Efficient Cell Delivery and Lipid-Specific Endosomal Escape by Supercharged Peptides

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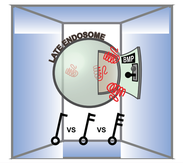
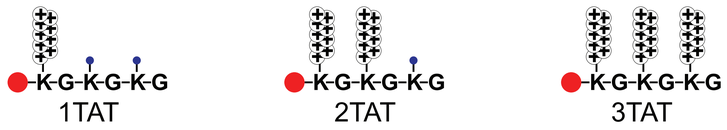


1 Research Background:

- Supercharged cell penetrating agents, such as the peptide TAT, have been employed as delivery tools for biologically-active macromolecules
- TAT and other delivery agents have proven greatly inefficient as a means of cargo delivery into the cytosolic space of cells (cargo has only been successfully delivered to ≤1% of total cells)
- This issue of inefficiency can largely be attributed to endosomal entrapment of delivery agents
- Studies have shown that supercharged molecules, or molecules with ≥ +0.75 charge / kDa, exhibit greater cell penetration activity by circumventing this issue
- The purpose of this study is to characterize the affect on cell penetration by increasing charge density as well as gain greater mechanistic insight into the process of endosomal escape.

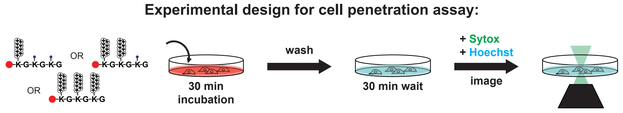
2 Synthetic design: branched TAT copies on a labeled, peptide scaffold

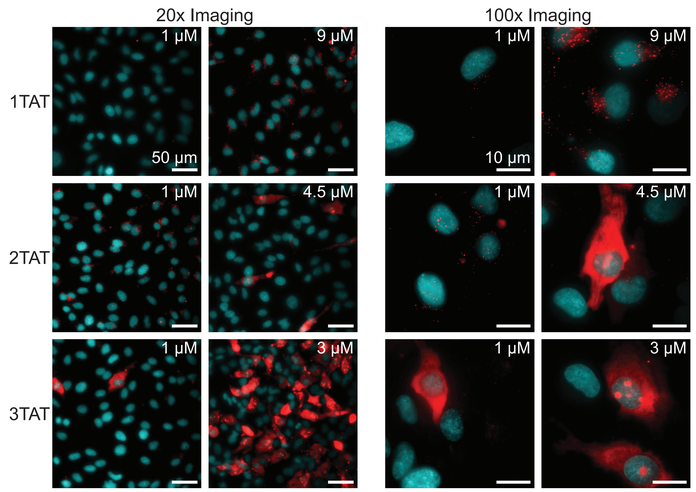


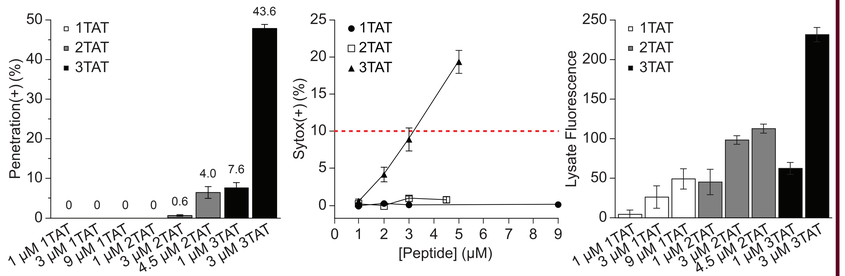
Peptide:	MW (Da):	Charge:	Charge / kDa:
1TAT	2448	9+	3.7
2TAT	3784	18+	4.8
3TAT	5122	27+	5.3

= TAMRA
= acetyl
= TAT
TAT = NH_3^+-GRKKRRQRRR

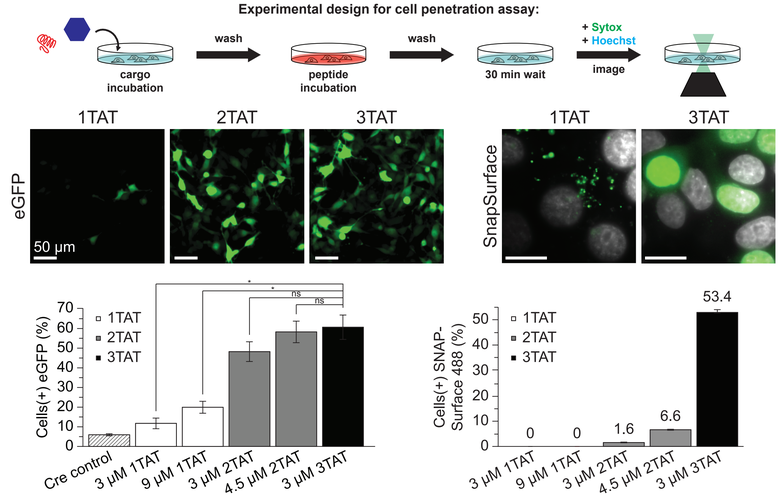
3 Cell penetration efficiency increases as a function of molecular charge density



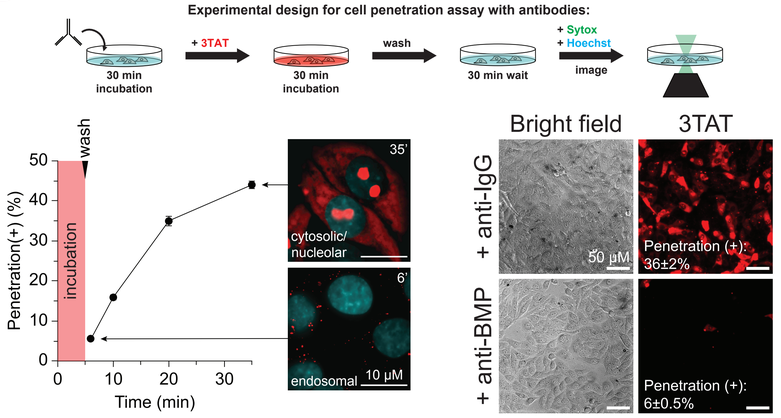




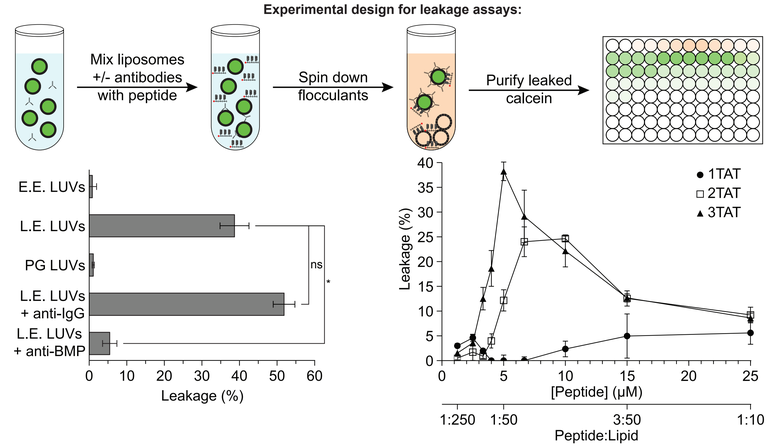
4 Delivery efficiency of macromolecular cargos increases as a function of charge density



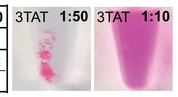
5 Supercharged peptides penetrate cells via endocytosis



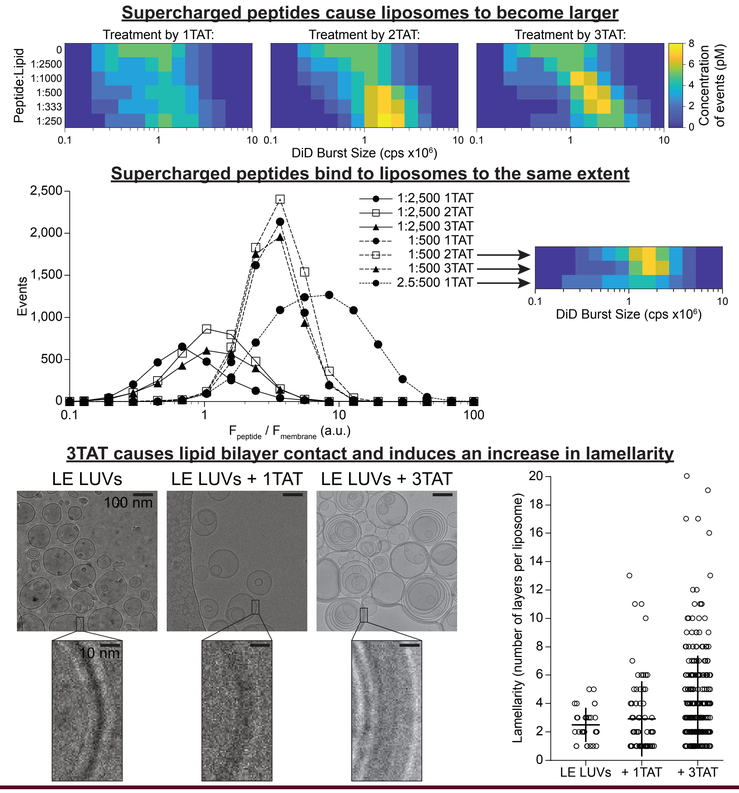
6 Supercharged peptides cause liposomes mimicking late endosomes to leak lumenal content



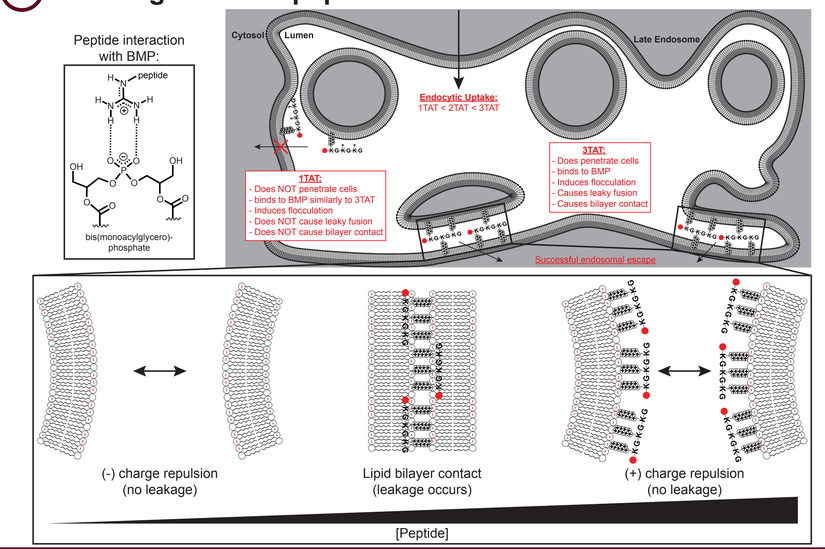
P:L	1:250			1:50			1:10			3:10	1.5:10
Peptide	1TAT	2TAT	3TAT	1TAT	2TAT	3TAT	1TAT	2TAT	3TAT	1TAT	2TAT
Zeta potential (mV)	-53.0	-50.4	-51.3	N/A	N/A	N/A	N/A	N/A	+39.7	+21.7	+35.2
Flocculation	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No



7 Supercharged peptides induce flocculation, increase vesicle lamellarity and cause lipid bilayer contact



8 Working model of peptide interaction with late endosomes



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10 References

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* These authors contributed equally to this work.
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