Structural Modeling of Protein Interactions by Analogy: Application to PSD-95

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Summary

We describe comparative patch analysis for modeling the structures of multi-domain proteins and protein complexes, and apply it to the PSD-95 protein. Comparative patch analysis is a hybrid of comparative modeling based on a template complex and protein docking, with a greater applicability than comparative modeling and a higher accuracy than docking. It relies on structurally defined interactions of each of the complex components, or their homologs, with any other protein, irrespective of its fold. For each component, its known binding modes with other proteins of any fold are collected and expanded by the known binding modes of its homologs. These modes are then used to restrain conventional molecular docking, resulting in a set of binary domain complexes that are subsequently ranked by geometric complementarity and a statistical potential. The method is evaluated by predicting 20 binary complexes of known structure. It is able to correctly identify the binding mode in 70% of complexes compared to 30% for protein docking. We applied comparative patch analysis to model the complex of the third PDZ domain and the SH3-GK domains in the PSD-95 protein, whose structure is unknown. In the first predicted configuration of the domains, PDZ interacts with SH3 leaving both the GMP-binding site of GK and the C-terminus binding cleft of PDZ accessible, while in the second configuration PDZ interacts with GK, burying both binding sites. We suggest that the two alternate configurations correspond to the different functional forms of PSD-95 and provide a possible structural description for the experimentally observed cooperative folding transitions in PSD-95 and its homologs. More generally, we expect that comparative patch analysis will provide useful spatial restraints for the structural characterization of an increasing number of binary and higher order protein complexes.

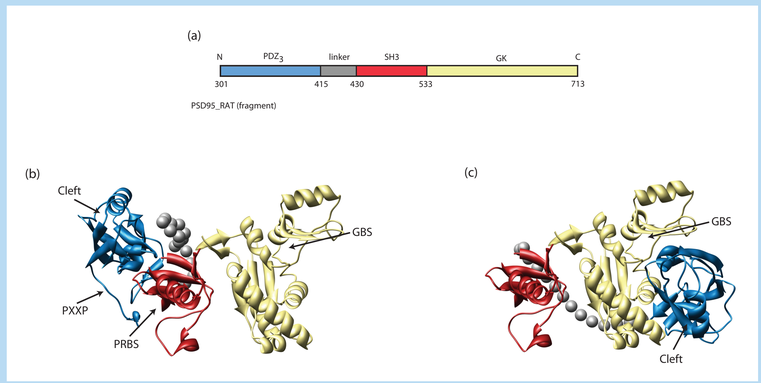
Application to PSD-95 protein

Comparative patch analysis was applied to model the tertiary structure of the core fragment of rat PSD-95 that includes the PDZ_3, SH3 and GK domains. We predicted two configurations, each suggesting a unique functional role.

1. In the first configuration, the hydrophobic cleft of the PDZ domain (Cleft) and the GMP-binding site (GBS) of the GK domain are both accessible.

2. In contrast, both binding sites are buried in the second configuration, by the interface between the PDZ3 and GK domains.

3. The PDZ_3 PXXP motif is in proximity to the SH3 proline-rich binding site (PRBS) in the first configuration, consistent with the classical SH3-PXXP motif recognition.

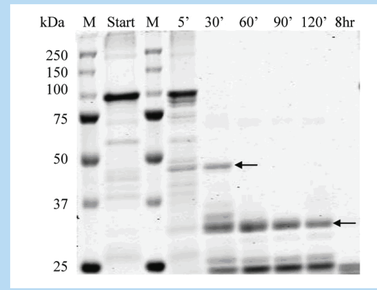


Limited proteolysis

1. Limited proteolysis of recombinant PSD-95 was carried out using Proteinase K.

2. A prominent ~48 kDa band at 30 minutes which corresponds to the PDZ_3-SH3-GK fragment.

3. Further digestion leads to appearance of a stable ~34 kDa band corresponding to the SH3-GK fragment.



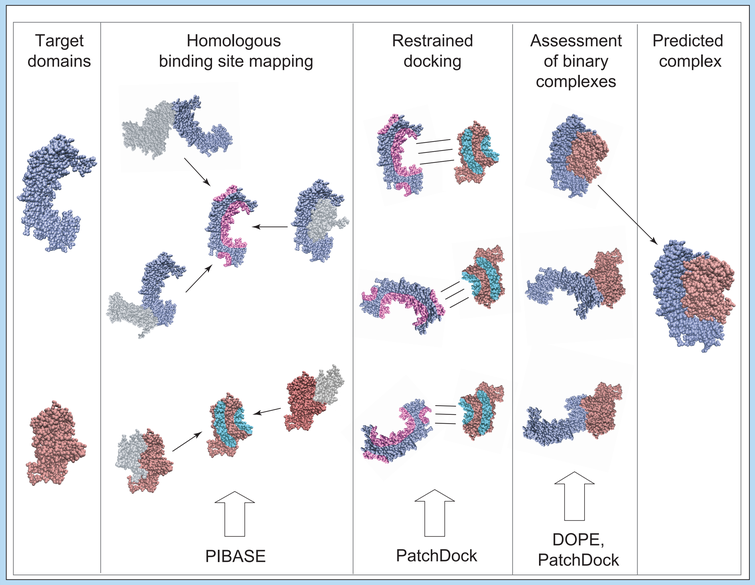
A two-functional-states hypothesis

- The two configurations point to an efficient regulatory mechanism for switching the functional state with a single interaction.

- This two-state model also provides a structural explanation for the change in binding affinity between the GK domain and MAP1A protein in the presence of the PDZ_3 domain [3]. The affinity is high when GK domain is alone, it is dramatically reduced when PDZ_3 domain is added, and it is recovered upon titration of a C-terminal peptide of CRIPT known to specifically interact with the hydrophobic cleft of PDZ_3.

Methods

Basic steps of comparative patch analysis approach



Evaluation

A benchmark set of 20 binary domain complexes was used to evaluate comparative patch analysis.

Evaluation results:

1. The overall structure was improved for **13** of the **20** complexes, compared to protein docking by PatchDock[2].

2 In **15** complexes, comparative patch analysis produced models with all-atom RMS error **<3** Å (only **6** complexes for docking).

Application to PDZ_3-SH3-GK fragment of PSD-95

1. Remove redundant binding sites (that share more than 95% of their residues) for PDZ_3, SH3, and GK domains and their homologs.

2. Apply comparative patch analysis to obtain a ranked ensemble of models.

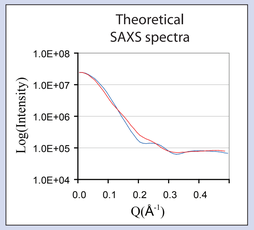
3. Remove from the ensemble those models that are not compatible with the 14-residue linker length between the PDZ_3 and SH3 domains.

Conclusions

Suggested experiments:

1. Site-directed mutagenesis of the interface residues in the first proposed state could be used together with pull-down assays to validate the predicted interaction interface.

2. The lack of accessibility of the GMP-binding site in the second state could be tested using nucleotide-binding assays.



3. We expect the experimentally obtained SAXS spectra to be helpful in distinguishing the two PSD-95 states, based on the difference in theoretically predicted SAXS spectra.

Future directions:

Comparative patch analysis will be further applied to model the entire structure of PSD-95 and other multidomain proteins and protein complexes in PSD.

References

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3. Brenman JE, Topinka JR, Cooper EC, McGee AW, Rosen J, *et al* (1998) *Prot. Sci.*, 13