

# Chemistry–A European Journal

Supporting Information

## **Molecular Rotational Correlation Times and Nanoviscosity Determined by $^{111\text{m}}\text{Cd}$ Perturbed Angular Correlation (PAC) of $\gamma$ -rays Spectroscopy**

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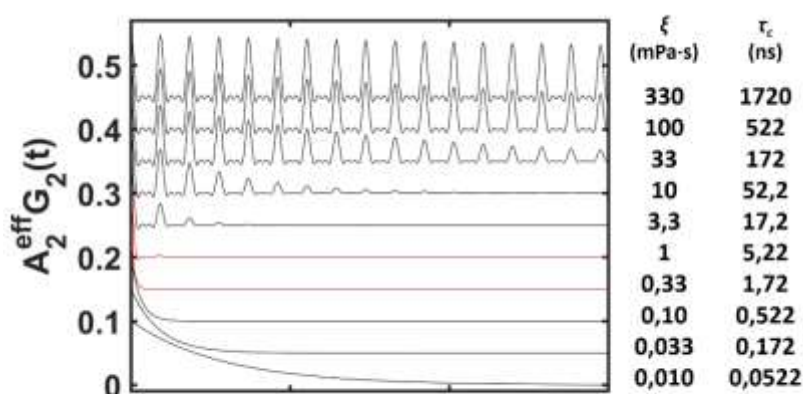
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## Upper and lower limits of measurable rates of dynamics by PAC spectroscopy

Here, we present a more general discussion of the upper and lower limits of measurable rotational correlation times and viscosities using PAC spectroscopy. At the extreme of low damping due to rotational diffusion, i.e., high viscosity, the damping is only measurable if data extending to long times in  $R(t)$ , *vide supra*, are recorded. Note that the error bar on each data point in  $R(t)$  increases as a function of time,  $t$ , see Figure 1A, simply because the decay of the intermediate nuclear level means that long times between the two  $\gamma$ -rays are unlikely events. Figure S1 illustrates the effect of rotational diffusion on simulated  $^{111}\text{mCd}$  PAC spectra, and clearly, the damping is not observable for very viscous solutions ( $\xi$  larger than around 300 mPa·s). Thus, a value of  $\lambda$  on the order of  $1 \mu\text{s}^{-1}$  (or correspondingly  $\tau_c$  on the order of 1000 ns) is approximately the lower limit of what may be determined using  $^{111}\text{mCd}$  PAC. In the current experiments, the Cd<sup>II</sup> binding site exhibits static line broadening with  $\delta$  around 2 %, and this also leads to damping of the PAC signal. PAC isotopes with longer lifetimes of the intermediate nuclear level in the  $\gamma$ - $\gamma$  cascade (such as  $^{204\text{m}}\text{Pb}$ ), may extend the lower limit of measurable  $\lambda$  to about  $0.3 \mu\text{s}^{-1}$ . Note that the lower limit of measurable  $\lambda$  is controlled by the lifetime of the intermediate nuclear level, because this dictates the longest time scale at which an adequate S/N for  $R(t)$  may be achieved.

The upper limit of  $\lambda$ , i.e., at low viscosity, within the slow reorientation time regime, depends on when the condition  $\omega_0\tau_c \gg 1$  (or equivalently  $\omega_0 \gg \lambda$ ) breaks down. With the PAC parameters indicated in the caption of Figure S1, the slow reorientation time regime is limited to  $\lambda$  smaller than about  $100 \mu\text{s}^{-1}$ , corresponding to  $\tau_c$  on the order of 10 ns and  $\xi$  around 2 mPa·s (for a molecule of molecular mass = 10000). In  $^{181}\text{Hf}$  and  $^{199\text{m}}\text{Hg}$  PAC spectroscopy, the recorded NQI frequencies are often higher,<sup>[1-4]</sup> and therefore,  $\lambda$  up to about  $300 \mu\text{s}^{-1}$  may be determined using these radioisotopes. Note that the upper limit of  $\lambda$  is controlled by the measured NQI frequency, because  $\omega_0\tau_c \gg 1$  must be fulfilled within the slow reorientation time regime. Of course, the limit is also controlled by  $\tau_c$ , and thus by the molecular mass of the  $^{111}\text{mCd}$  labelled molecule. Increasing the molecular mass to e.g., 100000 g/mol extends the measurable range to viscosities as low as 0.2 mPa·s, at the expense of loss of sensitivity to high viscosities.

Considerably higher values of  $\lambda$  may be determined in the fast reorientation time regime,  $\omega_0 \ll \lambda$ , as demonstrated by e.g., Danielsen *et al.* and Baudry *et al.* in Ref. [5,6], respectively. In this time regime, i.e., at low viscosity or for small molecules, the PAC signal is an exponentially decaying function, see Figure S1, and the rotational correlation time cannot be determined without prior knowledge of  $\omega_0$  and  $\eta$ , see equation (6). Therefore, to determine  $\lambda$ , the NQI parameters must first be determined in an independent experiment designed so that  $\omega_0 \gg \lambda$ , e.g., in a highly viscous, frozen solution or a precipitate, so that  $\omega_0$  and  $\eta$  can be measured. Then, assuming that the NQI is the same in the slow and in the fast fluctuation regimes, equation (6) may be used to determine  $\lambda$ . With  $\omega_0 = 340 \text{ Mrad/s}$  and  $\eta = 0.16$  (the NQI recorded in this work), the smallest detectable value of  $\tau_c$  is  $\sim 3 \text{ ps}$ , corresponding to an exponent in equation (6) of  $-0.001 \text{ ns}^{-1}$ . This is on the order of the rotational correlation time of a single water molecule in water,<sup>[7]</sup> provided only as a point of reference, since  $^{111}\text{mCd}$  cannot be used as a PAC probe in a water molecule. In this estimate, it was assumed that the exponentially decaying function,  $G_2(t)$ , should change from 1 at  $t=0$  to 0.7 after 500 ns. Note that the exponent scales with  $\omega_0^2\tau_c$ , so a decrease of  $\omega_0$  to e.g., 100 Mrad/s will decrease the largest value of  $\lambda$  that may be determined by a factor of about 10. Clearly, the fast reorientation time regime extends far beyond viscosities relevant to liquids, and even the gas phase with typical viscosities on the order of 0.02 mPa·s may be probed.

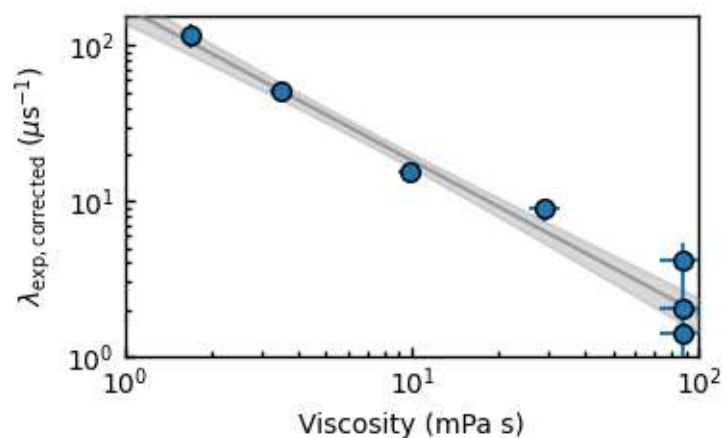


**Figure S1.** Simulated  $^{111}\text{mCd}$  PAC perturbation functions for a 10000 g/mol molecule labelled with  $^{111}\text{mCd}$  in solutions with different viscosities,  $\xi$ , and thus different rotational correlation times,  $\tau_c$ , (indicated on the right). The  $^{111}\text{mCd}$  PAC parameters are idealized based on the measured data for TRIL12AL16C ( $\omega_0 = 341$  Mrad/s,  $\eta = 0$ ,  $A_{\text{eff}} = 0.1$  and  $\delta = 0$ ), to allow for easy visual inspection of the damping.  $\tau_c$  is calculated according to the Stokes-Einstein-Debye approximation, see Table 1, using  $r_h = 3$  Å,  $\rho = 1.37$  g/cm<sup>3</sup>,  $T = 298.15$  K, and the PAC probe site is assumed to be rigid (no intramolecular dynamics). Perturbation functions below the red lines are calculated within the fast molecular reorientation approximation (low viscosity) and exhibit pure exponential decay. Perturbation functions above the red lines the perturbation functions are calculated within the slow molecular reorientation approximation (high viscosity) and exhibit damped oscillations. Perturbation functions indicated in red are in the intermediate time regime, where neither of the two approximations are valid.

## Reproducibility

Three repetitions including individual sample preparations,  $^{111}\text{mCd}$  PAC experiments, and data analysis, see Table 1, were made for the  $^{111}\text{mCd}$  PAC experiment with 55 % sucrose (viscosity of 88 mPa·s), added to give high viscosity, in order to test the reproducibility of the determination of  $\lambda_{\text{exp}}$ . This high viscosity experiment was selected for this test, because it is the most difficult case, both due to 1) minor variations in sample temperature will significantly affect the viscosity, because  $\xi(T)$  varies rapidly with temperature at high viscosity, and 2) the static line broadening,  $\delta \sim 0.02$ , accounts for a significant amount of damping under these conditions, and it is not obvious that the additional damping due to rotational diffusion would be fitted reliably. The values of  $\lambda_{\text{exp}}$  vary from 3.2(4) to 5.9(1.2)  $\mu\text{s}^{-1}$ , i.e., more than the statistical standard deviation derived from fitting data for each individual experiment, but it is encouraging that the variation of  $\lambda_{\text{exp}}$  is still within reasonable limits, even in this difficult case. The observed variation of the other PAC parameters may be due to minor differences in pH and temperature from one experiment to the next.

## Standard curve



**Figure S2.** Standard curve for rate of rotational diffusion versus viscosity as log-log plot  $\lambda_{\text{exp}}$ , corrected is the measured rate of dynamics,  $\lambda_{\text{exp}}$ , from which the rate of intramolecular dynamics, 1.8(5)  $\mu\text{s}$  is subtracted:  $\lambda_{\text{exp,corrected}} = \lambda_{\text{exp}} - 1.8 \mu\text{s}$ . Data points from  $^{111\text{m}}\text{Cd}$  PAC experiments are indicated as blue filled circles, and fit:  $\lambda_{\text{exp,corrected}} = 176\xi^{-0.99}$ . The shaded area corresponds to points within one standard deviation of the fitted line.

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