

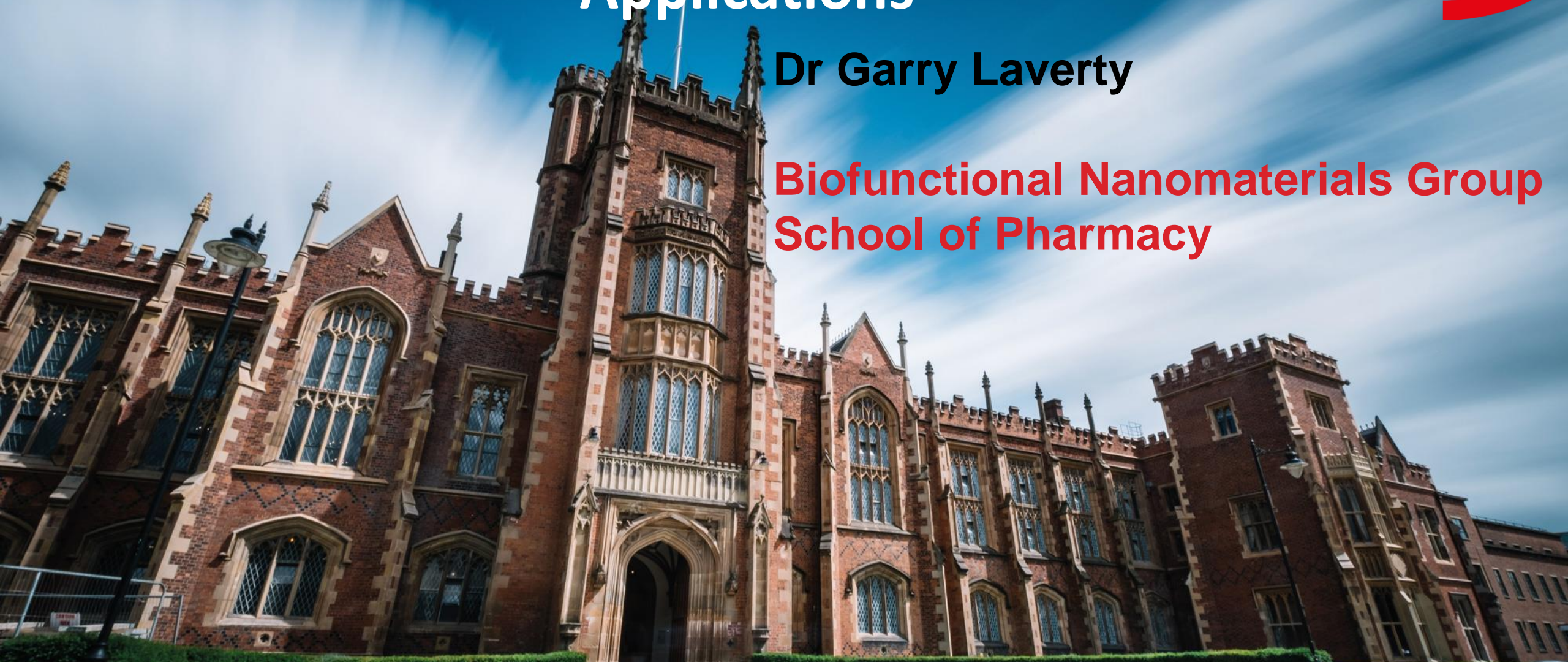


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BELFAST

# Peptide Hydrogel and Nanotubes for Drug Delivery and Biomaterial Applications

Dr Garry Laverty

Biofunctional Nanomaterials Group  
School of Pharmacy





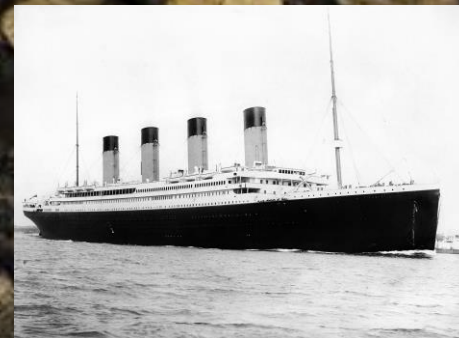
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**1<sup>st</sup> in the UK  
for Pharmacy**

**(The Times and Sunday Times  
Good University Guide 2021)**





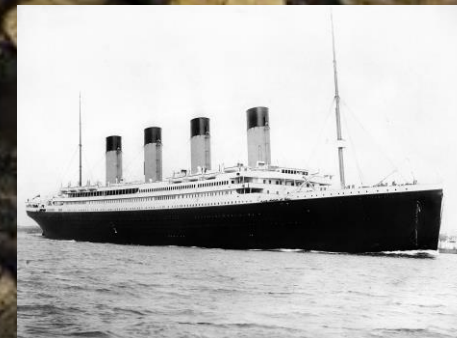
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# What are Peptide Nanomaterials?

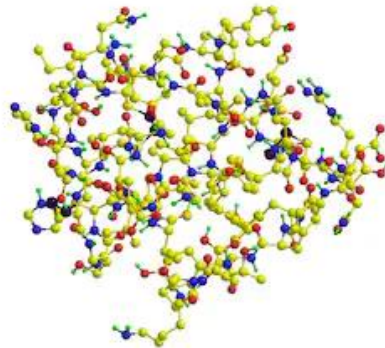
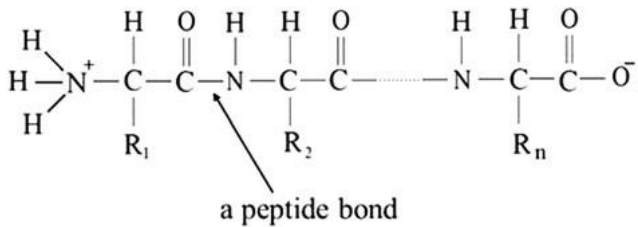
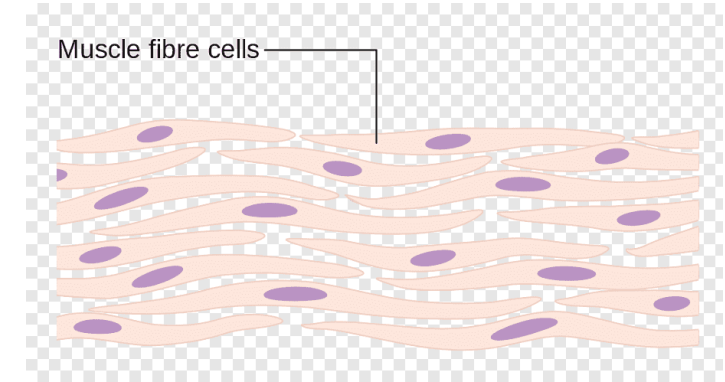
# What are Peptide Nanomaterials?



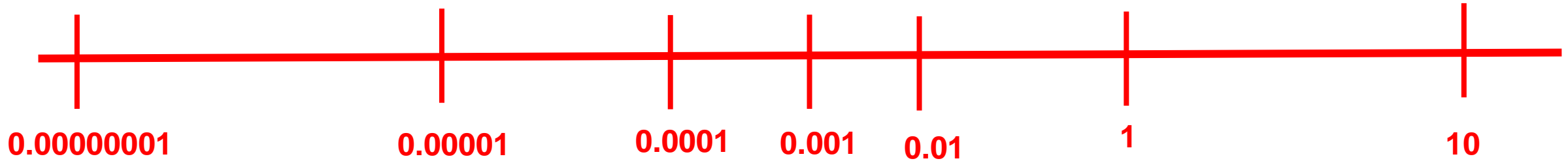
Peptide



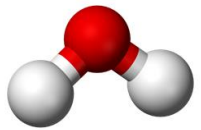
Protein



Size in centimetres



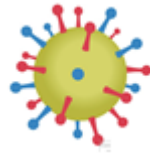
Water molecule



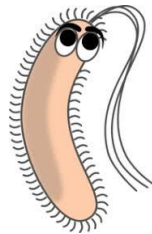
Peptide nanotube



Virus



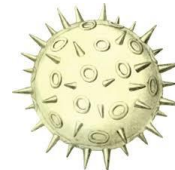
Bacteria



Red blood cell



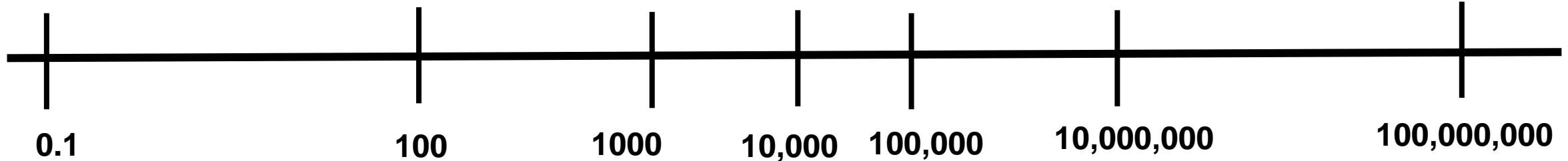
Pollen



Marble



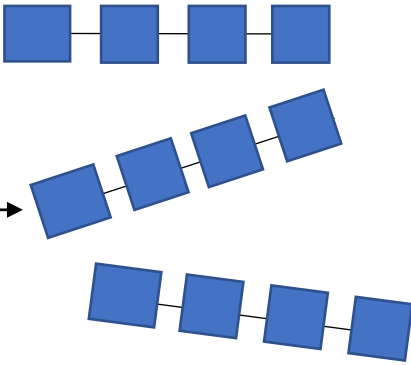
Tennis ball



Size in nanometres

# Peptide Nanomaterials: Core Technology

Self-assembled Peptides

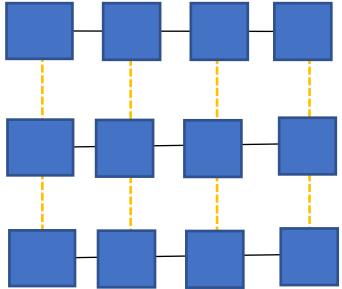


Short peptide sequences

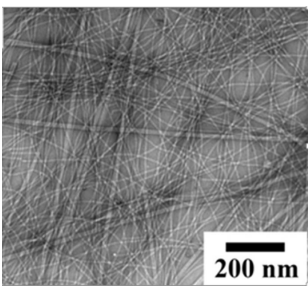
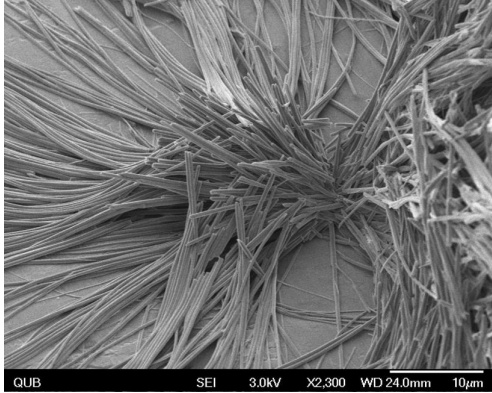
Non assembled

Stimuli

- pH
- Temperature
- Ionic Strength
- Specific enzymes

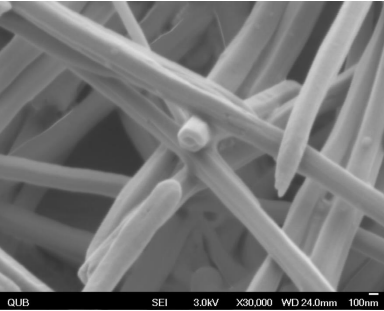


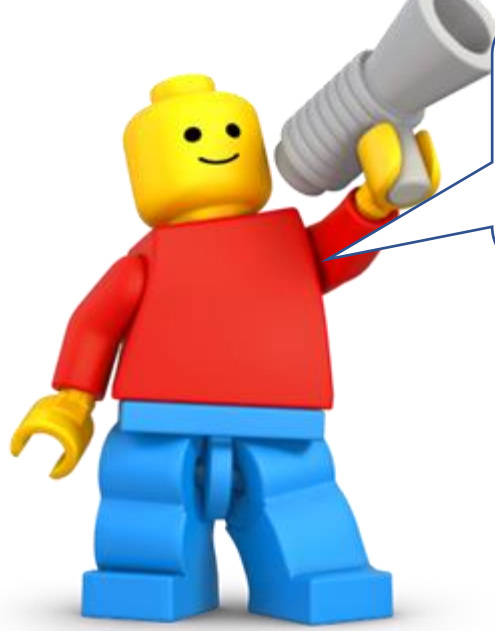
Self-assembly



Peptide Hydrogels

Peptide Nanotubes



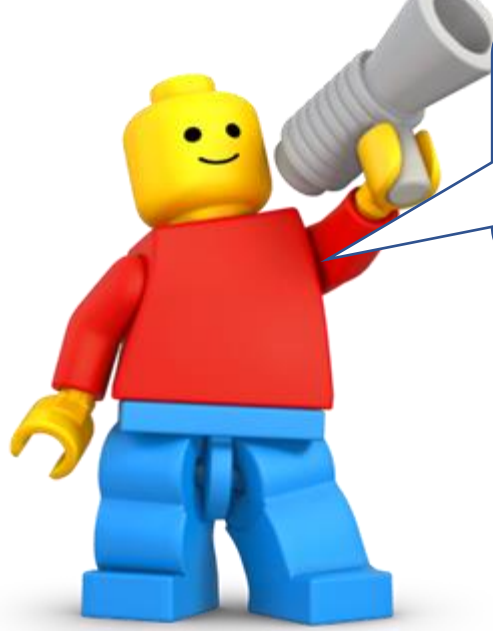


Biofunctional nanomaterials  
utilising the building blocks of  
life!

**What medical  
applications can we  
use these for?**







Biofunctional nanomaterials utilising the building blocks of life!

What medical applications can we use these for?



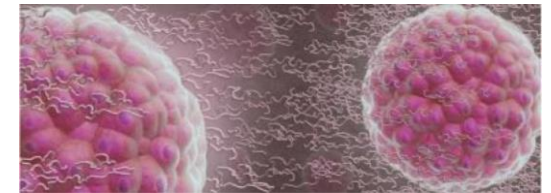
Infection and Medical Devices



Wound healing



Drug Delivery (*In situ* forming implants, blood brain barrier, cancer, bacteria)

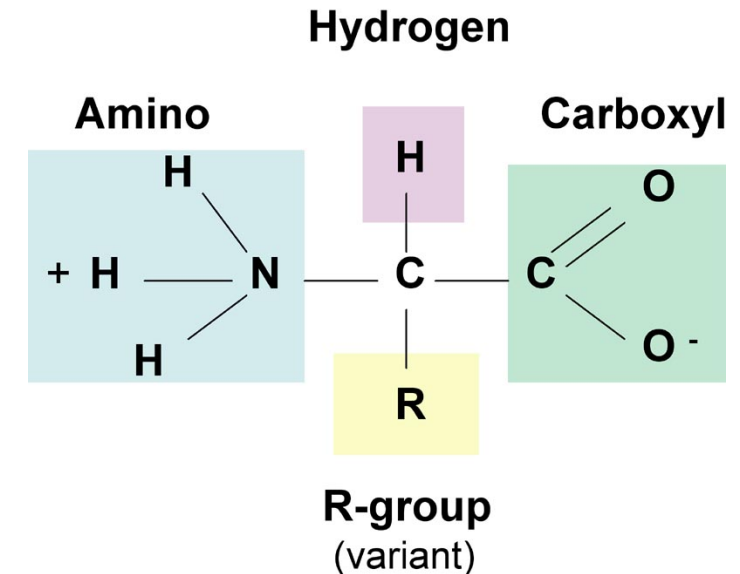


Stem Cells/Regenerative medicine

# Advantages of ultrashort peptides

- Ultrashort peptides (< 7 amino acids) → More **cost effective** → Upscale by Pharmaceutical Industry → Increased translational potential → Patient benefit
- Numerous advantages over current synthetic materials including:
  - **Increased chemical versatility**
  - **Minimal immunogenicity and enhanced biocompatibility**
  - **Tunable biodegradability**
  - **Tailored self-assembly/pharmacological properties (e.g. antimicrobial) in response to stimuli**

## Amino Acid Structure



# Why are there not more peptide medicines?

# Why are there not more peptide medicines?

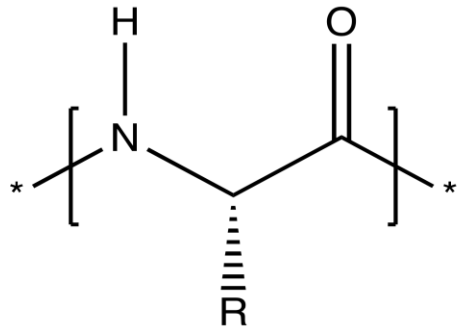
- Most peptides/proteins delivered topically or intravenously.

## Why?

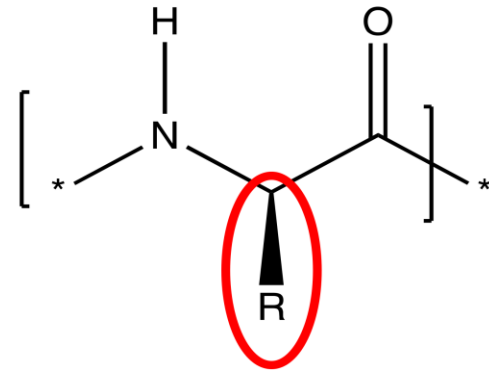
- Major disadvantage: Limited stability *in vivo*
  - pH
  - Proteolytic enzymes
- Balance between clinical efficacy and safety/toxicity
  - Relatively straightforward in lab vs difficulty in patients



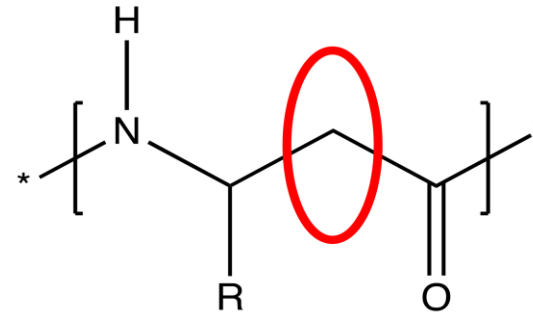
# Peptide-mimetics versus peptides



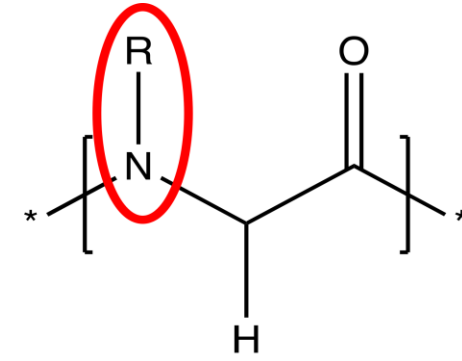
(i) L-peptide enantiomer



(ii) D-peptide enantiomer



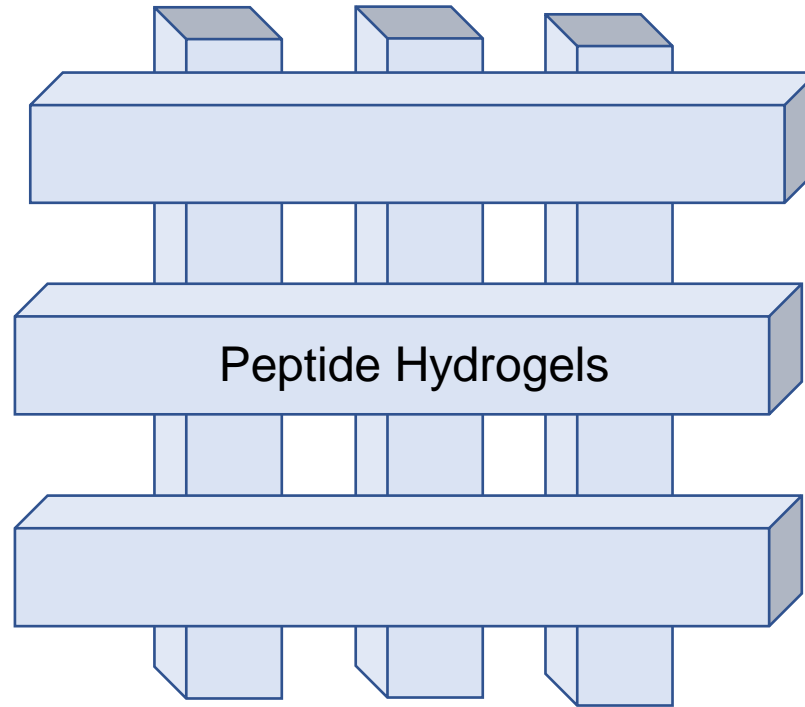
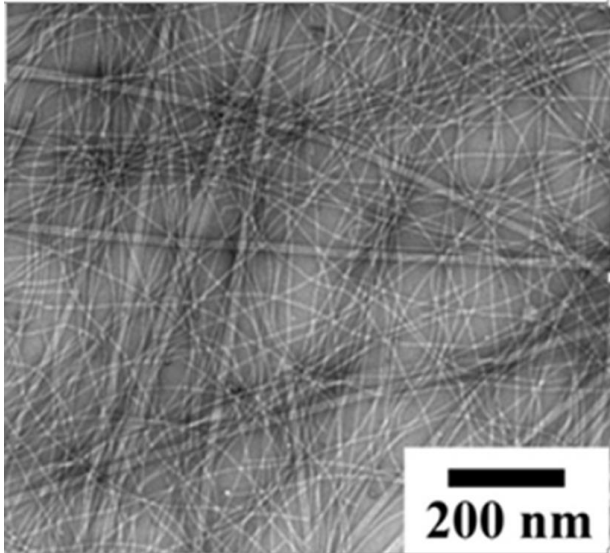
(iii)  $\beta$ -homo peptide



(iv) Peptoid

Structural differences (*red circle*) between L- $\alpha$ -peptides (i) and peptide-mimetics (ii-iv). ii) D-peptide is opposing stereoisomer of L-form. iii)  $\beta$ -homo peptides possess additional methylene ( $-\text{CH}_2$ ) within each unit. iv) peptoid R-group on nitrogen rather than  $\alpha$ -carbon.

# Peptide hydrogel nanomaterials



# Injectable peptide-mimetic hydrogel for sustained delivery of drugs

- Eradicating HIV/AIDs by 2030 remains a central goal of the World Health Organisation.
- Key to this addressing this challenge is overcoming patient medication adherence issues.
- Complicated antiretroviral regimens, including a commitment to daily intake of tablets.
- There is need for a convenient and effective long-acting formulation to deliver drugs over a sustained period e.g. 28 days.
- Multipurpose product: combined HIV + contraceptive

Structural overview of our enzyme responsive drug delivery implant

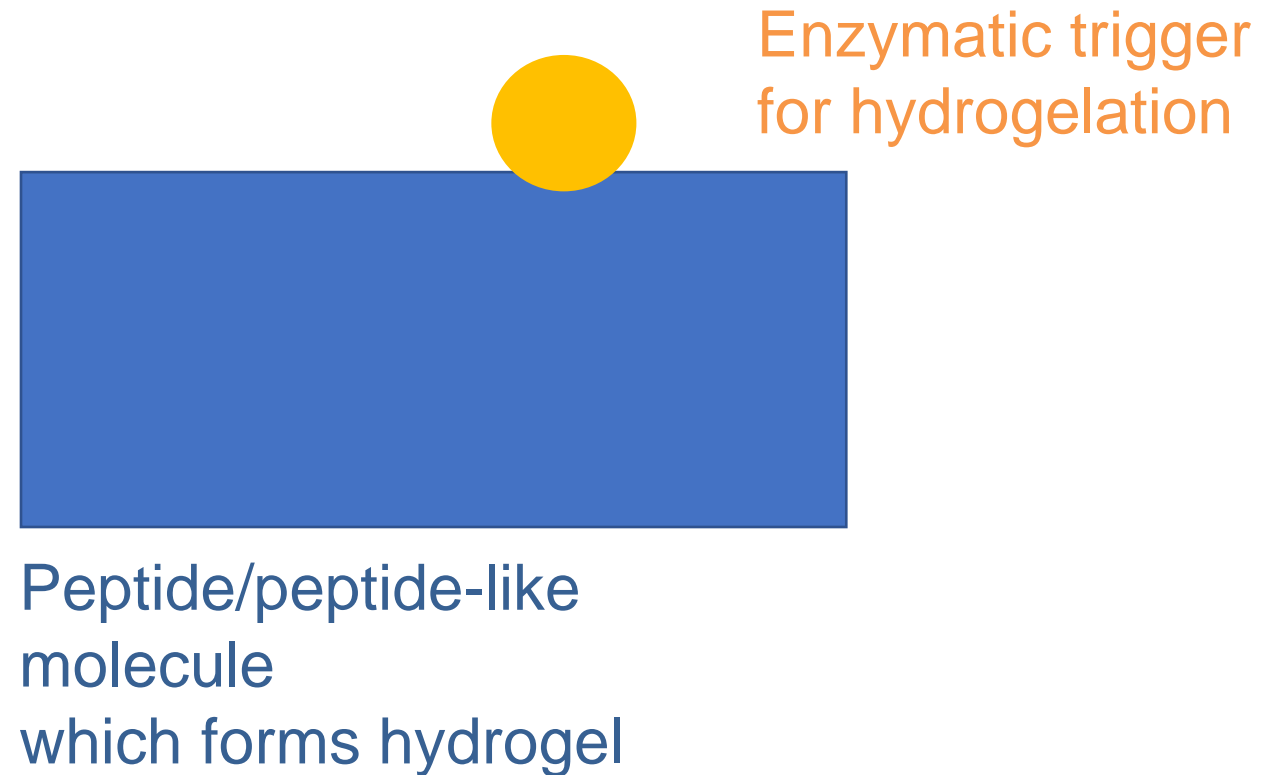


Peptide/peptide-like  
molecule  
which forms hydrogel

# Injectable peptide-mimetic hydrogel for sustained delivery of drugs

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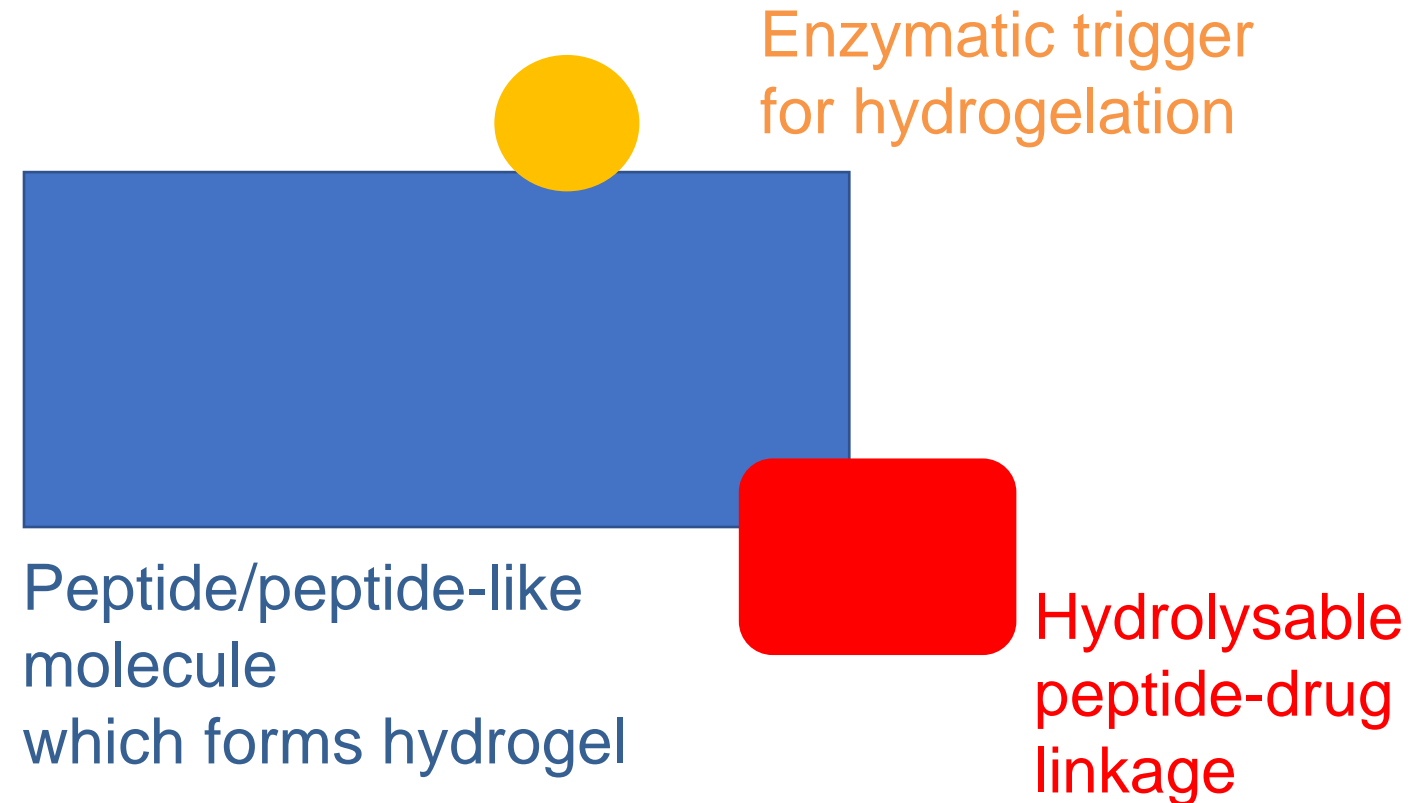




# Injectable peptide-mimetic hydrogel for sustained delivery of drugs

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Structural overview of our enzyme responsive drug delivery implant



# Peptide-mimetic hydrogelators for sustained delivery of drugs

- a) Soluble peptide-drug conjugate administered subcutaneously each month



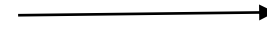
# Peptide-mimetic hydrogelators for sustained delivery of drugs

a) Soluble peptide-drug conjugate administered subcutaneously each month



Skin  
Subcutaneous  
tissue

Specific  
enzyme in  
tissue



cleaves  
hydrophilic  
group

b)

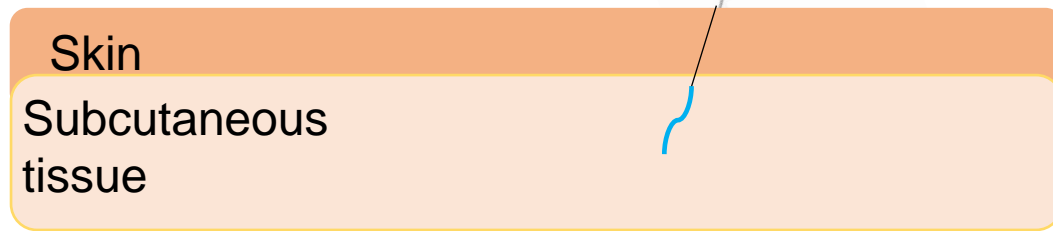
Skin  
Subcutaneous  
tissue



Formation of protease resistant  
hydrogel

# Peptide-mimetic hydrogelators for sustained delivery of drugs

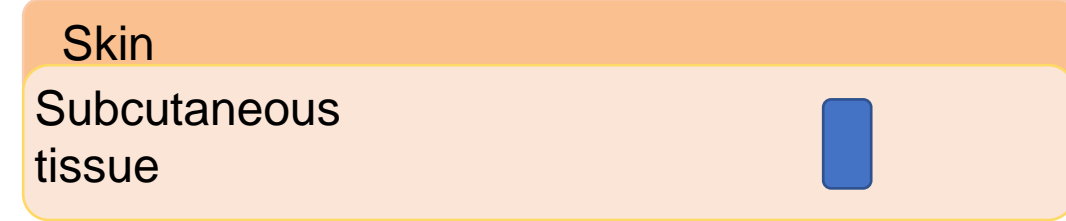
a) Soluble peptide-drug conjugate administered subcutaneously each month



Specific enzyme in tissue

cleaves hydrophilic group

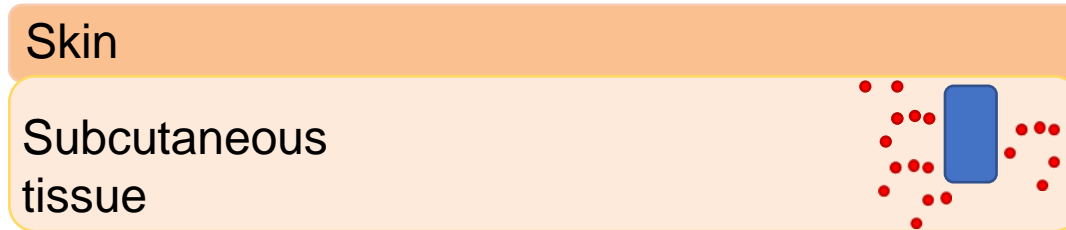
b)



Formation of protease resistant hydrogel

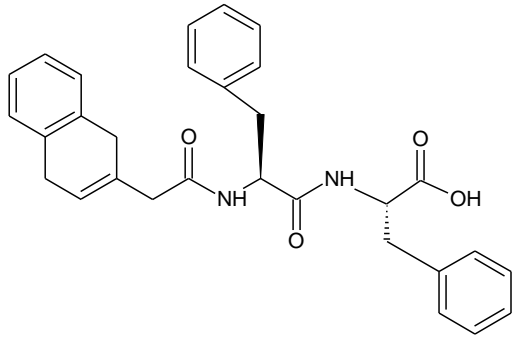
c)

Tissue environment, pH 7.4, 37°C  
Hydrolysis of drug-peptide linkage



Sustained release of conjugated drugs from peptide hydrogel

# L- $\alpha$ and D-peptide enantiomers NapFFKY(p)-OH



Naphthalene-phenylalanine-phenylalanine  
**NapFF-OH**



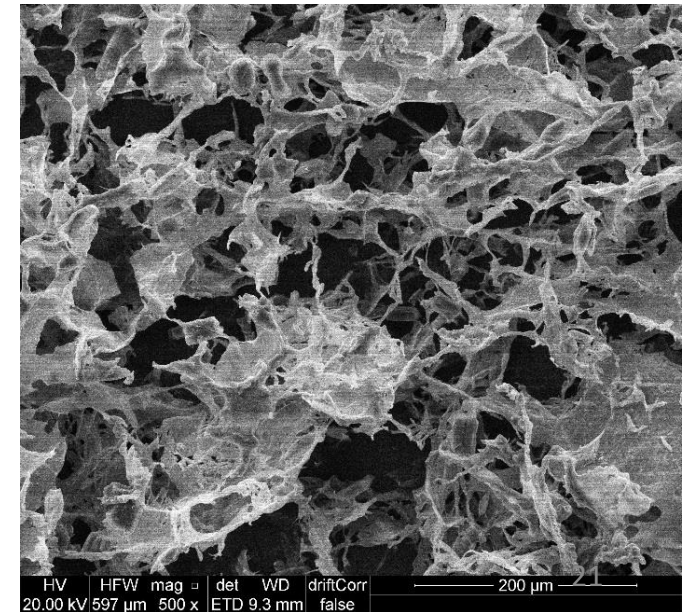
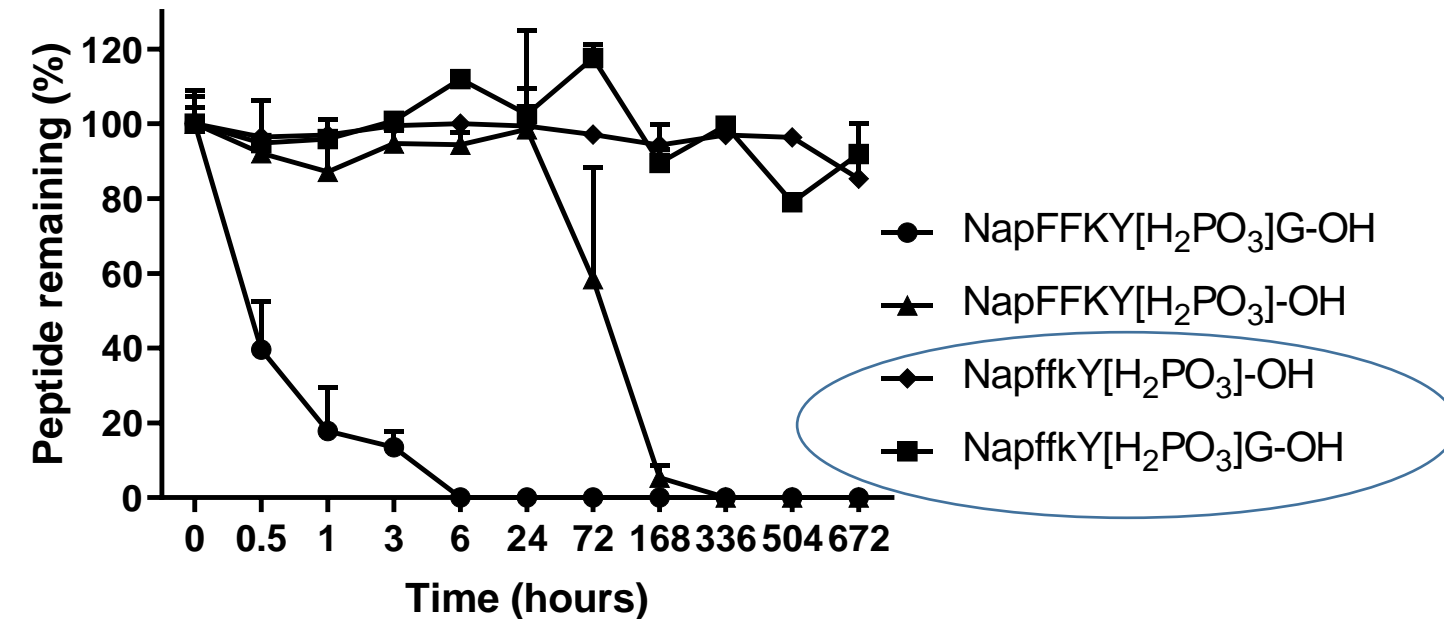
**Solution**  
(upon injection)

Phosphatase  
enzyme

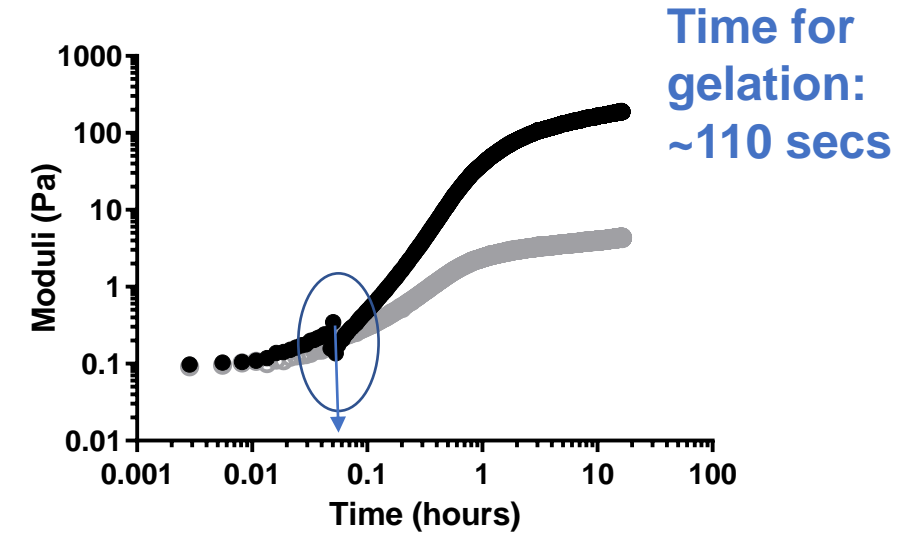
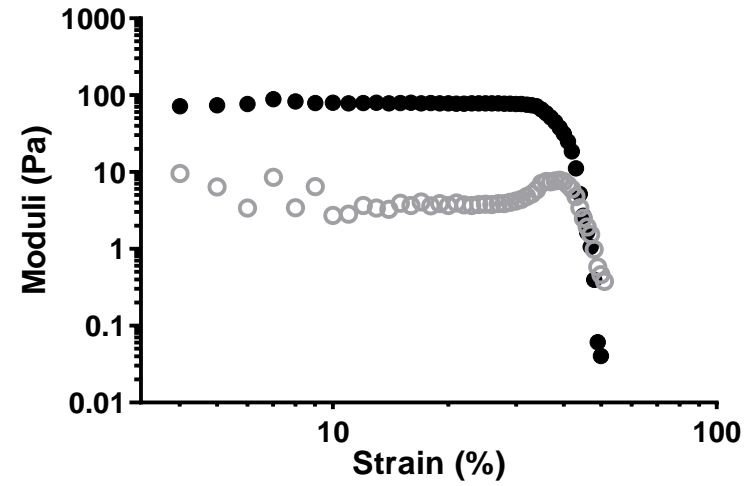
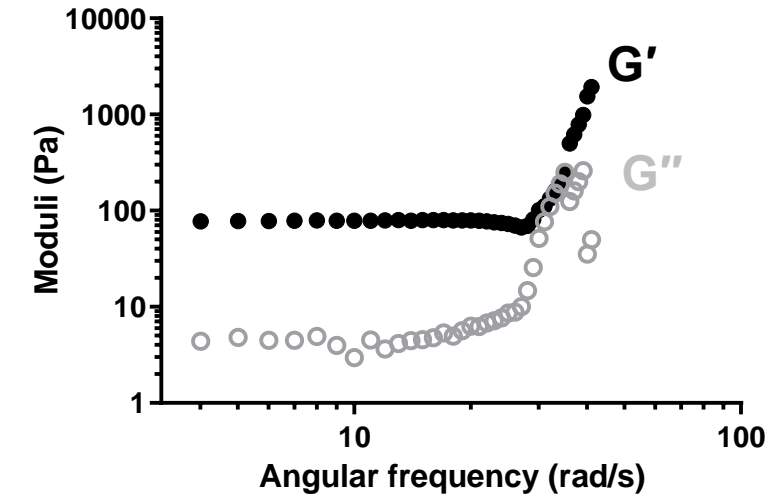


**Hydrogel**  
(after injection)

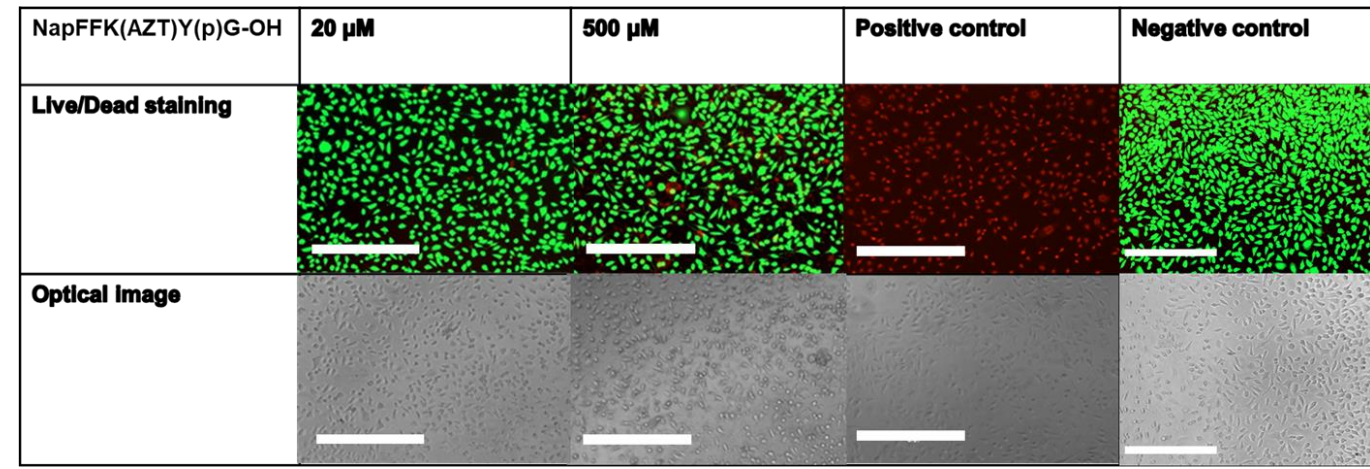
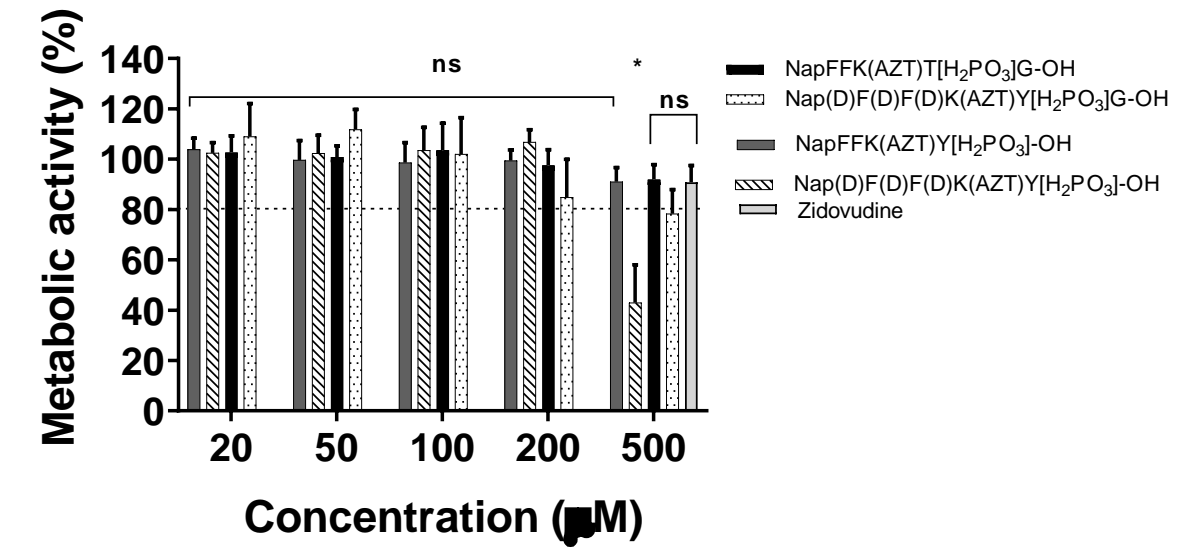
## Biostability: Proteinase K



# Rheology: Hydrogel formation 2% w/v Napffk(AZT)YG-OH.

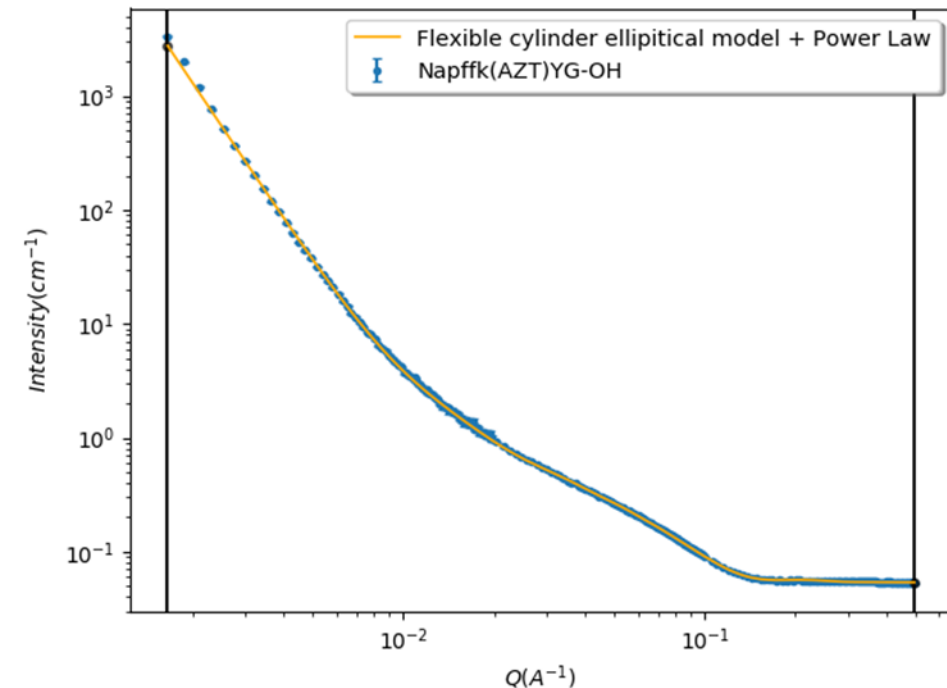


# Cell toxicity 24 hours: MTS cell viability and Live/Dead assays

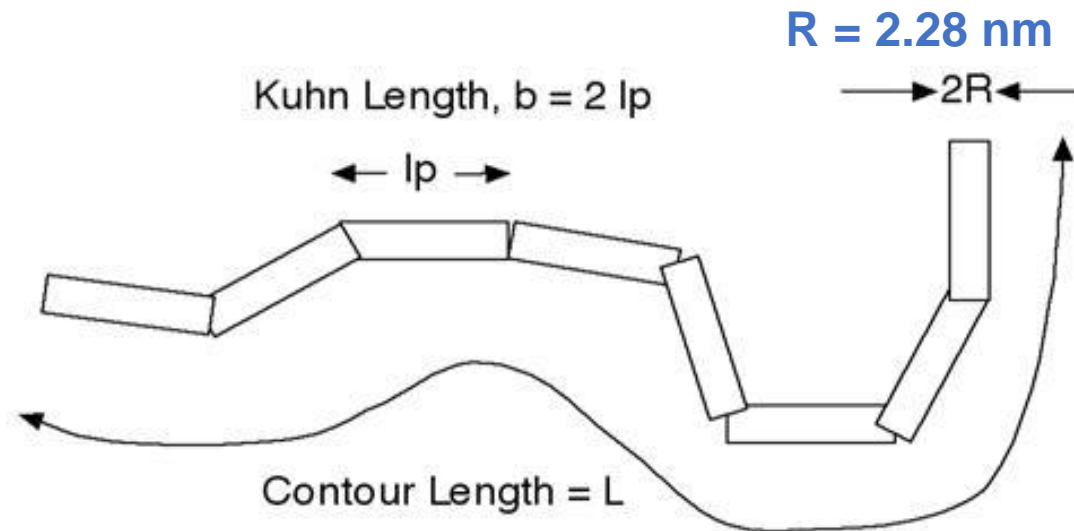


# Small Angle Neutron Scattering (SANS)

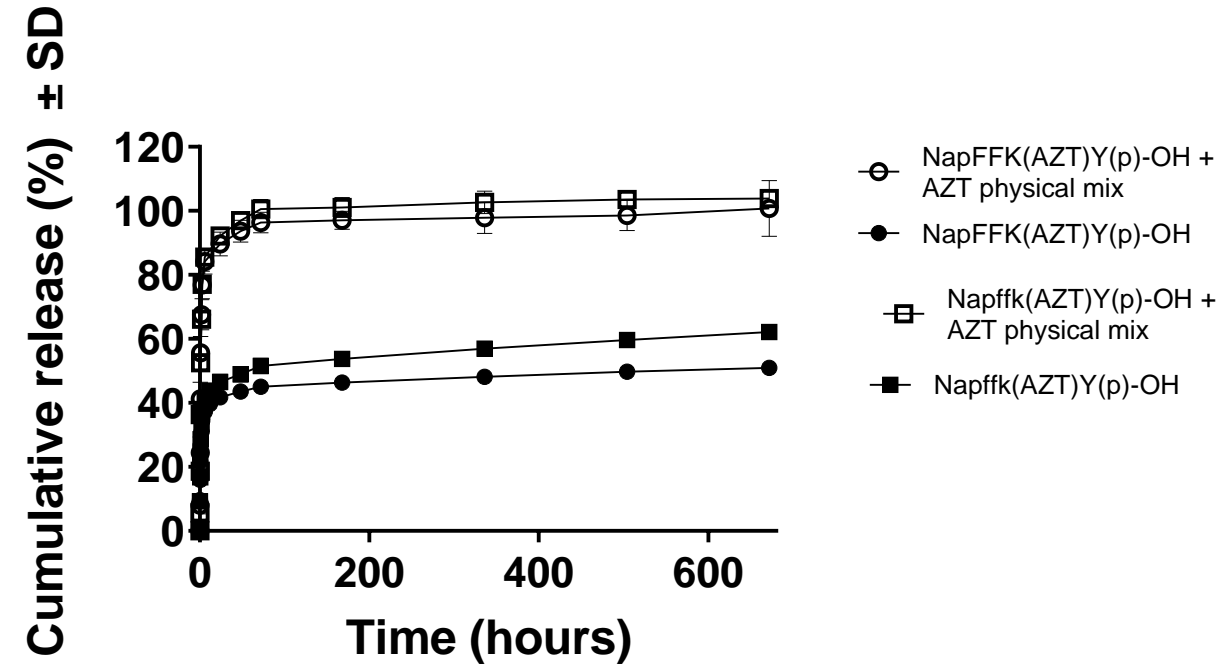
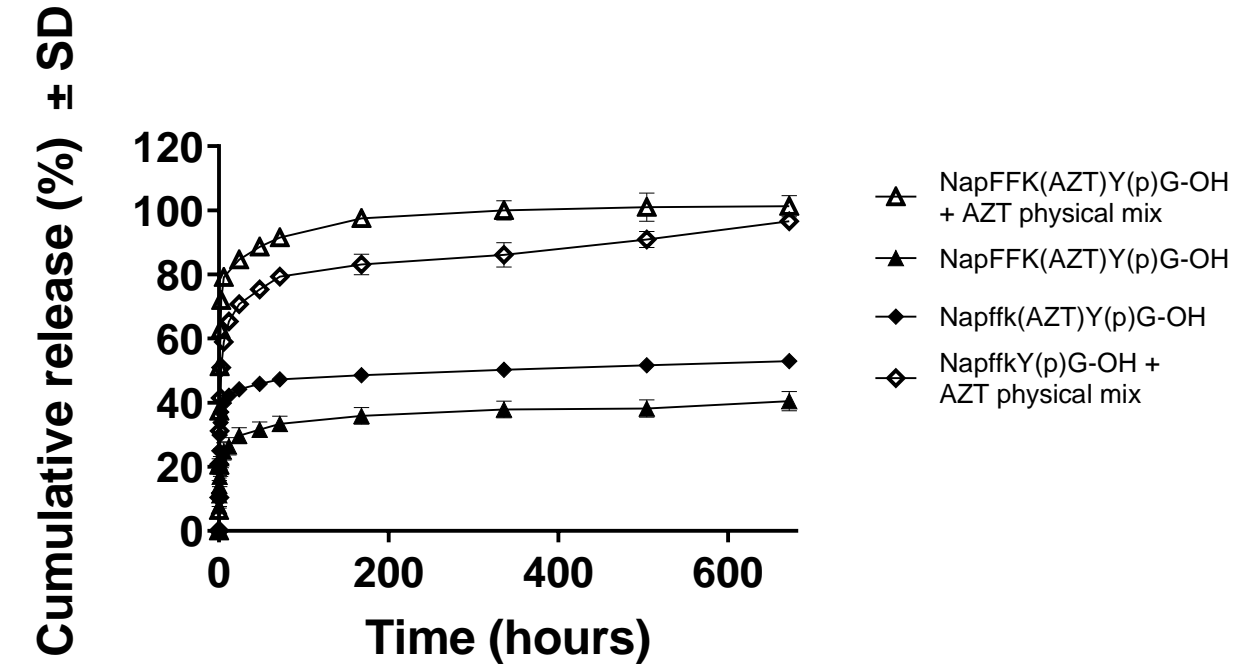
- A tool for structural characterisation of materials
- Can characterise materials at macroscopic level, modify peptide sequence and see impact
- From the structural information results we can determine whether the rheology drug release kinetics are based upon the fiber structure or the entanglement of those fibers



## Fits flexible cylinder elliptical model



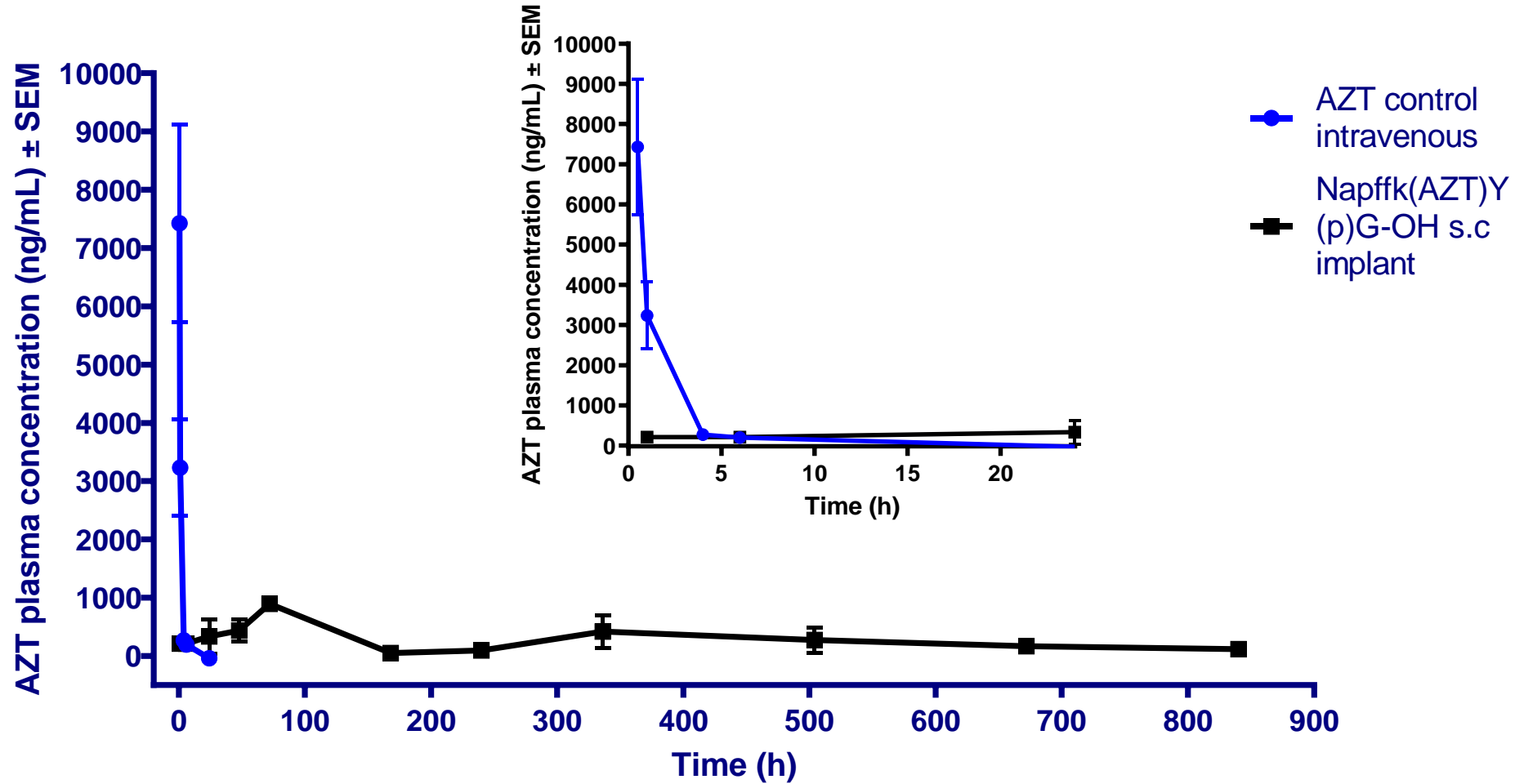
# ***In vitro* drug release 28 days: Chemically conjugated vs. physically mixed zidovudine (AZT)**



**Bust release significantly reduced in chemically conjugated vs. physically mixed zidovudine (AZT)**



# Drug release 28 days: Chemically conjugated vs. physically mixed zidovudine (AZT)



With  $IC_{50}$  range for AZT = 30 – 130 ng/mL for 35 days

# Advantages compared to current long-acting injectables

## Limitations of current long-acting injectable technologies

1) **Fast "burst" release** of drug upon administration (suspensions, microspheres, polymer implants)

2) Need for **surgery** (polymer implants)

3) Requires **large needles** (e.g. suspensions, microspheres)

## How our approach resolves this

1) Combination of hydrogel formation and breakage of peptide-drug bond = significant **reduction in "burst" release**

2) Soluble injection breaks down to **non-toxic products**

3) Formulation is fully soluble in water enabling use of **narrow bore needles**

# Advantages compared to current long-acting injectables

## Limitations of current long-acting injectable technologies

4) **Stability issues** upon storage/transport (suspensions)

5) **Limit on drug type and loading**, e.g. suspensions only allow water-insoluble drugs

6) **Persistent pain** for months after injection due to hydrophobic nature (oily liquids)

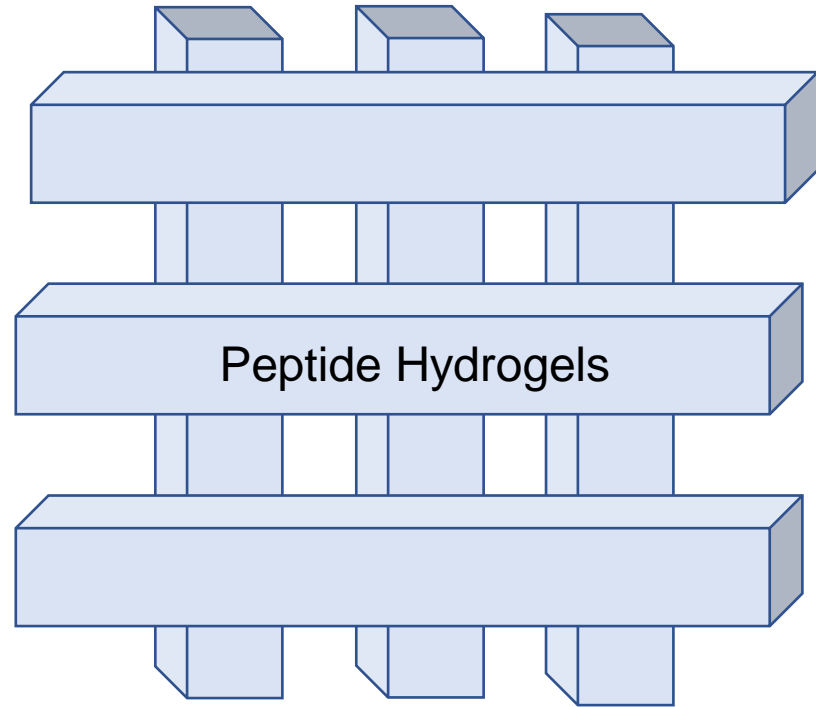
## How our approach resolves this

4) Can be transported as freeze-dried powder for mixing with water prior to injection = **increased stability**

5) Drug precisely attached to peptide = **increased drug loading**. Vast range of **hydrophobic** and **hydrophilic drugs** can be attached

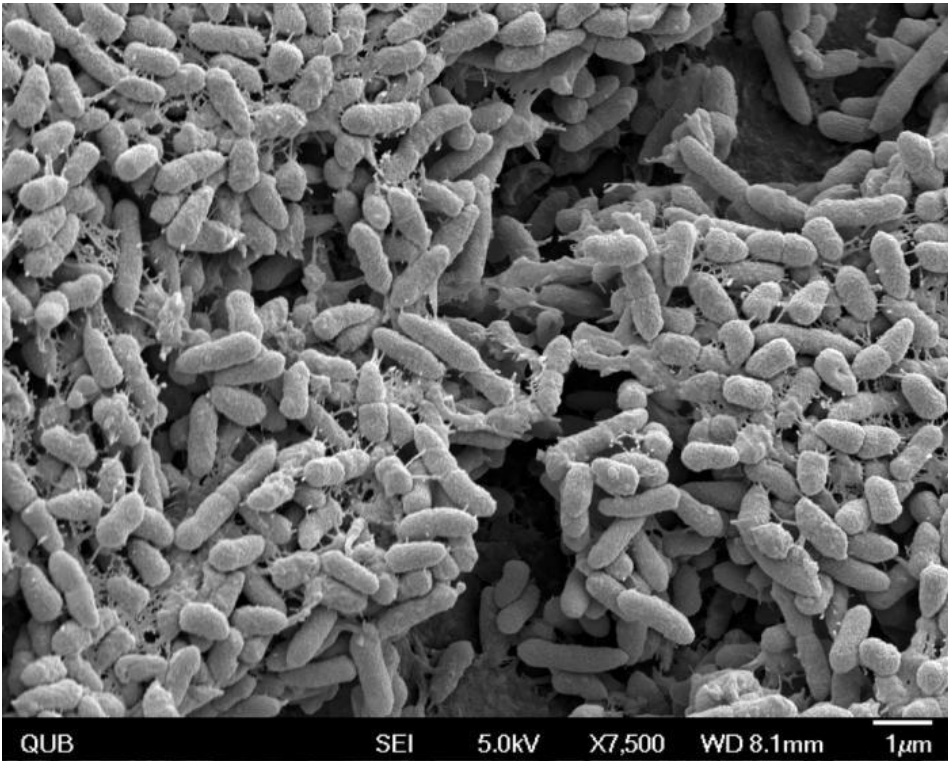
6) Aqueous, **water based solvent**, improved biocompatibility

# Peptide hydrogel applications

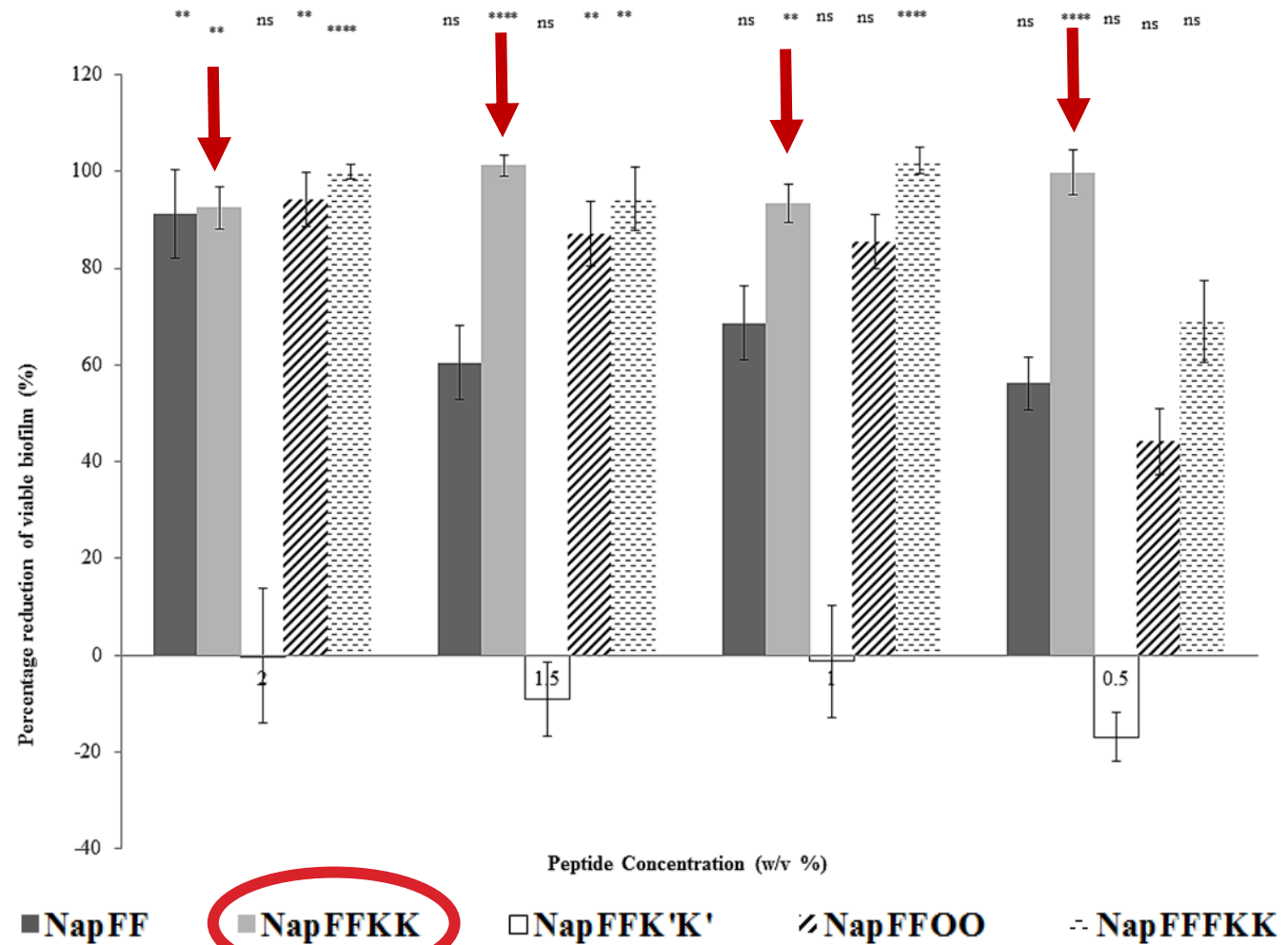


- **Diseases with medication adherence issues (e.g. HIV/AIDs, schizophrenia, Substance abuse)**
- **Cancer (intra-tumoral delivery)**
- **Ocular delivery**
- **Spinal/CNS delivery**
- **Vaccines: peptides as immune adjuvants, extended protection**
- **Infection**

# Antibiofilm efficacy



*SEM Pseudomonas aeruginosa, shown here attached to an implant surface, is one of many resistant microorganisms*



Percentage reduction of mature 24 hour *Staphylococcus aureus* (ATCC 29213) biofilm after 24 hour incubation with peptide hydrogels utilizing an alamarBlue assay (N= 8)

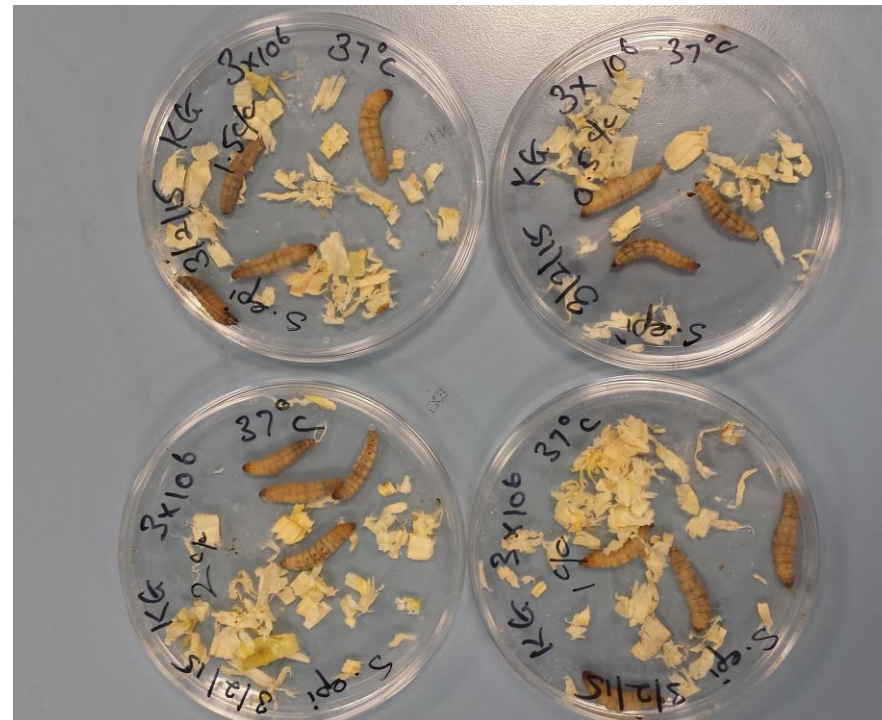
# Galleria mellonella (waxworm) assay



National Centre  
for the Replacement  
Refinement & Reduction  
of Animals in Research



Adult *Galleria mellonella* being inoculated via the pro-leg.



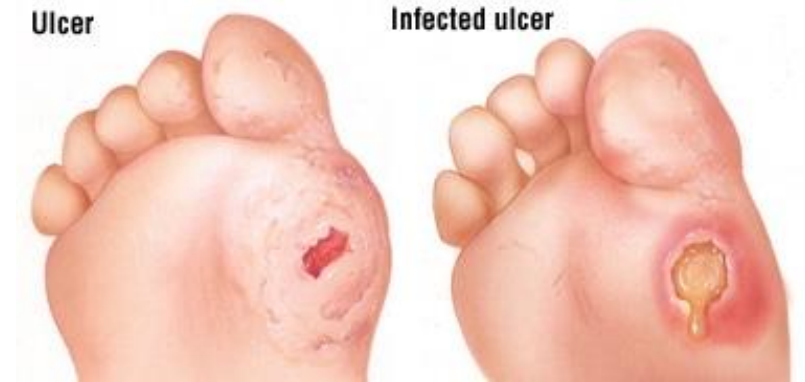
Non viable *Galleria mellonella*



Data demonstrates biocompatibility (NapFFKK-OH) and reduction in bacterial load with *S.aureus* (ATCC 29213) *S.epidermidis* (ATCC 35984), *E. coli* (NCTC 11303) and *Pseudomonas aeruginosa* (PAO1)

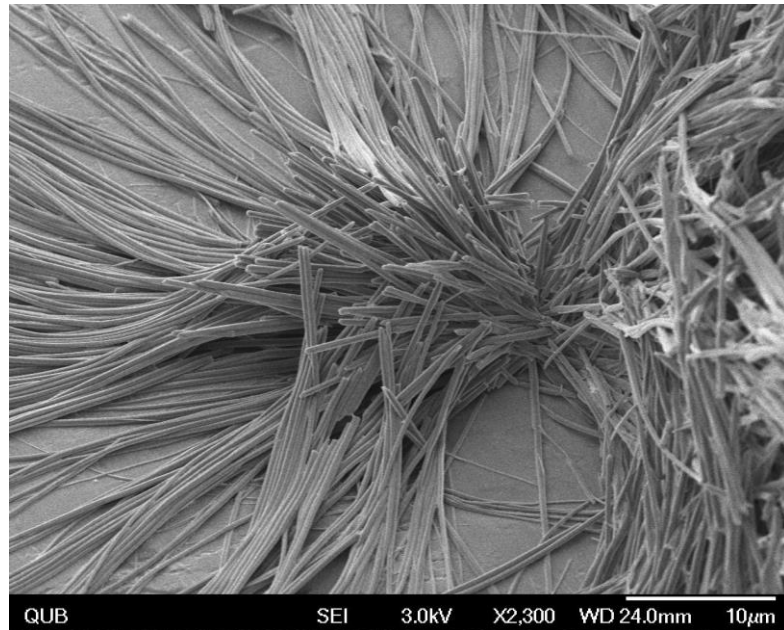
# Multifunctional NSAID-peptide hydrogels for the treatment of chronic wounds

- Chronic wounds: unable to heal fully or respond to treatment within **4 to 12 weeks**. E.g. diabetic ulcers.
- Latest UK estimates (2005-06): **575,600 patients annually**, cost to NHS: **£ 3.1 billion**, 3% of yearly healthcare expenditure.
- Differ from acute wounds = prolonged inflammation that prevents healing fully: Non steroidal anti-inflammatory drugs (NSAIDs) showing benefit.
- Optimal multifunctional peptide: **hydrogelating, biocompatible, antimicrobial, anti-inflammatory, pro-angiogenic**

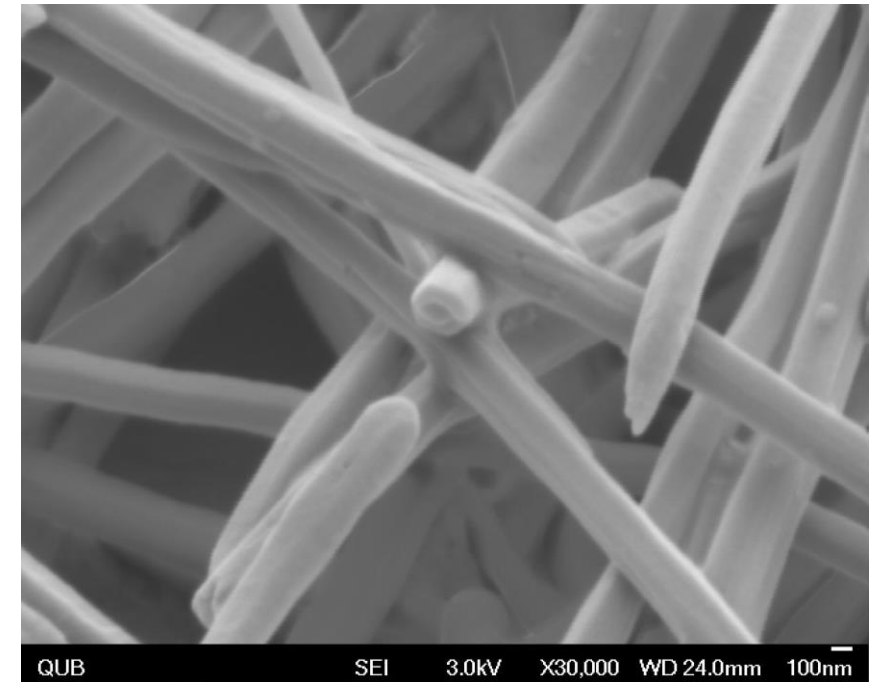


Prevention better than cure!!

# Peptide nanotubes

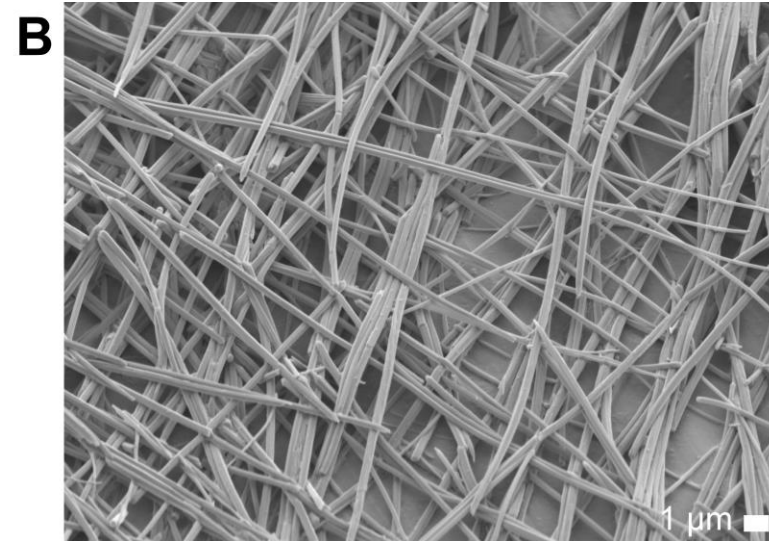
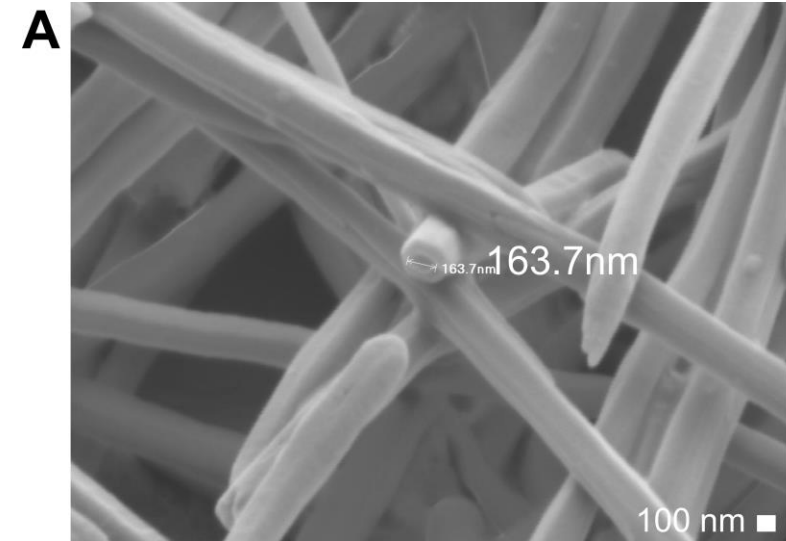


Peptide Nanotubes

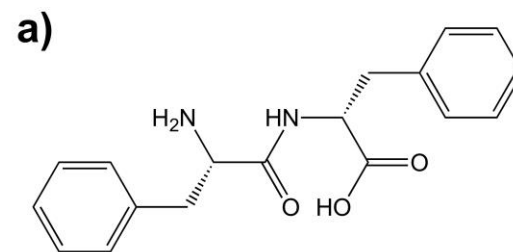




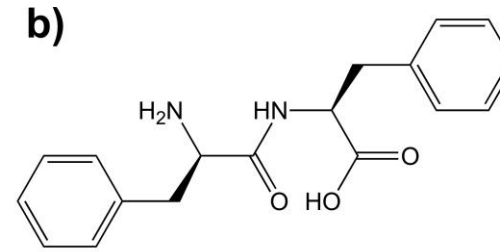
# SEM, nanosize and architecture



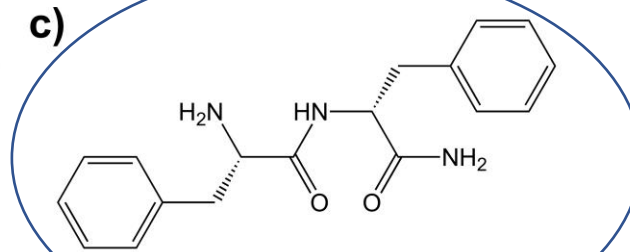
SEM images of 1 mg/mL  $\text{NH}_2\text{-FF-COOH}$  nanotube structures at (A) 30,000 $\times$  magnification, scale bar = 100 nm, (B) 4000 $\times$  magnification, scale bar = 1  $\mu\text{m}$ .



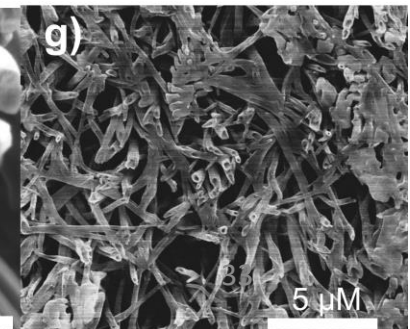
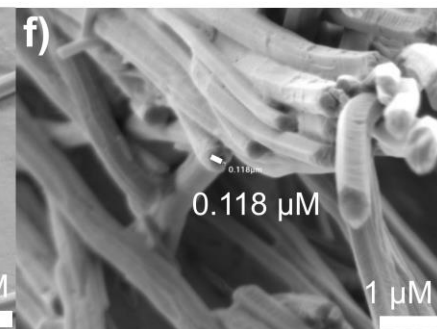
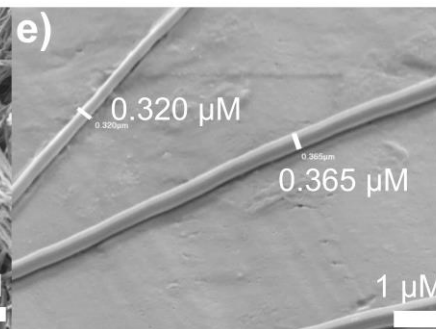
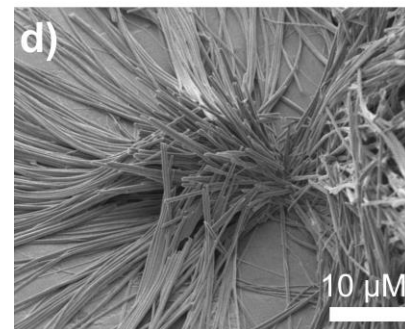
$\text{H}_2\text{N-FF-COOH}$



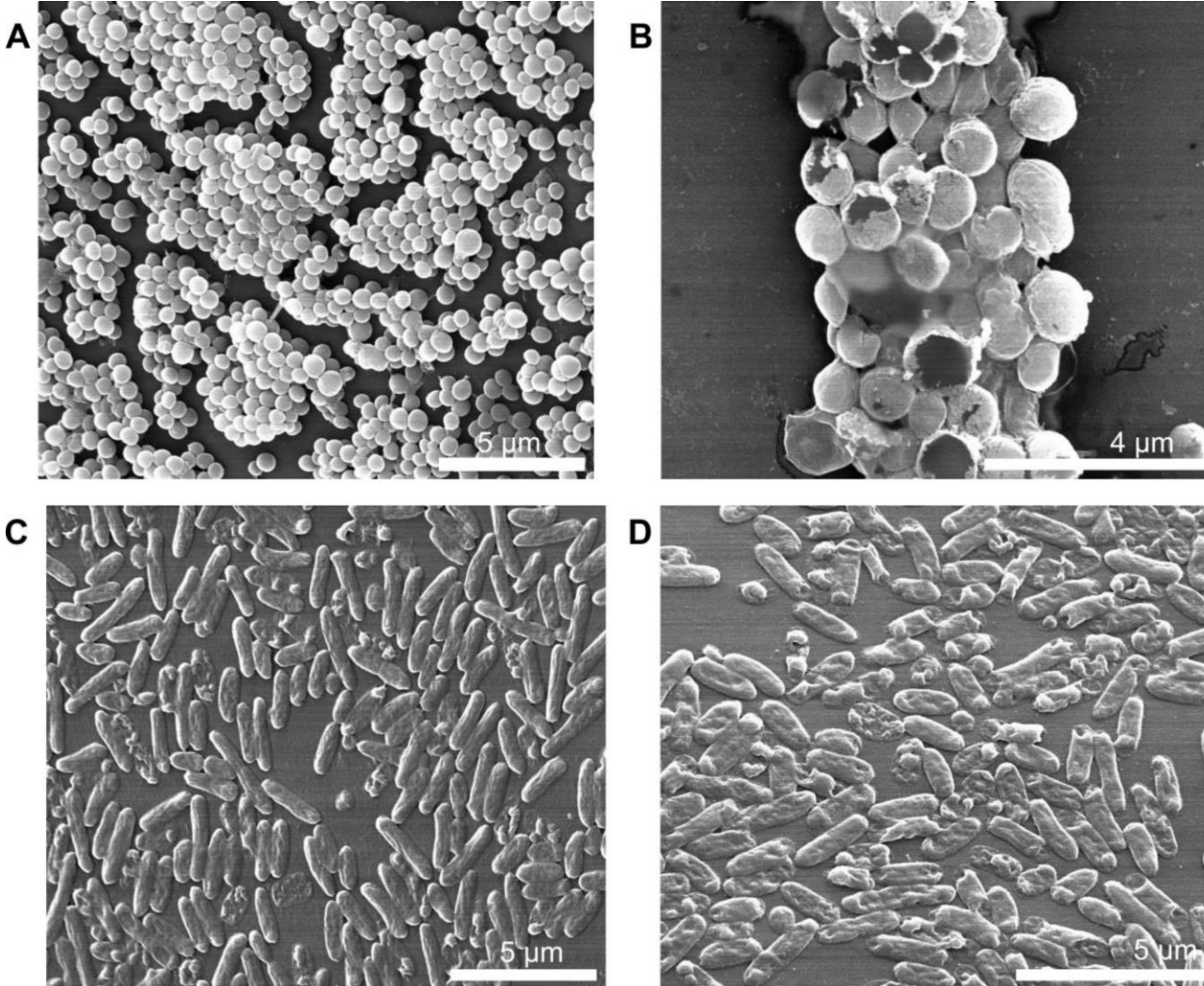
$\text{H}_2\text{N-ff-COOH}$



$\text{H}_2\text{N-FF-NH}_2$



# SEM analysis

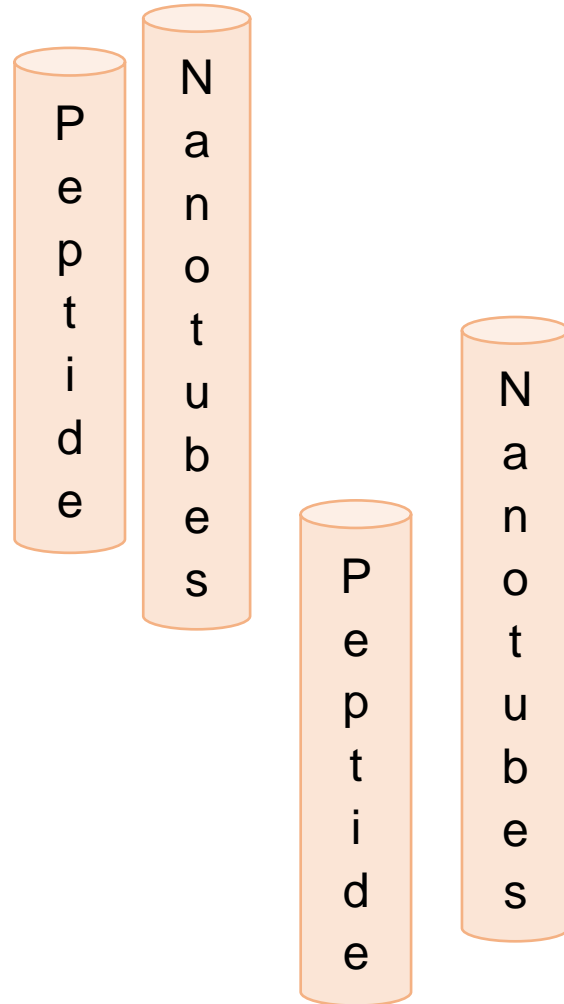


SEM images of **(A)** 15,000× magnification untreated 24 h mature *S. aureus* NCTC 10788 biofilm on MBEC peg, scale bar = 5 μm. **(B)** 30,000x magnification *S. aureus* NCTC 10788 biofilm after 24 h treatment with 2.5 mg/mL  $\text{NH}_2\text{-FF-COOH}$ , scale bar = 4 μm. **(C)** 15,000× magnification untreated 24 h mature *E. coli* ATCC 15597 on MBEC peg, scale bar = 5 μm. **(D)** 20,000× magnification mature *E. coli* ATCC 15597 biofilm after 24 h treatment with 2.5 mg/mL  $\text{NH}_2\text{-FF-COOH}$ , scale bar = 5 μm.

- Ion channel formation?
- Surfactant like action?

-Polyanionic alginate (*P. aeruginosa*)  
or colanic acid (*E.coli*)

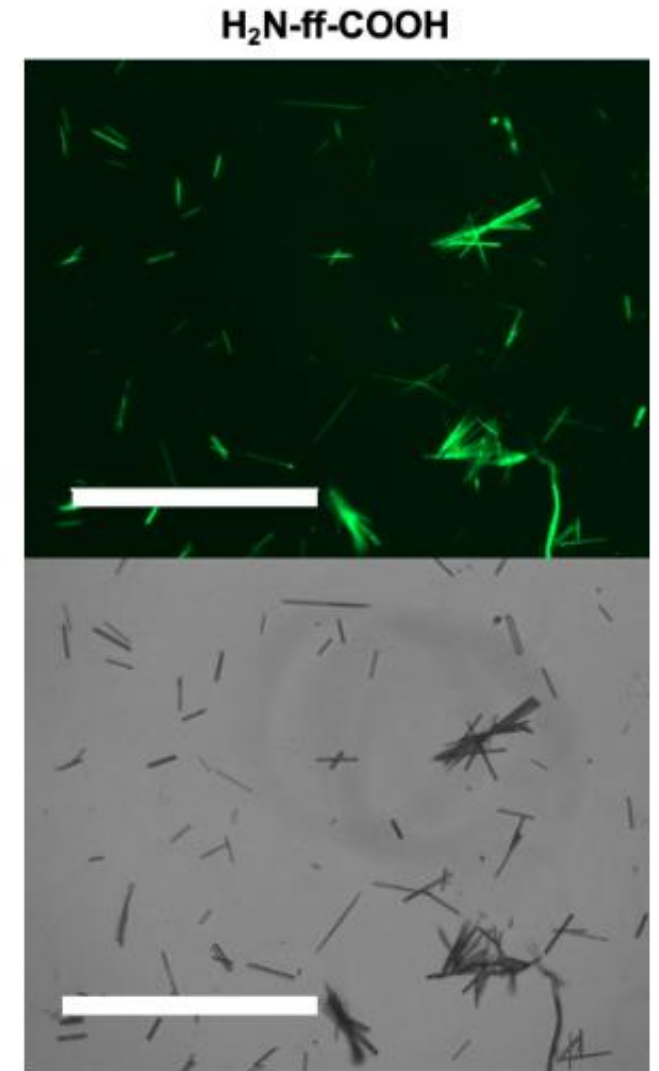
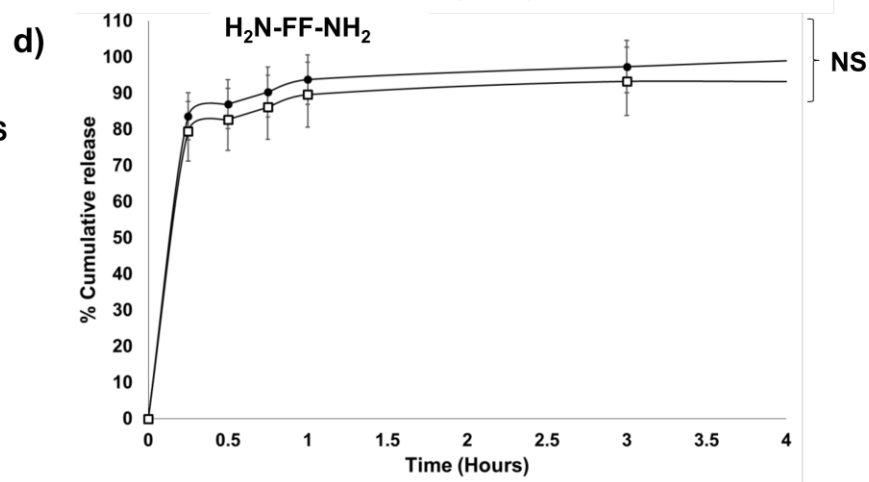
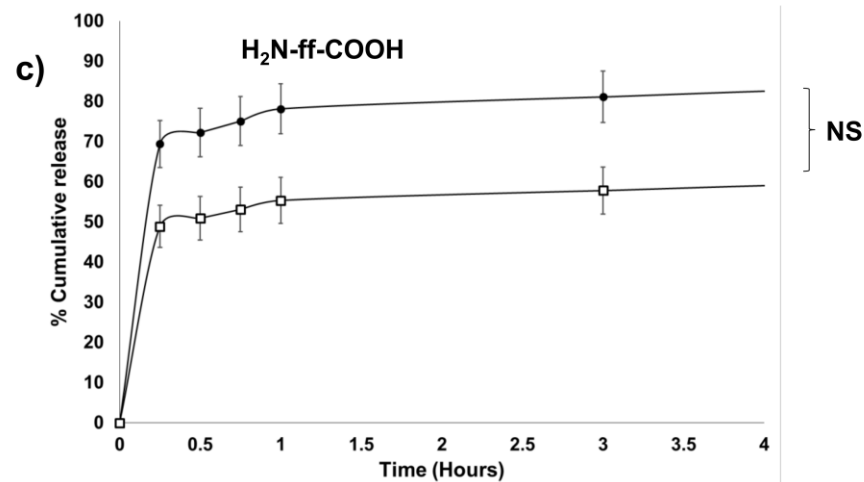
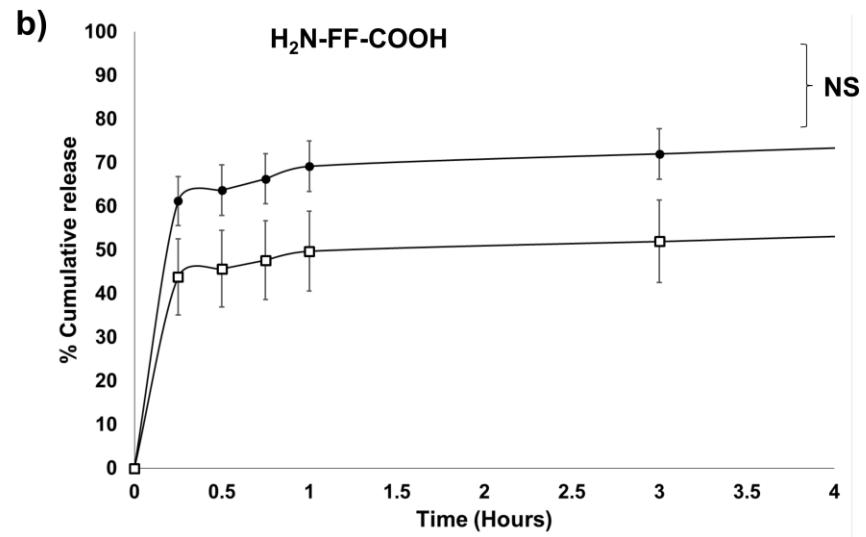
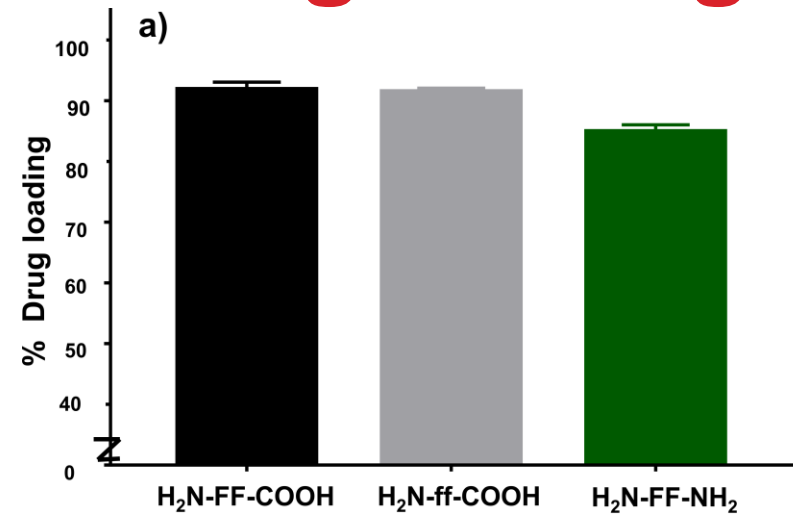
# Peptide nanotubes: Delivery across Biological Barriers



- **Outer Membrane Gram-negative bacteria**
- **Blood Brain Barrier**
- **Intracellular cancer delivery**



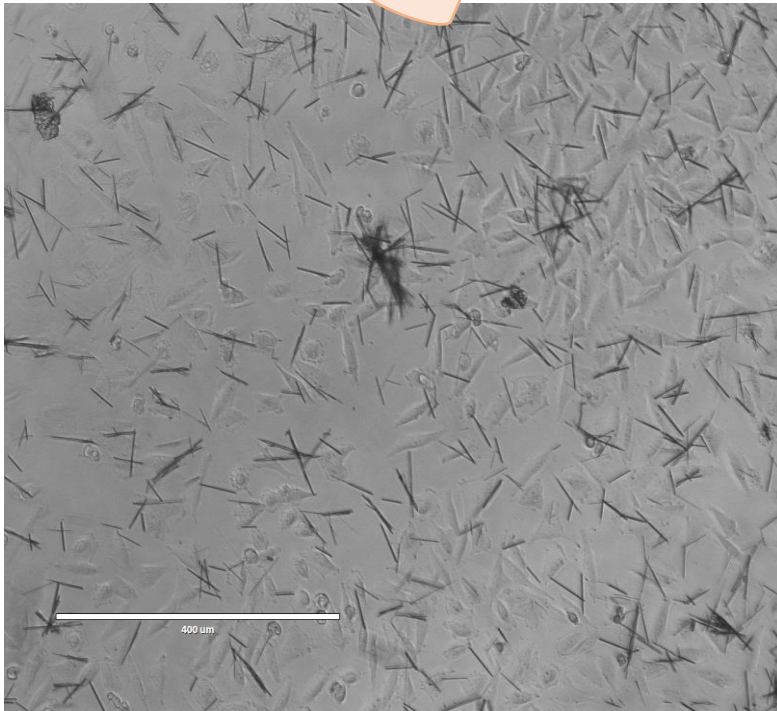
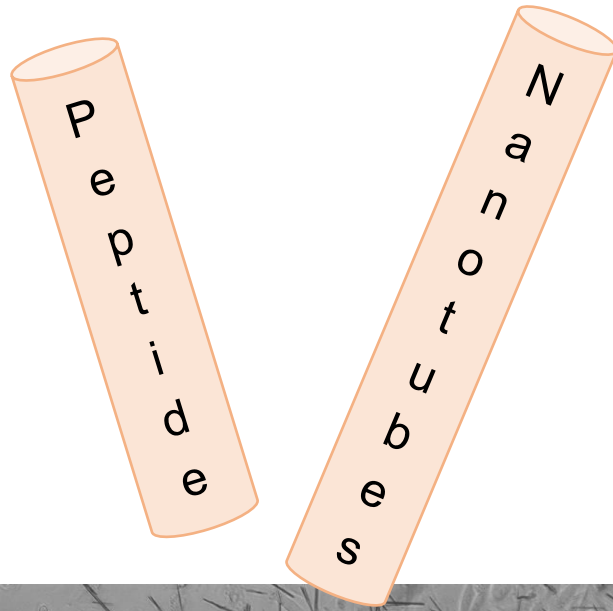
# Drug loading and release



Drug loading and cumulative release profile of dipeptide nanotubes. a) Percentage loading of model hydrophilic drug sodium fluorescein into 10mg/mL H<sub>2</sub>N-FF-COOH, H<sub>2</sub>N-ff-COOH and H<sub>2</sub>N-FF-NH<sub>2</sub> peptide nanotubes. b-d) Cumulative drug release over time (up to 4 hour timepoint) at pH 5.5 (white square) and pH 7.4 (black circle) from 10 mg/mL concentration of b) H<sub>2</sub>N-FF-COOH, c) H<sub>2</sub>N-ff-COOH and d) H<sub>2</sub>N-FF-NH<sub>2</sub>. Three replicates (n = 3) utilized for each study.

Porter, S. L. Coulter, S. M. Pentlavalli, S. Laverty, G.\* Pharmaceutical formulation and characterization of dipeptide nanotubes for drug delivery applications. *Macromolecular Bioscience*. 20, 2000115. *Macromol. Biosci.* 2020, 2000115.

# Peptide nanotubes: Challenges to overcome



- Reliance on Enhanced Permeation and Retention (EPR) effect
- A physiological trigger would be ideal :
  - Hypoxia
  - pH
  - Protease enzyme..... Unravel to deliver cargo
- Chemical changes (e.g. ligands) to these short (dipeptide) motifs impact self-assembly
- Size

# Biofunctional Nanomaterials Group

## Our Funders



**1<sup>st</sup> in the UK**  
for Pharmacy

(The Times and Sunday Times  
Good University Guide 2021)

**EPSRC**

Engineering and Physical Sciences  
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**MRC**

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Council



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- Dr Garry Laverty (Principal Investigator)
- Dr Sreekanth Pentlavalli (EPSRC/Wellcome Trust Research Fellow)
- Dr Sophie Gilmore (EPSRC Research Fellow)
- Dr Emily Cross (MRC Research Fellow)
- Yuming An (PhD student)
- Xin Huang (MPhil student)

- The Xu Group  
Brandeis University
- The Adams Lab  
University of Glasgow
- Ralf Schweins: ILL



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