Posters

Protein Dynamics II

2272-Pos
Collective Dynamics Underlying Allosteric Transitions
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Allostery is essential for regulation in many biological systems. In allosteric systems the binding affinity of an active site depends on the binding in a distant binding site. The information flow between these sites is communicated through a conformational change of the system. Usually two conformational states are distinguished: The T-state (tense) with a low binding affinity in the active site and the R-state (relaxed) with a high binding affinity. The collective motion responsible for the conformational change from the T- to R- state and vice versa is known as the allosteric transition.
In this work allosteric transitions in proteins are studied. From Molecular Dynamics (MD) simulations collective motions are extracted using Principal Component Analysis (PCA) and similar methods with the aim to identify the molecular determinants underlying allosteric transitions. As a first example system the chaperon complex GroEL/GroES is employed. For this system cooperativity within one ring and anticooperativity between the two GroEL rings is known to take place depending on the binding of nucleotides and binding of the co-chaperonin GroES.

2273-Pos
Dynamic Analysis of Phosphatase 1B WPD Loop
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Protein tyrosine phosphatase 1B (PTP1B) plays a key role as negative regulator of insulin and leptin signaling, and is therefore a major molecular target for the treatment of type II diabetes and obesity. WPD loop is a key element in the mechanism of PTP1B catalysis. In the apo form, WPD loop is usually in an "open" conformation, whereas it closes over the active site upon substrate binding. Here, targeted molecular dynamics (TMD) simulations are reported to examine the transition of the WPD loop between the open and closed states and as well as the effect of this motion on the PTP1B conformational activation mechanism. Our results indicate that WPD loop motion is governed by hydrophobic interactions between the WPD loop, loop 11 and α3 and α6 helices. The simulations are repeated in the presence and absence of the α7 helix. The hydrophobic interaction network is better maintained in the presence of the α7 helix, suggesting that the high mobility of α7 helix allows the transition between the open and closed states of the WPD loop. In addition, the formation of a hydrogen bond between the backbone oxygen of Trp-179 and the sidechain nitrogens of Arg-221 is observed to mediate the closure of WPD loop. Elucidating the detailed mechanism of PTP1B conformational activation will guide future drug design efforts toward type II diabetes and obesity.

2274-Pos
A Method to Study the Time-Dependent Remodeling of High Density Lipoproteins by Individual-Particle Electron Cryo-Tomography
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High density lipoprotein (HDL) levels are inversely associated with cardiovascular risk. HDL comprises numerous subclasses of particles which are diverse in terms of size, shape, structure and function. In terms of defining HDL as a therapeutic target, it is important to understand how the various subclasses undergo time-dependent rearrangements into functionally diverse sub-populations of particles.
The structure of HDL subclasses was examined after storage for several months to over one year at 4°C. The subclasses include discoidal reconstituted HDL (rHDL) 7.8, 8.4, and 9.6 nm in diameter and 9.3 nm spherical rHDL. As judged by SDS-PAGE, long-term storage did not affect the integrity of apolipoprotein (apo) A-I. However, long-term storage did result in the remodeling of some of the discoidal and spherical rHDL into large ($>$ 12nm) and very small particles ($<$7nm) that were not present in the original samples. To determine if the large particles were generated by fusion of the smaller particles, we used individual-particle electron cryo-tomography (IPET, see Lei Zhang and Gang Ren' abstract for detailed methodology) to reconstruct three-dimensional density maps of individual HDL particles. We observed large size discoidal rHDL 12nm, 17nm and 24nm in diameter. Discoidal rHDLs with partially merged surfaces, possibly fusion intermediates, were also apparent. Some of these intermediates were associated with very small particles (~6-7nm). Given that very small particles $<$7nm in diameter were not found in the original samples, but appeared after long-term storage, it is possible that the very small particles were generated and expelled from the fusion of the large discoidal particles. These observations suggest that HDL can rearrange into more energetically stable configurations by fusion. This approach provides a novel methodology to study the time-dependent HDL remodeling processes.

2275-Pos
Structure of 9.6nm Discoidal High-Density Lipoprotein Revealed by Individual-Particle Electron Tomography
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Human high-density lipoprotein (HDL) conveys excess cholesterol from peripheral tissues to the liver and steroidogenic organs for clearance during reverse cholesterol transport (RCT). The majority of HDL's ability to reverse or prevent the onset of cardiovascular disease is derived from HDL's mediation of RCT. HDL particles in vivo vary in size, shape, components, and biological functions. 9.6nm discoidal HDL (140-240 kDa) is the disk-shaped precursor of mature spherical HDL and contains phospholipids and two apolipoprotein A-I (apoA-I) molecules. Discoidal HDL is a critical intermediate between lipid-poor apoA-I and mature spherical HDL during HDL assembly as preferential substrate for lecithin:cholesterol acyl transferase (LCAT), the enzyme that loads cholesterol ester onto HDL. However, HDL structure determination is frustrated by the dynamic nature and heterogeneity of HDL.
Here we have studied the structure of 9.6nm discoidal HDL by individual-particle electron tomography (IPET), e.g. the combination of electron tomography (ET) and our newly developed local refinement reconstruction program (see details of the method in Lei Zhang and Gang Ren's abstract). Individual HDL particles were imaged by ET with tilt angles ranging from $-70°$ to $70°$ in steps of $1°$. By tracking each individual HDL particle image from the tilt series of micrographs after their contrast transfer function correction, we reconstructed three-dimensional (3D) density maps of each particle using our local-area refinement reconstruction program. By analysis of the 3D density maps, we propose a structural model for 9.6nm discoidal HDL that is in general agreement with the reported double-belt model. In our model, however, the two N-terminal domains are mainly alpha-helical, are oriented parallel to each other and fold back into the lipid-bilayer region near the center of disc. This research suggests the IPET is a novel method for the structure study of lipoproteins.

2276-Pos
Monitoring the Structural Changes of Conjugated Antibodies by High-Resolution Electron Microscopy and Individual-Particle Electron Tomography
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Peptides have promise as potent and selective drug candidates but have not succeeded primarily because of poor pharmacokinetics. The fusion of these peptides to our scaffold antibody has produced molecules, CovX-Bodies, which protect the peptide from renal elimination and/or enzymatic degradation. However, it is possible that the fusion process may in itself affect the intrinsic properties of the antibody scaffold (eg. effector function). Changes in function may relate to changes in structure. However, the structural study of highly dynamic and structurally heterogenous antibodies by X-ray crystallization and NMR is extremely difficult. These studies were designed to examine structural changes that may have occurred during the conjugation process.
Here, we studied the structural changes of antibodies with the high-resolution electron microscopy and individual-particle electron tomography (IPET, for details, see the abstract presented by Lei Zhang and Gang Ren). We found 1) the average angle between the Fab regions was 55 degrees +-15 in unconjugated antibodies, but 40 degrees +-10 in unconjugated antibodies and 2) the average sizes of the Fc domain were similar regardless of conjugation. However, 3) the Fc domain shape differed significantly depending on conjugation. The Fc domains of conjugated antibodies were significantly elongated ($>$30%). Using IPET, we reconstructed a dozen of three-dimensional density maps of individual conjugated and unconjugated antibodies. Comparing the maps, it showed that all three domains of conjugated antibodies were elongated after fusing with the drug. This example

demonstrates that this method could be a useful tool for monitoring the structural changes of antibodies.

2277-Pos
Determining the Dynamic Protein Structure by Individual-Particle Electron Tomography: An Individual Antibody Structure at a Nanometer Resolution
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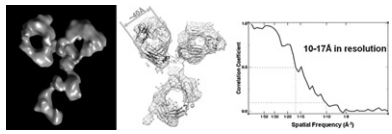
Antibodies are naturally dynamic, flexible, and structurally heterogeneous. However, the structural heterogeneity has made difficult the structural and functional study by current technologies, such as X-ray crystallography, NMR and single-particle electron microscopy.
Here, we report a method to study the antibody structure. The method, which we called individual-particle electron tomography (IPET), is the combination of current electron tomography (ET) technology with our reconstruction program for resolving the high-resolution structure of an individual particle (see our other abstracts). First, we used ET to image an individual antibody from a series of tilt angles. Then, we tracked the targeted antibody and windowed its images. Finally, we used our local refinement program to reconstruct the three-dimensional (3D) density map of the antibody (Figure). The map contained rich structural details, including the holes in the Fab domains, and was the highest resolution map ever obtained from an individual object (resolution was ~1nm). Comparing the maps from different antibodies, it allowed us to study antibody dynamics and mobility characteristics. Thus, we propose IPET as a novel method for the structural and functional study of the highly dynamic proteins.



2278-Pos
Structural Determination of Heterogeneous Protein by Individual-Particle Electron Tomography - Combination of Electron Tomography and Local Refinement Reconstruction Method for High-Resolution Structural Determination of Each Individual Protein Particle
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The dynamic nature and structural heterogeneity of proteins is essential to their function. However, the purification of these proteins, such as lipoproteins, liposomes, and antibodies, is difficult or even impossible. Heterogeneity prevents structural determination by current technologies, such as X-ray crystallization, NMR, and even single-particle electron cryo-microscopy (cryo-EM).
Here, we report a method to determine the structure of heterogeneous proteins by determining the structure of individual protein particle. This method, which we call individual-particle electron tomography (IPET), is the combination of current electron tomography (ET) technique with our reconstruction program. In brief, we used ET to image each individual protein particle from a series of tilt angles ranging from -70° to $+70^\circ$. Then, we reconstructed the 3D density map from each individual particle's images by searching each image's global center within the particle local area via an iteration and refinement algorithm and a set of dynamic filters and maskers. This method was particularly effective in processing the highly noisy and low-contrast cryo-EM images for generating the high resolution 3D density map. To elucidate our methodology and demonstrate its capabilities, we used a set of simulated cryo-EM data, a tilt series of images based on an individual transporter (~100kDa). The reconstructed 3D density map contains fine structural details, such as α-helices, at high resolution (beyond 10Å indicated by the Fourier shell correlation analysis). This program works robustly on both simulated data and real data (see our other abstracts). Considering IPET is the only method for high resolution structural determination based on an individual object of protein, we propose it as a novel method to study the structure of highly dynamic and heterogeneous proteins.

2279-Pos
Protein Aggregation as a Phase Transition Process
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In this model we attempt to mimic the aggregation pattern found in real proteins using a modified Ising model. Similar to a Blume-Campel model, we restrict the proteins to occupy discrete lattice points and define a pairwise interaction depending on the configuration of the protein (as in the Potts model). We define three specific conformational states: native, intermediate and unfolded and consider the aggregation process as a series of second-order phase transitions. In this framework we can analyze the zeros of the partition function analytically continued onto the complex plane, specifically the Yang-Lee and Fisher zeros. Using combinatorial arguments we can simplify the partition function and predict the occurrences of phase transitions as a function of the interaction potential and protein density.

2280-Pos
A Pharmacological Chaperon Preventing the Partial Unfolding of Prion Protein
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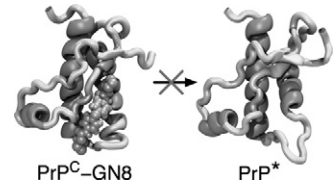
Prion diseases result from the conformational conversion of a normal prion protein (PrP^C) into an abnormal isoform (PrP^{Sc}). Recently, we discovered an anti-prion compound GN8 to interfere with the pathological conversion of prion protein; however, the mechanisms underlying the anti-prion activity remained unresolved. In this study, we provide the first evidence in supporting the chemical chaperon hypothesis that GN8 acts as a chaperon to stabilize the normal form of PrP^C. We performed a comparative study between different conformations of PrP^C with and without GN8 binding based on molecular dynamics (MD) simulations. We employed urea-driven unfolding simulations to determine if GN8 prevented the conformational conversion of PrP^C. One important finding was that GN8 efficiently suppresses local fluctuations and prevent the partial unfolding of PrP^C under denaturing perturbation. We concluded that GN8 inhibits the pathological conversion of PrP^C to PrP^{Sc} by suppressing the population of the intermediate state of PrP*. Our basic principle in this study constitutes a promising strategy with which to approach a dynamic-based drug design of therapeutic chemical chaperons for conformational diseases related to protein misfolding.



2281-Pos
Viscosity Effects on the Rates of Short Helical Peptide Conformational Dynamics in Neat Alcohol Solution
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We have used ^{13}C dynamic NMR spectroscopy to determine the rate constants for the conformational dynamics of a 3_{10} helical hexameric peptide, Z-$(Aib)_6$-OtBu (Aib = residue of alpha-aminoisobutyric acid). Because the strongly helix-promoting Aib residue is achiral, oligomers of Aib will form left- and right-handed helices with equal probability. Furthermore, these helices interconvert, through a large number of single bond rotations, between left- and right-handed 3_{10} helical forms on a timescale that is measurable via NMR spectroscopy. We have measured rate constants for this interconversion in a series of solvents of varying viscosities, including 1-, 2-, and 4-carbon alcohols, measured at temperatures between ~ 3°C and ~43 °C. Measurements in neat solvents is in contrast to the studies that seek to quantify the role of solvent viscosity using viscogens such as glycerol, ethylene glycol, and glucose added to dilute aqueous solutions of biopolymers. We have observed that, at low temperatures, the solvent viscosity limits the rate of the conformational dynamics of this peptide in a 1/eta fashion, consistent with Kramers' diffusional model of reaction dynamics in a viscous medium.

2282-Pos
Ultrafast Dynamics of Thermal Denaturation of Cytochrome-C with Two Dimensional Infrared Spectroscopy
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Native and thermally denatured heme axial methionine mutant cyt c552 from Hydrogenobacter thermophilus (Ht-M61A) is studied by various spectroscopic methods at various temperature points along the denaturation curve. Circular dichroism and steady state IR spectroscopy reveal that there are two distinct states corresponding to native and denatured populations of the protein. Ultrafast dyanmics are studied with 2-dimensional infrared echo spectroscopy (2D-IR) with the heme-bound CO as the vibrational probe. 2D-IR is sensitive to the global dynamics of the protein within the experimental window of ~100 ps. The spectral diffusion of the echo signal at different denaturation points is calculated, and the possible hydrophobic collapse in unfolding is discussed.