Insights from Elementary Hydrophobic Interactions into the Activation Enthalpy, Heat Capacity, Volume, and Compressibility of Protein Folding

Hue Sun Chan

Departments of Biochemistry, of Molecular Genetics, and of Physics
University of Toronto, Ontario M5S 1A8 Canada

http://biochemistry.utoronto.ca/chan/bch.html
Two-State-like Folding of Small, Single-Domain Proteins

1aps  2ci2  1urn
1lmb  1shf  1pgb
1wit  1csp  1ris
1psf  1div  1imq  1poh
Folding cooperativity means two-state-like folding/unfolding

Experimental criteria from:

- calorimetry: \( \frac{\Delta H_{\text{vH}}}{\Delta H_{\text{cal}}} \approx 1 \)
- chevron plots
- more direct probes of two-state behaviors

Q = fractional number of native contacts
The “Levinthal” Paradox was posed in response to the discovery of two-state protein folding by calorimetry

R. L. Baldwin (1994)  

“In 1968 I was listening to a seminar at Stanford by Cy Levinthal entitled ‘How to fold gracefully’. … Two years earlier, in 1966, Lumry, Biltonen and Brandts had argued persuasively that the reversible folding/unfolding reactions of small proteins follow a two-state model (U ↔ N, where U = unfolded, N = native) without observable intermediates. In his Stanford seminar, Cy Levinthal took their model and pushed it to a reductio ad absurdum. He made a simple calculation showing that it would take longer than the lifetime of the universe for a small protein to fold up by a random search of all possible conformations. Paul Flory was sitting next to me at his seminar, he nudged me and whispered ‘so there must be folding intermediates’.”
Folding Cooperativity and the Levinthal Paradox

- In view of the relevant historical context, a wide-open funnel is not a solution to the Levinthal paradox.
- As a simple consequence of polymer physics, intermediate conformations always exist but their populations need not accumulate during folding.

Generic protein properties such as folding cooperativity can be stringent constraints on modeling, providing important clues to the energetics underlying real protein behavior.
Hydrophobic Interaction is a Major Driving Force for Protein Folding

Kauzmann (1959), Dill (1990), etc.

Chan & Dill, Physics Today (1993)
Understanding Protein Energetics by Model Compound Transfers

Jacobsen & Linderstrøm-Lang, 1949; Schellman 1955; Kauzmann 1959; Tanford, 1970, ......
Limitations of Bulk-Phase Transfers

Implicit-solvent potentials and group additivity:

Many-body interactions and pairwise additivity:

Potential of Mean Force (PMF) for Pairwise Hydrophobic Interaction

- Free energy obtained by averaging over water degrees of freedom

**Figure from:** L. R. Pratt & D. Chandler, Theory of the hydrophobic effect. *J Chem Phys* 67, 3683-3704 (1977)
Two-body PMFs have important ramifications for understanding the energetics of protein folding


“A theoretical study of the hydrophobic effect (170, 171) indicates that the correlation between reduction in water-accessible surface area and transfer free energy is not general and should not be extended to folding intermediates (171) without further justification.”


- Differences between pair and bulk hydrophobic interactions.
Desolvation barriers enhance folding cooperativity

Native Topology (Contact Pattern) Dependent Folding Rates


*Can protein chain models capture this trend?*
Desolvation barrier effects are a likely contributor to the remarkable diversity in the folding rates of small proteins.


*Figure from:* Chan, Zhang, Wallin & Liu, *Annu Rev Phys Chem* (2011)
“Surprises” from pairwise hydrophobic interactions
Typically, Unfolded-to-Transition-State heat capacity change is negative for protein folding, but for the association of small nonpolar solutes in water, heat capacity change is positive around the desolvation free energy barrier.

**Length-scale dependence provides a possible probe for cooperativity?**

- $\Delta C_P$ highly non-monotonic
- *Not* well predicted by surface areas

*Shimizu & Chan, JACS (2001)*
Hydrophobic interactions among *small* hydrophobic solutes:

Robust $\Delta C_P > 0$ at the desolvation free energy barrier

“Δ $C_p$ Retardation”

Δ $C_p$ adopts native value only when the protein conformations have attained near-native compactness.

Pairwise Δ $C_p > 0$ at db provides a physical rationalization for this otherwise puzzling experimental phenomenon.

Folding Barriers

Imageries of Protein Folding

Traditional free energy profile

Nature of barrier different

Energy landscape

\[ \Delta G \]

free energy of conformational state

progress variable

“reaction coordinate”

free energy of a given conformation

high-dimensional conformational space

Each “coordinate” can represent collectively many different conformations.

\[ = \text{Potential of mean force (PMF), with solvent degrees of freedom pre-averaged.} \]

entropic & enthalpic components
Enthalpic barriers:

Non-Arrhenius folding rates, positive unfolded-to-transition state enthalpy changes at some temperatures

Does this mean that the folding landscape is not funnel-like?

A common misunderstanding of the funnel picture of protein folding

"... A funnel-like landscape assumes mainly entropic barriers, which is in contradiction to the experimental work that is displayed in table 1. All of these experiments show major enthalpic contributions to the free energy barriers. ..."
Enthalpy-Entropy Compensation at Desolvation

Temperature dependence of the potential of mean force (PMF)

Enthalpic barrier can be significantly higher than the desolvation free energy barrier

Moghaddam, Shimizu & Chan, JACS (2005); Liu & Chan, JMB (2005)
Local-nonlocal coupling

A hypothesized cooperative interplay between favorable nonlocal interactions and local conformational preferences


Merging and grouping of native-state hydrogen exchange isotherms, “foldons”


$\alpha$–helix association in water as a model for rate-limiting events in protein folding

A pair of 20-residue poly-alanine or poly-leucine helices

~3,800 water molecules

Simulated constant-pressure free energy of association (potential of mean force, PMF) at five temperatures

MacCallum, Moghaddam, Chan & Tieleman, PNAS (2007)
Enthalpic desolvation barriers of ~ 50 kJ/mol comparable to that of protein folding

Dramatic enthalpy-entropy compensation at the desolvation step leading to low or non-existent free energy barriers

At 25 deg C,

Enthalpic folding barrier height for

CI2 is ~ 30kJ/mol
(Oliveberg et al., 1995)

CspB is ~ 32kJ/mol
(Schindler & Schmid, 1996)
Enthalpic Folding Barrier ≠ Non-Funnel Landscape

Figure from: Chan, Zhang, Wallin & Liu, Annu Rev Phys Chem (2011)
Enthalpic barriers caused by steric dewetting – “large” parts of the protein coming together at the rate-limiting step

¿ Experimental correlation between activation volume and activation enthalpy?

MacCallum et al., Proc Natl Acad Sci USA (2007)
See also discussion in: Ferguson et al., J Mol Biol (2009)
Pressure Effects on Protein Folding
Pressure Effects on Protein Folding

- P. W. Bridgman (1914): Coagulation of albumen by pressure.


“*Denaturation by high pressure*. When a protein solution is subjected to high pressure, molecules of water are crushed into the protein molecule and cause denaturation.”


- Volume change upon unfolding is almost invariably negative
  [reviewed in Royer, *Biochim Biophys Acta* (2002)]

- Pressure-denatured state is compact, generally more compact than heat-denatured states

[see, e.g., Schroer et al. & Winter, *Biophys J* (2010)]
Hummer et al. (1998):

From pressure dependence of pairwise and triplet hydrophobic interactions to physics of pressure denaturation

“*This observation leads to our most significant conclusion regarding the mechanism of pressure denaturation: Pressure denaturation corresponds to the incorporation of water into the protein, whereas heat denaturation corresponds to the transfer of nonpolar groups into water*”

*Figure and quote from:* Hummer, Garde, García, Paulaitis & Pratt, *PNAS 95*, 1552-5 (1998)

- explains why slightly expanded structures ~ ssm are favored at high *Ps*

- $\Delta V^+(\text{ssm} \rightarrow \text{db}) \approx 3.8 \text{ ml/mol}$, and $\Delta V^+(\text{cm} \rightarrow \text{db}) \approx 1.6 \text{ ml/mol}$ [from $\partial (\text{PMF}) / \partial P$]
Observations relevant to the water penetration model of pressure denaturation

- Water penetration should occur at high pressure if the volume associated with the protein chain is larger inside the folded protein core than when it is partially or fully exposed to water.

- As far as $P-V$ effects are concerned, nonpolar solvents are not good models for protein cores ("protein volume paradox", Kauzmann 1987; Chalikian & Breslauer 1996).

- Partially but significantly water-exposed configurations at the cm position of two- or three-body PMFs are also not good models for protein cores that are sequestered from water [cf. $\Delta C_P(\xi)$].
Previous single-methane volume simulations showed good to reasonable agreements with experimental data on methane partial molar volume, heat capacity, compressibility and isobaric expansivity at $P = 1$ atm.  [Moghaddam & Chan, *J Chem Phys* (2007)]
Density of water around the two methanes

$P = 1$ atm

$P = 2,000$ atm
Pressure and Temperature Dependence of Two-Methane PMF

At low $T$, variation of $cm$ with $P$ non-monotonic, $cm$ destabilizes at high $P$.

At room $T$, $ssm$ destabilizes relative to $cm$ at high $P$. This is different from Hummer et al. (1998) and Ghosh et al. (2001).

- $ssm$ not stabilizing relative to $cm$ monotonically with increasing $P$.

Dias & Chan (2013)

- Most – although not all – trends are consistent with early simulations using 10 methanes + 508 TIP3P waters at 300K by Ghosh, García & Garde, JACS (2001).
Free Energy Components of Two-Methane PMF

Enthalpy-entropy compensation is prevalent.

Enthalpic stabilization of ssm at high pressure.

Appreciable $P\Delta V$ contribution to $\Delta H$ at high $P$, positive $P\Delta V$ near db, negative $P\Delta V$ near ssm.

- Where applicable, the trends observed here are consistent with an early study by Ghosh, García & Garde, *J Chem Phys* (2002).
Volume of Two-Methane Association: Pressure Dependence

- Spatial dependence strongly oscillatory.

Dias & Chan (2013)
A significant activation volume of folding suggests that “large” – not small – parts of the protein are coming together at the rate-limiting step of folding.

\[ \Delta V^\ddagger \approx 55 \text{ ml/mol} \]

*Experimental data from:

Dias & Chan (2013)
Pressure Dependence of the Volume of Two-Methane Association

- A clear trend of volume reduction with increasing $P$ near db, indicating positive compressibility for this “activation” volume.

- At high $P$, volume at cm is smaller than that at large separation and also smaller than that at ssm, suggesting that the cm type of partially exposed configurations are more favorable than the ssm type at high $P$ (?)
“Activation” volumes near db may be approximated by the void volumes encased by molecular surfaces defined by ~ water-size probes.

- commonly used $r_w = 1.4 \, \text{Å}$
  best fit at 1 atm $r_w \approx 1.77 \, \text{Å}$

- best-fit probe size depends on $P$

Molecular Surface or Connolly Surface

$$\text{MS} = \text{contact surface} + \text{reentrant surface}$$
Activation Volume of Unfolding can be Slightly Positive

Staphylococcal nuclease
pH 5.5, 21°C

Cold-shock protein CspB
pH 7.0, 25°C

**Figure from:** Vidugiris, Markley & Royer, *Biochemistry* **34**, 4909-4912 (1995)

**Figure from:** Jacob et al. & Schmid, *J Mol Biol* **318**, 837-845 (2002)
The Activation Volumes of Unfolding of some Staphylococcal Nuclease Mutants can be Significantly Negative

**Figures from:** Brun et al. & Royer, *Biochemistry* 45, 3473-3480 (2006)
At the point when folding is completed, the transient voids associated with the enthalpic barrier should collapse. Thus, the observation of a volume plateau suggests that, while the transient voids are collapsing, more “permanent” voids are being formed elsewhere in the protein due to heterogeneity in core packing.

In this perspective, both transient and permanent voids can contribute to the positive activation volume of folding. Transient voids may only account for a part but not necessarily the entire activation volume.
What can we learn from pairwise methane properties about pressure effects on protein folding?

- Void volume in the folded protein core is an essential factor in the thermodynamic equation, the cm configuration is not necessarily a good model for the folded state.

- Compressibility of the folded state is a key determinant of its stability under pressure; but this property may not be adequately reflected by the compressibility at cm.

- Pairwise methane data provide valuable insights into $P$-dependent transition-state and denatured/unfolded-state properties; but do not by themselves address the relative stabilities of the folded vs unfolded states. [What if the two-methane volume is smaller in the protein core than at ssm?]

Dias & Chan (2013)
Excess compressibility is highest near the desolvation barrier of two-methane association

\[
\Delta \kappa_T(\xi)/\rho^0 = -\left( \frac{\partial \Delta V(\xi)}{\partial P} \right)_T
\]

\[
\Delta \kappa_T \approx \Delta \kappa_S
\]
\[
\rho^0 = \text{density of water}
\]

→ Positive activation compressibility for protein folding?

- But this may not apply to “permanent” voids?
Early experiments (1960) using primitive techniques (by today’s standard) suggest that the unfolding transition state may be more compressible than the folded state.

### Table 2: Kinetics of denaturation of carbonylhemoglobin under pressure (pH 6.8)

<table>
<thead>
<tr>
<th>$P$ kg/cm²</th>
<th>Temperature °C</th>
<th>$k'$ sec⁻¹</th>
<th>$\Delta F^\neq$ kcal/mole</th>
<th>$\Delta H^\neq$ kcal/mole</th>
<th>$\Delta S^\neq$ cal/deg·mole</th>
<th>$\Delta V^\neq$ cc/mole</th>
</tr>
</thead>
<tbody>
<tr>
<td>6000</td>
<td>10</td>
<td>$4.5 \times 10^{-3}$</td>
<td>20</td>
<td>-29</td>
<td>-173</td>
<td>-99</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>$6.9 \times 10^{-4}$</td>
<td>21</td>
<td>-20</td>
<td>-144</td>
<td>-85</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>$3.9 \times 10^{-4}$</td>
<td>22</td>
<td>-17</td>
<td>-133</td>
<td>-68</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>$2.7 \times 10^{-4}$</td>
<td>22</td>
<td>-12</td>
<td>-116</td>
<td>-68</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>$8.1 \times 10^{-4}$</td>
<td>24</td>
<td>4</td>
<td>4</td>
<td>-47</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>$2.5 \times 10^{-3}$</td>
<td>24</td>
<td>4</td>
<td>4</td>
<td>-44</td>
</tr>
<tr>
<td>6500</td>
<td>15</td>
<td>$3.9 \times 10^{-3}$</td>
<td>20</td>
<td>-29</td>
<td>-171</td>
<td>-85</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>$1.5 \times 10^{-3}$</td>
<td>21</td>
<td>-21</td>
<td>-144</td>
<td>-68</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>$9.4 \times 10^{-4}$</td>
<td>22</td>
<td>-21</td>
<td>-144</td>
<td>-68</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>$1.9 \times 10^{-3}$</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>-47</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>$5.3 \times 10^{-3}$</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>-44</td>
</tr>
<tr>
<td>0</td>
<td>72.5</td>
<td>$7.0 \times 10^{-4}$</td>
<td>26</td>
<td>80</td>
<td>156</td>
<td>17</td>
</tr>
<tr>
<td>1000</td>
<td></td>
<td>$5.0 \times 10^{-4}$</td>
<td>26</td>
<td>73</td>
<td>136</td>
<td>17</td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td>$3.8 \times 10^{-4}$</td>
<td>26</td>
<td>70</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>3000</td>
<td></td>
<td>$5.8 \times 10^{-4}$</td>
<td>25</td>
<td>56</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>4000</td>
<td></td>
<td>$1.4 \times 10^{-3}$</td>
<td>25</td>
<td>42</td>
<td>49</td>
<td>-25</td>
</tr>
<tr>
<td>5000</td>
<td></td>
<td>$3.3 \times 10^{-3}$</td>
<td>24</td>
<td>37</td>
<td>38</td>
<td>-25</td>
</tr>
</tbody>
</table>

$$\Delta V^\neq = -RT \frac{d \ln k'}{dP}$$
Pressure “Chevrons”: Folding and unfolding arms are largely linear, indicating that activation compressibility is essentially zero. A pressure chevron arm that concaves upward is consistent with a positive activation compressibility.

Desolvation barrier (db) is an intrinsic feature of hydrophobic interactions; dbs are likely contributors to folding cooperativity.

The heat capacity of a typical folding transition state is comparable to that of two-helix association but has sign opposite to the db heat capacity for small nonpolar solutes, suggesting that large groups of residues approach one another cooperatively at the rate limiting step of folding.

Steric dewetting is a likely origin of enthalpic folding barriers, the existence of which can be consistent with a funnel picture of folding.

The maxima of two-methane excess volume and compressibility are near the db. For two-helix association, the excess volume and enthalpy peaks coincide, suggesting a significant contribution from transient voids to the positive folding activation volume. Concomitantly, “permanent” voids that persist in the folded state can contribute to the activation volume as well.

The sign of activation volume of unfolding hinges on the packing compactness of the folded state. Like enthalpy, volume change need not be monotonic along the folding pathway.
Coworkers

University of Toronto

Current group members:
Tao Chen • Loan Huynh
Tobias Sikosek • Jianhui Song

Former group members:
Artem Badasyan
Mikael Borg
Cristiano Dias
Allison Ferguson
Hüseyin Kaya
Michael Knott
Zhirong Liu
Maria Sabaye Moghaddam
Seishi Shimizu
Stefan Wallin
Zhuqing Zhang

University of Toronto

Prof. Alan R. Davidson
Arash Zarrine-Afsar
Prof. Julie D. Forman-Kay
Prof. G. Andrew Woolley
Prof. Régis Pomès

University of Münster

Prof. Erich Bornberg-Bauer
Richard Wroe
David Vernazobres
Tobias Sikosek

Baylor College of Medicine

Prof. E. Lynn Zechiedrich
Jennifer K. Mann

Supported by the Canadian Institutes of Health Research, Natural Sciences and Engineering Research Council of Canada & the Canada Research Chairs Program