Top-down trends in food analysis: towards nanotechnologies

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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”
Methods of analysis

Food safety and quality

Regulations
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).

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

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

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

DNA

- Methods of molecular biology
  - Nanotechnologies
  - Chromatography
  - Spectroscopy

Food Microbiology

- GMOs
- Food allergens

Food Authenticity
PNA probes for food DNA detection

Microarrays
SPR
CD
CE
HPLC

Colorimetric tests
Beacons

DNA
PNA

High tech → Low tech

HPLC
Beacons

Light Up Probes
The microarray technology approach

- MULTIPLEX PCR ANALYSIS
- Hybridization
- Deposition
- PNA PROBE LIBRARY DEDICATED TO FOOD
- 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

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<thead>
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<th></th>
<th>Lectin</th>
<th>Zein</th>
<th>MON810</th>
<th>RR</th>
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<th>GA21</th>
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<td>●●●●</td>
<td></td>
<td>●●●●●●</td>
<td>●●●●●●</td>
</tr>
</tbody>
</table>

In every sub-array: more than 1 thousand spots
PNA chip for RR-soy products

1) Soy seed

<table>
<thead>
<tr>
<th>MZ</th>
<th>SL</th>
<th>RR</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

2) Lecithin

<table>
<thead>
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<th>SL</th>
<th>RR</th>
<th>CP</th>
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</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

RR 125 bp

Lectin 157 bp
Detection of allergens in commercial products: hazelnut (Cor a1) and peanut (Ara h2)

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Breakfast cereals</td>
</tr>
<tr>
<td>2</td>
<td>Breakfast cereals with chocolate and hazelnut</td>
</tr>
<tr>
<td>3</td>
<td>Müesli snack with milk chocolate</td>
</tr>
<tr>
<td>4</td>
<td>Müesli snack with cocoa</td>
</tr>
<tr>
<td>5</td>
<td>Snack with cereals and cocoa</td>
</tr>
<tr>
<td>6</td>
<td>Cocoa and vanilla wafer</td>
</tr>
<tr>
<td>7</td>
<td>Hazelnut wafer</td>
</tr>
<tr>
<td>8</td>
<td>Biscuit topped with cocoa cream and milk chocolate</td>
</tr>
<tr>
<td>9</td>
<td>Milk chocolate</td>
</tr>
<tr>
<td>10</td>
<td>Chocolate for topping</td>
</tr>
<tr>
<td>11</td>
<td>Chocolate cream with hazelnut</td>
</tr>
</tbody>
</table>

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**

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

Detection limit: 50 fmol

<table>
<thead>
<tr>
<th>PNA Cor</th>
<th>PNA Ara</th>
<th>PNA Pru</th>
<th>Control</th>
</tr>
</thead>
</table>

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

PNA probes specificity
## Authenticity: Olive oil cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>60</th>
<th>120</th>
<th>183</th>
<th>198</th>
<th>345</th>
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</thead>
<tbody>
<tr>
<td>Biancolilla</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>Canino</td>
<td>A</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>G</td>
</tr>
<tr>
<td>Carolea</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
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<td>A</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
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<td>A</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>G</td>
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<tr>
<td>Leccino</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>R</td>
</tr>
<tr>
<td>Nocellara belice</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>R</td>
</tr>
<tr>
<td>Ogliarola leccese</td>
<td>T</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>G</td>
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<tr>
<td>Moraiolo</td>
<td>A</td>
<td>R</td>
<td>R</td>
<td>C</td>
<td>G</td>
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<tr>
<td>Bosana</td>
<td>A</td>
<td>R</td>
<td>R</td>
<td>C</td>
<td>G</td>
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<tr>
<td>Nocellara etnea</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>R</td>
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<tr>
<td>Arbequina</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>G</td>
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**SNPs in the Actin gene**

- **R = T o C**
- **S = G o C**
Olive variety identification by PNA microarrays

<table>
<thead>
<tr>
<th>DNA</th>
<th>PNA</th>
<th>A 60</th>
<th>T 60</th>
<th>C198</th>
<th>G198</th>
<th>CP</th>
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</thead>
<tbody>
<tr>
<td>T</td>
<td>A</td>
<td>60</td>
<td>60</td>
<td>C198</td>
<td>G198</td>
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"Ogliarola leccese"

<table>
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<th>DNA</th>
<th>PNA</th>
<th>A 60</th>
<th>T 60</th>
<th>C198</th>
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<tr>
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<td>60</td>
<td>C198</td>
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</table>

"Canino"

<table>
<thead>
<tr>
<th>DNA</th>
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<tbody>
<tr>
<td>T</td>
<td>A</td>
<td>60</td>
<td>60</td>
<td>C198</td>
<td>G198</td>
<td>CP</td>
</tr>
</tbody>
</table>

"Frantoio"
NOROVIRUS

Caliciviridae family: single strand RNA virus

Main responsible for non bacterial gastroenteritis

23 million cases per year estimated in USA

40% food-transmitted

water, mytilidae, fruits, vegetables

Not cultivable in vitro
Microarrays for Norovirus

Oligonucleotides

<table>
<thead>
<tr>
<th>Sample</th>
<th>Condition</th>
<th>Image</th>
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</thead>
<tbody>
<tr>
<td>GII_compl (10 nM)</td>
<td>Deposition control</td>
<td>![Image d) GI_GII_compl_Cy5 (10 nM)]</td>
</tr>
<tr>
<td>GI</td>
<td>Deposition control</td>
<td>![Image e) GI_GII_compl_Cy5 (10 nM)]</td>
</tr>
<tr>
<td>GI_compl_Cy5 (10 nM)</td>
<td>Deposition control</td>
<td>![Image f) GI_GII_compl_Cy5 (10 nM)]</td>
</tr>
<tr>
<td>GI_compl_Cy5 (90 nM) + GI_compl_Cy5 (10 nM)</td>
<td>Deposition control</td>
<td>![Image h) GI_GII_compl_Cy5 (90 nM) + GI_compl_Cy5 (10 nM)]</td>
</tr>
</tbody>
</table>

PCR products

<table>
<thead>
<tr>
<th>Sample</th>
<th>Condition</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>NoV GII sample 1</td>
<td>Deposition control</td>
<td>![Image g) NoV GII sample 1]</td>
</tr>
<tr>
<td>NoV GII sample 2</td>
<td>Deposition control</td>
<td>![Image i) NoV GII sample 2]</td>
</tr>
</tbody>
</table>

In collaboration with the Virology Unit of the University of Parma
PNA BEACON (STEMLESS)

Fluorophor

Quencher

carboxyfluorescein

DNA

Dabcyl
PNA BEACON FOR ROUNDUP READY-SOY

(F/F0)_{max} = 6.5

\[ \lambda_{EC} = 497 \text{ nm} \]

\[ \lambda_{EM} = 520 \text{ nm} \]
# Towards a complete “lab on a chip” system

- **PNA array**
- **Array with PNA beacon**
- **Labelled DNA**
- **Unlabelled DNA**
The “Lab on a chip” system

Sample inlet

Amplification Area using PCR

Separation Area

Detection Area
Surface Plasmon Resonance

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

SPR microfluidic platform based on Peptide nucleic acids (PNAs)
SPR microfluidic platform based on Peptide nucleic acids (PNAs)

Coverage: $5 \times 10^{12}$ 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'-TTTGGGAATTAGGGTTTTTTTTTTCGTCGAATAGCA-5' ssDNA-36mer-match

5'-GCAGCTTATCGT-3' Biotin

ssDNA-12merC₆Biotin

NPs
SPR microfluidic platform based on Peptide nucleic acids (PNAs)

10pM
Prion protein detection in attomole amounts


Prion protein

Reduction, alkylation, digestion → nanoLC/MS/MS

Detection of the specific peptide VVEQMCTTQYQK
Zeptomole and attomole detection of biotin-peptides by a dot-blot gold nanoparticle immunoassay


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?
Acknowledgments

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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)

A portable fluorometer for the rapid screening of M1 aflatoxin
C. Cucci, A.G. Mignani, C. Dall’Asta, R. Pela, A. Dossena

LED: 360 nm
Band-Pass Filter maximum: 450 nm
Future implementation: fiber optics.

Fig. 1. Schematic diagram of the fluorometer (left) and its possible implementation using fiber optics (right).
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:**
- DNA: $T_m = 50^\circ C$
- PNA: $T_m = 35^\circ C$

**Point-Mutation Recognition:**
- PNA-DNA > DNA-DNA

**15-mer:**
- Tm drop with a mismatch
  - PNA-DNA: $T_m: 69^\circ C \rightarrow 56^\circ C$
  - DNA-DNA: $T_m: 53^\circ C \rightarrow 49^\circ C$
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 ($\lambda_{ex}$ =497 nm $\lambda_{em}$ = 520 nm). Flow rate: 0.5 mL/min

$y = 42483x + 30291$

$R^2 = 0.9997$
PCR-FREE DNA analysis with DNA probes

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

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

\textbf{A 60} (11mer) \quad \text{Fluo-Glu-x xxx} \ A \ xxx xxx\text{-Lys-Lys(Dabcyl)-NH}_2

- \textit{Full match (Ogliarola, 13mer)}
- \textit{Mismatch (Canino e altri, 13mer)}
Bottom-up: building sensors on self-assembled monolayers (SAM)

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-159 hybridization taken in 10 nM 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 $\theta=57^\circ$] vs target concentration. The full curve corresponds to a fit according to the Langmuir isotherm with $K_A=3.8 \times 10^3 M^{-1}$. Typical $R^2$ after fitting is 0.999.