Supporting Information

Quantum Capacitance Based Amplified Graphene Phononics for Studying Neurodegenerative Diseases

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S1. EXPERIMENTAL METHOD

Rat CSF Samples. Male transgenic rats were euthanized using ketamine/xylazine administered through an intraperitoneal injection. Once the animal lost response to stimuli, it was positioned prone and the head was flexed downward at approximately 45-degrees. A 25-gauge needle attached to a 1cc syringe was percutaneously introduced into the cisterna magna and approximately 50-100µl of CSF was collected. CSF was frozen on dry ice and kept at -80±10°C. Rats (ages 130+/-10 days) were defined to be "early symptomatic" when weakness was observed in locomotion and at "end point" (ages 170+/-20 days) when the animals were not able to right themselves in less than 30 seconds when placed on their side.

Graphene Production and transfer. Pretreated Cu foil (99.8%, Alfa-Aesar, annealed, uncoated) was placed inside the standard 1-inch quartz tube of the home build LP-CVD system. The reaction chamber was evacuated to ~ 1.5 mTorr and flushed with 100 sccm of H_2 (99.9999% purity, Praxair) at total pressure of 650 mTorr for 20 minutes. The temperature was then increased to 1050°C with the 10 sccm H_2 for 25 minutes. This was followed by annealing of Cu foil at 1050°C to increase the grainsize and to smoothen the surface. Subsequently, 10 sccm of CH_4 (99.999% purity, Praxair) at partial pressure of 100 mTorr with 22 sccm H_2 (~160 mTorr) was introduced into the tube for 20 minutes. Following CH_4 exposure, the reaction chamber was cooled down to room temperature in 40 minutes (25°C/minutes). Through this

CVD process, graphene was found to be synthesized on both sides of the Cu foil. PMMA solution was spin coated on one side to mask the graphene while RIE process was used on the other side to remove the graphene present there. Cu from the PMMA/graphene/Cu system was etched using a dilute HNO_3 solution (1:3), ~1 hour. Afterwards, the PMMA/Graphene system was then transferred to two consecutive DI water baths (20 minutes each) to remove any residual ions or impurities and then picked up with SiO_2/Si chip. This chip was dried overnight and subsequently placed in an acetone bath for 10 mins to remove PMMA.

Raman Spectroscopy acquisition and data analysis. The Raman spectroscopic data was obtained using WITEC Raman Alpha 300-RA, with a laser excitation wavelength of 532 nm and an exposure time of ~5 min. All the graphene samples were probed using the 100X objective. The spot size was determined using the equation:

$$Spot\ size = \frac{1.22\lambda}{NA}$$

Where λ is the wavelength of the laser and NA is the numerical aperture (0.9 for 100X objective). Also, to analyze the Raman spectra, they were all normalized to the intensity of G peak and Lorentzian curve fit was used to custom fit the 2D peaks.

The Raman spectra of the CVD grown graphene transferred on SiO_2/Si is shown in Figure S1 depicting the D, G and 2D bands, along with the optical image. The 2D/G intensity ratio was calculated to be ~2.4 and the full width at half maximum (FWHM) of 2D peak was ~29.9 cm^{-1}.[1] These values correspond to graphene monolayer produced via CVD.[2] Further, the weak D peak indicates that the CVD grown graphene is of high quality (sp^2 C orbitals).[3]

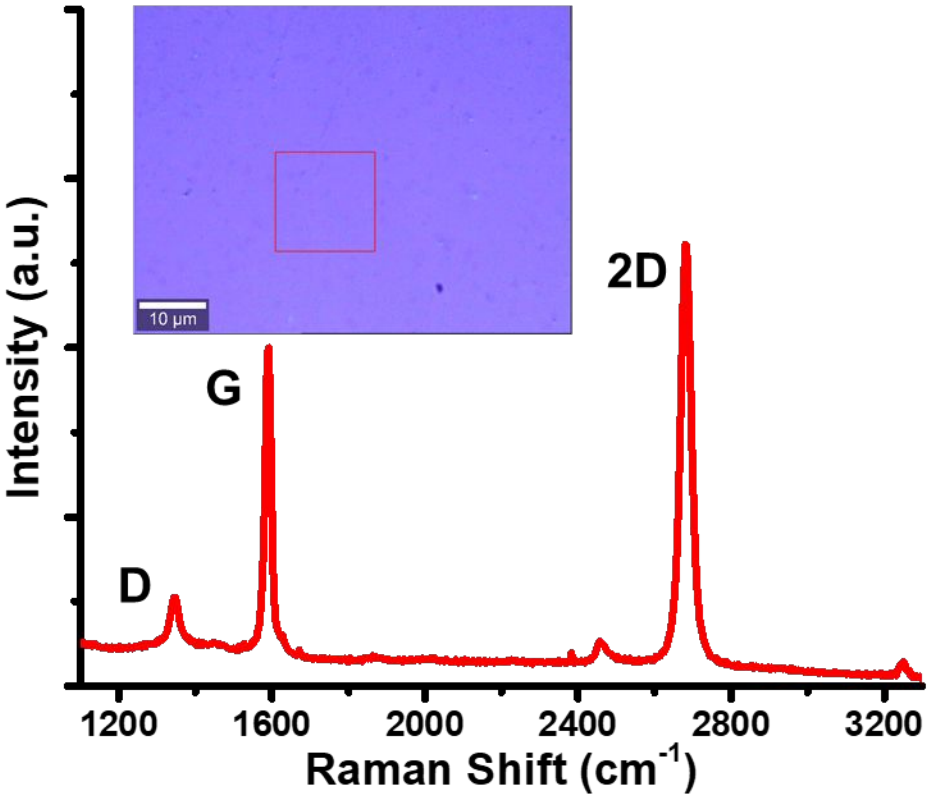

Figure S1. Raman Spectra of CVD graphene on SiO_2/Si substrate. (Inset) Optical image of graphene transferred SiO_2/Si substrate.

pH measurement. The pH of all the CSF samples were obtained using Oakton pH150.

Statistical Analysis. Student's t-test was performed throughout the study and a $p<0.05$ was used for statistical significance cutoff.

Average density estimation via quantum capacitance. The average of CSF's composite dipole moment (μ) were estimated via Hyperchem software. The composite dipole potential, originated from the charged surfaces of various proteins, lipids, nucleic acids and reactive oxygen species (present in CSF), is estimated as shown (using rectangular matrix):

$$V_{average} = \frac{\mu\rho\sin(45^o)}{4\varepsilon_o\pi}$$
$$\left(4\left\{\sum_{i=1}\left(\sqrt{(i\times d)^2+(r)^2}\right)^{-2}\right\}+8\left\{\sum_{i=1}\left(\sqrt{(i\times d\sqrt{2})^2+(r)^2}\right)^{-2}\right\}+4\left\{\sum_{i=1}\left(\sqrt{(i\times d\sqrt{5})^2+(r)^2}\right)^{-2}\right\}+\{r^{-2}\right.$$

where ε_o is vacuum permittivity (8.85 x 10^{-12} F/m), r is the distance from the molecules to the surface of graphene (~ 1 nm), $d = \sqrt{\frac{\sqrt{2}}{\rho}}$ is the distance of dipole of the charged molecules to the point of interest, i is the index (representing the distance of the dipole of the charged molecules and the point of interest), and r is vertical distance from the charged molecules to graphene surface (assumed to be 1 nm). Following this approach, the average density of the charged molecules interacting with the graphene lattice was estimated.

S2. Average dipole moment comparison for different sample groups

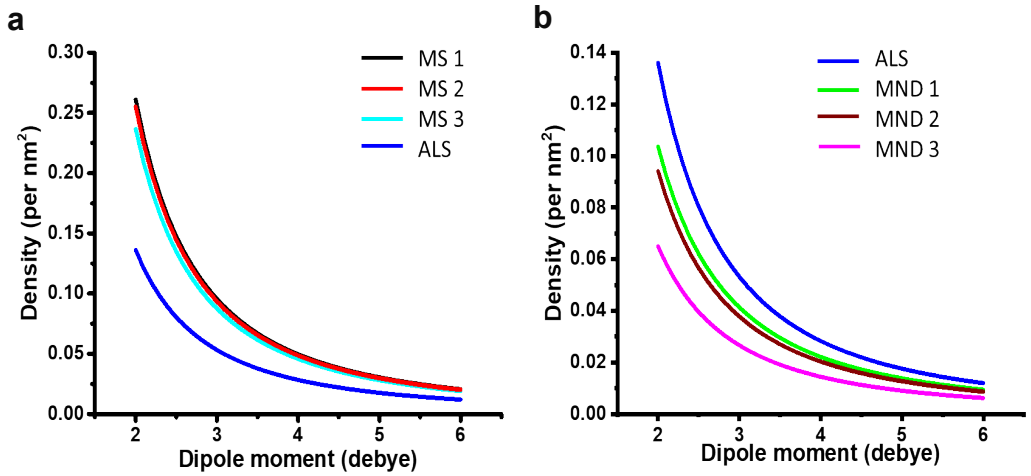

Figure S2. Calculated average induced dipole moment of human a) MS and b) MND CSF samples.

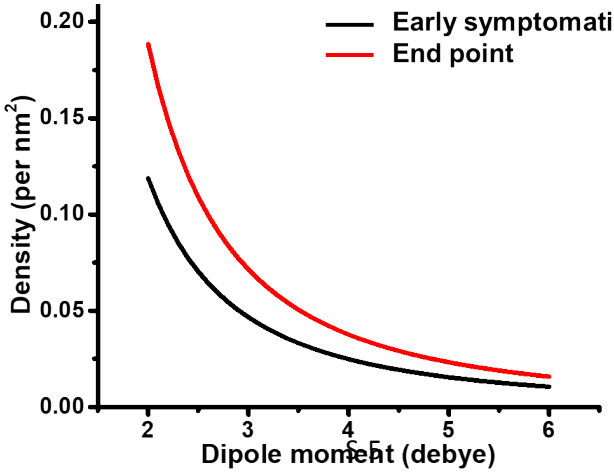

Figure S3. Calculated average induced dipole moment of CSF samples from rats at early symptomatic and end point stages.

REFERENCES:

(1) Hao, Y.; Wang, Y.; Wang, L.; Ni, Z.; Wang, Z.; Wang, R.; Koo, C. K.; Shen, Z.; Thong, J. T. L. Probing Layer Number and Stacking Order of Few-Layer Graphene by Raman Spectroscopy. *Small* **2010**, *6* (2), 195–200.

(2) Li, X.; Cai, W.; An, J.; Kim, S.; Nah, J.; Yang, D.; Piner, R.; Velamakanni, A.; Jung, I.; Tutuc, E.; Banerjee, S. K.; Colombo, L.; Ruoff, R. S. Large-Area Synthesis of High-Quality and Uniform Graphene Films on Copper Foils. *Science (80-.).* **2009**, *324* (5932), 1312–1314.

(3) Liu, L.; Ryu, S.; Tomasik, M. R.; Stolyarova, E.; Jung, N.; Hybertsen, M. S.; Steigerwald, M. L.; Brus, L. E.; Flynn, G. W. Graphene Oxidation: Thickness-Dependent Etching and Strong Chemical Doping. *Nano Lett.* **2008**, *8* (7), 1965–1970.