Supporting Information

Characterization of Protein-Ligand Interactions by SABRE

Ratnamala Mandal, Pierce Pham, and Christian Hilty*

Chemistry Department, Texas A&M University, 3255 TAMU, College Station, TX 77843, USA

*corresponding author. E-mail: chilty@tamu.edu

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1. Concentration Dependence of Signal Enhancement

A stock solution of 10 mM 4-amidinopyridine ligand with 2 mM precatalyst [Ir(IMeMes)(COD)]Cl was prepared in methanol-d_4. The stock solution was diluted to prepare samples of ligand concentrations 5 mM and 1.5 mM. The sample was hyperpolarized in the low-field of 6.5 mT with bubbling time of 30 s at 294 K. Signals were acquired at the 9.4 T high field after a manual transfer time of 4 s.

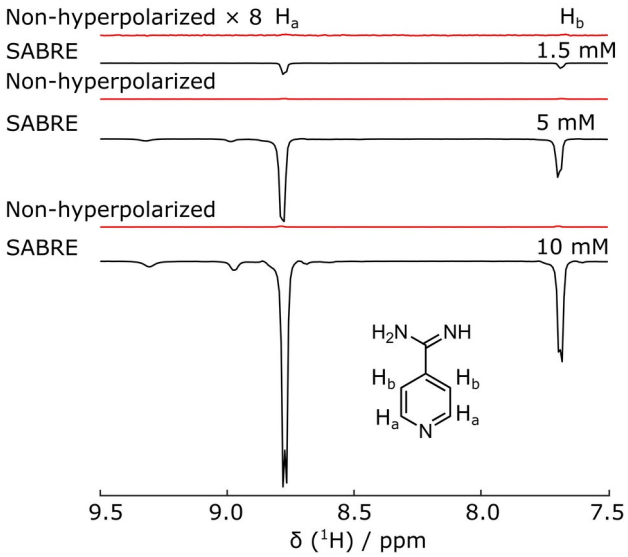

Figure S1. *Non-hyperpolarized (red -) and hyperpolarized (black -) spectra of 4-amidinopyridine at different ligand concentrations of 1.5 mM, 5 mM and 10 mM. The enhancements obtained were -87 and -34 for H_a and H_b at 1.5 mM concentration, -140 and -60 for H_a and H_b at 5 mM concentration and -230 and -110 for H_a and H_b at 10 mM concentration.*

2. Non-Hyperpolarized NMR Measurement of 4-amidinopyridine R_2 Relaxation

To determine the change in transverse relaxation rate for the ligand 4-amidinopyridine after binding to trypsin, the R_2 relaxation rate was determined from a pseudo-2D Carr-Purcell-Meiboom-Gill (CPMG) experiment.

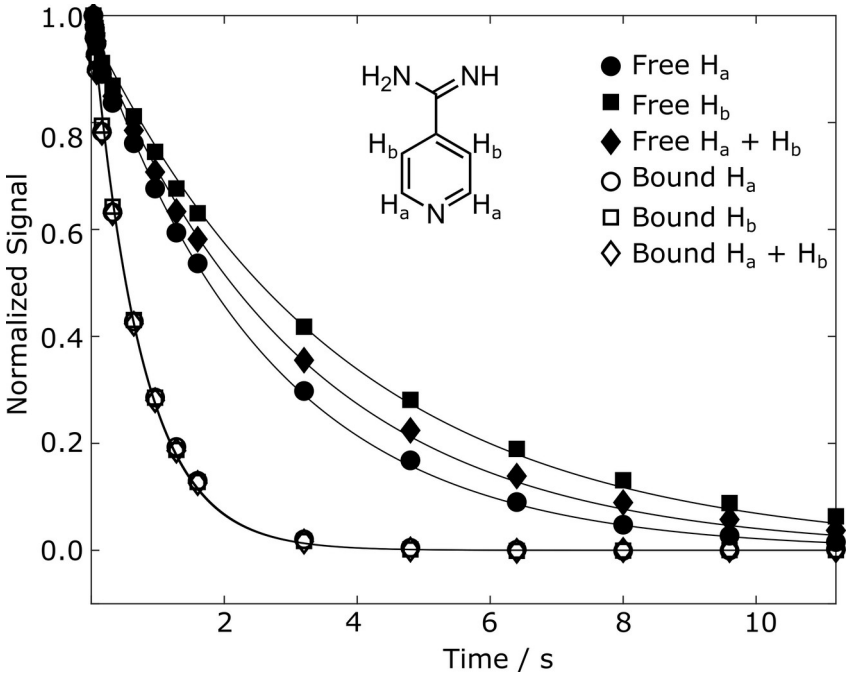

Figure S2. *R_2 rate determination of 8 mM 4-amidinopyridine without hyperpolarization in 50 mM aqueous sodium phosphate buffer. The filled symbols are for the free ligand and the open symbols for the ligand with trypsin. The rates of the free form are 0.38 s^{-1} for H_a (●), 0.26 s^{-1} for H_b (■), and 0.32 s^{-1} for both protons integrated together (♦). The rates of the bound forms are 1.35 s^{-1} for H_a (O), 1.36 s^{-1} for H_b (□), and 1.35 s^{-1} for the both protons integrated together (◊).*

3. Competitive Binding Experiment

To estimate the binding affinity of the ligand 4-amidinopyridine to trypsin, a competitive binding experiment was used according to a previously published protocol.[1] A non-hyperpolarized pseudo-2D CPMG experiment was measured to determine the changes in the R_2-relaxation rate of the reporter 4-(trifluoromethyl)benzene-1-carboximidamide (TFBC) ligand in the absence or presence of benzamidine or 4-amidinopyridine.

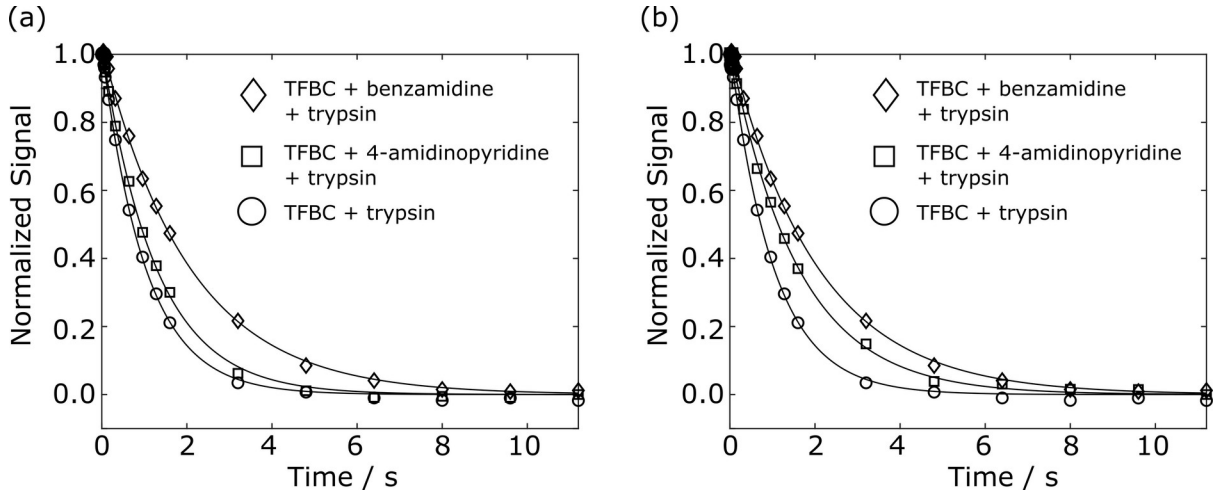

Figure S3. *Competitive binding experiment with samples prepared in 50 mM sodium phosphate buffer of (a) 1 mM TFBC and 18 µM trypsin (O), 1 mM TFBC, 400 µM 4-amidinopyridine and 18 µM trypsin (□), 1 mM TFBC, 400 µM benzamidine and 18 µM trypsin (◊), and (b) 1 mM TFBC and 18 µM trypsin (O), 1 mM TFBC, 800 µM 4-amidinopyridine and 18 µM trypsin (□), 1 mM TFBC, 400 µM benzamidine and 18 µM trypsin (◊).*

4. Control Experiment without Ligand

The signals of the ligand of interest and the chelating ligand appear in the same region. In the CPMG experiment as these peaks cannot be distinguished, a control experiment is performed where the experimental condition is same as in the binding experiment, but in the absence of the ligand. Below, a representative spectrum of the ligand and chelating ligand is shown, and result of the control experiment.

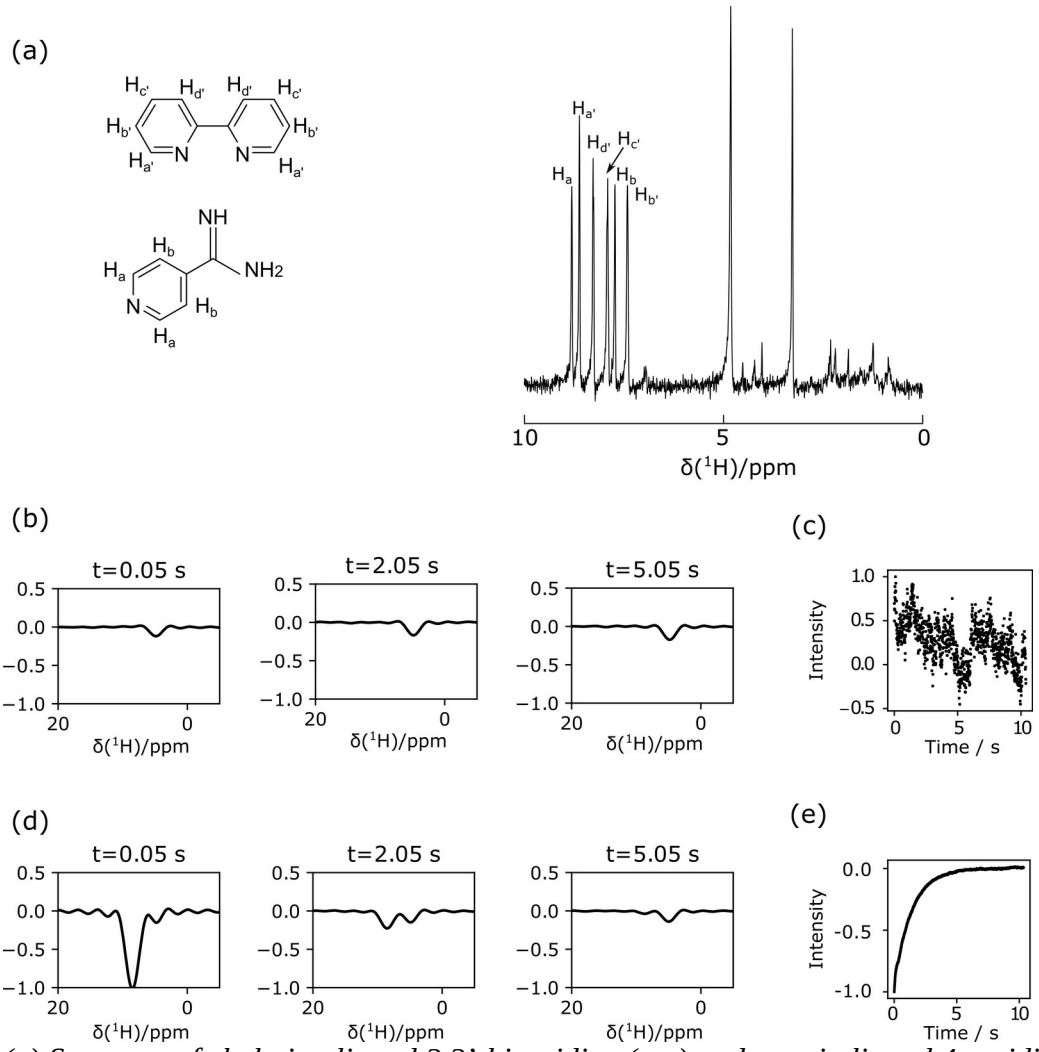

Figure S4. *(a) Structure of chelating ligand 2,2'-bipyridine (top) and trypsin ligand 4-amidinopyridine (bottom). (b) Non-hyperpolarized spectrum of ligand 4-amidinopyridine and chelating ligand 2,2'-bipyridine with catalyst in d_4-methanol. (b) Signal intensity from single-scan CPMG experiment in the absence of ligand 4-amidinopyridine but presence of 1.2 mM chelating ligand 2,2'-bipyridine (c) Fit from region corresponding to ligands. (d) Signal intensity from single-scan CPMG experiment in the presence of ligand 7.3 mM 4-amidinopyridine and 2.9 mM chelating ligand 2,2'-bipyridine. (e) Fit from region corresponding to ligands.*

5. Effect of 2,2’-bipyridine on Signal Enhancement

To test whether SABRE signal arises from the chelating agent 2,2’-bipyridine, the chelating agent was added to a sample after carrying out SABRE with only the ligand 4-amidinopyridine and the catalyst in methanol d_4.

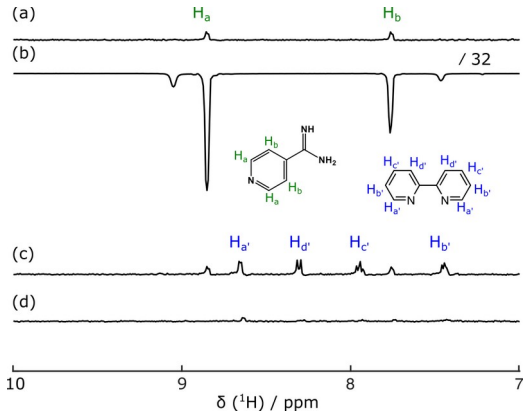

Figure S5. *(a) Non-hyperpolarized 400 MHz NMR spectra of 10 mM 4-amidinopyridine with Ir(IMeMes) catalyst in methanol-d_4. (b) SABRE hyperpolarized spectra of 4-amidinopyridine acquired at 9.4 T (400 MHz) after 30 s bubbling at 6.5 mT field and manual transfer time of 4 s. (c) Non-hyperpolarized 400 MHz NMR spectra of 10 mM 4-amidinopyridine with 2,2’-bipyridine and activated Ir(Me)(IMes) SABRE catalyst in methanol-d_4. (d) SABRE hyperpolarized spectra of 4-amidinopyridine in the presence of 2,2’-bipyridine acquired at 9.4 T after 30 s bubbling at 6.5 mT field and manual transfer time of 4 s.*

6. Effect of *para*-hydrogen Pressure on Signal Enhancement

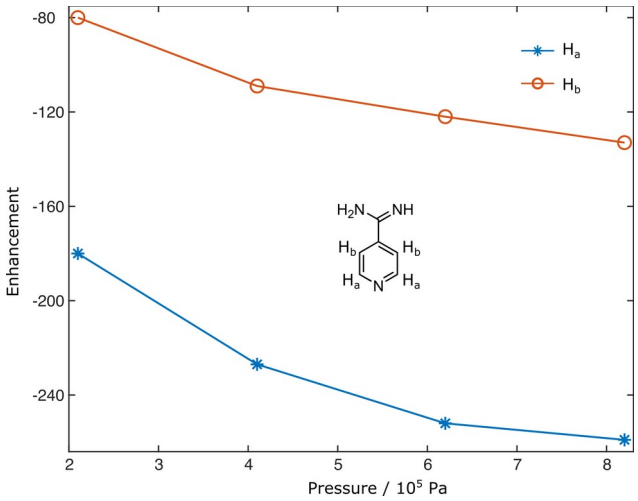

Figure S6. *Para-hydrogen pressure dependence of signal enhancement of 4-amidinopyridine with Ir(IMeMes) SABRE catalyst in methanol-d_4. The SABRE hyperpolarization was carried out at 6.5 mT by bubbling para-enriched hydrogen gas into the sample for 30 s. The NMR spectra were measured at 9.4 T after a manual transfer time of 4 s. The most negative number represents the highest enhancement.*

7. Trypsin Activity Assay

To check the activity of the trypsin, stopped-flow UV measurements were performed following a published protocol.[2] Briefly, a stock solution of 0.50 mM *N*-benzoyl arginine ethyl ester (BAEE) was prepared in 107 mM Tris buffer at pH=8.0 for the 0% methanol experiment, in 20% methanol and 80% Tris buffer for the 10% methanol experiment, and in 60% methanol and 40% Tris buffer for the 30% methanol case. The stock concentration of trypsin was 1.28 μM. The solutions of BAEE and trypsin were mixed in a cuvette at a ratio of 1:1 v/v, and the change in absorbance at 253 nm *vs*. time was recorded.

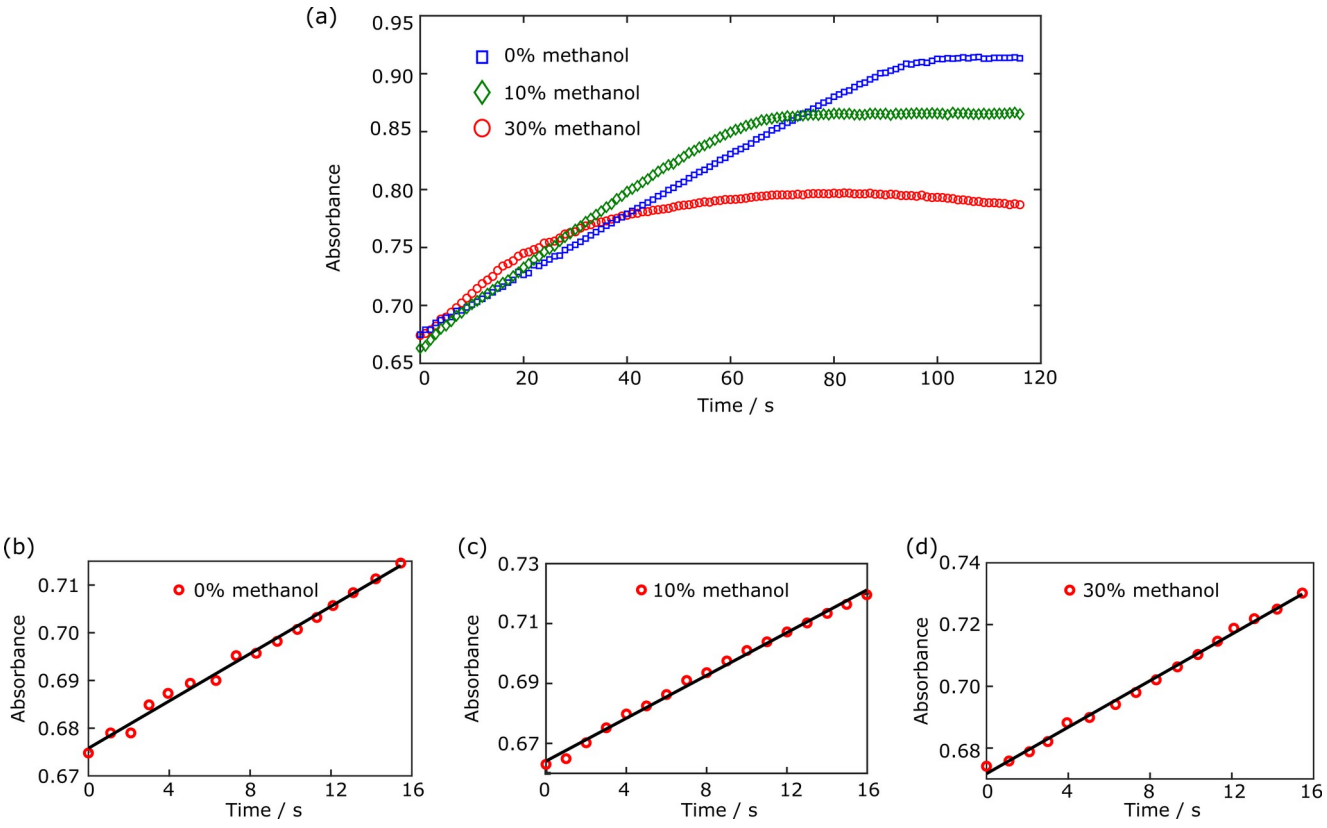

Figure S7. *(a) Change in Absorbance vs. time for the hydrolysis of BAEE catalyzed by trypsin, when the reaction was in 0% methanol (0.25 mM BAEE with 0.64 μM trypsin in Tris buffer) (□), in 10% methanol (0.25 mM BAEE with 0.64 μM trypsin in 90% Tris buffer and 10% methanol) (◊), and in 30% methanol (0.25 mM BAEE with 0.64 μM trypsin in 70% Tris buffer and 30% methanol) (O). (b) Fit of absorbance vs. time for the first 15 s of the reaction is in 0% methanol. The equation from the fit is y = 0.0024792 s^{-1}*x + 0.67579. (c) Fit of absorbance vs. time for the first 15 s of the reaction in 10% methanol and 90% Tris buffer. The equation from the fit is y = 0.0038 s^{-1}*x + 0.6717. (d) Fit of absorbance vs. time for the first 15 s of the reaction is in 30% methanol and 70% Tris buffer. The equation for the fit is y = 0.0035737 s^{-1}*x + 0.66409.*

8. List of Individual Experiments

The experimental parameters and the fitted R_2 relaxation rate for each experiment are summarized below in Table S1.

[ligand] / mM	[catalyst] / mM	[bipyridine] / mM	[protein] / mM	[ligand]/ [protein]	r_2 / s^{-1}
7.30	1.27	-	-	-	2.40
7.22	1.26	-	-	-	1.86
7.62	1.33	-	-	-	2.68
7.90	1.38	2.81	-	-	1.01
6.80	1.12	3.95	-	-	0.71
7.32	1.28	2.87	-	-	0.86
5.87	0.93	3.02	0.33	17.8	2.28
6.90	1.21	2.53	0.28	24.4	2.10
6.43	1.12	2.73	0.27	23.5	2.11
0.134	0.03	1.14	-	-	0.88
0.125	0.03	1.12	0.0072	17.4	1.89

9. References

1 Y. Kim and C. Hilty, *Angew. Chem. Int. Ed.*, 2015, **54**, 4941–4944.
2 A. M. J. Crowell, E. J. Stewart, Z. S. Take and A. A. Doucette, *Anal. Biochem.*, 2013, **435**, 131–136.