P2. 239 High-Throughput Analysis of Peptide Accumulation in Mammalian Cells

Sobika Bhandari, George Ongwae, Marcos M. Pires*

Department of Chemistry, University of Virginia, Charlottesville, Virginia, United States

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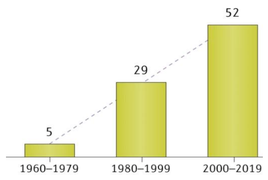
Background

Peptides as Potential Drugs

- Since the first commercial use of insulin in 1923, proteins and peptides have been emerged as potentially effective therapeutic scaffolds[1]
- However, a molecule's effectiveness as a therapeutic target is largely determined by its ability to reach its intended target



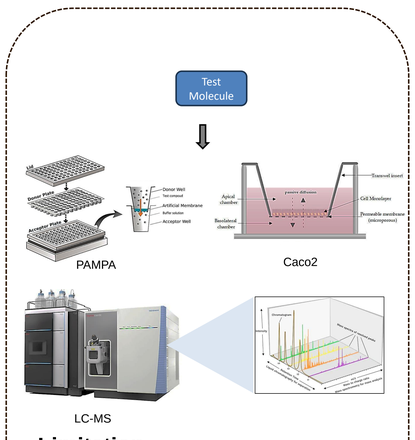
Peptide drug approvals[2]

Major Challenges

- Cell membrane acts as a **major barrier** for intracellular transport of biologically active molecules
- Given the **larger size**, peptides face difficulty entering the cytosol successfully instead, their delivery relies on endocytic pathway

How is Permeability Being Measured?

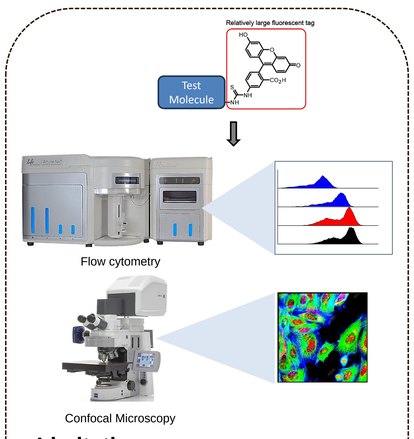
Tag-free assay



Limitation

Unable to distinguish sub-cellular localization[3]

Tag-based assay

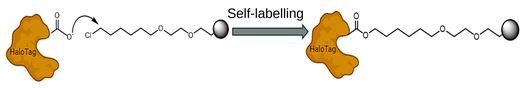


Limitation

Structural modification of molecule due to large fluorescent tag

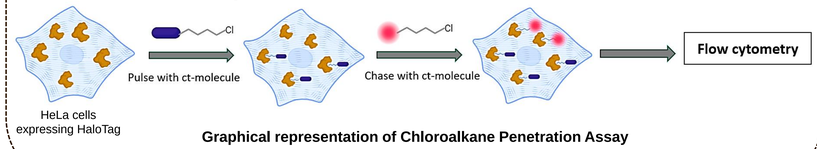
Chloroalkane Penetration Assay (CAPA)[4]

- Relies on genetically encoded stably expressed "**HaloTag**" GFP-fusion protein



Self-labelling of HaloTag protein to HaloTag ligands

- Pulse-chase assay

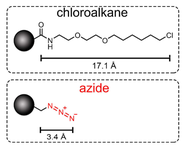


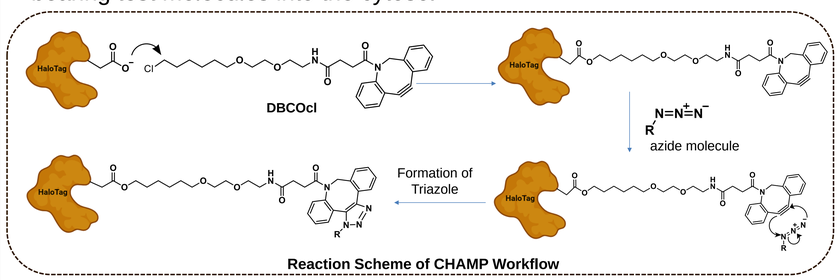
Graphical representation of Chloroalkane Penetration Assay

Limitation : Presence of long chloroalkane tag

Approach and Design

- Use a small azide tag,[5] comparatively less disruptive to molecules; named **CHAMP** (**Ch**loroalkane **A**zide-tagged **M**embrane **P**ermeability) Assay
- **DBCOcl** first binds irreversibly with HaloTag protein followed by SPAAC
- HaloTag-bound DBCO helps measure access of azide-bearing test molecules into the cytosol





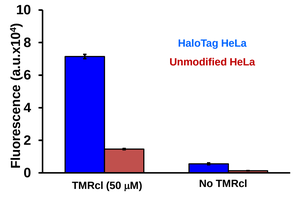
Reaction Scheme of CHAMP Workflow

CHAMP Assay Workflow

Results

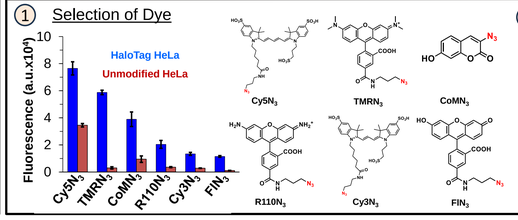
Expression of HaloTag in HeLa cells

- Unmodified HeLa cells not expressing HaloTag were used as a negative control
- Significant differences in signal confirmed chloroalkane-tagged fluorophore in the cytoplasm of mammalian cells is captured by the cells that express HaloTag protein

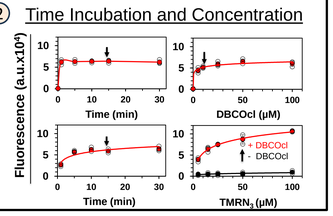


Parameter Optimization

① Selection of Dye



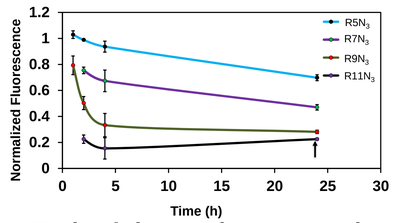
② Time Incubation and Concentration

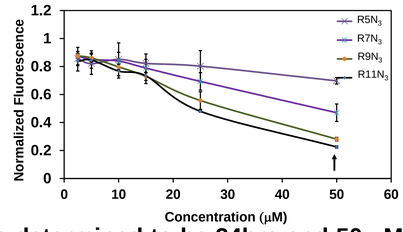


Results

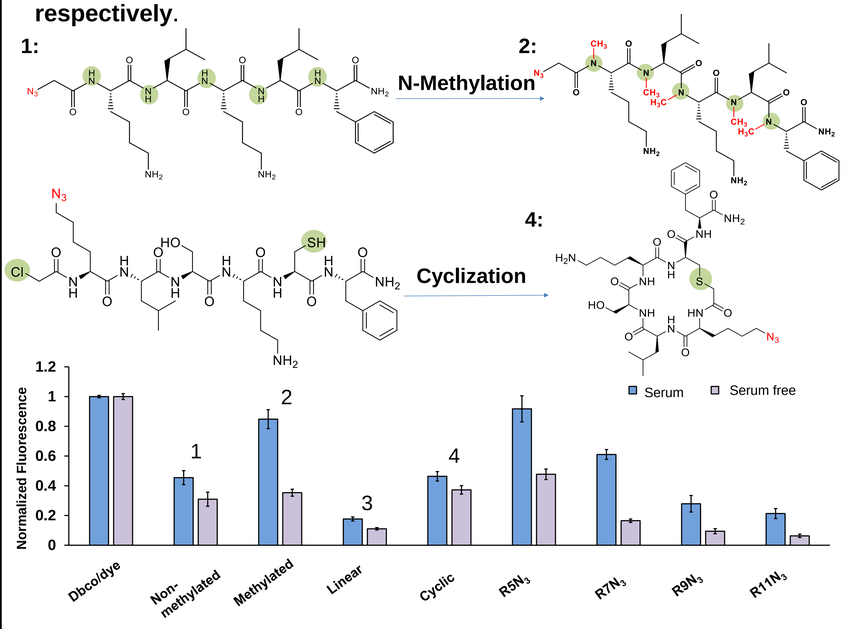
Assay Development with Peptides

- Peptides were synthesized using solid phase peptide synthesis (SPPS)
- Permeability of polyarginine peptides were analyzed to probe optimal assay conditions





- **Optimal time and concentration was determined to be 24hrs and 50 μM respectively.**



Conclusions

- Allows for comparative accumulation of azide-tagged peptides inside the cytosol of mammalian cells
- Significant decrease in signal corresponds to increase in accumulation of peptides in serum free media.
- Effective and high-throughput method to determine the cytosolic accumulation of peptides in mammalian cells.

References and Acknowledgements

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4. Kritzer J. A. et al *Science Direct; Elsevier*, **2024**, 81
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Pires research lab

NIH National Institutes of Health