Supplementary information

Influence of pH and salt on the ionization state and phase behavior of some aromatic acid derivatives of cholesterol at the air-aqueous interface

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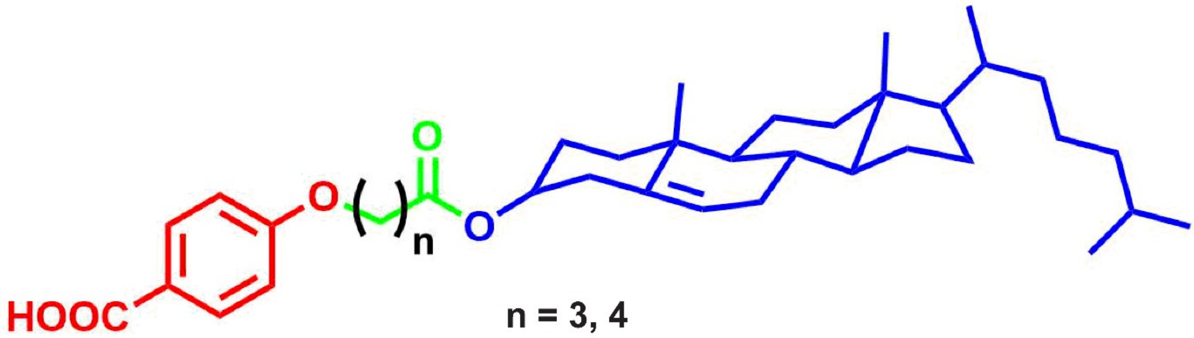

Fig S1. Chemical structure of aromatic acid derivative cholesteryl with different (n + 1) spacers. The hydrophilic aromatic acid group, spacer, and hydrophobic cholesteryl moiety are shown in different colors. The lengths of even (ChBA-C4) and odd (ChBA-C5) spacers are 2.6 and 2.7 nm, respectively.

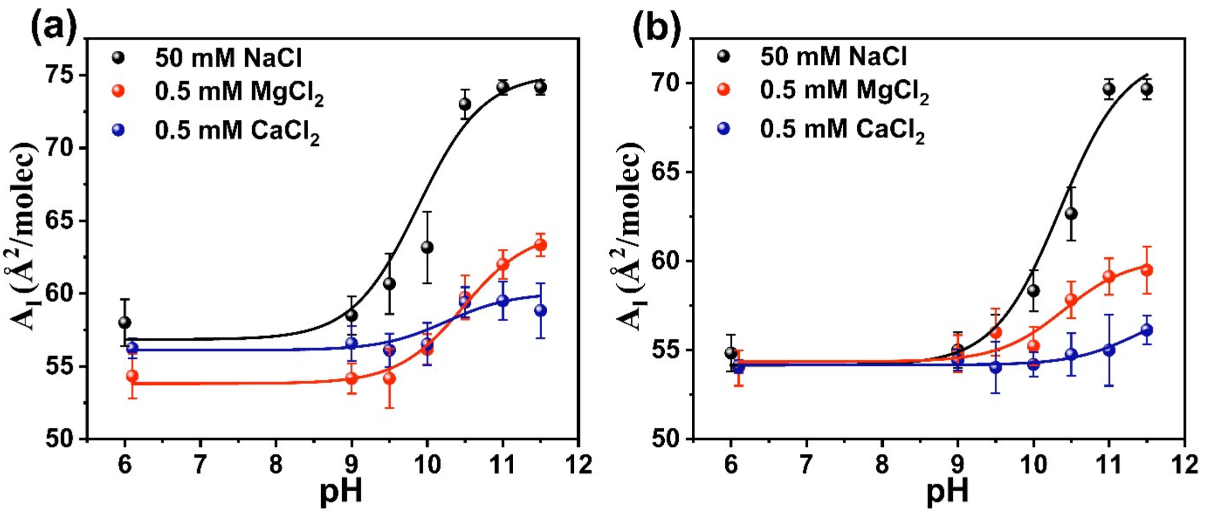

Fig S2. Variation of lift-off area per molecule (A_l) with pH of (a) ChBA-C4 and (b) ChBA-C5 films for subphase containing different concentrations of mono and divalent ions. Each data point represents the average of three independent measurements, with error bars indicating one standard deviation. The continuous curve represents a sigmoidal fit.

Table S1. Apparent surface pK_a (extracted by fitting the lift-off area per molecule, A_l, with pH) values of ChBA-C4 and ChBA-C5 for different mono and divalent ions in the subphase.

S.No	**Subphase**	**ChBA-C4**		**ChBA-C5**	
		Surface pK_a	**R^2**	**Surface pK_a**	**R^2**
1	pH change	10.8 ± 0.1 [1]	0.98	10.1 ± 0.3 [2]	0.94
2	NaCl (50 mM)	10.3 ± 0.2	0.98	9.9 ± 0.2	0.97
3	$MgCl_2$ (0.5 mM)	10.3 ± 0.2	0.94	10.5 ± 0.1	0.99
4	$CaCl_2$ (0.5 mM)	11.3 ± 0.6	0.91	10.3 ± 0.4	0.85

Hysteresis measurements: Compression-decompression (hysteresis) cycles were performed to assess the reversibility and kinetic stability of the ChBA derivatives. After initial compression to a target surface pressure of 10 mN/m, the monolayer was immediately expanded without equilibration, and the cycle was repeated three times. Hysteresis was evaluated based on peak area shifts, quantified by the displacement in molecular area at a target surface pressure. Experiments were carried out at both neutral pH and pH 11 subphases containing NaCl, $MgCl_2$, or $CaCl_2$.

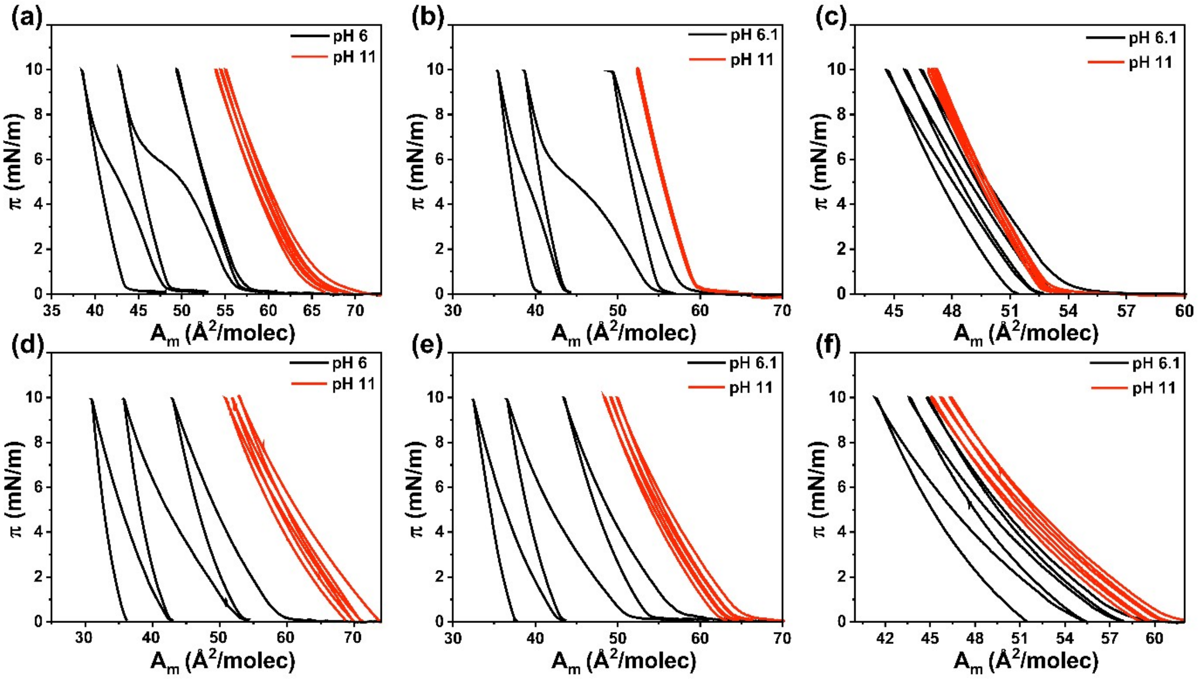

Fig S3. Compression-decompression cycles (three) of surface pressure - area per molecule (π-A_m) isotherms (298 K) of (a-c) ChBA-C4 and (d-f) ChBA-C5 derivatives at different pH. Here, the subphase contains (a, d) 50 mM NaCl, (b, e) 0.5 mM $MgCl_2$, and (c, f) 0.5 mM $CaCl_2$.

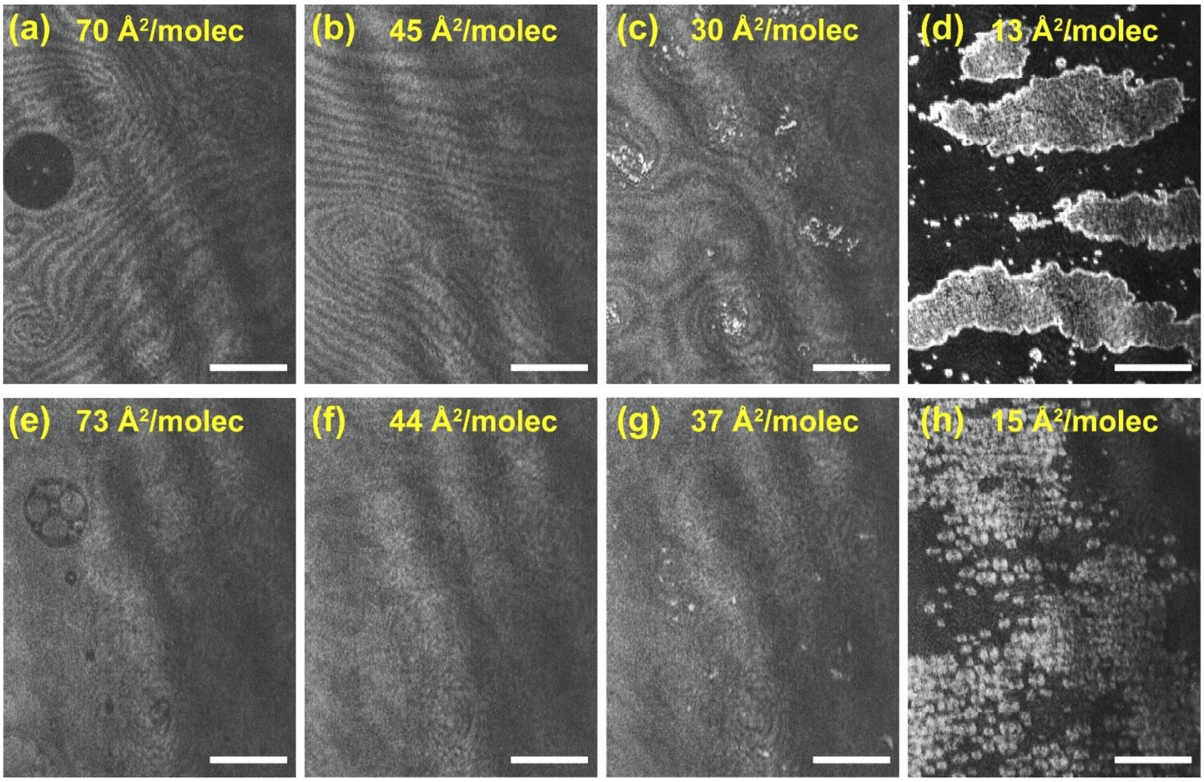

Fig S4. Brewster angle microscopy images of (a-d) ChBA-C4 and (e-h) ChBA-C5 derivatives with neutral pH (0.5 mM $MgCl_2$) solution obtained at different area per molecule. (a) Coexistence of gas (appearing dark), L_1 (grey region), and multilayer exhibiting fringe patterns, (e) Coexistence of gas (appearing dark), L_1 (grey region), and multilayer (brighter regions), (b) predominantly present multilayers (fringe pattern), (f) multilayers (bright regions) coexisting with L_1 phase (grey region) and (c, g) Nucleation of the crystallites (brighter spots) which coexist with multilayers, and (d, h) Coexisting multilayers with predominantly present 3-D crystallites. Scale bar represents 50 µm.

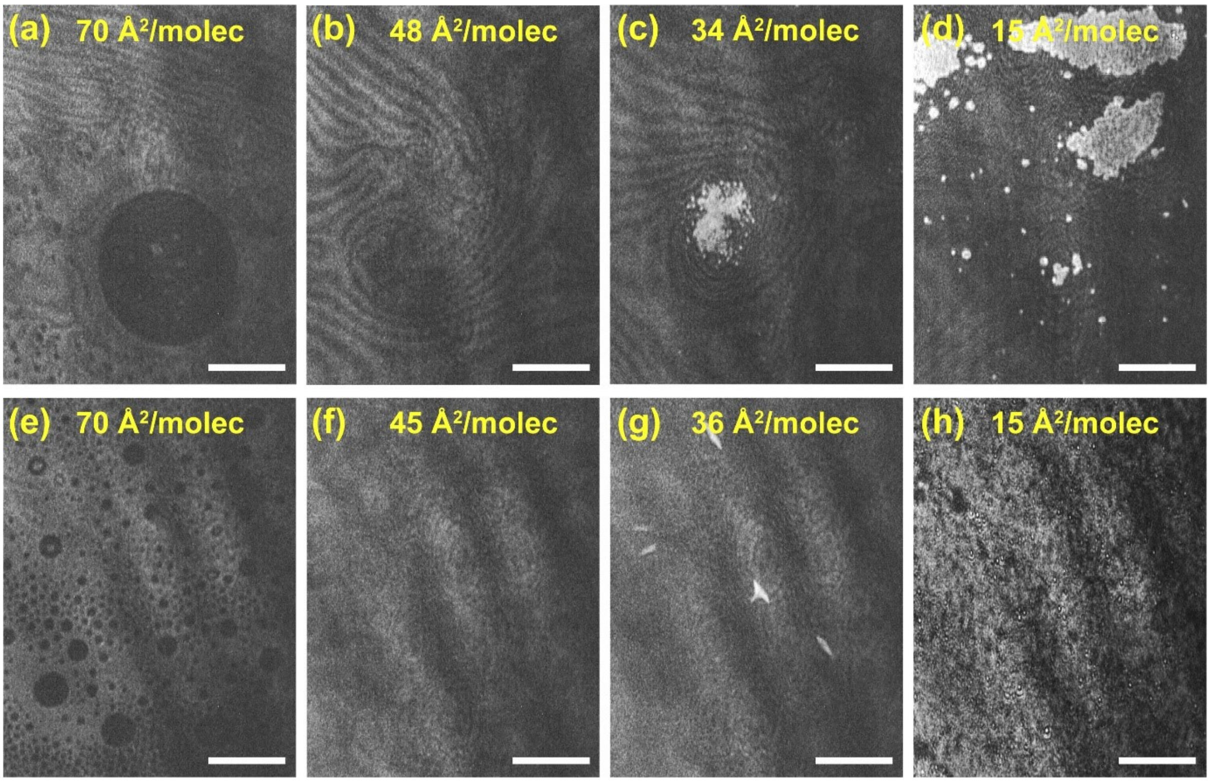

Fig S5. Brewster angle microscopy images of (a-d) ChBA-C4 and (e-h) ChBA-C5 derivatives with neutral pH (0.5 mM $CaCl_2$) solution obtained at different area per molecule. (a) Coexistence of gas (appearing dark), L_1 (grey region), and multilayer exhibiting fringe patterns, (e) coexistence of gas (appearing dark), L_1 (grey region), and multilayer (brighter regions), (b) predominantly present multilayers (fringe pattern), (f) multilayers (bright regions) coexisting with L_1 phase (grey region) and (c, g) Nucleation of the crystallites (brighter spots) which coexist with multilayers, and (d, h) Coexisting multilayers with predominantly present 3-D crystallites. Scale bar represents 50 µm.

References:

1. V. M. T. N. Moram, S. Y. Khatavi, C. V. Yelamaggad and P. Viswanath, Nature of self-assembled films and surface pK_a estimation of some aromatic acid derivatives of cholesterol at interfaces, *J Mol Liq*, 2025, **437**, 128423.
2. V. M. T. N. Moram, S. Y. Khatavi, C. V. Yelamaggad and P. Viswanath, pH-dependent self-assembly of an acid derivative of cholesterol at interfaces, *Colloids Surf A Physicochem Eng Asp*, 2024, **703**, 135247.