Leveraging Cell-Penetrating Peptides and Liposomes to Enhance Nanobody Therapy in Breast Cancer

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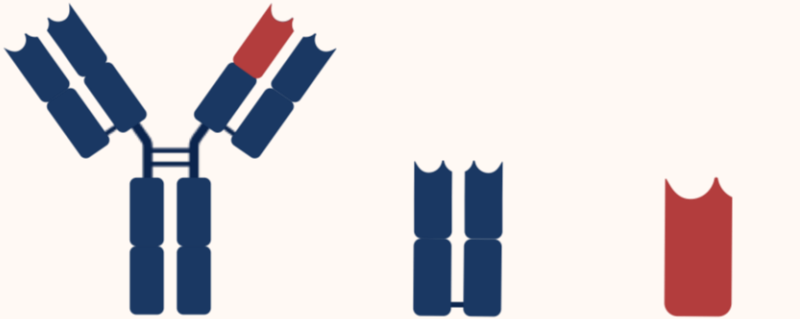
NEXT-GENERATION DELIVERY INNOVATIONS

Introduction



- Low molecular weight (15–30 kDa)
- Refolding after denaturation
- Production in procaryotes
- High target affinity
- Reduced immunogenicity

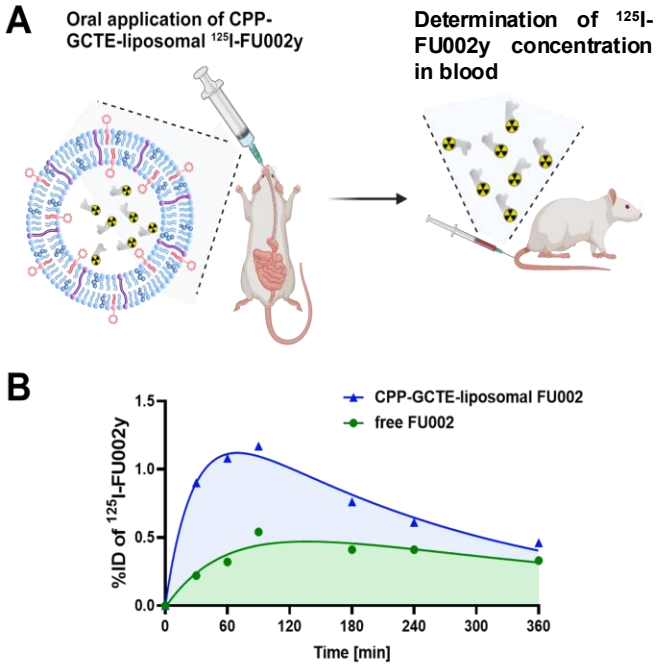
↓ Fast clearance and short half-life

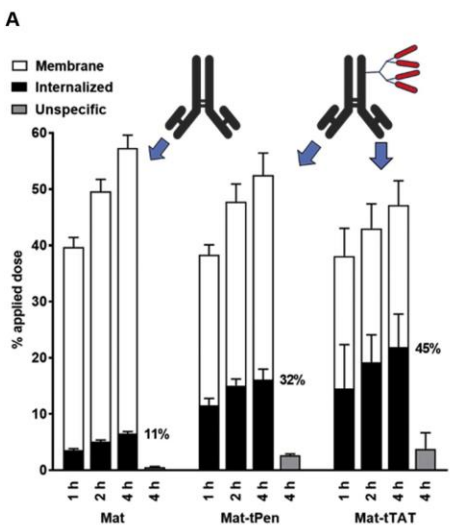
Two strategies are applied in this project:

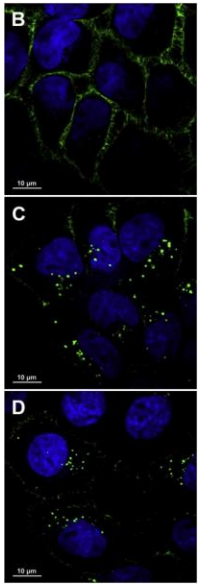
- Conjugation to CPPs
- Encapsulation in surface-modified liposomes

Introduction

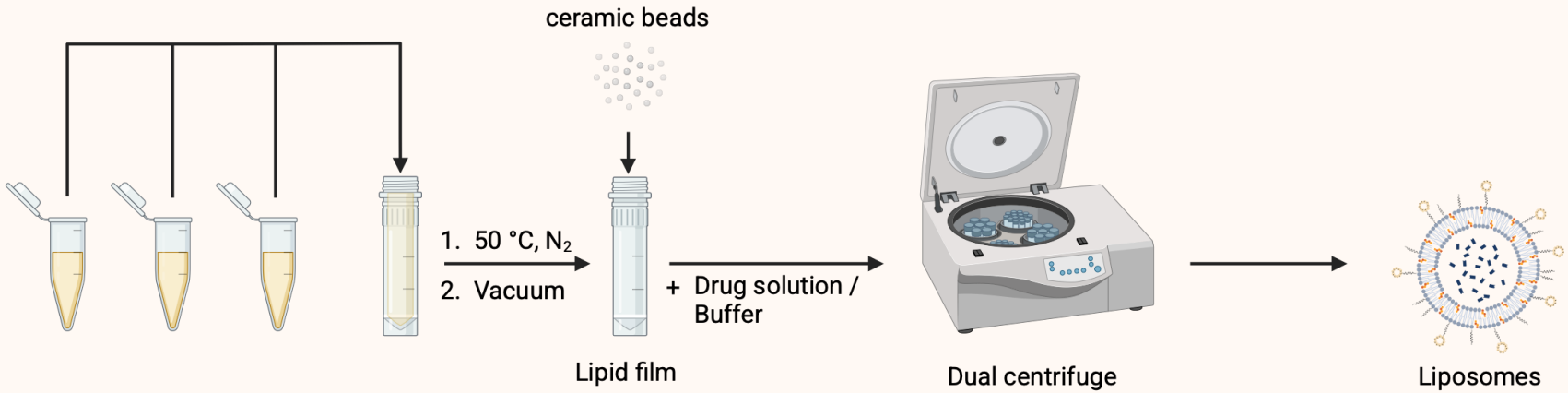
- Applicability of surface-modified liposomes
  (Werner et al. Adv. Healthcare Mater., 2024)
- Applicability of mAb-CPP-conjugates
  (Sauter et al., J control Release, 2020)





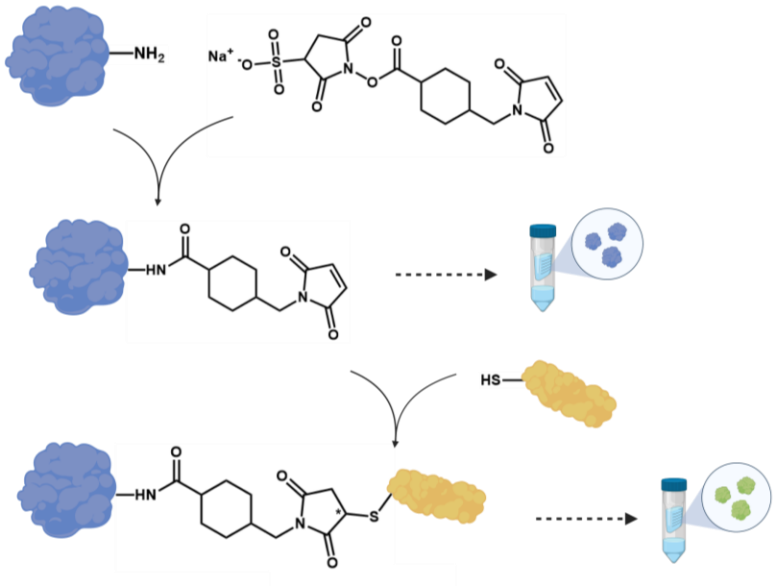


Part I: Liposomal preparation by dual centrifugation



DC: small, reproducible liposomes with high drug loading

Part II: Nanobody-CPP conjugates

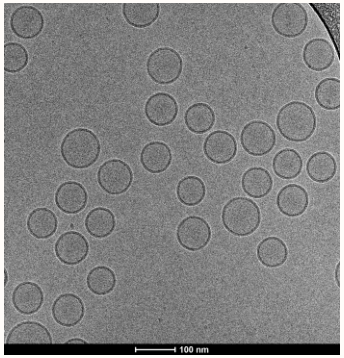


CPP = **C**ell-**P**enetrating-**P**eptide

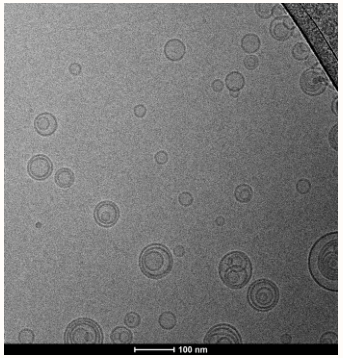
Enhance cellular interaction and uptake through cationic interaction

- → Rich in arginines or lysines
- → Non-toxic compound (*in vitro* and *in vivo*)
- → Produced with SPPS with an additional cysteine for maleimide coupling
- → Most important: Nona-arginine variations

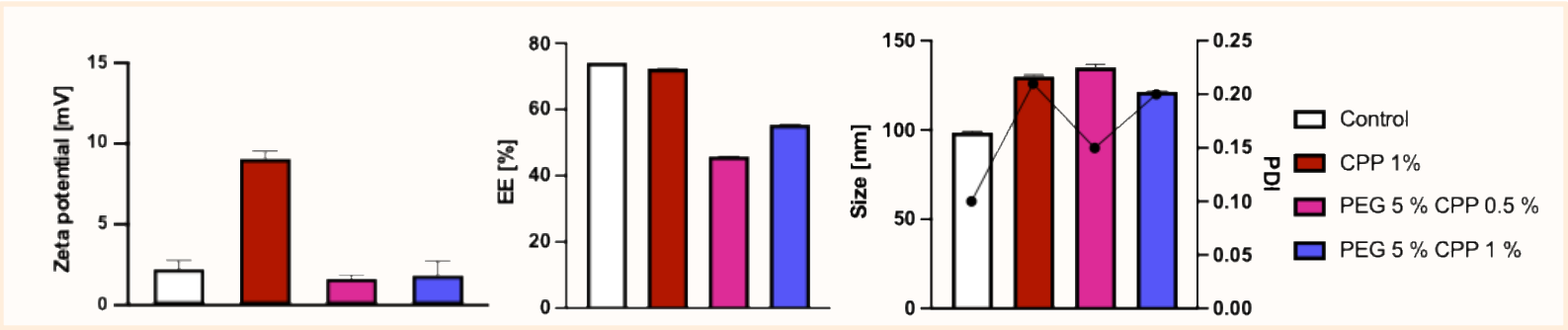
Part I: Liposomal characteristics



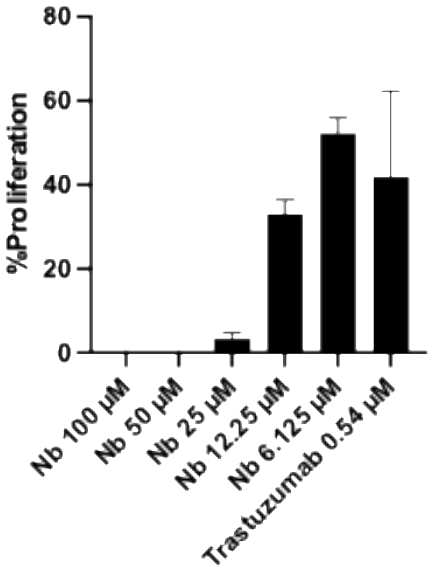
Control



CPP 0.5% PEG 5%

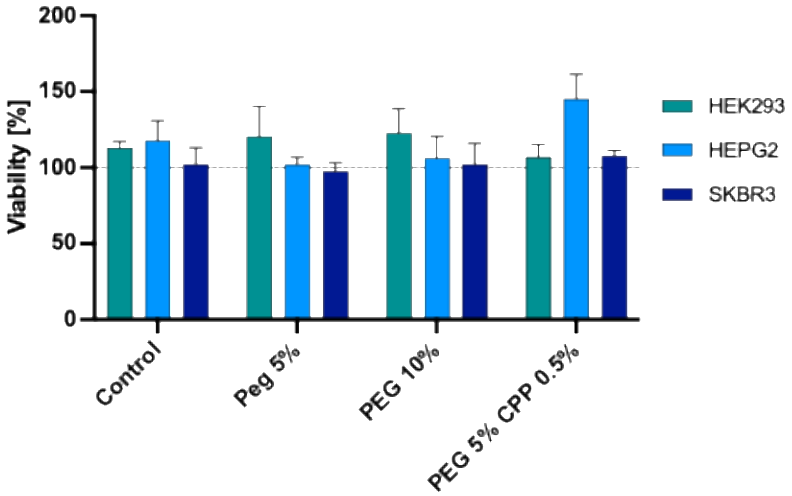


Liposomes: *In vitro* results



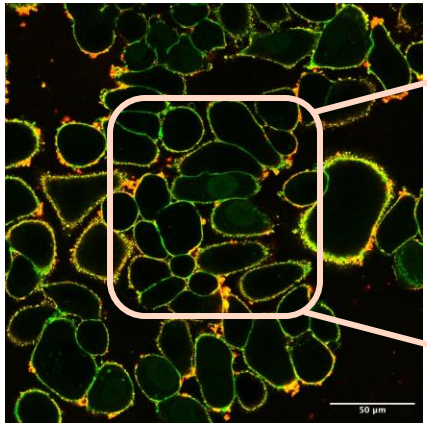
Key findings

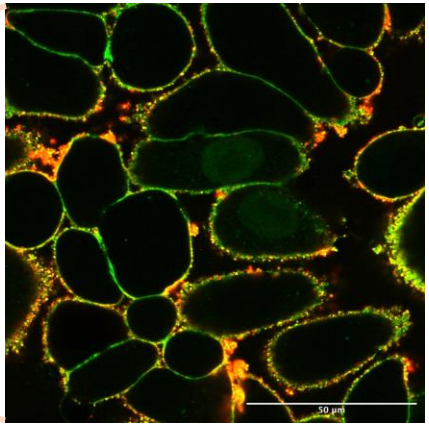
- → Nanobody inhibits proliferation on HER+ positive breast cancer cells
- → No cytotoxicity on kidneys/liver
- → Empty liposomes do not show any cytotoxicity



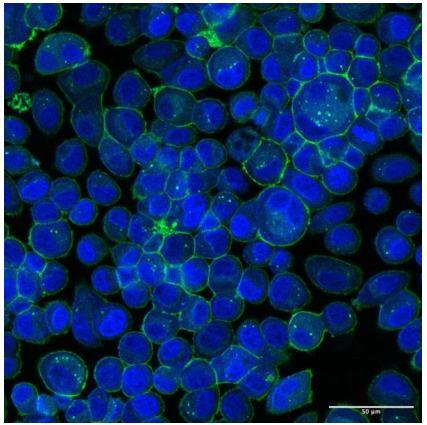
Liposomes: *In vitro* results
on HER2+ cells

PEG 5% CPP 0.5% + 50 µM Nb



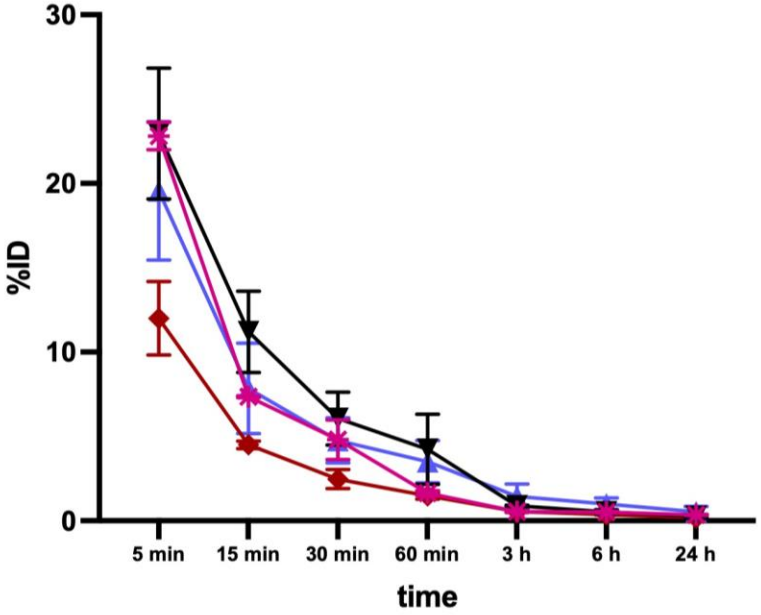


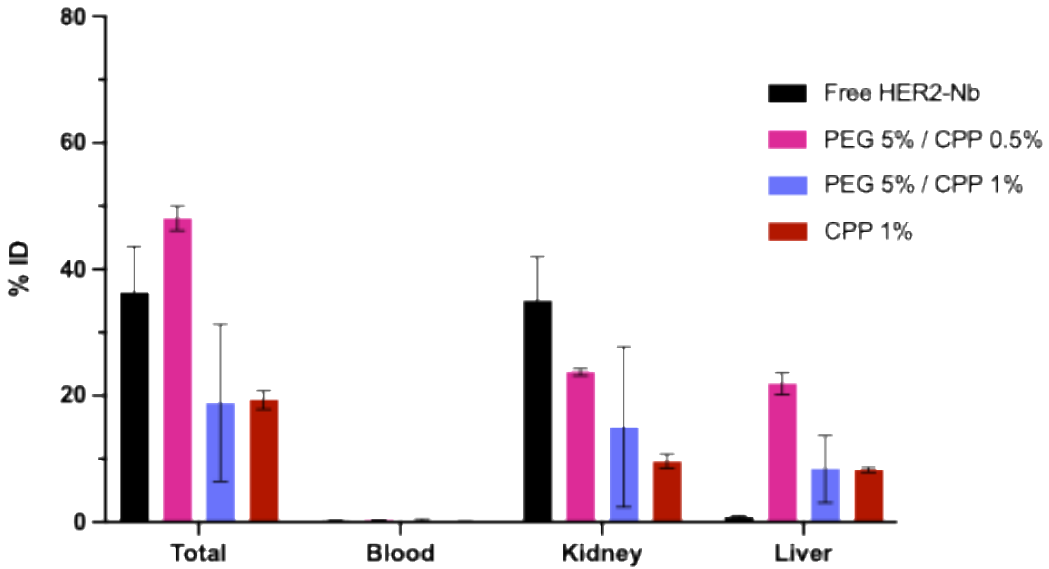
50 µM Nb



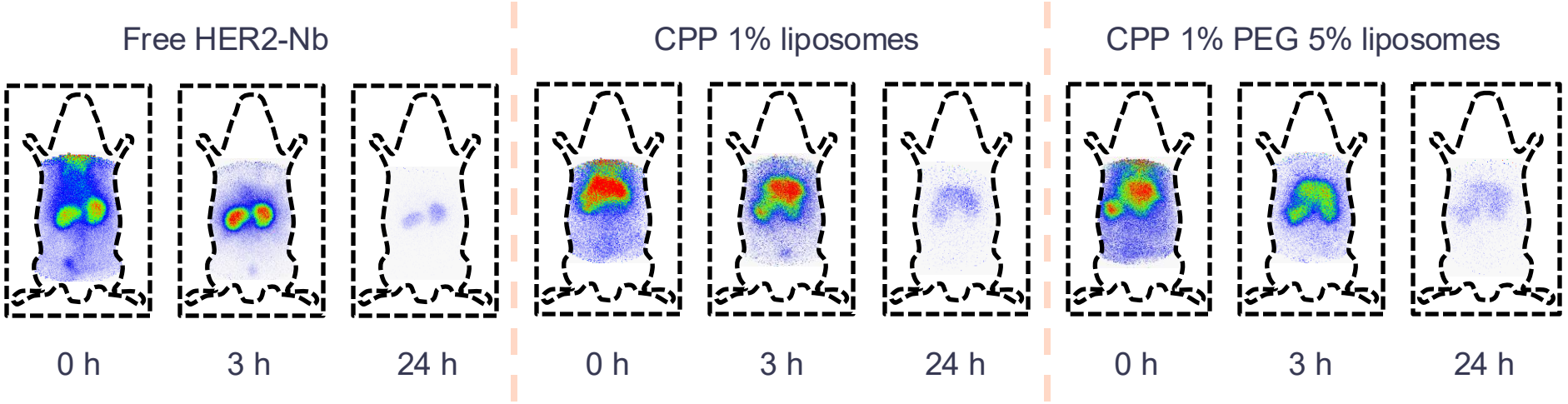
Liposomes: *In-vivo* rodent studies

Radiolabeling with 99mTc



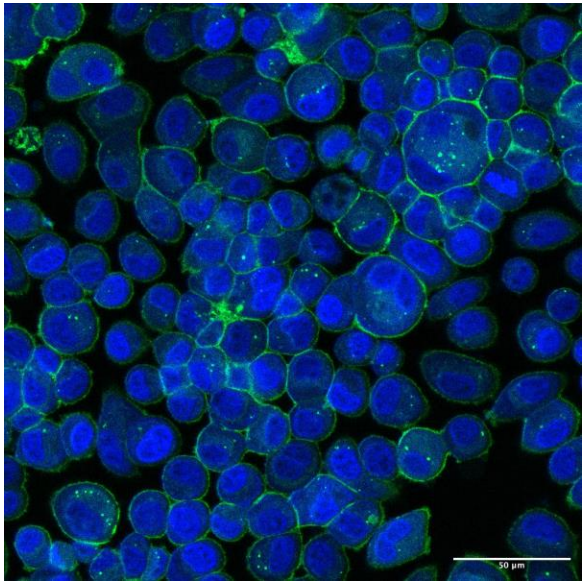


Liposomes: *In-vivo* rodent studies

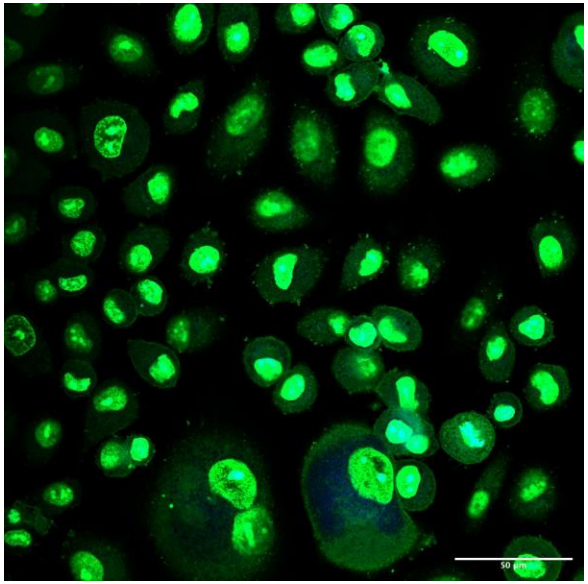


- Free protein gets rapidly cleared by the kidneys
- Liposomal formulations change biodistribution drastically

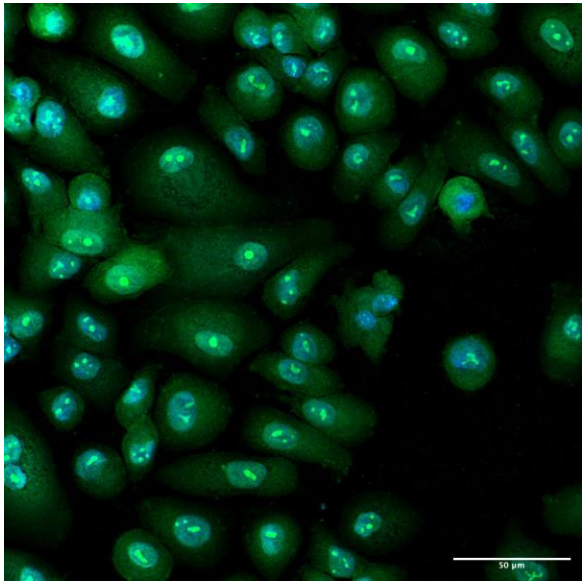
Part II: Nanobody-CPP conjugates: *In vitro* results



Nanobody



Nanobody-dR9

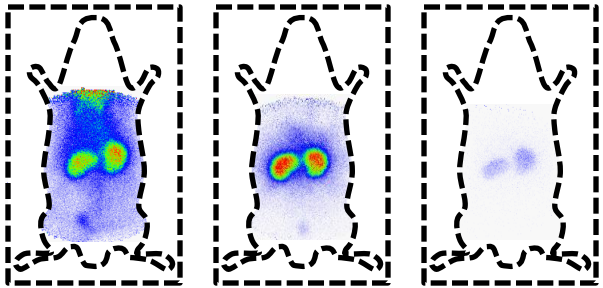


Nanobody-K9

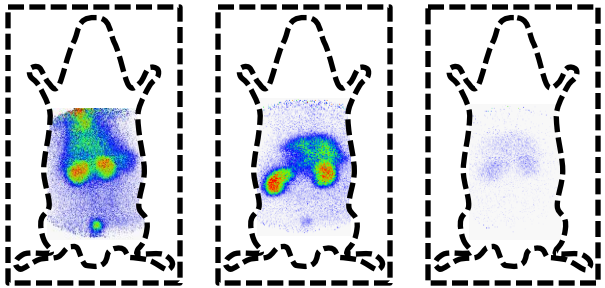
→ Cationic CPPs facilitate internalization

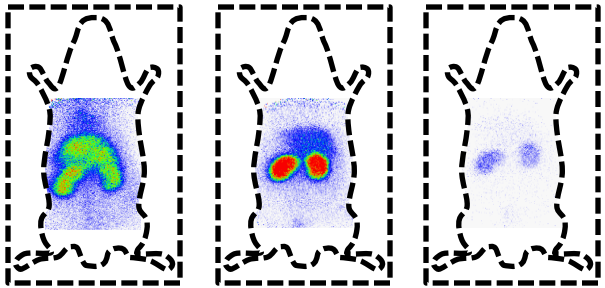
Conjugates: *In-vivo* rodent studies

Free HER2-Nb

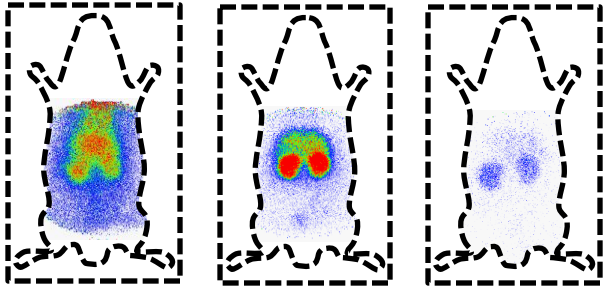


Nanobody-K9





Nanobody-E9



Nanobody-R9

Outlook

- → In-depth analysis of promising liposomal formulations (efficacy testing)
- → Uptake mechanism and efficacy testing of conjugates
- → Endosomal processing of conjugates
- → Qualitative analysis of conjugates *in-vivo*
- → HER2 Tumor model

Thank you for your attention!

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Phospholipid Research Center

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