Supporting Information:

Direct Observation of Nanosecond Water Exchange Dynamics at a Protein Metal Site

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Peptide synthesis and purification: Peptides used for this study were synthesized on an Applied Biosystems 433A solid phase peptide synthesizer-using standard Fmoc protocols,[1] and purified and characterized as previously reported.[2] Peptide stock solutions were prepared in double distilled water and concentrations were determined using Ellman's test.[3] All the solutions were degassed under argon atmosphere prior to experiments to prevent peptide oxidation.

[111m]Cd PAC spectroscopy: PAC spectroscopic data collection and sample preparation was performed as described previously.[4] The experiments were carried out in Copenhagen, Denmark, with [111m]Cd delivered by the University Hospital Cyclotron, except for one test experiment, which was carried out at ISOLDE, CERN. The experimental samples for PAC measurements at −20 °C, 1 °C, 20 °C, 35 °C and 50 °C contained 300 μM of peptide in 20 mM TRIS buffer with a Cd^{2+} to peptide ratio of 1:12. Sucrose was added to a final concentration of 55% (w/w) in order to reduce the rotational diffusion of the peptide. To account for the fact that the pK_a of TRIS buffer declines ~0.03 units per degree Celsius rise in temperature (vice versa for decrease in temperature), the pH of the samples were adjusted at room temperature so that the desired pH of 8.5±0.3 is achieved at the temperatures where the PAC measurements have been carried out. To ensure that the theoretical estimate is correct, pH has been measured at various temperatures with the whole setup equilibrated to that temperature.[5] This was done only once and the relation between pH and temperature was used in the rest of the sample preparation. 400 data points were used to fit the PAC spectra disregarding first 3-5 points due to systematic errors. The data analysis was carried out using standard methods for the data collected at −20 °C and 1 °C, where the two individual NQIs are observable (Table S1). Qualitatively the NQI parameters have the following meaning: ω_0 is proportional to the numerically largest diagonal element of the

diagonalised electric field gradient (EFG) tensor, V_{zz}, and thus a measure of the NQI strength; the asymmetry parameter, $\eta = (V_{xx}-V_{yy})/V_{zz}$, reflects the symmetry of the EFG tensor, and runs from 0 (axial symmetry) to 1; $\Delta\omega_0/\omega_0$ is a measure of the line broadening due to static variations of the probe site structure from one site to the next; τ_C is the rotational correlation time of the entire molecule in solution, *vide infra*; A is the amplitude of the signal. At 1 °C, 20 °C, 35 °C and 50 °C the data analysis required a different approach due to the influence of exchange dynamics, *vide infra* (the spectrum recorded at 1 °C can be analyzed by both methods).

Table S1: NQI parameters for TRIL16C and TRIL16CL23A. See the text and ref. 7 for a detailed discussion of the parameters.

t [°C]	ω_0 [rad/ns]	η	$\Delta\omega_0/\omega_0 \times 100$	$1/\tau_C$ [μs^{-1}]	A$\times$100	χ^2
TRI L16C						
−20	0.337(2)	0.24(1)	7.1(5)		8.2(5)	
				2(1)		1.29
	0.467(1)	0.16(1)	1.2(3)		2.3(2)	
1	0.338(1)	0.22(9)	4.9(4)		6.7(3)	
				3.8(4)		1.13
	0.459(2)	0.0(4)	3.1(5)		3.4(3)	
TRI L16CL23A						
−20	0.333(2)	0.26(1)	7.7(6)		6.3(4)	
				0.5(2)		1.28
	0.462(1)	0.163(6)	1.0(2)		2.4(2)	
1	0.335(2)	0.27(2)	6.9(9)		5.4(5)	
				4.1(5)		1.09
	0.455(1)	0.13(2)	2.4(4)		3.7(3)	

The simulations of the PAC spectra carried out to determine the lifetimes of the CdS_3O and CdS_3 species require a number of input parameters: dt (time per channel) in ns, w_iterations (number of iterations), lifetime of the two states in ns, frequency (ω_0), the asymmetry parameter (η), and Euler angles of the principal axis of the EFG (α, β, γ) in degrees for CdS_3O and CdS_3.[6] One therefore has to have prior knowledge about the NQI parameters (ω_0 and η, which were determined using the winfit program (kindly provided by Prof. Tilman Butz, University of Leipzig) for the PAC experiment carried out at $-20°C$, see Table S1). The parameter dt was chosen to be 0.562 ns, in order to correspond to the time per channel of the experimental setup, and thus give rise to the line width inherent to the PAC instrument. The number of iterations was chosen to be 10000 based on convergence tests. The PAC spectra at 1°C, 20°C, 35°C and 50°C were simulated as jumping between two states, CdS_3O and CdS_3, testing a range of different lifetimes, and $A_2G_2(t)$ for randomly oriented samples were calculated, scaled to the experimental effective amplitude, A_2^{eff} (which depends on the properties of the nuclear decay as well as the sample-detector geometry), multiplied by an exponential damping function reflecting rotational diffusion, *vide infra*, and finally optimized against the experimental data with a standard chi-square minimization procedure. From these analyses, the lifetime of the structures representing the two NQIs were extracted (Table 1 in the main text). It is important to emphasize that the statistical errors of ~1 ns are derived only from the statistical analysis, and errors due to assumptions in the model are not included. There are two central assumptions in the model used to simulate the PAC data: 1) the orientation of the electric field gradient tensor principal axis are the same for the CdS_3 and CdS_3O species. According to the semi-empirical BASIL model[7] (as well as to a simple point charge model for the ligands), the largest component of the EFG tensor, V_{zz}, lies perpendicular to the CdS_3 plane and in the same direction for the CdS_3O structure,

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assuming perfect trigonal planar and tetrahedral structures, respectively. Thus it seems plausible that the principal axis does not undergo significant change of direction between the two coordination geometries. Simulating the exchange dynamics with various orientations of the EFG tensor with changes up to 30 degrees of the Euler angles, indicates that this assumption may give rise to changes of the lifetimes of up to ~10 ns, 2) the PAC parameters (ω_0 and η) are temperature independent within the temperature range studied ($-20°C$ to $50°C$). Using data recorded at different temperatures for the reference peptides **TRI**L16Pen and **TRI**L12AL16C displaying pure CdS_3 and CdS_3O coordination geometries, respectively, we have estimated the changes of the NQI parameters for **TRI**L16C and **TRI**L16CL23A with temperature, assuming a linear change of the EFG diagonal elements within this limited temperature range, extrapolating from the recorded values at $-20°C$ (Table S2). The resulting change of the residence times (Table S3), may be compared to Table 1 in the main text.

Table S2: Estimated temperature dependence of NQI parameters, see the text for details.

	TRIL16C				TRIL16CL23A			
	CdS_3		CdS_3O		CdS_3		CdS_3O	
t (°C)	ω_0 (rad/ns)	η	ω_0 (rad/ns)	η	ω_0 (rad/ns)	η	ω_0 (rad/ns)	η
1	0.462	0.159	0.342	0.186	0.457	0.163	0.338	0.206
20	0.457	0.159	0.347	0.138	0.452	0.163	0.342	0.156
35	0.453	0.159	0.350	0.099	0.449	0.163	0.346	0.118
50	0.450	0.159	0.354	0.061	0.445	0.163	0.350	0.079

Table S3: Lifetimes of the CdS$_3$O (τ_1) and CdS$_3$ (τ_{-1}) species derived from the ^{111m}Cd PAC data, using temperature dependent NQI parameters, see the text for details.

t [°C]	TRIL16C		TRIL16CL23A	
	τ_1 [ns]	τ_{-1} [ns]	τ_1 [ns]	τ_{-1} [ns]
1	56	43	49	51
20	38	32	35	41
35	24	24	19	26
50	15	17	11	16

All changes of the residence times are less than 12 ns, as compared to those determined using temperature independent NQI parameters. We use the residence times determined using the temperature independent NQI parameters in the main text, because there may be a systematic error in transferring the temperature dependence of the NQI parameters from the reference peptides to **TRI**L16C and **TRI**L16CL23A. Thus, this analysis mainly serves to estimate the error on the determined residence times, which is then less than ~12 ns. It is noteworthy, that the trends, i.e. the changes in the lifetimes of the CdS$_3$O (τ_1) and CdS$_3$ (τ_{-1}) species, due to the L23A substitution is preserved despite the change in absolute values: The residence time of the metal ion bound water molecule is decreased upon the L23A substitution. In summary, the systematic errors may amount to ~20 ns on the absolute values of the lifetimes, but the trends are practically not affected, and consequently, the conclusions presented in the main text are valid.

The dynamics at the metal ion binding site is composed of local dynamics and the rotational diffusion of the entire peptide. The rotational correlation time, τ_c, was determined using the

reference peptide **TRI**L12AL16C at each temperature. In the following the symbol used for the viscosity is γ, in order to avoid confusing it with the asymmetry parameter derived from the PAC data. A linear correlation was assumed between τ_c and γ/T (where T is the absolute temperature), implicitly assuming a spherical shape of the tumbling molecule, for which $\tau_c = V\gamma/(kT)$ (where k is Boltzmann's constant and V is the volume of the molecule). The viscosity at each temperature was found using reference data,[8] and interpolated using a 5th order polynomium. Fitting τ_c (γ/T) to the experimentally determined values of τ_c provides more reliable values to be used in the data analysis. It also gives an estimate of the molecular volume V = 35787 Å^3. This is slightly larger, than the estimated volume of 32380 Å^3 based on the structure of the related peptide As(**CSL**9C)$_3$ (PBD code: 2JGO[9]), likely due to hydration. The values of $1/\tau_c$ used in the data analysis, assuming that the rotational diffusion is in the slow reorientation time regime, are presented in Table S4.

Table S4: Estimated inverse rotational correlation times for TRIL16C and TRIL16CL23A at the relevant temperatures (see the text for details).

t [ºC]	$1/\tau_c\ [\mu s^{-1}]$
1	3.4
20	13.0
35	15.5
50	16.6

The simulated PAC data were exponentially damped by multiplying with exp(-t/τ_c) before

comparing with the experimental data. Prior to the exponential damping, the simulated data were shifted to the baseline of the experimental data, and scaled to the amplitude of the experimental data.

^{113}Cd NMR spectroscopy

Variable temperature NMR spectra at temperatures 1 °C, 20 °C, 35 °C and 50 °C were collected on a Varian Inova 500 spectrometer (110.92 MHz for ^{113}Cd) equipped with a 5-mm switchable (sw) broadband probe and enhanced variable temperature (VT) unit with high-stability option and pre-conditioning unit. All the spectra were externally referenced to 0.1 M $Cd(ClO_4)_2$ solution in D_2O. A spectral width of 847 ppm (93,897 Hz) was sampled using a 4.5 µs 90° pulse with 0.05 s acquisition time, with no relaxation delay between scans. 30-35 mg of lyophilized peptide samples were kept under vacuum overnight prior to dissolving in 600 µL of 20 mM TRIS buffer in 15% D_2O which was degassed under argon. Peptide concentrations (determined using Ellman's test[3]) were 9-11 mM corresponding to the monomer. The required amount of 250 mM ^{113}Cd(NO_3)_2$ stock solution (prepared from 95% isotopically enriched ^{113}CdO obtained from Oak Ridge National laboratory) was added to the peptide solutions. pH adjustments were made at room temperature to account for the temperature-dependent pK_a change of TRIS to have desired pH of 8.5±0.3 at the temperatures of the measurements. The samples were prepared by dissolving 30-35 mg peptide in 250.5 µL of buffer so that the final volume of the solution after adding 306.1 mg sucrose was 500 µL. The density of peptide solution before addition of sucrose was assumed to be 1 g/mL. The pH of all the

solutions was adjusted using concentrated HCl and KOH. Attempts were made to keep the peptide samples under inert atmosphere; however the samples came into contact of air during pH

adjustments and data collection. All the spectra were collected for 3 hours and processed using MestreC.[10] The free induction decays were zero filled to double the number of original data points and treated with an exponential function with line broadening of 100 Hz prior to Fourier transform.

1. Chan, W. C.; White, P. D., *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*. Oxford Univ. Press: New York, 2000; p 346.
2. Dieckmann, G. R.; McRorie, D. K.; Tierney, D. L.; Utschig, L. M.; Singer, C. P.; O'Halloran, T. V.; Penner-Hahn, J. E.; DeGrado, W. F.; Pecoraro, V. L. *J. Am. Chem. Soc.* **1997,** *119*, 6195-6196.
3. Ellman, G. L. *Arch. Biochem. Biophys.* **1959,** *82*, 70-77.
4. Matzapetakis, M.; Farrer, B. T.; Weng, T.-C.; Hemmingsen, L.; Penner-Hahn, J. E.; Pecoraro, V. L. *J. Am. Chem. Soc.* **2002,** *124*, 8042-8054.
5. Hemmingsen, L.; Bauer, R.; Bjerrum, M. J.; Zeppezauer, M.; Adolph, H.; Formicka, G.; Cedergren-Zeppezauer, E. *Biochemistry* **1995,** *34*, 7145-7153.
6. Danielsen, E.; Jorgensen, L., Elkjr; Sestoft, P. *Hyperfine Interact.* **2002,** *142*, 607-626.
7. Hemmingsen, L.; Narcisz, K.; Danielsen, E. *Chem. Rev.* **2004,** *104*, 4027-4061.
8. Telis, V.; Telis-Romero, J.; Mazzotti, H.; Gabas, A. *Int. J. Food Prop.* **2007,** *10*, 185-195.
9. Touw, D. S.; Nordman, C. E.; Stuckey, J. A.; Pecoraro , V. L. *Proc. Natl. Acad. Sci.* **2007,** *104*, 11969-11974.
10. Cobas, J. C.; Sardina, F. J. *Concepts Magn. Reson.* **2003,** *19*, 80-96.