Suppression of proton mobility by hydrophobic hydration

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Proton transport in aqueous media is an extremely widespread and important process in both nature and technology. The energy household in cells, for instance, is based on the passage of protons across cell membranes through specialized proteins, proton pumps[1]. From a technological viewpoint, the main component of proton exchange membrane fuel cells is a hydrated exchange membrane made of Nafion™ where hydrogen dissociation takes place followed by migration of protons.[2,3] In bulk liquid water, the mobility of protons is more than an order of magnitude higher than can be explained on the basis of simple (particle) diffusion of the hydronium (H_3O^+) ion [4,5]. The fast motion of the proton charge through bulk liquid water involves the so-called Grotthuss mechanism [6], which involves an ongoing interconversion of covalent and hydrogen bonds between O and H atoms, leading to a net displacement of the positive charge [7]. Hence, in this mechanism, only the *charge* of the proton and not its *mass* is transported, which explains its high mobility. This picture of proton transport has recently been refined, by noting that the transfer of a proton from one water molecule to the next has non-local consequences for the hydrogen bonding arrangement of water molecules surrounding the proton [8,9]. The proton causes a structural rearrangement of the hydrogen bond network, and proton transfer requires the rearrangement of the hydrogen bonds of a significant number of water molecules [8,9].

While the fundamental principles of proton transport in bulk water have thus been established, more relevant aqueous proton transport processes occur in complex environments, which are less well understood. One important example is proton transport near hydrophobic moieties, relevant for proton transfer along (biological) membranes [10,11], in small embedded water pools within proteins [12], and through trans-membrane protein pores [13]. Here we investigate how the presence of hydrophobic groups affects the mobility of protons in water, using a combination of fluorescence microscopy and AC conductivity measurements. This work is motivated by recent studies of hydrophobic hydration that have revealed a dramatic reduction of the reorientation of water molecules for water next to methyl groups [14,15]. This effect was traced to the 'jump' mechanism of water reorientation, where water molecules are transiently 5-fold coordinated prior to rotating; the presence of a methyl group reduces the likelihood of 5-fold coordination and thereby strongly suppresses water reorientation [16]. The observation that water reorientation is essential for proton transfer[8,9] suggests that proton transfer is greatly affected when hydrophobic entities are present in solution – this hypothesis is tested here.

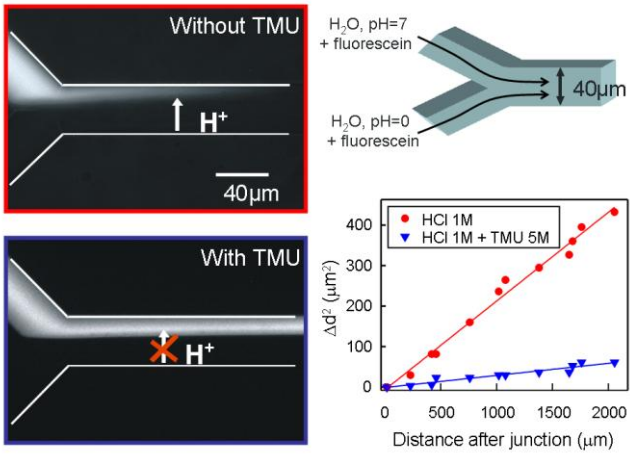

Figure 1. Fluorescence imaging microscopy of a microfluidic device demonstrating the dramatic effect of presence of hydrophobic tetramethylurea (TMU) on proton mobility. The device is a 2-way mixer (upper right) containing the fluorescent marker fluorescein in both channels. The image brightness corresponds to fluorescence intensity. An aqueous phase at pH=7 (upper channel) is mixed with an acidic phase at pH=0 (lower channel). The fluorescence is quenched at low pH - the lower channel appears dark. The upper left panel shows the results for pure water; the increase of the width of the dark region Δd is due to proton mobility, and increases with distance after junction (lower right). The lower left panel shows the results in the presence of 5M TMU. Analysis reveals a 10-fold reduction in proton mobility as a result of the presence of TMU.

Figure 1 shows how microfluidic flows can be used to quantify proton mobility. We record how the fluorescence of fluorescein is quenched as a result of proton transport across the channel of a microfluidic device, when a fluorescent low- and non-fluorescent high-pH phase are mixed in a two-way mixer (experimental details can be found in Supporting Information). The distance Δd between the center of the channel and the position of the sharply defined interface between fluorescent and non-fluorescent solutions is a measure for the distance over which the protons have traveled; this interface represents the position where the pH reaches the value of the pKa of fluorescein of 6.4. For diffusive proton motion, the square of Δd will vary linearly with distance from the junction x, as $\Delta d \approx \sqrt{Dt}$, with D the diffusion coefficient and t the time the two liquids have been in contact; for our system $t=x/U$, with U the flow speed in the channels. The slope of the lines in fig. 1 is therefore a direct measure of the proton diffusion coefficient. A

quantitative analysis of the data reveals a diffusion coefficient in the range of the 10^{-5} m^2/s for pure water.

Addition of the hydrophobic agent tetramethylurea (TMU) reveals that the proton diffusion coefficient decreases by *over a factor of 10* when 5M TMU is added to water. The reduction of proton mobility upon addition of TMU is not simply an effect of viscosity. The viscosity of a 5M TMU solution is only 1.35 times that of water (see supporting info). Fluorescence lifetimes imaging microscopy (FLIM) measurements performed on the same system allowed for the determination of the pH-dependent fluorescence lifetime, and were fully consistent with conclusions drawn from the intensity images.

Independent, quantitative conformation of this observation was obtained by AC (20 kHz) conductivity measurements. The conductivity σ is related to the diffusion coefficient D by: $\sigma = (D\, z^2\, e^2\, c\, N_A)/(k_B T)$ with the charge of the ion z=1, the elementary charge e, the speed of light c, Avogadro's number N_A and Boltzmann constant k_B and temperature T. The results are shown in Fig. 2, for a pH=1 solution of HCl, with varying amounts of TMU. For pure water, the proton diffusivity lies in the 10^{-5} range, in agreement with the microfluidic results obtained for 1M HCl solution.

Upon addition of TMU, the diffusivity is again observed to decrease by over an order of magnitude, in full agreement with the microfluidic experiments. Also shown in Fig. 2 are the same measurements with Urea. Urea serves as a reference to demonstrate that the simple decrease in the water volume fraction (resulting in the interruption of the water hydrogen-bonded network) has much less of an effect on proton mobility than the presence of the hydrophobic methyl groups.

The reduction of proton mobility upon addition of TMU can be understood as follows. Proton transport requires the rearrangement of a large number (~10's) of water molecules in the vicinity of the proton. A key aspect of this rearrangement is formed by the rotations of surrounding water molecules. It has previously been shown, using femtosecond time-resolved anisotropy measurements [13], that the reorientation of water molecules around methyl group is slowed down from 2.5 ps to over 10 ps. Moreover, a single methyl group can affect the reorientational dynamics of up to 5 O-H groups [13]. As such, the reorientation of water molecules in a 5M TMU solution is greatly suppressed. The dramatic effect of the presence of hydrophobic groups on proton transport can therefore be explained by the large effect of hydrophobic groups on water reorientation, in addition to the reduced effective water density in the TMU solution (by a factor of ~2). Together these effects account for the decrease of the proton diffusivity by an order of magnitude.

It has been proposed previously[17] that the reduction of proton mobility in solutions of isobutyric acid, another hydrophobic moiety, may be caused by a microscopic phase separation, giving rise to clustering that would serve to reduce the hydrogen-bond network connectivity, thus reducing the proton mobility. The results presented here show that clustering is not required to account for a significant decrease in proton mobility, as clustering in H_2O:TMU occurs only at very high TMU concentrations[12]. Likewise, the observed reduction of the proton mobility may well find its origin in the hydrophobic hydration of the isobutyric acid molecules.

Our results are of particular relevance for proton transfer in biological systems, where proton transfer near hydrophobic moieties of the membrane is essential for cellular function, and may account for previous observation of slowing down of proton transfer in confined media[3]. Moreover, these results identify a manner in which local proton transfer can be regulated by exposure of hydrophobic groups by e.g. a partial protein unfolding.

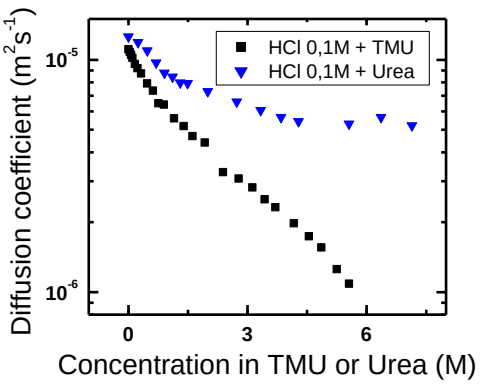

Figure 2. AC conductivity measurements provide proton diffusion coefficients in 0.1M HCl solutions with varying molarities of tetramethylurea (TMU) and Urea. Note the anomalously large effect of TMU on proton mobility, attributed to hydrophobic hydration of the TMU methyl groups.

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Supporting Information Available: Experimental section.

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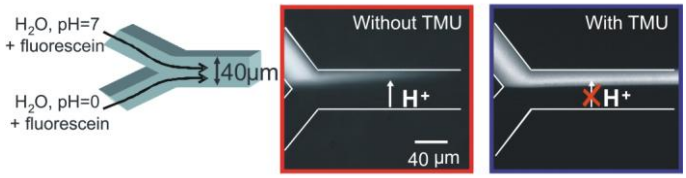

ABSTRACT FOR WEB PUBLICATION. Fluorescence microscopy and conductivity measurements reveal a remarkably strong effect of hydrophobic groups on the mobility of protons in water. The addition of 5 M of tetramethylurea (4 methyl groups per molecule) results in a reduction of the proton mobility by a factor of ~10: hydrophobic hydration strongly suppresses proton mobility. These observations demonstrate the collective nature of aqueous proton transport.