Probing the interaction of nanorods with various aspect ratios and amyloid beta Protein

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Human plasma. Blood was withdrawn from 10-15 different volunteers and collected into 10 ml K2EDTA coated tubes (BD Bioscience). Plasma was prepared following the HUPO BBB SOP guidelines.[1] Briefly, immediately after blood collection, each tube was inverted ten times to ensure mixing of blood with the EDTA, and subsequently centrifuged for ten minutes at 1300 g at 4 ?C. Equal volumes of plasma from each donor were collected into a secondary 50ml falcon tube and then centrifuged at 2400g for 15 minutes at 4 ?C. Supernatant was collected (leaving approximately 10% of the volume in the secondary tube) and it was then aliquoted into 1ml cryovials and stored at -80?C until use. The whole procedure did not take more than three hours. Following this procedure the plasma protein concentration is estimated to be ~80g/L in agreement with the literature.[2,3]

When plasma was used for experiments, it was allowed to thaw at room temperature and centrifuged for 3 min at 16.2kRCF. Thawed plasma was never re-frozen or re-thawed. All data presented are obtained using plasma from one donation session. The blood donation procedure was approved by the Human Research Ethics committee at University College Dublin.

Preparation of hard corona coated nanomaterials. In order to simulate *in vitro* and *in vivo* mediums, 10% and 100% different concentrations of plasma were used, respectively. Different types of nanomaterials were incubated at both predetermined plasma concentrations; in the case of *in vitro* simulation, plasma solutions were diluted with PBS keeping the final concentration of nanoparticles constant and equal to 0.1 mg/ml and 1

mg/ml, respectively. Nanomaterials were allowed to incubate with the plasma solutions at 37°C for one hour. To obtain hard protein corona complexes, after the incubation in plasma, the samples were centrifuged to pellet the nanomaterial-protein complexes and separated from the supernatant plasma. The pellet was then resuspended in 500 μl of PBS and centrifuged again for 3 minutes at 20,000 g at 15°C to pellet the particle-protein complexes. The standard procedure consists of three washing-steps before resuspension of the final pellet to the desired concentration. This treatment allows us to get rid of the proteins with low affinity for the NP surface (the soft protein corona).

Thioflavin T (ThT) assay. Amyloid Beta ($A\beta_{42}$) with sequence of MDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA was used in this study.

In order to prevent the depletion of Aβ42 from solution by the adherence of Aβ42 to the plates' chamber walls, the plates were coated by poly-L-Lysine (PLL). Briefly, the PLL was diluted to 15 μg/ml using Millipore ultra pure water. 300 μl of this solution was aliquoted into each well and incubated at room temperature for 60 minutes. The wells were then aspirated completely and rinsed with 10 times their volume (3 ml) of Millipore ultra pure water. The plates were allowed to dry at room temperature before use. 90 μl of (10 μM Aβ42 with 200 μM ThT (from a 2mM stock solution in water)) per well was incubated in the absence or presence of 10 μl of various nanomaterials, with different concentrations, per well at 37 °C and shaken at 700 rpm. Measurements were made at regular intervals (every 10 and 20 minutes, respectively) using a microplate reader with excitation and emission at 440 nm and 480 nm, respectively. Each experimental point is an average of the fluorescence signal of 8 wells (4 wells in each plate- we used 2 batches to be ensure that the data is reproducible) containing aliquots of the same solution (same particle and protein concentration). In order to be ensured about the suitability of ThT assay for various particles, the effects of ThT dye

with various nanoparticles in the absence of Aβ42 were probed and the results confirmed that there is no considerable interaction with various particles and ThT dye.

The obtained kinetic data were analyzed assuming the typical sigmoidal behavior in order to extract the kinetic parameters of the bimodal fibrillation processes. An empirical sigmoidal equation was used:

$$y = y_0 + \frac{y_{max} - y_0}{1 + e^{-(t - t_{\frac{1}{2}})k}} \quad (1)$$

Where y is the fluorescence intensity at time t, y_0 and y_{max} are the initial and maximum fluorescence intensities, respectively, $t_{1/2}$ is the time required to reach half the maximum intensity, and k is the apparent first-order aggregation constant. In addition, the lag time can be defined using the following equation:

$$\text{lagtime} = t_{1/2} - \frac{2}{k} \quad (2)$$

Results:

In the first step of this study, we probed the effects of carbon nanotubes (CNTs; : 10-40 nm and length: 0.1-10 μm), and various types of spherical nanoparticles (i.e. silica (100 nm) silica (200 nm), and polystyrene (100 nm)), in bare and protein coated states (human plasma concentration of 10% and 100%) for amyloid beta fibrillation process, using Thioflavin T assay and the results are presented in Figures 1 and 2.

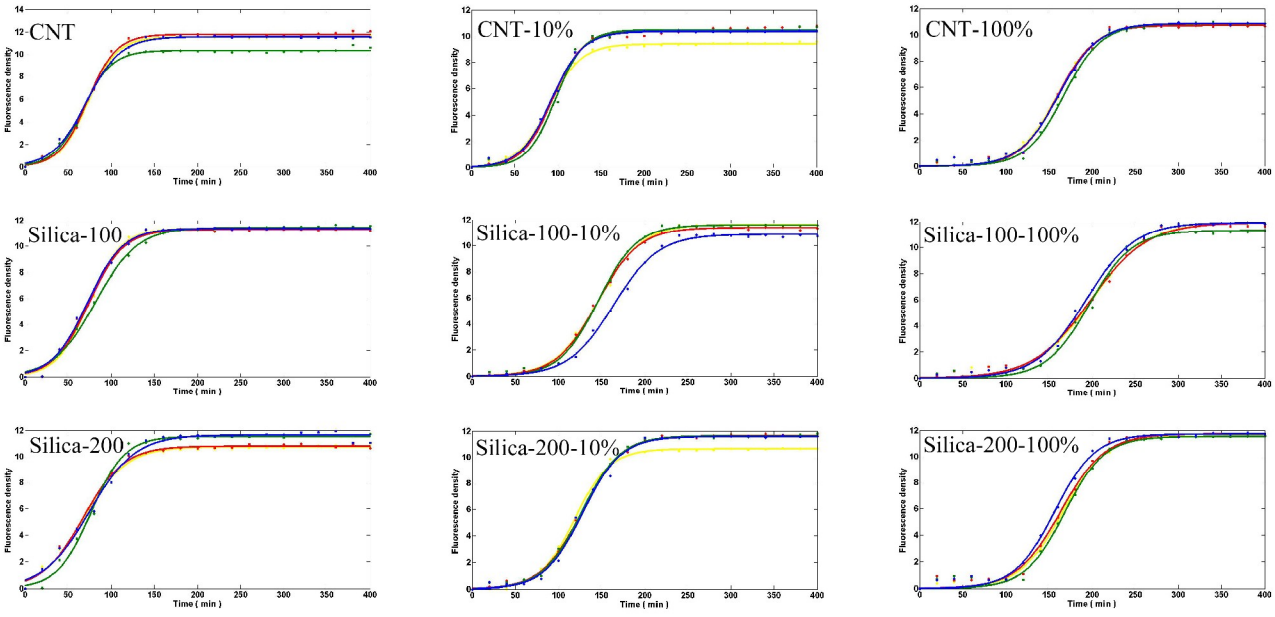
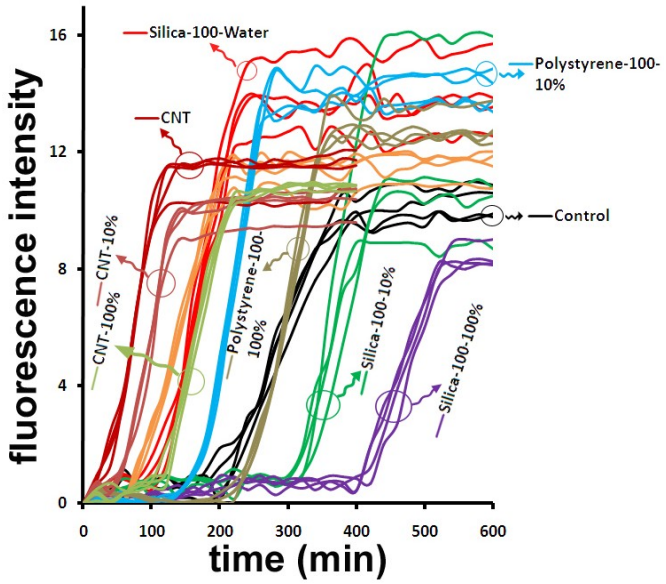
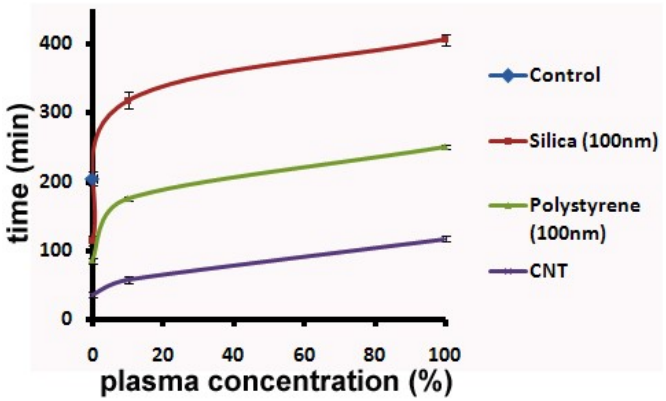


Figure 1: Examples of the fibrillization kinetics of various nanomaterials at bare and protein coated (both 10% and 100%)-states obtained from individual batches, showing the reproducibility of the results. The lines represent the best fittings to the equation 1.



(a)



(b)

Figure 2: Kinetics of Aβ fibrillation with and without protein coated nanoparticles. (a) Fibrilation kinetics of Aβ (concentration of 5 μM) in the absence and presence of various nanomaterials (both bare and protein coated) at 37 °C monitored by temporal development of ThT fluorescence intensity. (b) Calculated lag times of Aβ (concentration of 5 μM) in the absence and presence of various nanomaterials.

In order to obtain better understanding of the observed phenomenon, transmission electron microscopy (TEM) method was employed. Besides of the ThT assay results on the retardation of Aβ fibrillation in the presence of protein coated nanoparticles, the TEM images

(see Figure 3a-d) demonstrate that the oligomers and pre-fibrils were formed between and at the surface of protein coated particles, rather than formation of matured fibrils (see Figure 3a). On the basis of the obtained results, one can conclude that protein corona can reduce the catalysis effects of nanoparticles in the fibrillation process; this inhibitory effect is strongly dependent to the concentration of plasma proteins.

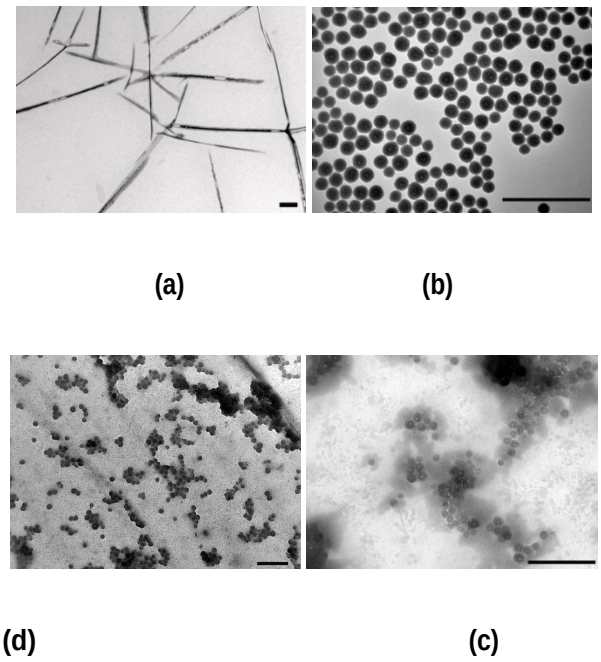


Figure 3: TEM images for samples prepared in the (a) absence and (c-f) presence of silica nanoparticles (100 nm; particles concentration of 1 mg/mL). (b) TEM mages of bare silica nanoparticles. (c) and (d) TEM images for samples which are obtained from interactions of Aβ with hard corona coated nanoparticles, with plasma concentration of 10% and 100%, respectively.

The whole story has been submitted to *Angwandte Chemie International Edition*.