Supplementary information

The rates of Cu(II)-ATCUN complex formation. Why so slow?

Radosław Kotuniak,[a] Paulina Szczerba,[a] Dobromiła Sudzik,[a] Marc J.F. Strampraad,[b] Peter-Leon Hagedoorn,[b] and Wojciech Bal*[a]

[a] Laboratory of Biological Chemistry of Metal Ions, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5a, 02-106 Warszawa, Poland. E-mail: wbal@ibb.waw.pl
[b] Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, The Netherlands.

Table of contents

Materials and Methods

Peptide Synthesis

For the purpose of this work 17 peptides were synthesized according to solid phase Fmoc strategy [S1] on a Prelude™ peptide synthesizer (Gyros Protein Technologies, Inc.). The TentaGel S RAM resin was used as a solid phase for Gly-Gly-His-amide, Gly-Gly-His-Gly-Gly-Gly-amide and Gly-Gly-His-Gly-amide peptides. For Gly-(N-Me)Gly-His and Ac-Gly-Gly-His peptides the TentaGel S PHB His(Trt) resin was employed. In case of Gly-Gly(N-Me)His, Gly-Gly-(Nτ-Me)His and Gly-Gly-(Nπ-Me)His peptides the dedicated modified histidine was diluted in the NMP/DIPEA solution and then manually esterified on a 2-chlorotrityl chloride resin with a mixture of DCM, methanol and DIPEA (80:15:5 volume ratio) as described in the protocol from ref. [S1]. After the synthesis the peptides were cleaved from the resin with a cocktail of TFA, TIS and water (95:2.5:2.5, volume ratio), except for Ac-Gly-Gly-His, which was acetylated with acetic anhydride prior to the cleavage reaction. At the end, the peptides were precipitated from the solution with cold diethyl ether and then lyophilized. The obtained peptides were purified by high-performance liquid chromatography (HPLC; Knauer) with UV−Vis detection at 220 nm on a C18 Eurospher II column (Knauer). The eluting solvent A was 0.1% TFA in water, and solvent B was 0.1% TFA in 90% acetonitrile. The purity of peptides was checked using electrospray ionization mass spectrometry (ESI-MS; Premier, Waters).

pH-metric titrations

The UV−Vis spectra were recorded on a Cary 50 Bio spectrophotometer (Varian) over the spectral range of 300−900 nm, and CD spectra were obtained on a J-815 CD spectropolarimeter (JASCO) at 250−800 nm at 25 °C in 1-cm-path-length quartz cuvettes (Hellma). The pH-metric titrations were performed with both types of detection for 1 mM peptide and 0.8 mM $CuCl_2$ water solutions. In all experiments the pH value was controlled by adding small amounts (~1 µl) of HCl or NaOH to obtain spectra in the approximate pH range 2-11 (for detailed values see captions under given figures in the main text and SI).

Diode-array stopped-flow technique

The reactions of Cu^{2+} ions with the N-methylated GGH analogues were studied using a SX20 stopped-flow spectrometer (Applied Photophysics Ltd.) equipped with a diode-array detector in the range 400-740 nm (in 1-cm-path-length quartz cuvette). The kinetic experiments for GGHG-am derivatives were studied on a SFM-300 diode-array stopped-flow apparatus (BioLogic) with the 400-900 nm detection range (in 0.5-cm-path-length quartz cuvette). The dead time of both instruments was 2 ms and the experiments were carried out at 25°C. The data were analysed using Originlab and KinTek Explorer [S2, S3]. We determined the reaction half-times ($t_{1/2}$) by fitting them to the one-exponential function.

Supplementary figures

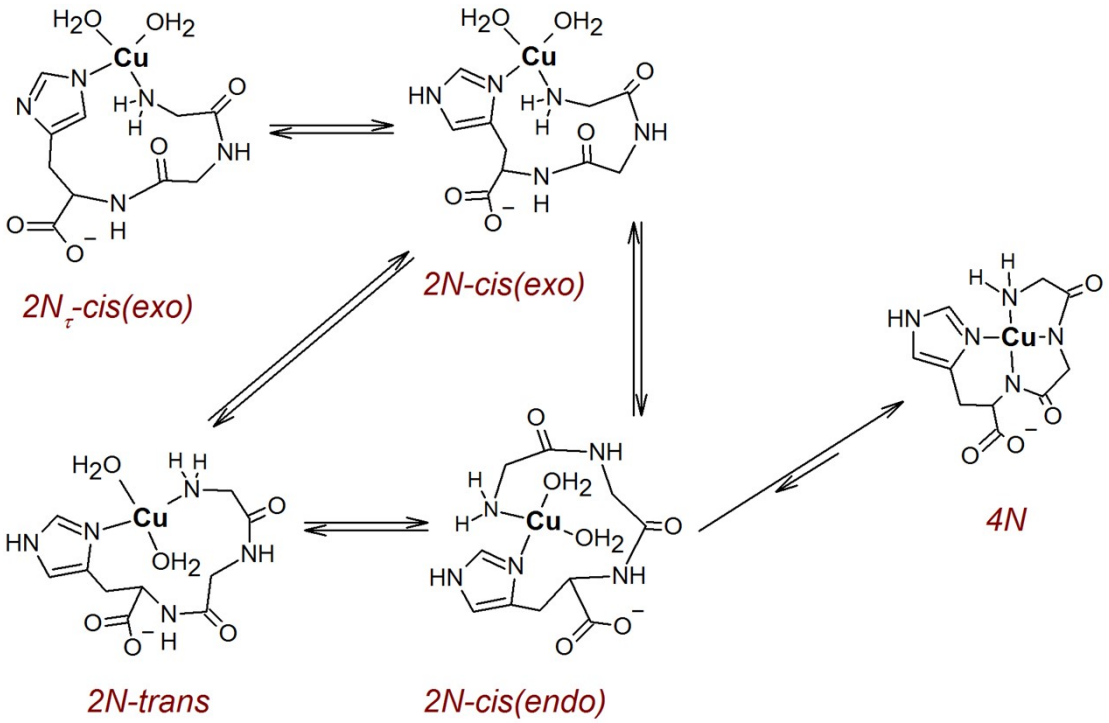

Figure S1. The postulated scheme of dynamic isomerisation of the IC species, based on ref. [S4].

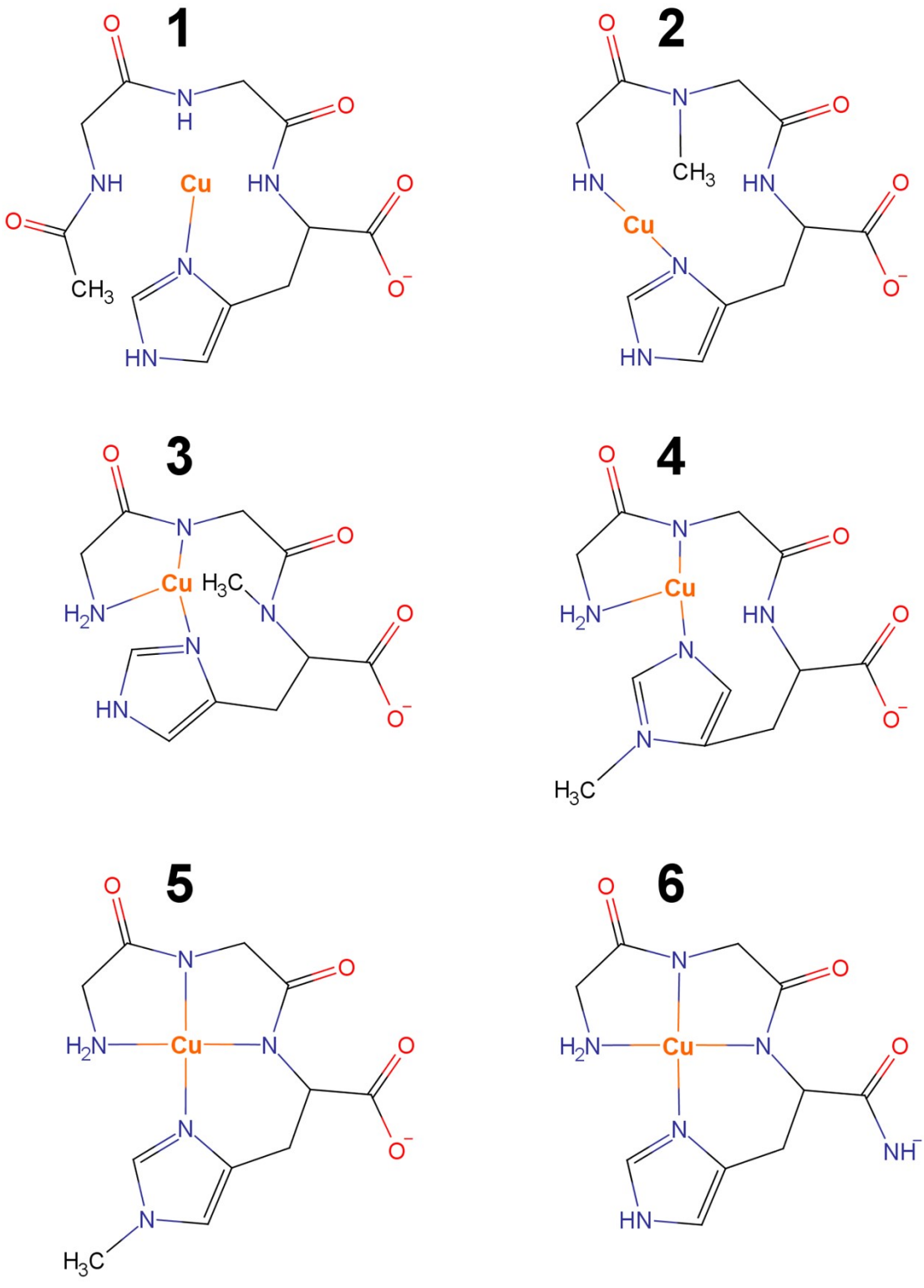

Figure S2. Structural visualization of the synthesized GGH analogues. According to numeric labels in Fig. 1 the structures represent 1 - Ac-Gly-Gly-His, 2 - Gly-(N-Me) Gly-His, 3 - Gly-Gly-(N-Me)His, 4 - Gly-Gly-(Nπ-Me)His, 5 - Gly-Gly-(Nτ-Me)His, 6 - Gly-Gly-His-amide in the presence of a Cu^{2+} ion. The Cu(II) bonding pattern reflects the main (or sole) species at pH 6, serving as an endpoint of stopped-flow experiments.

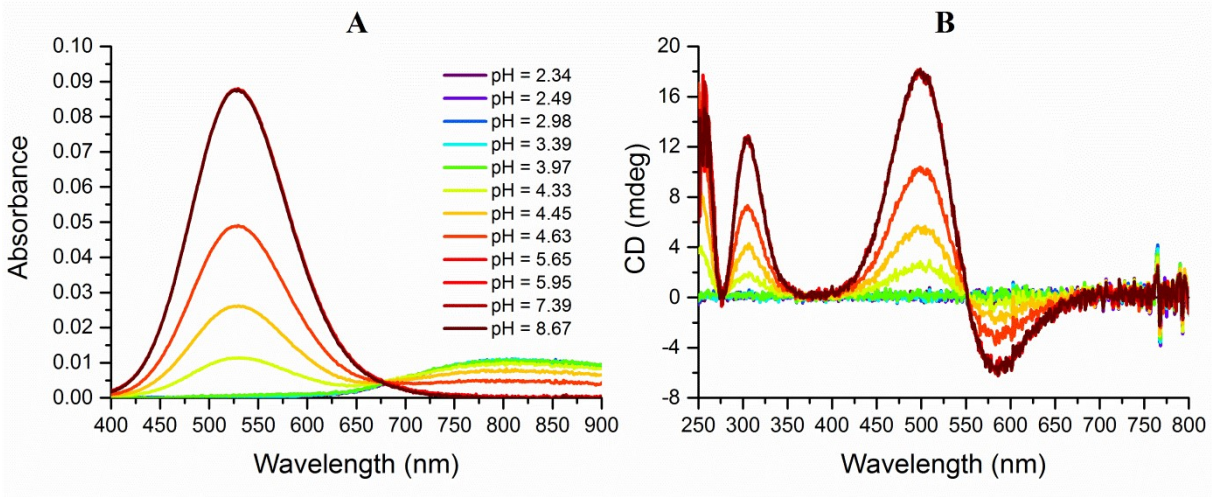

Figure S3. A) UV−Vis and B) CD pH-metric titrations of 1 mM GGH-am in the presence of 0.8 mM $CuCl_2$. The order of the spectra is rainbow-coded from purple to red and the pH values are identical for UV-Vis and CD titrations.

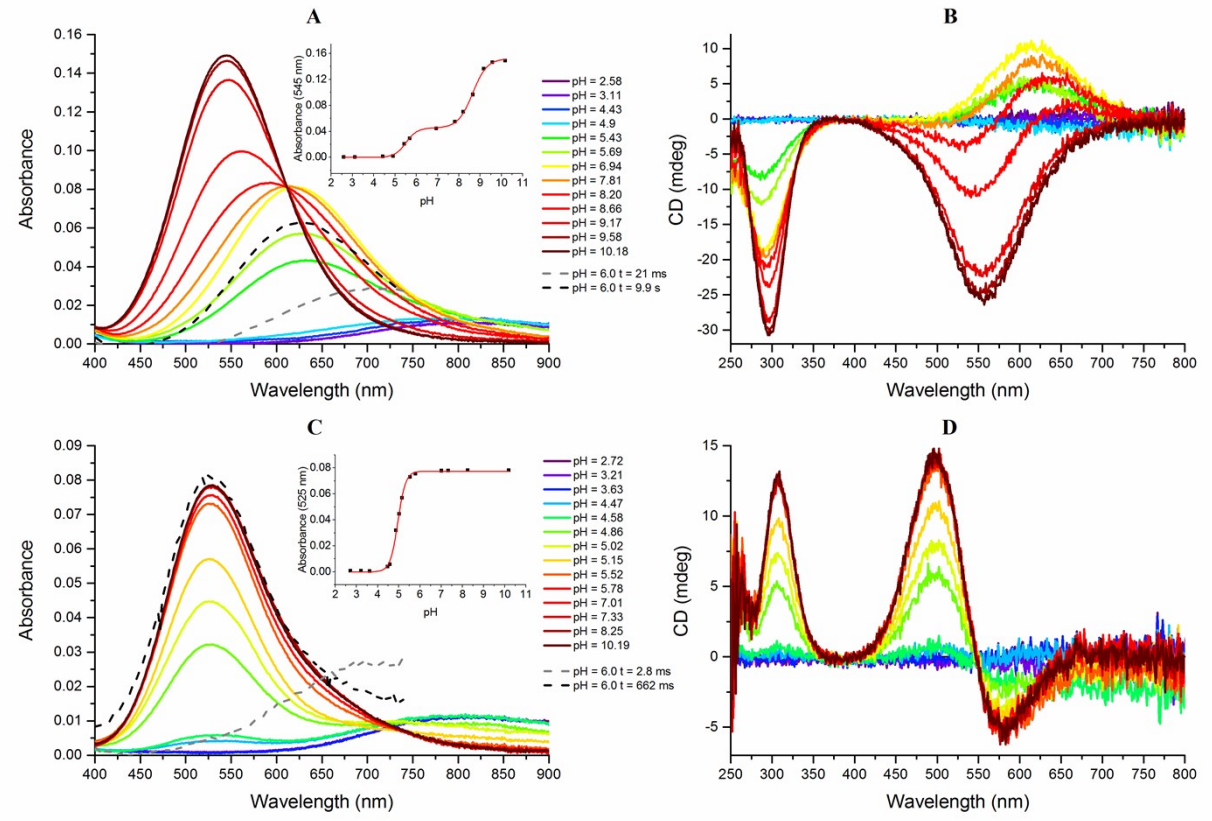

Figure S4. UV−Vis and CD pH-metric titrations of 1 mM GG(Nπ-Me)H (plots A,B) and GG(Nτ-Me)H (plots C,D) in the presence of 0.8 mM $CuCl_2$. The order of the spectra is rainbow-coded from purple to red (solid lines) and the pH values are identical for UV-Vis and CD titrations. The grey and black spectra (dashed lines) on UV-Vis panels are the diode-array spectra recorded at the beginning and at the end of the reaction, respectively. The insets in plots A and C are titration curves from the respective experiments. The absorbance signals from kinetic experiments were multiplied by a constant to match the concentrations used in titration experiment.

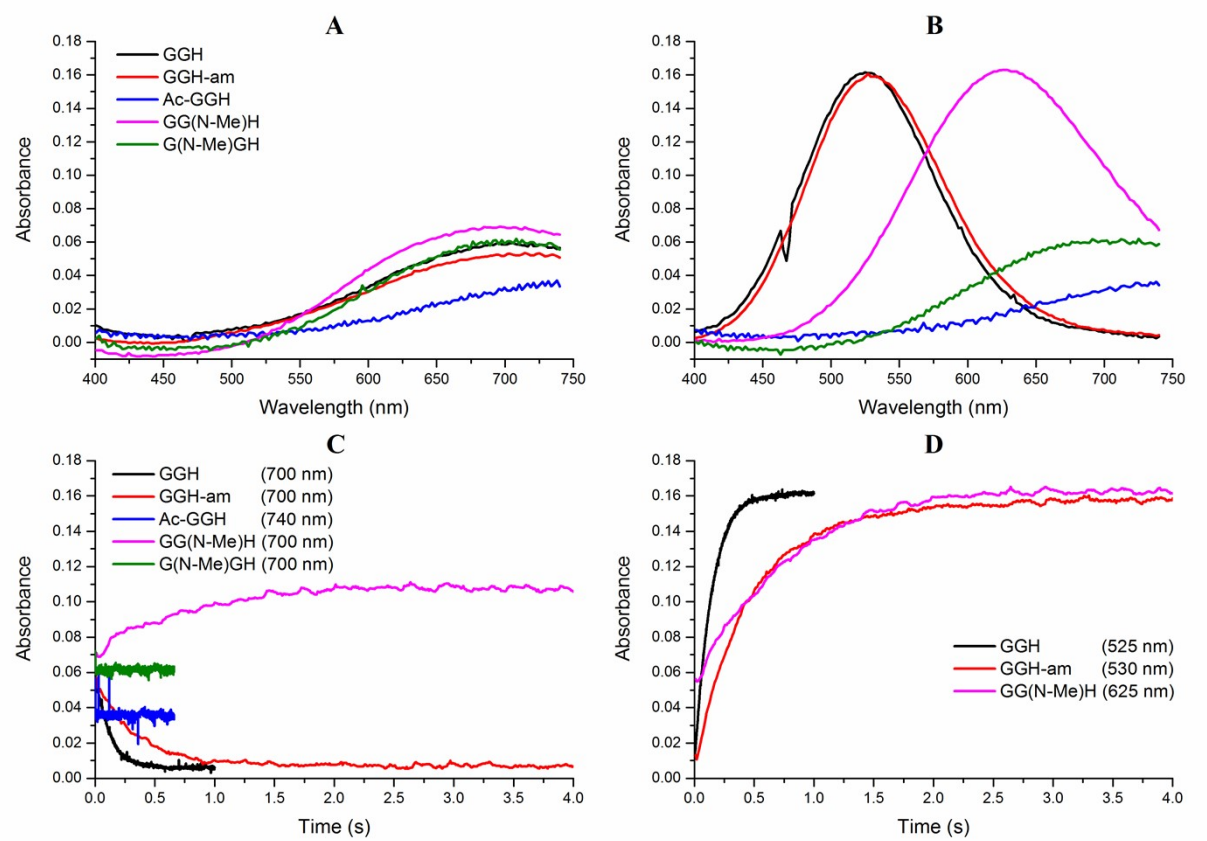

Figure S5. Diode-array stopped-flow experiments for 1.6 mM Cu(II) reacted with four different analogues (as outlined on the plot) in comparison to the reaction with GGH from ref. [S4] (black line). The upper plots show the kinetic spectra A) at the beginning and B) at the end of the reaction. The lower plots shows the kinetic curves for each reaction C) at the maximum of the initial spectrum and D) at the maximum absorbance of the final complex.

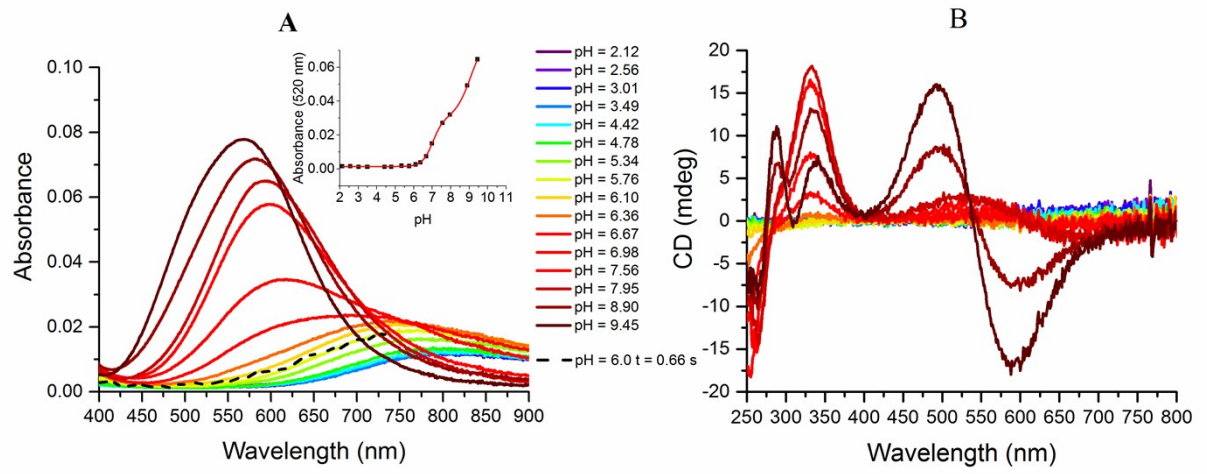

Figure S6. A) UV−Vis and B) CD pH-metric titrations of 1 mM Ac-GGH in the presence of 0.8 mM $CuCl_2$. The order of the spectra is rainbow-coded from purple to red (solid lines), and the pH values are identical for UV-Vis and CD titrations. The black spectra (dashed line) on UV-Vis panels are the diode-array spectra recorded at the end of the reaction, multiplied by a constant to match the concentrations used in titration experiment. Inset in plot A shows the pH titration generated for absorbance at 520 nm.

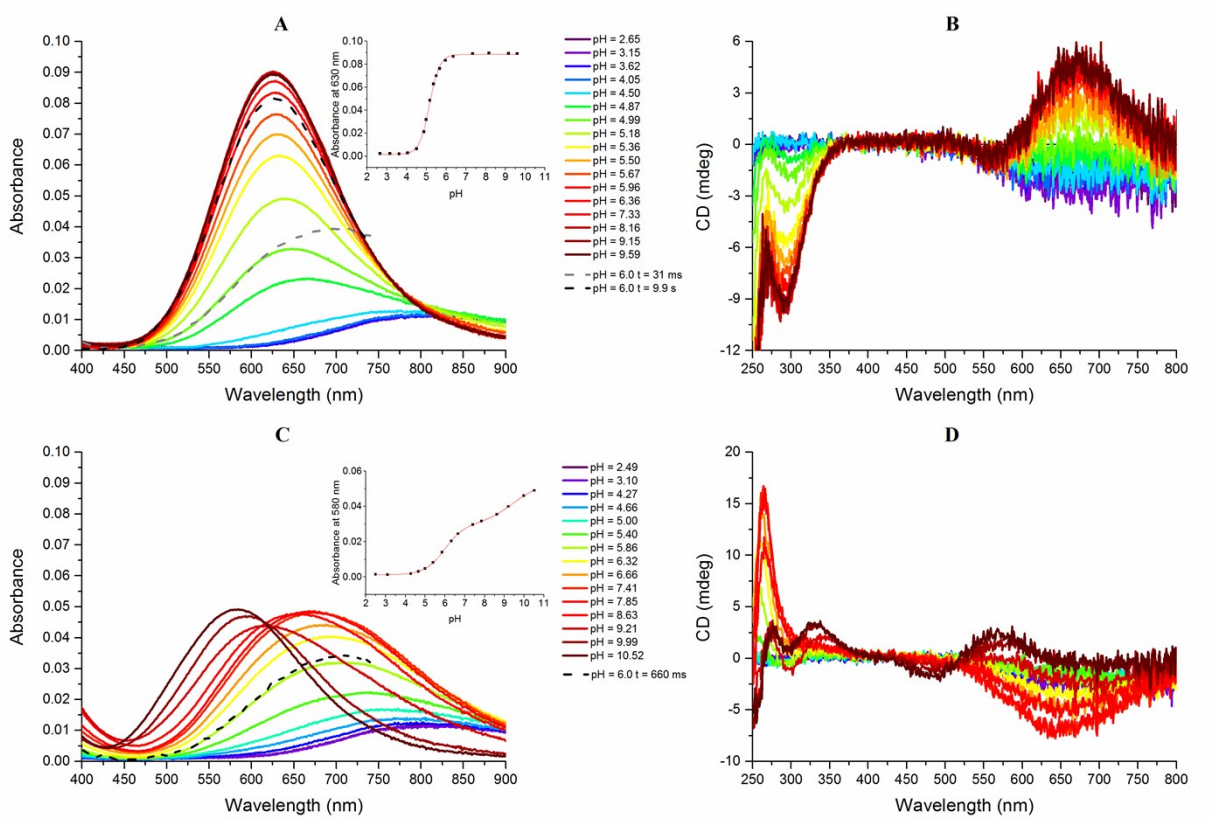

Figure S7. UV−Vis and CD pH-metric titrations of 1 mM GG(N-Me)H (panels A,B) and G(N-Me)GH (panels C,D) in the presence of 0.8 mM $CuCl_2$. The order of the spectra is rainbow-coded from purple to red (solid lines), and the pH values are identical for UV-Vis and CD titrations. The grey and black spectra (dashed lines) on UV-Vis panels denotes to diode-array spectra recorded at the beginning and at the end of the reaction, respectively. The insets in plots A and C are titration curves from the respectively experiments. The absorbance signals from kinetic experiments were multiplied by a constant to match the concentrations used in titration experiment.

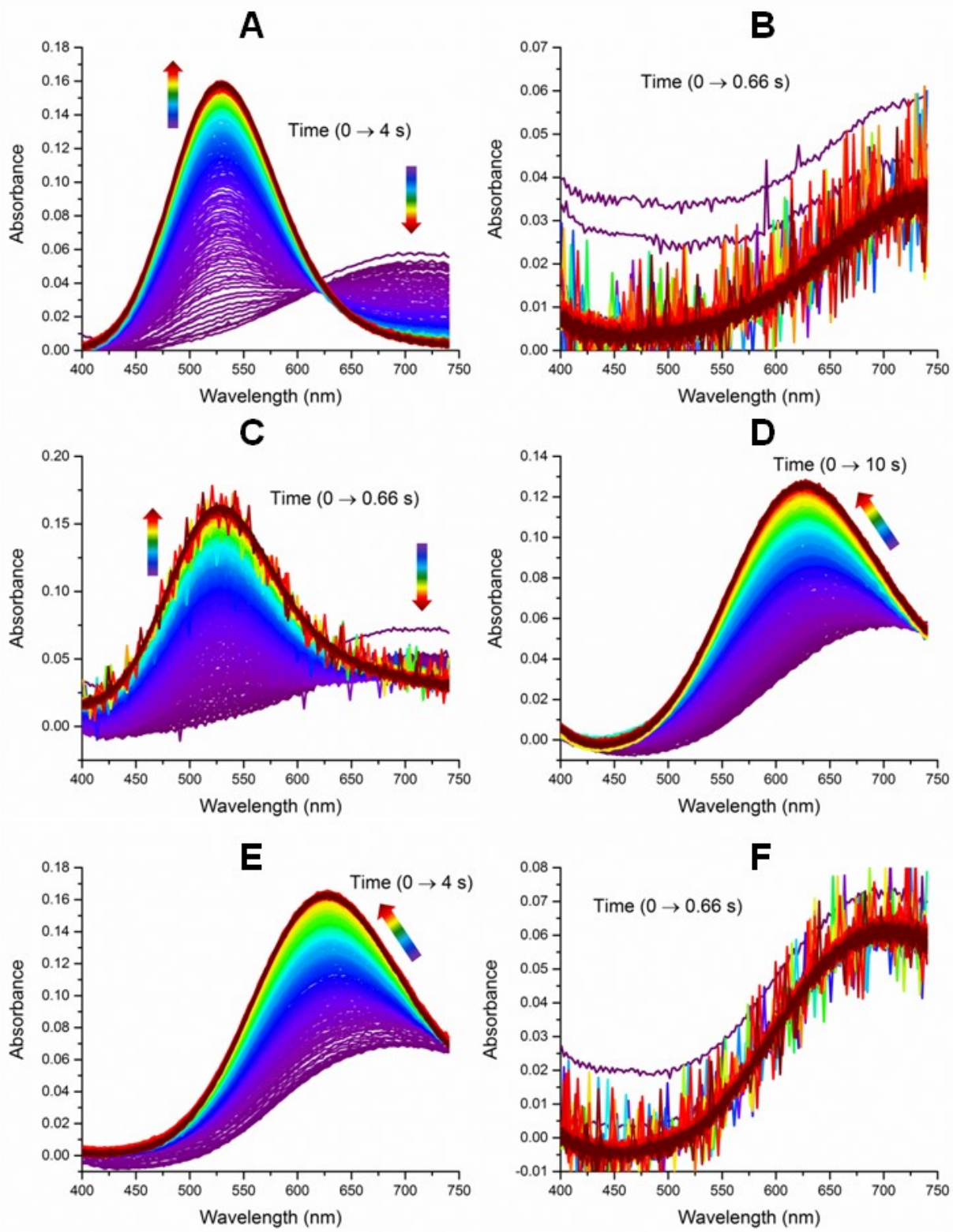

Figure S8. Diode-array stopped-flow experiments for mixing 3.2 mM Cu(II) and 4 mM peptide in 400 mM MES, pH 6.0; A) GGH-am, B) Ac-GGH, C) GG(Nτ-Me)H, D) GG(Nπ-Me)H, E) GG(N-Me)H, F) G(N-Me)GH. The time scale is rainbow-coded from purple to red and the direction of changes is indicated by the matching rainbow arrow.

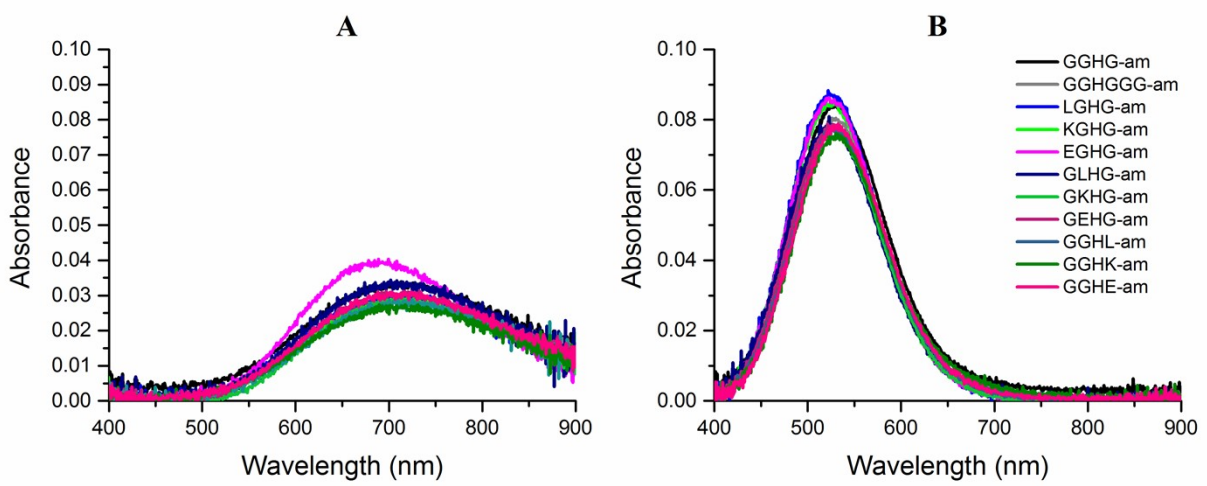

Figure S9. Kinetic spectra for mixing 3.2 mM Cu(II) with 4 mM GGHG-am (at 400 mM MES in pH = 6,0) and its derivatives recorded at A) the beginning and B) end of the reaction. The small deviations of the intensity of the spectra stem mostly from the diode-array detector baseline shift or slight differences in the Cu(II) concentrations, except of EGHG-am (see main text for details).

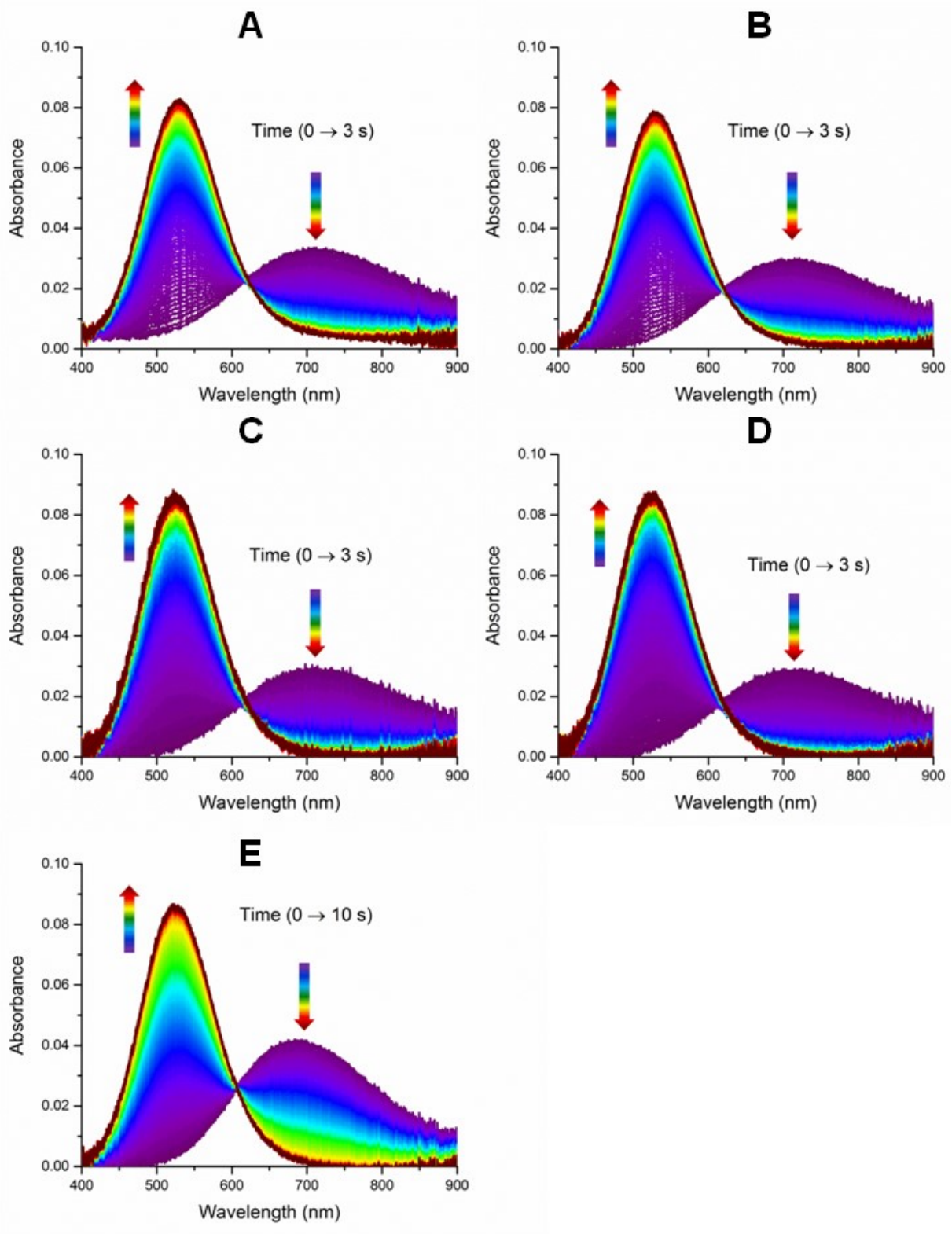

Figure S10. Diode-array stopped-flow experiments for mixing 3.2 mM Cu(II) and 4 mM peptide in 400 mM MES, pH 6.0; A) GGHG-am, B) GGHGGG-am, C) LGHG-am, D) KGHG-am, E) EGHG-am. The time scale is rainbow-coded from purple to red and the direction of changes is indicated by the matching rainbow arrow.

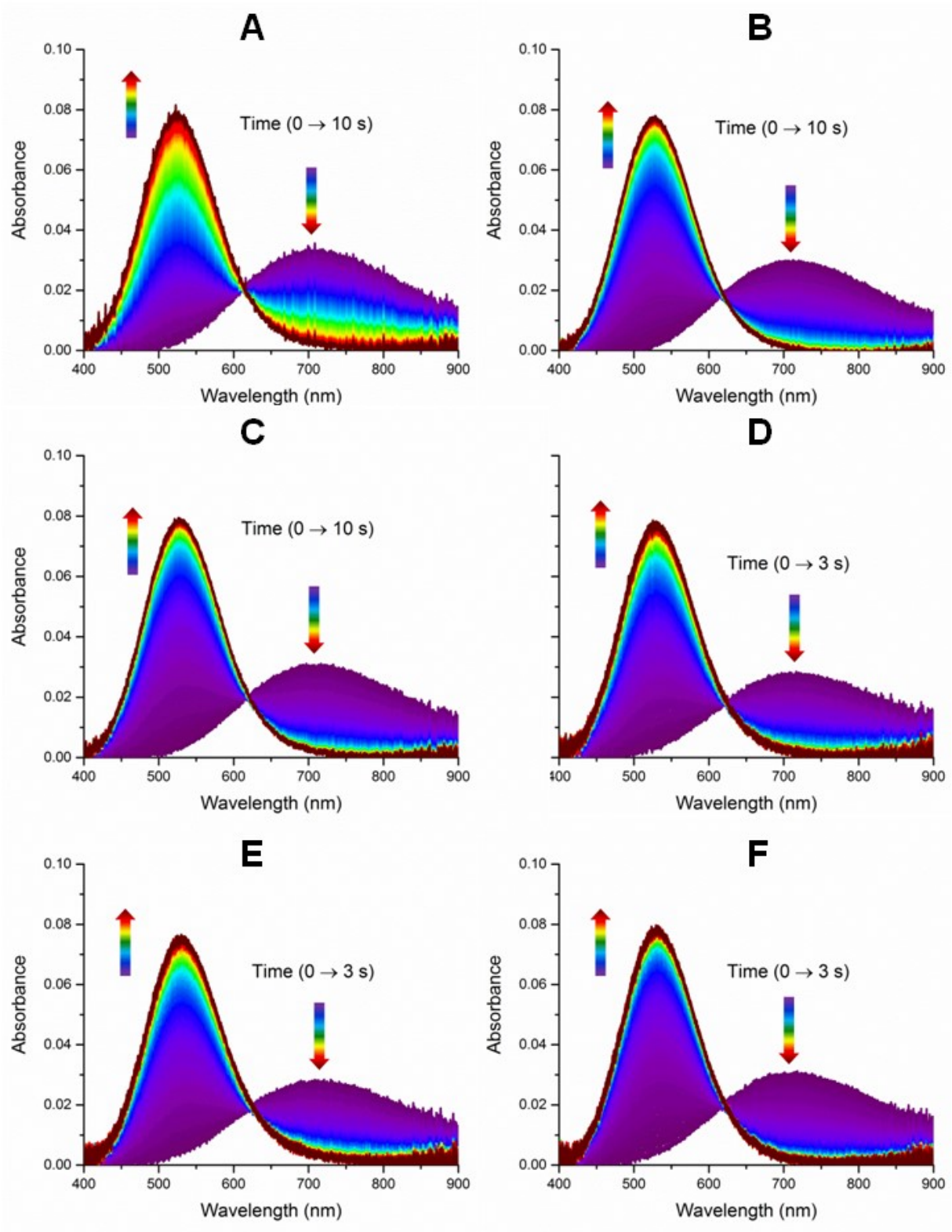

Figure S11. Diode-array stopped-flow experiments for mixing 3.2 mM Cu(II) and 4 mM peptide in 400 mM MES, pH 6.0; A) GLHG-am, B) GKHG-am, C) GEHG-am, D) GGHL-am, E) GGHK-am, F) GGHE-am. The time scale is rainbow-coded from purple to red and the direction of changes is indicated by the matching rainbow arrow.

References

[S1] W. C. Chan and P. D. White, Eds., Fmoc solid phase peptide synthesis: a practical approach, New York: Oxford University Press, 2000, 222.

[S2] K. A. Johnson, Z. B. Simpson, T. Blom, Anal. Biochem. 2009, 387, 20.

[S3] K. A. Johnson, Z. B. Simpson, T. Blom, Anal. Biochem. 2009, 387, 30.

[S4] R. Kotuniak, M. J. F. Strampraad, K. Bossak-Ahmad, U. E. Wawrzyniak, I. Ufnalska, P.-L. Hagedoorn and W. Bal, Angew. Chem., Int. Ed., 2020, 59, 11234–11239.