研究內容概要：

我們最近發展了蛋白質 ”摺疊-展開” 的易辛模型，在此模型中，蛋白質分子被視爲由彼此相互作用的 ”單元” 所組成的拓樸系綜，而每一個 “單元” 可以是摺疊態或是展開態。蛋白質在溶液中摺疊-展開的研究中，卡計、核磁共振、原子力顯微鏡、X 光繞射以及光譜實驗 (包括圓偏振雙色譜、螢光、共振拉曼散射) 已經被用來顯示由變性劑、pH 值、溫度等改變所造成的局部蛋白質結構變化。實驗上發現蛋白質 ”摺疊-展開” 的自由能變化通常取決於實驗的方法，以細胞色素 c 來說，用螢光、吸收光譜、共振拉曼散射以及圓偏振雙色譜定出的自由能彼此都不同，而且其 ”摺疊-展開” 的行爲也與用小角度 X 光散射、卡計以及原子力顯微鏡的結果不同。再者，在一定濃度的變性劑 (或溫度) 下，蛋白質摺疊 (或展開) 的分率對時間變化的動力學研究中，通常是顯示非指數型的衰變行爲，這與一般蛋白質 ”摺疊-展開” 的雙態模型預測的結果不同。

我們提出在蛋白質 ”摺疊-展開” 其熱力學與動力學的易辛模型，並且說明如何應用此模型來分析細胞色素 c、溶菌酶。結果顯示，某些實驗方法能夠提供蛋白質整體行爲的熱力學以及動力學資訊，然而，其他的方法只可以提供局部 ”群” 的行爲。

Thermodynamics and Kinetics of Protein Folding-Unfolding

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Recently we have developed an Ising model of protein folding-unfolding in which a protein is regarded as a topological ensemble of interacting units with each unit taking either folded or unfolded state. The thermodynamics and kinetics of protein folding – unfolding are determined by the elementary processes of protein-unfolding of the protein units through their unfold fraction or fold fraction inside proteins. With the folding – unfolding units built inside a protein, we can simply collect certain numbers of folding- unfolding units for forming groups (such as α – helix, β – sheet etc) in a protein to describe multi-group activities inside a protein. As a consequence, units in the same group fold or unfold collectively.

In the studies of protein folding – unfolding in solution, calorimetric experiment, NMR, AFM, X-ray diffraction and optical spectroscopies including circular dichroism, optical absorption, fluorescence and resonance Raman scattering have often been used to reveal local and/or structure changes of proteins induced by denaturants, PH, temperatures changes etc. It is found experimentally that the free energy changes of protein folding-unfolding usually depend on the experimental techniques used in the experiment. For example, for cytochrome c, the free energy changes determined by using fluorescence, optical absorption, RRS, and CD are different from each other, and their folding-unfolding behaviors are different from those determined by using low-angle XRD, differential calorimetry and AFM. Furthermore, in the kinetic studies the experimental curves of the folding (or unfolding) fraction of protein vs time for a given concentration of denaturants (or temperature) usually exhibit

non-exponential behaviors which are different from those predicted by the conventional two-state model of protein folding-unfolding.

Zipper Model

In the proposed model, one can view the cyt *c* protein as a collection of interacting foldon units. These units, in turn, are collections of amino acid residues in the secondary structures. The fact that the free energy of the folding-unfolding transition for the different residues of a given foldon unit is the same indicates a high level of cooperativity at the level of secondary structure. Thus, cyt *c* folding can be approximated as an "assembling" of the tertiary structure from two-state structural units (foldons). The conformation of the cyt *c* molecule is represented, then, by a set of binary variables:

$$\mathbf{x} = \{x_i\}, i = 1,..,N \; x_i \in (0,1) \tag{1}$$

where N=5 is the number of foldon units, $x_i = 1$ or $x_i = 0$ characterizing, respectively, the folded or unfolded state of the ith unit. The experimentally observed pathway of cyt *c* folding-unfolding via a series of partially unfolded intermediate conformations is presented schematically in Fig.1. The most recent HX experiments showed that the first three foldons fold by a stepwise sequential process, whereas the fourth and fifth do not follow a predetermined sequence. The assumed Hamiltonian,

which is (actually it is a free energy potential) reads:

$$H = \sum_{i=1}^{N} \varepsilon_i x_i + \sum_{i=1}^{N-1} j_i \prod_{k=1}^{i} x_k + j_N \prod_{k=1, k\neq N-1}^{N} x_k \quad , \tag{2}$$

where ε_i and j_i denote the free energy factors of the *i*th unit due to the solvent and coupling interactions, respectively. In other words j_i describes all cooperative effects, while ε_i is the sum of the residue-solvent effects over all residues of *i*th foldon. One can see that addition of the *i*th unit to the native structure is favored by coupling only if the zipper product $\prod_{k=1}^{i} x_k$ is equal to unity. The latter condition is fulfilled if all x_k units are in the native states, i.e. $x_k = 1, k = 1,..,i$. In this way, the sequence of cyt *c* folding is assumed to be governed by the nonadditive manybody interactions of foldons. According to the minimal frustration principle, only native coupling is taken into account by the Hamiltonian in Eq. (2). The j_N zipper product has been modified to account for the independent behavior of the fourth and fifth units.

For *N*=5, the summation over 32 possible states to find the partition function or the equilibrium averages of physical values can be done directly; for the general case, the analytical solution of the Zipper model can be used. By employing a substitution $x_i = (\sigma_i + 1)/2, \sigma_i \in (-1,1)$, the Zipper model is transformed into the GKI model:

$$H_{GKI} = -\sum_{i=1}^{N} \sigma_i E_i\left(\sigma_1,..,\sigma_{i-1,}\sigma_{i+1},...,\sigma_N\right) \quad .$$

(3)

For a system interacting with a heat bath and described by H_{GKI}, the master equation in the single flip approximation reads:

$$\begin{aligned}\frac{dP\left(x_1,..,x_i,..,x_N,t\right)}{dt} = &-\sum_i w\left(x_i \to 1-x_i\right)P\left(x_1,..,x_i,..,x_N,t\right) \\ &+\sum_i w\left(1-x_i \to x_i\right)P\left(x_1,..,1-x_i,..,x_N,t\right)\end{aligned} \quad ,$$

(4)

where $P\left(x_1,..,x_i,..,x_N,t\right)$ is the probability of occupying state $\mathbf{x} = \left\{x_1,..,x_i,..,x_N\right\}$ at time *t*, and the local flipping rate *w* is determined by the Fermi golden rule. In kinetic experimental measurements one observes the time evolution of an average of a physical value over all possible kinetic trajectories $\left\langle f(t)\right\rangle = \sum_{\mathbf{x}} f\left(\mathbf{x}\right)P\left(\mathbf{x},t\right)$. To analyze experimental results, it is especially important to know the time evolution of the folded fraction of the *i*th unit $\left\langle x_i(t)\right\rangle, i = 1,..,N$. The letter one is given by the solution of the set of differential equations:

$$\tau_i \frac{d}{dt}\langle x_i \rangle = \frac{1}{2} - \langle x_i \rangle + \frac{1}{2}\tanh\left(\frac{\langle E_i \rangle}{k_B T}\right),$$

(5)

where $\langle E_i \rangle$

$$\langle E_i \rangle = -\left(\varepsilon_i + \sum_{k \geq i}^{N-1} j_k \prod_{\substack{m=1 \\ m \neq i}}^{k} \langle x_m(t) \rangle + j_N \prod_{\substack{m=1 \\ m \neq i, N-1}}^{N} \langle x_m(t) \rangle\right)/2,\ i = 1,2,3$$
$$\langle E_i \rangle = -\left(\varepsilon_i + j_i \prod_{m=1}^{N-2} \langle x_m(t) \rangle\right)/2, \quad i = 4,5$$

,

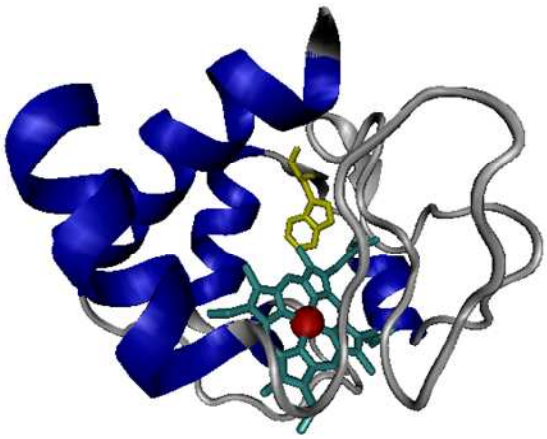
(6)

and τ_i depends on the heat bath interactions and $\langle E_i \rangle$. It should be noted that for heterogeneous structural units, Eq. (5) generally predicts multiphase nonexponential kinetics.

Conclusion

Using the Zipper model, the nonadditive manybody interactions of experimentally observed foldon units were introduced in a phenomenological way to describe the “on pathway” classical folding of cyt *c*. Such nonadditive interactions can play an important role in describing protein folding on a coarse-grained level.

Cytochrome c



Generated with Virtual Molecular Dynamics (VMD)
http://www.ks.uiuc.edu/Research/vmd/

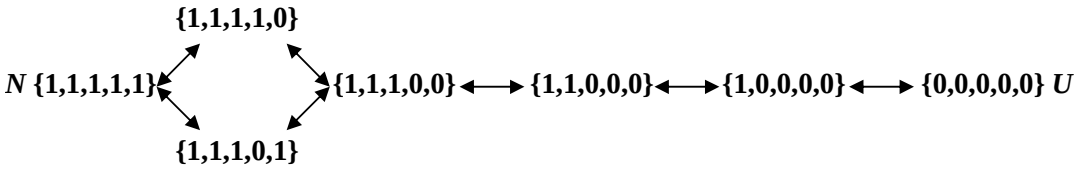


Fig. 1 The pathways of cyt *c* folding-unfolding via partially unfolded forms, as detected in HX experiments: *N*, the native fold; $\mathbf{x} = \{x_i\}$, the conformation of the partially unfolded state; *U*, the fully unfolded state.

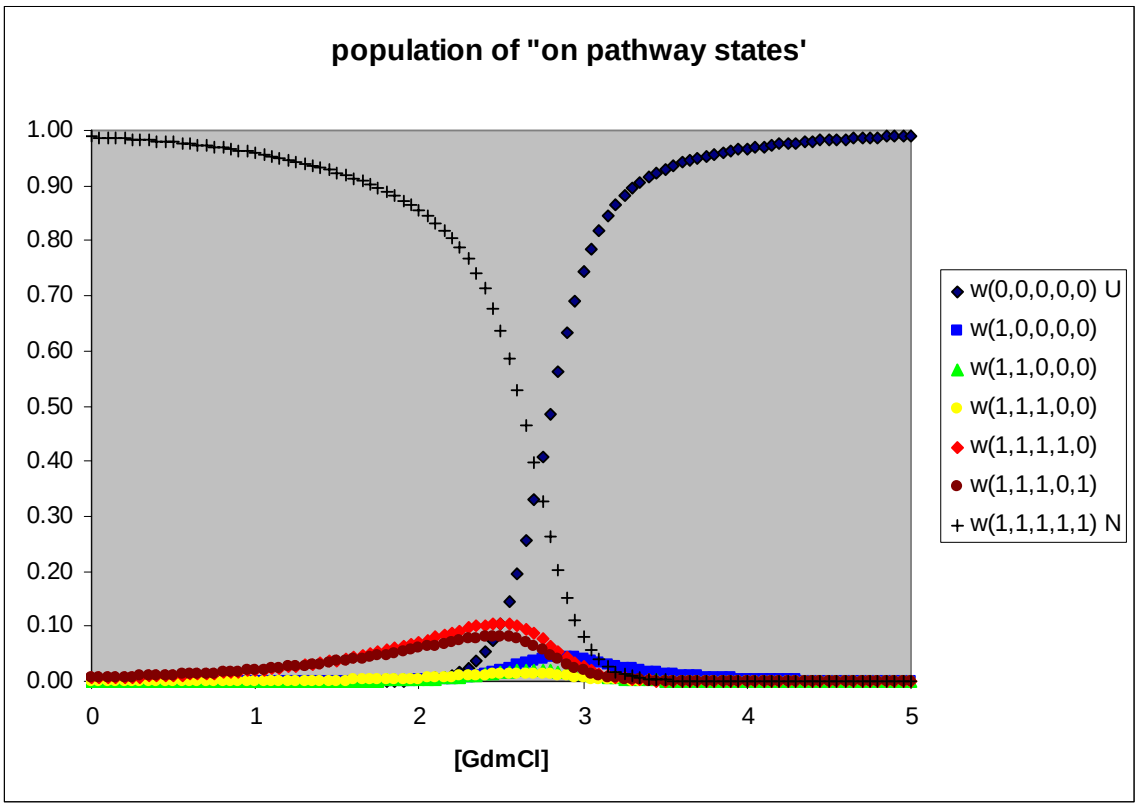


Fig. 2. Denaturant-dependent (GdmCl) probabilities of occupying the “on pathway” states (T = 300 K).

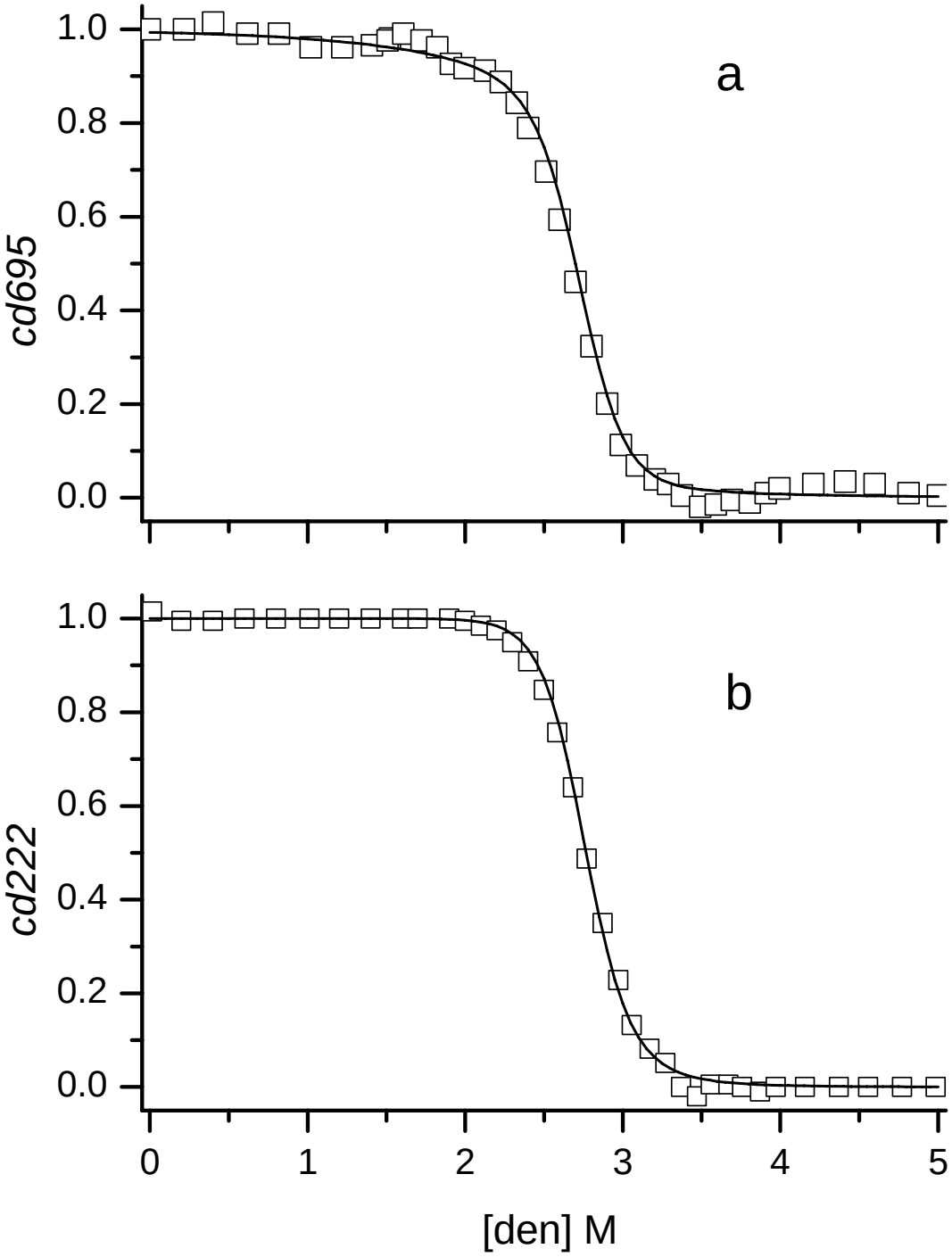


Fig. 3 The denaturant effect: comparison of the normalized optical signals due to the experimental measurements (scatters) and model calculations (solid lines) of cyt *c*

equilibrium folding-unfolding in GdmCl: **a,** *cd695*; **b,** *cd222*.

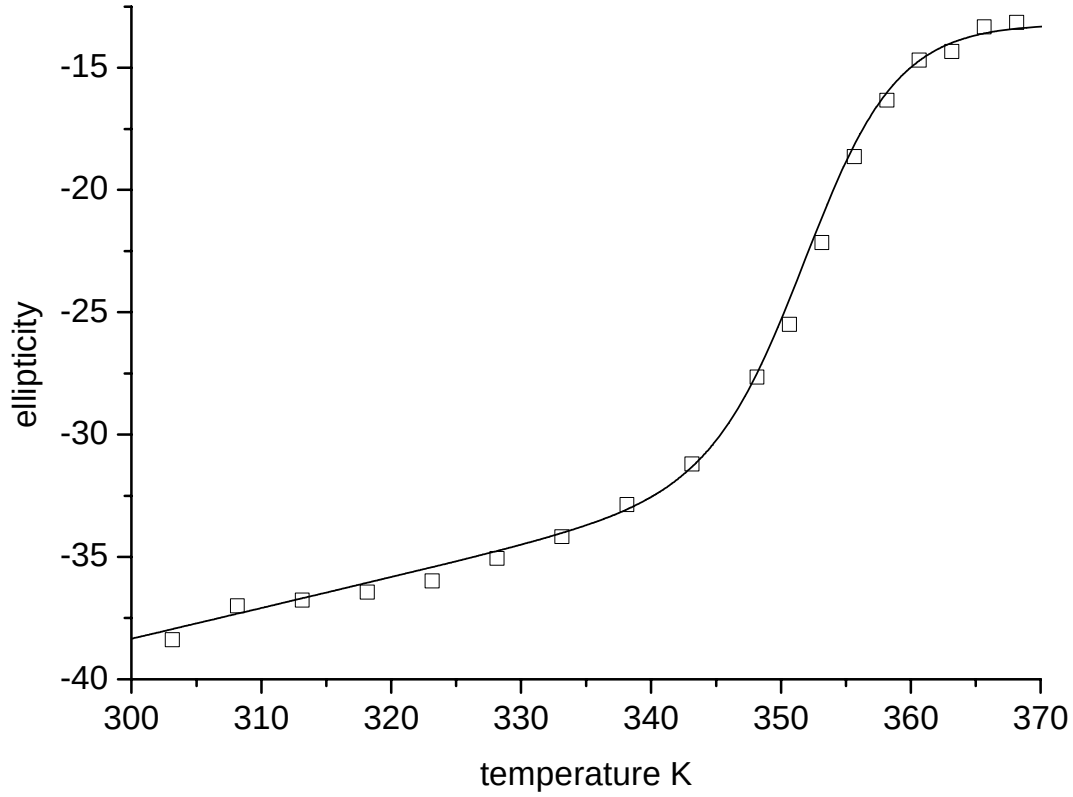


Fig. 4 The temperature effect: equilibrium unfolding of cyt *c* measured by *cd222* (scatter) and model calculations (solid line). The linear approximation for the native and unfolded signals was used in the calculations $f_{cd\,222}^{(N,U)}(T) = A^{(N,U)} + B^{(N,U)}T$ ($A^{(N)} = -76.0254, B^{(N)} = 0.12559; A^{(U)} = -9.8, B^{(U)} = -0.009$).

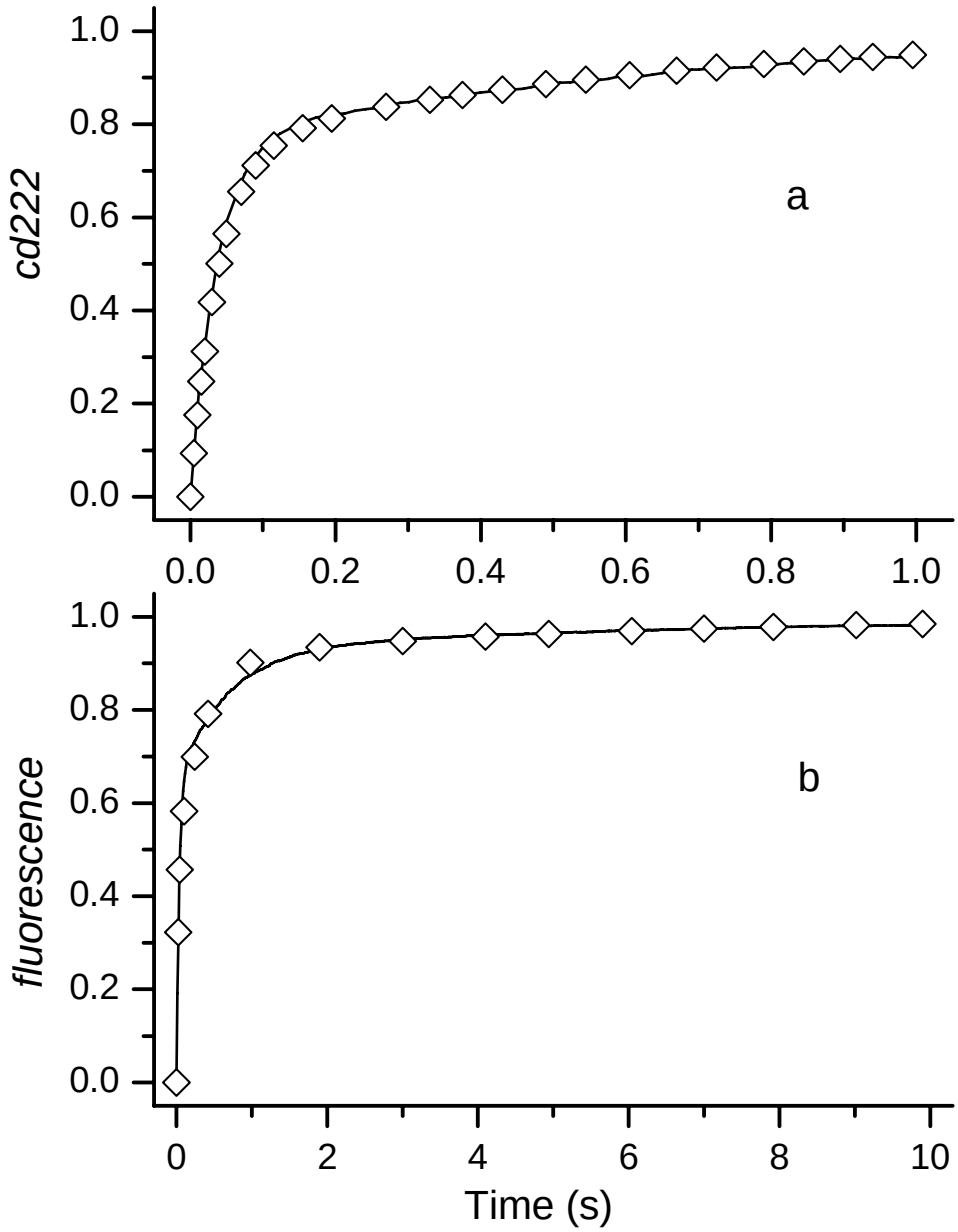


Fig. 5. Experimental (scatter) and model (solid lines) refolding kinetics of fully unfolded cyt *c* at 4.2 M [GdmCl] and by sudden dilution to 0.7 M [GdmCl]: **a,** normalized *cd222*; **b,** normalized fluorescence.

TABLE : Parameters of the foldon units.

i of the foldon unit	1	2	3	4	5
$j_i\ kJ/mol$	-11.7	-10.886	-5.86	-17.57	-7.58
$m_i\ kJ/(mol \cdot M)$	6.28	4.18	0.65	2.72	2.09
$\Delta S_i^{(0)}\ J/(mol \cdot K)$	171	-342	-71	-77.4	-93.7
$\Delta C_{p,i}\ kJ/(mol \cdot K)$	5.44	0	0	0	0
α_i^{cd222}	0.82	0.18	0	0	0
α_i^{fl}	0.7	0.24	0.06	0	0
α_i^{cd695}	0	0.	0.	1.	0