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Comment on "Self-assembling protein nanoparticles for cytosolic delivery of nucleic acids and proteins"

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Introduction

We read with great interest the study by Chaikof and colleagues published in Nature Biotechnology on the development of self-assembling protein nanoparticles (ENTER) for cytosolic delivery of nucleic acids and proteins (DOI:10.1038/s41587-025-02664-2). This innovative platform addresses critical challenges in nonviral delivery, particularly for gene editing and protein therapeutics, by enabling efficient intracellular transport while minimizing toxicity.

1. Modular design and optimization of the delivery system

The core strength of ENTER lies in its hierarchical modular design (Fig. 1a). Through four generations of iterative optimization, the researchers engineered elastin-like polypeptide (ELP) nanoparticles incorporating a hydrophilic corona, histidine-rich core, and endosomolytic peptides (EEPs). Notably, EEP13—discovered *via* machine-learning screening—demonstrated a 48% improvement in protein delivery efficiency compared to benchmark peptides (Fig. 2c). The V4-ELP-EEP13 system outperformed lipid nanoparticles (LNPs) in mRNA and Cre recombinase delivery across multiple cell lines (Fig. 5h), highlighting its versatility.

However, the study did not fully explore cell-type-specific uptake mechanisms. For example, do macrophages and hematopoietic stem cells differ in their endocytic pathways when interacting with ELP nanoparticles? Future studies could integrate receptor-mediated endocytosis markers (e.g., clathrin, caveolin) to elucidate these differences [1,2].

2. Endosomal escape mechanism and pH-responsive design

ENTER achieves endosomal escape through histidine protonation and EEP-mediated membrane disruption (Fig. 1c-f). Dynamic light scattering revealed ELP nanoparticle disassembly under acidic conditions, releasing EEP to destabilize endosomal membranes (Fig. 4g). This pH-responsive design minimizes free-peptide cytotoxicity while achieving up to 90% gene editing efficiency (Fig. 5h). Interestingly, siRNA delivery efficiency exhibited nonlinear dose dependence on ELP concentration (Fig. 4h), suggesting endosomal escape efficiency may be regulated by nanoparticle stability.

While TEM imaging (Fig. 1f) confirmed nanoparticle uniformity, real-time visualization of endosomal membrane disruption was absent. Future work could employ live-cell imaging to dynamically track ELP-endosome interactions and membrane perturbation [3].

3. Clinical translation potential and challenges

ENTER demonstrated remarkable *in vivo* efficacy: Intranasal administration achieved 25.4% tdTomato+ cells in large airway epithelium (Fig. 6b-e), surpassing LNPs (3.9% efficiency). Recombinant ELP production *via E. coli* offers scalability and reduced immunogenicity compared to viral vectors.

Key challenges remain for clinical translation: (1) Long-term toxicity data, especially regarding repeated dosing and immune responses, are lacking; (2) Lack of targeting moieties risks off-target effects in systemic administration; (3) Limited EEP sequence diversity may hinder cross-species applicability. Future studies should explore ligand conjugation (e.g., antibodies, glycans) for organ-specific delivery [4-7].

Conclusion

The ENTER system represents a groundbreaking advancement in nonviral gene delivery. Its modular design, efficient endosomal escape mechanism, and biocompatibility position it as a powerful tool for precision medicine. Future efforts should focus on optimizing targeting strategies, assessing long-term toxicity, and scaling production for clinical applications. ENTER's emergence opens new dimensions in therapeutic delivery, promising to transform gene editing and protein replacement paradigms.

Conflicts of interest

The authors declare no conflicts of interest.

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