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Improving Protein-ligand Modeling into Cryo-EM Data and the Use of Those Models in Drug Discovery Efforts

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Producing an accurate atomic model of protein-ligand interactions from the data generated by cryo-electron microscopy is often a challenging problem due to a combination of the noise in the experiment and the dynamic nature of protein-ligand binding. In order to address this problem we have developed ways to combine established computational modeling techniques with EM map potentials to create more accurate and more validated structural models of protein ligand binding.

Here we report on the incorporation of the OPLS3e force field with the VSGB2.1 implicit solvation model into popular Phenix package for real and reciprocal space model refinement. With the advent of the resolution revolution in cryo-electron microscopy, low resolution atomic refinement is more common, and complex force fields may aid in refinement by avoiding implausible structures, due to ligand strain or protein ligand contacts, permitted by the simpler restraints. Our results show significantly improved structure quality at lower resolution for X-ray refinement with reduced ligand strain, while showing only a slight increase in R-factors. For real space refinement of cryo-EM based structures, we find comparable quality structures and goodness-of-fit and reduced ligand strain. In addition, we explicitly show how structure quality is related with the map-model cross correlation as a function of data weight, and how it can be an insightful tool for detecting both over- and underfitting, especially coupled with accurate ligand energies. In addition to the tool, we will present its application to a structure-enabled drug discovery effort to identify modifications to streptogramin class antibiotics with improved resistance profiles.

We will also present modifications to our Glide ligand docking software to allow it to place ligands into unmodeled density. Combining this with the modified version of together can produce one or more poses that are consistent with both the experimental data and computational modeling at a range of resolutions for several ligand types. The pipeline is validated through several published cryoEM structures of complexes in different resolution ranges and various types of ligands. In all cases, at least one identified pose produced both excellent interactions with the target and agreement with the map.

We will also demonstrate using alchemical FEP calculations, along with the affinities of a series of congeneric compounds, to confirm a prospective protein ligand-pose in cases where some ambiguity remained in the atomic details of the protein-ligand binding site. These tools will be valuable for the robust identification and confirmation of ligand poses to enable structure-based drug discovery efforts enabled by cryo-EM.

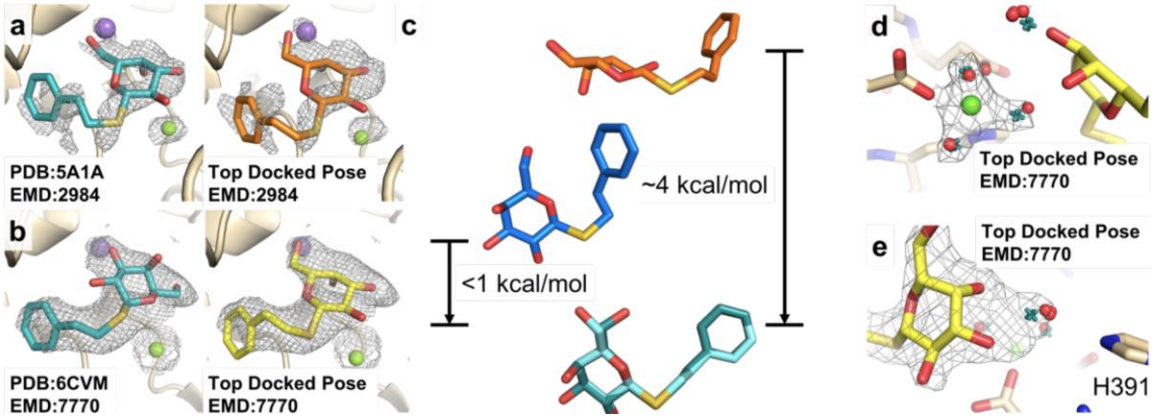

Figure 1. Results for PETG in beta-galactosidase. a, PDB:5A1A with its associated map EMD:2984. The deposited PDB pose is shown in teal, and the best pose obtained from GlideEM is shown in orange. The green and purple spheres correspond to magnesium and sodium ions, respectively. b, PDB:6CVM with its associated map EMD:7770. In teal, the deposited PDB pose and in yellow the best pose from GlideEM. c, A comparison of the deposited poses from PDB:5A1A (blue) and PDB:6CVM (orange) with the overlaid QM optimized geometries (teal). The energies reported are the difference between that conformation optimized with the O-C-S-C dihedral angle fixed and that conformation optimized without any fixed dihedrals. d, e, Results of JAWS calculations performed on the best GlideEM pose for the PDB:6CVM structure. Predicted water sites from triplicate simulations are depicted as red spheres, while real-space refined waters based on those positions are presented as teal crosses, and the map shown is EMD:7770.

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