SUPPORTING INFORMATION

Copper(II) Binding to the Intrinsically Disordered C-terminal Peptide of SARS-CoV-2 Virulence Factor Nsp1

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Contents

Materials and Methods

A synthetic 33-residue Nsp1-CT peptide (Ac-ELGTDPYEDFQENWNTKHSSGVTRELMRELNGG), a peptide with 3-nitrotyrosine (YNO_2) at position 154 (full-length Nsp1 numbering) (Ac-ELGTDP(YNO_2)EDFQENWNTKHSSGVTRELMRELNGG), and a peptide with H165 replaced by alanine (Ac-ELGTDPYEDFQENWNTKASSGVTRELMRELNGG) were obtained from Genscript Biotech Corporation. All peptides were prepared with N-terminal acetylation. All measurements were performed using 3-(N-morpholino)propanesulfonic acid buffer (MOPS, 20 mM). Circular dichroism (CD) spectra were recorded on an Aviv Model 430 spectropolarimeter. Fluorescence spectra were recorded on a modified Jobin Yvon Fluorolog-3 using two Ocean Optics QEPro CCD spectrometers as detectors spanning the 300-900 nm range. The excitation wavelength for tryptophan fluorescence spectra was 280 nm. The excitation source for measurements of tryptophan fluorescence decay kinetics was the fourth harmonic (266 nm, 10 ps, 10 Hz) of a regeneratively amplified passively mode-locked Nd:YAG laser. Tryptophan fluorescence was collected using reflective optics, filtered through a monochromator, and detected with a picosecond streak camera (Hamamatsu C5680).

Electron Paramagnetic Resonance (EPR) Spectroscopy CW-EPR spectroscopy. X-band CW-EPR spectra were obtained on a Bruker EMX spectrometer at a temperature of 77 K using a quartz liquid nitrogen immersion dewar on solutions prepared as frozen glasses in 30% glycerol, unless otherwise noted. EPR simulations were performed using the EasySpin software package in Matlab (The Mathworks, Inc).

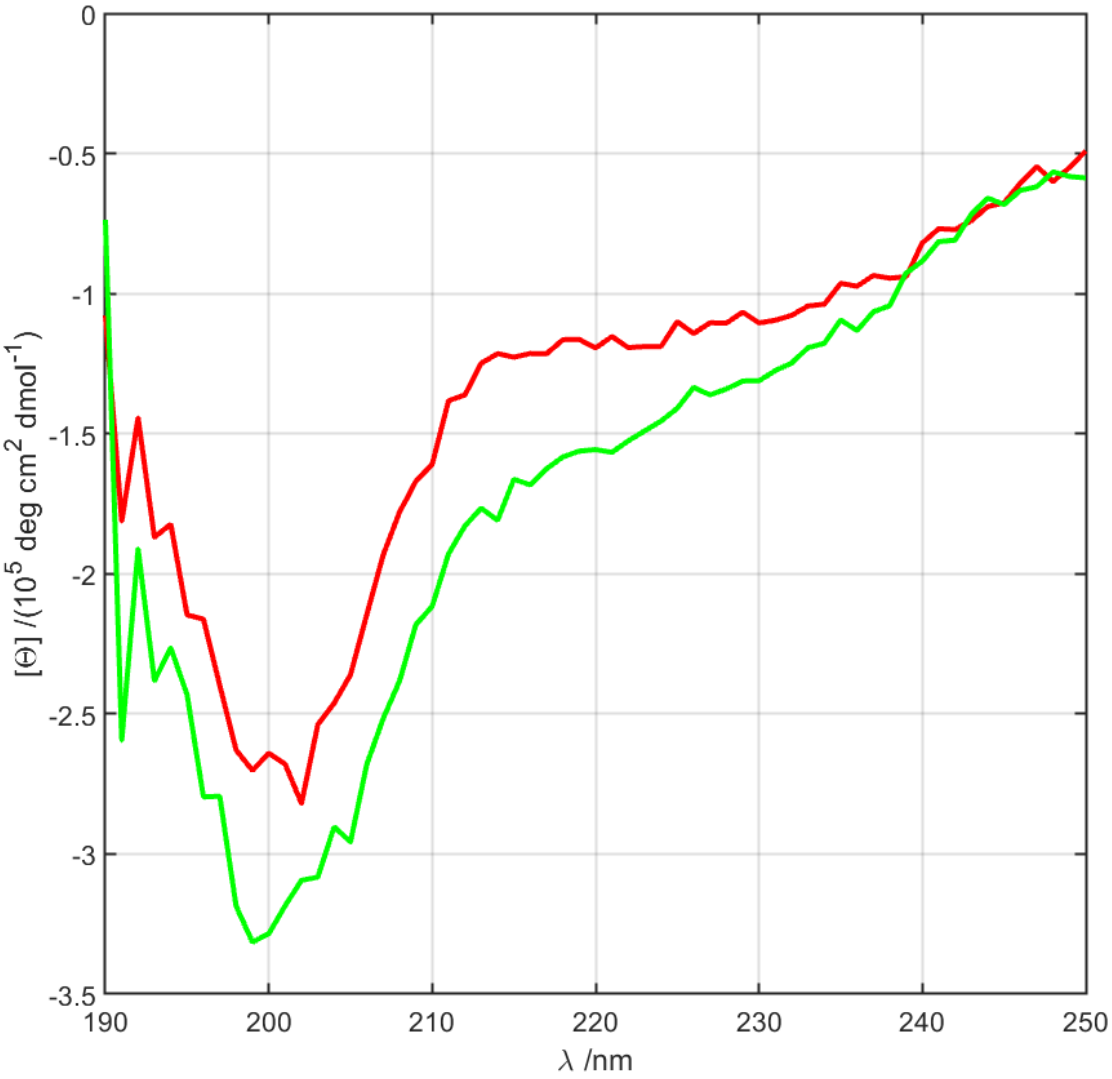


Figure S1- The far UV circular dichroism spectrum of Nsp1-CT (red, 30 μM, pH 6.5, MOPS 20 mM) contains a negative ellipticity minimum at 200 nm and a negative ellipticity shoulder near 230 nm. Addition of 1 equivalent of Cu(II) increases the ellipticity but does not indicate extensive α-helical structure.

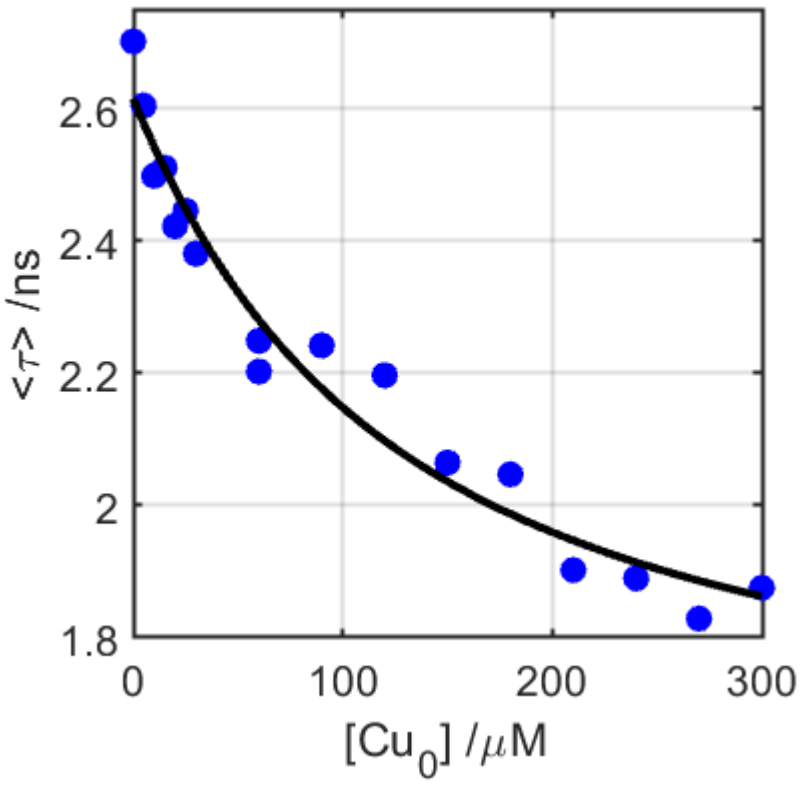
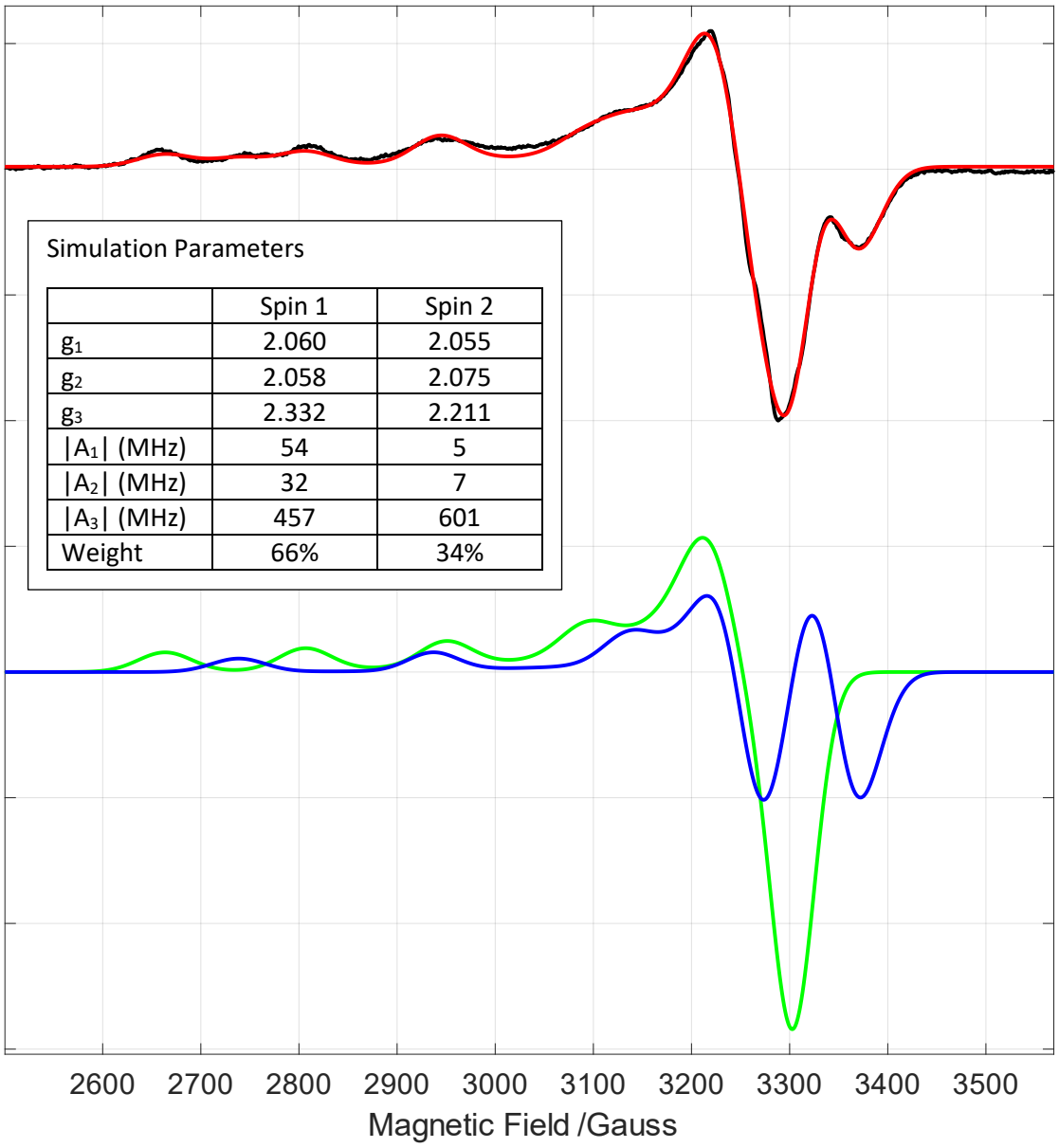
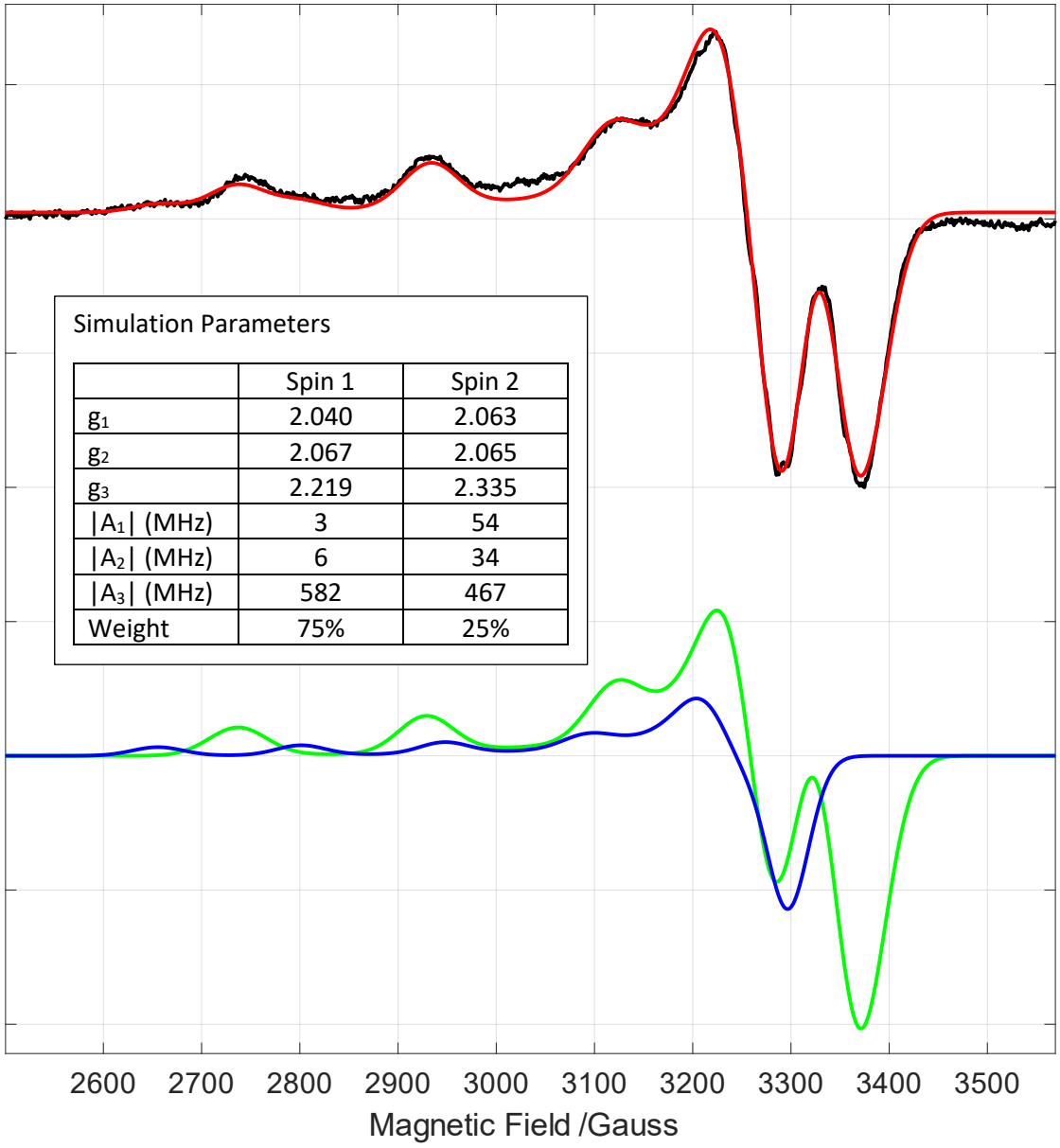


Figure S2. Variation in Nsp1-CT(H165A) W161 effective fluorescence decay time (<τ>) as a function of Cu(II) concentration. Solid line is a fit to a single binding site model with K_d = 106 μM ([Nsp1-CT(H165A)] = 30 μM, pH 7.5, MOPS 20 mM, 30% glycerol).



	Spin 1	Spin 2
g_1	2.060	2.055
g_2	2.058	2.075
g_3	2.332	2.211
$\|A_1\|$ (MHz)	54	5
$\|A_2\|$ (MHz)	32	7
$\|A_3\|$ (MHz)	457	601
Weight	66%	34%

Figure S3 – Cu(II):Nsp1-CT ([Cu] = [Nsp1-CT] = 100 μM, MOPS 20 mM, pH 6.5, 30% glycerol) X-band EPR spectrum (black) and simulation (red). Individual simulation components are shown in green (Spin 1) and blue (Spin 2). Acquisition parameters: MW frequency = 9.391-9.394 GHz, MW power = 2.2 mW, modulation amplitude = 8 Gauss, conversion time = 10.72 ms.



	Spin 1	Spin 2
g_1	2.040	2.063
g_2	2.067	2.065
g_3	2.219	2.335
$\|A_1\|$ (MHz)	3	54
$\|A_2\|$ (MHz)	6	34
$\|A_3\|$ (MHz)	582	467
Weight	75%	25%

Figure S4 – Cu(II):Nsp1-CT ([Cu] = [Nsp1-CT] = 100 μM, MOPS 20 mM, pH 7.5, 30% glycerol) X-band EPR spectrum (black) and simulation (red). Individual simulation components are shown in green (Spin 1) and blue (Spin 2). Acquisition parameters: MW frequency = 9.391-9.394 GHz, MW power = 2.2 mW, modulation amplitude = 8 Gauss, conversion time = 10.72 ms.

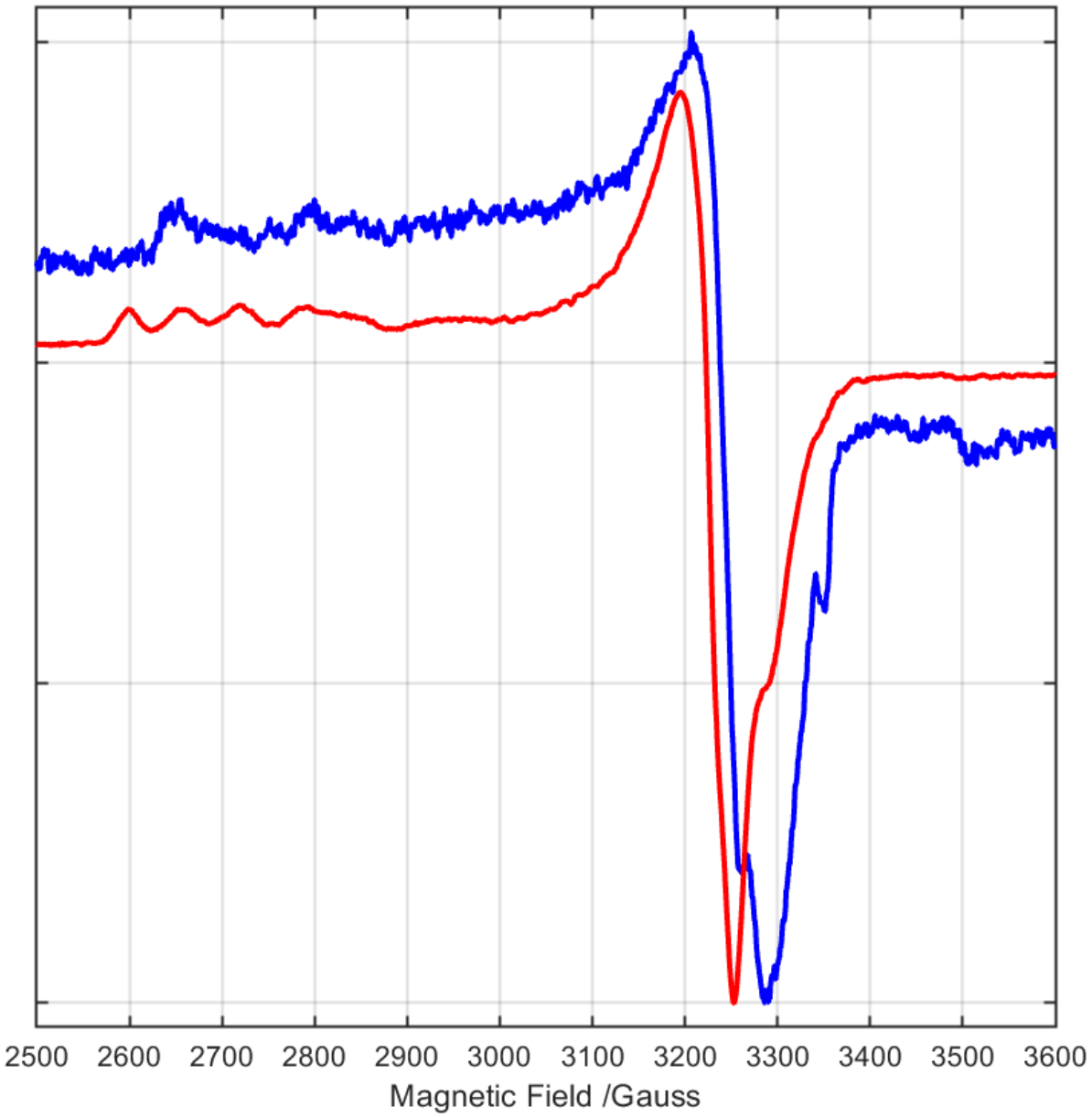


Figure S5 – Cu(II) MOPS ([Cu] = 100 μM, MOPS 20 mM, 30% glycerol) X-band EPR spectrum: pH 6.5 (red); pH 7.5 (blue). Acquisition parameters: MW frequency = 9.391-9.394 GHz, MW power = 2.2 mW, modulation amplitude = 8 Gauss, conversion time = 10.72 ms.

Table S1. Biexponential fitting parameters for Trp fluorescence decay kinetics in Nsp1-CT and Nsp1-CT-Y(NO_2)154.

Sample		k_{fit} (ns^{-1})[a]		Coefficients[a] (10^3 counts)
Nsp1-CT	k_1	1.3339	A_1	2.8434
	k_2	0.3072	A_2	4.7979
			A_∞	0.0583
Nsp1-CT-Y(NO_2)154	k_1	3.4125	A_1	3.3167
	k_2	0.6410	A_2	2.5583
			A_∞	0.0713

[a] Fluorescence decay function: $I(t) = A_1\exp(-k_1 \times t) + A_2\exp(-k_2 \times t) + A_\infty$