



santa`s chimney into the cell: intracellular delivery of proteins

technical journal club

17.12.2019

merve avar

introduction – why deliver proteins at all?

importance

- can be utilized as highly specific targets to interfere with pathways
- no genetic editing required
- lower side effects

challenges

- membrane impermeability
- high molecular weight (to small molecules)
- hydrophilicity

→ due to its potential, it is imperative to develop methods for intracellular protein delivery

leading the way in

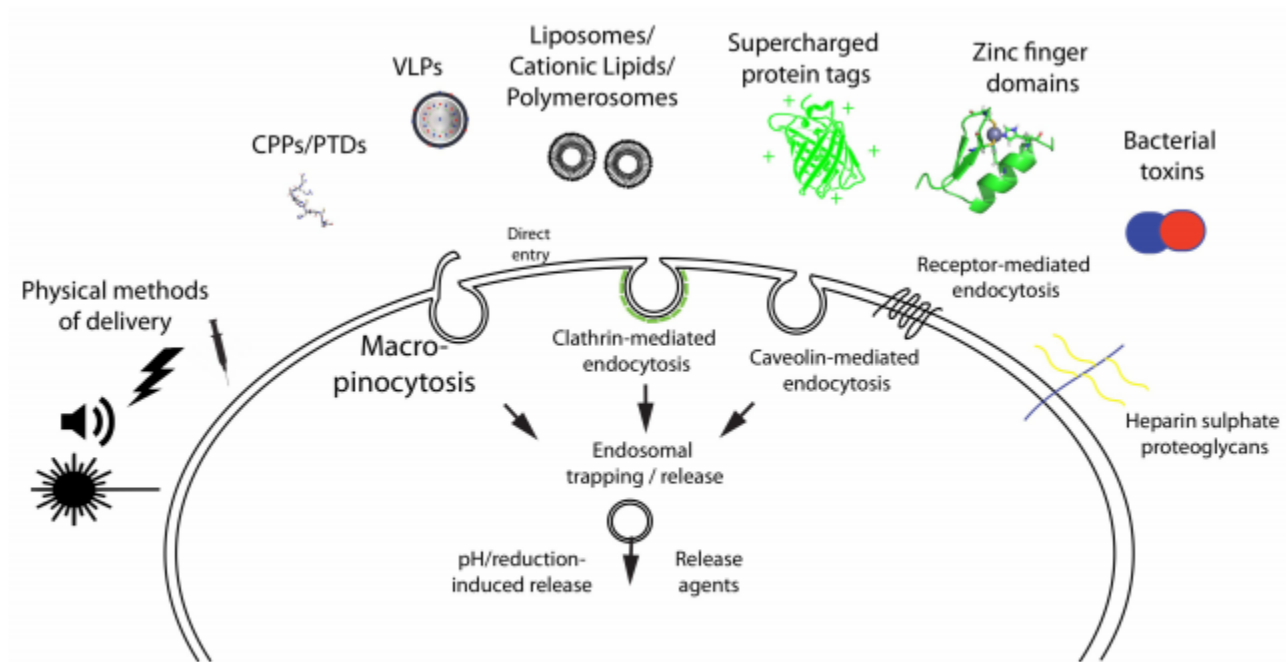


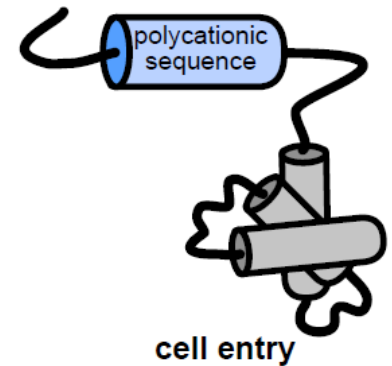
Figure 2. Methods for the direct delivery of proteins into cells. A variety of methods have been described for delivering functional protein to the cell interior and are illustrated along with potential mechanisms of uptake and endosomal release. CPP, cell-penetrating peptide; PTD, protein transduction domain; VLP, virus-like particle.

Miersch et. al., F1000

cargo encapsulation -> cellular internalization -> escape from endosomes -> cytosolic cargo release

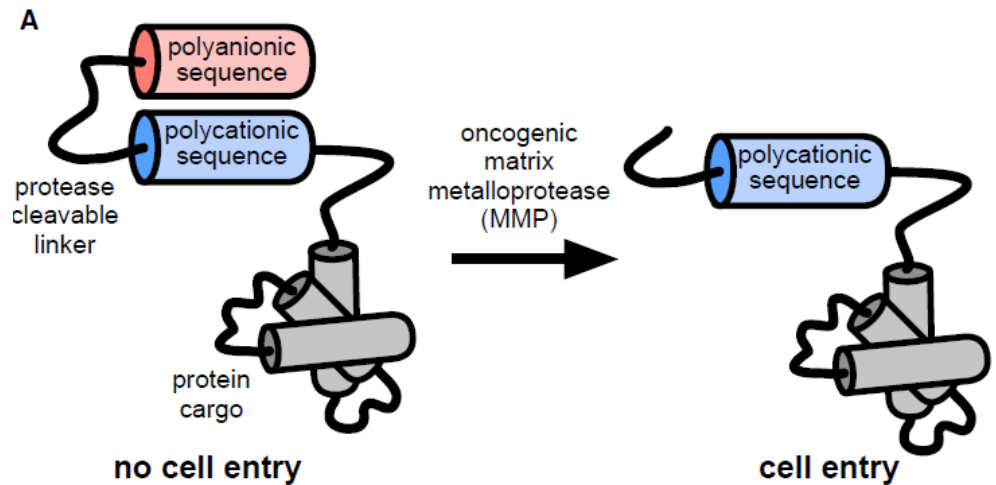
widely reported protein delivery methods

- cell-penetrating peptides
- protein resurfacing
- endosomolytic peptides
- toxin derived assemblies
- physical delivery methods
- gold particle complexes
- nanocomplexes



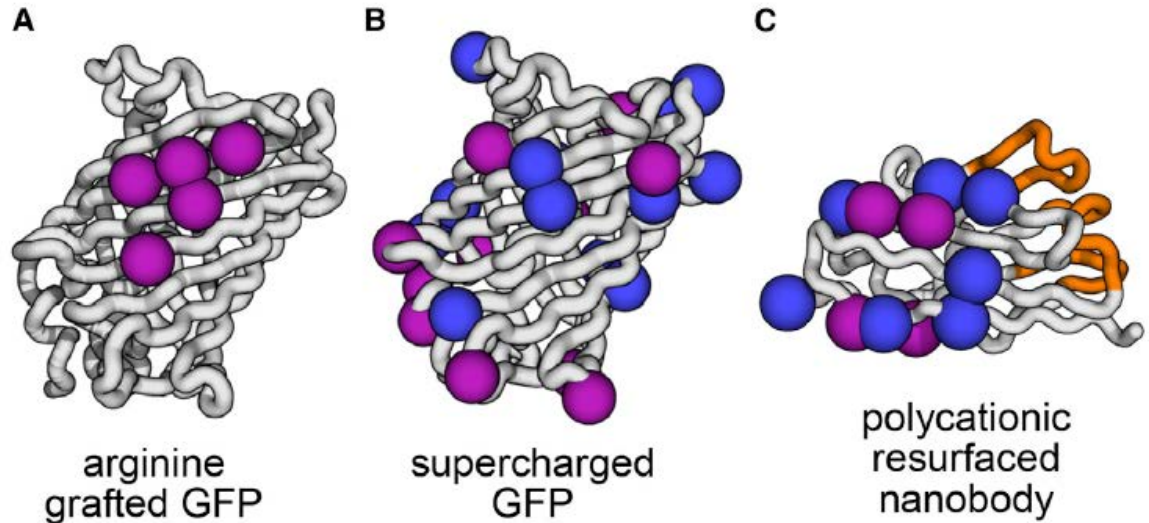
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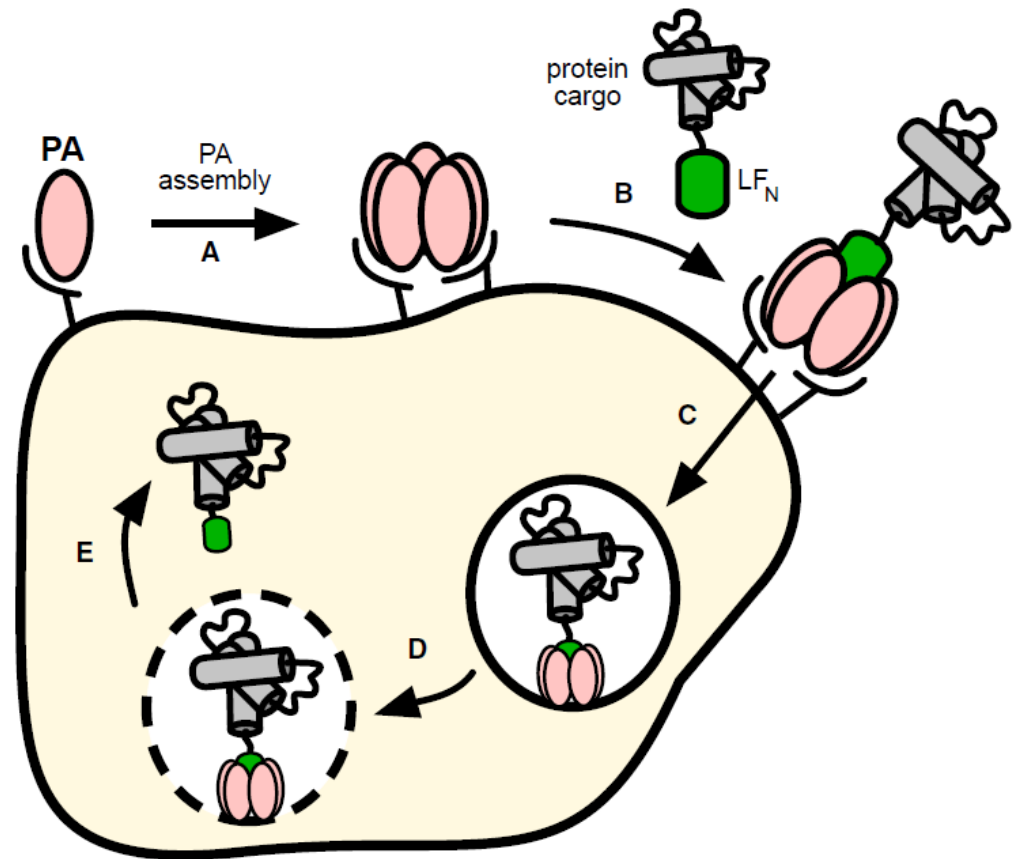
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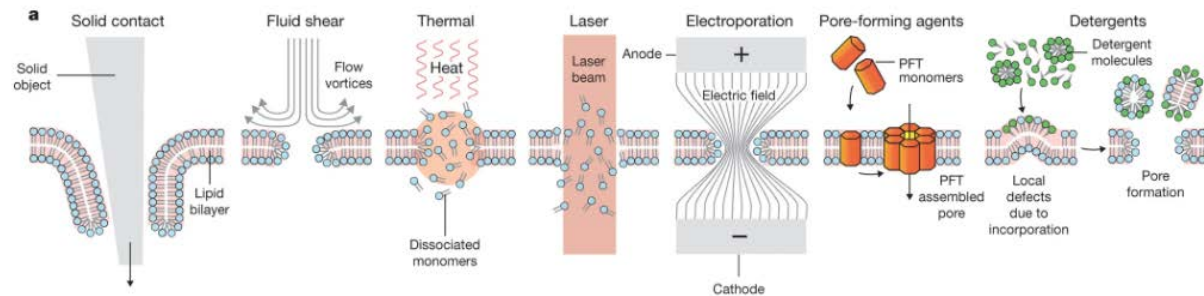
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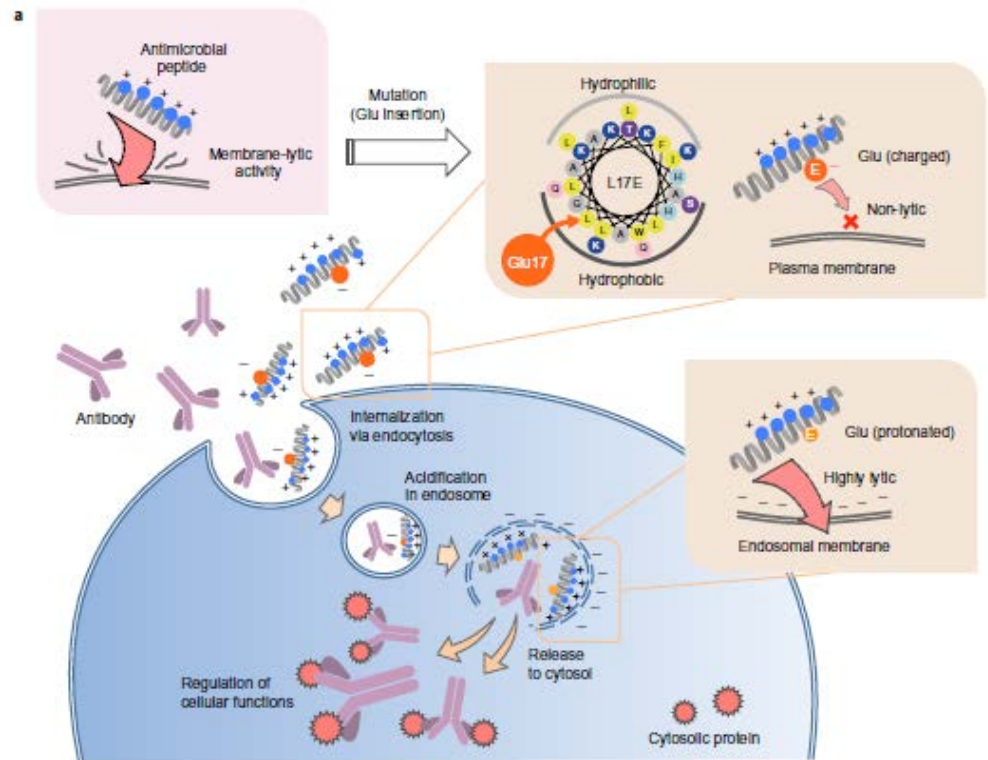
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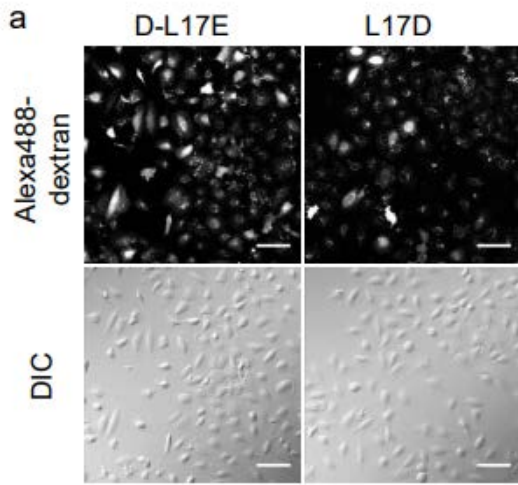
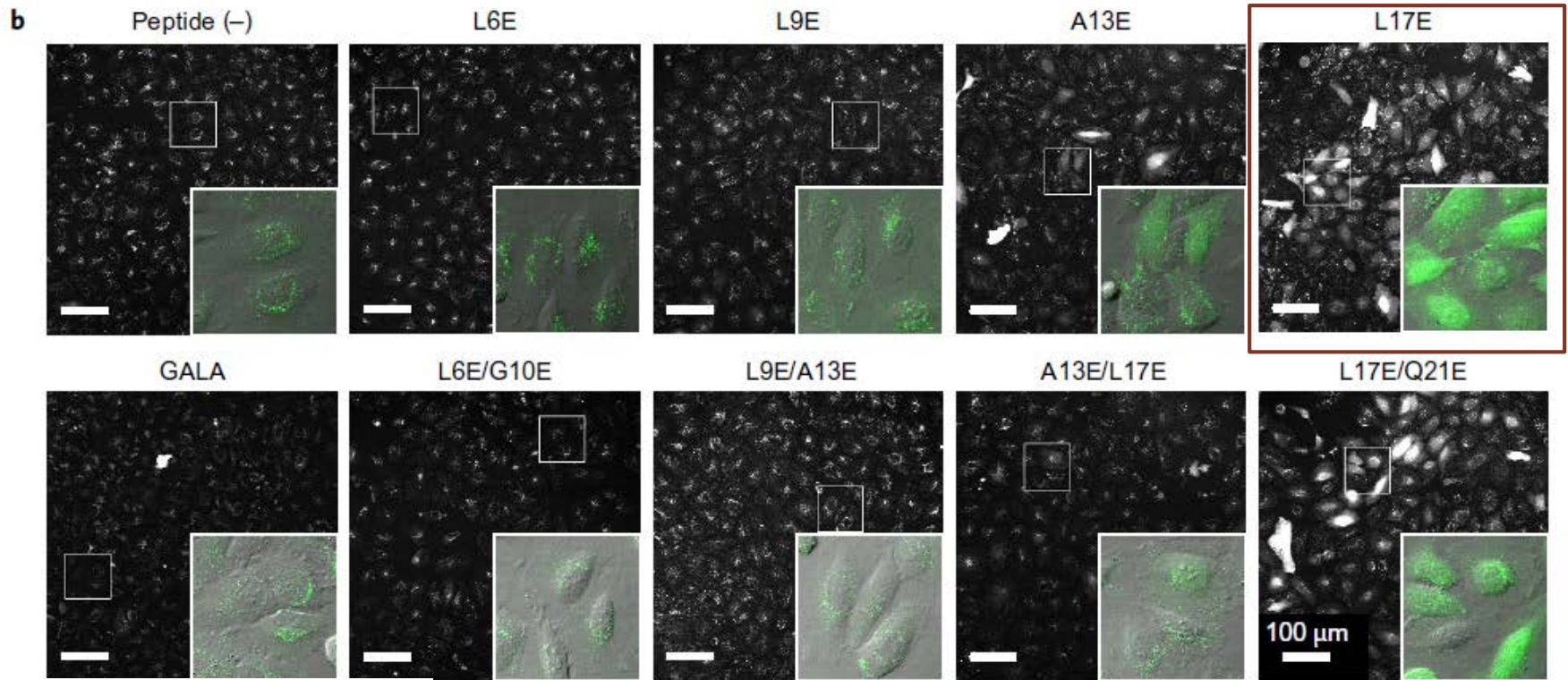
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Cytosolic antibody delivery by lipid-sensitive endosomolytic peptide

Misao Akishiba¹, Toshihide Takeuchi¹, Yoshimasa Kawaguchi¹, Kentarou Sakamoto¹, Hao-Hsin Yu¹, Ikuhiko Nakase^{1,2}, Tomoka Takatani-Nakase³, Fatemeh Madani⁴, Astrid Gräslund⁴ and Shiroh Futaki^{1*}

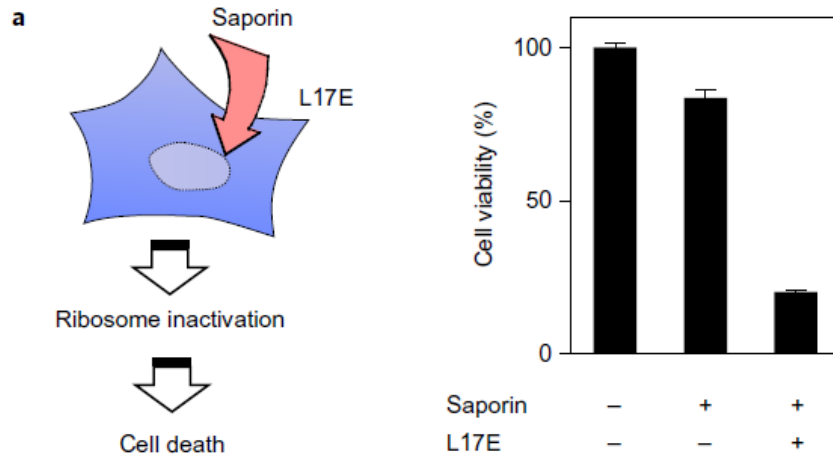
endosomolytic peptides



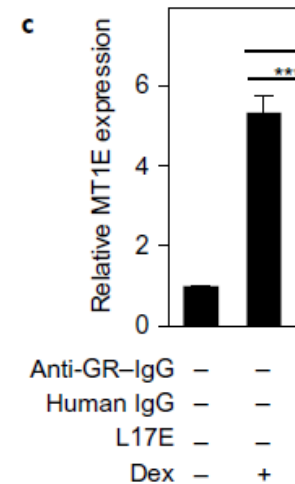
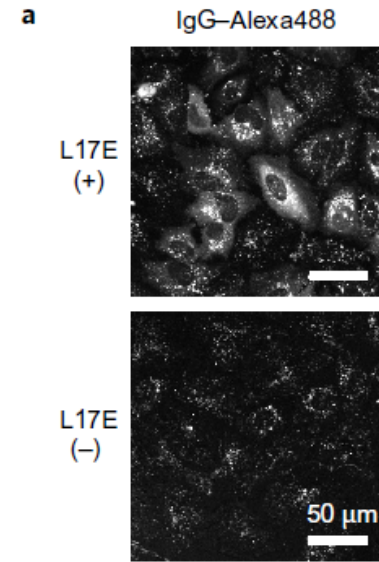
→ L17E best working peptide

→ does not require a transporter or a receptor

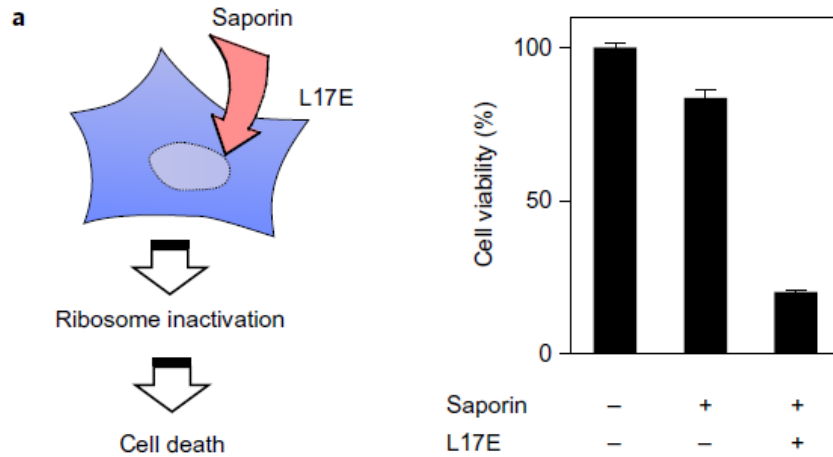
endosomolytic peptides- functionality of cargo



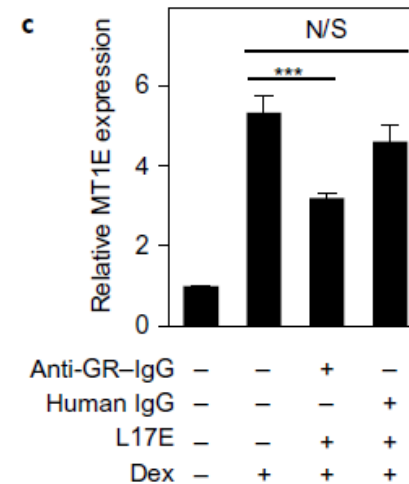
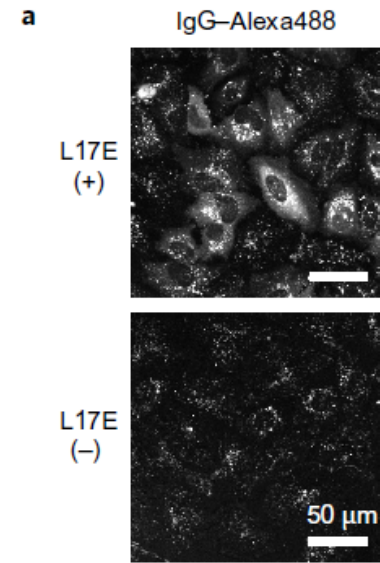
→ saporin induces cell death upon administration with L17E within 2 hours



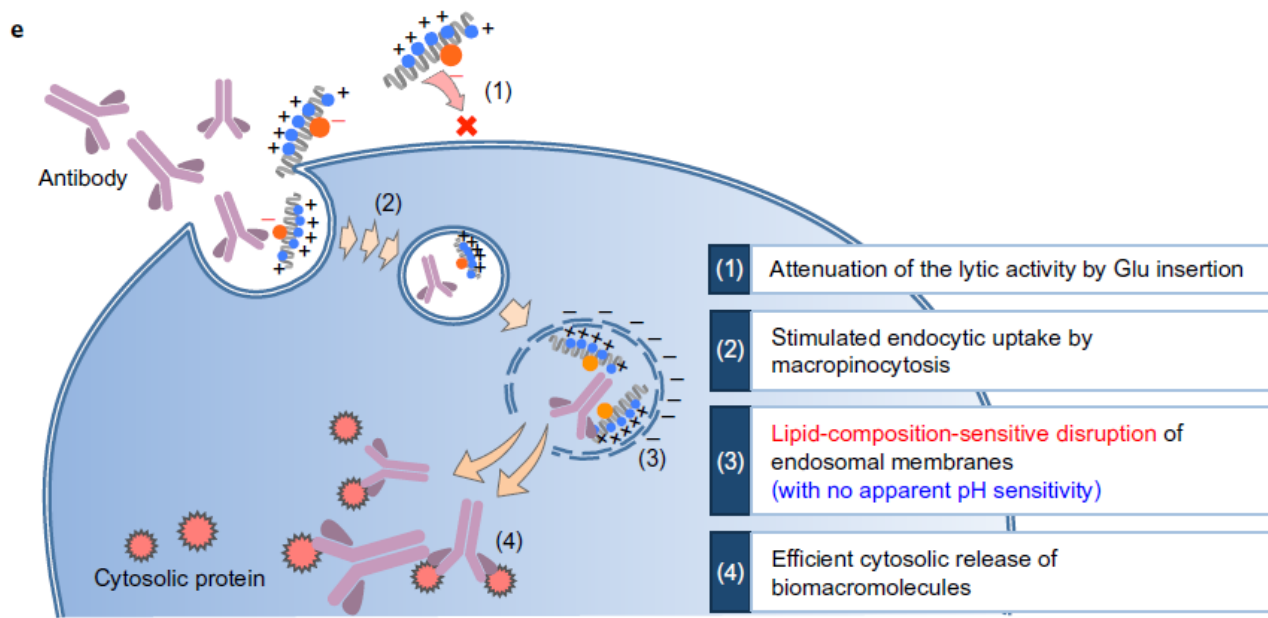
endosomolytic peptides- functionality of cargo



→ saporin induces cell death upon administration with L17E within 2 hours



endosomolytic peptides- pros and cons



advantages

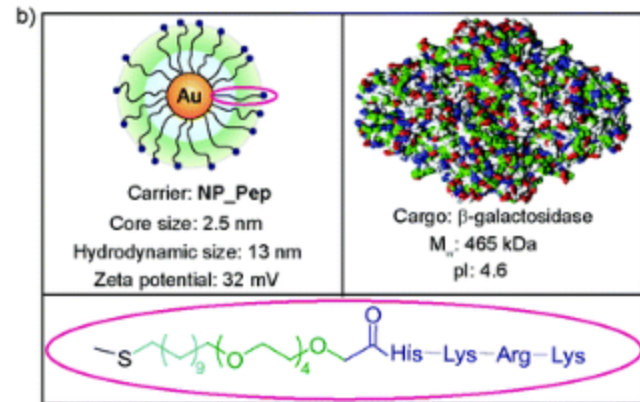
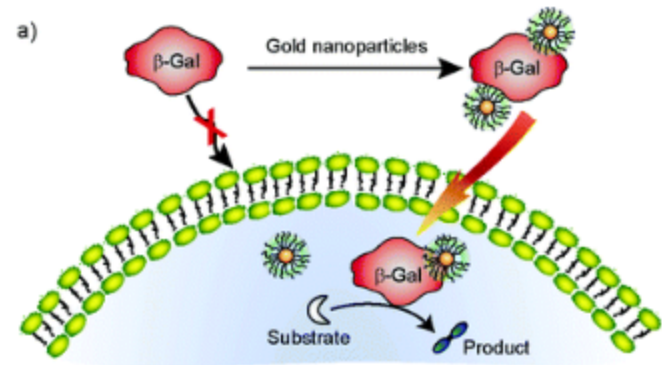
- easy to use
- does not require alterations to cargo
- reports on functionality of the delivered product
- mechanism proposed

shortcomings

- potential immuno response?
- types of cells not possible
- paper did not evaluate versatility of the system

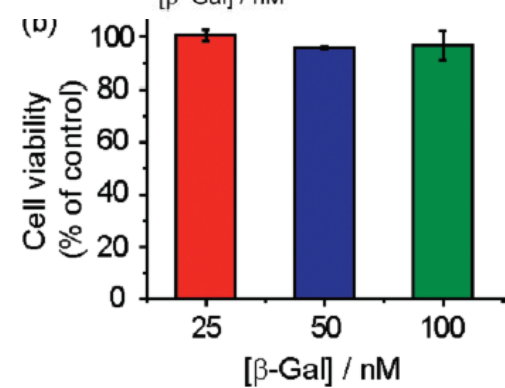
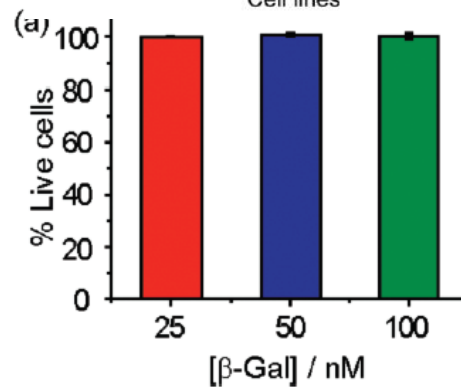
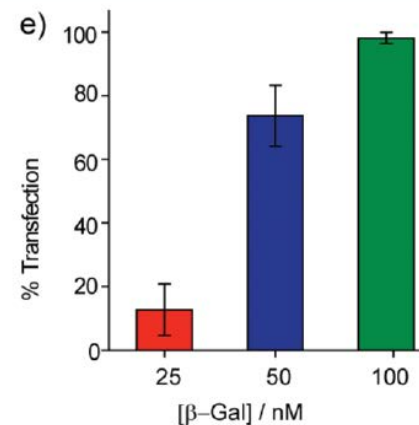
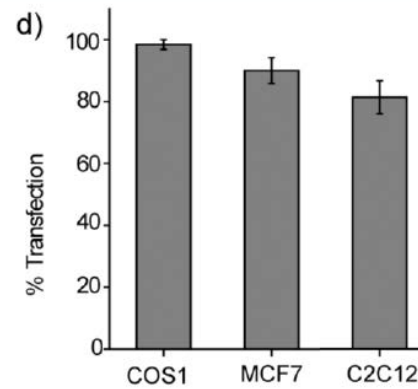
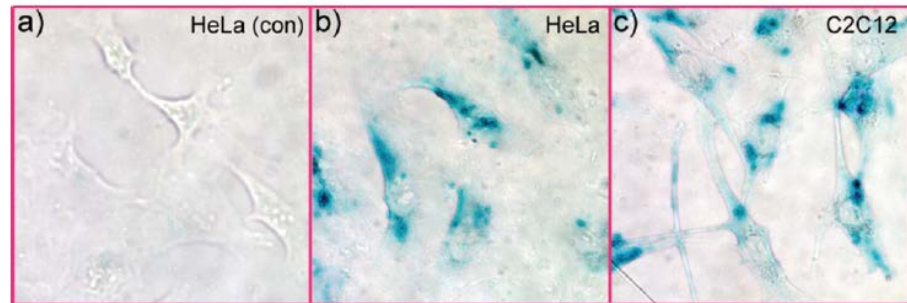
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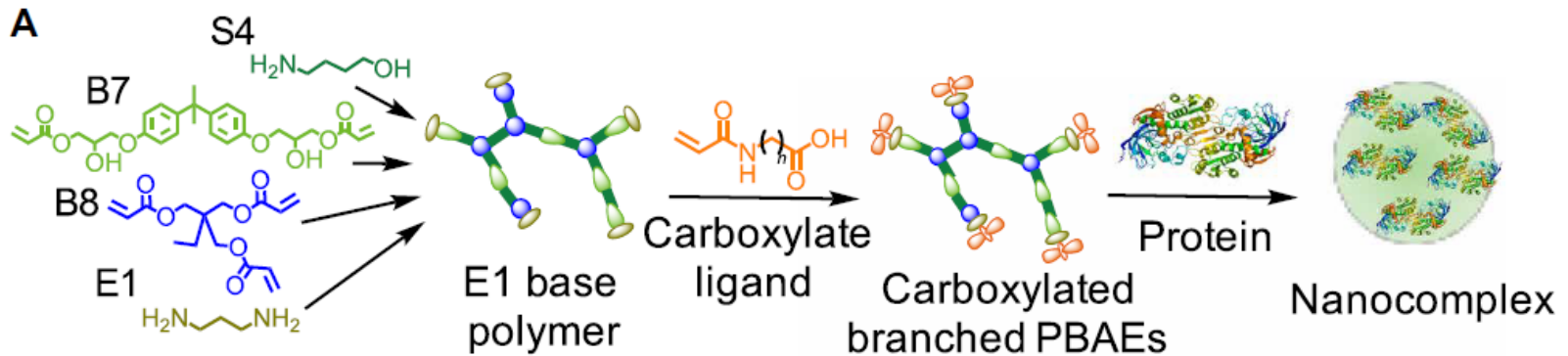
Carboxylated branched poly(β -amino ester) nanoparticles enable robust cytosolic protein delivery and CRISPR-Cas9 gene editing

Yuan Rui¹, David R. Wilson¹, John Choi², Mahita Varanasi¹, Katie Sanders¹, Johan Karlsson¹, Michael Lim^{2,3}, Jordan J. Green^{1,2,3,4*}

why important?

- self assembly with cargo, does not require chemical or biological alterations to the cargo or the carrier
- efficient in endolysosomal escape
- has wide applicability to carry proteins of differing size, pl
- applicable *in vivo*

assembly

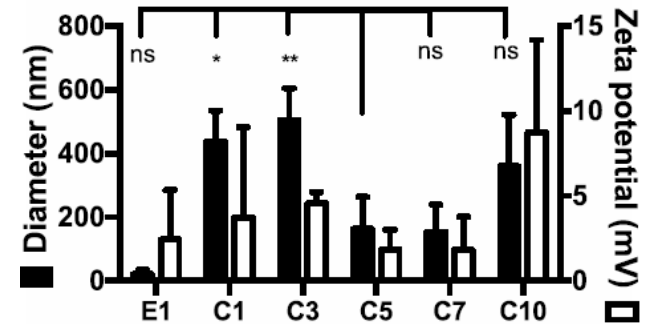
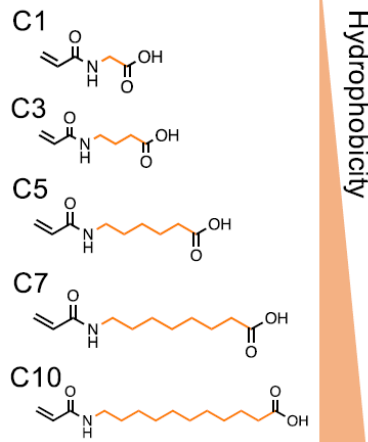


initial copolymerization

endcapping

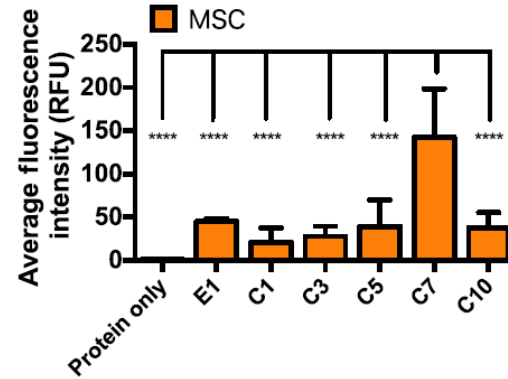
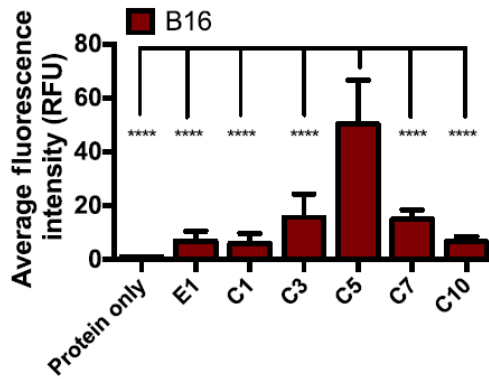
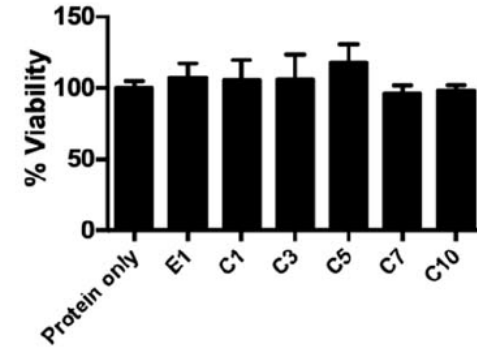
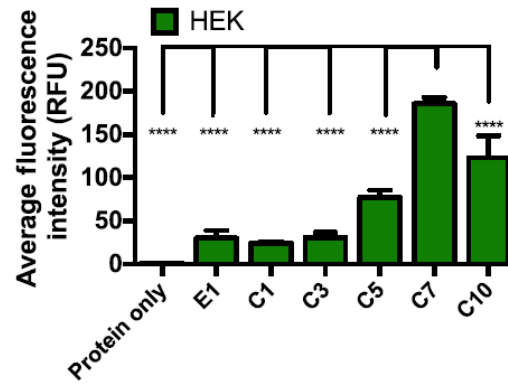
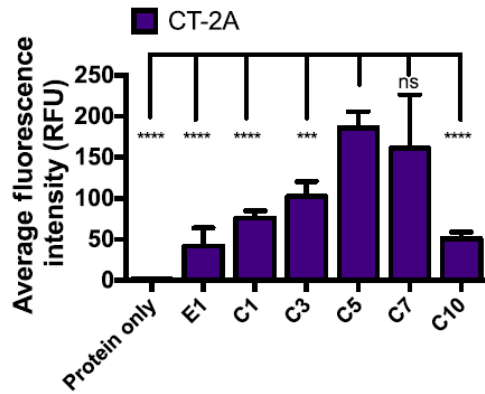
E1-base polymer:
amphiphilic and pH sensitive

carboxylated branched PBAE: supports hydrogen bond formation and salt bridges



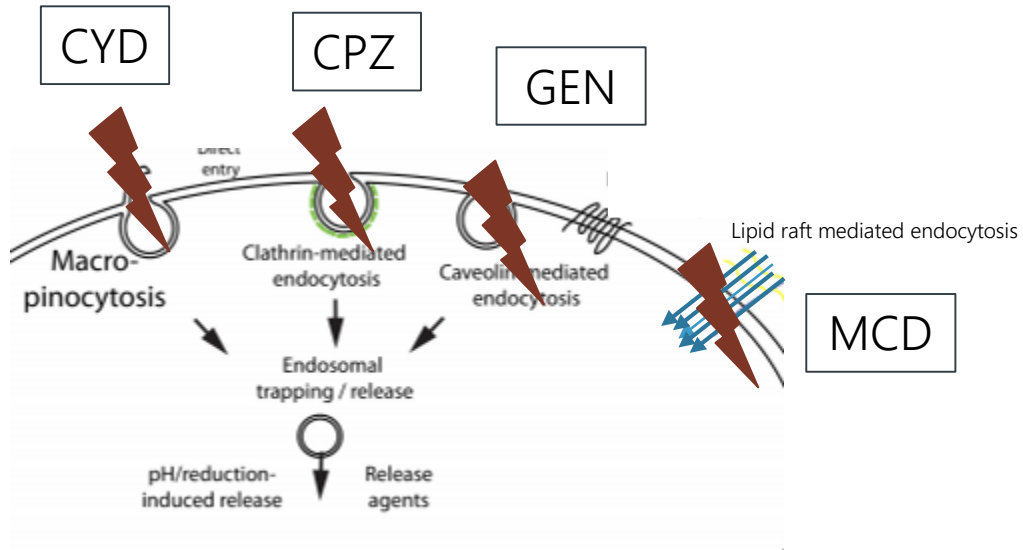
nanocomplex delivery into different cell lines

A



C5 and C7 in complex with FITC-BSA is taken up by different cell lines, and viability is not impaired

investigation of route of internalization



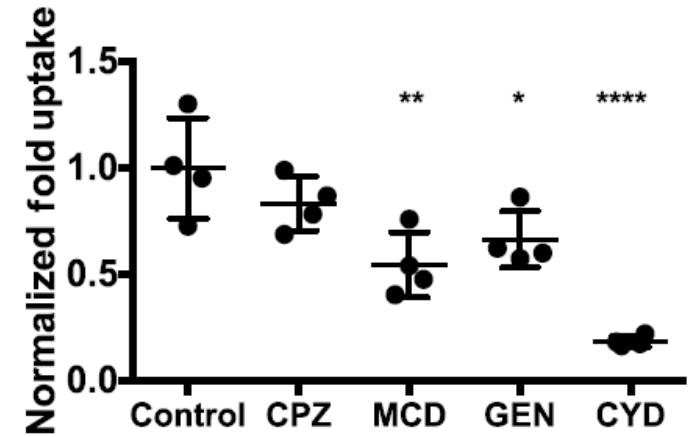
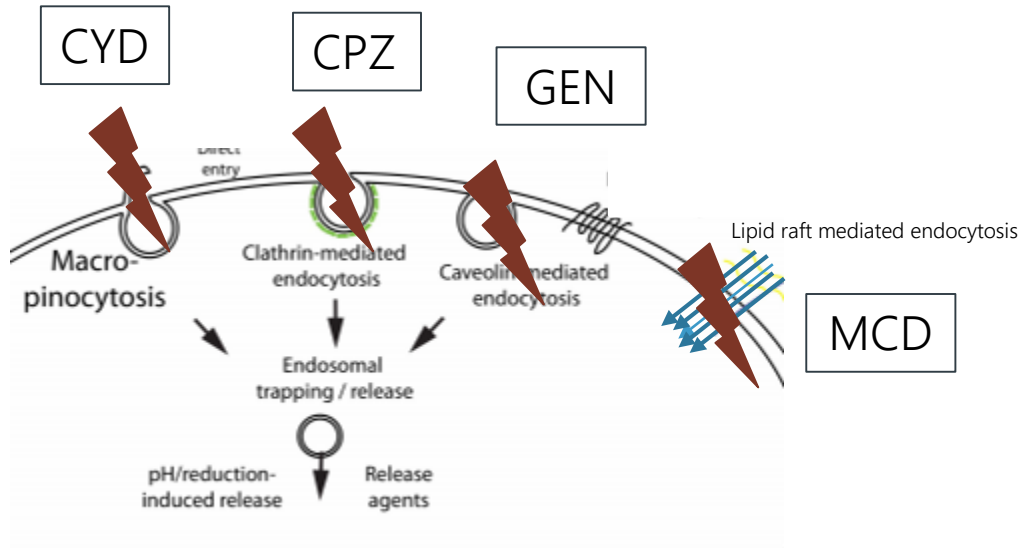
CPZ: chlorpromazine

GEN: genistein

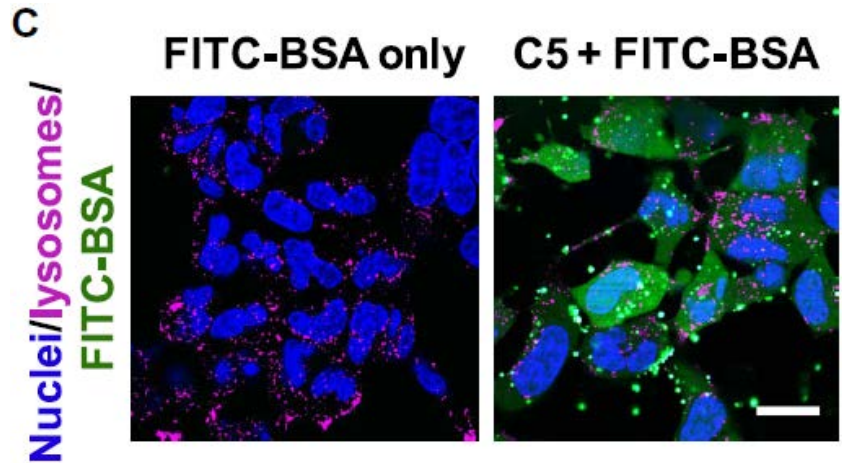
MCD: methyl-B-cyclodextrin

CYD: cytochalastin D

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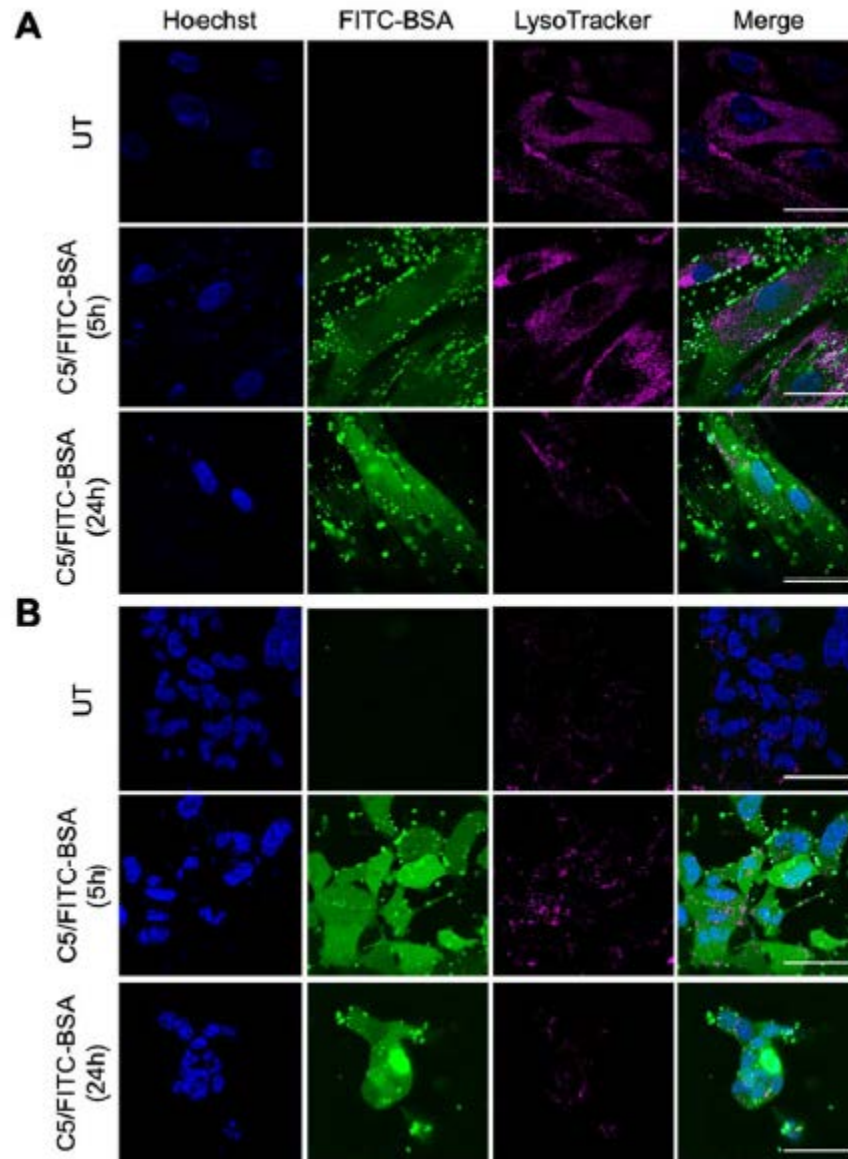


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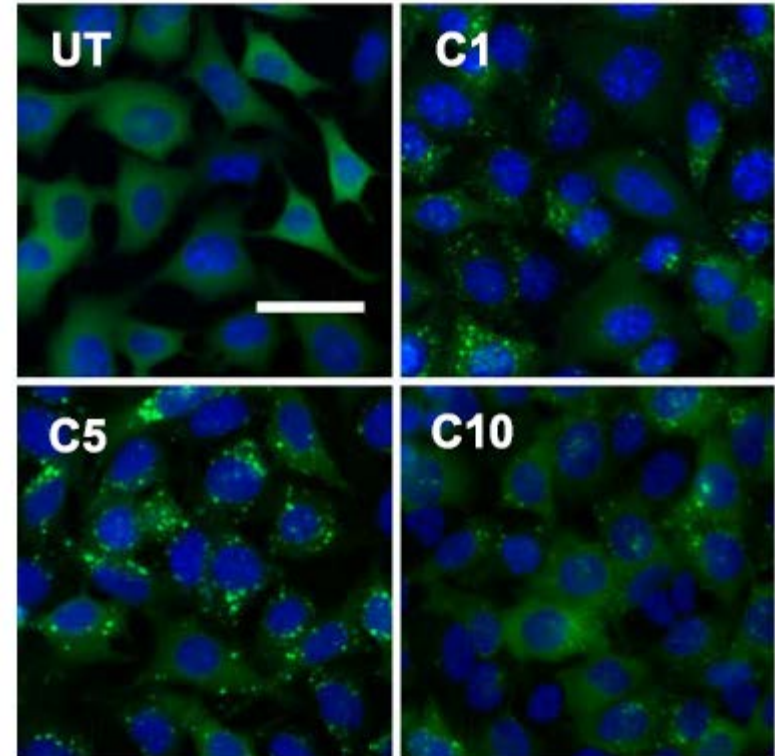
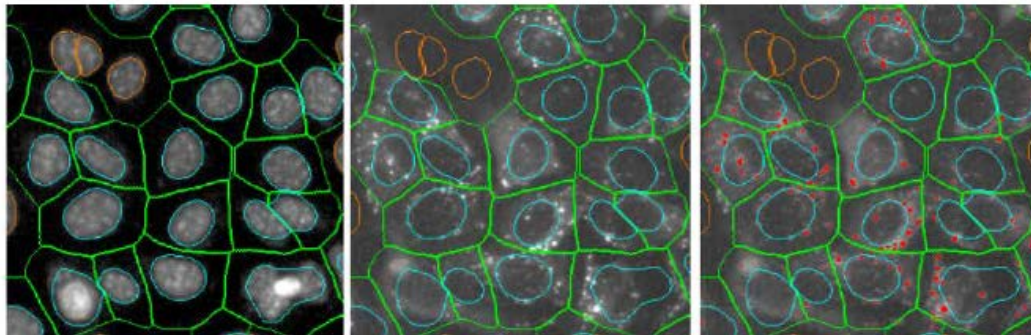
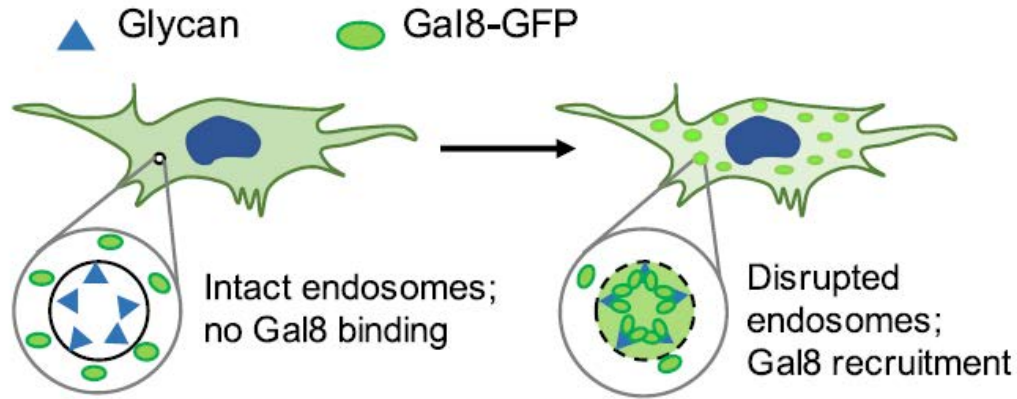
grand entry and great escape

human adipose derived
mesenchymal stem cells

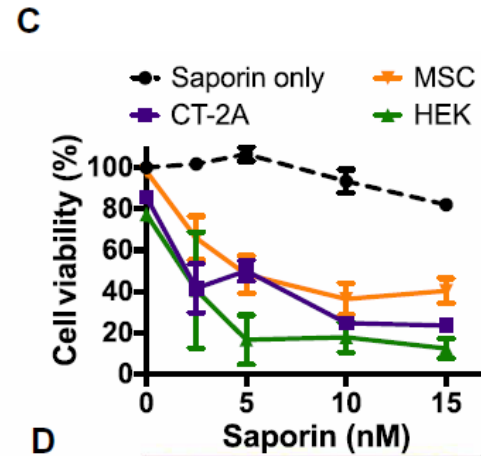
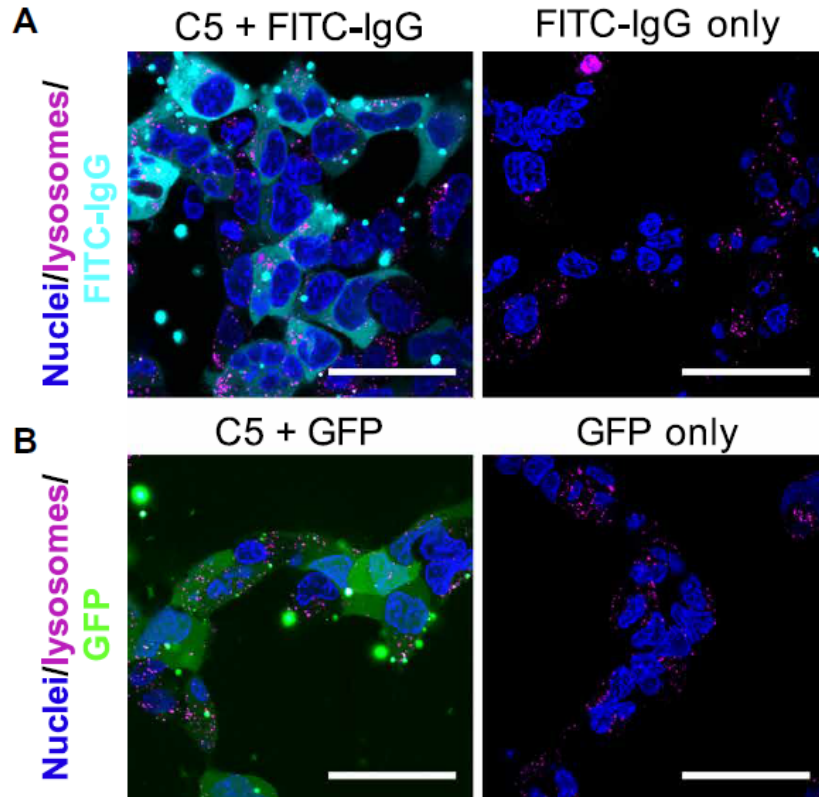


HEK293T cells

the great escape: from endosomes

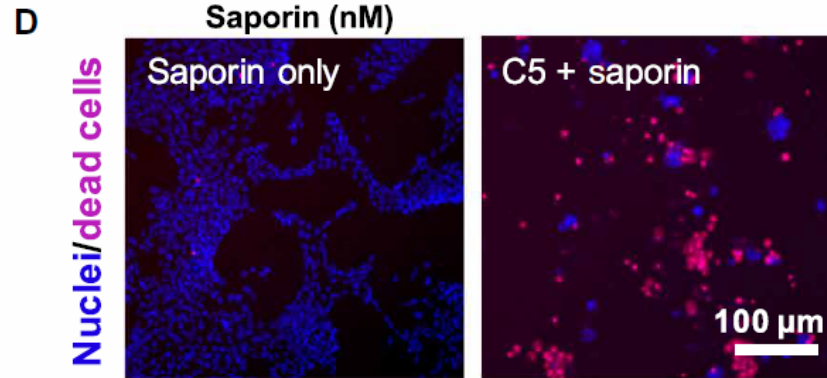


robustness of the delivery method

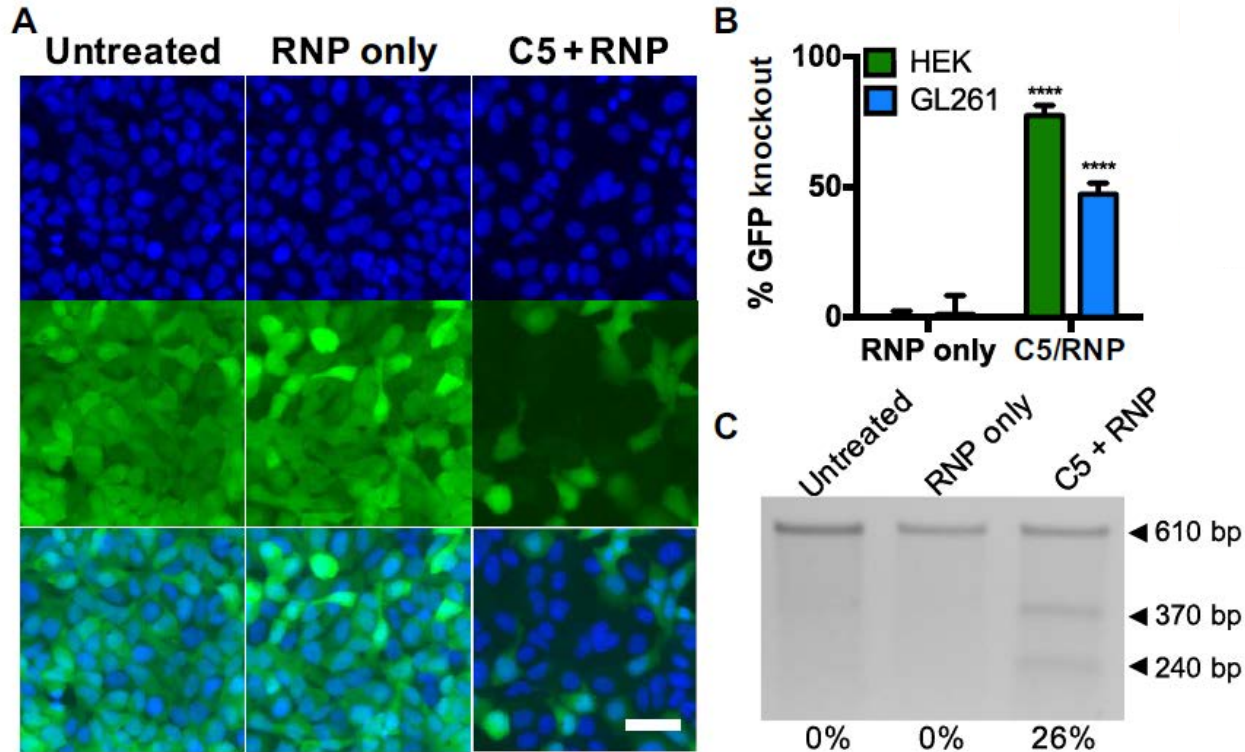


E

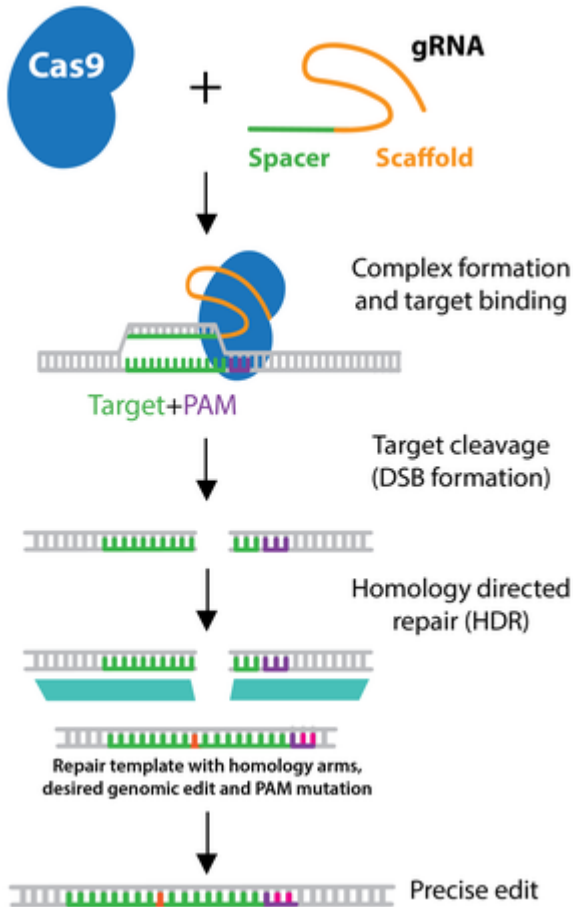
Protein	MW	pI
GFP	27 kDa	5.8
Saporin	29 kDa	9.5
BSA	66.5 kDa	4.7
IgG	150 kDa	6.6–7.2



CRISPR through nanocomplex RNP delivery



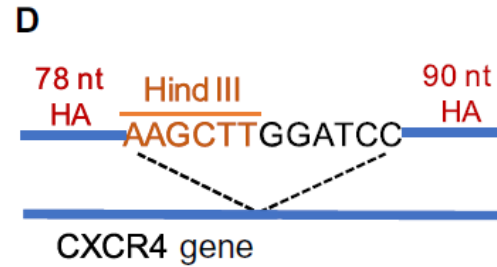
CRISPR through nanocomplex RNP delivery



self-assembly strategy

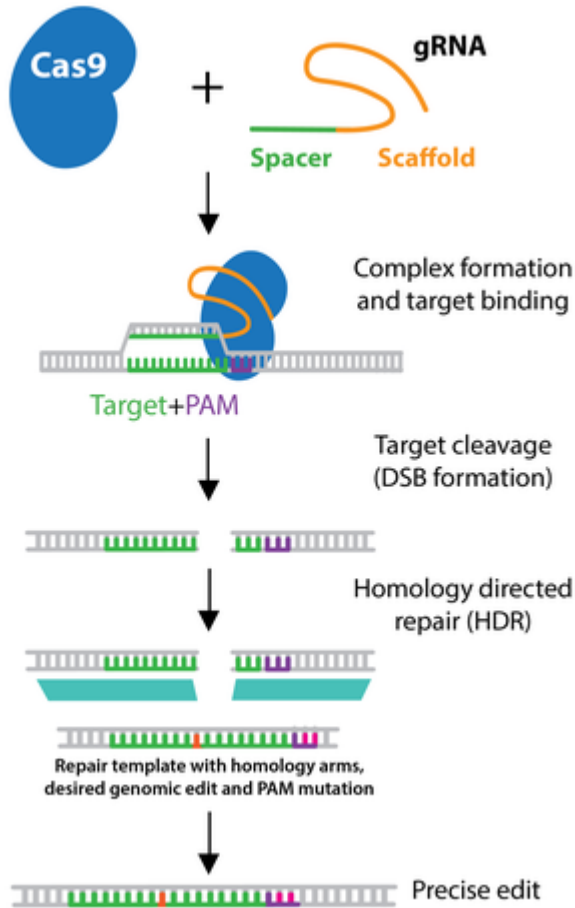
cas9 + sgRNA against CXCR4 → + ssDNA repair template → c5 polymer

edit strategy



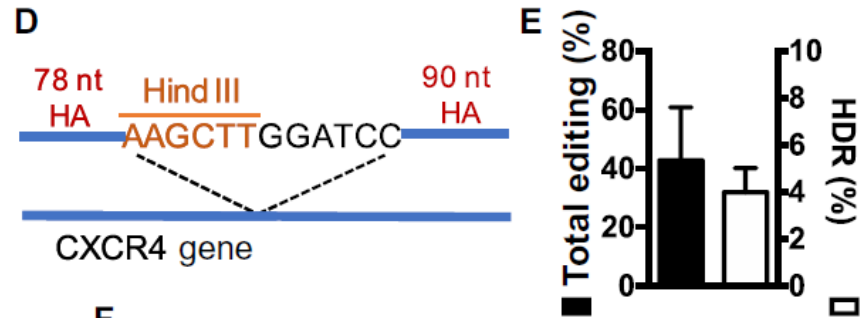
homology directed repair

CRISPR through nanocomplex RNP delivery



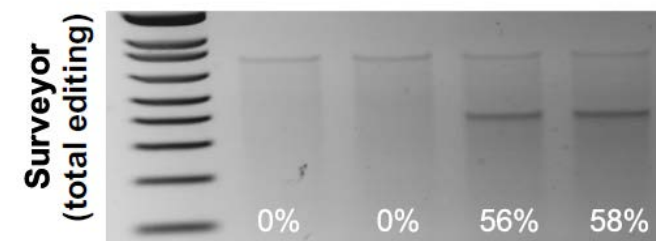
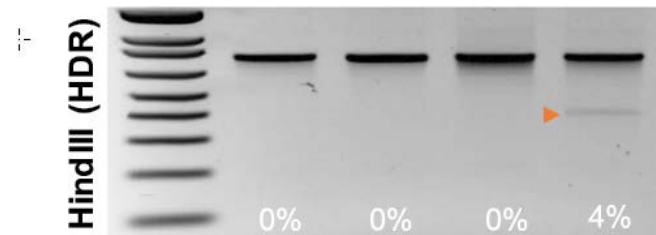
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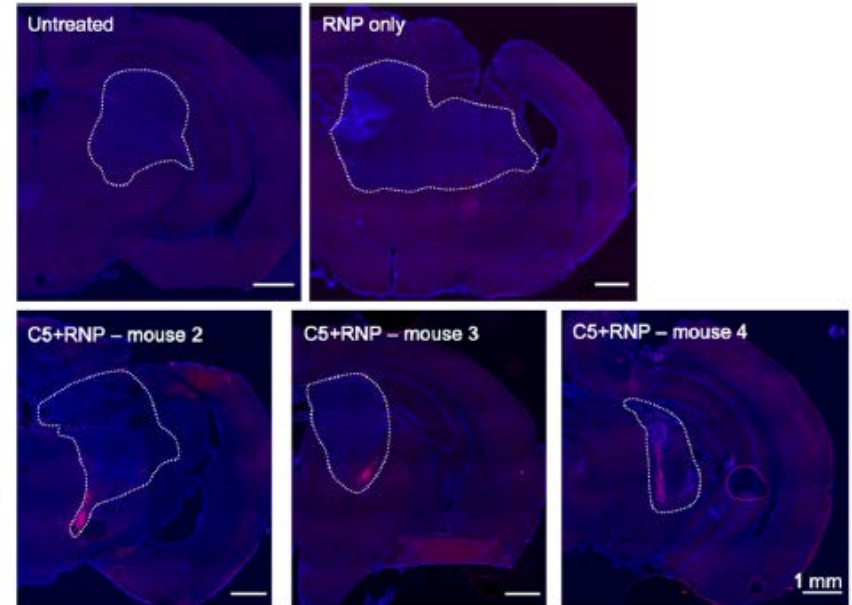
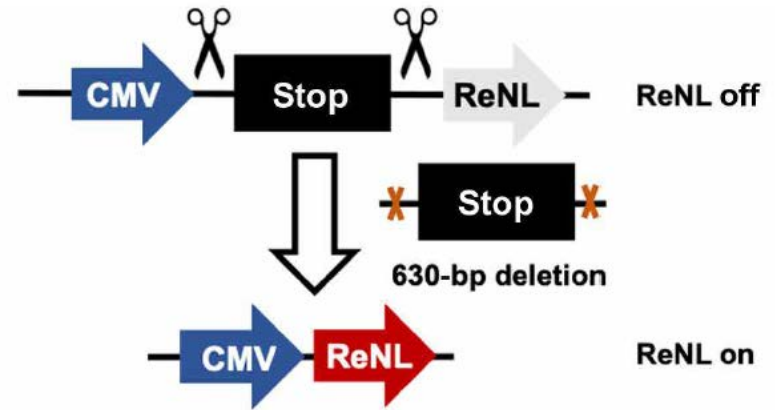
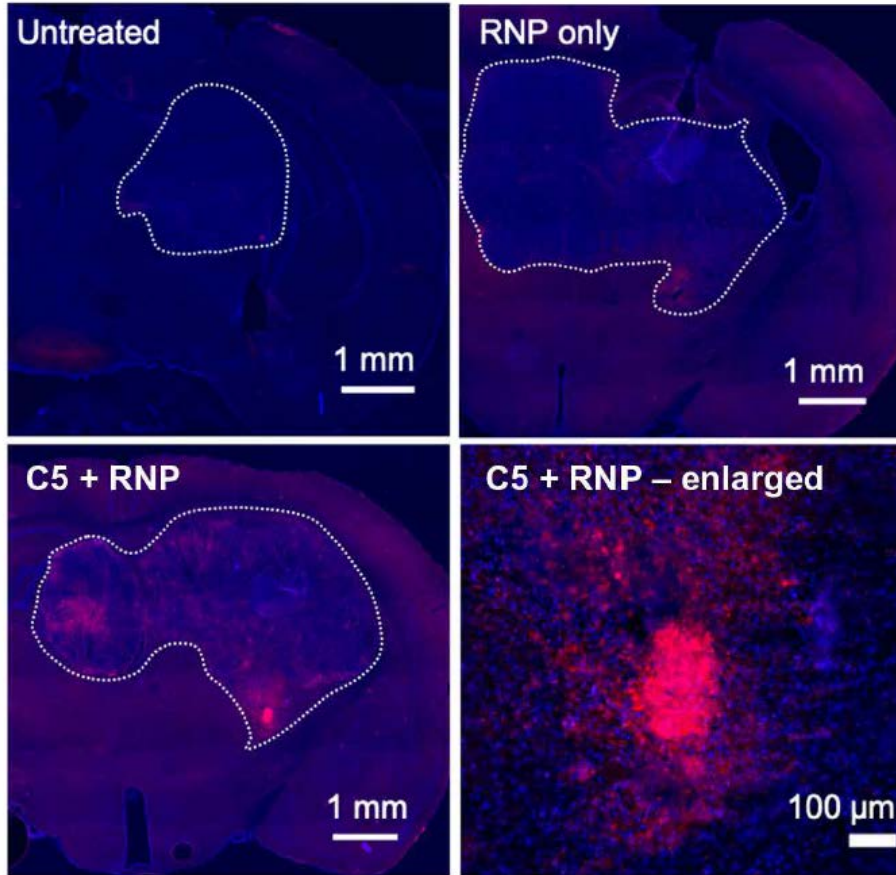
F

RNP	-	+	+	+
Donor DNA	-	+	-	+
C5 polymer	-	-	+	+



homology directed repair

in vivo CRISPR editing in murine glioma tumors – proof of principle



newest advance – advantages and shortcomings

advantages

- nanocomplexes are easy-to-use
- highly efficient in cargo and delivery of a wide range of proteins (differing in size and pI)
- perfect for in vitro use (storage and performance tested in comparison to commercially available products)
- (potential) capability of evading immune responses

shortcomings

- targeted delivery into certain types of cells not possible
- optimizations of in vivo delivery needed

Protein	Protein Characteristics		Nanoparticle Characteristics				
	MW	pI	Size (nm)	Zeta (mV)	Optimal Protein Dose	Optimal Polymer Dose (mg/mL)	Equivalent w/w
GFP	27 kD	5.8	150±50	9.9±0.7	300 ng	0.075	30
Saporin	29 kD	9.5	120±30	8.7±0.4	2.5-15 nM	0.075	2600-175
BSA	66.5 kD	4.7	160±60	5±1	300 ng	0.075	30
IgG	150 kD	6.6-7.2	120±20	-1±1	300 ng	0.075	30
Cas9	163 kD	9	180±10	12.3±0.2	690 ng	0.1	22



thank you for your attention and merry christmas!
(and hopefully a better new year)

publications referred to in this presentation and further reading:

- <https://www.frontiersin.org/articles/10.3389/fphar.2018.01208/full>
- <https://f1000research.com/articles/5-1947/v1>
- <https://www.sciencedirect.com/science/article/pii/S0168365914008165?via%3Dihub>
- <https://pubs.acs.org/doi/10.1021/acs.langmuir.6b04304>
- <https://advances.sciencemag.org/content/5/12/eaay3255.full>
- <https://www.ncbi.nlm.nih.gov/pubmed/28781125>
- <https://pubs.acs.org/doi/10.1021/ja907887z>
- <https://www.nature.com/articles/nmeth.2998>
- <https://www.nature.com/articles/nature19764>
- <https://www.nature.com/articles/nchem.2779.pdf>