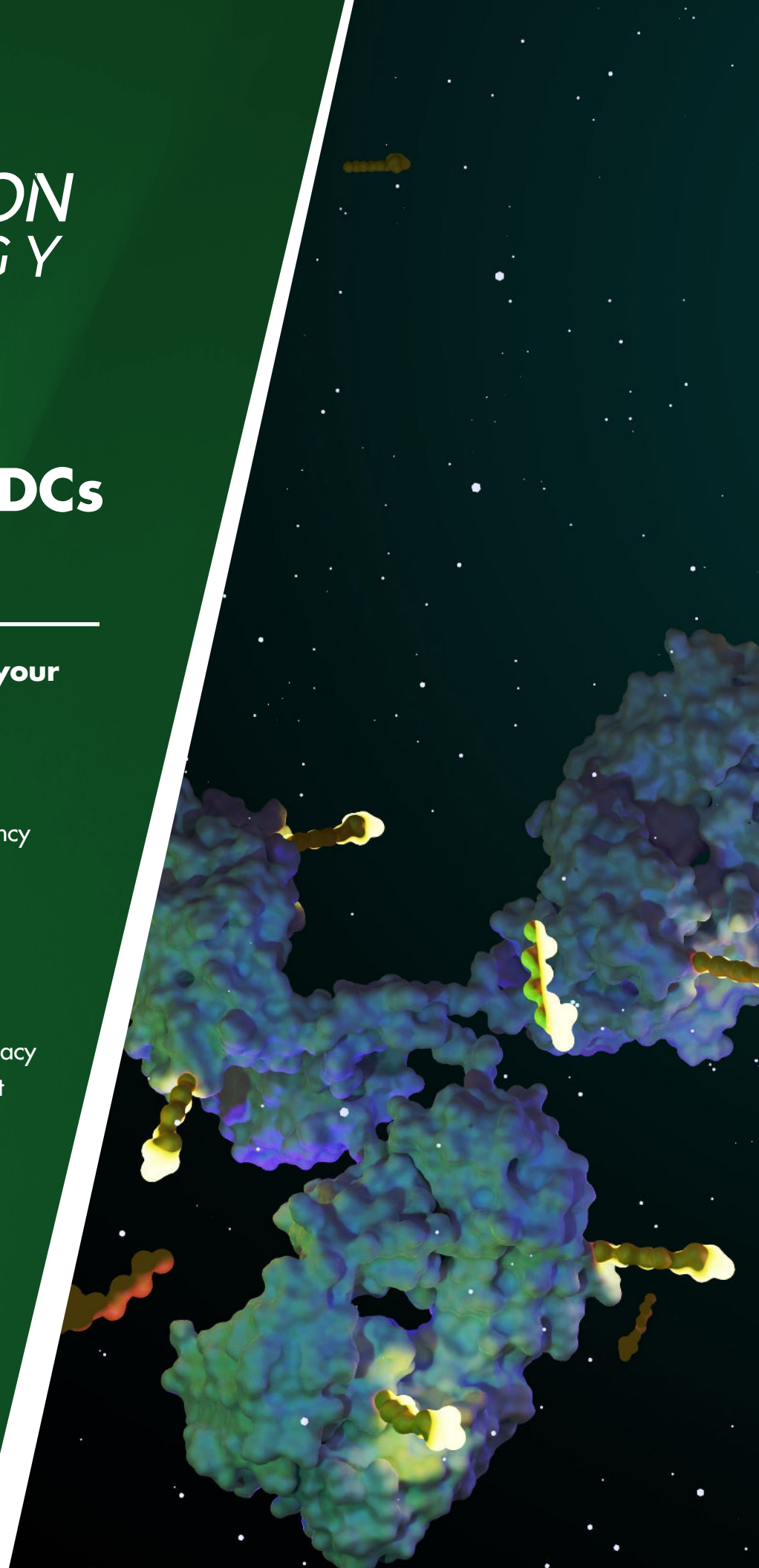
REACTION BIOLOGY

Advancing ADCs Together

Support for every step of your antibody drug conjugate discovery program

- Payload Evaluation and Selection
- Antigen Binding Specificity & Potency
- Internalization Assays
- Cytotoxicity & Safety
- Mechanism of Action & Efficacy
- Effector Function Assessment
- Safety Assays
- In-Vivo Pharmacokinetics and Efficacy
- GxP Quality & Potency Assessment



Unlock the Potential of ADCs with Reaction Biology

Integrated assay platforms and expert guidance to de-risk and optimize your breakthrough ADC candidates

Payload Evaluation and Selection

Selecting the right payload and linker directly influences therapeutic efficacy, safety, and mechanism of action. Evaluate critical payload potency, stability, linker release, and compatibility data to ensure optimal performance before or after conjugation.

- **OncoFlow-Profiler Panel:** Surfaceome profiling across 160+ cancer cell lines using flow cytometry platforms to identify actionable targets and enhance therapeutic precision, helping identify the most promising targets for payload optimization.
- **QIFIKIT:** Receptor density quantification using QIFIKIT and QuantiBright technologies to provide critical insights into target expression levels for optimized ADC design and model selection, ensuring payloads are matched to targes with the highest therapeutic potential.
- **ProLiFiler™ Cancer Cell Panel:** 160+ tumor cell lines across 15+ tumor types with CellTiter-Glo analysis for ADC efficacy across diverse cancer models, screening for payloads with the broadest and most effective anti-tumor activity.

Antigen Binding Specificity & Potency

Ensure your ADC maintains precise target engagement by optimizing antigen binding properties. Maximize therapeutic efficacy while minimizing off-target effects.

- **ImmunoAssays:** AlphaLisa, HRTF, Sandwich ELISA, MSD, and Luminex platforms with 32 conditions per target for assay development and quantitative binding analysis. These assays provide precise data to optimize payload binding and ensure accurate target engagement.
- **Protein Microarrays:** Proteome Profiler Human Phospho-RTK Array Kit (ARY001) and Human Phospho-Kinase Array Kit (ARY003) with LicorOdyssey™ CLx, capable of screening 40-50 targets per array, enabling HTS profiling for informed target selection and identification of the most potent antigen binding candidates.
- **Surface Plasmon Resonance (SPR):** Cytiva Biacore 8K and Biacore 8K+ with capacity for 50 KDs with specified concentration, 25 KDs with optimized concentration, 300 3-dose measurements, and 1000 single dose screens per week with real-time data on binding kinetics to ensure optimal antibody-target interactions.
- **Flow Cytometry:** Cytoflex S (Beckman), Fortessa (BD), and Sony spectral analyzers for cell surface binding confirmation, ensuring accurate target validation and characterization for specific antibody binding to intended target cells.

Internalization Assays

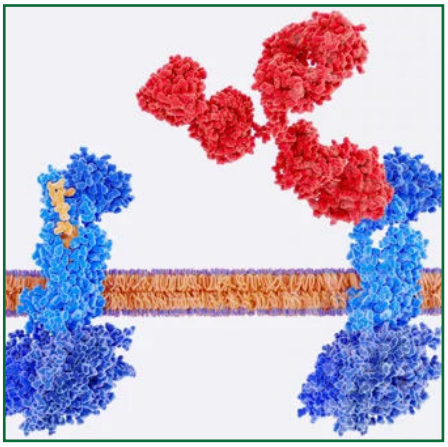
Evaluate how efficiently your ADC is internalized into target cells. Proper internalization is essential for delivering the payload to its intended site of action, ensuring therapeutic success.

- **pH-Sensitive Internalization:** Zenon-pHRodo labeling monitored by flow cytometry (endpoint) and Cytation Imager (real-time measurement) to track internalization dynamics and ensure efficient payload delivery to target cells.
- **High-Content Imaging:** Cytation 5 platform generating 160 data points per experiment with automated internalization scoring, providing detailed insights into ADC behavior and cellular interactions.
- **Real-Time Lysosomal Trafficking:** Comprehensive analysis across diverse cell lines and time points to ensure precise payload delivery and enhance understanding of internalization kinetics and efficacy.

Mechanism of Action & Efficacy

Optimize payloads selection and/or combination. Decipher how your ADC works at the cellular level and confirm its ability to kill cancer cells effectively. Ensure your ADC is optimized for maximum therapeutic impact and helps predict clinical outcomes.

- **MoA Bioinformatics:** Comparison to 2000+ reference drugs for mechanism prediction and pathway analysis to understand therapeutic action and optimization opportunities.
- **Cell Cycle Analysis:** Flow cytometry-based cell cycle profiling for mechanism-specific arrest patterns, revealing how your ADC disrupts cancer cell division, a critical step in understanding its efficacy.



Cytotoxicity & Safety

Understand the toxicological profile of your payload to ensure it retains its potency pre- and post-conjugation. Mitigate safety risks and ensures your ADC effectively kills cancer cells without excessive off-target toxicity.

- **Real-Time Cytotoxicity:** xCELLigence for continuous monitoring and CellTiter-Glo for endpoint measurements to determine IC50 across 8 concentrations in duplicate, providing real-time insights into ADC potency and effectiveness for an assessment for payload toxicity over time.
- **Fc-Mediated Cytotoxicity:** ADCC, CDC, and ADCP assays with xCELLigence, CellTiter-Glo, and flow cytometry to determine IC50 across 7 concentrations with PBMCs from 3 donors for robust evaluation of immune-mediated ADC activity, providing insights into how your ADC engages the immune system to kill cancer cells.
- **DNA Damage Assays:** ATM Cellular Phosphorylation Assay and γ-H2AX Foci Formation assay using Cytation 5 (80 conditions per 96-well plate) to identify payloads that induce DNA breaks, a key mechanism of cancer cell death.
- **Bystander Killing Effect:** Co-culture systems to evaluate payload diffusion and neighboring cell toxicity to illuminate off-target effects and minimize harm to healthy cells.
- **Non-Specific Cell Toxicity:** Measure protein expression in different primary cells, such as bone marrow, to assess potential off-target toxicity and mitigate impact to non-cancerous tissues.

In-Vivo Models

Translate your in vitro findings into physiologically relevant data. In vivo models provide critical insights into pharmacokinetics, biodistribution, and efficacy, helping you select the best ADC candidate.

- **Hollow Fiber Model:** Semi-permeable membrane encapsulated tumor cells with CellTiter-Glo viability measurements to bridge in vitro findings to in vivo testing, ensuring seamless translation of ADC efficacy data from the lab to animals.
- **Pharmacokinetic Studies:** Quantify ADC levels in blood and organ or tumor tissue using advanced platforms like MSD to evaluate absorption, distribution, metabolism, and excretion, ensuring optimal dosing and therapeutic efficacy.
- **Tailored Humanized Syngeneic Models:** Stable cell line engineering with human target expression (+/- luciferase), establishment of implanted tumor models, and efficacy studies using IVIS Lumina III bioluminescence imaging for predictive clinical insights.
- **Humanized Xenograft Models:** Human tumor models with engrafted human PBMCs, T cells, or NK cells monitored by flow cytometry and IVIS imaging to evaluate ADC performance in a clinically relevant immune environment.

GxP Quality & Potency Assessment

Validate the biological activity of your ADC with GxP-compliant potency assays. These assays ensure regulatory readiness and provide robust data to support clinical development.

- **Batch Release Testing:** Relative potency determination with statistical confidence intervals using platforms like Tecan Infinite M200 with fluorescence detection (530 nm excitation, 590 nm emission) to ensure consistent quality.
- **Potency Assays:** Standardized protocols to maintain ADC batch-to-batch consistency and meet stringent regulatory compliance.

CASE STUDY

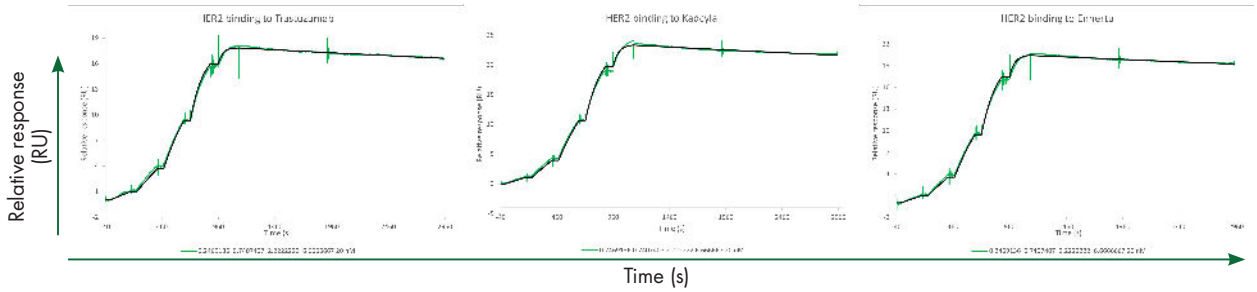
Payload-Dependent ADC Activity Profiles

Demonstrating How Different Payloads Drive Distinct Therapeutic Characteristics

Payload Evaluation and Selection

Reaction Biology conducted a comprehensive head-to-head comparison of two HER2-targeting ADCs, Kadcyla® (tubulin inhibitor) and Enhertu® (topoisomerase I inhibitor), to demonstrate how payload chemistry fundamentally drives ADC activity profiles. This study showcases our ability to generate actionable insights for payload selection and optimization during ADC discovery.

Key SPR & Proliferation Findings	
Kadcyla® (Tubulin Inhibitor Payload)	**Enhertu® (Topoisomerase I Inhibitor Payload)**
• **Rapid onset:** Significant growth inhibition within 24-48 hours • **HER2-dependent:** Strong activity in SK-BR-3 and SK-OV-3 cells (HER2+++) • **Lower Toxicity:** Minimal effect on HER2-low/ negative cells • **IC50 Range:** 1-10 nM in HER2+++ cells	• **Delayed onset:** Gradual growth inhibition over 72-168 hours • **Broader activity:** Active across multiple HER2 expression levels • **Higher Toxicity:** Significant activity even in HER2-low cells due to bystander effects • **IC50 Range:** 10-100 nM across cell panel



Binding Model	Target	Analyte	Chi² (RU²)	k_a (1/Ms)	k_d (1/s)	K_D (M)	R_{max} (RU)
1:1 Kinetic	Herceptin®	HER2	3.39E-02	1.08E−06	3.38E-05	3.14E-11	20.5
1:1 Kinetic	Kadcyla®	HER2	5.92E-02	1.05E−06	4.21E-05	4.01E-11	23.4
1:1 Kinetic	Enhertu®	HER2	3.39E-02	1.08E 06	3.38E-05	3.14E-11	20.5

Fig. 1: Surface Plasmon Resonance (SPR)

HER2 binding to Herzeptin® (Trastuzumab), Kadcyla®, and Enhertu® is depicted. Trastuzumab and ADCs were captured using on anti-Fc IgG immobilized on a Series S CM4 chip and data were collected on a Biacore 8K+ (Cytiva).

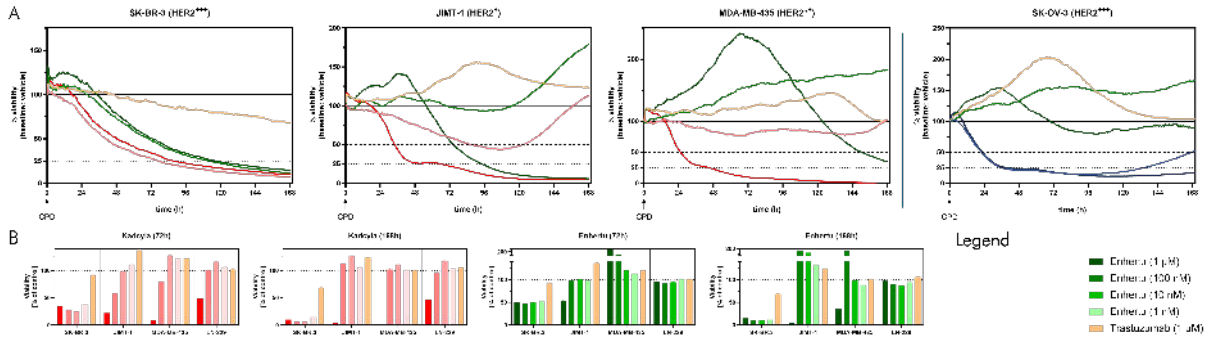


Fig. 2: Real-time cell analysis by measuring cell impedance with xCELLigence technology.

Breast cancer cells with different levels of HER2 expression (high / medium / low), an ovarian cancer cell line with high HER2 expression and a glioblastoma cell line without HER2 expression were plated on 96-well plates and treated with the indicated concentrations of Kadcyla® (trastuzumab emtansin), Enhertu® (trastuzumab deruxtecan) and Herceptin® (trastuzumab): (A) Time course of viability (measured twice per hour) compared to the untreated control up to day 7 (168h) for the two highest concentrations; (B) the graphical representation at day 3 (72h) and day 7 (168h) of all ADC concentrations tested.

Mechanistic Insights:

Kadcyla's mechanism of action was elucidated through Surface Plasmon Resonance (SPR) and proliferation assays. SPR revealed Kadcyla's distinct kinetic profile, demonstrating strict HER2 dependence due to its non-cleavable linker. Proliferation assays further confirmed its targeted activity, showing significant growth inhibition in HER2-positive cell lines (e.g., SK-BR-3 and SK-OV-3) with minimal effects on HER2-low or negative cells.

Together, these insights underscore the critical role of SPR and proliferation data in understanding payload-specific ADC mechanisms, enabling informed decisions for ADC optimization and development.

Partner with Reaction Biology and drive your ADC candidates towards clinical success

Leverage our integrated and end-to-end ADC capabilities to accelerate your drug discovery process:

- Identify and validate targets with advanced binding specificity, potency assays, and surfaceome profiling.
- Optimize lead candidates through comprehensive payload selection (including combination), mechanism studies, and cytotoxicity evaluations.
- Accelerate preclinical development with integrated in vitro and in vivo models for efficacy and safety assessment.
- Ensure batch-to-batch consistency with standardized potency assays and regulatory-compliant workflows.
- Streamline discovery to development with end-to-end assay integration and actionable insights at every stage.

Global Expertise, Tailored Solutions for Your ADC Needs

Start a project or learn more

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