

Effect of nanoparticles on the aggregation and amyloid formation of amyloid-beta peptide

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Introduction

Nanoparticles are becoming increasingly important in medicine and biotechnology. Moreover, due to the environmental pollution, the human body might be involuntarily exposed to nanoparticles in the everyday life. Proteins interacting with nanoparticles might undergo conformational alterations^{1,2} and nanomaterials can also affect the aggregation processes of proteins³.

Background

The A β peptides are known for forming amyloid aggregates in the brain in Alzheimer's disease. We used in-house expressed recombinant A β (1-42) to study the structure, aggregation kinetics and cytotoxicity of the peptide. We characterized nanoparticles of different materials and studied their effects on the structure and aggregation of A β .

Nanoparticles and Alzheimer's amyloid- β peptide

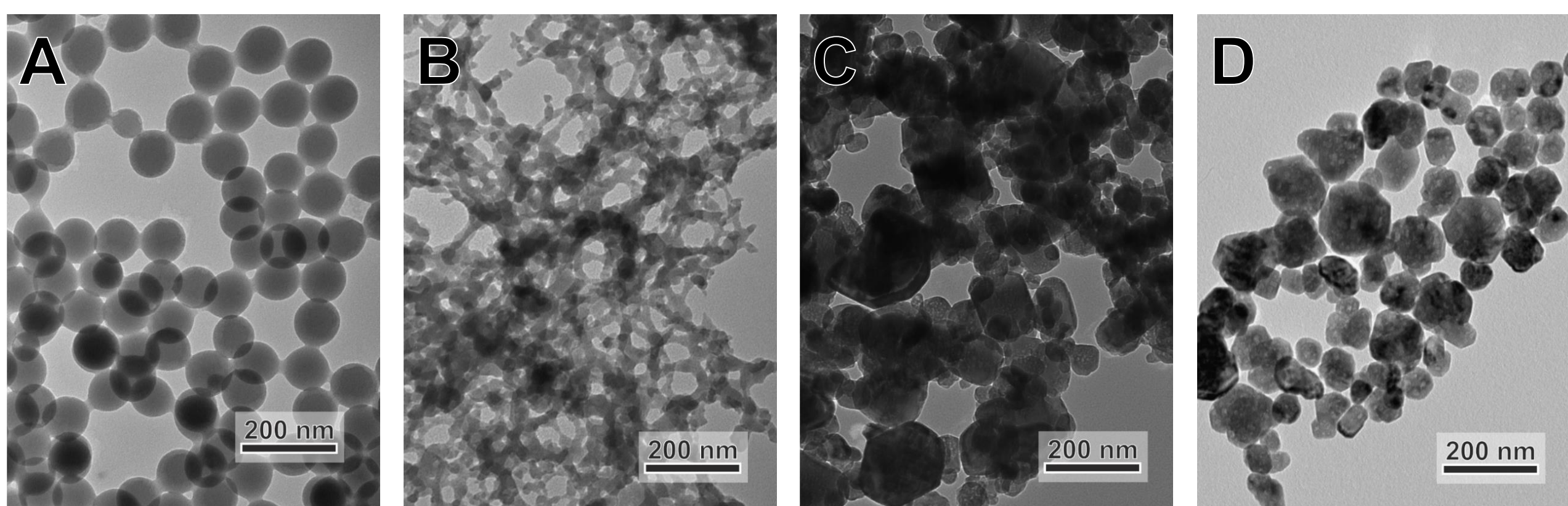


Fig. 1. TEM images of purchased nanoparticles of polystyrene (A), SiO₂ (B) and CaF₂ (C), and in-lab synthesized CaF₂ (D).

We purchased commercially available nanoparticles of polystyrene (PS), silica (SiO₂), and calcium-fluoride (CaF₂). Moreover, we synthesized CaF₂ nanoparticles in our lab. PS is a widely used material of plastic, and a frequent component in nano-sized environmental waste. SiO₂ and CaF₂ nanoparticles can be used in various biotechnological applications. CaF₂ came into our focus for being transparent in UV, therefore suitable for chiroptical spectroscopy measurements with proteins. Our studies are focusing on A β which can form micrometer long fibrils observable by transmission electron microscopy (TEM).

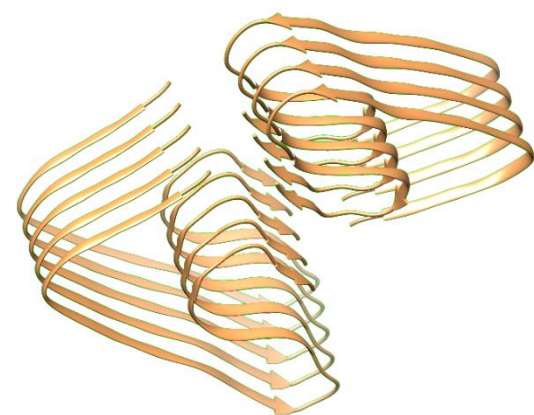


Fig. 2. Cryo-EM structure of A β (1-42) peptides forming a cross- β -sheet structured fibril. PDB: 5OQV

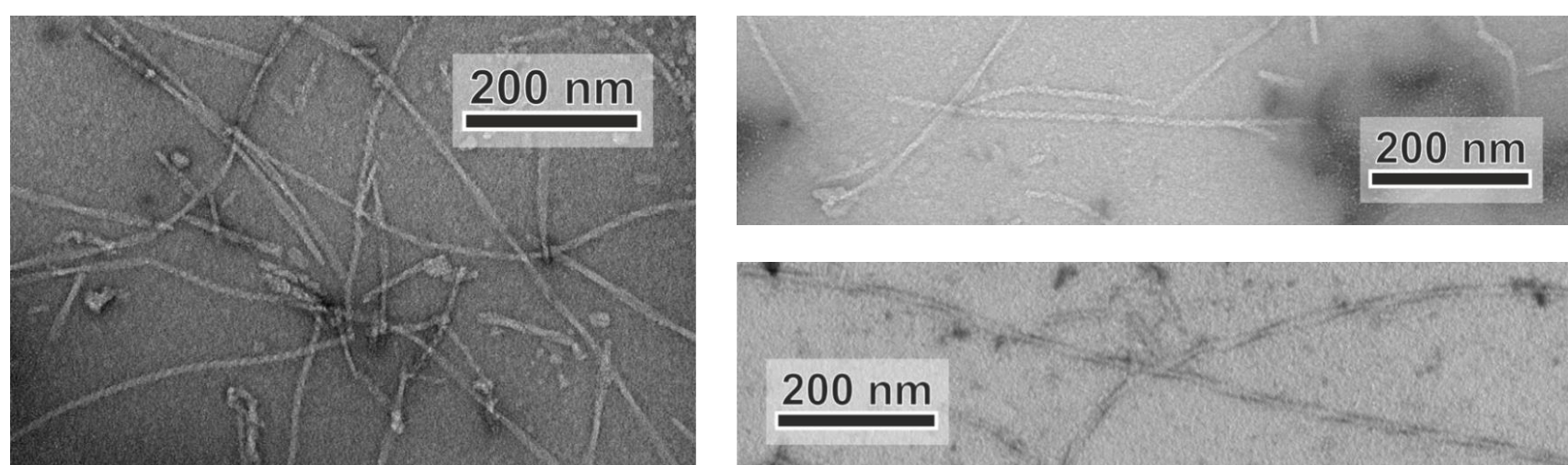


Fig. 3. TEM images of different morphological types of A β fibrils.

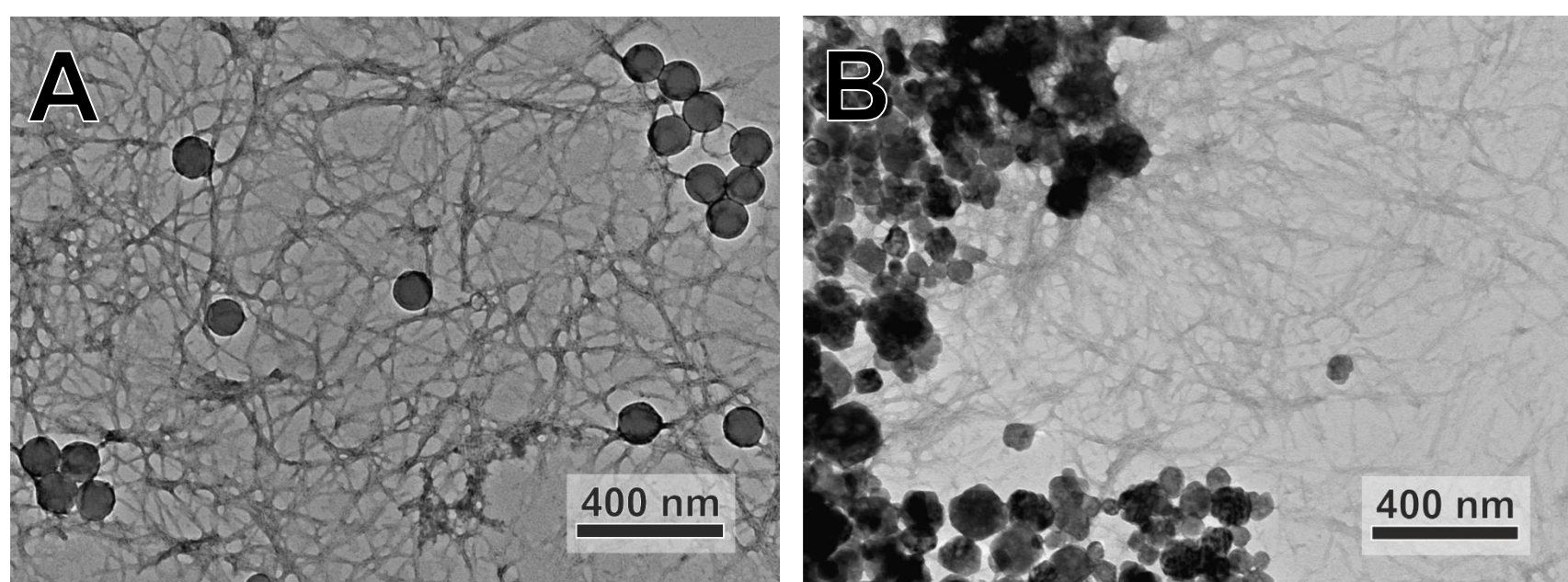


Fig. 4. TEM images of amyloid fibrils attached to PS (A) and CaF₂ (B) nanoparticles.

Effects of nanoparticles on A β

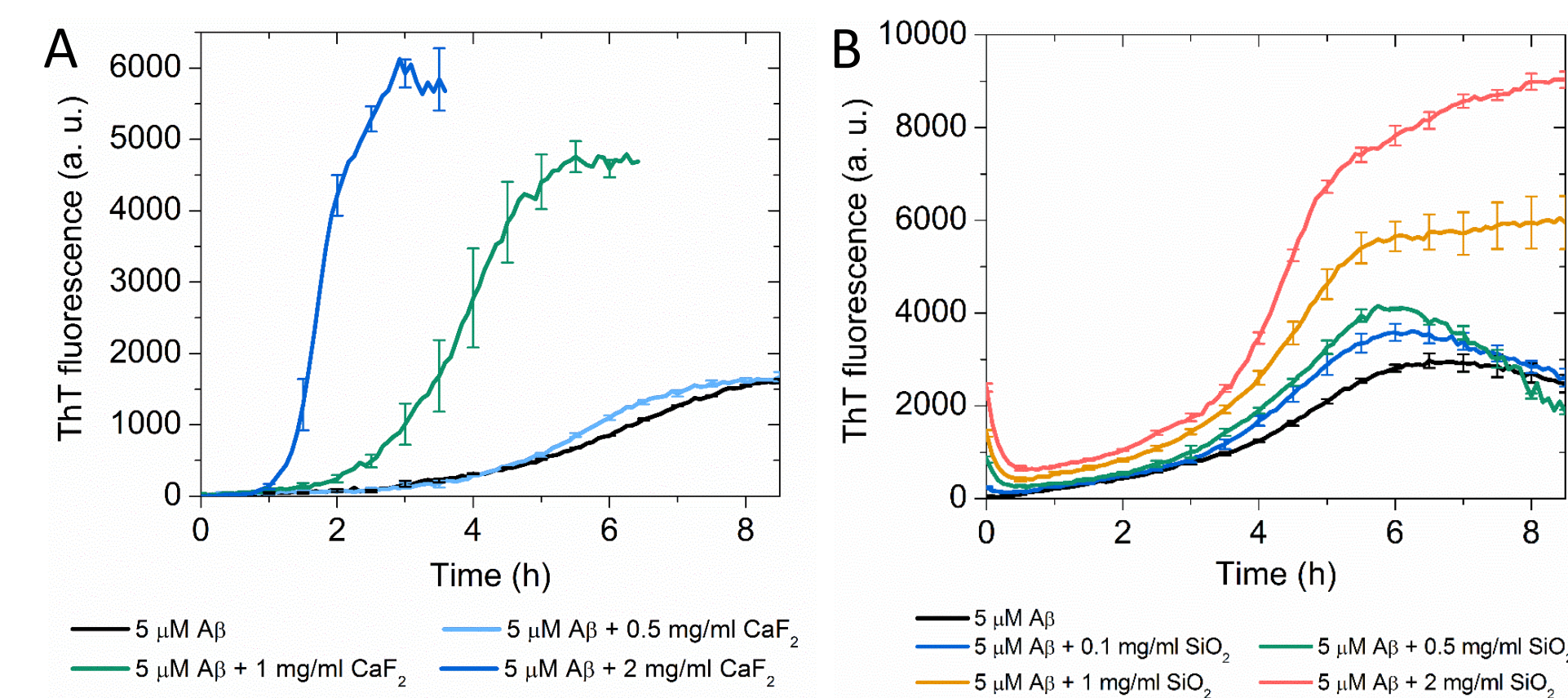


Fig. 5. Aggregation of A β followed by Thioflavin T (ThT) fluorescence, in the presence of CaF₂ (A) or SiO₂ nanoparticles (B) of different concentrations. Higher concentrations of nanoparticles resulted in higher fluorescence intensities in the case of both nanomaterials, and CaF₂ nanoparticles also shortened the lag phase.

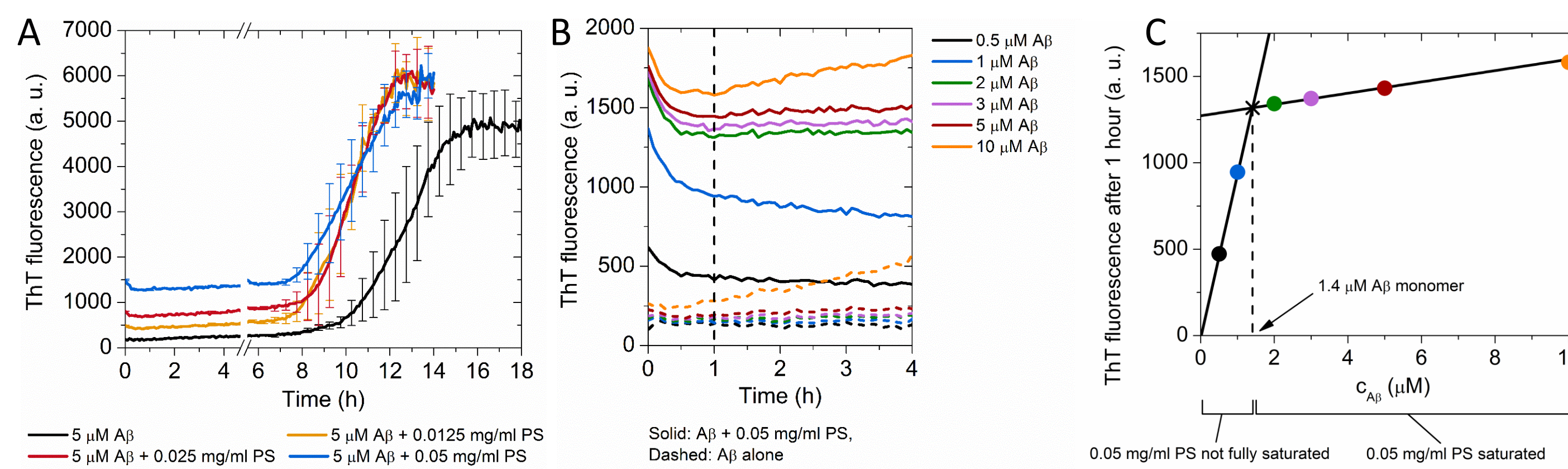


Fig. 6. ThT fluorescence of A β and PS nanoparticle samples. (A) The main effect of PS on A β of a constant concentration is that starting fluorescence intensities are elevated proportionally to PS concentration. (B) Different concentrations of A β at the initial phase of the aggregation time course (monomeric form is dominant). PS-bound A β monomers show an increased signal which is proportional to the A β concentration up to a saturation point, which is between 1 and 2 μ M. (C) By plotting ThT intensities measured at 1 hour, we found that 0.05 mg/ml PS can be saturated by $\sim$ 1.4 μ M monomeric A β .

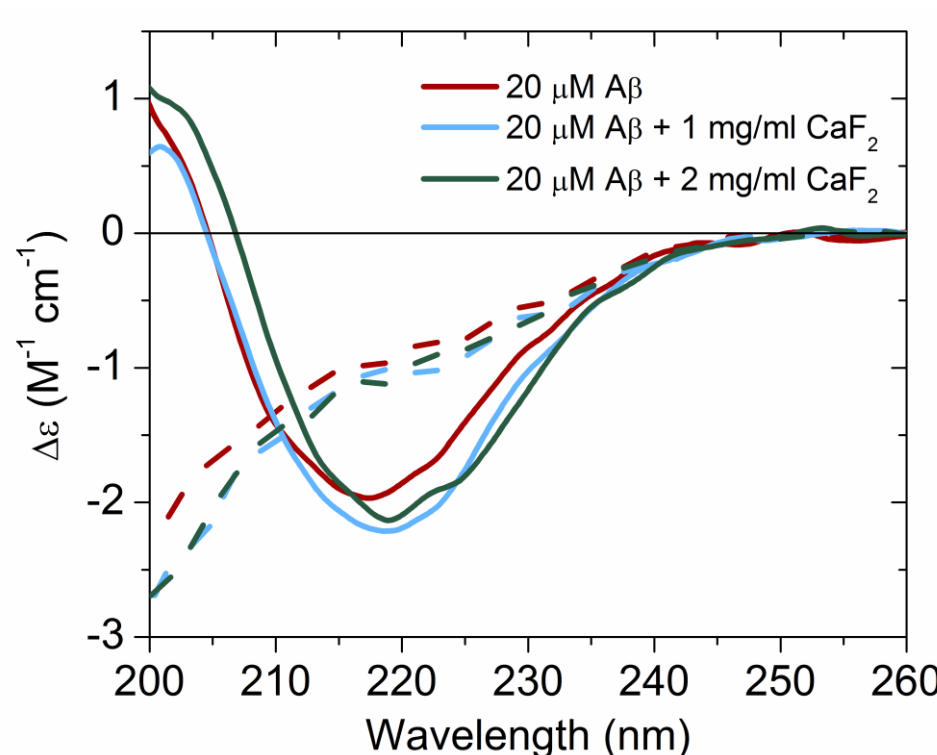


Fig. 7. CD spectra of A β in the presence of CaF₂ in different concentrations before (dashed) and after aggregation (solid).

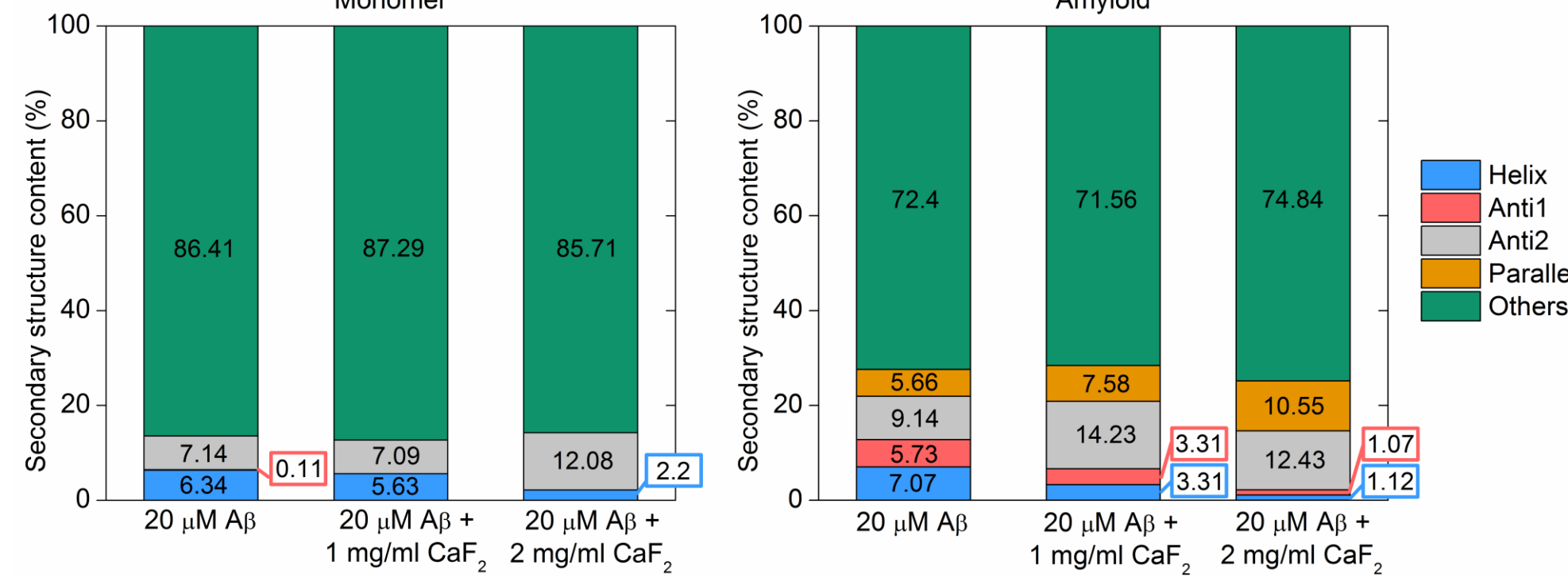


Fig. 8. Secondary structure content of A β monomer and amyloid in the presence of CaF₂ in different concentrations. Secondary structure content estimation was performed by the BeStSel method⁴⁻⁶. A β monomers are mainly disordered and amyloid form is rich in β -structures.

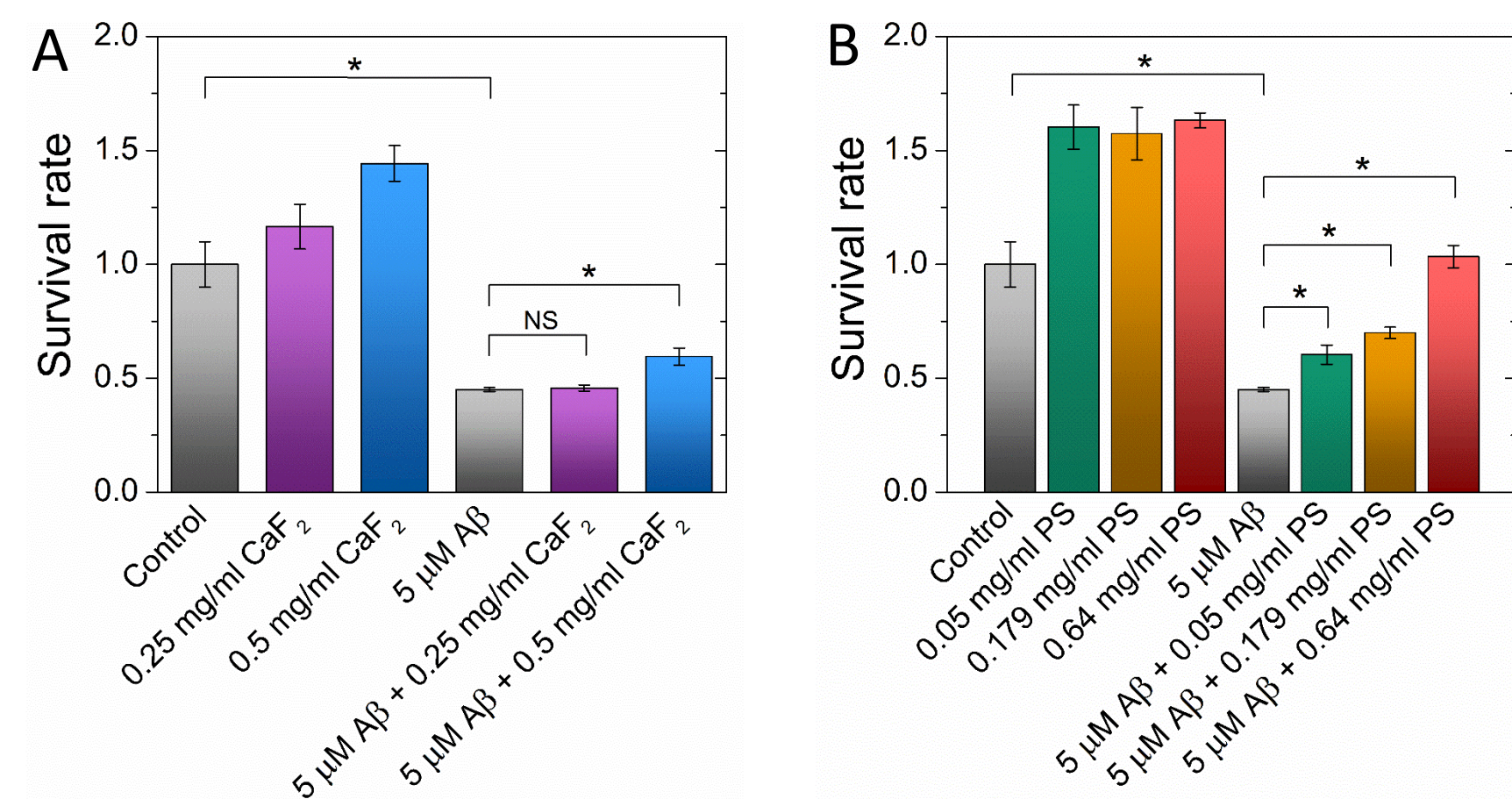


Fig. 9. Results of cytotoxicity measurements of nanoparticles, A β and their combinations on mHippoE-14 cell line by MTT assay. A β is highly toxic to cells, but its toxicity was significantly reduced when applied in combination with either CaF₂ (A) or PS (B) nanoparticles.

Number of technical replicates: 5
Statistical test: t-test
*: p $\leq$ 0.05

Conclusions

- CaF₂ nanoparticles shorten the lag time of A β aggregation. CaF₂ and SiO₂ nanoparticles increase ThT fluorescence of the amyloid form, showing the possibility that different types of fibrils might grow. PS nanoparticles can be saturated by A β monomers and particle-bound monomers show an elevated ThT signal.
- A β aggregated alone and in the presence of CaF₂ nanoparticles differ in β -structure composition. A β aggregated with CaF₂ contains a higher amount of parallel and relaxed antiparallel (Anti2) β -structure than A β fibrils formed alone. However, each type has significantly higher β -structure content than its corresponding monomer form.
- A β is remarkably cytotoxic on mouse hippocampal cells, but its toxic effect can be mitigated by CaF₂ or PS nanoparticles.

Acknowledgements

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