both side-chain rotamer preferences and for optimal amino acid sequence. These results pose a series of questions not just about fixed-charge, pairwise decomposable sequence design models, but also our novel polarizable, many-body optimization algorithms and how to use both approaches synergistically.

**1705-Plat**

**De Novo Design and Biophysical Characterization of an Affinity-Enhanced Protein Displaying the Structure of the Broadly Neutralizing HIV-1 2F5 Antibody Epitope**

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Current HIV-1 vaccine development approaches focus on stimulation of pan-neutralizing antibodies (pnAbs) that recognize functional sites on the HIV-1 Envelope spike. Although conserved, these neutralizing epitopes are masked or structurally dynamic, resulting in weak antibody response in natural infections. Only rare individuals develop an antibody response directed to them. Our goal is to engineer antigenic structures capable of exposing a stable conformation of these epitopes and thus inducing B-cell secretion of HIV-1 pnAbs. The Envelope membrane-proximal external region (MPER) is one of those target sites for the pnAbs 2F5, Z13 and 4E10. We postulated that conformationally stable structures are better immunogens and can elicit the production of better antibodies. To test this hypothesis we have engineered a protein with increased exposure of a stable conformation of the 2F5 epitope. We grafted the 2F5 epitope into a scaffold protein Top7 (Top7-2F5 chimera). Molecular dynamics (MD) simulations were used to engineer the grafting site and to assess protein stability. Circular dichroism analysis confirmed the remarkable structural stability of Top7-2F5. ELISA and surface plasmon resonance assays demonstrate that 2F5 pnAb showed a superior affinity to the Top7-2F5 (1.4e-9M) as compared to 9-mer (7.06e-8M) and 23-mer MPER (1.27e-8M) peptides. MD analysis indicated that the structure of the 9-mer residues grafted into the Top7 is 3-fold more likely to correspond to the native conformation as compared to 23-mer MPER peptide. Following, 2F5-specific memory B-cells from patient-derived PBMCs were identified by flow cytometry. The Top7-2F5 ability to activate the identified cells and to induce pnAbs secretion in vitro is currently being evaluated. These results show that MD simulations can help engineer vaccine antigens capable of enhancing protective antibody responses.

**1706-Plat**

**A Tool to Integrate User Expertise into Building Atomic Level Models for Large Biomolecular Systems**

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The amount of available structural data for large macromolecular multi-protein complexes that carry out critical cellular processes have continuously increased in recent years. This increase is mainly due to advances in cryo-electron microscopy (cryo-EM). However, structure analysis still face serious challenges in dealing with highly flexible or multi-conformational domains; in fact structures of the latter are often inaccessible to experiments alone. To tackle these challenges computational modeling can be employed in combining structural data from cryo-EM, X-ray crystallography, and NMR spectroscopy. Instead of following the common strategy in macromolecular modeling, namely automating the process of computer aided structure analysis to avoid human bias, we integrate instead user expertise into an interactive version of model building. In our respective tool, Rosetta/MDFF, a user is guided by experimental data and automated structure prediction. Our approach enabled us to resolve the missing segments of mechanistically crucial subunits of the 26S proteasome, a 2.5 MDa multi-subunit molecular machine, which is a key player in protein degradation in cells. Incorporating user expertise into model building, in particular, is useful for a system, as complex as the proteasome since automated procedures may fail in dealing with the characterization of complicated, ambiguous structural regions. The interactive feature of Rosetta/MDFF allows a user to manipulate structures during molecular dynamics flexible fitting (MDFF) by manually pulling them to the desired regions of density.

**1707-Plat**

**Residue Environment Score for Selecting Protein Structure Models and Protein-Protein Docking Models**

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In protein structure modeling area, it is critical to have an accurate scoring function that can select near-native structure models from a pool of alternative models. I have developed a novel scoring function that captures multi-residue environment in a protein model. The method called PRESCO (Protein Residue Environment SCO) uses a new representation of local amino acid environments in protein structures called the Side-chain Depth Environment (SDE). An SDE defines a local structural environment of a residue considering the coordinates and the depth of amino acids that locate in the vicinity of the side-chain centroid of the residue. The good performance of PRESCO was also proved in the last Critical Assessment of Techniques in Protein Structure Prediction (CASP 11), a world-wide protein structure prediction experiment. PRESCO method was ranked the top in the free modeling category among over 200 participated groups. (http://predictioncenter.org/casp11). In this presentation, I will further discuss that PRESCO performs well in recognizing near native complex structure models among other models in protein-protein docking prediction. I will show that PRESCO recognizes specific residue environment of residues at protein docking interface, which leads to successful distinction of near-native complex structure models from wrong models.

## Platform: Protein Assemblies

**1708-Plat**

**Specific Interaction of a Naturally Occurring Amyloidogenic Fragment of Streptococcus Mutans Adhesin P1 with Intact P1 on the Cell Surface Measured by Solid State Nuclear Magnetic Resonance Spectroscopy**

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The P1 adhesin of the cariogenic bacterium Streptococcus mutans is a cell surface-localized protein involved in sucrose-independent adhesion and colonization of the tooth surface. The immunoreactive and adhesive properties of S. mutans suggest an unusual functional quaternary ultrastructure comprised of intact P1 that is covalently attached to the cell wall interacting with non-covalently associated proteolytic fragments thereof, particularly the ~50-kDa C-terminal fragment C123 previously identified as Antigen II. S. mutans is capable of amyloid formation when grown in a biofilm and P1 is among its amyloidogenic proteins. The C123 fragment of P1 readily forms amyloid fibers in vitro suggesting it may play a role in the formation of functional amyloid during biofilm development. Using wild-type and P1-deficient strains of S. mutans, we have demonstrated that solid state NMR spectroscopy can be used to 1) globally characterize cell walls isolated from a Gram-positive bacterium; 2) quantify P1 localized on the cell surface; and 3) characterize the specific binding of heterologously expressed, isotopically-enriched C123 to cell wall-anchored P1. Our results lay the groundwork for future high-resolution characterization of the C123/P1 ultrastructure and subsequent adhesion and biofilm formation via NMR spectroscopy, and they support an emerging model of S. mutans colonization whereby quaternary P1-C123 interactions confer adhesive properties important to binding to immobilized human salivary agglutinin.

**1709-Plat**

**Regulation of PKR by RNA: Formation of Active and Inactive Dimers**

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PKR is a member of the eIF2$\alpha$ family of protein kinases that inhibit translational initiation in response to stress stimuli and functions as a key mediator of the interferon-induced antiviral response. PKR contains a dsRNA binding domain that bind to duplex regions present in viral RNAs, resulting in kinase activation and autophosphorylation. An emerging theme in the regulation of protein kinases is the allosteric linkage of dimerization and activation. The PKR kinase domain forms a back-to-back parallel dimer which is implicated in activation. We have developed a sensitive homo-FRET assay for kinase domain dimerization to directly probe the relationship between RNA binding, activation, and dimerization. In the case of perfect duplex RNAs, dimerization is correlated with activation and dsRNAs containing 30 bp or more efficiently induce kinase domain dimerization and activation. However, more complex duplex RNAs containing a 10-15 bp 2'-O-methyl RNA barrier produce kinase dimers but do not activate. Similarly, inactivating mutations within the PKR dimer interface that disrupt key electrostatic and hydrogen binding interactions fail to abolish dimerization. Our data support a model where activating RNAs induce formation of a back-to-back parallel PKR kinase dimer whereas