# Nanobodies as Affinity Capture Reagents

## Использование нанотел для связывания белковых комплексов

Roman Kotovich[1], Chloe Terestchenko[1], Anna Kogan[1,2], Krzysztof Łuszczyński[1,3], Marc Brillantes[1,4], Elizaveta Buianova[1,5], Daria Strelkova[1,6], Jill Trivedi[1,7], Natalia Ketaren[1,7]

*1 - School of Molecular and Theoretical Biology; 2 - Saint-Petersburg State University, Russia; 3 - Medical University of Warsaw, Poland; 4 - Rutgers School of Graduate Studies, NJ, USA; 5 - Skolkovo Institute of Science and Technology, Moscow, Russia; 6 - Carnegie Mellon University, PA, USA; 7 - The Rockefeller University, NY, USA*

***Higher affinity and thermally stable nanobodies result in a better capture of the Nup84 complex.***

***10 µg of nanobody per mg of beads are sufficient to capture the Nup84 complex.***

Для перевода на ***русский язык*** отсканируйте QR-код

***The goal is to use nanobodies as reagents to study protein protein interactions.***

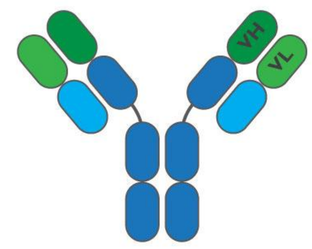
***Hypothesis**: when a nanobody is more stable, it will bind better to its antigen.*

**Aim 1**: Do certain properties of a nanobody affect its ability to perform in an affinity capture experiment?

↑ in affinity ⇒? ↑ in specificity

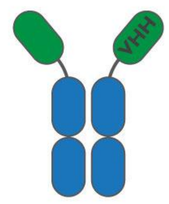
↑ in stability ⇒? ↑ in capturing target protein

**Aim 2**: What is the minimum amount of nanobody required to successfully capture a protein complex?




Conventional IgG

- Large (~150 kDa)
- Cannot make using standard lab techniques
- Expensive reagent
- Utilized in medicine



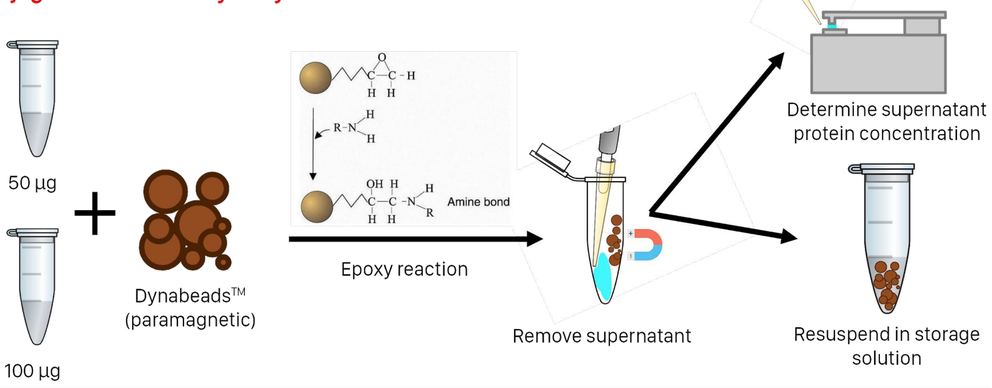

Heavy chain-only antibody

Nanobody

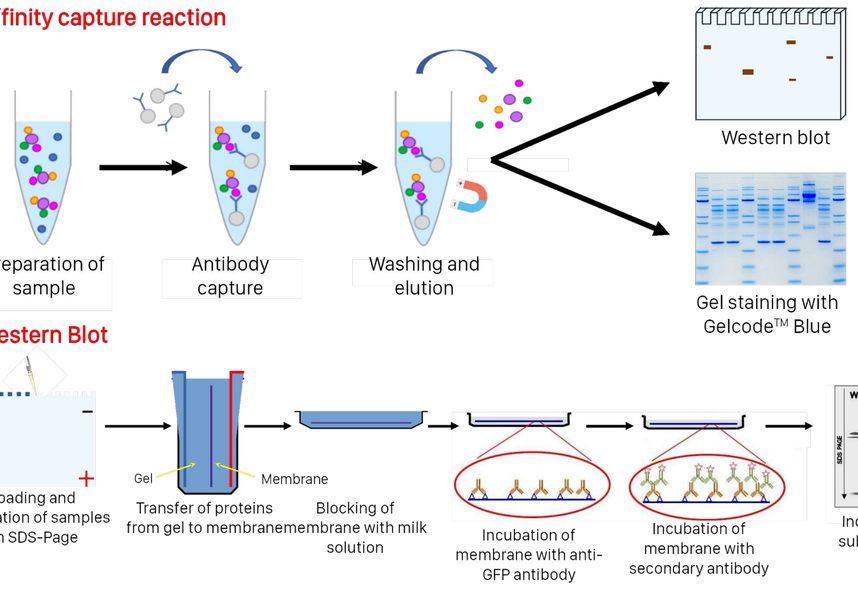
- Nanobody is small (~15 kDa)
- Easy to make in the lab
- Binds antigens like conventional antibodies
- Not enough is known just yet

# Methods

### Conjugation of nanobody to Dynabeads™



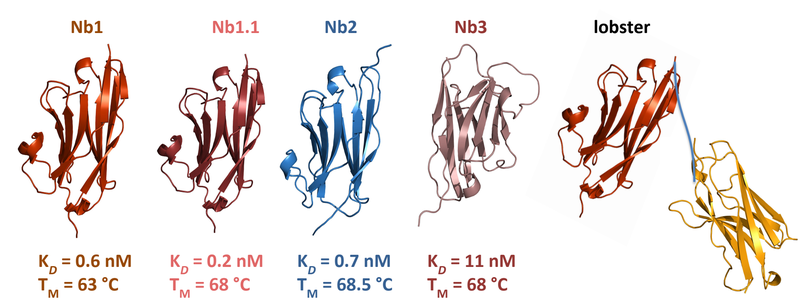

### Affinity capture reaction



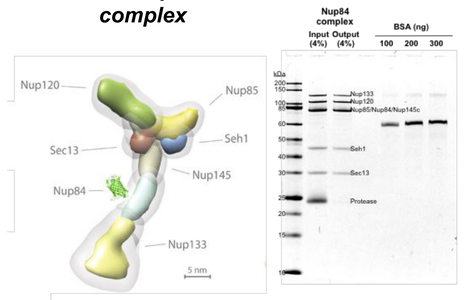

### Western Blot




***AIM1: Do certain properties of a nanobody affect its ability to perform in an affinity capture experiment?***

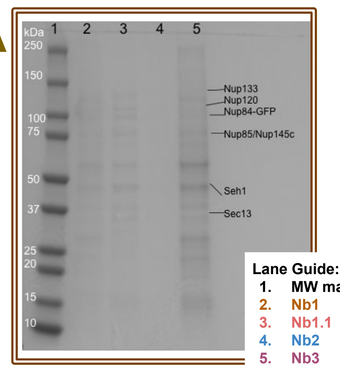



***The Nup84 complex***



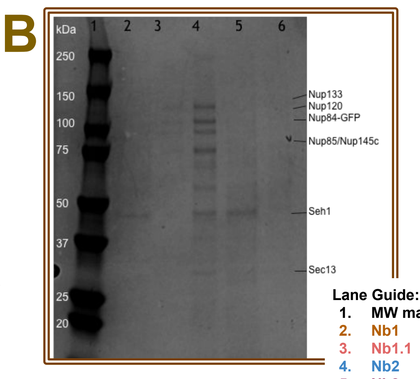

The Nup84 complex is a ~500 kDa complex that is a part of the largest complex in the cells – the nuclear pore complex.

**A**



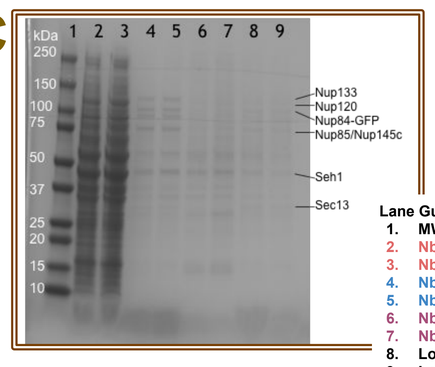

**Lane Guide:**
1. MW marker
2. Nb1
3. Nb1.1
4. Nb2
5. Nb3

**B**




**Lane Guide:**
1. MW marker
2. Nb1
3. Nb1.1
4. Nb2
5. Nb3
6. lobster

**C**




**Lane Guide:**
1. MW marker
2. Nb1.1 80 mg
3. Nb1.1 100 mg
4. Nb2 80 mg
5. Nb2 100 mg
6. Nb3 80 mg
7. Nb3 100 mg
8. Lobster 80 mg
9. Lobster 100 mg

**D**

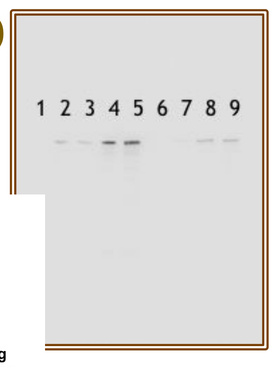



**Figure 1: Which nanobody captures the Nup84 complex the best?**

A. **Affinity capture experiment testing four different nanobodies [Nb1, Nb1.1, Nb2, Nb3]**
B. **Affinity capture experiment testing five different nanobodies [Nb 1, Nb1.1, Nb2, Nb3, lobster]**
C. **Affinity capture experiment of four different nanobodies with two different concentrations of starting material [80 mg & 100 mg]**
D. **Western blot of panel C.**

***AIM2:*** What is the minimum amount of nanobody required to successfully capture a protein complex?

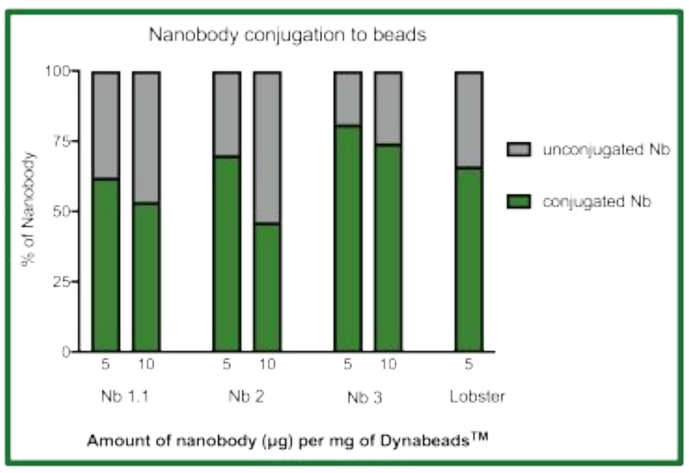
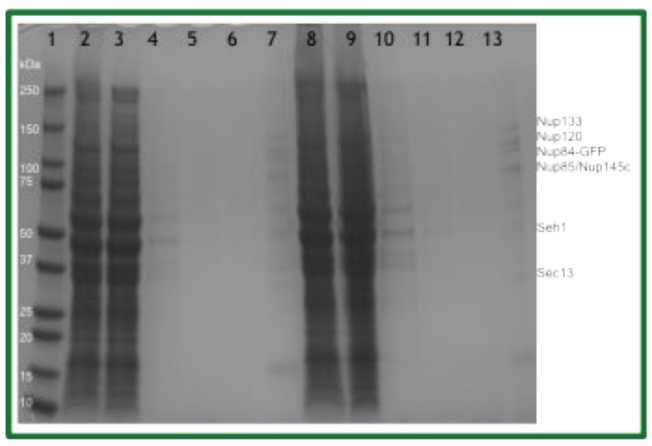



Figure 2: How much nanobody do we lose during the conjugation process?

We performed conjugation reactions with 4 different nanobodies using amounts of 50µg and 100µg (except for lobster Nb). The graph shows in grey the amount of nanobody not bound to the Dynabeads™ after the conjugation process which is no longer usable. In green is the amount of nanobody bound to the Dynabeads™.

**A**




**Lane Guide:**
1. MW marker
2. Input (50µg)
3. Flow-through (50µg)
4. Wash 1 (50µg)
5. Wash 2 (50µg)
6. Wash 3 (50µg)
7. Elution (50µg)
8. Input (100µg)
9. Flow-through (100µg)
10. Wash 1 (100µg)
11. Wash 2 (100µg)
12. Wash 3 (100µg)
13. Elution (100µg)

**B**

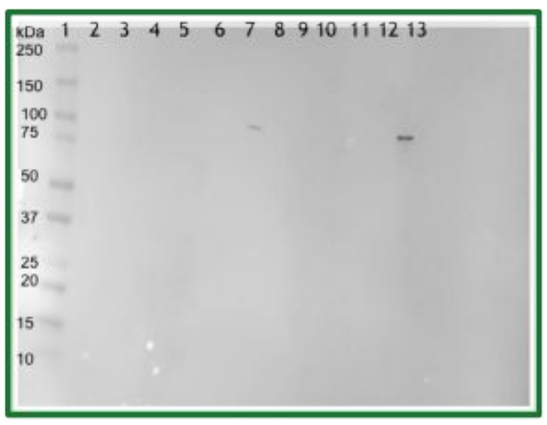



Figure 3: Does the amount of nanobody used during conjugation affect its ability to capture a protein complex?

A. Affinity capture reaction testing of Nb 1.1 conjugated using 50µg and 100µg Nb. Gel shows protein present in input, flowthrough, wash and Elution fractions.
B. Western Blot of panel A

## Discussion

***Aim 1:***

- Nbs with higher thermal stability and higher affinity (Nb1.1 & 2) captured the complex best.
- Nbs with low affinity, regardless of thermal stability (Nb3) did not capture the complex well.
- Our polyclonal Nb (lobster) did not capture the complex well. This may be because it could not access the 2nd epitope or the interaction was not stable.

***Aim 2:***

- Both 5 µg and 10 µg of nanobody per mg of beads were able to capture the complex.
- Increased amount of Nb captured more complex.

## Future Directions

- Replicate the experiments with promising findings to support their accuracy.
- Attempt to purify the entire nuclear pore complex with well performing nanobodies.
- Repeat these experiments with more nanobodies that have various characteristics to verify the results.

## Acknowledgements:

We would like to thank Ana Gutiérrez, Anna Almor for all their help with the wet lab materials and Katya Shuvalova for her help with the Western Blot.

**References:**

1.https://cores.ukb.uni-bonn.de/nanobodies/;2.https://www.thermofisher.com/order/catalog/product/14302D;
3.Fernandez-Martinez (2012) JCB196(4): 419