Full length amylin oligomer aggregation: insights from molecular dynamics simulation study and implications for design of aggregation inhibitors

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Table 1S Summery of simulated system of full length amylin single layer oligomer aggregate and the corresponding pentamer mutants.

ID	Sheet organization	# peptide/water mol./Cl⁻	Box size	T, K	T, ns
<i>Wild type insulin aggregate oligomer of different size</i>					
WT(SH1-ST1)	Parallel (<i>sheet1, strands1</i>)	535/10170/2	87.49×87.49×87.49	330	40
WT(SH1-ST2)	Parallel (<i>sheet1, strands2</i>)	1070/10593/4	89.10×89.10×89.10	330	40
WT(SH1-ST3)	Parallel (<i>sheet1, strands3</i>)	1605/11169/6	91.24×91.24×91.24	330	40
WT(SH1-ST4)	Parallel (<i>sheet1, strands4</i>)	2140/11833/8	93.60×93.60×93.60	330	40
WT(SH1-ST5)	Parallel (<i>sheet1, strands5</i>)	2675/12311/10	95.08× 95.08×95.08	330	40
<i>Single point glycine mutant (chain B) of insulin aggregate oligomer (SH1-ST10)</i>					
2G	Parallel (<i>sheet1, strands5</i>)	2659/12312/10	95.06×95.06×95.06	330	40
1034GG	Parallel (<i>sheet1, strands5</i>)	2599/12335/10	95.13×95.13×95.13	330	40
1626GG	Parallel (<i>sheet1, strands5</i>)	2549/12333/10	95.09×95.09×95.09	330	40
252829PPP	Parallel (<i>sheet1, strands5</i>)	2719/12310/10	95.12×95.12×95.12	330	40

Table 2S Clustering metric of the wildtype clusters per system

System	# cluster	DBI	pSF	SSR/SST	Rank	Critical distance	Cluster size	Cluster occupancy (%)
SH1-ST1	5	0.98	6204.31	0.83	1	3.27	3219	64.4
					2	3.77	1179	23.6
					3	4.01	310	6.2
					4	3.42	173	3.5
					5	3.12	119	2.4
SH1-ST2	5	1.32	10218.81	0.89	1	3.01	1466	29.3
					2	2.26	1192	23.8
					3	1.76	901	18.0
					4	2.47	767	15.3
					5	2.94	674	13.5
SH1-ST3	5	1.08	27928.93	0.96	1	1.25	2183	43.7
					2	1.11	1311	26.2
					3	1.27	619	12.4
					4	0.86	566	11.3
					5	1.46	321	6.4
SH1-ST4	5	1.45	12524.12	0.90	1	0.78	2551	48.3
					2	0.76	1870	35.4
					3	0.91	494	9.3
					4	0.90	211	4
					5	0.99	160	3
SH1-ST5	5	1.88	1665.49	0.67	1	0.74	1870	37.4
					2	0.80	1630	32.6
					3	0.77	860	17.2
					4	0.86	324	6.5
					5	0.86	315	6.3

Table 3S Clustering metric of the wildtype clusters per system

System	# cluster	DBI	pSF	SSR/SST	Rank	Critical distance	Cluster size	Cluster occupancy (%)
2G	5	1.49	7760.10	0.85	1	2.06	2292	45.3
					2	1.82	982	19.4
					3	1.78	834	16.5
					4	1.67	504	10.0
					5	1.67	448	8.9
1034G	5	1.62	1239.15	0.91	1	1.62	2047	40.6
					2	1.57	1536	30.5
					3	1.69	675	13.4
					4	1.73	531	10.5
					5	1.53	250	5.0
1626G	5	1.37	3509.93	0.73	1	2.01	1742	33.9
					2	1.82	1484	28.8
					3	1.78	990	19.2
					4	1.60	842	16.4
					5	1.29	86	1.70
252829G	5	1.42	9400.26	0880	1	1.31	1147	23.0
					2	1.28	1089	21.8
					3	1.38	1034	20.7
					4	1.19	895	17.9
					5	1.53	828	16.6

Figure S1 Schematic drawing of the setup used for estimating the stability of the amylin oligomer on the addition of monomer during the longitudinal growth.

WT, pentamer (initial)

WT, Pentamer (37.4%)

WT, Pentamer (32.6%)

A

Mutant, 2CG (initial)

Mutant, 2CG (45.3%)

B

Mutant, 1034GG (initial) Mutant, 1034GG (40.6%)

Mutant, 1034GG (30.5%)

C

Mutant, 1636GG (initial) Mutant, 12626GG (33.9%)

Mutant, 12626GG (28.8%)

D

Mutant, 252829PPP (initial) Mutant, 252829PPP (33.9%)

Mutant, 252829PPP (33.9%)

Mutant, 252829PPP (33.9%)

E

Figure S2. The initial fibril structure and the respective most populated clusters representative structures of the studied pentamer single layer models of the wildtype amylin and its mutants: (A) Wildtype, (B) Mutant, 2CG, (C) Mutant, 1034GG, (D) Mutant, 1626GG and (E) Mutant, 252829PPP (with sequence of pramlintide). The initial fibril conformations are shown in blue and the most populated cluster with the corresponding percentage (refers to cluster occupancy) are shown in magenta.

A

B

C

D

E

Figure S3. Mean β -sheet content detected in the (a) monomer (b) dimer (c) trimer (d) tetramer and (e) pentamer at 330 K. The portion of the β -sheet is plotted for each sequence position. Values were averaged over the entire simulation time and over all chains present in the oligomers. A value of 100% denotes that a particular residue adopted an extended conformation in all chains of the respective oligomer over the entire simulation time.

Figure S4 Free energy profile for the amylin oligomers of the wildtype amylin. The free energy was calculated using MMGBSA.

Figure S5 Profile of $\Delta\Delta G$ (where $\Delta\Delta G = \Delta G_n - \Delta G_3$, $n=2,3,4,5$): against the number of chains of amylin single layer oligomer aggregate nucleation fibrillation. The SH1-ST3 being assumed to be critical nuclei for the aggregates growth. At higher number of chains the oligomer is stable and energetically favorable.

Figure S6 Schematic representation of the initial fibril structure and the respective the two layers of the structure of the well-ordered hydrophobic core of HET-s(218– 289) amyloid fibrils (left half). This fold would explain all currently available data and is compatible with the primary structure of FgHET-s(218–289) as well (right). Circles representing positively or negatively charged residues are colored blue or red, respectively; polar but uncharged residues are green and non-polar residues are white.