



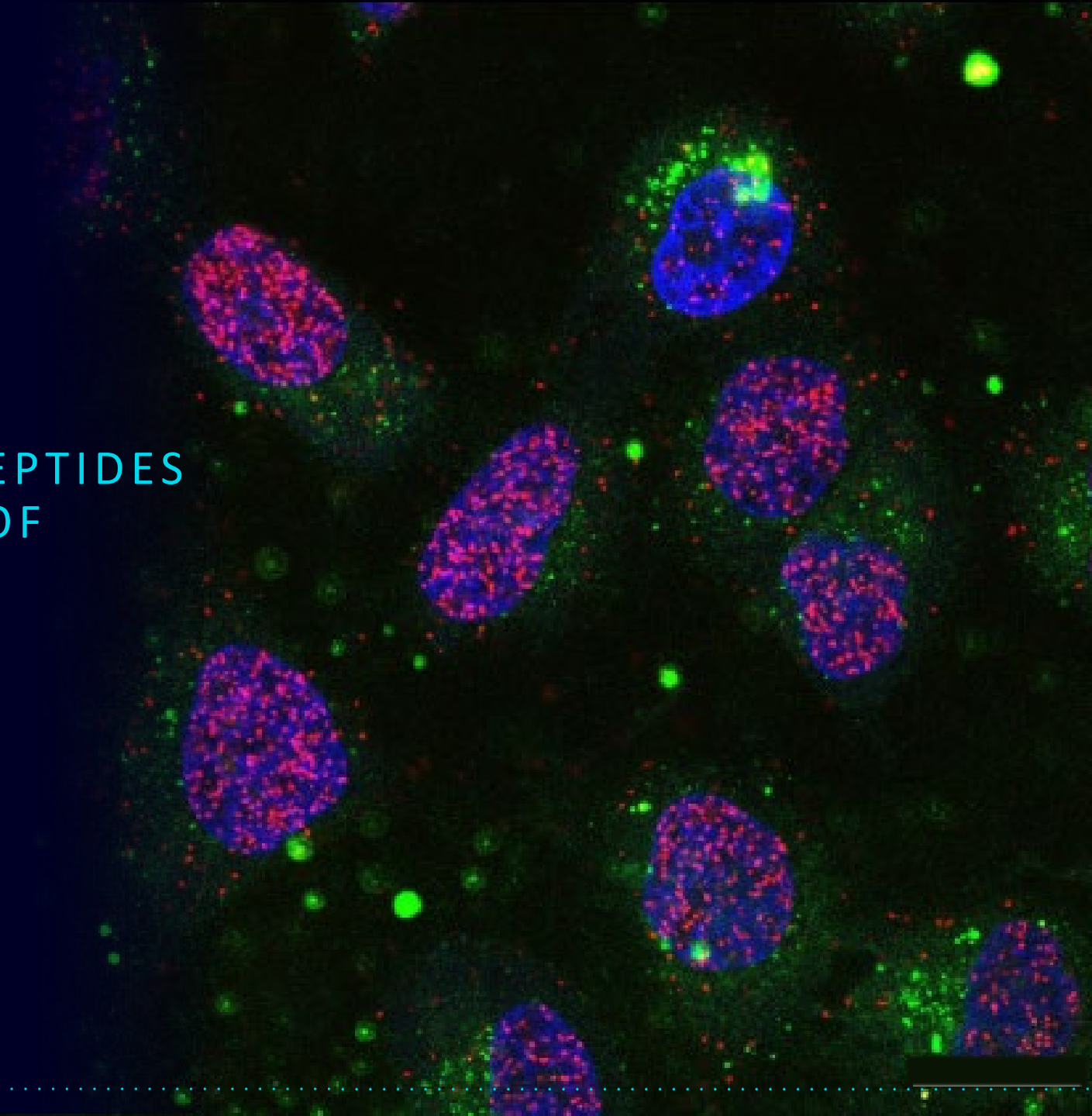
CANCER
RESEARCH
HORIZONS

FURTHER FASTER TOGETHER

PLATFORM TECHNOLOGY: TRIMERIC CELL-PENETRATING PEPTIDES FOR INTRACELLULAR DELIVERY OF MOLECULES

Non-confidential overview

May 2023



OPPORTUNITY OVERVIEW



Novel platform technology

- Utilises novel scaffold chemistry to deliver macromolecules to intracellular targets
- Proprietary methodology for facile preparation of pharmacologically active complexes



Broad Therapeutic and diagnostic potential

- Trimeric peptides can be complexed with IgG, ScFv, FAb, and oligonucleotide payloads
- Compatible with radiolabels for Tx and Dx applications



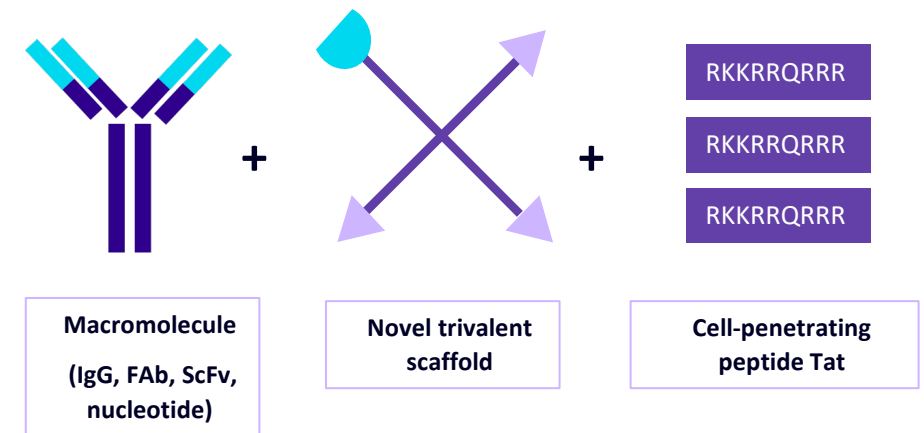
Ready to partner

- Developed by Prof Kate Vallis at University of Oxford and Dr Ole Tietz at Macquarie University
- Seeking licensing and/or collaboration partner to accelerate development

INTRACELLULAR DELIVERY OF MACROMOLECULES

The majority of the human proteome, and many “undruggable” targets are intracellular and cannot be accessed by macromolecule-based therapeutics

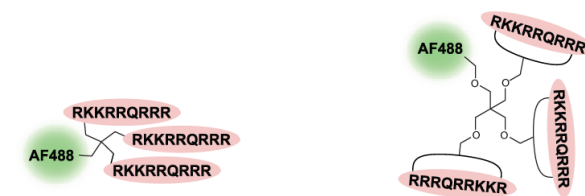
- This platform leverages the cell-penetrating peptide (CPP) HIV-tat (Tat) to provide a straightforward method for intracellular drug delivery
 - The cargo may be macromolecule such as an IgG antibody or antibody fragment, or nucleotide
- Previous CPP-based approaches have suffered from poor efficiency and required very high (>20uM) effective Ab concentrations in vitro
- The inventors have developed novel **trimeric cell penetrating peptides (tCPPs)** that are effective at low concentrations and can flexibly bind varied payloads



CPP CONFORMATION INFLUENCES INTRACELLULAR DELIVERY

Novel tCPPs more effectively penetrate cell membranes than monomeric CPPs

- In nature, Tat clusters at the cell membrane prior to endocytosis
- The inventors identified that trimers of Tat more effectively penetrate cell membranes than monomeric Tat, and created tCPPs based on novel small molecule scaffolds
- Novel tCPPs were further optimised by testing linear and cyclic conformations of Tat complexes
- Linear Tri-Tat A (**tri-Tat A**) and Trimeric cyclic tat-B (**tri-cTat B**) were taken forward for further development

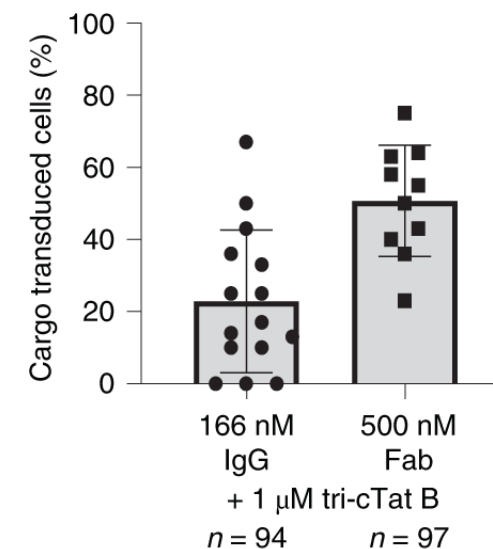
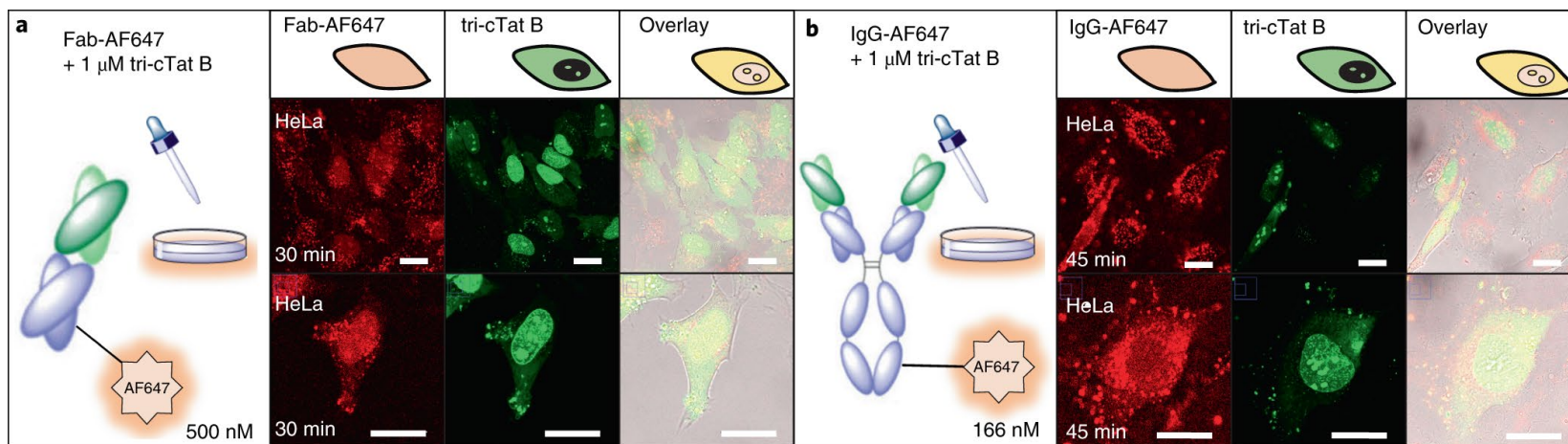


Tri-Tat A

Tri-cTat B

MACROMOLECULAR CARGOES ARE EFFECTIVELY DELIVERED INTRACELLULARLY WITH tri-cTat B

tri-cTat B effectively translocates non-specific mouse FAb or nonspecific IgG fragments into live HeLa cells at $<1\mu\text{M}$ IgG

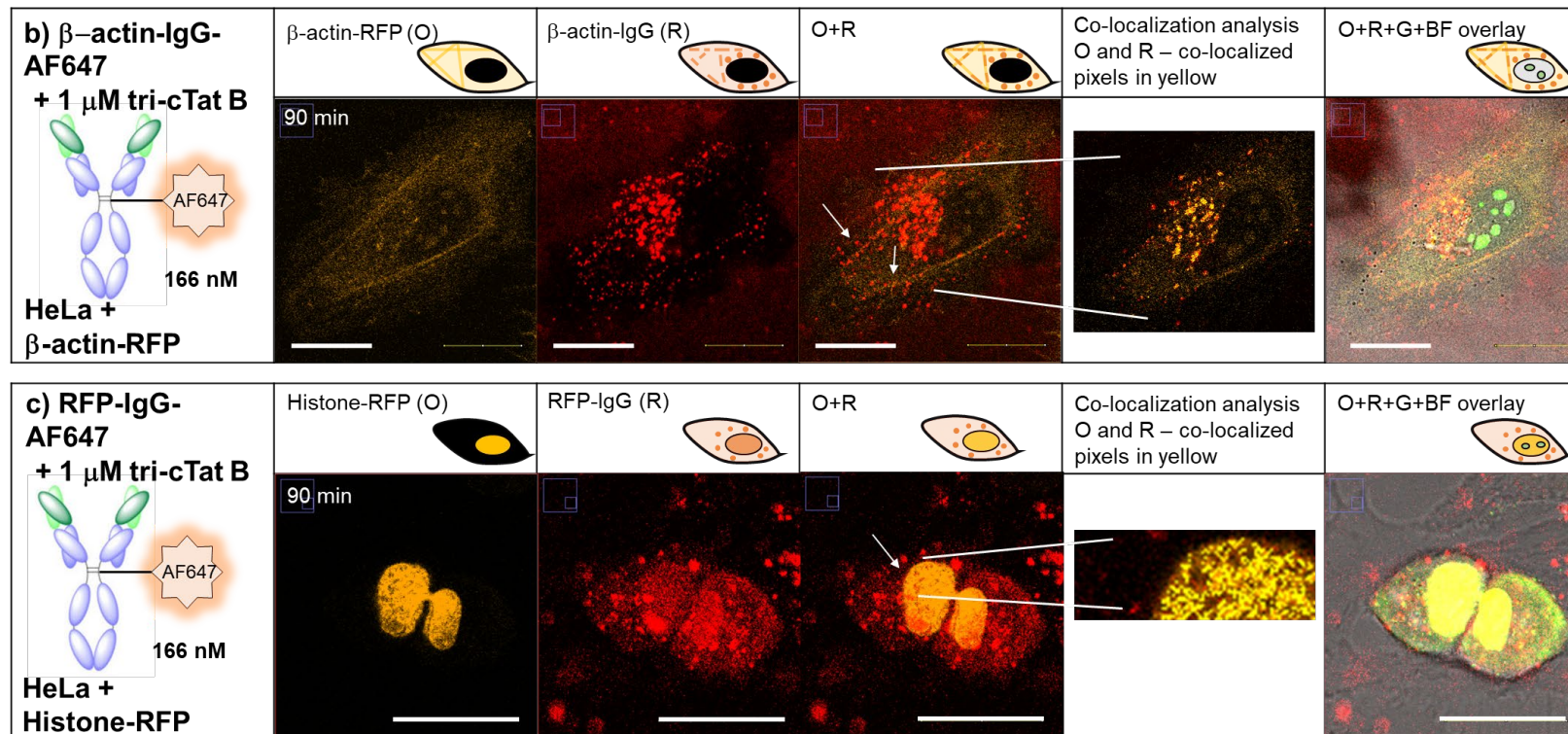


Live HeLa cells were incubated with tri-cTat B complexed with either FAb, or whole IgG and Alexa Fluor 647. Fluorescence from tCPP complexes is present in cytosol and nuclear compartments that would be impenetrable with naked FAb or IgGs.

Percentage of HeLa cells positive for homogenous cytosolic and nuclear cargo delivery, measured by AF647 fluorescence.

tri-cTat B DELIVERS FUNCTIONAL ANTIBODIES TO INTRACELLULAR TARGETS

tri-cTat B conjugated to functional antibodies demonstrated delivery into cytosolic and nuclear compartments in vitro

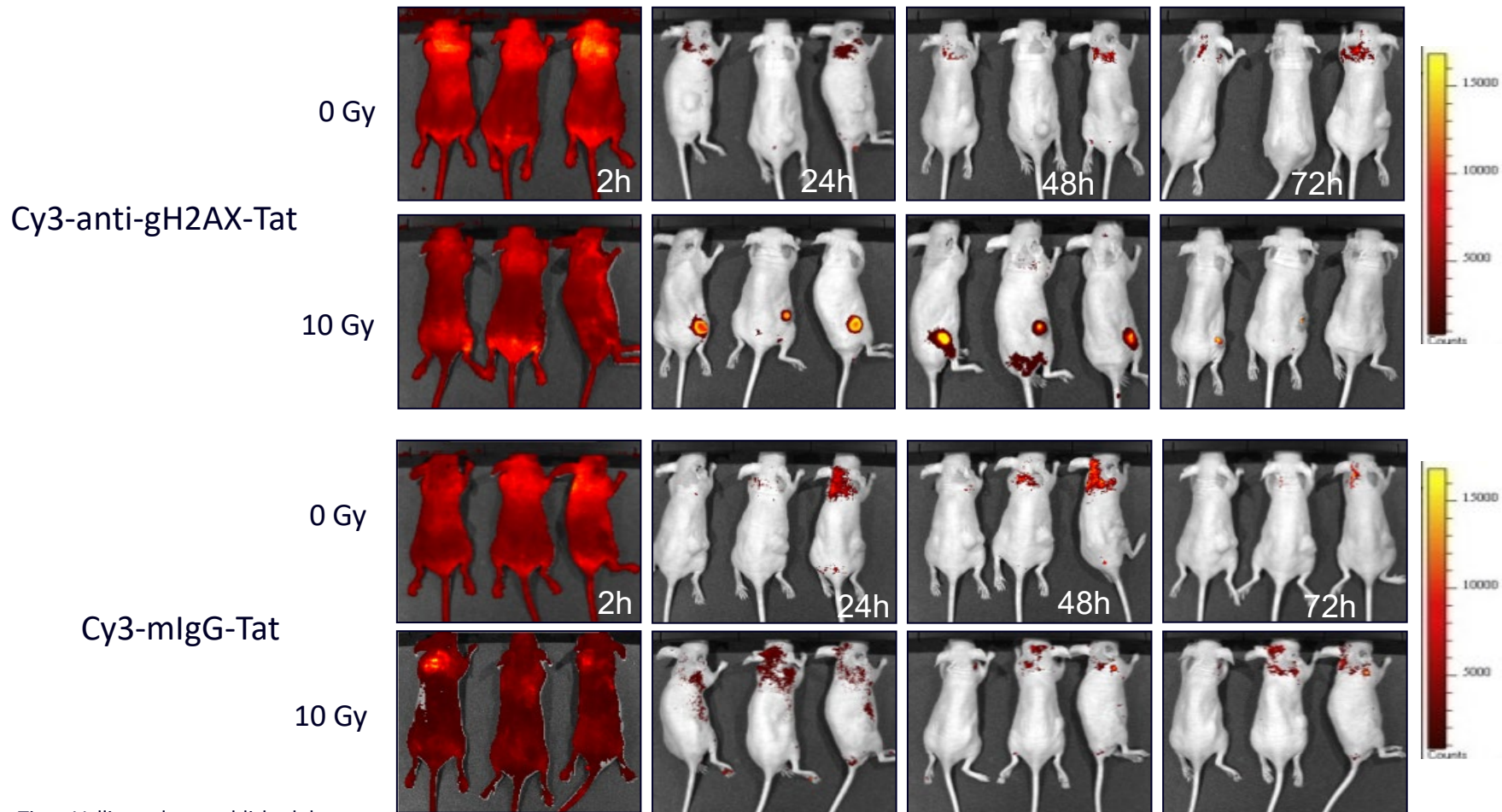


Live HeLa cells incubated with tCPP complexes conjugated to functional antibodies targeting β -actin (top) or histone (bottom) demonstrating targeting of cytosolic and nuclear epitopes, respectively.



SYSTEMICALLY ADMINISTERED IgG-tCPP CONJUGATES ACCUMULATE IN TUMOURS IN VIVO

Intra-tumoural accumulation of functional antitumour IgG-tCPPs in xenografts



Biodistribution time-course of cell-penetrating peptide Tat in MDA-MB-468 xenografts.

Specimens were sham-irradiated (0 Gy) or irradiated (10 Gy) post i.p. administration of anti- γ H2AX antibody linked to Tat (top row) or anti-mIgG (bottom row) antibody conjugated to Tat.

Source: Tietz, Vallis et al., unpublished data

PROJECT STATUS

Upcoming studies

- Optimising tCCP-FAb complexes to reach cytosolic targets (cytosolic IgGs already demonstrated)
- In vitro proof of concept of intracellular radiotherapeutic antibody delivery
- Delivery system optimisation (formulation and/or peptide biology)

Intellectual property

- Scaffold structure composition of matter and synthesis methodologies covered by PCT application PCT/GB2022/052093 filed in August 2022, and owned by Cancer Research Horizons
- tri-Tat A and tri-Tat B lead molecule chemistry (Further validation of tri-Tat A and tri-cTat B ongoing within priority year)
- Comprehensive data and know-how

SUMMARY

- Cell-penetrating peptide platform for intracellular delivery of functional macromolecules
- Novel chemical scaffold structure
- Proof of principle demonstrated
- Available for licensing and collaboration

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