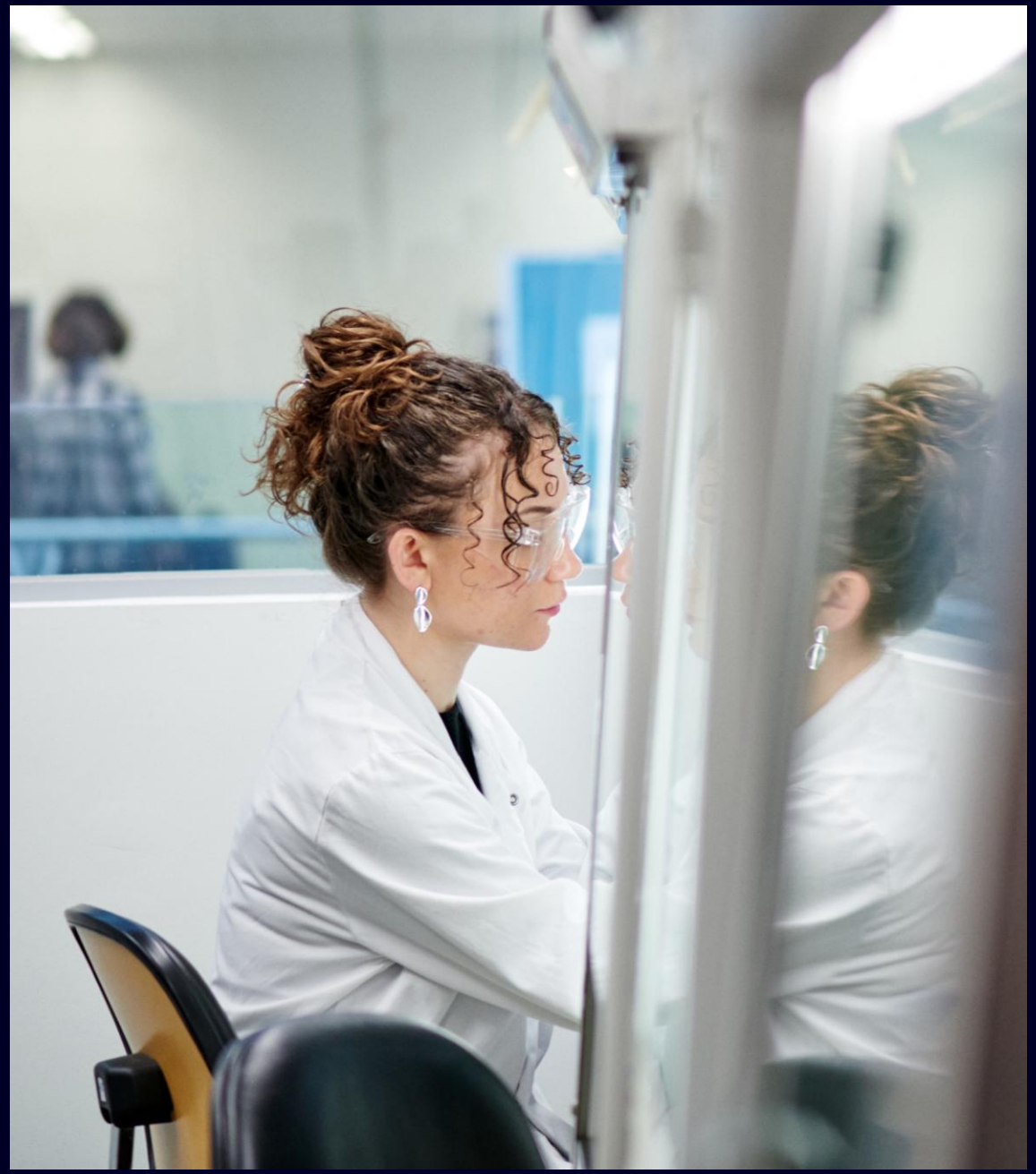


CANCER RESEARCH HORIZONS

MACQUARIE University

UNIVERSITY OF OXFORD

Trimeric cell-penetrating peptides for intracellular delivery

Non-confidential overview

FURTHER FASTER TOGETHER
We are beating cancer

Opportunity overview

CANCER RESEARCH HORIZONS



Platform technology

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



Broad tx/dx applications

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

Drug discovery R&D

- Use in target validation and MoA studies
- Use in screening peptides/macromolecules for function in cells



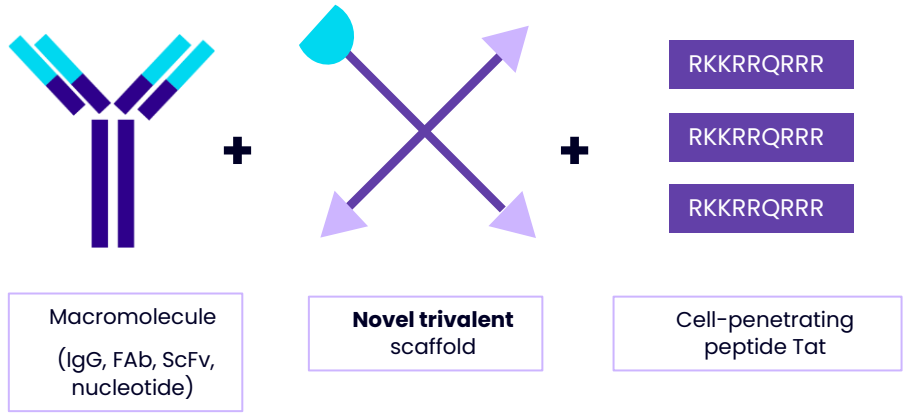
Partnering

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

Intracellular delivery

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

- This platform utilises the cell-penetrating peptide (CPP) HIV-tat (Tat) to provide a straightforward method for intracellular drug delivery
    - The cargo may be macromolecule such as IgG antibody or antibody fragment or oligonucleotide
- Previous CPP-based approaches have suffered from poor efficiency and required very high (>20uM) effective Ab concentrations in vitro
- The inventors have developed trimeric cell-penetrating peptides (tCPPs) that are effective at low concentrations and can flexibly bind varied payloads.
- **These tCPPs do not suffer from endosomal entrapment, unlike other CPPs**

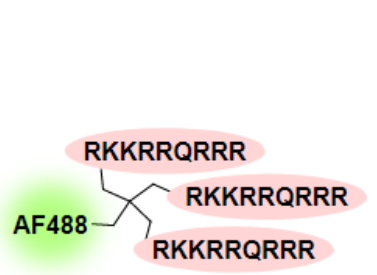


Conformation influences intracellular delivery

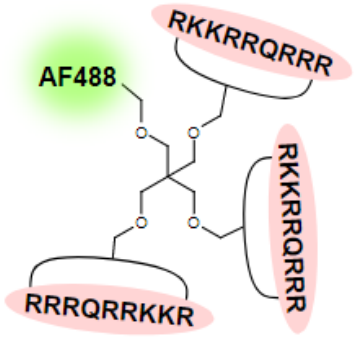
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Novel tCPPs more effectively penetrate cell membranes than monomeric CPPs

- 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-cTatB**) were taken forward for further development



Tri-Tat A

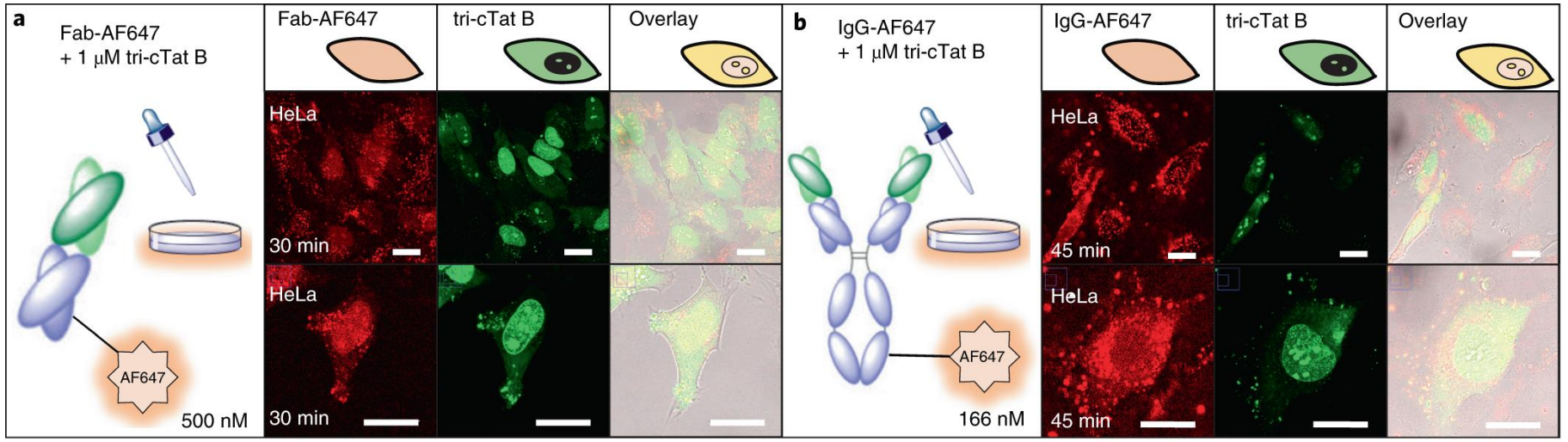


Tri-cTat B

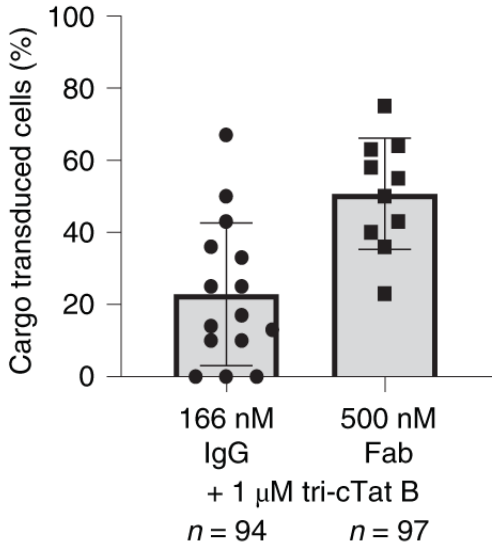
Intracellular delivery of cargoes - tri-cTat B

CANCER RESEARCH HORIZONS

tri-cTatB effectively translocates non-specific mouse Fab or nonspecific IgG fragments into live HeLA cells at <1μM IgG



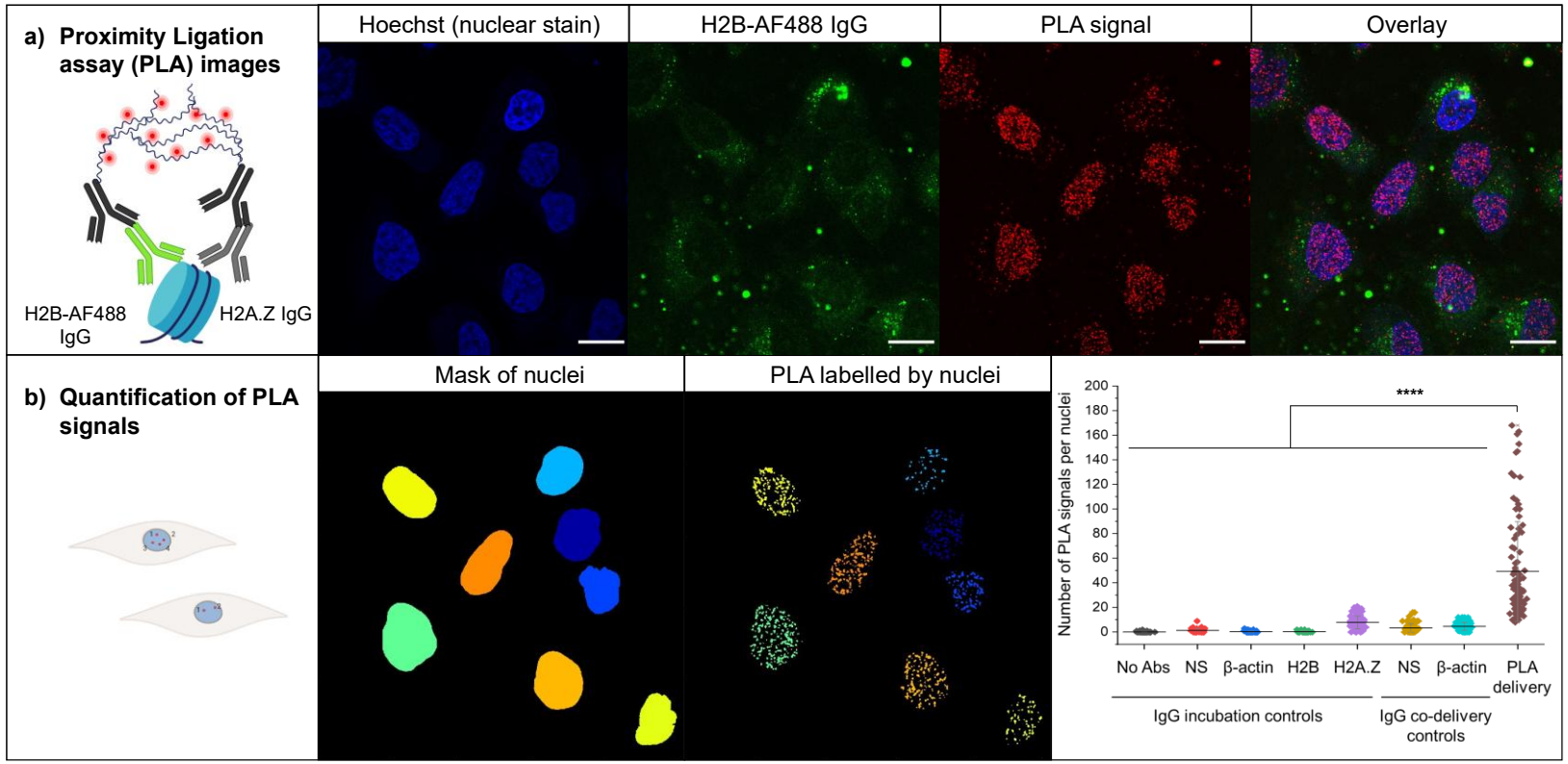
Live HeLa cells were incubated with tri-cTat B complexed with either FAb, or whole IgG and Alexa Fluor 647. Fluorescence from tCPP complexes is present in cytosol and nuclear compartments that would be impenetrable with naked Fab of IgG



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

Functional antibody delivery by tri-cTat B

Tri-cTat B co-delivery of functional antibodies demonstrate target engagement with Histone complexes in the nucleus



HeLa cells incubated with tCPP and functional antibodies targeting histone (H2B) demonstrating successful and specific target engagement in the nucleus by proximity ligation assay (PLA).

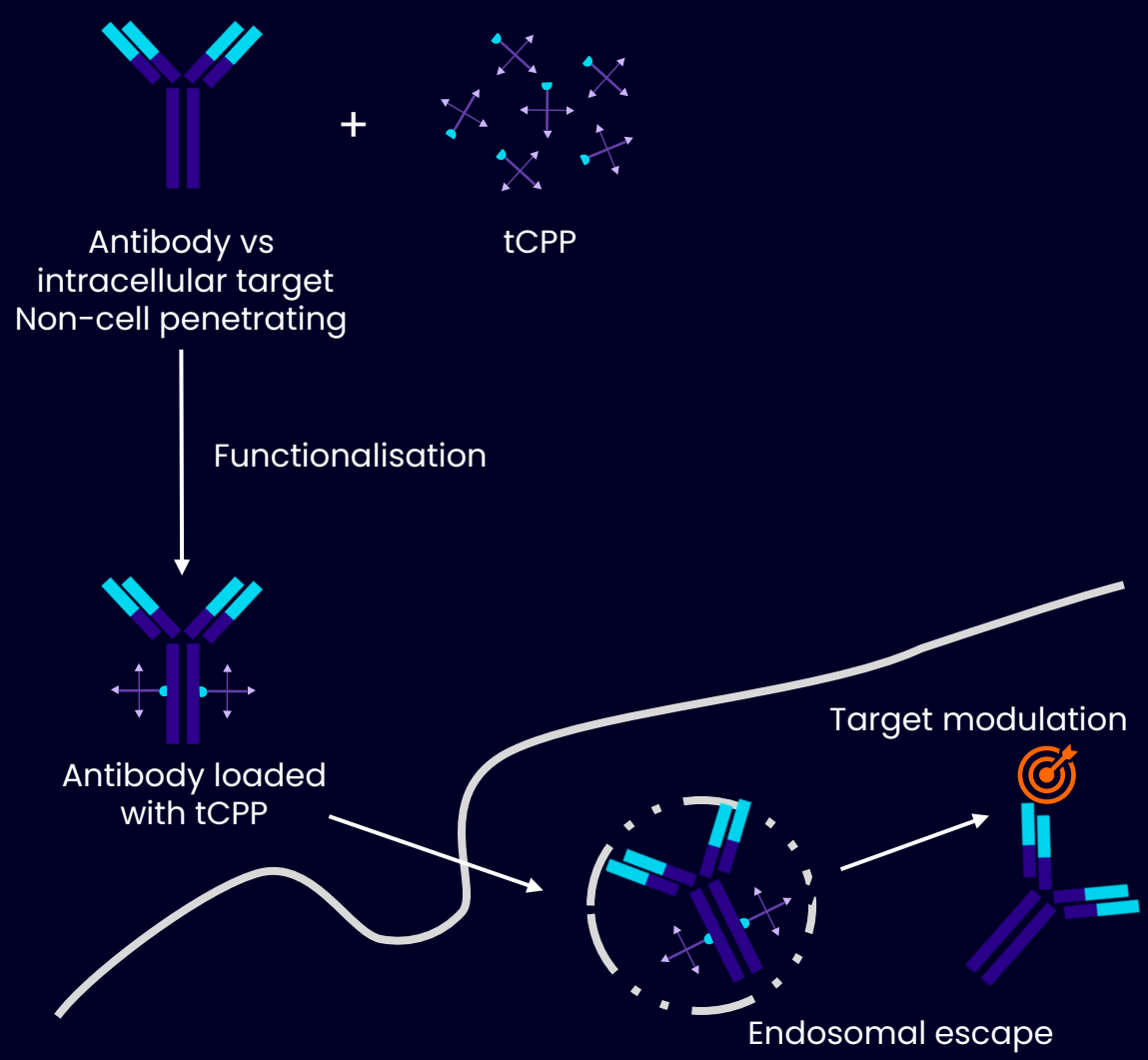
Tietz et al. *(2022), Nature Chemistry* **14**, 284–293

Data available under CDA

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Recent developments and ongoing work

- Two conjugation chemistries undergoing further optimisation
    - Rapid benchtop kit for R&D and assay development
    - Industry standard conjugation for development of therapeutics assets
- Ongoing program of work to further validate functional delivery of covalently linked cargoes in vivo
    - Development of biologics to treat cancer using tCPP
    - Development of biologics to treat neurodegenerative diseases using tCPP
    - Development of nucleic acid therapeutics using tCPP
    - In vivo biodistribution & stability of tCPPs



Summary

CANCER RESEARCH HORIZONS

Overview

- Novel platform technology for intracellular delivery of biological molecules including IgGs, ScFvs, and oligonucleotides
- Trimeric HIV-Tat based peptides for enhanced delivery
- Scaffold core chemistry claimed by composition of matter patent applications

Competition and differentiation

- Dramatically more efficient compared to previous approaches for intracellular delivery of macromolecules, thus decreasing effective concentrations needed to deliver a clinically relevant dose into the cell
- In vitro proof of concept achieved with IgGs, functional antibodies and DARPINs
- Systemic dosing of cell-penetrating peptides with tumour targeting monoclonal antibody cargoes leads to effective accumulation of these antibodies at tumour sites in vivo

Use cases



Mechanistic and target validation studies

- Use in R&D for probing biology of a target of interest
- Understanding functional impact of inhibition of given target by antibodies
- Labelling intracellular targets
- Mix and go: deliver by association



Drug discovery screening cascades

- Screening antibodies and/or peptide libraries for functional activity against intracellular targets
- Use to triage functional macromolecules prior to optimizing intrinsic cell penetration
- Mix and go, or creation of covalent libraries



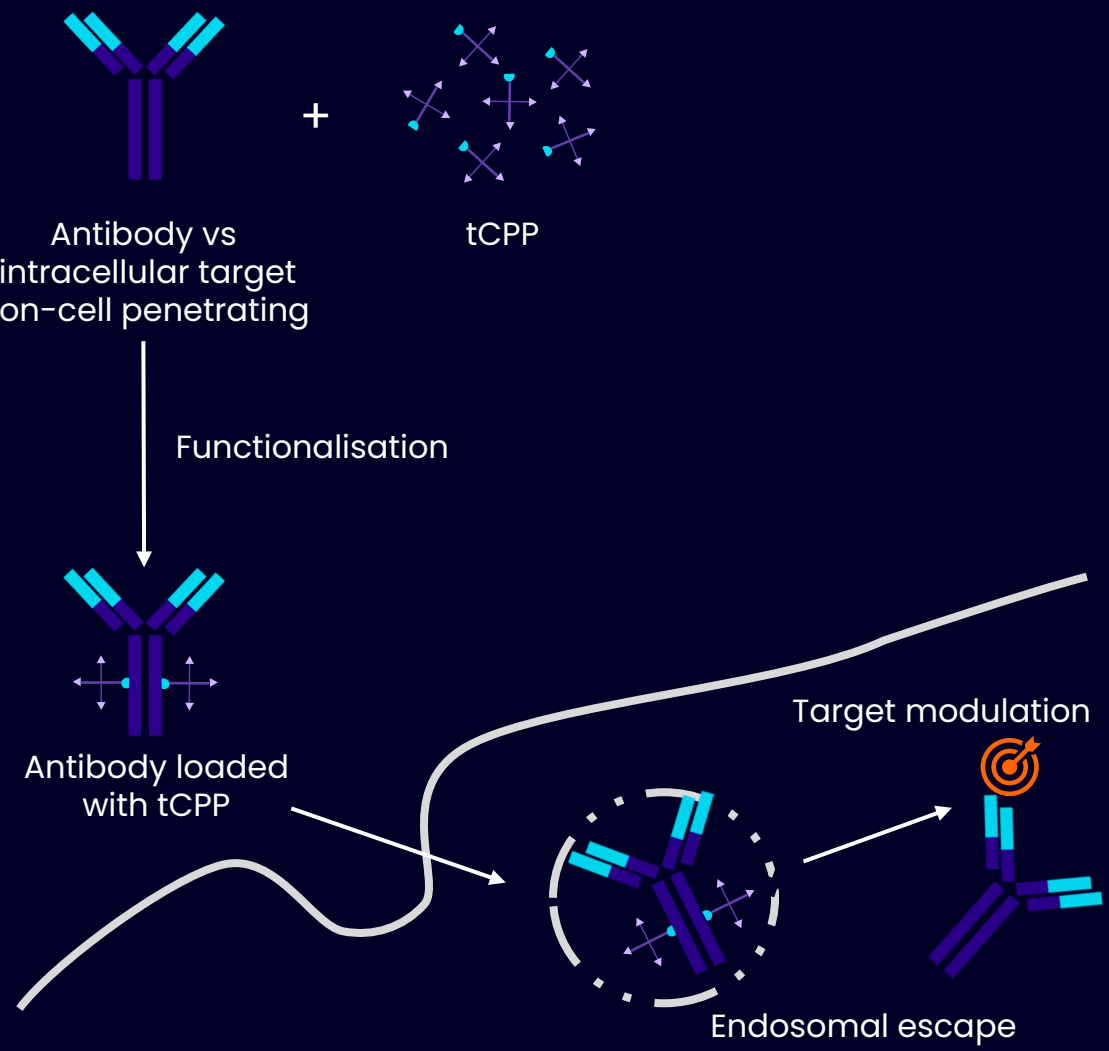
Intracellular delivery of therapeutics

- Covalent linkage to therapeutic antibodies against intracellular targets
- Opening door to therapeutic modulation of targets previously druggable only by small molecules

Use cases

Development of platform for antibodies vs intracellular targets

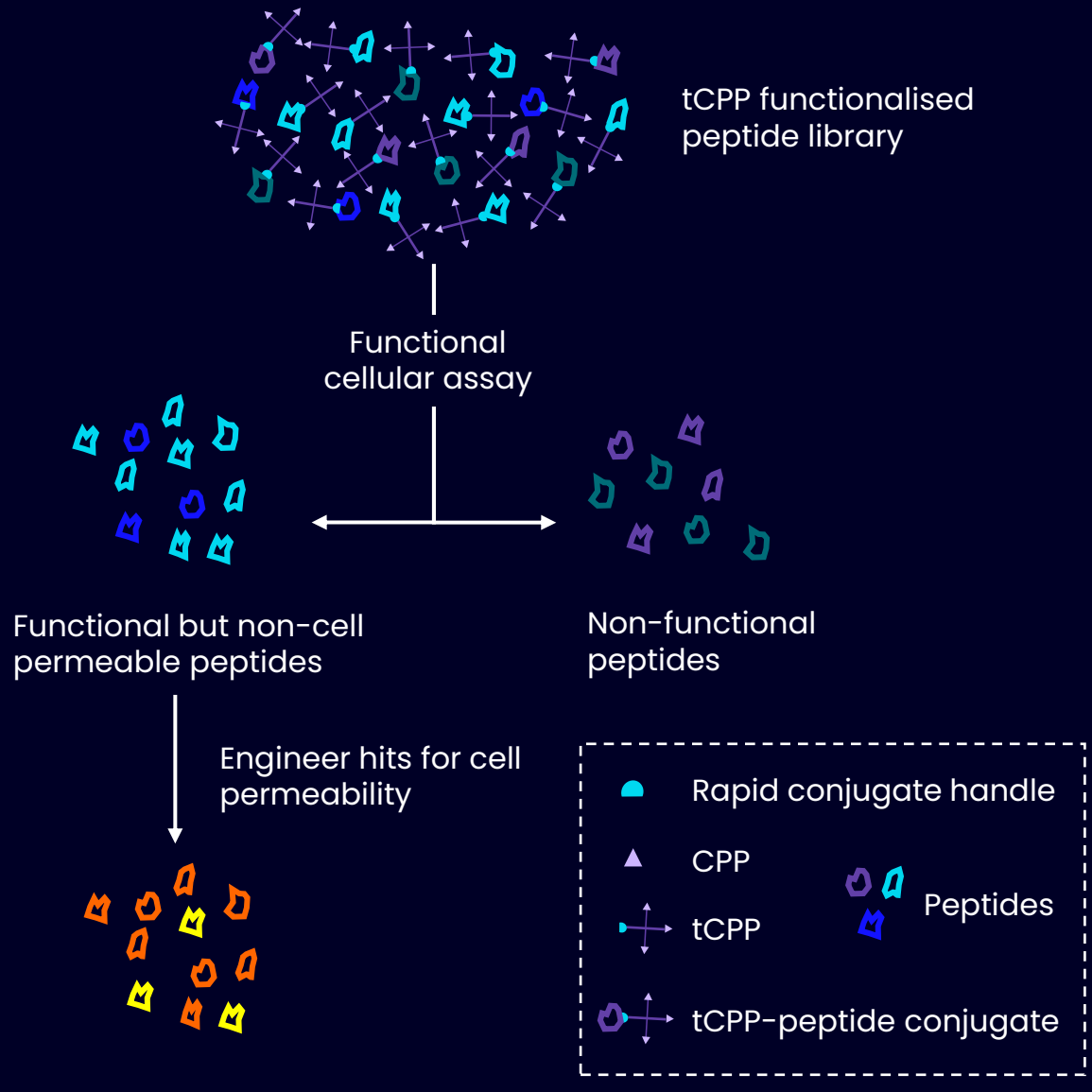
- We are developing specific chemistry to enable conjugation of tCPP to antibody modalities
- Proof of concept with antibody in R&D portfolio
- Develop together platform for creating cell-penetrating antibodies



Use cases

1) Developing a screening assay for peptides targeting intracellular targets

- Our tCPP technology offers an opportunity to screen libraries of non-cell permeable peptides for functional activity against intracellular peptides
- Create a high-throughput library to screen for functional peptides on a target-by target basis
    - Rapid benchtop conjugation protocol with no need for purification
- Select functional peptides and engineer these for intrinsic cell penetration



Thank you

For further information, please contact:

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