Electronic Supplementary Information:

The interaction of β2-microglobulin and gold nanoparticles: impact of coating, charge and size

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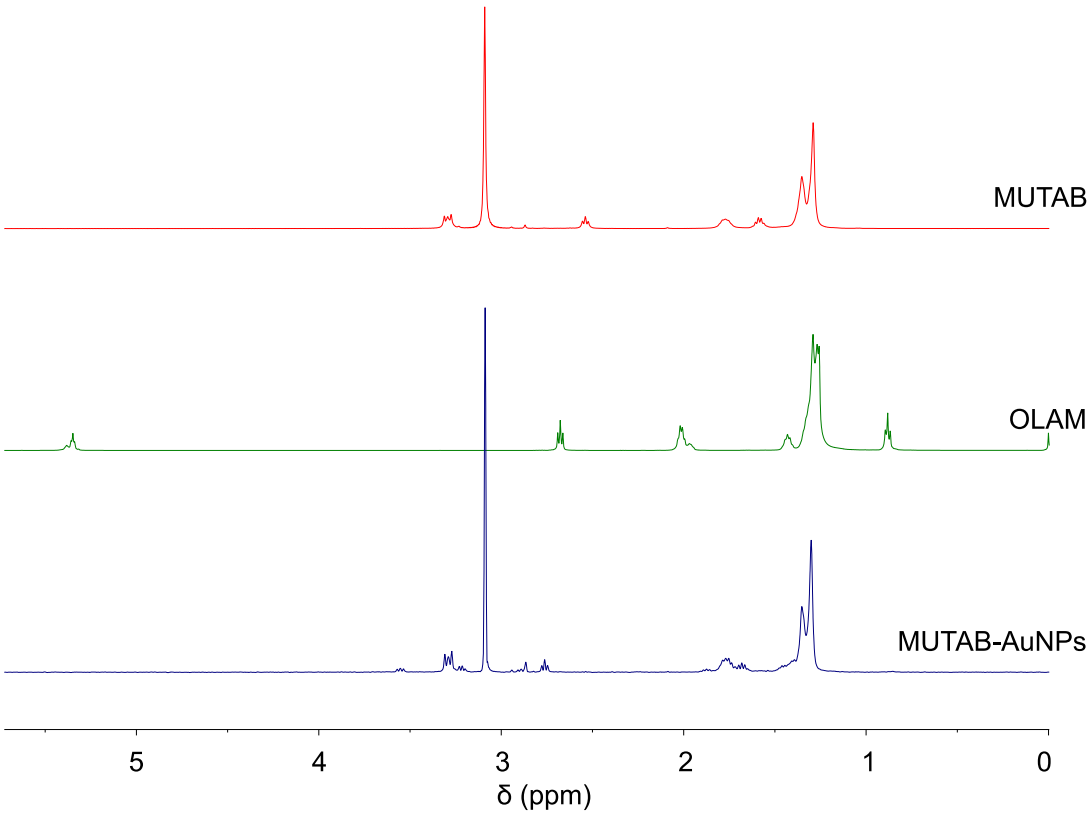

Fig. S1 ^{1}H NMR spectra of MUTAB in D_2O (red trace), OLAM in $CDCl_3$ (green trace) and MUTAB-AuNPs n D_2O (purple trace) indicating no presence of OLAM in MUTAB-AuNPs.

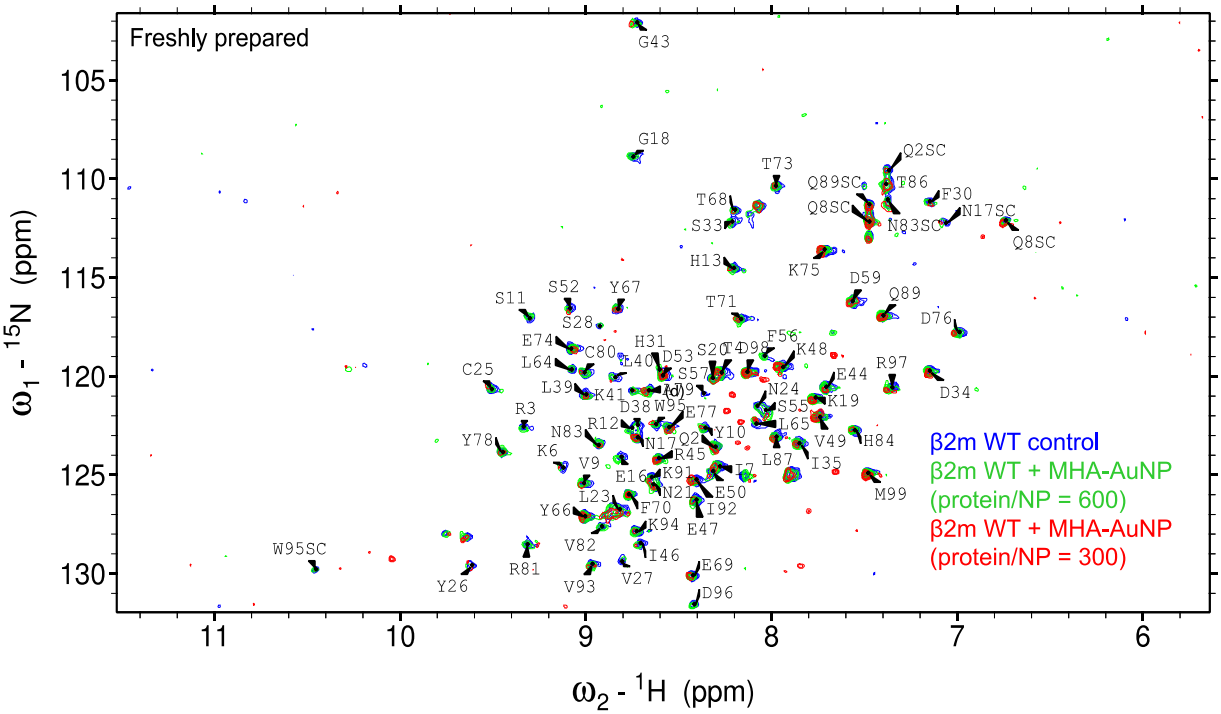

Fig. S2 Superimposition of β2m WT ^{15}N-^{1}H SOFAST HMQC spectra recorded at 500 MHz (^{1}H frequency) without (blue) and with MHA-AuNP (protein/NP = 600 in green and protein/NP = 300 in red). The corresponding backbone amide assignments are reported by single letter code and the side-chain amides are indicated by SC.

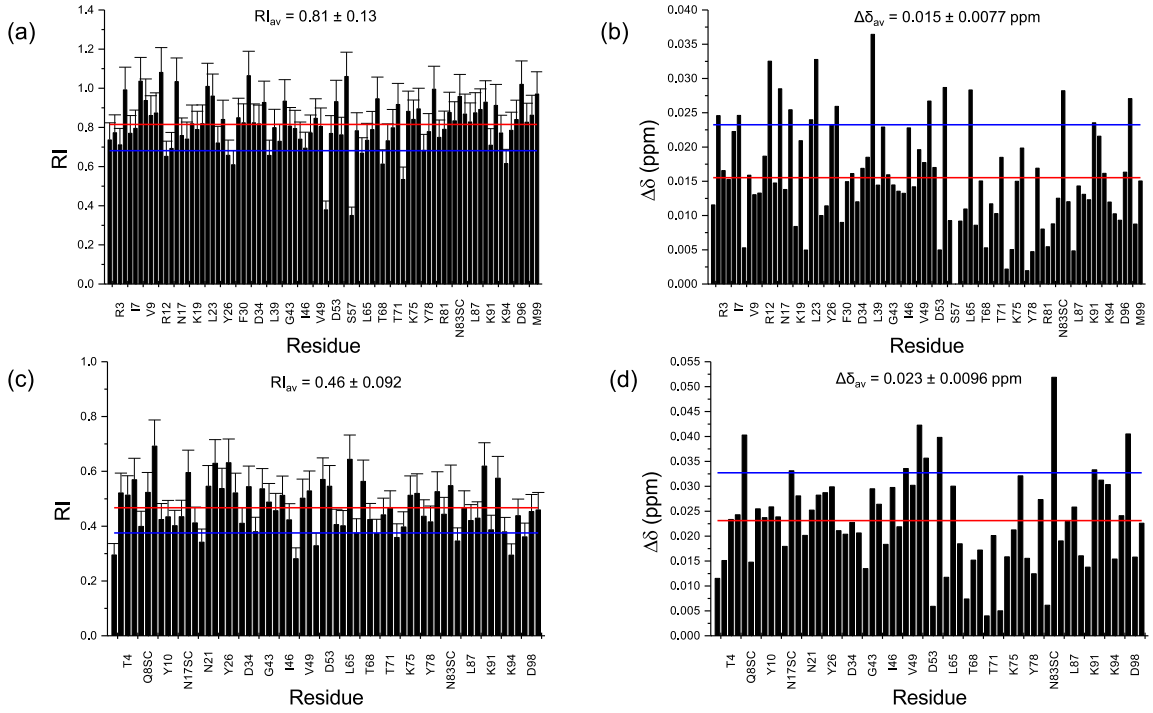

Fig. S3 a) and b) Bar plots of β2m amide cross-peak attenuations (RI) and combined chemical shift perturbations (Δδ), respectively, against the protein sequence, at protein/ MHA-AuNP = 600. c) and d) Bar plots of β2m amide cross-peak attenuations (RI) and combined chemical shift perturbations (Δδ), respectively, against the protein sequence, at protein/MHA-AuNP = 300. The horizontal lines indicate, respectively, the average values (red) and the displacement of one standard deviation (blue) above or below the average. To avoid graphic crowding, the abscissa labels of the panels were reported only every other three residues. Besides the observed backbone amides, also the following side-chain (SC) NH resonances were detected and included in the abscissa label list, according to the primary sequence order: Q2, Q8 (two separate signals), N17, N83, Q89, W95. The missing labels do not include the following unobserved or non-existing backbone NH connectivities: I1, P5, Q8, P14, A15, F22, G29, P32, E36, V37, N42, H51, L54, K58, W60, S61, F62, Y63, P72, V85, S88, P90. In addition to the listed residues, also R3, S11, R12, N24, V27, S28, H31, D38, L40, K41, F56, S57, L64, C80, R81, V82 and D96 were unobservable in the SOFAST HMQC maps from the solution with a higher NP concentration. It is worth highlighting that SOFAST experiments are very convenient to screen quickly the appearance of the two-dimensional NMR spectra, but they have a lower signal-to-noise ratio and non-uniform excitation artefacts compared to HSQC spectra acquired with same number of scans. To compromise between the need of minimising the measurement time and the necessity of quantification, acquisitions were typically limited to ~6-12 hours. The appreciable uncertainty on intensity measurements limited the confidence threshold for some of the observed changes. For instance, with a typical Δ(intensity) around 8%, all the values above unity in panel (a) in the spectrum recorded in presence of MHA-AuNPs should be considered with caution.

Table S1 Synopsis of WT β2m amides that proved most affected by the presence of MHA-AuNP, i.e. displaced more than one standard deviation from the average Δδ and RI values. The amides are grouped according to the secondary structure element location.

Structure region	Δδ outliers β2m/NP = 600	RI outliers β2m/NP = 600	Δδ outliers β2m/NP = 300	RI outliers β2m/NP = 300
N-term, A strand	I7		I7	
AB loop	R12, E16, N17SC	R12	N17SC	S20
B strand	N21SC, L23, V27	Y26, V27	L23	Y26
BC loop				
CC', C'D loops	D38	D38	K48	E47
D strand	E50	E50	E50, S52	E50
DE loop	S55	S57	S55	
E strand	L65	L64, T68	L65	T68
EF loop		T73		T73, E69
F strand	H84		N83SC, H84	H84
FG loop				
G strand, C-term	K91, R97	K94	K91	K94

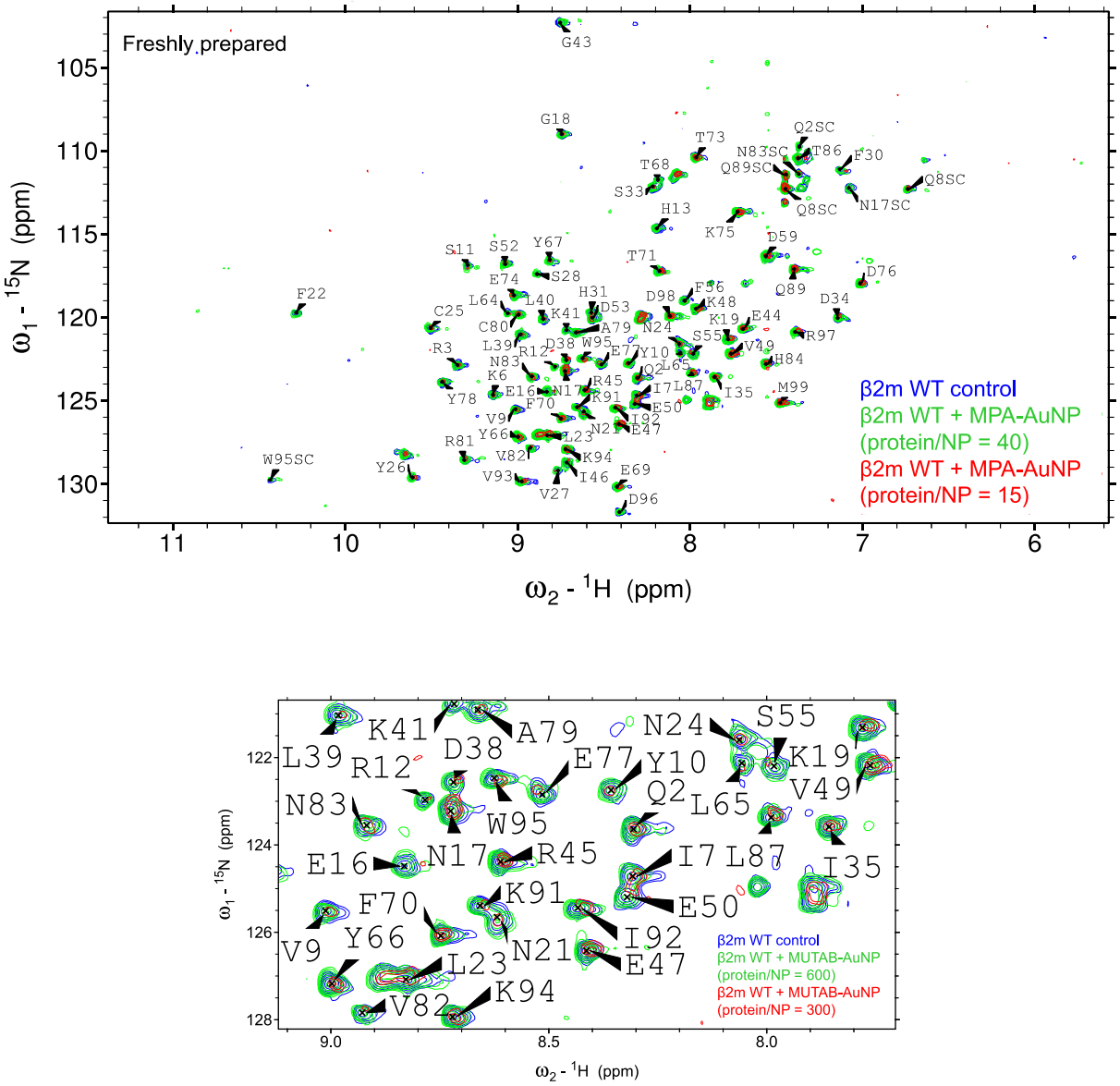

Fig. S4 Superimposition of β2m WT ^{15}N-^{1}H SOFAST HMQC spectra recorded at 500 MHz (^{1}H frequency) without (blue) and with MUTAB-AuNP (protein/NP = 600 in green and protein/NP = 300 in red). A partial magnification is reported below to illustrate overlay details. The corresponding backbone amide assignments are reported by single letter code and the side-chain amides are indicated by SC.

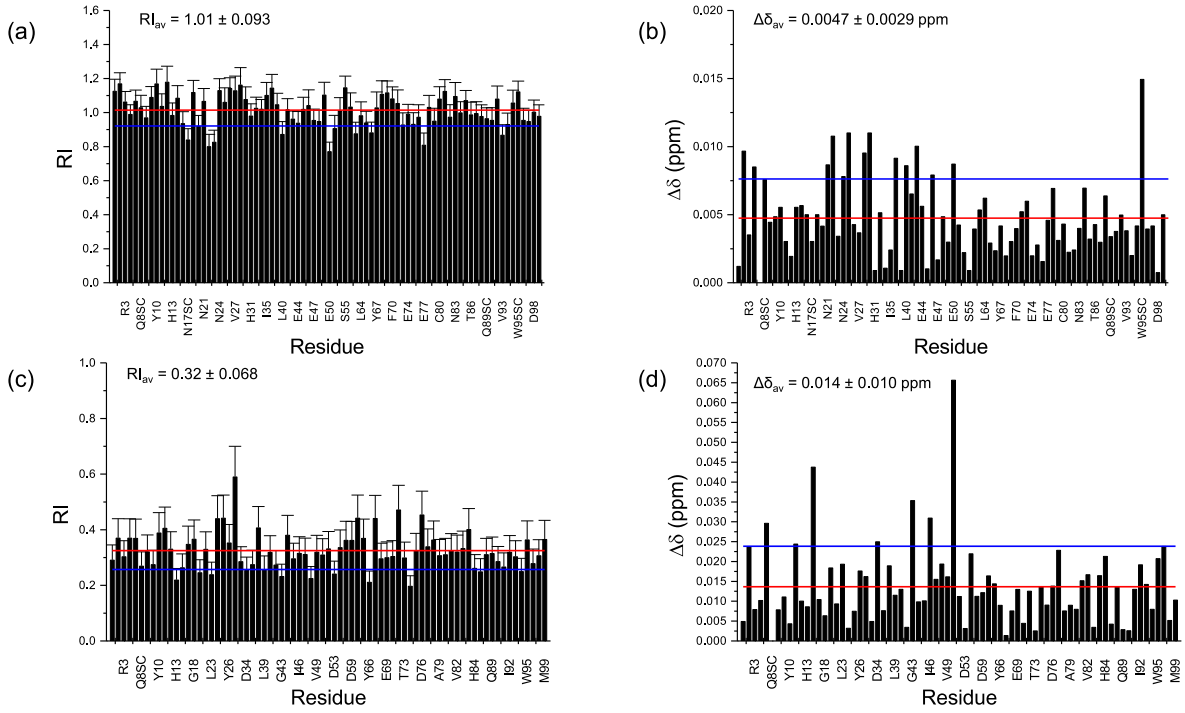

Fig. S5 a) and b) β2m amide cross-peak attenuations (RI) and combined chemical shift perturbations (Δδ), respectively, against the protein sequence, at protein/ /MUTAB-AuNP = 600. c) and d) β2m amide cross-peak attenuations (RI) and combined chemical shift perturbations (Δδ), respectively, against the protein sequence, at protein/ /MUTAB-AuNP = 300. The horizontal lines indicate, respectively, the average values (red) and the displacement of one standard deviation (blue) above or below the average. To avoid graphic crowding, the abscissa labels of the panels were reported only every other three residues. Besides the observed backbone amides, also the following side-chain (SC) NH resonances were detected and included in the abscissa label list, according to the primary sequence order: Q2, Q8 (two separate signals), N17, N83, Q89, W95. The missing labels do not include the following unobserved or non-existing backbone NH connectivities: I1, T4, P5, Q8, P14, A15, S20, G29, P32, E36, V37, N42, H51, L54, S57, K58, W60, S61, F62, Y63, P72, V85, S88, P90. In addition to the listed residues, also F22, S28, F30, H31 and D96 were unobservable in the spectrum of the solution with a higher NP concentration.

Table S2 Synopsis of WT β2m amides that proved most affected by the presence of MUTAB-AuNP, i.e. displaced more than one standard deviation from the average Δδ and RI values. The amides are grouped according to the secondary structure element location.

Structure region	Δδ outliers β2m/NP = 600	RI outliers β2m/NP = 600	Δδ outliers β2m/NP = 300	RI outliers β2m/NP = 300
N-term, A strand			I7, S11	
AB loop		N17SC, K19	E16	H13, K19
B strand	F22, C25	F22, L23		L23
BC loop	F30		D34	D34
CC', C'D loops		L40	G43, I46	G43
D strand		E50	E50	K48
DE loop		S52, D59		D53
E strand		Y66		
EF loop		E77		E74
F strand				
FG loop				T86
G strand, C-term	W95SC	V93	R97	K94

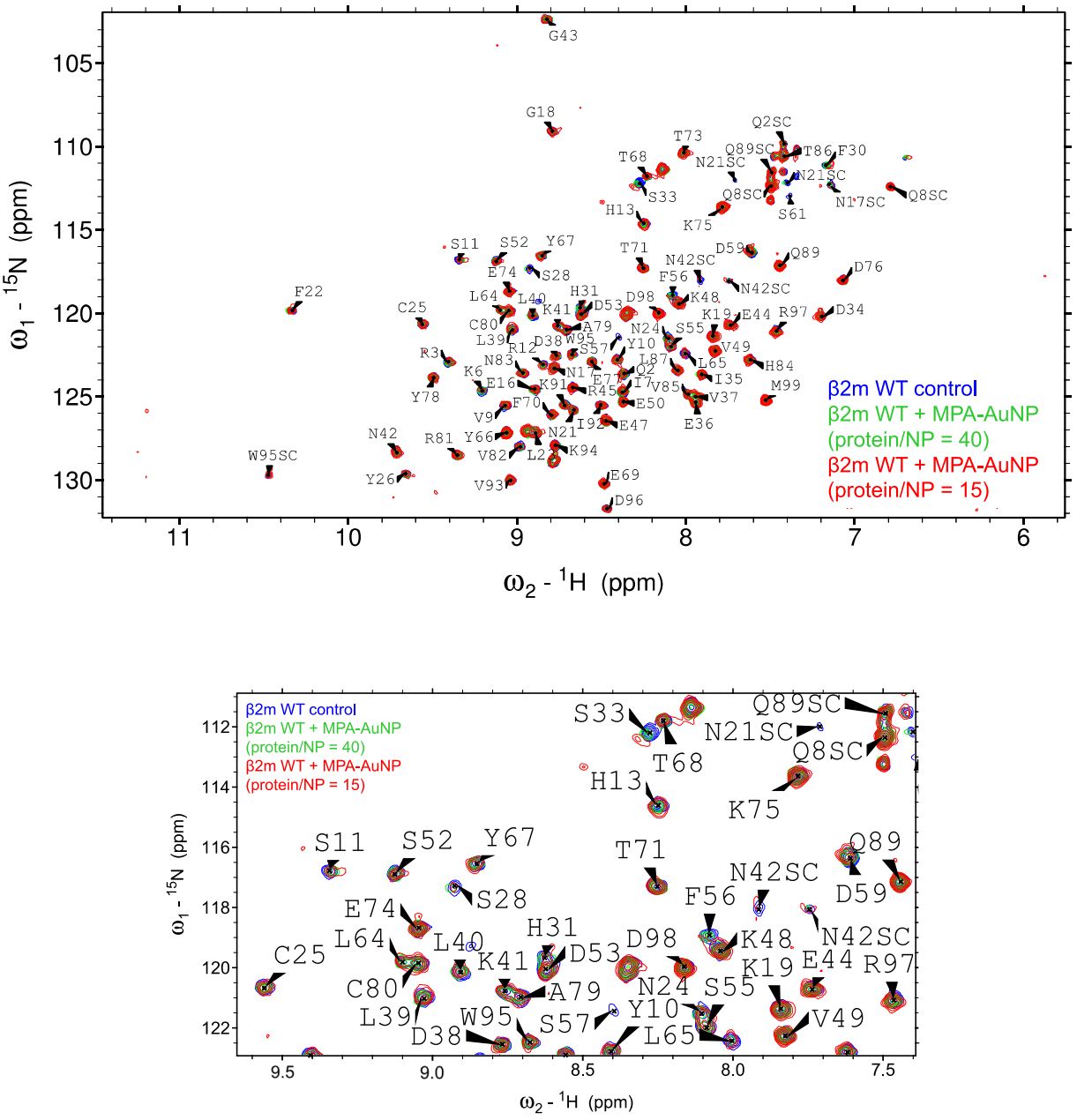

Fig. S6 Superimposition of β2m ^{1}H ^{15}N SOFAST HMQC spectra recorded at 500 MHz (^{1}H frequency) in absence of MPA-AuNPs (blue) and in presence of MPA-AuNPs with protein/NP ratio of 40 (green) and of 15 (red). A partial magnification is reported below to illustrate overlay details. The corresponding backbone amide assignments are reported by single letter code and the side-chain amides are indicated with SC.

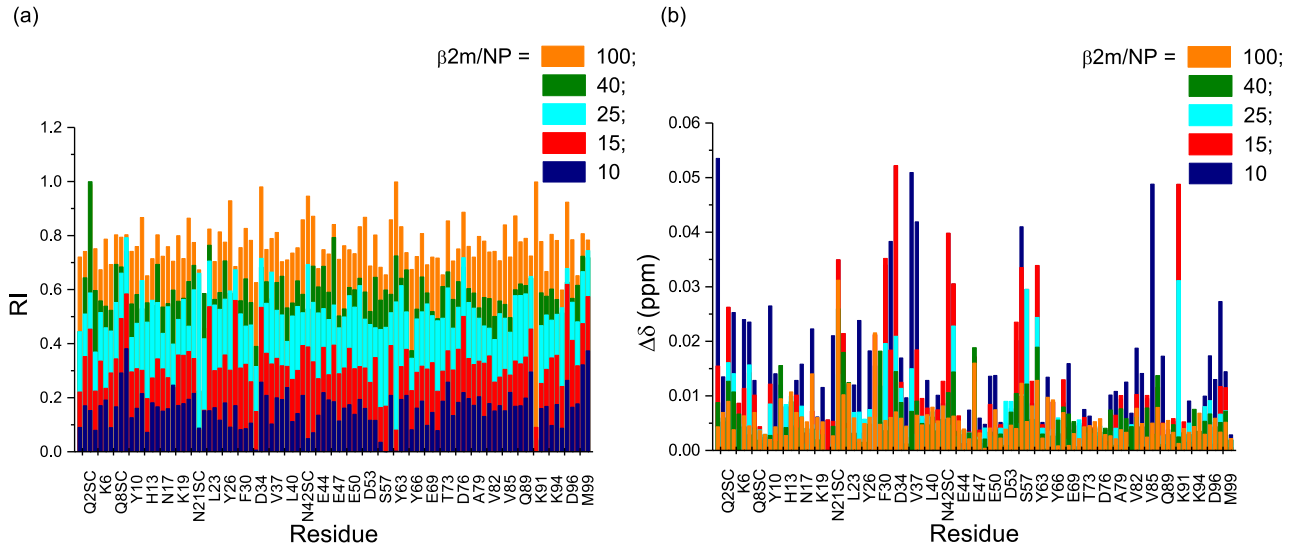

Fig. S7 a) and b) β2m amide cross-peak attenuations (RI) and combined chemical shift perturbations (Δδ), respectively, against the protein sequence, at different protein/MPA-AuNP ratios. Refer to color legend for the protein/MPA-AuNP ratios colour attributions. To avoid graphic crowding, the abscissa labels of the panels were reported only every other three residues. Besides the observed backbone amides, also the following side-chain (SC) NH resonances were detected and included in the abscissa label list, according to the primary sequence order: Q2, Q8, N17, N83, Q89, W95. The missing labels do not include the following unobserved or non-existing backbone NH connectivities: I1, P5, P14, G29, P32, L54, K58, D59, W60, F62, P72, S88, and P90. In addition to the listed residues, also R3, S11, R12, N24, V27, S28, H31, D38, L40, K41, F56, S57, L64, C80, R81, V82 and D96 were unobservable in the spectra of the solution with increasing NP concentrations.

Table S3 Synopsis of WT β2m amides that proved most affected by the presence of MPA-AuNP, i.e. displaced more than one standard deviation from the average Δδ and RI values. The amides are grouped according to the secondary structure element location.

Structure region	Δδ outliers	RI outliers
N-term, A strand	R3	R3
AB loop		
B strand		S28
BC loop	F30, H31, S33	F30, H31, S33
CC', C'D loops	E36, V37	
D strand		
DE loop	S55, F56, S57, S61	F56, S57, S61
E strand		L65
EF loop		
F strand		
FG loop		
G strand, C-term		

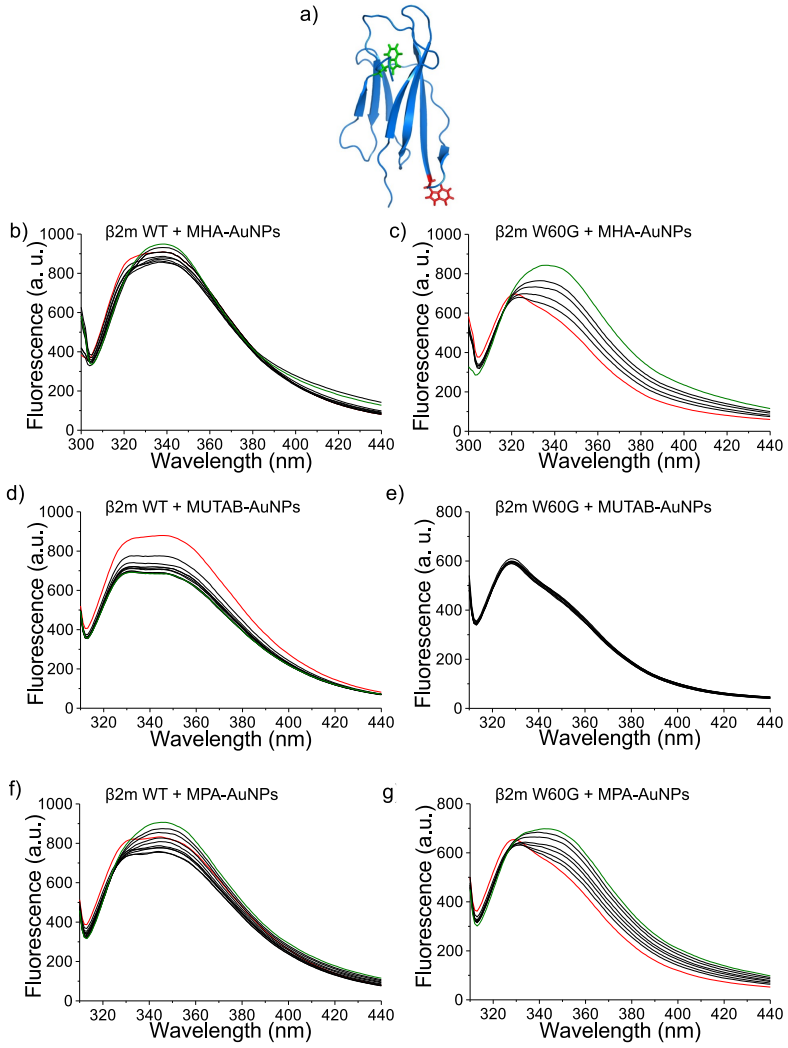

Fig. S8 a) Ribbon representation of β2m backbone solution structure[41] highlighting the different exposure of the two tryptophans of the molecule, with the solvent-exposed W60 (red) and the buried W95 (green). b) and c) Fluorescence quenching of 0.5 μM WT and W60G β2m, respectively, with MHA-AuNPs (protein/NP from 5000 to 500). d) and e) Fluorescence quenching of 0.5 μM WT and W60G β2m, respectively, with MUTAB-AuNPs (protein/NP from 5000 to 500). f) and g) Fluorescence quenching of 0.5 μM WT and W60G β2m, respectively, with MPA-AuNPs concentrations ranging from 0 to 55 nM. The control spectra are coloured in red, while the last titration points in green.