



## NanoSyrinx: Specific delivery of complex active biologics into target cells using nanosyringe drug delivery technology

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### Introduction

Biologics as therapeutics have been largely limited to extracellular targets due to their inability to cross cell membranes and effectively gain access to the cytosol or other intracellular compartments. NanoSyrinx is a discovery stage synthetic biology company creating a next-generation drug delivery technology for targeting intracellular pathways with biological molecules. Our protein-based technology is a modular system with genetically in-built specific targeting alongside delivery of protein payloads, allowing us to address a wide range of cellular functions such as selectively inducing cell death, regulating cellular pathways, and reprogramming gene expression. The purpose of this study is to show that nanosyringes self-assemble during production, including the protein payload, and selectively deliver cytosine deaminase (CD) enzyme directly into target cells which converts the 5-fluorocytosine (5-FC) pro-drug into its active, cytotoxic form, 5-fluorouracil (5-FU), resulting in cell death.

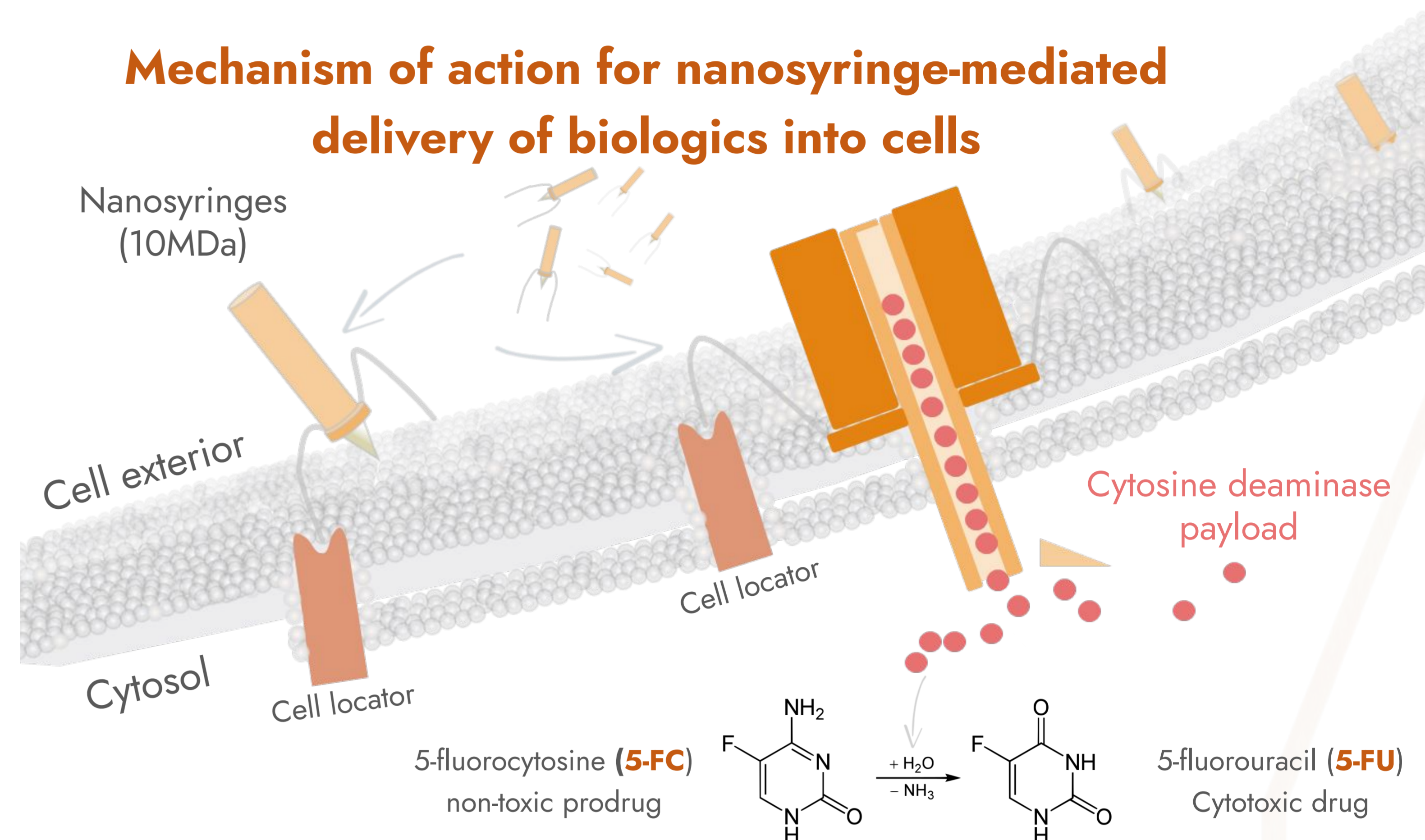
### Aims

- I. Produce nanosyringes loaded with cytosine deaminase (CD)
- II. Show binding of nanosyringes to target cells
- III. Show successful delivery of CD to target cells treated with 5-FC

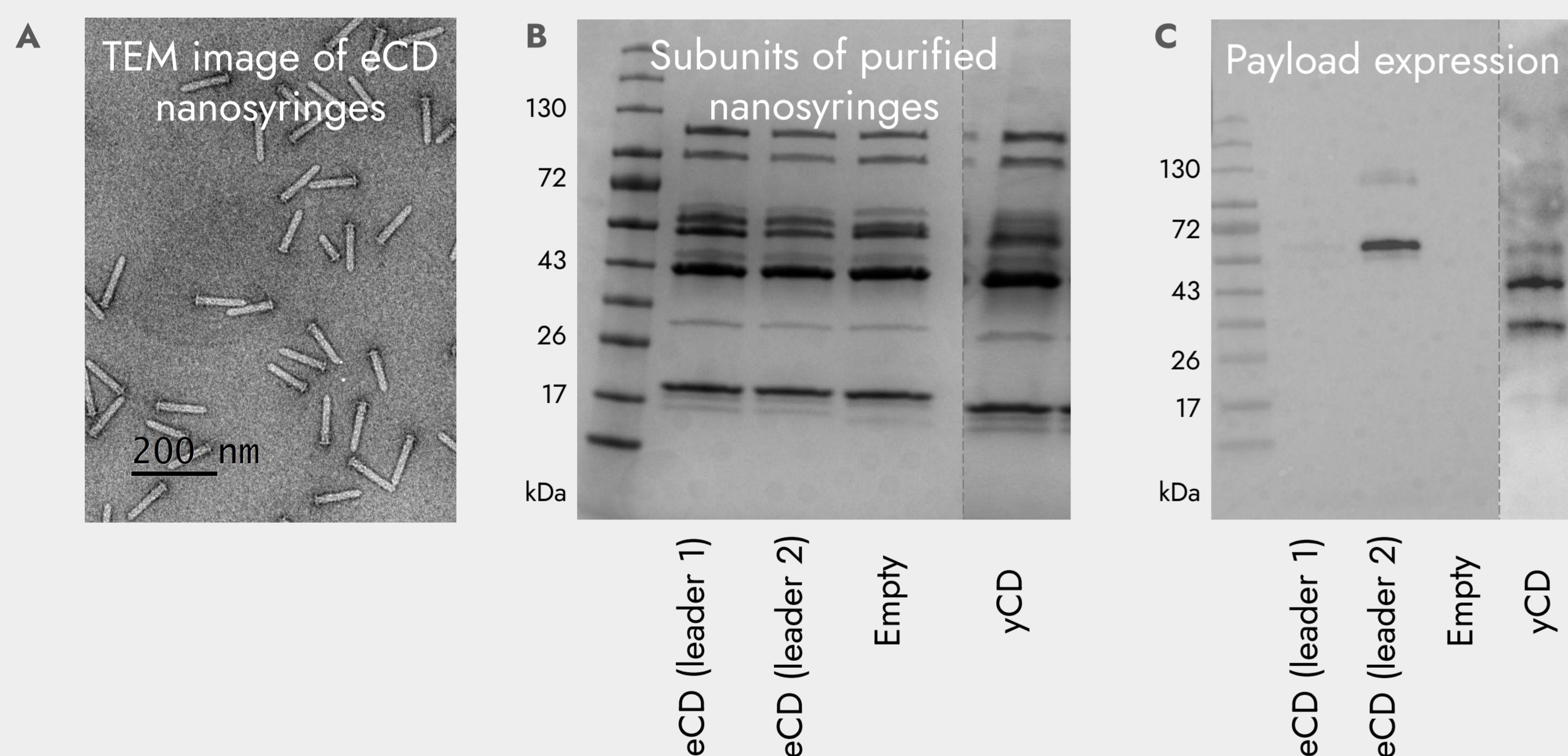
### Methods

CD derived from *S. cerevisiae* (yCD) or *E. coli* (eCD) were cloned into our nanosyringe plasmids. CD-loaded nanosyringes were then expressed in bacteria and purified. Two distinct loading peptide leader sequences were utilised to load the CD variants into nanosyringes. Binding of CD-loaded nanosyringes to two murine macrophage cell lines was assessed by flow cytometry. Cell viability was measured using LIVE/DEAD™ Viability/Cytotoxicity Kits for mammalian cells.

### Mechanism of action for nanosyringe-mediated delivery of biologics into cells

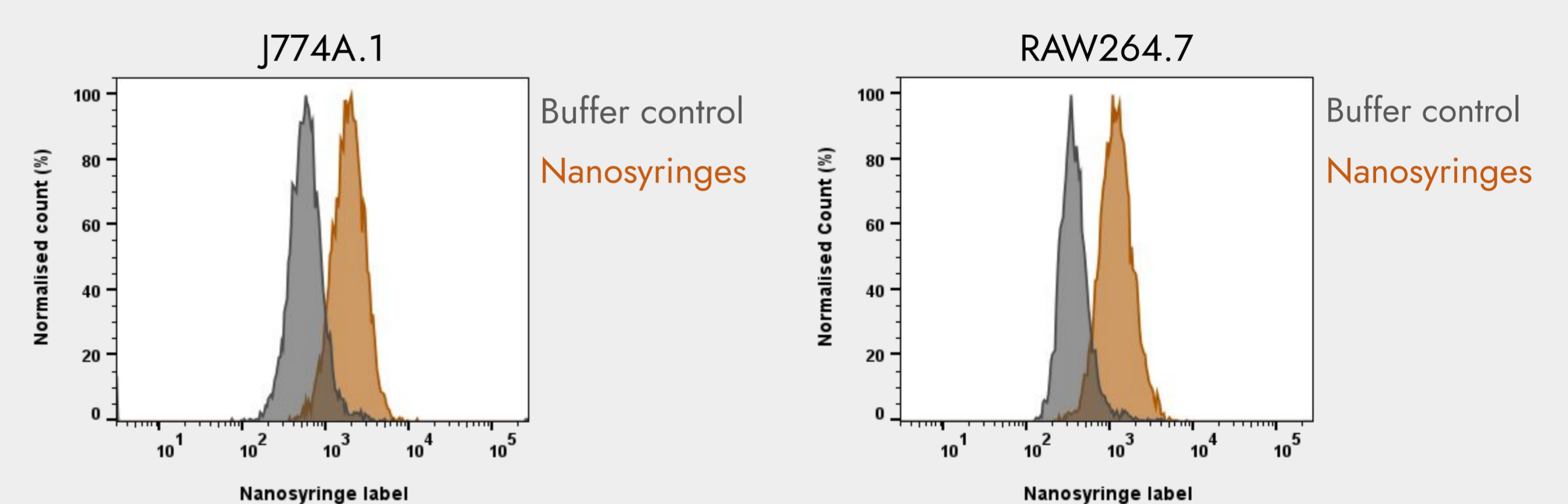


### Purification of CD-loaded nanosyringes



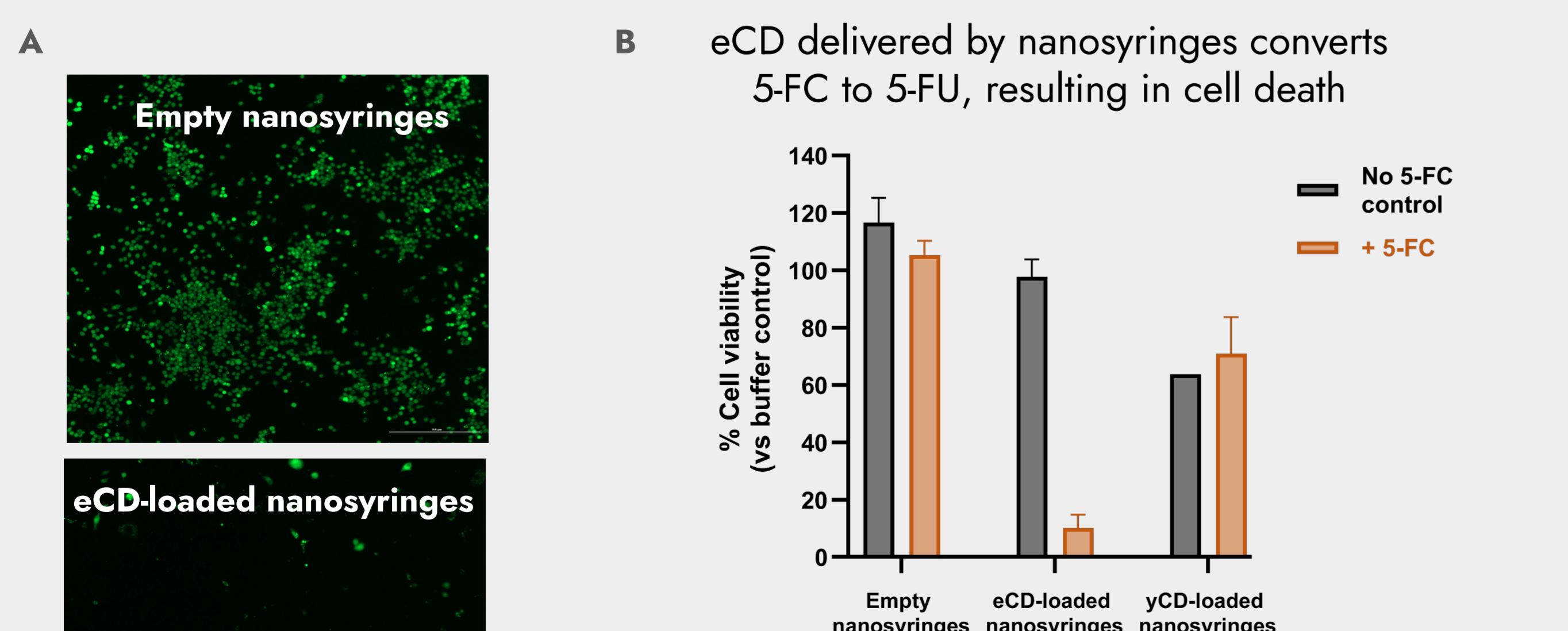
**Figure 1.** A. TEM images of purified nanosyringes loaded with CD enzyme (bar is 200nm). B. Protein stain of purified nanosyringes showing all 13 subunits. C. Payload stain in empty (no payload) and loaded nanosyringes containing either eCD (expressed using two unique loading leader sequences) or yCD.

### CD-loaded nanosyringes bind to murine macrophages



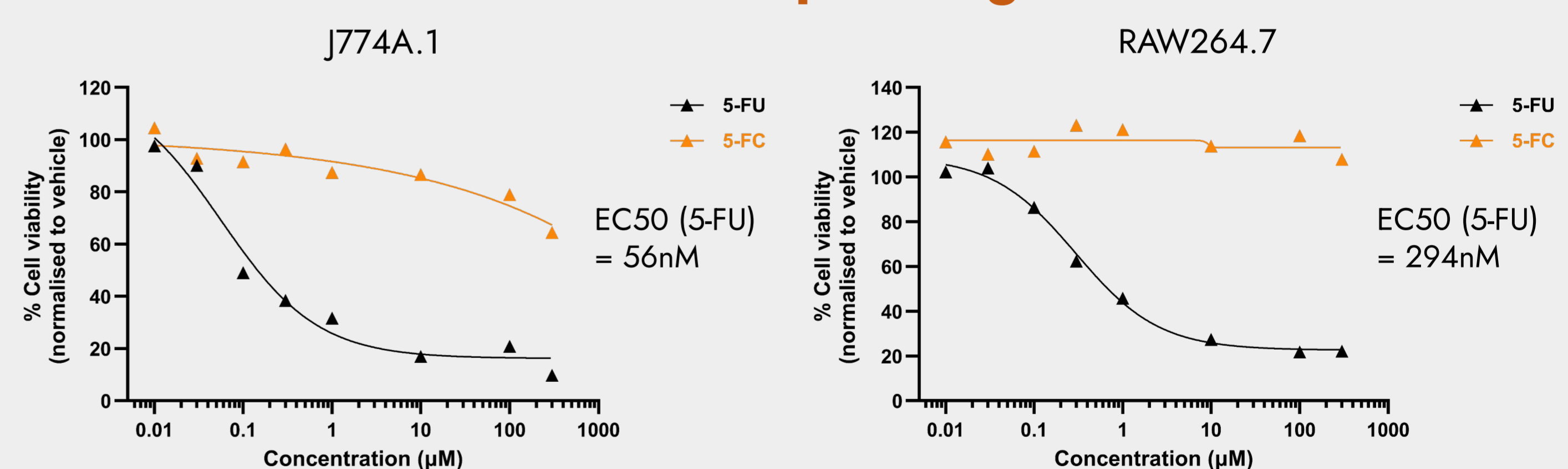
**Figure 2.** Flow cytometric analysis of mouse macrophages after incubation with labeled nanosyringes (orange) or buffer (grey)

### Nanosyringes deliver functional Cytosine Deaminase (CD) to murine macrophages resulting in cell death



**Figure 4.** A. Representative images of Calcein AM-positive RAW264.7 mouse cells after 72h incubation with either empty or eCD-loaded nanosyringes in the presence of 5-FC (bar is 300µm). B. Relative viability of cells after treatment with either empty or eCD/yCD-loaded nanosyringes in the presence or absence of 5-FC (n=3 except for yCD + No 5-FC Control).

### Murine macrophages are susceptible to 5-FU, but not the 5-FC prodrug



**Figure 3.** Susceptibility of murine macrophage cell lines to 5-FU and 5-FC, T = 72h.

### Conclusions

- I. We generated nanosyringes loaded with Cytosine Deaminase (CD) enzyme either from *S. cerevisiae* or *E. coli*, using two distinct peptide loading leader sequences. The leader sequence has a strong effect on the total amount of payload encapsulated into nanosyringes.
- II. We demonstrated that nanosyringes can deliver CD enzyme into cells where it can assemble into its active, multimeric form and convert the 5-FC pro-drug into the cytotoxic 5-FU drug, resulting in cell death.
- III. Additional data with other payloads and nanosyringes targeted to cell membrane proteins (data not shown) also highlights that the loading leader sequence should be chosen on a payload-by-payload basis.

### Acknowledgment and NanoSyrinx contact details

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