Analysis and Prediction of Protein Complex

Laboratory of Bioinformatics I
Module 2

Emidio Capriotti
http://biofold.org/

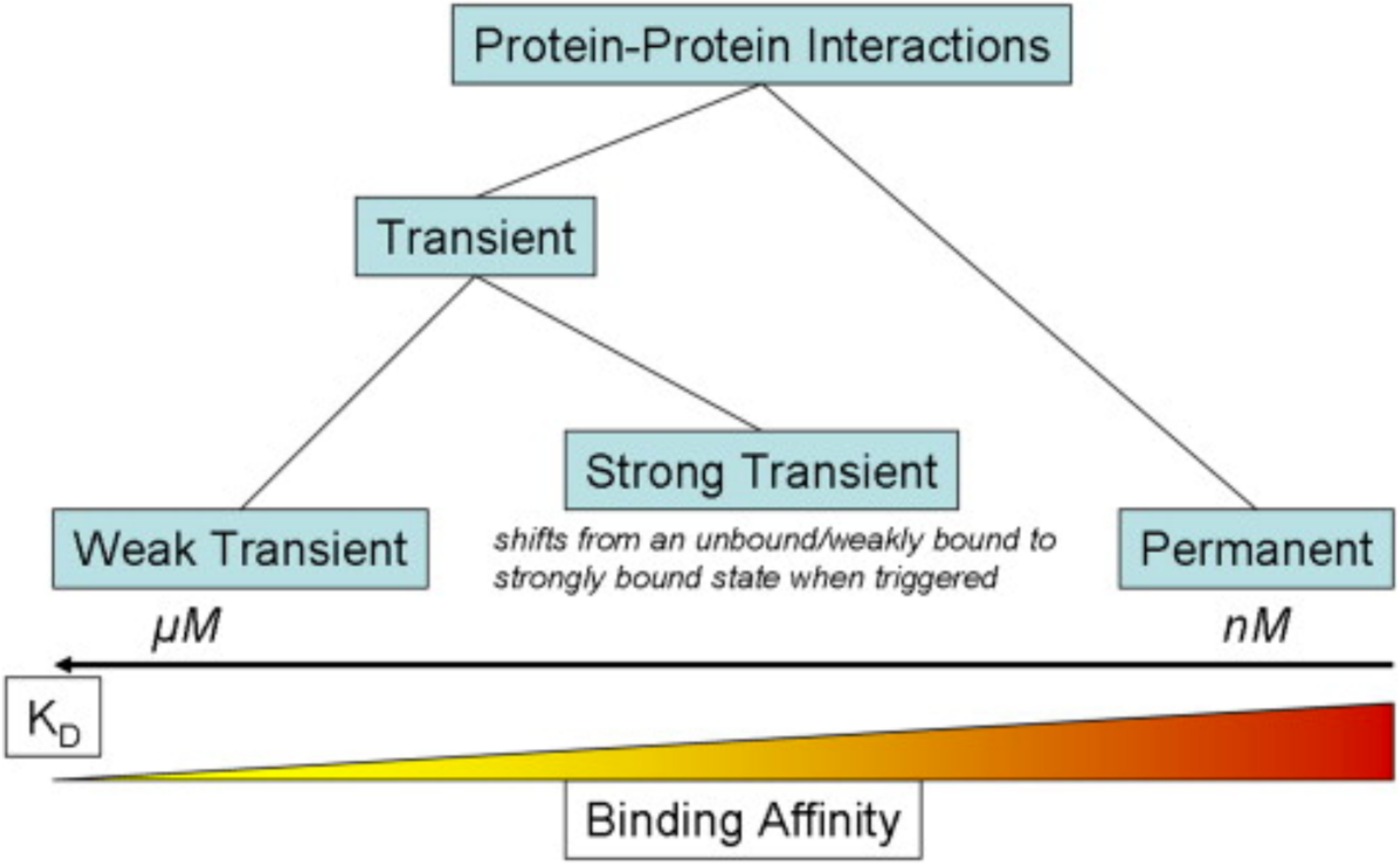
Biomolecules
Folding and
Disease

Department of Pharmacy and
Biotechnology (FaBiT)
University of Bologna

The Molecular Viewpoint

- The affinity of PPI varies from millimolar to picomolar, depending on the type of interaction and signaling needed (Chen et al. Protein Sci. 2013)
- Despite affinity varies over a wide range, proteins maintain a high degree of specificity for their partners
- Many proteins exhibit specificity for multiple partners (Reichmann et al. Curr. Opin. Struct. Biol. 2007).
- The nature of the interaction surface determines how proteins interact
- A detailed knowledge of the interaction surfaces of proteins and their energetics is necessary to understand the regulatory mechanisms of biochemical pathways (especially to modulate or block these pathways for therapeutic purposes)

Protein-Protein Interactions



Strong transient: This category includes interactions that are triggered/stabilised by an effector molecule or conformational change. An example is given by the Ras proteins, which form tight complexes with their partners when GTP-bound and only weak complexes when GDP-bound.

Perkins et al, Structure, 2010

Surface of Interaction (I)

- The area of PPI interfaces is large (1000 to 4000 Å^2)
- Standard-sized interfaces are 1200 to 2000 Å^2
- Short-lived and low-stability complexes -> smaller interfaces (1150–1200 Å^2)
- large surfaces (2000 to 4600 Å^2) ->
  - proteases and particular inhibitors
  - G-proteins and other components of the signal transduction system
- Protein-small molecule interaction surfaces have an area of 300 to 1000 Å^2.

Surface of Interaction (II)

- Surfaces of PPIs are generally flat and lack the grooves and pockets that are present at the surfaces of proteins that bind to small molecules.
- PPI surfaces are generally hydrophobic in nature.
- Only certain hydrophobic spots contribute to the free energy of binding and help to hold the two proteins together.
- Such regions are called hot spots.

Hot Spots

- Hot spots account for less than 50% of the contact area of PPI
- A region of protein surface is called a hot spot when replacement of an amino acid residue by alanine in that spot lowers the free energy of binding by at least 2 kcal/mol
- Analysis of the amino acid composition of hot spots shows that some residues are found more frequently in hot spots (Tyr, Trp, and Arg)
- The hot spots are surrounded by energetically less important residues that separate/prevent bulk water from hot spots

Analysis of Protein Complex

- identification of interface residues/hot spots
- details about the interface
  solvent accessible surface area, shape, complementarity between surfaces, residue interface propensities, hydrophobicity, segmentation and secondary structure, and conformational changes on complex formation
- assignment of protein function
- recognition of specific residue motifs

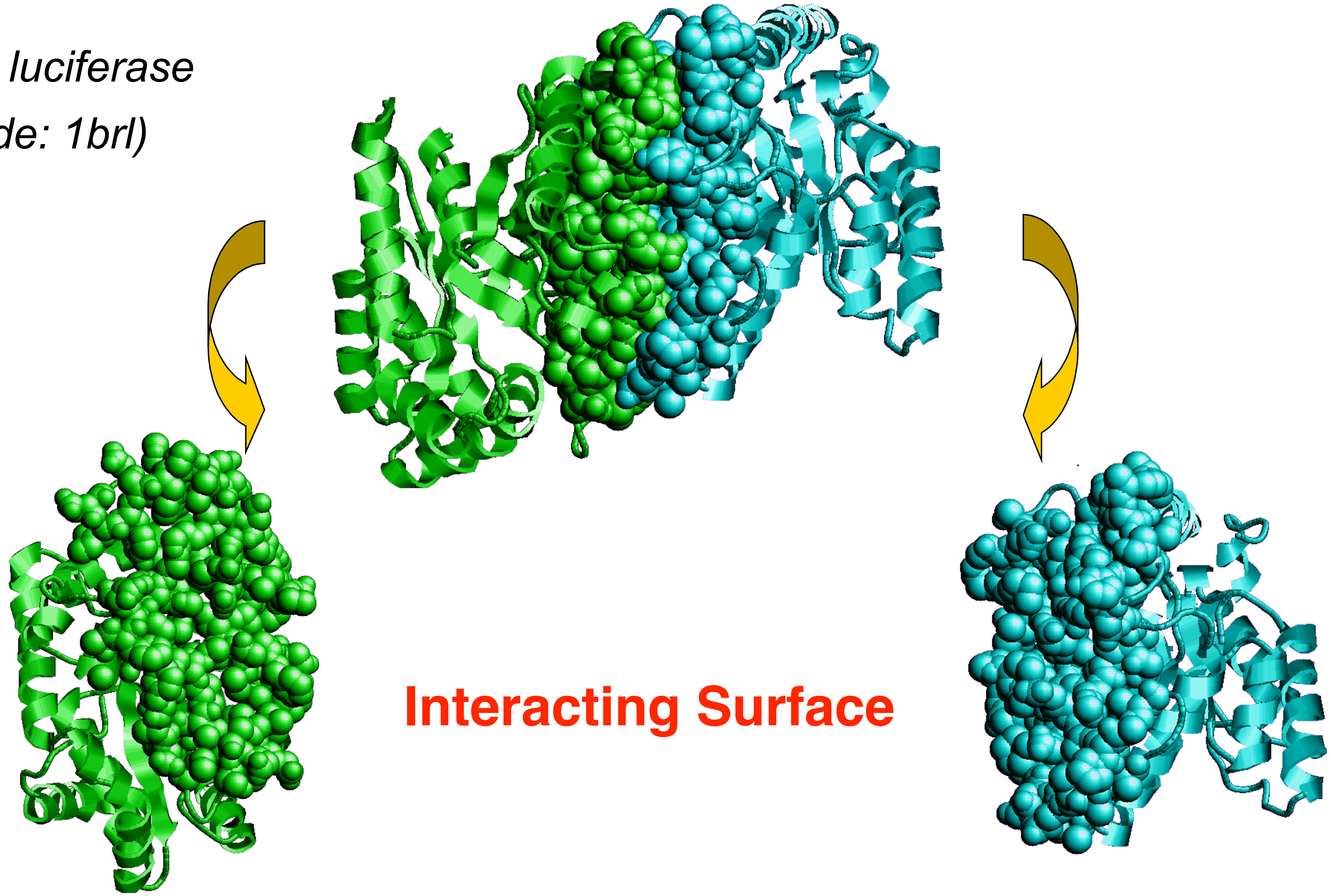
Structure PPI Data

- The most significant contribution to understanding the PPI surface comes from structural biology via X-ray crystallography or NMR as well as mutational studies
- Prediction of interaction/binding sites
- Prediction of protein-protein complexes

Interacting surface

Difference in Accessible Surface Area (ASA) between monomers and complex

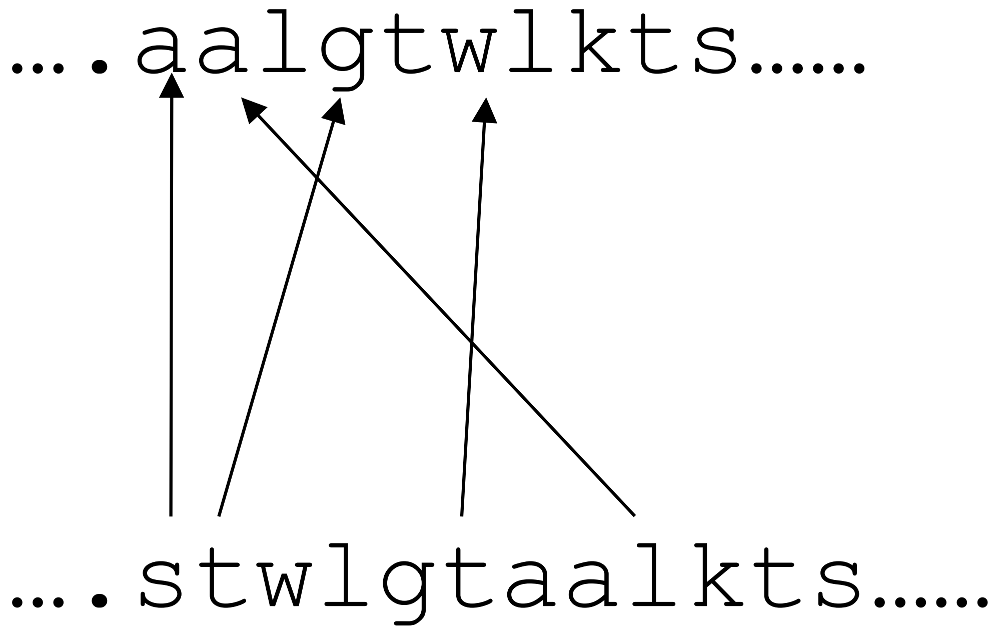
Bacterial luciferase
(PDB code: 1brl)



Interacting Surface

Prediction features

Protein Sequence

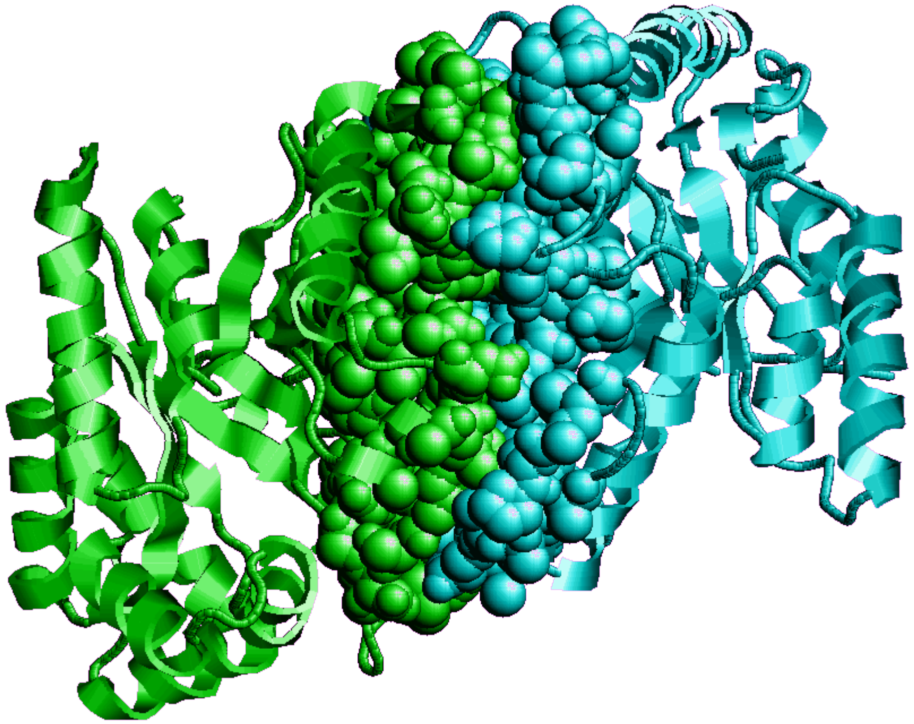

….aalgtwlkts……

….stwlgtaalkts……

+ Whole genome computation

- No exact location, No atomic description

Protein Structure



+ Exact location Atomic description

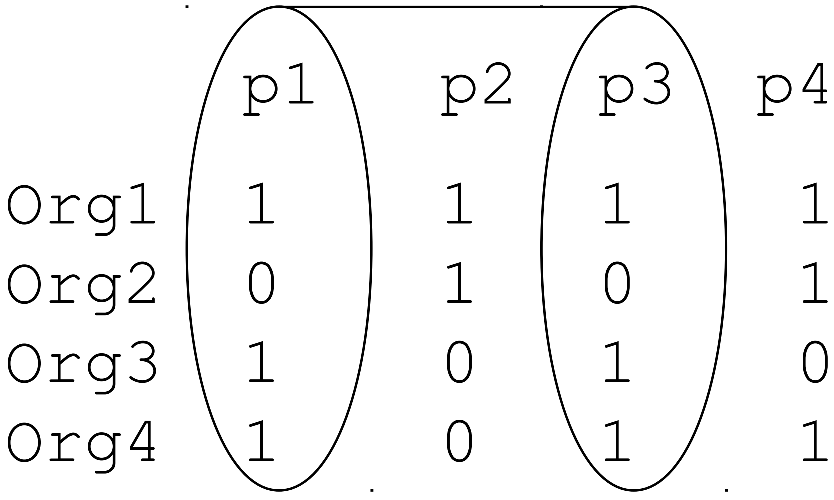
- Availability of the 3D coordinates

Three major problems

- Protein-Protein interaction networks: given a set of proteins, predict the possible partners
- Docking: given a pairs of proteins, known to interact, predict the geometry of the complex
- Protein-interaction sites: given a single protein, predict possible interacting regions

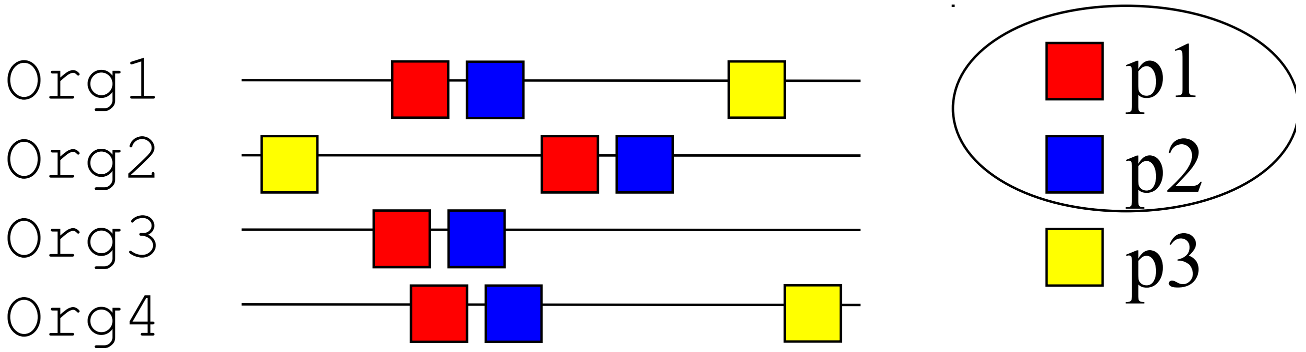
Sequence-based methods

Phylogenetic Profiling: interacting proteins should co-evolve and should have orthologs in closely related species.

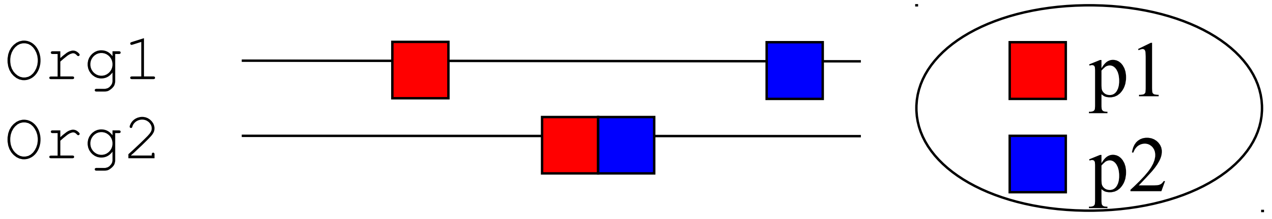


	p1	p2	p3	p4
Org1	1	1	1	1
Org2	0	1	0	1
Org3	1	0	1	0
Org4	1	0	1	1

Gene Neighborhood: interacting proteins and co-evolvig homologs tend to have close genomic locations.



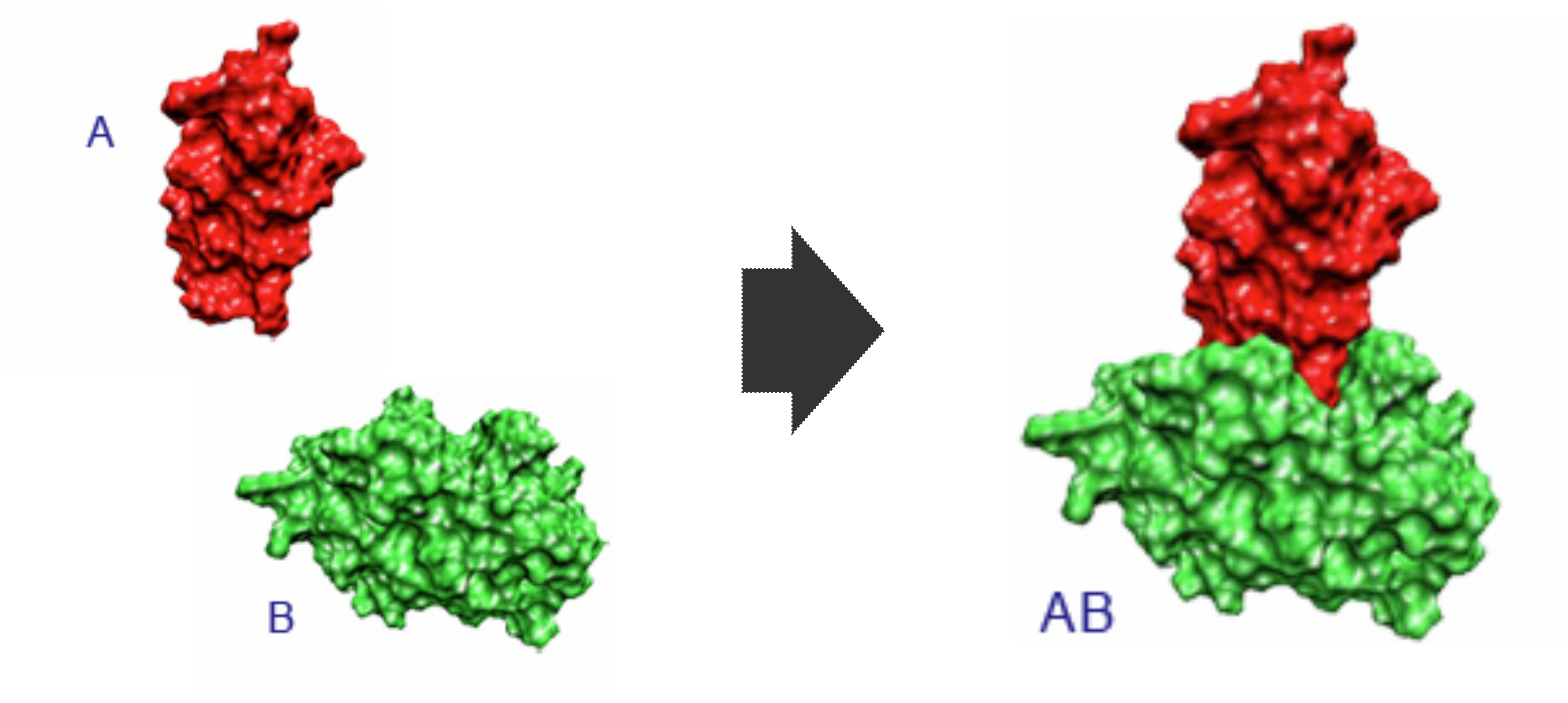
Gene Fusion: two proteins that interact may have homologs in other genomes that are fused into a unique protein



From wikipedia

Protein Docking

- Computational schemes that aims to **find the "best" matching between two molecules**, a **receptor** and a **ligand**
- The molecular docking problem can be defined as follows: **given the atomic coordinates of two molecules, predict their "correct" bound association**



Halperin et al. Proteins, 2002

Protein-Protein docking

- Used to model the quaternary structure of complexes formed by two or more interacting proteins

- It is the “gold standard” for prediction of PPIs

- It used to predict if two proteins interact and also how the interaction takes place ("mode" of binding)

- It is computationally very challenging and thus very unlikely to be applied for high throughput purposes.

Ngounou Wetie et al. Proteomics, 2013

What we can learn?

- Do proteins A (receptor) and B (ligand) bind *in vivo*?

If they do bind:

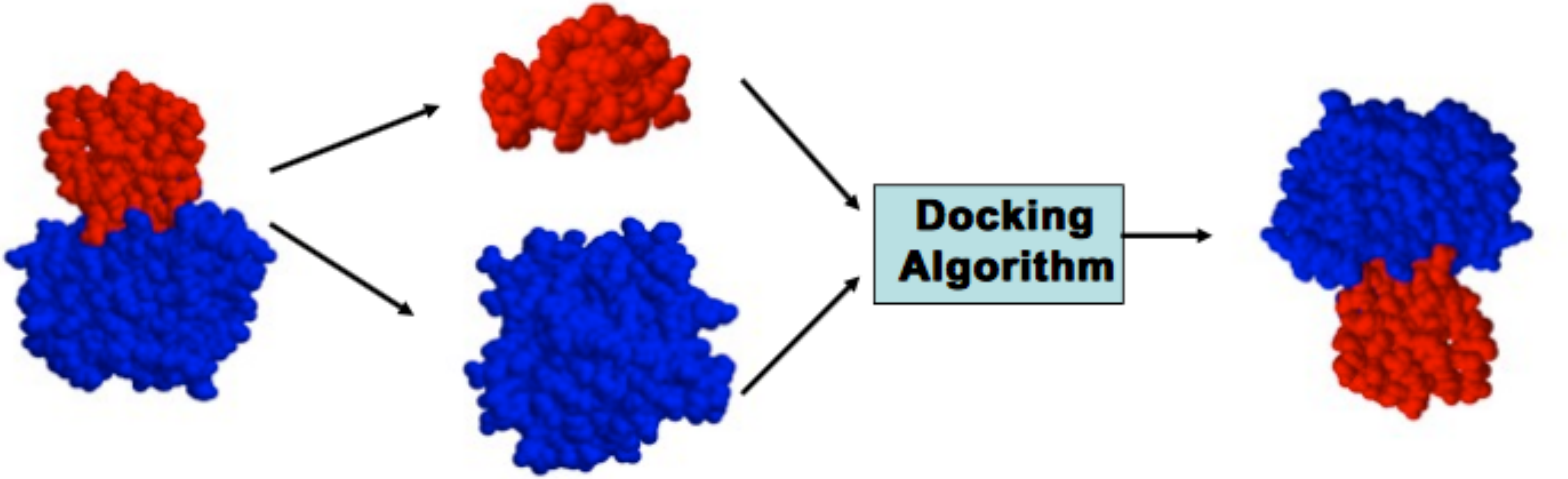
- What is the spatial configuration they adopt in their bound state?
- What is the structure of the protein complex (**near-native structure**) in atomic details ?
- How strong or weak is their interaction (which types of interactions are present)?
- What is the orientation that maximises the interaction, minimizing the energy of the complex?

If they don't bind:

- Would they bind if there was a mutation?

Bound docking

- Reconstruct a complex using the bound structures of the receptor and the ligand.
- After artificial separation of the receptor and the ligand, the goal is to reconstruct the native complex

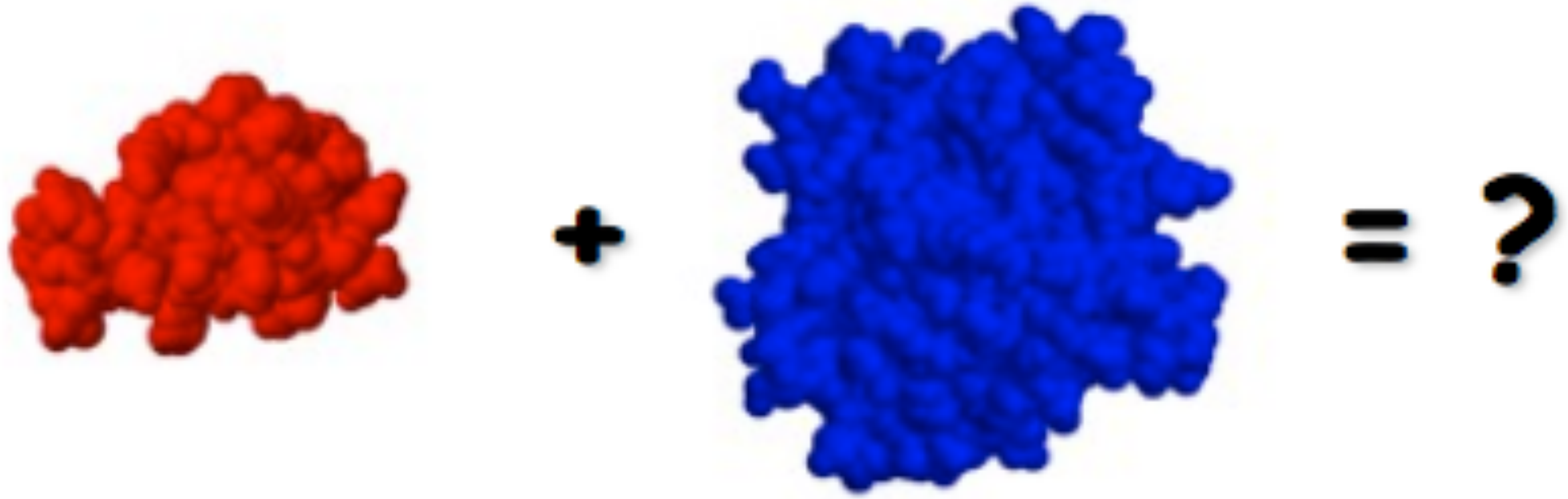


- No conformational changes are involved
- **Used to validate the algorithm**

Allegra Via, Systems Biology Course 2015

Predictive docking

- Schemes that attempt to reconstruct a complex using the unbound structures of the receptor and the ligand
- An "unbound" structure maybe a **native** structure, a **pseudo-native** structure, or a **modelled** structure
- **Native**: free in solution, in its uncomplexed state
- **Pseudo-native**: structure complexed with a molecule different from the one used for the docking



Halperin et al. Proteins, 2002

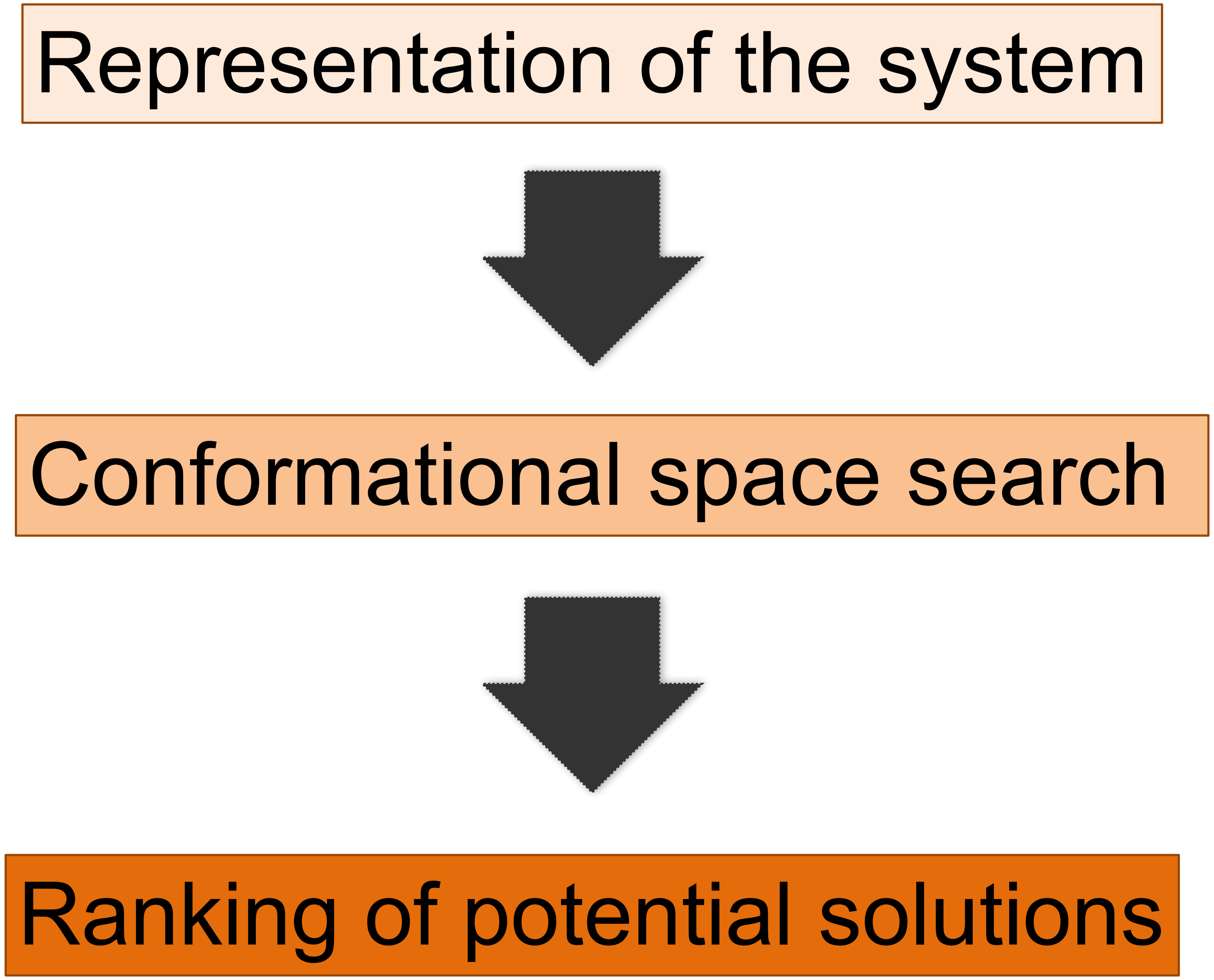
Why it is difficult?

- # of possible conformations are astronomical
  – thousands of degrees of freedom (DOF)

- Free energy changes are small
  – Below the accuracy of our energy functions

- Molecules are flexible
  – alter each other’s structure as they interact

Main docking steps



Representation of the system

Conformational space search

Ranking of potential solutions

Allegra Via, Systems Biology Course 2015

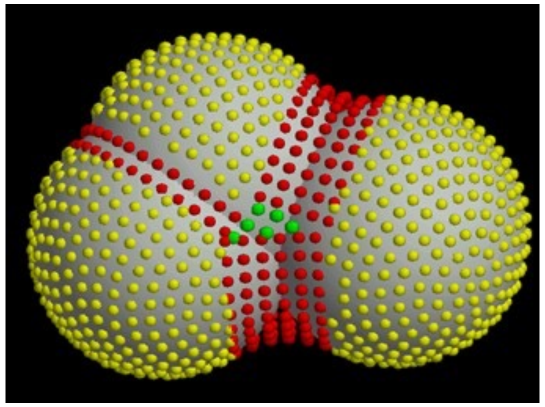
Systems representation

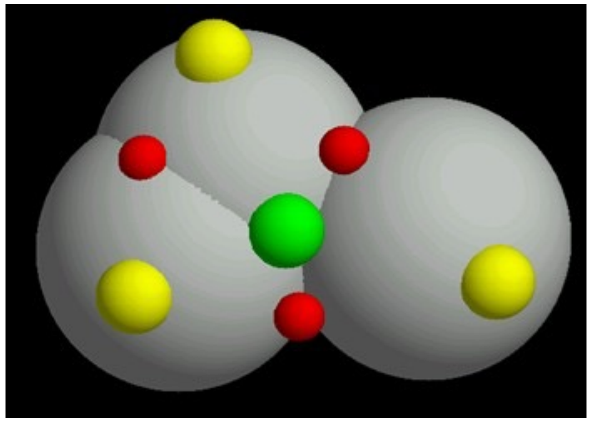
- Docking essentially simulates the interaction of the protein surface

- How do we define a protein surface?
  - Mathematical models (e.g. geometrical shape descriptors, a grid)
  - Static or dynamic treatment of the protein frame (rigid vs flexible)

- The choice of the system (surface) representation decides the types of conformational search algorithms, and the ways to rank potential solutions

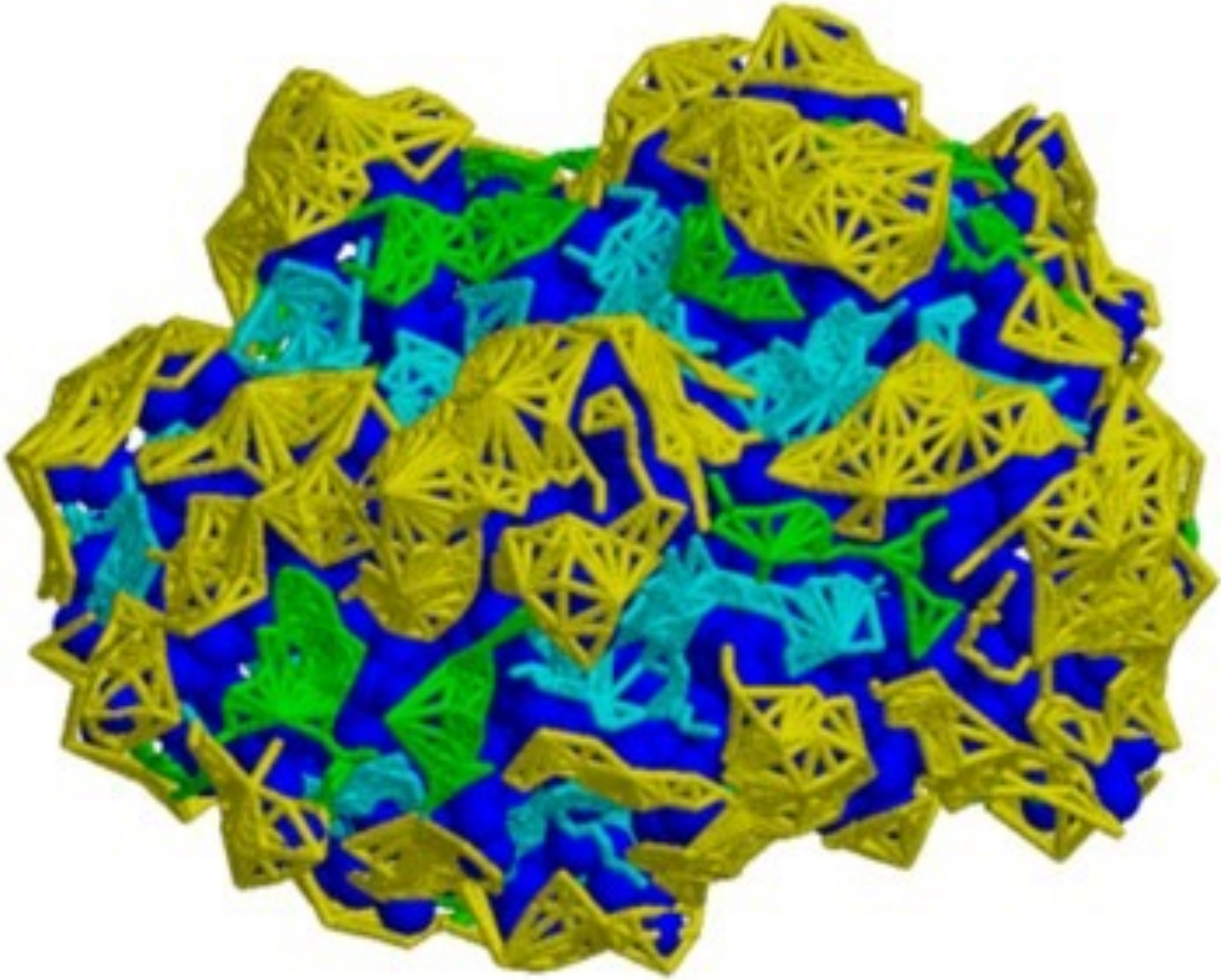
Surface representation





Patch detection

- Divide the surface into connected, non-intersecting, equal sized patches of critical points with similar curvature



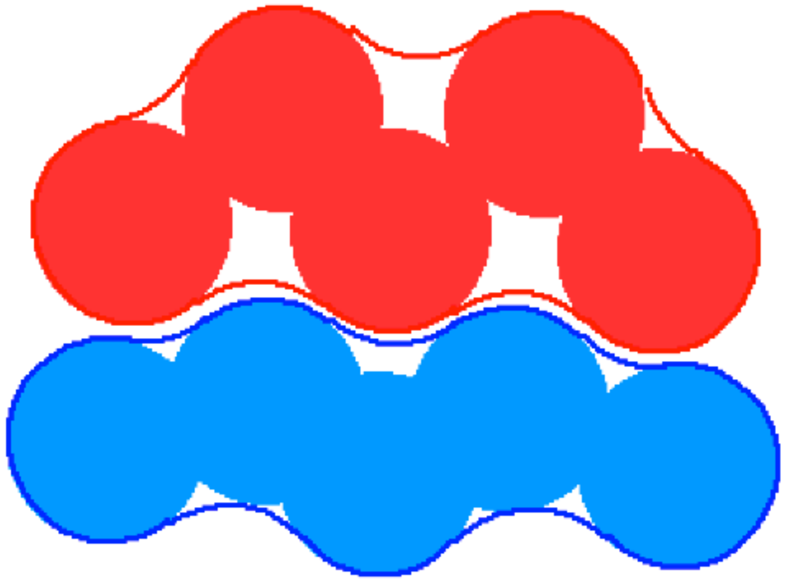
Yellow: knob patches
Cyan: hole patches
Green: flat patches
Blue: protein

Allegra Via, Systems Biology Course 2015

Molecular recognition

- Van der Waals
- Electrostatics
- Hydrophobic contacts
- Hydrogen bonds
- Salt bridges

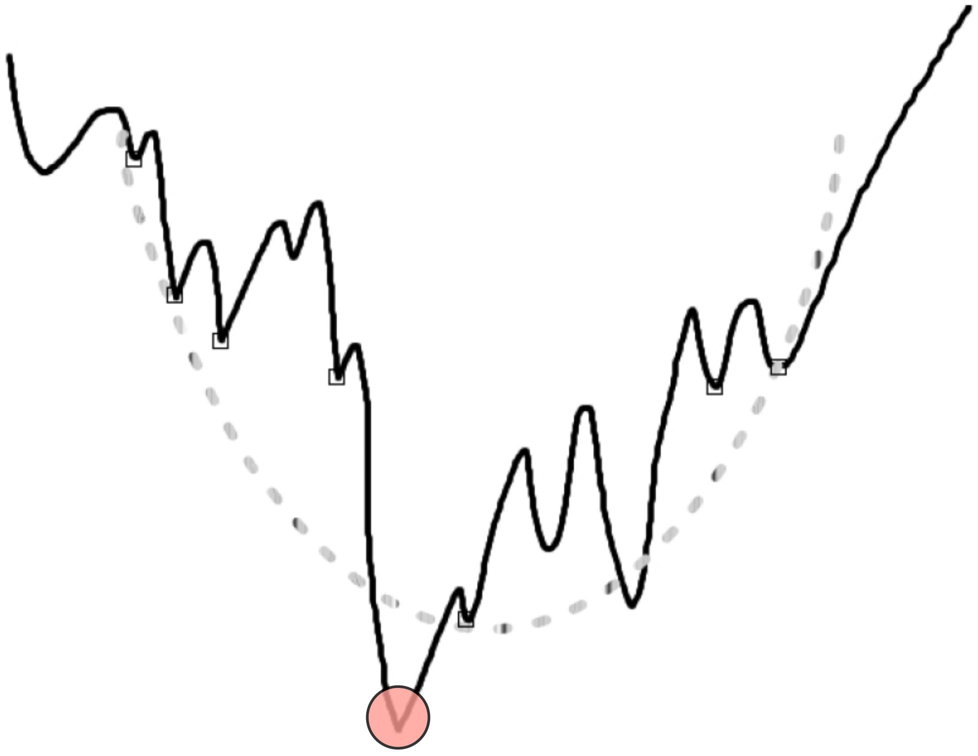
All interactions act at short ranges → surface complementarity is needed for tight binding



Efrat Mashiach, http://bioinfo3d.cs.tau.ac.il/

Conformational space

- Efficient search algorithm
- Speed and effectiveness in covering the relevant conformational space
- Computationally difficult - there are many ways to put two molecules together (3 translational + 3 rotational degrees of freedom)
- Goal: locate the most stable state (global minimum) in the energy landscape



Allegra Via, Systems Biology Course 2015

Docking types

- **Rigid body** is a highly simplistic model that regards the two proteins as two rigid solid bodies
  - fast → can explore the entire receptor and ligand surfaces
  - Less accurate
  - flexibility = "soft" belt into which atoms can penetrate

- The **semi-flexible** model is asymmetric; one of the molecules is considered flexible, while the receptor is regarded as rigid

- **Flexible** docking. Both molecules are considered flexible, though flexibility is limited or simplified
  - Slower
  - More accurate
  - Can model side-chain/backbone flexibility
  - highly reliable but too slow for extensive ligand docking

Minimization protocols

- scan of the entire solution space in a predefined systematic manner

  e.g., complete searches of all orientations between two rigid molecules by systematically rotating and translating one molecule about the other

- a gradual guided progression through solution space. Only part of the solution space is searched, or fitting solutions are generated.

  e.g., Monte Carlo, simulated annealing, molecular dynamics (MD), and evolutionary algorithms.

- Data-driven docking

  it uses the available information about binding site/interface residues .

Scoring the predictions

- A search algorithm may produce a large number of solutions (~10^9)
- Goal: discriminate between "correct" native solutions, i.e., with low RMSD from the crystal structure and others within reasonable computation time
- Good scoring function: fast enough to allow its application to a large number of potential solutions

  effectively discriminates between native and non-native docked conformations

  should include and appropriately weight all the energetic ingredients.

Allegra Via, Systems Biology Course 2015

Scoring parameters

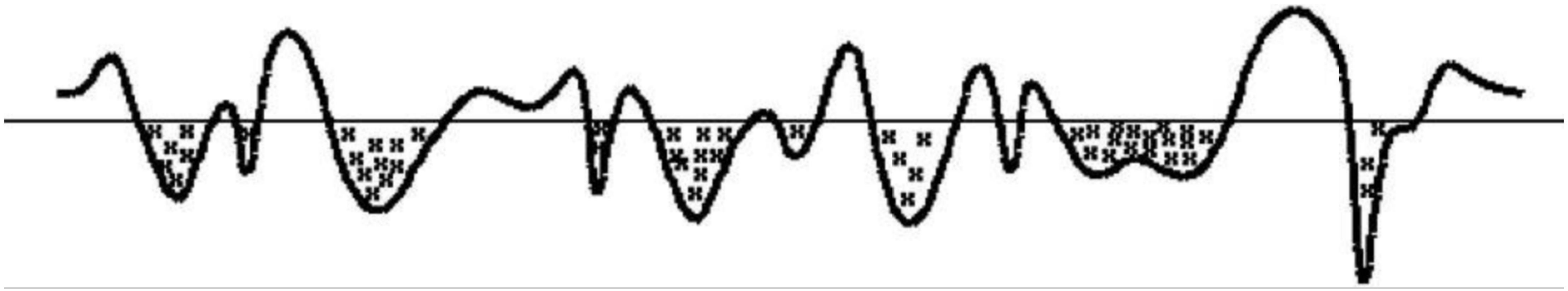
- Geometric complementarity - how to score complementarity is strongly coupled with the surface representation.
- Intermolecular overlap – tolerance to slight interface clashes and penalty for protein interior clashes (surface "belt" of nonpenalised penetration area)
- Intra-molecular overlap – when backbone flexibility is taken into account
- Hydrogen bonding
- Contact area: total interactions = hh + pp + hp (h = hydrophobic, p = polar)
- Pairwise aa and atom-atom contacts – empirical term derived form observed statistical frequency of aa contacts in X-ray proteins
- Electrostatic interactions and solvation energy

Knowledge-based scores

- Knowledge of the location of the binding site on one or both proteins drastically reduces the number of possible solutions
- Knowledge of the specific binding site residues reduces the search space even further
- Info about active site residues: site directed mutagenesis, chemical cross-linking, phylogenetic data
- Sometimes the binding site can be predicted
- For some families the major binding sites are known in advance (e.g. serine proteases and immunoglobulins)

Prediction clustering

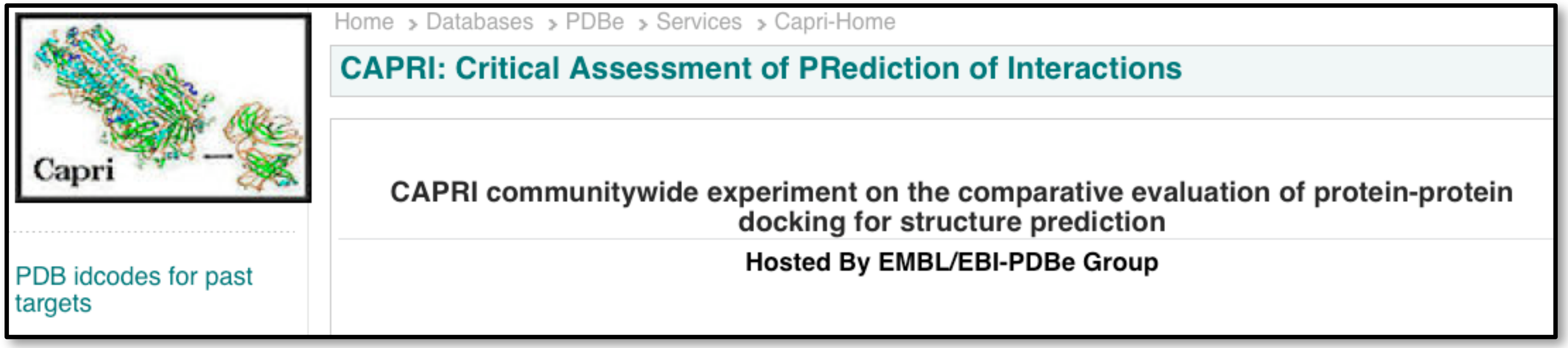
- **Events that occur in clusters are probably not random**
- The cluster with the largest number of low-energy structures is typically the native fold, the center of the most populated cluster being a structure near the native binding site
- Looking for large clusters is a major tool of finding near-native conformations



Kozakov et al, Biophys J, 2005

CAPRI Experiments

- CAPRI is a community-wide experiment in modelling the molecular structure of protein complexes
- CAPRI is a **blind prediction experiment** aimed at testing the performance of protein docking methods
- Rounds take place about every six months
- Each round contains between one and six target protein–protein complexes whose structures have been recently determined experimentally
- Targets are unpublished crystal or NMR structures of complexes, whose coordinates are held privately by the assessors, with the co-operation of the structural biologists who determined them
- The atomic coordinates of the two proteins are given to groups for prediction



CAPRI: Critical Assessment of PRediction of Interactions

CAPRI communitywide experiment on the comparative evaluation of protein-protein docking for structure prediction

Hosted By EMBL/EBI-PDBe Group

http://www.ebi.ac.uk/msd-srv/capri/

Conclusions (-)

- The *molecular docking problem* is far from being solved
- It is difficult to find very specific properties of protein-protein interfaces
- Results are generally poor with weakily interacting proteins
- Proteins are flexible and may undergo even large conformational changes upon binding
- Exhaustive space searches provide too many conformations
- Accurate interaction energies are too complicated to compute
- For most complexes the highest ranked structures are still false positives (high RMSD from the complex)
- No efficient method for reliable discrimination between correct solutions and FPs is currently available, in particular if the binding site is unknown
- Many FPs displaying good surface complementarity are far from the native complex

Conclusions (+)

- If the conformational change is limited to surface side-chain atoms, rigid body algorithms have been remarkably successful, even in absence of knowledge of the binding site
- Side-chain flexibility can be handled via a "soft" tolerance belt”
- Docking in steps" is a promising strategy: Initial rigid-body, entire surface algorithm followed by a dynamic method overcoming energy barriers
- Integration of experimental information produces reliable docking results
- Relatively easy for enzyme-inhibitor complexes
- Sometimes good results with antigen-antibody pairs

Some methods

- **HADDOCK** (software/web server).
  http://haddock.chem.uu.nl

- **CLUSPRO** (software/web server)
  http://cluspro.bu.edu

- **ICM-pro** (desktop-modeling environment)
  http://www.molsoft.com/protein_protein_docking.html

- **ROSETTADOCK** (software/web server)
  http://graylab.jhu.edu/docking/rosetta/
- http://rosettadock.graylab.jhu.edu/submit

- **GRAMM-X** (web server)
  http://vakser.bioinformatics.ku.edu/resources/gramm/grammx

- **PATCHDOCK/FIREDOCK** (software/web server)
  http://bioinfo3d.cs.tau.ac.il/PatchDock/

- **HEX** (software/web server)
  http://hexserver.loria.fr

Exercise

Download the DSSP file of the Bacterial luciferase (Vibrio harveyi) from the PDB (code: 1BRL)

- Generate the DSSP file for the protein complex and the isolated chains A and B
- Calculate the total solvent accessible area of the complex and isolated chains and calculate the surface of interaction for both chains.
- Given the size of the binding surface what kind of protein interaction it is expected?
- Find the residue at the interface and calculate the variation of relative solvent accessible area. Which residue are buried in the interacting surface?

Chain = col 12, AA = col 14, SS = col 17, Acc: cols 36-38, Phi: cols 104-109, Psi: cols 110-115