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Using Diffusion-Diffusion Exchange Spectroscopy to observe diffusion exchange in yeast

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Abstract

The permeability of cell membranes varies significantly across both healthy and diseased tissue, and changes in cell membrane permeability can occur during treatment response in tumours. Measurements of cell membrane permeability could therefore be useful for tumour detection and as biomarkers of treatment response in the clinic. As the diffusion of water across the cell membrane is directly dependent on cell membrane permeability, we have investigated the ability of diffusion-diffusion exchange spectroscopy to quantify the diffusion exchange of water in a suspension of yeast, as a first step towards its application in tumours.

Keywords

DEXSY, diffusion exchange, permeability, double diffusion encoding, 2D-ILT.

1. Introduction

Cell membrane permeability varies in both healthy and diseased tissues and has particular significance in oncology and neurology [1]. We present experimental data showing that diffusion-diffusion exchange spectroscopy (DEXSY) [2] can be used to probe and potentially quantify the exchange of water from intra- to extracellular compartments in a suspension of yeast, and how this changes over time. Our previous work used simulations of nerve tissue to demonstrate that the diffusion exchange of water can be measured using DEXSY, over a wide range of permeabilities, and that measured exchange increased monotonically with permeability [3,4]. In this study we investigate the ability of DEXSY to measure diffusion-exchange of water between the intra- and extracellular space, *in vitro*, in a suspension of yeast. We have used yeast as it is a well-defined and stable model for studying eukaryotic cells *in vitro* [5].

The DEXSY pulse sequence (**Figure 1**) incorporates two pairs of diffusion-encoding gradients, G_1 and G_2. The gradient pairs are separated by a mixing time, t_m, which allows diffusion exchange to occur. Data are processed using a 2D inverse Laplace transform (ILT), the signal from the DEXSY acquisition is ILT transformed to produce a distribution of diffusion coefficients; producing a 2D spectrum in which diffusivities encoded with G_1 are plotted against diffusivities encoded with G_2. Peaks that lie along the diagonal of these diffusion-diffusion exchange spectra represent spins exhibiting the same diffusivity during both sets of diffusion encoding gradients, whereas the off-diagonal peaks represent spins that have exchanged between two different diffusion environments [2, 6]. The signal equation for the DEXSY sequence is:

$$\frac{M}{M_0} = \sum p\left(D_1, D_2\right) e^{\left(-b_1 D_1\right)} e^{\left(-b_2 D_2\right)}$$

where subscripts correspond to parameters associated with either G_1 or G_2. The summation is across all spins in the system. For a pair of gradient pulses, $b = \gamma^2 \delta^2 G^2 (\Delta - \delta/3)$, where γ is the gyromagnetic ratio, G is the gradient strength, δ is the duration of the diffusion encoding gradient, Δ is the diffusion time; D is the distribution of diffusivities, M is the acquired signal M_0 is the signal acquired with no diffusion encoding gradients; p is the probability of the contribution to the signals from D_1 and D_2 [6].
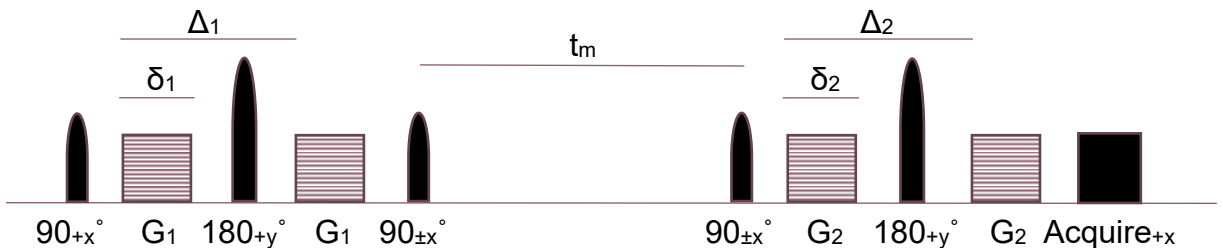

Figure 1: The DEXSY pulse sequence, imaging gradients not shown.

2. Methods

Our experimental data were acquired from a 9.4 T, 20 cm horizontal bore Varian MRI scanner. The sample consisted of a 15 ml falcon tube containing a mixture of 22 g l'hirondelle cake yeast in 10 ml of PBS (kept at approximately $22°$ C). The sample was agitated to ensure an even suspension at the start of the experiment.

A slice-selective DEXSY sequence was created by adding slice selective imaging gradients to the basic DEXSY pulse sequence shown in figure 1 and was used with the following parameters: number of averages = 4, δ = 15 ms, Δ = 17 ms, t_m = 200 ms, TR = 3000 ms, G_1 & G_2 = 0-720 mT/m in 16x16 uniformly-spaced steps. The measurement was repeated three times, with a duration of 102 minutes for each acquisition.

2D ILT software was kindly provided by Petrik Galvosas [7] and used to generate diffusion-diffusion exchange spectra from the raw data and the applied b values. The contribution due to imaging gradients was incorporated into the calculation of b values. The Diffusion Exchange Index (DEI) was defined as the ratio of exchange peaks to non-exchange peaks and was used as a semi-quantitative measure of exchange.

3. Results

Diffusion-diffusion exchange spectra are shown in **Figure 2a.** Peaks on the identity line (diagonal peaks), C & D, correspond to diffusing spins that remained within the same diffusion environment during both sets of diffusion encoding gradients, whilst off-diagonal peaks, A & B, correspond to diffusion exchange between environments. As intra- and extra-cellular compartments have different diffusivities [8], off-diagonal peaks were interpreted as evidence of exchange between intra- and extra-cellular compartments.

Diagonal peaks corresponding to spins remaining in the intracellular or extracellular compartments, were assigned based on their measured diffusivity (intracellular, $(0.032\pm0.006)\times10^{-9}$ m^2/s labelled as C; extracellular, $(1.0\pm3.0)\times10^{-9}$ m^2/s labelled as D). Both diagonal peaks were evident at all three time points (t=0, 102 and 204 minutes). Interestingly, intracellular diffusion peaks appeared to be split, which could correspond to vacuole and cytoplasm compartments [9]. A peak is also evident in the lower left corner of diffusion-diffusion spectra, which could correspond to a dot compartment representing the nucleus [8] or it could be an artifact of the inverse Laplace transform.

Two exchange (off-diagonal) peaks, labelled as A and B in **Figure 2a** were observed at the first two time points, but only one (B) was evident at t=204 minutes. Peak A corresponds to exchange from extra- to intracellular compartments; peak B corresponds to exchange from intra- to extracellular compartments. **Figure 2b** shows measurements of peak areas, which reveals changes with time. Peak A rapidly decreases with time, whilst B remains relatively stable; Peak C remains relatively constant whereas D decreases gradually with time. The apparent position of Peak A moves with time, this is probably due to a change in the rate of diffusion exchange between the two compartments over time, affecting the ILT [2].

The changes in the diffusion-diffusion exchange spectra observed over time in **Figure 2a** were reflected in DEI measurements, which were 0.0461±0.0006, 0.0371±0.0007 and 0.0303±0.00006 at t=0,102 and 204 minutes, respectively, indicating decreasing exchange with time (see **Figure 2c**). The cause of these changes is unclear but could be due to degradation of the sample
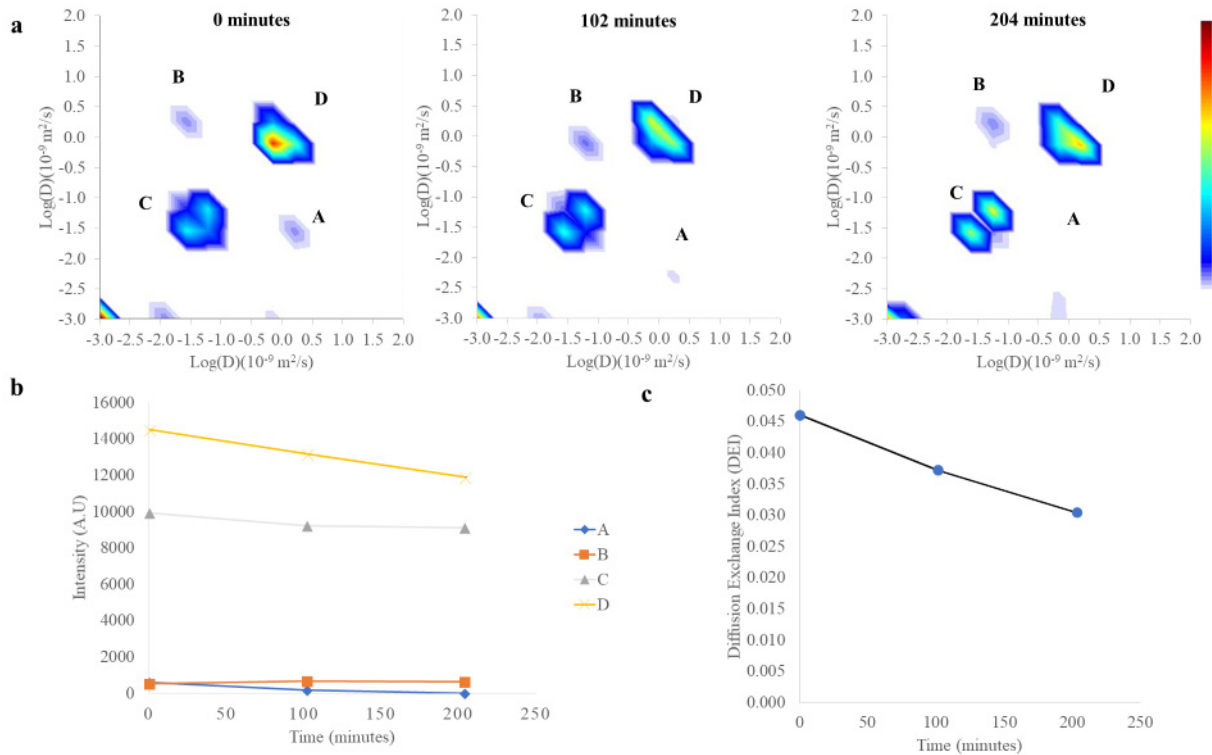
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Figure 2. a) Diffusion-diffusion spectra for the yeast suspension, at 0, 102 and 204 minutes into the experiment. Colour corresponds to the magnitude of the data (arb. units), which is scaled uniformly across the spectra. b) A graph showing the intensity of each peak assigned in (a) with time. c) A graph showing DEI (a measure of diffusion exchange) in the yeast suspension with time.

4. Conclusion

We have shown here that the diffusion exchange of water between intra- and extra-cellular compartments in yeast can be observed *in vitro* using DEXSY. Our results are in agreement with our previous *in silico* experiments, using simulated nerve tissue. Interestingly, our measurements of DEI indicated that exchange decreased with time during the experiment, presumably due to degradation of the sample resulting either from physiological changes in the yeast, sedimentation, or an as-yet undefined source of variation. Future work will aim to further investigate these changes. We also aim to establish whether DEXSY can be used to quantify and measure changes in diffusion exchange *in vivo*. Acknowledgements:

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