization of Bacterial DNA Segregation Machinery in a Cell-Free Reaction
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Bacterial plasmids have evolved segregation machineries to partition replicated DNA to the daughter cells at cell division. P1 phage lysogenizes as a low-copy-number plasmid in Escherichia coli. Its partition system consists of three components, a centromere-like region, parS, an adaptor protein that binds to the centromere, ParB and a partition ATPase, ParA. In general, it is known that a ParB/parS partition complex is formed when ParB oligomerize onto the centromere. This large nucleoprotein complex interacts with ParA and is thought to couple ATP hydrolysis to drive the movement and segregation of plasmids to opposite cell-halves. To understand ATP-driven DNA segregation, we reconstituted the P1 plasmid partition system in a cell-free reaction and visualized the spatiotemporal dynamics using TIRF microscopy. We coated a flow cell surface with non-specific DNA to mimic the bacterial nucleoid surface and flowed in the three-component reaction system. We found that ParA coats the artificial nucleoid creating a reference scaffold for plasmid movement. ParA assembles onto the ParB/parS complexes and anchors them onto the ParA-coated nucleoid surface. ParB stimulates ParA disassembly leading to vigorous Brownian motion of the plasmid as the complex loses bridging interactions with the nucleoid. The plasmid detaches from the nucleoid surface leaving a hole devoid of ParA, which is refilled rapidly with ParA rebinding onto the nucleoid. FRAP experiments demonstrate the dynamic exchange of proteins on the nucleoid surface and the partition complex. We present a Par partition model of ParB-stimulated ParA assembly/disassembly triggering dynamic instability leading to plasmid segregation and movement.

Neutron & X-Ray Scattering

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Accurate Structures, Conformations, and Assemblies of Macromolecules in Solution by X-Ray Scattering (SAXS) using Quality Control Parameters
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Conformations and assemblies of proteins, DNA, and RNA, plus their detailed structural chemistry, encode key information needed to define biological outcomes in cell biology. We are developing SAXS combined with crystallography as a premiere tool for defining macromolecular conformations and connections suitable to join proteins to pathways and at the proteomic scale1. Crystallography supplies unsurpassed structural detail for mechanistic analyses. Yet, advances in SAXS are making this technique increasingly powerful and robust for efficiently examining complexes in solution, as aided by interfaces allowing biologists to do these experiments2-3. Structures of flexible filaments of the XLF with XRCC4·Ligase IV4-5, the conformational and flexible Rad50 link to Mre116, as well as of DNA-PKcs in complex with Ku and DNA7 support the promise of SAXS for examining the assemblies and conformations of dynamic complexes in solution8. In principle, SAXS can provide reliable solution data on small and large macromolecules1-2. In practice, SAXS can be limited by problems in samples and analyses, which can be reduced or avoided2. SAXS has not been perceived as a legitimate structural technique largely due to previous limitations from data collection and interpretation. Our emerging results show that SAXS has great potential to provide accurate shapes, conformations, and assembly states in solution and inform biological functions in fundamental ways1-7. Our results furthermore show that many SAXS limitations can be overcome by improved sample characterization, quality control parameters and methods to assess model accuracy.

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Data Collection and Processing for Simultaneous Small- and Wide-Angle Protein Solution Scattering
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The procedures used at beamline X9 of National Synchrotron Light Source for collecting and processing protein solution scattering data are described. The scattering data are collected simultaneously on two CCD detectors, one (SAXS) located at the end of the ~2.5m-long flight tube and the other (WAXS) at ~0.4m from the sample in vacuum. A package of python scripts, named pyXS, are used to apply data corrections pertain to the scattering geometry and reduce the 2D SAXS and WAXS CCD images to a combined 1D curve spanning the full q-range, typically from 0.007 $Å^{-1}$ to 2.0 $Å^{-1}$. The subtraction of buffer scattering is performed based on either the intensity of water scattering peak near 2.0 $Å^{-1}$, or the combination of the protein volume fraction and transmitted beam intensity. Fluorescence from a salt solution is utilized to calibrate the X-ray energy as well as to determine the flat field response of the detector and the geometric corrections. Details of the data conversion and corrections, as well as example data, will be presented.

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Solution Conformation of Extracellular Matrix Proteins
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The extracellular matrix (ECM) is composed of large, multi-domain proteins with varying degree of shapes and post-translational modifications. Therefore, it is challenging to study such proteins with a single technique to gain reliable information about their size, shape and conformation. We have employed a multidisciplinary approach using a variety of techniques to investigate the solution conformation of laminin gamma-1 short arm, netrin-4 and G3 domain of agrin fused with human IgG-Fc (G3Fc). Briefly, the hydrodynamic properties of entire multi-domain proteins were studied using analytical ultracentrifugation, dynamic light scattering, and small angle X-ray scattering (SAXS). Further, the information from SAXS for entire multi-domain protein was then either used to determine ab initio structure or combined with high-resolution data for individual domains to obtain a detailed solution structure of desired multi-domain protein. Additionally, the SAXS models were verified by comparing the experimentally determined hydrodynamic parameters with the parameters calculated from solution structures. The ab initio structures for laminin gamma-1 short arm revealed an extended and curved assembly. We could also combine small portion of high-resolution structure from X-ray Crystallography data to obtain rigid-body models. Further, we found that the netrin4 has an elongated shape in solution using ab initio methods. Remarkably, we found that the G3Fc chimera is T-shaped and not Y-shaped like an antibody in solution by rigid body modelling. Thus, we conclude that by combining various methods, it is possible to explore large muldi-domain protein and protein-protein assemblies that will help enable us to understand structure-function relationship of these proteins with various forms of diseases.

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Structural Studies of Septin2G Amyloid Fibrils
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Septins are proteins from the GTP-binding family and participate in cell division cycle performing functions such as secretion and cytoskeletal division. They can also be found in neurodegenerative conditions as Alzheimer's and Parkinson's diseases, forming highly organized fiber-like aggregates known as amyloids. In this work, we used small angle x-ray scattering (SAXS) to investigate the formation and time evolution of septins aggregates under the influence of temperature and concentration.