Supporting Information

Charge Density-Dependent Modifications of Hydration Shell Waters by Hofmeister Ions

*Feng Guo, and Joel M. Friedman**

Department of Biophysics and Physiology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, New York, U.S.A. 10461

E-mail: jfriedma@aecom.yu.edu

Supplemental Figure Captions:

Supplemental Figure 1. Emission fluorescence from Gd^{3+} coordinated calmodulin with different gating delay time. (Excitation: 273nm. The integration time: 1000 us.) The figure shows that GVSBLS with gated acquisition eliminates interference from the intrinsic fluorescence of calmodulin. This is achieved because of the lifetime of Gd^{3+} luminescence (~1000 us) is much longer than much shorter lived intrinsic emission from Trp/Tyr/Phe.

Supplemental Figure 2. Normalized water OH stretching mode VSB for samples of 1mM $GdCl_3$ aqueous solution (—) and 1 mM calmodulin and 1 mM Gd^{3+} in 10 mM HEPES buffer at pH 7.0 (—). *Inset:* enlargement of normalized –OH stretching mode VSB

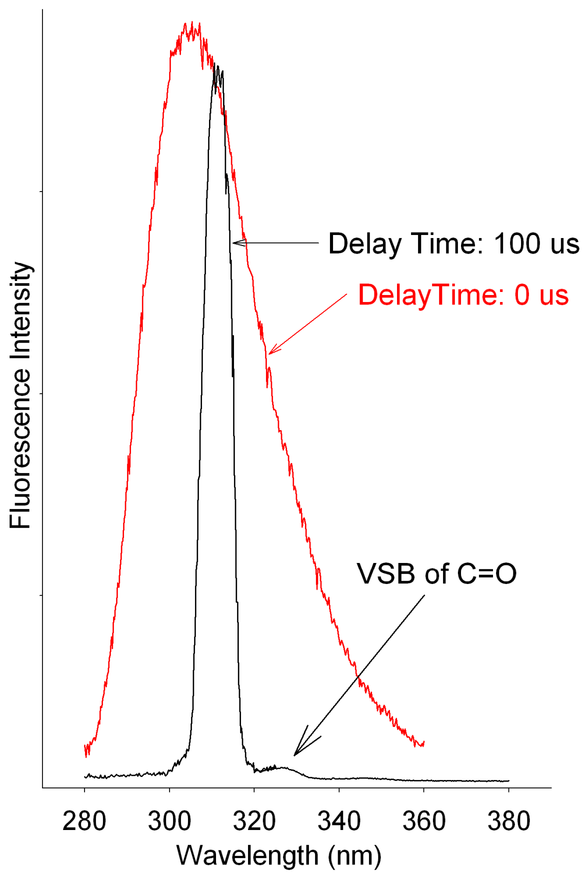
Supplemental Figure 3. Normalized water OH stretching mode VSB for samples of 1 mM Gd^{3+} aqueous solution (—), 1 mM mSE3 and 1 mM Gd^{3+} in 10 mM HEPES buffer at pH 7.0 in the absence of salts (—) and in the presence of 4.0 M $MgCl_2$ (—).

Supplemental Figure 4. Normalized stretching mode VSB for samples of 1 mM calmodulin and 1 mM Gd^{3+} in 10 mM HEPES buffer at pH 7.0 in the absence of salts (—) and in the presence of 4.0 M NaCl (—) and 4.0 M $MgCl_2$ (—). *Inset:* enlargement of normalized –OH stretching mode VSB

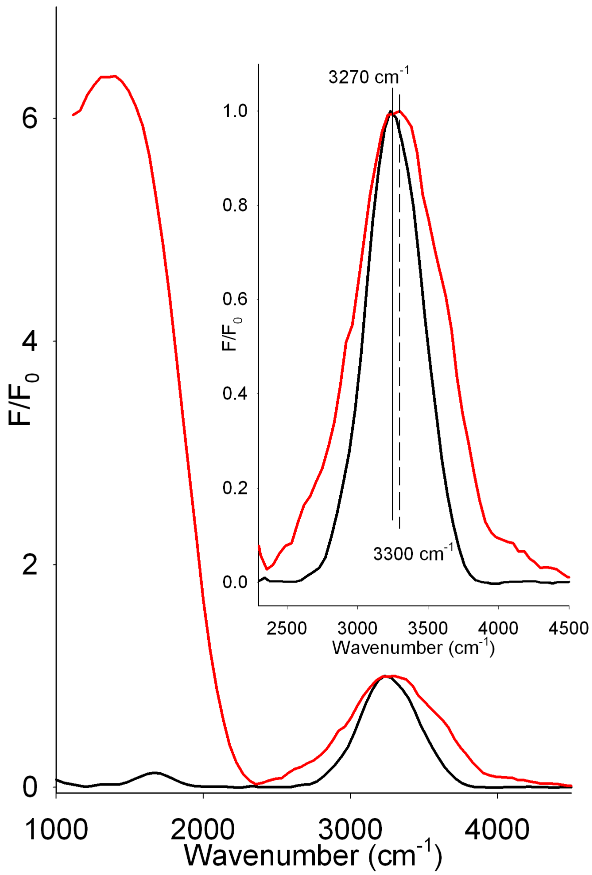
Supplemental Figure 5. Experimental Setchenow constants vs. OH stretch frequency shift obtained from GVSBLS of free Gd^{3+} with 1 M added chloride salts (r^2=0.780)

Supplemental Figure 6. Normalized stretching mode VSB for samples of 0.5 M $GdCl_3$ () and 0.5 M $GdCl_3$ with 4 M KCl in the absence () and presence of 1 M NaCl (); 1 M $MgCl_2$ () and 1 M $AlCl_3$ ().

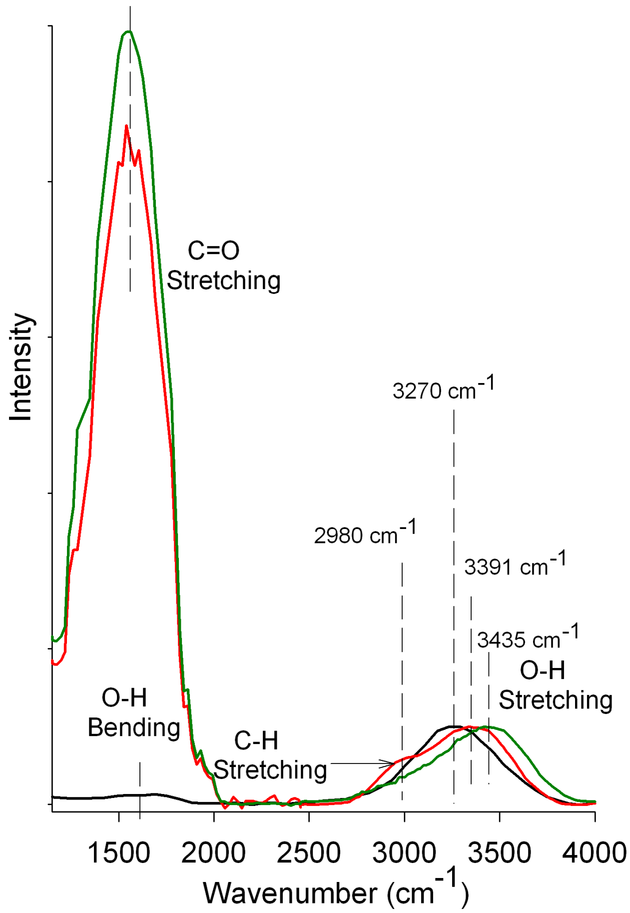
Supplemental Figures :



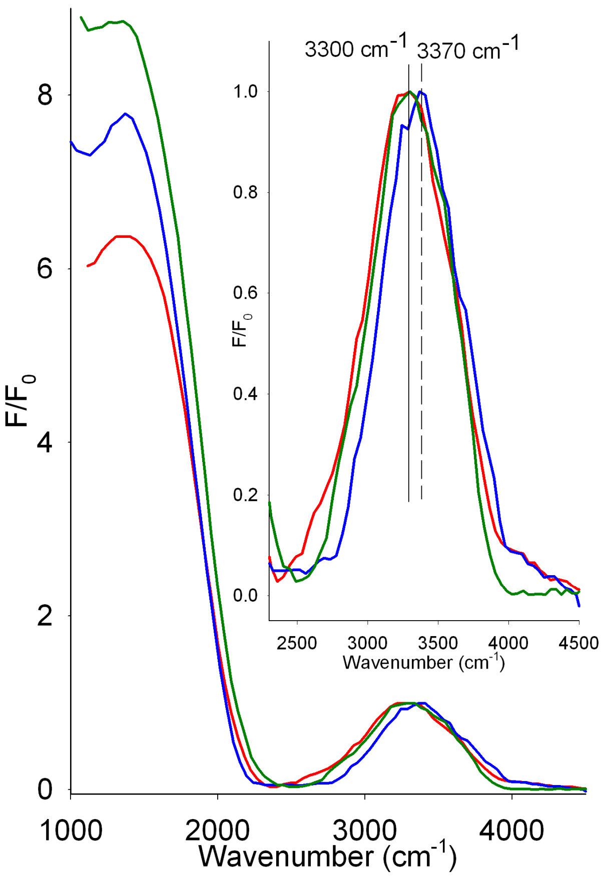
Supplemental Figure 1



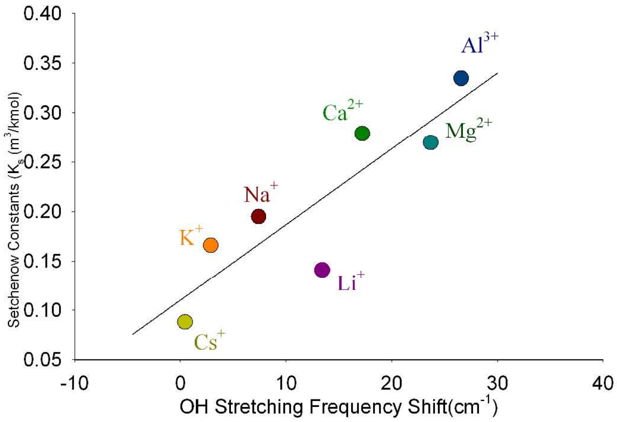
Supplemental Figure 2



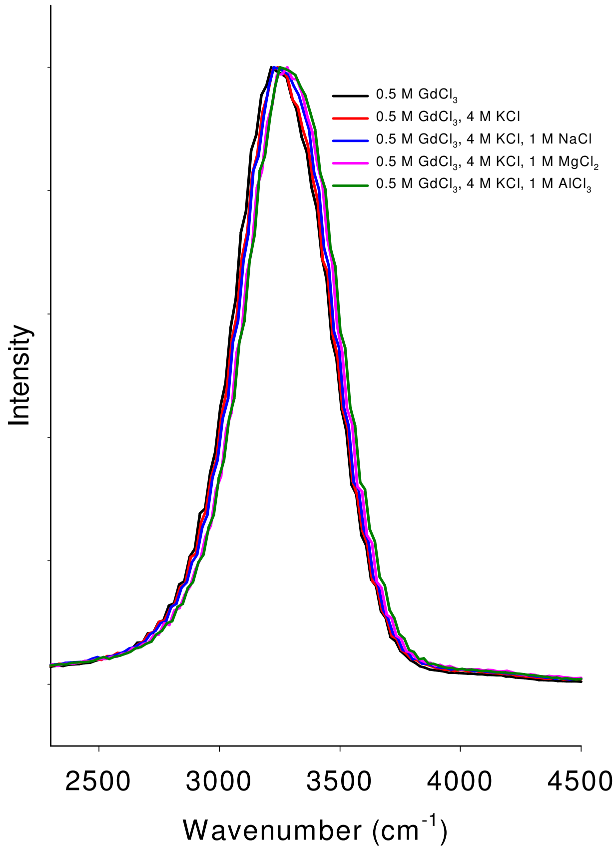
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6

Supplemental Table Captions:

Supplemental Table 1. Comparison of mSE3 and SE2 with Troponin C and general LBT sequence. Bold type indicates metal-coordinating residues. (unpublished results from Langdon Martin in the B. Imperiali Lab at MIT).

Supplemental Table 2. Summary of binding constants of Ca^{2+}, Gd^{3+} and Mg^{2+} to EDTA and calmodulin at pH 7.0.

Supplemental Table 3. The OH stretching VBS frequencies derived from aqueous solutions of 0.5 M $GdCl_3$, 80 mM $GdCl_3$+100 mM EDTA, 1 mM $GdCl_3$+1 mM calmodulin and 1 mM $GdCl_3$+1 mM mSE3 and (in parenthesis) the frequency shift for the different coordinated Gd^{3+} species relative to the frequency from free Gd^{3+}.

Supplemental Table 4. The OH stretching VBS frequencies derived from aqueous solutions of: i) 0.5 M $GdCl_3$ with 1 M Hofmeister series chloride and acetate cations; and ii)100 mM EDTA with 80 mM $GdCl_3$ in 10mM HEPES buffer at pH 7.0 with 1 M Hofmeister series chloride cations. In the parenthesis are the frequency shifts for the given sample in the presence of the added salt relative to the frequency for the sample in the absence of added salt.

Supplemental Table 5. OH stretching VSB frequency derived from 10 mM HEPES pH 7.0 aqueous solution samples of 0.5 M $GdCl_3$; 1 mM $GdCl_3$ + 1 mM calmodulin; 1 mM $GdCl_3$ + 1 mM mSE3; and 80 mM $GdCl_3$ + 100 mM EDTA with added $MgCl_2$ and NaCl and (in parenthesis) the relative frequency shift induced by addition of cations.

Supplemental Table 6. The OH stretching VSB frequency derived from: i) aqueous solutions of 0.5 M $GdCl_3$ with 1 M magnesium, potassium and sodium halide salts; ii) aqueous solutions of 100 mM EDTA and 80 mM $GdCl_3$ in 10 mM HEPES buffer at pH 7.0 with 1 M magnesium, potassium and sodium halide salts and iii) trehalose derived glass doped with 0.25 M $GdCl_3$ and 0.5 M magnesium, potassium and sodium halide salts. In parenthesis are the frequency shifts relative to the salt free sample.

Supplemental Table 7. OH stretching VSB frequency derived from samples of 0.5 M $GdCl_3$ and 0.5 M $GdCl_3$ with 4 M KCl in the absence and presence of 1 M NaCl; 1 M $MgCl_2$ and 1 M $AlCl_3$. In parenthesis are the relative frequency shifts induced by addition of salts. In bold parenthesis are the relative frequency shifts induced by addition of salts compared to 0.5 M $GdCl_3$ with 4 M KCl.

Supplemental Table 1

LBT numbering	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Troponin C	I	F	D	K	N	A	D	G	F	I	D	I	E	E	L	G	E
LBT	Y	I	D	T	N	N	D	G	W	Y	E	G	D	E	L	L	A
SE2	Y	I	D	T	N	N	D	G	W	Y	E	G	D	E	L	L	A
mSE3	Y	I	D	T	N	N	D	A	W	I	E	G	D	E	L	L	A

Supplemental Table 2

	EDTA	Calmodulin (binding site I/II)	mSE3
Ca^{2+}	$5.0x10^{10}$ M^{-1}*	$5x10^{3}$ M^{-1} **	--
Gd^{3+}	$2.3x10^{17}$ M^{-1}*	10^{7}-10^{8} M^{-1}***	10^{6} M^{-1}****
Mg^{2+}	$4.9x10^{8}$ M^{-1}*	10^{3} M^{-1}**	--

Available at http://www.cem.msu.edu/~cem333/EDTATable.html;

** [1];

*** [2];

**** Peptide binding constants are from unpublished data from Dr. Imperiali

Supplemental Table 3

	OH stretching frequency /cm^{-1} (relative frequency shift from free Gd^{3+})		
Free Gd^{3+} (0.5 M)	80 mM Gd^{3+} coordinated 100 mM EDTA	1 mM Gd^{3+} coordinated 1 mM calmodulin	1 mM Gd^{3+} coordinated 1 mM mSE3
3270 (--)	3310 (+40)	3300 (+30)	3391 (+121)

Supplemental Table 4

	OH stretching frequency /cm^{-1} (relative frequency shift from samples without salts)		
	Free Gd^{3+}		EDTA coordinated Gd^{3+}
	Cl^-	Acetate	Cl^-
Al^{3+}	3296.6 (+26.6)	--	3335 (+25)
Mg^{2+}	3291.3 (+21.7)	3302.5 (+32.5)	3334 (+24)
Ca^{2+}	3287.2 (+17.2)	3295.4 (+25.4)	3325 (+15)
Li^+	3283.4 (+13.4)	3286.1 (+16.1)	3319 (+9)
Na^+	3277.4 (+7.4)	3282.8 (+12.8)	3317 (+7)
K^+	3272.8 (+2.8)	3275.0 (+5.0)	3311 (+1)
Cs^+	3270.4 (+0.4)	3271.5 (+1.5)	3310.1 (+0.1)

Supplemental Table 5

Salts added	OH stretching frequency /cm^{-1} (relative frequency shift from samples without salts)			
	Free Gd^{3+} (0.5 M)	80 mM Gd^{3+} coordinated 100 mM EDTA	1 mM Gd^{3+} coordinated 1 mM calmodulin	1 mM Gd^{3+} coordinated 1 mM mSE3
$MgCl_2$ 1 M	3291 (+21)	3334 (+24)	3325 (+25)	3413 (+23)
$MgCl_2$ 2 M	3298 (+28)	3351 (+41)	3335 (+35)	--
$MgCl_2$ 4 M	3313 (+43)	3390 (+80)	3370 (+70)	3435 (+44)
NaCl 1 M	3278 (+8)	3317 (+7)	3301 (+1)	--
NaCl 2 M	3283 (+13)	3319 (+9)	3302 (+2)	--
NaCl 4 M	3288 (+18)	3323 (+13)	3305 (+5)	3394 (+3)

Supplemental Table 6

	OH stretching frequency /cm^{-1} (relative frequency shift from samples without salts)		
	Solution phase		Trehalose derived Glass
	Free Gd^{3+}	100 mM EDTA coordinated 80 mM Gd^{3+}	Free Gd^{3+}**
MgF_2*	3267(-3)	3309 (-1)	-
$MgCl_2$	3291 (+21)	3334 (+24)	3392 (+172)
$MgBr_2$	3294 (+24)	3335 (+25)	-
KF	3269.6 (-0.3)	3309. 9 (-0.1)	-
KCl	3273 (+3)	3311 (+1)	-
KBr	3275 (+5)	3312 (+2)	-
KI	3276 (+6)	3313 (+3)	-
NaF	3251 (-19)	3301 (-9)	3124 (-96)
NaCl	3277 (+7)	3317 (+7)	3190 (-30)
NaBr	3286 (+16)	3323 (+13)	-
NaI	3297 (+27)	3331 (+21)	-

*concentration is far less than 1 M, using the saturate solution instead.

** The vibration frequency of water from GVSBLS in glass is 3220 cm^{-1}

Supplemental Table 7

	OH stretching frequency /cm^{-1} (frequency shift from samples without salts) **(frequency shift from sample with 4 M KCl)**
--	3270 (--)
4 M KCl	3274.5 (+4.5) **(--)**
4 M KCl, 1 M $AlCl_3$	3299.4 (+29.4) **(+24.9)**
4 M KCl, 1 M $MgCl_2$	3294.9 (+24.9) **(20.4)**
4 M KCl, 1 M NaCl	3281.6 (+11.6) **(+7.1)**
1 M $AlCl_3$	3296.6 (+26.6)
1 M $MgCl_2$	3291.3 (+21.7)
1 M NaCl	3277.4 (+7.4)

(1) Tsai, M. D.; Drakenberg, T.; Thulin, E.; Forsen, S. *Biochemistry* **1987**, *26*, 3635-43.
(2) Snyder, E. E.; Buoscio, B. W.; Falke, J. J. *Biochemistry* **1990**, *29*, 3937-43.