

Vectorisation par nanobodies (VHHs)

Supramolecular Heterodimer Assembly for Nanoparticles Functionalization

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ICANS

INSTITUT DE CANCÉROLOGIE

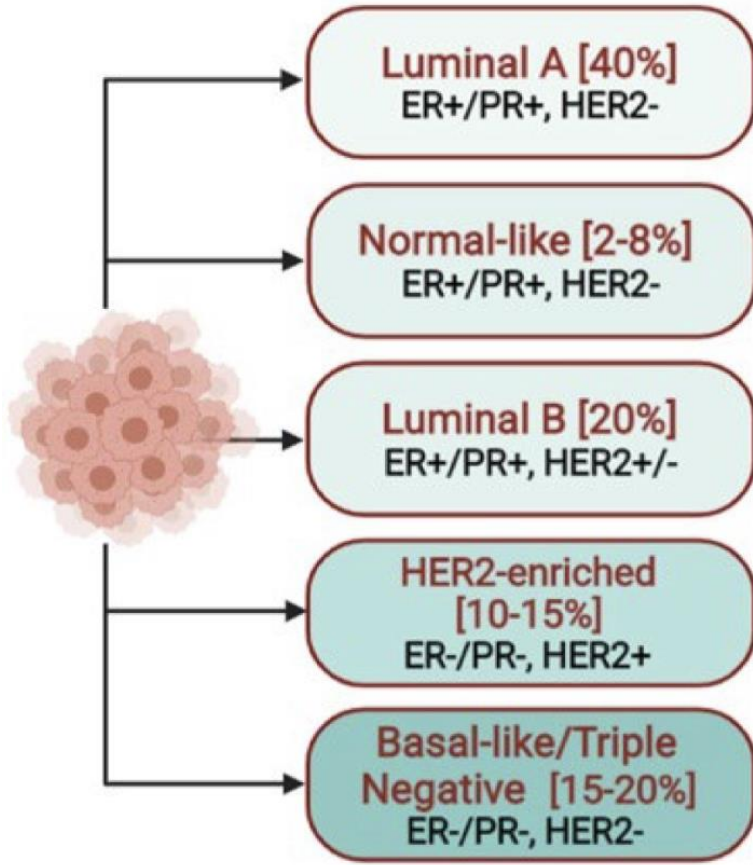
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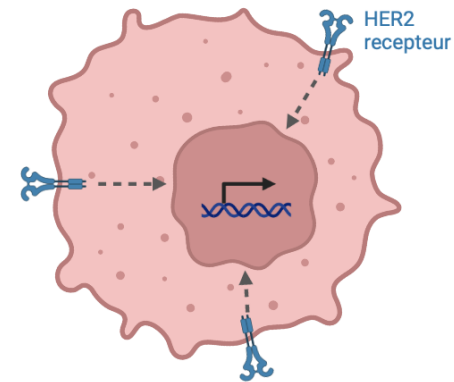
Introduction

Therapeutic target

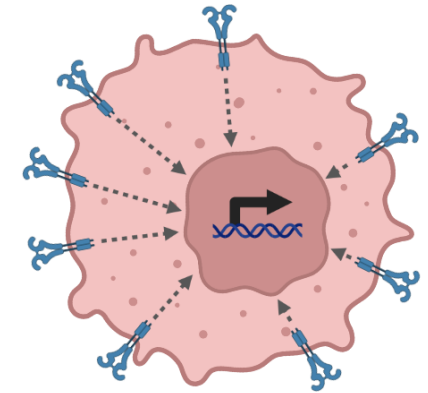
Different types of breast cancer



Healthy breast cells



Cancer breast cells



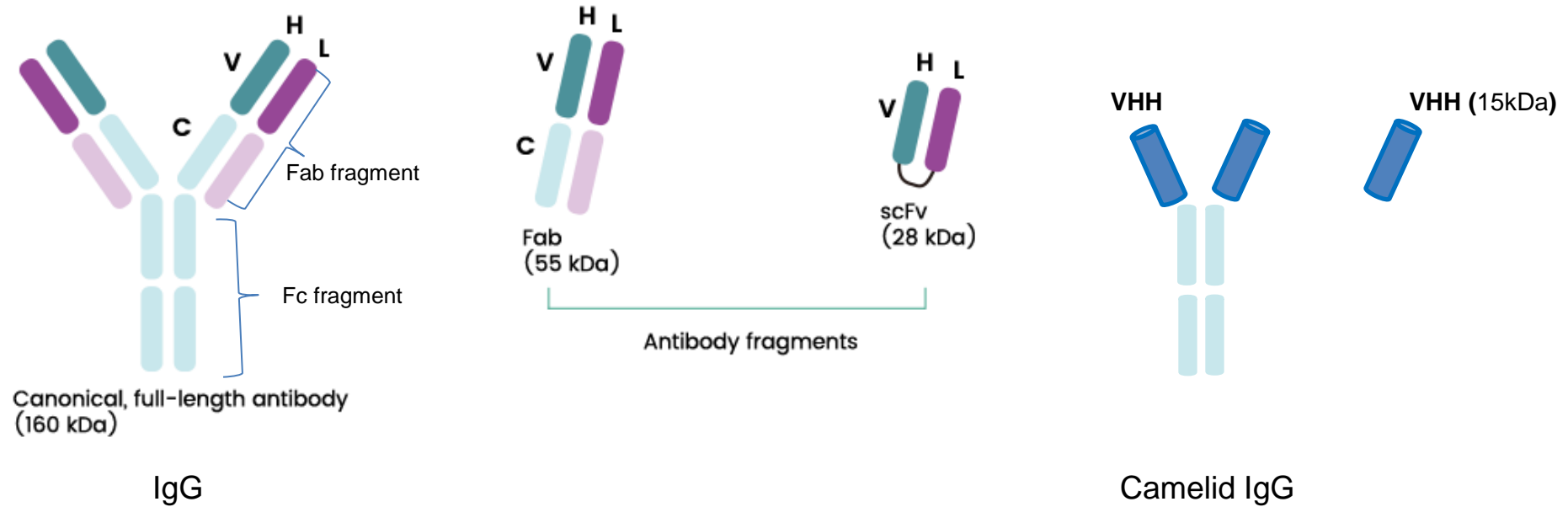
➔ Focusing on the breast cells cancer overexpressing HER2



Need to have a targeting strategy

Introduction

Therapeutic Antibodies

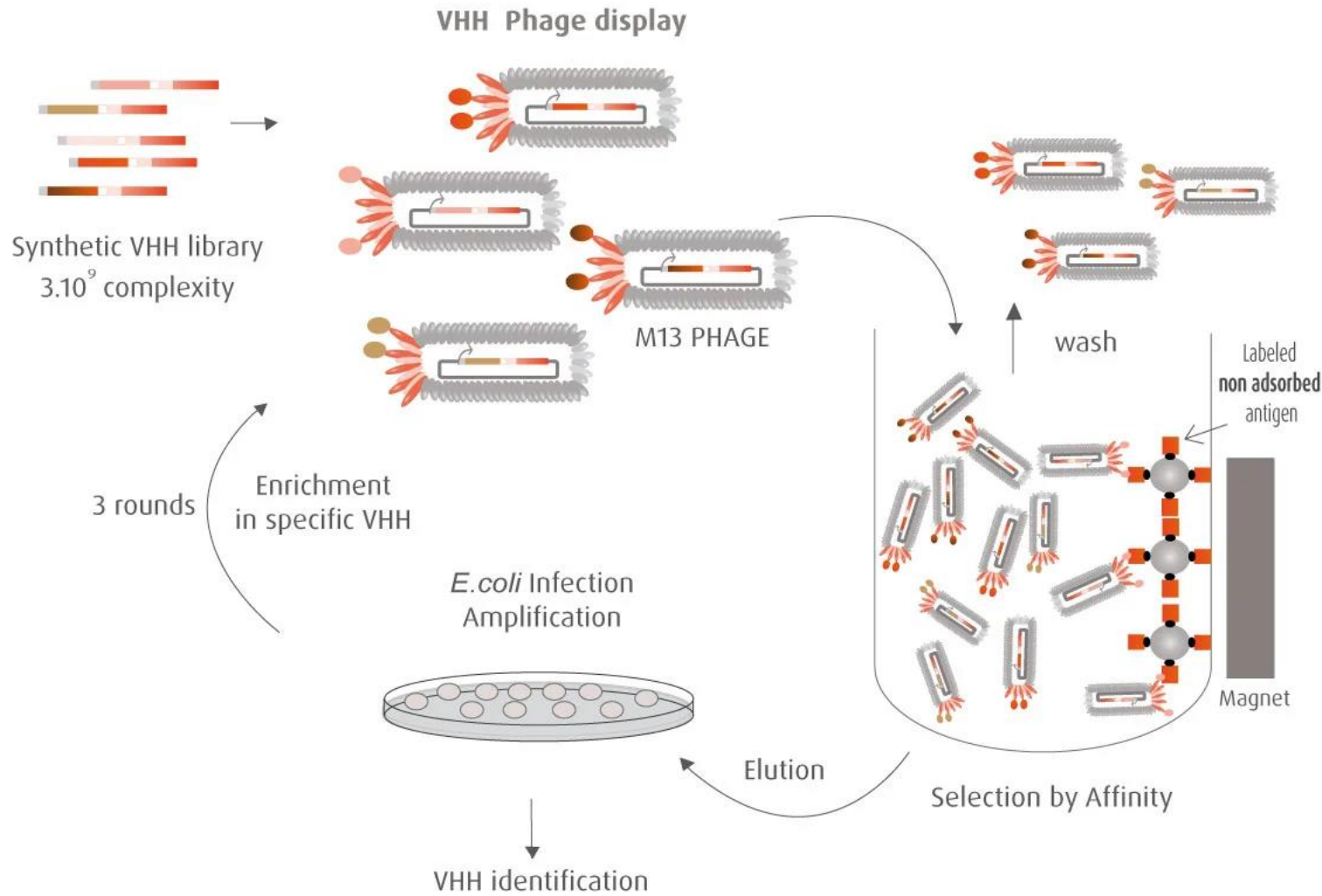


Why wanting to go for fragments or VHH

- Lower cost
- Higher affinity
- More versatility due to biotechnological advances such as VHH phage display

Introduction

VHH Phage display



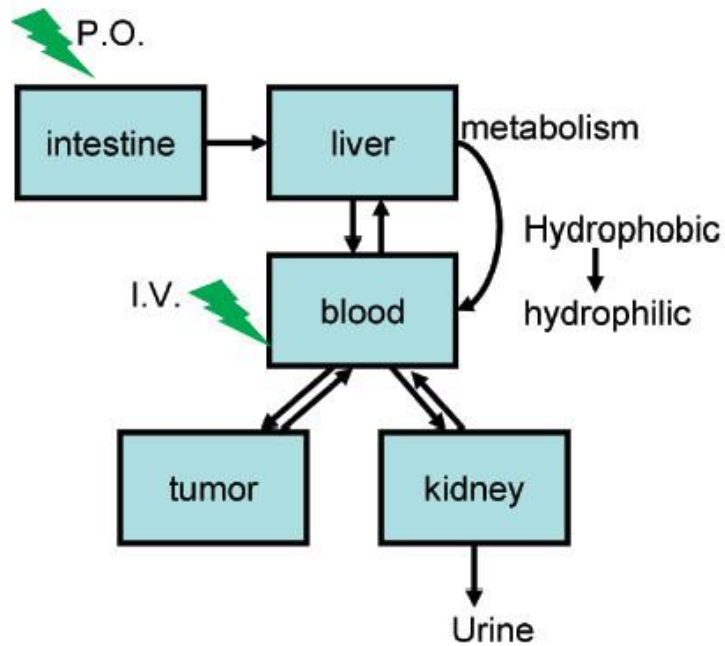
Advantages

- Fast screening for the most efficient VHH sequence
- Possibility to fuse the sequence with other proteins
- Possibility to Tag the sequence for further chemical modifications

Introduction

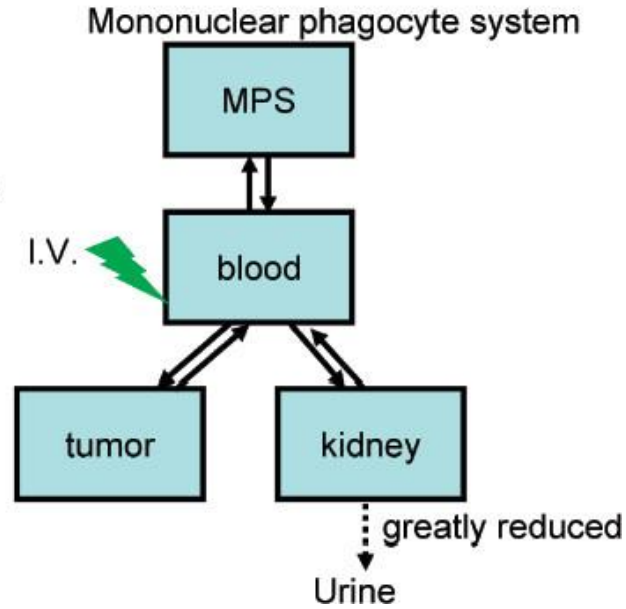
Why nanoparticle encapsulation is of utmost importance for drug delivery

Small molecular drug



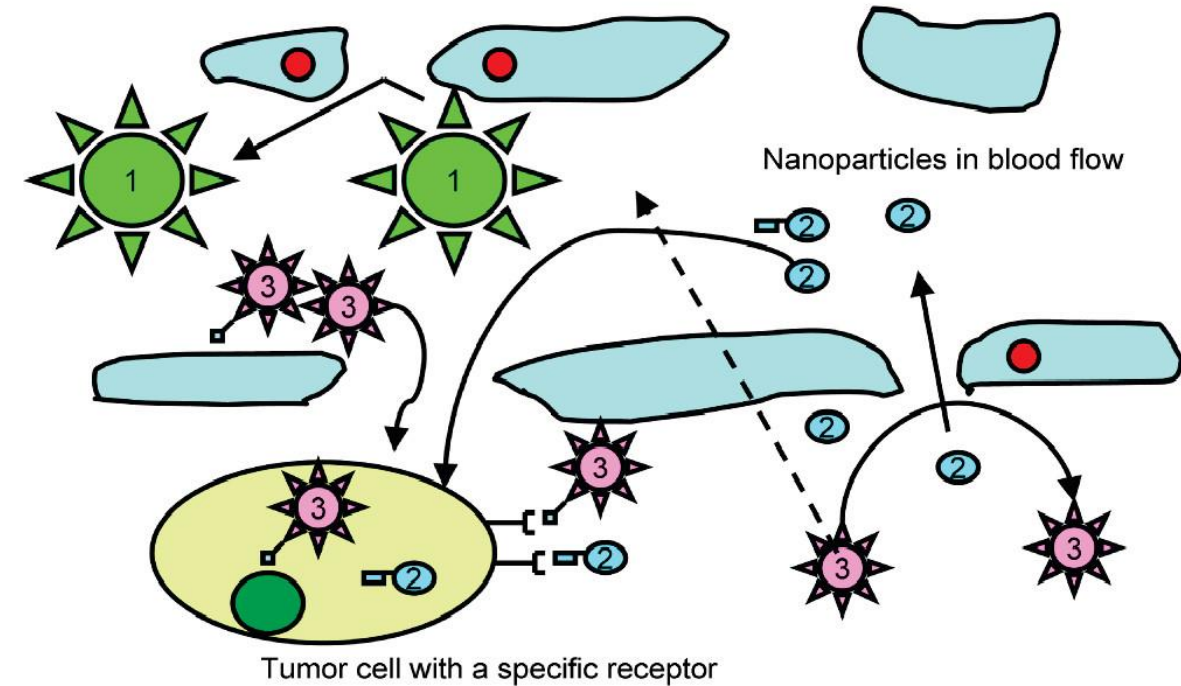
- Rapid clearance from the liver
- Low efficiency

Nanoparticles



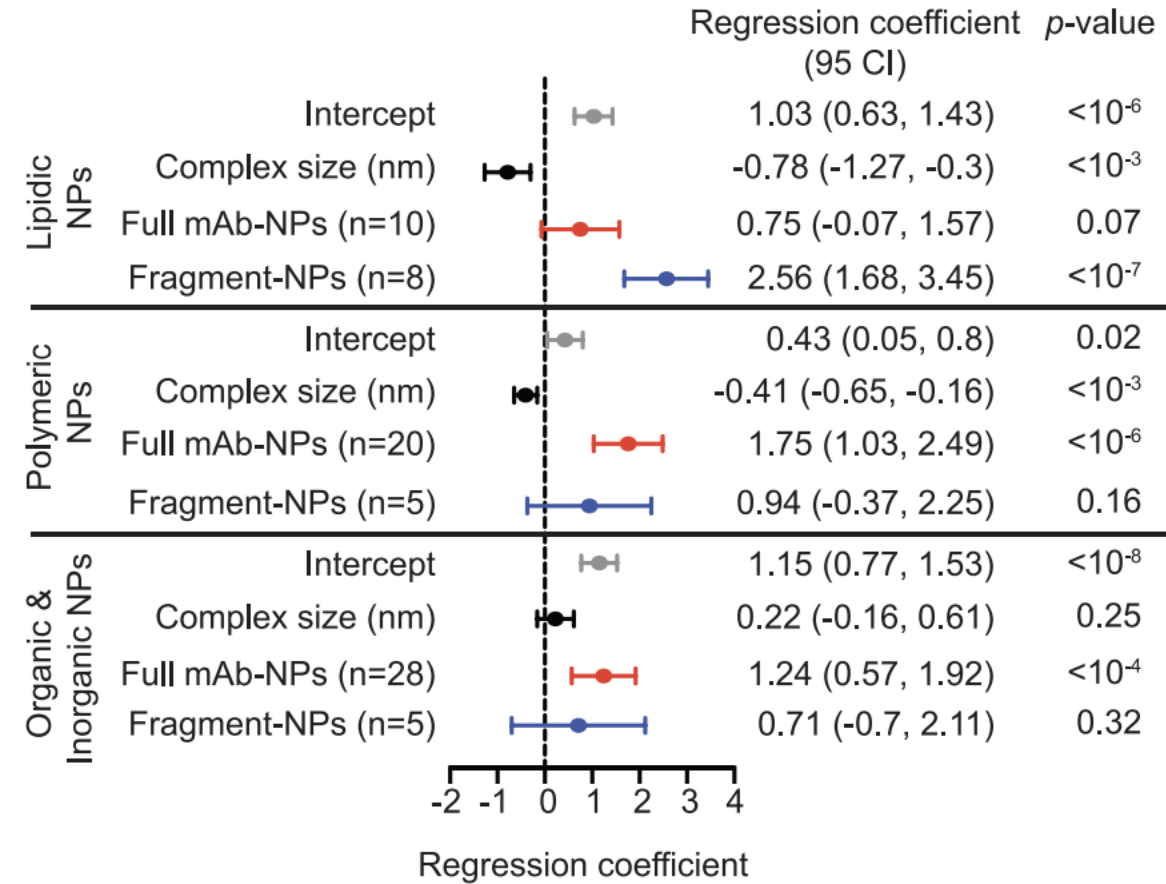
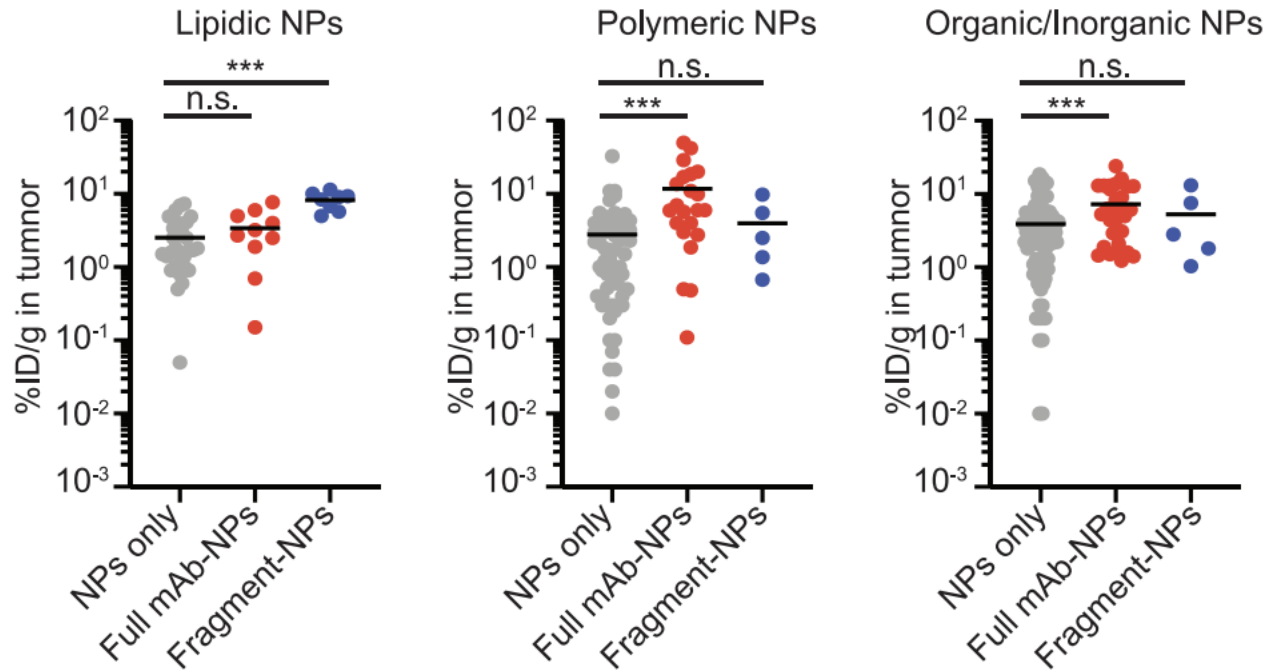
- PEGylation reduces MPS uptake
- Protection against the liver metabolizing enzyme
- Increased AUC for PK analysis between 3 to 36 times

Leaky endothelial wall with increased pore size in the tumor tissue

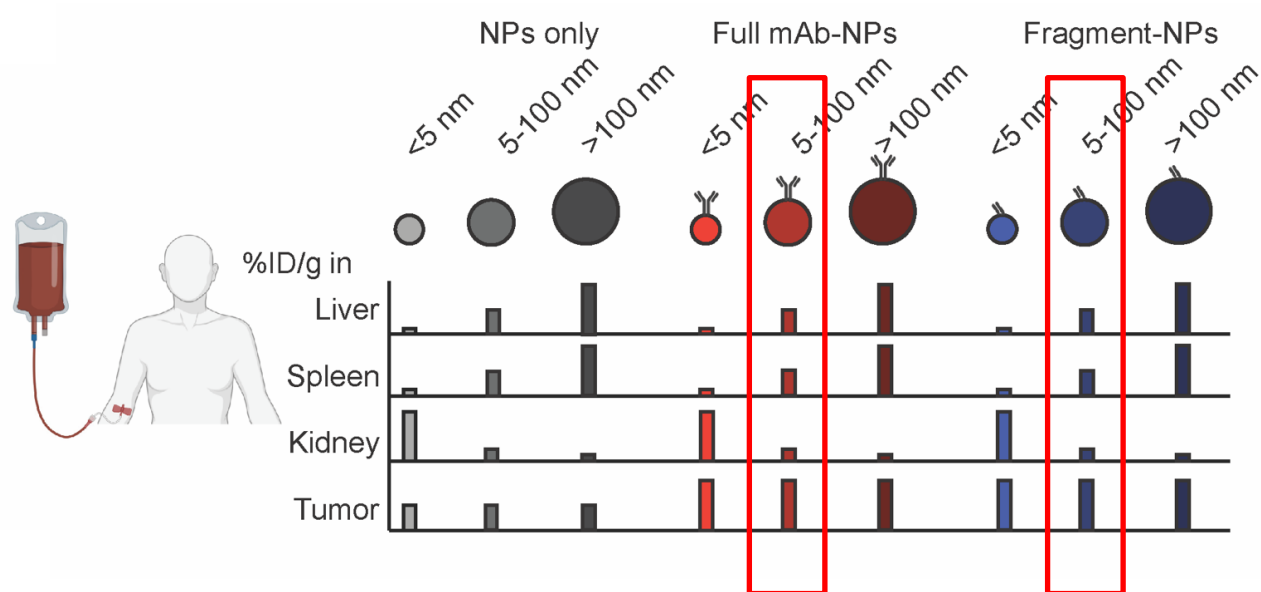
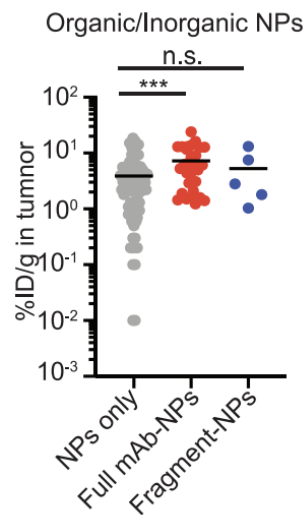
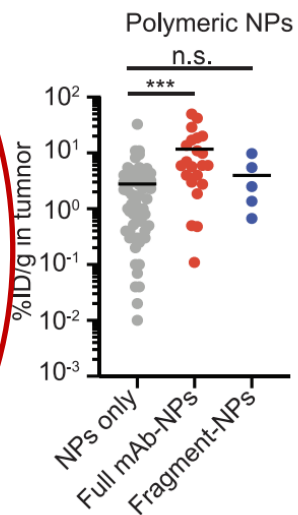
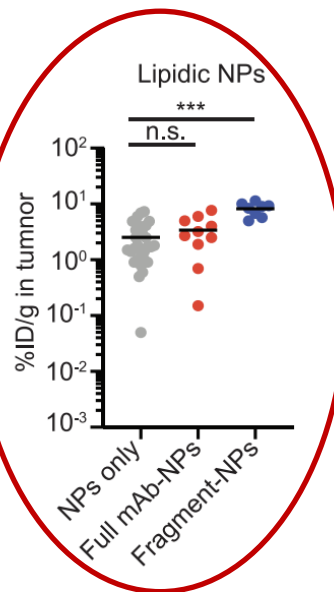


Better tumoral BD due to EPR effects

The NP material impacts the tumor uptake post-biofunctionalization

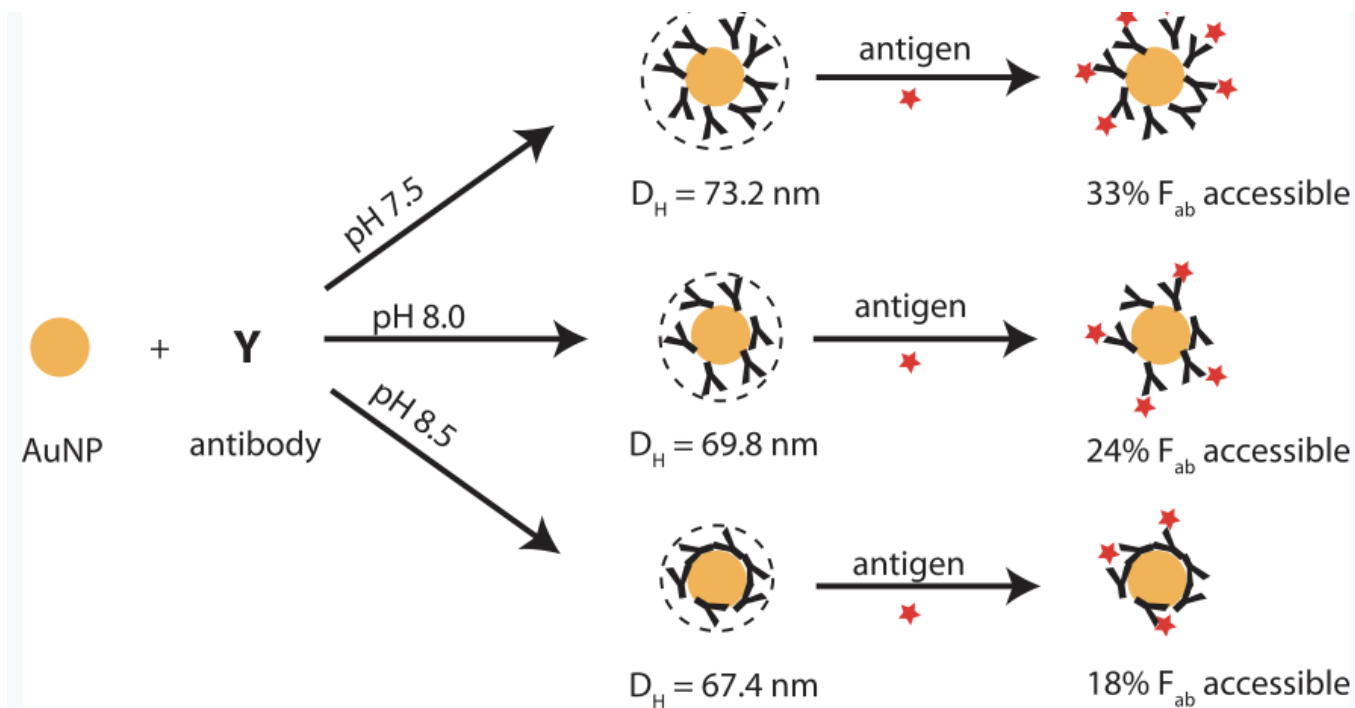


Interest of Antibody fragments (VHH)



**Same efficiency versus full mAb
But lower size**

Orientation of the VHH linked to Nanoparticle matters



Efficiency of the VHH is highly dependant on the orientation

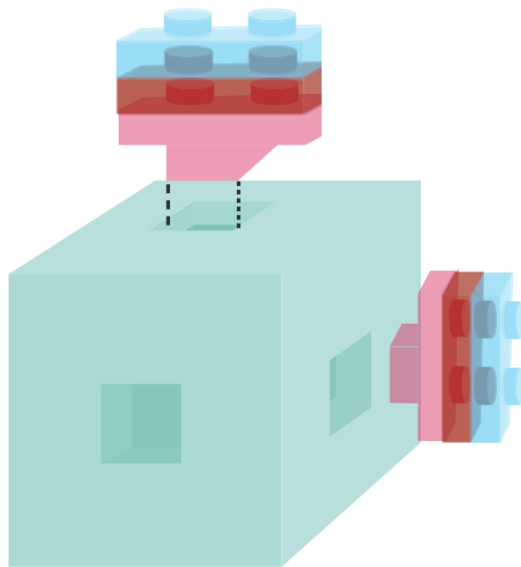
Therefore : need to think about

- Orientation
- Reproducibility of the synthesis
- Versatility of the targeting
- Modularity in the choice of the NP to address either therapeutic effects or diagnosis (Theragnostic)

The ideal construction : Lego

Schematic representation

Template of versatile targeting nanoparticles:



Components of the nanoparticle:



Organic or inorganic nanoparticle



K3



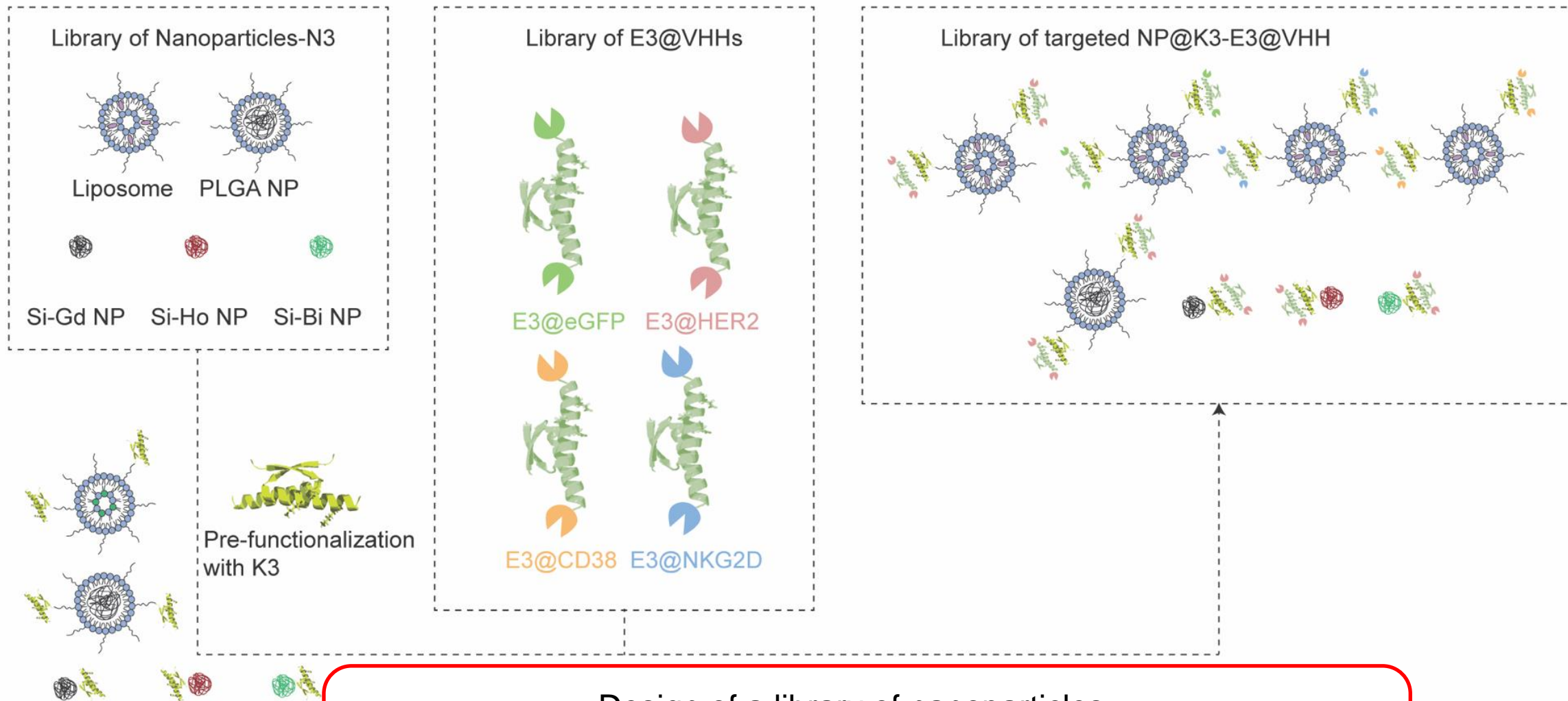
E3

Self assembling peptides derived from p53 self assembly tetramer



Single domain antibody (VHH)

Our ideal construction : Use of supramolecular assemblies

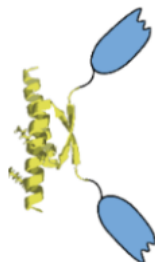


Design of a library of nanoparticles

Nanoparticle targeting both cancer or/and immune cells

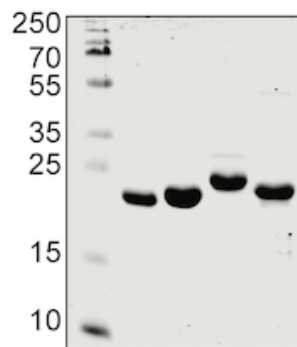
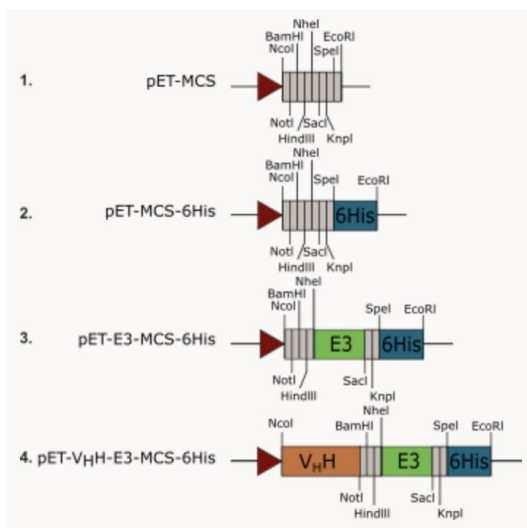
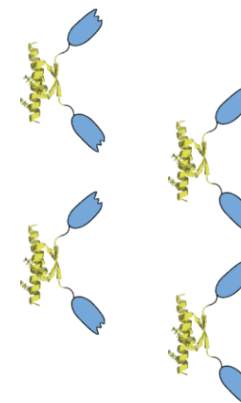
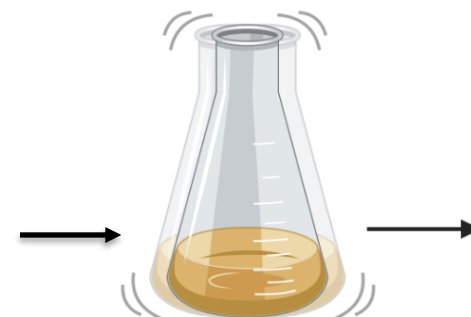
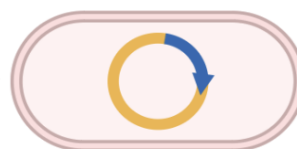
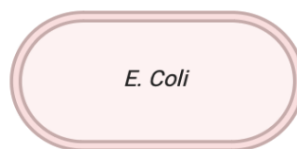
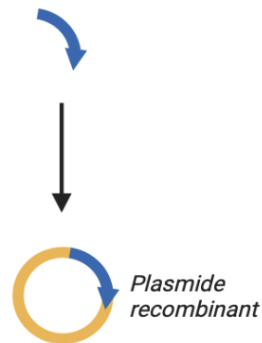
The E3-VHH building block

E3 peptide linked to NKG2D VHH:



U A C U C A A G U U C A

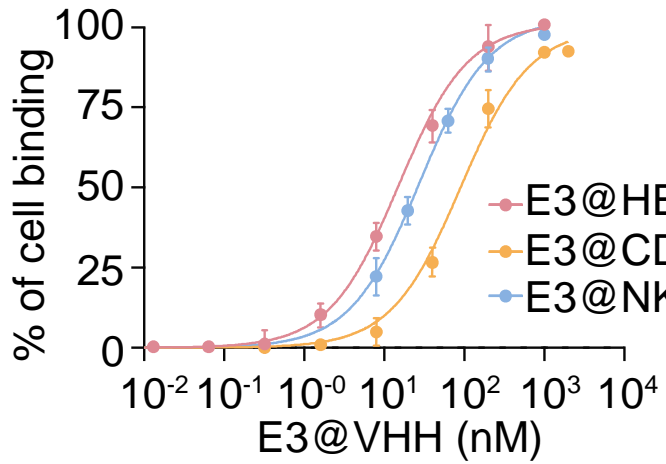
Collaboration with IGBMC



SDS PAGE gel

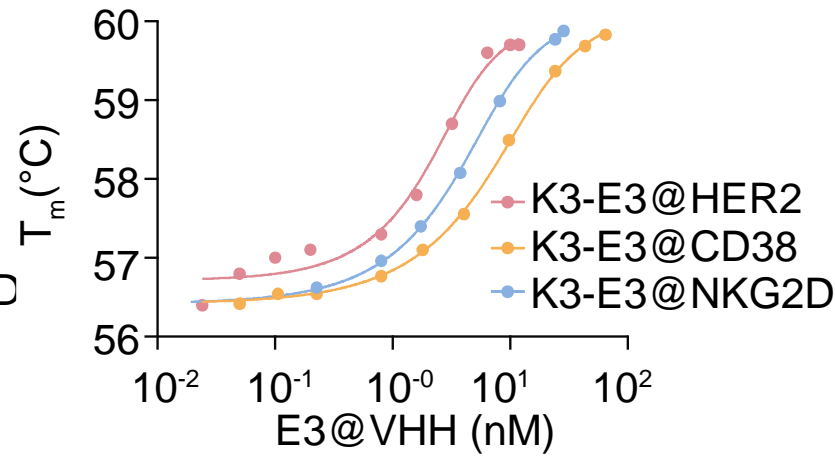
E3@eGFP
E3@HER2
E3@CD38
E3@NKG2D

The E3-VHH building block



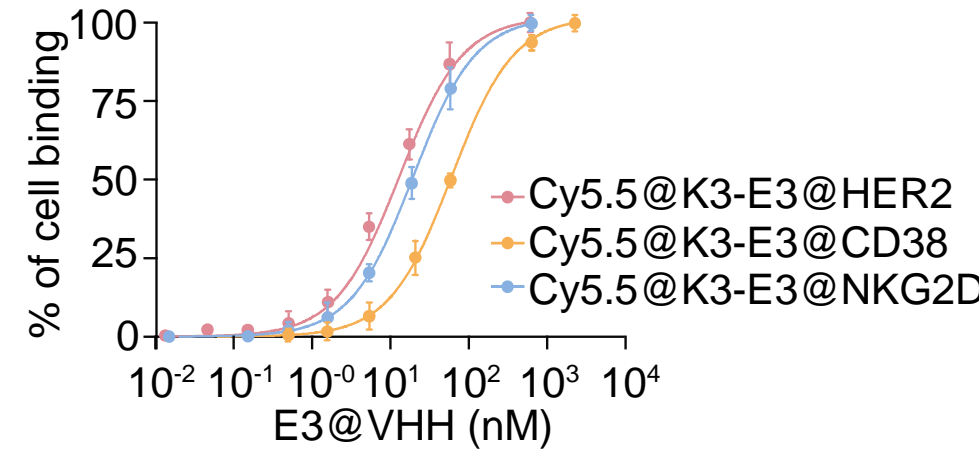
VHH specificity to Antigen

$10 < k_D < 50\text{nM}$



K3-E3@VHH auto assembly

$8 < k_D < 11\text{ nM}$

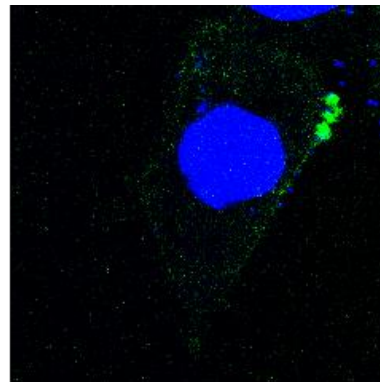
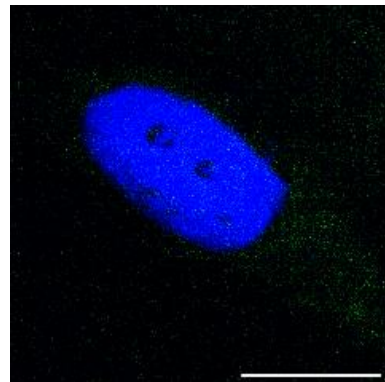


Hetero Tetramer@VHH specificity to Antigen

$10 < k_D < 50\text{nM}$

Cy5.5@K3-E3@HER2

MDA-MB_231 (Her2 low)



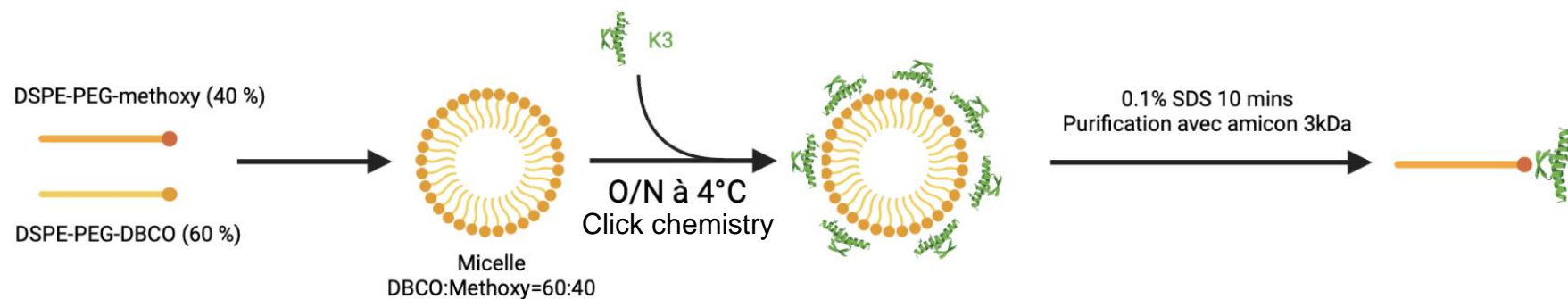
HCC1954 (Her2 high)

No loss of affinity
Keeps specificity

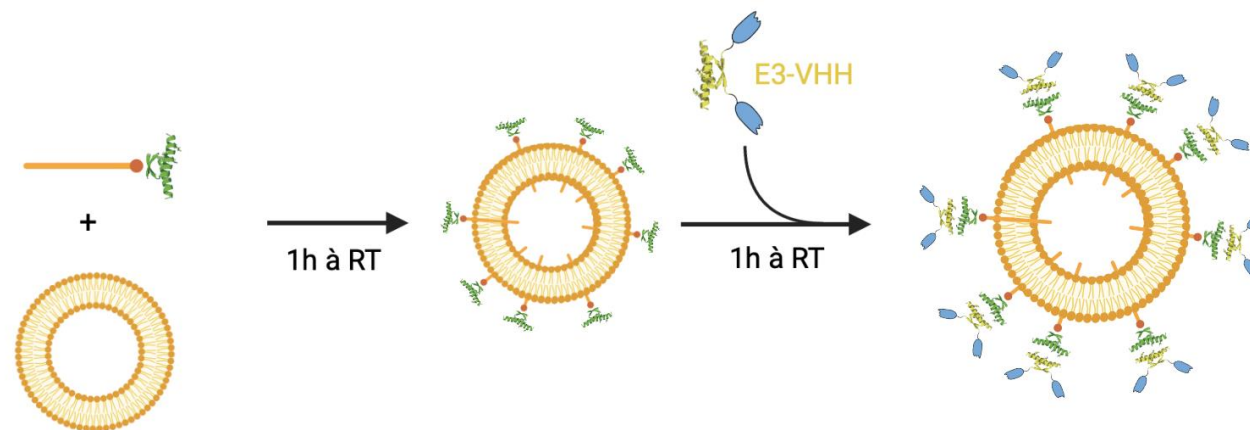
Our ideal construction : Use of supramolecular assemblies

Building of liposome based functionalized nanoparticle :

1st step :
Lipide-K3 formation

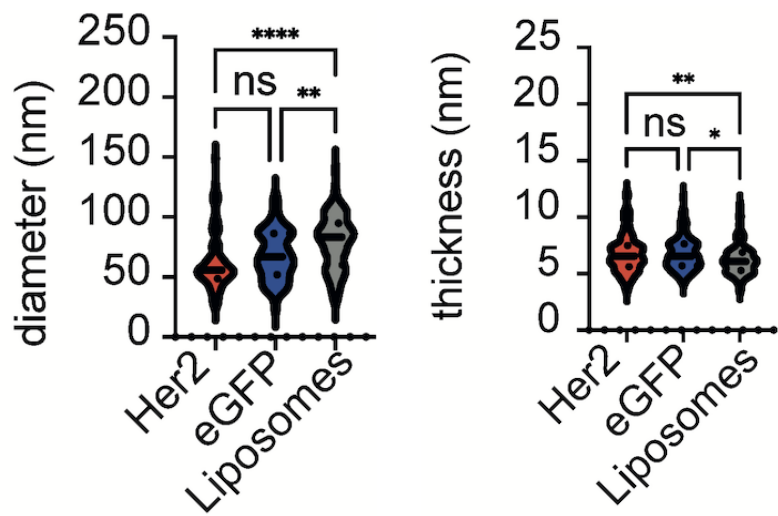
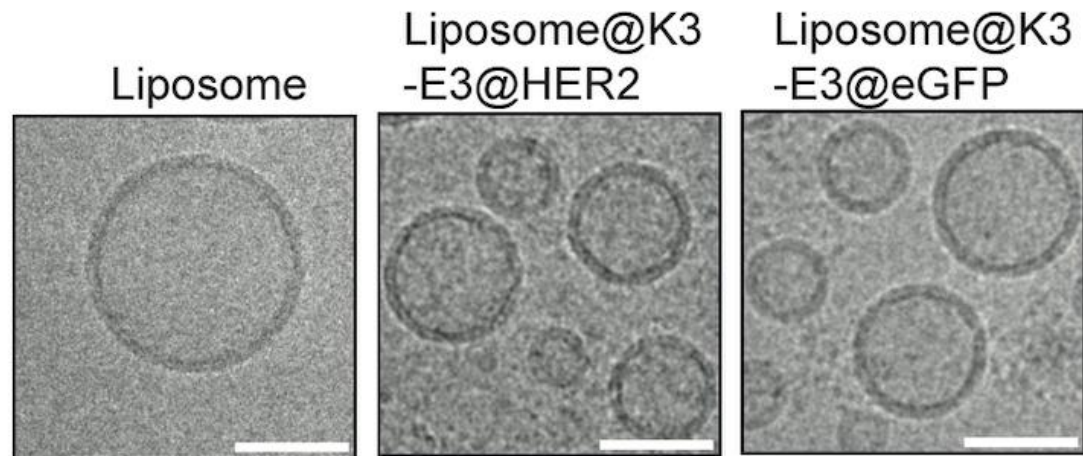


2nd step :
Nanoparticle Functionalization

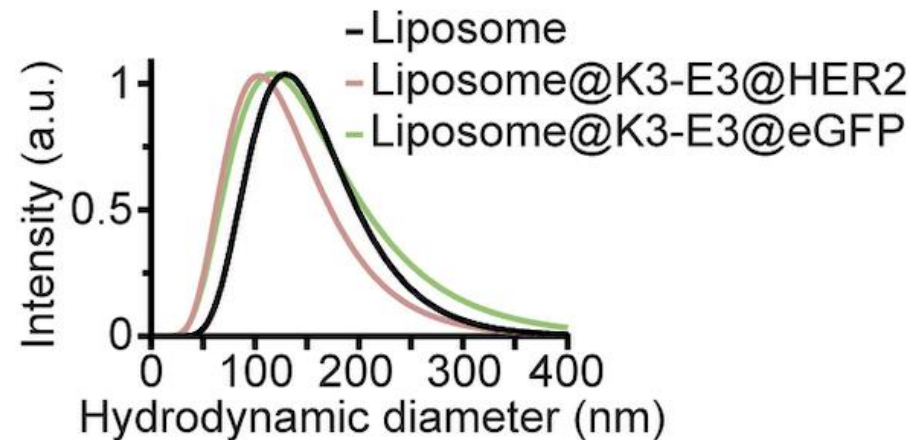


Liposome@K3-E3@VHH characterization

cryoTEM images of the liposomes

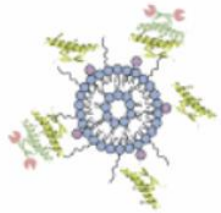


DLS measurements

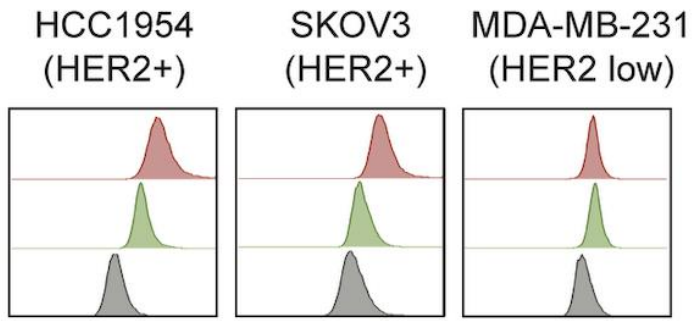


Average size around 100nm

In vitro specificity of the nanoparticles



Liposome@K3
-E3@HER2

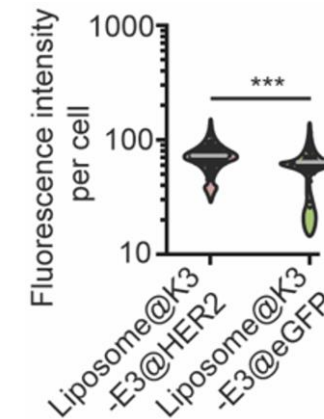
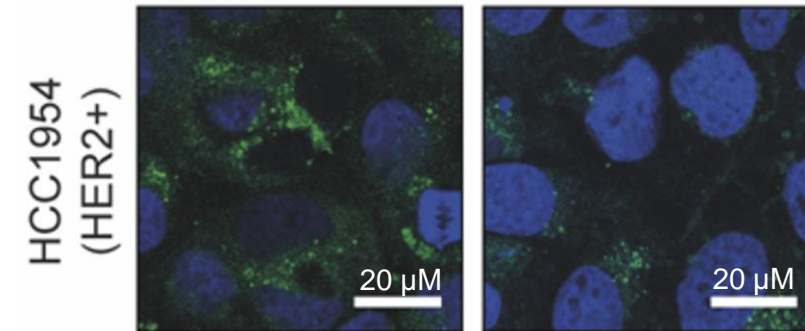


Liposome

- Untreated
- Liposome/K3-E3@HER2
- Liposome/K3-E3@eGFP

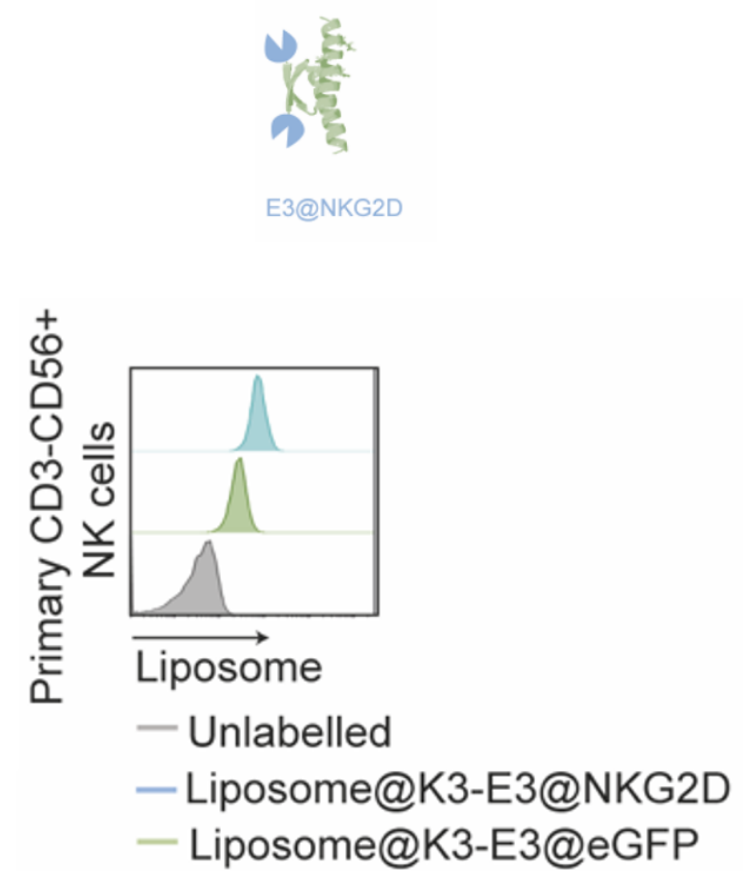
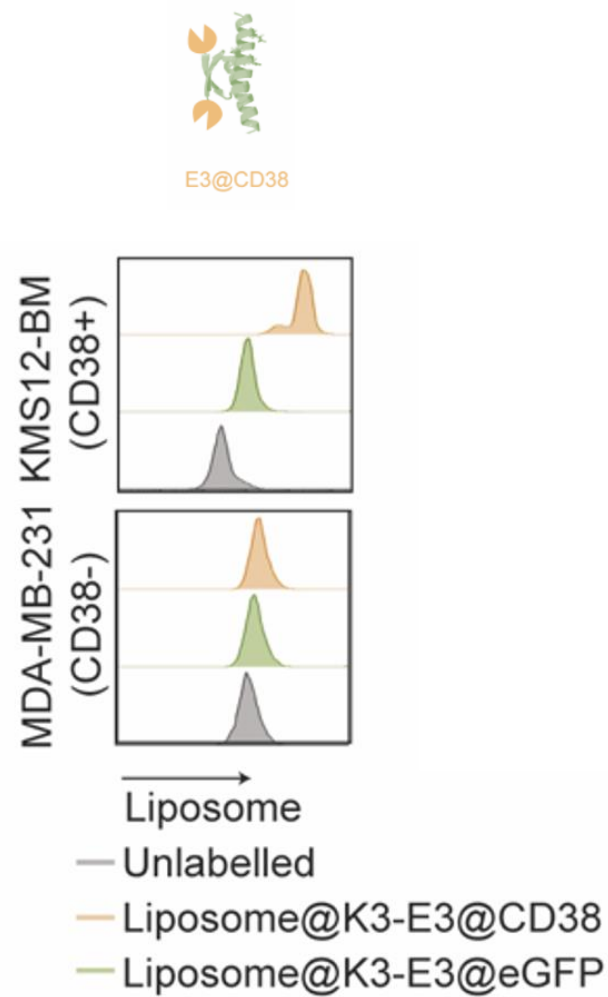
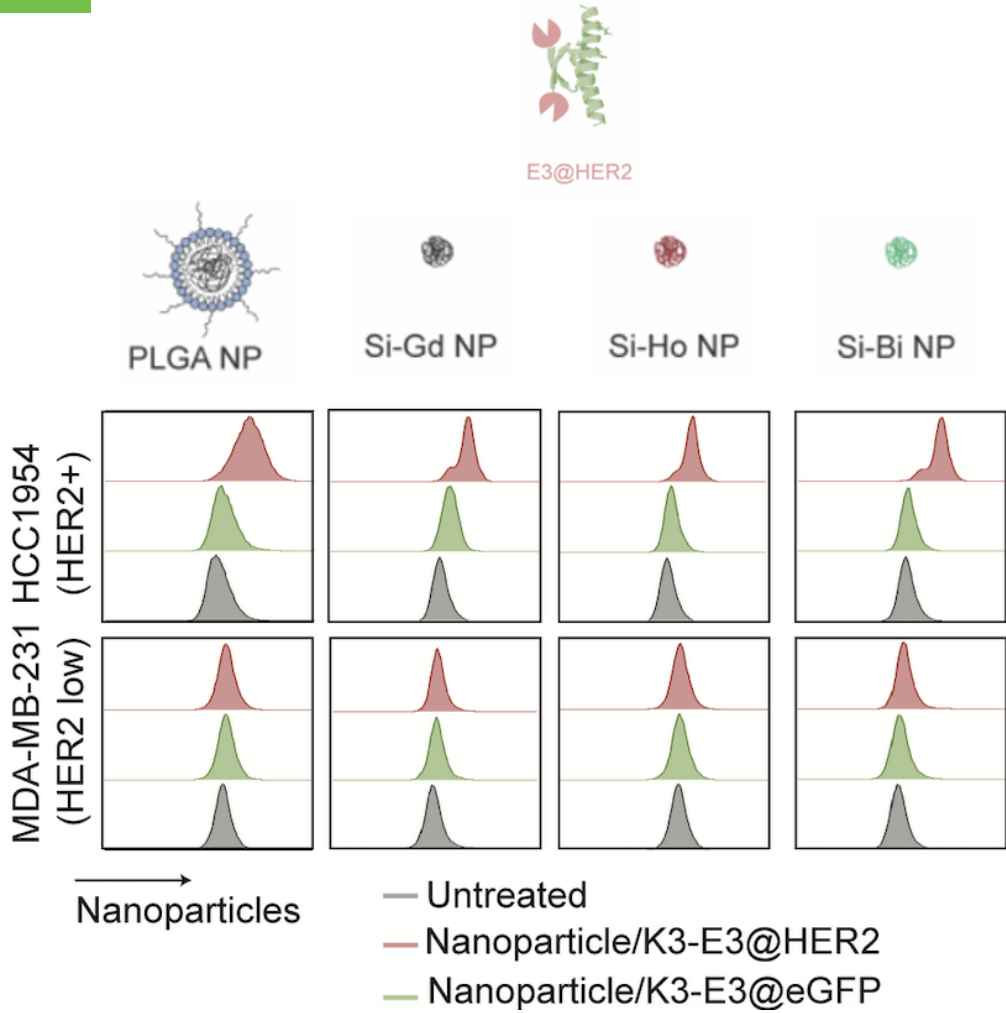
DAPI
Liposome@K3
-E3@HER2

DAPI
Liposome@K3
-E3@eGFP



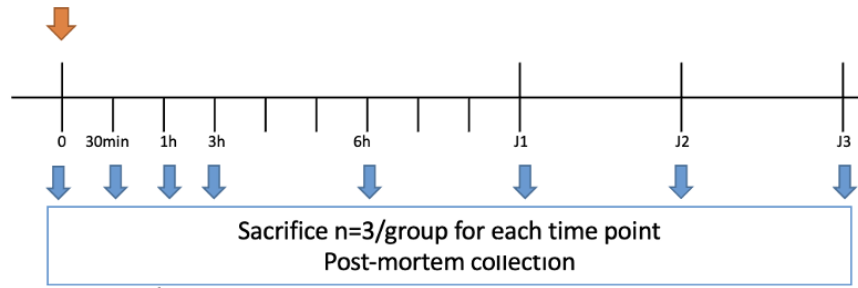
Better *in vitro* binding of the targeted liposome

In vitro specificity of the nanoparticles



In vivo experiment on mouse model

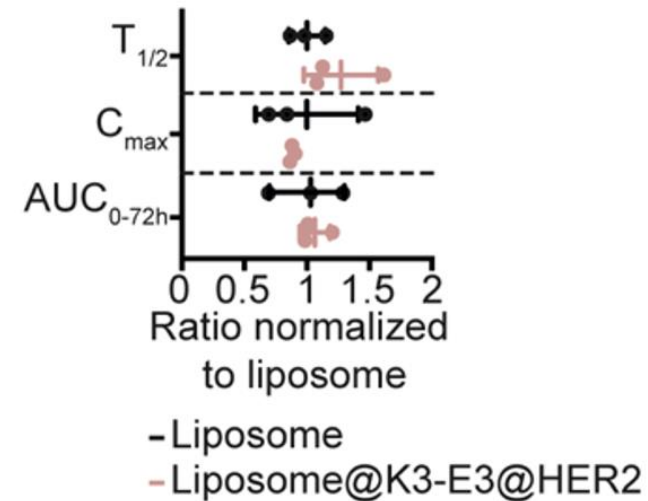
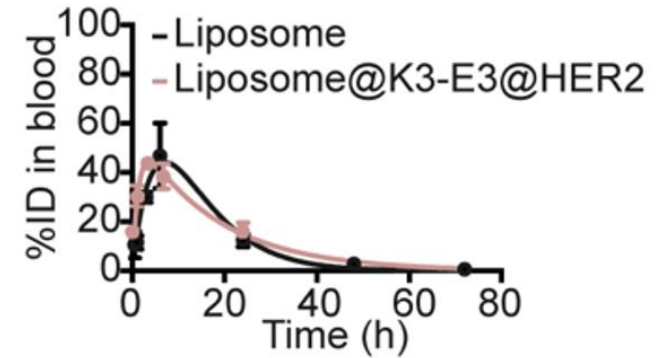
Liposome and
Liposome@K3-E3@HER2
injection



Blood
Liver Spleen
Lungs Kidneys

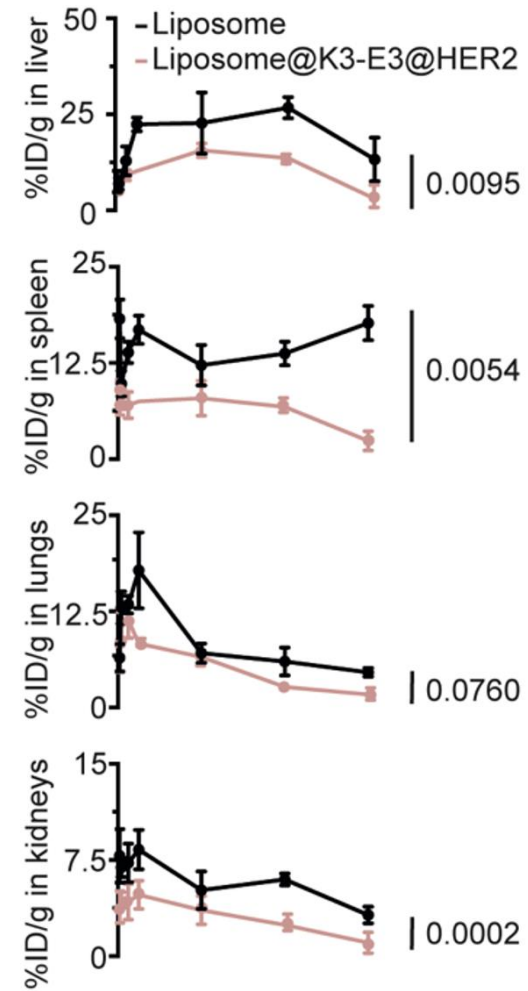
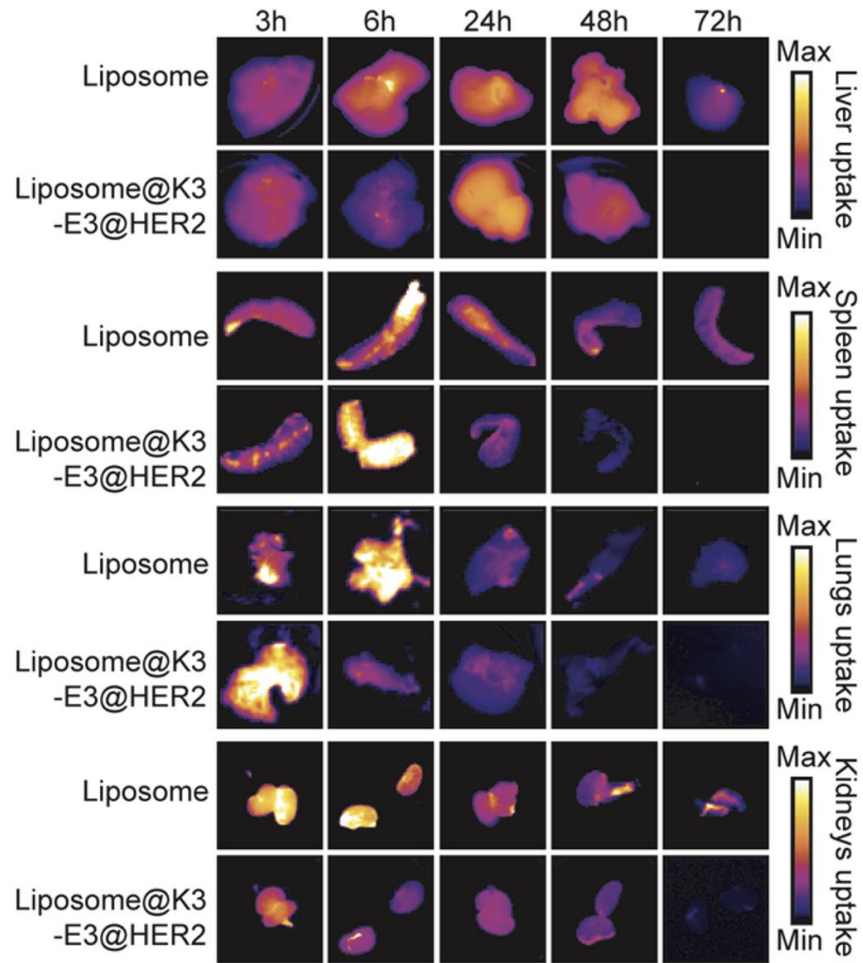


IP injection of
Liposomes



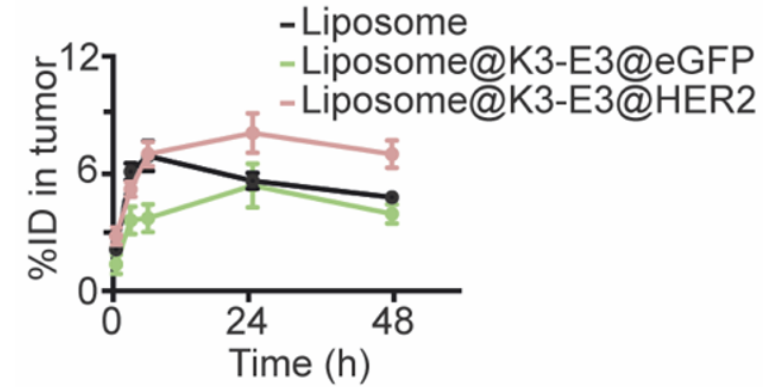
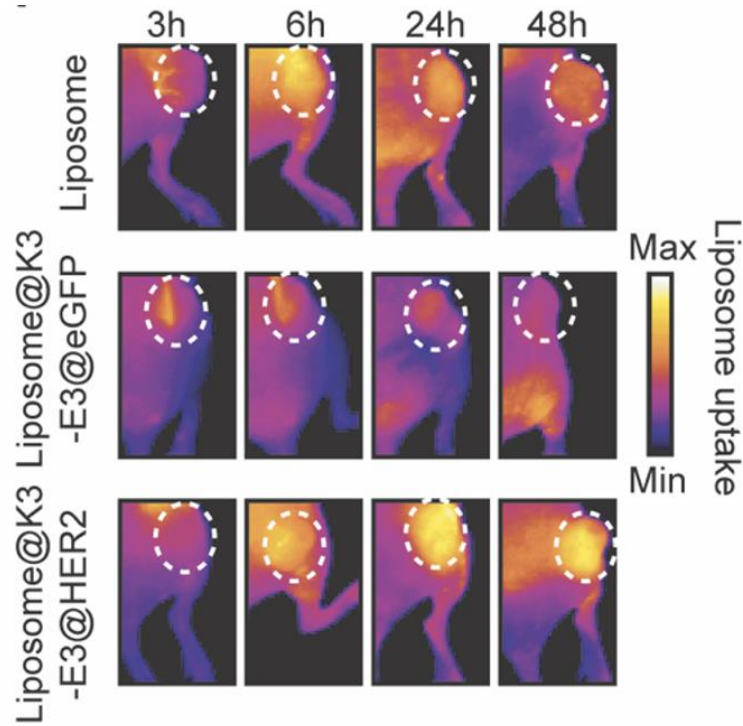
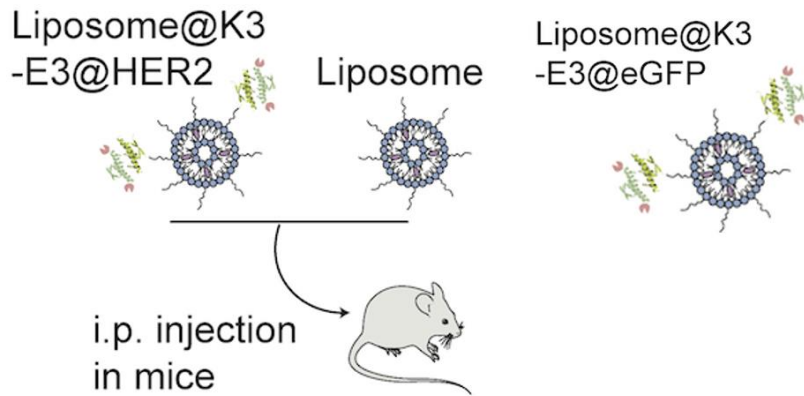
Same pharmacokinetic of targeted and non-targeted liposomes

In vivo experiment on mouse model

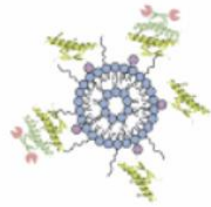


Same behaviour for the targeted and non-targeted liposomes

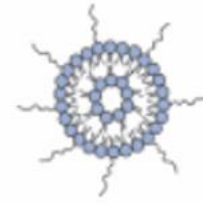
In vivo studies on HER2+ breast cancer mouse model



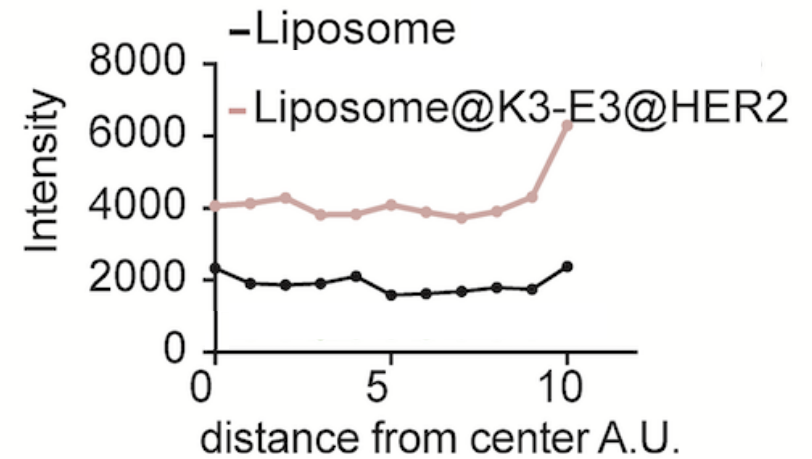
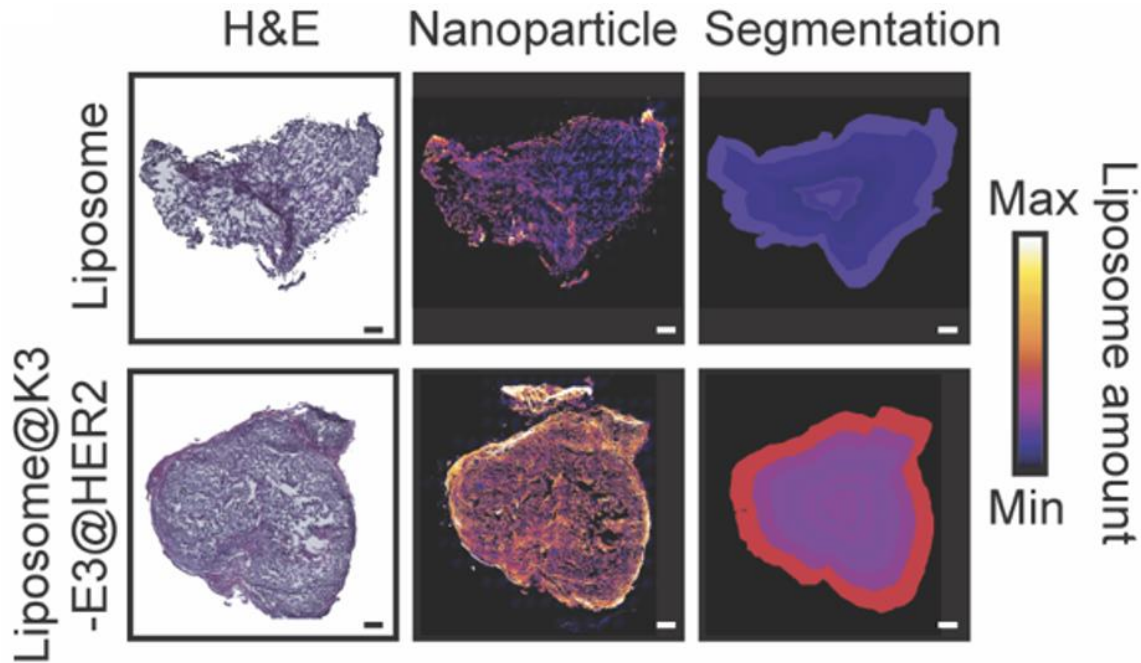
Ex vivo studies on HER2+ breast cancer mouse model



Liposome@K3
-E3@HER2

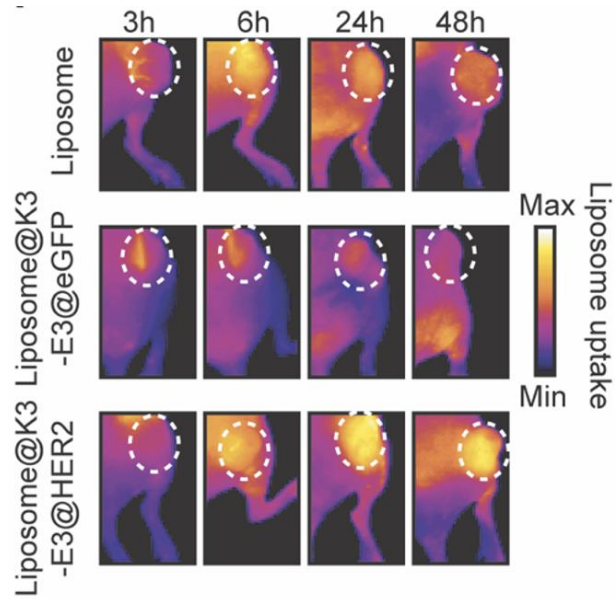


Liposome



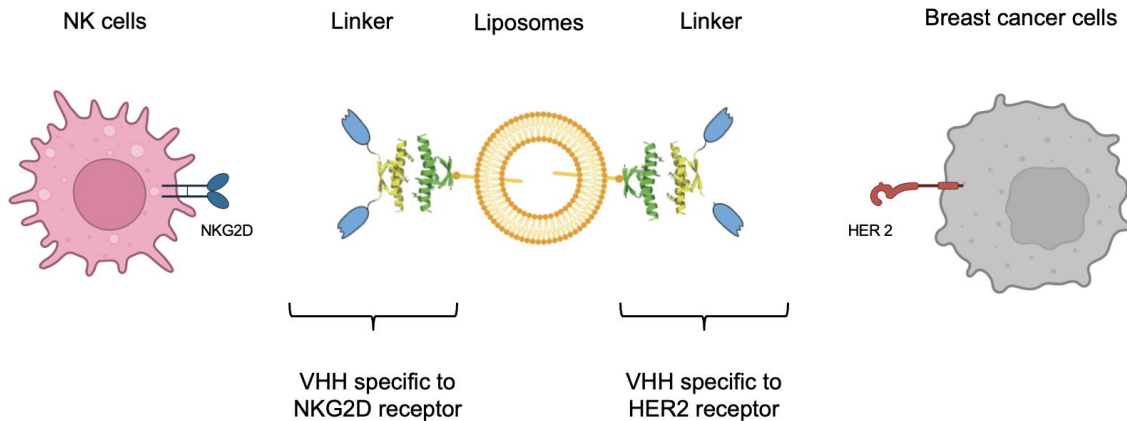
Better tumour penetration of targeted liposomes

Conclusion and perspectives on targeting tumor cells



Conclusion:

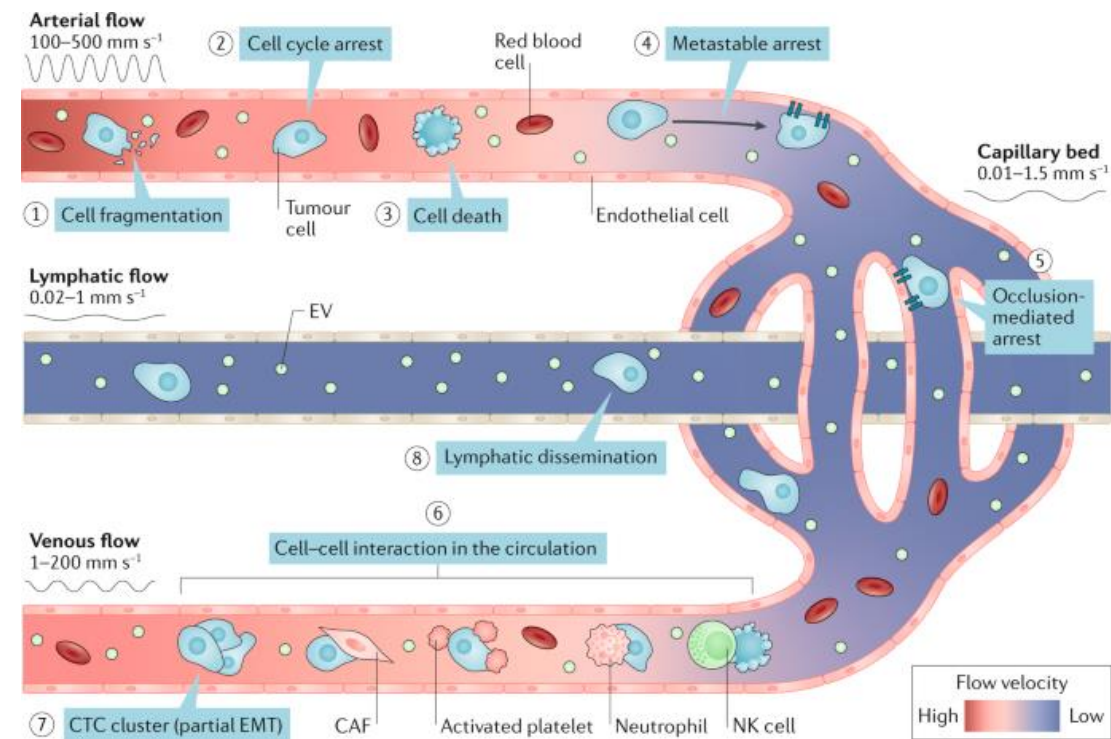
- Library of nanoparticle
- Library of VHH (targeted receptors)
- In vitro and in vivo proof of binding



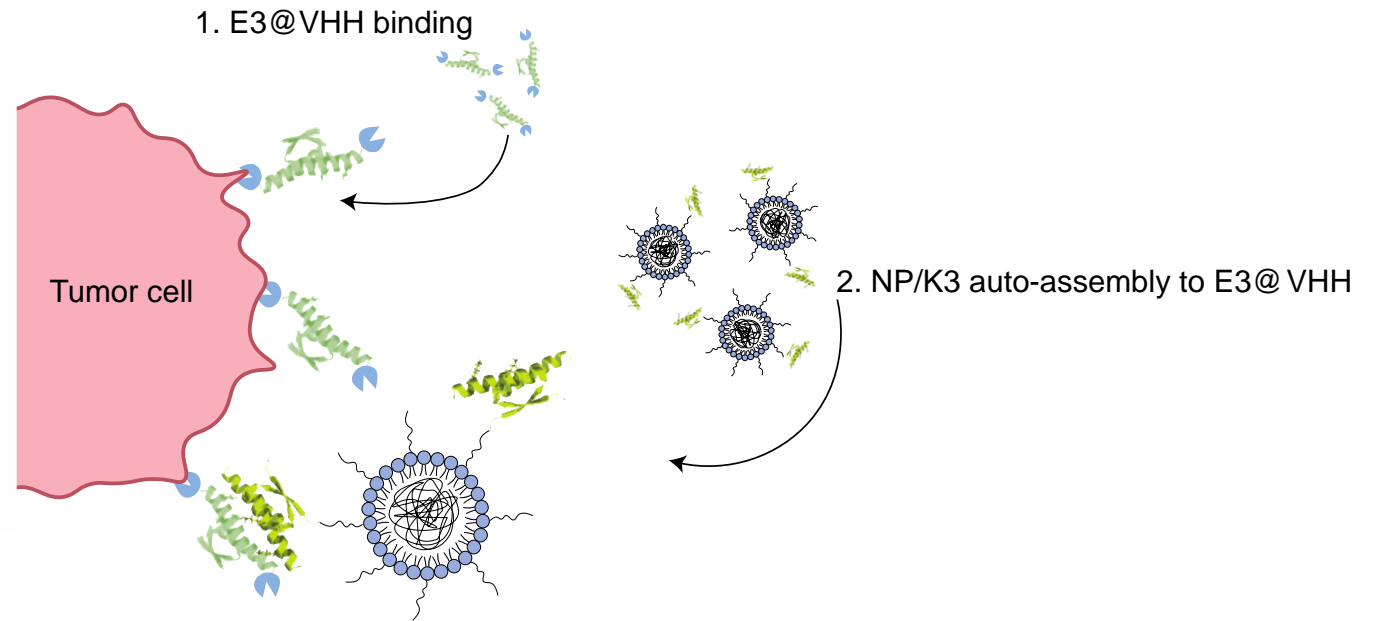
Perspectives:

- Targeting of immune cells
- Bi specific approach
- pDC NP

Diagnosis strategy this platform



Pre-targeting strategy

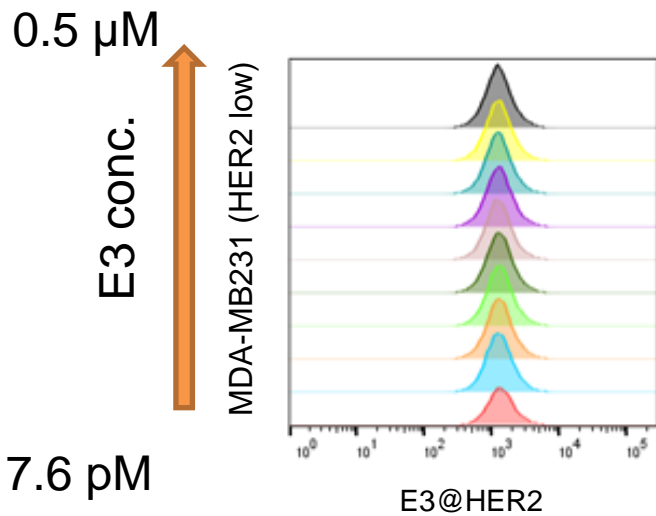
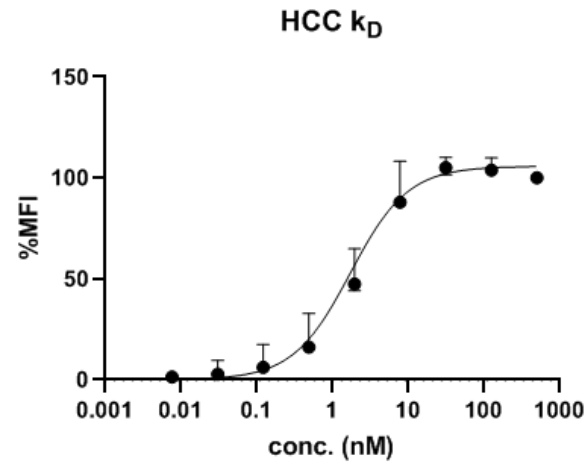
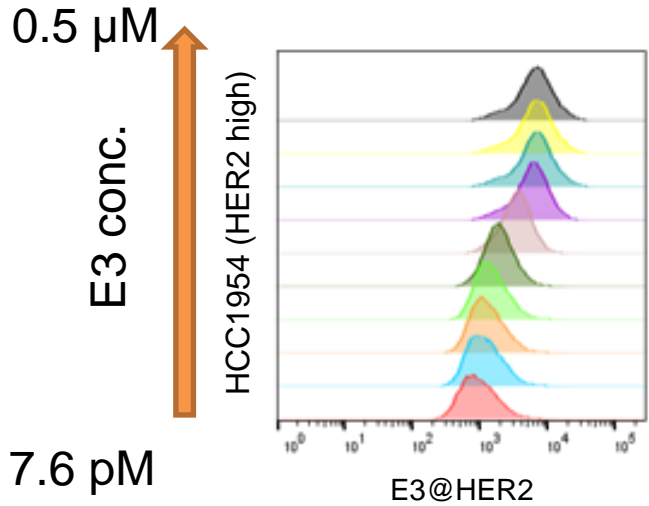


Follain G., et al. Nature Review Cancer 2020

Screening blood samples
Looking for CTCs

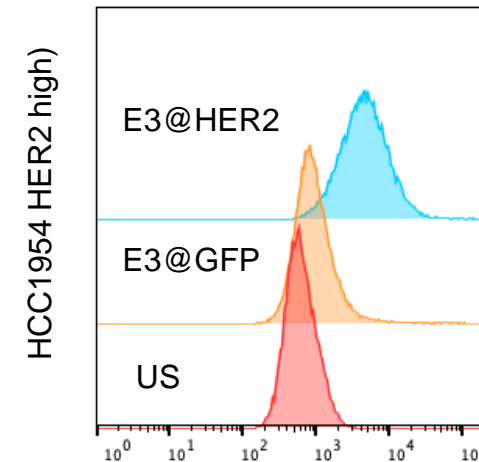
Diagnosis strategy this platform

S E P O S O P I T



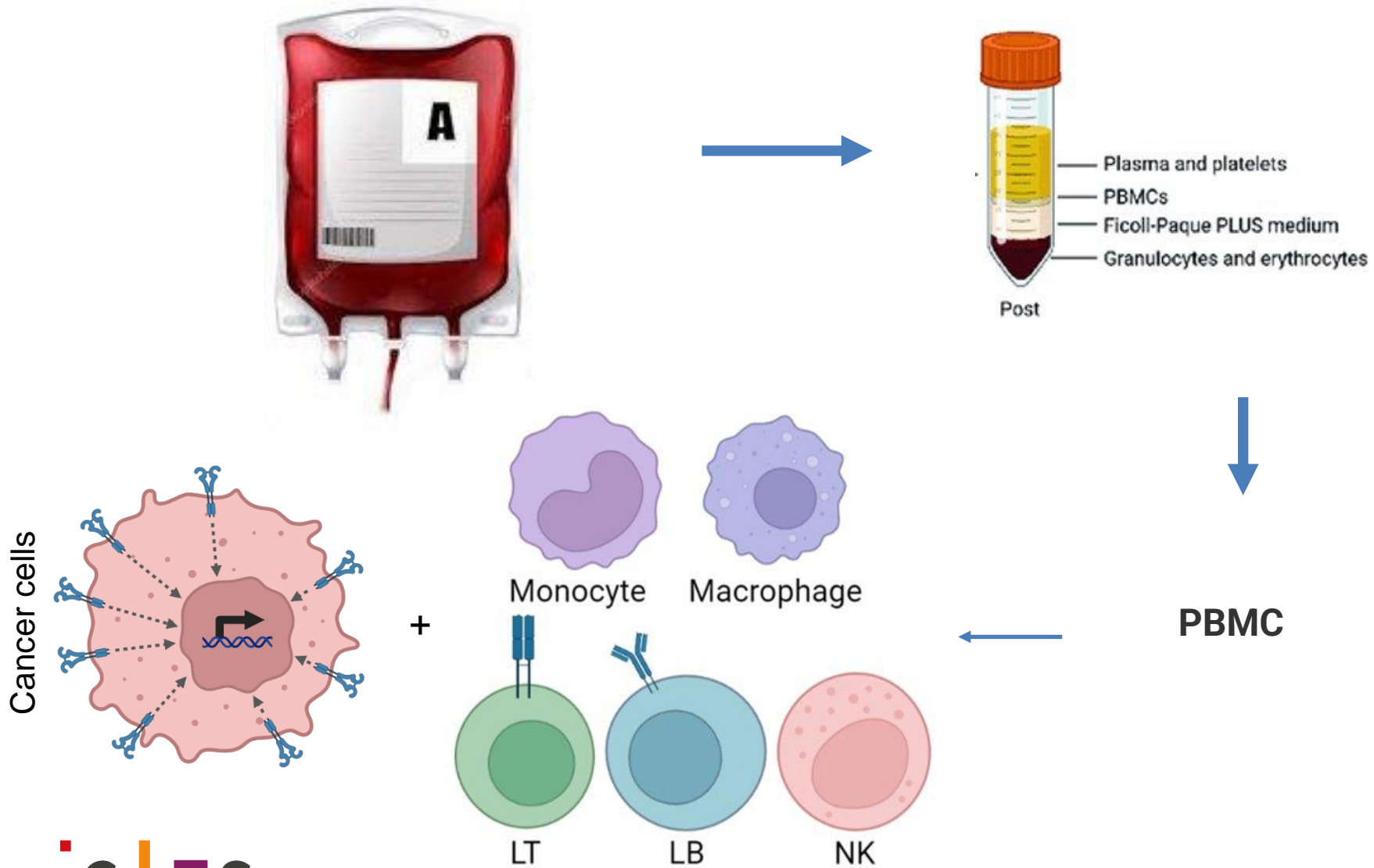
- E3-K3 association remains efficient $k_D=8nM$
- E3@VHH remains specific

P L G A



- No dependence on the carrier
- E3@VHH remains specific

Diagnosis strategy this platform

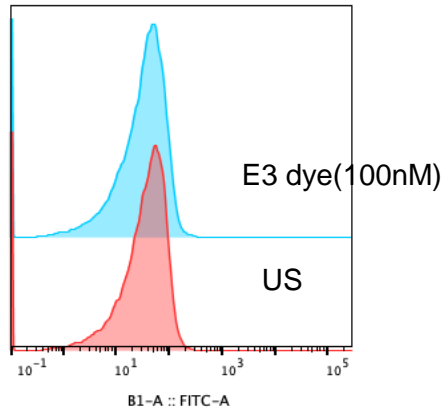


Questions:

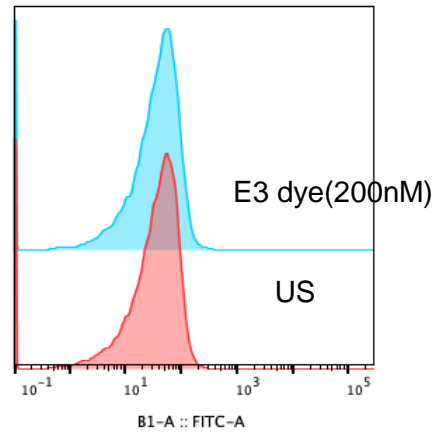
- Is the building block inert with regards to PBMC
- Is the targeting strategy efficient in « blood »

Diagnosis strategy this platform

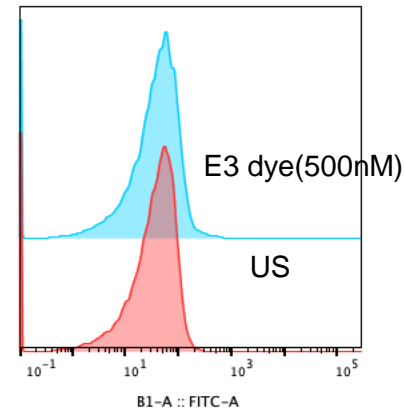
Interaction of Cy5.5@E3@VHH/K3@Nanoparticle with PBMC



Sample Name	Subset Name	Count	Median : B1-A
2023-03-12_PBMC SG113 0.1.0001.fcs	Single Cells	30026	20.1
2023-03-12_PBMC US.0001.fcs	Single Cells	30123	24.5



Sample Name	Subset Name	Count	Median : B1-A
2023-03-12_PBMC SG113 0.2.0001.fcs	Single Cells	30028	26.4
2023-03-12_PBMC US.0001.fcs	Single Cells	30123	24.5



Sample Name	Subset Name	Count	Median : B1-A
2023-03-12_PBMC SG113 0.5.0001.fcs	Single Cells	30006	26.4
2023-03-12_PBMC US.0001.fcs	Single Cells	30123	24.5

PBMC is not reacting with Dye E3-VHH.

Diagnosis strategy this platform

PBMC vs HCC ratio to find minimum detectable range

PBMC:HCC= 1:1
Theoretical: 50%
T/E mAb:50%
T/E E3VHH: 40%

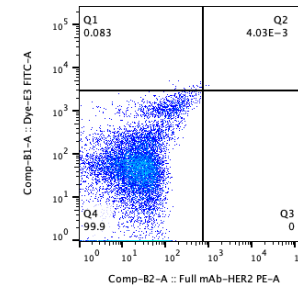
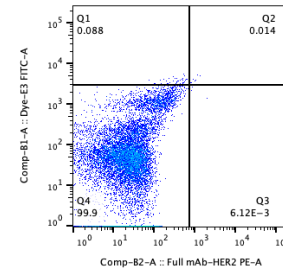
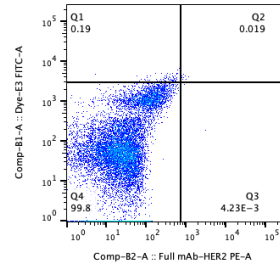
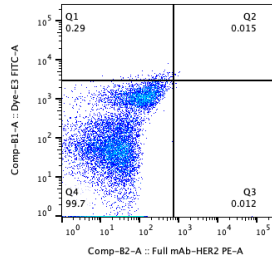
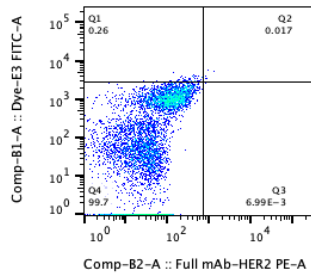
PBMC:HCC= 2:1
Expected: 33%
T/E mAb:36%
T/E E3VHH: 28%

PBMC:HCC= 3:1
Expected: 25%
T/E mAb:31%
T/E E3VHH: 23%

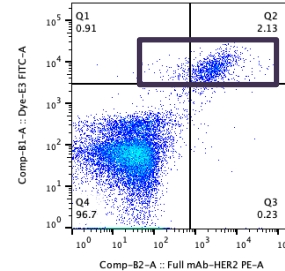
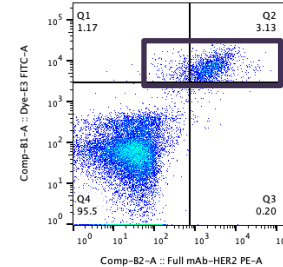
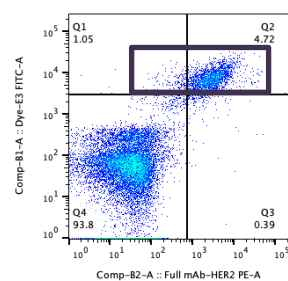
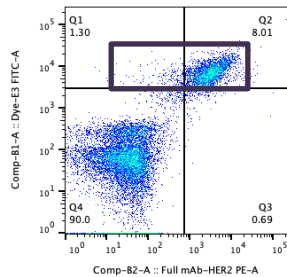
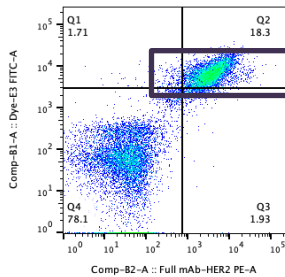
PBMC:HCC= 4:1
Expected: 20%
T/E mAb:28%
T/E E3VHH: 21%

PBMC:HCC= 5:1
Expected: 15%
T/E mAb:24%
T/E E3VHH: 20%

US



E3-V_HH-HER2



- Ratio is high due to signal/noise ratio of the detection
- Need to monitor Cancer cells differently

Conclusion and perspectives

Conclusion:

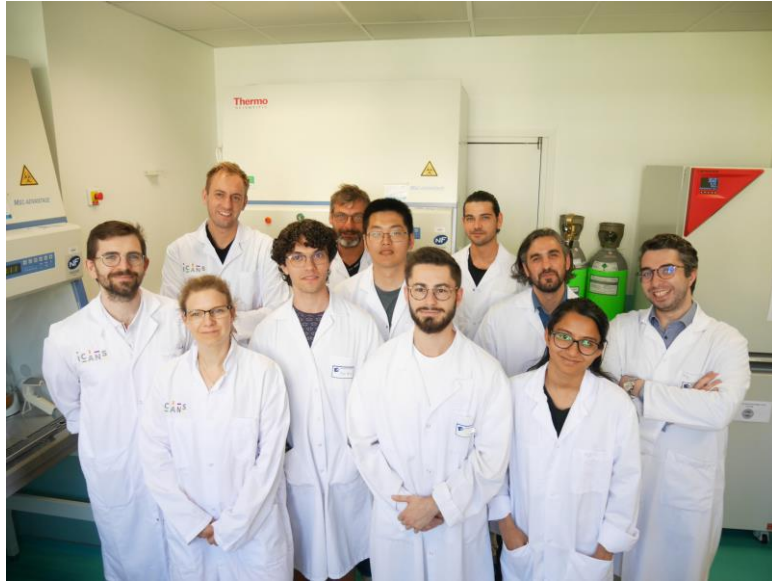
- Library of nanoparticle
- Library of VHH (targeted receptors)
- In vitro and in vivo proof of binding targeting
- Pretargeting for diagnosis –specific and no dependancy on the carrier

Perspectives:

- Targeting of immune cells
- Bi specific approach
- pDC@NP@immune cells

- Nanocarrier with high signal/noise ratio for the diagnosis

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