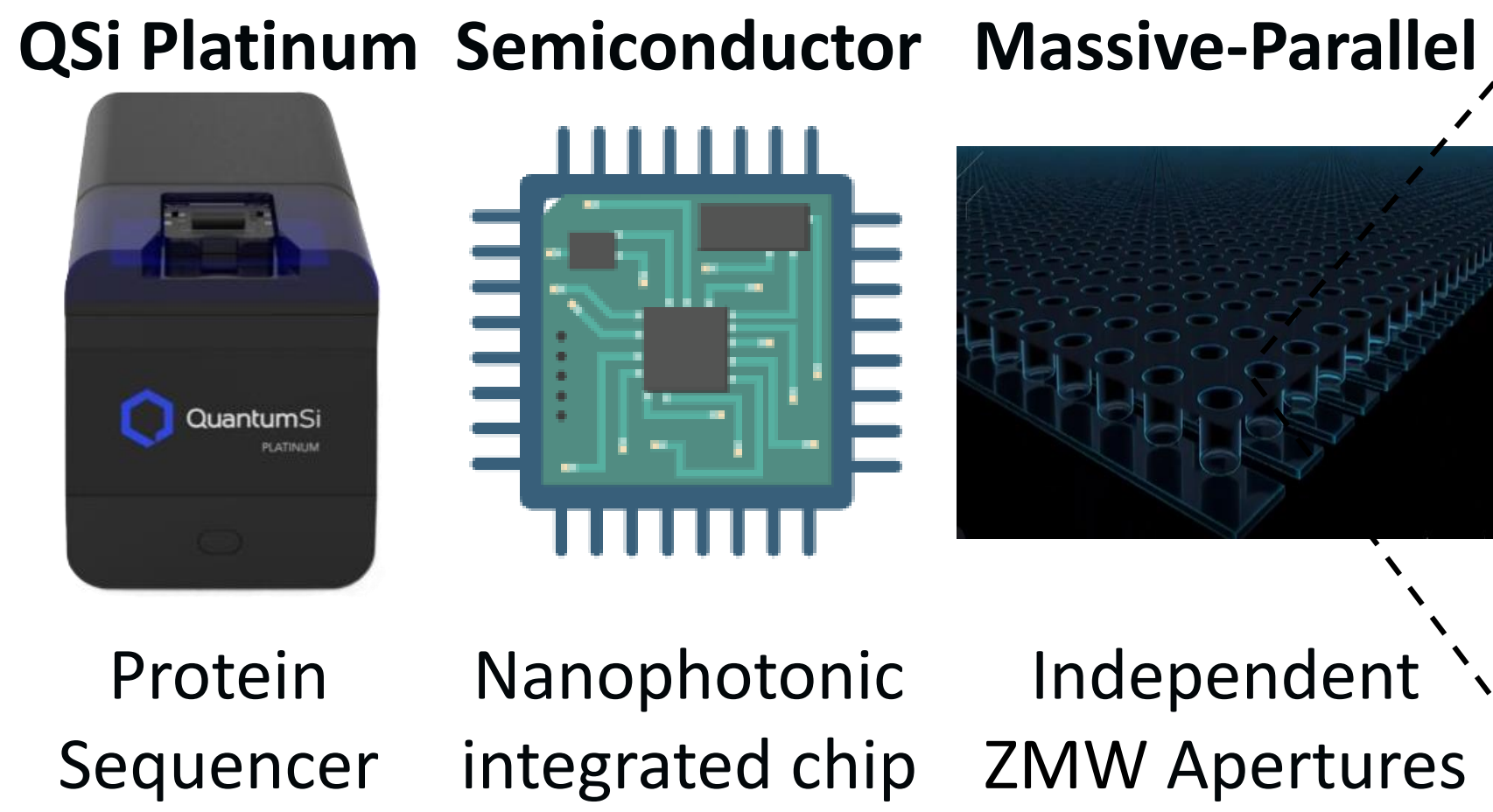


Multiplexing Nanobody Kinetics Measurements at the Single-Molecule Level

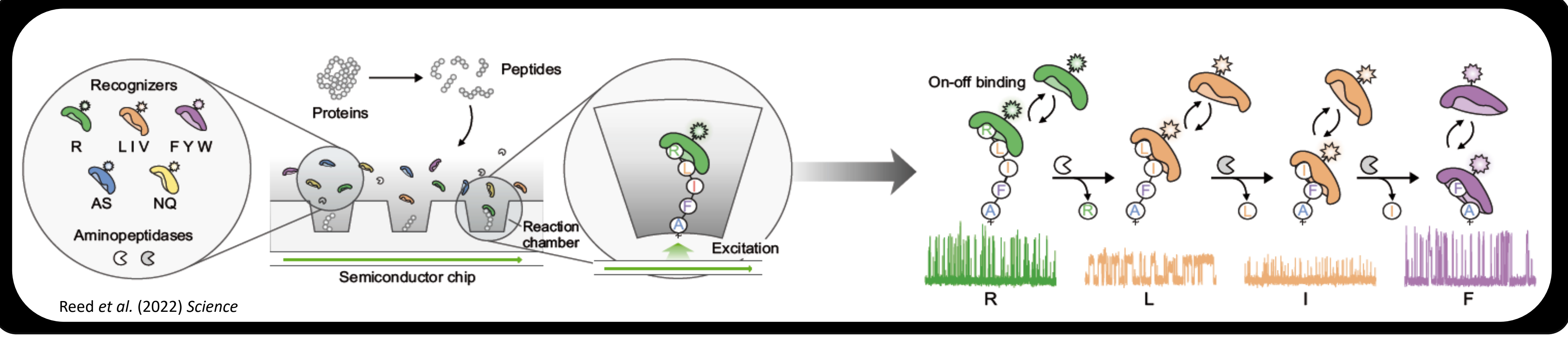
Ghada H. Mansour*¹, Sebastian Hutchinson², Eilyn Redheuil¹, Ahmed Rehan¹, Adeline Pichard-Kostuch², Marco Ribezzi-Crivellari², Andrew D. Griffiths¹

¹Laboratoire de Biochimie, ESPCI Paris, Université PSL, CNRS UMR 8231, Paris, France. ²Quantum-Si, France, Paris, France.

Poster Keys: NGPS (Next-Generation Protein Sequencing), ZMW (Zero-mode waveguide), BC (Barcode), PD (Pulse Duration), IPD (Inter-Pulse Duration), GFP (Green Fluorescent Protein)



Single Molecule Resolution of Next-Generation Protein Sequencing (NGPS)



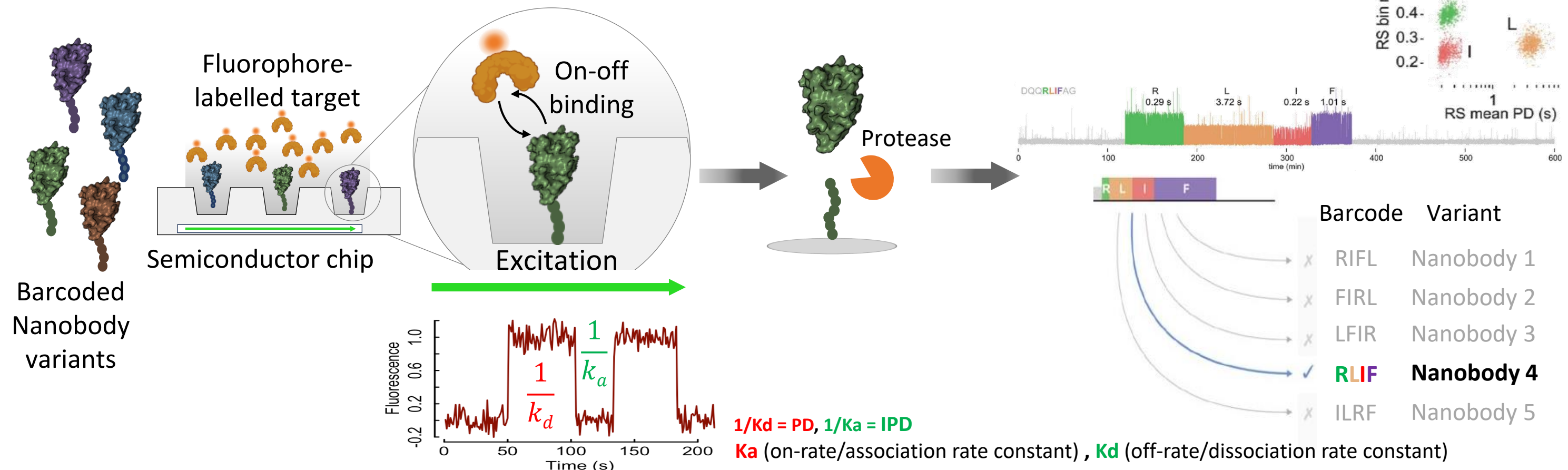
Abstract

Single-molecule methods provide useful insights into the intermolecular variations and functional differences of individual molecules and have revealed much about the complexity of biological processes. Today it is possible to monitor binding kinetics at the single molecule level in a highly parallel fashion using zero-mode waveguide (ZMW) arrays. This allows monitoring the binding/unbinding of fluorescently-labelled molecules on millions of immobilised targets simultaneously, obtaining a full kinetic description of their interactions, offering a complementary picture to classical techniques but also uncovering important details that are missed in bulk studies. In our work, we use ZMW arrays to study the binding kinetics of antibody-antigen interactions at the single-molecule level. Moreover, we demonstrate that it is possible to couple these binding kinetics measurements with single-molecule protein sequencing to multiplex kinetic analysis to panels of nanobodies. This new approach to the study of nanobodies will help us understand the sequence/function relationship in nanobodies and open new directions in nanobody affinity maturation.

Objective

On-chip single-molecule kinetic characterization of multiplexed nanobodies

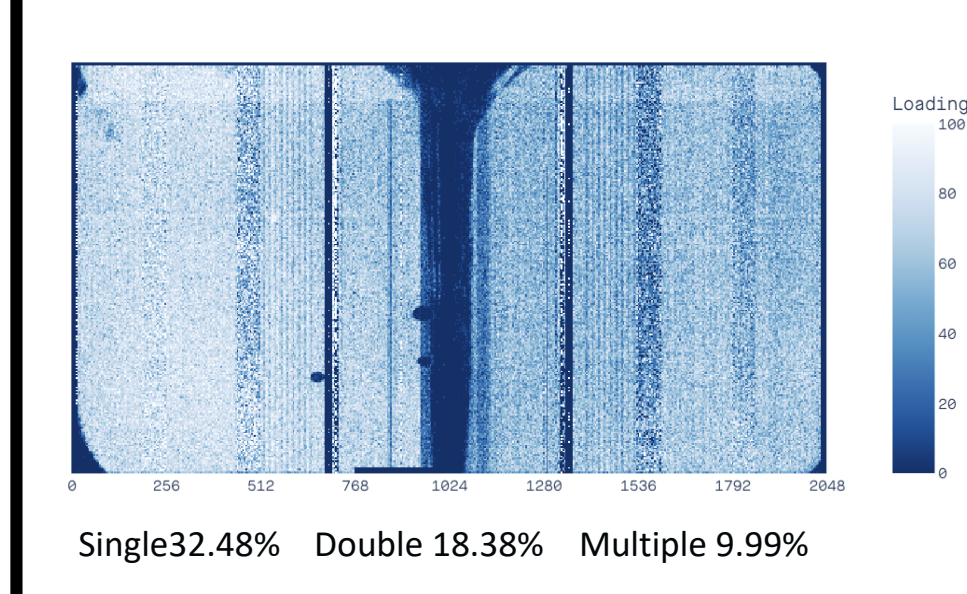
1. Single-molecule binding kinetics
2. Expose barcode
3. NGPS barcode



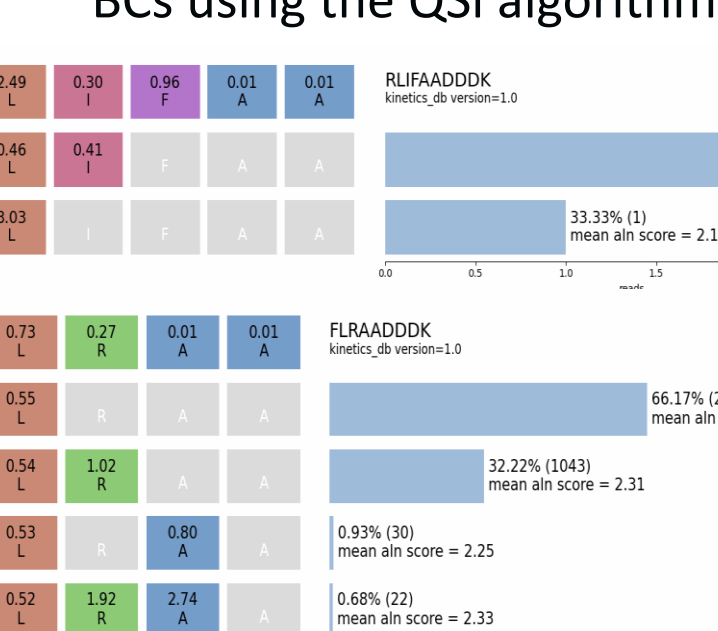
Results

Model Selection Data

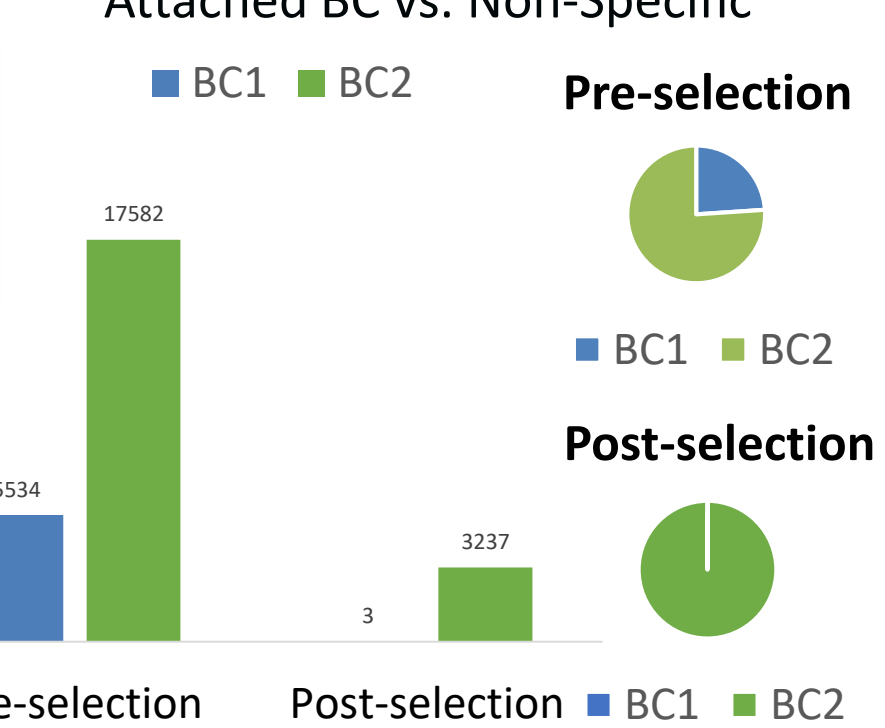
Simultaneous Imaging of Thousands of Molecules on a Semiconductor Chip



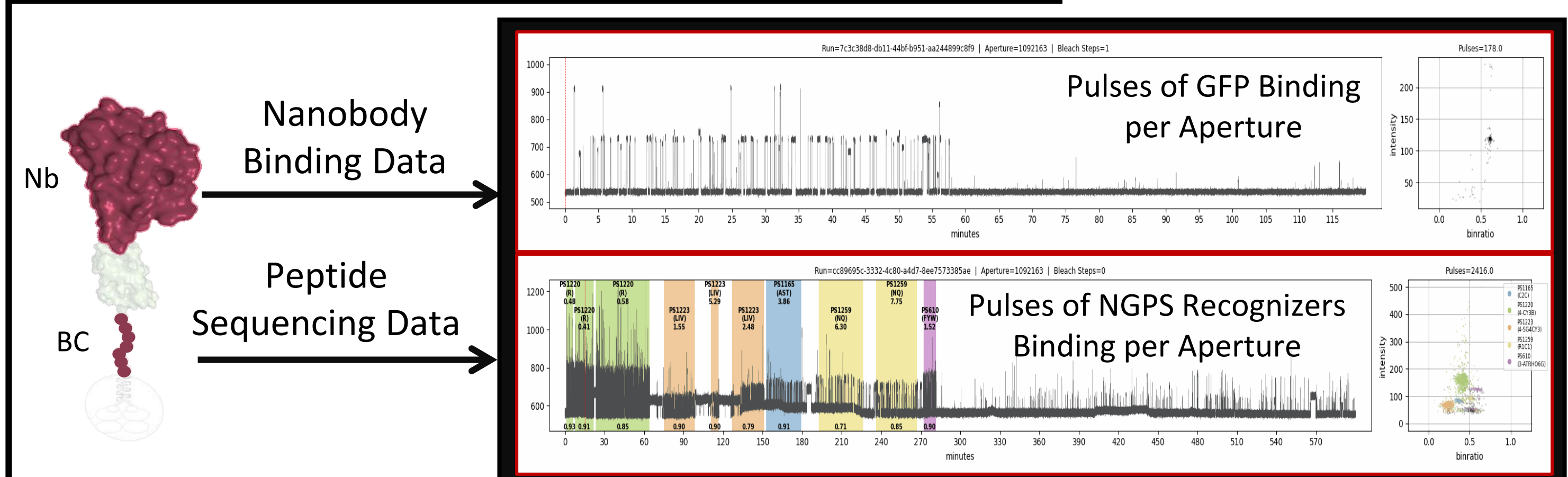
Read Alignments to Reference BCs using the QSi algorithm



Enrichment of Specific Nanobody-Attached BC vs. Non-Specific

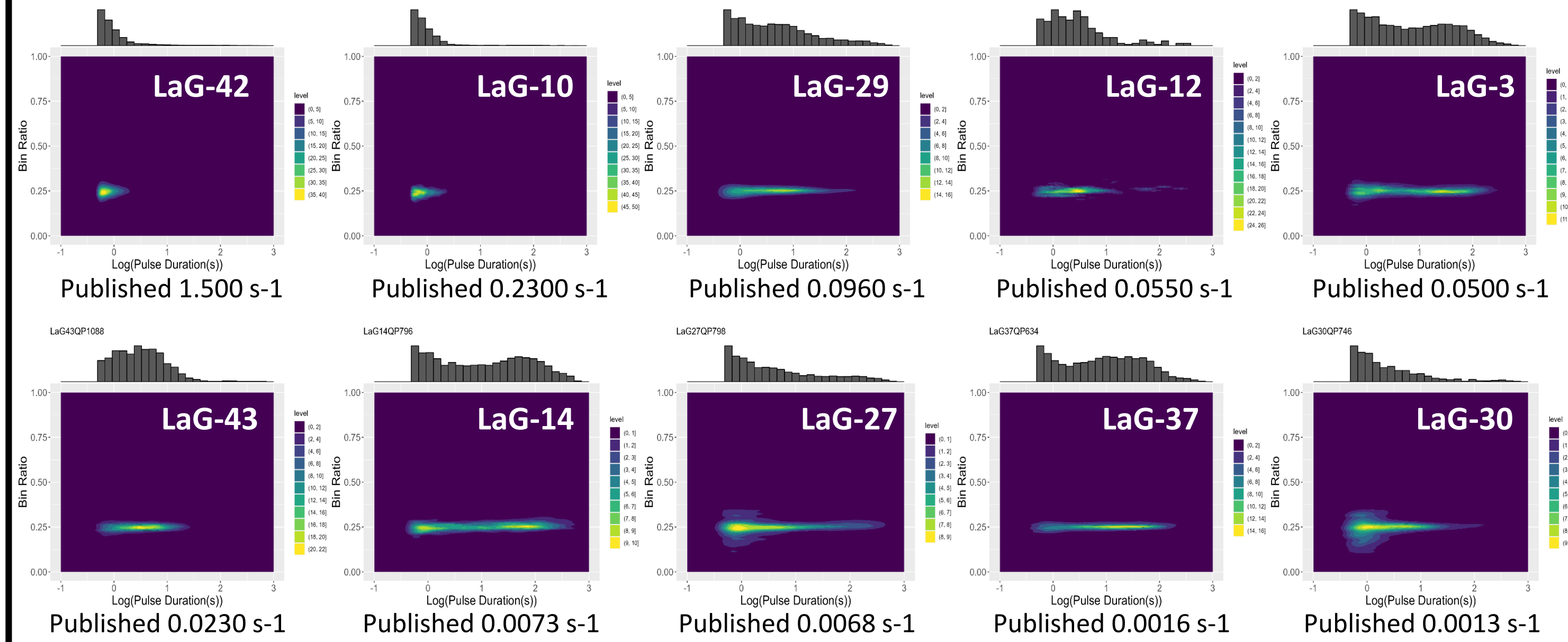


Single Molecule Data of Nanobody Binding Kinetics

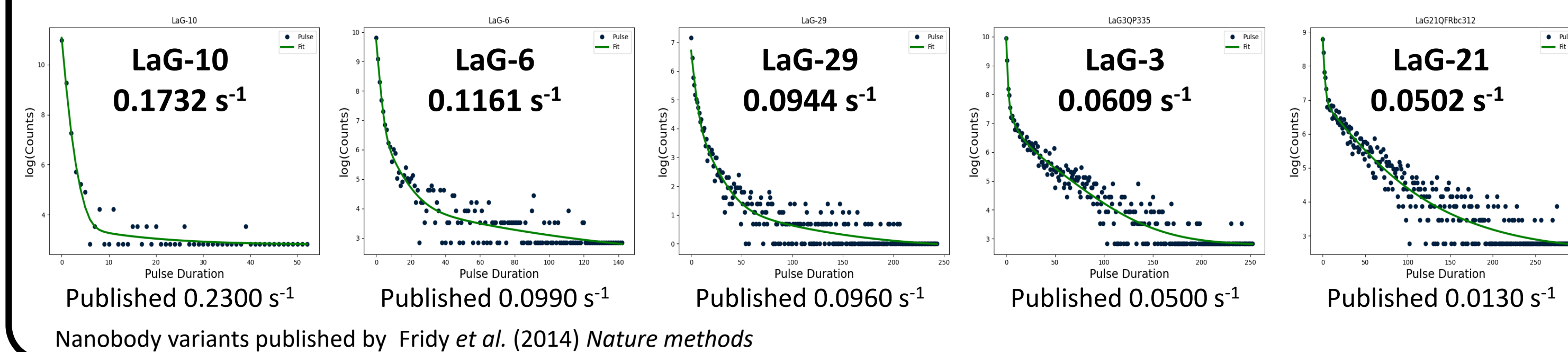


Pulse Duration (PD) of Nanobodies

Qualitative Analysis: Nanobodies with faster published Koff exhibit shorter PD on the platinum.

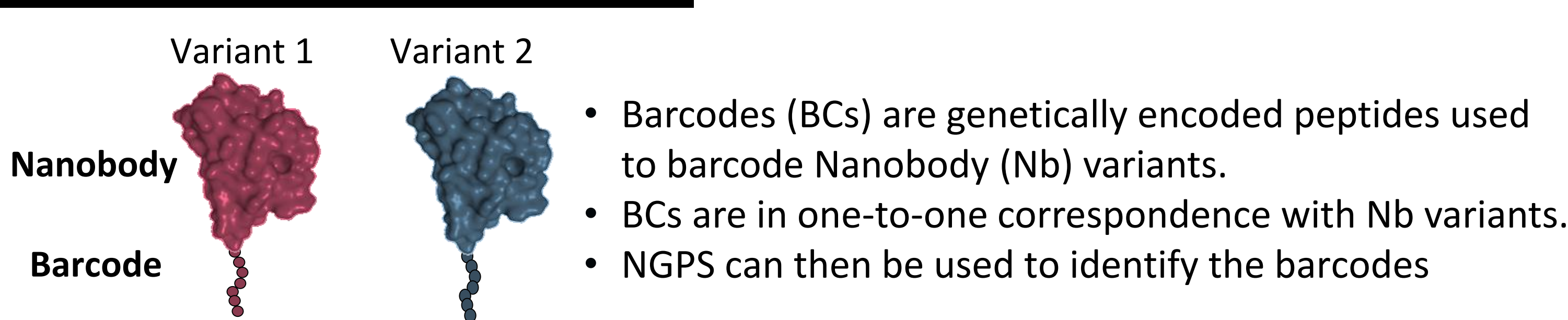


Quantitative Analysis: Some nanobodies show Koff consistent with literature findings.

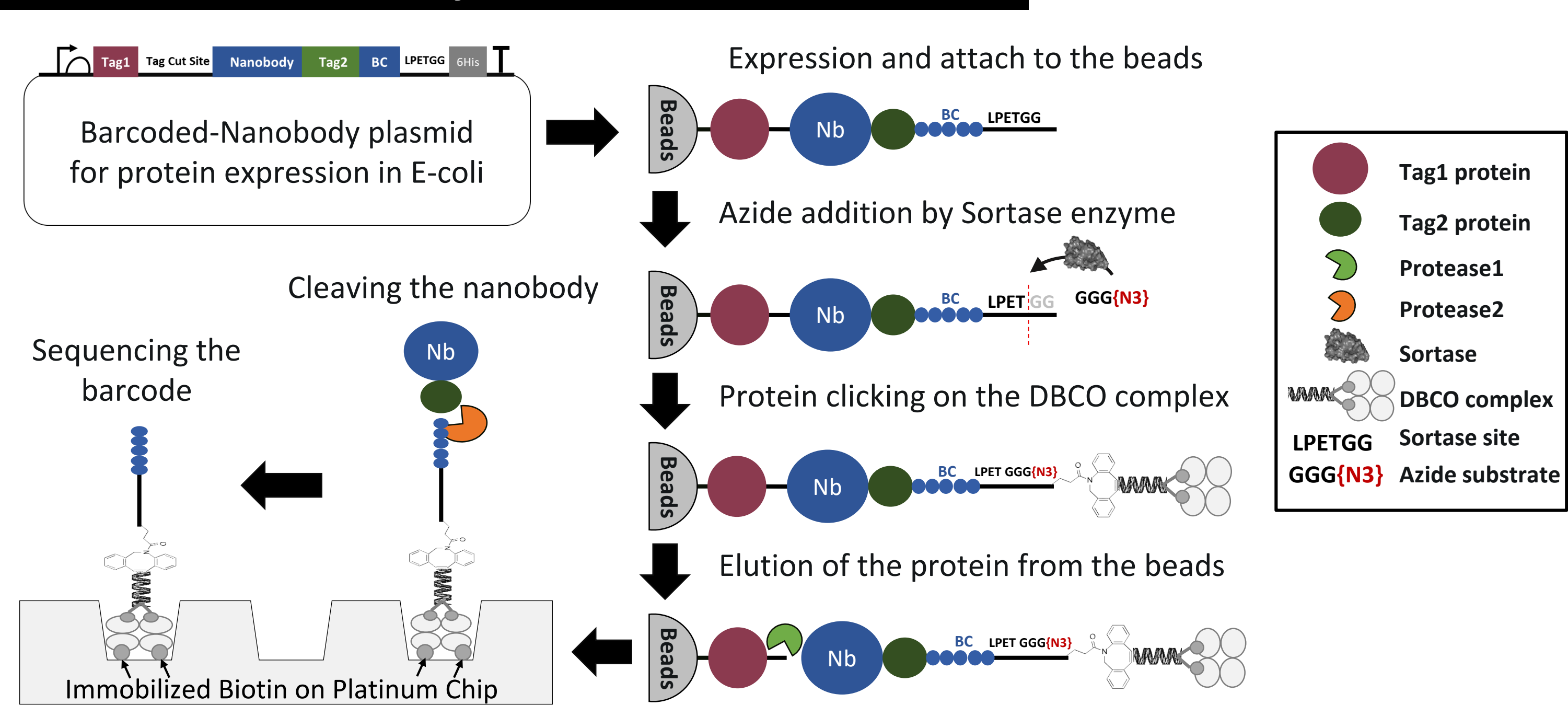


Methodology

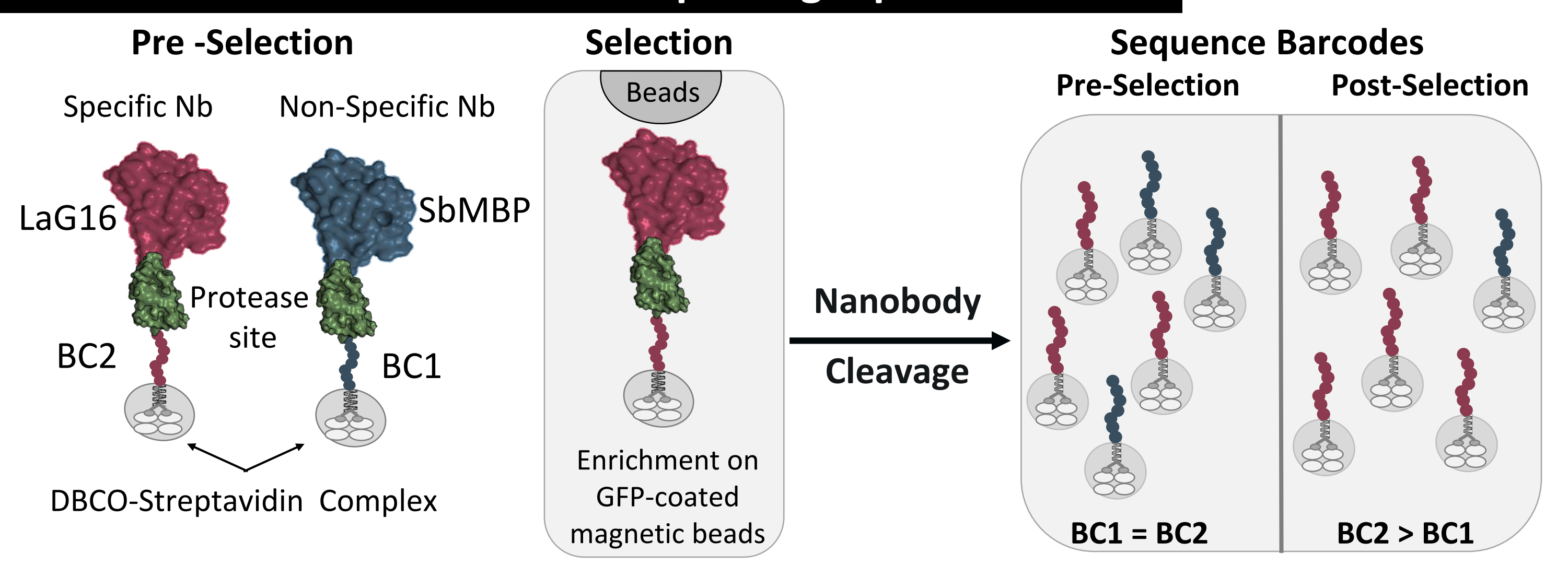
Peptide Barcodes for Multiplexing



Genetic-encoded Library for Barcoded Nanobodies



Model Selection for Proof of Concept. Target protein: GFP



LaG16 and SbMBP published by Zimmermann et al. (2018) *elife*

Conclusion

In summary, our model selection has generated highly promising results of multiplexing two different nanobodies and differentiating them based on their unique barcode. This allows us to successfully characterize the kinetics of multiplexed nanobody variants at the single-molecule level. The ability to multiplex and analyze these nanobodies individually offers an exciting approach to unravelling the complexities of protein interactions, thereby revolutionizing single-molecule studies in the field of proteomics.

Literature cited:

- Reed BD et al. Real-time dynamic single-molecule protein sequencing on an integrated semiconductor device. *Science*. 2022
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Correspondence:

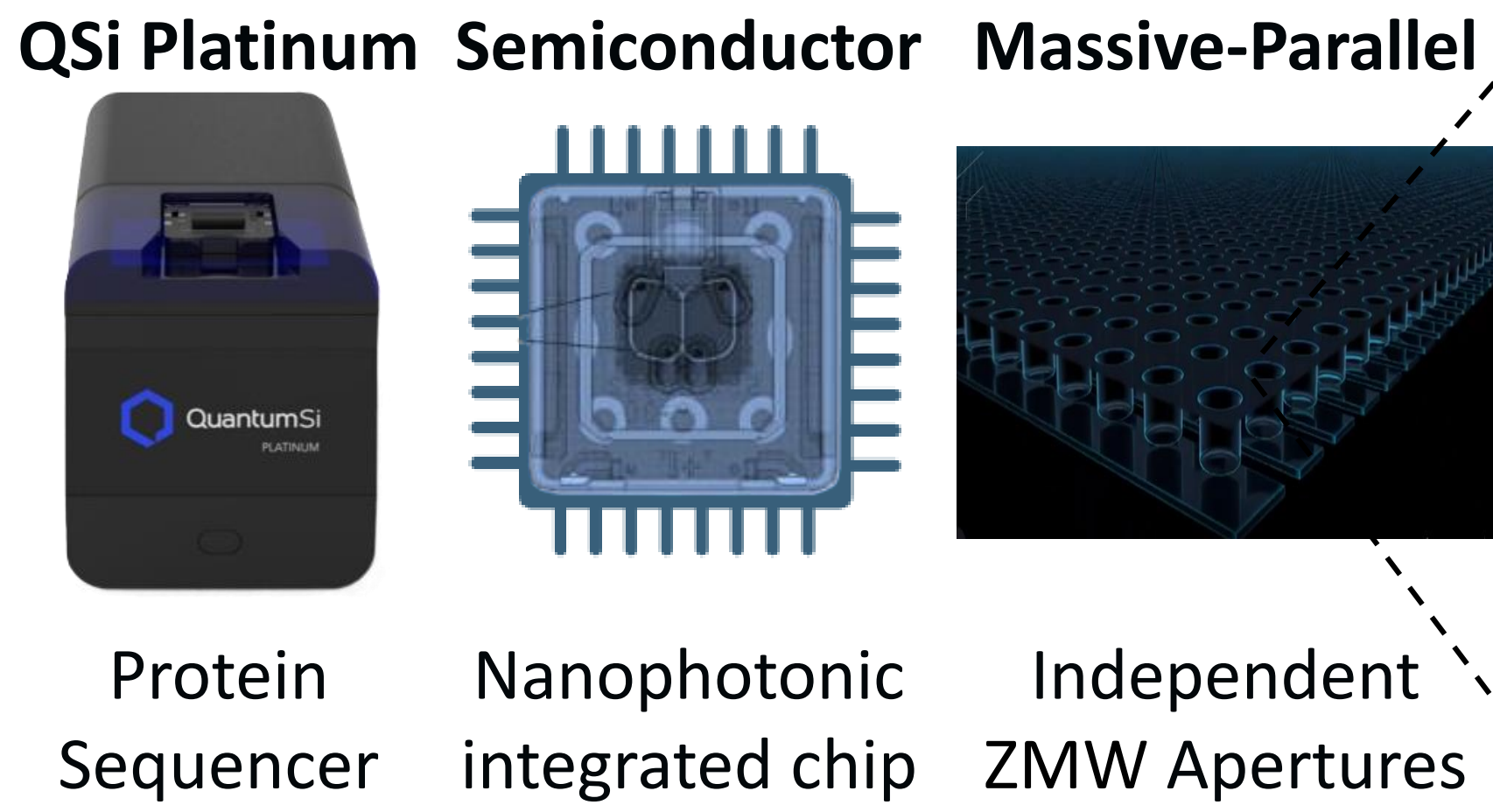
ghada.mansour@espci.fr, shutchinson@quantum-si.com, mribezzi@quantum-si.com

Multiplexing Nanobody Kinetics Measurements at the Single-Molecule Level

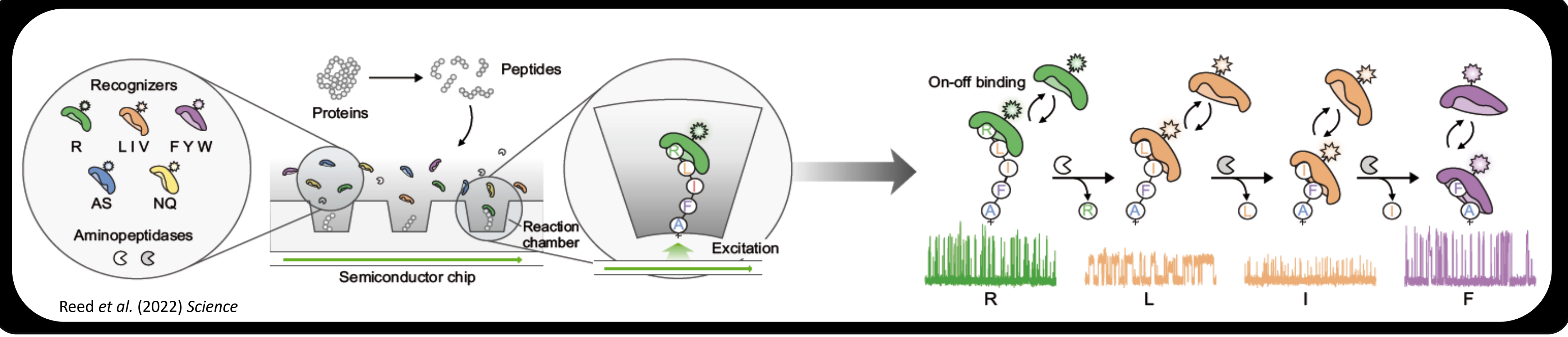
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Poster Keys: NGPS (Next-Generation Protein Sequencing), ZMW (Zero-mode waveguide), BC (Barcode), PD (Pulse Duration), IPD (Inter-Pulse Duration), GFP (Green Fluorescent Protein)



Single Molecule Resolution of Next-Generation Protein Sequencing (NGPS)

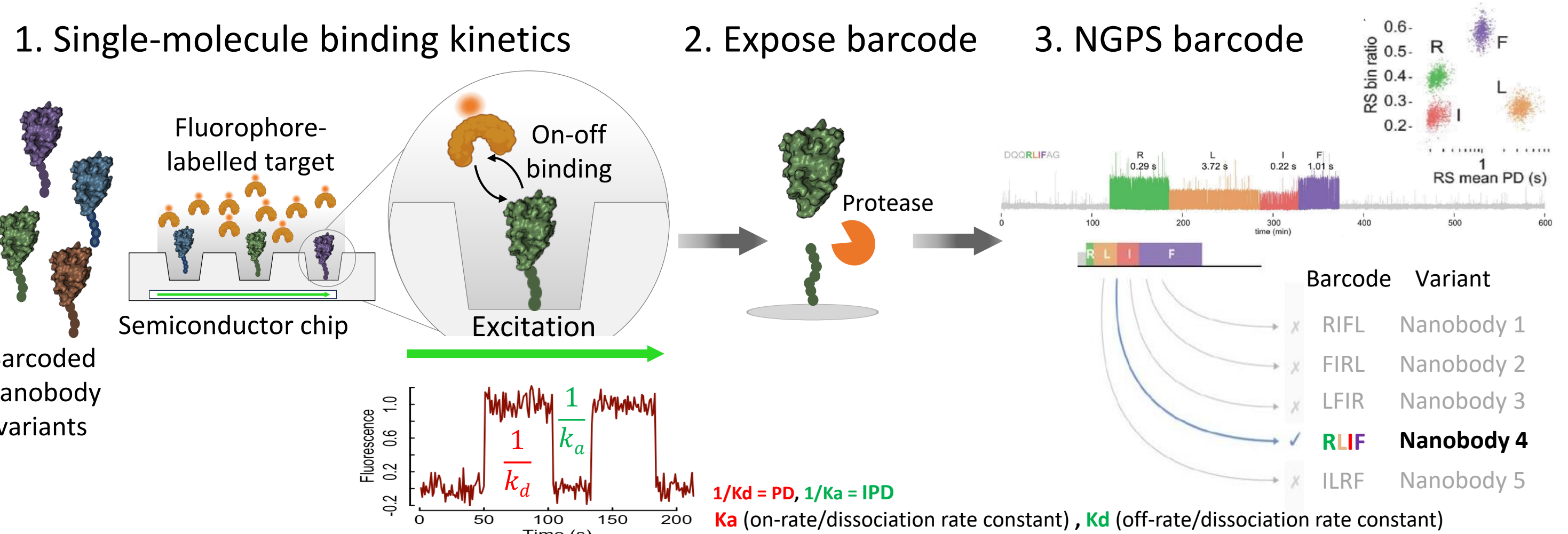


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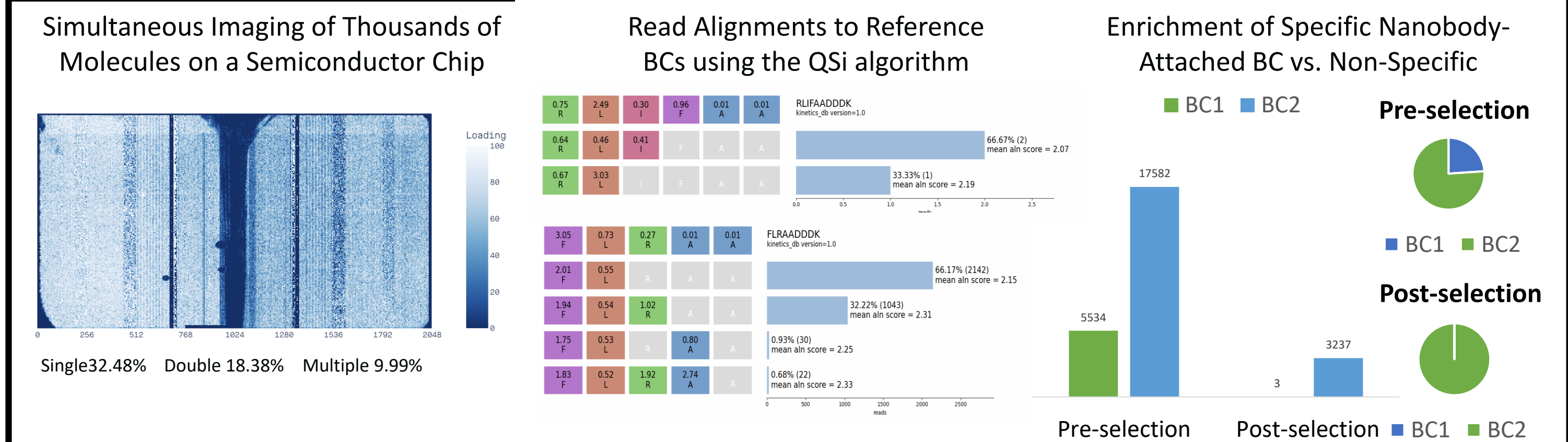
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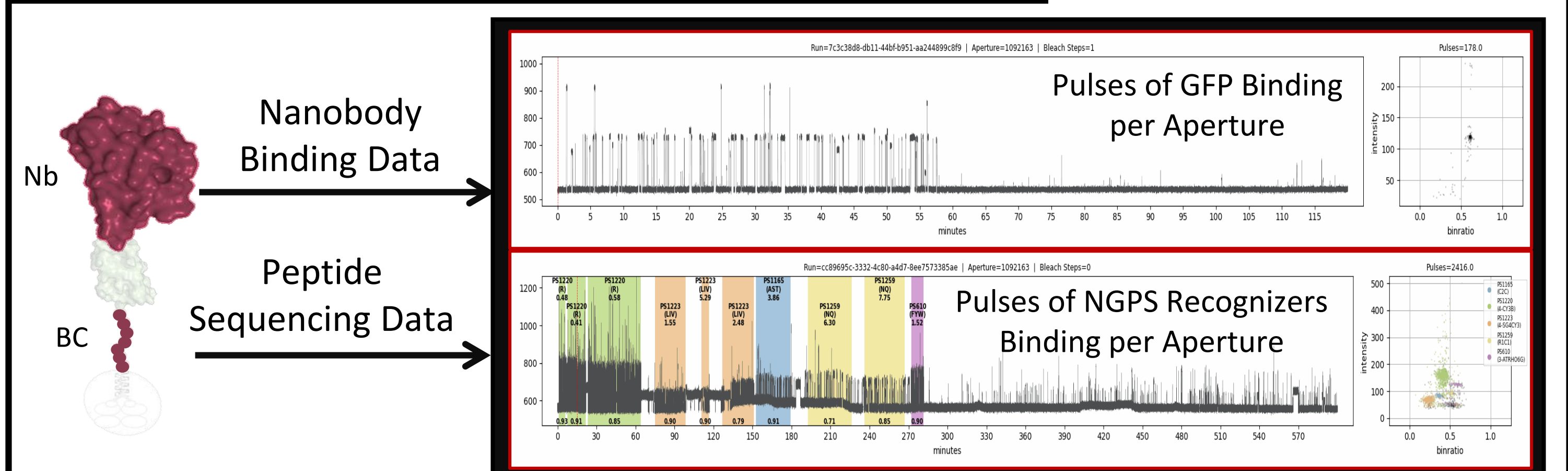


Results

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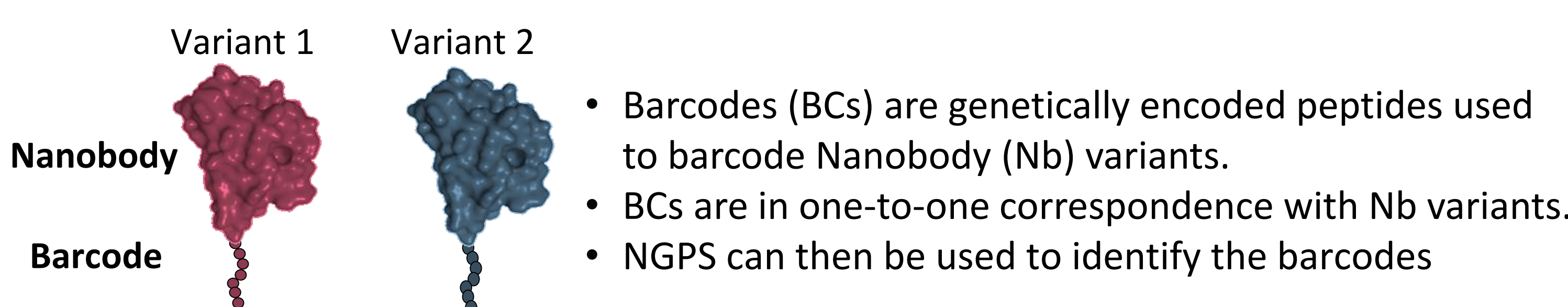


Single Molecule Data of Nanobody Binding Kinetics

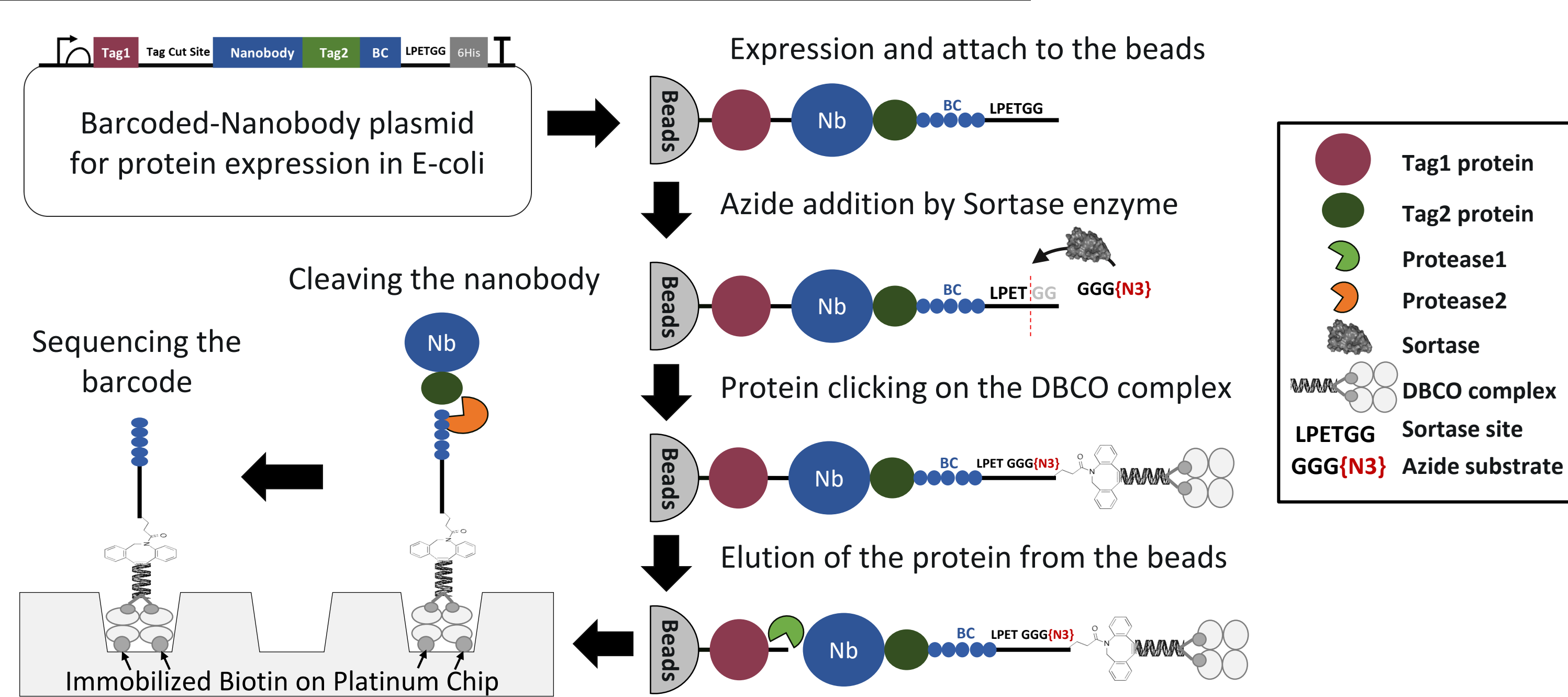


Methodology

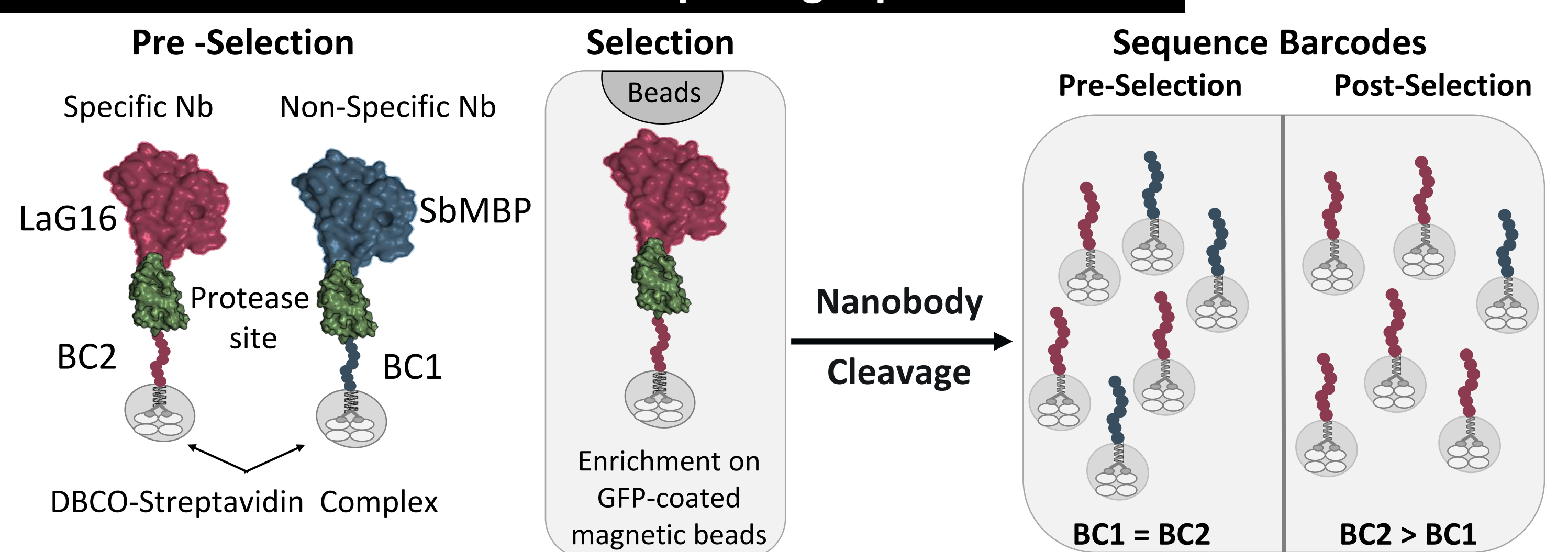
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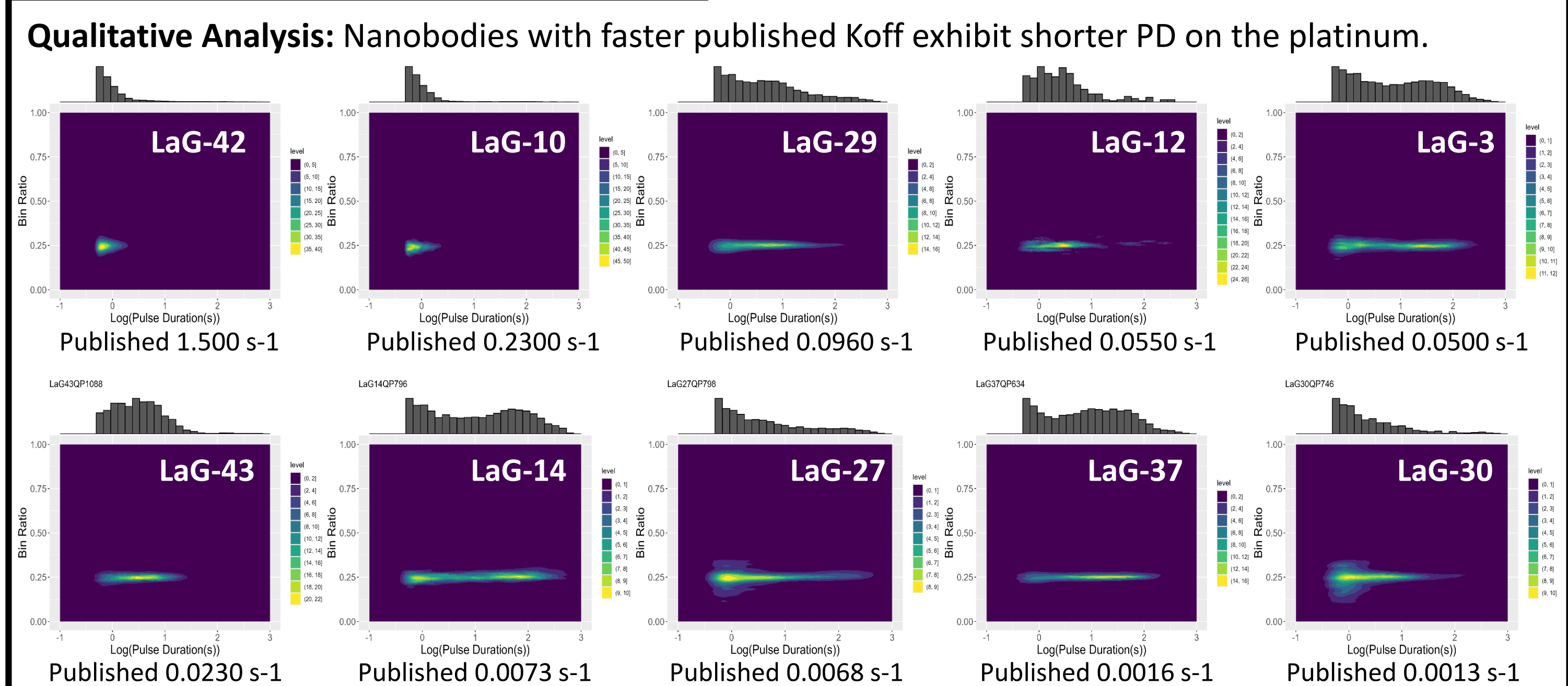
Genetic-encoded Library for Barcoded Nanobodies



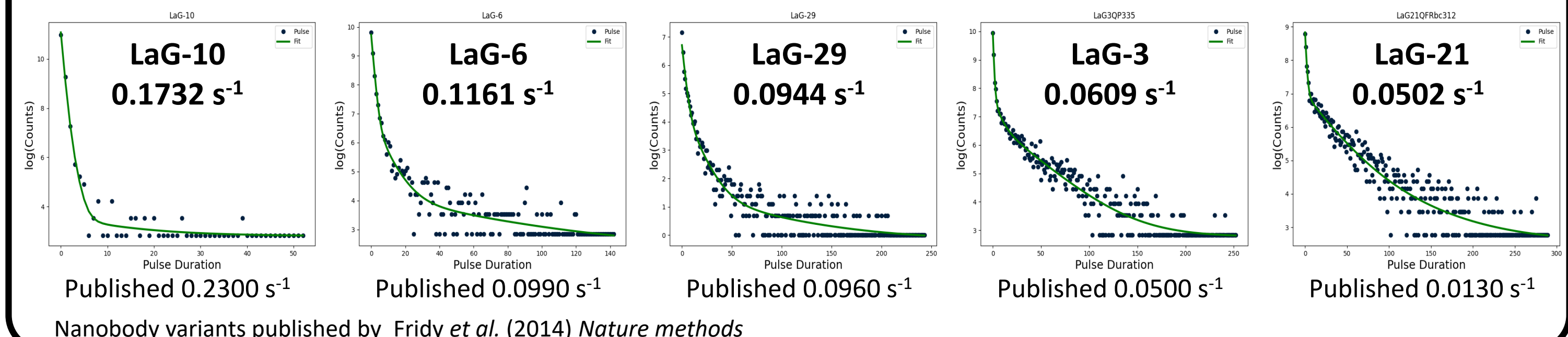
Model Selection for Proof of Concept. Target protein: GFP



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