

## Supporting Information

# Graphene Oxide Strongly Inhibits Amyloid Beta Fibrillation

*Morteza Mahmoudi<sup>1,2,3\*</sup>, Omid Akhavan<sup>4,5\*</sup>, Mehdi Ghavami<sup>1</sup>, Farhad Rezaee<sup>6</sup>, and Seyyed Mohammad  
Amin Ghiasi<sup>4</sup>*

<sup>1</sup>Department of Nanotechnology, Faculty of Pharmacy Tehran University of Medical Sciences, P.O. Box  
14155-6451, Tehran, Iran.

<sup>2</sup>Nanotechnology Research Center Faculty of Pharmacy Tehran University of Medical Sciences Tehran,  
P.O. Box 14155-6451, Tehran, Iran.

<sup>3</sup>Current Address: School of Chemical Sciences, University of Illinois at Urbana-Champaign, 600 South  
Mathews Avenue, Urbana, Illinois 61801

<sup>4</sup> Department of Physics, Sharif University of Technology, P.O. Box 11155–9161, Tehran, Iran.

<sup>5</sup> Institute for Nanoscience and Nanotechnology, Sharif University of Technology, P.O. Box 14588–  
89694, Tehran, Iran.

<sup>6</sup>Department for Experimental and Molecular Medicine, Academic Medical Center, University of  
Amsterdam, Amsterdam, The Netherlands

### *Synthesis of graphene oxide*

Natural graphite powder (particle diameter of  $\leq 20 \mu\text{m}$ , Fluka Inc.) was utilized as the starting raw material to synthesize graphite oxide suspension through a modified Hummers' method. The details of the applied method can be found elsewhere<sup>[1]</sup>. Then, the prepared graphite oxide powder was dispersed in deionized (DI) water to obtain an aqueous graphite oxide suspension with yellow-brownish color. After that, the aqueous suspension was centrifuged at 2000 rpm for 15 min and at 8000 rpm for 10 min to remove unexfoliated graphitic plates and tiny graphite particles, respectively. Graphene oxide suspension with concentration of  $\sim 0.1 \text{ mg/mL}$  was obtained by exfoliation of the filtered graphite oxide suspension through its sonication at frequency of 40 kHz and power of 150 watt for 30 min.

### *Material characterizations*

The surface topography of the GO sheets and amyloid fibrils were examined by atomic force microscopy (AFM, Digital Instruments NanoScope V) in tapping mode. The samples for the AFM imaging were prepared by drop-casting a diluted GO-containing suspension onto a cleaned Si(100) substrate. XPS was used to investigate the chemical states of the GO sheets. The data were obtained by a hemispherical analyzer equipped by an Al K $\alpha$  X-ray source ( $h\nu = 1486.6 \text{ eV}$ ) working at a vacuum better than  $10^{-7} \text{ Pa}$ . The C(1s) peak was deconvolution by using Gaussian components after a Shirley background elimination. The O/C atomic ratio was evaluated by using peak area ratios of C(1s) and O(1s) core levels and the effect of sensitivity factor (SF) of each element in XPS. The carbon structures of the GO sheets were investigated by Raman spectroscopy (HR-800 Jobin-Yvon) at room temperature using an Nd-YAG laser source operating at wavelength of 532 nm. The sample for Raman spectroscopy was prepared by drop-casting a GO suspension onto the Si substrate followed by heating at  $100^\circ\text{C}$  in air for 30 min.

**Preparation of hard corona coated GO sheets.** In order to simulate *in vitro* and *in vivo* mediums, 10% and 100% different concentrations of fetal bovine serum (FBS) were used, respectively. GO sheets were incubated at both predetermined FBS concentrations; in the case of *in vitro* simulation, FBS solutions were diluted with PBS keeping the final concentration of GO constant and equal to 0.01 mg/ml. GO sheets were allowed to incubate with the protein solutions at 37°C for one hour. To obtain hard protein corona complexes, after the incubation in FBS, the samples were centrifuged to pellet the GO-protein complexes and separated from the supernatant FBS. The pellet was then resuspended in 500 µl of PBS and centrifuged again for 30 minutes at 13,000 g at 15°C to pellet the sheets-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 GO surface (the soft protein corona). Before the final washing process, the samples were transferred into a low protein attachment Eppendorf tube followed by the last centrifugation. GO-sheets/protein corona complexes was then suspended in 1X loading buffer in the presence of DTT, boiled for 5 minutes under agitation and run on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

**SDS-PAGE.** In order to define the protein profiles of formed hard coronas on the surface of various silver NPs, 1D SDS-PAGE (12%) was employed. In this case, the hard corona coated GO-sheets were re-suspended 40 µL of fresh phosphate buffered saline (PBS; Sigma-Aldrich) followed by addition of 20 µL loading buffer, containing 10% DTT. Gels were scanned using a Biorad GS-800 calibrated densitometer scanner.

## Methods

**Differential Centrifugal Sedimentation.** Differential centrifugal sedimentation experiments were performed with a CPS Disc Centrifuge DC24000. The analyzer measures particle size distributions using centrifugal sedimentation within an optically clear spinning disc that is filled with fluid. Sedimentation is stabilized by a density gradient within the fluid, and the accuracy of the measured sizes is insured through the use of a known size calibration standard run immediately before each measurement. The use of a

biological sample with a large amount of proteins requires a new sucrose gradient to be prepared for each measurement. The concentration of the particles at each size is determined by continuously measuring the turbidity of the fluid near the outside edge of the rotating disc. The turbidity measurements are converted to a weight distribution by Mie theory light scattering calculations. The choice of the experimental parameters, namely the fluid gradient, the speed of rotation, etc. is based on the type of material being analyzed and the range of sizes being measured. For GO sheets, the non-sphericity of the instruments was fixed to 1.5. The primary information from the analytical disk centrifuge is the time taken for the particles to travel from the centre of the disk through a defined viscous sucrose gradient to a detector placed at the outer rim of the disk under a strong centrifugal force. For materials with homogenous density and simple shape one can directly relate this time to a particle size; this is the meaning of the size cited on the x-axis of all figures presented here, and hence it should be considered as an ‘apparent’ size in the case of aggregated particles (including dimers and trimers). Moreover, for the sake of clarity in the comparison of different samples, we chose to show the data as relative weight particle size distribution. The tallest peak (highest weight value) in the distribution is called the ‘base’ peak (has a value of 100%) and all other particle size (multimer) peaks are then normalized against this base peak to give a relative weight distribution.

**Thioflavin T (ThT) assay.** Amyloid Beta ( $A\beta_{40}$ ) was purchased from Sigma. In order to prevent the depletion of  $A\beta_{40}$  from solution by the adherence of  $A\beta_{40}$  to the plates’ chamber walls, the plates were coated by poly-L-Lysine (PLL). Briefly, the PLL was diluted to 15  $\mu\text{g/ml}$  using Millipore ultra pure water. 300  $\mu\text{l}$  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  $\mu\text{l}$  of (10  $\mu\text{M}$   $A\beta_{42}$  with 200  $\mu\text{M}$  ThT (from a 2mM stock solution in water)) per well was incubated in the absence or presence of 10  $\mu\text{l}$  of various GO sheets (i.e. bare and protein corona coated) per well at 37 °C and shaken at 700 rpm. Measurements were made at regular intervals (every 10 minutes) using a microplate reader with excitation and emission at 440 nm and 480 nm, respectively. Each experimental point is an

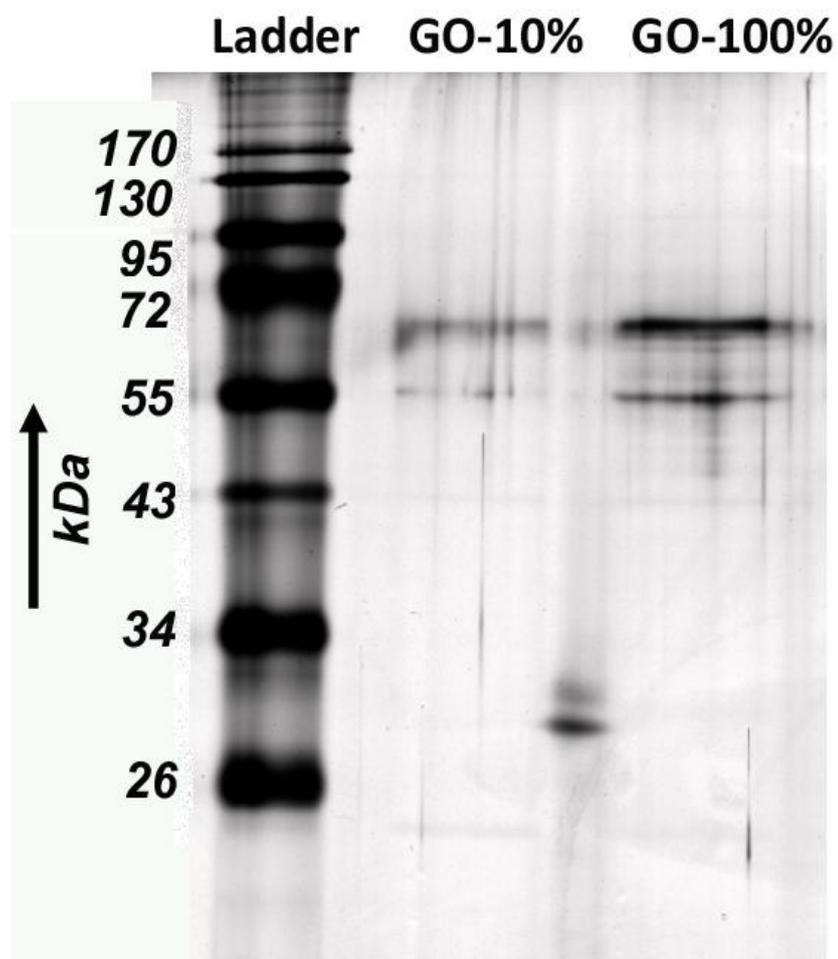
average of the fluorescence signal of 8 wells (4 wells in each plate- we used 2 batches to be ensure that the data is reproducible) containing aliquots of the same solution (same GO sheets and protein concentration). In order to be ensured about the suitability of ThT assay for various particles, the effects of ThT dye with GO sheets in the absence of A $\beta$ 40 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:

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

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:

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



**Figure S1:** SDS-PAGE gel of FBS proteins obtained from various (i.e. sphere, cube, wire, and triangle) silver NP-protein complexes free from excess plasma following incubation at different NPs concentrations. The molecular weights of the proteins in the standard ladder are reported on the both left and right for reference.

## References

- [1] A. Esfandiar, O. Akhavan, A. Irajizad, *Journal of Materials Chemistry* **2011**, *21*, 10907-10914.