Ion mobility spectrometry - mass spectrometry defines the oligomeric intermediates in amylin amyloid formation and the mode of action of inhibitors

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Supplementary Information

Oligomer Order	Charge State	hIAPP CCS (Å^2)	rIAPP CCS (Å^2)
1	2	513.3 (i)	509.7 (i)
1	2	606.1 (ii)	575.6 (ii)
1	3	681.6 (i)	737.3
1	3	790.4 (ii)	n/a
2	3	948.0 (i)	890.0 (i)
2	3	1019.8 (ii)	1037.7 (ii)
3	4	1252.3	1178.4
3	5	1346.3	1346.4
4	5	1537.7	1463.9
4	6	1614.7	1626.8
5	7	1927.7	1912.1
6	8	2202.6	2311.0

Table S1: Experimental CCSs of hIAPP and rIAPP oligomers. CCS of hIAPP and rIAPP oligomers measured using ESI-IMS-MS (50μM peptide), observed after 2 min incubation (37 ^{o}C, 600 rpm, 20 mM ammonium acetate buffer, pH 6.8). Experimental error is ± 5% for all cross sections measured by use of an IMS-MS calibration[43] over 3 experiments. (i) and (ii) denote different conformers of the same ion. Note that while CCS stated here do not change with experimental conditions i.e. high ionic strength, the relative populations of the conformers present are altered at different ionic strength.

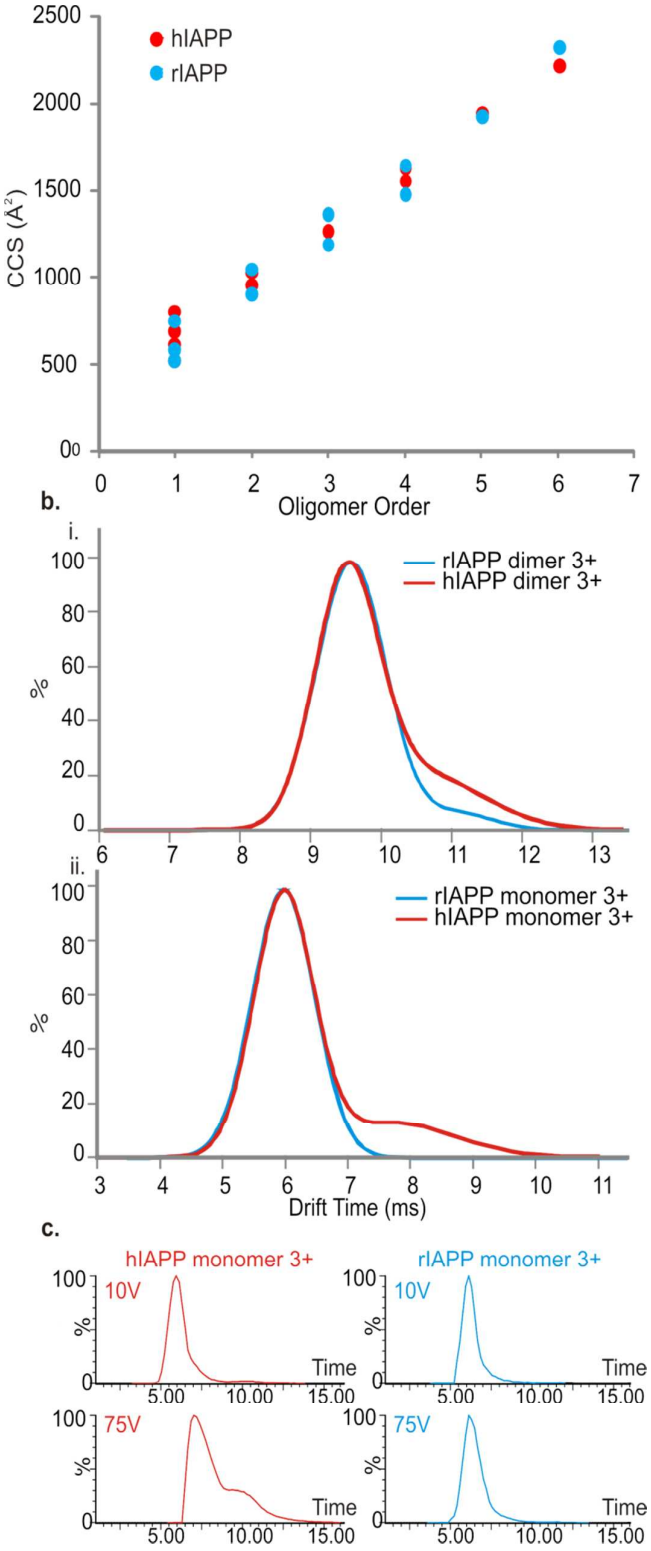

Figure S1: Comparisons of hIAPP and rIAPP oligomers. a) CCS of hIAPP oligomers (red) and rIAPP oligomers (blue) measured using ESI-IMS-MS plotted versus oligomer order. Experimental error is ± 5% for all CCS measurements. b) Arrival time distribution (ATD) of the hIAPP (red) and rIAPP (blue) 3+ dimer (i) and 3+ monomer (ii). c) ATDs of 3+ monomers of hIAPP and rIAPP at trap collision cell voltages 10 V and 75 V.

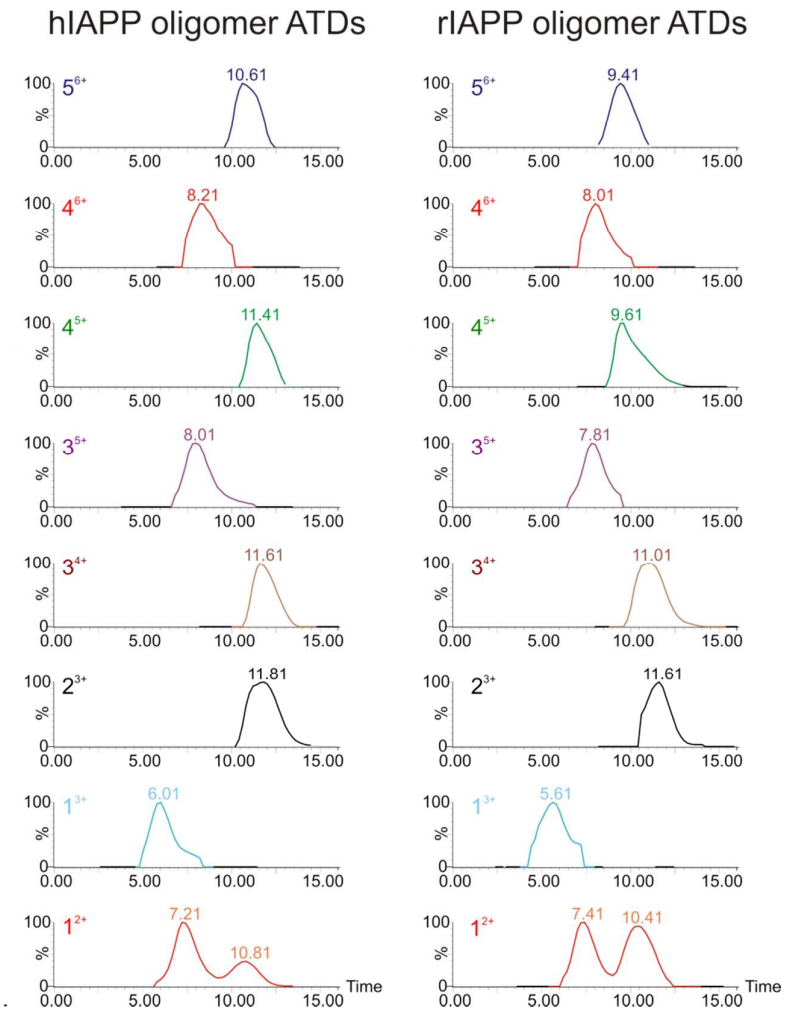

Figure S2: Comparisons of hIAPP and rIAPP monomer and oligomer arrival time distributions.

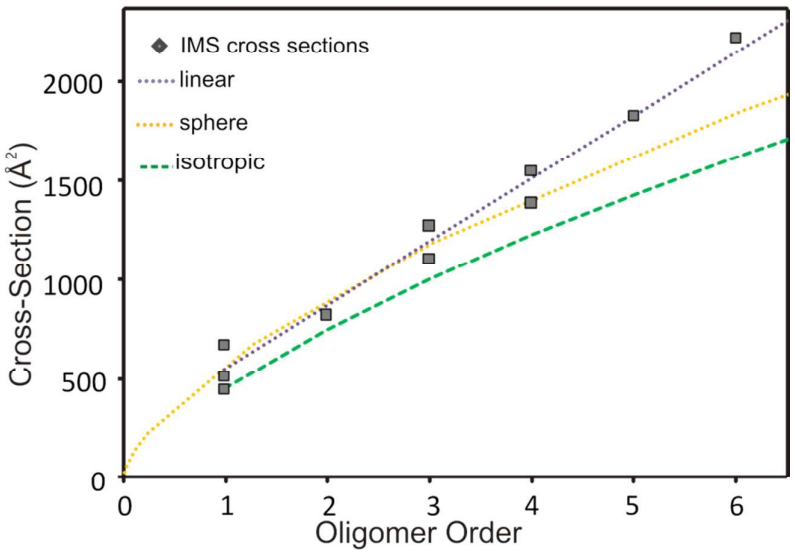

Figure S3: rIAPP oligomer CCS measurements. CCS of rIAPP oligomers measured using ESI-IMS-MS versus oligomer order for all charge states. The orange dashed line represents a spherical growth model based on the average density of a protein (0.44 Da/Å^3)[49]. The purple dashed line represents a linear growth model[48] and the green dashed line represents an isotropic growth model[48].

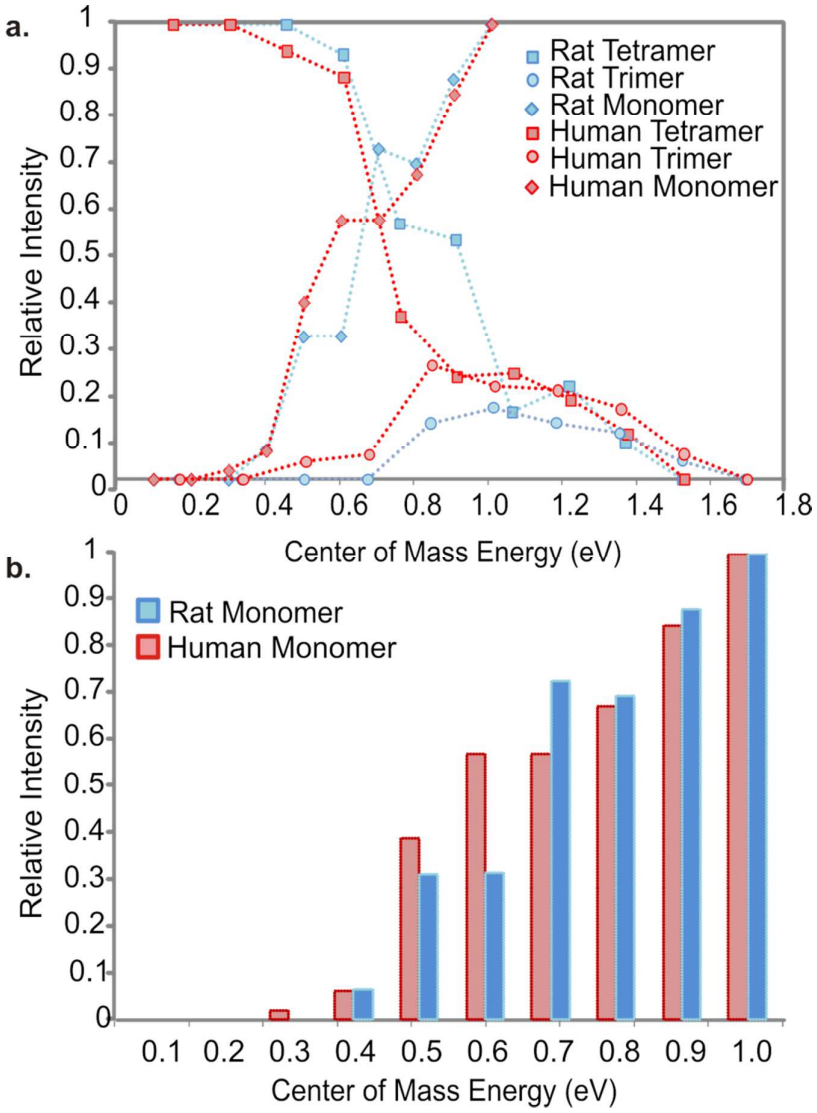

Figure S4. Differences between rIAPP and hIAPP tetramer stabilities in the gas-phase measured using CID. a) MS/MS collision induced dissociation (CID) of rIAPP (blue) and hIAPP (red) tetramer ions. Relative intensity of the 6+ tetramer ions (squares) of each peptide is plotted versus increasing ion-accelerating voltage into the transfer T-wave device (CID). Monomer ion intensity (diamonds) and trimer ion intensity (circles) increase as tetramer ions (squares) become dissociated. b) Bar charts showing appearance of hIAPP (red) and rIAPP (blue) monomer from dissociation of tetramer ions with increasing CID voltage.

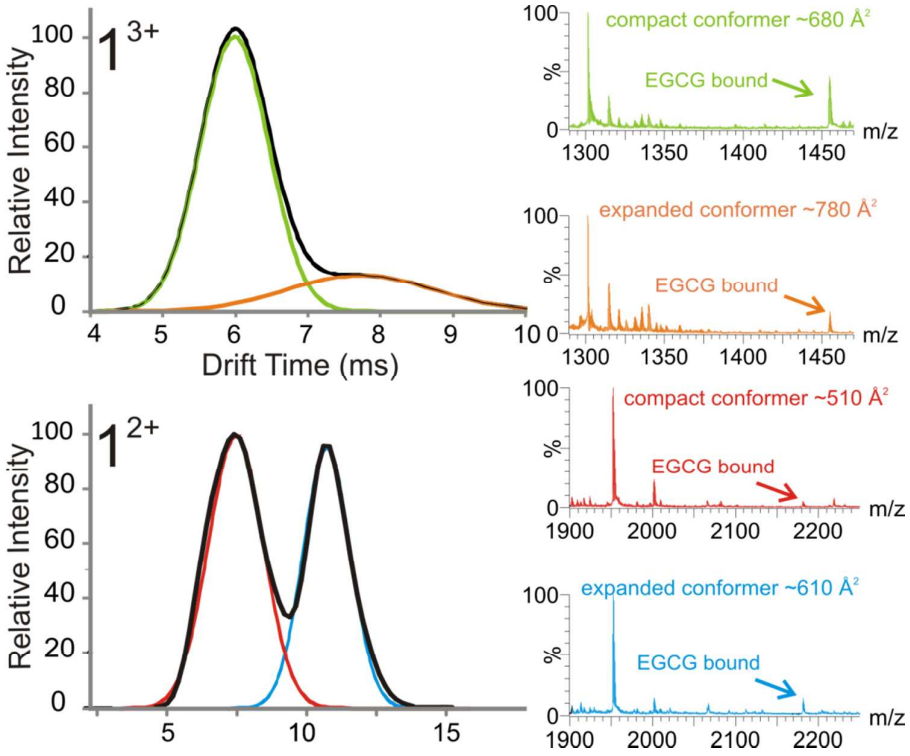

Figure S5. Arrival time distributions show that the 3+ monomer ions occupy two conformers (t_D = 6 & 8 ms) and the 2+ monomer ions also occupy two conformers (t_D = 7.6 & 10.6 ms). EGCG binds to the both the expanded conformer (orange/blue) and to the compact conformer (green/red) of both the 3+ and 2+ monomer ions.

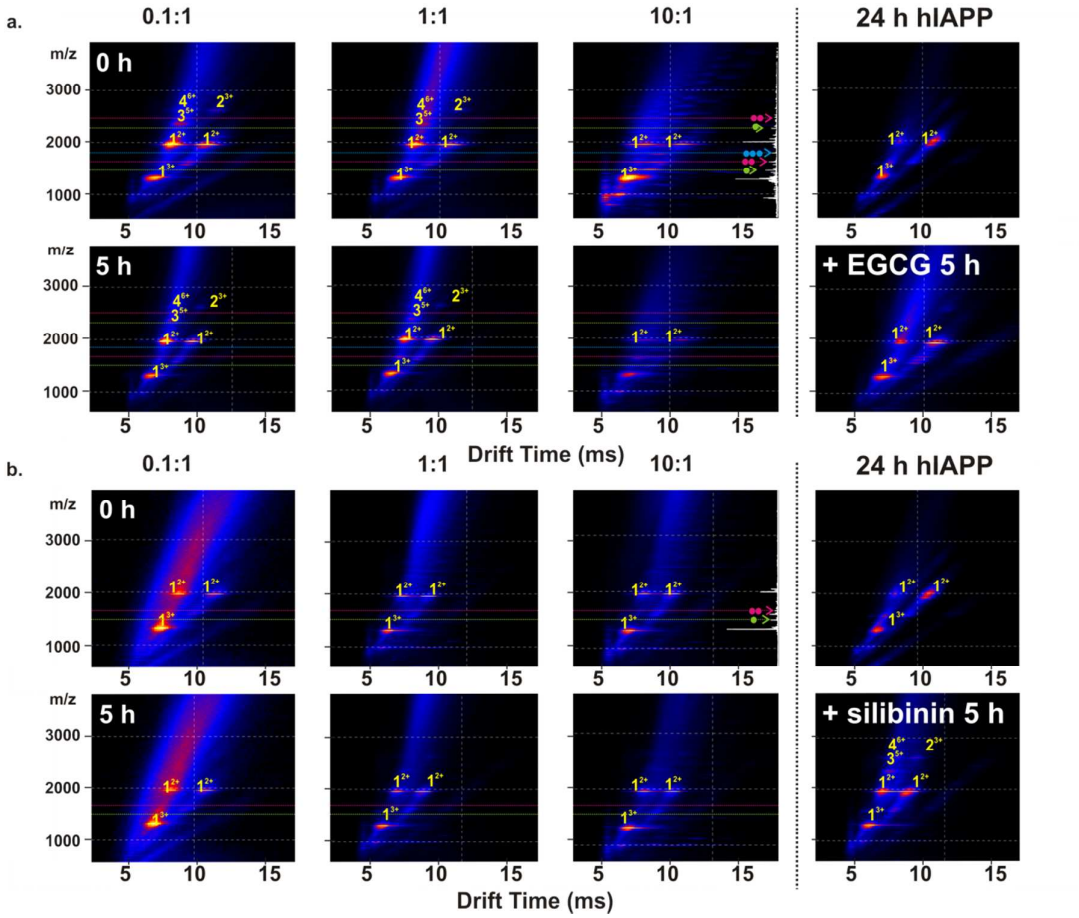

Figure S6: ESI-IMS-MS time courses of inhibition of hIAPP oligomer formation by EGCG and silibinin. Driftscope plots of hIAPP oligomers formed in the presence of 0.1:1, 1:1 or 10:1 molar ratios of (a) EGCG or (b) silibinin at t= 2 min and t= 5 hours. The right hand driftscope plots show hIAPP alone after 24 h incubation and after subsequent addition of a 5-fold molar excess of inhibitor (ammonium acetate buffer pH 6.8, 37^{o}C, 600 rpm).

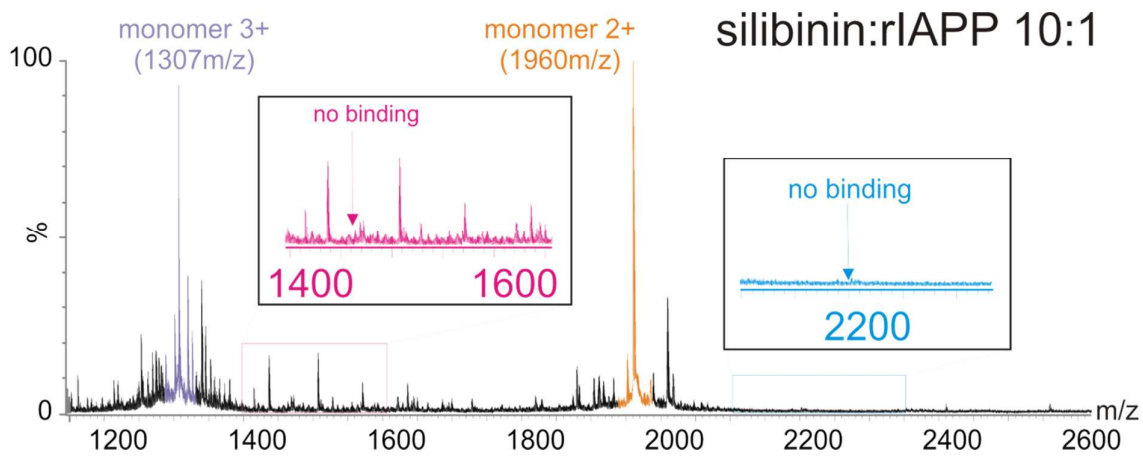

Figure S7. Positive ion ESI mass spectra showing absence of binding of silibinin molecules (added at 500 µM to 50 µM peptide) to the 3+ monomer ions (purple) and to the 2+ monomer ions (orange) at a molar ratio of silibinin:rIAPP of 10:1. Zoomed regions of the spectra at which bound peaks (arrow) would appear are inset.

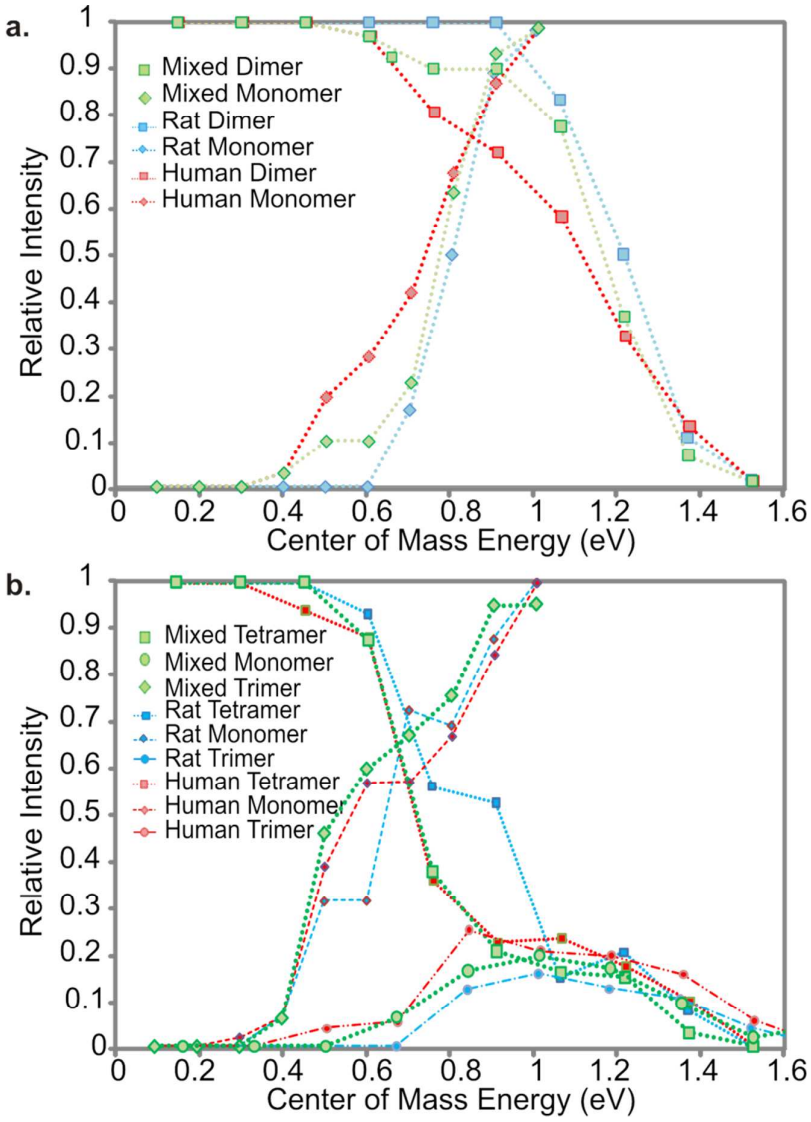

Figure S8: Differences between the gas-phase stabilities of hIAPP, rIAPP and hIAPP/rIAPP mixed oligomers, measured using CID. a) Collision induced dissociation of mixed (a) dimers and (b) tetramers. a) The relative intensity of the 3+ mixed dimer ions (squares) plotted against increasing ion-accelerating voltage into the transfer T-wave device. hIAPP (red) and rIAPP (blue) are also plotted for comparison. b) The relative intensity of the mixed 6+ tetramer ions (squares) plotted against transfer voltage. Monomer (circle) and trimer (diamonds) ions appear with increasing CID voltage and trimer ion intensity subsequently decreases again as they are dissociated to monomer. In both cases mixed oligomers show intermediate stability with respect to oligomers formed from hIAPP alone and rIAPP alone.