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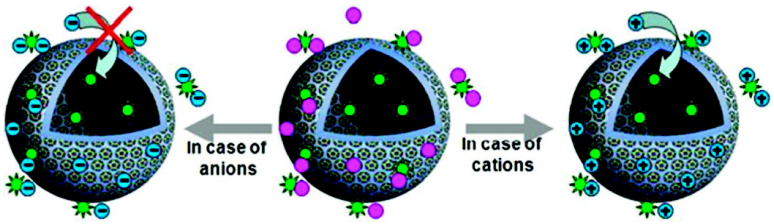

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Membranes Based on "Keplerate"-Type Polyoxometalates: Slow, Passive Cation Transportation and Creation of Water Microenvironment

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Ion transport across cell membranes is one of the most critical processes in living cells, which requires energy and specific carriers due to the existence of the hydrophobic lipid layer in the phospholipid bilayer structure. Specific proteins can provide this crucial function by serving as ion channels or carriers.[1]

The hydrophilic, nanoscaled polyoxometalates (POM) anions can form uniform, single-layer, vesicle-like, "blackberry" supramolecular structures in polar solvents via counterion-mediated attraction.[2,3] Their sizes, ranging from tens to hundreds of nanometers, can be accurately controlled by adjusting the charge density of macroions and/or the solvent quality.[3] The inter-POM hydrogen bonds provide a soft, membrane nature to the blackberry surface.[2b,c] The blackberry structure is also very stable and robust, as it does not fall apart even after being broken on a solid surface.[2a,d]

Here, we explore whether the nature of water (e.g., polarity or viscosity) inside blackberries is different from that in bulk solution and also whether the conserved and soft blackberry membrane is permeable to various ions (i.e., achieving passive transportation) by using cation-sensitive fluorophore chlorotetracycline (CTC) and two anion-sensitive fluorophores, 6-methoxyquinoline (6-MQ) and coumarin 1. We use 2.5-nm-sized, "Keplerate" $[Mo^{VI}_{72}Fe^{III}_{30}O_{252}(CH_3COO)_{12}\{Mo_2O_7(H_2O)\}_2\{H_2Mo_2O_8(H_2O)\}(H_2O)_{91}]$ ($\{Mo_{72}Fe_{30}\}$, Figure 1) clusters for this study.[4] $\{Mo_{72}Fe_{30}\}$ is a type of weak electrolyte; they are neutral clusters in crystals but become macroanions in water by deprotonating 7−8 H^+ from each cluster, and then form ~60-nm-diameter blackberries.[2c,3b] Each blackberry consists of >1000 $\{Mo_{72}Fe_{30}\}$ macroions on its surface, making a charged layer separating the inside environment from the bulk solution. There is no long-range ordered packing of POMs on the blackberry surface. The POMs do not touch each other due to the electrostatic repulsions; hence, many "channels" are formed on the blackberry surface.[2b,d] Single, spherical "Keplerate" POMs such as $\{Mo_{132}\}$ and $\{Mo_{72}Fe_{30}\}$ allow a limited number of small cations to move slowly across their surface pores and stay inside the clusters.[5] However, if blackberries are permeable, they have to use their "channels"—the space between POMs.

For a typical experiment, $\{Mo_{72}Fe_{30}\}$ crystals are dissolved in water containing coumarin 1, a good probe to scrutinize the environmental micropolarity and microviscosity of the solvent. Blackberry formation occurs slowly in solution and takes two weeks for the complete formation at 40 °C, as monitored by laser light-scattering measurements.[2d] We observe significant decreases in the fluorescence quantum yield (ϕ_f), fluorescence life time (τ_f), and the absorbance at λ_{max} (maximum absorbance) with increasing $\{Mo_{72}Fe_{30}\}$ concentration (Figure 2a). Prominent changes in the steady-state spectral properties are observed during the blackberry formation. A continuous blue-shift in both the absorption and fluorescence spectra for coumarin 1 is detected (Figure 2a insets), followed by an increase in the fluorescence intensity (Figure 2b), suggesting that some fluorophores experience a different environment during blackberry formation. A very similar blue-shift was

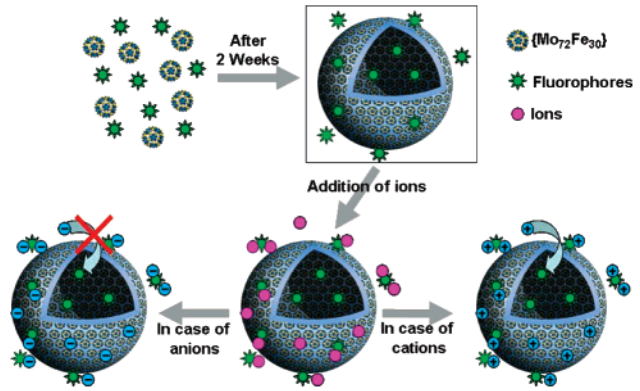

Figure 1. Formation of fluorophore-containing $\{Mo_{72}Fe_{30}\}$ blackberries in solution. The cations can slowly pass through the blackberry membrane but the anions cannot.

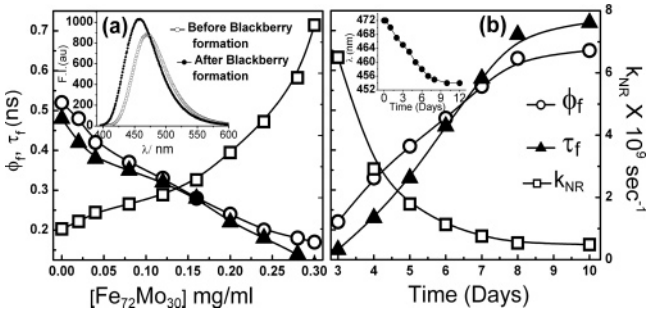

Figure 2. Change of ϕ_f, τ_f, and k_{NR} of coumarin 1 with (a) increase in $\{Mo_{72}Fe_{30}\}$ and (b) time, during the blackberry formation. (Inset) Change in fluorescence maxima during the blackberry formation.

observed in the time-resolved fluorescence studies (Figure 2b insets). The significant shift of 18 nm in the peak position with the increase in fluorescence intensity and τ_f indicate that some probes are in a different environment after the blackberry formation, i.e., the water inside blackberries is different from that in bulk solution. A slow component starts contributing to the increase of the average τ_f with the blackberry formation, which can be attributed to the gradual encapsulation of the fluorophores inside blackberries, since the regular component is unchanged during the process. No more changes are observed once the blackberry formation is completed.

More evidence comes from the time-resolved fluorescence anisotropy results.[6] The fluorophore did not show any time-resolved anisotropy before the blackberry formation. However, a biexponential decay [$r(t)$] of 0.23 is obtained after the blackberry formation, indicating an increase in the immediate viscosity around the probe, i.e. the fluorophores experience a different environment. Three different ambiances are observed, corresponding to three different states of fluorophores: in bulk solution, on the blackberry surface, and inside the blackberries. The initial decrease in ϕ_f and τ_f with the addition of $\{Mo_{72}Fe_{30}\}$ is attributed to the dynamic

10.1021/ja077715n CCC: $40.75

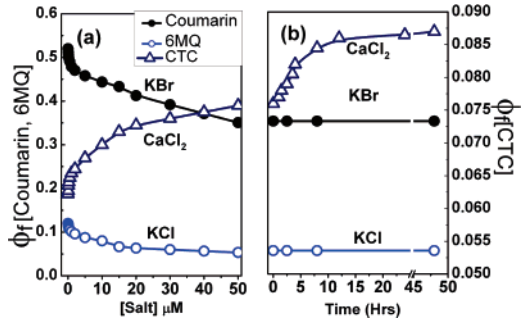

Figure 3. Change in fluorescence quantum yield of coumarin 1, 6-MQ, and CTC with addition of KBr, KCl, and $CaCl_2$, respectively. (a) Instantaneous change occurs with the addition of salts; (b) change in fluorescence quantum yield with time, once the addition of salt is stopped.

quenching of coumarin 1 by the single POMs, as the Stern−Volmer constants obtained from the steady-state and time-resolved results are essentially comparable. The continuous increase in the ϕ_f and τ_f during blackberry formation is followed by a decrease in the nonradiative rate constant k_{NR} (Figure 2b), suggesting that some radiative channels are blocked in restricted environments, i.e., inside the blackberries.[7]

A possible reason for the anomalous behavior of the blackberry-encapsulated water could be due to the large number of the hydrogen bonds formed close to POM anions.[8] Water in a nanoscaled confined environment behaves differently than in bulk solution.[9] The exciting discovery here is that its nature can be different even for a relatively large quantity. Over three million water molecules stay in each $\{Mo_{72}Fe_{30}\}$ blackberry (60−70 nm size); i.e. a different water microenvironment can be created in bulk solution with the help of self-assembled POM macroanions.

The cation transport across the blackberry shell can be monitored by encapsulating ion-sensitive fluorophores inside the blackberries.[10] The steady-state fluorescence and absorption spectra of two fluorophores, 6-MQ (for anions) and CTC (for divalent cations), are used for this study. When $\{Mo_{72}Fe_{30}\}$ crystals are dissolved in water containing fluorophores, the change in ϕ_f for both fluorophores (Figure 2S, Supporting Information [SI]) follows the same trend as that for coumarin 1, suggesting that some fluorophores (∼40%) are incorporated inside the blackberries.

Once the fluorophore-containing blackberries are formed, extra salts are added into the solution. The salt concentrations are controlled to up to 60 μM (7−8 times higher than the fluorophore concentration). An increase in the ϕ_f of CTC is obtained when divalent cations, e.g., Ca^{2+} or Mg^{2+}, are added to the blackberry solution (Figure 3a). CTC can bind to Ca^{2+} and Mg^{2+} with enhanced fluorescence by forming fluorescent chelates, and the fluorescence of the chelate itself is a function of the polarity of the environment.[11] Most divalent cations distribute around the large anionic blackberries,[3a] creating a favorable situation for the possible transport of cations across the blackberry membrane. Interestingly, we observe a continuous increase (for ∼12−14 h, Figure 3b) in ϕ_f of CTC with time when Ca^{2+} or Mg^{2+} are added, followed by a blue-shift (4 nm for Ca^{2+}, 5 nm for Mg^{2+}). The slow increase of ϕ_f with time indicates that some fluorophores cannot be accessed by the divalent cations when they are just added, but the change of the cation environment leads to more and more cation−fluorophore interactions. A reasonable explanation is that the Ca^{2+} or Mg^{2+} ions first quickly interact with the fluorophores in bulk solution and on the external blackberry surface, until all the initial available fluorophores are saturated. Then, more and more fluorophores become accessible to the divalent cations with time, indicating that the cations move slowly across the blackberry membrane and interact with the fluorophores present inside. This is strong evidence to show that the anionic blackberry membrane is permeable to small cations, without the help of any specific carriers. Furthermore, the curves (ϕ_f vs time) for Ca^{2+} and Mg^{2+} are almost identical, suggesting that the cation transport across blackberry membrane does not show obvious dependence on cationic size at least for small ions, since the channels are broad enough. Due to the different water environment the fluorophores inside blackberries generate signals at a lower wavelength. The actual spectrum contains signals from fluorophores both outside and inside the blackberries.

To test the permeability of the blackberry membrane to small anions, KCl and KBr are added into the blackberry solutions containing 6-MQ or coumarin 1, respectively, and an instant quenching of fluorescence is observed in both cases. When KCl or KBr is added, the anions instantly interact with those fluorophores present in the bulk phase and on the blackberry surface until all fluorophores are saturated. However, no change in either the fluorescence or absorption spectra for coumarin 1/KBr and 6-MQ/KCl solutions is observed with time which also rules out the leakage of the blackberry-encapsulated fluorophores. This observation indicates that the anions cannot go inside the blackberries, due to the strong electrostatic repulsion near blackberry surface. It also confirms that the initial instant change in the ϕ_f is purely due to the fluorophores present in bulk solution and on the external surface of the blackberries.

In summary, we have demonstrated that the microenvironment inside the self-assembled hydrophilic, inorganic POM macroions is different from that in bulk aqueous solutions. A water molecule becomes more viscous when residing in such relatively isolated microenvironments. The membranelike blackberry shell allows slow, passive transportation of small cations but not the anions.

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Supporting Information Available: Experimental details and additional graphics and references. This material is available free of charge via the Internet at http://pubs.acs.org.

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