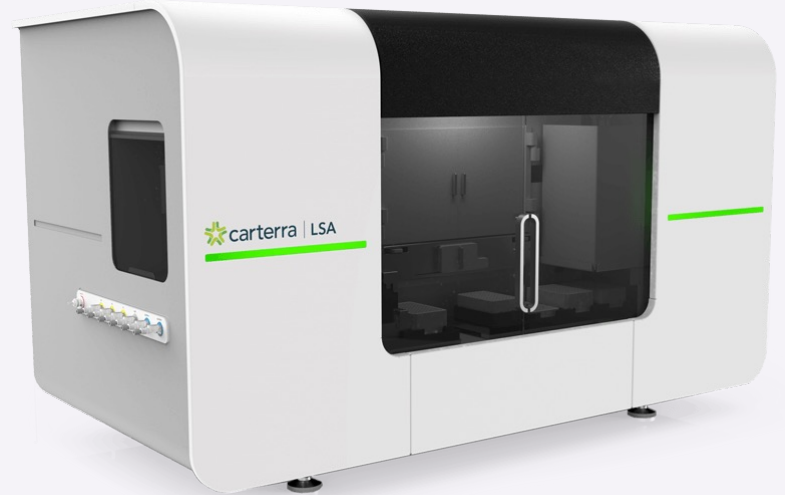


**sanofi**

Internal



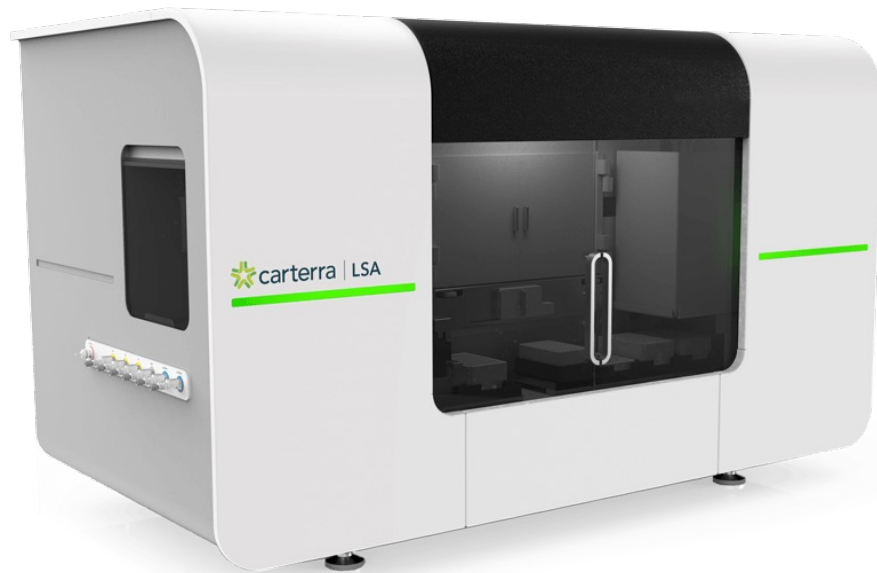
?



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[1] [3]

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# The Carterra LSA in NANOBODY® Drug Discovery

*Enabling a high-throughput  
binding assessment*

Heleen Vanhoolandt and Zara Frizell

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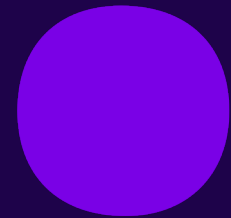
19-04-2023

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WHO ARE WE AND WHAT DO WE DO?
- 02 **Why the Carterra LSA?**  
ADVANTAGES IN OUR WORKFLOW
- 03 **Anti-FLAG Setup**  
OPTIMIZATION AND APPLICATION ON THE CARTERRA LSA
- 04 **Summary + What's Next?**

# 01 Introduction

WHO ARE WE? WHAT DO WE DO?

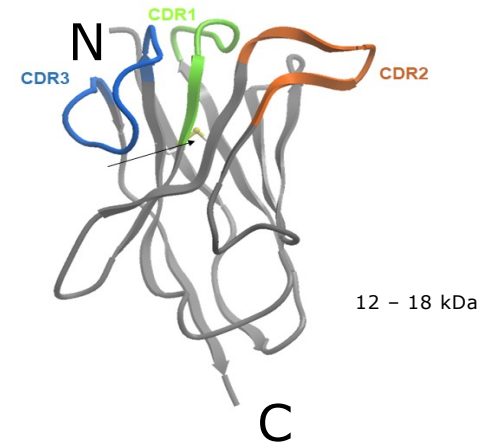
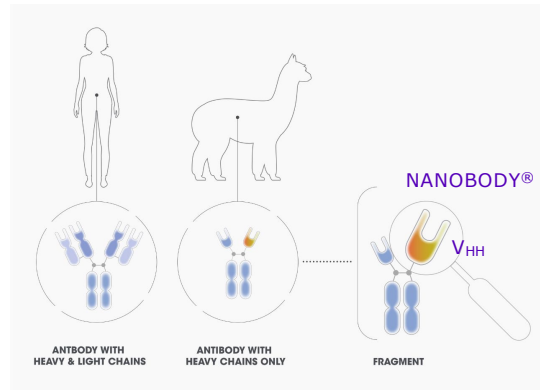


Internal

Who are we? What do we do?

# Sanofi Ghent and the NANOBODY<sup>®</sup> molecule/V<sub>HH</sub>

Sanofi Ghent



## NANOBODY<sup>®</sup> Research Platform

- Biological drugs
- Combining strength of Antibody based medicine and small molecule-based medicine
  - Antibody-based medicine: High specificity, lower toxicity, advantages in development
  - Small molecule-based medicine: easier to reach binding site / intrude target
  - Possibility to create multispecifics
- Various therapeutic indications: Inflammation & Immunology, Immuno-oncology, Rare Neurological Diseases

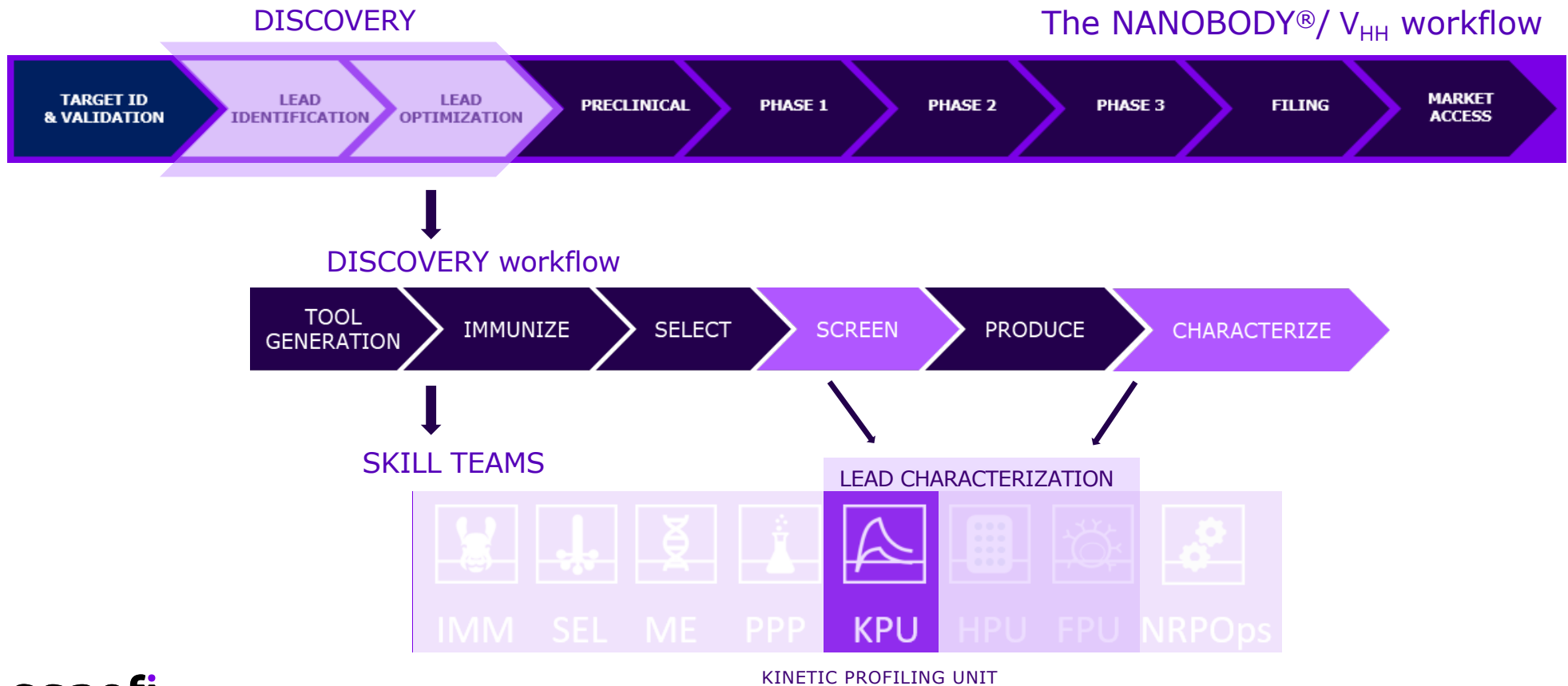
sanofi

[2] [5]

Internal

Who are we? What do we do?

# Who are we within the NANOBODY® Research Platform?



Who are we? What do we do?

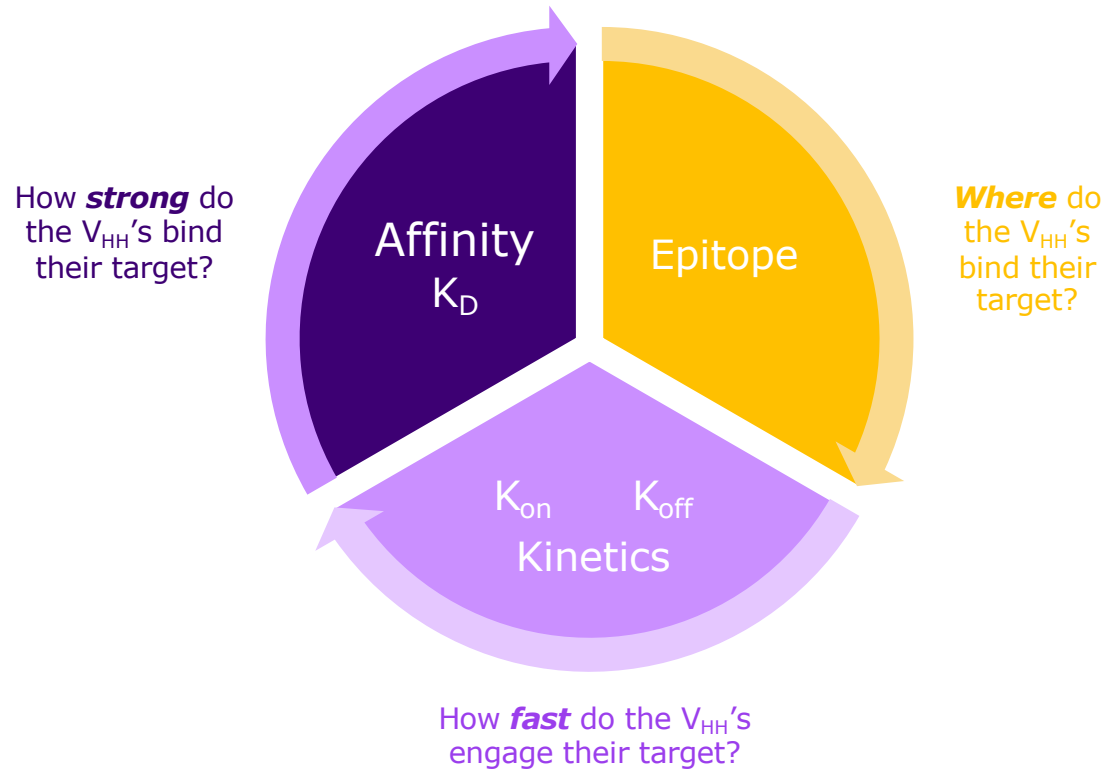
# Kinetic Profiling Unit

**What** do we assess?

Internal



KPU





Internal

Who are we? What do we do?

# Kinetic Profiling Unit

**How** do we assess?



KPU



Surface Plasmon Resonance (SPR)

Bilayer Interferometry (BLI)

- Kinetics ( $k_{on}$ ,  $k_{off}$ )
- Affinity ( $K_D$  [ $k_{on}$ ,  $k_{off}$ ])
- Epitope Binning



In-solution affinity



switchSENSE®

Who are we? What do we do?

# Kinetic Profiling Unit



KPU

**Kinetic profiling** within the discovery workflow

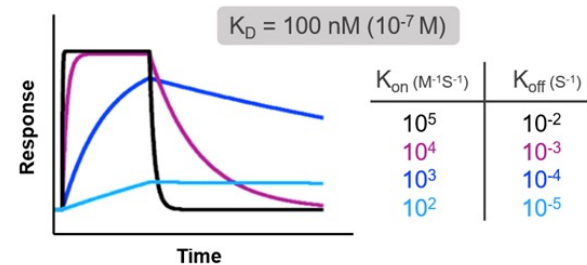
SCREENING



CHARACTERIZATION

- Periplasmic extract (peri) + concentration unknown  
→ Less time intensive to produce
- Confirm binding
- Know your  $k_{off}$  early on → early ranking

- Purified material + concentration known
- Epitope binning
- Affinity:  $V_{HH} \leftrightarrow$  target
- Affinity with kinetic rate constants ( $k_{on}$ ,  $k_{off}$ ) to select specific profiles → SPR



## 02 **Why the Carterra LSA?**

ADVANTAGES WITHIN OUR WORKFLOW

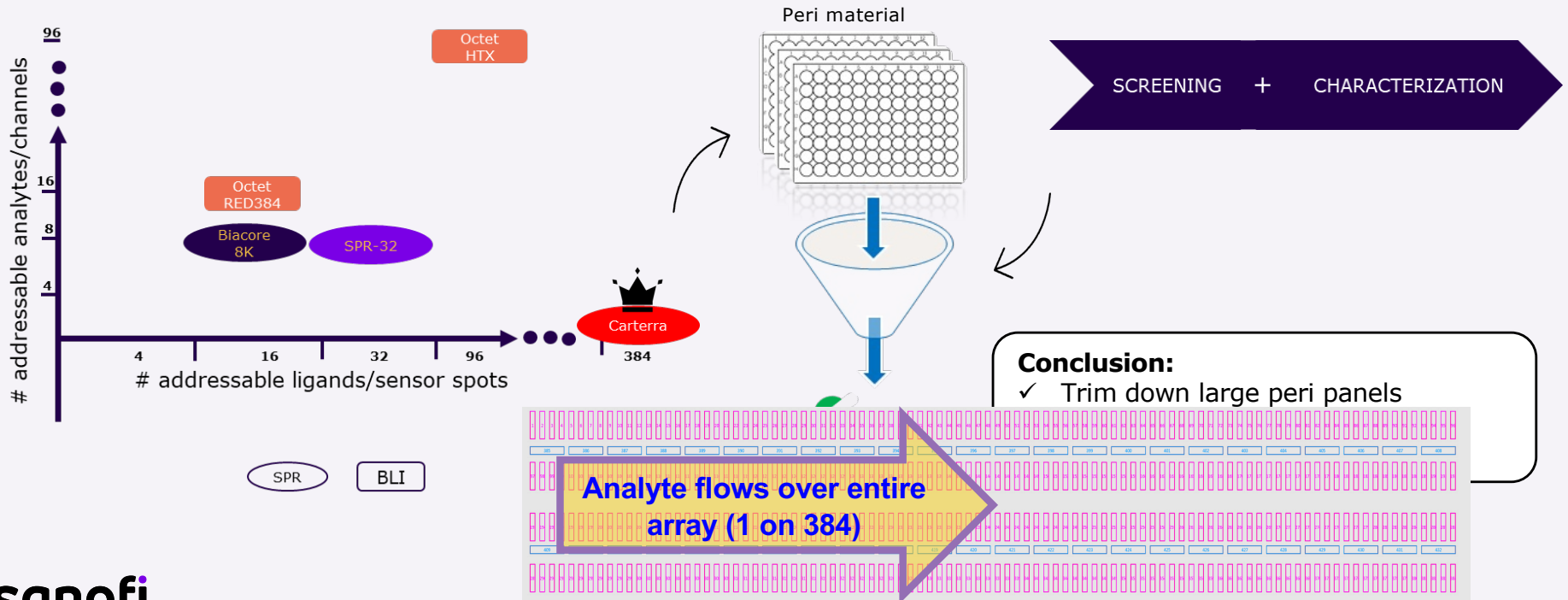


Advantages within our workflow

# The Carterra LSA

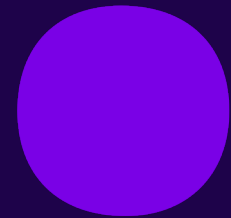
- Higher throughput → large lead panels

- Determine  $k_{on}/k_{off}$  (characterization) using peri (screening)



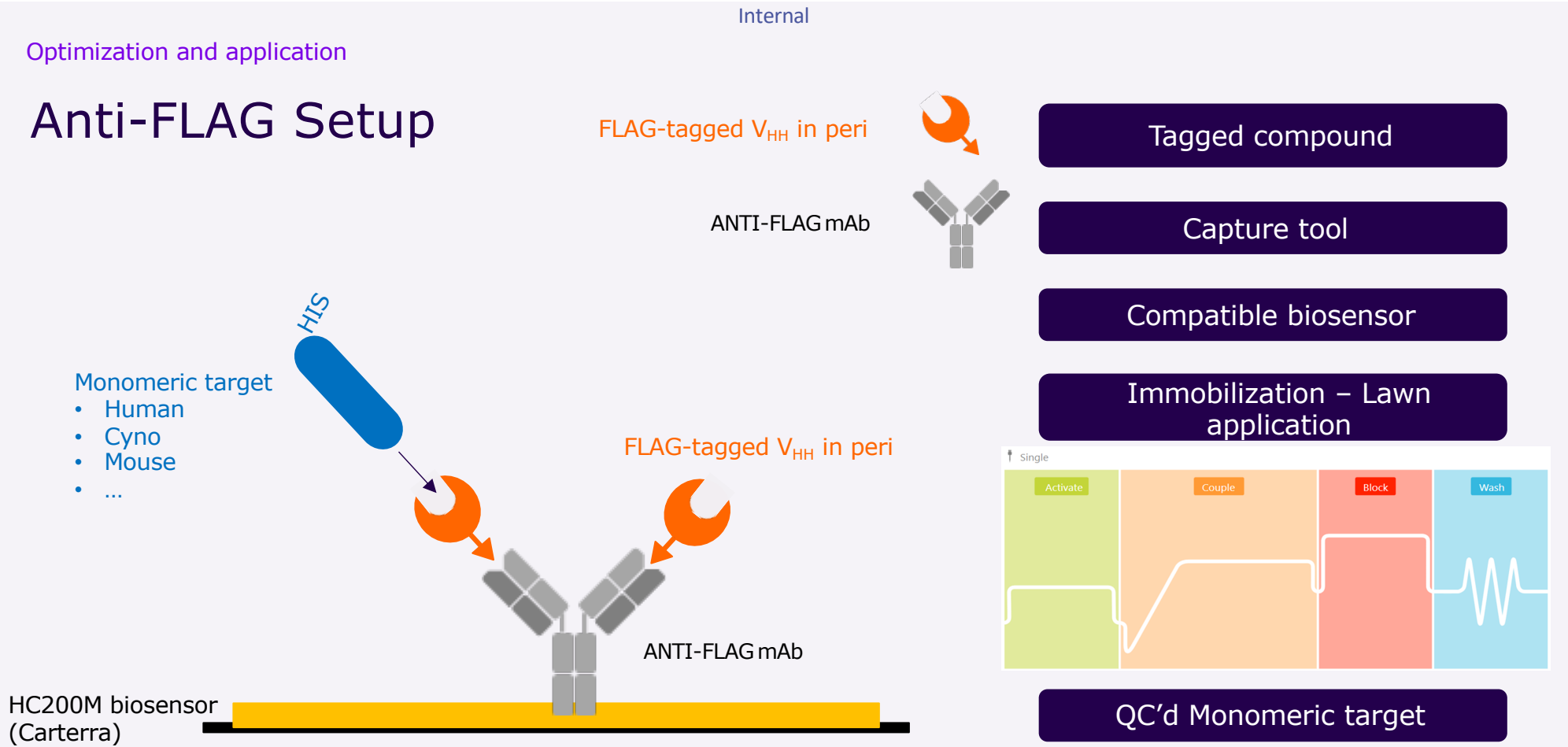
# 03 **Anti-FLAG Setup**

OPTIMIZATION AND APPLICATION ON THE CARTERRA LSA



Optimization and application

# Anti-FLAG Setup



Internal



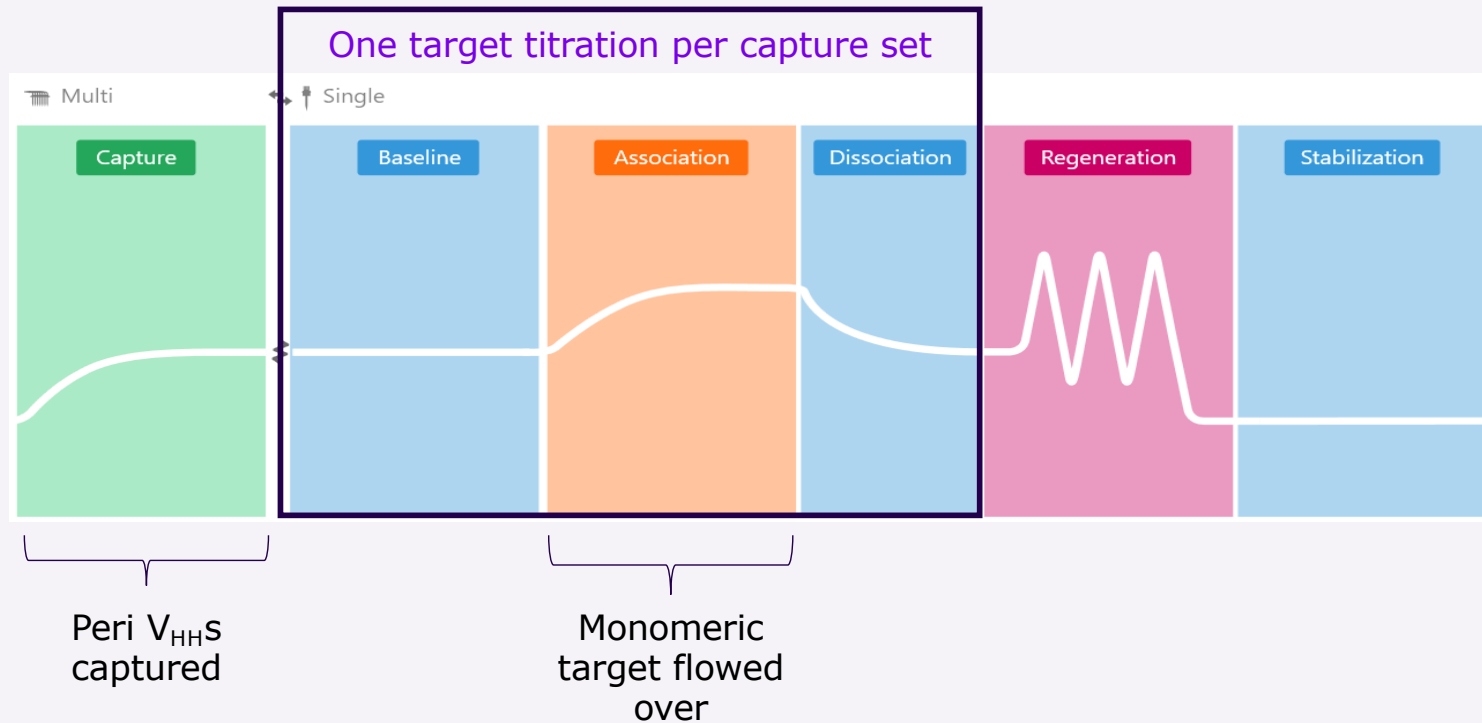
How do we use this  
setup to determine  
affinities?

Optimization and application

# Affinity Determination of $V_{HHs}$ in Periplasmic Extract

Protocol setup

## Capture Kinetics Application



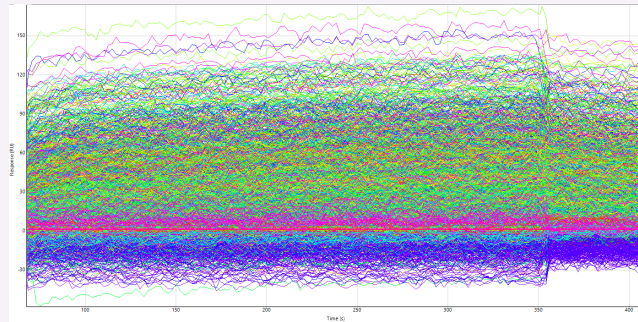


Optimization and application

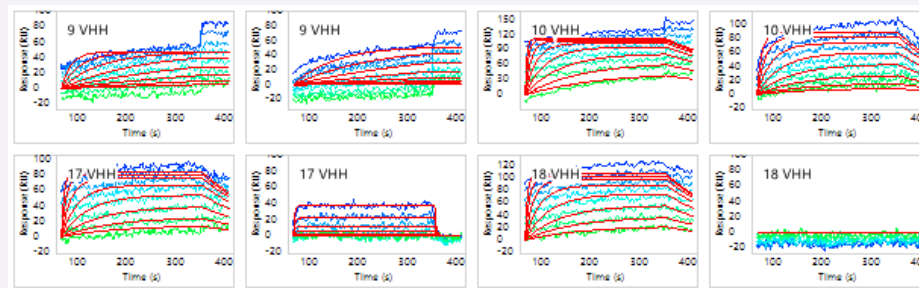
# Affinity Determination of V<sub>HH</sub>s in Periplasmic Extract

## Data Processing

Raw data




Double-referenced,  
Y-aligned (serial), cropped



Fitted using  $k_d k_a$   
model

Internal

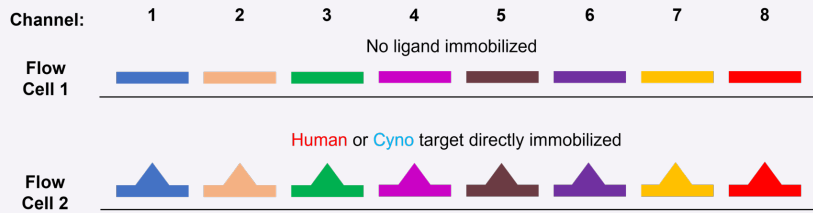
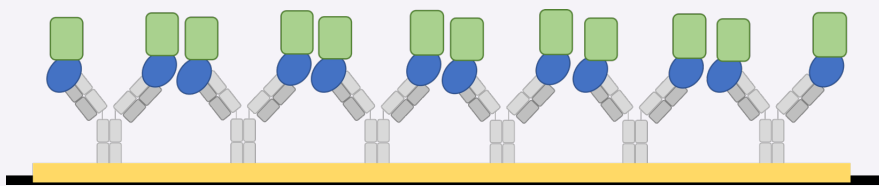
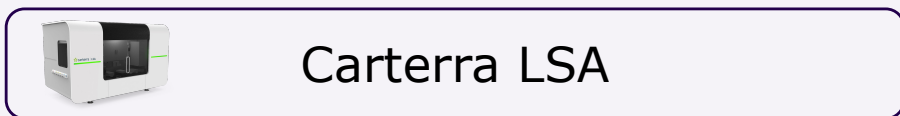


How did we validate  
the anti-FLAG  
setup?

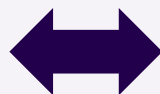
Optimization and application

# Validating the Anti-FLAG Setup

Data comparison between Cytospora and Biacore



Anti-FLAG capture setup  
 Targets flowed over as analytes  
 $V_{HH}$ s captured on chip surface

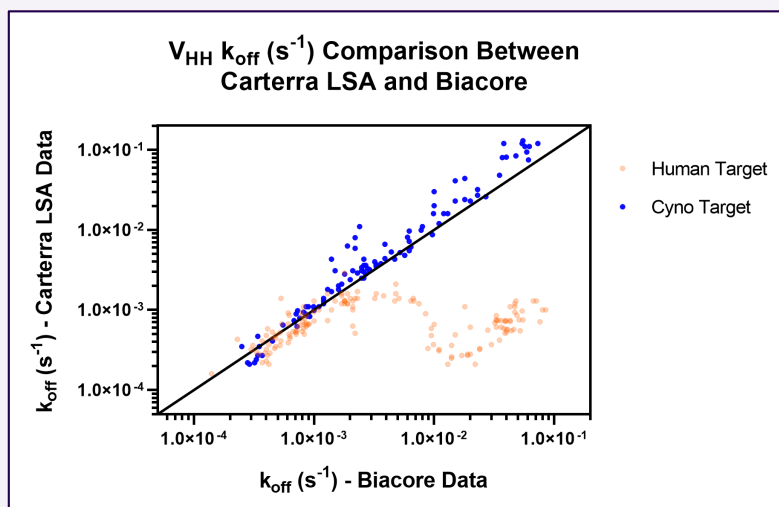


Direct setup  
 Targets directly immobilized  
 $V_{HH}$ s flowed over as analytes

# Carterra and Biacore $k_{off}$ Comparison

Value comparison and ranking

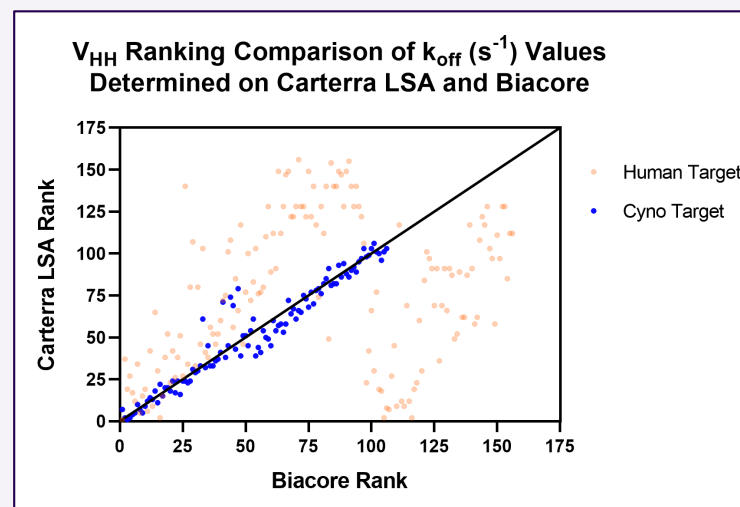
## $k_{off}$ Value Comparison



**Human** target later shown not to be monomeric (aSEC)

**sanofi**

## $k_{off}$ Rank Comparison



Ranking:

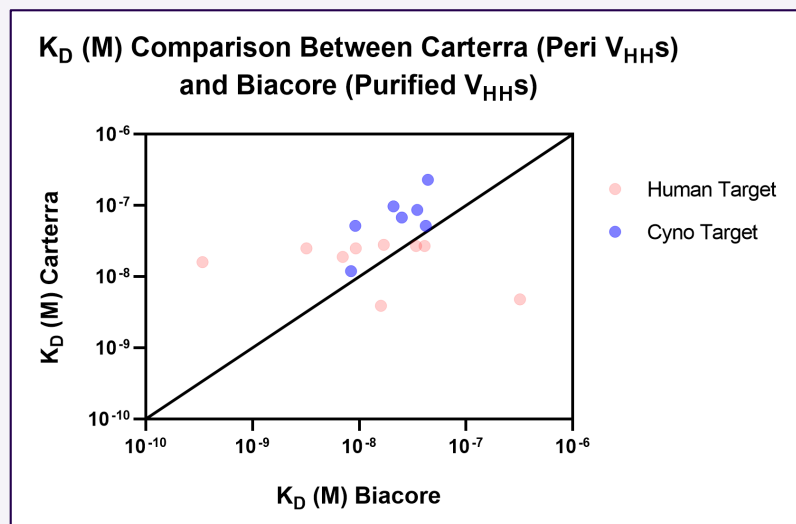
Increasing number

Faster  $k_{off}$

# Carterra and Biacore $K_D$ (M) Comparison

Value comparison and ranking

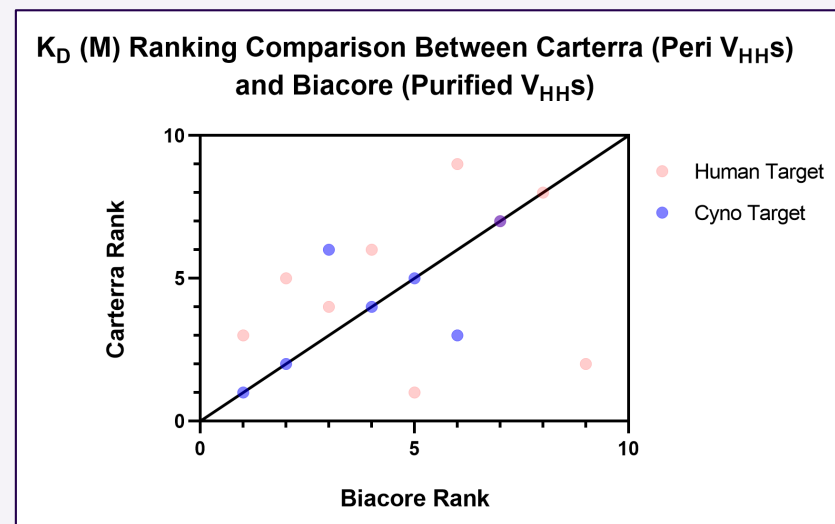
## $K_D$ (M) Value Comparison



Human target later shown not to be monomeric (aSEC)

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## $K_D$ (M) Rank Comparison



Ranking:

Increasing number

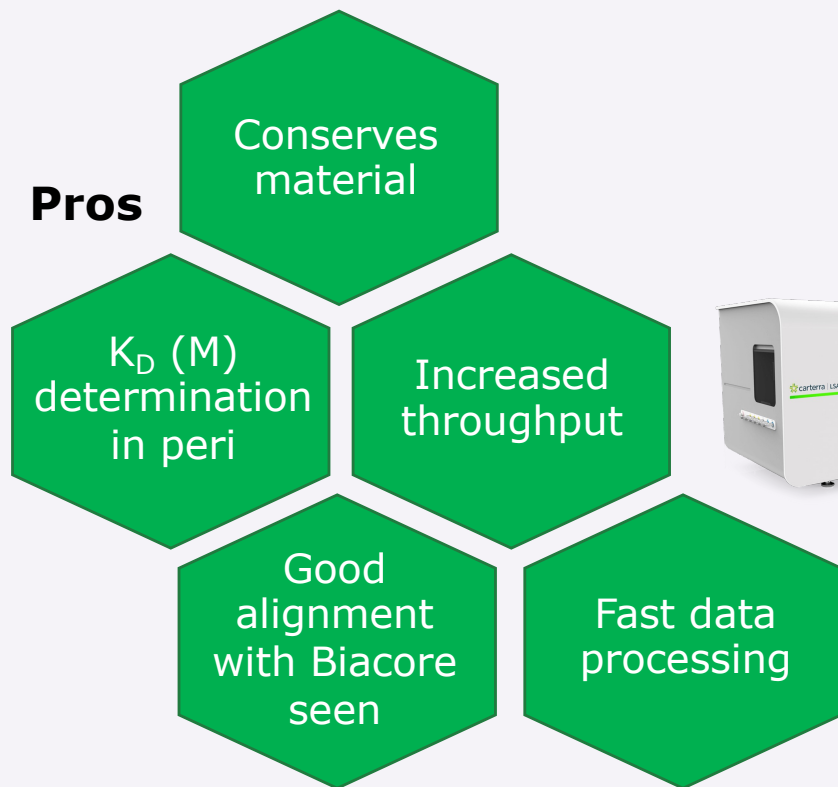
Decreasing affinity

## 04 Summary + What's Next?

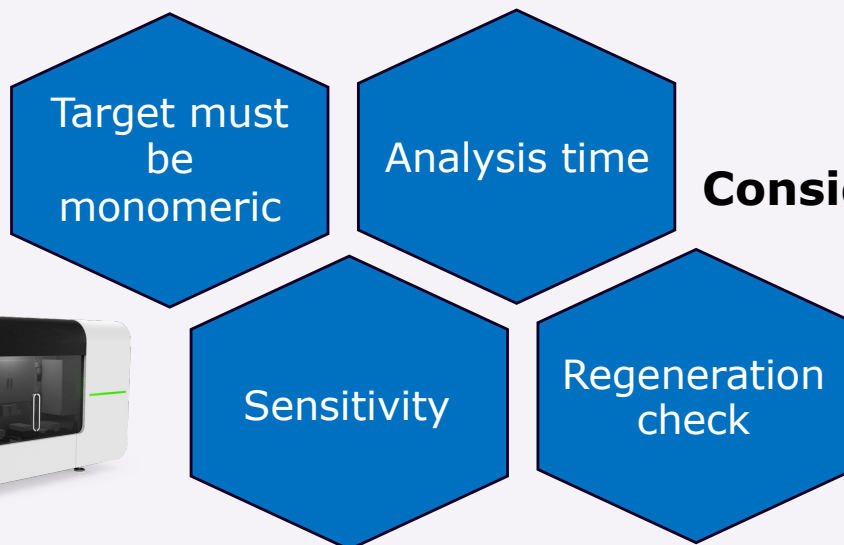


# Summary + What's Next?

## Pros



## Considerations



## Next Exploration

Epitope binning of  $V_{HH}$ s in periplasmic extract

# Acknowledgements



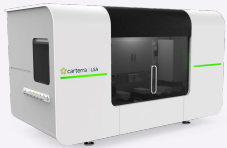
KPU

Arne Claeys for his heavy involvement in the development of the anti-FLAG setup

The KPU team members involved in running experiments and working to develop the anti-FLAG setup

Bjoern Niebel, Ine Storme, Evelyn De Tavernier and Natalia Sarmiento for data review and feedback

Bjoern Niebel for generating the figures related to kinetic profiling



Judicaël Parisot for the helpful feedback and tips when developing the anti-FLAG setup on the Carterra LSA

The whole Carterra team for additional tips and tricks for assays and care of the instrument



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2. Sanofi 2023, *NANOBODY® Technology Platform*, Sanofi, viewed 28<sup>th</sup> February 2023, <https://www.sanofi.com/en/science-and-innovation/research-and-development/technology-platforms/nanobody-technology-platform>
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Thank you for your attention!  
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