SUPPORTING INFORMATION

A cryptophane-based "turn-on" ^{129}Xe NMR biosensor for monitoring calmodulin

Brittany A. Riggle, Mara L. Greenberg, Yanfei Wang, Rebecca F. Wissner, Serge D. Zemerov, E. James Petersson, and Ivan J. Dmochowski*
Department of Chemistry, University of Pennsylvania, 231 South 34th St., Philadelphia, PA 19104, USA

CONTENTS

GENERAL INFORMATION

Instrumentation and Methods. ^{1}H NMR (500 MHz) data were obtained in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-*d6*) using a Bruker DMX 500 NMR spectrometer. Column chromatography was performed using silica gel (60 Å pore size, 40-75 μm particle size) from Sorbent Technologies. Thin layer chromatography (TLC) was performed using silica gel plates (60 Å pore size, Silicycle) with UV light at 254 nm as the detection method. MALDI-MS data were collected using a Bruker Ultraflex III TOF/TOF mass spectrometer. All HPLC purifications were performed on a Varian Prostar 210 system equipped with a quaternary pump and diode array detector. All air- and moisture-sensitive reactions were performed under inert atmosphere in glassware flamed under vacuum, using anhydrous dry solvents. Standard workup procedures involved multiple (~3) extractions with the indicated organic solvent, followed by washing of the combined organic extracts with water or brine, drying over Na_2SO_4 and removal of solvents *in vacuo*. All yields reported were determined after purification by column chromatography or reverse phase HPLC, unless otherwise noted. All data were collected using instruments in the Chemistry Department at the University of Pennsylvania.

Materials. Organic reagents and solvents were used as purchased from the following commercial sources: Sigma-Aldrich: N,N-diisopropylethylamine (DIPEA); copper (I) bromide; dimethyl sulfoxide (DMSO, anhydrous, 99.9%); Sigmacote®; Tris-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA). Fisher: acetone (HPLC grade); chloroform (CH_2Cl_3, HPLC grade); dichloromethane (CH_2Cl_2, HPLC grade); ethyl acetate (EtOAc, HPLC grade); hexanes (HPLC grade); hydrochloric acid; 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), methyl alcohol (MeOH, HPLC grade), perchloric acid (60%); potassium carbonate (anhydrous); sea sand (washed); sodium chloride (NaCl); sodium hydroxide (NaOH); sodium sulfate (anhydrous). Novabiochem (currently EMD Millipore; Billerica, MA, USA): 6-azidohexanoic acid; 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). MarCor: deionized (DI) water filtered (18 MΩ). Acros Organics: β-propiolactone (90%); cesium carbonate (Cs_2CO_3, 99.5%); chloroform-*d* ($CDCl_3$); 1,2-dibromoethane; 3,4-

dihydroxybenzaldehyde (97%); N,N-dimethylformamide (DMF, 99.8%, anhydrous, acrosseal); dimethylsulfoxide-*d6*, 4-hydroxy-3-methoxybenzyl alcohol (99%); propargyl bromide (80% solution in toluene); scandium(III) trifluoromethanesulfonate ($Sc(OTf)_3$, 95%); sodium borohydride ($NaHB_4$, powder, 98%); sodium hydride (NaH, 60% dispersion in mineral oil); tetrahydrofuran (THF, extra dry, over molecular sieves); triisopropylsilane (TIS). MG Industries (Linde Group, NJ): xenon gas (scientific grade).

SYNTHETIC PROCEDURES

Cryptophane Synthesis. Tripropargyl cryptophane was generated in a 6-step synthesis in 6.4% overall yield from two commercially available compounds, 3,4-dihydroxy-benzaldehyde and vanillyl alcohol as previously published.[1] Spectroscopic data agreed with literature values.[1]

Peptide Synthesis and Purification. FRRIAR peptide was synthesized using standard Fmoc-based solid phase peptide synthesis as described previously,[1] and then N-terminally modified with commercially available 6-azidohexanoic acid and purified by reverse-phase HPLC.[1] Alternatively, azido-FRRIAR was purchased from Anaspec as a purified white powder separated into 10 mg aliquots.

Solubilizing Linker Synthesis. 3-Azidopropionic acid was prepared from β-propiolactone by literature procedure and matched the reported ^{1}H NMR spectrum.[2]

3-azidopropionic acid (**4**). Sodium azide (4.5 g, 0.69 mmol, 1 eq) was dissolved in MilliQ water. β-propiolactone (4.4 mL, 0.069 mmol, 1 eq) was added dropwise and the reaction was allowed to stir at rt for 6 h. The reaction was neutralized with 1 M HCl and then extracted 3 times with diethyl ether. The organic layer was then dried over sodium sulfate and filtered through cotton. A clear oil in a 15% yield was recovered in DMSO, requiring no further purification.

FRRIAR-TUC Biosensor Synthesis. Copper (I)-catalyzed [3+2] azide-alkyne cycloaddition (CuAAC) between Azido-FRRIAR peptide and cryptophane and subsequent cycloaddition reaction between the cryptophane and 3-azidopropionic acid yielded FRRIAR-TUC biosensor.

FRRIAR-cryptophane (**3**). To conjugate the azido-FRRIAR peptide to the tripropargyl cryptophane (**1**) CuAAC was performed with modified conditions of those previously employed.[3-6] Firstly, 5.3 mg (1.0 eq) of **1** and 10 mg of **2** (0.8 eq) were dissolved in 1 mL of dry N,N-dimethylformamide (DMF) in a conical reaction vessel. The reaction mixture was degassed under stirring. In a separate vial, 16 mg (6 eq) of tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methylamine (TBTA) copper ligand was dissolved in 100 µL of DMF. A separate solution of 0.35 M copper (I) bromide (CuBr) in DMSO was also prepared. To the TBTA solution, 43 µL of CuBr (3 eq) was added, vortexing after addition. The reagent mixture was then added to the reaction vessel. The reaction vessel was again degassed and then covered with foil and allowed to stir for 3 h under nitrogen at rt. A small aliquot of reaction was removed (2 µL) and diluted in HPLC solvent mixture, 50:50 mixture of acetonitrile and water with 0.1% trifluoroacetic acid to 1 mL. To verify product formation, analytical reverse-phase HPLC was performed using a Grace C18 analytical column (250 × 4.6 mm, 5 µm beads) and monitored at 215 and 277 nm. The elution gradient was composed of two solvents: 0.1% aqueous TFA (solvent A) and a 0.1% solution of TFA in CH_3CN (solvent B). The column was equilibrated at 90% A for 5 min. The purification method then went from 90% A to 60% A over 3 min, then from 60% A to 22% A over 32 min, and finally decreased to 0% A over the next 5 min at a flow rate of 1 mL/min with a 1 mL injection volume. The FRRIAR-cryptophane conjugate eluted at 28.00 min, Figure S1. MALDI-MS m/z calculated for FRRIAR-cryptophane $C_{166}H_{225}N_{37}O_{33}$ ($M+H^+$) 3265.71; found 3265.76, Figure S2.

FRRIAR-trisubstituted ultrasensitive cryptophane (FRRIAR-TUC) (**5**). After 3 h the crude reaction (**3**) was then dosed with 6 µL 3-azidopropionic acid (**4**) (11 eq) and allowed to stir overnight while covered, to yield the FRRIAR-TUC biosensor. The reaction was diluted 10-fold in 50:50 ACN:H_2O and purified. Purification was achieved through reverse-phase HPLC employing a semi-preparative method and using a Grace C18 semi-preparative column (10 × 250 mm, 5 µm beads). The column was equilibrated at 90% A for 5 min. The purification method then went from 90% A to 55% A over 3 min, then from 55% A to 51% A over 7 min, and then from 51% A to 47% A over 13 min. This was followed by a gradient from 47% A to 40% A over 6 min and finally decreased to 0% A over the next min at a flow rate of 4 mL/min

with a 1 mL injection volume. The biosensor eluted at 20.30 min Figure S3. MALDI-MS m/z calculated for FRRIAR-TUC $C_{208}H_{289}N_{43}O_{61}$ ($M+H^+$) 3496.02; found 3495.99, Figure S4. The pure fractions were then collected and lyophilized to a white powder and dissolved in a minimal amount of DMSO and then diluted to desired concentrations in 10 mM HEPES at pH 7.2 with and without 1 mM $CaCl_2$.

Calmodulin Protein Expression and Purification. Calmodulin (CaM) protein was expressed and purified following previously established protocols.[7] *Escherichia coli* BL21(DE3) cells were transformed with a plasmid encoding the *Gallus gallus* CaM gene. Transformed cells were selected on the basis of ampicillin resistance. M9 minimal media (50 mL) supplemented with ampicillin (100 μg/mL) was inoculated with single colonies. A M9 salts solution (42.3 mM Na_2HPO_4, 22.0 mM KH_2PO_4, and 8.5 mM NaCl) was prepared and autoclaved. Autoclaved solutions of the following salts were added per liter of M9 salts: 10 mL of 10% NH_4Cl, 1 mL of 2 M $MgSO_4$, 1 mL of 15 mg/mL $FeCl_2$ (in 1.0 M HCl), 1 mL of 15 mg/mL $ZnCl_2$ (in acidified H_2O), and 2 mL of 10% Bacto™ Yeast Extract. The primary 50 mL culture was incubated at 37 °C with shaking at 250 rpm overnight. The cells were harvested at 5000 g for 15 min and the resulting pellet was resuspended in 1 L of M9 minimal media supplemented with ampicillin. The 1 L culture was incubated at 37 °C with shaking at 250 rpm until the absorbance at 600 nm reached an OD of 0.9 AU. The culture was induced with 1 mM isopropyl D-galactoside (IPTG), and then incubated at 25 °C for an additional 12 h. The cells were again collected at 5000 g for 15 min and the resulting pellet was suspended in 15 mL of resuspension buffer: 50 mM 3-(N-morpholino)propanesulfonic acid (MOPS), 100 mM KCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol, pH 7.5. The cells were lysed by sonication and cooled on ice for 5 min. $CaCl_2$ was added to the sonicated lysate to a final concentration of 5 mM prior to centrifugation for 20 min at 30,000 g, 4 °C. CaM was purified from the cleared cell lysate using a phenylsepharose CL-4B column (resin bed volume = 10 mL) with EDTA as eluent. The column was first equilibrated with 4 column volumes of Buffer A (50 mM Tris base, 1 mM $CaCl_2$, pH 7.5). After the clear cell lysate was loaded and allowed to pass through the resin, the column was washed with 4 column volumes of Buffer A, 4 column volumes of high-salt Buffer B (50 mM Tris base, 0.5 M NaCl, 0.1 mM $CaCl_2$, pH 7.5), and an additional 2 column volume washes of Buffer A to

restore low-salt conditions prior to elution. CaM was eluted with Buffer C (10 mM Tris base, 10 mM EDTA, pH 7.5) and collected in 4 mL fractions until absorbance at 280 nm was no longer detected. A second column purification was performed on the first batch of eluted fractions (re-saturated with $CaCl_2$ to a concentration of 20 mM) to obtain CaM in high purity. Column fractions were dialyzed against 10 mM ammonium bicarbonate (pH 8.0) and stored as a lyophilized powder at -20 °C. SDS-PAGE analysis was performed to analyze dialyzed CaM elution fractions.

CHARACTERIZATION METHODS

Electronic Circular Dichroism (ECD) Spectroscopy. For ECD studies, samples of FRRIAR peptide and FRRIAR-TUC were prepared in acetonitrile and water. HPLC-grade solvents were used to minimize observed light scattering from DMSO and HEPES from CaM binding buffer: 10 mM HEPES, 1 mM $CaCl_2$, pH 7.2 with 1% DMSO.[8] All ECD spectroscopy experiments were performed on the Aviv 410 CD spectrometer. Data were collected at 25 °C from 260-190 nm, with a 30 s averaging time, 1 nm wavelength step, 1 s averaging time, and 1 nm bandwidth. The samples in Figure 3 were prepared by dissolving lyophilized FRRIAR or FRRIAR-TUC in 1:1 MeCN:H_2O to 30 μM or 10 μL, respectively. The concentration was confirmed by measuring the absorbance at 280 nm, $\varepsilon_{280} = 6{,}970\ M^{-1}cm^{-1}$ [FRRIAR] and $\varepsilon_{280} = 16{,}970\ M^{-1}\ cm^{-1}$ [FRRIAR-TUC] and using an Agilent 89090A UV-visible spectrophotometer. Percent helicity increased from 11% to 37% after FRRIAR was conjugated to cryptophane (Table S1).

Data Analysis. The molar ellipticity was calculated from the observed ellipticity (mdeg) and has the units of deg cm^2 $dmol^{-1}$. The molar ellipticity is given by equation **S1** where *C* is the concentration of the peptide or biosensor, l is the path length of the cuvette-0.1 cm, and n_r is the number of residues-31.[9]

$$[\theta] = \frac{\theta_\lambda}{(C * l * n_r * 10)} \quad \text{(S1)}$$

The helical contents of FRRIAR and FRRIAR-TUC were determined from circular dichroism studies at 30 and 10 μM concentrations, respectively, and in accordance with literature precedent.[10] The concentration variation is a result of the large difference in extinction coefficients (6970 vs 16970 $M^{-1} \cdot cm^{-1}$). Data were normalized to molar ellipticity to allow for direct comparison. Studies at various concentrations demonstrated reproducible percent helicity values (data not shown). Helicity was calculated using the formulas **S2** and **S3** where n_r is the number of amino acids in the peptide, in this case 17. Racemic cryptophane was employed for these experiments, and thus did not contribute to the measured CD signal.[11] Data are shown in Table S1. These experiments were performed in 1:1 MeOH:H_2O because DMSO contributed to significant scattering as did the buffer to a lesser degree, Figure S9, black stars.[8] Comparative studies between FRRIAR in buffer and FRRIAR in 1:1 MeCN:H_2O demonstrated equivalent spectra, shown in Figure S9 where FRRIAR in buffer verse FRRIAR in MeCN:H_2O is shown in closed and open red circles, respectively and FRRIAR-TUC in buffer verse FRRIAR-TUC in MeCN:H_2O is shown in closed and open blue diamonds.

$$\%helicity = 100 * \frac{[\theta]_{222}}{^{max}[\theta]_{222}} \quad \text{(S2)}$$

$$^{max}[\theta]_{222} = -40000 * [1 - \frac{2.5}{n_r}] \quad \text{(S3)}$$

Table S1: α-helical content of FRRIAR vs FRRIAR-TUC in 1:1 MeCN:H2O

Compound	$-[\theta]_{222}$ (deg • cm^2 • $dmol^{-1}$)	Helicity (%)
FRRIAR	3,959	11
FRRIAR-TUC	12,596	37

Tryptophan Fluorescence Studies. All fluorescence studies with the peptide and CaM were carried out on a Photon Technology International (PTI) QuantaMaster™ 40 fluorescence spectrometer (Birmingham, NJ, USA). Samples containing 0, 7.5, 15, 22.5, or 30 μM CaM and 30 μM FRRIAR peptide were prepared in 10 mM HEPES, 1 mM $CaCl_2$, pH 7.2 with 1% DMSO and placed in the fluorimeter. Fluorescence spectra were collected at 25 °C in quartz cuvettes with a 1-cm path length. The samples

were excited at 295 nm and emission data were collected from 300-400 nm. For all spectra, the slit widths were 5 nm, scan rate was 60 nm/min, averaging time was 1 s, and the data interval was 1 nm. All fluorescence studies with the biosensor (FRRIAR-TUC) and CaM were prepared in the same fashion and carried out on Cary Eclipse Fluorescence Spectrophotometer from Agilent (formally Varian).

Hyperpolarized ^{129}Xe NMR Spectroscopy. Hyperpolarized (hp) ^{129}Xe was generated using spin-exchange optical pumping (SEOP) method with a home-built version of the previously commercially available Nycomed-Amersham (now GE) model IGI.Xe.2000 ^{129}Xe hyperpolarizer. A gas mixture of 89% helium, 10% nitrogen, and 1% natural abundance xenon (Linde Group, NJ) was used as the hyperpolarizer input. 795 nm circularly polarized diode laser was used for optical pumping of Rb vapor. ^{129}Xe was hyperpolarized to 10–15% then cryogenically separated, accumulated, thawed, and collected in controlled atmosphere valve NMR tubes (New Era). After hp Xe collection, NMR tubes were shaken vigorously to mix cryptophane solutions with hp Xe. All ^{129}Xe NMR measurements were carried out on a Bruker BioDRX 500 MHz NMR spectrometer (138.12 MHz frequency for ^{129}Xe), using a 5-mm BBO NMR probe. Sample temperature was controlled by VT unit on NMR spectrometer to 300 ± 1 K. Eburp1 shaped pulse was used to selectively excite Xe@FRRIAR-TUC biosensor peak. Spectra were averaged over 16 scans. A delay of 0.15 s was given between scans to allow for xenon exchange. All acquired NMR spectra were processed with 25 Hz Lorentz broadening. Chemical shifts were referenced to free xenon gas of 0 atm at 0 ppm, shown in Figure 5. The excitation bandwidth used for the selective pulse was 2000 Hz (14.5 ppm). When centered at 65 ppm, this pulse covers the chemical shift range where cryptophane and its derivatives have been previously observed. The center of the selective pulse was also varied to scan different frequency ranges, and no change in the spectrum were observed. With hard pulse excitation, the spectrum of the cage alone, apo-CaM with FRRIAR-TUC, and Holo-CaM with FRRIAR peptide only showed the Xe@aq peak. These control studies are shown in Figures 5a, S13, and S14. All NMR studies were performed in 10 mM HEPES, 1 mM $CaCl_2$, pH 7.2 with 1% DMSO.

Thermal Melt of Calmodulin. To confirm calcium had been removed from CaM to generate the apo form, temperature-dependent circular dichroism (CD) spectroscopy was performed following literature

protocol.[7] Calcium containing CaM (Ca^{2+}-CaM) is thermostable (T_m > 90 ºC). Thus, we measured the thermal unfolding of the apo-protein, which has reported T_m of 55 ºC.[12] CD data were obtained from approximately 30 μM protein sample monitored at 222 nm between 0 and 95 ºC using the variable temperature module with the Aviv 410 CD spectrometer. Data were collected every 1 ºC, using a 30 s averaging time, 2 min temperature equilibrium, and 1 nm band width. The resulting ellipticity (θ_D) measurements were converted to molar residue ellipticity values (θ) using equation **S1**, described above. The fraction folded (f_f) for apo-protein was determined using linear baselines to fit the low (θ_F) and high (θ_U) temperature data, equations **S4** and **S5**, respectively.

$$\theta_F = m_F T + b_F \quad \text{(S4)}$$

$$\theta_U = m_U T + b_U \quad \text{(S5)}$$

The entirety of the data range was then fit to equation **S6** where $K = e^{-(\Delta H - T\Delta S)/RT}$, where ΔH and ΔS are adjustable parameters and R = 8.3145 J•mol^{-1}•K^{-1}. The resulting plot is shown in Figure S6.

$$\theta = \theta_F(T) f_f(T) + \theta_U(T)(1 - f_f(T)) \quad \text{(S6)}$$

Where: $f_f = K/(1+K)$

Table S2: Calculated values for delta H and delta S from the thermal melt of apo calmodulin

	Calculated Values
ΔH	1.43 x 10^5 kJ • mol^{-1}
ΔS	444 J • mol^{-1} • K^{-1}

Gel Shift Assay. All gels were run on a Bio-Rad PowerPac™ Basic gel setup in 1x Tris-glycine solution (prepared from a dilution of commercially available Tris-Glycine 10x Solution for Electrophoresis in ddH_2O). Mini-protean®TGX™ precast gels, 4-15%, 30 μL/well from Bio-Rad. Initial characterization of CaM gel shift upon binding was achieved by preparing samples with 0, 10, or 20 μM FRRIAR with 10 μM CaM (final concentration) and is shown in Figure S6. For the FRRIAR-TUC titration gel samples containing 0, 2.5, 5, 7.5, or 10 μM FRRIAR-TUC (final concentration) and 10 μM CaM (final concentration) were prepared in 10 mM HEPES, 1 mM $CaCl_2$, pH 7.2 with 1% DMSO, Figure S7. For the apo/holo binding gel, 10 μM (final concentration) of either apo or holo CaM was combined with 10 μM

FRRIAR (final concentration), 10 μM FRRIAR-TUC, or buffer, Figure 2. For all gels, Native Tris-Glycine Sample Buffer 2x from Novex (25 μL) was combined with 25 μL of each sample. For all gels, samples were incubated for 1 min after prep before being loaded onto a 4-15% gradient gel. The gel box was put on ice and run at 120 V for 1.5 h before being stained with Coomassie brilliant blue stain mixture (20 min) and then destained. The gels were imaged on the Typhoon FLA 7000 gel imager.

FIGURES:

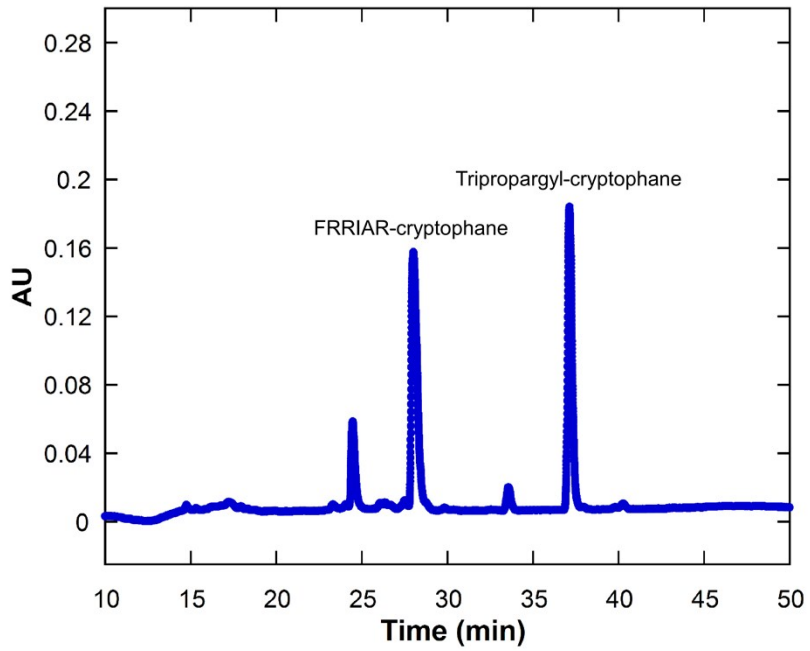

Figure S1: HPLC chromatogram of FRRIAR-cryptophane (**3**). UV absorbance monitored at 277 nm. Peak assignment based on MALDI-MS.

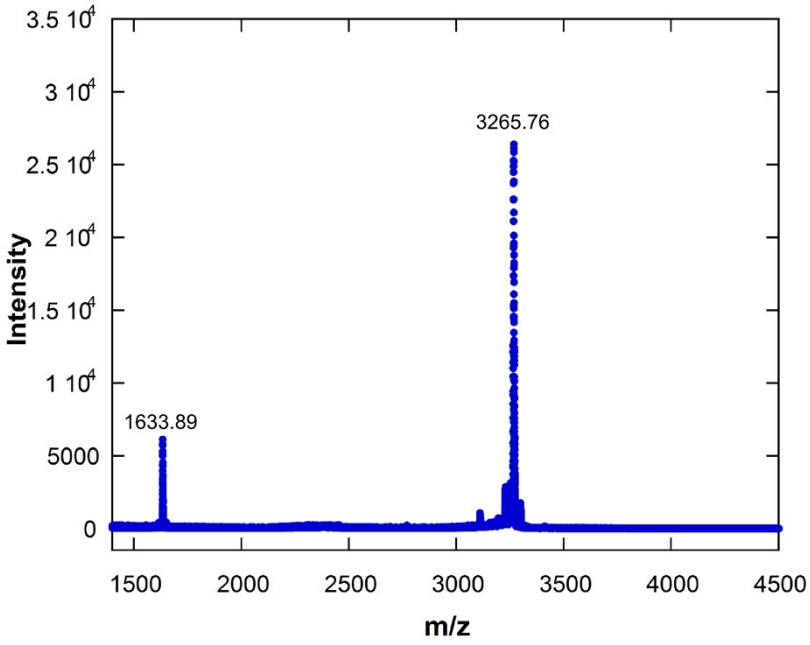

Figure S2: MALDI-MS spectrum of FRRIAR-cryptophane (**3**). Expected mass [M+H$^+$] 3265.71; found 3265.76.

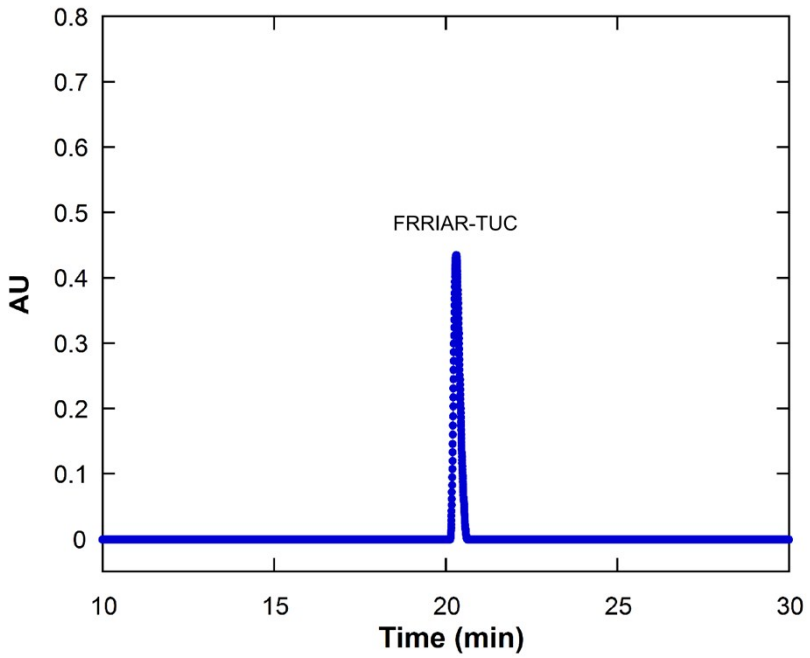

Figure S3: HPLC chromatogram of FRRIAR-TUC (**5**). UV absorbance monitored at 277 nm. Peak assignment based on MALDI-MS.

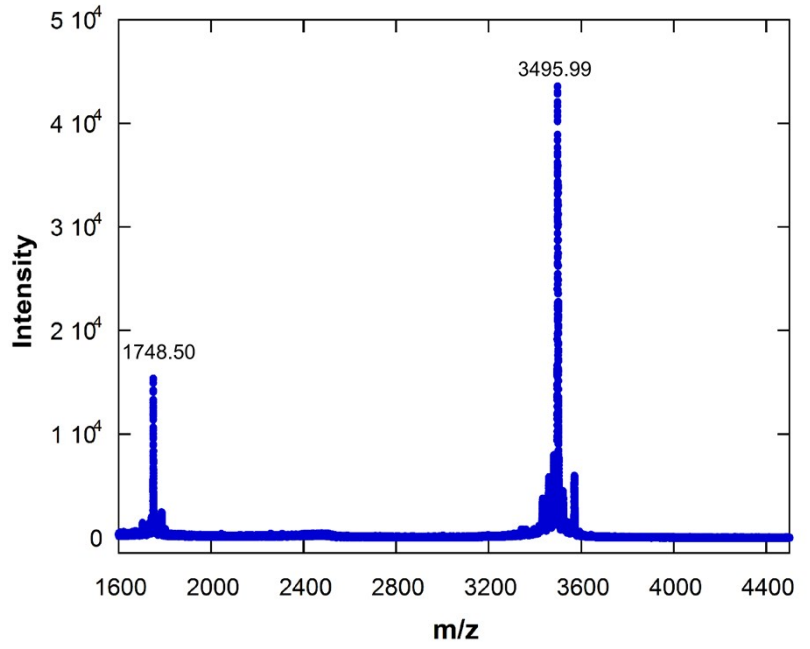

Figure S4: MALDI-MS spectrum of FRRIAR-cryptophane (**5**). Expected mass [M+H$^+$] 3496.02; found 3495.99.

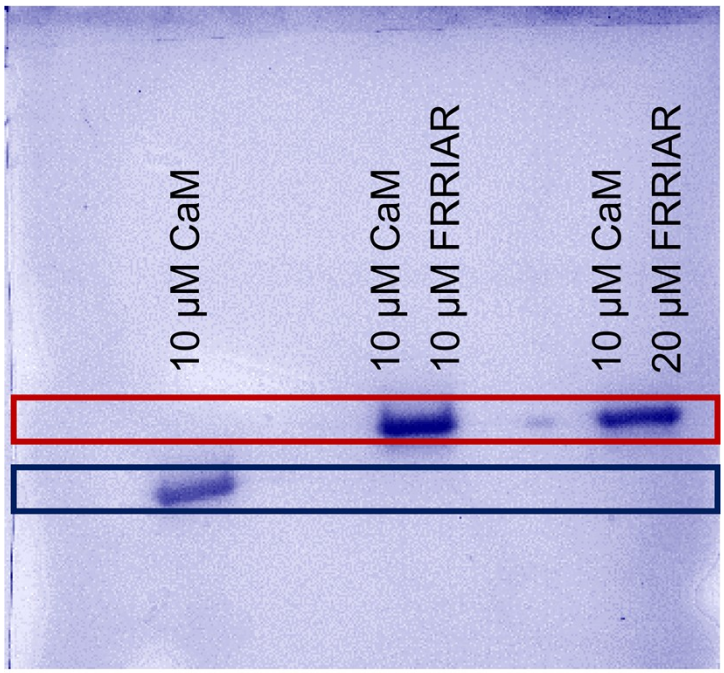

Figure S5: Native gel shift assay demonstrating retarded gel migration of CaM after binding FRRIAR and that it does so in 1:1 stoichiometry. Buffer: 10 mM HEPES, 1 mM $CaCl_2$, pH 7.2 with 1% DMSO.

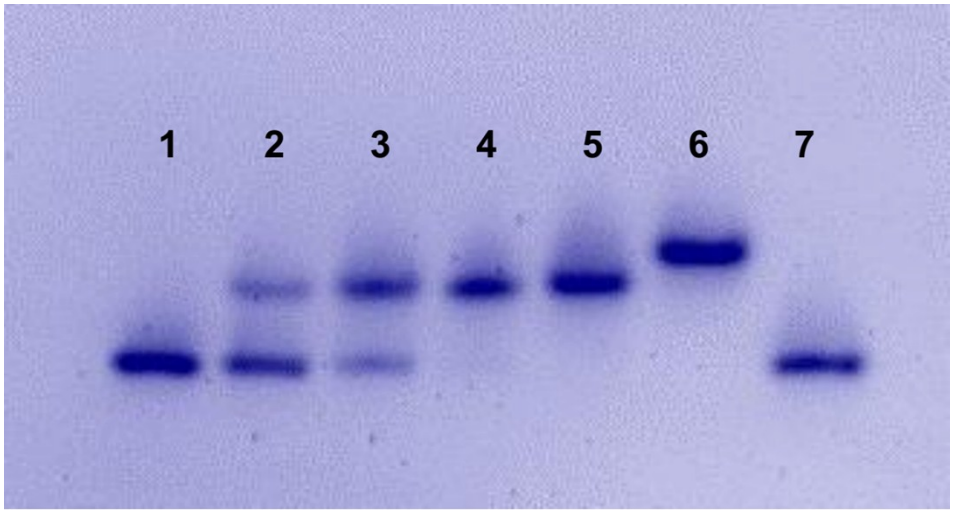

Figure S6: Native gel shift assay, where samples are all 10 µM CaM (final concentration) plus 1. buffer; 2. 2.5 µM FRRIAR-TUC; 3. 5.0 µM FRRIAR-TUC; 4. 7.5 µM FRRIAR-TUC; 5. 10 µM FRRIAR-TUC; 6. 10 µM FRRIAR peptide; and 7. buffer. Buffer: 10 mM HEPES, 1 mM $CaCl_2$, pH 7.2 with 1% DMSO.

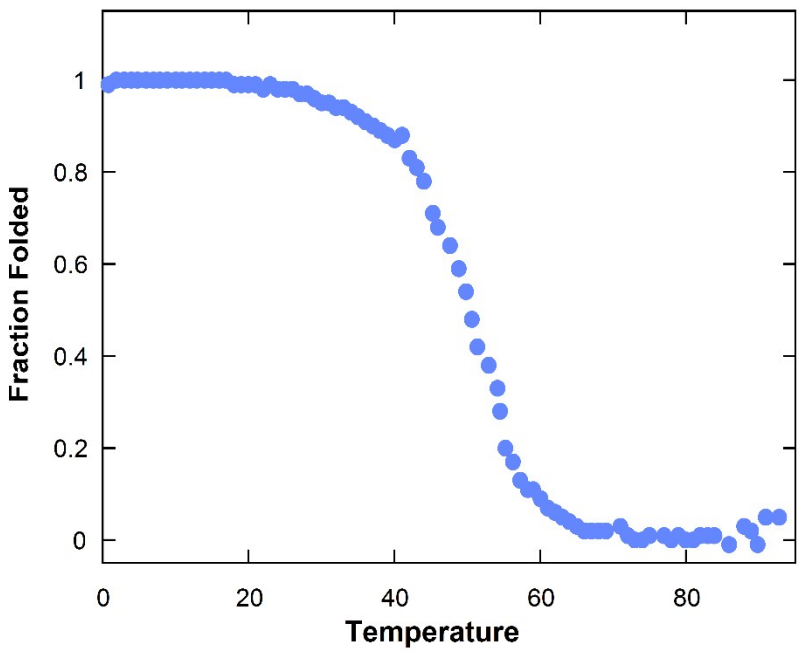

Figure S7: Temperature-dependent circular dichroism spectroscopy of 30 μM apo calmodulin. Buffer: 10 mM HEPES pH 7.2.

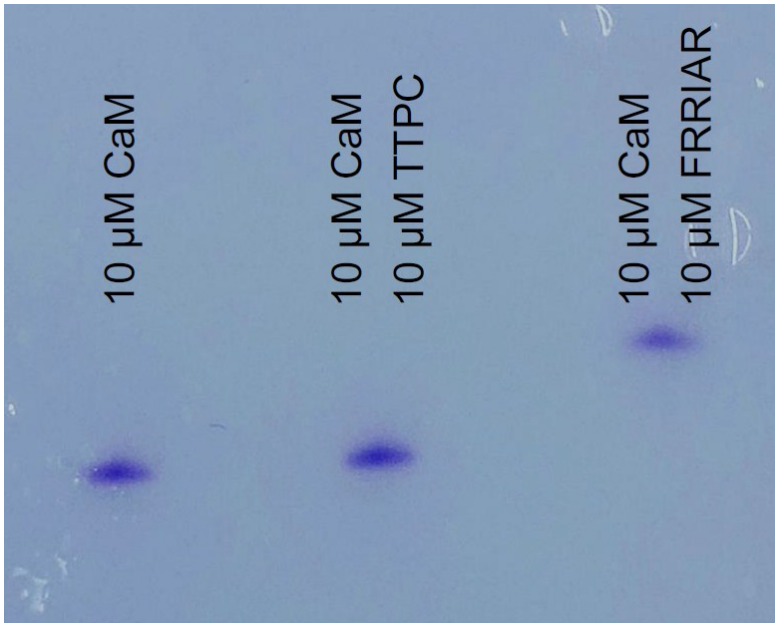

Figure S8: Control gel shift assay showing that the host cage, tris(triazole propionic acid) cryptophane-A derivative (TTPC), lane 2, does not generate the FRRIAR peptide-induced conformation of CaM, lane 3. Buffer: 10 mM HEPES, 1 mM $CaCl_2$, pH 7.2 with 1% DMSO.

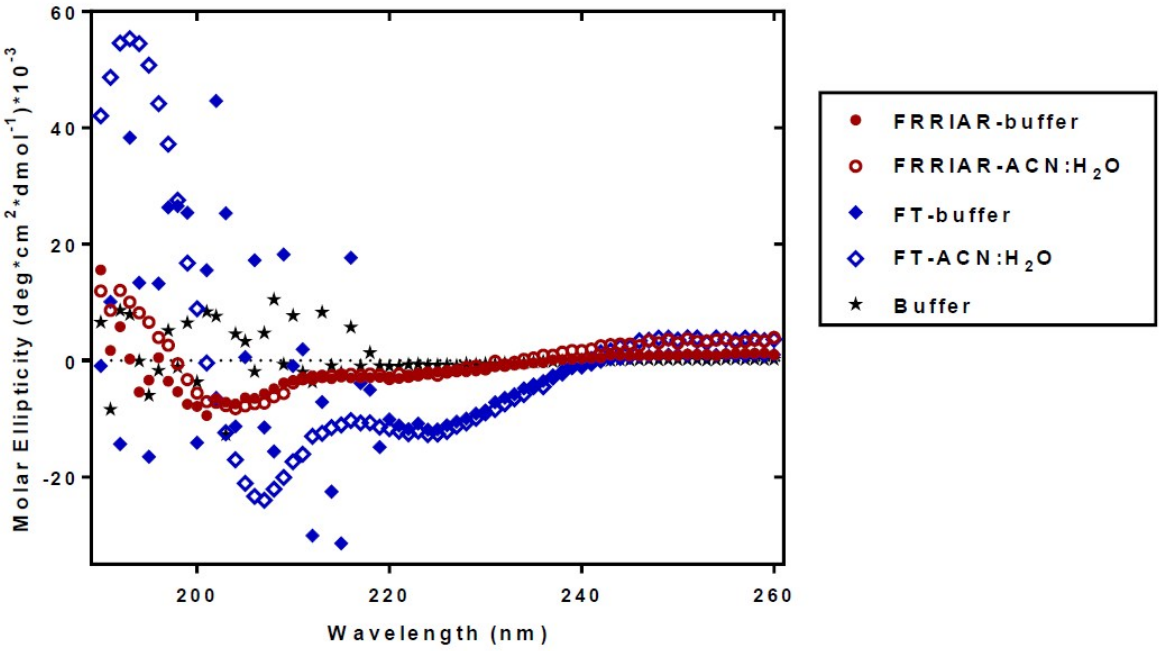

Figure S9: Circular dichroism of FRRIAR peptide (red traces) and FRRIAR-TUC (FT) (blue traces) in buffer (closed symbols) as compared to ACN:H_2O (open symbols) and CD of buffer alone (black trace). Buffer: 10 mM HEPES, 1 mM $CaCl_2$, pH 7.2 with 1% DMSO.

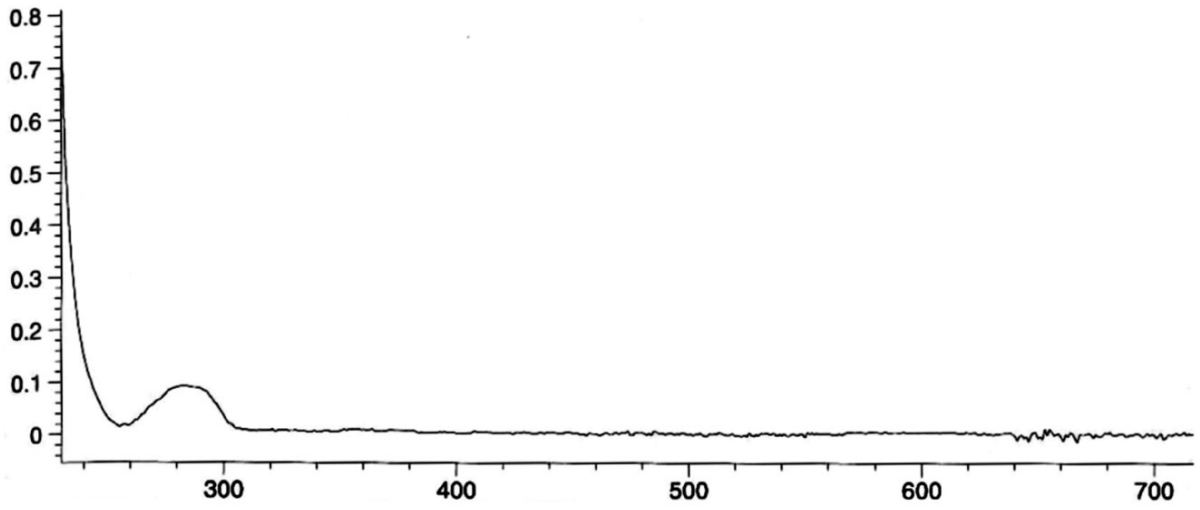

Figure S10: UV-vis spectrum of FRRIAR-TUC (diluted) after ^{129}Xe NMR. Sample concentration: 72.8 µM, $\varepsilon_{280} = 16{,}970\ M^{-1}\ cm^{-1}$. Buffer: 10 mM HEPES, 1 mM $CaCl_2$, pH 7.2 with 1% DMSO.

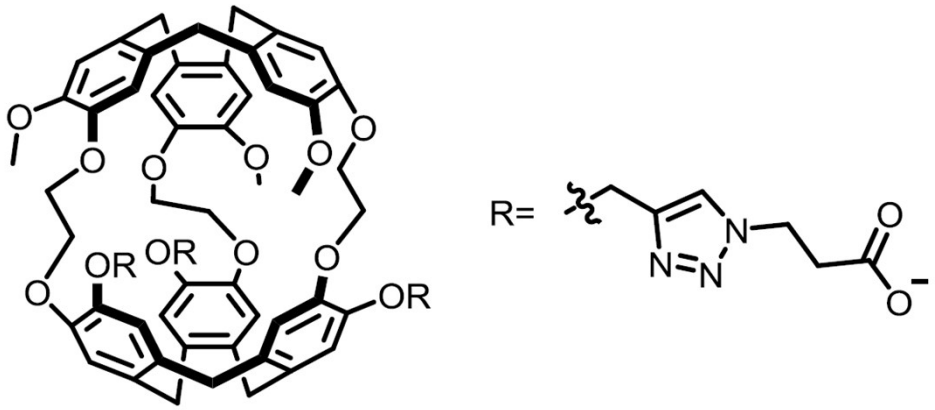

Figure S11: Structure of tris(triazole propionic acid) cryptophane-A derivative (TTPC), a water-soluble cryptophane with good xenon affinity at rt.[13, 14]

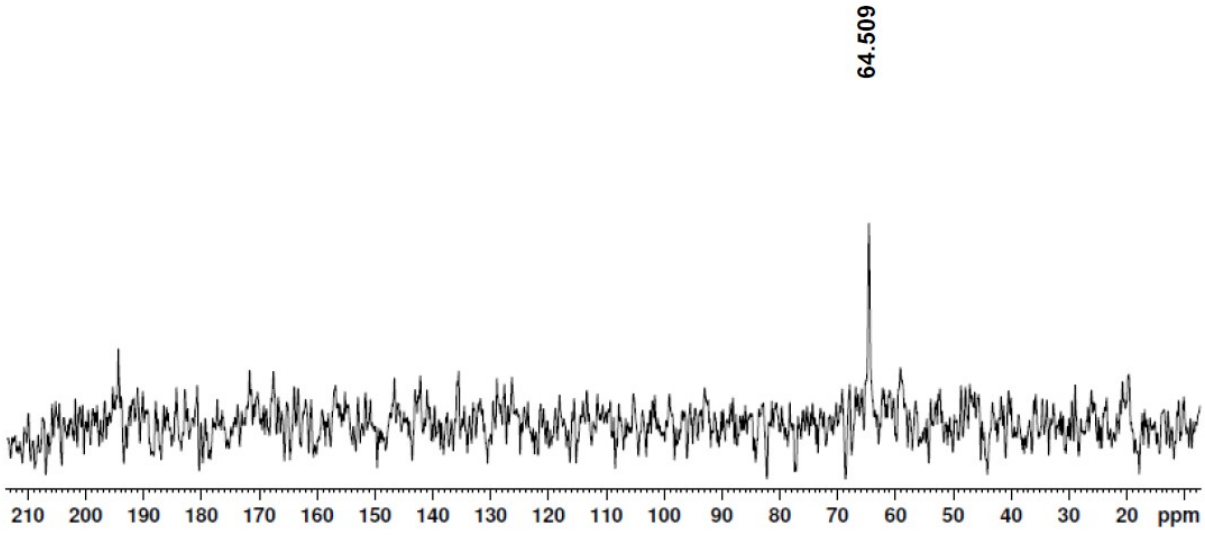

Figure S12: Xenon-129 NMR of tris(triazole propionic acid) cryptophane-A (TTPC), 70 µM. Spectrum acquired using a selective pulse centered at 61.5 ppm. A single peak, assigned to xenon encapsulated in TTPC, was observed at 64.509 ppm. Buffer: 10 mM HEPES, 1 mM $CaCl_2$, pH 7.2 with 1% DMSO.

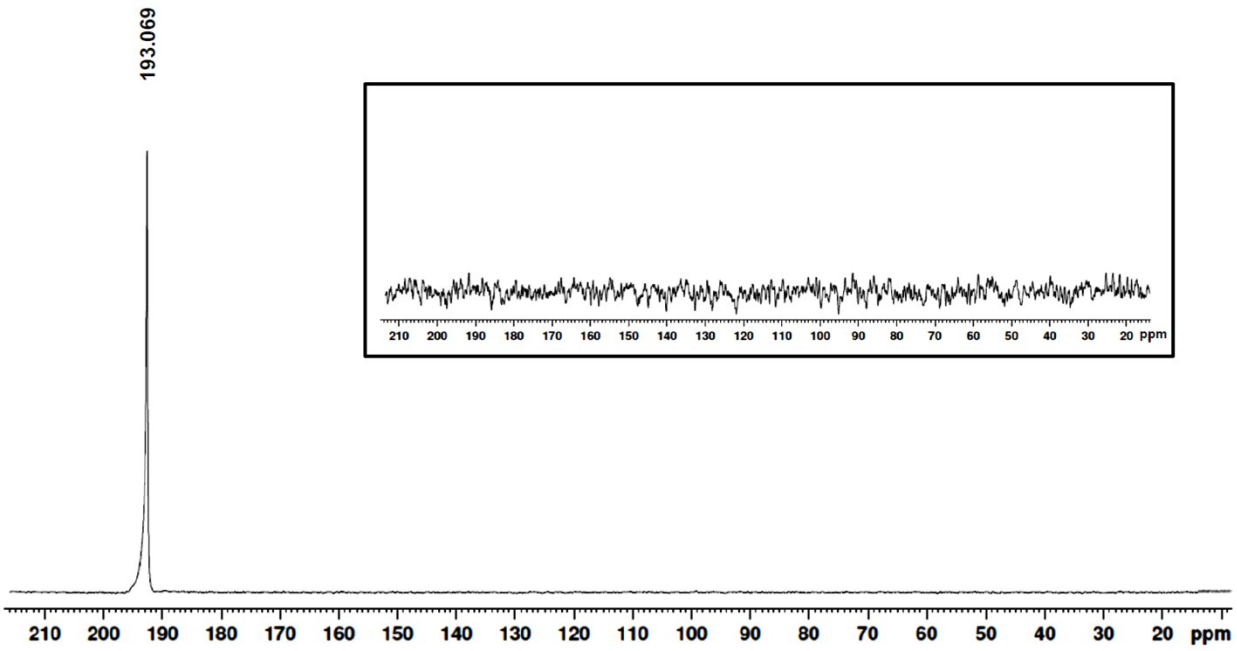

Figure S13: Xenon-129 NMR spectrum of apo-calmodulin (70 μM) with FRRIAR-TUC (70 μM) in 10 mM HEPES, pH 7.2 with 1% DMSO using a 90° hard pulse or selective pulse (inset) centered at 65.2 ppm. Only peak for Xe@H_2O (193.069 ppm) was observed.

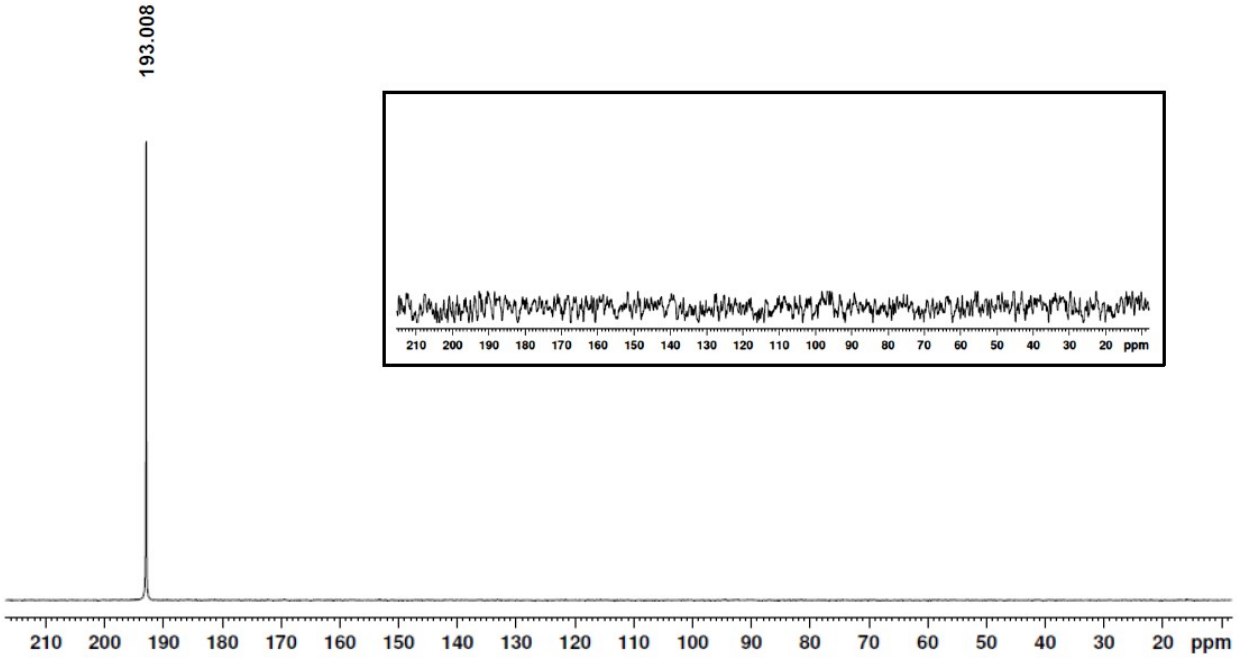

Figure S14: Xenon-129 NMR spectrum of holo-calmodulin (70 μM) and FRRIAR peptide (70 μM) in 10 mM HEPES, 1 mM $CaCl_2$, pH 7.2 with 1% DMSO using a 90° hard pulse or a selective pulse (inset) centered at 67.0 ppm. Only peak for Xe@H_2O (193.008 ppm) was observed.

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