Università degli Studi di Firenze

# Affinity-based biosensors (ABB) for anti-doping applications

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# Two classes of biosensors:

## Enzymatic (catalytic)

The biological element (enzyme) converts the substrate into a product

$$S \rightleftharpoons P$$

The transducer reveals **S** or **P**

## Affinity

The biological element (receptor) binds specifically the analyte leading to a complex

$$A + B \rightleftharpoons AB$$

The transducer reveals the **complex**

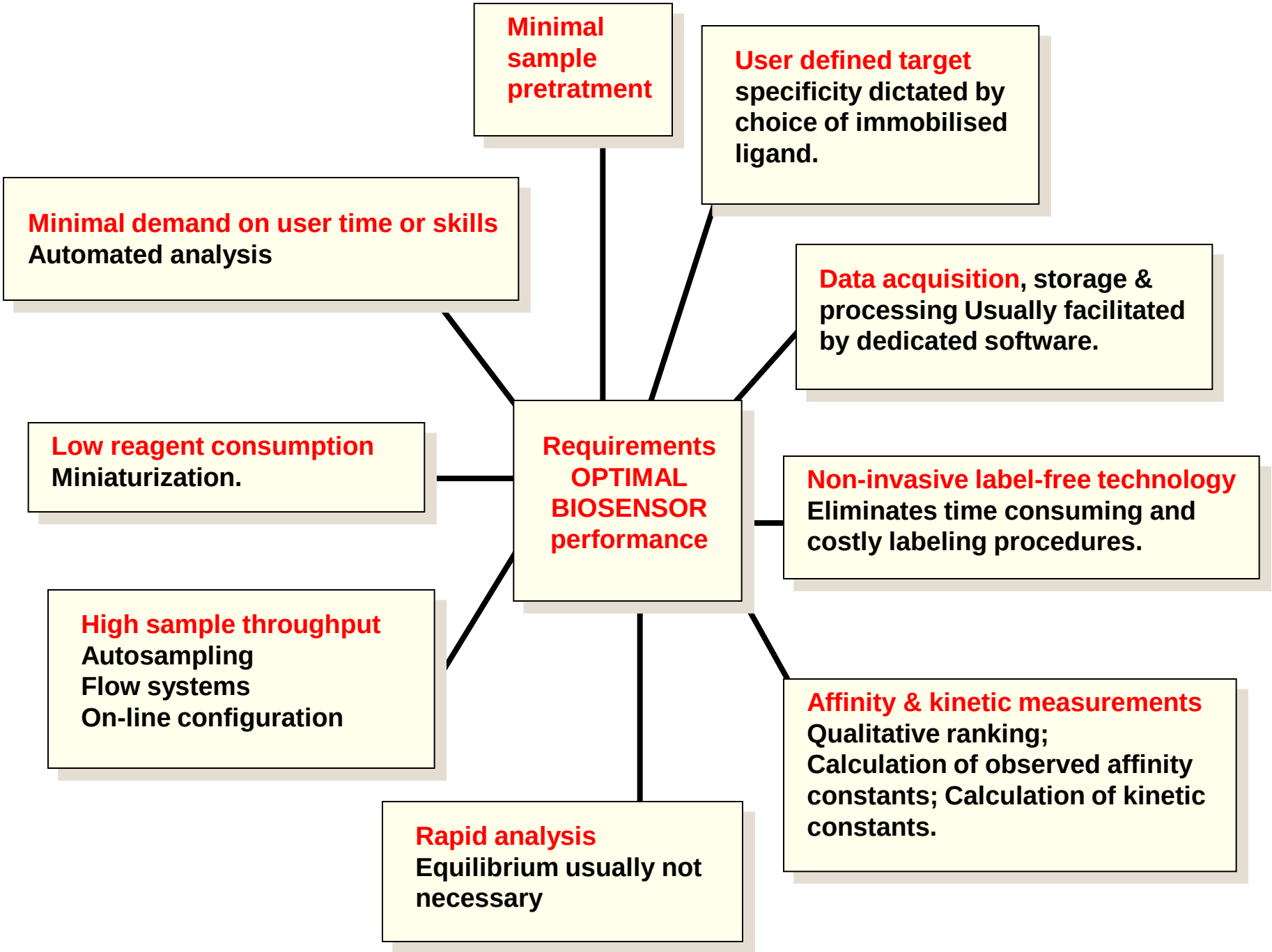
# BIOSENSORS

## Receptors

- Tissues
- Microorganisms
- Organelles
- Enzymes
- Cell receptors
- Antibodies
- Nucleic acids
- Biomimetic receptors (i.e. Oligopeptides, Aptamers, MIP)

## Transducers

- Electrochemical
- Optical
- Thermometric
- Piezoelectric
- …….


Minimal sample pretratment
User defined target specificity dictated by choice of immobilised ligand.
Minimal demand on user time or skills
Automated analysis
Data acquisition, storage & processing Usually facilitated by dedicated software.
Low reagent consumption
Miniaturization.
Requirements OPTIMAL BIOSENSOR performance
Non-invasive label-free technology
Eliminates time consuming and costly labeling procedures.
High sample throughput
Autosampling
Flow systems
On-line configuration
Affinity & kinetic measurements
Qualitative ranking;
Calculation of observed affinity constants; Calculation of kinetic constants.
Rapid analysis
Equilibrium usually not necessary

# Current Paradigm of Blood Glucose Monitoring

Prick finger or arm

Load lancet into launcher and reassemble launcher

Deposit blood drop on to test strip & insert strip


Read test strip

Dispose of materials

**Fully Integrated Sampling & Monitoring**

1-2 Minutes

*Courtesy of Prof. A.P.F. Turner*

**In the case of doping how Affinity-based biosensors (ABBs) can play a role?**

# Development of Affinity-Based Biosensors (ABBs)

- a) ***Hybridization sensor: direct DNA detection***

  Transgene detection for gene doping analysis

  1. Target DNA sequences

  Probe: DNA oligonucleotides;

  Transducers: Piezoelectric and Optical (i.e. SPR imaging)

- ***b) Interaction sensors: Immuno- and apta- sensors based on new receptors (APTAMERS)***

  2. Protein detection,

  Probe: Ab and/or Aptamers;

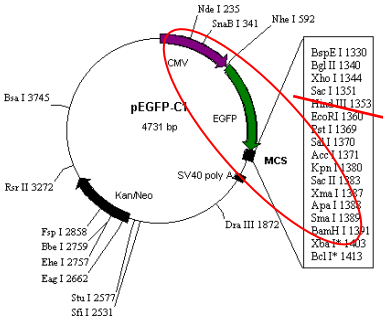
  Transducers: Electrochemical and Optical (SPR)

## 1. Target DNA sequence detection

DIRECT METHODS

- Analysis of recombinant products
- Analysis of exogenous nucleotidic sequences

of the transgene
of the gene cassette: markers, promoter





Vector cassette

INDIRECT METHODS

- Analysis of the induced secondary effects by the transgenosis event

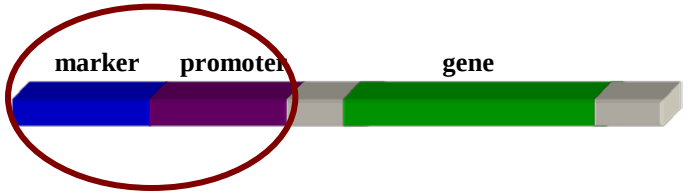
Azzazy et al, Analyst 2007, 132, 951-957

Bautina et al, 2008, J. Gene Med. 10, 3-20

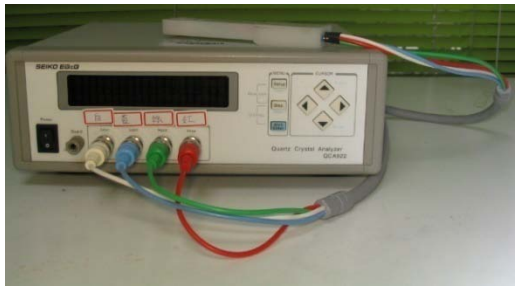
# Development of **piezoelectric DNA-based** biosensors for transgene detection

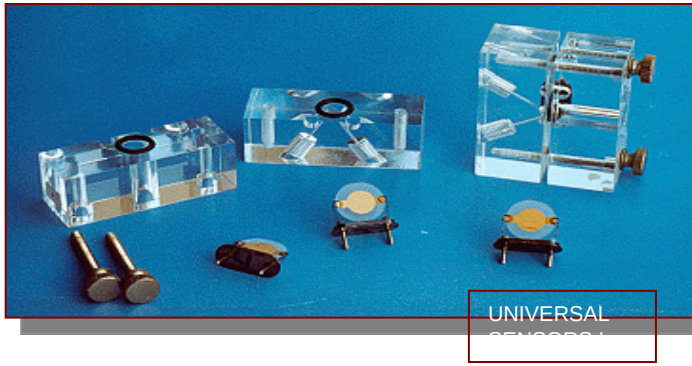
## Real-time, label-free analysis

Analysis of exogenous nucleotidic sequences



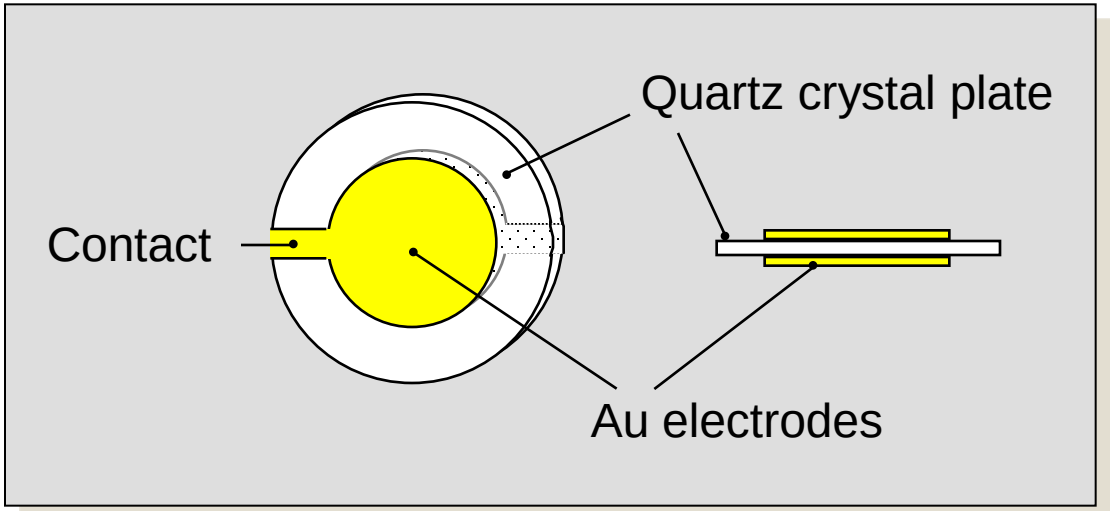

## QUARTZ CRYSTAL MICROBALANCE (QCM) - Principle -



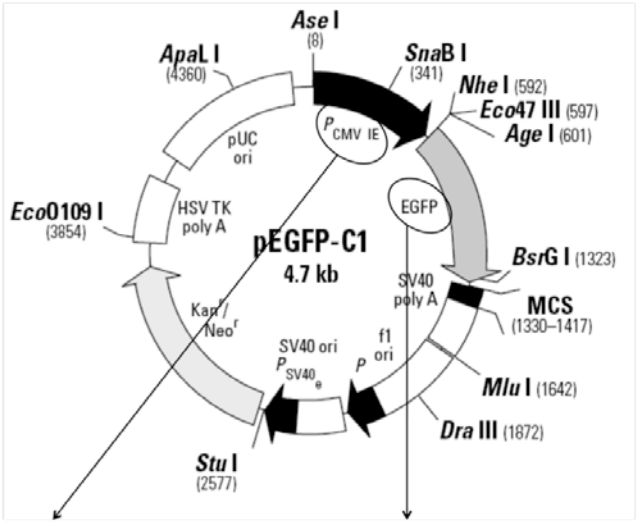


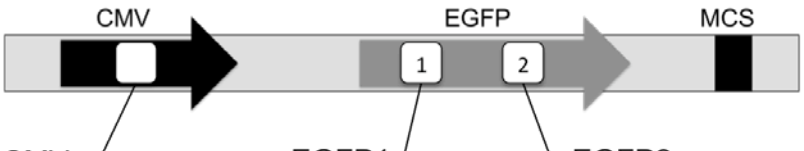
$$\Delta f = \frac{-2\ f_0^2}{A\sqrt{\rho_q \mu_q}}\Delta m$$

$$\Delta f = -2.26 \cdot 10^6\ f_0^2 \frac{\Delta m}{A}$$



# The model system: the plasmid EGFP-C1




CMV

```
TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA
TAGCCCATATATGGAGTTCCGCGTTACATAACTTACGG
TAAATGGCCCGCCTGGCTGACGGCCCAACGACCCCCG
CCCATTGACGTCAATAATGACGTATGTTCCCATAGTAAC
GCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGT
ATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTG
TATCATATGCCAAGTACGCCCCCTATTGACGTCAATGA
CGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGA
CCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTAT
TAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAG
TACATCAATGGGCGTGGATAGCGGTTTGACTCACGGG
GATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGT
TTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGT
CGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTA
GGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGG
TTTAGTGAACCGTCAGATCCGCTAGC
```

**CMV: fragment of 272 pb**

EGFP1 EGFP2

```
ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACG
GCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACG
GCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTC
GTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGC
ACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAG
GACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACC
GCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGA
GTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGG
TGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG
CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCC
AGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGT
GACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTCCGGACTCAGATCTCGA
GCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGATCTAGATA
```

**EGFP1: fragment of 187 pb**

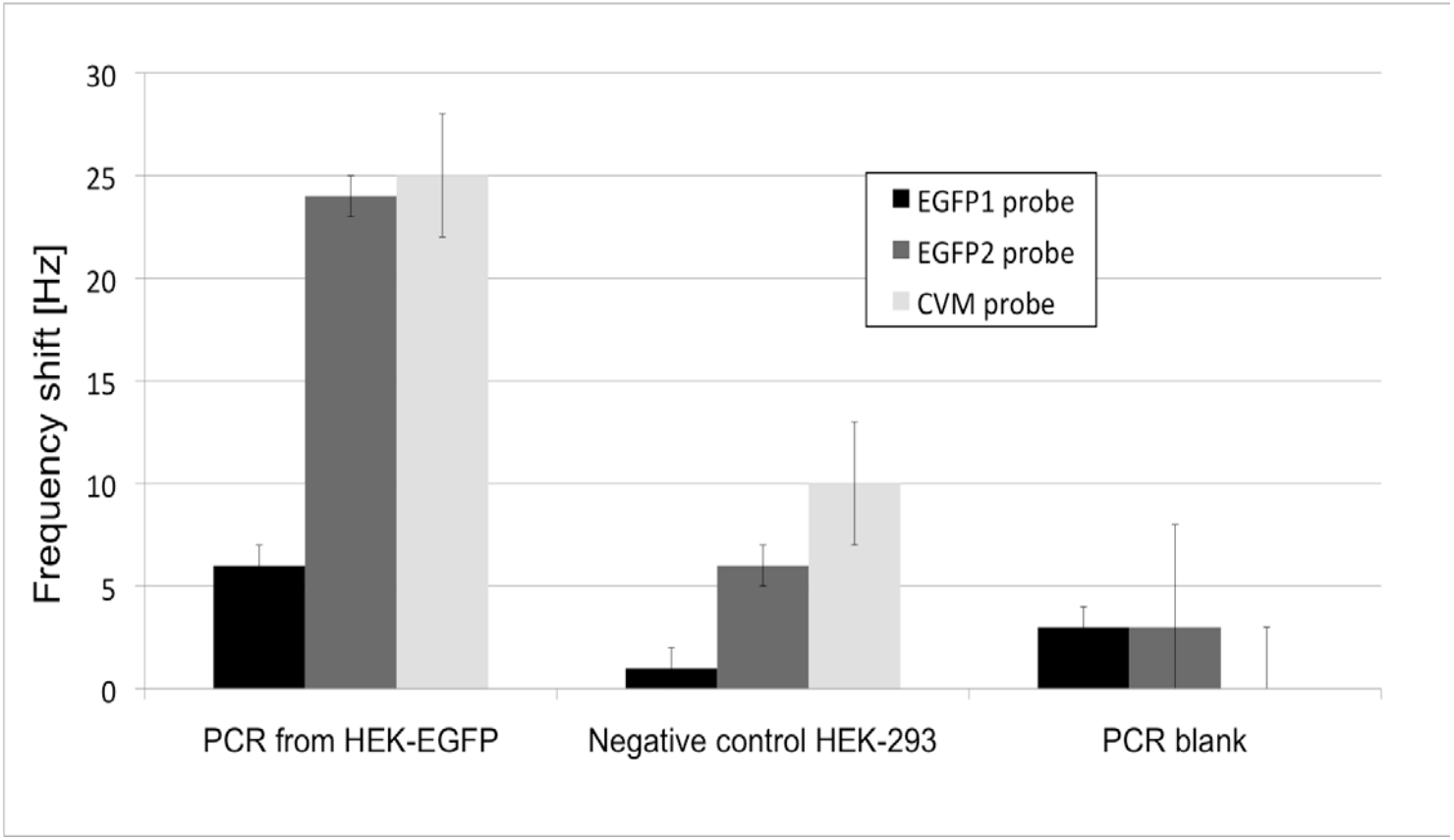
**EGFP2: fragment of 219 pb**

Plasmid EGFP-C1

Wide used vector for mammal transgenosis protocols and potentially useful for illegal purposes, i.e. GENE DOPING


Scarano S., M.M. Spiriti, G.Tigli, P. Bogani, M. Buiatti, M. Mascini, M. Minunni, Affinity Sensing for transgenes detection in anti-doping control, *Analytical Chemistry*, 2009, 81 (23), 9571-9577,

# Transgene detection of target sequences in DNA from Human transgenic cells (HEK-EGFP Human Embryogenic Kidney cells)




PCR Amplified DNA extracted from transgenic human cells (Human Hembryogenic Kidney HEK-EGFP), transformed with plasmid pEGFP-C1 and the relative control (non transformed cells HEK-293) are tested on EGFP and CMV biosensors carrying the three different probes EGFP1, EGFP2 and CMV. The concentration of the PCR amplicons was 220 nM for all the samples. Each sample was tested three times (n=3).

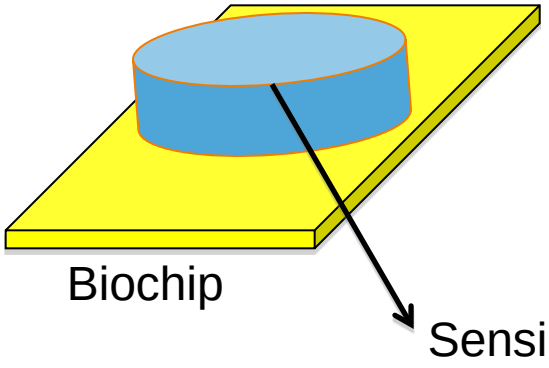
Scarano S., M.M. Spiriti, G.Tigli, P. Bogani, M. Buiatti, M. Mascini, M. Minunni, Affinity Sensing for transgenes detection in anti-doping control, *Analytical Chemistry*, 2009, 81 (23), 9571-9577

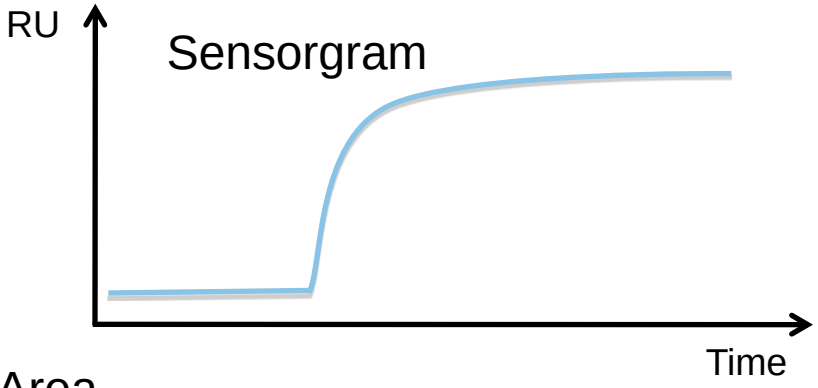
Moving to real-time, label free and

**multi-analyte simultaneous detection……..**

# SPR IMAGING (SPRI) FOR BIOSENSING: AN INNOVATIVE LABEL-FREE, MULTIARRAY PLATFORM FOR THE DETECTION OF BIOAFFINITY INTERACTIONS

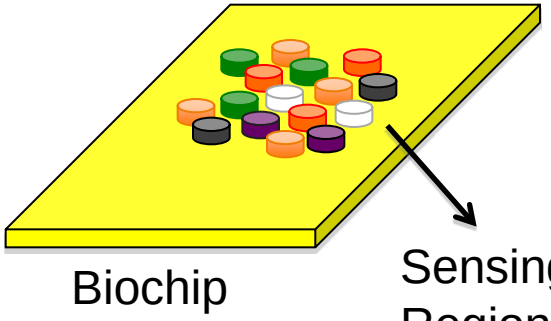
## Surface Plasmon Resonance (SPR)



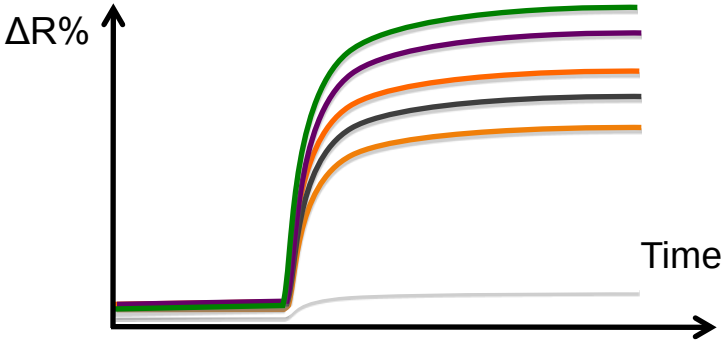




Array of Light Sensitive Diodes (LSD)

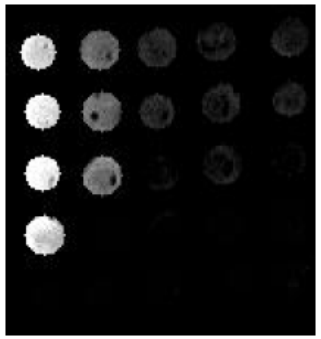
## SPR imaging (SPRi)




Sensing Areas:
Regions of Interest (ROIs)

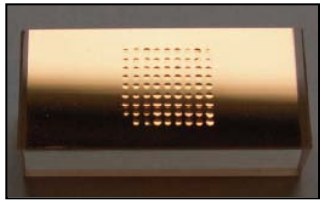


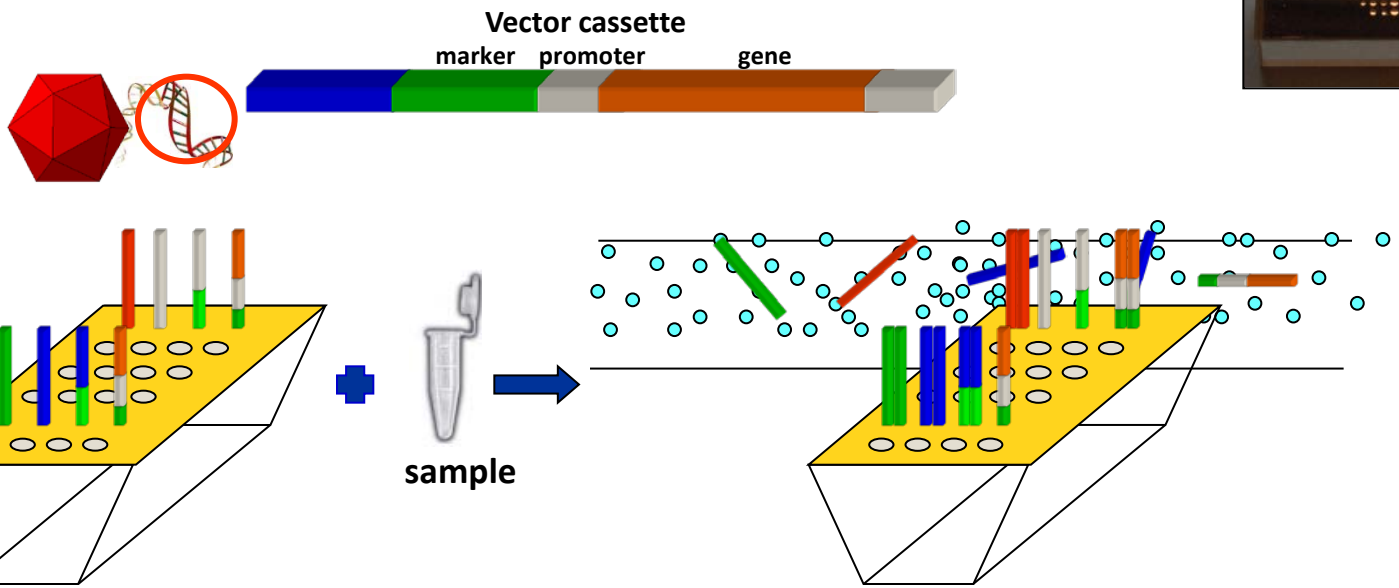

Charge Coupled Device (CCD)

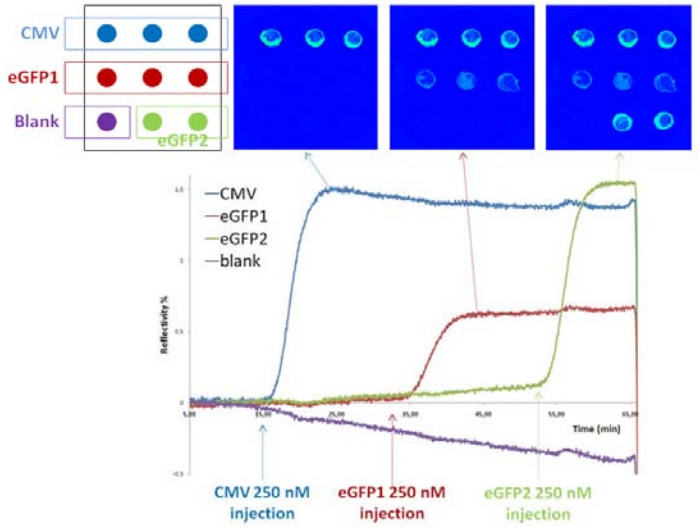


S. Scarano, M.Mascini, APF Turner and M.Minunni, Surface Plasmon Resonance Imaging for Affinity-Based Biosensors, *Biosensors and Biolectronics*, **2010,** 25, 5, 957-966

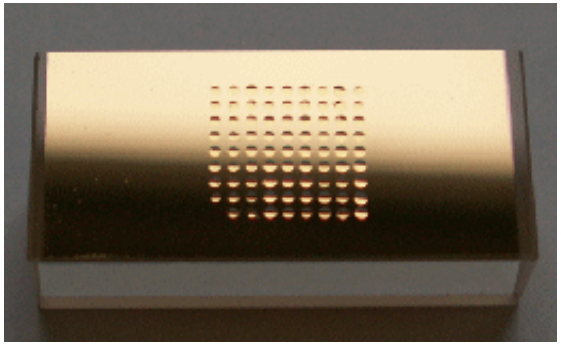
# Multiarray based on optical transduction Surface Plasmon Resonance imaging (SPR-i) with immobilized different probes for different targets.

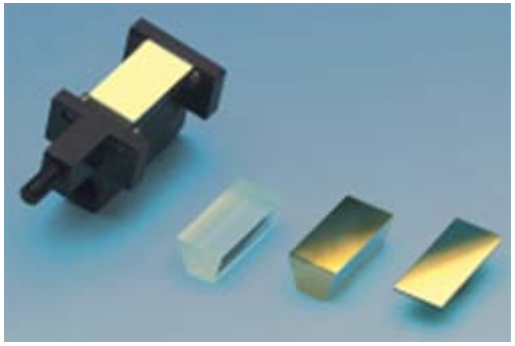




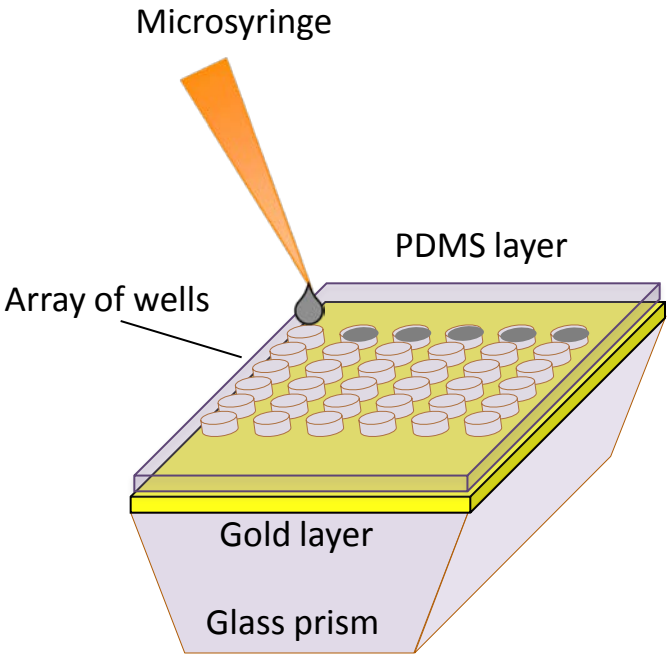
# Biochips and Array Preparation



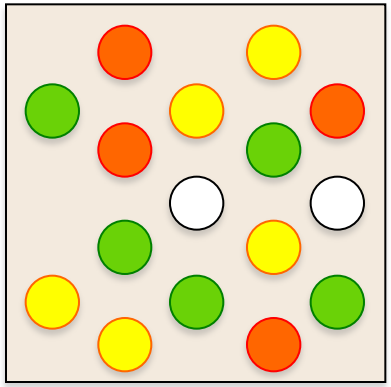


Resonance couplers:

Glass prisms or slides coated with gold




Biochip design



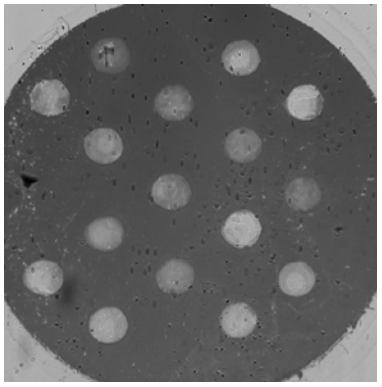
Probe 1

Probe 2

Probe 3

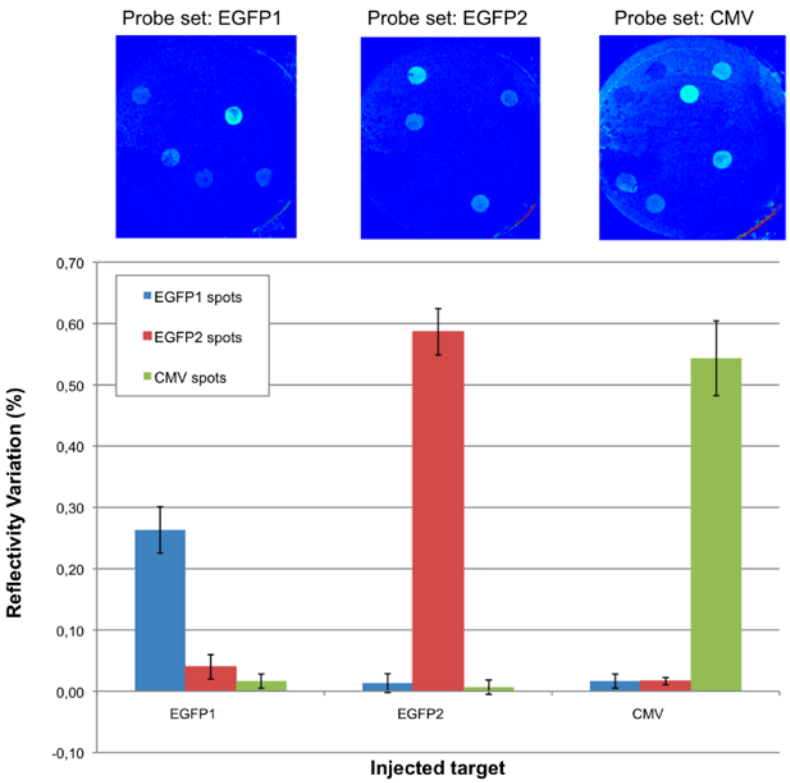
Negative controls

Bioreceptors Array



Biochip view by CCD before interaction

## Selectivity




## Calibration with synthetic oligonucleotides

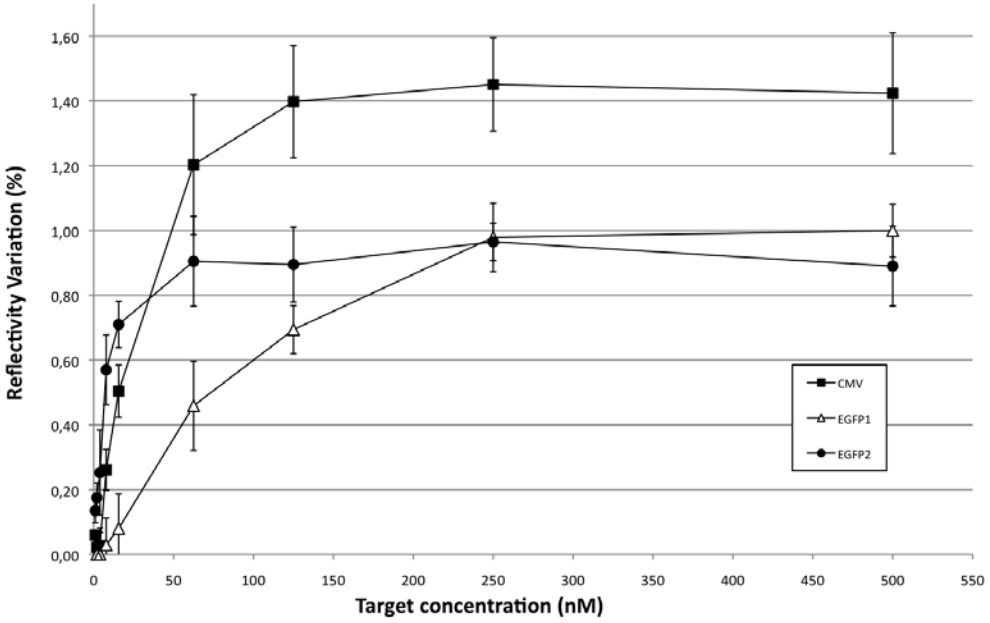



**Figure 2:** SPRi calibration curves with synthetic targets EGFP1, EGFP2 and CMV in the range 1-500 nM.

**Figure 1.** EGFP1, EGFP2, and CMV complementary targets were tested for their specificity toward each corresponding probe sequence. Regeneration steps among injections are required to recover the probe availability.

# Transgene detection of target sequences in DNA from Human transgenic cells (HEK-EGFP Human Embryogenic Kidney cells)

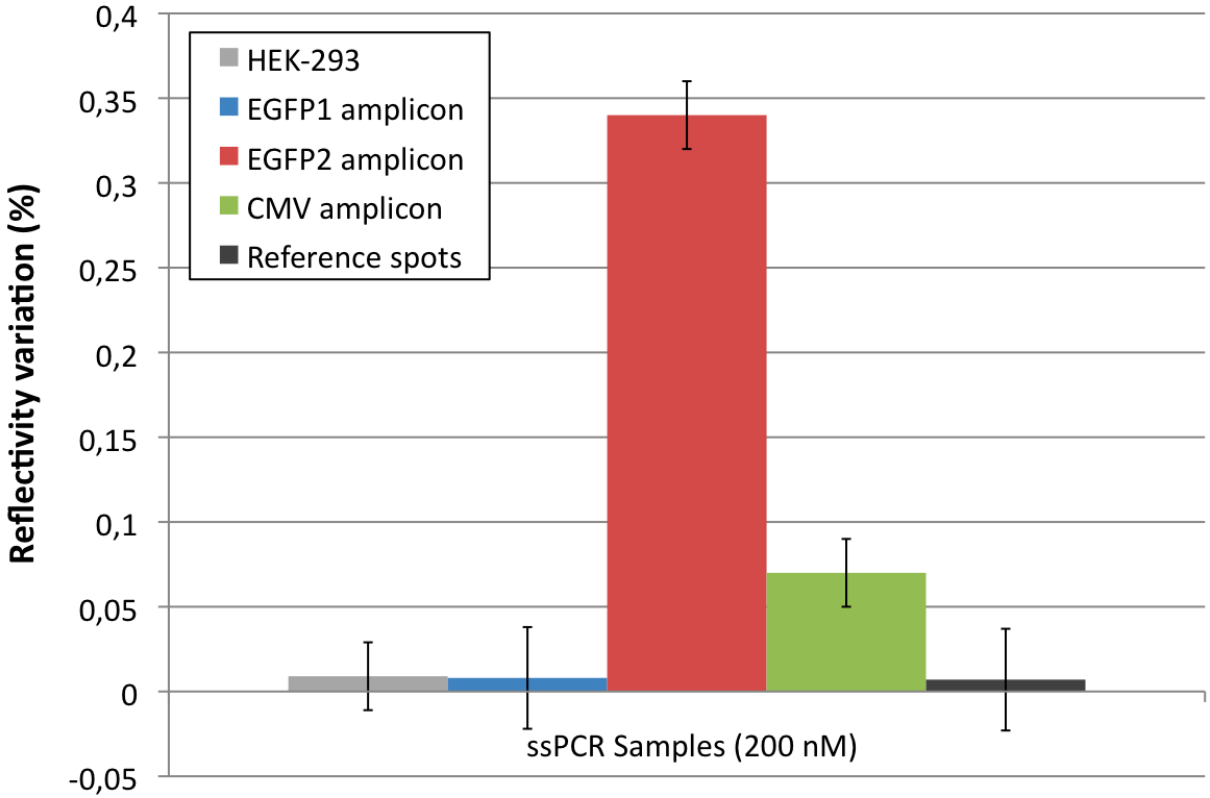



**Figure 3.** SPRi results on biotinylated amplicons after strands separation with microbeads. From left to right: 1) PCR amplicons from HEK-293 (negative control). The response is averaged on the three amplicons obtained with the three couples of primers; 2) amplicon from HEK-GFP cells containing EGFP1 target sequence, tested for the specific response on EGFP1 probe; 3) **amplicon from HEK-GFP cells containing EGFP2 target sequence, tested on EGFP2 probe;** 4) **HEK-GFP cells amplified with CMV primers couple, and tested on CMV probe;** 5) response on reference spots for all amplicons, averaged on the two spots of the array, and on all amplicons tested.

S. Scarano, M.L. Ermini, M.M. Spiriti, P. Bogani, M. Mascini and M. Minunni, Simultaneous detection of transgenes by Surface Plasmon Resonance imaging for gene doping detection *submitted to Analytical Chemistry* Manuscript ID: ac-2011-00877m,

# *b) Interaction sensors: Immuno- and apta- sensors*

Examples:

Protein detection:  EPO,  using immobilized Ab and

Aptamers for EPO

**Table 1** Proteins of interest for anti-doping purposes, their concentration in plasma, and entry in the UniProt database

| Protein | Protein mass (kDa) | Comment | PTM | Concentration | Detection | UniProt access no. |
|---|---|---|---|---|---|---|
| Ghrelin | 3.37 | | Octanoyl | 100–150 fmol/ml | Competition assay | Q9UBU3 |
| Sermorelin | 3.36 | | | | – | |
| ACTH | 4.64 | | $PO_3$ | 5–46 pg/ml | | P01189 |
| Insulin | 5.99 | SS dimer | | | MS, IA | P01308 |
| IGF-I | 7.75 | | | 110–330 ng/ml[a] | IA | P05019 |
| GHRH-1 | 12.45 | | Amidated | | – | P01286 |
| GHRH-2 | 12.35 | | Amidated | | | |
| GH-22 | 22.21 | | – | 0.01–5 ng/ml | IA-SPR | P01241-1 |
| GH-20 | 20.36 | Splice variant | – | 0.005–0.5 ng/ml | IA | P01241-2 |
| EPO | 18.48 | | 3N+1O | 0.5 ng/ml | IEF-WB | P01588 |
| LH-β | 13.29 | Hetero dimer | 1N | 0.16–0.068 μg/ml | IA-SPR | P01229 |
| LH-α | 10.30 | | 2N | | | P01215 |
| hCG-β | 15.52 | Hetero dimer | 2N+4O | 33 ng–1.6 μg/ml | IA | P01233 |
| hCG-α | 10.30 | | 2N | | | P01215 |
| PIIINP | 13.23 | | | 1.5–6 ng/ml | IA | P02461 |
| HIF1-α | 92.74 | | OH-Pro<br>NO-Cys | – | | Q16665 |
| HIF3-α | 72.79 | | OH-Pro | – | – | Q9Y2N7 |
| MGF | 2.89 | Splice variant | – | – | – | |
| PDGF A | 14.39 | | 1N | 250–370 pg/ml | IA | P04085 |
| PDGF B | 12.38 | | – | | | P01127 |
| PDGF C | 37.21 | Latent form | 2N | | | Q9NRA1 |
| PDGF D | 13.96 | | 1N | | | Q9GZP0 |
| PDGF AB | 26.77 | | 1N | | | |
| FGF 1 | 15.82 | | – | 54–109 pg/ml | IA | P05230 |
| FGF 2 | 16.40 | | – | 0.34–2.4 pg/ml | IA | P09038 |
| FGF 3 | 24.94 | | – | – | – | P11487 |
| FGF 4 | 19.28 | | – | – | – | P08620 |
| FGF 5 | 27.57 | | 1N | – | – | P12034 |
| FGF 6 | 18.97 | | 1N | – | – | P10767 |
| FGF 7 | 18.87 | | 1N | <30 pg/ml | IA | P21781 |
| FGF 8 | 24.19 | | 1N | – | – | P55075 |
| FGF 9 | 23.13 | | 1N | – | – | P31371 |
| FGF10 | 19.31 | | 2N | – | – | O15520 |
| FGF 11 | 24.99 | | – | – | – | Q92914 |
| FGF 12 | 27.38 | | – | – | – | P61328 |
| FGF 13 | 27.55 | | – | – | – | Q92913 |
| FGF 14 | 27.81 | | – | – | – | Q92915 |
| FGF 16 | 23.75 | | 1N | – | – | O43320 |
| FGF 17 | 22.50 | | 1N | – | – | O60258 |
| FGF 18 | 21.02 | | 2N | – | – | O76093 |
| FGF 19 | 21.42 | | – | 31–554 pg/ml | IA | O95750 |
| FGF 20 | 23.49 | | – | – | – | Q9NP95 |
| FGF 21 | 19.40 | | | n.d.–910 pg/ml | IA | Q9NSA1 |
| FGF 22 | 17.16 | | – | – | – | Q9HCT0 |
| FGF 23 | 25.32 | | 1O | – | – | Q9GZV9 |
| HGF-α | 53.65 | α/β SS dimer | 2N+1O | 670–2,000 pg/ml | IA | P14210 |
| HGF-β | 25.98 | | 2N | | | |

**Table 1** (continued)

| Protein | Protein mass (kDa) | Comment | PTM | Concentration | Detection | UniProt access no. |
|---|---|---|---|---|---|---|
| VEGF A | 23.88 | | 1N+3N6AcLys | 46–666 pg/ml | IA | P15692 |
| VEGF B | 19.35 | | – | | | P49765 |
| VEGF C | 13.10 | | 2N | | | P49767 |
| VEGF D | 13.10 | | 2N | | | O43915 |
| PlGF | 22.77 | | 2N | | | P49763 |

*PTM* post-translational modification, N,N- and O,O-linked glycosylation; *SPR* surface plasmon resonance; *IA* immunoassay; *IEF* isoelectric focussing; $PO_3$ phosphorylation; *NO* nitroso; *OH* hydroxylation; *MS* mass spectrometry; *Ac* acetylation; *n.d.* not detected; *ACTH* adrenocorticotropic hormone; *IGF* insulin-like growth factor; *GNRH* growth-hormone-releasing hormone; *GH* human growth hormone; *EPO* erythropoietin; *LH* luteinising hormone; *hCG* human chorionic gonadotropin; *PIIINP* procollagen III N-terminal propeptide; *HIF* hypoxia-inducible factor; *MGF* mechano growth factor; *PDGF* platelet-derived growth factor; *FGF* fibroblast growth factor; *HGF* hepatocyte growth factor; *VEGF* vascular endothelial growth factor; *PlGF* placental growth factor

[a] Subject age between 20 and 40 years

Anal Bioanal Chem
DOI 10.1007/s00216-011-4830-9

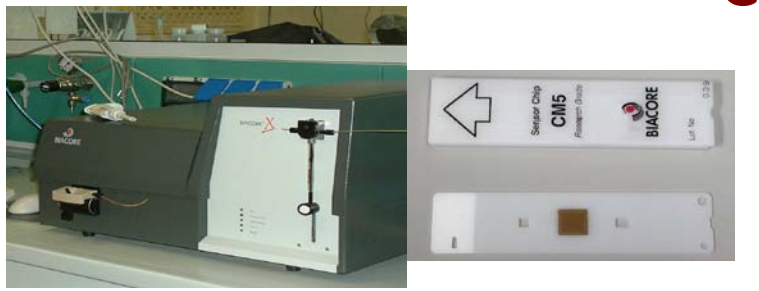
REVIEW

# Surface plasmon resonance in doping analysis

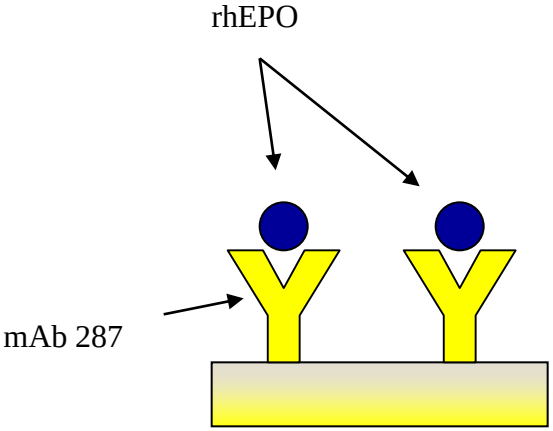
**R. Gutiérrez-Gallego · E. Llop · J. Bosch · J. Segura**

Hormone Abuse in sports; The antidoping perspective, Barroso O., Mazzoni I., Rabin O., *Asian J. Androl.*, **2008**, (3) 391-402

# Conventional SPR

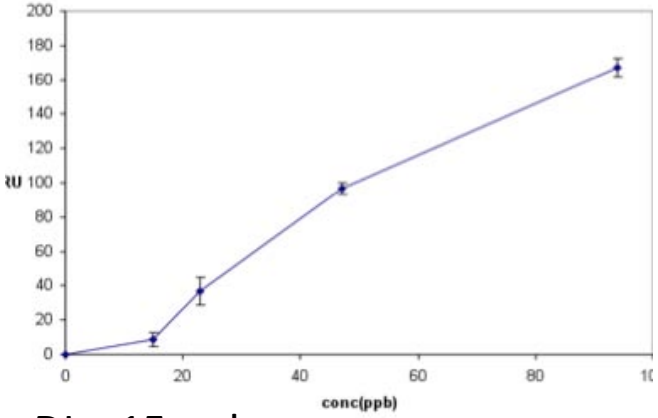



- mAb 287




Instrumentation BIAcore X™, CM5 Chip

## Calibration in buffer



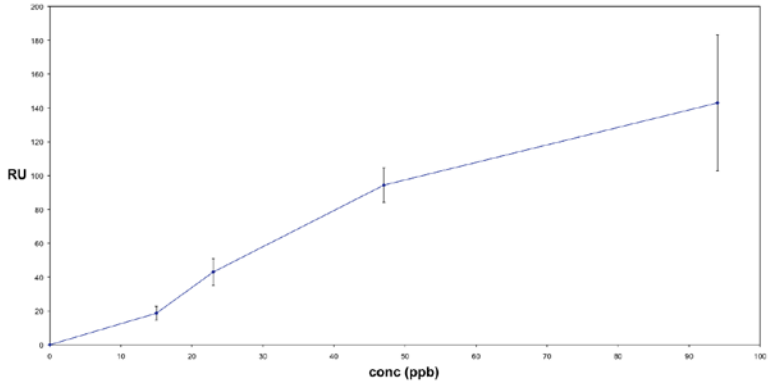

DL= 15 ppb

low selectivity: SrHEpo/S uHEpo=0,33

reproducibility: CVav%: 18%, $S_{HSA}$ ~ 0

| | Urine/ PBS | | |
|---|---|---|---|
| | 1/10 | 1/5 | 1/1 |
| blank | 3 RU | 12 RU | 18 RU |
| rHEpo 47 ppb | 85 RU | 91 RU | 91 RU |

## Calibration in spiked urine




DL= 23 ppb

low reproducibility: CV%: 20%

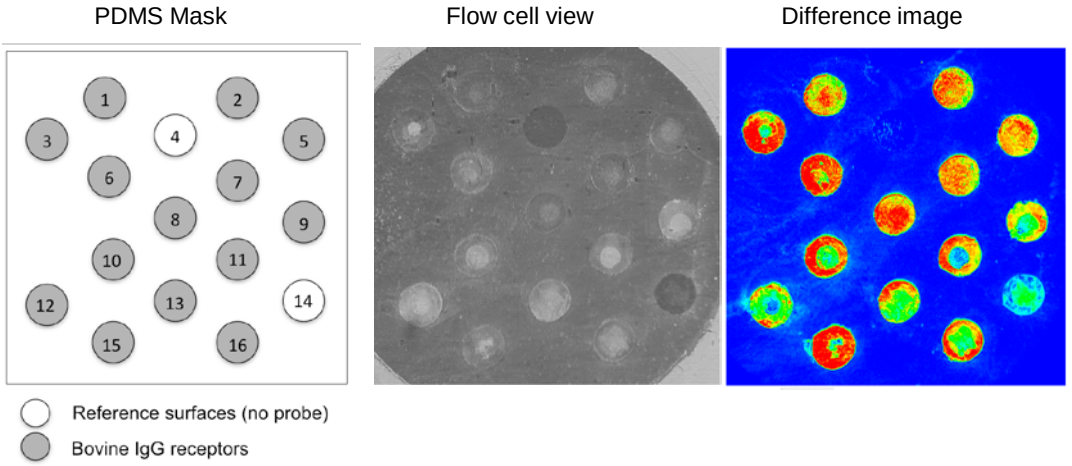
Interferences i.e. Surea ~ 0

- **Selecting a sample dilution**
- Different ration urine/PBS 20 mM (pH=7,4) tested alone or spiked with rHEpo

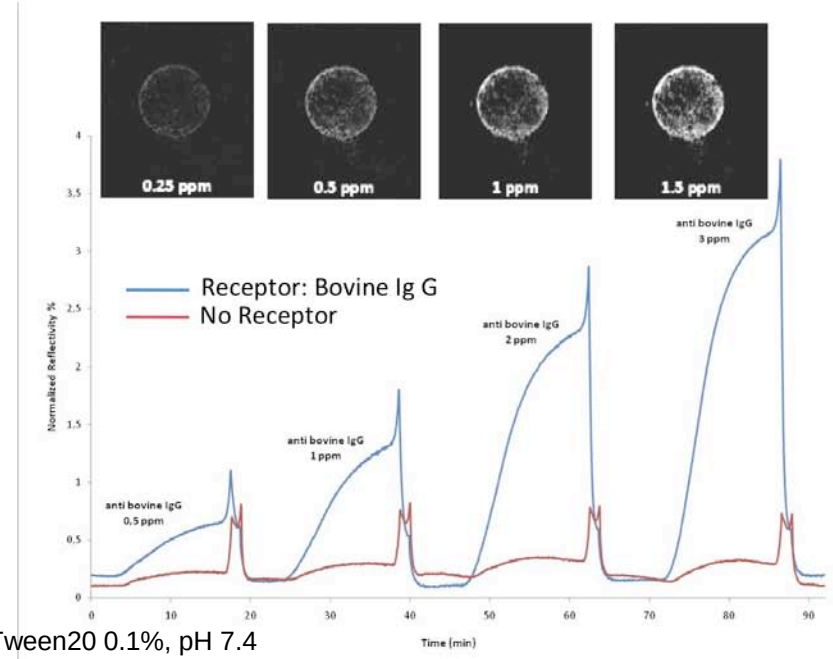
# Multi-analyte detection in real matrices: SPRi for proteins detection in Human Serum

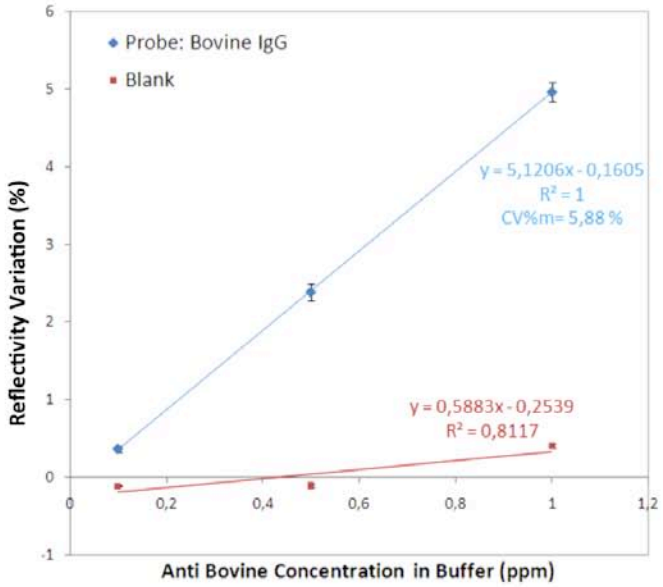
*Immobilization:*
Amino-coupling on SAM

*Matrices:*
Human serum




## Anti-Bovine IgG Detection in Buffer*

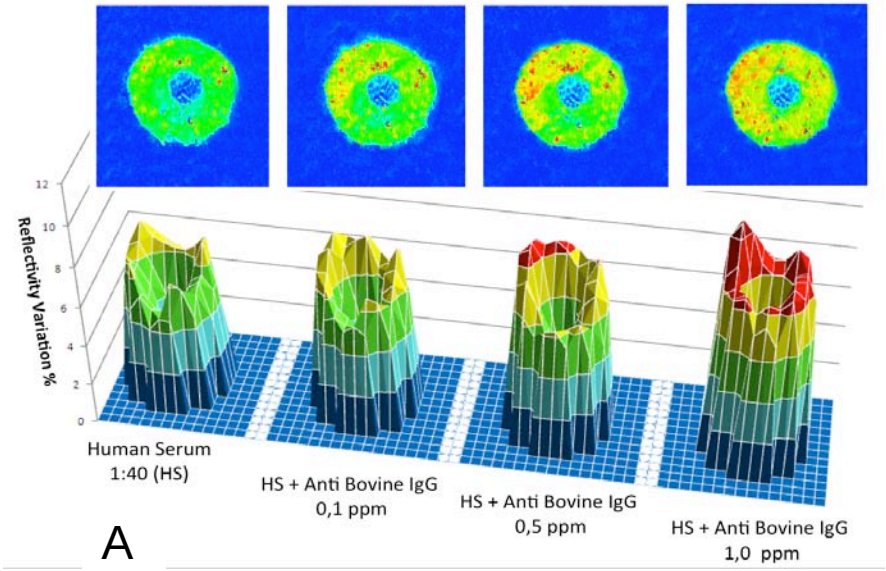


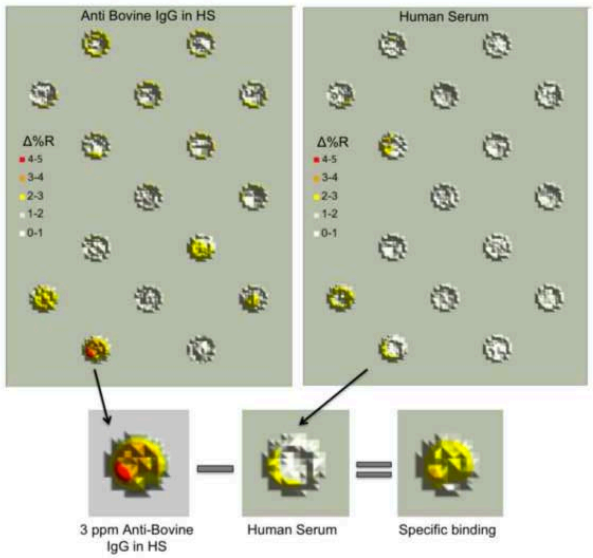

*HEPES 10 mM, Tween20 0.1%, pH 7.4

# Anti-Bovine IgG detection in Human Serum

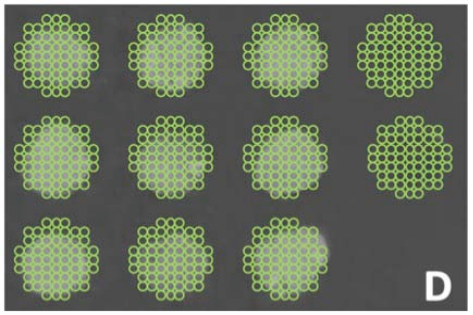
Dilution Factor: 1/40

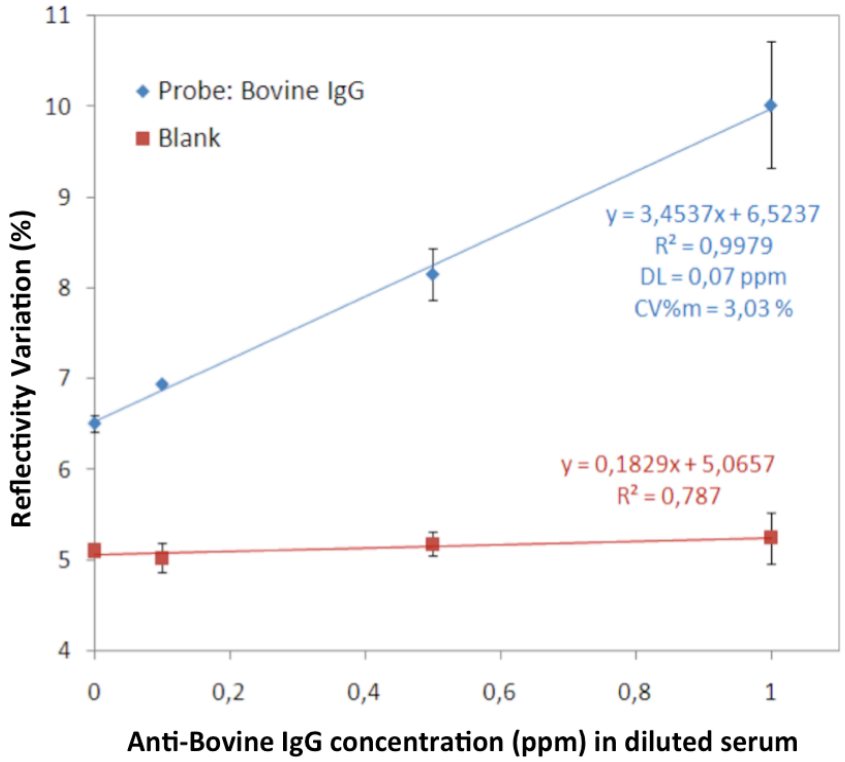



A




Our approach:
61 signals/spot




B

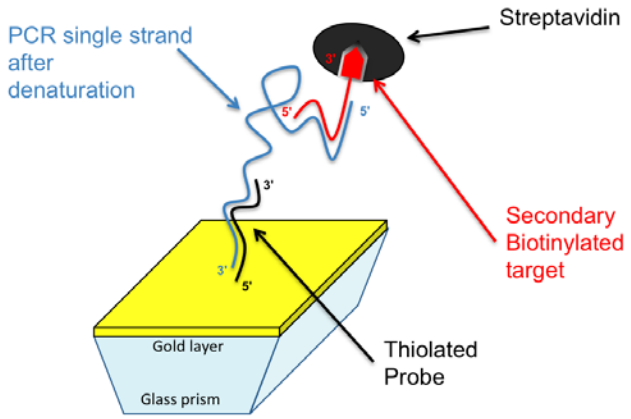

S. Scarano, C. Scuffi, M. Mascini, M. Minunni, Surface Plasmon Resonance Imaging (SPRi)-Based Sensing: A New Approach in Signal Sampling and Management, *Biosensors and Biolectronics*, 26 (4), 2010**,** 1380-1385;

S. Scarano, C. Scuffi, M. Mascini, M. Minunni, Surface Plasmon Resonance Imaging (SPRi)-based sensing for anti-Bovine Immunoglobulins detection in Human milk and serum, submitted to *Analytica Chimica Acta, 18 May 2011,* ACA-11-981

# Improving sensitivity  by molecular architectures….

# Amplification of the signal

## 1. By strepdavidin



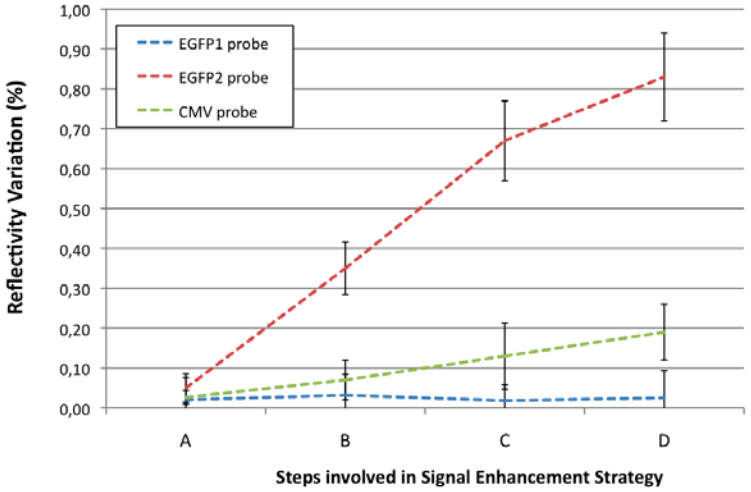
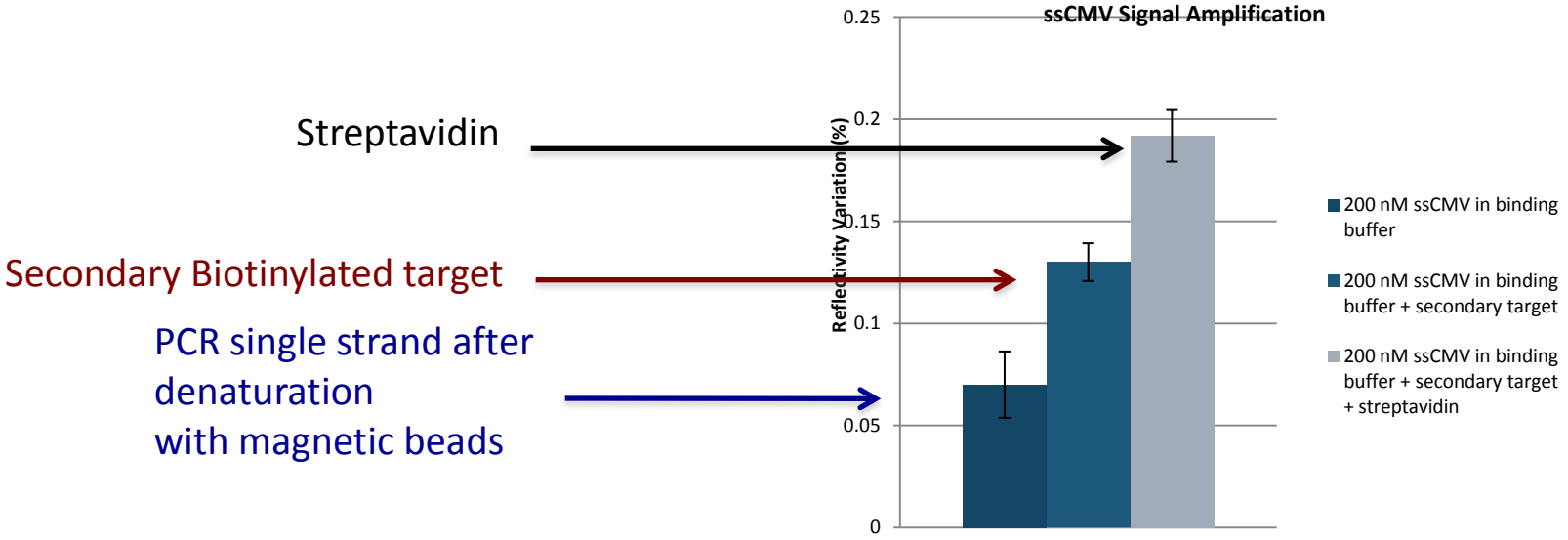
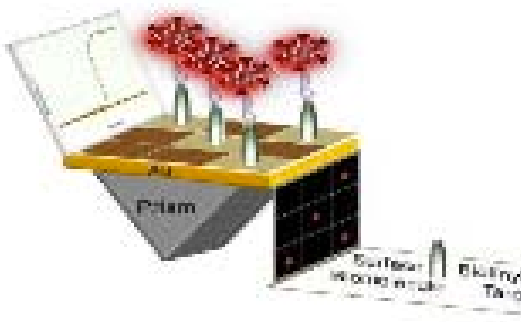




**Figure 6.** Sketched representation of the strategy followed for signal enhancement on PCR amplicons after their denaturation and subsequent binding on biochip (left), and results obtained (right). A) Injection of streptavidin alone; B) SPRi signals for the three PCR amplicons (200 nM); C) Injection of secondary biotinylated target at equimolar concentration; D) Streptavidin addition (equimolar to PCR samples) and relative increment of signals.



# 2. By Quantum Dots (QD) modified with streptavidin



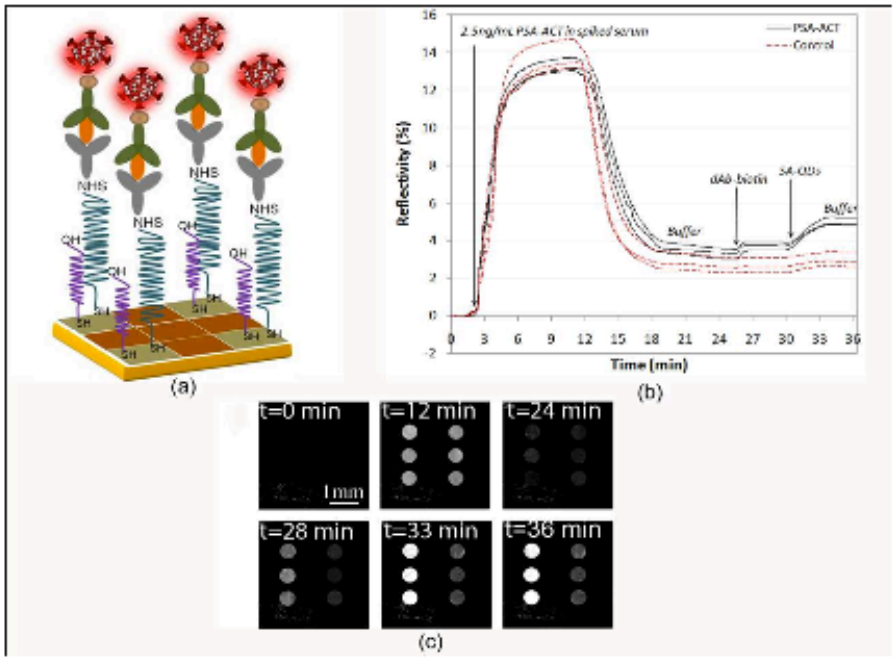
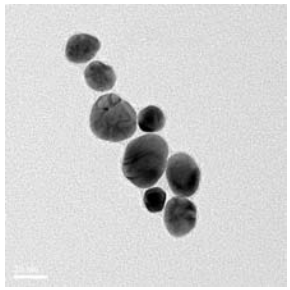
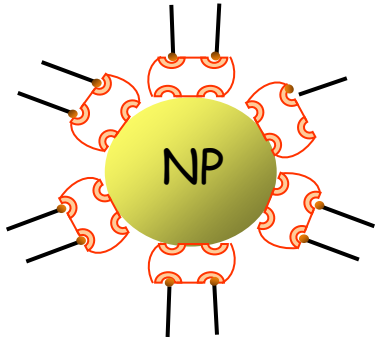



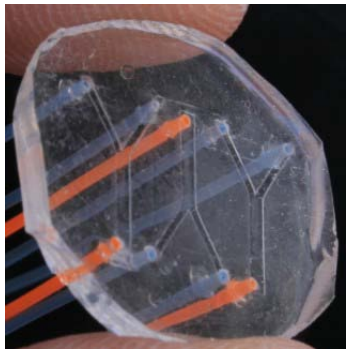

Figure 5. (a) Schematic representation of gold chip functionalization with PEG-COOH (blue) and PEG-OH (purple) followed by the addition of: capture antibody PSA-ACT complex (grey), PSA-ACT antigen (orange), biotinylated detection antibody PSA-ACT complex (green-brown) and streptavidin coated QDs (red). (b) SPRi kinetic curves for detection of PSA-ACT in spiked serum. (c) The corresponding difference images showing time-lapsed binding kinetics for initial buffer injection, PSA-ACT complex in spiked serum injection, buffer wash, dAb-biotin injection, SA-QD injection and final buffer wash on three spots of anti-PSA (left) and anti-IgG (right).

Lidija Malik, Marinella G. Sandroz and Maryam Tabrizian, Designed Biointerface Using Near Infra-Red Qunatum Dots for Ultrasensitive Surface Plasmon Resonance Imaging Biosensors,, *Analytical Chemistry,* Just accepted, DOI: 10.1021/ac200465m, **Publication date (Web) 23 May 2011**

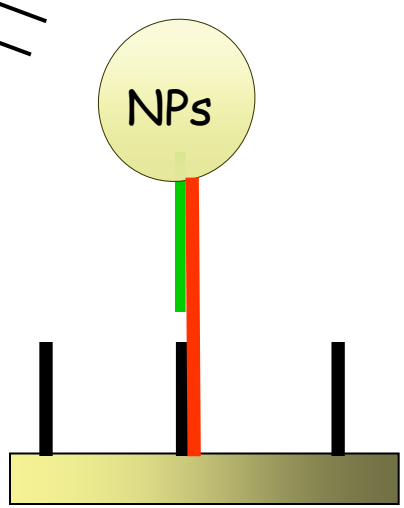
# 3. By gold Nanoparticle (NPs) modified with streptavidin

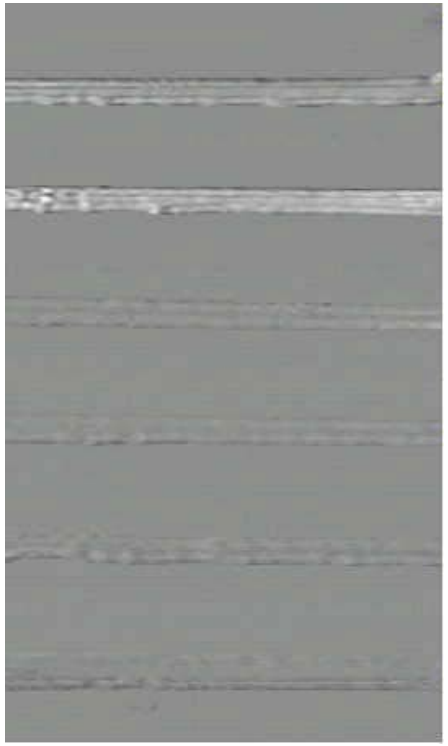




d=20 (±5) nm



PNA probe

Target DNA sequence

5'oligonucleotide 3'-Biotin — NPs

R.D'Agata. R.Corradini. C.Ferretti. L.Zanoli. M.Gatti. R.Marchelli. G.Spoto *Biosens. Bioelectron*. 25. 2010. 2095,
D'Agata. Corradini. Grasso. Marchelli. Spoto. *ChemBioChem*. 2008. 9. 2067.

*Courtesy of Prof. G. Spoto*

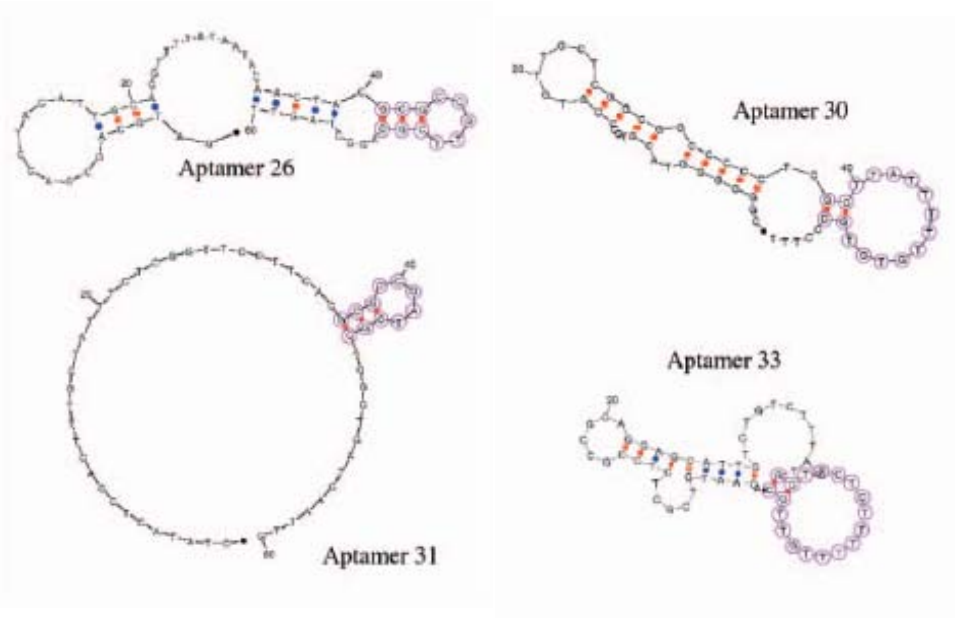
# Synthetic receptors

- Aptamers;

# *APTASENSORS: Interaction sensors*

## *Nucleic acid-based sensor (Probe: DNA or RNA)*

**Aptamers** are oligonucleotides (DNA or RNA molecules) that can bind with high affinity and specificity to a wide range of target molecules (proteins, peptides, drugs, vitamins and other organic or inorganic compounds).




They were “discovered” in 1990 by the development of an in vitro selection and amplification technique, known as SELEX (Systematic Evolution of Ligands by Exponential enrichment).

(Ellington et al., **Nature** 346, 818; Tuerk and Gold, **Science** 249, 505)

# Target molecules

## PROTEINS

Syrian golden hamster prion
Escherichia coli SelB
L-selectin
Tyrosine phosphatase
Ff gene 5
Thrombin
HIV-1 Tat
HIV-1 Rev
Vascular endothelial growth factor
Prostate specific antigen
Human IgE
Taq DNA polymerase
Iron regulatory protein
Human oncostatin M
Human neutrophil elastase
Human CD4 antigen
Lysozyme
C-reactive protein
Tumor necrosis factor a
NF-kB
Acetylcholine receptor
Thyroid transcription factor

## INORGANIC COMPOUNDS

Malachite green
$Mg^{2+}$

## ORGANIC COMPOUNDS

ATP
FMN
Theophylline
Organic dyes
Cocaine

## VITAMINS

Cyanocobalamin
Biotin

## DRUGS

Neomycin B
Streptomycin
Tobramycin
Tetracyclin
Kanamycine A
Dopamine

## TOXINS

Cholera toxin
Staphylococcal enterotoxin B

## POLLUTANTS AND CARCINOGENIC COMPOUNDS

4-chloroaniline
2,4,6-trichloroaniline
Pentachlorophenol
Methylenedianiline

## OTHERS

*Bacillus anthracis* spores

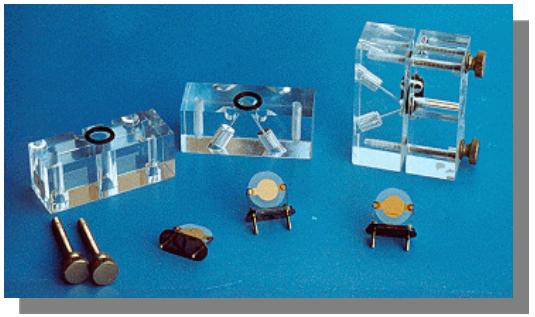
# Aptasensor with Piezoelectric detection

9.5MHz *Elbatech,*

**Table 2. Comparison between the Analytical Characteristics of the Sensor Obtained When Immobilizing the Biotinylated Aptamer with a PolyT Tail**

| immobilized aptamer concn | binding time (min) | linear regression | $R^2$ | CV (%) | cycles[a] |
|---|---|---|---|---|---|
| 1 $\mu$M | 20 | $y = -0.22x + 1.94$ | 0.933 | 35 | 14 |
| 1 $\mu$M thermal treatment | 20 | $y = -0.23x + 0.42$ | 0.977 | 21 | 18 |
| 0.5 $\mu$M thermal treatment | 20 | $y = -0.23x + 0.68$ | 0.988 | 14 | 18 |
| 0.5 $\mu$M thermal treatment | 30 | $y = -0.22x$ | 0.998 | 10 | 22 |

[a] Binding cycles performed without loosing in sensitivity



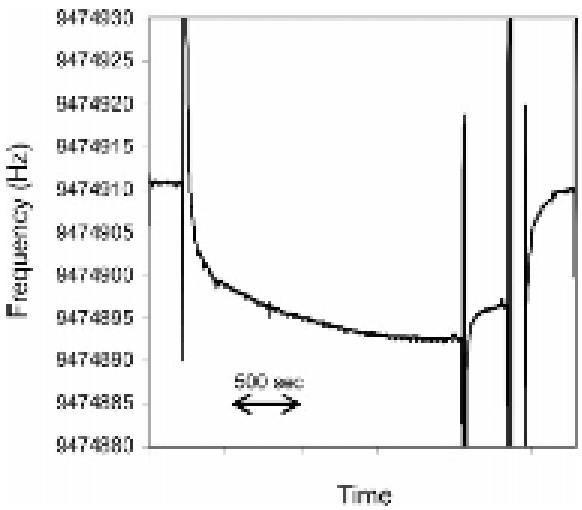



**Figure 1.** Typical binding curve (30 min) obtained with 50 nM thrombin interacting with the immobilized biotinylated aptamer.

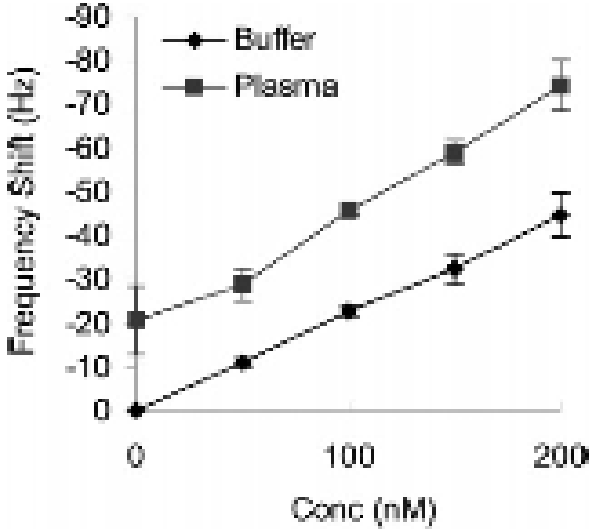



**Figure 2.** Calibration plot obtained with different concentrations of thrombin in buffer and plasma.


Luzi E., M. Minunni, S. Tombelli, M. Mascini, New trends in affinity sensing: aptamers for ligand binding, *Trends in Analytical Chemistry* (*TRAC*), **2003**, 22, 11

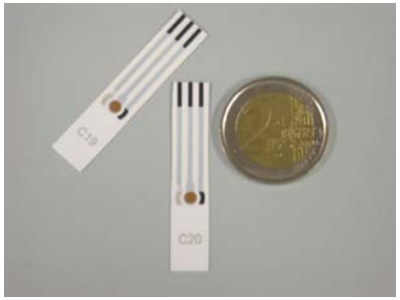
S. Tombelli, M. Minunni, M. Mascini, Analytical application of aptamers, *Biosensor and Bioelectronics,* 20 (12) 2424-2434, **2005**, 810-818

Bini, M. Minunni, S. Tombelli, S. Centi, M. Mascini, Analytical Performances of Aptamer-Based Sensing for Thrombin Detection. *Analytical Chemistry* **2009**, 79(7), 3016-3019;

# Aptasensor with Electrochemical detection

**Table 2. Comparison between Different Binding Buffers (Biacore CM5 Chip with Biotinylated 15-Mer Aptamer with PolyT Tail)**

| binding buffer | shift (thrombin 20 nM) |
|---|---|
| Tris 50 mM pH 7.8 | 261 ± 23 RU |
| Tris 50 mM pH 7.4, NaCl 140 mM KCl 5 mM, $MgCl_2$ 1 mM | 507 ± 81 RU |
| Tris 50 mM pH 7.4, NaCl 140 mM, $MgCl_2$ 1 Mm | 517 ± 72 RU |
| HEPES 10 mM pH 8.0 | 135 ± 21 RU |



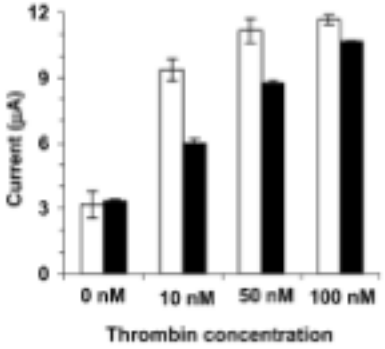



**Figure 5.** Results obtained with serum samples spiked with thrombin (black histograms) and comparison with the same concentrations tested in buffer (white histograms).

## Assay optimization by SPR

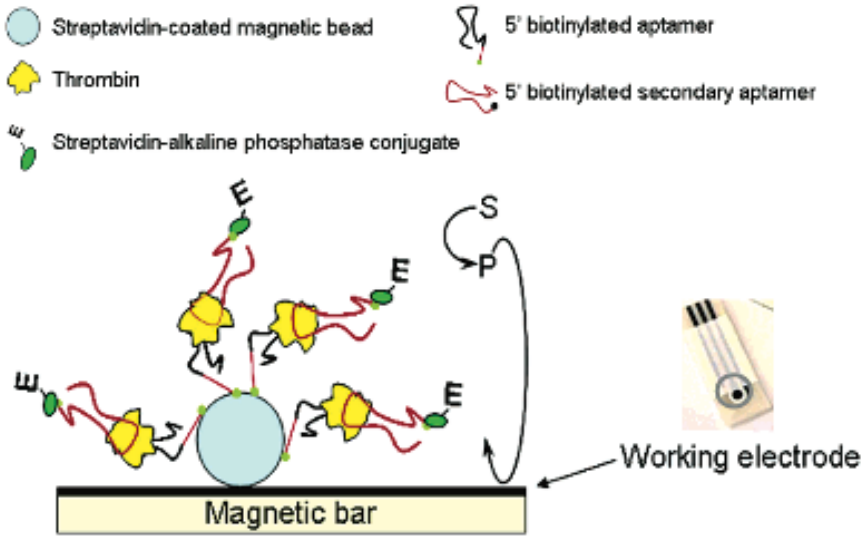



**Figure 1.** Scheme of the electrochemical sandwich assay coupled to magnetic beads.

S. Centi, S. Tombelli, M. Minunni, M. Mascini, Aptamer-Based Detection of Plasma Proteins by an Electrochemical Assay Coupled to Magnetic Beads, *Analytical Chemistry*, 79(4), 1466-1473, **2007**

Talanta 80 (2009) 985–990

Contents lists available at ScienceDirect

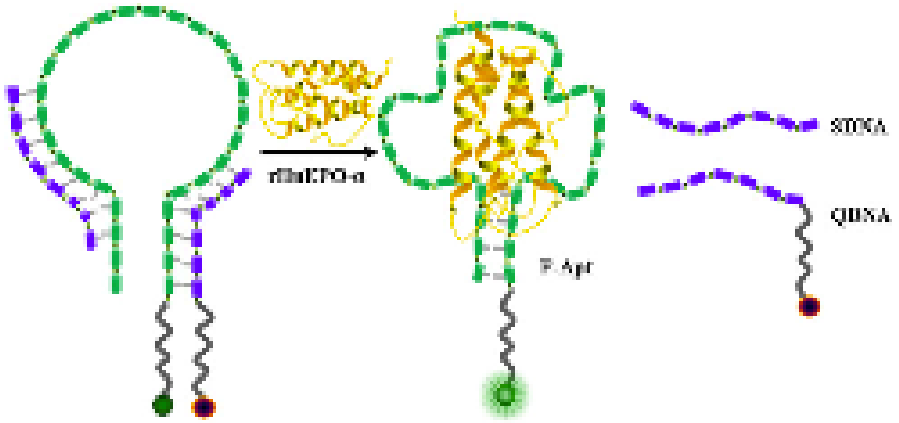
Talanta

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

# An aptameric molecular beacon-based "Signal-on" approach for rapid determination of rHuEPO-α

Zhaoyang Zhang[a,b], Lei Guo[a], Jijun Tang[a], Xingjie Guo[b], Jianwei Xie[a,*]

[a] Beijing Institute of Pharmacology and Toxicology, No. 27 Tai Ping Road, Hai Dian District, Beijing 100850, China
[b] School of Pharmacy, Shenyang Pharmaceutical University, No. 103 Wen Hua Road, Shen He District, Shenyang 110016, China




**Scheme 1.** Illustration of "Signal-on" mode using 807–35 nt aptamer.

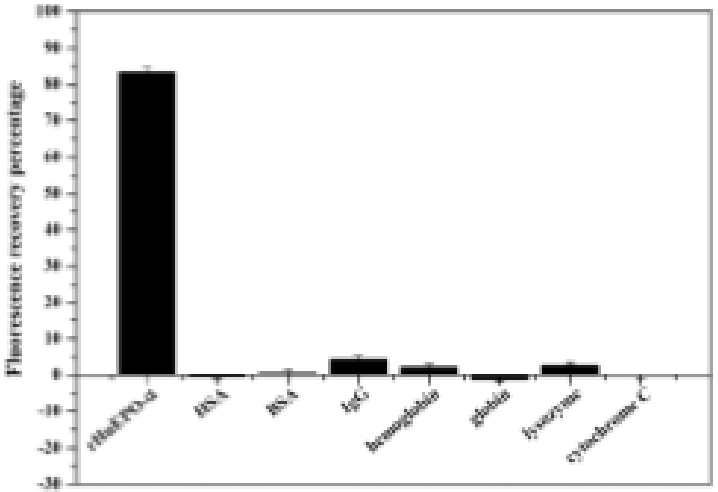


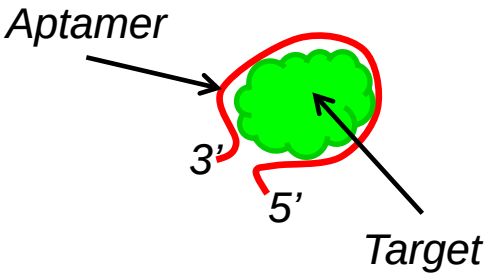

**Fig. 8.** Binding specificity of the "Signal-on" aptameric MB. Different proteins HSA, BSA, IgG, hemoglobin, globin, lysozyme and cytochrome C were compared with rHuEPO-α in their capability to bind with F-Apt in physiological buffer contained 5 mM $Mg^{2+}$ and 0.5 μM BSA. Other conditions are same as in Fig. 7. The concentration of rHuEPO-α is 200 nM, other proteins is fivefold of rHuEPO-α. Data are reported as mean ± SD values with RSD < 2.0% ($n = 3$).

## 4. Conclusion

Here we reported an aptameric MB-based probe for direct determination of rHuEPO-α in homogeneous physiological buffer for the first time. The structural switchable "Signal-on" strategy demonstrated here using 807–35 nt aptamer as a proof of principle, the effective signal transduction module showed a potential practical utility of our new selected ssDNA aptamer for rHuEPO-α detection with a LOD of 0.4 nM. Improved sensitivity can be achieved when coupling preconcentration procedures and signal amplification strategies in the further work. Aptamer 807–35 nt was also expected to be a powerful biosensor probe or a potential clinic diagnosis element to facilitate new methodological developments for rHuEPO-α, including *in vitro* diagnostics, or high-throughput screening of abused samples in doping control area.

Shen R., L. Guo, Z. Zhang, Q. Meng, J. Xie Highly Sensitive determination of recombinant human erytropoietin-α in aptamer-based affinity probe capillary electrophoresis with laser-induced fluorescence detection, J. of Chromatography A, 1217, 2010, 5635-5641

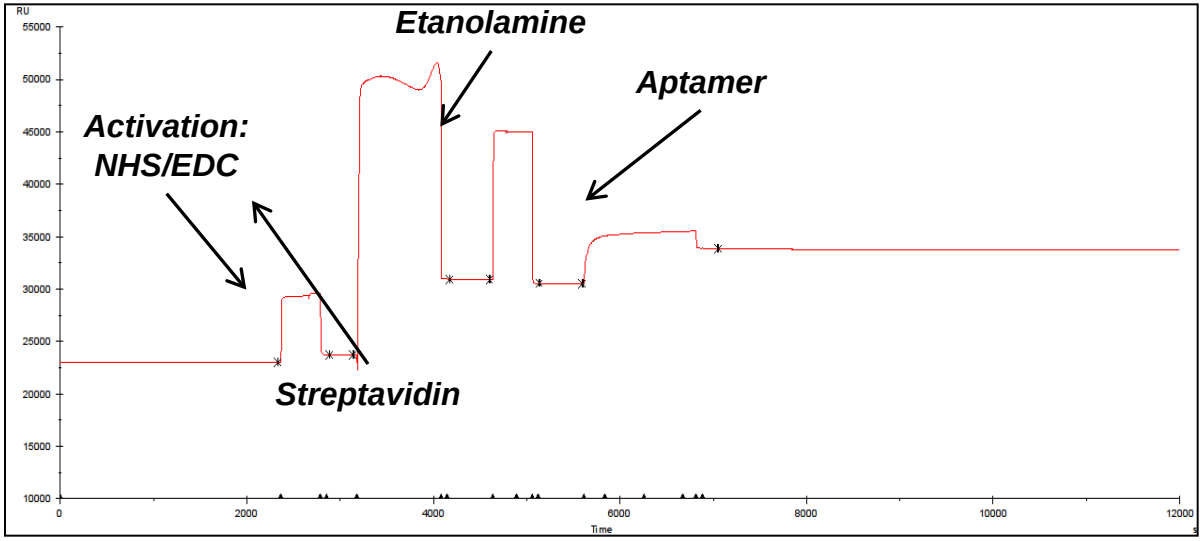
# APTASENSING for EPO: preliminary results




| Aptamer | Sequence | Modification |
|---|---|---|
| Apt1BioTEG 5' | 5'-BioTEG-**GGGGGCCAAGGATTCATGTGTTTGTTTAACAGCTAGCGCCGC**-3' | Biotina + tail trietilenglycol (TEG) in 5' |

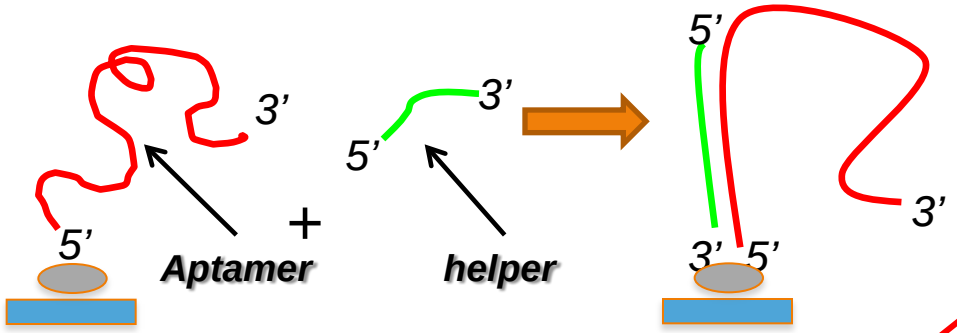
**Streptavidin immobilizated on chip CM5**

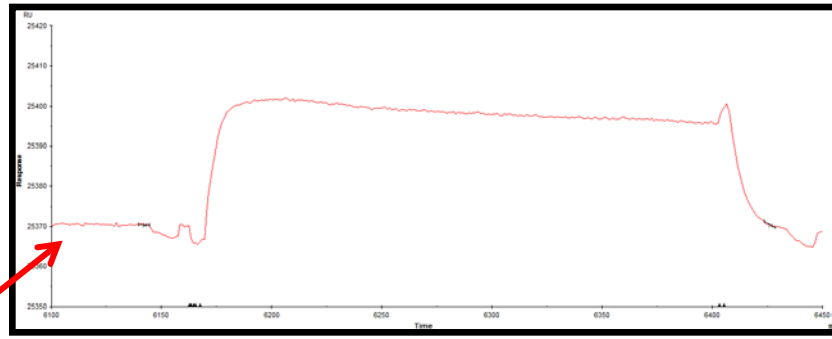
## IMMOBILIZATION OF THE BIOTINYLATED APTAMER

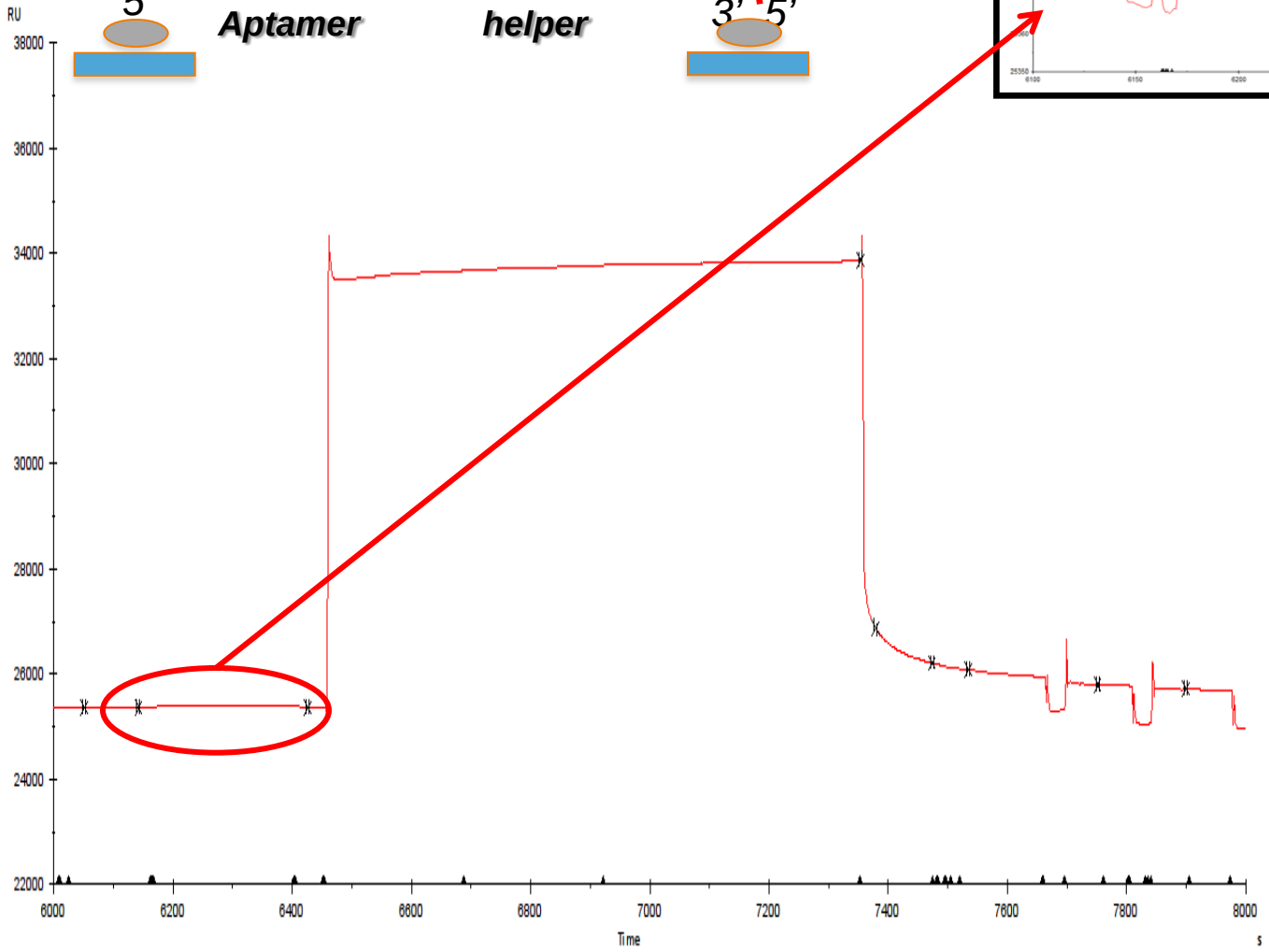



*Zhang Z., Guo L., Tang J., Guo X, Xie J., Talanta, 80, 985-990,2009;
*Brevetto U.S.A. n °6180348, 2001

# APTASENSOR






***rH-EPO 188 ppb after helper injetion: Non reproducible signal***

# CONCLUSIONS

• ABBs suitable for real-time, label-free, simultaneous analysis of target analytes (nucleic acids, proteins) with interesting features for screening purposes;

• Portable instrumentation,

• Possibility to improve detection limits,

• Fast analysis time (minutes);

• Increase the spectrum of analyte (new receptors);

• Analysis of complex matrices without sample pre-treatment except dilutions

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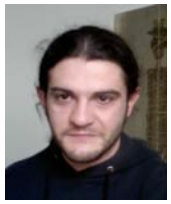

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