Application of FCCS in the Development of Bispecific Antibodies

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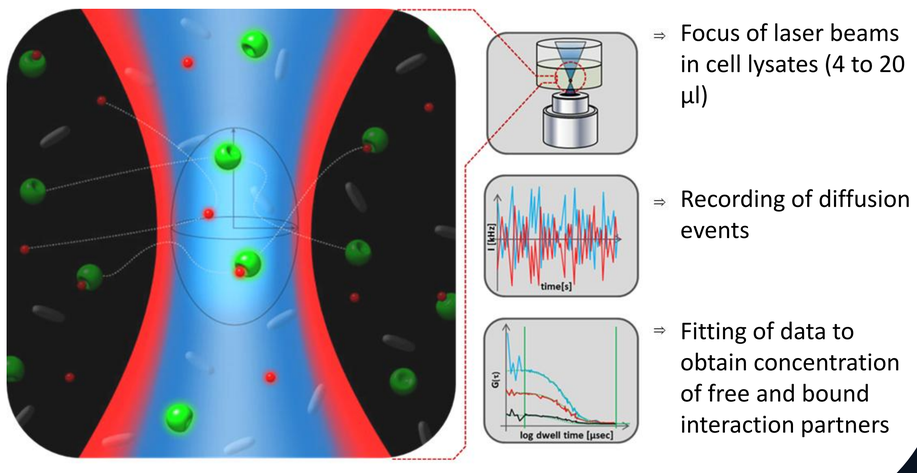
INTRODUCTION

Fluorescence Cross-Correlation Spectroscopy (FCCS) is a versatile platform technology for investigating molecular interactions under physiological conditions. The method has been successfully applied to small-molecule drugs, peptides, antibodies, membrane proteins, and nucleic acids to determine equilibrium binding constants and kinetic parameters.

FCCS assays can be performed in complex biological environments, including crude cell lysates, body fluids, living cells, blood plasma, and tissue homogenates. The technology provides a highly precise and information-rich readout, enabling the determination of molecular concentrations, molecular size, binding states, and complex half-life.

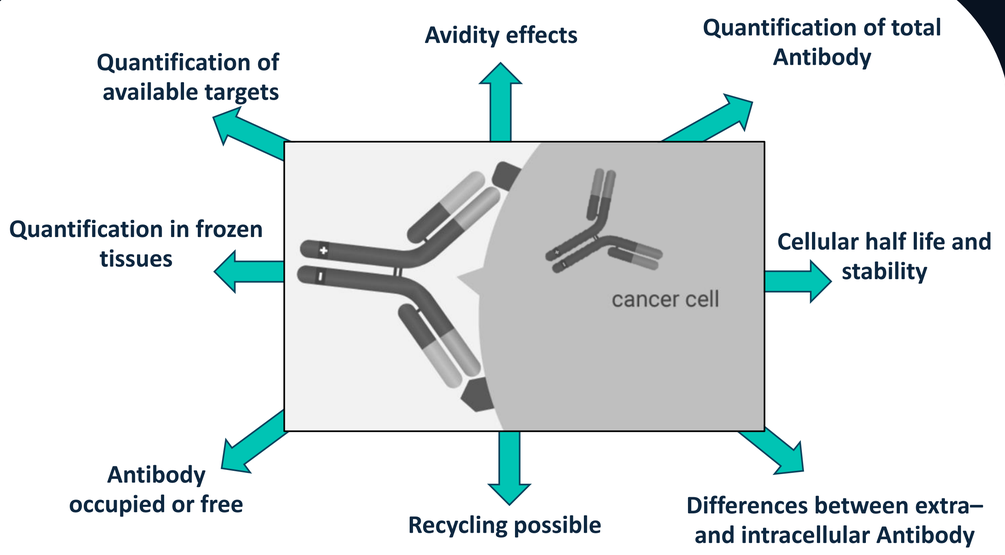


⇒ Focus of laser beams in cell lysates (4 to 20 µl)

⇒ Recording of diffusion events

⇒ Fitting of data to obtain concentration of free and bound interaction partners

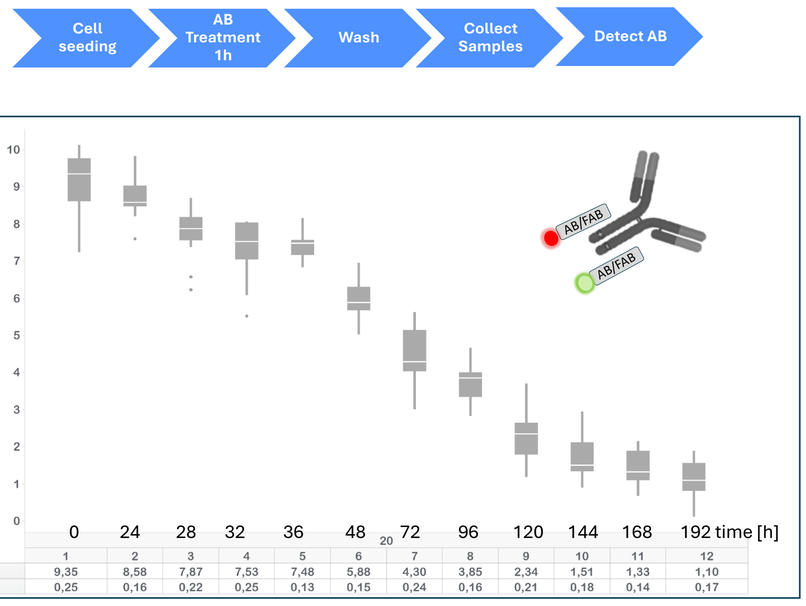
PROJECT

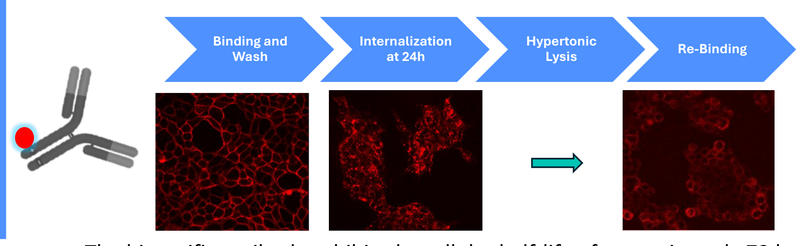


In this work, we focused on the characterization of a bispecific antibody by evaluating target occupancy in cells and tumor biopsy samples. The primary objective was to establish an assay platform capable of quantifying both the available concentration of each target and the fraction of target molecules occupied by the antibody in patient-derived needle biopsies. In addition, the platform was used to investigate the intracellular stability, trafficking, and fate of the antibody following cellular uptake.

Fluorescence Cross-Correlation Spectroscopy (FCCS) enables the quantitative assessment of target engagement and molecular interactions directly in biologically relevant systems. By providing precise measurements of target occupancy, binding dynamics, and intracellular antibody behavior, this approach generates valuable translational insights and supports data-driven decision-making throughout preclinical and clinical drug development.

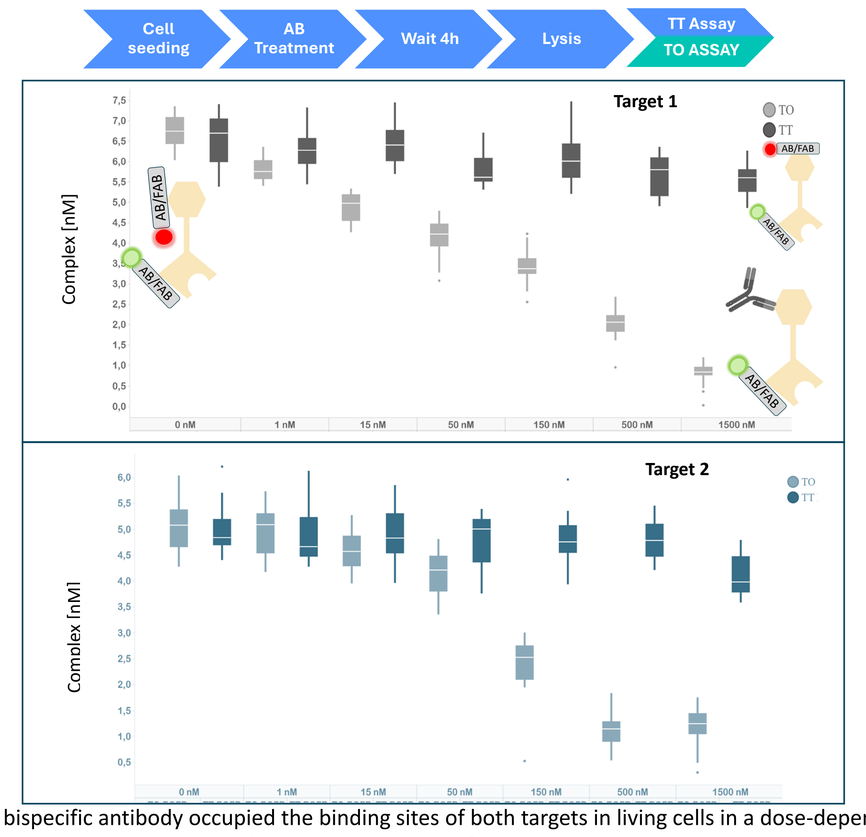
CELL HALF LIFE





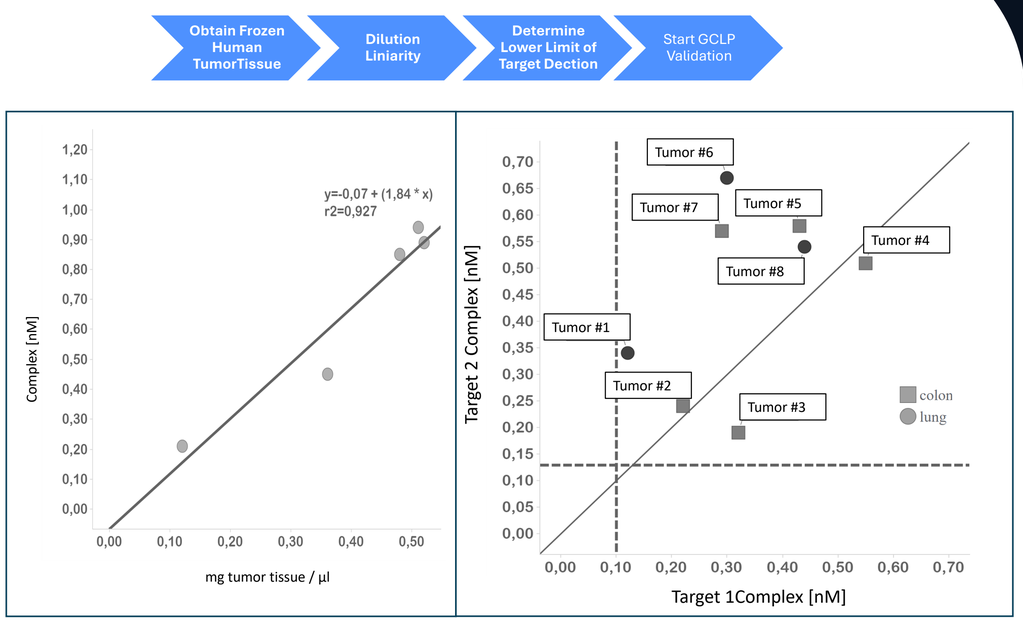
The bispecific antibody exhibited a cellular half-life of approximately 72 hours and retained its ability to bind both targets following internalization, demonstrating sustained intracellular stability and functionality.

TARGET OCCUPANCY



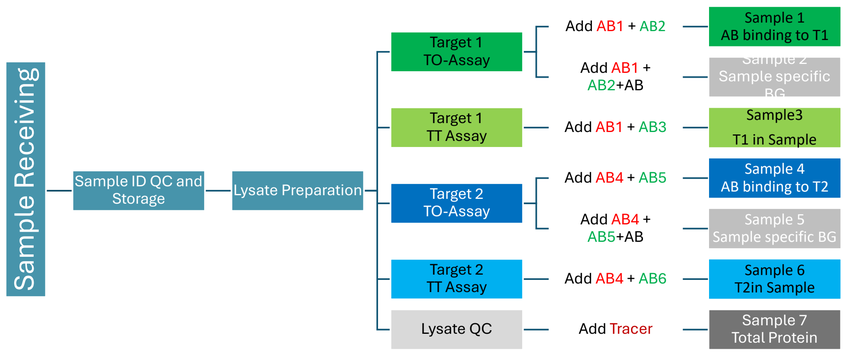
The bispecific antibody occupied the binding sites of both targets in living cells in a dose-dependent manner, demonstrating specific target engagement. A complementary assay enabled quantification of the absolute amount of available target independently of antibody binding. Theseassays provided a robust platform for assessing target occupancy and target availability in cells.

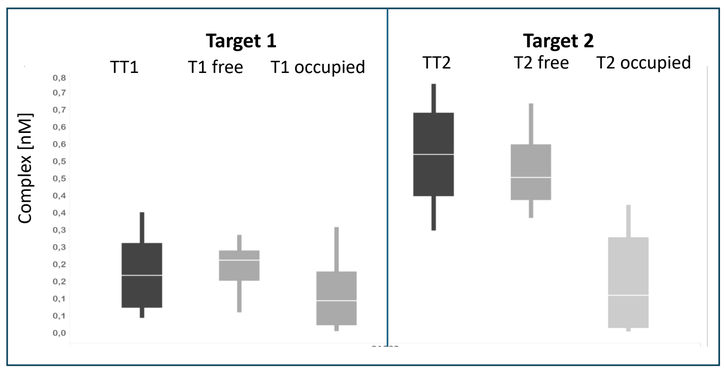
TUMOR DATA



Both targets could be detected in lysates from snap-frozen human tumor biopsies. Target expression levels varied between tumor types and individual patients. Importantly, as little as 1 mg of biopsy material was sufficient to perform both total target quantification and target occupancy measurements for both targets.

PATIENT WORKFLOW





A workflow was established to quantify both the absolute target concentration and target occupancy in patient-derived tumor biopsies with high statistical confidence. FCCS provides robust and quantitative data for monitoring in vivo target engagement of therapeutic agents, demonstrated here for a bispecific antibody.

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