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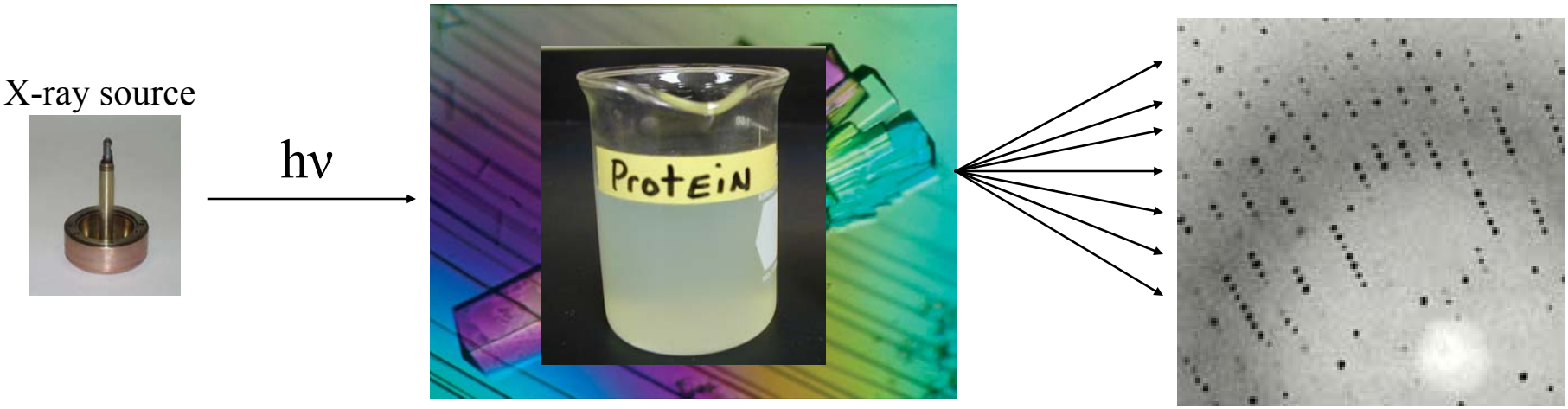
Designing Proteins

The Creative Potential of Enthalpy and Entropy

Proteins are chemical: they have defined atomic structures accessible through X-ray crystallography



Purify a protein → Grow a crystal of it → Put in X-ray beam

X-ray diffraction patterns show electron density, define atomic coordinates

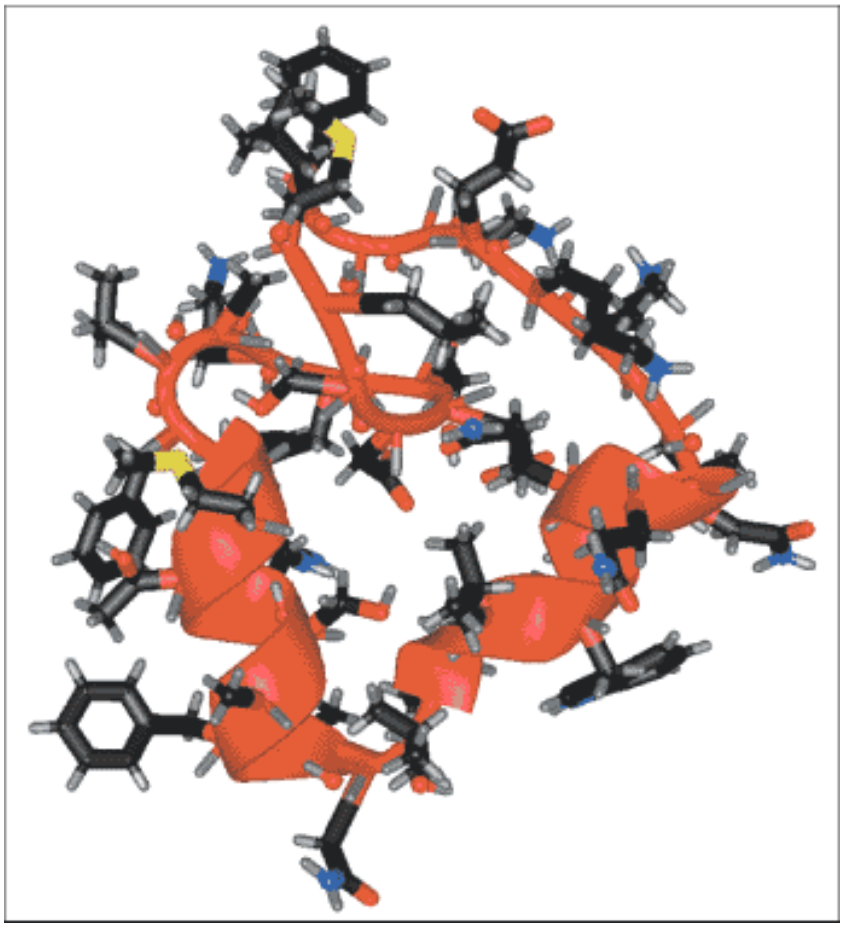
http://prism.mit.edu/X-ray/cuanode3.jpg http://www.bio.ph.ic.ac.uk/pxcourse/images/diff_pattern.sq.jpg
http://hasylab.desy.de/user_info/available_instruments/x_ray_protein_crystallography/index_eng.html

Atomic structures are beautiful and complex, proteins much more so

http://www.wellcomecollection.org/exhibitionsandevents/exhibitions/fromatomstopatterns/gallery/WTD039444.htm

The atomic coordinates of a protein allow for chemical/computational analysis and design

Figure 2 The structure of an intermediate state (at 350 nanoseconds) in one of the 500-nanosecond simulations



Protein structure

+ computer algorithms

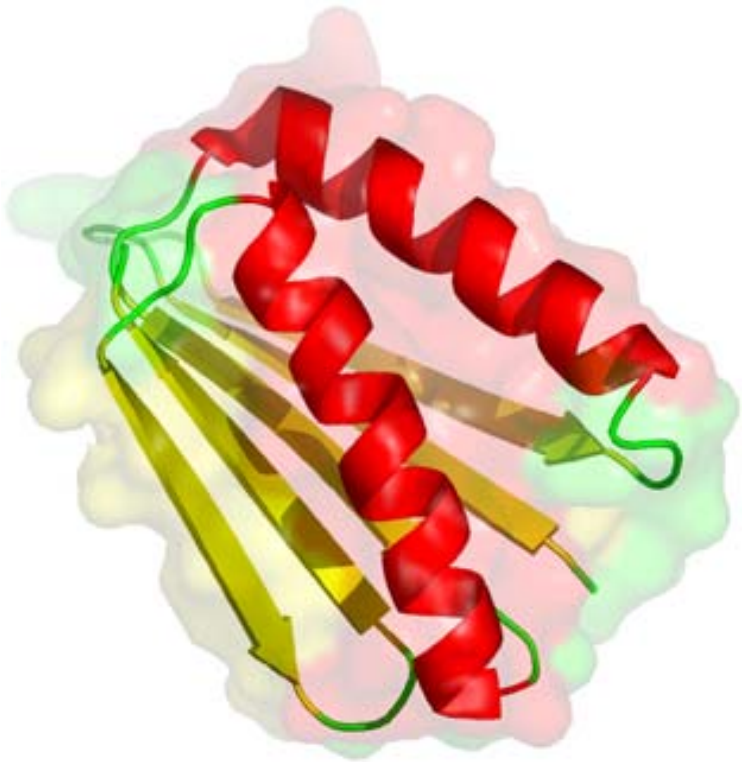
(molecular mechanics & force fields)

→ understanding which atoms are most strongly interacting

Protein chemistry's Holy Grail: The Protein Folding Problem

- Fix amino acid sequence → Find atomic coordinates
- Movie of computer simulation of villin folding
- Weeks of CPU time = a few milliseconds of a folding pathway
- Rosetta@home, Folding@home, FoldIt, etc.
- We are getting closer to the goal each year, but X-ray crystallography is still the trusted standard

The Inverse Protein Folding Problem (design) is a little easier



David Baker's Top7,
the first designed protein

- Fix atomic coordinates (of the protein backbone) → Fit in amino acids inbetween
- Instead of moving a backbone through space, we are filling in the gaps with amino acids

Fewer degrees of freedom mean this problem is more computationally tractable

Also, we can "stack the deck" and search for the strongest possible interactions

Protein chemists analyze Top7 in terms of enthalpy and entropy

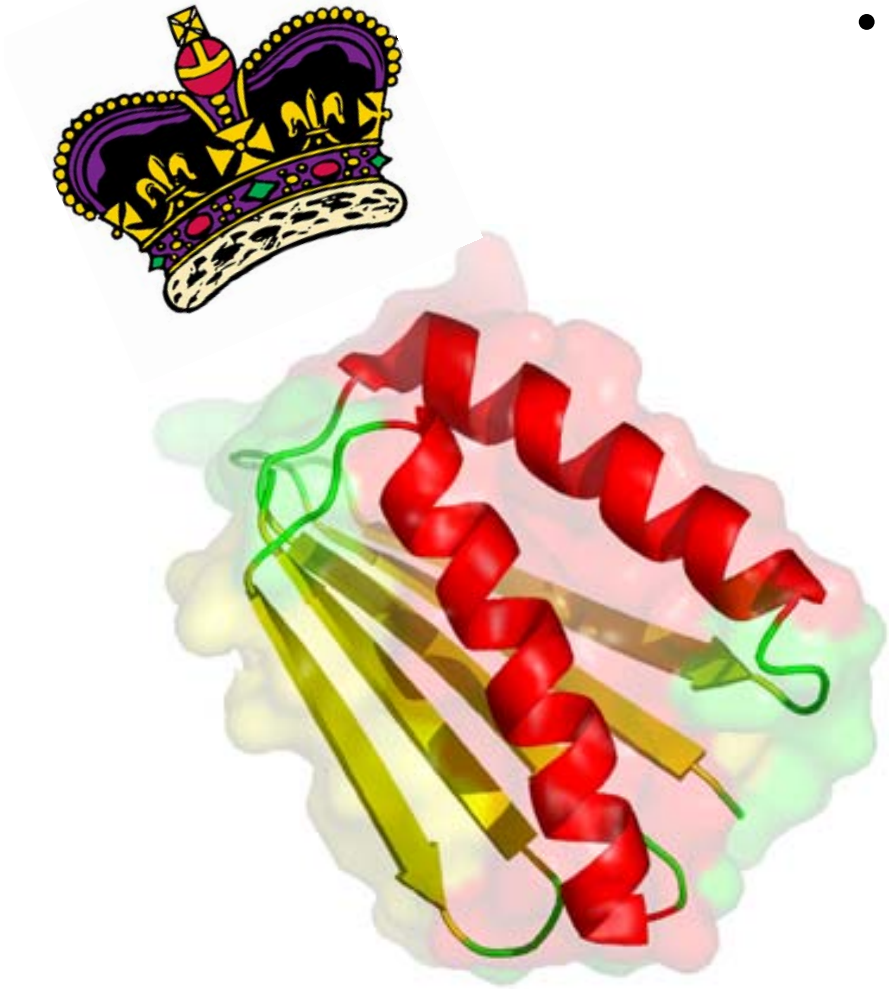
$$\Delta G = \Delta H - T\Delta S$$

ΔG = "free energy" = negative if a reaction will produce more products than reactants

ΔH = "enthalpy" = heat emitted or absorbed (forming strong bonds releases heat)

ΔS = "entropy" = degeneracy; isoenergetic states accessible to a system. $S = k \ln \Omega$

Extreme enthalpy holds Top7 together



- Rosetta optimizes good bonds, minimizes bad ones, in every location

(Rosetta knows little of entropy beyond an implicit solvation term)

Top7 has an excess of enthalpic stability:

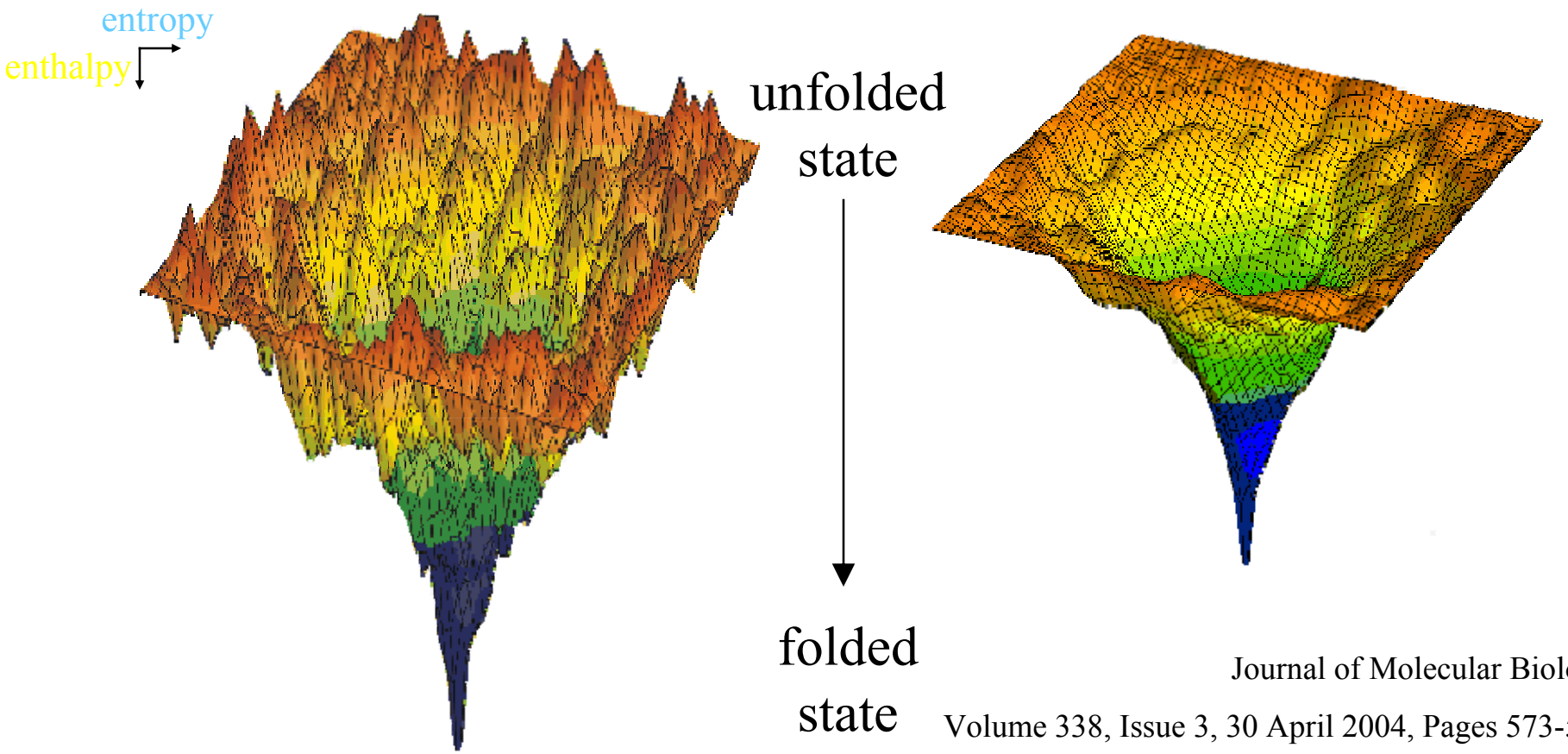
It does not unfold when boiled

Must add nearly saturating concentrations of unfolding chemicals to unfold it

The hidden costs of Top7's stability:
(i) A rough folding funnel and
(ii) persistent, dimerizing fragments

Rough "folding funnel" = Top7

Smooth funnel = natural protein



Journal of Molecular Biology
Volume 338, Issue 3, 30 April 2004, Pages 573-583

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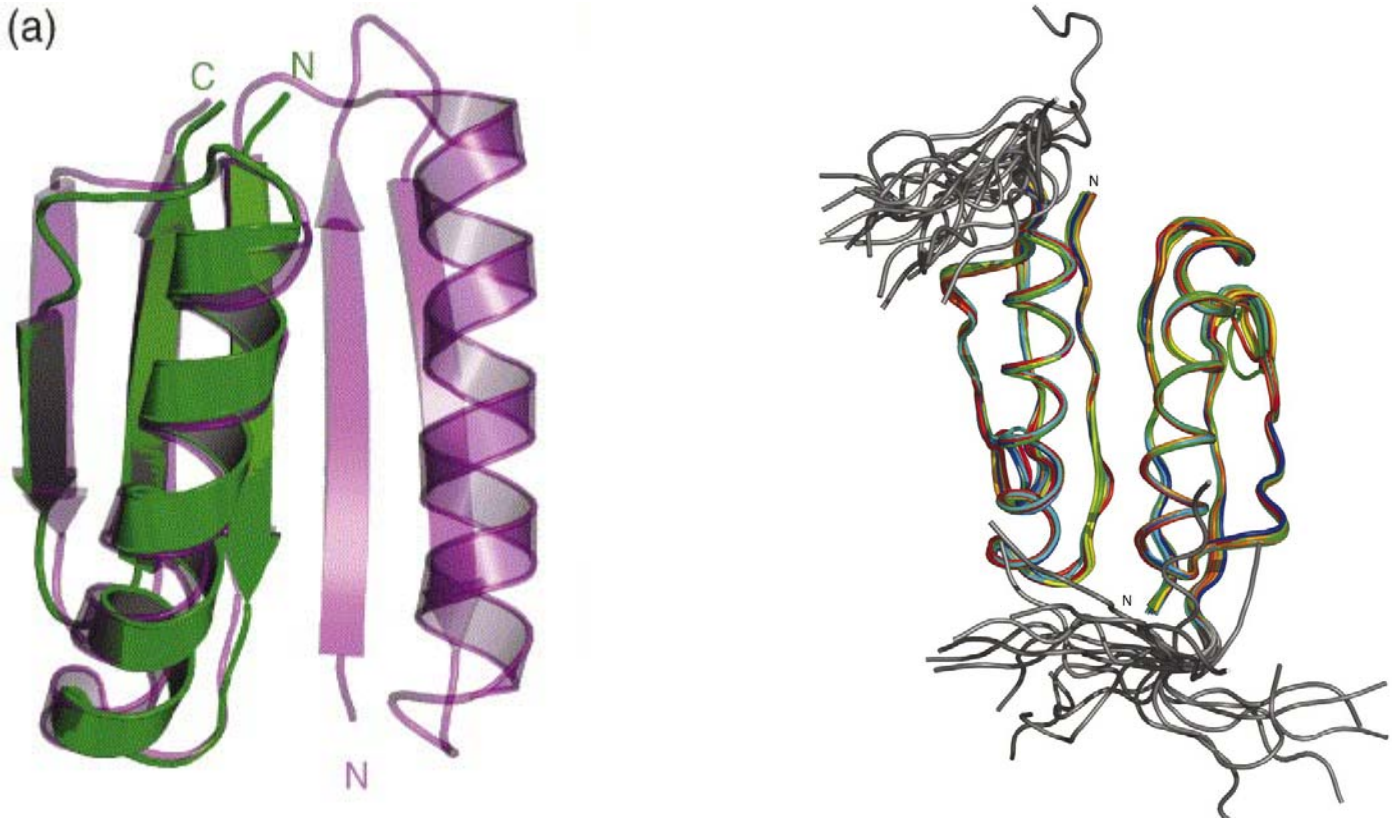
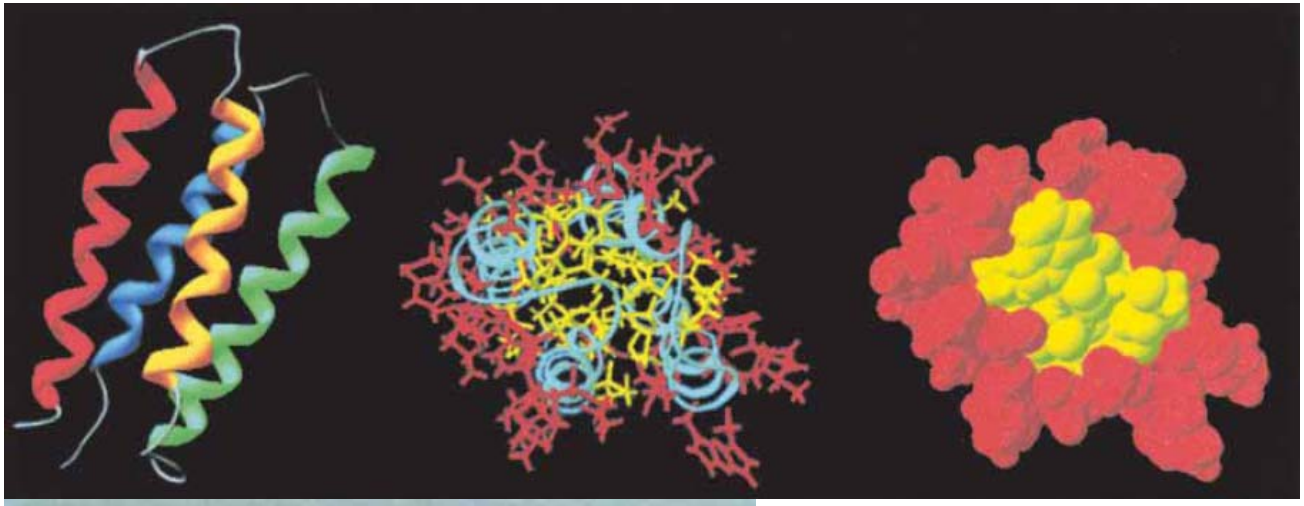
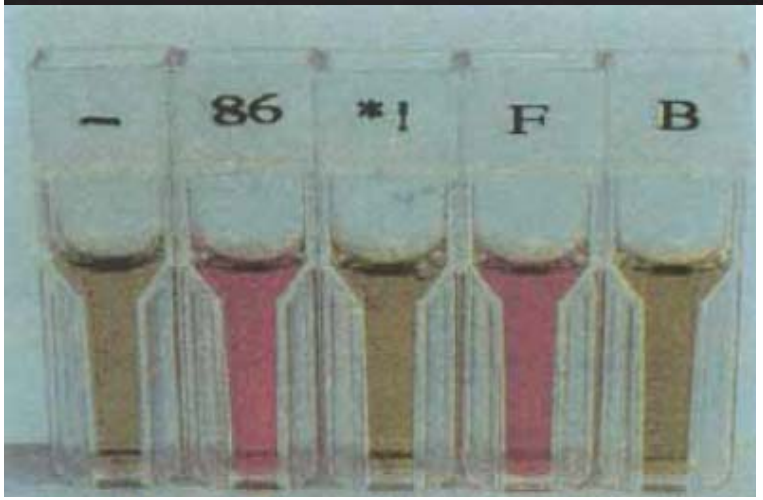


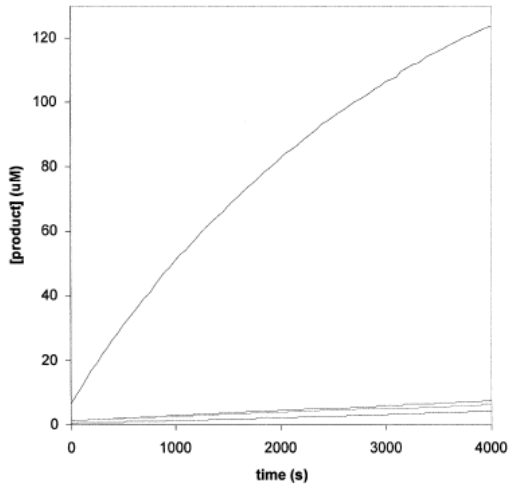
Journal of Molecular Biology
Volume 362, Issue 5, 6 October 2006, Pages 1004-1024

Michael Hecht's Lab: All proteins need is a little stability and they can catalyze reactions surprisingly well



Pattern random polar/non-polar amino acids to produce 4 semi-stable helices





Test for activities:

- Heme/CO binding
- Peroxidase
- Esterase
- Lipase*

*Not peer-reviewed yet

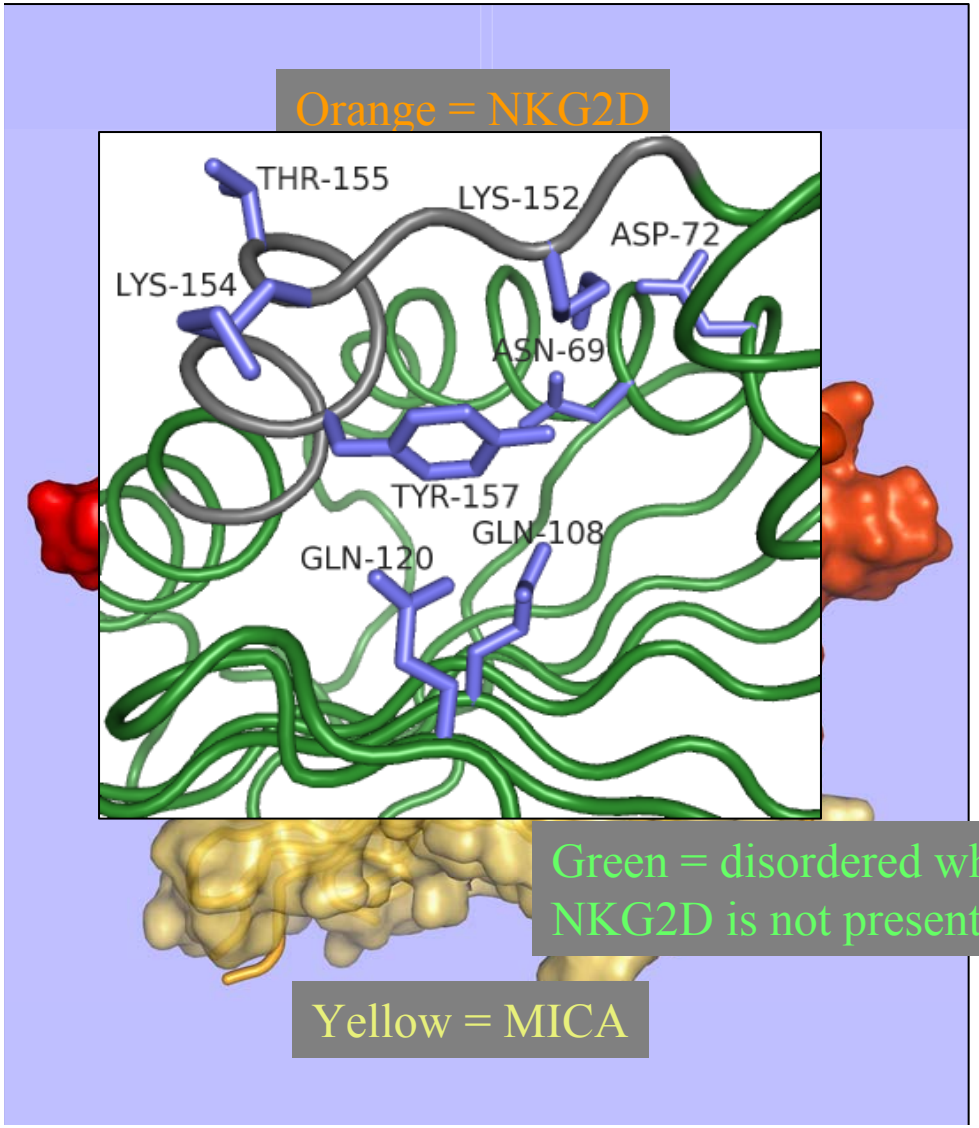
Protein Sci. 2004 13: 1711-1723

Protein Engineering, Design & Selection vol. 17 no. 1 pp. 67±75, 2004

The potential fruitfulness of the proteome

- Hecht's best "default" proteins are similar to the proteins observed in nature
  (without even trying)
- For some activities, "worse"-structured proteins are better catalysts
- Defined structure appears to be the pre-requisite for enzymatic activity
- "Moonlighting" proteins are likely
- Creative potential: A genome pregnant with possibilities

We can design protein-protein surfaces/interfaces as well as cores



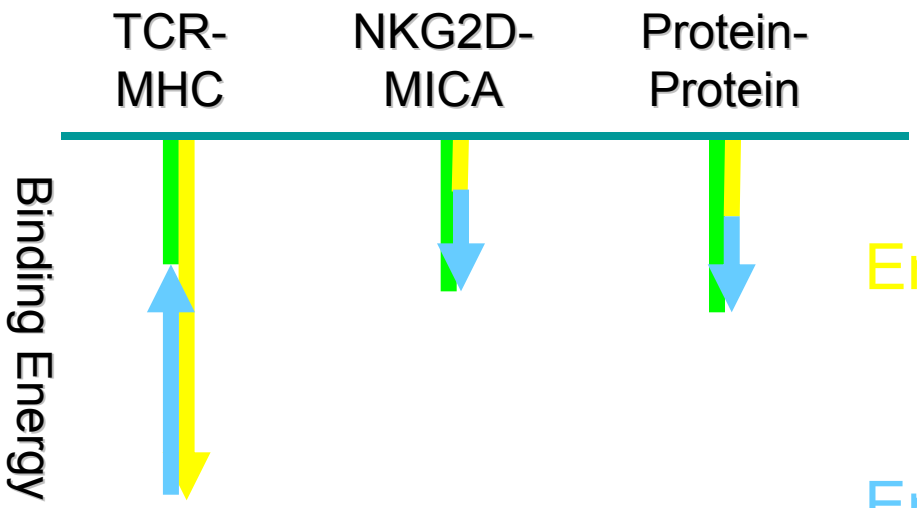
Our proteins: MICA & NKG2D

A section of MICA is disordered until NKG2D sits down on it

Our strategy: remove an entropy barrier by stabilizing interactions *beneath* the interface, inside MIC-A

- Better design scores meant our 8 chosen residues fit together better (better enthalpy)

The NKG2D/MICA interaction is driven by entropy as well as enthalpy



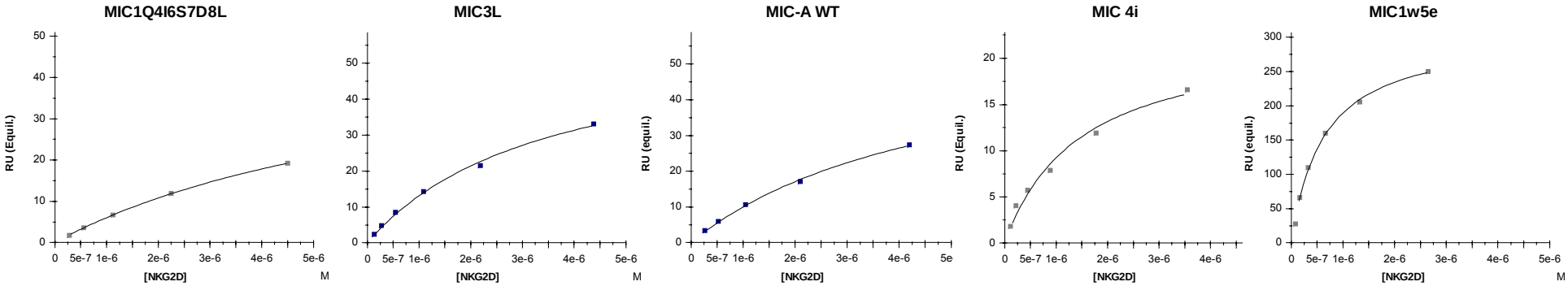
Measure thermodynamics of binding with surface plasmon resonance at different temperatures to construct van't Hoff plots

Enthalpy = MICA forms stronger bonds to NKG2D than to water

Entropy = water can assume more configurations when it's not next to MICA (squeezed out by NKG2D binding); Entropy makes this interaction (and others) happen

Protein-protein interaction data (n=30) taken from Stites (1997) *Chem. Rev.* 97: 1233-50.

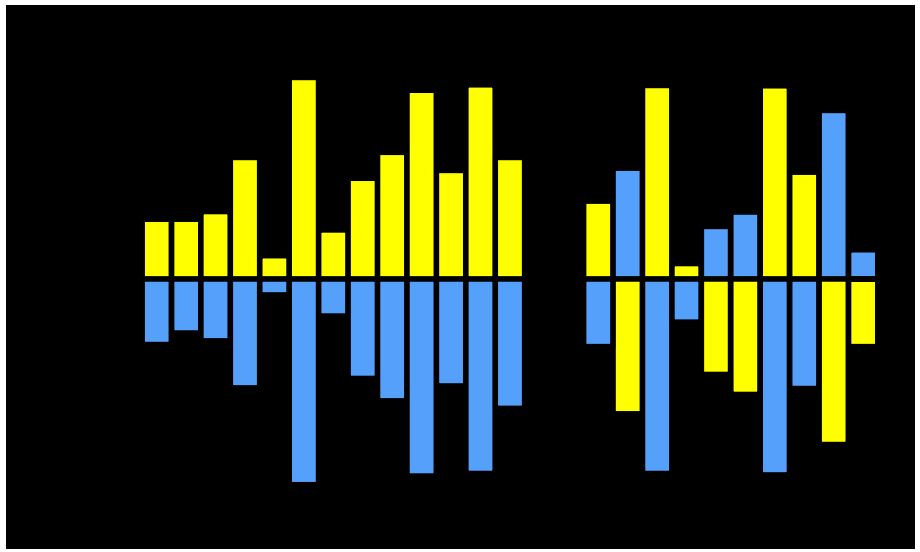
Decreasing the entropy of MIC-A didn't help, but increasing it did help



Better Design Scores

"normal"

Worse Design Scores



Entropy-enthalpy compensation: we stabilized entropy, but enthalpy overcompensated

Is some MIC-A disorder necessary for binding to its receptor?

Other examples where increasing entropy helps stabilize a protein interaction

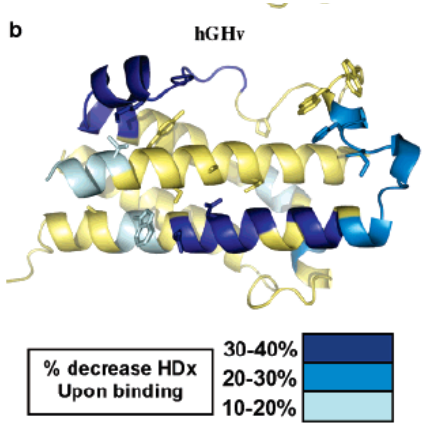
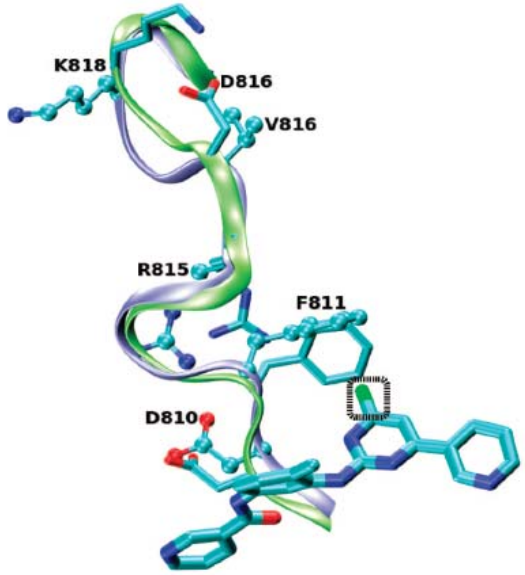


FIGURE 4: Ribbon diagram illustrating the percent decrease in H/D exchange upon binding for wt-hGH (a) and hGHv (b). Percent increase in exchange is color-coded according to legend.



- Increasing entropy of reactant protein: Kossiakoff's hGHv
- Increasing entropy of product complex: Fernandez's CI displacing a nearby loop
- Recall Hecht's flexible, default, multispecific proteins

Complex chemicals have complex mechanisms of interaction, and entropy can be harnessed to manipulate those mechanisms

Biochemistry **45**(28): 8488-98.

Mol Pharm. 2008 May-Jun;5(3):430-7.

Reclaiming Entropy from the "Dark Side"

Entropy is consistent, lawful, predictable, manipulable, even good.

"Without some disorder nothing can be alive."

- RJP Williams, *The Natural Selection of the Chemical Elements* (1996), p. 83

http://www.padawansguide.com/gifs/ghosts1.jpg (c) 1983 Lucasfilm

An argument against “stochastophobia” (the fear of entropy)

- 2 reasons for “stochastophobia”:
  a.) entropy (and math: $S = k \ln \Omega$) are complex concepts
  b.) it sounds like chance is the ultimate arbiter of life

- My own studies found 2 ways entropy makes good things happen:
  Water molecules disordering drives protein association
  We increased MICA’s configurations and observed better binding to NKG2D = better potential therapy

- Unpredictable molecular motions lead to predictable stochastic behavior of a population of molecules
  MIC-A binding NKG2D may be driven by randomness but it will always bind

Finding that entropy is good: Entropy is a cornerstone of creation

- If randomness can be reliably used in protein chemists' acts of sub-creation, might it also be relied upon in creation of species?

- Entropy: not a barrier, but an opportunity

"What remains indelibly remarkable, therefore, is ... the delicate blend of openness, constraint, and temporality that clothes the cosmos with drama. ... If nature is narrative, we must remark at how fortunate it is that adaptation and design are not comfortably complete."

  – John F. Haught, "Is Fine-Tuning Remarkable?" *Fitness of the Cosmos for Life* (Cambridge, 2008), p.45, 46.

Thank you!

- Thanks also to:
  - 5 years of biochemistry student researchers working on this project
  - Seattle Pacific University
  - Montana Research Endowment
  - The National Institutes of Health for funding our research
  - Owen Ewald (Classics, SPU) for the accurate translation of “stochastophobia”