TagLite technology: adding a new level to drug discovery analysis

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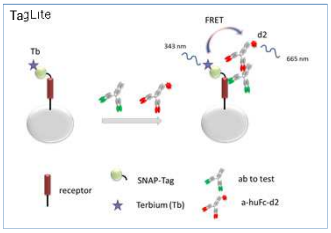
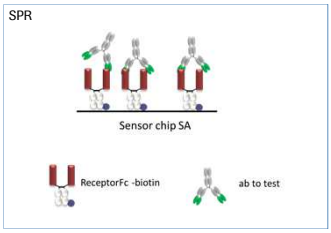
ABSTRACT

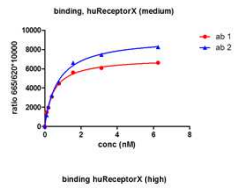
Antibody-based drugs currently represent one of the largest and fastest growing areas of protein therapeutics. This, along with the advent of protein engineering, has resulted in more potent and complex molecules being produced with enhanced effector functions, better affinity and safety, as well as improved targeting and greater efficacy. With the generation of such complex molecules comes the need for more stringent analytical assays in order to better characterize their potential as drug candidates. One important requirement of such an assay is the ability to determine the binding efficiency of an antibody to an antigen present on the cell surface, thus allowing for a more physiologically relevant assessment of the candidate than surface plasmon resonance (SPR) analysis alone. The introduction of the new TagLite technology by Cisbio now offers the opportunity to reliably and reproducibly measure protein-protein interactions on a living cell in an easy and user-friendly way. Furthermore, this technology presents a significant advantage over SPR and fluorescence activated cell sorting (FACS) in that it allows for the measurement of binding to cell surface targets in their natural, biological environment and native quaternary structure. Moreover, in contrast to FACS, no washing steps are required thus enabling the identification of low affinity binding partners. We demonstrate here the value of such a technology in helping to identify the best drug candidates through binding kinetics (KD determination, EC50) of antibodies to antigens on transfected cells, as well as epitope binning and ligand competition.

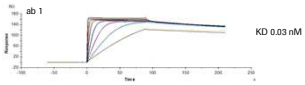
Binding assays

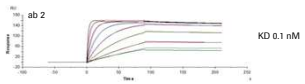
KD determination: TagLite versus SPR (Biacore)

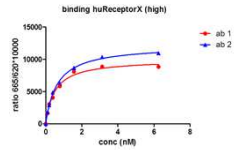








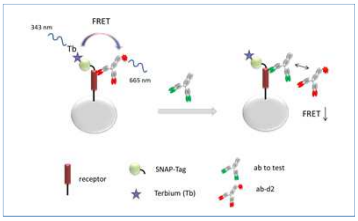


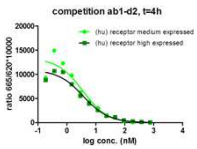


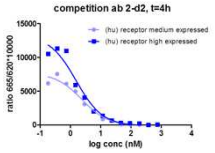
	TagLite		SPR	
	ab 1	ab 2	ab 1	ab 2
huReceptorX (medium expression)				
Kd (nM)	0.470	0.721	0.030	0.100
R square	0.997	0.998		
huReceptorX (high expression)				
Kd (nM)	0.496	0.633		
R square	0.991	0.997		

- Both binders bind with a similar avidity to the receptor.
- No difference between medium and high expressor cells.

Ki determination: competition assay versus binding assay (anti-huFc-d2)



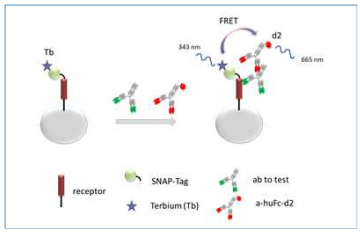


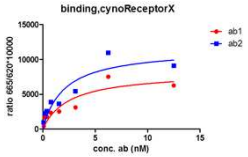


	ab 1-d2	ab 2-d2
(human) receptor medium expressed:		
Ki (nM)	2.820	1.324
R square	0.928	0.977
(human) receptor high expressed:		
Ki (nM)	2.950	0.855
R square	0.973	0.969

- Also in the competition assay very similar avidity was observed confirming the binding results.
- Due to the lack of a detection ab this format is superior to the binding assay format but requires the labeling of each ab.

Species cross reactivity (human versus cynomolgus)



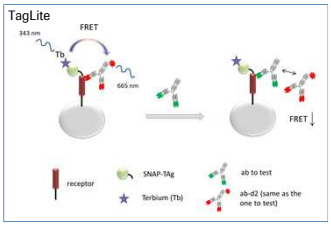


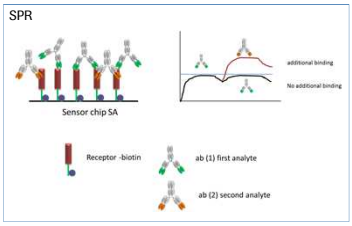
	ab 1	ab 2
(cynomolgus) receptor :		
Ki (nM)	2.503	2.076
R square	0.8237	0.8502

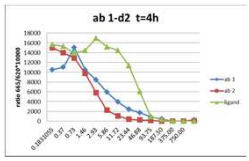
- Both binders cross react with cynomolgus.

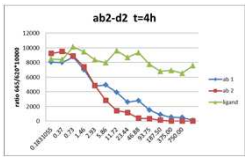
Epitope binning

Epitope binning









TagLite

bound first	ab 1	ab 2	ligand
ab 1	0	0	0
ab 2	0	0	1
0	cannot bind in addition		
1	can bind in addition		

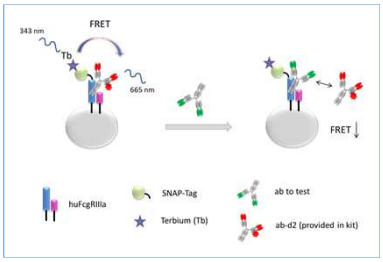
Biacore

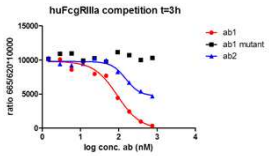
injected first	ab 1	ab 2	ligand
ab 1	0	0	0
ab 2	0	0	1
0	cannot bind in addition		
1	can bind in addition		

- Both antibodies compete each other suggesting that they recognise the same epitope but:
- Ab 1 (ligand blocking)
- Ab 2 (non-ligand blocking)
- They might recognise an overlapping epitope or steric hindrance occurs when one ab is bound.
- SPR and TagLite give the same results

FcR binding

HuFcgRIIIa binding TagLite (Fc knock out versus wt)





	ab 1	ab 2
IC50	91.28	151.9
R square	0.9818	0.9625

- All binders with wtFc bind to huFcgRIIIa with ab 1 showing a greater competition than the ab2.
- The Fc binding knock-out mutant does not show any FcgRIIIa binding as expected.

CONCLUSIONS

- **Avidity measurements can be carried out in two different formats:**
  - *1) detection with anti-huFc-d2*
  - → advantage: no additional labeling required
  - → disadvantage: bivalent binding of the detection ab might add an additional avidity effect
  - → saturation might not be reached due to conc. limitation of the ratio ab:detection ab
  - *2) competition assay*
  - → advantage: low affinity binders can be measured as high conc. of ab can be used
  - → disadvantage: same binder or binder recognising the same epitope has to be labeled.
  - →Both assays represent a useful tool to characterise the binding of antibodies to their antigen in a physiological environment
- **Species cross-reactivity:**
  - → Comparison of cross-reactive binding can be easily addressed.
- **Epitope binning:**
  - → the assay allows the determination of epitope bins in a physiological environment
  - → the results were in line with SPR results
- **FcR binding:**
  - → FcR binding kits provide an excellent and easy to use tool for FcgR binding and effector function prediction
  - → Also FcR abrogating mutants can be easily characterised in such a setting.
  - →The TagLite technology adds a new level to drug discovery technology by allowing an easy and quick characterisation of binders in a physiological environment.