Protein Folding - seeing is deceiving

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Protein Folding - seeing is deceiving

Covido ergo Zoom

- with apologies to Descartes

Protein Folding - seeing is deceiving

Recent paper

Protein Science (2021) 30: 1606-1616

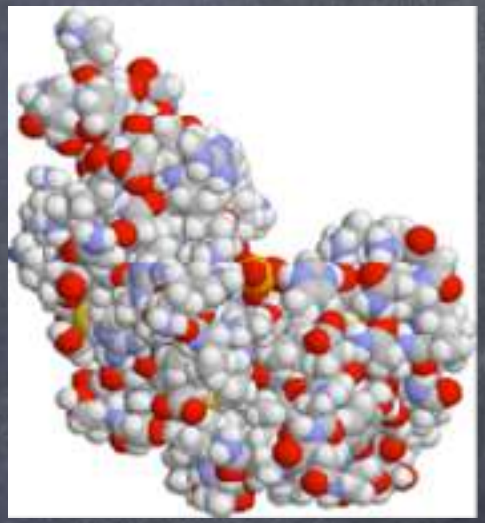
and further thoughts from

Biochemistry, "Protein folding from a physical-chemical perspective: entropy as organizer", invited Perspective, in preparation.

Protein Folding Problem

Ribonuclease A

LYS GLU THR ALA ALA ALA LYS PHE GLU ARG
GLN HIS MET ASP SER SER THR SER ALA ALA
SER SER SER ASN TYR CYS ASN GLN MET MET
LYS SER ARG ASN LEU THR LYS ASP ARG CYS
LYS PRO VAL ASN THR PHE VAL HIS GLU SER
LEU ALA ASP VAL GLN ALA VAL CYS SER GLN
LYS ASN VAL ALA CYS LYS ASN GLY GLN THR
ASN CYS TYR GLN SER TYR SER THR MET SER
ILE THR ASP CYS ARG GLU THR GLY SER SER
LYS TYR PRO ASN CYS ALA TYR LYS THR THR
GLN ALA ASN LYS HIS ILE ILE VAL ALA CYS
GLU GLY ASN PRO TYR VAL PRO VAL HIS PHE
ASP ALA SER VAL



Definition of the problem for this talk:

Predict conformation from sequence.

Protein Folding Problem

The stunning success of deep-learning artificial intelligence (AI) approaches has transformed the field.

Jumper, J., Evans, R., Pritzel, A., et al. (2021) Highly accurate protein structure prediction with AlphaFold, Nature 596, 583-589.

Tunyasuvunakool, K., Adler, J., Wu, Z., et al. (2021) Highly accurate protein structure prediction for the human proteome, Nature 596, 590-596.

Baek, M., DiMaio, F., Anishchenko, I., et al. (2021) Accurate prediction of protein structures and interactions using a three-track neural network, Science 373, 871-876.

Progress in Science

observation → pattern recognition → theory/models

Where are we in this progression?

observation: 50th year of the protein data bank (PDB)

pattern recognition: deep learning AI

theory/models: ??

Protein Folding Problem

observation → pattern recognition → theory/models

In imperfect analogy, protein structure prediction using AI is akin to Mendeleev's compilation of the periodic table of the elements prior to its eventual derivation from quantum mechanics (e.g., molecular orbital theory).

Outline of this seminar

The current paradigm
Excluding interactions
Entropy as organizer

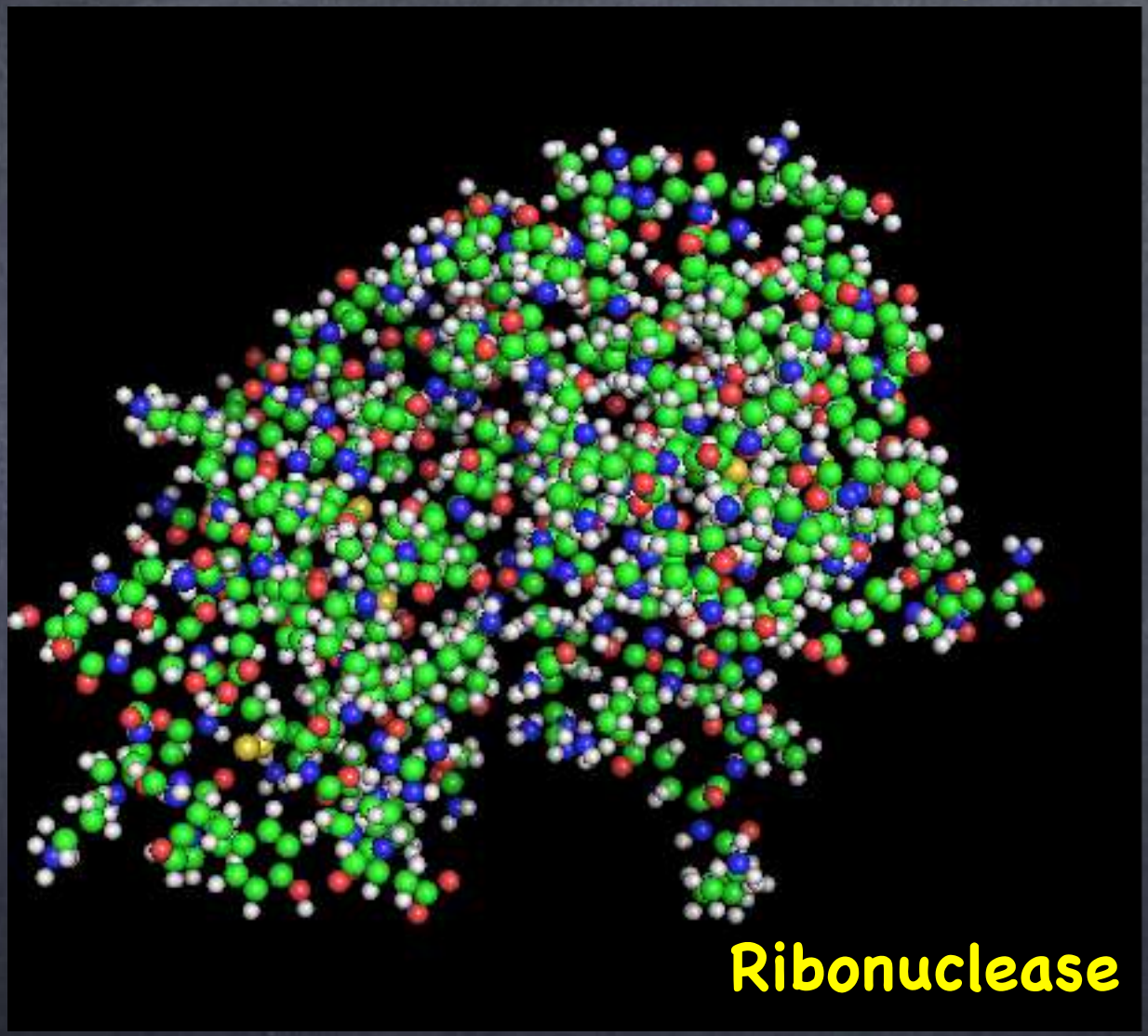
Framing the problem

Friedrich Nietzsche: what we see depends on the perspective from which we look.

Tony Schwartz (my dentist): if you're not looking for it, you don't see it.

In both politics and science, what we see depends on the perspective from which we look.

The current paradigm



Intuitively, what determines this conformation?

The current paradigm

Conditioning expectations about protein folding: many favorable interactions

The Anfinsen Hypothesis

What am I thinking?*

All backbones are the same. Side chains discriminate. The most energetically favorable constellation of interactions between and among the side chains corresponds to the native conformation.

U(nfolded) ⇌ N(ative)

*But, of course it doesn't matter: this is thermodynamics.

The current paradigm

The Anfinsen Hypothesis

$$U \rightleftharpoons N$$

Native state ≡ minimum free-energy conformer

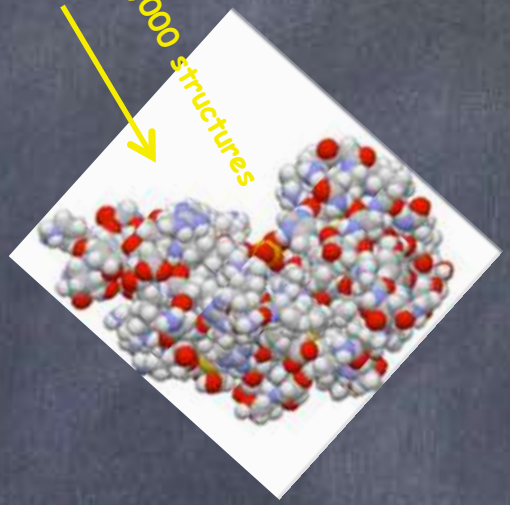
All backbones are the same - side chains discriminate

Haber & Anfinsen (1961) J Biol Chem 236:422-424

The current paradiam (sort of)

$$U \rightleftharpoons N$$

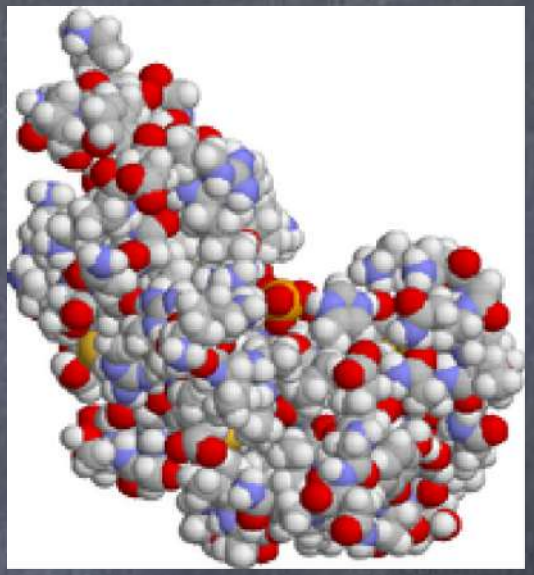
featureless
random coil



- Native state ≡ minimum free-energy conformer
- Unfolded state is a featureless landscape
- All backbones are the same - side chains discriminate
- Organizing interactions are visible in N

Current Paradigm - what the mechanism?

The "what you see is what you get" view.



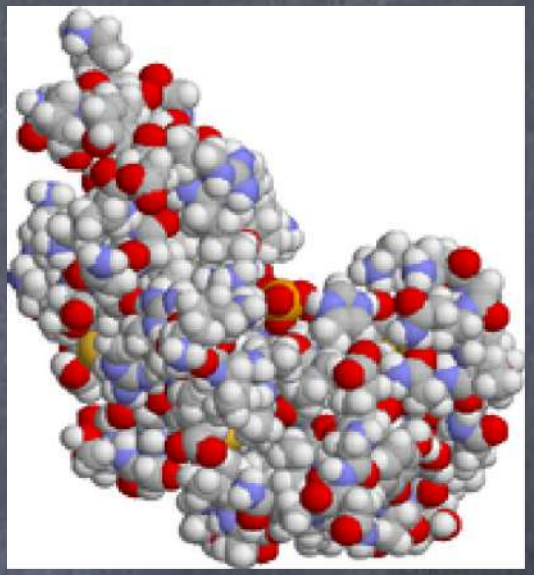
Force field minimization

$$\text{Protein} = \frac{A}{r^{12}} - \frac{B}{r^{6}} - \frac{\sum q_i q_j}{\varepsilon r_{ij}} - \text{H-bonds-torsions-dipoles ...}$$

Also: knowledge-based potentials, contact energies, Go models, lattice models ...

All are attractive (i.e. stabilizing) interactions

Current Paradigm - what's the mechanism?



The "what you see is what you get" view.

Force field minimization

$$\mathrm{Protein} = \frac{A}{r^{12}} - \frac{B}{r^{6}} - \frac{\sum q_i q_j}{\varepsilon r_{ij}} - \text{H-bonds-torsions-dipoles ...}$$

Also: knowledge-based potentials, contact energies, Go models, lattice models ...

but seeing is deceiving

Seeing is deceiving

Seeing is deceiving

Tim Noble and Sue Webster

Now for something completely different

Re-framing the question

Disfavored interactions

~~The what you see is what you get view, i.e. Organizing interactions are visible in N~~

Proposing instead that substantial organization results from elimination of unfavorable interactions - excluding interactions.

But first, what's an excluding interaction?

What's an Excluding interaction

Driving forces are attractive interactions. Excluding forces are disfavored interactions. They exclude high-energy interactions, reducing entropy loss on folding.

Knowledge-based potentials, contact energies, Gō models, lattice models ... all based on attractive interactions.

Two primary excluding forces:
(i) sterics
(ii) hydrogen-bond satisfaction

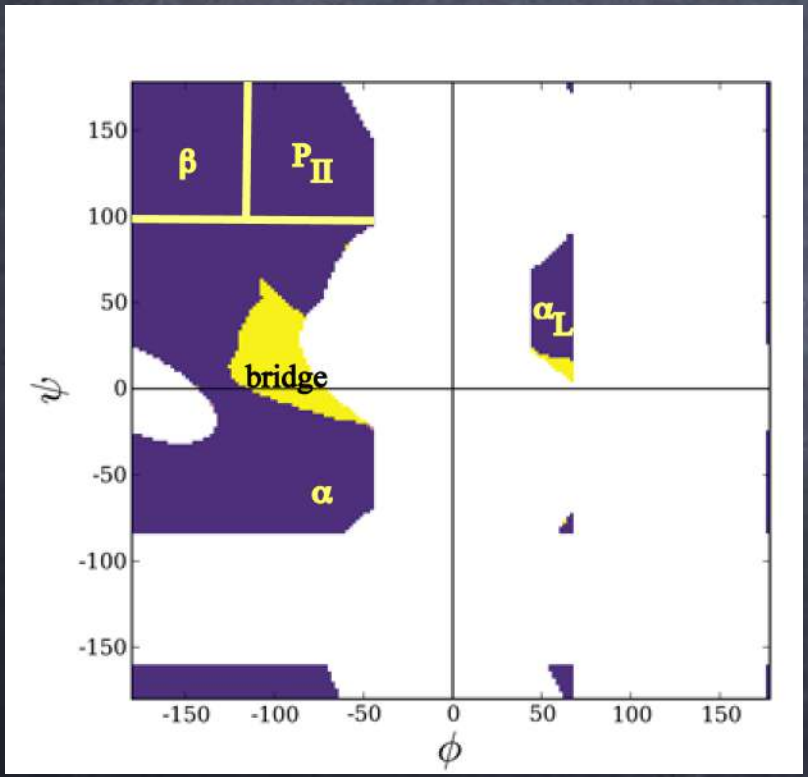
Excluding interactions are not visible in the X-ray structure

Excluding interactions

Condition expectations with a toy model

Excluding interactions are not visible in the X-ray structures

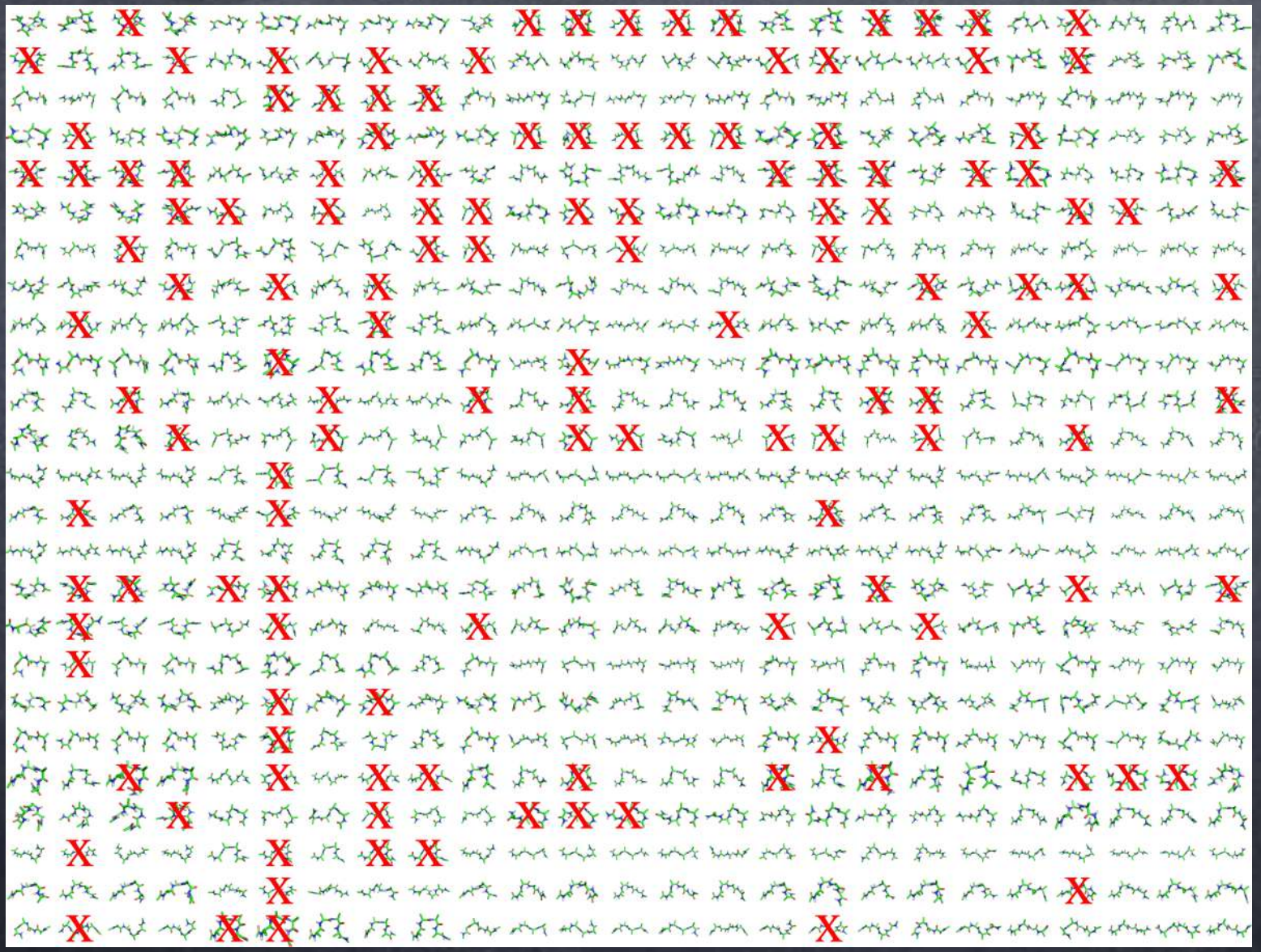
Exhaustive menu of Ala tetramers with a 5-state model



$5^4 = 625$ possible distinct conformers

Excluding interactions are not visible in the X-ray structure

Excluding interactions are not visible in the X-ray structure



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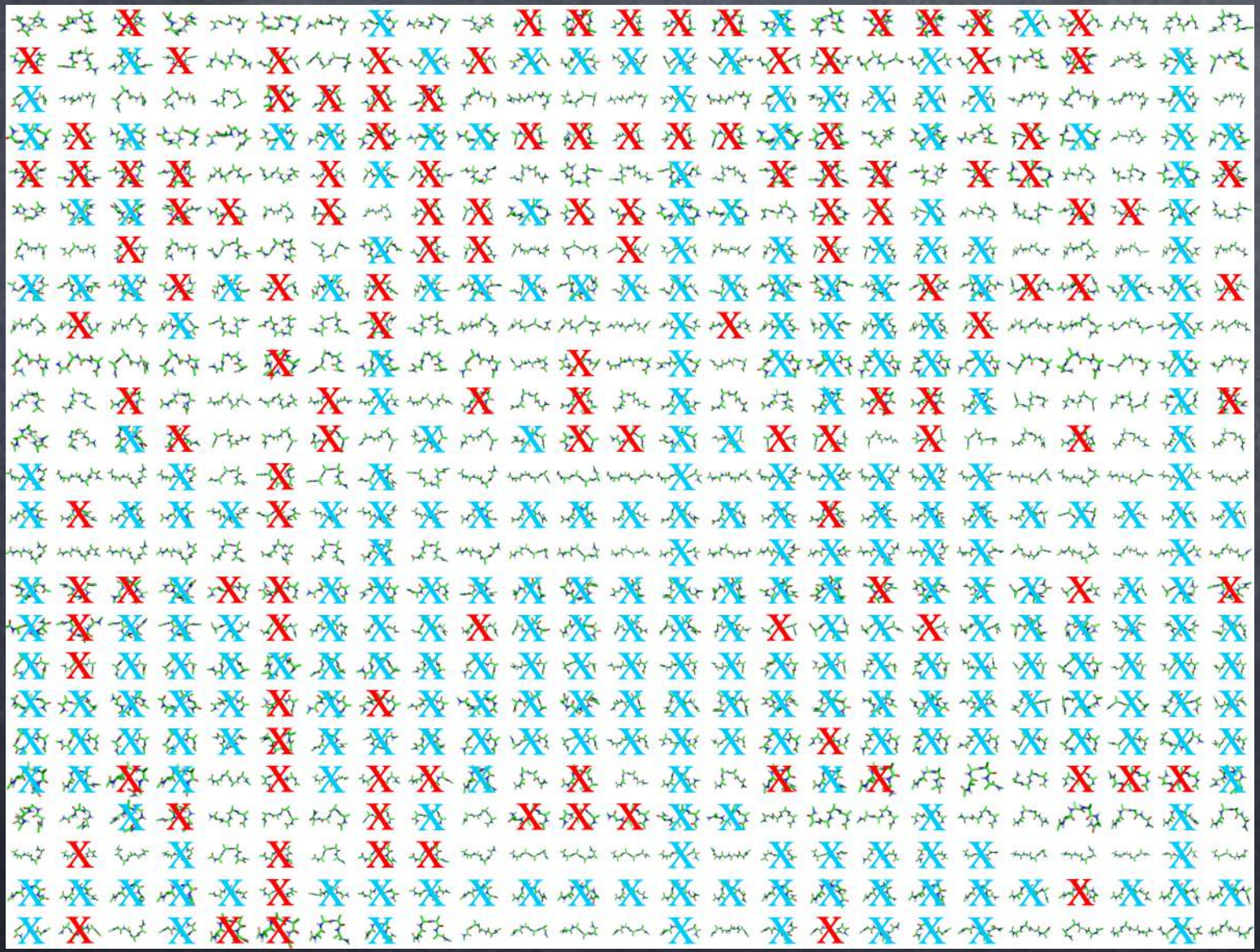
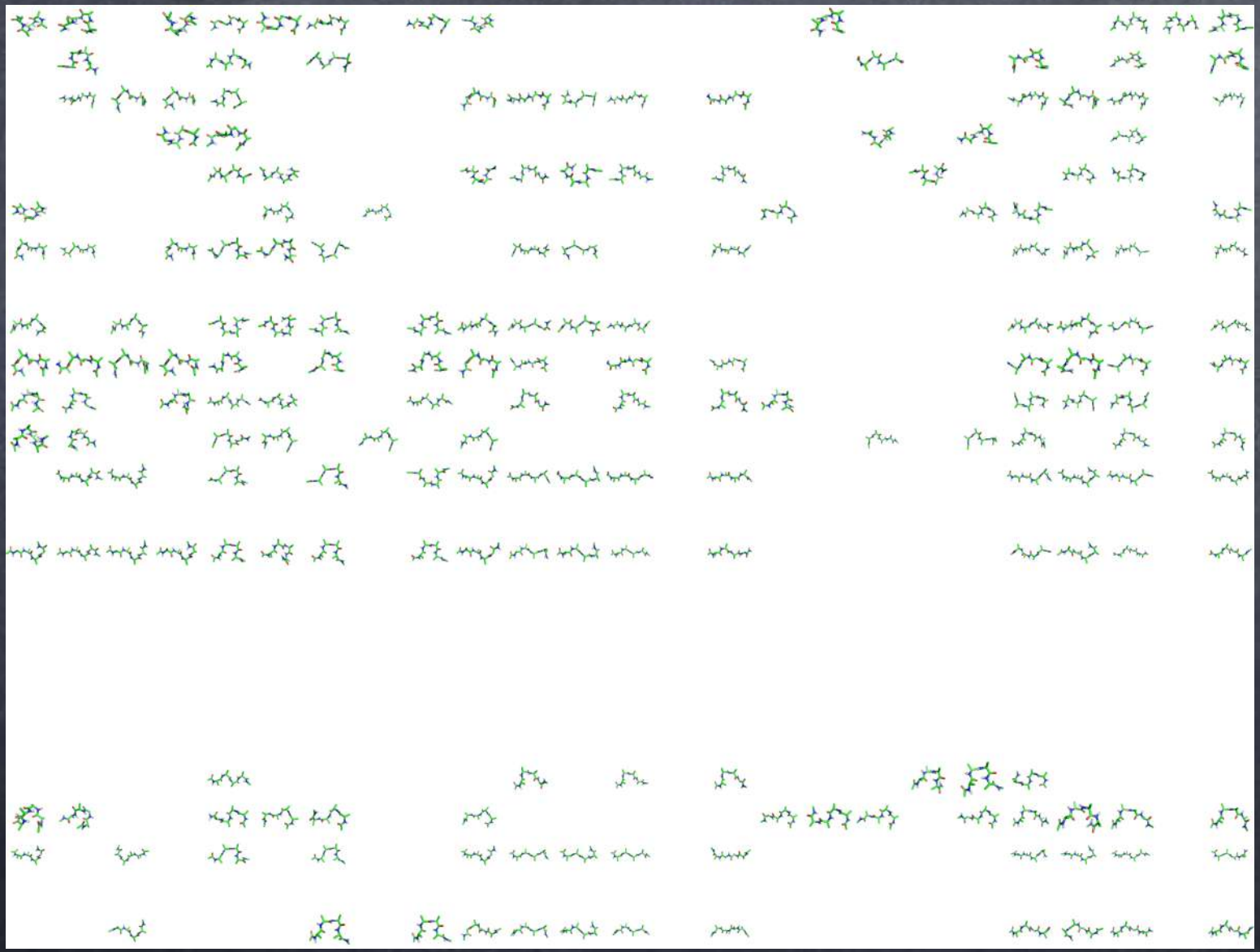


Excluding interactions are not visible in the X-ray structure



The organizing power of excluding interactions

Conformers with steric clashes or backbone polar groups that lack hydrogen bond partners make a negligible contribution to the overall thermodynamic population and are not visible in the native structure.

Excluding interactions are not visible in the X-ray structure

Not captured in knowledge-based potentials, contact energies, Go models, lattice models

What's an Excluding interaction

Two primary excluding forces:
(i) sterics
(ii) hydrogen-bond satisfaction

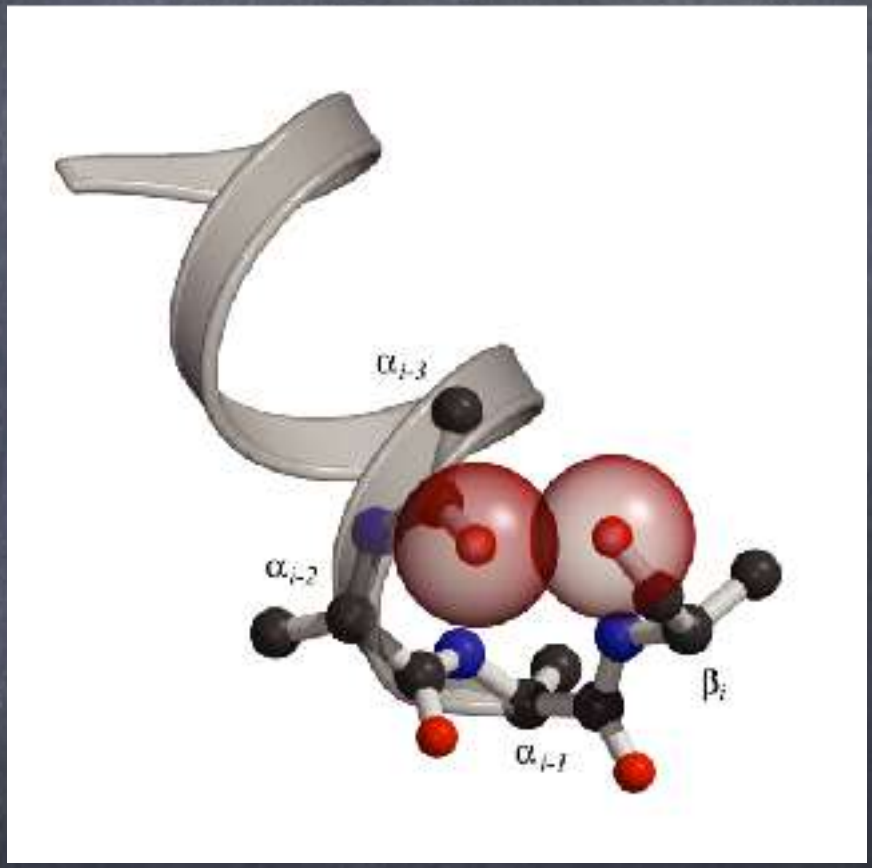
An example of each type of excluding force

Excluding interactions - sterics

The Flory isolated-pair hypothesis: The simplifying assumption that each φ,ψ pair is sterically independent of all but its adjacent chain neighbors.

Excluding interactions - sterics

An α-helix cannot be followed by a contiguous β-strand



Systematic local steric restrictions extend beyond adjacent chain neighbors.

Pappu, Srinivasan & GDR(2000) PNAS 97:12565-12570.
Fitzkee & GDR (2004) Protein Science 13: 633-639.
Fitzkee & GDR (2005) "J. Mol. Biol. 353: 873-887.

Excluding interactions - H-bond satisfaction

Protein Folding: current paradigm

$U \rightleftharpoons N$

All backbones are the same - side chains discriminate

The most energetically favorable constellation of interactions between and among the side chains corresponds to the native conformation.

Force field minimization

$$\mathrm{Protein} = \frac{A}{r^{12}} - \frac{B}{r^{6}} - \frac{\sum q_i q_j}{\varepsilon r_{ij}} - \text{H-bonds-torsions-dipoles ...}$$

Backbone-dominated model of folding

~~All backbones are the same—side chains discriminate~~

When a protein folds, many backbone polar groups are removed from solvent access. These groups must find intra-molecular hydrogen-bond partners. Why?

GDR et al, (2006) "A backbone-based theory of protein folding." Proc Nat. Acad. Sci. 103: 16623-16633.

Backbone-dominated model of folding

It's the 21st century and we're still unsure of how much a hydrogen bond is worth. But we do have a good idea that the energetic cost of a completely unsatisfied H-bond = ~+5 kcal/mol.

$$P_u = e^{\frac{-\Delta E_{hb}}{RT}} = 0.02\%$$

P_u - probability of an unsatisfied hydrogen bond
ΔE_{hb} - energy of a hydrogen bond (~ -5 kcal/mol)
R - gas constant
T - temperature

Fleming & GDR (2005) "Do all backbone polar groups in proteins form hydrogen bonds?" Protein Sci 14:1911-1917.

Panasik, Fleming & GDR (2005) "Hydrogen-bonded turns in proteins: The case for a recount" Protein Science 14: 2910-2914

Backbone-dominated model of folding

A backbone hydrogen bond may add little to the stability of the native state, but a completely unsatisfied backbone hydrogen bond would be dramatically destabilizing (+5 kcal/mol), shifting the U⇌N folding equilibrium far to the left,

rivaling the entire $\Delta G_{conformation}$ for a typical protein ≈ [-5, -15] kcal/mol.

Fleming & GDR (2005) "Do all backbone polar groups in proteins form hydrogen bonds?" Protein Sci 14:1911-1917.

Panasik, Fleming & GDR (2005) "Hydrogen-bonded turns in proteins: The case for a recount" Protein Science 14: 2910-2914

Backbone-dominated model of folding

$\Delta G_{conformation}$ for a protein ≈ [-5, -15] kcal/mol.

If a backbone polar group is satisfied by water when unfolded but left unsatisfied when folded, the U ⇌ N would be shifted far to the left.

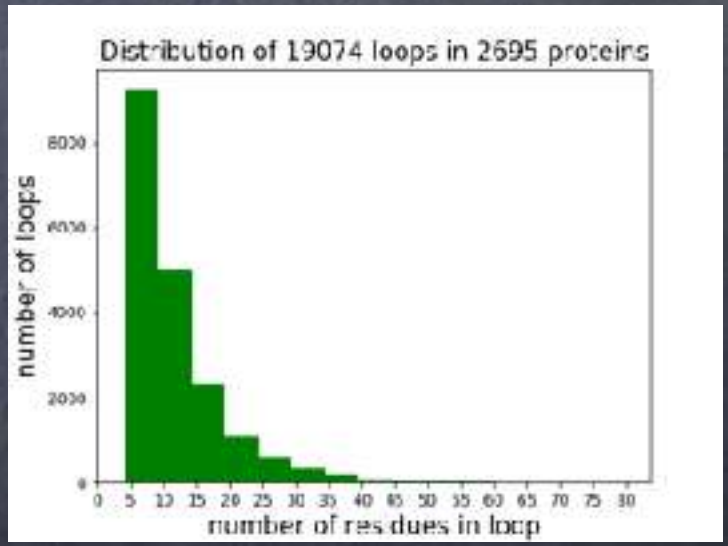
There are only two extensible hydrogen-bond-satisfying conformers: α-helix and β-strands. Necessarily, all proteins are built on scaffolds of these two hydrogen-bonded elements.

GDR et al, (2006) "A backbone-based theory of protein folding." Proc Nat. Acad. Sci. 103: 16623-16633.

How many distinct scaffolds are possible?

Using lysozyme (129 residues) as a template, a typical domain might have ~ 10 elements of α-helix and/or β-sheet = 2^{10} possibilities X complexity from interconnecting loops.

Interconnecting loops are typically short and therefore constraining.



∴ Only a few thousand scaffolds are possible

Backbone-dominated model of folding

~~All backbones are the same -- side chains discriminate~~

For a protein domain (e.g. lysozyme or ribonuclease), only a few thousand backbone scaffolds are possible (not some incomprehensibly large number).

- Chothia (1992) Proteins. One thousand families for the molecular biologist, Nature 357, 543-544
- Przytycka, Aurora & GDR (1999). "A protein taxonomy based on secondary structure." Nat Struct Biol 6(7): 672-682.

Backbone-dominated model of folding

Of thermodynamic necessity, proteins are built on scaffolds of α-helix and/or β-sheet, and only a few thousand backbone scaffolds are possible.

Backbone-dominated model of folding

~~All backbones are the same -- side chains discriminate~~

For a protein domain (e.g. lysozyme or ribonuclease), only a few thousand backbone scaffolds are possible (not some incomprehensibly large number).

side chains discriminate among these alternatives

Side chains discriminate among these alternatives

~~All backbones are the same -- side chains discriminate~~

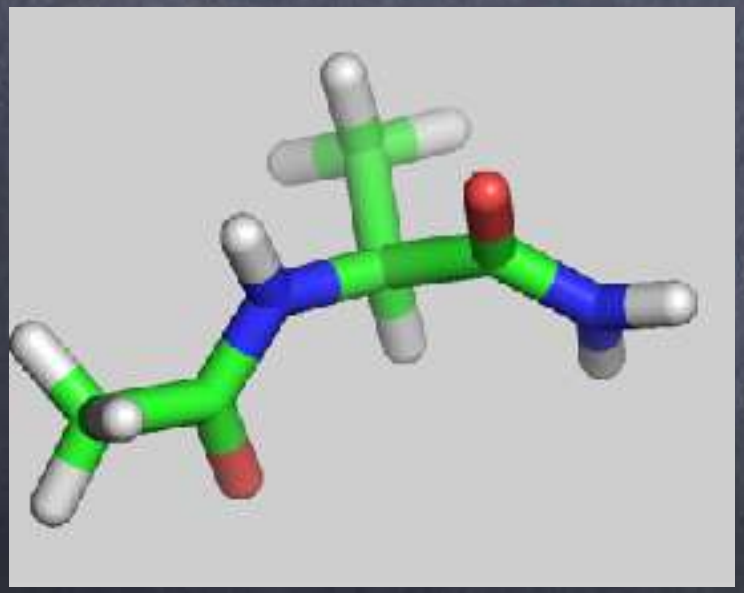
But even here, steric excluding interactions impose substantial restrictions.

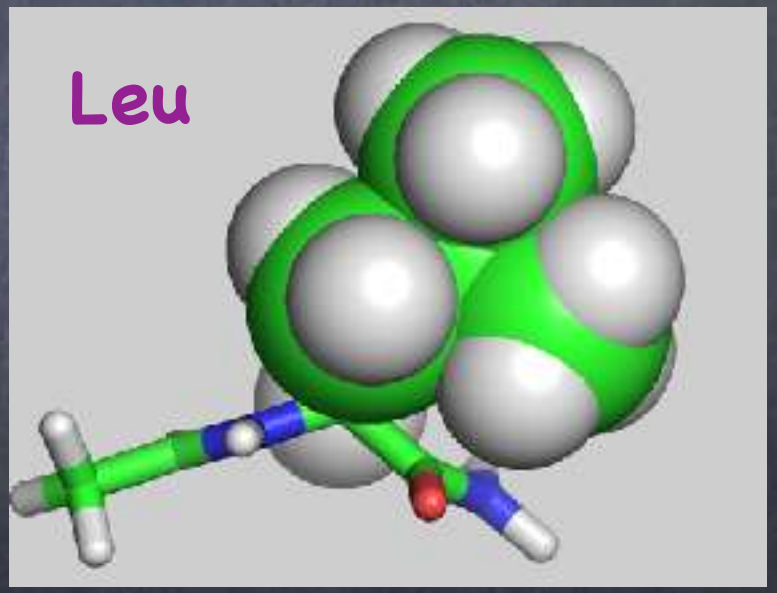
A Ramachandran-type map for side chains

Side chain conformational bias

local organization - sterics only

Blocked mono-peptides: CH3-CO-AA-NH2





GDR (2019) Ramachandran maps for side chains in globular proteins. Proteins 87:357-364.

A Ramachandran-type map for side chains

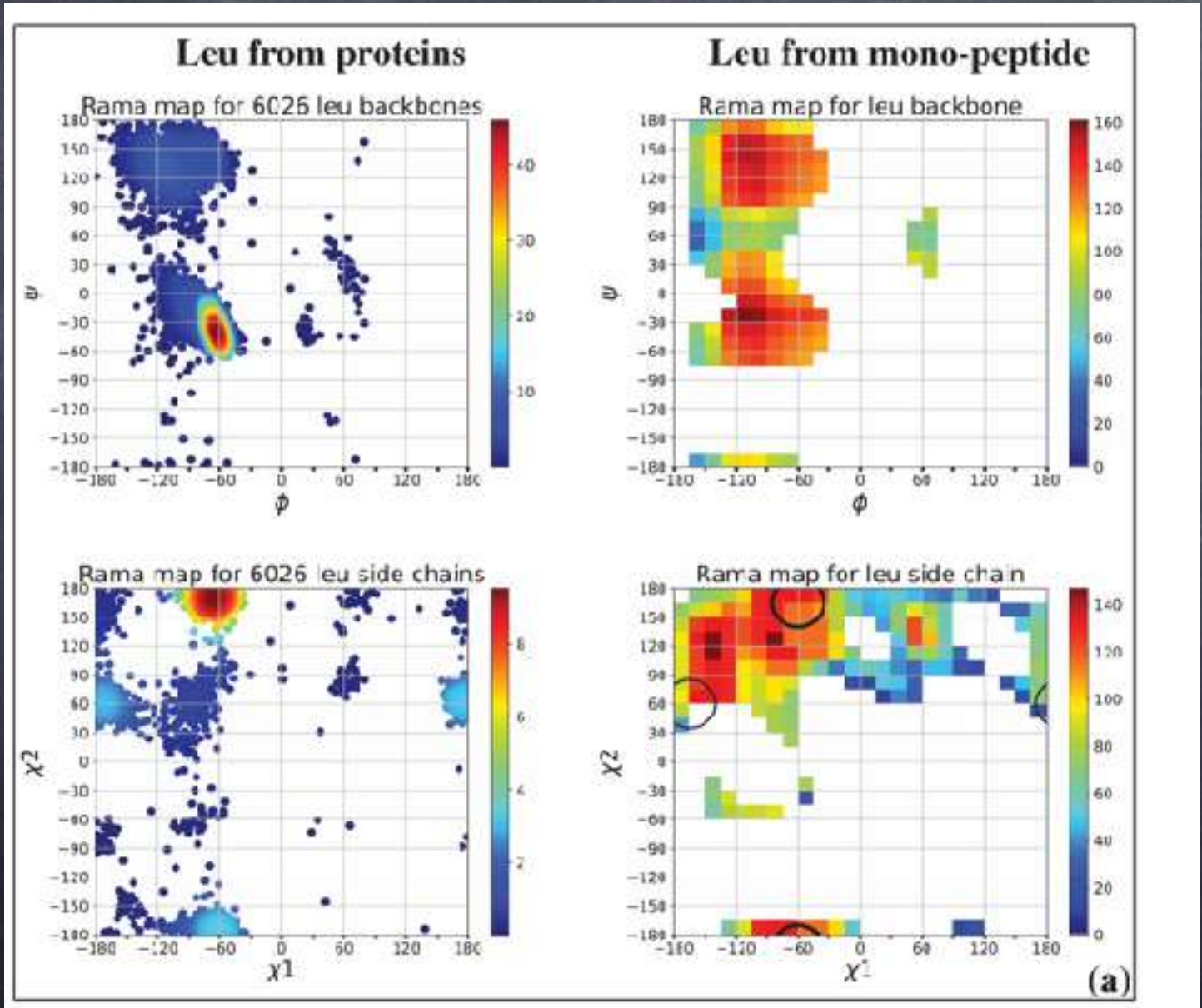
Side chain conformational bias
local organization - sterics only

Blocked mono-peptides: CH3-CO-AA-NH2

For each clash-free backbone conformation, generate the full range of side chains conformations: $\chi 1$ = [-180, 180], $\chi 2$ = [-180, 180]; exclude those with steric clashes. For each allowed side chain conformation, increment the backbone population count.

GDR (2019) Ramachandran maps for side chains in globular proteins. Proteins 87:357-364.

Excluding interactions - sterics



Backbone-dominated model of folding

A long-standing question:
Why don't individual molecules get stuck in meta-stable traps en route from U to N?

Answer: of thermodynamic necessity, proteins are built on scaffolds of α-helix and/or β-sheet, and only a few thousand backbone scaffolds are possible.

Entropy is the primary organizer in protein folding

Of thermodynamic necessity, globular protein are built on hydrogen bond-satisfied scaffolds of α-helices and/or β-strands. Scaffold folding is highly cooperative, not residue by residue. If not, dangling unsatisfied backbone polar groups would shift the $U \rightleftharpoons N$ equilibrium far to the left.

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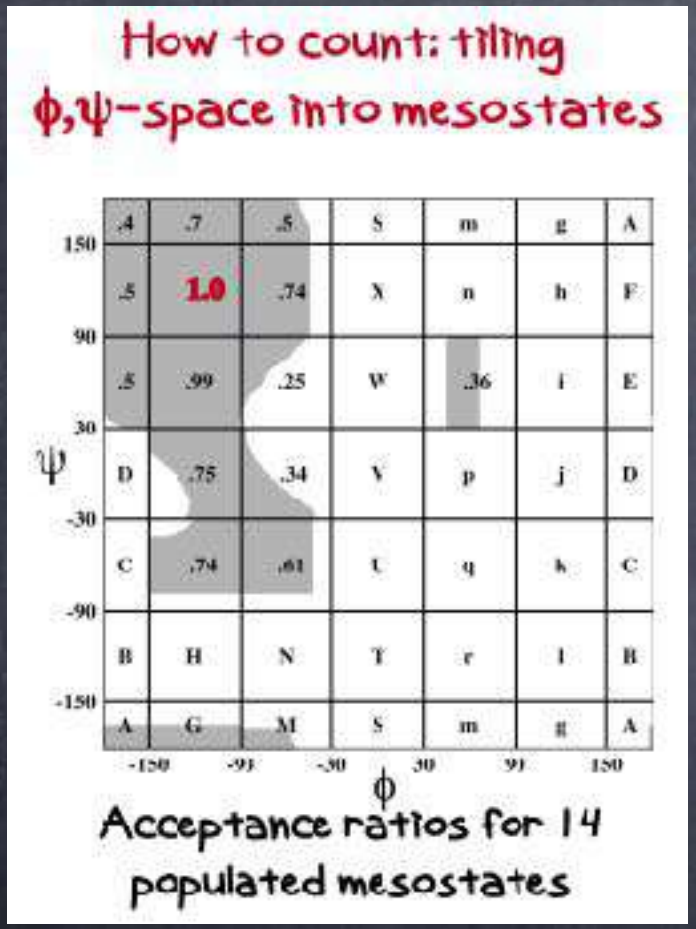
Under folding conditions, the selection of scaffold segments is limited to the two possible alternatives - α-helix or β-strand - implying a substantial degree of prior organization. If so, scaffold assembly of these pre-organized components comes at a dramatically reduced cost in conformational entropy.

Entropy is the primary organizer in protein folding



Tile space into a 60° x 60° grid of mesostates. 60% of the 14 allowed mesostates is accessible = 8.4 grid equivalents.

If residue by residue

$\Delta S_{folding}$ = - R ln(8.4/1) cal/mol/degree/residue

$\Delta G_{folding}$ = 1.28 kcal/mol/residue at 27°C

If segment by segment

$\Delta S_{segment}$ = - R ln(2/1) cal/mol/degree/segment

$\Delta G_{segment}$ = .42 kcal/mol/segment at 27°C

13 kcal/mol for a 10-residue segment if residue by residue

0.42 kcal/mol for a 10-residue segment if segment by segment

Hydrogen-bonding as a thermodynamic pivot

The big 3:

- Conformational entropy always favors U
- The hydrophobic effect always favors N
- Hydrogen-bonding favors U under unfolding conditions but favors N under folding conditions.

The thermodynamic-pivot hypothesis hinges on whether water is a poor solvent for the protein backbone.

In conclusion

Conformers with unsatisfied backbone hydrogen bonds will be culled, thereby rarefying the folding population and reducing the entropy cost of folding.

The ideas proposed here suggest the existence of a quintessential simplicity that underlies the apparent complexity of protein folding.

Thank you!