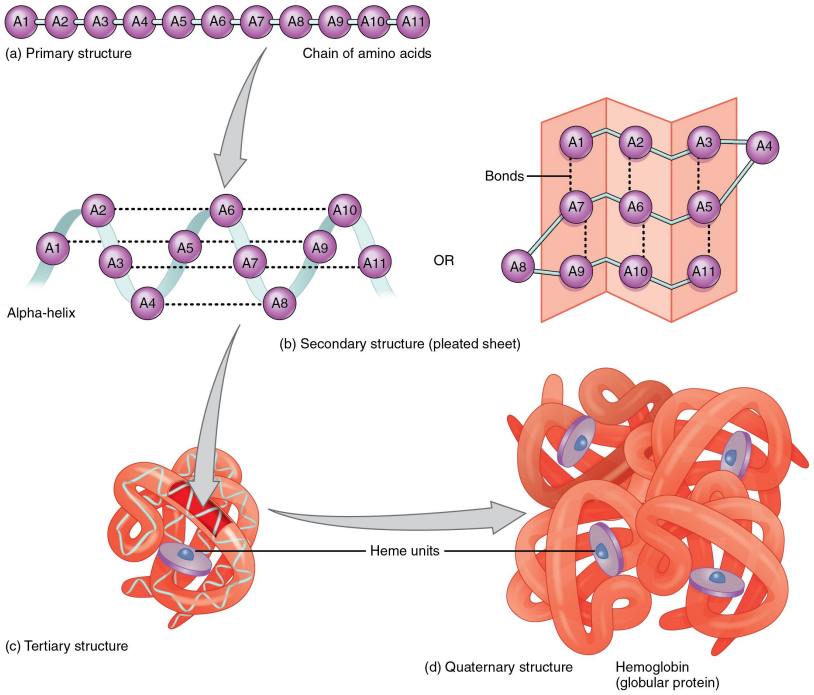
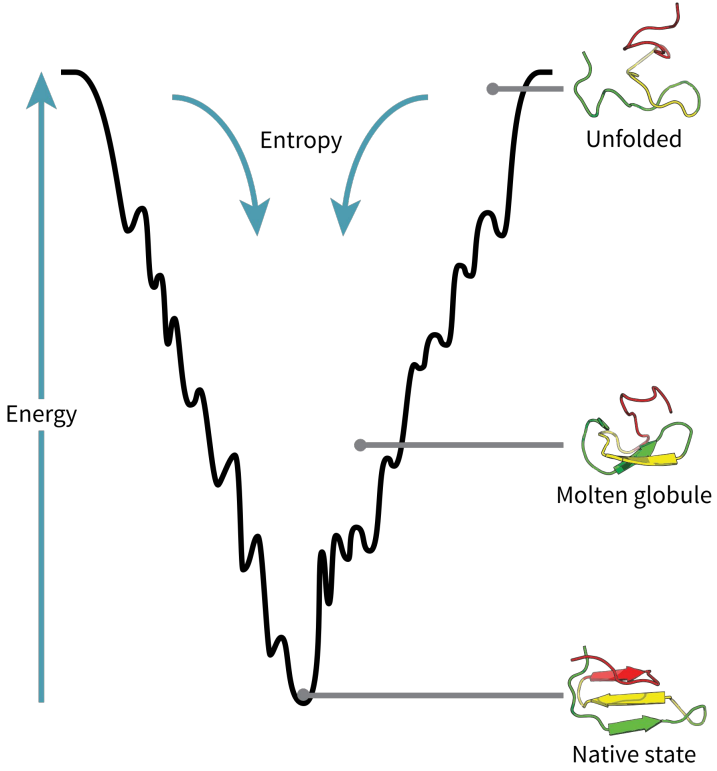
Week 12: Protein structure

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

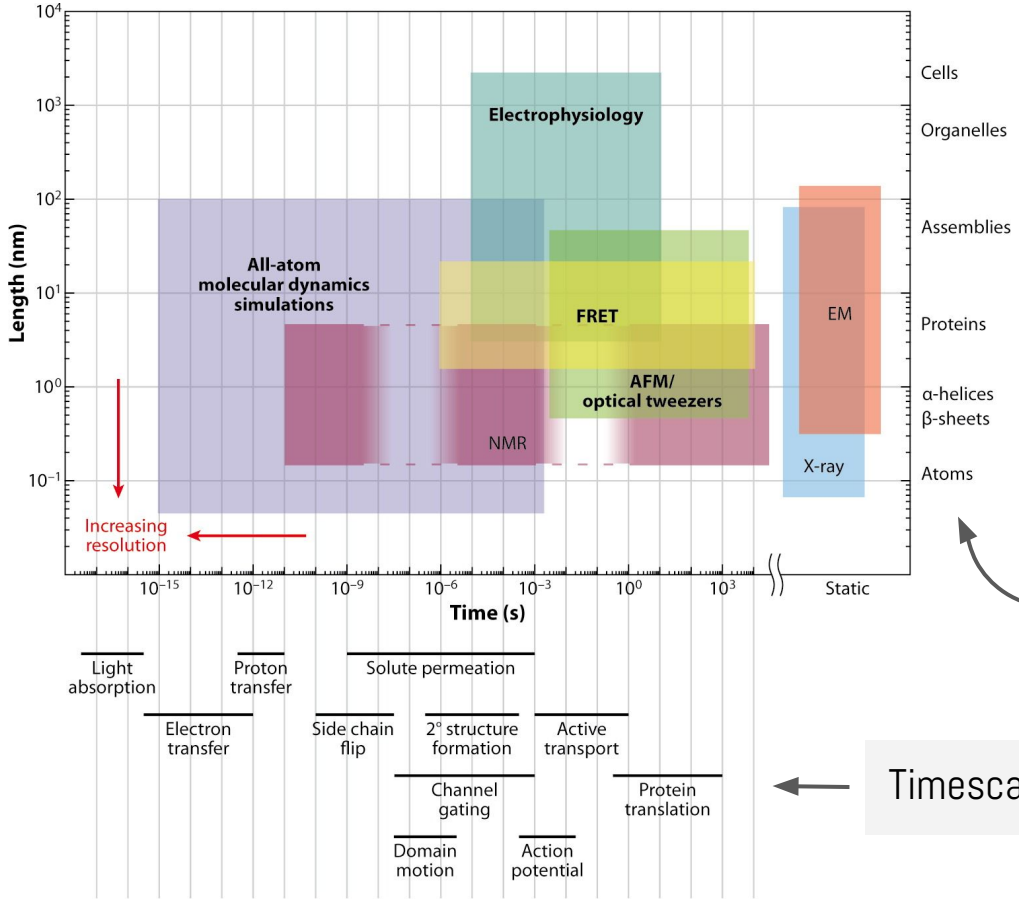
Proteins have 3D structures that are closely tied to their function





Wikipedia

Various experimental techniques to determine protein 3D strcuture



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

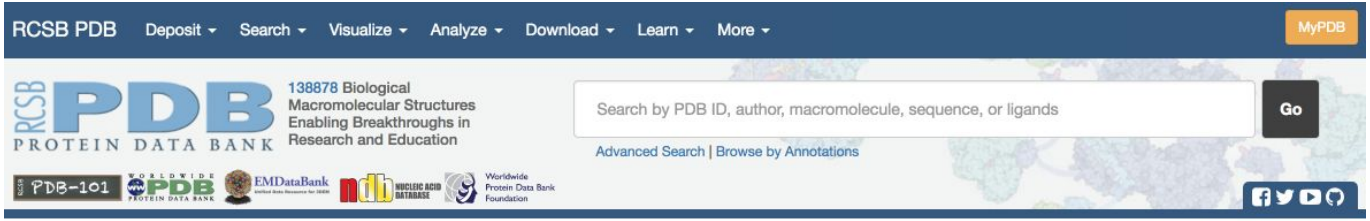
Spatial resolution of biological features

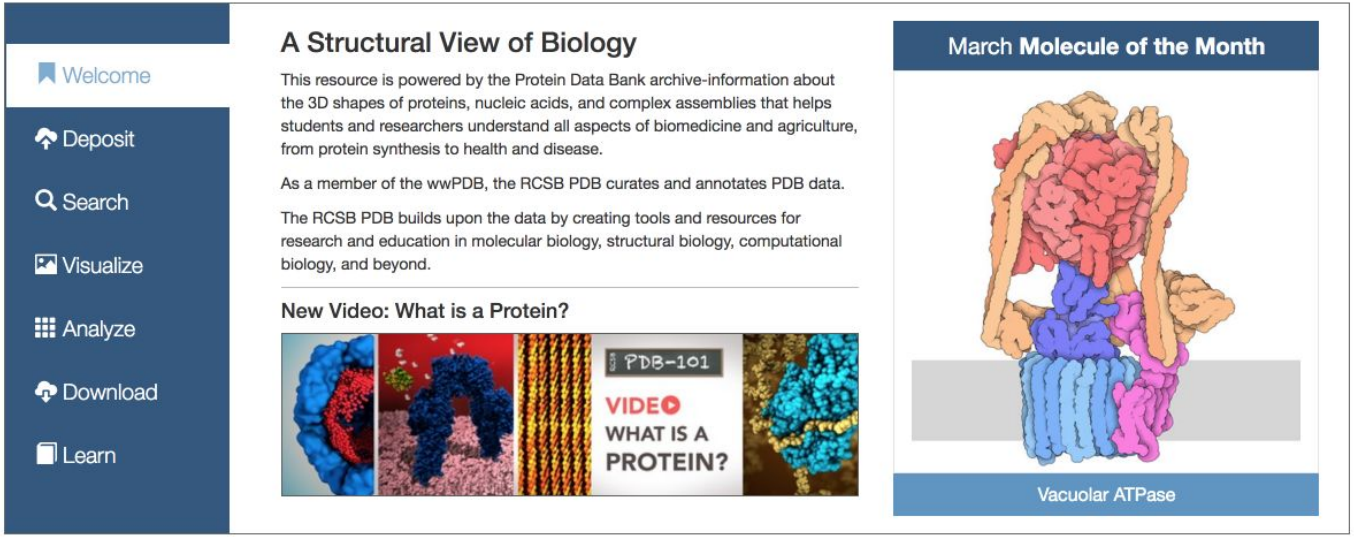
Timescales of molecular processes

Dror (2012) Annu. Rev. Biophys.

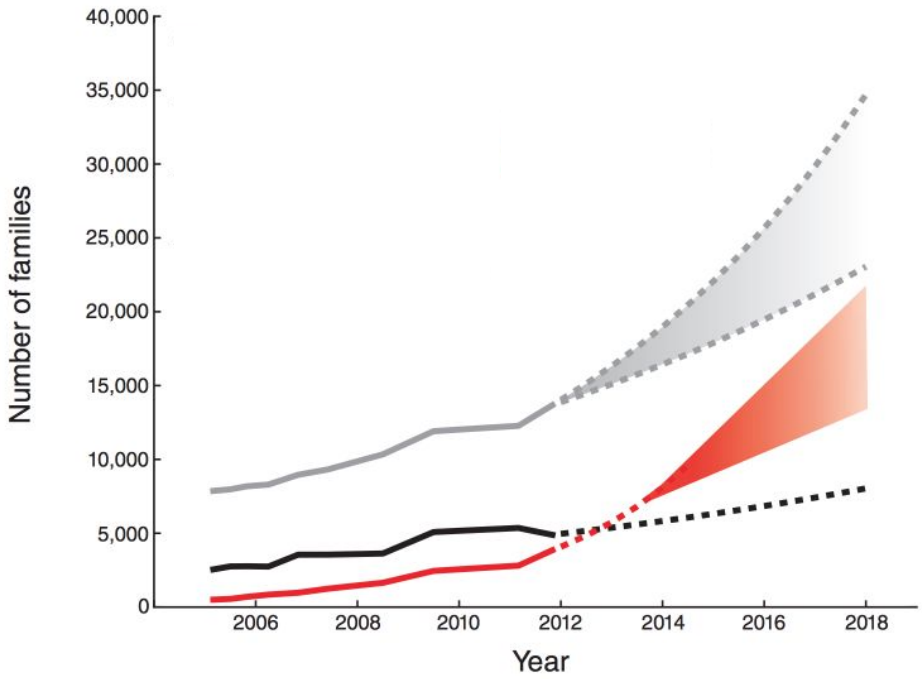
Protein Data Bank (PDB)

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





Experimental methods for 3D structure determination



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).

Experimental structure-determination
(Done one-by-one)

Marks (2012) Nat. Biotech.

Need computational methods to predict structure from sequence

1. Physics-based methods (*in silico* energy functions)
    a. Only works for small proteins *de novo*.
    b. Needs massive infrastructure
2. Knowledge-based (sequence similarity to proteins with known structures; homology modeling)
    a. Only works for small proteins *de novo*.
    b. This is true even with fragments.
3. Finding potential interactions between residues to then map to structure
    a. Takes advantage of a billion-year dataset

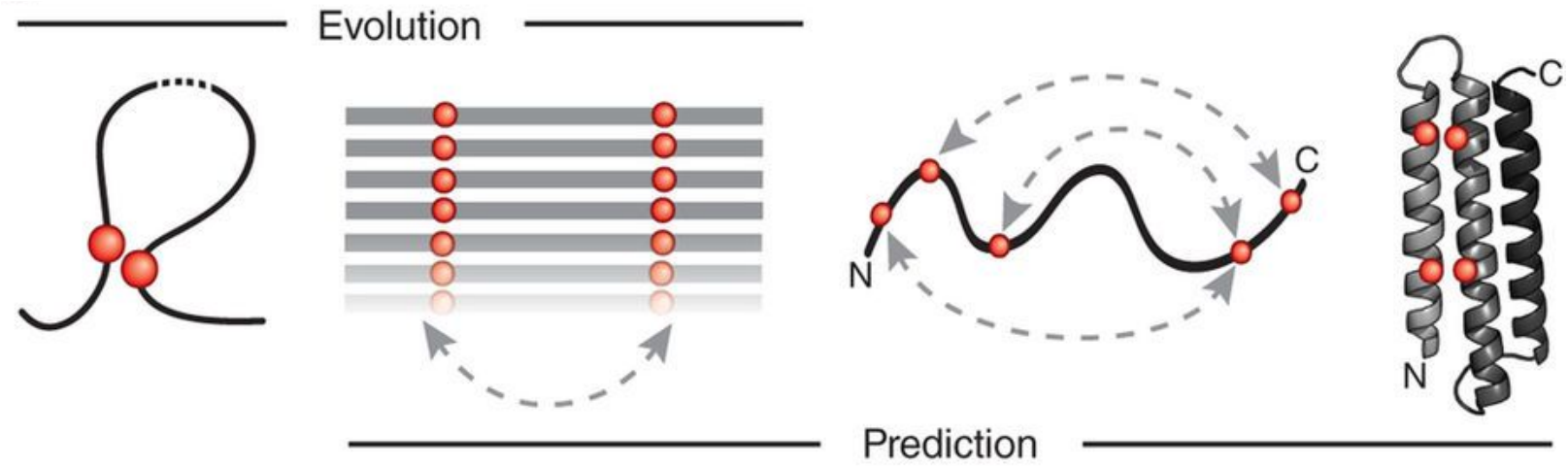
Contacts in structure leave a record in sequence

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

Visible record of residue covariation in related protein sequences.

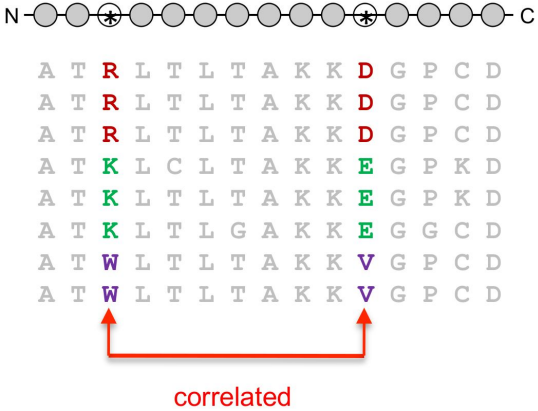
Inverse problem – inferring directly causative residue couplings (evolutionary couplings) from the covariation record – challenging due to transitive correlations & other confounding effects.

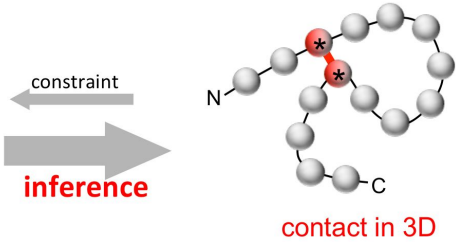
ECs can be used to predict the unknown 3D structure of a protein from a set of sequences alone.

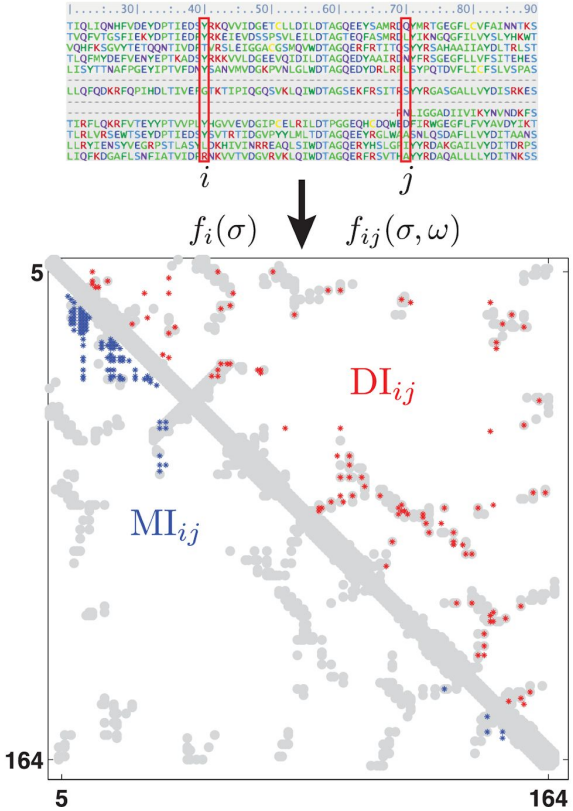


Marks (2012) Nat. Biotech.

Predicting protein 3D structure from sequence







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

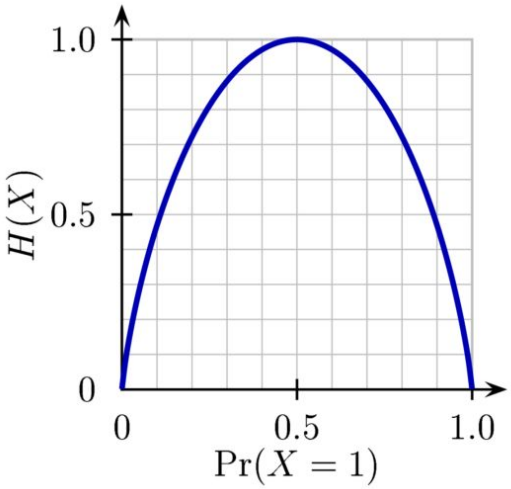
Capturing interactions based on mutual information

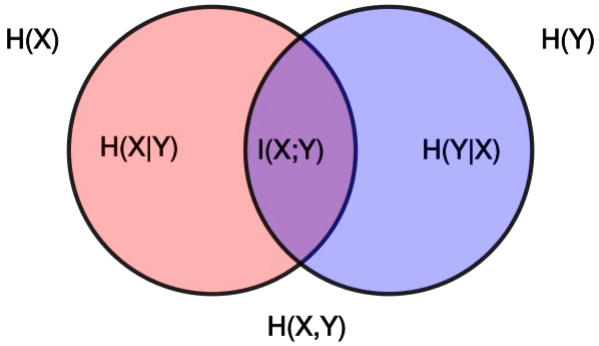
Entropy (H): the average amount of information produced by a stochastic source of data.

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

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

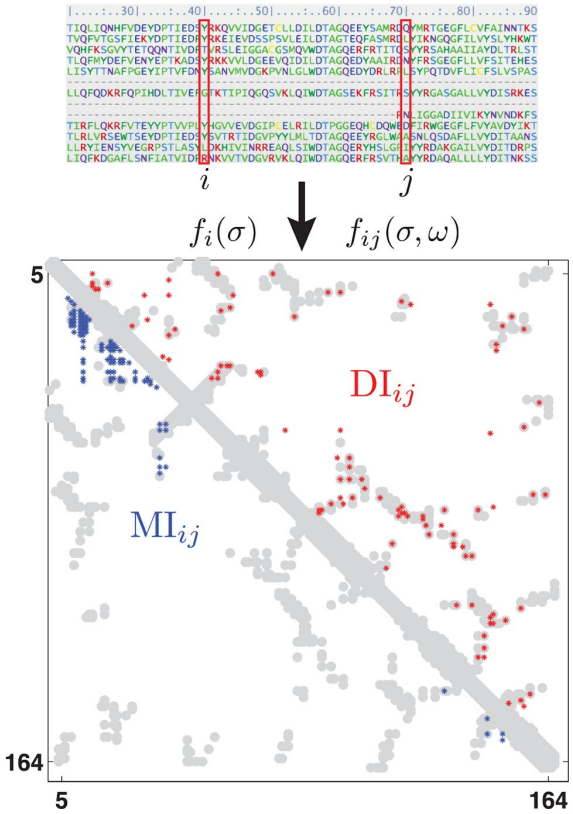
$$I(X;Y) = \sum_{x,y} p(x,y) \log \frac{p(x,y)}{p(x)p(y)} = \mathrm{H}(Y) - \mathrm{H}(Y|X)$$

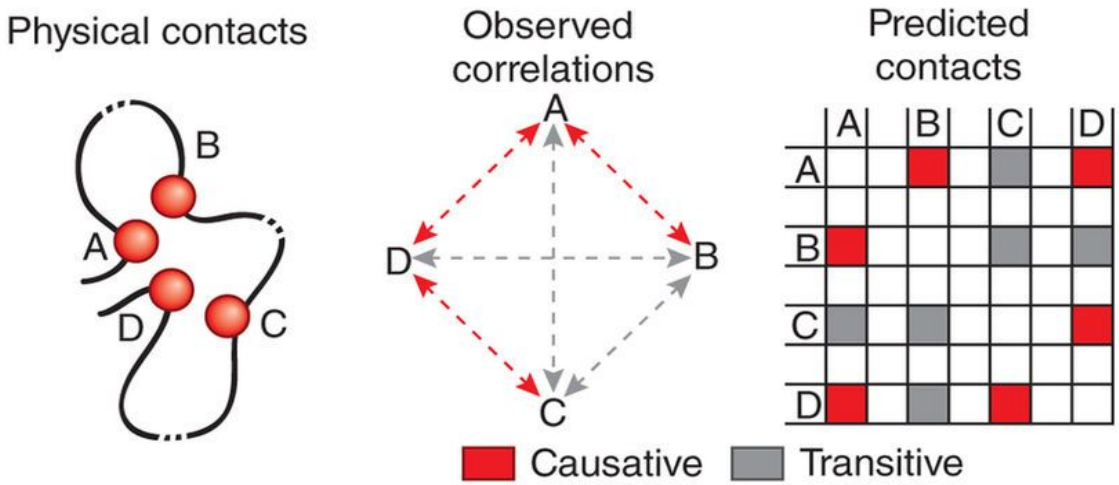




Wikipedia

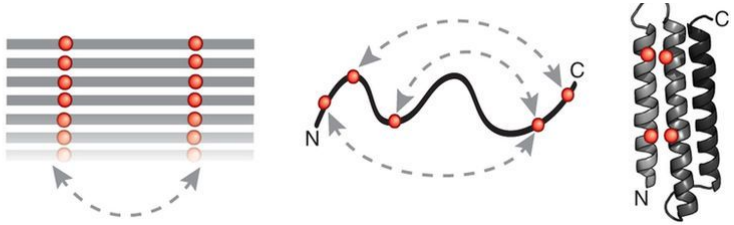
Capturing interactions based on mutual information





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

Capturing interactions using a global probability model



Build a global probability model that 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.

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.

- Each J_{ij} is a 20-by-20 matrix

Wikipedia

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The idea of <u>maximum-entropy</u>: 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.

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

The 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

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

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

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

Final step:

- average product correction (APC).

Wikipedia

Global probabilistic models of residue coupling (maximum-entropy)

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

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

Pairwise maximum-entropy distribution

Parameter inference

- pseudolikelihood maximization (PLM)

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

- 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

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

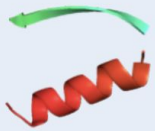
Pair scoring functions

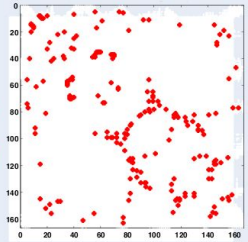
Stein (2015) PLoS Comp. Biol.

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

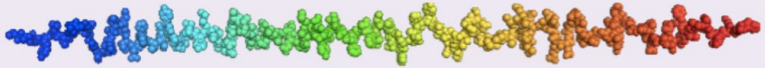
```
assign (resid 143 and name CA) (resid 123 and name CA)  4 4 3
assign (resid 16 and name CA) (resid 10 and name CA)  4 4 3
assign (resid 141 and name CA) (resid 82 and name CA)  4 4 3
assign (resid 129 and name CA) (resid 87 and name CA)  4 4 3
assign (resid 92 and name CA) (resid 11 and name CA)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



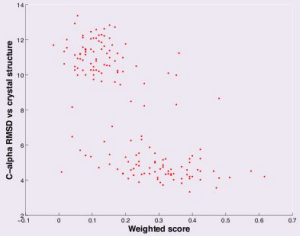


predicted contacts (EICs)

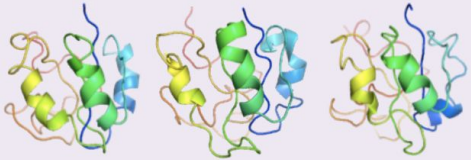
Start with extended structure
use **distance geometry** and **simulated annealing** with predicted constraints, EICs, to fold the chain



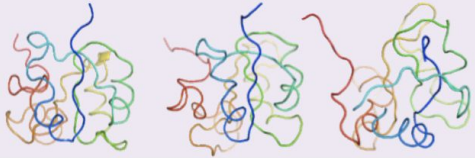
Rank predicted structures using quality measure of backbone alpha torsion and beta sheet twist



good scores

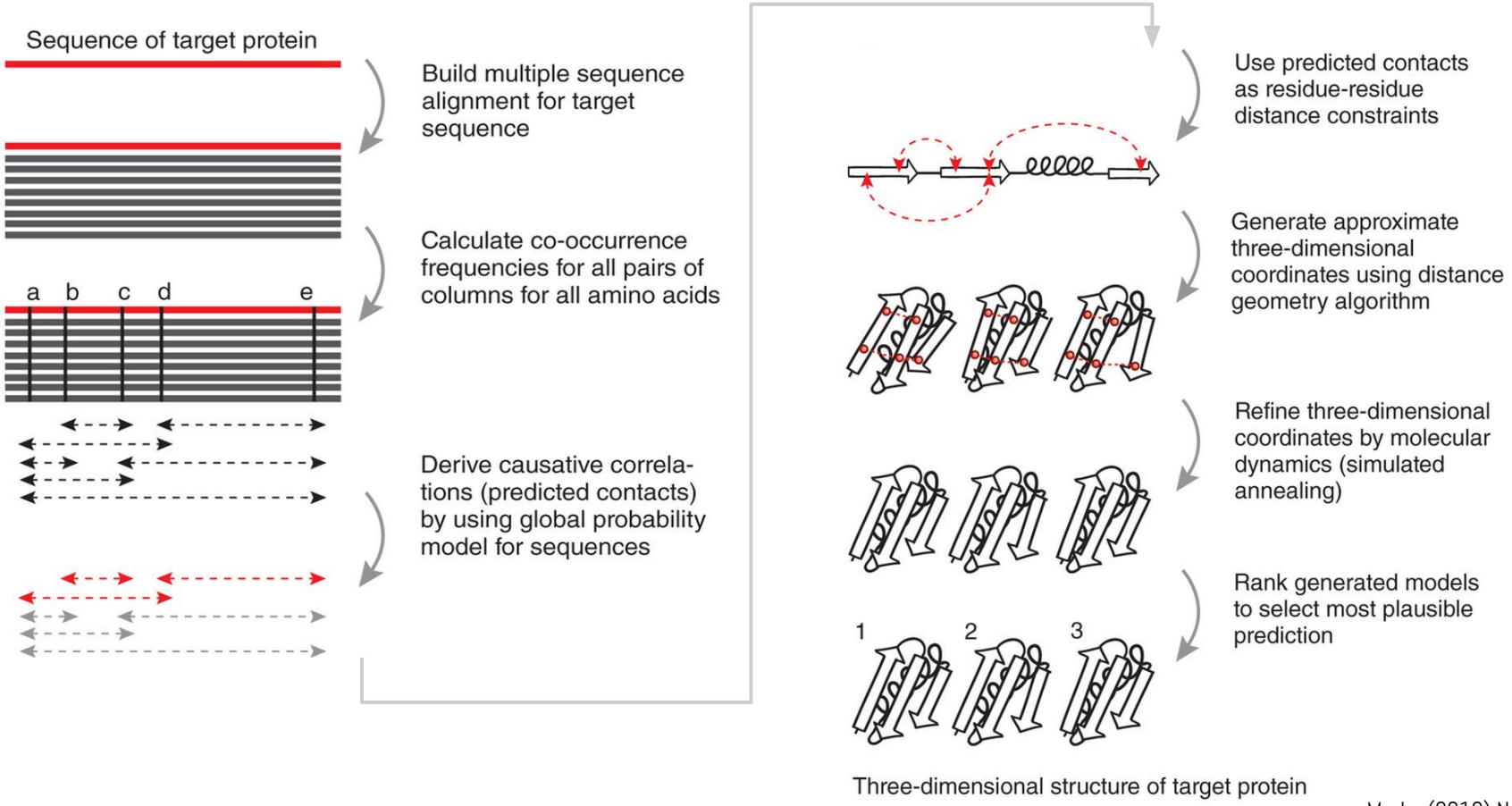


bad scores



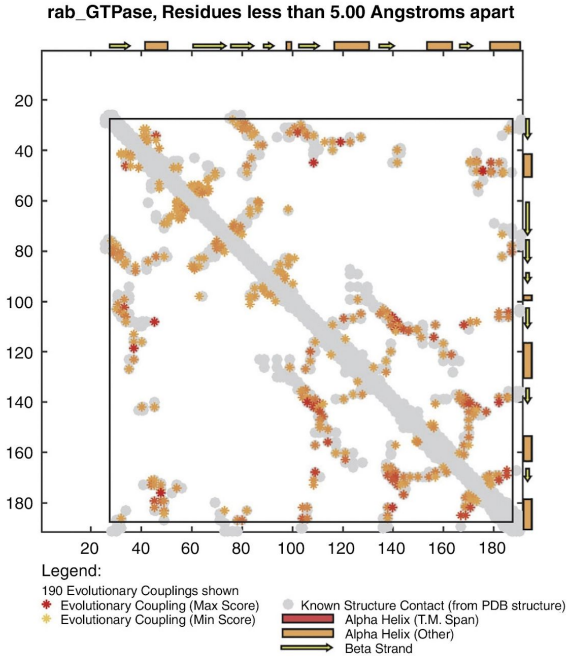
Marks (2011) PLoS One

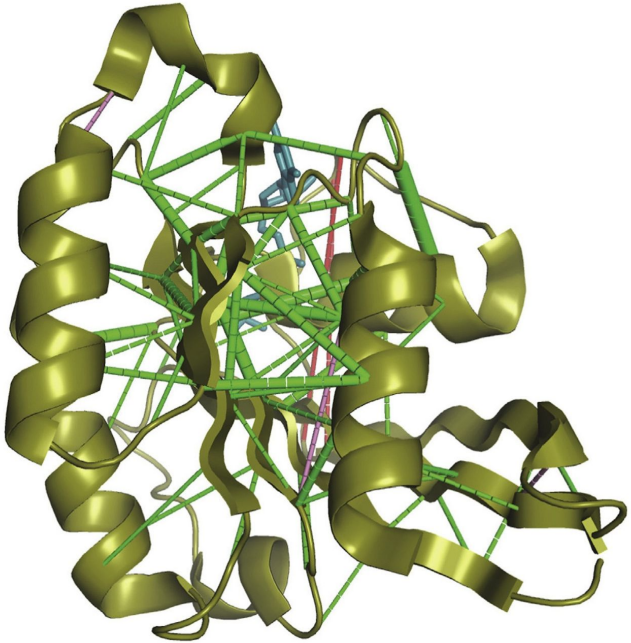
Predicting protein 3D structure from sequence



Marks (2012) Nat. Biotech.

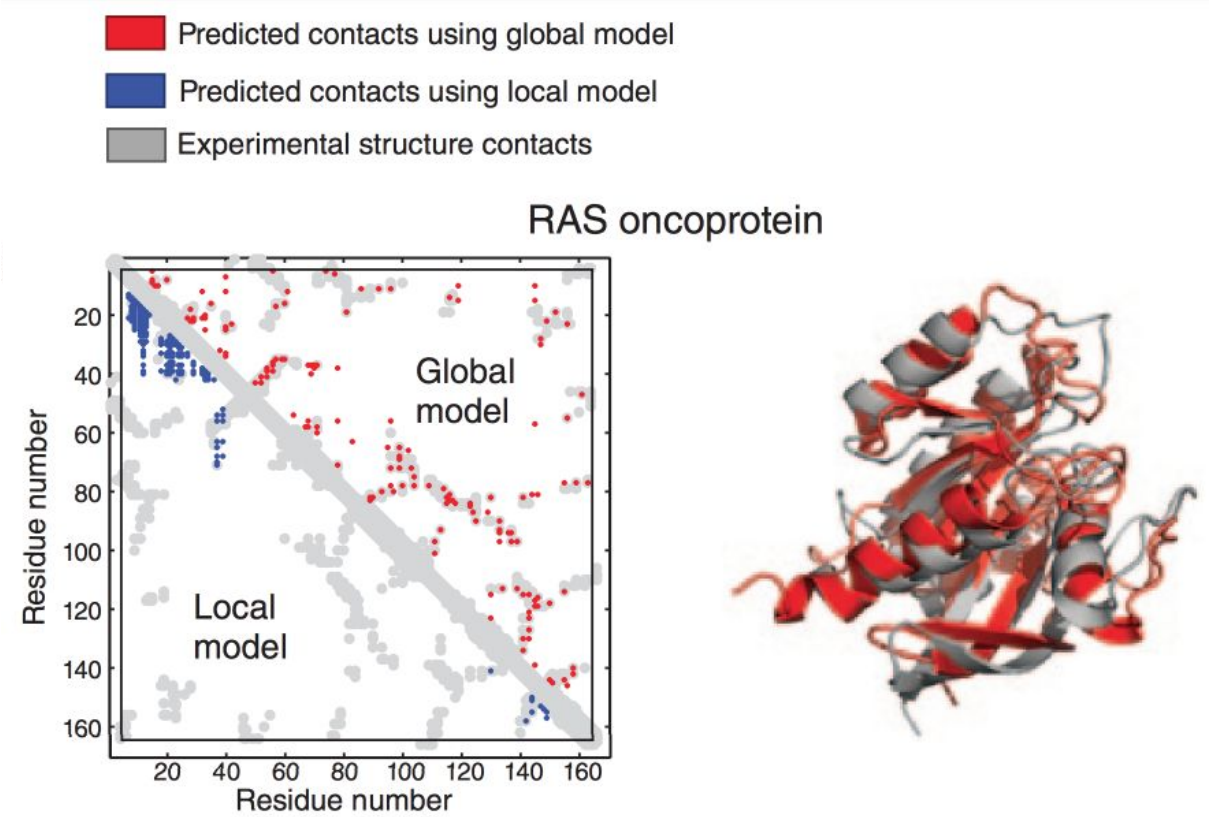
Predictions of 3D structures based on evolutionary coupling





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

Predictions of 3D structures based on evolutionary coupling



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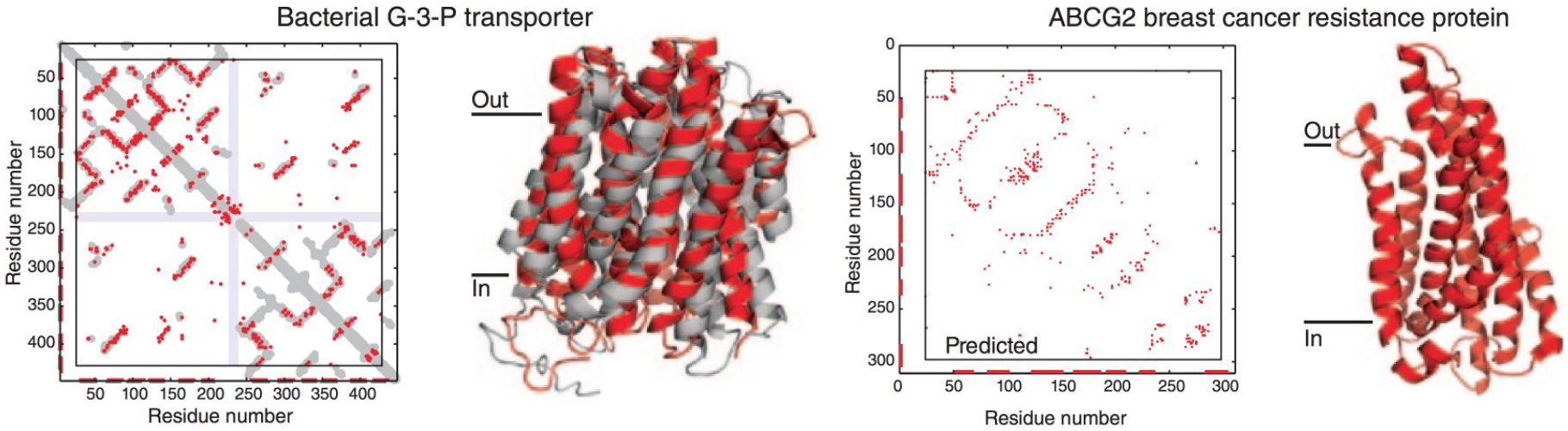

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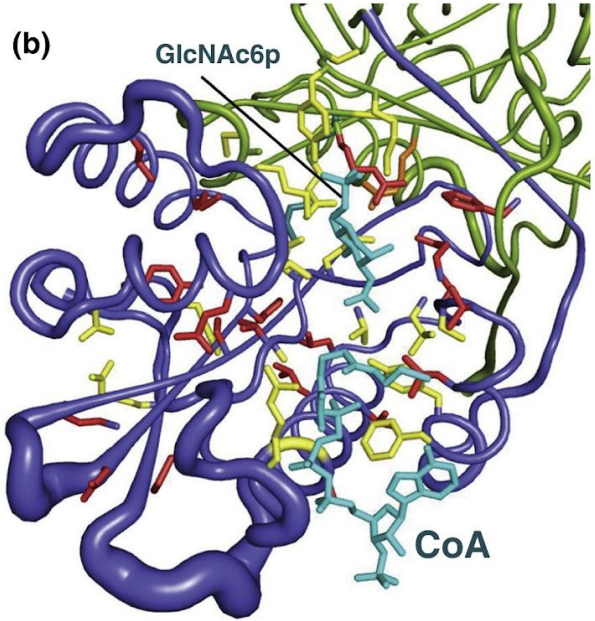
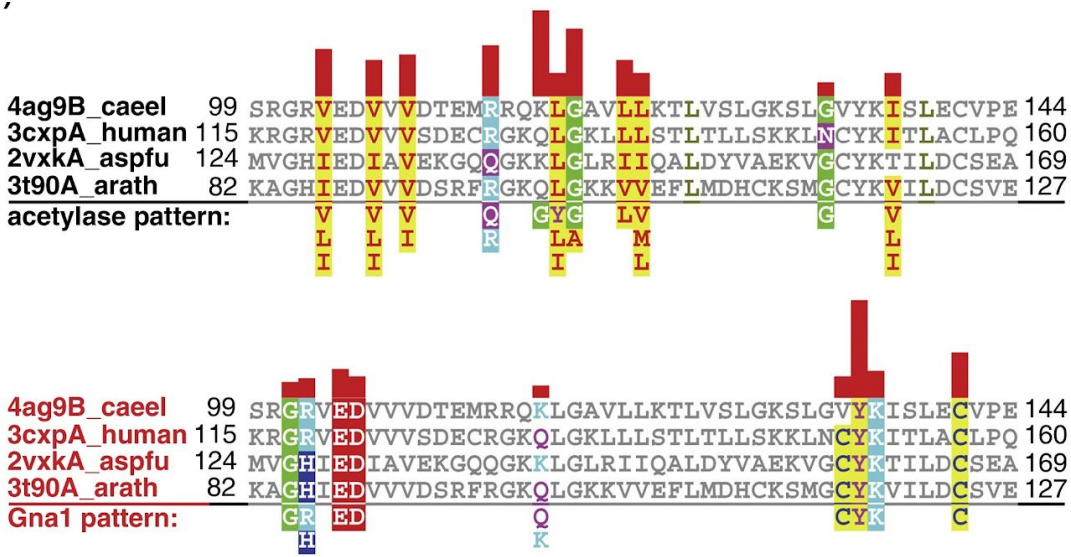


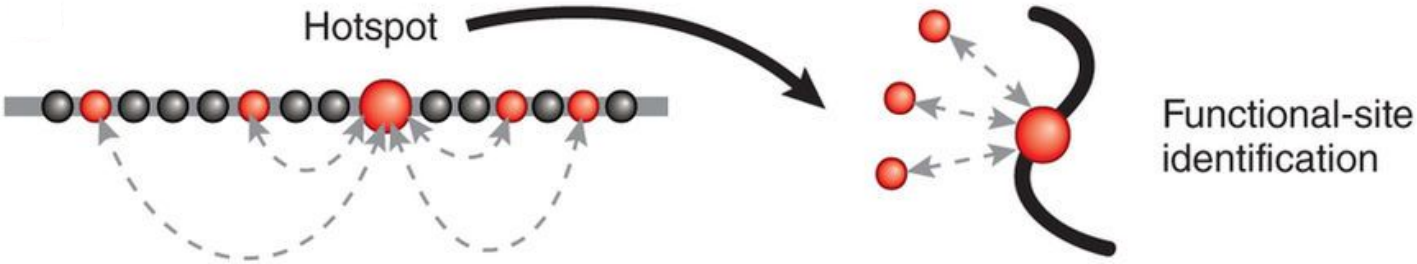


Newald (2016) Curr. Opin. Struct. Biol.

Detecting functional hotspots

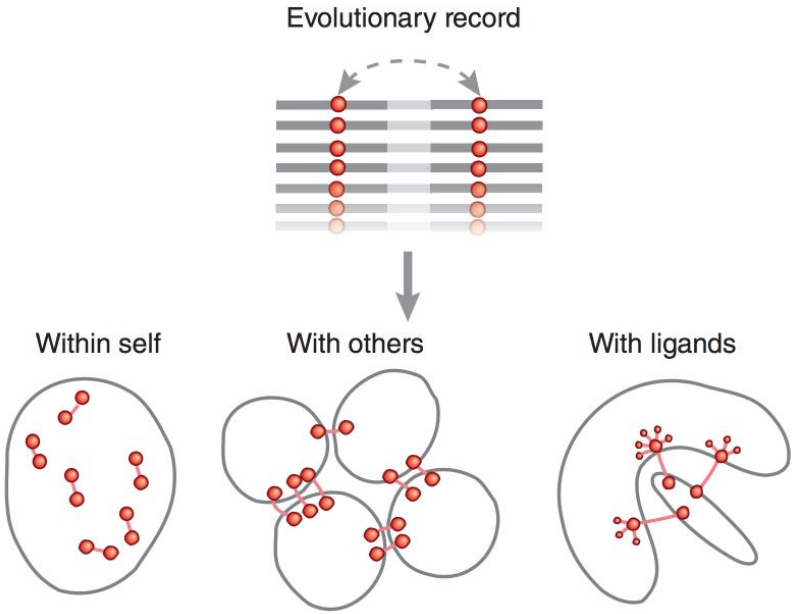
Residues subject to a high number of evolutionary pair constraints represent likely functional hotspots.

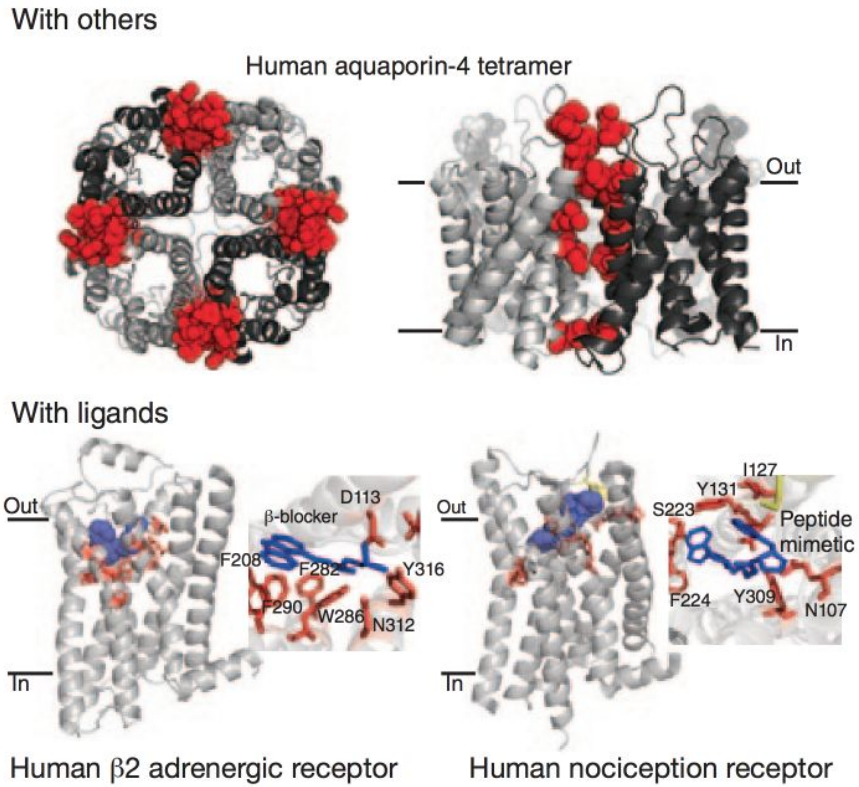
- Such highly constrained residues include residues in functional sites (for e.g., interaction with external ligands).
- Not detectable by analysis of single-residue conservation.



Marks (2012) Nat. Biotech.

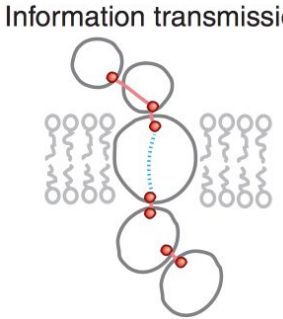
Predicting protein-protein & protein-ligand interactions

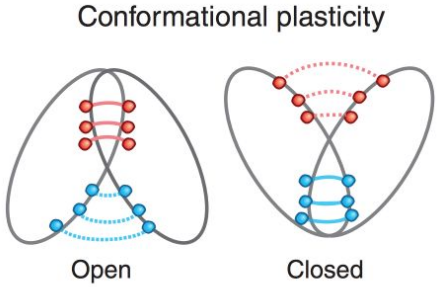


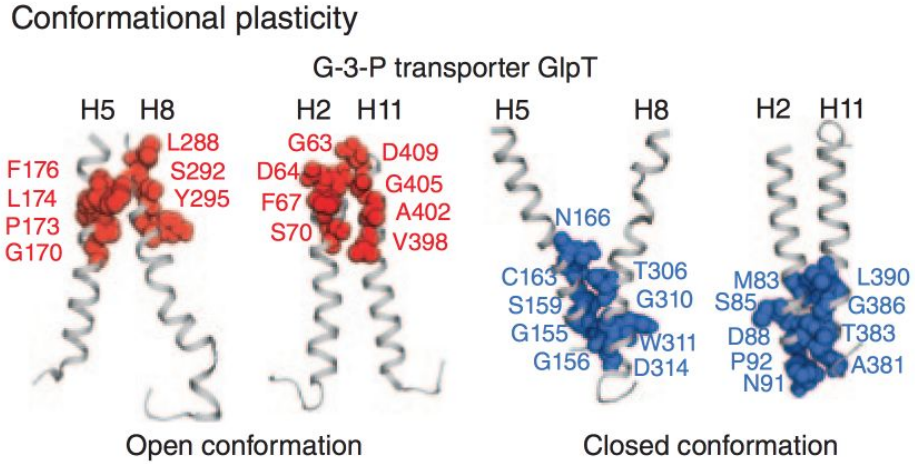


Marks (2012) Nat. Biotech.

Predicting conformational changes

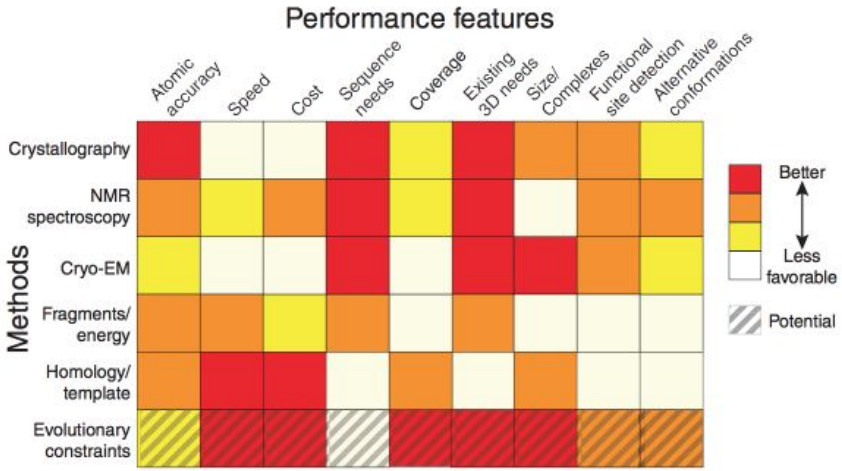


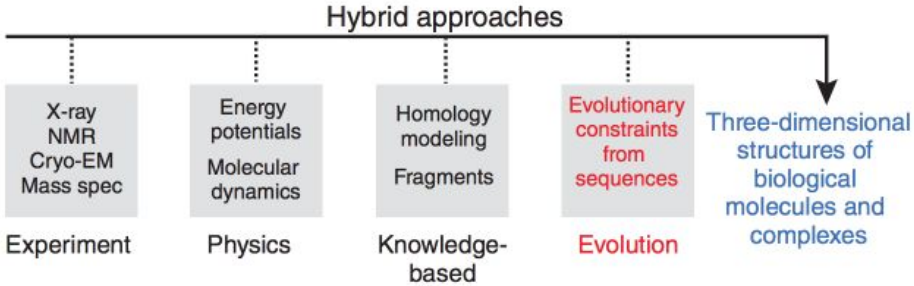




Marks (2012) Nat. Biotech.

Hybrid approaches for determining protein 3D structure





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