Use of a new water-soluble Zn sensor to determine Zn affinity for the amyloid-β peptide and relevant mutants

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SUPPLEMENTARY INFORMATION

1-Materials and methods.

Chemicals.

Reagents were commercially available and were used as received.
The Zn(II) ion source was $Zn(SO_4)(H_2O)_7$. A unique stock solution at 0.1 M was prepared.

Peptides.

Aβ16 peptide (sequence DAEFRHDSGYEVHHQK and referred to as Aβ in the following) and the modified counterparts (Ac-Aβ, Ac-DAEFRHDSGYEVHHQK; H6A-Aβ, DAEFRADSGYEVHHQK, H13A-Aβ, DAEFRHDSGYEVAHQK; H14A-Aβ, DAEFRHDSGYEVHAQK; D1N-Aβ, NAEFRHDSGYEVHHQK; E3Q-Aβ, DAQFRHDSGYEVHHQK; D7N-Aβ, DAEFRHNSGYEVHHQK; E11Q-Aβ, DAEFRHDSGYQVHHQK and Y10F-Aβ DAEFRHDSGYEVHHQK were bought from GeneCust (Dudelange, Luxembourg) with purity grade > 98%.
Stock solutions of the peptides were prepared by dissolving the powder in milliQ water (resulting pH ~ 2). Peptide concentration was then determined by UV-visible absorption of Tyr10 considered as free tyrosine (at pH 2, $(\varepsilon_{276}-\varepsilon_{296}) = 1410\ M^{-1}cm^{-1}$). For the Y10F-Aβ mutant, the absorption of the two Phe ($(\varepsilon_{258}-\varepsilon_{280}) = 390\ M^{-1}cm^{-1}$) was used. pH of the peptide stock solutions were then adjusted at a pH value the closest possible to pH 7.1 to avoid any pH drift upon addition of the peptide during the competition experiments. Indeed the K_a value of the L_2-Zn complex is highly dependent with pH, and as a direct consequence, the measurements must be (and have been) done under the very same pH conditions.

Ligand $Na_2H_2L_2$.

The sodium salt of the H_2L_2 ligand (*N,N′*-Bis[(5-sulfonato-2-hydroxy)benzyl]-*N,N′*-dimethyl-ethane-1,2-diamine) was prepared as previously described.[1] 0.1 M stock solution was prepared according to the molecular mass determined by elemental analysis (resulting pH ~8).

Methods.

UV-Vis spectra were recorded on an Agilent 8453 spectrometer at 25°C in 1 cm path length quartz cuvette.

Protonation and Zn binding constants. Equilibrium constants for protonation and Zn(II) complexation reactions with the ligand H_4L_2 were determined by protometric titrations in a 0.1 M KNO_3 aqueous medium. Protometric titrations were fully automated using an automatic titrator composed of a microprocessor burette Metrohm Dosimat 665, a metrohm 6 0234100 glass electrode with an incorporated Ag/AgCl reference and a pH-meter metrohm 713 connected to a computer. All measurements were performed within a thermoregulated cell at 25 °C under an argon stream to avoid the dissolution of carbon dioxide. An HNO_3 solution at exactly 10^{-2} M was used to calibrate the electrode. For a classical titration, a total of 120 to 150 points (volume of titrant, pH) was taken.
All the used commercial reagents were of the highest purity (> 99%) and were used without further purification. The stock solution of ligand was prepared in distilled water at a concentration of 1 mM in presence of 5 mM HNO_3. A $Zn(NO_3)_2$ solution was prepared at a concentration of 10^{-2} M in presence of 3.10^{-2} M HNO_3. Zn(II) concentration was precisely determined by ICP-AES (Liberty Series II, Varian). The titrating solution of carbonate-free base KOH was prepared from a standardized 1M solution (Prolabo).

Acidity constants for the ligand H_4L_2 were determined by titrating 20 mL of 0.75 and 1 mM ligand with 0.1 M KOH. The stability constants of Zn(II) with H_4L_2 were determined by titrating a solution of 0.83 mM Zn(II) and 1.0 mM ligand (1:1.2 metal:ligand molar ratio) with 0.1 M KOH. The protonation and formation constants were calculated from potentiometric data by HYPERQUAD[2,3] software and the computer program HYSS[3] was used to plot the species distribution curves in the pH range 2-12. Five independent titrations were used in determining the final values. The value of used pK_w was 13.77. The global acidity constants β_{0lh} of H_4L_2 and global stability constants β_{mlh} of Zn(II) complexes are defined by equations (1) and (2):

$$mM + lL + hH \rightleftharpoons M_mL_lH_h \qquad \text{(Eq. 1)}$$

$$\beta_{mlh} = \frac{[M_mL_lH_h]}{[M]^m[L]^l[H]^h} \qquad \text{(Eq. 2)}$$

in which m, l and h are values in the general ligand (m=0) and complex formula for $[M_mL_lH_h]$. M, L and H correspond to the metal ion, the ligand L, and the protons, respectively (in sake of clarity, the charges are omitted). The calculated uncertainties for log β and pKa were determined on the basis of the standard deviation.

Competition experiments between L_2 and Aβ peptides have been monitored by UV-Vis in a hepes buffer 50 mM. In a typical experiment, the ligand L_2 (60 µM, in theory), Zn(II) (50 µM, in theory) was mixed and 1 to 10 equivalents of peptide (compared to the theoretical Zn(II) concentration) added and the UV-Vis spectrum of each of the 12 samples recorded. To be in reproducible pH condition, the same stock buffer solution (adjusted to pH 7.1) was used for a given set of experiments. Two different sets of data, in which all the experiments were repeated 2 or 3 times for each peptide, were obtained with two different starting buffer stock solutions, and the results were coherent. All the competition experiments were performed at 25°C.

Analysis of the data were performed with a 2- step procedure for each experiment.

Step 1. Real concentration in L_2 was determined using absorbance at 252 nm, with a ε_{252} = 6130 M^{-1} cm^{-1} at pH 7.1. Then, real concentration in Zn added was determined using absorbance of the L_2-Zn complex at 252 nm and considering the formation of the L_2-Zn complex with a ε_{252} = 30000 M^{-1} cm^{-1}. The latter value was determined using Zn titration of L_2 (See Figure S1).

Step 2. Change in the absorbance at 252 nm was reproduced according to an in-house procedure using the above-determined L_2 and L_2-Zn concentrations as starting parameters.

Absorbance was calculated according to:

$$Abs = ([peptide] - [\alpha]) \cdot \varepsilon_{252nm}^{peptide} + [\alpha] \cdot \varepsilon_{252nm}^{peptide-Zn} + ([L_2] - [Zn] + [\alpha]) \cdot \varepsilon_{252nm}^{L_2} + ([Zn] - [\alpha]) \cdot \varepsilon_{252nm}^{L_2-Zn}$$

where α stands for the progression of the following reaction: peptide + Zn → peptide-Zn.

$$\alpha = \frac{-b + \sqrt{\Delta}}{2a}$$

with

$$b = \frac{K_d^{peptide-Zn}}{K_d^{L_2-Zn}} \cdot ([L_2] - [Zn]) + ([peptide] + [Zn])$$

$$\Delta = b^2 - 4 \cdot a \cdot c \; ; \qquad a = \frac{K_d^{peptide-Zn}}{K_d^{L_2-Zn}} - 1 \; ; \qquad c = -[peptide] \cdot [Zn]$$

$K_d^{peptide-Zn}$ was adjusted to obtain the best reproduction of the experimental data.

2. Zn titration of the L_2 ligand

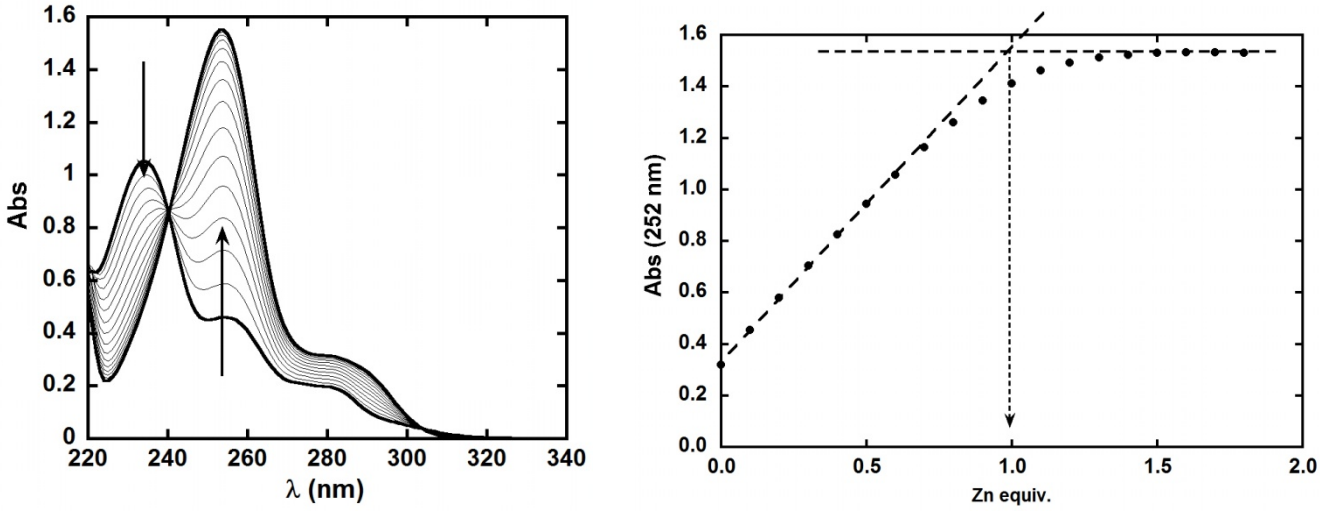


Figure S1. Titration curve of Zn in L_2. Evolution of the L_2 UV-Vis spectrum (left panel) and absorbance value at 252 nm (right panel) upon addition of increasing amount fo Zn. $[L_2]$ = 50 µM in hepes buffer 50 mM pH 7.1, ℓ= 1cm, T = 25°C.

The progress of the reaction at the stoichiometry point (SP) is given by $\frac{Abs(SP) - Abs_0}{Abs_{max} - Abs_0}$ and leads to an affinity value of the L_2 + Zn⟶ L_2-Zn reaction equals to $K_a = 1.4\ 10^6\ M^{-1}$.

3. Speciation diagram of L_2-Zn

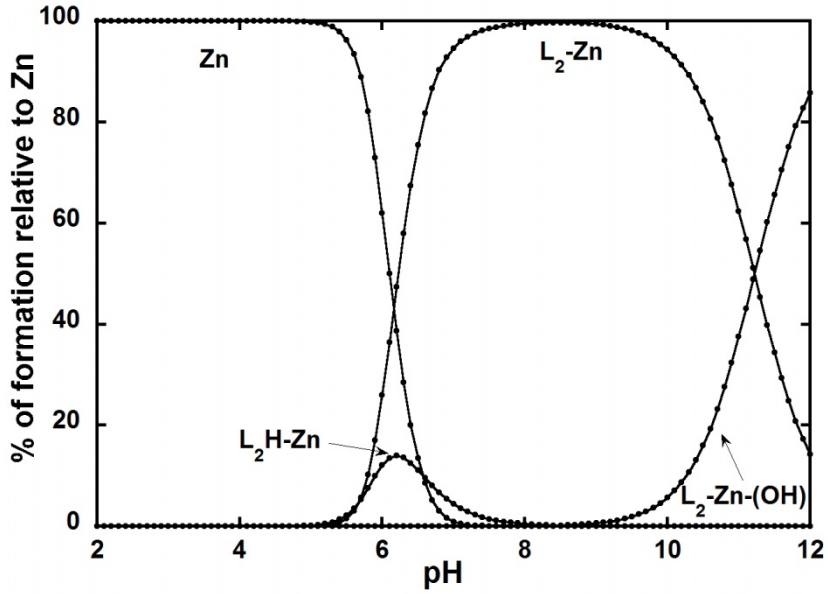


Figure S2: Solution speciation diagram for the L_2-Zn system with $[L_2]$ = 1 mM and $[L_2]$:$[Zn^{2+}]$ = 1.2:1. Charges are omitted for the sake of clarity.

Table S1. Logarithmic values of the overall acidity constants of the ligand (β01h) and of the overall stability constants of Zn(II) complexes (βm1h). (Standard deviation is indicated in parenthesis for the last digit.)

$\log \beta_{mlh}$	L_2	pKa
$\log \beta_{0l4}$	34.24 (7)	5.05
$\log \beta_{0l3}$	29.19 (6)	7.79
$\log \beta_{0l2}$	21.40 (4)	9.72
$\log \beta_{0l1}$	11.68 (4)	11.68
$\log \beta_{1l1}$	19.70 (4)	-
$\log \beta_{1l0}$	14.03 (1)	-
$\log \beta_{1l-1}$	2.81 (2)	-

[a] $\beta_{mlh} = \frac{[M_m L_l H_h]}{[M]^m [L]^l [H]^h}$ where $mM + lL + hH \rightleftharpoons MmLlHh$.

The dissociation constant at pH 7.1 was determined according to:

$$K_d = \frac{[M]\sum_h [LH_h]}{\sum_h [MLH_h]} = \frac{\sum_h \beta_{01h}[H]^h}{\sum_h \beta_{11h}[H]^h}$$

A K_a ($1/K_d$) value of 1.2 10^6 M^{-1} was found and used for further competition experiments with peptides.

4. UV-Vis spectra of the Y10F-Aβ16 competition with L_2

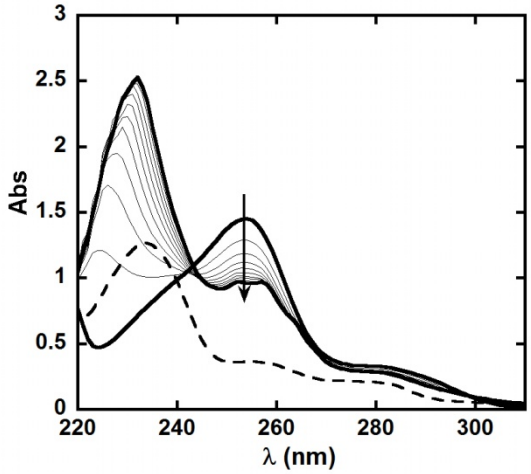


Figure S3. UV-Vis spectra of a solution of L_2 (dotted line), in presence of Zn (solid bold line) and after addition of increasing amount of Y10F-Aβ peptide (Absence of the absorption at 275 nm, compared to Figure 1 in the full text). The arrows indicate the variation of the UV-Vis spectra upon Aβ16 addition. [L2] = 60 µM, [Zn] = 50 µM, [Y10F-Aβ16] = 50-500 µM, Hepes buffer 50mM, pH 7.1, 25°C.

5. Competition data

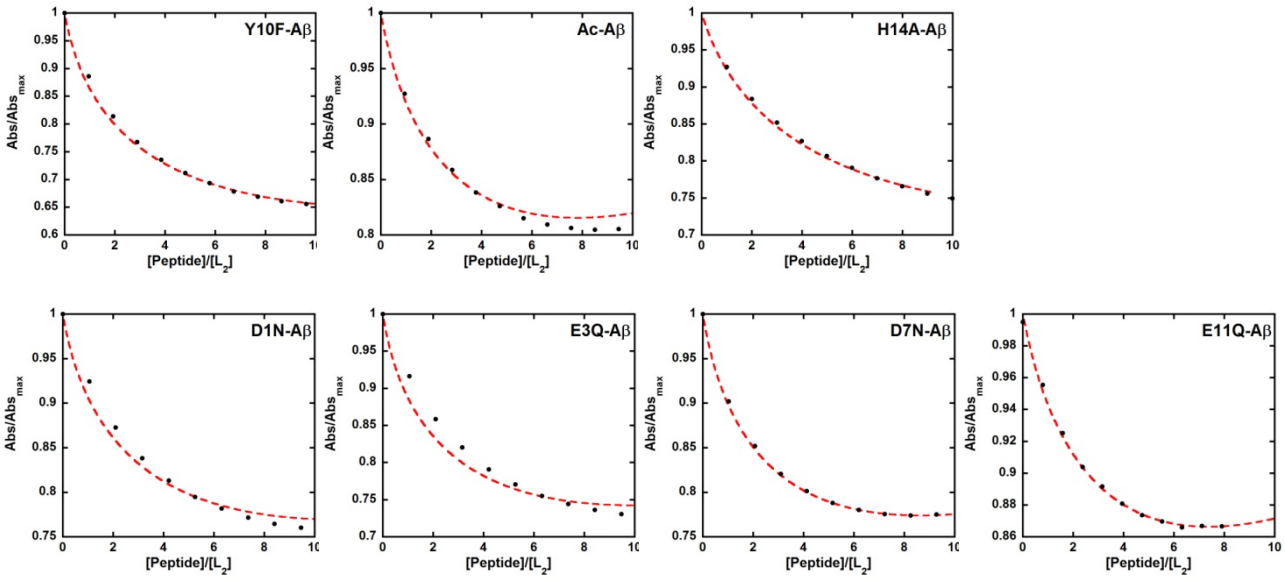


Figure S4. Experimental normalized Absorbance (dotted black points) and their reproduction (red lines) of L_2-Zn system upon addition of increasing equivalents of peptides. [L_2] = 60 µM, [Zn] = 50µM and [peptides] = 50 - 500 µM, Hepes buffer 50mM, pH 7.1, 25°C.

6-Error bar

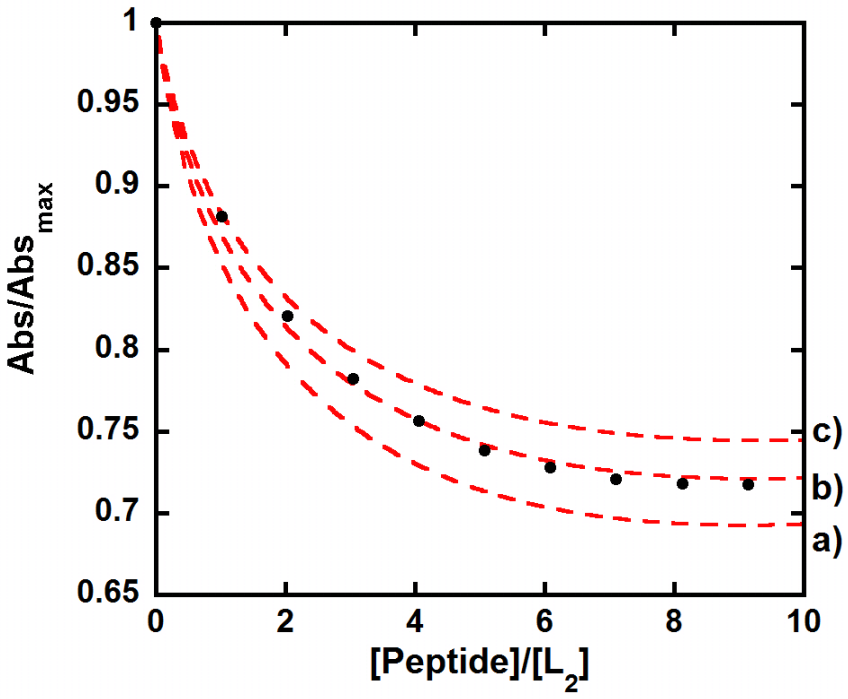
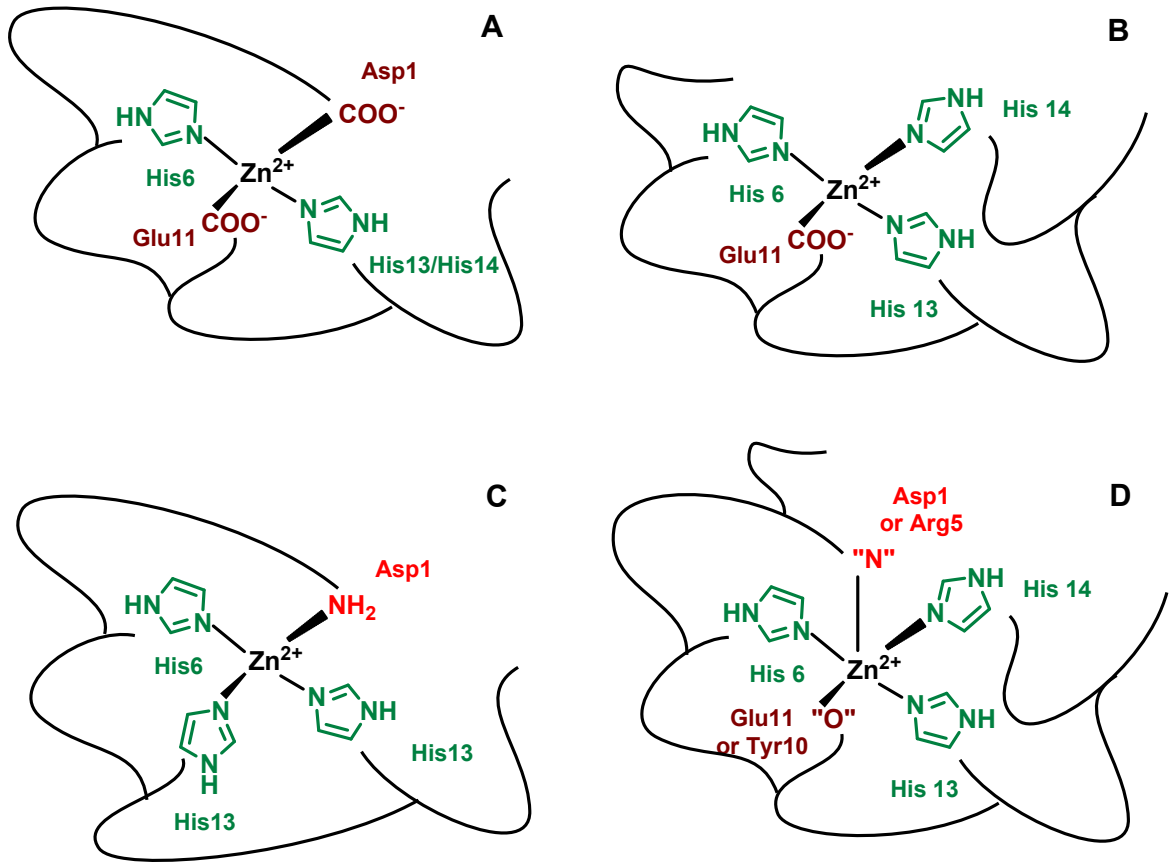


Figure S5. Experimental normalized Absorbance (dotted black points) and its reproductions (dotted red lines) of L_2-Zn system upon addition of increasing equivalents of Aβ16, with a) $K_a = 1.35\ 10^5\ M^{-1}$; b) $K_a = 1.08\ 10^5\ M^{-1}$; c) $K_a = 0.91\ 10^5\ M^{-1}$. $[L_2] = 60\ \mu M$, [Zn] = 50μM and [peptides] = 50 - 500 μM, Hepes buffer 50mM, pH 7.1, 25°C.

Figure S5 shows the sensibility of the fitting procedure. The best reproduction of the experimental data is curve b) obtained for $K_a = 1.08\ 10^5\ M^{-1}$. Curves a) and c) are calculated with ± 20% of difference in the K_a value and lead to unsatisfying reproduction of the experimental data. This thus indicates that the error bar on the K_a value are below 20%.

7-Zn coordination model



Scheme S1. Zn coordination model proposed here (A), from ref. 4 and 5 (B), ref. 6 (C) and ref. 7 (D).

References

1. Noël, S.; Perez, F.; Ladeira, S.; Sayen, S.; Guillon, E.; Gras, E.; Hureau, C. *J. Inorg. Biochem.* **2012,** *117*, 322-325.
2. Gans, P.; Sabatini, A.; Vacca, A., Talanta **1996**, 43, 1739-1753.
3. Alderighi, L.; Gans, P.; Ienco, A.; Peters, D.; Sabatini, A.; Vacca, A., Coord. Chem. Rev. **1999**, *184*, 311-318.
4. Zirah, S.; Kozin, S. A.; Mazur, A. K.; Blond, A.; Cheminant, M.; Ségalas-Milazzo, I.; Debey, P.; Rebuffat, S., *J. Biol. Chem.* **2006,** *281*, 2151-2161.
5. Damante, C. A.; Osz, K.; Nagy, N. V.; Pappalardo, G.; Grasso, G.; Impellizzeri, G.; Rizzarelli, E.; Sovago, I., Inorg. Chem. **2009**, *48*, 10405-10415.
6. Migliorini, C.; Porciatti, E.; Luczkowski, M.; Valensin, D., *Coord. Chem. Rev.* **2012,** *256*, 352-368.
7. Tôugu, V.; Palumaa, P., *Coord. Chem. Rev.* **2012,** *256*, 2219-2224.