

¹H Concentrations

Buffer

The buffer was prepared from d₈-glycerol and D₂O at a ratio of 70/30 (v/v). The ¹H concentration in the medium was calculated to be 0.8 M assuming 99 % of deuteration for d₈-Glycerol and 99.9 % for D₂O.

Flavodoxin

From Watt et al., the molecular volume for Flavodoxin is 18,200 Å³ (oxidized, room temperature)³. Using a molecular volume of 18.2 nm³ per molecule and a total of 969 protons per molecule a ¹H concentration of 88 M can be calculated (969/(18.2e-24 * N_A)).

At 85 % deuteration the ¹H concentration is reduced to 13 M

Average ¹H-¹H distances

Based on the knowledge of the ¹H concentration an average distance between ¹H nuclei can be calculated assuming a body-centered cubic packing:

$$r = \frac{\sqrt{3}}{2} \sqrt[3]{\frac{2}{N_A c}}$$

with r the distance, c the concentration and N_A the Avogadro number. The following distances were calculated:

Buffer	(0.8 M)	$r = 1.4$ nm
¹ H-FD	(88 M)	$r = 0.29$ nm
² H-FD	(13 M)	$r = 0.55$ nm

¹H-to-SQ and ¹H-to-Surface Distances in Flavodoxin

To calculate distances between protons in FD and the SQ radical, and distances between protons and the nearest TOTAPOL molecule, we took advantage of the high structural homology among flavodoxins from *D. vulgaris* (coordinate set 4FX2.pdb³) and *E. coli* (coordinate set 2HNB.pdb⁴). The former is a crystal structure providing locations for water molecules surrounding the FD molecule, whereas the latter is an NMR structure providing coordinates for all the ¹H atoms in FD. The two structures differ slightly with respect to the structure of surface loops but nonetheless share backbone rmsd of 1.36 Å (Figure S4A). This is adequate for our purpose, to simply estimate the distances of closest approach between the ¹H we observed, and each of SQ- or external TOTAPOL.

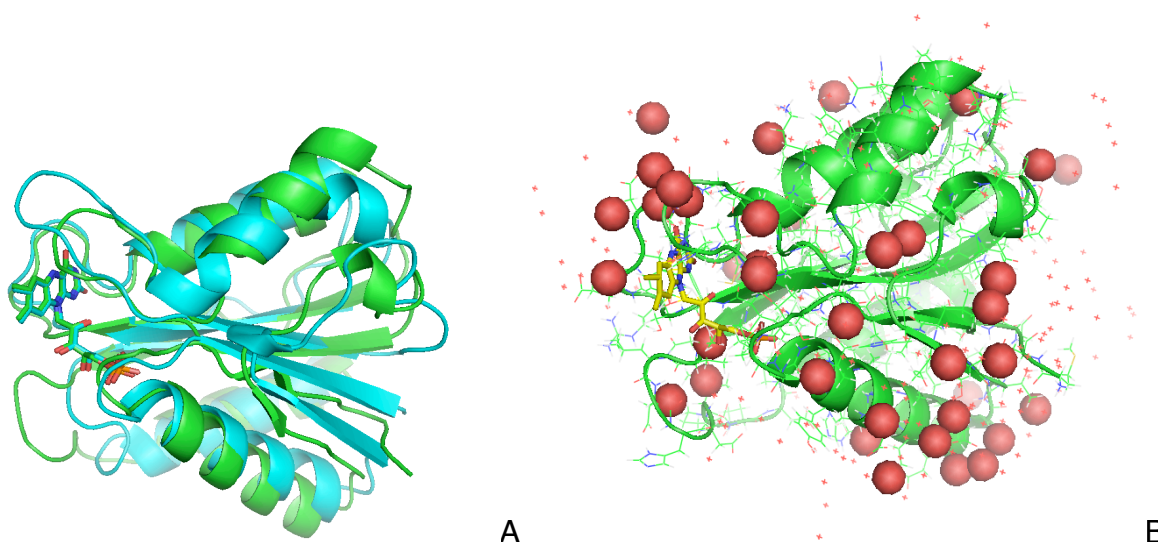


Figure S4: **A:** overlay of the FD structure from *D. vulgaris* (aqua) and the MioC FD from *E. coli* (green) based on coordinate files 4FX2.pdb³ and 2HNB.pdb model 21⁴, respectively. **B:** locations of water molecules surrounding FD 4FX2.pdb shown with the ribbon structure of model 21 of 2HNB.pdb after overlaying the latter on the 4FX2 structure (A). Water molecules with occupancies ≥ 0.8 are shown as red spheres and other water molecules as small red crosses. Figures were generated with Pymol⁵.

The coordinates of model 21 of 2HNB.pdb were overlain on those of 4FX2.pdb using the 'fit' tools in Swiss PdbViewer (<http://www.expasy.org/spdbv/>). The position of

the flavin was approximated to be the centre of the central ring, determined by calculating the average of the coordinates of the six atoms of the central ring: C4a, N5, C5a, C9a, N10 C10a. To estimate the distribution of distances to FD's surface, all water molecules with occupancies of ≥ 0.8 from the 4FX2.pdb coordinate set were added to the 2HNB2 coordinate set produced by overlaying the two FDs, as the coordinates of their O atoms (red spheres in **Figure S4B**). Because some patches of the FD surface do not have high-occupancy waters, our criterion could produce longer-than-actual distances to the nearest H₂O, but such an error would tend to reproduce the longer distance of closest approach expected of a larger molecule such as TOTAPOL.

For each FD ¹H atom the distance to the center of the flavin and the distance to each of the high-occupancy water molecules was calculated. For each ¹H, the minimum distance to a water molecule was determined and rounded to the nearest integer, as was the distance to the flavin center. The number of protons at a given distance was plotted vs. the distance, to produce the distance distribution given in **Figure 6** for each of distance to the centre of the flavin and distance to the closest high-occupancy water. The average distance to the closest high-occupancy water is 6.3 Å, the standard deviation of the distribution is 2.5 Å, and the mode of the distribution is 5 Å. The average distance to the centre of the flavin is 20.7 Å, the standard deviation of this distribution is 7.9, Å, and the mode of this distribution is 26 Å. Data from a total of 1081 ¹Hs are presented.

Figure S5:

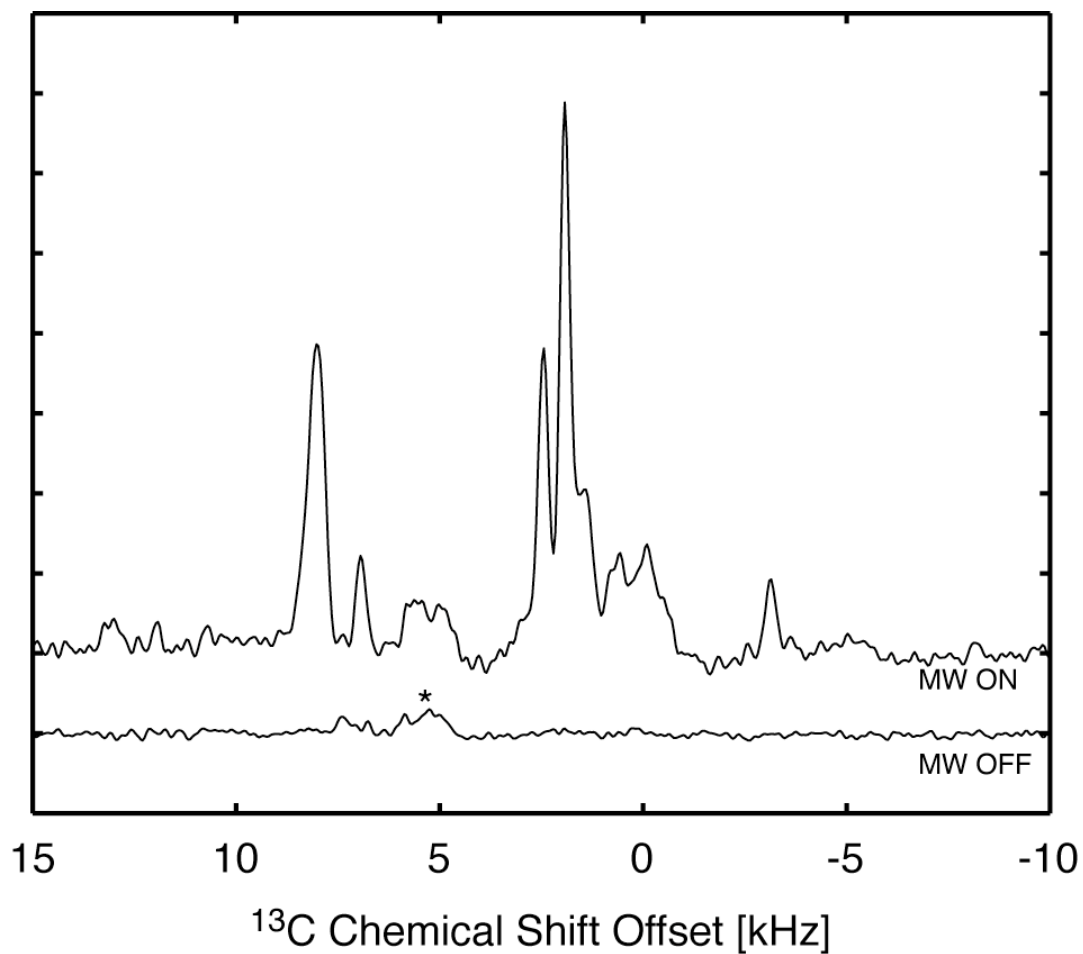


Figure S5: ^1H -DNP-enhanced ^{13}C spectrum of $\text{U-}^{13}\text{C}$ FD using the exogenous polarizing agent TOTAPOL. The estimated enhancement is $\epsilon > 100$. The signals observed in the MW OFF spectrum are ^{13}C impurities from the stator. The ^1H polarization is transferred to ^{13}C nuclei using a 0.7 ms cross-polarization step. Acquisition of the ^{13}C signal is performed under ^1H TPPM decoupling. The microwave polarization time was 20s and the spinning frequency was set to 5 kHz. Sample temperature: 90 K.

Figure S6: Comparison of buildup curves obtained via ^1H vs. ^{13}C detection

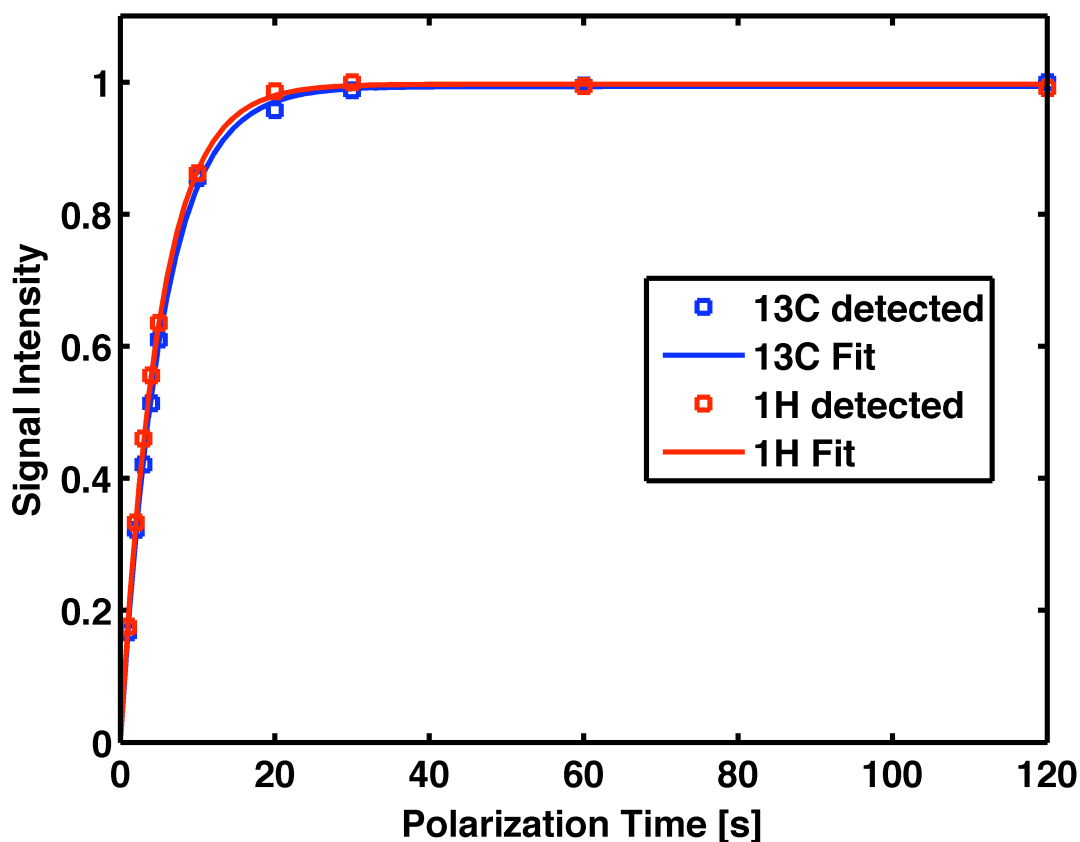


Figure S6: Comparison of direct ^1H detection and indirect ^1H detection through a CP step to ^{13}C . Sample was $[^2\text{H}, ^{13}\text{C}]$ -FD and the polarization agent was 10 mM TOTAPOL.

Both traces can be fitted using a mono-exponential recovery function of the form $y(t) = 1 - \exp(-t/\tau_B)$. Data shown here are already scaled by the steady state enhancement to facilitate visual comparison of the buildup kinetics. For both detection methods a build-up time of 5 s was determined.

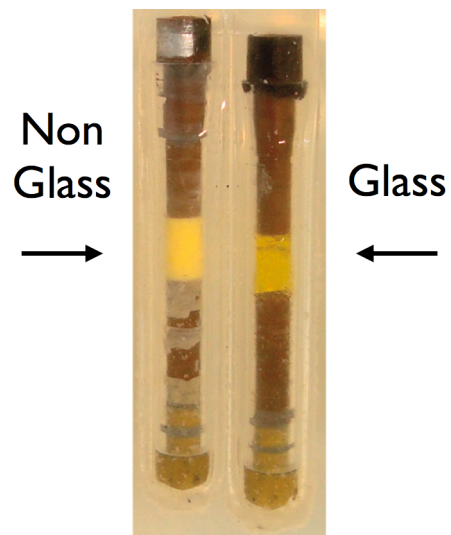


Figure S7: Comparison of a glassy sample (70% ^2H -glycerol: 30% $^2\text{H}_2\text{O}$ v:v) and a non-glass sample (12% ^2H -glycerol: 88% $^2\text{H}_2\text{O}$ v:v). Both contain 2 mM oxidized ^1H -FD and 10 mM TOTAPOL.

References for supplementary material

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