Supporting Information

Quantification of Protein Aggregation Rate and Quenching effect in amylin-inhibitors complex

Suparna Khatun[a], Anurag Singh[a], Debabrata Mandal[b], Amreesh Chandra[a,b] and Amar Nath Gupta[a]

[a]Department of Physics, Indian Institute of Technology Kharagpur-721302, India

[b]School of Nanoscience and Technology, Indian Institute of Technology Kharagpur-721302, India

S0. Sample purity

The purity of samples as reported by the high-performance liquid chromatography (HPLC) was 97% for amylin, 95% for A-β, 99% for resveratrol, 99.5% for curcumin. For citric acid, purity of 99.5% was bought from Sigma Aldrich.

S1. Hydrodynamic radius (R_H)

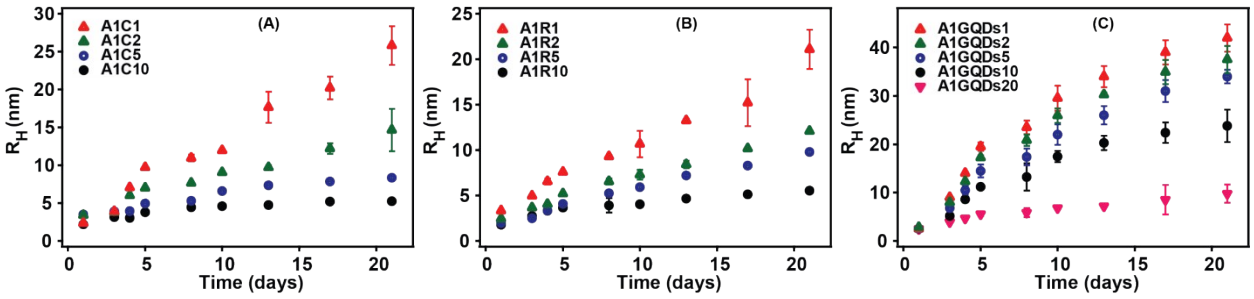


Fig S1. Hydrodynamic radius (R_H) of amylin-ligand complexes in PBS buffer at pH 6.5±0.1 as a function of time at temperature 25±0.5 °C for (A) amylin and curcumin complexes in the ratio A1C1, A1C2, A1C5 and A1C10 (B) amylin and resveratrol complexes in the ratio A1R1, A1R2, A1R5 and A1R10 (C) amylin and GQDs complexes in the ratio A1GQDs1, A1GQDs2, A1GQDs5, A1GQDs10 and A1GQDs20.

The hydrodynamic radius of the amylin-inhibitor complexes shown for infering the dose-dependent effect of the complexes on the formation of oligomers. It is clear that with increase in the concentration of the ligands, the hydrodynamic radius of the amylin-inhibitor complexes is decreasing, indicating that higher concentration of ligands facilitate the inhibition of amylin aggregation.

S2. Relative change in R_H for all the inhibitors

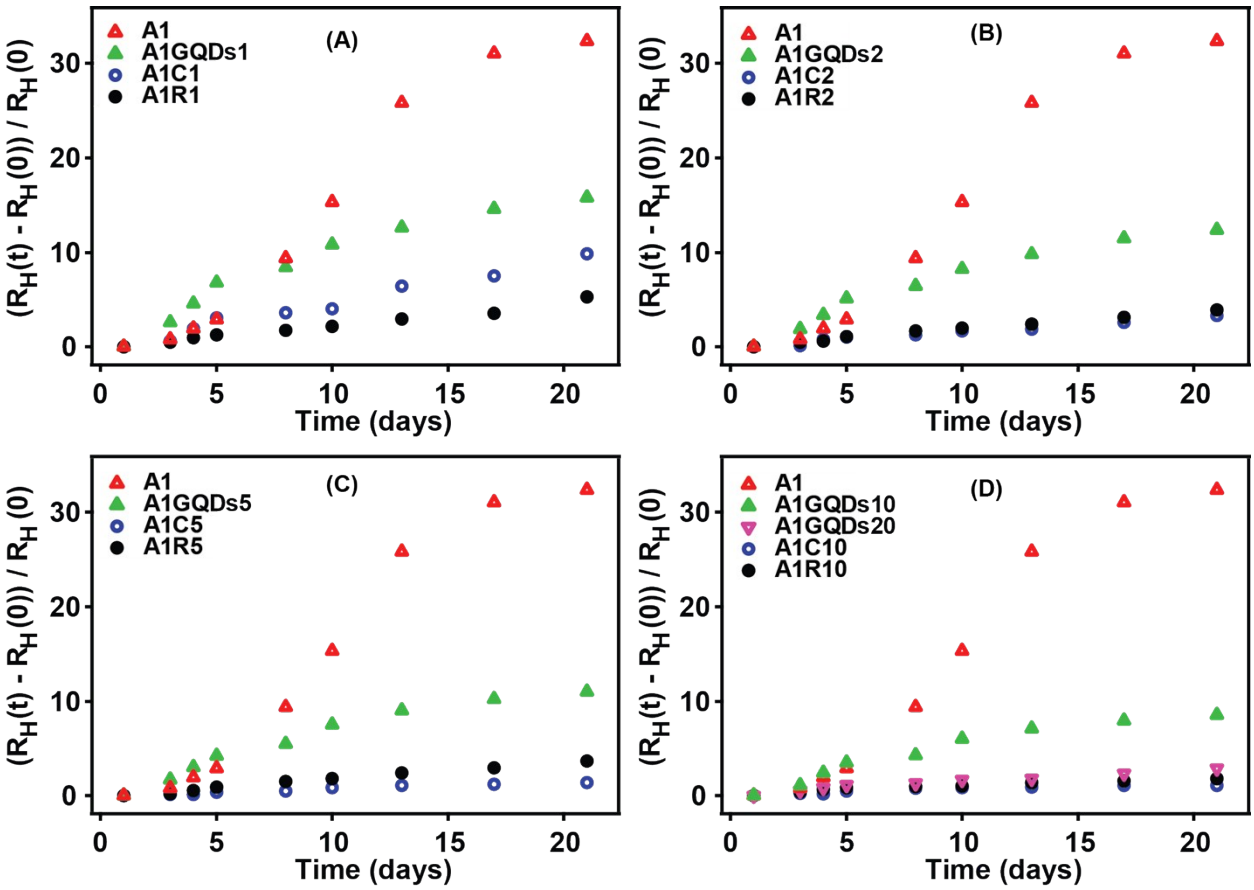


Fig S2. Changes in the hydrodynamic radius (R_H) of amylin-ligand complexes in PBS buffer at pH 6.5±0.1 as a function of time at temperature 25±0.5 ^{o}C for (A) bare amylin A1, A1GQDs1, A1C1 and A1R1 (B) bare amylin A1, A1GQDs2, A1C2 and A1R2 (C) bare amylin A1, A1GQDs5, A1C5 and A1R5 (D) bare amylin A1, A1GQDs10, A1GQDs20, A1C10 and A1R10.

The relative change in the hydrodynamic radius followed the sigmoidal behaviour for bare amylin and the amylin-inhibitor complexes. For initial stages of aggregation, the changes in the hydrodynamic radius for the amylin and GQDs complexes are greater than that of the bare amylin, indicating the promoting role of GQDs in amylin aggregation for initial stage of aggregation. With increase in time, the relative changes in the hydrodynamic radius are way smaller for amylin inhibitor complexes in comparison to the bare amylin, showing the capability of the used ligands for inhibition. Moreover, comparatively speaking, the changes in case of natural products are much smaller than that of the amylin and GQDs complexes, indicating that natural product derivatives are better inhibitors for amylin aggregation in comparison to GQDs under the experimental conditions.

S3. Rate constant for amylin-inhibitor complexes

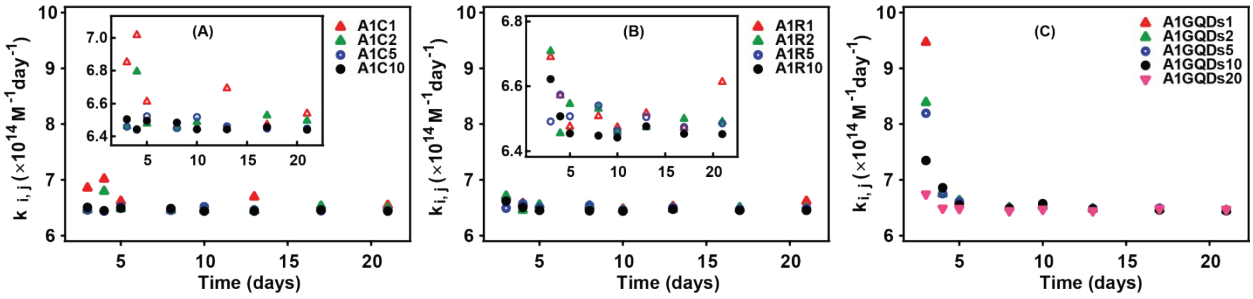


Fig S3. The rate constants for amylin-ligand complexes in PBS buffer at pH 6.5±0.1 as a function of time at temperature 25±0.5 °C for (A) amylin and curcumin complexes in the ratio A1C1, A1C2, A1C5 and A1C10 (B) amylin and resveratrol complexes in the ratio A1R1, A1R2, A1R5 and A1R10 (C) amylin and GQDs complexes in the ratio A1GQDs1, A1GQDs2, A1GQDs5, A1GQDs10 and A1GQDs20. The inset in the (A) and (B) panels are included to clearly show the variations in the rate constants for different amylin complexes with curcumin and resveratrol respectively.

Here, the rate constant for the same amylin-inhibitor complexes for different ligand concentrations are shown. In case of natural product derivatives, the rate constants are smaller at the initial stages itself and further decreases with increase in the ligand concentration, which is evident from the rate constants shown in the inset of Fig S3 (A) and (B). But, the same is not true for amylin-GQDs complexes. For amylin-GQDs complexes, the rate constants are higher at the initial stages of aggregation and further decrease with increase in the ligand concentration.

S4. Absorbance for A1GQDs10

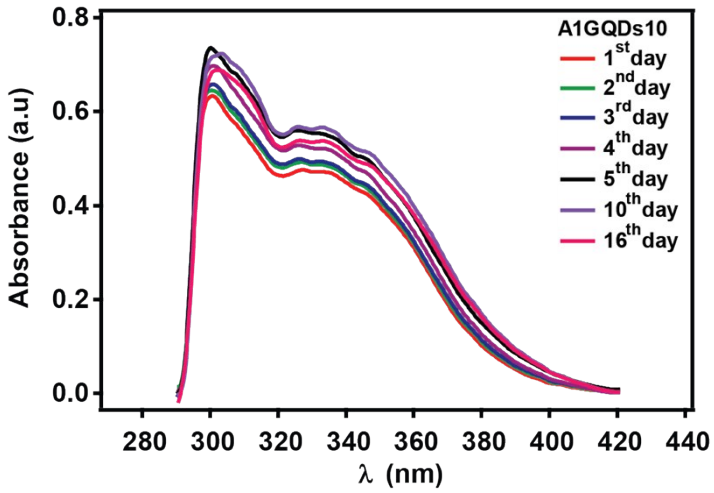


Fig S4. UV-Vis spectra from amylin and GQDs in molar ratio 1:10 in PBS buffer at pH=6.5±0.1 and at T=25±0.5 °C as a function of time.

S5. TEM Images

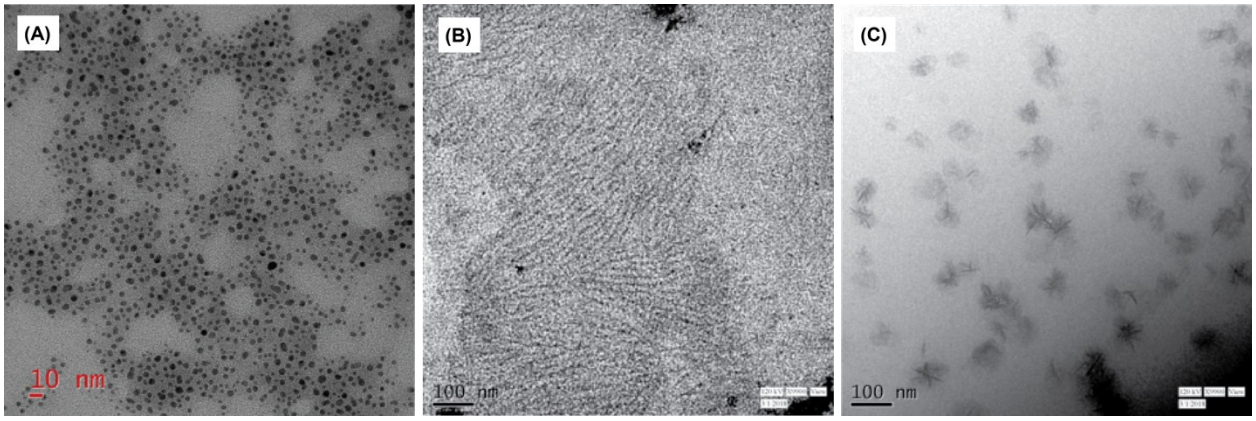
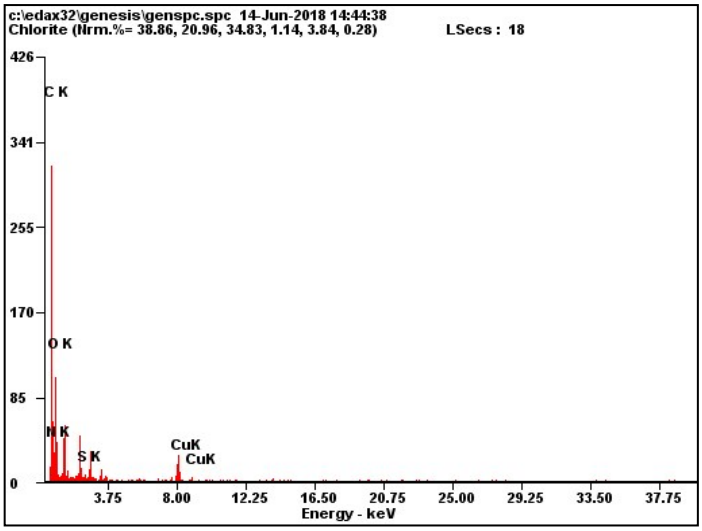


Fig S5. TEM images of amylin-inhibitors complex (A) Amylin and Curcumin (1:5) with average size 5-8 nm. (B) Amylin and GQDs (1:10) with thinner and shorter fibrils compare to amylin alone. (C) Fragment of fibrils observed in molar ratio of Amylin and Resveratrol (1:5).

S6. EDAX measurement for TEM images

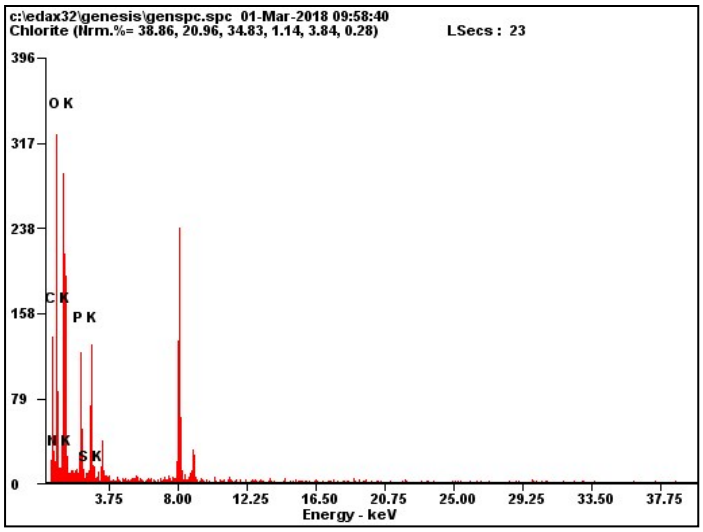
(a) Amylin (A1)



Element	Wt %	At %
C K	56.14	61.72
N K	17.85	16.83
O K	25.97	21.44
S K	00.03	00.01
CuK	00.02	00.00

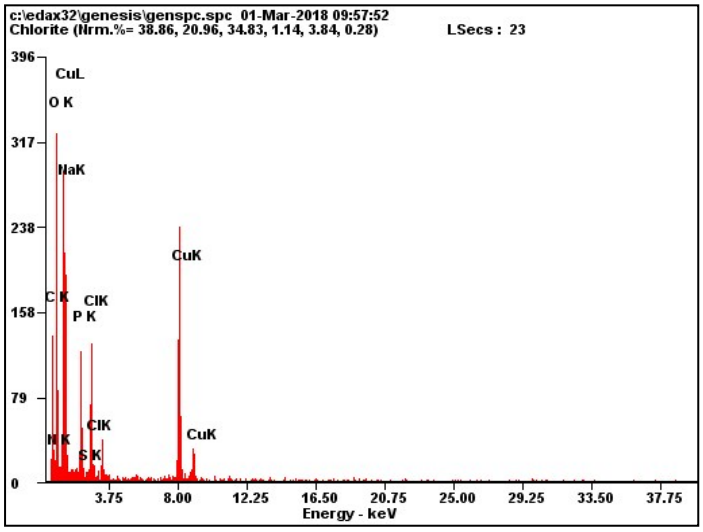
(b) Amylin-Curcumin complexes

(i) A1C5



Element	Wt %	At %
C K	36.95	43.64
N K	08.76	08.87
O K	52.75	46.78
P K	01.52	00.70
S K	00.03	00.01

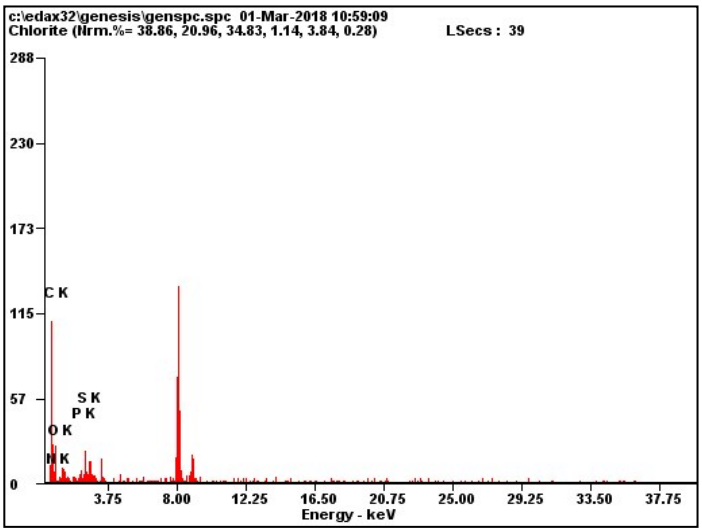
(ii) A1C10



Element	Wt %	At %
C K	37.38	45.79
N K	07.25	07.62
O K	41.58	38.24
NaK	11.45	07.33
P K	01.39	00.66
S K	00.02	00.01
ClK	00.75	00.31
CuK	00.18	00.04

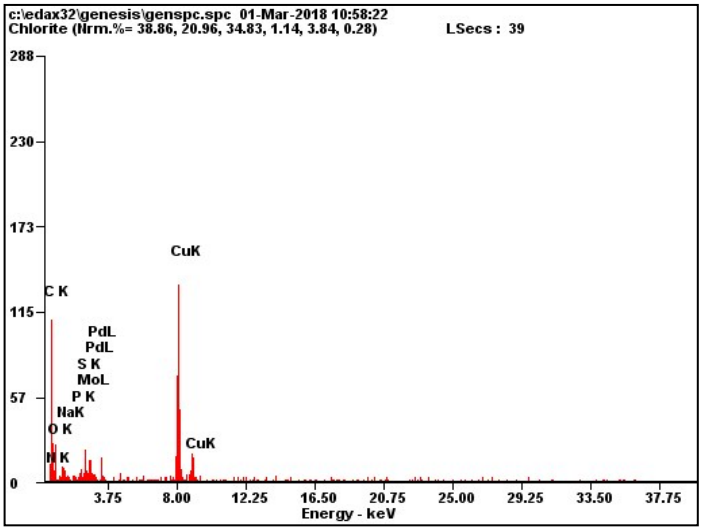
(c) Amylin-Resveratrol complexes

(i) A1R5



Element	Wt %	At %
C K	64.70	69.92
N K	15.22	14.10
O K	19.31	15.67
P K	00.25	00.11
S K	00.51	00.21

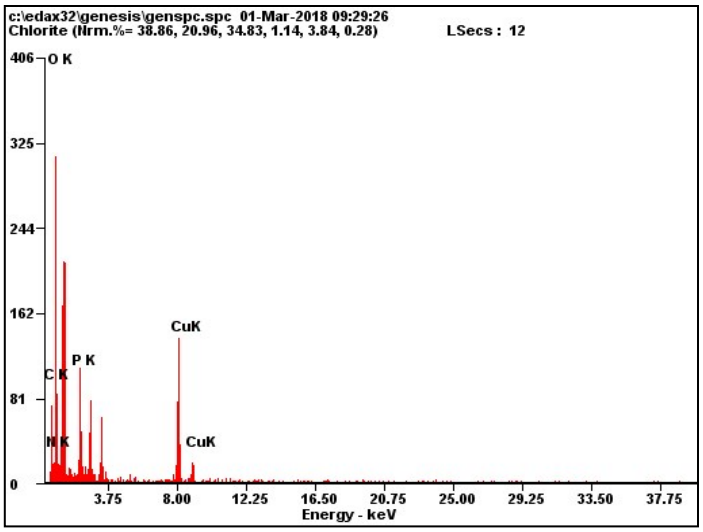
(ii) A1R10



Element	Wt %	At %
C K	64.47	70.64
N K	14.17	13.32
O K	17.77	14.61
NaK	01.82	01.04
P K	00.25	00.11
MoL	00.78	00.11
S K	00.21	00.09
PdL	00.19	00.02
CuK	00.35	00.07

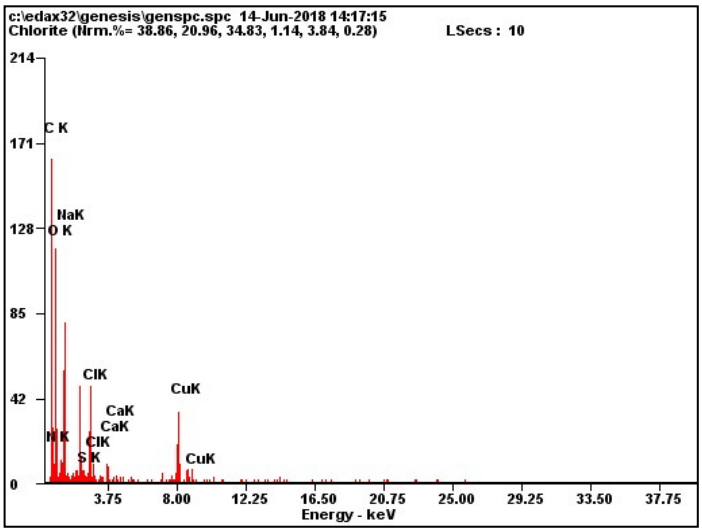
(d) Amylin-GQDs complexes

(i)A1GQDs10



Element	Wt %	At %
C K	27.07	33.09
N K	07.50	07.86
O K	63.33	58.10
P K	01.92	00.91
CuK	00.17	00.04

(ii) A1GQDs20



Element	Wt %	At %
C K	50.87	58.67
N K	09.28	09.18
O K	31.34	27.13
NaK	08.07	04.86
S K	00.03	00.01
ClK	00.34	00.13
CaK	00.03	00.01
CuK	00.04	00.01

References

1. S. Jha, J. M. Snell, S. R. Sheftic, S. M. Patil, S. B. Daniels, F. W. Kolling and A. T. Alexandrescu, *Biochemistry*, 2014, **53**, 300-310.
2. Y. Dong, J. Shao, C. Chen, H. Li, R. Wang, Y. Chi, X. Lin and G. Chen, *Carbon*, 2012, **50**, 4738-4743.
3. W. Schärtl, *Light scattering from polymer solutions and nanoparticle dispersions*, Springer Science & Business Media, 2007.