Nanobodies Workshop: Binder Recovery by In Silico and In Vitro Panning

Report of Contributions

Contribution ID: **3** Type: **Lecture**

Characteristics of Nanobody Libraries

Tuesday 23 September 2025 09:00 (1h 30m)

This introductory session provides a foundational overview of nanobody (VHH) libraries and their role in binder discovery. Participants will learn the key differences between immune libraries (from antigen-immunized animals), naïve or pre-immune libraries (from non-immunized sources), and synthetic libraries (constructed through targeted sequence design and CDR randomization). The session explores how library diversity is generated—through methods such as PCR amplification of VHH genes or synthetic variation—and how it influences library quality and downstream success. Common library sizes are discussed, as well as how different display platforms (e.g. phage, yeast, ribosome) are used to present libraries to antigens, with an emphasis on phage display.

Primary author: Prof. DE MARCO, Ario (University of Nova Gorica, Slovenia)

Presenter: Prof. DE MARCO, Ario (University of Nova Gorica, Slovenia)

Contribution ID: **4** Type: **Lecture**

Nanobody Isolation In Silico From Pre-Immune Libraries

Tuesday 23 September 2025 11:00 (1 hour)

Recent advances in high-throughput sequencing and structure prediction have enabled "virtual panning"of naïve nanobody (VHH) repertoires. This session reviews the core modules of computational nanobody discovery: repertoire profiling via MiXCR and clonotype clustering, homology modeling with NanobodyBuilder2 and ColabFold/Boltz1, and docking benchmarking of published VHH–antigen complexes using ZDock and Boltz-1 scored by different scoring strategies.
We'll showcase case studies from ongoing work—including clonotype analysis of a naïve llama library and redocking of SAbDab-derived VHH–antigen pairs to assess predictive performance.

Presenter: KROPIVSEK, Klara (University of Nova Gorica)

Contribution ID: **5** Type: **Practical**

Nanobody Isolation In Silico From Pre-Immune Libraries (hands-on)

Tuesday 23 September 2025 13:00 (1h 30m)

In the hands-on workshop, participants rotate through brief, guided exercises:

1. Processing VHH sequences and clustering clonotypes with MiXCR;
2. Building and comparing VHH models side-by-side using NanobodyBuilder2 vs. ColabFold/Boltz1;
3. Visualizing antigen–VHH interfaces;
4. Interpreting ZDock/Boltz-1 predictions via DockQ scores;
5. Exploring CDR motif distributions from MiXCR output. By session's end, attendees will understand each workflow component, its computational demands, and current limitations —enabling them to build their own in silico nanobody screening pipelines.

Presenter: KROPIVSEK, Klara (University of Nova Gorica)

Track Classification: In Silico Modelling and Design

Contribution ID: **6** Type: **Lecture**

De Novo Nanobody Design

Thursday 25 September 2025 09:00 (1h 30m)

Nanobodies (Nbs) from pre-immune libraries could exhibit deficiencies in biophysical properties, such as stability and affinity, necessitating an optimization step. This can be achieved either in vitro through controlled mutagenesis followed by another round of panning or in silico by proposing variants. This approach, which involves screening multiple single-site variants, may demand extensive experimental efforts. Additionally, negative epistatic effects can occur when combining multiple potentially beneficial single-site variants, where increased affinity variants may negatively impact stability.
Emerging data-driven Machine Learning (ML) approaches are promising in the field of de novo Nb design or redesign, as they can learn multidimensional constraints between protein structure, stability and binding capacity, while explicitly designing protein and non-protein biomolecules at atomic-level resolution.
Anyway, a small amount of data is available for training Nbs-specific methods and available models have been tested and validated mainly in the design of mini-binders of known protein biomarkers. In this workshop session an introduction to these methods and their expected accuracy on published data will be provided, with the possibility to try a couple of them through a Jupiter Notebook run over the internet browser in a simple case study. In the second part there will be a perspective and discussion on adapting such de novo protein design systems for nanobody CDRs (re)design tasks.

Presenter: Dr ORLANDO, Marco (University of Milano-Bicocca, Italy)

Track Classification: In Silico Modelling and Design

Contribution ID: **7** Type: **Practical**

Workshop Practical: Nanobody Panning Against a Target Antigen

Tuesday 23 September 2025 15:00 (2 hours)

In the practical part of the workshop, participants will gain hands-on experience with phage display-based nanobody panning. Working in small groups, they will perform a selection round using a nanobody (VHH) library and a chosen model antigen immobilized on a solid surface. The goal is to enrich for nanobody clones that specifically bind the target.
The workflow includes incubation of the library with the antigen, washing to remove non-binders, elution of bound phages, and infection of E. coli for amplification. If time allows, participants will also explore panning on live cells to simulate more complex and physiologically relevant selection environments.

Takeaway: Participants will understand and carry out the core steps of a nanobody selection campaign, gaining insight into the practical considerations and challenges of identifying specific binders from a large pre-immune or synthetic library.

Track Classification: Experimental Panning

Contribution ID: **8** Type: **Lecture**

Molecular Modelling Approaches for the Prediction of Nanobody Biophysical Properties

Thursday 25 September 2025 11:00 (1h 30m)

Nanobodies (VHHs) have proved to be valid substitutes of conventional IgG antibodies in basic research and diagnostics, and they are actively tested to confirm their therapeutic potential. Because of their size (~125 amino acids), significantly smaller than that of ordinary antibodies, VHHs can be modelled in silico in relatively short time with the current computational resources.
In this context, a number of computational modelling approaches, i.e. homology modelling, molecular docking, and molecular dynamics, will be reviewed in this session as useful tools able to predict and optimize the biophysical features of nanobodies.
Finally, we will address the problem of predicting how VHHs sequences can tolerate mutations by employing a simulation protocol based on all-atom molecular dynamics and whole-molecule docking.

Presenter: SOLER, Miguel A. (University of Udine, Italy)

Track Classification: Biophysics and Optimization

Contribution ID: **9** Type: **Lecture**

Keynote Lecture I

Monday 22 September 2025 16:00 (1 hour)

To be announced.

Contribution ID: **10** Type: **Lecture**

Nanobodies for Structural Studies: Crystallization Chaperones & Cryo-EM Stabilizers

Thursday 25 September 2025 13:30 (1h 30m)

Nanobodies, also known as single-domain antibody fragments derived from camelid heavy-chain antibodies, have emerged as valuable tools in structural biology. Due to their small size, high stability, and strong binding affinity, nanobodies serve effectively as crystallization chaperones, aiding in the formation of well-ordered crystals.
Their ability to recognize specific epitopes on target proteins can stabilize transient conformations, facilitating structural determination by X-ray crystallography. Nanobodies can also suppress conformational flexibility, which often hampers crystallization efforts, leading to higher-resolution structures. In cryo-electron microscopy (cryo-EM), nanobodies act as stabilizers by binding selectively to flexible or dynamic regions of macromolecules. This stabilization reduces conformational heterogeneity, resulting in clearer, higher-resolution reconstructions. Moreover, their small size enhances particle orientation diversity, improving data quality.
Nanobodies are also useful as fiducial markers or tags, aiding in particle alignment during image processing. Overall, they have revolutionized structural studies by enabling detailed biological insights into complex, dynamic systems that were previously challenging to analyze, making them indispensable tools in structural biology and drug discovery.

Presenter: Dr DE MARCH, Matteo (University of Nova Gorica, Slovenia)

Track Classification: Structural and Imaging Applications

Contribution ID: **11** Type: **Lecture**

Affinity Maturation

Friday 26 September 2025 09:00 (1h 30m)

More information to follow.

Presenter: Dr FORTUNA, Sara (Italian Institute of Technology, Italy)

Track Classification: Biophysics and Optimization

Contribution ID: **12** Type: **Lecture**

Nanobodies in Advanced Flourescent Microscopy

Friday 26 September 2025 11:00 (1h 30m)

Advanced microscopy techniques are continually redefining how we study biological systems, offering unprecedented spatial and temporal resolution. In super-resolution fluorescence microscopy, spatial resolution can reach the scale of tens of nanometers. However, as optical resolution improves, molecular labeling is increasingly becoming the limiting factor.
In this talk, we will focus on how nanobodies, small, monovalent, single-domain antibody fragments, can overcome key limitations of conventional labeling approaches such as fluorescent proteins and standard antibodies. Their compact size (~15 kDa) minimizes linkage error, while their monovalency prevents artificial clustering or crosslinking of target molecules, an essential feature for quantitative imaging and live-cell compatibility.
Nanobodies can be efficiently expressed in E. coli and customized with labeling tags, allowing for functionalization (organic dyes, click chemistry groups). This enables detailed biophysical characterization, including the determination of binding kinetics or saturation concentration, which are critical for developing robust, reproducible labeling protocols.
Finally, we will highlight validated, ready-to-use nanobody systems such as anti-GFP and anti-ALFA-tag binders, which offer plug-and-play solutions without the need for custom nanobody generation.

Presenter: Dr KALOUSKOVA, Barbora (TU Wien, Austria)

Track Classification: Structural and Imaging Applications

Contribution ID: **13** Type: **Short Talk**

PhD2PhD

Tuesday 23 September 2025 17:00 (1 hour)

Session in which PhD students present their current work or problem they want to develop, and get feedback from lecturers and peers.

Track Classification: PhD2PhD

Contribution ID: **14** Type: **Practical**

Workshop Practical: Nanobody Panning Against a Target Antigen

Wednesday 24 September 2025 09:00 (1h 30m)

In the practical part of the workshop, participants will gain hands-on experience with phage display-based nanobody panning. Working in small groups, they will perform a selection round using a nanobody (VHH) library and a chosen model antigen immobilized on a solid surface. The goal is to enrich for nanobody clones that specifically bind the target.
The workflow includes incubation of the library with the antigen, washing to remove non-binders, elution of bound phages, and infection of E. coli for amplification. If time allows, participants will also explore panning on live cells to simulate more complex and physiologically relevant selection environments.

Takeaway: Participants will understand and carry out the core steps of a nanobody selection campaign, gaining insight into the practical considerations and challenges of identifying specific binders from a large pre-immune or synthetic library.

Track Classification: Experimental Panning

Contribution ID: **15** Type: **Practical**

Workshop Practical: Nanobody Panning Against a Target Antigen

Wednesday 24 September 2025 11:00 (1h 30m)

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Takeaway: Participants will understand and carry out the core steps of a nanobody selection campaign, gaining insight into the practical considerations and challenges of identifying specific binders from a large pre-immune or synthetic library.

Track Classification: Experimental Panning

Contribution ID: **16** Type: **Practical**

Workshop Practical: Nanobody Panning Against a Target Antigen

Wednesday 24 September 2025 13:30 (2 hours)

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Takeaway: Participants will understand and carry out the core steps of a nanobody selection campaign, gaining insight into the practical considerations and challenges of identifying specific binders from a large pre-immune or synthetic library.

Track Classification: Experimental Panning

Contribution ID: **17** Type: **Practical**

Workshop Practical: Nanobody Panning Against a Target Antigen

Wednesday 24 September 2025 16:00 (1 hour)

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Takeaway: Participants will understand and carry out the core steps of a nanobody selection campaign, gaining insight into the practical considerations and challenges of identifying specific binders from a large pre-immune or synthetic library.

Track Classification: Experimental Panning

Contribution ID: **21** Type: **Short Talk**

PhD2PhD

Wednesday 24 September 2025 17:00 (1 hour)

Session in which PhD students present their current work or problem they want to develop, and get feedback from lecturers and peers.

Track Classification: PhD2PhD

Contribution ID: **22** Type: **Short Talk**

PhD2PhD

Friday 26 September 2025 13:30 (1 hour)

Session in which PhD students present their current work or problem they want to develop, and get feedback from lecturers and peers.

Track Classification: PhD2PhD

Contribution ID: **23** Type: **Lecture**

Keynote Lecture II

Thursday 25 September 2025 15:30 (1 hour)

To be announced.

Track Classification: Keynote Lecture

Contribution ID: **24** Type: **Lecture**

Wrap Up and Goodbye

Friday 26 September 2025 14:30 (30 minutes)

With Coffee