Does Deamidation affect Inhibitory Mechanisms towards Amyloid Protein Aggregation?

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Electronic Supplementary Information

Experimental Section

Sample preparation for mass spectrometry (MS). Wild-type hIAPP (Eurogentec Ltd., England) and the synthetic mutant hIAPP with iso-aspartic acid residue replacement at Asn-21, Asn-22, and Asn-35 ($(isoD)_3$hIAPP; Pepscan Company Ltd, The Netherlands) were dissolved in Milli Q H_2O (Direct-Q® 3 UV System, Millipore Corporation, US) with pH ~7.5 to 500 μM for storage (at -80°C) and samples were consumed within 2 weeks. (-)-epigallocatechin gallate (EGCG) were purchased from Sigma Aldrich Company Ltd, England and dissolved in Milli-Q H_2O to 500 μM for storage (at -80°C). Recombinant human insulin (hINS) (Sigma Aldrich Company Ltd, England) was dissolved in acidified Milli-Q H_2O (~pH 5.8) to 500 μM for storage (at -80°C), as performed by Susa *et al*.[1]

For the MS spectra of hIAPP with potential inhibitors, 50 μM solutions of hIAPP and $(isoD)_3$hIAPP were initially mixed with 50 μM solutions of EGCG or hINS in Milli-Q H_2O separately. The mixed solutions were then vortexed for half an hour at room temperature before further dilution by 5-fold with Milli-Q H_2O into 10 μM solutions. The pH value of all solutions were measured using a pH meter (Hanna; UK), the values were between pH 6.8 – pH 7.5. The solutions were then centrifuged at 14,000 rpm using a technico Maxi microcentrifuge (ThermoFisher; UK) for 5 minutes before MS analysis.

For MS relative quantification, 10 μM solutions of wild-type hIAPP and mutant $(isoD)_3$hIAPP were mixed with 10 μM solutions of EGCG or hINS in Milli-Q H_2O individually. A 200 μL aliquot was extracted from each solution and stored in -80°C while the remaining 300 μL solution was incubated at 37°C for 7 days. After 7 days, solutions stored at -80°C (regarded as fresh solutions) and solutions incubated at 37°C were centrifuged at 14,000 rpm for 1 hour to separate the soluble wild-type hIAPP and mutant $(isoD)_3$hIAPP from the insoluble aggregates (precipitate) in the solutions. Supernatant solutions were further diluted 10-fold with 49.5:49.5:1 H_2O/acetonitrile/formic acid and centrifuged at 14,000 rpm for 5 minutes prior to MS analysis.

Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) analysis. MS spectra of the early oligomers of the amyloid proteins with or without potential inhibitors were acquired using nano-electrospray ionisation (nESI) FTICR MS with a capillary voltage of 0.8 – 1.3 kV and a source temperature of 80°C. The nESI glass capillaries (World Precision Instruments, England) were pulled on a Sutter P-97 capillary puller instrument (One Digital Drive Novato, USA). Samples were loaded onto the glass capillaries and inserted with nickel chromium wire (Jacobs, USA) to ground the electric potential for ionisation.

For the MS relative quantification spectra of soluble amyloid proteins, an Apollo II electrospray ionisation (ESI) source MS (Bruker Daltonics ,Bremen, Gremany) with a capillary voltage of 4 – 5 kV was applied and the source temperature was set to 200°C. The ESI flow rate was optimised to 100 – 150 μL/h. 5 MS spectra were acquired for each solutions as technical replicates and standard deviation for each solutions was calculated.

All mass spectra were acquired on a 12 tesla solariX FTICR MS (Bruker Daltonics, Bremen, Germany) in positive ionisation mode. Sample ions were first accumulated from 0.1 – 1 seconds in the hexapole collision cell before being directed to an infinity ICR cell for excitation and detection.[2] All mass spectra were acquired with 4 mega point (22-bit) transients and were analysed using Bruker DataAnalysis 4.2 software MS (Bruker Daltonics, Bremen, Germany).

FTICR MS/MS analysis. For the collisionally activated dissociation tandem MS (CAD MS/MS) experiments, precursor ions were isolated using a quadrupole mass filter, collided with argon gas and accumulated for 1 – 3 seconds in the hexapole collision cell. The collision energies were varied from 0 – 12 V in order to generate the breakdown curves for the precursor ions which were composed of the amyloid protein with the bound molecule of interest. Fragments were then directed to the ICR cell for detection. 5 spectra were acquired for each collision energy level as technical replicates and standard deviation for each solutions was calculated.

For electron capture dissociation tandem MS (ECD MS/MS) experiments, precursor ions were first isolated with a wide mass-to-charge (*m/z*) isolation window (100 *m/z*) in the quadrupole and accumulated for 1-5 seconds in the collision cell. Ions were further transferred to the ICR cell and isolated with MULTI-CHEF isolation with an excitation power of 50 – 55%.[3] The precursor ions were then irradiated with 1.2 eV electrons from a 1.5 A heated hollow cathode and the fragments were detected by the ICR cell.

MS data analysis. All MS data were analysed using Bruker DataAnalysis 4.2 software MS (Bruker Daltonics, Bremen, Germany). The most intense isotopic peak from each fragment with signal-to-noise ratio (S/N) over 5 was manually matched with the assigned species. 5 fragments from each spectrum were chosen for the internal calibration of the spectrum and all fragments were then assigned with an uncertainty of 0.1 – 0.5 parts-per-million (ppm) (Table S1- S4†).

To study the binding strength between wild-type hIAPP and the potential inhibitors, the dissociation curves of each complex precursor ion, which composed of hIAPP and the binding molecule, were plotted. The areas of all the isotopic peaks from each

charge state of hIAPP, the complexes, and the potential inhibitors (if present) were measured using the Bruker DataAnalysis 4.2 software. The relative percentage of the complex was calculated as follow:

$$\text{Complex (\%)} = \frac{Isotopic\ peak\ areas\ of\ the\ complex}{Total\ isotopic\ peak\ areas\ of\ hIAPP + complex + binding\ molecule}$$

The dissociation curves for the complexes of hIAPP with different potential inhibitors were obtained by plotting the relative percentage of each complex calculated above against the applied CAD energies. The same calculation method was used to obtain the dissociation curves of the mutant $(isoD)_3$hIAPP with various potential inhibitors.

To study the long-term inhibition performance of each binding molecule against hIAPP aggregation, the MS spectra of the incubated wild-type hIAPP solutions (with/ without inducing the potential inhibitor) were compared to their corresponding fresh MS spectra which were obtained using the solutions stored at -80ºC. The absolute peak areas of the fresh and incubated solutions were first tested against a 2-tailed T-test to differentiate whether the peak areas between the fresh and incubated solutions were significant. The hypothesis applied in the 2-tailed T-test assumed there was no significant different between the fresh and incubated solutions, thus a p-value that was less than 0.05 (95% confidence) meant the two solutions were significantly different and vice versa. The percentage change of soluble hIAPP was then calculated as follows after the 2-tailed T-test:

$$\text{\% change} = \frac{(peak\ area\ of\ incubated\ solution - peak\ area\ of\ fresh\ solution)}{peak\ area\ of\ fresh\ solution}\ x\ 100\%$$

The same calculation method was applied to obtain the percentage change of the mutant $(isoD)_3$hIAPP experiments.

Transmission electron microscopy (TEM). TEM images of the incubated solutions, including the 10 μM solutions of the wild-type hIAPP and the mutant $(isoD)_3$hIAPP individually mixed with the 10 μM solutions of EGCG and hINS were acquired on a Jeol 2010F TEM which operated at 200 kV. 5 μL of the incubated solution was added onto a carbon coated grid (EM Resolutions, England) and settled for 1 minute, followed by using a filter paper to remove the excess aqueous solution. A 2% (w/v) uranyl acetate solution was then added onto the carbon grid and settled for 1 minute before removal by a filter paper. Multiple TEM images were acquired with magnification from x10,000 to x50,000. The contrast of the TEM images were auto-corrected by IrfanView 64 software (Irfan, Austria).

Thioflavin T (ThT) fluorescence reactivity. Fluorescence absorbance of the 50 μM solutions of the wild-type hIAPP and the mutant $(isoD)_3$hIAPP with various

potential inhibitors were measured using a GloMax®-Multi Detection System (Promega, USA) at room temperature. All samples were placed in a black 96 well-plate and mixed with a 100 μM ThT solution. Fluorescence absorbance for the 96 well-plate was measured every 45 minutes for 73 hours (99 sampled points) with excitation at 405 nm and emission measurement at 490 nm.[4, 5] The fluorescence absorbance were normalised to 1 using the maximum and the minimum readings. The lag phase of each sample was then calculated according to the previous studies.[6, 7]

The corrected ThT fluorescence absorbance of the wild-type hIAPP and the mutant $(isoD)_3$hIAPP solutions were obtained by using the absolute fluorescence absorbance obtained from the amyloid protein solutions minus the absolute fluorescence absorbance of the 100 μM ThT solution. The corrected ThT fluorescence absorbance of the mixtures of wild-type hIAPP/ mutant $(isoD)_3$hIAPP with various potential inhibitors were calculated using the absolute fluorescence absorbance obtained from the mixtures minus the absolute fluorescence absorbance of the corresponding potential inhibitor.

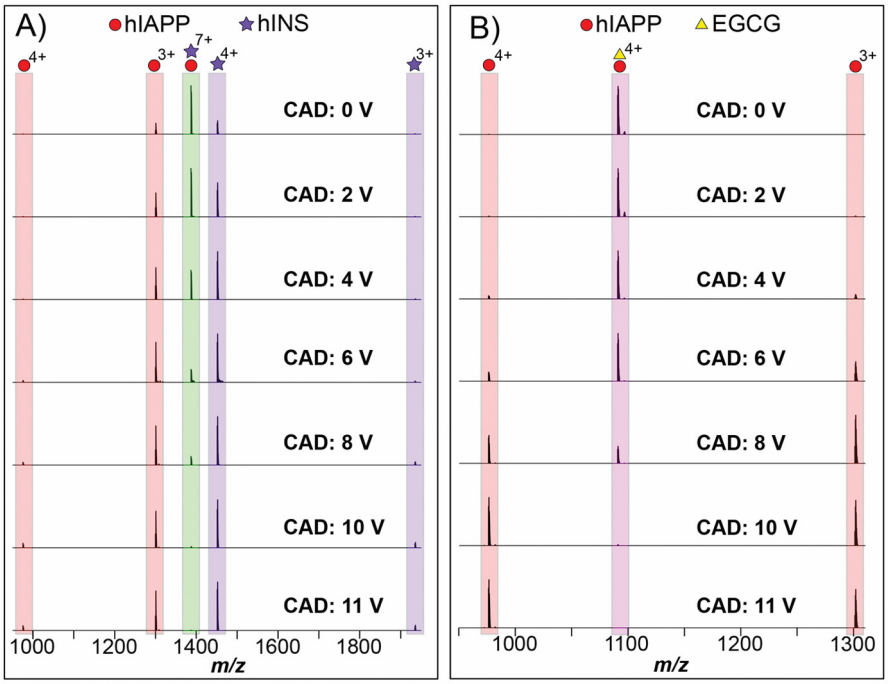

Fig S1. The representative CAD MS/MS spectra of (A) the 7+ charge state molecule ion of the hIAPP monomer unit with an intact hINS molecule and (B) the 4+ charge state molecular ion of the hIAPP monomer unit with an attachment of EGCG molecule.

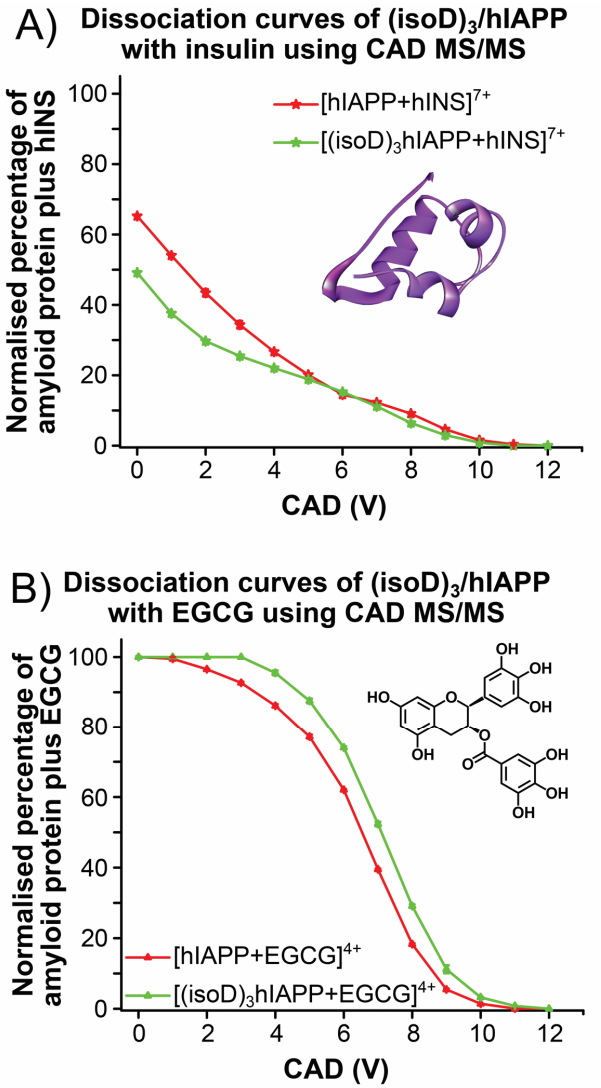

Fig S2. CAD MS/MS dissociation curves of (A) hIAPP/(isoD)3hIAPP with hINS and (B) hIAPP/(isoD)3hIAPP with EGCG. The 1:1 complex composed of an hIAPP/(isoD)3hIAPP and either a hINS or EGCG molecule inhibitor molecule was isolated and fragmented using increasing dissociation energies in a set of CAD MS/MS experiments until the complex was fully dissociated (Fig. S1). It was shown that hINS bound more strongly to hIAPP than the $(isoD)_3$hIAPP, with a higher percentage of the intact 7+ heterodimer complex being observed at each energy level for the hIAPP. In contrast, the non-specific inhibitor EGCG bound more strongly to the $(isoD)_3$hIAPP species rather than the hIAPP species, with a lower percentage of the intact 4+ complex being observed at each energy level for the hIAPP. The dissociation results obtained in the complexes of the polypeptides with hINS/EGCG show the gas phase stabilities of the complexes involve fragile, non-covalent interactions, and are significantly altered by the deamidation in the polypeptides, suggesting deamidation may affect the interaction mechanism between hIAPP and its potential inhibitors.

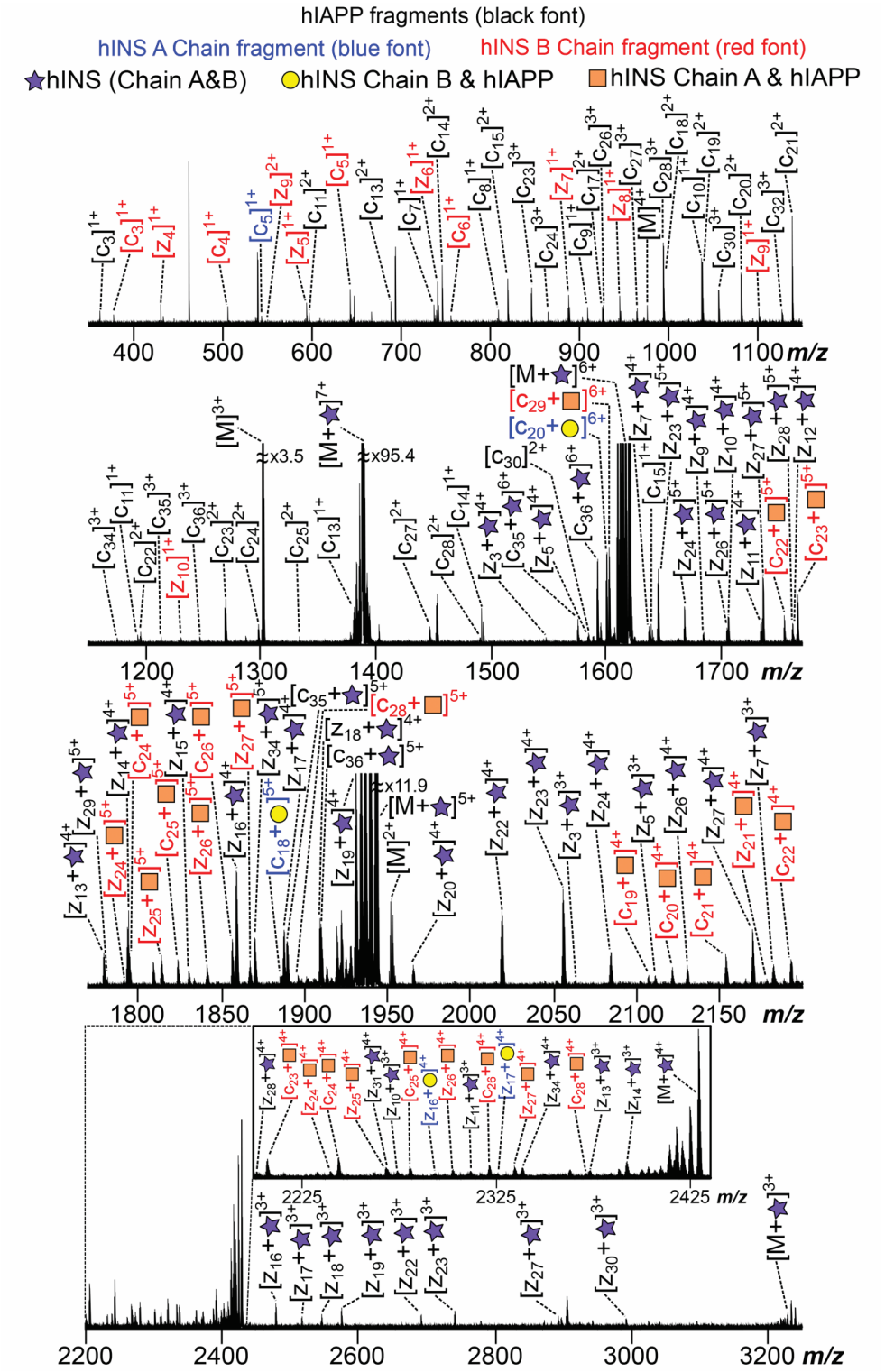

Fig S3. ECD spectrum of the 7+ charge state heterodimer (a hIAPP plus a hINS biomolecules). The assigned fragments are listed in the supporting information Table S1.

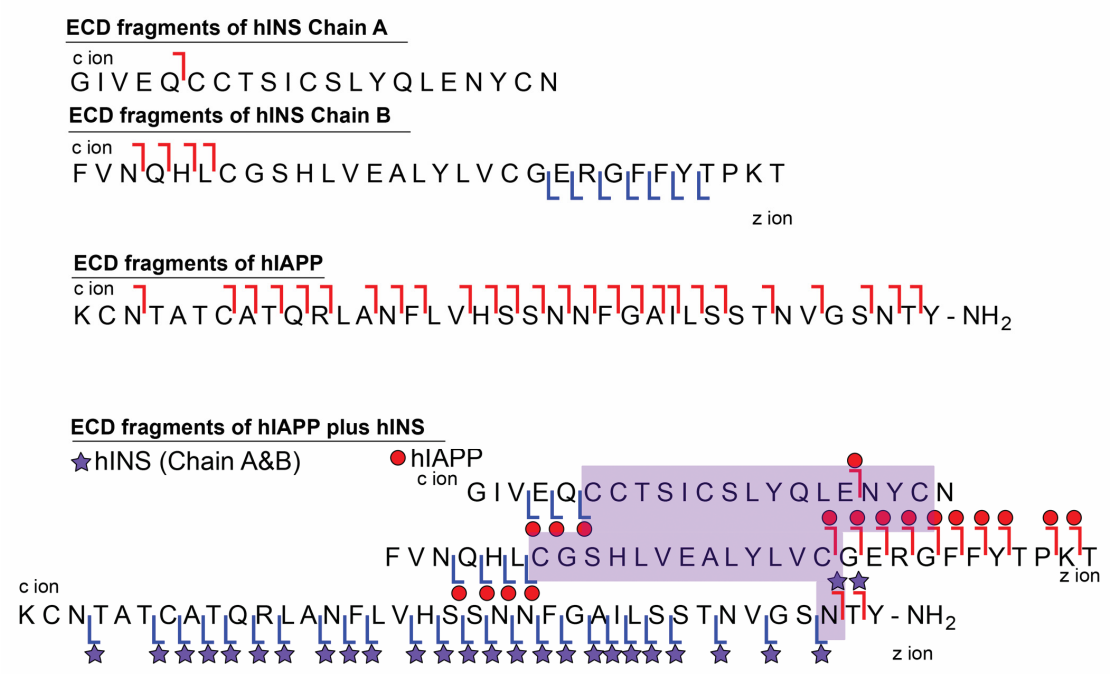

Fig S4. The summarised fragmentation map of the 7+ charge state heterodimer (a hIAPP plus a hINS biomolecules). The highlighted area (coloured in purple) indicates the proposed interaction region between the monomer unit of hIAPP and a hINS biomolecule.

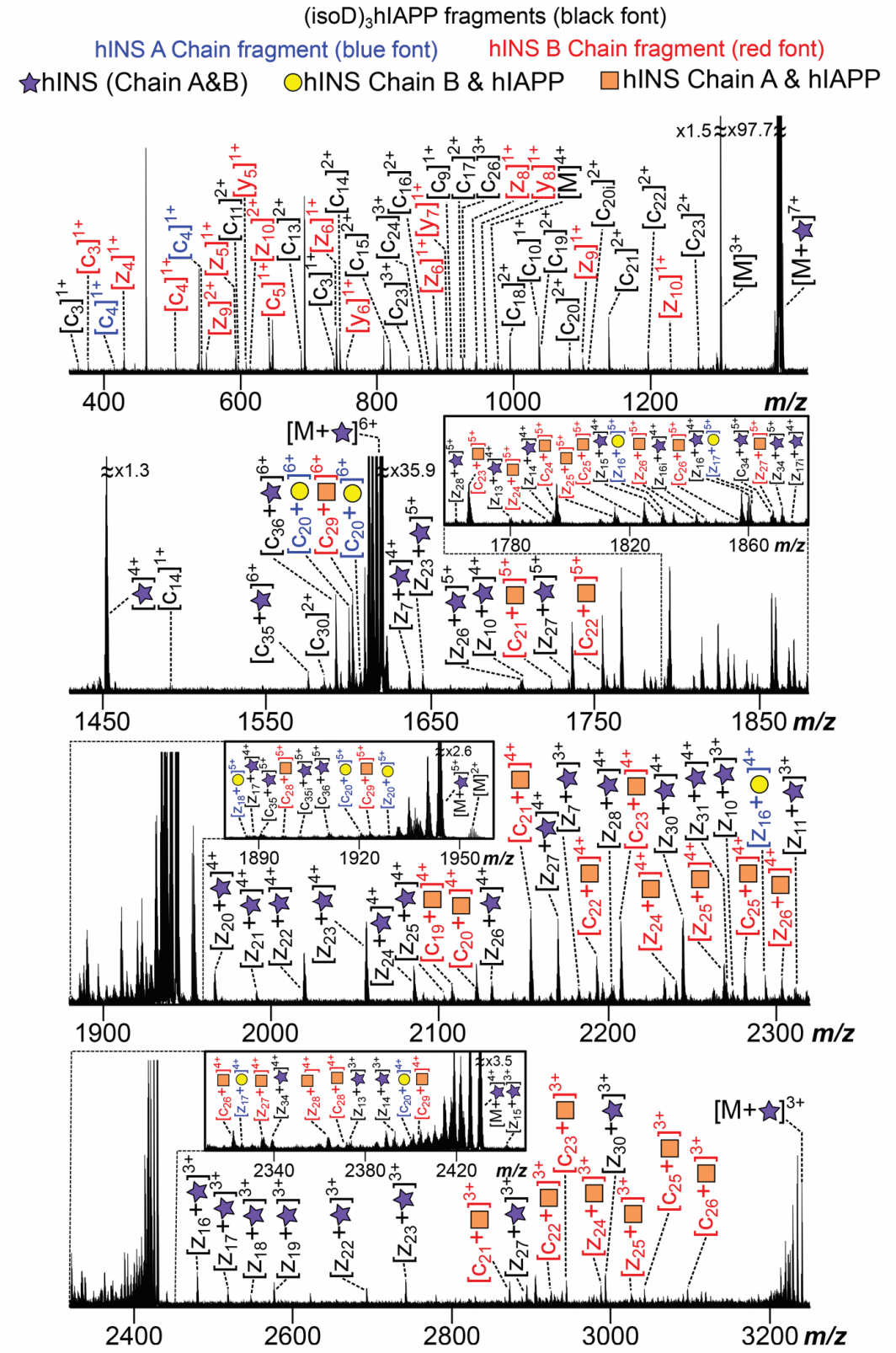

Fig S5. ECD spectrum of the 7+ charge state heterodimer (an $(isoD)_3$hIAPP plus a hINS biomolecules). The assigned fragments are listed in the supporting information Table S2.

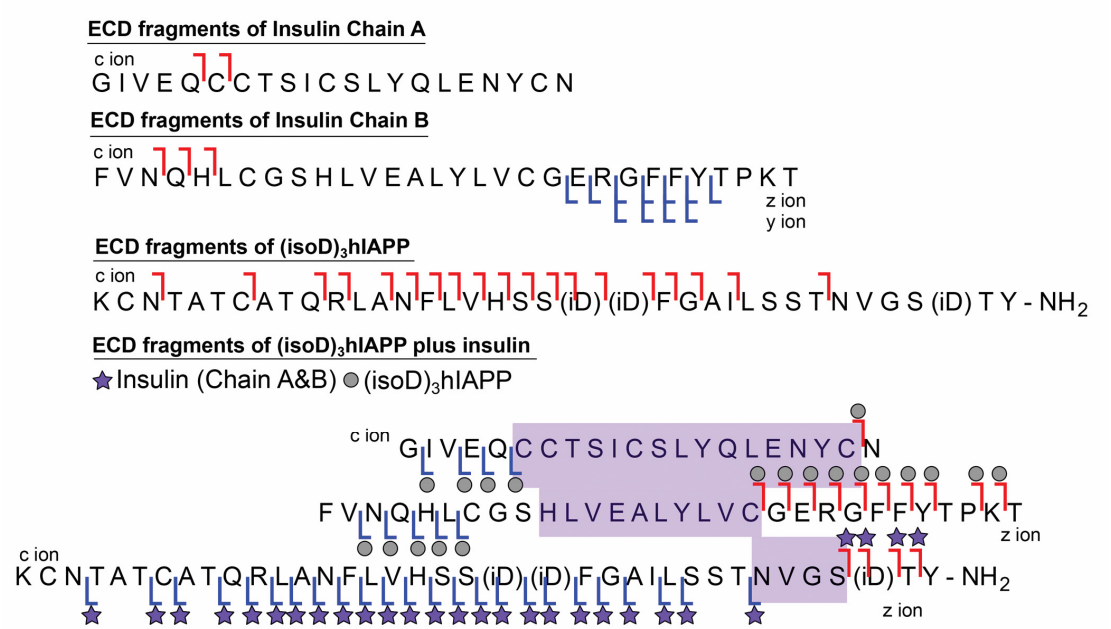

Fig S6. The summarised fragmentation map of the 7+ charge state heterodimer (an (isoD)$_3$hIAPP plus a hINS biomolecules). The highlighted area (coloured in purple) indicates the proposed interaction region between the monomer unit of (isoD)$_3$hIAPP and a hINS biomolecule.

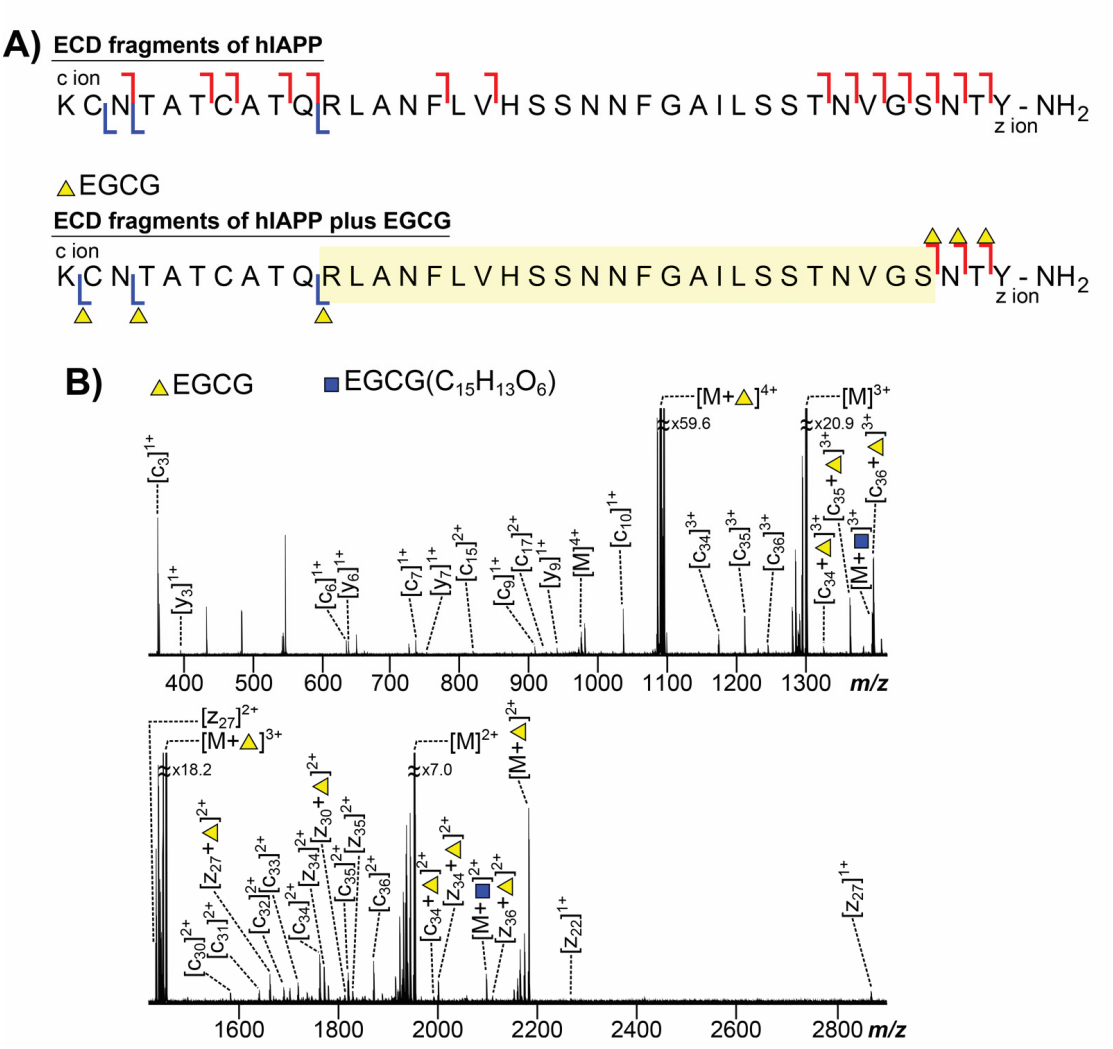

Fig S7. (A) The summarized ECD fragments of the 4+ charge state molecular ion of hIAPP monomer unit and an EGCG molecule. The highlighted area (coloured in yellow) indicates the proposed interaction region between the monomer unit of hIAPP and an EGCG molecule. (B) The ECD MS/MS spectrum of the 4+ charge state molecular ion of hIAPP monomer unit and an EGCG molecule. The assigned fragments are listed in the supporting information Table S3.

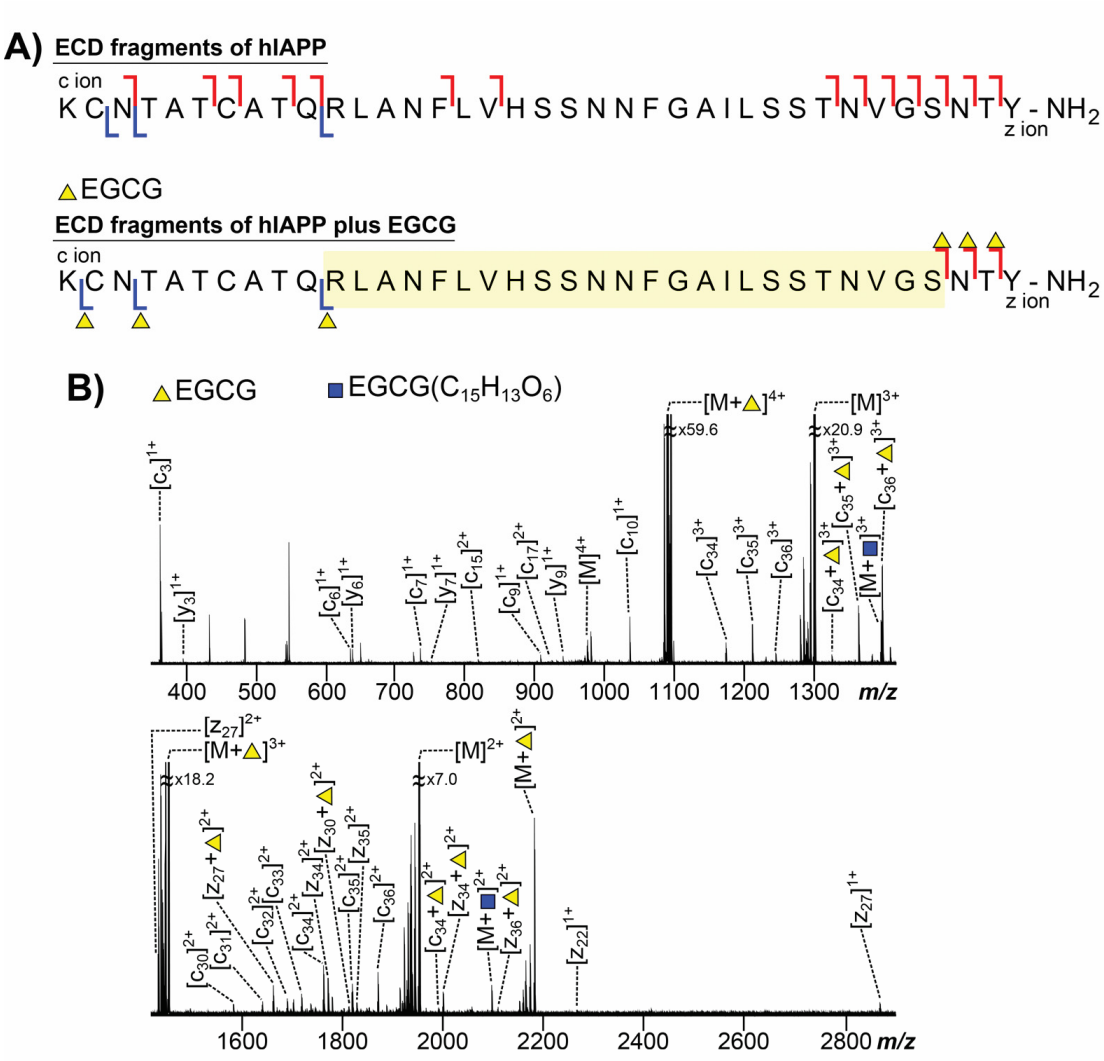

Fig S8. (A) The summarized ECD fragments of the 4+ charge state molecular ion of the mutant $(isoD)_3$hIAPP monomer unit and an EGCG molecule. The highlighted area (coloured in yellow) indicates the proposed interaction region between the monomer unit of the mutant $(isoD)_3$hIAPP and an EGCG molecule. (B) The ECD MS/MS spectrum of the 4+ charge state molecular ion of the mutant $(isoD)_3$hIAPP monomer unit and an EGCG molecule. The assigned fragments are listed in the supporting information Table S4.

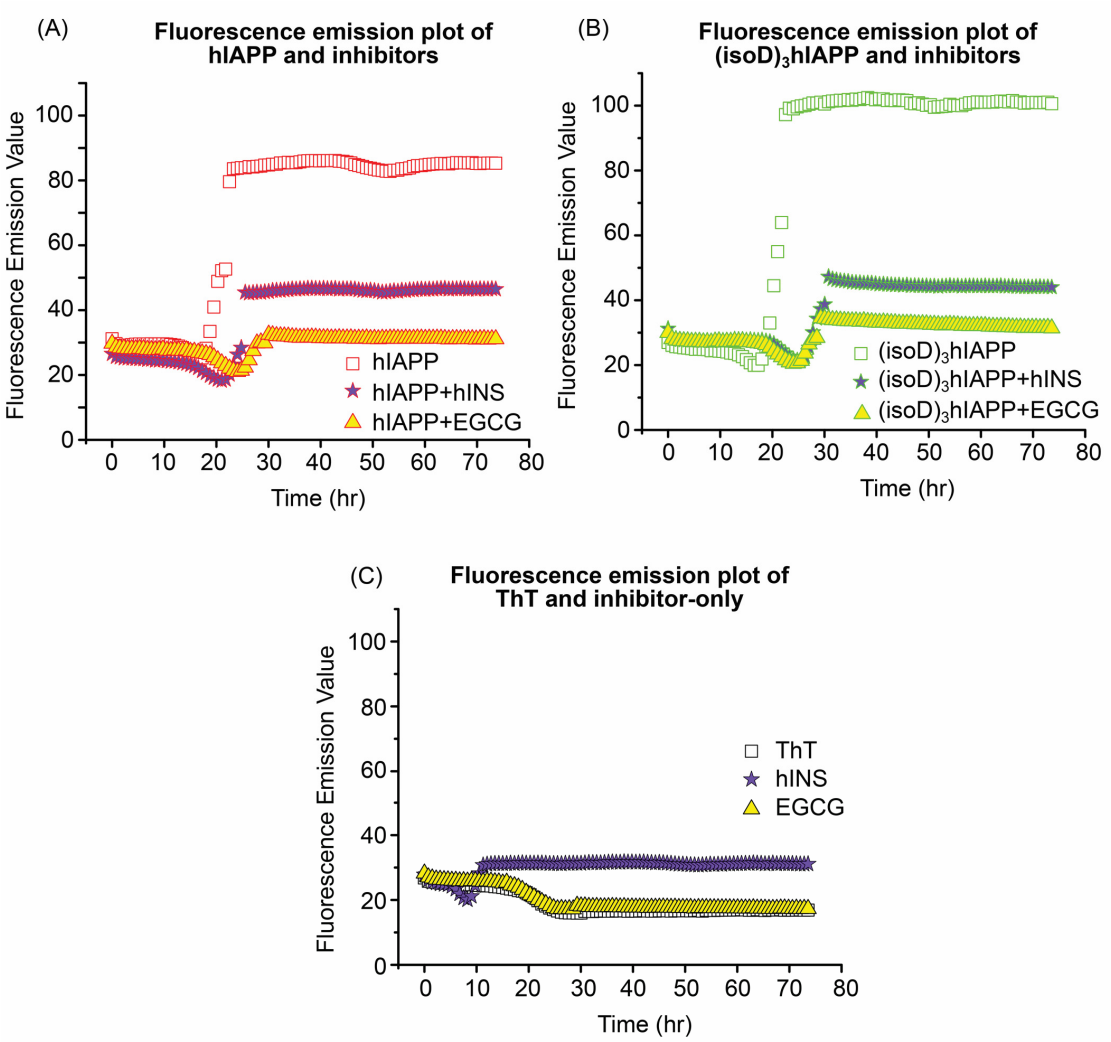

Fig S9. The fluorescence emission value of 50 μM (A) hIAPP and the equimolar potential inhibitors, (B) $(isoD)_3$hIAPP and the equimolar potential inhibitors, as well as (C) ThT solution and 50 μM potential inhibitors only.

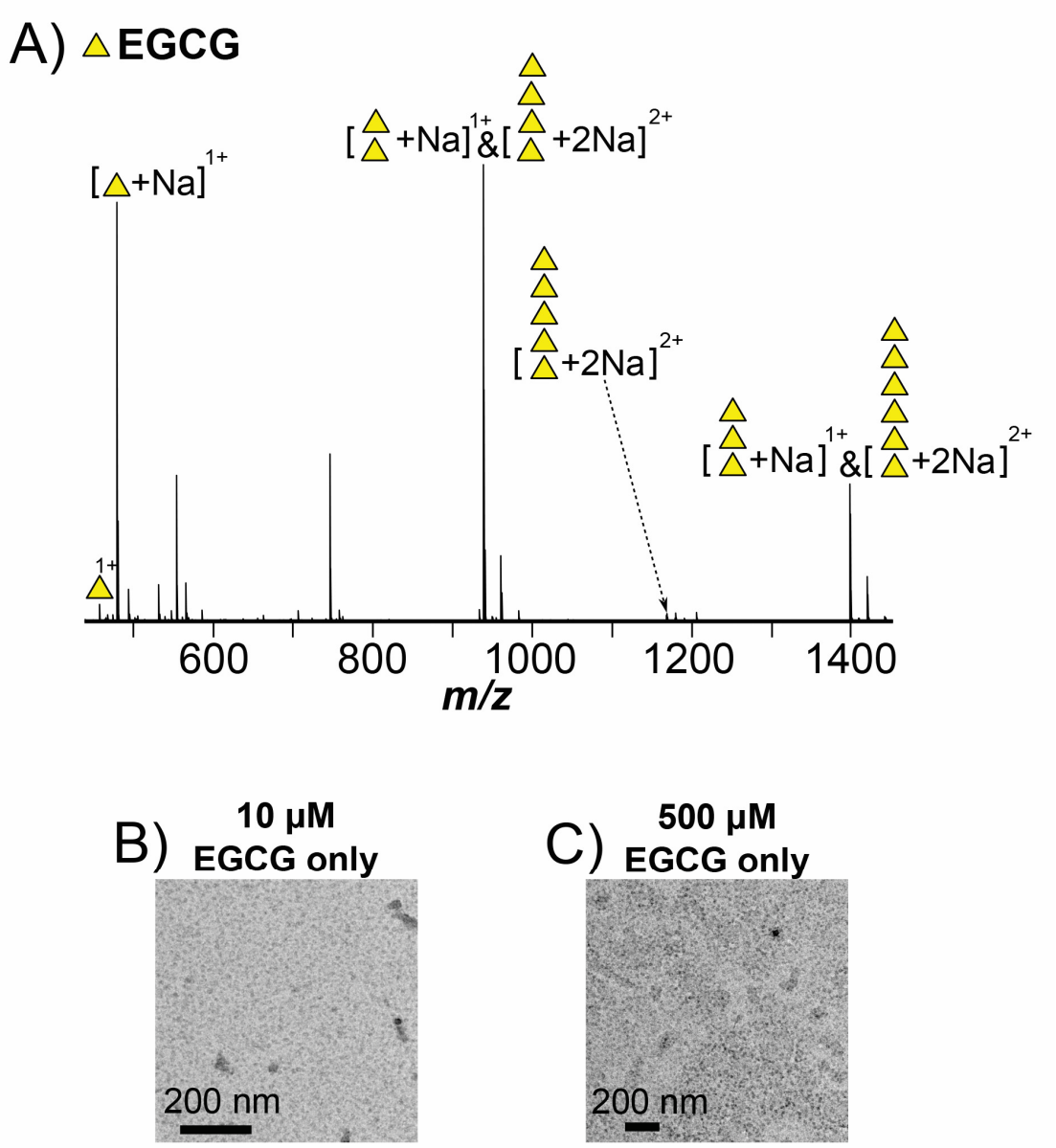

Fig S10. (A) The MS spectrum of the 10 μM aqueous solution of EGCG only. The EGCG solution was diluted to 50 μM and vortexed for half an hour, then the solution was further diluted to 10 μM and centrifuged at 14,000 rpm before MS analysis. Early oligomers, from monomer to hexamer, of EGCG were observed in the MS spectrum which indicates EGCG can rapidly aggregate in an aqueous solution. The TEM images of 1 week incubated solutions of (B) 10 μM EGCG solution only and (C) 500 μM EGCG solution only. Circular spots are observed in the TEM images with the addition of EGCG solution, the average diameter of the circular spots in the 10 μM EGCG solution is 9 nm and the average diameter of the spots in the 500 μM EGCG solution is 12 nm. The TEM images here demonstrate EGCG aggregates in the aqueous solution.

ECD fragments of the 7+ charge state heterodimer [hIAPP + hINS]

Sample	Fragment	Ion	Charge	Theoretical m/z	Experimental m/z	Error (ppm)
hIAPP only	hIAPP	M	2+	1952.444365	1952.445111	0.38
hIAPP only	hIAPP	M	3+	1301.295128	1301.295201	0.06
hIAPP only	hIAPP	M	4+	976.473865	976.473886	0.02
hIAPP only	hIAPP	c3	1+	363.180901	363.180902	0.00
hIAPP only	hIAPP	c7	1+	737.306908	737.306648	-0.35
hIAPP only	hIAPP	c8	1+	808.344022	808.343748	-0.34
hIAPP only	hIAPP	c9	1+	909.391701	909.391436	-0.29
hIAPP only	hIAPP	c10	1+	1037.450279	1037.450161	-0.11
hIAPP only	hIAPP	c11	1+	1193.551390	1193.551587	0.17
hIAPP only	hIAPP	c11	2+	597.279333	597.279053	-0.47
hIAPP only	hIAPP	c13	1+	1377.672568	1377.673361	0.58
hIAPP only	hIAPP	c13	2+	689.339922	689.339635	-0.42
hIAPP only	hIAPP	c14	1+	1491.715496	1491.716126	0.42
hIAPP only	hIAPP	c14	2+	746.361386	746.361106	-0.38
hIAPP only	hIAPP	c15	1+	1638.783910	1638.784576	0.41
hIAPP only	hIAPP	c15	2+	819.895593	819.895342	-0.31
hIAPP only	hIAPP	c17	2+	925.971832	925.971641	-0.21
hIAPP only	hIAPP	c18	2+	994.501288	994.501061	-0.23
hIAPP only	hIAPP	c19	2+	1038.017303	1038.017126	-0.17
hIAPP only	hIAPP	c20	2+	1081.533315	1081.532980	-0.31
hIAPP only	hIAPP	c21	2+	1138.554781	1138.554657	-0.11
hIAPP only	hIAPP	c22	2+	1195.576245	1195.576315	0.06
hIAPP only	hIAPP	c23	2+	1269.110452	1269.110634	0.14
hIAPP only	hIAPP	c23	3+	846.743643	846.743554	-0.11
hIAPP only	hIAPP	c24	2+	1297.621184	1297.620936	-0.19
hIAPP only	hIAPP	c24	3+	865.750796	865.750609	-0.22
hIAPP only	hIAPP	c25	2+	1333.641112	1333.641354	0.18
hIAPP only	hIAPP	c26	3+	926.790274	926.789975	-0.32
hIAPP only	hIAPP	c27	2+	1446.223805	1446.223638	-0.12
hIAPP only	hIAPP	c27	3+	964.484962	964.484964	0.00
hIAPP only	hIAPP	c28	2+	1490.241206	1490.241217	0.01
hIAPP only	hIAPP	c28	3+	993.829896	993.829891	-0.01
hIAPP only	hIAPP	c30	2+	1583.779674	1583.779878	0.13
hIAPP only	hIAPP	c30	3+	1056.523134	1056.523072	-0.06
hIAPP only	hIAPP	c32	3+	1127.560247	1127.560364	0.10
hIAPP only	hIAPP	c34	3+	1175.912156	1175.912589	0.37
hIAPP only	hIAPP	c35	3+	1213.258129	1213.258544	0.34
hIAPP only	hIAPP	c36	3+	1247.274945	1247.274420	-0.42
Insulin A Chain only	Insulin A Chain	c5	1+	544.308939	544.308688	-0.46
Insulin B Chain only	Insulin B Chain	c3	1+	378.213581	378.213589	0.02
Insulin B Chain only	Insulin B Chain	c4	1+	506.272159	506.271966	-0.38
Insulin B Chain only	Insulin B Chain	c5	1+	643.331071	643.330778	-0.46
Insulin B Chain only	Insulin B Chain	c6	1+	756.415135	756.414724	-0.54
Insulin B Chain only	Insulin B Chain	z4	1+	431.250026	431.249939	-0.20
Insulin B Chain only	Insulin B Chain	z5	1+	594.313354	594.313110	-0.41
Insulin B Chain only	Insulin B Chain	z6	1+	741.381768	741.381527	-0.33
Insulin B Chain only	Insulin B Chain	z7	1+	888.450182	888.450083	-0.11
Insulin B Chain only	Insulin B Chain	z8	1+	945.471646	945.471448	-0.21
Insulin B Chain only	Insulin B Chain	z9	1+	1101.572757	1101.573033	0.25
Insulin B Chain only	Insulin B Chain	z9	2+	550.786105	550.785891	-0.39
Insulin B Chain only	Insulin B Chain	z10	1+	1230.615350	1230.615841	0.40
hIAPP plus Insulin	hIAPP plus Insulin	M	3+	3238.855716	3238.854187	-0.47
hIAPP plus Insulin	hIAPP plus Insulin	M	4+	2429.141494	2429.142002	0.21
hIAPP plus Insulin	hIAPP plus Insulin	M	5+	1943.313046	1943.313443	0.20
hIAPP plus Insulin	hIAPP plus Insulin	M	6+	1619.427447	1619.427392	-0.03
hIAPP plus Insulin	hIAPP plus Insulin	M	7+	1388.223731	1388.222704	-0.74
hIAPP plus Insulin	Insulin A Chain	c20	6+	1599.754276	1599.754214	-0.04
hIAPP plus Insulin	Insulin A Chain	z16	4+	2293.064081	2293.064589	0.22
hIAPP plus Insulin	Insulin A Chain	z17	4+	2325.078733	2325.078653	-0.03
hIAPP plus Insulin	Insulin A Chain	z18	5+	1885.871364	1885.871611	0.13
hIAPP plus Insulin	Insulin B Chain	c19	4+	2107.482415	2107.483135	0.34
hIAPP plus Insulin	Insulin B Chain	c20	4+	2121.487385	2121.487270	-0.05
hIAPP plus Insulin	Insulin B Chain	c21	4+	2153.497521	2153.497499	-0.01
hIAPP plus Insulin	Insulin B Chain	c22	4+	2192.773330	2192.773427	0.04
hIAPP plus Insulin	Insulin B Chain	c22	5+	1754.219685	1754.219472	-0.12
hIAPP plus Insulin	Insulin B Chain	c23	4+	2207.781551	2207.781806	0.12
hIAPP plus Insulin	Insulin B Chain	c23	5+	1765.623980	1765.623979	0.00
hIAPP plus Insulin	Insulin B Chain	c24	4+	2244.548693	2244.547962	-0.33
hIAPP plus Insulin	Insulin B Chain	c24	5+	1795.236892	1795.236856	-0.02
hIAPP plus Insulin	Insulin B Chain	c25	4+	2280.814911	2280.815231	0.14
hIAPP plus Insulin	Insulin B Chain	c25	5+	1824.450207	1824.450622	0.23

Sample	Fragment	Ion	Charge	Theoretical m/z	Experimental m/z	Error (ppm)
hIAPP plus Insulin	Insulin B Chain	c26	4+	2321.580766	2321.581731	0.42
hIAPP plus Insulin	Insulin B Chain	c26	5+	1856.862493	1856.862979	0.26
hIAPP plus Insulin	Insulin B Chain	c28	4+	2371.356458	2371.355481	-0.41
hIAPP plus Insulin	Insulin B Chain	c28	5+	1896.883404	1896.883827	0.22
hIAPP plus Insulin	Insulin B Chain	c29	6+	1602.087178	1602.087134	-0.03
hIAPP plus Insulin	Insulin B Chain	z21	4+	2178.522447	2178.523282	0.38
hIAPP plus Insulin	Insulin B Chain	z24	4+	2239.285863	2239.286889	0.46
hIAPP plus Insulin	Insulin B Chain	z24	5+	1792.029854	1792.029364	-0.27
hIAPP plus Insulin	Insulin B Chain	z25	4+	2268.308542	2268.308948	0.18
hIAPP plus Insulin	Insulin B Chain	z25	5+	1814.245858	1814.245772	-0.05
hIAPP plus Insulin	Insulin B Chain	z26	4+	2302.573277	2302.573352	0.03
hIAPP plus Insulin	Insulin B Chain	z26	5+	1842.258864	1842.258245	-0.34
hIAPP plus Insulin	Insulin B Chain	z27	4+	2335.088870	2335.090524	0.71
hIAPP plus Insulin	Insulin B Chain	z27	5+	1867.871757	1867.871917	0.09
hIAPP plus Insulin	hIAPP	c35	5+	1889.887284	1889.887505	0.12
hIAPP plus Insulin	hIAPP	c35	6+	1575.240953	1575.240464	-0.31
hIAPP plus Insulin	hIAPP	c36	5+	1910.497623	1910.497296	-0.17
hIAPP plus Insulin	hIAPP	c36	6+	1591.581151	1591.581085	-0.04
hIAPP plus Insulin	hIAPP	z3	3+	2062.944772	2062.944905	0.06
hIAPP plus Insulin	hIAPP	z3	4+	1547.458964	1547.458220	-0.48
hIAPP plus Insulin	hIAPP	z5	3+	2110.962606	2110.963800	0.57
hIAPP plus Insulin	hIAPP	z5	4+	1583.722822	1583.722980	0.10
hIAPP plus Insulin	hIAPP	z7	3+	2182.667922	2182.668342	0.19
hIAPP plus Insulin	hIAPP	z7	4+	1637.251212	1637.252150	0.57
hIAPP plus Insulin	hIAPP	z9	4+	1683.520936	1683.521294	0.21
hIAPP plus Insulin	hIAPP	z10	3+	2274.037750	2274.037266	-0.21
hIAPP plus Insulin	hIAPP	z10	4+	1705.778658	1705.779637	0.57
hIAPP plus Insulin	hIAPP	z11	3+	2312.064759	2312.065994	0.53
hIAPP plus Insulin	hIAPP	z11	4+	1733.799170	1733.799257	0.05
hIAPP plus Insulin	hIAPP	z12	4+	1762.320723	1762.321406	0.39
hIAPP plus Insulin	hIAPP	z13	3+	2374.108297	2374.108691	0.17
hIAPP plus Insulin	hIAPP	z13	4+	1779.578921	1779.578564	-0.20
hIAPP plus Insulin	hIAPP	z14	3+	2392.781614	2392.782476	0.36
hIAPP plus Insulin	hIAPP	z14	4+	1793.834286	1793.834601	0.18
hIAPP plus Insulin	hIAPP	z15	4+	1831.102502	1831.102190	-0.17
hIAPP plus Insulin	hIAPP	z16	3+	2480.152623	2480.153239	0.25
hIAPP plus Insulin	hIAPP	z16	4+	1859.613225	1859.613843	0.33
hIAPP plus Insulin	hIAPP	z17	3+	2517.833081	2517.833039	-0.02
hIAPP plus Insulin	hIAPP	z17	4+	1888.624934	1888.625324	0.21
hIAPP plus Insulin	hIAPP	z18	3+	2547.177629	2547.178108	0.19
hIAPP plus Insulin	hIAPP	z18	4+	1909.881974	1909.882584	0.32
hIAPP plus Insulin	hIAPP	z19	3+	2575.520295	2575.521536	0.48
hIAPP plus Insulin	hIAPP	z19	4+	1932.391433	1932.391192	-0.12
hIAPP plus Insulin	hIAPP	z20	4+	1966.155222	1966.155322	0.05
hIAPP plus Insulin	hIAPP	z22	3+	2692.258282	2692.259678	0.52
hIAPP plus Insulin	hIAPP	z22	4+	2018.944275	2018.943853	-0.21
hIAPP plus Insulin	hIAPP	z23	3+	2741.615295	2741.615532	0.09
hIAPP plus Insulin	hIAPP	z23	4+	2055.460798	2055.461004	0.10
hIAPP plus Insulin	hIAPP	z23	5+	1644.769445	1644.769952	0.31
hIAPP plus Insulin	hIAPP	z24	4+	2084.723243	2084.723765	0.25
hIAPP plus Insulin	hIAPP	z24	5+	1667.578032	1667.578644	0.37
hIAPP plus Insulin	hIAPP	z26	4+	2130.752175	2130.752715	0.25
hIAPP plus Insulin	hIAPP	z26	5+	1704.402280	1704.402822	0.32
hIAPP plus Insulin	hIAPP	z27	3+	2893.705069	2893.704358	-0.25
hIAPP plus Insulin	hIAPP	z27	4+	2169.277708	2169.277893	0.09
hIAPP plus Insulin	hIAPP	z27	5+	1735.422057	1735.422001	-0.03
hIAPP plus Insulin	hIAPP	z28	4+	2201.542923	2201.543457	0.24
hIAPP plus Insulin	hIAPP	z28	5+	1761.033774	1761.033616	-0.09
hIAPP plus Insulin	hIAPP	z29	5+	1781.243313	1781.243788	0.27
hIAPP plus Insulin	hIAPP	z30	3+	2992.414504	2992.414832	-0.11
hIAPP plus Insulin	hIAPP	z31	4+	2270.063030	2270.063299	0.12
hIAPP plus Insulin	hIAPP	z34	4+	2338.598121	2338.598584	0.20
hIAPP plus Insulin	hIAPP	z34	5+	1871.078772	1871.078728	-0.02
					Average Error (ppm):	0.03
					Average Absolute Error (ppm):	0.23
					Standard Deviation:	0.29

Table S1. ECD MS/MS fragments of the 7+ charge state heterodimer (a hIAPP plus a hINS biomolecules).

ECD fragments of the 7+ charge state heterodimer [$(isoD)_3$hIAPP +hINS]

Sample	Fragment	Ion	Charge	Theoretical m/z	Experimental m/z	Error (ppm)
$(isoD)_3$ only	$(isoD)_3$	M	2+	1952.915077	1952.916573	-0.77
$(isoD)_3$ only	$(isoD)_3$	M	3+	1302.947539	1302.947937	-0.31
$(isoD)_3$ only	$(isoD)_3$	M	4+	976.961177	976.961469	-0.30
$(isoD)_3$ only	$(isoD)_3$	c3	1+	363.180902	363.180901	0.00
$(isoD)_3$ only	$(isoD)_3$	c7	1+	737.306908	737.306922	-0.02
$(isoD)_3$ only	$(isoD)_3$	c9	1+	909.391701	909.392223	-0.57
$(isoD)_3$ only	$(isoD)_3$	c10	1+	1037.450279	1037.450500	-0.21
$(isoD)_3$ only	$(isoD)_3$	c11	2+	597.279333	597.279056	0.46
$(isoD)_3$ only	$(isoD)_3$	c13	2+	689.339922	689.339936	-0.02
$(isoD)_3$ only	$(isoD)_3$	c14	1+	1491.715496	1491.715986	-0.33
$(isoD)_3$ only	$(isoD)_3$	c14	2+	746.361386	746.361328	0.08
$(isoD)_3$ only	$(isoD)_3$	c15	2+	819.895593	819.895563	0.04
$(isoD)_3$ only	$(isoD)_3$	c16	2+	876.437625	876.438141	-0.59
$(isoD)_3$ only	$(isoD)_3$	c17	2+	925.971832	925.972433	-0.65
$(isoD)_3$ only	$(isoD)_3$	c18	2+	994.501288	994.501468	-0.18
$(isoD)_3$ only	$(isoD)_3$	c19	2+	1038.017303	1038.017273	0.03
$(isoD)_3$ only	$(isoD)_3$	c20	2+	1082.034683	1082.035086	-0.37
$(isoD)_3$ only	$(isoD)_3$	c20i	2+	1110.032142	1110.032510	-0.33
$(isoD)_3$ only	$(isoD)_3$	c21	2+	1139.046789	1139.046837	-0.04
$(isoD)_3$ only	$(isoD)_3$	c22	2+	1196.560261	1196.560006	0.21
$(isoD)_3$ only	$(isoD)_3$	c23	2+	1270.094468	1270.093553	0.72
$(isoD)_3$ only	$(isoD)_3$	c23	3+	847.065404	847.065136	0.32
$(isoD)_3$ only	$(isoD)_3$	c24	3+	866.072559	866.072370	0.22
$(isoD)_3$ only	$(isoD)_3$	c26	3+	927.446285	927.446898	-0.66
$(isoD)_3$ only	$(isoD)_3$	c30	2+	1585.265090	1585.265442	-0.22
Insulin only	Insulin only	M	4+	1452.668486	1452.668366	0.08
Insulin Chain A only	Insulin Chain A	c4	1+	416.250361	416.250383	-0.05
Insulin Chain A only	Insulin Chain A	c5	1+	544.308939	544.308813	0.23
Insulin Chain B only	Insulin Chain B	c3	1+	378.213581	378.213611	-0.08
Insulin Chain B only	Insulin Chain B	c4	1+	506.272159	506.272103	0.11
Insulin Chain B only	Insulin Chain B	c5	1+	643.331071	643.330997	0.12
Insulin Chain B only	Insulin Chain B	y5	1+	609.324255	609.324225	0.05
Insulin Chain B only	Insulin Chain B	y6	1+	756.392669	756.392628	0.05
Insulin Chain B only	Insulin Chain B	y7	1+	903.461083	903.461012	0.08
Insulin Chain B only	Insulin Chain B	y8	1+	960.482547	960.482506	0.04
Insulin Chain B only	Insulin Chain B	z4	1+	430.242202	430.242196	0.01
Insulin Chain B only	Insulin Chain B	z5	1+	593.305531	593.305469	0.10
Insulin Chain B only	Insulin Chain B	z6	1+	740.373945	740.373860	0.11
Insulin Chain B only	Insulin Chain B	z7	1+	887.442485	887.442444	0.05
Insulin Chain B only	Insulin Chain B	z8	1+	944.463823	944.464273	-0.48
Insulin Chain B only	Insulin Chain B	z9	1+	1101.572757	1101.572855	-0.09
Insulin Chain B only	Insulin Chain B	z9	2+	550.786105	550.786068	0.07
Insulin Chain B only	Insulin Chain B	z10	1+	1230.615350	1230.615080	0.22
Insulin Chain B only	Insulin Chain B	z10	2+	615.307402	615.307277	0.20
$(isoD)_3$ Plus Insulin	$(isoD)_3$ Plus Insulin	M	3+	3240.173471	3240.173590	-0.04
$(isoD)_3$ Plus Insulin	$(isoD)_3$ Plus Insulin	M	4+	2430.129966	2430.130028	-0.03
$(isoD)_3$ Plus Insulin	$(isoD)_3$ Plus Insulin	M	5+	1944.103863	1944.103700	0.08
$(isoD)_3$ Plus Insulin	$(isoD)_3$ Plus Insulin	M	6+	1619.919462	1619.919053	0.25
$(isoD)_3$ Plus Insulin	$(isoD)_3$ Plus Insulin	M	7+	1387.929577	1387.929056	0.38
$(isoD)_3$ Plus Insulin	Insulin Chain A	c20	4+	2399.864385	2399.863176	0.50
$(isoD)_3$ Plus Insulin	Insulin Chain A	c20	5+	1921.096063	1921.096710	-0.34
$(isoD)_3$ Plus Insulin	Insulin Chain A	c20	6+	1601.080263	1601.080577	-0.20
$(isoD)_3$ Plus Insulin	Insulin Chain A	z16	4+	2294.553419	2294.553690	-0.12
$(isoD)_3$ Plus Insulin	Insulin Chain A	z16	5+	1834.841709	1834.841128	0.32
$(isoD)_3$ Plus Insulin	Insulin Chain A	z17	4+	2326.818517	2326.819567	-0.45
$(isoD)_3$ Plus Insulin	Insulin Chain A	z17	5+	1860.452849	1860.451866	0.53
$(isoD)_3$ Plus Insulin	Insulin Chain A	z18	5+	1886.662172	1886.661199	0.52
$(isoD)_3$ Plus Insulin	Insulin Chain A	z20	5+	1928.691886	1928.692517	-0.33
$(isoD)_3$ Plus Insulin	Insulin Chain A	z20	6+	1607.745126	1607.744601	0.33
$(isoD)_3$ Plus Insulin	Insulin Chain B	c19	4+	2107.468995	2107.467783	0.58
$(isoD)_3$ Plus Insulin	Insulin Chain B	c20	4+	2122.225339	2122.225238	0.05
$(isoD)_3$ Plus Insulin	Insulin Chain B	c21	3+	2872.314220	2872.315547	-0.46
$(isoD)_3$ Plus Insulin	Insulin Chain B	c21	4+	2154.486003	2154.485220	0.36
$(isoD)_3$ Plus Insulin	Insulin Chain B	c21	5+	1723.789056	1723.788772	0.16
$(isoD)_3$ Plus Insulin	Insulin Chain B	c22	3+	2924.347928	2924.350542	-0.89
$(isoD)_3$ Plus Insulin	Insulin Chain B	c22	4+	2193.512766	2193.512357	0.19
$(isoD)_3$ Plus Insulin	Insulin Chain B	c22	5+	1754.808920	1754.808280	0.36
$(isoD)_3$ Plus Insulin	Insulin Chain B	c23	3+	2943.689059	2943.689486	-0.15
$(isoD)_3$ Plus Insulin	Insulin Chain B	c23	4+	2208.018613	2208.018861	-0.11
$(isoD)_3$ Plus Insulin	Insulin Chain B	c23	5+	1766.214397	1766.214135	0.15
$(isoD)_3$ Plus Insulin	Insulin Chain B	c24	5+	1796.028862	1796.028287	0.32
$(isoD)_3$ Plus Insulin	Insulin Chain B	c25	3+	3043.070451	3043.072454	-0.66
$(isoD)_3$ Plus Insulin	Insulin Chain B	c25	4+	2281.803352	2281.805163	-0.79
$(isoD)_3$ Plus Insulin	Insulin Chain B	c25	5+	1825.041795	1825.041970	-0.10

Sample	Fragment	Ion	Charge	Theoretical m/z	Experimental m/z	Error (ppm)
$(isoD)_3$ Plus Insulin	Insulin Chain B	c26	3+	3096.089220	3096.088739	0.16
$(isoD)_3$ Plus Insulin	Insulin Chain B	c26	4+	2322.569218	2322.569675	-0.20
$(isoD)_3$ Plus Insulin	Insulin Chain B	c26	5+	1857.854878	1857.854278	0.32
$(isoD)_3$ Plus Insulin	Insulin Chain B	c28	4+	2372.094357	2372.093390	0.41
$(isoD)_3$ Plus Insulin	Insulin Chain B	c28	5+	1897.675376	1897.675157	0.12
$(isoD)_3$ Plus Insulin	Insulin Chain B	c29	4+	2403.615663	2403.616483	-0.34
$(isoD)_3$ Plus Insulin	Insulin Chain B	c29	5+	1923.294381	1923.294992	-0.32
$(isoD)_3$ Plus Insulin	Insulin Chain B	c29	6+	1602.913197	1602.913307	-0.07
$(isoD)_3$ Plus Insulin	Insulin Chain B	z24	3+	2988.366670	2988.367640	-0.32
$(isoD)_3$ Plus Insulin	Insulin Chain B	z24	4+	2240.773940	2240.773485	0.20
$(isoD)_3$ Plus Insulin	Insulin Chain B	z24	5+	1792.419876	1792.419161	0.40
$(isoD)_3$ Plus Insulin	Insulin Chain B	z25	3+	3026.397342	3026.396963	0.13
$(isoD)_3$ Plus Insulin	Insulin Chain B	z25	4+	2269.046508	2269.047066	-0.25
$(isoD)_3$ Plus Insulin	Insulin Chain B	z25	5+	1815.437480	1815.436826	0.36
$(isoD)_3$ Plus Insulin	Insulin Chain B	z26	4+	2303.561725	2303.562573	-0.37
$(isoD)_3$ Plus Insulin	Insulin Chain B	z26	5+	1842.448485	1842.448164	0.17
$(isoD)_3$ Plus Insulin	Insulin Chain B	z27	4+	2335.826840	2335.825940	0.39
$(isoD)_3$ Plus Insulin	Insulin Chain B	z27	5+	1868.260608	1868.260160	0.24
$(isoD)_3$ Plus Insulin	Insulin Chain B	z28	4+	2365.088898	2365.089350	-0.19
$(isoD)_3$ Plus Insulin	$(isoD)_3$	c34	5+	1867.672710	1867.672031	0.36
$(isoD)_3$ Plus Insulin	$(isoD)_3$	c35	5+	1890.878497	1890.878405	0.05
$(isoD)_3$ Plus Insulin	$(isoD)_3$	c35	6+	1575.731990	1575.731976	0.01
$(isoD)_3$ Plus Insulin	$(isoD)_3$	c35i	5+	1902.278035	1902.278605	-0.30
$(isoD)_3$ Plus Insulin	$(isoD)_3$	c36	5+	1910.887650	1910.887764	-0.06
$(isoD)_3$ Plus Insulin	$(isoD)_3$	c36	6+	1592.406283	1592.406213	0.04
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z7	3+	2182.995768	2182.994961	0.37
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z7	4+	1637.497139	1637.497312	-0.11
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z10	3+	2274.363773	2274.364133	-0.16
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z10	4+	1705.523604	1705.523105	0.29
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z11	3+	2312.058484	2312.058788	-0.13
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z13	3+	2374.100016	2374.099224	0.33
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z13	4+	1781.077386	1781.077523	-0.08
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z14	3+	2392.775341	2392.775875	-0.22
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z14	4+	1794.581369	1794.581723	-0.20
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z15	3+	2441.798178	2441.799229	-0.43
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z15	4+	1831.348497	1831.348918	-0.23
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z16-i	4+	1845.855821	1845.857090	-0.69
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z16	3+	2481.476328	2481.477078	-0.30
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z16	4+	1860.105241	1860.105782	-0.29
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z17-i	4+	1874.362038	1874.362807	-0.41
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z17	3+	2518.816823	2518.817651	-0.33
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z17	4+	1888.360880	1888.360677	0.11
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z18	3+	2547.493513	2547.491350	0.85
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z19	3+	2576.504196	2576.504353	-0.06
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z20	4+	1966.894700	1966.893921	0.40
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z21	4+	1991.409858	1991.409202	0.33
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z22	3+	2693.242048	2693.242231	-0.07
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z22	4+	2019.680886	2019.680124	0.38
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z23	3+	2742.264893	2742.265273	-0.14
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z23	4+	2056.698532	2056.698955	-0.21
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z23	5+	1645.359863	1645.359065	0.49
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z24	4+	2085.710560	2085.709268	0.62
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z25	4+	2103.219063	2103.218598	0.22
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z26	4+	2130.738494	2130.737027	0.69
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z26	5+	1704.792251	1704.792062	0.11
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z27	3+	2895.024600	2895.023753	0.29
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z27	4+	2170.516822	2170.517872	-0.48
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z27	5+	1736.212920	1736.212575	0.20
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z28	4+	2202.030375	2202.029056	0.60
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z28	5+	1762.025070	1762.025125	-0.03
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z30	3+	2994.066584	2994.066236	0.12
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z30	4+	2245.036214	2245.037564	-0.60
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z31	4+	2270.801007	2270.802349	-0.59
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z34	4+	2340.338033	2340.338894	-0.37
$(isoD)_3$ Plus Insulin	$(isoD)_3$	z34	5+	1871.468773	1871.468687	0.05
					Average Error (ppm):	-0.01
					Average Absolute Error (ppm):	0.27
					Standard Deviation:	0.34

Table S2. ECD MS/MS fragments of the 7+ charge state heterodimer (an $(isoD)_3$hIAPP plus a hINS biomolecules).

ECD fragments of the 4+ molecular ion [hIAPP + EGCG]

Sample	Fragment	Ion	Charge	Theoretical m/z	Experimental m/z	Error (ppm)
hIAPP only	hIAPP	M	2+	1952.446878	1952.445693	0.61
hIAPP only	hIAPP	M	3+	1301.965336	1301.965433	-0.07
hIAPP only	hIAPP	M	4+	976.223165	976.223115	0.05
hIAPP only	hIAPP	c3	1+	363.180901	363.180903	-0.01
hIAPP only	hIAPP	c6	1+	636.313372	636.313307	0.10
hIAPP only	hIAPP	c7	1+	737.306908	737.306793	0.16
hIAPP only	hIAPP	c9	1+	909.391701	909.392330	-0.69
hIAPP only	hIAPP	c10	1+	1037.450279	1037.450491	-0.20
hIAPP only	hIAPP	c15	2+	819.895593	819.895681	-0.11
hIAPP only	hIAPP	c17	2+	925.971832	925.972160	-0.35
hIAPP only	hIAPP	c30	2+	1584.281062	1584.280627	0.27
hIAPP only	hIAPP	c31	2+	1641.302522	1641.302312	0.13
hIAPP only	hIAPP	c32	2+	1690.335345	1690.334878	0.28
hIAPP only	hIAPP	c33	2+	1719.347463	1719.347399	0.04
hIAPP only	hIAPP	c34	2+	1762.362086	1762.362107	-0.01
hIAPP only	hIAPP	c34	3+	1175.912156	1175.912079	0.07
hIAPP only	hIAPP	c35	2+	1820.388715	1820.388850	-0.07
hIAPP only	hIAPP	c35	3+	1213.592384	1213.592237	0.12
hIAPP only	hIAPP	c36	2+	1870.411302	1870.411105	0.11
hIAPP only	hIAPP	c36	3+	1246.940685	1246.940038	0.52
hIAPP only	hIAPP	y3	1+	396.187761	396.187732	0.07
hIAPP only	hIAPP	y6	1+	639.309668	639.309634	0.05
hIAPP only	hIAPP	y7	1+	753.352596	753.353056	-0.61
hIAPP only	hIAPP	y9	1+	941.432304	941.432732	-0.45
hIAPP only	hIAPP	z22	1+	2266.104890	2266.104193	0.31
hIAPP only	hIAPP	z27	1+	2868.446331	2868.445815	0.18
hIAPP only	hIAPP	z27	2+	1434.726804	1434.726964	-0.11
hIAPP only	hIAPP	z34	2+	1771.360072	1771.360101	-0.02
hIAPP only	hIAPP	z35	2+	1828.381536	1828.381635	-0.05
hIAPP plus EGCG ($C_{15}H_{13}O_6$)	hIAPP	M	2+	2096.979994	2096.979343	0.31
hIAPP plus EGCG ($C_{15}H_{13}O_6$)	hIAPP	M	3+	1397.986480	1397.985924	0.40
hIAPP plus EGCG	hIAPP	M	2+	2181.990765	2181.990604	0.07
hIAPP plus EGCG	hIAPP	M	3+	1454.994485	1454.994299	0.13
hIAPP plus EGCG	hIAPP	M	4+	1090.995108	1090.994632	0.44
hIAPP plus EGCG	hIAPP	c34	2+	1991.404542	1991.405413	-0.44
hIAPP plus EGCG	hIAPP	c34	3+	1327.937130	1327.936824	0.23
hIAPP plus EGCG	hIAPP	c35	3+	1366.285572	1366.285710	-0.10
hIAPP plus EGCG	hIAPP	c36	3+	1399.969938	1399.969107	0.59
hIAPP plus EGCG	hIAPP	z27	2+	1663.265384	1663.264553	0.50
hIAPP plus EGCG	hIAPP	z30	2+	1813.337064	1813.337861	-0.44
hIAPP plus EGCG	hIAPP	z34	2+	2001.405284	2001.404286	0.50
hIAPP plus EGCG	hIAPP	z36	2+	2110.432358	2110.432229	0.06
				Average Error (ppm):		0.06
				Average Absolute Error (ppm):		0.24
				Standard Deviation:		0.31

Table S3. ECD MS/MS fragments of the 4+ charge state molecular ion (a hIAPP plus a EGCG molecules).

ECD fragments of the 4+ molecular ion of [$(isoD)_3$hIAPP + EGCG]

Sample	Fragment	Ion	Charge	Theoretical m/z	Experimental m/z	Error (ppm)
$(isoD)_3$ only	$(isoD)_3$	M	2+	1954.925496	1954.924942	0.28
$(isoD)_3$ only	$(isoD)_3$	M	3+	1302.279143	1302.279059	0.06
$(isoD)_3$ only	$(isoD)_3$	c3	1+	363.180901	363.180898	0.01
$(isoD)_3$ only	$(isoD)_3$	c7	1+	737.306908	737.306854	0.07
$(isoD)_3$ only	$(isoD)_3$	c8	1+	808.344022	808.344160	-0.17
$(isoD)_3$ only	$(isoD)_3$	c9	1+	909.391701	909.391865	-0.18
$(isoD)_3$ only	$(isoD)_3$	c10	1+	1037.450279	1037.450652	-0.36
$(isoD)_3$ only	$(isoD)_3$	c15	2+	819.895593	819.895799	-0.25
$(isoD)_3$ only	$(isoD)_3$	c17	2+	925.971832	925.972020	-0.20
$(isoD)_3$ only	$(isoD)_3$	c32	2+	1692.825783	1692.825730	0.03
$(isoD)_3$ only	$(isoD)_3$	c33	2+	1719.830087	1719.829403	0.40
$(isoD)_3$ only	$(isoD)_3$	c34	2+	1763.847504	1763.847939	-0.25
$(isoD)_3$ only	$(isoD)_3$	c34	3+	1176.234095	1176.234008	0.07
$(isoD)_3$ only	$(isoD)_3$	c34i	2+	1793.852388	1793.851868	0.29
$(isoD)_3$ only	$(isoD)_3$	c34i	3+	1195.569257	1195.569163	0.08
$(isoD)_3$ only	$(isoD)_3$	c35	2+	1821.864890	1821.863500	0.76
$(isoD)_3$ only	$(isoD)_3$	c35	3+	1214.242141	1214.241984	0.13
$(isoD)_3$ only	$(isoD)_3$	c36	2+	1872.388731	1872.388124	0.32
$(isoD)_3$ only	$(isoD)_3$	c36	3+	1248.927090	1248.927464	-0.30
$(isoD)_3$ only	$(isoD)_3$	y4	1+	484.203806	484.203809	-0.01
$(isoD)_3$ only	$(isoD)_3$	y5	1+	541.225270	541.225288	-0.03
$(isoD)_3$ only	$(isoD)_3$	y6	1+	640.293684	640.293720	-0.06
$(isoD)_3$ only	$(isoD)_3$	z28	2+	1500.232133	1500.232022	0.07
$(isoD)_3$ only	$(isoD)_3$	z30	2+	1586.775917	1586.774655	0.80
$(isoD)_3$ only	$(isoD)_3$	z34	2+	1772.836090	1772.836105	-0.01
$(isoD)_3$ only	$(isoD)_3$	z35	2+	1830.358982	1830.358487	0.27
$(isoD)_3$ plus EGCG ($C_{15}H_{13}O_6$)	$(isoD)_3$ plus EGCG ($C_{15}H_{13}O_6$)	M	2+	2098.957258	2098.957106	0.07
$(isoD)_3$ plus EGCG ($C_{15}H_{13}O_6$)	$(isoD)_3$ plus EGCG ($C_{15}H_{13}O_6$)	M	3+	1398.970505	1398.969616	0.64
$(isoD)_3$ plus EGCG	$(isoD)_3$ plus EGCG	M	2+	2184.469182	2184.468035	0.53
$(isoD)_3$ plus EGCG	$(isoD)_3$ plus EGCG	M	3+	1455.978516	1455.978501	0.01
$(isoD)_3$ plus EGCG	$(isoD)_3$ plus EGCG	M	4+	1091.482404	1091.482042	0.33
$(isoD)_3$ plus EGCG	$(isoD)_3$	c34	3+	1328.927284	1328.927366	-0.06
$(isoD)_3$ plus EGCG	$(isoD)_3$	c34i	3+	1348.262449	1348.262382	0.05
$(isoD)_3$ plus EGCG	$(isoD)_3$	c35	3+	1366.935462	1366.935379	0.06
$(isoD)_3$ plus EGCG	$(isoD)_3$	c36	3+	1401.288112	1401.288199	-0.06
$(isoD)_3$ plus EGCG	$(isoD)_3$	z27	2+	1664.239945	1664.239592	0.21
$(isoD)_3$ plus EGCG	$(isoD)_3$	z28	2+	1729.274622	1729.273583	0.60
$(isoD)_3$ plus EGCG	$(isoD)_3$	z30	2+	1814.311630	1814.311018	0.34
$(isoD)_3$ plus EGCG	$(isoD)_3$	z34	2+	2002.377420	2002.377122	0.15
$(isoD)_3$ plus EGCG	$(isoD)_3$	z35	2+	2060.401324	2060.400919	0.20
$(isoD)_3$ plus EGCG	$(isoD)_3$	z36	2+	2110.903378	2110.902434	0.45
				Average Error (ppm):		0.13
				Average Absolute Error (ppm):		0.22
				Standard Deviation:		0.28

Table S4. ECD MS/MS fragments of the 4+ charge state molecular ion (an $(isoD)_3$hIAPP plus a EGCG molecules).

Reference:

1. A. C. Susa, C. Wu, S. L. Bernstein, N. F. Dupuis, H. Wang, D. P. Raleigh, J. E. Shea and M. T. Bowers, *J. Am. Chem. Soc.*, 2014, **136**, 12912-12919.
2. P. Caravatti and M. Allemann, *Org. Mass Spectrom.*, 1991, **26**, 514-518.
3. L. De Koning, N. Nibbering, S. Van Orden and F. Laukien, *International journal of mass spectrometry and ion processes*, 1997, **165**, 209-219.
4. F. T. S. Chan, G. S. K. Schierle, J. R. Kumita, C. W. Bertoncini, C. M. Dobson and C. F. Kaminski, *Analyst*, 2013, **138**, 2156-2162.
5. T. Yang, X. H. Wang, C. L. Zhang, X. Ma, K. Wang, Y. Q. Wang, J. Luo, L. Yang, C. Yao and X. Y. Wang, *Chem. Commun.*, 2016, **52**, 2245-2248.
6. P. Arosio, T. P. Knowles and S. Linse, *Physical Chemistry Chemical Physics*, 2015, **17**, 7606-7618.
7. S. K. Shoffner and S. Schnell, *Physical Chemistry Chemical Physics*, 2016, **18**, 21259-21268.