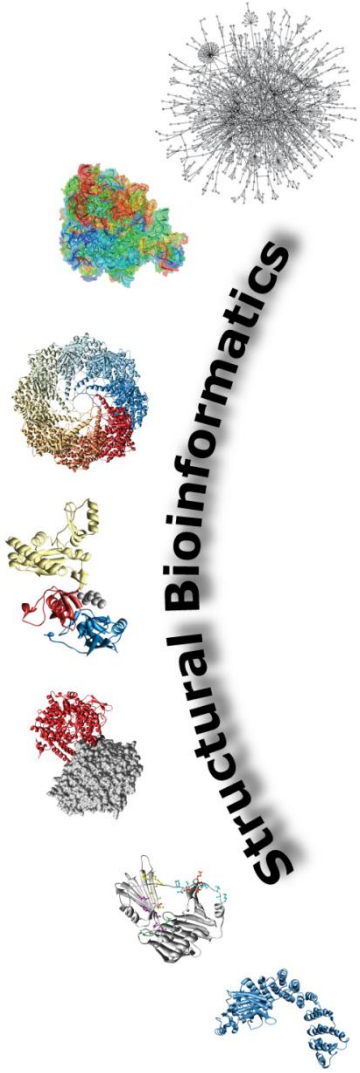
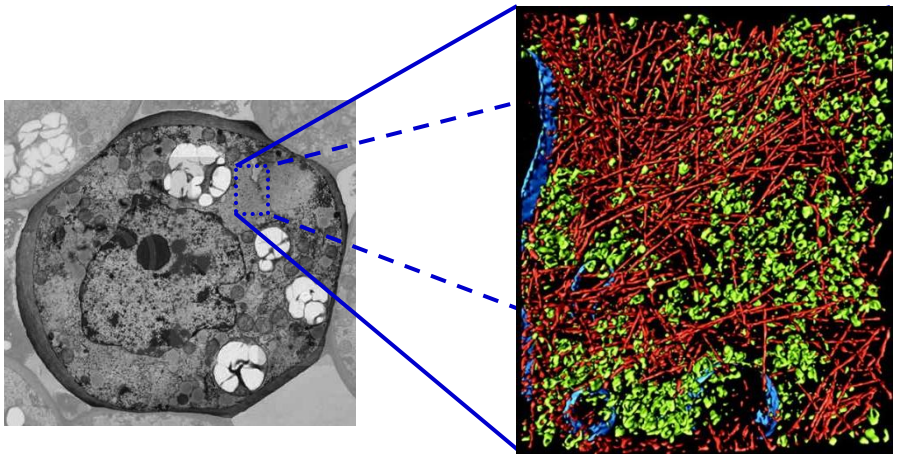


II8010. Lecture Materials

Dmitry Korkin, Ph.D.

Informatics Institute and
Department of Computer Science
University of Missouri, Columbia

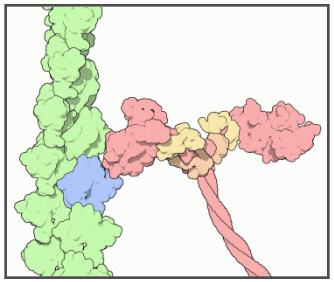
Protein assemblies are essential building blocks of a living cell



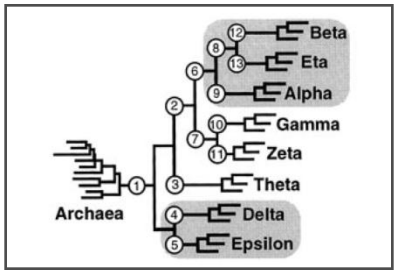
Cytoplasmic assemblies

- 1.7 interactions per protein
- 30,000 interactions in yeast
- proteins consist of ~2 domains

Function



Evolution



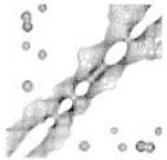
Medicine

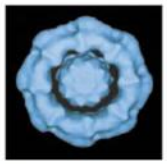
O. Medalia et al., Science (2002)

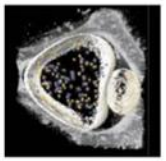
Methods for structural characterization of macromolecules

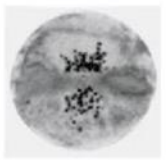
Experimental

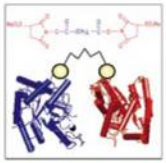

X-ray crystallography


NMR spectroscopy

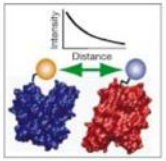

2D and single-particle EM


Electron tomography

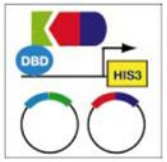

Immuno-electron microscopy


Chemical cross-linking

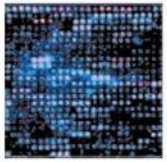

Affinity purification mass-spectroscopy



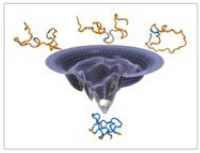
FRET



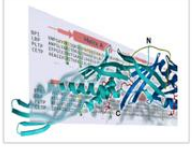
Yeast two-hybrid system

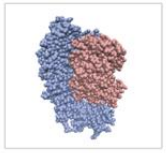

Gene/protein arrays

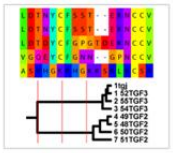
Computational


Ab-initio modeling

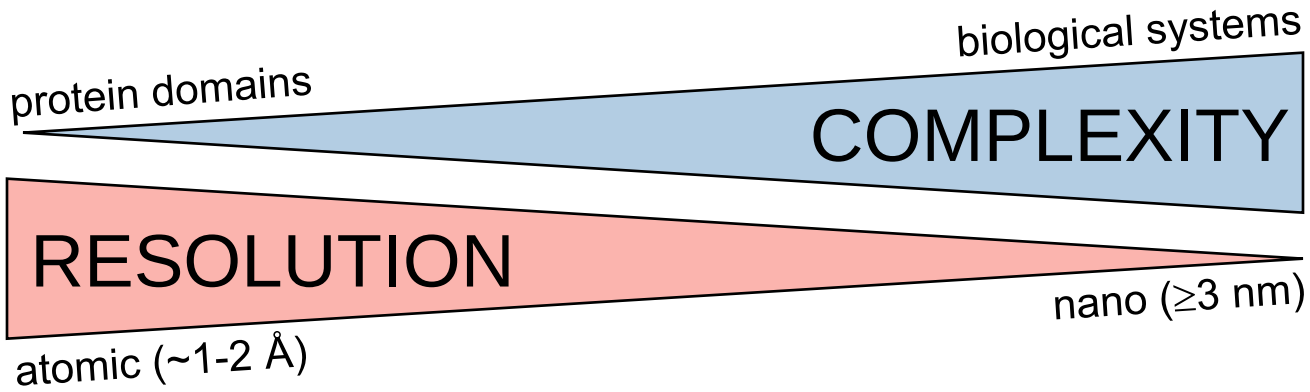

Comparative modeling


Protein threading


Molecular docking


Phylogenetic profiling

Knowledge-based information retrival

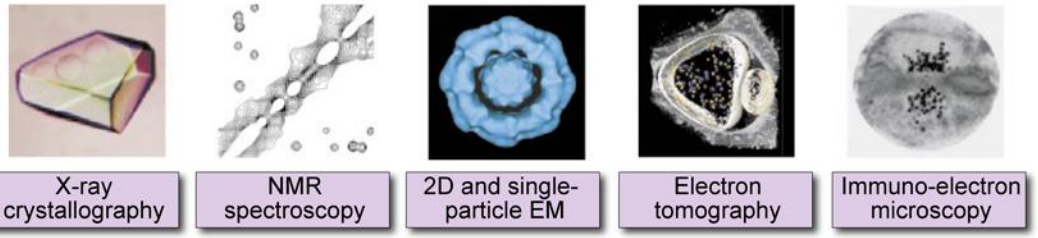


Russell RB, Alber F, Aloy P, Davis FP, Korkin D, Pichaud M, Topf M, Sali A, *Curr.Opin.Struct.Biol.*, 2004

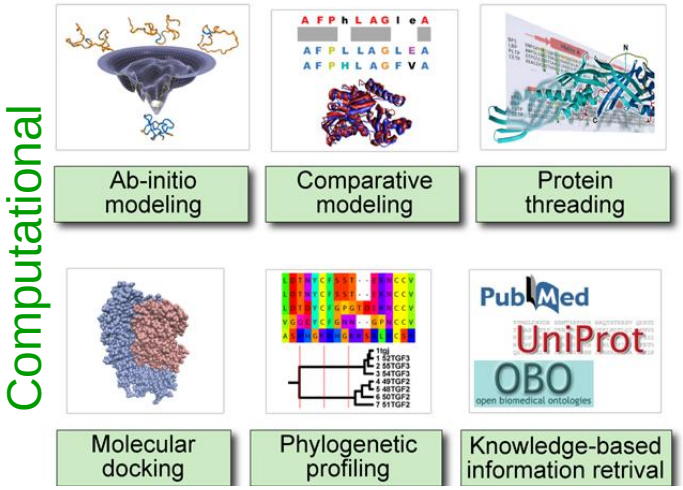
Alber F, Forster F, Korkin D, Topf M, Sali A, *Annu. Rev. Biochem.*, 2008, in press

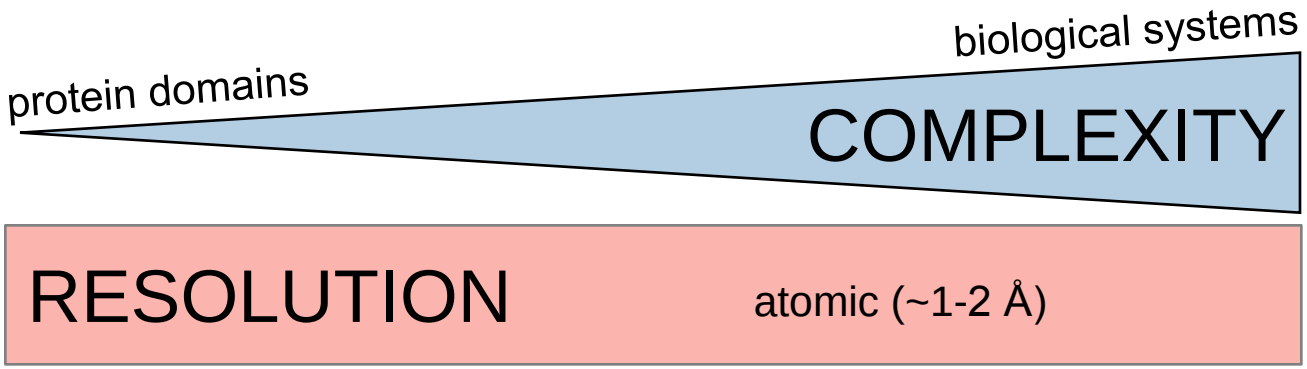
Methods for structural characterization of macromolecules

Experimental



Computational





Russell RB, Alber F, Aloy P, Davis FP, Korkin D, Pichaud M, Topf M, Sali A, *Curr.Opin.Struct.Biol.*, 2004

Alber F, Forster F, Korkin D, Topf M, Sali A, *Annu. Rev. Biochem.*, 2008, in press

Protein interactions in structural bioinformatics context

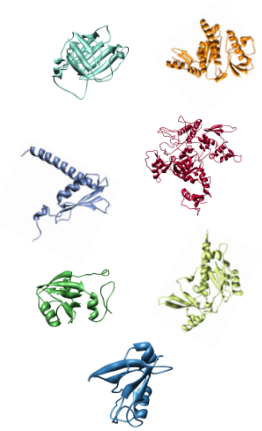
subunit sequences

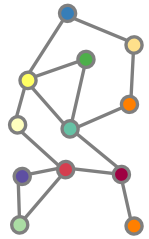
subunit structures & models

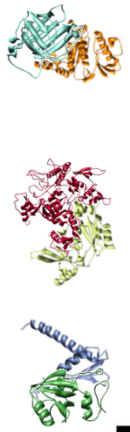
interaction network

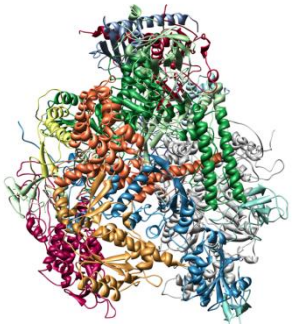
binary sub-complexes

assembly structure

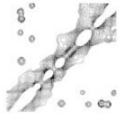








X-ray crystallography



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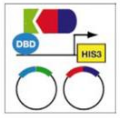

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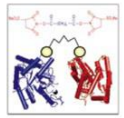
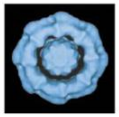
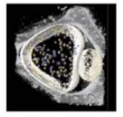


Chemical cross-linking



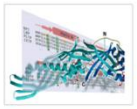
2D and single-particle EM



Electron tomography

Ab-initio modeling

Comparative modeling



Protein threading

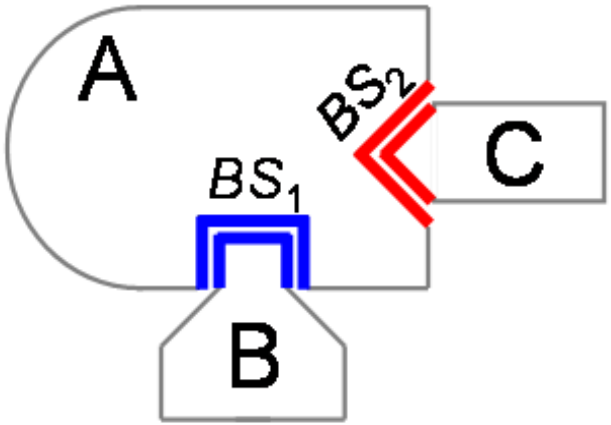
Protein–protein interaction vocabulary

- A protein–protein interaction (PPI), or binary protein interaction, is defined between any two structural subunits
- Structural subunits = small proteins, domains, peptides;
- Two residues (from two different subunits) are called the contact residues, if there is at least a pair of atoms, one from each residue that are in close proximity (usually ≤6Å)
- Two subunits interact if they have at least a pair of contact residues
- interaction = (S_1, S_2, Or)

Protein–protein interaction vocabulary

- Protein binding site for an interaction $I_{1=}$ (S_1, S_2, Or) is a set of all contact residues of either S_1 or S_2.

- Protein interface for an interaction $I_{1=}$ (S_1, S_2, Or) is a set of all pairs of contact residues, one from each protein binding site



How to characterize an interaction interface

- N of contact residues in a binding site
  - On average 20-30 residues per each binding site

- Buried surface = $Surf_1 + Surf_2 - Surf_{12}$
  - Usually >1100 $Å^2$
  - each of the interacting partners contributing at least 550 Å2 of complementary surface.
  - Average interface residue covers some 40 Å2.
  - dimers contribute 12% of their accessible surface area to the contact interface, trimers 17.4% and tetramers 20.9%.
  - variations are large

- Binding free energy
  - Energy required to dissociate two subunits

What causes two proteins interact?

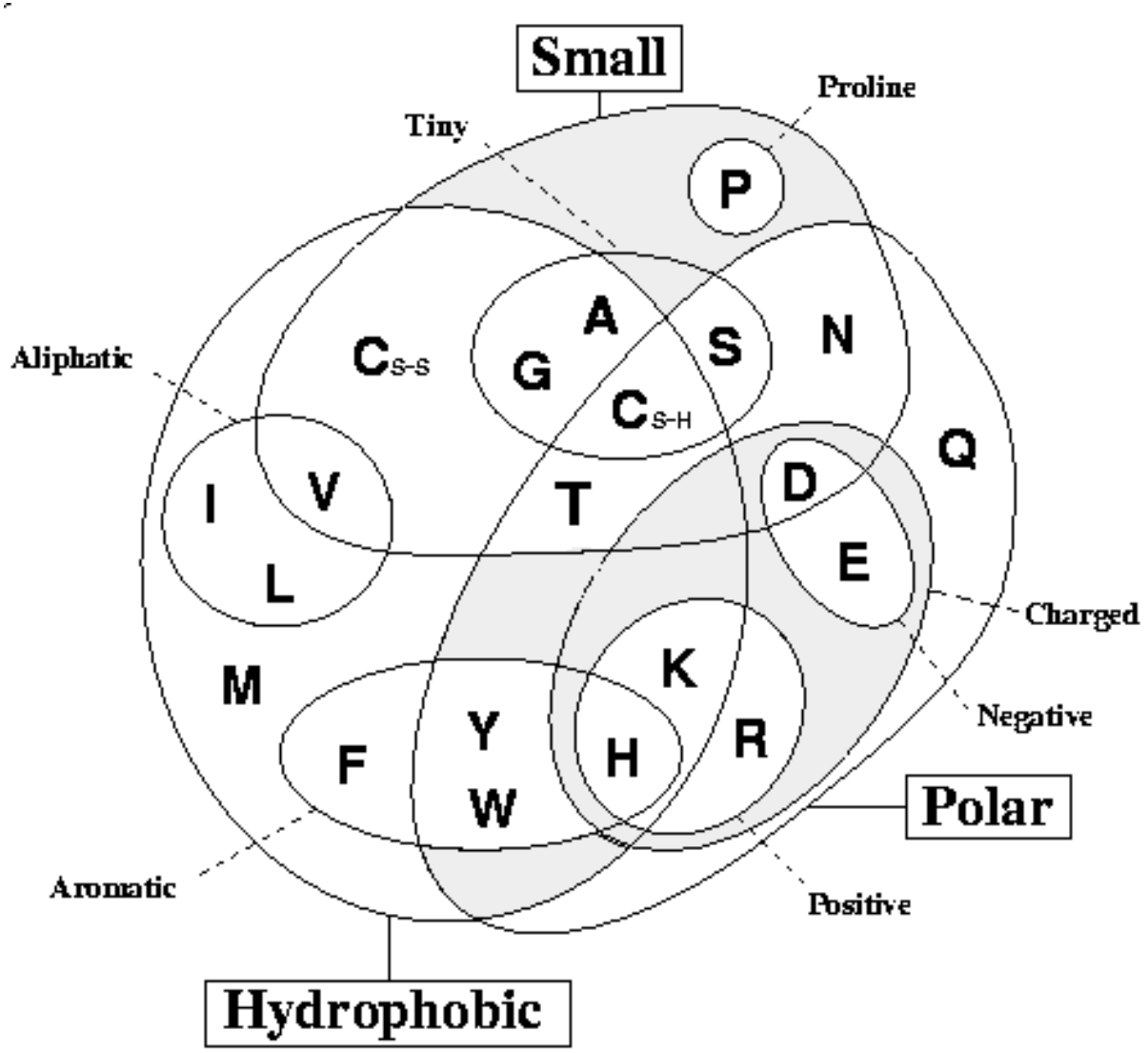
- Geometrical complementarity
  - Do they have to be completely complementary? Not necessarily!

- Physico-chemical complementarity
  - Electrostatic interactions
  - Hydrogen bonds
  - van der Waals attraction

- Interaction with water
  - Hydrophobic effect: Hydrophobic residues tend to be buried in the interface

A standard size interface (~ 1600 Å^2) buries about 900 Å^2 of the non-polar surface, 700 Å^2 of polar surface, and contains 10 (± 5) hydrogen bonds.

Amino acid residues. Basic classes



Electrostatic interactions and hydrophobic efeect

- The average protein-protein interface is not less polar or more hydrophobic than the surface remaining in contact with the solvent
- Water is usually excluded from the contact region
- Non-obligate complexes tend to be more hydrophilic in comparison, as each component has to exist independently in the cell.

Van der Waals interactions

- Van der Waals interactions occur between all neighboring atoms
- These interactions at the interface are no more energetically favorable than those made with the solvent
- However, they are more numerous, as the tightly packed interfaces are more dense than the solvent and hence they contribute to the binding energy of association.

Hydrogen bonds

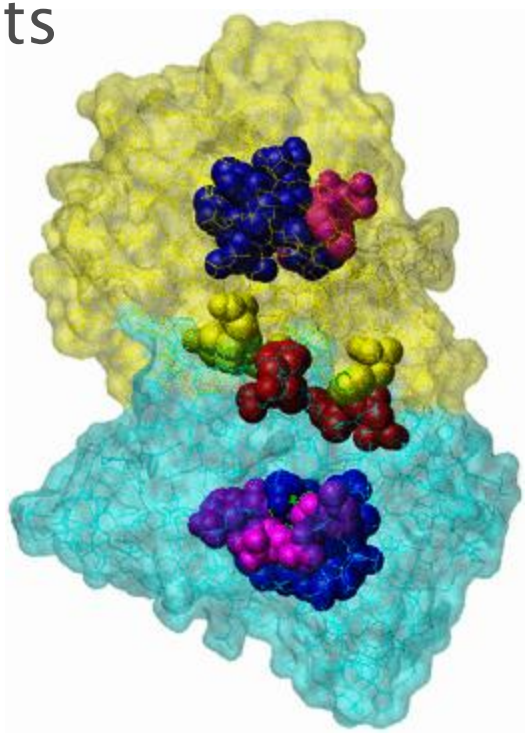
- Hydrogen bonds between protein molecules are more favorable than those made with water
- Interfaces in permanent associations tend to have fewer hydrogen bonds than interfaces in transient associations
- The number of hydrogen bonds is about 1 per 170 $Å^2$ buried surface
- In a set of reasonably stable dimers there are, on average, 0.9 to 1.4 hydrogen bonds per 100 $Å^2$ of contact area buried (interfaces covering > 1000 $Å^2$)
- The number of hydrogen bonds varies from 0 to 46
- Side-chain hydrogen bonds represent approximately 76-78% of the interactions.

Secondary structure of protein-protein interfaces

- Can vary drastically
- In one study the loop interactions contributed, on average, 40% of the interface contacts
- In another study (involving 28 homodimers), 53% of the interface residues were α-helical, 22% β -sheets, and 12% $\alpha\beta$, with the rest being coils

Hot spots

- Residues that make significant contribution to the binding free energy are generally clustered together
- The clusters are called the hot-spots
- Introduced by Jim Wells

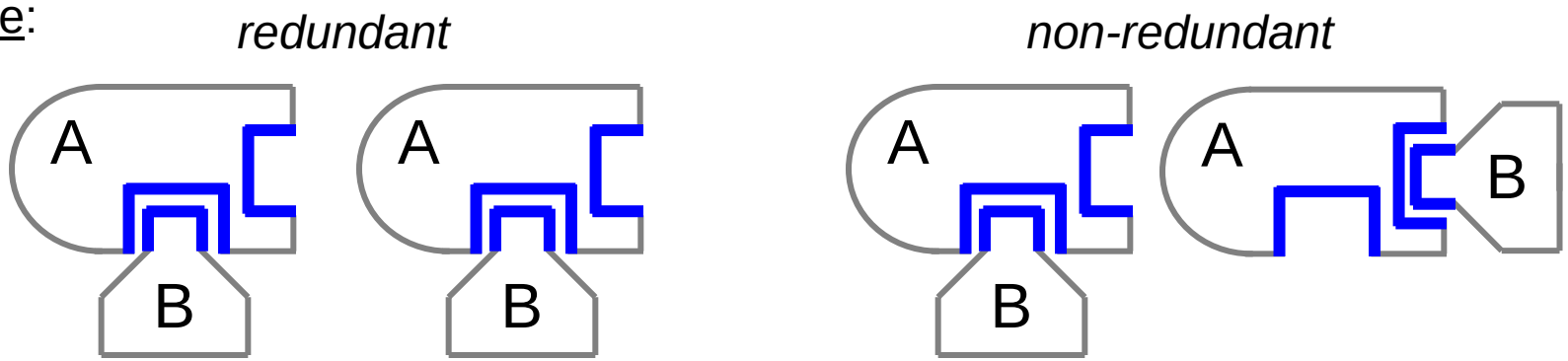


Redundant interactions

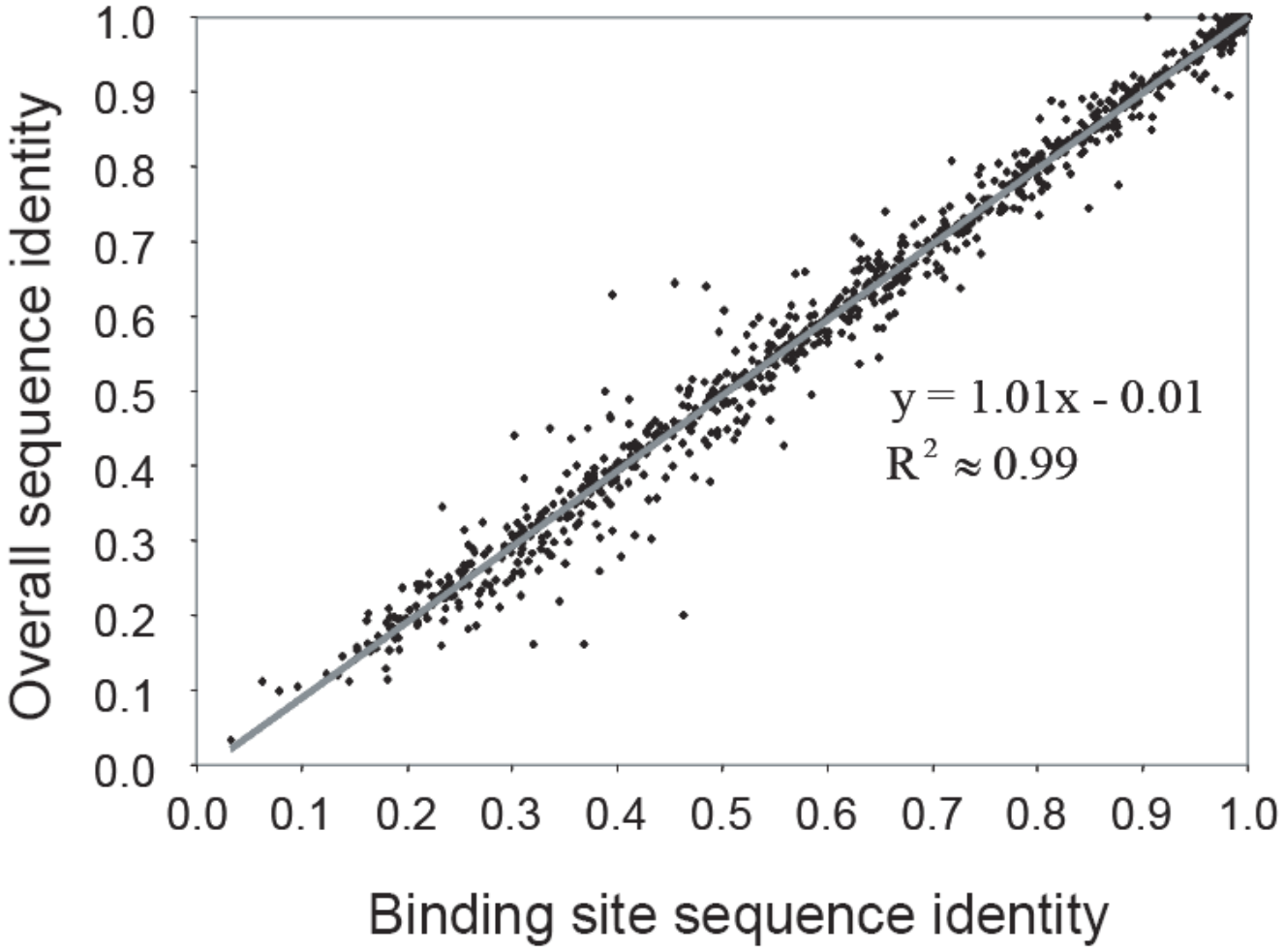
Definition. Two interactions, (A_1, B_1) and (A_2, B_2) are *redundant* if both pairs of partners are similar AND interfaces are similar :

1. Sequence identity between A_1 and A_2 is more then 90% ;
2. Sequence identity between B_1 and B_2 is more then 90%;
3. The interfaces of (A_1, B_1) and (A_2, B_2) are in the same PIBASE cluster.

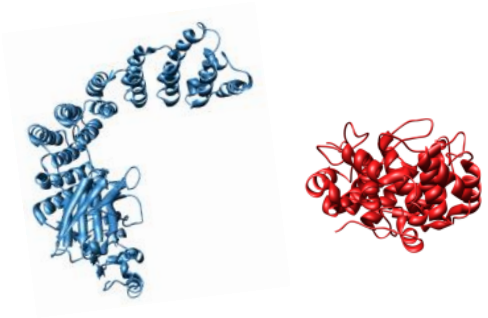
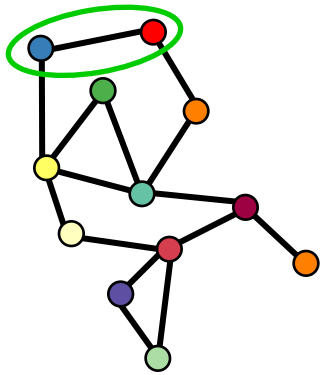
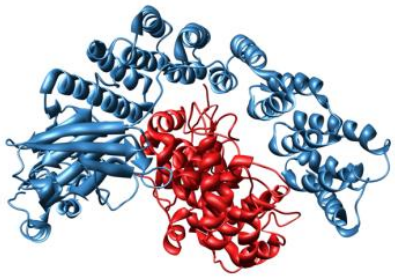
Example:



Are PPI interfaces more conserved in sequence than the rest of the protein?



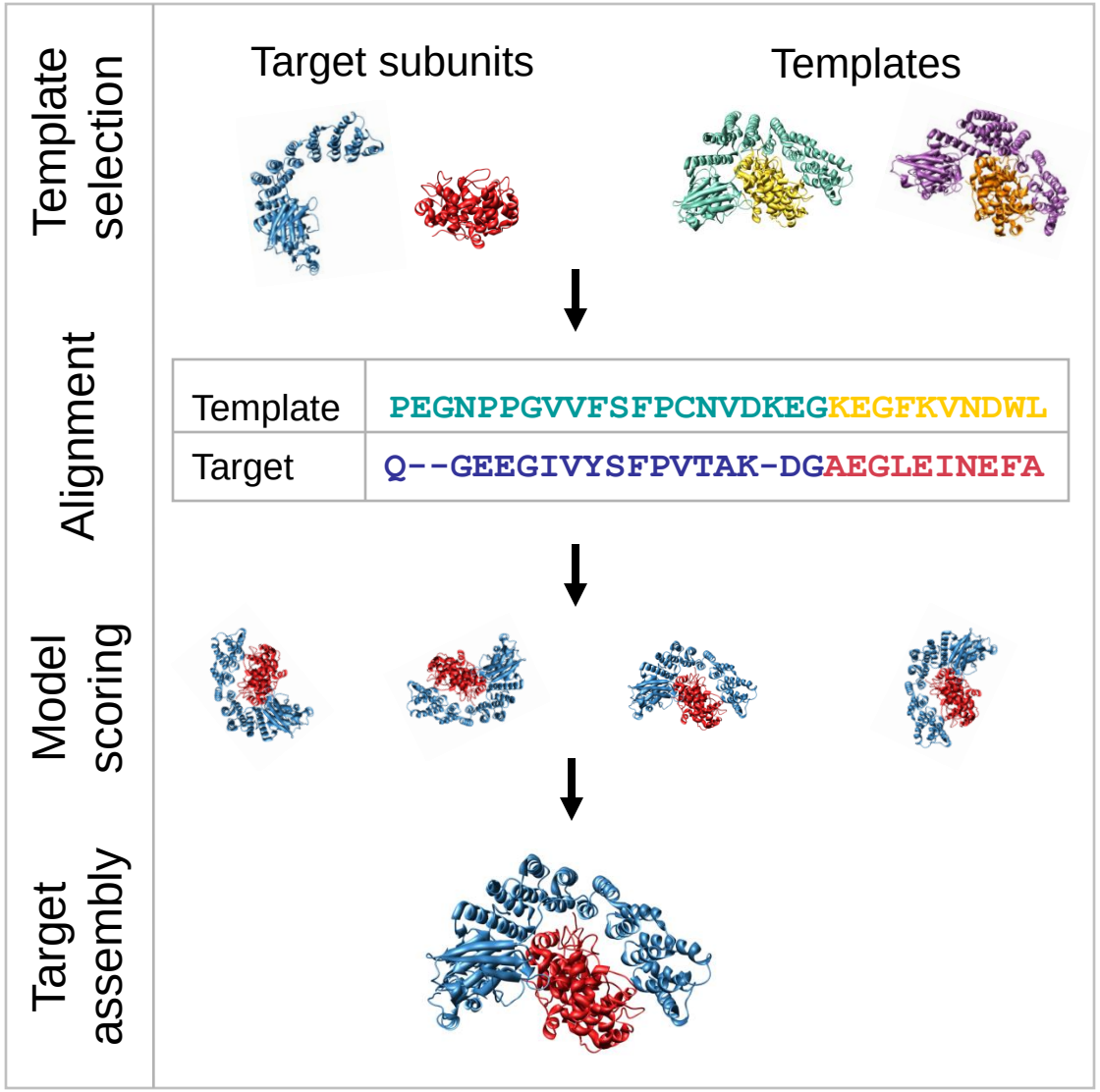
Modeling structures of subunit-subunit interactions

INPUT	Subunit structures	Binary interaction	OUTPUT	Assembly structure
				

Methods usually address three common aspects:

1. Efficient representation of structures
2. Comprehensive enumeration of all candidate models
3. Accurate selection of the native model

Homology modeling of protein assemblies



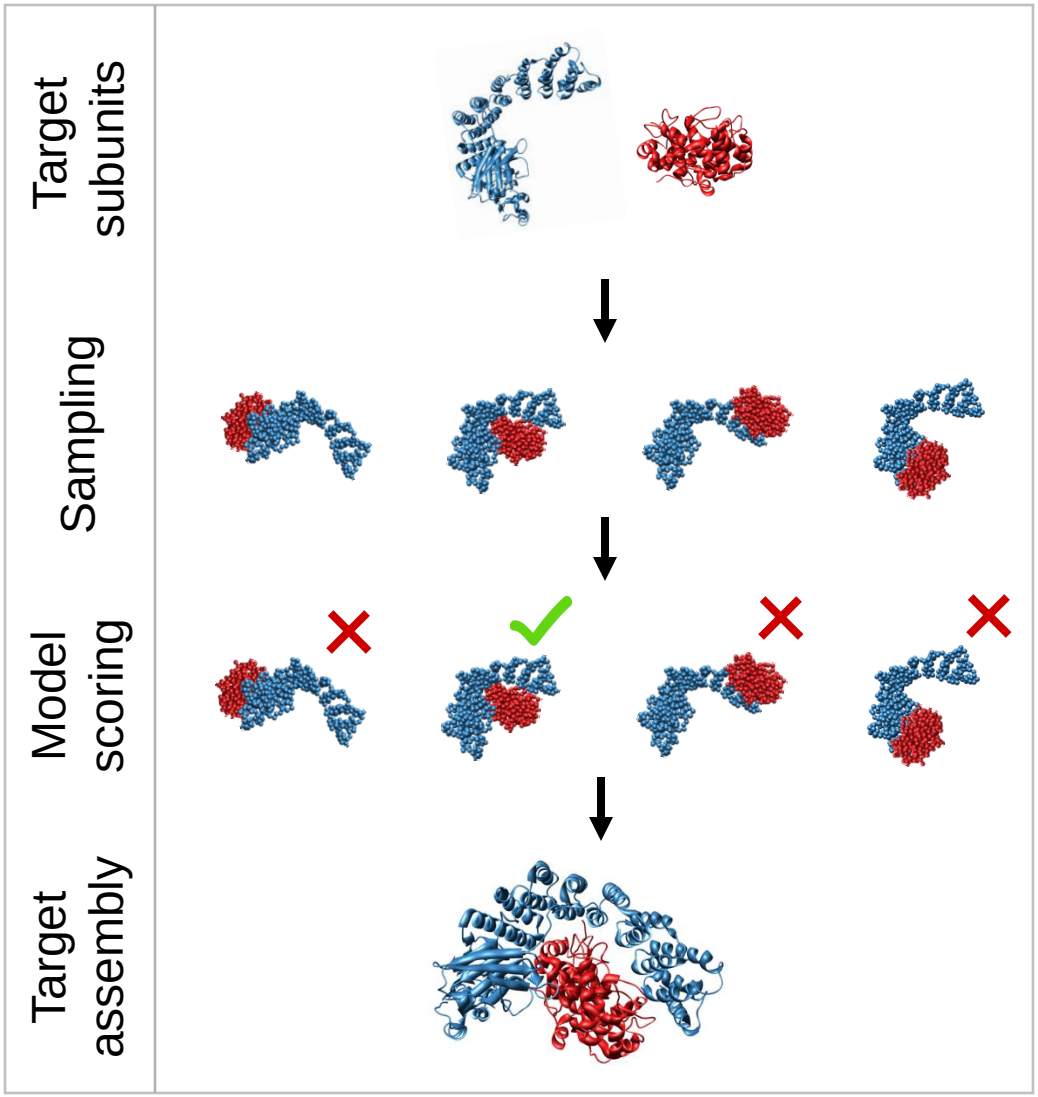
Pros:

- high accuracy
- fast

Cons:

- low coverage
- predicts only existing binding modes

Protein docking



Pros:

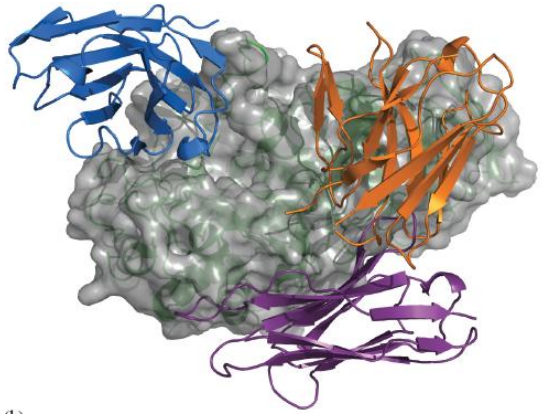
- high coverage
- can predict novel binding modes

Cons:

- low accuracy
- slow

Homology modeling: Can we improve it?

- What if we have only substructures as templates, not the entire structure?
- What if we have two structural templates that observe two different binding modes?



- Can we use additional data to improve the accuracy?

A recent approach addressing this questions

Nucleic Acids Research, 2006, Vol. 34, No. 10 ***2943–2952***
doi:10.1093/nar/gkl353

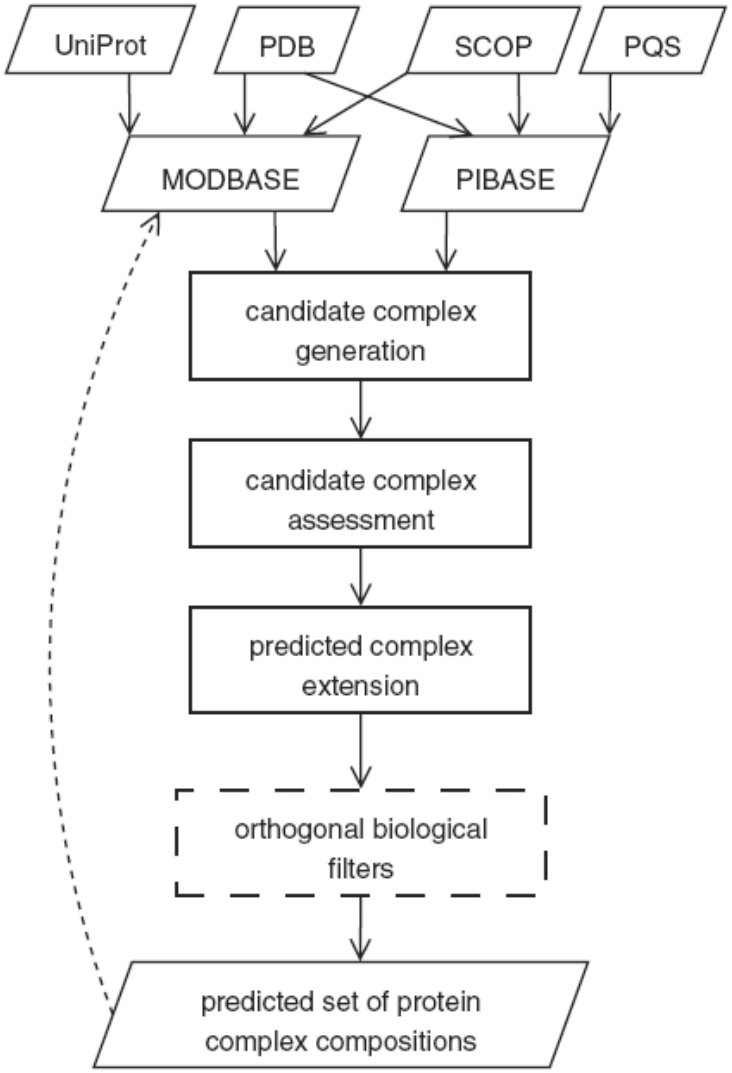
Protein complex compositions predicted by structural similarity

Fred P. Davis[1,2], Hannes Braberg[1,2], Min-Yi Shen[1,2], Ursula Pieper[1,2], Andrej Sali[1,2,*] and M.S. Madhusudhan[1,2,*]

[1]Department of Biopharmaceutical Sciences and [2]Department of Pharmaceutical Chemistry, California Institute for Quantitative Biomedical Research, University of California, San Francisco, 1700 4th Street, Byers Hall, San Francisco, CA 94143-2552, USA

Received March 15, 2006; Revised April 1, 2006; Accepted April 20, 2006

Methods flowchart



Statistical potential to evaluate protein interfaces

- A series of statistical potentials was built using the binary domain interfaces in PIBASE
- Extracted from structures at or above 2.5 s resolution, randomly excluding 100 benchmark interfaces
- 24 statistical potentials were built using different values of 3 parameters:
  - The contacting atom types (main chain–main chain, main chain–side chain, side chain–side chain or all)
  - The relative location of the contacting residues (inter- or intra-domain)
  - Distance threshold for contact participation

Statistical potential to evaluate protein interfaces

$$g_{ij} = \frac{\sum_{p=1}^{N} \sum_{c=1}^{\Delta n_{ij}^{(p)}(R_o)} \mathrm{cifa}_{ci,\,cj} n_p}{\sum_{p=1}^{N} n_{ij}^{(p)} \max\left(\mathrm{cifa}_{i,j}\right)}$$

$$\mathrm{cifa}_{x,\,y} = \min\left(\frac{\text{interacting atoms}_x}{\text{atoms}_x}, \frac{\text{interacting atoms}_y}{\text{atoms}_y}\right),$$

$$n_{ij}^{(p)} = \begin{cases} n_i^{(p)} n_j^{(p)} & \text{intra-domain potential.} \\ n_i^{(d1)} n_j^{(d2)} + n_i^{(d2)} n_j^{(d1)} & \text{inter-domain potential.} \end{cases}$$

$$w_{ij} = -\ln\left[\frac{g_{ij}}{\frac{1}{400}\sum_{k=1}^{20}\sum_{l=1}^{20} g_{kl}}\right].$$

i, j: residue types in protein *p*

Adding experimental data

		Experimental Overlap		
	Predicted	All	BIND	Cellzome
Binary Interactions				
experimental		19,424	13,191	6,942
Z-score ≤ -1.7	12,867	409	324	151
Z + Co-Function	6,808	390	311	145
Z + Co-Localization	4,606	278	220	102
Z + Co-Loc + Co-Func	3,387	270	217	97
Higher-Order complexes				
experimental		783	296	491
Z-score	12,702	66	54	35
Z + Co-Function	3,544	51	45	28
Z + Co-Localization	2,189	14	7	10
Z + Co-Loc + Co-Func	1,234	8	4	7

Protein docking

doi:10.1016/S0022-2836(03)00670-3 *J. Mol. Biol.* (2003) **331**, 281–299

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Protein–Protein Docking with Simultaneous Optimization of Rigid-body Displacement and Side-chain Conformations

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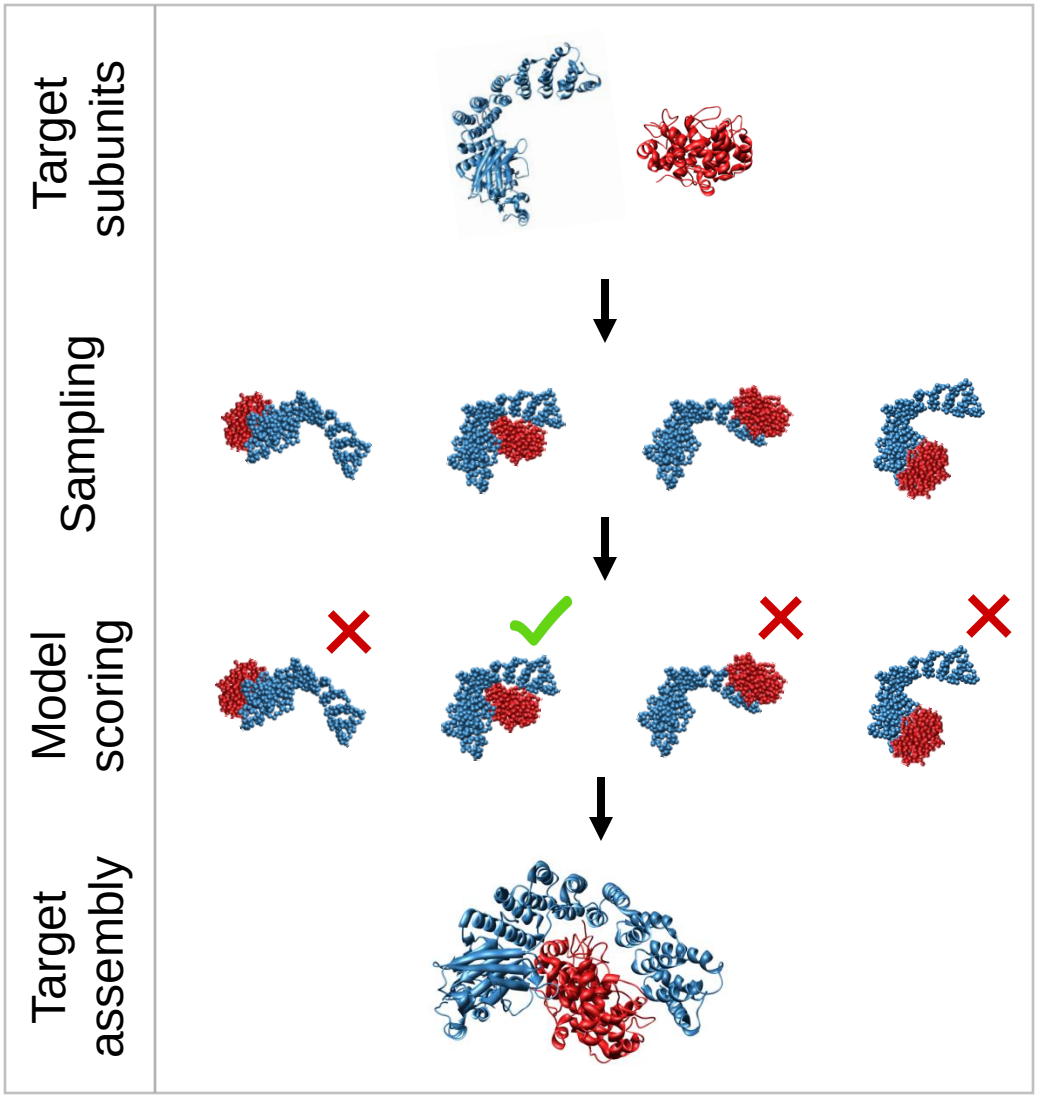
Protein docking



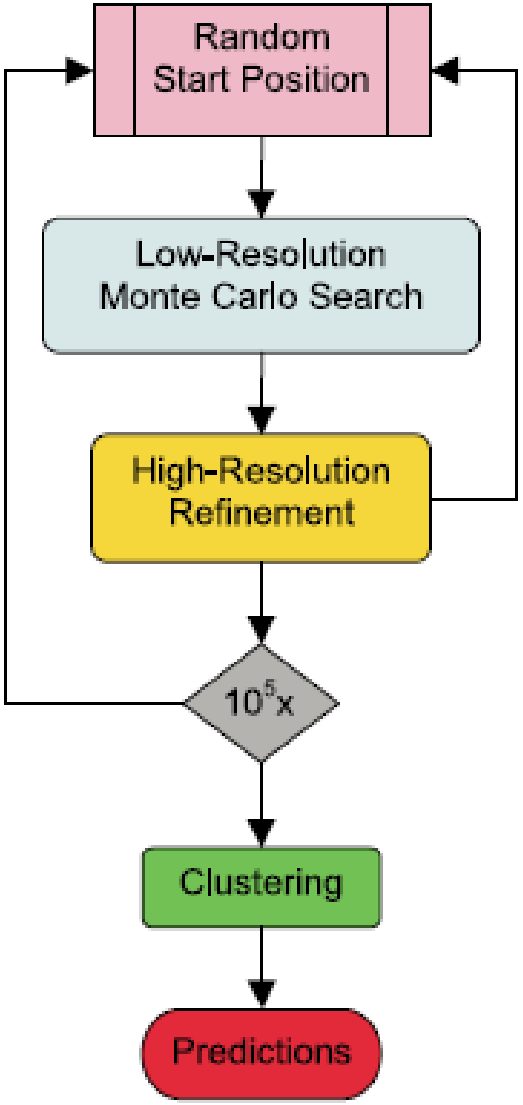
Pros:

- high coverage
- can predict novel binding modes

Cons:

- low accuracy
- slow

Docking protocol



Protocol details

1. Create a decoy set: start with random orientation of each partner + translation of one partner along the line of protein centers to create glancing contact

2. Perform Monte-Carlo simulation: 500 attempts of translating and rotating one partner around surface of another one. 50% acceptance rate. Each step is chosen randomly with a mean value of 0.7 Å (translation) and 5 (rotation)

3. Low-resolution residue scale potentials are calculated based on Bayesian expansion that estimates the probability of correctness for each decoy

4. High resolution refinement: explicit side-chains are added using a backbone-dependent rotamer packing algorithm; use fixed number of multiple rotamers; select an optimal configuration using simulated annealing Monte-Carlo search

Protocol details (contd.)

5. Rigid body is minimized using a full-atom scoring function

6. Select the best-scoring candidates and cluster them using pair-wise RMSD using hierarchical clustering algorithm with 2.5 Å clustering threshold

7. Clusters with the most members are selected as final

All-atom scoring function

Terms included:

• van der Waals interactions

• solvation using a pair-wise Gaussian solvent-exclusion model

• hydrogen bonding energies using an orientation-dependent function derived from high-resolution protein structures

• a rotamer probability term

• residue–residue pair interactions derived statistically from a database of protein structures

• a simple electrostatic term

•a surface area and atomic solvation term (for decoy discrimination only, due to the expense of calculation)

All-atom scoring function

General form of all-atom scoring function:

$$
\begin{aligned}
S = {} & w_{\text{atr}} S_{\text{atr}} + w_{\text{rep}} S_{\text{rep}} + w_{\text{sol}} S_{\text{sol}} + w_{\text{sasa}} S_{\text{sasa}} + w_{\text{hb}} S_{\text{hb}} \\
& + w_{\text{dun}} S_{\text{dun}} + w_{\text{pair}} S_{\text{pair}} + w_{\text{elec}}^{\text{sr-rep}} S_{\text{elec}}^{\text{sr-rep}} + w_{\text{elec}}^{\text{sr-atr}} S_{\text{elec}}^{\text{sr-atr}} \\
& + w_{\text{elec}}^{\text{lr-rep}} S_{\text{elec}}^{\text{lr-rep}} + w_{\text{elec}}^{\text{lr-atr}} S_{\text{elec}}^{\text{lr-atr}} \qquad (7)
\end{aligned}
$$

Weights are learned using a statistical approach: a logistic regression was used to define the weights that maximally separates good decoys from others

All-atom scoring function

General form of all-atom scoring function:

$$S = w_{\text{atr}}S_{\text{atr}} + w_{\text{rep}}S_{\text{rep}} + w_{\text{sol}}S_{\text{sol}} + w_{\text{sasa}}S_{\text{sasa}} + w_{\text{hb}}S_{\text{hb}} + w_{\text{dun}}S_{\text{dun}} + w_{\text{pair}}S_{\text{pair}} + w_{\text{elec}}^{\text{sr-rep}}S_{\text{elec}}^{\text{sr-rep}} + w_{\text{elec}}^{\text{sr-atr}}S_{\text{elec}}^{\text{sr-atr}} + w_{\text{elec}}^{\text{lr-rep}}S_{\text{elec}}^{\text{lr-rep}} + w_{\text{elec}}^{\text{lr-atr}}S_{\text{elec}}^{\text{lr-atr}} \quad (7)$$

Weights are learned using a statistical approach: a logistic regression was used to define the weights that maximally separates good decoys from others

A potential problem: what if the contribution of some members is not linear?

Results

Four classes of complexes:

- Enzyme/Inhibitor
- Antibody/Antigen
- Difficult
- Other

Two types of structural conformations:

- Semibound
- Unbound-unbound

Overview of correct predictions

Table 2. Correct predictions by interface type

	All			Unbound–unbound			Semibound		
	BB-p	XU-p	XU-g	BB-p	UU-p	UU-g	BB-p	BU-p	BU-g
Enzyme/inhibitor	21/22	18/22	17/22	15/16	12/16	11/16	6/6	6/6	6/6
Antibody/antigen	10/16	9/16	8/16	3/5	1/5	3/5	7/11	8/11	5/11
Other	5/10	5/10	3/10	1/4	1/4	0/4	4/6	4/6	3/6
Difficult	6/6	0/6	0/6	4/4	0/4	0/4	2/2	0/2	0/2
Total	42/54	32/54	28/54	23/29	14/29	14/29	19/25	18/25	14/25

How can we combine both approaches?

- Search locally, not globally
- Use known data about related proteins/assemblies
- Knowledge of subunit binding sites is crucial when associating them into assembly

Do similar proteins use similar binding sites?

Do similar proteins use similar binding sites?

Family *F* of protein homologs

↓

$\forall\ p_i \in F$: determine binding sites

↓

select a representative structure $p_R \in F$

↓

$\forall\ p_i \in F$: map its binding sites onto p_R

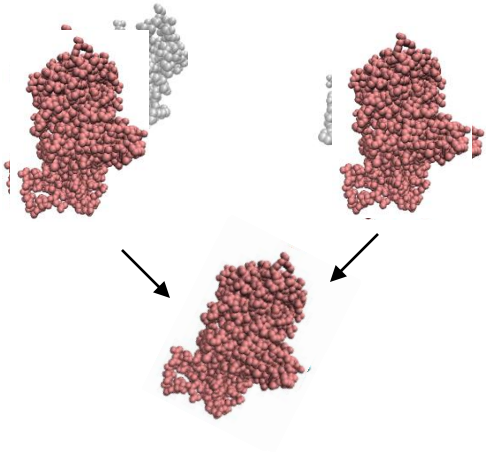
using structural alignment of p_i and p_R

↓

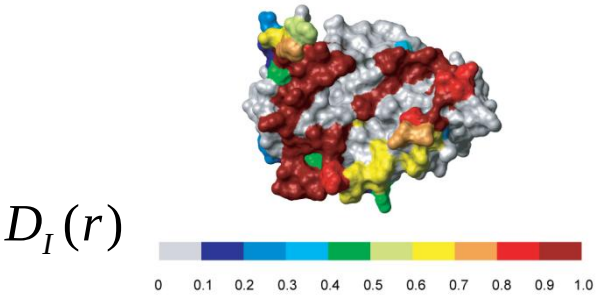
generate a random sample of binding sites for p_R

↓

calculate binding site localization



$$Loc = \frac{\sum_{r \in CR} D_I(r)}{N_{CR}}, \quad D_I(r) = \frac{N_{BS}(r)}{\max_r(N_{BS}(r))}$$



Yes: 72% of 1,847 families have binding sites with co-localization greater than expected by chance

Korkin D, Davis FP, Sali A, *Protein Sci.*, 2005

Comparative patch analysis

OPEN ACCESS Freely available online

PLoS COMPUTATIONAL BIOLOGY

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

Dmitry Korkin[1,2,3], Fred P. Davis[1,2,3], Frank Alber[1,2,3], Tinh Luong[4], Min-Yi Shen[1,2,3], Vladan Lucic[5], Mary B. Kennedy[4], Andrej Sali[1,2,3*]

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Basic steps of comparative patch analysis

Target domains

Korkin D, Davis FP, Alber F, Lucic V, Kennedy MB, Sali A., *PLoS Comput. Biol.*, 2006

Comparative patch analysis: Methods

- Protein binding sites were extracted from PiBASE (Davis FP and Sali A, 2005)
  - database of non-redundant protein interactions
  - proteins are clustered into families based on SCOP classification

- PatchDOCK was used for the local docking (Shneidman-Duhovny et al, 2005)
  - rigid body docking
  - restrained to maximize geometrical complementarity of the given binding sites

- Scoring function is composite:

$$f_{SCORE} = f_{DOPE} + f_{PATCHDOCK}$$

  - DOPE score: an atomic distance-dependent pairwise statistical potential (Shen MY and Sali A, 2006)

Comparative patch analysis: Computational challenges

Time complexity of the method:

$$T(N,M) = \underbrace{O(N) + O(M)}_{\text{alignment}} + \underbrace{O(NM)}_{\text{loc. docking}} + \underbrace{O(NM)}_{\text{scoring}}$$

N, *M* are the numbers of family members for the input subunits

- Running time for a benchmark set of 20 protein assemblies, with 50 non-redundant members on average: ~800 CPU-hours.

- *N* and *M* could be large (up to 3,000). Can we reduce them?

The number of binding sites can be reduced

Benchmark: Comparative patch analysis converges to a native configuration, if the residue overlap of the input and native binding sites is $\geq$ 75%

Idea: No need to try all sites with the high co-localization

Solution (work in progress):

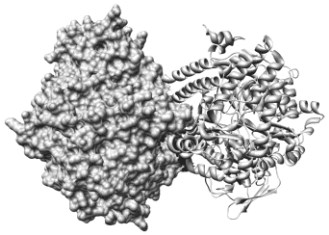
- cluster all binding sites based on their mutual overlap;
- use one representative binding site per cluster as the input to comparative patch analysis;

Performance on a benchmark set

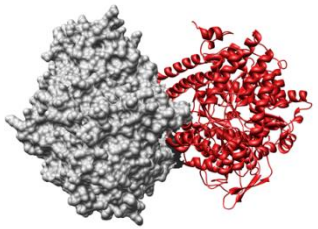
- benchmark set: 20 binary protein complexes (9 multidomain proteins, 11 protein assemblies)
- the method was evaluated using three different measures:

$$(1)\ O_B = \frac{1}{2}\left[\frac{N\ \ B_1^{pred} \cap B_1^{exp}}{N\ \ B_1^{pred} \cup B_1^{exp}} + \frac{N\ \ B_2^{pred} \cap B_2^{exp}}{N\ \ B_2^{pred} \cup B_2^{exp}}\right]; \quad (2)\ O_I = \frac{N(I_{pred} \cap I_{native})}{N(I_{pred} \cup I_{native})}; \quad (3)\ \textit{RMS error}$$

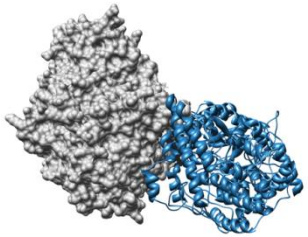
native



2 binding sites



1 binding site



conventional docking