**Supplementary Data**

"Push and Pull": a hypothesis to unify the physical and chemical unfolding of proteins

Guilherme A. P. de Oliveira[1] and Jerson L. Silva[1]

[1] Programa de Biologia Estrutural, Instituto de Bioquímica Médica Leopoldo de Meis, Instituto Nacional de Biologia Estrutural e Bioimagem, Centro Nacional de Ressonância Magnética Nuclear Jiri Jonas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

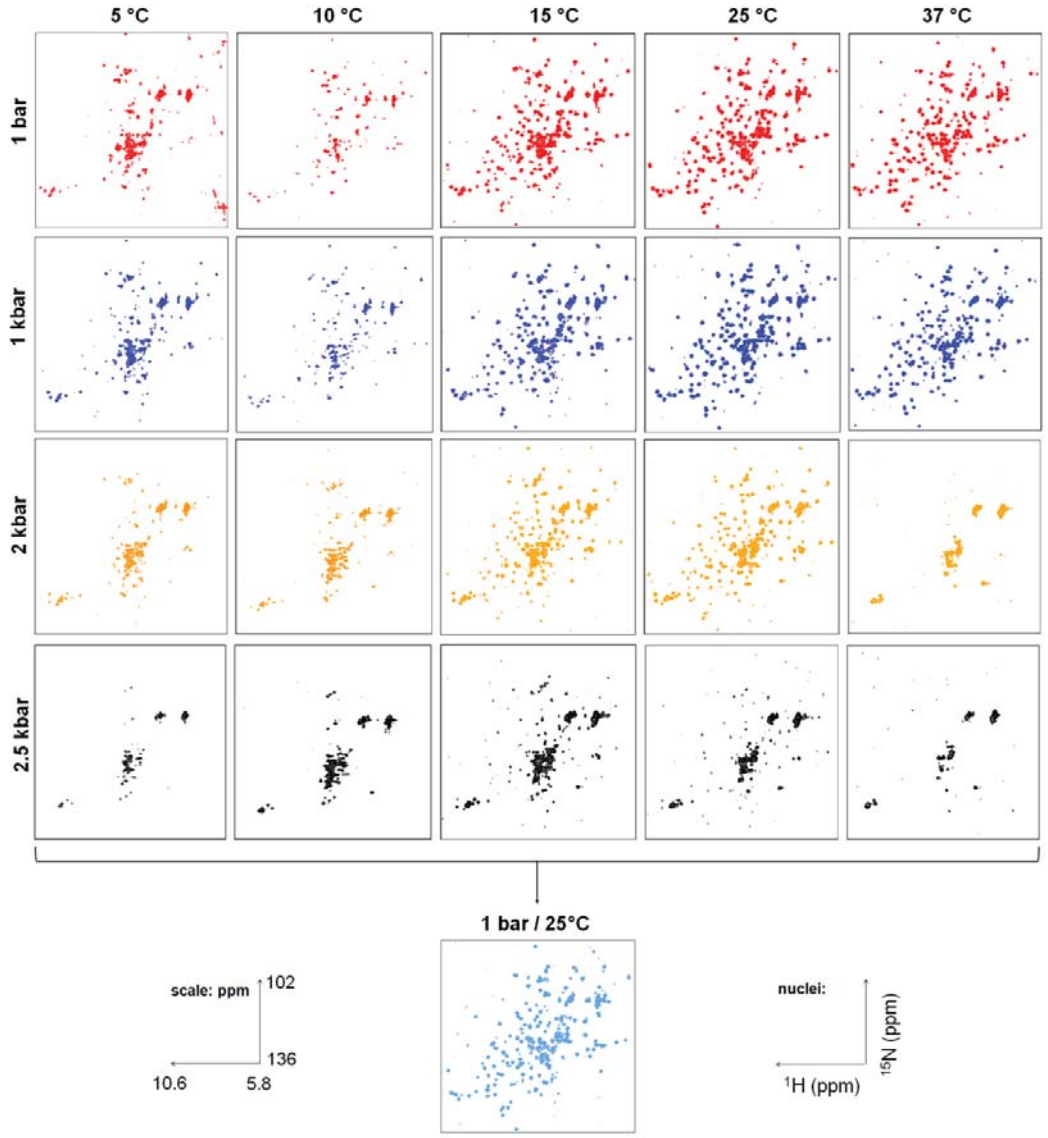


**Figure S1.** Representative $^{1}$H-$^{15}$N HSQC spectra of MpNep2 for various pressures at 5, 10, 15, 25 and 37°C. Refolding was observed for all studied conditions.

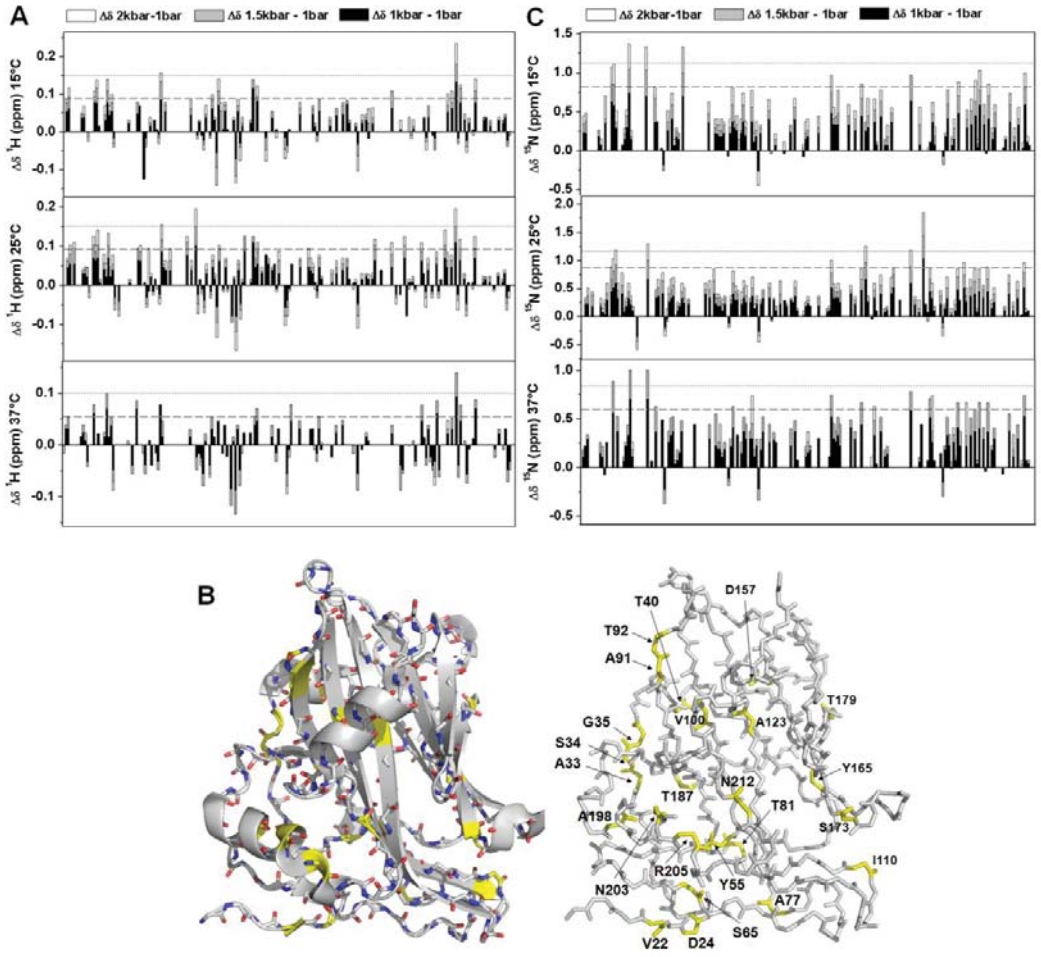


**Figure S2**. (A and C) Evaluation of $^{1}H$ and $^{15}N$ chemical-shift changes among residues at 15, 25 and 37°C and at different pressure ranges. (B) A crystal structure highlighting the most perturbed residues.

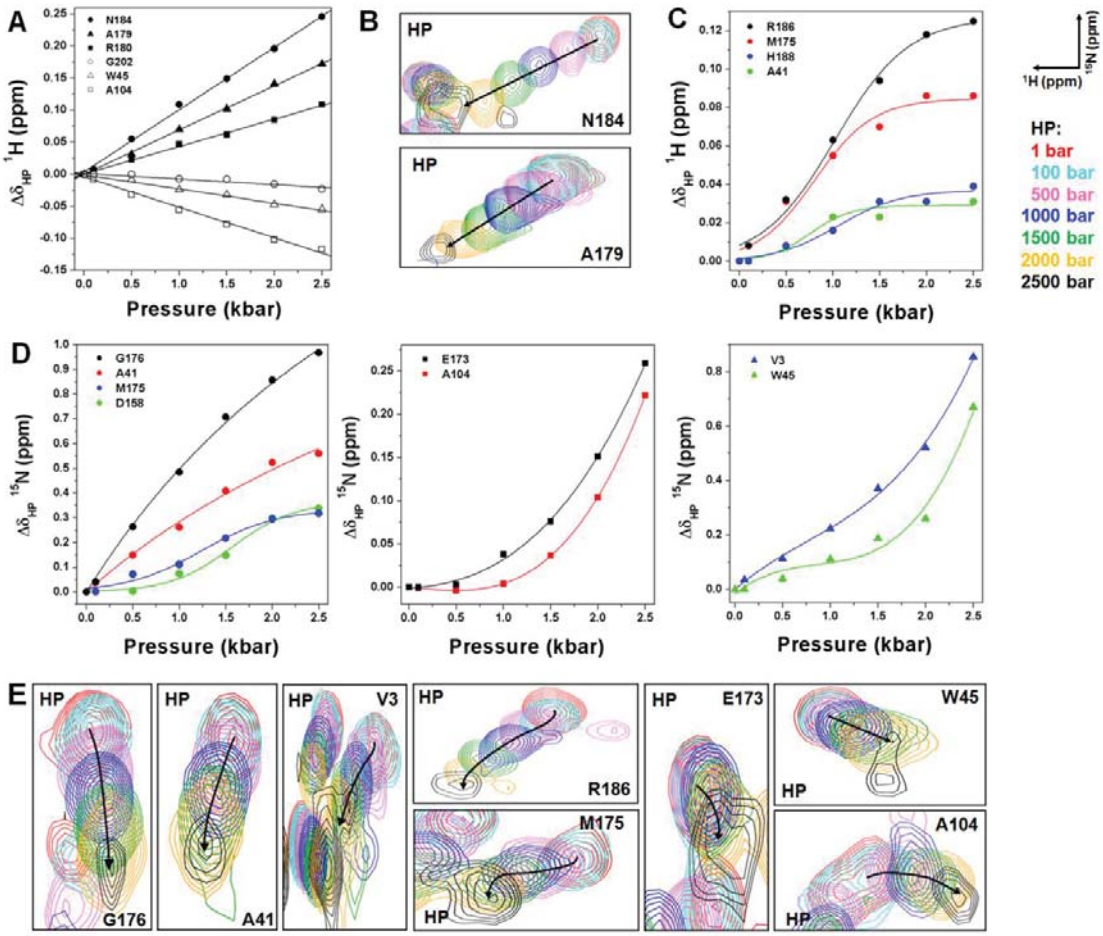


**Figure S3.** High pressure chemical shift dependence. (A-B) Representative resonances that exhibit a linear dependence of $\Delta\delta$ $^1$H chemical shifts for increasing pressure. (C, D, E) Representative resonances that exhibit a nonlinear dependence of $\Delta\delta$ $^1$H and $^{15}$N chemical shifts for increasing pressure.

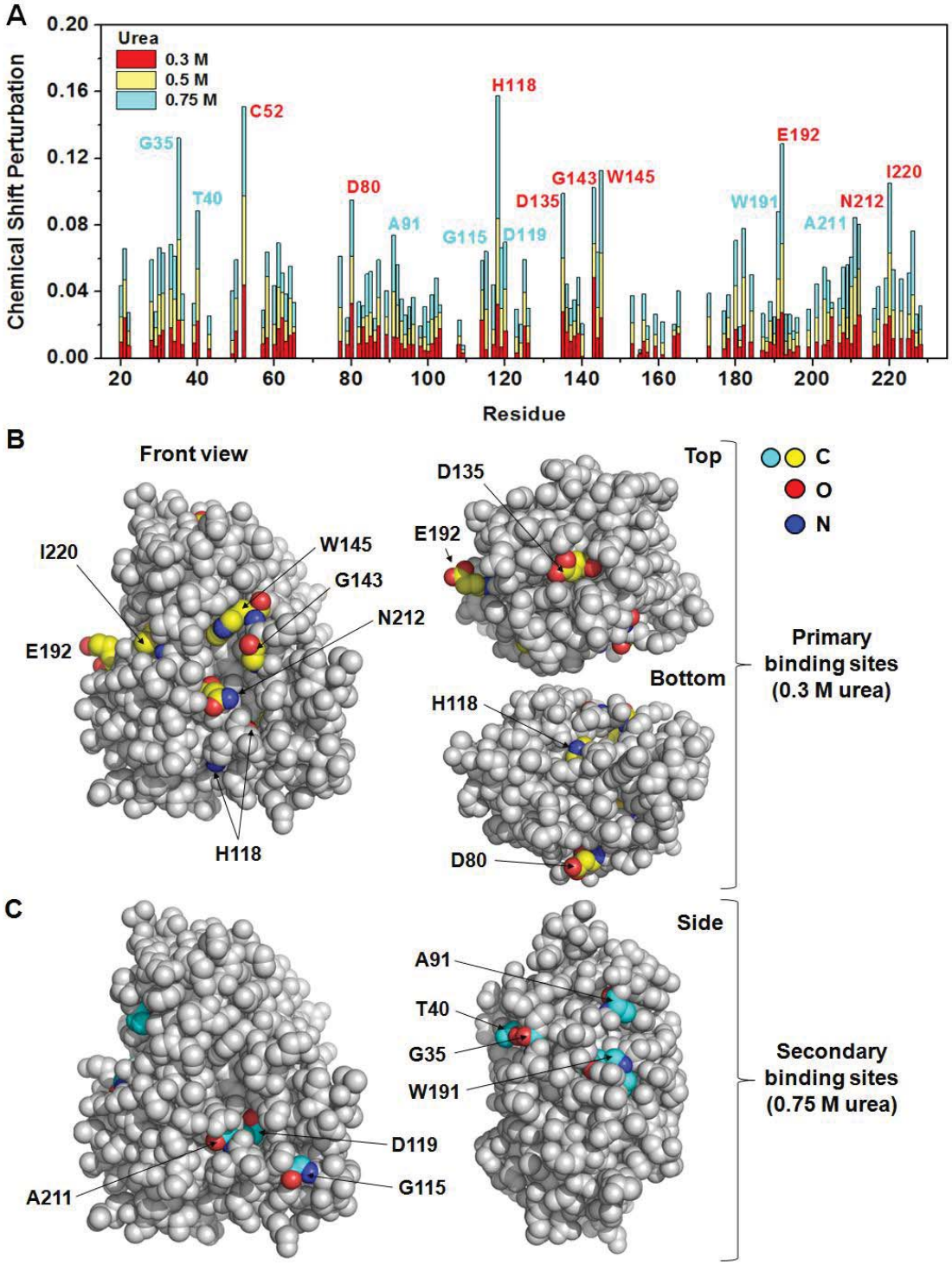


**Figure S4.** Chemical shift perturbation (CSP) analysis to monitor the preferential binding sites of urea to MpNep2. (A) CSP as a function of residues for three different concentrations of urea (0.3, 0.5 and 0.75 M) against free protein (0 M urea). The cut-off to define primary (one s.d. above the 0.3 M average, red) and secondary binding sites (one s.d. above the 0.75 M average, cyan) was the CSP average (α) among all residues plus one standard deviation (s.d.). α ± s.d. for CSP 0.3 M was 0.013 ± 0.01; for CSP 0.5 M was 0.016 ± 0.011 and for CSP 0.75 M was 0.02 ± 0.012. (B and C) Front, top, bottom and side views of the MpNep2 crystal structure (PBD: 3ST1) showing the primary (yellow) and secondary (cyan) binding sites of urea.

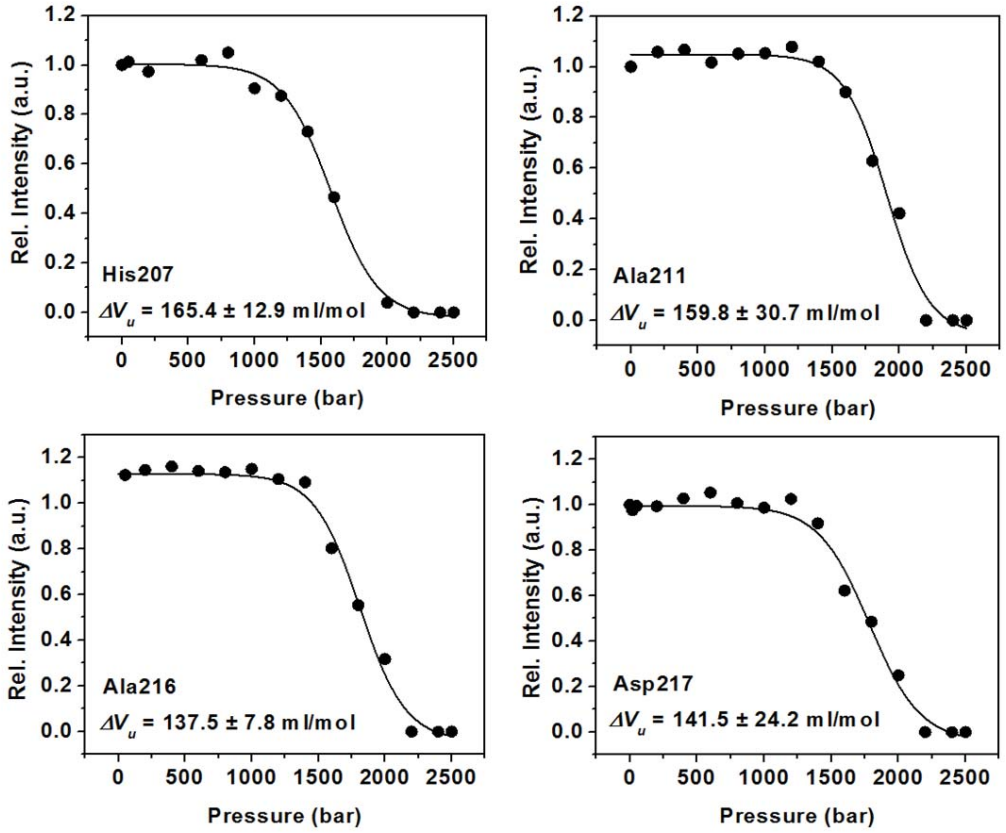


**Figure S5.** Crosspeak relative intensity vs. pressure profile obtained from $^{1}$H-$^{15}$N HSQC experiments at 37°C for H-N His207, Ala211, Ala216 and Asp217 resonances.

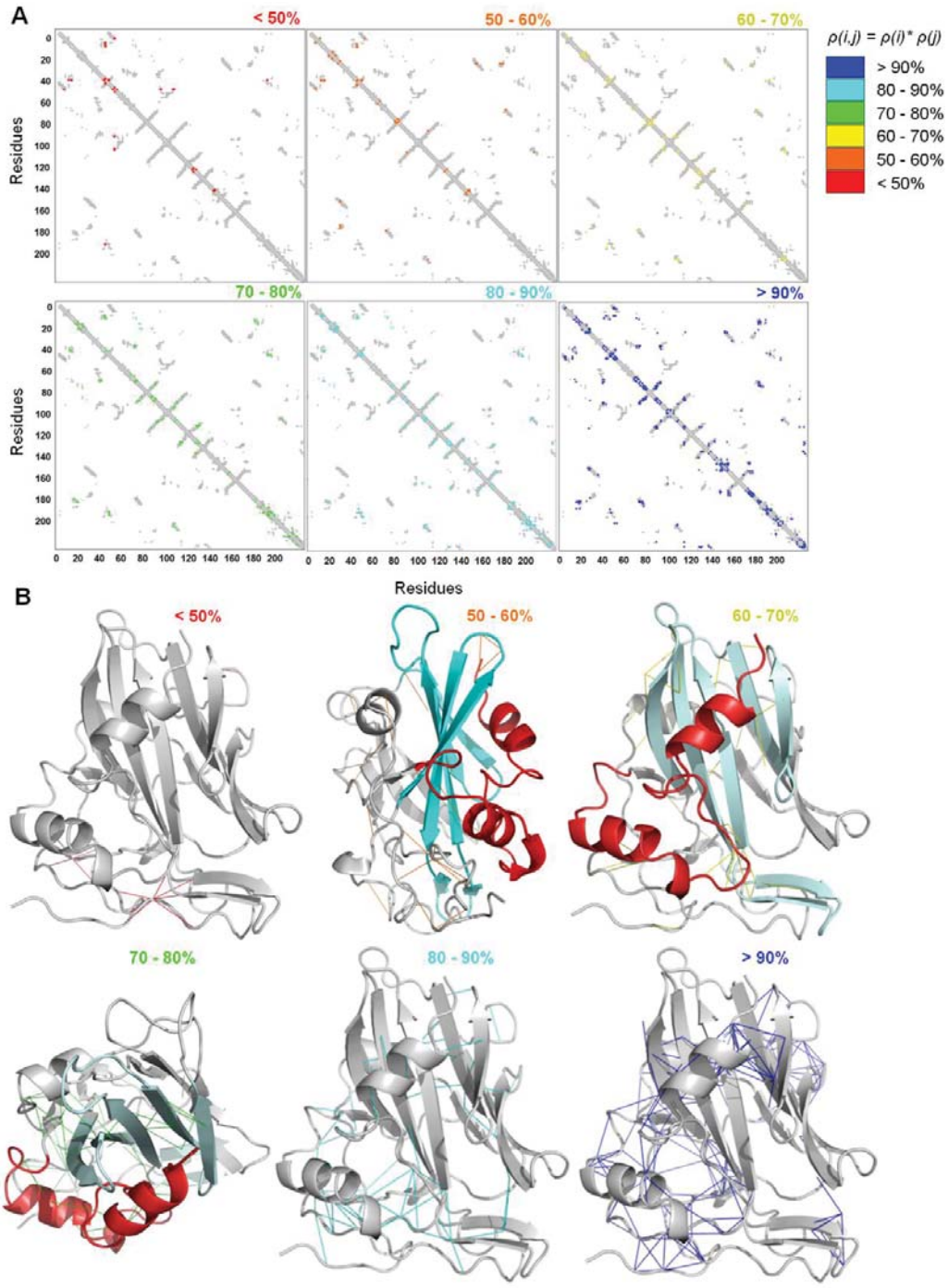


**Figure S6.** (A-B) Extended representation of the fractional contact maps at 1000 bar, 25°C and in the range between <50% and >90% of contact formation probability.

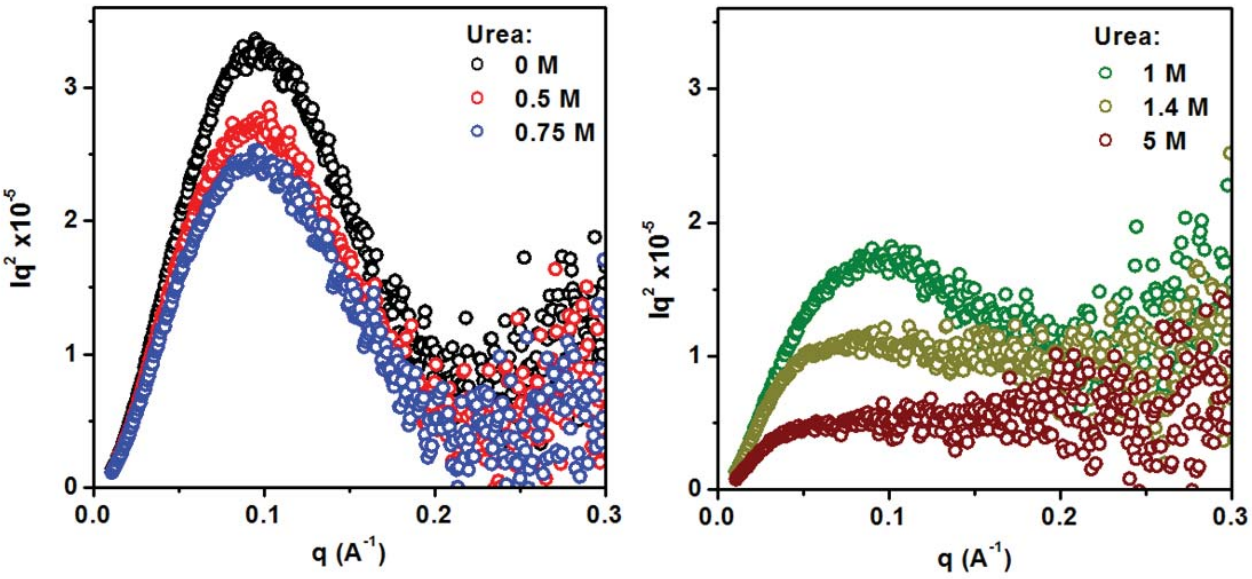


**Figure S7**. Kratky plots ($Iq^2$ as a function of $q$) are used to qualitatively distinguish between globular and disordered particles scattered in solution. Folded particles present a bell-shaped plot with a well-defined maximum of $Iq^2$ vs. $q$ (0, 0.5 and 0.75 M urea). Unfolded proteins show a slower intensity decay, reaching a plateau instead of a peak over a specific range of $q$, followed by a monotonic increase (1.4 and 5 M urea). The ensemble of partially unfolded particles leads to increased scattering at higher angles and intermediate behavior between the folded and random chain (1 M urea).

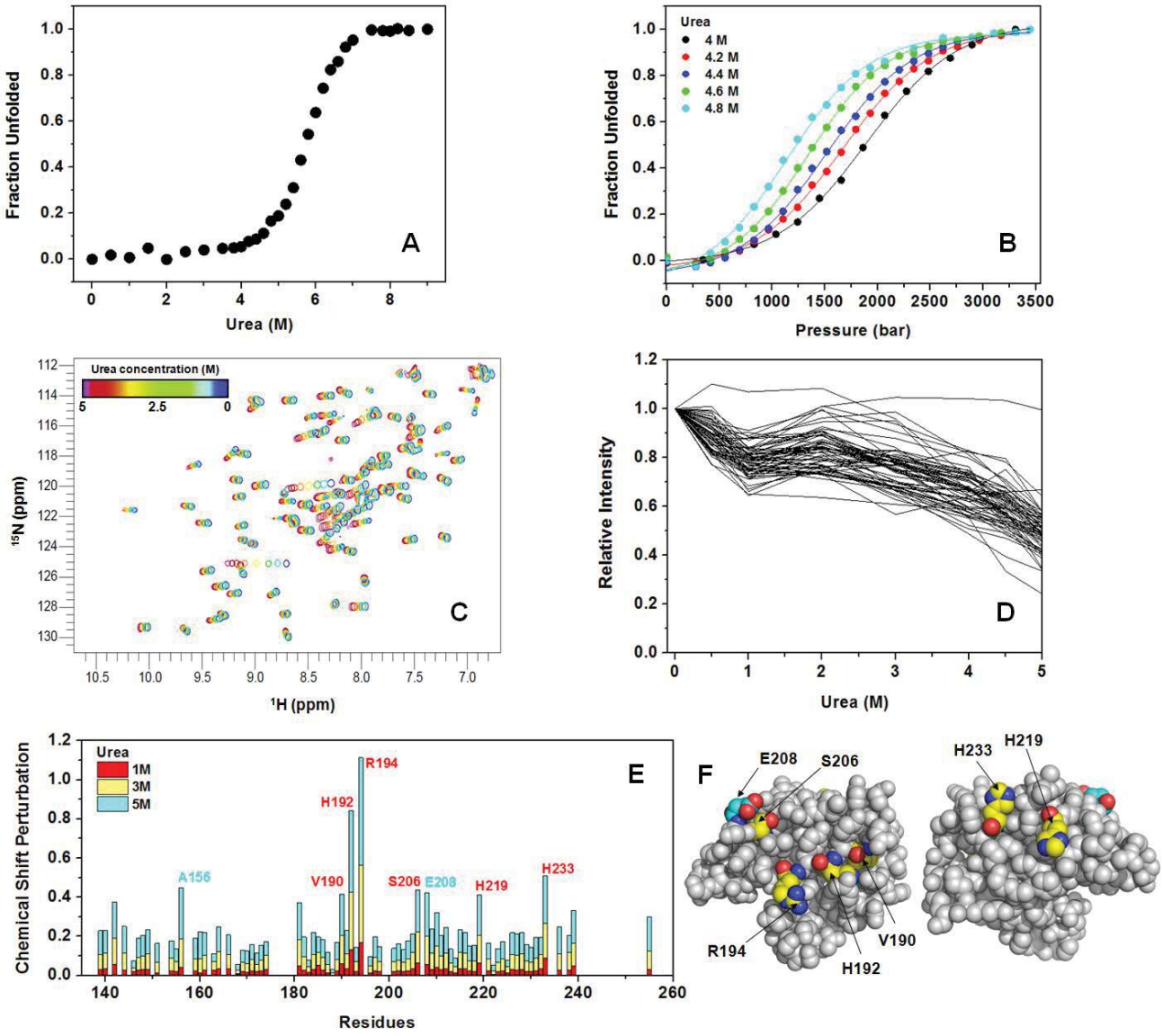


**Figure S8.** High pressure and urea spectroscopic studies on the SH2 domain. (A) Urea and (B) high pressure titrations at different sub-denaturing concentrations of urea monitored by Trp fluorescence. (C) $^{1}$H-$^{15}$N HSQC chemical shift dependence of SH2 upon increasing urea concentration. (D) Line broadening analysis of the response to increasing concentrations of urea. (E) Chemical shift perturbation (CSP) analysis to monitor the preferential binding sites of urea to the SH2 domain. CSP as a function of residues for three different concentrations of urea (1, 3 and 5 M) against free protein (0 M urea). $\alpha \pm$ s.d. for CSP 1 M was $0.031 \pm 0.024$, for CSP 3 M was $0.077 \pm 0.055$ and for CSP 5 M was $0.113 \pm 0.079$. (F) The SH2 crystal structure (PBD: 1OPK) showing the primary (yellow) and secondary (cyan) binding sites of urea. The atom color scheme is the same as that in Figure S5.

**Table S1.** Pressure-induced unfolding using sub-denaturing concentrations of urea reveal increased volume changes ($\Delta V_u$).

| Protein | Urea (M) | $\Delta V_u$ (mL/mol) | $\Delta V_u$ Behavior | Reference |
|---|---|---|---|---|
| MpNep2 | 0 | 96.1 ± 45.0 |  increase | This work |
| | 0.3 | 103.2 ± 26.8 | | |
| | 0.5 | 114.7 ± 26.1 | | |
| SNAse* | - | - | increase | 50, 51 |
| skTnC F29W N-Domain (1-90) **Apo** state | 2.0 | 30.19 ± 0.69 |  increase | 56, 57 |
| | 2.5 | 38.13 ± 1.73 | | |
| | 3.0 | 44.24 ± 1.68 | | |
| | 3.5 | 48.62 ± 2.03 | | |
| skTnC F29W N-Domain (1-90) **Holo** state | 5.0 | 36.74 ± 0.76 |  increase | 56, 57 |
| | 5.5 | 36.60 ± 1.40 | | |
| | 6.0 | 46.39 ± 1.12 | | |
| | 7.0 | 59.59 ± 0.73 | | |
| | 8.0 | 60.65 ± 3.95 | | |
| skTnC F105W C-Domain (88-162) **Holo** state | 4.0 | 44.24 ± 2.88 |  increase | 58 |
| | 5.0 | 44.16 ± 0.58 | | |
| | 6.0 | 51.27 ± 0.66 | | |
| | 7.0 | 58.79 ± 0.45 | | |
| | 8.0 | 61.47 ± 1.75 | | |
| skTnC F29W (1-161) **Apo** state | 2.0 | 21.31 ± 0.28 |  increase | 24 |
| | 3.0 | 27.11 ± 1.29 | | |
| | 3.5 | 34.52 ± 0.99 | | |
| | 4.0 | 44.90 ± 1.58 | | |
| skTnC F29W (1-161) **Holo** state | 5.0 | 25.89 ± 1.07 |  increase | 24 |
| | 5.5 | 39.44 ± 1.09 | | |
| | 6.0 | 44.08 ± 1.25 | | |
| | 7.0 | 47.69 ± 0.50 | | |
| skTnC F105W (1-161) **Holo** state | 4.0 | 39.97 ± 1.82 |  increase | 58 |
| | 5.0 | 46.57 ± 2.06 | | |
| | 6.0 | 57.84 ± 2.70 | | |
| | 7.0 | 57.43 ± 1.56 | | |
| SH2 | 4.0 | 60.60 ± 1.60 |  increase | This work |
| | 4.2 | 62.06 ± 1.23 | | |
| | 4.4 | 61.82 ± 1.83 | | |
| | 4.6 | 74.04 ± 1.83 | | |
| | 4.8 | 74.52 ± 2.23 | | |

*Systematic studies on wild-type and different SNAse mutants using high-pressure and Gdm-Cl