Electronic Supplementary Material (ESI) for Metallomics.

Supporting Information

Hierarchical Binding of Copper^{II} to N-truncated Aβ_{4-16} Peptide

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Materials

N-truncated $A\beta_{4\text{-}16}$ peptide was synthesized according to Fmoc strategy and purified by HPLC as described before.[1] Labeled $A\beta_{4\text{-}16}$ peptide containing HiLyte 488 fluorescent dye at the C-terminal lysine was purchased from AnaSpec (USA) via Cambridge Bioscience (UK). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution, Ethylenediaminetetraacetic acid (EDTA) solution were purchased from AppliChem GmbH (Germany). Sodium chloride (NaCl) was purchased from Sigma-Aldrich (UK) and dissolved in water to prepare a 2 M stock solution. Copper^{II} chloride dihydrate ($CuCl_2 \bullet 2H_2O$) was purchased from Alfa Aesar (USA) and dissolved in water to prepare a 20 mM stock solution. N-truncated $A\beta_{4\text{-}16}$ peptide was dissolved in 50 mM HEPES buffer solution (pH 7.5) containing 100 mM NaCl prior to experiments, and stored at -20 ℃. The concentration of N-truncated $A\beta_{4\text{-}16}$ was determined measuring the peak absorbance of the dye (ε = 70000 M^{-1} cm^{-1}). This measurement was performed with a Lambda 25 UV/Vis spectrometer (PerkinElmer, USA).

Experimental Sections

Stopped flow

All Cu^{II} binding measurements were performed on a KinetAsyst SF-610X2 stopped flow spectrophotometer (HI-TECH Scientific, UK). Samples were excited by a fibre-coupled MCLS1-473-20 diode laser at 473 nm (Thorlabs, USA). In double mixing experiments, fluorescence emission was filtered using a 515 nm long pass neutral density filter (Comar, UK) before being detected by a photon multiplier tube. Data points were recorded using a logarithmic time-scale sampling scheme. For each data point, a minimum of 10 traces were averaged. Time points below 2 ms were excluded due to the instrument dead time (~1 ms).

Data Analysis

The averaged raw curves were analyzed using OriginPro 2015 (OriginLab, USA). Each curve was fitted with equation (1) which was consisted of double exponential components applying Levenberg-Marquardt nonlinear regression analysis.

$$y = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_3 e^{-k_3 t} \tag{1}$$

In the analysis of Cu^{II} binding experiment, mean rate values of different HEPES concentration were calculated by equation (2), and errors were calculated by equation (3). The amplitude of the third component (A_3) was ignored in further analysis due to its negligibility. In order to determine Cu^{II} binding rate constant k_{on}, mean rate values of different HEPES concentration were empirically fitted with a parabola centred at zero, and the errors associated with this fitting were propagated by inverse error propagation.

$$mean\ k = \frac{A_1 k_1 + A_2 k_2 + A_3 k_3}{A_1 + A_2 + A_3} \tag{2}$$

$$\sigma_F^2 = (\frac{\partial F}{\partial A_1})^2 \cdot \sigma_{A_1}^2 + \left(\frac{\partial F}{\partial k_1}\right)^2 \cdot \sigma_{k_1}^2 + \left(\frac{\partial F}{\partial A_2}\right)^2 \cdot \sigma_{A_2}^2 + \left(\frac{\partial F}{\partial k_2}\right)^2 \cdot \sigma_{k_2}^2 + \left(\frac{\partial F}{\partial A_3}\right)^2 \cdot \sigma_{A_3}^2 + \left(\frac{\partial F}{\partial k_3}\right)^2 \cdot \sigma_{k_3}^2 \tag{3}$$

In the analysis of double mixing experiment, curves were globally fitted by sharing the rate values for Species I and Species II respectively.

UV-Vis spectroscopy

UV-Vis measurements were performed on a Cary 60 spectrophotometer at room temperature (between 20-25 °C) in the spectral range 200-800 nm, as well as Scanning Kinetics at 525 nm. A 500 mM EDTA was purchased from Sigma-Aldrich. A 500 mM potassium phosphate buffer at pH 7.4 was prepared by mixing potassium dihydrogen phosphate 99 % (KH_2PO_4) with potassium hydrogen phosphate 98 % (KH_2PO_4) in Milli-Q water. Stock solutions were further diluted to the desired concentration 100mM for the following experiments. First the Cu^{II}A$\beta_{4\text{-}16}$ complex was generated with a stoichiometric ratio Cu^{II}: A$\beta_{4\text{-}16}$, 0.9:1, (0.9 mM : 1 mM) to avoid the presence of free Cu^{II}. The copper transfer from Cu^{II}A$\beta_{4\text{-}16}$ to EDTA was checked in different EDTA concentrations. Total 39 experiments were performed: 4x 5 mM of EDTA, 3x 10 mM of EDTA, 3x 15 mM of EDTA, 5x 20 mM of EDTA, 4x 30 mM of EDTA, 5x 40mM of EDTA, 3x 50mM of EDTA, 3x 60 mM of EDTA, 3x 80 mM of EDTA, 3x 100 mM of EDTA, 3x 120 mM of EDTA. The fitting of the apparent dissociation rate was with an empirical exponential function in order to extrapolate k_{off}.

Supporting Figures

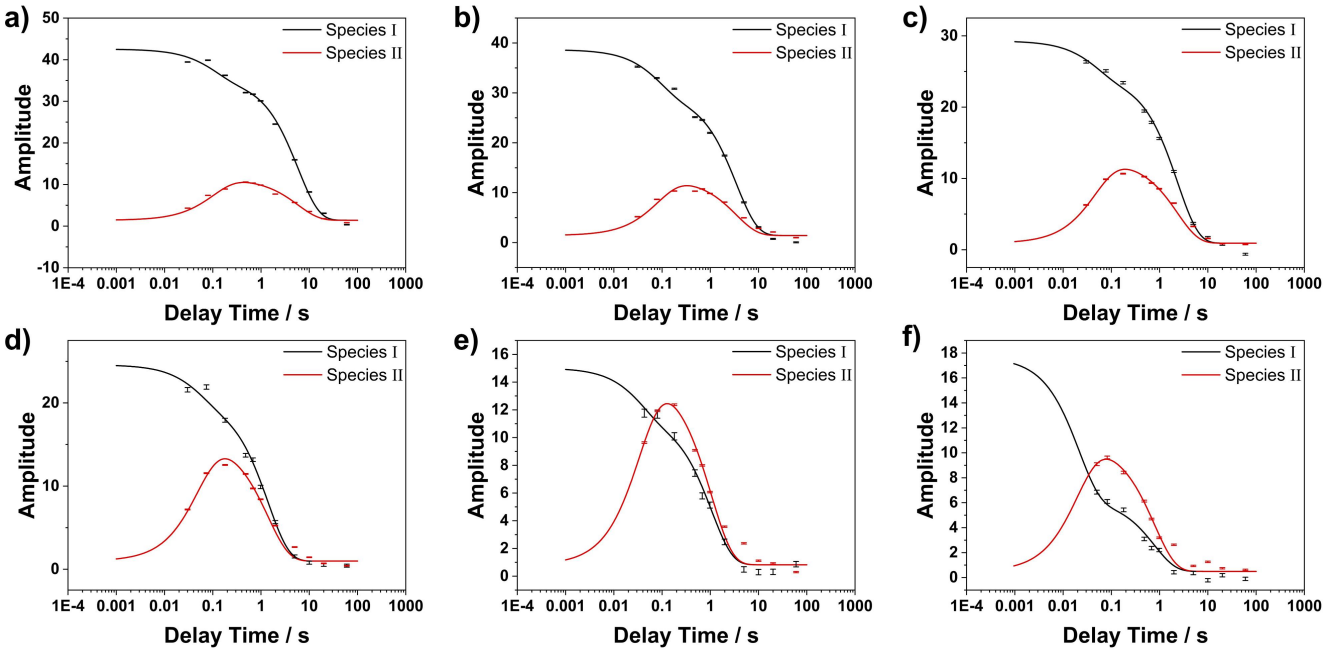

Figure S1. Evolutions of the two intermediate species are temperature dependent. From (a) to (f): 283 K, 288 K, 293 K, 298 K, 303 K, 308 K, respectively.

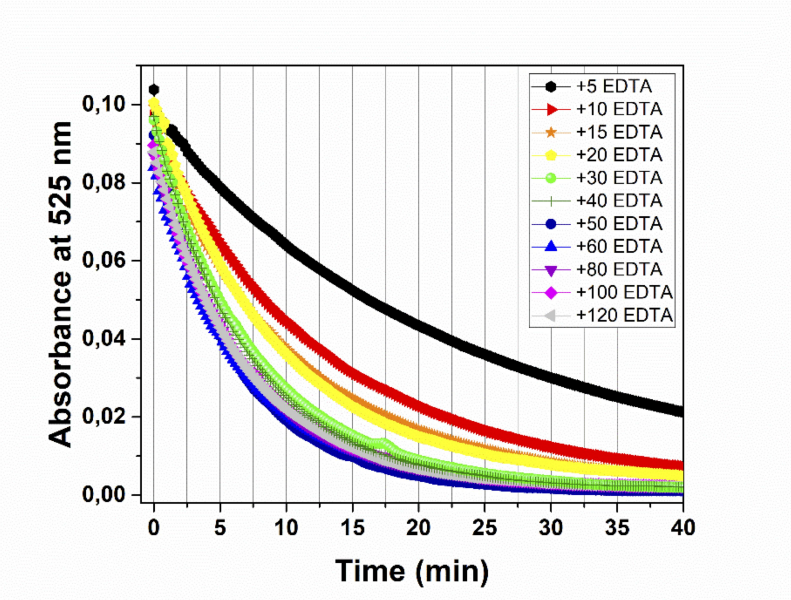

Figure S2. Kinetics of dissociation of $Cu^{II}A\beta_{4\text{-}16}$ after mixing with different ratios of EDTA was monitored by UV-Vis spectroscopy, by measuring absorbance at 525 nm, the maximum of the d-d band of the complex.[1]

Table S1. Rate constants of Cu^{II} binding process after ~2 s from Fig. 2a.

[Aβ], [Cu^{II}] / μM	0.50	0.75	1.0	1.5	2.0	2.5
k' / 10^{-1} s^{-1}	3.23(1)	2.38(2)	2.08(2)	2.63(1)	2.82(2)	2.96(1)

Reference

1 M. Mital, N. E. Wezynfeld, T. Frączyk, M. Z. Wiloch, U. E. Wawrzyniak, A. Bonna, C. Tumpach, K. J. Barnham, C. L. Haigh, W. Bal, S. C. Drew, *Angew. Chem.* 2015, **127**, 10606-10610; *Angew. Chem. Int. Ed.* 2015, **54**, 10460–10464.