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Supporting Material

Modulation of KvAP unitary conductance and gating by 1-alkanols and other surface active agents

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Supplemental Information

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Capacitance measurements

Here, we provide additional specific capacitance measurements to place our own estimates in the context of other data in the literature collected using different protocols and preparations. In essence, we adapted a method which is widely used to provide a qualitative, visual monitor of bilayer formation, based on the increase in capacitance accompanying membrane thinning. We compare decane- and hexadecane-based bilayers, and compare decane-containing bilayers with and without added cholesterol. Alkanols were added by perfusing the upper and lower chamber compartments with solutions containing the specified concentrations.

TABLE S1

Specific capacitance (Cm) measurements reported below are based on linear fits to capacitance vs. area measurements taken from continuous video recordings made during the bilayer thinning process (see Figs. S1, S2). Where conditions for data collection overlap, there is no substantial difference from the values obtained with the abbreviated protocol used to gather data for the full range of ethanol, propanol, and butanol concentrations (Fig. 2 in the main text). In the results tabulated here, ethanol (400 mM in aqueous solution) did not significantly change Cm in decane/POPE-POPG membranes, nor did the addition of heptanol (20 mM in aqueous solution) to hexadecane bilayers. Addition of cholesterol (11 mM in the bilayer-forming solution) significantly increased Cm of decane based bilayers. In general, hexadecane-based bilayers showed higher Cm than decane-based bilayers of similar lipid composition.

Lipid solvent & conditions	**Specific Capacitance*** ($\mu F\text{-}cm^{-2}$), mean±sem (n)	**Relative Cm** (value relative to decane control)	**Probability**
Decane	0.36±0.06 (3)	1.00	
Decane+Ethanol	0.38±0.01 (2)	1.06	0.81*
Decane+Cholesterol	0.56±0.04 (8)	1.56	0.03*
Decane+Cholesterol+Ethanol	0.63 (1)	1.75	n.d.
Hexadecane	0.63±0.02 (4)	1.75	0.005*
Hexadecane+Heptanol	0.65±0.06 (3)	1.81	0.73**

Comparisons, 2-tailed t-test: * vs decane control; ** vs hexadecane control; n.d., not determined. ***Absolute values of Cm might be ~1.3× higher than given here due to a deviation between the optical axis (normal to the partition in the bilayer chamber) and the axis of the hole; this would lead to systematic underestimates of bilayer areas, without affecting the Relative Cm.

Supplementary Figures – Legends

Fig. S1

Experimental protocol designed to measure specific capacitance in relatively rapidly thinning bilayers. Typically, but not invariably, thinning was more rapid when hexadecane, rather than decane, was used as the solvent, hence the use of this approach to collect the comparative data for Table S1.

A. Individual video images taken during the thinning process which yielded a bilayer covering most of the area of the hole. The bilayer area is demarcated below the dark wave front (the lipid- and solvent-containing torus – see green arrows), which is clearly visible in the first two frames (33% and 66% of the area as bilayer). In the last frame (~99% of area is bilayer), this boundary is barely resolved at the top right of the hole.

B. Current traces corresponding to the video frames in part A., showing the increase in capacitative charging current elicited by a saw-tooth voltage waveform (see Fig. S2) as the bilayer expands to occupy a larger and larger fraction of the area of the hole. The jump in the current amplitude occurring when the slope of the voltage reverses is proportional to the total capacitance, including the bilayer capacitance.

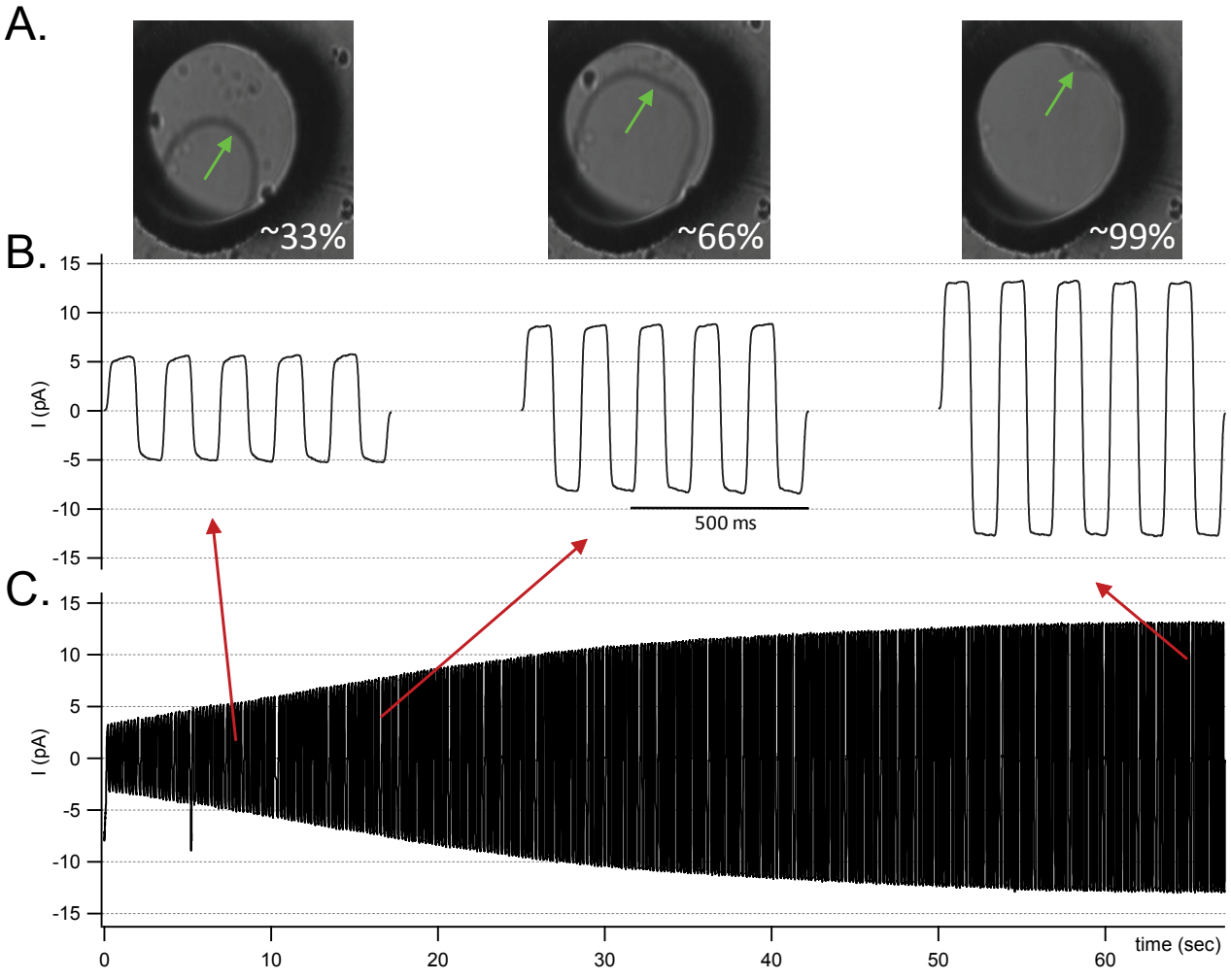
C. Continuous current record, whose envelope reflects the time course of the increase in capacitance as the lipid-solvent blob yields solvent to the torus around the edges of the hole, yielding a bilayer over most of the area of the hole. The starting "blob" capacitance, near t=0, approximates the summed capacitances of blob of lipid-solvent mixture which fills the whole before initiation of thinning, plus the parallel capacitance provide by the plastic partition surrounding the hole, and any stray capacitance in the circuit connections.

Fig S2

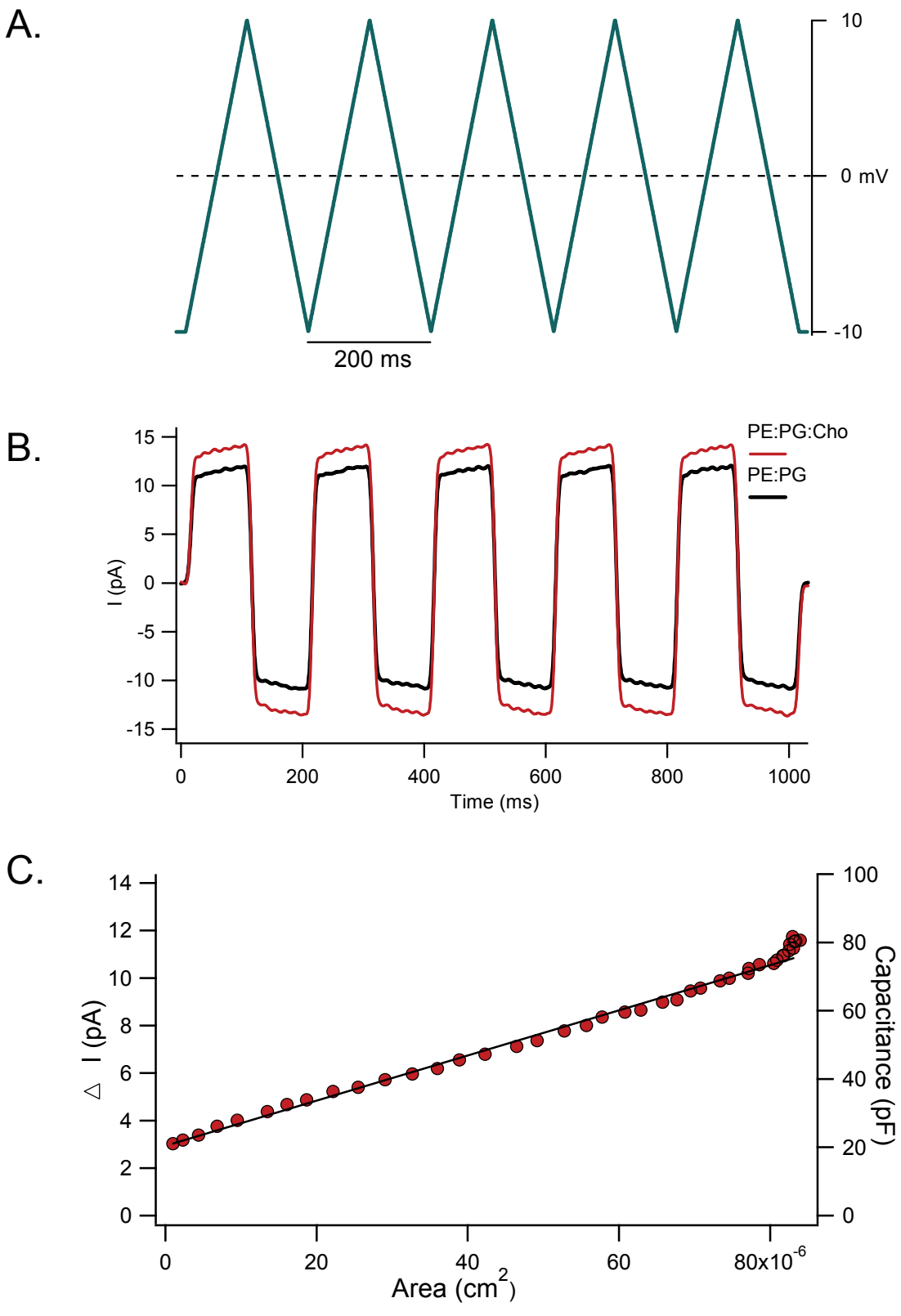
A. Voltage waveform used for capacitance measurements for rapidly thinning bilayers. The pattern shown was repeated episodically as rapidly as the acquisition system allowed, providing a quasi-continuous readout of capacitance changes (see Fig. S1, part C).

B. Superposed current traces corresponding to the voltage command in A. illustrating an increased in bilayer capacitance when cholesterol (11 mM) was present in the membrane.

C. Plot of the amplitude of the current step, elicited when the slope of the voltage changes from positive to negative, against measured bilayer area (see Fig. S1, part A). The slope of the line gives an estimate of area-specific capacitance. Deviations from linearity at the extreme(s) of the range of measured areas probably result from difficulties as the thinning front approaches the edge of the hole. For the results in Table S1, we used only experiments for which we obtained a continuous sequence of capacitance estimates over ≥40% of the visible area of the hole.



Supplementary – Fig. S1



Supplementary – Fig. S2