Size and Sequence and the Volume Change of Protein Folding

Jean-Baptiste Rouget[1], Tural Aksel[2], Julien Roche[1,3], Jean-Louis Saldana[1], Angel E. Garcia[3], Doug Barrick[2] and Catherine A. Royer[1*]

Supplementary Methods

The pressure and guanidine-HCl unfolding NW and NWC

In order to better constrain the parameters, both chemical and pressure denaturation profiles for the NW and NWC constructs were analyzed globally. Emission spectra for the NW and NWC constructs at 20 μM in 150 mM NaCl, 25 mM Tris-HCl, pH 8.0, 20C were recorded between 320 and 450 nm with an excitation at 290 nm. For each spectrum, average emission wavelength is calculated using the expression,

$$\langle\lambda\rangle = \left(\frac{\sum I_\lambda \lambda^{-1}}{\sum I_\lambda} \right)^{-1} \quad (1)$$

where I_λ is the intensity of emission at wavelength λ, which is also normalized to have its maximum and minimum values set to 1 and 0 respectively. To obtain ΔV_u, two-state unfolding model is fit to $\langle\lambda\rangle$ data via the following expression,

$$\langle\lambda\rangle([x], p) = \frac{(I_F - a_F p - b_F[x])\langle\lambda\rangle_F \langle F\rangle + I_U \langle\lambda\rangle_U (1-\langle F\rangle)}{(I_F - a_F p - b_F[x])\langle F\rangle + I_U (1-\langle F\rangle)} \quad (2)$$

with

$$\langle F\rangle = \left(1 + e^{-(\Delta G_u - m[x] - \Delta V p)/RT}\right)^{-1} \quad (3).$$

Here I_F and I_u correspond to the intensity values of the folded and unfolded state, respectively at 0 M Gdn-HCl and atmopsheric pressure, $\langle\lambda\rangle_F$ and $\langle\lambda\rangle_U$ are the average emission wavelength of the folded state and unfolded state, respectively, x is the GdnHCl concentration.. To facilitate the convergence of fitting and have higher confidence in the fitted value for ΔV_u, ΔG_u and m-values are constrained to their respective values obtained from CD GdnHCl unfolding experiments. To estimate 95% confidence interval for ΔV_u, 1000 steps of bootstrapping analysis was performed.

Table S1. Comparison of the Free Energies and m-values for the urea denaturation of Nank constructs from the pressure unfolding studies with previously reported values from urea melts. (Results for urea melts are from reference 22 in the main text.)

CD urea melt			Fluorescence urea melt			HP Fluorescence		
Construct	ΔG^{o}_{u} (kcal/mol)	m (kcal/l/mol)	Construct	ΔG^{o}_{u} (kcal/mol)	m (kcal/l/mol)	Construct	ΔG^{o}_{u} (kcal/mol)	m (kcal/l/mol)
1-7	6.65	2.85	1-7	6.78	2.91	1-7	7.0 ± 1.2	2.6 ± 0.5
1-6	2.85	1.76	1-6	3.01	1.85	1-6	2.8 ± 0.1	1.2 ± 0.1
1-5	2.69	1.73	1-5	2.84	1.67	1-5	2.7 ± 0.2	1.2 ± 0.1
3-7	1.81	1.58	3-7	1.70	1.67	3-7	2.8 ± 0.5	1.4 ± 0.4
2-7	4.96	2.36	2-7	5.05	2.44	2-7	6.7 ± 1.3	2.5 ± 0.6

Table S2. Equilibrium and Activation volume changes for the Nank deletion constructs

1-7 (ref 25 in main text)			1-6			1-5		
[Urea] (M)	ΔV^{o}_{f} (ml/mol)	ΔV^{*}_{f} (ml/mol)	[Urea] (M)	ΔV^{o}_{f} (ml/mol)	ΔV^{*}_{f} (ml/mol)	[Urea] (M)	ΔV^{o}_{f} (ml/mol)	ΔV^{*}_{f} (ml/mol)
2	44 ± 4.1	37.3	1.0	48 ± 5.3	65.8 ± 5.6	1.1	47 ± 9	57.9 ± 2.1
2.1		38.2	1.4		58.8 ± 2.9	1.3		50.1 ± 1.9
2.2		38.8	1.6		61.7 ± 3.1	1.5		51.6 ± 2.2
2.3		41.0	1.8		66.4 ± 4.5	1.7		61.0 ± 2.7
			2.0		72.5 ± 10.4	1.9		64.4 ± 6.6
$\langle \Delta V^{*}_{f} \rangle = 38.8 \pm 1.5$		$\langle \Delta V^{*}_{u} \rangle = -5$	$\langle \Delta V^{*}_{f} \rangle = 65.0 \pm 5.2$		$\langle \Delta V^{*}_{u} \rangle = +17$	$\langle \Delta V^{*}_{f} \rangle = 57.0 \pm 6.1$		$\langle \Delta V^{*}_{u} \rangle = +10$

2-7			3-7			4-7		
[Urea] (M)	ΔV^{o}_{f} (ml/mol)	ΔV^{*}_{f} (ml/mol)	[Urea] (M)	ΔV^{o}_{f} (ml/mol)	ΔV^{*}_{f} (ml/mol)	[Urea] (M)	ΔV^{o}_{f} (ml/mol)	ΔV^{*}_{f} (ml/mol)
1.9	55 ± 4.7	56.3 ± 2.5	1.1	51 ± 5.58	58.2 ± 2.5	1.5	39.4 ± 5	46.12 ± 1.2
2.1		52.6 ± 2.3	1.3		54.6 ± 1.9	1.75		44.09 ± 2.2
2.1		56.3 ± 1.7	1.5		57.9 ± 4.2	1.85		48.91 ± 1.6
2.3		59.8 ± 3.5						
$\langle \Delta V^{*}_{f} \rangle = 56.3 \pm 5.7$		$\langle \Delta V^{*}_{u} \rangle = +1.3$	$\langle \Delta V^{*}_{f} \rangle = 56.9 \pm 2.0$		$\langle \Delta V^{*}_{u} \rangle = +7$	$\langle \Delta V^{*}_{f} \rangle = 46.3 \pm 1.2$		$\langle \Delta V^{*}_{u} \rangle = +6.9$

Standard deviations of equilibrium volume changes correspond to the results of rigorous confidence limit testing, taking into account the correlation between fit parameters. Standard deviations on the activation volumes correspond to standard deviations of the mean of the results from the various urea concentrations.

Table S3. Temperature dependence of the equilibrium and activation volume changes for the Nank1-7, 2-7 and 3-7 constructs

1-7 (2.1 M urea , ref 25 in main text)				2-7 (values averaged over 5 [Urea] at each T)				3-7 (values averaged over 5 [Urea] at each T)			
T (C)	ΔV^{o}_{f} (ml/mol)	ΔV^{*}_{f} (ml/mol)	ΔV^{*}_{u} (ml/mol)	T (C)	ΔV^{o}_{f} (ml/mol)	ΔV^{*}_{f} (ml/mol)	ΔV^{*}_{u} (ml/mol)	T (C)	ΔV^{o}_{f} (ml/mol)	ΔV^{*}_{f} (ml/mol)	ΔV^{*}_{u} (ml/mol)
12	69.6 ± 5.5	57.7 ± 6	-16.5 ± 5	12	72.5 ± 6.7	59.9 ± 10	-12.1	12	55.4 ± 5.6	53.5 ± 4.6	-1.8 ± 4.5
16	52.3 ± 5.3	30.7 ± 0.9	-12.6 ± 1								
20	44.2 ± 4.7	38.2 ± 1.8	-4.2 ± 1.8	20	54.5 ± 4.7	59.5 ± 6	+5	20	51.5 ± 5.5	56.9 ± 2	5.4 ± 5
24	42.5 ± 7.5	43.6 ± 1.1	0.7 ± 1.7								
28	38.9 ± 4.2	60.8 ± 3.4	21.9 ± 8	28	33.8 ± 4.5	61.5 ± 10	+27	28	41.8 ± 6.5	38.4 ± 7	-3.4 ± 7
$\Delta\alpha$	-1.8 ± 0.3	0.5 ± 0.6	2.3 ± 0.5	$\Delta\alpha$	-2.4 ± 0.1	0.1 ± 0.09	2.5 ± 0.2	$\Delta\alpha$	-0.9 ± 0.2	-1 ± 0.8	-0.1 ± 0.6

Table S4. Equilibrium and Activation volume changes for the NAG2 and NAG5 variants

NAG2			
[Urea] (M)	ΔV^{o}_{f} (ml/mol)	ΔV^{*}_{f} (ml/mol)	k_{of}
0.5	54.4 ± 3.8	85.5 ± 2.8	0.09
0.8		78.8 ± 7.8	0.035
$\langle\Delta V^{*}_{f}\rangle = 82.3 \pm 5.3$		$\langle\Delta V^{*}_{u}\rangle = +23.4$	

NAG5			
[Urea] (M)	ΔV^{o}_{f} (ml/mol)	ΔV^{*}_{f} (ml/mol)	k_{of}
1.1	47 ± 5	37 ± 1	0.01
1.3		27 ± 1	0.005
1.5		35 ± 2	0.0044
$\langle\Delta V^{*}_{f}\rangle = 33 \pm 1.3$		$\langle\Delta V^{*}_{u}\rangle = -14$	

Standard deviations of equilibrium volume changes correspond to the results of rigorous confidence limit testing, taking into account the correlation between fit parameters. Standard deviations on the activation volumes correspond to standard deviations of the mean of the results from the various urea concentrations.

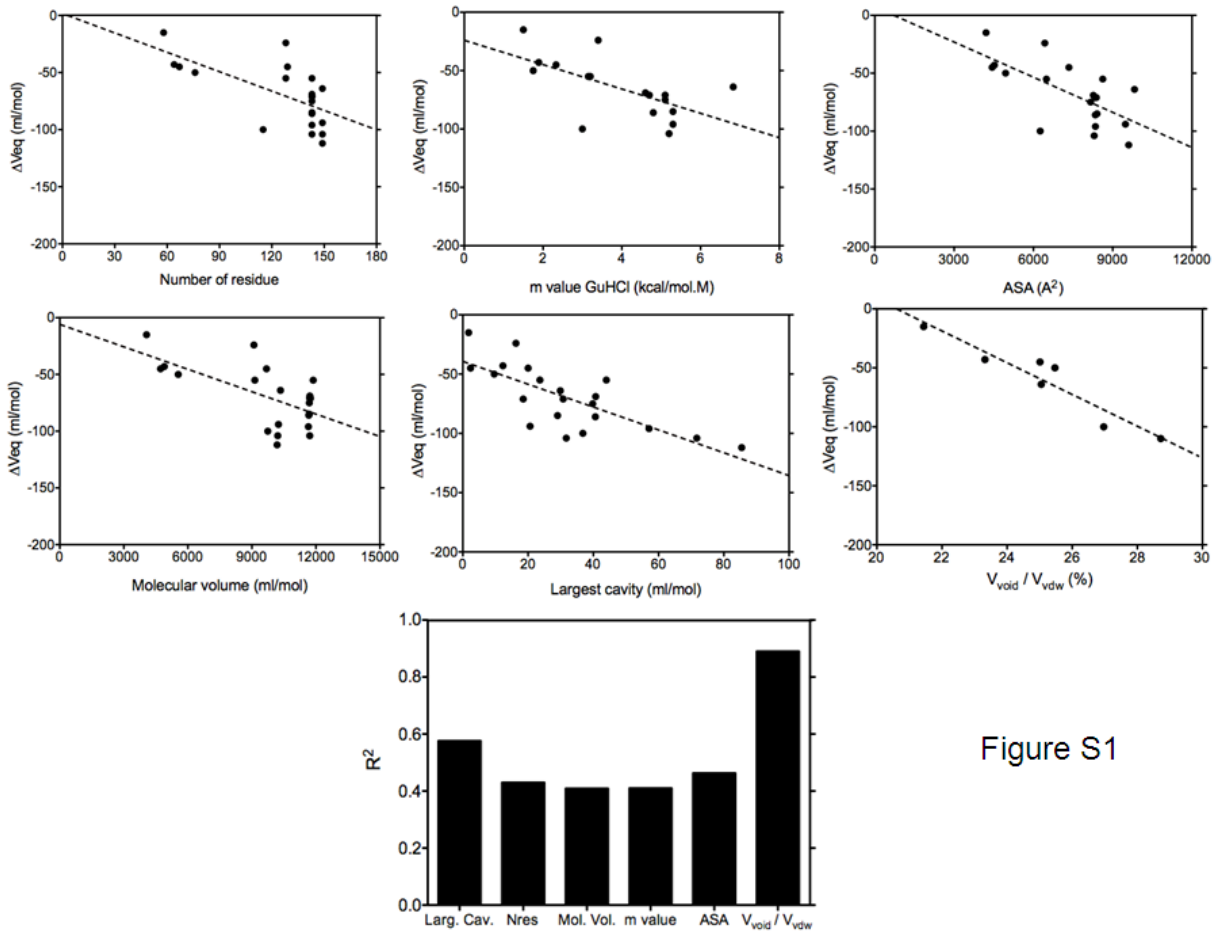

Figure S1

Figure S1. Correlation between the measured volume change upon unfolding ΔV_u and various structural or thermodynamic parameters. Data were collected for a series of monomeric proteins studied at approximately 20 degrees Celcius (reference 10, 12 -18 in the main text). Besides m-values and ΔV_u, non-experimental data were calculated from the available reference structures. Molecular volume as well as void volume, van der Waals (vdv) volume and the largest cavity volume were calculated using MCVol algorithm (reference 44 in the main text). The accessible surface area (ASA) was calculated using the g_sas algorithm from GROMACS 4.4 (reference 45 in the main text).

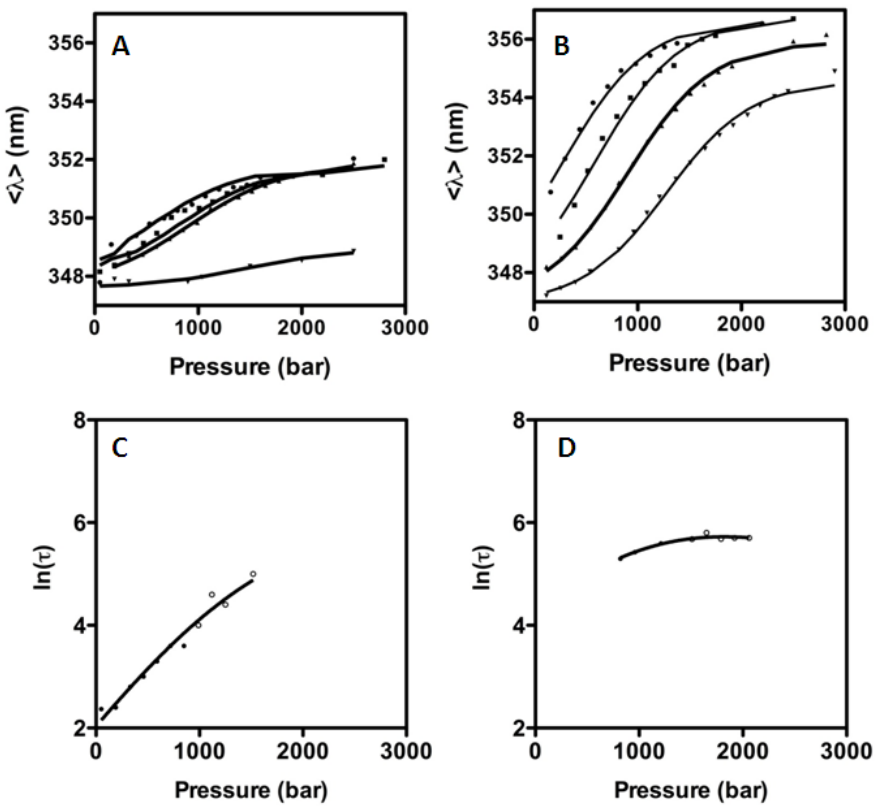

Figure S2

Figure S2. Pressure unfolding of the Nag2 (A and C) and Nag5 (B and D) mutants. A and B) Equilibrium unfolding profiles at 0.3, 0.5, 0.8 and 1M urea for A) Nag2 and 1.1, 1.3, 1.5 and 1.7 M urea for B) Nag5; C and D) Pressure dependence of the natural logarithm of the pressure-jump relaxation time for C) Nag2 at 0.5 M urea and D) Nag5 at 1.1 M urea. Data were obtained at 20°C in Tris buffer at pH 7.5 as for the variants in Figures 3 and 4 in the main text.

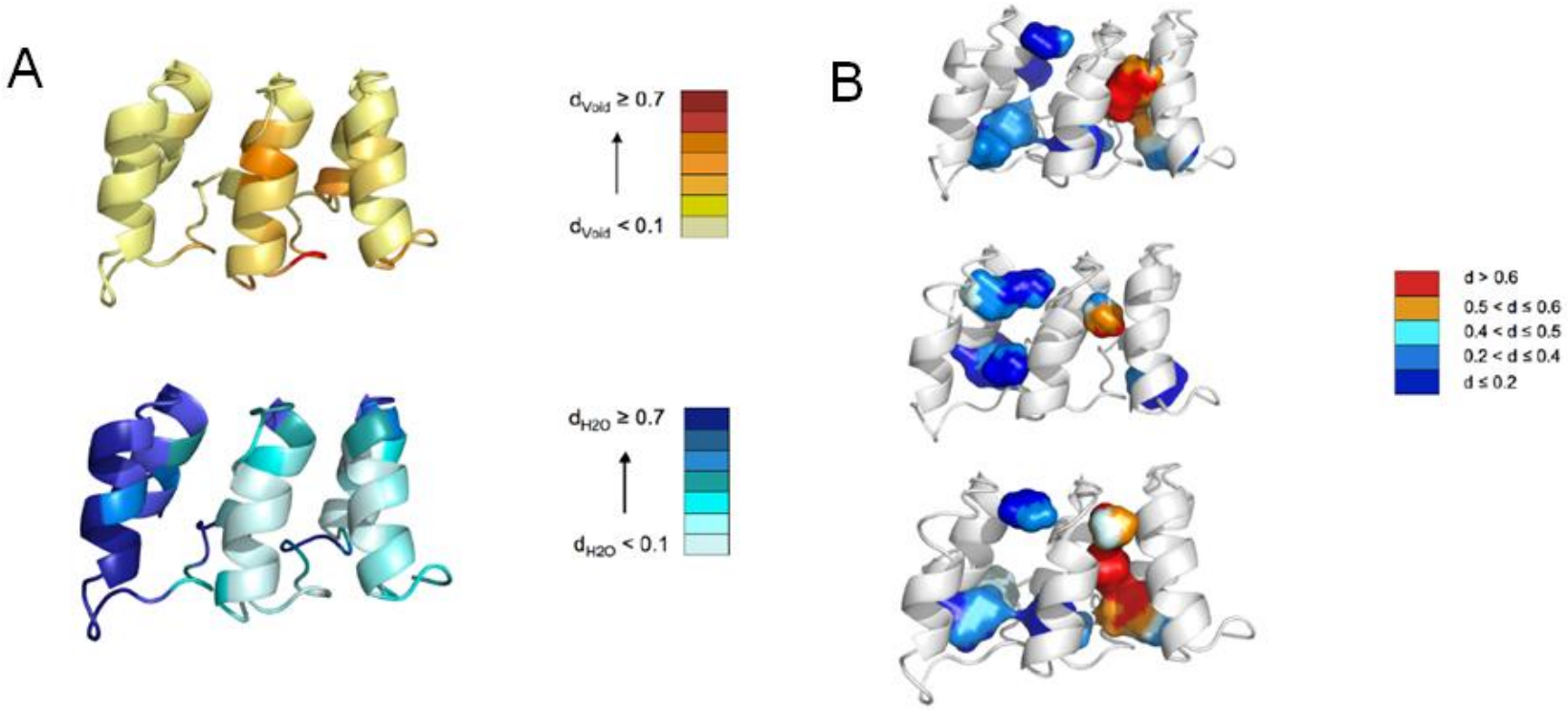

Figure S3

A) Top Figure Average void density, bottom figure average hydration density calculated as described in the main text. B) Representative configurations in which the cavities are colored according to the distance from the position in the cavity to the first water molecule. Atomic coordinates of the Nank 345 structure were extracted from the Nank full-length reference structure (1OT8.pdb) starting from GLN 80 to ALA 179. The protein was inserted in a cubic box and hydrated with 6440 SPC/E water molecules. 4 Na+ ions were added to neutralize the system. After energy minimization and equilibration steps, the resulting configuration was used as starting configuration for 10ns-long MD simulation. Configurations were saved every 10ps and the analysis methods for void density and water density calculations over the 1000 produced configurations were the same than for the full-length Nank simulation in the main text: