Electronic Supplementary Information

Determination of the Aggregate Binding Site of Amyloid Protofibrils Using Electron Capture Dissociation Tandem MS

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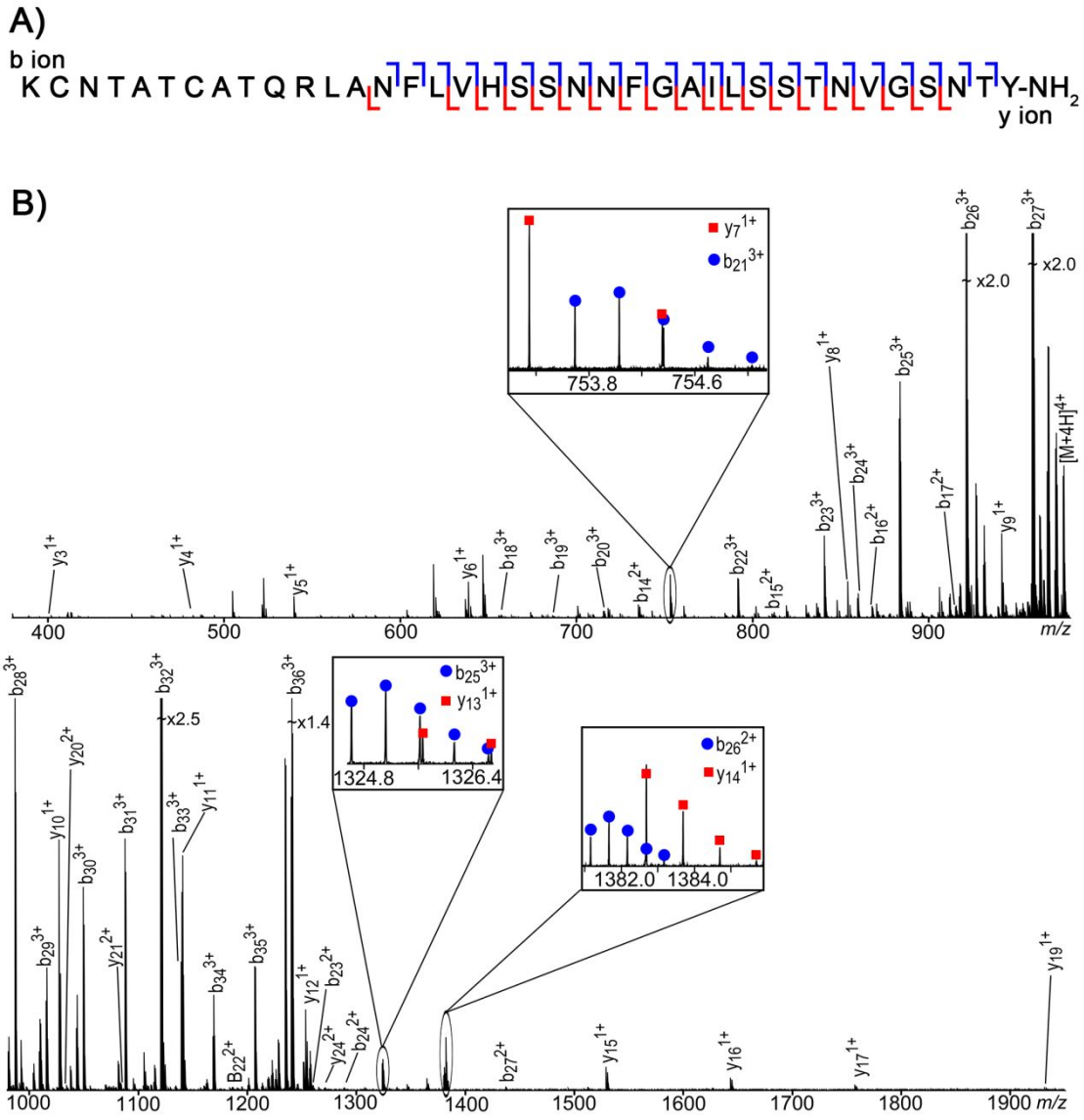

Figure S1. (A) Summarised b- and y-ion fragments observed in the (B) 18 V CAD mass spectrum of the 4+ charge state hIAPP monomer. The hIAPP sequence coverage from the CAD MS/MS was 65% and showed effective fragmentation and production of b and y ions from Asn-14 to Try-37; while no fragments were obtained from Lys-1 to Ala-13. Water and ammonia (NH_3) loss from fragment were not labelled in the spectrum, but were common. The inserts show examples of isotopic distribution peaks, i.e. the b_{25}^{2+} and y_{13}^{1+} ions, which must be resolved with high resolution mass spectrometry in order to accurately assign fragment ions. The mass difference between the second

isotopic peak of y_7 ion and the third isotopic peak of b_{21} ion was 0.0099 Da, and the resolving power at *m/z* 753 was 240,000. The assigned fragments are listed in the Supporting Information Table S-1.

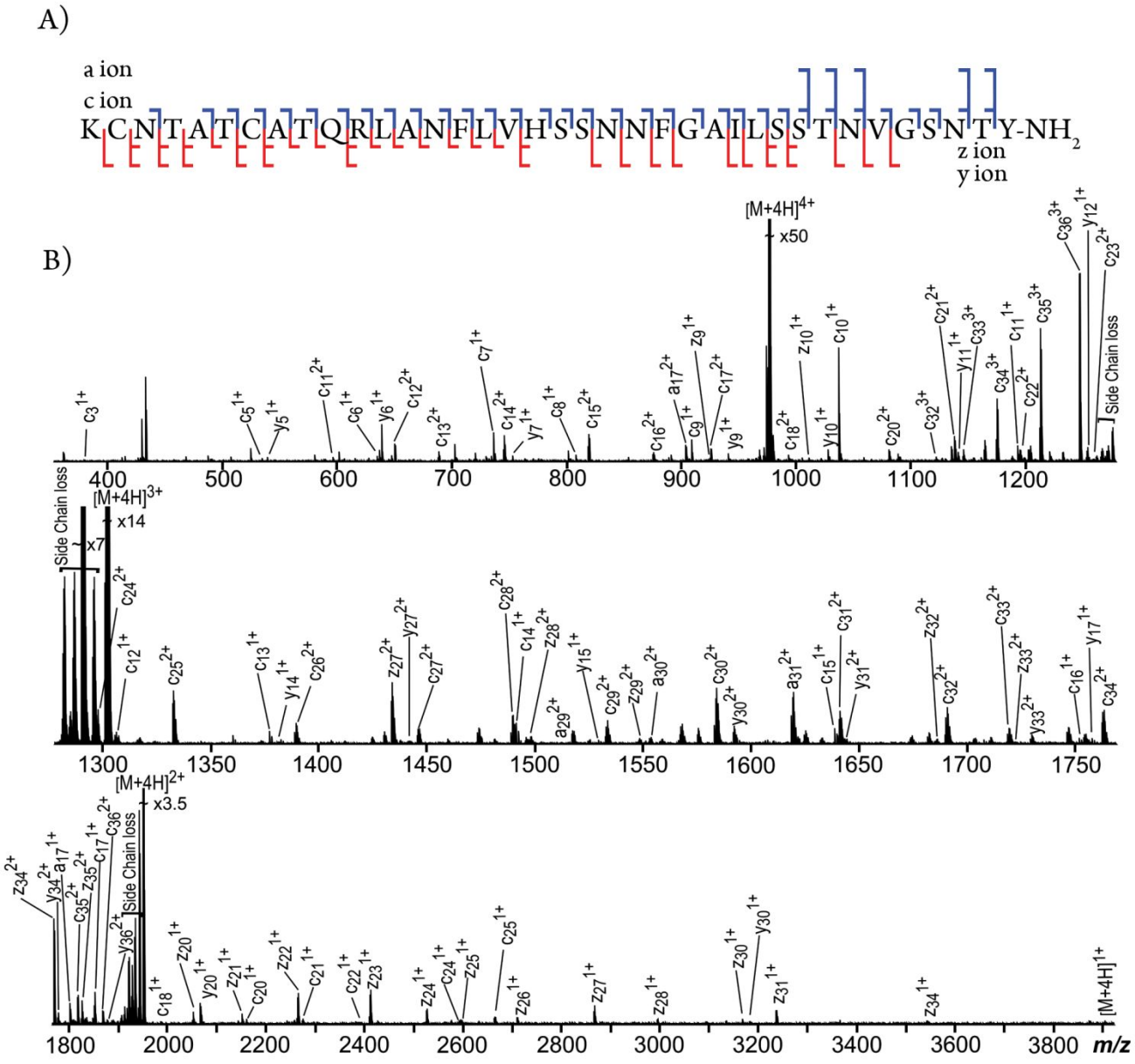

Figure S2. (A) Summarised a-, c-, y- and z-ion fragments observed in the (B) IR-ECD mass spectrum of the 4+ charge state hIAPP monomer. The hIAPP sequence coverage from the IR-ECD MS/MS was 100%. Amino acid side chain losses were not labelled in the spectrum, but were common. The assigned fragments are listed in the Supporting Information Table S-2.

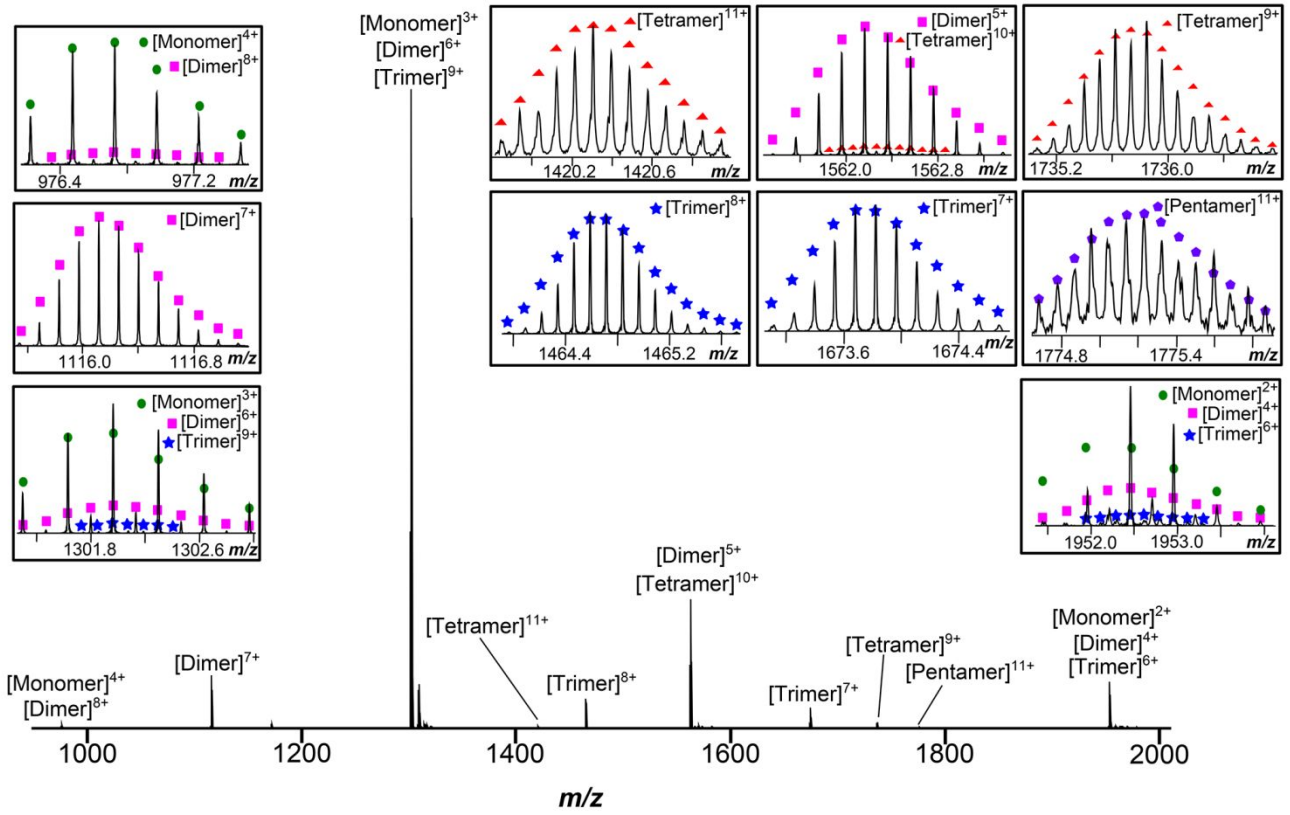

Figure S3. nESI mass spectrum of the early oligomers from 10 μM hIAPP aqueous solution only. hIAPP monomer (circle), dimer (square), trimer (star), tetramer (triangle), and pentamer (pentagon), were detected in the spectrum. The peak intensity decreased gradually with increasing oligomer size. Inset, enlarge regions of spectra are shown for the labelled species. Coloured shapes represent theoretical calculated species overlaid onto observed pattern.

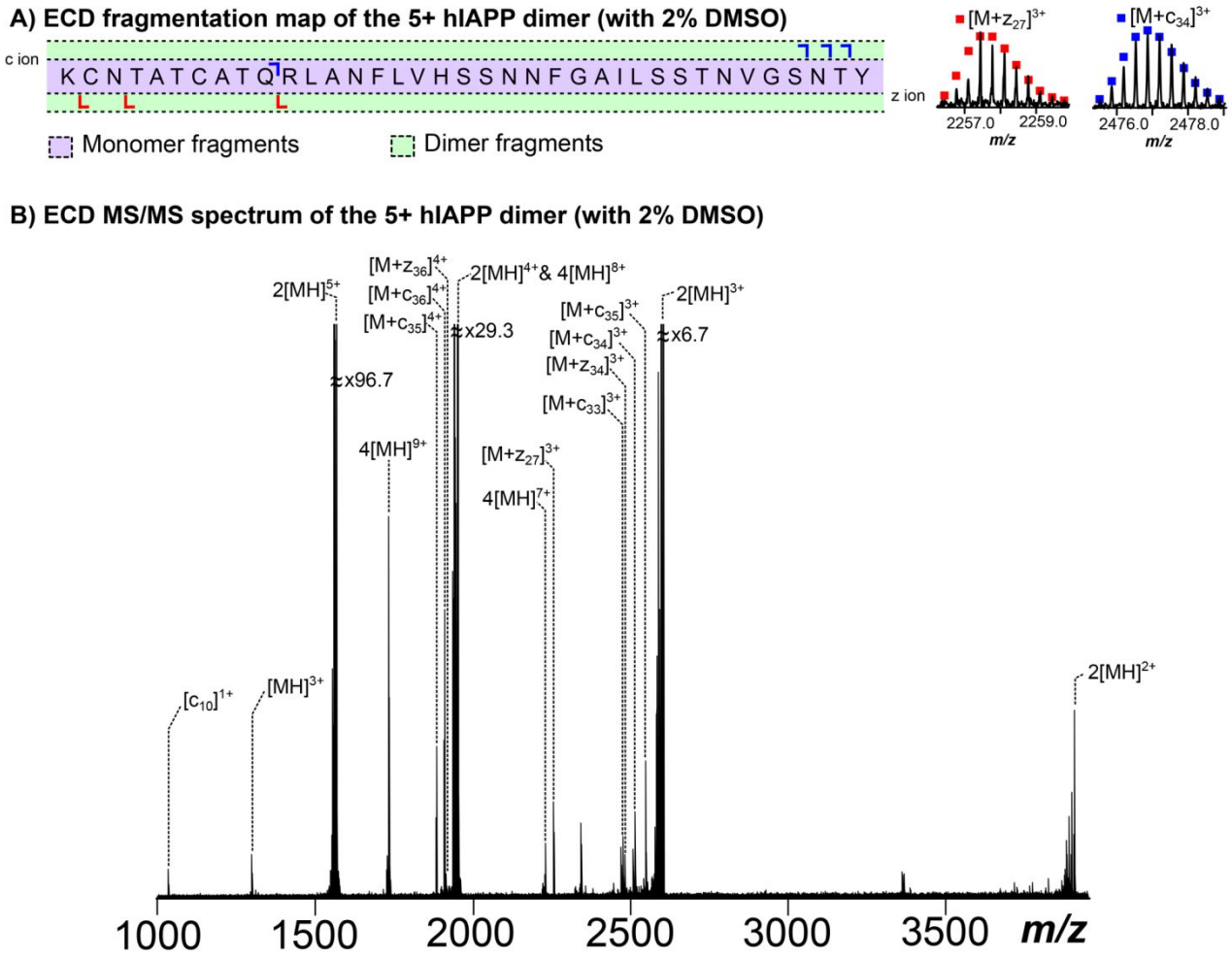

Figure S4. (A) Summarised c- and z-ion fragments observed in the (B) ECD mass spectrum of the 5+ charge state hIAPP dimer (in aqueous solution with 2% residual DMSO). The amino acid side chain losses were not labelled in the spectrum but were common. The isotopic distribution of the 10+ charge state hIAPP tetramer was present and overlapped with the 5+ charge state hIAPP dimer during isolation, and higher order oligomers, i.e. tetramer, are observed in the dimer ECD spectrum. The assigned fragments are listed in the Supporting Information Table S-3.

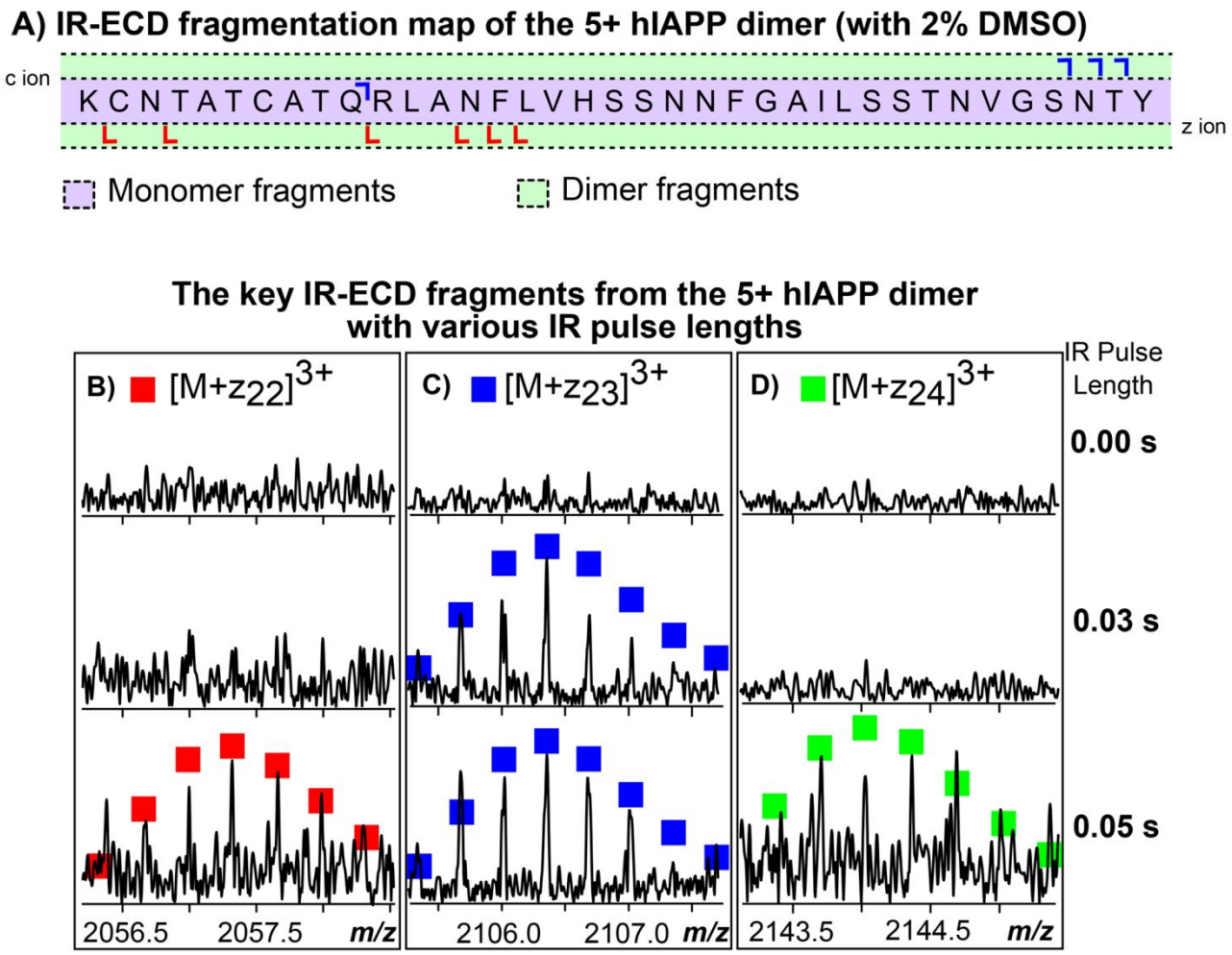

Figure S5. (A) Summarised c- and z-ion fragments observed in the IR-ECD MS/MS of the 5+ dimer hIAPP. Key IR-ECD fragments of (B) $[M+z_{22}]^{3+}$ (red), (C) $[M+z_{23}]^{3+}$ (blue), and (D) $[M+z_{24}]^{3+}$ (green) from the 5+ charge state hIAPP dimer with various IR pulse lengths. When no IR laser was applied, no dimer fragments from $M+z_{22}$ to $M+z_{24}$ were observed. When IR pulse length was set to 0.03 s, an extra dimer fragment, $M+z_{23}$, was shown (B). Dimer fragments from $M+z_{22}$ to $M+z_{24}$ were identified from the spectrum while IR pulse length was increased to 0.05 s ((B) and (D) respectively). Coloured squares indicate simulated isotopic distributions in spectra where the fragment was detected and assigned.

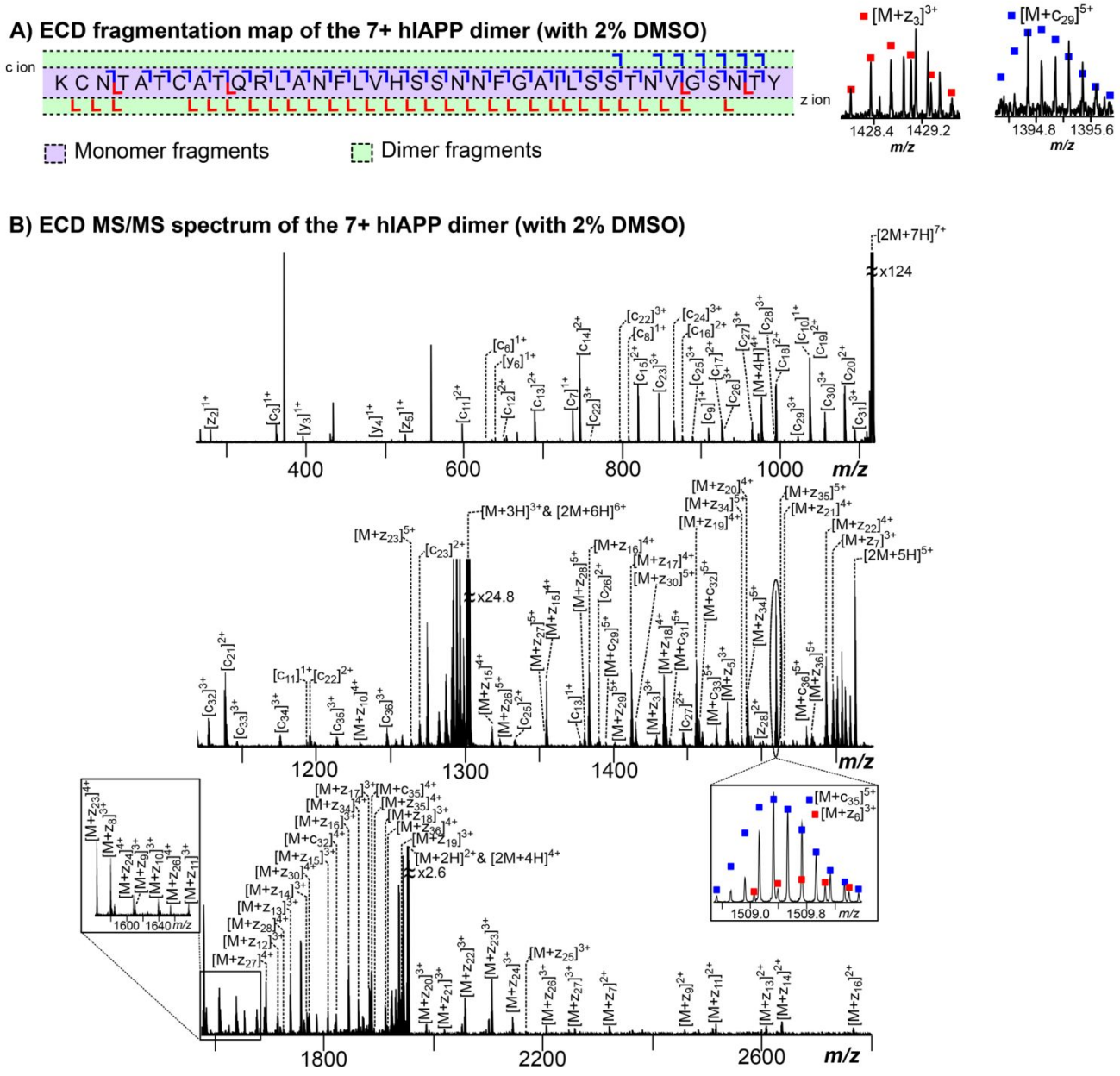

Figure S6. (A) Summary of c- and z-ion fragments observed in the (B) ECD mass spectrum of the 7+ charge state hIAPP dimer (in aqueous solution with 2% residual DMSO). The side chain losses were not labelled for clarity. An increased number of fragments are observed in the ECD mass spectrum of the 7+ charge state dimer than the 5+ charge state dimer spectrum. The assigned fragments are listed in the Supporting Information Table S-4.

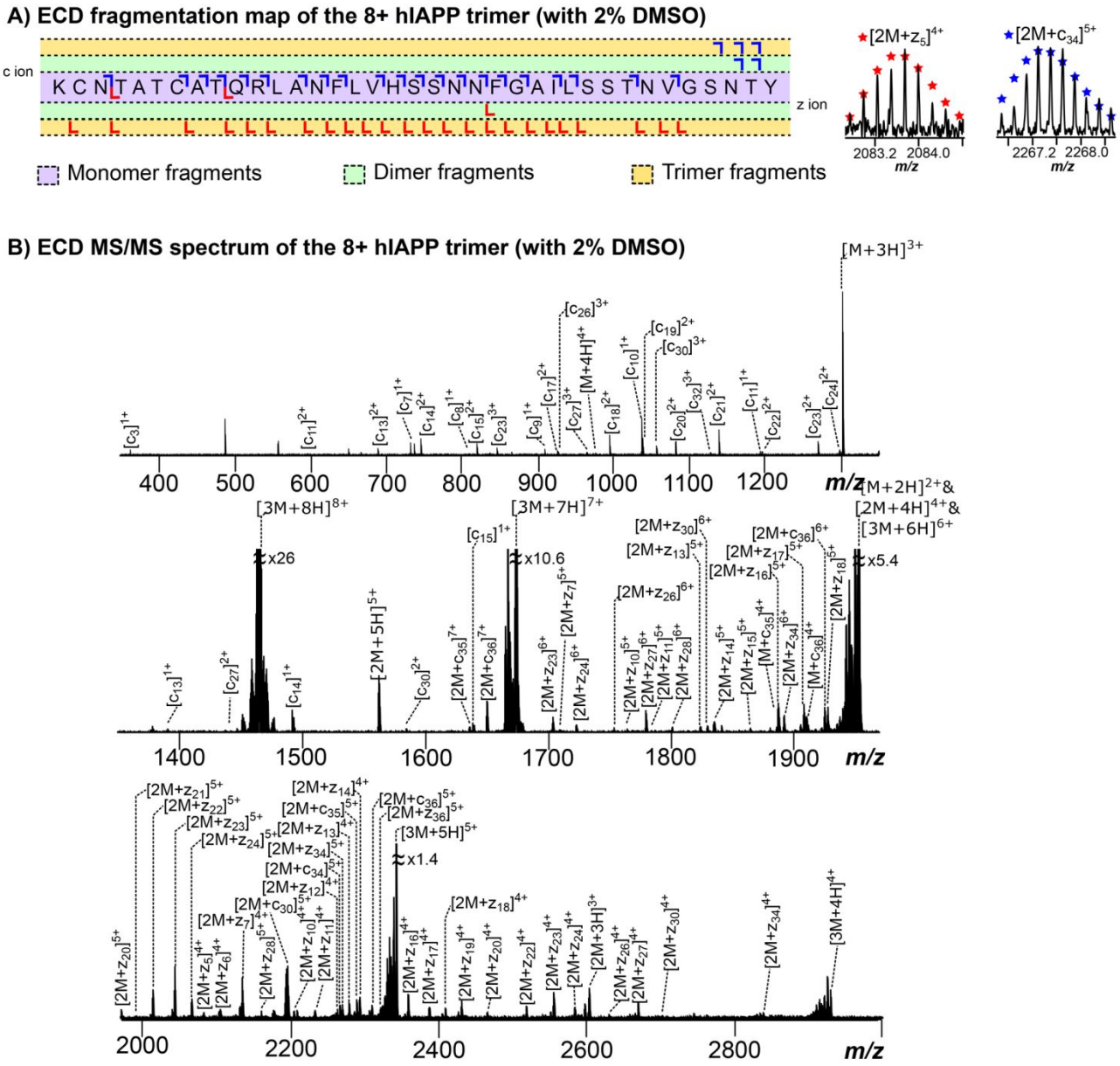

Figure S7. (A) Summary of c- and z-ion fragments observed in the (B) ECD mass spectrum of the 8+ charge state hIAPP trimer (in aqueous solution with 2% residual DMSO). The side chain losses were not labelled for clarity. The assigned fragments are listed in the Supporting Information Table S-5.

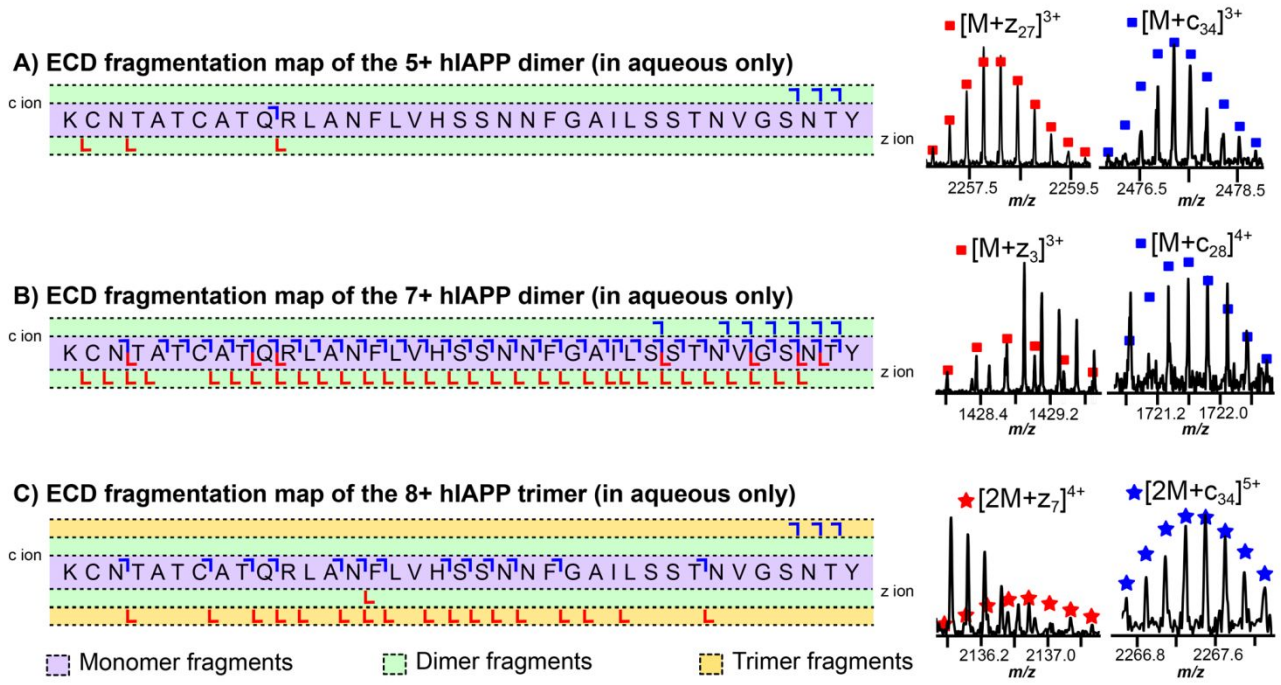

Figure S8. Summarised fragments observed in the ECD mass spectrum of the (A) 5+ charge state dimer, (B) 7+ charge state dimer, and (C) 8+ charge state trimer of hIAPP, in aqueous solution. Examples of the smallest assigned dimer c-and z-ions from the ECD MS/MS of the 5+ and the 7+ charge state dimers are inserted, respectively. Examples of the most critical trimer c- and z-ion from the ECD MS/MS of the 8+ charge state trimer are also inserted. Coloured shapes represent theoretical calculated species overlaid onto observed pattern. The assigned fragments are listed in the Supporting Information Tables S-6, S-7, and S-8.

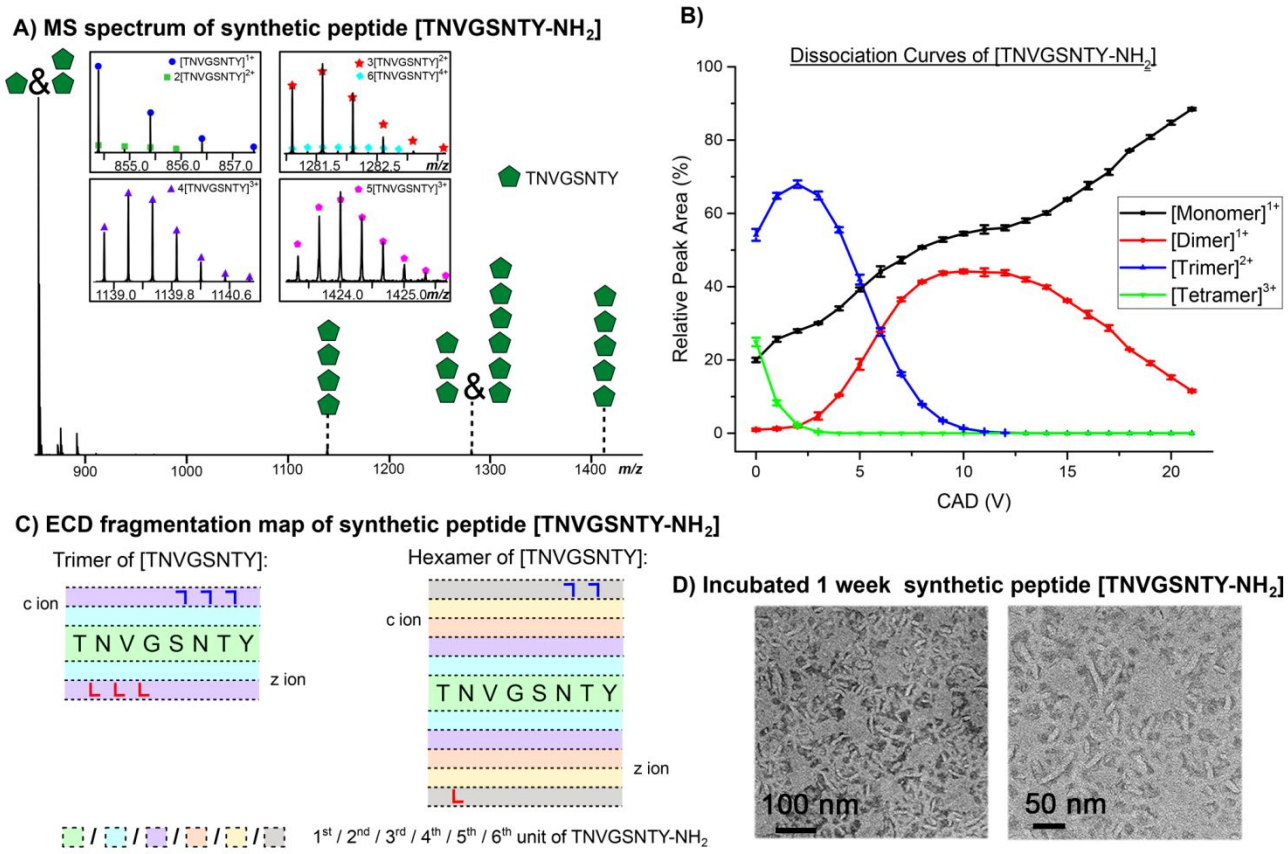

Figure S9. (A) Mass spectrum of the early oligomers of an eight-residue segment of hIAPP (^{30}TNVGSNTY^{37}-NH_2) in aqueous solution. (B) The relative peak areas of monomer (black), dimer (red), trimer (blue), and tetramer (green) during the dissociation of the 3+ charge state tetramer of the eight-residue segment of hIAPP in the CAD MS/MS. (C) Summary of the ECD MS/MS fragments of the 2+ charge state hIAPP segment in aqueous solution. (D) TEM images The TEM images of the 1-week 10 μM hIAPP segment solution. The scale bars for each TEM image are inset.

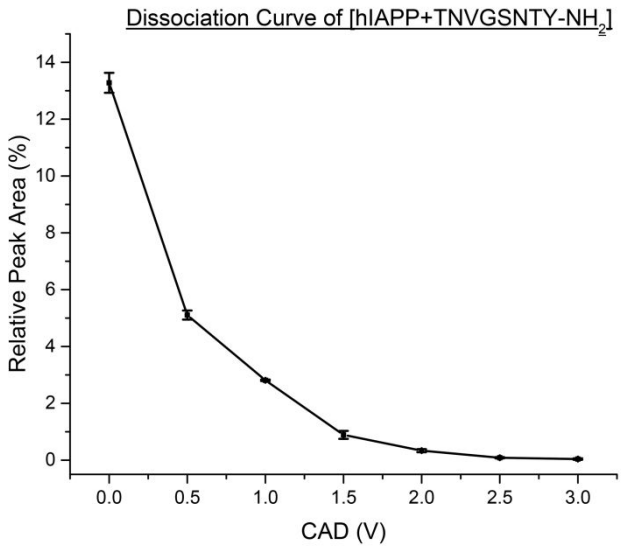

Figure S10. (A) The CAD curve of the 4+ charge state hIAPP and ^{30}TNVGSNTY^{37}-NH_2 in 1:1 at *m/z* 1190. Only 13% of the mixture was successfully isolated and detected by the MS when no CAD energy was applied, which indicated the interaction between the biomolecules was very weak. The mixture was completely dissociated when 3 V of additional CAD energy was applied.

The table of assigned CAD MS/MS fragments of the 4+ hIAPP monomer

Ion	Charge	Theoretical *m/z*	Experimental *m/z*	Error (ppm)
MH	4+	976.223168	976.223160	0.008
b14	2+	737.848058	737.848010	0.065
b15	2+	811.382265	811.382370	-0.129
b16	2+	867.824297	867.824300	-0.003
b17	2+	917.458504	917.458500	0.004
b18	2+	985.987960	985.988030	-0.071
b18	3+	657.661065	657.661150	-0.129
b19	3+	686.671741	686.671520	0.322
b20	3+	715.682418	715.682430	-0.017
b21	3+	753.696727	753.696740	-0.017
b22	2+	1187.062917	1187.063020	-0.087
b22	3+	791.711036	791.711020	0.020
b23	2+	1260.597124	1260.597440	-0.251
b23	3+	840.733841	840.733860	-0.023
b24	2+	1289.107856	1289.107970	-0.088
b24	3+	859.740996	859.740930	0.077
b25	2+	1324.626413	1324.626300	0.085
b25	3+	883.420034	883.420040	-0.007
b26	2+	1381.168445	1381.168610	-0.119
b26	3+	921.114722	921.114690	0.035
b27	2+	1437.710477	1437.710670	-0.134
b27	3+	958.809410	958.809420	-0.010
b28	3+	987.820086	987.820110	-0.024
b29	3+	1016.830762	1016.830790	-0.028
b30	3+	1050.513322	1050.513220	0.097
b31	3+	1088.527631	1088.527500	0.120
b32	3+	1121.550436	1121.550350	0.077
b33	3+	1140.557591	1140.557563	0.025
b34	3+	1169.568267	1169.568320	-0.045
b35	3+	1207.582576	1207.582550	0.022
b36	3+	1241.265136	1241.265380	-0.197
y3	1+	396.187761	396.187760	0.003
y4	1+	483.219790	483.219820	-0.062
y5	1+	540.241254	540.241230	0.044
y6	1+	639.309668	639.309610	0.091
y7	1+	753.352596	753.352530	0.088
y8	1+	854.400275	854.400260	0.018
y9	1+	941.432304	941.432240	0.068
y10	1+	1028.464333	1028.464310	0.022
y11	1+	1141.548397	1141.548340	0.050
y12	1+	1254.632461	1254.632790	-0.262
y13	1+	1325.669575	1325.669660	-0.064
y14	1+	1382.691039	1382.690920	0.086
y15	1+	1529.759453	1529.759470	-0.011
y16	1+	1643.802381	1643.802440	-0.036
y17	1+	1757.845309	1757.844940	0.210
y19	1+	1931.909367	1931.908680	0.356
y20	2+	1034.987778	1034.987630	0.143
y21	2+	1084.521985	1084.521550	0.401
y24	2+	1271.619688	1271.619710	-0.017
			Average Error:	0.014
			Absolute Averge Error	0.087
			Standard Deviation:	0.128

Table S1. List of the assigned CAD MS/MS fragments of the 4+ charge state hIAPP monomer.

The table of assigned IR-ECD MS/MS fragments of the 4+ hIAPP monomer

Ion	Charge	Theoretical *m/z*	Experimental *m/z*	Error (ppm)
MI	2+	1951.43911	1951.43866	0.231
MI	3+	1301.29518	1301.29502	0.123
MI	4+	976.22322	976.22328	-0.065
a17	1+	1807.93112	1807.93134	-0.120
a17	2+	903.96531	903.96539	-0.087
a29	2+	1511.75323	1511.75176	0.970
a30	2+	1561.77315	1561.77194	0.777
a31	2+	1618.79462	1618.79380	0.505
a35	3+	1198.58713	1198.58681	0.269
a36	3+	1232.26969	1232.26908	0.496
c3	1+	362.17313	362.17312	0.014
c5	1+	535.26574	535.26570	0.080
c6	1+	635.30568	635.30560	0.131
c7	1+	737.30696	737.30696	-0.004
c8	1+	808.34407	808.34406	0.014
c9	1+	909.39175	909.39152	0.253
c10	1+	1037.45033	1037.45041	-0.079
c11	1+	1193.55144	1193.55160	-0.135
c11	2+	597.27938	597.27939	-0.013
c12	1+	1306.63550	1306.63612	-0.472
c12	2+	653.82141	653.82139	0.037
c13	1+	1377.67262	1377.67320	-0.423
c13	2+	688.83606	688.83613	-0.104
c14	1+	1491.71555	1491.71590	-0.238
c14	2+	745.85752	745.85764	-0.158
c15	1+	1638.78396	1638.78399	-0.019
c15	2+	819.39173	819.39174	-0.013
c16	1+	1751.86802	1751.86790	0.070
c16	2+	875.93376	875.93381	-0.055
c17	1+	1850.93644	1850.93660	-0.088
c17	2+	925.46797	925.46763	0.366
c18	1+	1987.99535	1987.99514	0.105
c18	2+	993.99742	993.99771	-0.287
c19	1+	2075.02738	2075.02573	0.794
c20	1+	2162.05941	2162.05959	-0.085
c20	2+	1081.02945	1081.02974	-0.265
c21	1+	2276.10234	2276.10147	0.380
c21	2+	1138.05092	1138.05099	-0.063
c22	1+	2390.14526	2390.14589	-0.262
c22	2+	1195.07238	1195.07235	0.027
c23	2+	1268.60659	1268.60608	0.401
c24	1+	2594.23514	2594.23517	-0.011
c24	2+	1297.11732	1297.11750	-0.138
c25	1+	2665.27226	2665.27400	-0.655
c25	2+	1332.63588	1332.63575	0.096
c26	2+	1389.17791	1389.17764	0.194
c27	2+	1445.71994	1445.71981	0.091
c28	2+	1489.23596	1489.23572	0.159
c29	2+	1532.75197	1532.75136	0.399
c30	2+	1583.27581	1583.27540	0.260
c31	2+	1640.29728	1640.29653	0.454
c32	2+	1689.83148	1689.83118	0.179
c32	3+	1127.22604	1127.22587	0.148
c33	2+	1718.84613	1718.84536	0.446
c33	3+	1145.89725	1145.89698	0.236
c34	2+	1762.36214	1762.36211	0.017
c34	3+	1174.90793	1174.90780	0.107
c35	2+	1819.38360	1819.38333	0.151
c35	3+	1212.92224	1212.92203	0.170
c36	2+	1869.90744	1869.90769	-0.132
c36	3+	1246.94074	1246.94060	0.110
y5	1+	540.24130	540.24125	0.098
y6	1+	639.30972	639.30975	-0.056
y7	1+	753.35265	753.35263	0.020
y9	1+	941.43235	941.43252	-0.177
y10	1+	1028.46438	1028.46443	-0.047
y11	1+	1141.54845	1141.54859	-0.126
y12	1+	1254.63251	1254.63226	0.199
y14	1+	1382.69109	1382.69111	-0.016
y15	1+	1529.75950	1529.76034	-0.548
y17	1+	1757.84536	1757.84562	-0.149
y20	1+	2067.96050	2067.96070	-0.095
y27	2+	1441.73088	1441.73073	0.105
y30	1+	3182.59781	3182.59677	0.326
y30	2+	1591.80257	1591.80241	0.099
y31	2+	1643.30716	1643.30816	-0.609
y33	2+	1729.34956	1729.34844	0.645
y34	2+	1779.87340	1779.87317	0.127
y35	2+	1836.89486	1836.89417	0.376
y36	2+	1888.39945	1888.39807	0.732
z9	1+	924.40580	924.40610	-0.320
z10	1+	1011.43783	1011.43803	-0.195
z20	1+	2053.95743	2053.95716	0.131
z21	1+	2153.02584	2153.02496	0.410
z22	1+	2266.10991	2266.10964	0.118
z23	1+	2413.17832	2413.17751	0.336
z24	1+	2527.22125	2527.22156	-0.123
z25	1+	2598.25836	2598.25951	-0.441
z26	1+	2711.34243	2711.34193	0.183
z27	1+	2867.44354	2867.44323	0.107
z27	2+	1433.72152	1433.72140	0.083
z28	1+	2995.50212	2995.50392	-0.602
z28	2+	1497.75081	1497.75092	-0.075
z29	2+	1547.77074	1547.77091	-0.112
z30	1+	3167.58691	3167.58800	-0.344
z30	2+	1583.79321	1583.79456	-0.856
z31	1+	3236.60843	3236.60698	0.447
z32	2+	1685.31772	1685.31730	0.252
z33	2+	1721.84155	1721.84026	0.749
z34	1+	3541.71292	3541.71563	-0.766
z34	2+	1770.85621	1770.85563	0.327
z35	2+	1827.87767	1827.87733	0.188
			Average Error:	0.056
			Absolute Average Error:	0.245
			Standard Deviation:	0.326

Table S2. List of the assigned IR-ECD MS/MS fragments of the 4+ charge state hIAPP monomer.

The table of assigned ECD MS/MS fragments of the 5+ hIAPP dimer (in aqueous solution with 2% DMSO)

Ion	Charge	Theoretical *m/z*	Experimental *m/z*	Error (ppm)
MI	3+	1301.963507	1301.963571	0.049
2[MI]	5+	1561.352698	1561.352462	-0.151
2[MI]	4+	1951.691010	1951.691642	0.324
2[MI]	3+	2603.591637	2603.591864	0.087
2[MI]	2+	3905.889909	3905.887841	-0.529
4[MI]	9+	1735.727472	1735.727310	-0.093
4[MI]	7+	2231.936089	2231.935654	-0.195
c10	1+	1037.450279	1037.450296	0.016
M+c33	3+	2477.204351	2477.203234	-0.451
M+c34	3+	2515.218663	2515.218503	-0.064
M+c35	4+	1886.413782	1886.414164	0.203
M+c35	3+	2549.237172	2549.237617	0.175
M+c36	4+	1911.675709	1911.675955	0.129
M+z27	3+	2257.776545	2257.776772	0.101
M+z34	3+	2482.868916	2482.869352	0.176
M+z36	4+	1916.414523	1916.414374	-0.078
			Average Error:	-0.019
			Absolute Average Error:	0.176
			Standard Deviation:	0.231

Table S3. List of the assigned ECD MS/MS fragments of the 5+ hIAPP dimer ion (in aqueous solution with 2% residual DMSO). M represents one hIAPP unit.

The table of assigned ECD MS/MS fragments of the 7+ hIAPP dimer

Ion	Charge	Theoretical	Experimental	Error (ppm)
MI	4+	976.223165	976.223805	0.656
MI	3+	1301.295128	1301.295174	0.035
MI	2+	1952.444365	1952.444262	-0.053
2[MI]	7+	1116.112577	1116.111471	-0.991
2[MI]	6+	1301.462261	1301.462328	0.051
2[MI]	5+	1561.956388	1561.956346	-0.027
2[MI]	4+	1953.196034	1953.195933	-0.052
c3	1+	362.173076	362.173094	0.050
c5	1+	534.257869	534.258045	0.329
c6	1+	635.305548	635.305514	-0.054
c7	1+	737.306908	737.307100	0.260
c8	1+	808.344022	808.344287	0.328
c9	1+	909.391701	909.391934	0.256
c10	1+	1037.450279	1037.450719	0.424
c11	2+	597.279333	597.279334	0.002
c11	1+	1193.551390	1193.551623	0.195
c12	2+	653.821365	653.821234	-0.201
c13	2+	689.339922	689.340001	0.115
c13	1+	1378.675984	1378.675689	-0.214
c14	2+	746.361386	746.361455	0.092
c15	2+	819.895593	819.895640	0.057
c16	2+	876.437625	876.437762	0.156
c17	2+	925.971832	925.971982	0.162
c18	2+	994.501527	994.501605	0.078
c19	2+	1038.017303	1038.017169	-0.129
c20	2+	1081.533317	1081.533376	0.055
c21	3+	759.706520	759.706974	0.598
c21	2+	1139.056142	1139.056500	0.314
c22	3+	797.386589	797.386802	0.267
c22	2+	1196.077601	1196.077549	-0.043
c23	3+	846.409393	846.409492	0.117
c23	2+	1269.110452	1269.110945	0.388
c24	3+	865.416548	865.416691	0.165
c25	3+	889.429834	889.430058	0.252
c25	2+	1333.139741	1333.139860	0.089
c26	3+	926.790272	926.790897	0.674
c26	2+	1390.183152	1390.183739	0.422
c27	3+	964.484962	964.484859	-0.107
c27	2+	1447.226592	1447.226424	-0.116
c28	3+	993.829896	993.830644	0.753
c28	2+	1490.241206	1490.241252	0.031
c29	3+	1022.840573	1022.840462	-0.109
c29	2+	1533.757222	1533.758500	0.833
c30	3+	1056.188874	1056.189084	0.199
c30	2+	1584.281062	1584.280913	-0.094
c31	3+	1094.203184	1094.203179	-0.005
c31	2+	1641.302522	1641.302019	-0.306
c32	3+	1127.225988	1127.225763	-0.200
c32	2+	1691.338138	1691.338733	0.352
c33	3+	1146.567401	1146.567371	-0.026
c33	2+	1720.350316	1720.350904	0.342
c34	3+	1176.246205	1176.246590	0.327
c34	2+	1762.362086	1762.362122	0.020
c35	3+	1213.258129	1213.258089	-0.033
c35	2+	1820.386340	1820.386545	0.113
c36	3+	1247.274945	1247.275773	0.664
c36	2+	1870.408779	1870.409286	0.271
y2	1+	282.144833	282.144822	-0.039
y3	1+	396.187761	396.187745	-0.040
y4	1+	483.219829	483.219862	0.068
y6	1+	639.309668	639.309557	-0.174
y7	1+	753.352596	753.352769	0.230
y8	1+	854.400275	854.400522	0.289
y30	2+	1592.303929	1592.304003	0.046
z2	1+	266.126109	266.126109	0.000
z5	1+	525.230353	525.230345	-0.015
z28	2+	1498.756089	1498.757430	0.895
z34	2+	1771.861482	1771.862629	0.647
M+c29	5+	1394.680928	1394.680459	-0.336
M+c31	5+	1438.300318	1438.300930	0.426
M+c32	5+	1457.511616	1457.510861	-0.518
M+c32	4+	1822.140215	1822.139354	-0.473
M+c33	5+	1469.519461	1469.519063	-0.271
M+c34	5+	1486.323445	1486.323359	-0.058
M+c35	5+	1509.332474	1509.331626	-0.562
M+c35	4+	1886.665738	1886.665069	-0.355
M+c36	5+	1529.942864	1529.942434	-0.281
M+z3	3+	1428.350791	1428.350757	-0.024
M+z5	3+	1476.704568	1476.704885	0.215
M+z6	3+	1509.058958	1509.059320	0.240
M+z7	3+	1547.741690	1547.741830	0.090
M+z7	2+	2320.607666	2320.607643	-0.010
M+z8	3+	1581.088313	1581.089390	0.681
M+z9	3+	1610.769064	1610.769547	0.300
M+z9	2+	2459.168869	2459.170923	0.835
M+z10	4+	1229.584072	1229.584641	0.463
M+z10	3+	1639.445612	1639.445934	0.196
M+z11	3+	1676.471867	1676.471173	-0.414
M+z11	2+	2515.206996	2515.206528	-0.186
M+z12	3+	1714.835007	1714.834497	-0.297
M+z13	3+	1738.514047	1738.514068	0.012
M+z13	2+	2607.771504	2607.772357	0.327
M+z14	4+	1317.890129	1317.890715	0.445
M+z14	3+	1757.521201	1757.520755	-0.254
M+z14	2+	2635.780806	2635.780280	-0.200
M+z15	4+	1355.158480	1355.158464	-0.012
M+z15	3+	1807.212338	1807.212131	-0.115
M+z16	4+	1382.917263	1382.917504	0.174
M+z16	3+	1844.892492	1844.892443	-0.027
M+z16	2+	2767.339383	2767.339020	-0.131
M+z17	4+	1412.430522	1412.430040	-0.341
M+z17	3+	1882.906802	1882.906777	-0.013
M+z18	4+	1433.436703	1433.436422	-0.196
M+z18	3+	1912.251644	1912.251179	-0.243
M+z19	4+	1455.695966	1455.695701	-0.182
M+z19	3+	1941.264102	1941.263992	-0.057
M+z20	4+	1489.960695	1489.960191	-0.338
M+z20	3+	1985.946098	1985.945390	-0.357
M+z21	4+	1514.727806	1514.727036	-0.508
M+z21	3+	2019.971451	2019.971805	0.175
M+z22	4+	1543.000167	1542.999155	-0.656
M+z22	3+	2058.000340	2058.000870	0.258
M+z23	5+	1264.014214	1264.014352	0.109
M+z23	4+	1579.013956	1579.014330	0.237
M+z23	3+	2106.354800	2106.354957	0.075
M+z24	4+	1607.776644	1607.776314	-0.205
M+z24	3+	2144.034920	2144.034484	-0.203
M+z25	4+	1626.287918	1626.287893	-0.015
M+z25	3+	2168.382333	2168.383232	0.415
M+z26	5+	1323.246008	1323.245622	-0.292
M+z26	4+	1654.308308	1654.307969	-0.205
M+z26	3+	2206.411232	2206.412492	0.571
M+z27	5+	1354.466227	1354.466407	0.133
M+z27	4+	1693.584213	1693.584379	0.098
M+z27	3+	2258.112487	2258.114584	0.929
M+z28	5+	1380.679464	1380.678457	-0.729
M+z28	4+	1725.598859	1725.599307	0.260
M+z29	5+	1401.089476	1401.088932	-0.388
M+z30	5+	1415.296902	1415.296093	-0.572
M+z30	4+	1768.870673	1768.870285	-0.219
M+z34	5+	1490.724486	1490.724988	0.337
M+z34	4+	1862.904657	1862.904749	0.049
M+z35	5+	1513.332634	1513.332401	-0.154
M+z35	4+	1891.665973	1891.665087	-0.468
M+z36	5+	1533.733951	1533.734428	0.311
M+z36	4+	1916.915649	1916.915513	-0.071
			Average Error:	0.052
			Absolute Average Error:	0.257
			Standard Deviation:	0.337

Table S4. List of the assigned fragments from the ECD mass spectrum of the 7+ charge state hIAPP dimer. M represents one hIAPP unit.

The table of assigned ECD MS/MS fragments of the 8+ hIAPP trimer (in aqueous solution with 2% DMSO)

Ion	Charge	Theoretical *m/z*	Experimental *m/z*	Error (ppm)
MI	4+	976.473865	976.473629	-0.242
MI	3+	1301.295128	1301.295286	0.121
[MI]	2+	1951.940453	1951.941145	0.355
2[MI]	5+	1562.356263	1562.357084	0.525
2[MI]	4+	1952.693510	1952.694205	0.356
2[MI]	3+	2603.925582	2603.925690	0.041
3[MI]	8+	1464.081791	1464.080743	-0.716
3[MI]	7+	1673.809188	1673.808501	-0.410
3[MI]	6+	1952.944506	1952.944143	-0.186
3[MI]	5+	2343.733943	2343.733157	-0.335
3[MI]	4+	2929.417034	2929.416922	-0.038
c3	1+	362.173076	362.173076	0.000
c7	1+	737.306908	737.306719	-0.256
c8	1+	808.344022	808.344125	0.127
c9	1+	909.391701	909.391441	-0.286
c10	1+	1037.450279	1037.450094	-0.178
c11	2+	597.279333	597.279295	-0.064
c11	1+	1193.551390	1193.551988	0.501
c13	2+	689.339922	689.339629	-0.425
c13	1+	1377.672568	1377.673088	0.377
c14	2+	746.361386	746.361097	-0.387
c14	1+	1491.715496	1491.715765	0.180
c15	2+	819.895593	819.895366	-0.277
c15	1+	1638.783910	1638.784035	0.076
c17	2+	925.971832	925.971525	-0.332
c18	2+	994.501288	994.501262	-0.026
c19	2+	1038.017303	1038.016936	-0.354
c20	2+	1081.533317	1081.533089	-0.211
c21	2+	1138.554781	1138.554661	-0.105
c22	2+	1195.576245	1195.576197	-0.040
c23	3+	846.409393	846.409301	-0.109
c23	2+	1269.110452	1269.110459	0.006
c24	2+	1297.621184	1297.620988	-0.151
c26	3+	926.790274	926.790027	-0.267
c26	2+	1890.183152	1890.183166	0.007
c27	3+	964.484962	964.484299	-0.687
c27	2+	1446.725192	1446.726334	0.789
c30	3+	1056.188874	1056.188926	0.049
c30	2+	1584.782465	1584.781451	-0.640
c32	3+	1127.560247	1127.560294	0.042
M+c35	4+	1886.161825	1886.160904	-0.488
M+c36	4+	1911.675709	1911.674109	-0.837
2M+c30	5+	2195.464155	2195.464715	0.255
2M+c34	5+	2267.699988	2267.699908	-0.035
2M+c35	7+	1635.791881	1635.791842	-0.024
2M+c35	6+	1908.423016	1908.422376	-0.335
2M+c35	5+	2289.906164	2289.907005	0.367
2M+c36	7+	1650.370440	1650.369669	-0.467
2M+c36	6+	1925.264301	1925.264063	-0.124
2M+c36	5+	2310.318271	2310.317485	-0.340
M+z23	3+	2106.687071	2106.687266	0.093

Ion	Charge	Theoretical *m/z*	Experimental *m/z*	Error (ppm)
2M+z5	4+	2083.497355	2083.498318	0.462
2M+z6	4+	2108.012511	2108.012947	0.207
2M+z7	5+	1709.019096	1709.019944	0.496
2M+z7	4+	2137.526829	2137.527322	0.231
2M+z10	5+	1764.642859	1764.643131	0.154
2M+z10	4+	2205.802305	2205.802133	-0.078
2M+z11	5+	1787.059229	1787.060986	0.983
2M+z11	4+	2235.076894	2235.077545	0.291
2M+z12	4+	2262.344371	2262.343377	-0.439
2M+z13	5+	1824.083932	1824.083442	-0.269
2M+z13	4+	2280.355611	2280.355687	0.033
2M+z14	5+	1835.287772	1835.287996	0.122
2M+z14	4+	2294.610979	2294.609671	-0.570
2M+z15	5+	1864.500986	1864.500270	-0.384
2M+z16	5+	1887.510052	1887.510169	0.062
2M+z16	4+	2359.888843	2359.889136	0.124
2M+z17	5+	1910.719554	1910.719165	-0.204
2M+z17	4+	2388.650128	2388.649501	-0.262
2M+z18	5+	1927.925508	1927.924985	-0.271
2M+z18	4+	2409.405841	2409.405482	-0.149
2M+z19	4+	2431.915600	2431.916230	0.259
2M+z20	5+	1972.944156	1972.943782	-0.190
2M+z20	4+	2466.181719	2466.182499	0.316
2M+z21	5+	1992.557390	1992.557247	-0.072
2M+z22	5+	2015.374671	2015.374524	-0.073
2M+z22	4+	2519.971551	2519.971167	-0.152
2M+z23	6+	1703.824077	1703.823394	-0.401
2M+z23	5+	2044.587908	2044.587680	-0.112
2M+z23	4+	2555.736390	2555.735772	-0.242
2M+z24	6+	1722.998291	1722.997816	-0.276
2M+z24	5+	2067.598059	2067.598981	0.446
2M+z24	4+	2584.496336	2584.495916	-0.163
2M+z26	6+	1753.685163	1753.686125	0.549
2M+z26	5+	2104.622773	2104.623063	0.138
2M+z26	4+	2630.777217	2630.776164	-0.400
2M+z27	6+	1779.367869	1779.367588	-0.158
2M+z27	5+	2135.842994	2135.842285	-0.332
2M+z27	4+	2669.803880	2669.802875	-0.376
2M+z28	6+	1801.212170	1801.210973	-0.665
2M+z28	5+	2161.254244	2161.254600	0.165
2M+z30	6+	1829.558849	1829.559634	0.429
2M+z30	4+	2745.842000	2745.843566	0.570
2M+z34	6+	1892.414312	1892.414141	-0.090
2M+z34	5+	2270.496380	2270.495936	-0.196
2M+z34	4+	2838.121979	2838.121431	-0.193
2M+z36	5+	2314.508068	2314.506366	-0.735
			Average Error:	-0.067
			Absolute Average Error:	0.280
			Standard Deviation:	0.343

Table S5. List of the assigned fragments from the ECD mass spectrum of the 8+ charge state hIAPP trimer ion (in aqueous solution with 2% residual DMSO). M represents one hIAPP unit.

The table of assigned ECD MS/MS fragments of the 5+ hIAPP dimer (in aqueous solution only)

Ion	Charge	Theoretical *m/z*	Experimental *m/z*	Error (ppm)
[MI]	3+	1302.297541	1302.297961	0.323
2[MI]	5+	1561.553258	1561.552850	-0.261
2[MI]	4+	1952.693410	1952.695277	0.956
2[MI]	3+	2603.927471	2603.928753	0.492
2[MI]	2+	3906.890716	3906.890042	-0.173
4[MI]	9+	1735.727472	1735.727516	0.025
4[MI]	7+	2231.792930	2231.793086	0.070
c10	1+	1037.450279	1037.450277	-0.002
M+c34	3+	2477.204233	2477.204139	-0.038
M+c35	4+	1886.413773	1886.413868	0.050
M+c35	3+	2515.552587	2515.553103	0.205
M+c36	4+	1912.176753	1912.176749	-0.002
M+c36	3+	2549.237059	2549.237578	0.204
M+z27	3+	2257.442353	2257.441339	-0.449
M+z34	3+	2482.868892	2482.868192	-0.282
M+z36	4+	1916.665083	1916.666074	0.517
			Average Error:	0.102
			Absolute Average Error:	0.253
			Standard Deviation:	0.350

Table S6. List of the assigned ECD MS/MS fragments of the 5+ charge state hIAPP dimer (in aqueous solution only). M represents one hIAPP unit.

The table of assigned ECD MS/MS fragments of the 7+ hIAPP dimer (in aqueous solution only)

Ion	Charge	Theoretical *m/z*	Experimental *m/z*	Error (ppm)
[MI]	3+	1301.295128	1301.295155	0.021
[MI]	2+	1951.439053	1951.439594	0.277
2[MI]	7+	1116.112578	1116.112111	-0.418
2[MI]	6+	1302.131432	1302.130613	-0.629
2[MI]	5+	1562.557828	1562.556711	-0.715
2[MI]	4+	1953.197422	1953.197083	-0.174
2[MI]	3+	2604.597466	2604.599266	0.691
a17	2+	904.468923	904.469305	0.422
c3	1+	362.173076	362.173079	0.008
c5	1+	534.257869	534.257718	-0.283
c6	1+	635.305548	635.305497	-0.080
c7	1+	737.306908	737.306894	-0.019
c8	1+	808.344022	808.343993	-0.036
c9	1+	909.391701	909.391626	-0.082
c10	2+	519.228778	519.228684	-0.181
c10	1+	1037.450279	1037.450421	0.137
c11	2+	597.279333	597.279269	-0.107
c11	1+	1193.551390	1193.552256	0.726
c12	2+	653.821365	653.821253	-0.171
c13	2+	689.339922	689.339879	-0.062
c13	1+	1377.672568	1377.672392	-0.128
c14	2+	746.361386	746.361366	-0.027
c14	1+	1491.715496	1491.715364	-0.088
c15	2+	819.895593	819.895629	0.044
c16	2+	876.437625	876.437799	0.199
c17	2+	925.971832	925.971871	0.042
c18	2+	994.501288	994.501399	0.112
c19	2+	1038.017303	1038.017367	0.062
c20	2+	1081.533317	1081.533380	0.058
c21	3+	759.372279	759.372332	0.070
c21	2+	1138.554781	1138.554898	0.103
c22	3+	797.386589	797.386535	-0.068
c22	2+	1195.576245	1195.576726	0.402
c23	3+	846.409393	846.409348	-0.053
c23	2+	1269.110452	1269.110661	0.165
c24	3+	865.416548	865.416434	-0.132
c25	3+	889.095586	889.095288	-0.335
c26	3+	926.790274	926.790073	-0.217
c26	2+	1389.681773	1389.681581	-0.138
c27	3+	964.484962	964.484562	-0.415
c27	2+	1446.223805	1446.224334	0.366
c28	3+	993.495638	993.495502	-0.137
c28	2+	1490.241206	1490.242028	0.552
c29	3+	1023.174607	1023.174961	0.346
c29	2+	1534.759192	1534.760089	0.584
c30	3+	1056.188874	1056.188728	-0.138
c30	2+	1584.281062	1584.281747	0.432
c31	3+	1094.203184	1094.203292	0.099
c31	2+	1641.305046	1641.305402	0.217
c32	3+	1127.225988	1127.225585	-0.358
c32	2+	1691.841749	1691.841673	-0.045
c33	2+	1146.567401	1146.568161	0.663
c33	2+	1719.347463	1719.347416	-0.027
c34	2+	1175.578077	1175.578159	0.070
c34	2+	1762.362091	1762.362565	0.269
c35	3+	1213.926469	1213.926076	-0.324
c35	2+	1819.884937	1819.885330	0.216
c36	3+	1247.608904	1247.608555	-0.280
c36	2+	1871.410938	1871.411570	0.338
y3	1+	396.187761	396.187739	-0.056
y4	1+	483.219790	483.219859	0.143
y5	1+	540.241254	540.241149	-0.194
y6	1+	639.309668	639.309731	0.099
y7	1+	753.352596	753.352333	-0.349
y8	1+	854.400275	854.400411	0.159
y9	1+	941.432304	941.432472	0.178
z2	1+	266.126109	266.126109	0.000
z3	1+	381.176861	381.176838	-0.060
z5	1+	525.230353	525.230286	-0.128
z27	2+	1434.726804	1434.726864	0.042
z28	2+	1498.252176	1498.252684	0.339
M+c28	4+	1722.092874	1722.092573	-0.175
M+c31	5+	1437.899490	1437.898369	-0.780
M+c32	5+	1457.713182	1457.712567	-0.422
M+c32	4+	1822.641179	1822.641257	0.043
M+c33	5+	1469.319041	1469.319187	0.099
M+c33	4+	1835.895847	1835.896338	0.267
M+c34	5+	1486.523886	1486.523255	-0.424
M+c34	4+	1857.903038	1857.903286	0.133
M+c34	3+	2477.206107	2477.206784	0.273
M+c35	6+	1257.944941	1257.944384	-0.443
M+c35	5+	1509.132031	1509.131650	-0.252
M+c35	4+	1886.413773	1886.413722	-0.027
M+c35	3+	2515.888529	2515.889693	0.463
M+c36	6+	1274.619189	1274.618504	-0.537
M+c36	5+	1530.344824	1530.344143	-0.445
M+z3	3+	1428.016652	1428.017814	0.814
M+z4	3+	1457.361471	1457.361442	-0.020
M+z5	3+	1476.370423	1476.369607	-0.553
M+z5	2+	2214.553213	2214.553546	0.150
M+z6	3+	1510.061465	1510.061814	0.231
M+z7	3+	1547.741690	1547.741300	-0.252
M+z7	2+	2321.108897	2321.107527	-0.590
M+z8	3+	1581.758350	1581.758113	-0.150
M+z9	3+	1610.434934	1610.434541	-0.244
M+z10	4+	1230.085200	1230.085218	0.015
M+z10	3+	1639.445612	1639.445189	-0.258
M+z11	3+	1676.471867	1676.471280	-0.350
M+z12	3+	1714.835007	1714.834779	-0.133
M+z13	3+	1738.514047	1738.513671	-0.216
M+z13	2+	2607.267432	2607.267132	-0.115
M+z14	4+	1318.391358	1318.390730	-0.476
M+z14	3+	1757.855327	1757.854802	-0.299
M+z14	2+	2636.279353	2636.279372	0.007
M+z15	4+	1355.158481	1355.158135	-0.255
M+z15	3+	1806.878157	1806.877692	-0.257
M+z16	4+	1383.669106	1383.669214	0.078
M+z16	3+	1844.892468	1844.892459	-0.005
M+z16	2+	2767.338976	2767.339318	0.124
M+z17	4+	1412.179946	1412.179831	-0.081
M+z17	3+	1882.906778	1882.907494	0.380
M+z17	2+	2824.861593	2824.862515	0.326
M+z18	4+	1433.937956	1433.938030	0.052
M+z18	3+	1911.917458	1911.917980	0.273
M+z19	4+	1455.695966	1455.695755	-0.145
M+z19	2+	2910.888569	2910.887702	-0.298
M+z20	4+	1489.960695	1489.961248	0.371
M+z20	3+	1986.280293	1986.280702	0.206
M+z21	4+	1514.727806	1514.727172	-0.419
M+z21	3+	2019.971374	2019.972282	0.450
M+z22	4+	1542.998831	1542.998568	-0.170
M+z22	3+	2057.331957	2057.332668	0.346
M+z23	5+	1264.214693	1264.214468	-0.178
M+z23	4+	1579.765949	1579.765670	-0.177
M+z23	3+	2105.352124	2105.352029	-0.045
M+z23	2+	3160.033644	3160.033403	-0.076
M+z24	4+	1608.276681	1608.276576	-0.065
M+z24	3+	2144.034920	2144.034716	-0.095
M+z25	4+	1626.035962	1626.035200	-0.469
M+z25	3+	2168.382264	2168.382341	0.036
M+z26	5+	1323.847526	1323.846832	-0.524
M+z26	4+	1654.306958	1654.306403	-0.335
M+z26	3+	2206.078772	2206.078781	0.004
M+z27	5+	1354.666757	1354.666205	-0.407
M+z27	4+	1693.584213	1693.583638	-0.340
M+z27	3+	2258.112467	2258.112424	-0.019
M+z28	5+	1380.679464	1380.679001	-0.335
M+z28	4+	1726.100055	1726.099418	-0.369
M+z29	5+	1400.889004	1400.888206	-0.570
M+z29	4+	1750.860782	1750.860731	-0.029
M+z30	5+	1414.895940	1414.895086	-0.604
M+z30	4+	1768.620062	1768.620698	0.360
M+z30	3+	2357.824324	2357.824847	0.222
M+z33	4+	1837.893258	1837.892451	-0.439
M+z34	5+	1490.123150	1490.122540	-0.409
M+z34	4+	1862.654075	1862.654369	0.158
M+z34	3+	2483.204833	2483.203905	-0.374
M+z35	5+	1512.931737	1512.932144	0.269
M+z35	4+	1891.164808	1891.165443	0.336
M+z36	5+	1533.331957	1533.330732	-0.799
M+z36	4+	1916.915620	1916.916114	0.258
			Average Error:	-0.044
			Absolute Average Error:	0.248
			Standard Deviation:	0.311

Table S7. List of the assigned ECD MS/MS fragments of the 7+ charge state hIAPP dimer (in aqueous solution only). M represents one hIAPP unit.

The table of assigned ECD MS/MS fragments of the 8+ hIAPP trimer (in aqueous solution only)

Ion	Charge	Theoretical *m/z*	Experimental *m/z*	Error (ppm)
[MI]	3+	1301.295128	1301.295525	0.305
[MI]	2+	1951.940453	1951.941533	0.553
2[MI]	5+	1562.356263	1562.356241	-0.014
2[MI]	4+	1952.693510	1952.693825	0.161
2[MI]	3+	2603.591528	2603.591786	0.099
3[MI]	8+	1464.582971	1464.582582	-0.266
3[MI]	7+	1673.952355	1673.951942	-0.247
3[MI]	6+	1952.944506	1952.944697	0.098
3[MI]	5+	2343.533517	2343.534255	0.315
3[MI]	4+	2929.417034	2929.416863	-0.058
c3	1+	363.180901	363.180901	0.000
c7	1+	737.306908	737.306979	0.096
c9	1+	909.391701	909.391753	0.057
c10	1+	1037.450279	1037.450632	0.340
c13	2+	689.339922	689.339981	0.086
c13	1+	1377.672568	1377.672453	-0.083
c14	2+	746.361386	746.361488	0.137
c14	1+	1491.715496	1491.716356	0.577
c15	2+	819.895593	819.895671	0.095
c15	1+	1638.783910	1638.784787	0.535
c18	2+	994.501288	994.501941	0.657
c19	2+	1038.017303	1038.017693	0.376
c20	2+	1081.533317	1081.533296	-0.019
c21	2+	1138.554781	1138.554888	0.094
c23	2+	1269.110452	1269.110367	-0.067
c30	3+	1056.857180	1056.857694	0.486
M+c14	4+	1349.651986	1349.652295	0.229
M+z23	3+	2105.685906	2105.684670	-0.587
2M+c34	5+	2267.699988	2267.699294	-0.306
2M+c35	7+	1635.791881	1635.790912	-0.592
2M+c35	6+	1908.423016	1908.423197	0.095
2M+c35	5+	2290.106592	2290.106669	0.034
2M+c36	7+	1650.370440	1650.369713	-0.441
2M+c36	6+	1925.264301	1925.265117	0.424
2M+c36	5+	2310.517700	2310.518213	0.222
2M+z7	4+	2136.773804	2136.773786	-0.008
2M+z11	4+	2234.575828	2234.573911	-0.858
2M+z13	4+	2280.103655	2280.102968	-0.301
2M+z14	5+	1835.287772	1835.287864	0.050
2M+z14	4+	2294.109849	2294.110242	0.171
2M+z16	5+	1887.510052	1887.510259	0.110
2M+z16	4+	2359.387700	2359.387809	0.046
2M+z17	5+	1910.719554	1910.718763	-0.414
2M+z17	4+	1388.149011	1388.149681	0.483
2M+z18	5+	1927.925508	1927.924537	-0.504
2M+z18	4+	2410.157588	2410.156797	-0.328
2M+z19	4+	2431.414510	2431.414302	-0.086
2M+z20	5+	1972.944156	1972.943100	-0.535
2M+z22	5+	2015.374671	2015.375993	0.656
2M+z22	4+	2519.469038	2519.470008	0.385
2M+z23	6+	1703.824077	1703.824082	0.003
2M+z23	5+	2044.587908	2044.588728	0.401
2M+z23	4+	2556.488124	2556.488923	0.313
2M+z24	6+	1722.998291	1722.999253	0.558
2M+z24	5+	2067.598059	2067.597329	-0.353
2M+z24	4+	2583.995165	2583.994963	-0.078
2M+z26	5+	2104.822114	2104.821212	-0.429
2M+z27	6+	1779.367869	1779.367687	-0.102
2M+z27	5+	2135.842994	2135.842633	-0.169
2M+z27	4+	2670.556970	2670.556856	-0.043
2M+z28	5+	2161.453606	2161.454306	0.324
2M+z30	6+	1829.558849	1829.558150	-0.382
2M+z34	6+	1892.414312	1892.413093	-0.644
2M+z34	5+	2271.499727	2271.499425	-0.133
			Average Error:	0.024
			Absolute Average Error:	0.275
			Standard Deviation:	0.348

Table S8. List of the assigned fragments from the ECD mass spectrum of the 8+ charge state hIAPP trimer ion (in aqueous solution only). M represents one hIAPP unit.

Ion	Charge	Theoretical *m/z*	Experimental *m/z*	Error (ppm)
2MH+c5	1+	2182.024503	2182.024212	-0.133
2MH+c6	1+	2297.075256	2297.074902	-0.154
2MH+c7	1+	2399.126351	2399.124459	-0.789
2MH+z5	1+	2232.011932	2232.011937	0.002
2MH+z6	1+	2330.076933	2330.074927	-0.861
2MH+z7	1+	2444.119860	2444.119499	-0.148
5MH+c6	3+	1620.088832	1620.088636	-0.121
5MH+c7	3+	1654.441566	1654.442594	0.621
5MH+z7	3+	1669.439658	1669.439893	0.141
3[MH]	2+	1281.096770	1281.096595	-0.137
3[MH]	1+	2561.185513	2561.186263	0.293
6[MH]	4+	1282.350153	1282.349911	-0.189
6[MH]	3+	1708.797730	1708.798167	0.256
MH	1+	854.400272	854.400285	0.015
			Average Error:	-0.086
			Absolute Average Error:	0.276
			Standard Deviation:	0.387

Table S9. List of the assigned fragments from the ECD mass spectrum of the 2+ charge state trimer of the hIAPP segment (^{30}TNVGSNTY^{37}-NH_2). M represents one hIAPP unit.

Ion	Charge	Theoretical *m/z*	Experimental *m/z*	Error (ppm)
[hIAPP]	3+	1301.295034	1301.295128	0.072
[hIAPP]	2+	1952.946261	1952.945652	-0.312
[hIAPP+TNVGSNTY]	4+	1189.571414	1189.571482	0.057
[hIAPP+TNVGSNTY]	3+	1586.095401	1586.095367	-0.021
[hIAPP+TNVGSNTY]	2+	2379.645083	2379.644330	-0.316
[TNVGSNTY]	1+	854.400272	854.400332	0.070
c3	1+	363.180901	363.180902	0.003
c7	1+	737.306908	737.306884	-0.033
c9	1+	909.391701	909.392057	0.391
c10	1+	1037.450279	1037.450525	0.237
c35	2+	1820.386836	1820.386597	-0.131
c ion of TNVGS	1+	476.246339	476.246325	-0.029
c ion of TNVGSN	1+	590.289267	590.289294	0.046
c ion of TNVGSNT	1+	691.336946	691.336919	-0.039
hIAPP+z ion of SNTY	2+	2185.541566	2185.540981	-0.268
hIAPP+z ion of GSNTY	3+	1476.370423	1476.369560	-0.585
hIAPP+z ion of GSNTY	2+	2214.553924	2214.552379	-0.698
hIAPP+z ion of VGSNTY	3+	1509.057288	1509.057173	-0.076
hIAPP+z ion of VGSNTY	2+	2263.586505	2263.586224	-0.124
TNVGSNTY+c32	2+	2118.536699	2118.536594	-0.050
TNVGSNTY+c33	2+	2147.049813	2147.050779	0.450
TNVGSNTY+c34	3+	1459.708148	1459.707114	-0.708
TNVGSNTY+c34	2+	2190.561113	2190.561344	0.105
TNVGSNTY+c35	3+	1497.386516	1497.386130	-0.258
TNVGSNTY+c35	2+	2246.581754	2246.581291	-0.206
TNVGSNTY+c36	3+	1531.405017	1531.405418	0.262
TNVGSNTY+c36	2+	2298.111132	2298.111262	0.057
TNVGSNTY+z27	2+	1861.421286	1861.421295	0.005
TNVGSNTY+z30	2+	2011.492975	2011.493855	0.437
TNVGSNTY+z34	2+	2198.056564	2198.055447	-0.508
			Average Error:	-0.072
			Absolute Average Error:	0.219
			Standard Deviation:	0.297

Table S10. List of the assigned fragments from the ECD mass spectrum of the 4+ charge state of [hIAPP + ^{30}TNVGSNTY^{37}-NH_2] species.