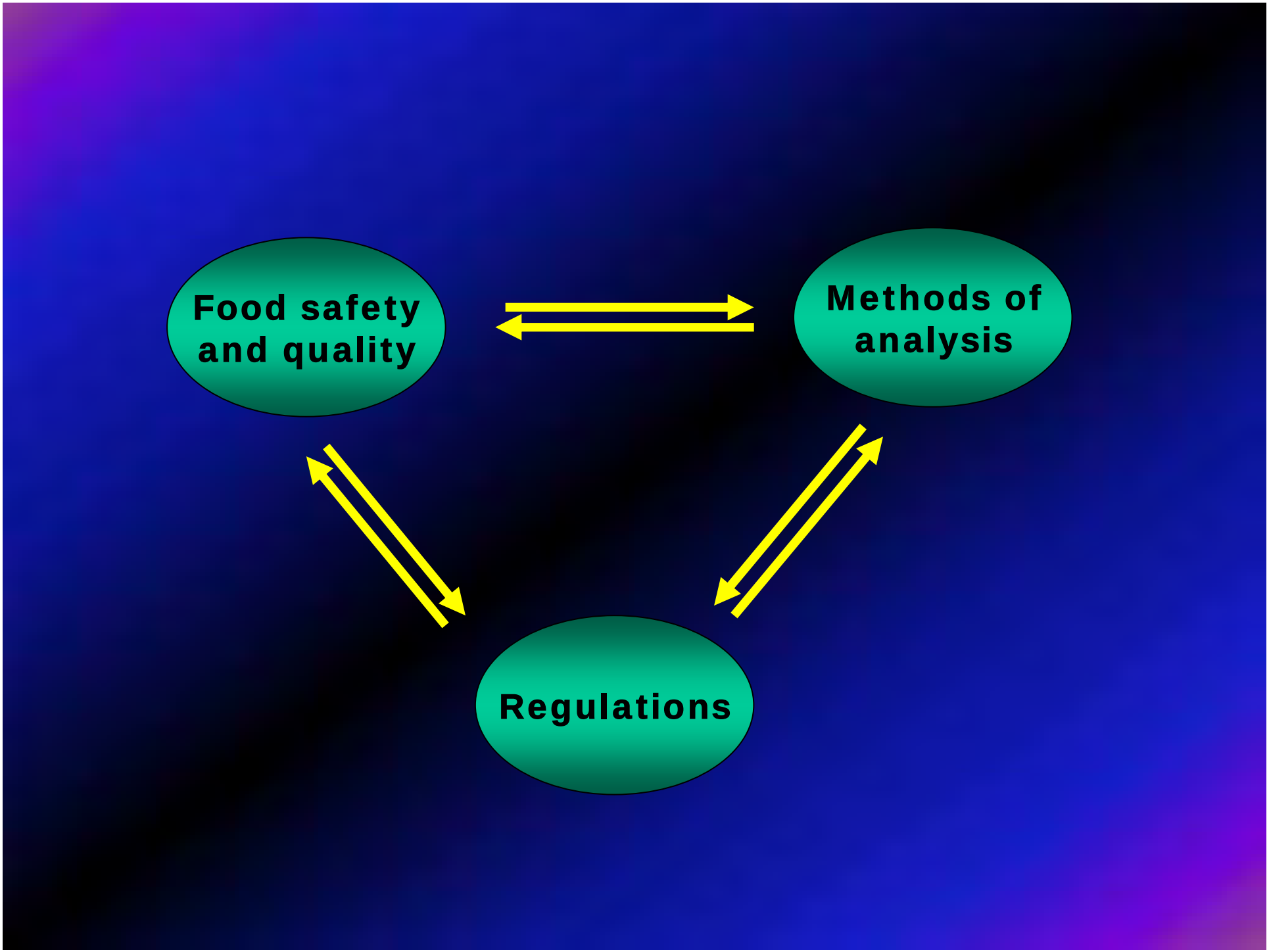
Top-down trends in food analysis: towards nanotechnologies

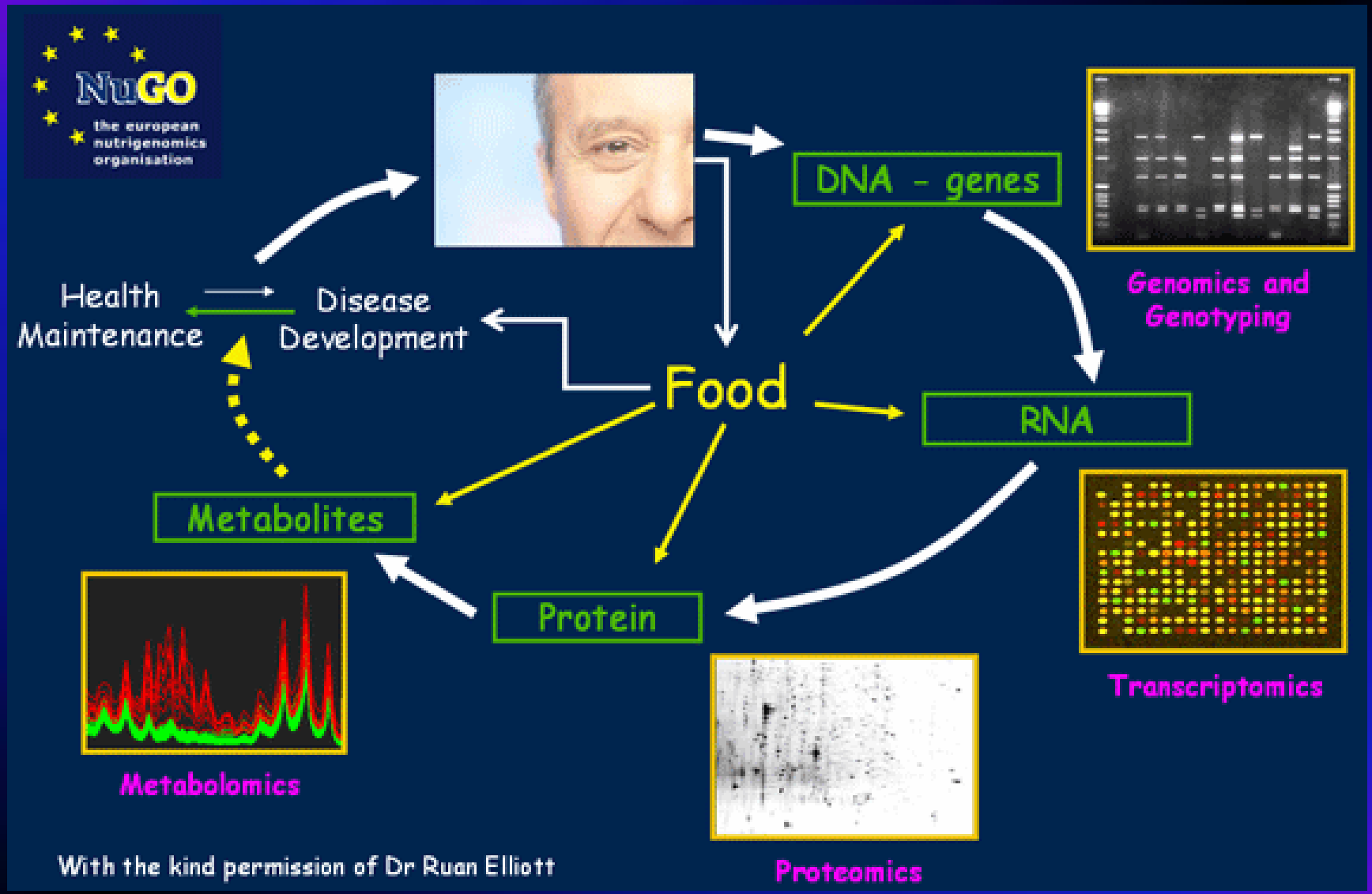
Rosangela Marchelli

Department of Organic and Industrial Chemistry
Faculty of Agriculture
University of Parma

EU White Paper on Food Safety 2000

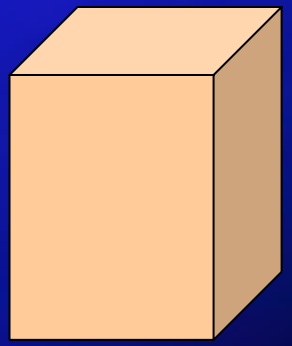
- "…there has been an enormous development, in the past decades, both in the methods of food production and processing and the controls required to ensure that acceptable safety standards are being met"

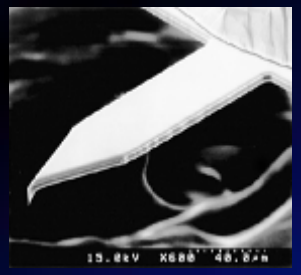

Food safety and quality
Methods of analysis
Regulations


NuGO
the european nutrigenomics organisation
DNA - genes
Genomics and Genotyping
Health Maintenance
Disease Development
Food
RNA
Metabolites
Protein
Transcriptomics
Metabolomics
Proteomics
With the kind permission of Dr Ruan Elliott

Top-down and bottom-up approaches

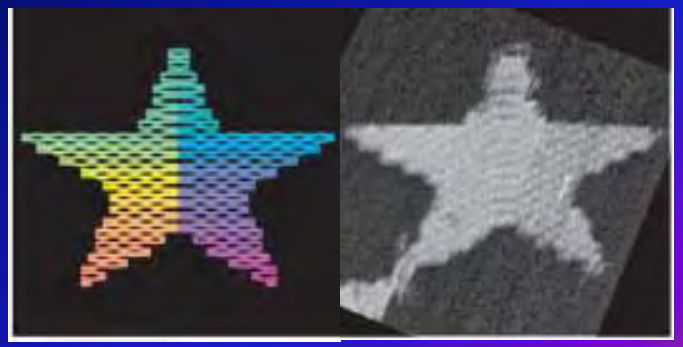
"Top-down" – building something by starting with a larger piece and carving away material (like a sculpture)





AFM tip, used to manipulate, image and measure atomic scale features.

"Bottom-up" – building something by putting together smaller pieces (like building a car engine).



DNA "origami"

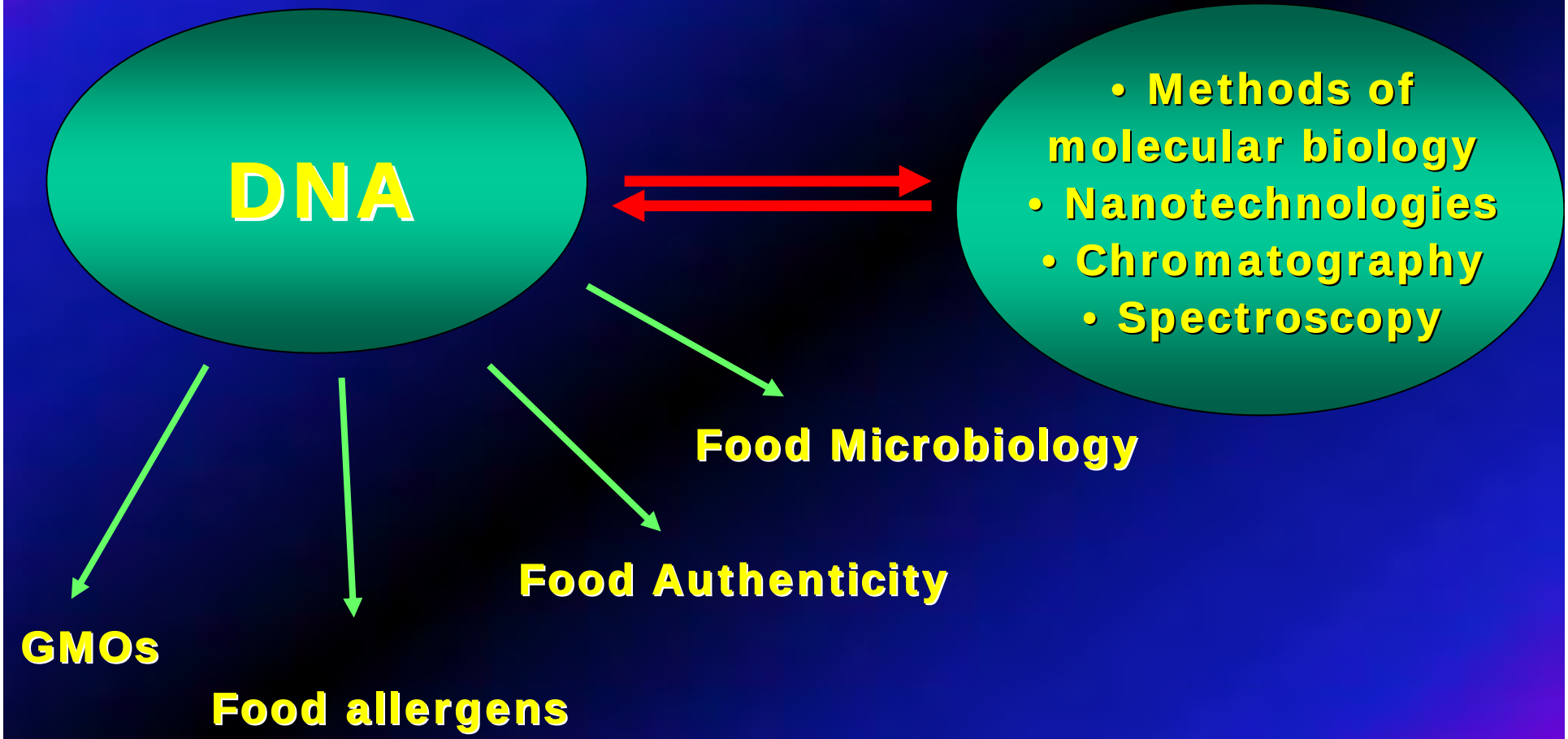
Why is “micro and nano” good?

- Portable
- Lighter

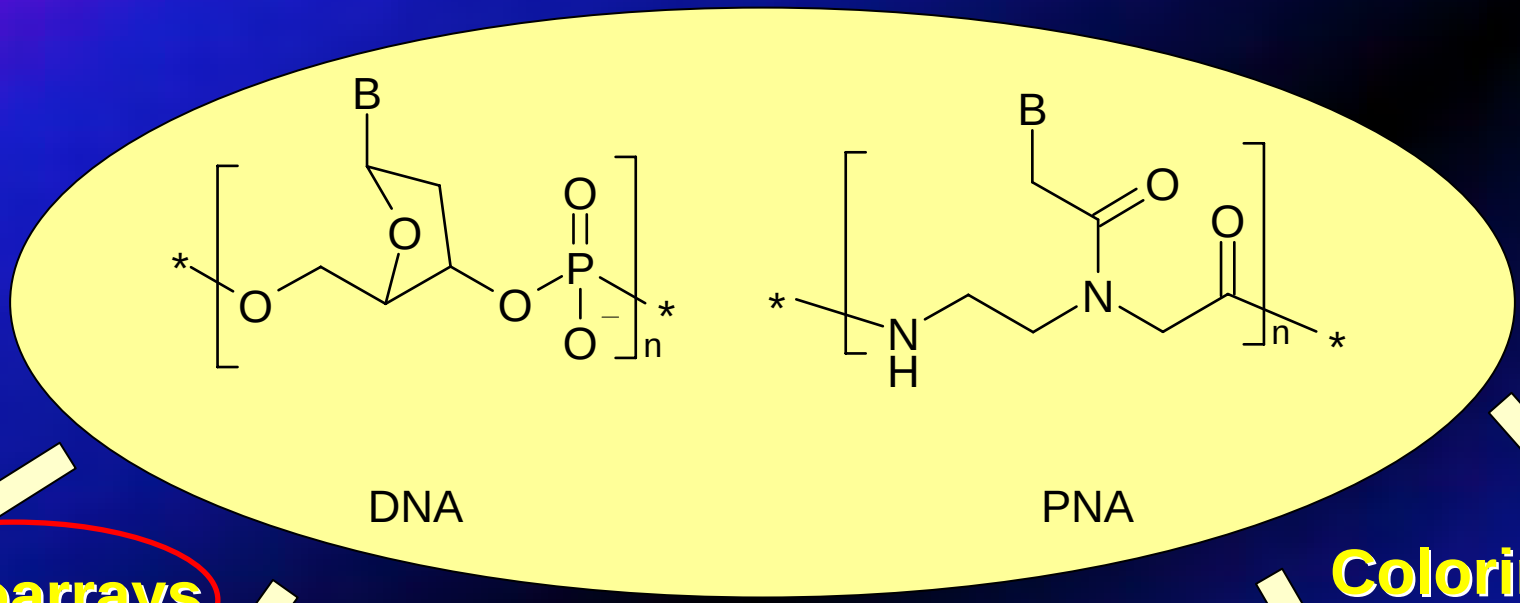
- Faster

- Lower environmental impact (reagents/solvents)
- Cheaper
- More energy efficient
- Electronic reading allowing smart communication strategies
- Different properties at very small scale

New methods based on genomics



PNA probes for food DNA detection



DNA

PNA

Microarrays

SPR

CD

CE

HPLC

Light Up Probes

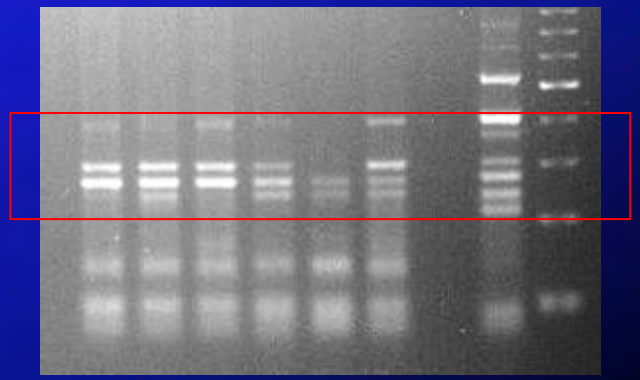
Beacons

Colorimetric tests

High tech

Low tech

The microarray technology approach

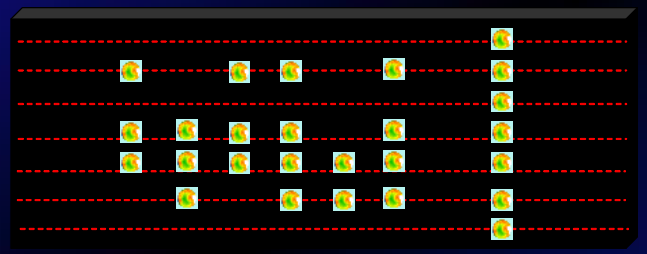


MULTIPLEX PCR
ANALYSIS

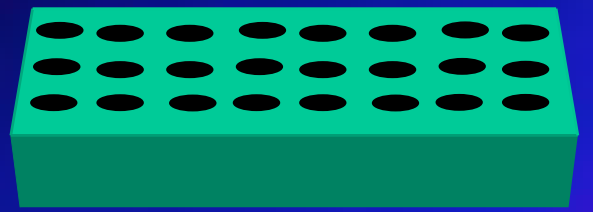
Hybridization

PNA PROBE LIBRARY
DEDICATED TO FOOD

Deposition

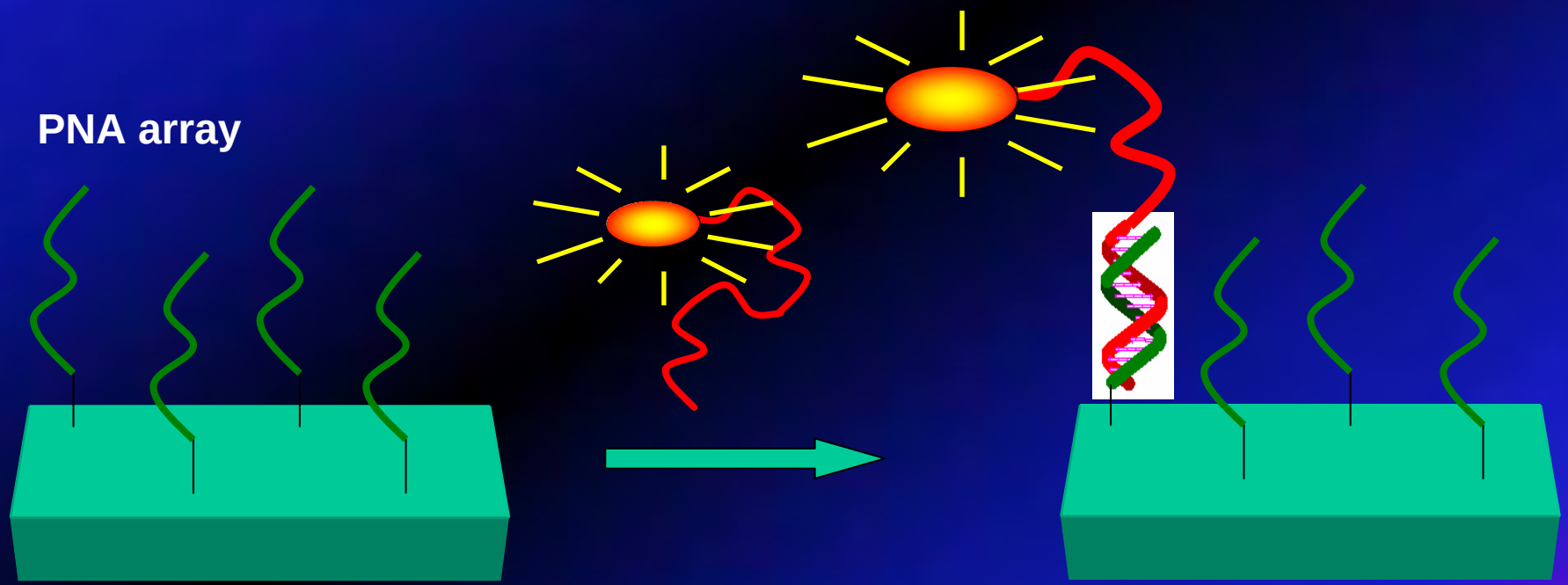


MICROARRAY

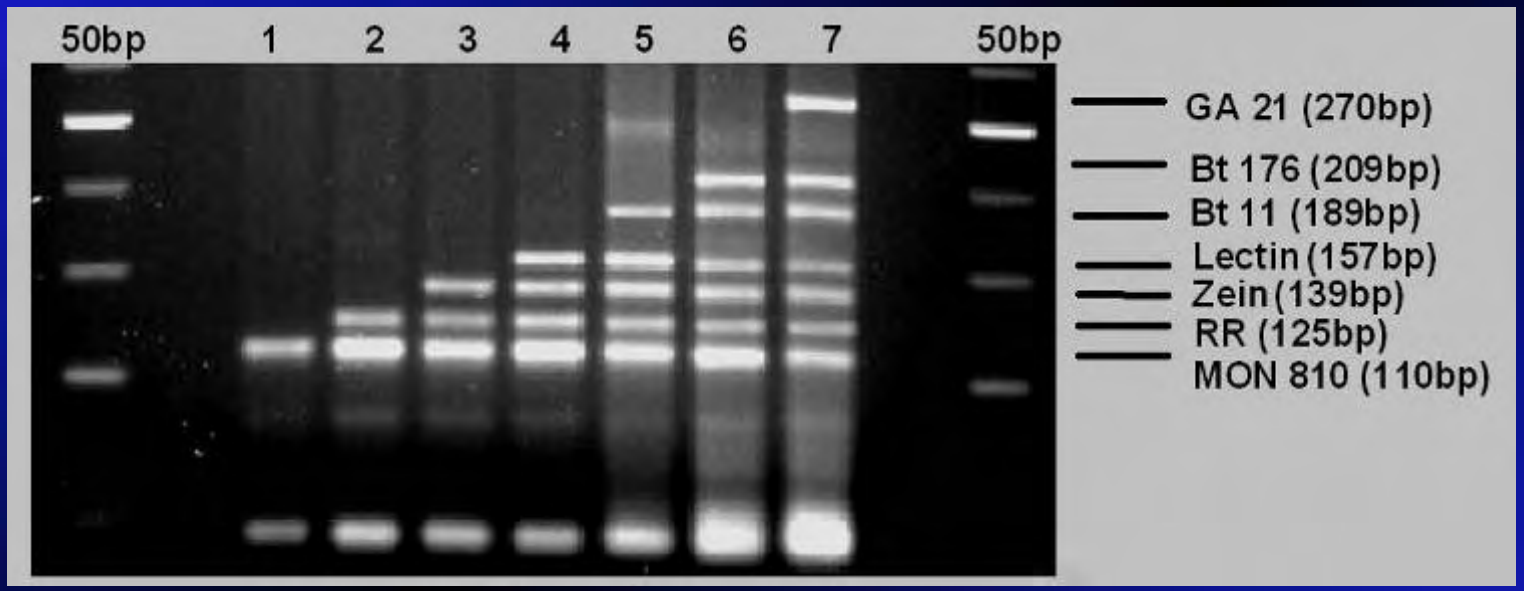


MICROARRAYS

1. Probe design and synthesis
2. Probe deposition
3. Hybridization with the target DNA

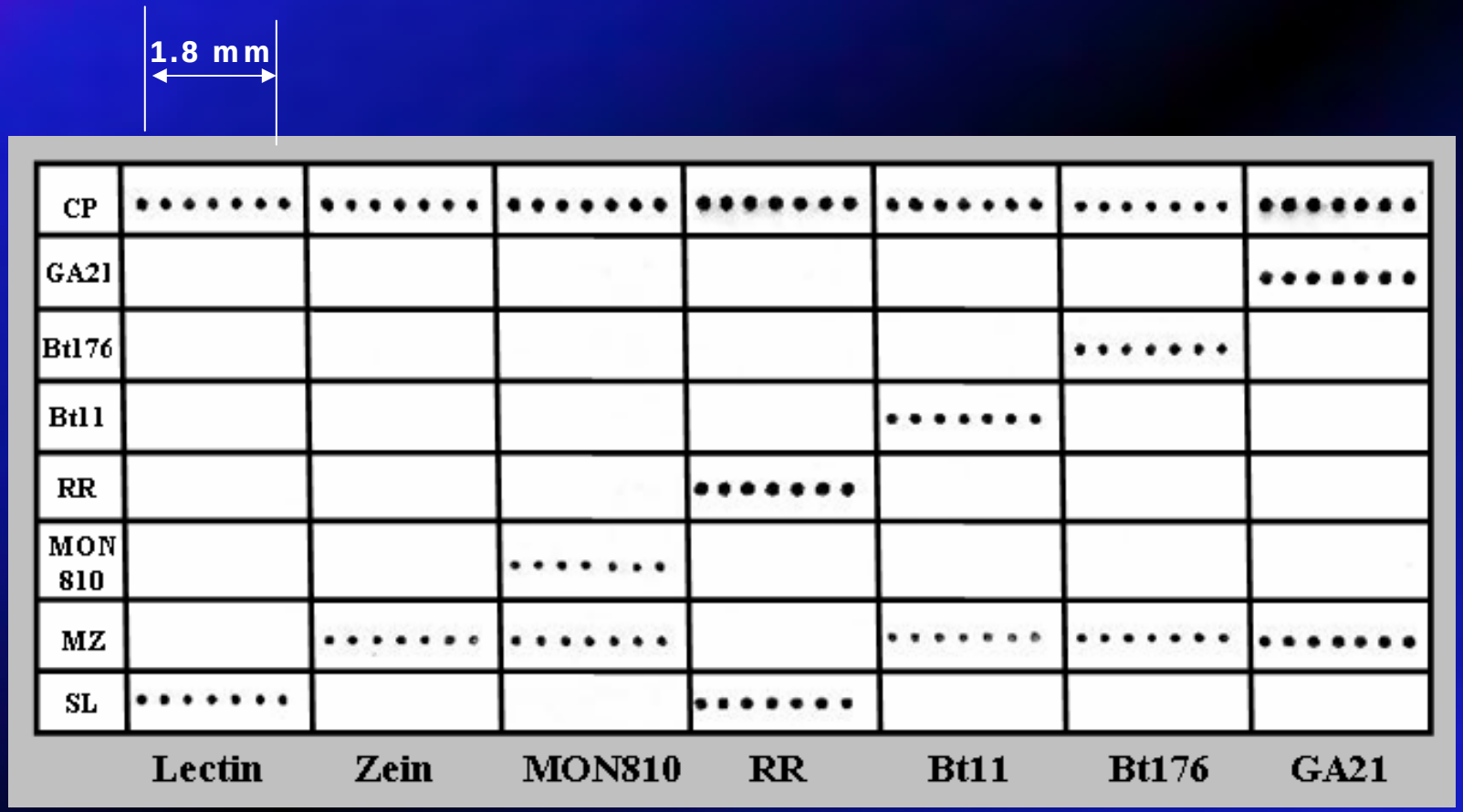


GMOs: development of a PCR multiplex



Germini A., Zanetti A., Salati C., Rossi S., Forre C., Schmid S., Fogher C., Marchelli, R. *Journal of Agricultural and Food Chemistry*, 2004, 52, 3275-3280

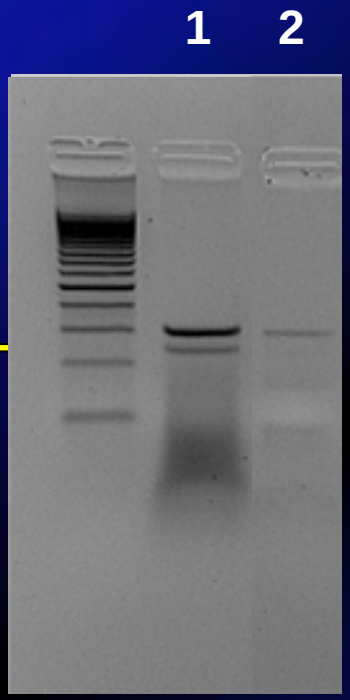
Specificity hybridisation test



Germini A., Rossi S., Zanetti A., Corradini R., Fogher C., Marchelli R. *Journal of Agricultural and Food Chemistry*, 2005, 53, 3958-3962.


In every sub-array: more than 1 thousand spots
0.8 cm

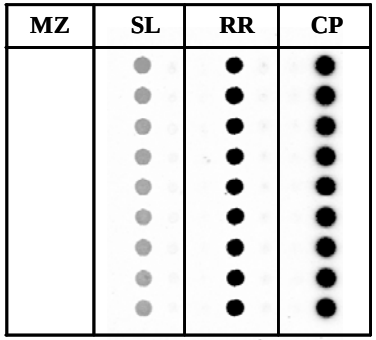
PNA chip for RR-soy products



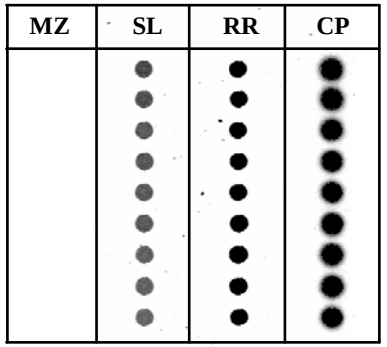
RR 125 bp

Lectin
157 bp

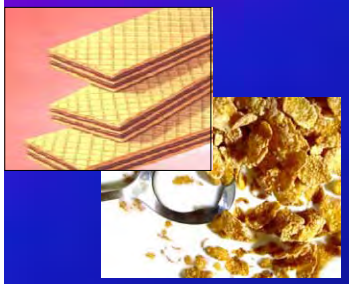
1) Soy seed



2) Lecithin

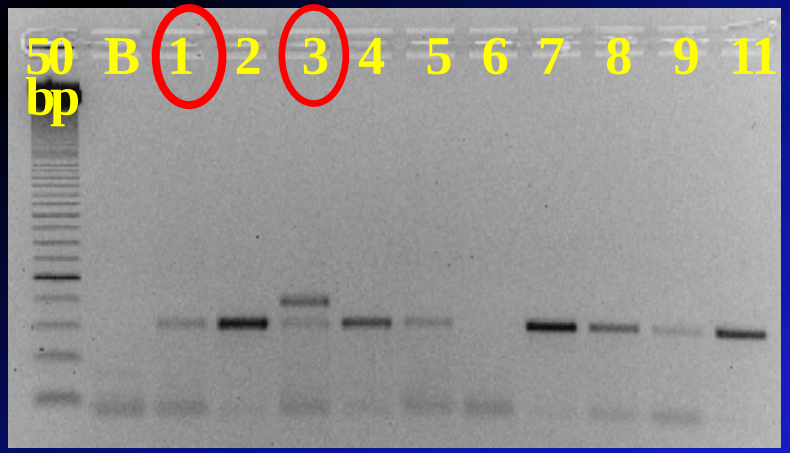


Detection of allergens in commercial products: hazelnut (Cor a1) and peanut (Ara h2)



Sample	Product
1	Breakfast cereals
2	Breakfast cereals with chocolate and hazelnut
3	Müesli snack with milk chocolate
4	Müesli snack with cocoa
5	Snack with cereals and cocoa
6	Cocoa and vanilla wafer
7	Hazelnut wafer
8	Biscuit topped with cocoa cream and milk chocolate
9	Milk chocolate
10	Chocolate for topping
11	Chocolate cream with hazelnut

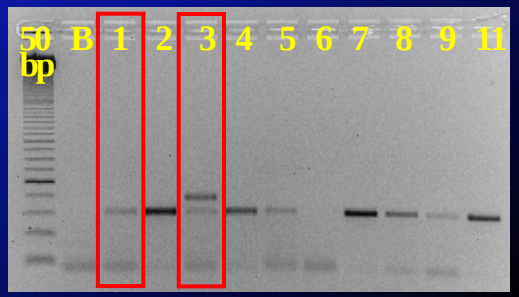
PCR analyses performed on several foodstuff claiming to contain or to not contain hazelnut as ingredient or possible contaminants.



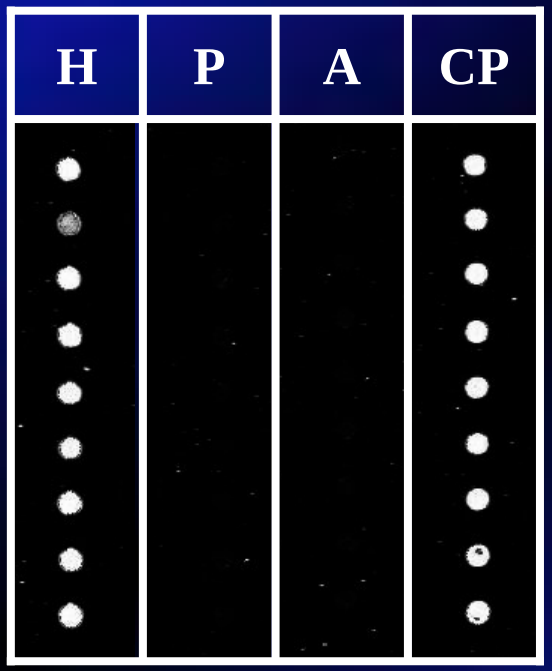
Hazelnut Cor a 1 (156bp) and Peanut Ara h 2 (201bp) amplicons.

Limit of detection : 50pg DNA.

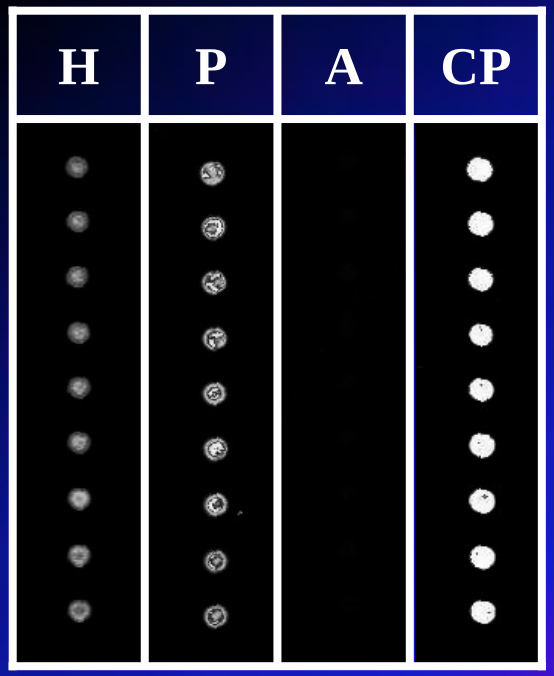
PNA - microarray detection of hazelnut and peanut DNA in commercial products



Sample 1. Breakfast cereals

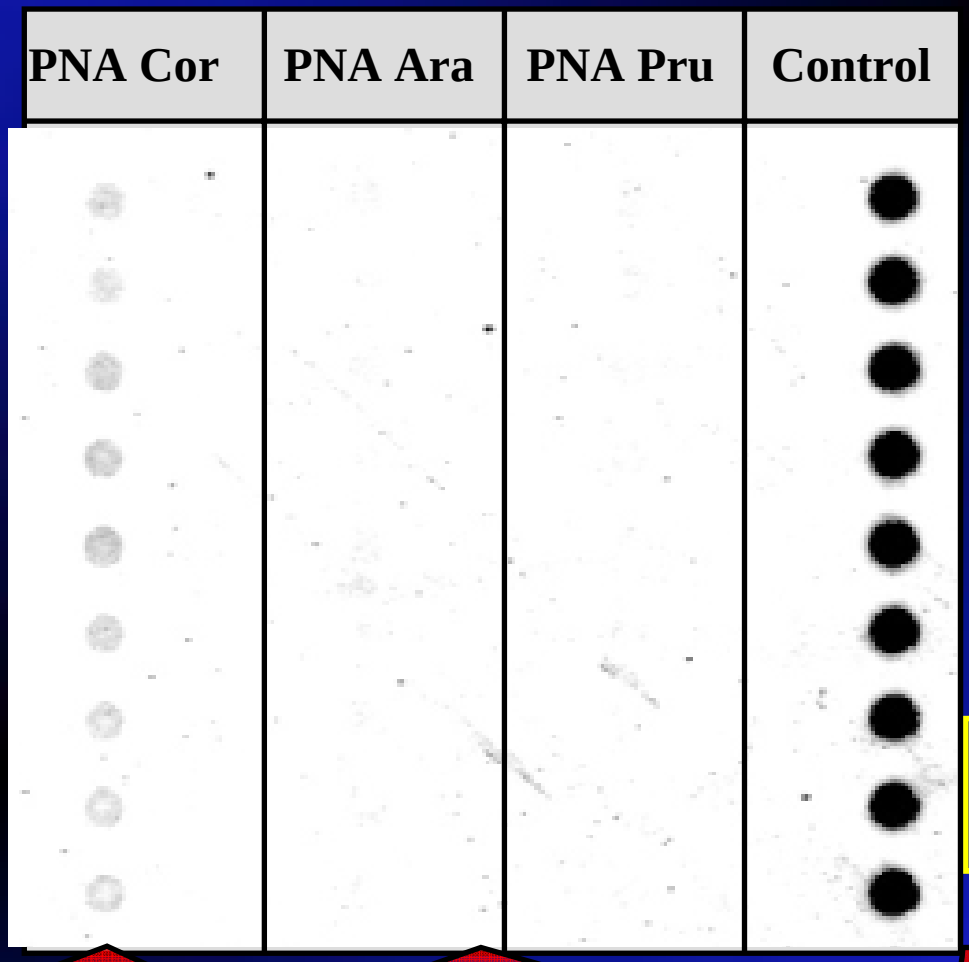


Sample 3. Muesli snack



Rossi S., Scaravelli E., Germini A., Corradini R., Fogher C., Marchelli R. *European Food Research Technology*, 2006, 223, 1-6

Detection of hazelnut oil (5%) in extra-virgin olive oil



- Cor a 1 Hazelnut
- Ara h 2 Peanut
- Pru du 2.1 Almond

Detection limit 50fmol

PNA probes specificity

Authenticity: Olive oil cultivars

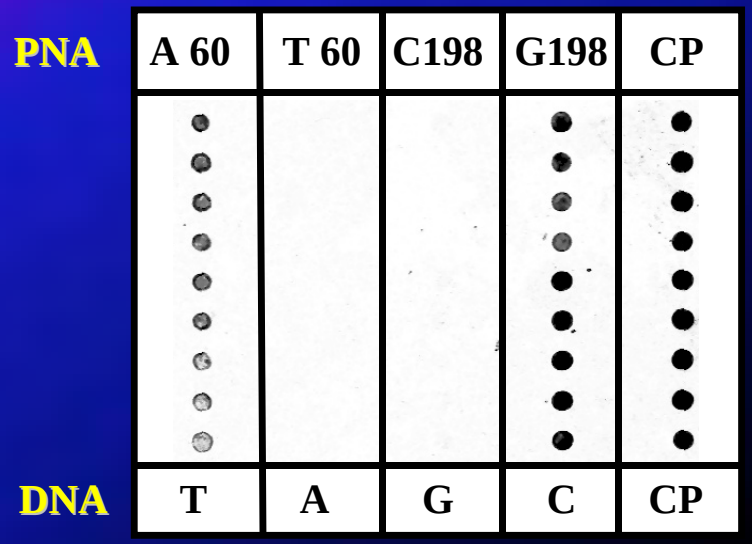
Cultivar	60	120	183	198	345
Biancolilla	A	A	G	C	G
Canino	A	R	R	S	G
Carolea	A	A	G	C	G
Coratina	A	A	G	C	G
Frantoio	A	A	G	C	G
Leccino	A	A	G	C	R
Nocellara belice	A	A	G	C	R
Ogliarola leccese	T	A	G	C	G
Moraiolo	A	R	R	C	G
Bosana	A	R	R	C	G
Nocellara etnea	A	A	G	C	R
Arbequina	A	A	G	C	G

SNPs in the Actin gene

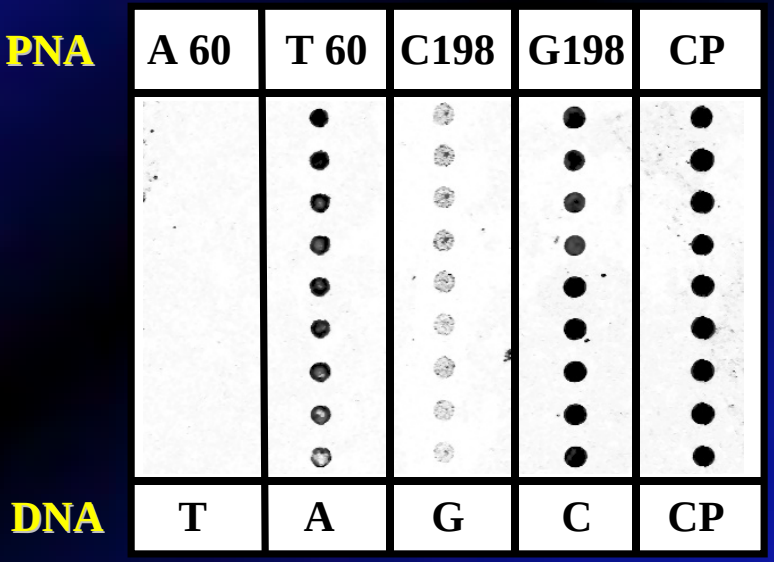
R = T o C
S = G o C

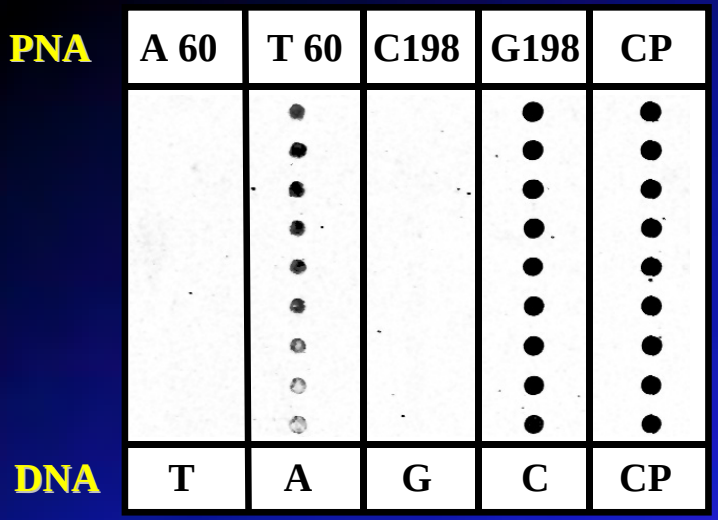
Olive variety identification by PNA microarrays

"Ogliarola leccese"



"Canino"

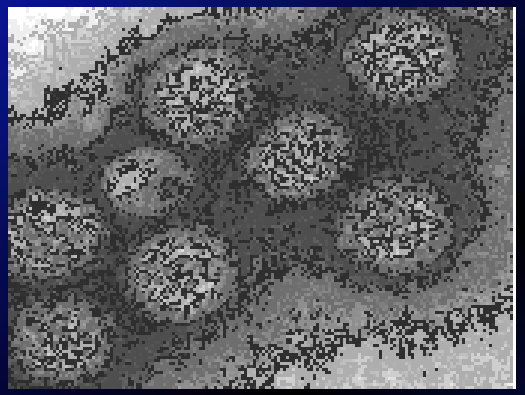




"Frantoio"

NOROVIRUS

Caliciviridae family: single strand **RNA virus**



Main responsible for non bacterial gastroenteritis

23 milion cases per year estimated in USA

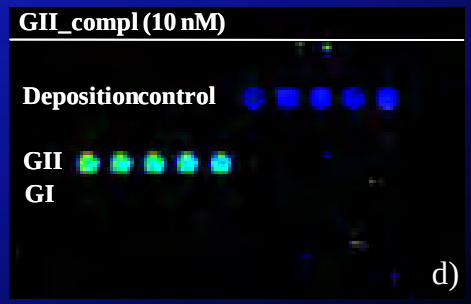
40% food-transmitted

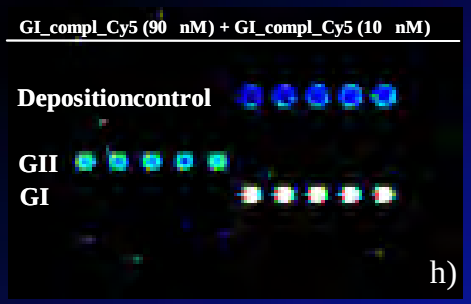
water, mytilidae, fruits, vegetables

Not cultivable in vitro

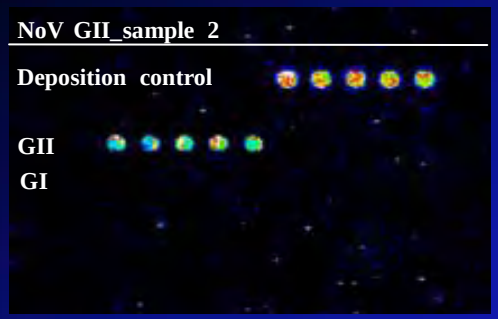
Microarrays for Norovirus

Oligonucleotides



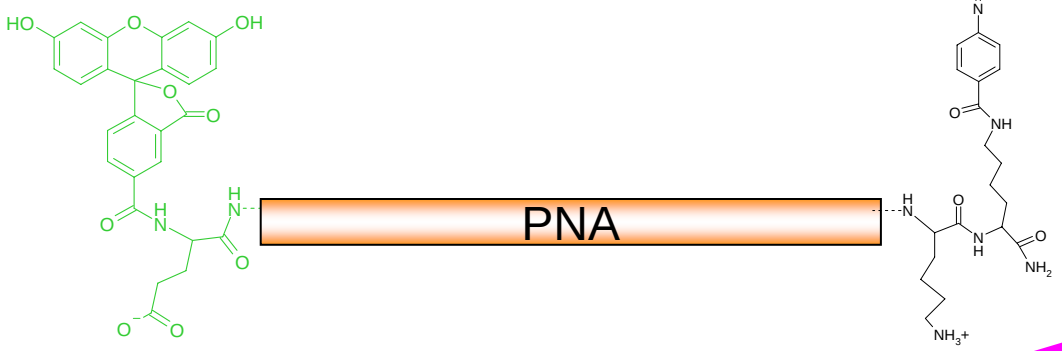


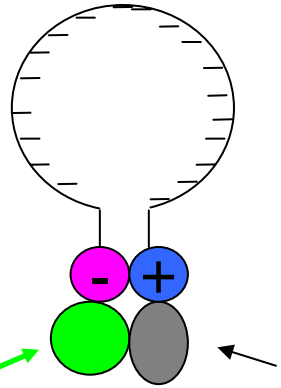
PCR products



In collaboration with the Virology Unit of the University of Parma

PNA BEACON (STEMLESS)



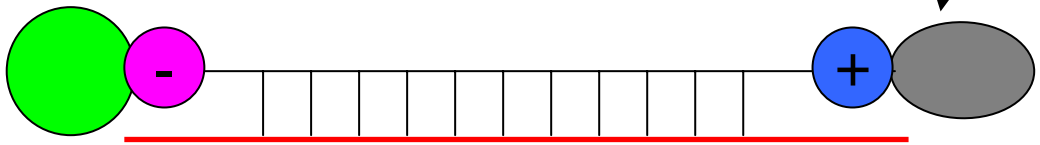


Fluorophor

Quencher

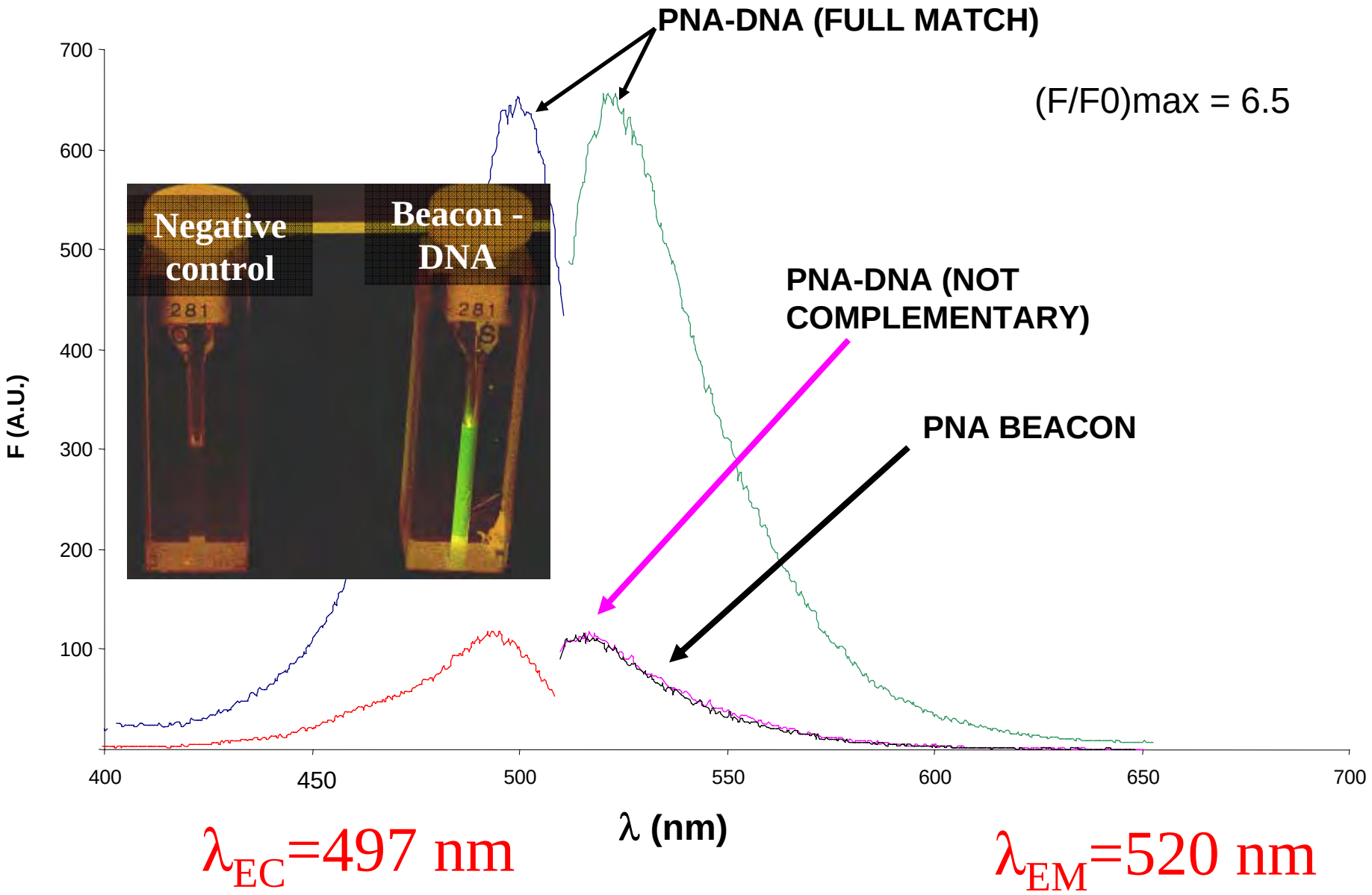
carboxyfluorescein

Dabcyl



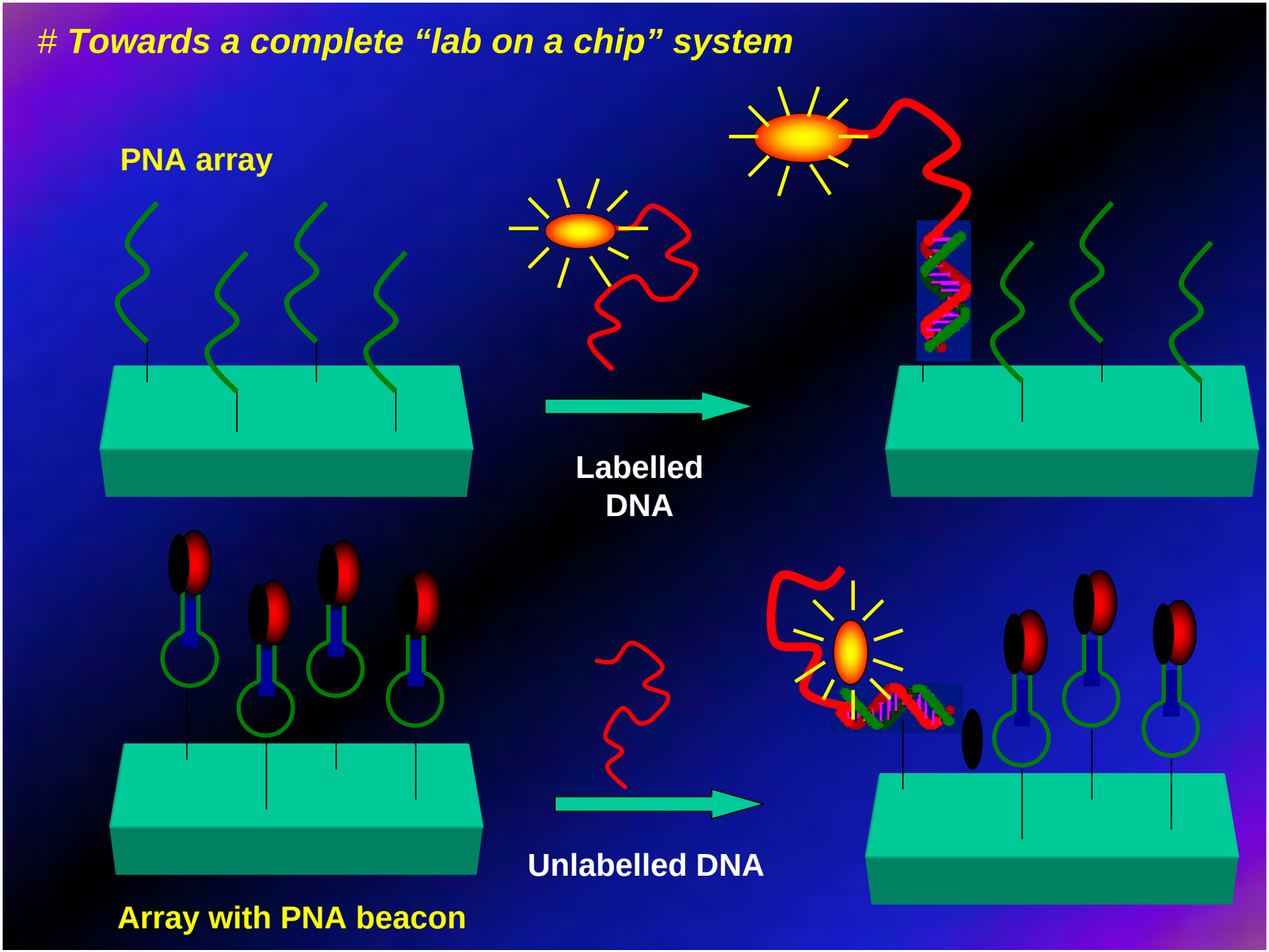
DNA

PNA BEACON FOR ROUNDUP READY-SOY

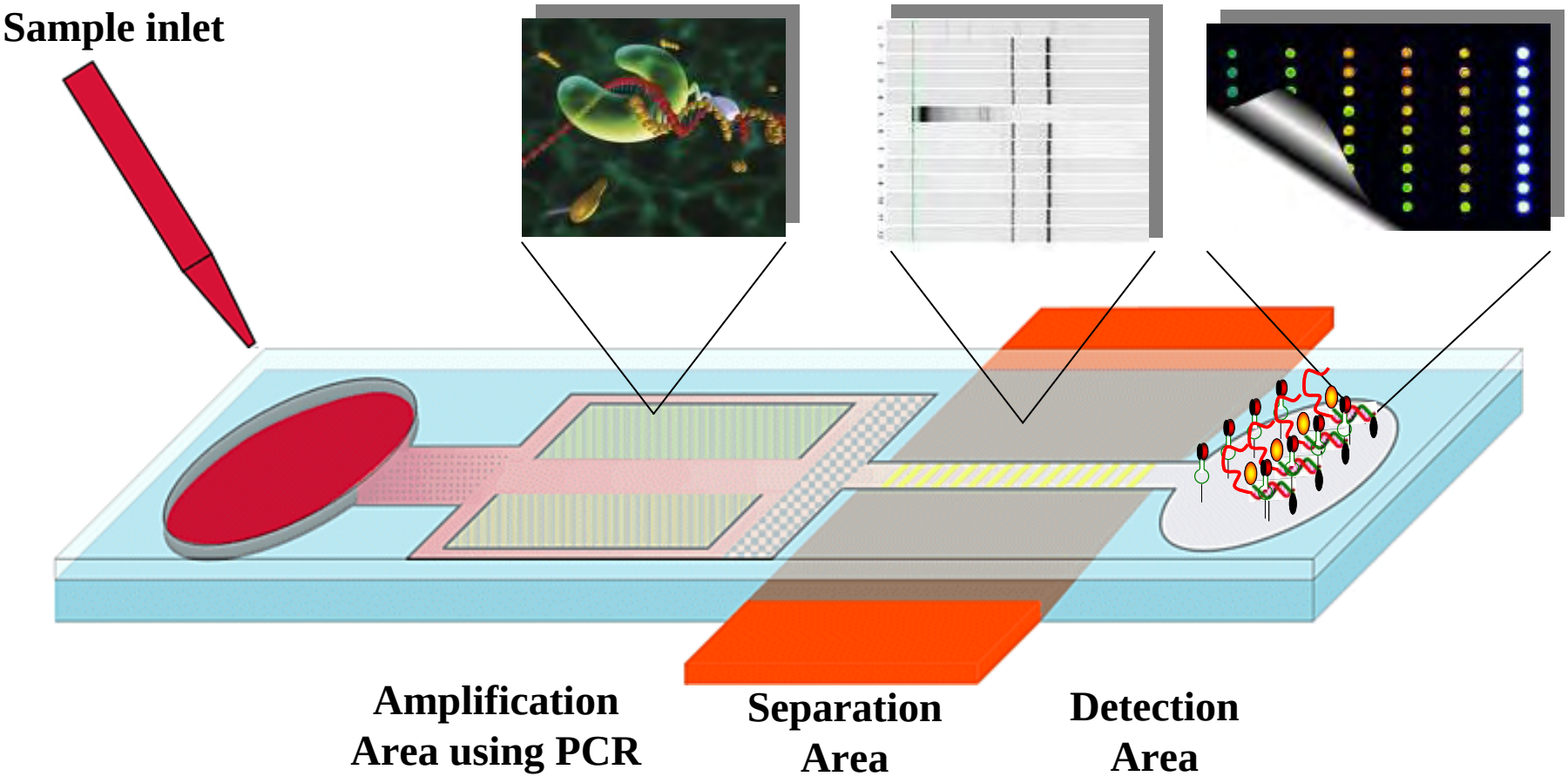


λ_{EC}=497 nm

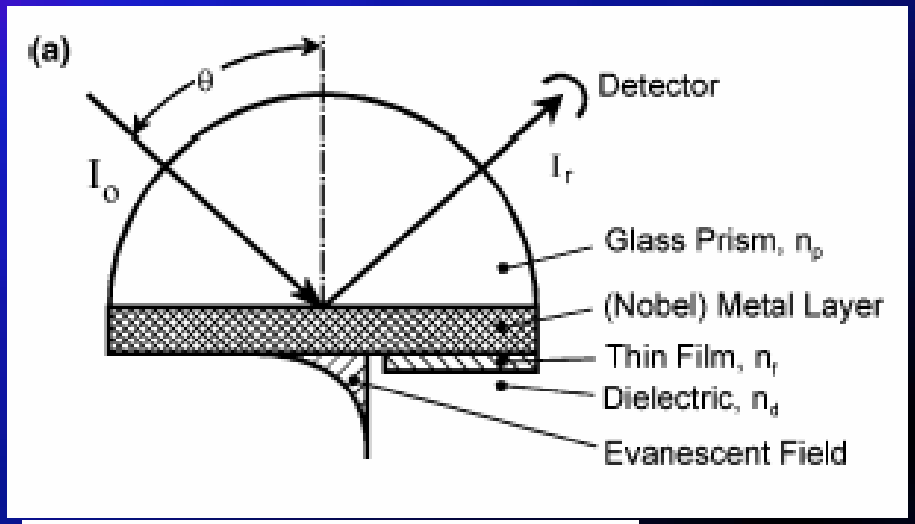
λ_{EM}=520 nm

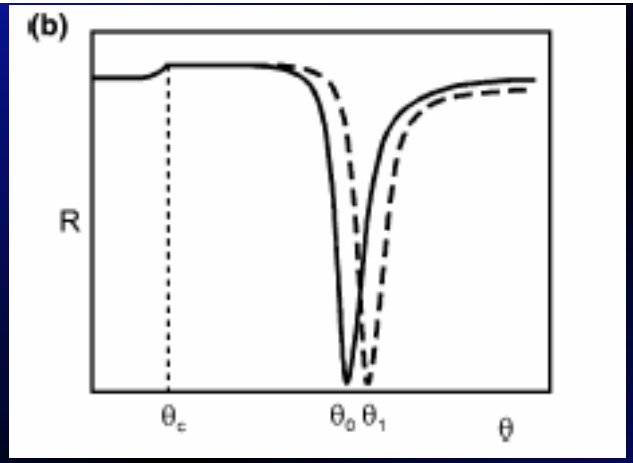

Towards a complete “lab on a chip” system
PNA array
Labelled DNA
Unlabelled DNA
Array with PNA beacon

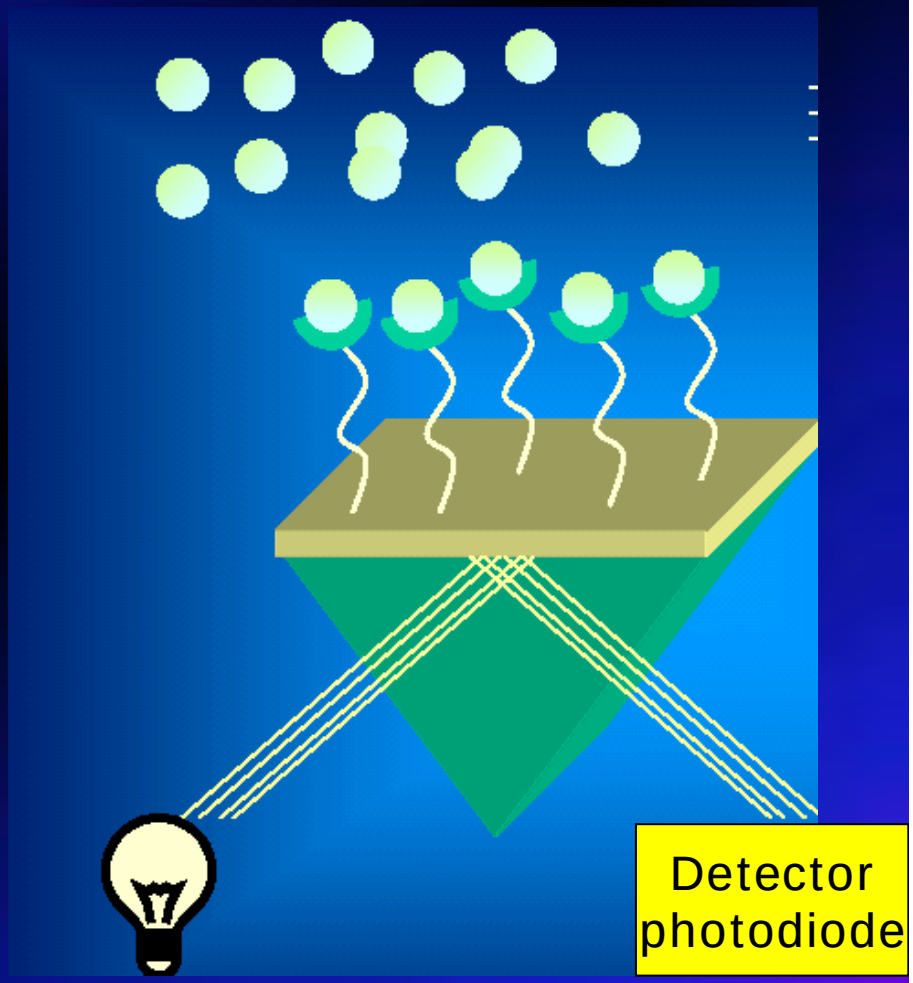
The “Lab on a chip” system



Surface Plasmon Resonance

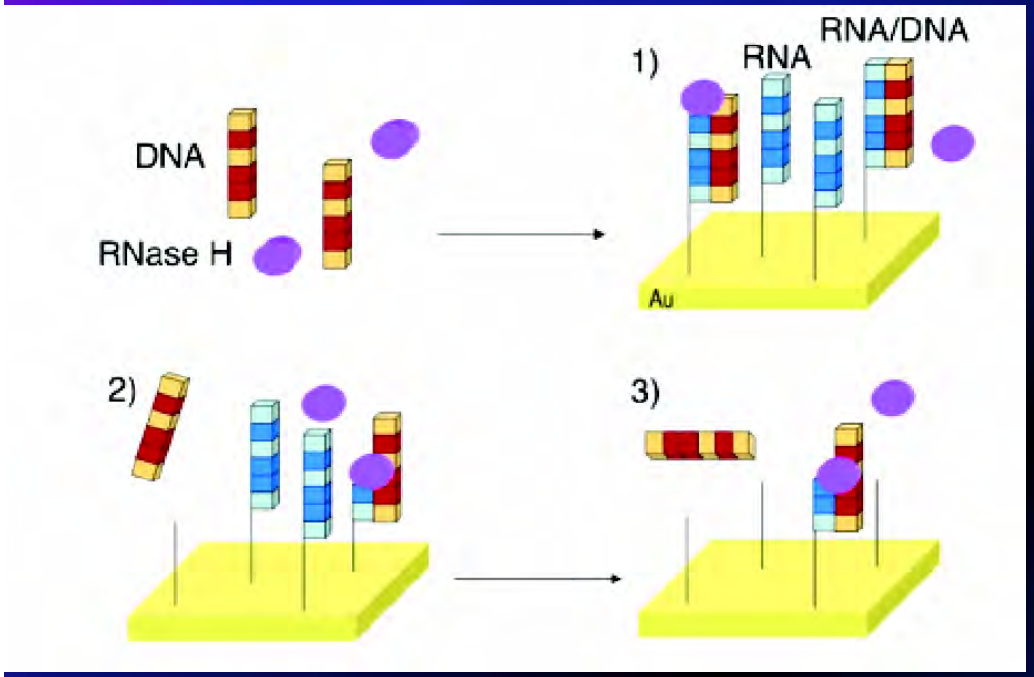


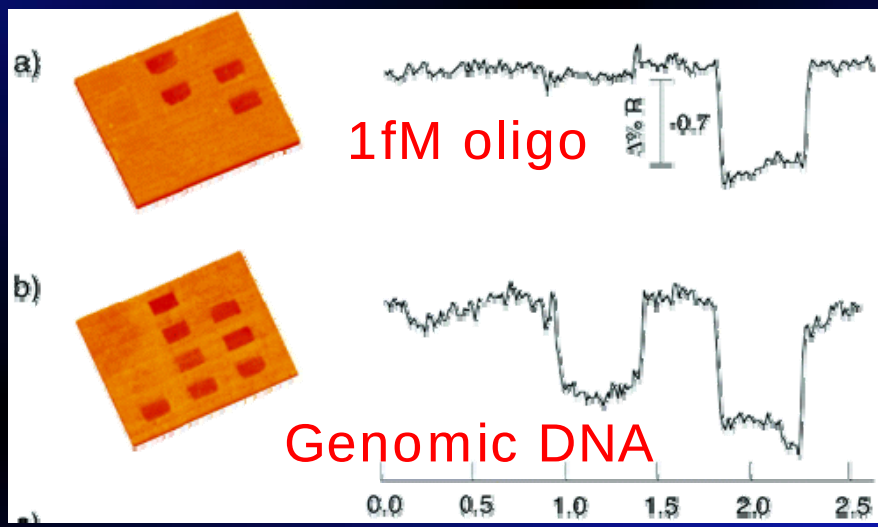




Typical reflectivity scan (R versus incident angle θ) before (full curve) and after the deposition of a thin film (broken curves).

PCR-free DNA Detection

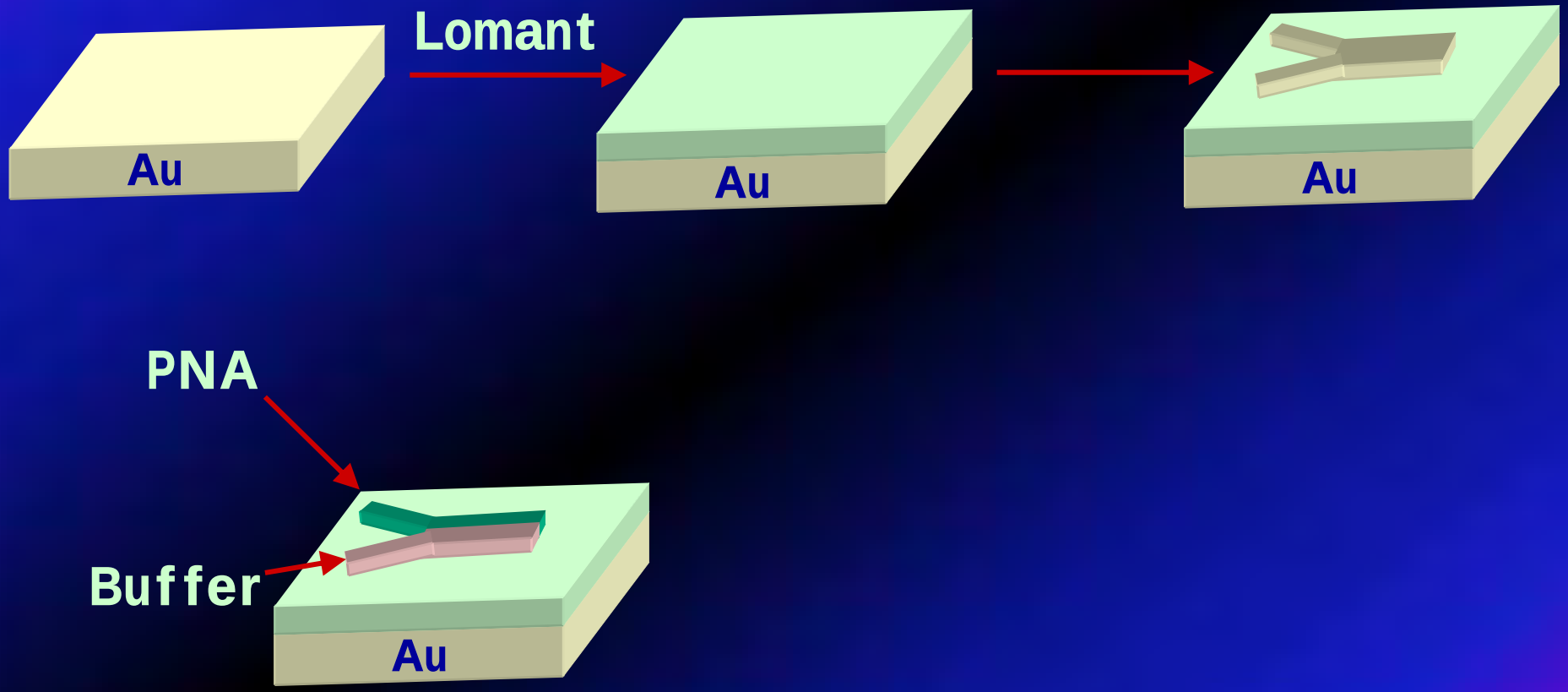




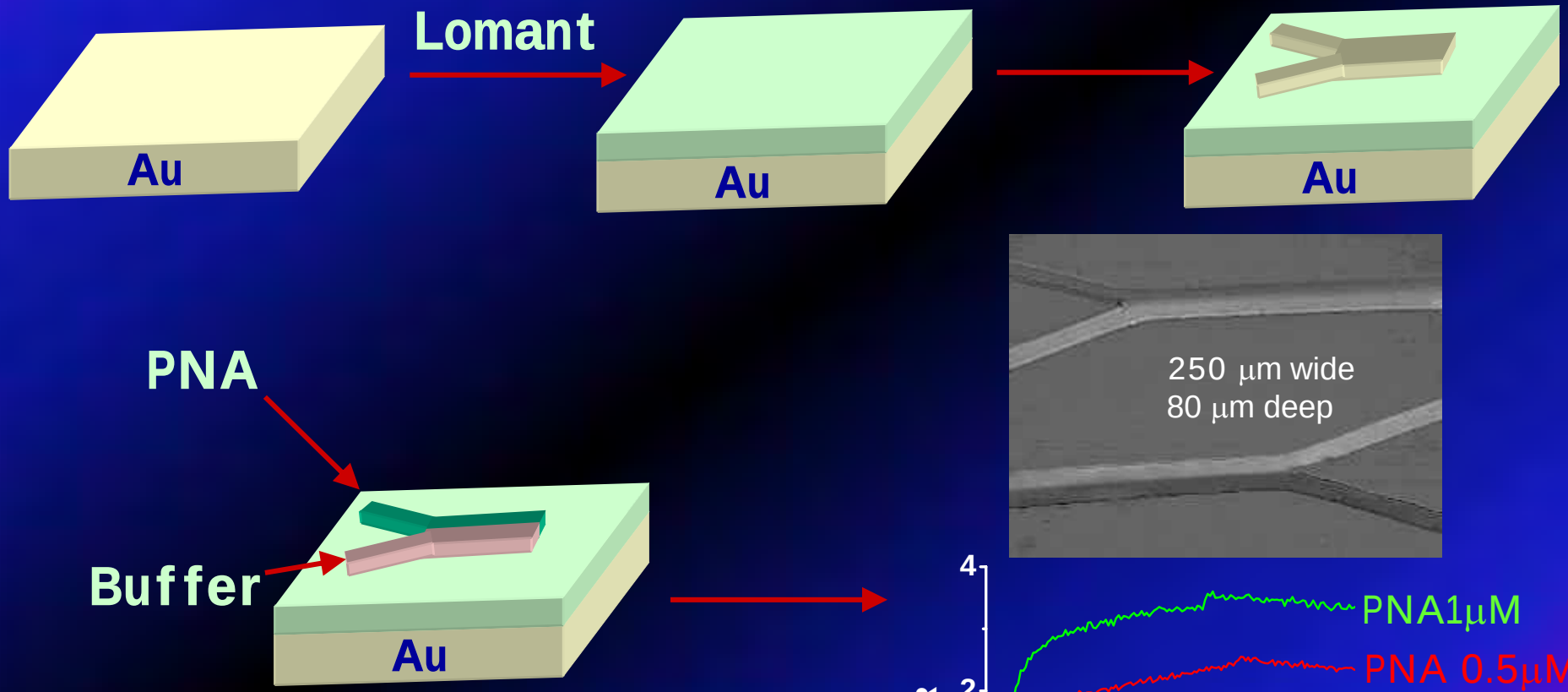
Scheme of PCR-free detection of genomic DNA using a RNAseH amplification of the SPR signal depletion. Detection limit of 10^{-15}M was attained

T.T. Goodrich, H.J. Lee, R.M. Corn *J. AM. CHEM. SOC.* 2004, *126*, 4086-4087

SPR microfluidic platform based on Peptide nucleic acids (PNAs)



SPR microfluidic platform based on Peptide nucleic acids (PNAs)



Coverage: 5×10^{12} molecule cm^{-2}

In collaboration with G. Spoto (University of Catania)

SPR microfluidic platform based on Peptide nucleic acids (PNAs)

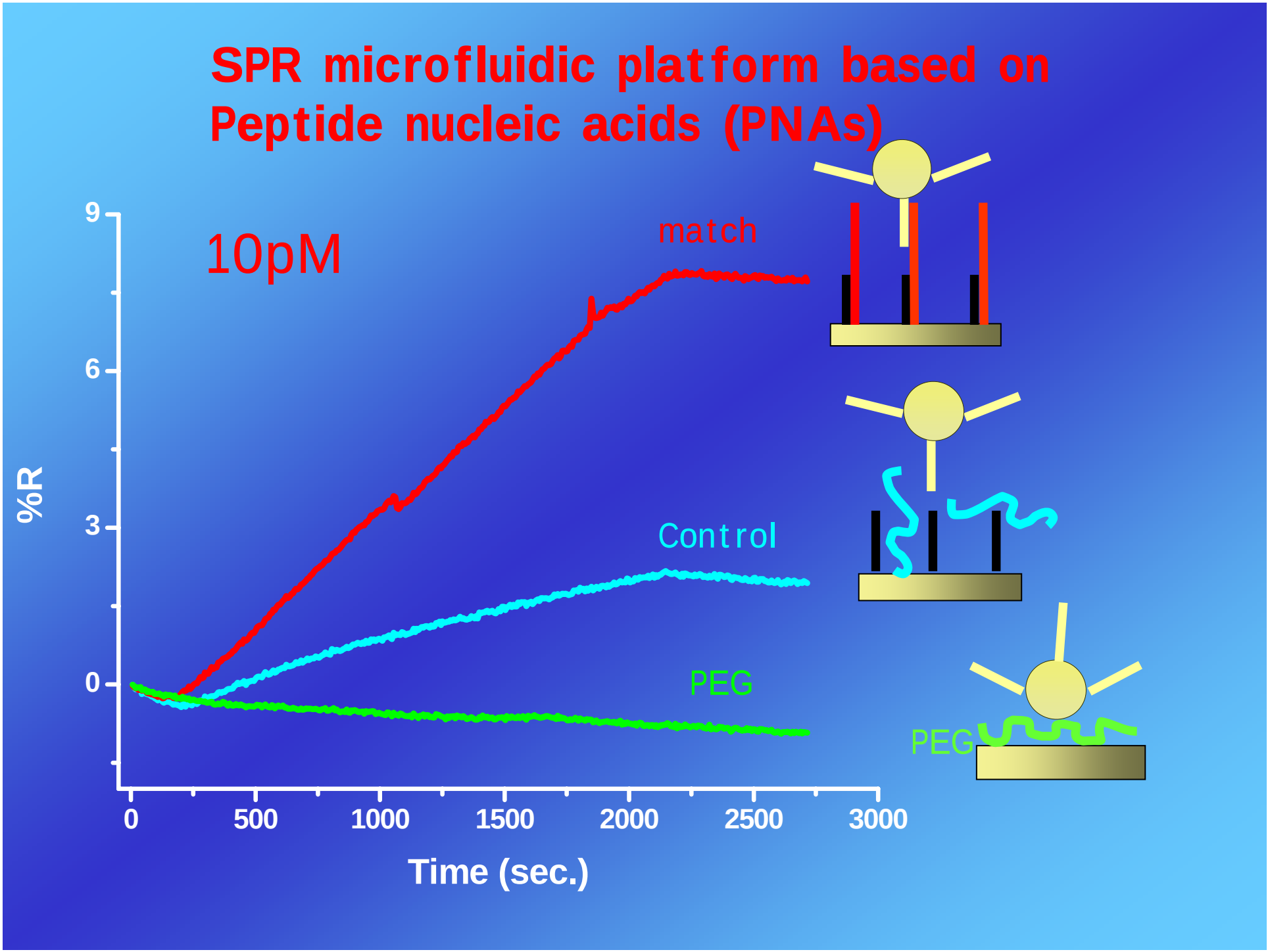
5'-LL-AAACCCTTAATCCCA-3' PNA-15mer

3'-TTTGGGAATTAGGGTTTTTTTTTTTCGTCGAATAGCA-5'
ssDNA-36mer-match

5'-GCAGCTTATCGT-3'-Biotin
ssDNA-12merC_6Biotin

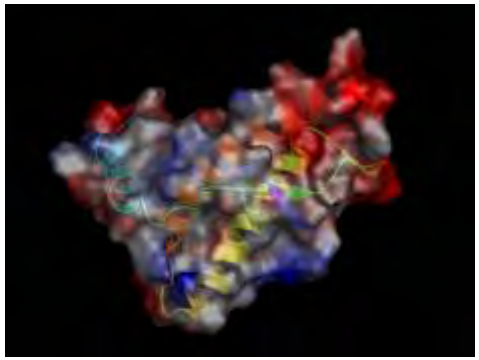
NPs

SPR microfluidic platform based on Peptide nucleic acids (PNAs)



Prion protein detection in attomole amounts

Onisko et al., J.Am.Soc.Mass Spec., 2007, 18, 1070-1079



Prion protein

Reduction, alkylation, digestion

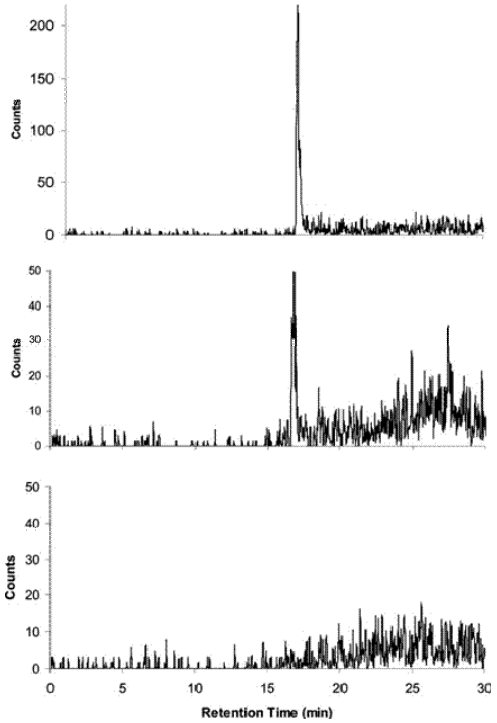
nanoLC/MS/MS

Detection of the specific peptide
VVEQMCTTQYQK

900 attomol

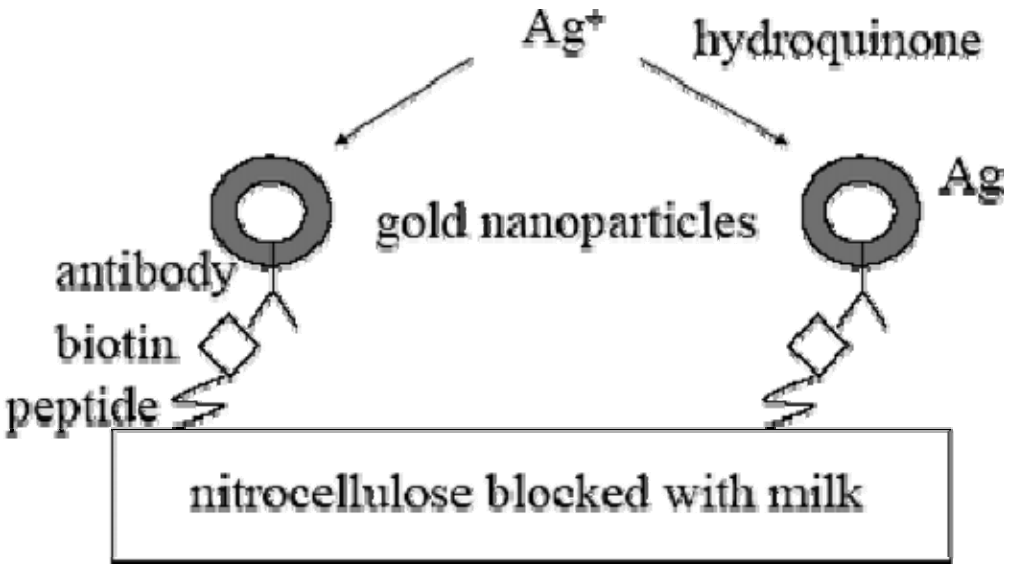
300 attomol

0 attomol

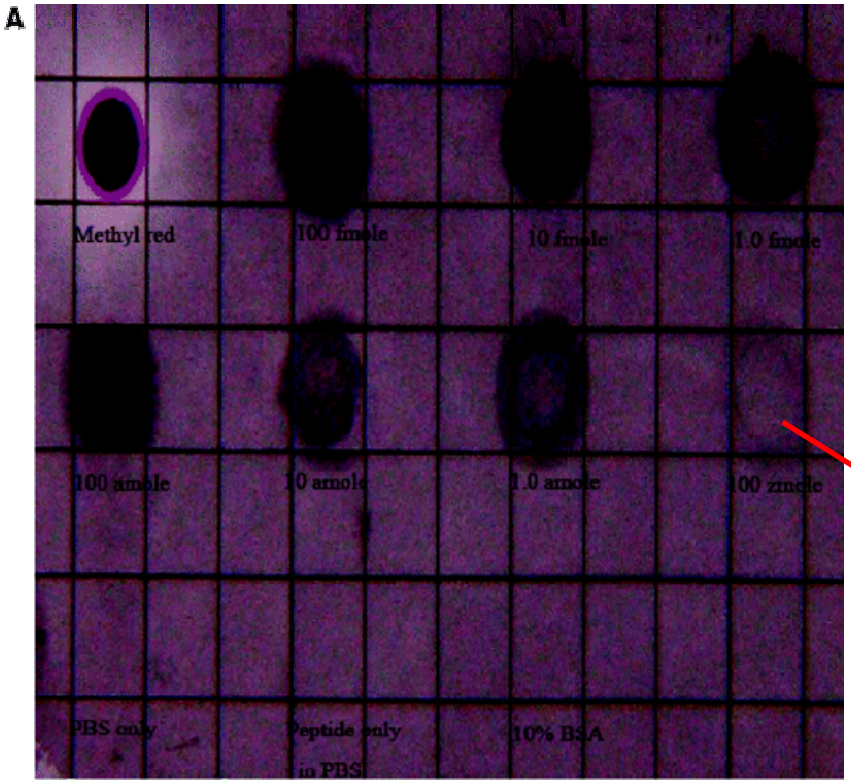


Zeptomole and attomole detection of biotin-peptides by a dot-blot gold nanoparticle immunoassay

Hou S.Y. Et al., Analytical Chemistry, 2007, 79, 980-985



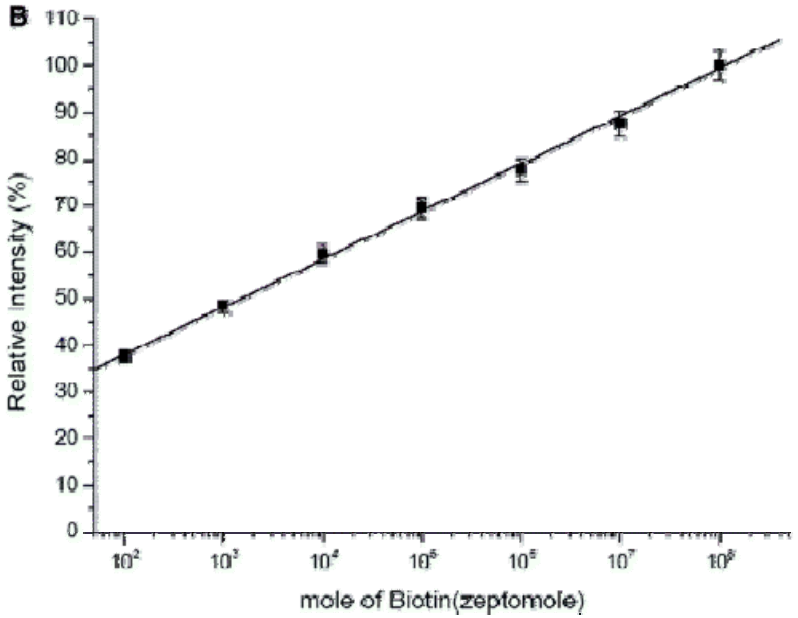
peptide sequence: SGQSWRPQGRFG



Detection of biotin-peptide with silver enhancement.

(A) Dot images for different levels of the biotin-peptide after silver enhancement.

100 zeptomoles



B) Detection curve for the biotin-peptide

Conclusions

- **The trend in food analysis is to devise micro(nano) systems, which can provide affordable, portable, fast and user friendly equipments**
- **Nanotechnologies allow to push down the detection limits almost to “nothing”**
- **How low should the legal limits be set in food analysis ?**

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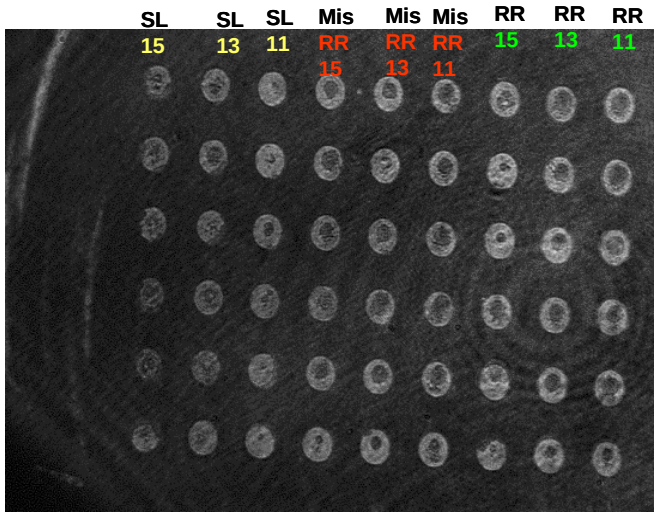
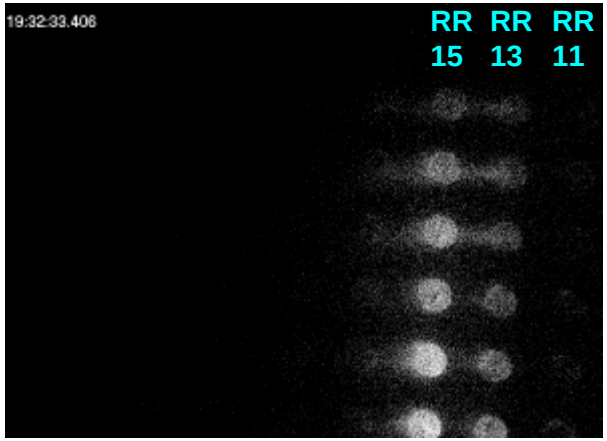
Alessandro Calabretta
Alessandro Tonelli
Valeria Cavatorta
Mattia Mangia

Surface Plasmon Microscopy



SPM image of Array B against air. All Parma PNAs (1μM probe concentration)



SPFM image of the array after hybridisation with 1% RR125 GMO target solution

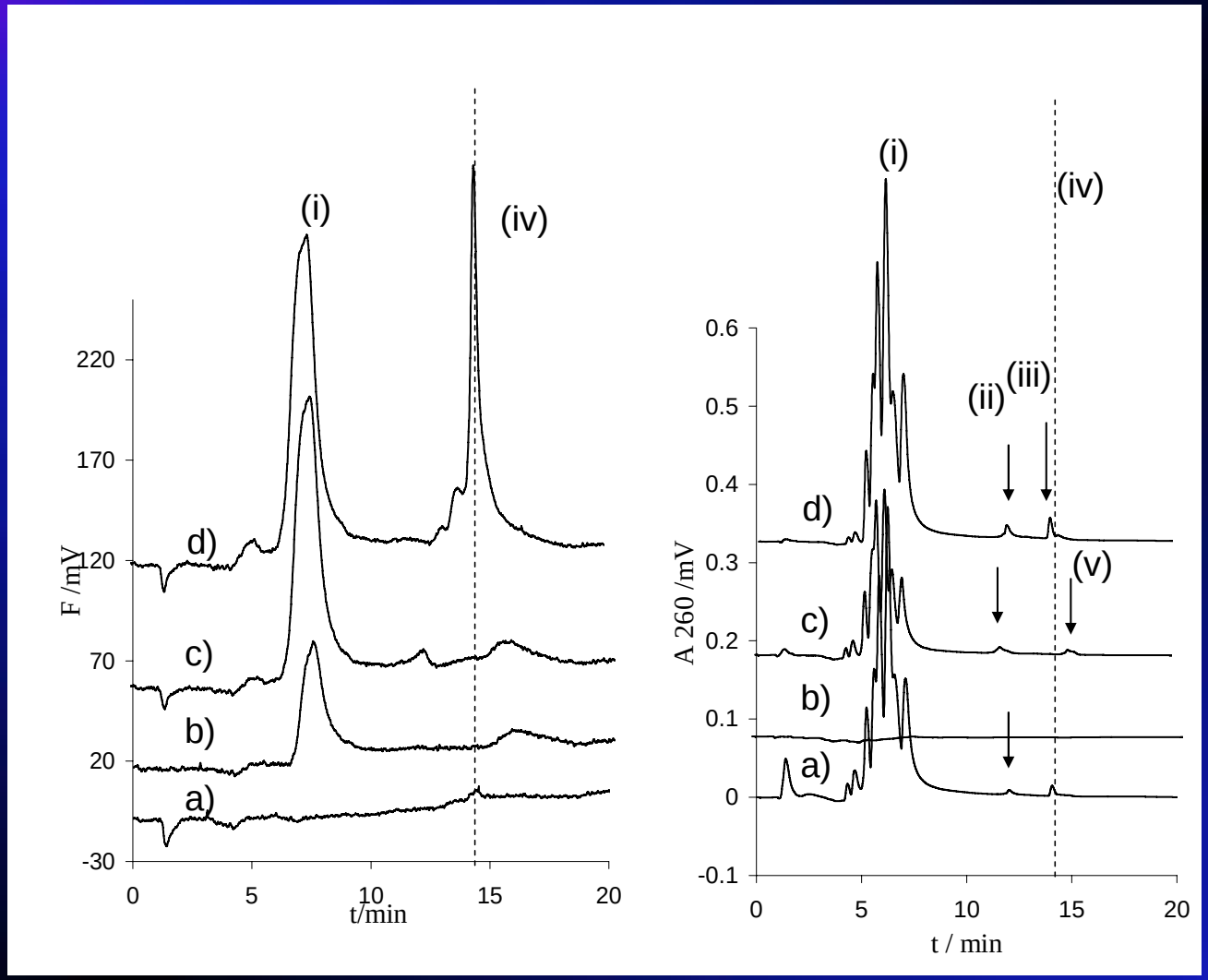
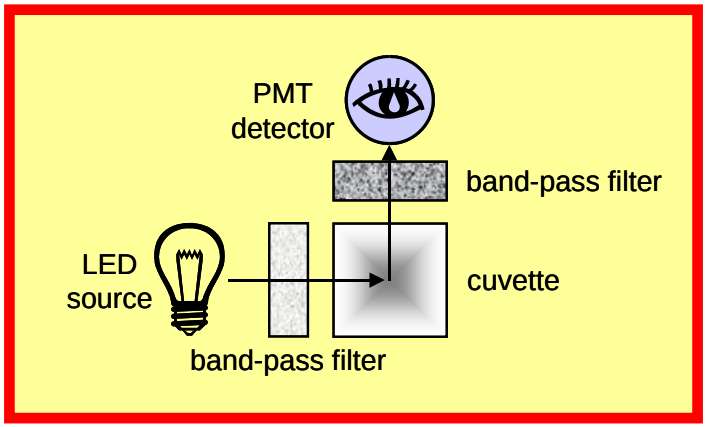
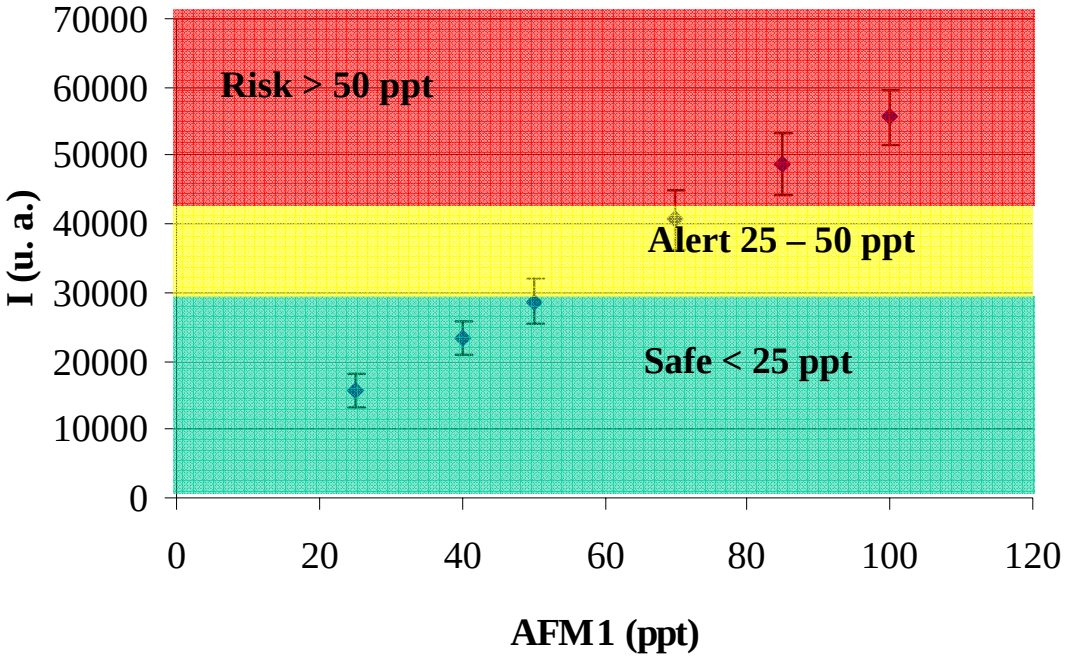
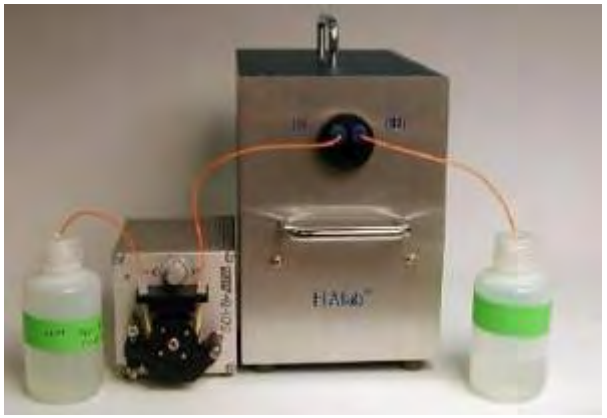


Figura 14. Tracciato IE-HPLC con detector a fluorescenza (lex = 497 nm, l em = 520 nm, sinistra) e UV (260 nm, destra) relativo a : a) amplificato di PCR da solo; b) beacon RR-soia (1 mM) solo; c) beacon RR-soia (1 mM) + amplificato di PCR aspecifico; d) beacon RR-soia (1 mM) + amplificato di PCR specifico. I vari picchi sono stati identificati come: i) beacon e componenti della reazione di PCR; ii) prodotti di amplificazione aspecifici (primer-dimers); iii) prodotto di PCR 79-mer (soia RR) iv) ibrido PNA beacon-DNA; v) prodotto di PCR aspecifico da 201bp (controllo negativo).). Colonna: TSK-gel DNA NPR; eluenti: A = TRIS 0.02M, pH = 9.0, B = NaCl 1M in eluent A. Gradiente lineare: from 100%A to 100% B in 20 min; flusso: 0.5 mL/min.

Aflatoxins: FIAlab PMT-FL Detector (Fluorescence In-flow Assay)







LED: 360 nm
Band-Pass Filter maximum: 450 nm

A portable fluorometer for the rapid screening of M1 aflatoxin
C. Cucci, A.G. Mignani, C. Dall'Asta, R. Pela, A. Dossena
Sensors and Actuators B, 2007, in press

Future implementation: fiber optics.

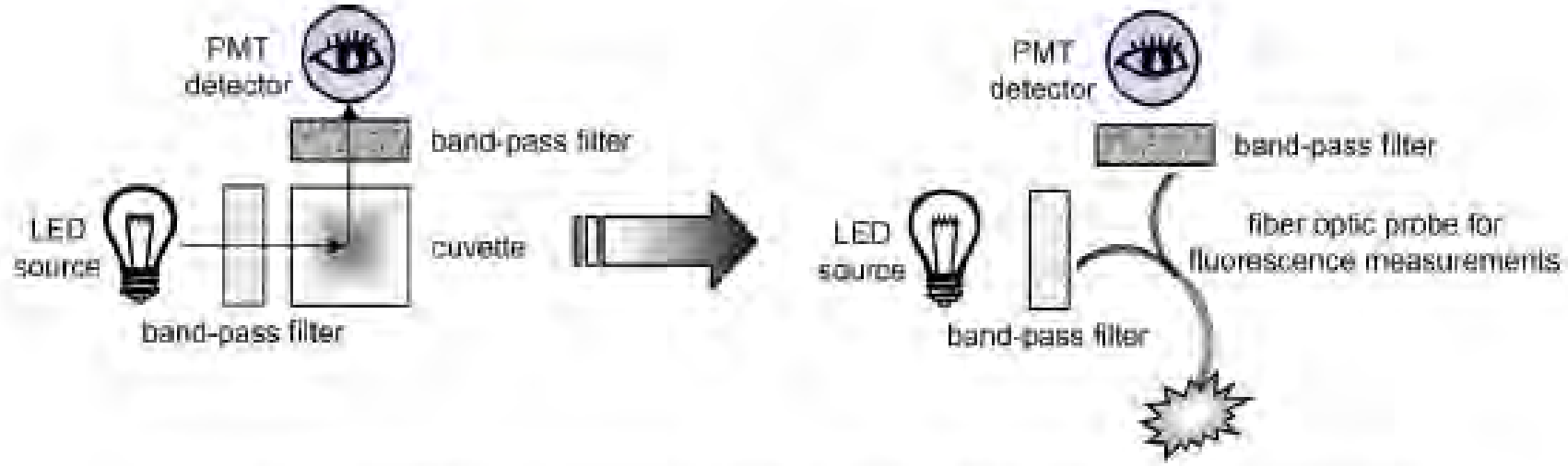
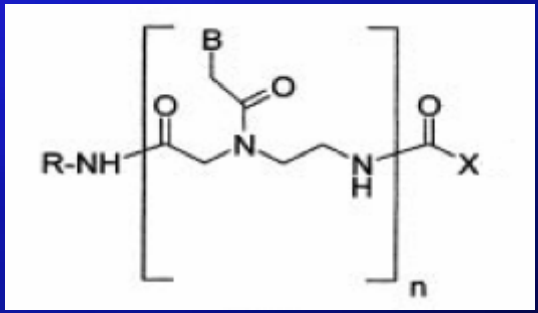
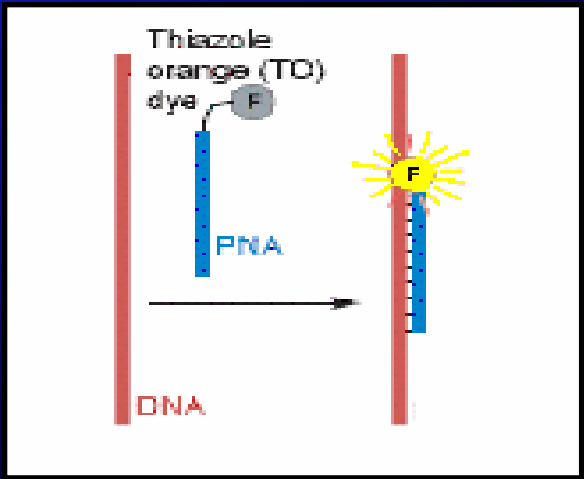


Fig. 1. Schematic diagram of the fluorometer (left) and its possible implementation using fiber optics (right).

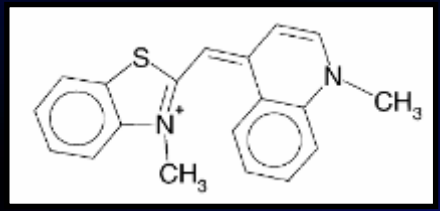
LightUp® Probes

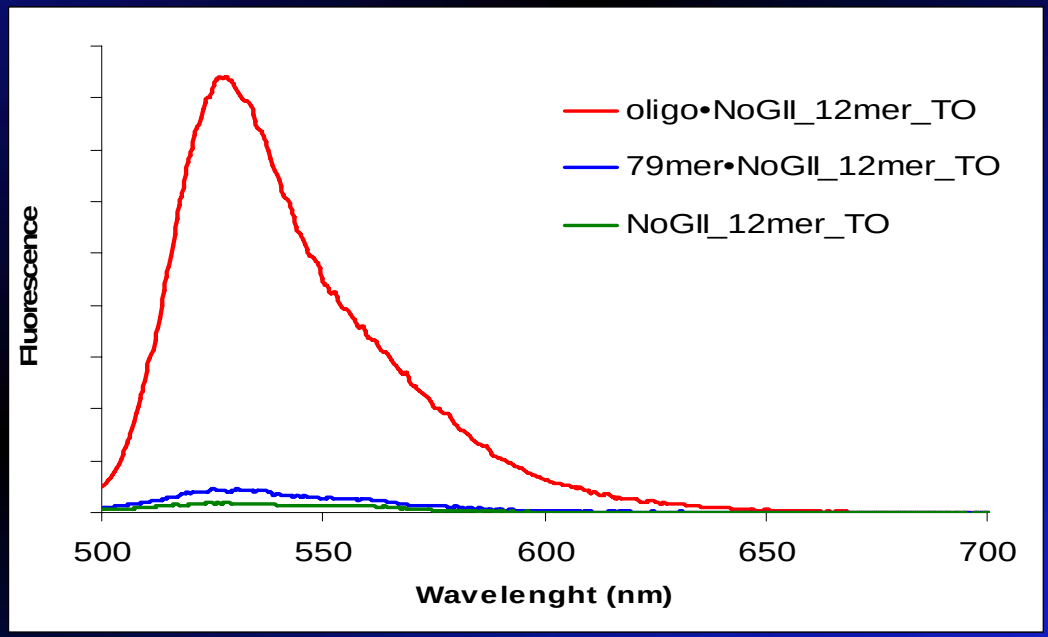
PNA





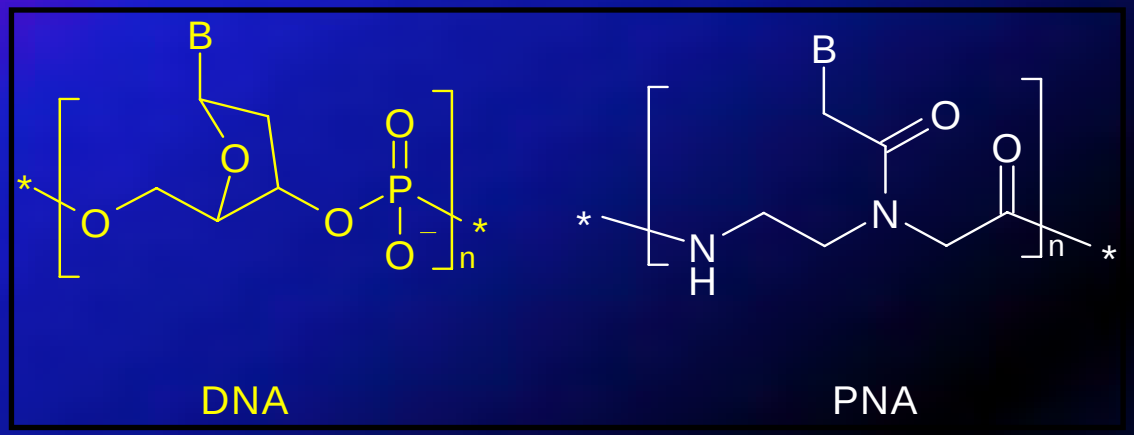
Thiazole Orange (TO)

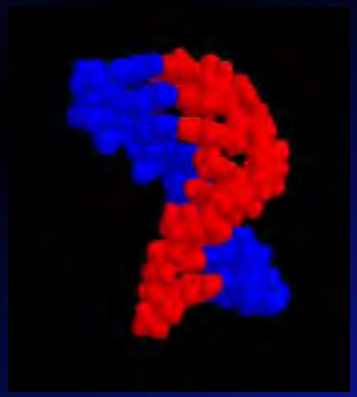




Fluorescence emission spectra of free NoGII_12mer_TO (green line) and PNA_12mer_TO in the presence of non-complementary (blue line) and complementary (red line) DNA oligonucleotide. Samples were analyzed at a concentration of 1 µM. Excitation wavelength: 470 nm. All samples were heated at 95 °C for 3 min and spectra were acquired after 5 min at 45 °C

Identification of specific DNA sequences by Peptide Nucleic Acids (PNAs)





DUPLEX STABILITY:

PNA-DNA >>DNA-DNA

10-mer: **Tm = 50°C** **Tm = 35°C**

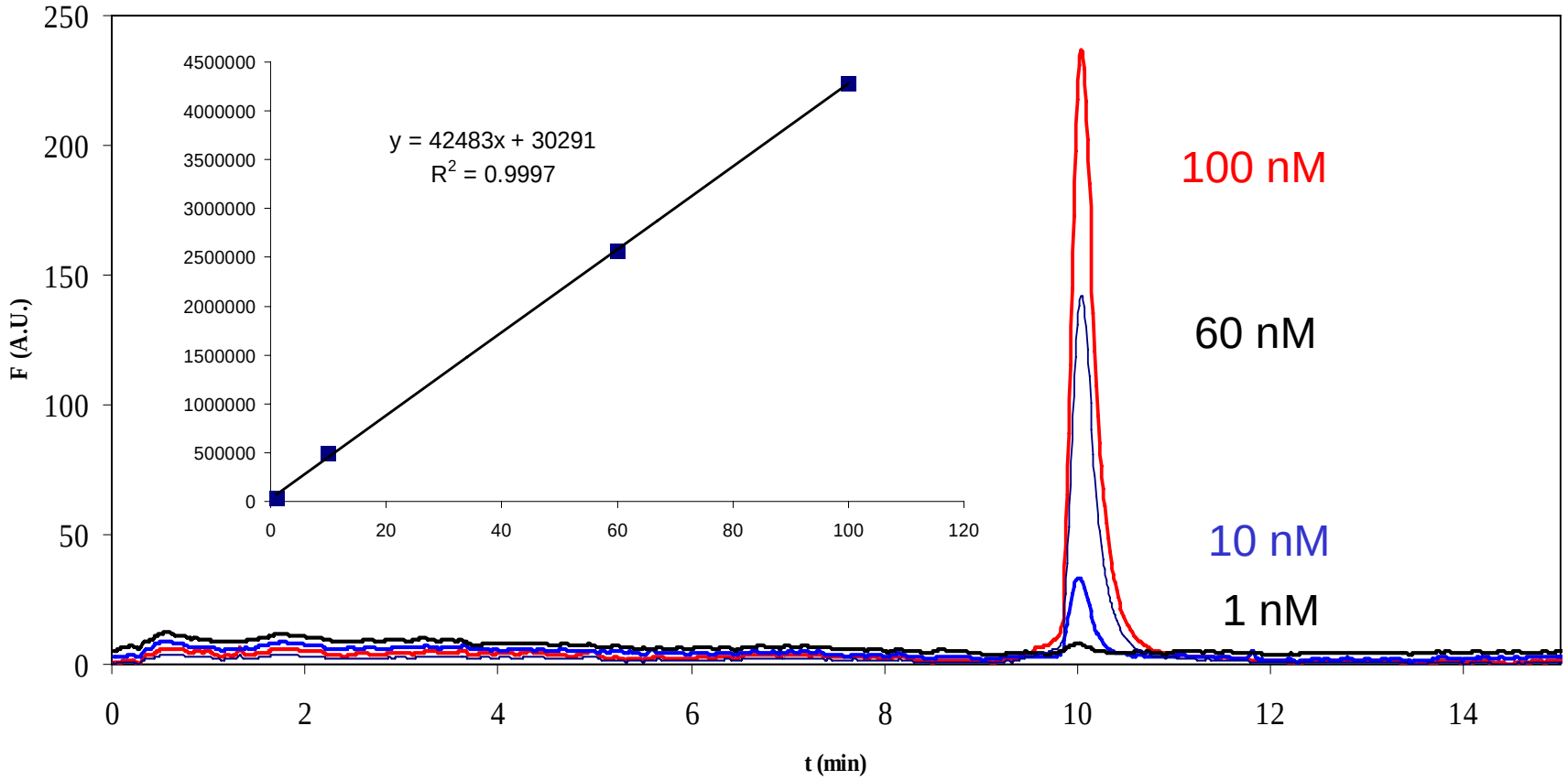
POINT-MUTATION RECOGNITION:

PNA-DNA > DNA-DNA

15-mer: Tm drop with a mismatch **Tm: 69°C -> 56°C** **Tm: 53°C -> 49°C**

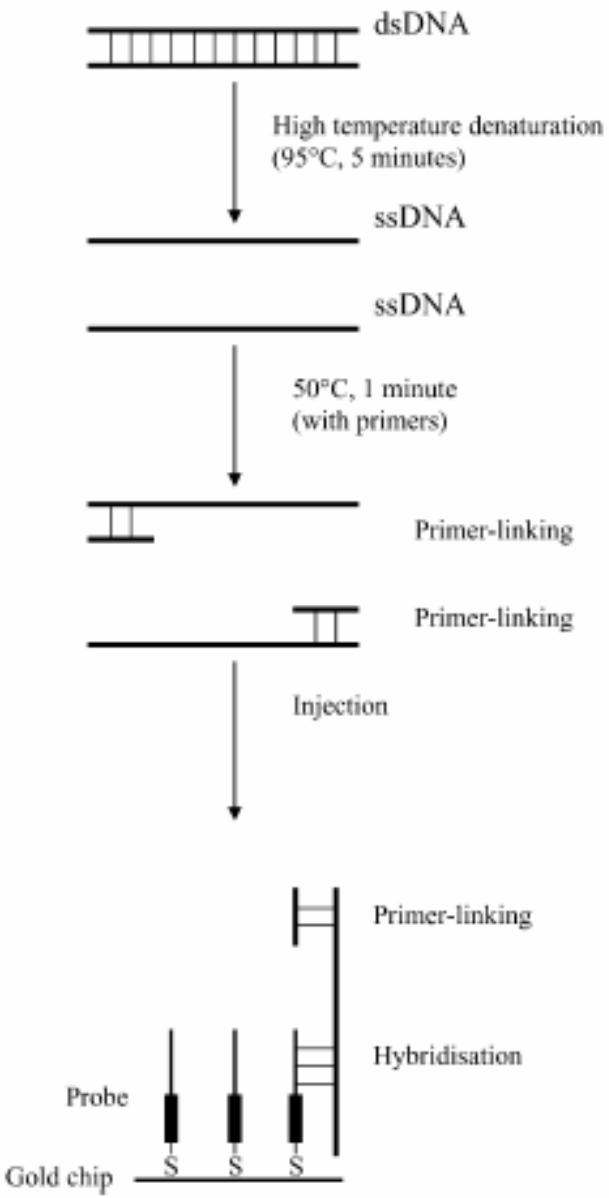
GMOs

Quantitative analysis of unlabelled DNA by RR-beacon/IE HPLC



Conditions: column TSK-gel DEAE-NPR 4.6mm x 7.5cm. Gradient elution from 100%A (tris, pH = 9.0) to 100% B (1M NaCl in eluent A) in 20 min. Fluorescence detector (λex =497 nm λem= 520 nm).Flow rate: 0.5 mL/min

PCR-FREE DNA analysis with DNA probes



35S genomic DNA detection from GM *Nicotiana glauca*, by piezoelectric sensing

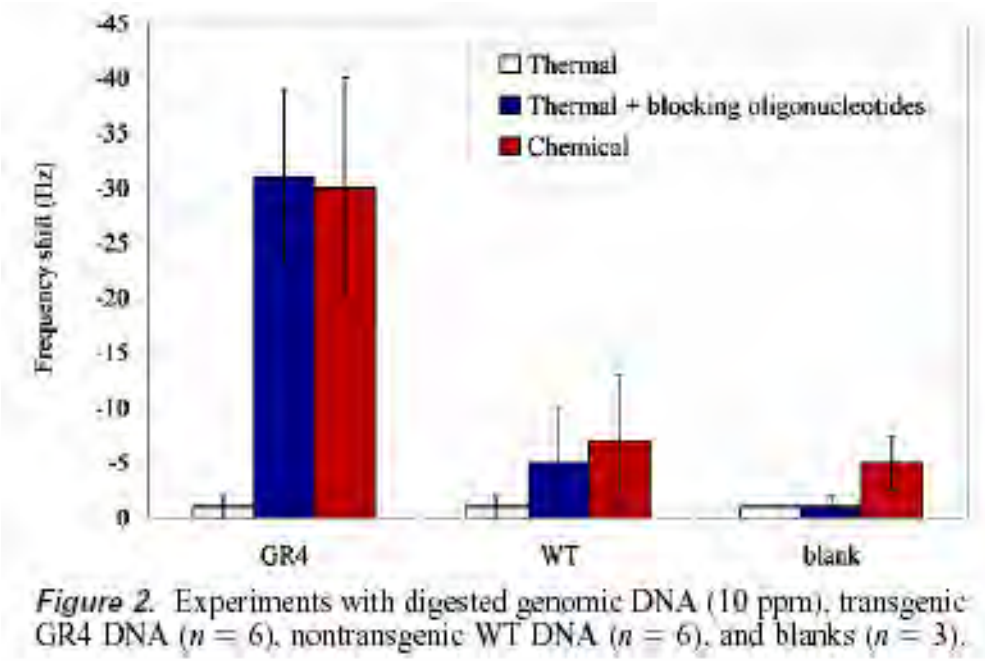
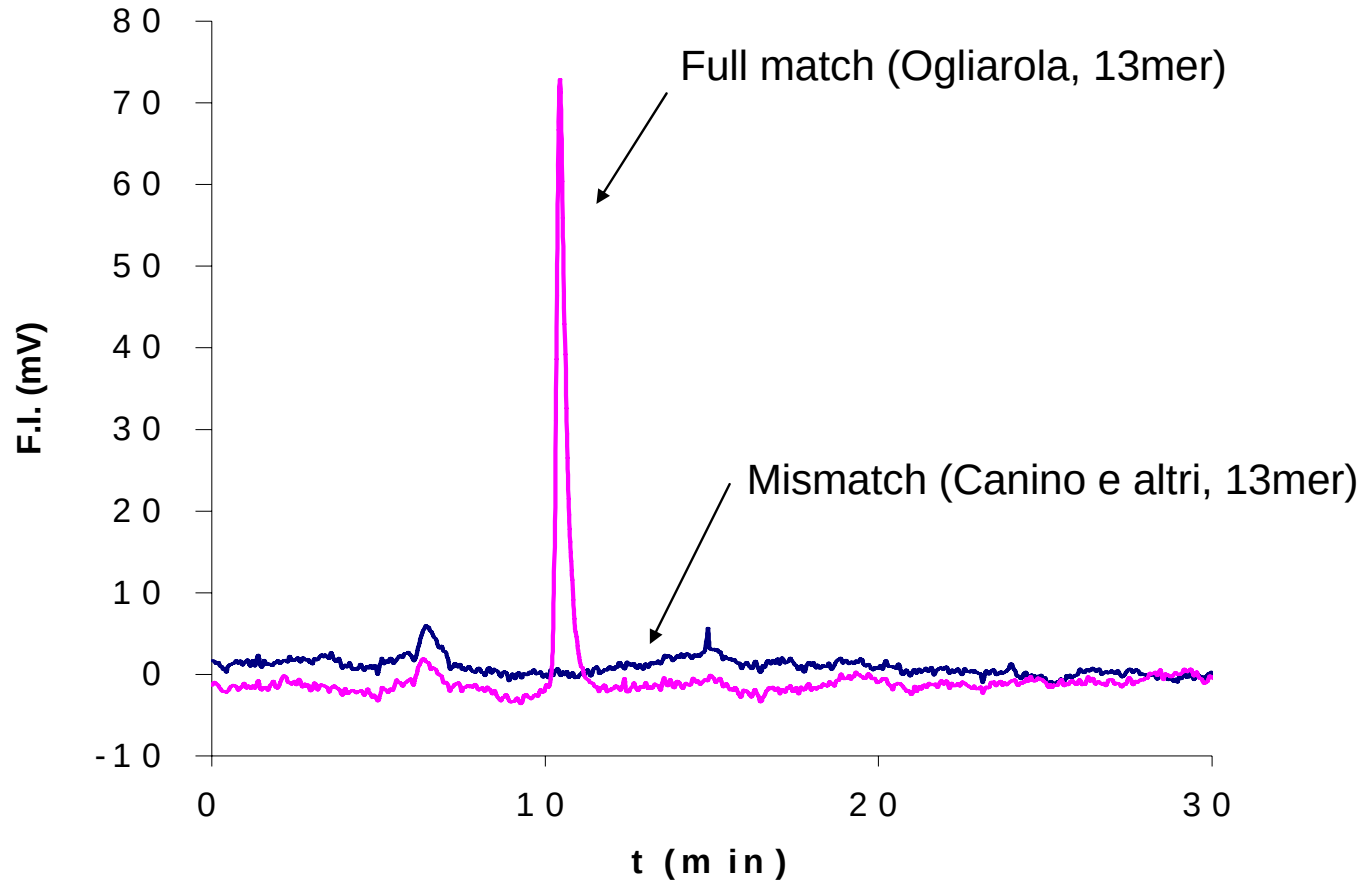


Figure 2. Experiments with digested genomic DNA (10 ppm), transgenic GR4 DNA ($n = 6$), nontransgenic WT DNA ($n = 6$), and blanks ($n = 3$).

Minunni M., Tombelli S., Fonti J, Spiriti M.M., Mascini M., Bogani P., Buiatti M.
Journal of the American Chemical Society, 2005, 127, 7966-7967

PNA beacon

A 60 (11mer) **Fluo-Glu-x xxx A xxx xxx-Lys-Lys(Dabcyl)-NH_2**



Bottom-up: building sensors on self-assembled monolayers (SAM)

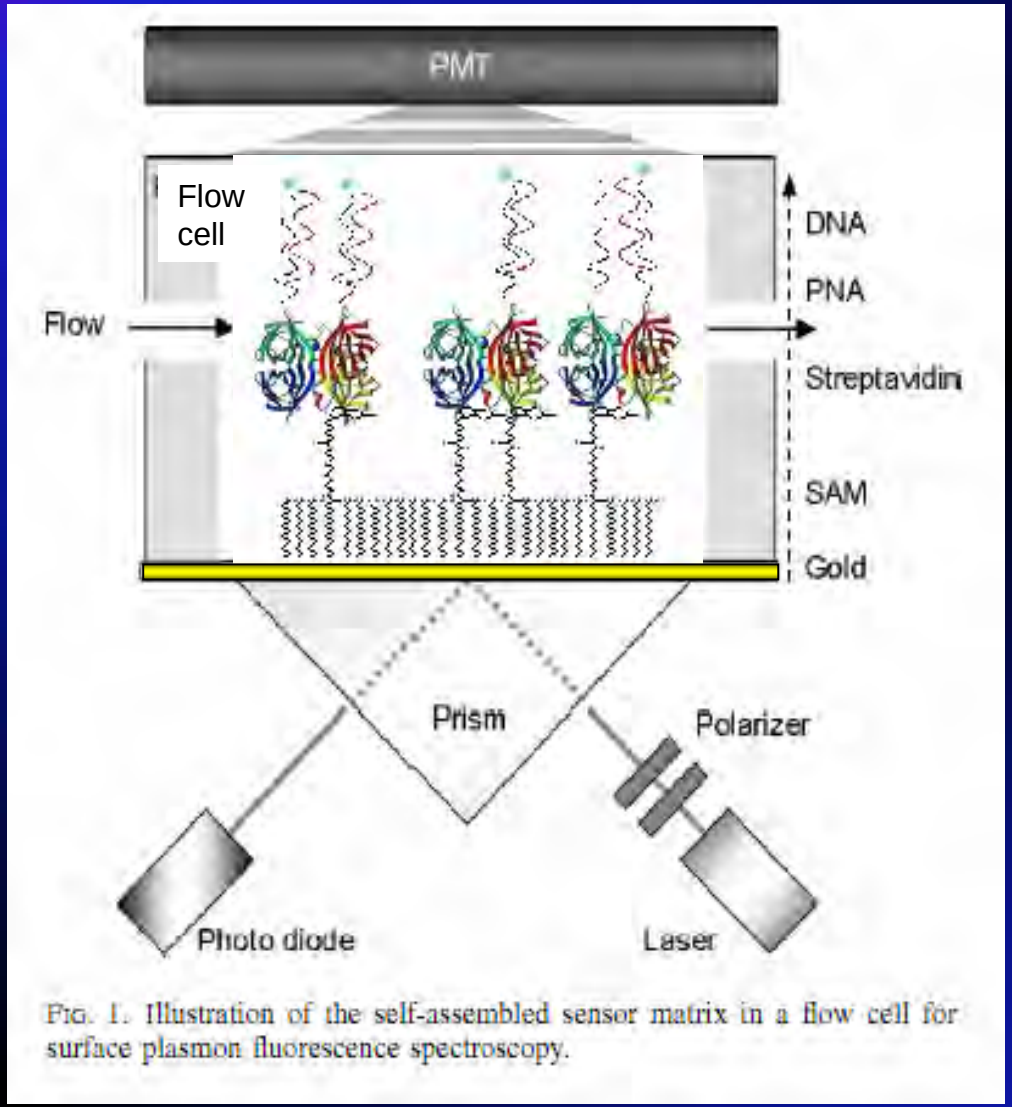


FIG. 1. Illustration of the self-assembled sensor matrix in a flow cell for surface plasmon fluorescence spectroscopy.

Surface-Plasmon Fluorescence Spectroscopy (SPFS)

Measures:

Affinity
Kinetics
Concentration
Arraying/Imaging is possible

Selective detection of RR-soy PCR

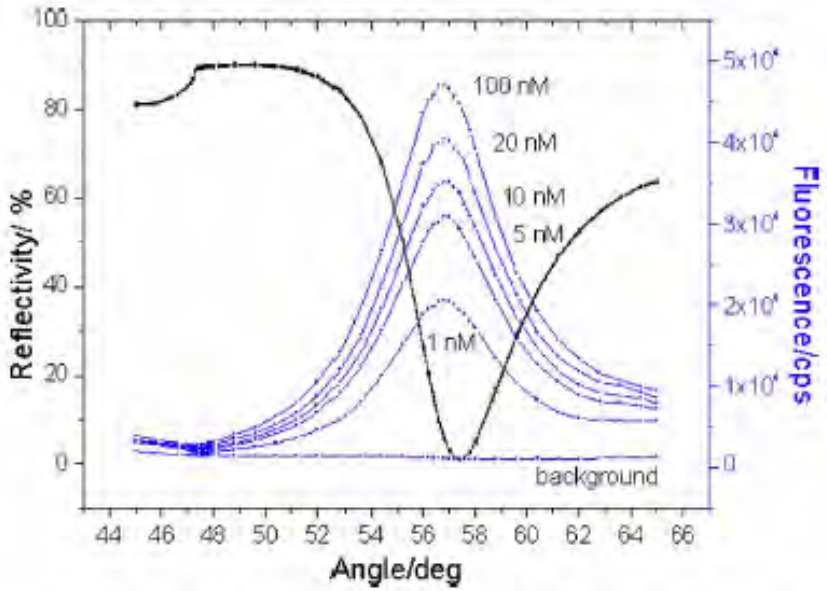


FIG. 7. Angular scans for PNA/DNA RR-169 hybridization taken in 10 mM phosphate buffer after saturation of the fluorescence was reached for 1, 5, 10, 20, and 100 nM target solutions, respectively, together with background fluorescence intensity recorded after rinsing. Typical R^2 after fitting is 0.999.

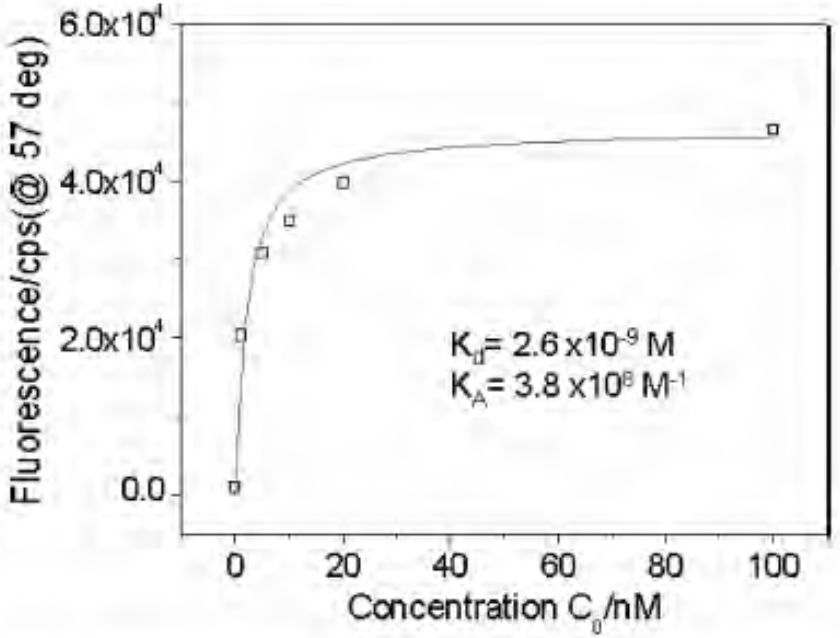


FIG. 8. Maximum fluorescence intensity [taken from Fig. 7(b) at $\theta=57°$] vs target concentration. The full curve corresponds to a fit according to the Langmuir isotherm with $K_A=3.8\times10^8 M^{-1}$. Typical R^2 after fitting is 0.999.