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Abstract: 187P

Single-molecule genotyping of thousands of variants

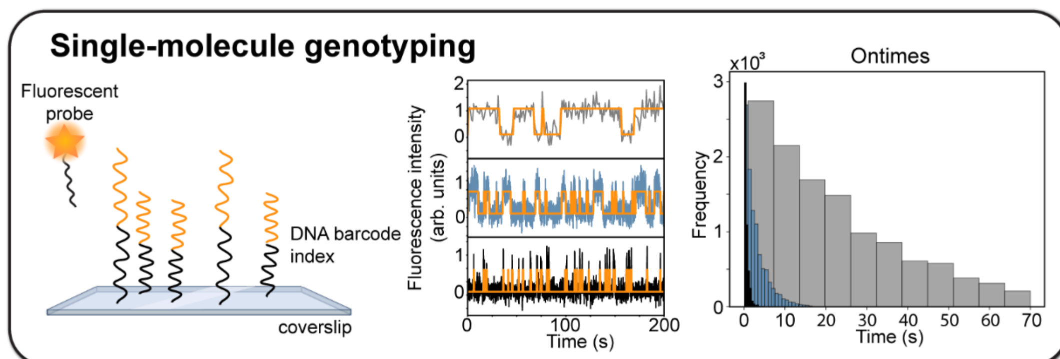
Stefan H. Mueller[1,2], Lisanne M. Spenkelink[1,2], David M. Grimson[1,2], Gillian Lee[3], Aidan C. Fitch[1,2], Sebastian Hutchinson[3], Marco Ribezzi-Crivellari[3], Andrew D. Griffiths[3], Antoine M. van Oijen[1,2]

[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.

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. Our method uses DNA-based barcodes consisting of multiple single-stranded DNA indices. The hybridisation kinetics are strongly dependent on the oligo length. We use single-molecule total internal reflection fluorescence microscopy (smTIRF) characterise the "kinetic fingerprint" of thousands of molecules simultaneously using Hidden-Markov modelling (see figure). Combining this with multi-colour TIRF this allows identification of up to 10000 individual targets.



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.