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Small^{Micro}

Supporting Information

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Nanoscale Heterogeneity of the Molecular Structure of Individual hIAPP Amyloid Fibrils Revealed with Tip-Enhanced Raman Spectroscopy

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Nanoscale heterogeneity of the molecular structure of individual hIAPP amyloid fibrils

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Figure S1. STEM images of hIAPP amyloid fibrils formed in bulk solution. Fibrils were formed in a) 50 mM phosphate buffer at pH=2.0; b) 5 mM phosphate buffer at pH=7.8. Scale bars are 500 nm.



Figure S2. AFM topography images of hIAPP fibrils formed in bulk solution and deposited and dried on a mica surface. Fibrils were formed in a) 50 mM phosphate buffer at pH=2.0; b) 5 mM phosphate buffer at pH=7.8. Scale bars are 1 μ m and AFM height calibration bar is 20 nm.



Figure S3. Spectra showing examples of amide I bands assigned to 1) unordered or α -helical structures; 2) β -sheet structures; 3) mixed structures or 4) suppressed amide I band. 5) Reference spectrum measured on glass.

Table S1: Vibrational modes of amide I structure bands, amino acid side chains and functional groups.^[1-4]

	Wavenumber
Unordered/α-helical	$1630-1655 \text{ cm}^{-1}$
β-sheet	$1660-1680 \text{ cm}^{-1}$
Histidine (His)	1494 cm^{-1} , 1331 cm $^{-1}$, 1183 cm $^{-1}$

Phenylalanine (Phe)	1038 cm^{-1} , 1010 cm $^{-1}$
Tyrosine (Tyr)	856 cm^{-1} , 826 cm^{-1}
Cysteine (Cys)	803 cm^{-1} , 767 cm $^{-1}$, 745 cm $^{-1}$, 692 cm $^{-1}$, 674 cm $^{-1}$, 668 cm $^{-1}$
Amide (NH_2^+/NH_3^+)	$1144 \text{ cm}^{-1}, 1080 \text{ cm}^{-1}$
Carbonyl (C=O)	$1660-1705 \text{ cm}^{-1}, 1400 \text{ cm}^{-1}$





(black).

Table S2. Peak fitting results of A	TR/FT-IR spectra for hIAPP	fibrils formed at pH=2.0.
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Peak position	Relative area	Assignment
1621 cm^{-1}	30%	β-sheet
1626 cm ⁻¹	36%	β-sheet
1661 cm ⁻¹	28%	β-turn
1677 cm ⁻¹	6%	β-sheet

Table S3. Peak fitting results of ATR/FT-IR spectra for hIAPP fibrils formed at pH=7.8.

Peak position	Relative area	Assignment
1626 cm ⁻¹	59%	β-sheet
1660 cm^{-1}	32%	β-turn
1675 cm^{-1}	9%	β-sheet



Figure S5. Relative propensity plots of amino acid residues for unordered/ α -helical structure (blue), β -sheets (red), mixed structures (purple) and suppressed amide I bands (green) for hIAPP fibrils formed a) in bulk at pH=2.0; b) in bulk at pH=7.8; c) at a lipid interface.

	Bulk fibrils pH=2.0	Bulk fibrils pH=7.8	Fibrils at lipid monolayer
Unordered/a	49%	77%	68%
β-sheet	36%	7%	11%
Mixed	15%	16%	21%
His	2%	8%	11%
Phe	18%	11%	6%
Tyr	6%	11%	7%
Cys	23%	17%	21%
	50/	120/	*
C=0	3%	12%0	-15
NH_{2}^{+}/NH_{3}^{+}	7%	11%	14%

Table S4. Overview of measured percentages of structure and amino acid residues.

* COOH/COO was not analyzed in samples containing lipids, because of the overlap of bands.

Table S5. Overview of the number of active amide I and suppressed bands and measuredpercentages of structure and amino acid residues per grid for bulk fibrils formed at pH=2.0.

	Grid 1	Grid 2	Grid 3	Grid 4	Grid 5	Grid 6
# active amide I	34	52	61	99	25	8
# suppressed	13	4	11	0	10	6
Unordered/α	85%	63%	10%	39%	96%	75%
β-sheet	0%	17%	62%	53%	0%	13%
Mixed	15%	19%	28%	8%	4%	13%
His	2%	4%	3%	0%	6%	0%
Phe	2%	11%	11%	28%	40%	0%
Tyr	4%	9%	0%	0%	14%	57%
Cys	28%	73%	17%	4%	3%	21%
С=О	15%	4%	7%	0%	3%	7%

NH2 ⁺ /NH3 ⁺	19%	4%	11%	0%	9%	0%

	Grid 1	Grid 2	Grid 3	Grid 4	Grid 5	Grid 6	Grid 7
# active amide I	16	36	46	46	4	35	21
# suppressed	10	16	19	10	4	0	2
Unordered/a	69%	89%	89%	33%	50%	100%	100%
β-sheet	6%	3%	2%	22%	50%	0%	0%
Mixed	25%	8%	9%	46%	0%	0%	0%
His	4%	15%	11%	4%	13%	0%	4%
Phe	12%	13%	11%	23%	0%	0%	0%
Tyr	8%	6%	5%	2%	88%	29%	17%
Cys	8%	6%	9%	16%	50%	23%	61%
С=О	0%	13%	25%	9%	50%	3%	0%
NH2 ⁺ /NH3 ⁺	8%	15%	11%	16%	0%	3%	9%

Table S6. Overview of the number of active amide I and suppressed bands and measured percentages of structure and amino acid residues per grid for bulk fibrils formed at pH=7.8.

Table S7. Overview of the number of active amide I and suppressed bands and measured percentages of structure and amino acid residues per grid for fibrils formed at the lipid interface.

	Grid 1	Grid 2	Grid 3	Grid 4
# active amide I	39	31	15	53
# suppressed	28	30	13	78
Unordered/α	64%	77%	67%	66%
β-sheet	13%	10%	7%	11%
Mixed	23%	13%	27%	23%
His	12%	8%	14%	12%
Phe	10%	8%	11%	1%

Tyr	9%	5%	11%	7%
Cys	12%	16%	11%	30%
NH ₂ ⁺ /NH ₃ ⁺	15%	21%	7%	11%
Lipids	25%	8%	7%	11%

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