

# Origami with strings: protein folding by computer

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# Outline of Talk

- 🦖 What is Biomolecular Engineering? Bioinformatics?
- 🦖 What is a protein?
- 🦖 The folding problem and variants on it:
  - Local structure prediction
  - Fold recognition
  - Comparative modeling
  - “Ab initio” methods
  - Contact prediction



# What is Biomolecular Engineering?

Engineering **with**, **of**, or **for** biomolecules. For example,

**with**: using proteins as sensors or for self-assembly.







**of**: protein engineering—designing or artificially evolving proteins to have particular functions

**for**: designing high-throughput experimental methods to find out what molecules are present, how they are structured, and how they interact.



# What is Bioinformatics?

Bioinformatics: using computers and statistics to make sense out of the mountains of data produced by high-throughput experiments.

-  Genomics: finding important sequences in the genome and annotating them.
-  Phylogenetics: “tree of life”.
-  Systems biology: piecing together various control networks.
-  DNA microarrays: what genes are turned on under what conditions.
-  Proteomics: what proteins are present in a mixture.
-  Protein structure prediction.



# What is a protein?

- 🦖 There are many abstractions of a protein: a band on a gel, a string of letters, a mass spectrum, a set of 3D coordinates of atoms, a point in an interaction graph, . . . .
- 🦖 For us, 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.
- 🦖 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?

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MTMSRRNTDA ITIHSILDWI EDNLESPLSL EKVSEKSGYS 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.

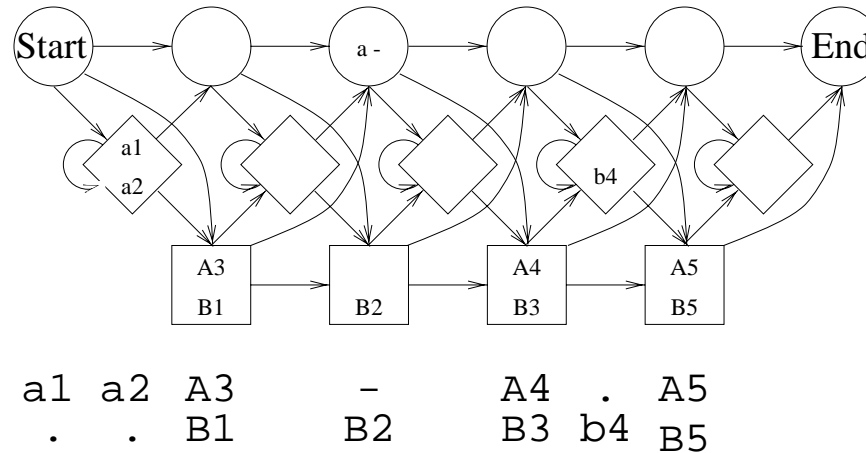


# Hidden Markov Models

- 🦖 *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

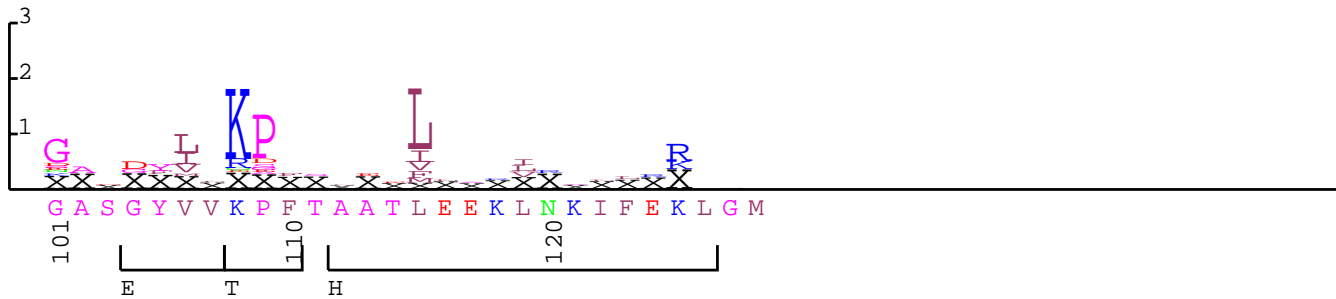
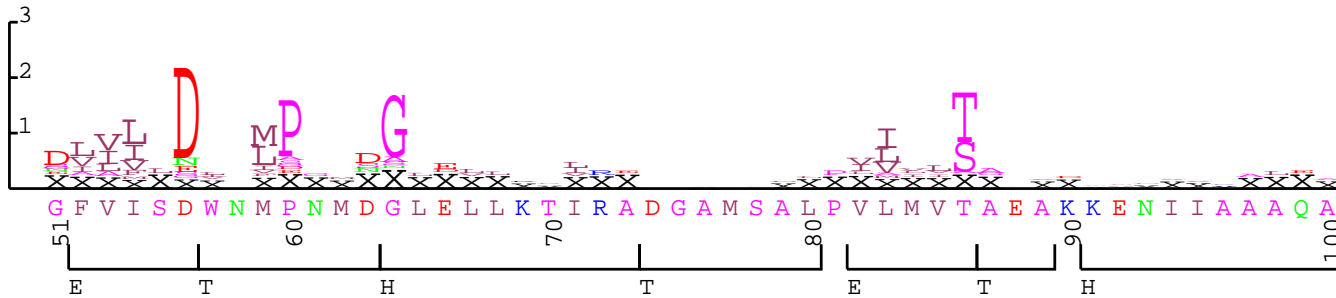
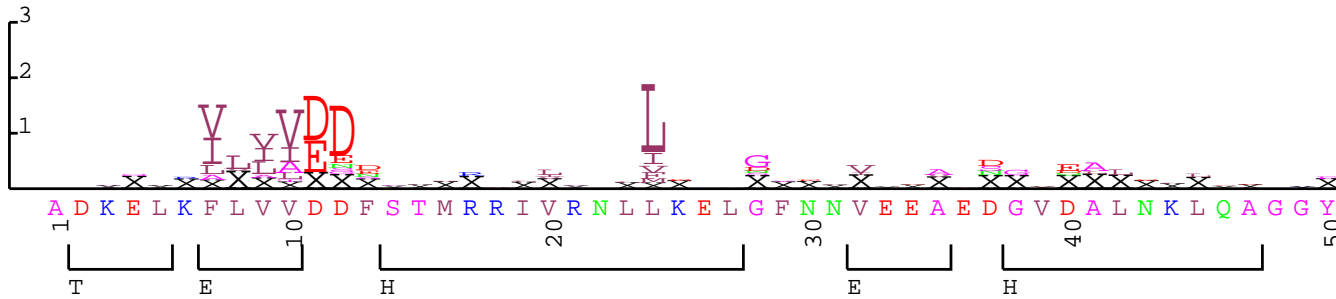


- 🦖 Circles are null states.
- 🦖 Squares are *match states*, each of which is paired with a null *delete state*. We call the match-delete pair a *fat state*.
- 🦖 Each fat state is visited exactly once on every path from Start to End.
- 🦖 Diamonds are *insert states*, and are used to represent possible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



# What is single-track HMM looking for?

nostruct-align/3chy.t2k w0.5



# 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.



# CASP Competition Experiment

- 🦖 Everything published in literature “works”
- 🦖 CASP set up as true blind test of prediction methods.
- 🦖 Sequences of proteins about to be solved released to prediction community.
- 🦖 Predictions registered with organizers.
- 🦖 Experimental structures compared with solution by assessors.
- 🦖 “Winners” get papers in *Proteins: Structure, Function, and Bioinformatics*.



# 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  $\sum \log(P(\text{correct output}))$ .

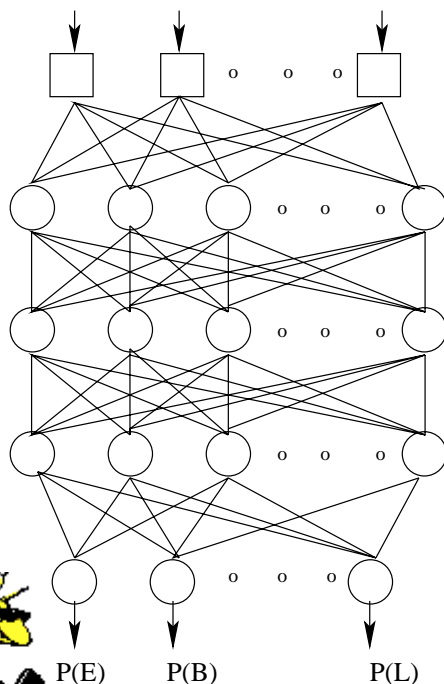




# Neural Net

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



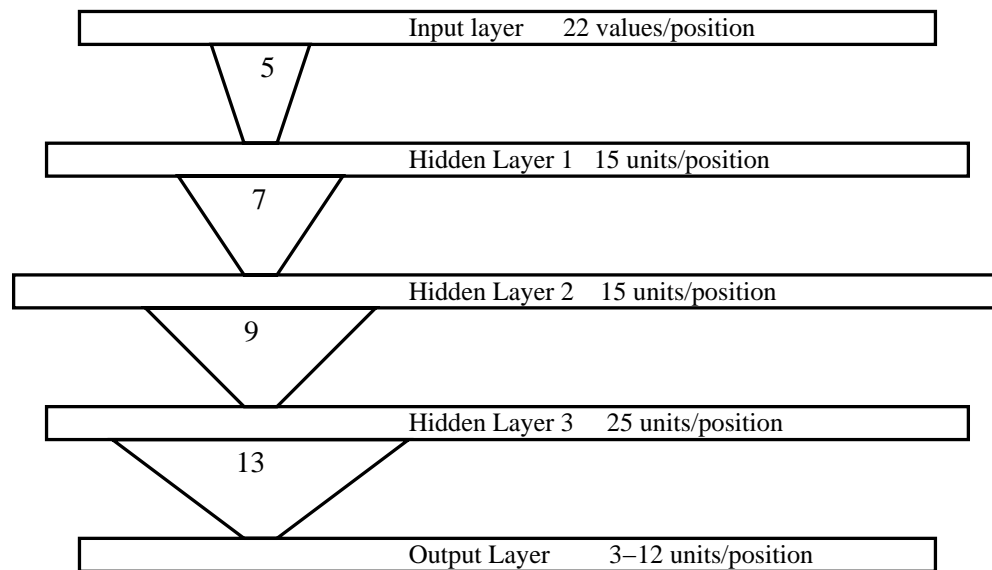
**Inputs**

**Hidden Layer 1**

**Hidden Layer 2**

**Hidden Layer 3**

**Output Layer**



# DSSP

🦖 DSSP is a popular program to define secondary structure.

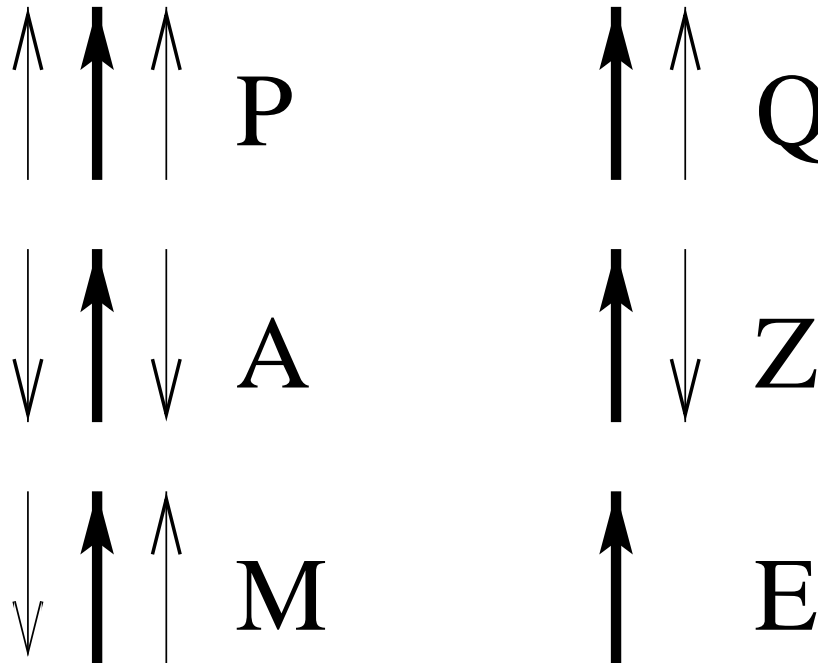
🦖 7-letter alphabet: EBGHSTL

- E =  $\beta$  strand
- B =  $\beta$  bridge
- G =  $3_{10}$  helix
- H =  $\alpha$  helix
- I =  $\pi$  helix (very rare, so we lump in with H)
- S = bend
- T = turn
- L = everything else (DSSP uses space for L)



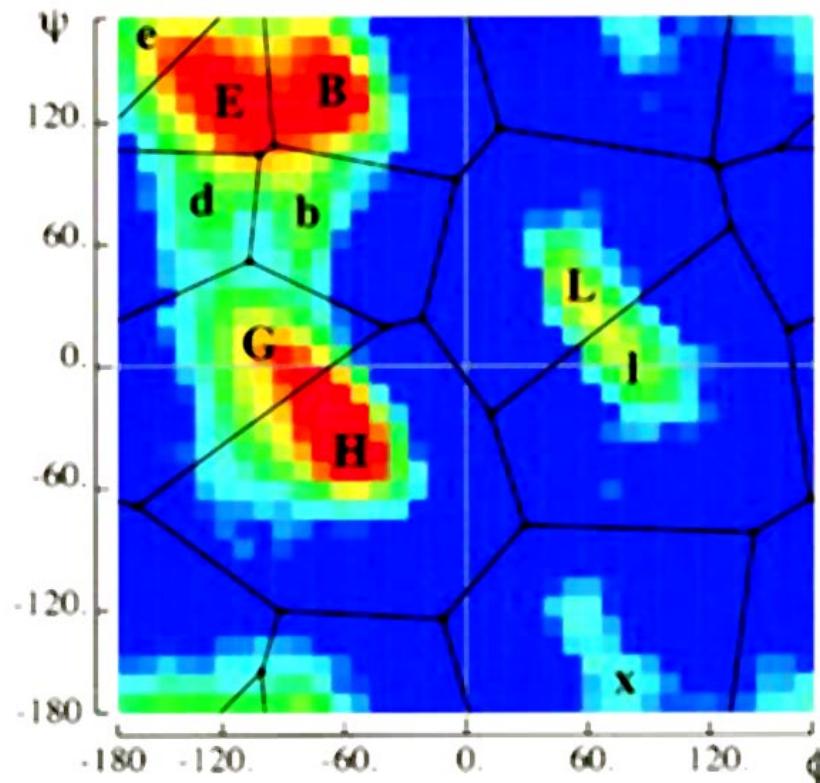
# STR: Extension to DSSP

- 🦖 Yael Mandel-Gutfreund noticed that parallel and anti-parallel strands had different hydrophobicity patterns, implying that parallel/antiparallel can be predicted from sequence.
- 🦖 We created a new alphabet, splitting DSSP's E into 6 letters:



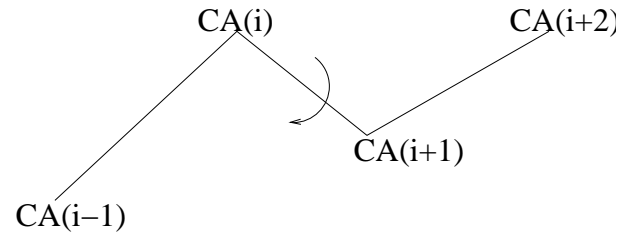
# HMMSTR $\phi$ - $\psi$ alphabet

- 🦖 For HMMSTR, Bystroff did k-means classification of  $\phi$ - $\psi$  angle pairs into 10 classes (plus one class for cis peptides).
- 🦖 We used just the 10 classes, ignoring the  $\omega$  angle.

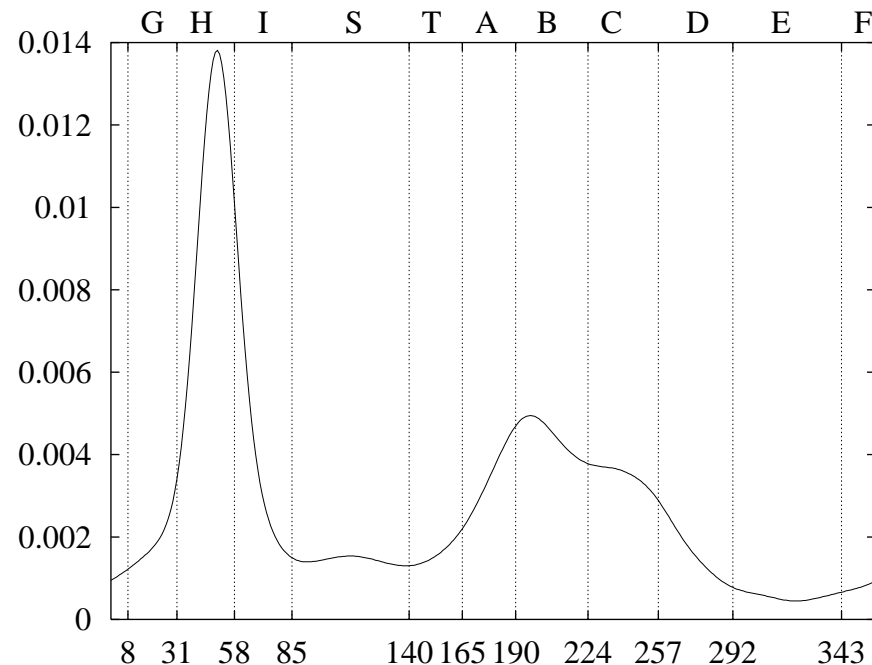


# ALPHA11: $\alpha$ angle

🦒 Backbone geometry can be mostly summarized with one angle per residue:

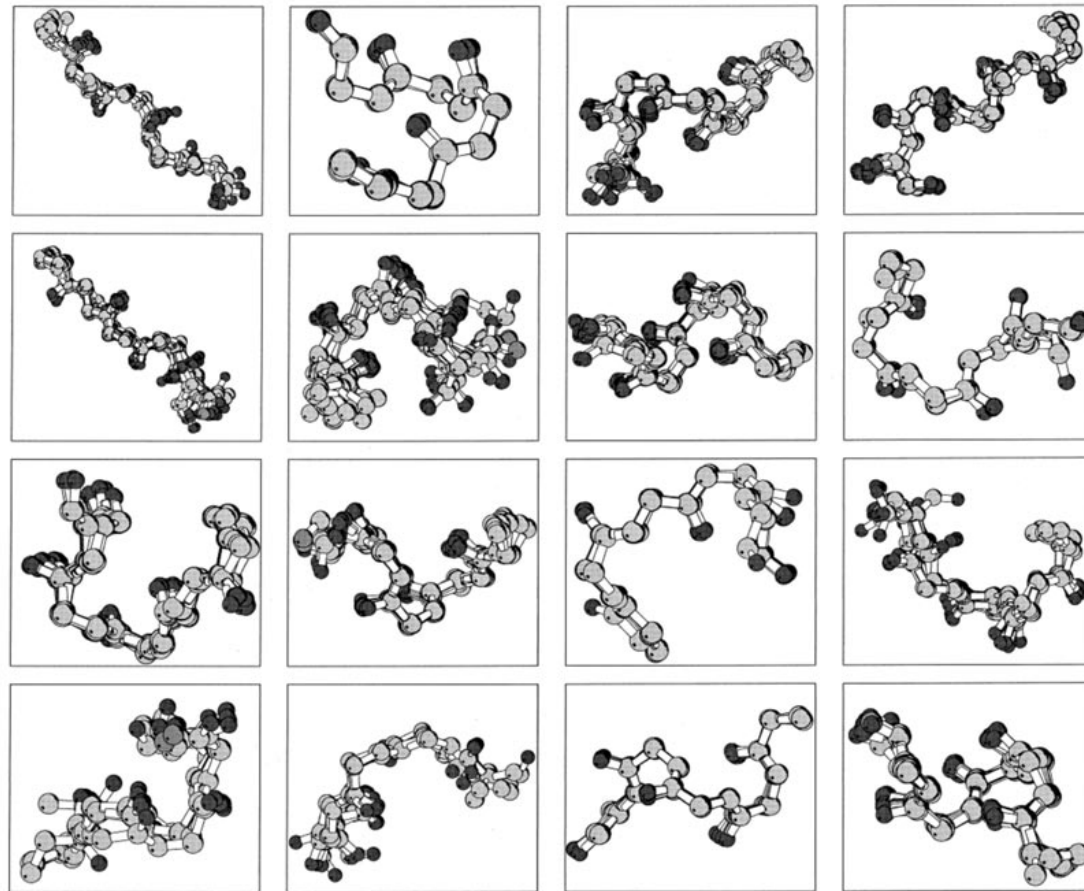


🦒 We discretize into 11 classes:



# de Brevern's Protein Blocks

Clustered on 5-residue window of  $\phi$ - $\psi$  angles:



# Burial alphabets

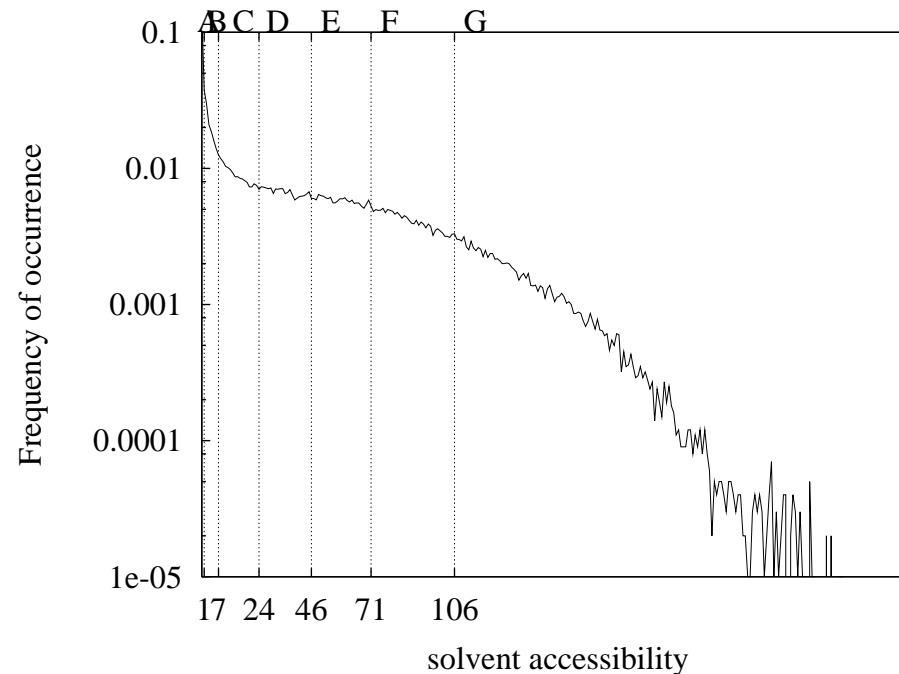
Our second set of investigations was for a sampling of the many burial alphabets, which are discretizations of various accessibility or burial measures:

- 🐉 solvent accessible surface area
- 🐉 relative solvent accessible surface area
- 🐉 neighborhood-count burial measures



# Solvent Accessibility

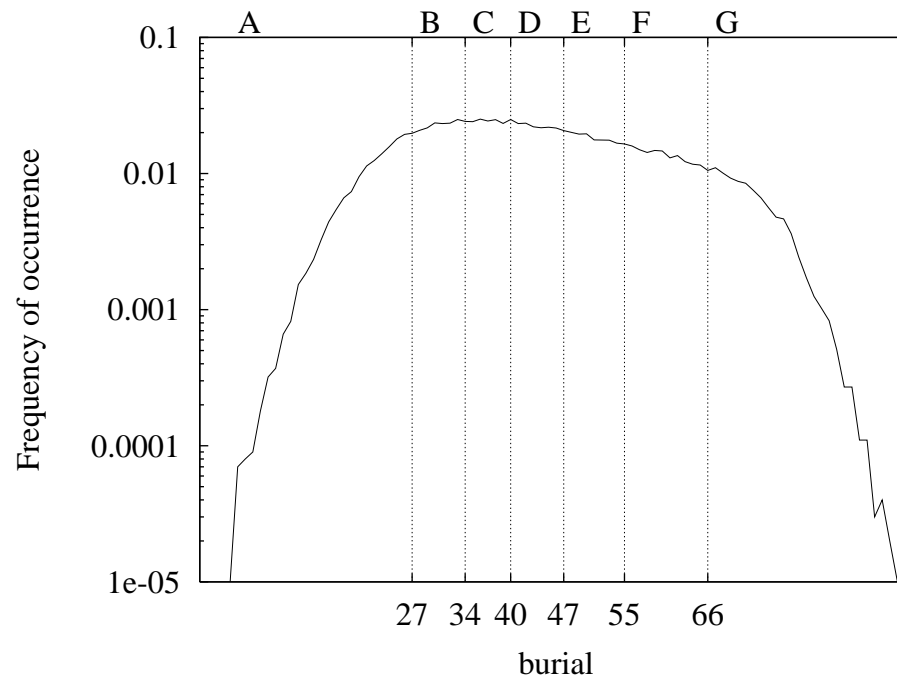
- 🦖 Absolute SA: area in square Ångstroms accessible to a water molecule, computed by DSSP.
- 🦖 Relative SA: Absolute SA/ max SA for residue type (using Rost's table for max SA).





# Burial

- Define a sphere for each residue.
- Count the number of atoms or of residues within that sphere.
- Example: center =  $C_{\beta}$ , radius =  $14\text{\AA}$ , count =  $C_{\beta}$ , quantize in 7 equi-probable bins.



# Mutual Information

- 🦖 Mutual information between two random variables (letters of alphabet):

$$MI(X, Y) = \sum_{i,j} P(i, j) \log \frac{P(i, j)}{P(i)P(j)},$$

- 🦖 We look at mutual information between different alphabets at same position in protein. (redundancy)
- 🦖 We look at mutual information with one alphabet between corresponding positions on alignments of sequences.



# Information Gain

Information gain is how much more we know about a variable after making a prediction.

$$I(X) = \text{average} \log \frac{\hat{P}_i(X_i)}{P_0(X_i)}$$

$\hat{P}_i$  is predicted probability vector for position  $i$

$X_i$  is actual observation at position  $i$

$P_0$  is background probability vector



# Conservation and Predictability

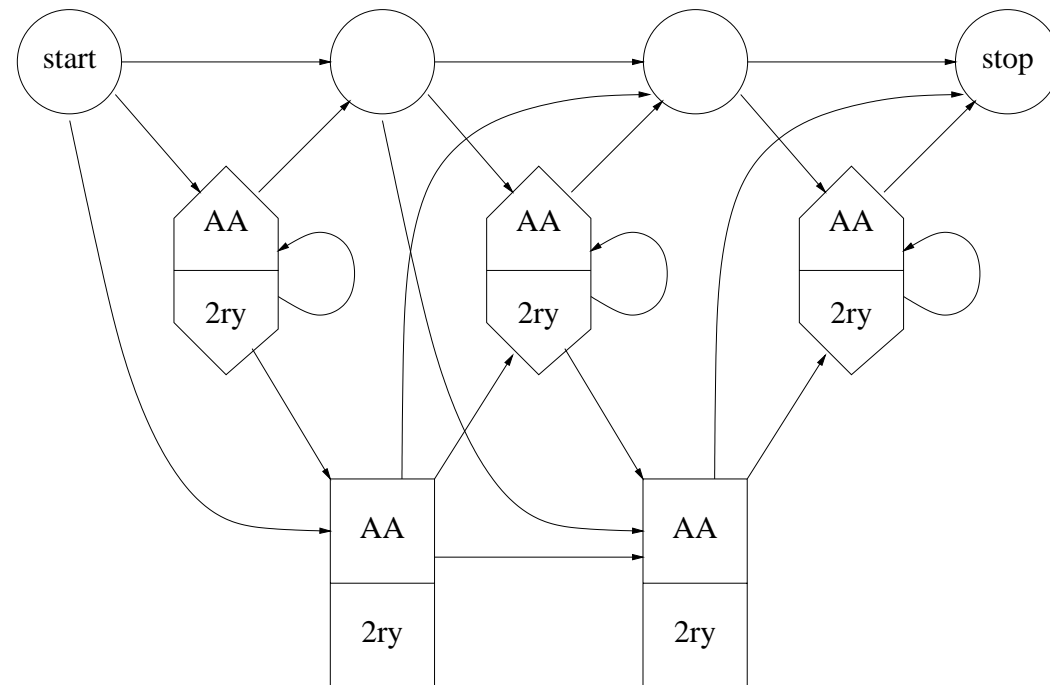
Name	alphabet		MI with AA	conservation	predictability	
	size	entropy		mutual info	info gain per residue	$Q_{ A }$
str	13	2.842	0.103	<b>1.107</b>	1.009	0.561
protein blocks	16	3.233	0.162	0.980	<b>1.259</b>	0.579
stride	6	2.182	0.088	0.904	0.863	0.663
DSSP	7	2.397	0.092	0.893	0.913	0.633
stride-EHL	3	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	<b>0.682</b>	0.502	
CB-14	7	2.786	0.106	0.667	<b>0.525</b>	
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	



# Multi-track HMMs

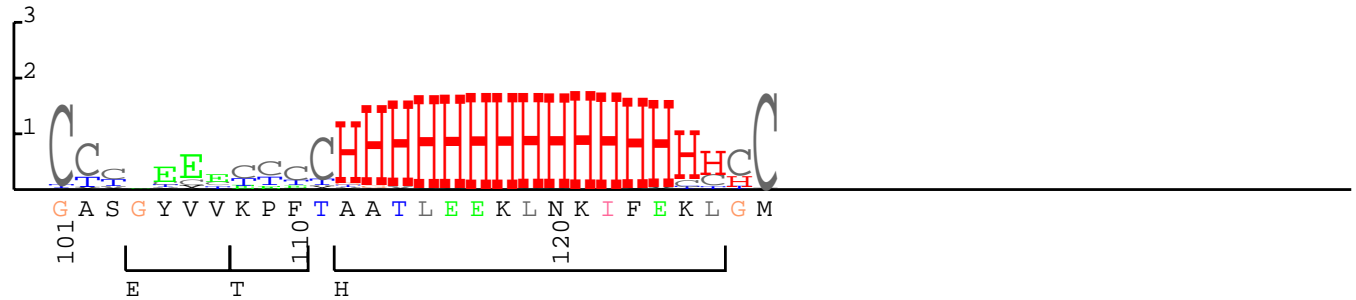
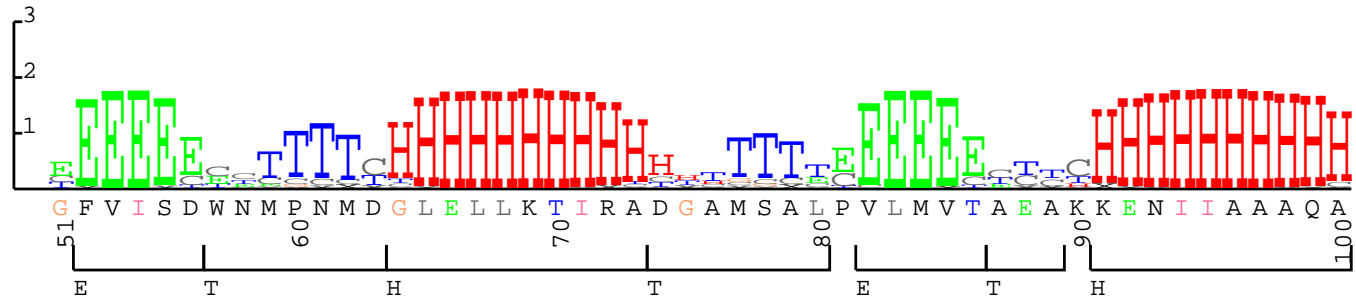
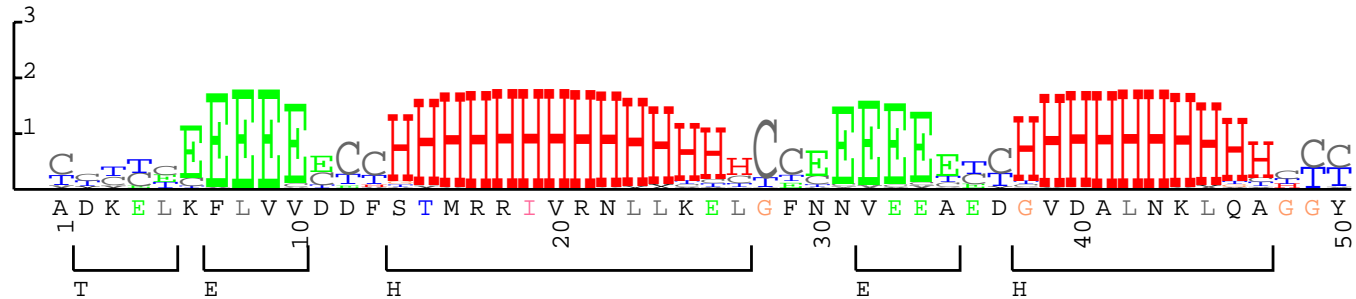
We can also use alignments to build a two- or three-track target HMM:

- 🦖 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.



# What is second track looking for?

nostruct-align/3chy.t2k EBGHTL



# 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.



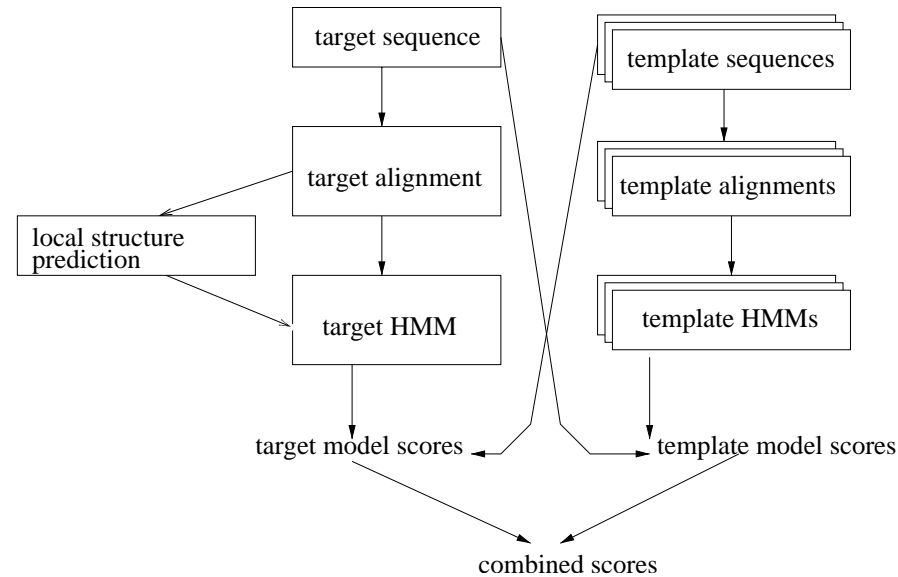
# Template-library Fold Recognition

- 🦖 Build an HMM for each protein in the template library, based on the template sequence (and any homologs you can find).
- 🦖 The HMM library has over 12,000 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.






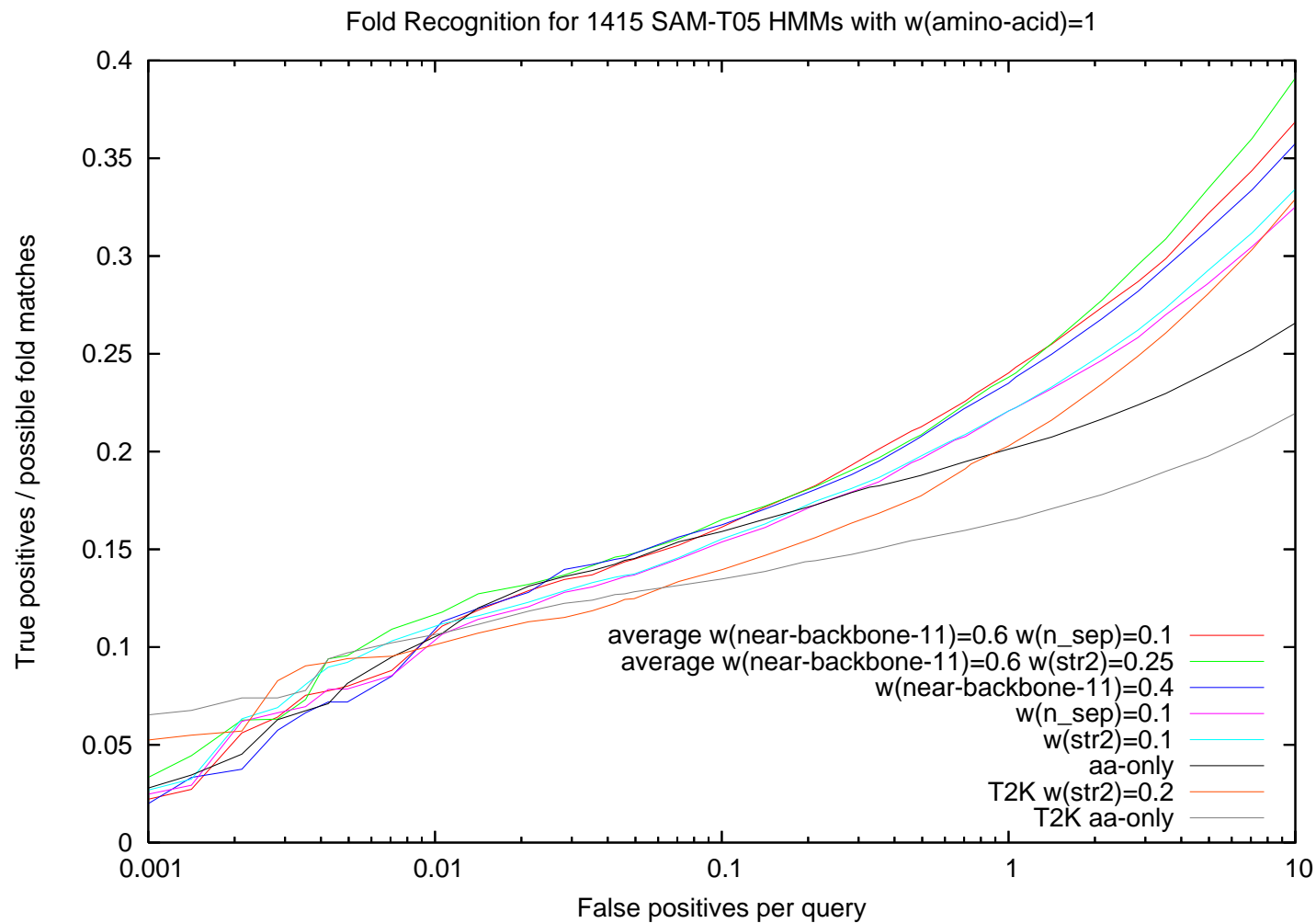
# Combined SAM-Txx method



- 🐼 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).

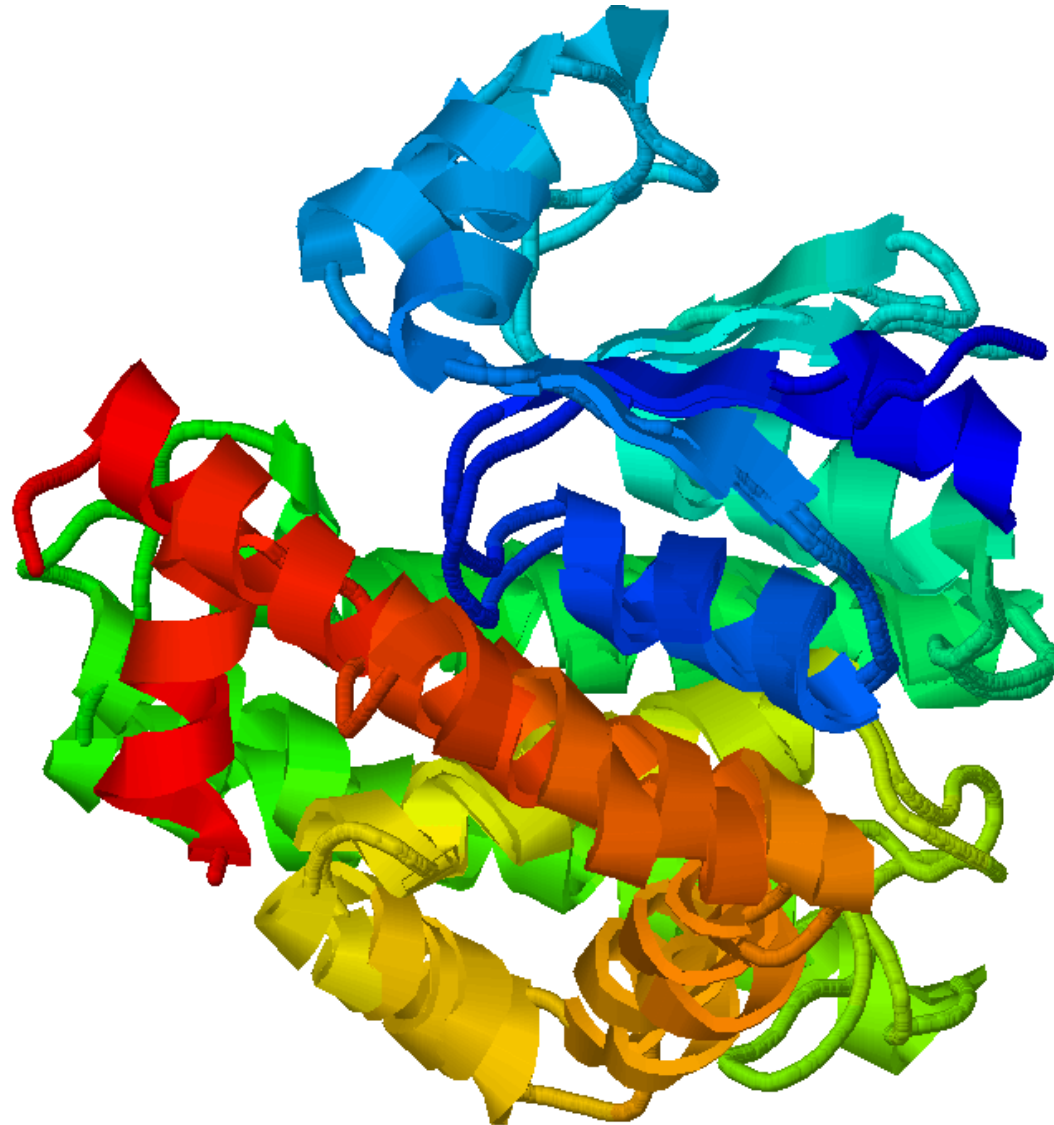
 [http://www.soe.ucsc.edu/research/compbio/SAM\\_T06/T06-query.html](http://www.soe.ucsc.edu/research/compbio/SAM_T06/T06-query.html)

# Fold recognition results



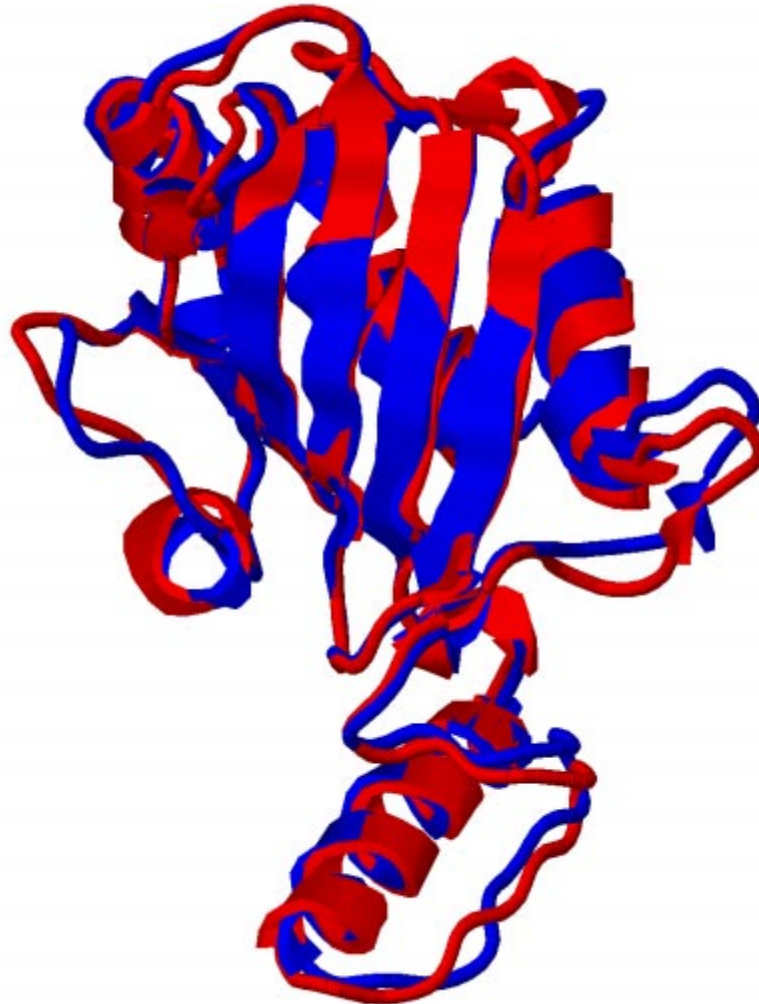
# Comparative modeling: T0232

RMSD= 5.158Å all-atom, 4.463Å  $C_{\alpha}$



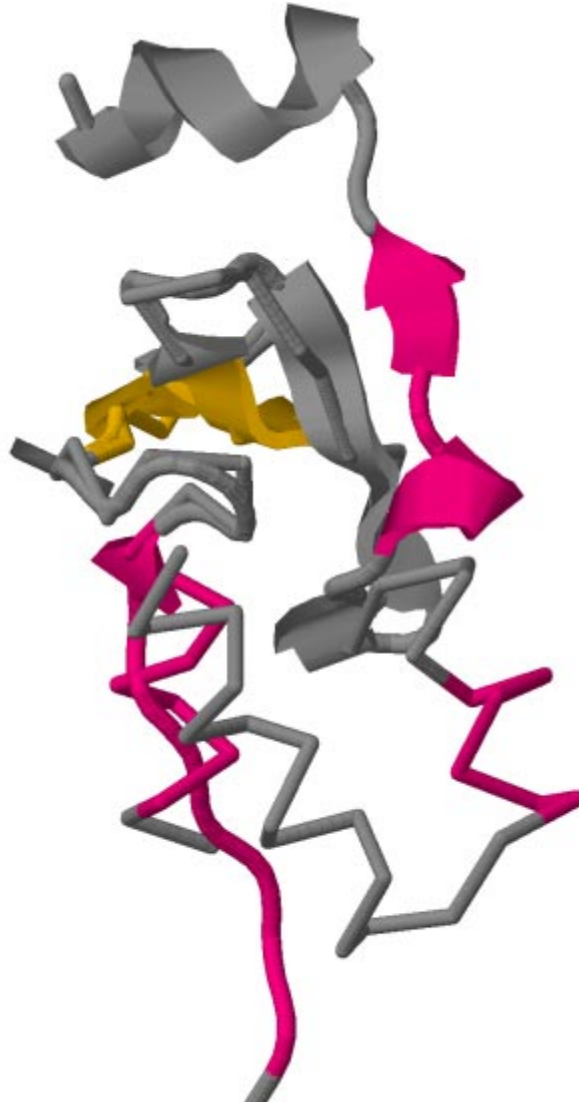
# T0298 domain 2 (130–315)

RMSD= 2.468Å all-atom, 1.7567Å  $C_{\alpha}$ , GDT=82.5%  
best model 1 submitted to CASP7 (red=real)



# Comparative modeling: T0348

RMSD= 11.8 Å  $C_{\alpha}$ , GDT=58.2% (cartoon=real)  
best model 1 by CASP7 GDT, Robetta1 slightly better.



# 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 backbone 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, rotamer preference, radius of gyration, constraints, ...



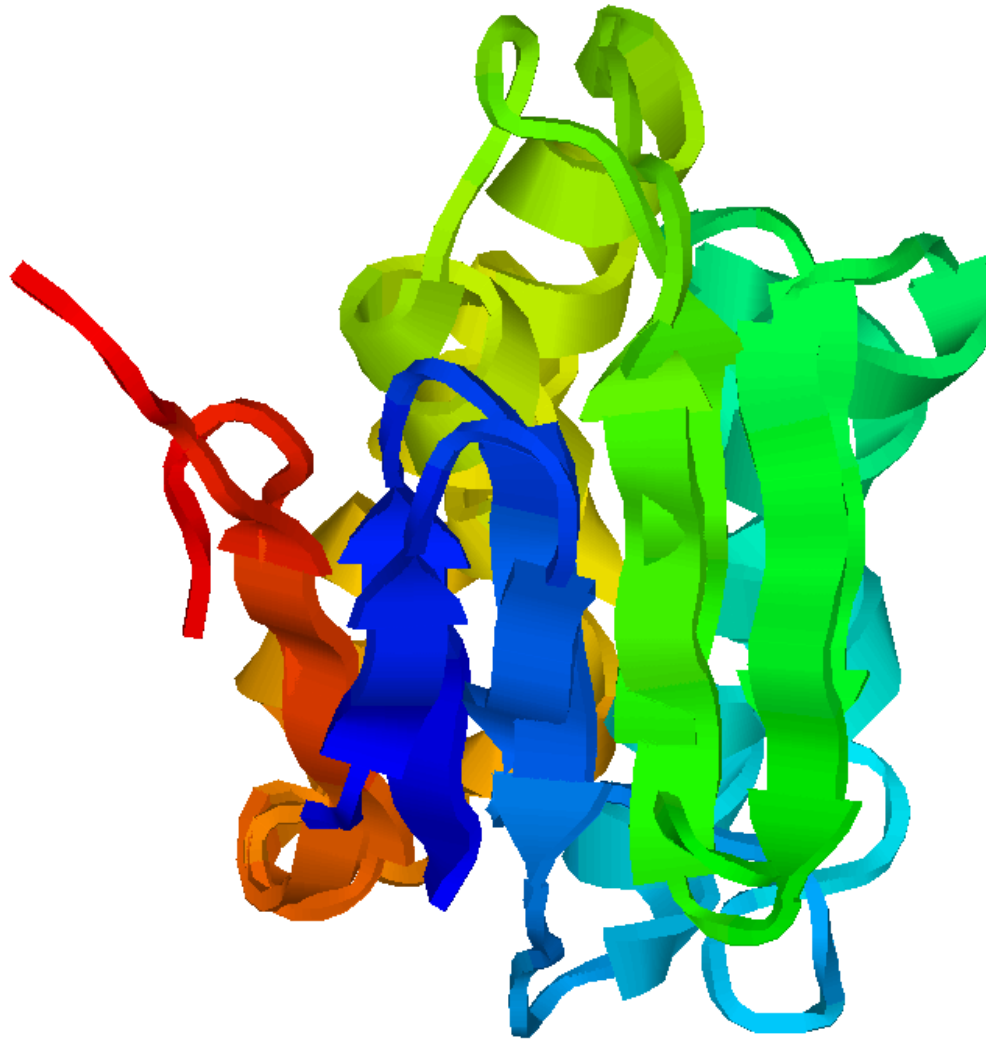


# Target T0201 (NF)

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



# Target T0201 (NF)



# Contact prediction

- 🦖 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 rank of  $\log(\text{e-value})$  within protein.
- 🦖 Feed  $\log(\text{e-values})$ , log rank, contact potential, joint entropy, and separation along chain for pair, and amino-acid profile, predicted burial, and predicted secondary structure for each residue of pair into a 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:

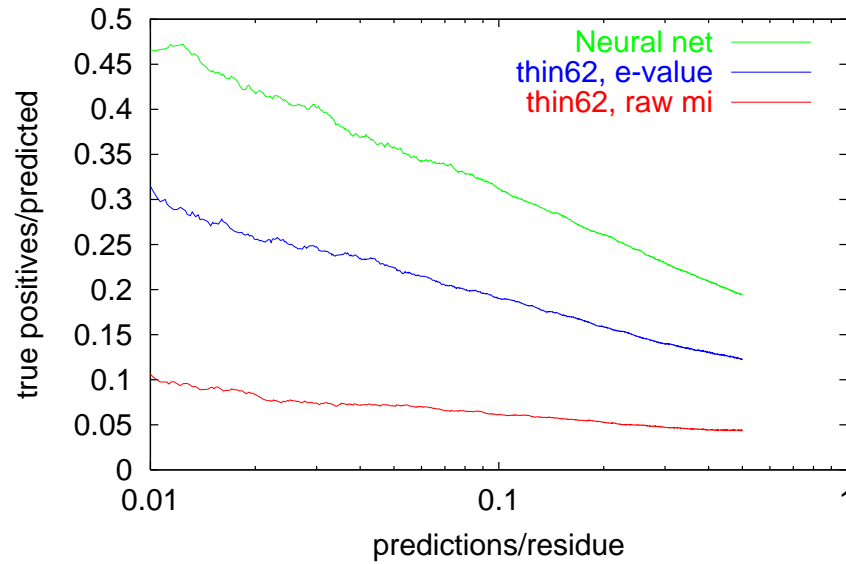
$$\frac{\sum \frac{\chi(i, j)}{\text{Prob}(\text{contact} | \text{separation} = |i - j|)}}{\sum 1}$$

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

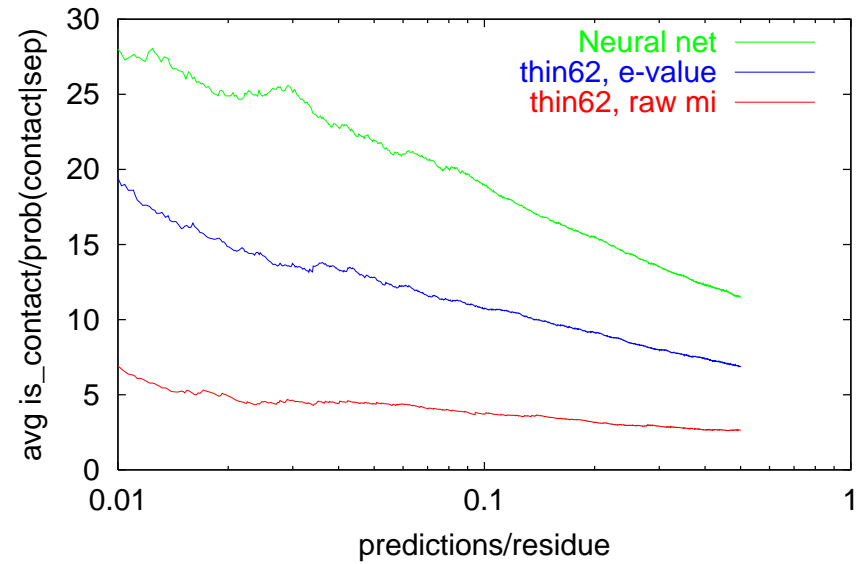


# Contact prediction results

Accuracy of contact prediction, by protein



Weighted-accuracy of contact prediction, by protein

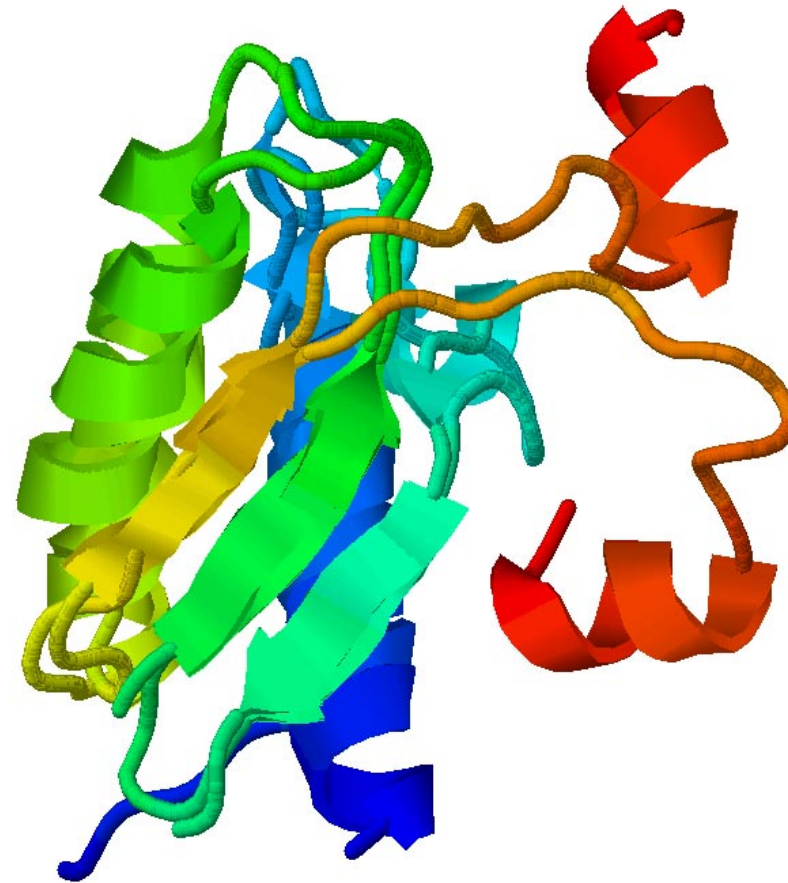


# Target T0230 (FR/A)

- 🦖 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.

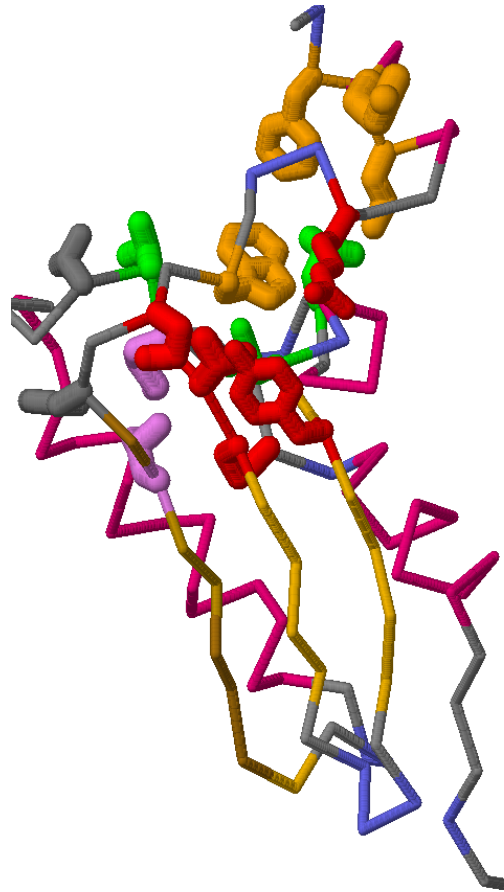


# Target T0230 (FR/A)



# Target T0230 (FR/A)

Real structure with contact predictions:





# Web sites

## **These slides:**

<http://www.soe.ucsc.edu/~karplus/papers/origami-with-strings-jan-2007>

## **SAM-T06 prediction server:**

[http://www.soe.ucsc.edu/research/compbio/SAM\\_T06/T06-query.html](http://www.soe.ucsc.edu/research/compbio/SAM_T06/T06-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>

