

SIMULTANEOUS LABEL-FREE ANALYSIS OF 1485 ANTIBODY-ANTIGEN INTERACTIONS

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INTRODUCTION

Protein-protein interactions (PPIs) are crucial for most cellular and biochemical processes and represent emerging drug targets. The demand for high throughput screenings is rising. Here, we characterise the binding of an anti-FLAG antibody to 1485 spots of FLAG epitope variants by using the label-free single colour reflectometry (SCORE). This technology enables a precise, fast, and reliable characterisation of PPIs representing a unique combination of high density peptide microarrays with label-free read-out.

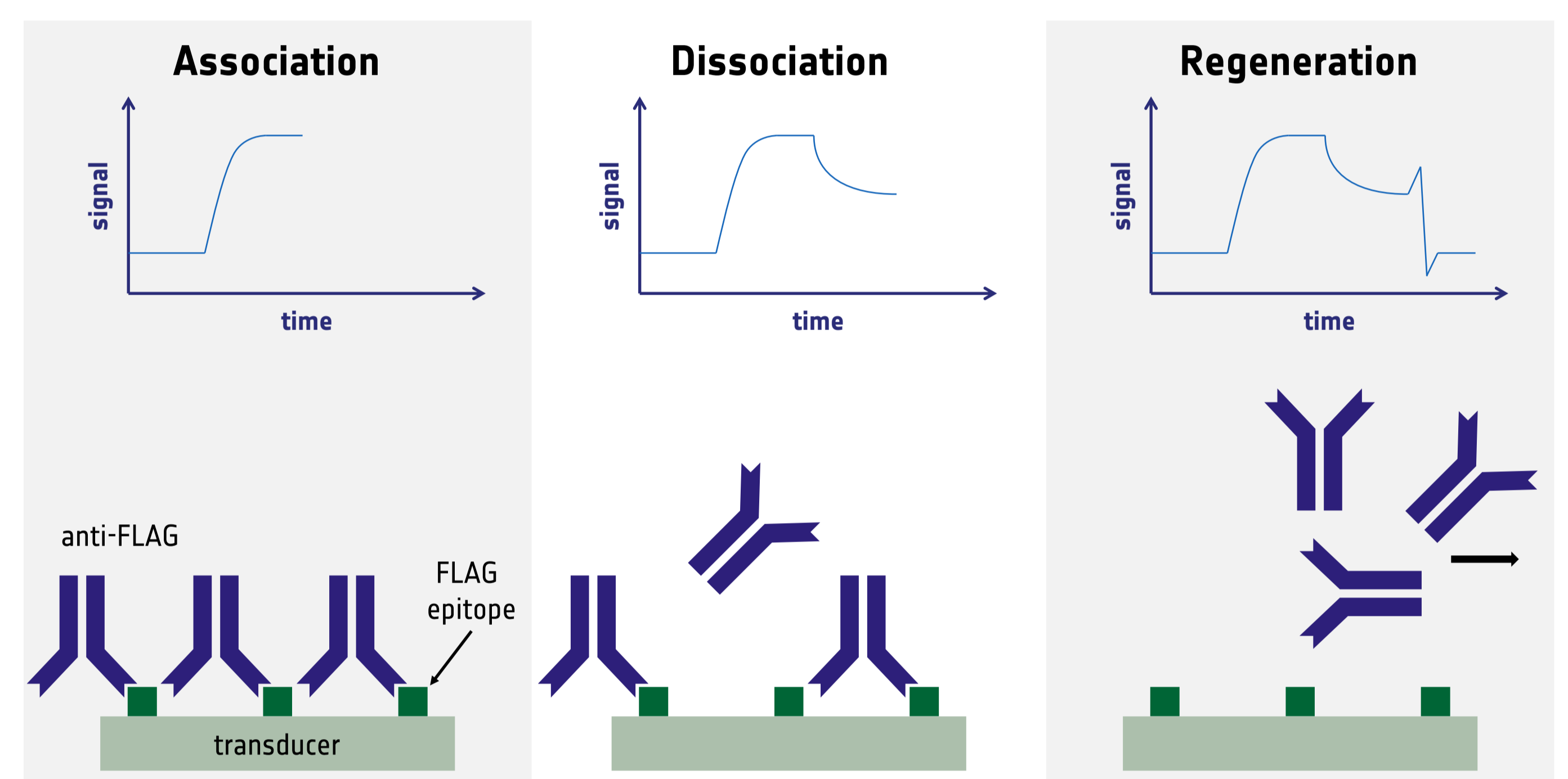
TECHNOLOGY

Biametrics' proprietary SCORE technology featured in the b-screen LB 991 system (Berthold Technologies, Bad Wildbad, Germany) implements the interference of monochromatic light (no fluorescence labels needed) in order to detect biomolecular interactions. Ligands can easily be immobilised onto a biopolymer-coated glass-type transducer. Specific binding of analyte molecules changes the reflection coefficient of the bilayer and is directly monitored in real-time by SCORE. High throughput measurements with up to 22,500 interactions are feasible in one single run.

SUMMARY

- High density microarrays combined with label-free read-out
- Simultaneous detection of 1485 interactions in one run
- Fast, reliable, and exact determination of kinetic parameters

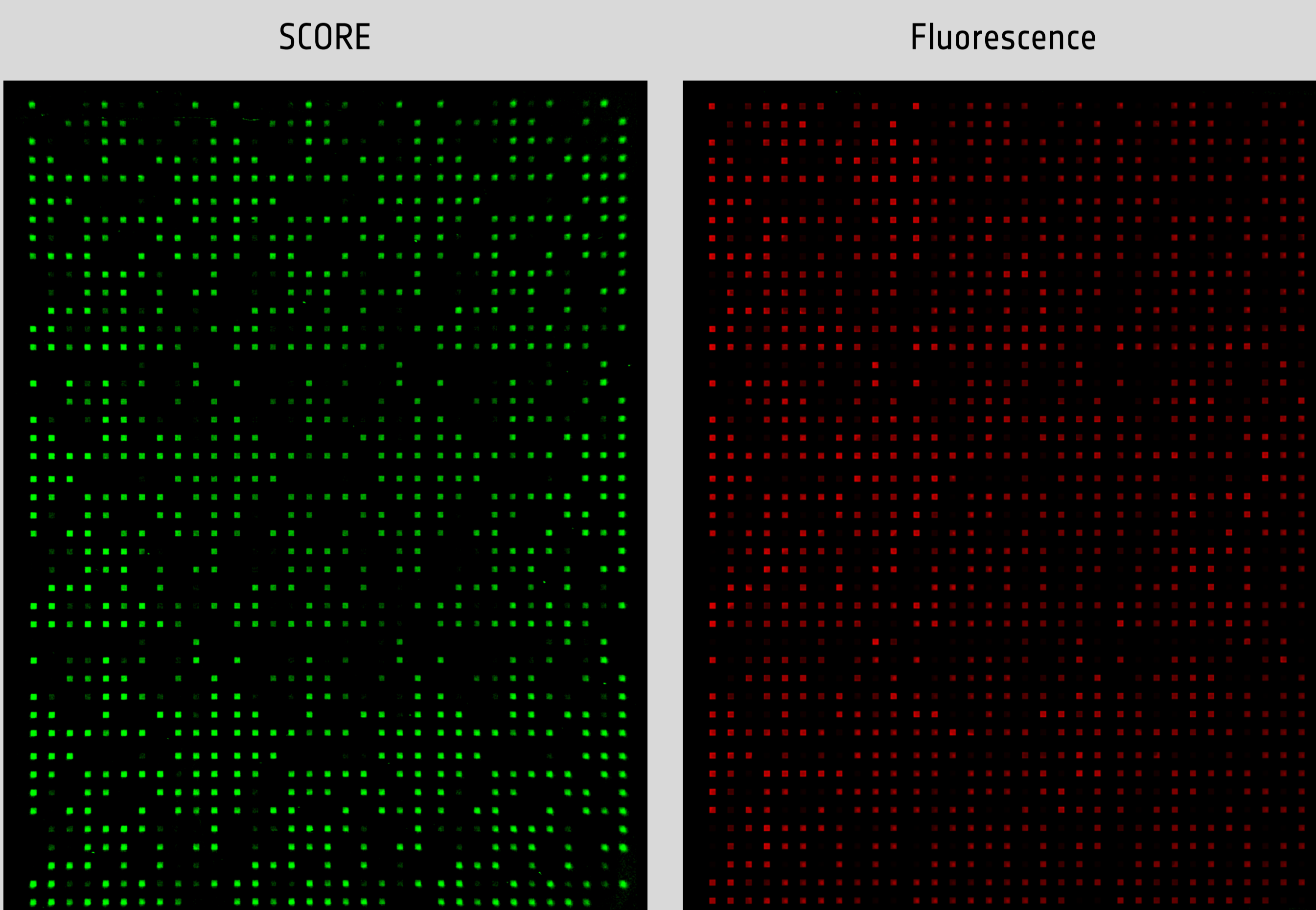
ASSAY FORMAT



- High density peptide array (1485 rectangular spots) of FLAG epitope variants
- Anti-FLAG antibody binds to each individual spot
- Regeneration procedure makes one transducer reusable multiple times

RESULTS

Comparable data for SCORE and fluorescence analysis



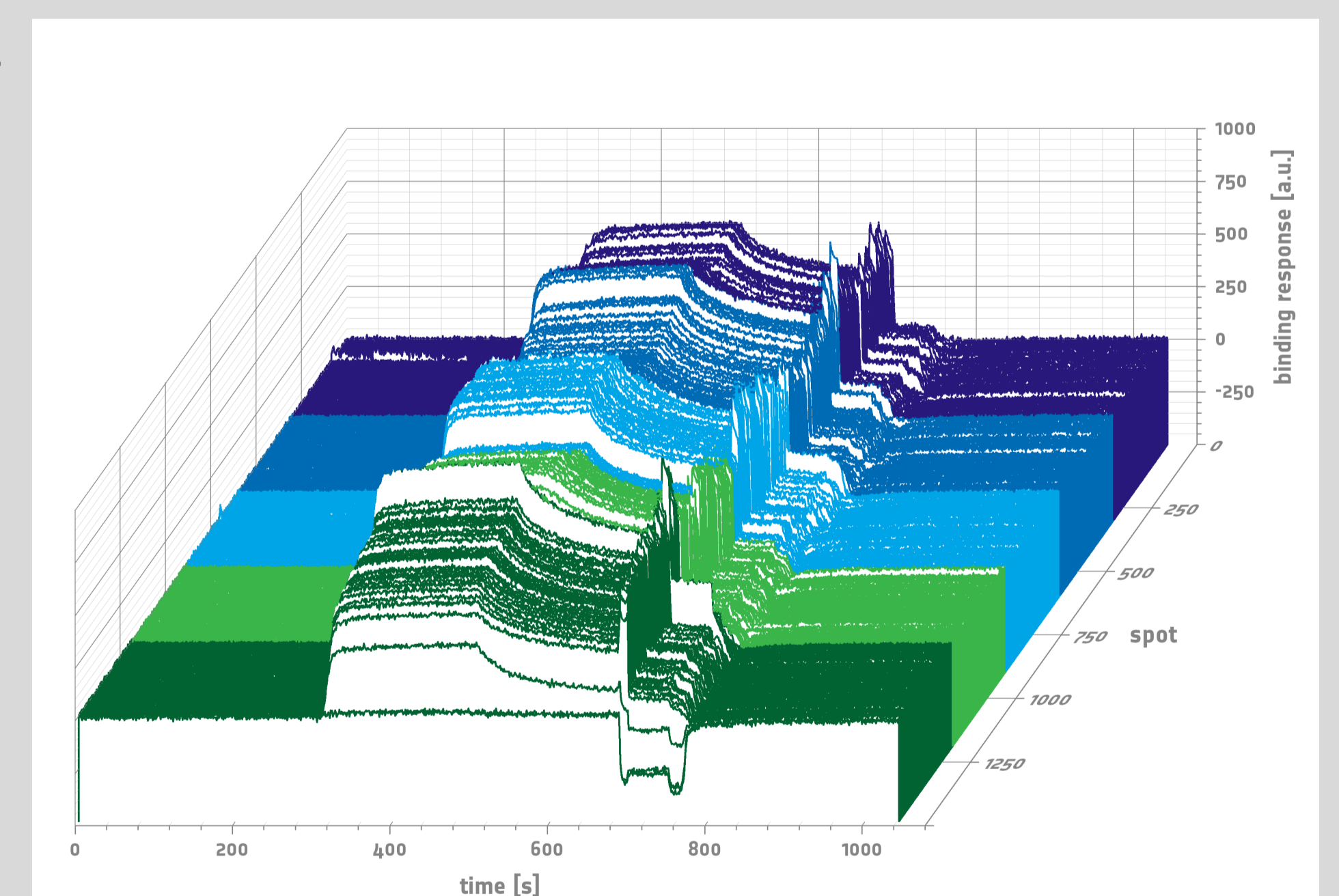
Label-free detection of anti-FLAG antibody binding using the SCORE technology (left) yields similar results as the fluorescent detection using the anti-FLAG-Cy3 antibody (right). A high density peptide microarray of FLAG epitope variants was generated on SCORE glass-type transducer through photolithographic immobilisation.

Watch me!

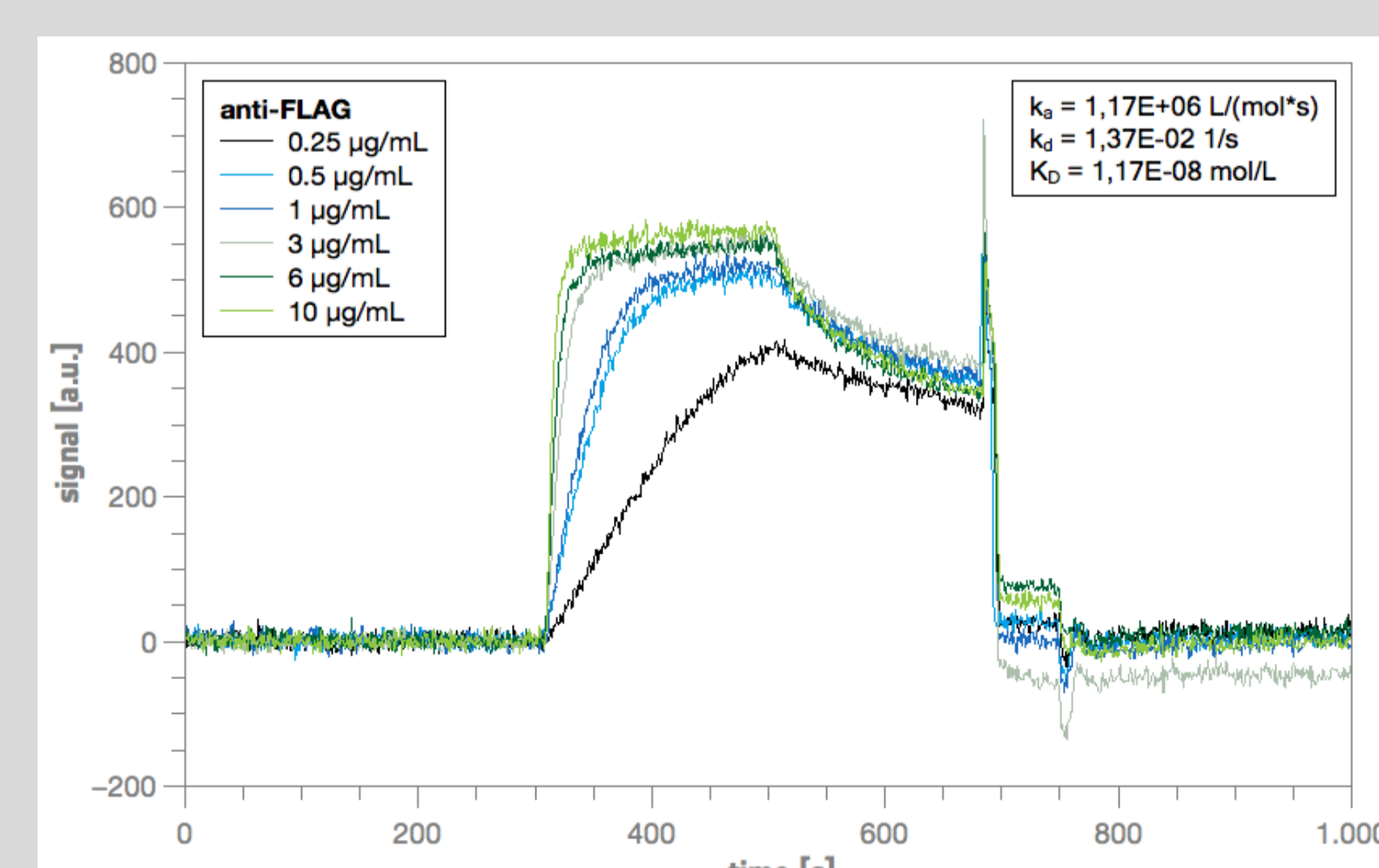


Real-time binding curves for each spot

Simultaneous detection of 1485 protein interactions. Binding of anti-FLAG to FLAG epitope variants was monitored by SCORE. Each individual spot from the high density microarray (see image left) results in a binding curve.



Full kinetic evaluation for each binding event



Binding curves of one representative FLAG epitope are shown for different concentrations of anti-FLAG. Full kinetic information was extracted within minutes for all 1485 spots with the integrated b-nd evaluation software.