



# Bio**guide**

innovative technologies



# Protein characterization and kinetics instruments



**Flow-Induced Dispersion Analysis (FIDA)** is an innovative technique used to characterize biomolecular interactions, with applications in drug discovery, biopharmaceutical development, and diagnostics. By analysing the dispersion of molecules in a flow field, FIDA enables precise measurements of binding affinities and kinetic profiles without requiring extensive sample preparation or large quantities of reagents. This method is highly sensitive, capable of detecting subtle interactions in complex biological samples, and is versatile enough to study a range of analytes, from small molecules to large proteins and nucleic acids.



**Nanophotonic Evanesence Field Sensing** advanced optical sensing technology designed to detect biomolecular interactions with high sensitivity and specificity. Utilizing nanophotonic waveguides, this technique harnesses the evanescent field to monitor molecular binding events at the sensor surface in real time. Ideal for drug development, diagnostics, and molecular research, it provides quantitative data on binding affinities and kinetics with minimal sample requirements. This innovative approach is capable of detecting low-abundance biomolecules and offers a streamlined, label-free alternative to traditional assays, significantly enhancing the accuracy and efficiency of bioanalytical measurements.



**Biosensing Instrument Inc. specializes** in the development of surface plasmon resonance (SPR) instruments for real-time biomolecular interaction analysis. Their high-performance SPR systems are widely used in drug discovery, life sciences, and material research, providing precise measurements of binding kinetics, affinity, and specificity. Known for their innovative designs and user-friendly software, Biosensing Instrument's products cater to both academic and industrial researchers, offering solutions for studying small molecules, proteins, nucleic acids, and even cells. With a focus on sensitivity and versatility, the company continues to advance SPR technology to meet the evolving needs of biosensing applications.

# Live cell kinetics instruments



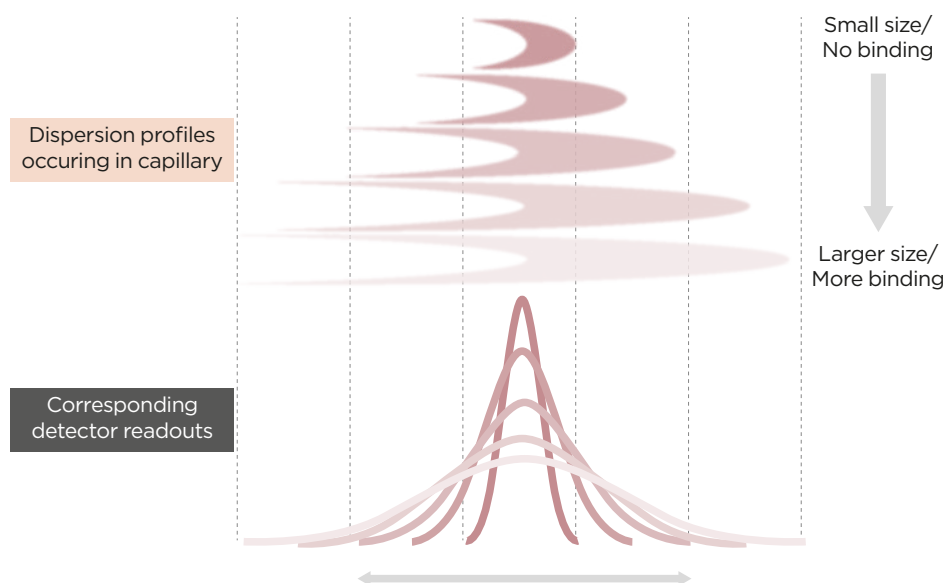
**Surface Plasmon Resonance Microscopy (SPRM)** is a cutting-edge technique that integrates surface plasmon resonance (SPR) with high-resolution optical microscopy to enable label-free, real-time monitoring of molecular interactions at the interface of biological samples or material surfaces. Unlike traditional SPR, which measures averaged binding events, SPRM offers spatially resolved imaging, providing insights into localized interactions at the single-cell level, with high sensitivity to changes in refractive index near the surface. This allows for the analysis of dynamic molecular processes such as receptor-ligand binding, protein-protein interactions, and cellular adhesion. SPRM is a powerful tool in areas like drug discovery, immunology, cell biology, and material science, enabling detailed studies of molecular kinetics and interaction mechanisms in real-time and with minimal sample preparation.



**LigandTracer** is an advanced biosensing technique that allows real-time kinetic analysis of molecular interactions directly on living cells. Unlike traditional endpoint assays, LigandTracer continuously monitors the binding and dissociation of labeled ligands to their target receptors, providing valuable insights into affinity, kinetics, and specificity. This method is particularly useful in drug development, antibody research, and cellular receptor studies, offering a non-invasive and dynamic approach to studying molecular interactions under physiological conditions. By using a rotating cell dish and a sensitive detection system, LigandTracer minimizes background noise and enables high-quality kinetic measurements, making it a powerful tool in biomedical and pharmaceutical research.

# Flow-Induced Dispersion Analysis

Protein Kinetics



# Flow-Induced Dispersion Analysis

## Specific applications:

### Membrane Proteins

- Binding based on molecular size and fluorescence
- Detect both strong and weak binding
- Study membrane protein kinetics in crude samples

### Ternary complex formation and degradation:

- Quantitative determination of ternary complex formation,
- Determination of all construct components bindings, including cooperativity
- Determination of the bound fraction of the protein of interest to the ternary complex

### Lipid and extracellular vesicles

- As an in-solution, non-immobilization technology FIDA enables characterization of interactions on surface of large particles
- Study LNPs directly in serum, thus obtain clinically relevant data
- No buffer constraints – FIDA users have wide research design flexibility

### Cryo-EM

- Key sample properties identified in just 4 minutes
- Upfront rejection of samples of suboptimal quality samples reduces costs and saves time spent on samples that are not suited for Cryo-EM or droplet formation
- Informed troubleshooting

### Engineered Antibodies

- FIDA users identify single best clone, saving time and sample material
- Characterisation directly in fermentation media results in a streamlined workflow and early-stage exclusion of non-functional clones

### CRISPR

- Detection of weak and strong cas-gRNA interactions
- Quantitative K<sub>d</sub> and R<sub>h</sub> measurement

### Amyloid Fibrils

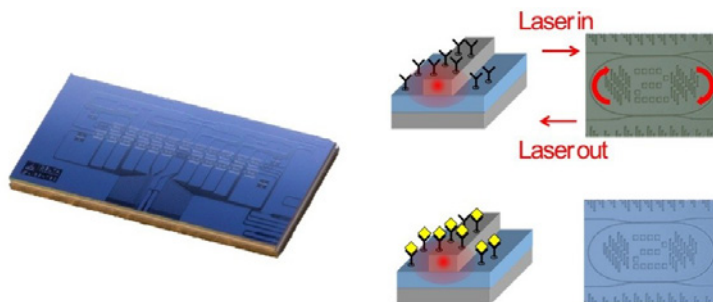
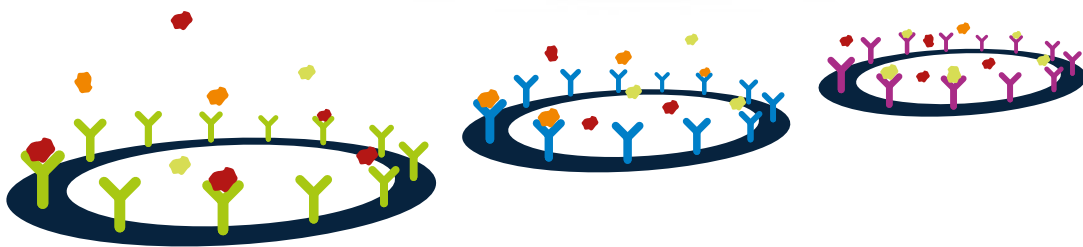
- Distinguish between binding to monomers, small oligomers and fibrils without any size bias – highly valuable for drug discovery
- Determine the size of fibrils
- Work on patient-derived fibrils (e.g. Cerebral Spinal Fluid)
- Nanoliter to microliter sample sizes

### Biomolecular condensates - LLPS

- FIDA users don't receive yes or no responses. Instead, they receive droplet count and relative size measures, which allows them to fast-track their research through quantitative data
- Measure light-phase concentration at the various conditions of droplet formation

# Nanophotonic Evanescent Field Sensing

Protein Kinetics



Photonic Chip

Difference in binding – Difference in refractive index

# Nanophotonic Evanescence Field Sensing

## How much?

- Concentration measurements
- Biological active concentration

## How specific?

- Yes/No data
- Epitope mapping
- Design of affinity pairs
- Isotyping
- Ligand fishing
- Buffer formulation
- Multi-site binding analysis

## How strong?

- Equilibrium analysis
- Equilibrium dissociation constant ( $K_D$ )
- Relative ranking of analytes
- Affinity plot
- Weak and strong interactions

## How fast?

- Kinetic rate analysis
- Association and dissociation
- $k_d / k_a = K_D$

## Specific applications:

### Membrane Proteins

- Biosensing & Diagnostics
- Disease detection
- Biomarker identification

### Environmental Sensing

- Chemical contamination
- Gas detection

### Nanomaterial Characterization

- Surface analysis
- Nanomaterial properties

### Molecular & Cellular Sensing

- Single-cell analysis
- Drug development

### Immunosensing & Vaccine Development

- Antigen-antibody binding
- Immune response)

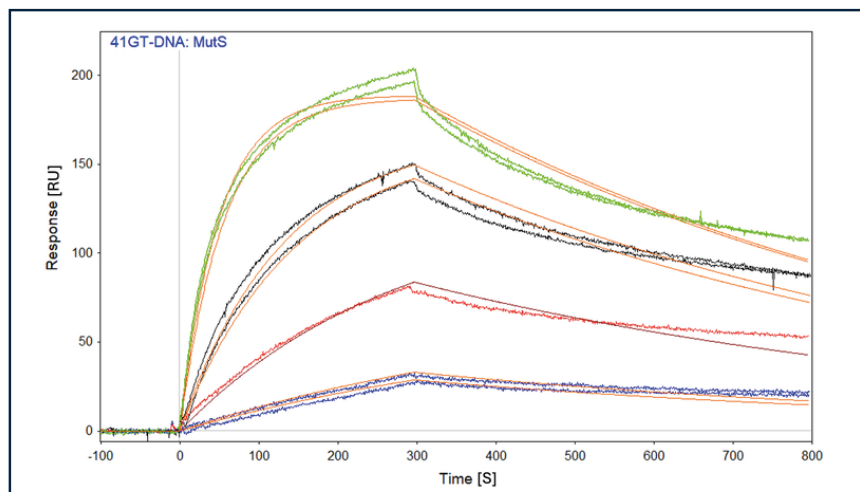


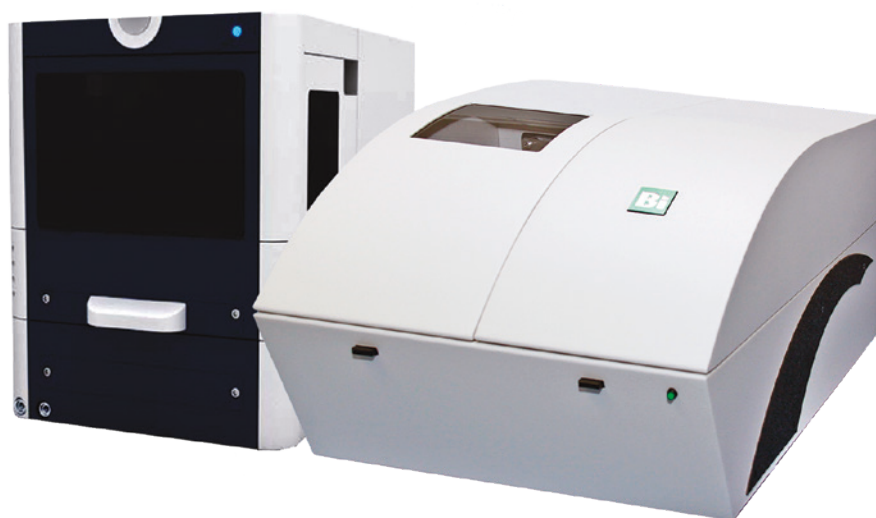
Figure 3: Multi-cycle kinetic experiment of MutS binding (From bottom to top 25, 50, 100, 200 nM) to the 41GT oligonucleotide. The smooth straight lines are the fitting over the measured curves.

	$k_a$	$k_d$	$K_D$	Rmax	Chi2
	( $\times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ )	( $\times 10^{-3} \text{ s}^{-1}$ )	( $\times 10^{-9} \text{ M}$ )	(RU)	
1:1 model	5.65	1.35	23.9	202	5.1

Table 1: Results of the fitting for the protein binding to the 41GT oligonucleotide.

# Surface Plasmon Resonance

Protein Kinetics



Instrument	BI-2500	BI-4500
Number of Channels	3-channel	5-channel
Throughput	Medium	High
Sensitivity	High	Higher
Applications	Small molecules, proteins, nucleic acids	High-throughput screening, complex binding studies
Best For	Academic & industrial research	Advanced research & drug discovery
Automation	Semi-automated	More automation capabilities



# Surface Plasmon Resonance

The BI-4500 is essentially an upgraded version of the BI-2500, offering more channels for higher throughput, improved sensitivity, and better automation features, making it ideal for demanding biosensing applications. The BI-2500, on the other hand, is a solid choice for standard interaction studies with great sensitivity at a lower throughput.

## Specific applications:

### Drug Discovery and Development

- Ligand-Receptor Binding Studies
- Pharmacokinetics

### Biochemical and Immunoassays

- Detection of Antibodies and Antigens
- Biomarker Identification

### Environmental Monitoring (Gas-SPR)

- Gas-SPR (Gas Phase Detection)
- Chemical Contaminants in the Air

### Biosensing for Healthcare (Point-of-Care Diagnostics)

- Point-of-Care Diagnostics
- Clinical and Laboratory Applications

### Gas Phase and Surface Interaction Studies (Gas-SPR)

- Gas-SPR (Gas Phase Interactions)
- Catalysis and Gas Sensors

### Electrochemical SPR (EC-SPR) in Energy and Biosensing

- Electrochemical Impedance Spectroscopy
- Energy Storage and Conversion
- Electrochemical Sensing

### Material Science and Nanotechnology

- Nanomaterial Characterization
- Surface Functionalization

### Food Safety and Quality Control

- Pathogen Detection
- Detection of Adulterants and Toxins

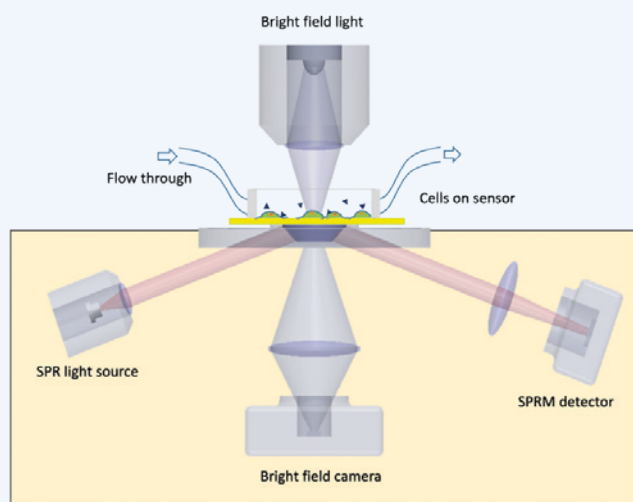
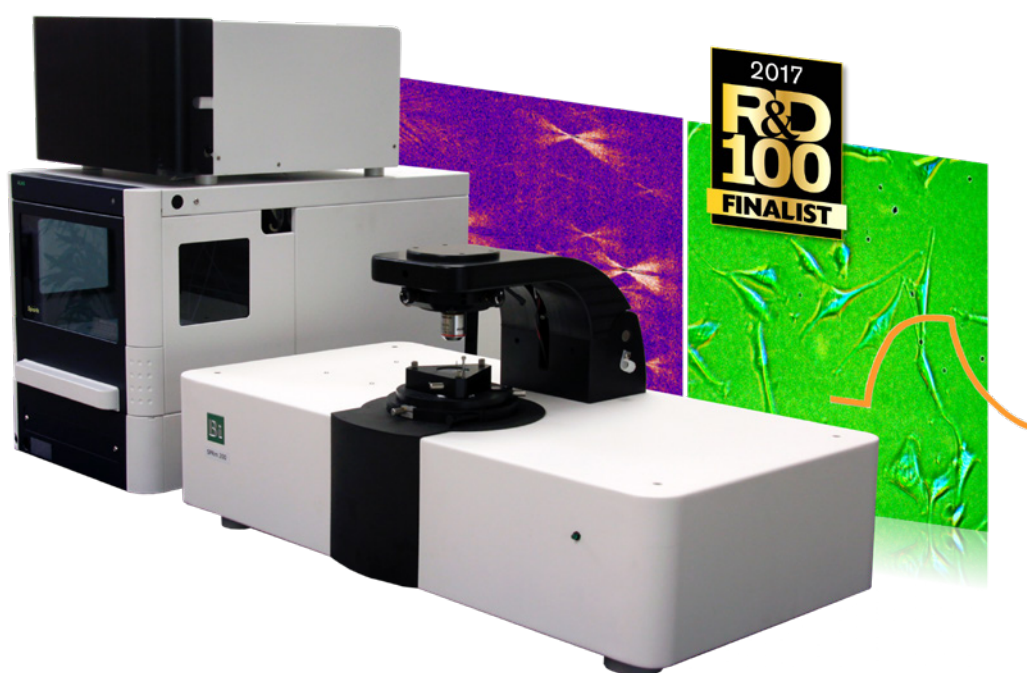
### Structural Biology and Protein Interaction Studies

- Binding Kinetics

# Surface Plasmon Resonance Microscopy

## SPRm-200

Live cell kinetics



# Surface Plasmon Resonance Microscopy

- **Detection Method:** Label-free
- **Best for:** Pharma Industry, Cell biologists, Antibody manufacturers
- **Key Benefit:** Non-Invasive and Live-Cell Compatible, Single-Cell Resolution, Real-Time Monitoring, High Spatial Resolution, Minimal Sample Preparation

## Specific applications:

### Drug Discovery

- For real-time monitoring of ligand-receptor binding and pharmacokinetics

### Cancer Research

- Studies antibody-cancer cell receptor interactions and immuno-oncology therapies

### Immunology & Vaccine Development

- Assesses antibody-antigen binding and immune cell interactions for vaccine and immunotherapy research

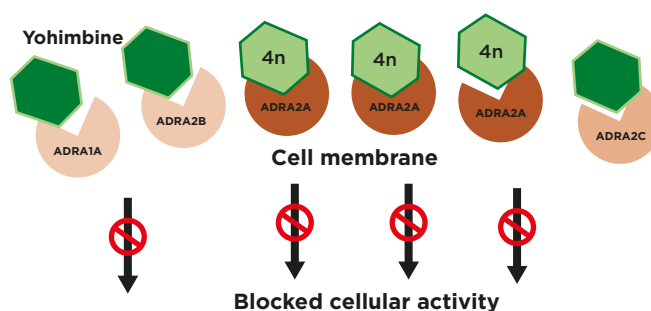
### Cell Biology

- Monitors cell receptor activation, migration, adhesion, and signaling

## Binding Activities of Yohimbine Analogues on ADRA2A Overexpressing Live Cells

#163

Millions of sepsis-related deaths are reported every year, which makes acute septic shock one of the leading causes of death in intensive care units around the world. Septic shock is characterized by multiple organ failure, which is generally initiated by the activation of alpha-2A adrenergic receptor ( $\alpha$ 2A adrenoceptor, ADRA2A) in Kupffer cells. These effects can be reversed upon infusion of ADRA2 antagonists such as yohimbine (nonselective) or BRL-44408 maleate (ADRA2A selective). Furthermore, yohimbine has demonstrated significant anti-inflammatory and antifibrotic activity in both in vitro (hepatic endothelial and stellate cells and hepatocytes) and in vivo (hepatic inflammation/fibrosis) models.



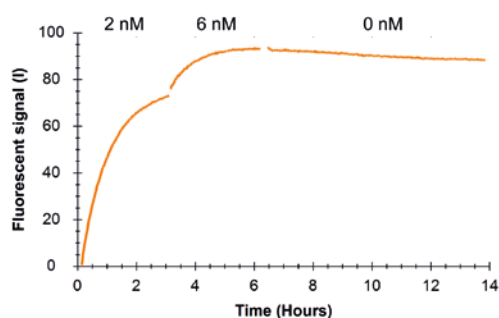
# LigandTracer Green

- **Detection Method:** Interchangeable LED-based fluorescence detector
- **Best For:** Researchers using fluorescently labeled ligands (e.g., FITC, Alexa Fluor dyes)
- **Key Benefit:** Non-radioactive, highly sensitive detection with minimal background interference

## Application Example

### Characterize therapeutic antibodies

LigandTracer was used to study the affinity and kinetics of the therapeutic antibodies cetuximab (Erbix), trastuzumab (Herceptin) and pertuzumab (Perjeta) binding to epidermal growth factor receptors expressed on tumor cells. The interactions were monitored for many hours, suitable for the characterization of the slow on ( $k_a$ ) and off ( $k_d$ ) rates of these high affinity antibodies. In fact, time to equilibrium when incubating 1 nM of these antibodies range from 10 h to 39 h. Information about interaction kinetics or dynamics can be useful for understanding drug residence time and appropriate dose.



Interaction	$k_a$ ( $M^{-1}s^{-1}$ )	$k_d$ ( $s^{-1}$ )	$K_D$ (pM)
Cetuximab - EGFR	$9.5 \times 10^4$	$1.9 \times 10^{-6}$	20
Trastuzumab - EGFR	$1.8 \times 10^4$	$7.1 \times 10^{-6}$	394
Pertuzumab - EGFR	$7.1 \times 10^4$	$3.1 \times 10^{-6}$	43

## Specific applications:

- Monitor the dynamics of protein-cell interactions in real-time, both during incubation and after wash
- Flexible platform compatible with many applications with protein binding to receptors on living cells being the most common
- Derive the affinity, on/off-rate, specificity and dose/time dependent target occupancy

# LigandTracer White

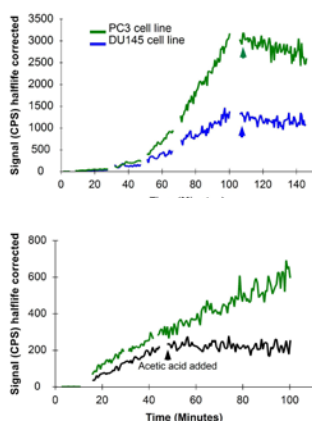
- **Detection Method:** Solid state electron/positron ( $\beta^-/\beta^+$ ) detector
- **Best For:**  $\beta^-$ ,  $\beta^+$  particles (50 keV and higher) emitted by radionuclides suitable for radiotherapy and PET-imaging purposes
- **Key Benefit:** Ultra-sensitive detection, ideal for low-abundance targets where fluorescence may not be viable

## Application Example

### In vitro kinetics evaluation and uptake

The uptake and retention of [11C]-acetate was studied in two parallel titration assays on two different cancer cell lines, PC3 (green) and DU145 (blue), in a humidified incubator at 37 °C, using LigandTracer White. Cells were incubated with increasing concentrations of [11C]-acetate followed by replacement of the incubation solution with medium (indicated by arrows). Uptake of [11C]-acetate was higher in PC3 cells, which are known to be more aggressive than DU145 cells.

In a competition assay, two experiments were performed in parallel on PC3 cells under the same experimental conditions as above. The uptake of 1 MBq [11C]-acetate by cells (black and green) was monitored for 15 min. Thereafter, excess amount of non-labeled acetic acid was added to one of the dishes (black) which reduced the uptake of [11C]-acetate by PC3 cells considerably.



## Specific applications:

- Flexible platform compatible with a large number of applications with radioligands binding to receptors on live cells being the most common
- Derive the affinity, on/off-rate, specificity, dose/time dependent target occupancy and cellular uptake/retention
- Oncology research (e.g., radiolabeled antibodies targeting tumor cells)

# LigandTracer Yellow

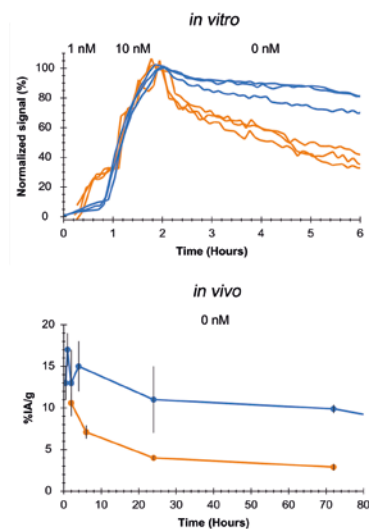
- **Detection Method:** High energy  $\gamma$  scintillator-based detector
- **Best For:** Tumor models validation in medical imaging
- **Key Benefit:** Suitable for detection of all radionuclides used in PET/SPECT imaging

## Application Example

### Validate tumor models in medical imaging

Uptake and retention of  $^{111}\text{In}$ -labeled anti-HER2 affibody ABY-025 was monitored on SKOV3 cells (expressing large amounts of HER2, blue) and DU-145 cells (expressing moderate amounts of HER2, orange) in a humidified incubator at 37 °C, with LigandTracer (upper graph). The data was compared with in vivo measurements of SKOV3 (blue) and DU-145 (orange) tumor xenografts in mice (lower graph).

The retention of tumor associated activity was three times longer for SKOV3 than for DU-145, both in vitro and in vivo. LigandTracer Yellow can therefore be a valuable tool for estimation of the in vivo outcome and optimization of animal experiments.



## Specific applications:

- Detect ligand-cell interactions and cellular uptake in real-time using PET/SPECT tracers
- Flexible platform compatible with a large number of applications with radioligand binding to receptors on live cells being the most common. Derive the affinity, on/off-rate, specificity and dose/time dependent target occupancy



# LigandTracer Grey

- **Detection Method:** Solid state  $\gamma$  and X-ray detector
- **Best For:** Entry-level users performing standard kinetic measurements
- **Key Benefit:** Cost-effective solution for fundamental research utilizing  $^{125}\text{I}$

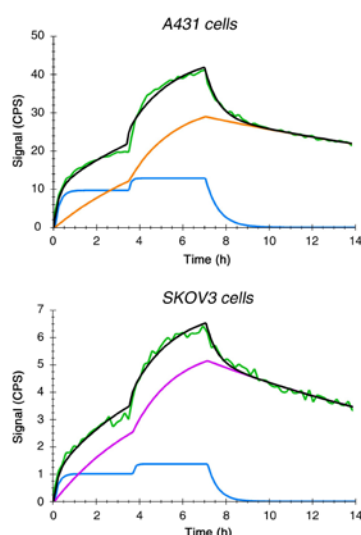
## Application Example

### Explore biological mechanisms in greater detail

The interaction between the epidermal growth factor (EGF) and its receptor (EGFR) was detected on A431 and SKOV3 cells (green). By fitting the LigandTracer data to a mathematical model (black\*) it was possible to decipher the kinetics and affinity of the binding of EGF to EGFR monomers (blue\*), EGFR homodimers (orange\*) and EGFR-HER2 heterodimers (purple\*).

It was observed that the homodimer interaction was only present on A431 cells (EGFR+++, HER2+) and that the heterodimer interaction only occurred on SKOV3 cells (EGFR+, HER2+++). Such improved understanding of dimerization patterns and allosteric modulation can provide important clues about the underlying biology.

*\*Fitted curves*



### Specific applications:

- Monitor the dynamics of protein-cell interactions in real-time, using proteins labeled with low energy  $\gamma$ -emitting radionuclides such as  $^{125}\text{I}$
- Derive the affinity, on/off-rate, specificity and dose/time dependent target occupancy





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