Tau protein

Fibril formation associated with various “tau-opathies” (Alzheimer’s, Parkinson’s)
Strategy for intervention
Introduction to protein design

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How can we find an inhibitory peptide??

• “Manually” (ie. look at the structure)
• Directed evolution
• Computational methods
Directed evolution

- Amplify
- Mutagenize
- Sub-clone
- Transform
- Screening
- Selection
- Surviving clones
- High performing clones
- Isolate mutant genes
- Gene of interest
- Vector and gene
- Bacterial host cell
Computational protein design

- Protein sidechains exhibit strong conformational preferences: “rotamers”

- Given some backbone coordinates, find the rotamers which are most compatible with this backbone
Protein design protocol

• Optimization:
  • At each residue to be designed, build a set of allowed rotamers
  • Search through this set of rotamers
  • At each step, use a physics-based energy function to evaluate the “fitness” of the currently selected set of rotamers

• Take the sequence from the structure with the best energy!
What’s the “best” structure?

• The energy function will dictate how “good” we think a given conformation is - and hence which sequence we choose!

• Philosophy: if we capture the physical forces which makes known protein structures work, ours will work too
Energy function

• What physical forces should our energy function capture?
  • Packing
  • Hydrogen bonding
  • Electrostatics
  • Solvation
  • Conformational preferences (helix propensities, etc.)
Searching through rotamers

- Dead-end elimination
  - Desmet et al., Nature (1992)
  - Branch-and-bound
  - Solutions (combinations of rotamers) are clustered, and whole clusters are discarded if not optimal
  - Clusters of good solutions are further subdivided
  - Analogous to dynamic programming, in which “good” solutions are explored further
  - Pro: guaranteed to find global minimum
  - Con: difficult to formulate problem, requires strict pairwise additivity
Searching through rotamers

• Monte Carlo search
  • Now common, has broadly replaced DEE
  • Typically implemented with simulated annealing program
  • Stochastic - *not guaranteed to find global minimum* (is this a problem?)
  • Pro: easy implementation, extensible to other problems (e.g. multistate design)
Application to a protein-protein interface

Prediction: the resulting proteins will bind more tightly than the starting proteins!
Stabilizing proteins

Dahiyat and Mayo, PNAS (1997)
“De novo” design of a complete protein

Zif268
(a naturally-occurring zinc finger domain)

FSD-1
.designed using the Zif268 scaffold, with no zinc!)}
“De novo” design of a complete protein

Dahiyat and Mayo, Science (1997)
Searching in sequence space

- 28 residues, 20 amino acids for each

- \(20^{28} = 2.7 \times 10^{36}\) possibilities

- If they had evaluated 1000 sequences per second, it would have taken \(8.5 \times 10^{25}\) years
The energy landscape

Rugged energy landscape

Flat energy landscape

Smooth energy landscape

THIS IS WHAT’S OBSERVED
Flexible backbone design: Top7

Key development: iterate between (fixed backbone) design and (fixed sequence) structure-prediction
A novel fold - Top7

Kuhlman et al.,
A novel fold - Top7

• This fold hasn’t yet been observed in Nature.

• **Hypothesis:** there’s nothing special about it, we just haven’t seen it yet!

• **Future:** build scaffolds as needed for other design projects (with great accuracy!)
Back to our case study....
Tau protein

Fibril formation associated with various “tau-opathies” (Alzheimer’s, Parkinson’s)
Strategy for intervention
D-peptides

- Not degraded
- Non-immunogenic

Hydrogen bonding pattern is preserved, but all sidechain interactions must be redesigned
Assaying fibril formation
Assaying fibril formation

Tau alone

Tau + designed peptide
16 years of protein design

- **Then:** improved packing in small regions of proteins

- **Now:** novel binding reagents, enzymes, protein-protein interfaces, allosteric switches, DNA recognition sites

- **Future:** applications abound!