Supporting Information for

Molecular Concentration Determination Using Long-Interval Chemical Exchange Inversion Transfer (CEIT) NMR Spectroscopy

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List of Supplementary Content

Experimental Description

Sample preparation. The cryptophane-A derivative CrA-$(COOH)_6$ was purchased from a commercial supplier and used without purification. The structure of CrA-$(COOH)_6$ (Figure S1) is shown in the supporting information. As a stock solution, 1.22 mg CrA-$(COOH)_6$ was dissolved in 10 mL deionized water, and the pH was adjusted through addition of NaOH solution (1 M). The solution was then diluted to different concentration levels (10.5 nM, 105.0 nM, 1.05 μM, 10.5 μM, 100.0 μM, 2.5 μM, 4.9 μM, 7.2 μM and 9.3 μM) for ^{129}Xe NMR experiments.

Hyperpolarized ^{129}Xe NMR. Hyperpolarized ^{129}Xe was generated with the spin exchange optical pumping technique using a commercial hyperpolarizer system (verImagin Healthcare, Wuhan, China). A xenon gas mixture consisting of 2% Xe (natural abundance), 10% N_2, and 88% He was used. The temperature in the pumping cell was 418 K, and the gas mixture pressure was 65.7 psi. The average ^{129}Xe spin polarization was approximately 10%.

All NMR experiments were conducted on a 400 MHz wide-bore NMR spectrometer (Bruker Avance, Ettlingen, Germany) using a 10 mm BBO probe. A 10 mm tube containing the test sample was placed in magnet with the temperature controlled at 297 K. The sample was directly bubbled for 20 s at a flow rate of 0.12 standard liters per minute, which was followed by a 3 s delay to allow the sample to remain stable before acquisition.

The signals were referenced to the signal of free xenon in solution (0 ppm).

Supplementary Information Text

Section S1. Derivation of xenon host molecule concentration expression

The dynamics of magnetization in the free Xe pool, $\mathrm{M_{Xe}(t)}$, and in the host molecule bound Xe, $\mathrm{M_{CXe}(t)}$, was described during the periods of delay time t by Bloch–McConnell equations restricted to the following:[1]

$$\frac{\mathrm{dM_{Xe}(t)}}{\mathrm{dt}} = \mathrm{M_{Xe}^{0}R_{1Xe}} - (\mathrm{k_{on}} + \mathrm{R_{1Xe}})\mathrm{M_{Xe}(t)} + \mathrm{k_{off}M_{CXe}(t)} \tag{A1}$$

$$\frac{\mathrm{dM_{CXe}(t)}}{\mathrm{dt}} = \mathrm{M_{CXe}^{0}R_{1CXe}} + \mathrm{k_{on}M_{Xe}(t)} - (\mathrm{k_{off}} + \mathrm{R_{1CXe}})\mathrm{M_{CXe}(t)} \tag{A2}$$

The thermal equilibrium magnetizations were denoted as $\mathrm{M_{Xe}^{0}}$ and $\mathrm{M_{CXe}^{0}}$. The longitudinal relaxation rates were denoted as $\mathrm{R_{1Xe}}$ and $\mathrm{R_{1CXe}}$. The inverse lifetimes of free Xe and Xe in the host molecule bound pools were denoted as $\mathrm{k_{off}}$ and $\mathrm{k_{on}}$, respectively. To obtain a simple expression, some reasonable assumptions were made. First, the constant terms $\mathrm{M_{Xe}^{0}}$ and $\mathrm{M_{CXe}^{0}}$ were negligible because of the very low thermal polarization compared with the hyperpolarized magnetizations $\mathrm{M_{Xe}(t)}$ and $\mathrm{M_{CXe}(t)}$ prevailing during the experiments. Second, the longitudinal relaxation rate for xenon bound to host molecule, $\mathrm{R_{1CXe}}$, was less than a tenth of 1 hertz and can be completely ignored in (A2) in comparison with the dissociation rate $\mathrm{k_{off}}$, which was of the order of tens of hertz in aqueous solution and even higher in organic solvents. Disregarding $\mathrm{R_{1Xe}}$ against $\mathrm{k_{on}}$ in (A1) was not generally justified because of the smallness of $\mathrm{k_{on}}$, particularly at low host concentrations. The systems (A1) and (A2) became the following:

$$\frac{\mathrm{dM_{Xe}(t)}}{\mathrm{dt}} = -(\mathrm{k_{on}} + \mathrm{R_{1Xe}})\mathrm{M_{Xe}(t)} + \mathrm{k_{off}M_{CXe}(t)} \tag{A1'}$$

$$\frac{\mathrm{dM_{CXe}(t)}}{\mathrm{dt}} = \mathrm{k_{on}M_{Xe}(t)} - \mathrm{k_{off}M_{CXe}(t)} \tag{A2'}$$

The differential equations (A1') and (A2') were solved.

$$\mathrm{M_{Xe}(t)} = -\mathrm{C_2}\mathrm{e}^{(\mathrm{t}\lambda_1)}\frac{\mathrm{d}-\lambda_1}{\mathrm{c}} - \mathrm{C_1}\mathrm{e}^{(\mathrm{t}\lambda_2)}\frac{\mathrm{d}-\lambda_2}{\mathrm{c}} \tag{A3}$$

$$\mathrm{M_{CXe}(t)} = \mathrm{C_2}\mathrm{e}^{(\mathrm{t}\lambda_1)} + \mathrm{C_1}\mathrm{e}^{(\mathrm{t}\lambda_2)} \tag{A4}$$

In which, $\mathrm{c} = \mathrm{k_{on}}$, $\mathrm{d} = -\mathrm{k_{off}}$, C_1 and C_2 were constants.

$$\lambda_1 = \frac{-\mathrm{k_{on}} - \mathrm{R_{1Xe}} - \mathrm{k_{off}} + \sqrt{(\mathrm{k_{off}} - \mathrm{k_{on}} - \mathrm{R_{1Xe}})^2 + 4\mathrm{k_{off}k_{on}}}}{2} \tag{A5}$$

$$\lambda_2 = \frac{-\mathrm{k_{on}} - \mathrm{R_{1Xe}} - \mathrm{k_{off}} - \sqrt{(\mathrm{k_{off}} - \mathrm{k_{on}} - \mathrm{R_{1Xe}})^2 + 4\mathrm{k_{off}k_{on}}}}{2} \tag{A6}$$

Considering that $\mathrm{k_{off}} \gg \mathrm{k_{on}} > 0$ and $\mathrm{k_{off}} \gg \mathrm{R_{1Xe}} > 0$, we obtained $(\mathrm{k_{off}} - \mathrm{R_{1Xe}} + \mathrm{k_{on}})^2 \gg 4\mathrm{k_{on}R_{1Xe}}$, and the expressions of λ_1 and λ_2 were simplified into the following:

$$\begin{aligned}\lambda_1 &= \frac{-(\mathrm{k_{on}} + \mathrm{R_{1Xe}} + \mathrm{k_{off}}) + \sqrt{(\mathrm{k_{off}} - \mathrm{R_{1Xe}} - \mathrm{k_{on}})^2 + 4\mathrm{k_{on}}\mathrm{k_{off}}}}{2} \\ &= \frac{-(\mathrm{k_{on}} + \mathrm{R_{1Xe}} + \mathrm{k_{off}}) + \sqrt{(\mathrm{k_{off}} - \mathrm{R_{1Xe}} + \mathrm{k_{on}})^2 + 4\mathrm{k_{on}}\mathrm{R_{1Xe}}}}{2} \\ &\approx \frac{-(\mathrm{k_{on}} + \mathrm{R_{1Xe}} + \mathrm{k_{off}}) + \sqrt{(\mathrm{k_{off}} - \mathrm{R_{1Xe}} + \mathrm{k_{on}})^2}}{2} \\ &\approx -\mathrm{R_{1Xe}} \end{aligned} \tag{A5'}$$

$$\begin{aligned}\lambda_2 &= \frac{-\left(\mathrm{k_{on}} + \mathrm{R_{1Xe}} + \mathrm{k_{of}}_{f}\right) - \sqrt{(\mathrm{k_{off}} - \mathrm{R_{1Xe}} - \mathrm{k_{on}})^2 + 4\mathrm{k_{on}}\mathrm{k_{off}}}}{2} \\ &= \frac{-\left(\mathrm{k_{on}} + \mathrm{R_{1Xe}} + \mathrm{k_{of}}_{f}\right) - \sqrt{(\mathrm{k_{off}} - \mathrm{R_{1Xe}} + \mathrm{k_{on}})^2 + 4\mathrm{k_{on}}\mathrm{R_{1Xe}}}}{2} \\ &\approx \frac{-(\mathrm{k_{on}} + \mathrm{R_{1Xe}} + \mathrm{k_{off}}) - \sqrt{(\mathrm{k_{off}} - \mathrm{R_{1Xe}} + \mathrm{k_{on}})^2}}{2} \\ &\approx -(\mathrm{k_{on}} + \mathrm{k_{off}}) \end{aligned} \tag{A6'}$$

Before the free chemical exchange process started, t=0, and both $\mathrm{e}^{(\mathrm{t}\lambda_1)}$ and $\mathrm{e}^{(\mathrm{t}\lambda_2)}$ equaled 1. $\mathrm{M_{Xe}}(0)$ and $\mathrm{M_{CXe}}(0)$ were the magnetizations of free Xe and host molecule bound Xe at t=0. Thus, equation (A3) and (A4) changed into the following:

$$\mathrm{M_{Xe}}(0) = -\mathrm{C_2}\frac{-\mathrm{k_{off}}-\lambda_1}{\mathrm{k_{on}}} - \mathrm{C_1}\frac{-\mathrm{k_{off}}-\lambda_2}{\mathrm{k_{on}}} \tag{A3'}$$

$$\mathrm{M_{CXe}}(0) = \mathrm{C_2} + \mathrm{C_1} \tag{A4'}$$

Then, we achieved the expressions of constant $\mathrm{C_1}$ and $\mathrm{C_2}$, as follows:

$$\mathrm{C_1} = \frac{\mathrm{M_{Xe}}(0)\mathrm{k_{on}} - \mathrm{M_{CXe}}(0)(\mathrm{k_{off}} - \mathrm{R_{1Xe}})}{\mathrm{R_{1Xe}} - \mathrm{k_{on}} - \mathrm{k_{off}}} \tag{A7}$$

$$\mathrm{C_2} = \frac{\mathrm{M_{Xe}}(0)\mathrm{k_{on}} + \mathrm{M_{CXe}}(0)\mathrm{k_{on}}}{-\mathrm{R_{1Xe}} + \mathrm{k_{on}} + \mathrm{k_{off}}} \tag{A8}$$

As shown in Figure S2, the magnetization of free Xe and host molecule bound Xe after the *n*th 180° inversion pulse were denoted as $\mathrm{M_{Xe}^{(n)}}(0)$ and $\mathrm{M_{CXe}^{(n)}}(0)$ respectively. The magnetizations of free Xe and host molecule bound Xe after delay time t for free exchange were respectively denoted as $\mathrm{M_{Xe}^{(n)}}(\mathrm{t})$ and $\mathrm{M_{CXe}^{(n)}}(\mathrm{t})$. Furthermore, we defined a parameter $\beta^{(n-1)}$, and $\mathrm{M_{CXe}^{(n)}}(0) = -\beta^{(n-1)}\mathrm{fr}\,\mathrm{M_{Xe}^{(n)}}(0)$, fr was the ratio between host molecule bound Xe and free Xe. The expressions of $\mathrm{M_{Xe}^{(n)}}(\mathrm{t})$ and $\mathrm{M_{CXe}^{(n)}}(\mathrm{t})$ in equation (A3) and (A4) became the following:

$$\begin{aligned}\mathrm{M_{Xe}^{(n)}}(\mathrm{t}) &= -\mathrm{C_2}\mathrm{e}^{(\mathrm{t}\lambda_1)}\frac{\mathrm{d}-\lambda_1}{\mathrm{c}} - \mathrm{C_1}\mathrm{e}^{(\mathrm{t}\lambda_2)}\frac{\mathrm{d}-\lambda_2}{\mathrm{c}} \\ &\approx -\frac{\mathrm{M_{Xe}^{(n)}}(0)\mathrm{k_{on}} + \mathrm{M_{CXe}^{(n)}}(0)\mathrm{k_{on}}}{-\mathrm{R_{1Xe}} + \mathrm{k_{on}} + \mathrm{k_{off}}}\mathrm{e}^{(\mathrm{t}(-\mathrm{R_{1Xe}}))}\frac{-\mathrm{k_{off}} - (-\mathrm{R_{1Xe}})}{\mathrm{k_{on}}} \\ &\quad - \frac{\mathrm{M_{Xe}^{(n)}}(0)\mathrm{k_{on}} - \mathrm{M_{CXe}^{(n)}}(0)(\mathrm{k_{off}} - \mathrm{R_{1Xe}})}{\mathrm{R_{1Xe}} - \mathrm{k_{on}} - \mathrm{k_{off}}}\mathrm{e}^{\left(\mathrm{t}(-(\mathrm{k_{on}}+\mathrm{k_{off}}))\right)}\frac{-\mathrm{k_{off}} - \left(-(\mathrm{k_{on}} + \mathrm{k_{off}})\right)}{\mathrm{k_{on}}} \\ &\approx -\frac{\mathrm{M_{Xe}^{(n)}}(0)\mathrm{k_{on}} + \left(-\beta^{(n-1)}\mathrm{fr}\,\mathrm{M_{Xe}^{(n)}}(0)\right)\mathrm{k_{on}}}{-\mathrm{R_{1Xe}} + \mathrm{k_{on}} + \mathrm{k_{off}}}\mathrm{e}^{(-\mathrm{tR_{1Xe}})}\frac{-\mathrm{k_{off}} + \mathrm{R_{1Xe}}}{\mathrm{k_{on}}} \\ &\quad - \frac{\mathrm{M_{Xe}^{(n)}}(0)\mathrm{k_{on}} - \left(-\beta^{(n-1)}\mathrm{fr}\,\mathrm{M_{Xe}^{(n)}}(0)\right)(\mathrm{k_{off}} - \mathrm{R_{1Xe}})}{\mathrm{R_{1Xe}} - \mathrm{k_{on}} - \mathrm{k_{off}}}\mathrm{e}^{(-\mathrm{t}(\mathrm{k_{on}}+\mathrm{k_{off}}))}\frac{\mathrm{k_{on}}}{\mathrm{k_{on}}} \\ &\approx -\frac{\mathrm{M_{Xe}^{(n)}}(0)\mathrm{fr}\,\mathrm{k_{off}} - \beta^{(n-1)}\mathrm{fr}\,\mathrm{M_{Xe}^{(n)}}(0)\mathrm{fr}\,\mathrm{k_{off}}}{-\mathrm{R_{1Xe}} + \mathrm{fr}\,\mathrm{k_{off}} + \mathrm{k_{off}}}\mathrm{e}^{(-\mathrm{tR_{1Xe}})}\frac{-\mathrm{k_{off}} + \mathrm{R_{1Xe}}}{\mathrm{fr}\,\mathrm{k_{off}}} \\ &\quad - \frac{\mathrm{M_{Xe}^{(n)}}(0)\mathrm{fr}\,\mathrm{k_{off}} + \beta^{(n-1)}\mathrm{fr}\,\mathrm{M_{Xe}^{(n)}}(0)(\mathrm{k_{off}} - \mathrm{R_{1Xe}})}{\mathrm{R_{1Xe}} - \mathrm{fr}\,\mathrm{k_{off}} - \mathrm{k_{off}}}\mathrm{e}^{(-\mathrm{t}(\mathrm{k_{on}}+\mathrm{k_{off}}))} \\ &\approx -\frac{\mathrm{M_{Xe}^{(n)}}(0) - \beta^{(n-1)}\mathrm{fr}\,\mathrm{M_{Xe}^{(n)}}(0)}{-\mathrm{R_{1Xe}} + \mathrm{fr}\,\mathrm{k_{off}} + \mathrm{k_{off}}}\mathrm{e}^{(-\mathrm{tR_{1Xe}})}(-\mathrm{k_{off}} + \mathrm{R_{1Xe}}) \\ &\quad - \frac{\mathrm{M_{Xe}^{(n)}}(0)\mathrm{fr}\,\mathrm{k_{off}} + \beta^{(n-1)}\mathrm{fr}\,\mathrm{M_{Xe}^{(n)}}(0)(\mathrm{k_{off}} - \mathrm{R_{1Xe}})}{\mathrm{R_{1Xe}} - \mathrm{frk_{off}} - \mathrm{k_{off}}}\mathrm{e}^{(-\mathrm{t}(\mathrm{k_{on}}+\mathrm{k_{off}}))}\end{aligned}$$

$$\begin{aligned}
&\approx \frac{M_{Xe}^{(n)}(0) - \beta^{(n-1)}\mathrm{fr}\, M_{Xe}^{(n)}(0)}{-R_{1Xe} + \mathrm{fr}\, k_{off} + k_{off}} e^{(-tR_{1Xe})}(k_{off} - R_{1Xe}) \\
&\qquad + \frac{M_{Xe}^{(n)}(0)\mathrm{fr}\, k_{off} + \beta^{(n-1)}\mathrm{fr}\, M_{Xe}^{(n)}(0)(k_{off} - R_{1Xe})}{-R_{1Xe} + \mathrm{fr}\, k_{off} + k_{off}} e^{(-t(k_{on}+k_{off}))} \\
&\approx M_{Xe}^{(n)}(0)\frac{1-\beta^{(n-1)}\mathrm{fr}}{-R_{1Xe}+\mathrm{fr}\, k_{off}+k_{off}} e^{(-tR_{1Xe})}(k_{off} - R_{1Xe}) + M_{Xe}^{(n)}(0)\frac{\mathrm{fr}\, k_{off}+\beta^{(n-1)}\mathrm{fr}(k_{off}-R_{1Xe})}{-R_{1Xe}+\mathrm{fr}\, k_{off}+k_{off}} e^{(-t(k_{on}+k_{off}))}
\end{aligned} \tag{A9}$$

$$\begin{aligned}
M_{CXe}^{(n)}(t) &= C_2 e^{(t\lambda_1)} + C_1 e^{(t\lambda_2)} \\
&\approx \frac{M_{Xe}^{(n)}(0)k_{on} + M_{CXe}^{(n)}(0)k_{on}}{-R_{1Xe} + k_{on} + k_{off}} e^{(t(-R_{1Xe}))} + \frac{M_{Xe}^{(n)}(0)k_{on} - M_{CXe}^{(n)}(0)(k_{off} - R_{1Xe})}{R_{1Xe} - k_{on} - k_{off}} e^{\left(t\left(-(k_{on}+k_{off})\right)\right)} \\
&\approx \frac{M_{Xe}^{(n)}(0)\mathrm{fr}\, k_{off} + \left(-\beta^{(n-1)}\mathrm{fr}\, M_{Xe}^{(n)}(0)\right)\mathrm{fr}\, k_{off}}{-R_{1Xe} + \mathrm{fr}\, k_{off} + k_{off}} e^{\left(t(-R_{1Xe})\right)} \\
&\qquad + \frac{M_{Xe}^{(n)}(0)\mathrm{fr}\, k_{off} - \left(-\beta^{(n-1)}\mathrm{fr}\, M_{Xe}^{(n)}(0)\right)(k_{off} - R_{1Xe})}{R_{1Xe} - \mathrm{fr}\, k_{off} - k_{off}} e^{\left(t\left(-(k_{on}+k_{off})\right)\right)} \\
&\approx M_{Xe}^{(n)}(0)e^{\left(t(-R_{1Xe})\right)}\mathrm{fr}\left(\frac{k_{off}-\beta^{(n-1)}\mathrm{fr}\, k_{off}}{-R_{1Xe}+\mathrm{fr}\, k_{off}+k_{off}} + \frac{k_{off}+\beta^{(n-1)}(k_{off}-R_{1Xe})}{R_{1Xe}-\mathrm{fr}\, k_{off}-k_{off}} e^{\left(t\left(R_{1Xe}-(k_{on}+k_{off})\right)\right)}\right)
\end{aligned} \tag{A10}$$

Considering that $k_{off} \gg R_{1Xe} > 0$, we achieved $(k_{off} - R_{1Xe}) \approx k_{off}$ and $\mathrm{fr}\, k_{off} + k_{off} - R_{1Xe} \approx (1 + \mathrm{fr})k_{off}$. In addition, $\frac{1}{\mathrm{fr}+1} \approx 1$, because $1 \gg \mathrm{fr}$. The expression (A9) and (A10) was further simplified into the following:

$$\begin{aligned}
M_{Xe}^{(n)}(t) &\approx M_{Xe}^{(n)}(0)\frac{1-\beta^{(n-1)}\mathrm{fr}}{(1+\mathrm{fr})k_{off}} e^{(-tR_{1Xe})}k_{off} + M_{Xe}^{(n)}(0)\frac{\mathrm{fr}\, k_{off} + \beta^{(n-1)}\mathrm{fr}\, k_{off}}{(1+\mathrm{fr})k_{off}} e^{(-t(k_{on}+k_{off}))} \\
&\approx M_{Xe}^{(n)}(0)\frac{1-\beta^{(n-1)}\mathrm{fr}}{(1+\mathrm{fr})} e^{(-tR_{1Xe})} + M_{Xe}^{(n)}(0)\frac{\mathrm{fr} + \beta^{(n-1)}\mathrm{fr}}{(1+\mathrm{fr})} e^{(-t(k_{on}+k_{off}))} \\
&\approx M_{Xe}^{(n)}(0)e^{(-tR_{1Xe})}\left(\frac{1 + \mathrm{fr} - \mathrm{fr} - \beta^{(n-1)}\mathrm{fr}}{(1+\mathrm{fr})} + \frac{\left(1+\beta^{(n-1)}\right)\mathrm{fr}}{(1+\mathrm{fr})} e^{(-t(k_{on}+k_{off}-R_{1Xe}))}\right) \\
&\approx M_{Xe}^{(n)}(0)e^{(-tR_{1Xe})}\left(1 - \left(1+\beta^{(n-1)}\right)\mathrm{fr} + \left(1+\beta^{(n-1)}\right)\mathrm{fr}\, e^{(-t(k_{on}+k_{off}-R_{1Xe}))}\right) \\
&\approx M_{Xe}^{(n)}(0)e^{(-tR_{1Xe})}\left(1 - \left(1+\beta^{(n-1)}\right)\mathrm{fr}\left(1 - e^{(-t(k_{on}+k_{off}-R_{1Xe}))}\right)\right)
\end{aligned} \tag{A9'}$$

$$\begin{aligned}
M_{CXe}^{(n)}(t) &\approx M_{Xe}^{(n)}(0)e^{\left(t(-R_{1Xe})\right)}\mathrm{fr}\left(\frac{\left(1-\beta^{(n-1)}\mathrm{fr}\right)k_{off}}{(1+\mathrm{fr})k_{off}} + \frac{\left(1+\beta^{(n-1)}\right)k_{off}}{-(1+\mathrm{fr})k_{off}} e^{\left(t\left(R_{1Xe}-(k_{on}+k_{off})\right)\right)}\right) \\
&\approx M_{Xe}^{(n)}(0)e^{\left(t(-R_{1Xe})\right)}\mathrm{fr}\left(\frac{\left(1-\beta^{(n-1)}\mathrm{fr}\right)}{(1+\mathrm{fr})} - \frac{\left(1+\beta^{(n-1)}\right)}{(1+\mathrm{fr})} e^{\left(t\left(R_{1Xe}-(k_{on}+k_{off})\right)\right)}\right) \\
&\approx M_{Xe}^{(n)}(0)e^{\left(t(-R_{1Xe})\right)}\mathrm{fr}\left(\frac{(1+\mathrm{fr}) - \left(1+\beta^{(n-1)}\right)\mathrm{fr} - \left(1+\beta^{(n-1)}\right)e^{\left(t\left(R_{1Xe}-(k_{on}+k_{off})\right)\right)}}{(1+\mathrm{fr})}\right) \\
&\approx M_{Xe}^{(n)}(0)e^{\left(t(-R_{1Xe})\right)}\mathrm{fr}\left(1 - \left(1+\beta^{(n-1)}\right)\left(\mathrm{fr} + e^{\left(t\left(R_{1Xe}-(k_{on}+k_{off})\right)\right)}\right)\right)
\end{aligned} \tag{A10'}$$

We defined another parameter $\alpha^{(n)}$ as follows:

$$\begin{aligned}
\alpha^{(n)} = \frac{M_{CXe}^{(n)}(t)}{\mathrm{fr}\, M_{Xe}^{(n)}(t)} &\approx \frac{M_{Xe}^{(n)}(0)\mathrm{fr}\, e^{-R_{1Xe}t}\left(1 - \left(1+\beta^{(n-1)}\right)\left(\mathrm{fr} + e^{t(R_{1Xe}-k_{on}-k_{off})}\right)\right)}{\mathrm{fr}\, M_{Xe}^{(n)}(0)e^{-R_{1Xe}t}\left(1 - (1+\beta^{(n-1)})\mathrm{fr}(1 - e^{t(R_{1Xe}-k_{on}-k_{off})})\right)} \\
&\approx \frac{\left(1-\left(1+\beta^{(n-1)}\right)\left(\mathrm{fr}+e^{t\left(R_{1Xe}-k_{on}-k_{off}\right)}\right)\right)}{\left(1-\left(1+\beta^{(n-1)}\right)\mathrm{fr}\left(1-e^{t\left(R_{1Xe}-k_{on}-k_{off}\right)}\right)\right)}
\end{aligned} \tag{A11}$$

In an off-resonance experiment, $M_{Xe}^{(n)}(0) = M_{Xe}^{(n-1)}(t)e^{-R_{1Xe}t_p}$ and $M_{CXe}^{(n)}(0) = \alpha^{(n)}\mathrm{fr}\, M_{Xe}^{(n-1)}(t)e^{-R_{1Xe}t_p} = -\beta^{(n)}\mathrm{fr}\, M_{Xe}^{(n-1)}(t)e^{-R_{1Xe}t_p}$. Thus, $\beta^{(n)} = -\alpha^{(n)}$. We obtained an iterative expression of free Xe magnetization that was related to the *n*th 180° inversion pulse-delay to the *(n-1)*th under arbitrarily delay time t, as follows:

$$\mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n})}(\mathrm{t}) \approx \mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n}-1)}(\mathrm{t})\mathrm{e}^{-\mathrm{R}_{1\mathrm{Xe}}\mathrm{t}_{\mathrm{p}}}\mathrm{e}^{(-\mathrm{t}\mathrm{R}_{1\mathrm{Xe}})}\left(1-\left(1-\alpha^{(\mathrm{n}-1)}\right)\mathrm{fr}\left(1-\mathrm{e}^{(-\mathrm{t}(\mathrm{k}_{\mathrm{on}}+\mathrm{k}_{\mathrm{off}}-\mathrm{R}_{1\mathrm{Xe}}))}\right)\right) \tag{A12}$$

$$\mathrm{M}_{\mathrm{CXe}}^{(\mathrm{n})}(\mathrm{t}) \approx \mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n}-1)}(\mathrm{t})\mathrm{e}^{-\mathrm{R}_{1\mathrm{Xe}}\mathrm{t}_{\mathrm{p}}}\mathrm{e}^{(\mathrm{t}(-\mathrm{R}_{1\mathrm{Xe}}))}\mathrm{fr}\left(1-\left(1-\alpha^{(\mathrm{n}-1)}\right)\left(\mathrm{fr}+\mathrm{e}^{\left(\mathrm{t}\left(\mathrm{R}_{1\mathrm{Xe}}-(\mathrm{k}_{\mathrm{on}}+\mathrm{k}_{\mathrm{off}})\right)\right)}\right)\right) \tag{A13}$$

$$\alpha^{(\mathrm{n})} \approx \frac{\left(1-\left(1-\alpha^{(n-1)}\right)\left(\mathrm{fr}+\mathrm{e}^{\mathrm{t}\left(\mathrm{R}_{1\mathrm{Xe}}-\mathrm{k}_{\mathrm{on}}-\mathrm{k}_{\mathrm{off}}\right)}\right)\right)}{\left(1-\left(1-\alpha^{(n-1)}\right)\mathrm{fr}\left(1-\mathrm{e}^{\mathrm{t}\left(\mathrm{R}_{1\mathrm{Xe}}-\mathrm{k}_{\mathrm{on}}-\mathrm{k}_{\mathrm{off}}\right)}\right)\right)} \tag{A14}$$

With sufficient delay time for free chemical exchange, we obtained $\mathrm{e}^{\mathrm{t}(\mathrm{R}_{1\mathrm{Xe}}-\mathrm{k}_{\mathrm{on}}-\mathrm{k}_{\mathrm{off}})} \approx 0$. Thus, we obtained $\alpha^{(\mathrm{n})} \approx 1$ for arbitrarily 180° inversion pulse-delay number n. We further simplified $\mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n})}(t)$ and $\mathrm{M}_{\mathrm{CXe}}^{(\mathrm{n})}(t)$ into the following:

$$\mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n})}(\mathrm{t}) \approx \mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n}-1)}(\mathrm{t})\mathrm{e}^{-\mathrm{R}_{1\mathrm{Xe}}\mathrm{t}_{\mathrm{p}}}\mathrm{e}^{(-\mathrm{t}\mathrm{R}_{1\mathrm{Xe}})} \tag{A15}$$

$$\mathrm{M}_{\mathrm{CXe}}^{(\mathrm{n})}(\mathrm{t}) \approx \mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n}-1)}(\mathrm{t})\mathrm{e}^{-\mathrm{R}_{1\mathrm{Xe}}\mathrm{t}_{\mathrm{p}}}\mathrm{e}^{(\mathrm{t}(-\mathrm{R}_{1\mathrm{Xe}}))}\mathrm{fr} \tag{A16}$$

Then, we obtained the magnetization expression of free Xe after the *n*th 180° inverse pulse-delay unit in an off-resonance experiment, as follows:

$$\mathrm{M}_{\mathrm{Xe\_off}}(\mathrm{t}) \approx \mathrm{M}_{\mathrm{Xe}}^{(0)}(0)\mathrm{e}^{-\mathrm{n}\mathrm{R}_{1\mathrm{Xe}}(\mathrm{t}_{\mathrm{p}}+\mathrm{t})} \tag{A17}$$

However, in an on-resonance experiment, $\mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n})}(0) = \mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n}-1)}(\mathrm{t})\mathrm{e}^{-\mathrm{R}_{1\mathrm{Xe}}\mathrm{t}_{\mathrm{p}}}$ and $\mathrm{M}_{\mathrm{CXe}}^{(\mathrm{n})}(0) = -\alpha^{(\mathrm{n})}\mathrm{fr}\,\mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n}-1)}(\mathrm{t})\mathrm{e}^{-\mathrm{R}_{1\mathrm{Xe}}\mathrm{t}_{\mathrm{p}}} = -\beta^{(\mathrm{n})}\mathrm{fr}\,\mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n}-1)}(\mathrm{t})\mathrm{e}^{-\mathrm{R}_{1\mathrm{Xe}}\mathrm{t}_{\mathrm{p}}}$. Thus, $\beta^{(\mathrm{n})} = \alpha^{(\mathrm{n})}$. We obtained an iterative expression of free Xe magnetization that was related to the *n*th 180° inversion pulse-delay to the *(n-1)*th under arbitrarily delay time t, as follows:

$$\mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n})}(t) \approx \mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n}-1)}(\mathrm{t})\mathrm{e}^{-\mathrm{R}_{1\mathrm{Xe}}\mathrm{t}_{\mathrm{p}}}\mathrm{e}^{(-\mathrm{t}\mathrm{R}_{1\mathrm{Xe}})}\left(1-\left(1+\alpha^{(n-1)}\right)\mathrm{fr}\left(1-\mathrm{e}^{(-\mathrm{t}(\mathrm{k}_{\mathrm{on}}+\mathrm{k}_{\mathrm{off}}-\mathrm{R}_{1\mathrm{Xe}}))}\right)\right) \tag{A18}$$

$$\mathrm{M}_{\mathrm{CXe}}^{(\mathrm{n})}(\mathrm{t}) \approx \mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n}-1)}(\mathrm{t})\mathrm{e}^{-\mathrm{R}_{1\mathrm{Xe}}\mathrm{t}_{\mathrm{p}}}\mathrm{e}^{(\mathrm{t}(-\mathrm{R}_{1\mathrm{Xe}}))}\mathrm{fr}\left(1-\left(1+\alpha^{(n-1)}\right)\left(\mathrm{fr}+\mathrm{e}^{\left(\mathrm{t}\left(\mathrm{R}_{1\mathrm{Xe}}-(\mathrm{k}_{\mathrm{on}}+\mathrm{k}_{\mathrm{off}})\right)\right)}\right)\right) \tag{A19}$$

$$\alpha^{(\mathrm{n})} \approx \frac{\left(1-\left(1+\alpha^{(n-1)}\right)\left(\mathrm{fr}+\mathrm{e}^{\mathrm{t}\left(\mathrm{R}_{1\mathrm{Xe}}-\mathrm{k}_{\mathrm{on}}-\mathrm{k}_{\mathrm{off}}\right)}\right)\right)}{\left(1-\left(1+\alpha^{(n-1)}\right)\mathrm{fr}\left(1-\mathrm{e}^{\mathrm{t}\left(\mathrm{R}_{1\mathrm{Xe}}-\mathrm{k}_{\mathrm{on}}-\mathrm{k}_{\mathrm{off}}\right)}\right)\right)} \tag{A20}$$

With sufficient delay time for free chemical exchange, we obtained $\mathrm{e}^{\mathrm{t}(\mathrm{R}_{1\mathrm{Xe}}-\mathrm{k}_{\mathrm{on}}-\mathrm{k}_{\mathrm{off}})} \approx 0$. Thus, we obtained $\alpha^{(\mathrm{n})} \approx 1$ for arbitrarily 180° inversion pulse-delay number n. We further simplified $\mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n})}(t)$ and $\mathrm{M}_{\mathrm{CXe}}^{(\mathrm{n})}(t)$ into the following:

$$\mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n})}(\mathrm{t}) \approx \mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n}-1)}(\mathrm{t})\mathrm{e}^{-\mathrm{R}_{1\mathrm{Xe}}\mathrm{t}_{\mathrm{p}}}\mathrm{e}^{(-\mathrm{t}\mathrm{R}_{1\mathrm{Xe}})}(1-2\mathrm{fr}) \tag{A21}$$

$$\mathrm{M}_{\mathrm{CXe}}^{(\mathrm{n})}(\mathrm{t}) \approx \mathrm{M}_{\mathrm{Xe}}^{(\mathrm{n}-1)}(\mathrm{t})\mathrm{e}^{-\mathrm{R}_{1\mathrm{Xe}}\mathrm{t}_{\mathrm{p}}}\mathrm{e}^{(\mathrm{t}(-\mathrm{R}_{1\mathrm{Xe}}))}\mathrm{fr}(1-2\mathrm{fr}) \tag{A22}$$

Then, we obtained the magnetization expression of free Xe after the *n*th 180° inverse pulse-delay unit in an on-resonance experiment, as follows:

$$\mathrm{M}_{\mathrm{Xe\_on}}(\mathrm{t}) \approx \mathrm{M}_{\mathrm{Xe}}^{(0)}(0)\mathrm{e}^{-\mathrm{n}\mathrm{R}_{1\mathrm{Xe}}(\mathrm{t}_{\mathrm{p}}+\mathrm{t})}(1-2\mathrm{fr})^{\mathrm{n}} \tag{A23}$$

According to the definition of CEITR, we obtained the expression of CEITR, as follows:

$$\mathrm{CEITR} = \frac{\mathrm{M}_{\mathrm{Xe\_on}}(\mathrm{t})}{\mathrm{M}_{\mathrm{Xe\_off}}(\mathrm{t})} = (1-2\mathrm{fr})^{\mathrm{n}} \tag{A24}$$

Then, we achieved the ratio between host molecule bound Xe and free Xe, as follows:

$$\mathrm{fr} = \frac{1-\mathrm{CEITR}^{\frac{1}{\mathrm{n}}}}{2} \tag{A25}$$

Finally, the host molecule concentration [HM] was quantified with the obtained fr, the binding constant K, and the free Xe concentration [Xe] [2]

$$[\mathrm{HM}] = \mathrm{fr}\left(\frac{1}{\mathrm{K}}+[\mathrm{Xe}]\right) \tag{A26}$$

The fr was calculated from the CEIT experiment with formula (A25). The binding constant K was obtained by NMR spectroscopy method as shown in the next part. The free Xe concentration was achieved by the solubility of Xe in water and partial pressure of Xe with formula [Xe]=[Xe Solubility]·[Xe partial pressure].

Section S2. Measurement of the binding constant of CrA-(COOH)$_6$

The ratio fr of xenon in the host to free xenon was measured from peak integrals in the direct ^{129}Xe NMR spectrum of 2.5 μM CrA-(COOH)$_6$ solution (Figure S3). Noting that [HM] = [unoccupied HM]

+ [Xe@HM], in which [HM] stands for the host molecule concentration, [unoccupied HM] and [Xe@HM] were the concentrations of unbound and bound xenon host molecule, respectively. We rearranged the binding constant K as follows [2-3]:

$$\mathrm{K} = \frac{[\mathrm{Xe@HM}]}{[\mathrm{Xe}]([\mathrm{HM}] - [\mathrm{Xe@HM}])} = \frac{\mathrm{fr}}{[\mathrm{HM}] - \mathrm{fr}[\mathrm{Xe}]}$$
$$= \frac{0.0036}{2.500 - 0.0036 \times \left(4.4 \times 1000 \times \left(\frac{65.7}{14.696} \times 0.02\right)\right)}\ \mu\mathrm{M}^{-1}$$
$$\approx 3322\ \mathrm{M}^{-1}$$

Using the solubility of xenon in water[4] (~4.4 mM/atm 297 K) and the known amount of host added, we then calculated the binding constant K. The direct spectrum was integrated to determine fr, as shown below.

Section S3. Analysis of the standard deviation of concentration determination

As shown in equation (A26), the concentration of the xenon host molecule was proportional to fr with the known binding constant and free xenon concentration. While for fr, the standard deviation was obtained as follows:

$$\sigma_{\mathrm{fr}}^2 = \left(\frac{\partial \mathrm{fr}}{\partial \mathrm{CEITR}}\right)^2 \sigma_{\mathrm{CEITR}}^2 = \left(\frac{\mathrm{CEITR}^{\frac{1}{n}-1}}{2\mathrm{n}}\right)^2 \sigma_{\mathrm{CEITR}}^2 \tag{A27}$$

The relative deviation of fr was shown to be:

$$\mathrm{RD}_{\mathrm{fr}} = \frac{\sigma_{\mathrm{fr}}}{\mathrm{fr}} = \frac{\mathrm{CEITR}^{\left(\frac{1}{n}-1\right)}}{\mathrm{n}\left(1-\mathrm{CEITR}^{\frac{1}{n}}\right)} \sigma_{\mathrm{CEITR}} \tag{A28}$$

The σ_{CEITR} term was determined by the noise level during the measurement. The minimum value of term $\frac{\mathrm{CEITR}^{\left(\frac{1}{n}-1\right)}}{n\left(1-\mathrm{CEITR}^{\frac{1}{n}}\right)}$ could be obtained when $\mathrm{CEITR} = \left(1 - \frac{1}{n}\right)^n \rightarrow e^{-1} \approx 0.368$. Thus, when CEITR is e^{-1} or close to it, the minimum $\mathrm{RD}_{\mathrm{fr}}$ will be obtained. To obtain a minimum R_{fr}, an optimal cycle number $\mathrm{N}_{\mathrm{opt}}$ can be calculated using a measured CEITR under cycles number N by the formula below:

$$\mathrm{N}_{\mathrm{opt}} = -\frac{\mathrm{N}}{\ln(\mathrm{CEITR})} \tag{A29}$$

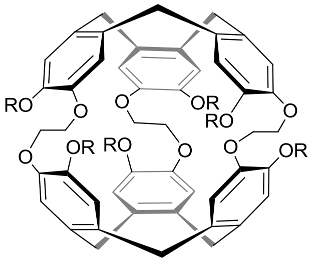

Figure S1. The structure of CrA-$(COOH)_6$. R=-CH_2COOH.

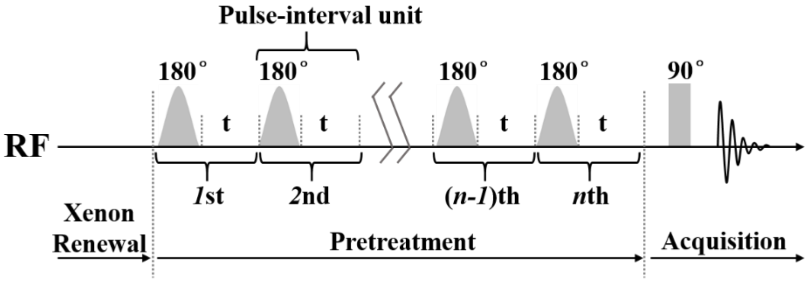

Figure S2. The CEIT pulse sequence diagram. The 180° pulses are selective inversion pulses, t is the interval time between 180° pulses, and n is the number of pulse-interval unit repeat time.

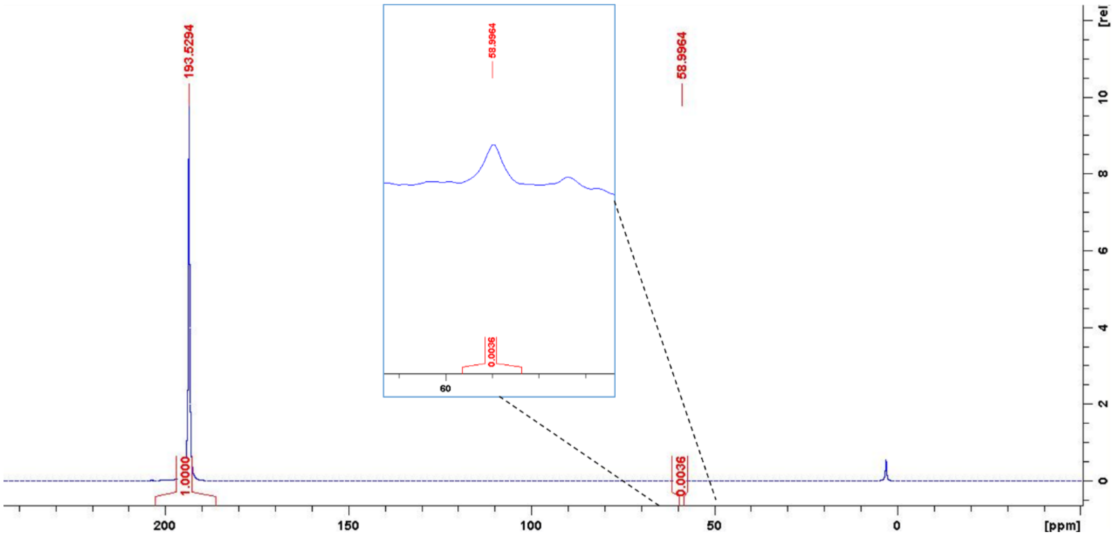

Figure S3. 2.5 μM CrA-(COOH)$_6$ ^{129}Xe NMR spectroscopy with NS=64.

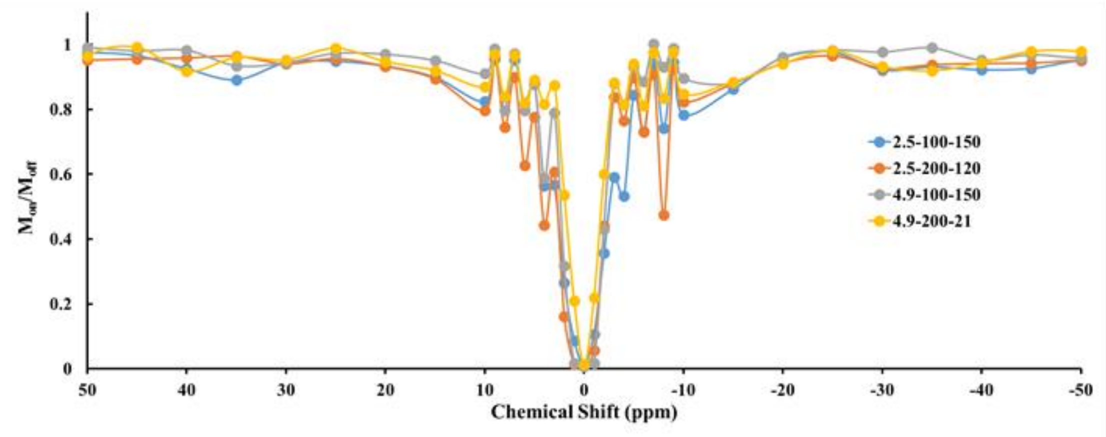

Figure S4. The local CEIT z-spectra achieved with different experimental settings (blue: 2.5 μM, 100 cycles, t_d=150 ms; red: 2.5 μM, 200 cycles, t_d=120 ms; grey: 4.9 μM, 100 cycles, t_d=150 ms; orange: 2.5 μM, 200 cycles, t_d=21 ms).

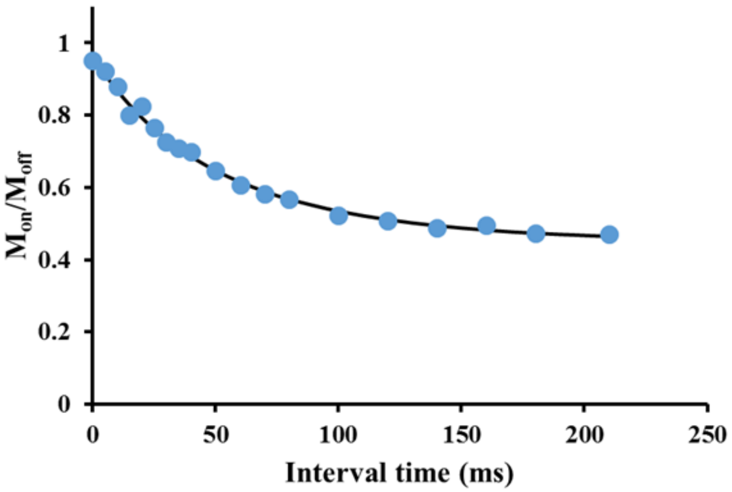

Figure S5. The exchange rate fitting of 2.5 μM CrA-$(COOH)_6$ at 297 K and 65.7 psi. The fitted exchange rate k is about 15.35 Hz using the following equation ($\mathrm{y} = \mathrm{A}\left(1 - 2B\left(1 - e^{-k(t-C)}\right)\right)^{100}$, data: blue squares, fit: black line).

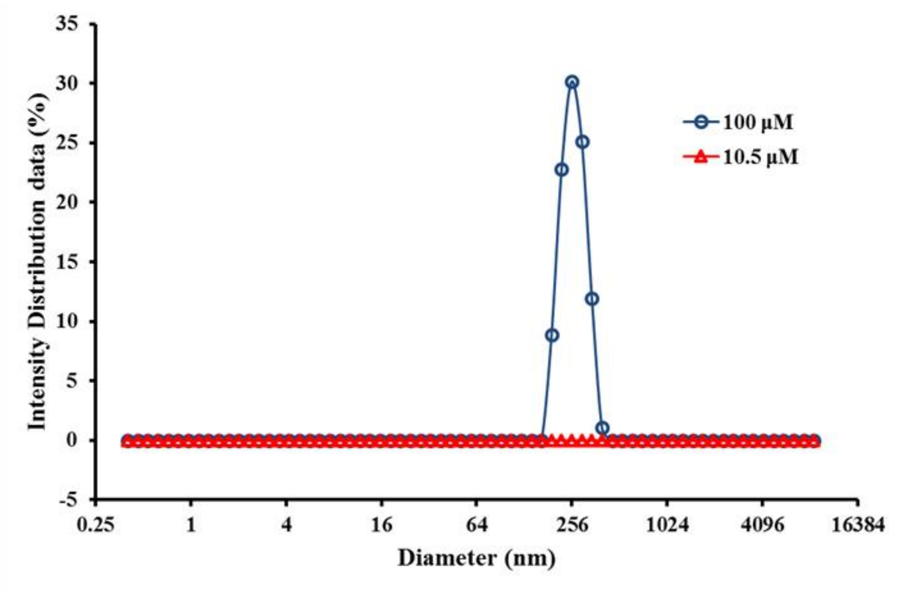

Figure S6. DLS data showing size distribution by number in aqueous solution at 298 K of 100.0 µM CrA-$(COOH)_6$ (average diameter=357.0 nm, PdI=0.463) and 10.5 µM CrA-$(COOH)_6$ (no aggregation observed).

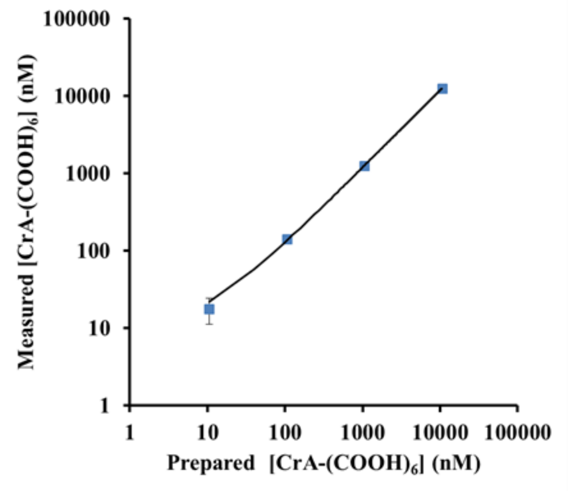

Figure S7. The comparison of the measured CrA-$(COOH)_6$ concentrations and prepared CrA-$(COOH)_6$ concentrations ranges from 10.5 nM to 10.5 μM. The number of cycles *N* of concentration from low to high are 800, 800, 200 and 50, respectively. The measured CrA-$(COOH)_6$ concentrations agree well with the prepared CrA-$(COOH)_6$ concentrations (y=1.182x+9.654, R^2=1.000).

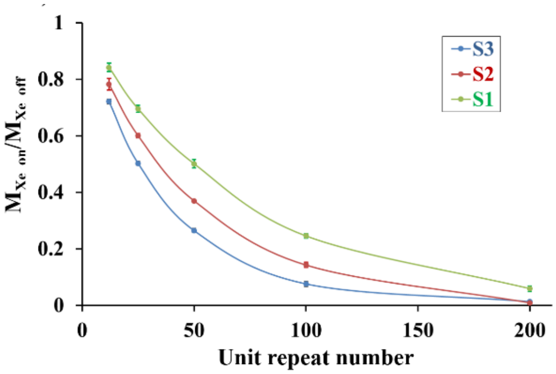

Figure S8. The CEIT effect of sample S1 (green), S2 (red), S3 (blue) under variable unit repeat numbers (200, 100, 50, 25, and 12 cycles, t_d=150 ms).

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