Proton-Detected 2D Radio Frequency Driven Recoupling Solid-state NMR Studies on Micelle-associated Cytochrome-b_5

Manoj Kumar Pandey[1], Subramanian Vivekanandan[1], Kazutoshi Yamamoto[1], Sangchoul Im[2], Lucy Waskell[2] and Ayyalusamy Ramamoorthy[*1]

[1]Biophysics and Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109-1055

[2]Department of Anesthesiology, University of Michigan and VA Medical Center, Ann Arbor, Michigan 48105

[*]To whom correspondence should be addressed (ramamoor@umich.edu)

Figure S1. Two-dimensional ^{1}H-^{1}H RFDR and NOESY pulse sequences used in this study.

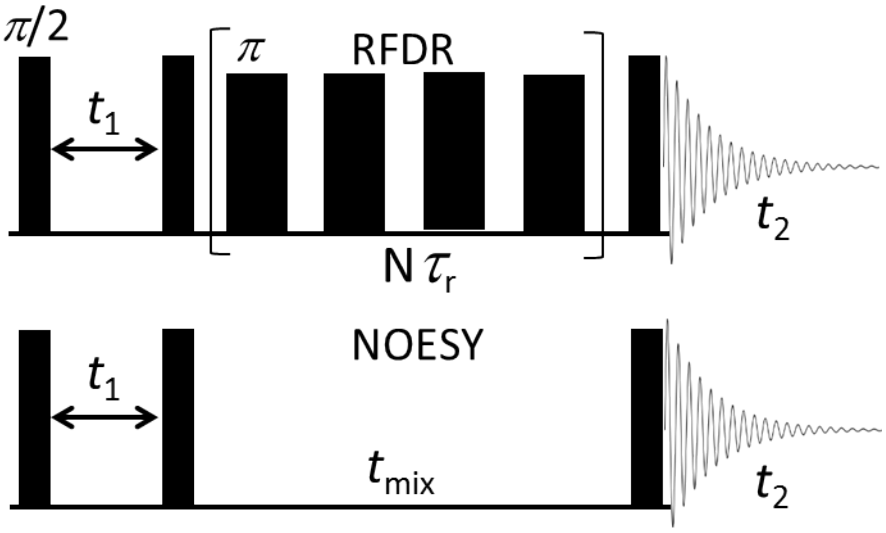

Figure S2. Superimposed 2D ^{1}H-^{1}H RFDR spectra of cytb$_5$ in the presence (black) and absence (blue) of DPC micelles recorded at mixing times of 25, 50, 100, 150, 200 and 300 ms, showing the alpha-side chain ^{1}H-^{1}H chemical shift correlations. More cross-peaks are observed in the presence of DPC micelles. This observation indicates the presence of unaveraged ^{1}H-^{1}H dipolar couplings in cytb$_5$ due to its interaction with DPC micelles that restricts the isotropic motion of the protein.

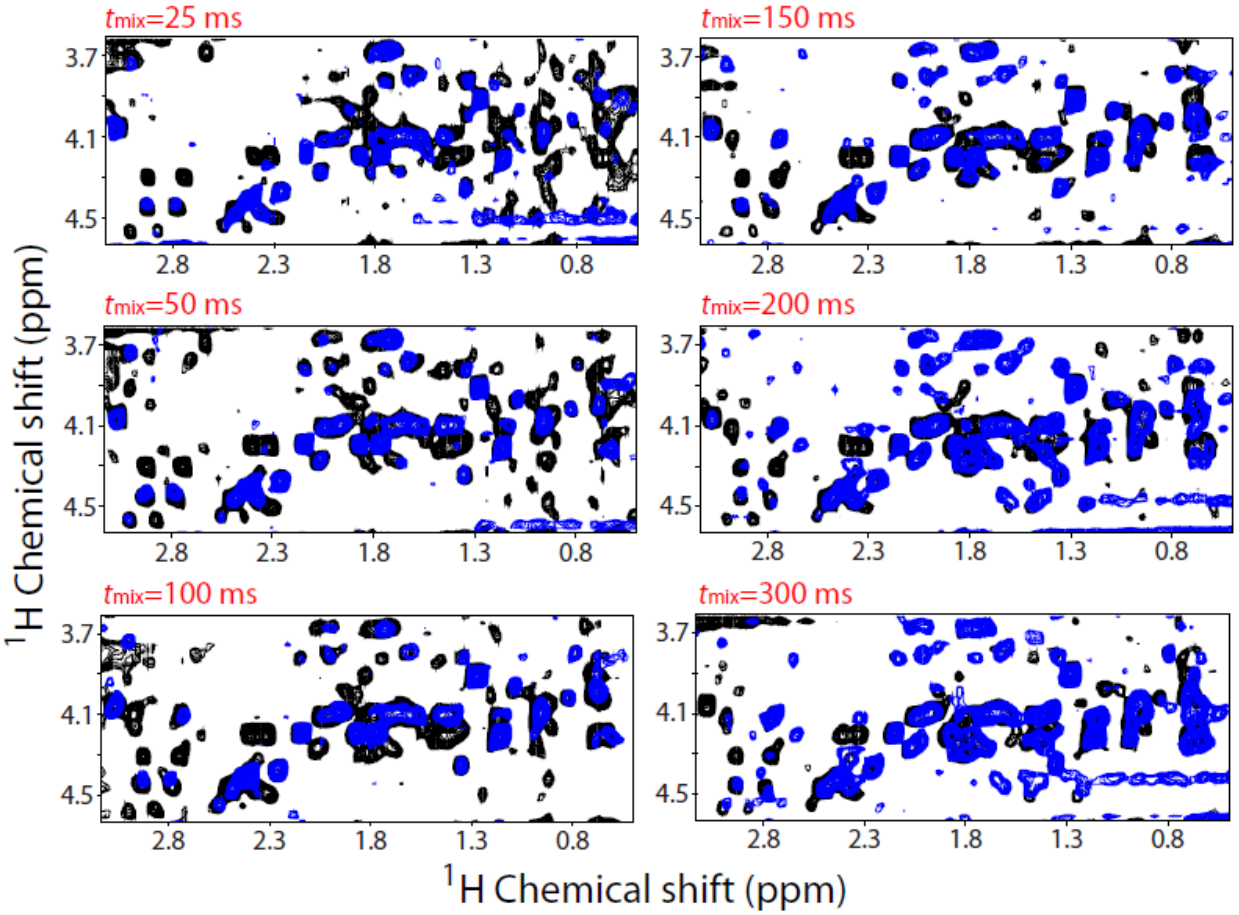

Figure S3. Superimposed 2D ^{1}H-^{1}H NOESY (red) and RFDR (black) spectra of cytb$_5$ incorporated in DPC micelles recorded at mixing times of 25, 50, 100, 150, 200 and 300 ms, showing the chemical shift correlation of aromatic protons.

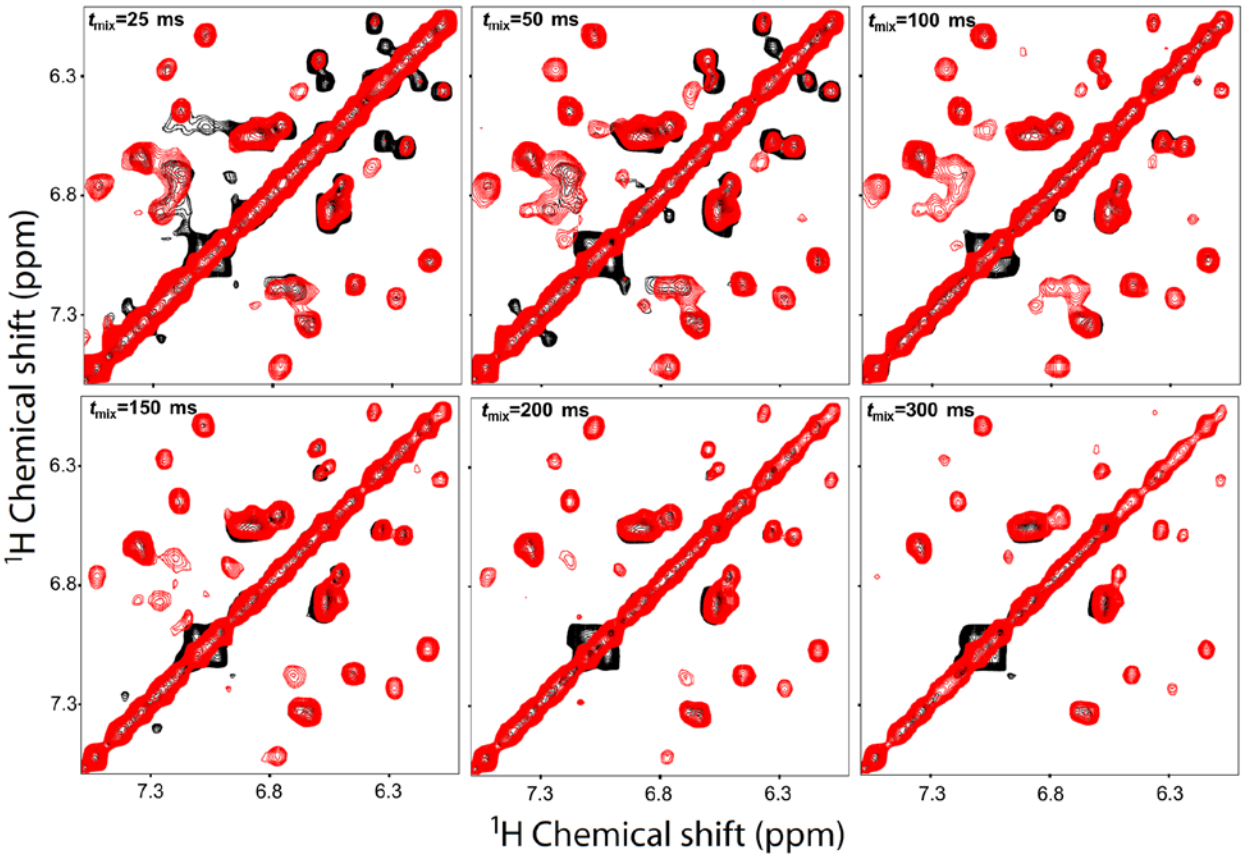

Table S1. Relaxation parameters obtained from the simulation of experimentally measured cross-peak intensities of $cytb_5$ in the absence of DPC micelles.

Residues	RFDR		
	1/T1* (s^{-1})	R (s^{-1})	I_0
$K39H_{\varepsilon}$-H_{β}	1	7	15
$R52H_{\delta}$–$Q54H_{\gamma}$	0.5	11	4
$M96H_{\alpha}$-$H_{\beta 1}$	4.7	5	18
$E53H_{\alpha}$-$H_{\gamma 2}$	1.5	7	3
$D71H_{\alpha}$-$H_{\beta 2}$	2.5	7	10
$D6H_{\alpha}$-$H_{\beta 1}$	2.5	7	10