

## Electronic Supplementary Information

### The protonation state and binding mode in a metal coordination complex from the charge measured in solution by electrophoretic NMR

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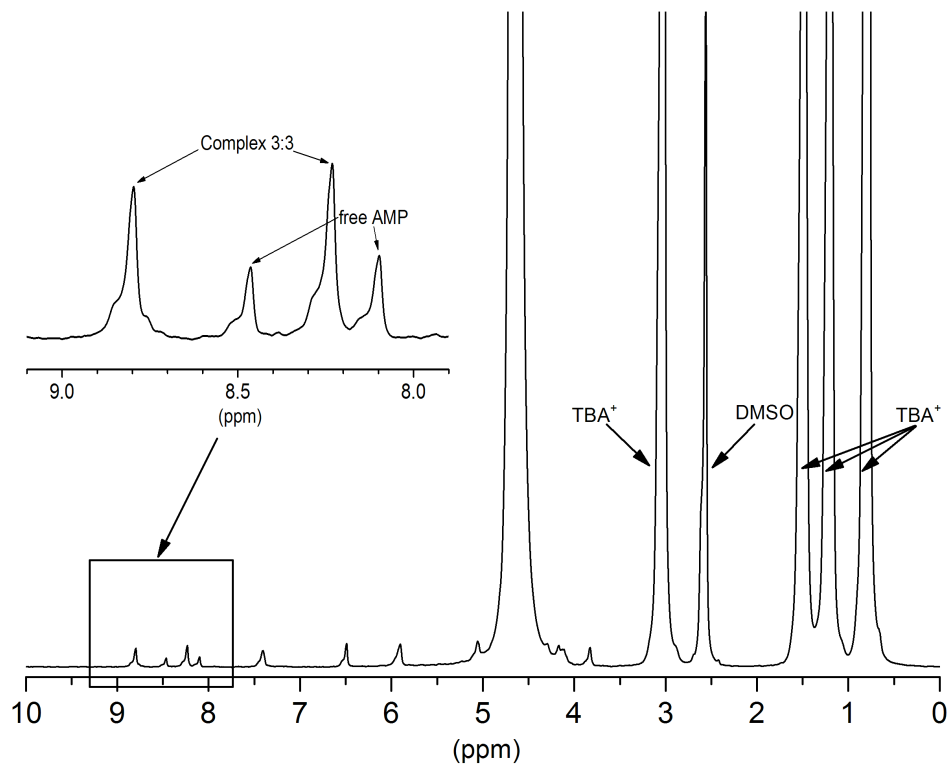
#### Samples

The adenosine 5'-monophosphate (AMP) monohydrate (>97%, Sigma-Aldrich), uranyl nitrate (99%, Merck), tetrabutylammonium hydroxide (TBA-OH) 30-hydrate (>99%, Sigma-Aldrich) and dimethylsulfoxide (DMSO, 99,5 %, Merck) were used as received and all solutions prepared in D<sub>2</sub>O (99.9 % D, Isotec Inc). Beside the ones presented in the paper, two additional test samples (Samples 4 and 5) were prepared, by the procedure as given in the main text, in order to establish whether or not TBA<sup>+</sup> associates to AMP. Both samples contained 45 mM TBA-OH in D<sub>2</sub>O with either 2 mM AMP monohydrate (Sample 4) or 10 mM AMP monohydrate (Sample 5).

#### <sup>1</sup>H NMR experiments

All NMR experiments were performed on a Bruker Avance 500 spectrometer at 298 K, equipped with a 5 mm high resolution probe (Bruker BBI) with a gradient coil capable to supply 0.5 T/m maximum gradient strength. The <sup>1</sup>H NMR spectrum obtained in Sample 2 is shown in Fig. S1.

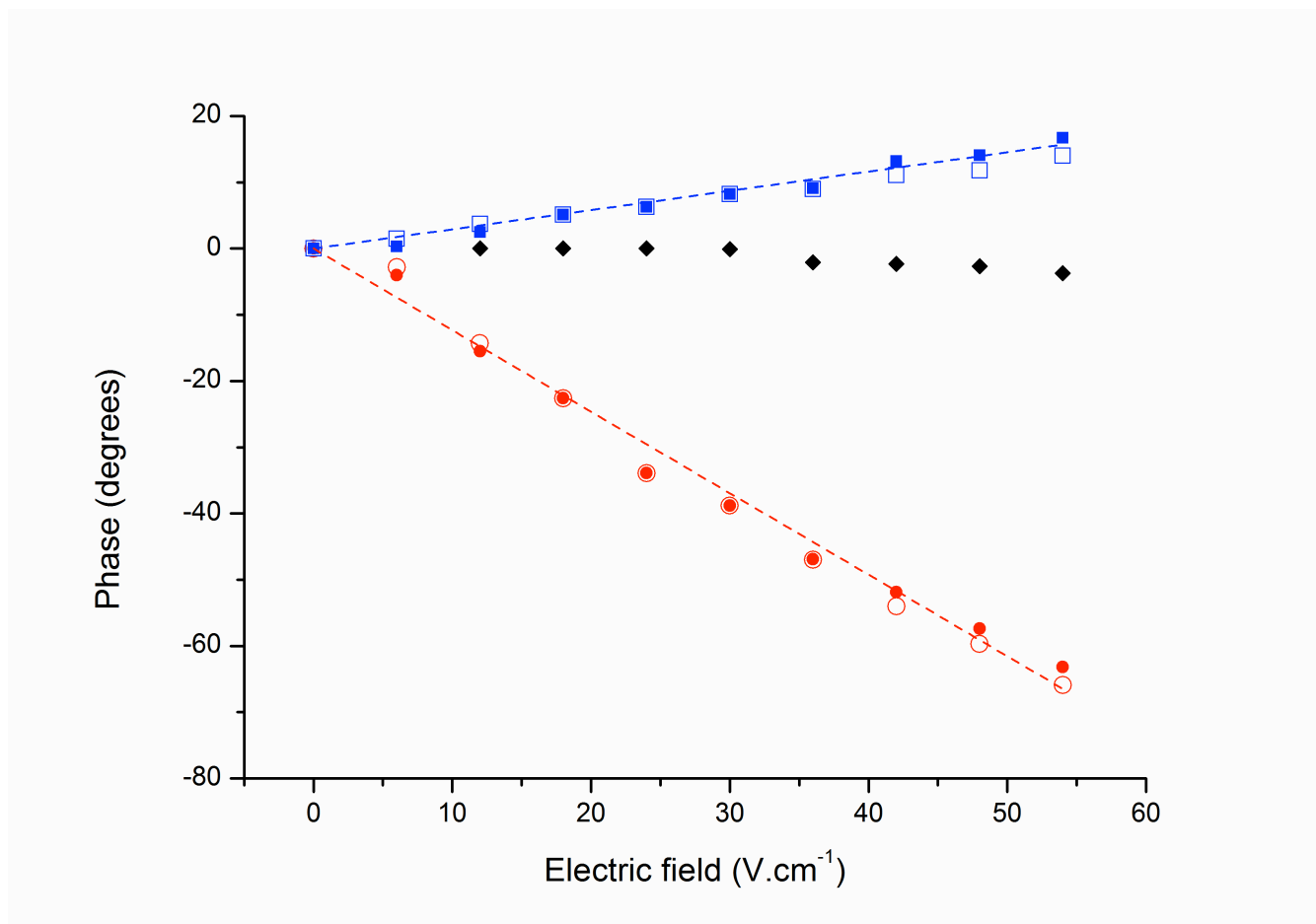
Diffusion experiments were performed using the stimulated echo experiment. Parameters used in the diffusion experiments in Samples 1-5 were  $\Delta = 140$  ms and  $\delta = 4$  ms. The gradient strength  $g$  was incremented linearly from 0.005 up to 0.39 T/m in 24-32 steps. For sample 6,  $\Delta = 100$  ms was used and  $g$  was incremented from 0.005 to 0.39 T/m in 32 steps. The gradient strength was calibrated using the known <sup>1</sup>H diffusion coefficient<sup>3</sup> of HOD in heavy water  $D = 1.902 \cdot 10^{-9}$  m<sup>2</sup>/s.



**Fig. S1** The <sup>1</sup>H NMR spectrum of Sample 2 as recorded in the eNMR cell. The magnetic field homogeneity in the sample is clearly somewhat worse than customary in conventional high resolution NMR probes, which is caused by the presence of electrodes in the sample cell<sup>1</sup>. The inset shows the magnified spectral region containing the heteroaromatic AMP peaks for Complex 3:3 and free ligands. The full assignment of the other AMP peaks is provided elsewhere<sup>2</sup>.

In the eNMR experiments, a double stimulated echo pulse sequence with bipolar electrophoretic voltages<sup>4</sup> was used. The electrophoretic displacement period  $\Delta$  was set to 200 ms, the gradient pulse duration  $\delta$  was 1 ms and  $g$  was 0.13 T/m. The voltage was stepped from 0 V in 10 equal steps up to 200 V. The phase shifts obtained as the function of applied electrophoretic voltage are shown in Fig. S2 for an eNMR experiment in Sample 2. The 5 mm eNMR cell used and the generation and control of electrophoretic voltages has been described elsewhere.<sup>1</sup> The distance between the palladium electrodes was approximately 35 mm (distance calibrated<sup>1</sup> by eNMR experiments in a aqueous solution (with D<sub>2</sub>O) of 10 mM tetramethylammonium bromide). All sample volumes for eNMR measurements were 500  $\mu$ l and to each sample was added 1  $\mu$ l of DMSO, which was used as the

uncharged reference. A slight temperature increase due to Joule heating (in the order of 0.1 - 0.2 K, established in separate experiments in isoconductive samples containing molecules with temperature dependent  $^1\text{H}$  chemical shift) was noted in the eNMR experiments. The obtained diffusion coefficients were corrected for this effect by exploiting the known temperature dependence of the viscosity of  $\text{D}_2\text{O}$ <sup>5</sup>.



**Fig. S2** The phase shifts of the  $^1\text{H}$  NMR signals of Complex 3:3 (○),  $\text{TBA}^+$  (□) and DMSO (◆) with increasing electric field strength  $E$  in sample 2. Linear fits of the phase shifts relative to the DMSO reference phases (● for Complex 3:3 and ■ for  $\text{TBA}^+$ ), as demonstrated here for the  $\text{TBA}^+$  data, provide the respective electrophoretic mobilities.<sup>1</sup>

The results presented in Table S1 show that the  $\text{TBA}^+$  cation does not significantly bind to free AMP, as the effective charge of  $\text{TBA}^+$  in Samples 4 and 5 is roughly the same as that in Sample 3. The concentration of Complex 3:3 in Samples 1 and 2 (see Table 1) were determined by measuring the integral intensities of free AMP and Complex 3:3 lines in  $^1\text{H}$  spectra recorded with a relaxation delay of 10 seconds.

**Table S1.** Diffusion coefficients  $D$ , electrophoretic mobilities  $\mu$ , and effective charges  $z$  of AMP and TBA<sup>+</sup> in Samples 4-5.

		AMP			TBA <sup>+</sup>		
Sample	pD	$D$ (10 <sup>-11</sup> m <sup>2</sup> /s)	$\mu$ (10 <sup>-8</sup> m <sup>2</sup> /Vs)	$z$	$D$ (10 <sup>-11</sup> m <sup>2</sup> /s)	$\mu$ (10 <sup>-8</sup> m <sup>2</sup> /Vs)	$z$
Sample 4	13	35.8	-2.39	-1.71 <sup>a</sup>	40.8	1.06	0.66
Sample 5 <sup>b</sup>	13	36.1	-2.49±0.09	-1.79±0.09 <sup>a</sup>	40.0±0.24	0.97±0.04	0.62±0.03

<sup>a</sup>Free AMP.

<sup>b</sup>Standard deviation calculated from results in three repeated experiments.

### Effective charge in fast exchange

We analyze the situation where an ion is distributed between two pools, one (pool identified as “free”) that contains free ions in solution and the other (“bound”) that is for ions that bind to other, larger objects (particle, complex, macromolecule). We assume that there exists fast exchange between these two pools – in other words, NMR experiments measure average molecular properties. Molecules in either pool are characterized by their own respective experimental parameters. Hence, we have distinct self-diffusion coefficients  $D_{free}$  and  $D_{bound}$  and distinct electrophoretic mobilities  $\mu_{free}$  and  $\mu_{bound}$ . We also assume that the solution is dilute which means that binding of the ions does not change the properties of the ions in the free pool.

Because of fast exchange both diffusion and electrophoretic NMR experiments measure population averages of the parameters

$$D_{av} = (1-p)D_{free} + pD_{bound} \quad (S1a)$$

$$\mu_{av} = (1-p)\mu_{free} + p\mu_{bound} \quad (S1b)$$

where  $p$  is the fraction of ions bound. Hence, the measured effective charge defined as

$$z_{av} = \frac{\mu_{av}}{\alpha D_{av}} \quad (S2)$$

with  $\alpha = e/k_B T$  as scaling parameter (see Eq. 1) can be expressed as

$$z_{av} = \frac{\mu_{av}}{D_{av}} \frac{k_B T}{e} = \frac{\frac{1-p}{D_{bound}} z_{bound} + \frac{p}{D_{free}} z_{free}}{\frac{1-p}{D_{bound}} + \frac{p}{D_{free}}} \quad (\text{S3a})$$

where

$$z_{free} = \frac{\mu_{free}}{\alpha D_{free}} \quad \text{and} \quad z_{bound} = \frac{\mu_{bound}}{\alpha D_{bound}} \quad (\text{S3b})$$

are the pool-specific effective charges. Often, one is interested in obtaining  $p$  which can be derived on the conventional manner as given in Eqs. 3a and 3b in the main text.

### Counterion association and the nominal charge

In the main text, we state that the plausible mechanism of  $TBA^+$  association to Complex 3:3 is electrostatic. One could argue for other, not electrostatic mechanism of association. If that were the case, one could not exclude  $TBA^+$  ions carrying their  $OH^-$  counterions with them while associating. In this hypothetical case, Eq. (4) in the main text should be replaced by

$$z_C = z_{nominal} + z_{free} \frac{p \cdot [TBA^+]}{[Complex\ 3:3]} \quad (\text{S4})$$

which results (with  $z_{free}$  taken from Table 2) in having slightly different nominal charges for Complex 3:3, see Table S2. The conclusions are not altered.

**Table S2.** Same as Table 3 in the main text, but having  $z_{nominal}$  calculated from Eq.S4 instead of Eq.4 in the main text.

Sample	$p$ (%)	$z_{nominal}$
1	7.7±0.8/6.2±2	-4.7±0.3/-4.5±0.4
2	7.2±0.7/5.2±1.7	-6.5±0.4/-6.0±0.5

## References

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