Protein (name, PDB id, etc.)

Variants (position, substitution)

Experimental method (technique, pH, salt concentrations, T, etc. used)

Stability data (∆G and/or Tm of WT and mutant, or corresponding ∆∆G and/or ∆Tm and any indication stabilizing/destabilizing)

**Stability 01 (PMID: 24945274)**

Applied to HET-s this would mean that a change of the stability of the β-solenoid may alter heterokaryon incompatibility, spontaneous prion formation, prion propagation and spore killing (Figure 2, Figure 3, Tables 1 and 2). In order to get insights into the individual contribution of the amino acid side chains to the stability of the HET-s prion, Ala variants K229A, I231A, V239A, Q240A, L241A, N262A, V264A, V267A, E272A, S273A, G278A, F286A, D288A and F286A/W287A were measured by a fibril denaturation assay using GuHCl (Figure 5 and Table 3) following the concept by Santoro and Bolen to study protein folding and unfolding [61]… The positive ΔΔG value for K229A of 1.5 kcal/mol indicates that the salt bridge between K229 and E265 does not play a favorable stability effect on the β-solenoid structure. Similarly, the replacement E272A (removing the E272-R236 salt bridge) results in a positive stability effect of approximately 2 kcal/mol. The amino acid replacements in the hydrophobic core at position V239, L241, V264 also had a 1 kcal/mol positive effect on stability V267 had no effect.

**Stability 02 (PMID: 25184759)**From Table 1, the ΔΔGU values for P19A and P18A are −11.8 and ca. −1.2 kJ/mol, respectively. The data appearing in Table 1 provide multiple determinations for a number of single site mutations and for acidification (pH 2.5 stabilities versus those at pH 7). We designate the acidification effect as ΔpH: ΔpH = 3.13 ± 0.61 kJ/mol (over 10 observed cases). Three single site mutation effects, all fold-destabilizing, are also observed in three or more instances: Δ(S14A) = 5.55 ± 0.43 (n = 3), Δ(R16nva) = 5.5 ± 1.6 (n = 4), and Δ(P17A) = 2.37 ± 0.46 kJ/mol (n = 3). All of these are based on the ΔGU300K values in Table 1. A P12W mutation was also examined in multiple situations (ΔΔGUmut = −1.7 – +2.9 kJ/mol); no single value can be given for this mutation, vide infra. The most destabilizing single site mutation for the Trp-cage, other than P19A and Y3A, is the S14A mutation. The basis for this is now established. In all cases, Hγ of the serine hydroxyl forms an H-bond to O=C-Gly11. In the crystal structure23 of a cyclic Trp-cage that lacks the R16/D9 salt bridge due to the pH employed for crystallization, the S14Hγ/O=C-Gly11 interaction is clearly present and the Ser-Oγ accepts an H-bond from R16HN (the amide NH). In analogues with an R16/D9 salt-bridge, the Arg side chain wrapping around the indole ring places R16Hε, a side chain NH, in position for H-bonding to Ser-Oγ either directly or via a tightly associated water molecule.23,27a These H-bonding interactions are required to allow the burial of the polar S14 side chain in the hydrophobic core, and they contribute a net stabilization of the cage fold. As a result, even though the introduction of an additional alanine in the 310 helix might be expected to provide some net stabilization to this secondary structure element, an S14A mutation is fold-destabilizing (ΔΔGU = −5.5 ± 0.4 kJ/mol).

Table 1. Thermodynamic parameters for wild-type and mutants of mnemiopsin obtained by analysis of equilibrium denaturation curves of [Fig. 2](https://www.sciencedirect.com/science/article/pii/S1386142516300208?via%3Dihub" \l "f0010); a and b, using Eq. [(1)](https://www.sciencedirect.com/science/article/pii/S1386142516300208?via%3Dihub" \l "fo0005).

| Variants | Equilibrium parameters of fluorescence measurements[a](https://www.sciencedirect.com/science/article/pii/S1386142516300208?via%3Dihub#tf0005),[b](https://www.sciencedirect.com/science/article/pii/S1386142516300208?via%3Dihub#tf0010) | | | Equilibrium parameters of CD measurements[a](https://www.sciencedirect.com/science/article/pii/S1386142516300208?via%3Dihub#tf0005),[b](https://www.sciencedirect.com/science/article/pii/S1386142516300208?via%3Dihub#tf0010) | | |
| --- | --- | --- | --- | --- | --- | --- |
| m-Value[a](https://www.sciencedirect.com/science/article/pii/S1386142516300208?via%3Dihub#tf0005) | [Urea]50% | ΔG(H2O)[a](https://www.sciencedirect.com/science/article/pii/S1386142516300208?via%3Dihub#tf0005) | m-Value[a](https://www.sciencedirect.com/science/article/pii/S1386142516300208?via%3Dihub#tf0005) | [Urea]50% | ΔG(H2O)[a](https://www.sciencedirect.com/science/article/pii/S1386142516300208?via%3Dihub#tf0005) |
| WT | 4.14 ± 0.005 | 3.43 ± 0.004 | 14.20 ± 0.004 | 4.93 ± 0.003 | 3.63 ± 0.002 | 17.92 ± 0.003 |
| R39K | 4.19 ± 0.008 | 3.79 ± 0.007 | 15.90 ± 0.006 | 5.08 ± 0.005 | 3.87 ± 0.004 | 19.65 ± 0.007 |
| S128G | 3.36 ± 0.003 | 3.71 ± 0.003 | 12.46 ± 0.004 | 4.70 ± 0.009 | 3.48 ± 0.007 | 16.34 ± 0.008 |
| V183T | 4.45 ± 0.003 | 3.56 ± 0.002 | 15.86 ± 0.004 | 5.11 ± 0.008 | 3.80 ± 0.006 | 19.40 ± 0.006 |

**Stability 03 (PMID: 27616457)**

Table 3. Thermodynamic parameters for WT brazzein and mutants obtained by analysis of equilibrium denaturation curves of Fig. 9 using equation (3).

| **Protein variants** | [**a**](https://www.sciencedirect.com/science/article/pii/S0300908416301730?via%3Dihub#tbl3fna)**ΔG(H2O)**[**b**](https://www.sciencedirect.com/science/article/pii/S0300908416301730?via%3Dihub#tbl3fnb) | [**a**](https://www.sciencedirect.com/science/article/pii/S0300908416301730?via%3Dihub#tbl3fna)**m-value**[**b**](https://www.sciencedirect.com/science/article/pii/S0300908416301730?via%3Dihub#tbl3fnb) | **[Urea]50%**[**b**](https://www.sciencedirect.com/science/article/pii/S0300908416301730?via%3Dihub#tbl3fnb) |
| --- | --- | --- | --- |
| WT | 1.97 ± 0.09 | 0.52 ± 0.02 | 3.79 ± 0.22 |
| A19D | 1.73 ± 0.07 | 0.60 ± 0.03 | 2.88 ± 0.18 |
| A19K | 1.51 ± 0.05 | 0.52 ± 0.02 | 2.90 ± 0.15 |
| A19G | 2.03 ± 0.09 | 0.44 ± 0.03 | 4.61 ± 0.37 |

In quantitative analysis of urea denaturation curves by fitting of experimental data to equations [(1)](https://www.sciencedirect.com/science/article/pii/S0300908416301730?via%3Dihub#fd1), [(2)](https://www.sciencedirect.com/science/article/pii/S0300908416301730?via%3Dihub" \l "fd2), [(3)](https://www.sciencedirect.com/science/article/pii/S0300908416301730?via%3Dihub" \l "fd3). The free energy change of protein in unfolding reaction at different concentration of urea is theoretically extrapolated to the zero concentration of urea by fitting experimental data to equation [(3)](https://www.sciencedirect.com/science/article/pii/S0300908416301730?via%3Dihub#fd3) and reported as ΔG (H2O).

**Stability 04 (PMID: 28250938)**

Wild-type FimH and the two mutants were expressed in the protease-deficient E. coli HM 125, extracted from the periplasm and purified to homogeneity (Fig. 1▸ a). Their exact molecular weights were confirmed by ESI-MS (Supplementary Fig. S1). Identical CD spectrum profiles (Fig. 1▸ b) indicate that the mutants in the native conformation retain a similar secondary structure to the wild type (WT). Furthermore, the stability of the mutants is preserved, as verified by GdmCl-unfolding experiments. The conformational transition curves of the WT and the mutants show superimposable profiles, with similar transition midpoint values (Fig. 1▸ c). The free energy of folding and the folding cooperativity for the WT and the Y48A and Y137A mutants indicate that the Y48A mutant is the least stable protein, with the lowest folding cooperativity, whereas FimH may even be stabilized by the Y137A mutation (Table 2▸).

Table 2. Stability and folding cooperativity of FimH tyrosine-gate mutants as derived from GdmCl-induced unfolding (Fig. 1▸ c)

All energies are given in kJ mol−1.

WT FimH Y48A FimH Y137A FimH

Free energy of folding −50.00 ± 3.07 −44.05 ± 2.37 −53.71 ± 4.53

Folding cooperativity 18.17 ± 1.11 15.95 ± 0.85 19.63 ± 1.64

**Stability 05 (PMID: 29637772)**

Here, we show that the D187N mutant gelsolin in a Ca2+ depleted, low pH-activated, open conformation could assemble into amyloidogenic oligomers without necessarily undergoing the specific proteolytic step. Although both wild-type (WT) and mutant proteins exhibit closely overlapping globular shapes at physiological conditions, the latter exhibits subjugated actin depolymerization, loss of thermodynamic stability, and folding cooperativity.

The relative structural stabilities of the two proteins were studied by thermal unfolding at pH 7. Far-UV CD spectra (210 nm) were monitored as a function of increasing temperature. Both WT and mutant gelsolin unfolded with a single transition over the temperature range of 20–90 °C (Figure 4A). Both of the proteins started to lose native secondary structure (change in 210 nm band) above 45 °C and precipitated into large visible aggregates beyond 60 °C with an apparent loss of CD signal. Since the thermal refolding curves were irreversible, a reliable estimate of free energy (ΔG) could not be determined. The resulting curves were analyzed, and Tm (melting temperature) was calculated. Tm is the temperature where half of any protein population is denatured and thus can be used for a comparative estimate of stability between two proteins. The Tm values of WT and mutant gelsolin were found to be ∼54 and 51 °C, respectively, indicating a destabilizing effect of the D187N point mutation (Table 2).

**Stability 06 (PMID: 23861663)**

Thirteen mutants that have relatively more negative ΔΔG were tested experimentally, including V25I, T30M, A33Y, T50M, T54Y, A81M, V88L, V90I, L106M, N107F, D109E, V120I, and N124F. Melting temperatures of the wild-type (WT) and mutants were determined (see Materials and Methods) and are listed in Table 2. The WT FbFP has a Tm of 42.8°C, indicating a moderate thermostability. The temperature fluorescence plot suggests at 37°C, 25% of fluorescence is already lost compared to that of 14°C which is the starting temperature of measurements (Figure 1). Eight of the tested mutants displayed improved stability (Table 2), whereas four mutants are less stable and one mutant L106M loses fluorescence at room temperature, presumably because L106 points to the active site and the mutant might perturb the FMN binding pocket.

**Stability 07 (PMID: 26307947)**

To evaluate unfolding phenomenon and thermodynamic stability, thermal denaturation studies of

proteins were performed using CD spectroscopy. Thermal and chemical denaturation studies revealed that mutants Arg658Cys and Ile738Val have a decrease in Tm and ∆G than the wild type. In silico studies of BARD1 BRCT (568-777) and mutant protein indicate loss in structural compactness on the Ile738Val mutant. The transition mid-point( Tm) calculated for BARD1 BRCT (568-777) wild- type, Arg658Cys and Ile738Val mutant protein were 40.6°C, 41.7°C and 47.8°C respectively. The

changes in free energies corresponding to the denaturation were: ∆G°H2o 9.6 ± 0.32 kcal/mol,

7.3± 0.12 kcal/mol, 7.6±.42 kcal/mol respectively. It is therefore, concluded that BARD1 BRCT (568-777) wild- type and Ile738Val mutant proteins have higher thermal stability than Arg658Cys (Figure: - 4B, 4C, 4D). Fluorescence spectroscopy was used to monitor unfolding at 283 K induced by GuHCl. For chemical denaturation of BARD1 BRCT (568-777) wild- type, and Arg658Cys, Ile738Val mutant proteins, thermodynamic parameters were calculated by plotting average fluorescence emission wavelength against GuHcl concentration (Figure: -3B). ΔG° H2O for BARD1 BRCT, Arg658Cys, and Ile738Val were 7.19 ± .36 kcal/mol, , 6.29 ± .41 kcal/mol, 6.8 ± .21 kcal /mol respectively.

**Stability 08 (PMID: 16501221)**

To determine the effects of the substitutions on protein stability, we examined the urea‐induced equilibrium unfolding of the RICK‐CARD mutants by monitoring changes in fluorescence emission and far‐UV CD in the presence of urea‐containing buffers (Fig. 3). Two probes were monitored in the case of fluorescence emission. Samples were excited at 280 nm to monitor the fluorescence emission of tryptophanyl and tyrosinyl residues, or samples were excited at 295 nm to monitor tryptophanyl fluorescence emission. It has been shown that folding intermediates may show different fluorescence emission properties with use of these two probes (Clark et al. 1993; Bose and Clark 2001). The equilibrium folding of wild‐type RICK‐CARD is well described by an apparent two‐state folding mechanism (ΔG = 3.0 ± 0.1 kcal mol−1, m = 1.27 ± 0.06 kcal mol−1 M−1 at pH 8 and 25°C) (Chen and Clark 2003), and representative data are shown in Figure 3 for comparison with the mutants. The results show that two mutants (P87A and P85A/P87A) are described by an apparent two‐state equilibrium folding process. In agreement with the CD spectra (Fig. 2), which show a loss in secondary structure, the proteins are less stable than is wild‐type RICK‐CARD. The mutation of P87 to alanine (Fig. 3C) was the least perturbing (ΔG = 2.4 ± 0.1 kcal mol−1, m = 1.59 ± 0.08 kcal mol−1 M−1 at pH 8 and 25°C), although the m‐value is significantly larger than that of wild‐type RICK‐CARD. Overall, the data for this mutant are consistent with a well‐formed native structure in the absence of denaturant. This agrees with the far‐UV CD spectra (Fig. 2) that showed the P87A substitution to be the least perturbing. In addition, a two‐state process best described the data for the double mutant, P85A/P87A (ΔG = 1.2 ± 0.1 kcal mol−1, m = 0.62 ± 0.08 kcal mol−1 M−1 at pH 8 and 25°C) (Fig. 3F); however, the large urea‐dependent baseline in the pretransition suggests that the native ensemble consists of a mixture of native and non‐native conformations. This also agrees with the CD spectra (Fig. 2), which show a decrease in secondary and tertiary structure for this mutant.

**Stability 09 (PMID: 29132128)**

The stability of both FF domains resulted considerably perturbed by CySO3H formation (Fig. 3A and B), with a decrease in FF-WT-OX Tm of ≈ 20 K respect that of FF-WT, and a reduction > 20 K in the case of the R18A-OX-Tm relative to FF-R18A (supplementary Table 2). These data were corroborated by thermal denaturation followed by H-NMR1. Reduction of the native signal dispersion is observed at lower temperatures in oxidized than in non-oxidized species both for FF-WT and FF-R18A (supplementary Fig. 2). In all cases the reaction was reversible.

The chemical unfolding of the FF domains was analyzed at pH 5.7 and 298 K. The urea denaturation curves at equilibrium were obtained recording the changes in molar ellipticity at 222 nm (Fig. 3C) and Trp intrinsic fluorescence at 360 nm (Fig. 3D) at increasing denaturant concentrations. The thermodynamic values were calculated assuming a two-state model according to the unfolding single cooperative transition shown by all FF domains (Table 1). The ΔGF-U of FF-WT-OX measured by fluorescence and CD spectroscopy corresponds to ≈ 1.5 kcal/mol with a [Urea]50% of ≈ 2 M, in contrast to the ≈ 5.0 kcal/mol and [Urea]50% of ≈ 6 M exhibited by FF-WT. Reliable thermodynamic values could not be approximated for FF-R18A-OX since it unfolds at very low urea concentrations (< 1 M). In FF-WT, CySO3H reduces ΔGF-U by ≈ 3.5 kcal/mol. Because FF-R18A ΔGF-U ≈ 3.5 kcal/mol is not surprising that FF-R18A-OX is unstable even in the absence of denaturant.

**Stability 10 (PMID: 26880334)**

Thermodynamic and kinetic data for wild-type hPin1 WW and mutants thereof. Hydrophobic core 2 lies on the ligand-binding face of the three-stranded β-sheet and is formed by the side chains of residues R14, Y23, and F25 (Fig. 1a). These residues are only moderately conserved in WW domains, presumably because hydrophobic core 2 contributes to stability and ligand binding. Ala mutations of residues 14, 23, and 25 in hPin1 WW, although severely destabilizing the native state (∆∆ Gf ~ 9 kJ/mol) (Fig. 1c), allow for folding into the native-state under the most favorable folding conditions (4 °C).