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Abstract: 102O

Single-molecule genotyping of thousands of variants

Stefan H. Mueller[1,2], Lisanne M. Spenkelink[1,2], David M. Grimson[1,2], Gillian Lee[3], Aidan C. Fitch[1,2], Sebastian Hutchinson[3], Katie Croce[4], Evan McCormack[4], Omer Ad[4], Marco Ribezzi-Crivellari[3], Andrew D. Griffiths[3], Antoine M. van Oijen[1,2]

[1]Molecular Horizons and School of Chemistry and Molecular Bioscience, University of Wollongong, Wollongong, NSW 2522, Australia.
[2]Illawarra Health & Medical Research Institute, Wollongong, NSW 2522, Australia
[3]Laboratoire de Biochimie, Institute of Chemistry, Biology and Innovation (CBI), UMR8231, ESPCI Paris, PSL Research University, 75231 Paris Cedex 05, France.
[4]Quantum-Si, Guilford, Connecticut, United States.

High-throughput screening allows rapid testing of thousands to millions of samples for biological activity. Current screening methods are based on ensemble readouts such as binding affinity purification and fluorescence sorting. These readouts are not well suited for the characterisation of complex, multi-parametric molecular phenotypes. Moreover, these screening methods use measurements based on the average activity of large numbers of molecules. This averaging makes it impossible to resolve the underlying 'microscopic' phenotypes such as heterogeneity in binding kinetics, or fluctuations in the rate of catalytic activity.

Single-molecule microscopy methods are ideal to characterise complex phenotypes and to measure heterogeneity. However, to date there are no single-molecule genotyping methods that allow for the simultaneous determination of the genotype of thousands of variants.

We have developed a novel sequencing-by-hybridisation approach that allows single-molecule genotyping of 10^3–10^4 variants (Figure 1). Our method uses DNA-based barcodes consisting of multiple single-stranded DNA indices. Sets of fluorescent hybridisation probes, complementary to the different barcode indices can be used to read out these barcodes. The hybridisation kinetics of the probes depend strongly on the index length. Repeated measurement of probe binding will result in accurate characterisation of hybridisation kinetics, thereby allowing the reliable distinction between each index length. Total internal reflection fluorescence microscopy (TIRF) allows simultaneous single-molecule genotyping of thousands of molecules per field of view.

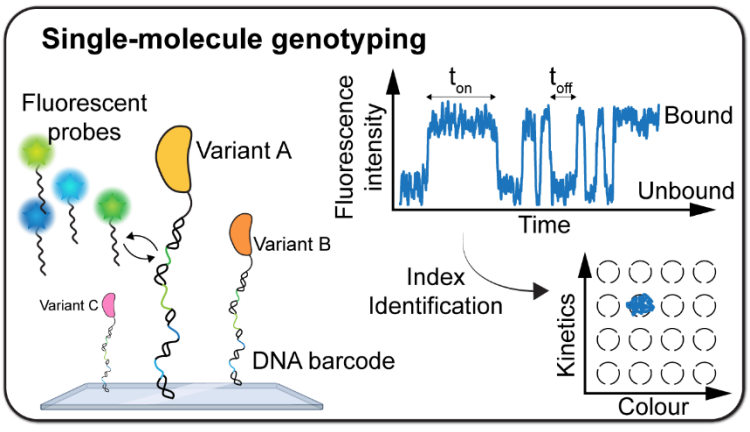

Figure 1: Schematic representation of single-molecule sequencing by hybridisation approach

The DNA-based barcodes can be uniquely attached to variants within the screen. As a proof of concept, we use SNAP-display to attach barcodes to a small library of antibodies. We characterise both genotype and phenotype of these antibodies in the same experiment.