Disulfide-Stabilized Tumor-Targeted Cyclic Peptide Nanotubes

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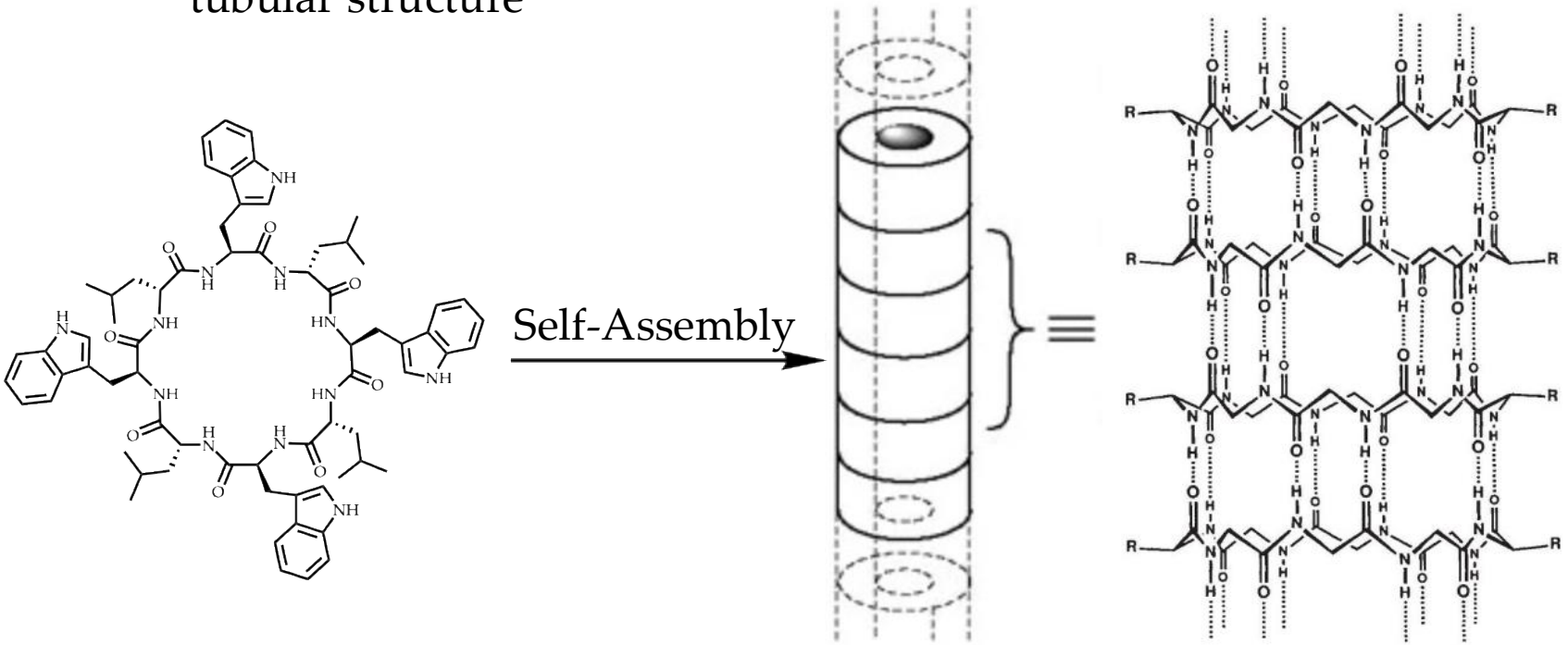
Abstract: Peptide-based materials are desirable materials due to their biocompatibility, structural tunability, and the ability to incorporate unnatural amino acids into their structure. Cyclic peptide nanotubes (cPNTs) fall into the category of peptide-based nanomaterials, composed of cyclic peptide subunits which form nanotubes through intermolecular hydrogen bonding. cPNTs have high functionalization potentials and exhibit enhanced cell permeation. When cPNTs insert parallel to cell membranes, this causes disruption of the membrane and cell death. In this study, we prepared disulfide-stabilized tumor-targeted cPNTs to enhance cytotoxicity using peptide nanomaterials. We report the synthesis of tumor-targeted cPNTs from cyclic peptide monomers tagged with varying amounts of the tumor-homing peptide tLyp-1. cPNTs were prepared using pH-triggered cPNT self-assembly and visualized using transmission electron microscopy (TEM) confirming the formation of cPNTs of on average 7.5 ± 1.2 nm diameter and 81.6 ± 33.7 nm length. Additional characterization by Fourier transform infrared spectroscopy (FTIR) and dynamic light scattering (DLS) is currently underway as well as cytotoxicity studies in U87MG glioblastoma cells. Tubular nanomaterials exhibit ideal pharmacokinetic profiles and as such, tumor-targeted cPNTs hold untapped potential as a new class of therapeutic nanomaterials.

Keywords: Peptide-based materials; nanomaterials; cyclic peptide nanotubes; tumor-targeting

Introduction

- Cyclic peptide nanotubes (cPNTs) are a form of peptide-based nanomaterial
- cPNTs are made of alternating D/L flat cyclic peptide monomers which stack through the formation of a hydrogen bond network to form into a tubular structure

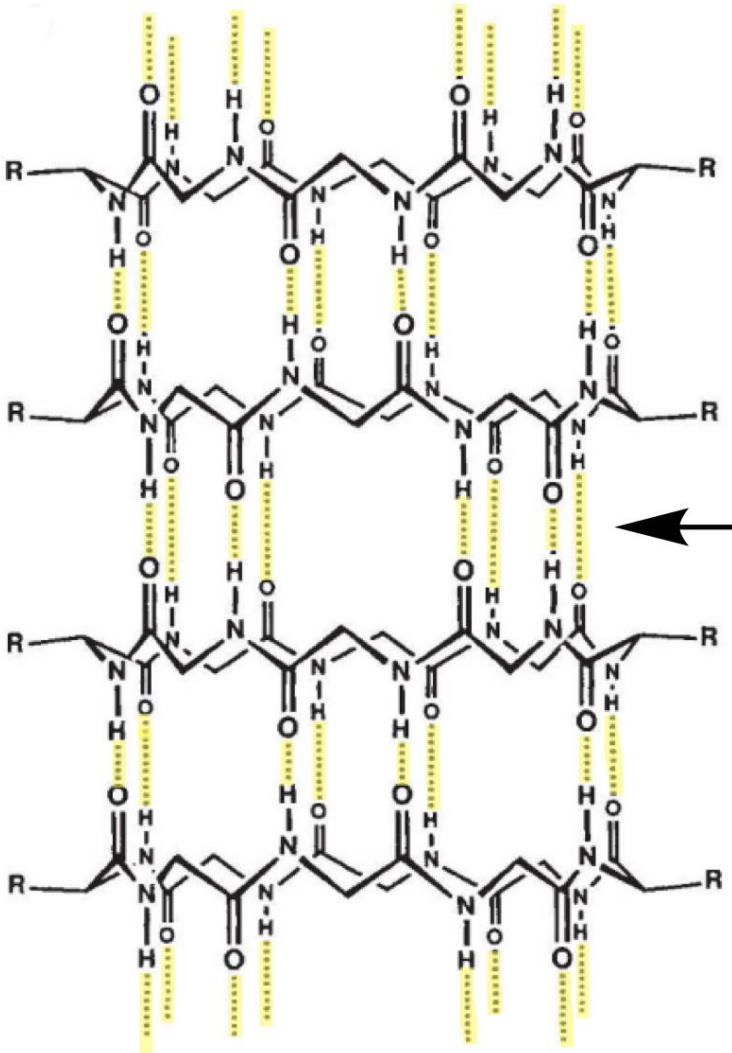


Cyclic Peptide Monomer

Cyclic Peptide Nanotube

Adapted from Ghadiri MR et al. Nature. 1993 Dec; 366:324-7, and Fernandez-Lopez S et al. Nature. 2001 Jul 26; 412:452-5.

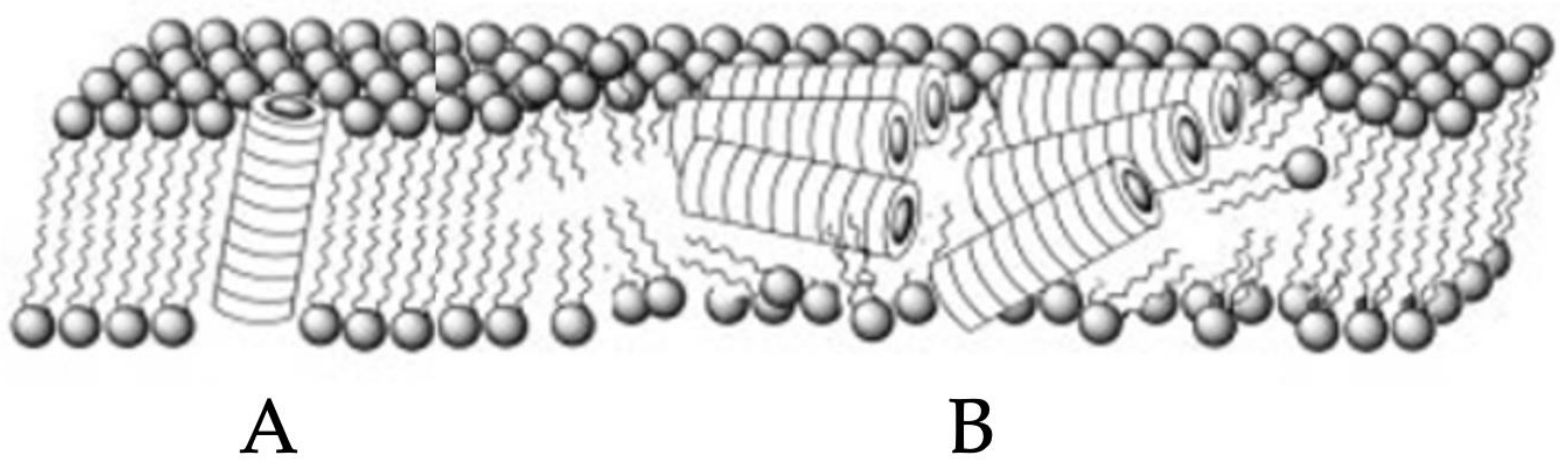
cPNT Hydrogen Bonds



Adapted from Ghadiri MR et al. Nature. 1993 Dec; 366:324-7

cPNT Applications

- cPNTs have shown antiviral activity, and can be used for drug delivery and targeted imaging
- They can also insert perpendicular to cell membranes (A), forming channels, or parallel to cell membranes (B), leading to cell death
  - We plan to encourage cPNTs to disrupt the cell membrane similar to B and exploit cPNTs as therapeutic nanomaterials



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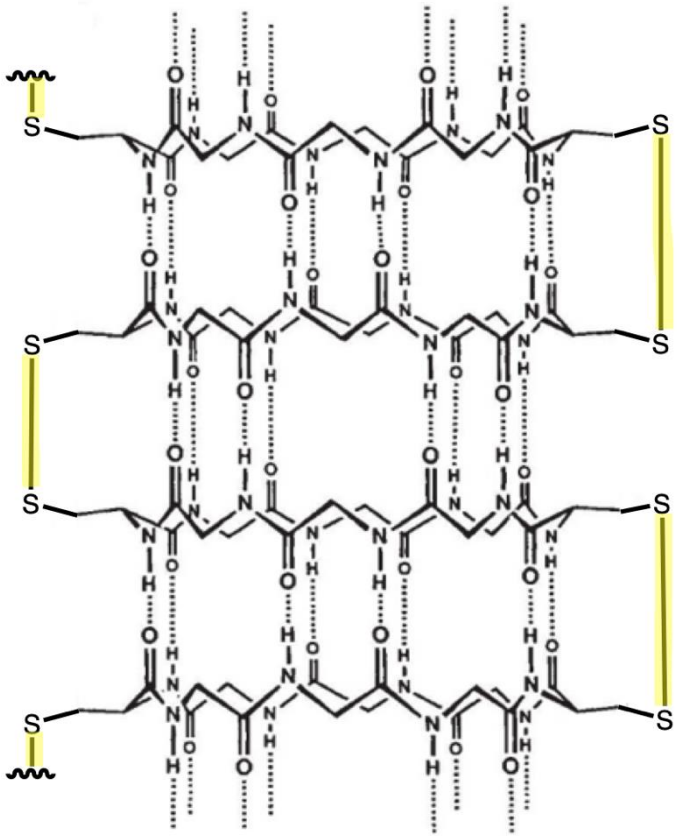
Hartgerink JD et al. Journal of the American Chemical Society. 1996 Jan;118:43–50.
Chapman R et al. Australian Journal of Chemistry. 2010;63:1169.

Disulfide Bond Covalent Tethers

- Being held together by only hydrogen bonds, this can make cPNTs fragile in biological systems
  - By the addition of covalent disulfide bond tethers, we aim to further stabilize cPNTs

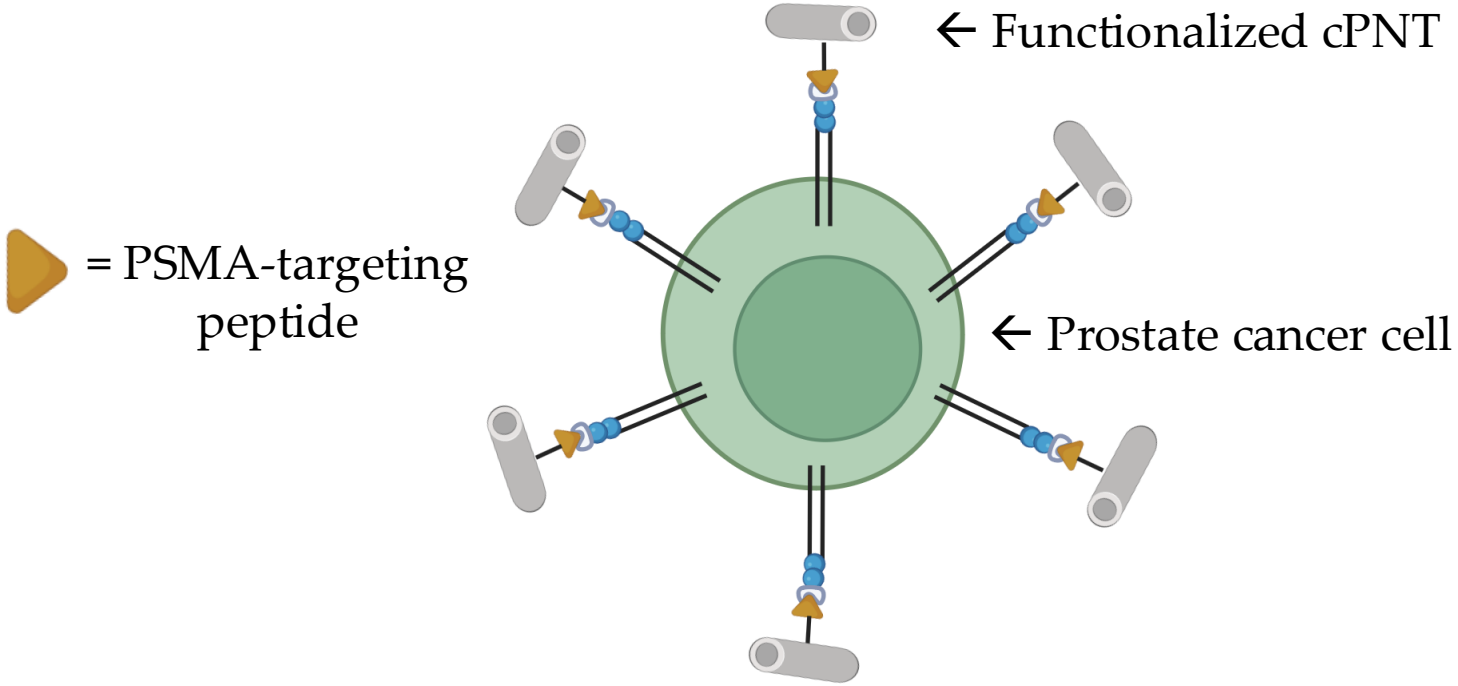


Disulfide bond tethers

Adapted from Ghadiri MR et al. Nature. 1993 Dec; 366:324-7.

Tumor-Targeting cPNTs

- Once stabilized, we aim to target the cPNTs to cancer cells, leading to targeted cell death
  - There are only few examples of cell targeted cPNTs using prostate-specific membrane antigen (PSMA) targeting peptides. One such example includes functionalizing cPNTs with PSMA to be used for prostate cancer imaging



Adapted from Fátima, S et al. Nanoscale. 2024 Nov.
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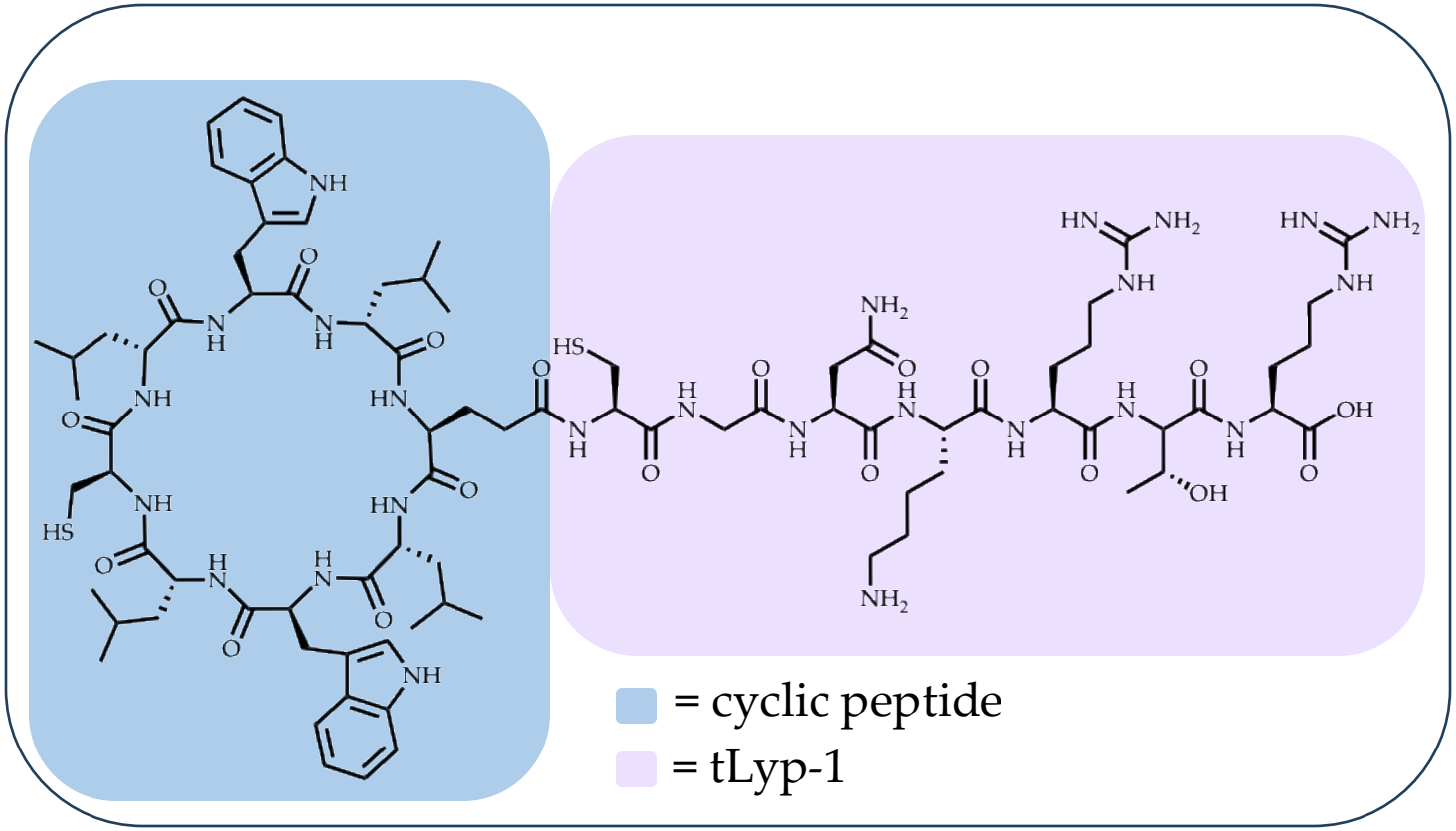
 Fátima, S et al. Nanoscale. 2024 Nov.

tLyp-1 cPNTs

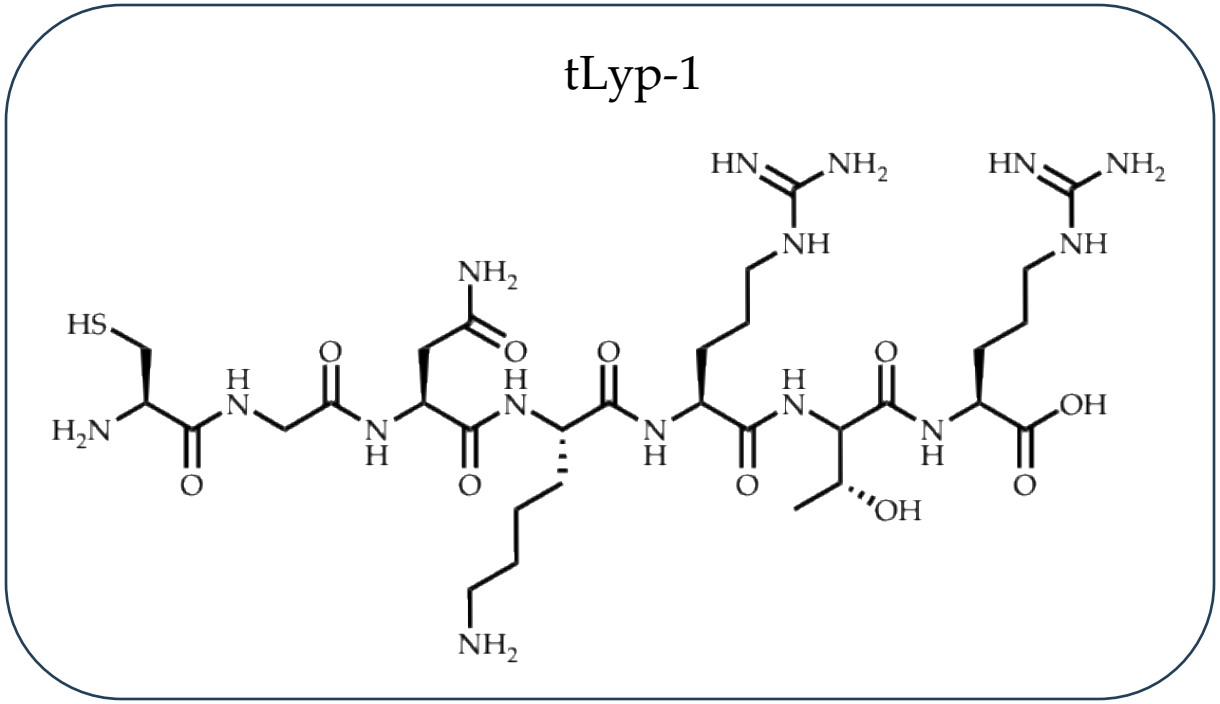
- We chose to functionalize our nanotubes by appending tLyp-1 to our cyclic monomers



Larue, L et al. Bioorganic Chemistry. 2023 Jan;130:106200.
Jubb, A. M et al. The Journal of Pathology. 2012 Jan;226:50–60.

tLyp-1

- tLyp-1 is tumor-homing, cell permeable, as well as aids in solubility of cyclic peptide monomers
- Has been shown to target the neuropilin-1 (NRP-1) co-receptor, shown to affect tumor cell proliferation and pathological angiogenesis, typically seen in aggressive cancers such as breast, renal, and lung cancers



Larue, L et al. Bioorganic Chemistry. 2023 Jan;130:106200.
Jubb, A. M et al. The Journal of Pathology. 2012 Jan;226:50–60.

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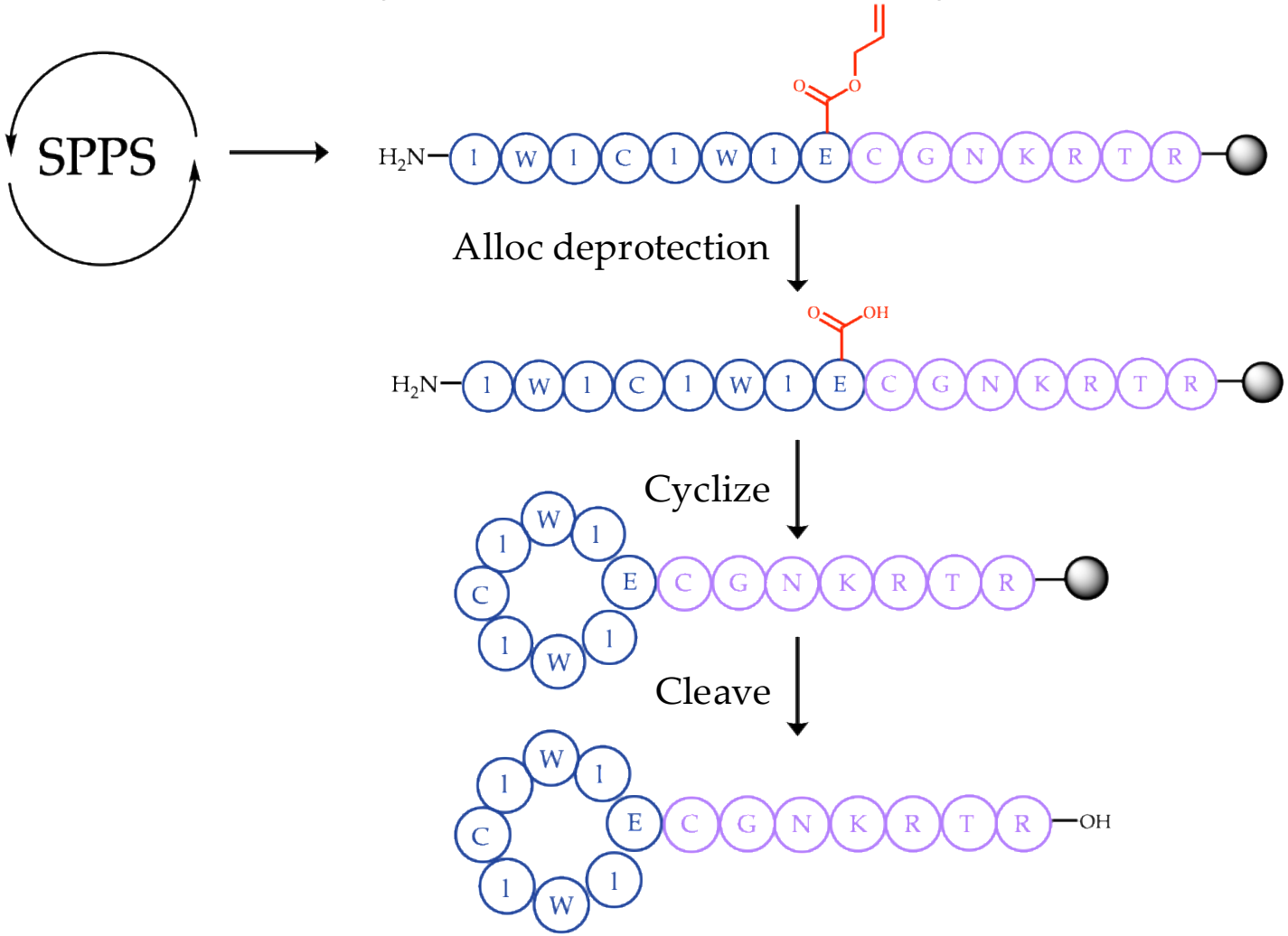
Methods: tLyp-1 functionalized cyclic peptide monomers



$[M+H]^+$ calc = 1874.28 m/z
$[M+H]^+$ found = 1874.4 m/z

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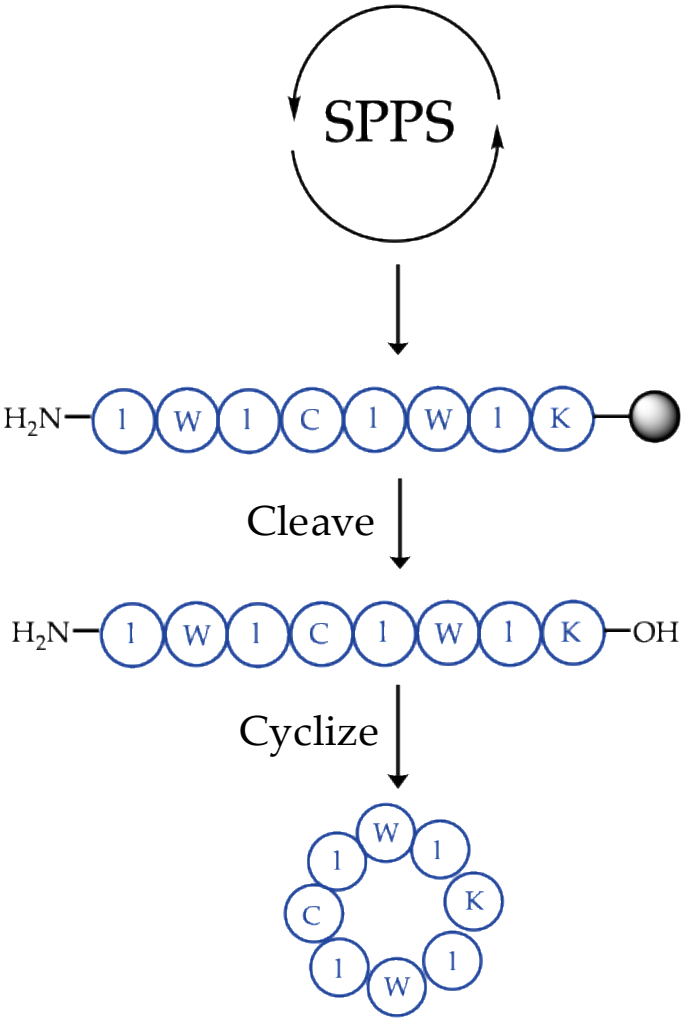
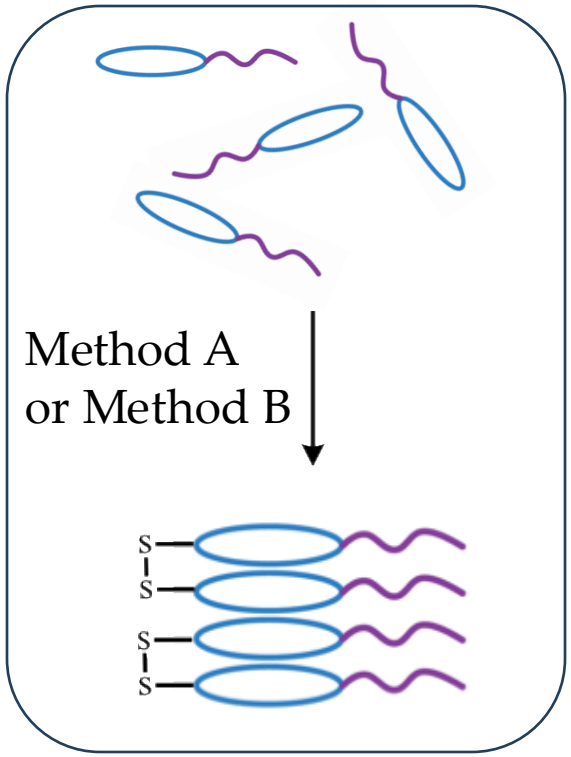
Unfunctionalized (Control) cPNTs

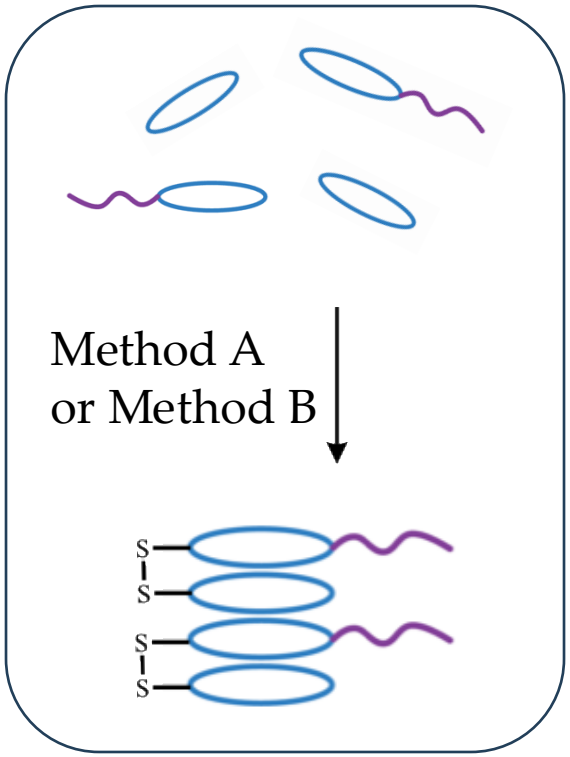


$[M+H]^+$ calc = 1056.5 m/z
$[M+H]^+$ found = 1056.60 m/z

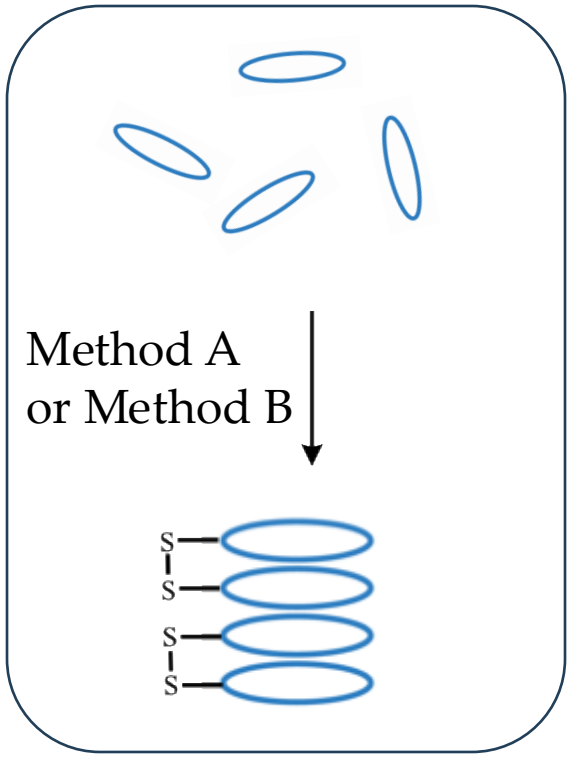
Self-Assembly of cPNTs



Fully Functionalized



50/50 Functionalized



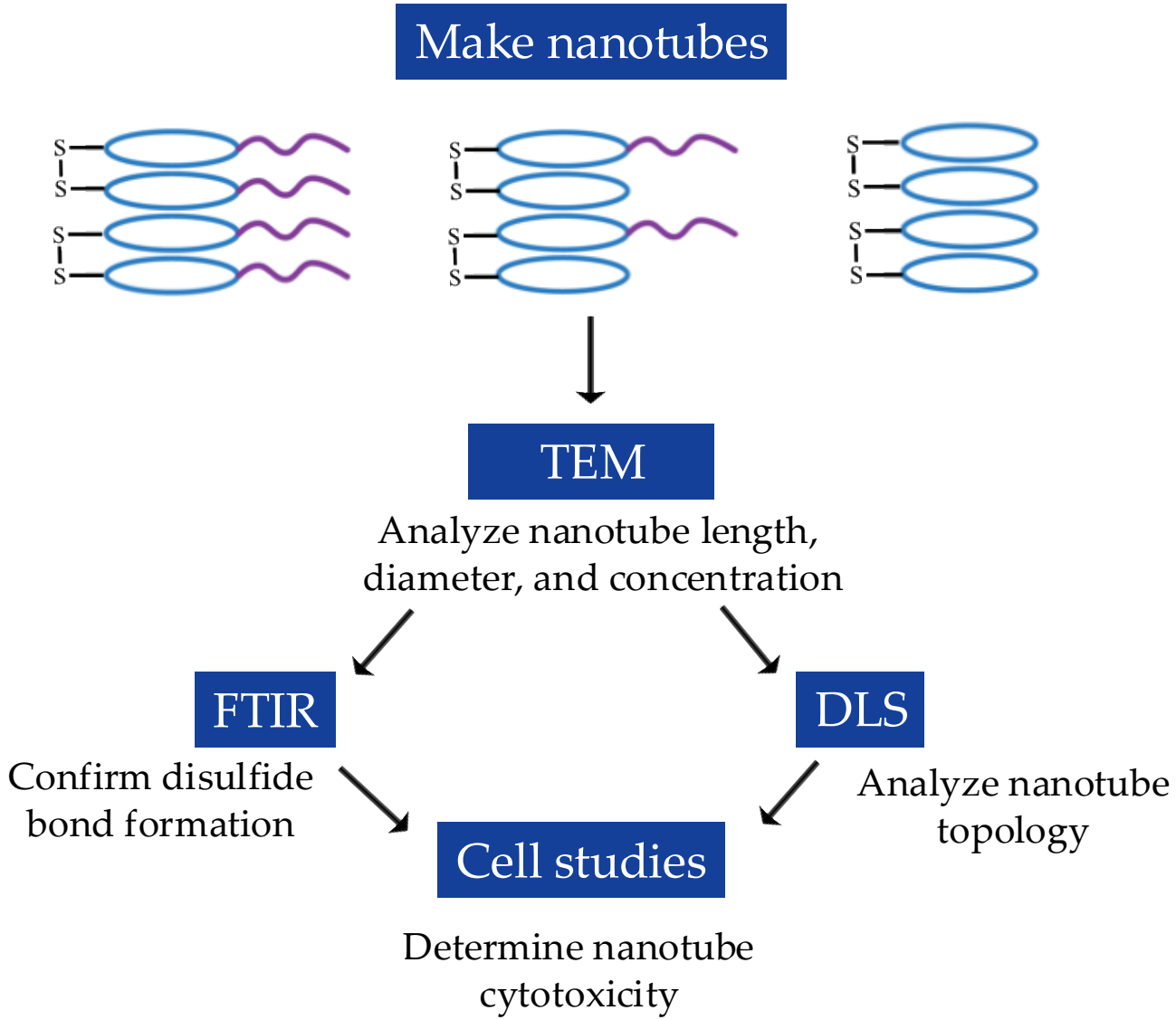
Unfunctionalized
"Control"

Assembly Methods:

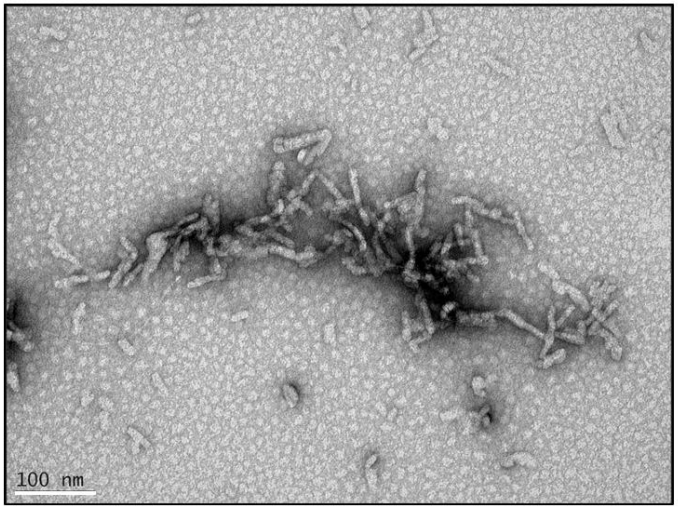
A: 1M NaOH + 0.13 M TFA to pH 1

B: 0.13 M TFA + 1 M NaOH to pH 14

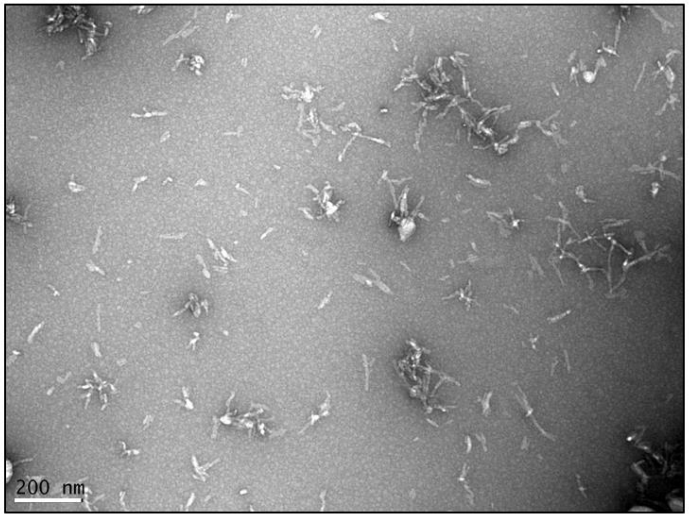
Workflow



Results: Fully Functionalized cPNTs



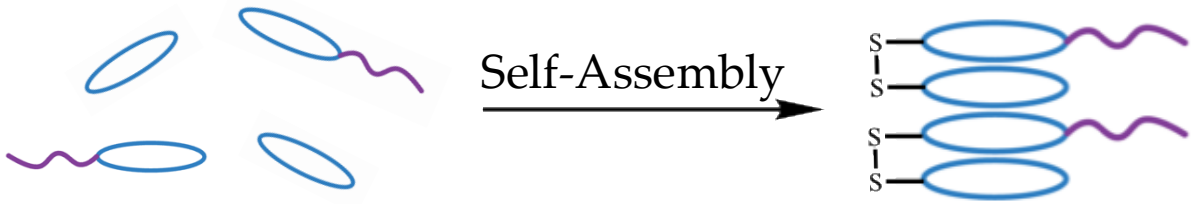
TEM A

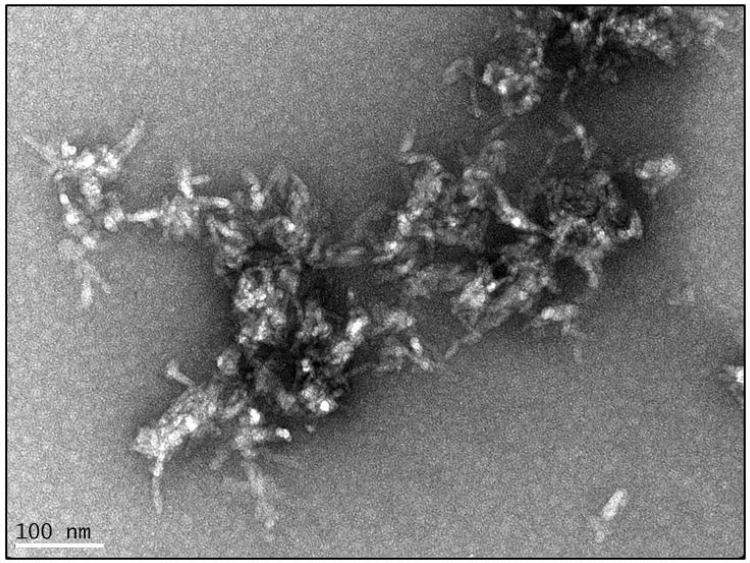


TEM B

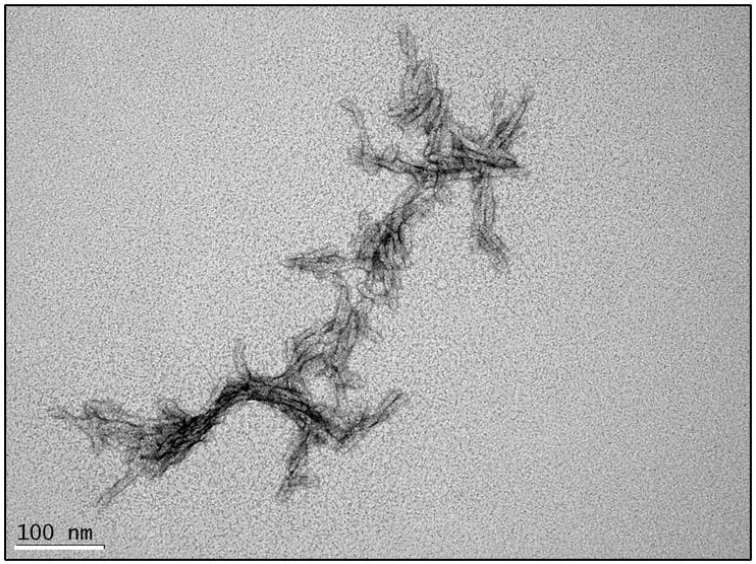
Method	Average Length	Average Diameter
A	74.7 ± 37.8 nm	7.6 ± 1.2 nm
B	109.0 ± 39.6 nm	7.4 ± 1.2 nm

50/50 Functionalized cPNTs





TEM A



TEM B

Method	Average Length	Average Diameter
A	75.0 ± 32.8 nm	7.5 ± 1.0 nm
B	67.7 ± 24.7 nm	7.4 ± 1.2 nm

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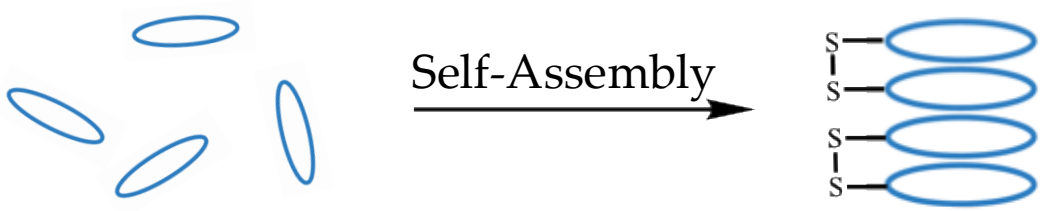
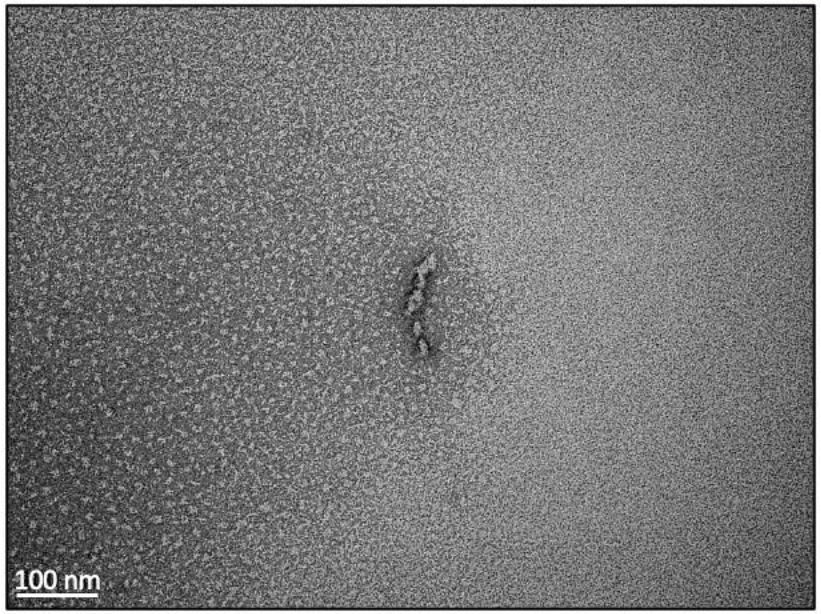
Unfunctionalized cPNTs



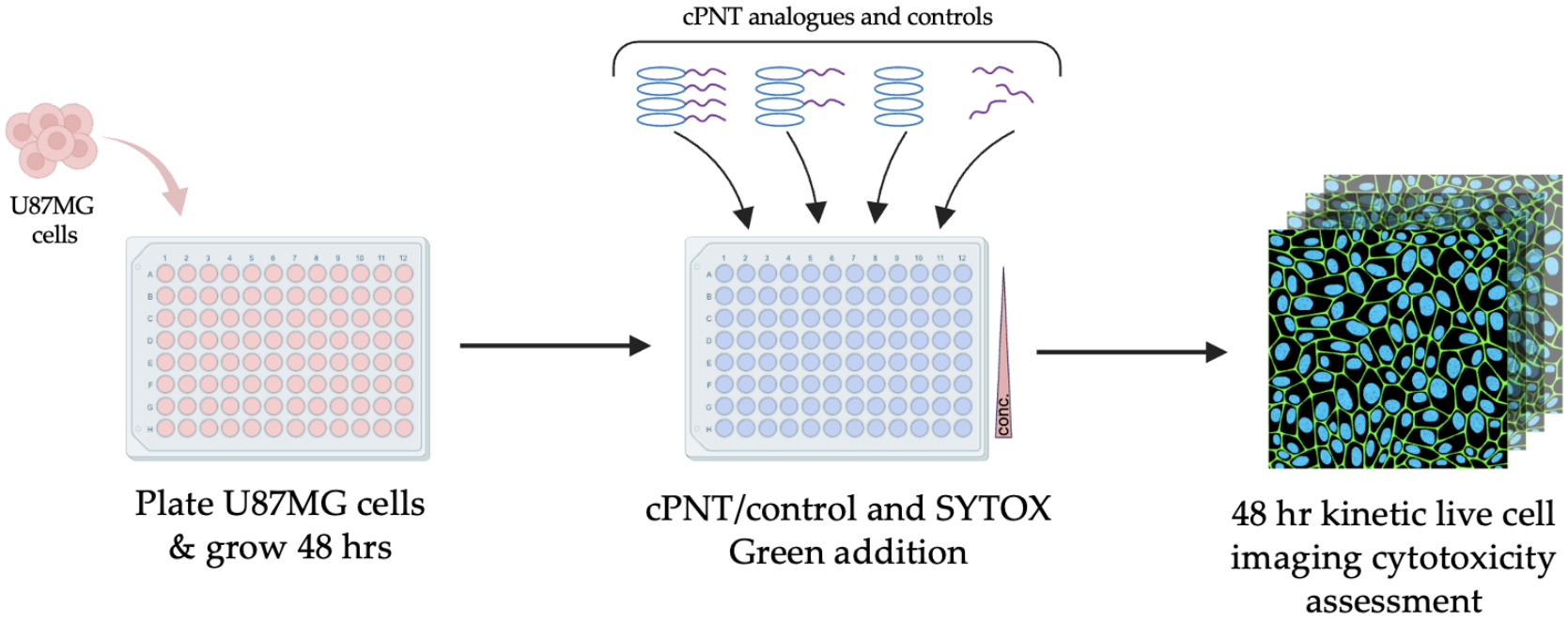


TEM B

Method	Average Length	Average Diameter
A	N/A	N/A
B	N/A	N/A

Cytotoxicity Evaluation

- Now that we can successfully synthesize nanotubes, preliminary cell work is underway. U87MG cells are known to upregulate NRP-1 therefore we have chosen them for evaluation



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Hu, B et al. Oncogene. 2008 Mar;26:5577-5586.

Conclusions

- While cell work, FTIR, and DLS is still on-going, it is promising to see nanotube formation while functionalized with the tumor-targeting peptide tLyp-1
- More work will be done to enable cPNT formation of bare cyclic peptides, likely by changing solvent conditions to improve solubility during cPNT formation
- Moving forward, cell work with our three nanotube conditions compared to linear tLyp-1 alone will be compared in U87MG glioblastoma cells to assess cellular toxicity

Conflicts of Interest

The authors declare no conflicts of interest

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