

LigandTracer®

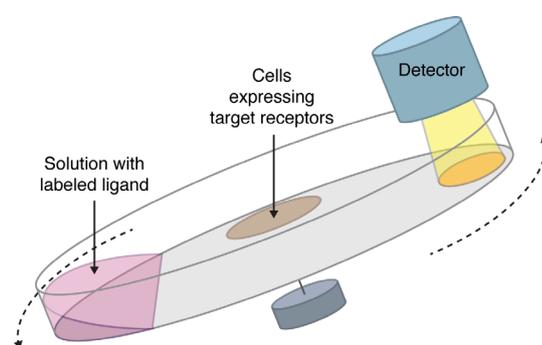
Understanding how your ligand interacts with living cells

LigandTracer® monitors molecular interactions on living cells in real-time. The instrument is particularly suited to follow protein binding to cell-surface receptors, and allows to measure on- and off-rates as well as affinities.

LigandTracer Technology

Target cells are seeded in a local part of a circular cell dish and are allowed to attach firmly to the dish surface. This is typically obtained by seeding cells on a circular spot or in a tilted dish and placing the dish in the incubator for a few hours. After attachment, the dish can be left overnight in the incubator with additional medium in a horizontal position to let cells grow towards a confluent layer.

To start a LigandTracer experiment, the dish is placed on an inclined rotating support with fresh medium. After establishing a baseline signal, liquid containing a labeled (fluorescent or radioactive) ligand (e.g. a protein or a small synthetic molecule) is added. A detector, mounted over the elevated part of the dish, measures the signal intensity from both the cell area and a reference area (cell free or another cell line), which enables a continuous subtraction of the background signal from the cell line of interest every revolution. As the ligand interacts with the cells over time, an increasing or declining response is generated, representing the association and dissociation rates of the interaction.



The curve can be fitted to interaction models, which provide information on kinetic properties and the affinity of the interaction. Deriving interaction properties from the measured interaction curves does not rely on the absolute magnitude of the response but on the curve shape in relation to the ligand concentration in the dish, which makes the method independent of receptor or label quantities. For accurate analysis of interaction dynamics and affinity, a typical experiment is performed with two or three consecutive steps with increasing ligand concentrations. This results in unique, concentration dependent curvatures during the binding phase.

As experiments are performed in disposable cell dishes and cells or liquid do not come in contact with any part of the instrument the instrument is free of maintenance.



Available instrument models

The different LigandTracer models are separated by the type of label they can detect. LigandTracer Green monitors fluorescence with detectors available for different excitation and emission wavelengths covering the most commonly used fluorophores. There are also three instrument models available for radiolabeled ligands, measuring low and high energy gamma radiation (LigandTracer Grey and LigandTracer Yellow, respectively) as well as beta radiation (LigandTracer White).

Application Example

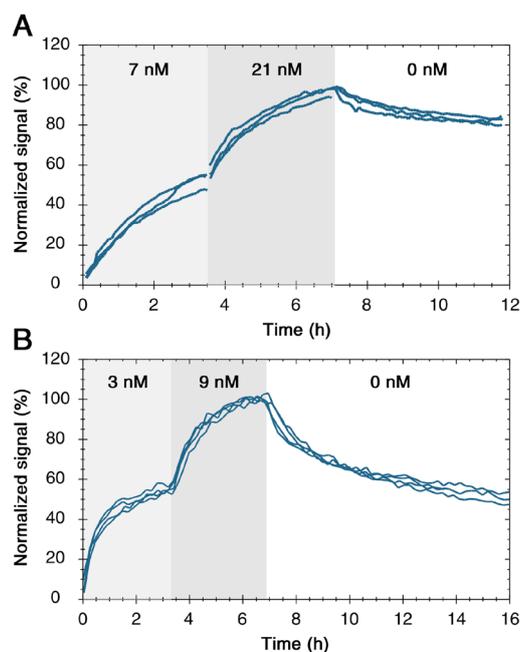
Materials and methods

SKOV3 cells expressing the HER2 receptor and A431 cells expressing the epidermal growth factor receptor (EGFR) were seeded in local areas of cell culture dishes at least one day prior to measurement. Following a short baseline measurement with 3 ml of fresh cell culture medium, FIBA*-bound trastuzumab and Iodine-125 labeled EGF were added to SKOV3 and A431 cells and their binding were monitored for 3.5 h for each concentration in LigandTracer Green (Fig. A) and LigandTracer Grey (Fig. B), respectively. The solutions were then replaced with fresh cell culture medium to measure retention.

*FIBA = Fluorescent monovalent anti-IgG binding antibody

Results

Recorded association and dissociation traces: replicates of the FITC-trastuzumab – HER2 interaction on SKOV3 cells (Fig. A, n = 3) and of the ¹²⁵I-EGF – EGFR interaction on A431 cells (Fig. B, n = 4) are very similar, confirming the robustness of the assay.



Conclusions

LigandTracer offers a straightforward and reproducible method for detecting protein interactions on living cells over minutes, hours, or even days. The use of living cells takes you one step closer to *in vivo* situations and is helpful for understanding biological processes, such as the uptake of drugs and the binding of imaging agents, as well as how cells integrate environmental clues into their signaling.

Reference and protocols

1. Björke H et. al. *Automated, high-resolution cellular retention and uptake studies in vitro*. Appl Radiat Isot. 2006. 64(8):901-905.
2. Björkelund H et. al. *Comparing the epidermal growth factor interaction with four different cell lines: intriguing effects imply strong dependency of cellular context*. PLoS ONE. 2011. 6(1): e16536.
3. Bondza S et. al. *Conjugation effects on antibody-drug conjugates: Evaluation of interaction kinetics in real time on living cells*. Mol Pharm. 2014. 11(11):4154-4163.