Molecular Folding

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A Minimal Protein Folding Model To Measure Hydrophobic and CH–π Effects on Interactions between Nonpolar Surfaces in Water

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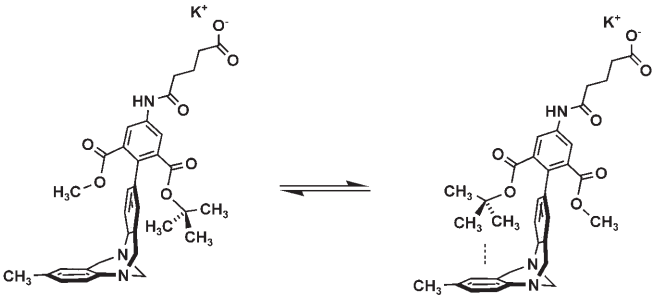
Predicting the pathways of protein folding and quantifying the relative thermodynamic stability of intermediate and final states along these pathways constitute two of the most important challenges in modern chemistry. Such predictions are difficult because the desired relative free-energy differences among the solvated intermediates depend importantly on the sum of numerous weak intramolecular forces that contribute to each folded state, on the partition functions representative of these states, and on the effects of water and co-solutes upon these interactions. Pauling's hydrogen-bonding motifs[1] and the canonical hydrophobic effect[2] have been joined by a new generation of weak intermolecular forces—each of which has been proposed as a potential contributor to protein folding and/or drug–receptor binding. Such forces include a variety of aromatic interactions that may be divided among neutral CH–π interactions,[3] ion–π interactions,[4] and OH–π interactions[5] together with CH–O interactions,[6] the venerable salt bridge,[7] and various halogen bonds.[8]

The validation of computational methods for predicting folding behavior requires accurate and precise experimental data in well-defined contexts.[9,10] A decade ago, we introduced a "molecular torsion balance" for measuring folding energies to quantify the CH–π interaction and to examine the effect of electron-withdrawing and electron-donating substituents on this force.[11] We concluded that the edge-to-face aromatic interaction was driven principally by London dispersion forces and that substituents had little effect on the magnitude of the interaction.[12,13] The average folding energy found in our model for edge-to-face aromatic interactions in organic solvents was 0.3 kcal mol^{-1} and methyl aryl π-face interactions led to slightly higher folding energies, 0.5 kcal mol^{-1}. The balance we used incorporated a methyl group counterpoised with an ester. This required that we correct folding energies because they may have been affected by dipole moment and solvation differences between ester groups and methyl groups. In addition, our original balance was not water soluble and it was therefore not possible to measure the effects of water on folding energies.

The measurement method we describe herein improves on our earlier methods. The experiments evaluate equilibria of the type illustrated in Scheme 1 and Figure 1. In these new torsion balances, as in our original studies, rotation about the biphenyl bond is slow enough that individual signals for folded and unfolded states are observable.[14,15] Here, two esters are counterpoised and the rotation process exchanges the position of only two alkyl groups. In the illustration, the exchange is between a methyl group and a *tert*-butyl group. No correction for dipole moment change is required. Furthermore, we have incorporated a water-solubilizing group on the axis of rotation, a location that minimizes any effects of this group on the folding equilibria.

In the folding event, as the larger group moves from the *exo* to the *endo* position, the solvent-accessible nonpolar surface area of the molecule is reduced and water is expelled.



Scheme 1. Representative chemical structures for the folding models presented herein. The unfolded (left) and folded (right) conformations for *tert*-butyl ester **10b**.

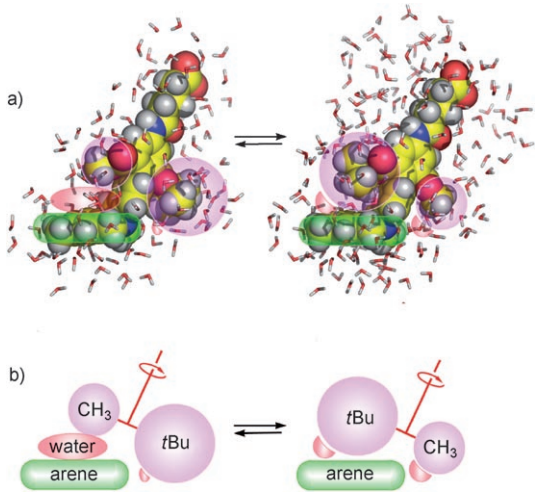


Figure 1. a) Representation of solvated *tert*-butyl ester **10b** in unfolded (left) and folded (right) conformations. b) Simplified representation illustrating the changes in hydration surrounding the *tert*-butyl (*t*Bu) and arene surfaces caused by folding.

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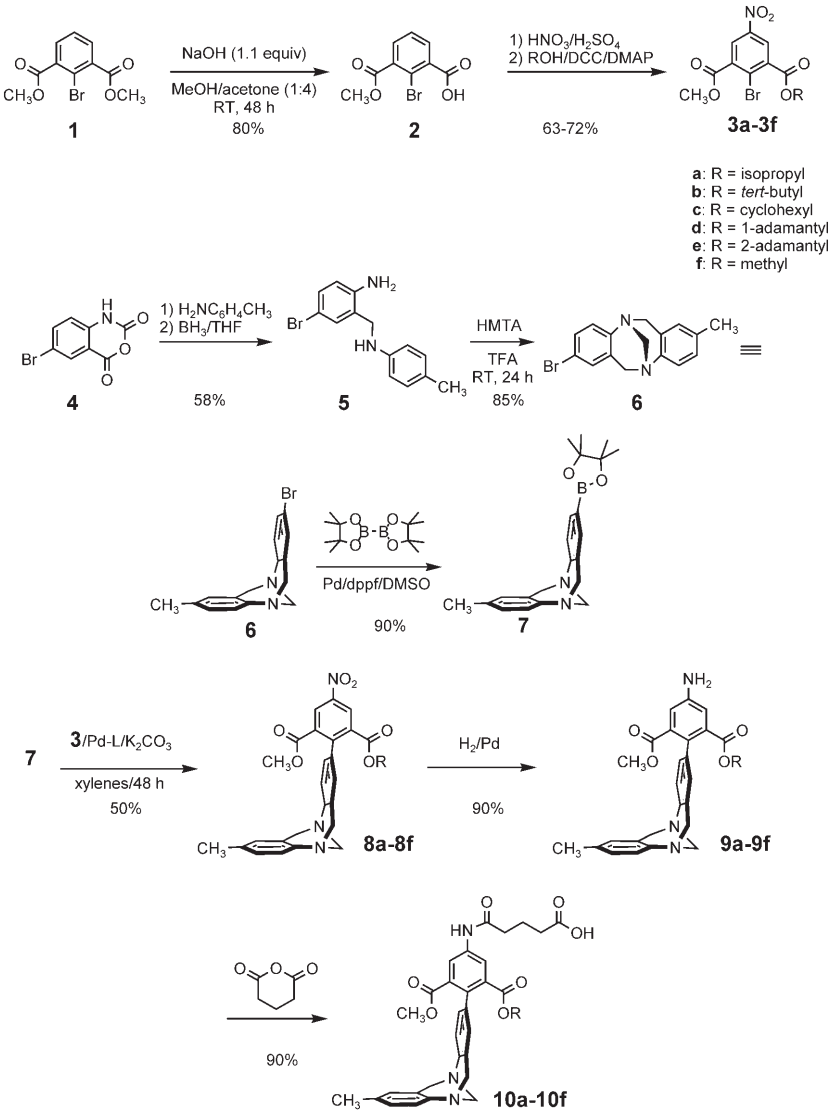
At the same time, the *tert*-butyl group establishes contact with the arene ring (Figure 1). An X-ray diffraction study of a similar isopropyl ester established the methyl–arene distance (C to centroid) to be 3.1 Å.[12]

The mutual-transfer free energy associated with this exchange of positions of the methyl and *tert*-butyl group is defined exactly by the equilibrium constant for the exchange. This method of examining transfer energies reduces ambiguities (for example, uncertainties in activity-coefficient assignments) that are associated with traditional phase-transfer free-energy studies[16] and is more direct than attempts to measure weak hydrophobic association effects through intermolecular association studies.[17] The experimental results are also immediately addressable by free-energy perturbation calculation methods.[18]

The synthesis of the required water-soluble torsion balances features the controlled hydrolytic desymmetrization of diester **1** to afford hemiester **2**[19] (Scheme 2). Nitration and esterification of the hemiester provided the unsymmetrical diesters **3a**–**3e**, and nitration of **1** provided the symmetrical ester **3f**. Pinacolatoboronate dibenzodiazocine **7** was readily obtained by using our unsymmetrical Tröger's base synthesis method[20] and was completed with a Pd-catalyzed boronation.[21,22] The two portions of the torsion balance were united through a Suzuki[23] reaction to provide nitro diesters **8a**–**8f**. Reduction of the nitro group and treatment of the resulting anilines **9a**–**9f** with glutaric anhydride provided the final carboxylic acids **10a**–**10f**. The acids were soluble in D_2O that contained Cs_2CO_3 or K_2CO_3.[24]



Scheme 2. Synthesis pathway for the water-soluble torsion balances **10a**–**10f**. DCC = 1,3-dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine, DMSO = dimethyl sulfoxide, dppf = 1,1′-bis(diphenylphosphanyl)ferrocene, HMTA = hexamethylenetetramine, TFA = trifluoroacetic acid.

The free-energy changes associated with folding in $CDCl_3$, $\Delta G_{fold}(CDCl_3)$, for the nonpolar esters **8a**–**8e** and **8a**–**8e** (Table 1) were in the range expected based on our prior studies.[11,12] They were independent of the substituent (nitro, amino, or amide) at the position *meta* to the esters (on the rotation axis), which is where we planned to introduce a polar group to enhance water solubility. Folding energies in water, $\Delta G_{fold}(D_2O)$, of **10a**–**10e** were higher compared with organic solvents but mirrored the trend observed in organic solvents: $\Delta G_{cyclohexyl} > \Delta G_{isopropyl} > \Delta G_{tert\text{-}butyl}$.

In the absence of experimental or theoretical evidence to the contrary, we must expect the CH–π dispersion interaction energy to be the same in chloroform and water,[11,12] and one may logically define the difference between folding in nonpolar solvents and water $(\Delta G_{fold})D_2O-(\Delta G_{fold})CDCl_3$ to be a measure of the hydrophobic contribution to folding in water. This difference increases with the size of the alkyl group: 0.22 kcal mol^{-1} for the isopropyl ester to nearly 0.35 kcal mol^{-1} for the adamantyl ester torsion balance.

The influence of nonpolar surface exposure on folding in water may be quantified by the excess solvent free-energy parameter γ, which is defined as the excess free energy per square angstrom of the nonpolar–water interface.[25,26] The magnitudes of γ reported in prior work range from 7 to 200 cal mol^{-1} Å^2. To specify a value of γ based on our data requires calculation of the change in exposed surface area in the unfolded and folded states. This change in the area of the nonpolar-surface–water interface can be combined with our measurement of the hydrophobic effect $((\Delta G_{fold})D_2O-(\Delta G_{fold})CDCl_3)$ to evaluate the microscopic excess free energy, γ.

We calculated γ by determining the solvent-accessible surface areas (ASA) for folded and unfolded torsion balances. Depending on the nature of the molecule, 100 to 50000

Table 1: Folding data for diesters **8**, **9**, and **10** at 298 K.[a]

Entry	Ester	R	ΔG_{fold} ($CDCl_3$) [kcal mol^{-1}]	ΔG_{fold} (D_2O) [kcal mol^{-1}]
1	**8a**	$(CH_3)_2HC$-	0.50	i[b]
2	**9a**	$(CH_3)_2HC$-	0.50	i[b]
3	**10a**	$(CH_3)_2HC$-	0.50[c]	0.72
4	**8b**	$(CH_3)_3C$-	0.65	i[b]
5	**9b**	$(CH_3)_3C$-	0.65	i[b]
6	**10b**	$(CH_3)_3C$-	0.65[c]	0.92
7	**8c**	cyclohexyl	0.36	i[b]
8	**9c**	cyclohexyl	0.36	i[b]
9	**10c**	cyclohexyl	0.36[c]	0.67
10	**8d**	1-adamantyl	0.36	i[b]
11	**9d**	1-adamantyl	0.36	i[b]
12	**10d**	1-adamantyl	0.36[c]	0.68
13	**8e**	2-adamantyl	0.55	i[b]
14	**9e**	2-adamantyl	0.55	i[b]
15	**10e**	2-adamantyl	0.55[c]	0.9
16	**8f**	H_3C-	0.0	i[b]
17	**9f**	H_3C-	0.0	i[b]
18	**10f**	H_3C-	0.0[c]	0.0

[a] Free-energy change upon folding calculated from the observed equilibrium constant determined by integration and NMR line-shape analysis. Samples were at 0.1 mM concentration. [b] Not soluble. [c] The methyl ester of the free acid was used.

starting geometries were generated and geometry optimizations were carried out on each (MMFF and Eng–Huber force fields). The ASA for conformations lying within 1 kcal mol^{-1} of the global minimum were Boltzmann averaged to provide an average ASA for the ensemble of conformations contributing to the folding states.

The values of the calculated γ ranged from 5 to 30 cal mol^{-1} Å^2. These values of γ lie in the low end of the range that was expected based on prior work. The breadth of the calculated values arises owing to uncertainties in the calculation of the surface areas for small molecules, which is where we are attempting to see the finest details of the hydrophobic effect. We look forward to creating molecular torsion balances that evaluate larger nonpolar surface changes. It will be especially interesting to evaluate future data in comparison with predictions made by the Lum-Chandler-Weeks theory of hydrophobicity. An intriguing aspect of this important theory is that the value of γ is expected to change with the area of the nonpolar surface—water in contact with small nonpolar surfaces is predicted to have a lower excess energy (per square angstrom) than water in contact with more extensive nonpolar surfaces, a prediction not opposed to our results.[27]

This study demonstrates that even very small models of proteins are influenced by the effects of water on folding. We have described the synthesis and initial evaluation of a water soluble molecular torsion balance that exhibits two-state folding. We find that despite the small changes in solvent-accessible area that accompany folding, the effect of water on folding is clearly evident. The new torsion balance we present herein can serve as a versatile tool for precise quantitative studies of other important effects on folding and drug binding, including halogen bonds, cation–π interaction, and salt bridges.

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