SUPPLEMENTAL MATERIAL FOR:

Cholesterol modifies huntingtin binding to, disruption of, and aggregation on lipid membranes*

Xiang Gao[1,§], Warren A. Campbell IV[2,§], Maxmore Chaibva[1], Pranav Jain[1], Ashley E, Leslie[1], Shelli L. Frey[2], and Justin Legleiter[1,3,4]

[1]The C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, WV 26505, USA; [2]Department of Chemistry, Gettysburg College, Gettysburg, PA 17325, USA; 3 NanoSAFE, PO Box 6223, West Virginia University, Morgantown, WV, 26506, USA 4the Center for Neurosciences, West Virginia University, Morgantown, WV 26505, USA.

*Running title: *Cholesterol modifies the interaction of huntingtin with lipid membranes*

[§] X.G. and W.A.C. contributed equally to this work

To whom correspondence should be addressed:
Justin Legleiter, Department of Chemistry, West Virginia University, 217 Clark Hall, Morgantown, WV 26506, USA; Phone: 304-293-0175; Email: justin.legleiter@mail.wvu.edu

The impact of cholesterol on TBLE bilayers.

To understand why the addition of cholesterol to TBLE membranes would affect htt peptide insertion, individual surface pressure versus mean molecular area isotherms were measured for a series of binary mixtures of TBLE and cholesterol and additivity plots constructed (Supplemental Figure 1). Comparison of the mean molecular area of the binary mixtures to that of a theoretical mean area consistent with ideal mixing of the two components provides evidence that exogenous cholesterol causes condensation or a stiffening of the monolayer (Supplemental Figure 1B) which may affect the ability of the htt peptide to insert into the layer.

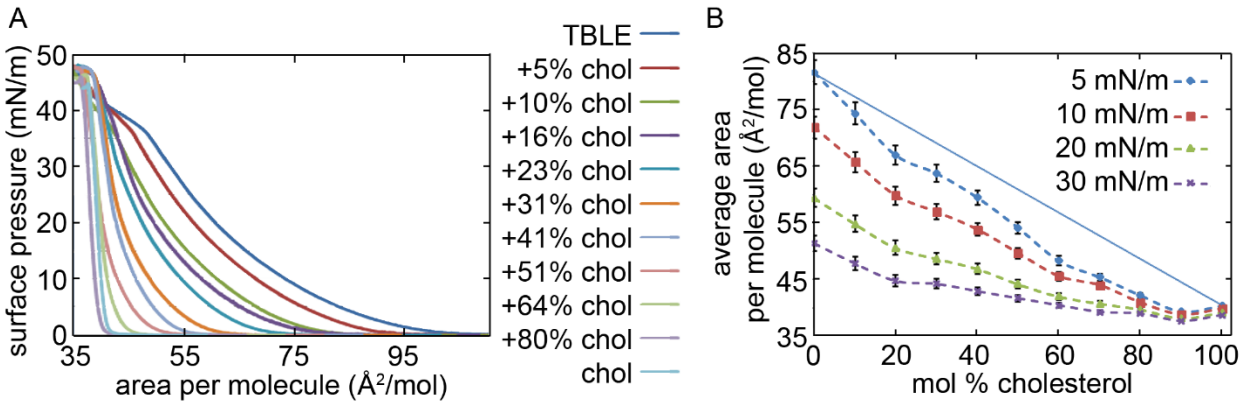
Surface pressure versus molecular area isotherms were measured for TBLE, cholesterol, and binary TBLE/cholesterol monolayers at the air/buffer interface at 30ºC. Supplemental Figure 1A shows the overlap of the resulting isotherms. The isotherm of TBLE shows that it is expanded compared to cholesterol as TBLE lifts-off at a higher area per molecule (~ 100 $Å^2$/mol) and remains in the liquid expanded phase until well above physiological surface pressures, likely due to a high percentage of unsaturated lipid components. Due to its smaller size and planar molecular structure, cholesterol lifts-off at ~ 40 $Å^2$/mol and is condensed until collapse.

To determine how the individual components of TBLE and cholesterol interact with one another in the mixture and affect the material properties of the membrane, the mean molecular area was measured as a function of monolayer composition of a given surface pressure.[1] Supplemental Figure 1B shows the plot of experimental mean molecular area at 5, 10, 20, and 30 mN/m versus the mol% percentage of cholesterol in the TBLE/cholesterol monolayer. The so-called "additivity plot" can indicate possible deviations from ideal mixing. At each surface pressure, a straight line (connecting the area per molecule of the pure components; only shown here at 5 mN/m for clarity) represents "ideal" mixtures with the theoretical area, A_{mix}, given by the additivity equation

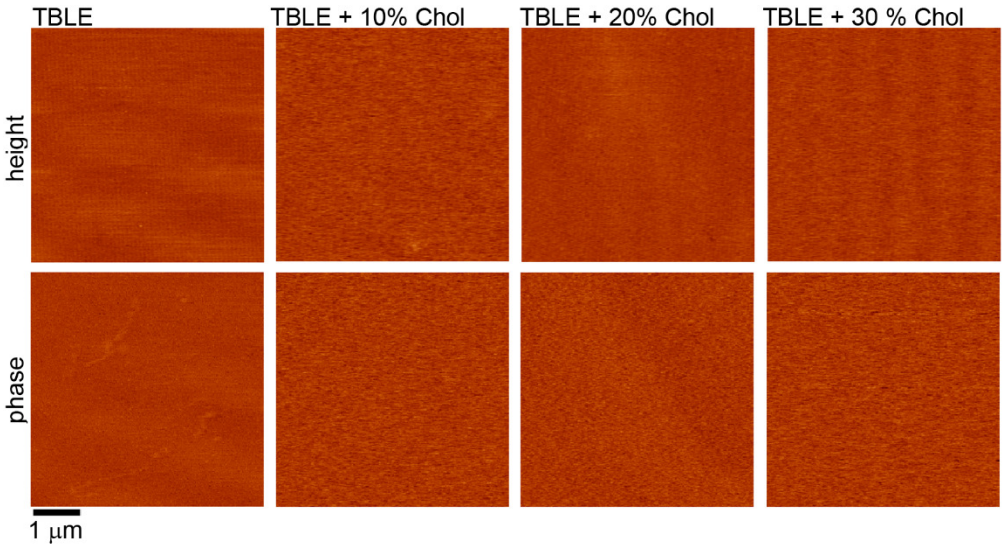
$$A_{mix} = X_{chol}A_{chol} + A_{TBLE}(1-X_{chol}) \qquad (3)$$

where X_{chol} is the molar fraction of cholesterol and A_{chol} and A_{TBLE} are the mean molecular areas of cholesterol and TBLE, respectively, at the corresponding surface pressure, estimated from isotherms of their respective pure monolayers. If the additivity plot follows the ideal mixing line, this indicates a miscible or completely homogenous film where the components mix, but do not interact. It can also indicate that the two components are immiscible, essentially patches of one component in a monolayer of the other. Deviations from the ideal mixing line are evidence of miscibility with molecular interactions between the components.

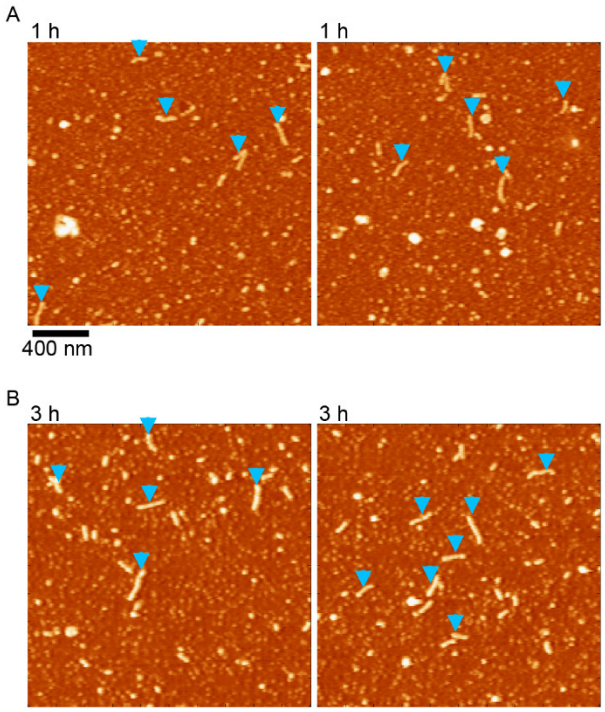
The average molecular areas in Supplemental Figure 1B clearly show that the binary mixtures of TBLE and cholesterol are at a smaller molecular area or more condensed compared to the ideal system. This indicates specific condensing molecular interactions between the components upon mixing, essentially a stiffening of the monolayer.



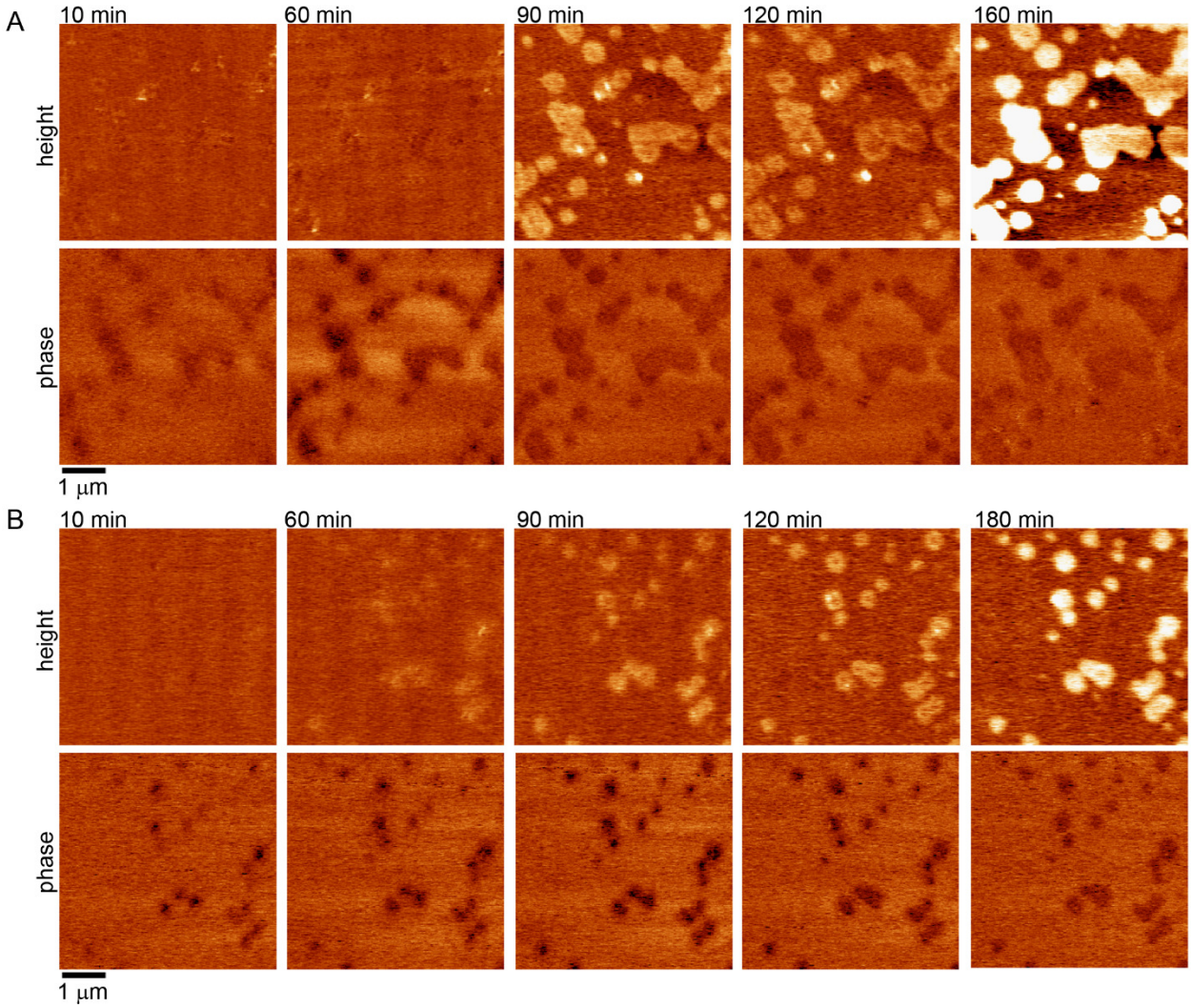
Supplemental Figure 1. A) Monolayer compression isotherms of pure TBLE, pure cholesterol, and binary mixtures of 5, 10, 16, 23, 31, 41, 51, 64, 80 wt% cholesterol at 30ºC. B) Mean area per molecule in mixed monolayers of TBLE and cholesterol at surface pressures of 5, 10, 20, and 30 mN/m. Mol% and average area per molecule was calculated assuming an average TBLE molecular weight of 850 g/mol. The solid line at 5 mN/m represents values calculated by the additivity rule and corresponds to ideal mixtures. Dashed lines are added to guide the eye.



Supplemental Figure 2. AFM height and phase images taken in solution of continuous, supported lipid bilayers of TBLE, TBLE + 10% cholesterol, TBLE + 20% cholesterol, and TBLE + 30% cholesterol prior to exposure to any htt-exon1(51Q).



Supplemental Figure 3. AFM height images of htt-exon1(51Q) aggregates that formed after (A) 1h and (B) 3 h at 20 μM in the absence of lipids. Samples were deposited on mica for imaging. Blue arrows indicated short fibrils. Scale bar applies to all images.



Supplemental Figure 4. Sequential AFM height and phase images taken in solution of supported TBLE bilayers containing (A) 20% or (B) 30% exogenously added cholesterol exposed to htt-exon1(51Q).

Captions for Supplemental Movies:

Supplemental Movie 1. Sequential AFM height images taken in solution of a pure supported TBLE bilayer exposed to htt-exon1(51Q).

Supplemental Movie 2. Sequential AFM height images taken in solution of a supported TBLE bilayer enriched with 10% cholesterol exposed to htt-exon1(51Q).

REFERENCE

1. Gaines, G. L. (1966) In Interscience Monographs on Physical Chemistry: Insoluble Monolayers at Liquid-Gas Interfaces. (Priogine, I. ed.), Interscience, New York. pp 281-300