PhD Open Days

Breaking the Vicious Cycle: Multifunctional Peptides Targeting the RANK-TRAF6 Interaction

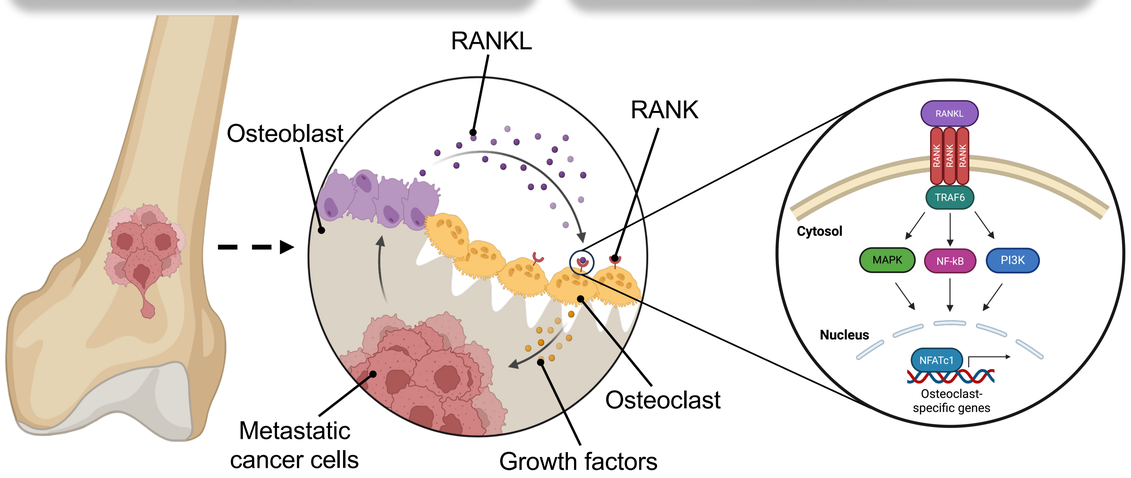
PHD PROGRAMME IN BIOENGINEERING AND BIOSCIENCES

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1. Background

Most **cancer deaths** are prompted by **incurable metastasis**, with **bone** being one of the **most frequent metastasis sites**[1].

The bone undergoes **constant remodelling** during an individual's lifetime. This **remodelling** is **disrupted** in **metastasis**[1].



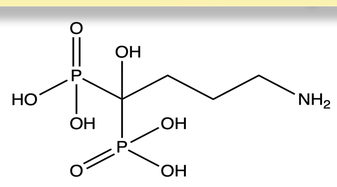
The vicious cycle of bone metastasis and its signalling pathways

Cancer cells induce **osteoblasts** to secrete receptor activator of nuclear factor kappa B ligand (**RANKL**), which binds to its receptor, **RANK**, in osteoclasts[1].

Through tumour necrosis factor receptor associated factor 6 (**TRAF6**), this interaction increases **osteoclast proliferation**, promotes **bone resorption** and **secretion of growth factors**[1].

2. Proposal

Develop **bone-seeking peptides** that **disrupt** the **RANK/TRAF6** axis and thus **hinder osteoclastogenesis** and **metastatic potential**



Bone-targeting unit

A bisphosphonate **(alendronate)** with **high affinity** for **bone mineral**[2]

Fragment A

Cathepsin K-sensitive linkers

Cathepsin K is an abundant **protease** in the **bone microenvironment**[3]

Fragment B

Cell-penetrating peptides (CPPs)

Peptide sequences able to promote **cell entry** of different cargoes[4]

Fragment C

RANK-derived sequences

Bind to **TRAF6** and **disrupt** the **RANK/TRAF6 interface**[5]

Linker1 / **Linker2** / **Linker2** + **CPP1** / **CPP2** →

PepA	PEG2-Linker1-Ahx-CPP1-NH2
PepB	PEG2-Linker2-Ahx-CPP1-NH2
PepC	PEG2-Linker3-Ahx-CPP1-NH2
PepD	PEG2-Linker1-Ahx-CPP2-NH2
PepE	PEG2-Linker2-Ahx-CPP2-NH2
PepF	PEG2-Linker3-Ahx-CPP2-NH2

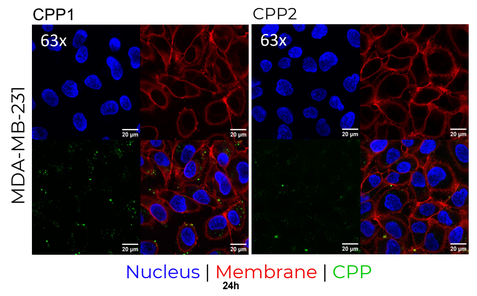
PEG – Polyethylene glycol **Ahx** – 6-aminohexanoic acid

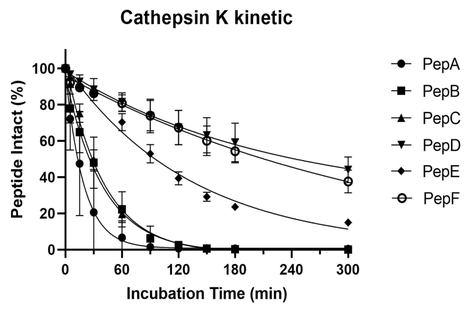
CPP1 / **CPP2** + **1A** / **1B** / **4A** / **4B** →

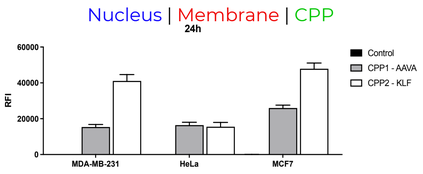
2A	CPP1-1A-NH2	**3B**	CPP2-1B-NH2
2B	CPP1-1B-NH2	**5A**	CPP1-4A-NH2
3A	CPP2-1A-NH2	**5B**	CPP1-4A-NH2

3. Results and Conclusions

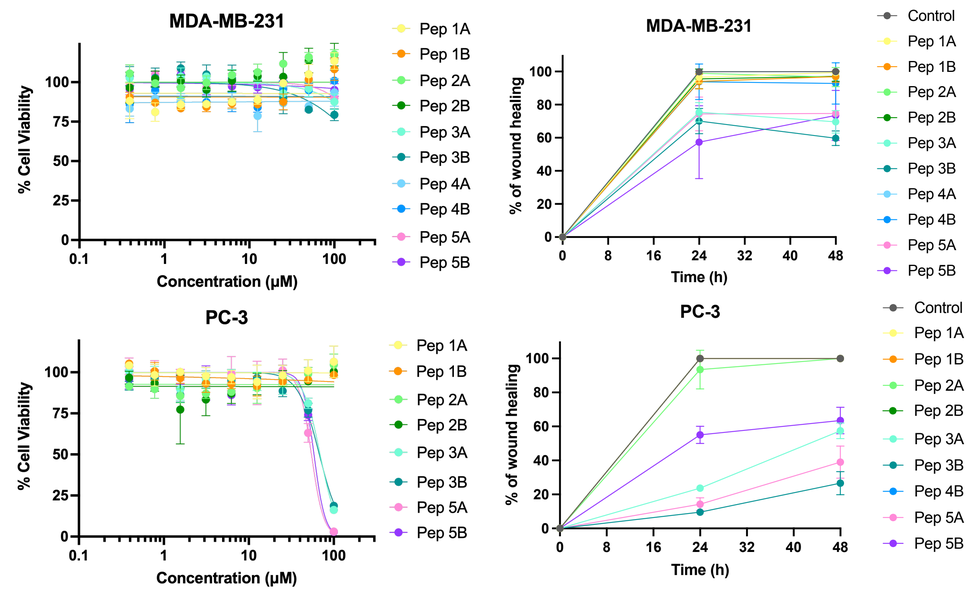
Cell Internalization and Cathepsin K Cleavage





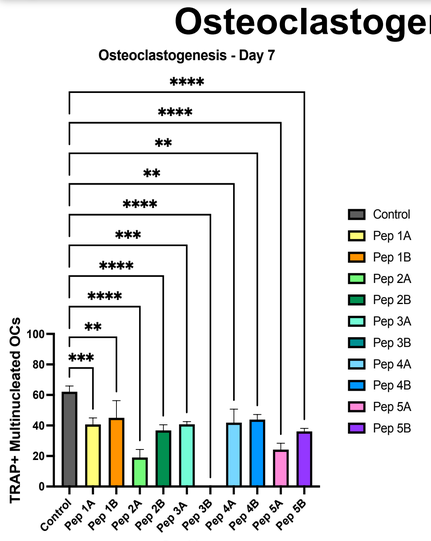


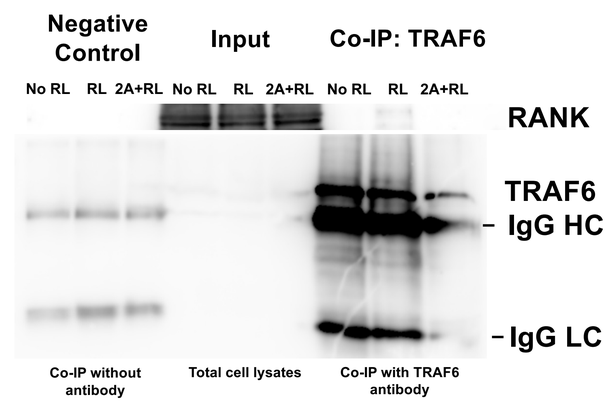
Both CPPs are **internalized; peptides A, B and C** show **faster cleavage kinetics** than D, E and F.

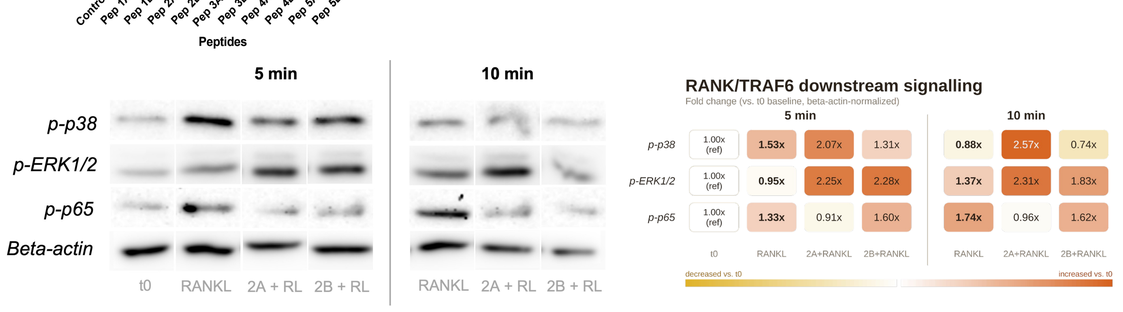


Only **3A, 3B, 5A and 5B** show **slight cytotoxicity** and also **impair wound healing** in **prostate cancer cells** – likely **reflecting cytotoxicity, not an anti-migratory effect**.

Osteoclastogenesis and TRAF6 Signalling







2A and 5A show the **strongest inhibition of osteoclastogenesis**; **3B** is **cytotoxic** to **monocytes. 2A disrupts RANK/TRAF6** and seems to **reduce p65 phosphorylation**.

Conclusions

- **Cargo release depends on the CPP sequence, not on the linker type.**
- **2A and 5A reduce osteoclastogenesis; 2A also disrupts RANK/TRAF6.**
- **Linker 1 and 2A are the lead candidates for the full conjugate.**

REFERENCES

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3. Qian, D. *et al.* Curr. Oncol. 29.8: 5963-5987 (2022).
4. Zorko, M. *et al.* Adv. Drug Deliv. Rev. 180: 114044 (2022).
5. Park, J. H. *et al.* Mol. Cells. 40.10: 706-713 (2017).

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