

Proteine ricombinanti per studi
strutturali.

studio dell'interazione
proteina/proteina e
proteina/ligando

PPI : metodi di caratterizzazione quantitativa

SPR

Studio dell'interazione proteina/proteina e proteina/ligando :

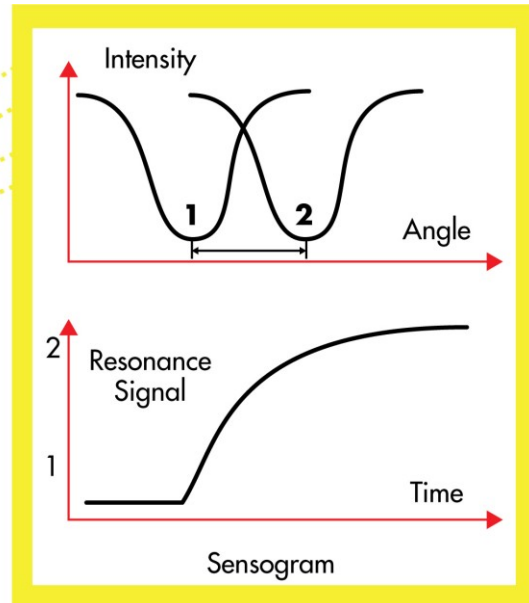
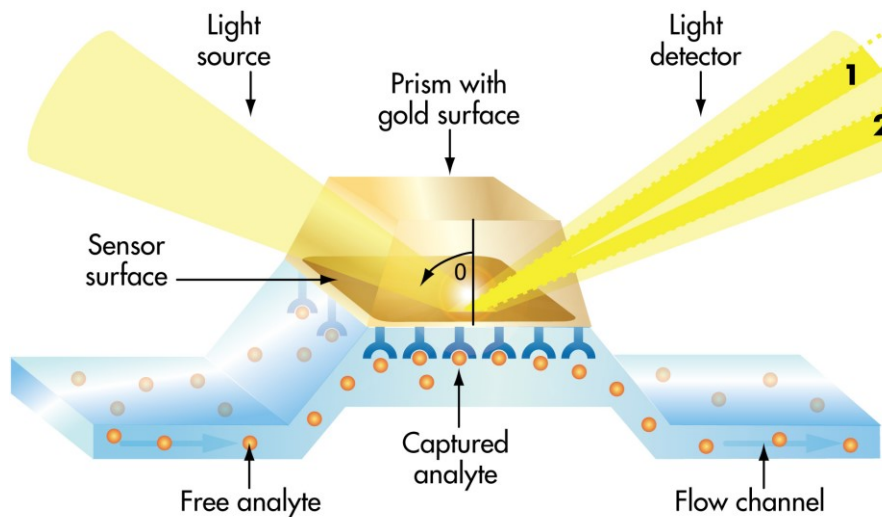
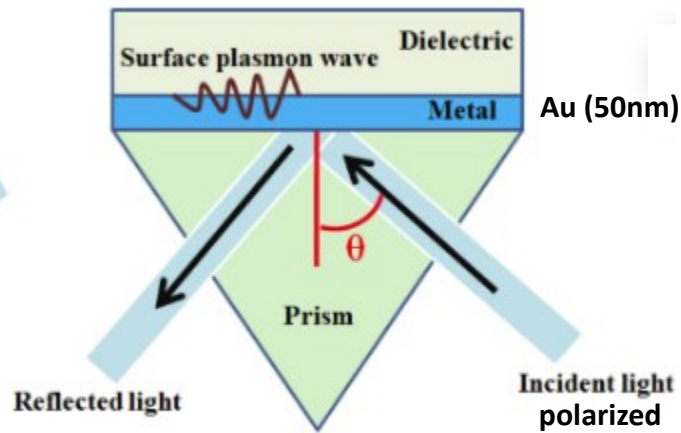
SPR (Surface Plasmon Resonance): basi

Risonanza \leftrightarrow
momento dei fotoni = momento del SP

MINIMO nell'INTENSITA' al θ_{SPR}

$\Delta m \rightarrow \Delta IR \rightarrow \Delta \theta_{SPR}$

$1RU = (10^{-4})^\circ = 1pg/mm^2$



Studio dell'interazione proteina/proteina e proteina/ligando :

SPR : basi

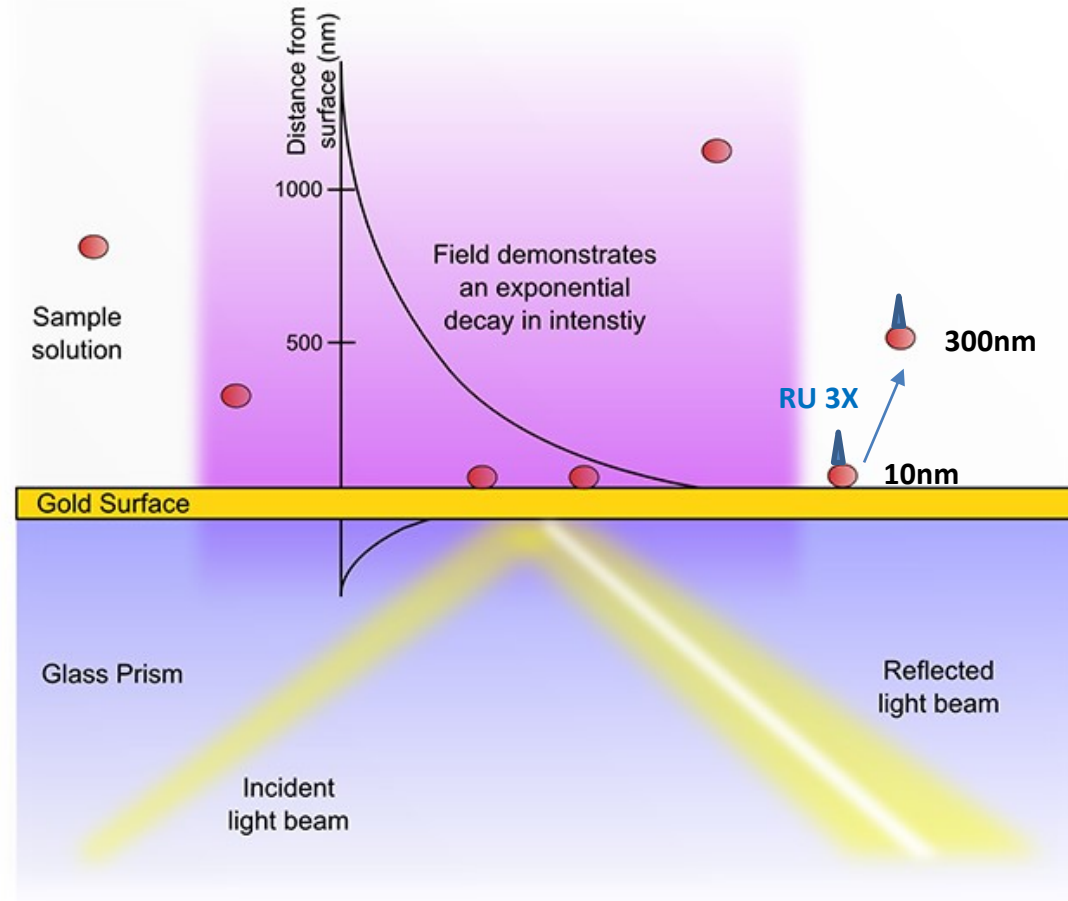
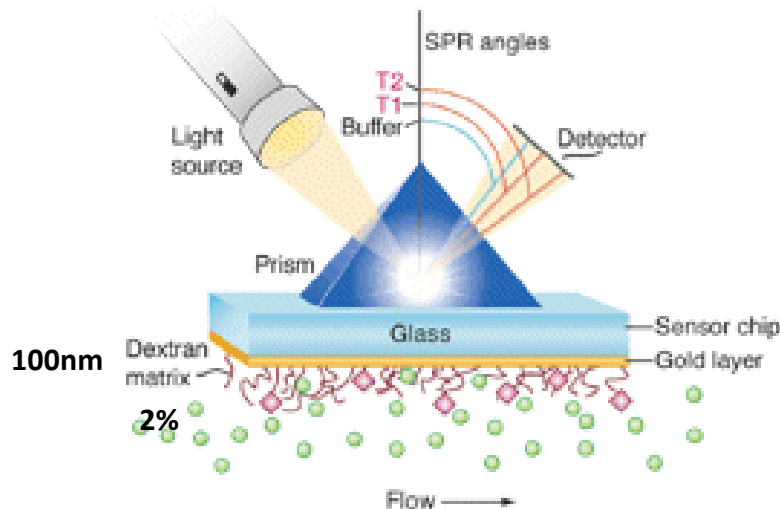
SP influenza la componente \perp del campo elettrico evanescente

→ calo esponenziale

$d = \text{decay length} \approx \frac{1}{2} \lambda$ (= d in cui I cala a $1/e \approx 37\%$)

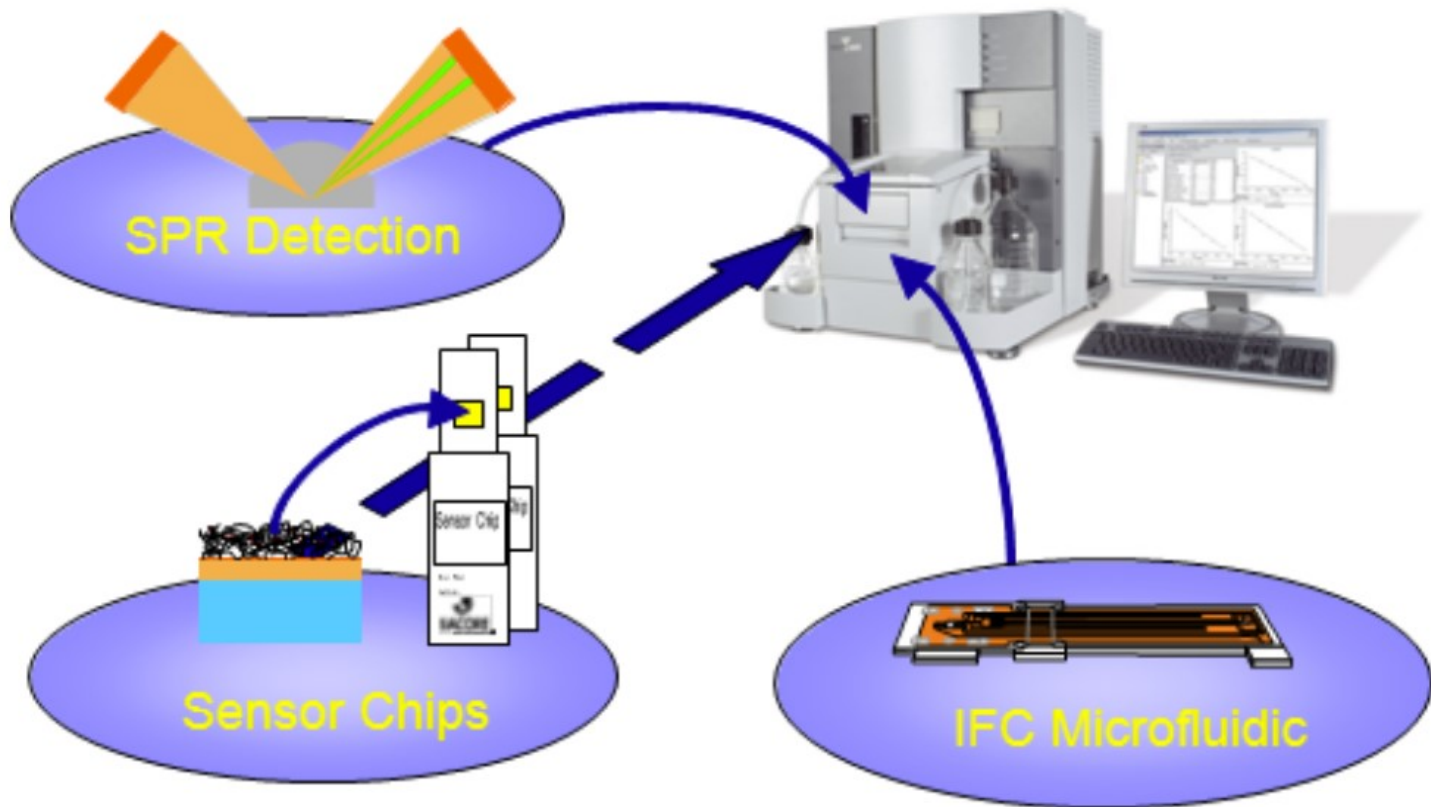
$\lambda = 600-800\text{nm}$

→ Δm misurabili entro 300-400nm



Studio dell'interazione proteina/proteina e proteina/ligando :

SPR : strumentazione



Studio dell'interazione proteina/proteina e proteina/ligando :

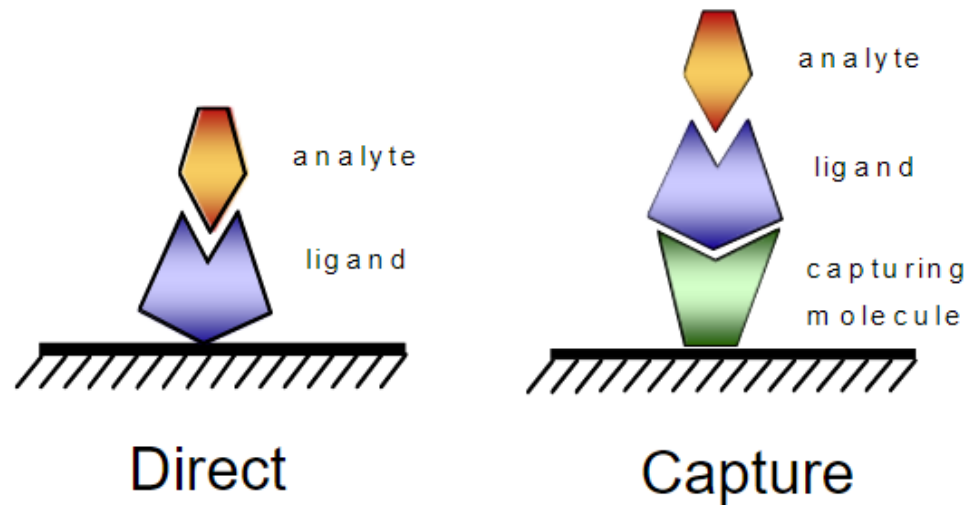
SPR : basi

GE Healthcare
Life Sciences



Studio dell'interazione proteina/proteina e proteina/ligando :

SPR : Immobilizzazione



Covalent coupling of Regeneration down to	ligand	capture molecule
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Orientamento

casuale

univoco

Densità

elevata

minori

Rigenerazione

necessaria

non necessaria

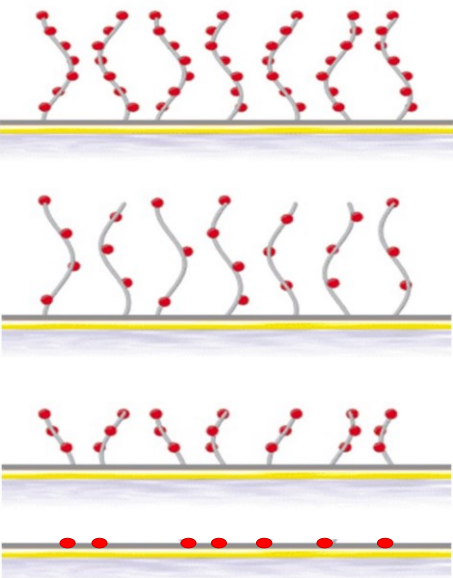
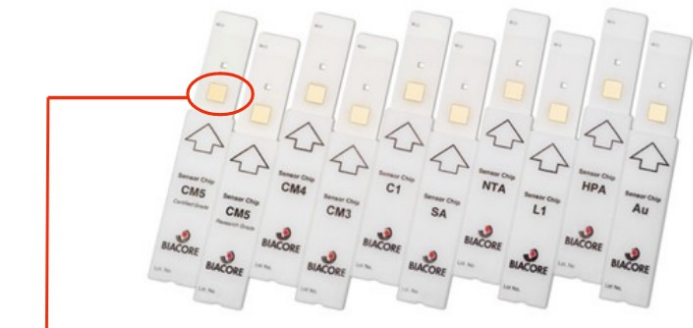
Purezza/stabilità

necessaria

non necessaria

Studio dell'interazione proteina/proteina e proteina/ligando :

SPR : chips



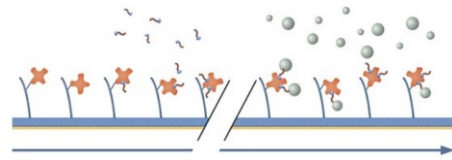
CM5

CM7

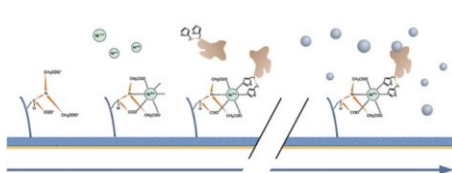
CM4

CM3

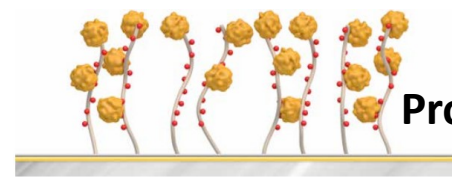
C1



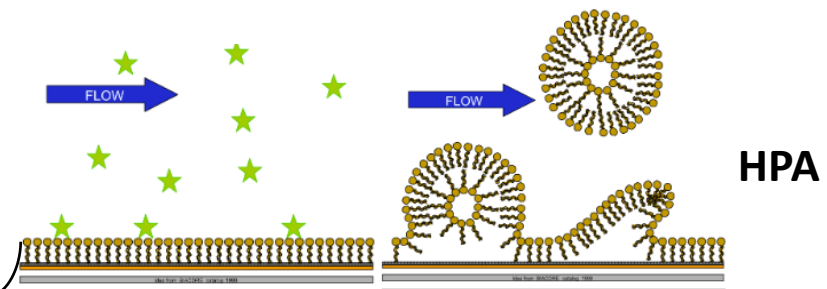
SA



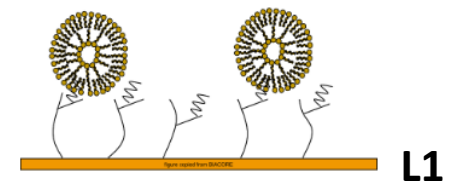
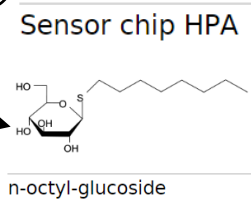
NTA



Protein A/G/L

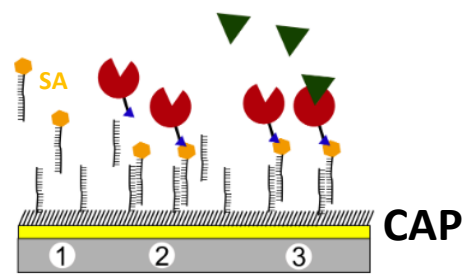


HPA



L1

Sensor chip L1

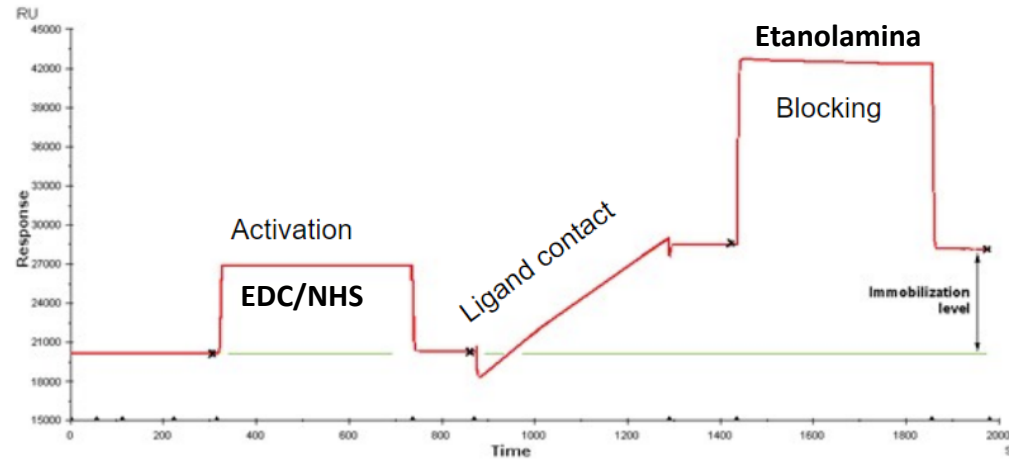
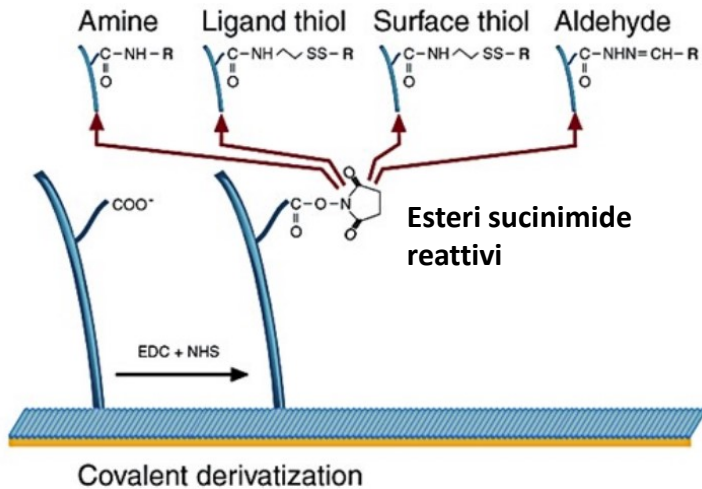


CAP

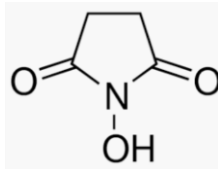
CAP sensor chip

Studio dell'interazione proteina/proteina e proteina/ligando :

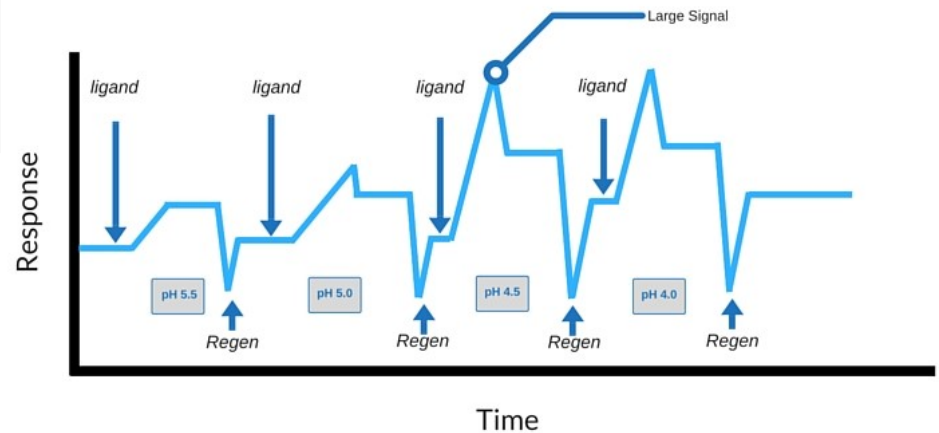
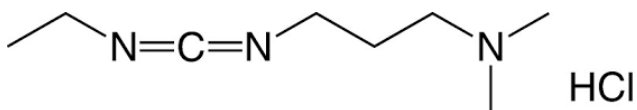
SPR : Immobilizzazione diretta



N-Hydroxy succinimide (NHS)

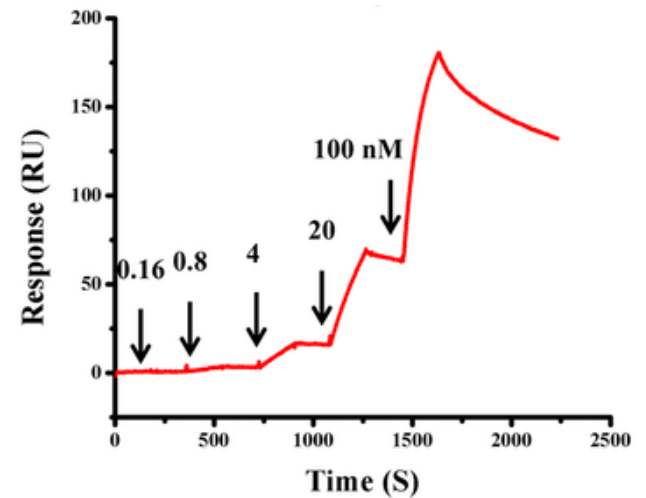
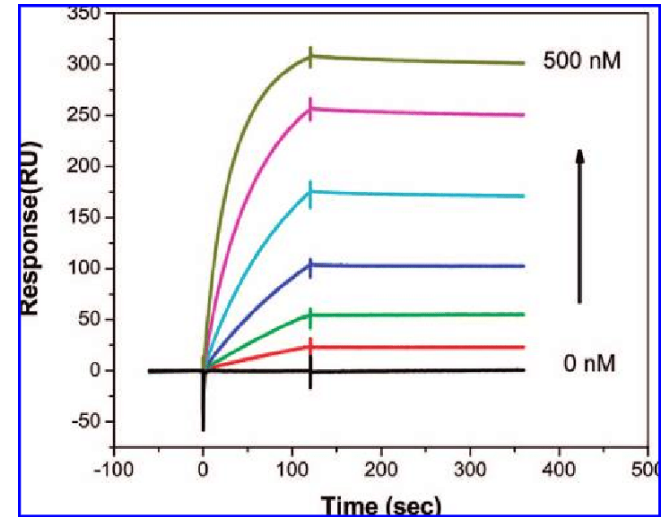
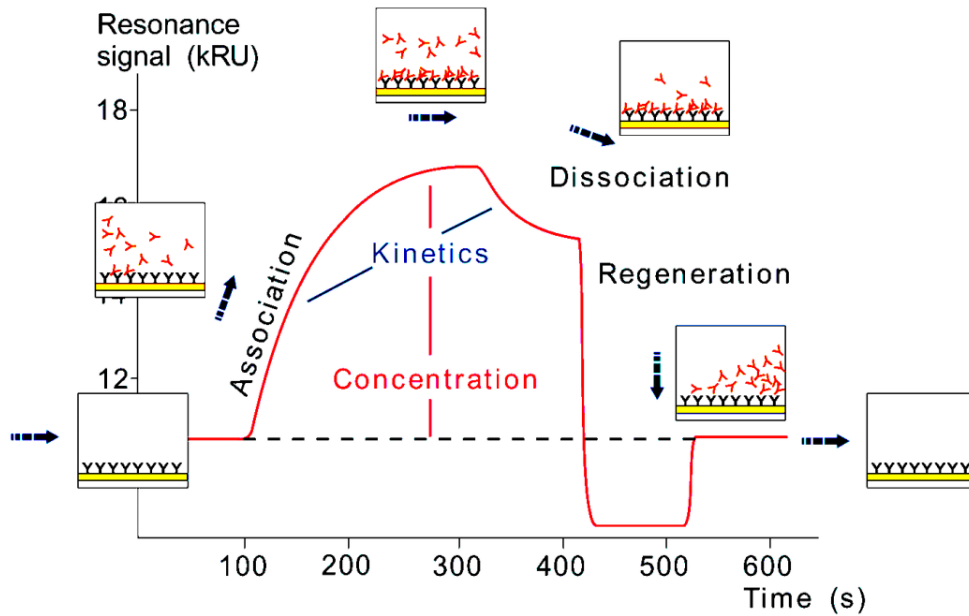


1-Ethyl-3-(3-dimethyl amino propyl) carbo di imide (EDC)



Studio dell'interazione proteina/proteina e proteina/ligando :

SPR : iniezione/rigenerazione

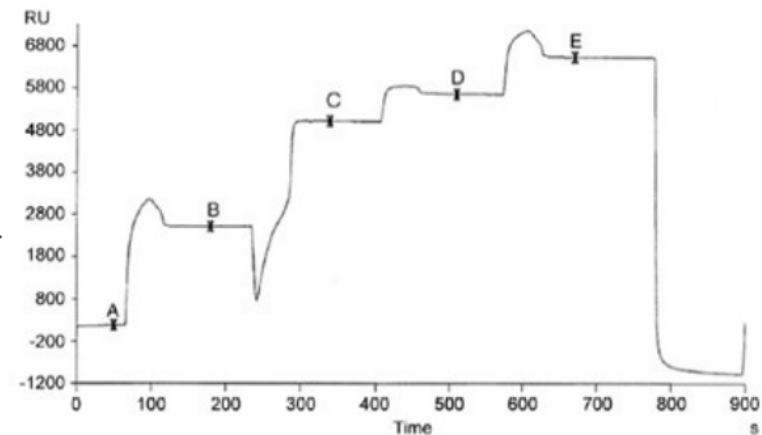
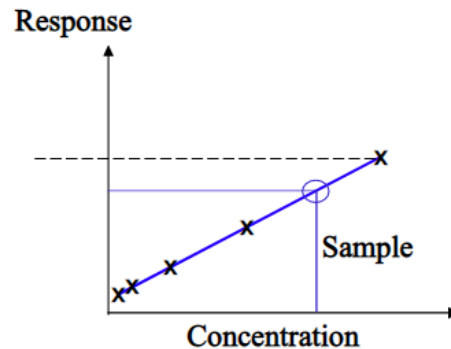
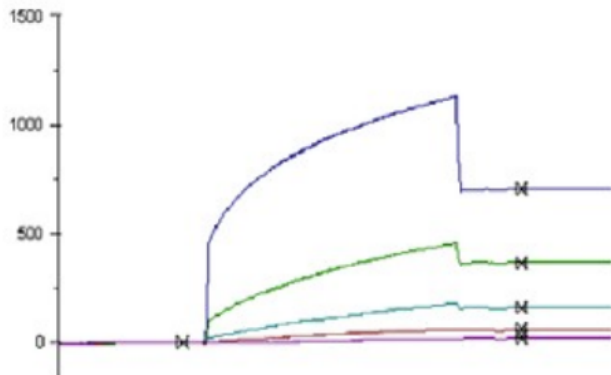
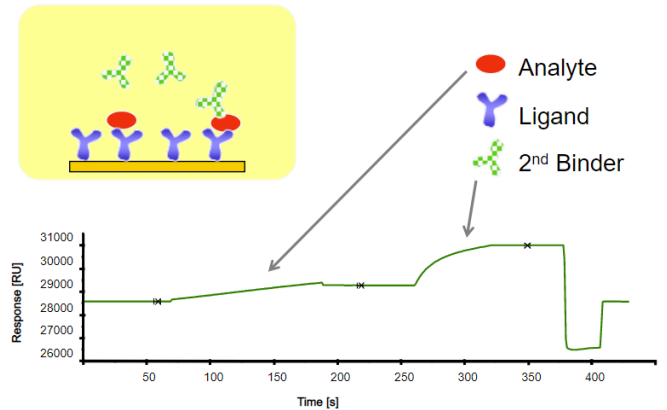
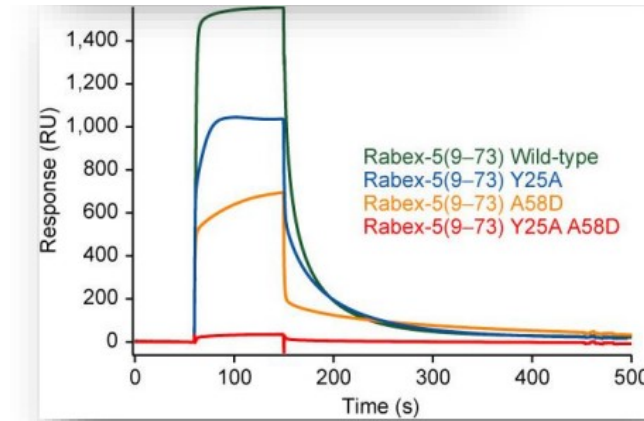


Studio dell'interazione proteina/proteina e proteina/ligando :

SPR : applicazioni

SENZA STUDI CINETICI:

- Specificità
- Binding multiplo
- Concentrazione attiva



Sample matrix for
calibration curve

==

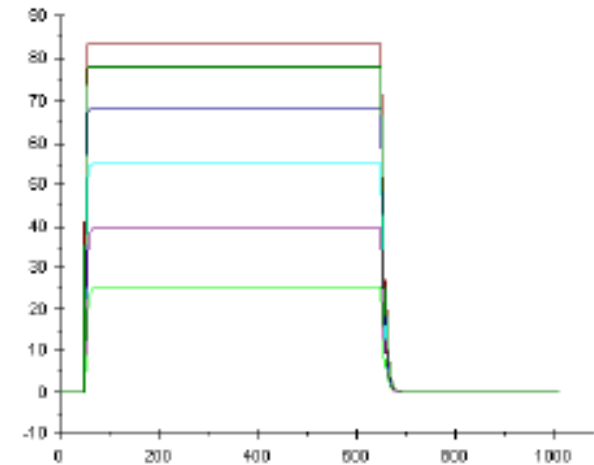
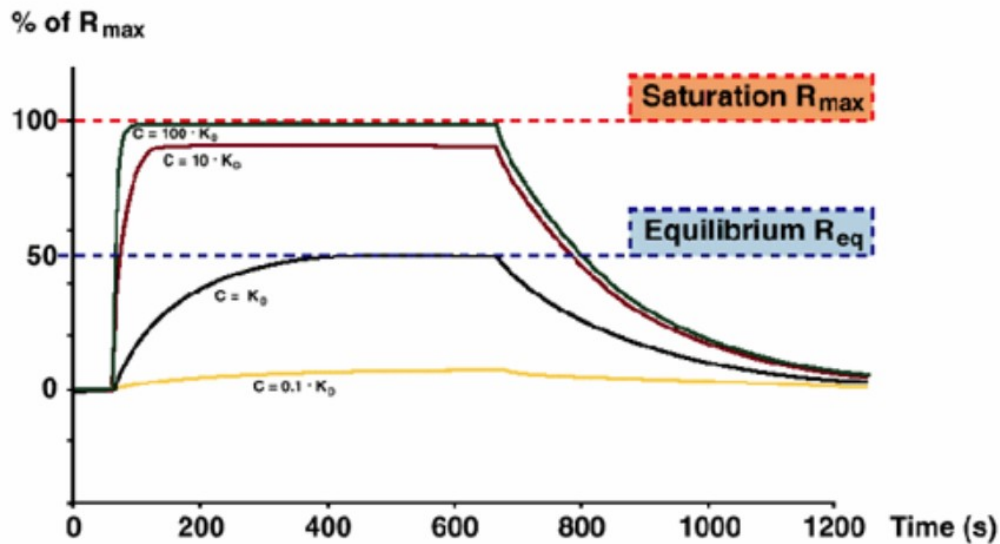
Sample matrix for
unknown samples

Studio dell'interazione proteina/proteina e proteina/ligando :

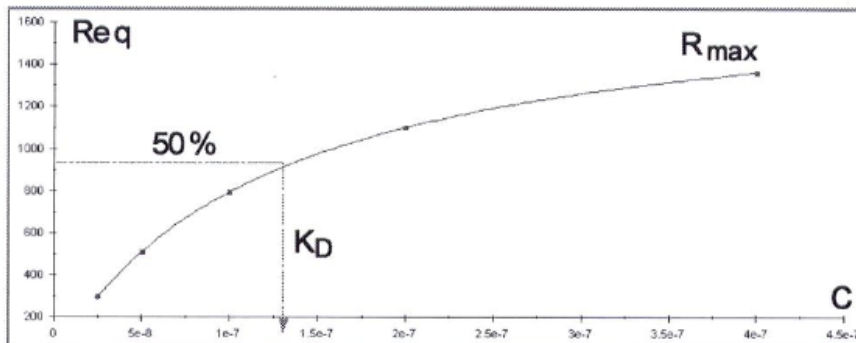
SPR : applicazioni

SENZA STUDI CINETICI: AFFINITA'

Misure allo stato stazionario (per interazioni veloci)



Affinity only



Studio dell'interazione proteina/proteina e proteina/ligando :

SPR : applicazioni

STUDI CINETICI: velocità del legame

k_{on} (riconoscimento): range $10^{-3} - 10^7$ 1/s

k_{off} (stabilità): range $10^{-1} - 10^{-6}$ 1/MS

$K_D = k_{off}/k_{on}$ (affinità): range $10^{-5} - 10^{-12}$ M

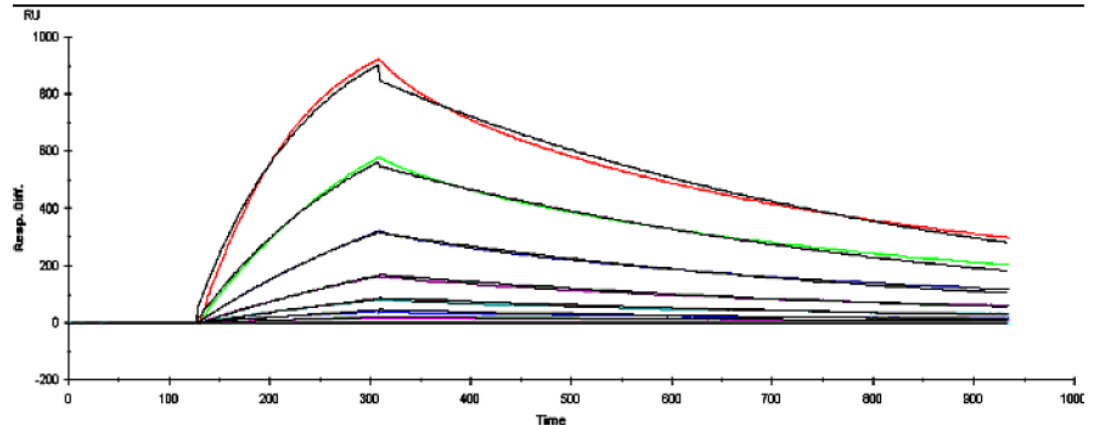
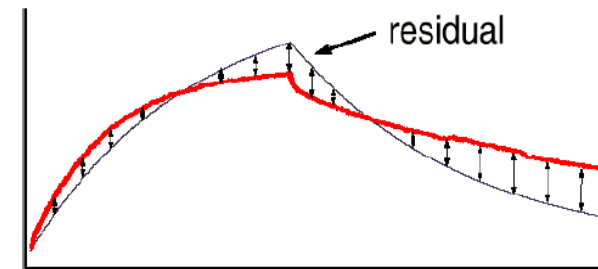
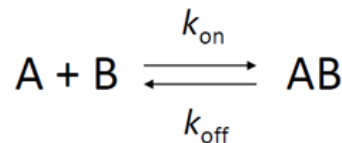
A: analita in soluzione

AB: complesso

B: ligando ($[B]_{tot} = R_{max}$, $[B]_{free} = R_{max} - R$)

k_{on} k_{off} K_D da global fitting con modello

$$\frac{dR}{dt} = k_{on} c (R_{max} - R) - k_{off} R$$

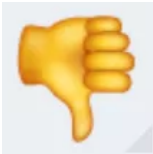


Studio dell'interazione proteina/proteina e proteina/ligando :

SPR



HT
Anche su estratti totali
Bassa quantità di Ligando
Label free
Real time
Flessibile
Accoppiabile con MS
 k_{on} : range $10^{-3} - 3 \times 10^9$ 1/Ms (Proteine), $10^{-3} - 5 \times 10^7$ 1/Ms (Piccoli ligandi)
 k_{off} : range $1 - 10^{-6}$ 1/s

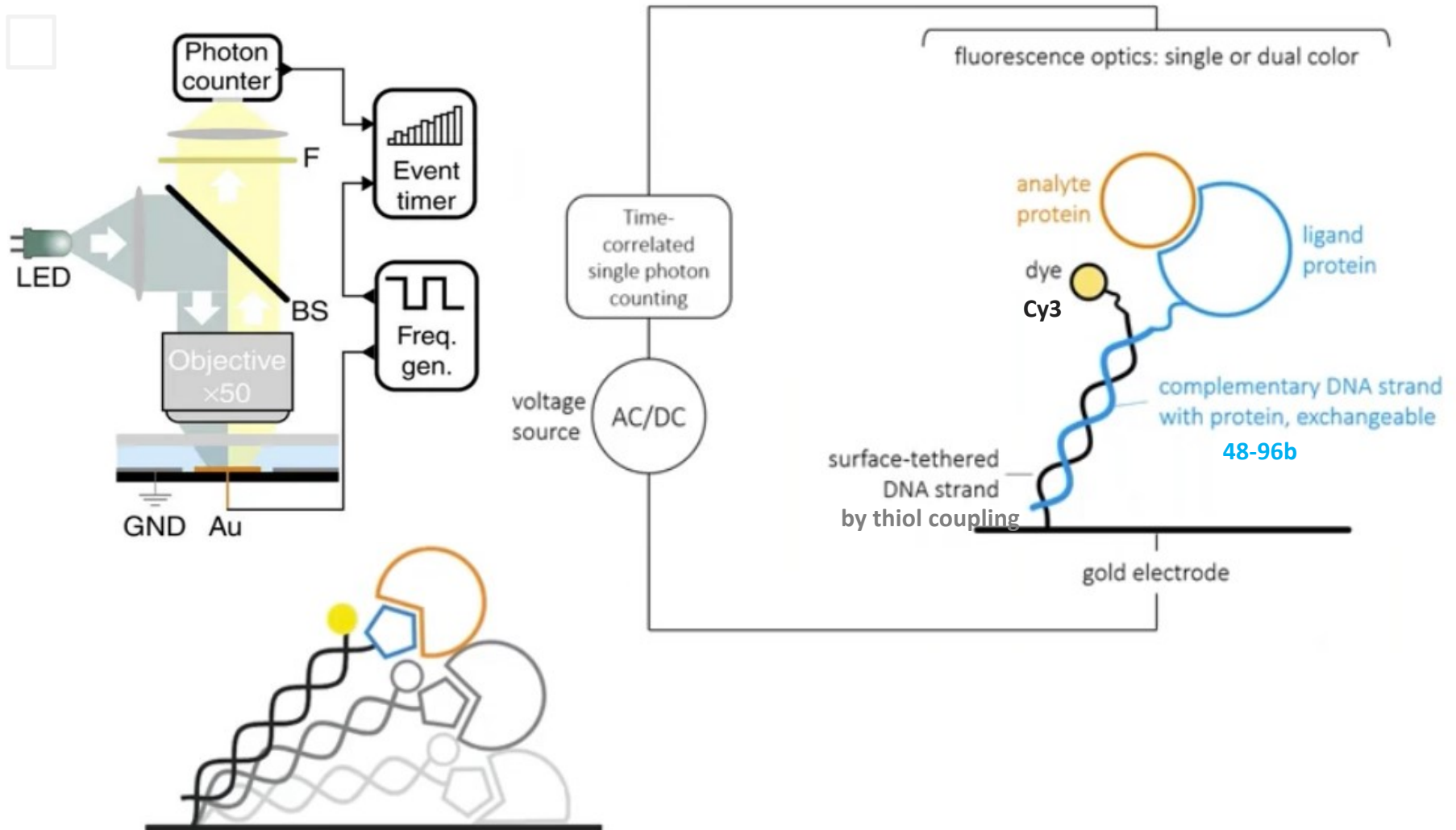


Immobilizzazione
Interazioni aspecifiche
Problemi di rigenerazione
Cinetiche di dissociazione molto veloci

Electrically switchable nanolevers

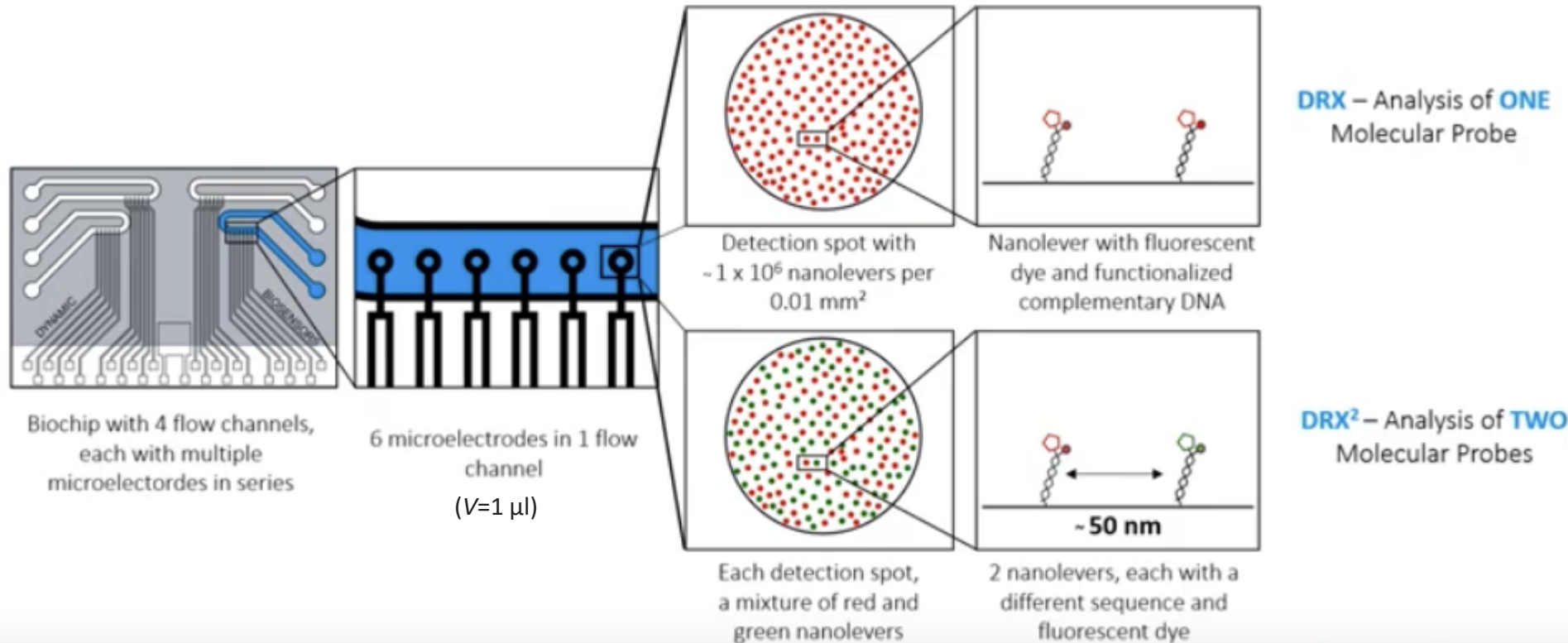
Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers: basi



Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers: basi

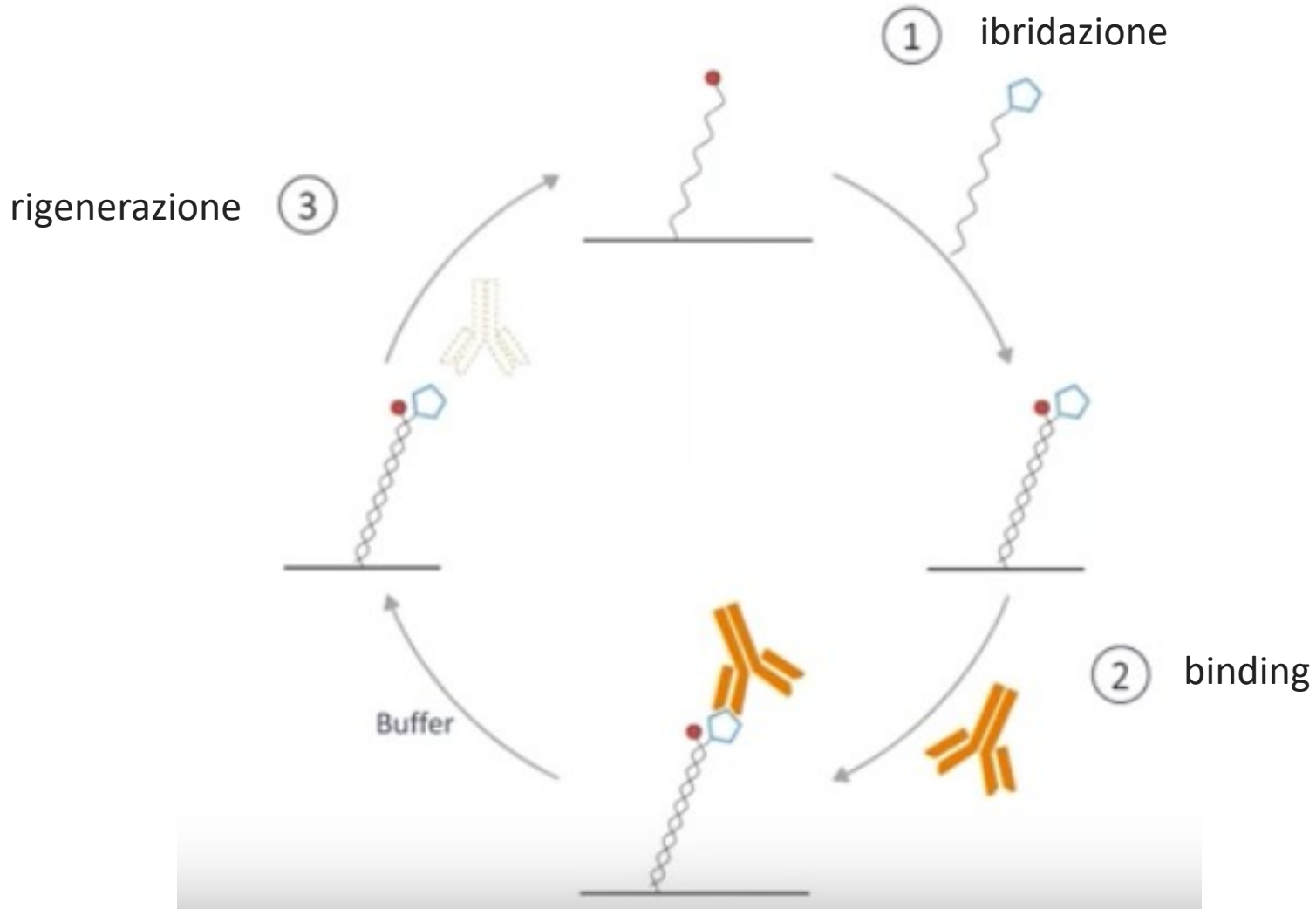


Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers

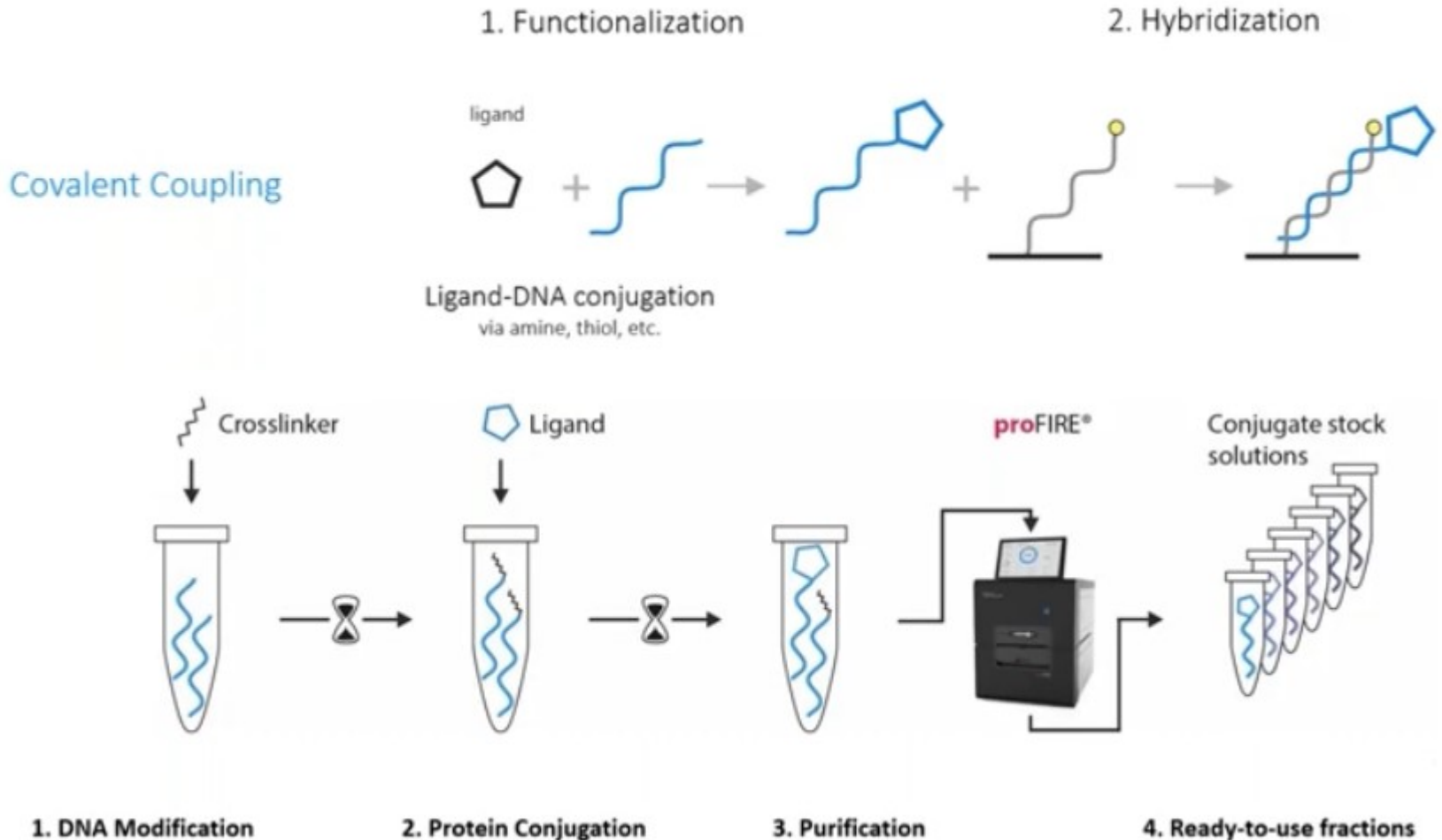
Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers: ciclo sperimentale



Studio dell'interazione proteina/proteina e proteina/ligando :

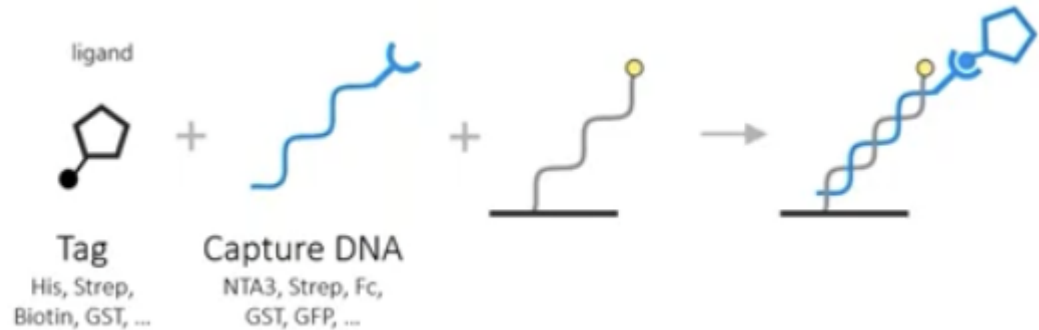
Electrically switchable nanolevers: immobilizzazione



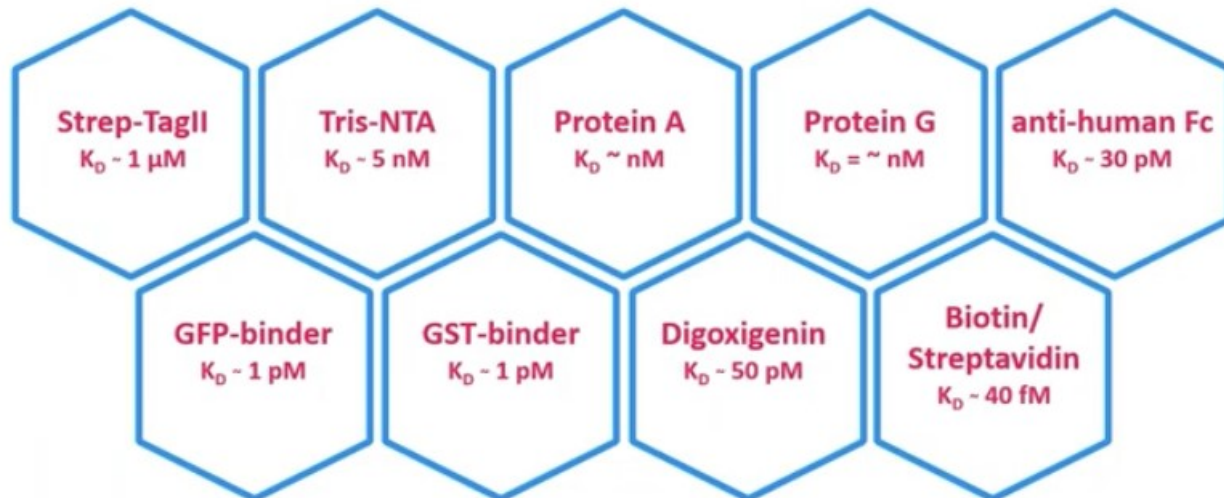
Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers: immobilizzazione

Capture via tag



Affinity-based Immobilization Methods



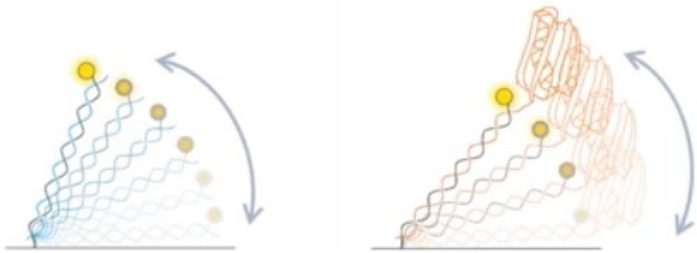
Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers: dimensioni/conformazione

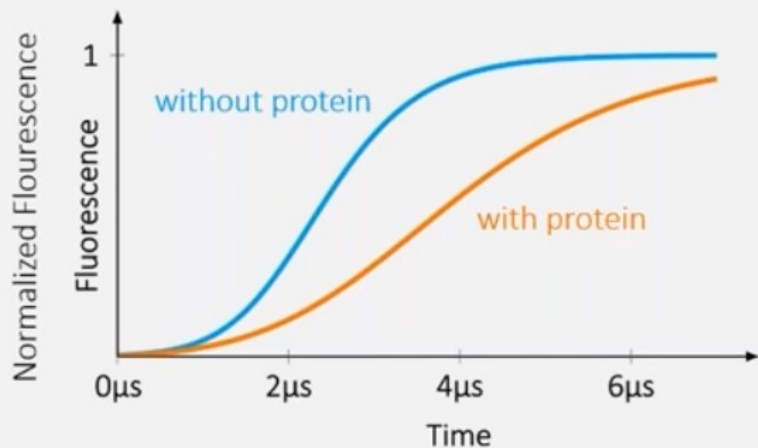
Ligando → influenza attrito idrodinamico/ movimento delle nanoleve

D_H (Accuratezza di 0.1nm)

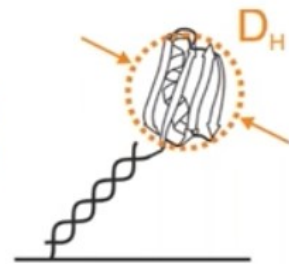
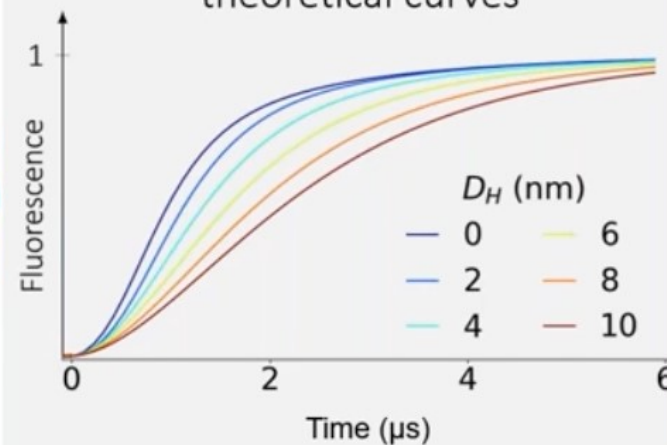
Modificazioni conformazionali



Experimental switching curves

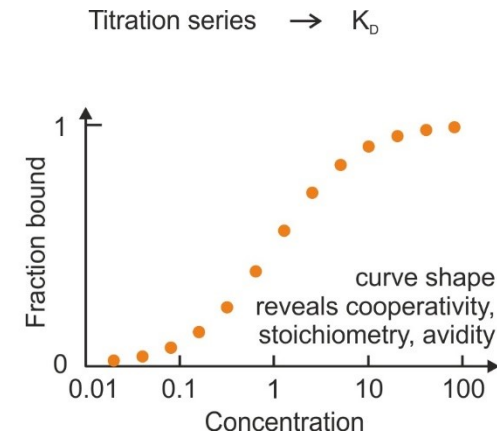
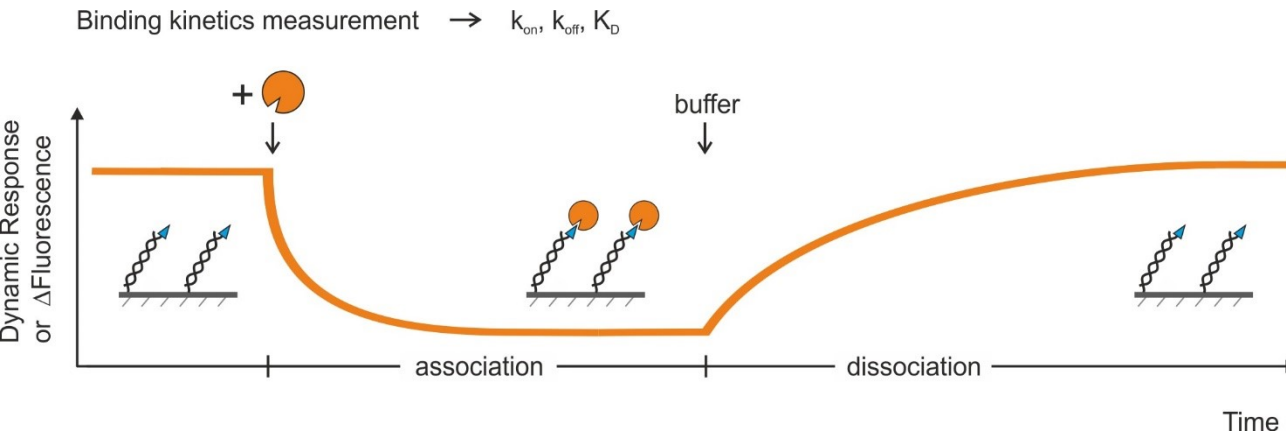


Comparison with library of theoretical curves



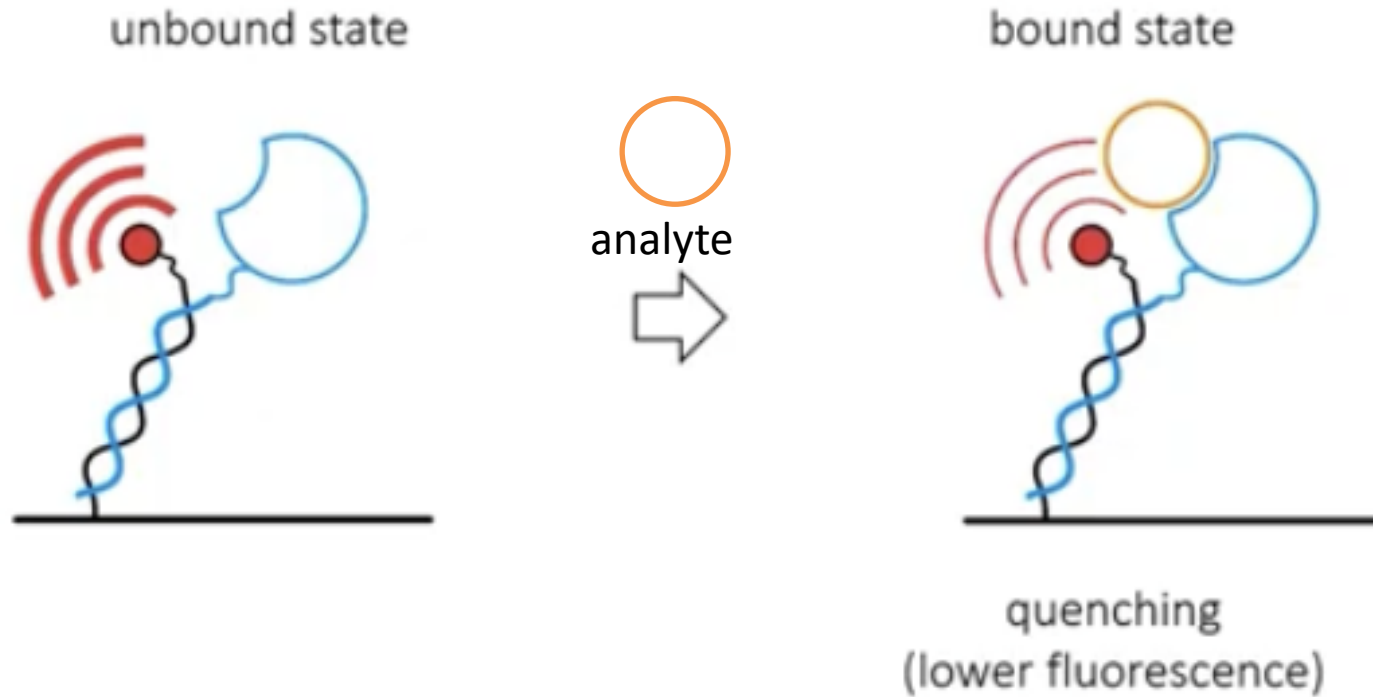
Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers: analisi della cinetica del legame



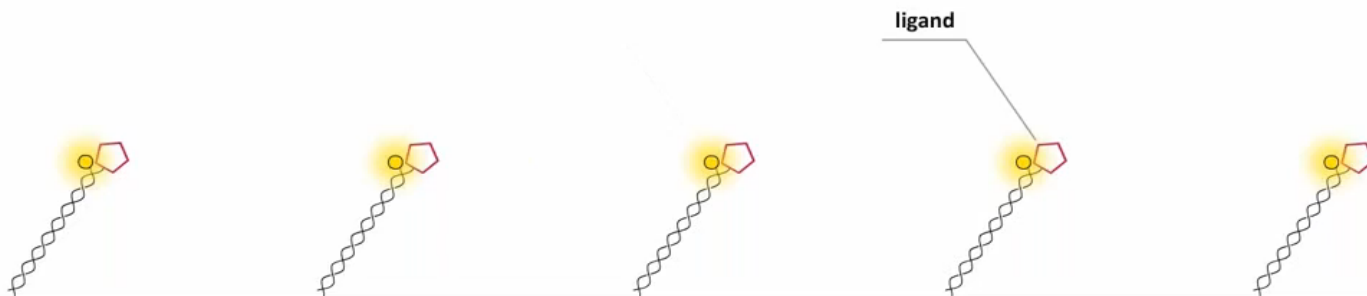
Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers: static mode



Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers: static mode



Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers: dynamic mode

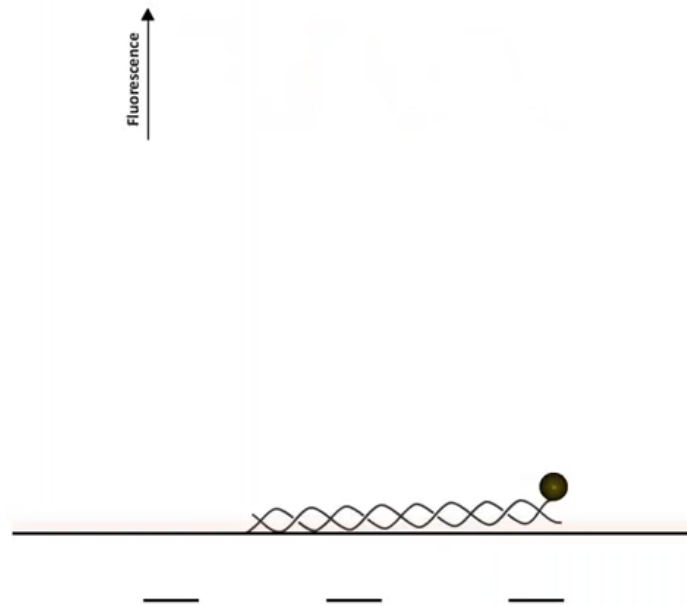


Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers: dynamic mode

Measurement Modes (using Electrically Switchable DNA Nanolevers)

DYNAMIC



Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers: dynamic mode



← flow channel →

↑
gold electrode

Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers: strumentazione

switchSENSE® Analyzer – DRX & DRX²



Autosampler Features

Capacity = 48 vials OR 96-well plate
Temperature control = 8°C – 40°C

Integrated Microfluidics and Liquid Handling

Flow rates up to **4000 µL/min**

Electro-optical measurement readout

Time-Correlated Single Photon Counting
(TCSPC)

Automated

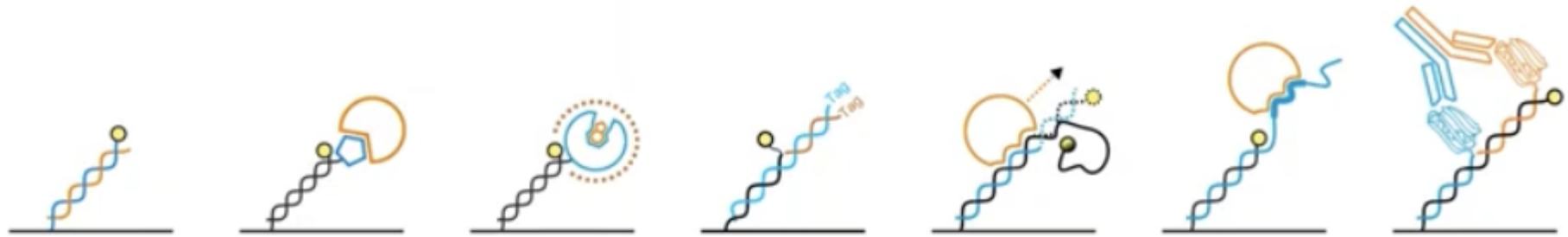
72 h operation

Sample measurement temperature

= 8°C – 75°C

Studio dell'interazione proteina/proteina e proteina/ligando :

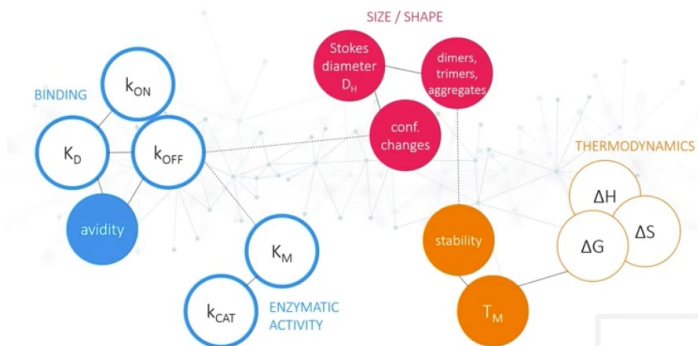
Electrically switchable nanolevers: applicazioni



Interaction Analysis & Biophysical Analysis

Affinity, avidity
 Kinetics
 Inhibitor screening
 Protein diameter
 Conformational changes
 Melting temperature
 Thermodynamics
 Polymer./nuclease activity
 Bispecific antibodies

K_A, K_D , fM sensitivity
 k_{ON}, k_{OFF}
 IC_{50}
 D_H
 $\Delta D_H = 0.1 \text{ nm}$
 T_M
 $\Delta H, \Delta S, \Delta G$
 k_{CAT}, K_M, k_{EXO}
 Avidity



Studio dell'interazione proteina/proteina e proteina/ligando :

Electrically switchable nanolevers:



Versatilità/Flessibilità

HT

Anche su estratti totali

Bassa quantità di Ligando

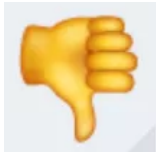
Label free

Real time

k_{on} : range $10^3 - 10^8$ 1/Ms

k_{off} : range $1 - 10^{-6}$ 1/s

K_D : range 50 fM – 1 mM



Interazioni aspecifiche

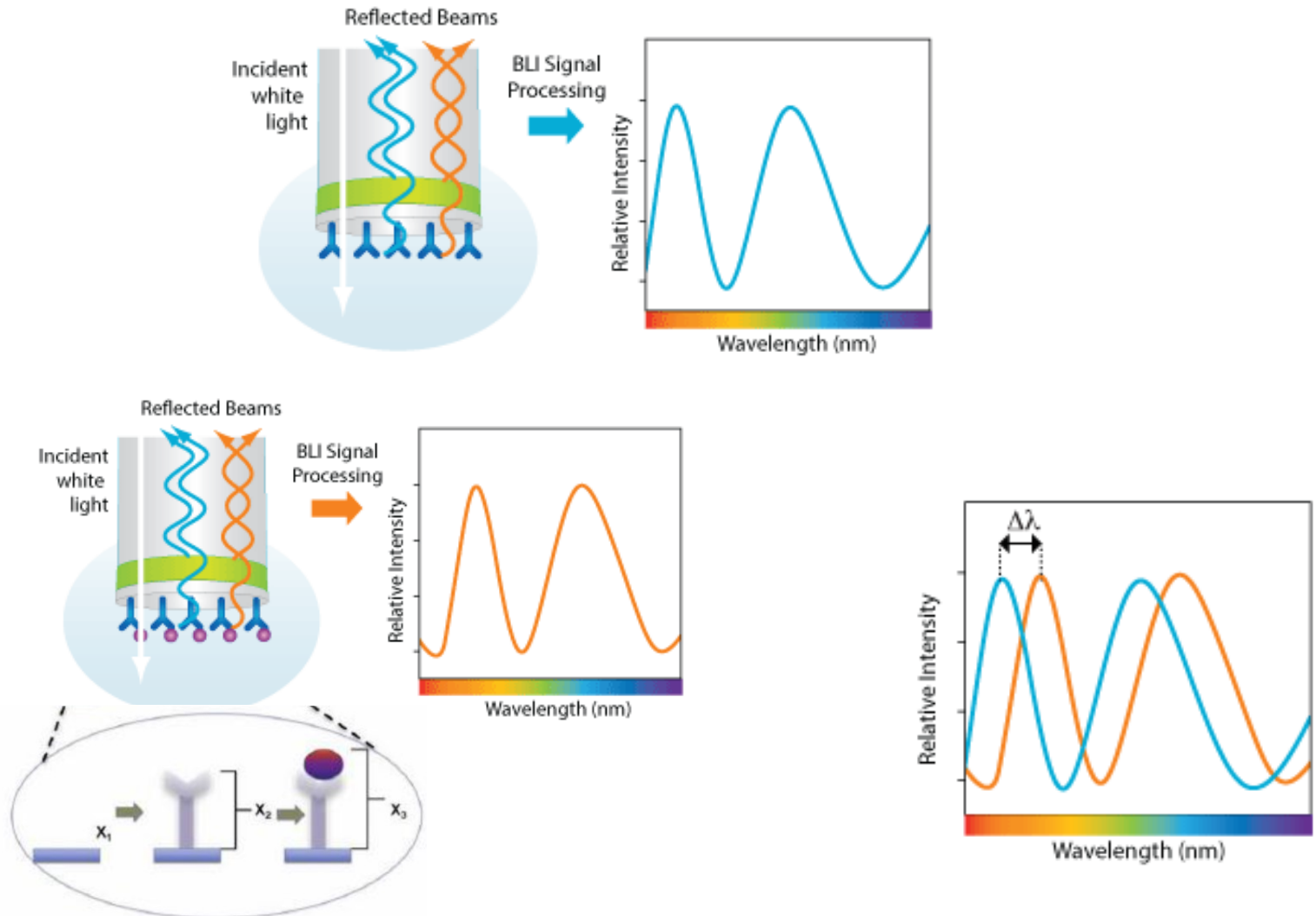
Problemi di coniugazione con il DNA

Non adatto a piccole molecole

BioLayer Interferometry (BLI)

Studio dell'interazione proteina/proteina e proteina/ligando :

Bi-layer interferometry (BLI): basi



Studio dell'interazione proteina/proteina e proteina/ligando :

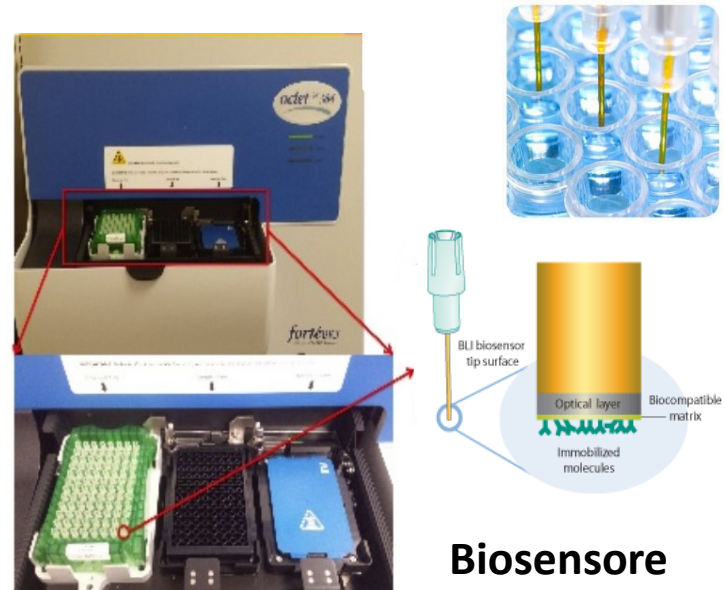
Bi-layer interferometry (BLI): strumentazione



Manuale



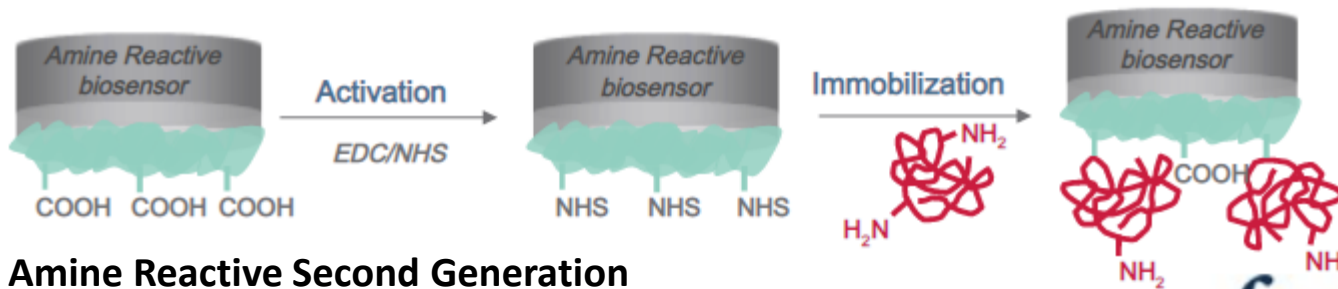
Automatico



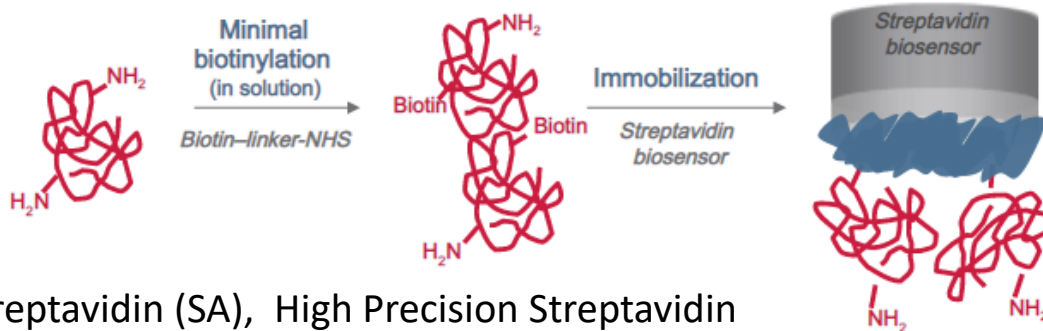
Biosensore

Studio dell'interazione proteina/proteina e proteina/ligando :

Biolayer interferometry (BLI): Biosensori



Amine Reactive Second Generation

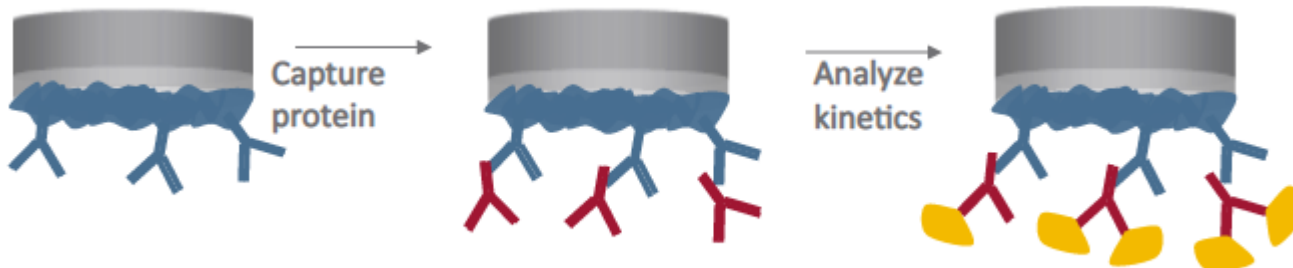


Streptavidin (SA), High Precision Streptavidin (SAX), Super Streptavidin (SSA)

CAPTURE

Ni-NTA (NTA)
Anti-Penta-HIS, Anti-HIS (HIS2)

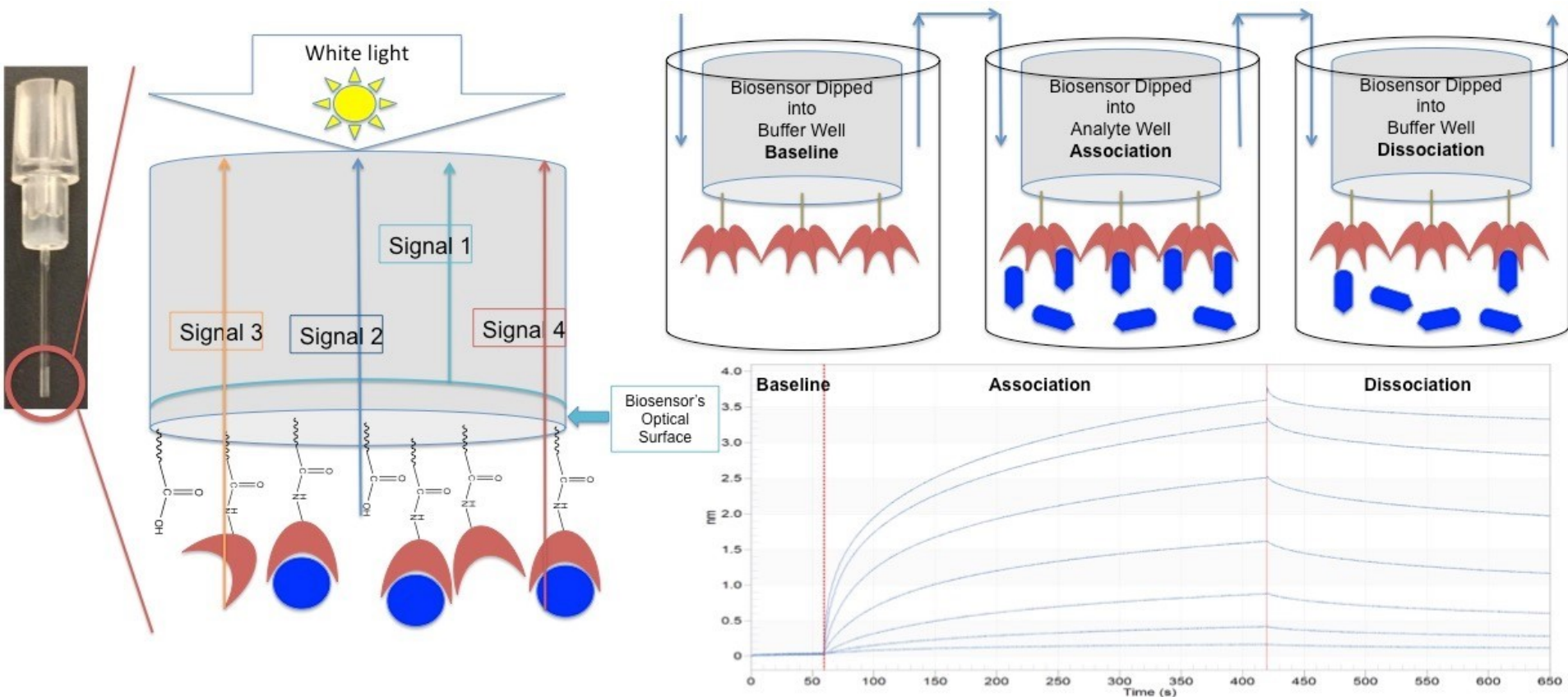
Anti-GST (GST)



Protein A/G/L
Anti-Human IgG Fc,
Anti-Human Fab-CH1,
Anti-Murine IgG Fv,

Studio dell'interazione proteina/proteina e proteina/ligando :

Biolayer interferometry (BLI):esperimento



Studio dell'interazione proteina/proteina e proteina/ligando :

Biolayer interferometry (BLI):



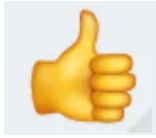
made by freemake.com

Dctet® Assay Platform: High-quality Protein Kinetics and Quantitation

Fluidics-free BLI biosensors for superior performance
and unrivaled flexibility



Studio dell'interazione proteina/proteina e proteina/ligando :



HT/veloce

Anche su estratti totali

Bassa quantità di Ligando

Label free

Real time

Flessibilità

Non serve rigenerare

k_{on} : range $10^1 - 10^7$ 1/Ms (apparenti)

k_{off} : range $10^{-1} - 10^{-6}$ 1/s (apparenti)

K_D : range $10^{-3} - 10^{-11}$ 1/s



Interazioni aspecifiche

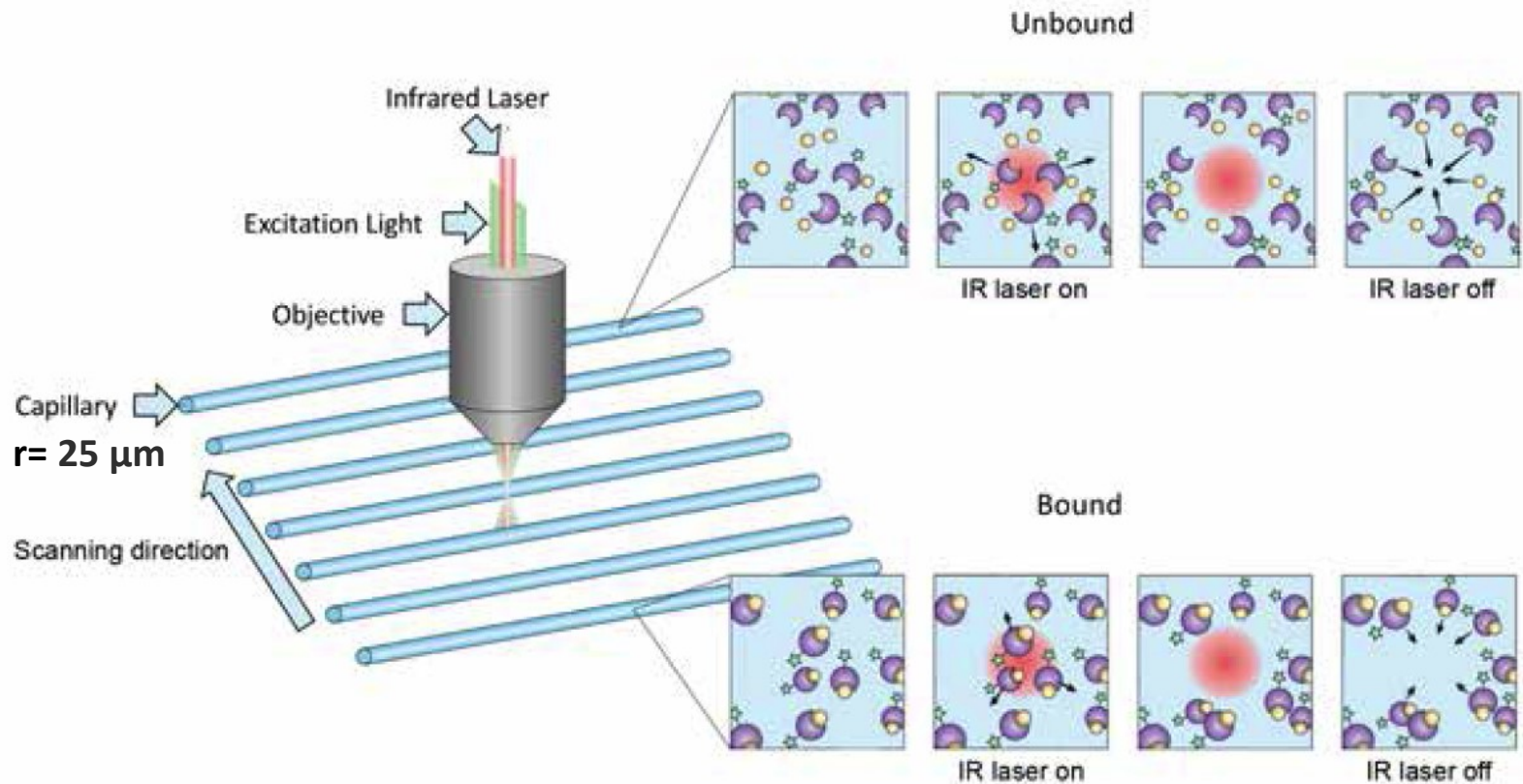
Non affidabile per cinetiche molto veloci o molto lente (evaporazione)

Dipendente dalle dimensioni dell'analita (nominalmente fino a 0.15kDa)

MicroScale Thermophoresis

Studio dell'interazione proteina/proteina e proteina/ligando :

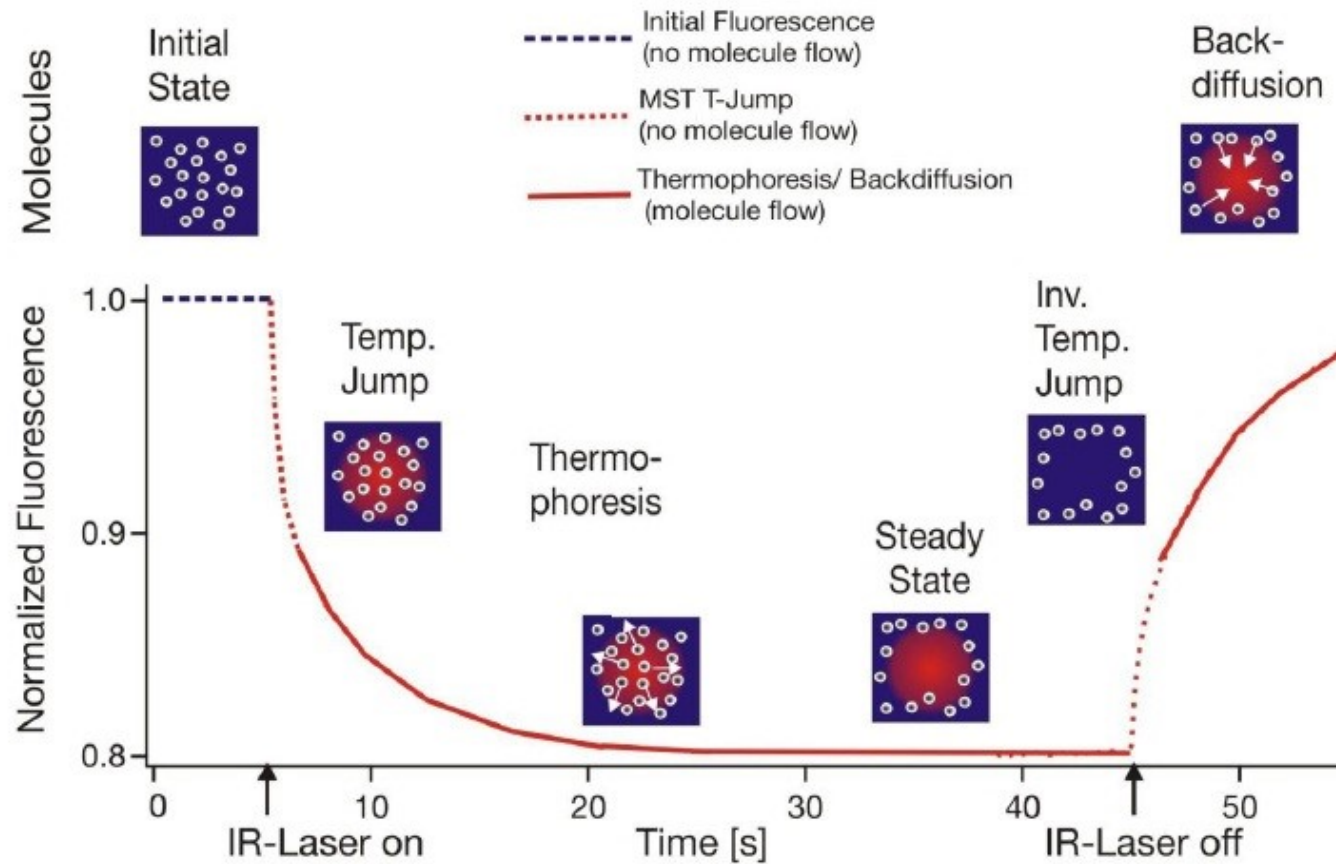
MicroScale Thermophoresis (MST): basi



Gradiente di $T = 2-6 \text{ }^\circ\text{C}$

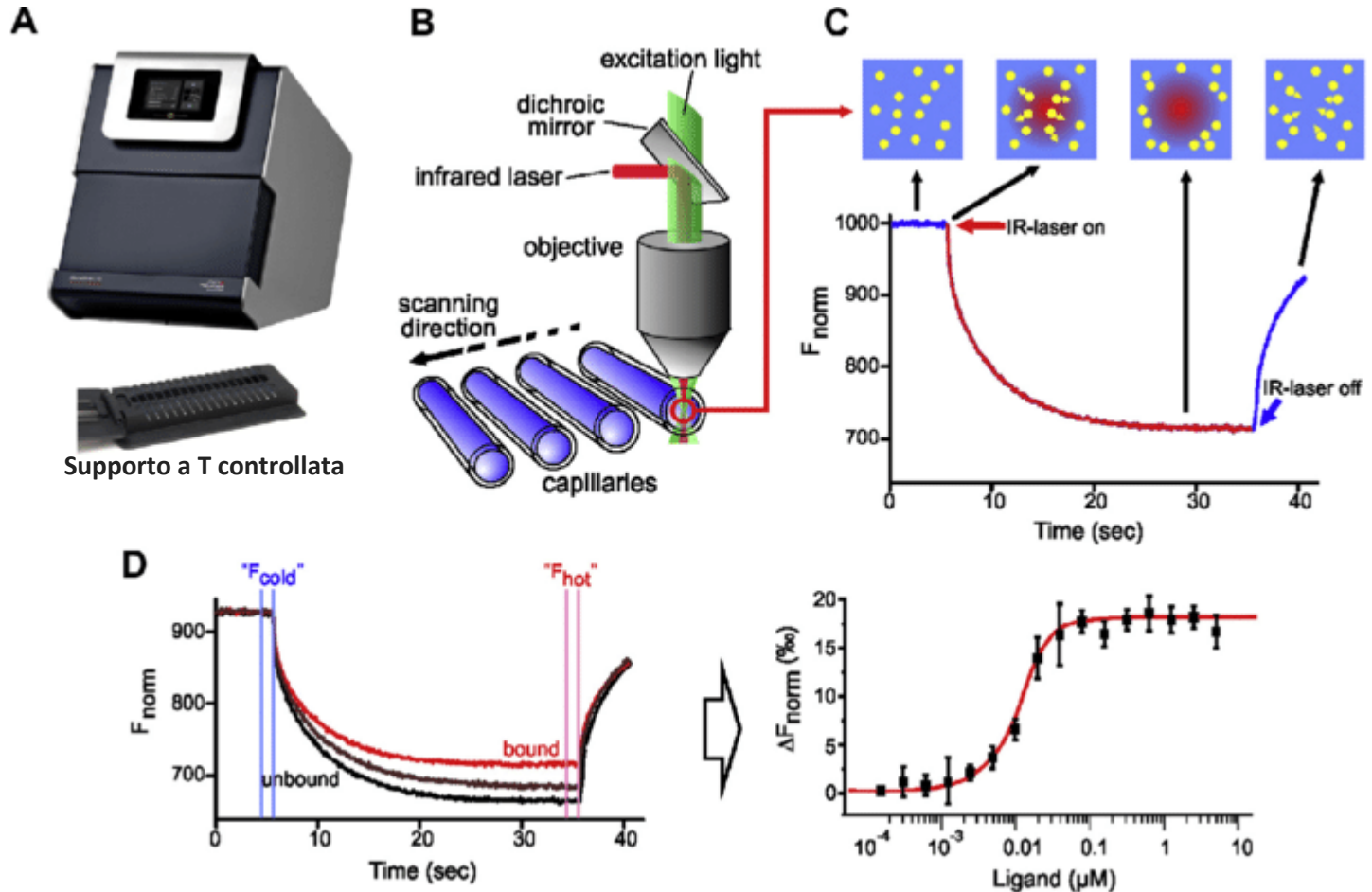
Studio dell'interazione proteina/proteina e proteina/ligando :

MicroScale Thermophoresis (MST): basi



Studio dell'interazione proteina/proteina e proteina/ligando :

MicroScale Thermophoresis (MST): **esperimento**



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MicroScale Thermophoresis (MST): **esperimento**

1 **MARCATURA CON SONDE FLUORESCENTI:** a seconda del detector

- **Pico RED detector** (excitation λ : 600-650 nm): [fluoroforo] \geq 50 pM

Per acidi nucleici: Cy5

Per proteine: RED-Tris-NTA (per his tagged proteins)

AlexaFluor647, NT647 da coniugare per amine o thiol coupling

Kd range: pM – mM

- **Nano BLUE detector** (excitation λ : 460-490 nm): [fluoroforo] \geq 5 nM

Per proteine:

fluoresceina, AlexaFluor488, NT495, GFP

Kd range: nM – mM

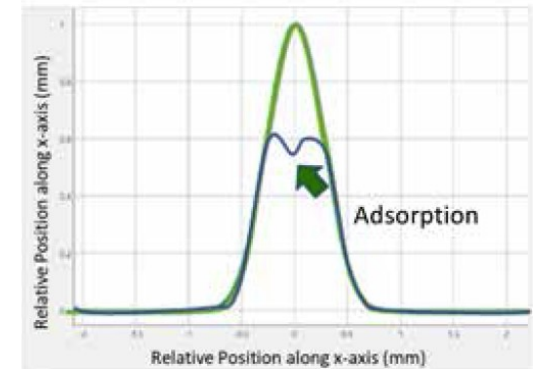
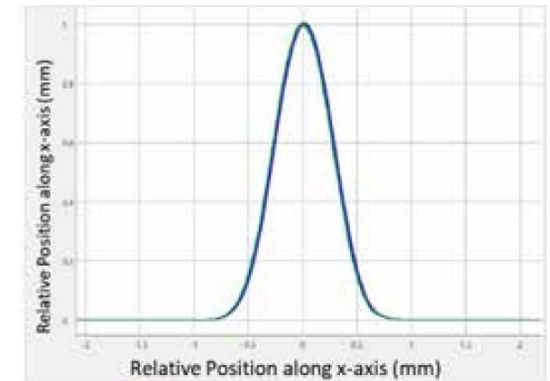
2 **SCELTA DEL SISTEMA TAMPONE**

3 **CONTROLLO DEL LIVELLO DI FLUORESCENZA**

Scansione di 2 capillari con la molecola marcata:

Livello di fluorescenza

Problemi di adsorbimento

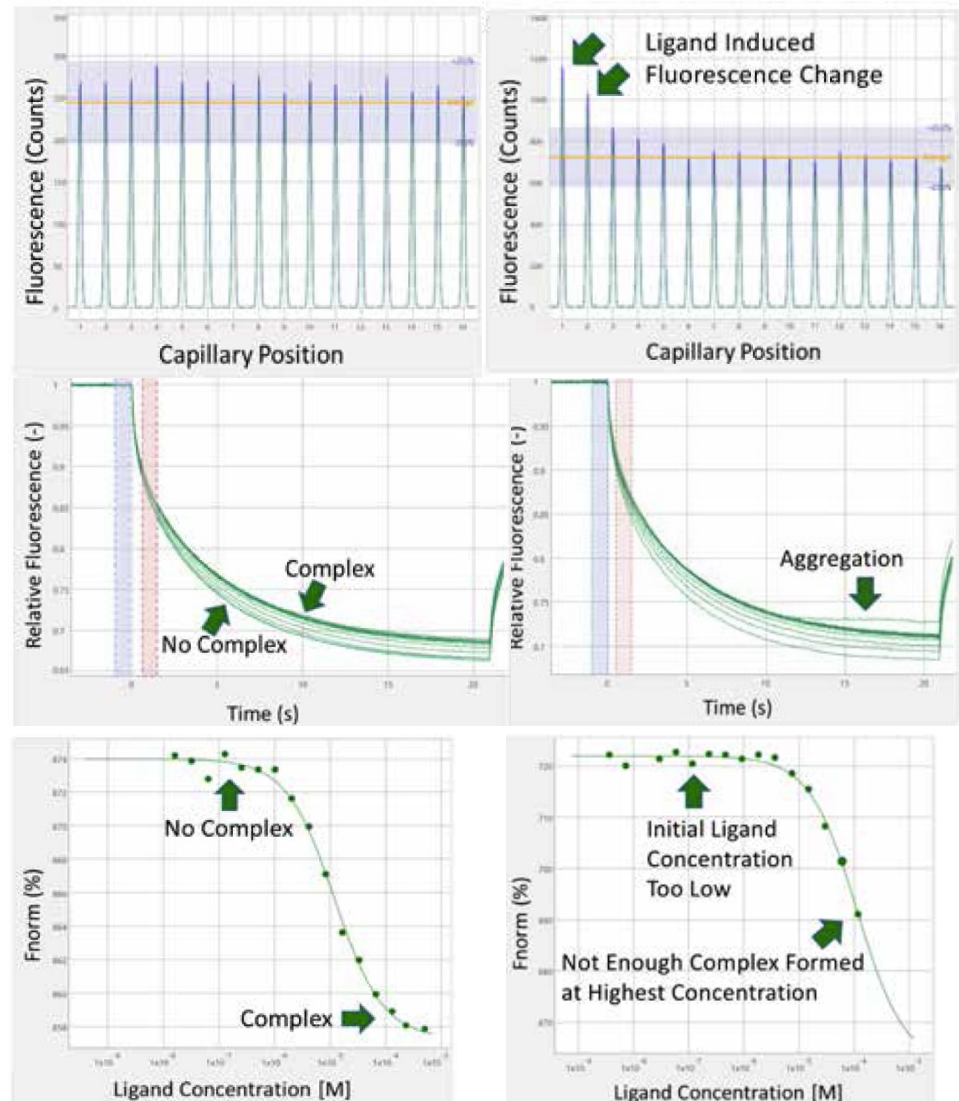


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MicroScale Thermophoresis (MST): **esperimento**

4. BINDING AFFINITY TEST

- Diluizioni seriali in 20 μL totali trasferite nei capillari
- Scansione dei capillari per paragonare il livello di fluorescenza
- Profilo MST durante il test
- Binding curves



Studio dell'interazione proteina/proteina e proteina/ligando :

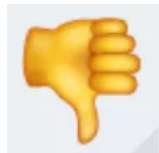
MicroScale Thermophoresis (MST): **esperimento**

Studio dell'interazione proteina/proteina e proteina/ligando :

MicroScale Thermophoresis (MST):



Veloce (anche formarti HT)
Bassa quantità di Target
Non richiede immobilizzazione
Ampia applicabilità
Ampia compatibilità di tamponi
 K_D : range $10^{-3} - 10^{-9}$ M



Richiede marcatura
Interazioni aspecifiche
Nessuna informazione cinetica
poco adatto per PLI

