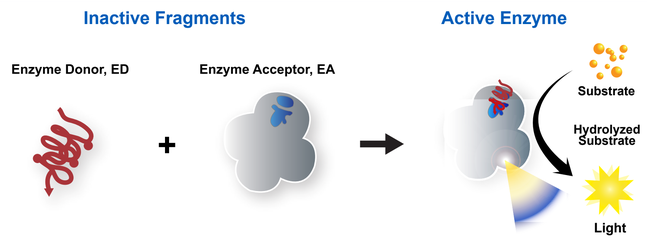
Detection of EGFR, HER2, HER3 and HER4 Hetero- and Homodimerization Using EFC-based Cellular Assays

Abhishek Saharia, Ph.D., Jennifer Lin-Jones, Hyna Dotimas, Sangeetha Gunturi, Abha Srivasta, Tom Wehrman, Ph.D., and Jane Lamerdin, Ph.D.
DiscoveRx Corporation, Fremont, CA 94538-3142

Abstract

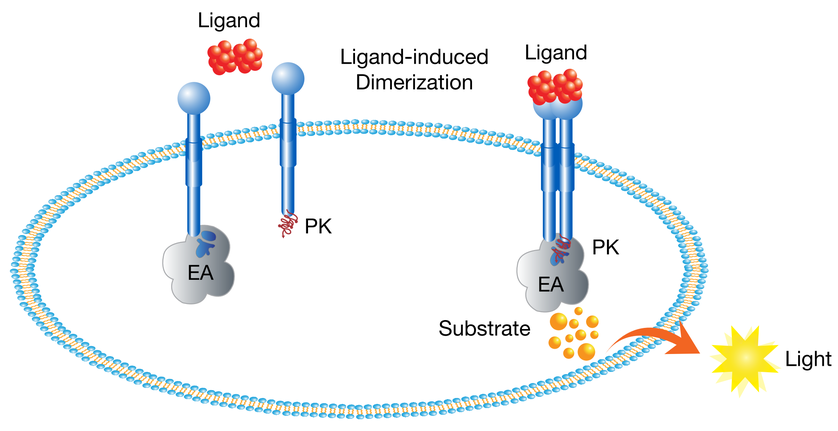
Ligand-induced receptor dimerization is the first functional step in receptor activation, representing the most proximal, functional read-out for receptor activation. Here we present a novel application of the Enzyme Fragment Complementation system to monitor receptor-receptor interactions at the surface of intact cells, applicable to diverse receptor types such as Interleukin receptors, BMP receptors and cytokine receptors. For the purpose of this poster, we will focus on the HER family of receptors. It is well understood that the HER family proteins can dimerize with the other members of its family leading to a complicated oncogenic signaling cascade. Surprisingly, existing cellular assays have been unable to faithfully monitor these interactions proximally in a drug discovery setting. We present EFC-based cellular assays that monitor EGFR homodimerization, EGFR-ErbB2 and ErbB2-ErbB3 heterodimerization events, amongst others. The high signal to noise ratio, serum tolerance and reproducibility make these assays applicable to a diverse range of applications for the identification and development of therapeutic small molecules and biologics, including screening, functional characterization, QC lot release assays and neutralizing antibody studies. Additionally, some data will be presented on how these assays can be used to determine potency of bi-specific antibodies.

Enzyme Fragment Complementation



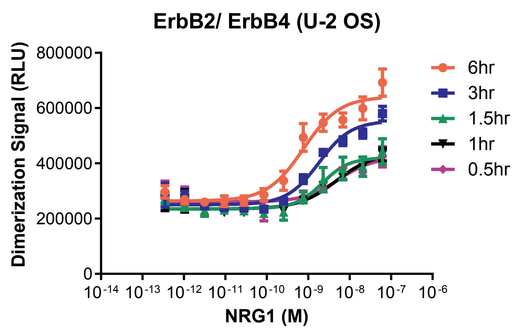
Enzyme Fragment Complementation (EFC) technology consists of the β-galactosidase (β-gal) enzyme, split into two inactive components, the enzyme donor peptide (ED) and enzyme acceptor (EA). When brought together in close proximity, ED complements with EA forming active β-gal. The active enzyme will give a chemiluminescent signal in the presence of substrate providing a highly amplified signal, and therefore, a very sensitive assay.

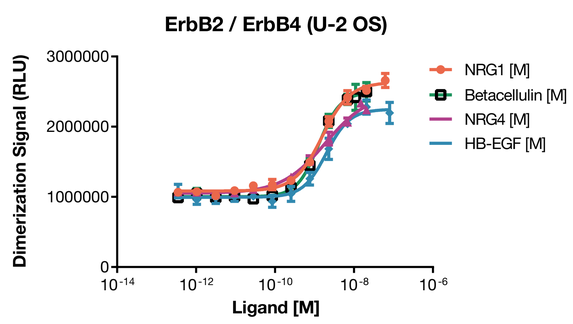
How PathHunter Dimerization Assays Work



Using EFC, assays have been created to measure receptor heterodimerization, homodimerization or co-receptor recruitment. The two target receptors are tagged with ProLink™ (PK) or Enzyme Acceptor (EA). Upon ligand-induced activation, the receptors naturally dimerize forcing the two β-gal components to complement and create an active enzyme. Active β-gal generates a chemiluminescent signal in the presence of substrate, signaling the formation of receptor dimers.

ErbB2/ErbB4 heterodimerization & ErbB4 homodimerization assays

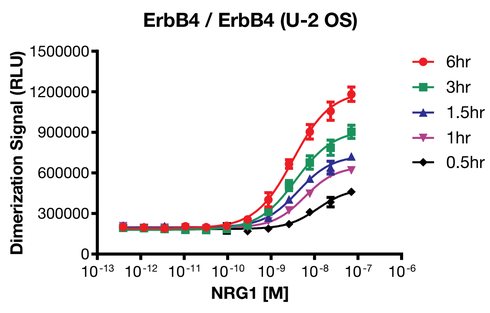


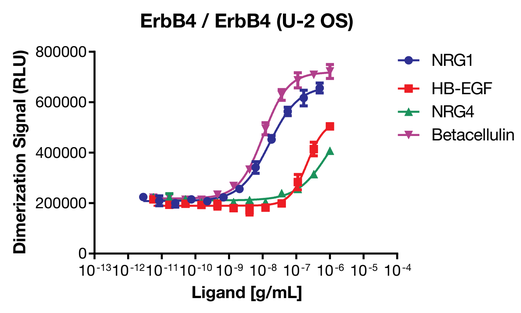


Time point, hr	0.5	1	1.5	3	6
EC_{50} (nM)	5.5	4.7	2.2	1.8	0.77
S/B	1.4	1.6	1.6	2.0	2.3

Ligand	NRG1β	Betacellulin	HB-EGF	NRG4
EC_{50} (nM)	1.56	1.28	2	1.9
S/B	2.4	2.4	2.0	2.3

Ligand-induced dimerization of ErbB2 and ErbB4 receptors. (Left Panel) Time course of NRG1-induced ErbB2/ErbB4 heterodimerization. U-2 OS cells stably expressing the extracellular domains of the ErbB2 receptor fused to the PK tag and ErbB4 fused to the EA tag were seeded 24 hr before treatment with NRG1b for 0.5- 6 hr in dose response format. Robust dimerization is detected within 3 hr of ligand incubation, with EC_{50} plateauing by 6 hr (Right Panel) Ligand profile for ErbB2/ErbB4 heterodimer assay. Note all ligands are essentially equipotent in the ErbB2/ErbB4 assay (compare to ErbB4 homodimer), demonstrating that ErbB2 can enhance the binding of NRG4 and HB-EGF to ErbB4 (relative to ErbB4 homodimer). These data are consistent with reports in the literature that NRG1b and NRG4 are ~equipotent towards ErbB4 in cells over-expressing ErbB2.



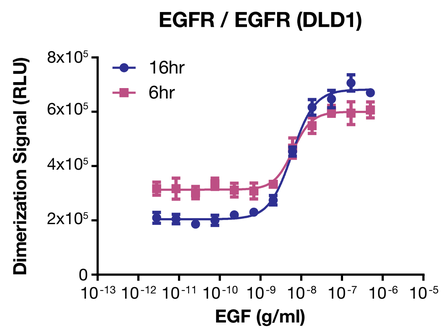


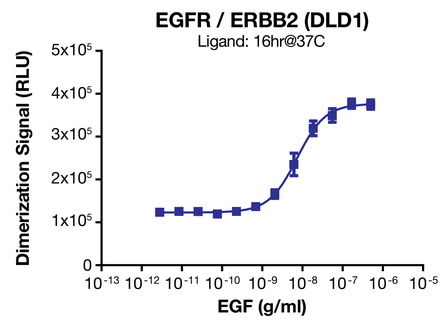
Time point, hr	0.5	1	1.5	3	6
EC_{50} (nM)	11.8	5.9	4.2	3.7	3.2
S/B	2.4	3.0	3.4	4.7	5.9

Ligand	NRG1β	Betacellulin	HB-EGF	NRG4
EC_{50} (nM)	2	1.4	28	123
S/B	3.0	3.3	2.3	1.8

Ligand-induced dimerization of ErbB4 receptors. (Left Panel) Time course of NRG1-induced ErbB4 homodimerization. U-2 OS cells stably expressing the extracellular domain of the ErbB4 receptor fused to the PK or EA tags were seeded 24 hr before treatment with NRG1 for 0.5- 6 hr in dose response format. Robust dimerization is detected within 1 hr of ligand incubation, with EC_{50} plateauing by 6 hr (Right Panel) Ligand profile for ErbB4/ErbB4 homodimer assay. ErbB4/ErbB4 –expressing cells were treated with the indicated ErbB4 ligands for 6 hr at 37C. Observed EC_{50}s for NRG1 and betacellulin are in good agreement with cell-based ligand binding affinities described in the literature (1.5-5nM).

EGF-induced homo- and hetero-dimerization of EGFRs



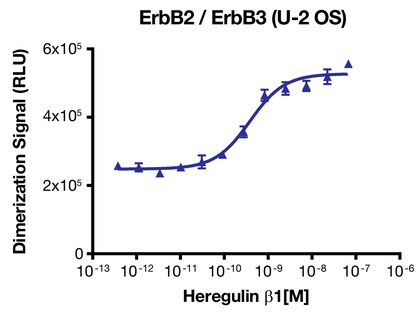


Time point, hr	6	16
EC_{50}, ng/mL	6.3	5.9
S/B	2.0	3.0

Time point, hr	16
EC_{50}, ng/mL	7.3
S/B	3.0

Ligand-induced dimerization of EGFR and ErbB2 receptors. (**A**) Time course of EGF-induced EGFR/EGFR homodimerization. DLD1 cells stably expressing the extracellular domains of the EGF receptor fused to the ProLink and EA tags were seeded 24 hr before treatment with EGF for 6 or 16 hr in dose response format. Assay window improves substantially with 16 hr incubation with ligand. (**B**) EGF-induced heterodimerization of EGFR and ErbB2. DLD1 cells stably expressing the extracellular domains of the EGF receptor fused to EA and ErbB2 fused to PK were seeded 24 hr before treatment with EGF for 16 hr in dose response format. As expected (since ErbB2 does not bind ligand), the EC_{50} for EGF is in good agreement with that observed for the EGFR homodimer.

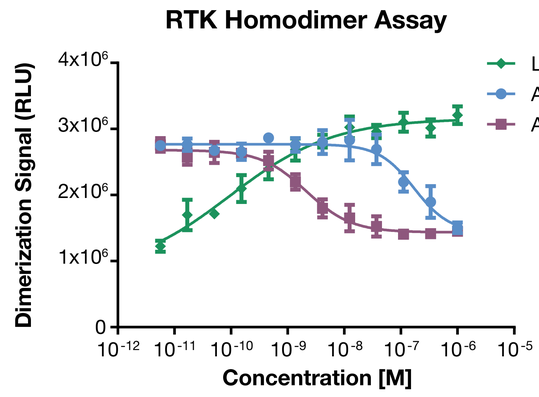
Heregulin-induced heterodimerization of ErbB2/ErbB3 complexes



Time point, hr	6
EC_{50}, nM	0.35
S/B	2.6

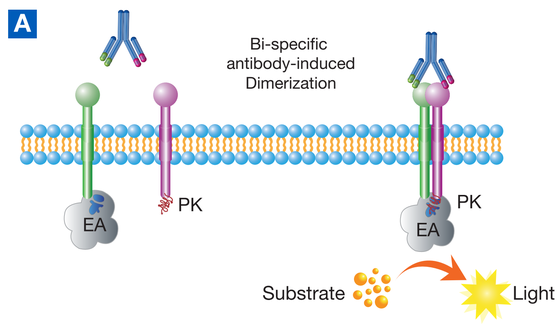
Ligand-induced dimerization of ErbB2 and ErbB3 receptors. U-2 OS cells stably expressing the extracellular domains of the ErbB2 receptor fused to the ProLink and ErbB3 fused to EA were seeded 24 hr before treatment with Heregulinβ1 (NRG1) for 6 hours in dose response format.

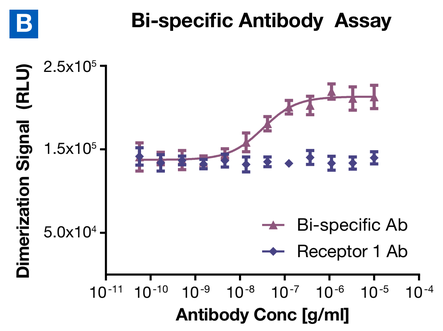
Use of dimerization assays to characterize anti-receptor antibodies



Blocking receptor dimerization with anti-receptor antibodies. Cells expressing an RTK homodimer were allowed to adhere overnight, then treated with two different anti-receptor antibodies for 6 hr in the presence of an EC_{80} dose of ligand. The presence of anti-receptor antibody produced an inhibitory response by blocking the RTK homodimer formation with varying efficacy.

Use of dimerization assays to characterize bi-specific antibodies





(**A**) The dimerization assay format has also been successfully used to monitor the potency of bi-specific antibodies. The two target receptors are tagged with PK or EA and expressed in the same cell line. Upon binding the bi-specific antibody, the receptors are brought in close proximity, forcing the two β-gal components to complement and create an active enzyme, which will generate signal in the presence of substrate. These assays can be used for lot release testing of bi-specific antibodies as they can determine whether both the arms of an antibody are functional. (**B**) Antibody-induced receptor dimerization assay. Cells expressing an RTK heterodimer were allowed to adhere overnight, then treated with two different antibodies for 6 hr; one antibody specific to one receptor in the heterodimer, the other antibody is a bi-specific antibody that should induce receptor dimerization (purple triangles).

Summary & Conclusions

We present here a novel cell-based assay platform for the quantitation of ligand-induced receptor dimerization utilizing our well established Enzyme Fragment Complementation system. We demonstrate that the technology harnesses the native biology of receptor dimerization and is applicable across diverse receptor types, with specific examples demonstrated for the HER family of receptors. Further, we have shown that the pharmacology of ligand binding is consistent with reports in the literature. As demonstrated, these assays have been successfully used to identify inhibitors of receptor dimerization. Intriguingly, our success in creating assays for bi-specific antibodies have garnered much interest within the field as these assays have been used to create potency and lot release assays for the bi-specific antibodies. This platform represents a unique tool to address a wide variety of applications for the development of biologics, including mechanism of action studies, QC lot release and neutralizing anti-drug antibody assays for anti-ligand, anti-receptor and bi-specific antibodies. These robust assays have also been used to identify small molecules that block ligand-receptor interactions.

tel | 1.866.448.4864 tel | +44.121.260.6142 e | info@discoverx.com

DiscoveRx
www.discoverx.com