

Recenti sviluppi nei biosensori plasmonici per la rivelazione ultrasensibile di polimorfismi a singolo nucleotide in biopsia liquida

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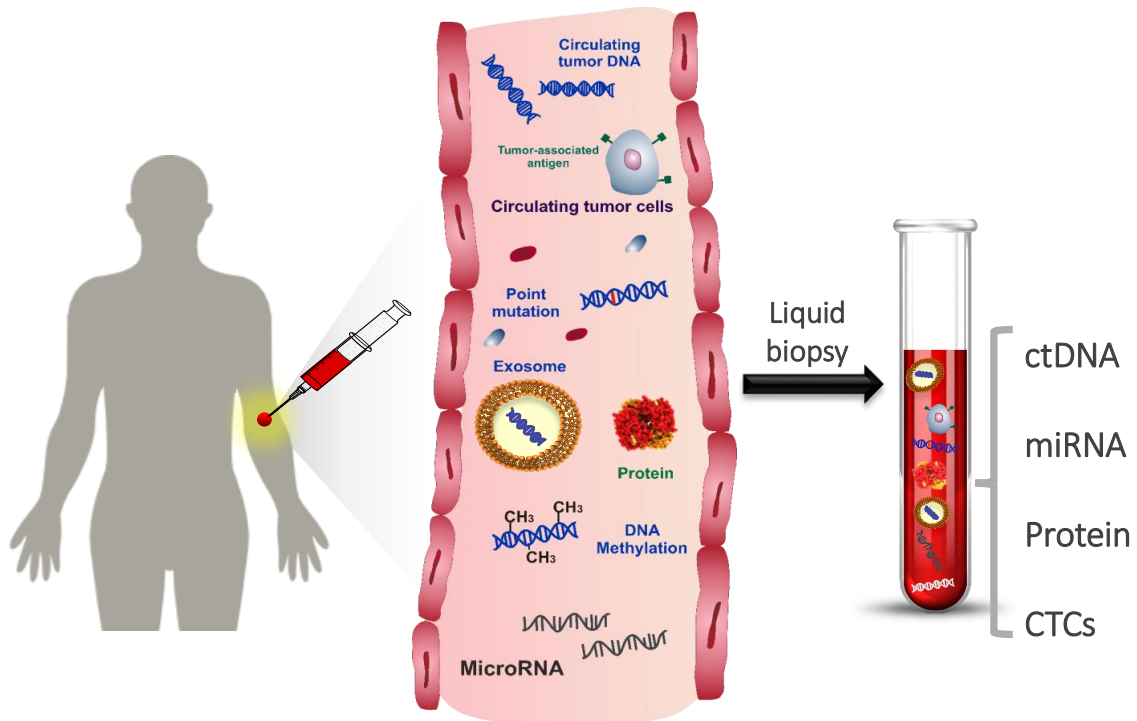
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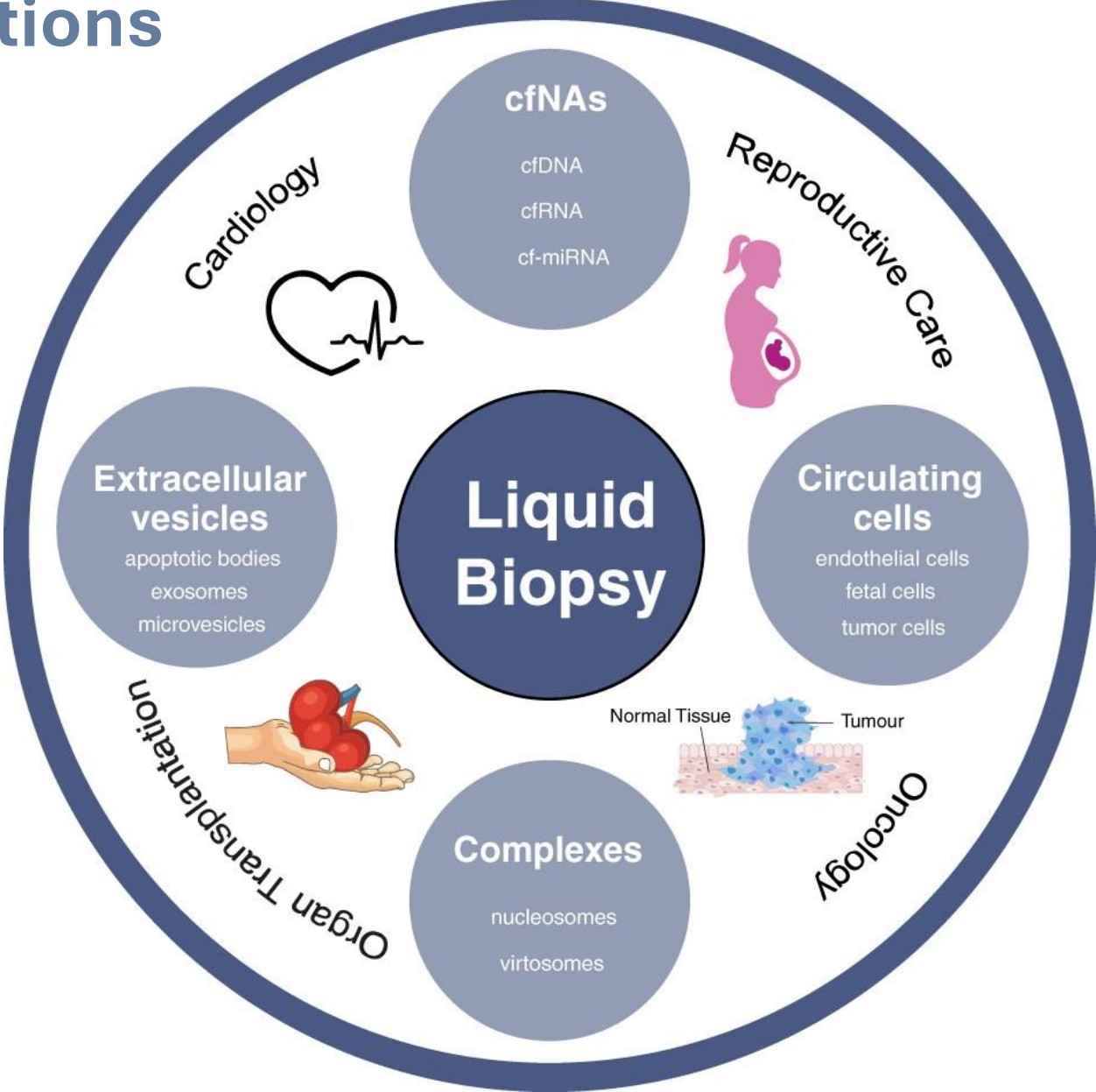
**Nuove strategie di ricerca integrata su Salute, Alimentazione e Ambiente,
XV Convegno Nazionale INBB**

Liquid biopsy

Liquid biopsy is a minimally invasive method that uses blood or bodily secretions to **detect circulating biomarkers** related to the **tumour's genetic makeup**.



Clinical applications



Circulating tumor DNA (ctDNA)



Chromosomal abnormalities



Epigenetic modifications



Point mutations



Translocations



Amplifications and deletions

Detection

Advantages

Disadvantages



NGS

- Cost-effective large screening
- Multiple variants detection
- Screening of unknown mutations

- Bioinformatics
- Longer turnaround time (7-28 days)
- Cost (\$\$\$-\$\$\$\$) per sample
- Error potential
- Lower sensitivity/specificity
- Large sample volume (~ 1.5 mL)

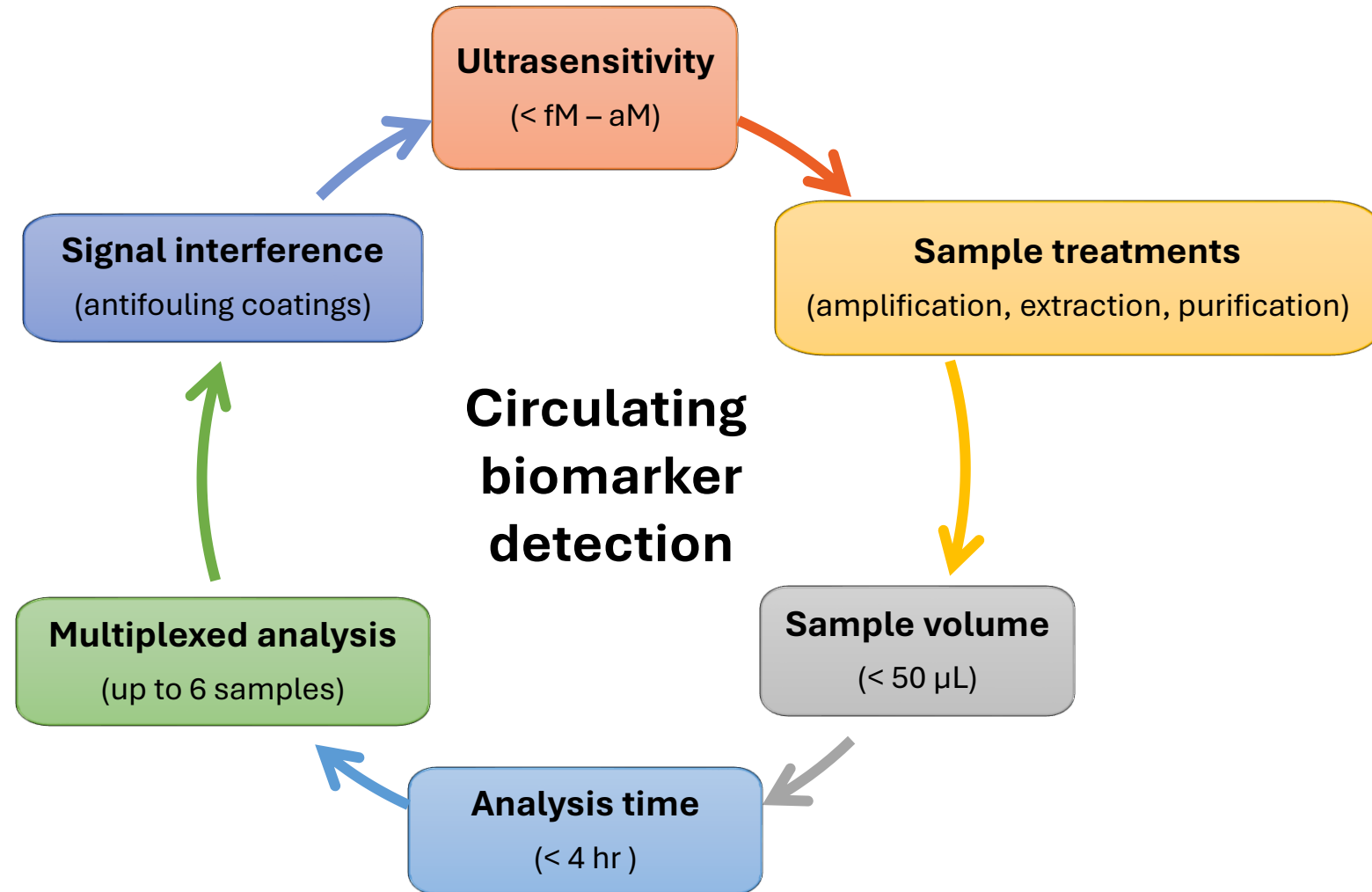


Droplet digital PCR

- High sensitivity/specificity (MAF < 0.1%)
- Absolute quantification
- Long-term patient monitoring
- Short turnaround time (3 – 6 hr)
- Cost (\$) per sample
- Small sample volume (< 40 µL)

- Droplet variability/reproducibility
- Cross-contamination risks
- Analysis of known mutations

Advanced liquid biopsy technology



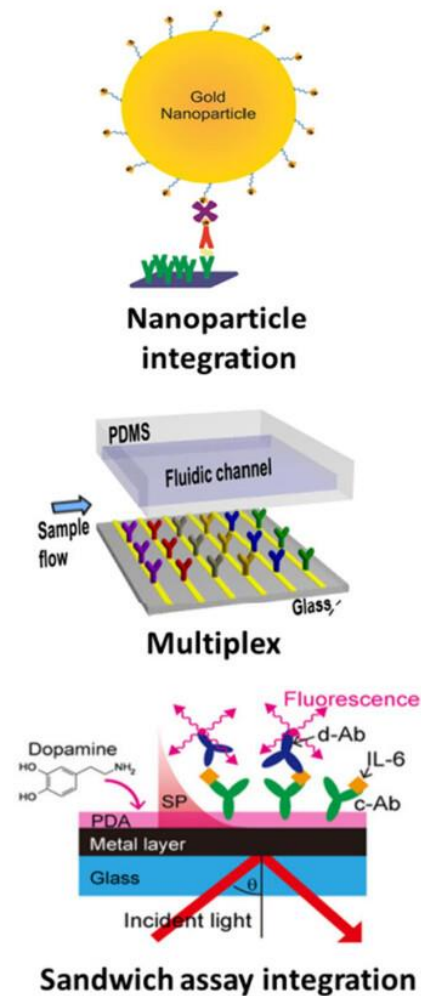
Key advances in plasmonic biosensor technologies

Advances in SPR biosensors for biomarker detection

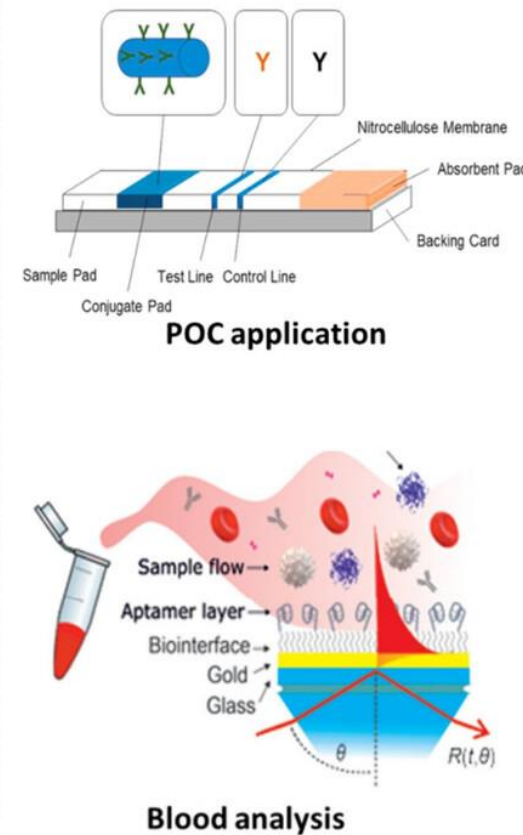
Instrumental development



Sensor development



Application development



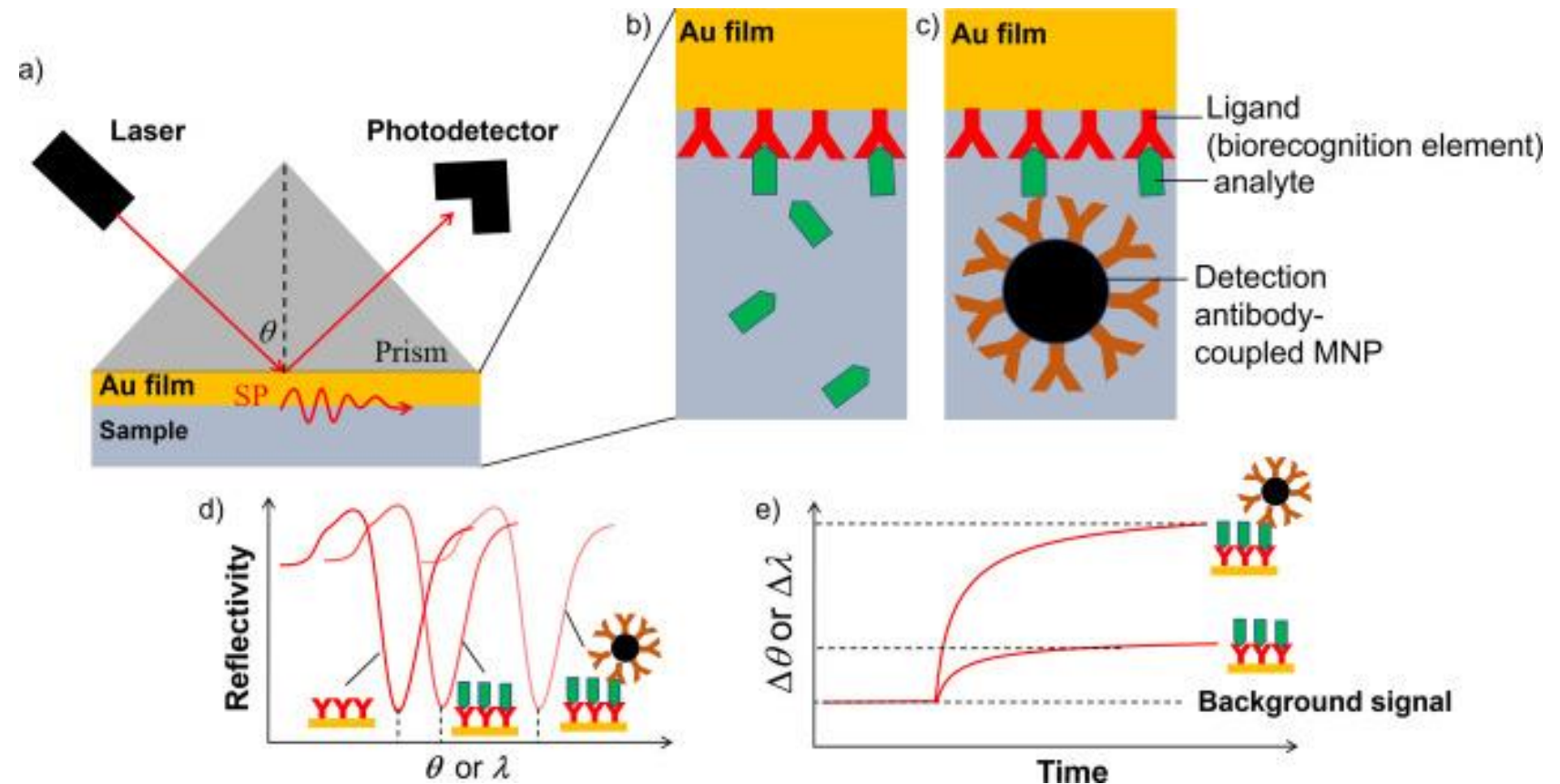
Superparamagnetic particles



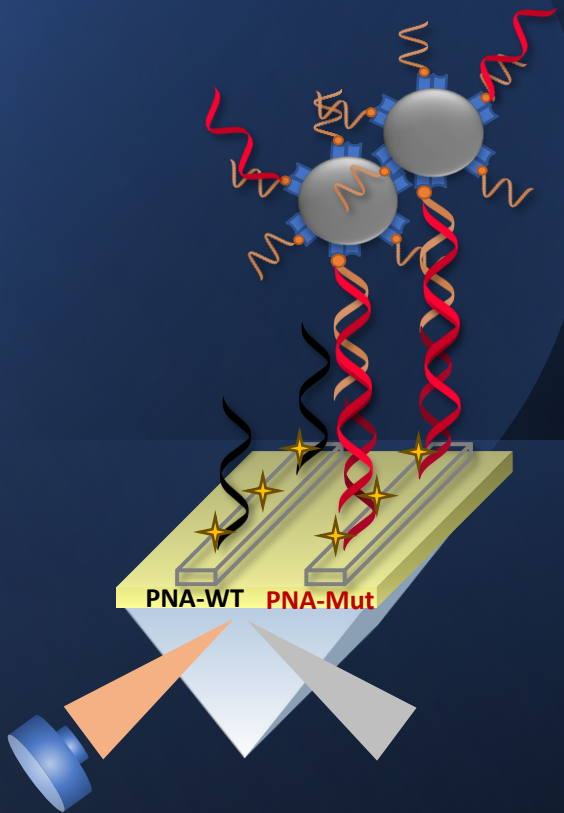
- monodisperse
- no magnetic memory
- rapid separation under magnetic field

... in SPR assay...

- ✓ Signal amplification
- ✓ Analyte delivery



Superparamagnetic bead-based SPR sensor

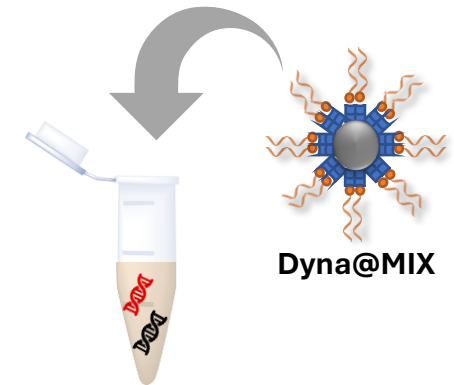


- Cancer diagnosis
- SNPs in KRAS gene

Colorectal cancer

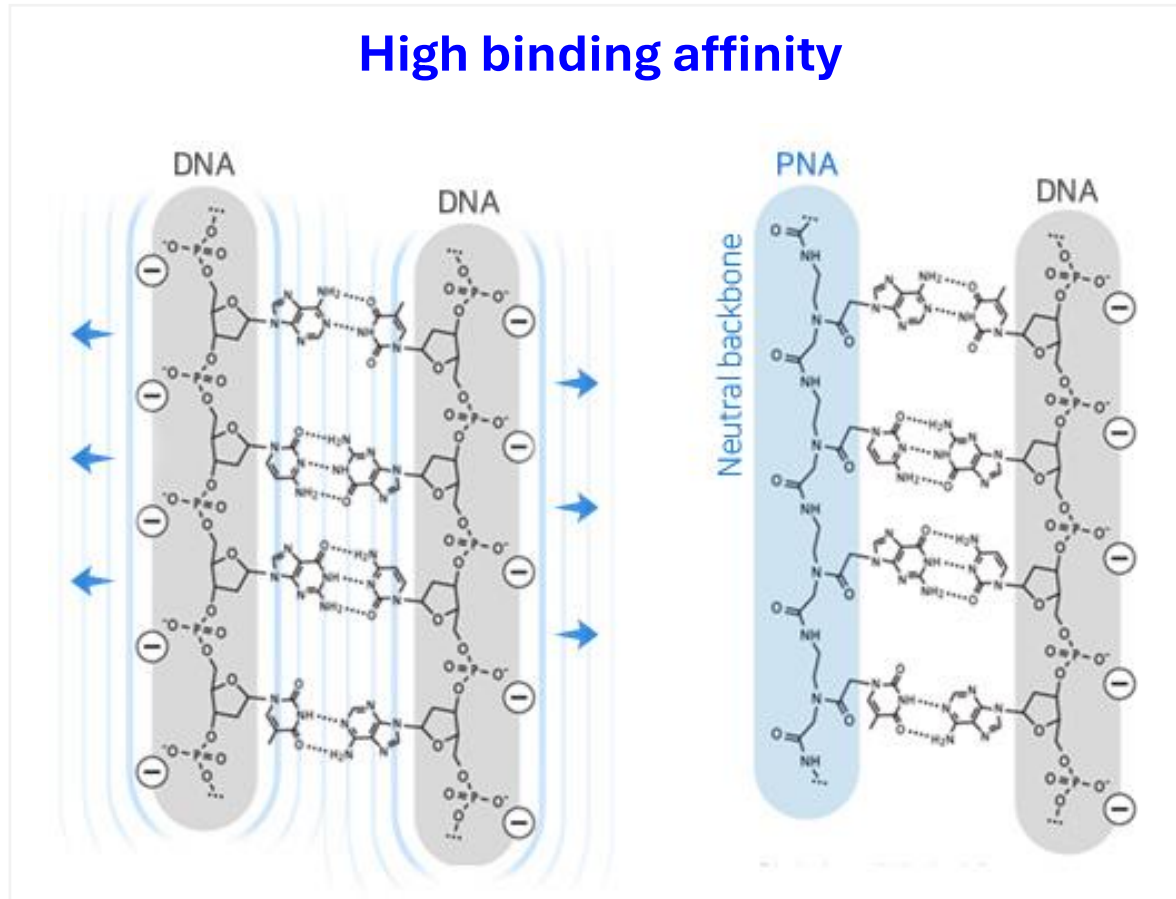
DNA Wild-type (WT)

DNA Mutated
(p.G13D)
(p.G12A)
(p.G12V)
(p.G12R)



PNA probes: FEATURES & ADVANTAGES

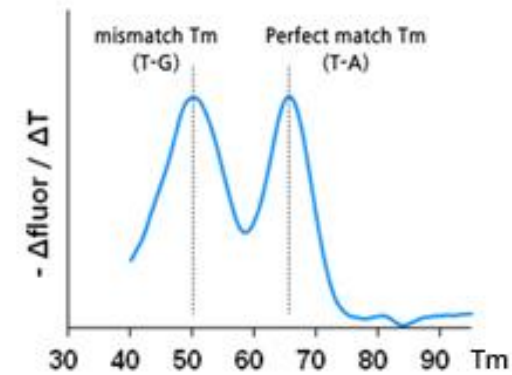
High binding affinity



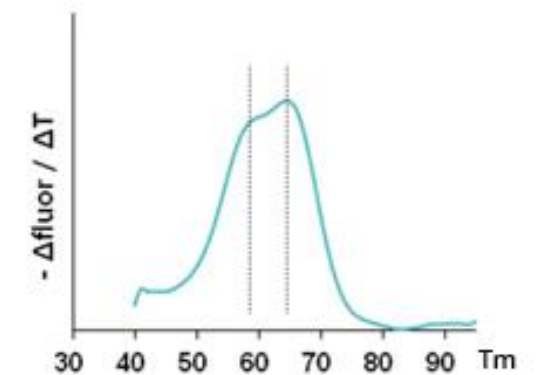
High specificity and sensitivity



1-point mismatch



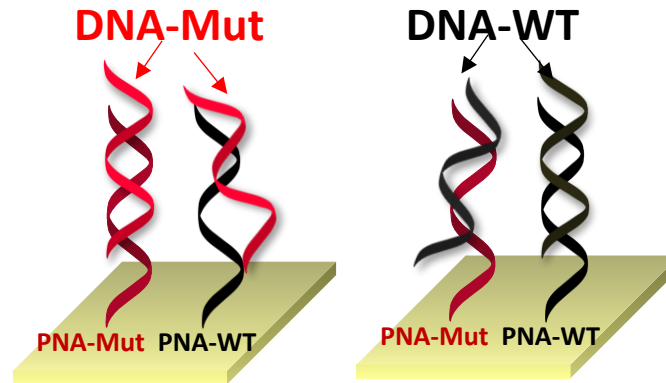
PNA probe



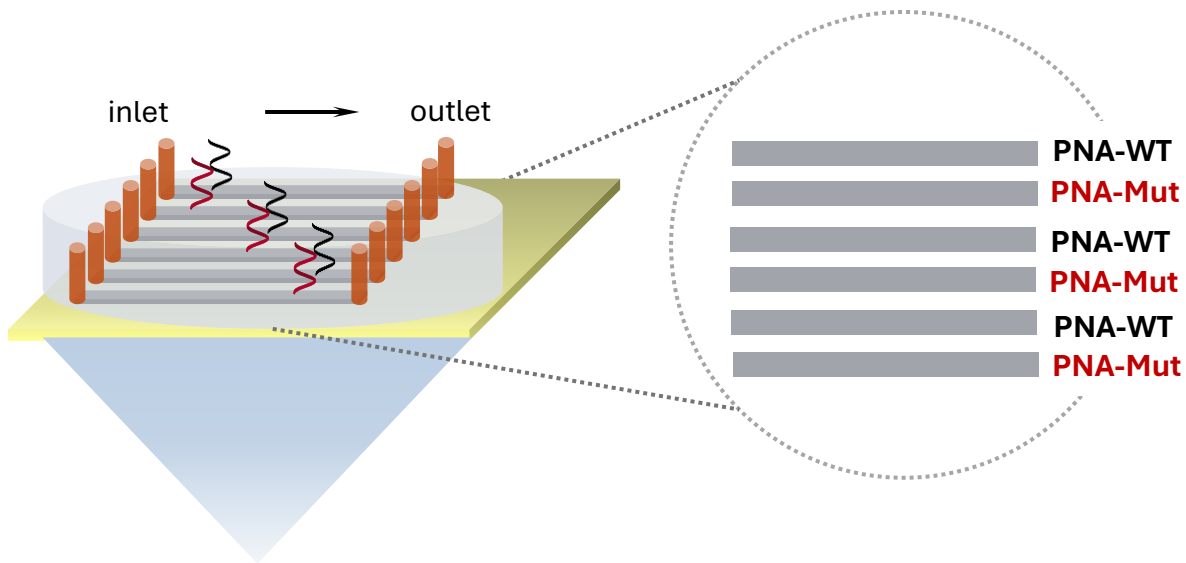
DNA probe

PNA probe

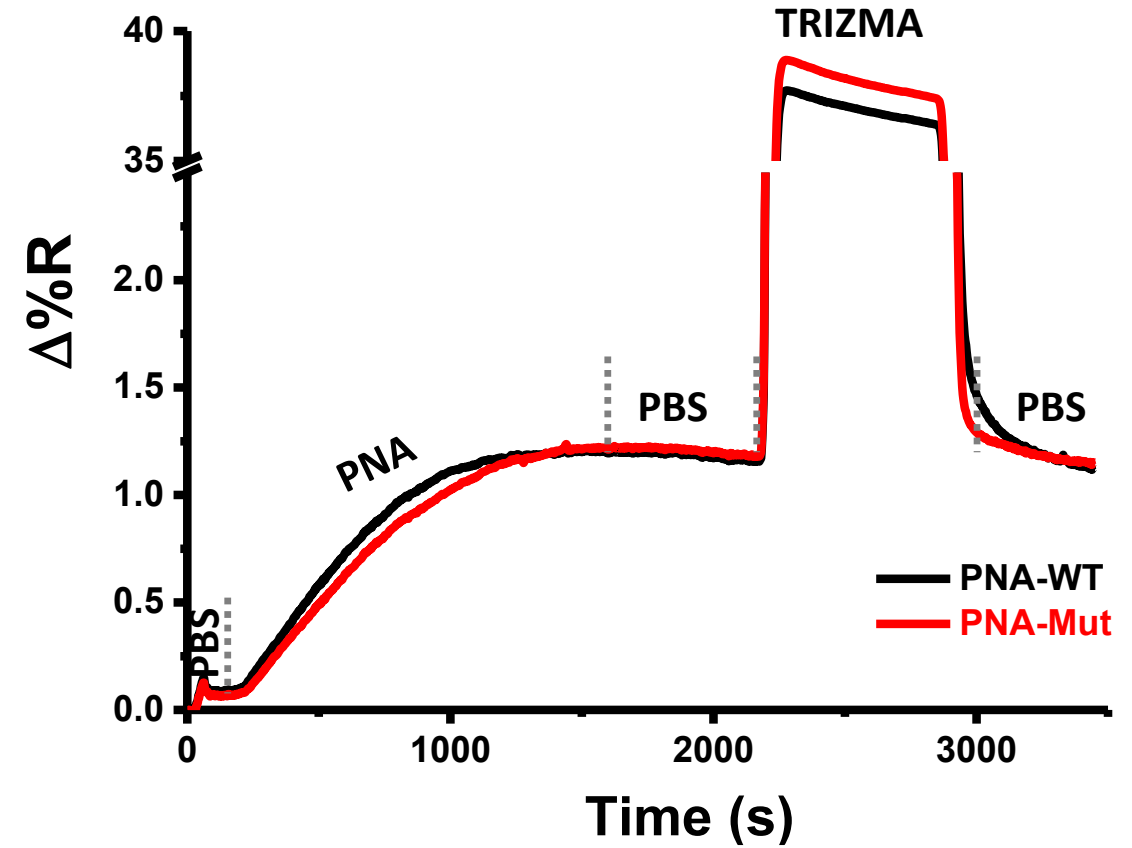
Microfluidic device



Multi-analytes detection



SPRI binding curve



Collected plasma sample

Capturing DNA sequences

Incubation/Isolation

Normal (WT)
Mutated (p.G13D)
(p.G12A)
(p.G12V)
(p.G12R)

KRAS gene related to colorectal cancer

Step 1

Step 2

Dyna@MIX

PNA immobilization probes on plasmonic sensor

Light source

CCD camera

PNA

PNA-WT

PNA-Mut

Dyna@MIX-DNA

Plasmonic SNP detection

Injection into SPR flow system

PNA-WT PNA-Mut

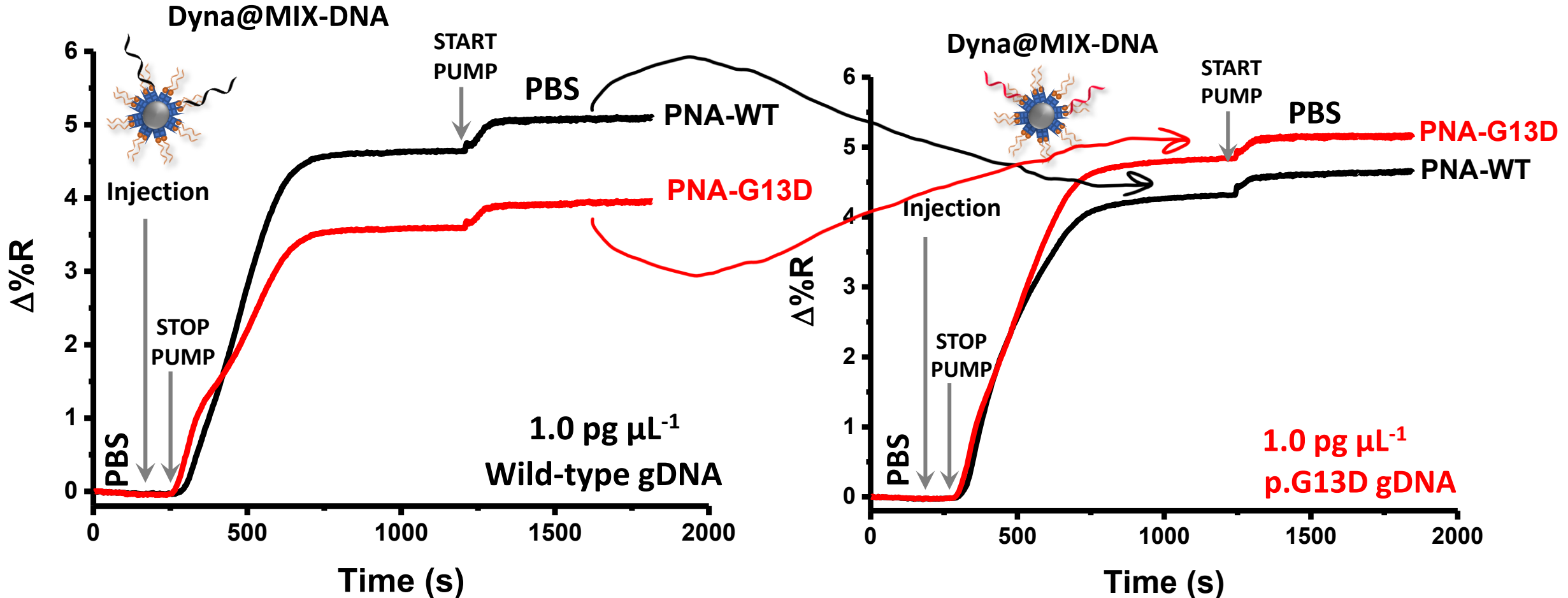
Superparamagnetic bead- based SPR sensor

Genomic DNA sequences at $1 \text{ pg } \mu\text{L}^{-1}$ ($< 300 \text{ copies mL}^{-1}$)
from HT29 (wild-type, WT) and LoVo (G13D KRAS mutation, p.G13 D) cell lines

Colorectal cancer diagnosis



20 μL sample volume



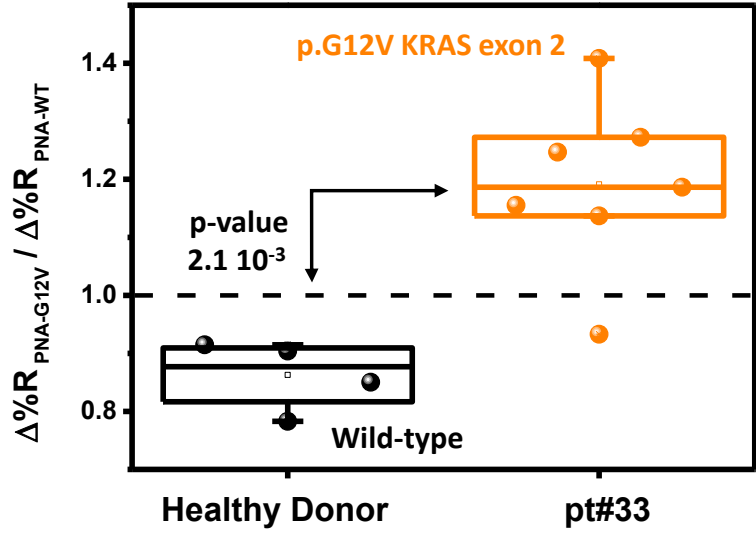
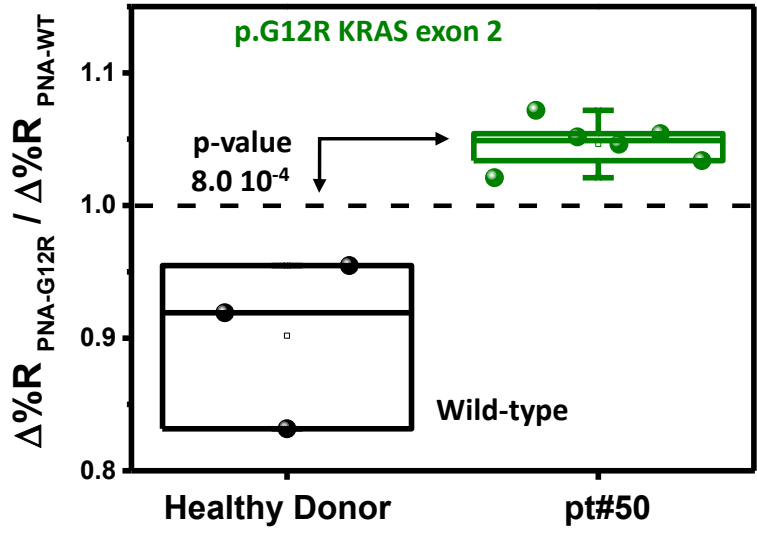
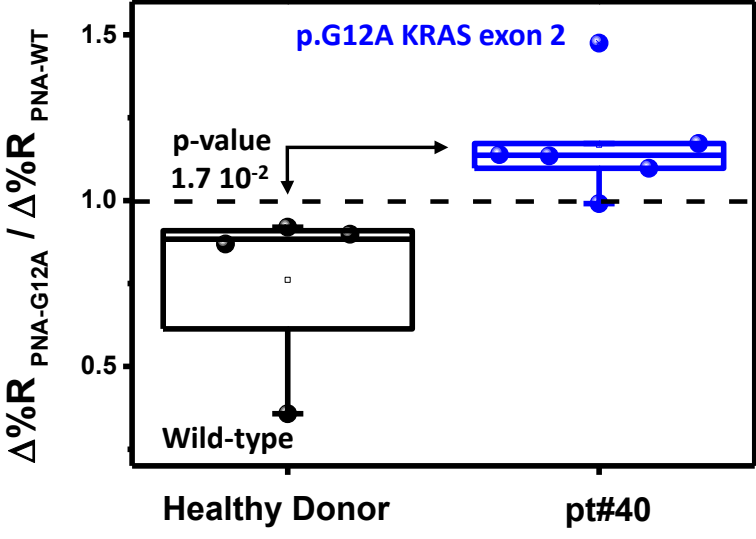
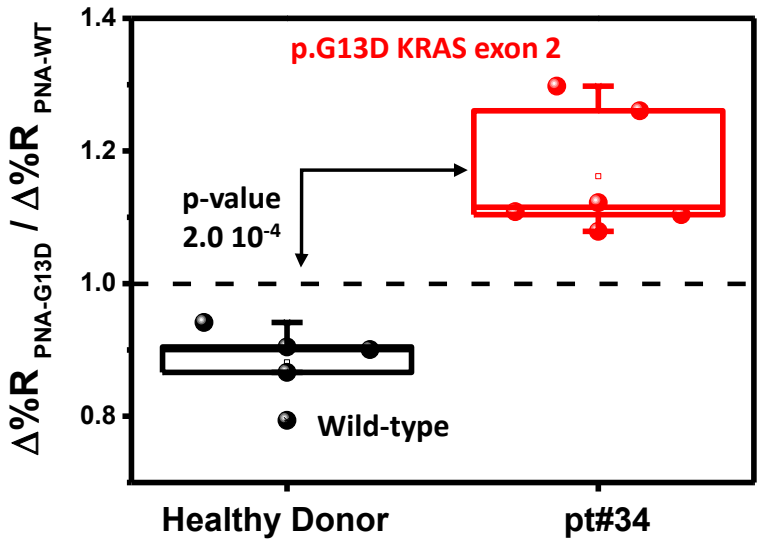
Liquid biopsy

Sensitivity

~0.5 aM

Sample volume

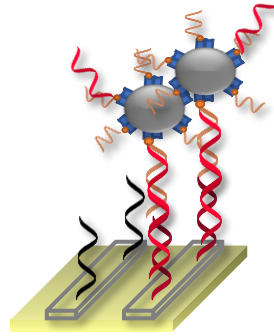
20 μ L



Summary

Colorectal cancer diagnosis

Superparamagnetic bead-based SPR sensor



Sensitivity
Sample treatments
Sample volume
Analysis time
Multiplexed analysis
Signal interference
Workflow analysis

0.5 aM
NO
20 μ L
1 hr 40 min
Up to 6 channels
NO
2

Future applications :

- Polyfunctional beads for multi-targeted analysis
- Clamps

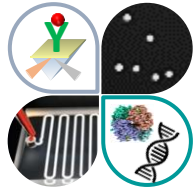
Acknowledgments



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Eruption on 14th August 2023