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Tuning the selective interaction of lysozyme and serum albumin on a carboxylate modified surface

Bedabrata Saha,[a] Jiban Saikia,[b] and Gopal Das[a,b]

[a] *Centre for the Environment, Indian Institute of Technology Guwahati, Assam, India*

[b] *Department of Chemistry, Indian Institute of Technology Guwahati, Assam, India*

Supplementary information

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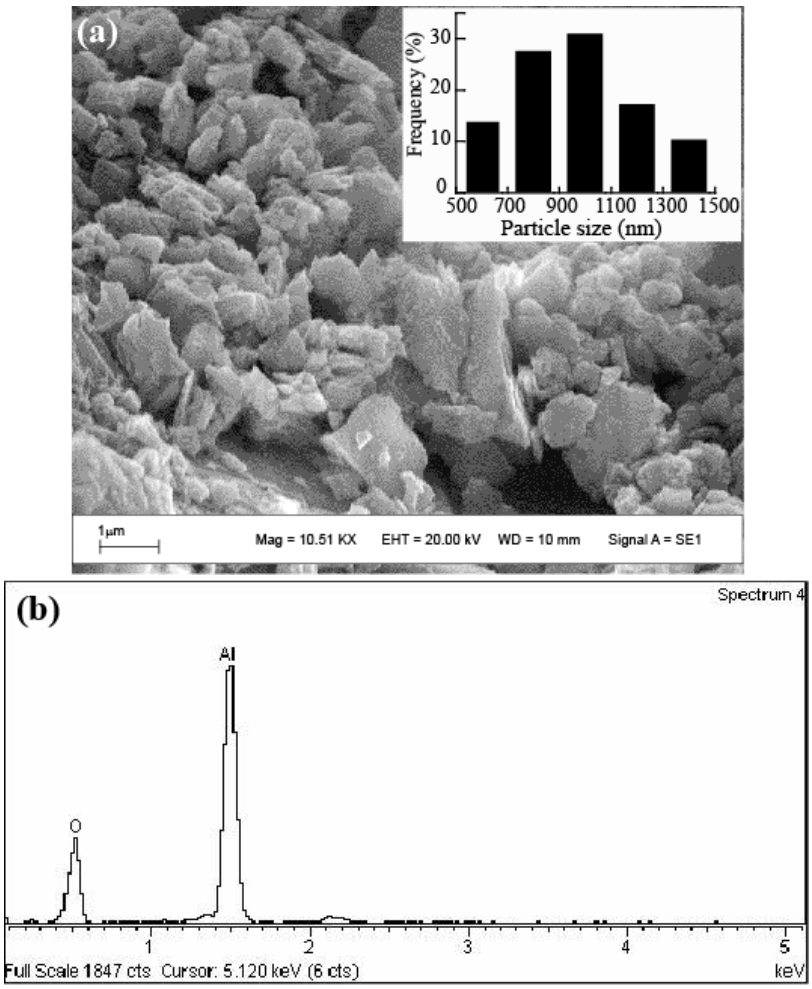

Fig S1. (a) SEM images of commercially obtained alumina particles reveals the irregular shape of the particles. Moreover, these alumina particles having an wide size distribution (inset) with an average particle size of ~1 μm. (b) Characterization of alumina particles by EDX spectra taken during the SEM analysis reveals the presence of Al and O.

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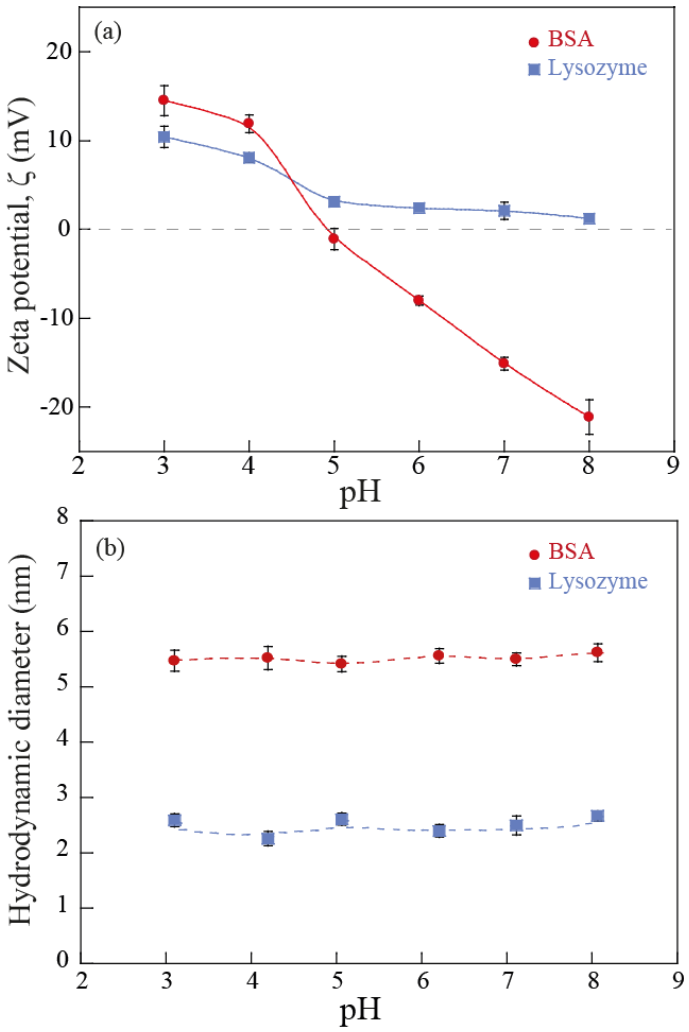

Fig S2. (a) Zeta potential measurement of BSA and lysozyme in 10 mM phosphate buffer as a function of pH. (b) Dynamic light scattering (DLS) measurements also reveal an average hydrodynamic diameter of ~5.5 nm for BSA and ~2.5 nm for lysozyme in solution in this pH range. This represents fairly monomeric forms of the protein molecules in agreement with the previously reported data. Also the protein molecules don't get aggregated in this pH range studied, as there is no visible change in hydrodynamic radius was observed.

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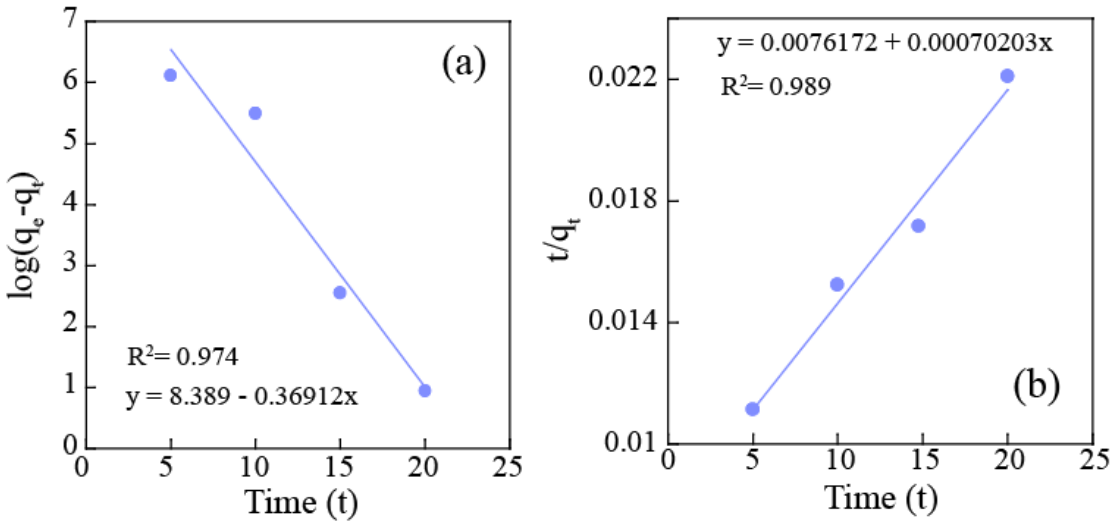

Fig S3. (a) First order and (b) second order kinetic model analysis of lysozyme adsorption on TMA coated surface at pH 7.4 and 25^{o}C. Adsorption kinetics could be better described with second order model.

Table S1. Kinetic rate constants and correlation coefficient of lysozyme adsorption on TMA coated surface.

	First order kinetics		Second order kinetics	
	k_1 (L min^{-1})	R^2	k_2 (g mg^{-1} min^{-1})	R^2
Lysozyme	0.850	0.974	6.4×10^{-5}	0.983

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Fig S4. Freundlich isotherm model of lysozyme adsorption on TMA coated surface. However, the isotherm data fitted better in Langmuir model compared to Freundlich model as discussed in the main text.

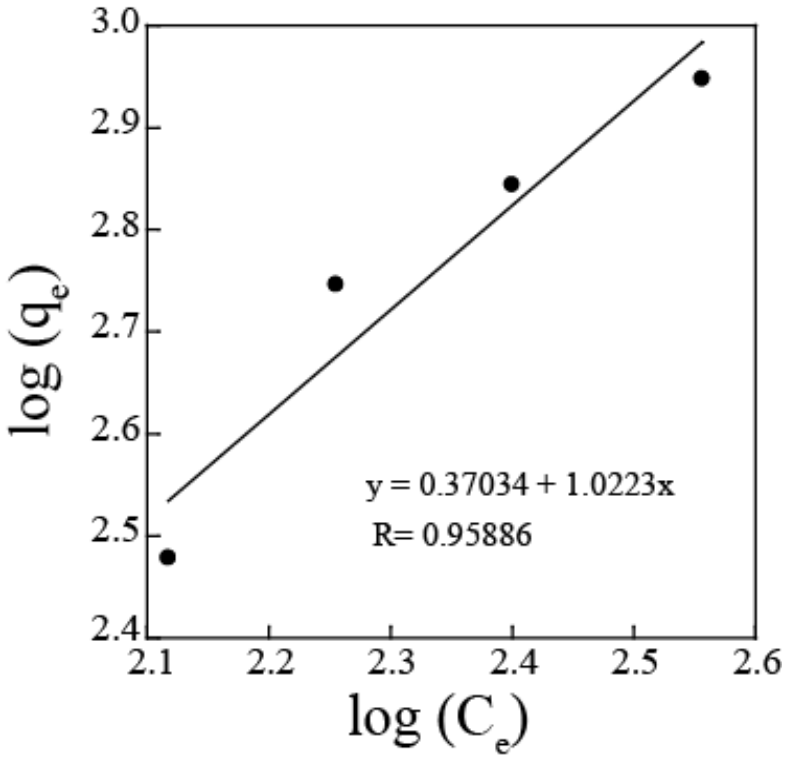

Table S2. Langmuir and Freundlich isotherm parameters of lysozyme adsorption on TMA coated surface.

	Langmuir isotherm				Freundlich isotherm		
	$\Gamma_{Lys,m}$ (mg m^{-2})	b (L mg^{-1})	R^2	R_L	K_f (mg m^{-2})	n	R^2
Lysozyme	2.17	0.0021	0.998	0.86	2.344	0.980	0.958

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Fig S5. The standard average enthalpy change (ΔH^o) and entropy change (ΔS^o) of lysozyme adsorption as function of temperature, was calculated from the Van't Hoff equation[1] given by,

$$\ln(K_c) = (\frac{\Delta S^o}{R}) - (\frac{\Delta H^o}{R})\frac{1}{T}$$

Where, K_c is the equilibrium stability constant, can be calculated from the ratio of lysozyme concentration at solid surface (C_s, mmol m^{-2}) and at aqueous solution (C_e, mmol mL^{-1}).

A linearized plot between ln(K_c) and 1/T is plotted below using the above equation, which was used to calculate the values of ΔH^o and ΔS^o. The average change in entropy (ΔS^o) for lysozyme adsorption was found to be -7.3 J K^{-1} mol^{-1}. The negative value of ΔH^o (-14.9 KJ mol^{-1}) also supports the exothermic nature of the adsorption process.

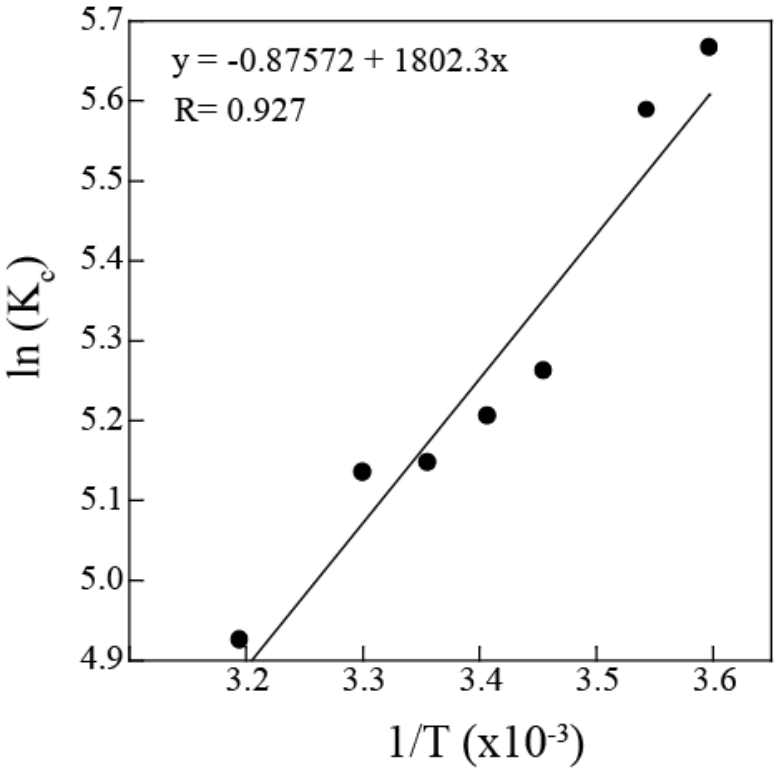

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Table S3. Secondary structural composition of hen egg white lysozyme (HEWL) in solution (PBS, pH 7.4) as measured at different temperature. The helical content of native remains almost same throughout the temperature range. However, the helical content of adsorbed lysozyme at 40oC decrease to some extent at ~19%, which mostly due to the additional interaction with the solid surface during adsorption process.

Lysozyme solution at different temperature	***α-Helix (%)***	***β-Sheet (%)***	***Rest (%)***
5°C	~25	~28	~47
25°C	~25	~32	~43
40°C	~24	~30	~46

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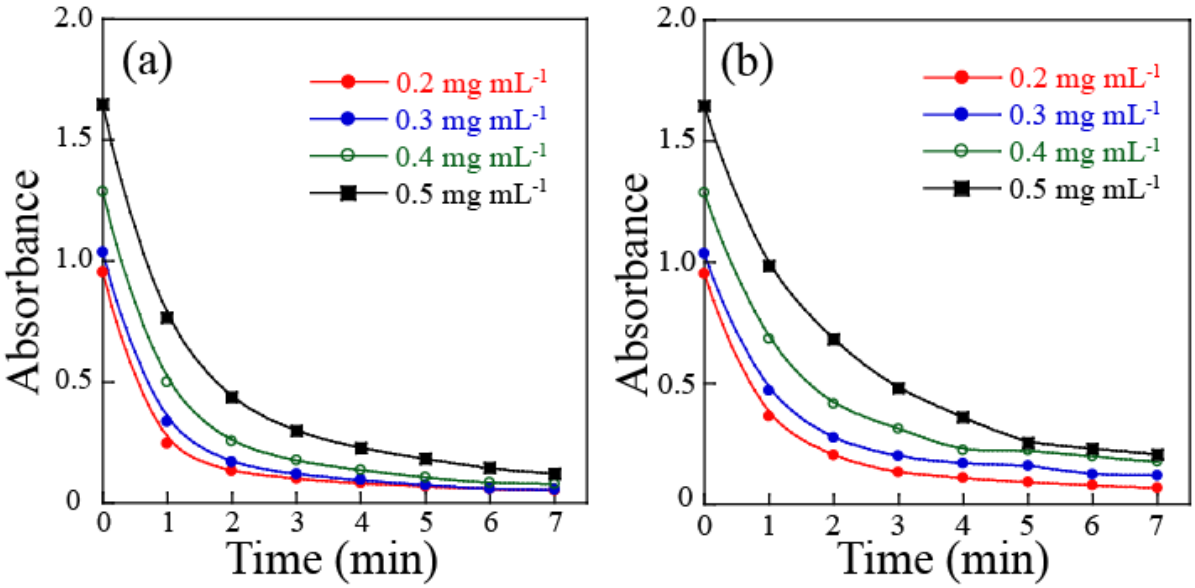
Fig S6. Activity of (a) free and (b) adsorbed lysozyme with different concentration of *M. lysodeikticus* (substrate) solution (0.2-0.5 mg mL^{-1}). The ordinate represents the decay in absorbance due to the lysis of *M. lysodeikticus* by free and adsorbed lysozyme measured for 7 min. We achieve high retention of enzymatic activity of surface lysozyme (~98% of native enzyme), which also persists in different harsh conditions.



Reference:

1. P. Atkins, de Paula, J. Physical Chemistry, 8th ed.; W.H. Freeman & Co., 2006