

## SUPPORTING INFORMATION

### Ascorbate oxidation by Cu(Amyloid- $\beta$ ) complexes: determination of the intrinsic rate as function of alterations in the peptide sequence reveals key residues for ROS production

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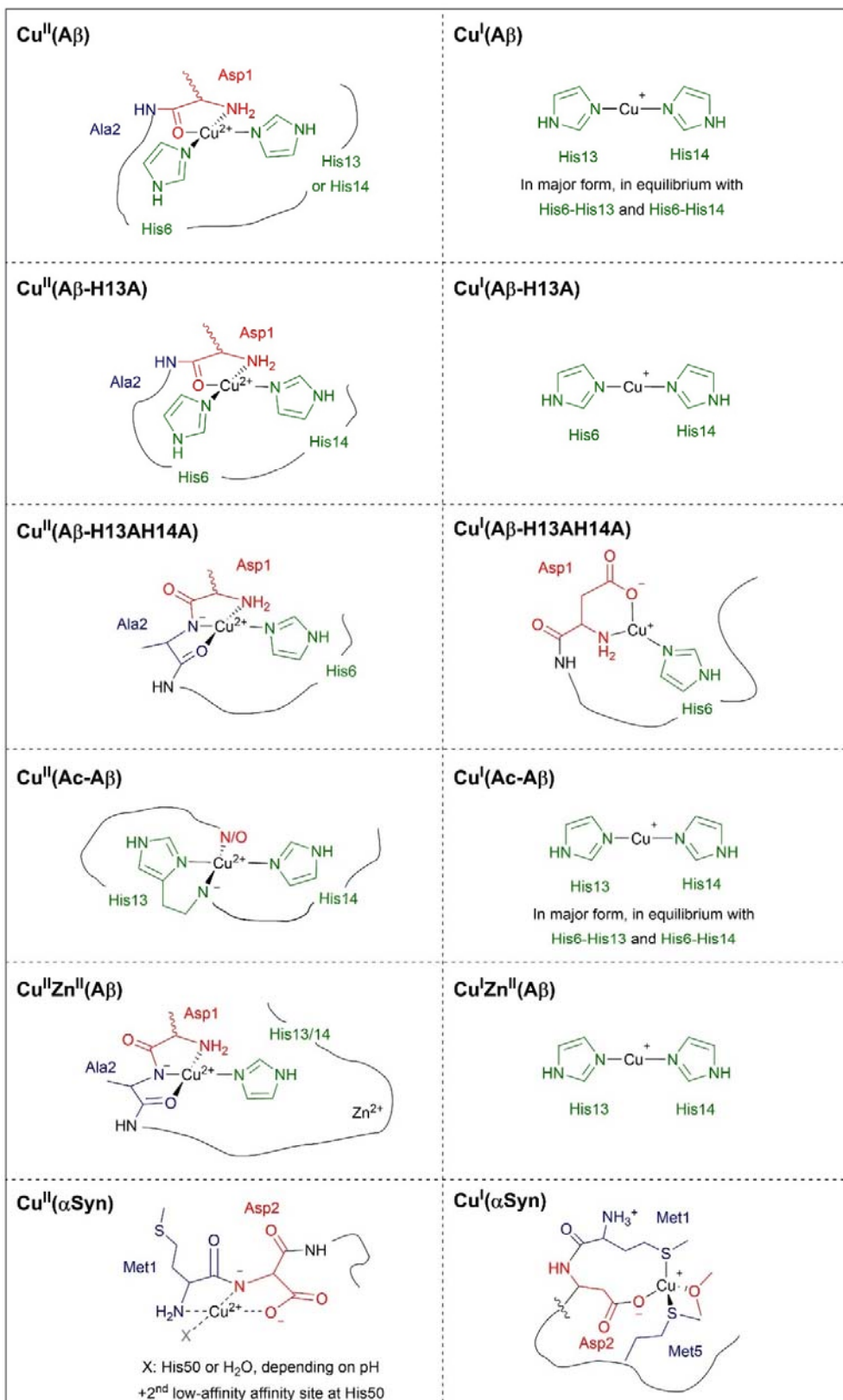
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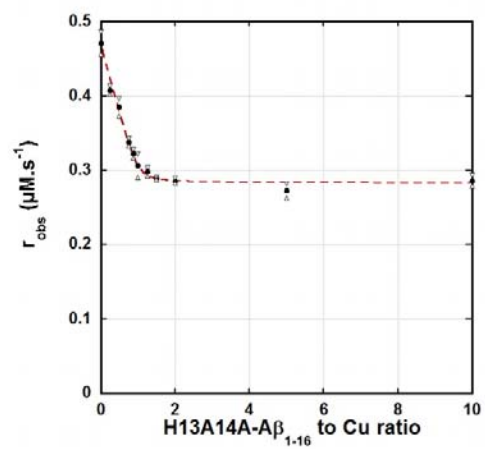
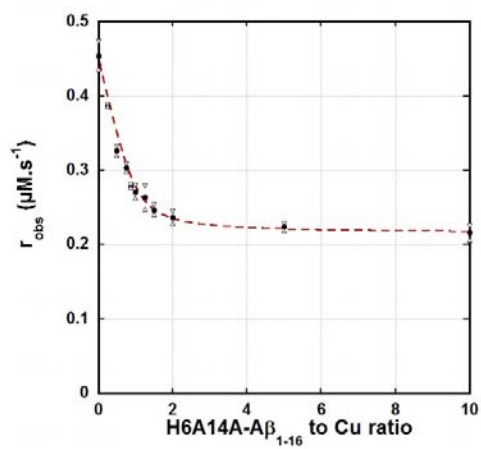
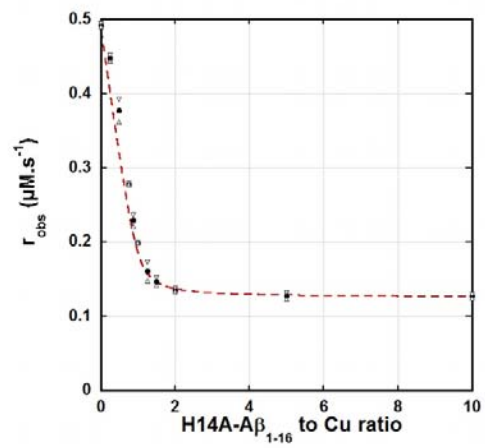
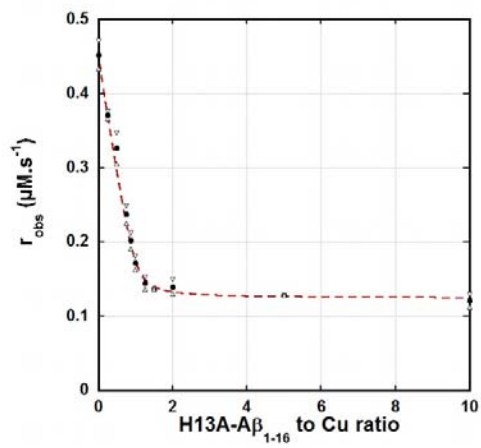
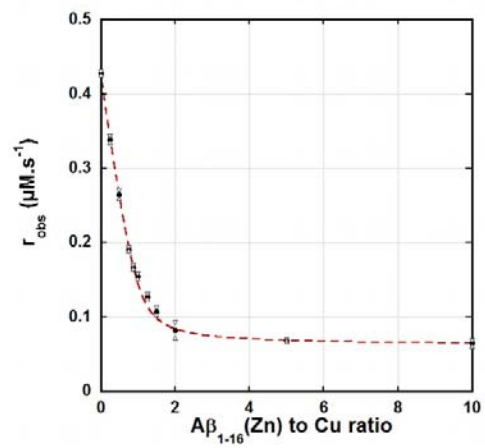
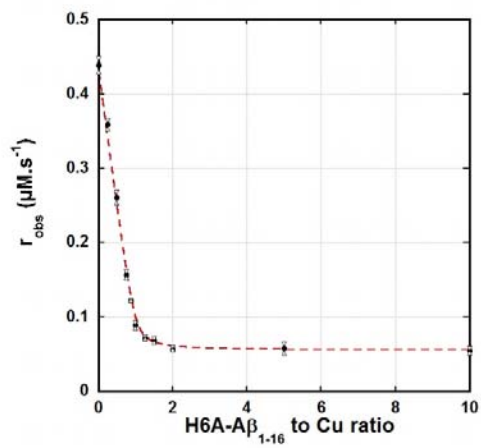
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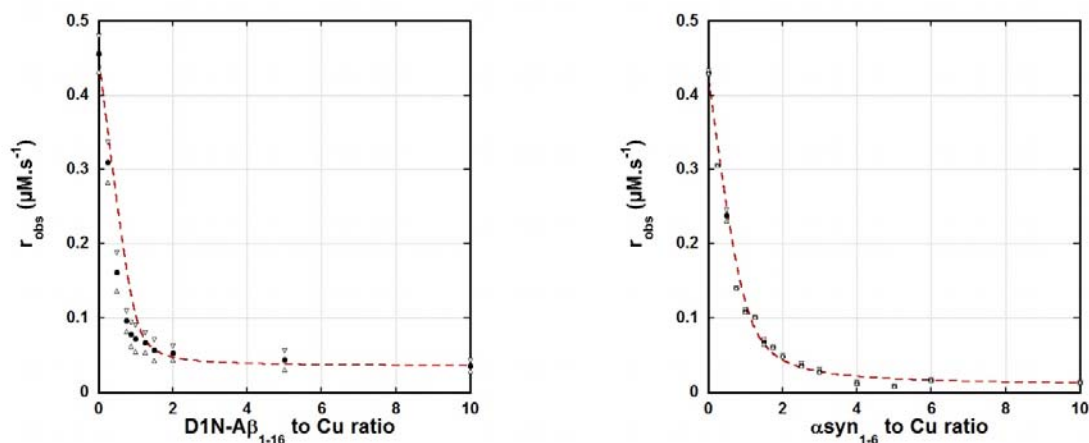
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**Figure S-1.** Main coordination sites for Cu(II) and Cu(I) at physiological pH for some of the different peptides used in this work.





**Figure S-2.** Initial molar ascorbate oxidation rate as a function of peptide to Cu ratio for the various peptides studied here. Dots correspond to experimental data (given as mean  $\pm$  S.D.,  $n = 3$ ), best fits are given as a dashed line. HEPES 0.05 M, pH 7.4; [Cu] = 10  $\mu$ M, [Asc] = 100  $\mu$ M. For fitting values, see Table 1.

For the D1N peptide, it is worth noting that the curves cannot be explained if a simple equilibrium between bound and unbound Cu is considered. This is for instance illustrated by the fact that at Cu to peptide ratio of 2, the rate of ascorbate oxidation is lower than the mean between the rate of free Cu and the intrinsic rate of Cu(D1N-A $\beta_{1-16}$ ). Thus, the simple two states model used for the other peptides is not sufficient. Because at the pH of the experiments, D1N binds Cu(II) in its N-terminal part, leaving two His available for binding to Cu(I), it is proposed that a 2:1 Cu:peptide complex is present in solution. This means that most all the points before the 1:1 stoichiometry cannot be used. Points at very high peptide to Cu ratio will however give access to the  $r_i^P$  value. Evaluation of the  $K^P$  is more complex and only a rough estimation can be obtained using only the values at ratio higher than 1:1.

### Evaluation of $K^P$ and $r_i^P$ values.

A short Kaleidagraph program was made *in house* to obtain theoretical curves and adjustments of parameters were done to best reproduce the experimental data.

Theoretical curves are calculated according to:

$$c2 = 1/(C_0 * K^P) + c1 + 1 ; \quad (1)$$

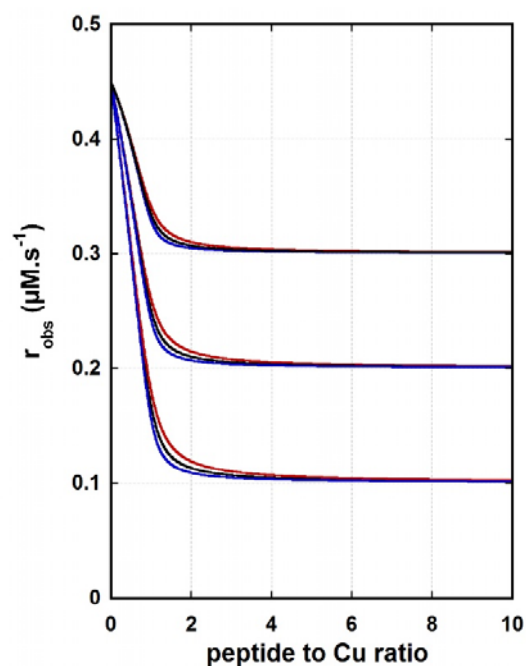
$$c3 = c2 * c2 - 4 * c1 ; \quad (2)$$

$$c4 = (c2 - \text{sqrt}(c3)) / 2 ; \quad (3)$$

$$c5 = r^f * ((1 - c4) - \text{corr} * (1 - c4)^2 * C_0) + r_i^P * c4 ; \quad (4)$$

where  $c1$  is a column with values varying from 0 to 10 by step of 0.01;  $C_0$  is the initial Cu concentration,  $K^P$ ,  $r_i^P$ ,  $r^f$  and  $\text{corr}$  are defined in the text.  $C_i$ ,  $i = 2-4$  correspond to the resolution of the second order equation given by  $K^P C_0 = \frac{\alpha}{(1-\alpha)(s-\alpha)}$  (5) and  $c4$  to the parameter  $\alpha$ .  $c5$  is the theoretical curve.

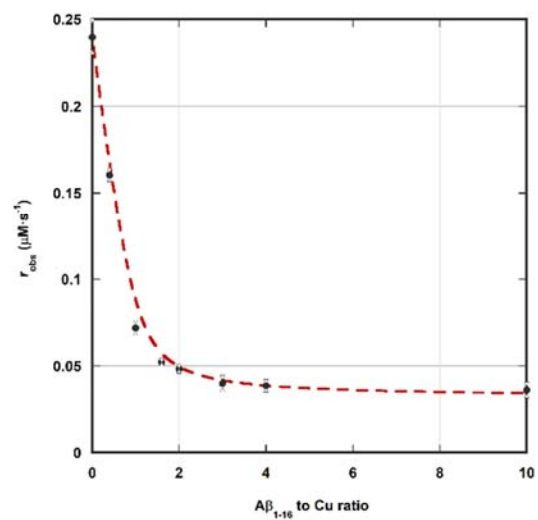
While the  $r_i^P$  value is strongly constrained by the values of  $r_{obs}$  at high peptide to Cu ratio, the  $K^P$  value is more difficult to determine with a high precision. To illustrate this point, we plot the theoretical curves with varying  $K^P$  ( $2 \times 10^6$ ,  $3 \times 10^6$  and  $4.5 \times 10^6 \text{ M}^{-1}$ ) for three given values of  $r_i^P$  (0.1, 0.2 and  $0.3 \mu\text{M}^{-1}$ ) in the figure below.



**Figure S-3.** Theoretical curves of  $r_{obs}$  as a function of  $K^P$  and  $r_i^P$  0.1  $\mu\text{Ms}^{-1}$ ; middle panel:  $r_i^P = 0.2 \mu\text{Ms}^{-1}$  and right panel:  $r_i^P = 0.1, 0.2$  and  $0.3 \mu\text{Ms}^{-1}$ .

Red lines:  $K^P = 2 \times 10^6 \text{ M}^{-1}$ ; black lines:  $K^P = 3 \times 10^6 \text{ M}^{-1}$  and blue lines:  $K^P = 4.5 \times 10^6 \text{ M}^{-1}$ .  $r^f = 0.52 \mu\text{Ms}^{-1}$ ,  $\text{corr} = 14 \times 10^3 \text{ M}^{-1}$ ,  $[\text{Cu}] = 10 \mu\text{M}$ ,  $[\text{Asc}] = 100 \mu\text{M}$ .

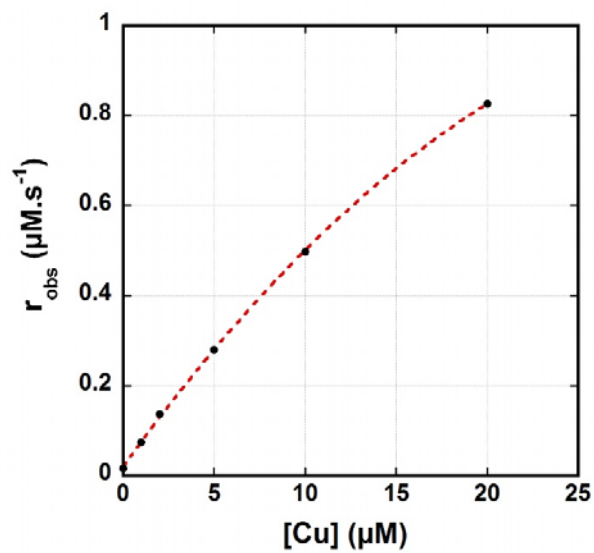
It is important to note here that a solution to improve the precision on the  $K^P$  value would have been to decrease the working concentration in Cu. We studied the Cu(A $\beta$ ) at  $[\text{Cu}] = 5 \mu\text{M}$  and found the same  $K^P$  and  $r_i^P$  values. But we observed that while increasing the theoretical precision on the  $K^P$  value, the error due to measurements increased (the  $r_{obs}$  measured becoming lower) as well, leading to an annulation of the potential benefit.



**Figure S-4.** Initial molar ascorbate oxidation rate as a function of peptide to Cu ratio for Aβ<sub>1-16</sub> at [Cu] = 5 μM.

Dots correspond to experimental data (given as mean ± S.D., n = 3), best fit is given as a dashed line ( $K^P = 2$ ;  $r_i^P = 58$  nMs<sup>-1</sup>). HEPES 0.05 M, pH 7.4; [Cu] = 5 μM, [Asc] = 100 μM.

Another important note is about the evaluation of the *corr* parameter. We decided to set its value and determined it by the quadratic fitting of the data  $r_{obs} = f([Cu])$  plotted in Figure S5. The *corr* parameter is the ratio between the first and second coefficient of the quadratic function.



**Figure S-5.** Curve of  $r_{obs}$  as a function of [Cu] (black dots) and its quadratic fitting. [Asc] = 100 μM, [HEPES] = 0.05 M, pH 7.4.