Supporting Information

A Method for Estimation of Plasma Protein Binding Using Diffusion Ordered NMR Spectroscopy (DOSY)

Rachel Taylor,[1] Thomas Swift,[2] David Wilkinson,[3] and Kamyar Afarinkia[1,4]*

Correspondence to: kamyar.afarinkia@uwl.ac.uk

[1] *Institute of Cancer Therapeutics, University of Bradford, Richmond Road, BD7 1DP, United Kingdom.*

[2] *Polymer and Biomaterials Laboratories, School of Chemistry and Bioscience, University of Bradford, Richmond Road, BD7 1DP, United Kingdom.*

[3] *Labcorp Drug Development, Otley Rd, Harrogate, HG3 1PY, United Kingdom.*

[4] *School of Biomedical Sciences, University of West London, Saint Mary's Road, London W5 5RF, United Kingdom.*

Setup of the Dosy Experiment

The DOSY spectra were collected using a Bruker Spectrospin 400 Ultrashield NMR spectrophotometer operating at 400 MHz, with samples maintained at 298.2 K via a condensed gas feed (400 lph). ^{1}H DOSY NMR measurements were set up with 16 gradient scans with 16 N repeat samples across each gradient. All measurements were initiated using Bruker TopSpin (version 3.7) with IconNMR automation. The Bruker pulse sequence ledbpgp2s (LED with bipolar gradient pulse pair, 2 spoil gradients) without modification (**Figure S1**) was used to acquire date.

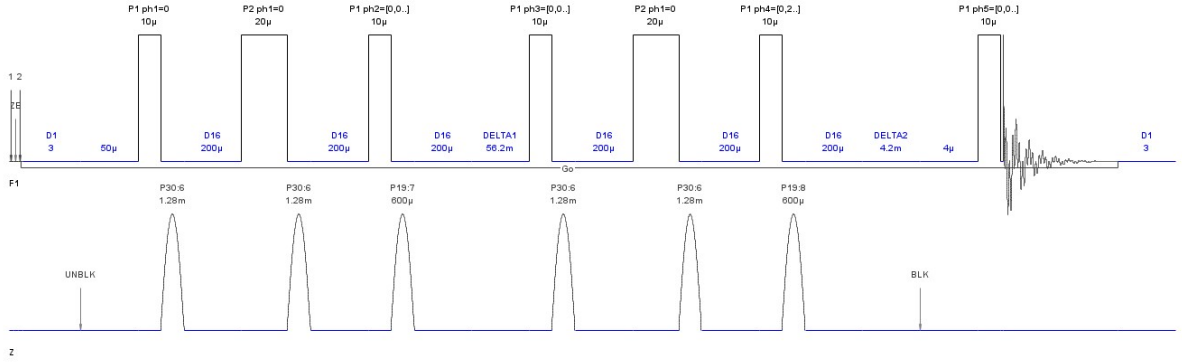

Figure S1: Pulse sequence ledbpgp2s used in DOSY experiments

*Reference: Wu, D.H.; Chen, A.D.; Johnson, C.S. (**1995**) "An Improved Diffusion-Ordered Spectroscopy Experiment Incorporating Bipolar-Gradient Pulses" J. Magn. Reson. Ser. A 115(2), 260-264.*

Full Summary of Diffusion NMR experiments and Exemplar Raw Data

^{1}H NMR of Caffeine, BSA and mixtures thereof produces a variety of peaks visible between 1 and 8 ppm which were of relevance to the study as shown in **Figure S2**.

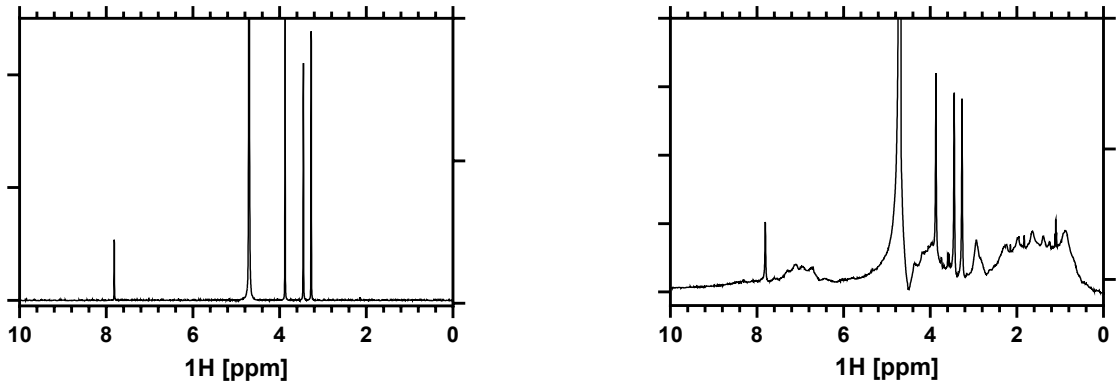

Figure S2 – ^{1}H NMR of pure Caffeine (left) and a Caffeine : BSA 3 :1 ratio (right)

BSA, Caffeine, and mixtures thereof were analysed by DOSY NMR and found to diffuse at a rate of 6.54 x 10^{-11} m^2 /S and 6.19 x 10^{-10} m^2 /S respectively (fitting to peaks δ 1.63 and δ 3.87 with 16 gradient steps as shown in **Figures S3**, **S4** and **S5**).

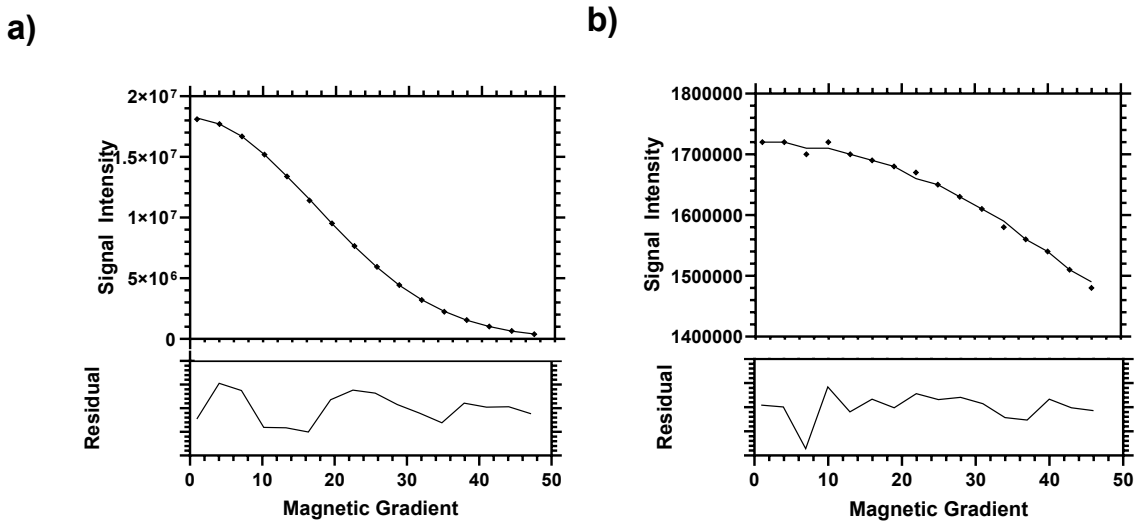

Figure S3 – Diffusion Decay of Pure Compounds. A) Caffeine: Diffusion decay (peaks = raw data, line = applied fit and std. deviation residuals below) of pure Caffeine in D_2O δ 3.87 pm. γ 26752 rad/(s*Gauss), δ 0.0015800 s and Δ 0.059900 s. Calculated D = 6.19E-10 ± 3.010e-12 m^2/s. b) BSA: Diffusion decay (peaks = raw data, line = applied fit and std. deviation residuals below) of pure BSA in D_2O δ 1.63 pm. γ 26752 rad/(s*Gauss), δ 0.0015800 s and Δ 0.059900 s. Calculated D = 6.54E-11 ± 2.846e-12 m^2/s

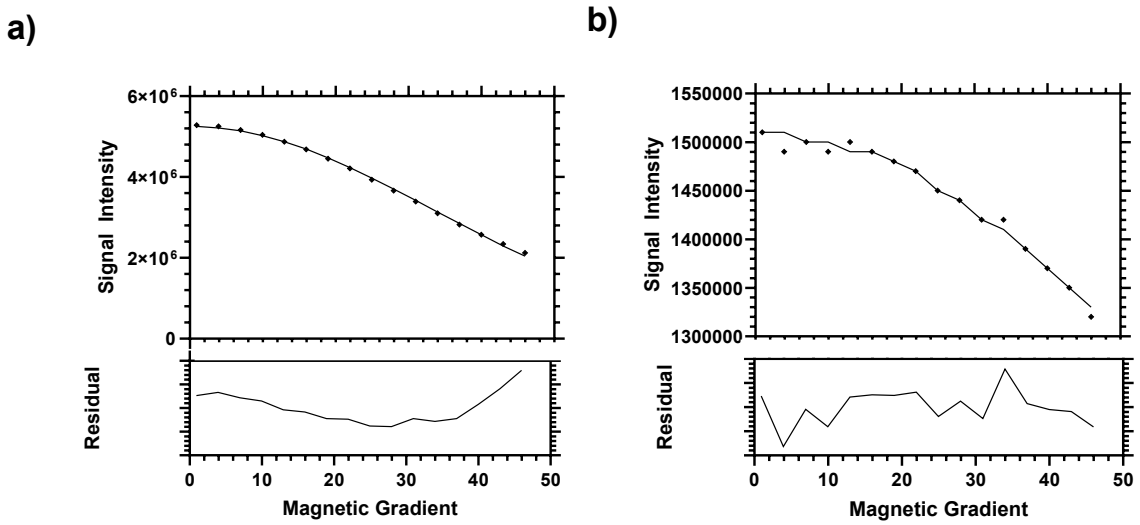

Figure S4 – Diffusion Decay of Caffeine and BSA in a 3 : 1 Ratio. A) Caffeine: Diffusion decay (peaks = raw data, line = applied fit and std. deviation residuals below) of Caffeine in 3 :1 BSA ratio δ 3.87 pm. γ 26752 rad/(s*Gauss), δ 0.0015800 s and Δ 0.059900 s. Calculated D = 5.25E-10 ± 8.416e-12 m^2/s. b) BSA: Diffusion decay (peaks = raw data, line = applied fit and std. deviation residuals below) of BSA in 3 :1 mixture δ 1.64 pm. γ 26752 rad/(s*Gauss), δ 0.0015800 s and Δ 0.059900 s. Calculated D = 5.62E-11 ±4.114e-12 m^2/s

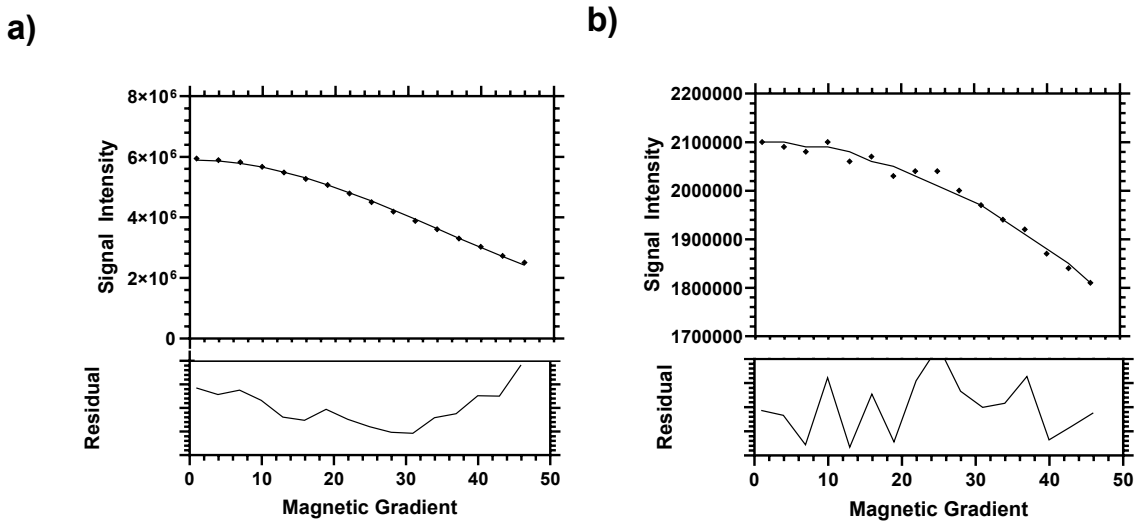

Figure S5 – Diffusion Decay of Caffeine and BSA in a 10 : 1 Ratio. A) Caffeine: Diffusion decay (peaks = raw data, line = applied fit and std. deviation residuals below) of Caffeine in 10 :1 BSA ratio δ 3.87 pm. γ 26752 rad/(s*Gauss), δ 0.0015800 s and Δ 0.059900 s. Calculated D = 4.08E-10 ± 8.865e-12 m^2/s. b) BSA: Diffusion decay (peaks = raw data, line = applied fit and std. deviation residuals below) of BSA in 10 :1 mixture δ 1.64 pm. γ 26752 rad/(s*Gauss), δ 0.0015800 s and Δ 0.059900 s. Calculated D = 6.69E-11 ± 4.9633e-12 m^2/s