Magic-Angle Spinning Solid-State NMR Spectroscopy of Nanodisc–Embedded Human CYP3A4†

Supporting Information

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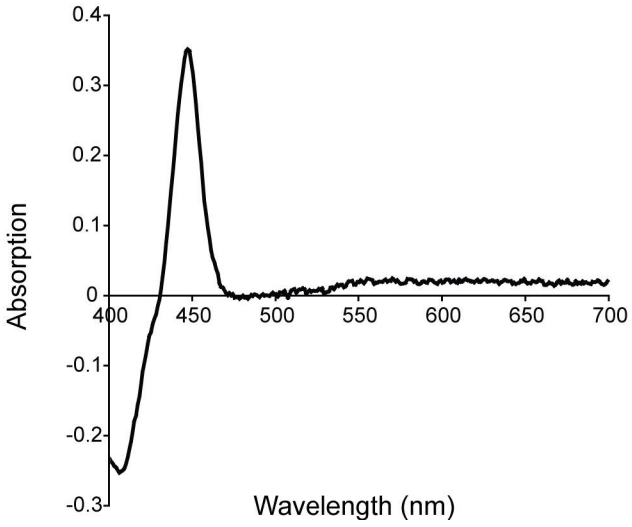

Figure S1. CO difference spectrum of purified ^{13}C, ^{15}N labeled CYP3A4. The spectra were acquired using a Cary Bio 300 UV-Vis spectrophotometer (Varian, Lake Forest, CA) in dual-beam mode. A spectrum was acquired on a sealed cuvette containing 800 μl of deoxygenated 15 mM potassium phosphate buffer with dithionite and 200 μl purified CYP3A4. To this, 400 μl of a dithionite containing, CO saturated deoxygenated 15 mM potassium phosphate buffer was added, and another spectrum was acquired. The spectra were corrected for the difference in concentration, and the first spectrum was subtracted from the second one to yield presented CO difference spectrum. All of the purified CYP3A4 is in 450 form.

Integrity of Precipitated CYP3A4 in Nanodiscs.

To confirm that CYP3A4 remained active in the precipitated Nanodiscs, sample of CYP3A4 in Nanodiscs identical to that on which SSNMR data were acquired was prepared and concentrated to ~15 μM. To this sample bromocriptine was added in excess causing a high-spin shift (Figure S2A), and the sample was then mixed with equal volume of 40% PEG 3350, and incubated overnight at 4°C. The sample was spun down, and the supernatant was removed. The pellet was then placed on a thin layer (0.1 mm) quartz cuvette, and a spectrum was acquired using a Cary Bio 300 UV-Vis spectrophotometer (Varian, Lake Forest, CA) in dual-beam mode. Although we have been able to test the integrity of resuspended CYP3A4 Nanodisc containing 20% glycerol (Figure 1, main text), it was necessary to confirm that the pellet of the samples with and without glycerol was indeed functionally identical. Thus, we also analyzed the pellet of bromocriptine-CYP3A4 Nanodiscs with 20% glycerol (Figure 2SD), confirming that the two samples are the same.

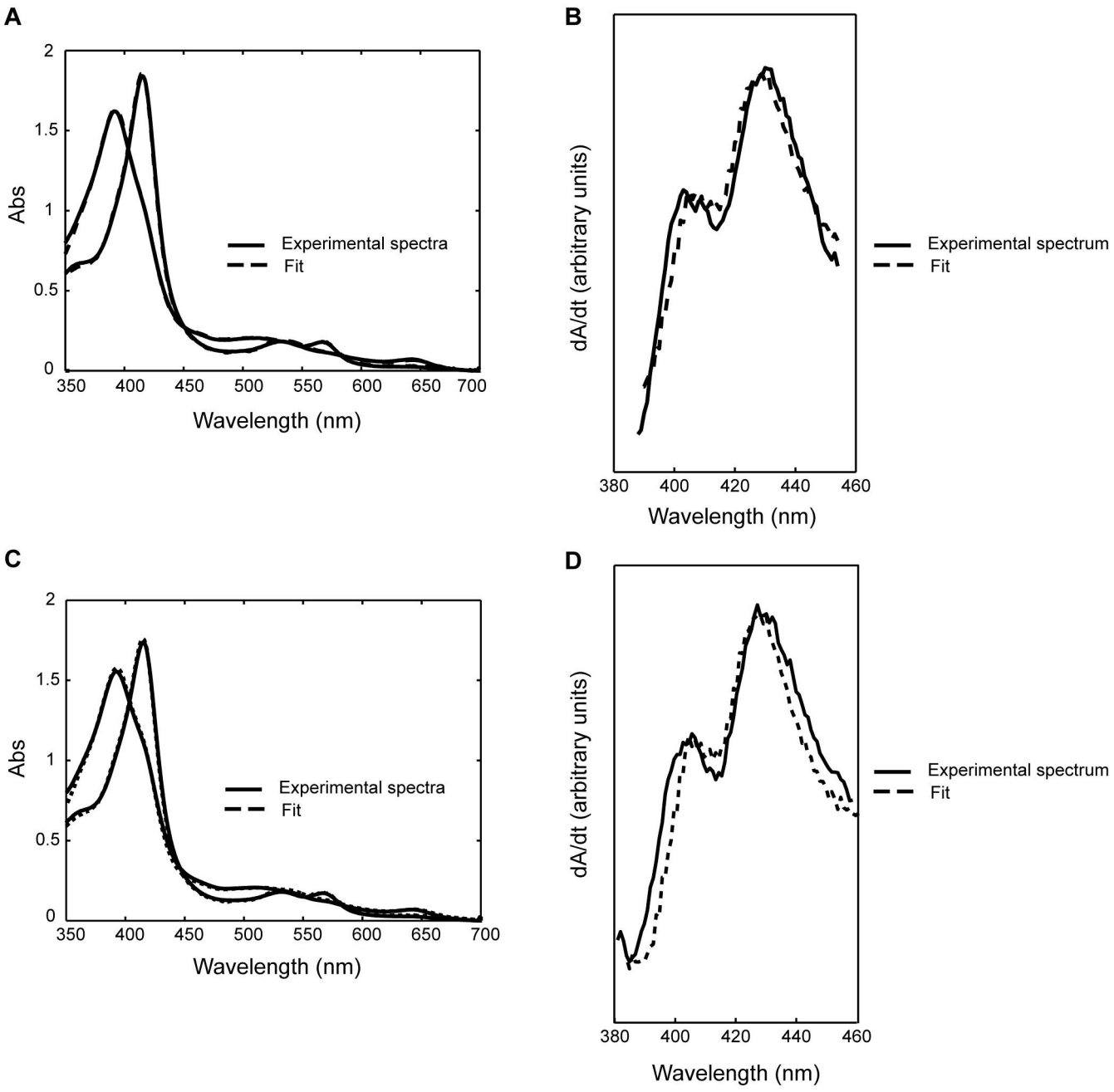

Figure S2. A) Low to high-spin shift of CYP3A4 in Nanodiscs due to bromocriptine binding (full line), and linear combinations of two reference spectra corresponding to pure high-spin and low-spin states (dashed line). B) First derivative of the bromocriptine-bound CYP3A4 Nanodisc pellet. C) and D) Same as A) and B), respectively, of a sample containing 20% glycerol. Due to

the nature of the sample, the spectrum had high scattering. The first derivative of the experimental spectrum (Figure S2B, full line) was fitted with the linear combination of the first derivatives of two reference spectra corresponding to the pure high-spin and low-spin CYP3A4. The result of this fit is shown in Figure S2B (dashed line), with the calculated fraction of high-spin ~ 75%. Similar fit of the experimental spectrum (Figure S1A, dashed line) prior to precipitation indicated ~90% high-spin shift. The high percentage of high-spin, caused by bromocriptine binding to CYP3A4 directly indicates that CYP3A4 is still active in the precipitated state. The same analysis was done on a sample containing 20% glycerol (Figure S2 C and D), concluding that samples with and without 20% glycerol are the same.

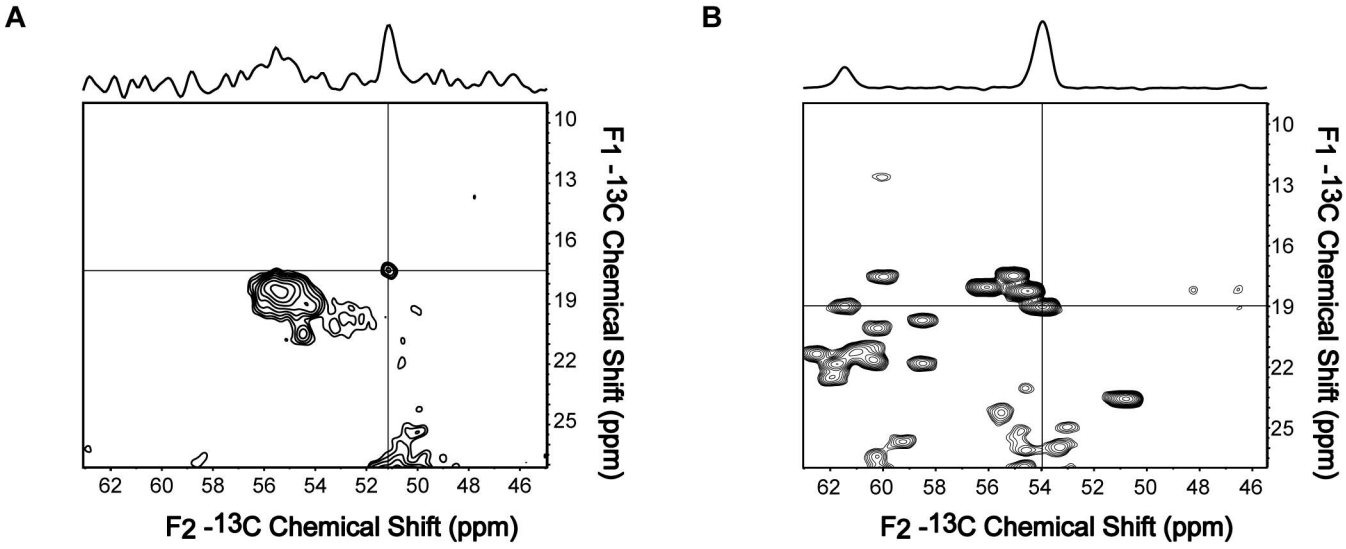

Figure S3. Comparison of line widths between CYP3A4 in a Nanodisc and microcrystalline preparation of β1 Immunoglobulin Binding Domain of Protein G (GB1). Both spectra were acquired on a 600 MHz InfinityPlus four-channel Varian NMR spectrometer. A) 2D ^{13}C-^{13}C chemical shift correlation spectrum of [U-^{13}C, ^{15}N] CYP3A4 (~3.5 mg) in a Nanodisc. The data were acquired with SPC-5 recoupling scheme (other acquisition parameters noted in the main

text). The data were processed with 0.195 ppm (30 Hz) net line broadening (Lorentzian-to-Gaussian apodization in both dimensions), and zero filled to 8192 (F2) x 2048 (F1). B) 2D ^{13}C-^{13}C chemical shift correlation spectrum of [U-^{13}C, ^{15}N] GB1 (~18 mg) with 5 ms DARR mixing time. The data were processed with 0.195 ppm (30 Hz) net line broadening (Lorentzian-to-Gaussian apodization in both dimensions), and zero filled to 16384 (F2) x 8192 (F1) complex points. Data for GB1 were acquired with 1.8 ms ^{1}H-^{13}C CP contact time, 70 kHz ^{1}H TPPM decoupling (7.1 μs, 17°), 15 ms t_1 acquisition time (1200 x 12.5 μs) with TPPI detection, 32 ms t_2 acquisition time (2560 x 12.5 μs), 2 s pulse delay. Indirect dimensions were not compared since they were limited by digital resolution.

TABLE S1. Resonance positions and line widths of 15 randomly selected crosspeaks in 2D ^{13}C-^{13}C chemical shift correlation spectrum with 200 ms DARR mixing time[a]

ω_1-^{13}C (ppm)	ω_2-^{13}C (ppm)	ω_1 FWHM[b] (Hz)	ω_2 FWHM[b] (Hz)
63.4	50.0	139	75
62.9	30.4	118	83
53.8	22.4	149	93
39.0	61.2	168	103
16.6	26.2	91	75
53.6	41.8	125	125
18.2	29.2	134	92
18.6	59.3	126	68
49.8	62.3	105	77
37.3	21.7	106	82
53.9	25.8	144	84
33.3	51.4	153	98
23.6	37	115	84
37.1	27.6	151	95
60.9	34.3	137	105

[a] The data were acquired with a similar set of parameters as shown in Figure 3 caption of the main text, with the exception of DARR mixing time.
[b] Data were processed with 30 Hz net line broadening (Lorentzian-to Gaussian apodization). The full width at half-maximum height (FWHM) was determined using Sparky (T.D. Goddard and D.G. Kneller, SPARKY 3, University of California, San Francisco).