2019. 7. 24 高階醫藥研發轉體與研究平台實作研習營

局部區域表面電漿共振LSPR (Localized Surface Plasmon Resonance)

Speaker : 林俐吟 & 游季禹

nicoya

BioSafe 俊欣生技

1. 什麼是表面電漿共振SPR (Surface Plasmon Resonance)?

2. SPR的重要性

3. 新一代的局部區域表面電漿共振LSPR (Localized Surface Plasmon Resonance)

4. 應用實例分享

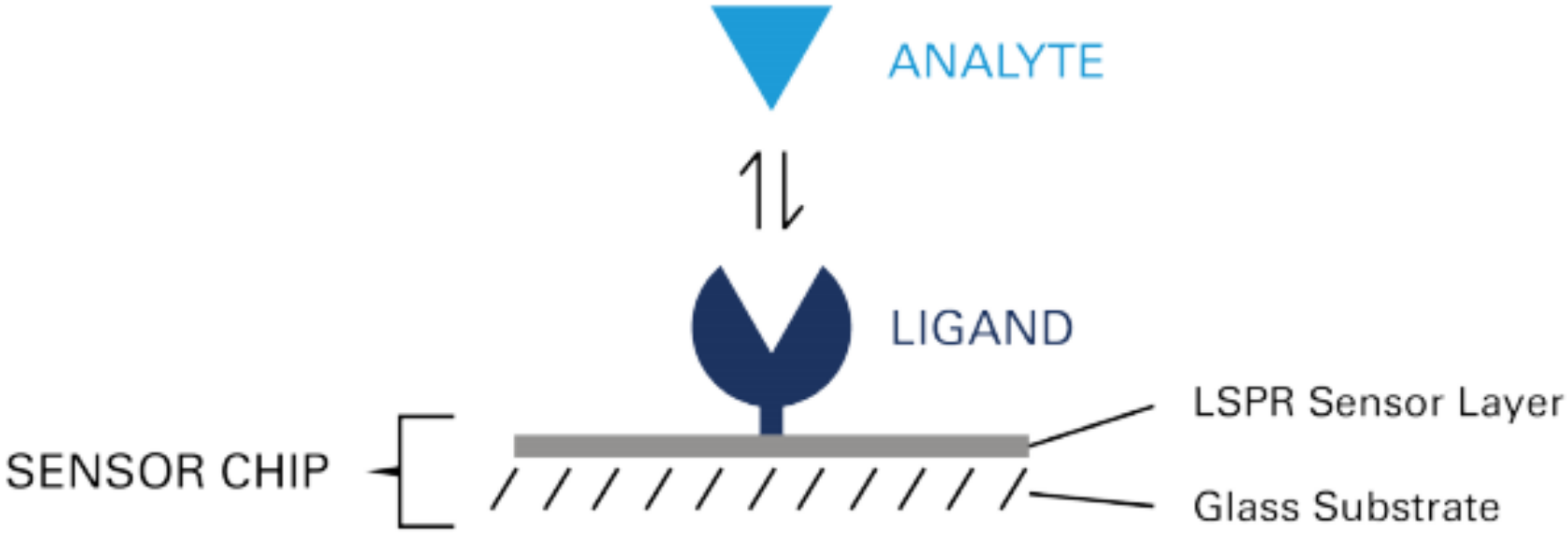
5. LSPR操作流程

6. LSPR上機實作

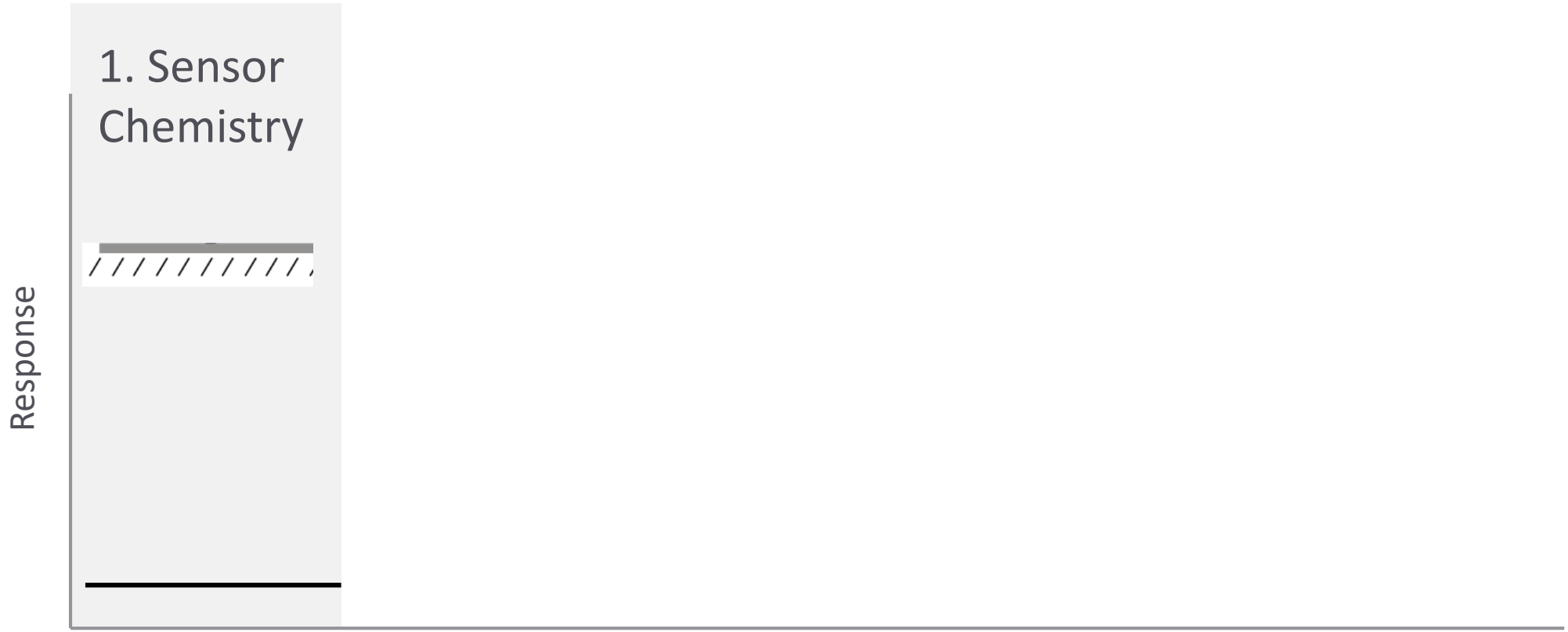
1. 什麼是表面電漿共振SPR (Surface Plasmon Resonance)?

Surface Plasmon Resonance

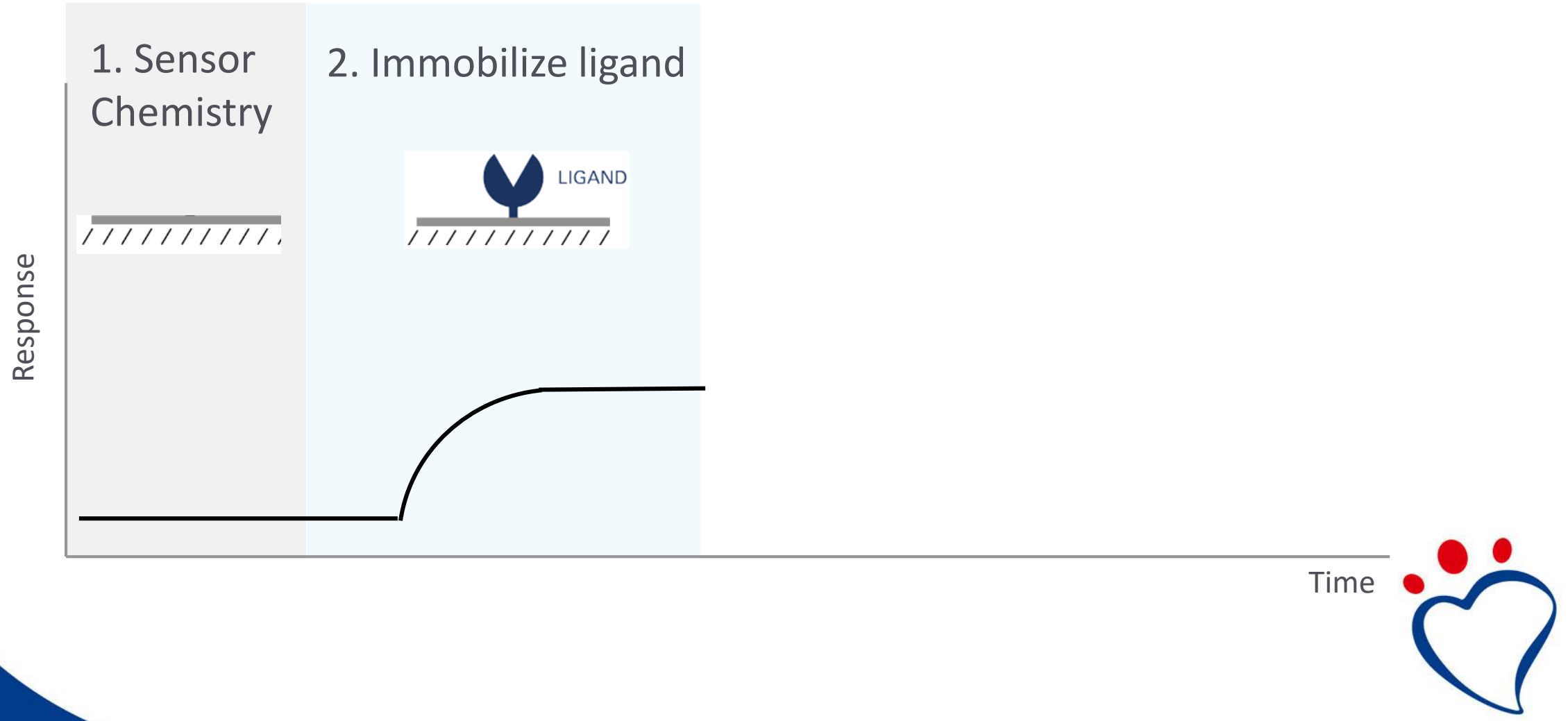
Label-Free Interaction Analysis



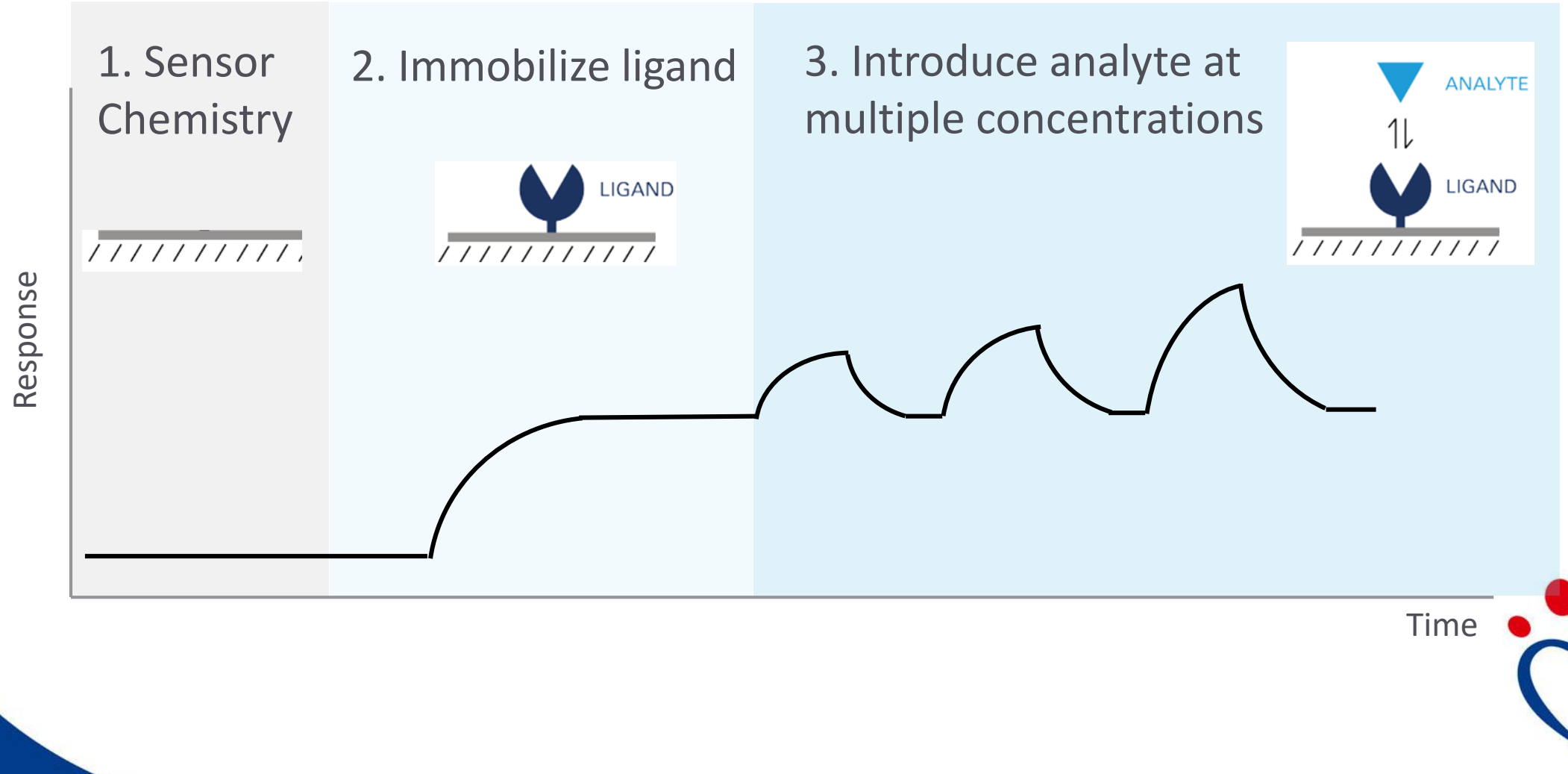
General Overview of an SPR Experiment



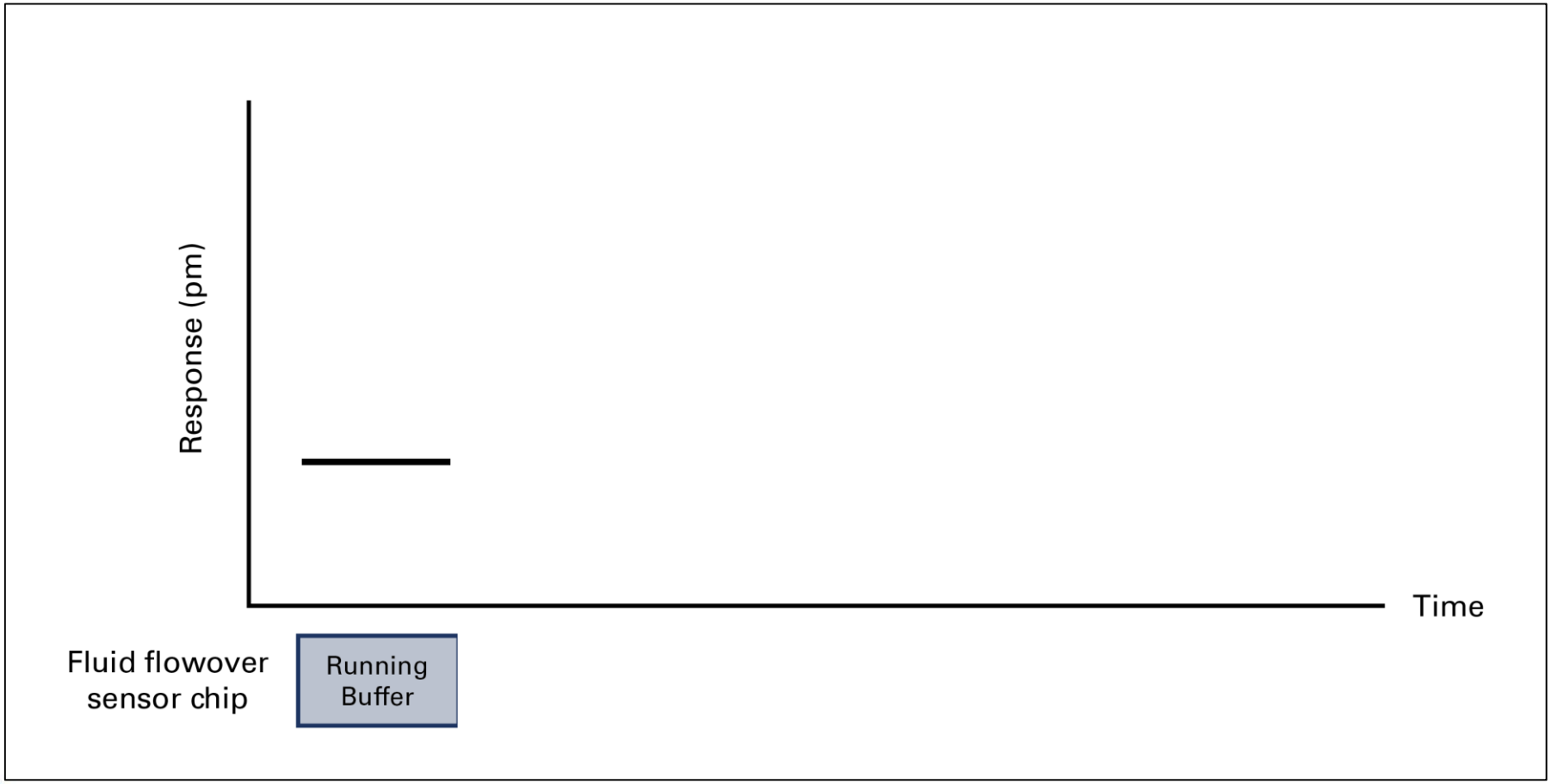
General Overview of an SPR Experiment

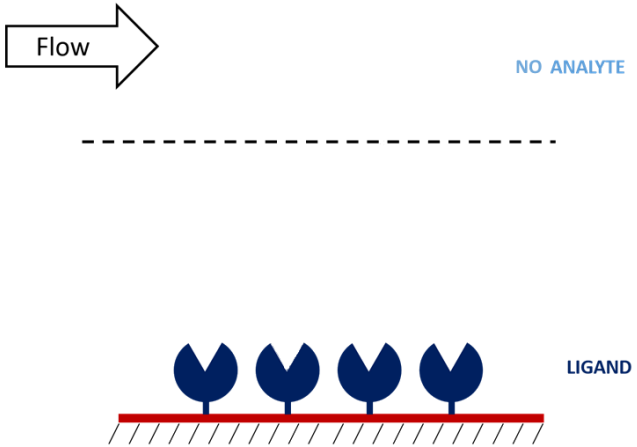


General Overview of an SPR Experiment



Binding Kinetics: Analyte Binding Curve





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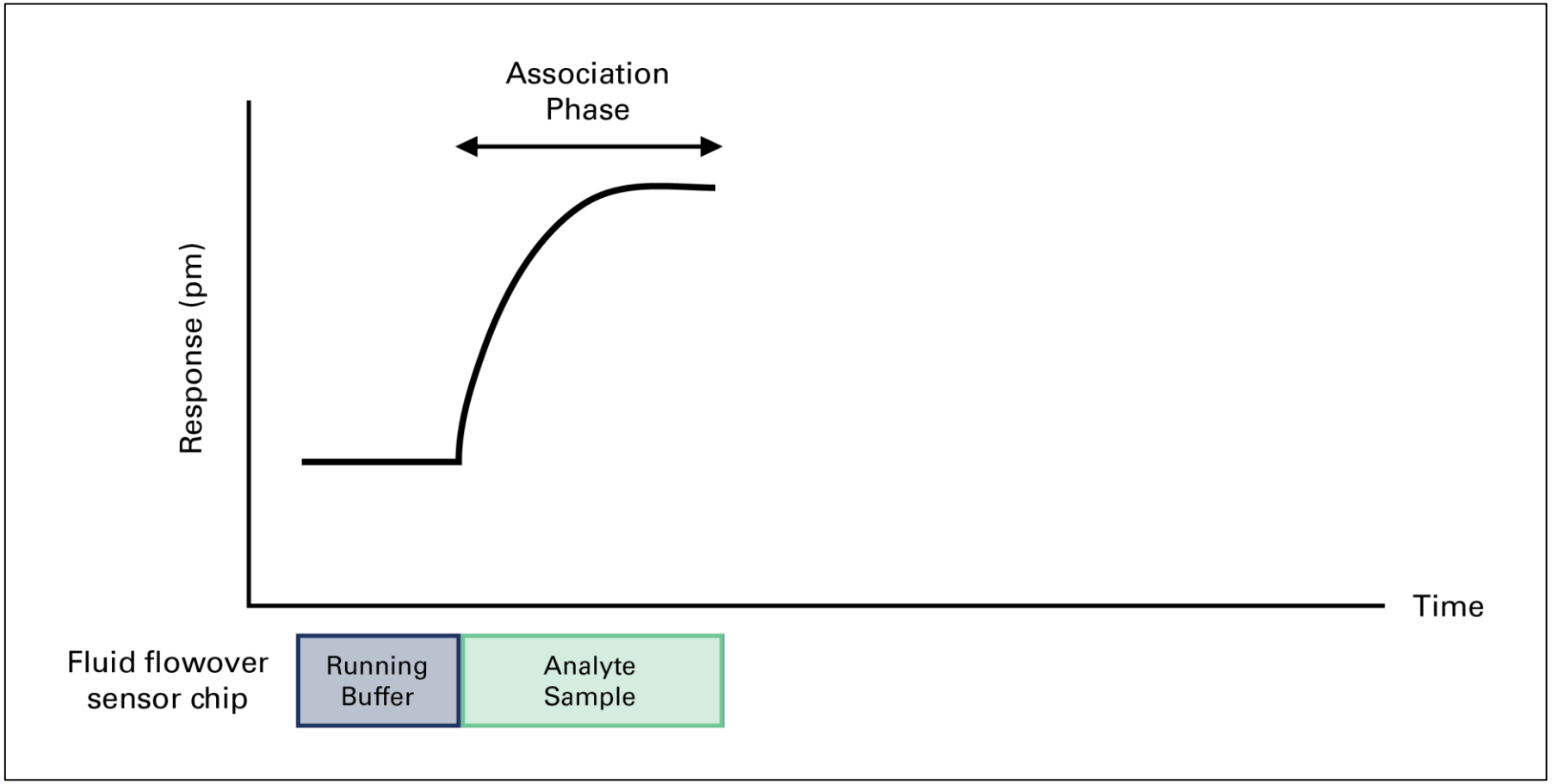

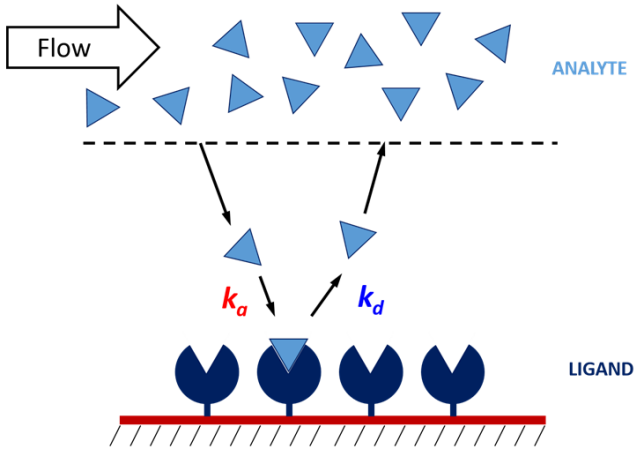

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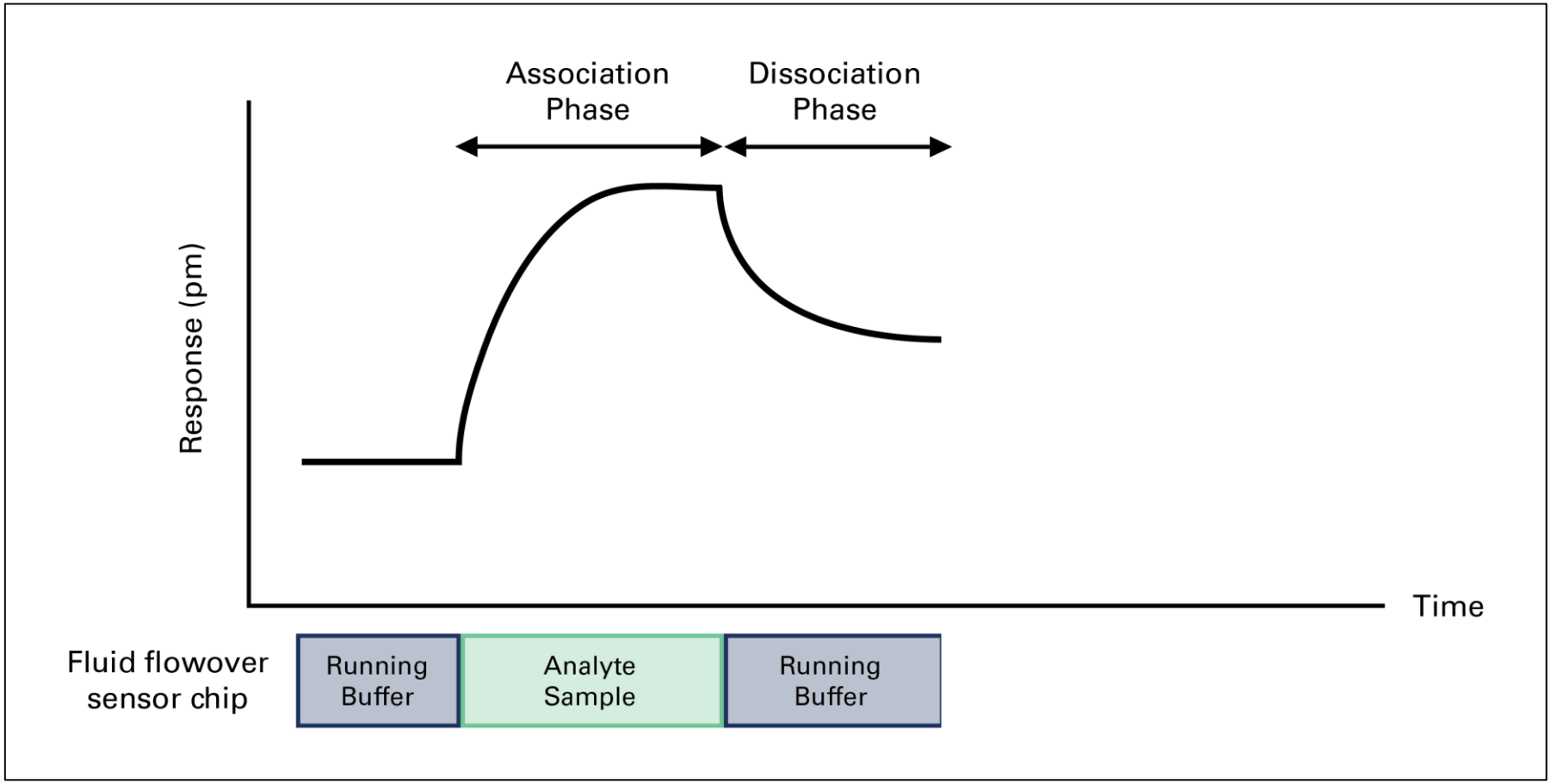
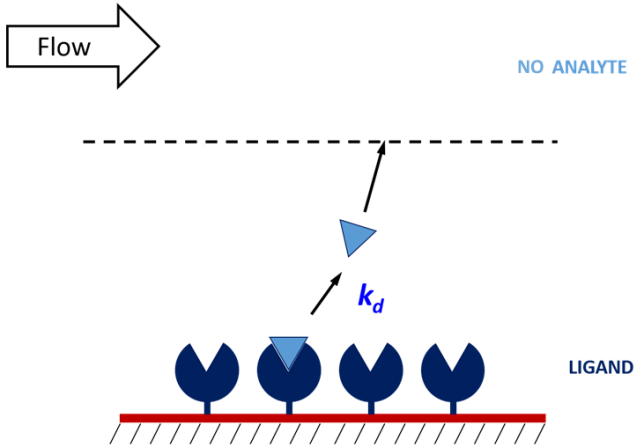
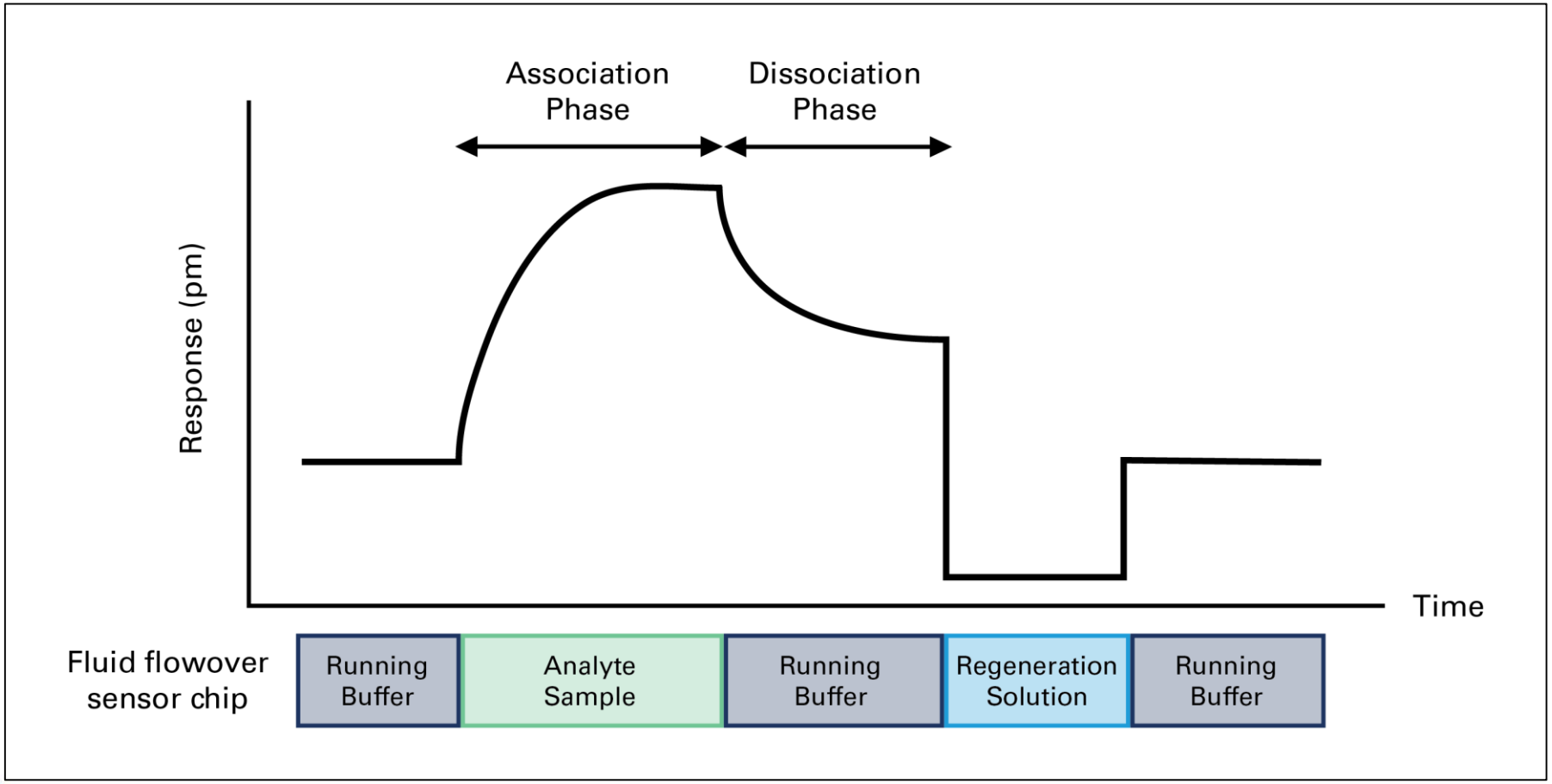
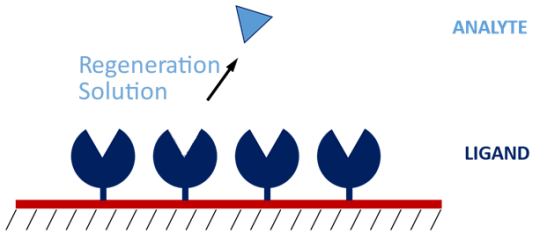


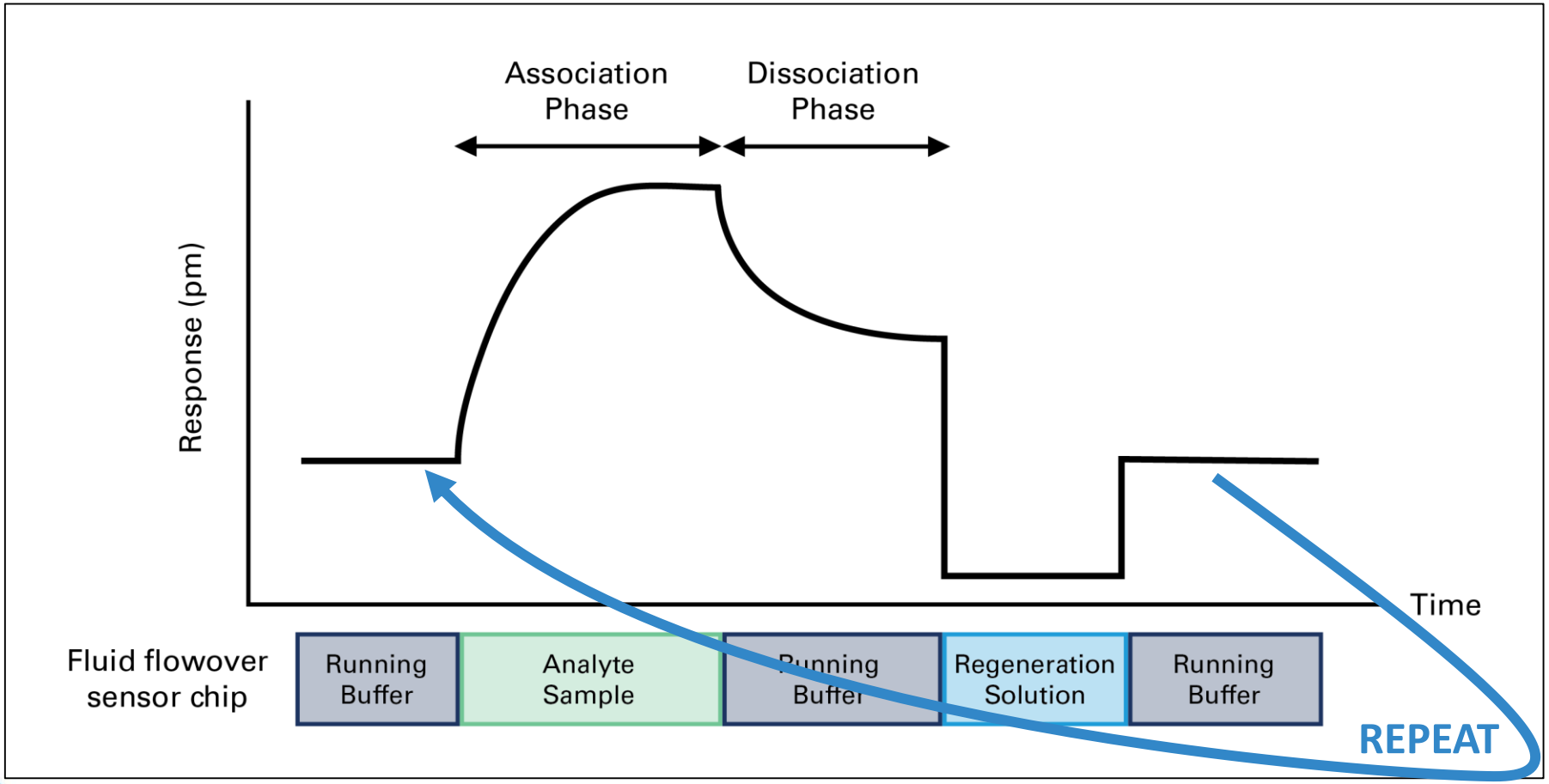


Binding Kinetics: Analyte Binding Curve





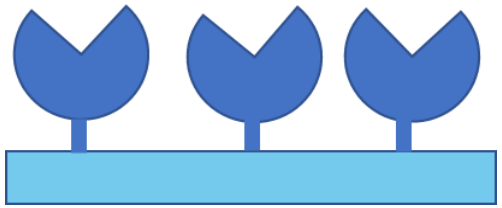
Binding Kinetics: Analyte Binding Curve



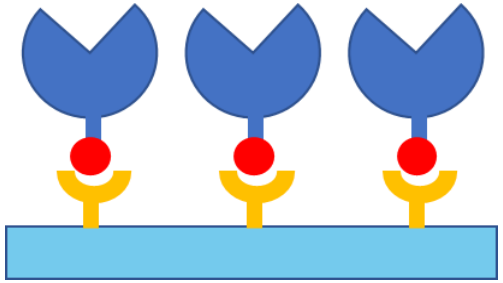
- Repeat 3 to 5 different concentrations of analyte

Sensor Chips

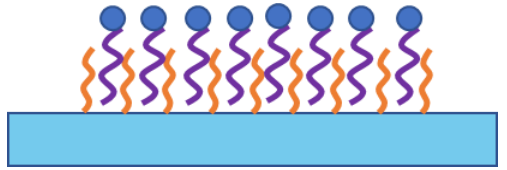
Covalent Coupling

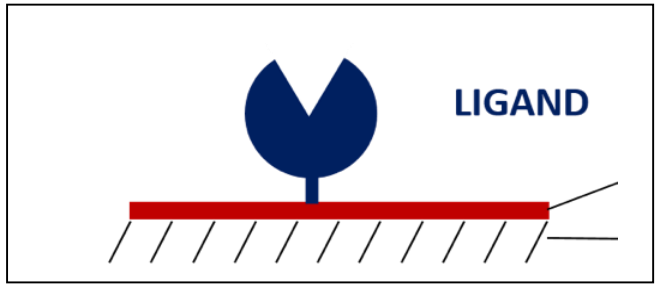


Capture Coupling



Lipid Capture

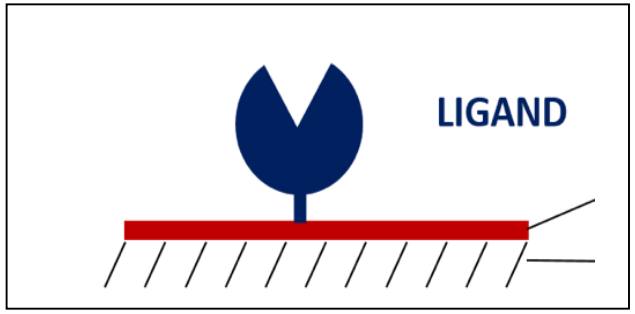




Sensor Chips

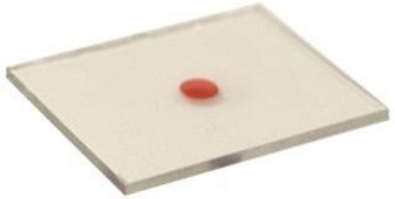
Covalent Coupling

- Carboxyl
- Amine
- Thiol



Capture Coupling

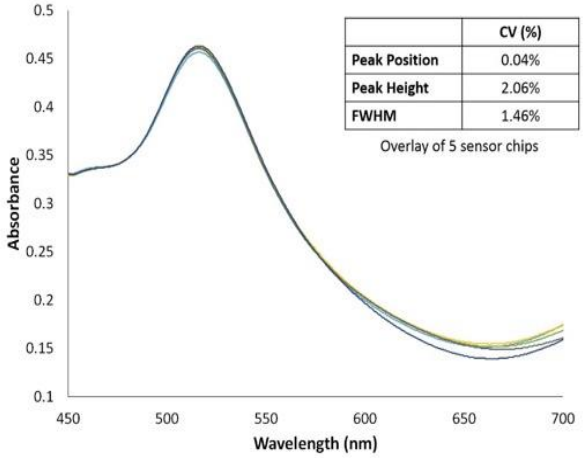
- Streptavidin/Biotin
- NTA
- GST
- Protein A



High sensor repeatability

Lipid Capture

- Liposome (LIP)
- Hydrophobic (MEM)



SENSOR CHIP	COATED FOR IMMOBILIZATION OF:
Carboxyl	any amine group via EDC/NHS
NTA	his-tagged targets
Streptavidin	biotin tagged targets
Biotin	streptavidin coupled targets
GST	GST-tagged biomolecules/proteins
Protein A	IgG based antibodies
Amine	any carboxyl group via EDC/NHS
Liposome Binding (LIP)	liposomes/membrane proteins
Hydrophobic (MEM)	lipid monolayer
Thiol	maleimide-taged ligands
Standard Gold	non-functionalized and perfect for custom surface chemistry development and ligands with thiol groups

Applications

Binding Kinetics/Equilibrium Constants:

- Protein-Protein
- Protein-Antibody
- Protein-Nucleic Acid
- Protein-Lipid
- Protein-Carbohydrates
- Protein-Small Molecule
- Protein-Peptide
- Protein-Aptamer
- DNA, RNA
- Virus particles
- Cells & bacteria
- Nanoparticles
- Polymers

Materials science

Detection

Quantification

Nanomaterial characterization

2. SPR的重要性

Yes/No Binding

FP

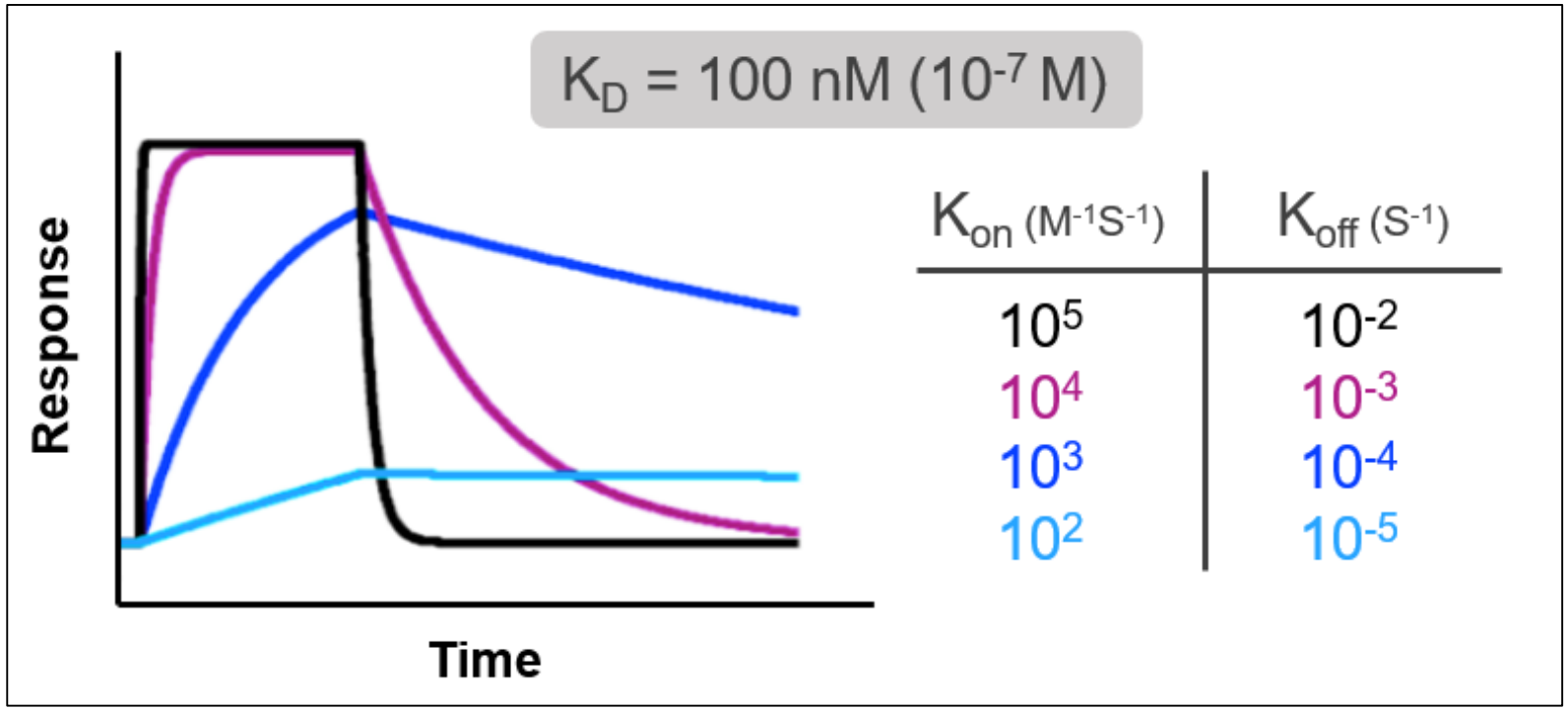
EMSA

Pull down

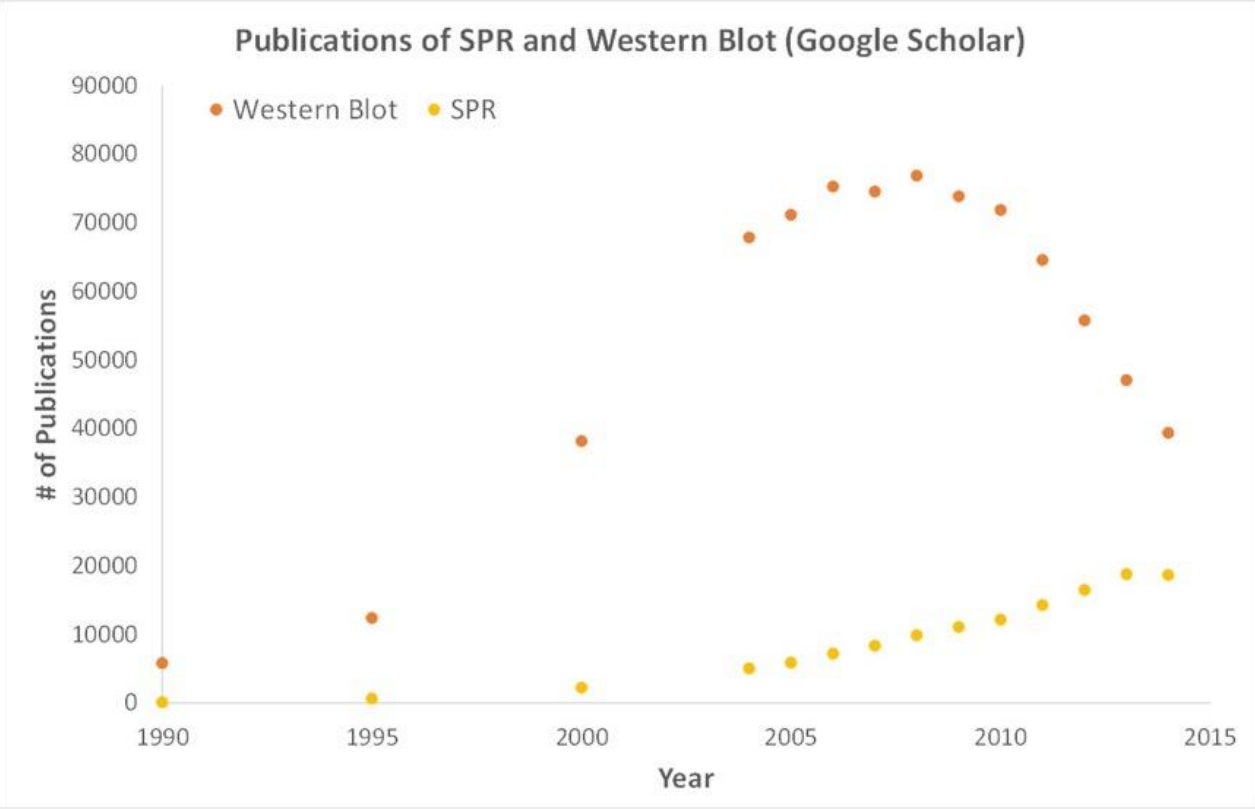
ELISA

SPR uncovers what's underneath the surface

Affinity Doesn't Tell the Whole Story



Different interactions can have the same affinity.



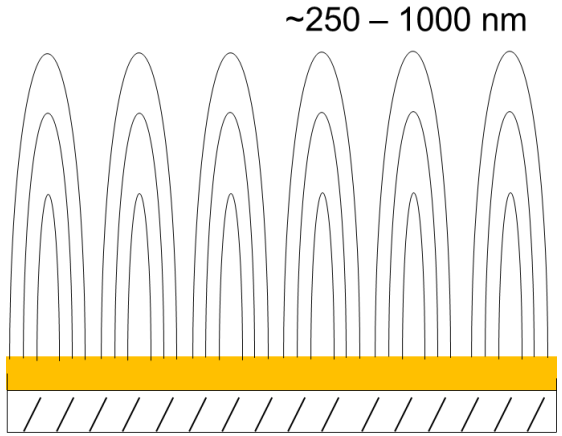
Scientific publications involving **Surface Plasmon Resonance (SPR)** have increased drastically over the years.

3. 新一代的局部區域表面電漿共振LSPR (Localized Surface Plasmon Resonance)

Traditional SPR Measurement Technique

Sensor Surface:

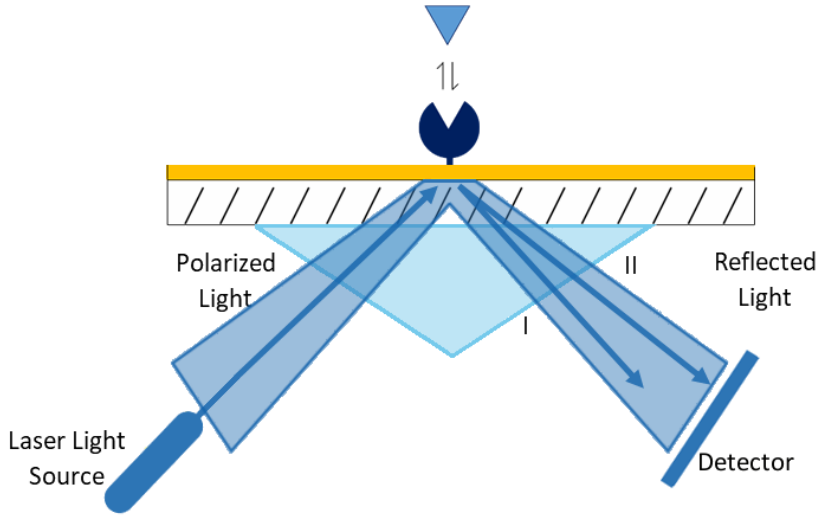
- Thin film of gold
- Surface plasmon resonance

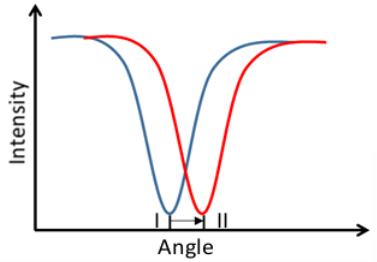


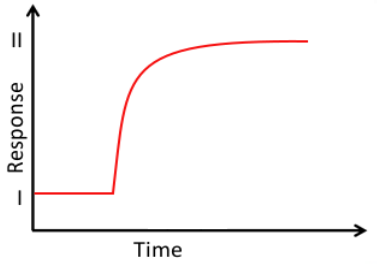
Traditional SPR

Detection:

- Change in refractive index
- Reflectance angle



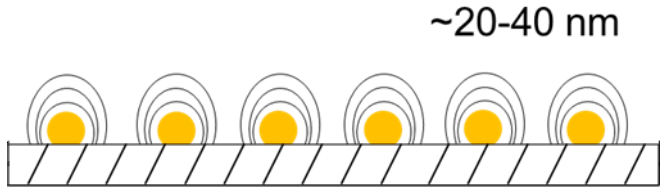




LSPR Measurement Technique (OpenSPR)

Sensor Surface:

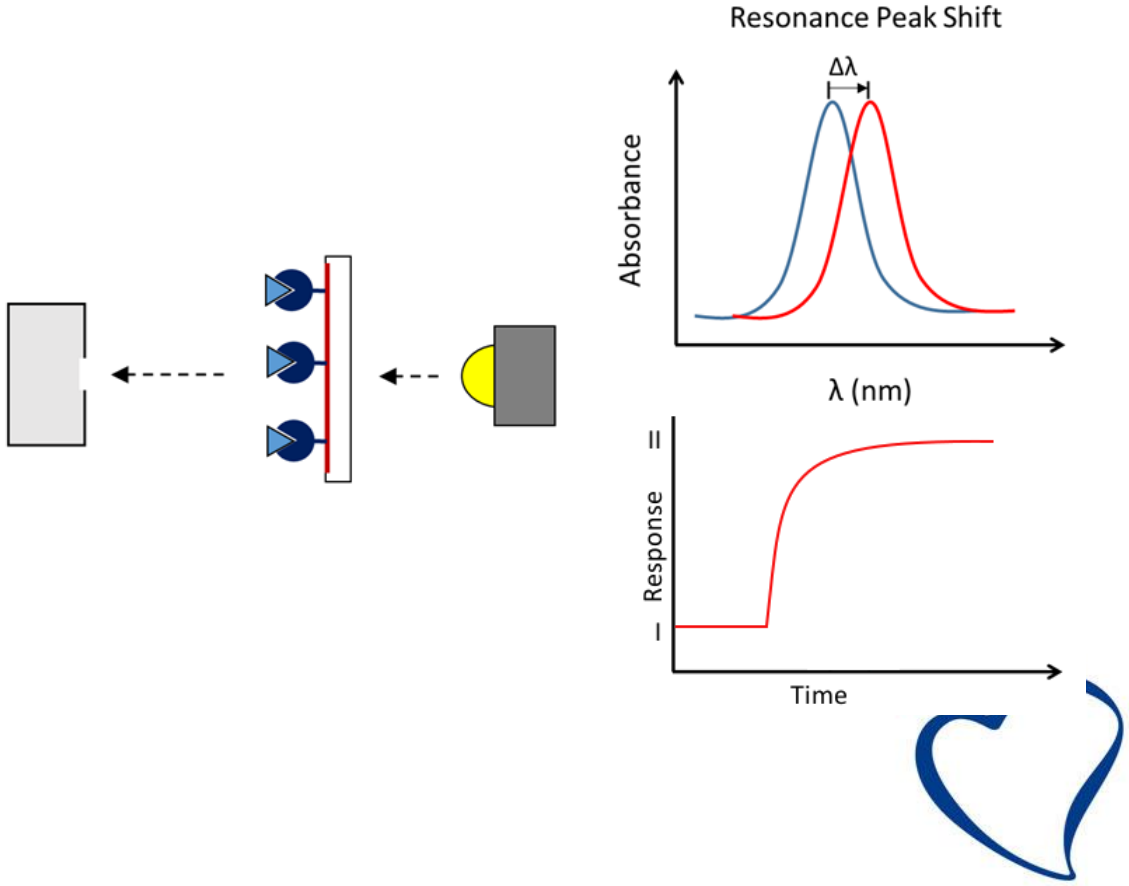
- Nano-structured gold
- ***Localized*** Surface plasmon resonance



LSPR

Detection:

- Change in refractive index
- Absorbance (change in peak position)



LSPR

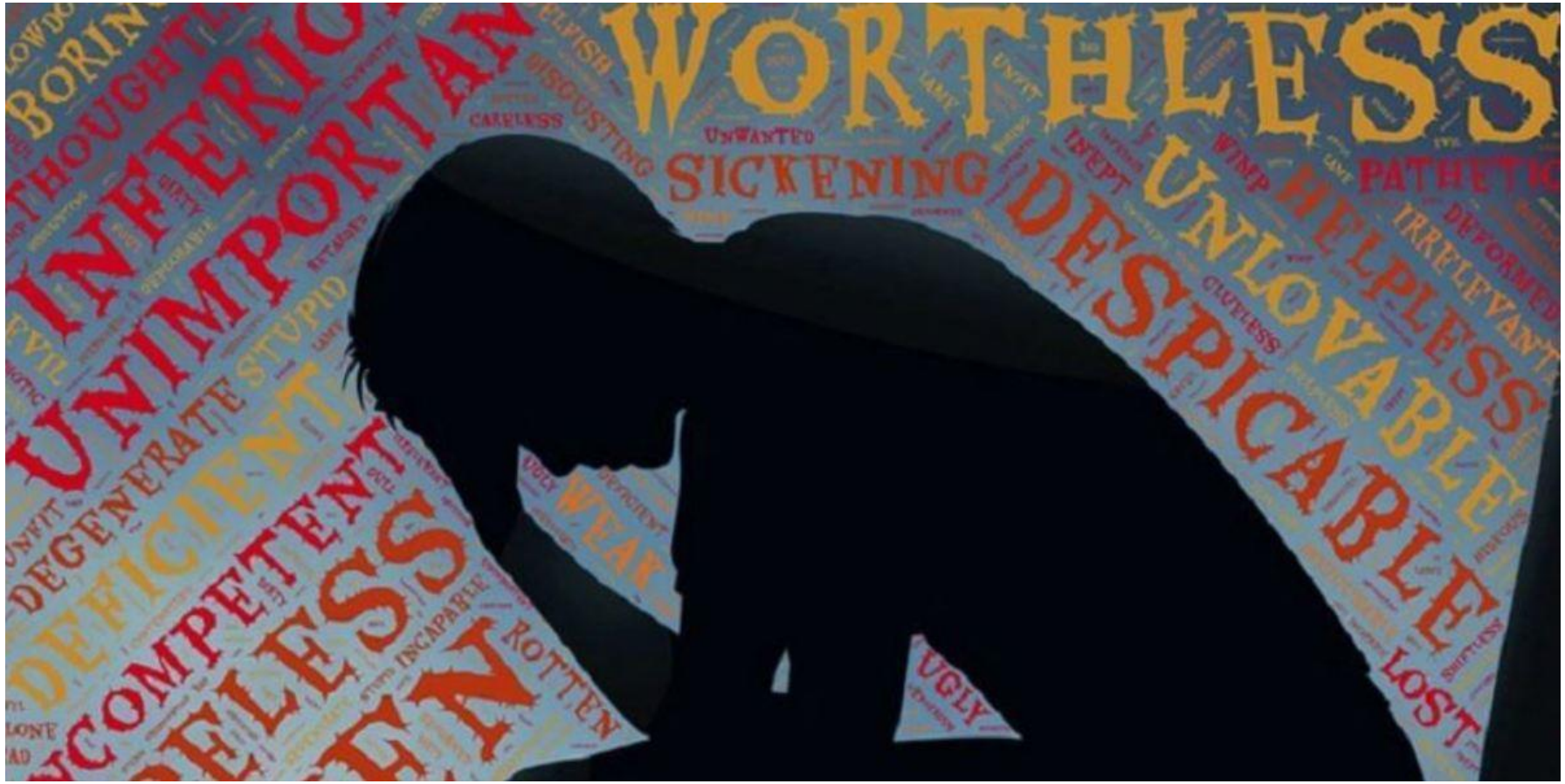
- Bench Top instrument
- User friendly
- Save time
- Low-Maintenance

SPR

- High throughput

4. 應用實例分享

抗憂鬱藥的旅程



International Immunopharmacology 67 (2019) 119–128

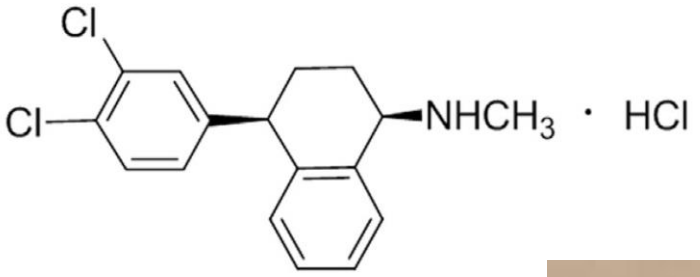
Contents lists available at ScienceDirect

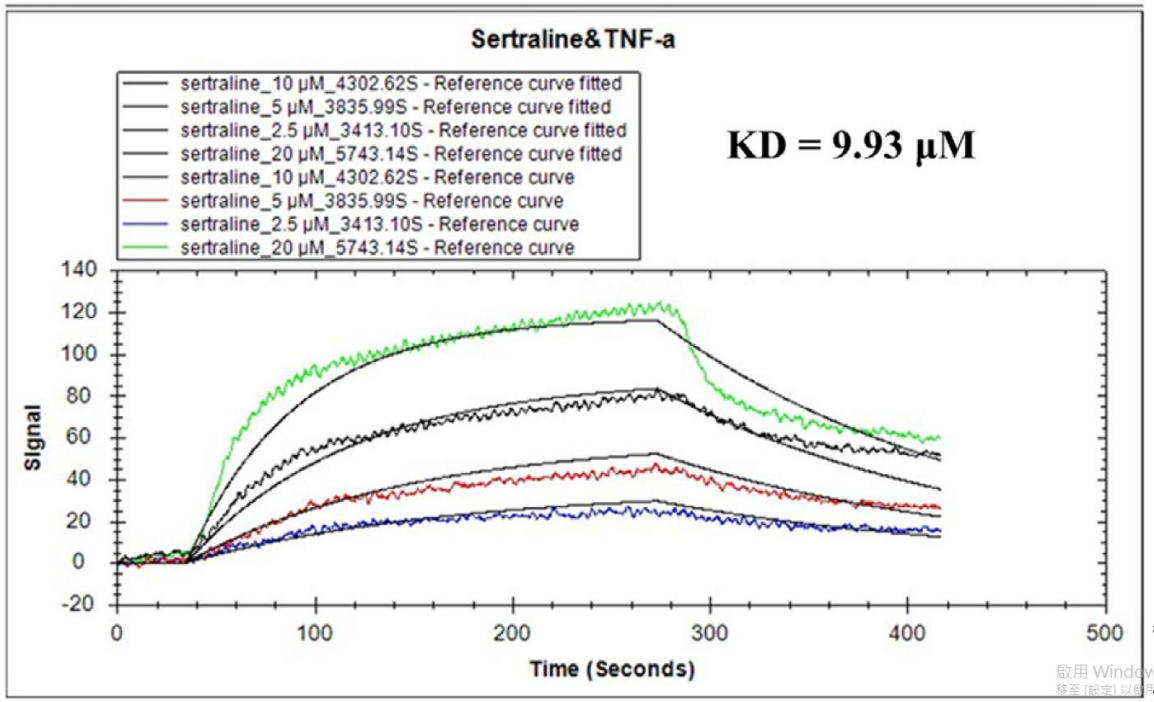
International Immunopharmacology

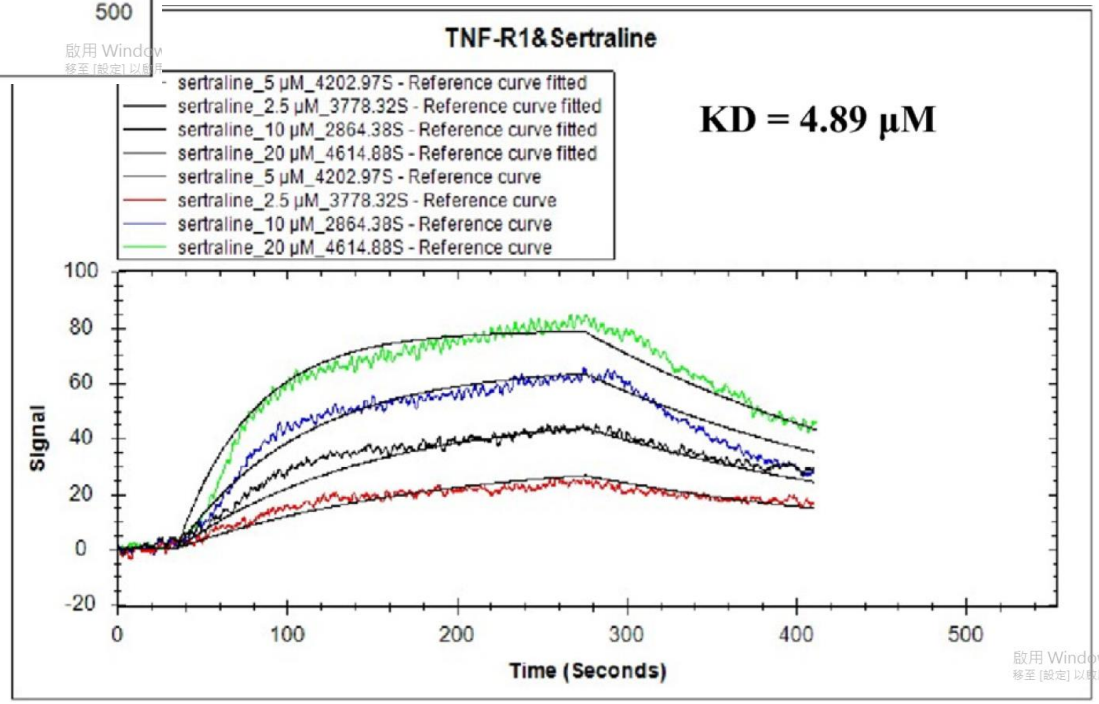
journal homepage: www.elsevier.com/locate/intimp

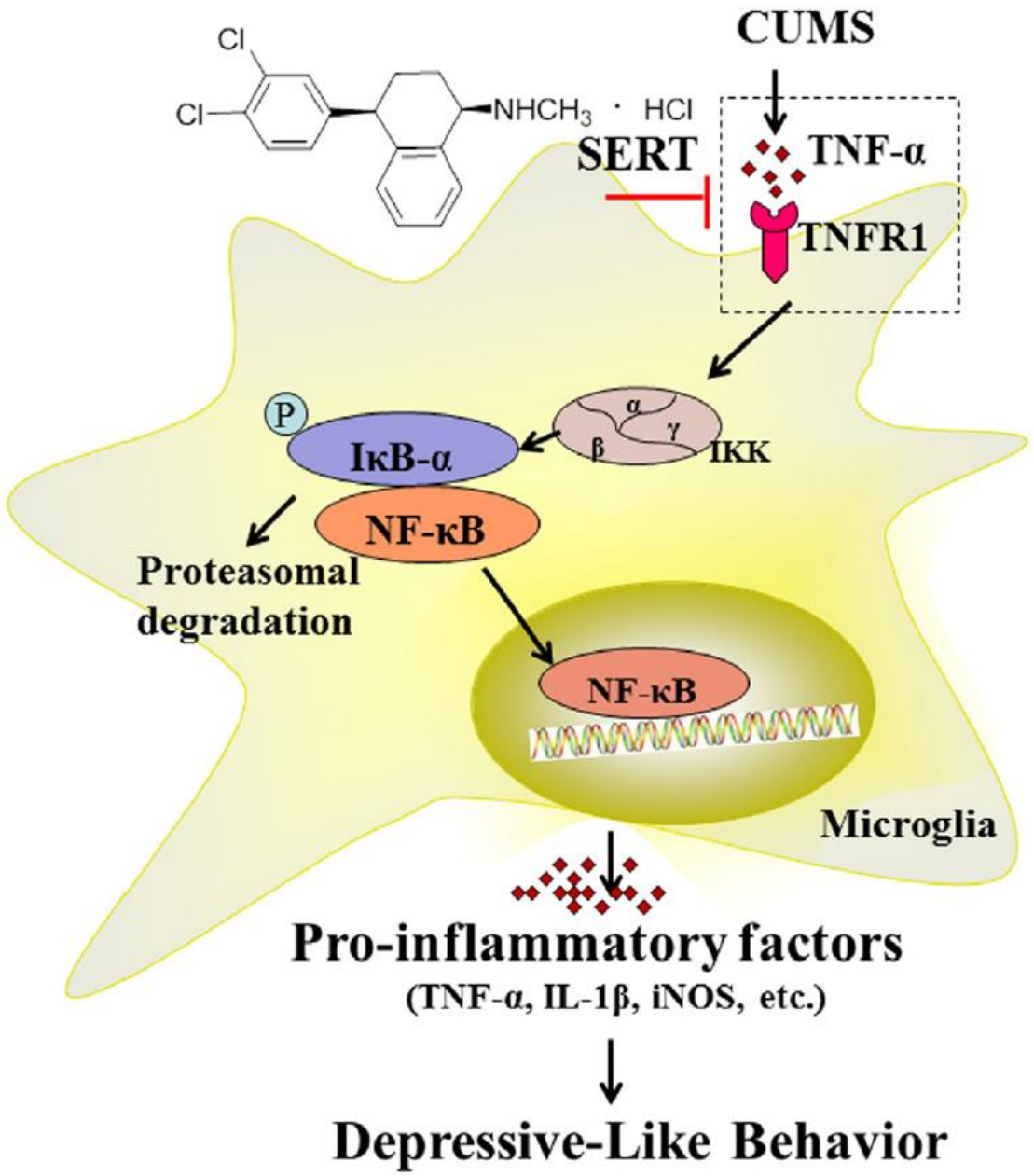
Sertraline ameliorates inflammation in CUMS mice and inhibits TNF-α-induced inflammation in microglia cells

Ying Lu[a,1], Xiang Xu[a,1], Tong Jiang[a], Lan Jin[a], Xu-Dong Zhao[a], Jia-Hui Cheng[a], Xue-Jun Jin[a], Juan Ma[a], Hu-Nan Piao[b,*], Lian-Xun Piao[a,*]


Cl
Cl
NHCH3 · HCl

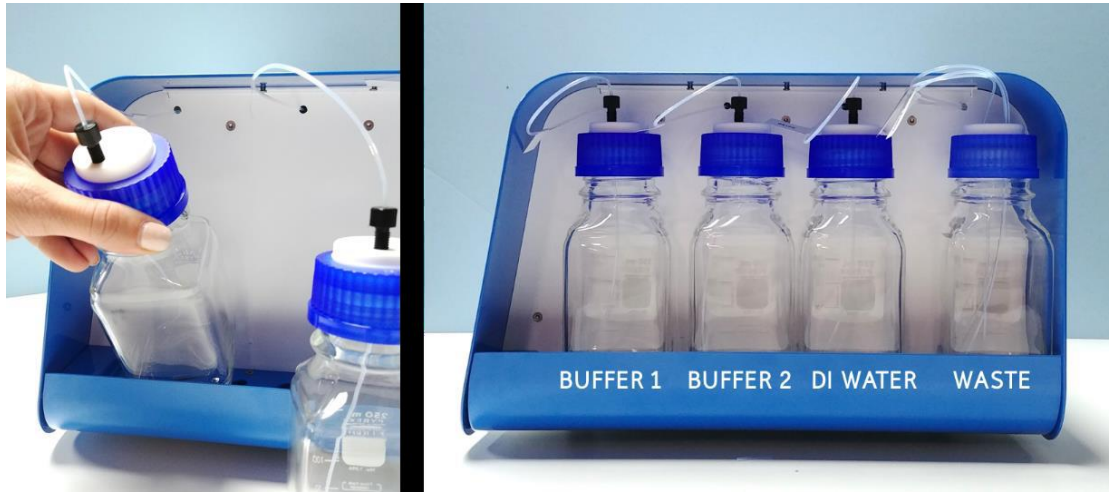

Sertraline&TNF-a
sertraline_10 μM_4302.62S - Reference curve fitted
sertraline_5 μM_3835.99S - Reference curve fitted
sertraline_2.5 μM_3413.10S - Reference curve fitted
sertraline_20 μM_5743.14S - Reference curve fitted
sertraline_10 μM_4302.62S - Reference curve
sertraline_5 μM_3835.99S - Reference curve
sertraline_2.5 μM_3413.10S - Reference curve
sertraline_20 μM_5743.14S - Reference curve
KD = 9.93 μM
Signal
Time (Seconds)


TNF-R1&Sertraline
sertraline_5 μM_4202.97S - Reference curve fitted
sertraline_2.5 μM_3778.32S - Reference curve fitted
sertraline_10 μM_2864.38S - Reference curve fitted
sertraline_20 μM_4614.88S - Reference curve fitted
sertraline_5 μM_4202.97S - Reference curve
sertraline_2.5 μM_3778.32S - Reference curve
sertraline_10 μM_2864.38S - Reference curve
sertraline_20 μM_4614.88S - Reference curve
KD = 4.89 μM
Signal
Time (Seconds)


Cl
Cl
NHCH3 · HCl
CUMS
SERT
TNF-α
TNFR1
P
IκB-α
α
β
γ
IKK
NF-κB
Proteasomal
degradation
NF-κB
Microglia
Pro-inflammatory factors
(TNF-α, IL-1β, iNOS, etc.)
Depressive-Like Behavior

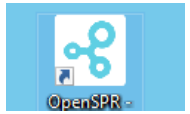
LSPR操作流程

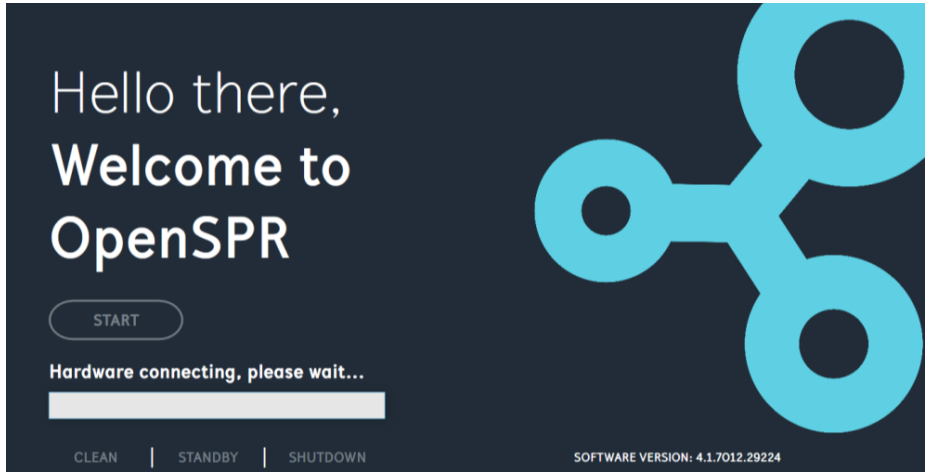
1. 放置實驗所需之buffer以及DI water

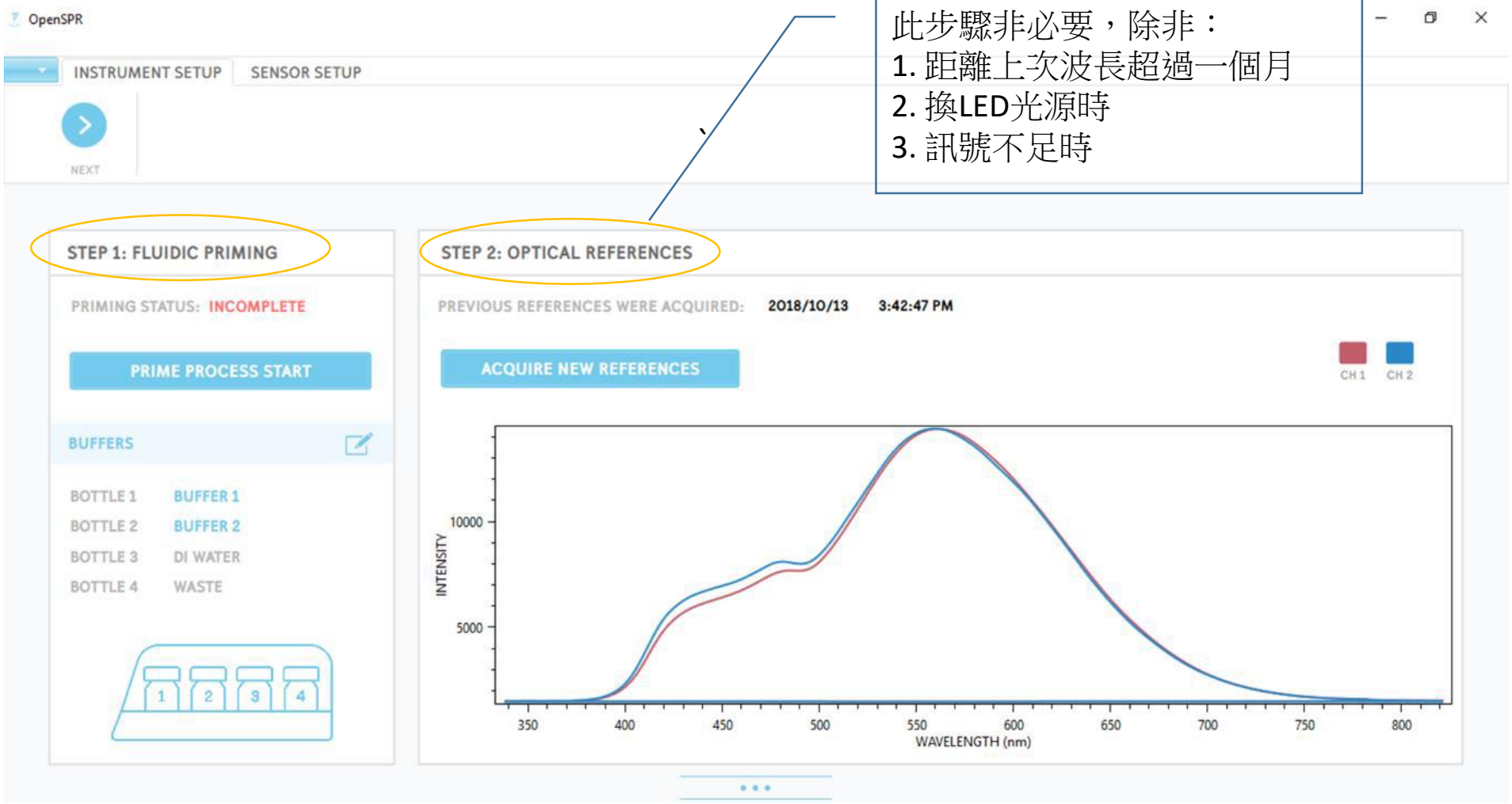


2. 開啟儀器電源

3. 開啟電腦桌面之軟體

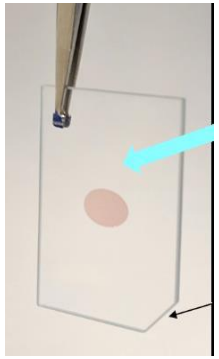






此步驟非必要，除非：
1. 距離上次波長超過一個月
2. 換LED光源時
3. 訊號不足時

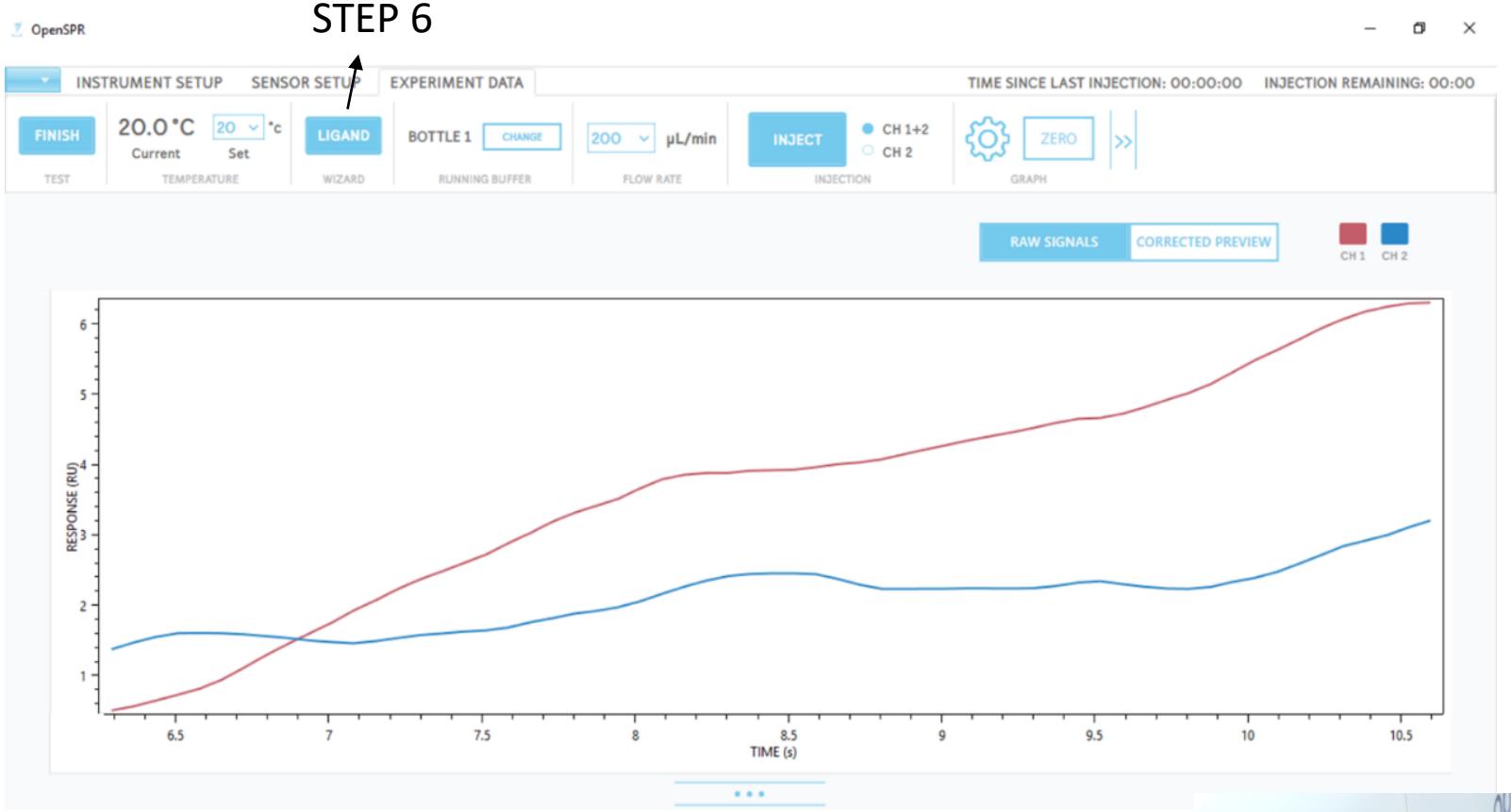
STEP 5

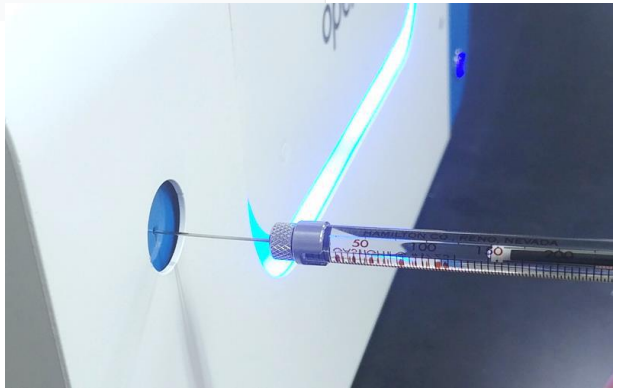


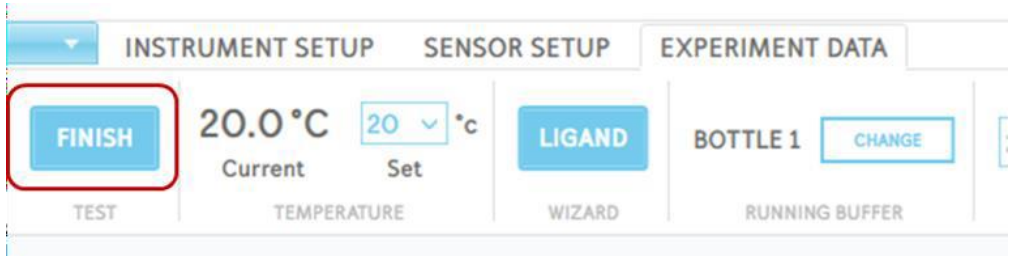
Front sensor surface

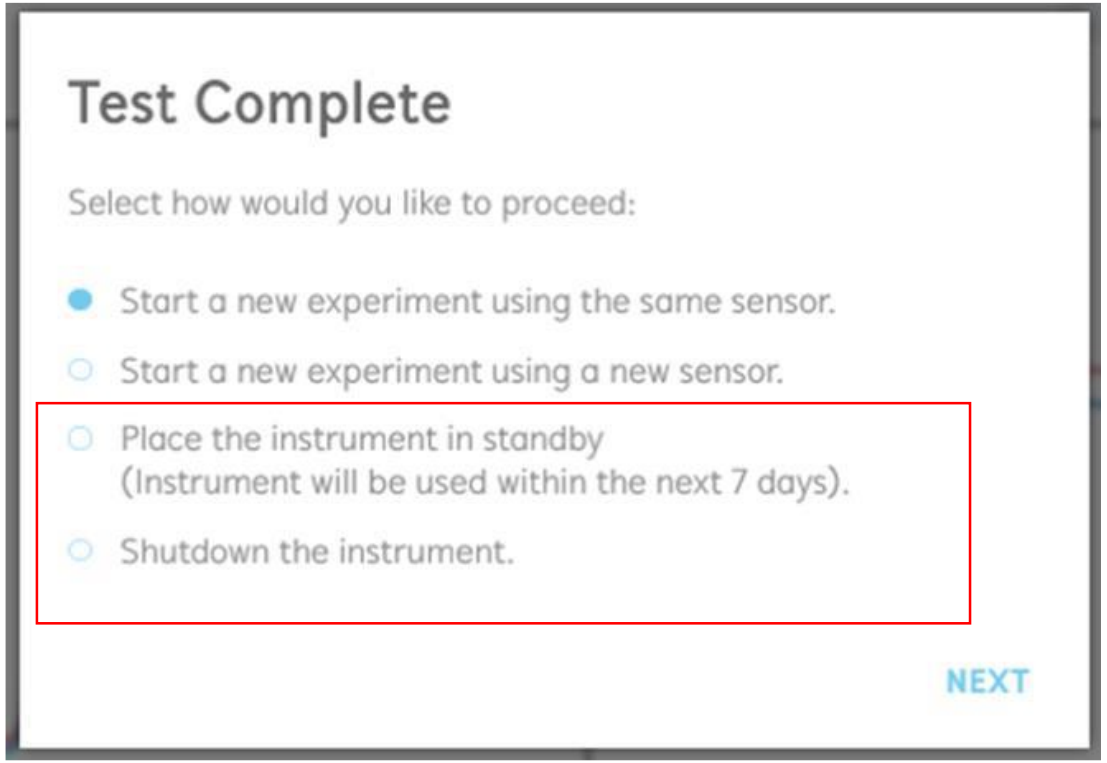
Cut corner of the glass positioned in the bottom right

STEP 6


OpenSPR
INSTRUMENT SETUP
SENSOR SETUP
EXPERIMENT DATA
TIME SINCE LAST INJECTION: 00:00:00
INJECTION REMAINING: 00:00
FINISH
TEST
20.0 °C
Current
20
Set
°c
TEMPERATURE
LIGAND
WIZARD
BOTTLE 1
CHANGE
RUNNING BUFFER
200
µL/min
FLOW RATE
INJECT
CH 1+2
CH 2
INJECTION
ZERO
GRAPH
RAW SIGNALS
CORRECTED PREVIEW
CH 1
CH 2
RESPONSE (RU)
TIME (s)




INSTRUMENT SETUP
SENSOR SETUP
EXPERIMENT DATA
FINISH
20.0 °C
Current
20
Set
°c
LIGAND
BOTTLE 1
CHANGE
TEST
TEMPERATURE
WIZARD
RUNNING BUFFER


Test Complete
Select how would you like to proceed:
Start a new experiment using the same sensor.
Start a new experiment using a new sensor.
Place the instrument in standby
(Instrument will be used within the next 7 days).
Shutdown the instrument.
NEXT

Thank you for your attention!

nicoya

BioSafe
俊欣生技