

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.

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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 $\alpha 3$ and $\alpha 6$ helices. The simulations are repeated in the presence and absence of the $\alpha 7$ helix. The hydrophobic interaction network is better maintained in the presence of the $\alpha 7$ helix, suggesting that the high mobility of $\alpha 7$ 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.

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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.

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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.

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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 heterogeneous 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 $\pm$ 15 in unconjugated antibodies, but 40 degrees $\pm$ 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