

## Comment on "A Theory of Macromolecular Chemotaxis" and "Phenomena Associated with Gel–Water Interfaces. Analyses and Alternatives to the Long-Range Ordered Water Hypothesis"

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*J. Phys. Chem. B* **2013**, *117*. DOI: 10.1021/jp302587d*J. Phys. Chem. B* **2013**, *117*. DOI: 10.1021/jp302589y■ RESPONSE TO "A THEORY OF  
MACROMOLECULAR CHEMOTAXIS"

The presence of large aqueous zones that exclude substances from the vicinity of various surfaces has become increasingly recognized. Two recent volumes have shown that such exclusion phenomena may address a number of previously unresolved issues in biology.<sup>1,2</sup> With demonstrations of exclusion from nonbiological surfaces as well, interest in these phenomena has been rapidly broadening. This is reflected in conferences in which these phenomena are given a voice, and also in increasingly numerous keynote lectures worldwide (<http://faculty.washington.edu/ghp/>).

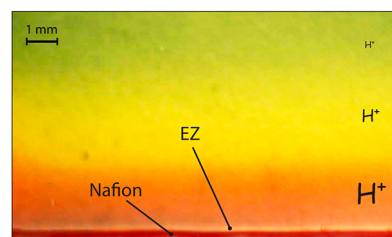
The two papers by Schurr et al.,<sup>3,4</sup> my colleagues from our own university's department of chemistry, reflect this broadening interest. Schurr et al. have taken the trouble to prepare two extensive documents offering an alternative view of the nature and origin of this so-called exclusion zone (EZ). The authors do not challenge the EZ's existence. Rather, they make a laudable attempt to fit the phenomenon with an explanation based on known chemical features such as ion gradients and coupled fluxes.

I reply because I am not convinced that their explanation fits all the cases in which EZs are observed, or perhaps any of them. I present six straightforward arguments that in my opinion refute the proposed mechanism.

(1) If chemical gradients create the EZ, then abolishing those gradients should eliminate the EZ. We imposed flow of water and microspheres inside Nafion tubes. Even with rapid flow (linear velocities up to 50 000  $\mu\text{m/s}$ ), which should wash away any near-surface gradients, the EZs continued to persist.<sup>5</sup> Hence, the EZs seem unrelated to near-surface gradients.

(2) Light profoundly expands the EZ.<sup>6</sup> Reversible expansions by as much as 10 times are easily realizable. These expansions are wavelength dependent, and they occur with weak light (whose induced temperature increase amounts to less than 1  $^{\circ}\text{C}$ ). The proposed theory offers no plausible reason for any such light-induced expansion.

(3) Since washing out any putative chemical gradients fails to eliminate the EZ (see above), a related question is whether such gradients exist at all. Schurr et al. consider potential pH and salt gradients. In the case of Nafion, with no salt added to the water and many associated protons, the proposed gradients should exist mainly in the form of pH gradients. The experiment in Figure 1 uses dyes to probe for pH gradients next to the Nafion surface. Red color indicates  $\text{pH} < 3$ , i.e., high proton concentration. One can see that elevated proton levels are indeed present in the image, but they appear *beyond* the EZ;

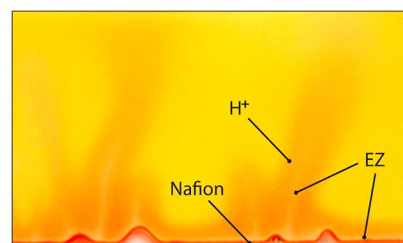


**Figure 1.** Proton distribution beyond the exclusion zone (viewing the wide face of a narrow chamber). The EZ excludes the pH-sensitive dye. The pH value immediately beyond the EZ is 3 or below (red-orange).<sup>9</sup>

the EZ itself shows *no color*: the EZ apparently excludes the dye (as it excludes many other molecules; see below). The theory of Schurr et al. would place this color gradient not beyond the EZ but *within* the EZ.

(4) Chemical gradients are proposed to lie in the immediate vicinity of the material surface. Correspondingly, the EZs should lie in that same vicinity, eventually diminishing in size as the gradients subside. EZs actually *grow* with time; they grow in the form of long dendrites projecting from the main EZ (Figure 2). The EZs keep growing, to lengths documented up to 1 m, and persist up to 2 weeks.<sup>7</sup> When cut midway with a laser, their distal ends persist. Interpreting these features in terms of near-surface gradients seems challenging.

(5) If chemical gradients bear responsibility for creating EZs, then surfaces that do not generate chemical gradients should not exhibit this phenomenon. We showed, in an early paper,<sup>8</sup>



**Figure 2.** Similar to Figure 1, taken at a lower magnification and later time. Note dendritic EZ extensions, which may continue to grow. Note also zones of low pH (red) surrounding each vertical EZ extension, demonstrating low pH beyond, not within, the EZ.<sup>9</sup>

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that monolayers—*single* self-assembled molecular layers in Nanopure water—are able to build exclusion zones on the order of 50  $\mu\text{m}$  wide. Monolayers are unlikely to create and sustain extensive chemical gradients.

Besides monolayers, many other surfaces unlikely to create chemical gradients succeed in creating EZs. They include aluminum,<sup>9</sup> cellulose acetate,<sup>10</sup> plasma-cleaned glass, photo-activated titanium dioxide, and *n*-silicon,<sup>11</sup> and thin layers of polyethylene glycol.<sup>12</sup> We also find large EZs next to noble metals after passing current through them.<sup>13,14</sup> In this variety of materials, the common feature is the juxtaposed water, not the hypothesized chemical gradients.

EZs are also evident next to biological surfaces, including muscle,<sup>8</sup> vascular endothelium and xylem,<sup>11</sup> rabbit eye lens,<sup>15</sup> and various additional biological surfaces.<sup>16</sup> In no case has it been shown that these surfaces are the particularly strong ion exchangers proposed to create the chemical gradients.

(6) While the proposed theory focuses on microspheres, microspheres are not the only species excluded. Also excluded are proteins, an array of particles and solutes, and many dyes<sup>11</sup>—including the pH-sensitive dyes shown in Figures 1 and 2, whose molecular weights are as low as  $\sim 100$ . Any theory purporting to explain the presence of an EZ based on the character of a single excluded entity, while many others of differing character are similarly excluded, can hardly claim success. The mass ratio of a 1  $\mu\text{m}$  microsphere to a 1 nm dye molecule, for example, computes to about 1 000 000 000. An adequate theory would need to explain why substances of vastly different charge and mass get excluded, and also explain why they get excluded with nearly similar dynamics.

The proposed theory fails to explain many EZ basics. On the other hand, I do not wish to exclude the possibility that chemical gradients may form next to certain surfaces that produce EZs, but this hypothesis has to be tested with experiments. Schurr et al. did test their hypothesis with their own experiments; the reader is encouraged to examine their results (presented just before their Discussion) and judge the extent to which their experimental results support their theory. For the six reasons given above, their theory cannot be a universal explanation for all situations in which EZ phenomena appear.

Our interpretation, based on the many findings from our laboratory and those of others, rests on a reorganization of EZ water molecules into a liquid crystalline form.<sup>11</sup> Incident electromagnetic energy drives that reorganization. The ordered zone profoundly excludes solutes, and may grow to macroscopic dimensions under appropriate conditions.

Anyone interested in visualizing the EZ for themselves can do so in a simple way: Place an ordinary ion-exchange bead (copper color) onto a glass slide with white paper beneath to enhance visualization. Larger beads make visualization easier. Dissolve 5 mg of Evans blue dye into 50 mL of water. Place several droplets of the dye solution around the bead, letting the droplets coalesce with the bead at the center; remove excess solution—the liquid should just cover the bead. Illuminate from above at 45°. Use magnifying glass if desired. A shell-like, dye-excluding EZ on the order of 0.5 mm thick should appear within a minute or two.

While physical chemists may feel uneasy with the notion of long-range ordering of water molecules, evidence for that point of view is comprehensively reviewed in a recent book.<sup>11</sup> Many physical features of the EZ, including viscosity, density, thermal emission, spectral absorption, etc., differ from the respective

features of bulk water. The book knits together many observations from diverse laboratories into a framework, whose predictive power accounts not only for diverse laboratory observations but also for common everyday phenomena whose explanations have heretofore been lacking. It provides a starting point for anyone seriously interested in exploring this phenomenon, or exploring water in general.

#### ■ RESPONSE TO “PHENOMENA ASSOCIATED WITH GEL–WATER INTERFACES. ANALYSES AND ALTERNATIVES TO THE LONG-RANGE ORDERED WATER HYPOTHESIS”

In the first paper of this pair,<sup>3</sup> Schurr et al. proposed a theory to account for the presence of an exclusion zone. In the second paper,<sup>4</sup> Schurr provides reasons why he believes such a theory is necessary. His approach consists mainly in critically examining the early evidence adduced to support the original interpretation, centered on the ordering of water.<sup>8,17</sup> We welcome this challenge, for our interpretation brings potentially far-reaching consequences for all science, involving water and beyond. If our interpretation is to be taken seriously, it must remain robust to challenge.

Schurr sets the stage with several broad assertions, including two that are crucial to our argument: (a) evidence for ordered water in the exclusion zone is lacking, especially evidence for refractive index difference and birefringence, and (b) in biology, ordered water would give rise to features never observed. Since Schurr dismisses evidence we regard as relevant, I have no choice but to address those arguments. The reader may then make an informed decision.

**(a). Direct Evidence for Ordered Water.** The aqueous region next to various hydrophilic surfaces was recently explored by two groups with extensive experience in optics.<sup>18–20</sup> Both groups found that the refractive index of the interfacial zone next to Nafion and hydrogels was higher than that of the bulk water beyond, by up to 10%. The highly refractile zone extended some 50  $\mu\text{m}$  from the material surface. Thus, the EZ has a higher refractive index than bulk water.

The latter group further reported that the interfacial zone was birefringent (although they attribute the birefringence to features other than water). This finding follows preliminary evidence of birefringence from our own group,<sup>7</sup> as well as evidence of birefringence obtained from biological organisms<sup>21</sup>—the latter author concluding that interfacial water exists in liquid crystalline form.

Probing the structure of interfacial water by electron diffraction, a Harvard group showed that the substantial volume of water trapped inside large protein vesicles was ordered. The diffraction pattern showed hexagonal order.<sup>22</sup> Thus, ice-like water may account for the observed birefringence.

These conclusions about the nature of interfacial water are not entirely new. More than 60 years ago, an exhaustive review by Henniker cited more than 150 papers demonstrating long-range effects of interfaces on the adjacent fluids.<sup>23</sup> Those long-range effects appear not only in water but were also common in other liquids. The extent of these surface-induced effects was stated to reach up to “hundreds of micrometers” from the interface.

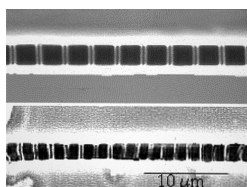
Thus, evidence from both older and newer studies concurs: surfaces induce long-range reordering of juxtaposed liquid molecules. In the case of water, the ordering may be hexagonal.

The tightly packed (and therefore highly refractile) hexagonal array of water molecules may constitute the exclusion zone, the tight packing responsible for the observed exclusion.

**(b). Biological Evidence and Consequences of an Ordered Water Zone.** To reinforce the claim of “no evidence”, Schurr invokes biology. He seems unaware of the work carried out by Nobelist Albert Szent-Gyorgyi (considered the father of modern biochemistry) and the lifetime of work carried out by Gilbert Ling and his colleagues ([www.GilbertLing.org](http://www.GilbertLing.org)). Ling’s five books and numerous publications spanning seven decades have provided extensive evidence for long-range ordering of water in biological environments. It was this wealth of evidence that first drew us into the field. Indeed, our own evidence merely augments the large body of evidence produced by Ling and his colleagues.

Schurr attempts to reinforce his no-evidence position by quoting a study that shows unaltered diffusion inside the cell. However, the outcome of a single study seems insufficient to challenge the ample evidence to the contrary. Some of the leaders in the cell biology field report substantially diminished diffusion in cells, by up to *several orders of magnitude*.<sup>25–30</sup> Cell biologists recognize that the cell is crowded with macromolecules; the mean intermolecular distance is equivalent to a lineup of approximately seven water molecules;<sup>24</sup> since virtually all cell water is therefore interfacial, the diminished diffusivity that is widely reported seems unsurprising.

Schurr further opines that, if isolated cells were surrounded by ordered EZs, then the differing refractive indices ought to produce halos (bright borders) around those cells. He asserts, however, “...halos have never been reported for either hydrophilic gels or biological tissues of any kind.” Yet halos are routinely seen in images of living cells and tissues. Figure 3



**Figure 3.** Single muscle myofibrils from honeybee flight muscle (top) and rabbit cardiac muscle (bottom) examined by phase-contrast microscopy. Note the white halos alongside each myofibril.

provides a representative example. A literature search will turn up countless additional examples. Thus, Schurr’s argument that exclusion zones cannot surround cells because halos are absent belies the voluminous evidence to the contrary.

Schurr goes on to dismiss the idea of a shell of ordered water by asserting that any such barrier would preclude the necessary exchange across the cell boundary. In fact, the barrier merely *slows* the exchange: plant and animal physiologists have long known about this “unstirred layer” of slowed exchange around cells and tissues (for a review, see Pollack and Clegg<sup>16</sup>). The issue of blockage vanishes if the EZ layer contains localized breaches, which are confirmed experimentally.<sup>10</sup> Such “holes in the dam” constitute the necessary conduits for exchange, their narrowness accounting for the exchange’s reported slowness.

In sum, the dismissal of the ordered water hypothesis based on “lack of evidence” ignores the voluminous evidence coming from a large body of published papers spanning over a century. That evidence has provided a good rationale for past studies, and continues to provide a rationale for further studies.

Schurr continues his argument with a critique of the (mostly early) evidence produced in our laboratory. Admittedly, we lacked the experience at the outset to avoid all interpretational pitfalls. Even so, I believe it is fair to say that we have accumulated considerable evidence in support of the ordered water interpretation, and I will show next that our experimental evidence stands up at least to the four specific areas of criticism of that interpretation.

**I. Microsphere Exclusion.** Schurr speculates that chemical gradients cause microsphere exclusion. The response to paper I<sup>3</sup> offered six reasons why this argument does not apply. Among them: vigorous washout of those putative chemical gradients leaves substantial exclusion zones, and surfaces that could not reasonably produce chemical gradients bear substantial exclusion zones. Thus, Schurr’s theoretical speculations fail to conform to experimental evidence.

Further, Schurr focuses his argument on microspheres. Microspheres, however, are not the only excluded species. Excluded substances include carbon particles, colloidal gold, quantum dots, and a wide assortment of dyes including even ordinary food colorings.<sup>31</sup> The focus on microspheres thus misleads; it belies the experimental fact that exclusion is far more general, and that any proposed mechanism must address that generality.

**II. Long Range Electrostatic Potential Gradients.** Again, Schurr argues for chemical gradients. However, a proposal of this sort rests on the presumption that the EZ comprises water that can sustain those gradients, i.e., bulk water. However, the data presented above show otherwise: the EZ is *not* bulk water. Hence, the chemical gradients proposed to create the electrical potential gradients may not exist at all.

Schurr further argues that the concept of net charge inside the EZ violates the principle of electroneutrality, i.e., that net charge cannot be sustained over any reasonable length of time. Anyone watching MIT professor Walter Lewin’s stunning Kelvin water dropper demonstration ([http://www.youtube.com/watch?v=oY1eyLEo8\\_A&feature=related](http://www.youtube.com/watch?v=oY1eyLEo8_A&feature=related)), where separated bodies of water eventually discharge onto one another, will immediately see that large bodies of water *can* bear net charge. If any doubt remains, then the experience of getting electric shocks from touching certain kinds of drinking water (which my colleagues and I have personally experienced) eliminates such doubt. Extensive theoretical constructs arguing that these phenomena should *not* occur, no matter how elegant or sophisticated, cannot compete with the simple experience demonstrating that they *do* occur.

A practical outcome of the separation of charge between the EZ and bulk water is the generation of electrical energy—derived from incident photon energy. We have been pursuing this application with impressive preliminary results.

**III. Infrared Imaging.** EZs radiate less infrared energy than bulk water.<sup>8</sup> This result should not surprise, for the liquid crystalline makeup of the EZ implies fewer charge displacements and hence less radiant emission. Schurr argues for alternative explanations, depending on certain geometric uncertainties. His arguments merit consideration; however, similar results appear in experiments with fully defined geometric circumstances, namely, in vertically oriented Nafion tubes immersed in a water bath, the tubes extending to the full height of the bath. In this ambiguity-free geometry, the EZ annuli surrounding the tube show similarly diminished emission.<sup>32</sup> Thus, a consistent interpretation fits both geo-



metries; namely, the EZ generates relatively less infrared energy because of its more stable liquid crystalline nature.

**IV. NMR Imaging.** Again, Schurr proposes an alternative interpretation consistent with the chemical gradient theory. His arguments stem from certain technicalities of NMR experiments, technicalities upon which experts themselves often disagree because of the technique's inherent complexity. On the other hand, the images in question unambiguously show that the T2 value in the EZ differs from the T2 value in the bulk. Therefore, the conclusion stands that respective zones are physically distinct.

In sum, the four issues of challenge fail to derail the original line of interpretation, that the EZ is a distinct, ordered structure that excludes solutes. Ample evidence for this interpretation (which Schurr seems to have missed) lends strength to this view.

Regarding the additional critiques summarized in paper I,<sup>3</sup> an implicit response appears in my new book,<sup>11</sup> which presents the full spate of evidence for water ordering and its consequences. I hope the reader may be willing to examine that evidence before drawing judgment. The book goes on to show how an understanding of the nature of interfacial water creates a foundation for interpreting many heretofore poorly understood features of water.

## AUTHOR INFORMATION

### Notes

The authors declare no competing financial interest.

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