Biosensor Technologies: SPR, BLI and DNA Nanolevers 2019

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Biosensors




- Biophysics has a range of instruments based on biosensors
- Biosensors detect the interaction between a macromolecule attached to a solid chip surface (ligand) and a macromolecule in solution (analyte)
- Different physical changes at the surface can be used to monitor the interaction

Range of Experiments

- Is there an interaction?
  -binding partners
- Equilibrium analysis
  - determination of dissociation constant (K_d)
  - mutational analysis
- Kinetic rate analysis
  - determination of the on- and off- rate constants (k_a and k_d) to understand the dynamics of the system
- Size analysis?
  - determination of the diameter of the interacting partner

What is the relevance of binding kinetics?

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

$$K_d = \frac{k_d}{k_a}$$

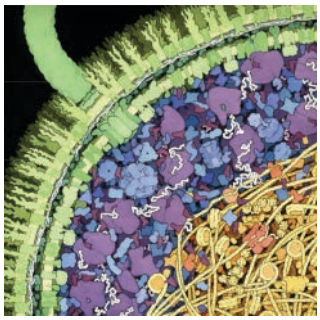
- The cell is a dynamic system
- On-rates are concentration dependent

The level of binding is not just related to the affinity

- Off-rates are independent of concentration

  Related to half-life

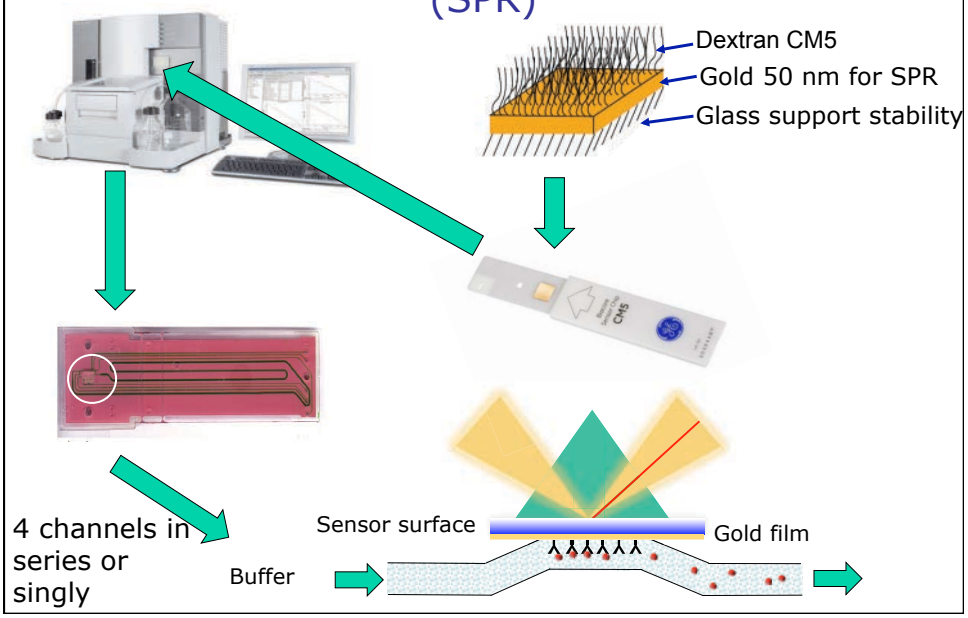
- The same affinity can be resolved into different on and off rates for different interactions - dissect different mechanisms



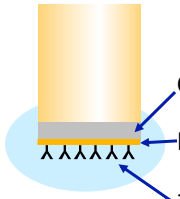
Biosensors

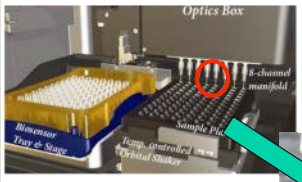
dynamic BIOSENSORS
PLEXERA
Excellence in Surface Plasmon Resonance BioNavis
BIO-RAD
PALL ForteBio
NanoSPR DEVICES
sensiQ TECHNOLOGIES
nicoya LIFESCIENCES
HORIBA Scientific
BIOPTIX
Bi Biosensing Instrument
BIACORE
Affinité Instruments
Reichert TECHNOLOGIES Life Sciences
AMETEK ULTRA PRECISION TECHNOLOGIES
Q-Sense
sensia

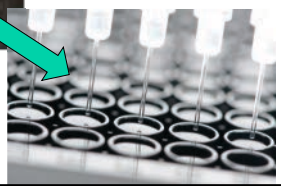
Format: Biacore-Surface Plasmon Resonance (SPR)

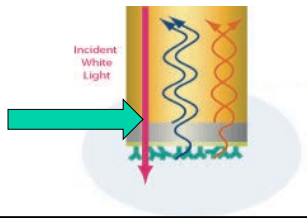


Format: Octet - Bio-Layer Interferometry (BLI)

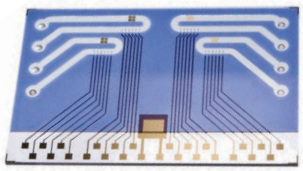


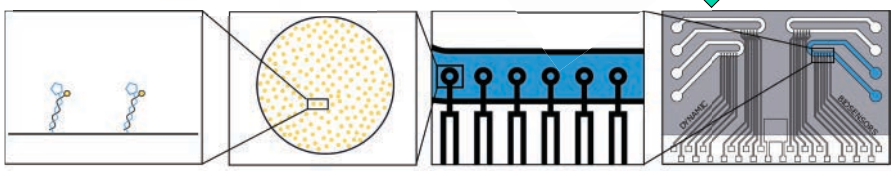






Format: switchSense- DNA-nanolevers





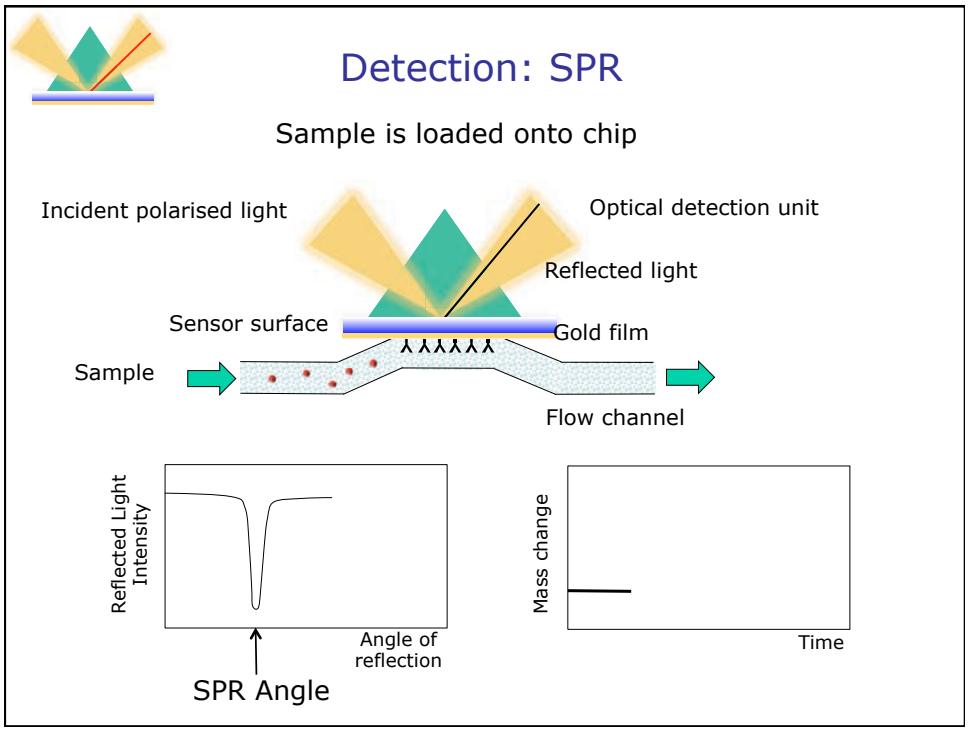
Nanolever with fluorescent dye and functionalized complementary DNA

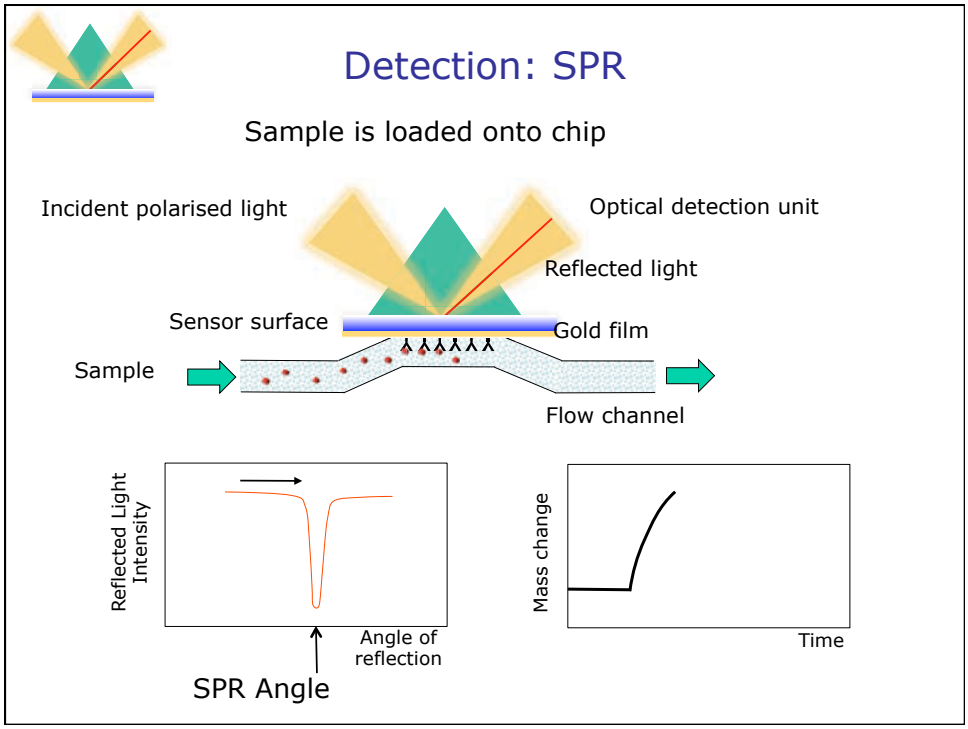
Detection spot with ~ 10^4 - 10^6 nanolevers per 0.01 mm²

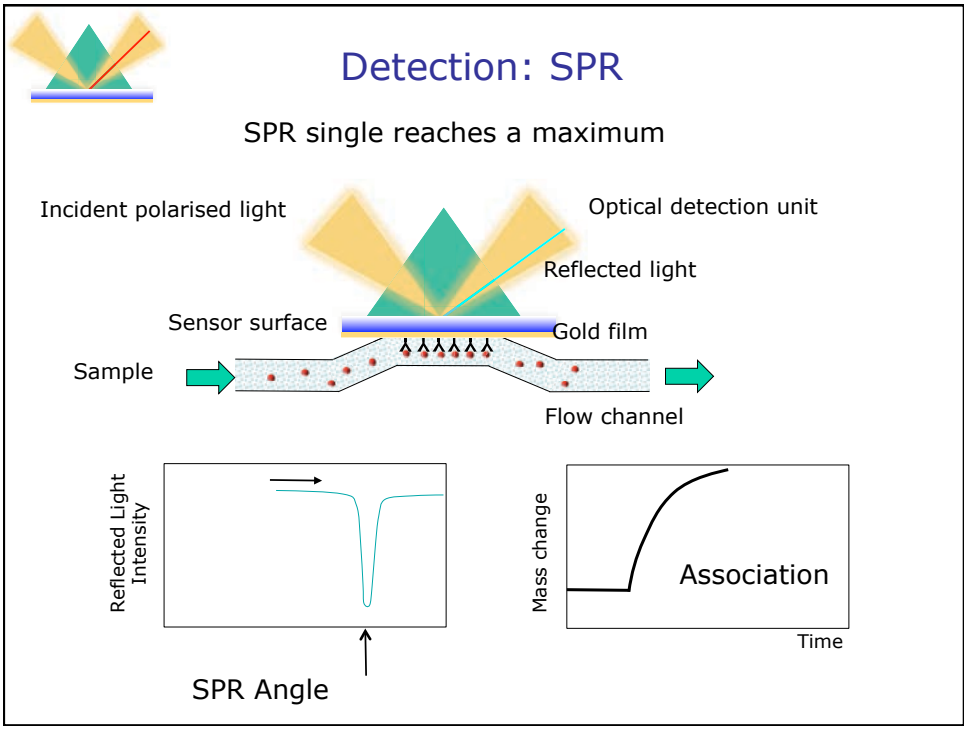
6 microelectrodes in 1 flow channel

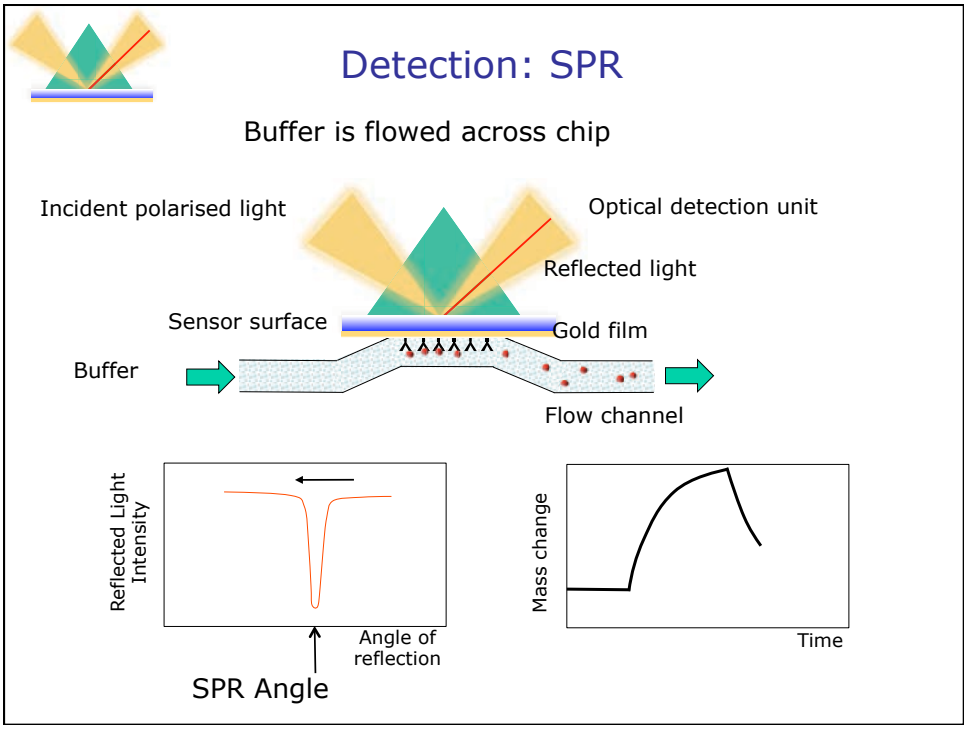
Biochip with 4 flow channels, each with multiple microelectrodes in series

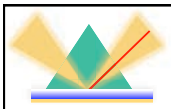
dynamic BIOSENSORS


Detection: SPR
Sample is loaded onto chip
Incident polarised light
Optical detection unit
Reflected light
Sensor surface
Gold film
Sample
Flow channel
Reflected Light Intensity
Angle of reflection
SPR Angle
Mass change
Time


Detection: SPR
Sample is loaded onto chip
Incident polarised light
Optical detection unit
Reflected light
Sensor surface
Gold film
Sample
Flow channel
Reflected Light Intensity
Angle of reflection
SPR Angle
Mass change
Time

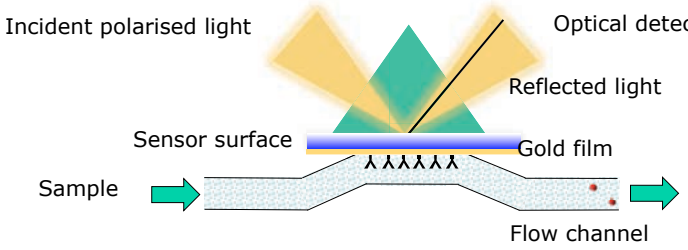

Detection: SPR
SPR single reaches a maximum
Incident polarised light
Optical detection unit
Reflected light
Sensor surface
Gold film
Sample
Flow channel
Reflected Light Intensity
Mass change
Association
Time
SPR Angle

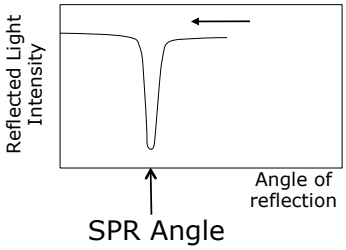

Detection: SPR
Buffer is flowed across chip
Incident polarised light
Optical detection unit
Reflected light
Sensor surface
Gold film
Buffer
Flow channel
Reflected Light Intensity
Mass change
Angle of reflection
Time
SPR Angle

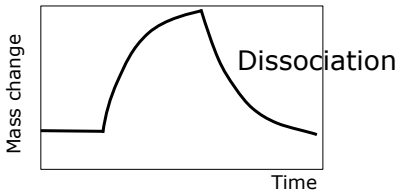


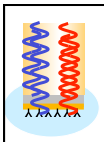
Detection: SPR

SPR signal drops to baseline level

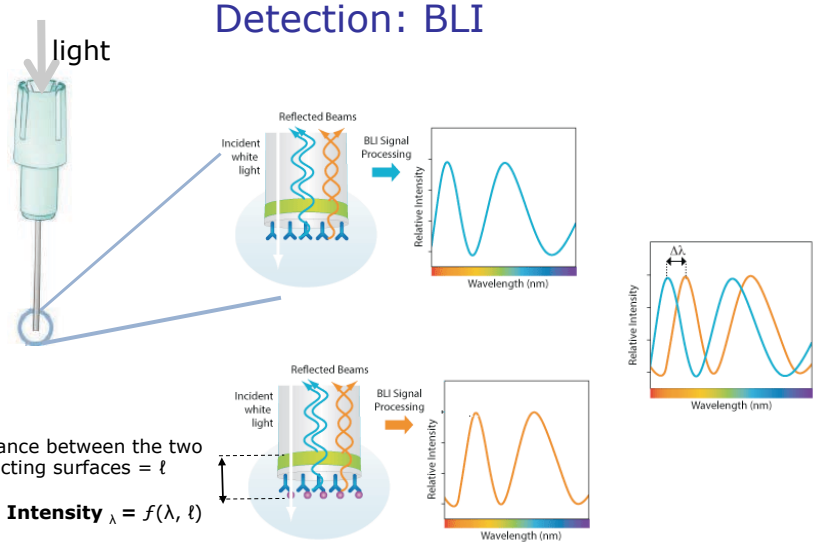


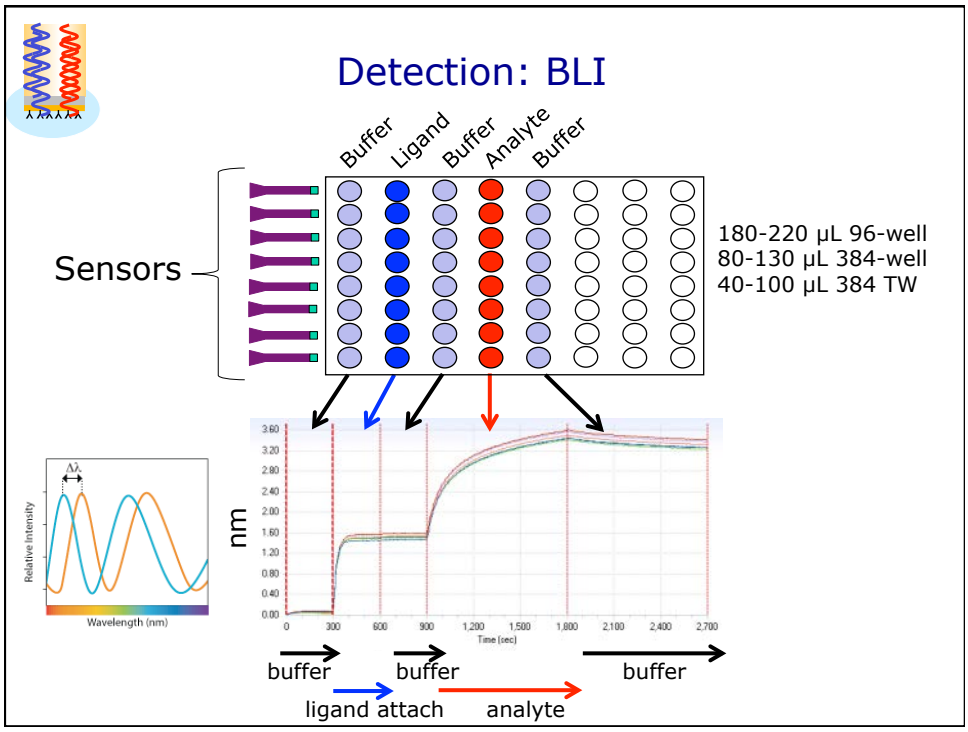


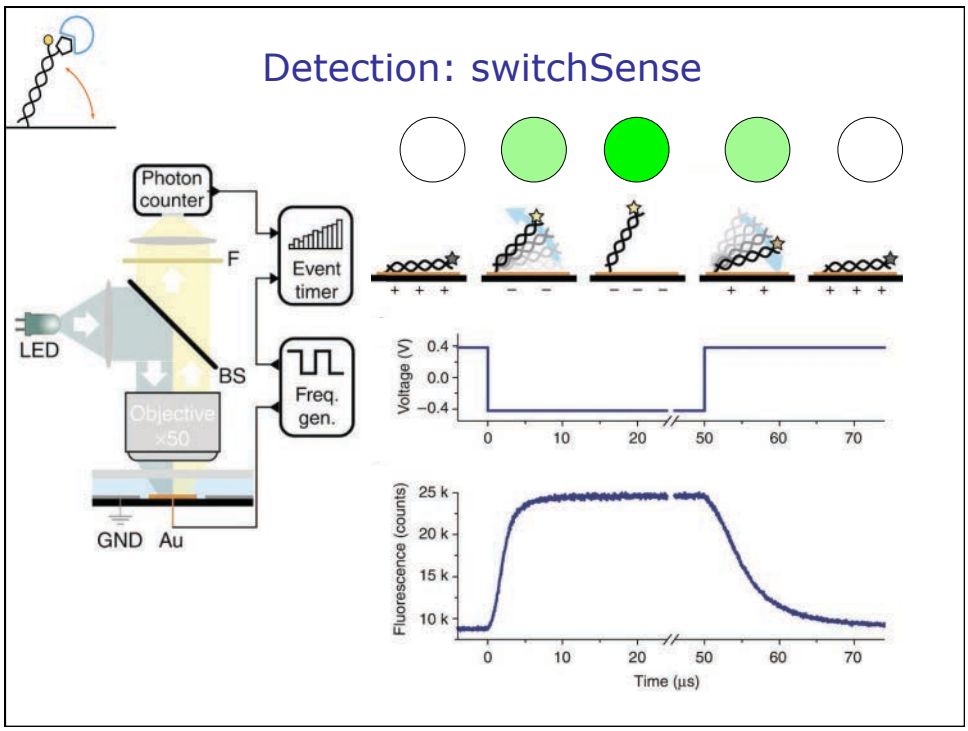


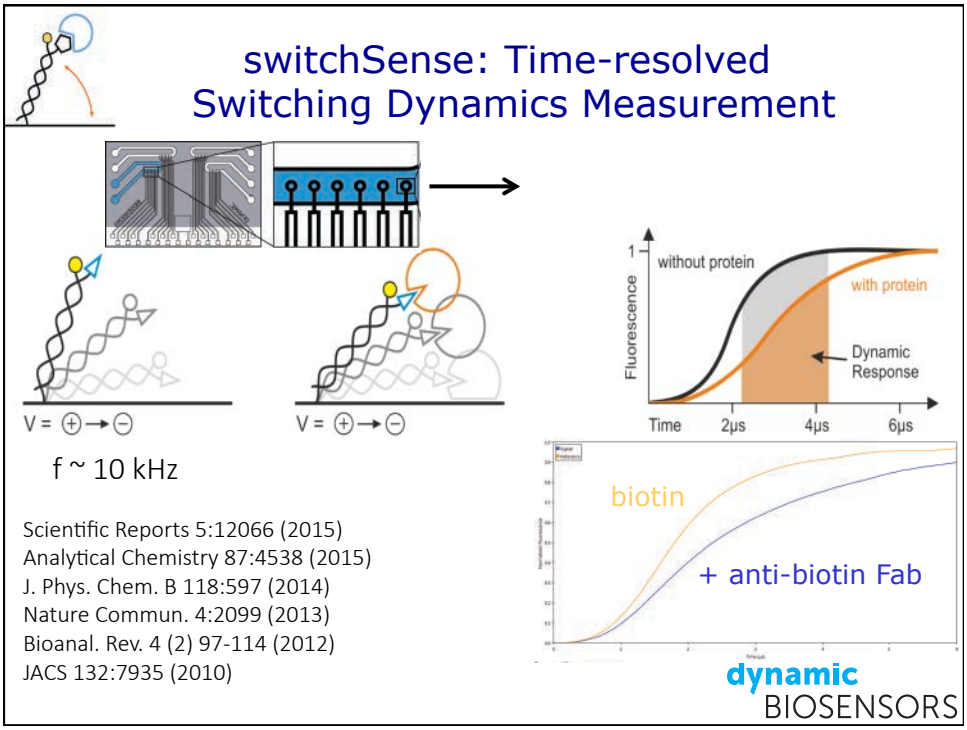


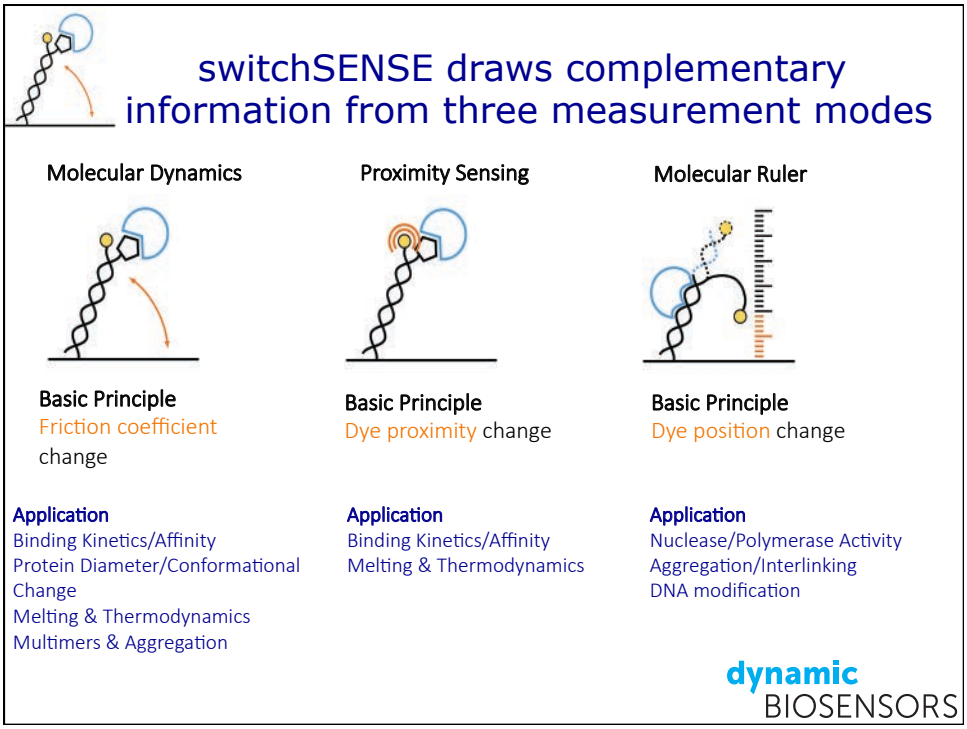
Detection: BLI



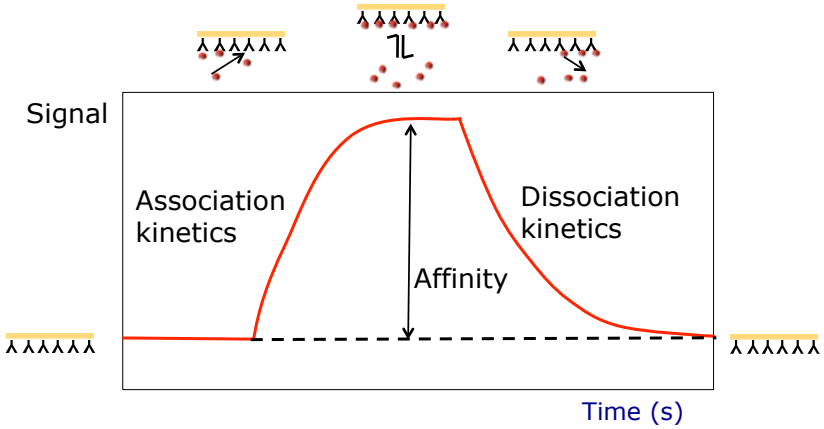

Detection: BLI
Buffer
Ligand
Buffer
Analyte
Buffer
Sensors
180-220 µL 96-well
80-130 µL 384-well
40-100 µL 384 TW
Relative Intensity
Wavelength (nm)
nm
Time (sec)
buffer
buffer
buffer
ligand attach
analyte


Detection: switchSense
Photon counter
F
Event timer
LED
BS
Freq. gen.
Objective ×50
GND
Au
Voltage (V)
Fluorescence (counts)
Time (µs)


switchSense: Time-resolved
Switching Dynamics Measurement
V = ⊕→⊖
V = ⊕→⊖
f ~ 10 kHz
Scientific Reports 5:12066 (2015)
Analytical Chemistry 87:4538 (2015)
J. Phys. Chem. B 118:597 (2014)
Nature Commun. 4:2099 (2013)
Bioanal. Rev. 4 (2) 97-114 (2012)
JACS 132:7935 (2010)
1
Fluorescence
without protein
with protein
Dynamic
Response
Time
2µs
4µs
6µs
biotin
+ anti-biotin Fab
dynamic
BIOSENSORS


switchSENSE draws complementary
information from three measurement modes
Molecular Dynamics
Basic Principle
Friction coefficient
change
Application
Binding Kinetics/Affinity
Protein Diameter/Conformational
Change
Melting & Thermodynamics
Multimers & Aggregation
Proximity Sensing
Basic Principle
Dye proximity change
Application
Binding Kinetics/Affinity
Melting & Thermodynamics
Molecular Ruler
Basic Principle
Dye position change
Application
Nuclease/Polymerase Activity
Aggregation/Interlinking
DNA modification
dynamic
BIOSENSORS

Biosensor Kinetic Data



SPR: Resonance units : 1000 RU = 1 ng of bound protein/mm^2 of chip surface area
1 RU = 1 x 10^{-4}°

BLI: nm :wavelength shift and not size!

SwitchSense: rate of fluorescence recovery- dynamics of switching (dru)

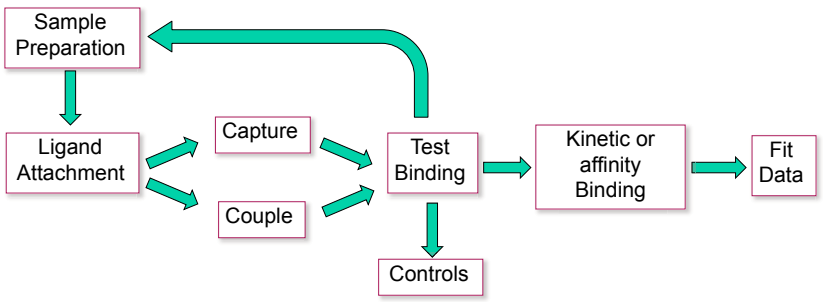
Comparison of Biosensors

	SPR	BLI	SwitchSense
Types of experiment	Affinity/ Kinetics	Affinity/Kinetics	Affinity/Kinetics/ Sizing/DNA enzyme kinetics/ conformation
Dynamic range	No limit ?	>150 Da	?
Affinities	pM to mM	10 pM – 1mM	50 fM – 1 mM
Association rates	10^3 –10^7 $M^{-1}s^{-1}$	10^2 –10^7 $M^{-1}s^{-1}$	10^3 – 10^8 $M^{-1}s^{-1}$
Dissociation rates	10^{-5} –1 s^{-1}	10^{-6} –10^{-1} s^{-1}	10^{-6} –1 s^{-1}
Temperature control	4-45 °C	Ambient to 40 °C	8-75 °C
Throughput	96 well plate	96 or 384 plate	96 well plate
Sizing accuracy	n.a.	n.a.	0.1 nm

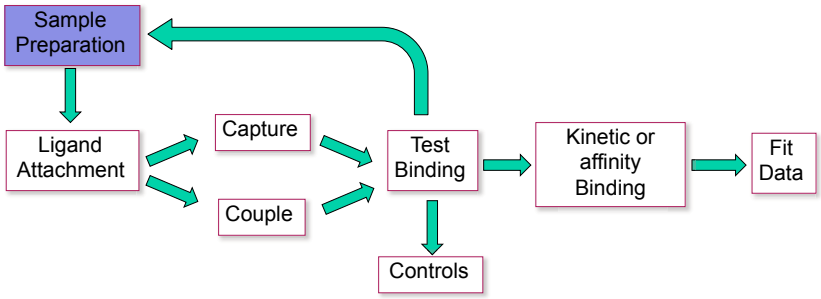
Comparison of Biosensors: Issues

- Cost: Capital cost and cost per experiment (chips *vs* sensors)
- Artifacts of immobilisation
  " God made the bulk; surfaces were invented by the devil" Wolfgang Pauli
- Non-specific binding (care with controls)
- Buffers: Biacore sensitive to RI changes, switchSense is salt sensitive
- Real limits to detection: response rate and total assay time
  - fast rates or very slow off-rates may not be accurately detected
  - switchSense can measure very long dissociations
- Fitting issues:
  - Some high-affinity interactions with slow dissociation rates can be difficult to analyse

Biosensor Workflow

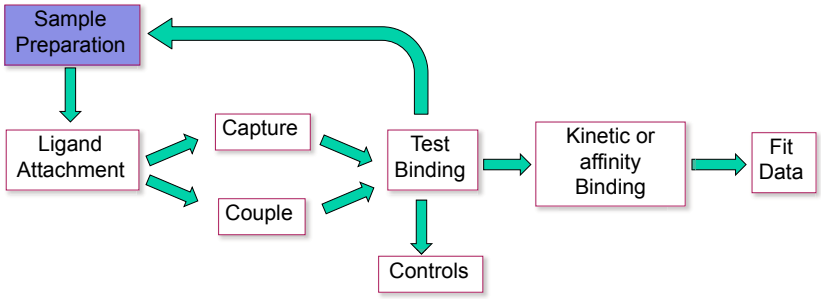


Sample preparation: Buffers

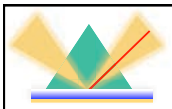


- Buffers ensure filtered and degassed (Biacore 2000)
- Compatible with system (salt affects switchSENSE switching)
- Avoid Tris-HCl if coupling *via* amides
- Detergent? DMSO?
- For SPR: match buffers for running buffer and analyte to minimise effect of refractive index changes

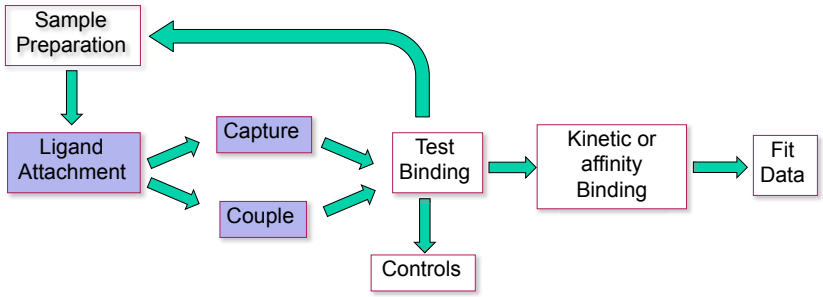
Sample preparation: ligand and analyte



- Protein aggregates removed: spin, SEC
- Concentrations accurate (UV, AAA)
- Ligand *vs* analyte? Ideally, compare binding interaction with either attached:
  - Economic, Specificity, Aggregation or Valency issues



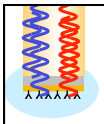
Sample preparation: how much ligand for SPR?



For kinetics, immobilise a low amount of ligand (R_{max} 100 – 200RU)
For equilibrium, immobilise enough to get good response.

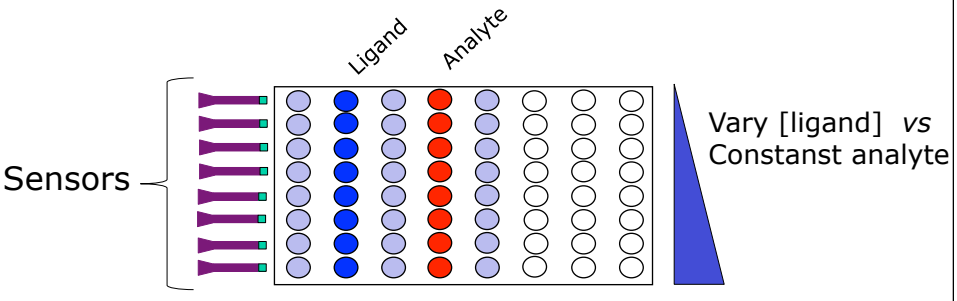
$$R_L = R_{\max} \bullet \frac{Mwt_L}{n \bullet Mwt_A}$$

R_L is amount ligand bound, R_{max} is the maximum response, n is stoichiometry, Mwt is the molecular weight of analyte A and ligand L

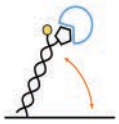


Sample preparation: how much ligand for BLI?

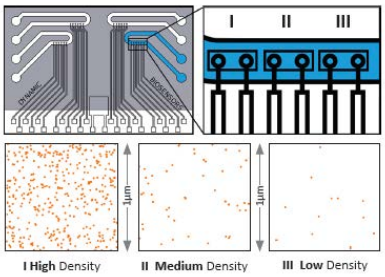
- BLI
  - Trial dilution series from 100 -200 nM ligand
  - Plate-based run in parallel
  - Use analyte concentration 80% for max response
  - Aim for up to 1 nm over 5 min

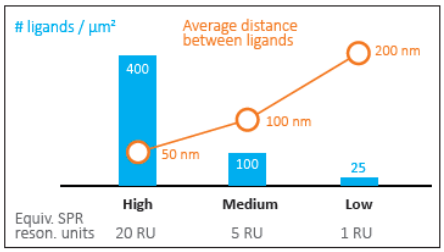


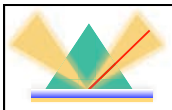
Sample preparation: how much ligand for switchSense?



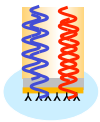
- SwitchSense
  - 100-500 nM DNA-coupled ligand
  - If avidity an issue need lower level
  - can vary by competition or reducing lever density by electrical desorption
  - use different density chips:

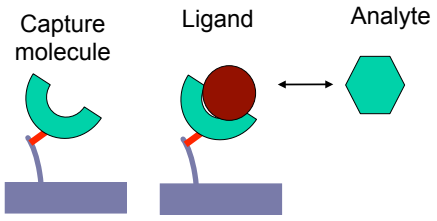






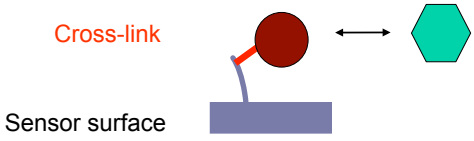
SPR/BLI: Ligand Attachment





Capturing Methods

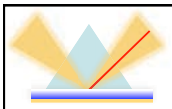
- Streptavidin for Biotin
- Specific Ab
- Anti-GST for GST
- NTA- for His-tagged proteins
- Protein A for IgG



Couple Methods

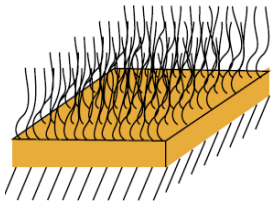
- Amine coupling
- Thiol coupling

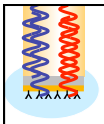
Advantages/Disadvantages?



SPR Immobilisation -CHIPS with Everything

Biosensor	Application
Immobilization	
• CM5	the standard chip
• C1	multivalent or very large analytes (flat carboxymethylated surface)
• CM3	large analytes (shortened dextran matrix)
• CM4	high non-specific binders (low degree of carboxylation)
• CM7	LMW analytes (denser x 3 immobilisation)
• PEG	alternative to dextran based surfaces, flat surface good for very large or multivalent binding partners
Affinity Tag Capture	
• SA	biotinylated ligands
• L1	lipid membrane components
• HP	hydrophobic for lipid membranes
• NTA	his-tagged proteins
Antibody-Specific Capture	
• Protein A	Fc region of antibodies
• Protein L	wide range of antibody fragments



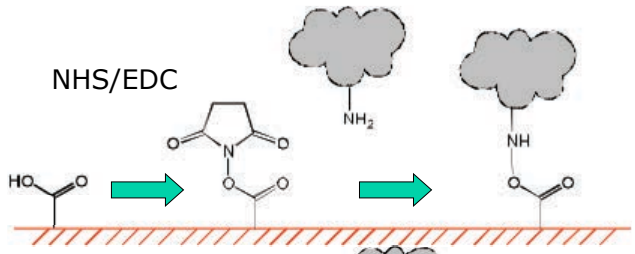


Octet Immobilisation Sensors with Everything

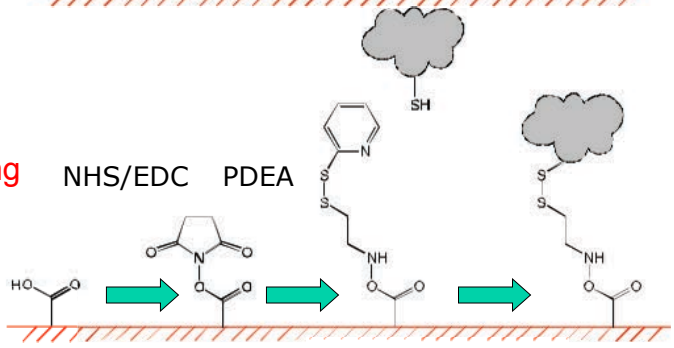
Biosensor	Application
Immobilization	
• Amine Reactive 2nd Gen (AR2G)	Covalent coupling to reactive amine groups
• Aminopropylsilane (APS)	Adsorption to hydrophobic moieties
Affinity Tag Capture	
• Streptavidin (SA)	Biotinylated ligands
• Super Streptavidin (SSA)	Biotinylated ligands (high-density surface)
• Anti-FLAG (FLG)	FLAG-tagged recombinant proteins
• Anti-GST (GST)	GST-tagged recombinant proteins
• Anti-Penta HIS (HIS)	HIS-tagged recombinant proteins
• Anti-Penta HIS 2nd Gen (HIS2)	HIS-tagged recombinant proteins
• Ni-NTA (NTA)	HIS-tagged recombinant proteins
Antibody-Specific Capture	
• Anti-Human IgG Fc Capture (AHC)	Human IgG Fc region, kinetic analysis
• Anti-Human IgG Fc Capture (AHQ)	Human IgG Fc region, quantitation
• Anti-Mouse Fc Capture (AMC)	Mouse IgG1, 2a & 2b Fc regions, kinetic analysis
• Anti-Mouse Fc Capture (AMQ)	Mouse IgG1, 2a & 2b Fc regions, quantitation
• Anti-Human Fab-CHI (FAB)	Fab-CH1 domains of human IgG
• Protein A (ProA)	Quantitation of various species IgG
• Protein G (ProG)	Quantitation of various species IgG
• Protein L (ProL)	Quantitation of IgG via kappa light chain

Immobilisation - Chemistry

Amine Coupling

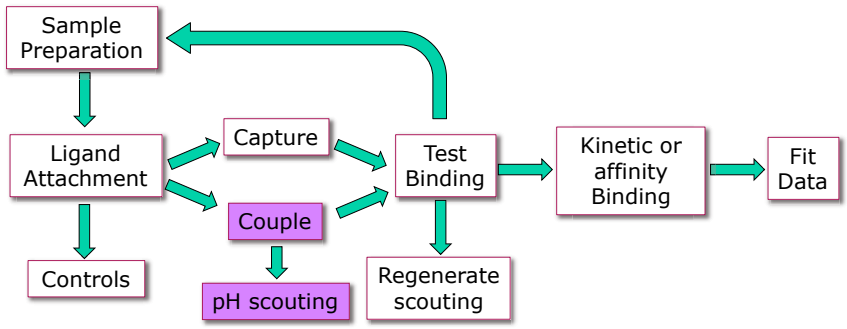


Thiol Coupling

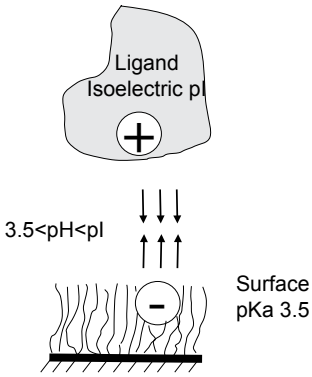


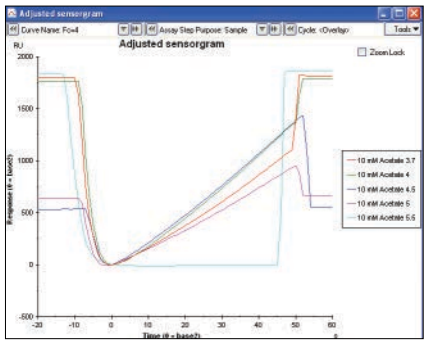
Alternatives e.g. via maleimide

pH Scouting for amine coupling



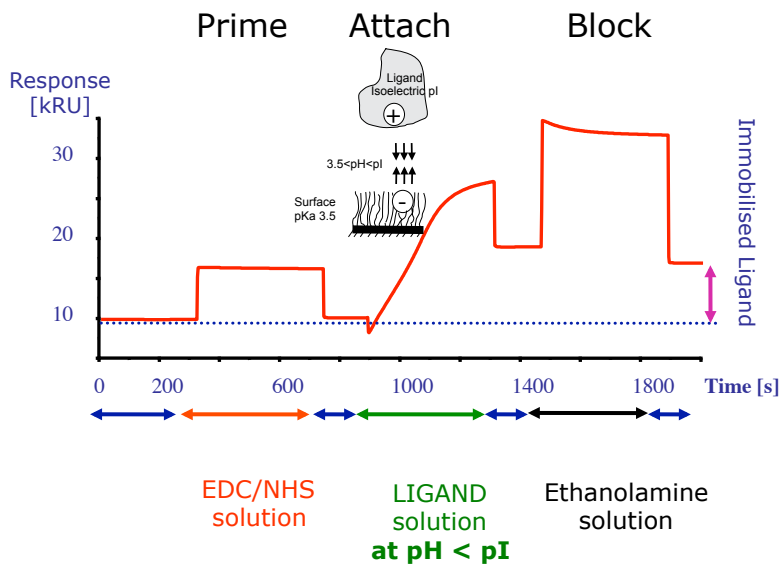
pH Scouting for amine coupling





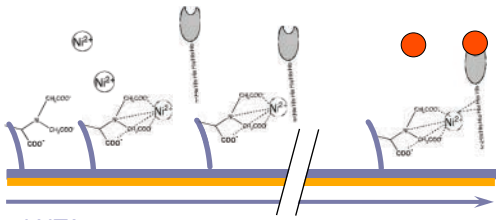
- Look for linear traces
- Calculate potential R_L *vs* injection time *vs* pH

SPR Amine Coupling



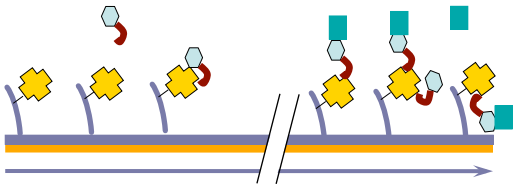
SPR Immobilisation - Tagged Proteins

Sensor Chip NTA



Pre-immobilized NTA
Nickel chelation
Capture of his-tagged protein
Binding of analyte

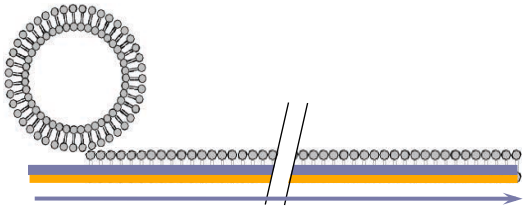
Sensor Chip SA



Stepavidin coated surface
Binding of biontinylated ligand
Binding of analyte

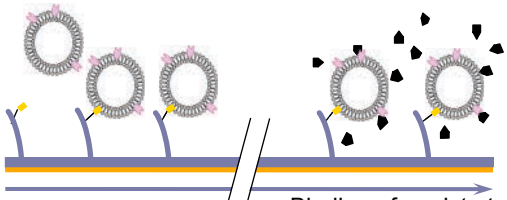
SPR Immobilisation – Membrane Applications

Sensor Chip HPA



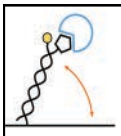
Hydrophobic surface
Coating with user defined liposomes

Sensor Chip L1



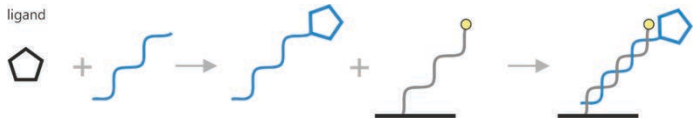
Immobilisation of liposomes

Binding of analyte to receptor



switchSense Ligand Attachment

Functionalization of the complementary DNA



To link a ligand to the complementary DNA strand, there are various coupling methods:

Chemical:

Amine reactive group

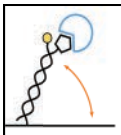
Thiol reactive group

Click Chemistry

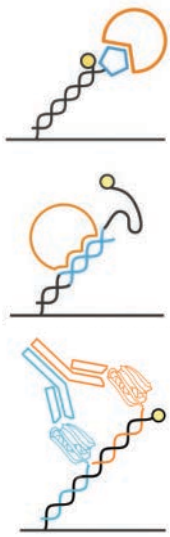
Capture tags:

Tris-NTA, Biotin, Streptavidin, Digoxigenin, Strep-Tactin® XT

Anti-LC-kappa (Murine) Anti-IgG-Fc Rabbit, Protein A, Protein G, GST-binding, GFP-binding

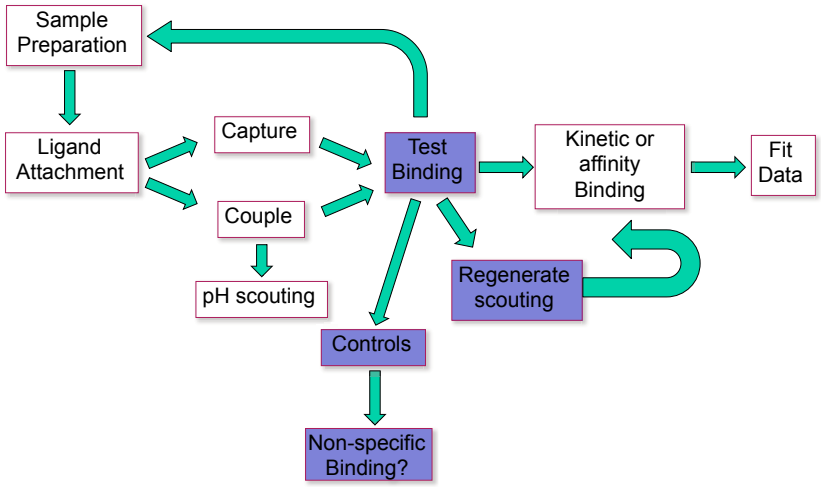


switchSense Swings for all

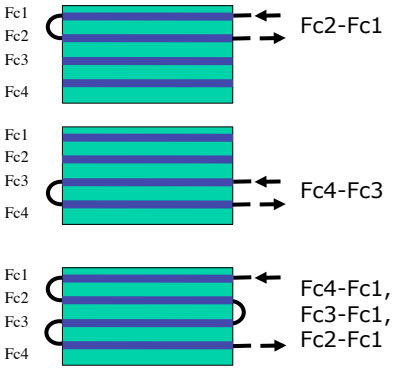


- Multi-purpose Biochips (MPC) 48 and 96 bp
  - Binding Kinetics
  - Binding Affinity
  - Protein Diameter
  - Conformational Change
  - Melting & Thermodynamics
  - Multimers & Aggregation
- Enzymatic Biochips
  - Nuclease & Polymerase Activity
  - 54 or 80 bp
  - 3’ of fluorophor strand attached to chip surface
- Bifunctional Biochips
  - Bispecific Binders & Avidity (7 mm or 14 mm)

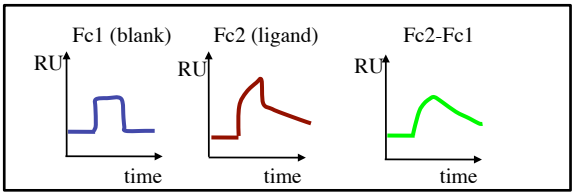
Controls and regeneration



SPR: Controls Reference Channel



- Channels in series for reference subtraction e.g. Fc2 (ligand)-Fc1 (control) subtraction or Fc4-Fc3, or (Fc4, Fc3, Fc2)-Fc1
- Treat as active ligand bound channel
- Immobilise a non-interacting control biomolecule that mimics physical properties of ligand e.g. a mutant that doesn't bind, a random DNA sequence



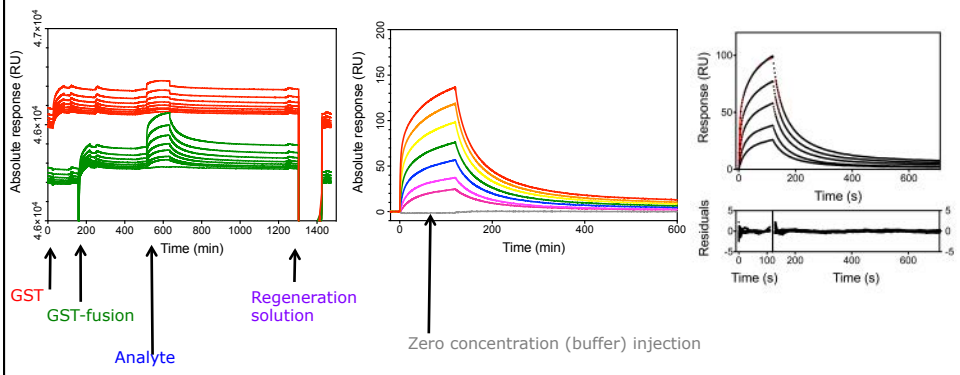
SPR: Controls Reference Channel

Anti-GST immobilised on Fc1 and Fc2 Chip

Raw data:
FC1 ligand GST
FC2 ligand GST-fusion

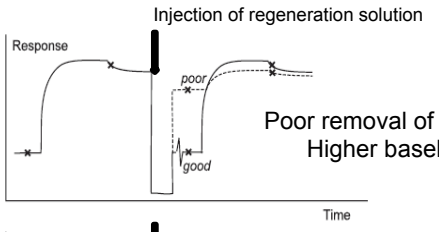
Reference corrected

Buffer corrected and fitted

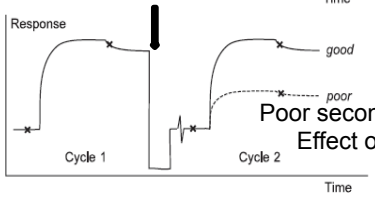


SPR Regeneration

One chip is used for many binding experiments
Therefore need to remove any residual analyte by regenerating the surface:



Poor removal of analyte
Higher baseline



Poor second response
Effect on ligand

Regenerating scouting:

salt (1M NaCl)

acid (pH 2.5)

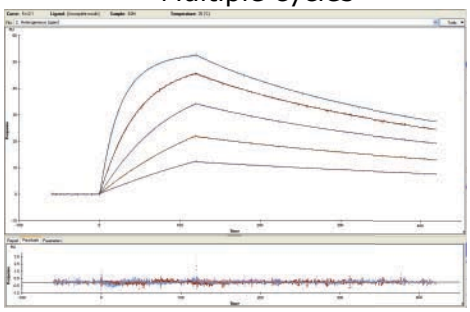
alkali (pH 11)

detergent

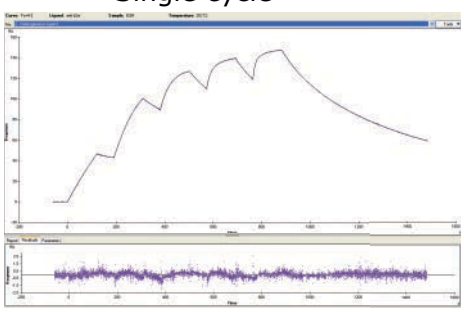
Can avoid problems using single cycle kinetics on T200
Or use BLI sensors in parallel

SPR Multiple vs Single Cycle Kinetics

Multiple cycles

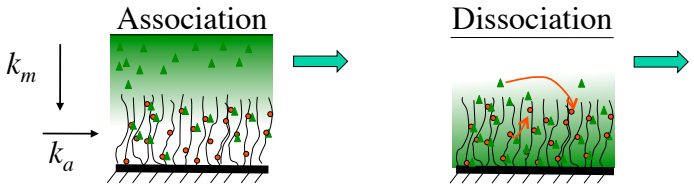


Single cycle



Fit	ka1 (1/Ms)	kd1 (1/s)	KD1 (M)	ka2 (1/Ms)	kd2 (1/s)	KD2 (M)	Rmax1 (RU)	Rmax2 (RU)	Chi² (RU²)
MCK	1.10E+06	0.002957	2.69E-09	5.17E+06	0.003214	6.22E-10	34.72	21.74	0.0222
SCK	7.46E+05	0.004182	5.61-09	5.90E+06	0.002374	4.03E-10	38.44	115.8	0.109

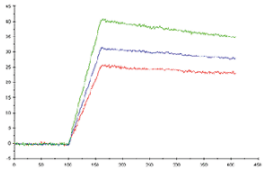
SPR Mass transport problems



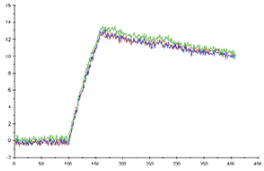
- During interactions analyte has to diffuse into or out of the dextran layer
- Mass transport problems occur when analyte is consumed faster than the flow can replenish it during association
- During dissociation, if analyte isn't removed by flow then it can rebind.
- k_m dependent on cube-root of flow rate

SPR Mass transport problems

Compare rates at different flow rates 5, 10, 20 µl/min



Mass transport effect



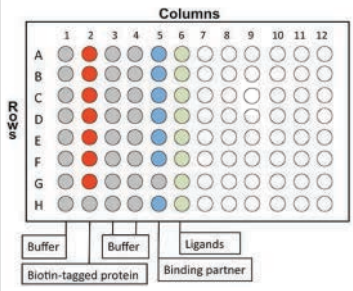
No effect of Mass transport

Overcome mass transport:

- Lowest amount of immobilized ligand
- Higher flow rates (e.g. 30 µl/min)

- Fit data using model that includes mass transport variable (caution: may be difficult to fit, don't invoke unless there is a mass transport effect)

BLI Controls



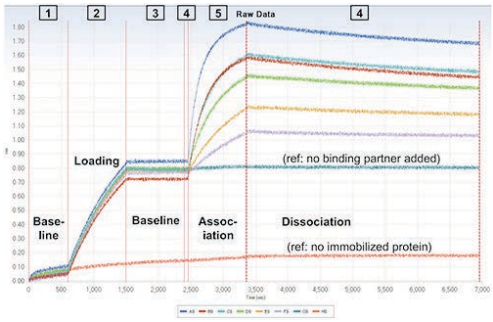
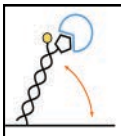


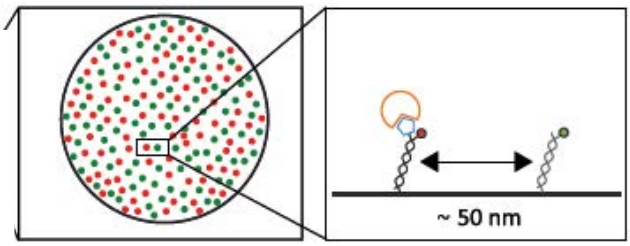
Plate-based

In series: use zero concentration of analyte and no ligand
In parallel: use another row of sensors but don't load ligand and repeat cycle

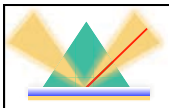
Naman B. Shah and Thomas M. Duncan J Vis Exp. 2014; (84): 51383.



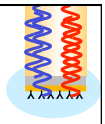
SwitchSense DRX^2 controls



Compare non-specific binding to ligand addressed nanolever in one channel with controls hybidrized with control strand in other colour channel. If non-specific change buffer add detergent etc…..



Non-specific binding



When interaction with surface is greater than the interaction with ligand:
-large non-specific response swamps specific interaction

Change Experimental Design:

- Change Chip e.g CM4 instead of CM5
- Use PEG-Chip
- Cross-link dextran surface with 5-10 mM aminomethyl-PEG prior to ligand
- Change pH of running buffer especially if basic protein
- Increase salt concentration of buffer 150–500 mM (physiological concentrations and higher)

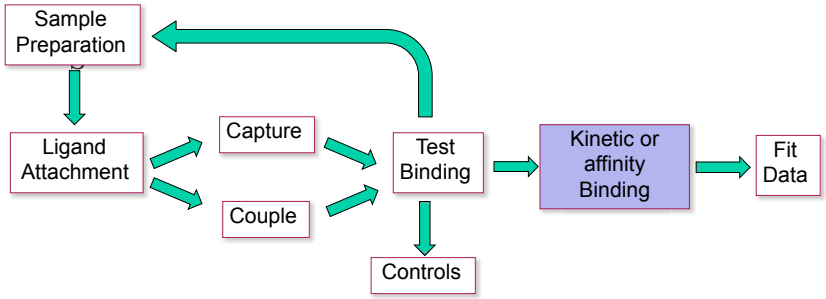
Additivies

- non-ionic detergents such as Tween-20 (P-20) 0.005% and above
- Blocking agents such as casein, PEG or gelatin, BSA (up to 1–2%)
- EDTA (3 mM)
- NSB Reducer soluble carboxyl methyl dextran (0.1 – 10 mg/ml)

48

- For streptavidin surfaces lock surface with e.g. biotin, biocytin

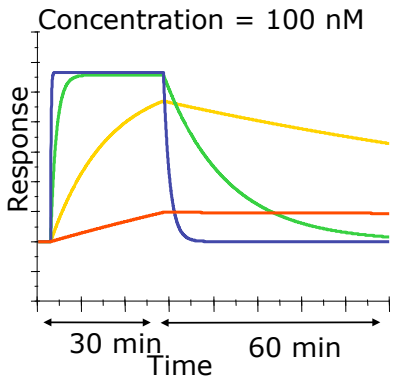
Affinity and Kinetics



- At least five concentrations for kinetics, more for affinity
- Adequate concentration range (above and below Kd)
- Concentration in duplicate or triplicate
- Include one or two zero-concentration

Signal shape depends on kinetics

- All 4 analytes have the same affinity K_d = 10 nM = 10^{-8} M
- The binding kinetic constants vary by 4 orders of magnitude



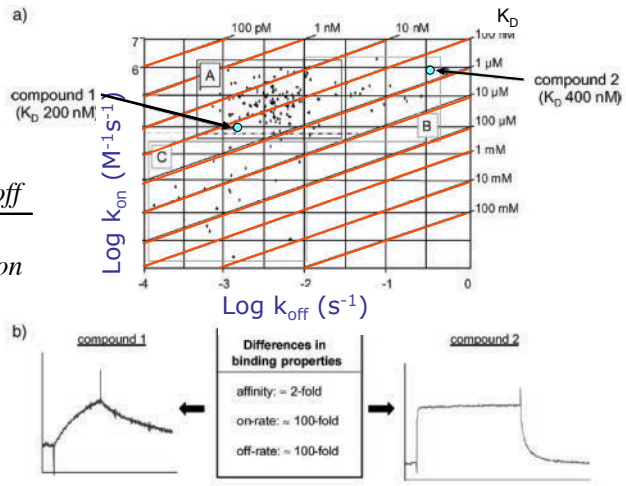
k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})
10^6	10^{-2}
10^5	10^{-3}
10^4	10^{-4}
10^3	10^{-5}

$$K_d = \frac{k_d}{k_a}$$

- Analytes with slow off-rates occupy the target for a longer time
- Observed on-rate is also concentration dependent

Dynamic Space: Small Molecule Screening

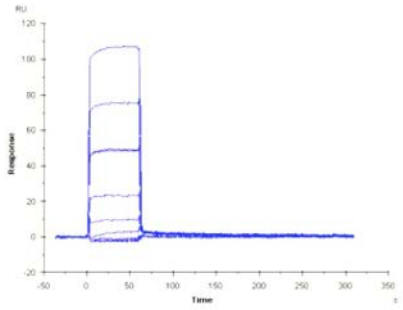
$$K_d = \frac{k_{off}}{k_{on}}$$



Huber (2005) J. Mol. Recogn.

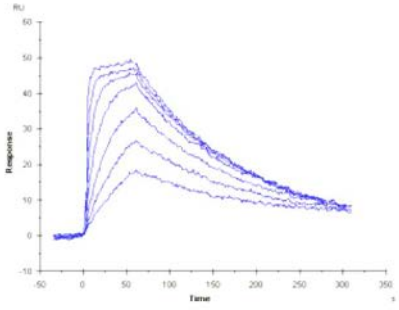
Kinetics determine Sensogram analysis

Steady-state



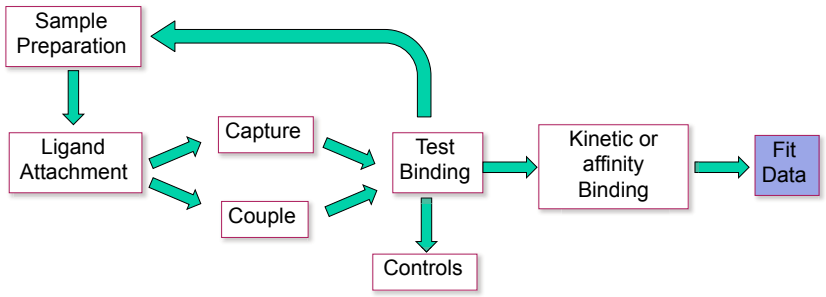
Fast on, fast off
Analyse Req vs [Analyte]

Kinetics



Slow on, slow off
Equilibrium not reached,
Analyse kinetics

Now to Fit

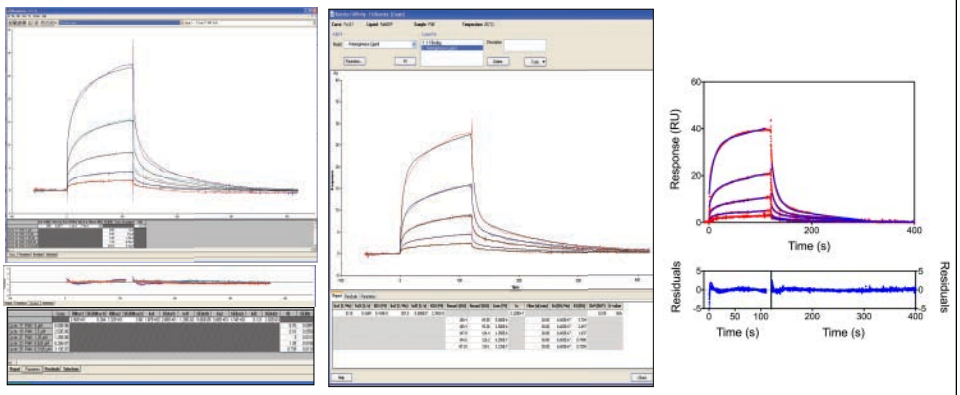


Before the analysis

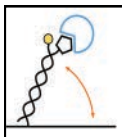
- Subtract reference binding
- Double reference by subtracting zero analyte concentration (buffer control)

Determining kinetics and K_d from SPR

BiaEval | T200 Evaluation | Prism



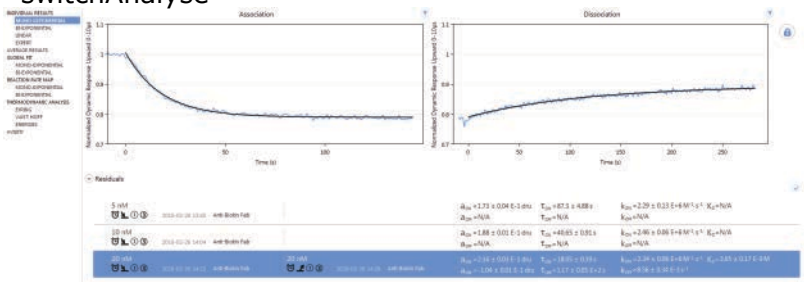
See Data Fitting lecture for more information



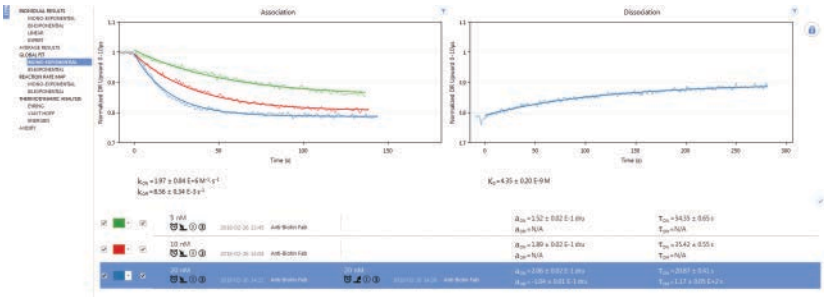
Determining kinetics and K_d from switchSense

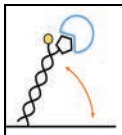
switchAnalyse

Local

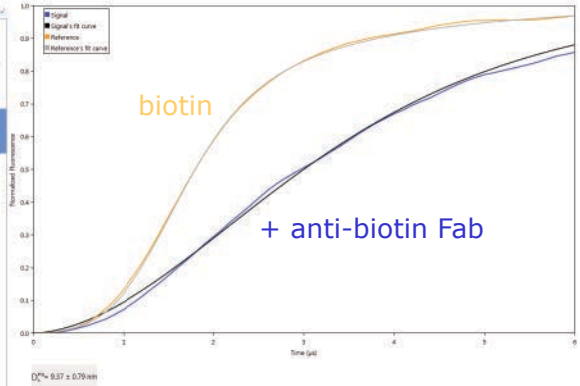


Global



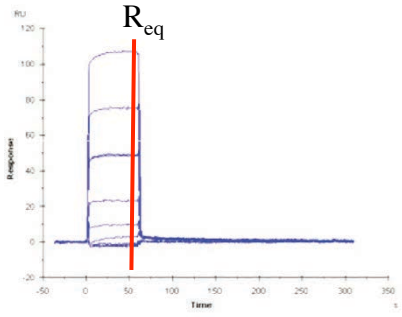


Determining size from switchSense

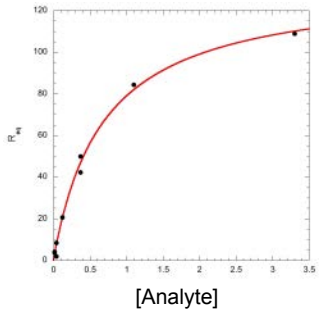


Fit using steady-state model

Steady-state



Plot R_{eq} vs [Analyte]

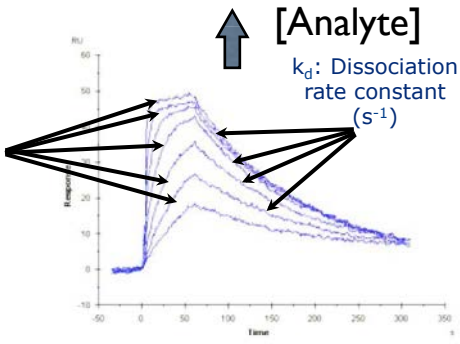


$$R_{eq} = \frac{C \bullet K_a \bullet R_{\max}}{C \bullet K_a + 1}$$

where C is the [Analyte], K_a is association constant ($1/K_d$)

Determining kinetics and K_d

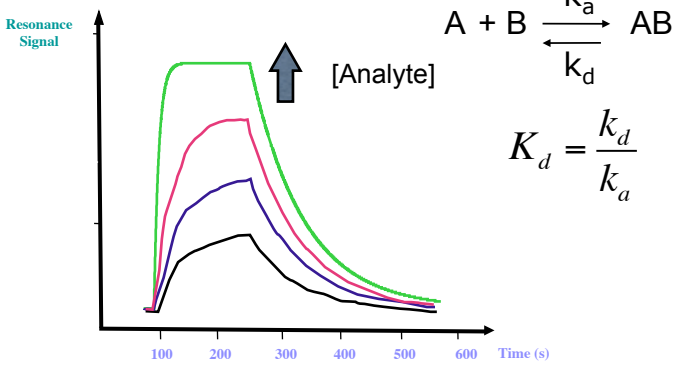
k_a: Association rate constant (M^{-1} s^{-1})
+
k_d: Dissociation rate constant (s^{-1})



Which model?

Drift - linear component due to instrument or non-specificity
Bivalent - analyte or ligand has two binding sites
Heterogenous ligand - parallel reactions
Conformational Change - two step reaction

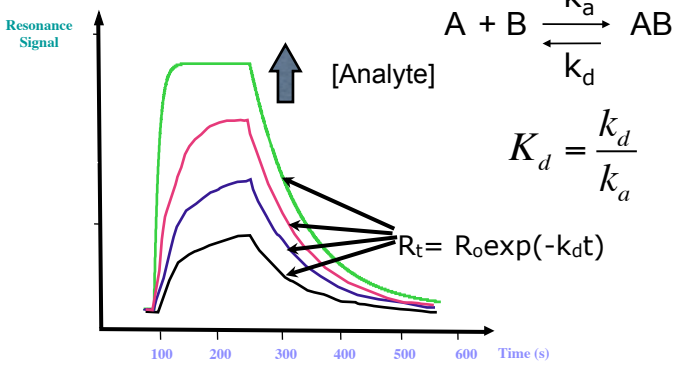
Determining kinetics and K_d



$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

$$K_d = \frac{k_d}{k_a}$$

Determining kinetics and K_d

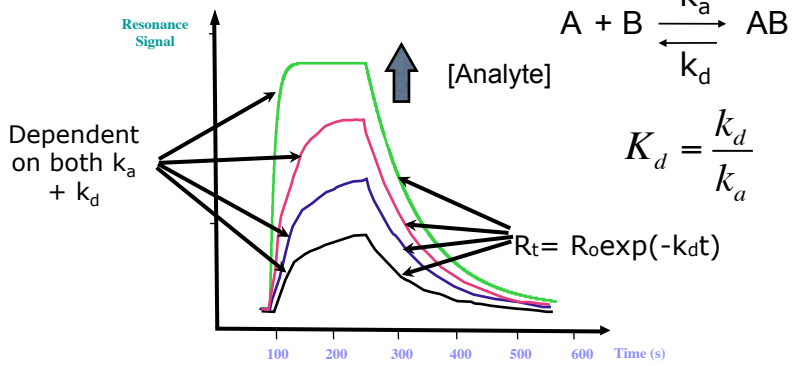


$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

$$K_d = \frac{k_d}{k_a}$$

$R_t = R_o \exp(-k_d t)$

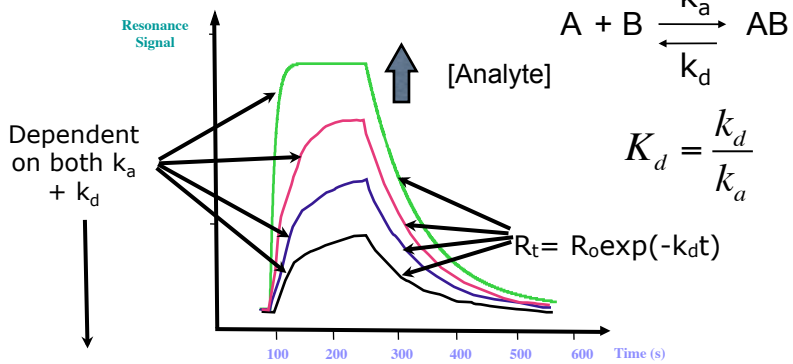
Determining kinetics and K_d



$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

$$K_d = \frac{k_d}{k_a}$$

Determining kinetics and K_d



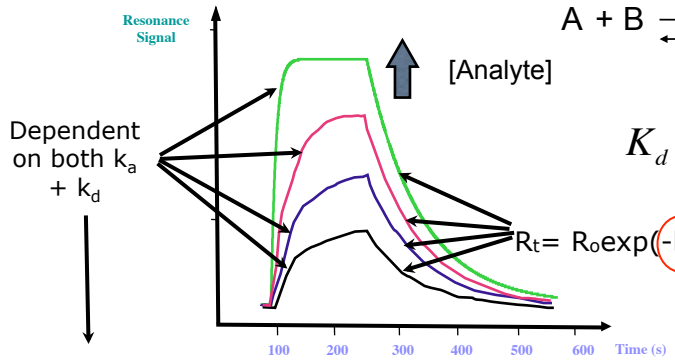
$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

$$K_d = \frac{k_d}{k_a}$$

$$R_t = \left(\frac{C \bullet k_a \bullet R_{\max}}{C \bullet k_a + k_d} \right) \bullet \left(1 - \exp(-(C \bullet k_a + k_d) \bullet t \right)$$

where C is concentration of analyte and R_{max} is the maximum response.

Determining kinetics and K_d



$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

$$K_d = \frac{k_d}{k_a}$$

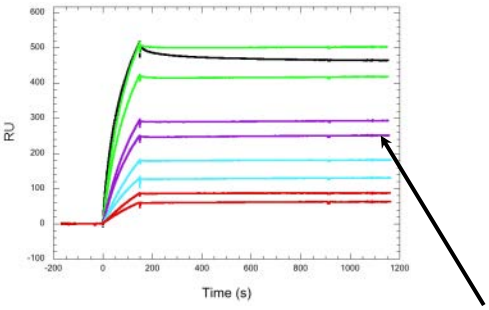
$R_t = R_o \exp(-k_d t)$

$$R_t = \left(\frac{C \bullet k_a \bullet R_{max}}{C \bullet k_a + k_d} \right) \bullet \left(1 - \exp(-(C \bullet k_a + k_d) \bullet t\right)$$

where C is concentration of analyte and R_{max} is the maximum response.

What if kd is wrong?

Global analysis of interactions can lead to errors in analysis



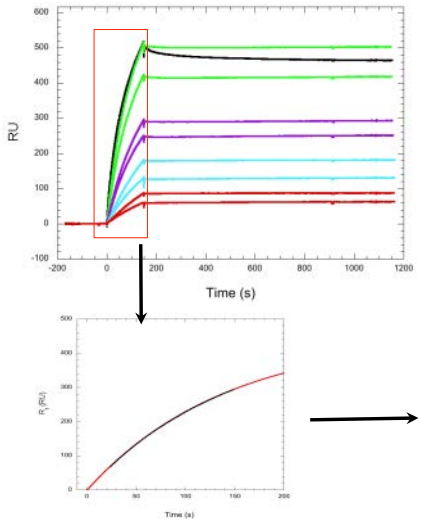
$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

$$K_d = \frac{k_d}{k_a}$$

Global fit gives poor fitting for k_d very low value

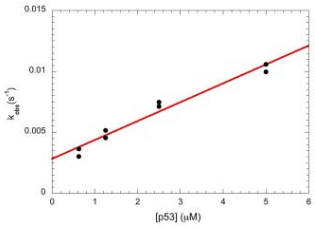
Estimated K_d approximately 30 nM

Kinetic analysis of interaction can overcome problems in global analysis



$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

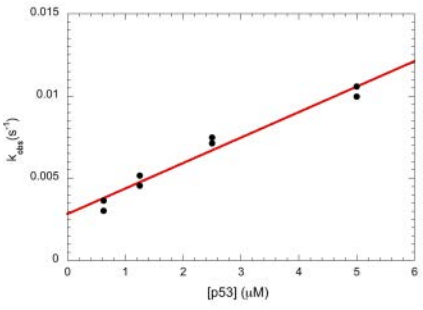
$$K_d = \frac{k_d}{k_a}$$



$R_t = R_I + R_{max}(1-\exp(k_{obs} * t))$

$k_{obs} = k_a[\text{Analyte}] + k_d$

Kinetic analysis of interaction can overcome problems in global analysis



$k_{obs} = k_a[\text{protein}] + k_d$

k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_d (μM)
1.5×10^3	2.8×10^{-3}	1.8

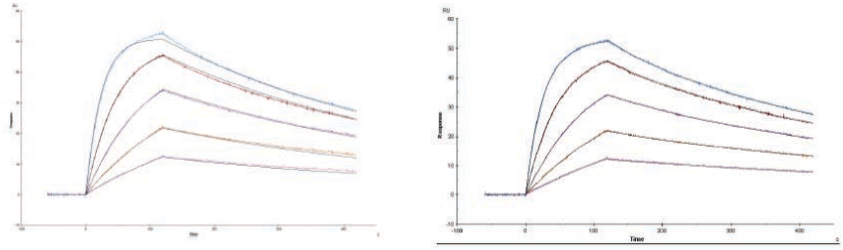
$K_d = k_d/k_a$

K_d=2.2 μM by ITC

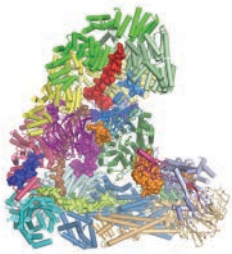
Tidow *et al*. (2006)

The Analysis

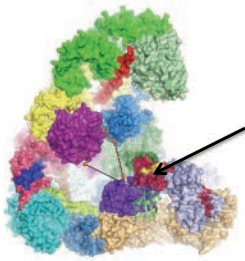
- Make a choice of evaluation model
- Try the simplest first
- Is the fit acceptable?
  access by residuals, standard error of parameters, Chi^2
- Don't invoke a complicated model just because the fit looks better
- Are the results relevant?



SPR Project



Cdh1



UbcH10-Ub
(E2 ligase)

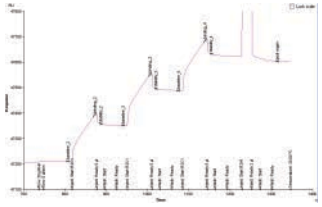
APC/C
E3 Ring–cullin ligase
20 subunits

How does activation of APC/C affect the affinity for the E2 UbcH10?

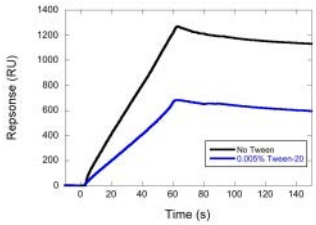
Attach biotin-UbcH10 to SA Chip and flow across apo- and ternary APC/C

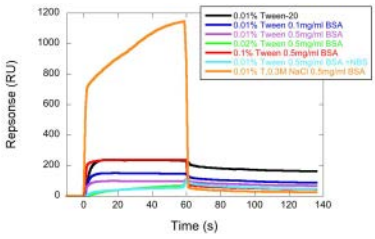
SPR Workflow

Immobilisation UbcH10-biotin on Streptavidin Chip



Test Interaction with APC/C



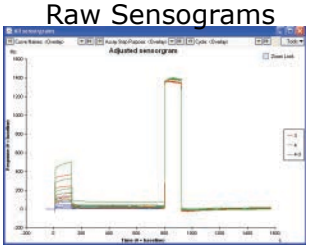


High background binding to chip.
Look at non-specific binding stability with different additives e.g.
NSB
Tween-20
BSA
NaCl

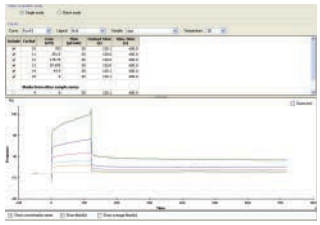
SPR Workflow

Kinetics Wizard

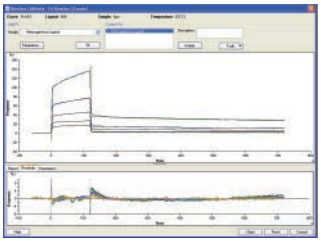
Raw Sensograms



Processed data

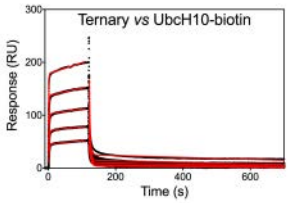


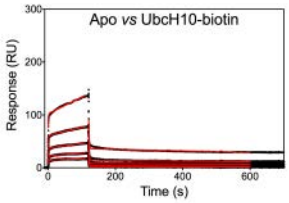
Fit data



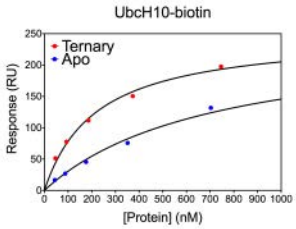
Compare Results

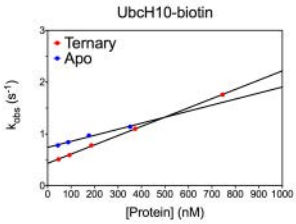
Kinetics





Equilibrium



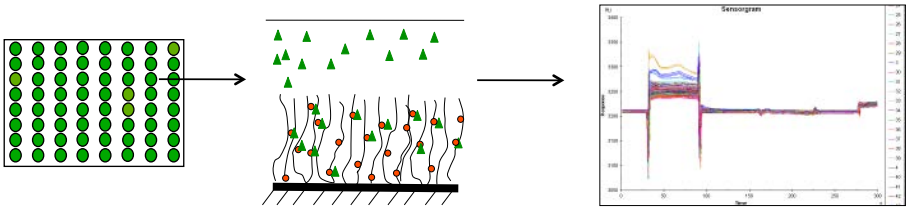


k_{obs}

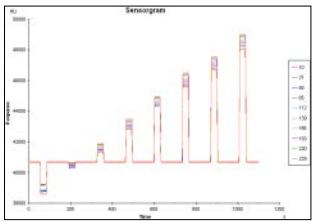
Ligand	Analyte	k_{off} (s^{-1})	k_{on} (M^{-1} s^{-1})	K^{eq}_d (nM)	K^{kin}_d (nM)
UbcH10	Ternary	0.42 ± 0.02	1.78 ± 0.2 x 10^6	215	236 ± 29
	Apo	0.75	1.1 ± 0.1 x 10^6	703	682 ± 83

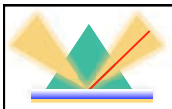
Chang *et al.* (2014) Nature

SPR Ligand Screening



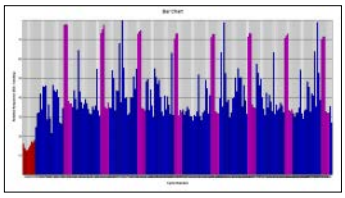
- Very small changes in mass/RU relative to control surface
- Need T200
- 96 or 384 well-format
- Up to three proteins in parallel
- Take care of DMSO – use solvent correction



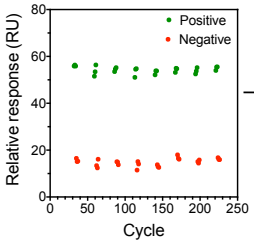


SPR Ligand Screening

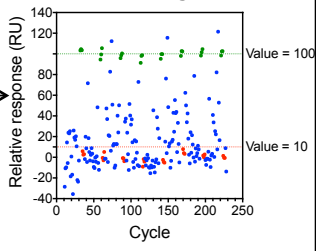
Binding



Controls



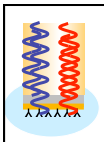
Ranking



High Z'

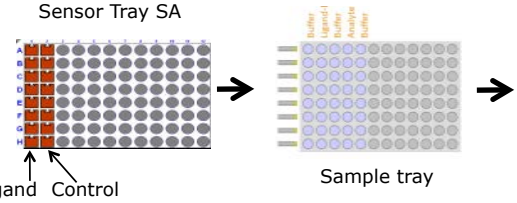
- Take care of baselines – positive and negative binding controls
- Ideal to have binding controls e.g. another protein
- Workflow built into evaluation software

(data from Javier Garcia-Nafria)

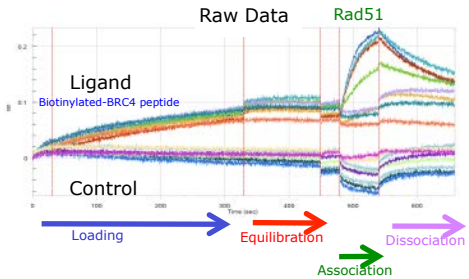


BLI in action: BRC4 peptide vs Rad 51

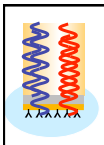
Sensor Tray SA



Raw Data

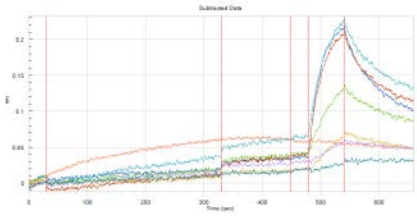


Data from Joe Maman, Dept of Biochemistry, University of Cambridge

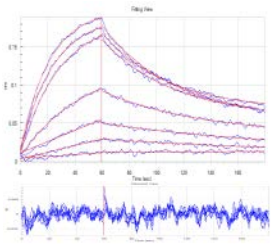


BLI in action: BRC4 peptide vs Rad 51

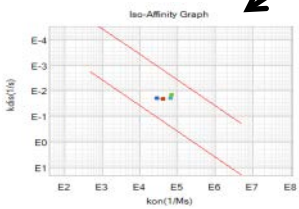
Corrected Data



Fit Data



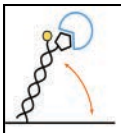
Results



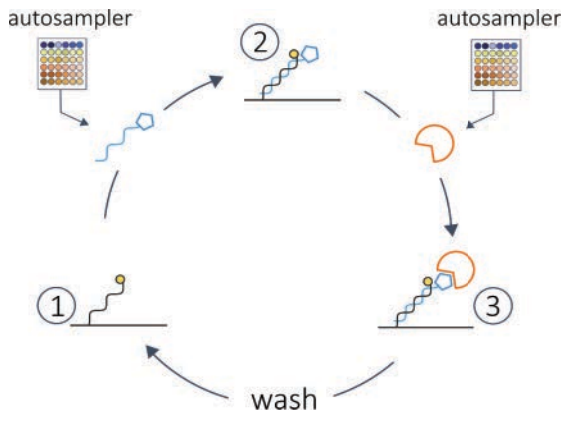
residuals

$$K_d = \frac{k_{diss}}{k_{on}} = 43nM$$

Data from Joe Maman, Dept of Biochemistry, University of Cambridge



switchSense in action



1 → 2

Functionalization of the **switch**SENSE biochip

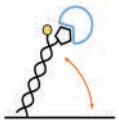
2 → ⇠

Interaction measurement

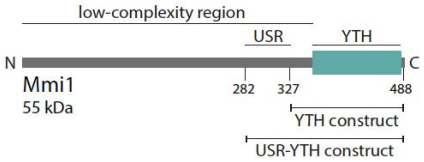
3 → 1

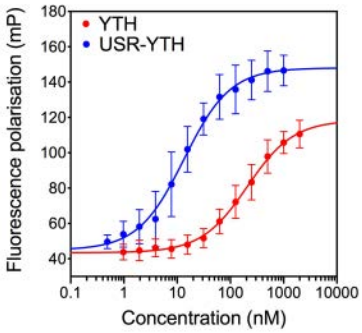
Wash away the ligand-cNL analyte complex

dynamic BIOSENSORS



SwitchSense in action



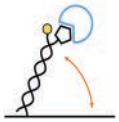


How does the USR increases the affinity of interaction of the Mmi1 YTH domain with RNA?

- Synthesize RNA/DNA hybrid as ligand

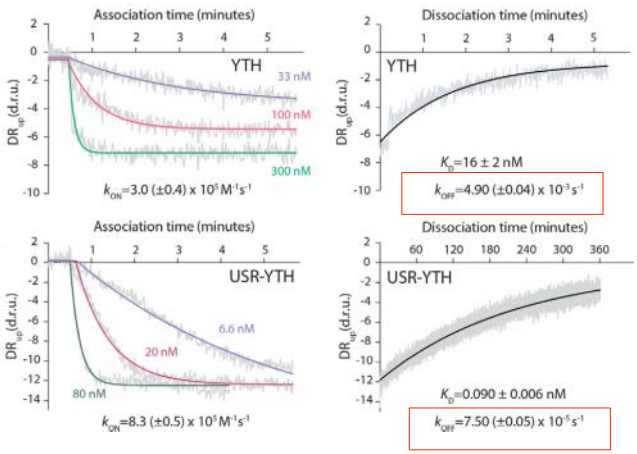
- Flow proteins over ligand on SwitchSense

Stowell *et al. (2018)* J. Biol. Chem.



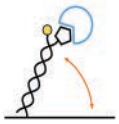
SwitchSense in action

How does the USR increases the affinity of interaction of the Mmi1 YTH domain with RNA?

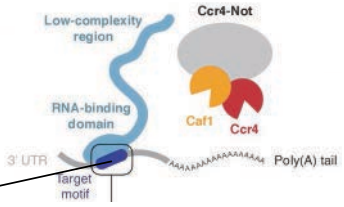


USR decreases k_{off} by 2 orders of magnitude

Stowell *et al. (2018)* J. Biol. Chem.



switchSense in action



Puf3 target RNA

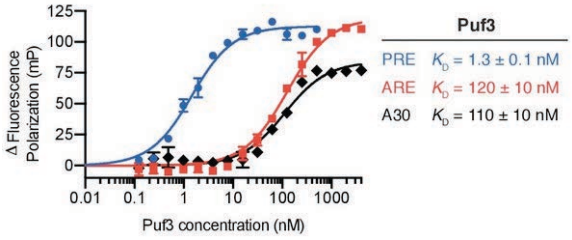
5′ PRE A30 poly(A) 3′

Zfs1 target RNA

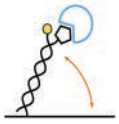
5′ ARE A30 poly(A) 3′

5′ A30 poly(A) 3′

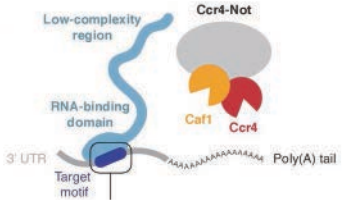
5′-6FAM-labeled RNA



Webster *et al.* (2019) eLife



SwitchSense in action



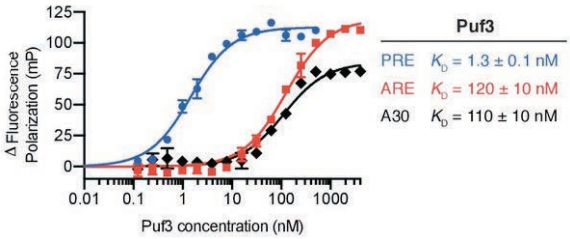
Puf3 target RNA

5′ PRE A30 poly(A) 3′

Zfs1 target RNA

5′ ARE A30 poly(A) 3′

5′ A30 poly(A) 3′



Why does Puf3 bind tighter to PRE sequence?

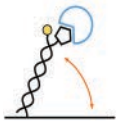
What is the effect on deadenylase activity of Ccr4-Not complex?

- Synthesize Puf3 target RNA/DNA as a ligand

  5′ PRE RNA DNA 3′

- Flow Puf3^{PUM} protein over ligand on SwitchSense

Webster *et al.* (2019) eLife



SwitchSense in action

How does target sequence effect activity?

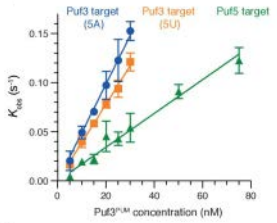
SwitchSense Puf3 target RNA/DNA

Association

5′ RNA DNA 3′

Puf3 target (5A) ACCUGUA**A**AUAGGCG–[*DNA]
Puf3 target (5U) ACCUGUA**U**AUAGGCG–[*DNA]
Puf5 target **AGU**UGUAU**G**UAGGCG–[*DNA]
Poly(A) [A]15–[*DNA]

Flow Puf3 protein over ligand on SwitchSense

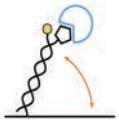


RNA	k_{on} ($\times 10^6$ $M^{-1}s^{-1}$)	$T_{1/2}$ at 20 nM (s)
Puf3 target (5A)	4.9 ± 0.1	7.0 ± 0.1
Puf3 target (5U)	3.9 ± 0.1	8.8 ± 0.1
Puf5 target	1.7 ± 0.1	20.1 ± 0.7
Poly(A)	0.73 ± 0.03	47 ± 2

RNA target sequence increases k_{on} (~7 fold)

RNA target with A at position 5 slightly increases to k_{on} compared to U

Webster *et al.* (2019) eLife



SwitchSense in action

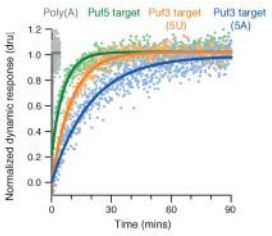
How does target sequence effect activity?

SwitchSense Puf3 target RNA/DNA

Dissociation

5′ RNA DNA 3′

Puf3 target (5A) ACCUGUA**A**AUAGGCG–[*DNA]
Puf3 target (5U) ACCUGUA**U**AUAGGCG–[*DNA]
Puf5 target **AGU**UGUAU**G**UAGGCG–[*DNA]
Poly(A) [A]15–[*DNA]



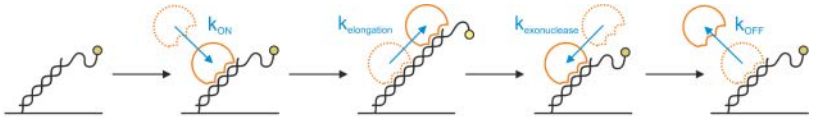
RNA	k_{off} ($\times 10^{-4}$ s^{-1})	$T_{1/2}$ (min)
Puf3 target (5A)	8.1 ± 0.3	14.2 ± 0.5
Puf3 target (5U)	16 ± 2	7.2 ± 0.8
Puf5 target	28 ± 5	4.3 ± 0.7
Poly(A)	4000 ± 1000	0.03 ± 0.01

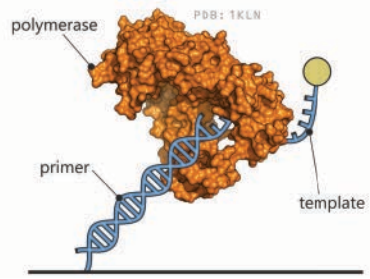
- Poly(A) without target sequence RNA rapidly dissociates
- RNA target with A at position 5 has lower k_{off} and hence increased $T_{1/2}$ x 2 compared to U and x 3 compared to Puf5 target

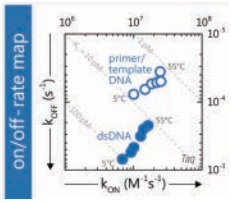
Webster *et al.* (2019) eLife

switchSENSE provides one Workflow for Polymerase DNA/RNA Interaction Measurements

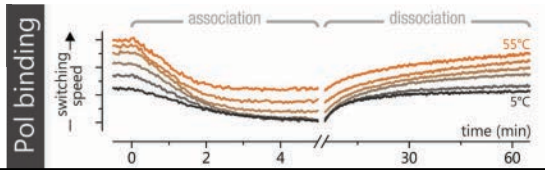
Different primer/template combinations use modular sequence exchange







k_{on}, k_{off}, K_D temperature dependence



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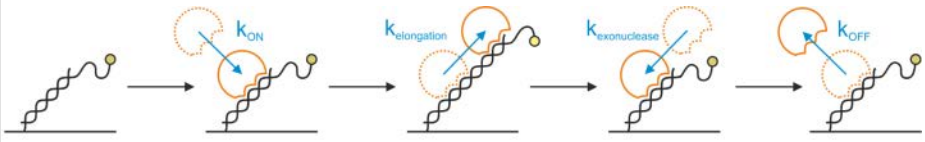
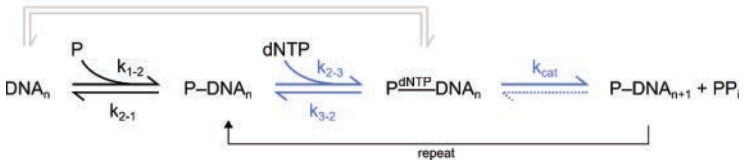
Polymerase Characterization with switchSENSE





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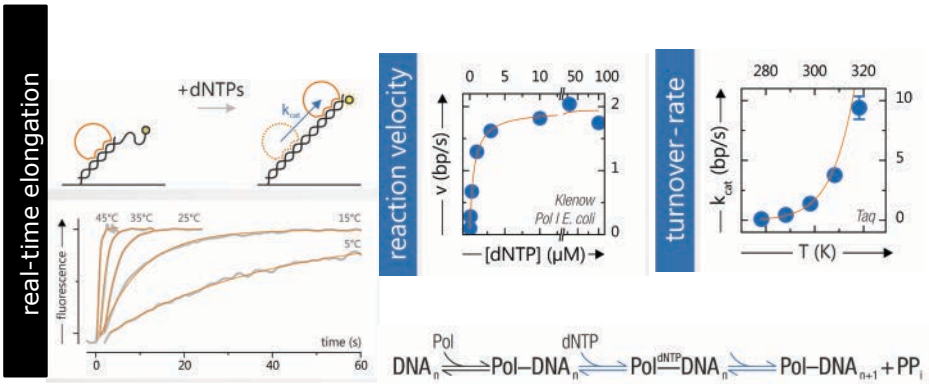
switchSENSE Measures the Polymerase Activity in Basepairs per Second & Determines the Michaelis Constant K_M



Michaelis Constant K_M = 0.58 µM

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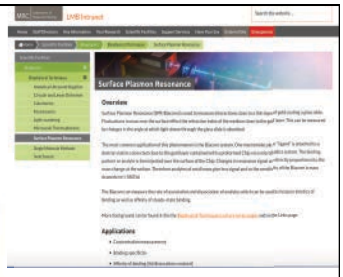
Further Information?



SPR
http://www.biacore.com/
http://www.sprpages.nl/

switchSense
http://www.dynamic-biosensors.com/switchsense/

Octet
http://www.fortebio.com/octet-platform.html

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