A population shift between sparsely-populated folding intermediates determines amyloidogenicity

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Supplementary Methods

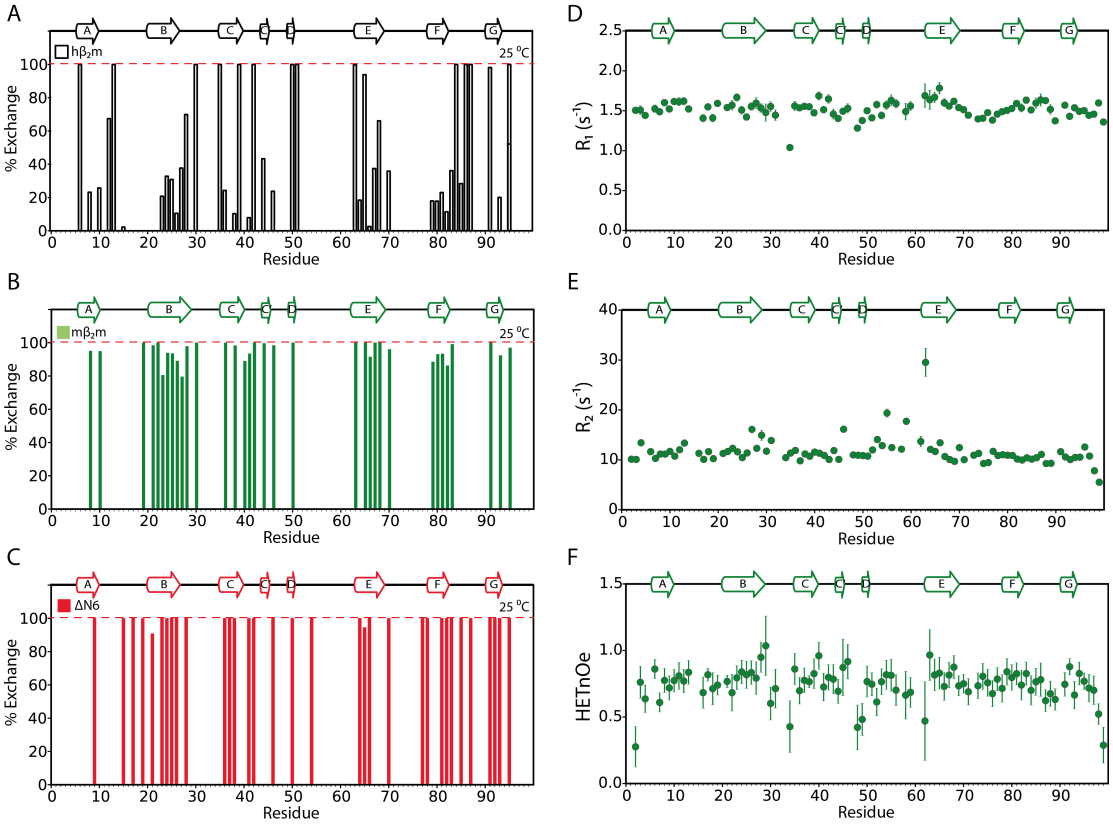
Analysis of NUS spectra

In traditional real-time NMR experiments a series of stand-alone 2D or 3D spectra are acquired. Fast pulsing techniques have allowed a significant decrease on the length of these experiments but limitations still exist. For example, the reaction rate has to be known or estimated in order to decide on the length of each spectrum. On the other hand, in a NUS experiment, only a fraction of the points in the indirect dimension is collected and therefore much better resolution can be achieved both in the reaction time and spectral frequency dimensions. After acquisition of a NUS experiment the peaks can be reconstructed using the following model:

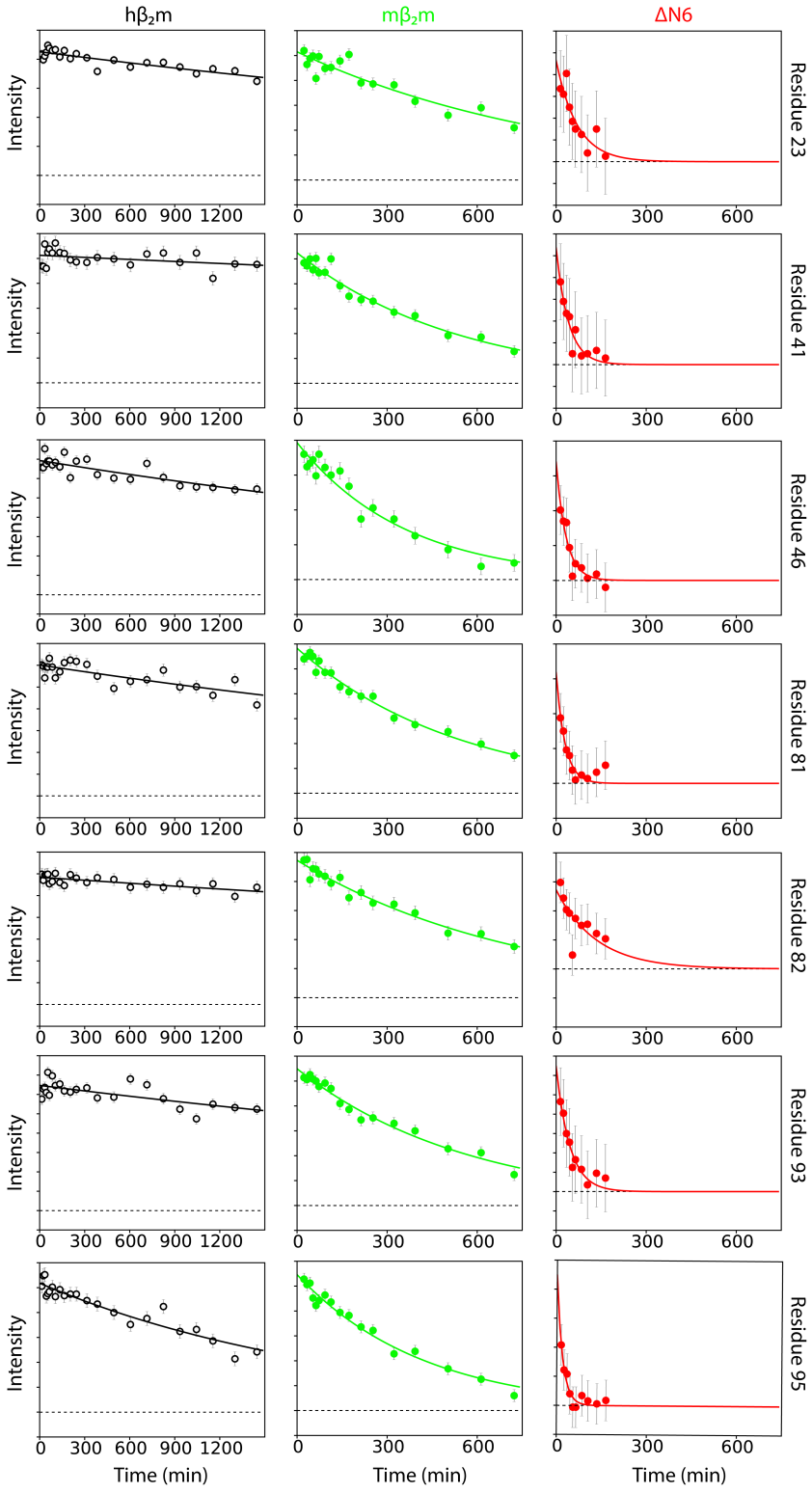
$$S^k = \sum_i a_i F_i^H \otimes F_i^{CO} \otimes F_i^N$$

where the model spectrum (in this case HNCO) can be reconstructed using a number of components (peaks). Each of these components can be described by three parameters: an amplitude α_i and three peak shapes F_i^H, F_i^{CO}, F_i^N creating the model for multi-dimensional decomposition (MDD). Assuming that the peaks do not change position and shape, the model can be used for co-processing over a set of spectra of the same type (e.g. 2D BEST-TROSY HSQCs) or of different type (e.g. 2D BEST-TROSY HSQC with HNCA+ and HNCO+). The co-processing increases sensitivity and allows further improvement the reaction time resolution. Then the individual spectra can be extracted during the processing step using the appropriate time window [1]. Here, we used a sliding window which got larger as the reaction progressed to emphasise the early intermediate species.

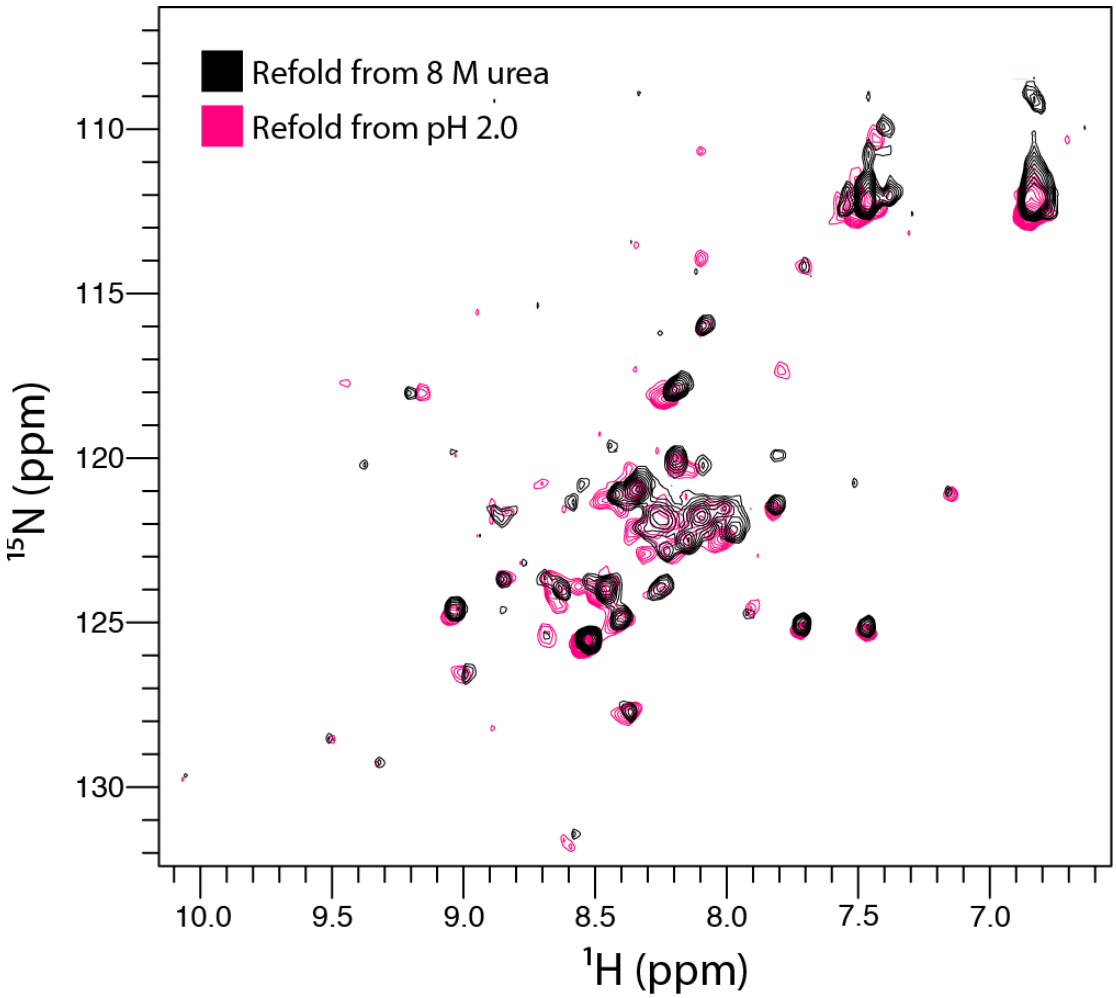
Supplementary Figures



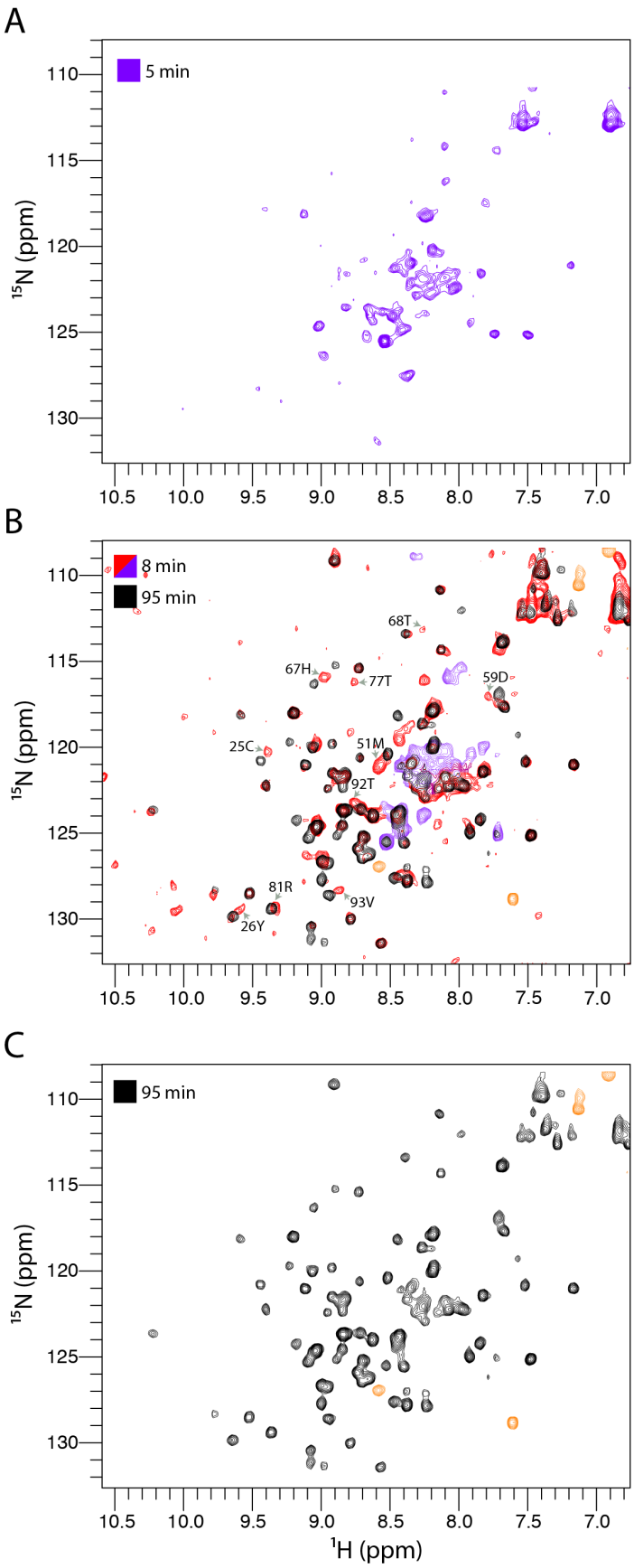
Supplementary Figure 1. Dynamics of the native state of mβ$_2$m, hβ$_2$m and ΔN6. (A) Per cent hydrogen exchange of hβ$_2$m after 1500 min in 10 mM sodium phosphate pH 6.2, 25 °C. (B) As in (A) but for mβ$_2$m. (C) As in A but for ΔN6. To calculate the intensity after 1500 min H/D exchange data were fitted to single exponentials and the fitted value at 1500 min was divided by the initial intensity at t=0. (D) Longitudinal T_1 relaxation (R_1=1/T_1) for 80 μM mβ$_2$m at pH 6.2. (E) Transversal R_2 relaxation rates (R_2=1/T_2) for 80 μM mβ$_2$m at pH 6.2. (F) {^{1}H}-^{15}N heteronuclear nOe rates for 80 μM mβ$_2$m at pH 6.2.



Supplementary Figure 2. Kinetic stability of β_2m variants. Representative hydrogen exchange profiles for the amide hydrogen of different residues in hβ_2m, mβ_2m and ΔN6 at 25 °C and pH 6.2 (see Figure S1). Zero is shown as a dashed line.

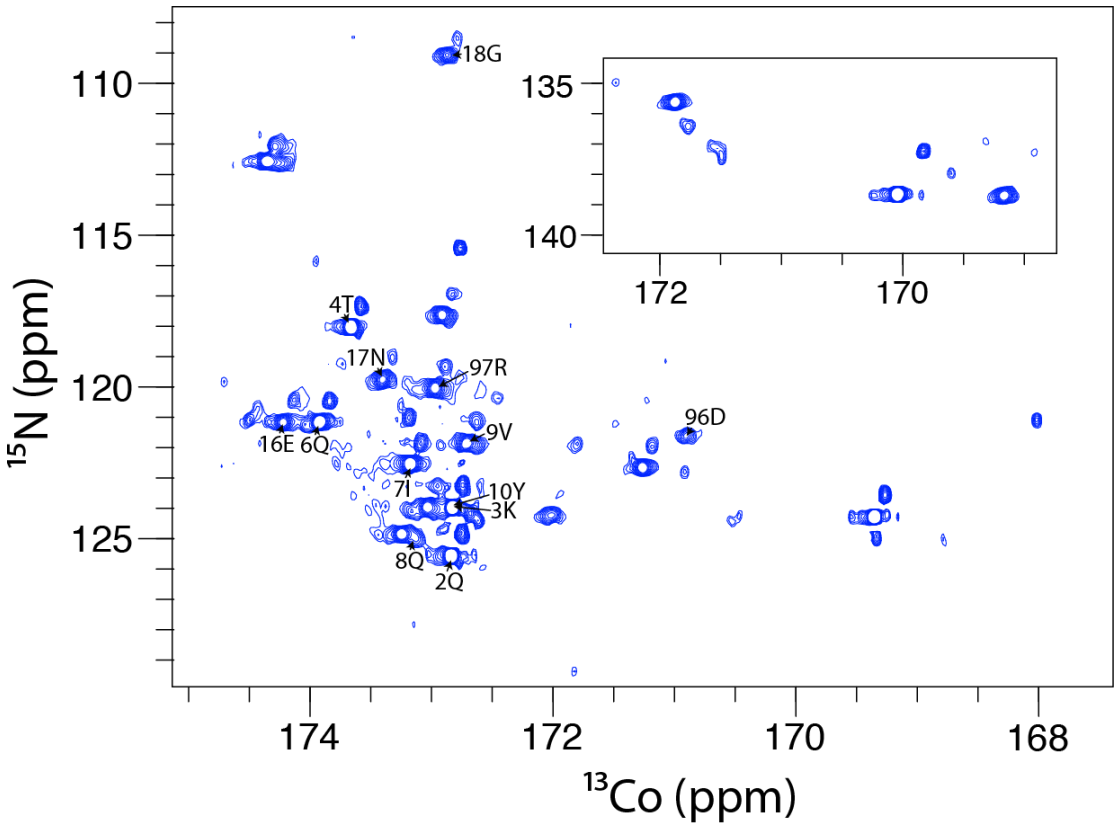


Supplementary Figure 3. Folding of mβ$_2$m from pH 2.0 or 8 M urea. The ^{1}H-^{15}N SOFAST-HSQC spectrum of mβ$_2$m collected 3 min after refolding was initiated from 10 mM sodium phosphate pH 2.0 (pink) or from 10 mM sodium phosphate pH 6.2, 8 M urea (residual 0.8 M urea –black).

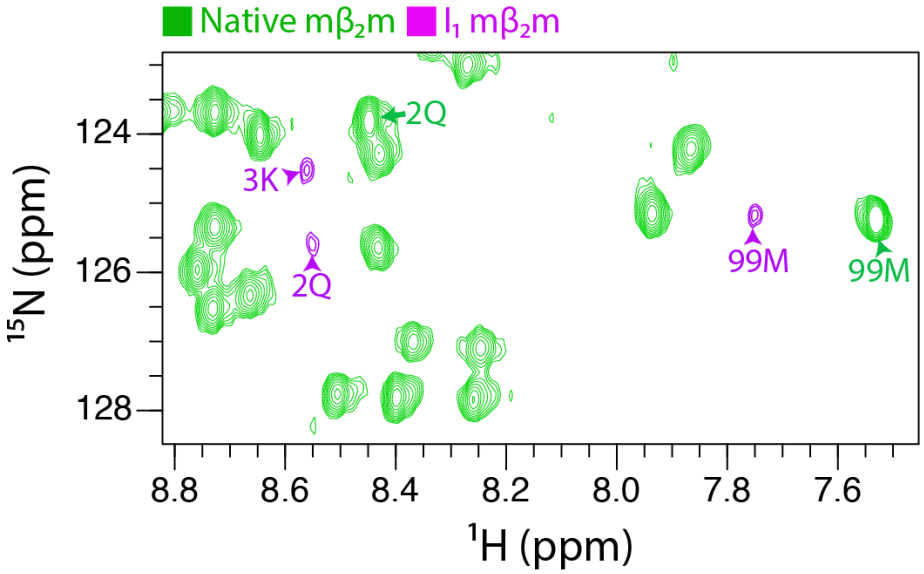


Supplementary Figure 4. Additional states populated during the folding of mβ$_2$m. (A) The ^{1}H-^{15}N SOFAST-HSQC spectrum of mβ$_2$m collected 3 min after

refolding was initiated by urea dilution. (B) The ^{1}H-^{15}N SOFAST-HSQC spectrum of mβ_2m collected 8 min after refolding was initiated (red/purple) overlayed with the spectrum collected after 95 min (black). Peaks in the 8 min spectrum that correspond to the molten-globule-like state shown in (A) were colored purple. Peaks with small chemical shift changes relative to the native state are annotated on the spectrum. (C) The ^{1}H-^{15}N SOFAST-HSQC spectrum of mβ_2m collected 95 min after refolding was initiated.



Supplementary Figure 5. Characterizing the molten-globule of mβ$_2$m. Direct ^{13}C-detected CON spectrum of 1.3 mM mβ$_2$m at pH 3.6. Assignments of the molten-globule-like intermediate (I_1) are shown in black. The proline region is shown as inset.



Supplementary Figure 6: The I_1 state is populated under native conditions. The ^{1}H-^{15}N HSQC spectrum of $m\beta_2m$ at pH 6.2. Resonances of the native state are colored green, while resonances of the I_1 state are shown in magenta.

Supplementary Tables

	ΔG^{o}_{unf} (kJ/mol)	M^{o}_{unf} (kJ/mol/M)
$m\beta_2m$	-10.7 ± 0.5	4.8 ± 0.2
$h\beta_2m$	-23.1 ± 1.0	4.7 ± 0.2
ΔN6	-11.4 ± 0.4	3.5 ± 0.1
$m\beta_2m$ I_1	~ -4.8	~ 4.3
$h\beta_2m$ I_T	-9.57 ± 0.54	6.26 ± 0.33

Supplementary Table 1. Unfolding free energies and M values for $m\beta_2m$, $h\beta_2m$ and ΔN6. Values were calculated by fitting equilibrium unfolding data shown in Figure 2A to a two state model (pH 6.2, 25°C). Unfolding free energies and M values for $m\beta_2m$ I_1 were calculated by fitting stopped-flow data (see Methods, pH 6.2, 37°C). Data for $h\beta_2m$ I_T were taken from Jahn *et. al.*, [2] (pH 7.0, 25°C).

Residue	Atom	Shift (ppm)
1Ile	Ca	61.011
1Ile	Cb	38.777
1Ile	C	172.928
2Gln	H	8.529
2Gln	N	125.781
2Gln	Ca	55.382
2Gln	Cb	29.713
2Gln	C	172.845
3Lys	H	8.483
3Lys	N	124.033
3Lys	Ca	56.153
3Lys	Cb	33.229
3Lys	C	173.689
4Thr	H	8.259
4Thr	N	117.972
4Thr	Ca	59.679
5Pro	Cb	32.167
5Pro	Ca	63.010
5Pro	C	173.898
6Gln	Ca	55.586
6Gln	Cb	29.629
6Gln	H	8.397
6Gln	N	121.522
6Gln	C	173.168
7Ile	Ca	60.858
7Ile	Cb	38.870
7Ile	H	8.249
7Ile	N	122.950
7Ile	C	173.203
8Gln	Ca	55.419
8Gln	Cb	29.961
8Gln	H	8.464
8Gln	N	125.021
8Gln	C	172.636
9Val	Ca	61.993
9Val	H	8.097
9Val	N	122.163
9Val	H	8.123
9Val	N	121.662
9Val	Cb	32.720
9Val	C	172.964
10Tyr	Ca	57.548
10Tyr	H	8.306
10Tyr	N	124.204
15Pro	Cb	32.038
15Pro	C	174.284
16Glu	Cb	30.189
16Glu	Ca	56.442
16Glu	H	8.598
16Glu	N	121.611
16Glu	C	173.655
17Asn	Cb	38.975
17Asn	Ca	53.151
17Asn	H	8.482
17Asn	N	119.821
17Asn	C	172.936
18Gly	Ca	45.143
18Gly	H	8.386
18Gly	N	109.086
95Trp	Cb	29.546
95Trp	Ca	56.837
95Trp	C	170.922
96Asp	Cb	41.207
96Asp	Ca	53.903
96Asp	H	8.102
96Asp	N	121.864
96Asp	C	172.969
97Arg	H	7.823
97Arg	Cb	36.205
97Arg	Ca	55.915
97Arg	N	120.222
97Arg	C	172.942
98Asp	Ca	54.466
98Asp	H	8.309
98Asp	N	121.137
98Asp	C	172.431
99Met	Ca	57.032
99Met	H	7.782
99Met	N	125.022

Supplementary Table 2: Chemical shift assignments of the backbone atoms of the I_1 state of mβ_2m at pH 6.2.

Supplementary References

(1) Mayzel, M.; Rosenlöw, J.; Isaksson, L.; Orekhov, V. Y. *J Biomol NMR* **2014**, *58*, 129–139.
(2) Jahn, T. R.; Parker, M. J.; Homans, S. W.; Radford, S. E. *Nat Struct Mol Biol* **2006**, *13*, 195–201.