Measuring Nanobody Kinetics at the Single-Molecule Level

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ABSTRACT

Antibodies are a paradigm for high-affinity, protein-based binding reagents and are extremely important in biotechnological, diagnostic, and therapeutic applications. Of special interest are nanobodies, recombinant variable domains from heavy-chain-only antibodies. Nanobodies have several advantages: their small molecular weight, superior solubility and stability and clearance rate. One particular use of nanobodies is their use in imaging, which requires tailoring affinity and specificity for their targets. Despite their benefits, nanobodies have only recently been used in single-molecule assays, e.g. PAINT imaging, which require careful tuning of their kinetic properties.

In this work we demonstrate the detection of nanobody-antigen binding at the single-molecule level. We first demonstrate the measurement of interaction kinetics between an immobilised GFP target and a LaG-16 antibody in solution, with similar results to bulk-derived kinetics constant. We then demonstrate the same kind of experiments in a more complex situation with multiple nanobodies.

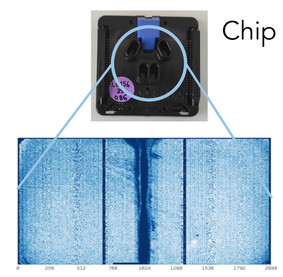
INTRODUCTION

Next-generation protein sequencing™ is a transformational tool for protein science which enables new proteomic discoveries in human health and disease, such as biomarker detection and characterization with single-molecule resolution.

Quantum-Si has developed the world's first next-generation protein sequencer™. The workflow involves immobilization of peptides in individual apertures of a zero-mode waveguide (ZMW) array on an integrated semiconductor chip[1]. Fluorescently labelled N-terminal amino acid (NAA) recognizers bind on-off to NAAs and aminopeptidases sequentially cleave and expose each NAA for recognition. The lifetime and intensity and order of binding events result in a kinetic signature that is used to determine the peptide sequence.

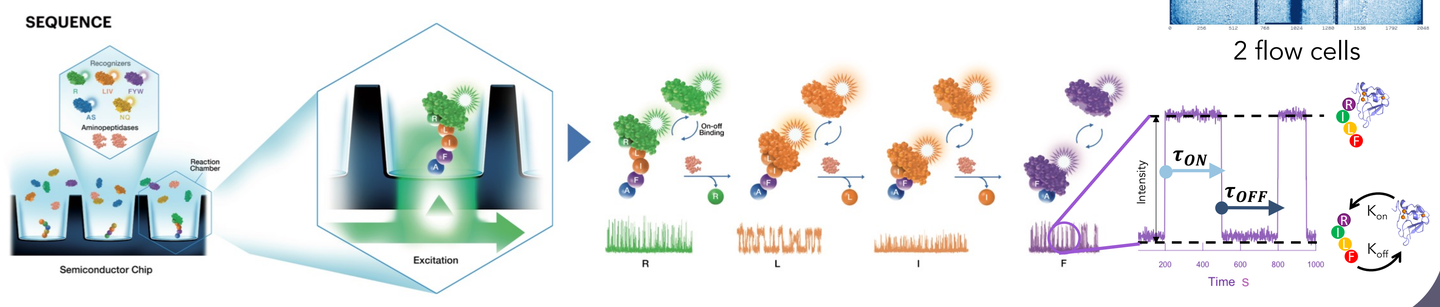
We demonstrate the use of this technology to study nanobody kinetics with single-molecule resolution.

Platinum™



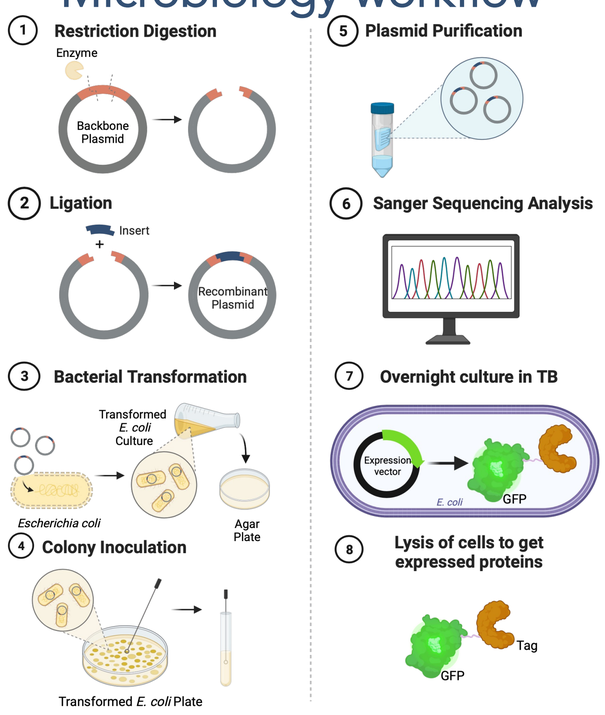
Chip

2 flow cells

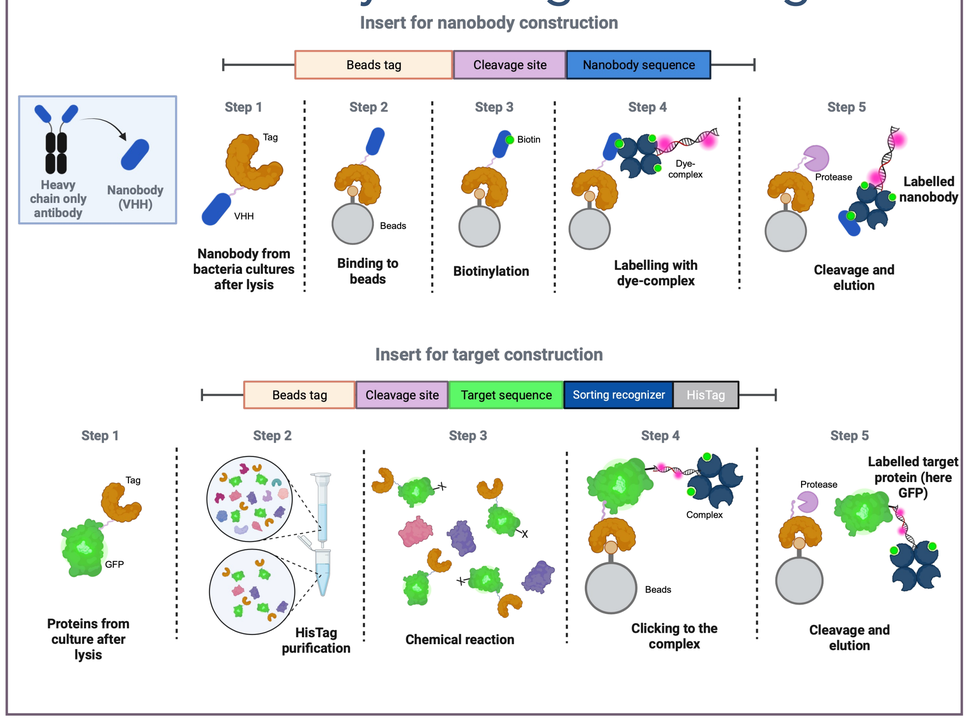


METHODS

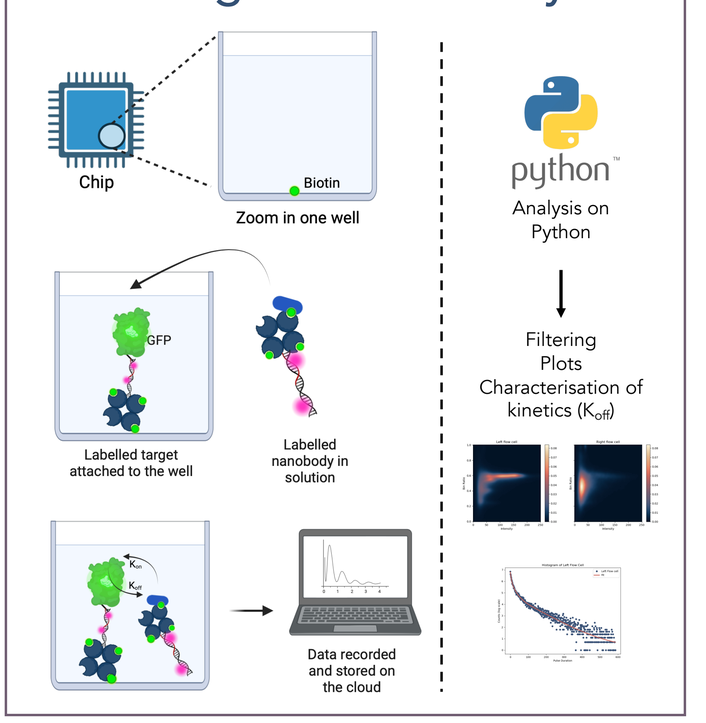
Microbiology workflow



Nanobody and target labelling

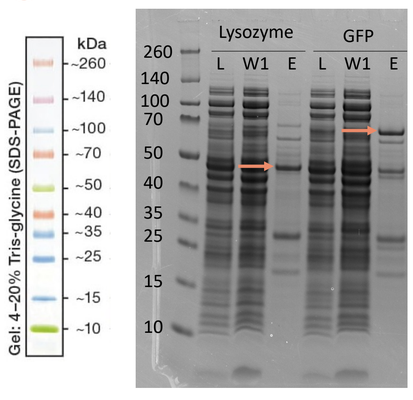


Loading and data analysis



PRELIMINARY RESULTS

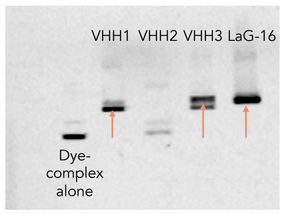
1 Target preparation :



Protein SDS-Page gel 4-12% Bis-Tris of 2 targets after HisTag purification :
Lysozyme : 50 kDa
GFP : 62,46 kDa

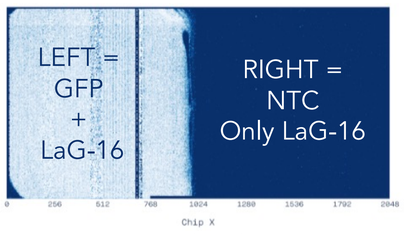
L: lysis
W1: 1st wash
E : elution

2 Labelling of the nanobody :



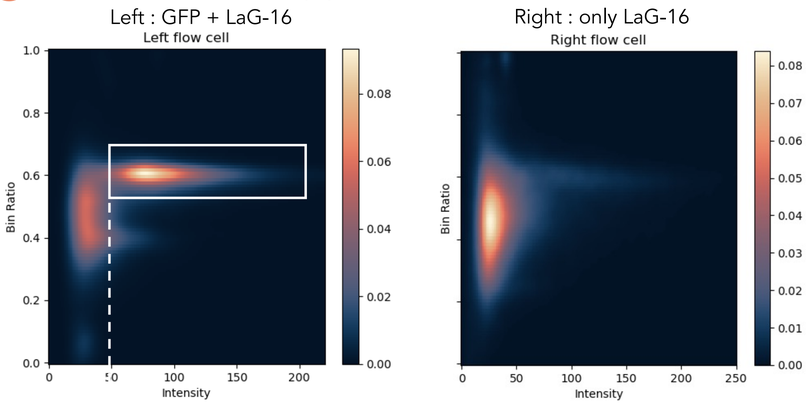
TBE gel with fluorescent imaging (no ladder visible):
A shift in size from the dye-complex alone to the VHH associated with the dye complex allows for the validation of the correct labelling of the VHHs.
Here VHH2 is not labelled while the others are.

3 Loading of the chip :

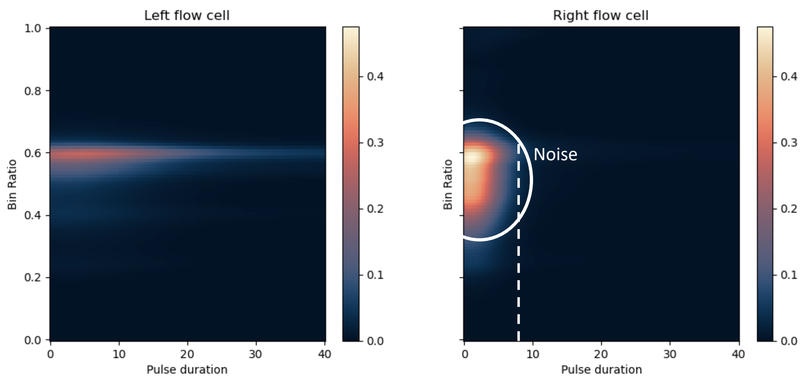


Loading of the flow cells
Nothing loaded on the right one.

4 Data analysis : Heatmap plotting and filtering

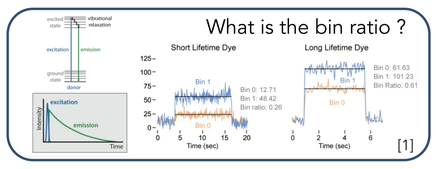


Heatmap 1: Intensity vs. Bin ratio

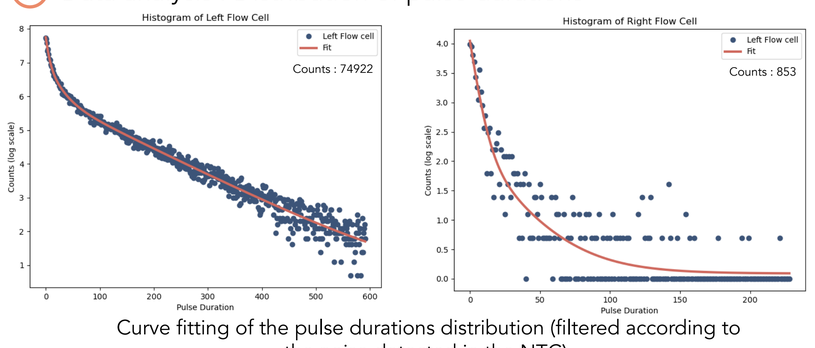


Heatmap 2: Pulse duration vs. Bin ratio

Filtering on:
- the intensity > 43
- the pulse duration > 8s
- the bin ratio between 0,51 and 0,66.

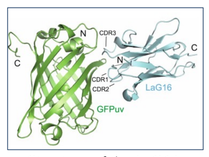


5 Data analysis : Distribution of pulse durations



Curve fitting of the pulse durations distribution (filtered according to the noise detected in the NTC).

6 Measurement of the K_{off}



Structure of the LaG16-GFP complex[2]

Experiments	K_{off} 1	K_{off} 2	K_{off} 3
NTC	0,17	0,034	x
#1	0,17	0,044	0,0079
#2	0,20	0,047	0,0086
#3	0,49	0,063	0,0080

The distributions of the pulse durations for the experiments with LaG-16 in solution interacting with GFP on the chip can be fitted with 3 exponentials functions, showing the presence of 3 distinct biological events.
The kinetics of GFP/LaG-16 are linked to the third constant because the NTC experiment only contains 2-exponential fits, which are also observable in the GFP/LaG-16 experiments.

From the literature: K_{off} for the GFP/LaG-16 is $1{,}1.10^{-3}\ s^{-1}$ [3].

Conclusions and perspectives

Thanks to this proof-of-concept, we measured the kinetics of the interaction between the GFP and the nanobody LaG-16 with reproductible results. The K_{off} has a 8-fold difference with the literature, it can be linked to the presence of the dye-complex (streptavidin) on the LaG-16. We made the same experiment with another target and will now try with full-chain antibodies (IgG) to increase the range of application of this technique.

[1] Brian D. Reed et *al.* Science, 2022, 378 (6166) 186–192.
[2] Ziyue Zhang et *al.* Sci Rep, 2020; 10: 6239.
[3] Peter C Fridy et *al.* Nat Methods 11, 1253-11260 (2014).

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