Insights into the N-terminal Cu(II) and Cu(I) binding sites of the human copper transporter CTR1

*Yulia Shenberger, Ortal Marciano, Hugo E. Gottlieb, and Sharon Ruthstein**

The Department of Chemistry, Faculty of Exact Science, Bar Ilan University, Ramat-Gan, Israel, 5290002

*corresponding author: sharon.ruthstein@biu.ac.il Tel. 972-3-7384329 , Fax. 972-3-7384053

Supporting Information

Figures S1-S9

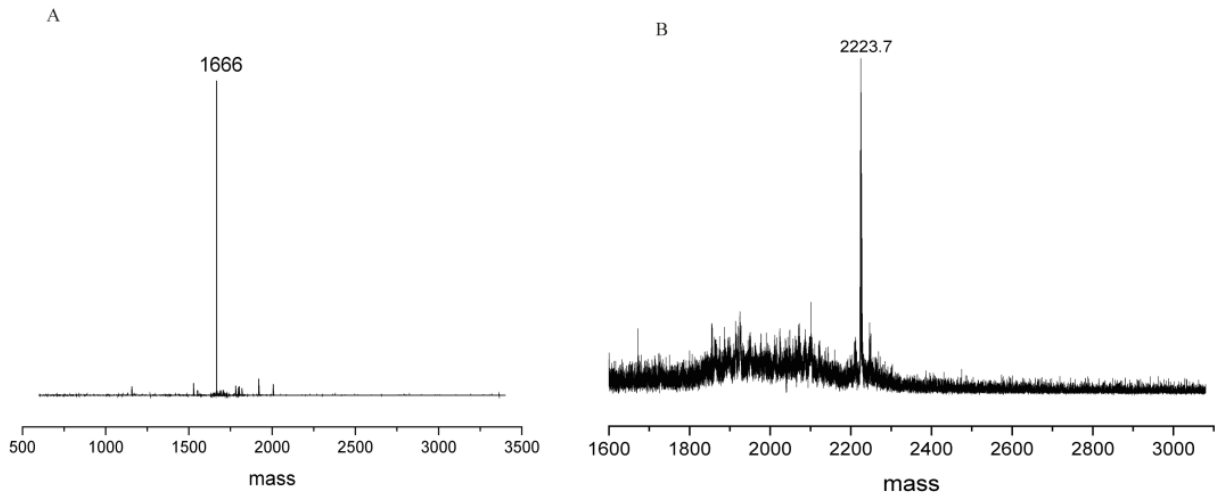

Figure S1: A. Mass spectra of Pep1 (1666 Da). B. Mass spectra of Pep7 (2224 Da).

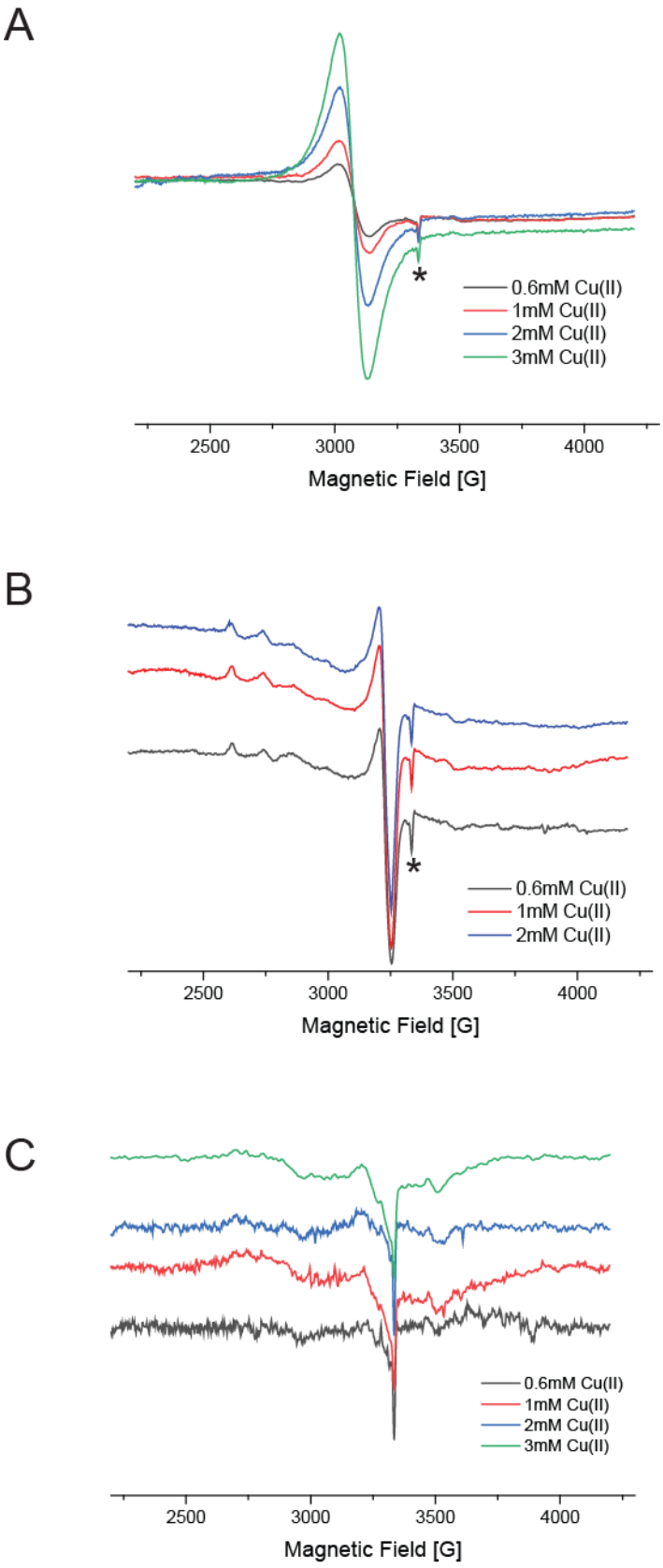

Figure S2: LT CW-EPR spectra of free Cu(II) in **A**. water. **B**. KPi buffer, and in **C**. HEPES buffer. In HEPES, Cu(II) is less dissolved, characterized by low SNR. The asterisk sign marks a signal originated from the quartz capillary.

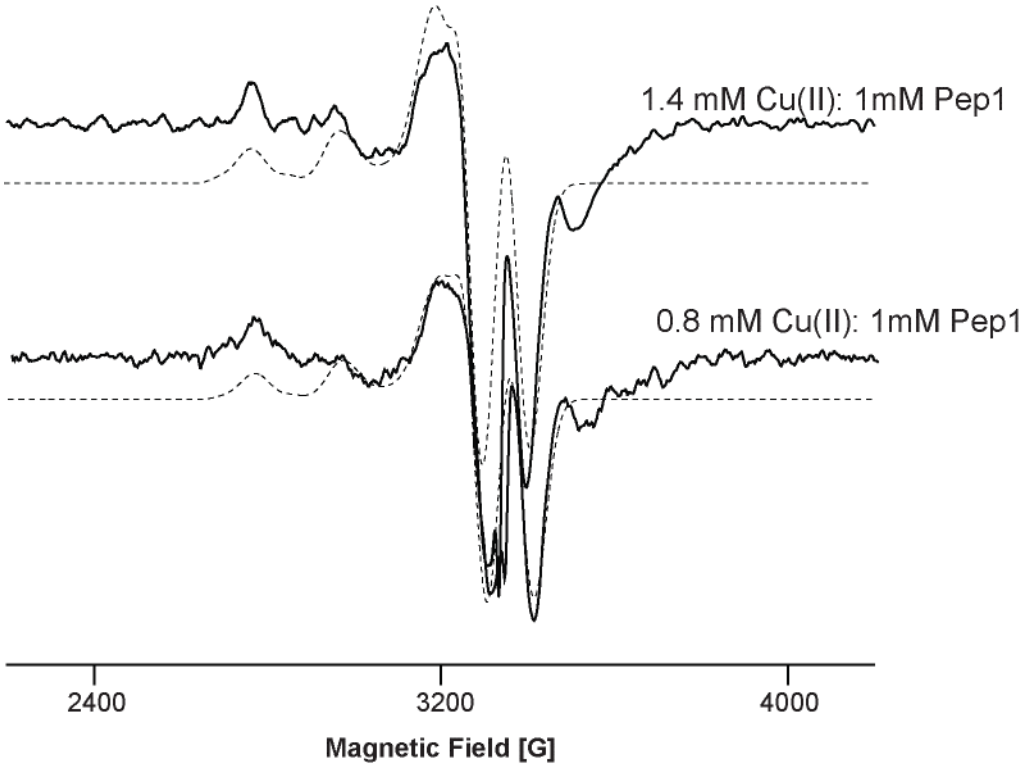

Figure S3: LT CW-EPR spectra (solid lines) and simulated data (dashed lines) for 1.4mM and 0.8mM Cu(II) in the presence of 1mM Pep1 in HEPES buffer. The EPR spectra were simulated with two species: 4O and 4N coordination. Where the parameters for 4N coordination are: $g_{\|}$=2.18 ± 0.005, $A_{\|}$=215 ± 2.0 G. In 0.8mM Cu(II), 95% are in 4N coordination. In 1.4mM Cu(II), 92% are in 4N coordination.

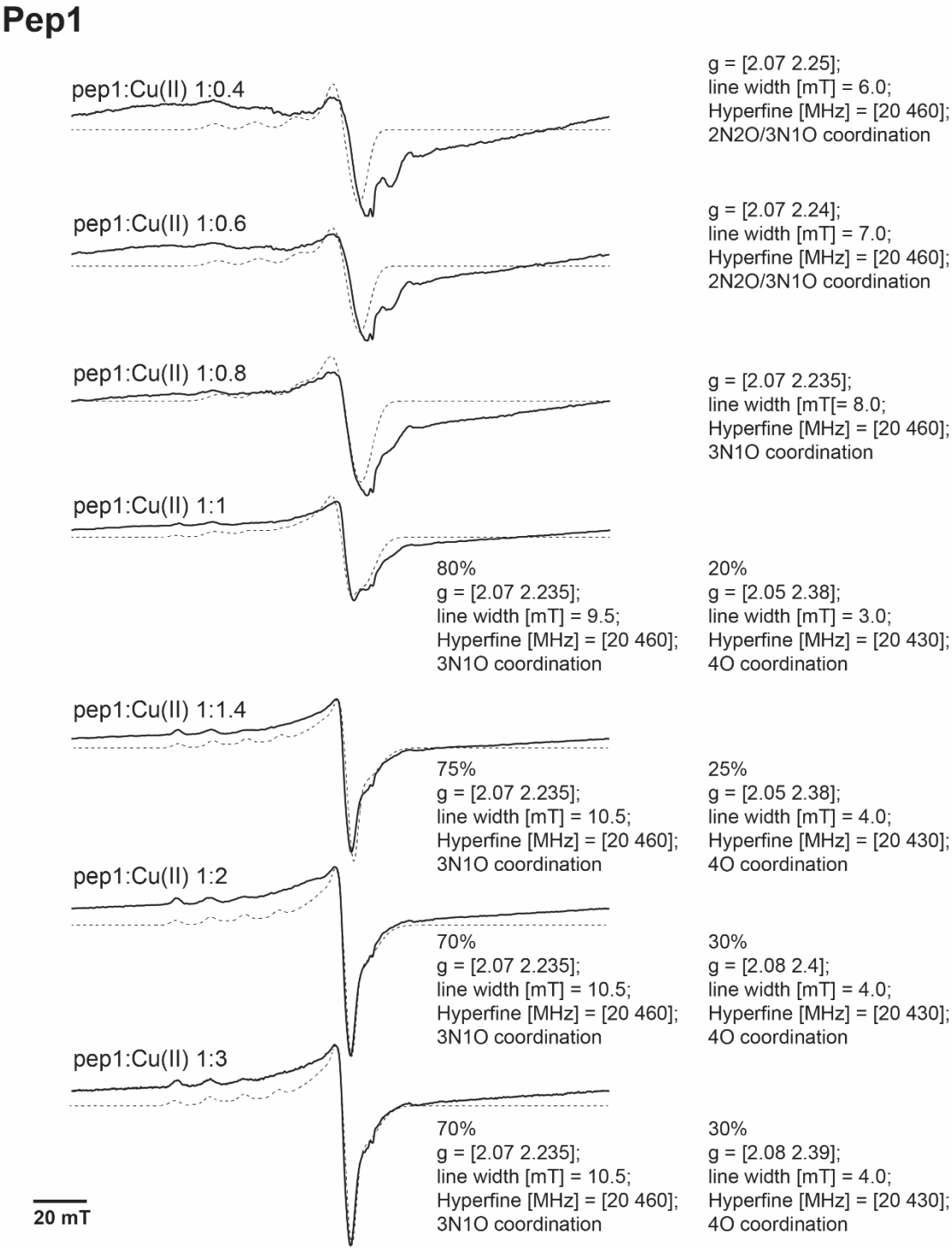

Figure S4: LT CW-EPR spectra (solid lines) and simulated data (dashed lines), and the parameters derived from the simulations for [Pep1]:[Cu(II)] ratio of 1:0.4, 1:0.6, 1:0.8, 1:1, 1:1.4, 1:2, 1:3, using the Easyspin tool box (pepper function).

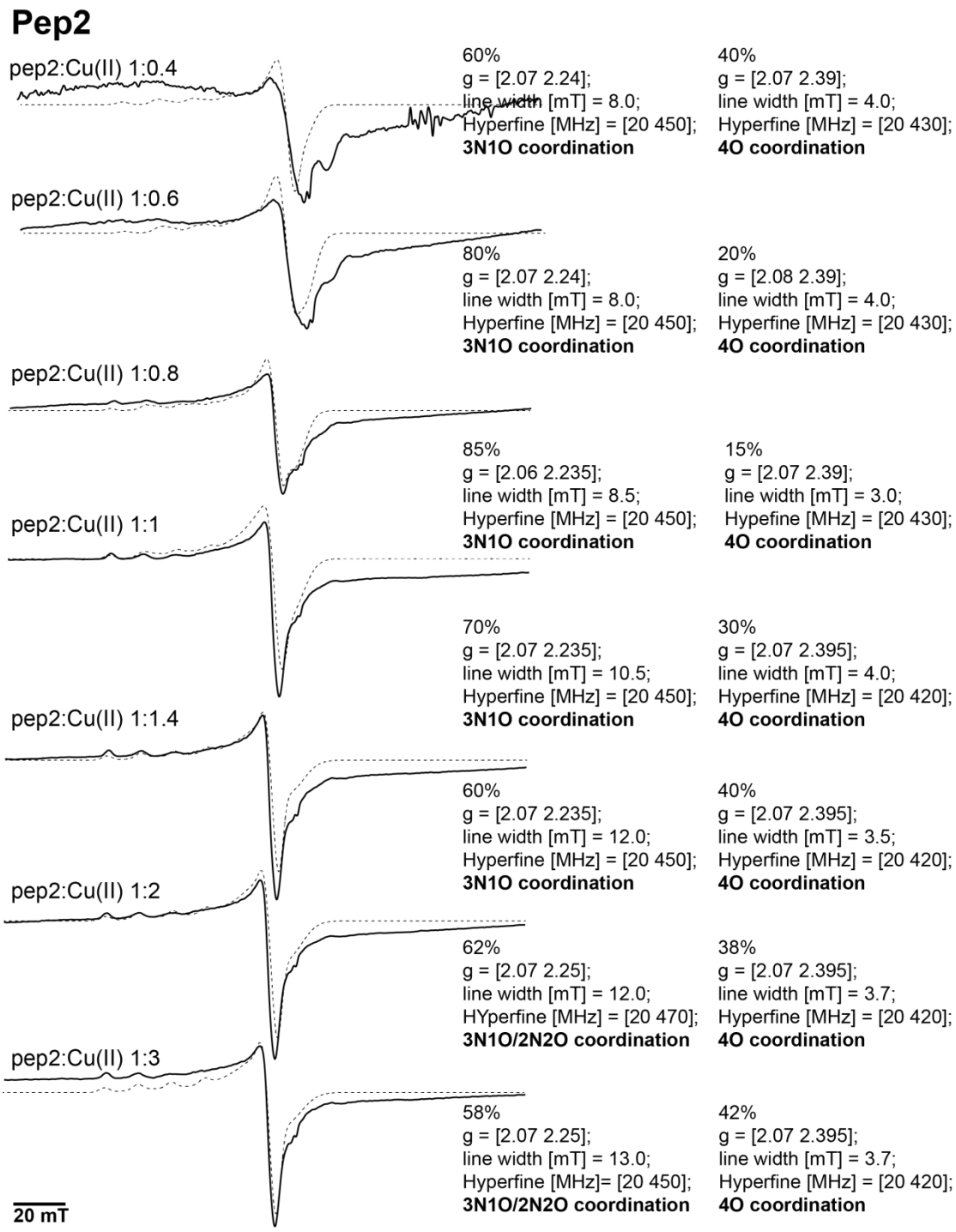

Figure S5: LT CW-EPR spectra (solid lines) and simulated data (dashed lines), and the parameters derived from the simulations for [Pep2]:[Cu(II)] ratio of 1:0.4, 1:0.6, 1:0.8, 1:1, 1:1.4, 1:2, 1:3, using the EasySpin toolbox (pepper function).

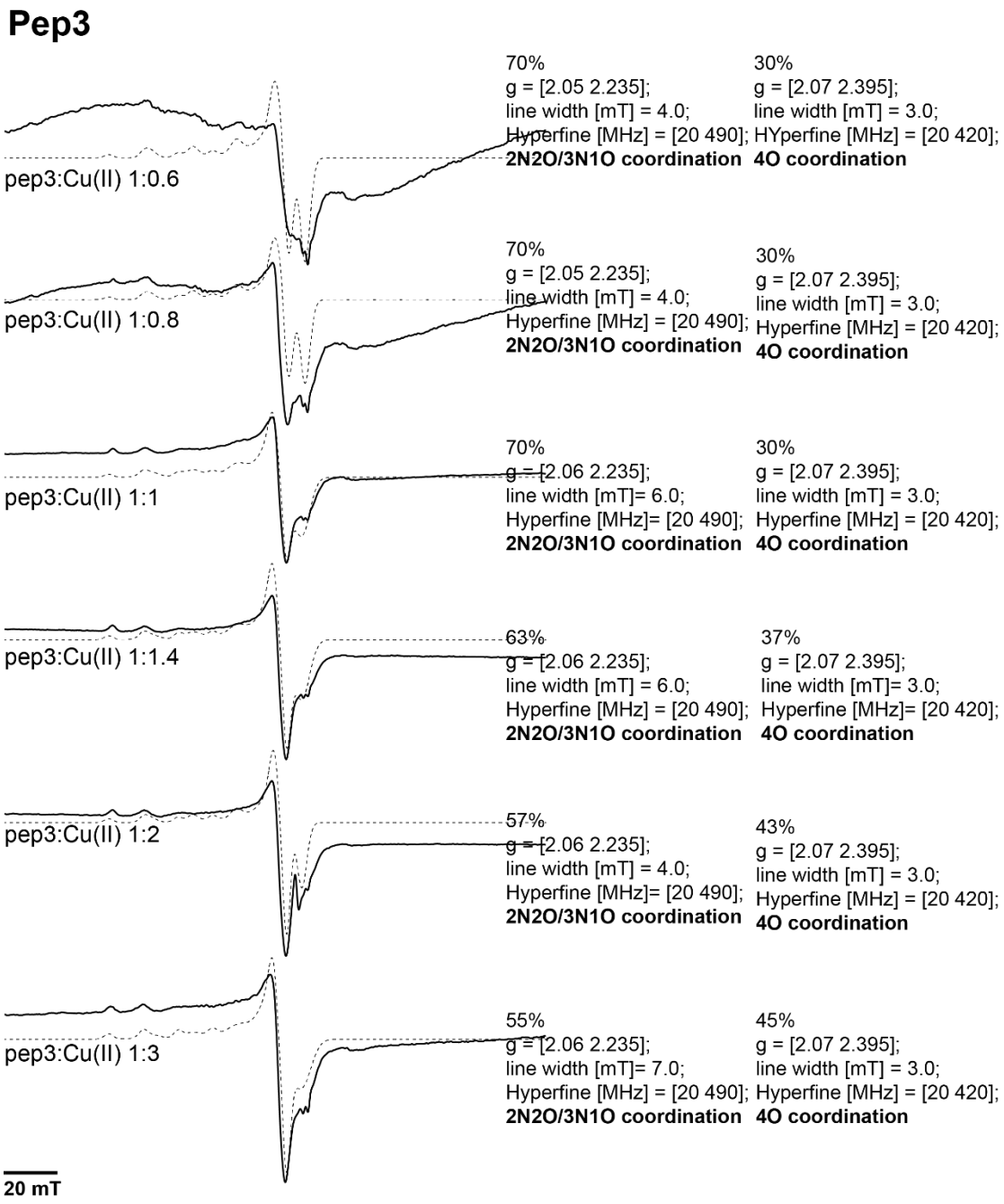

Figure S6: LT CW-EPR spectra (solid lines) and simulated data (dashed lines), and the parameters derived from the simulations for [Pep3]:[Cu(II)] ratio of 1:0.4, 1:0.6, 1:0.8, 1:1, 1:1.4, 1:2, 1:3, using the EasySpin toolbox (pepper function).

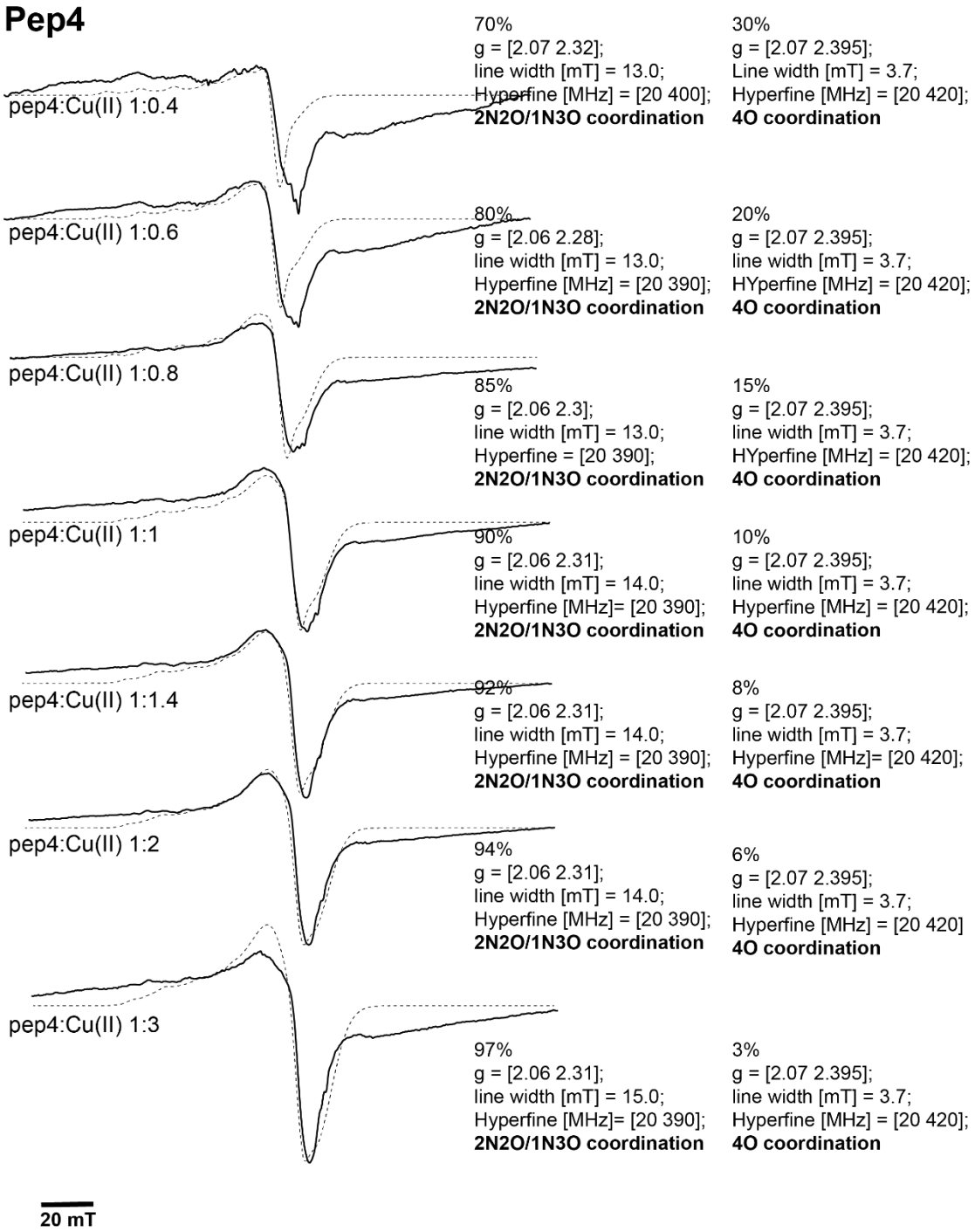

Figure S7: LT CW-EPR spectra (solid lines) and simulated data (dashed lines), and the parameters derived from the simulations for [Pep4]:[Cu(II)] ratio of 1:0.4, 1:0.6, 1:0.8, 1:1, 1:1.4, 1:2, 1:3, using the EasySpin toolbox (pepper function).

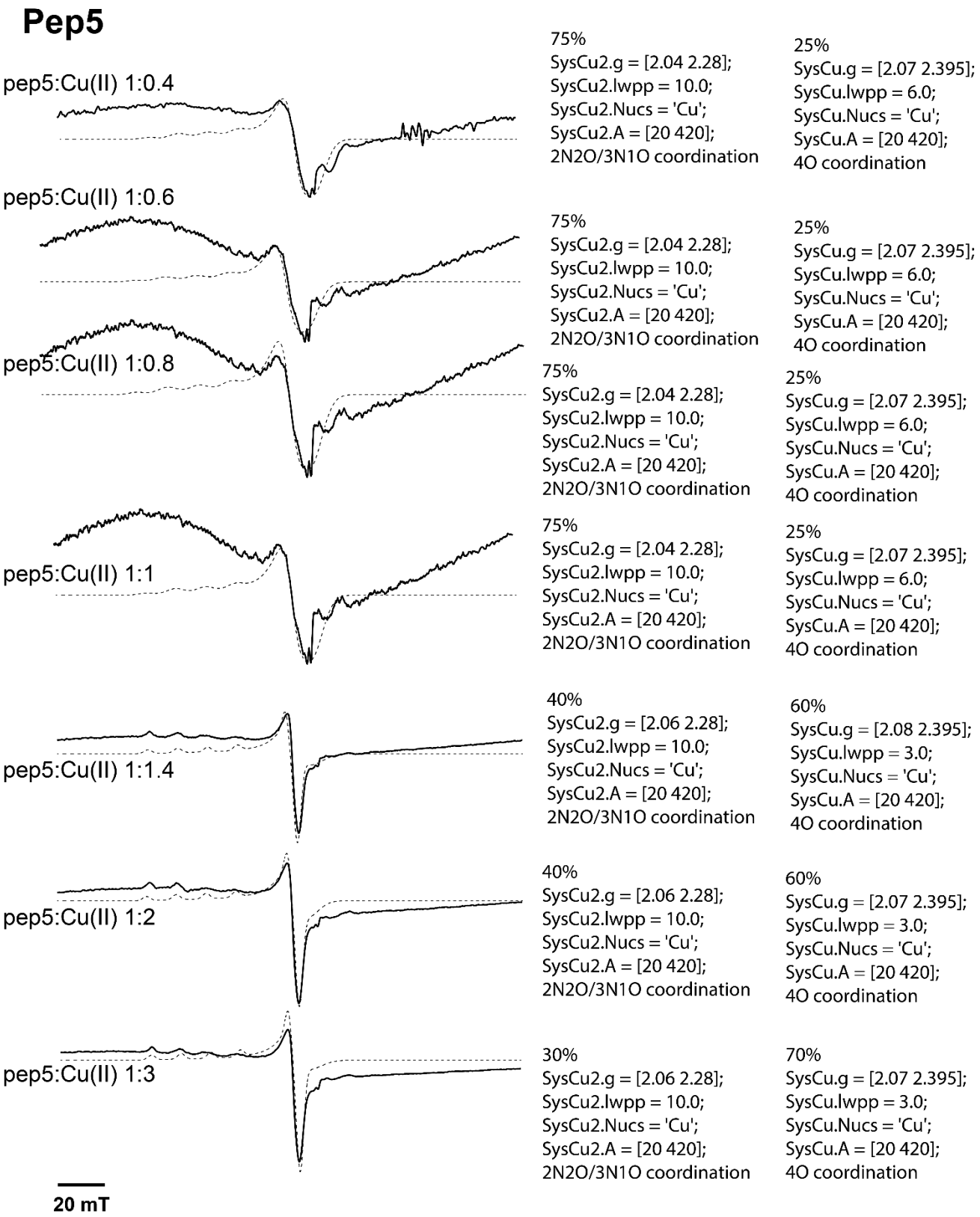

Figure S8: LT CW-EPR spectra (solid lines) and simulated data (dashed lines), and the parameters derived from the simulations for [Pep5]:[Cu(II)] ratio of 1:0.4, 1:0.6, 1:0.8, 1:1, 1:1.4, 1:2, 1:3, using the EasySpin toolbox (pepper function).

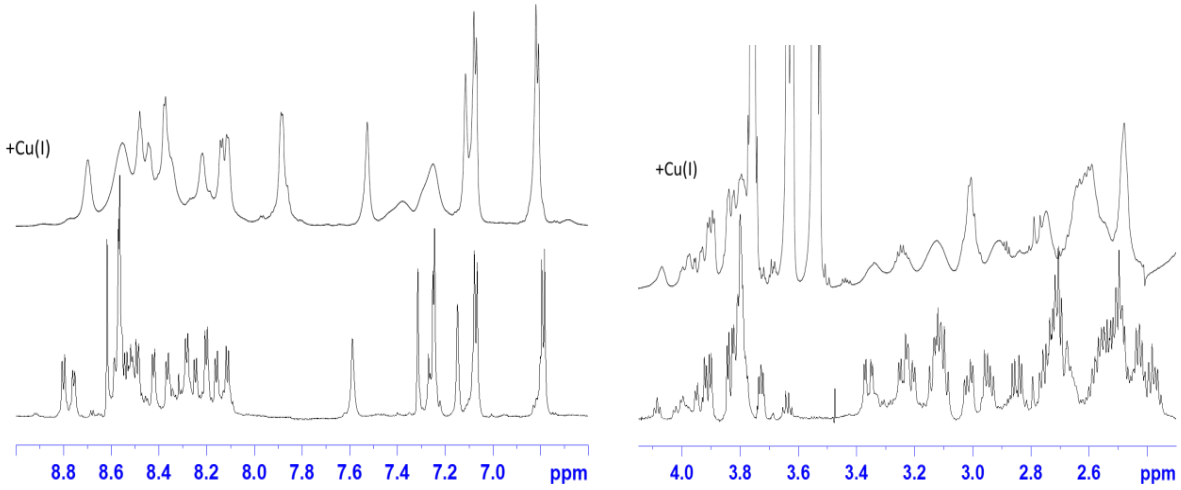

Figure S9: A region of the ^{1}H NMR spectra of Pep1_WT (lower spectra), and in the presence of Cu(I) (upper spectra).