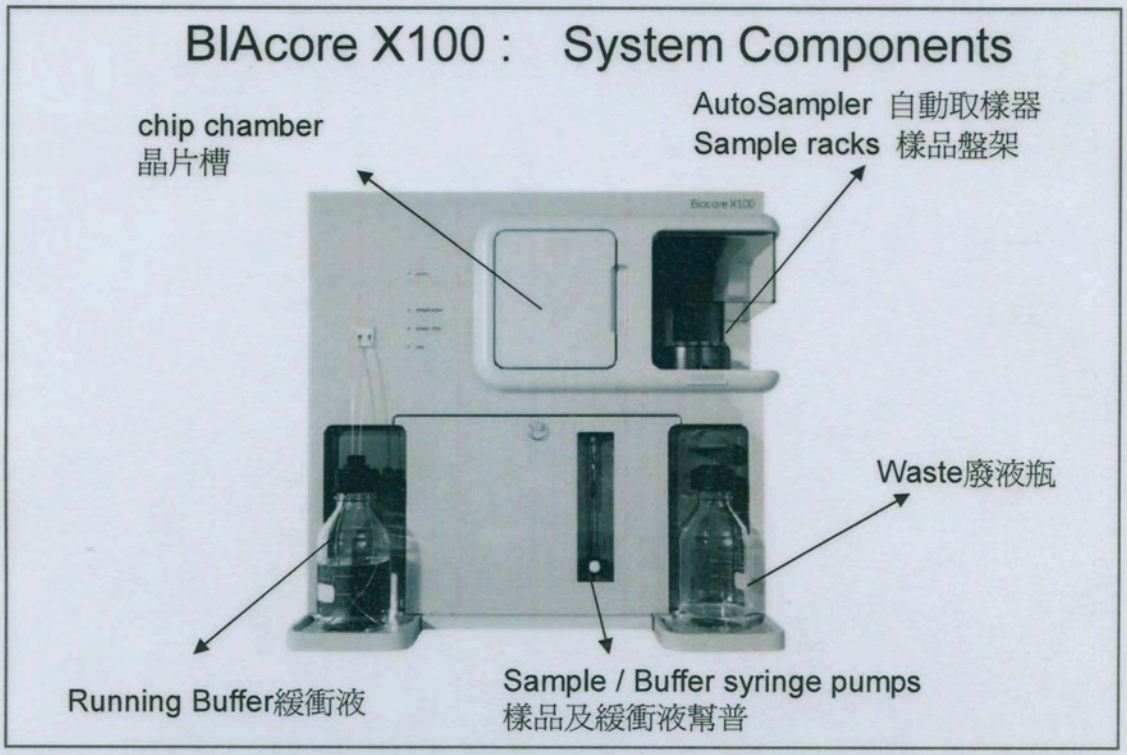
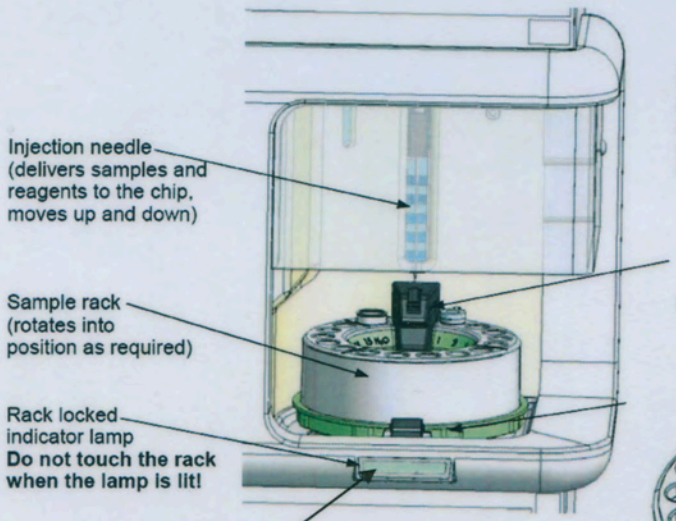
BIAcore X100 簡易操作

系統組件簡介





啓動系統：

1. 啓動 Biacore X100 主機 (總按鈕位於左側後下方)，電腦及列印機。
   待主機面版上 TEMP 指示燈停止閃爍，即溫度穩定。

2. 緩衝液需預先以. 0.22uM 濾膜過濾，Degas，經冷藏緩衝液需回室溫再使用。

3. 啓動 BIAcore X100 Control Software:
   點取 Start Menu。於 BIA Program Menu 選擇 BIAcore X100 Control Software。

軟體登入：

4. 鍵入 User Name 和 Password，按下 OK 登入。

插入晶片：

5. 將緩衝液放置 Buffer tray，插入幫泵導管。放置廢液回收瓶。

6. 若系統內尚未插入晶片，螢幕則顯示 Dock 視窗。插入晶片（步驟 7-10）。
   若系統內尚有晶片，請先退出晶片再插入新的晶片（步驟 13-14）

7. 向下掀開晶片阜遮蓋，抽出晶片座槽滑套。

8. 將晶片平放置槽內，箭頭指向儀器端，並確認晶片底部有對準到座槽上的導引針。

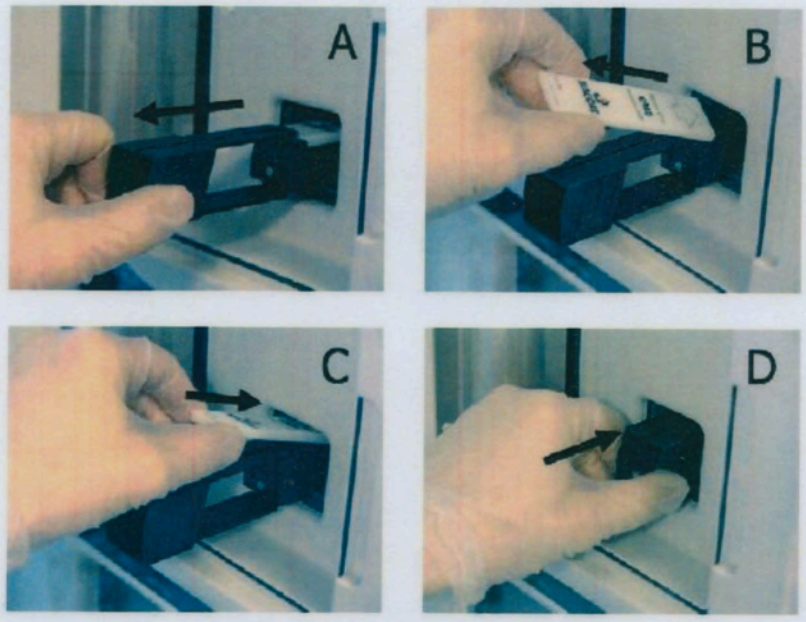
9. 將座槽滑套收回，蓋起遮蓋。

10. 點選 Dock。Dock 步驟完畢後，Sensor Chip 指示燈轉爲穩定亮燈狀態。

11. 若主機面版 Sensor Chip 指示燈爲閃爍狀態，則表示儀器內已插入一晶片，但尚未 Dock。
    此時可參考步驟 13-14 取出。

12. 螢幕顯示 PRIME 視窗，點選 START，啓動緩衝液灌充步驟。Prime 需約 7 分鐘。
    按 Exit 退出。

✧ 每日初次使用機器時，或在更換 running buffer 後，可重複 Prime 2 -3 次，已確保 Running buffer 徹底致換。



取出晶片：

13. 若系統內尚有 docked chip，螢幕則可能顯示兩種對話窗，Standby 窗:表示有連續緩衝液流動的待機狀態，按 Stop 停止緩衝液流速。；若顯示 Prime 視窗，則表示無流速狀態，直接點選 No。

14. 選 Tools :Undock., 按 Undock。待 Sensor Chip 指示燈轉爲閃爍狀態後，掀開遮蓋，取出晶片。

設定自動取樣機樣品盤規格：

15. 點選  Load Samples，待"Rack Lock"燈號熄滅後，將樣品盤退出。

16. 擺放好樣品後，按下 OK 鍵，樣品盤會自動轉動讀取樣品位置。

17. "Rack Lock" 燈號亮起時，即固定好樣品盤，使用儀器中請勿任意拿取。

✧ 本系統雖然具備樣品架自動偵測功能，使用者更換樣品時，請務必再次重新按下 Load Samples 讓機器讀取樣品位置，確定樣品狀況。

反應訊號之常態化 (Signal Normalisation)：

18. 建議使用時：當使用新晶片或溫度設定更便。選 Tools: More Tools: Normalize。

19. 取 120 µl BIAnormalisating solution 70%加入 1.5 ml 塑膠瓶，放置 Position1 位置。

20. 按照對話視窗的指示操作，開啓 normalisation 步驟，校正偵測器，normalisation 需約 8 分鐘。

BIAcore X100 定期保養

爲了確保系統性能以及實驗結果的品質，請落實以下定期維護清洗步驟。
BIAcore X100 已內建所有系統清洗程式。程式可由 Tools: More Tools 取得。

所需要耗材：
- Biacore Maintenance Kit type 1 (BR-1006-66)：系統清洗試劑組
- Maintenance Chip （系統附件）：在執行 Desorb 及 Sanatize 等清洗步驟時，需使用清洗專用的晶片 maintenance chip。使用前檢查晶片正背面，必要時可先用清水洗後擦拭乾淨使用。

每 日 保 養 ：

實驗前：
過濾，degas 緩衝液以 0.22um 濾片過濾，並 degas 後使用。
Prime 系統 3 -5 次

實驗後：
取出實驗晶片，放入清洗專用晶片，用二次水 Prime 系統。
若需 4 天內使用機器，執行 Tools : Standby，否則執行:Tools: Shutdown。
取出晶片，執行 Undock 後關機。

每 週 保 養：

系統清洗：
執行；Tools : More Tools: Desorb。
Prime x3 次後使用。 （Prime 後執行 Standby 效果更佳。）

每 月 保 養：

系統清洗：
執行；Tools : More Tools: Desorb。
執行 Tools : more Tools: Sanitize。
Prime x3 次後使用。 （Prime 後執行 Continous Flow 效果更佳。）

系統性能檢測：
執行：Tools : More Tools: System Check.
使用新的 CM5 chip 來執行。

BIAcore X100 buffer 注意事項

有機溶劑溶液請先參閱使用手冊，嚴禁未經測試或不相容的溶液使用在 Biacore X100 儀器上 !!

晶片須在系統 Undocked 情況下始能取出晶片 !

Real-Time Biomolecule Interaction Analysis
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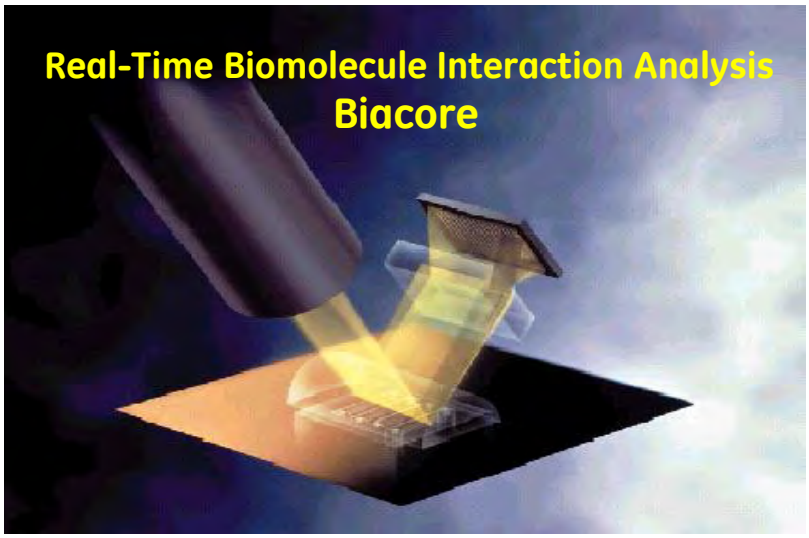

李宗樹 Forest.LEE@ge.com
GE Healthcare Life Sciences

GE imagination at work

BIACORE

Content

- Introduce to Interaction
  - SPR, ITC, DSC
  - Kinetics and Affinity
- Introduce to Biacore
- Applications
- Experimental Design (Workflow)
- Maintenance and Resource

Biacore™ data in almost 10 000 scientific publications

Basic and applied research in the fields of

- Cancer
- Neurobiology
- Immunology
- Infectious diseases
- Functional proteomics
- Cell signaling
- Vaccines
- Selection and characterization of binding reagents
- Drug discovery

and many more….

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Biophysics Increases Data Confidence and Elucidates Mechanism of Action

1. Confirm **stability**	Understanding of stability crucial to ensure suitable conditions for interaction analysis
2. Confirm **interactions**	Multiple techniques are very useful in characterizing all aspects of an interaction
Stoichiometries	Interacting pairs or multimeric complexes **(N)**
Binding strengths	**Affinities** range from mM to below pM **(K_A, K_D)**
Interaction forces	Hydrogen bonds, electrostatic interactions, hydrophobic effects
Reaction rates	Association **rate** constants 10^3 to 10^9 $M^{-1}s^{-1}$ **(ka)** Dissociation **rate** constants 10^{-5} to >1 s^{-1} **(kd)**

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Workflow for Label-Free Characterization of Biomolecules

Biomolecule stability -> Interaction analysis and kinetics -> Thermodynamics

MicroCal Differential Scanning Calorimetry (DSC) systems

Biacore Surface Plasmon Resonance (SPR) systems

MicroCal Isothermal Titration Calorimetry (ITC) systems

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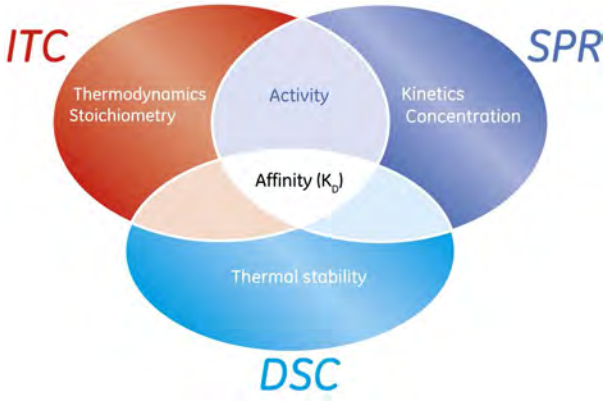
Measure and characterize biomolecule structure, function and activity



Obtain a wide range of critical, binding and stability related data to make your conclusions and discovery/development decisions with confidence

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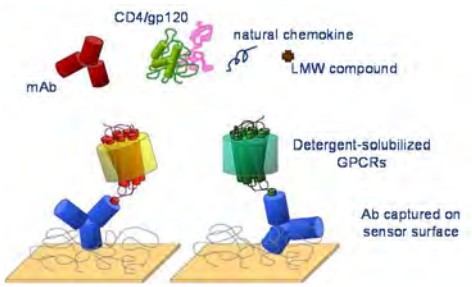
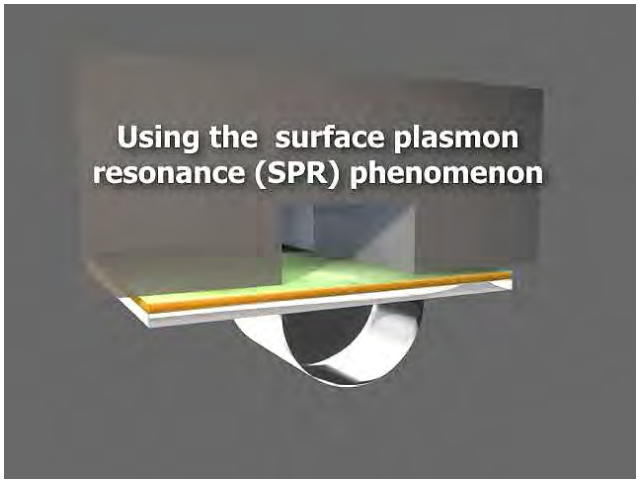
Analysis of a wide range of biomolecular interactions

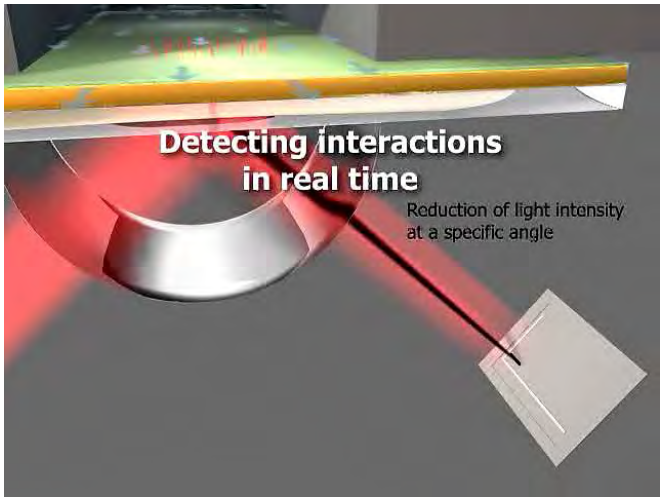
- Proteins
- Nucleic acids
- Lipids & membrane-associated molecules
- Carbohydrates
- LMW compounds (>200 Da)
- Whole cells
- Viruses/bacteria



See how it works -SPR



Monitoring interactions in real time



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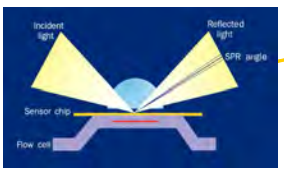
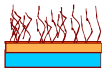
Cornerstones of Biacore® SPR Technology



SPR (Surface Plasmon Resonance) Detection System



Gold-Dextran Surfaces

Microfluidic System (IFC)

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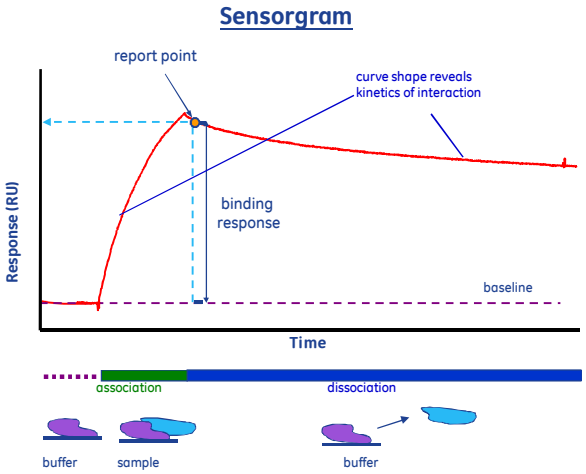
Data output from label-free interaction analysis



Yes/no binding?

Specificity of binding?

How strong?

How fast?

How much?

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Comprehensive information from one system

•Analyze molecular interactions in real time and obtain a wide range of critical, binding-related data:

Detect

Yes/No

Identify

Specificity

Binding partners

Epitope mapping

Characterize

Affinity

Kinetics

Active Concentration

Thermodynamics

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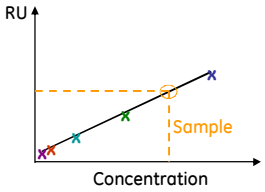
Concentration analysis in Biacore™

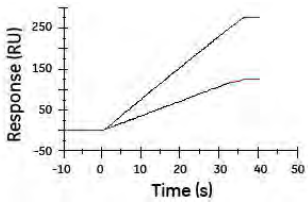
Concentration analysis using a calibration curve

- The sample concentration is read from the calibration curve
- Suitable for analyses where calibration/standard sample is available



CFCA (Calibration-free concentration analysis)

- The sample concentration is derived from the initial binding rate
- Perform concentration analysis when calibration/standard is not available
- Validate the specified concentration of standard



imagination at work

What are kinetics and affinity?

Kinetics

- How fast do things happen? – Time-dependent
- Association – how fast molecules bind
- Dissociation – how fast complexes fall apart
- Kinetics determine whether a complex forms or dissociates within a given time span

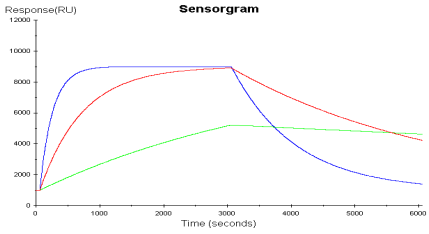
Affinity

- How strong is a complex? – Time-independent
- Affinity determines how much complex is formed at equilibrium (steady state where association balances dissociation)

imagination at work

What is the relevance of binding kinetics?

- The cell is a dynamic system – rarely in equilibrium
- The same affinity can be resolved into different on and off rates for different interactions
  - Kinetic data reveal more information



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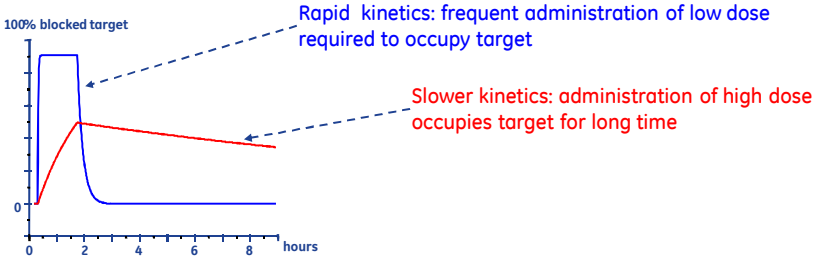
Kinetic properties have therapeutic consequences

- Kinetic properties affect multiple aspects of drug function (e.g. pharmacokinetics, dosing)



This information is critical to therapeutic performance of drugs!

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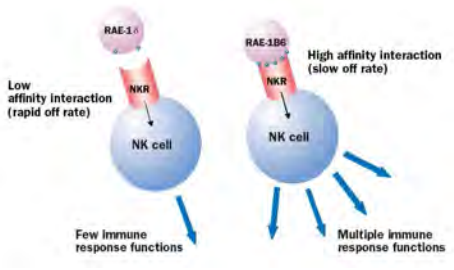
Kinetics properties determine function

- Biological processes are *dynamic*
- Kinetic data enables
  - Linking of structure to function
  - Better understanding of biological mechanisms



Carayannopoulos et al (2001) *Eur J Immunol*, 32, 597-605

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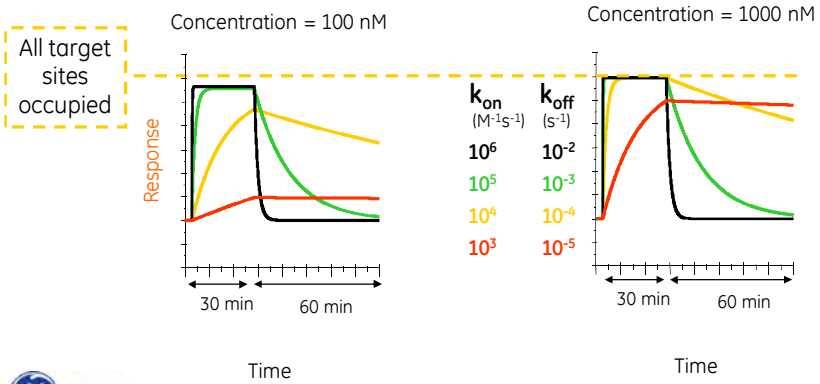
Same affinity but different kinetics

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



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Rate constants

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

- Association rate: $\frac{d[AB]}{dt} = k_a \cdot [A] \cdot [B]$

  Ms^{-1} $M^{-1}s^{-1}$ M M

  On-rate is concentration dependent

- Dissociation rate: $-\frac{d[AB]}{dt} = k_d \cdot [AB]$

  Ms^{-1} s^{-1} M

  Off-rate is concentration independent

kd= 0.01 s^{-1}= 1% of the complexes decays per second

imagination at work

Equilibrium Constants

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

- Equilibrium association constant:

$$K_A = \frac{[AB]}{[A] \cdot [B]} \quad [M^{-1}]$$

High K_A = High affinity

- Equilibrium dissociation constant

$$K_D = \frac{[A] \cdot [B]}{[AB]} \quad [M]$$

High K_D = Low affinity

$$K_A = \frac{1}{K_D}$$

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Equilibrium and kinetic constants are related

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

– Association rate: $\frac{d[AB]}{dt} = k_a \cdot [A] \cdot [B]$

– Dissociation rate: $-\frac{d[AB]}{dt} = k_d \cdot [AB]$

– At equilibrium:
Association = Dissociation $k_a \cdot [A] \cdot [B] = k_d \cdot [AB]$

– The equilibrium constant: $K_A = \frac{[AB]}{[A] \cdot [B]} = \frac{k_a}{k_d}$ $K_D = \frac{[A] \cdot [B]}{[AB]} = \frac{k_d}{k_a}$

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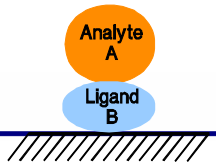
Kinetics in Biacore



In BIACORE at any time t: $[A]_t = C$
$[AB] = R$
$[B]_o = R_{max}$ thus $[B]_t = R_{max} - R$

A A A A A A A A A A A A A A A A
B B B B B B B B B B B B B B B B

R_{max} at saturation

A A A A A A A A
B B B B B B B B B B B B B B B B

$R = [AB]_t$

A A A A A A A A
B B B B B B B B B B B B B B B B

$[B]_t = R_{max} - R$

We do not need to know the "real" concentration of ligand or complex

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Rate and affinity in Biacore terms

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

$$\frac{d[AB]}{dt} = k_a \cdot [A] \cdot [B] - k_d \cdot [AB]$$

$$\frac{dR}{dt} = k_a \cdot C \cdot [R_{max} - R] - k_d \cdot R$$

RU/s $M^{-1}s^{-1}$ M RU s^{-1} RU

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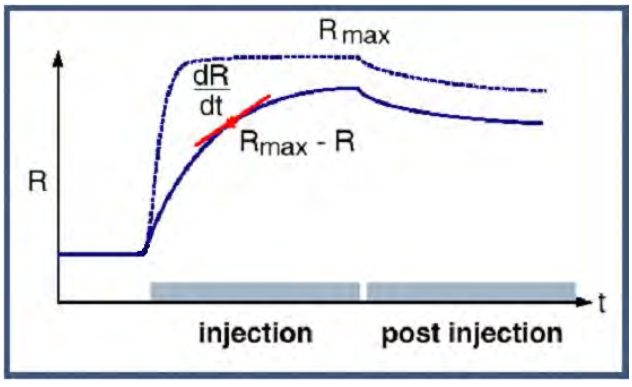
The net rate equation terms in a sensorgram



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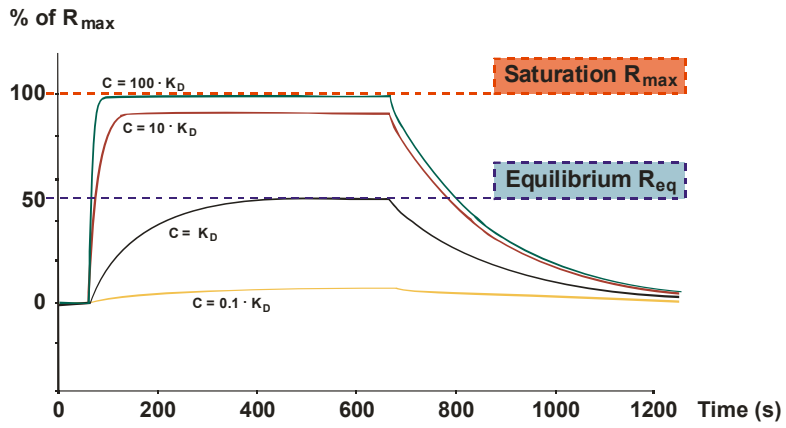
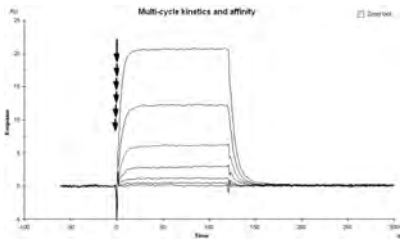
Information in a sensorgram - equilibrium

- The relationship between R_{max}, R_{eq} and K_D



Multi-cycle kinetics and affinity

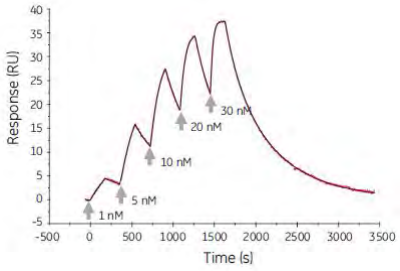
- Analyte concentration series
- Each concentration in a separate cycle
- Zero concentration + replicates
- With capture, new ligand for each cycle
- Regeneration requirement



- Dependent on consistent surface performance between cycles
- Cannot be used if regeneration is difficult

Single cycle kinetics and affinity

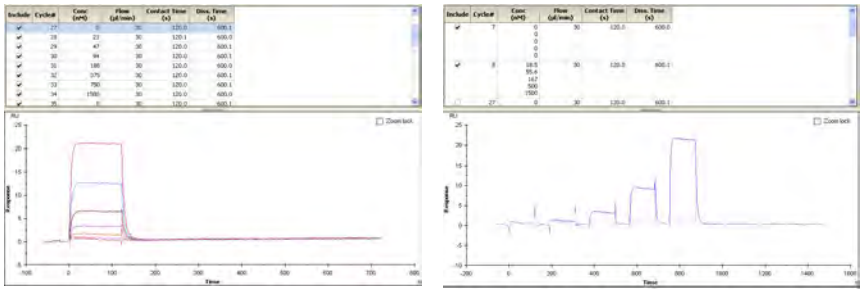
- Analyte concentration series
- Up to 5 concentrations in a single cycle
- Single dissociation phase
- No replicates
- With capture, single ligand injection
- No regeneration



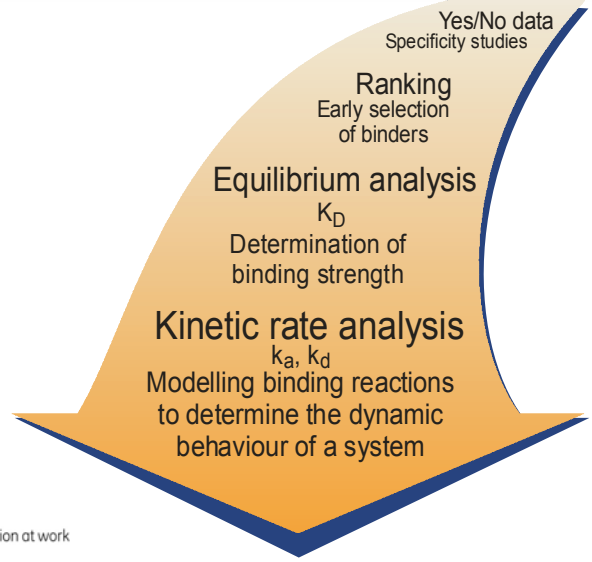
- Reduced risk of inconsistent surface performance
- Less need for assay development (regeneration optimization)
- Most useful when regeneration conditions can't be found

The two approaches give the same results

- Evaluation handles both multi- and single-cycle
  - Applies to Biacore X100 Evaluation Software and Biacore T100 Evaluation Software version 2.0 or later
- Multi- and single cycle can be evaluated together



Decide on what information you want



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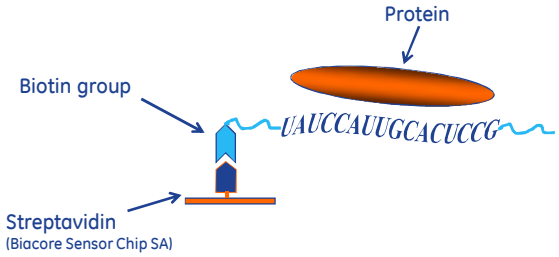
Unraveling the mechanisms of RNA-binding protein functions using kinetic analysis



Acknowledgements to Dr. Ite Laird-Offringa, USC, LA, USA

Park S. et al, (2000), Moll Cell Biol 20: 4765-72
Katsamba et al, (2001), Biol Chem 276: 21476 -81

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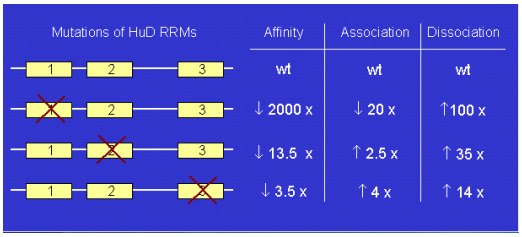
BIACORE

Functional roles of RRMs revealed

– Multiple RNA recognition motifs (RRMs) in HuD
- HuD: neuron-specific protein that binds to AU-rich motifs in mRNAs - involved in post-transcriptional gene regulation
- HuD contains three consensus RRMs, but affinity studies suggest that only two are functionally important



Kinetic studies

major RRM

small contributions to affinity, significant effect on complex stability (shown by k_d changes)

•Functional roles of RRM 2 & 3 remained masked until revealed by the rate constant analysis

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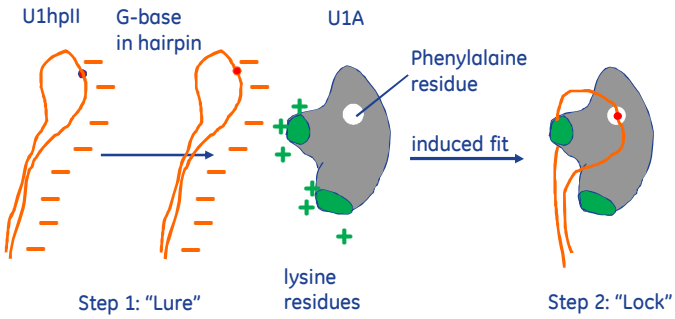
Model of U1A-U1hpII binding

- Kinetic studies led the authors to propose a two-step model for U1A binding to RNA:



Detailed kinetic determinations made the derivation of this new two-step "lure and lock" model for RNA-protein binding possible

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Characterization of the membrane-binding properties of two peripheral proteins by SPR: A molecular basis for calcium-dependent subcellular targeting mechanisms

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Background

- Understanding cellular signaling pathways is a vital component of many areas of biomedical research
- One important feature is translocation of signal proteins to specific subcellular locations
- Peripheral membrane proteins (PMPs) are a particularly important group of molecules in this regard
- Many PMPs contain a C2 (protein kinase C conserved 2) domain - involved in Ca-dependent subcellular targeting

Stahelin *et. al.* have attempted to explain the subcellular targeting mechanisms of PMPs in terms of their membrane-specific kinetic & affinity properties, using Biacore

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Peripheral membrane proteins

- Cytosolic phospholipase A_2 (cPLA$_2$)
- lipolytic enzyme of pharmacological significance
- contains a C2 domain
- preferentially binds to phosphatidylcholine (PC)
- translocates to the perinuclear region in response to Ca^{2+}

- Protein kinase C-α (PKC-α)
- serine/threonine kinase, signal transduction molecule
- contains a C2 domain
- preferentially binds to phosphatidylserine (PS)
- translocates to the plasma membrane in response to Ca^{2+}

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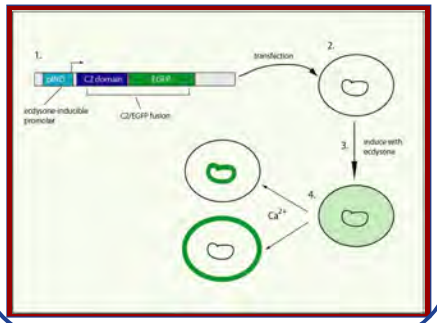
Experimental methods

SPR kinetic & affinity studies

- model membranes (enriched in PS or PC to mimic plasma or perinuclear membranes) attached as coated vesicles to L1 sensor surfaces
- wild-type and mutant C2 domains from cPLA$_2$ and PKC-α used as analytes - K_D, k_a & k_d values derived
- C2 mutations assessed for affects on both general phospholipid binding and phospholipid specificity

Live cell imaging of subcellular translocation

- how does the SPR data correlate with cellular behavior?



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Results (1): SPR

Protein	Affinity PS	Affinity PC	Affinity PS:PC
PKC-α-C2	14 nM	150 nM	10.7
T251A	↓ 18 x	↓ 16 x	9.6
N189A	↓ 5 x	≈ 1	1.9

- The C2 domain shows an 11-fold preference for PS (plasma membrane mimic)
- Almost entirely due to a slower dissociation rate for PS
- T251A mutation reduces general phospholipid affinity
- N189A mutation significantly reduces phospholipid selectivity

Protein	Affinity PC	Affinity PS	Affinity PC:PS
$cPLA_2$ -C2	11 nM	120 nM	10.9
L39A	↓ 191 x	↓ 10 x	0.6
Y96A	↓ 209 x	↓ 21.6	1.1

- The C2 domain shows an 11-fold preference for PC (perinuclear membrane mimic)
- Almost entirely due to a faster association rate for PC
- L39A mutation reduces PC selectivity (primarily via k_d)
- Y96A mutation reduces PC selectivity (via both k_d and k_a)

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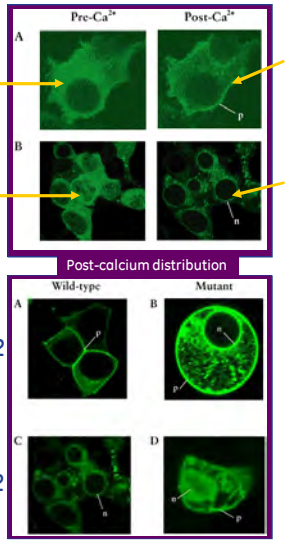
Results (2): C2 domain targeting

PKC-α: pre-activation

$cPLA_2$: pre-activation



wt PKC-α-C2

wt $cPLA_2$ -C2

translocation to plasma membrane

translocation to perinuclear membranes

In live cell imaging experiments, the C2 domains of the two PMPs behaved as predicted from the SPR data

- in "domain swap" experiments, subcellular targeting was directed entirely by the C2 domain (not the rest of the PMP)

N189A mt → loss of selectivity

Y96A mt → loss of selectivity

SPR-defined mutants with loss of selectivity show same behavior in live cell experiments

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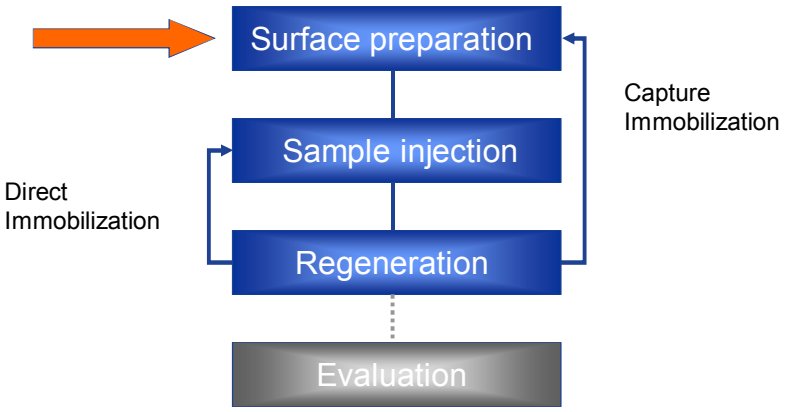
General steps in Biacore Assays



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Surface preparation

- What to immobilize?
- How to immobilize?
- What immobilization level is appropriate?
- Which Sensor Chip is suitable?

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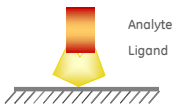
Surface preparation
What to immobilize?

- Considerations
  - Molecular weight of interactants
  - Tagging of interactants
  - Functional groups
  - Purity
  - Valency (number of binding sites)
  - Binding activity of immobilized interactant must be retained
  - pI
  - Available amount
  - Assay requirements

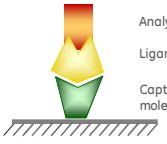
Surface preparation
How to immobilize?

- Direct immobilization
  - Covalent chemistry
  - Often heterogenous orientation
  - Higher binding capacity



Examples
- Amine coupling
- Ligand thiol coupling
- Surface thiol coupling
- Maleimide coupling
- Aldehyde coupling

- Capture approach
  - Orientation-specific
  - Selective capture from crude samples
  - Lower binding capacity



Examples
- Streptavidin - Biotin
- Anti-mouse Ig – MAb
- Anti-GST - GST
- NTA – 6His
- Anti-His – 6His
- Anti-FLAG - FLAG

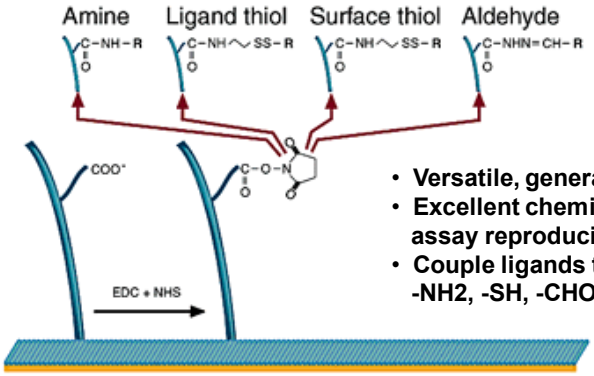
Choice of immobilization strategy dependent on ligand properties

Unstable ligand	→	Capture
Impure ligand	→	Capture
Covalent coupling results in loss of activity	→	Try other functional groups (e.g. Thiols)
Acidic ligands	→	Capture or alternative chemistry (e.g. Thiol coupling)
Regeneration is difficult	→	Capture

Sensor chip surfaces

Sensor Chips : CM5

The most versatile chip available

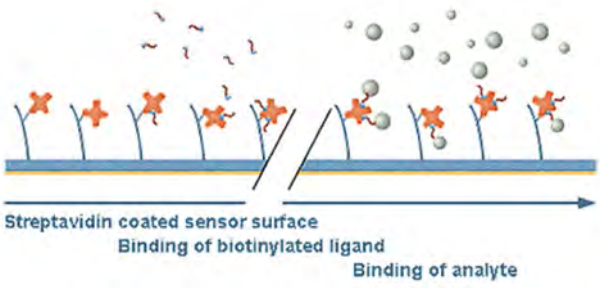


- **Versatile, general purpose matrix**
- **Excellent chemical stability and assay reproducibility**
- **Couple ligands to CM groups via -NH2, -SH, -CHO, -OH or -COOH**

Covalent derivatization of Sensor Chip CM5 for immobilization of ligand to carboxymethylated (CM) dextran surface

Sensor chip : SA

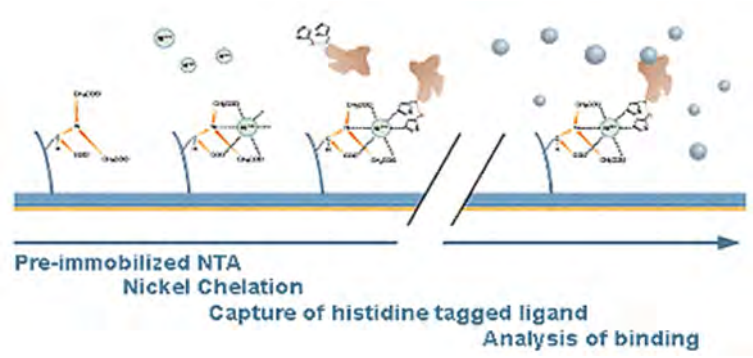
Captures biotinylated ligands, such as peptides, proteins and DNA



Sensor Chip SA has a carboxymethylated dextran matrix that is pre-immobilized with streptavidin. It is used for capturing biotinylated ligands such as peptides, proteins and DNA

Sensor Chip: NTA

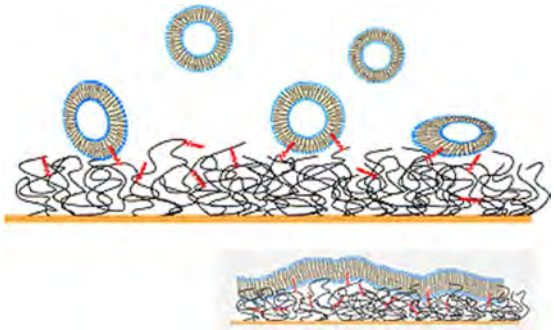
Capture of histidine-tagged ligands via metal chelation



Sensor Chip NTA has a carboxymethylated dextran matrix that is pre-immobilized with NTA*. It is used for capture of histidine-tagged ligands via metal chelation.

Sensor Chip: L1

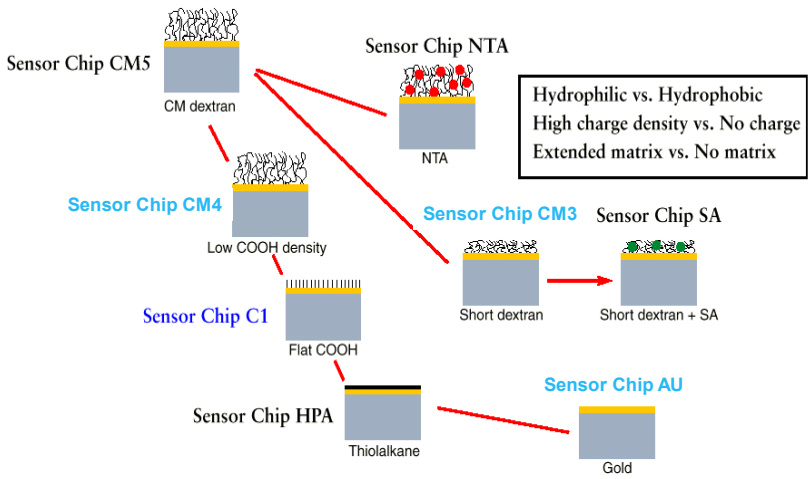
Capture liposomes rapidly and reproducibly



Pioneer Chip L1 has a carboxymethylated dextran matrix that has been modified with lipophilic substances. It is used for the rapid and reproducible capture of liposomes.
Whilst HPA allows for monolayer studies, L1 allows work with

bilayers for the study of trans-membrane proteins.

Sensor Chip Selection

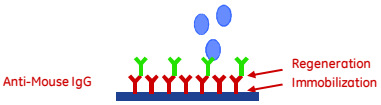


Sensor chips – an overview

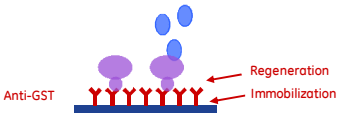
Sensor Chip CM5:	The most versatile chip
Sensor Chip CM4:	For low R_{max} and for reducing non-specific binding from e.g. crude sample matrix
Sensor Chip CM3:	For low immobilization levels and work with cells, viruses and high molecular weight analytes
Sensor Chip C1:	For work with cells and particles and when dextran matrix is not desired
Sensor Chip SA:	For capture of biotinylated ligands
Sensor Chip NTA:	For capture of His-tagged ligands
Sensor Chip HPA:	For looking at lipid monolayers interacting with membrane binding biomolecules
Sensor Chip L1:	For capture of liposomes with retention of lipid bilayer structure

Capture kits for a wide range of analyses

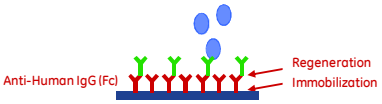
- Mouse Antibody Capture Kit



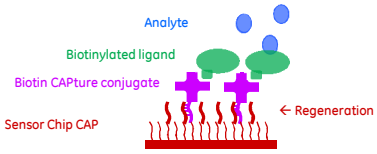
GST Capture Kit



Human Antibody Capture Kit



- Biotin CAPture Kit



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Immobilization – Example

Optimal Coupling pH Scouting :

(pre-concentration tests)

AMINE COUPLING

- Activation of functional groups
- Coupling of ligands
- Blocking

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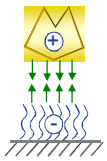
Preconcentration Tests

- Ligand is concentrated at the sensor surface by electrostatic attraction

pH < 3.5

Too low pH: no surface-charge, no electrostatic attraction

3.5 < pH < pI



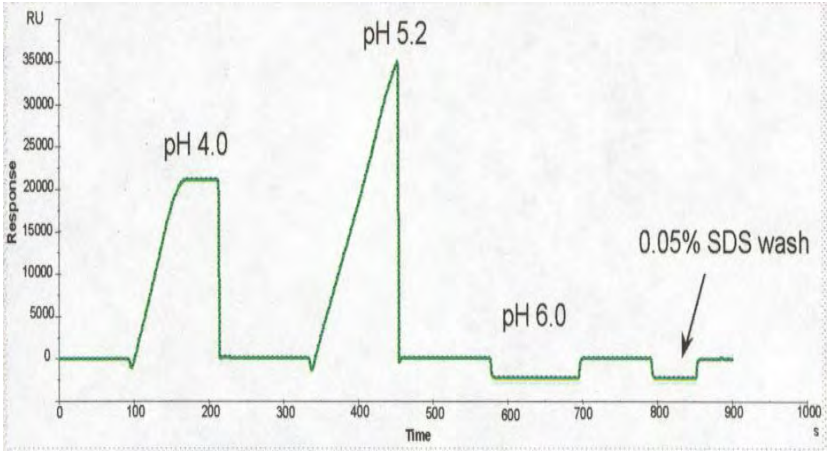
Appropriate pH: electrostatic attraction occurs

pH > pI

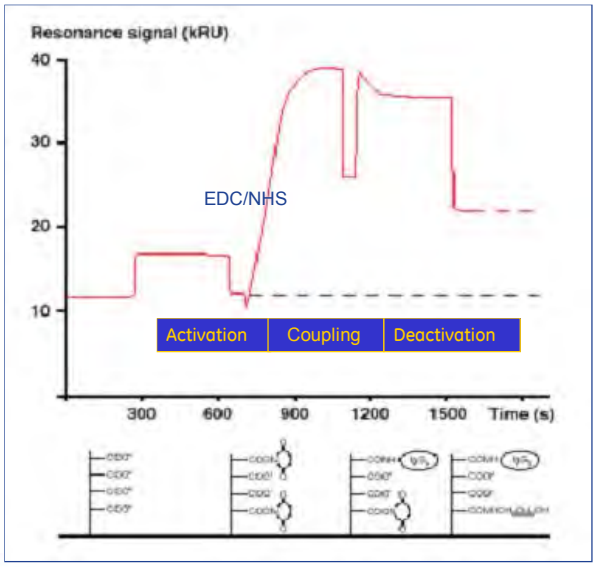
Too high pH: electrostatic repulsation

Preconcentration Tests

- Inject ligand diluted in buffers with different pH
- Gives information regarding suitable immobilization conditions



Amine Coupling



Tris buffer

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Immobilization levels

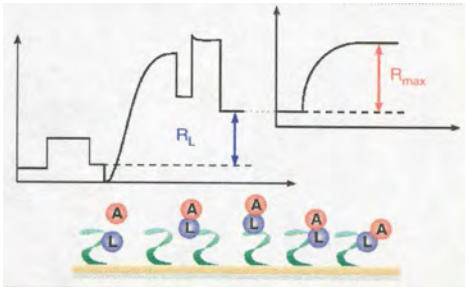
- The binding capacity of the surface depends on the immobilization level
- Different applications require different immobilization levels
- R_{max} describes the binding capacity of the surface

$$R_{max} = \frac{\text{analyte MW}}{\text{ligand MW}} \times R_L \times S_m$$

R_L = the immobilization level

S_m = the stoichiometric ratio

- The theoretical R_{max} is often higher than the experimental R_{max}



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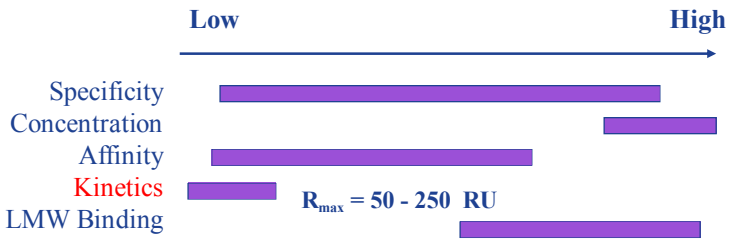
How Much to Immobilize ?

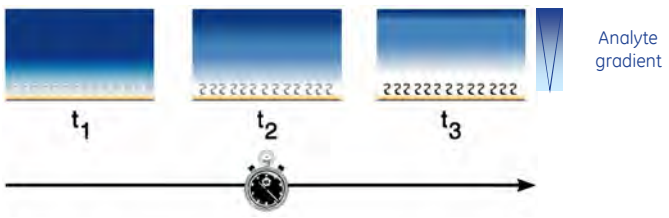
Immobilization Level is Application Specific

$$R_{max}\ (RU) = (MW_A / MW_L) \times R_L \times S_m$$



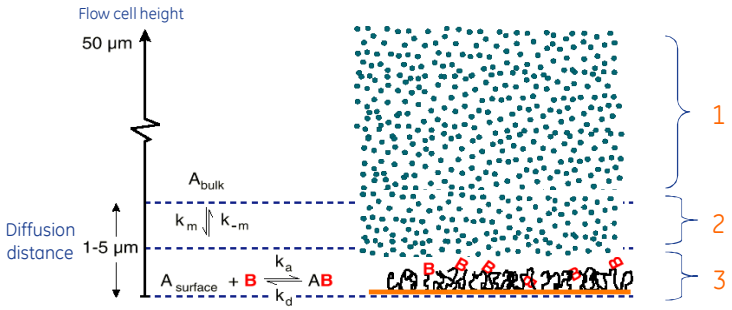
What is mass transport?

- Diffusive mass transport
  - Simple example in a static system



- Over time, analyte concentration at the surface will be depleted and a gradient will be generated through the liquid layer

Analyte consumption & supply



1. Analyte supplied by convection (continuous flow)
2. Diffusion becomes increasingly important as the flow rate reduces closer to the surface
3. Biomolecular interaction processes at the ligand/analyte interface

BIA Exercise : How much to immobilize ?

$$R_{max} = (MW_A / MW_L) \times R_L \times S_m$$

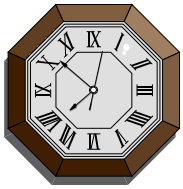
$$R_L = R_{max} (1/ S_m) (MW_L / MW_A)$$

- MW_A = 11800 Da (beta2 microglobulin)
- MW_L = 150,000 Da (anti-beta2-microglobulin)
- S= 2
- R_{max} = 50-250 RU

Solve for R_L

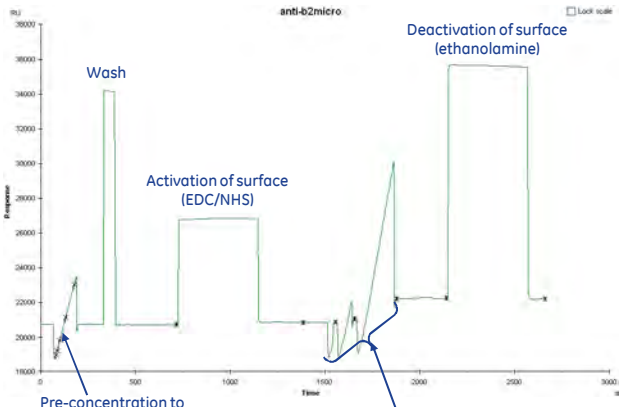
Controlling Your Immobilization

- **Protein Contact Time**
  - slope of the preconcentration curve
  - efficiency of the coupling residues
  - manual injection
- **Activation Time**
  - amount bound is linearly related to activation time
- **Protein Concentration**
  - Not easy to predict

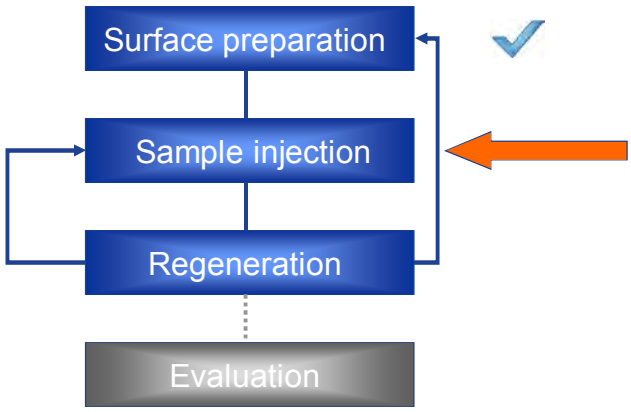


Targeting wizard to control immobilization level

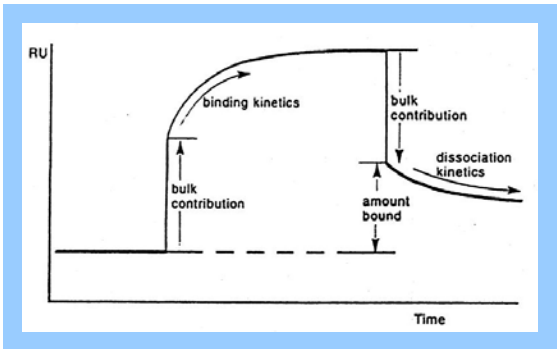
Remove the guesswork!



General steps in Biacore Assays



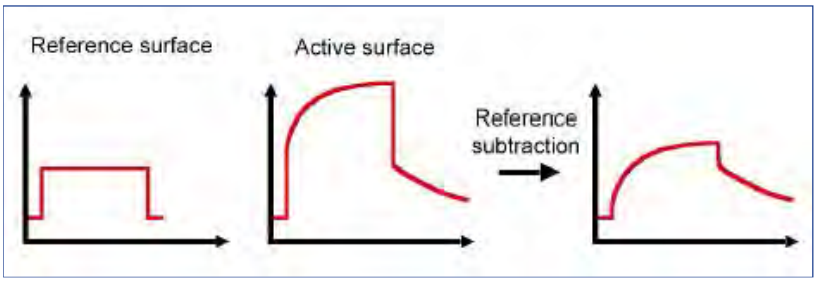
BIA Terminologies



Bulk Responses

SPR responses caused by the difference in bulk refractive index between the sample and the running buffer

Use of Reference Surfaces



Know Your Analyte

Buffer

- match running and sample buffers to reduce bulk effect
- If samples require organic solvents to aid solubility
  - Check solvent compatibility with relevant section in instrument handbook

Purity and Solubility

- is the sample homogeneous ?
- Does the biomolecule aggregate easily?

Information from other techniques

- incorporate into experimental design
- useful in data interpretation

Concentration range to use

- establish specificity : test with high analyte concentration
- kinetic and affinity experiments: use a range of concentrations

Nonspecific binding ?

Is there nonspecific binding ?

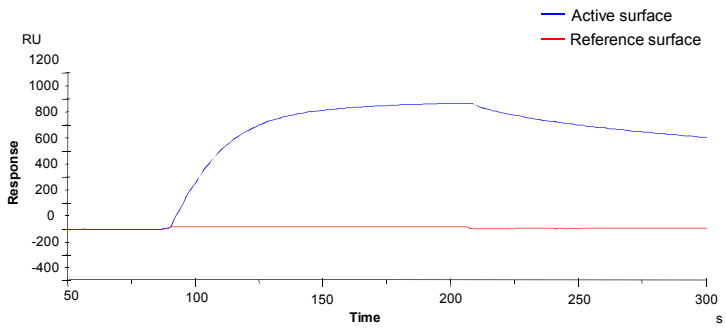
➔ If YES, to what ?
- **Use a control surface**
- **Use a blank (sample buffer) injection**

➔ try to control with alterations to sample and running buffer
- **pH, ionic strength, detergent**

➔ pioneer chips : reduce non-specific binding

Test the surface
non-specific binding test

- Initial injection before starting the "real" assay
- Use a generous concentration of analyte
- The sensorgram yields useful information on the interaction
- Useful to assess levels of non-specific binding to the reference surface



Design of reference surfaces

- Unmodified surface
  - Is acceptable as a reference surface in many cases
  - To check for non-specific binding to the dextran matrix
- Activated-deactivated surface
  - Treating the surface with the immobilization procedure, but omitting the ligand
  - Decreases the negative charge on the surface and may reduce non-specific binding
- Surface immobilized with dummy ligand
  - A protein that does not bind the analyte may be immobilized to approx. the same level as the ligand to mimic the active surface as closely as possible

Preparation of Sample and Buffers

Preparation of Ligand

- purity at least 95% pure by silver stain
- homogeneity
- concentrations: 20-200ug/ml

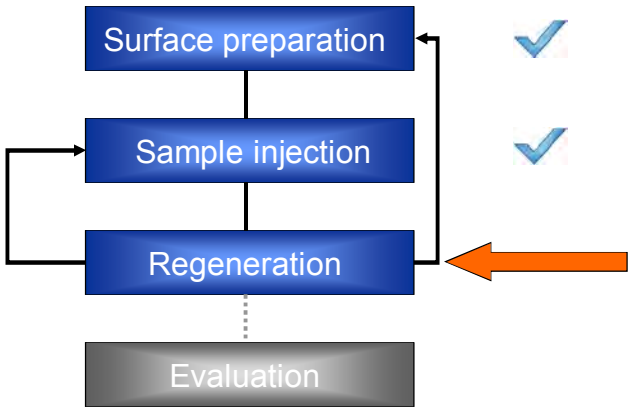
Preparation of Analyte

- concentrations ;mg/ml for accurate dilutions to, around KD values
- volumes :50 – 100 ul per injection
- match analyte buffer with running buffer

Preparation of Running Buffer

- filtered 0.22 um
- degassed
- include appropriate ions for binding interactions

General steps in Biacore Assays

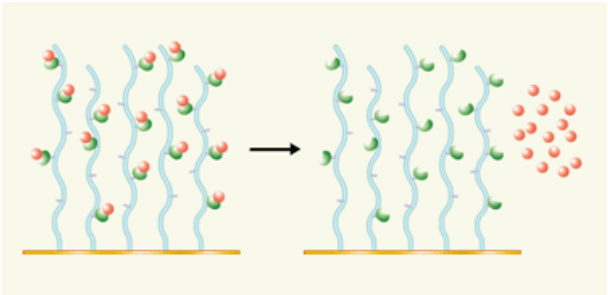


GE imagination at work

BIACORE

Regeneration

- Removes bound analyte completely from the surface
- The activity of the surface must remain unaffected
- Efficient regeneration is crucial for high-quality data



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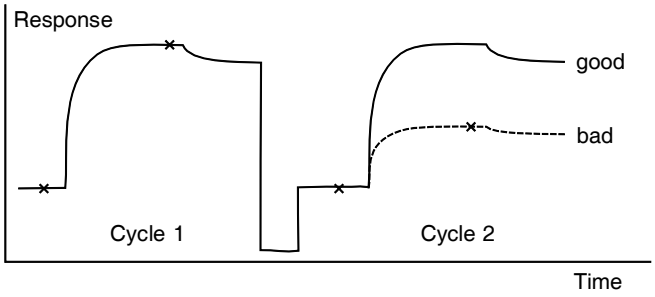
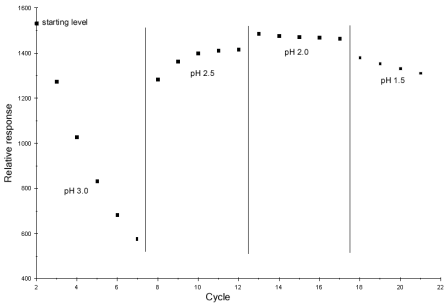
Testing regeneration conditions



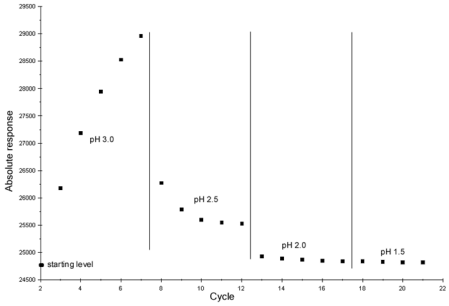
- Efficient regeneration removes all bound analyte
- A second injection of analyte reveals whether the ligand is still fully active
- Repeated cycles of analyte and regeneration injections are required to fully assess the conditions selected

Interpreting trends in analyte binding & baseline responses

Analyte binding response



Baseline response



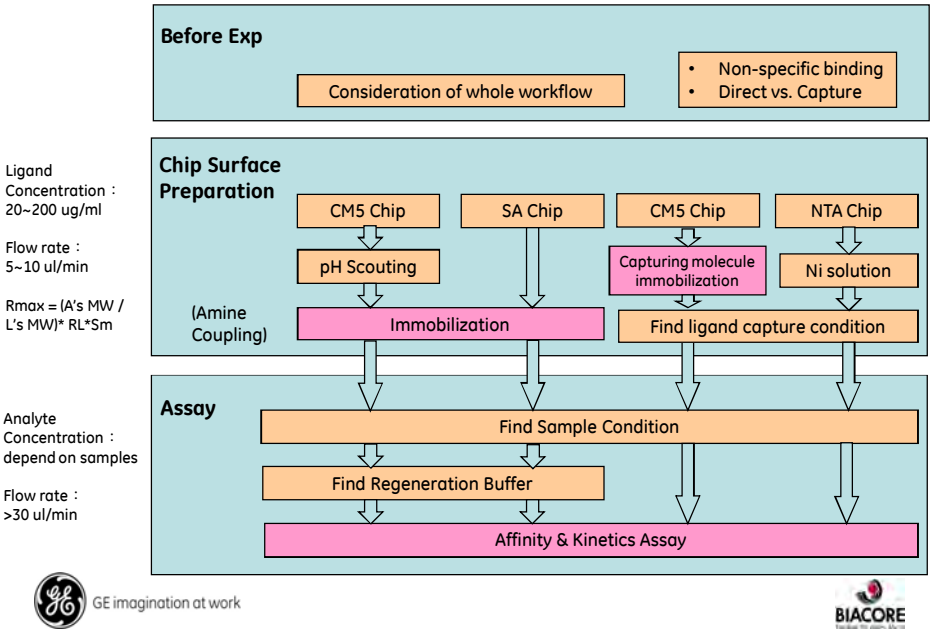
Regenerations

	Acidic	Basic	Hydrophobic	Ionic
Strong	PH<2 Glycine/HCl Formic Acid H3PO4	PH>10 NaOH KOH	50% ethylene glycol	6M Guanidin chloride
Intermediate	PH< 2 – 2.5 Glycine/HCl Formic Acid H3PO4	PH 9 – 10 Glycine/NaO NaOH KOH	40% ethylene glycol	2M MgCl2 4M KCl 3M KSCN
Weak	PH >2.5 Glycine/HCl Formic Acid H3PO4	PH < 9 HEPES / NaOH	25% ethylene glycol	1M NaCl

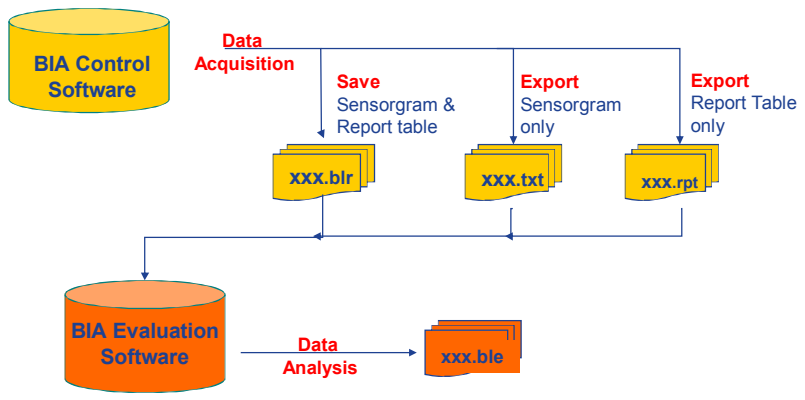
Regeneration Strategies

- Start with mild conditions
- Increase flow rate to 50-100 uL/min
  - **Follow injection with EXTRACLEAN**
- Use short contact times with pH extremes
  - **0.5-2 min, multiple short injections**
- Use longer contact times with high ionic strength
  - **2-5 min, one long injection**
- Test analyte binding in between trials
- Monitor baseline - may vary for first few trials

Summary of Experimental Design



BIA files and formats



GE Healthcare

Biacore

Maintenance – Why?

- Careful and thorough maintenance is essential for the performance of your Biacore system
- Many reported "problems" arise because of poor instrument maintenance routines
- Reproducibility may be affected by poor instrument maintenance

Follow the recommended system maintenance procedures!

Daily maintenance

- Filter and degas buffers
- Use Prime to flush the system
- Leave the instrument in Standby mode between experiments

Weekly maintenance

- Cleaning the liquid system
  - Run Desorb from the menu Tools: Working Tools
- Removal of salt residues
  - Rinse connector block and injection port on site with deionized water
  - Wipe injection needle and vial dislodger with wet tissue
- Inspection of pumps
  - Inspect pump tips for leakage
  - Inspect pump tips for contamination
  - If cleaning becomes necessary, follow instructions under Tools: Service Tools: Syringe/Tip

Monthly maintenance

- Cleaning the liquid system
  - Run Desorb from the menu Tools: Working Tools
  - Run Sanitize from the menu Tools: Working Tools
- Checking system performance
  - Run System Check from the menu Tools: Test Tools

How do I leave my Biacore system after the experiment?

- The system will be left for <4 days:
  - Use Standby to maintain the buffer flow until the next experiment

- The system will be left for >4 days:
  - Perform Shutdown

Storage of Sensor Chips (immobilized)

Storage conditions/ stability depend on the nature of attached ligand

Wet Storage :

- remove support from cassette
- Insert in buffer (50ml centrifuge tubes) at 4C
- before use: wipe off excess buffer from plastic support and BACK of gold chip, Do NOT touch surface, reinsert PROPERLY into cassette.

Dry Storage:

- Wrap cassette in saran wrap and store at 4C .
- Before use: re equilibrate to room temperature.

Biacore Web Site

http://www.biacore.com/lifesciences/index.html

- Download softwares
- Download manual/instruction
- Webinar
- Reference database
- On-line training course
- Product information

Thank you for your attention