

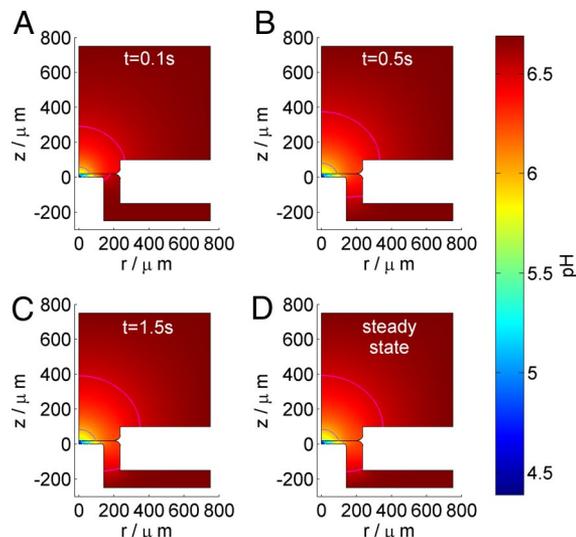
## Reply to Missner *et al.*: Timescale for passive diffusion across bilayer lipid membranes

A fundamental flaw in the analysis of Missner *et al.* (1) is evident from inspection of the equation presented in their letter, in which the overwhelming mass transport resistance for passive diffusion is from an unstirred layer on the *trans* side of the membrane. This is not a realistic representation of the experimental situation in a microelectrochemical system, where 2-dimensional diffusion greatly enhances mass transport, leads to the establishment of steady-state conditions on a rapid timescale (2–4), and allows the measurement of fast processes (2, 5, 6).

In Fig. 1 we show snapshots of the time-dependent pH response, obtained by simulation for the parameters pertinent to acetic acid (see ref. 2), after switching on an anodic electrode current of 5 nA (see figure 7 in ref. 2 for the axisymmetric cylindrical geometry of our experiments). There is no discernible difference in the profiles for 1.5 s and steady state. Thus, when Missner *et al.* (1) calculate that 1,200 s would be needed to achieve a steady state, they are wrong by  $\approx 3$  orders of magnitude. The experiments reported were at steady state, and it is entirely appropriate to solve the steady-state mass transport problem described (2). Fig. 1 also highlights the step in pH at the membrane boundary, indicating a clear kinetic barrier that can be measured by our technique; Missner *et al.* are incorrect when they claim that the membrane resistance reported would be difficult to detect because they have grossly underestimated mass transport rates.

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**Fig. 1.** Finite element simulations of the pH profile (in the axisymmetric cylindrical coordinate system similar to that defined in figure 7 of ref. 2) for an ultramicroelectrode-confocal microscopy measurement of membrane permeation (2). The simulations are for acetic acid permeation through a lipid bilayer at different times after the application of a 5-nA anodic current to the ultramicroelectrode. The electrode is at a distance of 20  $\mu\text{m}$  from the lipid bilayer membrane. The data demonstrate that the system rapidly reaches a steady state. In all frames the contours represent lines of equal pH (5, 5.5, 6, and 6.5). The profiles correspond to times of 0.1 s (A), 0.5 s (B), 1.5 s (C), and steady state (D). Details of the simulation were as given in ref. 2. For A–C the time-dependent version of the diffusion equation was solved; i.e., equation 5 in ref. 2, with the right hand side replaced with  $\partial c/\partial t$ .

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The authors declare no conflict of interest.

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