Bioinformatics Methods

http://users.soe.ucsc.edu/~karplus/papers/

Ravenna-methods-sep-2011.pdf

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Outline of Talk

- What is Biomolecular Engineering? Bioinformatics?
- Pattern Recognition in Bioinformatics
- Protein Structure Prediction and Protein Design
- Genome Assembly

What is Biomolecular Engineering?

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

with: using proteins (or DNA, RNA, . . .) 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.

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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 and RNA-seq: what genes are turned on under what conditions.
- Proteomics: what proteins are present in a mixture.
- Protein structure prediction.
- …

Classification and Parsing Problems

Many problems in bioinformatics can be viewed as classification or parsing or segmentation problems:

- Diagnosis: What type of cancer does a particular DNA or RNA come from? What viruses are infecting a patient?
- Prognosis: What are the chances of 5-year survival for a cancer patient? What drug treatments will be most effective?
- Which bases in a genome are part of non-coding RNA genes? promoters? 5’ UTR? exons? introns? 3’ UTRs?
- Which residues in a protein are parts of α-helices? β-sheets? turns? other local structures?
- What family does a protein belong to?
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Data is noisy

Biological data from wet-lab work is usually very noisy—sequence data from a sequencing machine has a 1–15% error rate.

- The error rate depends on the sequencing technology, the position in the fragment being sequenced, the type of base, the location on the sequencing chip, who prepared the DNA library, when the machine was last serviced, …
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Systematic error

Errors are not white noise, but often systematic biases that stem from unknown causes.

- Shotgun sequencing assumes that DNA is randomly fragmented and that all positions for the DNA are equally likely, but sequencer output shows a much wider range of coverage than that model implies.
- Some DNA overrepresented (perhaps adjacent to fragile points in DNA).
- Some DNA underrepresented or missing entirely (perhaps not amplified in PCR steps).

Biased sampling

- Biologists often study problems that interest the community, so there may be a huge amount of data for one organism or protein, and little or none for a closely related one.
- Training data may all come from one, non-representative example.
- Of 15 million protein sequences in the “non-redundant” database, 490,000 (3%) are from HIV virus strains, though there are only a dozen HIV proteins.
- Training and testing sets often need to be “thinned” to remove too-similar examples.
- Failure to thin, resulting in unrealistically good performance estimates, it the most common newbie mistake.

Too Much Data/Too Little Data

- We often have so much data that we can't fit it all in the computer at once. (A genome assembly may start from 250E9 bases of sequence data.)
- Labeled data is scarce and expensive—determining correct classification often requires time-consuming, expensive, and error-prone wet-lab work.
- Lots of unlabeled data, only a little labeled data, and labeled data has a high rate of mislabeling.
- It is common to do a combination of supervised and unsupervised learning, to try to get value from both the labeled and the unlabeled data.

Outline for proteins

- What is a protein?
- The folding problem and variants on it:
  - Local structure prediction
  - Fold recognition
  - Secondary structure prediction
  - Hidden Markov Models
  - Contact prediction
  - CASP experiment

What is a protein?

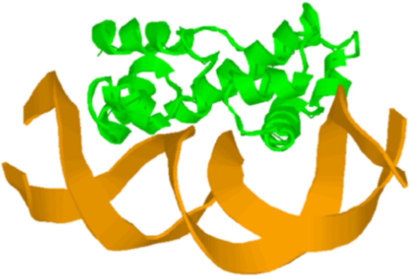
- There are many abstractions of a protein: a band on a gel, a string of letters, a mass spectrum, a set of 3D coordinates of atoms, a point in an interaction graph, … .
- For us, a protein is a long skinny molecule (like a string of letter beads) that folds up consistently into a particular intricate 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.

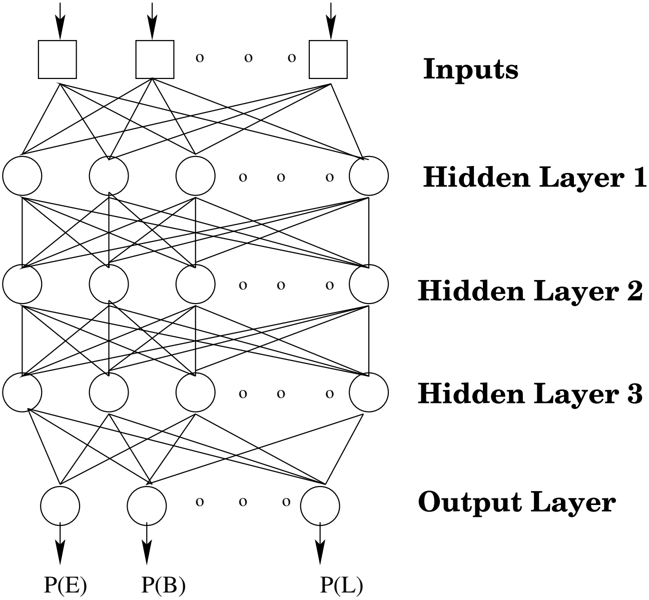
New-fold prediction

- 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.
- One popular choice is a 3-valued helix/strand/other alphabet. 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. (Random forests also doing well.)
- Using Conditional Random Fields can improve sampling of sequences, without improving accuracy on individual residues.

Neural Net Structure



Inputs

Hidden Layer 1

Hidden Layer 2

Hidden Layer 3

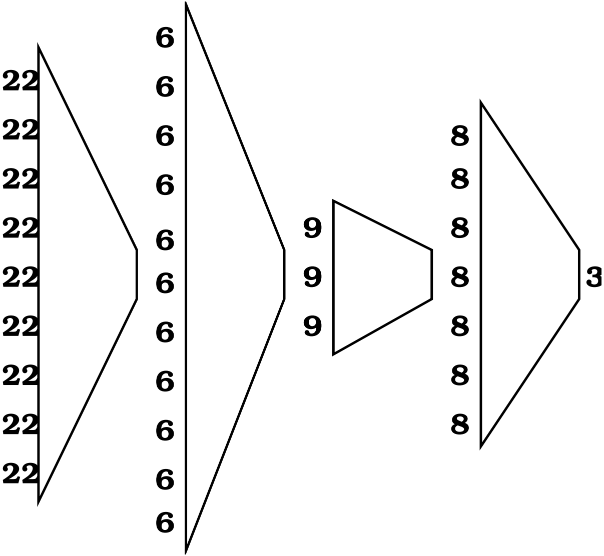
Output Layer

P(E)

P(B)

P(L)

Neural Net Windowing



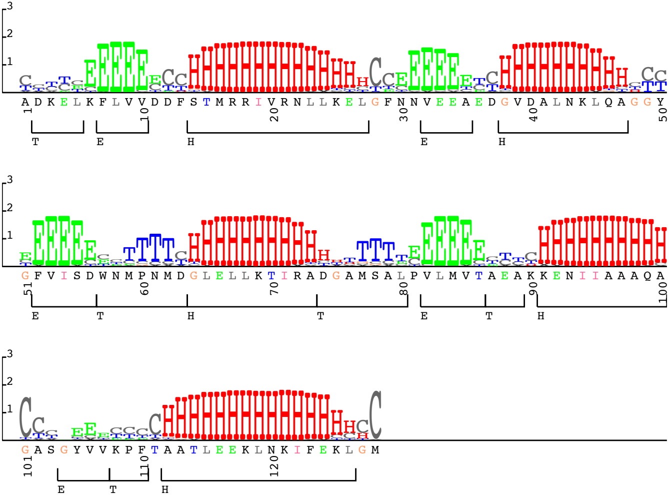
Local Structure Alphabets

- What local properties do we choose?
- We want properties that are well-conserved through evolution, easily predicted, and useful for finding and aligning templates.
- We have investigated many alphabets.
- Current favorites are str2, a 13-state secondary-structure alphabet that distinguishes between different β strands, and near-backbone-11, an 11-state burial alphabet.

Sequence logos (NN)

Summarize local structure prediction:

nostruct-align/3chy.t2k EBGHTL



Fold recognition

- Do iterative search to find similar sequences in databases of other proteins
- Use multiple sequence alignment to do local structure prediction.
- Build HMM that has multiple tracks (amino-acid and local structure alphabets).
- Search PDB using final HMM.

Fold recognition

- Do iterative search to find similar sequences in databases of other proteins:
  - Make a Hidden Markov Model from sequence or alignment.
  - Use HMM to search for similar sequences.
  - Retrain HMM on new set (or representative subset).
  - Align sequences using HMM.
  - Repeat.
- Use multiple sequence alignment to do local structure prediction.
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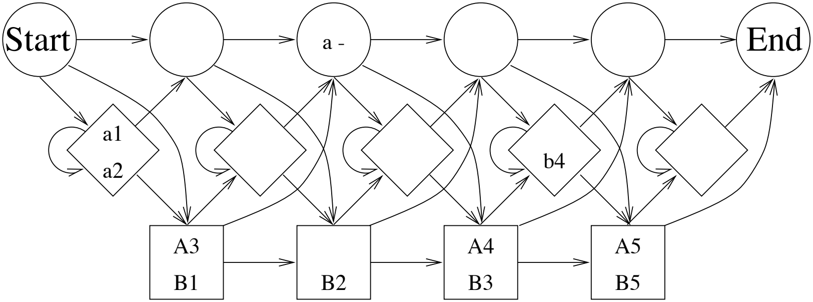
- Use multiple sequence alignment to do local structure prediction.
- Build HMM that has multiple tracks (amino-acid and local structure alphabets).
- Search PDB using final HMM.
  - Look for similar sequences in database of solved protein structures.
  - Use multi-track HMM to align target to solved structures.

Profile HMM

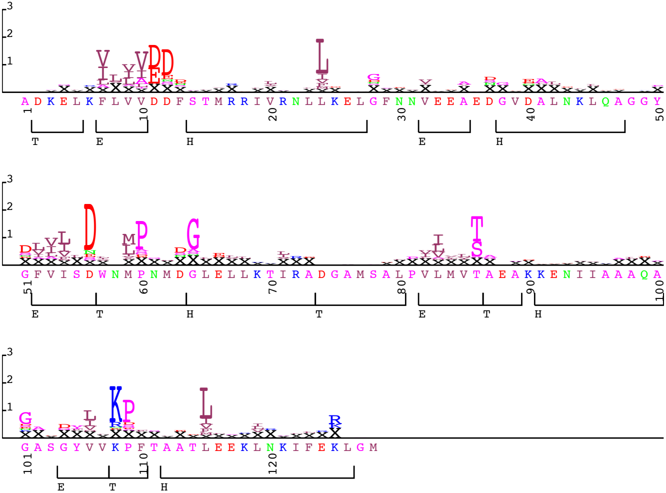


```
a1 a2 A3    -    A4  .  A5
 .  . B1   B2    B3 b4  B5
```

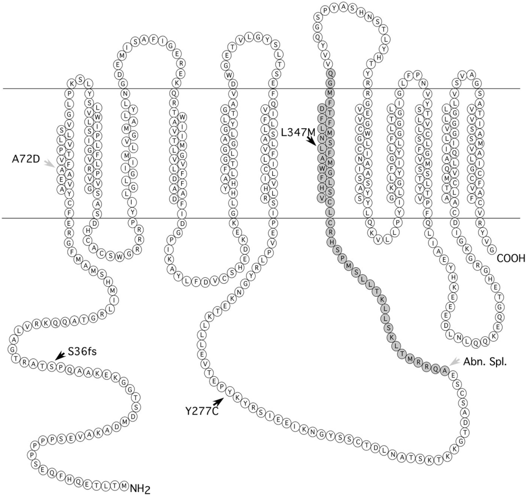
Sequence logos (MSA)

Summarize multiple alignment:

nostruct-align/3chy.t2k w0.5



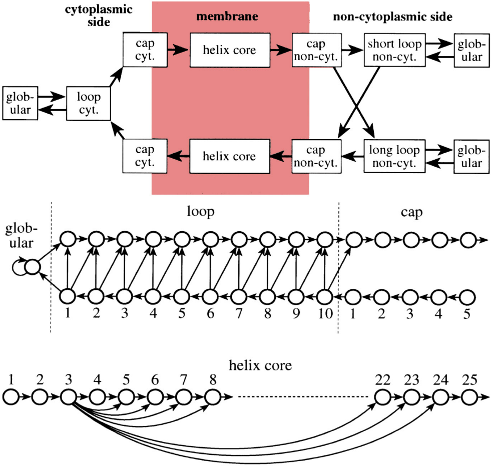
Transmembrane Helices



`http://www.genetics.org/content/175/2/867/F3.large.jpg`

Gunnarsson et al. *Genetics* February 2007 vol. 175 no. 2 867-877 doi: 10.1534/genetics.106.063107

TMHMM (a non-profile HMM)



Anders Krogh, Bjön Larsson, Gunnar von Heijne, and Erik L.L Sonnhammer. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *Journal of Molecular Biology* Volume 305, Issue 3, 19 January 2001, Pages 567–580 doi:10.1006/jmbi.2000.4315

Contact prediction

- Predict that residues separated along the chain are close in 3-space.
- 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(e-value) within protein.
- Feed log(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.

Full 3D modeling

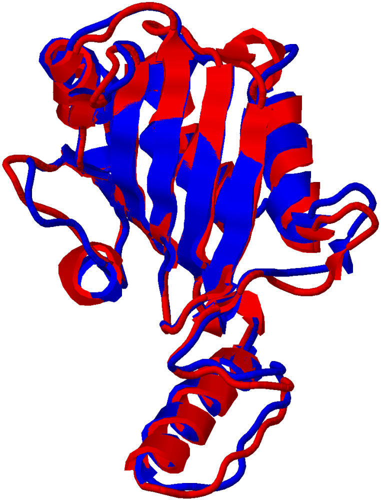
- Copy backbone atoms from aligned PDB file
- Copy fragments from shorter alignments to other PDB files.
- Combine randomly.
- Stochastic search to optimize “energy” function, which may include constraints from alignments, predicted contacts, local structure prediction, ...

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.*

T0298 domain 2 (130–315)

RMSD= 2.468Å all-atom, 1.7567Å C_α, GDT=82.5%
best model 1 submitted to CASP7 (red=real)



Computational Protein Design

- Train neural nets to take local-structure inputs and provide amino-acid outputs.
- Use RosettaDesign to design sequences, constrained by neural net outputs.
- Target applications: specific binding of carbon nanotubes, mimics for AGRP (agouti-related protein) binding to different melanocortin receptor.

Outline of genome assembly

- What is a genome?
- What sequencing technologies are currently used?
- The assembly problem
- Algorithms for assembly

What is a genome?

- Complete sequence of all DNA in a cell (exceptions for plasmids, viruses, organelles).
- Varies from cell to cell, so we usually approximate to get a “typical” genome.
- Usually want an *annotated genome* which has genes and other features labeled and indexed.

Current sequencing technologies

- Sequencing by size sorting
- Sequencing by ligation
- Sequencing by replication
- Single-molecule sequencing

Sequencing by size sorting

- Need pure sample: many copies of one DNA molecule.
- Generate “prefixes” of DNA, with known last base.
  - Maxam-Gilbert sequencing (obsolete): cuts DNA at specific base.
  - Sanger sequencing: copies DNA stopping at specific base.
  - Hood variant: copies DNA using fluorescent label for last base.
- Measure lengths of prefixes by electrophoresis.
- About $1.50/read, 800–1200 bases/read
- Error rate about 0.05% (1 in 2000)

Sequencing by ligation

- Only 1 platform (SOLiD)
- Shreds DNA, then does emulsion PCR to get beads with pure DNA fragments.
- Ligates small stretch of DNA to template.
- Unusual “color-space” reads. Color encodes 2 bases, but only 4 colors:

0 (blue):	AA, GG, CC, TT
1 (green):	AC, GT, CA, TG
2 (yellow):	AG, GA, CT, TC
3 (red):	AT, GC, CG, TA

- Takes a week to process a sample
- Get about 200–300 million 50-base reads.
- Error rate about 1.6%

Sequencing by replication

- Bases added one at a time, with detector to tell whether a base is added (or which base is added).
- Pyrosequencing (454)
- Illumina/Solexa (Genome Analyzer)
- Ion Torrent

Pyrosequencing (454 machine)

- After shearing and size-selecting DNA, attach to beads.
- Do emulsion-PCR to get a polony on each bead.
- Put beads into one-bead wells in picotiter plate.
- Do polymerization with one base type at a time.
- Use light emission to determine how many copies of base are added to end of chains.
- 1,000,000 reads, 500–1000 bases/read
- about $3k for a run
- Error rate about 0.9%
- When several bases in a row are identical, determining exactly how many bases of that type were present can be difficult. (homopolymer errors)

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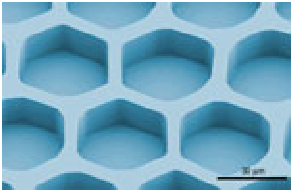

Nature Biotechnology 21, 1425–1427 (2003)

doi:10.1038/nbt1203-1425

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Illumina/Solexa

- Polonies grown as spots on a slide rather than separate beads.
- One base at a time reading, all 4 bases read at once (different color fluorophores).
- ≈ 5 billion 2×100-long paired-end reads.
- Error rate about 1.5%

Ion Torrent

- small, cheap machine (about $50,000)
- Electronic readout, no fluorescent molecules, no optics
- medium throughput, fast, low cost per run
- same homopolymer problems as 454 technology
- reads under 100 long

Single-molecule sequencing

- Several new technologies that don't require amplifying DNA:
  - Pacific Bioscience (SMRT)
  - Helicos Bioscience (Helicos)
  - nanopores
- All have super high error rates (10–20%).
- Same molecule must be read repeatedly to get useful data.
- PacBio occasionally gets very long reads, but various tricks are needed, making data analysis difficult.

Characteristics of data

platform	reads/run	read length	error rate	cost per base
Sanger	1–384	500–1200	very low	very high
454	1e6	500–1000	low	medium
Illumina	4e9	2 × 100	high	low
SOLiD	300e6	50	high	low

Different data representations

- base space
- flow space (454, Ion Torrent)
- color space
- Each sequencer and each program uses different data formats and different quality information.

The assembly problem

- Jigsaw puzzle with millions of pieces that overlap.
- Need much more DNA sequence than target genome (generally 15–100×)
- Want to end up with single sequence for each chromosome

Problems

- Sequence data is noisy.
- Repeats can have identical sequences in different parts of genome.
- DNA sample may have variations within sample.
- Data is huge (larger than computer memory).

Algorithms for assembly

- Overlap-consensus graph (needs long reads)
- de Bruijn graph (has trouble with high error rates and long reads)

Overlap consensus

- Each node is a single read. Edges represent overlaps between the end of one read and the beginning of another.
- Clusters of connected nodes can be used to build consensus contigs.
- Overlap must be large enough to be unique location in genome, or chimeric contigs can get built.
- Finding overlaps is expensive part.
- Clusters have to be broken where continuation of contig is ambiguous, so repeats tend to be represented by single consensus contig.
- Best method for 454 and Sanger data.

de Bruijn graph

- Each node is a k-mer. Edges connect window $[i, i+k)$ to window $[i+1, i+k+1)$ of read, and have counts of occurrence.
- Each read becomes a path in the graph.
- Contigs build from strongly supported paths.
- Errors create "bubbles" and "dead-ends" that need to be merged into main paths.
- No need to find overlaps, but graphs get huge.

Web sites

These slides:

`http://users.soe.ucsc.edu/~karplus/papers/Ravenna-methods-sep-2011.pdf`

UCSC bioinformatics info:

`http://www.bme.ucsc.edu/`

SAM-T08 prediction server: `http://compbio.soe.ucsc.edu/SAM_T08/T08-query.html`

CASP2 through CASP8—all our results and working notes:

`http://users.soe.ucsc.edu/~karplus/casp2/`

...

`http://users.soe.ucsc.edu/~karplus/casp8/`

Banana Slug Genomics wiki:

`http://banana-slug.soe.ucsc.edu/`