

**WORKSHOP
ON MODELLING OF SPECIFIC
MOLECULAR RECOGNITION
PROCESSES**

**ALTERNATIVE BINDING MODES OF PROLINE-RICH PEPTIDES
BINDING TO THE GYF DOMAIN**

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Recognition of proline-rich sequences plays an important role for the assembly of multi-protein complexes during the course of eukaryotic signal transduction and is mediated by a set of protein folds that share characteristic features. The GYF (glycine-tyrosine-phenylalanine) domain is known as a member of the super-family of recognition domains for proline-rich sequences. Recent studies on the complexation of the CD2BP2-GYF domain with CD2 peptides showed that the peptide adopts an extended conformation and forms a polyproline type II helix involving residues Pro4 - Pro7 [1]. R/K/GxxPPGxR/K is the key signature for the peptides that bind to the GYF domain [2]. In our combined theoretical and experimental study, we show that the peptides adopt a polyproline II helical conformation in the unbound form as well as in the complex. By molecular dynamics simulations we identify a novel binding mode for the G8W mutant and the wild-type peptide (shifted by one proline in register). In contrast, the conformation of the peptide mutant H9M remains close to the experimentally derived wild-type GYF-peptide complex. Possible functional implications of this altered conformation of the bound ligand are discussed in the light of our experimental and theoretical results.

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TARGET FLEXIBILITY IN MOLECULAR RECOGNITION

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Induced-fit effects are well known in the binding of small molecules to proteins and other macromolecular targets [1]. Protein kinases are particularly flexible proteins, so that such effects should ideally be considered in attempts at structure-based inhibitor design [2]. This workshop lecture will outline some recent progress in methods for including target flexibility in computational studies of molecular recognition. A focus will be the "relaxed complex method," in which ligands are docked to an ensemble of conformations of the target, and the best complexes are re-scored to provide predictions of optimal binding geometries [1, 3, 4].

More information can be found at <http://mccammon.ucsd.edu/>

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**MULTIPLE MOLECULAR RECOGNITION MECHANISMS.
CYTOCHROME P450 – A CASE STUDY**

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Biomolecular recognition is complex. The balance between the different molecular properties that contribute to molecular recognition, such as shape, electrostatics, dynamics and entropy, varies from case to case. This, along with the extent of experimental characterization, influences the choice of appropriate computational approaches to study biomolecular interactions. Here, I will present computational studies of cytochrome P450 (P450) enzymes and their interactions with small molecules and with other proteins. These interactions exemplify some of the diversity of molecular determinants of binding affinity and specificity observed for proteins.

P450s are heme protein monooxygenases that play a crucial role in the metabolism of the majority of clinical drugs. Thus, any drug design procedure should include consideration of the interactions of the designed compounds with P450s. P450s are also involved in the synthesis and degradation of numerous important endogenous compounds in many species of micro-organisms, plants and animals. P450s display a range of intermolecular interactions:

- Interactions with membrane: Prokaryotic P450s are soluble proteins whereas most eukaryotic P450s are membrane-bound enzymes.
- Interaction with proteins: Electrons are transferred to P450s *via* electron transfer proteins. These may consist of a two-iron-sulfur cluster ferredoxin shuttle protein and a soluble reductase or a membrane-bound NADPH reductase alone. P450s may also interact with other P450s and form oligomers.
- Interactions with small molecules: P450s act on different substrates, sometimes simultaneously binding more than one substrate molecule, and the specificity varies widely across the P450s.

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- Interactions *via* covalent attachment: Post-translational modification by phosphorylation and ubiquitination affects P450 structural and catalytic properties.

We have studied P450s by a combination of protein modeling, docking and simulation techniques. Modeling of mammalian P450s gives insights into the location and effects of post-translational modifications. Docking simulations of ferredoxin-P450 binding provide a new model for protein-protein complexation, which, unlike previous models, is consistent with new data from FTIR measurements (A. Kariakin, C. Jung, unpublished data). Molecular dynamics simulations of ligand egress from the buried active sites of P450s show differences in substrate access and product egress routes between membrane-bound mammalian P450s and soluble bacterial P450s that highlight the adaptability of the P450 fold to the requirements of differing cellular locations and substrate specificity profiles [1-3].

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**RAPID PROTEIN-LIGAND DOCKING USING SOFT MODES FROM
MD SIMULATIONS TO ACCOUNT FOR PROTEIN DEFORMABILITY:
APPLICATION TO PROTEIN KINASE-LIGAND INTERACTIONS**

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Most current docking approaches to predict the binding geometry of protein-ligand complexes assume a rigid receptor structure. However, protein-ligand complex formation can lead to changes in the receptor protein conformation that are sterically necessary to accommodate a bound ligand. An approach will be presented that allows relaxation of the protein conformation in precalculated soft flexible degrees of freedom during receptor-ligand docking [1]. The soft modes can be obtained by principal component analysis of a molecular dynamics simulation of the receptor protein in the unbound form. A simple penalty function for deformations in the soft flexible modes is used to limit receptor protein deformations during docking. This avoids a costly recalculation of the receptor energy by summing over all receptor atom pairs at each step.

The approach may provide a computationally efficient way to approximately account for receptor flexibility during docking of large numbers of putative ligands and putative docking geometries. Application of the approach to test systems and protein kinase ligand interactions will be presented.

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