Contact order revisited: Influence of protein size on the folding rate

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Abstract

Guided by the recent success of empirical model predicting the folding rates of small two-state folding proteins from the relative contact order (*CO*) of their native structures, by a theoretical model of protein folding that predicts that logarithm of the folding rate decreases with the protein chain length L as $L^{2/3}$, and by the finding that the folding rates of multistate folding proteins strongly correlate with their sizes and have very bad correlation with *CO*, we reexamined the dependence of folding rate on *CO* and L in attempt to find a structural parameter that determines folding rates for the totality of proteins. We show that the *Abs_CO* = *CO* × *L*, is able to predict rather accurately folding rates for both two-state and multistate folding proteins, as well as short peptides, and that this *Abs_CO* scales with the protein chain length as $L^{0.70 \pm 0.07}$ for the totality of studied single-domain proteins and peptides.

Keywords: Protein folding kinetics; two-state kinetics; multistate kinetics; contact order; protein size; protein topology; rate of folding

Many proteins fold and unfold by a simple two-state transition lacking observable intermediates at any solvent conditions (Jackson 1998). Many other proteins exhibit a more complicated multistate transition; namely, they have observable folding intermediates under physiological conditions. However, the boundary between these two groups of proteins is not as well defined.

It is known that some proteins can be switched from two-state to multistate folding, and vice versa, by point mutations or even by changing conditions such as the salt concentration or temperature (Jackson 1998). In addition, multistate folding is observed only far from the point of thermodynamic equilibrium between the native and denatured states, whereas, close to this point, all proteins fold without any observable intermediates (Privalov 1979; Jackson 1998; Finkelstein and Ptitsyn 2002).

Small two-state folding proteins have attracted particular attention of experimentalists and theorists. It was demonstrated that the logarithms of in-water folding rates of these proteins correlate with their gross topological parameter called relative contact order (*CO*; Plaxco et al. 1998b). The latter is defined as

$$CO = \frac{1}{L \cdot N} \sum_{}^{N} \Delta L_{ij}, \qquad (1)$$

where N is the number of contacts (within 6 Å) between nonhydrogen atoms in the protein, L is the length of the protein in amino acid residues, and ΔL_{ij} is the number of residues separating the interacting pair of nonhydrogen atoms (adjacent residues are assumed to be separated by one residue, etc.).

CO is a renormalization of the perhaps more intuitive measure, absolute contact order (*Abs_CO*),

$$Abs\_CO = \frac{1}{N} \sum_{}^{N} \Delta L_{ij} = CO \times L, \qquad (2)$$

which, however, was found to be less correlated than CO with folding rates of the two-state folders (Plaxco et al. 1998b; Grantcharova et al. 2001).

The CO was invented to compare differences in topology (rather than in size) between proteins of different length. This parameter is small for proteins stabilized mainly by local interactions and is large when residues in a protein interact frequently with partners far away in the protein sequence. The latter should lead to slower folding (Plaxco et al. 1998b; Fersht 2000). Indeed, negative correlation between the CO and the logarithm of folding rates was found to be very strong, ~ -0.8 (Plaxco et al. 1998b; Fersht 2000) for two-state folding proteins (which also holds for all two-state folding proteins studied to date; Fig. 1, circles).

However, examining a whole set of proteins studied to date (Table 1), we see that CO, although it still gives good results for two-state folding proteins, fails to predict the folding rates of short peptides and large multistate folding proteins (Fig. 1). It seems the reason is that CO takes into account topology only and pays no explicit attention to the protein size.
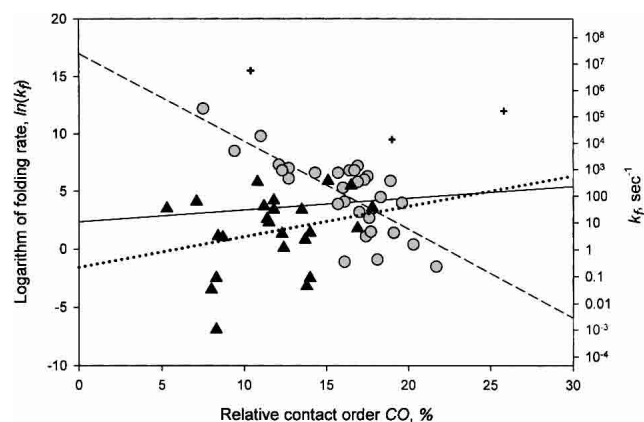
Figure 1. Natural logarithm of observed folding rate in water, $ln(k_f)$, versus relative contact order (CO) for various proteins and peptides: proteins having two-state folding kinetics at all the denaturant concentrations (circles), proteins having multistate folding kinetics in water (and at low denaturant concentrations; triangles), and short peptides (crosses). The figure includes peptides and proteins listed in Table 1; CO is computed after Equation 1 from the PDB coordinates (Bernstein et al. 1977). If several folding rates are observed for some protein (see Table 1), $ln(k_f)$ is the mean value of their natural logarithms. The dashed line represents the best linear fit for two-state folders only (the negative correlation coefficient is as significant as -0.75; the fitted dependence is $y = 16.94 - 0.76x$); the dotted line represents the best linear fit for multistate folders only (the correlation coefficient is $+0.26$; namely, it has the opposite sign compared with that for the two-state folders; $y = -1.55 + 0.26x$); the solid line represents the best linear fit for the totality of all peptides and proteins presented (the correlation coefficient is insignificant, $+0.10$ only; $y = 2.37 + 0.10x$).

A number of basic correlations between protein size and folding rate have been suggested (Thirumalai 1995; Gutin et al. 1996; Finkelstein and Badretdinov 1997a,b). All of them stress that, as might be expected, folding rate decreases monotonically with protein size, but all indicate different scaling laws for this decrease. It should be noted that some recent simulations of folding of off-lattice protein models with simplified potentials (Koga and Takada 2001) indicate that the logarithms of protein folding rate decrease with the chain length as $L^{0.61 \pm 0.18}$, which is in accordance with both Finkelstein and Badretdinov's (1997a,b) and Thirumalai's (1995) theories.

It has been shown, however, that the protein size by itself determines folding rates of only multistate folding proteins and fails to predict those for two-state folders (Galzitskaya et al. 2003): For multistate folders, the negative correlation between L^P (L being the number of residues in the chain and P a free parameter) and the logarithm of folding rates is as high as -0.80 in the broad range of power P from zero to one, whereas for two-state folders any correlation between folding rate and size is virtually absent.

This study is aimed to develop a general parameter for predicting the protein folding rates of two-state folding proteins, multistate folding proteins, and small peptides. This general estimate, if found, would be useful for two reasons: (1) Attribution of proteins to two-state or multistate folders is somewhat arbitrary, at least for proteins which can be switched from the two-state to the multistate behavior by point mutations or changing solvent conditions, and (2) it is useful to estimate the folding rate of a protein when one does not know a priori if it is two-state or multistate folding protein.

Results and Discussion

The simplest way to obtain such a parameter is to take into account both the protein topology and its size, that is, to combine a length-based theory with empirical topology effect (Plaxco et al. 1998b). Here we describe such a combination.

Specifically, a theory of Finkelstein and Badretdinov (1997a,b) predicted that in a vicinity thermodynamic midtransition, folding rates of all single-domain proteins should decrease with their lengths, L as $exp[-(0.5 \div 1.5) L^{2/3}]$, and where the size-independent coefficient $C = 0.5 \div 1.5$ depends on the topology of the protein: C is close to 0.5 when a protein is stabilized mainly by local interactions, so that semifolded protein does not contain closed loops protruding from the folding nucleus, and C is close to 1.5 when a protein has many long-range contacts, so that many closed loops protrude from the nucleus. Later it was shown (Galzitskaya et al. 2001) that the range $k_f = exp(0.5L^{2/3}) \times 10ns \div exp(1.5L^{2/3}) \times 10ns$ is valid for

Table 1. *List of proteins and polypeptides*[a]

No.	Protein	Reference	PDB	L	ln(k_f)	CO, %	Abs_CO
1	α-helix[b]	Thompson et al. 1997	—[b]	21	15.5	10.4	2.2
2	β-hairpin[c]	Munoz et al. 1997	1PGB	16	12	25.8	4.1
3	WW domain[d]	Jager et al. 2001	1PIN	34	9.5	19.0	6.5
4	E3/E1-binding domain of dihydrolipoyl acyltransferase[e]	Spector and Raleigh 1999	2PDD	41	9.8	11.0 ± 0.4	4.5 ± 0.2
5	ACBP	Kragelund et al. 1995	2ABD	86	6.6	14.3 ± 0.3	12.3 ± 0.3
6	Cytochrome b562[f]	Wittung-Stafshede et al. 1999	256B	106	12.2	7.5	7.9
7	Colicin E9 immunity protein	Ferguson et al. 1999	1IMQ	86	7.3	12.1	10.4
8	λ-Repressor	Burton et al. 1996	1LMB	80	8.5	9.4	7.5
9	Fibronectin ninth FN3 module	Plaxco et al. 1997	1FNF	90	−0.9	18.1	16.3
10	Twitchin	Clarke et al. 1999	1WIT	93	0.4	20.3	18.9
11	Tenascin (short form)	Clarke et al. 1997	1TEN	90 (89)	1.1	17.4	15.4
12	SH3 domain (a-spectrin)	Viguera et al. 1996	1SHG	62 (57)	1.4	19.1	10.9
13	SH3 domain (src)	Grantcharova and Baker 1997	1SRL	64 (56)	4	19.6	11.0
14	SH3-domain (PI3 kinase)[g]	Guijarro et al. 1998	1PNJ	90 (86)	−1.1	16.1	13.9
15	SH3-domain (fyn)	Plaxco et al. 1998a	1SHF	67 (59)	4.5	18.3	10.8
16	Photosystem I accessory protein	P. Bowers and D. Baker, unpubl.	1PSF	69	3.2	17.0	11.7
17	CspB (*Bacillus subtilis*)	Schindler et al. 1995	1CSP	67	7.0	16.4	11.0
		Perl et al. 1998			6.5		
18	CspB (*B. caldolyticus*)	Perl et al. 1998	1C9O	66	7.2	7.5	7.9
19	CspB (*Thermatoga maritima*)	Perl et al. 1998	1G6P	66	6.3	17.5 ± 0.4	11.4 ± 0.3
20	CspA	Reid et al. 1998	1MJC	69	5.3	16.0	11.0
21	Cyclophilin A	Ikura et al. 2000	1LOP	164	6.6	15.7	25.7
22	DNA-binding protein[h]	Guerois and Serrano 2000	1C8C	63	7	12.7	8.0
23	IgG binding domain of streptococcal protein L[i]	Kim et al. 2000	1HZ6	62	4.1	16.1	10.0
24	Protein G	McCallister et al. 2000	1PGB	57 (56)	6	17.3	9.7
25	FKBP12	Main et al. 1999	1FKB	107	1.5	17.7	18.9
26	Ci2	Jackson and Fersht 1991	2CI2	64	3.9	15.7	10.0
27	Activation domain procarboxypeptidase A2	Villegas et al. 1995	1AYE	80	6.8	16.7	13.4
28	Spliceosomal protein U1A[j]	Silow and Oliveberg 1997	1URN	102 (96)	5.8	16.9	16.2
29	Muscle-AcP[k]	Van Nuland et al. 1998a	1APS	98	−1.5	21.7 ± 0.6	21.2 ± 0.6

The columns in this table are as follows: Protein, name of protein; Ref, reference to the original article on folding and unfolding kinetics; PDB, Protein Data Bank entry (Bernstein et al. 1977); L, number of residues in the protein used in the experimental study, and (in parentheses) the number of residues that have defined three-dimensional coordinates and contribute to the relative contact order () calculations; ln(k_f), natural logarithm of the experimental folding rates in the water; and *Abs_CO*, absolute contact order.

[a] The list of single-domain proteins and peptides that lack both disulfide bonds and covalent bonds to ligands is taken from Galzitskaya et al. 2003). If folding of some protein was investigated at different temperatures, the experiment at the temperature closest to 25°C is presented in the Table; we took the slowest phase that is not considered as *cis/trans* proline isomerization phase in the original paper. If the three-dimensional structure of a protein whose folding was studied experimentally was absent in PDB, but PDB contains the structure of its mutant or very close homolog, the latter was used in our *CO* calculations; this is mentioned in a corresponding footnote. If several PDB entries are available for some protein, the best refined full-length X-ray structure is used in our *CO* calculation; in the absence of X-ray structure, the averaged NMR structure is used; in the absence of such, *CO* was averaged over all NMR models (in this case, the standard deviation is given). Nos. 1–3 indicate short peptides; 4–33, proteins with two-state folding within the whole range of experimental conditions; and 34–57, proteins with multistate folding in water.

[b] There is no PDB entry for the Ala-rich 21-residue α-helix studied; the ideal (Ala)_{21} α-helix was used in our contact order calculation.

[c] *ln(k_f)* value in water refers also to the midtransition point at 24°C

[d] Small WW domain consisting of one β-sheet is considered as a peptide. *ln(k_f)* value refers to the temperature 41.7°C.

[e] *ln(k_f)* value is the investigators' extrapolation of folding rate to 25°C.

[f] Two-state folding is assumed by long extrapolation made by investigators.

[g] Although the investigators of the experimental paper reported that the SH3 domain from PI3 kinase is 84 amino acids long, it was actually refolded by them with the additional two N-terminal residues and four C-terminal residues. The latter four are absent in the PDB entry.

all the studied peptides and single-domain proteins of a great variety of lengths, topologies, and folding behaviors.

Although Finkelstein and Badretdinov did not give an algorithm to compute their coefficient, *C*, from protein structure, it is clear that a physical sense of *C* is similar to those of the *CO* of Plaxco et al. Both are small for proteins with local contacts (i.e., α-helical proteins), and both are large for proteins with predominantly long-range contacts, which cannot avoid having many loops in a semifolded state. Therefore, the values of *C* and *CO* should correlate.

The simplest combination of *CO* and *L*, which seems to follow from theories of Plaxco et al. and Finkelstein and Badretdinov, may look like $CO \times L^{2/3}$. However, because we observe that *CO* is not a chain length–independent parameter (as the value *C* of Finkelstein and Badretdinov should be) but anticorrelates with the chain length, *L* (Fig. 2), for totality of proteins and peptides, we summarize *CO* and *L* in a general parameter, the "size-modified contact order" (SMCO), as

$$SMCO = CO \times L^{P} \tag{3}$$

Table 1. *List of proteins and polypeptides*[a]

No.	Protein	Reference	PDB	L	$ln(k_f)$	CO, %	Abs_CO
30	S6	Otzen and Oliveberg 1999	1RIS	101 (97)	5.9	18.9	18.4
31	*His*-containing phosphocarrier protein	Van Nuland et al. 1998b	1POH	85	2.7	17.6	15.0
32	N-terminal domain from L9	Kuhlman et al. 1998	1DIV	56	6.1	12.7	7.1
33	Villin 14T	Choe et al. 1998	2VIK	126	6.8	12.3	15.4
34	Apomyoglobin[l]	Cavagnero et al. 1999	1A6N	151	1.1	8.4	12.7
35	Colicin E7 immunity protein	Ferguson et al. 1999	1CEI	87 (85)	5.8	10.8	9.2
36	Cro protein	Laurents et al. 2000	2CRO	71 (65)	3.7	11.2	7.3
37	P16 protein	Tang et al. 1999	2A5E	156	3.5	5.3	8.3
38	Twitching Ig repeat 27	Fowler and Clarke 2001	1TIT	89	3.6	17.8	15.8
39	CD2, 1st domain	Parker et al. 1997	1HNG	98 (95)	1.8	16.9	16.0
40	Fibronectin tenth FN3 module	Cota and Clarke 2000	1FNF	94	5.5	16.5	15.5
41	IFABP from rat	Burns et al. 1998	1IFC	131	3.4	13.5	17.7
42	ILBP[m]	Dalessio and Ropson 2000	1EAL	127	1.3	12.3 ± 0.5	15.7 ± 0.6
43	CRBP II	Burns et al. 1998	1OPA	133	1.4	14.0	18.7
44	CRABP I	Burns et al. 1998	1CBI	136	−3.2	13.8	18.8
45	tryptophan synthase α-subunit[n]	Ogasahara and Yutani 1994	1QOP	268 (267)	−2.5	8.3	22.3
46	GroEL apical domain (191–345)	Golbik et al. 1998	1AON	155	0.8	13.7	21.2
47	Barstar[o]	Schreiber and Fersht 1993	1BRS	89	3.4	11.8	10.5
48	Che Y	Munoz et al. 1994	3CHY	129 (128)	1	8.7	11.2
49	Ribonuclease HI[p]	Parker and Marqusee 1999	2RN2	155	0.1	12.4	19.3
50	DHFR (dihydrofolate reductase)[q]	Jennings et al. 1993	1RA9	159	4.6	14.0	22.3
51	tryptophan synthase β₂-subunit[n]	Goldberg et al. 1990	1QOP	396 (390)	−6.9	8.3	32.5
52	N-terminal domain from PGK	Parker et al. 1995	1PHP	175	2.3	11.5	20.2
53	C-terminal domain from PGK[r]	Parker et al. 1996	1PHP	219	−3.5	8.0	17.4
54	Barnase	Matouschek et al. 1990	1BNI	110 (108)	2.6	11.4	12.3
55	T4 lysozyme[s]	Parker and Marqusee 1999	2LZM	164	4.1	7.1	11.6
56	Ubiquitin[t]	Khorasanizadeh et al. 1996	1UBQ	76	5.9	15.1	11.5
57	Suc 1[u]	Schymkowitz et al. 2000	1SCE	113 (101)	4.2	11.8	11.9

[h] The folding of mutant protein Y34W was studied experimentally; we used the available PDB structure of wild type in our calculation of *CO*.

[i] The folding of mutant protein Y47W was studied experimentally; we used the available PDB structure of this mutant in our calculation of *CO*.

[j] The folding of mutant protein F56W was studied experimentally; we used the available PDB structure of mutant Y31H/Q36R in our calculation of *CO*.

[k] The folding of mutant protein C21S was studied experimentally; we used the available PDB structure of wild type protein in our calculation of *CO*.

[l] We used the available PDB structure of a holoform of myoglobin (but without heme) in our calculation of *CO*.

[m] We used the available PDB structure of mutant protein T118S from pig in our calculation of *CO* instead of the wild type protein from rat

[n] The folding of protein from *Escherichia coli* was studied experimentally. We used the available PDB structure of the same protein from *Salmonella typhimurium* in our calculation of *CO*.

[o] The folding of mutant protein C40A/C82A was studied experimentally; we used the available PDB structure of this mutant in our calculation of *CO*.

[p] The folding of mutant protein C13A/C63A/C133A was studied experimentally; we used the available PDB structure of wild type protein in our calculation of *CO*.

[q] The folding of wild type protein was studied. We used the available PDB structure of mutant protein N37D in our calculation of *CO*. $ln(k_f)$ value refers to the summary rate of two parallel pathways of refolding of DHFR.

[r] The folding of mutant protein W290Y was studied experimentally. We used the available PDB structure of wild type protein in our calculation of *CO*.

[s] The folding of Cys-free mutant was studied experimentally. We used the available PDB structure of wild-type protein in our calculation of *CO*.

[t] The folding of bovine protein F45W mutant was studied experimentally. We used the available PDB structure of WT human protein in our calculation of *CO*.

[u] There is only a strand-exchanged form of suc1 dimer in PDB. We used a concatenation of fragment 2–88 of chain C and fragment 89–102 of chain A as a tentative structure of monomeric protein in our calculation of *CO*.

One can see that $P = 0$ corresponds to $SMCO = CO$, whereas $P = 1$ corresponds to $SMCO = Abs\_CO$.

The correlation of $SMCO$ and $ln(k_f)$, depending on the power P value, is presented in the inset in Figure 3. One can see that although any $P > 0.7$ results in approximately the same correlation for the totality of proteins and peptides, the best correlation is achieved at $P \approx 1$, that is, when $SMCO \approx Abs\_CO$. The correlation of Abs_CO and $ln(k_f)$ is presented in Figure 3.

It should be mentioned, however, that for the two-state folders, the best $ln(k_f)$–to–$SMCO$ correlation is achieved when $P = 0 \div 0.5$ rather than 1 (Fig. 3, inset).

However, this difference between the scaling laws observed for two-state folders and the other proteins correlates, to a certain extent, with the finding (Fig. 2) that CO is independent on the chain length for the two-state folders, whereas it decreases with the chain length, L, in proportion to $L^{-0.4}$ for multistate folders, and for the totality of proteins and peptides, CO decreases with their chain length, L, in proportion to $L^{-0.30 \pm 0.07}$ on the average.
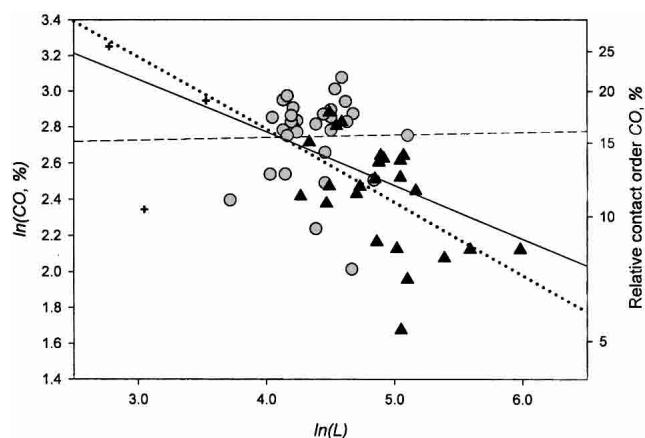
Figure 2. Logarithm of relative contact order versus logarithm of chain length. See legend to Figure 1 for specification of the symbols and other details. The dashed line represents the best linear fit for two-state folders only (the correlation coefficient is 0.02; $y = 2.68 + 0.01x$); the dotted line represents the best linear fit for multistate folders only (the correlation coefficient is -0.54; $y = 4.41 - 0.40x$); the solid line represents the best linear fit for the totality of all peptides and proteins (the correlation coefficient is -0.50; $y = 3.95 - 0.30x$). The linear regression coefficients 0.01, -0.40, and -0.30 are determined with errors ± 0.16, ± 0.13, and ± 0.07, respectively.

It is noteworthy that CO scales namely as $L^{-0.30 \pm 0.07}$ for the totality of proteins and peptides (Fig. 2, dashed line). This means that the value $Abs\_CO = CO \times L$ (which has
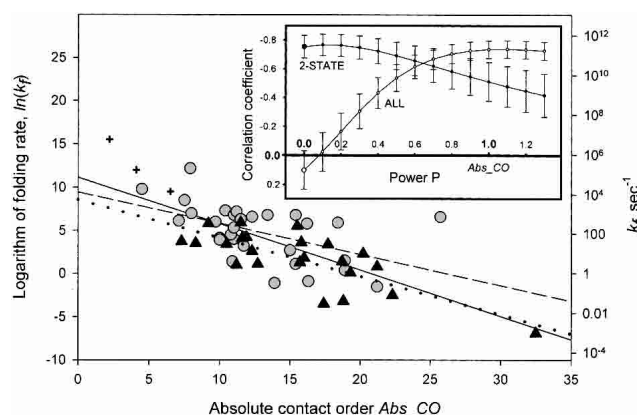
Figure 3. Logarithm of observed folding rate in water $ln(k_f)$ versus $Abs\_CO = CO \times L$. See legend to Figure 1 for specification of the symbols and other details. The dashed line represents the best linear fit for two-state folders only (the fitted dependence is $y = 9.44 - 0.36x$; the correlation coefficient is -0.51); the dotted line represents the best linear fit for multistate folders only (the fitted dependence is $y = 8.56 - 0.44x$; the correlation coefficient is -0.78); the solid line represents the best linear fit for the totality of all peptides and proteins presented (the fitted dependence is $y = 11.15 - 0.54x$; the correlation coefficient is -0.74). (*Inset*) Correlation coefficients between the logarithm of experimental folding rate in water and the value of $CO \times L^P$ depending on the value of power P. Error bars, standard errors in correlation coefficients. The curve "2-STATE" concerns the two-state folding proteins only, and the curve "ALL" concerns the totality of all studied peptides and proteins; the curve for the multistate folding proteins is not shown because it is close to the curve "ALL" up to the standard error in correlation coefficients.

the highest correlation with $ln[k_f]$ for the totality of proteins and peptides; Fig. 3, inset) scales with the chain length as $L^{0.70 \pm 0.07}$. This is in a very good concordance with a general scaling law $L^{2/3}$ predicted by Finkelstein and Badretdinov (1997a,b; although the Thirumalai's [1995] scaling law $L^{0.5}$ has only a little worse correlation with experiment, and thus, cannot be ruled out; Fig. 3, inset), and agrees with an empirical scaling $L^{0.61 \pm 0.18}$ resulting from simplified off-lattice folding simulations of Koga and Takada (2001).

Acknowledgments

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