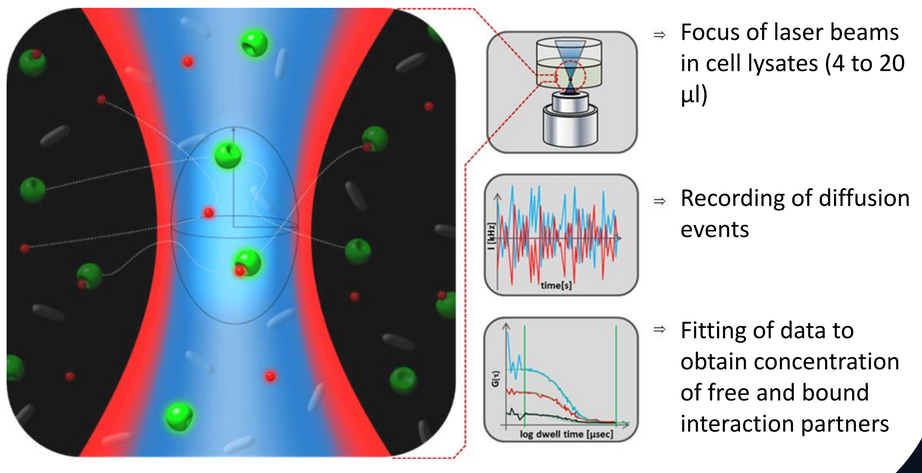
Application of FCCS in the Development for Small Molecules

Prof. Dr. Uwe Jacob; Dr. Stefan Hannus,
Dr. Frank Becker
Intana Bioscience GmbH, Planegg, Germany

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.

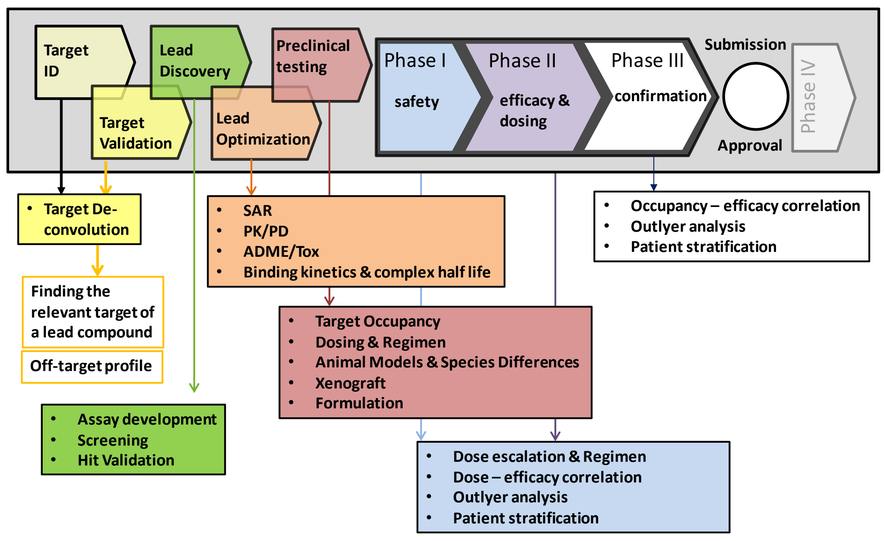


⇒ Focus of laser beams in cell lysates (4 to 20 µl)

⇒ Recording of diffusion events

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

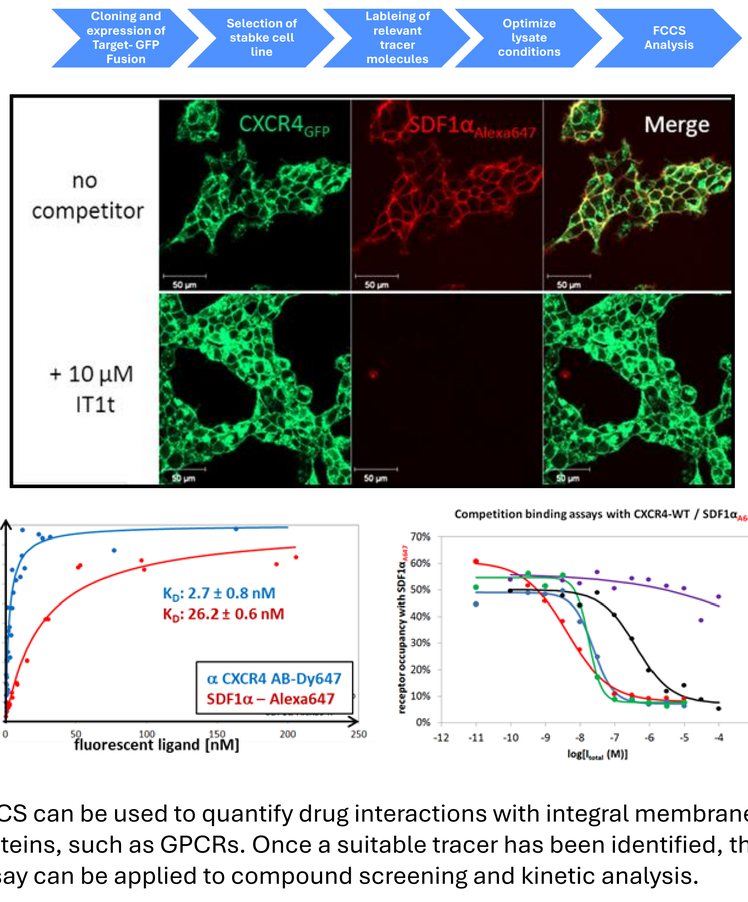
PROJECTS



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.

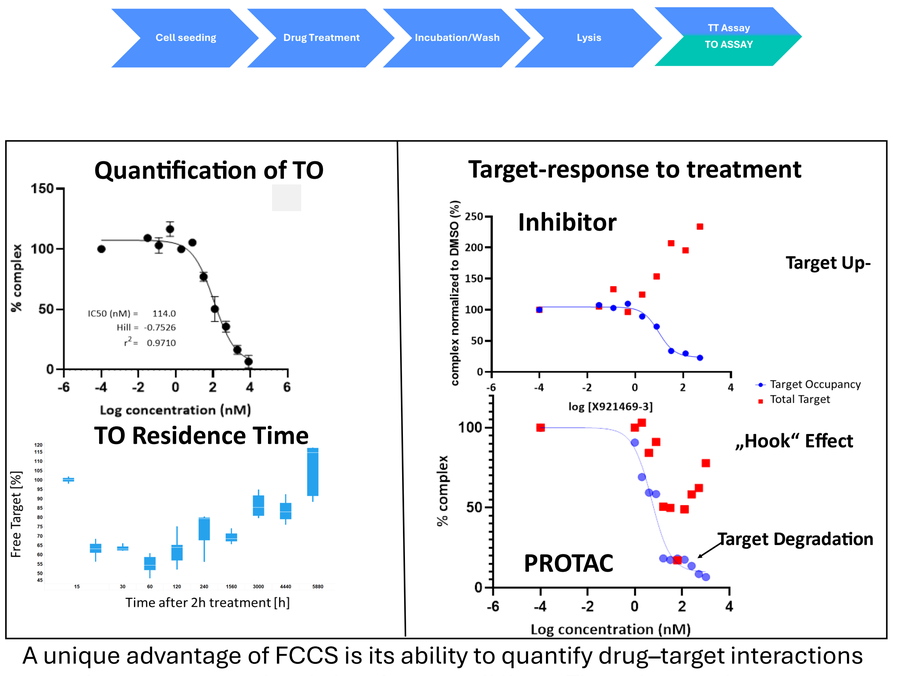
In this work, we present examples of projects in which FCCS experiments were used to quantify drug–target complexes in various sample types and address critical questions throughout the drug discovery value chain.

GPCR



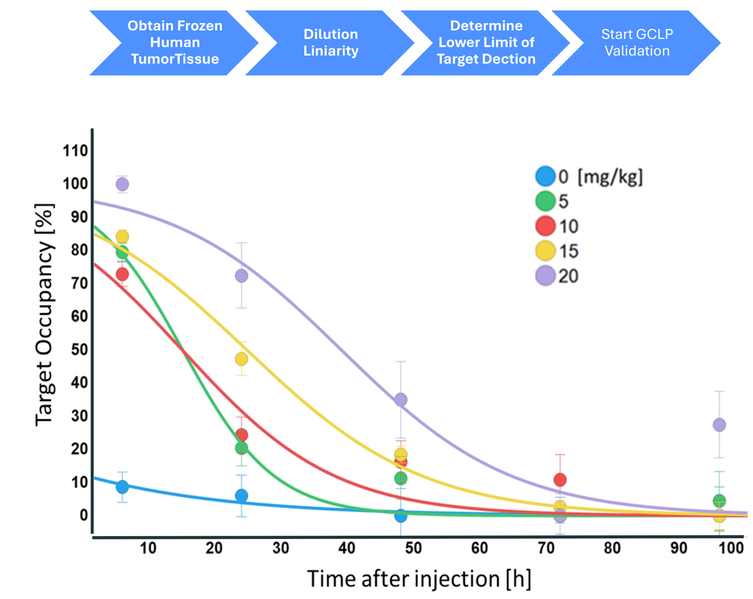
FCCS can be used to quantify drug interactions with integral membrane proteins, such as GPCRs. Once a suitable tracer has been identified, the assay can be applied to compound screening and kinetic analysis.

TARGET OCCUPANCY



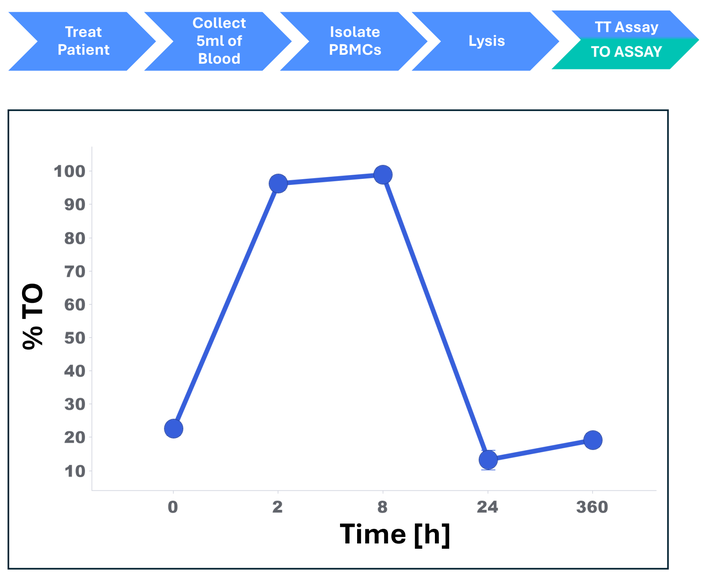
A unique advantage of FCCS is its ability to quantify drug–target interactions at endogenous target levels in relevant cell lines. These interactions are measured using a fluorescently labeled, target-specific antibody together with a tracer that binds to the drug-binding pocket. This approach enables quantification of the fraction of occupied target, or target occupancy (TO), at a defined time point. When combined with an additional total-target assay (TT assay), which uses two antibodies recognizing distinct epitopes on the target, FCCS provides a powerful platform for monitoring cellular responses to drug treatment.

ANIMAL DATA



FCCS can be used to characterize the pharmacodynamic effects of a drug in vivo. In this case, tumor samples were collected from animals following a single administration of the drug at defined doses. Samples obtained at different time points were analyzed by FCCS, providing a detailed profile of the dose-dependent duration and half-life of target engagement in the organism. These data can support rational study design and enable correlations between antitumor efficacy and target-occupancy levels.

PATIENT WORKFLOW



Personalized medicine strategies in drug discovery and development will benefit from assay platforms that enable detailed quantification of drug–target complexes in patient samples. FCCS has the potential to support clinical teams in monitoring target engagement and its duration in patients using liquid biopsies.
By enabling specific and precise measurement of drug–target interactions in clinical samples, FCCS may help demonstrate pharmacodynamic activity, support patient stratification, and relate therapeutic benefit to the biophysical interaction of a drug with its intended target.

Contact: frank.becker@intana.de; +49-151 22340536; www.intana.de