# Monitoring the Formation of Amyloid Oligomers Using Photoluminescence Anisotropy

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## 1. Supplementary Figures

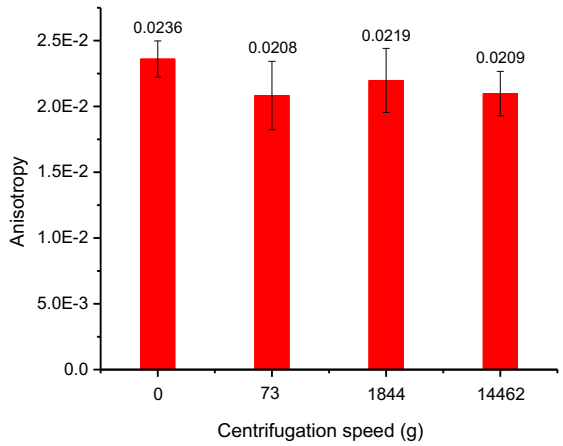


**Figure S1.** Anisotropy of $[Ru(bpy)_2(dpqp)]^{2+}$ with Aβ oligomers incubated for 72 h at room temperature subjected to different centrifugation speeds. Increasing centrifugation speed from 73 to 14462g does not significantly affect the anisotropy.

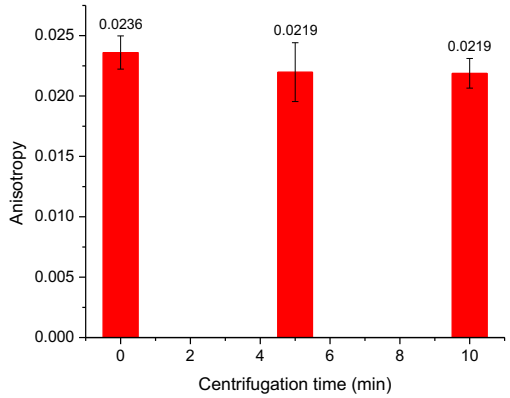


**Figure S2.** Anisotropy of $[Ru(bpy)_2(dpqp)]^{2+}$ with Aβ oligomers incubated for 72 h at room temperature and centrifuged at 1844g for different times intervals (0, 5 and 10 minutes). The results indicate that increasing the centrifugation time from 5 to 10 minutes does not have a marked effect in the anisotropy.

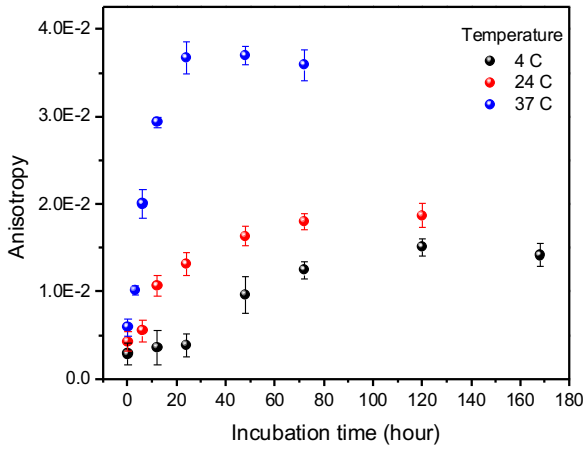


**Figure S3.** Monitoring the formation of Aβ oligomers at different temperatures using the photoluminescence anisotropy of $[Ru(bpy)_2(dpqp)]^{2+}$.

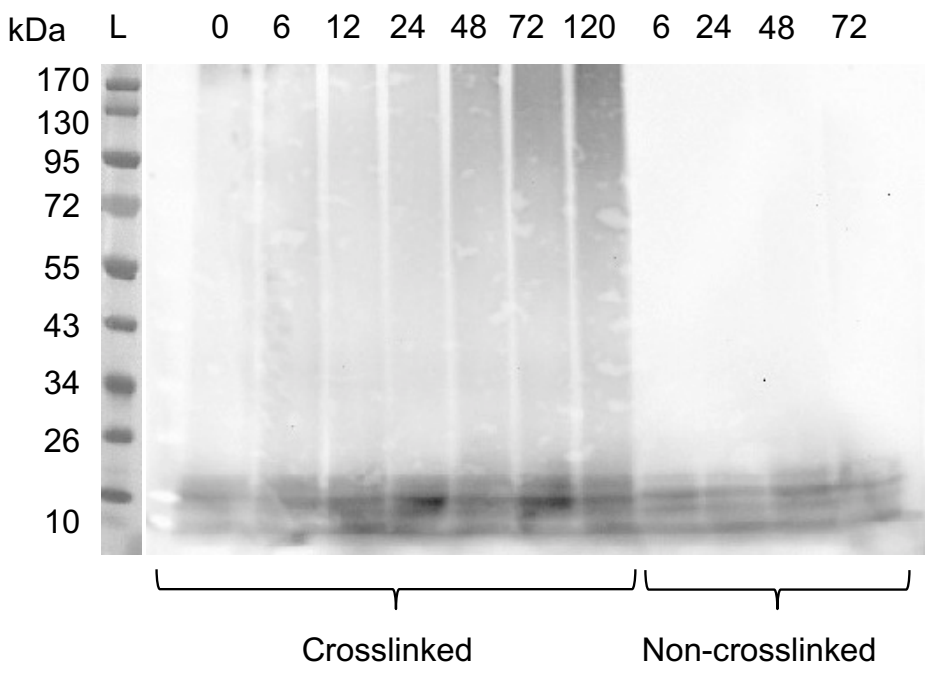


**Figure S4.** Western-Blot of Aβ oligomers crosslinked with glutaraldehyde. The samples were heated at 70˚C before putting them in the wells.

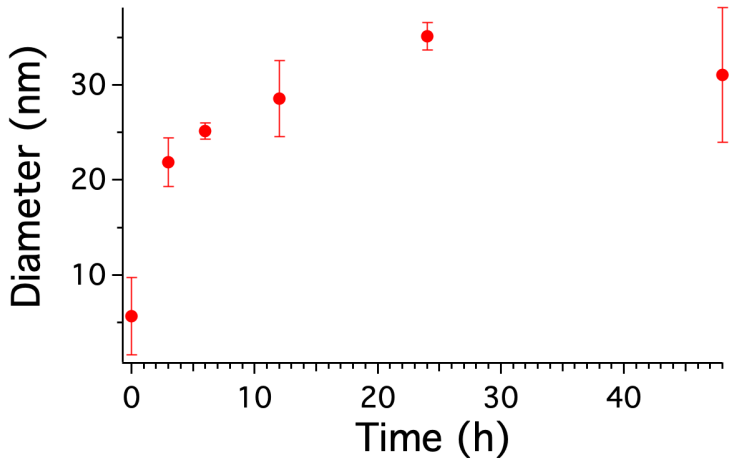


**Figure S5.** Determination of the size of Aβ oligomers using dynamic light scattering (DLS).

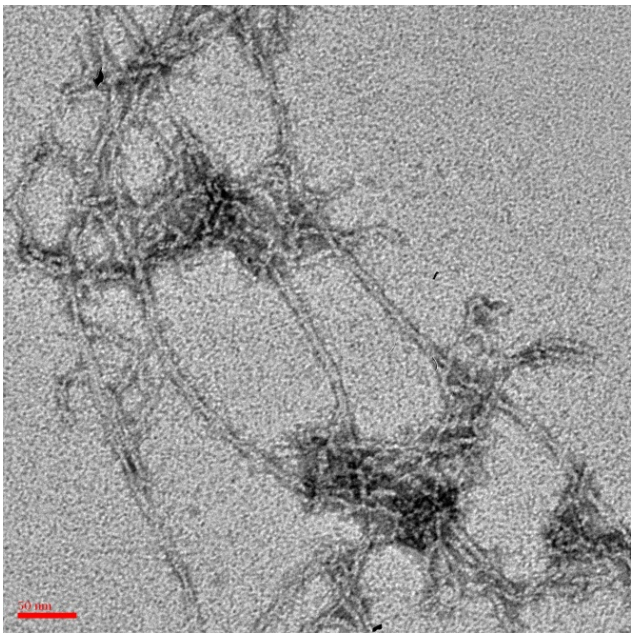

**Figure S6.** Aβ straight fibrils formed after incubation of the 72h mixture at 600 rpm and 37 °C. Long fibrils can be seen together with short curved fibrils formed during the quiescent incubation. Scale bar = 50 nm.

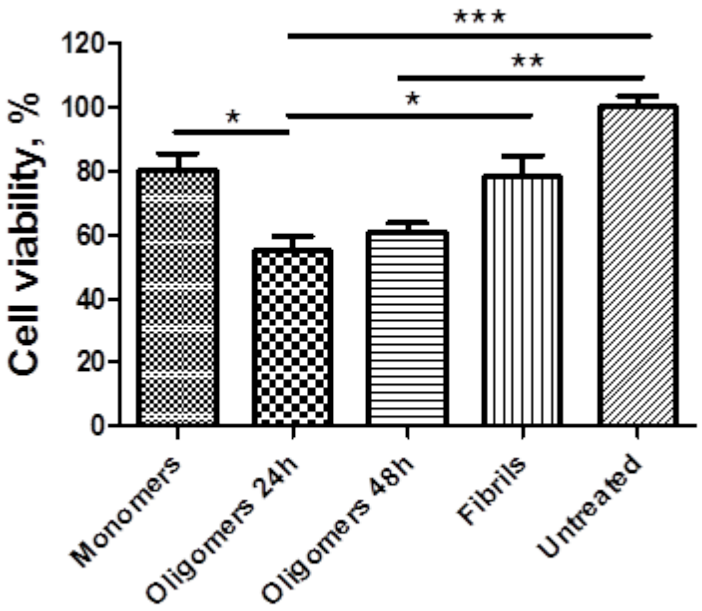


**Figure S7.** Aβ neurotoxicity assay. N2a cells were incubated for 24 h with Aβ in different conformations and cell viability was measured using MTT. Values and error bars are average and standard deviation of three measurements. The data was analyzed by one way ANOVA ($p=0.0005$), Tukey's post hoc test (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

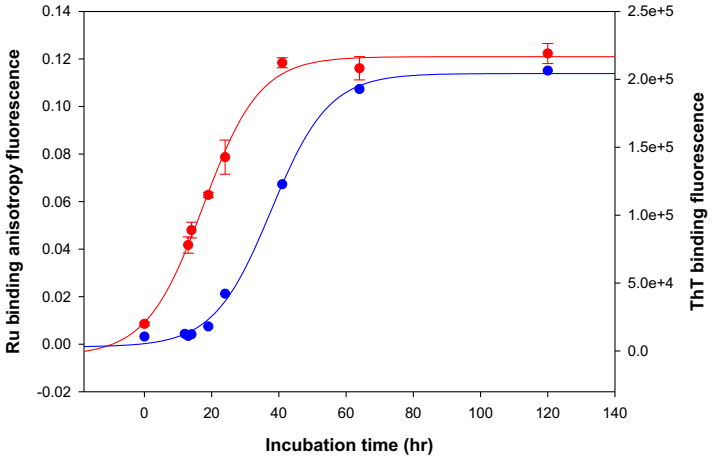


**Figure S8.** Real-time monitoring of αS oligomers using photoluminescence anisotropy of $[Ru(bpy)_2(dpqp)]^{2+}$ (red circles) and the emission of ThT (blue circles).

**Appendix 1. Derivation of Equation 4**

We start by rewriting equation 3 as:

$$r = f_f r_f + f_b r_b \qquad \text{(S1)}$$

where $r_f$ and $r_b$ are the anisotropy of free and bound $[Ru(bpy)_2(dpqp)]^{2+}$ respectively, and $f_f$ and $f_b$ are the fractional intensity of free and bound $[Ru(bpy)_2(dpqp)]^{2+}$ respectively. Assuming that the quantum yield of $[Ru(bpy)_2(dpqp)]^{2+}$ does not change significantly in the two different environments then:

$$f_f = \frac{[Ru_f]}{[Ru_f]+[Ru_b]} = \frac{[Ru_f]}{[Ru]} \qquad \text{(S2)}$$

$$f_b = \frac{[Ru_b]}{[Ru_f]+[Ru_b]} = \frac{[Ru_b]}{[Ru]} \qquad \text{(S3)}$$

Where $[Ru_f]$ and $[Ru_b]$ are the concentration of free and bound $[Ru(bpy)_2(dpqp)]^{2+}$ respectively. We define now that the sum of the free and bound species is equal to the total concentration:

$$[Ru] = [Ru_f] + [Ru_b] \qquad \text{(S4)}$$

$$[A\beta] = [A\beta_f] + [A\beta_b] \qquad \text{(S5)}$$

where [Ru] and [Aβ] is the total concentration of $[Ru(bpy)_2(dpqp)]^{2+}$ and Aβ binding sites respectively. We will now solve equation S1 in terms of [Ru] and [Aβ]. Rearranging eq S1 we get:

$$r = (1 - f_b)r_f + f_b r_b \qquad \text{(S6)}$$

$$r = r_f + f_b(r_b - r_f) \qquad \text{(S7)}$$

And taking $f_b$ from equation S3 gives:

$$r = r_f + (r_b - r_f)\frac{[Ru_b]}{[Ru]} \quad \text{(S8)}$$

The equilibrium dissociation constant can be calculated by:

$$Ru \cdots A\beta \leftrightharpoons Ru_f + A\beta_f \quad \text{(S9)}$$

Where $Ru_f$ represents free $[Ru(bpy)_2(dpqp)]^{2+}$ and $A\beta_f$ represents a free Aβ oligomer binding site. $Ru \cdots A\beta$ is the $[Ru(bpy)_2(dpqp)]^{2+}$ bound to an $A\beta$ binding site ($[Ru \cdots A\beta] = [Ru_b] = [A\beta_b]$).

The equilibrium dissociation constant ($k_d$) can be written as:

$$k_d = \frac{[Ru_f][A\beta_f]}{[Ru\cdots A\beta]} = \frac{[Ru_f][A\beta_f]}{[Ru_b]} \qquad \text{(S10)}$$

Rearranging this equation gives

$$[Ru_b]k_d = [Ru_f][A\beta_f] \qquad \text{(S11)}$$

Using equations S4 and S5 to substitute for $[Ru_f]$ and $[A\beta_f]$:

$$[Ru_b]k_d = ([Ru] - [Ru_b])([A\beta] - [Ru_b]) \quad \text{(S12)}$$

$$[Ru_b]^2 - (k_d + [A\beta] + [Ru])[Ru_b] + [A\beta][Ru] = 0 \quad \text{(S13)}$$

Solving this quadratic equation gives:

$$[Ru_b] = \frac{(k_d+[Ru]+[A\beta]) - \sqrt{(k_d+[Ru]+[A\beta])^2 - 4[A\beta][Ru]}}{2} \quad \text{(S14)}$$

Substituting eq S14 in eq S8 results in:

$$r = r_f + \frac{r_b - r_f}{2[Ru]}\left((k_d + [Ru] + [A\beta]) - \sqrt{(k_d + [Ru] + [A\beta])^2 - 4[A\beta][Ru]}\right) \quad \text{(S15)}$$

[Aβ] is not the total concentration of Aβ, but rather the total concentration of [Aβ] binding sites. To relate the total concentration of Aβ to the total concentration of Aβ binding sites we made the assumption that binding sites occurs only in oligomers (rather than in unstructured random coil monomers) and that there is one binding site per oligomer (one site model). The concentration of Aβ forming oligomers is then $[A\beta]_T$ - $[A\beta]_m$, where $[A\beta]_m$ is the concentration of monomers in solution and $[A\beta]_T$ is the total concentration of Aβ. Finally, the concentration of oligomer binding sites can be estimated by dividing by an approximate number of Aβ units (n) forming oligomers. The final equation is then:

$$r = r_f + \frac{r_b - r_f}{2[Ru]}\left(\left(k_d + [Ru] + \frac{[A\beta]_T - [A\beta]_m}{n}\right) - \sqrt{(k_d + [Ru] + \frac{[A\beta]_T - [A\beta]_m}{n})^2 - 4\frac{[A\beta]_T - [A\beta]_m}{n}[Ru]}\right) \quad (S16)$$

where $\frac{[A\beta]_T - [A\beta]_m}{n}$ is the approximate concentration of Aβ oligomers.