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From Self-Assembly to Function: Tunable Peptide Fibrils as Nanocarriers

Milena Czeszejko[1], Justyna Sawicka[1], Ewa Wieczerzak[1], Daria Sowik[2], Patrycja Rachubik[3], Tomasz Kulesza[3], Kamila Kitowska[4], Rafał Sądej[4], Zuzanna Pietralik-Molińska[5], Maciej Kozak[5], Olena Ivashchenko[6], Sylwia Rodziewicz-Motowidło[1]

[1]Department of Biomedical Chemistry, Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland [2]Department of Food Chemistry and Biotechnology, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland [3]Mirosław Mossakowski Medical Research Institute, Polish Academy of Sciences, Laboratory of Molecular and Cellular Nephrology, Gdańsk, Poland [4]Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk, Gdańsk, Poland [5]Department of Macromolecular Physics, Institute of Physics, Adam Mickiewicz University, Poznań, Poland [6] NanoBioMedical Centre, Adam Mickiewicz University in Poznań, Poznań, Poland

milena.czeszejko@phdstud.ug.edu.pl

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Katedra Chemii Biomedycznej

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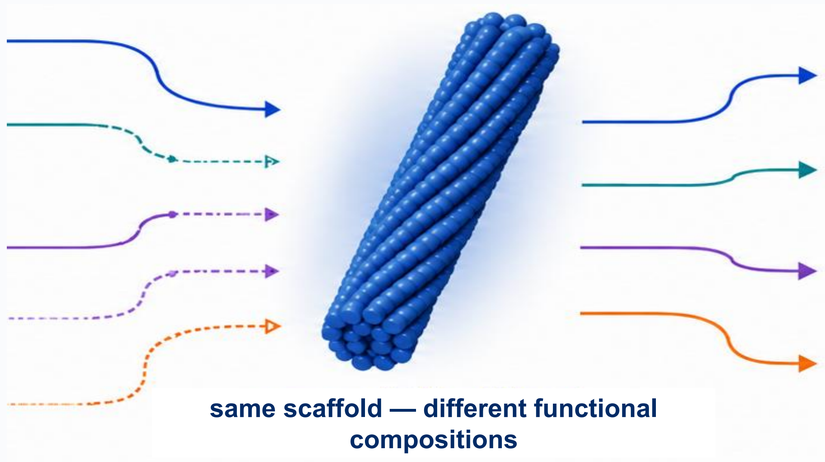
1 MODULAR DESIGN OF THE S7 FIBRIL PLATFORM

One fibrillogenic sequence enables multiple structurally and functionally distinct fibril systems.

Molecular building blocks

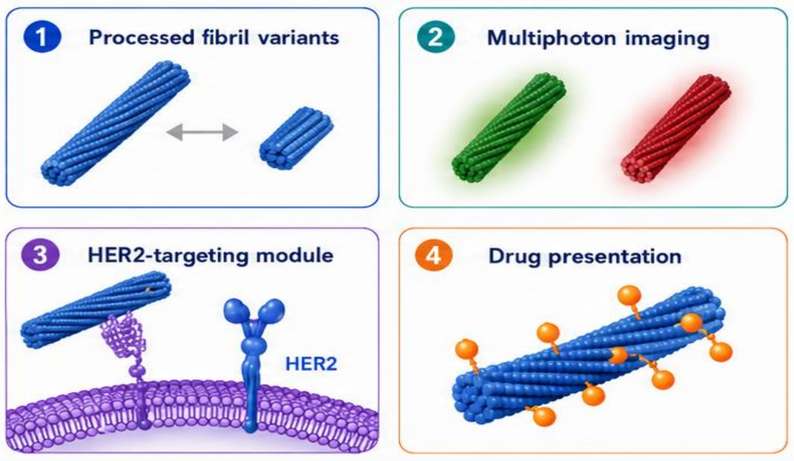
1. **fibrillogenic scaffold** s7: GQAGIVV
2. **Fluorescently labeled** S7 variants — PyTAP, BODIPY
3. **Targeting module** Af-PEG-s7, Af-SS-Cys-s7, (Cys-s7) = s8
4. **Therapeutic module** s7-MMAE

Ratio-controlled co-assembly



same scaffold — different functional compositions

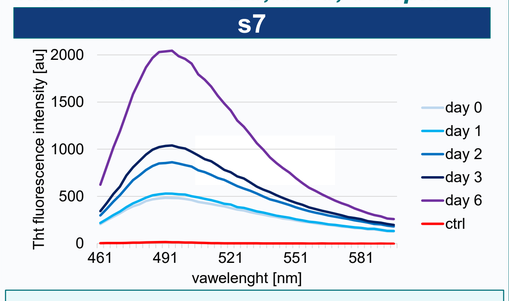
Tunable functional outputs



2 FIBRILLIZATION KINETICS

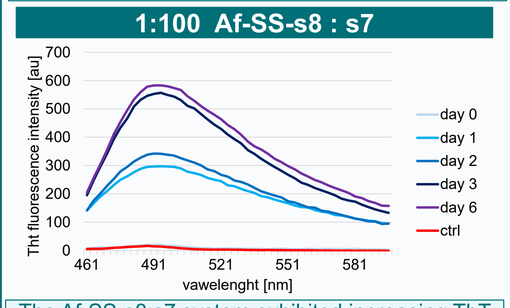
Time-resolved ThT fluorescence was used to monitor the formation of β-sheet-rich assemblies during 6 days of incubation in PBS, 37°C, 300rpm.

s7



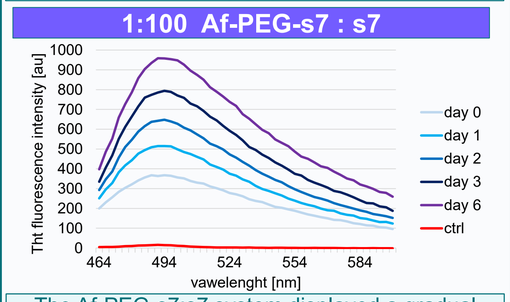
s7 showed a pronounced time-dependent increase in ThT fluorescence, with the highest signal observed on day 6, indicating progressive formation and maturation of ThT-binding fibrillar structures.

1:100 Af-SS-s8 : s7



The Af-SS-s8:s7 system exhibited increasing ThT fluorescence up to day 3, followed by only a minor change on day 6, suggesting that the ThT response reached an apparent plateau earlier than for s7 alone.

1:100 Af-PEG-s7 : s7

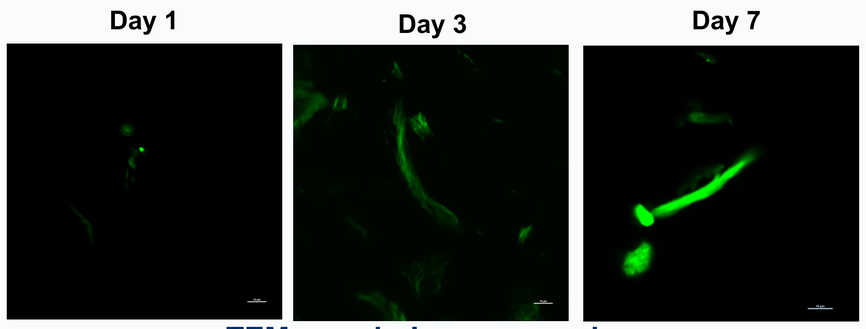


The Af-PEG-s7:s7 system displayed a gradual increase in ThT fluorescence throughout the incubation period. The final signal remained lower than for s7 alone but higher than for the Af-SS-s8-containing system.

3 FLUORESCENT TRACKING OF s7 FIBRILLIZATION

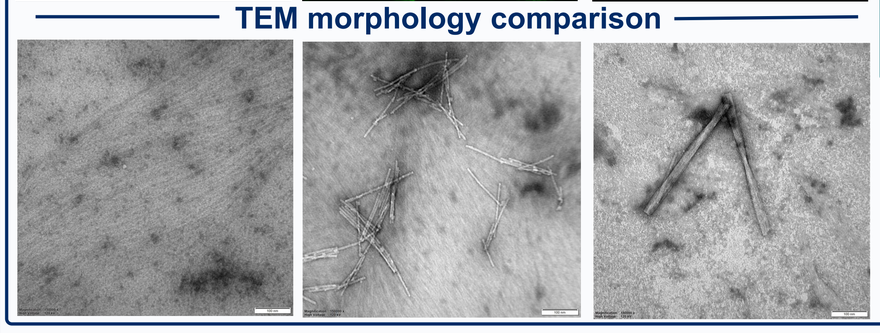
Fluorescently labelled s7 variants enable time-resolved and multichannel visualization of fibril formation.

A. Time-resolved fibrillization of BODIPY-s7



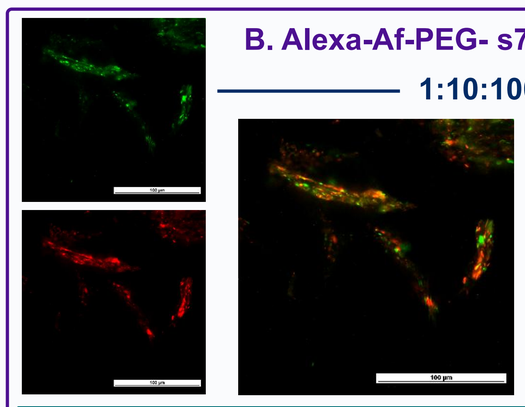
BODIPY-labelled s7 was monitored by confocal microscopy after 1, 3, and 7 days of fibrillization. A progressive increase in fluorescence intensity along fibrillar structures was observed over time, indicating ongoing fibril formation and maturation.

TEM morphology comparison



TEM imaging confirmed that the fluorescent structures corresponded to peptide fibrils.

B. Alexa-Af-PEG- s7 : PyTAP-s7 : s7 mixed systems



Mixed fibrillar systems composed of AlexaFluor-s7, PyTAP-s7, and unlabelled s7 were analysed after 7 days of fibrillization. Among the tested compositions, the 1:10:1000 ratio provided the most effective confocal imaging, with numerous well-defined fibrils visible in both fluorescence channels. The signal pattern was consistent with the co-presence of both labelled peptide variants within the fibrillar network.

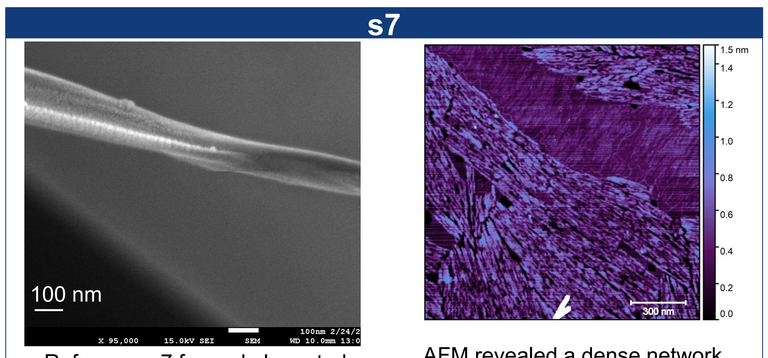
TEM analysis showed that fibril morphology depended on the proportion of unlabelled s7. At the 1:10:1000 ratio, the fibrils were shorter (approximately 200 nm), whereas at the 1:10:5000 ratio, longer fibrils (approximately 1 µm) were observed. In both cases, the fibril thickness remained comparable.

4 NANOSCALE MORPHOLOGY OF FUNCTIONALIZED FIBRILS

Affibody-containing systems retain fibrillar morphology while exhibiting linker-dependent structural organization.

A. cryo-SEM B. AFM

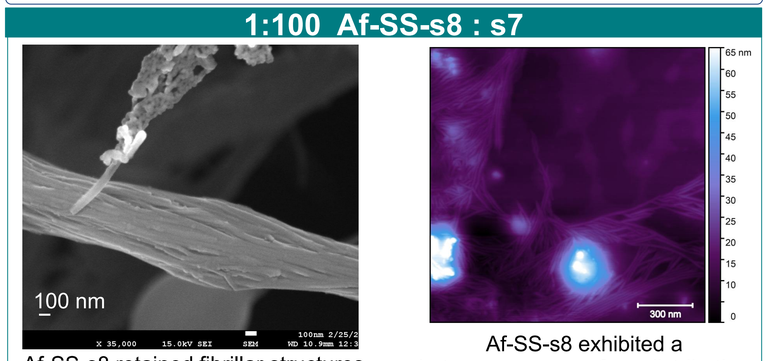
s7



Reference s7 formed elongated, continuous fibrillar structures.

AFM revealed a dense network of elongated reference fibrils.

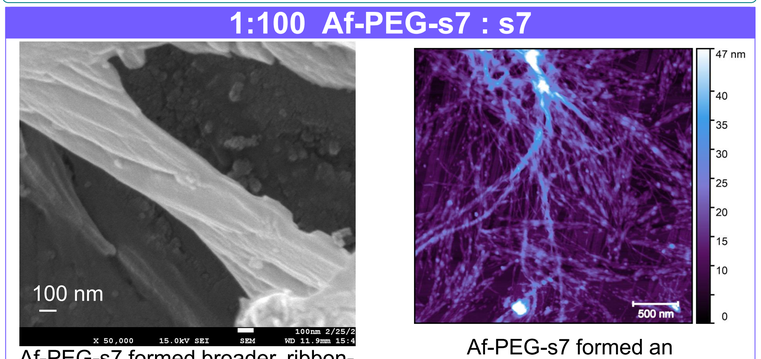
1:100 Af-SS-s8 : s7



Af-SS-s8 retained fibrillar structures with increased morphological heterogeneity.

Af-SS-s8 exhibited a heterogeneous surface distribution of fibrillar structures.

1:100 Af-PEG-s7 : s7



Af-PEG-s7 formed broader, ribbon-like fibrils with a pronounced tendency to bundle.

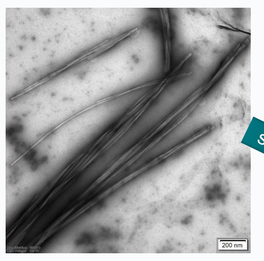
Af-PEG-s7 formed an interconnected fibrillar network with pronounced lateral association.

Cryo-SEM and AFM were used as complementary techniques to compare the nanoscale morphology of reference s7 fibrils and affibody-functionalized s8 systems. Both affibody-functionalized systems retained fibrillar morphology. The disulfide-linked conjugate was more heterogeneous, whereas the PEG-linked system showed pronounced ribbon formation and bundling.

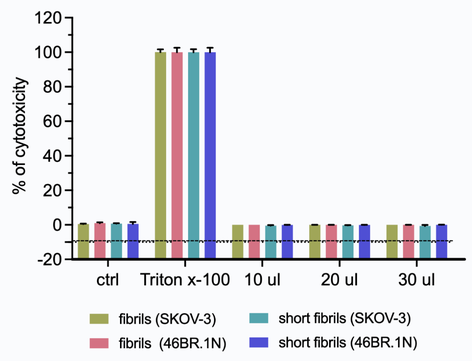
5 BIOLOGICAL READOUTS OF THE s7 PLATFORM

Sonicated s7 fibrils remained cytocompatible, while the mode of MMAE presentation influenced the morphology of BT-474 spheroids.

A. Cytocompatibility of sonicated fibrils

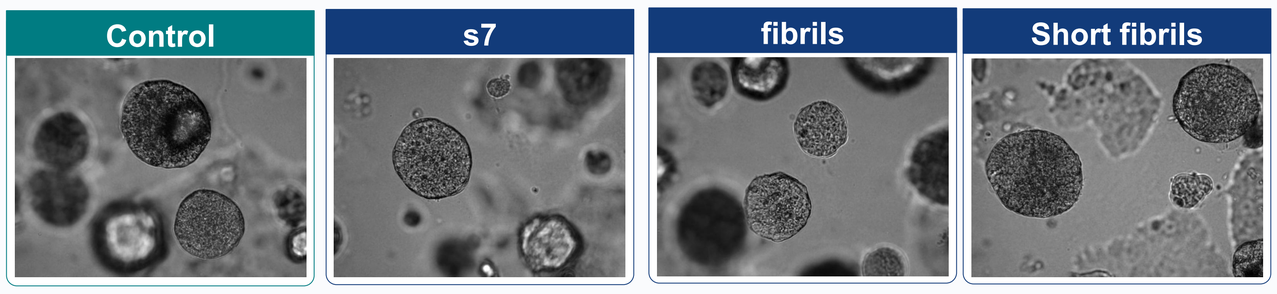


Amplitude = 40%, pulse cycle 2/3s on/off, 2 x 7,5 min



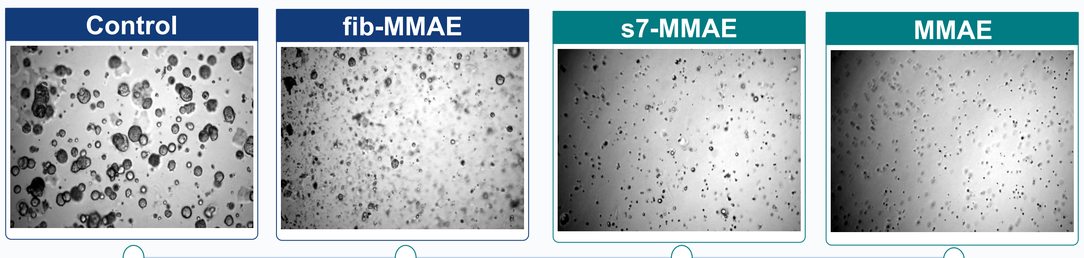
SKOV-3 cells and 46BR.1N human fibroblasts were incubated with non-sonicated and sonicated s7 fibrils. Cytotoxicity was assessed using an LDH-release assay. Neither fibril preparation caused a substantial reduction in cell viability under the tested conditions, indicating that sonication-induced fragmentation did not increase cytotoxicity.

B. Effect of the Investigated Systems on BT-474 Spheroid Morphology



BT-474 spheroids were cultured in Matrigel and treated with 10 µM s7, long s7 fibrils, or short s7 fibrils for 19 days. Neither s7 nor its fibrillar forms induced marked morphological changes compared with the control.

C. MMAE presentation in BT-474 spheroids



BT-474 spheroids were exposed to free MMAE, monomeric s7-MMAE, or fibrillar MMAE-containing systems and analysed by bright-field microscopy. The extent of spheroid disruption depended on the mode of drug presentation. Free MMAE produced the strongest morphological effect, whereas peptide-associated MMAE showed a reduced response, consistent with lower immediate drug accessibility.

6 KEY FINDINGS AND OUTLOOK

Summary of the modular s7 fibril platform and next experimental steps

KEY FINDINGS

1. **s7 scaffold** — Forms structurally defined fibrillar assemblies suitable for modular functionaization
2. **Affibody system** — Retain fibrillar morphology, while linker chemistry influence supramolecular organization
3. **Fluorescent modules** — Eneble time-resolved and multichannel visualization of s7 fibrils
4. **Sonication** — Remain cytocompatible with SKOV-3 cells and 46BR.1N fibroblasts.
5. **Biological performance** — s7 fibrils did not markedly alter BT-474 spheroid morphology, whereas MMAE presentation influenced the spheroid response.

OUTLOOK

- **Integrate functional modules** — Combine targeting, imaging, and therapeutic modules within a single fibrillar construct.
- **Optimize module composition** — Define optimal module ratios and linker chemistry for controlled fibril organization.
- **Assess nanocarrier performance** — Evaluate stability, drug accessibility, and biological activity of the complete nanocarrier.

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