Modeling protein main-chain flexibility in ligand docking and screening: *How much flexing is tolerated?*

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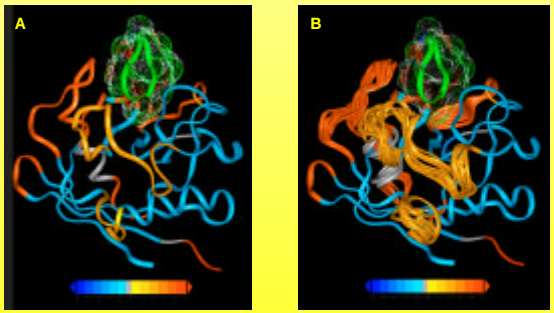
Analysis of conformational changes upon complex formation for a representative set of 39 pairs of ligand-free and ligand-bound structures showed that about 50% of the proteins undergo substantial main-chain and side-chain conformational changes when binding their ligands [1]. We are combining three tools, **SLIDE** for flexible ligand screening and docking, and **FIRST** and **ROCK** for predicting and sampling protein and ligand flexibility, in order to assess how much flexibility is allowed in protein–ligand recognition while still maintaining the specificity of interaction between molecules. This is the first approach, aside from molecular dynamics simulation, to model protein main–chain flexibility for ligand docking, with the advantage of sampling significantly diverse conformers of the protein and ligand.

The human **cyclophilinA (CypA) – cyclosporin** complex serves as an excellent model system for this study for the following reasons:

1. As a peptidyl cis–trans isomerase, **CypA** has to be flexible enough to accommodate both the cis and the trans conformations of its substrate.
2. The ligand, **cyclosporin**, is a peptide–like circular molecule with many rotational degrees of freedom. The same method used to model main–chain flexibility of the target protein can be applied to model the flexibility of this peptidyl ring.

We use the graph-theoretic algorithm, **FIRST** [2], to predict the intrinsic flexibility of the target protein based on the hydrogen–bond and hydrophobic interaction network of a single 3-dimensional structure.

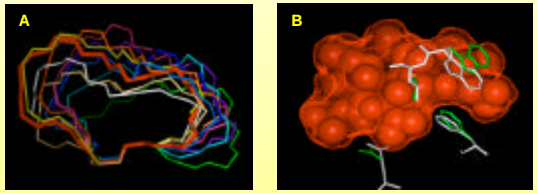


*A. The ribbon diagram of the human **CypA** (PDB code 1bck) colored by the flexibility index as calculated by **FIRST**, where flexibility increases from blue to red, with blue to gray being rigid and yellow to red flexible. The **cyclosporin** molecule, which is an inhibitor of **CypA**, is shown as a green ribbon covered by its dotted Connolly surface.*

*B. The ten most distinct **CypA** conformers out of the set of 500 generated by ROCK.*

1. Betts M.J., Sternberg M.J., Protein Engineering 12: 271, 1999
2. Jacobs D.J., Radar A.J., Kuhn L.A., Thorpe M.F., Proteins 44: 150, 2001

Based on these predictions, we create alternative semi–continuous protein main-chain conformations using the program **ROCK** [3] which employs a Monte Carlo approach (presented in a related poster by Ming Lei). The unique ability of **ROCK** to generate ring conformers is also employed to sample the conformational space available to the circular, peptide–like **cyclosporin** molecule. A database of 5000 small–step (2° angle rotation) conformers are generated starting with the **cyclosporin** conformation found in the X–ray structure of the **CypA–cyclosporin** complex 1bck.

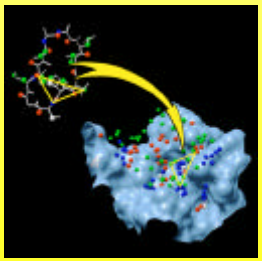


*A. Traces of the backbones of a wide range of **cyclosporin** conformers generated with **ROCK**.*

*B. Side–chain rotations performed by **SLIDE** upon docking **cyclosporin** (red spheres) into the active site of **CypA**. The position of the side chains from the X–ray structure are shown in white and their position after docking are shown in green.*

The ten most distinct main chain conformers of **CypA** (out of the 500 generated ones) and the 5000 ring conformers of **cyclosporin** are used as input to our docking and screening tool, **SLIDE** [4], which models protein side-chain motion during docking. **SLIDE** also models full ligand flexibility except for the case when the ligand is a ring.

*A template consisting of 100–150 points is used to describe the shape and chemistry of the binding site. **SLIDE** matches ligand interaction–point triplets to template triangles. Upon finding a good match, the ligand is docked into the binding site by least–square–fitting. Side– chain flexibility is modeled by rotating protein side–chains and flexible ligands parts to resolve van der Waals overlaps. The successful docking is scored based on the number of hydrogen bonds and hydrophobic complementarity between the protein and the ligand.*

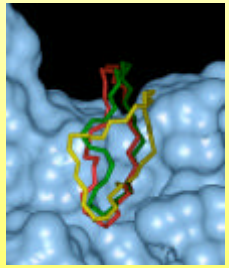


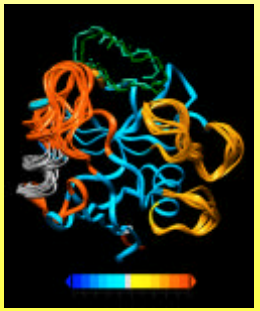
3. Thorpe, M.F., Lei, M., Rader, A.J., Jacobs, D.J., Kuhn, L.A., J. Molec. Graphics and Modeling 19: 60-69, 2001
4. Schnecke V., Kuhn L.A., Perspectives in Drug Discovery and Design 20: 171, 2000

178 different **cyclosporin** conformers could be successfully docked into the binding site of the crystal–structure **CypA** (1bck). The docking was classified as successful if the same part of the ligand was docked into the same area of the more rigid bottom of the binding site as seen in the crystal structure of the protein–ligand complex. The deviations of these conformers from the starting structure were measured by their backbone RMS deviations upon superposition onto the X–ray structure conformer and they ranged from zero up to 1.73 Å.

The conformer of **cyclosporin** seen in the 1bck protein–ligand complex was docked to its receptor with an RMSD of 0.49 Å compared to the crystal structure position. In addition, this **cyclosporin** conformer could be successfully docked (<2.0 Å RMSD) to six out of the ten most diverse **CypA** conformers. These are the ones with the smallest backbone RMSD values (1.038 – 1.30 Å) compared to the crystal structure from the set of most diverse ten with distances between individual backbone atom pairs up to 3.0 Å.

*Examples of **cyclosporin** conformers docked into the active site of **CypA**. The Connolly surface of the binding site is shown in blue, magenta is the original crystal structure conformer, purple is a conformer with a 0.8 Å backbone RMSD and the molecule in yellow is the conformer with the largest backbone RMSD of 1.7 Å that could still be docked reasonably well by SLIDE to the crystal structure **CypA**.*





*The ribbon diagram of the six **CypA** conformers that allowed successful docking of **cyclosporin** together with the backbones of two docked **cyclosporin** molecules: the ones having the smallest (0.49 Å) and largest (1.55 Å) RMSD compared to the X–ray structure position. Protein backbone deviations up to 3.0 Å in the loop regions surrounding the binding site can be observed in these conformers.*

Conclusions

Our results indicate that main–chain flexing in human **cyclophilin A** with displacements up to 3.0 Å is tolerated without hindering the protein's capacity to recognize and bind its ligand in a correct orientation. The ligand can flex even more: backbone displacements up to 4.5 Å are seen between conformers of **cyclosporin** that dock correctly, with the main conformational differences involving residues not buried deeply in the binding site of **cyclophilin A**.