

Effects of nanoparticles on the amyloid formation and cytotoxicity of the amyloid-beta peptide

Éva Moussong¹, Márton Péter Nyiri¹, Nikoletta Murvai¹, Judit Kun^{1,2}, Attila Kovács^{1,2}, Tamás Molnár¹, András Micsonai^{1,2}, József Kardos^{1,2}

¹Department of Biochemistry, Institute of Biology, ELTE Eötvös Loránd University, Budapest H-1117, Hungary

²ELTE NAP Neuroimmunology Research Group, Department of Biochemistry, Institute of Biology, ELTE Eötvös Loránd University, Budapest H-1117, Hungary

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

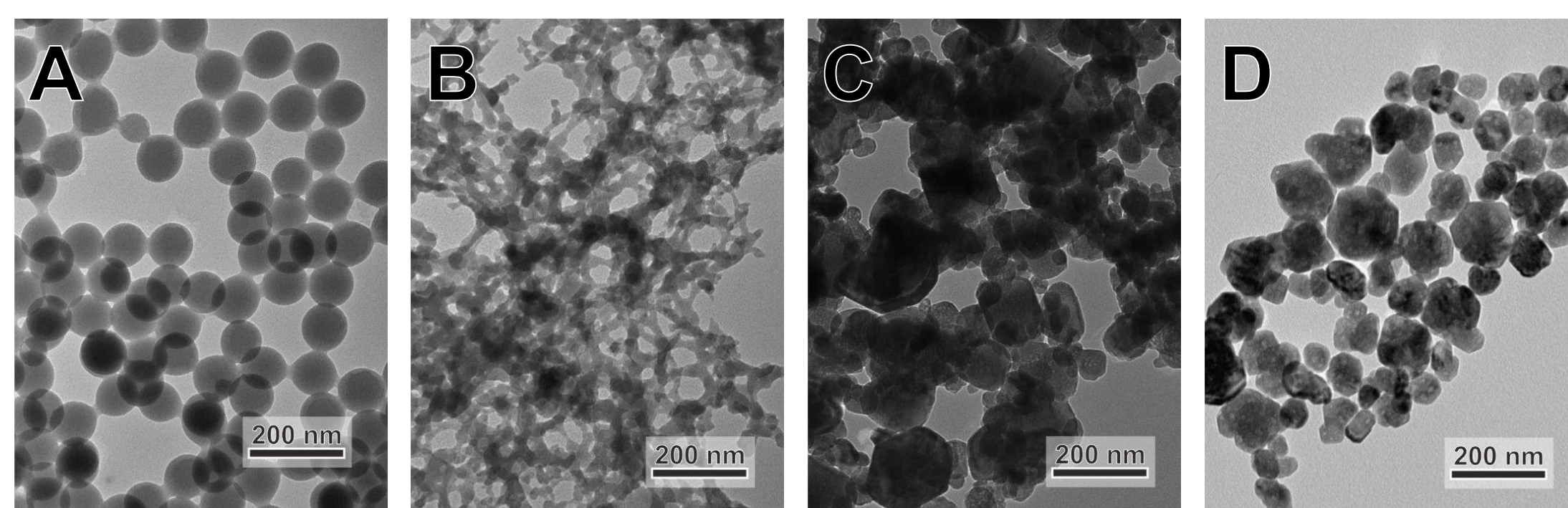


Fig. 1. TEM images of purchased nanoparticles of polystyrene (A), SiO₂ (B) and CaF₂ (C), and in-lab synthesized CaF₂ (D).

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 in the plastic industry, 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 several hundreds of nanometers long fibrils observable by transmission electron microscopy (TEM).

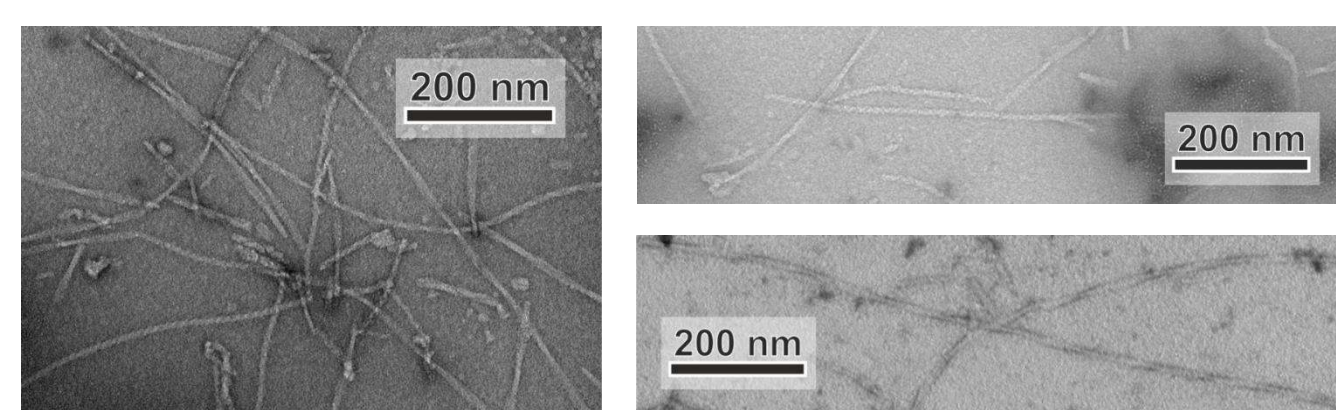


Fig. 2. TEM images of different morphological types of A β fibrils.

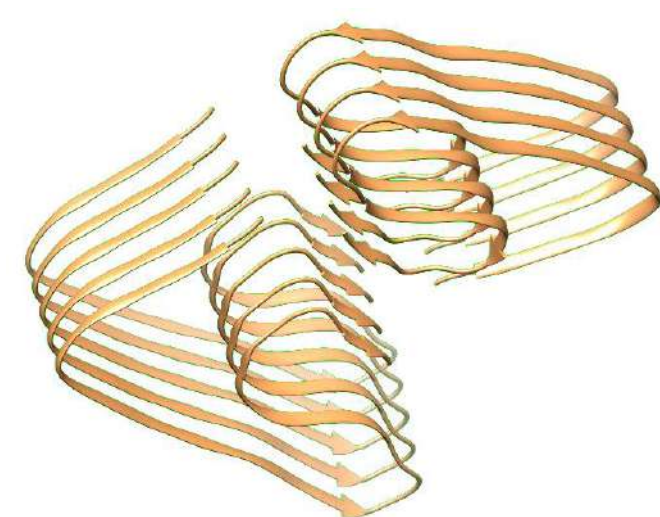


Fig. 3. Cryo-EM structure of A β (1-42) peptides forming a cross-beta sheet structured fibril. PDB: 5OQV

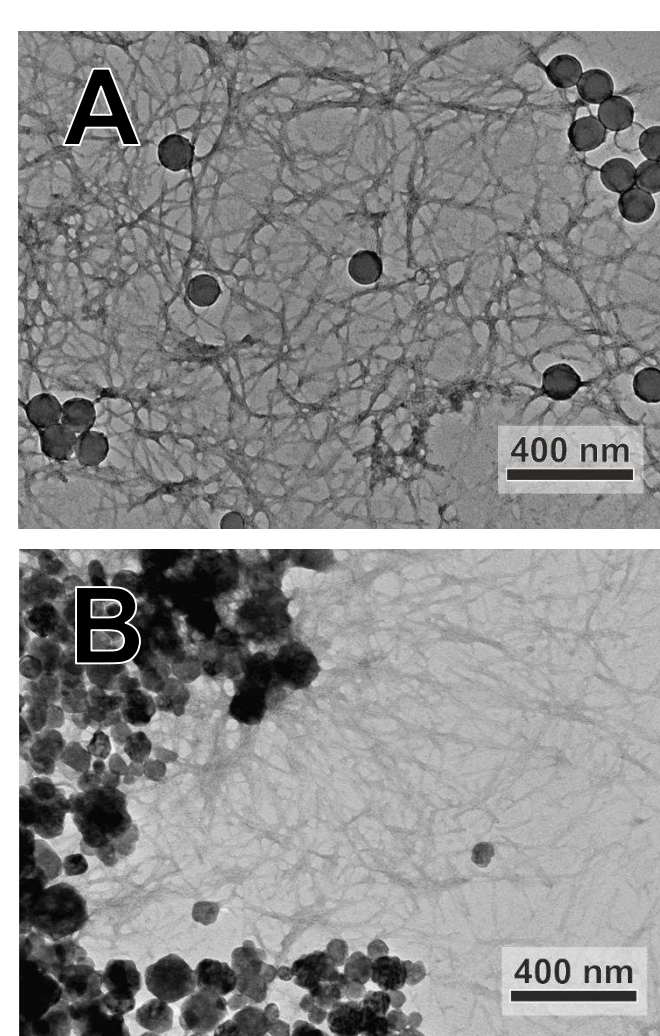


Fig. 4. TEM images of amyloid fibrils attached to PS (A) and CaF₂ (B) nanoparticles.

Effects on A β

ThT fluorescence

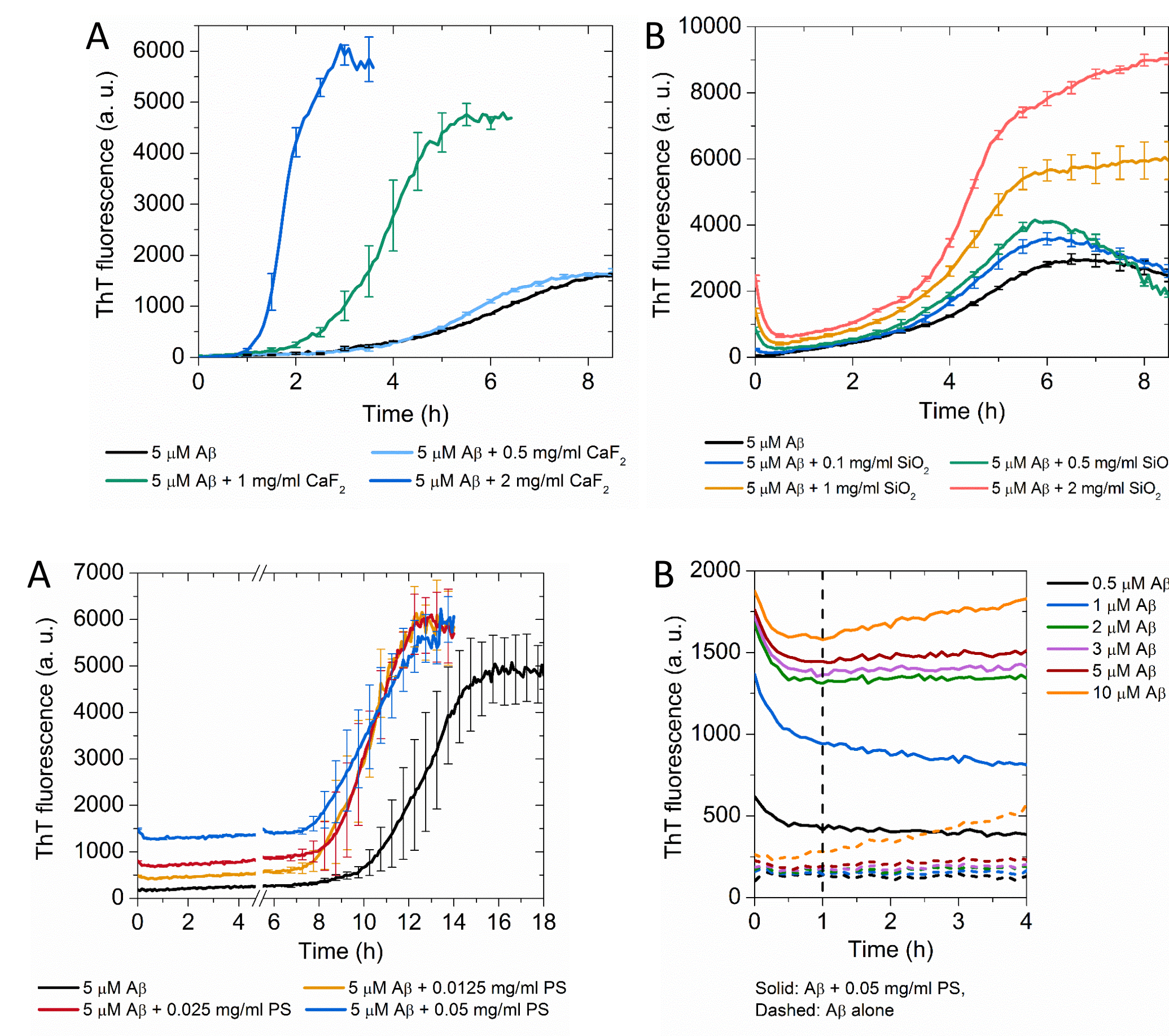


Fig. 5. Aggregation of A β followed by Thioflavin T (ThT) fluorescence, in the presence of CaF₂ (A) or SiO₂ nanoparticles (B) of different concentrations. Higher concentrations of nanoparticles resulted in higher fluorescence intensities in the case of both nanomaterials, and CaF₂ nanoparticles also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

Fig. 6. ThT fluorescence of samples containing A β and PS nanoparticles. (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 starting phase of the aggregation time course (monomeric form is dominant). PS-bound A β monomers show an increased signal which is proportionate 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 .

CD spectroscopy

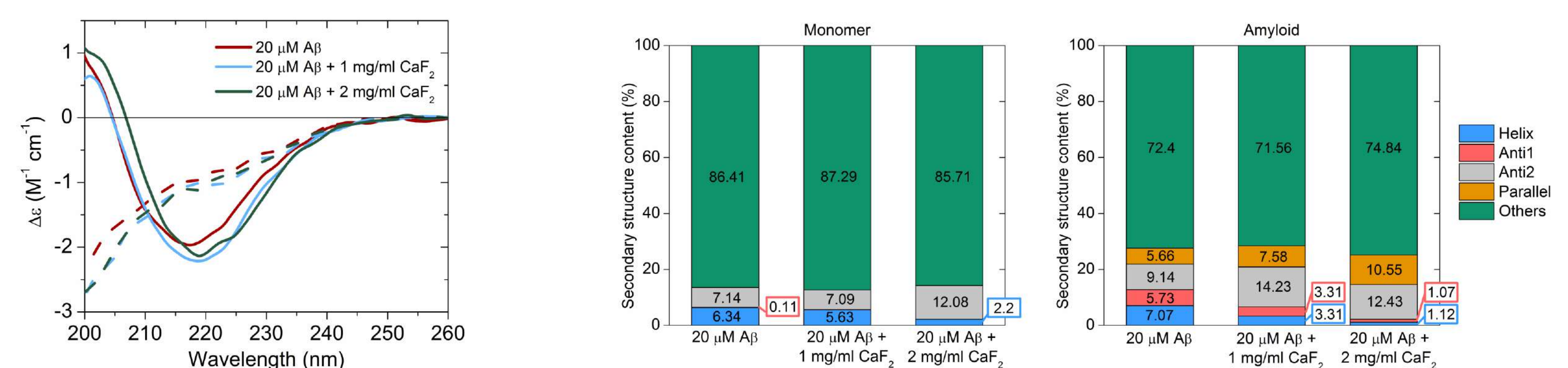


Fig. 7. CD spectra of A β in the presence of CaF₂ in different concentrations before (dashed) and after aggregation (solid).

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 beta structures.

Cytotoxicity

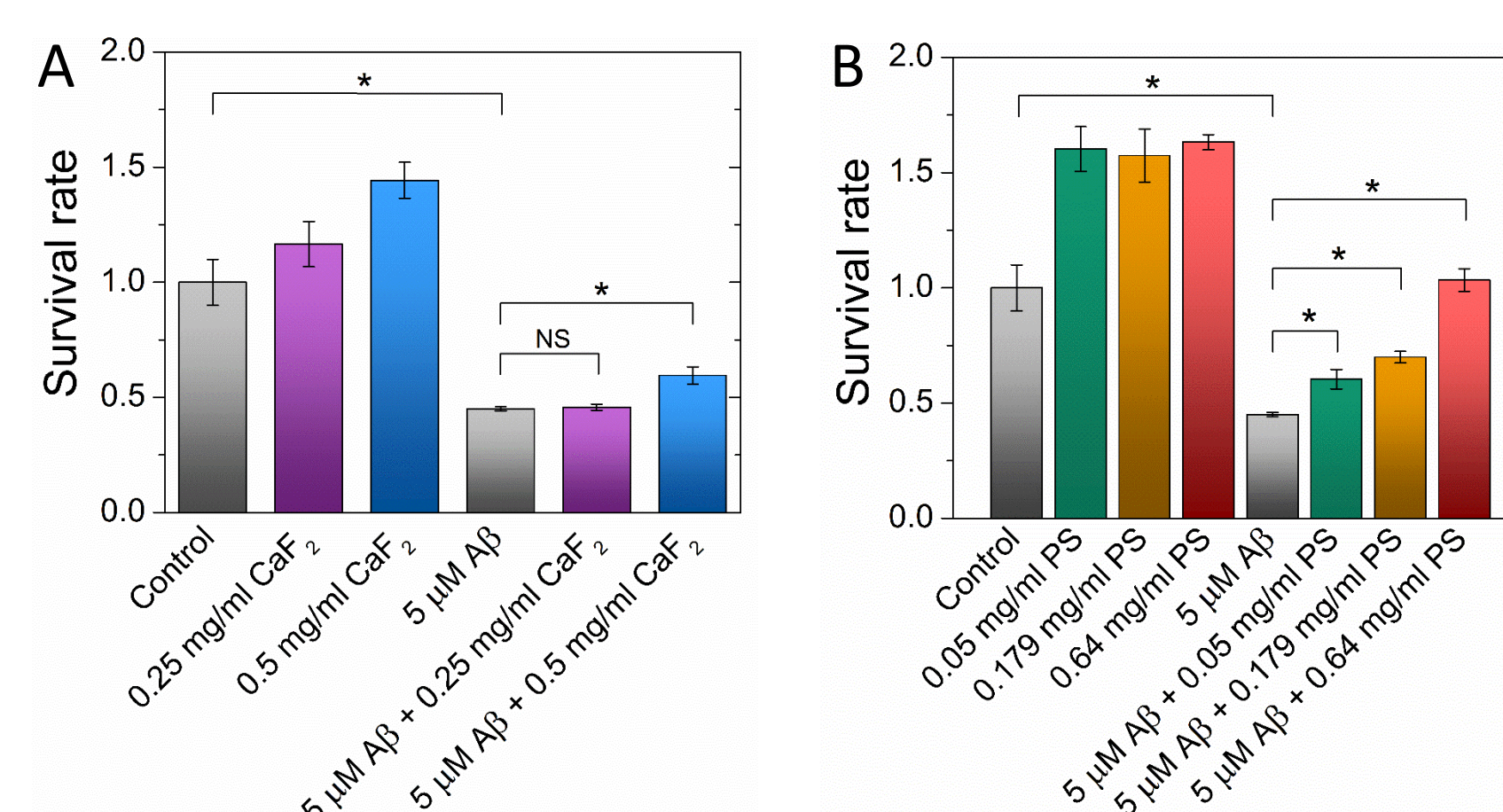


Fig. 9. Results of cytotoxicity measurements of nanoparticles, A β and their combinations on mHippoE-14 cell line by MTT assay. A β is highly toxic to cells, but its toxicity was significantly reduced when applied in combination with either CaF₂ (A) or PS (B) nanoparticles.

Number of technical replicates: 5
Statistical test: t-test
*: p < 0.05

Conclusions

- CaF₂ and SiO₂ nanoparticles shorten the lag time of A β aggregation and increase final ThT fluorescence, showing the possibility that different types of fibrils might form. 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 left-hand-twisted 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

This work is supported by the National Research, Development and Innovation Office of Hungary (grants PD135510 and K138937) and the Gedeon Richter Talentum Foundation (grant „Richter Gedeon Kiválósági PhD Ösztöndíj”).



References

- Karlsson, M., Mårtensson, L. G., Jonsson, B. H., & Carlsson, U. (2000). Adsorption of human carbonic anhydrase II variants to silica nanoparticles occur stepwise: binding is followed by successive conformational changes to a molten-globule-like state. *Langmuir*, 16(22), 8470-8479.
- Gagner, J. E., Lopez, M. D., Dordick, J. S., & Siegel, R. W. (2011). Effect of gold nanoparticle morphology on adsorbed protein structure and function. *Biomaterials*, 32(29), 7241-7252.
- John, T., Gladysz, A., Kubell, C., Martin, L. L., Risselada, H. J., & Abel, B. (2018). Impact of nanoparticles on amyloid peptide and protein aggregation: a review with a focus on gold nanoparticles. *Nanoscale*, 10(45), 20894-20913.
- Micsonai, A., Wien, F., Kernya, L., Lee, Y. H., Goto, Y., Réfrégiers, M., & Kardos, J. (2015). Accurate secondary structure prediction and fold recognition for circular dichroism spectroscopy. *Proceedings of the National Academy of Sciences*, 112(24), E3095-E3103.
- Micsonai, A., Wien, F., Bulváki, É., Kun, J., Moussong, É., Lee, Y. H., ... & Kardos, J. (2018). BeStSel: a web server for accurate protein secondary structure prediction and fold recognition from the circular dichroism spectra. *Nucleic Acids Research*, 46(W1), W315-W322.
- Micsonai, A., Moussong, É., Wien, F., Boros, E., Vadász, H., Murvai, N., ... & Kardos, J. (2022). BeStSel: webserver for secondary structure and fold prediction for protein CD spectroscopy. *Nucleic Acids Research*.