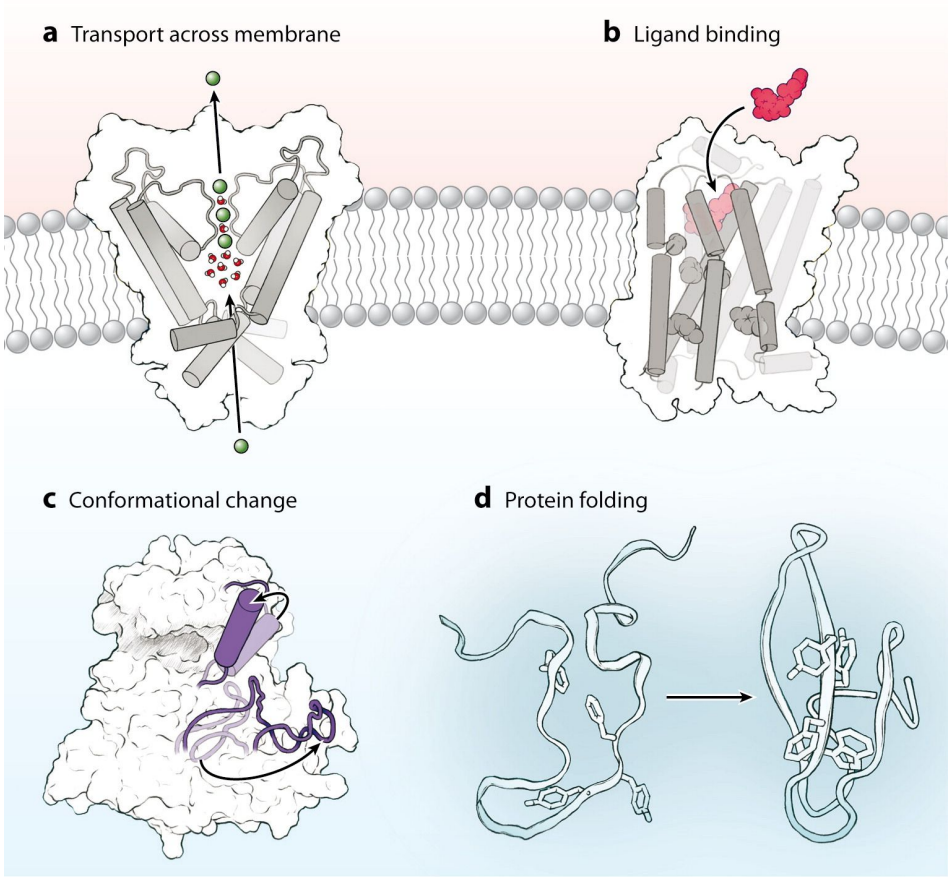
Lectures 21-22: Protein structure

- **Molecular dynamics**
- Amino-acid coevolution
  - Mutual information
  - Maximum entropy modeling

Molecular dynamics (MD) simulations = Computational microscope

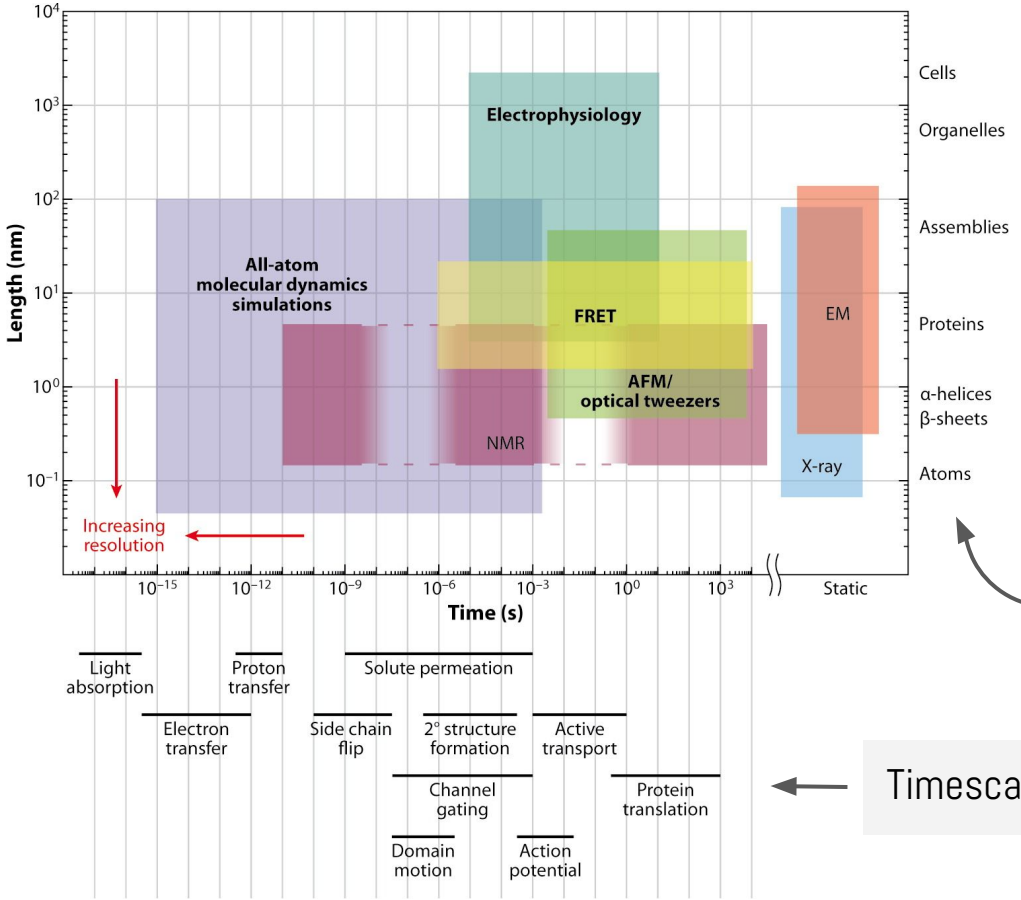


MD simulations reveal the workings of biomolecular systems at a spatial and temporal resolution that is often difficult to access experimentally.

- Positions and velocities of atoms are computed using Newton's laws of motion.

Dror (2012) Annu. Rev. Biophys.

Spatiotemporal resolution of various techniques



Spatial resolution of biological features

Timescales of molecular processes

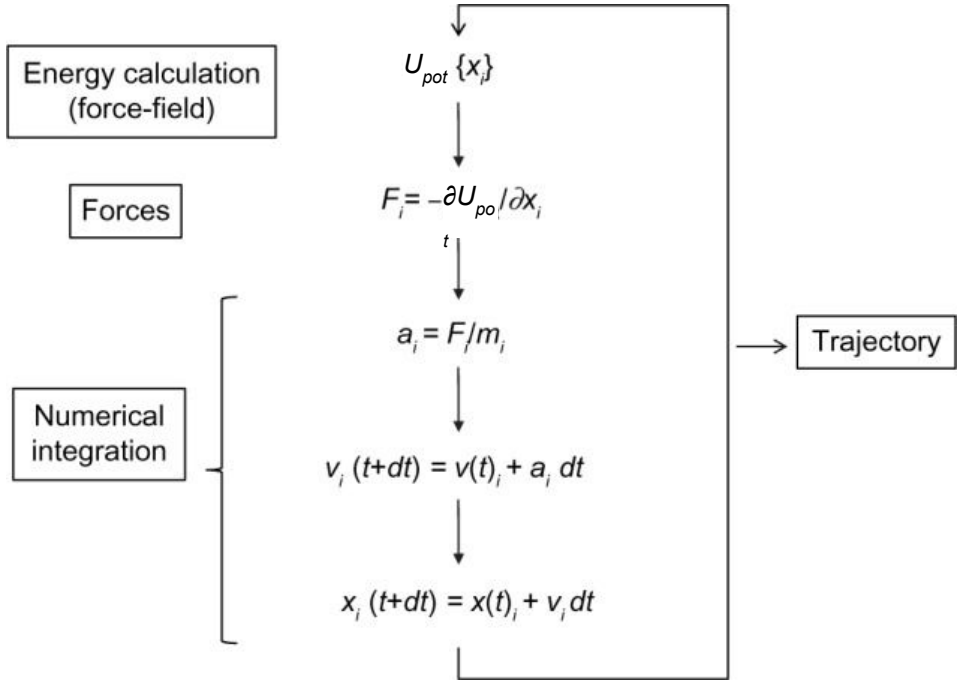
Data on single molecules (as opposed to only on ensembles) are in boldface.

- AFM, atomic force microscopy
- EM, electron microscopy
- FRET, Forster resonance energy transfer
- NMR, nuclear magnetic resonance

Dror (2012) Annu. Rev. Biophys.

Molecular dynamics (MD) simulations = Computational microscope

The basic MD algorithm.



The simulation output – the trajectory – is an ordered list of 3N atom coordinates for each simulation time (or snapshot).

U_{pot}: potential energy

t: simulation time

dt: iteration time

For each spatial coordinate of the N simulated atoms (i):

- x: atom coordinate
- F: forces component
- a: acceleration
- m: atom mass
- v: velocity.

Hospital (2016) Adv. Appl. Bioinform. Chem.

Force field and the energy function

The potential energy of *N* interacting atoms $U(\mathbf{r}_1, \ldots, \mathbf{r}_N)$ is a function of their positions $\mathbf{r}_i = (x_i, y_i, z_i)$.

The force acting upon *i*th atom is determined by the gradient (vector of first derivatives) with respect to atomic displacements:

$$\mathbf{F}_i = -\nabla_{\mathbf{r}_i} U(\mathbf{r}_1, \cdots, \mathbf{r}_N) = -\left(\frac{\partial U}{\partial x_i}, \frac{\partial U}{\partial y_i}, \frac{\partial U}{\partial z_i}\right)$$

Find the positions $r_i(t + \Delta t)$ at time $t + \Delta t$ in terms of the already known positions at time t.

Verlet algorithm:

$$\mathbf{r}_i(t + \Delta t) \cong 2\mathbf{r}_i(t) - \mathbf{r}_i(t - \Delta t) + \frac{\mathbf{F}_i(t)}{m_i}\Delta t^2$$

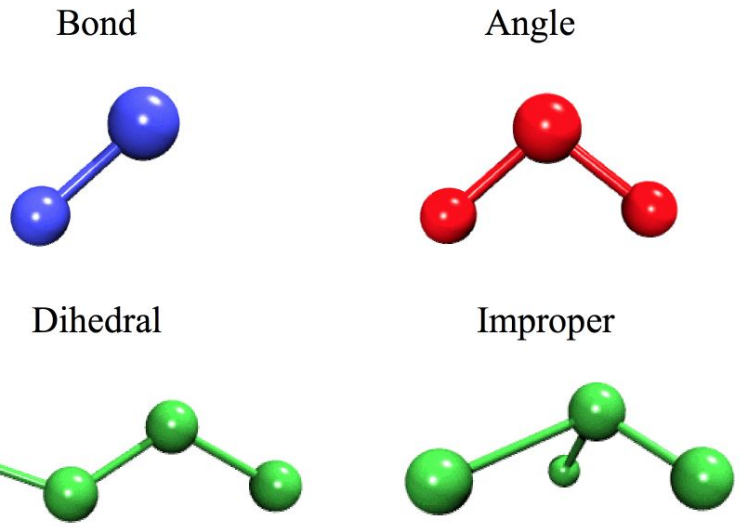
Force field: energy function used to compute the forces acting on atoms (due to interatomic interactions) during an MD simulation.

Force field and the energy function

$$U(\vec{R}) = \underbrace{\sum_{bonds} k_i^{bond}(r_i - r_0)^2}_{U_{bond}} + \underbrace{\sum_{angles} k_i^{angle}(\theta_i - \theta_0)^2}_{U_{angle}} +$$

$$\underbrace{\sum_{dihedrals} k_i^{dihe}[1 + \cos(n_i\phi_i + \delta_i)]}_{U_{dihedral}} +$$

$$\underbrace{\sum_i \sum_{j \neq i} 4\epsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right] + \sum_i \sum_{j \neq i} \frac{q_i q_j}{\epsilon r_{ij}}}_{U_{nonbond}}$$



U_{bond}: oscillations about the equilibrium bond length

U_{angle}: oscillations of 3 atoms about an equilibrium angle

$U_{dihedral}$: torsional rotation of 4 atoms about a central bond

$U_{nonbond}$: non-bonded energy terms (electrostatics and Lenard-Jones)

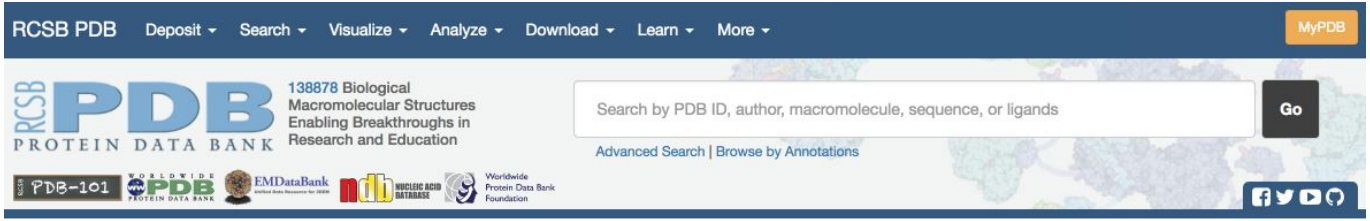
Force field: energy function used to compute the forces acting on atoms (due to interatomic interactions) during an MD simulation.

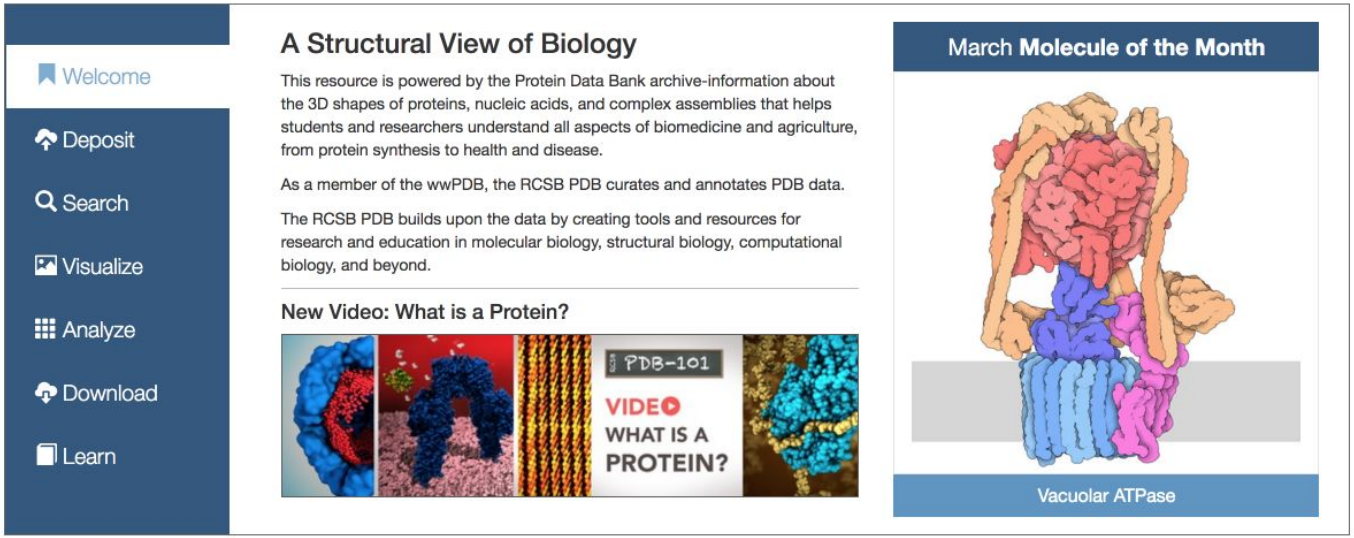
Steps in a typical MD simulation

1. Prepare molecule: Read in pdb and psf file
2. Minimization: Reconcile observed structure with force field used (T = 0)
3. Heating: Raise temperature of the system
4. Equilibration: Ensure system is stable
5. Dynamics: Simulate under desired conditions (NVE, NpT, etc); Collect your data
6. Analysis: Collect your data; Evaluate observables (macroscopic level properties); Or relate to single molecule experiments.

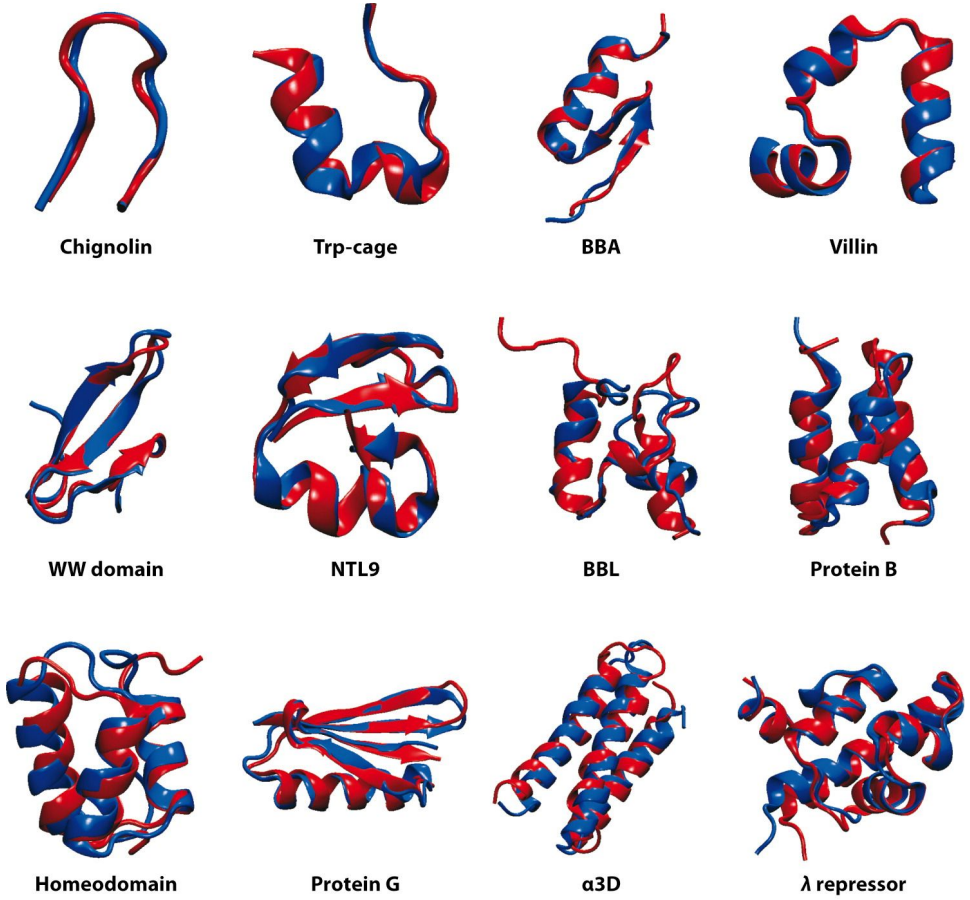
Protein Data Bank (PDB)

www.rcsb.org: 3D shapes of proteins, nucleic acids, and complex assemblies.





Simulations of structurally diverse proteins



Simulations with a single force field.

- 12 structurally diverse proteins fold spontaneously to a structure (blue) closely resembling that determined experimentally (red).
- Simulation snapshots chosen automatically based on a clustering analysis that did not exploit knowledge of the experimental structure.
- Total simulation time per protein: 104 – 2,936 μs – allowing observation of at least 10 folding & 10 unfolding events for each protein.

Dror (2012) Annu. Rev. Biophys.

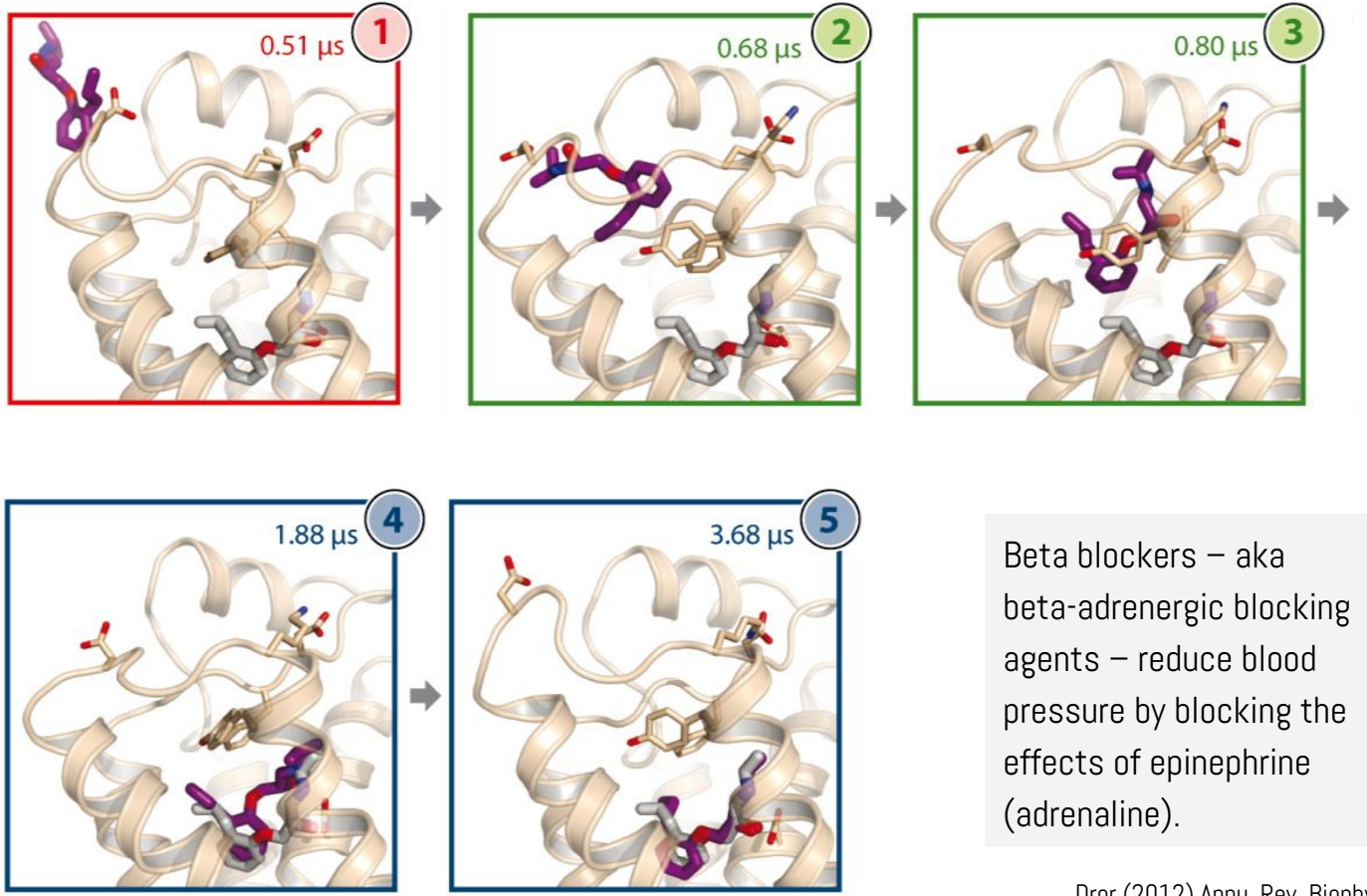
Beta-blockers binding spontaneously to the β2-adrenergic receptor

Metastable Intermediate stages of beta blocker binding.

1: Ligand moves from bulk solvent…

2, 3: … into the extracellular vestibule, and finally…

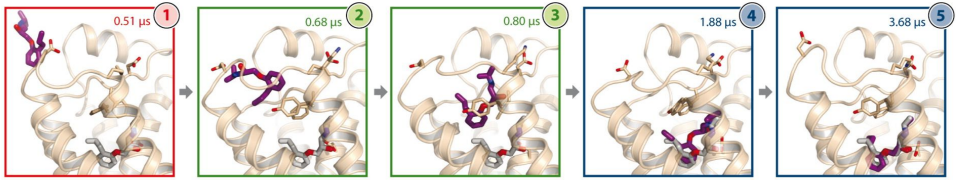
4, 5: … into the binding pocket.

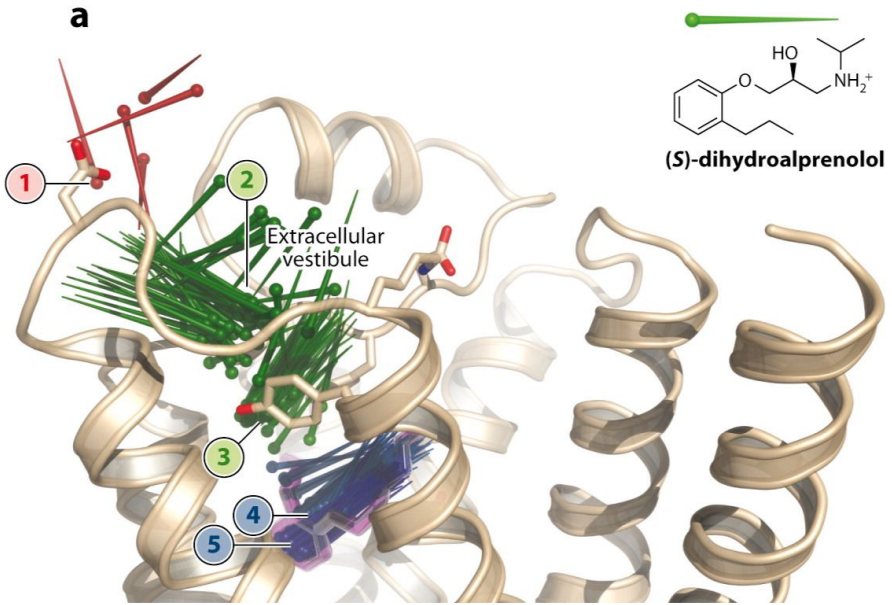


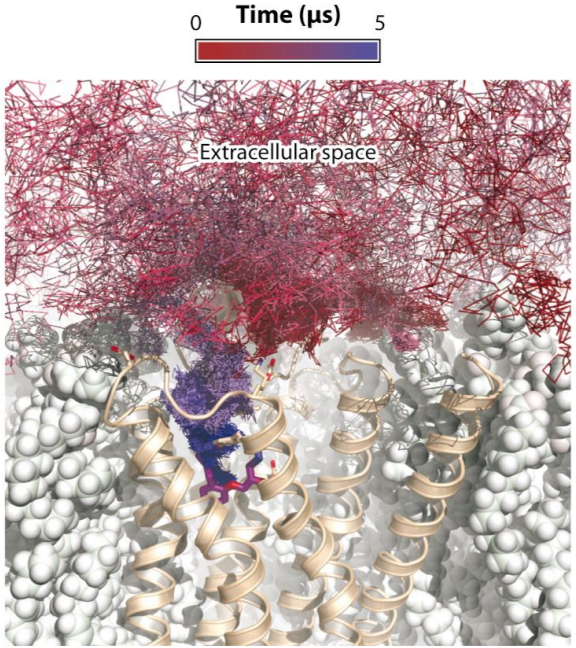
Beta blockers – aka beta-adrenergic blocking agents – reduce blood pressure by blocking the effects of epinephrine (adrenaline).

Dror (2012) Annu. Rev. Biophys.

Beta-blockers binding spontaneously to the β2-adrenergic receptor



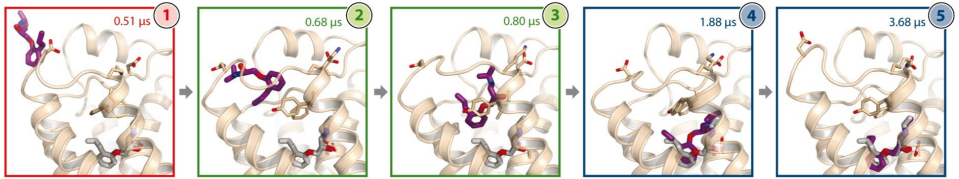


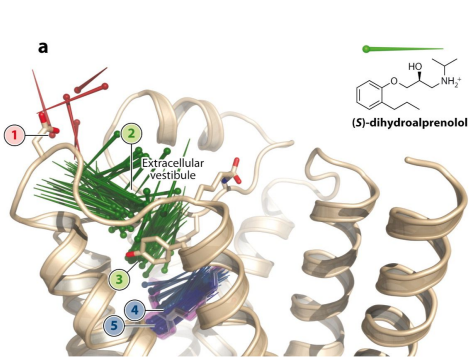


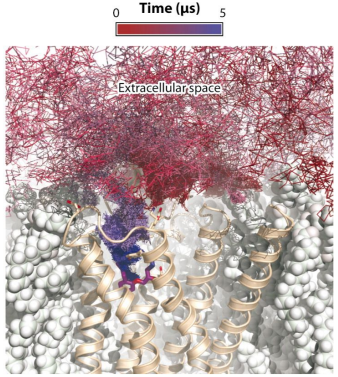
Pins: successive positions

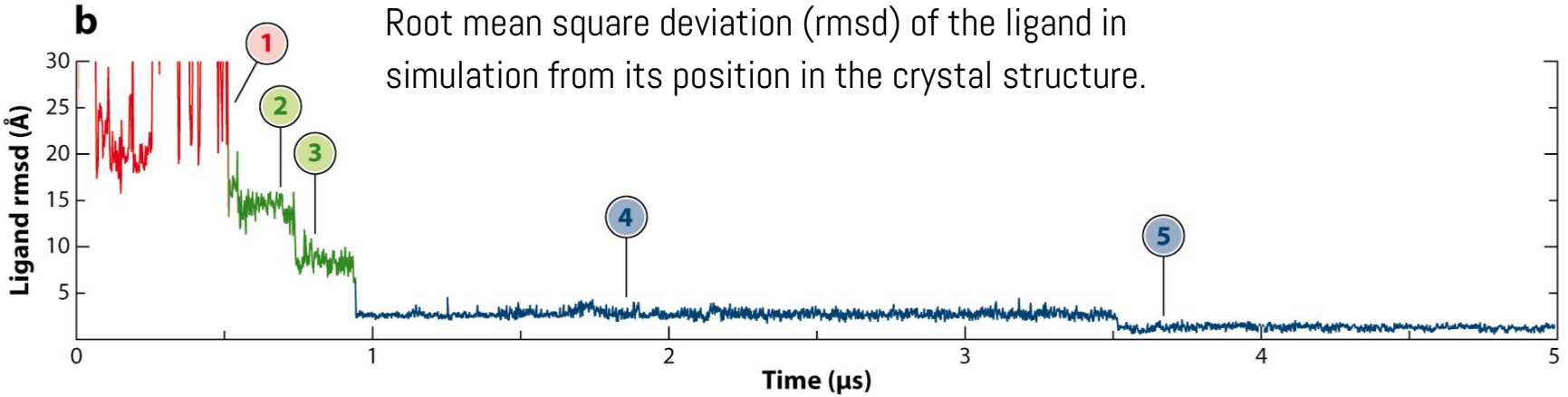
Dror (2012) Annu. Rev. Biophys.

Beta-blockers binding spontaneously to the β2-adrenergic receptor









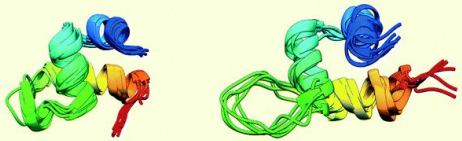
Root mean square deviation (rmsd) of the ligand in simulation from its position in the crystal structure.

Dror (2012) Annu. Rev. Biophys.

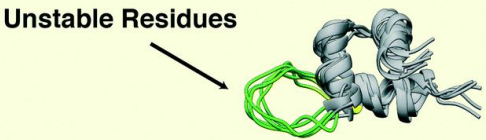
MD simulations for protein design

A. Modulating Protein Stability

I. Perform simulations in native and nonnative environments



II. Inform designs by analyzing the dynamics of unstable residues

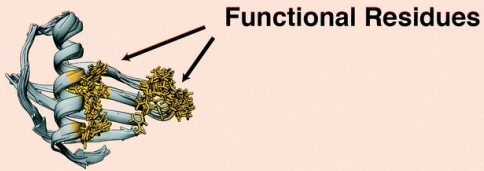


III. Perform simulations of designs to determine the impact of mutations on protein stability

B. Engineering Functional Regions

I. Perform simulations that capture functional dynamics

II. Inform designs by analyzing the dynamics of functional residues



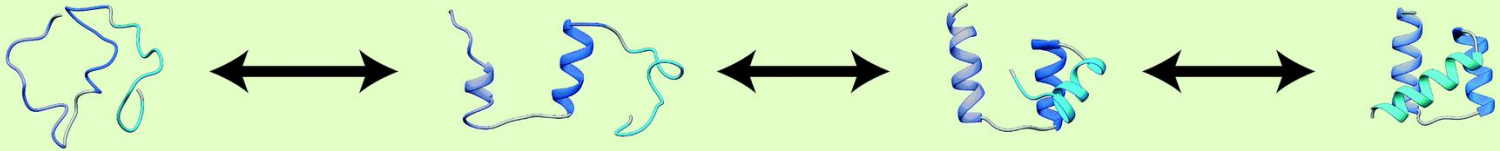
III. Perform simulations of designs to assess the impact of mutations on function regions

Childers & Daggett (2017) Mol. Syst. Des. Eng.,

MD simulations for protein design

C. Insights From Folding Pathways

I. Simulate the unfolding/folding pathway and partition the trajectory into conformational states



Denatured State	Folding Intermediates	Transition State	Native State

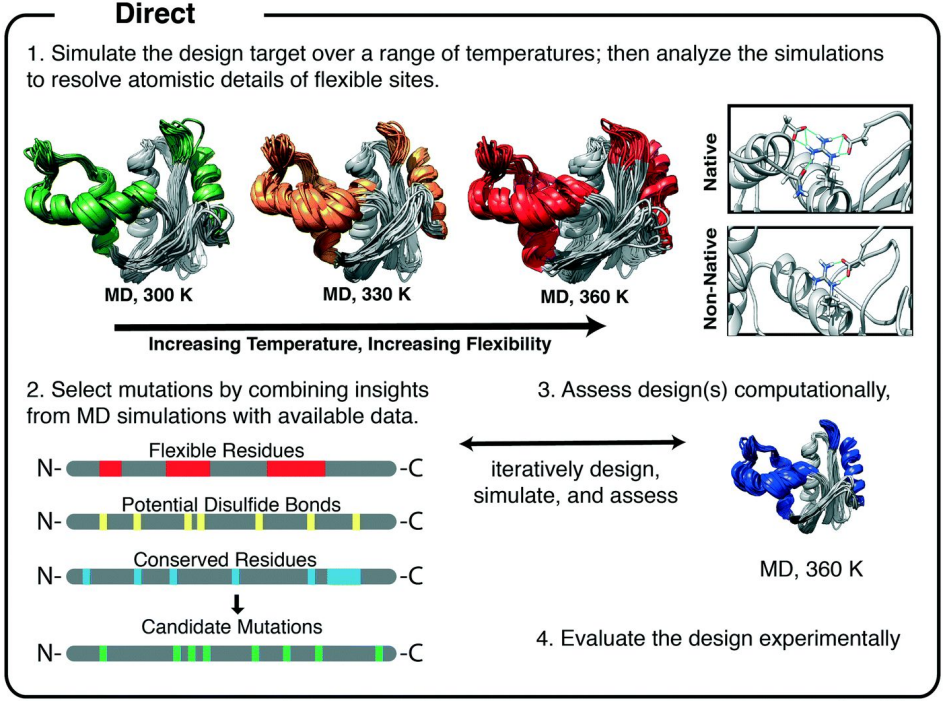
II. Inform designs with insights from specfic conformations or transitions along the folding pathway

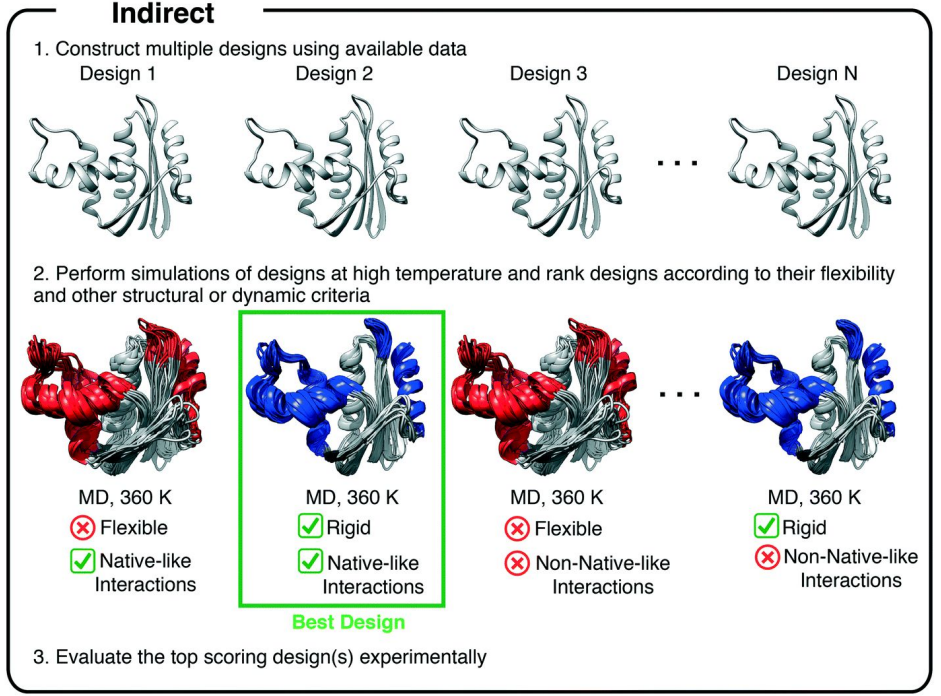
Destabilize the Denatured State	Stabilize Folding Intermediates	Design Fast Folding Variants	Probe / Alter the Folding Pathway

III. Perform simulations of designs to assess the impact of mutations on the folding landscape

Childers & Daggett (2017) Mol. Syst. Des. Eng.,

MD simulations for protein design





Childers & Daggett (2017) Mol. Syst. Des. Eng.,

Virtual screening: docking & MD simulations

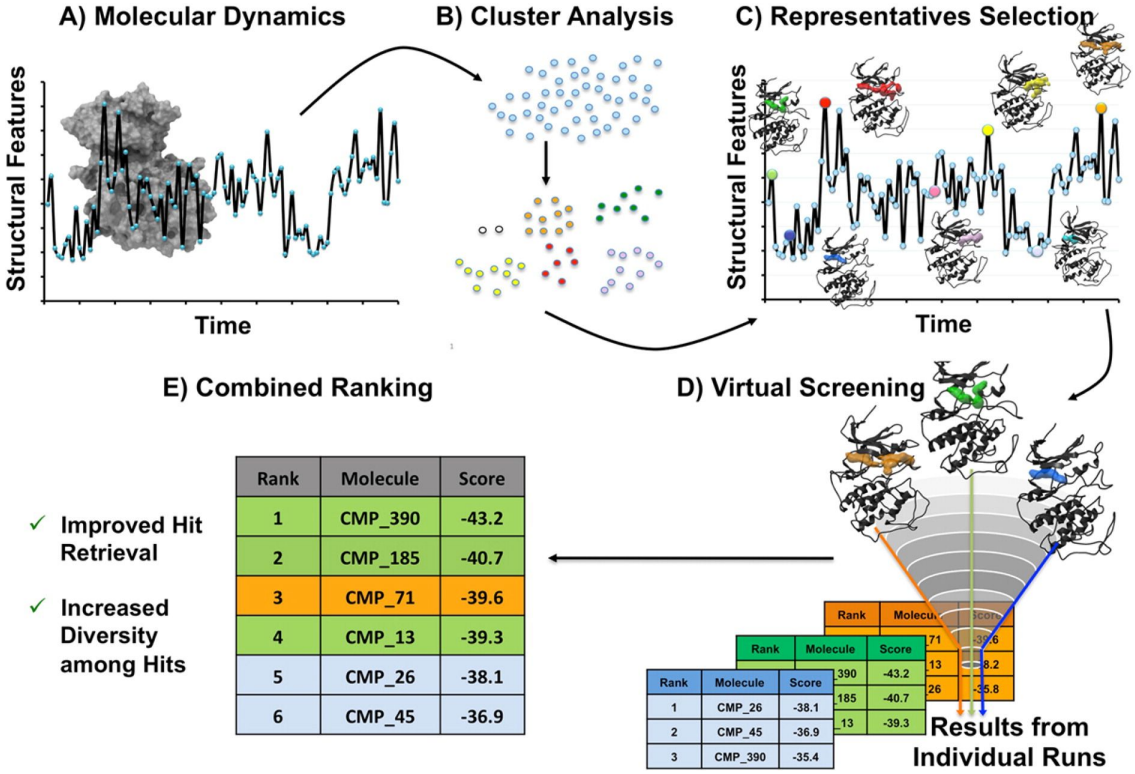
(A) Use MD trajectory to explore the receptor conformational space.

(B) Extract several snapshots from the trajectory; clustering to eliminate redundancy.

(C) From each cluster, select a representative structure (e.g., medoid).

(D) Carry out virtual ligand screening independently at each representative conformation.

(E) Return activity predictions by independent runs and combine together in a global ranking.



De Vivo (2016) J. Med. Chem.

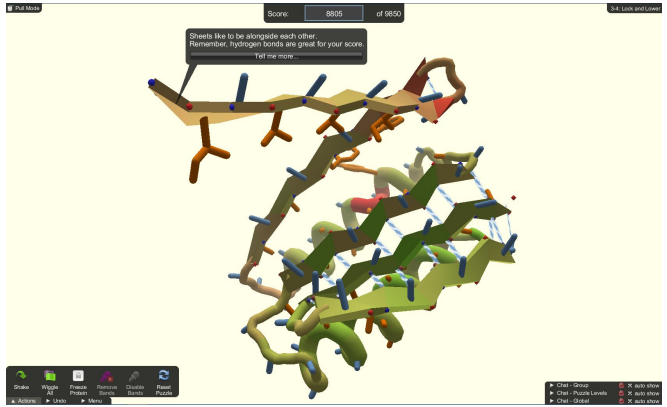
Distributed computing & Crowdsourcing

Folding@home: folding.stanford.edu

- Distributed computing project for MD simulations (e.g., protein folding, computational drug design).
- Uses the idle resources of personal computers owned by volunteers from all over the world.

Foldit: fold.it

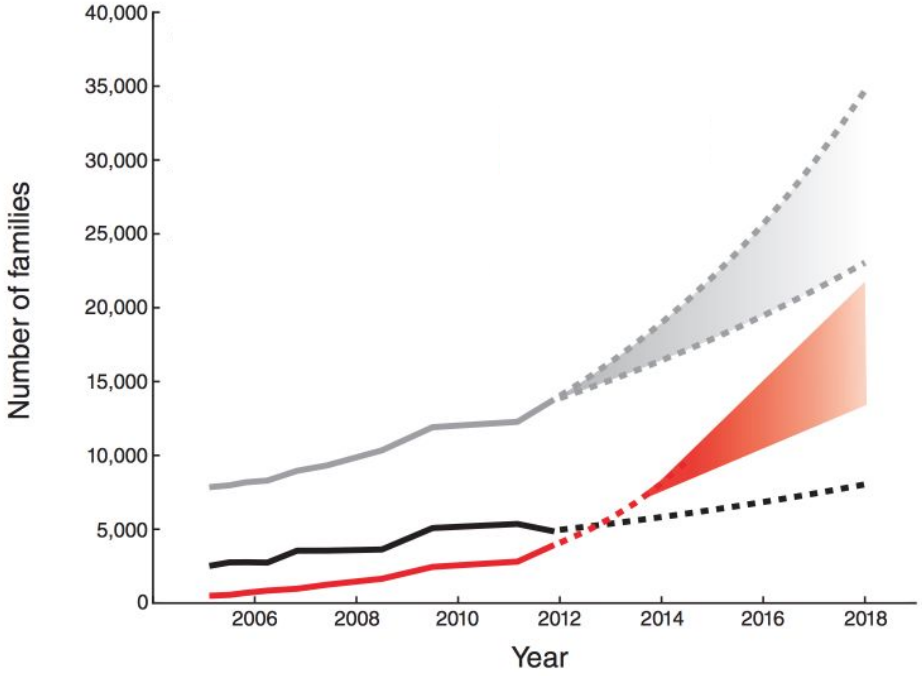
- An online game that poses complex puzzles about how proteins fold.
- Helped solve the structure of a protein-sniping enzyme critical for reproduction of the AIDS virus within 3 weeks; Identified targets for drugs to neutralize it.



Lectures 21-22: Protein structure

- Molecular dynamics
- **Amino-acid coevolution**
  - Mutual information
  - Maximum entropy modeling

Direct vs. indirect interactions



New protein families being discovered by high-throughput sequencing

Experimental structure-determination

Marks (2012) Nat. Biotech.

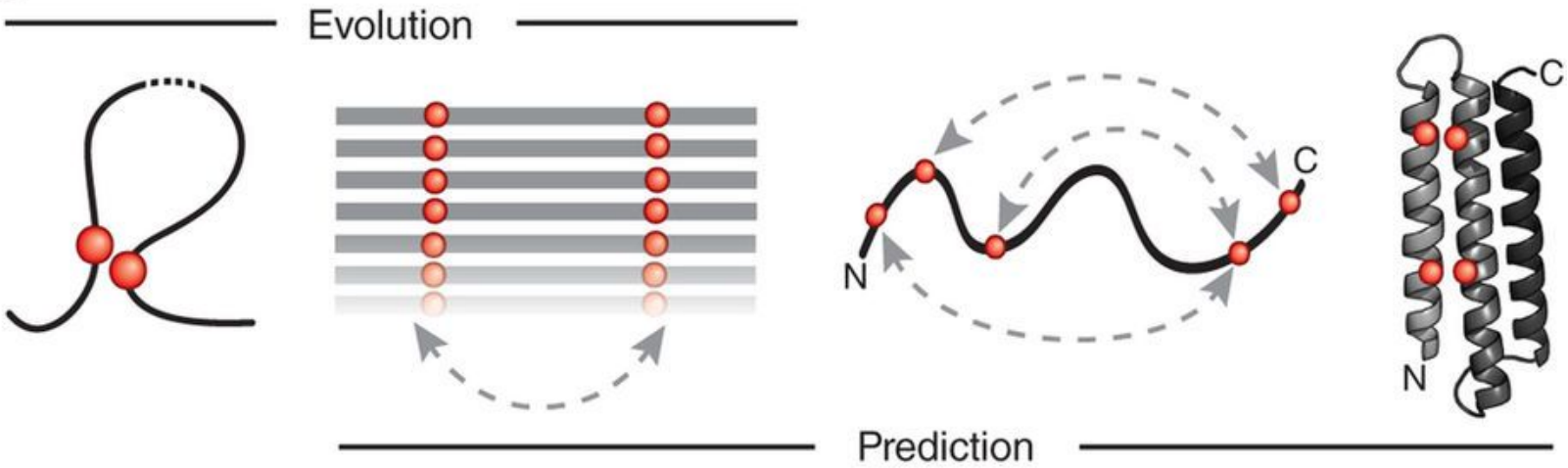
Predicting protein 3D structure from sequence

Evolutionary pressure to maintain favorable interactions b/w physically interacting AA residues in 3D.

Visible record of residue covariation in related protein sequences.

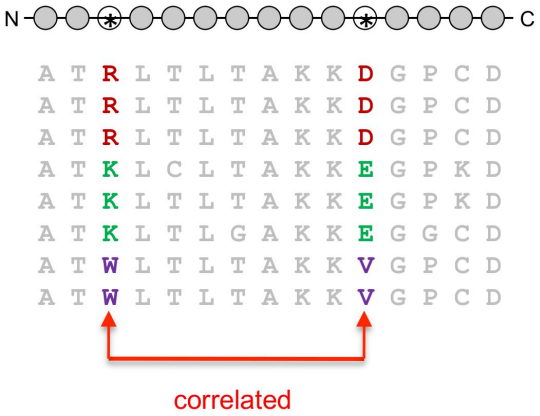
Inverse problem – inferring directly causative residue couplings (evolutionary couplings) from the covariation record – challenging due to transitive correlations & other confounding effects.

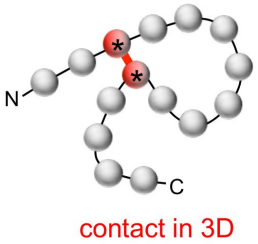
ECs can be used to predict the unknown 3D structure of a protein from a set of sequences alone.

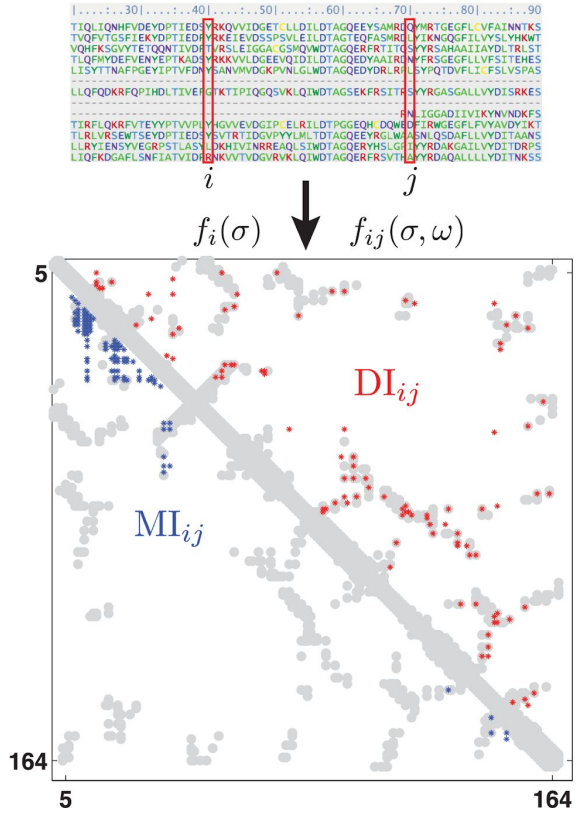


Marks (2012) Nat. Biotech.

Predicting protein 3D structure from sequence







Marks (2011) PLoS One; Marks (2012) Nat. Biotech.
Stein (2015) PLoS Comp. Biol.

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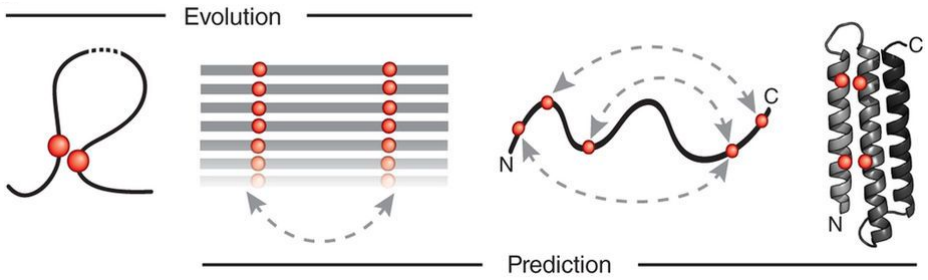


Growth in sequence databases from massively parallel sequencing.

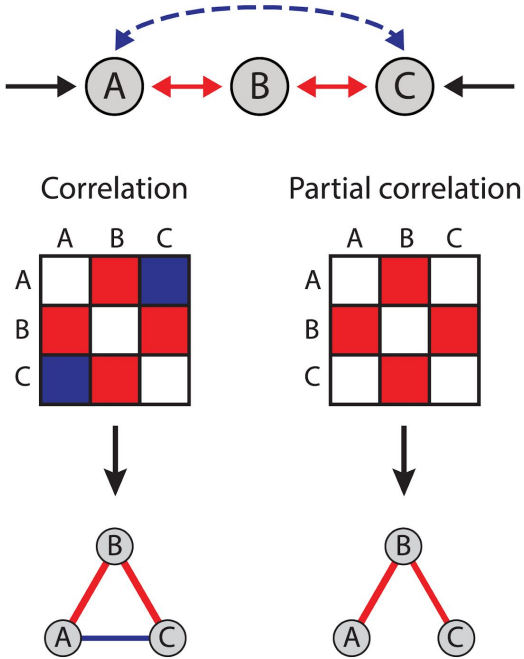
- Availability of sufficient sequences of sufficient diversity.
- Known protein families are growing in size from a few sequences to many thousands of sequences (advances in DNA sequencing tech).

Reduction of conformational search space by cooperative probability models.

- Global probability models account for the fact that interactions along an entire protein chain are mutually interdependent in a way that is inherently cooperative.
- Pair interactions are modified by interactions with other parts of the system and cannot be factored (probabilities are not a simple product of independent terms).
- Compared with molecular dynamics simulations, statistical approaches are many orders of magnitude more efficient in reducing a huge conformational search space to manageable proportions.

Marks (2012) Nat. Biotech.

Direct vs. indirect interactions



Stein (2015) PLoS Comp. Biol.

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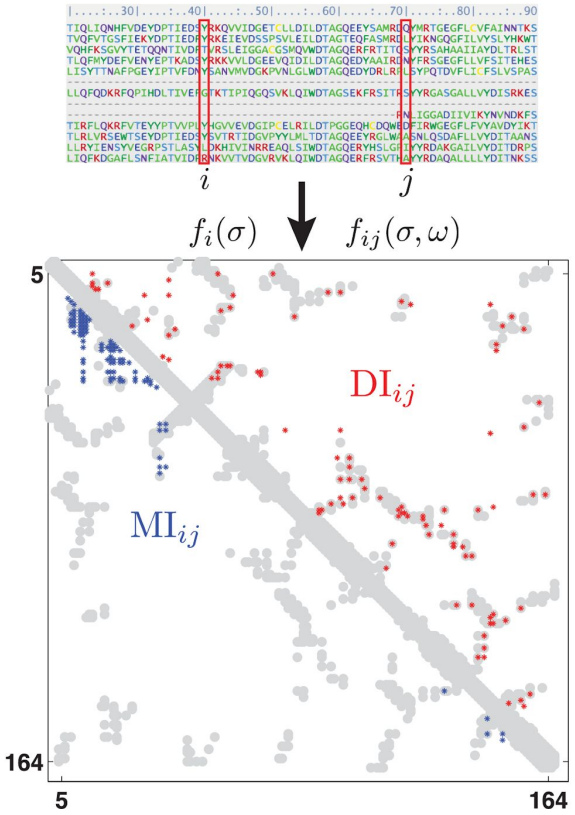
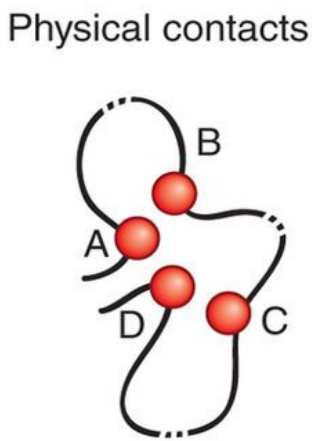
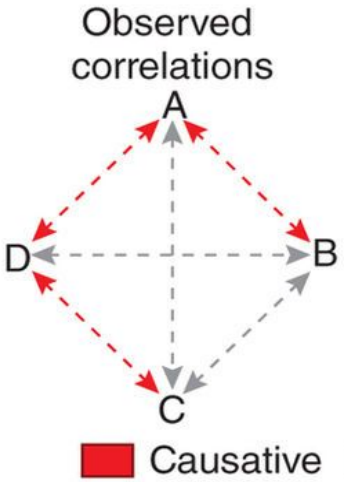
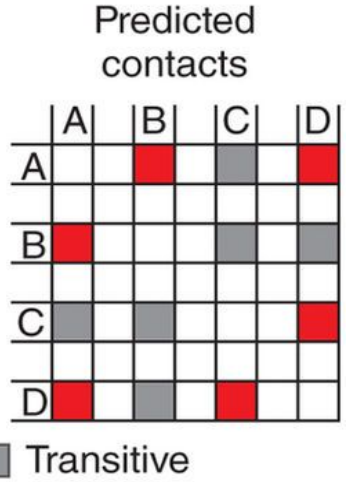








Causative Transitive

Marks (2011) PLoS One; Marks (2012) Nat. Biotech.
Stein (2015) PLoS Comp. Biol.

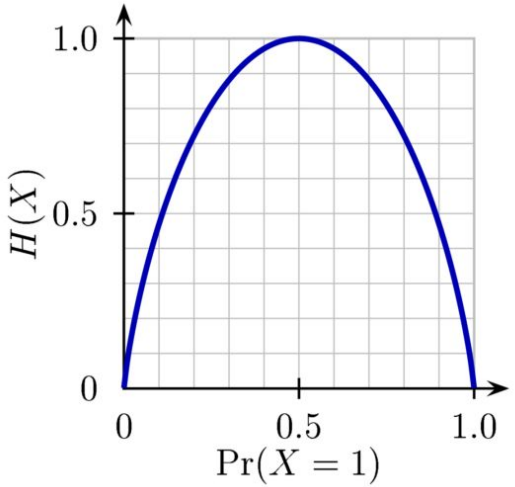
Information theory

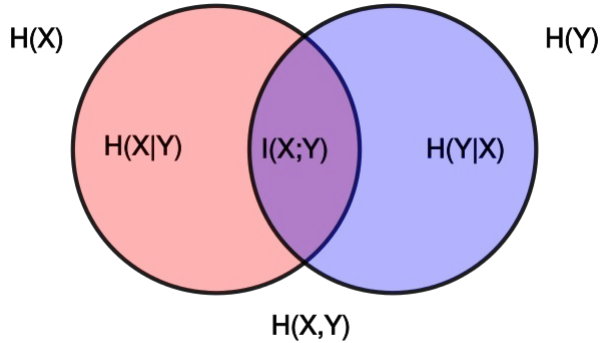
Entropy (H): the average amount of information produced by a stochastic source of data.

Mutual information: MI two random variables **I(X, Y)** quantifies the amount of information obtained about one random variable, through the other random variable.

$$\mathrm{H}(X) = -\sum_{i=1}^{n} \mathrm{P}(x_i) \log_b \mathrm{P}(x_i)$$

$$I(X;Y) = \sum_{x,y} p(x,y) \log \frac{p(x,y)}{p(x)p(y)} = \mathrm{H}(Y) - \mathrm{H}(Y|X)$$





Wikipedia

Global probabilistic models of residue coupling (maximum-entropy)

$$\mathbf{x} = (x_1, \ldots, x_L) \in \Omega^L$$

Pairwise maximum-entropy distribution

$$P(x_1, \ldots, x_L) = \frac{1}{Z} \exp\left(\sum_i h_i(x_i) + \sum_{i<j} e_{ij}(x_i, x_j) \right)$$

Parameter inference

- mean-field (MF)

$$e_{ij}^{\mathrm{MF}}(\sigma, \omega) = -\left(C^{-1}\right)_{ij}(\sigma, \omega)$$

- sparse maximum-likelihood (SML)

$$e_{ij}^{\mathrm{SML}}(\sigma, \omega) = -\left(C_{1,\lambda}^{-1}\right)_{ij}(\sigma, \omega)$$

- pseudolikelihood maximization (PLM)

$$\left\{ \boldsymbol{h}^{\mathrm{PLM}}(\boldsymbol{\sigma}), \boldsymbol{e}^{\mathrm{PLM}}(\boldsymbol{\sigma}, \boldsymbol{\omega}) \right\} = \underset{\boldsymbol{h}(\boldsymbol{\sigma}), \boldsymbol{e}(\boldsymbol{\sigma}, \boldsymbol{\omega})}{\arg\min} \left\{ -\ln l_{\mathrm{PL}} + \lambda_{\boldsymbol{h}} \|\boldsymbol{h}\|_2^2 + \lambda_{\boldsymbol{e}} \|\boldsymbol{e}\|_2^2 \right\}$$

Pair scoring functions

- direct information

$$\mathrm{DI}_{ij} = \sum_{\sigma,\omega} P_{ij}^{\mathrm{dir}}(\sigma, \omega) \ln \left(\frac{P_{ij}^{\mathrm{dir}}(\sigma, \omega)}{f_i(\sigma) f_j(\omega)} \right)$$

- Frobenius norm

$$\|e_{ij}\|_{\mathrm{F}} = \left(\sum_{\sigma,\omega} e_{ij}(\sigma, \omega)^2 \right)^{1/2}$$

- average product-corrected Frobenius norm

$$\text{APC-FN}_{ij} = \|e_{ij}\|_{\mathrm{F}} - \frac{\|e_{i\cdot}\|_{\mathrm{F}} \|e_{\cdot j}\|_{\mathrm{F}}}{\|e_{\cdot\cdot}\|_{\mathrm{F}}}$$

Stein (2015) PLoS Comp. Biol.

Global probabilistic models of residue coupling (maximum-entropy)

$a = (a_1, a_2 . . , a_N)$ A sequence made of monomers a_i taking values from a given alphabet

$$P(a|J,h) = \frac{1}{Z} \exp\left(\sum_{i=1}^{N-1} \sum_{j=i+1}^{N} J_{ij}(a_i, a_j) + \sum_{i=1}^{N} h_i(a_i)\right)$$

Probability of a sequence within the model.

$h(a_i)$: parameters that represent the propensity of symbol to be found at a certain position.

$J(a_i, a_j)$: represent an interaction, quantifying how compatible the symbols at both positions are with each other.

Wikipedia

Global probabilistic models of residue coupling (maximum-entropy)

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$$P(a|J,h) = \frac{1}{Z} \exp\left(\sum_{i=1}^{N-1} \sum_{j=i+1}^{N} J_{ij}(a_i, a_j) + \sum_{i=1}^{N} h_i(a_i)\right)$$

$$\begin{aligned} F[P] = &- \sum_a P(a) \log P(a) \\ &+ \sum_{i<j} \sum_{x,y} \lambda_{ij}(x,y)\Big(P_{ij}(x,y) - f_{ij}(x,y)\Big) \\ &+ \sum_i \sum_x \lambda_i(x)\Big(P_i(x) - f_i(x)\Big) \\ &+ \Omega\left(1 - \sum_a P(a)\right). \end{aligned}$$

The idea of maximum-entropy: For a given set of sample covariances and frequencies, the model represents the **distribution with the maximal entropy** of all distributions reproducing those covariances and frequencies.

The unique distribution ***P*** that maximizes the functional to the *left*.

$f_i(a)$: frequency of finding symbol ***a*** at position ***i***.

$f_{ij}(a, b)$: frequency of finding symbols ***a*** & ***b*** at positions ***i*** and ***j*** in the same sequence.

Wikipedia

Global probabilistic models of residue coupling (maximum-entropy)

$$a = (a_1, a_2 .., a_N)$$

$$P(a|J,h) = \frac{1}{Z} \exp\left(\sum_{i=1}^{N-1} \sum_{j=i+1}^{N} J_{ij}(a_i, a_j) + \sum_{i=1}^{N} h_i(a_i)\right)$$

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$$F_i = \frac{1}{N} \sum_{j \neq i}^{N} F_{ij}$$

$$F_{ij}^{APC} = F_{ij} - \frac{F_i F_j}{F}$$

$$F = \frac{1}{N^2 - N} \sum_{i,j,i \neq j}^{N} F_{ij}$$

The idea of maximum-entropy: For a given set of sample covariances and frequencies, the model represents the **distribution with the maximal entropy** of all distributions reproducing those covariances and frequencies.

The unique distribution ***P*** that maximizes the functional to the *left*.

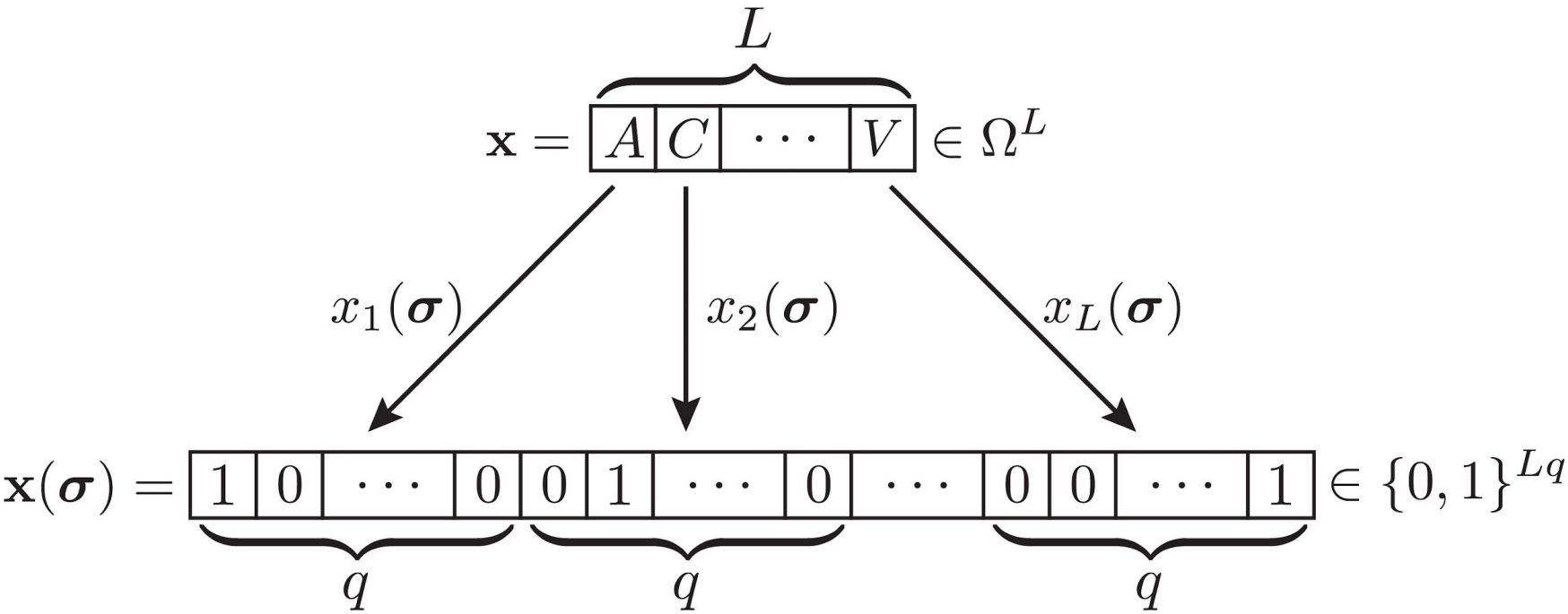
Final step:

- average product correction (APC).

Wikipedia

Global probabilistic models of residue coupling

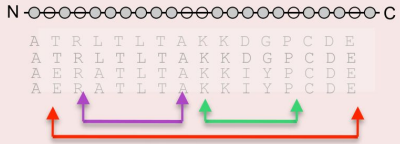
Binary embedding of amino acid sequence

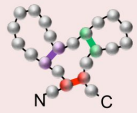


Stein (2015) PLoS Comp. Biol.

Global probabilistic models of residue coupling (maximum-entropy)

Align evolutionary diverged sequences





Calculate covariance matrix for each pair of sequence positions for all pairs of amino acids (A,B)

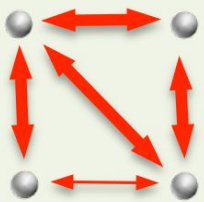
$$C_{ij}(A,B) = f_{ij}(A,B) - f_i(A)P_j(B)$$

$$C_{ij}^{-1}(A,B) = -e_{ij}(A,B)_{i \neq j}$$

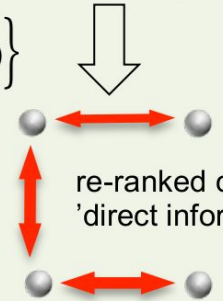
Identify maximally informative pair couplings using **statistical model** of entire protein to infer residue-residue co-evolution

$$P_{ij}^{Dir}(A,B) = \frac{1}{Z}\exp\left\{e_{ij}(A,B) + \tilde{h}_i(A) + \tilde{h}_j(B)\right\}$$

$$DI_{ij} = \sum_{A,B=1}^{q} P_{ij}^{Dir}(A,B)\ln\frac{P_{ij}^{Dir}(A,B)}{f_i(A)f_j(B)}$$



high ranking **transitive** 'indirect correlations'



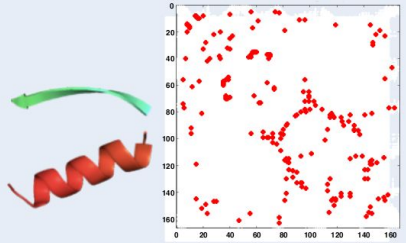
re-ranked correlations 'direct information' = DI

Marks (2011) PLoS One

From contacts to structure

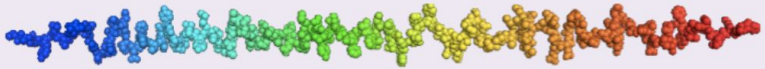
Analyze the highest scoring pairs to produce ranked list of residue pairs which we predict to be close in 3D space. Use these pairs as predicted close “evolutionary inferred contacts” , EICs, in folding calculations

```
assign (resid 143 and name CA) (resid 123 and name CA)  4 4 3
assign (resid 16 and name CA) (resid 10 and name CA)  4 4 3
assign (resid 141 and name CA) (resid 82 and name CA)  4 4 3
assign (resid 129 and name CA) (resid 87 and name CA)  4 4 3
assign (resid 92 and name CA) (resid 11 and name CA)  4 4 3
assign (resid 116 and name CA) (resid 81 and name CA)  4 4 3
```

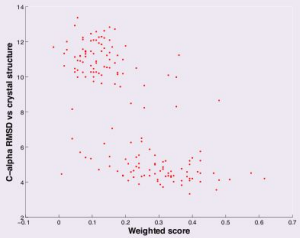


predicted contacts (EICs)

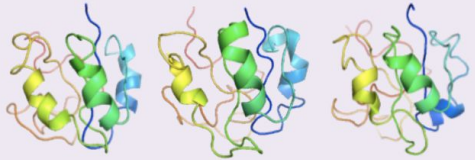
Start with extended structure
use **distance geometry** and **simulated annealing** with predicted constraints, EICs, to fold the chain



Rank predicted structures using quality measure of backbone alpha torsion and beta sheet twist



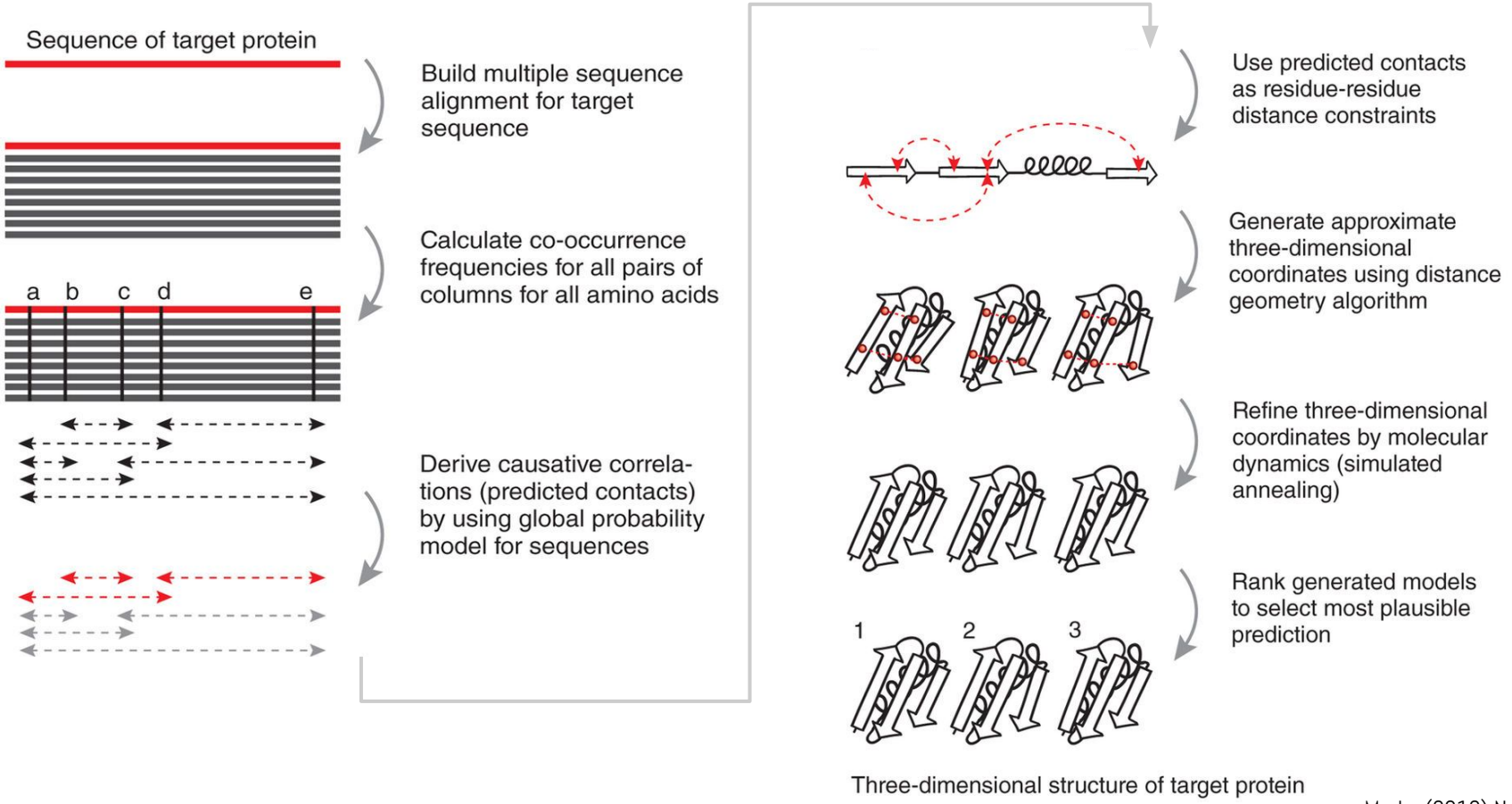
good scores



bad scores

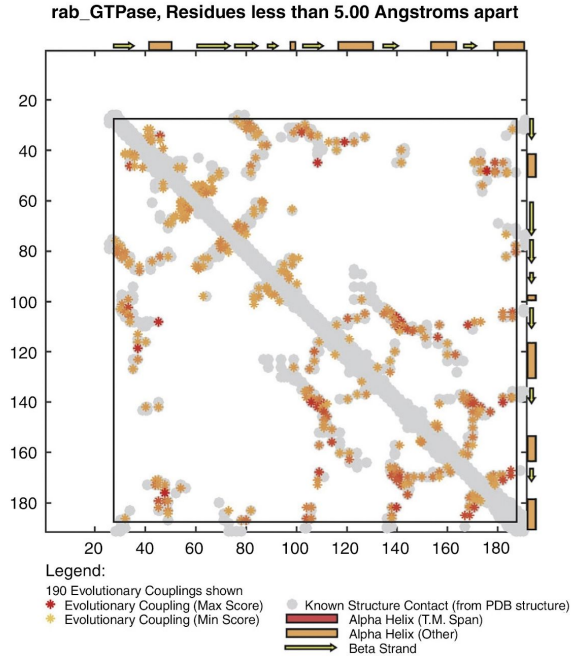
Marks (2011) PLoS One

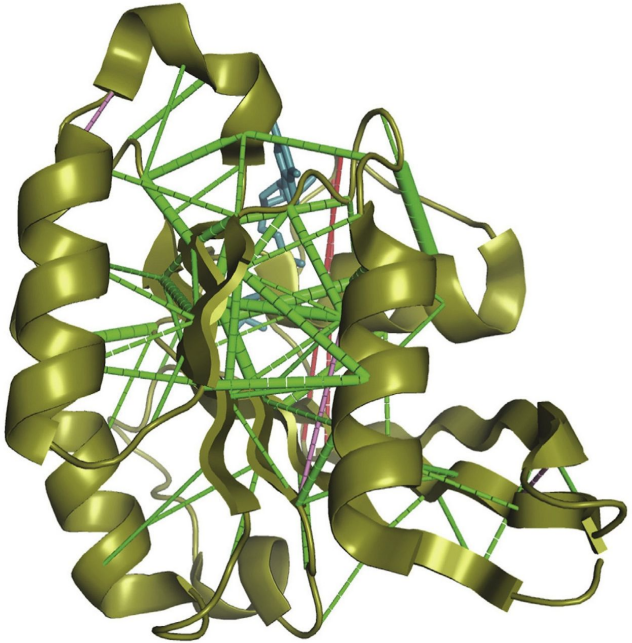
Predicting protein 3D structure from sequence



Marks (2012) Nat. Biotech.

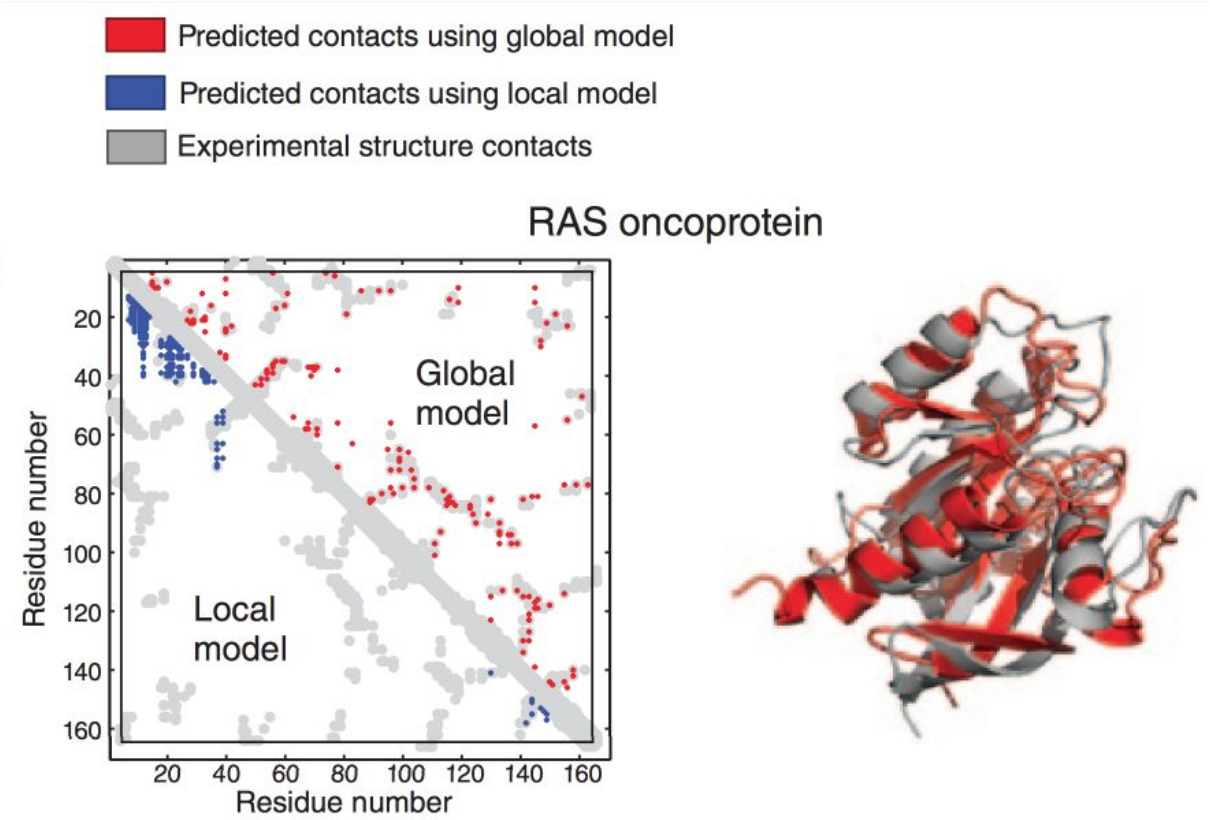
Predictions of 3D structures based on evolutionary coupling





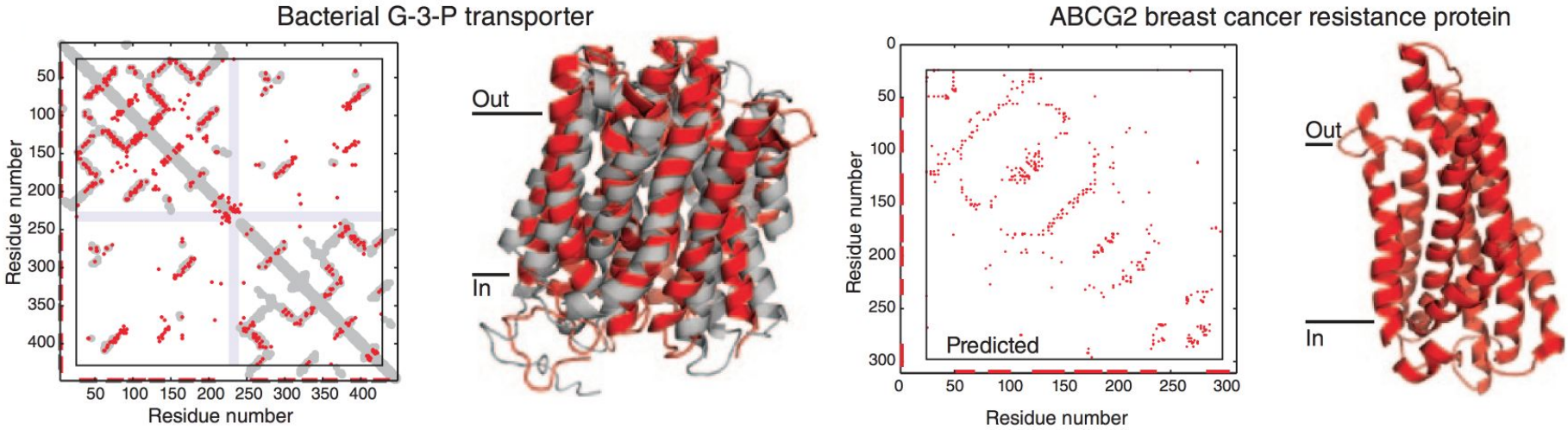
Newald (2016) Curr. Opin. Struct. Biol.

Predictions of 3D structures based on evolutionary coupling



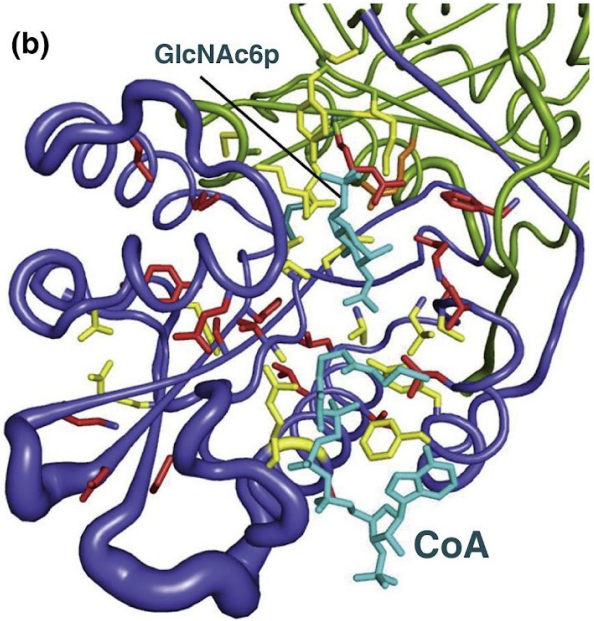
Marks (2012) Nat. Biotech.

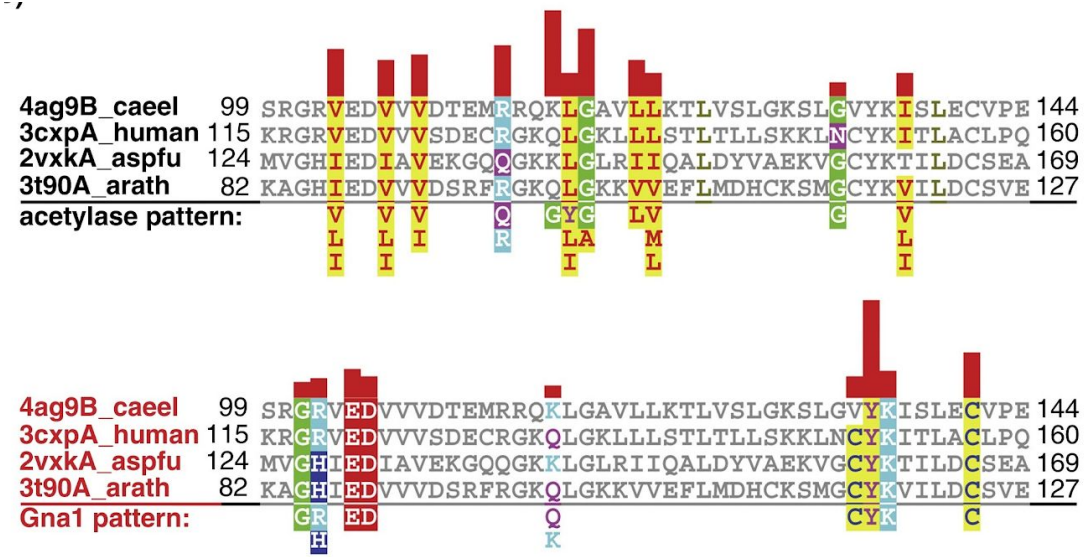
Predictions of 3D structures based on evolutionary coupling



Marks (2012) Nat. Biotech.

Predictions of 3D structures based on evolutionary coupling



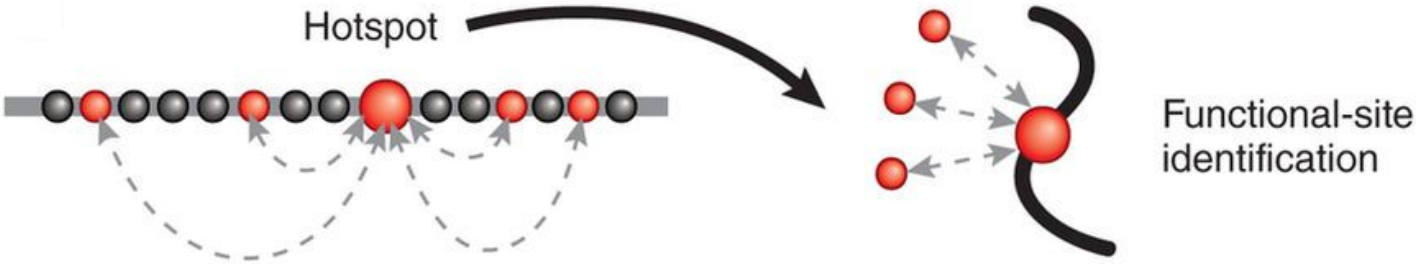


Newald (2016) Curr. Opin. Struct. Biol.

Detecting functional hotspots

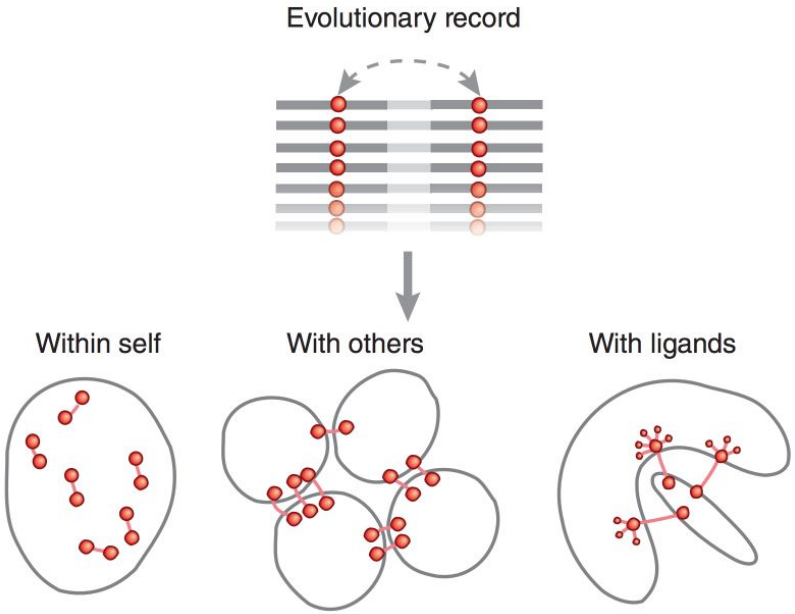
Residues subject to a high number of evolutionary pair constraints represent likely functional hotspots.

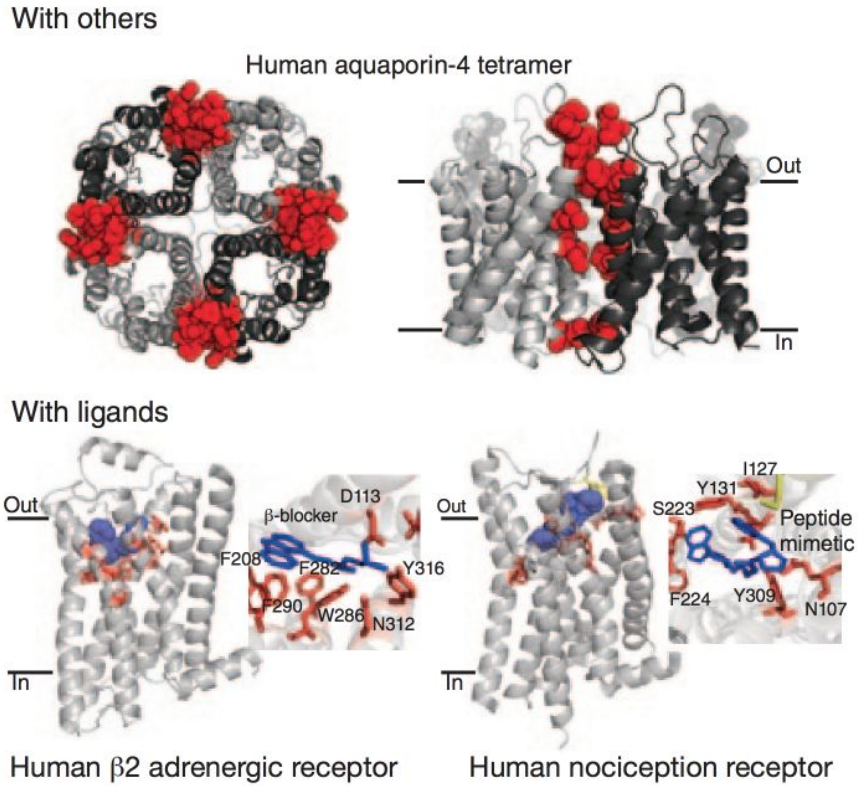
- Such highly constrained residues include residues in functional sites (for e.g., interaction with external ligands).
- Not detectable by analysis of single-residue conservation.



Marks (2012) Nat. Biotech.

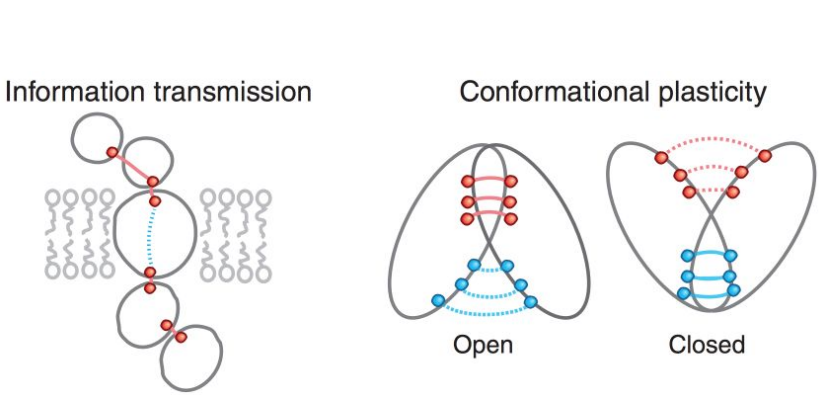
Predicting protein-protein & protein-ligand interactions

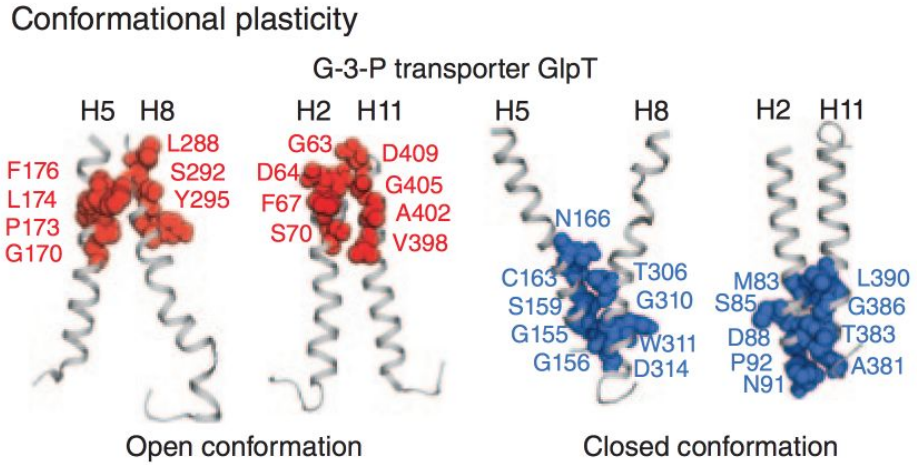




Marks (2012) Nat. Biotech.

Predicting conformational changes





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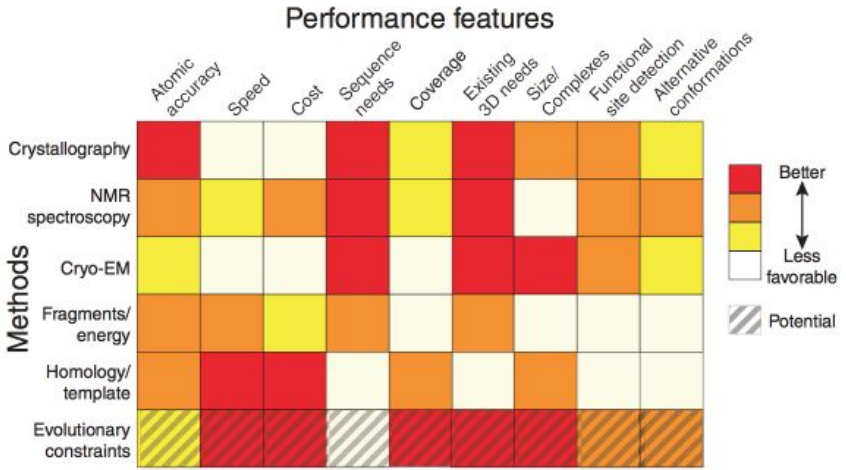
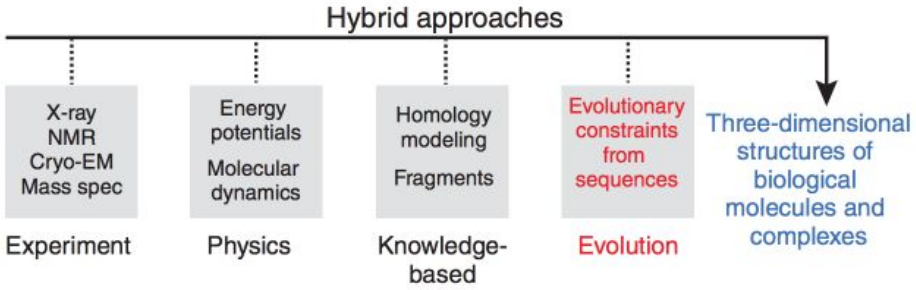
Hybrid approaches for determining protein 3D structure





Marks (2012) Nat. Biotech.