Supporting information for

Rapid Assessment of Human Amylin Aggregation and its Inhibition by Copper(II) Ions by Laser Ablation Electrospray Ionization Mass Spectrometry with Ion Mobility Separation

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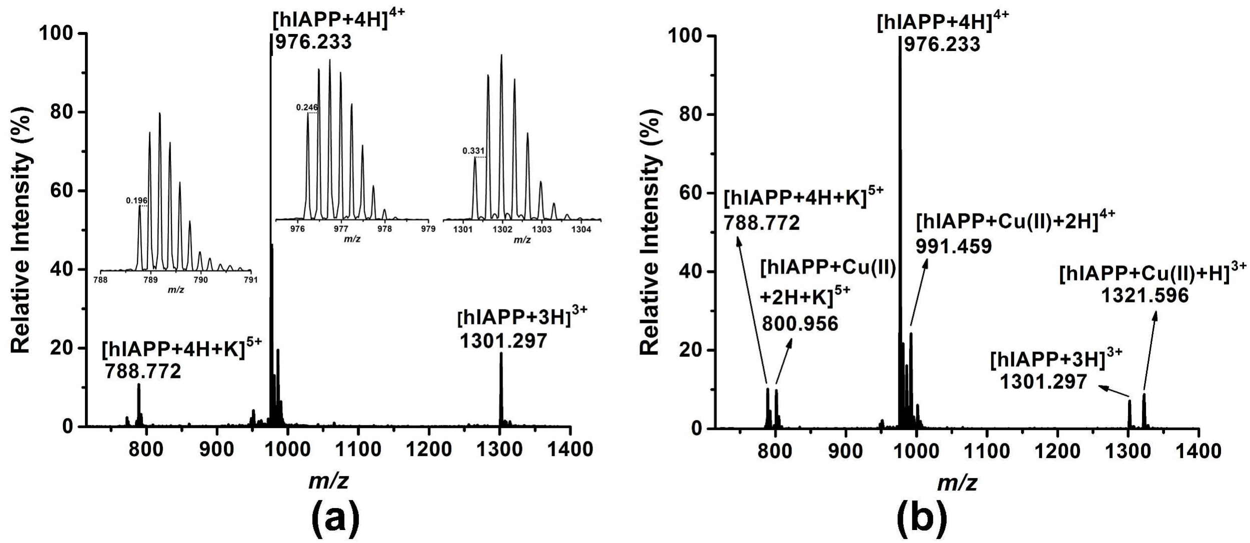

Figure S1. Mass spectra of (a) 20 μM hIAPP solution and (b) the copper adducts of hIAPP acquired by LAESI-IMS-MS. The copper adducts were produced by incubating the hIAPP solution with 40 μM $CuCl_2$ solution for 8 h. The insets in (a) show the isotope distribution patterns of the quintdruply, quadruply, and triply charged hIAPP ions.

(a) Synthesized hIAPP

KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY-NH_2

1 5 10 15 20 25 30 35

(b) $hIAPP_{H18A}$

KCNTATCATQRLANFLVASSNNFGAILSSTNVGSNTY-NH_2

(c) $hIAPP_{8\text{-}37}$

ATQRLANFLVHSSNNFGAILSSTNVGSNTY-NH_2

Figure S2. Amino acid sequence of (a) the synthetic human amylin (hIAPP), (b) amylin with residue 18, histidine, substituted by alanine ($hIAPP_{H18A}$), and (c) hIAPP fragment 8-37 ($hIAPP_{8\text{-}37}$).

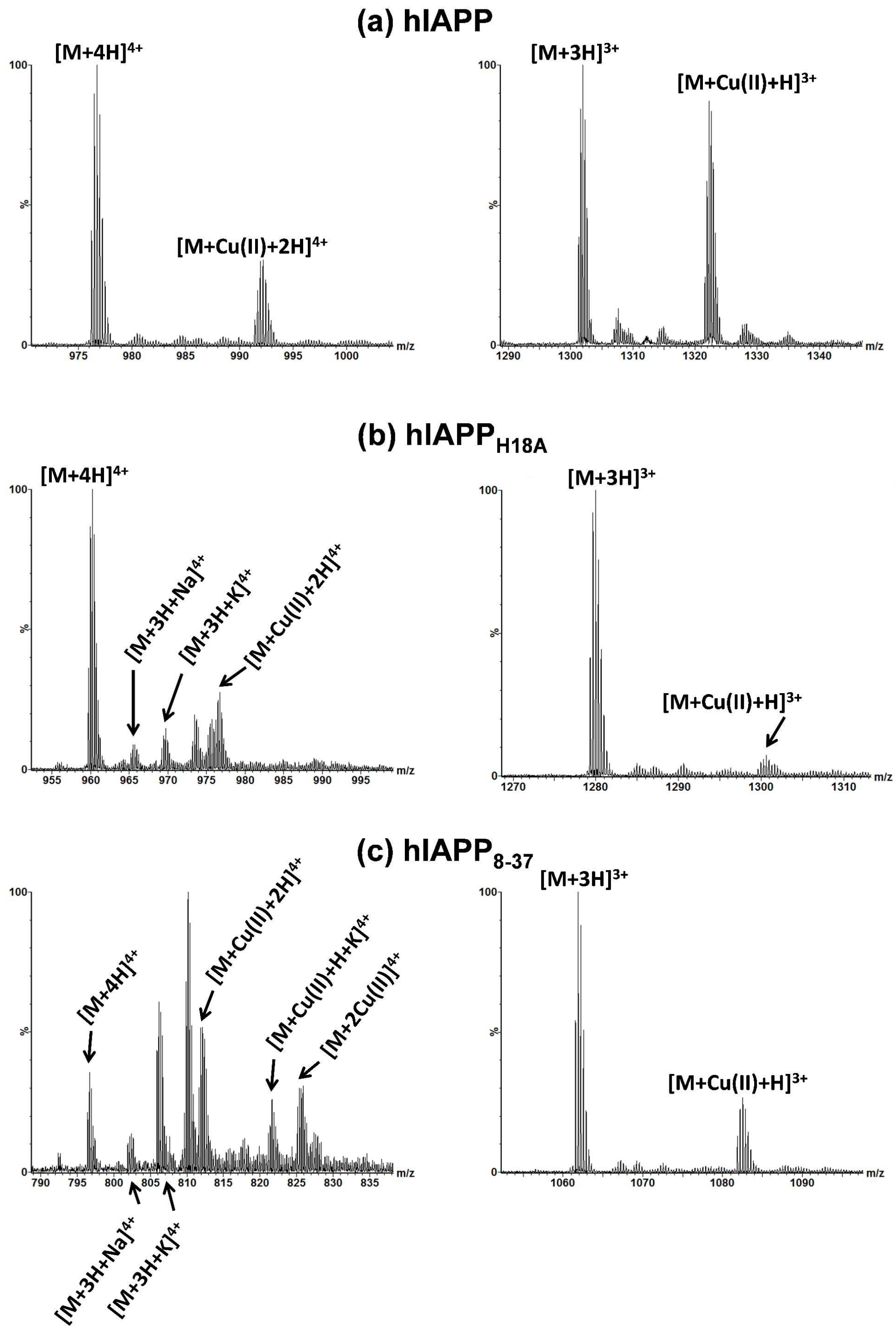

Figure S3. Quadruply and triply charged molecular and quasi-molecular ions in LAESI mass spectra from (a) hIAPP, (b) $hIAPP_{H18A}$, and (c) $hIAPP_{8-37}$ solutions in the presence of Cu^{2+}.

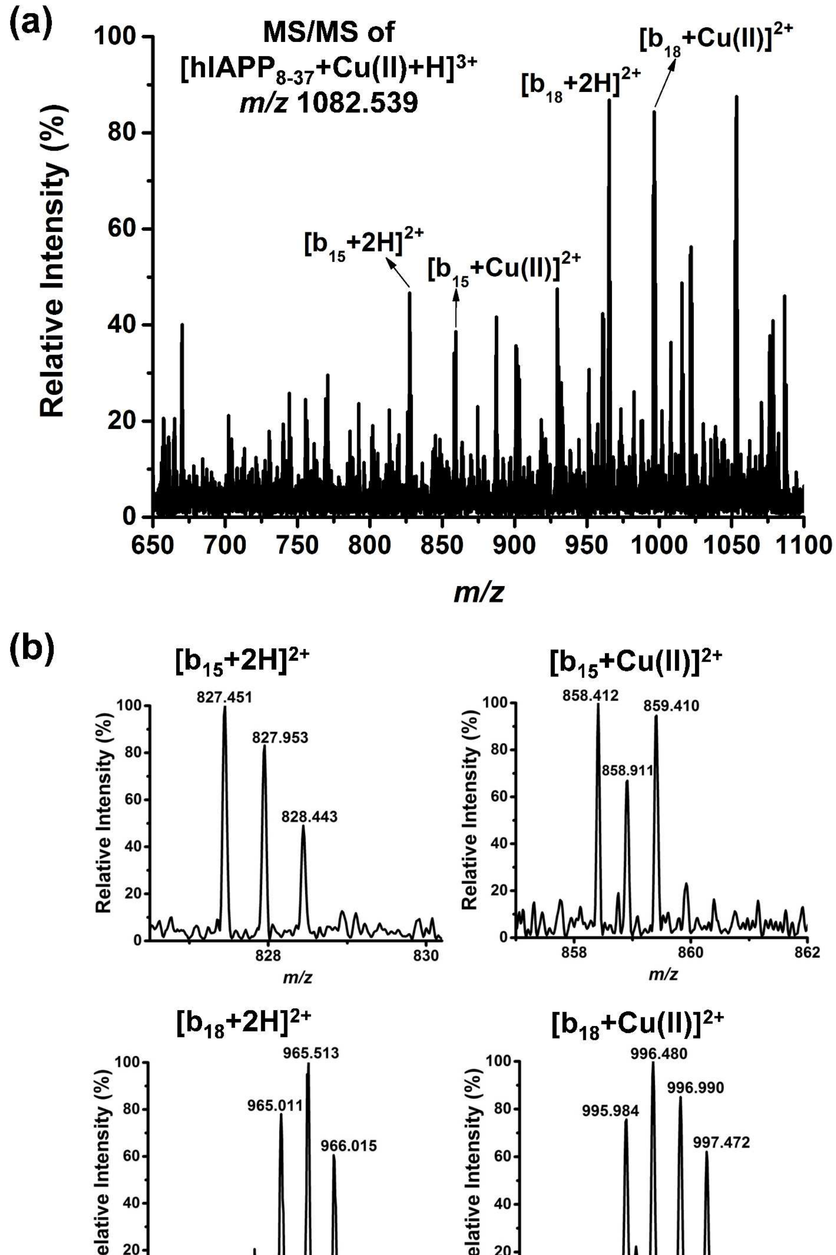

Figure S4. (a)Tandem mass spectrum of $[hIAPP_{8\text{-}37}+Cu(II)+H]^{3+}$ at *m/z* 1082.5 shows a Cu(II) adduct for fragments b_{18} and possibly b_{15}. (b) Isotope distribution patterns of fragment ions $[b_{15}+2H]^{2+}$, $[b_{15}+Cu(II)]^{2+}$, $[b_{18}+2H]^{2+}$, and $[b_{18}+Cu(II)]^{2+}$.

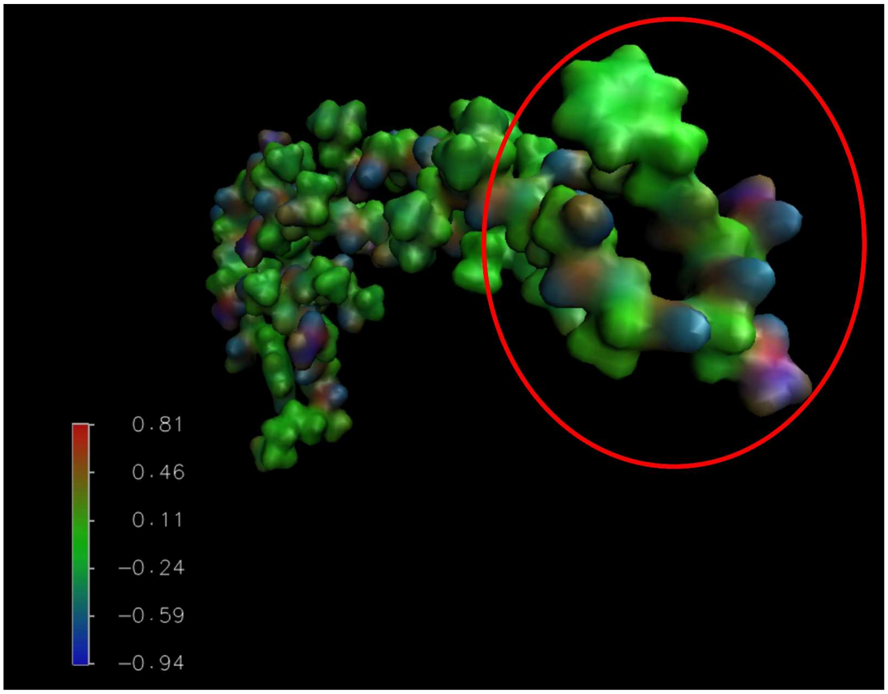

Figure S5. Simulated β-hairpin structure of the triply charged hIAPP is color coded by the partial charges on each atom. The red circle highlights the -HSSNN- residues at the loop of the β-hairpin, forming a negatively charged pocket that offers a potential coordination site for the Cu(II) ions.[1] The simulated structure of the amylin ion was kindly provided by Professor Michael T. Bowers of the Department of Chemistry and Biochemistry at the University of California, Santa Barbara.

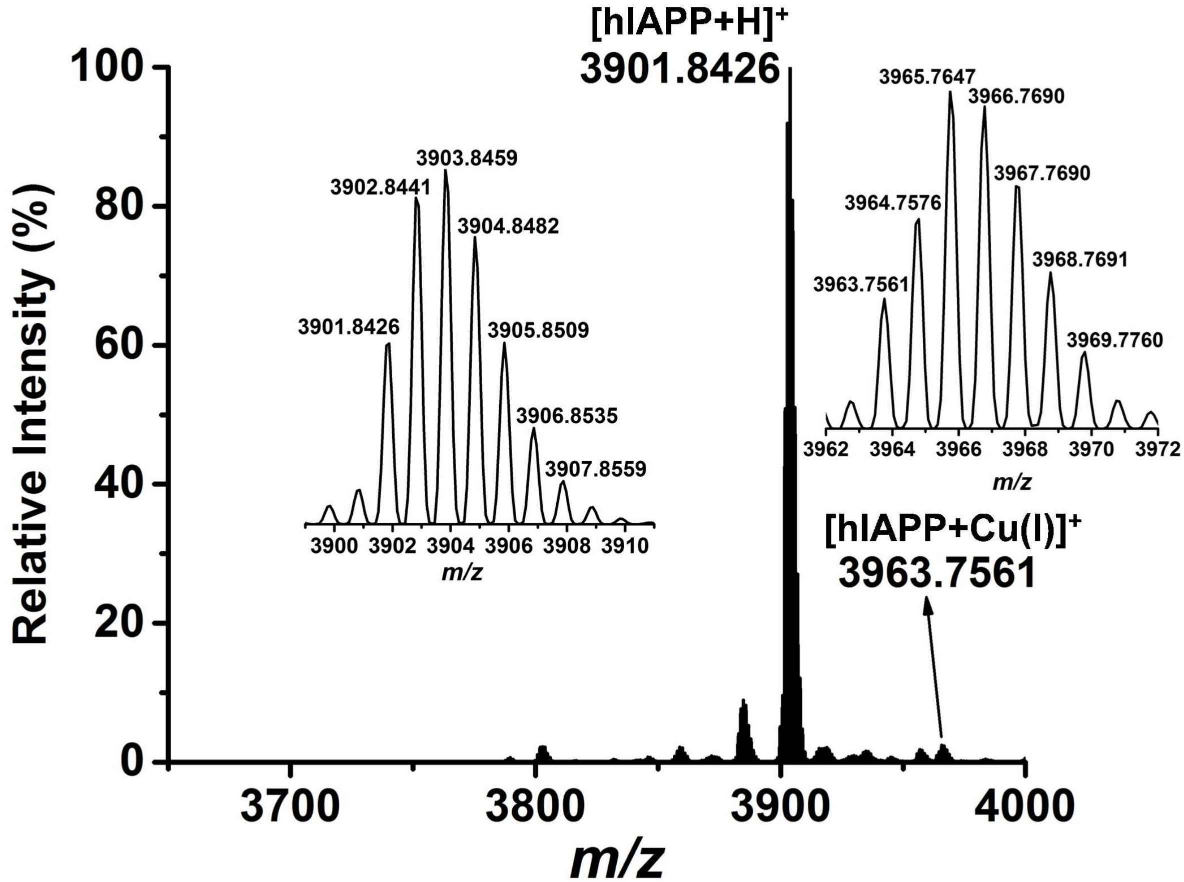

Figure S6. Mass spectrum of human amylin incubated with copper(II) obtained by MALDI-MS. The copper adduct peak shows that the Cu (II) is reduced to Cu(I), in agreement with a previous study.[2]

Table S1. Comparison of measured and calculated *m/z* and isotope distributions for (a) quadruply and (b) triply charged hIAPP-Cu adducts. The ions are distinguished based on the copper isotopes, ^{63}Cu and ^{65}Cu, carbon isotopes, ^{12}C and ^{13}C, and charge states, Cu(I) and Cu(II).

(a) 4+ hIAPP

	Measured hIAPP-Cu		Calculated hIAPP-Cu(I)				Calculated hIAPP-Cu(II)			
	m/z	**Relative intensity (%)**		***m/z***	**Δ *m/z* (mDa)**	**Relative intensity (%)**		***m/z***	**Δ *m/z* (mDa)**	**Relative intensity (%)**
M	991.459	35.3	${}^{12}C_{165}H_{264}N_{51}O_{55}S_2{}^{63}Cu(I)$	991.707	-248	36.4	${}^{12}C_{165}H_{263}N_{51}O_{55}S_2{}^{63}Cu(II)$	991.455	-4	36.4
M+1	991.706	72.2	${}^{12}C_{164}{}^{13}CH_{264}N_{51}O_{55}S_2{}^{63}Cu(I)$	991.957	-251	74.7	${}^{12}C_{165}{}^{13}CH_{263}N_{51}O_{55}S_2{}^{63}Cu(II)$	991.705	-1	74.7
M+2	991.954	97.9	${}^{12}C_{163}{}^{13}C_2H_{264}N_{51}O_{55}S_2{}^{63}Cu(I)$ and	992.207	-253	99.8	${}^{12}C_{163}{}^{13}C_2H_{263}N_{51}O_{55}S_2{}^{63}Cu(II)$ and	991.955	1	99.8
			${}^{12}C_{165}H_{264}N_{51}O_{55}S_2{}^{65}Cu(I)$	992.198	-244		${}^{12}C_{165}H_{263}N_{51}O_{55}S_2{}^{65}Cu(II)$	991.946	-8	
M+3	992.201	100.0	${}^{12}C_{162}{}^{13}C_3H_{264}N_{51}O_{55}S_2{}^{63}Cu(I)$ and	992.457	-256	100	${}^{12}C_{162}{}^{13}C_3H_{263}N_{51}O_{55}S_2{}^{63}Cu(II)$ and	992.205	4	100
			${}^{12}C_{164}{}^{13}CH_{264}N_{51}O_{55}S_2{}^{65}Cu(I)$	992.448	-247		${}^{12}C_{164}{}^{13}CH_{263}N_{51}O_{55}S_2{}^{65}Cu(II)$	992.196	-5	
M+4	992.463	80.1	${}^{12}C_{161}{}^{13}C_4H_{264}N_{51}O_{55}S_2{}^{63}Cu(I)$ and	992.707	-244	79.4	${}^{12}C_{161}{}^{13}C_4H_{263}N_{51}O_{55}S_2{}^{63}Cu(II)$ and	992.455	-8	79.4
			${}^{12}C_{163}{}^{13}C_2H_{264}N_{51}O_{55}S_2{}^{65}Cu(I)$	992.698	-235		${}^{12}C_{163}{}^{13}C_2H_{263}N_{51}O_{55}S_2{}^{65}Cu(II)$	992.446	-17	
M+5	992.710	50.6	${}^{12}C_{160}{}^{13}C_5H_{264}N_{51}O_{55}S_2{}^{63}Cu(I)$ and	992.957	-247	52	${}^{12}C_{160}{}^{13}C_5H_{263}N_{51}O_{55}S_2{}^{63}Cu(II)$ and	992.705	-5	52
			${}^{12}C_{162}{}^{13}C_3H_{264}N_{51}O_{55}S_2{}^{65}Cu(I)$	992.948	-238		${}^{12}C_{162}{}^{13}C_3H_{263}N_{51}O_{55}S_2{}^{65}Cu(II)$	992.696	-14	
M+6	992.958	27.2	${}^{12}C_{159}{}^{13}C_6H_{264}N_{51}O_{55}S_2{}^{63}Cu(I)$ and	993.207	-249	29	${}^{12}C_{159}{}^{13}C_6H_{263}N_{51}O_{55}S_2{}^{63}Cu(II)$ and	992.955	-3	29
			${}^{12}C_{161}{}^{13}C_4H_{264}N_{51}O_{55}S_2{}^{65}Cu(I)$	993.198	-240		${}^{12}C_{161}{}^{13}C_4H_{263}N_{51}O_{55}S_2{}^{65}Cu(II)$	992.946	-12	
M+7	993.205	10.6	${}^{12}C_{158}{}^{13}C_7H_{264}N_{51}O_{55}S_2{}^{63}Cu(I)$ and	993.457	-252	14.1	${}^{12}C_{158}{}^{13}C_7H_{263}N_{51}O_{55}S_2{}^{63}Cu(II)$ and	993.205	0	14.1
			${}^{12}C_{160}{}^{13}C_5H_{264}N_{51}O_{55}S_2{}^{65}Cu(I)$	993.448	-243		${}^{12}C_{160}{}^{13}C_5H_{263}N_{51}O_{55}S_2{}^{65}Cu(II)$	993.196	-9	

(b) 4+ hIAPP

	Measured hIAPP-Cu		Calculated hIAPP-Cu(I)				Calculated hIAPP-Cu(II)			
	m/z	Relative intensity (%)		*m/z*	Δ *m/z* (mDa)	Relative intensity (%)		*m/z*	Δ *m/z* (mDa)	Relative intensity (%)
M	1321.596	43.6	$^{12}C_{165}H_{263}N_{51}O_{55}S_{2}{}^{63}Cu(I)$	1321.940	-344	36.4	$^{12}C_{165}H_{262}N_{51}O_{55}S_{2}{}^{63}Cu(II)$	1321.604	8	36.5
M+1	1321.929	76.7	$^{12}C_{164}{}^{13}CH_{263}N_{51}O_{55}S_{2}{}^{63}Cu(I)$	1322.273	-344	74.7	$^{12}C_{165}{}^{13}CH_{262}N_{51}O_{55}S_{2}{}^{63}Cu(II)$	1321.937	8	74.7
M+2	1322.262	100.0	$^{12}C_{163}{}^{13}C_{2}H_{263}N_{51}O_{55}S_{2}{}^{63}Cu(I)$ and	1322.606	-344	99.8	$^{12}C_{163}{}^{13}C_{2}H_{262}N_{51}O_{55}S_{2}{}^{63}Cu(II)$ and	1322.270	8	99.8
			$^{12}C_{165}H_{263}N_{51}O_{55}S_{2}{}^{65}Cu(I)$	1322.598	-336		$^{12}C_{165}H_{262}N_{51}O_{55}S_{2}{}^{65}Cu(II)$	1322.261	-1	
M+3	1322.596	96.9	$^{12}C_{162}{}^{13}C_{3}H_{263}N_{51}O_{55}S_{2}{}^{63}Cu(I)$ and	1322.940	-344	100.0	$^{12}C_{162}{}^{13}C_{3}H_{262}N_{51}O_{55}S_{2}{}^{63}Cu(II)$ and	1322.604	8	100.0
			$^{12}C_{164}{}^{13}CH_{263}N_{51}O_{55}S_{2}{}^{65}Cu(I)$	1322.931	-335		$^{12}C_{164}{}^{13}CH_{262}N_{51}O_{55}S_{2}{}^{65}Cu(II)$	1322.595	-1	
M+4	1322.929	79.3	$^{12}C_{161}{}^{13}C_{4}H_{263}N_{51}O_{55}S_{2}{}^{63}Cu(I)$ and	1323.273	-344	79.4	$^{12}C_{161}{}^{13}C_{4}H_{262}N_{51}O_{55}S_{2}{}^{63}Cu(II)$ and	1322.937	8	79.4
			$^{12}C_{163}{}^{13}C_{2}H_{263}N_{51}O_{55}S_{2}{}^{65}Cu(I)$	1323.264	-335		$^{12}C_{163}{}^{13}C_{2}H_{262}N_{51}O_{55}S_{2}{}^{65}Cu(II)$	1322.928	-1	
M+5	1323.263	49.1	$^{12}C_{160}{}^{13}C_{5}H_{263}N_{51}O_{55}S_{2}{}^{63}Cu(I)$ and	1323.606	-343	52.0	$^{12}C_{160}{}^{13}C_{5}H_{262}N_{51}O_{55}S_{2}{}^{63}Cu(II)$ and	1323.270	7	52.0
			$^{12}C_{162}{}^{13}C_{3}H_{263}N_{51}O_{55}S_{2}{}^{65}Cu(I)$	1323.598	-335		$^{12}C_{162}{}^{13}C_{3}H_{262}N_{51}O_{55}S_{2}{}^{65}Cu(II)$	1323.261	-2	
M+6	1323.596	25.6	$^{12}C_{159}{}^{13}C_{6}H_{263}N_{51}O_{55}S_{2}{}^{63}Cu(I)$ and	1323.940	-344	29.0	$^{12}C_{159}{}^{13}C_{6}H_{262}N_{51}O_{55}S_{2}{}^{63}Cu(II)$ and	1323.604	8	29.0
			$^{12}C_{161}{}^{13}C_{4}H_{263}N_{51}O_{55}S_{2}{}^{65}Cu(I)$	1323.931	-335		$^{12}C_{161}{}^{13}C_{4}H_{262}N_{51}O_{55}S_{2}{}^{65}Cu(II)$	1323.595	-1	
M+7	1323.930	11.3	$^{12}C_{158}{}^{13}C_{7}H_{263}N_{51}O_{55}S_{2}{}^{63}Cu(I)$ and	1324.273	-343	14.1	$^{12}C_{158}{}^{13}C_{7}H_{262}N_{51}O_{55}S_{2}{}^{63}Cu(II)$ and	1323.937	7	14.1
			$^{12}C_{160}{}^{13}C_{5}H_{263}N_{51}O_{55}S_{2}{}^{65}Cu(I)$	1324.264	-334		$^{12}C_{160}{}^{13}C_{5}H_{262}N_{51}O_{55}S_{2}{}^{65}Cu(II)$	1323.928	-2	

Table S2. Measured 5+ hIAPP dimer collision cross sections (CCS_{meas}) are compared to the dimer CCS_{est} estimated from the CCS_{meas} of 4+ hIAPP monomers using the isotropic selfassembly model (see text).

	(4+) Monomer CCS_{meas} (Å^2)	**(5+) Dimer CCS_{meas} (Å^2)**	**Dimer CCS_{est} (Å^2)**	**Δ Dimer CCS (%)**
Conformer 1	594.2	Not detected	943.2	N/A
Conformer 2	683.8	Not detected	1085.5	N/A
Conformer 3	747.8	1139.6	1187.1	4.0

REFERENCES

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(2) Zhang, J.; Frankevich, V.; Knochenmuss, R.; Friess, S. D.; Zenobi, R. *J. Am. Soc. Mass Spectrom.* 2003, *14*, 42-50.