

Direct NMR detection of the unstable “red product” from reaction between nitroprusside and mercaptosuccinic acid

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1. Synthetic details

Materials. Chemicals were purchased from Sigma-Aldrich unless stated otherwise. These materials were used as received without further purification. 2-mercaptosuccinic acid (MSA, $\text{HOOCCH}(\text{SH})\text{CH}_2\text{COOH}$), sodium nitroprusside (SNP, $\text{Na}_2[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]\cdot 2\text{H}_2\text{O}$), sodium hydroxide (NaOH), sodium nitrite (NaNO_2), ^{15}N -labeled sodium nitrite ($\text{Na}^{15}\text{NO}_2$, 98% ^{15}N), ^{17}O -labeled water (H_2^{17}O , 41.1% ^{17}O , purchased from CortecNet), ethylenediaminetetraacetic acid (EDTA, $\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_8$), sodium carbonate (Na_2CO_3), sodium hydrogen carbonate (NaHCO_3), sodium chloride (NaCl), potassium cyanide (KCN), and ion-exchange resin (Amberlite IR-120, strongly acidic form).

Synthesis of ^{15}N -labeled SNP. $\text{Na}_2[\text{Fe}(\text{CN})_5(^{15}\text{NO})]\cdot 2\text{H}_2\text{O}$ was prepared in aqueous solution by mixing commercial SNP (260 mg) with 1.1 molar equivalents of NaOH and 1.9 molar equivalents of $\text{Na}^{15}\text{NO}_2$. The reaction mixture was kept at room temperature for 10 min, following by addition of 285 μL of 4 M acetic acid. The reaction mixture was concentrated to a paste on rotary evaporator. The residual material was dissolved in 2 mL of 4 M acetic acid and the solvent was evaporated on rotary evaporator. This process was repeated once. The residue was treated with 1,4-dioxane (4 mL); solid material was collected by filtration, washed with ethanol (2×3 mL), dried under vacuum, to give the title compound as red powder (153 mg, 59%). The ^{15}N enrichment in the product was 60%. ^{15}N NMR (50.6 MHz, D_2O): 371 ppm (ref. to liquid NH_3); ^{13}C NMR (125.6 MHz, D_2O), 134.8 ppm (eq) and 132.8 ppm (ax).

Synthesis of ^{17}O -labeled SNP. $\text{Na}_2[\text{Fe}^{\text{II}}(\text{CN})_5(\text{N}^{17}\text{O})]\cdot 2\text{H}_2\text{O}$ was prepared by mixing 265 mg of commercial SNP and 35.6 mg of NaOH in 300 μL of H_2^{17}O (41.1% ^{17}O). A few grains of Amberlite IR-120 (strongly acidic form) were gradually added to the reaction mixture and the progress of this reaction was monitored via ^{17}O NMR spectroscopy. Upon reaching the equilibrium, the mixture was then treated with 1,4-dioxane (4 mL) and the precipitates were collected via filtration and dried under vacuum. The ^{17}O -enrichment of the product was 20%. ^{17}O NMR (67.7 MHz, D_2O): 419 ppm (ref.

to water), ^{13}C NMR (125.6 MHz, D_2O): 134.8 ppm (eq) and 132.8 ppm (ax).

Synthesis of ^{17}O -labeled $\text{NaN}^{17}\text{O}_2$. NaNO_2 (0.8 g) and a few grains of Amberlite IR-120 (strongly acidic form) were placed in a round-bottom flask, to which H_2^{17}O (41.1%, 1 mL) was subsequently added. A cap was immediately placed on the flask, which was then sealed with parafilm. The mixture was stirred slowly for 20 min until a clear solution was obtained and this solution was kept at room temperature overnight. This solution was then heated at 75 °C for 2 h. The solution was subsequently lyophilized. The ^{17}O -enrichment of the product was 18%. ^{17}O NMR (67.7 MHz, D_2O): 661 ppm (ref. to water).

Synthesis of $[\text{Fe}^{\text{II}}(\text{CN})_5\text{N}(\text{O})\text{SR}]^{3-}$. A 200 mM MSA in D_2O buffer solution (0.05 M sodium carbonate, 0.1 M NaCl, 0.5 mM EDTA and 12 mM KCN; pH 11) was added to SNP (1 molar equivalents). In solution the complex exhibits a characteristic absorption band at $\lambda_{\text{max}} = 526$ nm in its UV/Vis spectrum. The corresponding ^{15}N - and ^{17}O -labeled complexes were prepared in the same fashion, except that ^{15}N - and ^{17}O -labeled SNPs were used. All solutions were freshly prepared.

2. NMR data analysis

The 2D ^1H - ^1H EXSY (NOESY) spectra of the MSA-SNP reaction mixture were analyzed with ExsyCalc (Mestrelab Research). At each temperature, two 2D spectra were recorded mixing times of 4 and 10 ms. The integrations of the cross and diagonal peaks were then used to determine the exchange rates (k_{d} and k_{f}) between the RP and MSA free ligand.

Table S1. Experimental equilibrium constants (K_{eq})^a and ligand exchange rates (k_{d} and k_{f}) of the reaction between NP and MSA at different temperatures from 1D ^1H NMR and 2D EXSY NMR spectra. The initial concentration was 50 mM for NP and MSA.

Temperature (K)	[RP]/[MSA]	K_{eq}	k_{d} (s^{-1})	k_{f} (s^{-1})
278	1 : 0.21	548.8	49.1	7.3
283	1 : 0.21	548.8	67.1	11.8
288	1 : 0.34	231.8	86.6	20.2
293	1 : 0.46	138.0		
298	1 : 0.54	105.6	113.2	62.0
303	1 : 0.94	43.9		
308	1 : 1.19	30.9		
313	1 : 4.20	5.9		

^a $K_{\text{eq}} = [\text{RP}]/([\text{NP}][\text{MSA}])$.

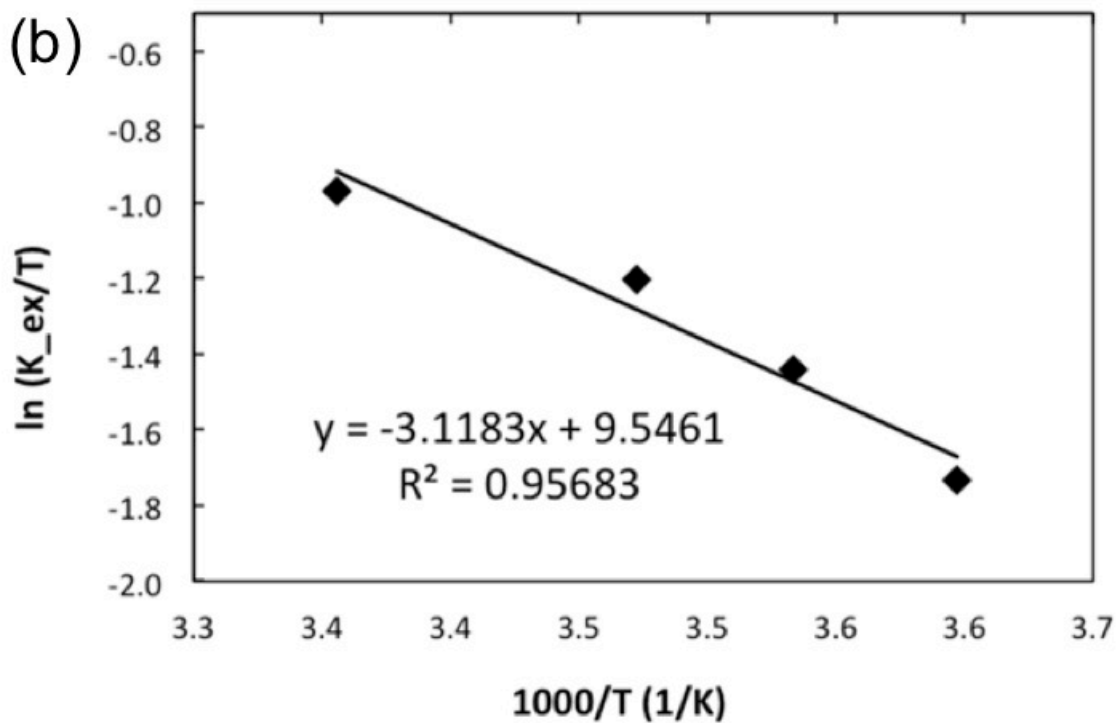
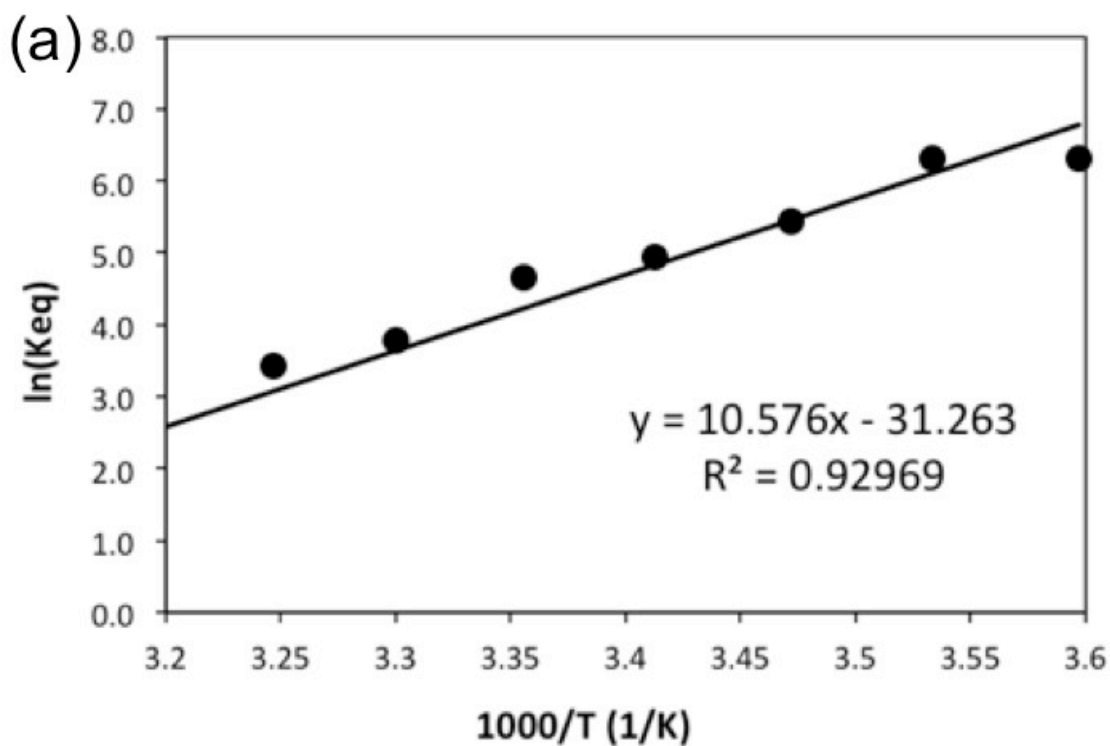


Figure S1. (a) Arrhenius plot of the experimental thermodynamic data and (b) Eyring plot of the experimental kinetic data. All data are given in Table S1.

Table S2. Summary of ^{13}C chemical shifts (in ppm) observed for NP, RP, RSNO, and RSSR.

Carbon	NP	RP	RSNO	RSSR ^a
C1 (COOH)	—	179.4	176.7	179.4
C2 (CH)	—	55.1	46.4	53.8, 53.5
C3 (CH ₂)	—	37.8	37.2	40.6, 40.5
C4 (COOH)	—	176.7	175.8	178.7
CN _{eq}	134.8	167.6	—	—
CN _{ax}	132.8	162.8	—	—

^aSpectra were recorded in reaction buffer (0.05 M sodium carbonate, 0.1 M NaCl, 0.5 mM EDTA and 12 mM KCN; pH 11).

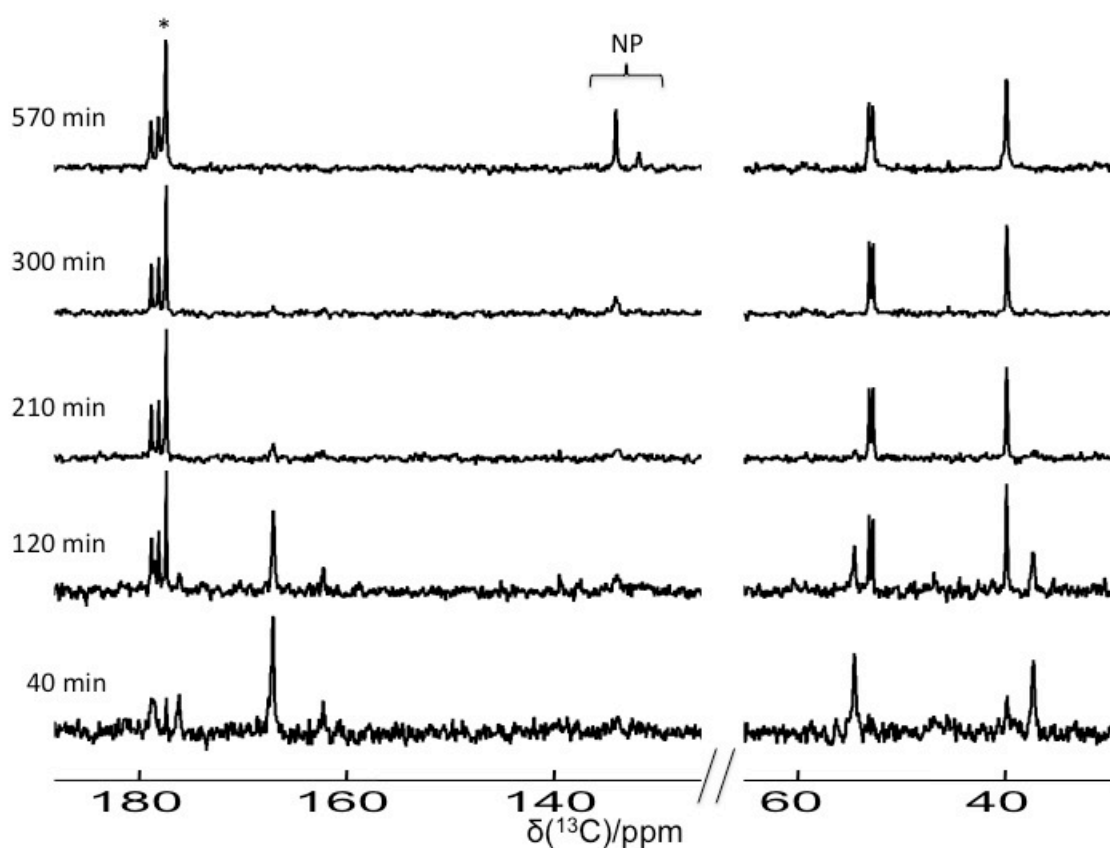


Figure S2. Decomposition of RP to RSSR monitored by ^{13}C NMR. Note that the slow regeneration of NP was due to the oxidation of paramagnetic $[\text{Fe}(\text{CN})_5\text{NO}]^{3-}$ (so invisible in the NMR spectra) by O_2 . The signal marked by * is due to $[\text{Fe}(\text{CN})_6]^{4-}$.