Supplementary Information

Fractal self-assembly and aggregation of human amylin

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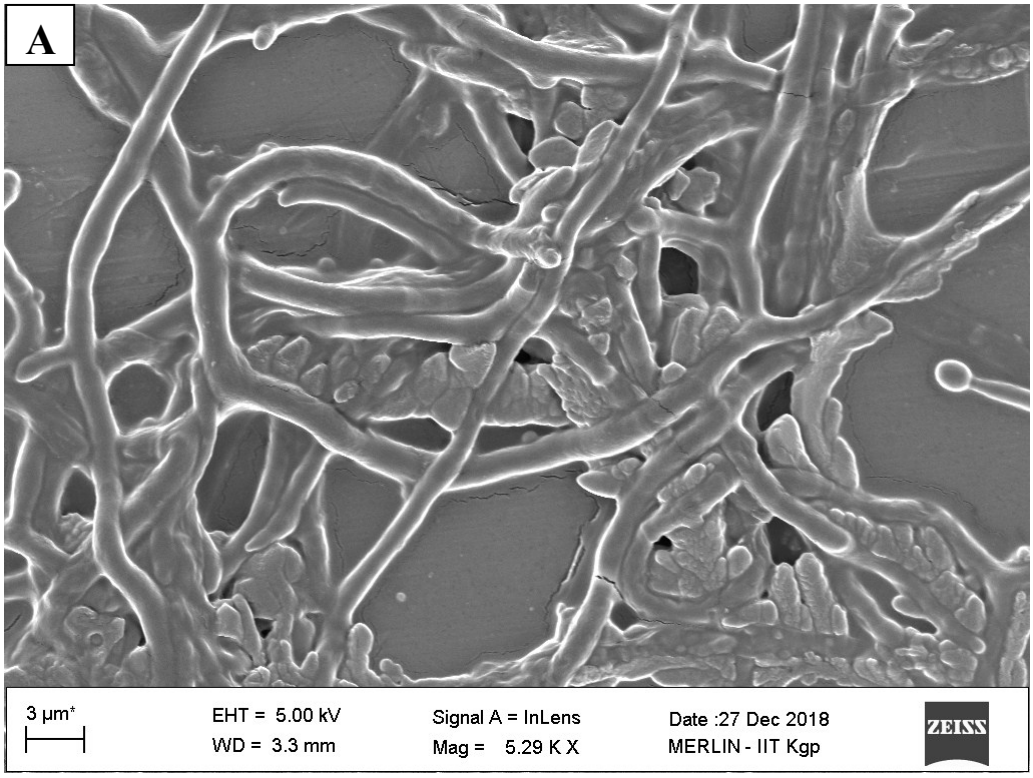
S1:

The freshly prepared amylin solution was kept at pH 6.5±0.1 and at a fixed temperature of 25.0±0.5 ºC for 15 days to observe the matured fibrils. These matured fibrils were observed through SEM (Fig S1 (A)). The solution was sonicated for 10 minutes, and when drop-casted on the glass substrate, fractal-like morphologies along with the fibrils were observed (shown in Fig. S1(B)). The solution containing matured fibrils were sonicated for 30 minutes, and fractal-like morphologies along with fibrils were observed, shown in Fig. S1(C)).



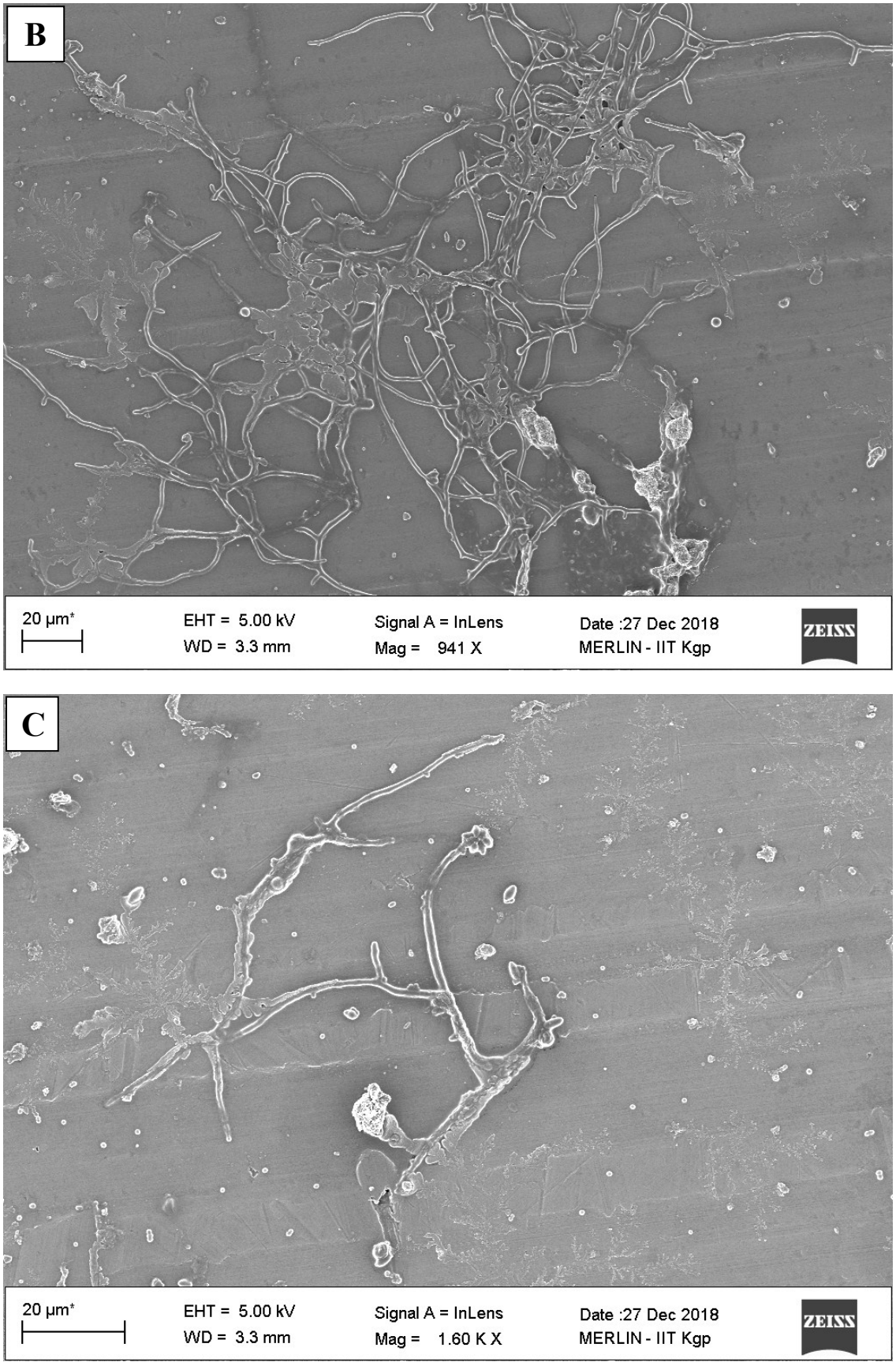


Fig. S1. The SEM images of the solution containing- (A) matured fibrils, (B) matured fibrils after 10 min sonication along with fractal-like structure, and (C) similar fractal-like structures with smaller fibrils of the solution after 30 min sonication.

S2:

The blank surface was obtained for DI water at pH 6.5±0.1, as shown in Fig S2 (A); and fractal-like morphologies were obtained from PBS through optical microscope shown in Fig S2 (B). The fractal dimension of the fractal structure formed from PBS was 1.86±0.05. The compositions of the used PBS (1X) includes NaCl (137 mM), KCl (2.7 mM), Na_2HPO_4 (10 mM) and KH_2PO_4 (1.8 mM). The pH of the prepared PBS (1X) was 7.3±0.1, but the pH of the final protein solution in PBS buffer was at pH 6.5±0.1, and the experiments were performed at this pH.

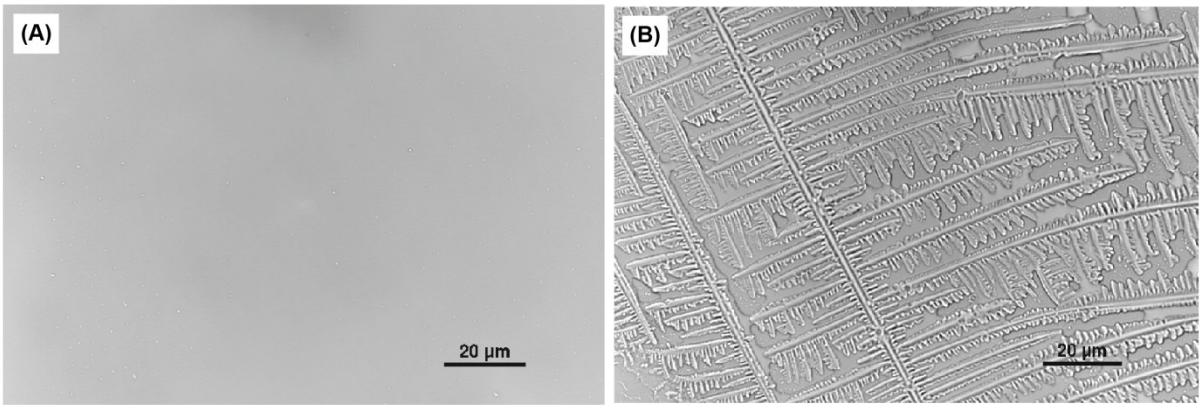
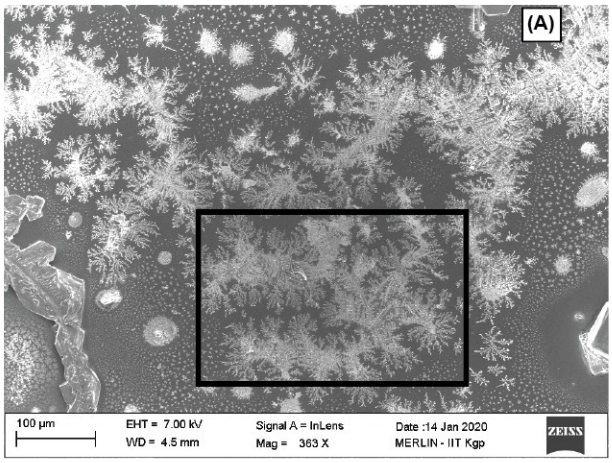
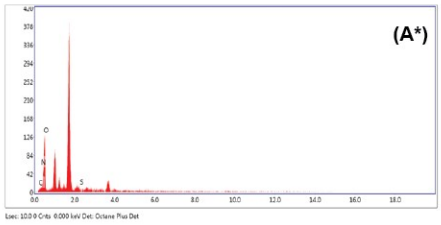


Fig. S2. The optical microscopy images of -(A) DI water and (B) PBS (1X).

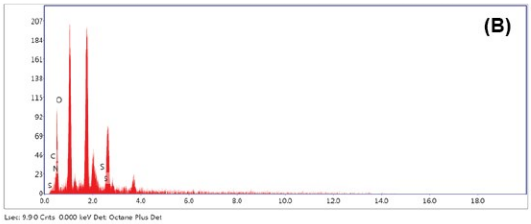
S3:

The EDAX analysis was done on SEM images obtained for amylin in PBS buffer at ~1µM concentration at pH 6.5±0.1 (shown in Fig S3 (A-B)) and pH 2.5±0.1 (shown in Fig S3 (C)). The significant presence of both nitrogen and sulphur at pH 6.5±0.1 and 2.5±0.1 confirmed the presence of amylin protein in the fractal morphologies.

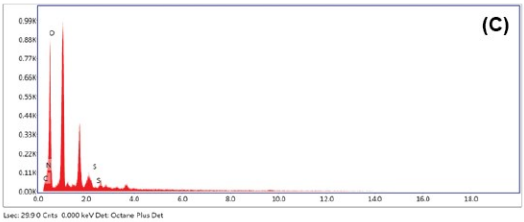




Element	Weight %	Atomic %	Net Int.	Error %
C K	7.10	9.15	11.27	24.18
N K	17.79	19.69	29.55	17.94
O K	71.59	69.57	192.34	10.32
S K	3.32	1.59	20.93	38.86



Element	Weight %	Atomic %	Net Int.	Error %
C K	7.47	9.85	8.87	29.10
N K	18.20	20.49	24.05	19.37
O K	67.24	66.27	154.19	11.32
S K	7.09	3.39	41.39	5.93



Element	Weight %	Atomic %	Net Int.	Error %
C K	5.28	6.78	19.22	12.02
N K	14.38	16.22	59.25	10.08
O K	76.52	75.11	585.25	8.28
S K	3.82	1.89	62.73	6.23

Fig. S3. The SEM image of amylin in PBS buffer at ~1µM concentration at (A) pH 6.5±0.1 and (A*) shows the result of the EDAX analysis of the selected area shown with black rectangles in (A); (B) The EDAX analysis of Figure 4 (B) at pH 6.5±0.1 and (C) The EDAX analysis of Figure 4 (C) at pH 2.5±0.1.

S4:

The Hydropathy and helical wheel plot of amylin

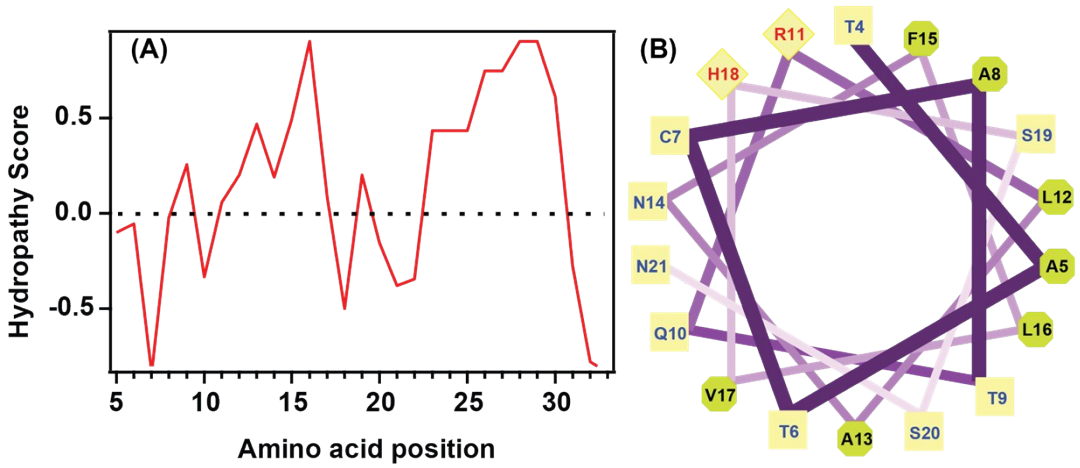


Fig. S4. (A) The hydropathy plot for human amylin reveals the presence of three major hydrophobic patches which may drive the fractal self-assembly and aggregation of human amylin and, (B) the helical wheel plot, indicating the unbiased distribution of hydrophobic residues (green color) over the helix.

The PROTSCALE software (http://us.expasy.org/tools/protscale.html) of Expert Protein Analysis system, Swiss Institute of Bioinformatics, Basel was used for hydropathy calculations on the primary structure of human amylin. The Kyte-Doolittle amino acid scale[1] with a window size of 9 amino acids and a linear weight-variation model was used for the calculations. The pepwheel software (http://www.bioinformatics.nl/cgi-bin/emboss/pepwheel) was used for revealing the positions of the hydrophobic residues on the human amylin (4-21 residues) having the helical secondary structure.

S5:

Docked structures

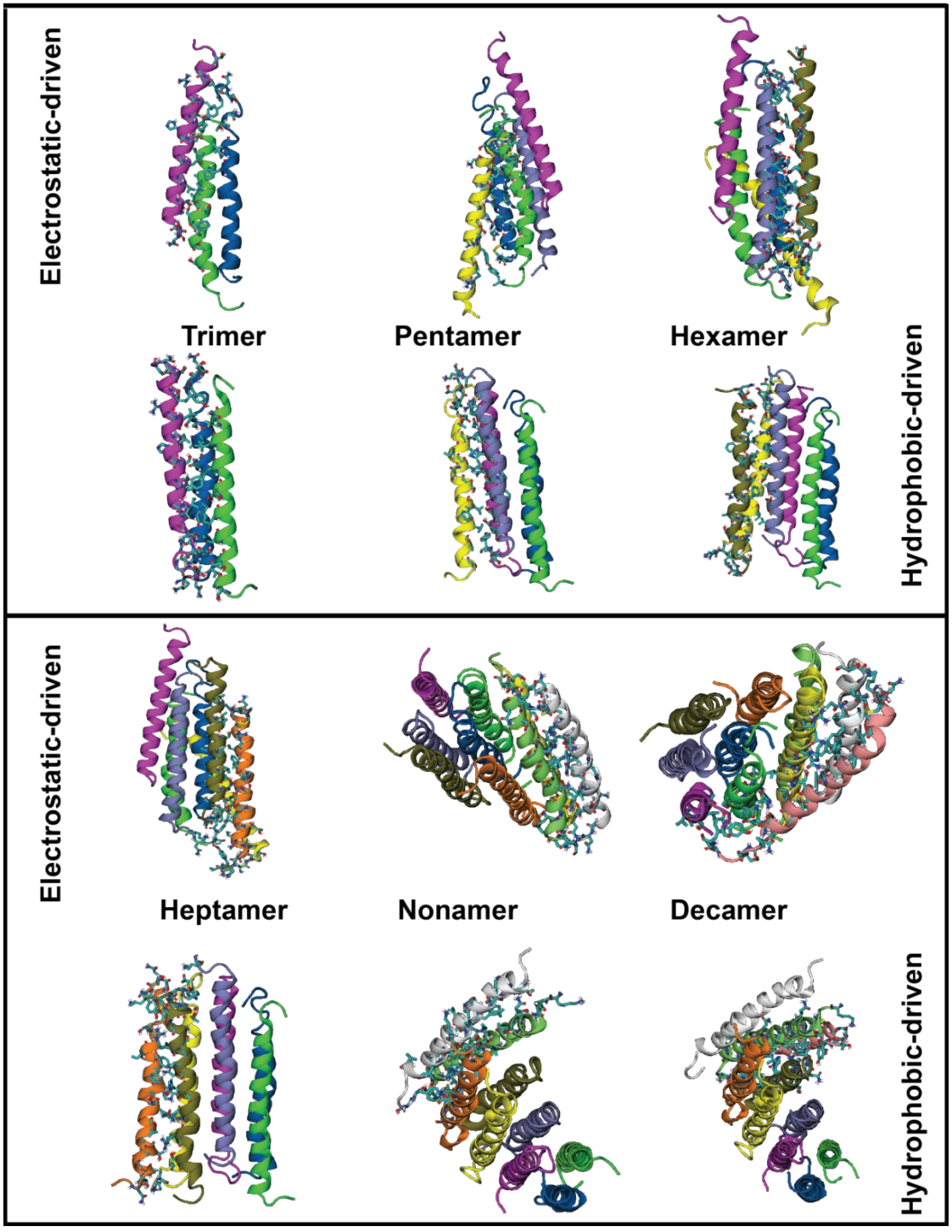


Fig. S5. The electrostatics-driven and the hydrophobic-driven docked structures of human amylin (trimer, pentamer, hexamer, heptamer, nonamer and decamer) obtained by docking the solution NMR structure of the α-helix structure of human amylin bound with micelle[2] (PDB ID: 2KB8) using ClusPro[3] web server.

S6:

Interface residues for electrostatic-driven docking

Table S6. The electrostatics and the hydrophobic residues in the interface of the docked structures obtained from electrostatic-driven docking using ClusPro server. The columns with alphabets represent the primary sequence of human amylin. The residues in green color are the polar/ionic residues and the residues in blue color are the hydrophobic residues. The polar/ionic and the hydrophobic residues, which are not colored, were not in the interface and were used to calculate the percentage of residues on the solvent-accessible surface area (SASA) of the docked structures. The columns containing the numbers represented the stage of the docking when that particular residue was included in the interface.

K	1	K	2	K		K	4	K	7	K		K	7	K	8	K	9	K	10
C	1	C	8	C		C	6	C	7	C		C	7	C	8	C	9	C	10
N	1	N	2	N		N		N	9	N		N	7	N	9	N	9	N	10
T	1	T	2	T	3	T	4	T	7	T	6	T	7	T		T	9	T	10
A	3	A	3	A		A		A	9	A		A		A		A		A	
T	5	T	5	T		T	6	T	9	T		T		T		T		T	
C	1	C	8	C	3	C	4	C	7	C	6	C	7	C	8	C	9	C	10
A	1	A	2	A	3	A	4	A	5	A	6	A	7	A	8	A	9	A	10
T	3	T	3	T	4	T		T	7	T		T	8	T		T		T	
Q	4	Q	5	Q		Q	6	Q	9	Q		Q		Q	9	Q		Q	
R	1	R	2	R	3	R	4	R	5	R	6	R	7	R	8	R	9	R	
L	1	L	2	L	3	L	4	L	5	L	6	L	7	L	8	L	9	L	10
A	5	A	3	A	4	A		A	8	A	7	A	8	A	9	A		A	
N	4	N	5	N		N	6	N	9	N	6	N		N	8	N	9	N	
F	1	F	2	F	3	F	4	F	5	F	6	F	7	F	8	F	9	F	10
L	1	L	2	L	3	L	4	L	5	L	6	L	7	L	8	L	10	L	10
V	5	V	3	V	4	V		V	8	V	7	V	8	V	9	V		V	
H	1	H	2	H	3	H	4	H	5	H	6	H	7	H	8	H	9	H	10
S	1	S	2	S	3	S	4	S	5	S	6	S	7	S	8	S	9	S	10
S	3	S	3	S	4	S	4	S	8	S	7	S	7	S	8	S	10	S	
N	5	N	3	N		N	6	N	9	N		N	8	N	9	N	9	N	
N	1	N	2	N	3	N	4	N	5	N	6	N	7	N	8	N	9	N	10
F	1	F	2	F	3	F	4	F	5	F	6	F	7	F	8	F	9	F	10
G	3	G	3	G	4	G		G	8	G		G	7	G		G	10	G	
A	7	A	3	A	10	A	6	A	10	A	6	A	8	A	8	A		A	
I	1	I	2	I	3	I	4	I	5	I	6	I	7	I	8	I	9	I	10
L	2	L	2	L	3	L	4	L	5	L	6	L	7	L	8	L	9	L	10
S	5	S	3	S		S		S	8	S		S	7	S	9	S		S	
S	1	S	2	S	10	S	6	S	5	S	6	S		S	8	S	9	S	10
T	1	T	2	T	3	T	4	T	5	T	6	T	7	T	8	T	9	T	10
N	2	N	2	N	4	N	4	N	5	N	7	N	7	N	8	N	9	N	10
V	1	V	2	V	10	V	6	V	9	V	6	V	7	V	9	V	10	V	10
G		G	2	G		G	6	G		G	6	G	7	G	8	G		G	10
S	2	S	2	S	3	S	4	S	5	S	6	S	7	S	8	S	9	S	10
N	2	N	2	N	3	N	4	N	5	N	6	N	7	N	8	N	9	N	
T	2	T	2	T	3	T	4	T	5	T	6	T	7	T	8	T	9	T	10
Y	1	Y	2	Y	3	Y	4	Y	5	Y	6	Y	7	Y	8	Y	9	Y	10

S7:

Interface residues for hydrophobic-driven docking

Table S7. The electrostatics and the hydrophobic residues in the interface of the docked structures obtained using hydrophobic-driven docking in ClusPro server. The columns with alphabets represent the primary sequence of human amylin. The residues in green color are the polar/ionic residues and the residues in blue color are the hydrophobic residues. The polar/ionic and the hydrophobic residues which are not colored were not in the interface and were used to calculate the percentage of residues on the SASA of the docked structures. The columns containing the numbers represented the stage of the docking when that particular residue was included in the interface.

K	2	K		K	3	K	5	K	6	K	7	K	9	K	9	K		K	
C		C		C	5	C	5	C	6	C	7	C	9	C	9	C		C	
N	1	N		N	3	N		N	6	N	6	N	7	N	9	N		N	
T	1	T	2	T	3	T	4	T	6	T	6	T	7	T	10	T	9	T	10
A		A	3	A	3	A		A	5	A	6	A	7	A		A		A	10
T		T		T	4	T		T		T	6	T	7	T	9	T		T	
C	2	C	2	C		C	4	C	6	C	7	C	9	C	10	C		C	10
A	1	A	2	A	3	A	4	A	5	A	7	A	7	A	8	A	9	A	10
T		T	3	T	3	T		T	5	T	6	T	7	T	8	T	9	T	
Q		Q		Q	4	Q	5	Q		Q	10	Q	7	Q	9	Q		Q	
R	1	R	2	R	3	R	4	R	6	R	7	R	9	R	8	R	9	R	10
L	1	L	2	L	3	L	4	L	5	L	6	L	7	L	8	L	9	L	10
A		A	4	A	4	A		A	5	A	6	A	7	A	8	A	9	A	
N	2	N		N	5	N	5	N	7	N	7	N	9	N	10	N		N	10
F	1	F	2	F	3	F	4	F	5	F	6	F	8	F	8	F	9	F	10
L	1	L	2	L	3	L	4	L	5	L	6	L	7	L	8	L	9	L	10
V		V		V	5	V	6	V		V	6	V	7	V	9	V	9	V	
H	1	H		H	3	H	4	H	7	H	7	H	8	H	10	H	9	H	10
S	1	S	2	S	3	S	4	S	5	S	6	S	7	S	8	S	9	S	10
S		S	3	S	4	S		S	5	S	6	S	7	S	8	S	9	S	
N		N	4	N	5	N	6	N	7	N	8	N	7	N	9	N		N	10
N	1	N	2	N	3	N	5	N	5	N	6	N	8	N	8	N	9	N	10
F	1	F	2	F	3	F	4	F	5	F	6	F	7	F	8	F	9	F	10
G		G	4	G	5	G		G	5	G	10	G	7	G	9	G		G	
A	2	A	2	A	3	A	6	A	7	A	8	A	9	A	10	A		A	10
I	1	I	2	I	3	I	4	I	5	I	6	I	7	I	8	I	9	I	10
L	1	L	2	L	3	L	4	L	5	L	6	L	7	L	8	L	9	L	
S	2	S	4	S	4	S	6	S	5	S	8	S	7	S	9	S		S	10
S	1	S	2	S	3	S	4	S	7	S	6	S	7	S	10	S	9	S	10
T	1	T	2	T	3	T	4	T	5	T	6	T	7	T	8	T	9	T	10
N	1	N	2	N	3	N		N	5	N	6	N	7	N	8	N		N	
V	2	V		V	4	V	5	V	7	V	8	V	7	V	10	V	9	V	10
G	2	G	2	G	3	G		G	7	G	8	G		G	10	G		G	10
S	1	S	2	S	3	S	4	S	5	S	6	S	7	S	8	S		S	
N	1	N	2	N	3	N	4	N	5	N		N	7	N	8	N		N	10
T	1	T	2	T	3	T	4	T	5	T	6	T	7	T	8	T	9	T	10
Y	1	Y	2	Y	3	Y	4	Y	5	Y	6	Y	7	Y	8	Y	9	Y	10

References

1. J. Kyte and R. F. Doolittle, *Journal of molecular biology*, 1982, 157, 105-132.
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