Cell-Based BRET Assays for Quantifying Antibody Mediated Blocking of Receptor-Ligand Interactions

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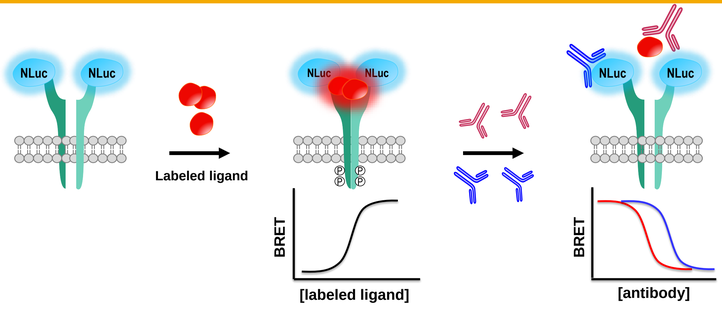
1. Introduction

Antibody-based therapeutics have become a major trend in the drug discovery pipeline. Often these antibodies are generated to recognize particular ligands or receptors in order to block their interactions. To screen for such antibodies, we have developed a cell-based assay that rapidly quantifies ligand binding to a specific receptor on the cell surface. The assay utilizes bioluminescence resonance energy transfer (BRET) to detect the interaction of a fluorescently-labeled ligand with a receptor genetically tagged with a small and exceptionally bright NanoLuc® luciferase. Subsequently, the capacity of antibodies to block this interaction is quantified through the reduction in BRET signal.

Fluorescently-labeled ligands are commonly generated by random chemical modification of lysine residues. However, this approach routinely results in heterogeneous ligand populations that may exhibit variable interference in the interactions with their cognate receptors. We addressed this by developing an alternative labeling method that is both stoichiometric to minimize population variability and site specific to minimize perturbation of receptor binding. Using this method we generated three growth factors EGF, PDGF-B and VEGF-A_{165} that were quantitatively labeled at the N-terminus and retained their biological function and binding affinity to their cognate receptors. In contrast, the random labeling resulted with significantly decreased bioactivity and binding affinity that was correlated to the number of labeled lysine residues.

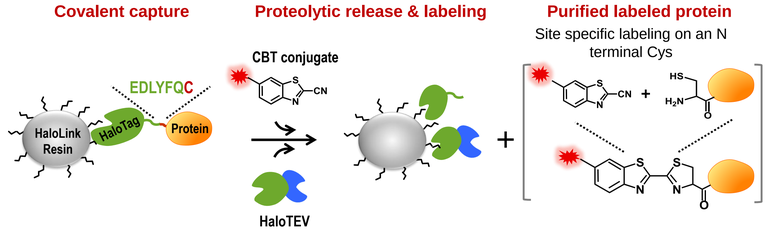
Here, we used these site specifically labeled growth factors and their cognate receptor tyrosine kinases (RTK), which are important drug targets in cancer biology, to demonstrate the applicability of this cell-based assay for determining blocking efficacy of numerous therapeutic and research antibodies. This homogenous assay can be implemented as an early screening tool of biologics blocking efficacy and should be able to significantly advance antibody discovery work flows.

2. Measuring Antibody Blocking Efficacy by NanoBRET™



Highly specific: specificity governed by tight distance constraints (<5nM)
Signal strength: combination of NanoLuc® high emission intensity and a proper fluorophore
Live-cell assay: binding interactions are interrogated in a relevant cellular context
Homogeneous screen for blocking efficacy of therapeutic antibodies

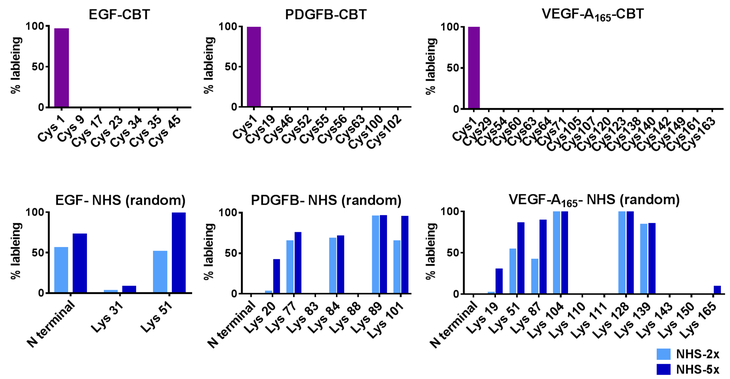
3. Site Specific Labeling of Receptors Ligands



Streamlined strategy for protein purification & labeling

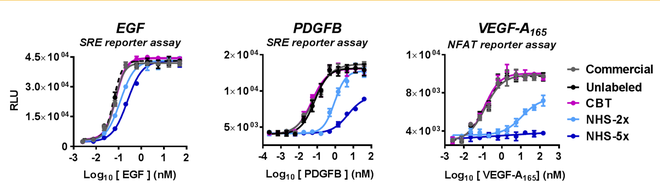
- **HaloTag® purification for efficient capture**
- **2-cyanobenzothiazol (CBT) for rapid chemical condensation**
- **Single-step purification of proteins that are labeled on an N-terminal cysteine residue.**

4. LC-MS/MS Analysis of Labeled Growth Factors



- **CBT labeling is site specific and quantitative (≥ 95% labeling)**
- **Random labeling is heterogeneous**

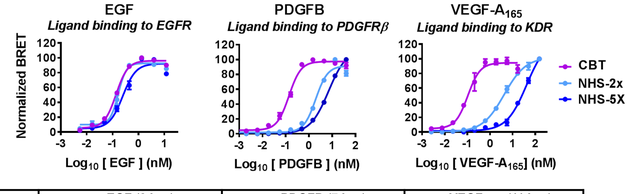
5. Influence of Labeling on Bioactivity



	EGF (2 Lys)		PDGFB (7 Lys)		VEGF-A_{165} (11 Lys)		
Labeling	EC50 (nM)	Reduced affinity	EC50 (nM)	Reduced affinity	EC50 (nM)	Reduced affinity	
Commercial	0.06		0.07		0.10		0.01 - 0.1nM
Unlabeled	0.07	—	0.07	—	0.13	—	
CBT	0.07	—	0.05	—	0.12	—	
NHS-2X	0.12	2 X	0.90	13 X	11.0	100 X	0.1 - 1.0nM
NHS-5x	0.25	4 X	5.00	71 X	N.D.	N.D.	1.0 - 10nM

- **Site-specific CBT labeling has no influence on bioactivity.**
- **Random labeling leads to significant influence on bioactivity, which is correlated with the labeling degree**

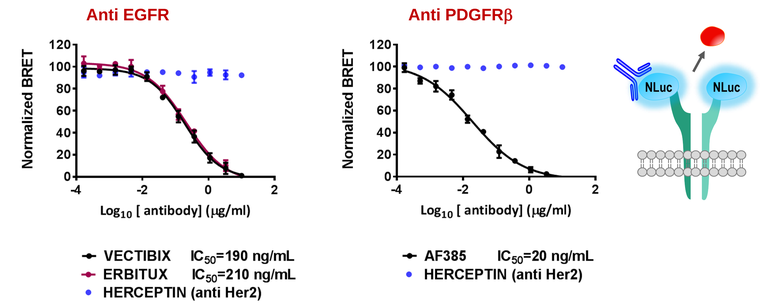
6. Influence of Labeling on Ligand Binding



	EGF (2 Lys)		PDGFB (7 Lys)		VEGF$_{165}$a (11 Lys)		
Labeling	EC50 (nM)	Reduced affinity	EC50 (nM)	Reduced affinity	EC50 (nM)	Reduced affinity	
CBT	0.14		0.14		0.1		0.1 - 1.0nM
NHS-2X	0.17		2.00	14 X	4.0	40 X	1.0 - 10nM
NHS-5x	0.25	2 X	7.00	50 X	42.0	400 X	10 - 100nM

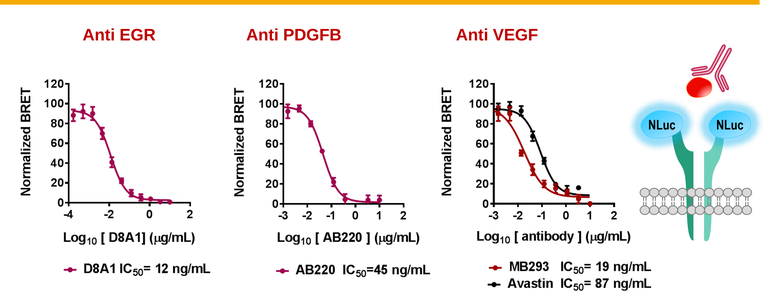
- **Site-specific CBT labeling has no influence on binding affinity**
- **Random labeling leads to significant influence on binding affinity, which is correlated with the labeling degree**

7. Measuring Blocking Efficacy of Anti-Receptor Antibodies



- The therapeutic antibodies VECTIBIX and ERBITUX & research antibody AF385, specifically blocked the binding of CBT labeled EGF and PDGFB with their cognate receptor
- Herceptin an anti-HER2 antibody used as a negative control, as expected did not block these interactions

8. Measuring Blocking Efficacy of Anti-Ligand Antibodies



- The research antibodies D8A1, AB220 & MB293 and the therapeutic antibody Avastin specifically blocked the binding of CBT labeled EGF, PDGFB and VEGF–A_{165} with their cognate receptor.

9. Conclusions

Homogeneous NanoBRET™ assay to quantitate antibodies blocking efficacy

- **Cell-based assay:** cell surface receptor-ligand interactions are interrogated in a relevant cellular context
- **Rapid quantification:** direct measurements of antibodies capacity to block the interaction between a fluorescently labeled ligand and its cognate receptor that is genetically fused to NanoLuc®
  - **NanoLuc®:** small and exceptionally bright
  - **Labeled ligands:** labeled on a single N-terminal cysteine residue without any significant influence on their biological activity

The NanoBRET™ assay can be implemented as an early screening tool of biologics blocking efficacy

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