Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

Understanding How Charge and Hydrophobicity influence Globular Protein Adsorption to Alkanethiol and Material Surfaces

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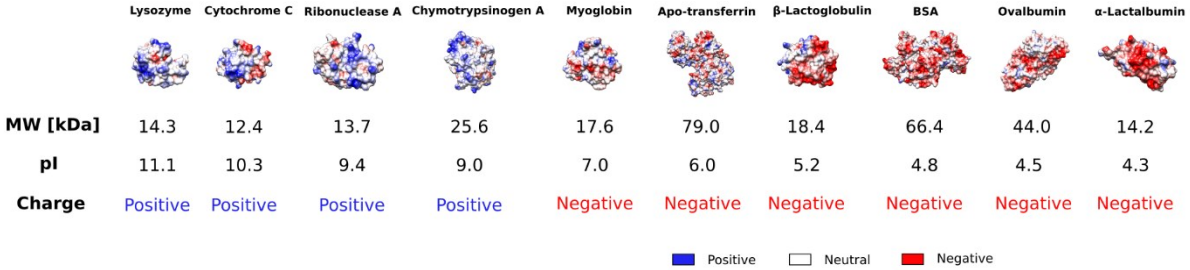

Fig. S1. Summary of the physical properties of the ten globular proteins. Surface rendered images depicting surface exposed charge residues together with molecular weight, isoelectric point (pI) and the net charge on the protein. Surface rendered images of proteins were created using Chimera 1.10.2 and available files from the protein data bank.

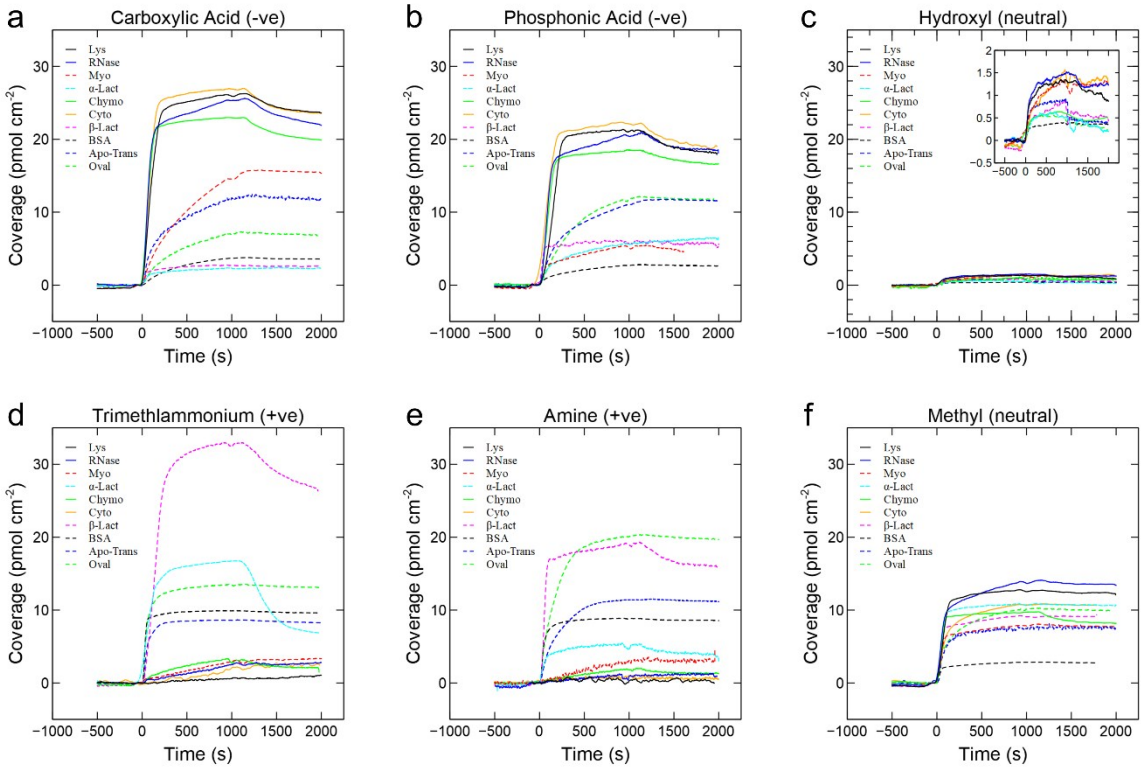

Fig. S2. Protein adsorption of ten globular proteins to six alkanethiol monolayer surfaces measured by Quartz Crystal Microbalance. **a-f,** Adsorption coverage versus time measured for all 60 protein-surface combinations using the Quartz Crystal Microbalance. Baseline obtained in pure buffer and then exchanged for protein solution at t = 0 s. Protein replaced with buffer at t = 1080 s (representative plots).

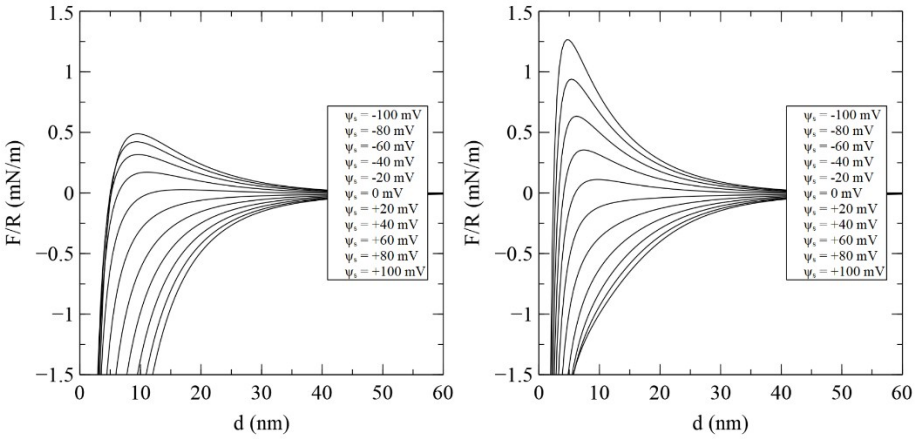

Fig. S3. Simulated force between a charged sphere of radius R and a flat plate in a 1mM monovalent electrolyte. Solution was simulated by solving the non-linear Poisson Boltzmann equation using a numerical method implemented in Matlab R2014a. Boundary conditions were Constant surface potential (left) and constant charge (right) with a Hamaker constant of $1.2 \times 10^{-19} J$. The potential of the sphere was held at ψ_p = -41mV and the plate was varied between -100mV to +100mV. The simulation is in good agreement with the work of Hillier *et al.* [1].

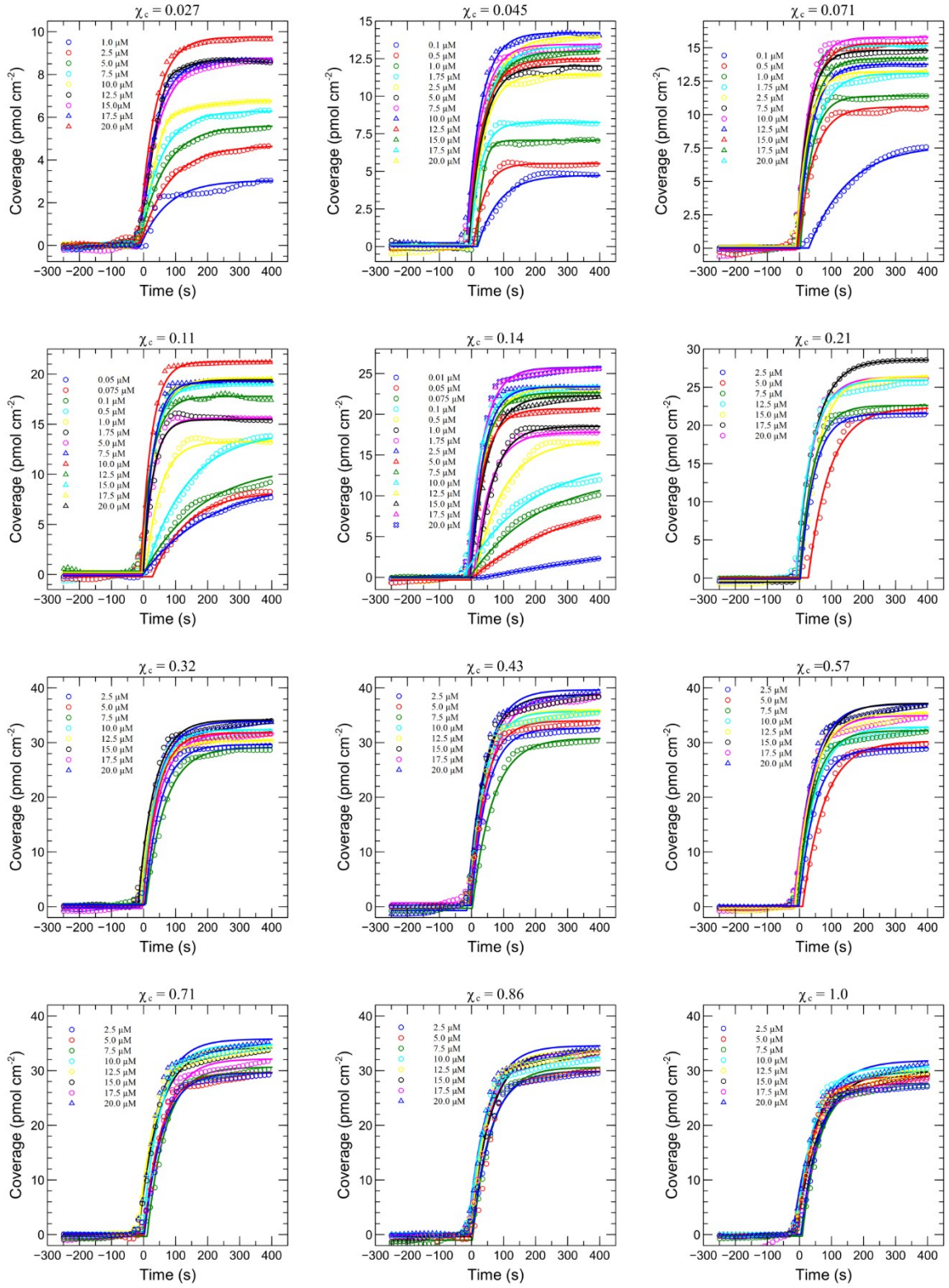

Fig. S4. Coverage versus time plots corresponding to the data shown in Fig. 4c. Solid lines are fits to the Langmuir equation of general form $m = A(1 - e^{-\gamma t})$, where $A = \frac{\beta}{\gamma}$, $\beta = k_a C m_\infty$ and $\gamma = k_a C + k_d$.

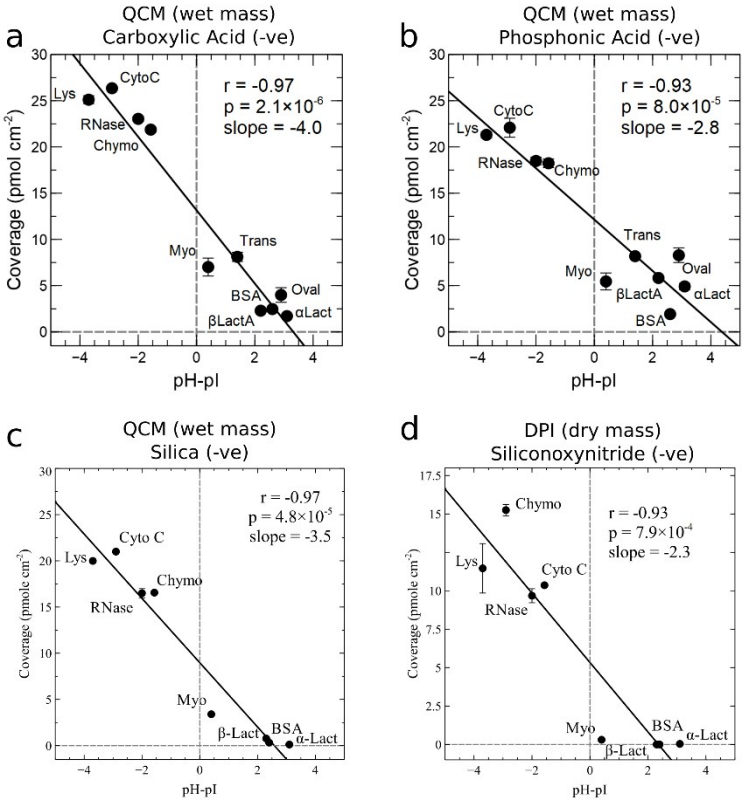

Fig. S5. General trends for protein adsorption to negatively charged surfaces are in agreement for all surfaces studied and independent of measurement technique. a) carboxylic acid terminated alkanethiol on gold; b) Phosphonic acid terminated alkanethiol on gold; c) Silica; d) siliconoxynitride. The general trend is the same when measured by QCM or DPI, except we see a reduced magnitude for DPI measured as expected due to the measurement of 'dry mass' as opposed to 'wet mass'.

Tables

Protein	pI	Reference
Lysozyme	11.1	[2]
Cytochrome C	10.3	[3]
Ribonuclease A	9.4	[2]
Chymotrypsinogen A	9.0	[4]
Myoglobin	7.0	[3]
Apo-transferrin	6.0	[5]
β-Lactoglobulin	5.2	[6]
Bovine Serum Albumin	4.8	[5]
Ovalbumin	4.5	[4]
α-Lactalbumin	4.3	[2]

Supplementary Table 1 | Isoelectric points of globular proteins used in this study together with references.

	dD/(dF/n) ($\times 10^7$)					
	COOH	H_2PO_3	OH	$N(CH_3)_3$	NH_2	CH_3
Lysozyme	-0.17 ± 0.07	0.01 ± 0.02	0.82 ± 0.26	-0.69 ± 1.46	0.31 ± 0.3	0.07 ± 0.01
Cyto-C	-0.08 ± 0.01	0.06 ± 0.07	0.11 ± 0.51	0.38 ± 0.25	-0.69 ± 0.00	-0.01 ± 0.07
RNase	-0.01 ± 0.06	0.06 ± 0.03	1.41 ± 1.15	0.33 ± 0.31	-0.29 ± 0.24	-0.15 ± 0.16
Chymo	-0.02 ± 0.01	-0.01 ± 0.01	-0.26 ± 0.69	0.46 ± 0.07	-1.02 ± 0.48	-0.04 ± 0.25
Myoglobin	0.18 ± 0.08	-0.21 ± 0.51	-0.44 ± 1.63	0.48 ± 0.11	0.09 ± 0.12	0.10 ± 0.04
Apo-Trans	0.71 ± 0.07	0.64 ± 0.01	1.33 ± 0.4	0.22 ± 0.04	0.31 ± 0.04	0.36 ± 0.06
β-Lact	0.32 ± 0.05	0.02 ± 0.03	0.37 ± 0.44	0.09 ± 0.06	0.01 ± 0.04	0.03 ± 0.03
BSA	0.66 ± 0.01	0.22 ± 0.05	0.23 ± 0.71	0.08 ± 0.01	0.17 ± 0.03	0.12 ± 0.02
Ovalbumin	1.75 ±0.21	1.12 ± 0.06	0.5 ± 0.17	0.23 ± 0.01	0.3 ± 0.00	0.56 ± 0.11
α-Lact	-0.00 ± 0.09	0.01 ± 0.05	0.36 ± 0.21	0.02 ± 0.04	-0.15 ±0.04	0.01 ± 0.01

Supplementary Table 2 | Confirmation that the Sauerbrey equation is valid. Data corresponding to Fig. 2, frequency (F) and dissipation (D) sampled at t = 400s. When dD/(dF/n) $\ll 4 \times 10^{-7}$ the Sauerbrey equation is valid.

	Frequency, dF/n (Hz)					
	COOH	H_2PO_3	OH	$N(CH_3)_3$	NH_2	CH_3
Lysozyme	-19.7 ± 0.7	-17.0 ± 0.2	-1.4 ± 0.3	-0.6 ± 0.5	-1.2 ± 0.7	-8.6 ± 0.7
Cyto-C	-18.7 ± 0.2	-14.5 ± 1.3	-0.3 ± 0.2	-2.9 ± 0.9	-0.2 ± 0.3	-8.3 ± 0.7
RNase	-17.7 ± 0.4	-14.5 ± 0.2	-1.3 ± 0.2	-1.2 ± 0.3	-0.9 ± 0.5	-8.5 ± 1.5
Chymo	-31.6 ± 0.3	-25.8 ± 0.3	-0.6 ± 0.4	-3.2 ± 0.4	-1.0 ± 0.15	-13.0 ± 1.2
Myoglobin	-7.1 ± 1	-5.3 ± 0.9	-0.7 ± 0.3	-1.9 ± 0.3	-1.5 ± 0.2	-6.4 ± 1.0
Apo-Trans	-36.9 ± 2.6	-36.6 ± 0.7	-3.7 ± 1.2	-40.0 ± 0.9	-41.6 ± 0.5	-34.1 ± 3.8
β-Lact	-2.6 ± 0.3	-6.3 ± 0.6	-0.28 ± 0.5	-24.9 ± 3.6	-21.5 ± 1.2	-15.1 ± 5.9
BSA	-9.3 ± 0.2	-7.5 ± 0.8	-0.45 ± 0.7	-35.4 ± 0.4	-32.8 ± 0.8	-11.3 ± 1.0
Ovalbumin	-9.4 ± 2.4	-20.4 ± 1.8	-1.7 ± 0.7	-31.0 ± 0.4	-41.6 ± 1.2	-17.2 ± 2.3
α-Lact	-1.3 ± 0.1	-3.7 ± 0.2	-0.4 ± 0.1	-13.0 ± 1.2	-4.2 ± 0.5	-8.6 ± 0.3

Supplementary Table 3 | Raw frequency data corresponding to Fig. 2 sampled at t = 400s, n = 5.

	Mass (ng cm^{-2})					
	COOH	H_2PO_3	OH	$N(CH_3)_3$	NH_2	CH_3
Lysozyme	357.0 ± 6.9	303.9 ± 4.3	25.5 ± 4.2	7.1 ± 0.5	0.8 ± 1.9	146.2 ± 14.8
Cyto-C	326.4 ± 2.3	273.1 ± 8.8	13.1 ± 1.8	41.9 ± 15.9	8.6 ± 1.4	146.5 ± 11.7
RNase	314.3 ± 3.8	252.0 ± 5.3	19.9 ± 2.0	15.0 ± 3.2	8.3 ± 5.6	146.2 ± 25.0
Chymo	559.0 ± 6.4	466.1 ± 6.3	11.7 ± 2.6	48.2 ± 4.2	14.9 ± 3.7	232.8 ± 22.1
Myoglobin	121.1 ± 16.5	95.9 ± 16.4	15.7 ± 2.2	28.8 ± 4.0	24.0 ± 0.5	112.4 ± 16.4
Apo-Trans	648.8 ± 41.4	647.8 ± 12.6	61.3 ± 12.2	700.6 ± 17.3	738.9 ± 10.2	521.0 ± 28.4
β-Lact	41.4 ± 1.4	105.8 ± 2.2	8.6 ± 2.4	442.3 ± 60.7	370.9 ± 21.0	160.6 ± 13.5
BSA	163.3 ± 5.3	126.0 ± 1.1	16.0 ± 3.0	635.2 ± 6.9	586.6 ± 9.5	196.3 ± 15.0
Ovalbumin	176.4 ± 36.1	365.3 ± 34.6	23.9 ± 6.1	553.3 ± 12.7	741.0 ± 15.2	305.4 ± 38.3
α-Lact	24.3 ± 2.5	69.2 ± 4.6	6.1 ± 1.4	228.8 ± 5.4	62.7 ± 2.6	153.4 ± 4.8

Supplementary Table 4 | Mass data corresponding to Fig. 2 sampled at t = 400s, n = 5.

	Molar coverage (p mol cm^{-2})					
	COOH	H_2PO_3	OH	$N(CH_3)_3$	NH_2	CH_3
Lysozyme	25.0 ± 0.5	21.3 ± 0.3	1.8 ± 0.3	0.5 ± 0.0	0.1 ± 0.1	10.2 ± 1.0
Cyto-C	26.3 ± 0.2	22.0 ± 0.7	1.1 ± 0.1	3.4 ± 1.3	0.7 ± 0.1	11.8 ± 0.9
RNase	23.0 ± 0.3	18.4 ± 0.4	1.5 ± 0.2	1.1 ± 0.2	0.6 ± 0.4	10.7 ± 1.8
Chymo	21.8 ± 0.3	18.2 ± 0.3	0.5 ± 0.1	1.9 ± 0.2	0.6 ± 0.1	9.1 ± 0.9
Myoglobin	6.9 ± 0.9	5.5 ± 0.9	0.9 ± 0.1	0.6 ± 0.2	1.4 ± 0.0	6.4 ± 0.9
Apo-Trans	8.2 ± 0.5	8.2 ± 0.2	0.8 ± 0.2	8.9 ± 0.2	9.4 ± 0.1	6.6 ± 0.4
β-Lact	2.3 ± 0.1	5.8 ± 0.1	0.5 ± 0.1	24.0 ± 3.3	20.2 ± 1.1	8.7 ± 0.7
BSA	2.5 ± 0.1	1.9 ± 0.0	0.2 ± 0.0	9.6 ± 0.1	8.8 ± 0.1	3.0 ± 0.2
Ovalbumin	4.0 ± 0.8	8.3 ± 0.8	0.5 ± 0.1	12.6 ± 0.3	16.8 ± 0.3	6.9 ± 0.9
α-Lact	1.7 ± 0.2	4.9 ± 0.3	0.4 ± 0.1	16.1 ± 0.4	4.4 ± 0.2	10.8 ± 0.3

Supplementary Table 5 | Molar coverage data corresponding to Fig. 2 sampled at t = 400s, n = 5.

References

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