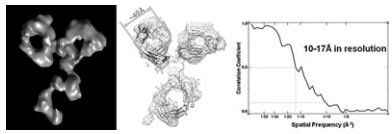
demonstrates that this method could be a useful tool for monitoring the structural changes of antibodies.

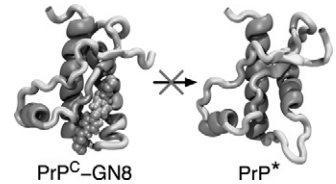
2277-Pos
Determining the Dynamic Protein Structure by Individual-Particle Electron Tomography: An Individual Antibody Structure at a Nanometer Resolution
Lei Zhang, **Gang Ren**.
University of California, San Francisco, San Francisco, CA, USA.
Antibodies are naturally dynamic, flexible, and structurally 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
Lei Zhang, **Gang Ren**.
University of California, San Francisco, San Francisco, CA, USA.
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
Travis A. Hoppe, Jian-Min Yuan.
Drexel University, Philadelphia, PA, USA.
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
Norifumi Yamamoto, Kazuo Kuwata.
Gifu University, Gifu, Japan.
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
Matthew A. Kubasik.
Fairfield University, Fairfield, CT, USA.
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
Jean K. Chung[1], Megan C. Thielges[1], Sara Bowman[2], Kara Bren[2], Michael D. Fayer[1].
[1]Stanford University, Stanford, CA, USA, [2]University of Rochester, Rochester, NY, USA.
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.