

Label-Enhanced SPR Improves the Detectability of Label-Free Surface Plasmon Resonance Analysis 100x

Anders Hanning, Episentec
Drug Discovery '15, Telford, 2-3 September 2015

Episentec - Better Biosensors
www.episentec.com

Label-Enhanced SPR

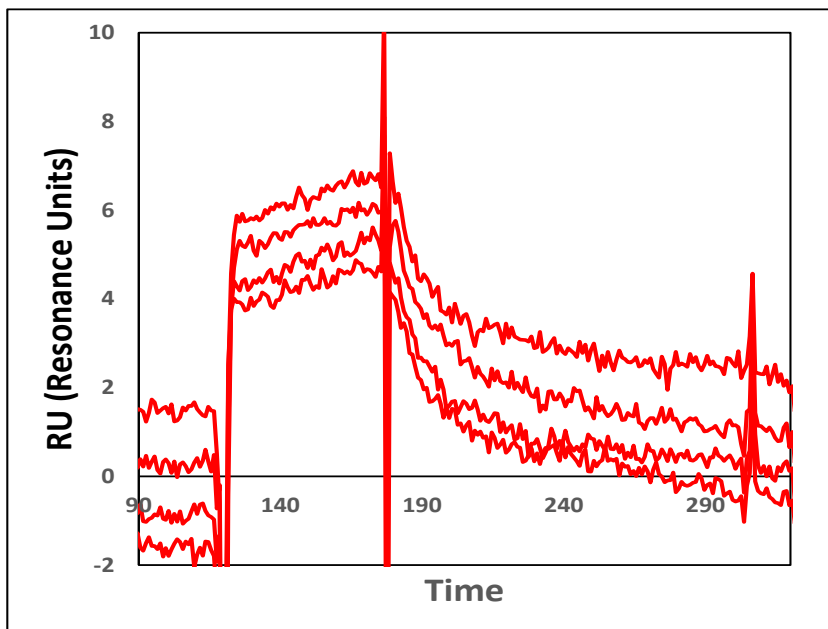
Dye-labelling combined with SPR dip shape analysis

- ▶ Enhanced sensitivity: 100x
- ▶ Enhanced specificity: 100%



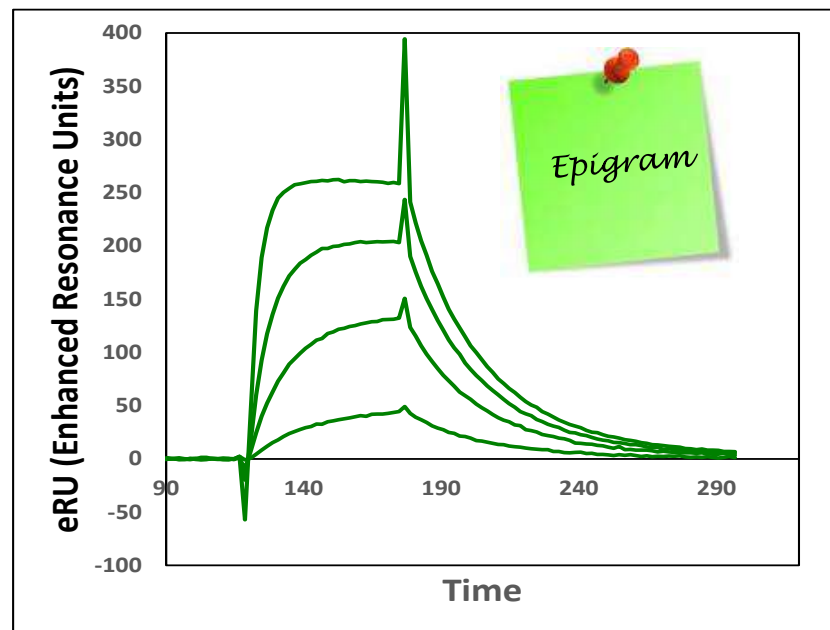
Small molecules: Sensitivity

Conventional SPR



15-50-100-500 μM

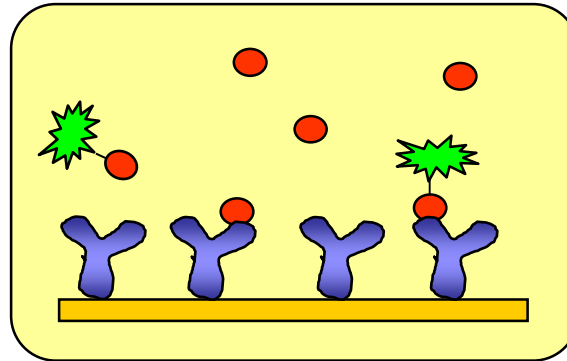
Label-Enhanced SPR



1-5-15-50 μM

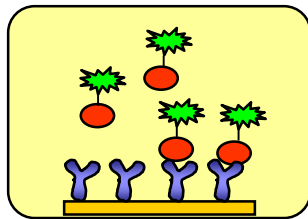
CA II immobilized on a CM5 sensor chip (Biacore 2000), sulfanilamide substrate

Formats

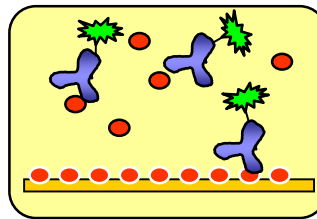


**Label-free interaction
with
label-enhanced readout**

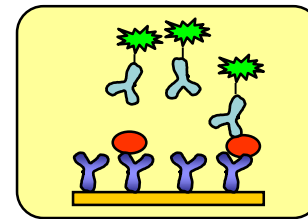
Competition



Direct binding

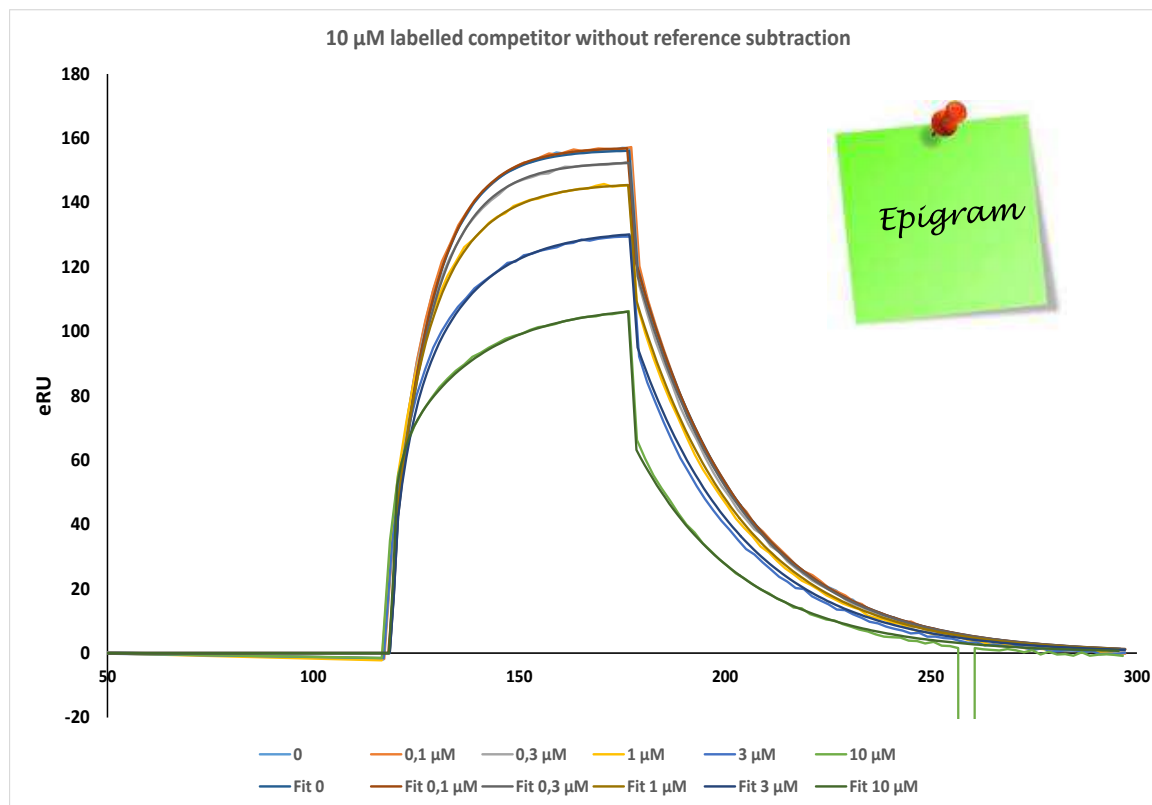


Inhibition



Sandwich

Small molecules: Competition

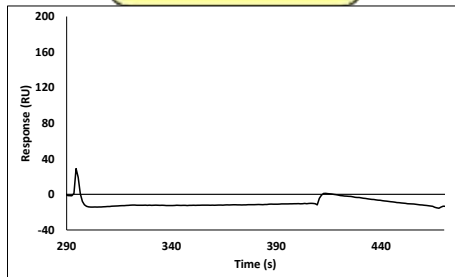
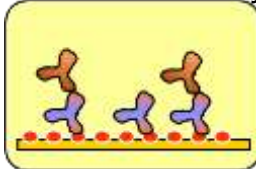


	1st run	2nd run	Mean
Sulfanilamide			
k_a ($\text{M}^{-1}\text{s}^{-1}$)	32 800	30 800	31 800
k_d (s^{-1})	0,146	0,145	0,146
K_D (μM)	4,44	4,72	4,58
Competitor			
k_a ($\text{M}^{-1}\text{s}^{-1}$)	6 600	6 700	6 600
k_d (s^{-1})	0,038	0,038	0,038
K_D (μM)	5,8	5,7	5,8

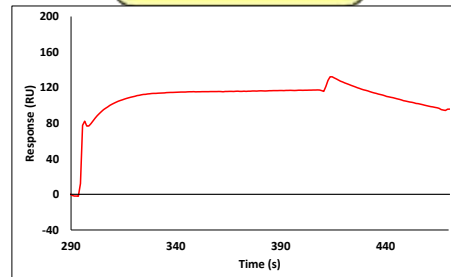
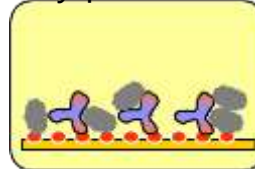
CA II immobilized on a CM5 sensor chip (Biacore 2000)

Proteins: Specificity

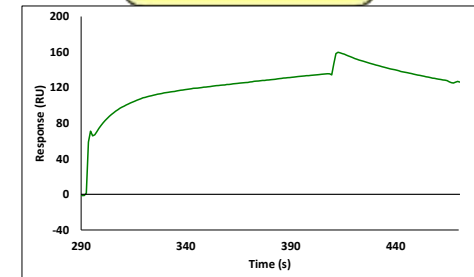
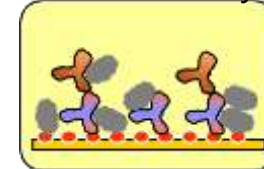
Native 2° Ab only



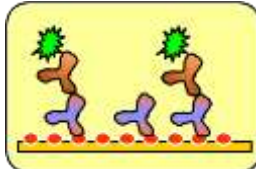
Sticky protein only



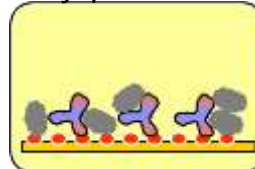
Native 2° Ab + sticky protein



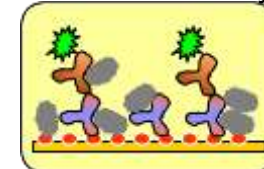
Labelled 2° Ab only



Sticky protein only



Labelled 2° Ab + sticky protein



Label-Enhanced SPR

Dye-labelling combined with SPR dip shape analysis

- ▶ Enhanced sensitivity: 100x
- ▶ Enhanced specificity: 100%



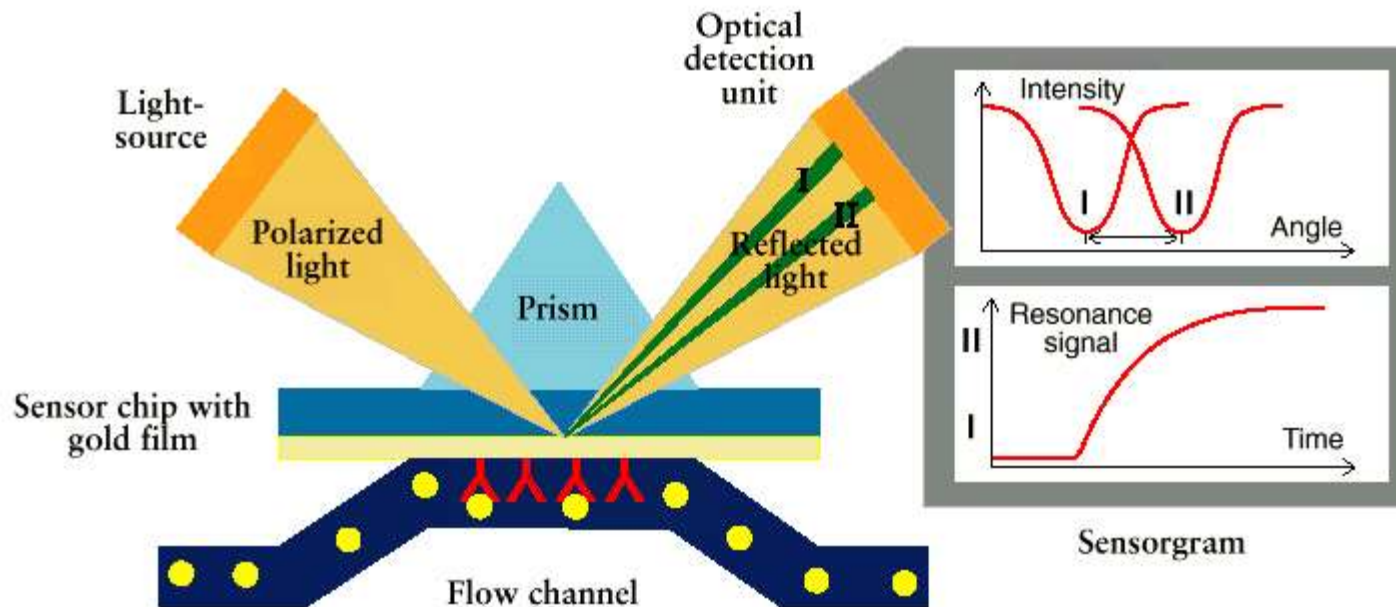
Extra slides

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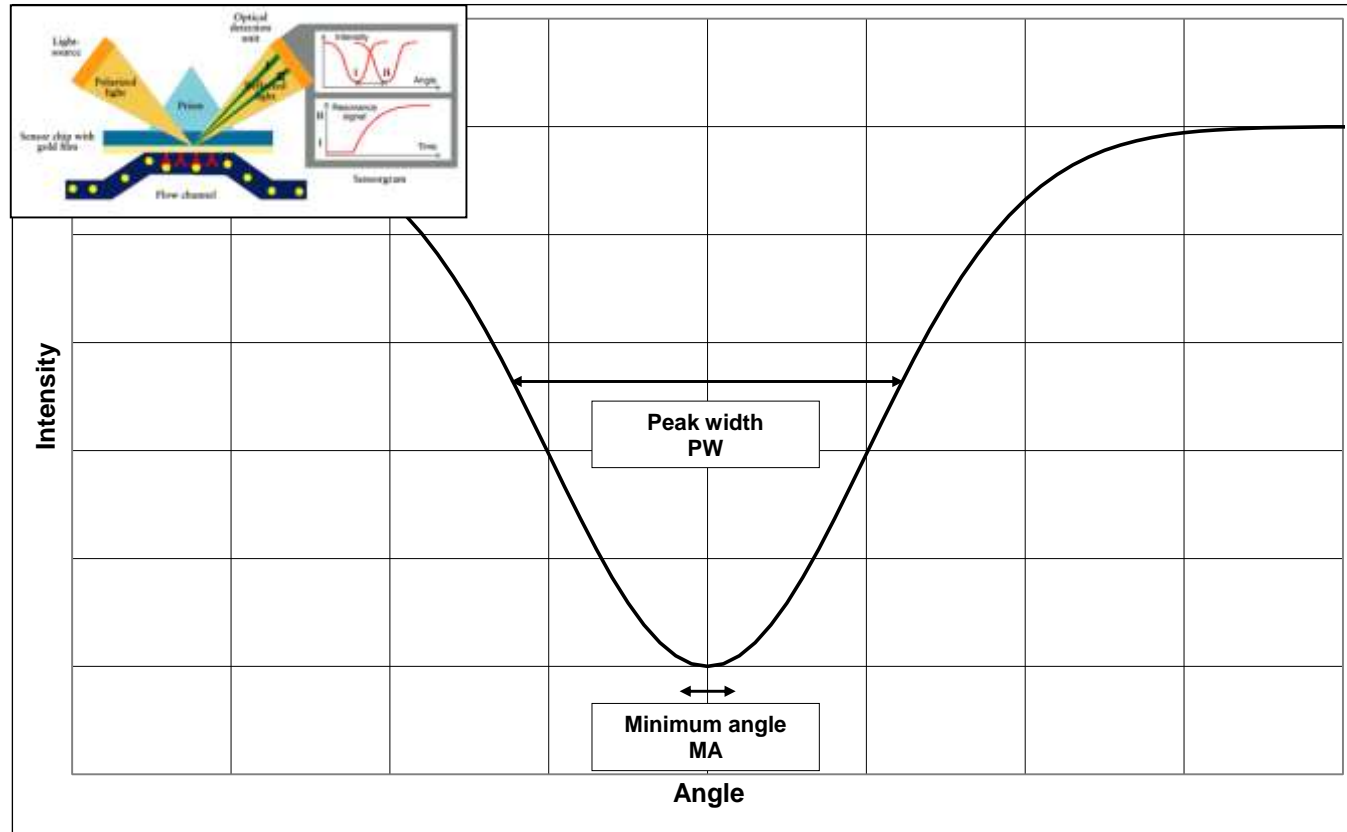
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Basic SPR

- Surface Plasmon Resonance is most common optical biosensor, e.g. Biacore.
- Measures changes in refractive index (RI) close to a solid sensor surface.
- Light is reflected off a gold coated sensor chip. At a certain angle – depending on the sample RI – light is absorbed. This angle is monitored in real time.



SPR dip shape analysis

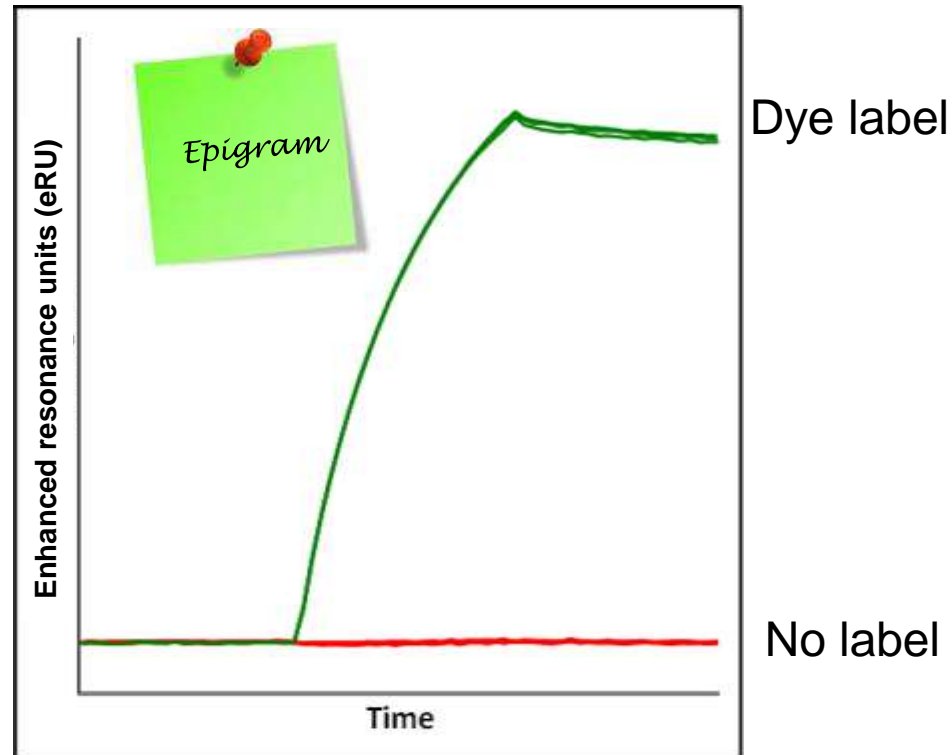


Dip shape depends on refractive index and absorbance.

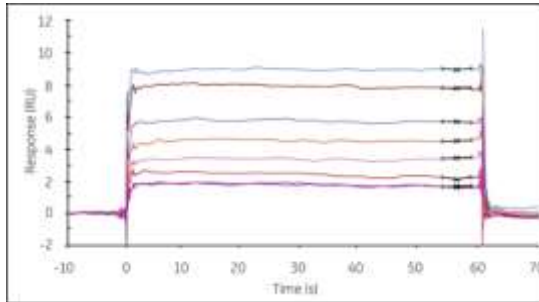
Label-Enhanced SPR

Enhanced sensorgram or 'epigram'.

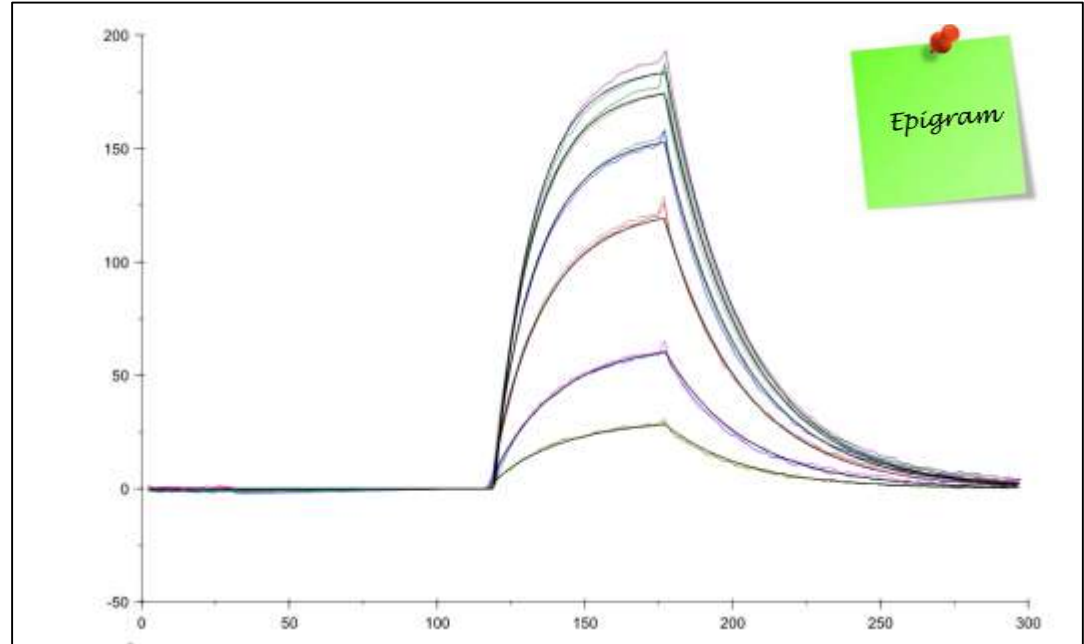
Enhanced resonance units or eRU.



Fragment kinetics



	MESA	Comp.
k_a ($M^{-1}s^{-1}$)	890	7800
k_d (s^{-1})	0.35	0.037
K_D (μM)	390	4.8



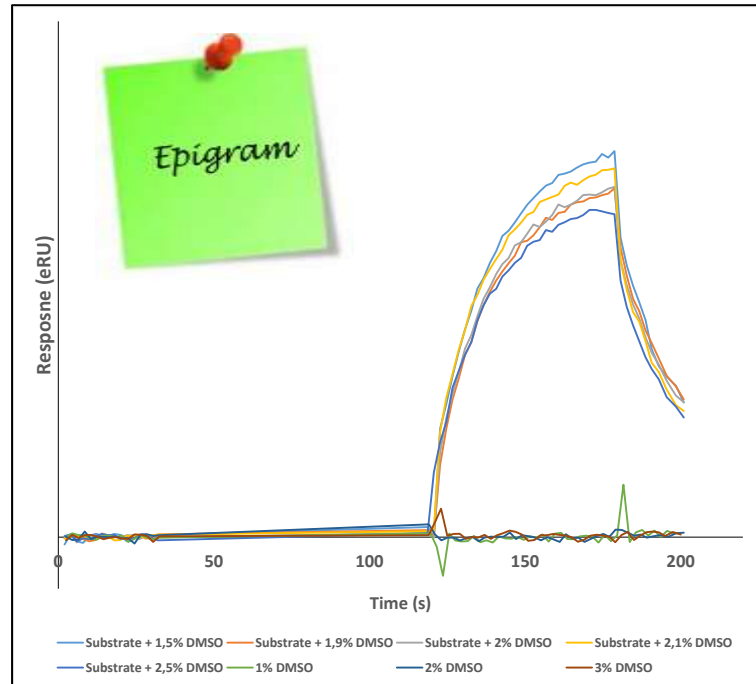
MeSA, 95 Da on CAII

Left panel: T200, 7000 RU immobilized, 31-2000 μM MeSA [1].

Right panel: Biacore™ 2000, 1000 RU CAII immobilized, 40-4000 μM MeSA, 5 μM labelled competitor.

1. Biacore T200 Data file 28-9794-15 AA. Biacore is a trademark of GE Healthcare companies. © 2014 General Electric Company. Reproduced by permission of the owner.

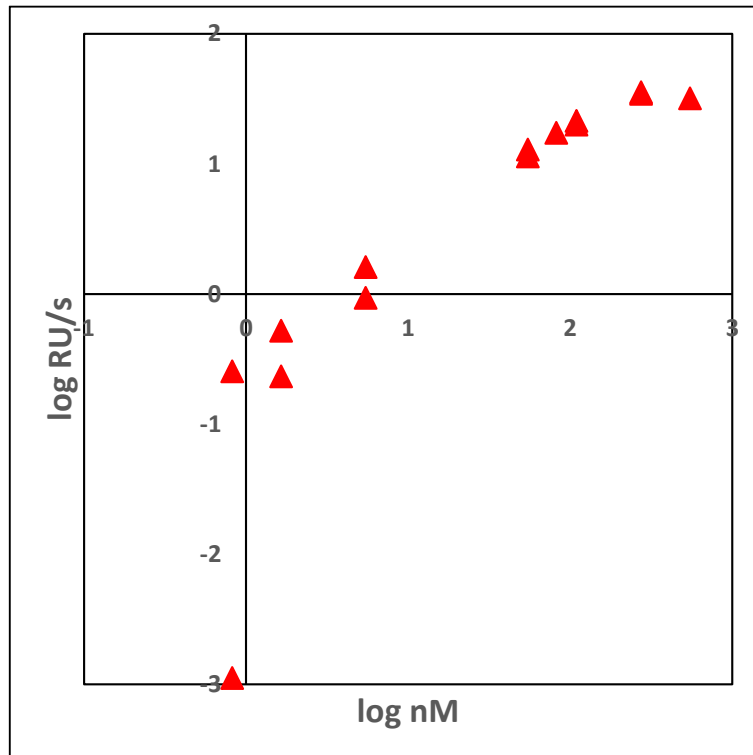
Elimination of bulk effects



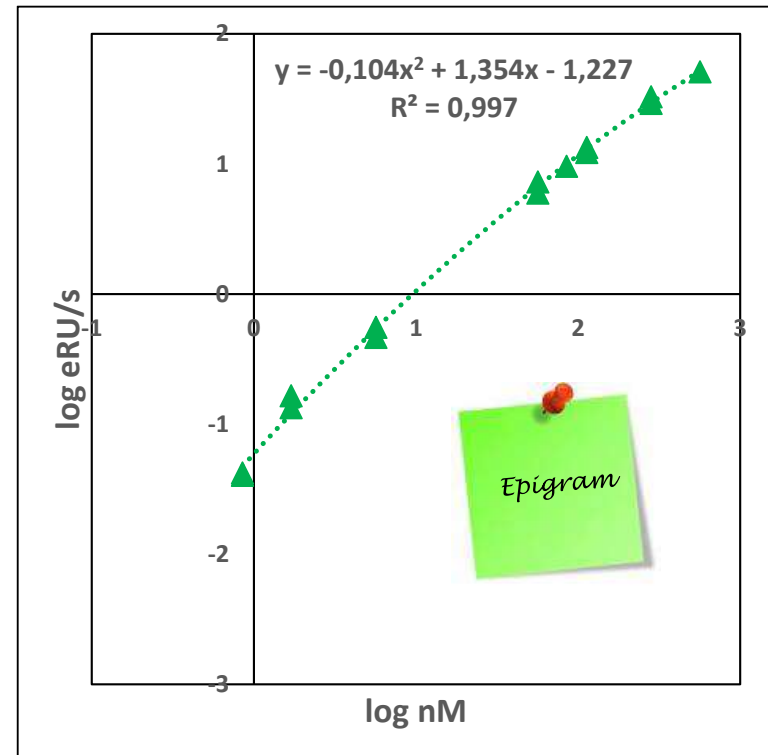
Biacore™ 2000, CM5 chip, 1000 RU CAII immobilized. Substrate: labelled AEBSA, 2.5 μM.
DMSO added 1-3%. No reference channel subtraction. The bulk disturbance is attenuated 100 000x.

Proteins: Dynamic range

Conventional SPR



Label-Enhanced SPR



30 kDa protein, direct binding, Biacore 2000

Method comparison

	Label-Free	Labelled	Labelled
Technology	SPR (DPI, QCM,...)	Dye-labelled SPR	Fluorescence (ELISA, radioactivity,...)
Sensitivity	Medium	High	Very high
Specificity	None	High	High
Problems with temperature, pressure, and concentration variations	Yes	No	No
Real time measurement and kinetics	Yes	Yes	No
Problems with quantum yield, quenching, bleaching	No	No	Yes
Monitoring of all reaction steps	Yes	No	No
Requires label	No	Yes	Yes

The best of both worlds combined in one instrument

B2 Series dyes for Biacore™

Dye	Charge	Aqueous solubility	Functionality
B20	Neutral, zwitterionic	Low	Carboxyl NHS ester Amine Maleimide
B21	One negative net charge	Moderate	
B22	Two negative net charges	Soluble	
B23	Three negative net charges	High	



EpiGrammer software

Calculates enhanced sensorgrams, 'epigrams'

- 100x S/N ratio increase
- 100% specificity

New 2.1 version with improved dip shape algorithms

- Improved response linearity
- Improved rejection of large disturbances
- Improved attenuation of DMSO disturbances: 100 000x S/N increase

