Supporting Information

Mechanistic insights into lysozyme interaction with MWCNTs and cholinium-based ionic liquids: A tripartite IL–NP–protein interaction study supported by molecular dynamics simulations

Pannuru Kiran Kumar[1], Indrani Jha[2,3], Anjeeta Rani[2,4], Sheldon Sookai,[5] Indra Bahadur[1*], Pannuru Venkatesu[2*]

[1]Department of Chemistry, and Material Science Innovation & Modelling (MaSIM) Research Focus Area, North-West University (Mafikeng Campus), Mmabatho 2735, South Africa

[2]Department of Chemistry, University of Delhi, Delhi 110 007, India

[3]Department of Applied Sciences, SET, Manav Rachna International Institute of Research and Studies, (Deemed to be University), Faridabad-121004, India

[4]Shaheed Rajguru College of Applied Sciences for Women, University of Delhi, Delhi – 110096, India

[5]Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, PO WITS 2050 Johannesburg, South Africa

Methods

Spectroscopic methods of MWCNTs and Lys in different concentrations of ILs

Ultraviolet-visible absorption spectra of MWCNTs and Lys in the presence of different concentrations of cholinium-based ILs were recorded from 250 to 800 nm by means of a double beam UV-visible spectrophotometer (UV-1800, Shimadzu Co., Japan) at room temperature 25 °C. Circular dichroism spectroscopy (CD) spectroscopic studies were performed using a PiStar-180 spectrophotometer (Applied Photophysics, U.K.) equipped with a Peltier system for temperature control. The protein's concentration was 1.0 mg/mL for far UV-CD and near UV-CD and each spectrum was collected by averaging three spectra. Cary Eclipse fluorescence spectrofluorimeter was used for measuring Steady state fluorescence emission spectra at 25 °C using the excitation wavelength at 295 nm. All emission spectra were taken using a slit width of the excitation and emission at 5 nm and 10 nm, respectively, between 310 and 400 nm.

To assess the hydrodynamic diameter (d_H) of MWCNTs and Lys in the presence of different concentrations of cholinium-based ILs, the Zetasizer Nano ZS90 dynamic light scattering (DLS) instrument (Malvern Instruments Ltd., UK) is used. The instrument is equipped with a fixed wavelength of 633 nm and 4 Mw He-Ne laser. A quartz sample cell containing 1.0 ml of

a filtered sample is sealed with a Teflon- coated screw cap to protect from air and dust. The Brownian motions of particles are detected by DLS and correlated to the particle size. The data collected was analyzed using the Malvern Zetasizer software version 7.01.

Table 1S Comparison of hydrodynamic diameter (d_H) of Lys in the presence of varying concentrations of MWCNTs.

Conc. of MWCNTs /μL	**Hydrodynamic diameter (d_H) of Lys/ (nm)**
Pure Lys	4.18
0.006	3.49
0.008	5.08
0.010	5.58
0.012	6.1

Table 2S Hydrodynamic diameter (d_H) of Lys in different concentrations of ILs, determined by DLS at pH 7.41 and T =25 °C.

Conc. of ILs/ M	**Hydrodynamic diameter (d_H) of Lys (nm)**	
	[ChCl]	**[ChAc]**
Pure Lys	4.18	4.18
0.01	3.65	4.29
0.1	3.95	4.42
1.0	4.74	5.28
2.0	4.76	8.51
3.0	6.87	9.46

Table 3S Hydrodynamic diameter (d_H) of the mixture of fixed MWCNTs (0.012 μL) and Lys (1 mg/ml) in different concentrations of [ChCl] and [ChAc], determined by DLS at pH 7.41 and T =25 °C.

Conc. of ILs and MWCNTs	Hydrodynamic diameter (d_H) of Lys (nm)	
	[ChCl]	[ChAc]
Pure Lys	4.18	4.18
0.01M +0.012 mI MWCNTs	6.61	8.59
1.0 M +0.012 mI MWCNTs	6.87	11.63
3.0 M +0.012 mI MWCNTs	16.17	19.13