www.biosensall.com
collabdtna@domaintherapeutics.com

bioSensAll™: Opening Up New Frontiers in RTK Drug Discovery

F. Gross#, A. Mancini#, G. Dugast#, B. Breton#, S. Schann*

#Domain Therapeutics NA Inc., 7171 rue Frederick-Banting, Montreal, H4S 1Z9, CANADA
* Domain Therapeutics SA, Bd Sébastien Brant 67400 Strasbourg-Illkirch, FRANCE

Introduction

bioSensAll™ is a proprietary live-cell BRET-based biosensor platform that allows the real time monitoring of signal transduction pathways of cell surface receptors, including **Receptor Tyrosine Kinases (RTKs)**.

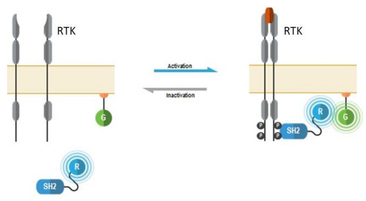
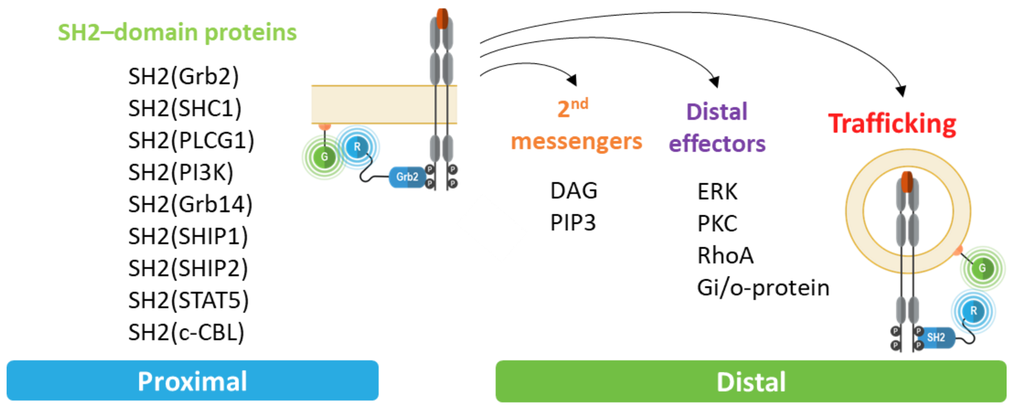


Figure 1: bioSensAll RTK assay principle

RTKs bind to a variety of signaling molecules and regulate many critical processes, including cell survival, proliferation, motility and differentiation. Importantly, dysregulated RTK function can lead to the development of numerous types of cancer.
Consequently**, RTKs are prime targets for new anti-cancer agents**. Yet, our understanding of the complex biology of RTKs remains incomplete and **their full therapeutic potential is largely underexploited.**

Most of the methodologies currently used to study RTK activation and screen for anti-RTK drug candidates revolve around RTK kinase activity which overlook key determinants of ligand therapeutic efficacy, including kinetics, localization and pathway-biased signaling.

BRET-based biosensor platform



Results: Real-Time Monitoring of EGFR Activation

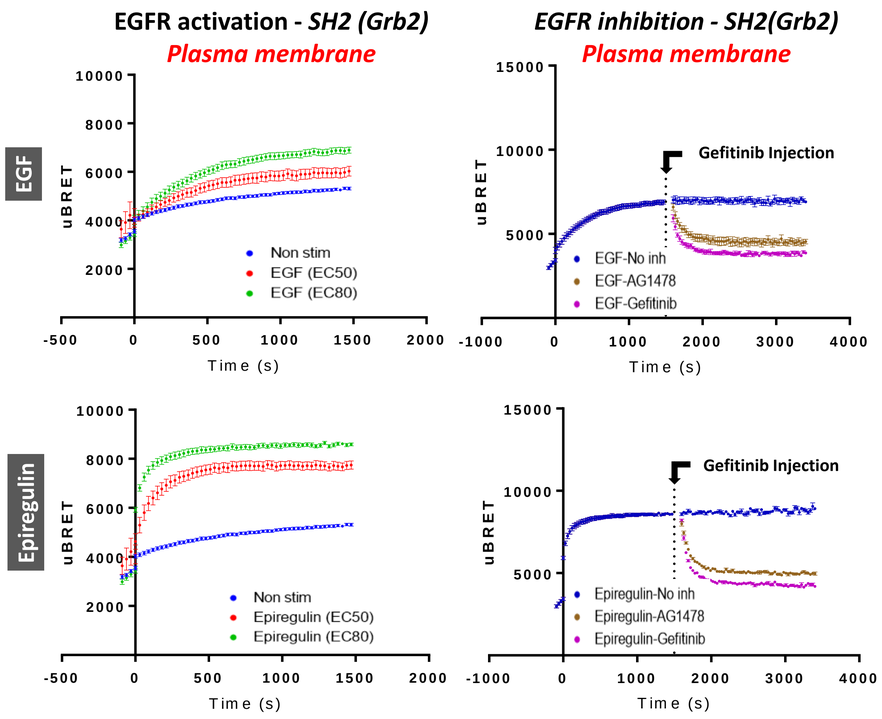


Figure 2: Activation of EGFR upon EGF / Epiregulin stimulation & Inhibition with Gefitinib

Kinetics of SH2(Grb2) recruitment to the plasma membrane differ in response to EGF and epiregulin stimulation. Indeed, epiregulin-induced SH2(Grb2) recruitment was more efficacious (~25%) and displayed faster kinetics relative to that observed with EGF ($t_{1/2}$ = 31.3 sec vs. 253.8 sec, respectively).

The plasma membrane recruitment of SH2(Grb2) in response to both agonists was sustained for at least 60 minutes and was rapidly **reversed** (within 7 mins) by the EGFR kinase inhibitor Gefitinib (Iressa®).

Theses results show the capacity to **assess ligand dynamics** on timescales ranging from milliseconds to hours.

Results: Localization & biased signaling

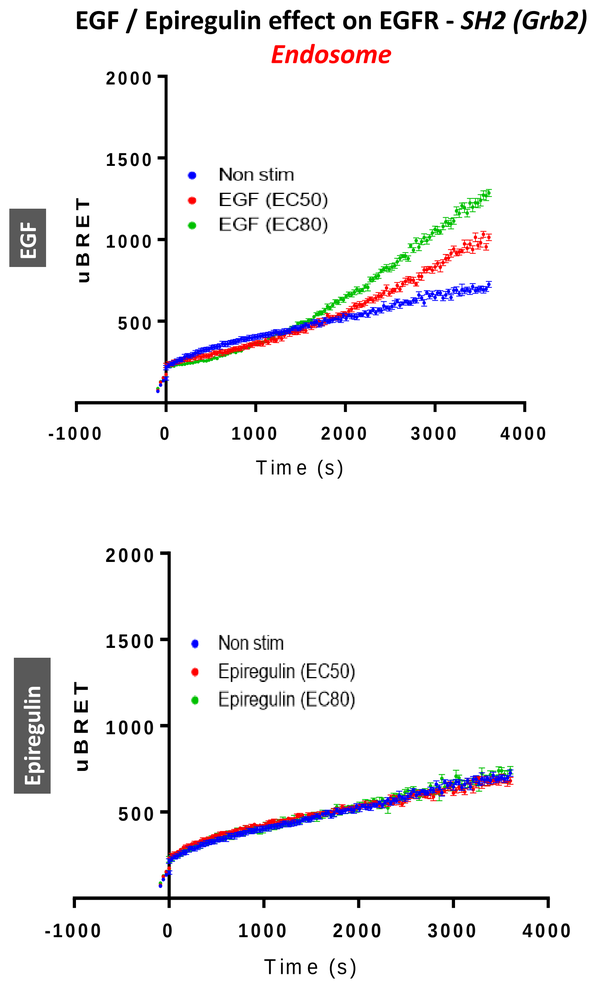


Figure 3: Effects of EGF and Epiregulin on EGFR internalization

Striking differences were observed in agonist-induced trafficking of the SH2(Grb2) biosensor to early endosomes.

Notably, EGF, but not epiregulin, promoted a gradual time-dependent increase in SH2(Grb2) levels in the **early endosome** compartment.
These results are consistent with previously published data demonstrating that EGF, but not epiregulin, promotes EGFR internalization.

This phenomenon, known as **bias,** demonstrates the capacity of the platform to clearly depict the unique **signalling signature** induced by different EGFR ligands.

Results: Non-canonical G protein Activation by EGFR & EGFR mutants

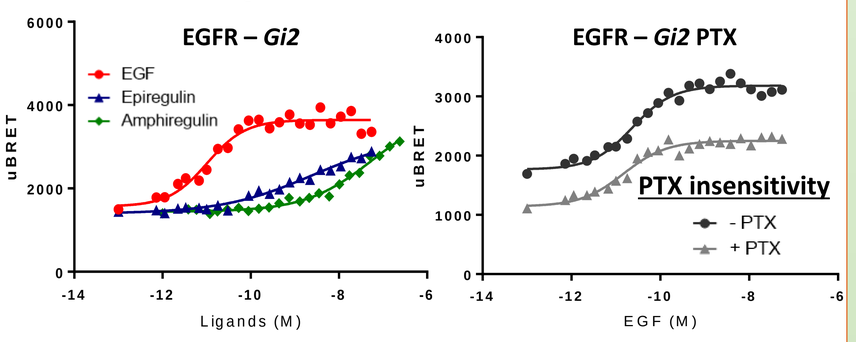


Figure 4: Recruitment of Gi2 with different ligands & effect of pertussis toxin (PTX)

Observed **G protein activation** with 3 endogenous ligands of EGFR is **GPCR independent (i.e., PTX insensitive),** and occurs via a different mechanism than with GPCRs.

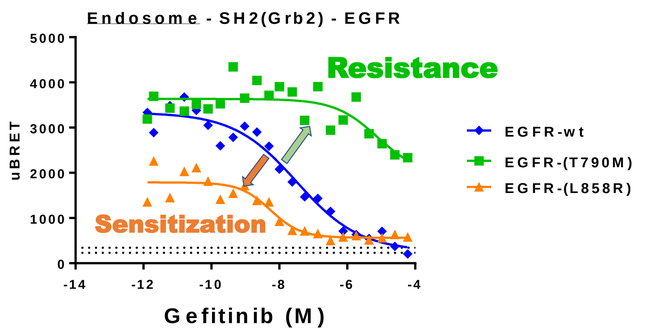


Figure 5: Effect of EGFR point mutations on receptor sensitivity to Gefitinib

The effect of **EGFR point mutations** on the receptor's sensitivity to inhibition by Gefitinib was tested. Both **resistance and sensitization** to Gefitinib were observed. These results are in accordance with previously published reports on the same mutations.

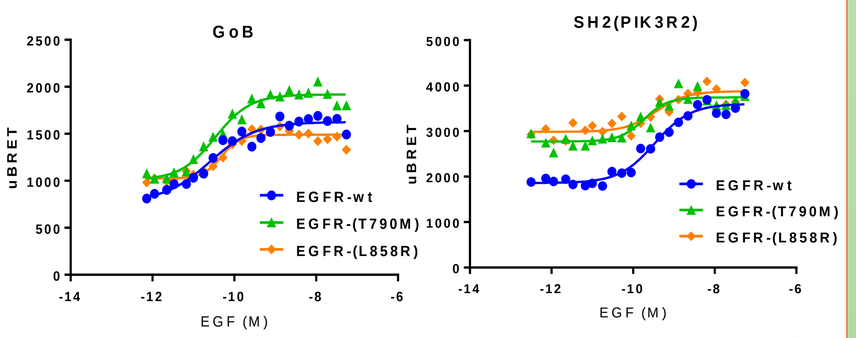


Figure 6: Effect of EGFR point mutations on the recruitment of GoB vs SH2(PIK3R2) EGFR constitutive activity

EGFR mutations were shown to have different signaling signatures:

- T790M increases the efficacy of EGFR G protein recruitment;
- Both T790M and L858R mutations significantly increased the constitutive activity of EGFR in engaging the SH2(PIK3R2) sensor. The ability to measure such phenomena could enable screening campaigns based on new criteria.

In addition to traditional *in vitro* kinase assays, bioSensAll™ provides the following advantages in RTK drug discovery:

Realtime Kinetics

- Ligand K_{on}/K_{off} measurement
- Latency of action of modulators
- Reversal of RTK's activation

Spatiotemporal analysis

- Ligand capacity to induce RTK internalization
- Localization of RTK interacting SH2 protein

Multiple Signaling Pathway analyses

- Ligand profiling signature
- Non-canonical G Protein activation
- RTK mutant profiling

bioSensAll™ unique combination of adaptability, HTS compatibility and real time kinetic capabilities across multiple effector pathways can help generate the quality of data required to enhance drug discovery in the field of RTKs