Origami with strings: protein folding by computer

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Outline of Talk

- The folding problem and variants on it:
  - Fold recognition
  - Local structure prediction
  - Ab initio methods
  - Comparative modeling
- Results

What is a protein?

- A protein is a long skinny molecule (like a string of letter beads) that folds up consistently into a particular intricate shape.
- The individual “beads” are amino acids, which have 6 atoms the same in each “bead” (the *backbone* atoms: N, H, CA, HA, C, O).
- The final shape is different for different proteins and is essential to the function in our bodies.
- The protein shapes are important, but are expensive to determine experimentally.

Folding Problem

The *Folding Problem*:
If we are given a sequence of amino acids (the letters on a string of beads), can we predict how it folds up in 3-space?

```
MTMSRRNTDA ITIHSILDWI EDNLESPLSL EKVSERSGYS KWHLQRMFKK
ETGHSLGQYI RSRKMTEIAQ KLKESNEPIL YLAERYGFES QQTLTRTFKN
YFDVPPHKYR MTNMQGESRF LHPLNHYNS
```

↓

Too hard!

Fold-recognition problem

The *Fold-recognition Problem*:
Given a sequence of amino acids A (the *target* sequence) and a library of proteins with known 3-D structures (the *template* library),
figure out which templates A match best, and align the target to the templates.

- The backbone for the target sequence is predicted to be very similar to the backbone of the chosen template.
- Progress has been made on this problem, but we can usefully simplify further.

Remote-homology Problem

The *Homology Problem*:
Given a target sequence of amino acids
and a library of protein *sequences*,
figure out which sequences A is similar to and align them to A.

- No structure information is used, just sequence information. This makes the problem easier, but the results aren't as good.
- This problem is fairly easy for recently diverged, very similar sequences, but difficult for more remote relationships.

New-fold prediction

- What if there is *no* template we can use?
- We can try to generate many conformations of the protein backbone and try to recognize the most protein-like of them.
- Search space is huge, so we need a good conformation generator and a cheap cost function to evaluate conformations.

Secondary structure Prediction

- Instead of predicting the entire structure, we can predict local properties of the structure.
- What local properties do we choose?
- We want properties that are well-conserved through evolution, easily predicted, and useful for finding and aligning templates.
- One popular choice is a 3-valued helix/strand/other alphabet—we have investigated many others. Typically, predictors get about 80% accuracy on 3-state prediction.
- Many machine-learning methods have been applied to this problem, but the most successful is neural networks.

Predicting Local Structure

- Want to predict some local property at each residue.
- Local property can be emergent property of chain (such as being buried or being in a beta sheet).
- Property should be conserved through evolution (at least as well as amino acid identity).
- Property should be somewhat predictable (we gain information by predicting it).
- Predicted property should aid in fold-recognition and alignment.
- For ease of prediction and comparison, we look only at discrete properties (alphabets of properties).

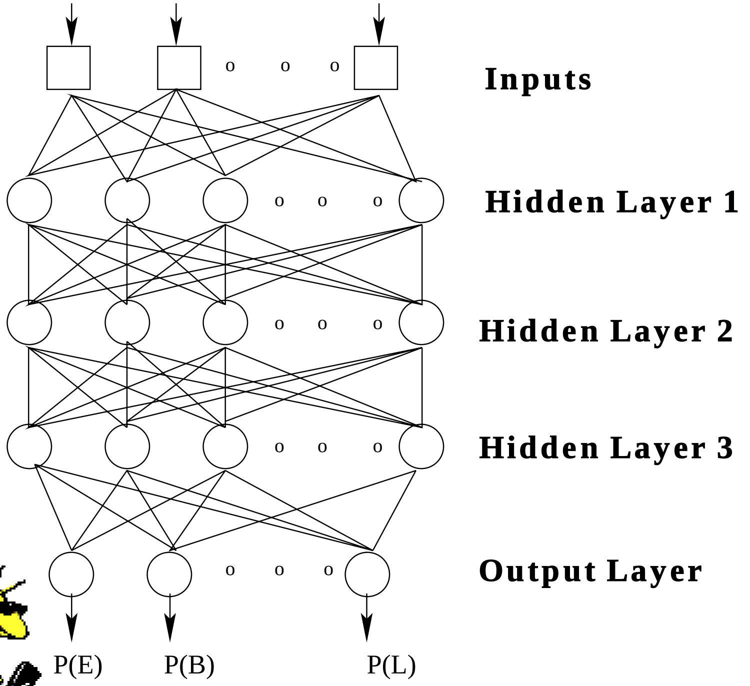
Using Neural Net

- We use neural nets to predict local properties.
- Input is profile with probabilities of amino acids at each position of target chain, plus insertion and deletion probabilities.
- Output is probability vector for local structure alphabet at each position.
- Each layer takes as input windows of the chain in the previous layer and provides a probability vector in each position for its output.
- We train neural net to maximize $\sum \log(P(\text{correct output}))$.

Neural Net

Typical net has 4 layers and 6471 weight parameters:

input/pos	window	output/pos	weights
22	5	15	1665
15	7	15	1590
15	9	15	2040
15	13	6	1176



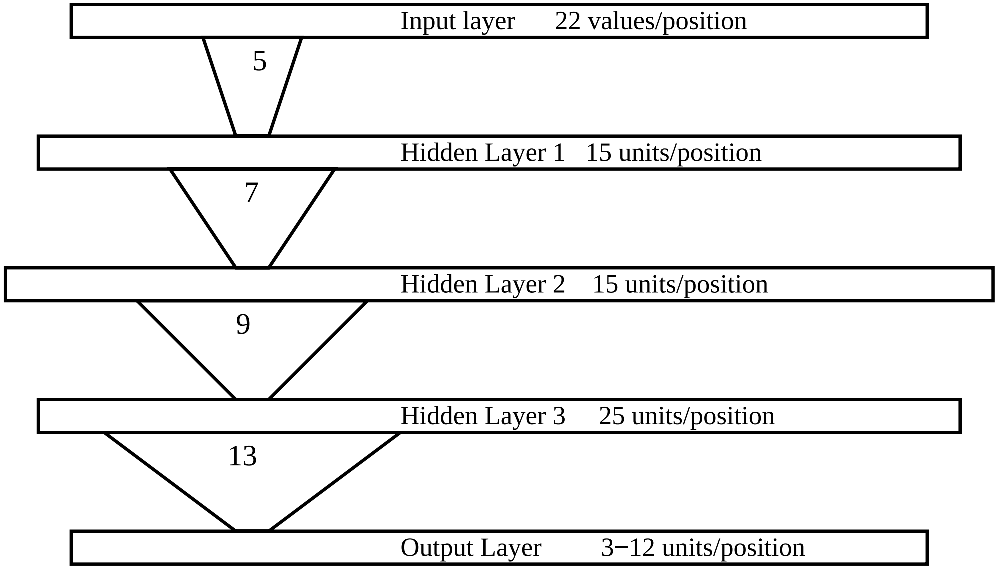
Inputs

Hidden Layer 1

Hidden Layer 2

Hidden Layer 3

Output Layer



Input layer 22 values/position

Hidden Layer 1 15 units/position

Hidden Layer 2 15 units/position

Hidden Layer 3 25 units/position

Output Layer 3−12 units/position

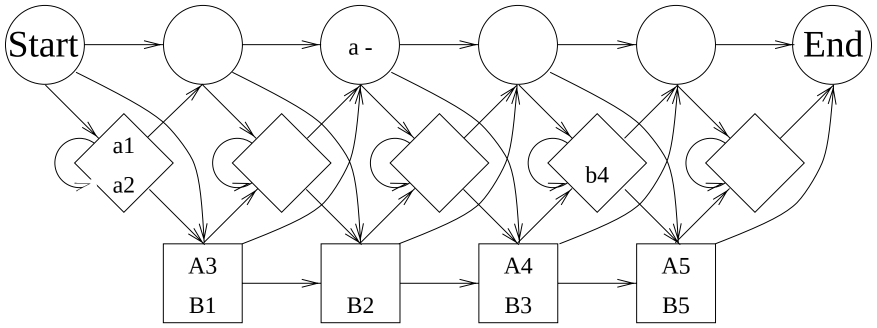
Conservation and Predictability

				conservation	predictability	
	alphabet		MI		info gain	
Name	size	entropy	with AA	mutual info	per residue	$Q_{\|A\|}$
str	13	2.842	0.103	**1.107**	1.009	0.561
protein blocks	16	3.233	0.162	0.980	**1.259**	0.579
stride	6	2.182	0.088	0.904	0.863	0.663
DSSP	7	2.397	0.092	0.893	0.913	0.633
stride-EHL	3	1.546	0.075	0.861	0.736	0.769
DSSP-EHL	3	1.545	0.079	0.831	0.717	0.763
CB-16	7	2.783	0.089	**0.682**	0.502	
CB-14	7	2.786	0.106	0.667	**0.525**	
CB-12	7	2.769	0.124	0.640	0.519	
rel SA	7	2.806	0.183	0.402	0.461	
abs SA	7	2.804	0.250	0.382	0.447	

Hidden Markov Models

- *Hidden Markov Models* (HMMs) are a very successful way to capture the variability possible in a family of proteins.
- An HMM is a stochastic model—that is, it assigns a probability to every possible sequence.
- An HMM is a finite-state machine with a probability for emitting each letter in each state, and with probabilities for making each transition between states.
- Probabilities of letters sum to one for each state.
- Probabilities of transitions out of each state sum to one for that state.
- We also include *null states* that emit no letters, but have transition probabilities on their out-edges.

Profile Hidden Markov Model



```
a1 a2 A3    -    A4  .  A5
 .  .  B1   B2   B3 b4  B5
```

- Circles are null states.
- Squares are *match states*, each of which is paired with a null *delete state*. We call the match-delete pair a *fat state*.
- Each fat state is visited exactly once on every path from Start to End.
- Diamonds are *insert states*, and are used to represent possible extra amino acids that are not found in most of the sequences in the family being modeled.

How is HMM built?

Overview of method for building a target HMM, given a single sequence (or a seed alignment):

loop: Construct a profile HMM with one fat state for each letter of sequence (or column of multiple alignment).

find: Find sequences in a large database of protein sequences that cost little with M. This is the *training set*.

Retrain M (using forward-backward algorithm) to re-estimate all probabilities, based on the training set.

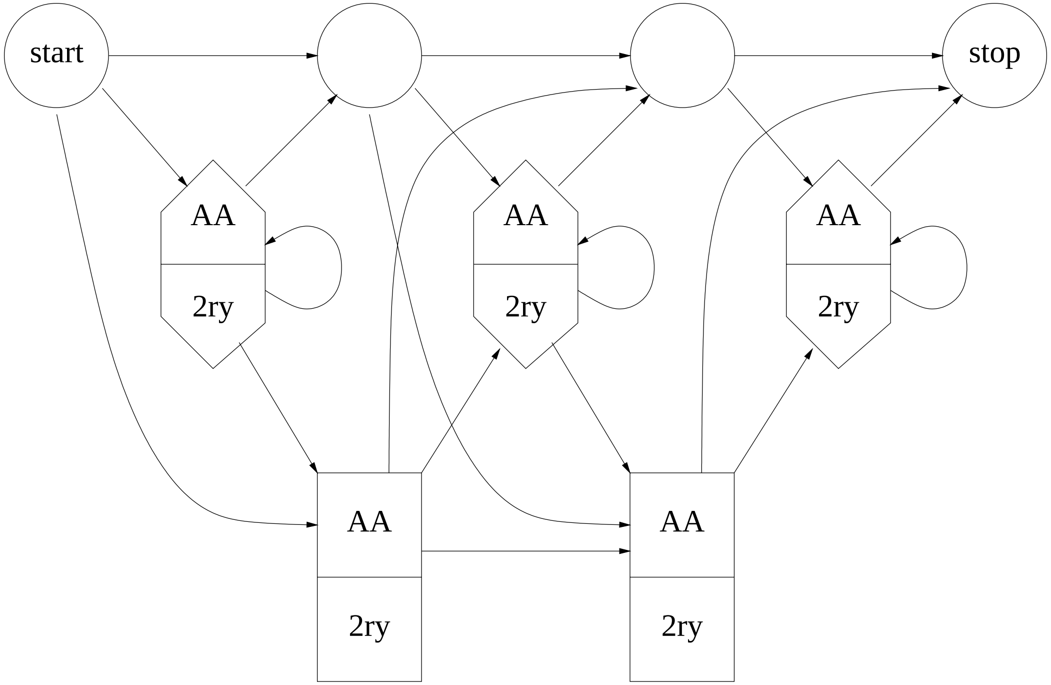
Make a multiple alignment (using Viterbi algorithm) of all sequences in the training set. The multiple alignment has one alignment column for each fat state of the HMM.

Repeat from *loop*, with thresholds in step *find* loosened.

Multi-track HMMS

We can also use alignments to build a two- or three-track target HMM:

- Amino-acid track (created from the multiple alignment).
- Local-structure track(s) with probabilities from neural net.
- Can align template (AA+local) to target model.



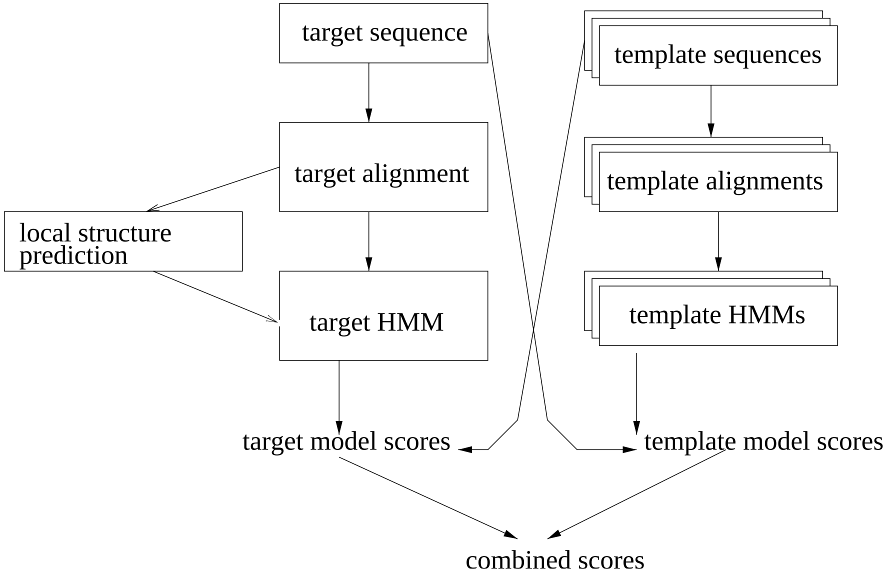
Target-model Fold Recognition

- Find probable homologs of target sequence and make multiple alignment.
- Make secondary structure probability predictions based on multiple alignment.
- Build an HMM based on the multiple alignment and predicted 2ry structure (or just on multiple alignment).
- Score sequences and secondary structure sequences for proteins that have known structure (all sequences for AA-only, 8,000-11,000 representatives for multi-track).
- Select the best-scoring sequence(s) to use as templates.

Template-library Fold Recognition

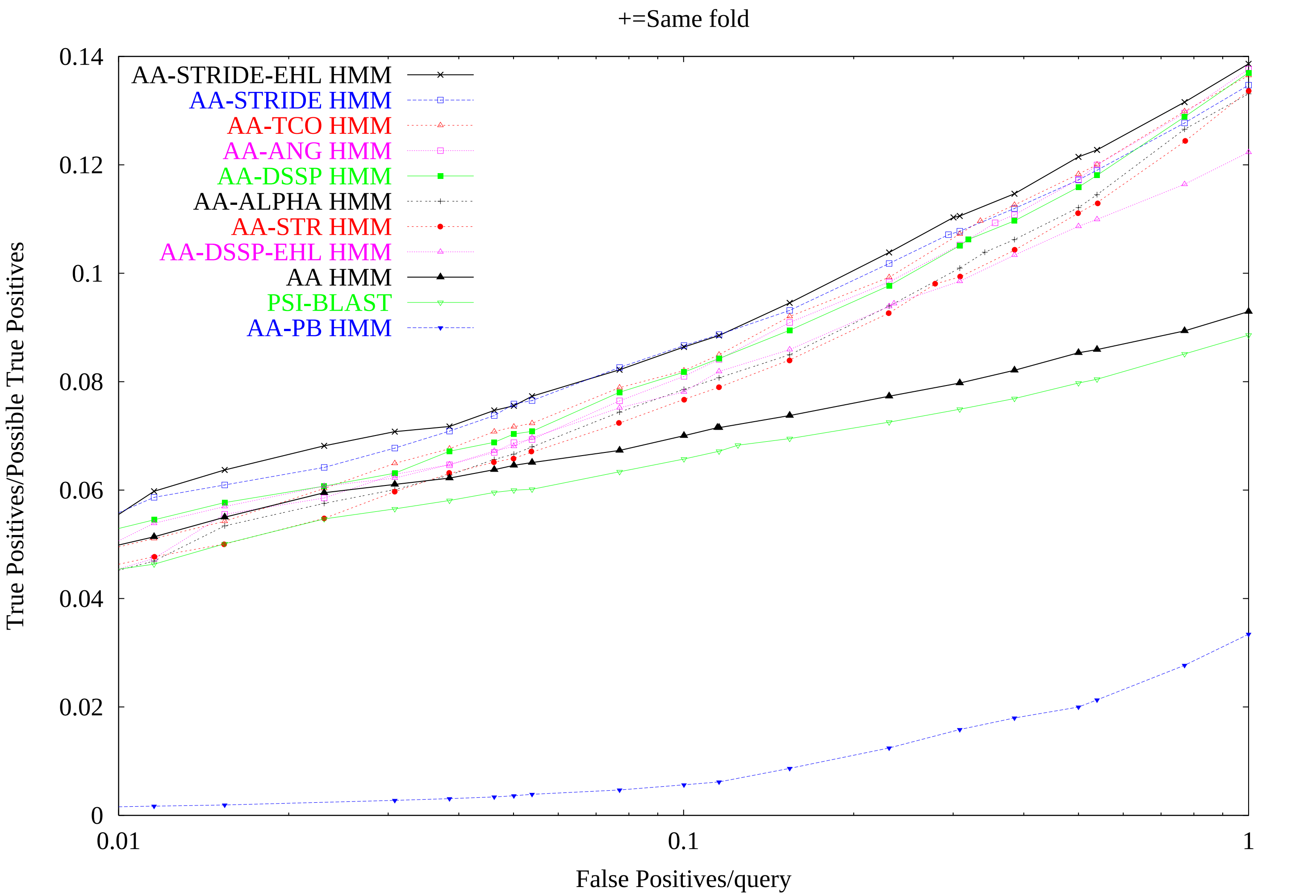
- Build an HMM for each protein in the template library, based on the template sequence (and any homologs you can find).
- The T2K library has over 11,000 templates from PDB.
- For the fold-recognition problem, structure information can be used in building these models (though we currently don't).
- Score target sequence with all models in the library.
- Select the best-scoring model(s) to use as templates.

Combined SAM-T02 method



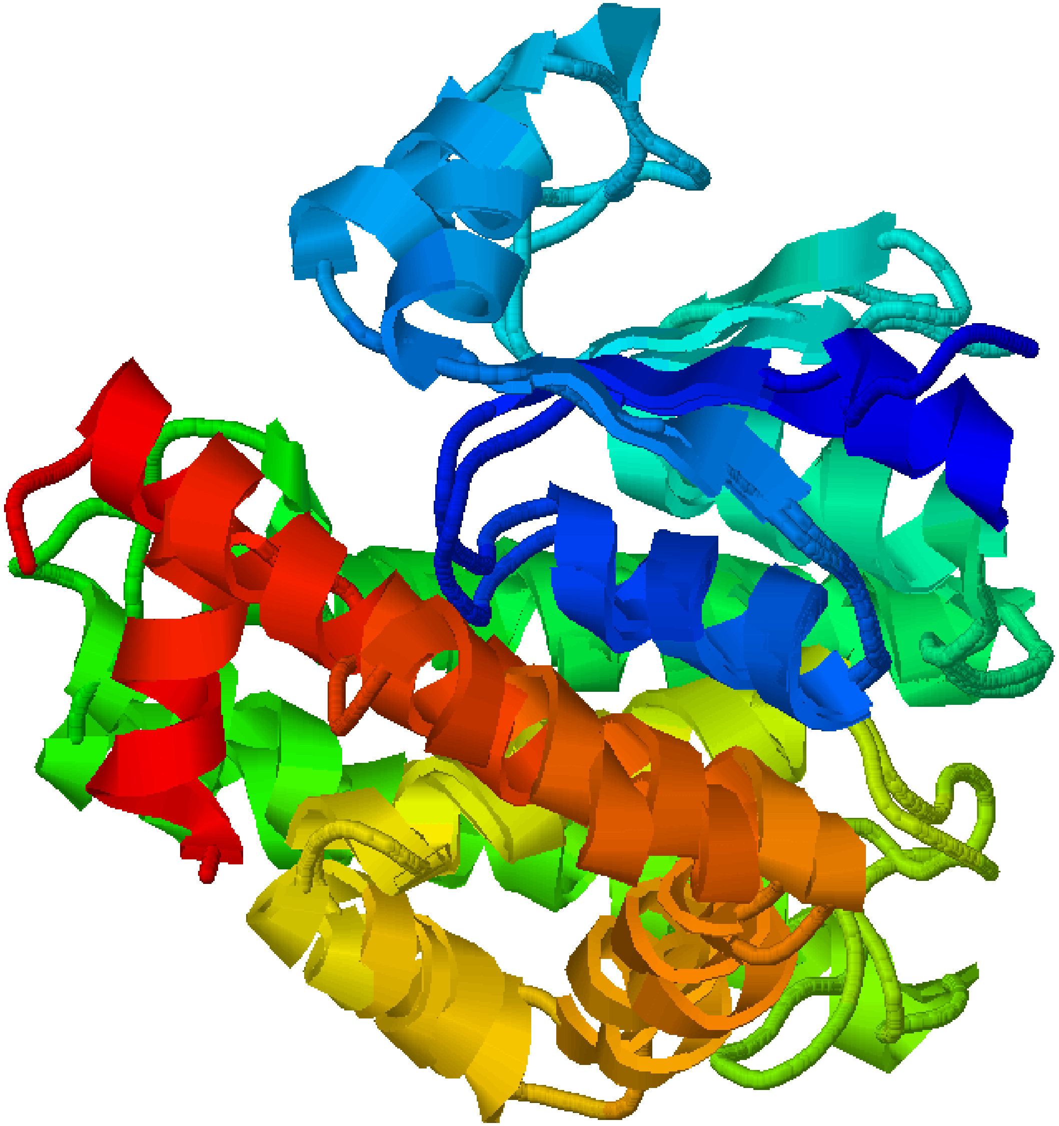
- Combine the costs from the template library search and the target library searches using different local structure alphabets.
- Choose one of the many alignments of the target and template (whatever method gets best results in testing).
- `http://www.soe.ucsc.edu/research/compbio/HMM-apps/T02-query.html`

Fold recognition results



Comparative modeling: T0232

RMSD= 5.158Å all-atom, 4.463Å C_α



Fragment Packing

- Fragment packing was introduced by Simon and Baker’s Rosetta program.
- It provides intelligent conformation generation for new folds.
- Rosetta conformation is contiguous chain.
- New conformations are created by randomly replacing fragment of backbone with different fragment (from library), keeping chain contiguous.
- Stochastic search by simulated annealing.

Undertaker

- Undertaker is UCSC’s attempt at a fragment-packing program.
- Named because it optimizes burial.
- Representation is 3D coordinates of all heavy atoms (not hydrogens).
- Can replace fragments (a la Rosetta) or full alignments—chain need not remain contiguous.
- Conformations can borrow heavily from fold-recognition alignments, without having to lock in a particular alignment.
- Use genetic algorithm with many conformation-change operators to do stochastic search.

Fragfinder

Fragments are provided to undertaker from 3 sources:

- Generic fragments (2-4 residues, exact sequence match) are obtained by reading in 500–1000 PDB files, and indexing all fragments.
- Long specific fragments (and full alignments) are obtained from the various target and template alignments generated during fold recognition.
- Medium-length fragments (9–12 residues long) for every position are generated from the HMMs with `fragfinder`, a new tool in the SAM suite.

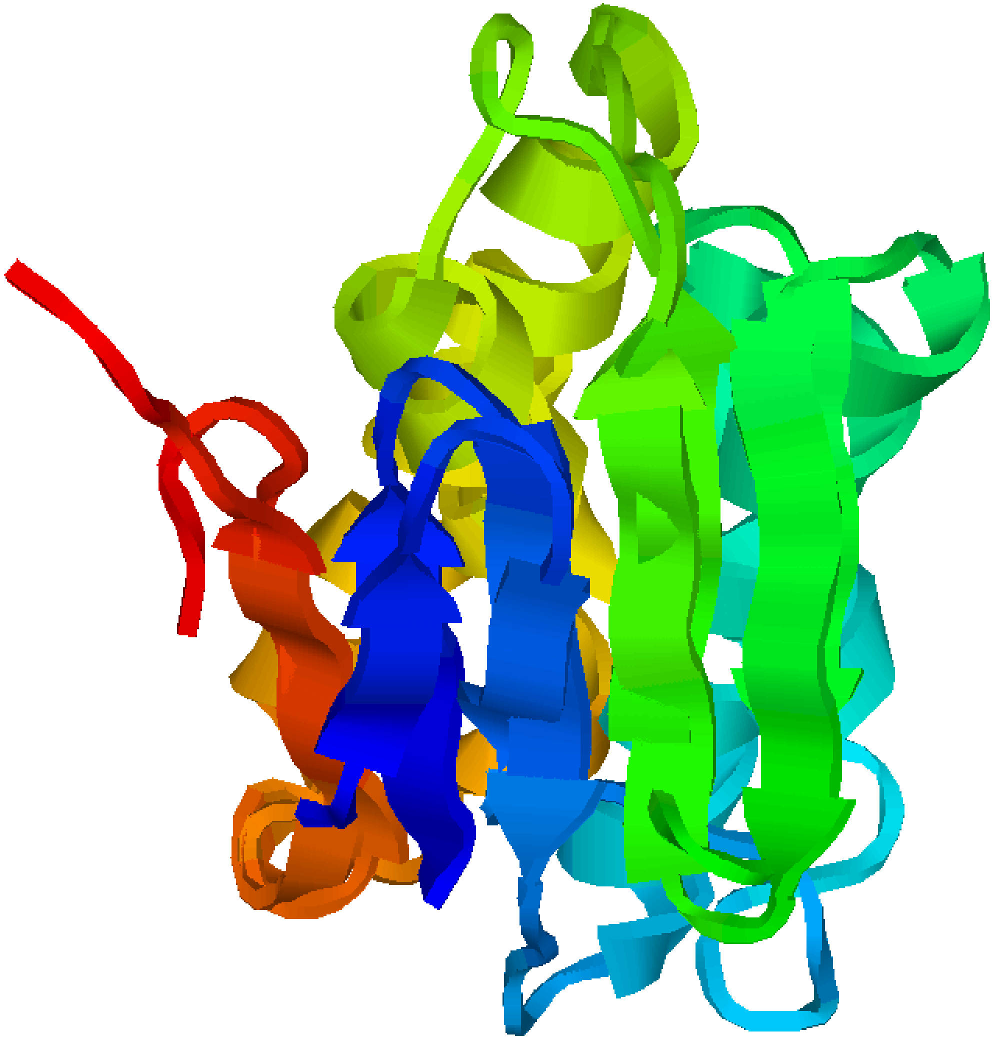
Cost function

- Cost function is modularly designed—easy to add or remove terms.
- Cost function can include predictions of local properties by neural nets.
- Clashes and hydrogen bonds are important components.
- There are over 40 cost function components available: burial functions, disulfides, contact order, radius of gyration, constraints, ...

Target T0201 (NF)

- We tried forcing various sheet topologies and selected 4 by hand.
- Model 1 has right topology (5.912Å all-atom, 5.219Å C_α).
- Unconstrained cost function not good at choosing topology (two strands curled into helices).
- Helices were too short.

Target T0201 (NF)



Contact prediction: new in 2004!

- Use mutual information between columns.
- Thin alignments aggressively (30%, 35%, 40%, 50%, 62%).
- Compute e-value for mutual info (correcting for small-sample effects).
- Compute z-score of log(e-value) within protein.
- Feed e-values, z-scores, conservation, amino-acid profile, separation along chain into neural net.

Evaluating contact prediction

Two measures of contact prediction:

- Accuracy:

$$\frac{\sum \chi(i,j)}{\sum 1}$$

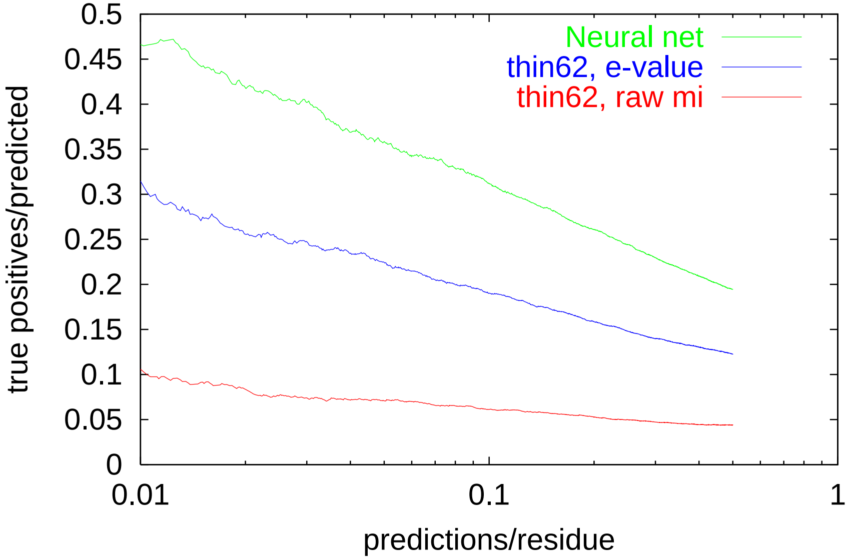
(favors short-range predictions, where contact probability is higher)

- Weighted accuracy:

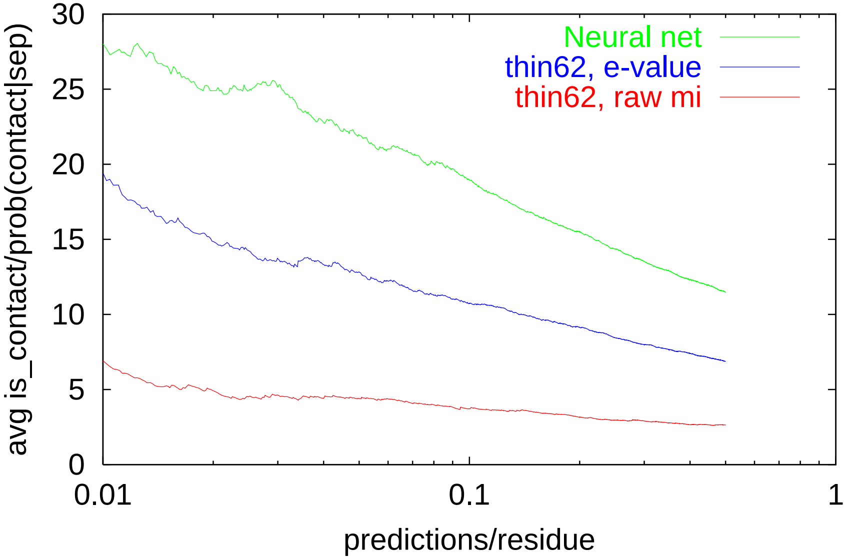
$$\frac{\sum \frac{\chi(i,j)}{\text{Prob}\left(\text{contact}\middle|\text{separation}_{=|i-j|}\right)}}{\sum 1}$$

(1 if predictions no better than chance based on separation).

Contact prediction results



Accuracy of contact prediction, by protein

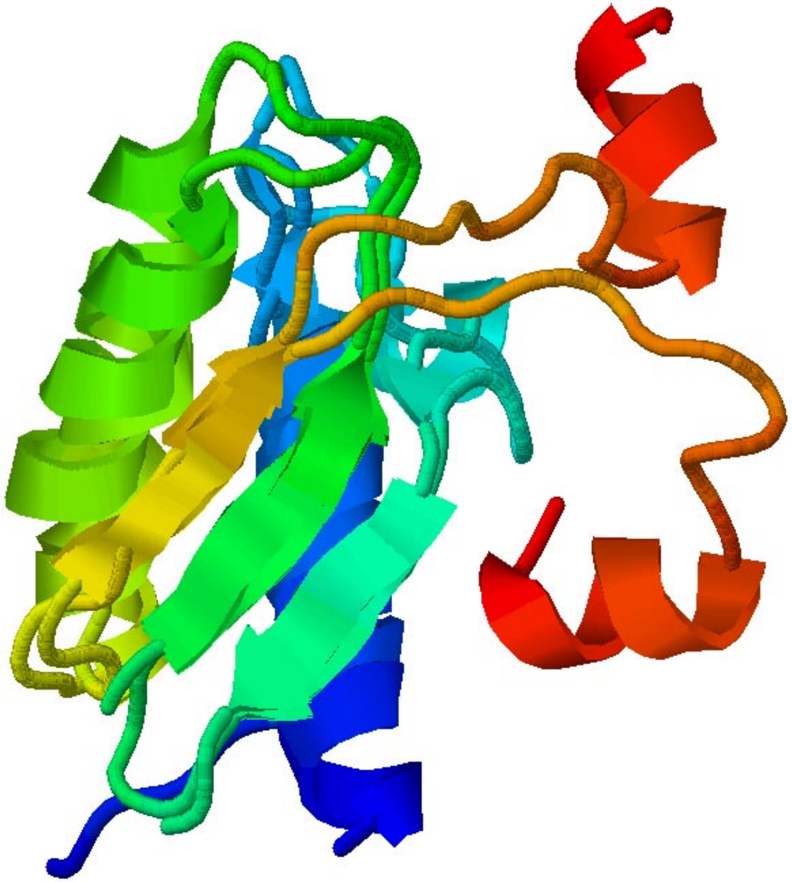


Weighted-accuracy of contact prediction, by protein

Target T0230 (FR/A)

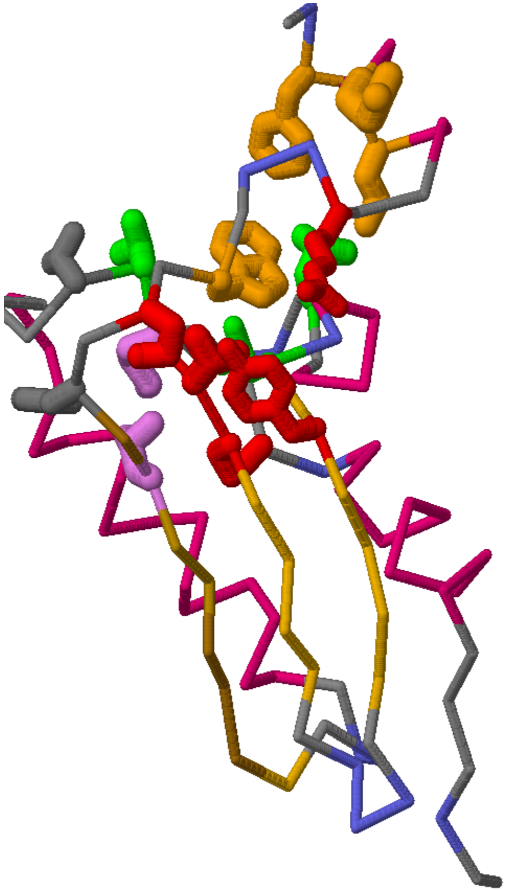
- Good except for C-terminal loop and helix flopped wrong way.
- We have secondary structure right, including phase of beta strands.
- Contact prediction helped, but we put too much weight on it—decoys fit predictions better than real structure does.

Target T0230 (FR/A)



Target T0230 (FR/A)

Real structure with contact predictions:



Web sites

These slides:

http://www.soe.ucsc.edu/~karplus/papers/origami-with-strings-upr-2005

SAM-T02 prediction server:

http://www.soe.ucsc.edu/research/compbio/HMM-apps/T02-query.html

CASP6 all our results and working notes:

http://www.soe.ucsc.edu/~karplus/casp6/

Predictions for all yeast proteins:

http://www.soe.ucsc.edu/~karplus/yeast/

UCSC bioinformatics (research and degree programs) info:

http://www.soe.ucsc.edu/research/compbio/

SAM tool suite info:

http://www.soe.ucsc.edu/research/compbio/sam.html