Combining protein and peptide biomaterials with laser technologies towards tissue engineering scaffolds

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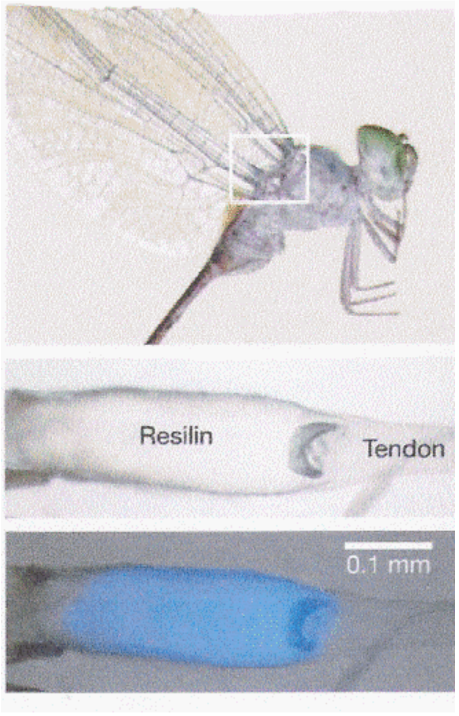
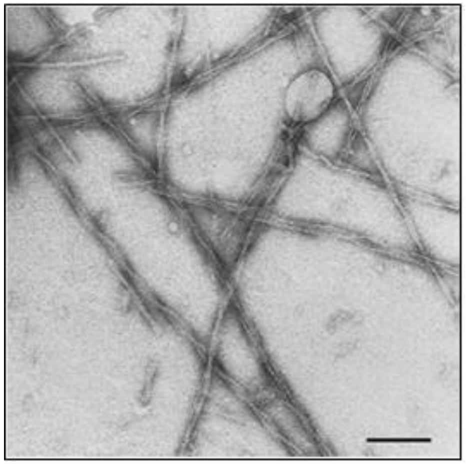
Can we exploit the
self-assembly

of natural biological materials

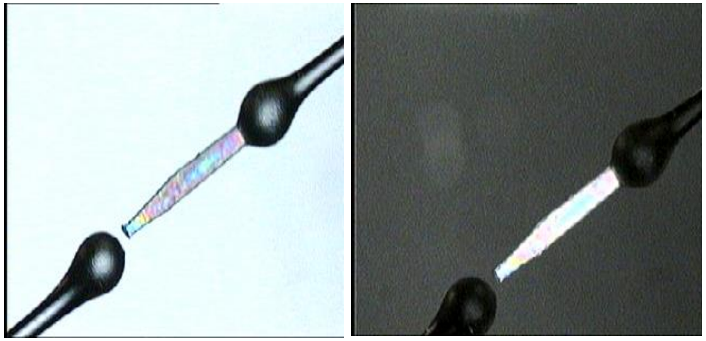
Towards the design
of novel biomaterials
from the nano-
to the macro-scale??

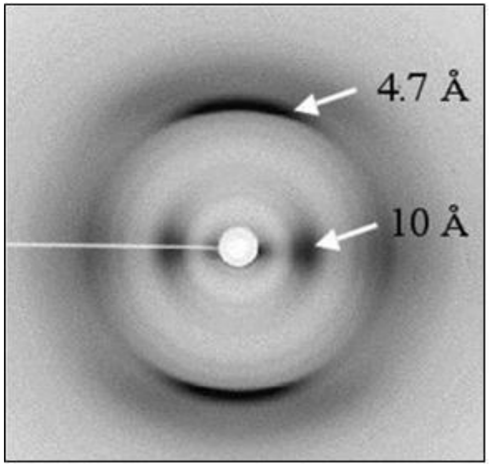


Protein and peptide fragments can self-assemble into "amyloid-type" fibrils



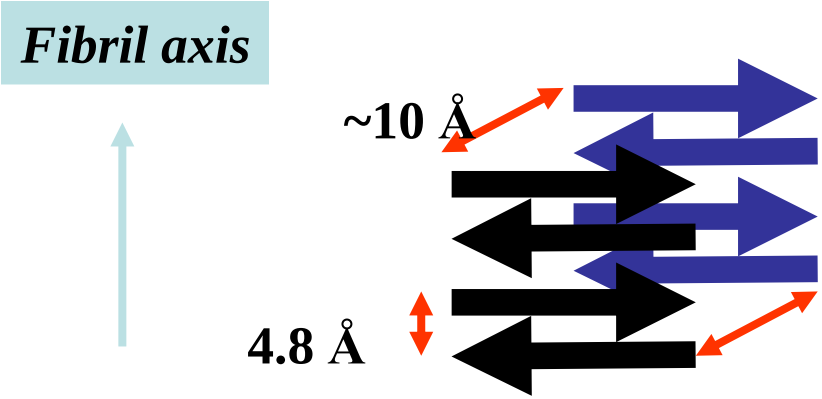
TEM Analysis
(bar:100nm)





Cross-beta model
for amyloid fibrils

X-ray fiber diffraction
pattern for amyloid fibrils



Amyloid fibrous assemblies

- **Amyloid assemblies can also be formed by proteins and peptides that are not associated with disease**

  They are also found in natural fibers / materials (insect chorions, curli fibers, egg stalks, etc)

Chrysopa (Green lacewing fly)
egg stalks (Geddes et al. 1968)

Fibrous nanostructured objects:

-promising for potential integration in future generations of nano-micro devices

-carbon nanotubes most investigated

-however, limitations: (high production costs, extreme manufacturing conditions)

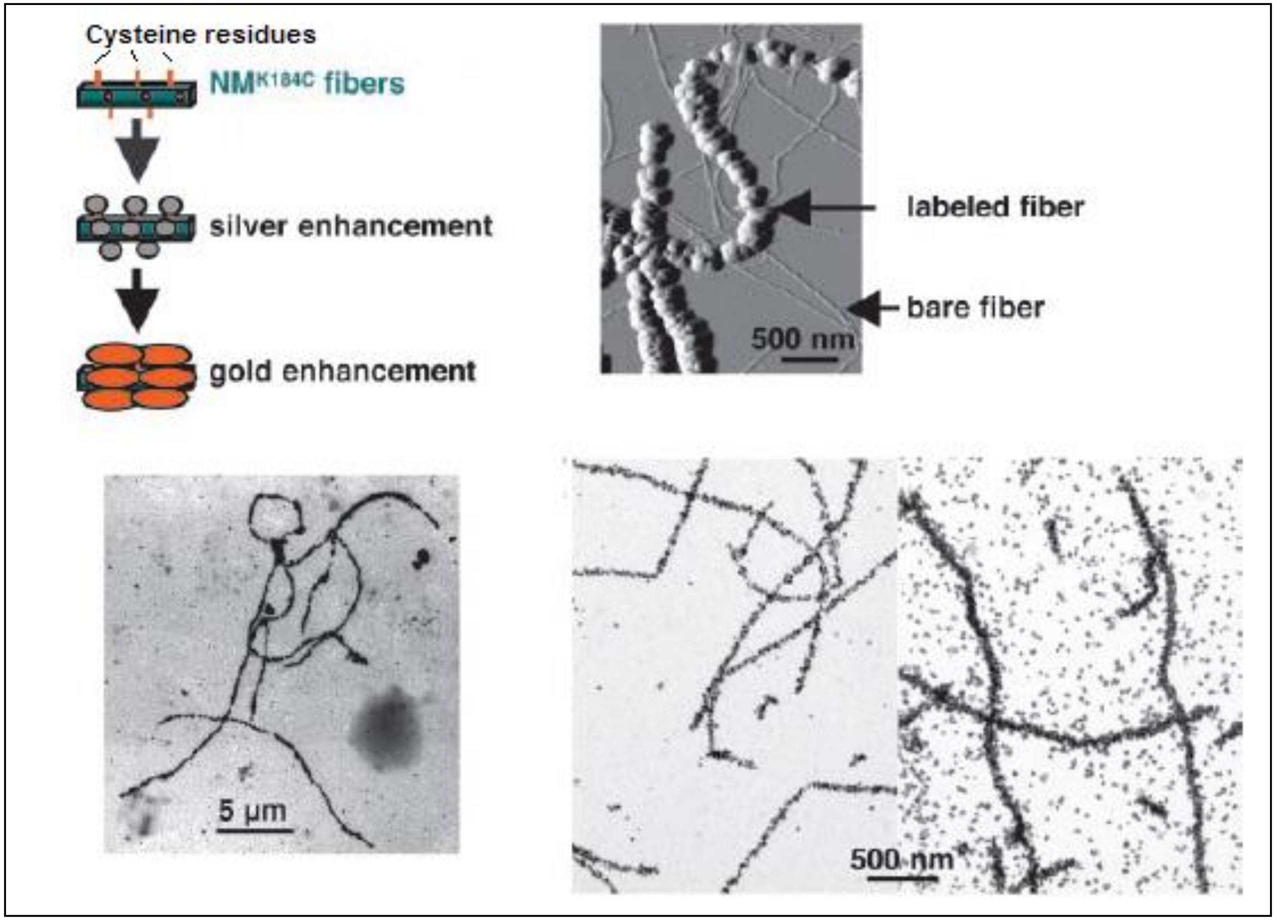
Advantages of biological counterparts (DNA, peptides, proteins):

-Spontaneous self-assembly in mild conditions

-ease of functionalization (possibility of modifications *at the sequence level*)

Self-Assembling peptides as templates for technological applications

A fragment from the yeast *Saccharomyces cerevisiae* Sup35 protein that forms amyloid fibrils was used for nanowire fabrication



Susan Lindquist group

Scheibel et al., PNAS 2003, 100: 4527

Natural assemblies as a source of inspiration for technological applications

Natural assemblies can inspire

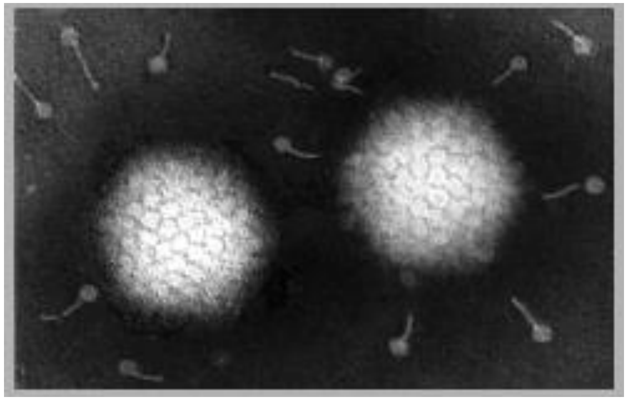
the specific design of

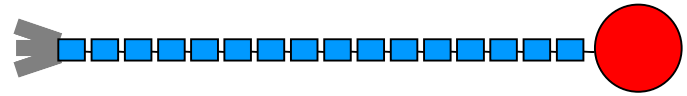
biological building blocks in order to

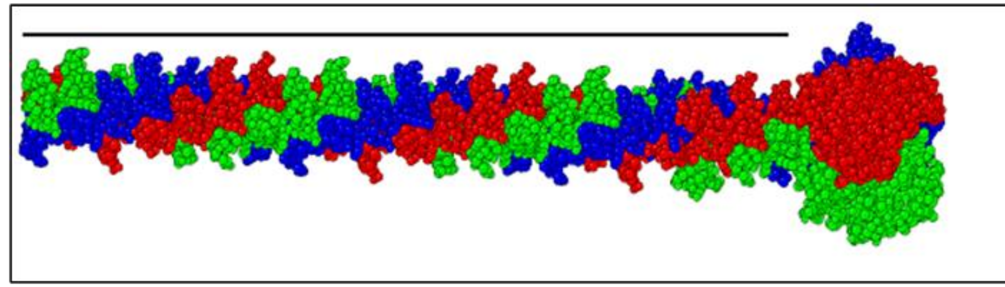
fabricate supramolecular structures

with advanced functionalities

Adenovirus fiber as a self-assembly system







Model for the full-length fiber based on the crystal structure of a stable fragment with 4 repeats

[van Raaij et al. Nature 1999, 401:935]

Properties of adenovirus fiber:

1. **Long and thin shaft (30 nm) gives the virus ‘reach’**
2. **Trimeric, beta-structured**
3. **Extremely stable proteins: resistant to SDS, temperature, denaturants**

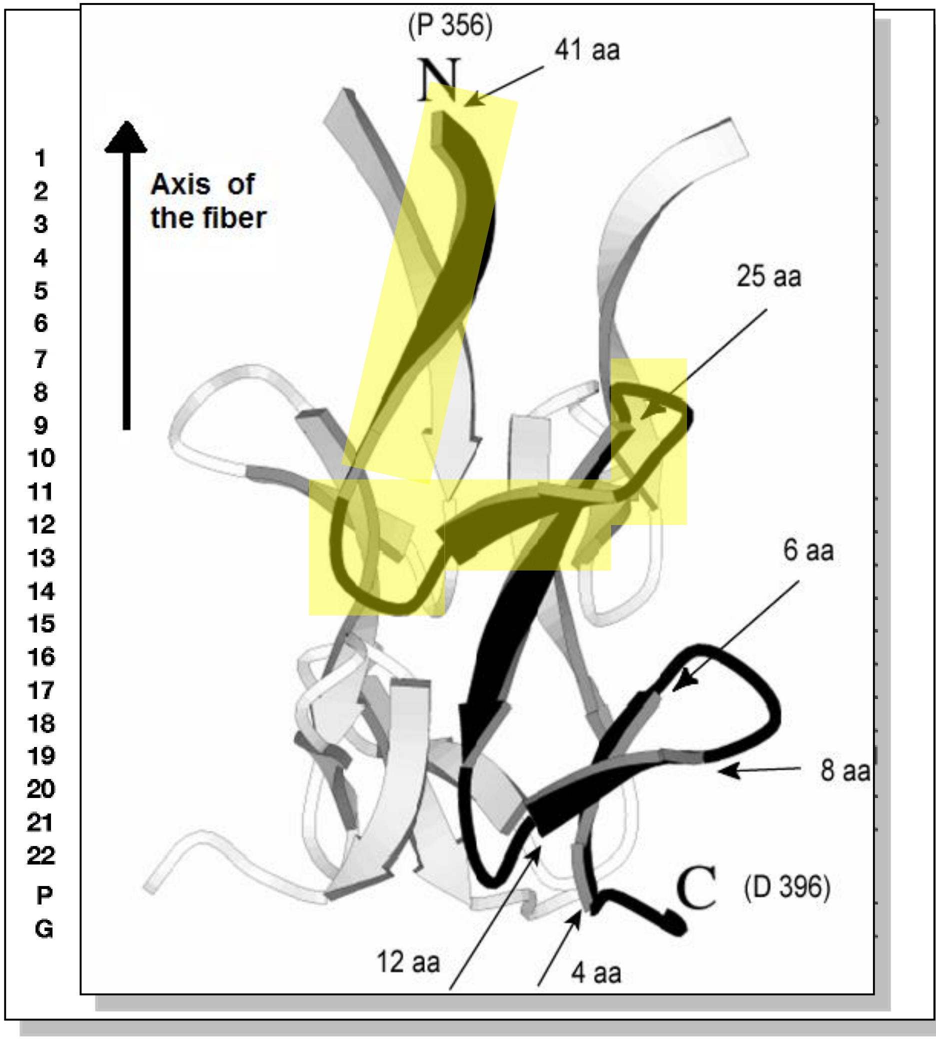
4 Fibrous shaft built from sequence repeats (blue squares)

Adenovirus fiber as a self-assembly system

Each fiber monomer contains 582aa

1. The fibrous shaft is built from a <u>repeating sequence motif</u> with a *hydrophobic* aminoacids (ORANGE and GREEN residues) alternating with *hydrophilic* ones and glycine or proline at conserved positions (PURPLE residues)

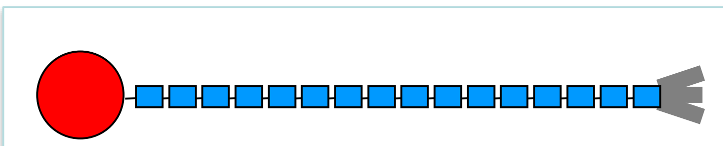
2. <u>The basic repeat fold</u> contains a beta-strand almost <u>parallel</u> to the axis of the fiber followed by a beta turn and another beta strand which runs at an angle of 45° relative to the fiber axis. The repeats are joined by a solvent exposed loop

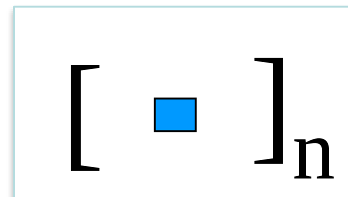


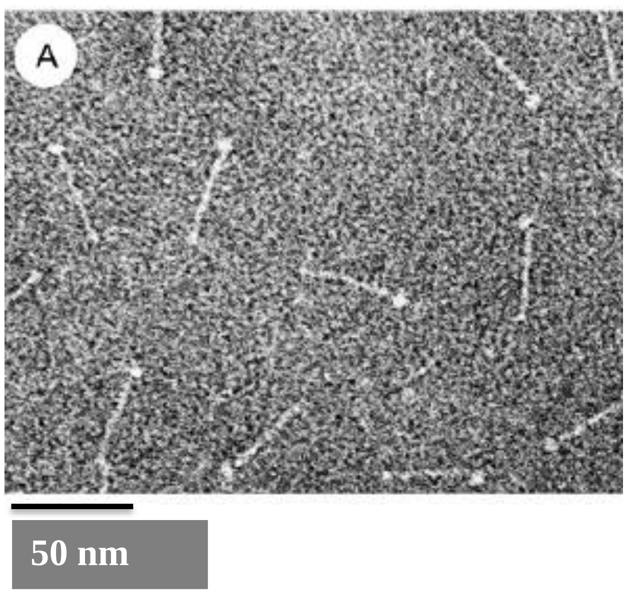
Papanikolopoulou et al., J. Biol. Chem. 2005, 280 : 2481

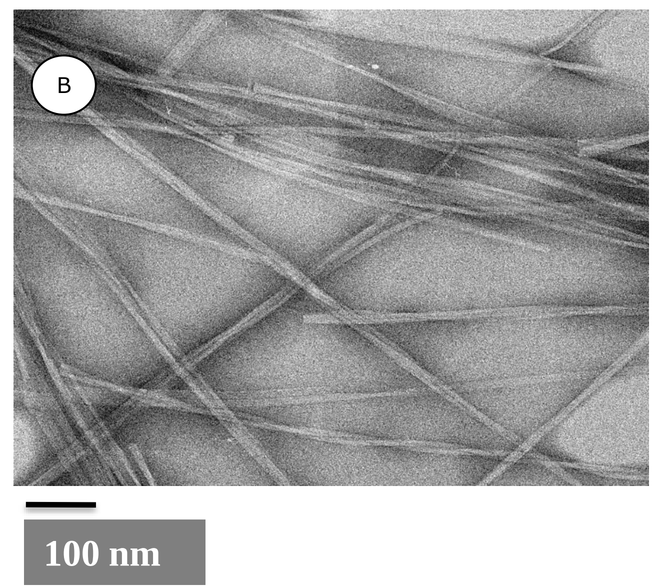
Peptide sequences as a self-assembly system

Identification of minimal peptides corresponding to natural building blocks (blue squares, octapeptide NSGAITIG) that can self-assemble into amyloid-type fibrils







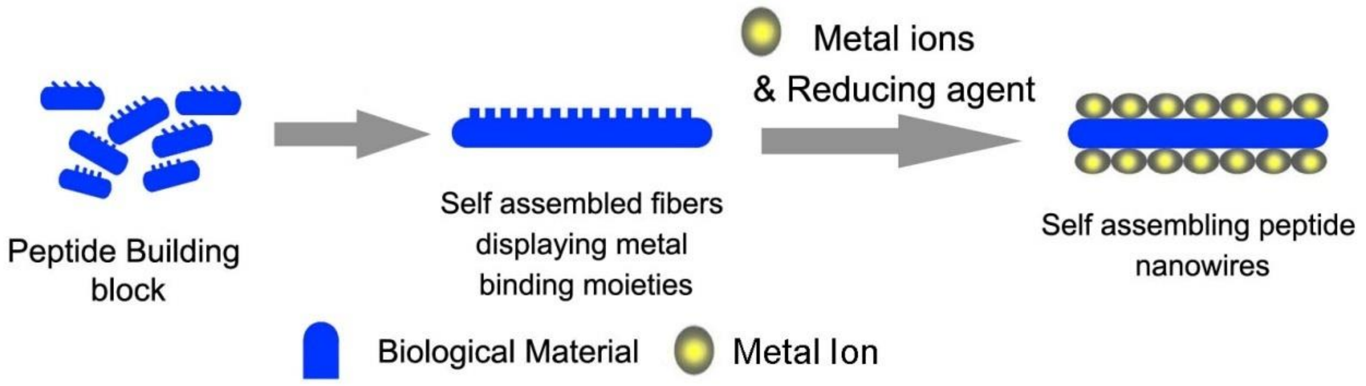


Papanikolopoulou et al., J. Biol. Chem. 2005, 280 : 2481

Can we rationally modify these fibrous building blocks towards specific functions?

eg. templating of inorganic materials

targeting sequences for metallization,
Calcium binding,
cell attachment _etc_



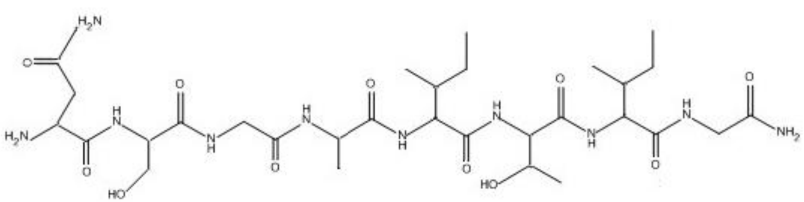
- **The N-S-G-A-I-T-I-G peptide self-assembles into amyloid fibrils with cross-beta structure**

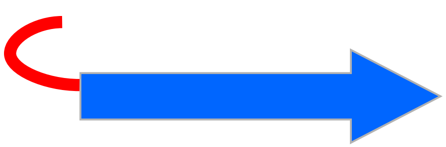
- **The beta strands are perpendicular to the fibril axis**

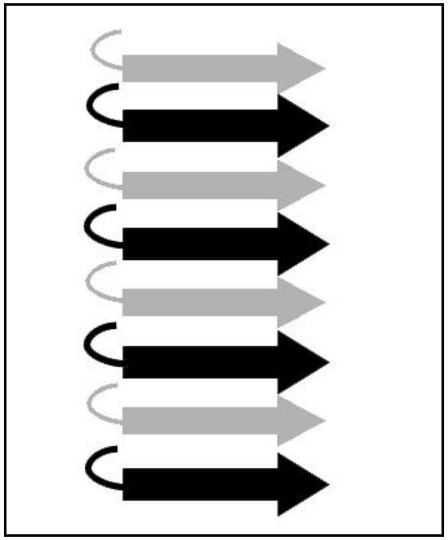
- **The peptide arrangement can be parallel or anti-parallel**

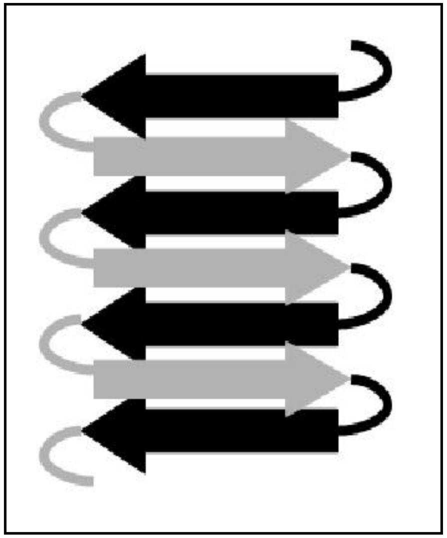
- **Molecular Dynamics simulations suggest that the N-S residues stay exposed out of the amyloid fibril core**

(Tamamis et al., J. Phys. Chem. B 2009, 113:15639)



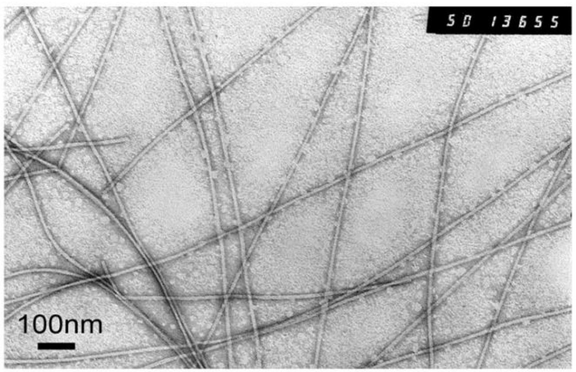




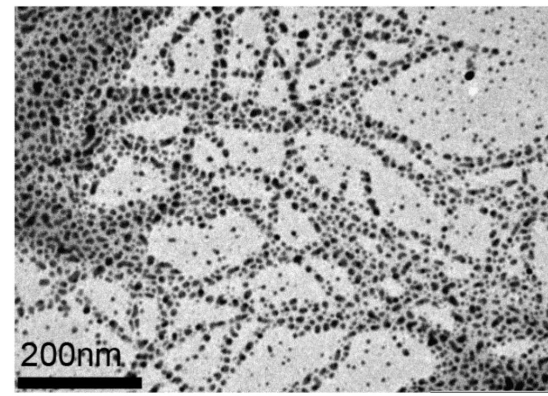


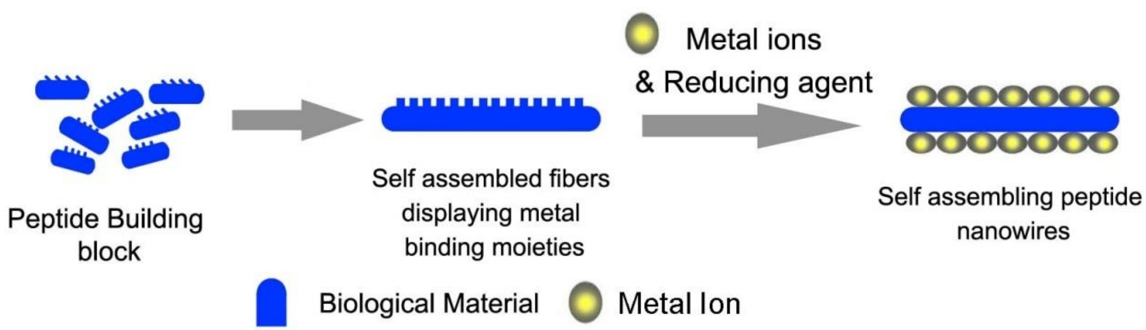
Rational modification of these building blocks towards specific functions

eg. templating of inorganic materials
(metal nanoparticles, silica, Qdots, calcium)
cell attachment *etc*



1) Kasotakis et al., 2009, Biopolymers 92:164-72
2) Kasotakis, Mitraki, 2012, Biopolymers, 98:501
3) Kasotakis et al., 2014, App. Phys.A., 116:977

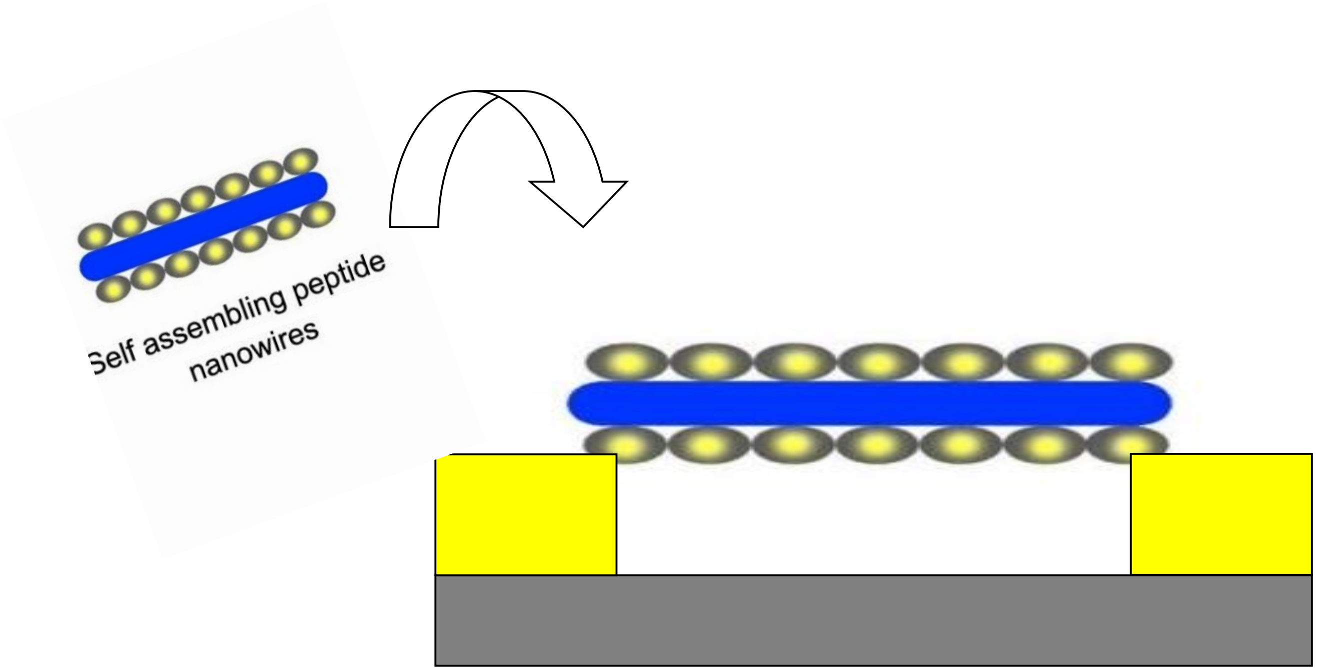




Can we control the positioning of fibers accurately, in 2- or 3-dimensions at the micro / nano scale???

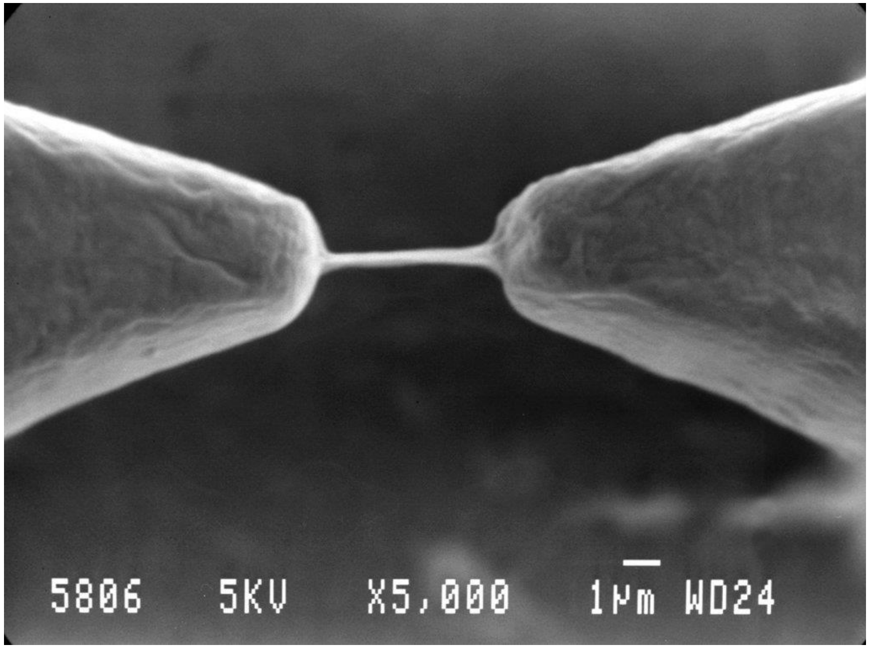
Directed positioning is a major challenge

For integration of self-assembling fibrous materials in devices



Development of inter-disciplinary approaches

**towards controlled positioning on surfaces
and integration in devices**

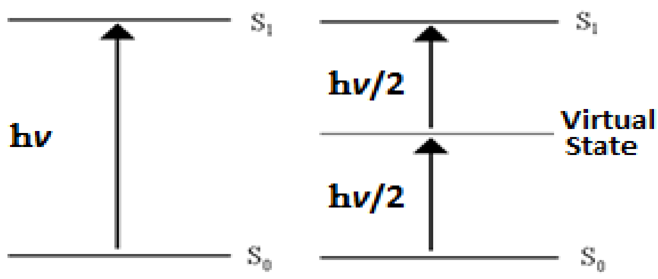


Example: peptide fibrils positioned on a hybrid organic-inorganic material using femtosecond Laser Technologies

V. Dinca et al., NanoLetters 2008, 8:538

(collaboration with Dr. M. Farsari et al., IESL, FORTH)

Direct Laser Writing (DLW)
Multi-photon polymerization technique (MPP)



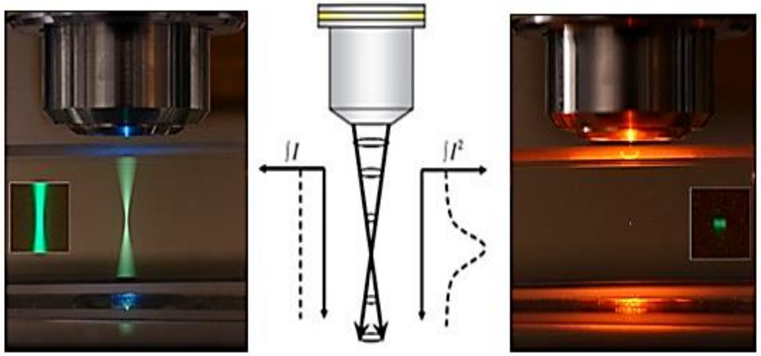
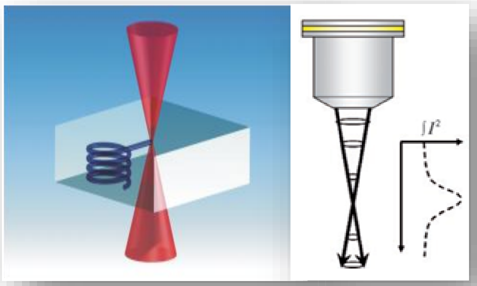


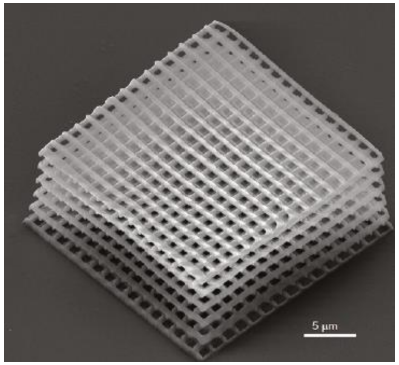
Image by Steve Ruzin and Holly Aaron, UC Berkeley

Hybrid Photostructurable Material

+



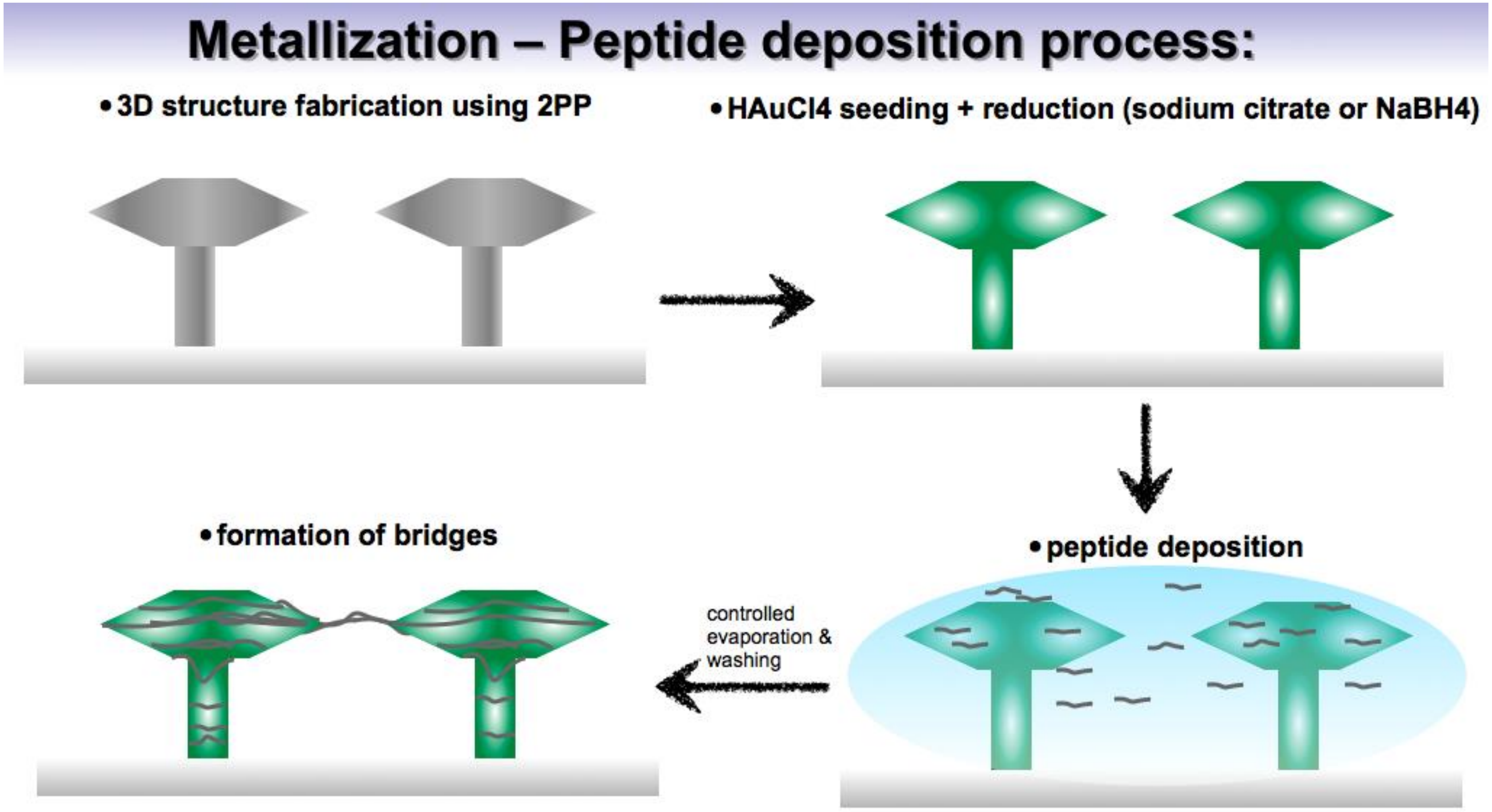
NIR fs pulses



- *Highly attractive and promising 3D microfabrication technology*
- *Real 3D Writing (no mask, mold or stamp)*
- *Complex Micro- & Nano- structures*

Sakellari et. al, 2012, ACSNano, 3: 2302

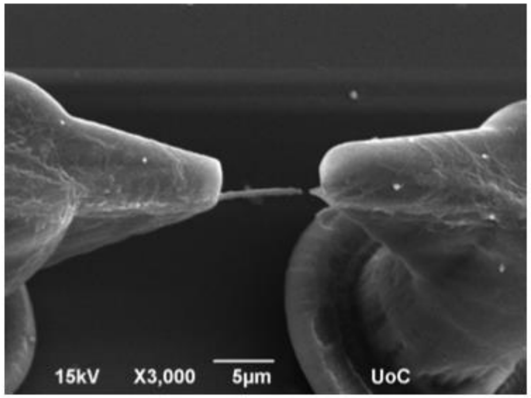
Positioning of metal-binding peptides on 3D structures fabricated using a composite sol-gel with metal binding sites

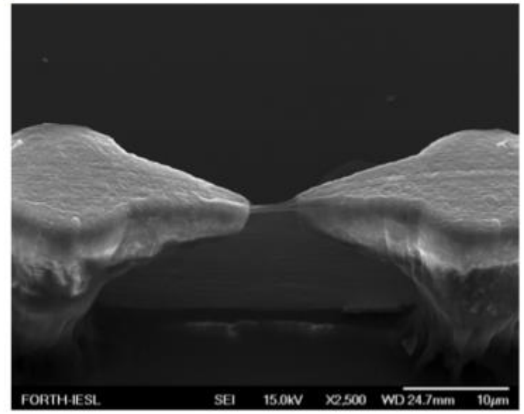


Hybrid organic-inorganic materials designed by Prof. Maria Vamvakaki

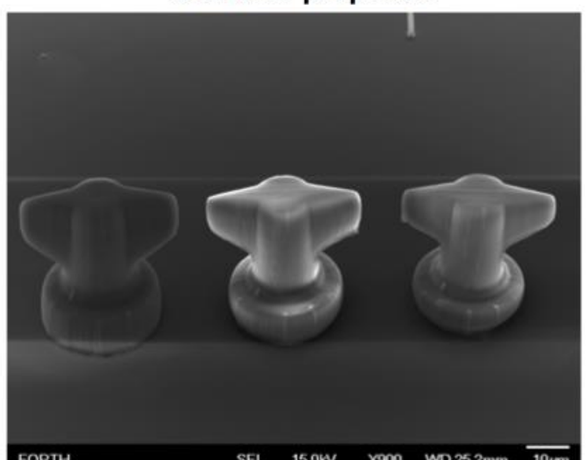
Positioning of Cysteine-containing peptides on 3D nanostructures fabricated using a composite sol-gel with metal binding sites

Cysteine containing peptide





control peptide



Can we develop « scaffold-on-scaffold » approaches?

ie.

Combine the advantages of top-down fabrication
(eg. control of scaffold geometry and micro-nanotopography)

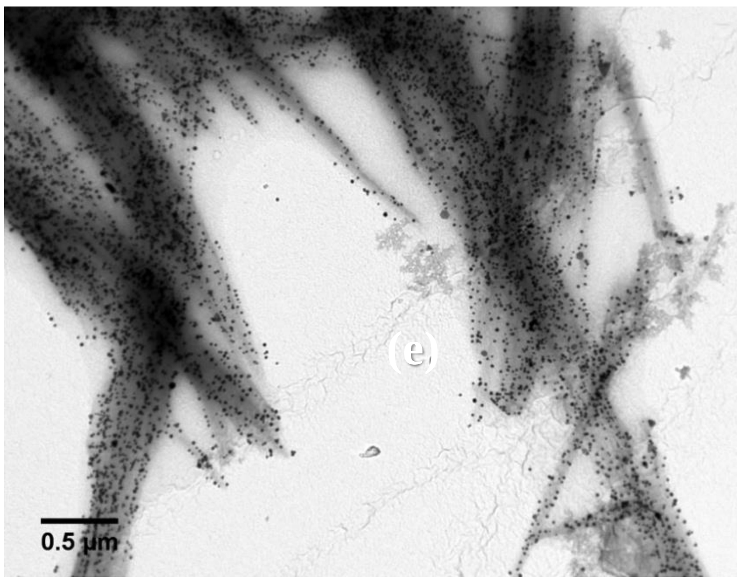
With

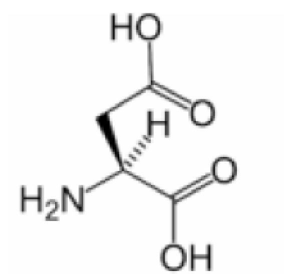
Bottom-up design possibilities of
Self-assembling peptides?

Design of bifunctional aspartate-containing self-assembled peptides

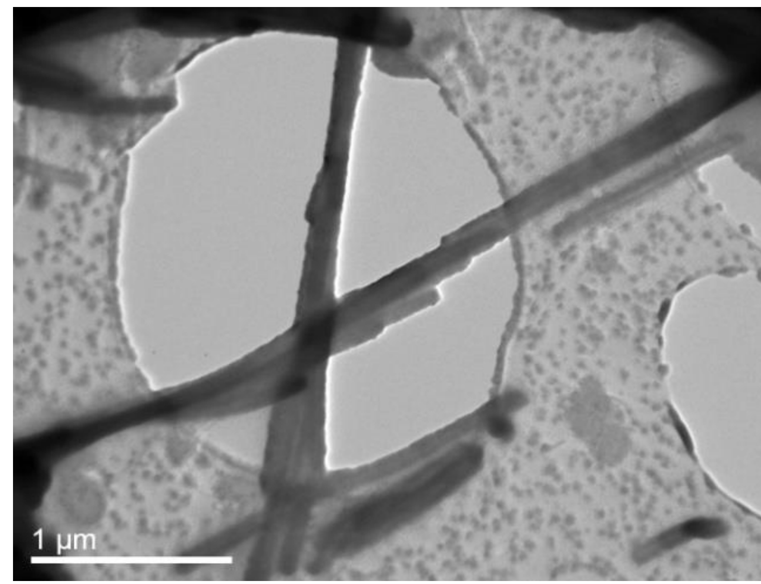
Peptide DDSGAITIG forms fibrils that:

a) Bind to metal nanoparticles
b) nucleate calcium phosphates on their surface (important for bone and teeth regeneration)





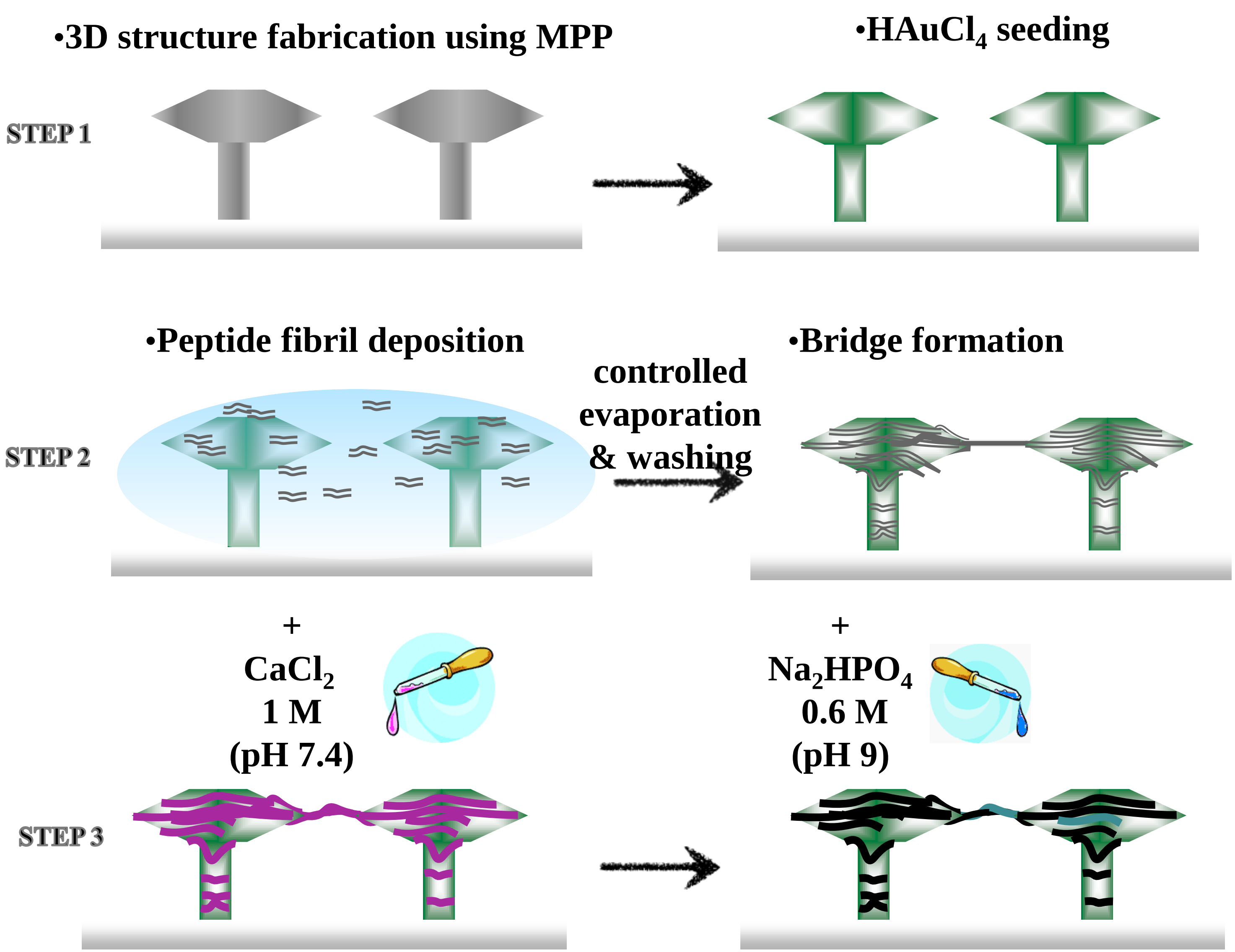
L-Aspartic acid
(Asp / D)



a) DDSGAITIG peptide fibrils after incubation with gold tetrachloroaurate solution and reduction with 1% citric acid

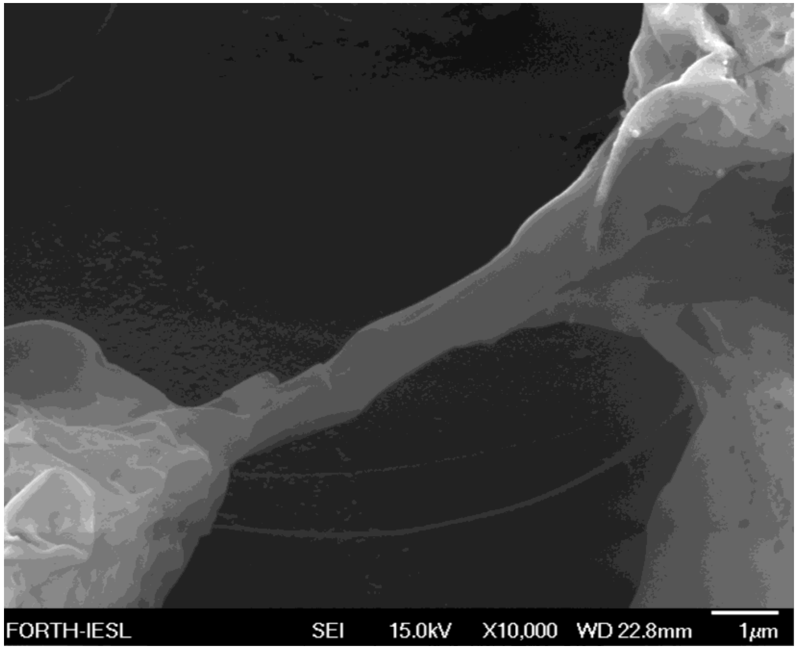
b) DDSGAITIG peptide fibrils after incubation with $CaCl_2$ and Na_2HPO4 on a holey grid

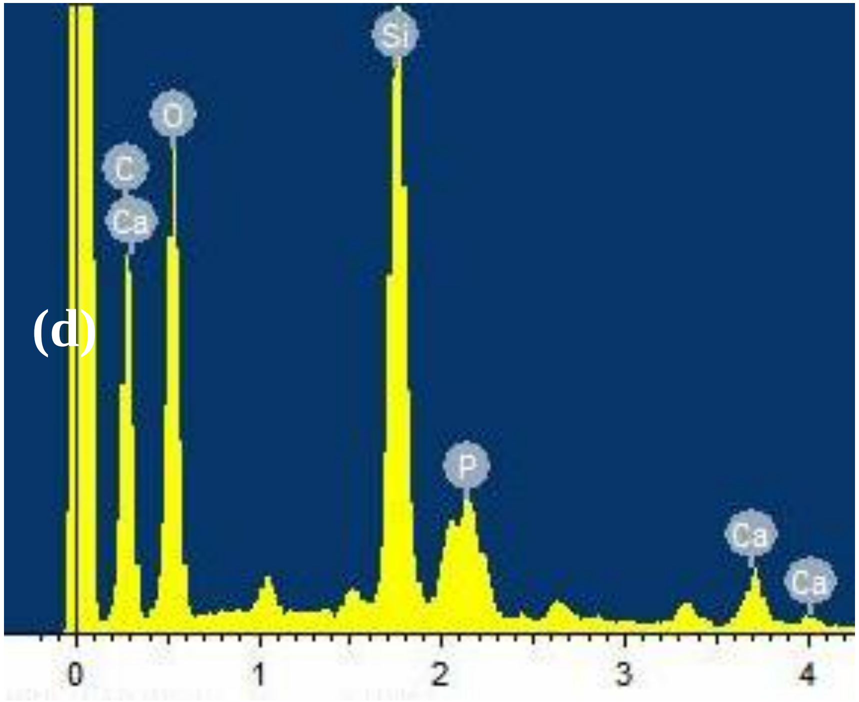
Development of a "Scaffold-on-Scaffold" strategy



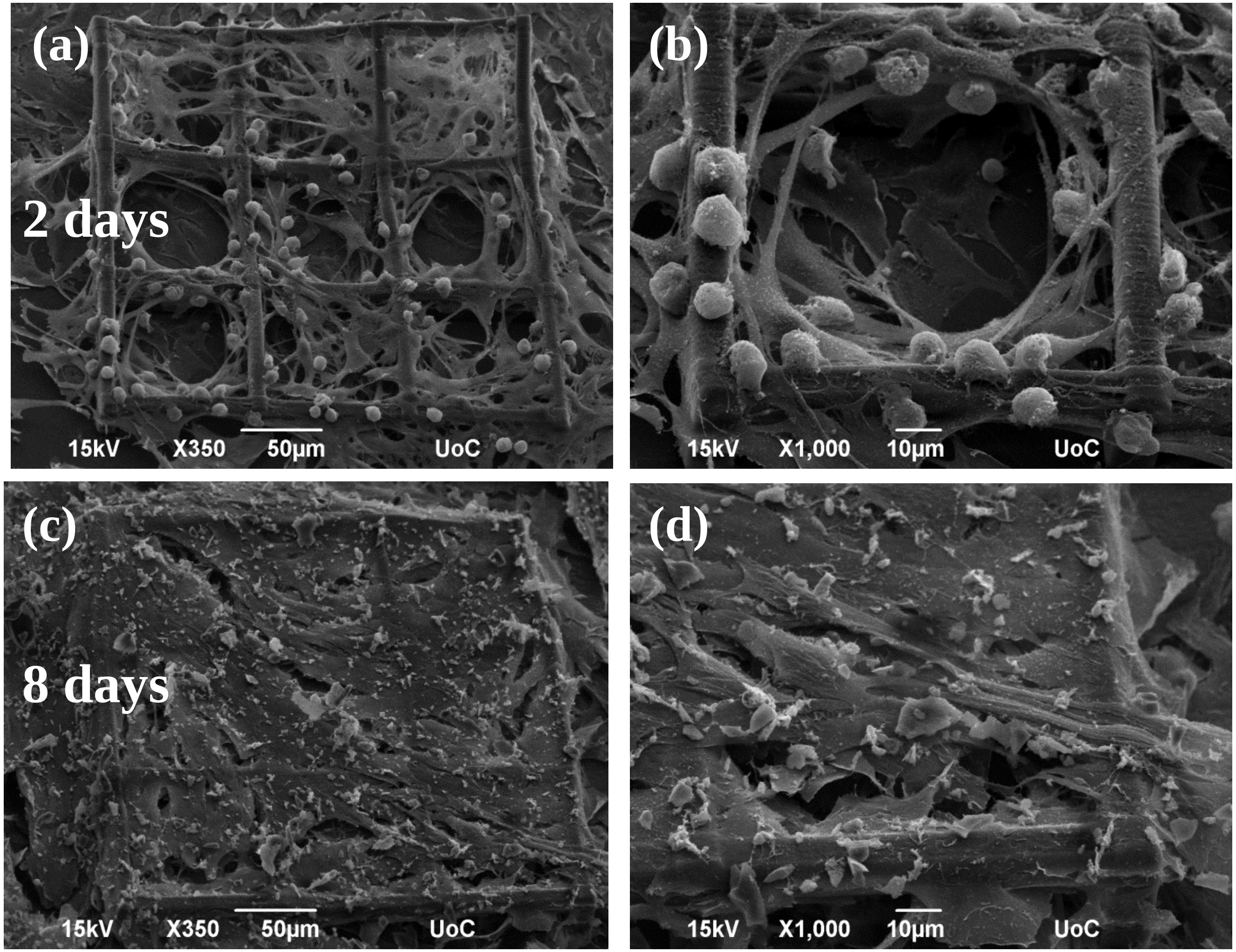
Terzaki et al., Biofabrication 2013, 5:045002

Peptide bridges positioned on the hybrid scaffold, covered with calcium phosphates
And EDX pattern on the bridge





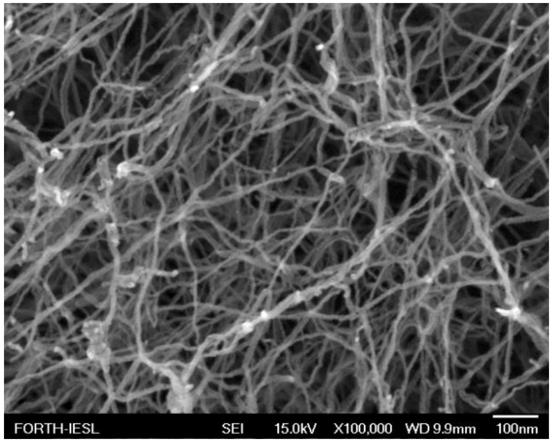
SEM images of pre-osteoblastic cells cultured onto DDSGAITIG peptide-functionalized and Calcium mineralized 3D woodpile-shaped scaffolds

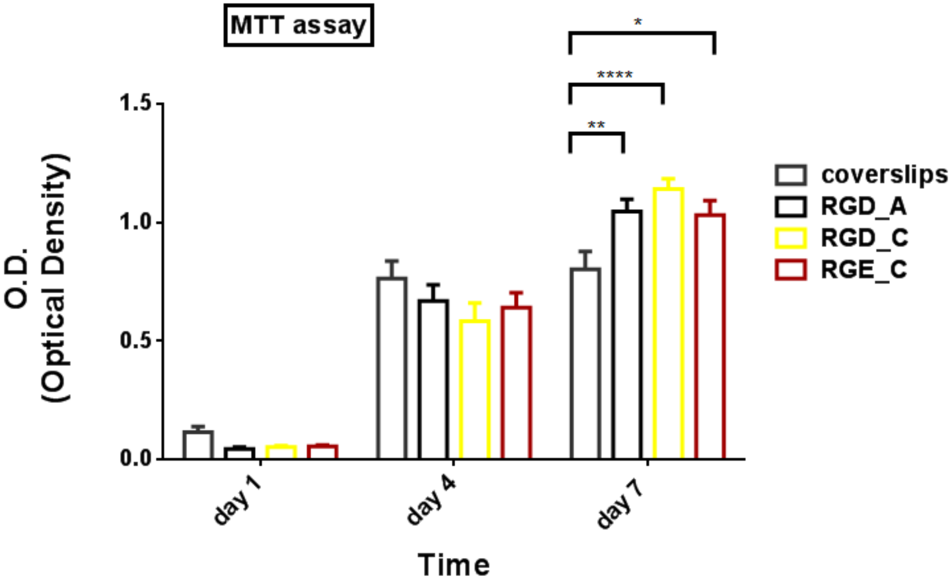


Terzaki et al., Biofabrication 2013, 5:045002 (with M. Chatzinikolaidou)

Designer peptides containing cell attachment (RGD) motifs as scaffolds for tissue engineering: theoretical and experimental studies

RGD-SGAITIG-C self-assembling peptides



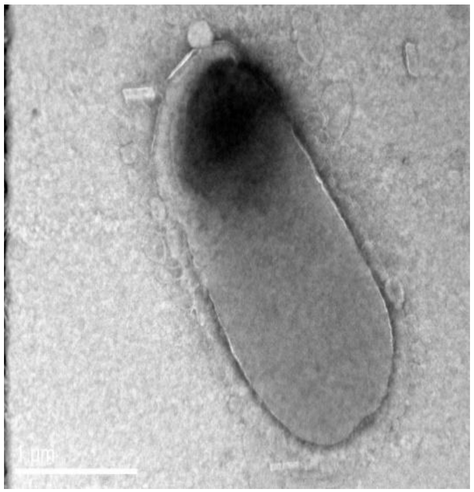


Loo et al., Adv. Healthcare Mat., 2015, 16: 1557

Deidda et al., ACS Biomat. Sci. and Eng. 2017, 7: 1404

Jonnalaggada et al., Mol. Syst. Design and Eng. 2017, 2: 321

Can we produce self-assembling fibrous materials in the macro-scale, using microbial cell factories (eg. recombinant production in bacteria)?



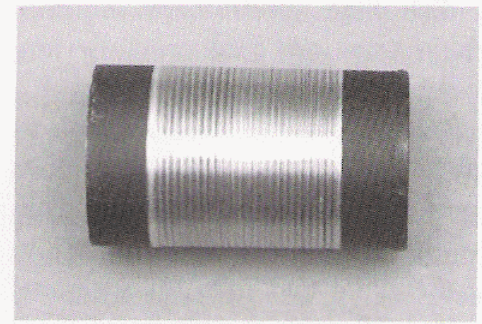
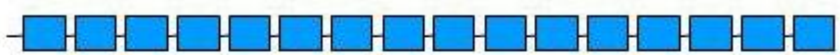


Fig. 5 Spider silk fiber spun from DP-1B protein produced in *E. coli*. Fiber was spun from HFIP solution by J. P. O'Brien (DuPont) essentially according to Lock (1992).

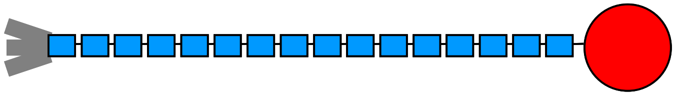


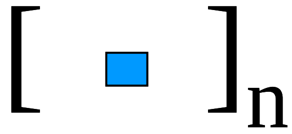
"RECOMBINAMER"

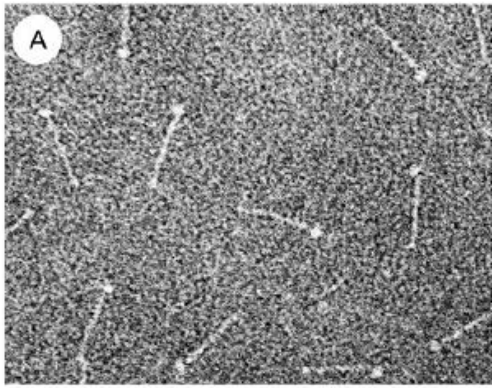
Concatamers, based on the minimal self-assembling building blocks

production in quantities will allow to use electrospinning and other methods used mainly for polymers

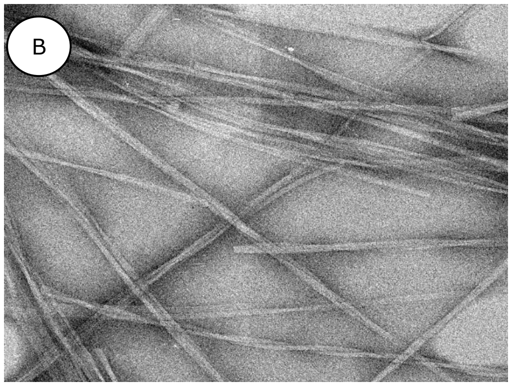
Next important challenge: recombinant production of self-assembling fibrous materials





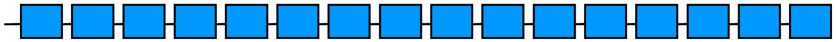


50 nm



100 nm

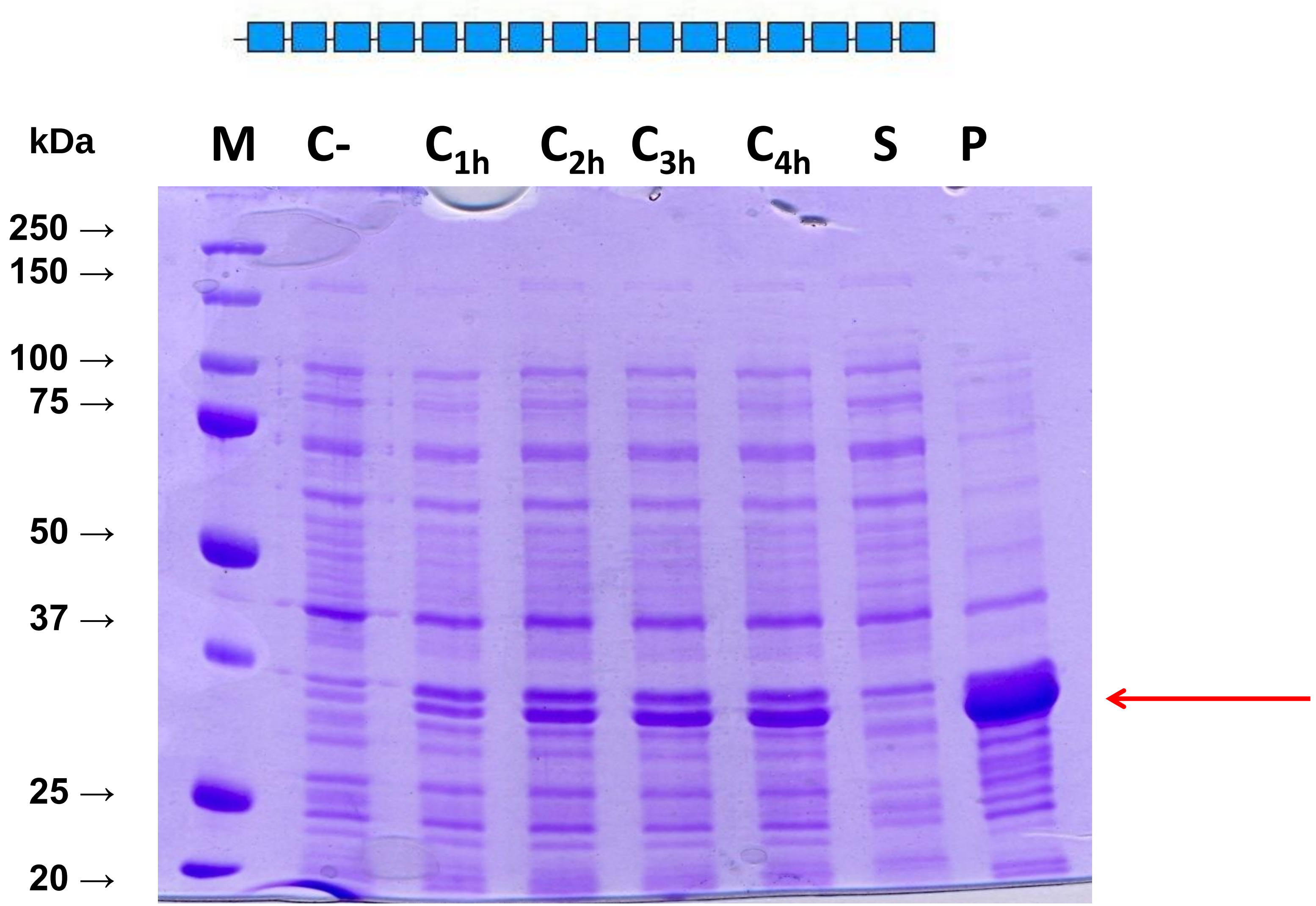
C



1) *Proteins composed of various lengths of the natural shaft*

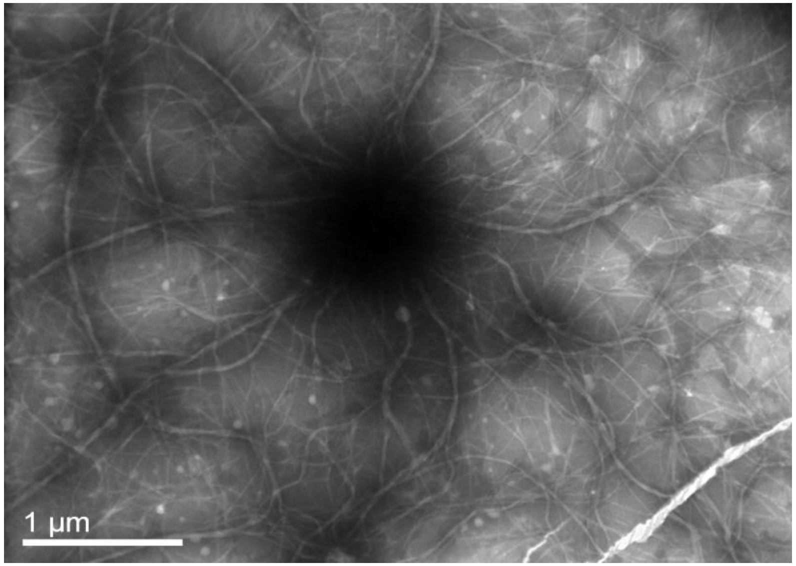
2) *Concatamers based on the minimal self-assembling building blocks*

Expression of the full-length shaft in *E.coli* (residues 61-392)

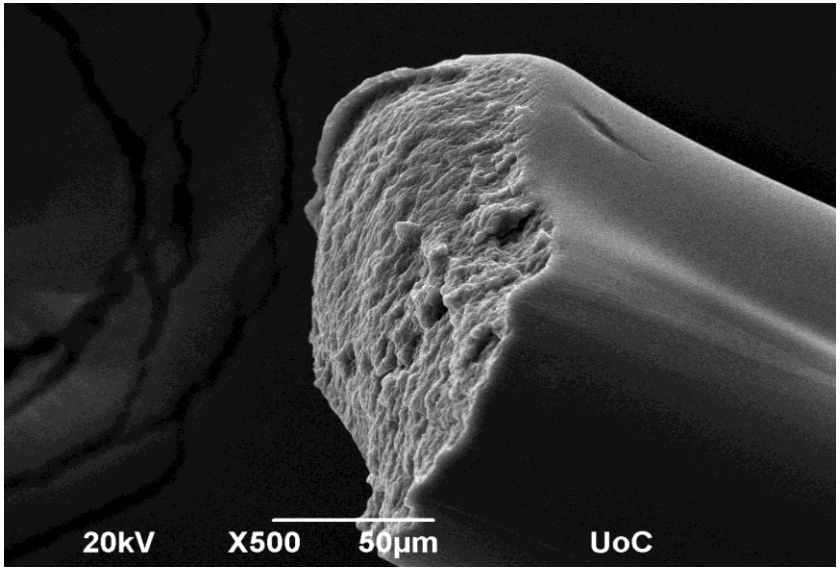


Lane M: molecular mass marker, ***Lane C-:*** non induced bacteria, ***Lane*** C_{1h}***:*** bacteria after 1h of induction, ***Lane*** C_{2h}***:*** bacteria after 2h of induction, ***Lane*** C_{3h}***:*** bacteria after 3h of induction, ***Lane*** C_{4h}***:*** bacteria after 4h of induction, ***Lane S:*** Supernatant of lysates, ***Lane P:*** pellet of lysates.

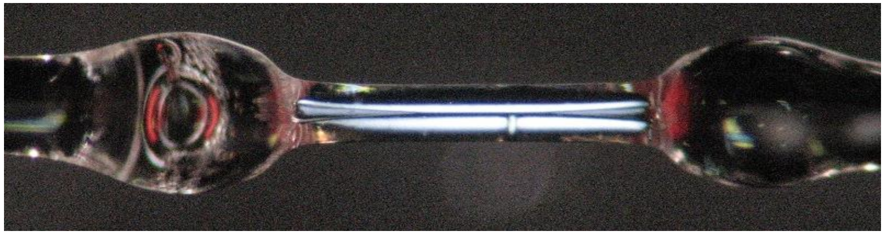
Processing of fibers from the purified proteins that self-assemble into amyloid-type fibrils



Protein is produced in form of “Inclusion bodies” consisting of amyloid-type fibrils



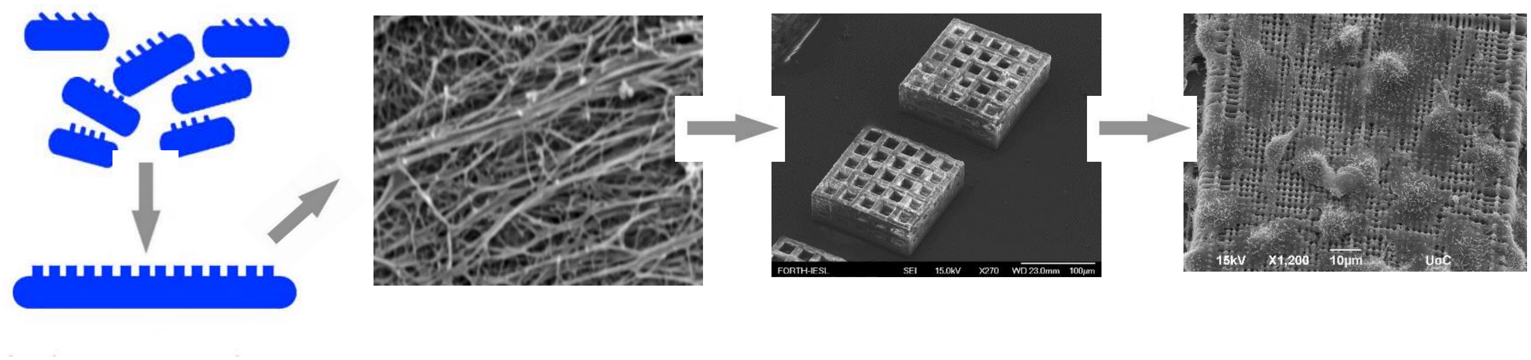
Fiber rod drawn from purified protein



Protein fiber drawn between two glass rods, viewed under crossed polars

Ariadni Prigipaki, PhD work

Can we photostructure protein and peptide-only materials and use them as scaffolds?



Peptide and protein Building blocks

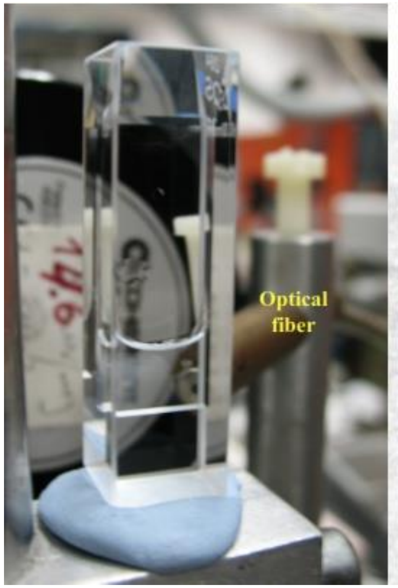
self-assembled matrices

3D photofabrication

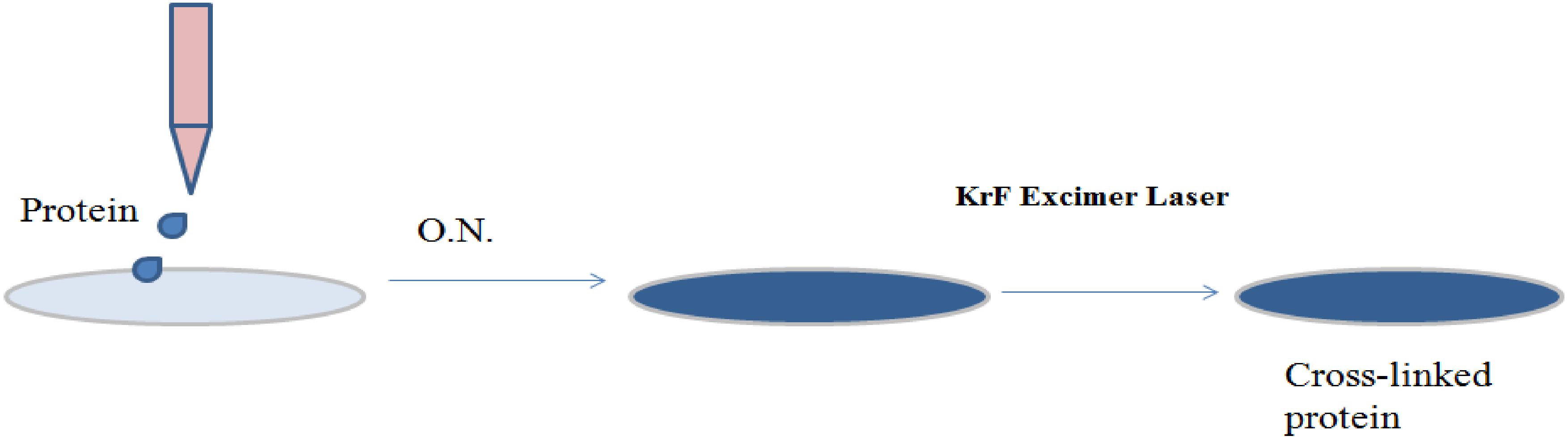
cell attachment on scaffolds

(collaboration with Dr. A. Selimis and Dr. A. Ranella, IESL, FORTH)

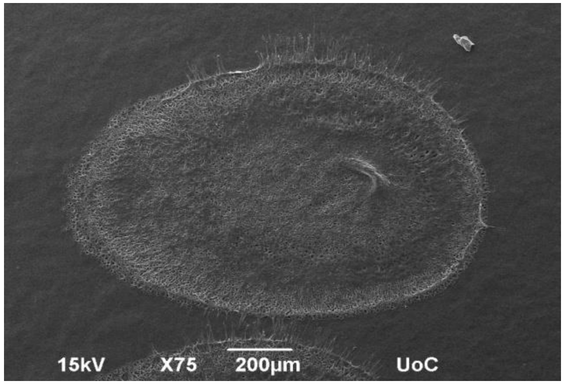
Cuvette Preparation

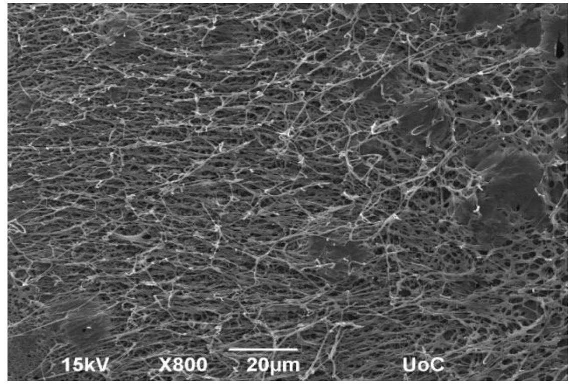


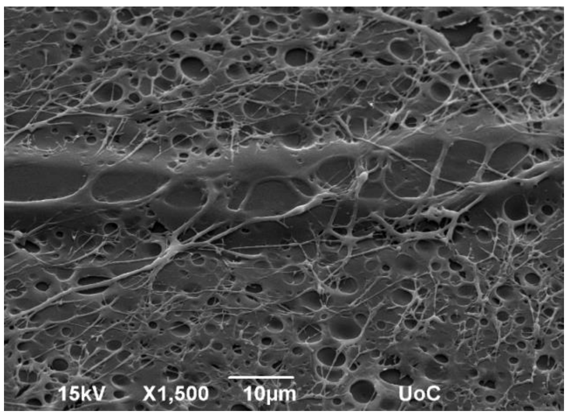
Film Preparation

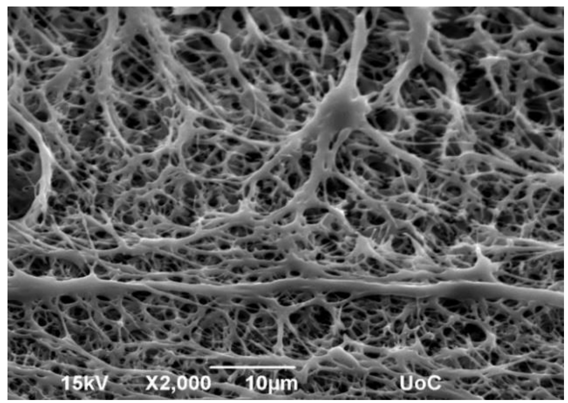


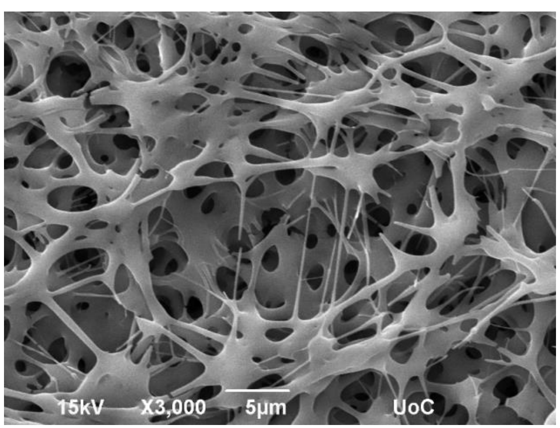
Irradiated area 300 mJ/cm^2

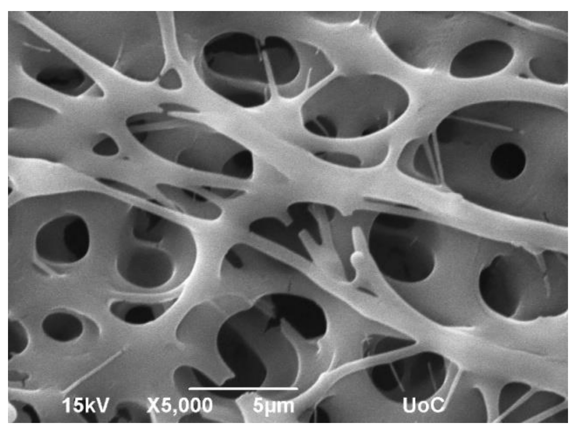






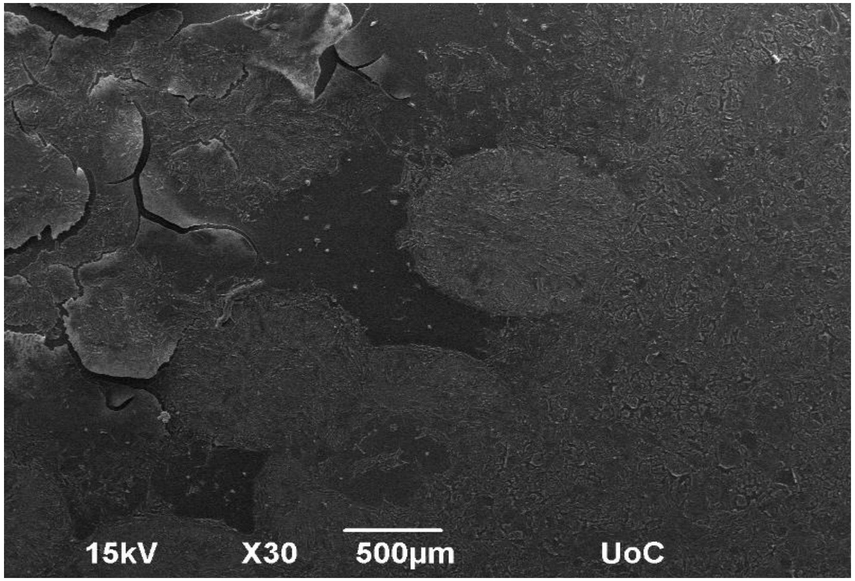


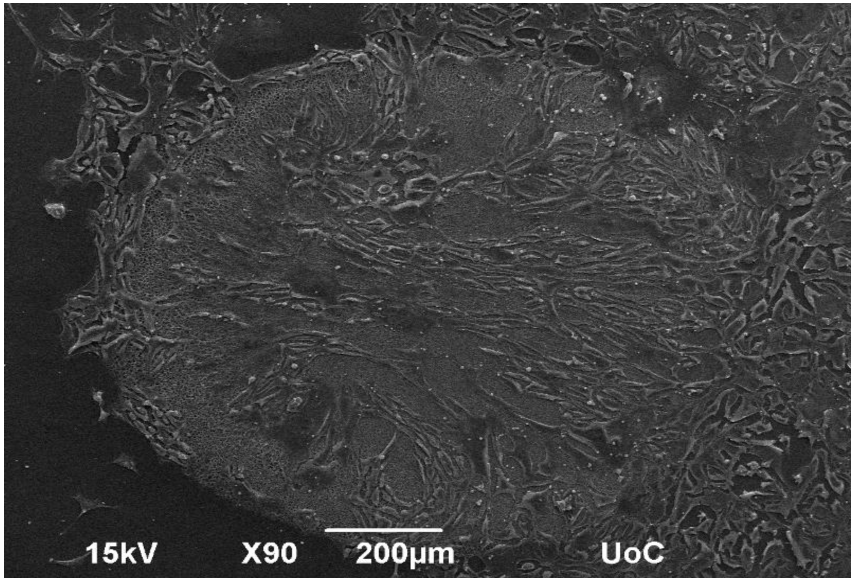


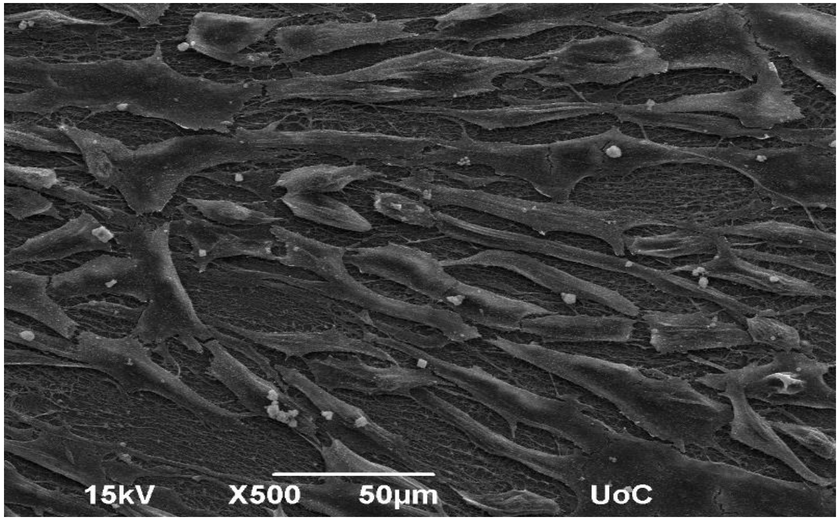


protein (2.5mg/ml)

Protein Scaffolds with Fibroblasts

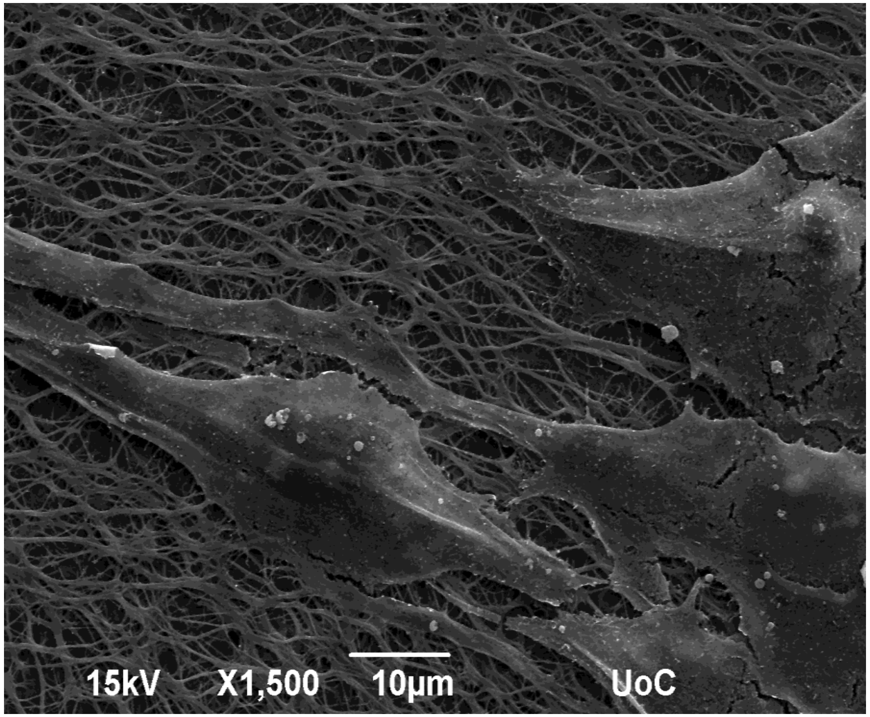


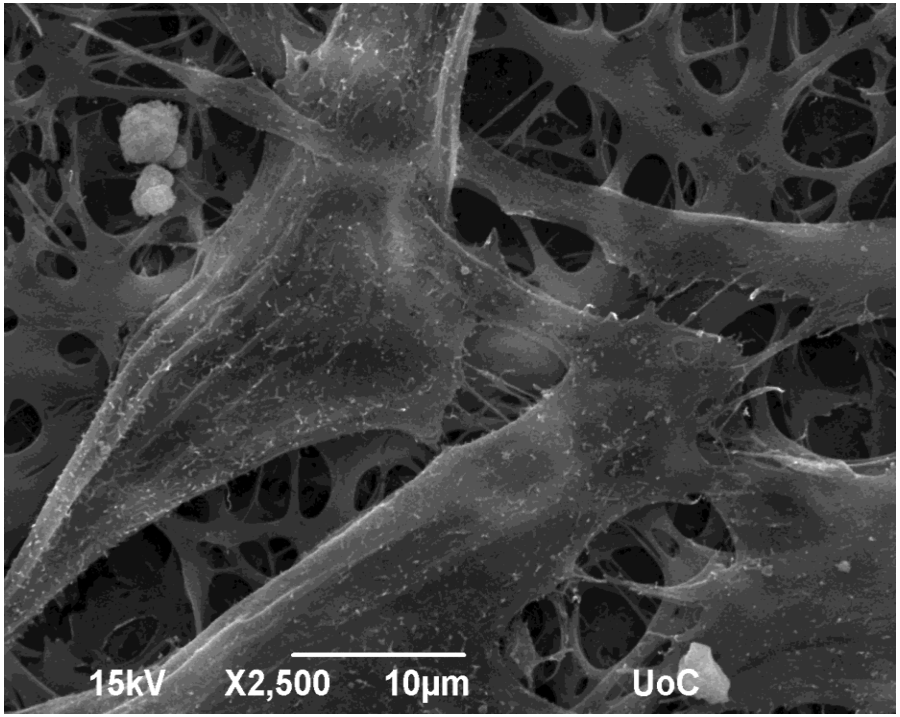




SEM image of the irradiated area of the protein film with one pulse (30ns) of the KrF excimer laser (248nm) at a fluence of 300 mJ/cm^2 (2.5mg/ml of protein - 40μl deposited, o/n drying at RT), covered with fibroblast cells (NIH/3T3 cells, 10^5 cells/ml, 72h of culture).

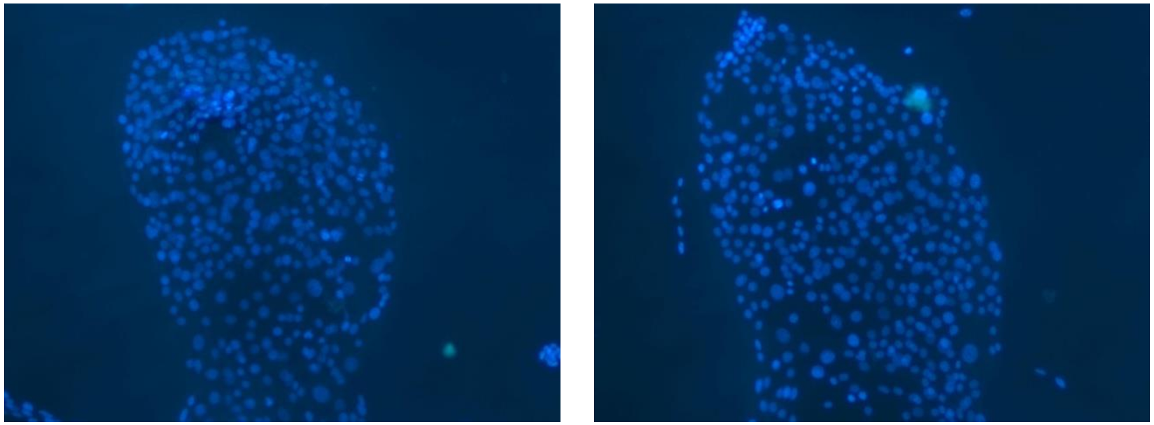
Protein Scaffolds with Fibroblasts



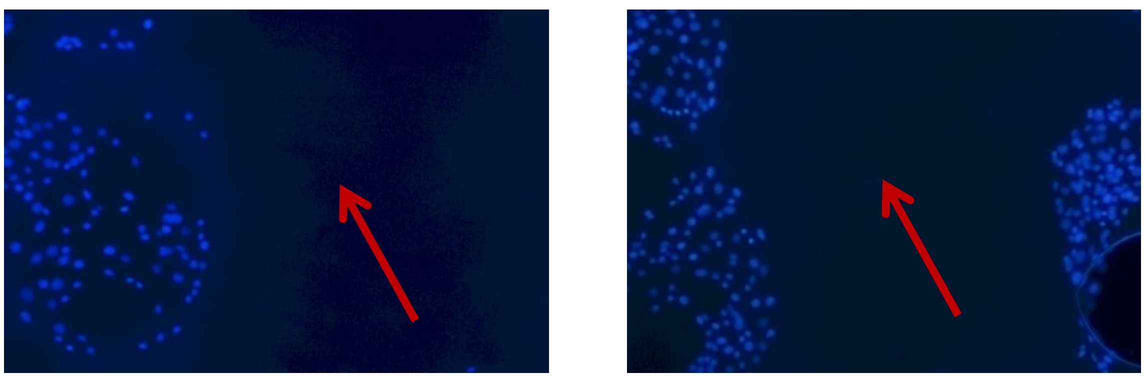


Cells attach selectively to the irradiated areas

Protein Scaffolds with Fibroblasts - UV Microscope DAPI DAY 5

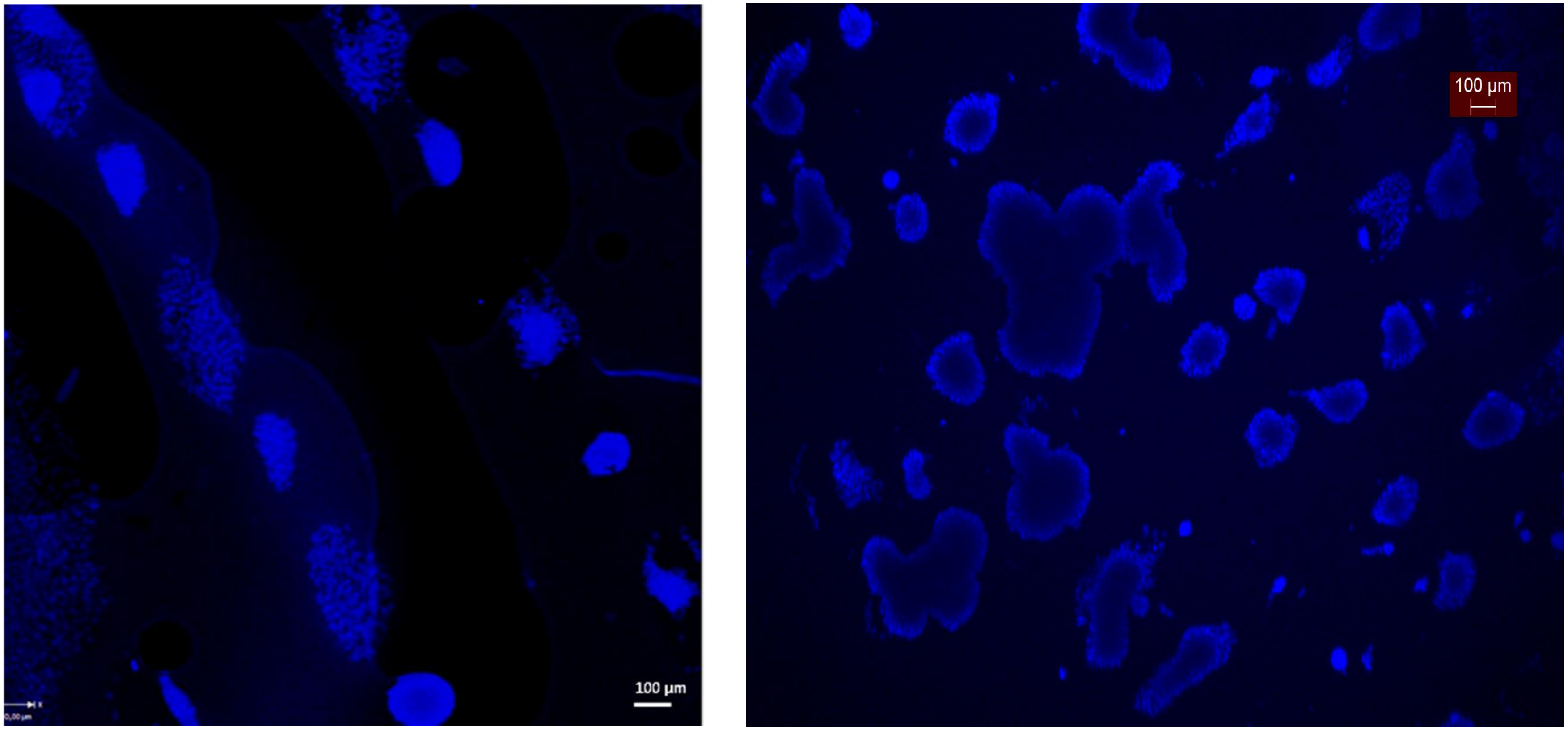


irradiated area



Non - irradiated area (red arrow)

Protein Scaffolds with Fibroblasts
Selective Cell Patterning



Fluorescence microscopy images of fibroblasts on laser patterned surfaces after 3 days of culture-DAPI staining.

Prigipaki et al., Biofabrication, 2017: 9, 045004

Summary:

Rational design of bio-nanomaterials
from the nano- to the macroscopic scale

using building blocks inspired from
Natural folds and hard-won biochemical knowledge

and
Development of inter-disciplinary approaches

Towards combining these bottom-up design possibilities
With top-down biofabrication technologies

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