Electronic Supplementary Material (ESI) for Materials Advances.

Concentrated aqueous dispersions of low-defect few-layer thick graphene using surface active ionic liquid for enhanced enzyme activity

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Supporting Information

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Annexure S1:

S1.1: Materials: Benzimidazole (>98), 1-bromobutane (>99), sodium hydride (>99) and tetrahydrofuran (anhydrous, >99) were obtained from Sigma and used without further purification. Hexane, ethyl acetate, chloroform, methanol, diethyl ether, dichloromethane, and acetone (AR Grade) has been procured from SD fine chemicals Ltd, Mumbai, India. All the measurements were performed in the Millipore water having resistivity of 18Ω. A Detailed description of the procedure followed for the synthesis of SAILs and their characterization using the NMR and Mass spectrophotometer is given in annexure S2. Cellulase from *Trichoderma reesei* (ATCC 26921), sodium carboxymethyl cellulose, Rochelle salt and dinitrosalicylic acid (98%), have been purchased from Sigma.

S1.2: Exfoliation and characterization of Graphene

The ability of SAIL $[C_4C_{12}Ebzm][Cl]$, in a wide concentration range (0.1 mmol L^{-1} to 15 mmol L^{-1}), ranging from below *cac* to above *cac*, to exfoliate graphene from graphite, mediated via low energy ultrasonic waves was tested in aqueous medium. The powdery graphite at a reasonably high concentration (4 mg ml^{-1}) was dispersed in aqueous solutions of SAIL followed by bath sonication using sonicator (Citizon CUB2.5, Power 50W, Frequency 40 KHz) for one hour at room temperature. The colloidal graphene-SAIL solution was kept for 24 hrs followed by centrifugation at 1000 rpm. Excess graphite was filtered off and the amount of exfoliated graphene was calculated by weighing the filtered un-exfoliated graphite as already mentioned in previous reports.[1] Therefore, from the calculations, the efficiency of the proposed method was found to be 75 % with the mass ratio of 3:1 for exfoliated and un-exfoliated graphite. The remaining supernatant was used for further characterization. UV-visible absorption spectra of thus obtained graphene supernatant were measured using UV-Vis spectrophotometer (UV-1800 SHIMADZU) in the wavelength range of 300-800 nm by using quartz cuvette having path length of 1 cm. Zeta-potential measurements of graphene dispersions were performed using light scattering apparatus (Zeta-sizer, nanoseries, nano-ZS), Malvern Instruments, equipped with a built-in temperature controller having an accuracy of ±0.1 K at a scattering angle of 173° using dip cell (ZEN-212). Raman spectra of graphite powder and exfoliated graphene after drying for 24 hours were recorded on Renishaw Raman Spectrophotometer in the range of 1000-300 cm^{-1} using Ar-ion laser at a wavelength of 514 nm. X-ray diffraction patterns of graphite powder and exfoliated graphene were acquired on a SHIMADZU MAXIMA B70000 using a Cu-target in the

2θ range of 5-80°. Morphology and the lattice parameters of obtained graphene were investigated on JEM-2100 Transmission Electron Microscope (TEM) working at 200 kV. Atomic force microscopy (AFM) was recorded in tapping mode with a NanoscopeIIIa (Veeco). Samples were drop casted on freshly prepared mica surface. Commercially available phosphorous doped silicon tips (Veeco, MPP-11100) were used as probes. 2D $^{1}H-^{1}H$ NOESY experiments were performed on a Brüker Ascend 500 spectrometer (AVANCE III HD console) with water suppression in a 10% D_2O− 90% H_2O mixture using a 5 mm BBO (broad-band observe) double-channel probe equipped with z-gradients. Phase sensitive ^{1}H homonuclear 2D NOESY NMR experiment was recorded with experimental parameters: fid size 4096 : F2 and 128 : F1, numbers of scan : 32, mixing time of 500 ms and the data obtained was processed using Topspin NMR software. Steady-state fluorescence were carried out using a Perkin Elmer LS-55 spectrophotometer having a quartz cuvette of unit path length. In order to demystify the exfoliation behavior of graphene driven by SAIL, we computed the absorption free energies employing periodic wave density functional theory simulations. These types of calculations were carried out using Quantum Expresso package[2] interfaced with plumed software[3] with a plane wave basis set with 130 Ry kinetic cut off. The exchange-correlation effects (XC) were accounted with Perdew−Burke−Ernzerhof (PBE)[4] formulation of generalized gradient approximation (GGA) approach. The Brellouin Zone was sampled using 16 x 16 x 1 Monkhorst-Pack grid[5] and Methfessel–Paxton[6] smearing of 0.0037 Ry was used during our computations. These settings were verified to achieve energy convergence of 1 meV per atom. All the calculations are spin polarized. The adsorption energies of all the adsorbates were computed employing the equation:

$$E_{ad} = E_{M/S} - E_M - E_S$$

Where $E_{M/S}$ is the total energy of adsorbed molecule on graphene, E_M represents the energy of adsorbed molecule, and E_S is the energy of graphene surface.

S1.3: Adsorption of cellulase on graphene

Exfoliated graphene from the colloidal dispersion was separated by centrifugation at 8000 rpm for 30 minutes followed by washing with methanol for at least 5 times. Removal of the SAIL from separated graphene has been confirmed by Thermogravimetric Analysis (TGA) (Figure S4), which was carried out using HITACHI STA7200 Thermal Analysis System under N_2

atmosphere at a heating rate of 10 ^{o}C min^{-1} in the temperature range of 25 to 500 ^{o}C. 25 uM L^{-1} of cellulase has been adsorbed on graphene in aqueous medium with and without SAIL, having 3 mg /ml concentration of graphene using stock solution of cellulase in acetate buffer of 4.5 pH. Same concentration of cellulase has been used to prepare aqueous solution of cellulase in acetate buffer and SAIL (1 mmol L^{-1}). Activity of cellulase in different mediums was detected using dinitrosalicylic acid (DNS) sugar assay test employing carboxymethyl cellulose (CMC) as substrate. DNS reagent has been prepared using the method described in literature.[7] Firstly, standard calibration curve was obtained using DNS analysis. Cellulase in different mediums (400 µl) and 1% CMC (700 µl) solutions were incubated for 20 minutes at 45 ^{o}C followed by addition of 200 µl DNS reagent. The solutions were then heated for 5 minutes at 90 ^{o}C on a water bath. Due to the reduction of nitro group of DNS to the amino group by sugar produced during hydrolysis of CMC, the solution turned deep red in color. Then 100 µl of 40% solution of Rochelle salt was added to stabilize the color of solution. Solutions were cooled to room temperature and then diluted 5 times to record absorbance at 546 nm. Further, the standard calibration curve was used to get the concentration of sugar and activity of cellulase was calculated using the standard equation given in Annesure S2. Secondary (2^{o}) structure of cellulase in different mediums and native cellulase was monitored using circular dichroism (CD) spectroscopy on Jasco J-810 spectrometer using a cuvette of path length 1 mm at a scan speed of 50 nm/min. Ternary structure of cellulase present in different systems has been characterized using steady state fluorescence spectroscopy. Intrinsic fluorescence of cellulase has been recorded on Perkin Elmer LS-55 spectrophotometer having a quartz cuvette of unit path length using 280 nm excitation wavelength. AFM of the native cellulase and the cellulase adsorbed on graphene was recorded in tapping mode on Anton Parr Tosca 400 series. Samples were drop casted on freshly prepared mica surface.

Annexure S2:

Synthetic procedure for the preparation of [C_4C_{12}Ebzm][Cl]:

The SAIL [C_4C_{12}Ebzm][Cl] under investigation was prepared by procedure mentioned in our previous report.[8] Solution of benzimidazole (50 mmol) in THF was added to a suspension of oil-free sodium hydride (100 mmol) in THF under nitrogen atmosphere at 25° C. 1-bromobutane (50 mmol) in THF was added dropwise to it and the mixture was stirred overnight at 60 °C. Solvent was removed using rotary evaporator and the product was extracted with dichloromethane (50 mL × 3). *N*-butylbenzimidazole was purified using column chromatography (EtOAc/*n*-hexane = 1/20). The other intermediate dodecyl 2-chloroacetate required for the synthesis of SAIL was synthesized in similar manner as prepared in our earlier report.[9] The resulting intermediate n-butylbenzimidazole (5 mmol) was allowed to react with the dodecyl 2-chloroacetate (5 mmol) for 12 hours at 80°C. The obtained crude reaction mixture was washed with diethyl ether and hexane. The product obtained was dried using rotary evaporator and characterized using ^{1}H NMR.

[C_4C_{12}Ebzm][Cl]: ^{1}H NMR (500 MHz, $CDCl_3$, δ-ppm) 0.883 (t, 3H, terminal-C*H*$_3$), 1.009 (t, 3H, N-CH_2-CH_2-CH_2-C*H*$_3$), 1.262 (br s, 18H, (-C*H*$_2$-)$_9$), 1.480 (m, 2H, -N-CH_2-CH_2-C*H*$_2$-CH_3), 1.670 (m, 2H, -COO-CH_2-C*H*$_2$-CH_2-CH_2-), 2.065 (m, 2H, -N-CH_2-C*H*$_2$-CH_2-C*H*$_3$), 4.217 (t, 2H, -COO-C*H*$_2$-CH_2-CH_2-), 4.576 (t, 2H, -N-C*H*$_2$-CH_2-CH_2-C*H*$_3$), 5.766 (s, 2H, -N^+-C*H*$_2$-COO-CH_2-), 7.582 (m, 1H, -N-C=C*H*-CH=CH-CH=C-N^+-), 7.654 (m, 1H, -N-C=CH -C*H*=CH -CH=C-N^+-), 7.676 (m, 1H, -N-C=CH CH=C*H*-CH=C N^+-), 7.728 (m, 1H, N-C=CH CH=CH C*H*=C N^+-), 11.536 (s, 1H, N-C*H*-N^+-).

Enzyme (Cellulase) Activity (IU)

$$IU = \frac{c}{M \times t \times V}$$

Where *c* is concentration of sugar released, *M* is molecular weight of sugar, t is time of incubation and *V* is volume of enzyme used.[10]

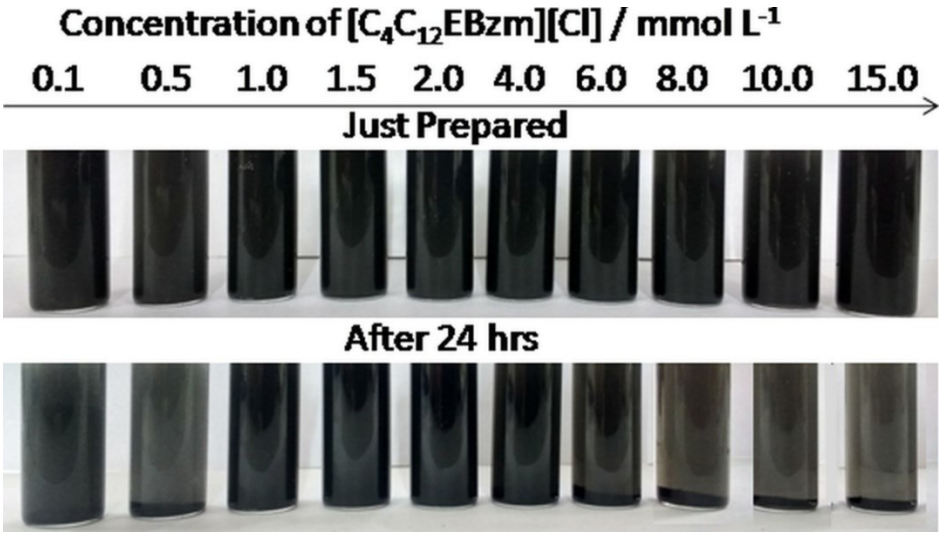

Fig. S1: Photographs showing SAIL mediated dispersion of graphene-flakes in aqueous solutions.

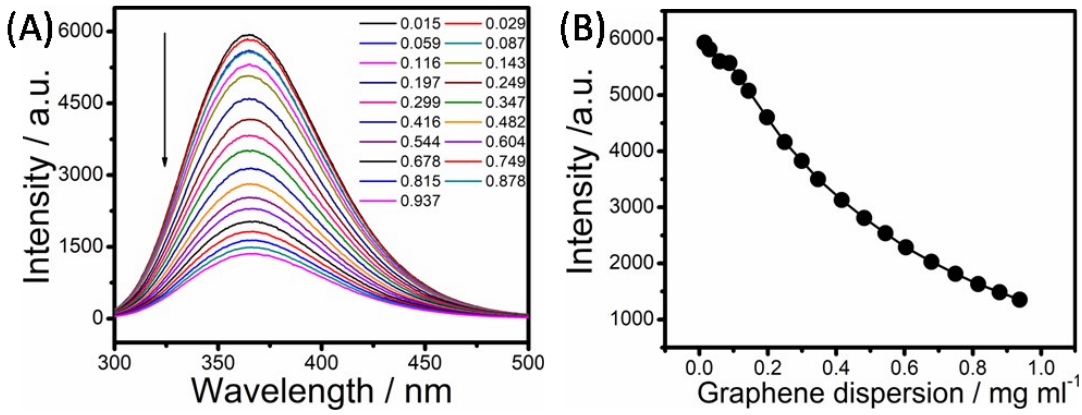

Fig. S2: (A) Fluorescence spectra of $[C_4C_{12}EBzm][Cl]$ (1 mmol L^{-1}) in presence of Graphene; (B) Change in fluorescence intensity of $[C_4C_{12}EBzm][Cl]$ as a function of graphene dispersion.

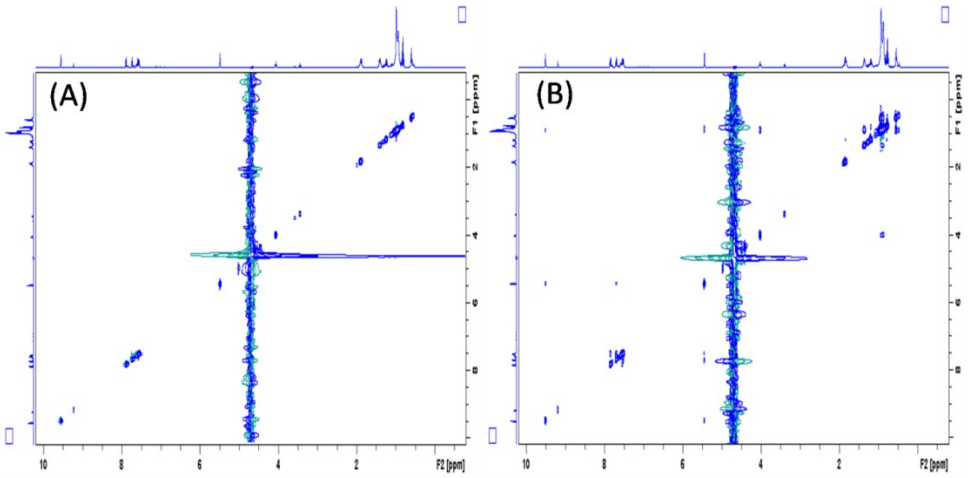

Fig. S3: 2D ^{1}H–^{1}H NOESY spectra of an aqueous solution of the $[C_4C_{12}EBzm][Cl]$ (A) without and (B) with graphene at 1 mmol L^{-1} concentration of $[C_4C_{12}EBzm][Cl]$.

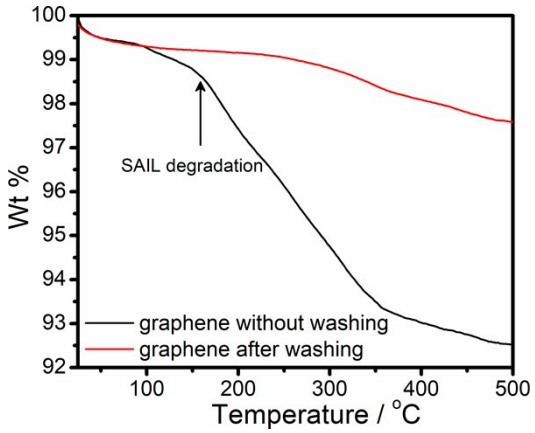

Fig. S4: TGA of exfoliated graphene-flakes after separation from the dispersion and SAIL. (No peak corresponding to SAIL in the plot of graphene after washing suggest the recovery of graphene without any contamination)

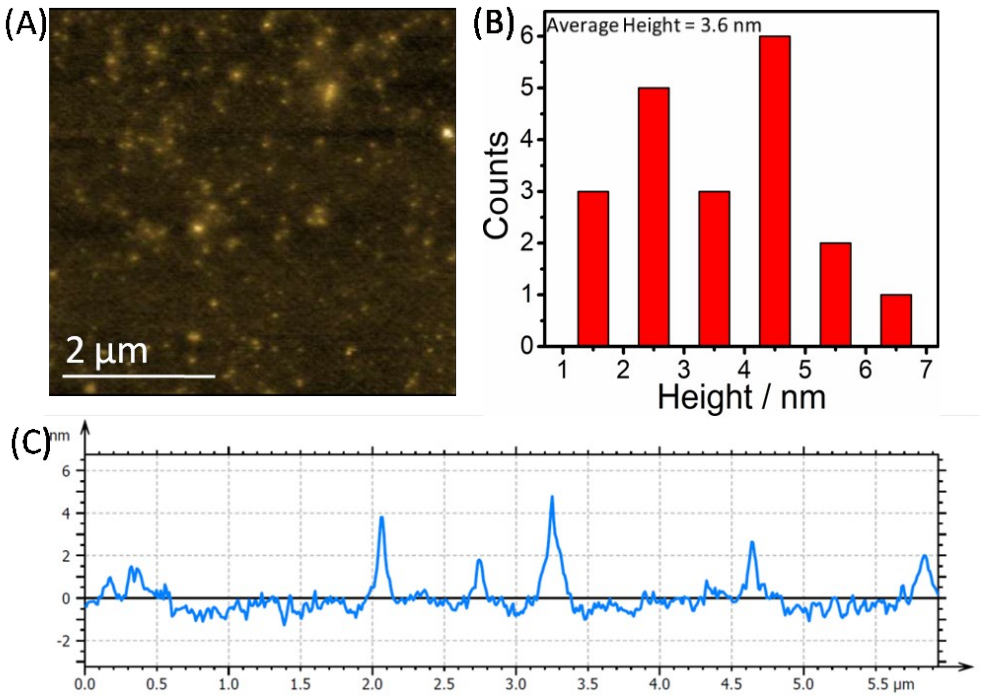

Fig. S5: (A) AFM image of the native cellulase; (B) Height profile; (C) surface topology vs. distance profile of native cellulase.

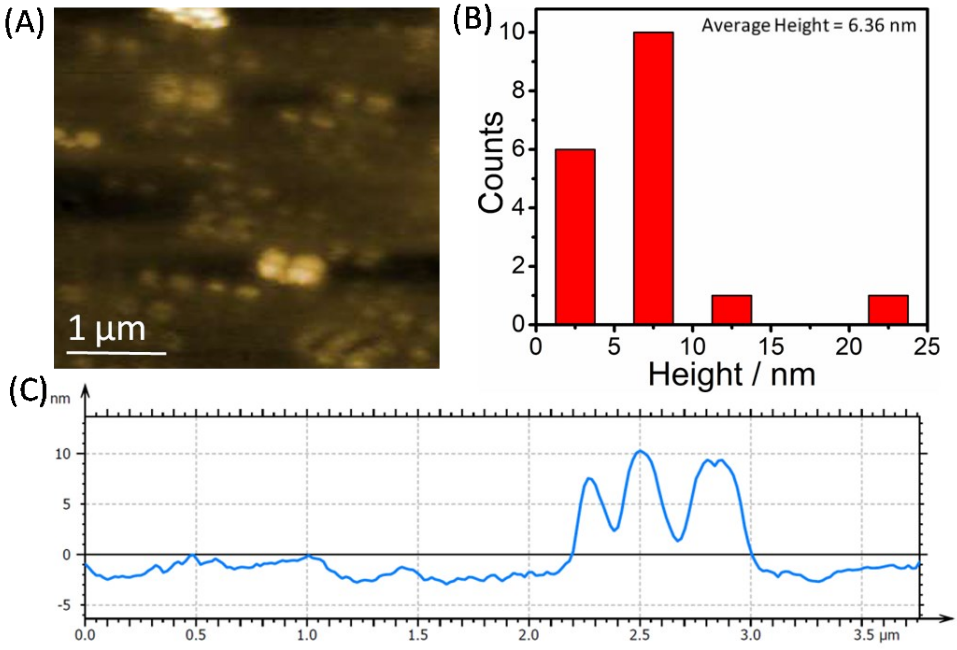

Fig. S6: (A) AFM image of the cellulase adsorbed on graphene (C@G); (B) Height profile and (C) surface topology vs. distance profile of C@G. (Height profile of the obtained graphene with cellulase (Fig. S5B) having thickness around 6.36 nm indicates the adsorption of cellulase (thickness ≈ 3 to 6 nm, Fig. S4) on graphene).

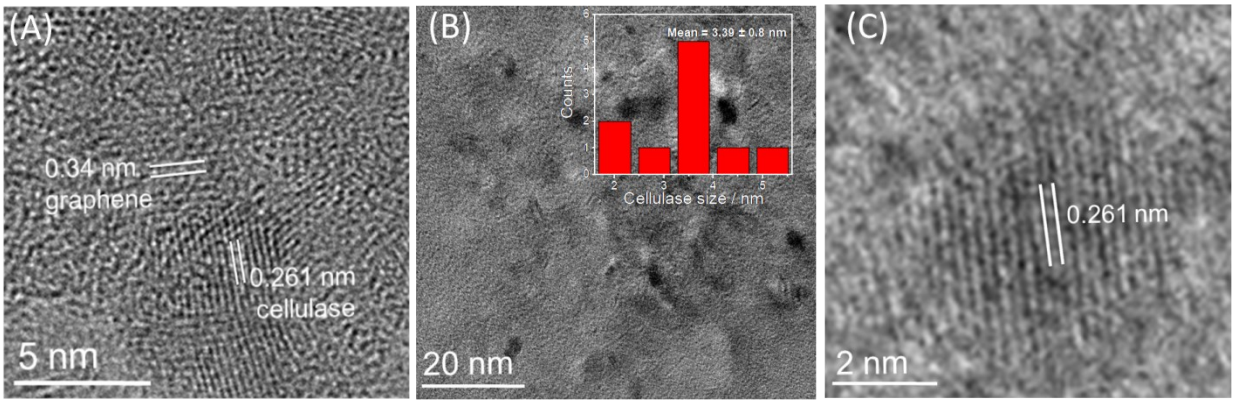

Fig. S7: (A) High resolution TEM image of cellulase adsorbed on graphene; (B) TEM image of native cellulase; (C) High resolution TEM image of native cellulase. (Inset of Fig. B shows the histograms showing the mean particle diameter along with standard deviation)

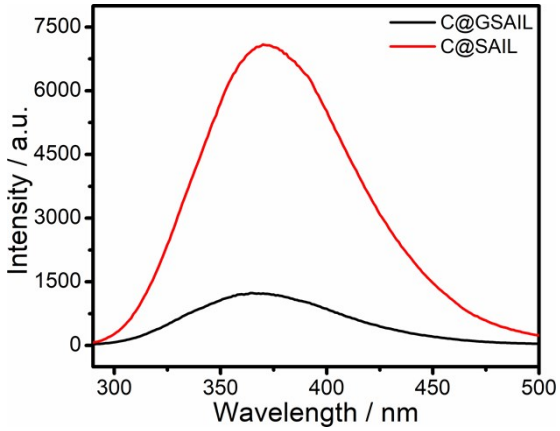

Fig. S8: Fluorescence spectra of cellulase in buffer in presence of SAIL and SAIL-graphene.

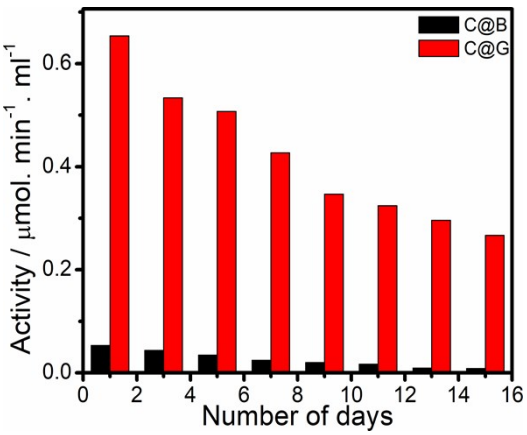

Fig. S9: Activity of cellulase in buffer and in graphene dispersion.

References

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