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Label-free, real-time biosensors for biomolecular interactions in complex samples **– Determine kinetics in crude samples and directly on the cell surface with Attana QCM!**

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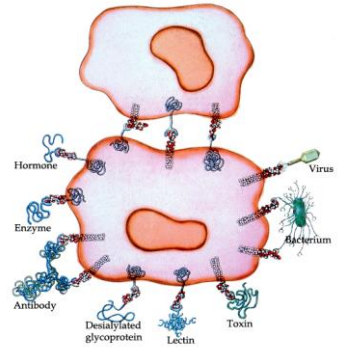
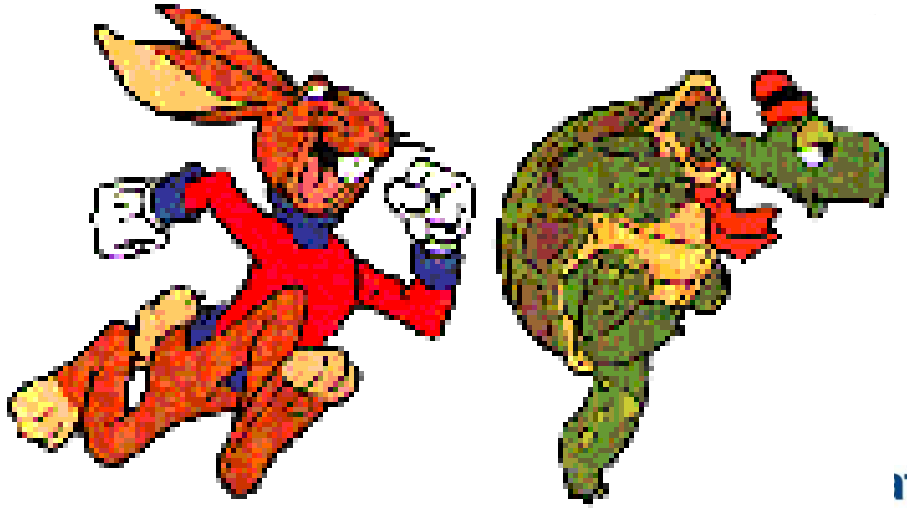
A novel tool for characterization of molecular interactions on cell surfaces

Combined biochemical and cell based kinetic antibody characterization bridges the gap between biochemical analyses and early in vivo animal pharmacology

Teodor Aastrup, PhD
Executive Vice President and co-founder Attana AB



- **Topics**
  - Quartz Crystal Microbalance QCM
  - Kinetics *vs.* equilibrium
  - Cell based *vs.* biochemical assays
  - Summary



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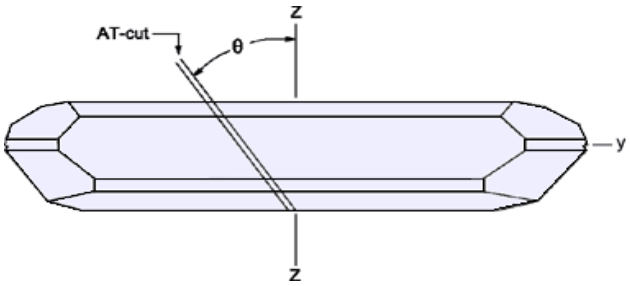
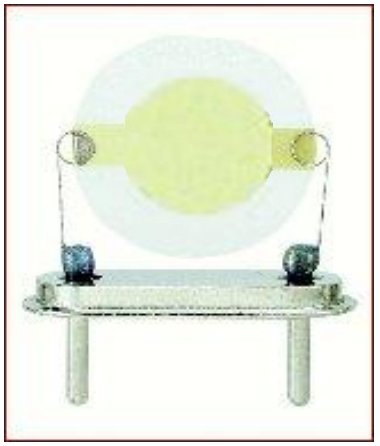
Quartz Crystal Microbalance QCM

Quartz



AT-cut



Sensor Crystal

Sensor Chip

QCM – found in phones, watches and computers

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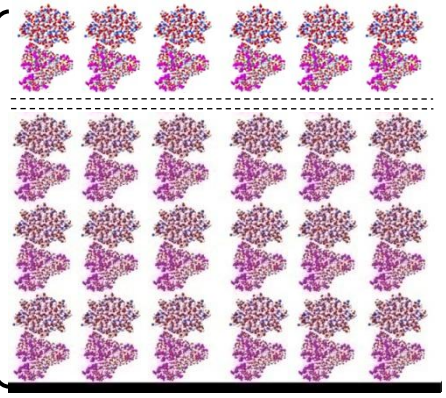
Attana QCM proximity independent

> 2 µm

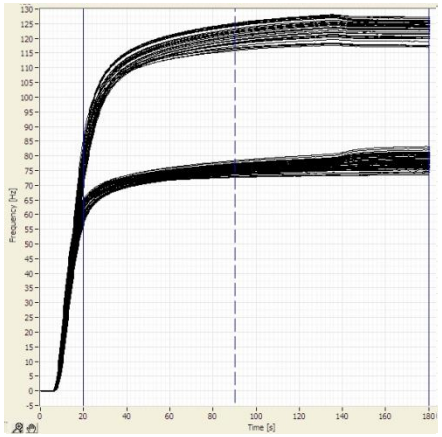
> 300 injections



Bt-BSA

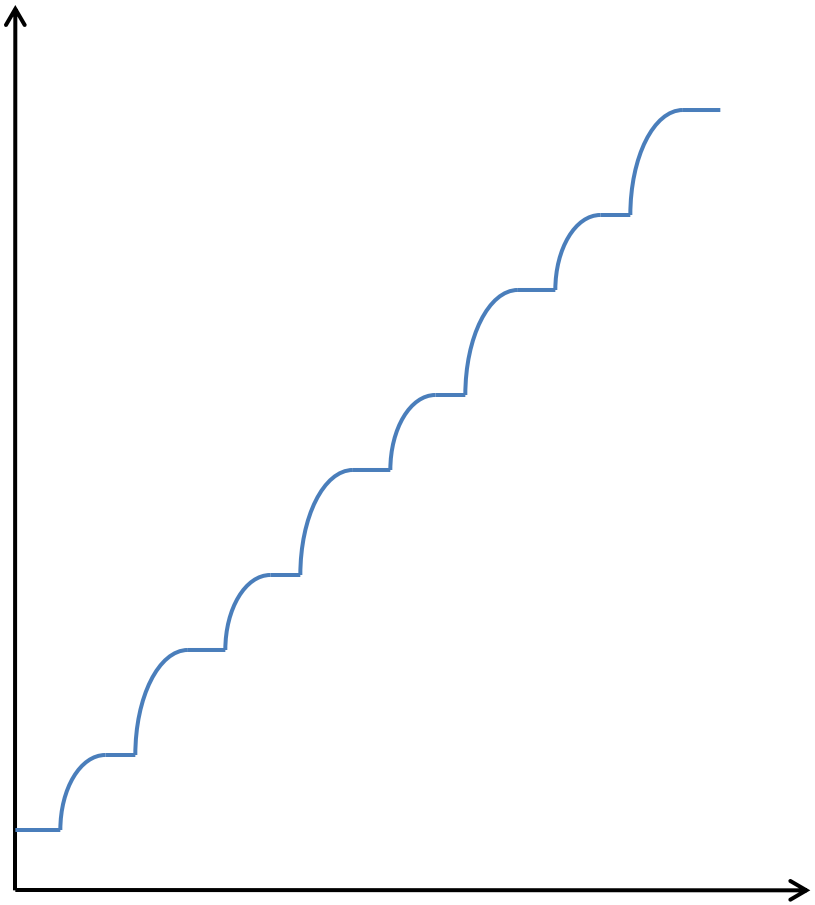
Streptavidin

Biotin surface



Bt-BSA

Streptavidin



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Affinity determination dependent on technology

System	Biochemical affinity /nM	Cell based affinity /nM	Affinity ration
Erbicin	50	5	10
ERB-hRNase	30	4,5	7
ERB-hcAb	7	1	7
Herceptin	0.1	5	0.02
Z_{wt}/IgG	92	2.1	43
Z_{N28A}/IgG	104	2.8	37
Z_{K35A}/IgG	169	3.7	46

"Differential binding of human immunoagents and Herceptin to the ErbB2 receptor"
Troise et al. FEBS Journal 275 (2008) 4967–4979 a 2008 The Authors Journal compilation a 2008 FEBS
"Evaluation of Staphylococcal Cell Surface Display and Flow Cytometry
for Postselectional Characterization of Affinity Proteins in
Combinatorial Protein Engineering Applications"
Löfbom et al. APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2007, p. 6714–6721 Vol. 73, No. 21

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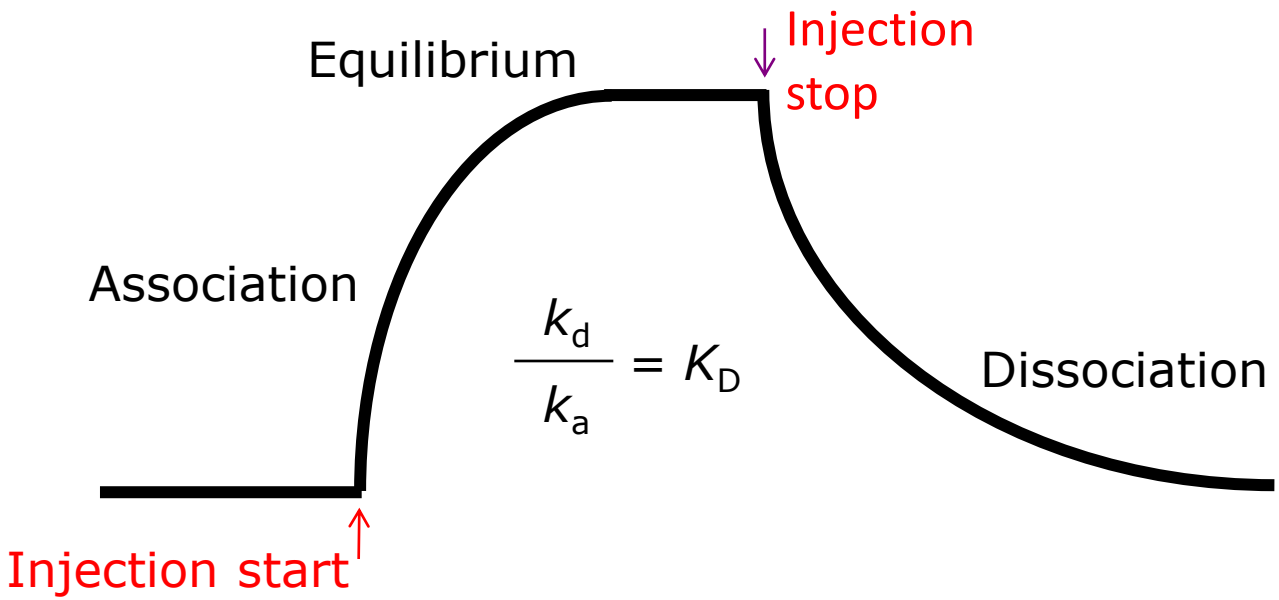
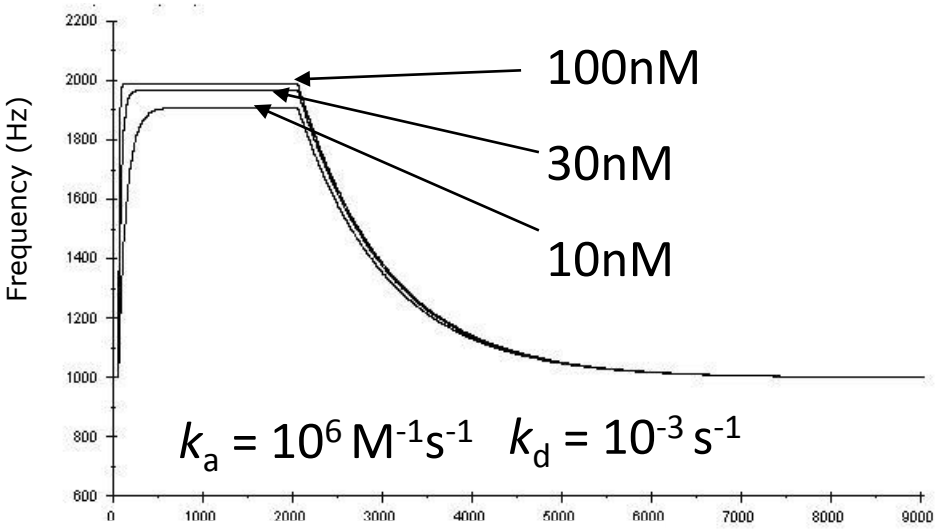
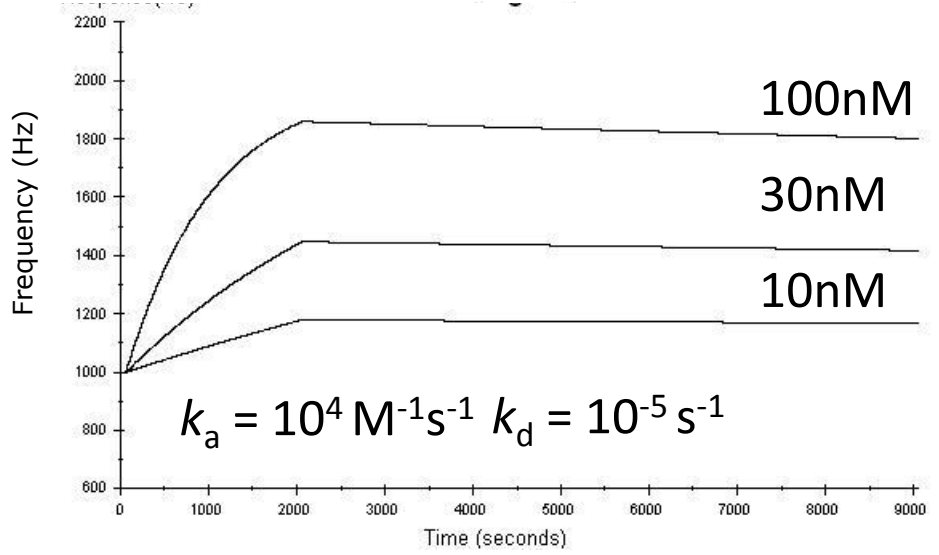
Kinetics *vs.* equilibrium: Kinetic rate constants and affinity

$$A+B \xrightarrow{k_a} AB \qquad A+B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB \qquad A+B \xleftarrow[k_d]{} AB$$



Kinetics *vs.* equilibrium: Importance of kinetic profile for application





Which compound is the best choice for use as:

a) Cancer treatment?

b) Sleeping pill?

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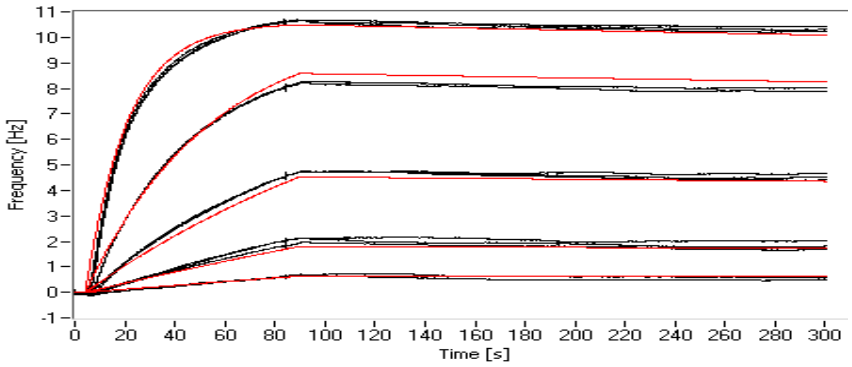
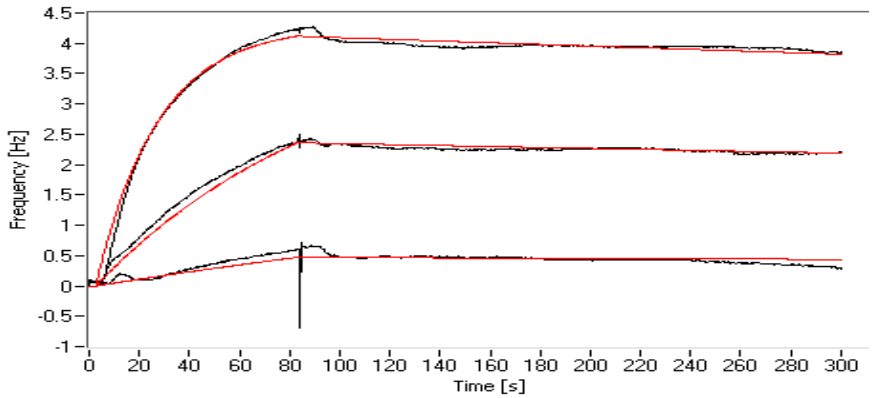
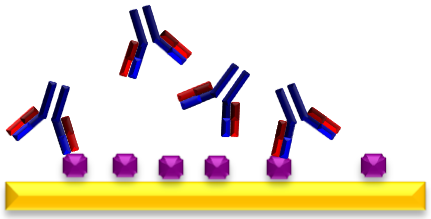
Kinetics *vs.* equilibrium: Importance of kinetic profile for application



k_a= 3.34E^5 M^{-1} s^{-1}
k_d= 1.19E^{-4} s^{-1}



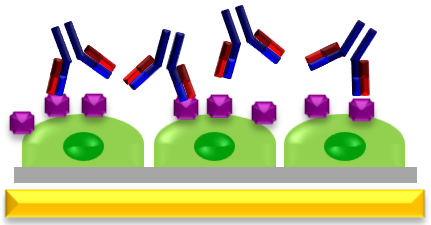
k_a= 1.3E^5 M^{-1} s^{-1}
k_d= 3.2E^{-4} s^{-1}



Biochemical assay

K_D= 0.36 nM

Biochemical Her2-Traztuzumab			Cell-based Her2-Traztuzumab		
k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
3.34E^{+5}	1.19E^{-4}	0.36E^{-9}	1.3E^{+5}	3.2E^{-4}	2.4E^{-9}



Cell based

K_D= 2.4 nM

Lower affinity by low accessibility of receptors

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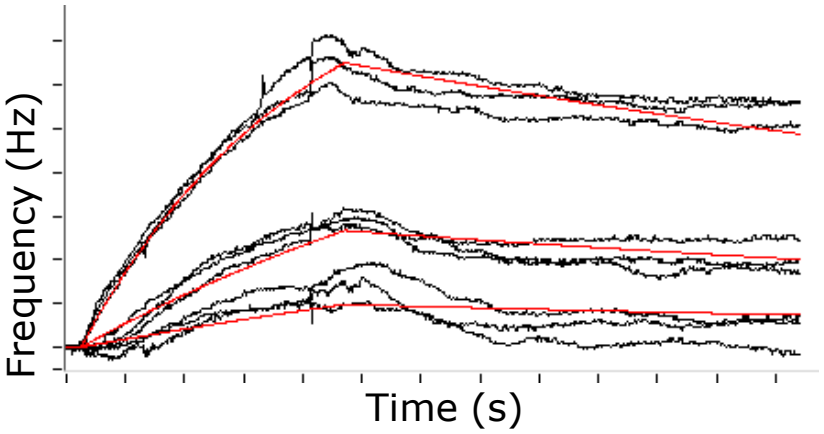
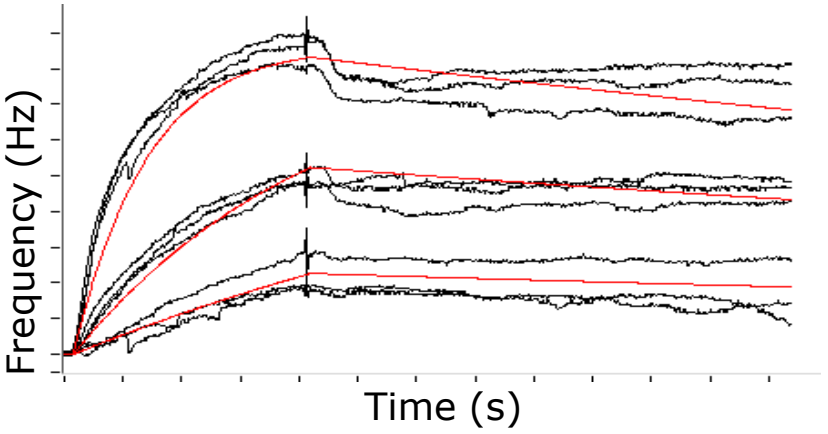
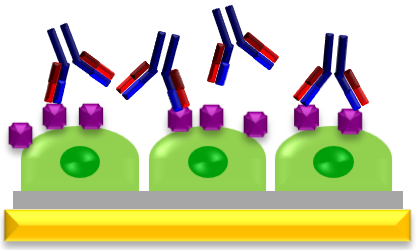
Cell based vs. biochemical assays: Dependence of environment



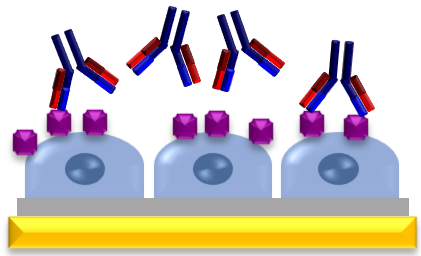




BT474

K_D= 21 nM

BT474 Her2-Traztuzumab			SKOV Her2-Traztuzumab		
k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
$9.1E^{+4}$	$1.9E^{-3}$	$21E^{-9}$	$3.2E^{+5}$	$1.2E^{-3}$	$3.9E^{-9}$



SKOV

K_D= 3.9 nM

Affinity dependent on accessibility of receptors

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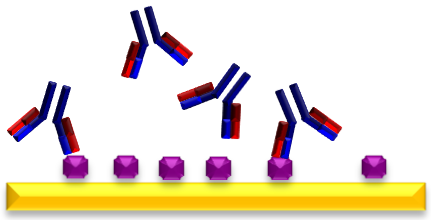
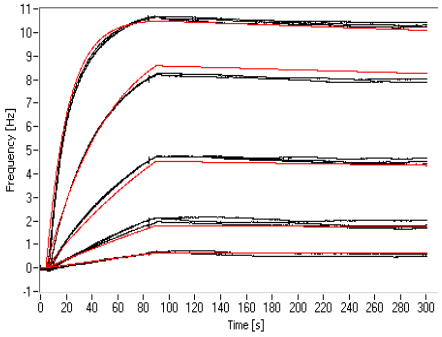
Cell based vs. biochemical assays: Off-target interactions

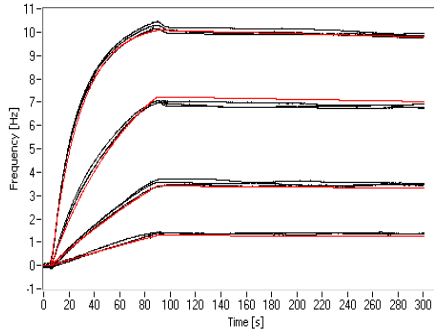


Biochemical assay

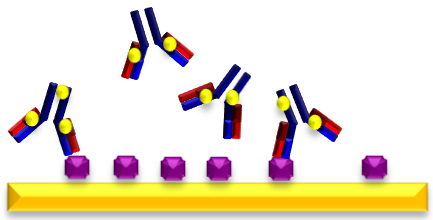
K_D= 0.36 nM



k_a= 3.34E^5 M^{-1} s^{-1}
k_d= 1.19E^{-4} s^{-1}

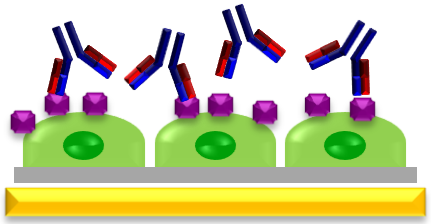


k_a= 7.28E^5 M^{-1} s^{-1}
k_d= 2.27E^{-4} s^{-1}



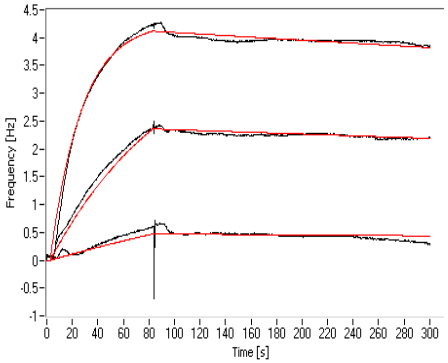
Biochemical assay

K_D= 0.31 nM

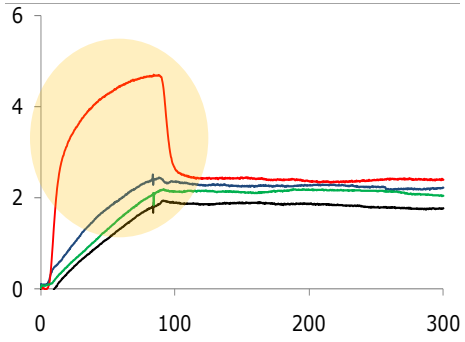


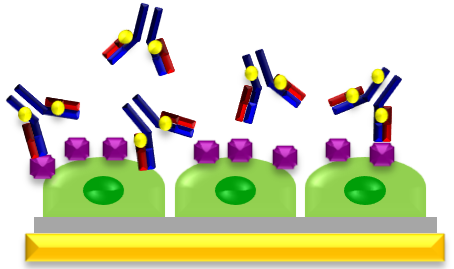
Cell based assay

K_D= 2.4 nM



k_a= 1.3E^5 M^{-1} s^{-1}
k_d= 3.2E^{-4} s^{-1}





Cell based assay

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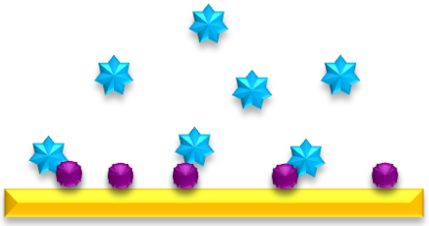
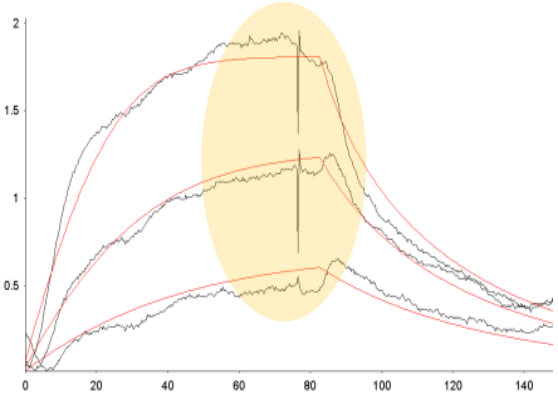
Cell based vs. biochemical assays: Clustering of receptors

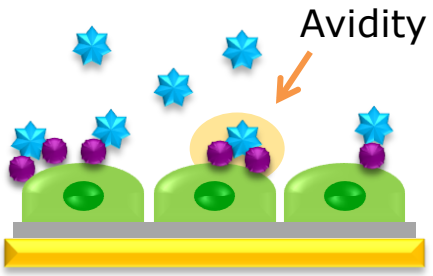


Biochemical assay

K_D: 1µM

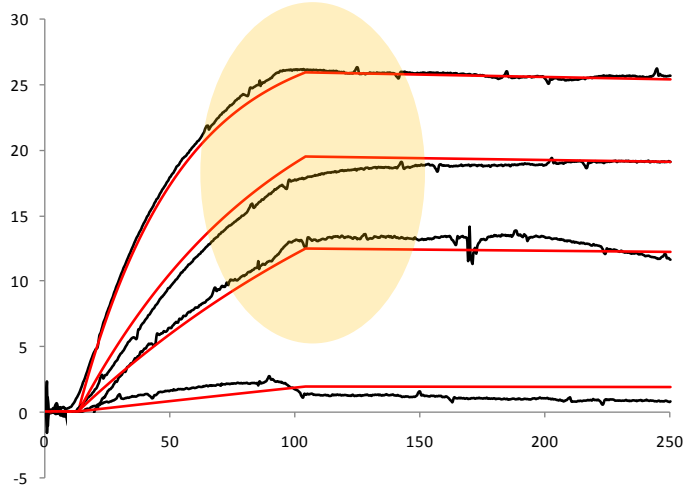


k_a= 2.5E^4 M^{-1} s^{-1}; k_d=2.6E^{-2} s^{-1}



Cell based assay

K_D: 13 nM



k_a= 8.9 E^3 M^{-1}s^{-1}; k_d= 1.2E^{-4} s^{-1}

100 times slower dissociation rate on cells than biochemical thanks to avidity

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Acknowledgement

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All co-workers at Attana AB, Stockholm

Dr. Sture Lindgren and co-workers at Sahlgrenska Academy, Göteborgs universitet, Gothenburg

Summary

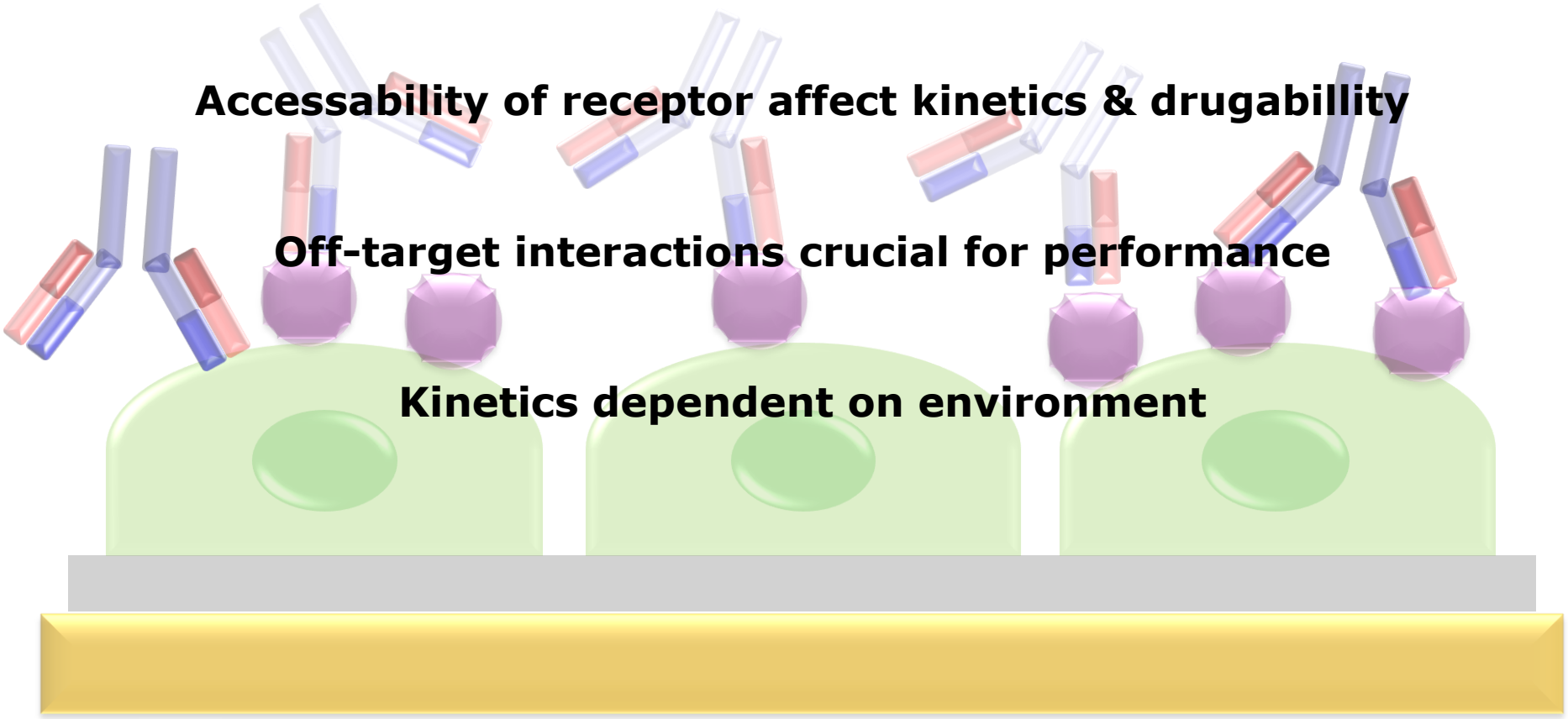
Full binding profiling enables new and important information

Full binding profile reveal interaction dynamics

Accessability of receptor affect kinetics & drugabillity

Off-target interactions crucial for performance

Kinetics dependent on environment



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