Proton Dynamics in Protein Mass Spectrometry

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Supporting Information

Supplementary Text S1

MD simulations of Aβ(1-16) peptide in aqueous solution. The MD simulations of Aβ(1-16) in solution appeared to be equilibrated after ~800 ns. This is suggested by the plots of the backbone heavy atoms root mean square deviations (RMSD) as a function of simulated time (Figure S4). To assess the convergence of the simulated trajectories from the last 200 ns, we considered their projections on the top essential dynamical spaces obtained from a standard covariance analysis. Following Hess's criterion[1], these projections were next compared with those expected for a random reference. The observed negligible overlap (i.e. cosine content close to 0, see Table S5) confirms adequate sampling of Aβ(1-16) conformations around the equilibrium position. Averaged structural properties were then calculated based on the last 200 ns trajectories. Notably, the majority of the

calculated structural properties are similar to the ones obtained from NMR structures (Table S3)[1]. This demonstrates the accuracy of our MD simulations in solution. Comparing experimental CCS (539 ± 16 Å^2) with the one obtained from MD simulations in water (average CCS values ranging from 575 ± 36 Å^2 to 596 ± 32 Å^2), we can conclude that Aβ(1-16) becomes more compact under MS conditions. This is consistent with previous observations on proteins[6-8] and DNAs.[9] The most representative structure was identified by cluster analysis[10] over the combined equilibrated trajectories from the four independent simulations. Water molecules were removed for following gas-phase simulations.

Supplementary Text S2

Determination of the lowest-energy protonation states of Aβ(1-16) peptide in the gas phase. The most probable protonation states identified by the MC/MD protocol (Table S4) were validated by predicting the experimentally measured maximum charge state (q=4+) of the peptide (Figure S2). Following a procedure developed by some of us,[11-12] we estimated the maximum charge state by calculating the intersection of the apparent gas-phase basicity (GB_{app}) fitted line as a function of the peptide net charge with the line of solvent GB. The intersection occurs at q=3.8+ (Figure S1), well matching the experimentally measured maximum charge state (q=4+) for Aβ(1-16).

References

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[1]The only disagreement was in a slightly underestimation of the content of 3_{10}-helix in the peptide. This has been also reported in previous literatures.[2-3] Indeed, 3_{10}-helix is currently underestimated by force field-based MD.[4-5]

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Supplementary Tables

Table S1. Hess's cosine content analysis of the four principal components (PC1-PC4) obtained from mass weighted covariance analysis[1] for the equilibrated MD trajectories of Aβ(1-16) in the gas phase at the main charge state. MD_gas1 to MD_gas3 (see Supplementary Text S2) are the gas-phase MD simulations on the lowest-energy protonation state predicted by MC/MD calculations.

ID	Cosine content		
	MD_gas1	MD_gas2	MD_gas3
PC1	0.0002	0.0004	0.0004
PC2	0.0001	0.0004	0.0006
PC3	0.0002	0.0009	0.0004
PC4	0.0002	0.0007	0.0003

Table S2. Average structural properties from the equilibrium trajectories of MD simulations of Aβ(1-16) in the gas phase at the main charge state. From left to right: simulation time (ms); collision cross section (CCS in Å^2); radius of gyration (R_g in Å); solvent accessible surface area (SASA in Å^2); distance between Cα atoms of N- and C-terminal residues (D_{NT-CT} in Å); number of hydrogen bonds in peptide (HB); number of contacts between heavy atoms with a cut-off of 5 Å (Cont in Å); occupancies of secondary structural elements (coil, bend, turn and helix in %). Standard deviations are reported in parenthesis. MD_gas1 to MD_gas3 (see Supplementary Text S2) are the gas-phase MD simulations on the lowest-energy protonation state predicted by MC/MD calculations, with different microscopic initial conditions.

System	Time	CCS	R_g	SASA	D_{NT-CT}	HB	Cont	Coil	Bend	Turn	Helix
MD_gas1	0.129	531 (15)	7.1 (0.1)	1623.8 (48.2)	14.1 (2.0)	18.5 (1.9)	3038.9 (67.7)	48.2 (9.4)	13.8 (6.0)	17.4 (7.4)	18.1 (11.2)
MD_gas2	0.120	516 (18)	7.0 (0.1)	1550.4 (50.4)	13.5 (1.4)	17.8 (2.5)	3020.4 (73.9)	40.0 (6.3)	33.9 (9.1)	21.4 (5.8)	0.1 (1.4)
MD_gas3	0.120	513 (17)	7.0 (0.1)	1540.0 (48.9)	15.6 (1.4)	18.8 (2.1)	2987.6 (58.9)	37.6 (6.6)	42.5 (6.9)	18.8 (9.4)	0.4 (2.5)

Table S3. Average structural properties from the equilibrium trajectories of MD simulations of Aβ(1-16) in water and the ones from NMR study.[13] From left to right: simulation time (μs); CCS (Å^2); R_g in (Å); SASA (Å^2); D_{NT-CT} (Å); HB; Cont (Å); occupancies of secondary structural elements (coil, bend, turn and helix in %). Standard deviations are reported in parenthesis. MD1 to MD4 are the classical MD simulations in aqueous solution with different microscopic initial conditions (see Supplementary Text S1).

System	Time	CCS	R_g	SASA	D_{NT-CT}	HB	Cont	Coil	Bend	Turn	Helix
NMR	-	604 (29)	8.9 (0.1)	1910.3 (119.2)	15.4 (4.6)	9.7 (2.0)	2553.7 (118.9)	49.1 (6.2)	18.1 (8.8)	19.7 (8.4)	12.5 (12.3)
MD1	1.0	596 (32)	8.9 (0.1)	1850.0 (80.8)	15.2 (5.5)	10.2 (2.0)	2649.5 (82.3)	51.3 (13.1)	21.3 (11.3)	22.5 (13.1)	4.4 (7.5)
MD2	1.0	580 (43)	8.7 (0.1)	1790.5 (70.9)	13.8 (5.4)	10.1 (2.6)	2696.7 (112.3)	51.9 (14.4)	18.8 (10.6)	23.8 (11.9)	3.8 (8.1)
MD3	1.0	575 (36)	8.5 (0.1)	1760.7(78.5)	12.9 (5.5)	9.6 (2.2)	2697.9 (110.8)	47.5 (11.5)	25.5 (8.2)	16.3 (9.1)	8.5 (8.7)
MD4	1.0	580 (32)	8.5 (0.1)	1750.5 (77.6)	12.8 (5.7)	9.9 (2.1)	2667.6 (87.2)	32.5 (9.3)	47.2 (10.1)	13.7 (7.9)	6.4 (4.5)

Table S4. The lowest-energy protonation states for charge states from 0 to 4+. The positive and neutral charged residues are indicated by "+" and "0", respectively.

Charge state	Number of charged amino acids	D1	E3	R5	H6	D7	E11	H13	H14	K16
0	0	0	0	0	0	0	0	0	0	0
1+	1	0	0	+	0	0	0	0	0	0
2+	2	0	0	+	0	0	0	0	0	+
3+	3	0	0	+	0	0	0	0	+	+
4+	4	0	0	+	+	0	0	+	0	+

Table S5. Hess's cosine content analysis of the four principal components (PC1-PC4) obtained from mass weighted covariance analysis[1] for the equilibrated MD trajectories of Aβ(1-16) in water. MD1 to MD4 are the classical MD simulations in aqueous solution with different microscopic initial conditions (see Supplementary Text S1).

ID	Cosine content			
	MD1	MD2	MD3	MD4
PC1	0.0041	0.0063	0.0054	0.0089
PC2	0.0062	0.0038	0.0025	0.0023
PC3	0.0003	0.0009	0.0008	0.0021
PC4	0.0070	0.0074	0.0004	0.0085

Table S6. Occupancy of H-bonds between ionizable residues' side chains in our MD simulations.

Donor Res	Acceptor Res	Occupancy
HIS14 (H14)	GLU3 (E3)	60%
GLU3 (E3)	HIS13 (H13)	69%
ARG5	ASP1	4%
ARG5	ASP7	9%
ASP7	GLU11	1%
LYS16	HIS6	9%
ASP1	ASP7	3%
HIS6	ASP1	4%

Supplementary Figures

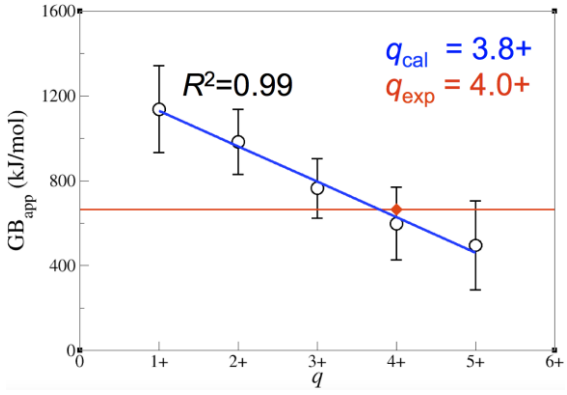

Figure S1. Prediction of the maximum charge state of Aβ(1-16). Apparent gas-phase basicity (GB_{app}) values (in kJ/mol) were calculated for the lowest energy protonation states of Aβ(1-16) (blue line and black cycle symbols). Standard deviation from the average is given as error bars. The red horizontal line indicates the GB of water (660.3 kJ/mol taken from Ref. [14]). The experimental maximum charge state is shown by red solid diamond.

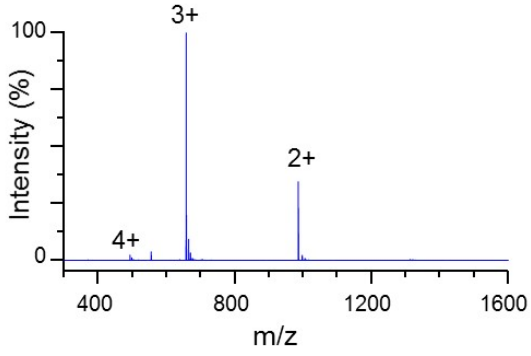

Figure S2. Nano-ESI-MS spectrum in positive-ion mode of 1 mM Aβ(1-16) in 10 mM ammonium acetate pH 7.4 acquired on the Qstar Elite (AB Sciex) instrument.

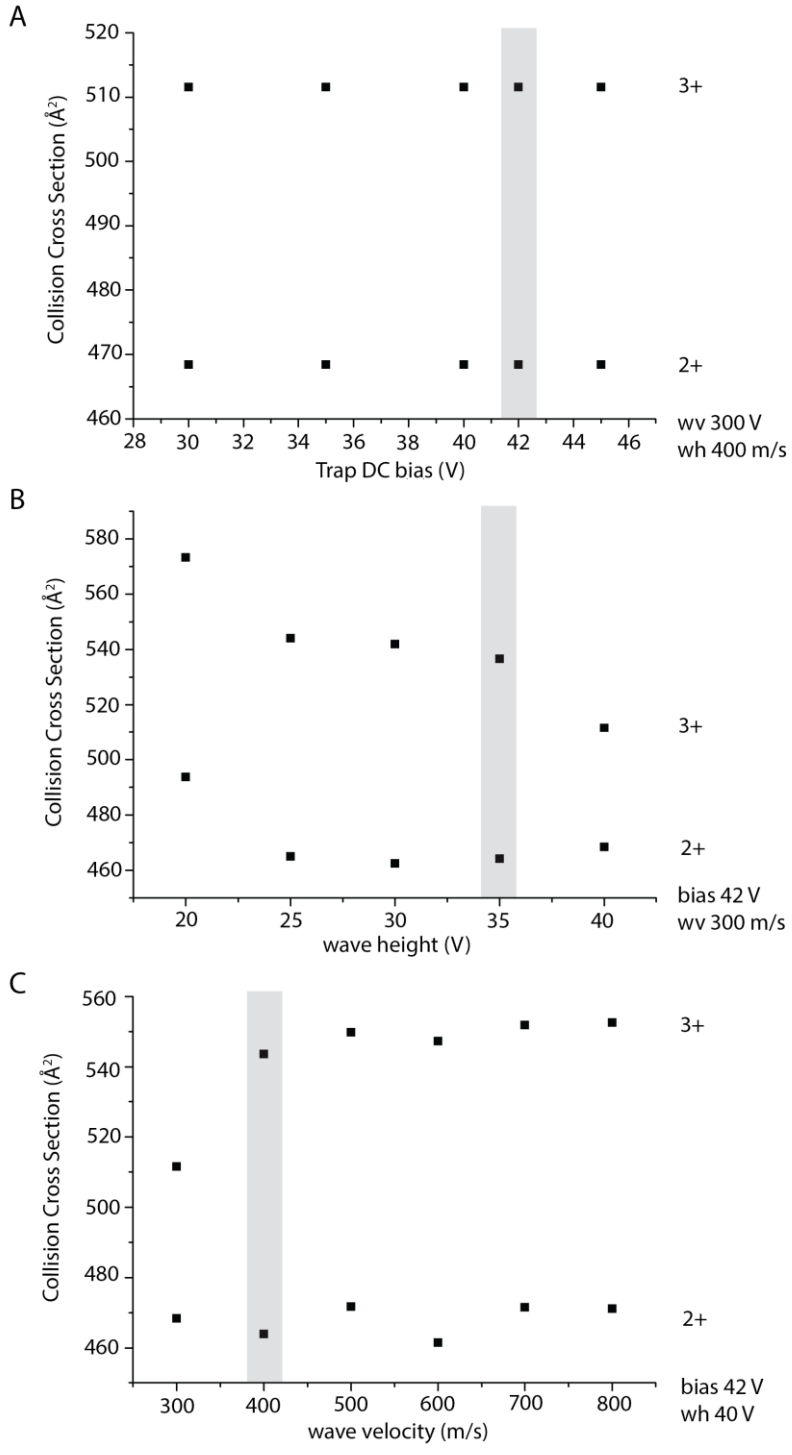

Figure S3 Determining influence of the trap DC bias (A) wave height (B) and wave velocity (C) on the experimental collision cross section of Aβ(1-16). Grey areas indicate the values that were chosen for the final collision cross section determination for Aβ(1-16).

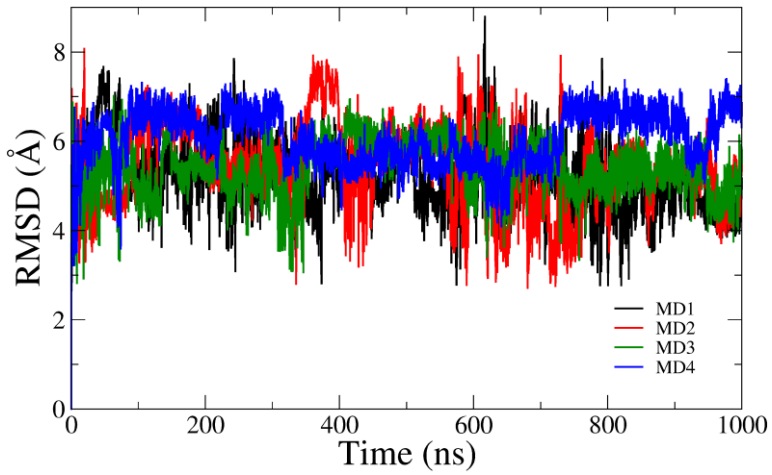

Figure S4. Root mean square deviations (RMSD) of backbone atoms from the starting conformations of Aβ(1-16) during four independent 1000 ns-long MD simulations in water. MD1 to MD4 are the classical MD simulations in aqueous solution with different microscopic initial conditions (see Supplementary Text S1).

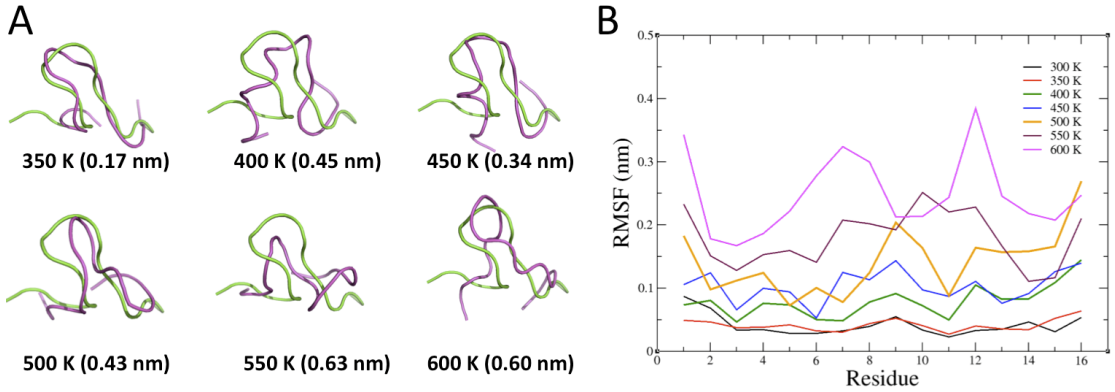

Figure S5. Determination of the temperature to carry out MC/MD simulations. **(A)** Superposition of the lowest energy configuration at 300 K (green) with that at other temperatures (magentas). RMSDs of backbone atoms are indicated in parentheses. **(B)** Root mean square fluctuation (RMSF) plotted for side chain atoms of the peptide from 5 ns-long MD simulations at various temperatures.

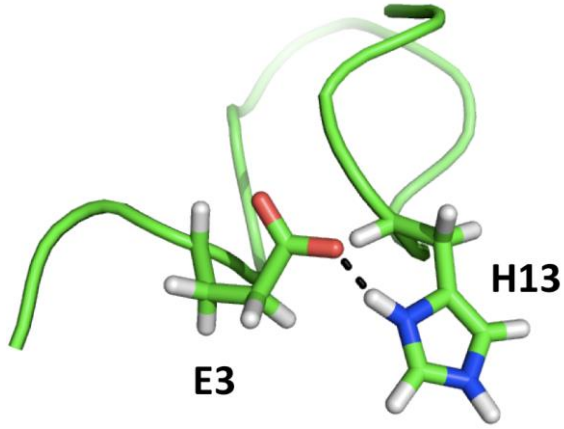

Figure S6. Cartoon representation of Aβ(1-16) structure obtained from QM/MM simulations after proton transfer from E3 to H13. Hydrogen bond is indicated by dashed line.

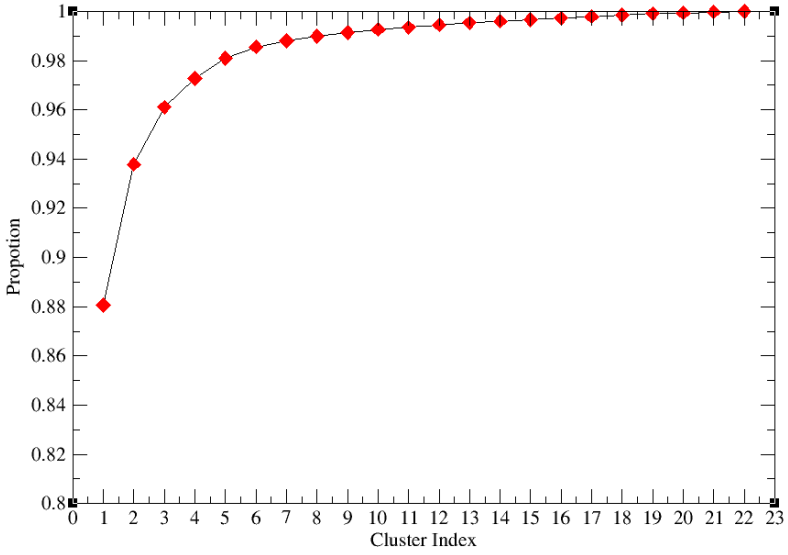

Figure S7. Cumulative proportion of each clusters over the conformational space sampled in the MD. The first three clusters covered almost all (96%) of the conformations.

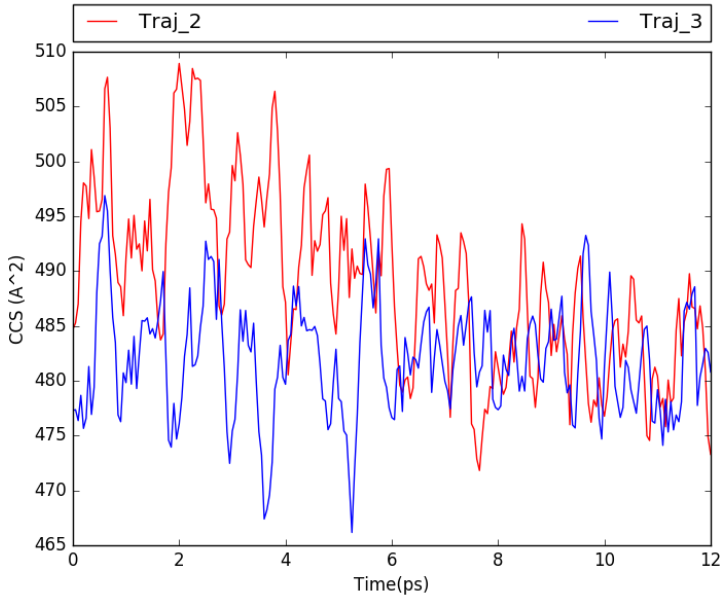

Figure S8. Time evolutions of the CCS values of protein in the second (Traj_2) and the third (Traj_3) QM/MM simulations.