Nanosecond dynamics at protein metal sites: An application of perturbed angular correlation (PAC) of γ-rays spectroscopy

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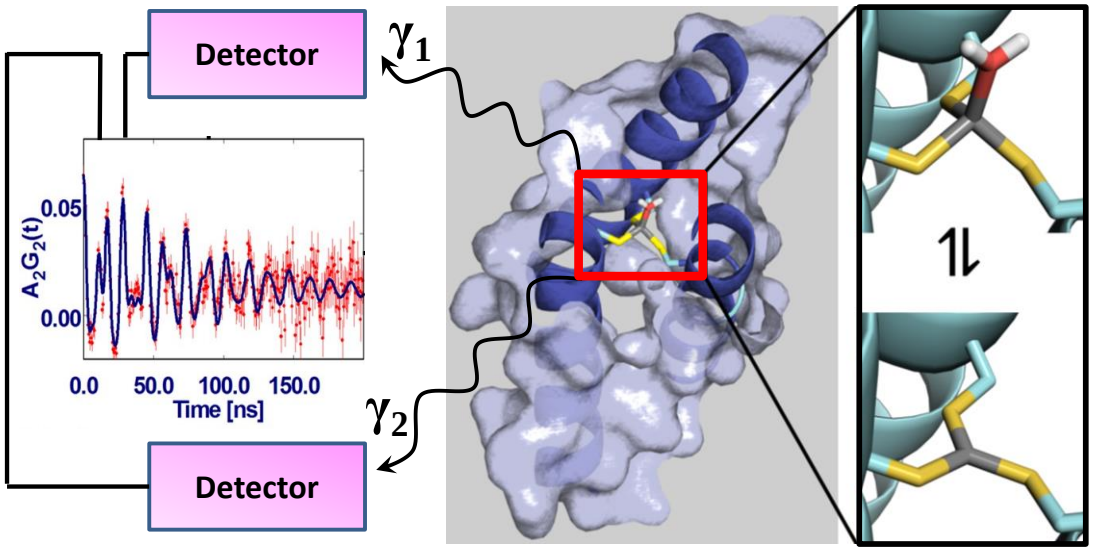

Metalloproteins are essential to numerous reactions in nature, and constitute approximately one third of all known proteins. Molecular dynamics of proteins has been elucidated with great success both by experimental and theoretical methods, revealing atomic level details of function involving the organic constituents on a broad spectrum of time scales. However, the characterization of dynamics at biomolecular metal sites on nanosecond time scales is scarce in the literature. The aqua ions of many biologically relevant metal ions exhibit exchange of water molecules on the nanosecond time scale or faster, often defining their reactivity in aqueous solution, and this is presumably also a relevant time scale for the making and breaking of coordination bonds between metal ions and ligands at protein metal sites. Ligand exchange dynamics is critical for a variety of elementary steps of reactions in metallobiochemistry, e.g. association and dissociation of metal bound water, association of substrate and dissociation of product in the catalytic cycle of metalloenzymes, at regulatory metal sites which require binding and dissociation of metal ions, as well as in the transport of metal ions across cell membranes or between proteins involved in metal ion homeostasis.

In Perturbed Angular Correlation of γ-rays (PAC) spectroscopy the correlation in time and space of two γ-rays emitted successively in a nuclear decay is recorded, reflecting the hyperfine interactions of the PAC probe nucleus with the surroundings. This allows for characterization of molecular and electronic structure as well as nanosecond dynamics at the PAC probe binding site. Herein, selected examples describing the application of PAC spectroscopy in probing the dynamics at protein-metal sites are presented, including 1) exchange of Cd^{2+} bound water in de novo designed synthetic proteins, and the effect of remote mutations on metal site dynamics, 2) dynamics at the β-lactamase active site, where the metal ion appears to jump between the two adjacent sites, 3) structural relaxation in small blue copper proteins upon $^{111}Ag^{+}$ to $^{111}Cd^{2+}$ transformation in radioactive nuclear decay, 4) metal ion transfer between two HAH1 proteins with change in coordination number, and 5) metal ion sensor proteins with two co-existing metal site structures.

With this work we hope to make our modest contribution to the field and perhaps spur additional interest in dynamics at protein metal sites, which we consider to be severely underexplored. Relatively little is known about detailed atomic motions at metal sites, for example, how ligand exchange processes affect protein function, and how the amino acid composition of the protein may control this facet of metal site characteristics. We also aim to provide the reader with a qualitative impression of the possibilities offered by PAC spectroscopy in bioinorganic chemistry, especially when elucidating dynamics at protein metal sites, and finally present data that may serve as benchmarks on a relevant time scale for development and tests of theoretical molecular dynamics methods applied to biomolecular metal sites.

Considerable effort has been devoted to explore the dynamics of organic constituents of proteins as well as the role of solvent molecules. This has led to fundamental discoveries, and a detailed atomic level characterization of many facets of protein function.[1-6] Metal ion containing proteins represent about one third of all proteins,[7-9] and some of the most abundant and important bio-metals are Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Cu^{+}/Cu^{2+}, and Zn^{2+}, with functions in all classes of proteins, and in essential processes such as catalysis, regulation of gene transcription, muscle contraction, signaling, photosynthesis and many others.[10,11] The aqua ions of all these metal ions display water exchange rates on the ns time scale, often defining their reactivity in aqueous solution,[12-14] implying that metal site dynamics on this time scale is central to protein function. Unfortunately, it is experimentally difficult to accurately characterize dynamics at the metal sites within proteins, on the nanosecond time scale, although very interesting results have been achieved in recent years.[15-21] In theoretical chemistry, classical molecular dynamics simulations have been employed massively to explore protein dynamics up to the millisecond time scale,[6] but often suffer from inadequate description of metal sites, where polarization and charge transfer effects as well as the making and breaking of metal–ligand bonds is nontrivial to parameterize properly.[22] On the other hand, *ab initio* quantum chemistry and first principle DFT based molecular dynamics is yet to reach the nanosecond range.[23]

PAC spectroscopy is a hyperfine interaction technique, which provides information on local metal site structure and on dynamics occurring on the nanosecond time scale, by measurement of the nuclear quadrupole interaction (NQI).[1,24]. In the following sections we will briefly present the theoretical background of PAC spectroscopy and provide selected examples of PAC spectroscopy applied to explore nanosecond dynamics at protein metal sites.

A. Theory of PAC Spectroscopy

Here we concisely describe the principles of PAC spectroscopy. For more comprehensive accounts of the technique and applications in biochemistry, the reader is referred to the literature[1,25]. PAC spectroscopy belongs to the family of techniques, such as Mössbauer spectroscopy, electron spin resonance, NMR, and NQR, where the hyperfine interactions of the nuclear magnetic and/or quadrupole moments with extranuclear magnetic

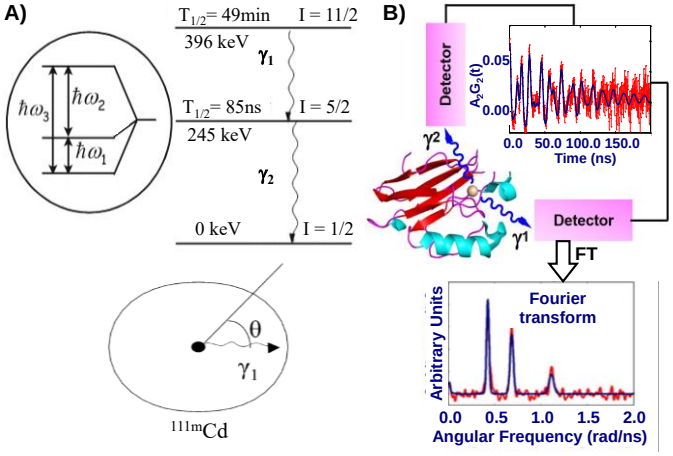

Figure 1. Overview of PAC spectroscopy. A) Right top panel: Schematic presentation of the decay of a typical PAC probe; ^{111m}Cd. Left top panel: The energy splitting of the intermediate level in an axially symmetric EFG (η = 0). Bottom panel: Angular correlation of the two γ-rays emitted in the decay of ^{111m}Cd (see text for details). B) Top: The angle and time between γ_1 and γ_2 is measured. Typically, several millions of such γ_1-γ_2 coincidences are recorded and analyzed, giving the plot in the upper right part of the figure. The analytical expression for $G_2(t)$ is known, see the text. Bottom: For visualization the data are Fourier transformed, directly providing the three frequencies reflecting the energy splitting of the intermediate nuclear energy level. Notice that $\omega_3=\omega_1+\omega_2$, and that for randomly oriented molecules, the first peak is the most intense. Reproduced with

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fields and/or electric field gradients (EFGs) are measured. Here we only present examples of the so-called nuclear quadrupole interactions (NQI) between the electric quadrupole moment of the PAC probe nucleus and the EFG from the surroundings. The EFG is a signature of the local metal site electronic and molecular structure and dynamics, and gives rise to hyperfine splitting of the nuclear energy levels, as shown explicitly for the intermediate (I = 5/2) level in the decay scheme for ^{111m}Cd – a typical PAC probe – in Figure 1A (top panel). It is this energy splitting that is recorded in PAC spectroscopy.

For a nucleus with spin I = 5/2, the intermediate level is split into three sublevels, with transition frequencies ω_1, ω_2, and ω_3 (Figure 1A), where $\omega_3 = \omega_1 + \omega_2$. The PAC isotopes referred to in this work all have the nuclear spin I = 5/2 in the intermediate nuclear energy level that defines the observable PAC pattern and frequency spectra (for other spin values see references[26,27]). In a PAC experiment the three frequencies ω_1, ω_2, and ω_3 are determined as a fingerprint of local structure. If the EFG changes with time, the energy splitting becomes time dependent, and the half-life of the intermediate level (85 ns for ^{111m}Cd), dictates the time scale on which the experiment is sensitive to dynamics. The hyperfine splitting is very small (~10 neV), and not recorded directly as energy differences of emitted γ-rays, but a different property of the nuclear decay is exploited. Due to conservation of angular momentum in the nuclear decay, there will be an angular correlation of the two γ-rays emitted (Figure 1A bottom panel). The distance from the center to the ellipsoid indicates the relative probability of emission of the second γ-ray (γ_2) at a given angle, θ, with respect to the first γ-ray (γ_1), if γ_1 is emitted as indicated in the figure. For ^{111m}Cd, the second γ-ray is most likely to be emitted in the same or opposite direction, whereas it is least likely to be emitted in the direction perpendicular to the first γ-ray. In PAC spectroscopy such γ-cascades are used. Detection of the first γ-ray in a given direction will result in an anisotropic probability distribution for emission of the second γ-ray, and typically the second γ-ray is detected as a function of angle, 180° and 90°, (and time) with respect to the detection of the first γ-ray. The angular anisotropy established in this way accounts for the angular correlation (AC) of the PAC acronym. The angular correlation is perturbed if the nucleus experiences magnetic and / or quadrupole hyperfine fields while it is in the intermediate nuclear level, leading to the full acronym PAC. That is, if the nucleus interacts with extranuclear fields while it is in the intermediate nuclear energy level (after emission of the first γ-ray, but before emission of the second γ-ray), the angular correlation is perturbed. The perturbation is manifested as an oscillation in time of the probability of detecting the second γ-ray in a given direction with respect to the first. In a semi-classical picture, the oscillation may be thought of as a consequence of nuclear spin precession caused by the hyperfine interactions, in analogy to spin precession in NMR spectroscopy. It is the measurement of this time dependence (spin precession), that allows for determination of the three frequencies ω_1, ω_2, and ω_3 (Figure 1B).

For most applications in biochemistry the molecules are randomly oriented. Under these conditions, and in the case of a static NQI (i.e. a time independent electric field gradient (EFG)), the so-called perturbation function is for I = 5/2 is given by:

$$G_2(t) = a_0 + a_1 \cos(\omega_1 t) + a_2 \cos(\omega_2 t) + a_3 \cos(\omega_3 t)$$

a_i and ω_i depend on only two parameters, the NQI strength typically given as ν_Q or ω_0 which are proportional to $|V_{zz}|$ and the axial asymmetry

parameter $\eta = (V_{yy}-V_{xx})/V_{zz}$, where V_{xx}, V_{yy}, and V_{zz} are the diagonal elements of the EFG tensor in the principal axis system, ordered such that numerically $|V_{zz}| \geq |V_{yy}| \geq |V_{xx}|$. Quantum chemical calculations of the EFG are often applied in the interpretation of experimental data.[1,28-31]

If the EFG is not static but changes within the PAC analysis time from a few to hundreds of ns, the perturbation function changes in a characteristic manner, as described in the following sections. The time dependence of the EFG may for example originate from rotational diffusion of the entire molecule to which the PAC isotope is bound, or from conformational dynamics of the molecule[1,32]. If this motion is slow ($1/\tau_c << \omega_0$), the perturbation function is exponentially dampened by $\exp(-t/\tau_c)$, where τ_c is the rotational correlation time of the molecule. If the motion is fast ($1/\tau_c >> \omega_0$), the perturbation function becomes $G_2(t) = \exp(-2.8\omega_0^2\tau_c(1+\eta^2/3)t)$, i.e. an exponentially decaying function with no reminiscence of oscillations. A different physical origin of the dynamics may be chemical exchange, i.e. exchange between two (or more) coordination geometries for the PAC probe. In analogy to NMR spectroscopy, chemical exchange may appear in different time regimes: slow exchange, where the signal from both species is clearly discernible, intermediate exchange where considerable line broadening affects the PAC spectra, or in fast exchange, where a single NQI is recorded, with parameters dictated by the EFGs of the two (or more) coexisting species and their relative population. The time scale on which NMR spectroscopy is sensitive to exchange dynamics is µs – ms, while PAC spectroscopy is inherently sensitive to ns dynamics, dictated by the lifetime of the intermediate level in the nuclear decay. Therefore, the two techniques are often very useful in combination, as will be evident from some of the examples, *vide infra*.

Advantages and limitations originate from the fact that radioisotopes are required in PAC-spectroscopy. It is a highly sensitive nuclear coincidence technique requiring only about 2×10^{10} - 10^{11} radioactive nuclei of the probe element to obtain a good spectrum, thus allowing for experiments at very low concentrations (<nM). Unfortunately, the number of long lived (days) isotopes suitable for PAC being performed at home laboratories is small. A number of short lived (hours or less) radioisotopes exist which exhibit the required nuclear properties suitable for PAC spectroscopy.[1] A list of selected isotopes that have been or might be used in PAC spectroscopy is provided in the supporting information, see Table S1. Experiments with short lived isotopes must be conducted close to a production facility, such as ISOLDE,[33] where a broad spectrum of radioisotopes is produced, or be near a nuclear reactor or a cyclotron properly equipped for production of specific radioisotopes.[1]

B. Applications of PAC Spectroscopy

In this section we present selected examples of applications of PAC spectroscopy with a focus on work from the PAC group in Copenhagen. The interested reader may find other highly interesting work on metal site dynamics in biomolecules in the literature.[1,34-37]

1. Water exchange dynamics at the metal site of de novo designed TRI peptides

Synthetic de novo designed proteins of the TRI family [general sequence Ac-G-(LKALEEK)$_{4-5}$-G-NH_2] have been studied extensively by PAC and NMR spectroscopy.[38-44] They offer a scaffold within which various heavy metal binding sites rich in thiol residues can be designed. In one such peptide, TRIL16C, a metal ion binding site with

three cysteines is formed. ^{111m}Cd PAC spectroscopy was applied to TRIL16C,[42,43] revealing two NQIs which were interpreted as CdS_3 (41 %) and CdS_3O (59 %) coordination geometries (Figure 2A), respectively. In contrast, ^{113}Cd NMR spectroscopy showed only one resonance (Figure 2, left panel part A), implying that the

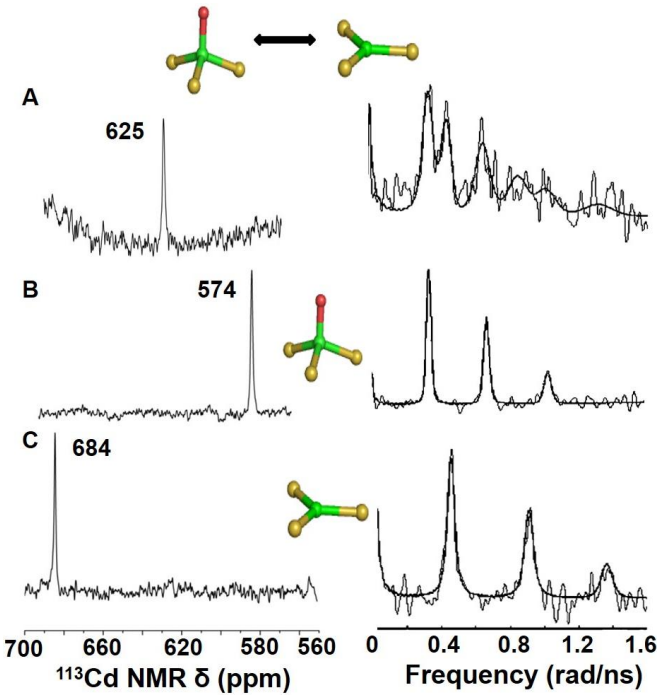

Figure 2. ^{113}Cd NMR (left panel) and ^{111m}Cd PAC (right panel) spectra of A) $Cd(TRIL16C)_3^-$, B) $Cd(TRIL12A16C)_3^-$, and C) $Cd(TRIL16Pen)_3^-$. Reproduced with permission from ref. 42 Copyright 2006 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

two coordination geometries display slow exchange on the PAC time scale and fast exchange in NMR spectroscopy. This example is particularly illustrative, because a further design effort resulted in peptides exhibiting pure CdS_3O (TRIL12AL16C) and pure CdS_3 (TRIL16Pen) coordination geometries, respectively, as determined by PAC spectroscopy (Figure 2 B and C).[42] As expected the ^{113}Cd NMR resonance of the original TRIL16C peptide falls in between the two pure species, and the relative population of the two species derived from the NMR spectra and the PAC spectra agree, as an independent test, supporting the above interpretation of exchange dynamics.

Recently, we extended this work to determine the rate of exchange dynamics of the water bound to Cd^{2+} in TRIL16C, and tested the effect of a remote amino acid substitution (L23A) on the metal site structure and dynamics, comparing the TRIL16C and TRIL16CL23A peptides (Figure 3).[2]

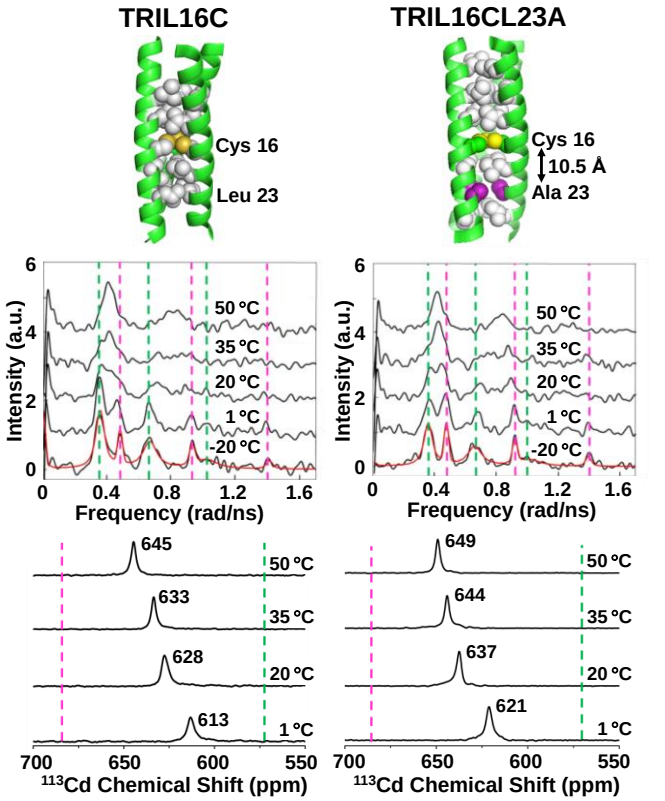

Figure 3. ^{111m}Cd PAC and ^{113}Cd NMR data for the TRIL16C (left column) and TRIL16CL23A (right column) proteins. Reproduced with permission from ref. 2. Copyright 2017 American Chemical Society.

The derived lifetimes of both the CdS_3 and the CdS_3O structures are tens of nanoseconds at room temperature for both the peptides. At the lowest temperature of a temperature series (-20 °C), PAC signals for both the CdS_3 and CdS_3O structures

were clearly discernable (Figure 3 D and E), indicating that H_2O exchange is slow. With an increase in temperature, the PAC signals broadened and at 35 °C coalesced into a single signal, indicating that water exchange was taking place in the intermediate exchange regime. With further increase in temperature to 50 °C the PAC signal again exhibited smaller line width, indicating water exchange approaching the fast exchange regime. Both the equilibrium and kinetic data derived from the PAC spectra suggested that the CdS_3O structure was destabilized in the TRIL16CL23A peptide, as compared to the TRIL16C peptide, indicating that the remote mutation of L23A ~10 Å away from the Cd^{2+} site perturbed the relative association and dissociation rate of water exchange in these two peptides. Observations from the PAC data were corroborated by a ^{113}Cd NMR series (Figure 3), which also showed that with an increase in temperature, the equilibrium population of the CdS_3 structure was stabilized compared to the CdS_3O structure. From the PAC data, the individual NQI for both the peptides were very similar, indicating that the metal site structure was essentially unaltered, even though the water exchange dynamics was perturbed by the remote L23A amino acid mutation.

2. β-lactamase – jumping metal ion between two adjacent metal sites

Metallo-β-lactamases (MBLs) catalyze the hydrolytic cleavage of the C-N bond of β-lactam antibiotics, and constitute one of the most important bacterial defense systems against such drugs. MBLs depend on Zn^{2+} ions for their function, and two adjacent metal ion binding sites have been identified (Figure 4).[45]

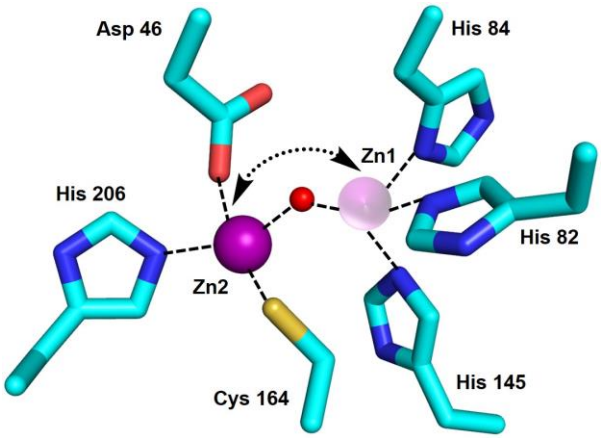

Figure 4. Proposed exchange of active site Zn^{2+} between two metal binding sites on the ns-μs time scale in β-lactamase. The figure is made in PyMOL[46] based on the PDB ID 2BMI.[45]

In a study combining ^{111m}Cd PAC and NMR spectroscopy, the metal ion binding sites in *Bacillus cereus* were investigated.[47] In the presence of less than one equivalent Cd^{2+} per enzyme, only one ^{113}Cd NMR resonance was observed, whereas two different NQIs were identified in the PAC data. This may be rationalized as slow exchange on the PAC time scale and fast exchange on the NMR time scale. Additionally the ^1H-NMR resonances of the imidazole nitrogen protons of both sites were not observed under the same experimental conditions, while they were observed for the species with two Cd^{2+} bound, further supporting an exchange dynamics hypothesis on the time scale from about 100 ns to 10 μs, presumably reflecting that the metal ion "jumps" between the two metal sites on this time scale (Figure 4). Interestingly, EXAFS data for binding of Zn^{2+} to the mononuclear enzyme indicates fractional population of a species with sulfur coordination.[48] This implies, that the dynamics at the metal site may also occur for the native metal ion. Thus, it is likely that these

dynamic features at the metal ion binding site of the resting enzyme reflect an inherent function of dynamics, although it has not yet been demonstrated to occur during catalysis.

3. ^{111}Ag to ^{111}Cd transformation in radioactive decay, and corresponding structural relaxation in azurin and plastocyanin

The electron transporters azurin and plastocyanin are so-called blue copper proteins.[49] It is commonly hypothesized that the coordination geometry is similar in the Cu^{+} and the Cu^{2+}-bound species, and therefore that structural reorganization accompanying the change of oxidation state is small and occurs rapidly (Figure 5).

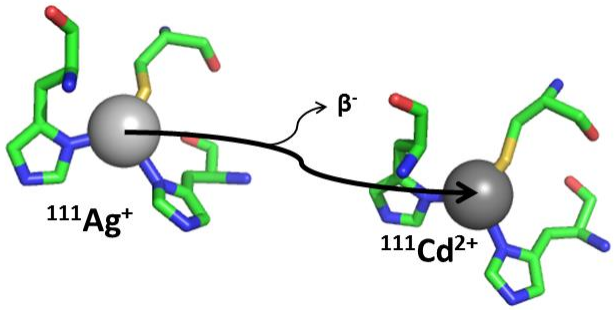

Figure 5. Model of structural re-organization of the metal site in azurin or plastocyanin upon the β^- decay of ^{111}Ag to ^{111}Cd. Immediately after the nuclear decay, the metal site structure is still that of Ag^{+}, and the PAC spectroscopic data indicate that relaxation to the Cd^{2+} coordination geometry occurs on the time scale of ~10-100 ns, see the text for details.[50,51]

Both ^{111}Ag and ^{111m}Cd PAC experiments were conducted for these proteins.[50,51] In the context of protein dynamics, the rate constant for structural relaxation from the Ag^{+} coordination geometry to the Cd^{2+} coordination geometry may be assessed, because the ^{111}Ag radioisotope decays to ^{111}Cd, and the recorded NQI becomes time dependent if the structural relaxation occurs on the ns time scale. For azurin, analysis of two different time windows of the PAC spectrum gave an estimate of the rate of the structural transformation of $(1.1 \pm 0.5)\cdot 10^{7}$ s^{-1} at 1 °C and $(1.8 \pm 0.8)\cdot 10^{7}$ s^{-1} at 25°C. This corresponds to a relatively small energy barrier of about 30 kJ/mol for the relaxation process, supporting the above hypothesis.[50]

Plastocyanin displayed similar behavior, i.e. a change of the NQI during the initial ~200 ns after emission of the first γ-ray.[51] The structural relaxation occurs at rates, which are about a factor of four higher than those derived for azurin at temperatures from 1 °C to 30 °C. The higher rates may reflect a more flexible metal site in plastocyanin than in azurin, a notion that is supported by different line broadening of the PAC spectra from the two proteins, clearly indicating greater structural variability at the metal site of plastocyanin.[51,52] It is also interesting to note that the relaxation from the Ag^{+} to the Cd^{2+} coordination geometry is not observed when plastocyanin is in complex with photosystem I, indicating that the metal site structure is affected by the plastocyanin-PSI interaction, presumably via His87.[51]

The structural reorganization accompanying a radioactive decay involving a transformation from one element to another is often considered a disadvantage of the PAC technique, but in the examples mentioned here, it is turned to an advantage. Notice that most of the other applications presented in this work refer to isomeric decays, where no elemental transmutation occurs.

4. Metal ion transfer by HAH1

HAH1 is a metallochaperone protein that is involved in Cu^{+} trafficking in humans. HAH1 also strongly coordinates Hg^{2+} at a Cys-X-X-Cys motif at the surface of the protein. Characterization of the solution coordination chemistry of Hg^{2+}-bound HAH1 may contribute to the understanding the kinetics and thermodynamics of metal transfer and

exchange between HAH1 and its partner proteins. Therefore, solution behavior of Hg^{2+}-HAH1 was investigated using ^{199m}Hg PAC and ^{199}Hg NMR spectroscopy in the pH range 7.5-9.4.[53] As expected, at pH 7.4 HAH1 formed a monomeric 2-coordinate linear HgS_2 bis-thiolato complex, while at pH 9.4 Hg^{2+}-induced self-association of HAH1 was observed, leading to the formation of a homodimeric HAH1 complex exhibiting what may be a mixture of 3-coordinate HgS_3 and 4-coordinate HgS_4 structures, see Figure 6.

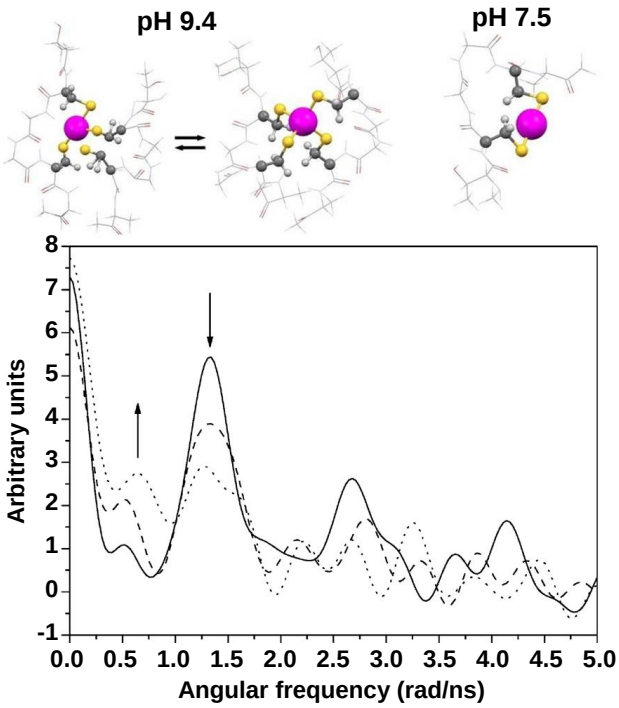

Figure 6: ^{199m}Hg PAC data for the HAH1 protein with 0.5 eq. of Hg^{2+} at different pH values. The arrows indicate spectral changes upon changing pH from 7.5 to 9.4. At pH 7.5 the signature of a HgS_2 coordination geometry is observed. At higher pH a lower frequency signal appears, reflecting higher coordination number in a complex where the metal ion bridges two proteins, possibly co-existing 3- and 4-coordinate Hg^{2+}, representing different steps in the process of transfer of the metal ion from one protein to another.

Exchange was observed between two or more (presumably HgS_3 and HgS_4) species, which was fast on the NMR time scale (ms) but slow to intermediate on the PAC time scale (ns). Thus, various structures expected to be present during metal ion transfer were identified, and the rate of exchange between them was estimated. Furthermore, the results highlight a plausible role of HAH1 in the detoxification of toxic Hg^{2+} in humans. Fast ligand exchange reactions by HAH1 can favor kinetically controlled pathways of metal detoxification overcoming the thermodynamic trapping by metallothioneins. Such kinetic aspects of metal ion regulation controlled by fast ligand exchange reactions are important to understand the molecular bases of detoxification of Hg^{2+}.

5. Exchanging coordination geometries in metal ion sensors

CadC is a metalloregulatory protein belonging to the ArsR/SmtB family of metal sensing proteins. In the absence of Cd^{2+} CadC binds to DNA and presumably suppresses expression of downstream genes responsible for extrusion of Cd^{2+} from cellular environment.[54] To probe the metal site structure of CadC, model systems have been prepared based on a three-helix bundle protein α_3D.[39,55,56] The correlation between ^{113}Cd NMR and ^{111m}Cd PAC data suggested that Cd^{2+} existed as a mixture of two 4-coordinate species CdS_4 and CdS_3O (O = exogenous water molecule) which were in slow exchange on the PAC times scale, but in fast exchange on the ^{113}Cd NMR time scale. It was proposed that similar structures exist and exchange in CadC, see Figure 7, and this model coherently accounts for ^{113}Cd NMR, EXAFS and UV absorption data reported for CadC.[54,56]

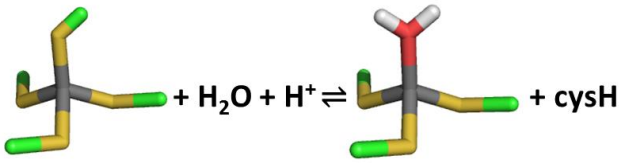

Figure 7. Model of proposed coordination geometries in equilibrium at the Cd^{2+} binding site of CadC, with four cysteines coordinating in one of the species, CdS_4, and upon dissociation of a cysteine and binding of a water molecule a CdS_3O site is formed (cysH is protonated cysteine).

Such rapid ligand exchange phenomena may facilitate the Cd^{2+} transfer from small molecules such as glutathione to CadC. Similarly, a ^{111}Ag PAC study has demonstrated the presence two co-existing species in Ag^{+}-bound BxmR.[57] The ^{111}Ag and ^{111m}Cd PAC spectra recorded for the Ag^{+}- and Cd^{2+}-substituted BxmR, respectively, are different, suggesting that relaxation to the Cd^{2+}-structure was slow and thus did not interfere with observation of the Ag^{+}-bound BxmR structure. Taken together therefore, it seems likely that the rapid exchange in metal ion coordination of these metal-sensing proteins is one way to facilitate the binding and removal of the metal ion from the metal site.

C. Summary and outlook

Structural data on protein metal sites are abundant, but characterization of nanosecond dynamics, how it may be an integral facet of function, and how it is controlled by the protein matrix, are still largely unresolved problems. With the current selection of examples, we hope to contribute to this field, and perhaps the reader will see potential applications of PAC spectroscopy in their own research, or the opportunity to use the data to calibrate and test theoretical methods that appropriately describe metal site dynamics. PAC spectroscopy has so far mainly been applied to characterize structure at the PAC probe site, but since the time window of nanoseconds is exactly where it is sensitive to dynamics, and this appears to be a central time scale to protein metal sites, we hope to break new ground over the coming years in this direction. The major limitation of the technique is availability and production of radioisotopes with appropriate nuclear properties for PAC spectroscopy. In this work we have described experiments conducted with ^{111m}Cd, ^{111}Ag, and ^{199m}Hg and the interested reader is referred to the literature for more comprehensive lists of PAC isotopes.[1,33,58] Although this work describes protein metal sites, the technique is not limited to such systems, and may be extended, for example, to well defined binding of water (or other) molecules to surfaces, and there may additionally be several applications that we have not yet envisaged.

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E. Biographical Information

Saumen Chakraborty is an Assistant Professor at the Chemistry and Biochemistry Department of the University of Mississippi. He earned his Ph.D. in Chemistry from the University of Michigan and postdoctoral trainings at the University of Illinois and the Los Alamos National Laboratory. His research interests are bioinorganic chemistry and nanomaterials.

Stavroula Pallada is conducting her PhD studies (at CERN, University of Copenhagen, and Democritus University of Thrace) on development of nuclear spectroscopic techniques at radioactive ion beam facilities, and applications in chemistry and biochemistry.

Jeppe T. Pedersen has experience within bioinorganic chemistry, and is currently senior research scientist at Novo Nordisk A/S conducting research within formulation of biopharmaceuticals.

Joao G. Correia is a senior Principal Researcher from Instituto Superior Técnico, University of Lisbon. JGC has worked 25 years at ISOLDE-CERN developing and applying nuclear radioactive techniques to material's science (nanoscopic) research.

Attila Jancsó is assistant prof. at the Department of Inorganic and Analytical Chemistry, University of Szeged. Over the past 15 years his research has been focused on metal ion-protein interactions approached via studies of oligopeptide models.

Lars Hemmingsen is assoc. prof. at the Department of Chemistry, University of Copenhagen, with expertise in PAC spectroscopy and quantum chemical calculations of spectroscopic properties, mainly focusing on applications in biochemistry.

F. Supporting Information

Table S1: Isotopes used or with potential use in PAC spectroscopy.

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