Red Blood Cell Assisted Protein Delivery (RAPiD): Transfer of Protein Cargo from Red Blood Cell to Vascular Surfaces

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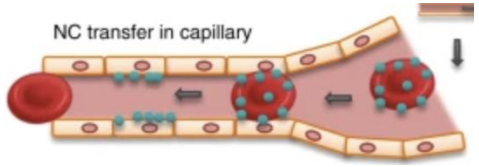
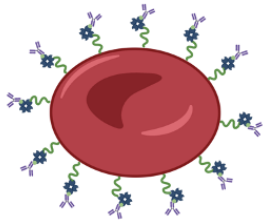
&THERAPEUTICS

Outline

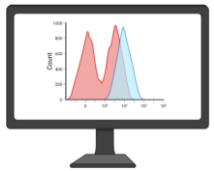
- Red blood cell-hitchhiking (RH) for nanocarriers (NCs) targeted delivery



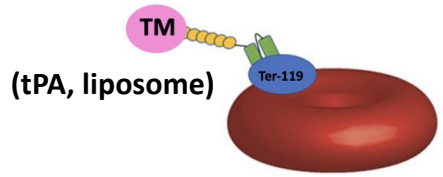
- **Biotin/streptavidin (SA) bridge: RBC-assisted protein delivery (RAPiD) to vascular surface**

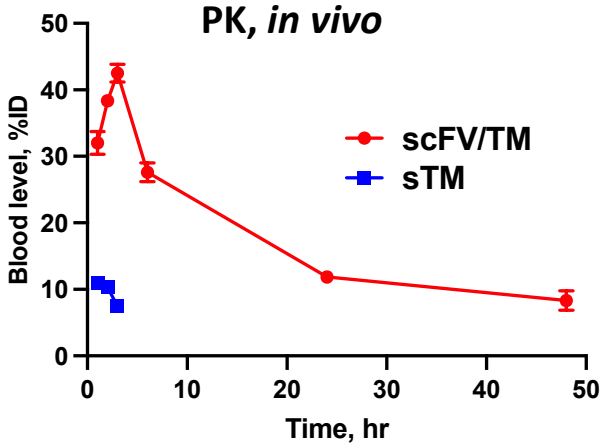


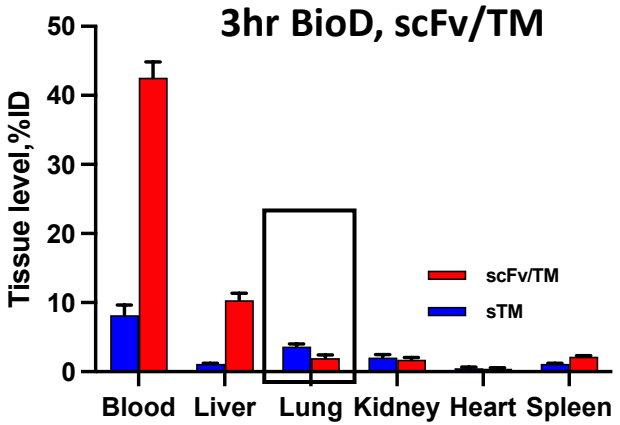
- Potential application



Affinity binding of antibody/scFv to RBC surface prolongs cargo circulation



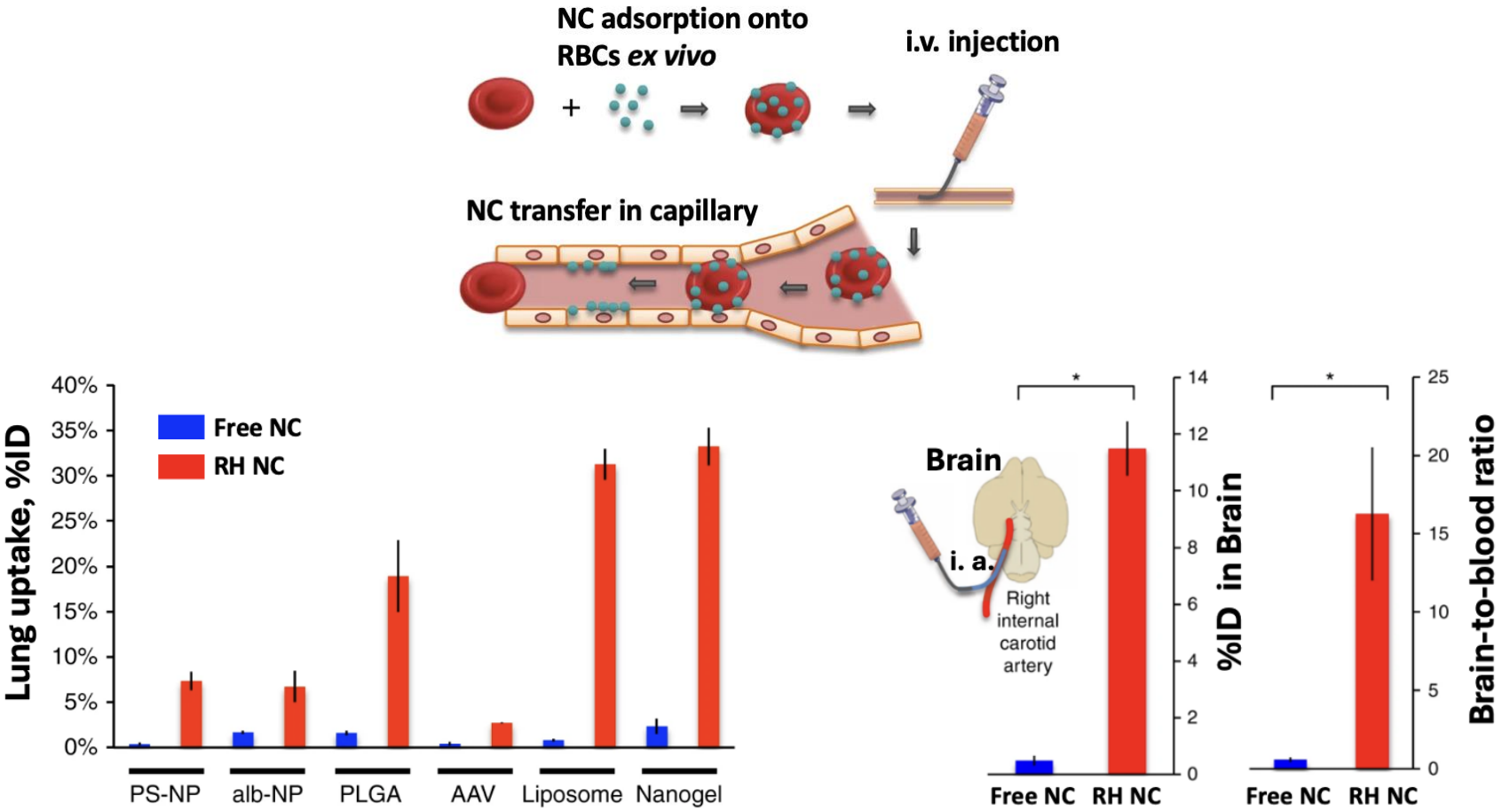




TM: thrombomodulin
scFv: single-chain variable fragment

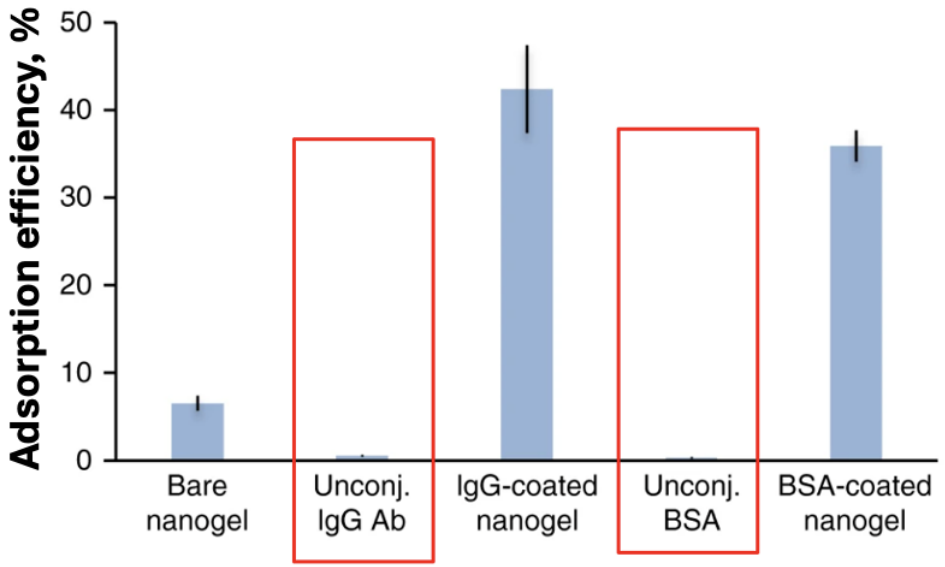
Zaitsev S., et al., JPET, 2010
Zaitsev S., et al., Blood, 2012

RBC-hitchhiking (RH) boosts delivery of NCs to targeted organs: orders of magnitude improvement



Brenner J., et al., Nat Commun., 2018

Free IgG or BSA fails to adsorb onto RBCs, preventing hitchhiking delivery of protein cargo



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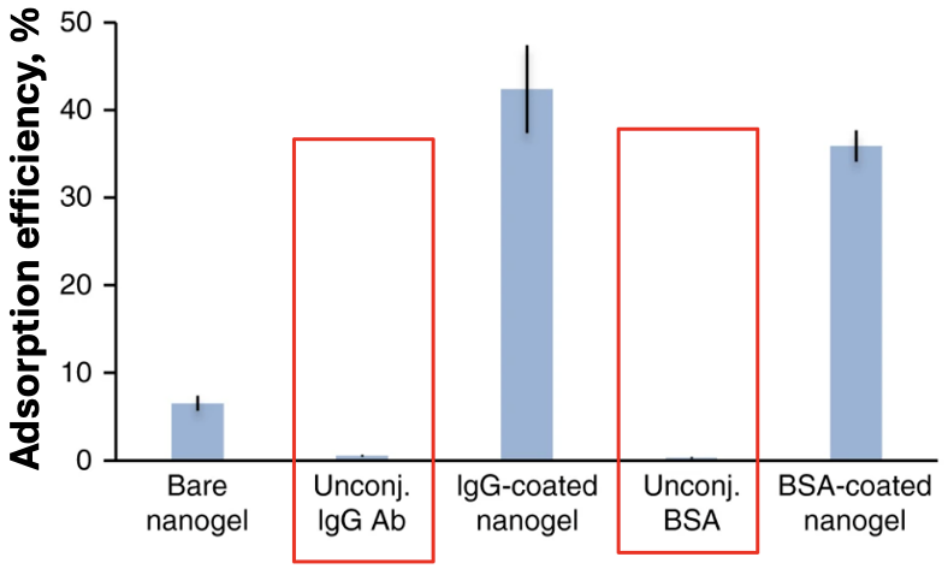
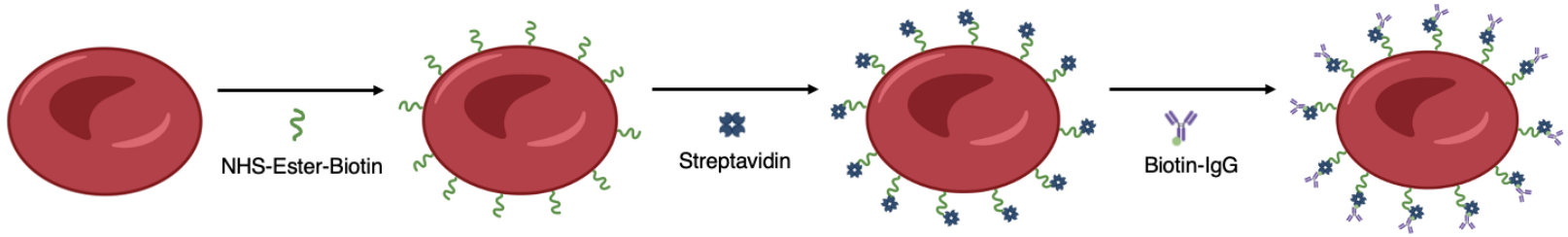


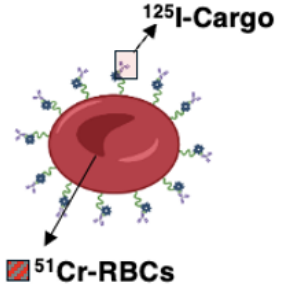
Is it possible to hitchhike protein cargos on RBCs for organ delivery using other conjugation strategies?

Brenner J., et al., Nat Commun., 2018

RBC-IgG conjugation via biotin/Streptavidin (SA) bridge



Gentle RBC biotinylation (5 µM) shows cargo organ retention: *depending on the first-pass organ (RAPiD)*



RBC-b/SA/IgG
(biotin, 5uM)

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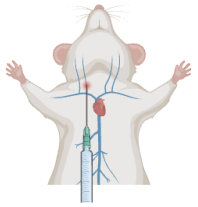
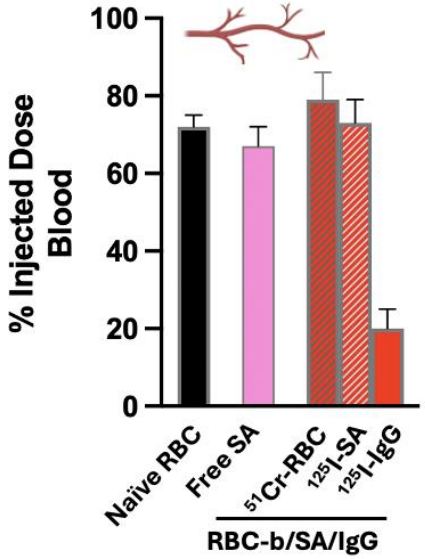
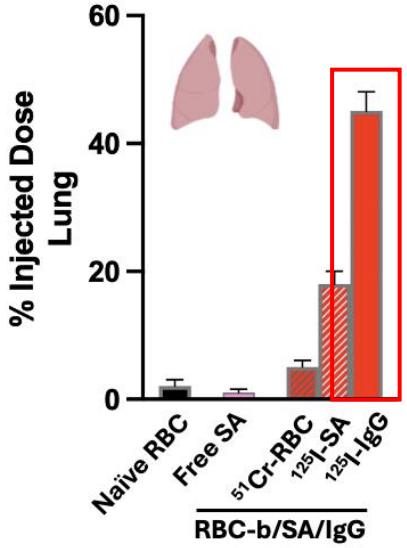
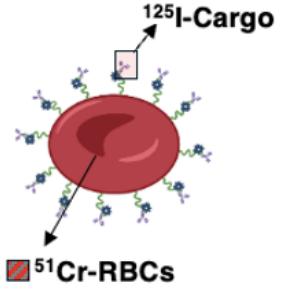


Jugular i.v







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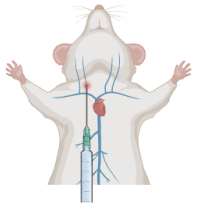

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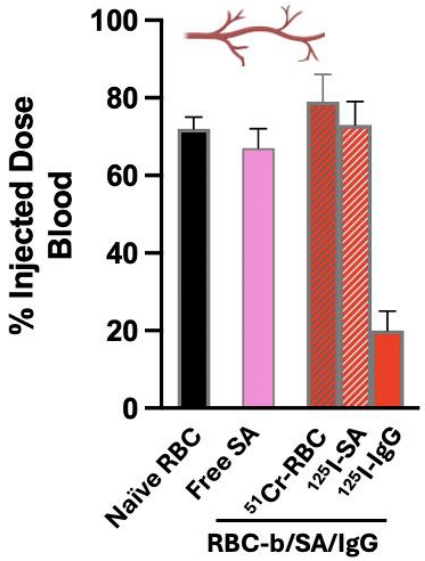

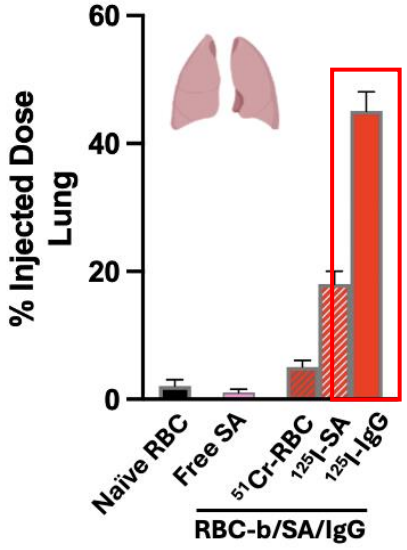

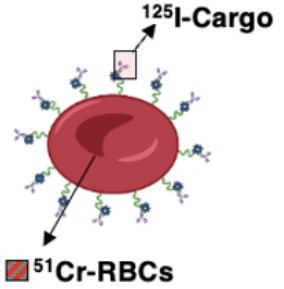

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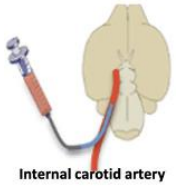
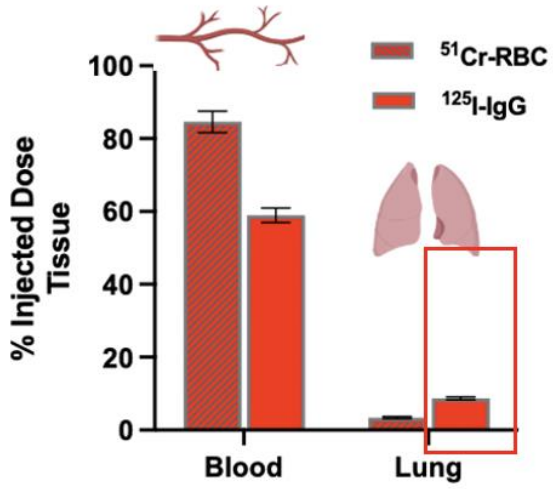
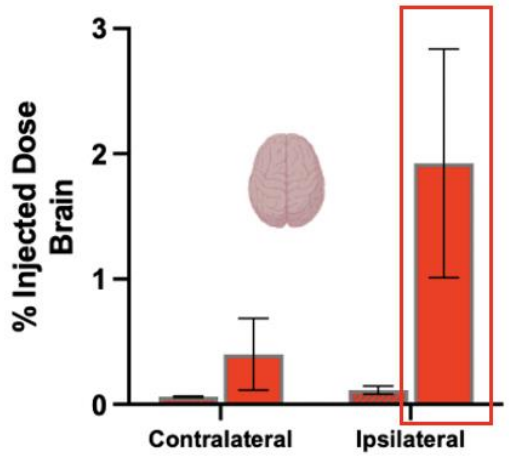
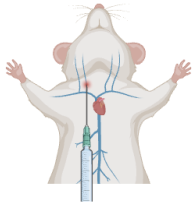


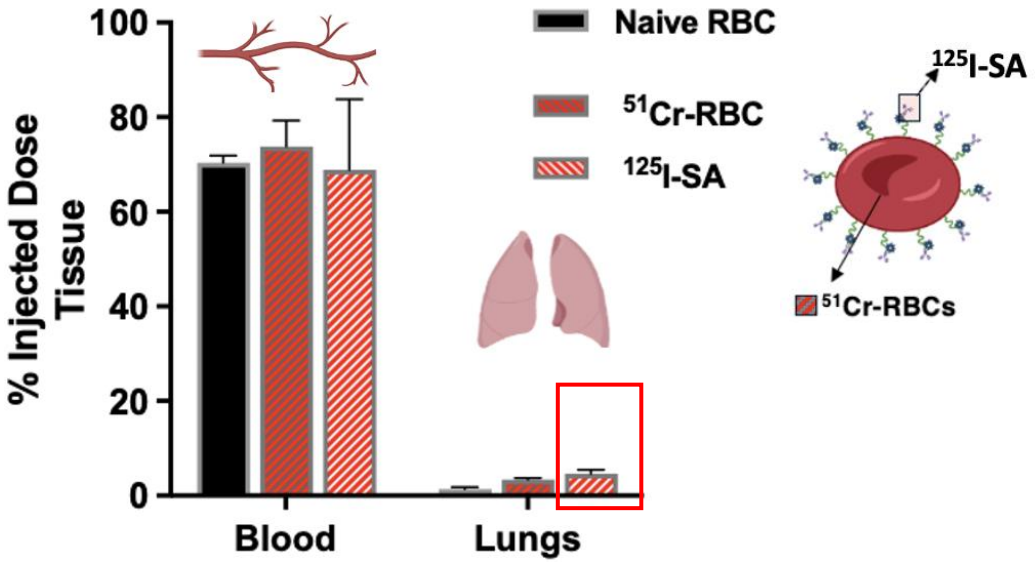


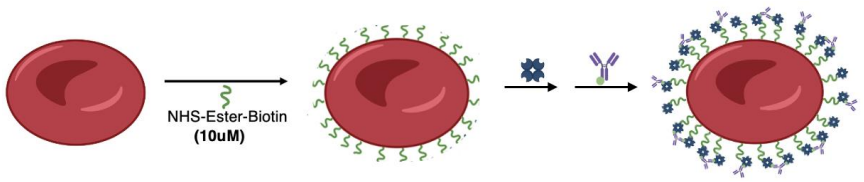


Higher RBC biotinylation (10µM) markedly diminishes lung cargo retention



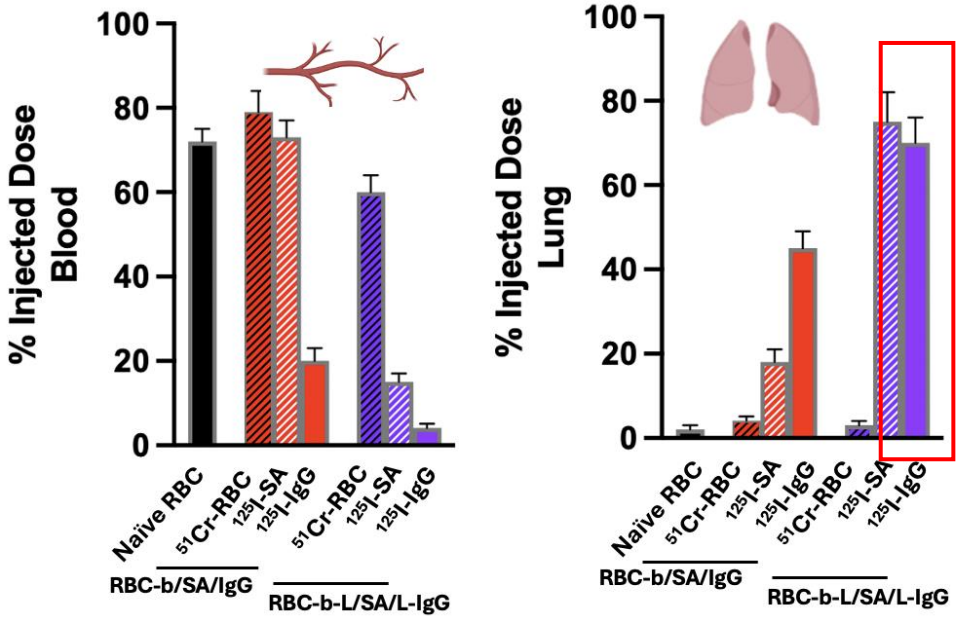
Jugular i.v

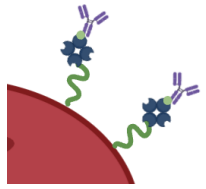




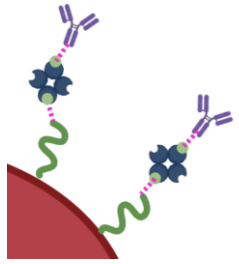
RAPiD cargo transfer

- **Enhanced by longer biotin linker**

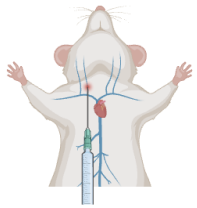




RBC-b/SA/IgG



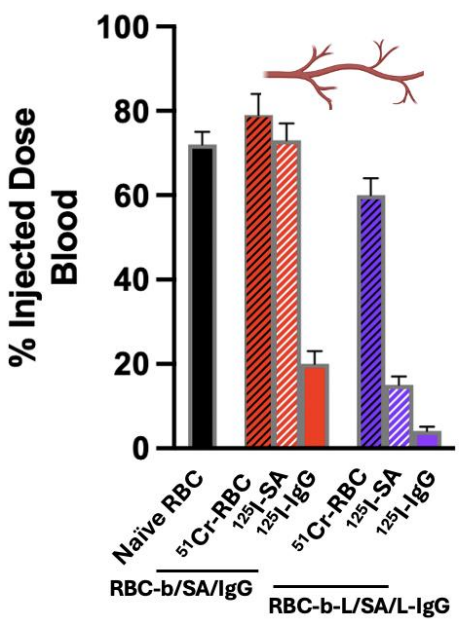
RBC-b-L/SA/L-IgG
L: linker

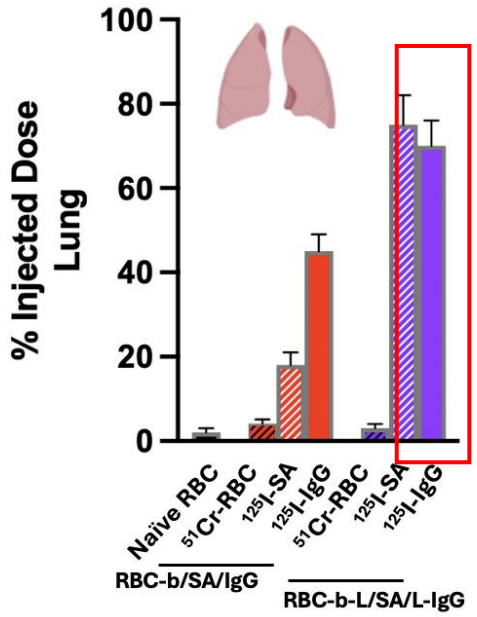


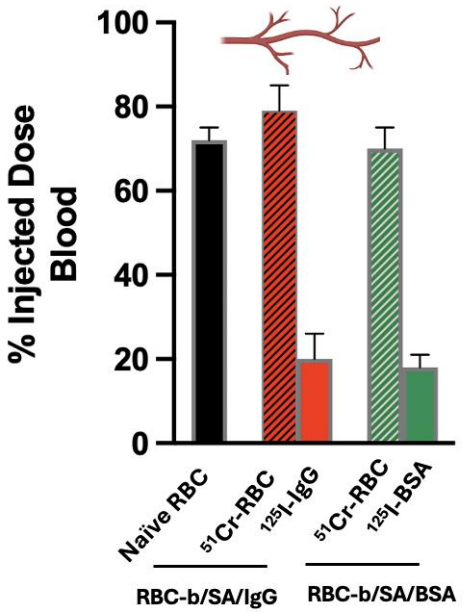
Jugular i.v

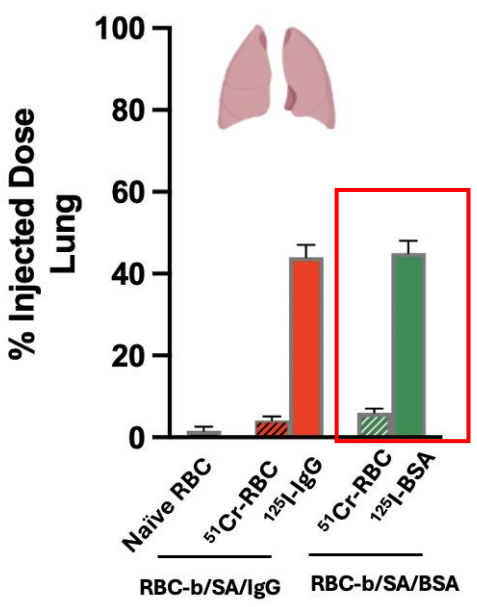
RAPiD cargo transfer

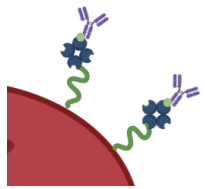
- **Enhanced by longer biotin linker**
- **Fc-independent**



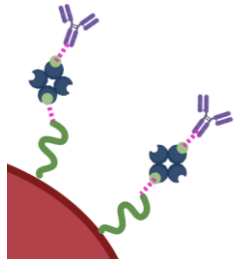




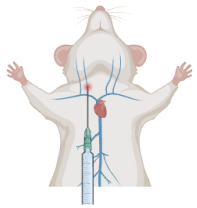




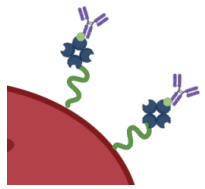
RBC-b/SA/IgG



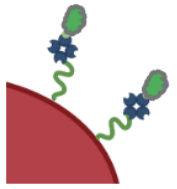
RBC-b-L/SA/L-IgG
L: linker



Jugular i.v



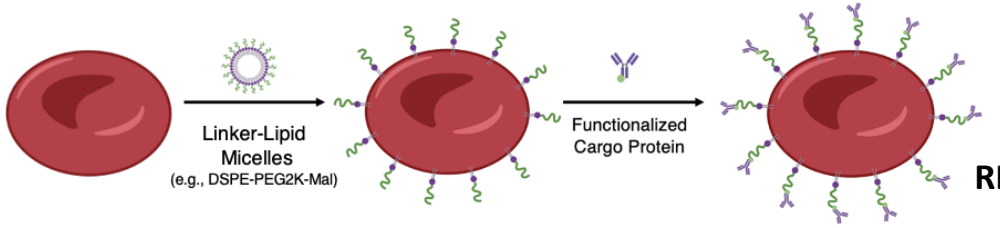
RBC-b/SA/IgG



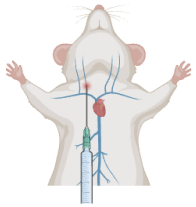
RBC-b/SA/BSA

Is biotin/SA linkage the deciding factor for the RAPiD cargo transfer?

Protein cargo conjugated to RBC via lipid painting shows the minimal retention in lung



RBC-lipid-IgG



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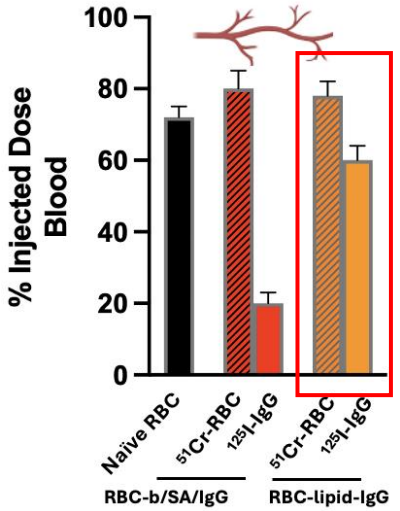
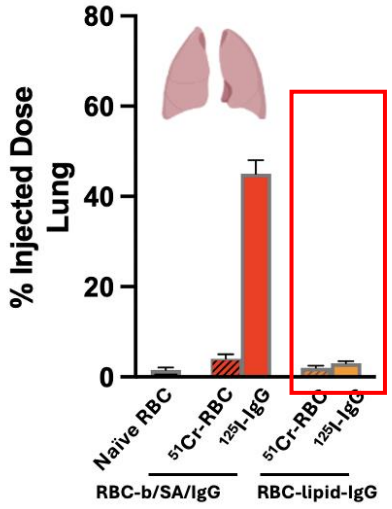
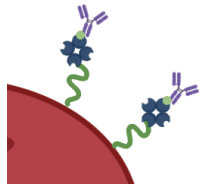
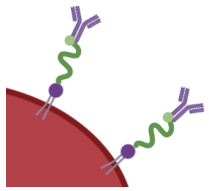






RBC-b/SA/IgG



RBC-lipid-IgG

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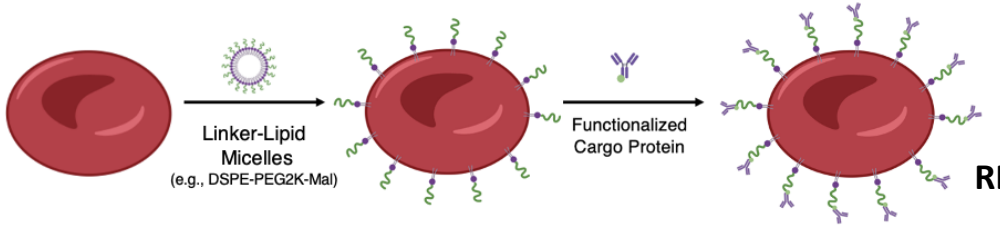

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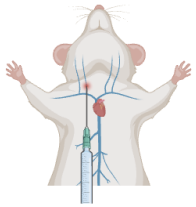

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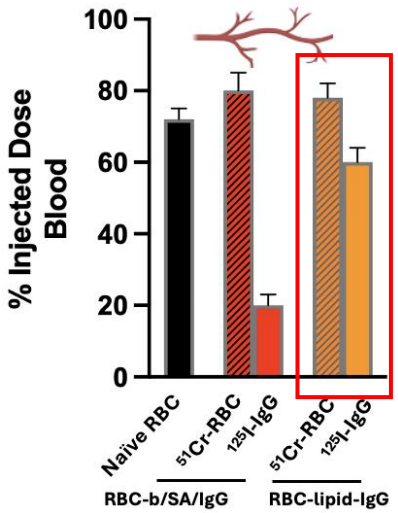
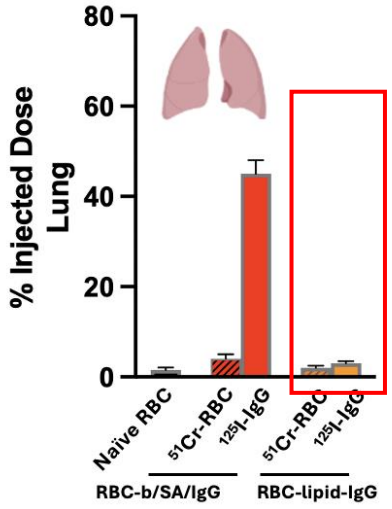
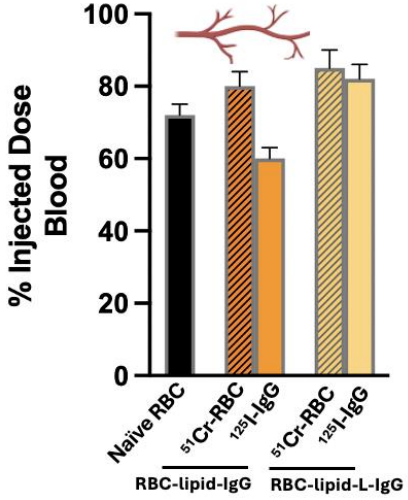
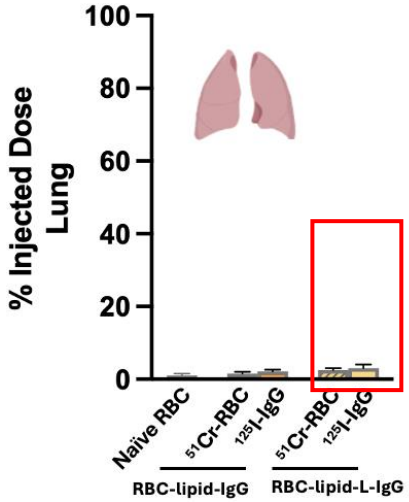
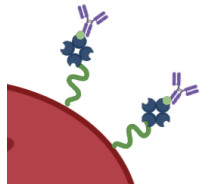


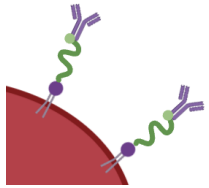




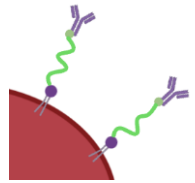




RBC-b/SA/IgG

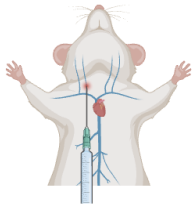


RBC-lipid-IgG

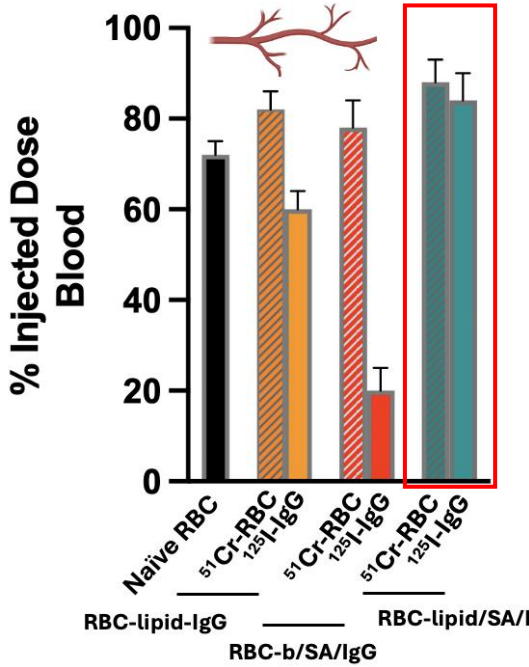


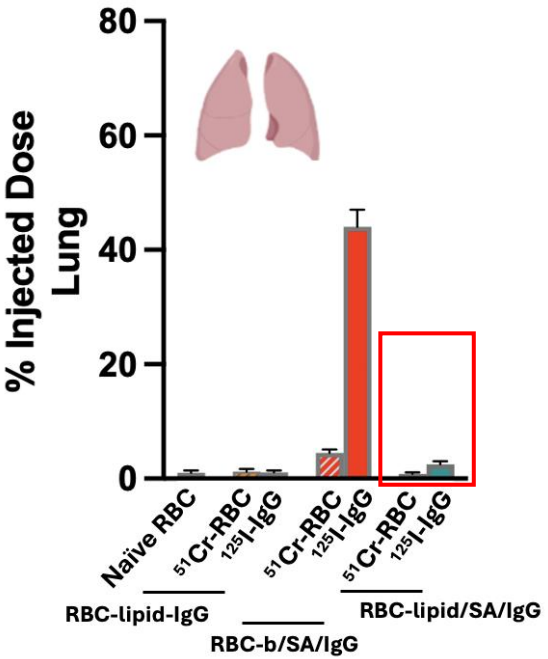
RBC-lipid-L-IgG
L: PEG 5000
linker

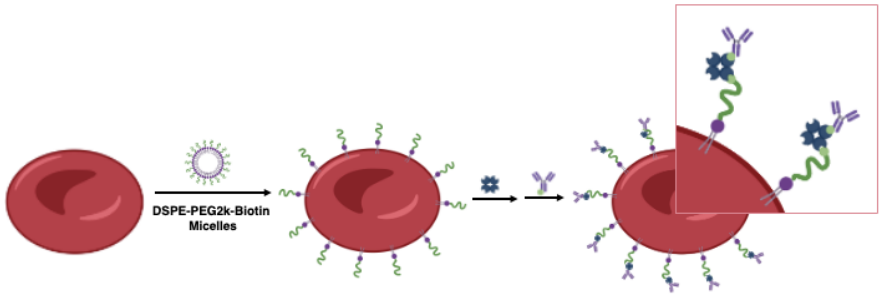
Hybrid structure of $RBC_{lipid\text{-}Biotin}$/SA/IgG-b shows no lung transfer

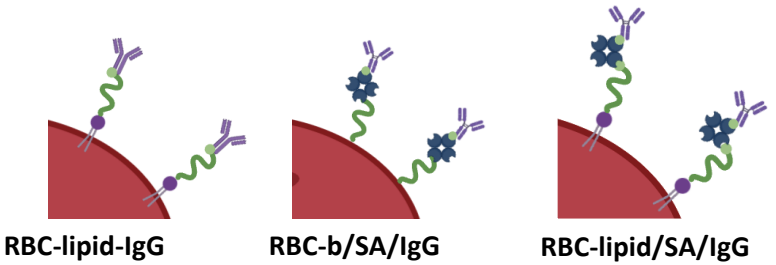


Jugular i.v









Is biotin/SA linkage the deciding factor for the RAPiD cargo transfer?

Conclusion

- The **biotin/SA platform** enables **RAPiD** transfer of protein cargo to downstream vasculature while preserving carrier RBC integrity;
- RAPiD cargo transfer can be controlled by modulating the **parameters** of biotin/SA bridge;
- RAPiD transfer accommodates **diverse protein cargoes**, including tPA and TM, underscoring its potential for inflammatory vascular disease applications.
- Next, we will identify the RBC membrane components that are critical for the RAPiD event.

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