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Measuring Molecular Exchange for Water in a Yeast Cell Suspension through NMR Diffusometry

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1. Introduction

NMR and diffusion has been used to determine molecular exchange for quite some time. It started with the Kärger model [1] using the echo attenuation of an ordinary pulsed-field-gradient spin echo (PGSE) to fit to a model including exchange between two domains with different diffusion coefficient. It continued with the Diffusion EXchange Spectroscopy (DEXSY) [2], which makes a correclation between two PGSEs with a mixing time inbetween through an inverse Laplace- transformation. Here we present a NMR Diffusometry technique that takes the advantages of the previous methods (the precision, like the Kärger model, is obtained through fitting the data to equations, and the directness to fast determine if exchange is present, in the same way as the DEXSY) but it requires significantly less time and mathematical processing.

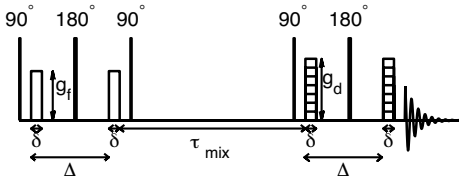

Fig 1: The FEXSY-sequence, containing two PGSEs with a mixing time in between. During the first PGSE the gradient strength used is constant, except for a first step to determine the equilibrium value (see Fig 2), (working as a filter) while for the other the gradient strength is changing (thus working as an indicator of fast and slow diffusion). Here also the different parameters used are indicated.

The new method, Filter EXchange SpectroscopY (FEXSY), is a modification of DEXSY by reducing the number of dimensions and instead taking well known simple equations (see Fig 1 and 2). This makes it possible to reduce the number of data points so that an appropriate amount can be obtained in just over 5 minutes.

This time reduction makes it ideal to measure samples which change over time, for example living cells, or samples that are available for a limited amount of time, for example humans or animals in a clinical environment. In combination with imaging it should be ideal to use in a MRI-scanner to identify diseases that effect the exchange time of the cells.

2. Experimental Results

The method was tried out on a sedimented yeast suspension. For these experiments δ was 5 ms, Δ was 9.1 ms and τ_{mix} was changed logarithmically between 10 and 400 ms. For the times where the filtering part was used g_f was set to 0.48 T/m and g_d was logarithmically spaced between 0.024 and 2.41 T/m.

The sample was measured at different temperatures (all temperatures between 5 and 32°C). The result of this can be seen in Fig 3, which verifies the method through its small error bars.

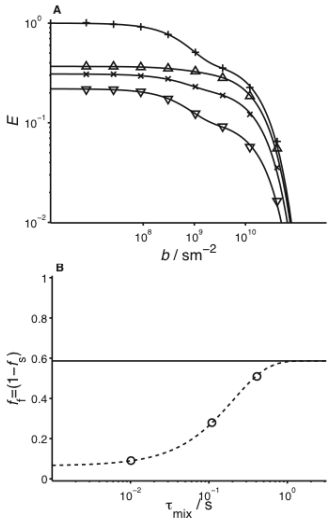

Fig 2: An example of the data obtained with the FEXSY pulse sequence obtained for a sedimented yeast suspension at 30°C. (A) The actual normalized data along with the fitted curves for the normalized echo attenuation $E = f_s(\tau_{mix})*\exp(-bD_s)+(1- f_s(\tau_{mix}))*\exp(-bD_f)$, where $f_s(\tau_{mix})$ is the fraction of slow component (depending on the mixing time τ_{mix} used), b is the echo attenuation factor equal to $(\gamma g_d \delta)^2(\Delta-\delta/3)$ and D_f and D_s are the fast and slow diffusion coefficients measured, respectively. The pluses marks the first step, without any filter gradient and $\tau_{mix}\approx 10$ ms, while the other markers indicate different mixing times using filter gradient, $\tau_{mix}\approx 10$ ms (triangles up), $\tau_{mix}\approx 100$ ms (crosses) and $\tau_{mix}\approx 400$ ms (triangles down). (B) The fitted values for the function $f_s(\tau_{mix})=f_s^e-(f_s^e-f_s^0)*\exp(-k\tau_{mix})$ where f_s^e is the equilibrium value for the slow component (indicated by the solid line), f_s^0 is the value for the slow component $\tau_{mix} = 0$ and k is the exchange rate for intra- and extracellular molecular exchange.

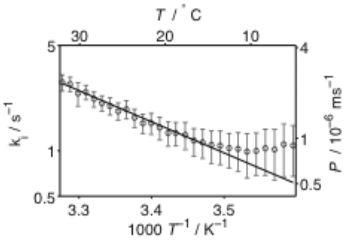

Fig 3: The temperature dependence of the exchange rate, and thus the permeability. k_i, the inverse of the life time for a water molecule to be inside the cells is calculated through $k_i = k/(1+K^{-1})$, where K is the equilibrium constant for the water molecules to pass between the intra- and extarcellular compartment. The permeability, P, is obtained by $P=Rk_i/3$, where R is the radius of the cells (here presumed to be 2.5 μm and independent of temperature).

The circles indicate the fitted exchange times while the solid line indicates the Arrhenius equation with an activation energy of 39±5 kJ/mol.

3. Conclusion

We present a new method to measure molecular exchange using diffusion NMR. Compared to former techniques it is less data and processing intensive and thus less time consuming. The technique was verified by measuring how the exchange time varies with temperature for a sedimented yeast suspension.

References

[1] J. Kärger, Adv. Colloid Interface Sci. 23 (1985) 129-148.
[2] P.T. Callaghan, I. Furó, J. Chem. Phys. 120 (2004) 4032-4038