SUPPORTING INFORMATION

Lanthanide-dependent coordination interactions in lanmodulin: a 2D IR and molecular dynamics simulations study

Stephanie Liu[†], Emily R. Featherston[‡], Joseph A. Cotruvo, Jr. *[‡], and Carlos R. Baiz**[†]

† Department of Chemistry, University of Texas at Austin, Austin, TX 78712

‡ Department of Chemistry, The Pennsylvania State University, University Park, PA 16802

Corresponding Authors: *juc96@psu.edu, **cbaiz@cm.utexas.edu

S1. Supplemental Data

The 2D IR spectra which were used to create the pump slice amplitude figures in the main text are provided below. More detailed information regarding 2D IR data collection and interpretation are provided in previous publications.¹



Figure S1. 2D IR spectra of WT and $4P_2A$ in the unbound (Apo) and Ca^{2+} bound state. The carboxylate region is magnified by a factor of 10 to highlight the low amplitude features. Both WT and $4P_2A$ exhibit a single broad feature in the Apo state, whereas significant differences in the carboxylate region appear in the Ca²⁺-bound state.



Figure S2. 2D IR spectra of WT and $4P_2A$ coordinated to a series of Ln^{3+} ions. The early Ln^{3+} ions are shown in red, while the late Ln^{3+} ions are shown in black. The carboxylate region is magnified by a factor of 10 to highlight low amplitude features. Differences in the WT spectra indicate WT LanM's ability to distinguish between early and late Ln^{3+} ions. The mutant $4P_2A$ displays more similar spectra for early and late Ln^{3+} ions.



Figure S3. Amide I subtracted carboxylate region spectra of WT and $4P_2A$ LanM, showing the individual Gaussian fitted peaks within the broader absorption feature. Spectra were normalized to the amide I peak area in the 1619-1690 cm⁻¹ region of the spectrum, which has been subtracted out to isolate the carboxylate region. Experimental lineshapes are composed of monodentate (blue) and unbound (red) carboxylate populations. Gaussian fitting was performed by a least squares minimization using the MATLAB package of programs.



Figure S4. Histograms showing the carbon-ion distance for coordinating carboxylate ligands in WT and $4P_2A$ extracted from MD trajectories. A cut-off distance of 0.4 nm (vertical dashed line) was chosen as the boundary between bound and unbound ligands. The majority of the population is concentrated between 0.2 and 0.4 nm in two distinct sub-populations, described in the main text.



Fig S5. RMSD of WT (top) and $4P_2A$ (bottom) LanM after least squares fit to the first frame of each 50 ns trajectory.



Fig S6. Ion-Oxygen RDF of WT (top) and $4P_2A$ (bottom) LanM.



Fig S7. Ion-water RDF of WT (top) and 4P₂A (bottom) LanM.

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	EF 1		EF 2		EF 3	
	WT	4P ₂ A	WT	$4P_2A$	WT	4P ₂ A
Ca^{2+}	7.03	7.00	7.10	7.66	7.59	7.01
Early Ln ³⁺	9.99	10.00	9.95	9.93	9.92	9.98
Late Ln ³⁺	9.00	9.46	9.00	9.00	9.00	9.01

Table S1. Coordination number for each binding site in WT and $4P_2A$ LanM, defined by the number of oxygens within 0.3 nm of each bound metal ion.

References

 Edington, S. C.; Gonzalez, A.; Middendorf, T. R.; Halling, D. B.; Aldrich, R. W.; Baiz, C. R. Coordination to Lanthanide Ions Distorts Binding Site Conformation in Calmodulin. *Proceedings of the National Academy of Sciences* 2018, 201722042. https://doi.org/10.1073/pnas.1722042115.