

## Supplementary Information

### Link Between Affinity and Cu(II) Binding Sites to Amyloid- $\beta$ Peptides Evaluated by a New Water-Soluble UV-Visible Ratiometric Dye with a Moderate Cu(II) Affinity

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## 1- CHARACTERIZATION OF THE LIGAND AND OF THE Cu(L) COMPLEX

### Characterization of $H_3Et_2L$ . 3,4-(diethyloxamate)benzoic acid

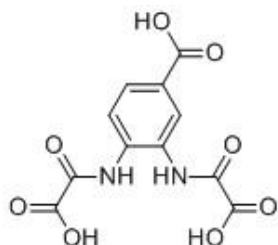
$^1H$  NMR (400 MHz, DMSO)  $\delta$  13.06 (s, 1H), 10.50 (d,  $J = 18.9$  Hz, 2H), 8.12 (d,  $J = 2.0$  Hz, 1H), 7.85 (dd,  $J = 8.5, 2.0$  Hz, 1H), 7.78 (d,  $J = 8.5$  Hz, 1H), 4.32 (qd,  $J = 7.1, 1.5$  Hz, 4H), 1.32 (td,  $J = 7.1, 1.9$  Hz, 6H).  $^{13}C$  NMR (101 MHz, DMSO)  $\delta$  166.26, 160.00, 155.77, 155.57, 133.88, 129.13, 128.23, 127.26, 126.99, 124.96, 62.61, 62.53, 13.79, 13.77. IR ( $cm^{-1}$ ): 3300(m), 3215(w), 1760(m), 1690(s), 1611(w), 1595(m), 1530(m), 1491(w), 1444(w), 1410(w), 1388(m), 1370(w), 1312(w), 1257(w), 1225(m), 1175(s), 1125(w), 1014(m), 945(w), 900(w), 862(m), 845(w), 820(w), 809(w), 774(s), 745(w), 707(w), 640(w), 608(w), 561(w), 542(m), 496(w), 458(m), 378(w), 328(m). Elemental analysis (%) calculated for  $C_{15}H_{16}N_2O_8$  ( $M_r = 352.29$  g mol $^{-1}$ ): C 51.13, H 4.57, N 7.95. Found: C 51.14, H 4.43, N 8.00.

### Characterization of $Na_{1.5}H_{3.5}L \cdot 2.2H_2O$

$^1H$  NMR (400 MHz, DMSO)  $\delta$  10.48 (d,  $J = 18.1$  Hz, 2H), 8.14 (d,  $J = 1.7$  Hz, 1H), 7.88 – 7.75 (m, 1H).  $^{13}C$  NMR (101 MHz, DMSO)  $\delta$  166.45, 161.67, 161.65, 158.74, 158.62, 134.31, 129.16, 127.71, 127.01, 126.72, 124.22. IR ( $cm^{-1}$ ): 3272(w), 3194(s), 1659(s), 1607(w), 1529(m), 1492(w), 1442(w), 1359(m), 1292(s), 1226(w), 1183(m), 1098(w), 928(w), 904(w), 841(w), 819(w), 757(m), 727(w), 707(w), 506(w), 452(m). Elemental analysis (%) calculated for  $C_{11}H_{10.9}N_2Na_{1.5}O_{10.2}$  ( $M_r = 368.79$  g mol $^{-1}$ ): C 35.82, H 2.97, N 7.59. Found: C 35.89, H 3.02, N 7.50.

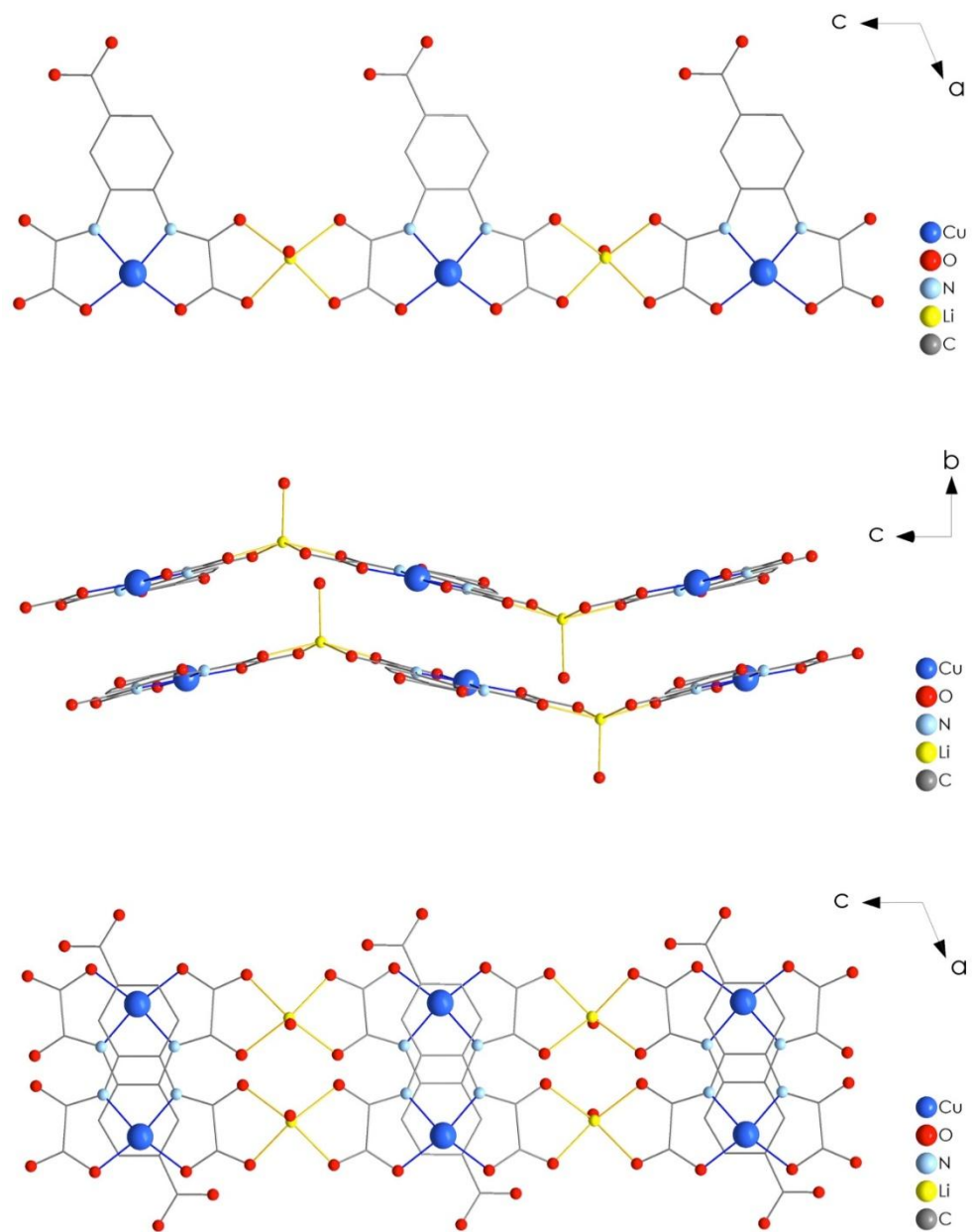
### Characterization of $Li_3[Cu(L)] \cdot 5.5H_2O$ , $Cu(L)$ .

IR ( $cm^{-1}$ ): 3298(bd), 1611(s), 1577(s), 1539(m), 1444(w), 1369(s), 1319(s), 1256(w), 1210(w), 1086(w), 981(w), 875(w), 787(m), 557(w), 448(w). Elemental analysis (%) calculated for  $C_{11}H_{14}CuLi_3N_2O_{13.5}$  ( $M_r = 474.6$  g mol $^{-1}$ ): C 27.83; H 2.97; N 5.90. Found: C 27.95; H 2.45; N 5.86.



**Scheme S1.** Acidic form of the ligand,  $H_5L$

Supramolecular arrangement/Crystal packing. On the oxamate groups, the remaining carbonyl functions chelate lithium ions. As a result, these ions link two adjacent complexes, generating thus a corrugated chain of complexes along the crystallographic  $c$  axis. The chains are further stacked along the crystallographic  $b$  axis (see Figure S1). See Table S1 and S2 for crystallographic data and for selected bond lengths and angles.



**Figure S1.** Crystal packing of Cu(L).

**Table S1. Crystallographic data.**

Formula <sup>a</sup>	C <sub>11</sub> H <sub>5</sub> CuLi <sub>3</sub> N <sub>2</sub> O <sub>9</sub>
<i>F.W.</i> [g mol <sup>-1</sup> ]	393,53
Crystal system	Monoclinic
Space group	<i>C</i> 2/ <i>c</i>
<i>a</i> [Å]	26.3214(15)
<i>b</i> [Å]	6.7217(4)
<i>c</i> [Å]	20.4183(11)
$\alpha$ [°]	90
$\beta$ [°]	112.590(3)
$\gamma$ [°]	90
<i>V</i> [Å <sup>3</sup> ]	3335.3(3)
<i>Z</i>	8
<i>T</i> [K]	200(1)
$\lambda$ [Å]	1,54178
$\rho_{calc}$ [g cm <sup>-3</sup> ]	1,567
$\mu$ (CuK $\alpha$ ) [mm <sup>-1</sup> ]	2,293
Measured reflections	16560
Unique reflections	2923
<i>R</i> <sub>int</sub>	0.0216
Reflections I>2 $\sigma$ (I)	2514
Parameters	271
Restraints	6
<i>R</i> <sub>1</sub> <sup>b</sup> [I>2 $\sigma$ (I)]	0.0720
<i>wR</i> <sub>2</sub> <sup>c</sup> [I>2 $\sigma$ (I)]	0.2432
GOF	1.128
Largest residuals [eÅ <sup>3</sup> ]	-0.626 ; 1.658

<sup>a</sup> Including solvate molecules.

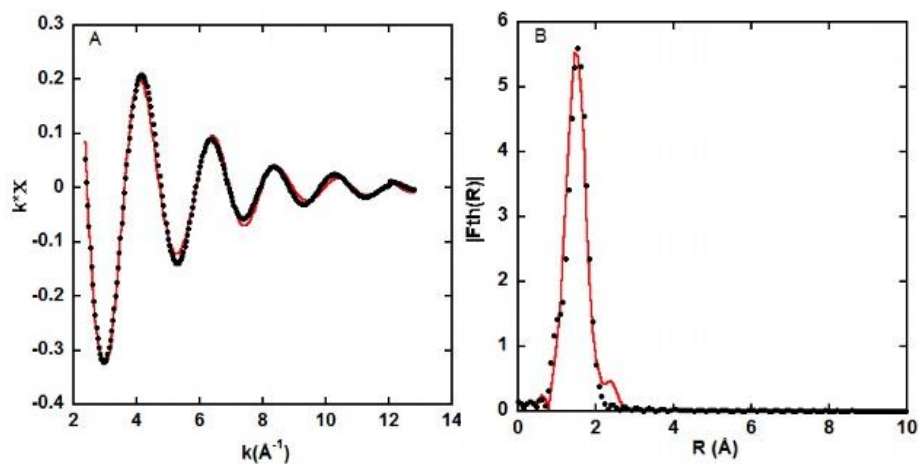
$$^b R_1 = \sum ||F_0| - |F_c|| / \sum |F_0|$$

$$^c \omega R_2 = [\sum (\omega(F_0^2 - F_c^2)^2) / \sum (\omega(F_0^2)^2)]^{1/2}$$

**Table S2. Selected bond lengths (Å) and angles (°).**

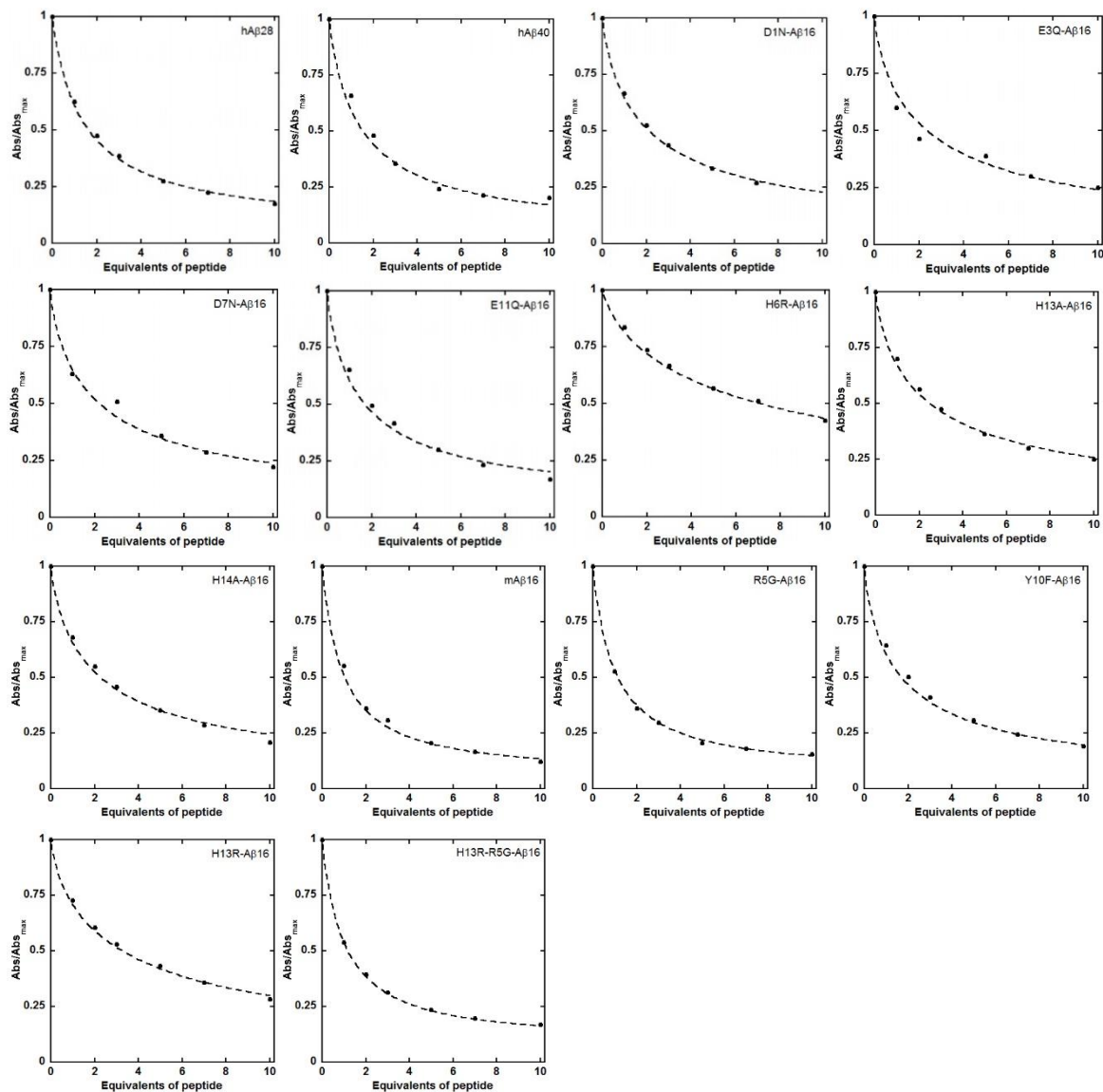
Cu1—O1	1.9588(34) Å	Cu1—N1	1.9061(55) Å
Cu1—O4	1.9642(51) Å	Cu1—N2	1.9127(37) Å
N1—Cu1—N2	83.35 (20) °	O1—Cu—O4	106.26(16) °
N1—Cu1—O1	85.28(17) °	N2—Cu1—O4	85.13(18) °

## 2- EXAFS PARAMETERS



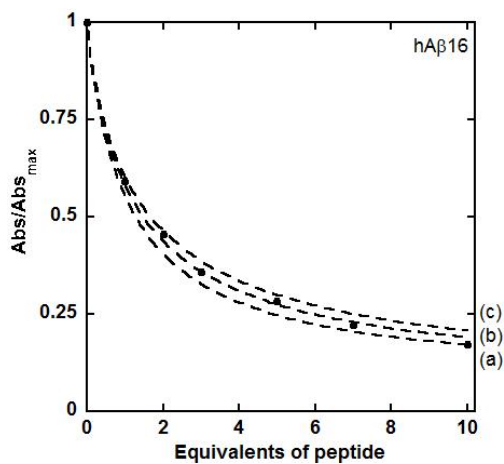
**Figure S2.** Cu K-edge EXAFS data fitting. Experimental data (dotted black points) and best fit (red lines) of the Cu(L) EXAFS signal, before and after the Fourier Transform, panels A and B respectively. [L] = 1 mM, [Cu(II)] = 0.9 mM, [HEPES] = 0.1 M, pH = 7.0, T = 20K.

### 3- COMPETITION DATA



**Figure S3.** Experimental absorbance (points) and the best fits (dotted lines) of the Cu(L) system upon addition of peptides. [L] = 50  $\mu$ M, [Cu(II)] = 45  $\mu$ M, [peptides] = 0 to 500  $\mu$ M, [HEPES] = 0.1 M, pH = 7.1, T = 25°C (where mentioned).

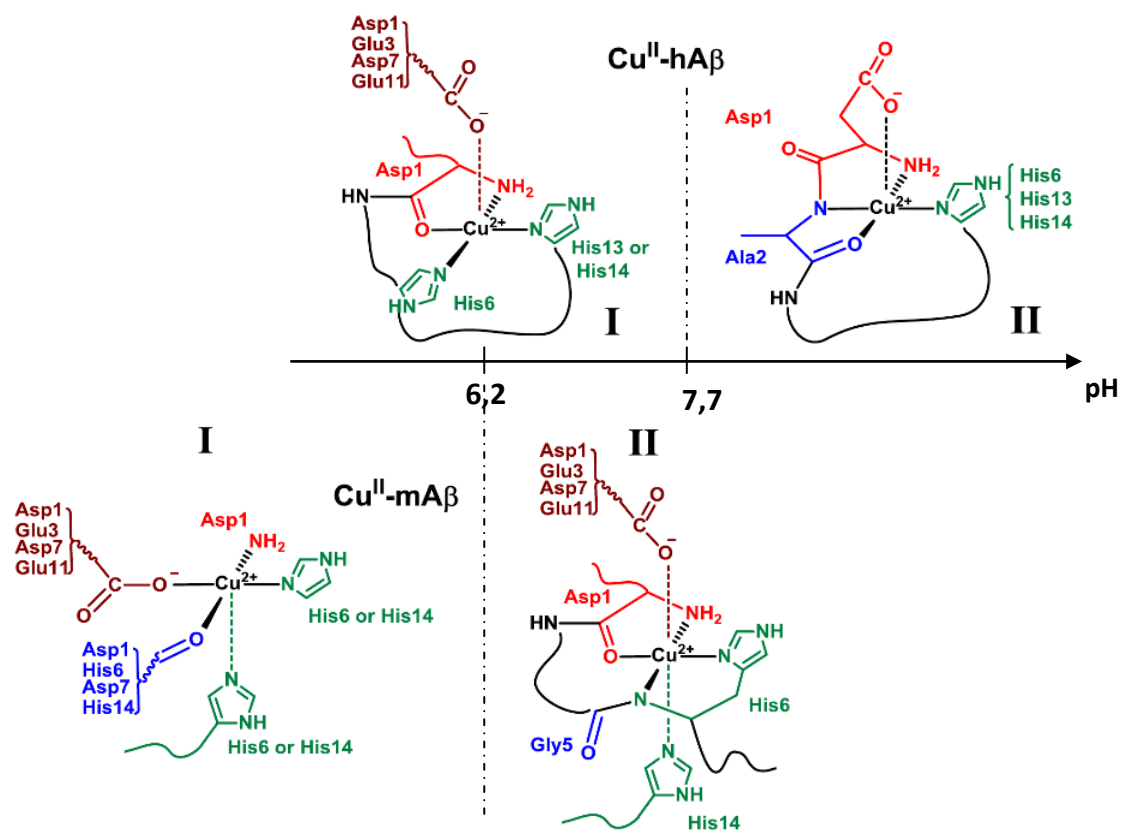
#### 4- ERROR BAR



**Figure S4.** Experimental absorbance (points) and its fits (dotted lines) of the Cu(L) system upon addition of increasing equivalents of hAβ<sub>16</sub>, with (a)  $K_a = 2.04 \cdot 10^9$ , (b)  $K_a = 1.61 \cdot 10^9$ , (c)  $K_a = 1.34 \cdot 10^9$ . [L] = 50 μM, [Cu(II)] = 45 μM, [hAβ<sub>16</sub>] = 0 to 500 μM, [HEPES] = 0.1 M, pH = 7.1, T = 25°C.

The accuracy of the fitting procedure is shown in the Fig. S4. The best fit is the (b) one and the curves (a) and (c) are calculated with  $\pm 20\%$  of difference in the  $K_a$  value. The fits (a) and (c) are not so closed to the best reproduction of the experimental data, so the error bar on the  $K_a$  value is below 20%.

## 5- Cu COORDINATION MODEL



Scheme S2. Cu(II) coordination model for the human and the murine Aβ peptides.