

Measuring Ion-Pairing and Hydration in Variable Charge Supramolecular Cages with Microwave Microfluidics*

Authors: Angela C. Stelson¹, Cynthia M. Hong^{2,3}, Mitchell C. Groenenboom⁴, Charles A. E. Little¹, James C. Booth¹, Nathan D. Orloff¹, Robert G. Bergman^{2,3}, Kenneth N. Raymond^{2,3}, Kathleen A. Schwarz⁴, F. Dean Toste^{2,3}, Christian J. Long¹

Affiliations:

¹: Communications Technology Laboratory, National Institute of Standards and Technology, Boulder, CO 80305 USA

²: Chemical Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA

³: Department of Chemistry, University of California, Berkeley, California 94720, United States

⁴: Materials Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, MS 20899 USA

Keywords: microwave microfluidics, ion-pairing, metal-organic cages, supramolecular chemistry

Address correspondence to Angela Stelson (angela.stelson@nist.gov)

*Certain commercial equipment, instruments, or materials are identified in this paper to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose. This paper is an official contribution of NIST; not subject to copyright in the US.

Abstract

Metal-organic supramolecular cages can act as charged molecular containers that mediate reactions, mimic enzymatic catalysis, and selectively sequester chemicals. The hydration of these cages plays a crucial role in their interactions with other species (for example, substrates, counterions, and guest molecules). However, the interactions of water molecules and counterions are challenging to detect by standard analytical chemistry techniques, but can be detected with microwave microfluidics. Here, we use microwave microfluidics to measure the hydration and ion pairing of two metal-organic cage assemblies that are isostructural but have different overall anionic charge (12- for $K_{12}(Ga_4L_6)$, 8- for $K_8(Si_4L_6)$). We supplement our measurements with density functional theory calculations to compare binding site energies on model metal-organic cage vertices. Unexpectedly, we find that the $K_8(Si_4L_6)$ cage is more strongly hydrated and forms a distinct ion pair species from the $K_{12}(Ga_4L_6)$ cage. We also find that the cage dynamics can be described as four di- and trianionic vertices rather than an overall anionic charge. We evaluate multi-ion species and distinct ion pair solvations as possible sources for differences in ion dynamics and hydration. Broadly, this work highlights the utility of microwave microfluidics to elucidate the consequences of charge states on metal-organic complexes in solution.

Manuscript

Introduction

Cavity-bearing supramolecular cages offer unprecedented opportunities for chemical control at the molecular level. These structures feature internal microenvironments that recognize and encapsulate molecular guests, act as enzyme-mimetic catalysts, and unlock new modes of reactivity.¹ A common design motif for such supramolecular catalysts are self-assembled metal-organic cages that consist of organic ligands coordinating cationic metal ions to generate a variety of charged, polyhedral architectures. These cages are commonly identified by the counterions, metal vertex atoms, and ligands (e.g., $K_{12}Ga_4L_6$) that describe the overall stoichiometry and charge of the system (see Fig. 1a as an example).

The reactivities of supramolecular catalysts are influenced by parameters such as cavity size, overall charge, and solvent exclusion. However, the impact of dynamics in solution on reactivity is largely not characterized.^{2–4} Poorly understood issues include the distribution of charge throughout these supramolecular systems, as well as the localization and rates of ion-pairing interactions.^{5–9} These factors are often ignored in the design of supramolecular cages because they are not well understood, despite the fact that they have consequences for chemical applications.^{4,6,10} Quantifying interactions between cages, solvent, and counterions would clarify the role that solvation and counterions play in guest association/dissociation and catalysis. Furthermore, pairing measurements with computational models could extend the rational design of cages

beyond geometric and architectural considerations of the cage itself to include noncovalent solvent and ion interactions.¹¹

The challenge for synthetic chemists is that noncovalent interactions (*e.g.*, coulombic, hydrogen bonding, and solvophobic effects) in solution are difficult to measure directly with traditional analytical chemistry techniques. Some solvation and ionic interactions in metal-organic cage systems were indirectly investigated by combining independent measurement techniques such as isothermal calorimetry (ITC), nuclear magnetic resonance (NMR) spectroscopy, and ultraviolet/visible spectroscopy.^{12–16} However, measurements of counterion interactions remain scarce, and indirect measurements are limited to strong ionic interactions in solution and specific counterion chemistries.¹⁷

Previous microwave (0.1 GHz to 100 GHz) dielectric spectroscopy studies of salt solutions have characterized the solvent-mediated ion pairing and hydration of ions in solution.^{18–20} Bulk-fluid dielectric spectroscopy can also measure hydration and ionic interactions in complex biomolecular systems including proteins, DNA, and cells.^{21–23} Microwave-microfluidic measurements enable on-chip measurements for nanoliter fluid volumes over a wide frequency range (0.4 MHz to 100 GHz). These measurements quantify the dipolar interactions of cages and their counterions in solution, as well as changes to the cooperative water relaxation and the hydration of cages and counterions.²⁴

Here, we investigate the ion pairing and hydration of $K_{12}(Ga_4L_6)$ and $K_8(Si_4L_6)$ metal-organic cages (Fig. 1a). Both structures are tetrahedral with six organic ligands (L = N,N'-bis(2,3-dihydroxybenzoyl)-1,5-diaminonaphthalene) acting as struts to link four cationic metal vertices (Ga(III) and Si(IV)) (Fig. 1b).² These two cage systems are

isostructural, but in comparison to $K_{12}(Ga_4L_6)$, each vertex of $K_8(Si_4L_6)$ has its charge reduced from trianionic (Ga(III)-triscatecholates) to dianionic (Si(IV)-triscatecholates). This chemistry lowers the overall anionic charge and potassium counterion number for each host from twelve to eight for the $K_{12}(Ga_4L_6)$ and $K_8(Si_4L_6)$ cages, respectively. The structural similarity of the cages affords the unique opportunity to not only study the solution-phase dynamics of each cage, but to isolate the impact of varied overall charge on the cage dynamics and ion pair formation (Fig. 1c and 1d). Here, we consider multiple solvation states for ion pairs (Fig. 1c) and multiple ions on each vertex (Fig. 1d) as possible ion-pairing configurations.

To characterize the hydration and ion pairing in solution, we used broadband (40 kHz to 110 GHz) microwave microfluidic measurements. Microwave microfluidic measurements provide critical advantages in measuring supramolecular systems over traditional dielectric spectroscopy. These advantages include smaller sample volumes and a broader frequency range. The broad frequency range allows microwave microfluidics to accurately correct for both the low-frequency effects associated with the electrical double layer at the electrode surface and the high-frequency effects from water relaxation. Ion-pairing measurands are typically small in comparison to these effects, so accurate corrections are critical to capture the ion-pair dynamics in supramolecular systems.

To characterize the ion-pair dynamics, we fit the frequency dependence of the experimentally-determined electrical capacitance and conductance with a model that included electrode effects, ion-pairing relaxation, and water relaxation (See Methods and SI Device Fabrication). From these fit parameters, we calculated the association rate of

cage-ion pair formation and the hydration numbers of the cages. We then compared the results to Debye-Hückel theory and found that the metal-organic cages are well described by ion-solution theory in the low-concentration limit when each of the metal vertices of the cage were treated as independent ion-pairing sites.²⁵ Density functional theory calculations of the charged cage vertices showed that the binding energy for $K_{12}(Ga_4L_6)$ to form a multi-ion complex is similar for the binding energy of a single ion-pair in the $K_8(Si_4L_6)$ cage. Our analysis also shows that the $K_8(Si_4L_6)$ cage is more strongly hydrated than the $K_{12}(Ga_4L_6)$ cage. Taken together, our measurements and models suggest that the increase in hydration in the $K_8(Si_4L_6)$ cage can be explained by the reduction of ions associated with the charged vertices.

Microwave Microfluidics Measurements

We used a microwave microfluidics device (Fig. S1) to measure the broadband electrical properties of aqueous solutions of $K_{12}(Ga_4L_6)$ and $K_8(Si_4L_6)$ cages over a range of concentrations. The device consisted of microfluidic channels with integrated coplanar waveguides (CPWs) of varying length. We measured each of these devices (top-down view in Fig. S1a) with a vector network analyzer (VNA) with microwave probes to determine the raw complex-scattering parameters (S-parameters).²⁴ The S-parameters were calibrated and used to extract the distributed circuit parameters of the transmission line: R_0 and L_0 , (resistance and inductance per unit length associated with the metallic conductors) and C_{tot} and G_{tot} , (effective capacitance and conductance of the materials in the gap between the center signal line and the ground planes on either side). We present calibrated fluid data as C_f and G_f as these quantities are related to the real and imaginary parts of the fluid permittivity (ϵ' and ϵ''), respectively:

$$\varepsilon' = (C_f - C_{air})k_{geom} + \varepsilon_0, \quad (1)$$

$$\varepsilon'' = \frac{G_f}{\omega} k_{geom}, \quad (2)$$

where C_{air} is the per-unit-length capacitance of an air-filled channel, ε_0 is the permittivity of free space, and k_{geom} is a geometric constant which is determined from device structure. While equations (1) and (2) allow us to convert capacitance C_f and scaled conductance $\frac{G_f}{\omega}$ directly to permittivity values, we used permittivity only to describe intrinsic fluid properties and do not present measured data in terms of permittivity. We used equations (1) and (2) to convert extracted admittance to permittivity after the effects of the electrical double layer (EDL) that forms on the surface of the electrodes had been accounted for.

We determined the distributed conductance and capacitance of microwave microfluidic devices for room-temperature ($25\text{ }^\circ\text{C} \pm 2\text{ }^\circ\text{C}$) measurements of air, de-ionized water (DI water), and a range of concentrations of $\text{K}_{12}(\text{Ga}_4\text{L}_6)$ and $\text{K}_8(\text{Si}_4\text{L}_6)$ metal-organic cages (Figs. 2a-d). The distributed capacitance of the air-loaded microfluidic line remained constant as a function of frequency, while the line loaded with DI water had a larger capacitance at low frequencies and a relaxation (a peak in the conductance paired with a drop in the capacitance) at $\sim 20\text{ GHz}$. This water relaxation has been commonly observed in aqueous solutions and was attributed to the cooperative relaxation of water molecules.^{26,27} The water relaxation was also present in the aqueous cage solution samples. For the aqueous cage solutions at low frequencies (below 10 MHz), we see an additional peak in the conductance and corresponding drop in the capacitance, which we attribute to the relaxation of the electrical double layer at the surface of our electrodes

(Fig. 2).^{24,28} The weak ionpairing relaxation is approximately two orders of magnitude smaller than the water relaxation, and is not easily visible on this plot. Notably, the broad frequency range afforded by microwave microfluidics is required to measure ion pairing in these cages systems because the ion-pairing relaxations extend below the frequency range typically covered by dielectric spectroscopy studies of ion-pairing.¹⁸ To extract the signals associated with each dipole in the fluid, we developed a frequency-dependent circuit model to fit the multiple relaxations across the frequency spectrum (see Methods and SI for more detail).

Fitting Procedure

To extract physical values from the broadband electrical data, we developed a circuit model to describe total admittance (inverse of impedance) $Y_{tot} = G_{tot} + i\omega C_{tot}$ of the cages in solution (see Fig. S7 for circuit diagram):

$$\frac{1}{Y_{tot}} = \frac{2}{Y_{EDL}} + \frac{1}{Y_f}, \quad (3)$$

where Y_{EDL} and Y_f are the admittances of the EDL and fluid, respectively. The model for the EDL is described in detail in the SI and elsewhere.²⁴ The fluid admittance can be described as four parallel distributed circuit components:

$$Y_f = Y_{IP} + Y_w + G_\sigma + i\omega C_\infty = i\omega \frac{C_{IP}}{1+(i\omega\tau_{IP})} + i\omega \frac{C_w}{1+(i\omega\tau_w)} + G_\sigma + i\omega C_\infty, \quad (4)$$

where C_∞ is the capacitance of the suspension at frequencies far above the relaxation of water, C_w is the dipolar contribution of the water, G_σ is the conductance due to cages and counterions, and C_{IP} is the dipolar contribution of the weak ion pairing. The time constants τ_w and τ_{IP} correspond to the relaxation times of the water and the cage-counterion pair, respectively. To fit these responses with Debye models, we performed a nonlinear least-squares fit to extract Y_{tot} (see Methods section for fit methodology). We plot all extracted

physical parameters (C_{IP} , G_{σ} , etc.) versus the stoichiometric ionic strength I of the solutions:

$$I = \frac{1}{2} \sum [c_i] z_i^2, \quad (5)$$

where c is the molar concentration of the solution. We use the ionic strength to account for the differences in charge distribution in the solutions, and to compare more directly to Debye-Hückel theory of salt solutions. We use the extracted fit parameters for each ionic strength solution to quantify and compare the hydration and ion pairing dynamics in the two cage systems.

DFT Models of Cage Vertices

We designed a model system consisting of a single cage vertex with a truncated ligand to evaluate binding sites for potassium ions and estimate the binding energies of the ion pairs formed by K⁺ ions and the vertices of the Ga and Si cages. We note that this model does not evaluate types of ion-pairing, because it does not include entropic effects and solvent effects, but rather provides a framework to understand the relative charge effects of the cage vertex environments. We find the lowest energy configurations between the K⁺ ions, which we designate K1 (2 equivalent sites) and K2 (schematic representation in Fig. 1d, molecular model in Fig. S2). We note that the equivalent K1 site is located inside the cage cavity, and the steric effects of the cage ligand are not considered in this simplified model. We calculate the density-functional theory (DFT) energies of contact ion pairs for model cage vertices (Table S3) and find that the Ga cage ion pairing energies are much larger than those of the Si cage for the K1, K1 equivalent, and K2 positions. The DFT binding energy with a second potassium added to the

K12(Ga4L6) system (i.e., 2-contact ion pair) is energetically comparable to that of a single K1 bound to the K8(Si4L6) cage (i.e., 1-contact ion pair).

Hydration of K₈(Si₄L₆) and K₁₂(Ga₄L₆)

The water relaxation at ~20 GHz is affected by the hydration of the cages and counterions. We calculated the apparent number of irrotationally bound (immobilized and not participating in the water relaxation) and displaced solvent molecules Z_{IB}^{ap} from Equation 6:

$$Z_{IB}^{ap} = \frac{c_s^0 - c_s^{ap} - V_{cage} * c}{c}, \quad (6)$$

where c_s^0 is the analytical concentration of water at 25 °C, c is the concentration of the cages, c_s^{ap} is the apparent concentration of the water, and V_{cage} is the volume of the cage in solution (see SI for calculation).^{19,29} The apparent concentration can be calculated as:

$$c_s^{ap} = \frac{2\varepsilon'(c)+1}{2\varepsilon'(0)+1} \times \frac{\varepsilon'(0)}{\varepsilon'(c)} \times \frac{c_s^0(0)}{C_w(0)} C_w(c), \quad (7)$$

where $\varepsilon'(c)$ is the relative permittivity at the low frequency limit (excluding the effects of the electrical double layer and constant phase element, see SI Fitting the EDL for more details) for a sample of cages with concentration c , $\varepsilon'(0)$ is the relative permittivity of DI water, $c_s^0(0)$ is the analytical concentration of water molecules (5.55×10^4 mol/m³ at 25 °C), and $C_w(c)$ and $C_w(0)$ are the capacitive contributions of the water loss relaxation in the cage and DI water samples, respectively.³⁰ To calculate the number of water molecules displaced by the volume of the cage, we estimated the volume of a cage in solution to be $\sim(321 \pm 82) \text{ \AA}^3$ or $\sim(10.7 \pm 2.8)$ water molecule equivalents for both K₁₂(Ga₄L₆) and K₈(Si₄L₆) cages) by including a solvation layer (see SI Determination of Cage Parameters). We subtracted the molar volume of the cages from $c_s^0(0)$ to determine

the average number of irrotationally bound water molecules to the cage surface. Previous hydration studies found that potassium ions are not hydrated in solution.³¹ Hence we attributed all irrotationally bound water molecules to the interior and exterior of the cage.

To calculate the true hydration number Z_{IB} of the cages, we first accounted for the effect of the EDL on the apparent hydration number Z_{IB}^{ap} (Fig. 3). The electrode geometry used in these measurements produced electric fields concentrated on the surface of the electrode, and these measurements are sensitive to changes in the charge distribution of the EDL.³² The calculated Z_{IB}^{ap} values (calculated from Eq. 1) are approximately linearly dependent on concentration in this regime. To calculate the true hydration number Z_{IB} , we applied a linear fit incorporating propagated errors in Z_{IB}^{ap} ³³ and used the value at infinite dilution (Z_{IB}).²⁰ The error bars extracted on these fits included propagated errors from all the fit parameters included in the calculation of Z_{IB} .

We found that the $K_{12}(Ga_4L_6)$ cage is less hydrated than the $K_8(Si_4L_6)$ cage with estimated Z_{IB} values at infinite dilution of 20.4 ± 5.5 and 52.7 ± 5.5 water molecules for $K_{12}(Ga_4L_6)$ and $K_8(Si_4L_6)$ cages, respectively. Using a spherical water model and a tetrahedral approximation for the cages,³⁴ we estimate that the number of water molecules required to make a complete hydration shell are ~50 to 70 for both the $K_8(Si_4L_6)$ and $K_{12}(Ga_4L_6)$ cages. This calculation suggests that the $K_{12}(Ga_4L_6)$ cages have only partial hydration shells while the $K_8(Si_4L_6)$ cages are almost fully hydrated. This result contrasts with salt solutions, where larger charge is strongly correlated with higher hydration. A possible reason for this difference could be the presence of additional ion-paired K^+ ions on the $K_{12}(Ga_4L_6)$, which would block water binding sites and reduce the charge of the multi-ion species, making water binding less energetically favorable. More

strongly hydrated salts tend to have lower overall solubilities, and this trend holds true for the cages as well, where $K_8(\text{Si}_4\text{L}_6)$ has a lower solubility in water than the $K_{12}(\text{Ga}_4\text{L}_6)$ cages.³⁵ The steeper slope of Z_{IB}^{ap} (Fig. 3) for $K_8(\text{Si}_4\text{L}_6)$ cages further supports the conclusion that the $K_8(\text{Si}_4\text{L}_6)$ cage is more hydrated, as the larger hydrated cages would extend the thickness of the EDL and increase the EDL-related systematic error. For a deeper understanding of the environment of the cages in solution, we analyzed (below) complementary physical parameters associated with the ion pairing and ionic conductivity in solution.

Ionic Conductivity of $K_8(\text{Si}_4\text{L}_6)$ and $K_{12}(\text{Ga}_4\text{L}_6)$

The bulk fluid conductance due to ions G_σ was leveraged to compare the cages in solution to Debye-Hückel theory of simple electrolytes. For both cages, G_σ varied linearly with both ionic strength I (Fig. 4) and concentration c (Fig. S3). This result agrees with Debye-Hückel predictions for a strong, fully dissociated electrolyte at low concentrations. However, while the overall ionic strengths measured in this experiment are low compared to conductivity studies of simple electrolyte solutions, the solutions were close to the limit of solubility of the cages in water. At high concentrations of cages, we did not observe the deviations from linearity predicted by Debye-Hückel theory, which predicts that conductivity is proportional to the square root of the ion concentration.²⁵ One explanation is that the concentrations required for these deviations to be detectable within our measurements could not be reached due to solubility limits of the cages. $K_{12}(\text{Ga}_4\text{L}_6)$ had a smaller slope as a function of ionic strength as well as absolute concentration c (Fig. S3) than $K_8(\text{Si}_4\text{L}_6)$, indicating a lower overall mobility per charge in solution. This result indicates that the more charged $K_{12}(\text{Ga}_4\text{L}_6)$ cage is binding more ions to reduce the

effective concentration of free ions in solution. This finding agrees with the DFT calculations that predict similar DFT energies between the charged vertex for two counterions for the $K_{12}(Ga_4L_6)$ cage, versus a single counterion for the $K_8(Si_4L_6)$ cage. The lower ionic conductivity of the $K_{12}(Ga_4L_6)$ cages in conjunction with the lower hydration state suggests that the $K_{12}(Ga_4L_6)$ environments in solution could result in different equilibrium ion pair types, and we further validated this hypothesis with the analysis of the ion-pairing relaxations.

Ion Pairing of $K_8(Si_4L_6)$ and $K_{12}(Ga_4L_6)$

We observed a single ion-pairing relaxation for both cages with magnitude C_{IP} (Fig. 5). The magnitude of the dipolar contribution increased with ionic strength, and—considering that we expect C_{IP} to be 0 F/m when I is 0 mMol—the relationship between C_{IP} and I appeared to be nonlinear. Dielectric spectroscopy studies of highly charged lanthanide salts (La^{3+} and Eu^{3+} salts) displayed multiple relaxations, indicating a thermal equilibrium of multiple types of ion pairs (contact and solvent-separated ion pairs).²⁰ In contrast, we observed a single relaxation in the highly charged cage system, and fits allowing for multiple ion pairs converged on the same relaxation frequency and did not improve overall goodness of fit.

The presence of a single relaxation indicates that the vertices of the cages could be treated as distinct (though potentially correlated) ion-pairing sites. We did not treat the cage as a single anionic point charge because this implies that highly anionic species (12^- and 8^-) have a single, highly favored counterion pairing state. Because ion pairs are the product of stepwise additions of ions (Fig. 1c), the presence of a single ion pairing state within the sensitivity of our measurement implies that a particular pairing reaction step

has a dramatically more favorable free energy of formation than any other step. Multiple Debye relaxations consistent with multi-step ion pairing processes are seen in atomic salts carrying 3⁺ charges, indicating that treating the cage as a highly anionic point charge with a single relaxation is unreasonable.^{20,36} In contrast, treating each vertex as an independent anionic charge with steric effects from the surrounding organic ligands would account for the presence of a single, dominant ion pair in each cage. This treatment is consistent with previously untested assumptions that the distribution of charge in both the K₈(Si₄L₆) and K₁₂(Ga₄L₆) cages is localized to the vertices.

The capacitance C_{IP} of the ion-pair relaxation (Fig. 5) represents the dipolar contribution of the ion pairs to the overall relative permittivity of the solution. The magnitude of C_{IP} can be related to the concentration of ion pairs $[c_{IP}]$:

$$[c_{IP}] = \frac{(\epsilon' + (1 - \epsilon')A_{IP})}{\epsilon'} \times \frac{3k_B T \epsilon_0}{N_A} \times \frac{1}{\mu_{eff}^2} k_{geom} C_{IP}. \quad (8)$$

Here, ϵ' is the permittivity of the solution, A_{IP} is the shape factor of the ion pair ($1/3$ for a spherical approximation), k_B is the Boltzmann constant, T is the temperature, ϵ_0 is the permittivity of free space, N_A is the Avogadro constant, μ_{eff} is the effective dipole moment of the ion pair³⁰, and k_{geom} is a geometric factor relating the measured capacitance with the dielectric constant of the fluid (see Methods). The effective dipole moment μ_{eff} is:

$$\mu_{eff} = \sqrt{g} \frac{\mu_{IP}}{(1 - \alpha_{IP} f_{IP})}, \quad (9)$$

where μ_{IP} is the true dipole moment of the ion pair, α_{IP} is the polarizability of the dipole, f_{IP} is the reaction field factor, and g is the Kirkwood correlation factor.³⁶ To a first approximation, we assume that $\frac{\sqrt{g}}{(1 - \alpha_{IP} f_{IP})}$ is similar in magnitude for both cages due to their similar chemistries and structures. The nonlinearity of C_{IP} (decreasing slope at

higher I) could not be fully accounted for with the nonlinearity in $[C_{IP}]$ ($\frac{A_{IP}}{\epsilon'}$ varies by ~1.3 % among all cage samples) and implies a nonlinear relationship between the concentration of cages and paired counterions.

Comparing Ion Pair Dynamics

The $K_8(Si_4L_6)$ cage displayed larger C_{IP} values than the $K_{12}(Ga_4L_6)$ cage, both as a function of ionic strength and concentration. This C_{IP} result implies that either the $K_8(Si_4L_6)$ cage ion pair has a much higher concentration in solution ($[C_{IP}]$), or that the dipole moment is larger for the $K_8(Si_4L_6)$ case, despite a smaller charge on the metal site. A high concentration of the $K_8(Si_4L_6)$ cage ion pair is unlikely, due to the lower number of counterions in solution, and the higher ionic conductivity as a function of concentration. Rather, we infer a larger dipole moment, not originating from a change in charge but through a different pairing mechanism (e.g., a differently solvated pair or a different number of bound ions, Fig. 1c-d). Complementary work on $K_{12}(Ga_4L_6)$ cages by use of a combination of NMR, UV/vis spectroscopy and ITC has previously characterized the association of large ammonium guests and counterions to the cage exterior *via* contact ion pairs, as those techniques are unable to detect K^+ ion pair interactions.¹² These findings agree with our Z_{IB} and G_σ data, which also suggest that the $K_{12}(Ga_4L_6)$ forms contact ion pairs, albeit with potassium counterions rather than large, strongly bound ammonium ions. The larger C_{IP} values of $K_8(Si_4L_6)$ suggest that solvent-ion pairs should be considered in this case.

One test to determine the type of ion pair in the $K_8(Si_4L_6)$ cage is to compare two methods of calculating the association constant K_a from the measured data. If the association constants computed with these two methods disagree for a candidate type of

ion pair, then the candidate is inconsistent with the measurements. The association constant can be related to $[c_{IP}]$ as follows:

$$K_a = \frac{[c_{IP}]}{[cage\ charge\ sites][K^+]} = \frac{[c_{IP}]}{(4[c] - [c_{IP}])(P \times [c] - [c_{IP}])} , \quad (10)$$

where P is the number of potassium ions per cage [twelve for $K_{12}(Ga_4L_6)$, eight for $K_8(Si_4L_6)$]. A table of candidate pairing mechanisms and the association constants computed with this method is provided in Supporting Information Table S2. K_a can also be related to the rate constants of the reaction:

$$K_a = \frac{k_1}{k_{-1}}, \quad (11)$$

where k_1 and k_{-1} are the formation and decay rates of the ion pair (k_{CIP} , k_{SIP} , k_{2SIP} , etc.) respectively.

The formation and decay rates of the ion pair can be estimated from the ion-pairing time constant τ_{IP} . The time constant τ_{IP} is defined by:

$$\frac{1}{\tau_{IP}} = \frac{1}{\tau_{or}} + \frac{1}{\tau_{ch}}, \quad (12)$$

where τ_{or} is the re-orientation time constant of the ion pair, and τ_{ch} is the chemical relaxation rate:^{18,29,37}

$$\frac{1}{\tau_{ch}} = k_{-1} + k_1(M_{cage}[c] - 2[c_{IP}]) \quad (13)$$

and M_{cage} is the multiplier associated with each cage type (12 for $K_8(Si_4L_6)$, 16 for $K_{12}(Ga_4L_6)$). If $[c] \gg [c_{IP}]$, then the equation becomes:

$$\frac{1}{\tau_{ch}} = k_{-1} + k_1(M_{cage}[c]) . \quad (14)$$

In addition to helping us estimate rate constants, the τ_{IP} measurements allowed us to test for the presence of multiple types of ion pairs and transitions between different

types of ion pair. A linear relationship between I and $\frac{1}{\tau_{ch}}$ indicates that the detected relaxation represents a single ion-pairing type. In contrast, a change in slope as a function of ionic strength would indicate a transition from 1-solvent ion pair to 1-contact ion pair, for example.^{18,29,38} We used the slope of the linear fit to estimate the formation rate k_1 , $[(1.06 \pm 0.10) \times 10^6 \text{ mMol/s and } (1.53 \pm 0.29) \times 10^6 \text{ mMol/s for the K}_{12}(\text{Ga}_4\text{L}_6) \text{ and K}_8(\text{Si}_4\text{L}_6) \text{ cages, respectively}]$. These formation rates are slightly smaller than those observed in simple salt solutions, and very fast on the timescale of large guest or substrate association and reaction.^{2,39,40} This indicates that, indeed, most ion-pairing interactions are rapid pre-equilibria to supramolecular phenomena.

The higher formation rate of the $\text{K}_8(\text{Si}_4\text{L}_6)$ cage further supported the argument that the $\text{K}_8(\text{Si}_4\text{L}_6)$ and $\text{K}_{12}(\text{Ga}_4\text{L}_6)$ cages form distinct ion-pairing species. Because the $\text{K}_8(\text{Si}_4\text{L}_6)$ cage carries lower overall charge than the $\text{K}_{12}(\text{Ga}_4\text{L}_6)$ cage, we expect a higher formation rate in the $\text{K}_{12}(\text{Ga}_4\text{L}_6)$ cages. However, this difference can be explained by multi-ion complexes such as a 2-contact ion pair configuration, where the binding of an additional K^+ ion reduces the overall charge. The intercepts of the linear fits were approximately an order of magnitude lower than typical salt solutions $((1.12 \pm 0.10) \times 10^9$ and $(0.93 \pm 0.11) \times 10^9$ for the $\text{K}_{12}(\text{Ga}_4\text{L}_6)$ and $\text{K}_8(\text{Si}_4\text{L}_6)$ cages, respectively). A reasonable explanation is that the re-orientation rate of the ion pair is greatly reduced by the larger mass of the cage and produces a smaller overall value of $\frac{1}{\tau_{or}} + k_{-1}$. Both cages have similar intercepts within the uncertainty of the fit, and we conclude that the decay rate and re-orientation dynamics of both ion pairs are similar.

Using the τ_{IP} data in Fig. 5b, we found that the ion-pair formation and decay rates of $\text{K}_8(\text{Si}_4\text{L}_6)$ and $\text{K}_{12}(\text{Ga}_4\text{L}_6)$ were quite similar within the uncertainty of the measurements,

resulting in a relative association rate ($\frac{K_a(Ga)}{K_a(Si)}$ from Eq. S7) of approximately 0.35. This, in comparison with the estimates from Table S2, suggests that the ion pairs measured in the $K_8(Si_4L_6)$ system have more solvent molecules than the $K_{12}(Ga_4L_6)$ ion pair to yield $\frac{\mu_{eff}^2(Si)}{\mu_{eff}^2(Ga)} > 1$. Complementary analysis of $K_{12}(Ga_4L_6)$ suggests the presence of contact ion pairs in solution for bulkier counterion chemistries, and we conclude that 2-contact ion pairs are likely present in this system due to the low solvation state and ionic conductivity of the $K_{12}(Ga_4L_6)$ cage.¹² The single hydration shell calculated for the $K_8(Si_4L_6)$ cages suggest a 1-solvent shared ion pair while the relative K_a values suggest a 2-solvent pair to best match the K_a values from the formation and decay rates (Table S1, S2, and Fig. S4). We note that the discrepancies in these calculations may be due to the simplistic models used to construct the vertex dipole moments. We also note that in order to develop more sophisticated models, one would need data from measurement techniques that—like microwave microfluidics—probe steric and solvent effects in solutions.

Conclusion

In this report, we demonstrated the electrical detection of ion-pairing interactions in two metal-organic cages by microwave microfluidics. These findings are necessary for a complete understanding of charged supramolecular catalysts, as they shed light into the fast pre-equilibria of supramolecular phenomena. Our measurements demonstrate that both cages possess a single concentration-dependent Debye-type dielectric relaxation, which we attributed to ion-pairing at the vertices of the cage. We found that, unexpectedly, the $K_8(Si_4L_6)$ cage is more hydrated in solution than its more charged counterpart $K_{12}(Ga_4L_6)$. Additionally, the differences in ionic conductivity and DFT binding energies between the cages support the presence of a multi-ion complex (2-contact ion

pair) in the $K_{12}(Ga_4L_6)$ cage. The differences in ion-pair capacitance suggest solvent-separated ion pairs (1- or 2-solvent ion pair) in the $K_8(Si_4L_6)$ cages. Studying ion-pairing interactions is critical to understanding fundamental solution dynamics in supramolecular systems and developing design parameters for noncovalent interactions in solution. We found that broadband electrical characterization with microwave microfluidics can provide fresh insights into ion-pairing interactions in complex chemical systems, offering a new tool for characterization of supramolecular catalysts. These microwave microfluidic measurements have the potential to elucidate the direct impacts of solvation and ion-pairing on guest molecule association and enzyme-like catalysis. To achieve this, our work highlights the need to couple microwave microfluidics measurements and advanced computational models that explicitly account for charge-based, entropic, and solvent effects.

Methods

Preparation of Cage Samples

The $K_{12}(Ga_4L_6)$ and $K_8(Si_4L_6)$ cages were prepared and isolated according to literature procedures.² A stock solution of $K_{12}(Ga_4L_6)$ was prepared at approximately 10 mM, which was accurately calibrated by 1H NMR. The calibration was performed by mixing a 100 μL aliquot of the stock solution with a 500 μL aliquot of D_2O containing 5 mM sodium tosylate as an internal standard. From this accurate concentration, serial dilutions were performed to obtain aqueous samples of $K_{12}(Ga_4L_6)$, which were then sealed under vacuum in glass ampules until measured.

The relatively low solubility of $K_8(Si_4L_6)$ in pure water limited the concentration regime which could be measured. To maximize the concentration of $K_8(Si_4L_6)$, a 35 mg cage sample was suspended in 1 mL of degassed water, sonicated for one hour, then allowed to stand overnight. The resulting cloudy solution was centrifuged and the supernatant was filtered with a 0.2 micron syringe filter. This saturated stock solution was calibrated by 1H NMR. The calibration was performed by mixing a 100 μL aliquot of the stock solution with a 500 μL aliquot of D_2O containing 5 mM of sodium tosylate as an internal standard. From this concentration, serial dilutions were performed to obtain aqueous samples of $K_8(Si_4L_6)$, which were then sealed under vacuum in glass ampules until measured.

References

- (1) Yoshizawa, M.; Klosterman, J. K.; Fujita, M. Functional Molecular Flasks: New Properties and Reactions within Discrete, Self-Assembled Hosts. *Angew. Chemie - Int. Ed.* **2009**, *48* (19), 3418–3438.
- (2) Hong, C. M.; Morimoto, M.; Kapustin, E. A.; Alzakhem, N.; Bergman, R. G.; Raymond, K. N.; Toste, F. D. Deconvoluting the Role of Charge in a Supramolecular Catalyst. *J. Am. Chem. Soc.* **2018**, *140*, jacs.8b01701.
- (3) Hart-Cooper, W. M.; Zhao, C.; Triano, R. M.; Yaghoubi, P.; Ozores, H. L.; Burford, K. N.; Toste, F. D.; Bergman, R. G.; Raymond, K. N. The Effect of Host Structure on the Selectivity and Mechanism of Supramolecular Catalysis of Prins Cyclizations. *Chem. Sci.* **2015**, *6* (2), 1383–1393.
- (4) Cullen, W.; Misuraca, M. C.; Hunter, C. A.; Williams, N. H.; Ward, M. D. Highly Efficient Catalysis of the Kemp Elimination in the Cavity of a Cubic Coordination Cage. *Nat. Chem.* **2016**, *8* (3), 231–236.
- (5) Evans, N. H.; Beer, P. D. Advances in Anion Supramolecular Chemistry: From Recognition to Chemical Applications. *Angew. Chemie - Int. Ed.* **2014**, *53* (44), 11716–11754.
- (6) Grommet, A. B.; Nitschke, J. R. Directed Phase Transfer of an Fe^{II}₄L₄ Cage and Encapsulated Cargo. *J. Am. Chem. Soc.* **2017**, *139* (6), 2176–2179.
- (7) Yi, S.; Brega, V.; Captain, B.; Kaifer, A. E. Sulfate-Templated Self-Assembly of New M₄L₆ Tetrahedral Metal Organic Cages. *Chem. Commun.* **2012**, *48* (83), 10295.
- (8) Rizzuto, F. J.; Wu, W.; Ronson, T. K.; Nitschke, J. R. Peripheral Templatation

- Generates an M II 6 L 4 Guest-Binding Capsule. *Angew. Chemie* **2016**, *128* (28), 8090–8094.
- (9) Bruns, C. J.; Fujita, D.; Hoshino, M.; Sato, S.; Stoddart, J. F.; Fujita, M. Emergent Ion-Gated Binding of Cationic Host-Guest Complexes within Cationic M12L24molecular Flasks. *J. Am. Chem. Soc.* **2014**, *136* (34), 12027–12034.
 - (10) Bolliger, J. L.; Ronson, T. K.; Ogawa, M.; Nitschke, J. R. Solvent Effects upon Guest Binding and Dynamics of a Fe^{II}₄L₄ Cage. *J. Am. Chem. Soc.* **2014**, *136* (41), 14545–14553.
 - (11) Custelcean, R. Anion Encapsulation and Dynamics in Self-Assembled Coordination Cages. *Chem. Soc. Rev.* **2014**, *43* (6), 1813–1824.
 - (12) Sgarlata, C.; Mugridge, J. S.; Pluth, M. D.; Zito, V.; Arena, G.; Raymond, K. N. Different and Often Opposing Forces Drive the Encapsulation and Multiple Exterior Binding of Charged Guests to a M4L6 Supramolecular Vessel in Water. *Chem. - A Eur. J.* **2017**, *23* (66), 16813–16818.
 - (13) Tobey, S. L.; Anslyn, E. V. Studies into the Thermodynamic Origin of Negative Cooperativity in Ion-Pairing Molecular Recognition. *J. Am. Chem. Soc.* **2003**, *125* (36), 10963–10970.
 - (14) Valderrey, V.; Escudero-Adán, E. C.; Ballester, P. Highly Cooperative Binding of Ion-Pair Dimers and Ion Quartets by a Bis(Calix[4]Pyrrole) Macrotricyclic Receptor. *Angew. Chemie - Int. Ed.* **2013**, *52* (27), 6898–6902.
 - (15) Sambrook, M. R.; Beer, P. D.; Wisner, J. A.; Paul, R. L.; Cowley, A. R.; Szemes, F.; Drew, M. G. B. Anion-Templated Assembly of Pseudorotaxanes: Importance of Anion Template, Strength of Ion-Pair Thread Association, and Macrocyclic Ring

- Size. *J. Am. Chem. Soc.* **2005**, *127* (7), 2292–2302.
- (16) Pluth, M. D.; Tiedemann, B. E.; van, H. H.; Nunlist, R.; Raymond, K. N. Diffusion of a Highly Charged Supramolecular Assembly: Direct Observation of Ion Association in Water207. *Inorg.Chem.* **2008**, *47* (5), 1411–1413.
- (17) Schalley, C. A. *Analytical Methods in Supramolecular Chemistry*; 2012.
- (18) Buchner, R.; Capewell, S. G.; Hefter, G. T.; May, P. M. Ion-Pair and Solvent Relaxation Processes in Aqueous Na₂SO₄ Solutions. *J. Phys. Chem. B* **1999**, *103* (7), 1185–1192.
- (19) Solutions, A.; Wachter, W.; Kunz, W.; Buchner, R.; Regensburg, D.-; Hefter, G.; Dse, C.; V, M. U. Is There an Anionic Hofmeister Effect on Water Dynamics ? Dielectric Spectroscopy Of. **2005**, 8675–8683.
- (20) Friesen, S.; Krickl, S.; Luger, M.; Nazet, A.; Hefter, G.; Buchner, R. Hydration and Ion Association of La³⁺ and Eu³⁺ Salts in Aqueous Solution. *Phys. Chem. Chem. Phys.* **2018**, *20*, 8812–8821.
- (21) Basey-Fisher, T. H.; Hanham, S. M.; Andresen, H.; Maier, S. A.; Stevens, M. M.; Alford, N. M.; Klein, N. Microwave Debye Relaxation Analysis of Dissolved Proteins: Towards Free-Solution Biosensing. *Appl. Phys. Lett.* **2011**, *99* (23), 10–13.
- (22) Ermilova, E.; Bier, F. F.; Hölzel, R. Dielectric Measurements of Aqueous DNA Solutions up to 110 GHz. *Phys. Chem. Chem. Phys.* **2014**, *16* (23), 11256.
- (23) Tros, M.; Zheng, L.; Hunger, J.; Bonn, M.; Bonn, D.; Smits, G. J.; Woutersen, S. Picosecond Orientational Dynamics of Water in Living Cells. *Nat. Commun.* **2017**, *8* (1), 1–7.

- (24) Little, C. A. E.; Orloff, N. D.; Hanemann, I. E.; Long, C.; Bright, V. M.; Booth, J. C. Modeling Electrical Double-Layer Effects for Microfluidic Impedance Spectroscopy from 100 KHz to 110 GHz. *Lab Chip* **2017**, *17*, 2674–2681.
- (25) Wright, M. *An Introduction to Aqueous Electrolyte Solutions*; 2007.
- (26) Jeong, J. Y.; Jang, J.; Patra, A.; Eom, K.; Park, I.; Yang, Y.; Kim, S.; Di Tommaso, D.; Park, G. S. Is Fast Relaxation Water Really a Free Water? *Int. Conf. Infrared, Millimeter, Terahertz Waves, IRMMW-THz* **2017**, 3–4.
- (27) Chekalin, N. V.; Shakhparonov, M. I. The Mechanism of Dielectric Relaxation in Water. *J. Struct. Chem.* **1968**, *9* (5), 789–790.
- (28) Bazant, M. Z.; Thornton, K.; Ajdari, A. Diffuse-Charge Dynamics in Electrochemical Systems. *Phys. Rev. E - Stat. Nonlinear, Soft Matter Phys.* **2004**, *70* (2 1), 1–24.
- (29) Buchner, R.; Samani, F.; May, P. M.; Sturm, P.; Hefter, G. Hydration and Ion Pairing in Aqueous Sodium Oxalate Solutions. *ChemPhysChem* **2003**, *4* (4), 373–378.
- (30) Cavell, E. A. S. Dielectric Relaxation in Non Aqueous Solutions. *Trans. Faraday Soc.* **1971**, *67* (1), 2225.
- (31) Chen, T.; Hefter, G.; Buchner, R. Dielectric Spectroscopy of Aqueous Solutions of KCl and CsCl. *J. Phys. Chem. A* **2003**, *107* (20), 4025–4031.
- (32) Little, C. A. E.; Orloff, N. D.; Hanemann, I. E.; Long, C. J.; Bright, V. M.; Booth, J. C. Modeling Electrical Double-Layer Effects for Microfluidic Impedance Spectroscopy from 100 KHz to 110 GHz. *Lab Chip* **2017**, *17* (15), 2674–2681.
- (33) York, D.; Evensen, N. M.; Martínez, M. L.; De Basabe Delgado, J. Unified

Equations for the Slope, Intercept, and Standard Errors of the Best Straight Line.

Am. J. Phys. **2004**.

- (34) Buchner, R.; Hefter, G. Interactions and Dynamics in Electrolyte Solutions by Dielectric Spectroscopy. *Phys. Chem. Chem. Phys.* **2009**, *11* (40), 8984.
- (35) Hünenberger, P.; Reif, M. *Single-Ion Solvation: Experimental and Theoretical Approaches to Elusive Thermodynamic*; 2011.
- (36) Schrödle, S.; Wachter, W.; Buchner, R.; Hefter, G. Scandium Sulfate Complexation in Aqueous Solution by Dielectric Relaxation Spectroscopy. *Inorg. Chem.* **2008**, *47* (19), 8619–8628.
- (37) Buchner, R.; Barthel, J. Kinetic Processes in the Liquid Phase Studied by High-Frequency Permittivity Measurements. *J. Mol. Liq.* **1995**, *63* (1–2), 55–75.
- (38) Barthel, J.; Kleebauer, M.; Buchner, R. Dielectric Relaxation of Electrolyte Solutions in Acetonitrile. *J. Solution Chem.* **1995**, *24* (1), 1–17.
- (39) Levin, M. D.; Kaphan, D. M.; Hong, C. M.; Bergman, R. G.; Raymond, K. N.; Toste, F. D. Scope and Mechanism of Cooperativity at the Intersection of Organometallic and Supramolecular Catalysis. *J. Am. Chem. Soc.* **2016**, *138* (30), 9682–9693.
- (40) Pluth, M. D.; Raymond, K. N. Reversible Guest Exchange Mechanisms in Supramolecular Host-Guest Assemblies. *Chem. Soc. Rev.* **2007**, *36* (2), 161–171.

Figures and captions

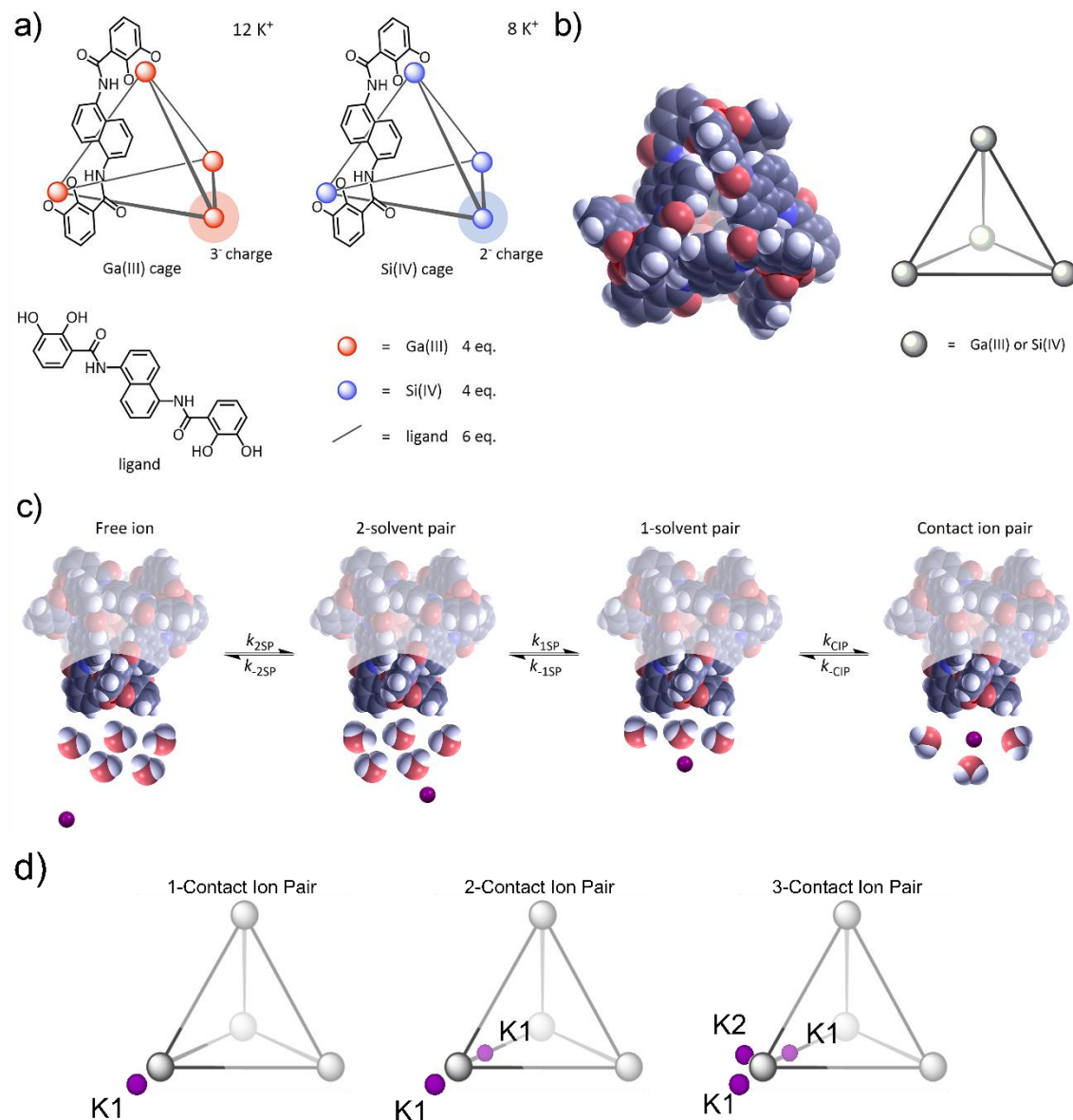


Fig. 1: Schematic representation of isostructural metal-organic cages $\text{K}_{12}(\text{Ga}_4\text{L}_6)$ and $\text{K}_8(\text{Si}_4\text{L}_6)$ with one ligand of six shown for clarity (a). A representative crystal structure of the cages with a schematic shown in the same orientation (b). Step-wise formation of close contact ion pairs *via* freely solvated potassium cations, 2-solvent pairing, and 1-solvent pairing of potassium cations at each cage vertex. (c). Schematic representation

of step-wise ion pair formation on the vertex charge center of the cage. Reaction rates for each step are labelled. (d). Schematic representation of different contact ion pair configurations on the vertex charge center of the cage filling position K1 (1-contact ion pair), K1 equivalent position (2-contact ion pair), and K2 (3-contact ion pair, only considered for the $K_{12}(Ga_4L_6)$ case).

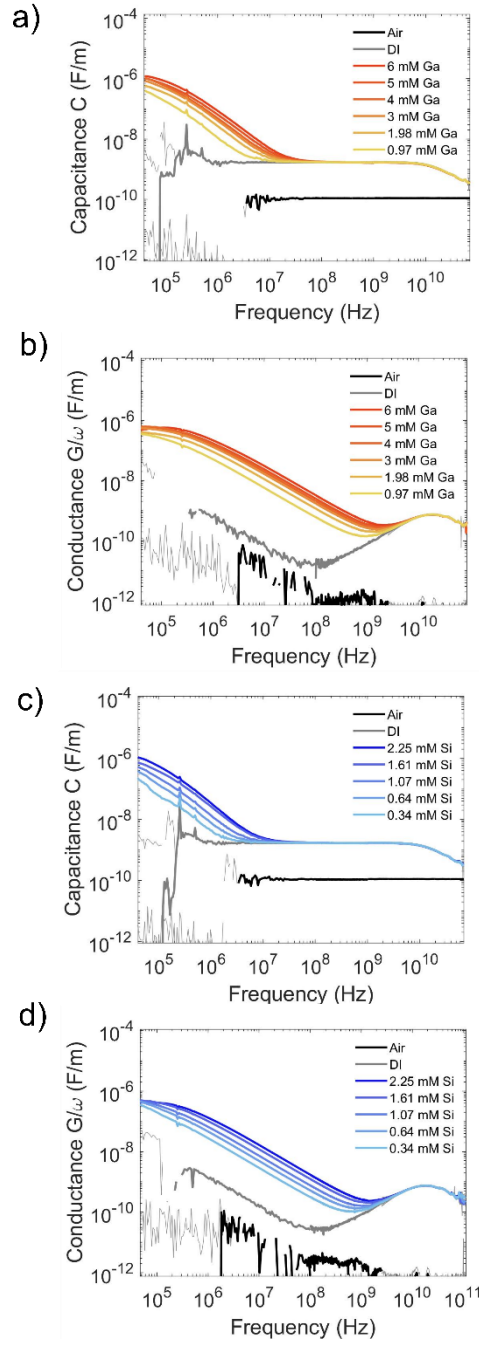


Fig. 2: Calibrated (a, c) distributed capacitance C_{tot} and (b, d) distributed conductance $\frac{G_{tot}}{\omega}$ for air, DI water, and a range of concentrations of (a, b) $K_{12}(Ga_4L_6)$ cages (0.97 mM to 6 mM) and (c, d) $K_8(Si_4L_6)$ cages (0.34 mM to 2.25 mM).

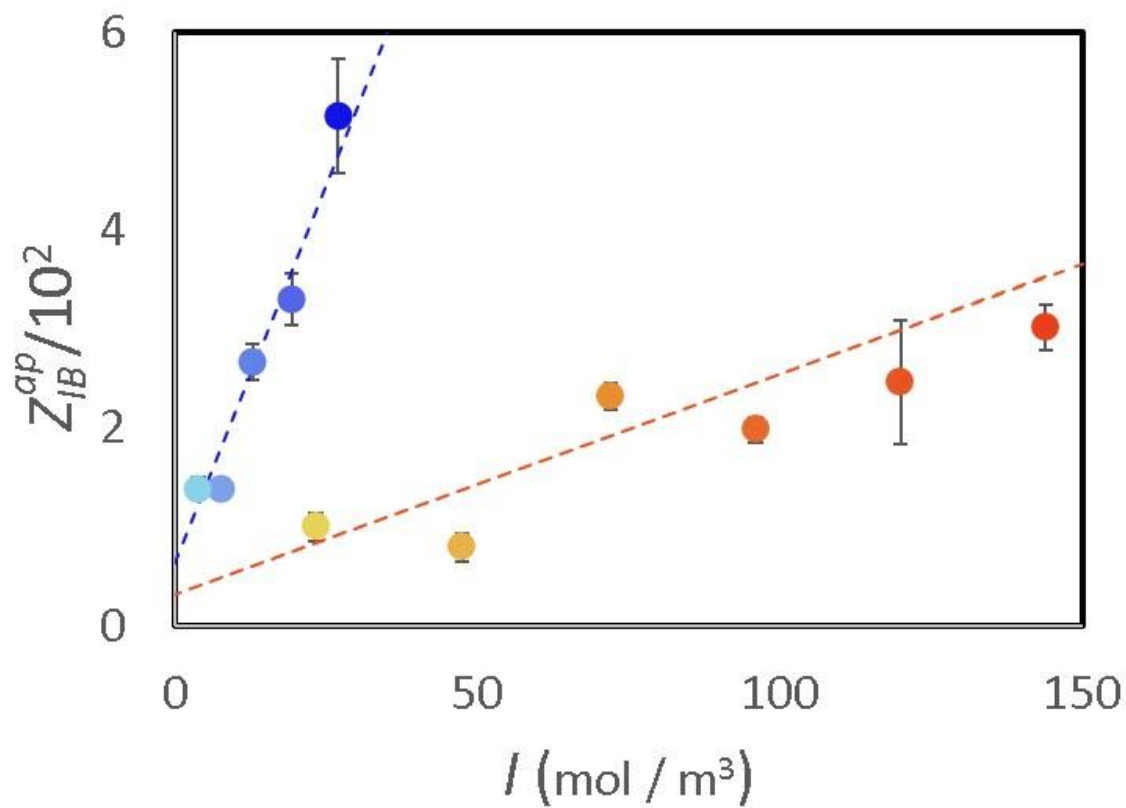


Fig. 3: Hydration number Z_{IB}^{ap} as a function of stoichiometric ionic strength I for $K_{12}(Ga_4L_6)$ (red and orange points, $R^2 = 0.82$) and $K_8(Si_4L_6)$ cages (blue points, $R^2 = 0.81$). Error bars represent 95 % confidence intervals propagated from fit parameters of C_{tot} and G_{tot} .

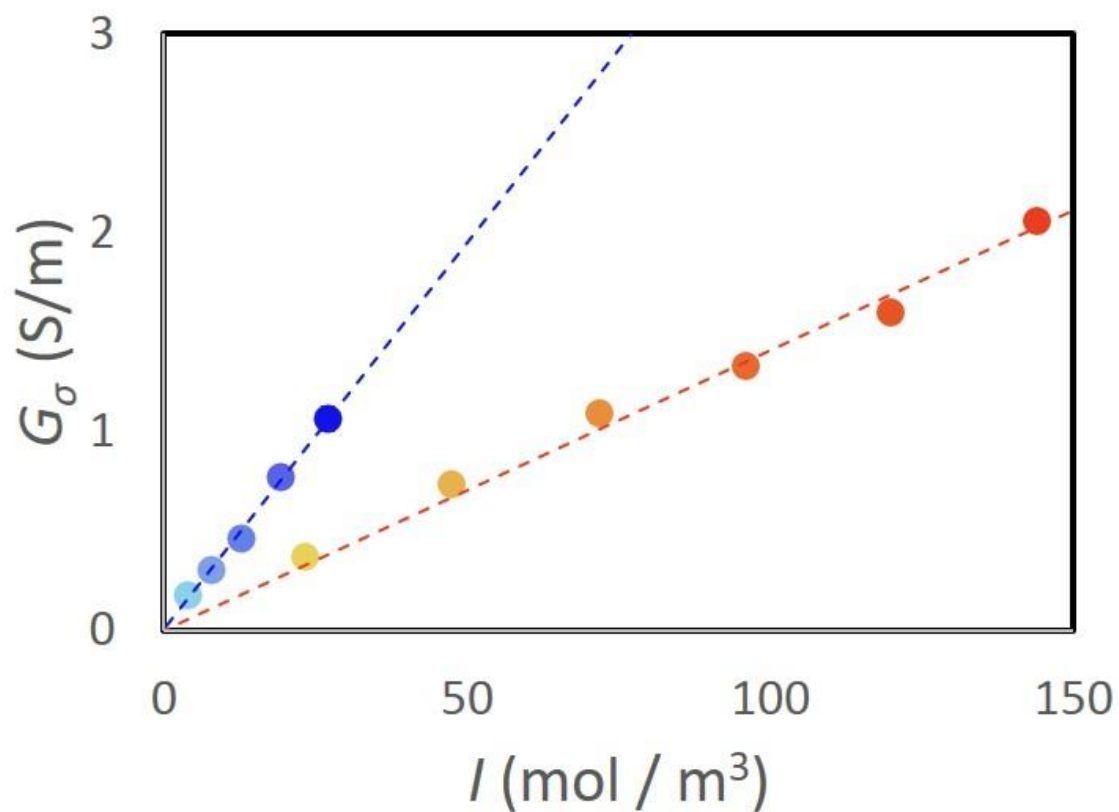


Fig. 4: Ionic conductivity as a function of stoichiometric ionic strength I for $K_{12}(\text{Ga}_4\text{L}_6)$ (red and orange points, $R^2 = 0.99$) and $K_8(\text{Si}_4\text{L}_6)$ cages (blue points, $R^2 = 0.99$). Error bars represent 95 % confidence intervals (smaller than point size).

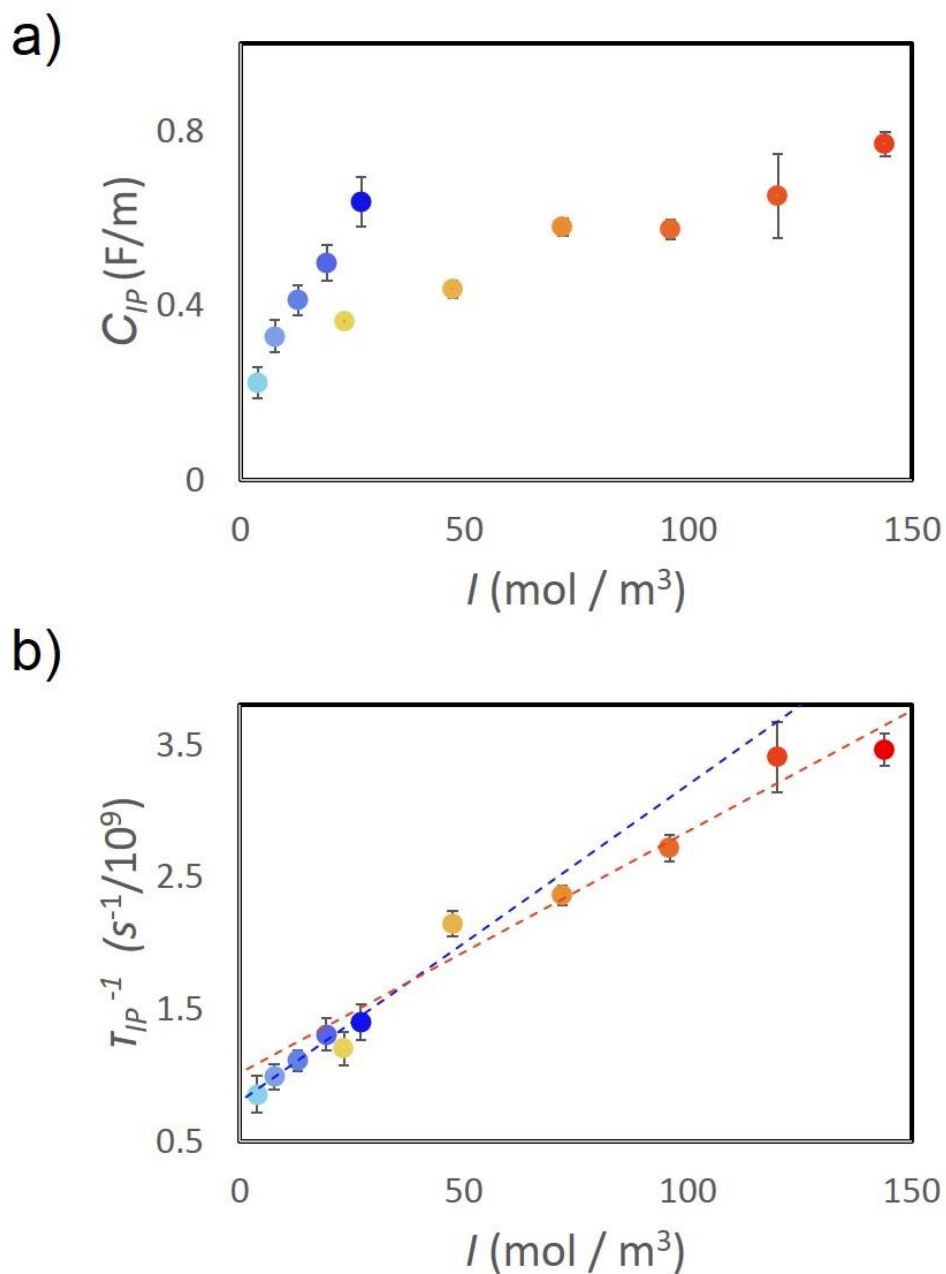


Fig. 5: a) Dipolar contribution of ion pairs C_{IP} as a function of stoichiometric ionic strength I for $K_{12}(Ga_4L_6)$ cages (red and orange points) and $K_8(Si_4L_6)$ cages (blue points). Error bars represent 95 % confidence intervals. b) Relaxation times of ion pairs τ_{IP} as a function of stoichiometric ionic strength I for $K_{12}(Ga_4L_6)$ cages (red and orange points, R^2

= 0.94) and $K_8(\text{Si}_4\text{L}_6)$ cages (blue points, $R^2 = 0.97$). Error bars represent 95 % confidence intervals.