Electronic Supplementary Information

Distinct interfacial ordering of liquid crystals observed by protein-lipid interactions that enabled the label-free sensing of cytoplasmic protein at liquid crystal-aqueous interface

Manisha Devi,[‡] *Indu Verma,*[‡] *and Santanu Kumar Pal**

Department of Chemical Sciences, Indian Institute of Science Education and Research Mohali (IISERM), Knowledge City, Sector-81, SAS Nagar, Mohali 140306, Punjab, India.

***Corresponding author**

Email: skpal@iisermohali.ac.in, santanupal.20@gmail.com

[‡]Joint first author

Table of Contents

Materials

Chemicals such as 4-cyano-4'-pentylbiphenyl (5CB) liquid crystal (LC), lysophosphatidic acid (LPA), bovine serum albumin (BSA), lactoferrin (Lf), lysozyme (Lys), thioflavin T (ThT), *N*,*N*-dimethyl-*n*-octadecyl-3-aminopropyltrimethoxysilyl chloride (DMOAP), hydrochloric acid (HCl), sodium hydroxide (NaOH), tris buffered saline (TBS) (pH 7.4), sodium dodecyl sulfate (SDS) and hexadecyltrimethylammonium bromide (CTAB) were purchased from Sigma-Aldrich (St. Louis,MO). Sulfuric acid (H_2SO_4), chloroform and hydrogen peroxide (H_2O_2, 30% *w/v*) were purchased from Merck (Mumbai, India). 1,2-dilauroyl-*sn-glycero*-3-phosphocholine (DLPC) and 1,2-dioleoyl-*sn-glycero*-3-phospho-(1′-myo-inositol-4′,5′-bisphosphate) ammonium salt (PIP_2) were purchased from Avanti Polar Lipids, Inc (Alabaster, AL). Ethanol was obtained from Changshu Hongsheng Fine Chemical Co., Ltd. Deionization of distilled water (DI water) source was performed by a Milli-Q-system (Millipore, Bedford, MA). Glass microscopic slides (Fisher's Finest Premium Grade) were purchased from Fischer Scientific (Pittsburgh, PA). Gold transmission electron microscopy (TEM) grids (20 μm thickness, 50 μm wide bars, 283 μm grid spacing) were obtained from Electron Microscopy Sciences (Fort Washington, PA). Silicone isolator (4.5 mm in diameter and 1.7 mm in depth) was received as a gift from Dr. Yang of Tshingua University.

Juxtamembrane (residue 645-660, sequence of our interest) of epidermal growth factor receptor (JM-EGFR) was purchased from Genxbio Health Sciences Pvt. Ltd. The sequence of protein is "RRRHIVRKRTLRRLLQ".

Experimental methods

Preparation of aqueous solutions of phospholipids and proteins

Stock solutions of different proteins

Accurately weighted solid proteins (BSA, Lf and Lys) were dissolved in 1 mM TBS (pH 7.4) to prepare stock solution (1 mM) of different proteins which were further diluted to required concentration with 1 mM TBS. An aqueous solution of JM-EGFR was prepared by diluting the stock solution (26 μM) in 1 mM TBS.

Stock solutions of different amphiphiles

Dispersion of phospholipids (DLPC and LPA) vesicles were prepared according to the reported procedure.[1] The details are reproduced below for reader's convenience.

"Briefly, the dispersion of DLPC dissolved in chloroform (25 mg mL^{-1}) was taken in round bottom flasks. The chloroform was evaporated under high vacuum for 3 h which results in the formation of lipid thin film along the inner walls of the flask. A stream of nitrogen was passed over the lipid film for 30 min. The dried lipid was resuspended in exact amount of TBS buffer (1 mM) to achieve the final concentration (1 mg mL^{-1}) of stock solution. The solution was vortexed for 1 min resulting in cloudy solution indicating the formation of large multilamellar vesicles (LMV). Subsequent sonication of lipid suspension using a probe ultrasonicator (1 × 15 min at 25 W) resulted in a clear solution which was used for further experiments."

LPA vesicles were prepared by dissolving accurately weighed LPA in minimum amount of solvent (chloroform:MeOH, 3:1, *v/v*). After this, similar method was followed as stated above for preparation of vesicles of DLPC. The stock solutions of PIP_2, CTAB and SDS were prepared by dissolving accurately weighed solid in TBS buffer.

Note: All solutions and lipid vesicles were prepared freshly.

Pretreatment of glass slides with DMOAP

Glass slides were cleaned with piranha solution and subsequently modified with DMOAP according to previously reported procedure.[2-4]

Preparation of LC films and optical cells

LC films and optical cells were prepared according to the previously reported methods.[3-5] The details are reproduced below for reader's convenience.

"The cleaned TEM gold grids were placed on the DMOAP modified glass surface. Then, uniform LC film of 20 μm thickness was prepared by dispensing 1 μL of 5CB onto the gold grid (20 μm thick).[3,4] A silicon isolator was placed on the glass slide and pressed to adhere to the glass slide, to avoid the leakage of LC."[5]

For experiments of protein adsorption at lipid laden LC-aqueous interface, first LC film was incubated with 30 μL of aqueous solution of lipid/surfactant for 5 min followed by addition of approximately 2 μL of protein from a stock aqueous solution to obtain a desired final concentration. For fluorescence microscopy, the above mentioned procedure was repeated for protein (final concentration of protein was 300 nM) adsorption followed by addition of 5 μL of stock solution of ThT (final concentration of ThT in well was 20 μM) and incubated for 15 min. The aqueous phase (which contains excess of lipid, protein and THT) was exchanged with 1 mM TBS to remove the background fluorescence.

Polarized optical microscopy and epifluorescence microscopy

The details of the instrument are taken from our previously reported papers.[3,4] The details are reproduced below for reader's convenience.

"The orientation of LCs was analysed under a Zeiss polarizing microscope (Scope. A1) which is equipped with crossed polars with objectives of magnification 50X and 100X. For fluorescence imaging, the samples were viewed under a Zeiss (Scope. A1) fluorescence microscope using a fluorescence filter cube with a 460 nm excitation filter and a 534 nm emission filter. Images were obtained with an AxioCam camera. Average grayscale intensity of the images obtained from polarized microscope was measured by processing atleast 4 squares of TEM grid using ImageJ free access software (developed by U. S. National Institutes of Health, Bethesda, MD)."[3,4]

Circular dichroism measurements

The details of the instrument are taken from our previously reported papers.[3] The details are reproduced below for reader's convenience.

"The circular dichroism (CD) experiments were performed on a Chirascan spectrophotometer (Applied Photophysics, U.K.) in a scan range of 200−280 nm and step size of 1 nm. A quartz cell (1 mm path length) was used for recording the CD spectra of solution. For each sample, the spectra were averaged over 5 scans and were corrected against the buffer signal using the ProData software provided with the CD instrument."[3] Aqueous solutions of phospholipids (DLPC/LPA) and proteins (JM-EGFR, BSA, Lf and Lys) were freshly prepared in 1 mM TBS (pH 7.4). Phospholipids (DLPC/LPA) were incubated with respective proteins for 1 h before recording the CD spectra.

CD is an excellent tool in the field of structural biology to investigate the secondary structure of proteins.[6] In CD spectra, the relatively large negative values of ellipticity correspond to α-helical structure and smaller negative ellipticity values correspond to β-sheet. α-helical structures are highly stable and regular with well-defined angles ϕ and ψ, hence produce very intense CD spectra with characteristic positive band at 193 nm and large negative bands at 222 nm and 208 nm. However, β-sheet structures have both parallel and antiparallel orientations of adjacent strands and different twists, therefore, angles ϕ and ψ vary considerably, hence proteins with β-sheet structures produce less intense CD spectra with a characteristic positive band at 195 nm and a negative band at 218 nm. A random coil (disordered protein) shows very low ellipticity above 210 nm and negative bands near 195 nm.[6]

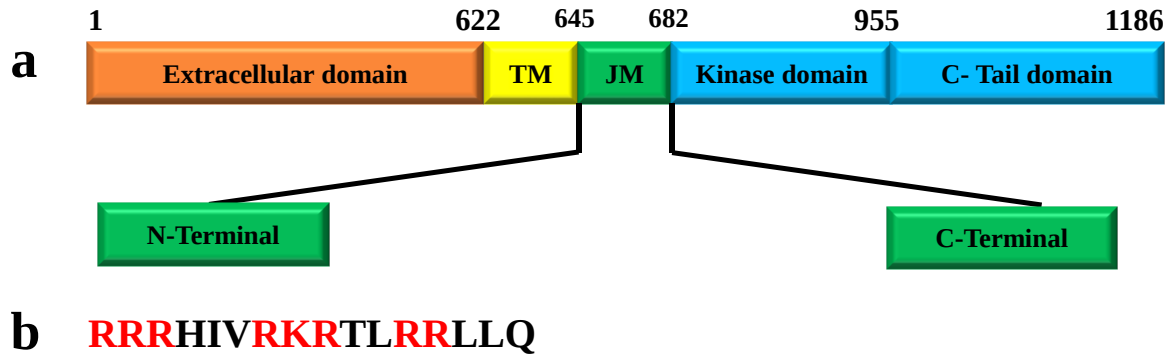


Fig. S1. (a) Schematic representation of topological structure of different domains of EGFR (1-1186). (b) Amino acid sequence of N-terminal portion of juxtamembrane (JM) (sequence of our interest). Amino acids denoted in red color represent basic amino acids.

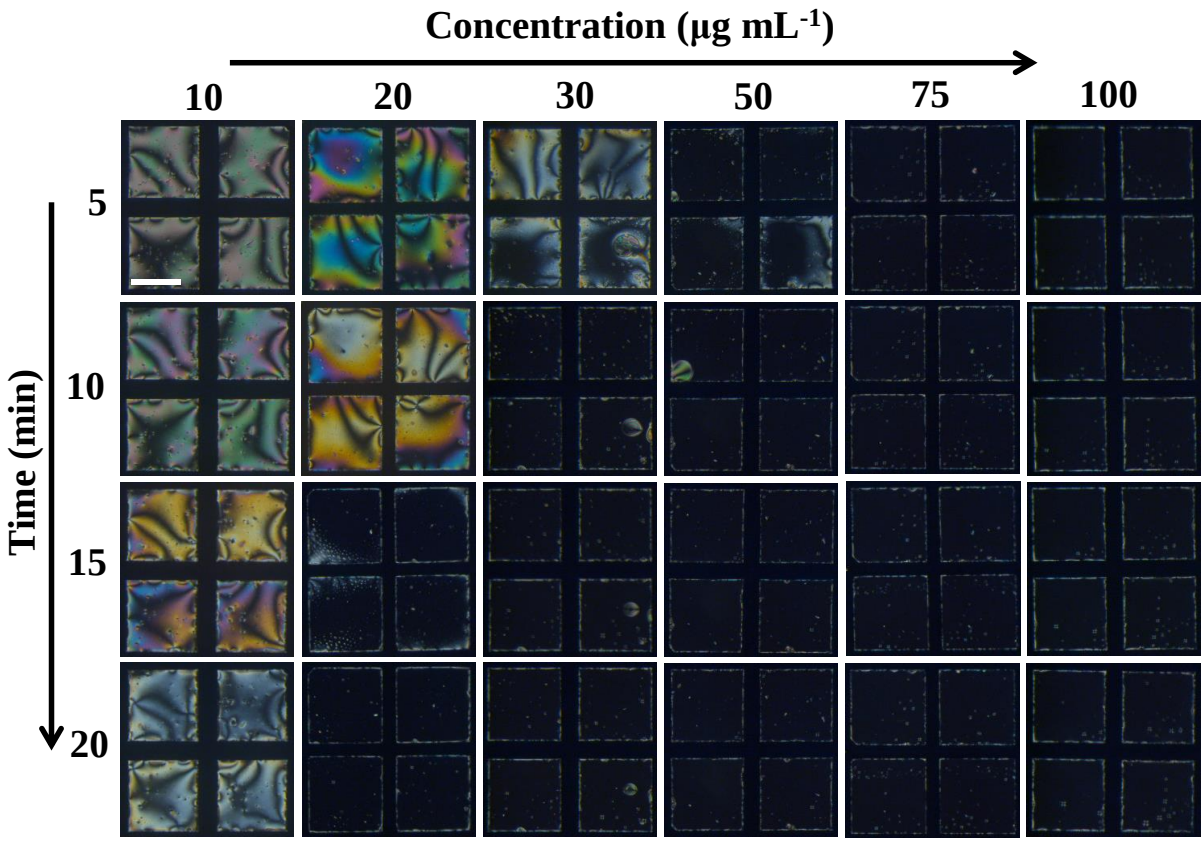


Fig. S2. Polarized optical microscopy images of the LC-aqueous interface in contact with different concentrations of DLPC (10-100 μg mL^{-1}) at different time intervals. Scale bar = 200 μm.

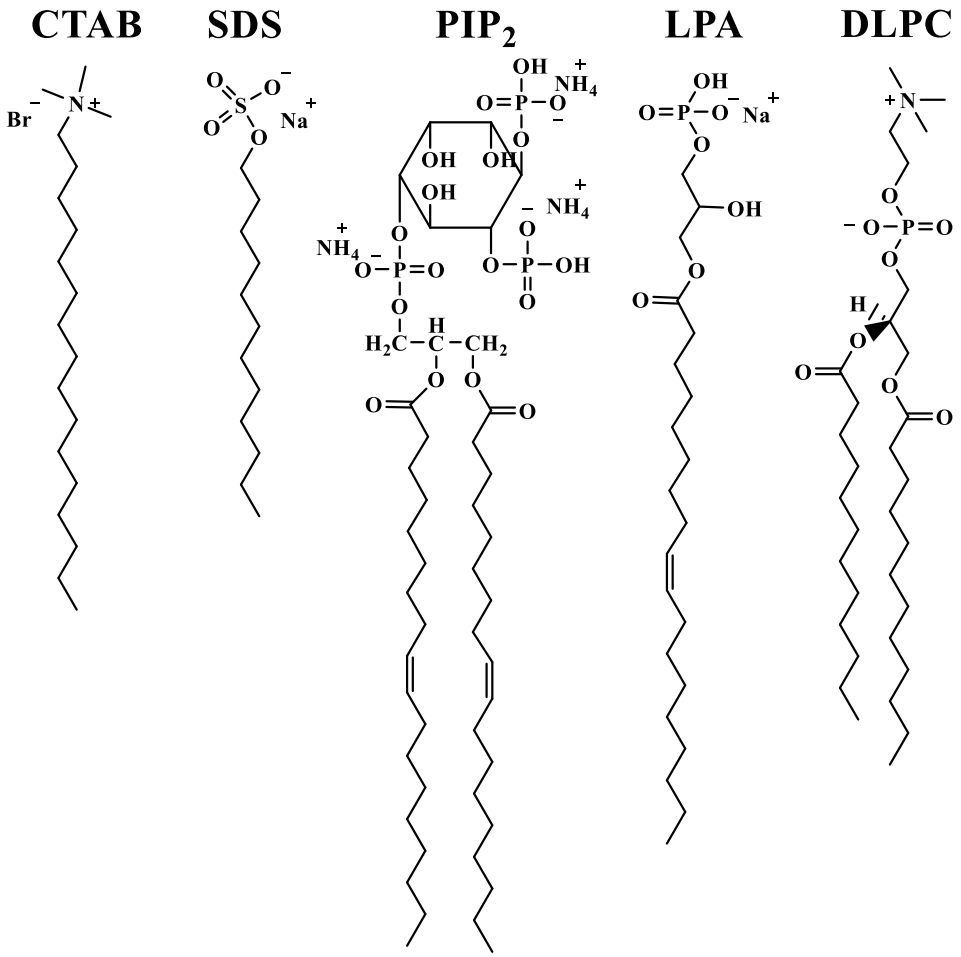


Fig. S3. Chemical structures of surfactants and lipids used in present study.

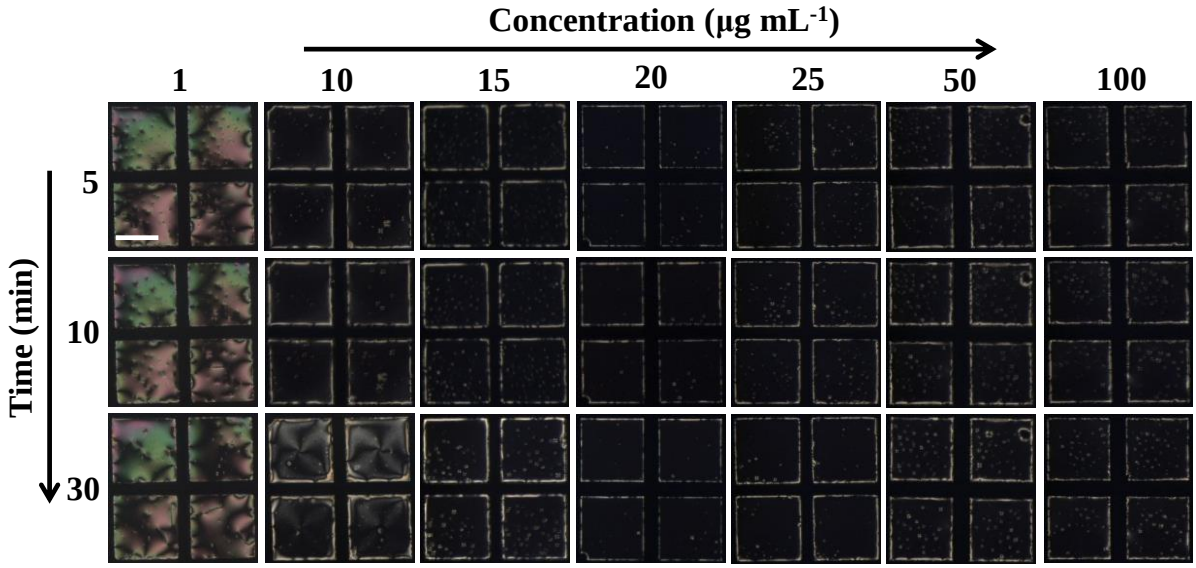


Fig. S4. Polarized optical microscopy images of the LC-aqueous interface in contact with different concentrations of LPA (1-100 μg mL^{-1}) at different time intervals. Scale bar = 200 μm.

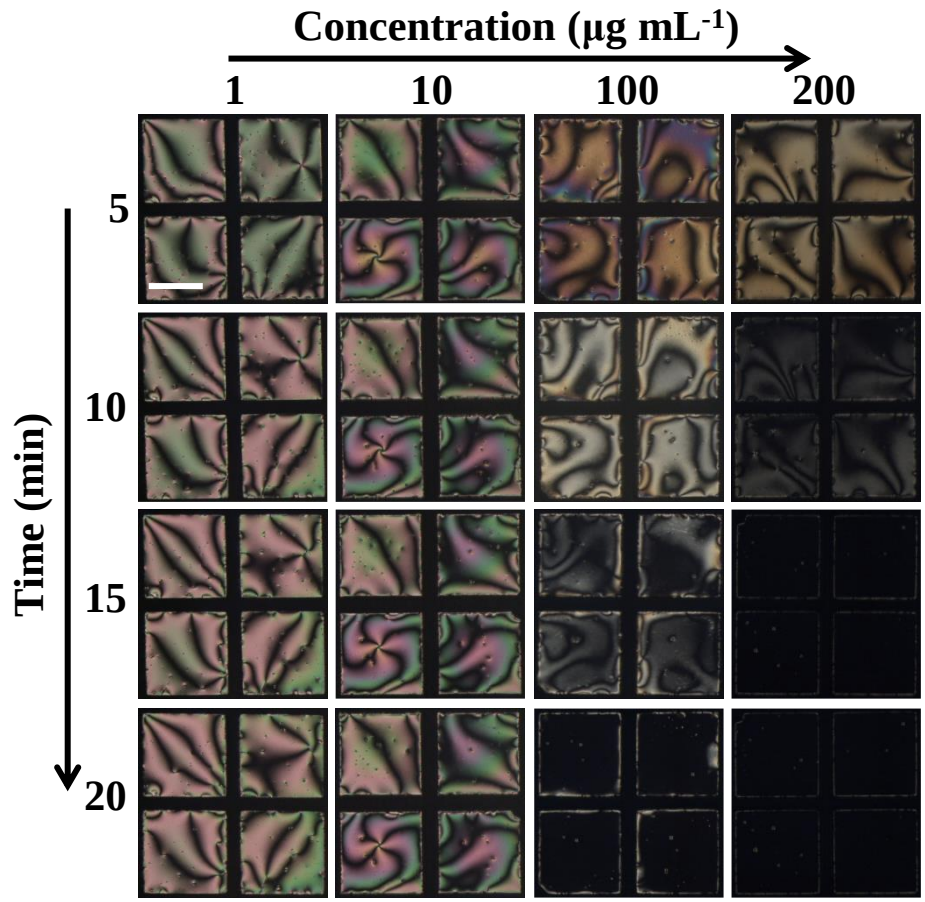


Fig. S5. Polarized optical microscopy images of the LC-aqueous interface in contact with different concentrations of PIP_2 (1-200 μg mL^{-1}) at different time intervals. Scale bar = 200 μm.

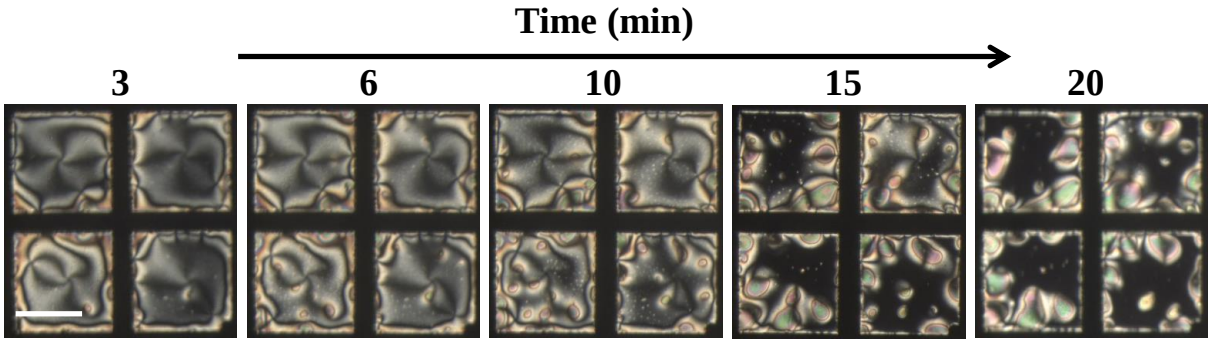


Fig. S6. Polarized optical microscopy images of the LC-aqueous interface in contact with PIP_2 (200 μg mL^{-1}) followed by addition of JM-EGFR (1 μM). Scale bar = 200 μm.

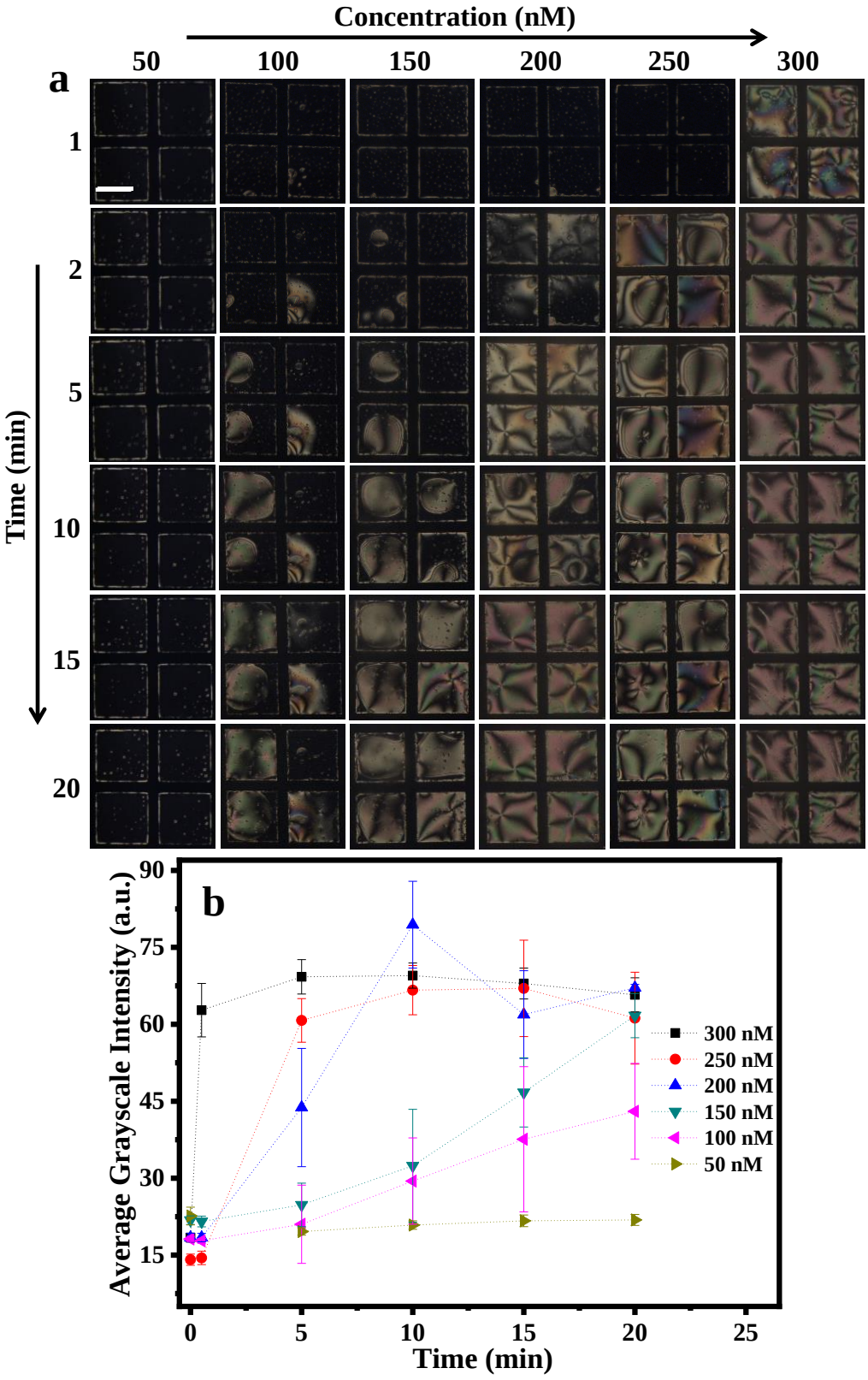


Fig. S7. (a) Polarized optical microscopy images of LPA laden LC-aqueous interface followed by introduction of different concentrations of JM-EGFR. The time indicated on left side follows after addition of JM-EGFR. (b) Average grayscale intensity of polarized optical microscopy images of LPA laden LC-aqueous interface in contact with different concentrations of JM-EGFR (50-300 nM). Scale bar = 200 μm.

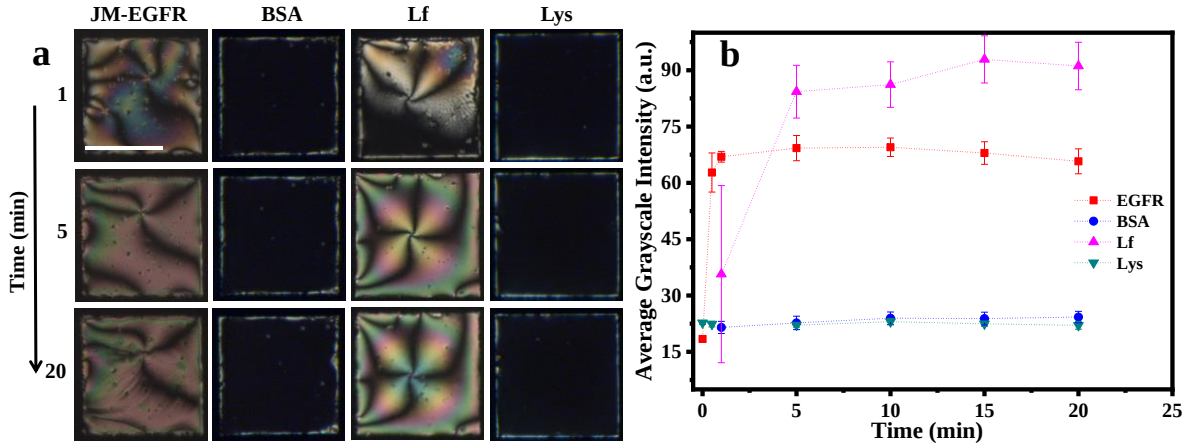


Fig. S8. (a) Polarized optical microscopy images of LPA laden LC-aqueous interface followed by introduction of 300 nM of different proteins (JM-EGFR, BSA, Lf and Lys) at different time intervals. (b) Average grayscale intensity of POM images of LPA laden LC-aqueous interface in contact with different proteins (300 nM). Scale bar = 200 μm.

Circular dichroism (CD) measurements:

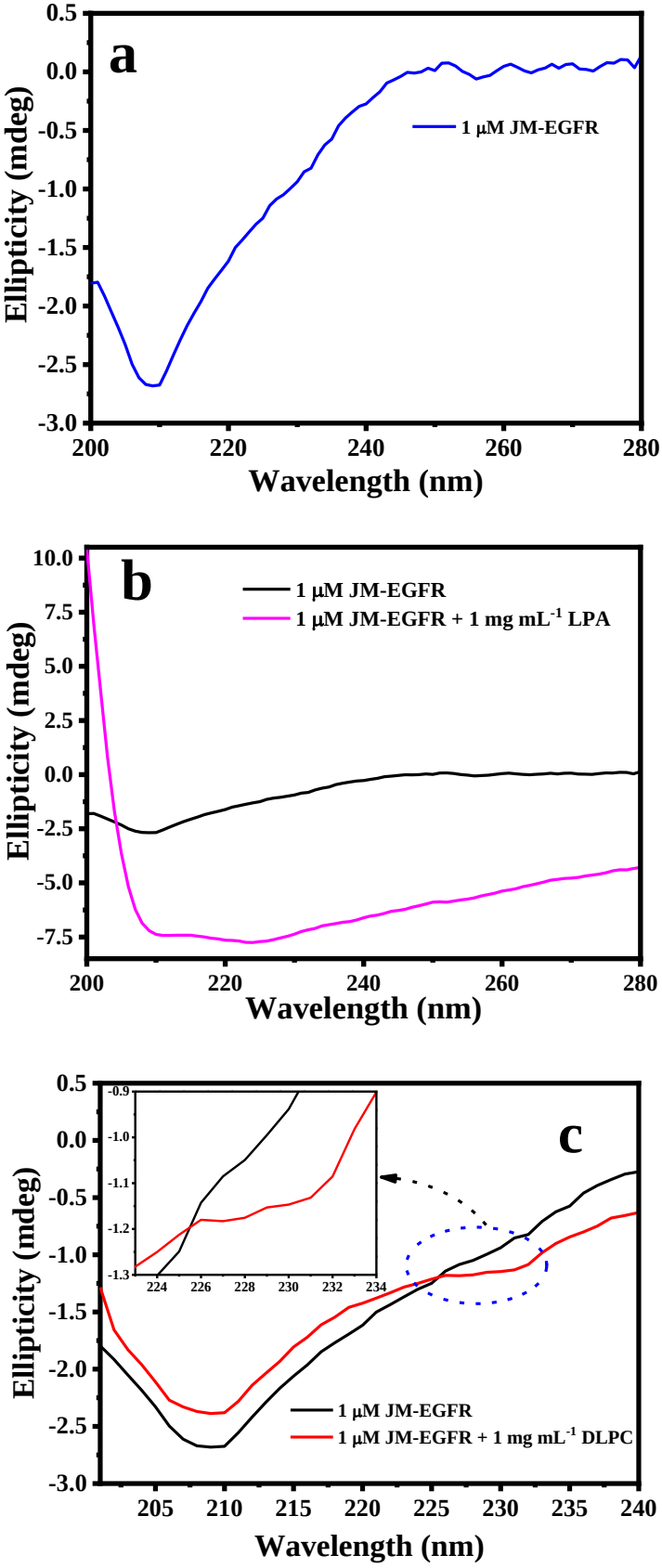
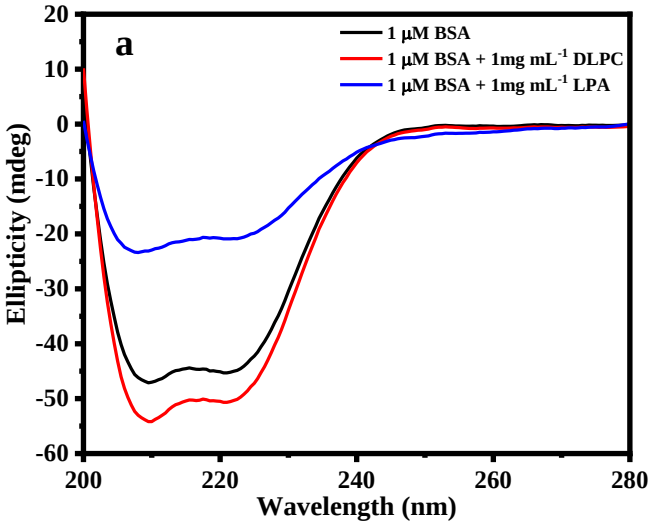
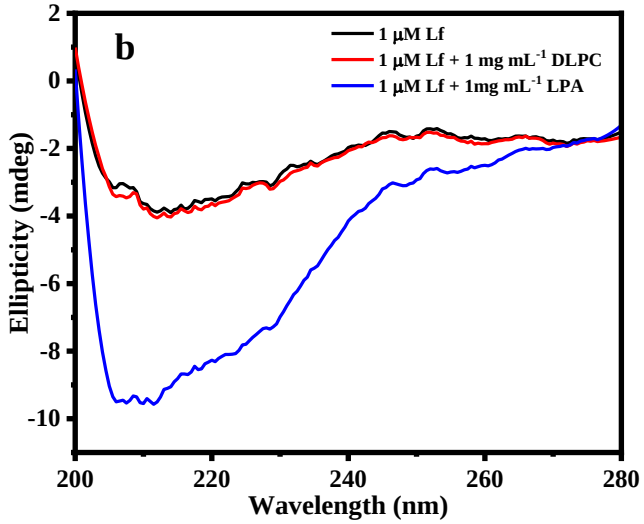


Fig. S9. CD spectra of (a) 1 μM JM-EGFR, (b) 1 μM JM-EGFR in presence and absence of 1 mg mL^{-1} LPA, and (c) 1 μM JM-EGFR in presence and absence of 1 mg mL^{-1} DLPC.

We have also recorded the CD spectra of control proteins (BSA, Lys, and Lf) in presence of DLPC and LPA in bulk solution (Fig. S10). We observed negligible change in the positions of representative peaks of BSA, Lf and Lys in the presence of DLPC and LPA. This suggests that there may not be any major conformational change in the native secondary structure of these proteins in the presence of DLPC and LPA. However, a considerable change was observed in the ellipticity of all these control proteins in presence of LPA as compared to that of DLPC. This suggests that there is less interaction of control proteins with DLPC as compared to LPA which is partially consistent with the observations at LC-aqueous interface.





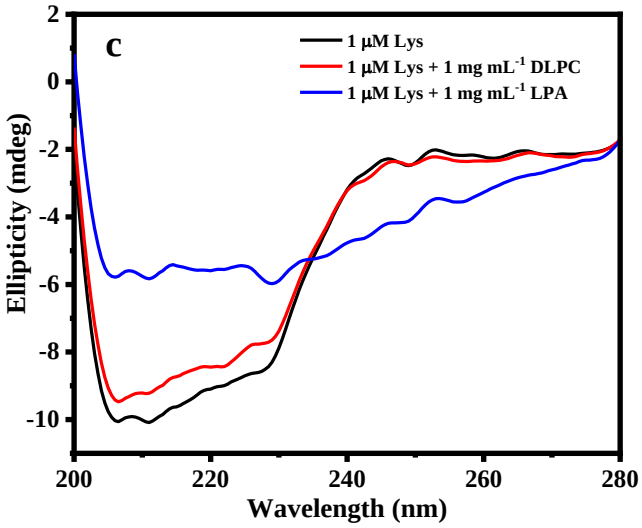


Fig. S10. CD spectra of (a) 1 μM BSA in absence and presence of 1 mg mL^{-1} DLPC and LPA, (b) 1 μM Lf in absence and presence of 1 mg mL^{-1} DLPC and LPA, and (c) 1 μM Lys in absence and presence of 1 mg mL^{-1} DLPC and LPA.

Table S1. Structural and physiological properties of proteins used in present study.

Protein	**Isoelectric point**	**Charge at experimental pH used in this work**	**Molecular weight**	**Secondary structure**	**References**
JM-EGFR	12.88	positive	23 KDa	Random coil	7-9
BSA	4.5	negative	66.4 KDa	α-helix = 60 %	10-12
Lys	11.16	positive	14.3 KDa	α-helix = 34.72 %, β-sheet = 11.72 %, overall = α-helix rich	13-15
Lf	8.7	positive	80 KDa	α-helix = 18.0 %, β-sheet = 57.1 %, overall = β-sheet rich	16, 17

References

1. D. Das and S. K. Pal, *ChemistrySelect*, 2017, **2**, 4779.
2. J. M. Brake, M. K. Daschner, Y.-Y. Luk and N. L. Abbott, *Science*, 2003, **302**, 2094**.**
3. I. Verma, M. Devi, D. Sharma, R. Nandi and S. K. Pal, *Langmuir*, 2019, **35**, 7816.
4. I. Verma, S. L. V. Selvakumar and S. K. Pal, *J. Phys. Chem. C*, 2020, **124**, 780.

5. X. Yang, Y. Tian, F. Li, Q. Yu, S. F. Tan, Y. Chen and Z. Yang, *Langmuir*, 2019, **35**, 2490.
6. L. Whitmore and B. A. Wallace, *Biopolymers*, 2007, **89**, 395.
7. K. Choowongkomon, M. E. Hobert, C. He, C. R. Carlin and F. D. Sönnichsen, *J. Biomol. Struct. Dyn.*, 2004, **21**, 813.
8. T. Sato, P. Pallavi, U. Golebiewska, S. McLaughlin and S. O. Smith, *Biochemistry*, 2006, **45**, 12704.
9. Y. Wang, J. Gao, X. Guo, T. Tong, X. Shi, L. Li, M. Qi, Y. Wang, M. Cai, J. Jiang, C. Xu, H. Ji and H. Wang, *Cell Research*, 2014, **24**, 959.
10. T. J. Peters, All about albumin: biochemistry, genetics, and medical applications. San Diego (CA): Academic Press; 1995.
11. A. J. Esfahlan, A. Qstadrahimi, R. J. Esfahlan, L. Roufegarinejad and R. Amarowicz, *Int. J. Biol. Macromol.*, 2019, **138**, 602.
12. J. Tian, J. Liu, Z. Hu and X. Chen, *Bioorg. Med. Chem.*, 2005, **13**, 4124.
13. L. Sheng, J. Wang, M. Huang, Q. Xu and M. Ma, *Int. J. Bio. Macromol.*, 2016, **92**, 600.
14. M. Kamshad, M. J. Talab, S. Beigoli, A. Sharifirad and J. Chamani, *J. Biomol. Struct. Dyn.*, 2019, **37**, 2030.
15. G. Gao and P. Yao, *J. Polym. Sci. A Polym. Chem.*, 2008, **46**, 4681.
16. X. Y. Wang, H. Y. Guo, W. Zhang, P. C. Wen, H. Zhang, Z. R. Guo and F. Z. Ren, *J. Dairy Sci.*, 2013, **96**, 33.
17. M. Zhang, B. Fang and L. Chen, *Chem. Eng. Trans.*, 2015, **46**, 1399.