Better than Chance: the importance of null models

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Outline of Talk

- What is Biomolecular Engineering? Bioinformatics?
- What is a protein?
- The folding problem and variants on it.
- What is a null model (or null hypothesis) for?
- Example 1: is a conserved ORF a protein?
- Example 2: is residue-residue contact prediction better than chance?
- Example 3: how should we remove composition biases in HMM searches?

What is Biomolecular Engineering?

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

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

of: protein and RNA engineering—designing or artificially evolving proteins or RNA 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”, ancestral genome reconstruction.
- Systems biology: piecing together various networks of molecular interactions.
- 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?

```
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.
- We can also try to predict local properties (e.g., secondary structure or burial) or contact between residues.

Scoring (Bayesian view)

- A model M is a computable function that assigns a probability $\mathsf{P}(A \mid M)$ to each sequence A.
- When given a sequence A, we want to know how likely the model is. That is, we want to compute something like $\mathsf{P}(M \mid A)$.
- Bayes Rule:

$$\mathsf{P}\left(M \,\middle|\, A\right) = \mathsf{P}\left(A \,\middle|\, M\right) \frac{\mathsf{P}(M)}{\mathsf{P}(A)} \; .$$

- Problem: $\mathsf{P}(A)$ and $\mathsf{P}(M)$ are inherently unknowable.

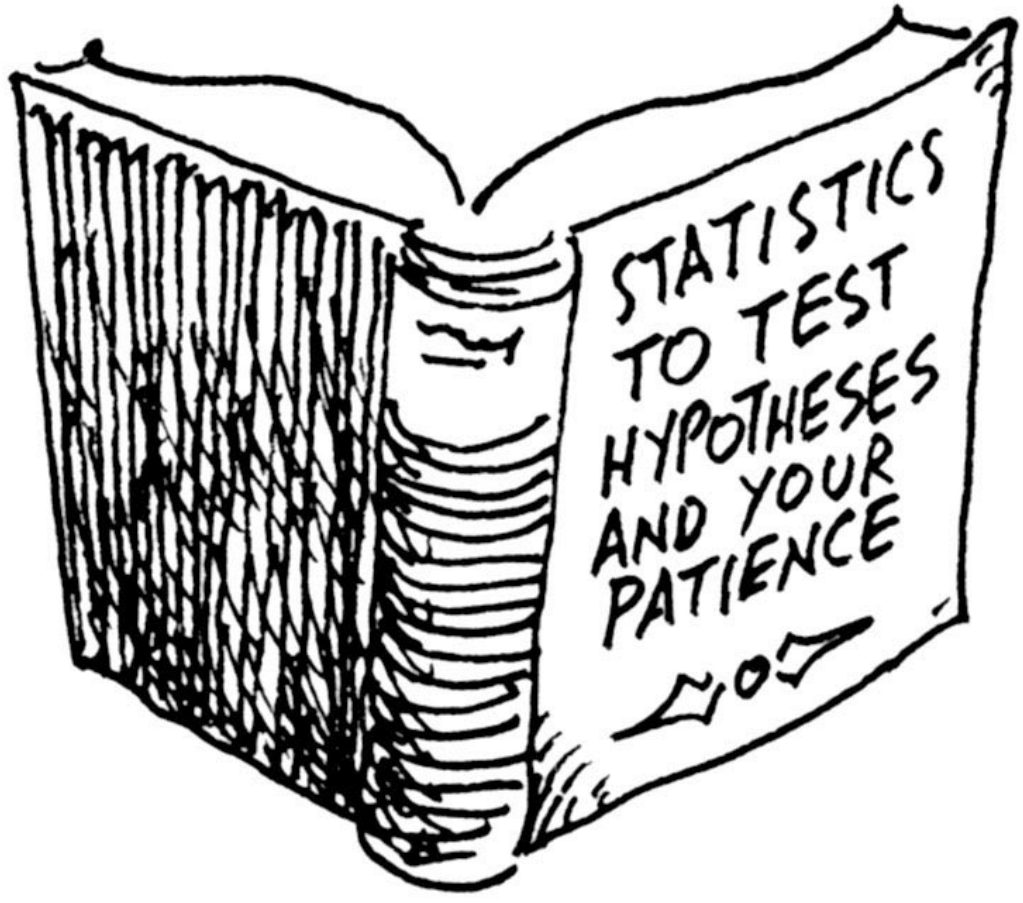
Null models

Standard solution: ask how much more likely M is than some *null hypothesis* (represented by a *null model* N):

$$\frac{\mathsf{P}\left(M \mid A\right)}{\mathsf{P}\left(N \mid A\right)} = \frac{\mathsf{P}\left(A \middle| M\right)}{\mathsf{P}\left(A \middle| N\right)} \frac{\mathsf{P}(M)}{\mathsf{P}(N)} .$$

posterior odds | likelihood ratio | prior odds

Test your hypothesis

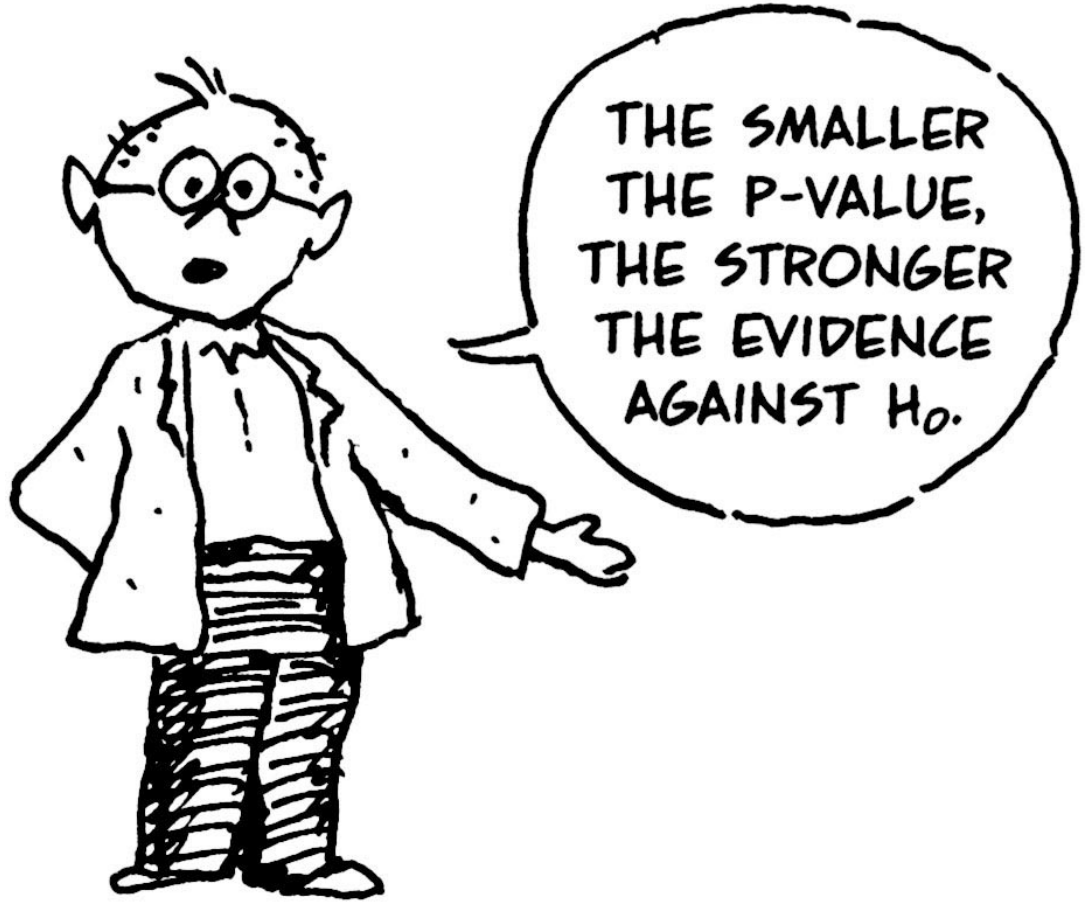


Thanks to Larry Gonick *The Cartoon Guide to Statistics*

Scoring (frequentist view)

- We believe in models when they give a large score to our observed data.
- Statistical tests (p-values or E-values) quantify how often we should expect to see such good scores “by chance”.
- These tests are based on a null model or null hypothesis.

Small p-value to reject null hypothesis



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Statistical Significance (2 approaches)

Markov's inequality For any scoring scheme that uses

$$\ln \frac{\mathsf{P}\left(\text{seq} \mid M\right)}{\mathsf{P}\left(\text{seq} \mid N\right)}$$

the probability of a score better than T is less than e^{-T} for sequences distributed according to N.

Parameter fitting For "random" sequences drawn from some distribution other than N, we can fit a parameterized family of distributions to scores from a random sample, then compute P and E values.

Null models

- P-values (and E-values) often tell us nothing about how good our hypothesis is.
- What they tell us is how bad our null model (null hypothesis) is at explaining the data.
- A badly chosen null model can make a very wrong hypothesis look good.

Example 1: long ORF

- A colleague found an ORF in an archæal genome that was 388 codons long and was wondering if it coded for a protein and what the protein's structure was.
- We know that short ORFs can appear "by chance".
- So how likely is this ORF to be a chance event?

Null Model 1

- DNA is undergoing no selection at all
- G+C content bias. (GC is 36.7%, AT is 63.3%.)
- Probability of stop codon
  TAG= 0.3165*0.3165*0.1835=0.0184, TGA=0.0184,
  TAA=0.0317, so p(STOP)=0.0685.
- P(388 codons without stop) = $(1 - p(\text{STOP}))^{388}$ = 1.1e-12
- E-value in a 3 Megabase genome is about 3.3e-6.
- We can easily reject the null hypothesis!

Null Model 2

- I forgot to tell you: this ORF is on the opposite strand of a known 560-codon ribosomal gene.
- What is the probability of this long an ORF, on opposite strand of known gene?
- Generative model: simulate random codons using the codon bias of the organism, take reverse complement, and see how often ORFs 388-long or longer appear.
- Taking 100,000 samples, we get estimates of P-value in the range 3e-05 to 6e-05.
- There are about 3000 genes, giving us an E-value of 0.09 to 0.18.

Null Model 3

- We can do the same sort of simulation, but restrict the codons to ones that would code for exactly the same protein on the forward strand.
- Now we get P-value of around 0.01 for long ORFs on the reverse strand of genes coding for this protein.

Protein or chance ORF?

Thanks to Larry Gonick *The Cartoon Guide to Statistics*

Not a protein

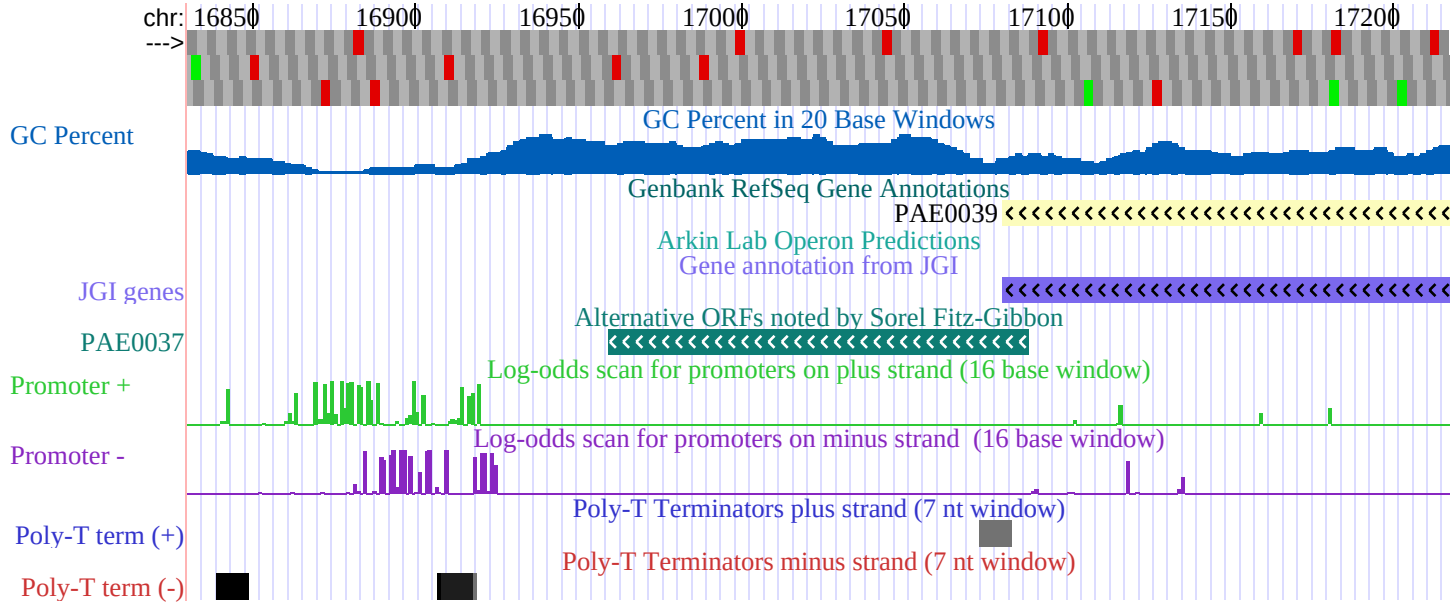
+ A tblastn search with the sequence revealed similar ORFs in many genomes.
– All are on opposite strand of homologs of same gene.
– “Homologs” found by tblastn often include stop codons.
– There is no evidence for a TATA box upstream of the ORF.
– No strong evidence for selection beyond that explained by known gene.

Conclusion: it is rather unlikely that this ORF encodes a protein.

Example 1b: another ORF

- pae0037: ORF, but probably not protein gene in

  Pyrobaculum aerophilum



- Promoter on wrong side of ORF.
- High GC content (need local, not global, null)
- Strong RNA secondary structure.

Example 2: contacts

- Is residue-residue contact prediction better than chance?
- Early predictors (1994) reported results that were 1.4 to 5.1 times “better than chance” on a sample of 11 proteins.
- But they used a uniform null model:

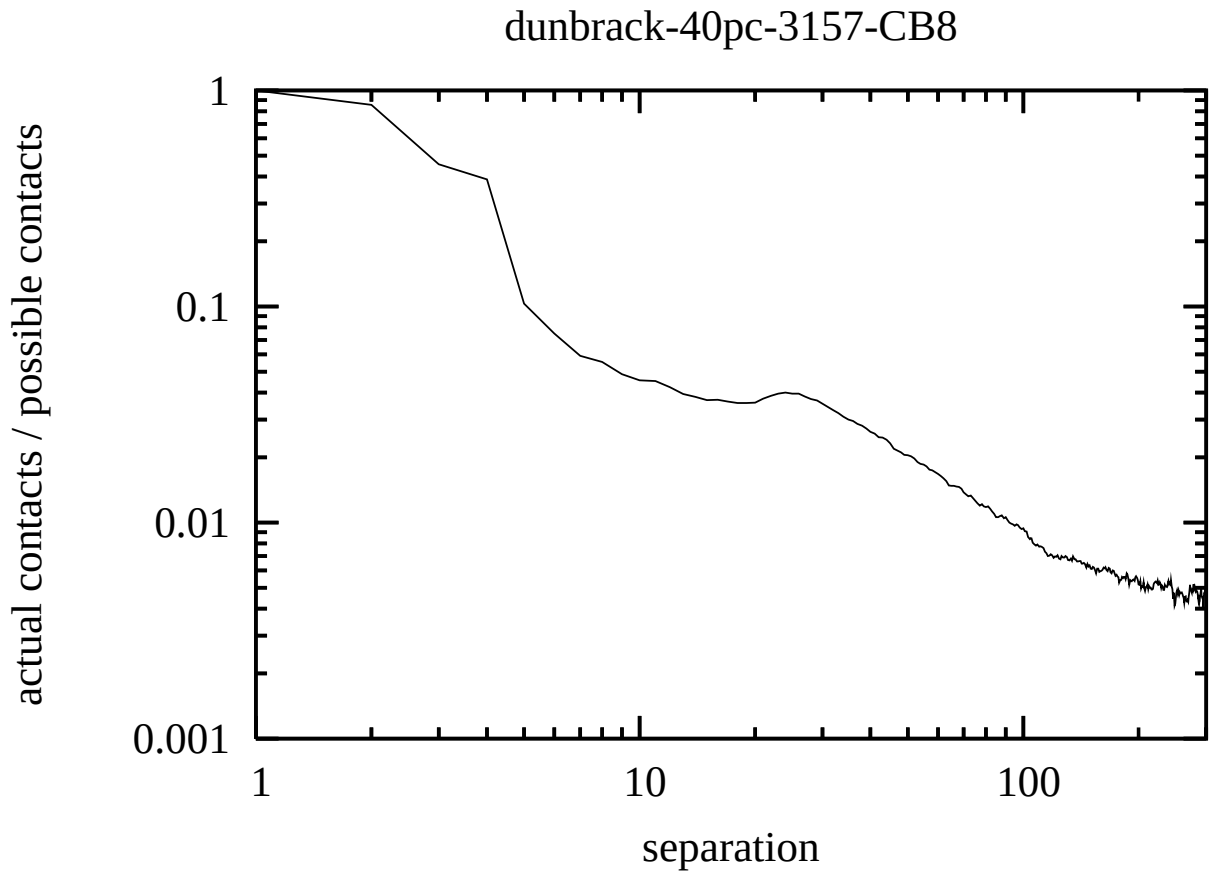
$$\mathrm{P}(\text{residue } i \text{ contacts residue } j) = \text{constant} \; .$$

- A better null model:

$$\mathrm{P}\left(\text{residue } i \text{ contacts residue } j\right) \;\; = \;\; \mathrm{P}\left(\text{contact} \;\middle|\; \text{separation} = |i-j|\right) \; .$$

P(contact|separation)

Using CASP definition of contact, CB within 8 Å, CA for GLY.



Can get accuracy of 100%

- By ignoring chain separations, the early predictors got what sounded like good accuracy (0.37–0.68 for L/5 predicted contacts)
- But just predicting that i and $i+1$ are in contact would have gotten accuracy of 1.0 for even more predictions.
- More recent work has excluded small-separation pairs, with different authors choosing different thresholds.
- CASP uses separation ≥ 6, ≥ 12, and ≥ 24, with most focus on ≥ 24.

Evaluating contact prediction

Two measures of contact prediction:

- Accuracy:

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

- Weighted accuracy:

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

$= 1$ if predictions no better than chance, independent of separations for predicted pairs.

Separation as predictor

If we predict all pairs with given separation as in contact, we do much better than uniform model.

sep	$P\left(\text{contact} \,\middle|\, \lvert i-j\rvert = \text{sep}\right)$	$P\left(\text{contact} \,\middle|\, \lvert i-j\rvert \geq \text{sep}\right)$	"better than chance"
6	0.0751	0.0147	4.96
9	0.0486	0.0142	3.42
12	0.0424	0.0136	3.13
24	0.0400	0.0116	3.46

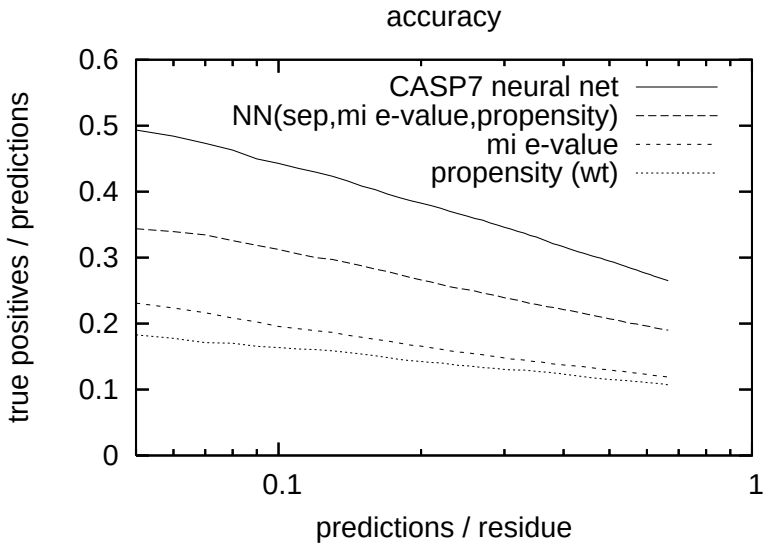
CASP7 Contact prediction

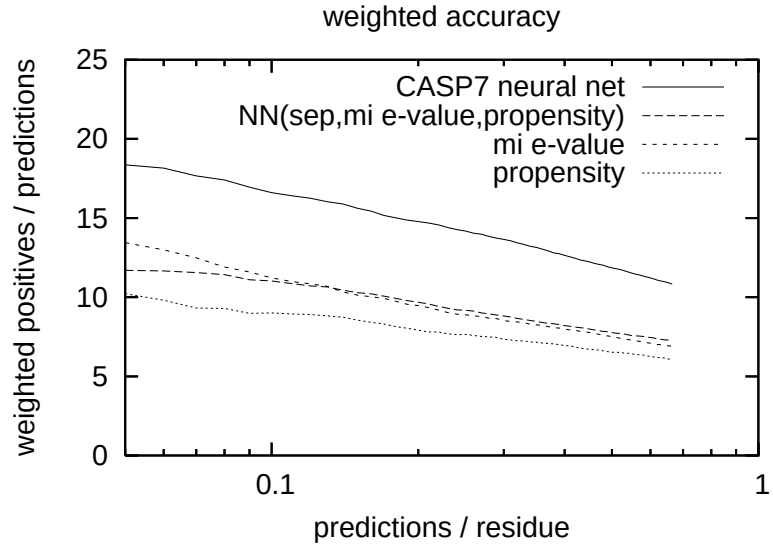
- Use mutual information between columns of thinned alignment ($\leq 50\%$ identity)
- Compute e-value for mutual info (correcting for small-sample effects).
- Compute rank of e-value within protein.
- Feed log(e-value), log(rank), contact potential, joint entropy, and separation along chain for pair, and amino-acid profile, predicted burial, and predicted secondary structure for window around each residue of pair into a neural net.

Now doing better

separation ≥ 9

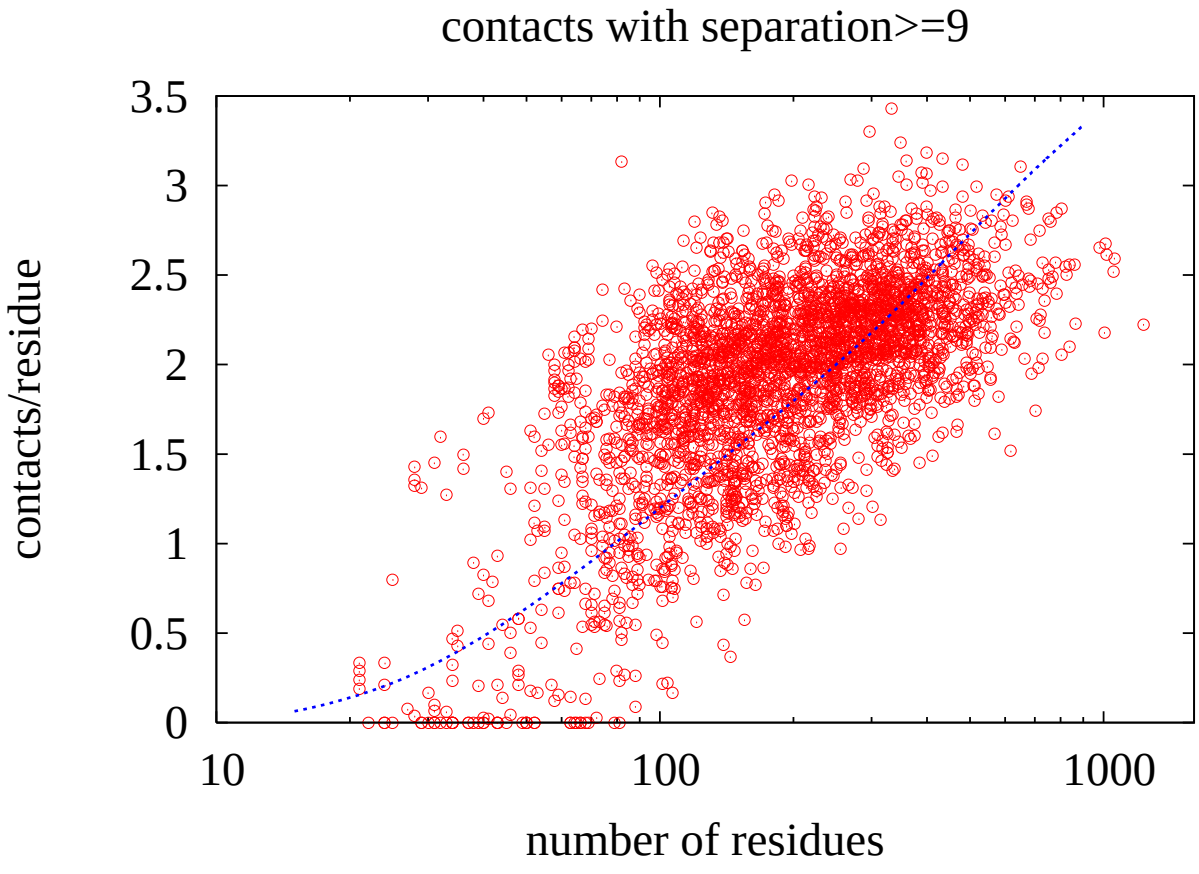
Predictions/residue taken separately for each protein.





Contacts per residue

We can also use our null model to predict the number of contacts per residue (which is not a constant).



Example 3: HMM

- Hidden Markov models assign a probability to each sequence in a protein family.
- A common task is to choose which of several protein families (represented by different HMMs) a protein belongs to.

Standard Null Model

- Null model is an i.i.d (independent, identically distributed) model.

$$\mathsf{P}\left(A \,\middle|\, N, \mathsf{len}\,(A)\right) = \prod_{i=1}^{\mathsf{len}(A)} \mathsf{P}(A_i)\ .$$

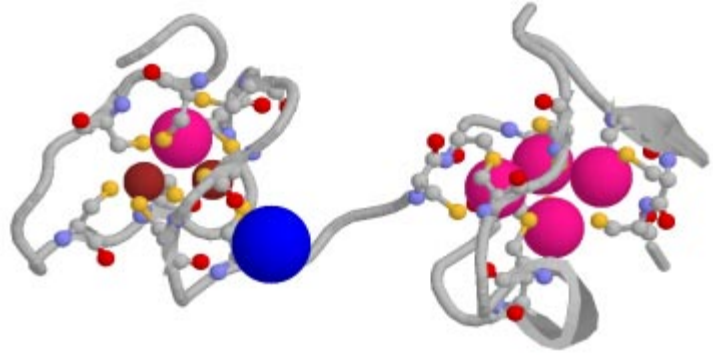
$$\mathsf{P}\left(A \,\middle|\, N\right) \;=\; \mathsf{P}(\text{sequence of length } \mathsf{len}\,(A)) \prod_{i=1}^{\mathsf{len}(A)} \mathsf{P}(A_i)\ .$$

Composition as source of error

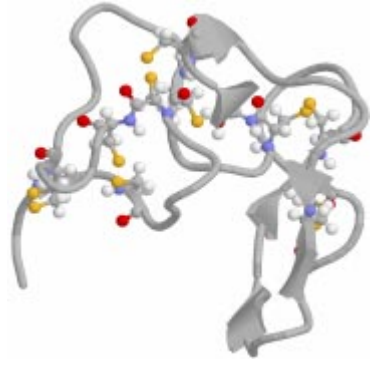
- When using the standard null model, certain sequences and HMMs have anomalous behavior. Many of the problems are due to unusual composition—a large number of some usually rare amino acid.
- For example, metallothionein, with 24 cysteines in only 61 total amino acids, scores well on any model with multiple highly conserved cysteines.

Composition examples

Metallothionein Isoform II (4mt2)

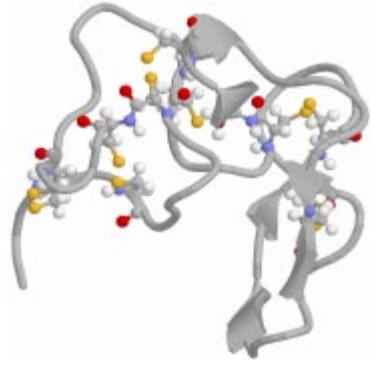


Kistrin (1kst)

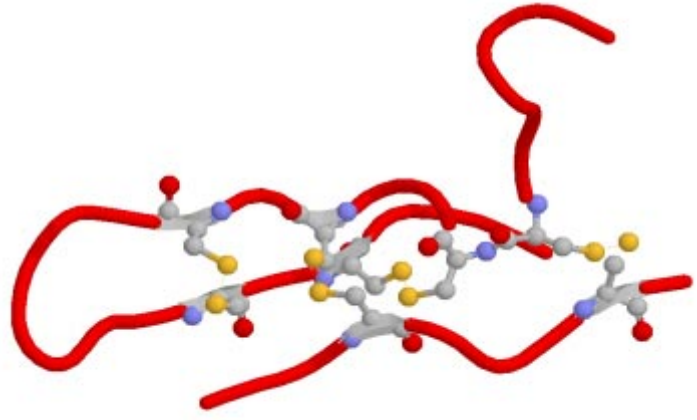


Composition examples

Kistrin (1kst)



Trypsin-binding domain of Bowman-Birk Inhibitor (1tabI)



Reversed model for null

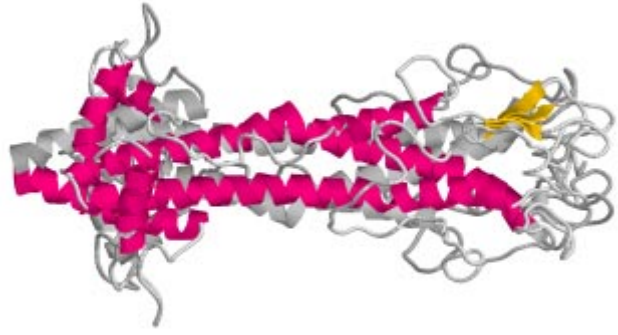
- We avoid this (and several other problems) by using a reversed model M^r as the null model.
- The probability of a sequence in M^r is exactly the same as the probability of the reversal of the sequence given M.
- This method corrects for composition biases, length biases, and several subtler biases.

Helix examples

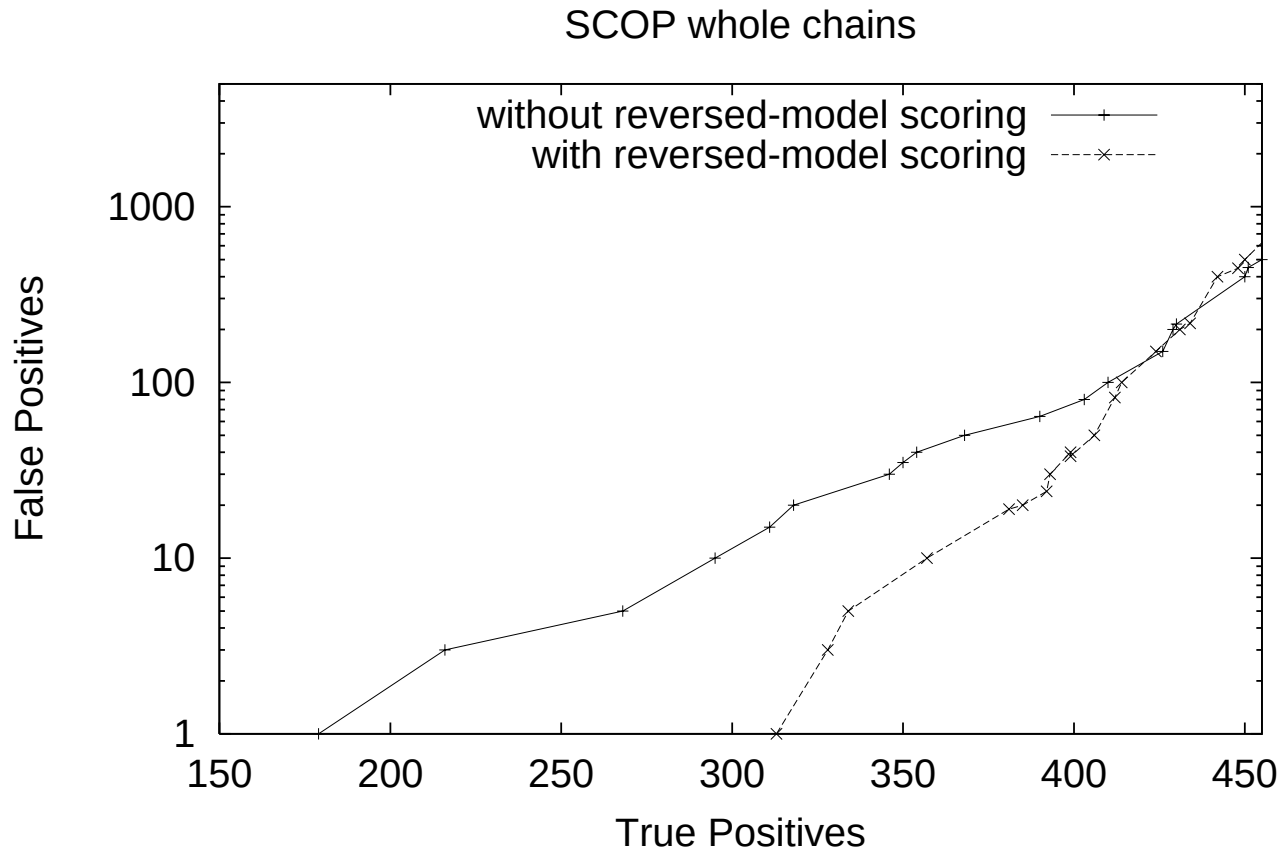
Tropomyosin (2tmaA)

Colicin Ia (1cii)

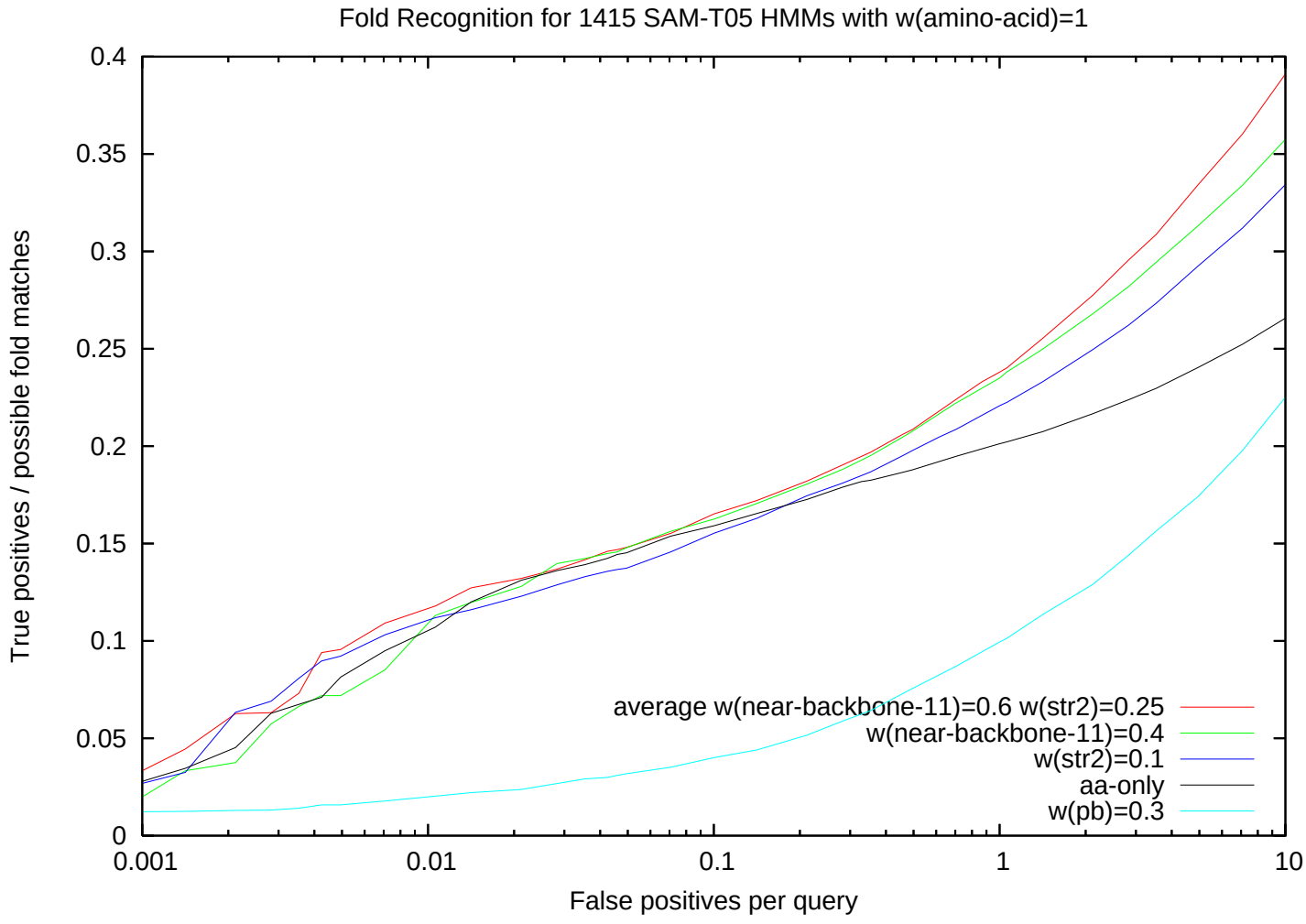
Flavodoxin mutant (1vsgA)



Improvement from reversed model



Fold recognition results



Take-home messages

- Base your null models on biologically meaningful null hypotheses, not just computationally convenient math.
- Generative models and simulation can be useful for more complicated models.
- Picking the right model remains more art than science.

Web sites

List of my papers: `http://www.soe.ucsc.edu/~karplus/papers/`

These slides: `http://www.soe.ucsc.edu/~karplus/papers/better-than-chance-sep-07.pdf`

Reverse-sequence null: Calibrating E-values for hidden Markov models with reverse-sequence null models. *Bioinformatics*, 2005. 21(22):4107–4115; `doi:10.1093/bioinformatics/bti629`

Archæal genome browser: `http://archaea.ucsc.edu`

UCSC bioinformatics (research and degree programs) info:

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