From biomarkers to biosensors: an unexpected journey

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ABSTRACT

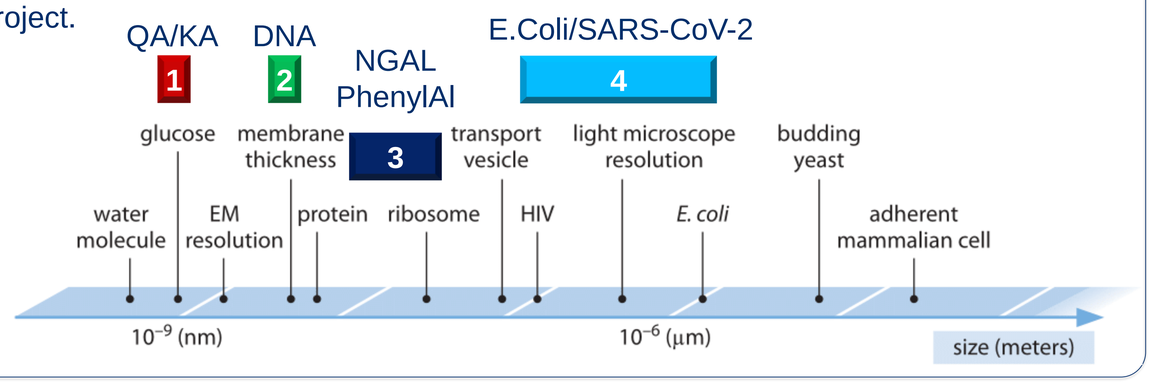
The identification of biomarkers of a disease or a pathological condition is the base for the development of any biosensor technology, either for its diagnosis or monitoring. Nevertheless, the journey between these two points is not really well-known and often its true complexity results unexpected.

Beside presenting the advances in the development of several biosensing strategies toward preliminary biomarkers, we show here the advantages and disadvantages of different technologies in terms of versatility for biomarkers changes. Furthermore, we give insights about the needs to successfully and rapidly develop specific and sensitive biosensors for Point-of-Care (PoC) testing for clinical applications.

ACLF BIOMARKERS

MICROB-PREDICT did not come to an official list of biomarkers yet. Therefore, we selected some molecules correlated with the pathology in literature (preliminary biomarkers) and a set of molecules of broader interest representing different classes. The aim of the latter being to cover most of the possible analyte types and to obtain biosensing platforms to be adapted to the biomarkers which will be defined in the next phases of the project.

Preliminary biomarkers:	**Analyte class**
• Quinolinic and Kynurenic acids (QA/KA)	small molecules
• Neutrophil Gelatinase-Associated Lipocalin (NGAL)	protein
Additional analytes:	**Analyte class**
• Phenylalanine	aminoacid
• SARS-CoV-2 virus	whole virus
• E.Coli	whole bacteria/DNA



FROM BIOMARKERS TO BIOSENSORS

Biosening technologies are conserved for analytes belonging to the same class (DNA, proteins, small molecules, etc.)

Optimization with putative analytes allows to save time in the development of biosensors for unknown biomarkers

Each new analyte needs whole new characterizations of the respective biosensor:

- New bioreceptors (commercially available? To be developed?)
- Different sample characteristics (Interference? Purification steps?)
- Stability/shelf-life
- Detection performance (LOD, sensitivity, linearity)

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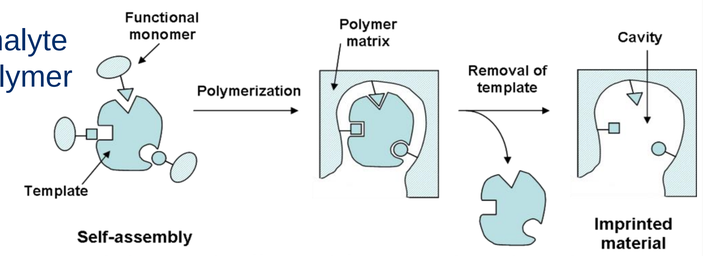
MIP-BASED BIOSENSORS

Biosensing mechanism: The analyte is used to template to shape a polymer and then removed. The polymer becomes selective toward the template.

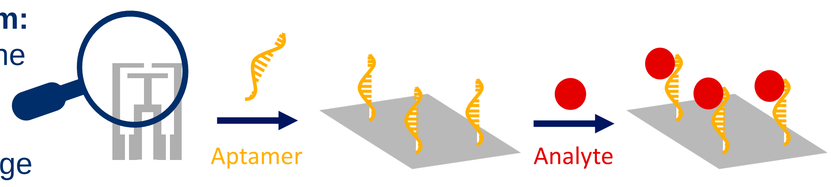


Pros/Cons: no need of a bioreceptor and theoretically stable over time but the polymer-template mixture and the polymerization conditions are long and difficult to optimize. Typically low reproducibility.

Flexibility: The change of the analyte often implies the change of the polymer or of the polymerization conditions. New optimization needed.

APTAMER-BASED BIOSENSORS

Biosensing mechanism: Aptamers selected for the analyte immobilized on the sensor. Binding causes detectable change of the properties.



Electrochemical or optical detection

Pros/Cons: aptamers are cheaper and more stable than antibodies. Standard functionalization protocols available. Sensitive to unspecific binding

Flexibility: Aptamers selection is not trivial and may be difficult for particular molecules.

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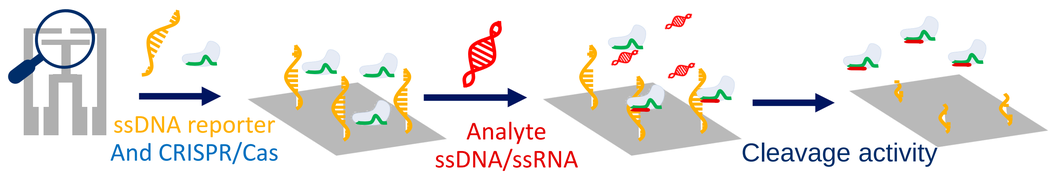
CRISPR-BASED BIOSENSORS



Biosensing mechanism: CRISPR/Cas enzyme activated by gRNA sequence acquire unspecific cleavage activity in presence of the gRNA complementary strand.

ssDNA cutting on sensor cause a detectable change.

Pros/Cons: Biosensor functionalization independent from the analyte.Sensitive to unspecific binding. Amplification needed and laborious.

Flexibility: Very high, gRNA direct the specificity.

ANTIBODY-BASED BIOSENSORS

Biosensing mechanism: Binding of the analyte to the antibody on the sensor produce a change of its properties. The detection can be label-free or labelled.

Pros/Cons: Specific and well-characterized but expensive, and laborious.

Flexibility: New bioreceptor needed for a new analyte.

CONCLUSIONS

Each biosensing technology grants different advantages and is characterized by specific limitations. Even if the development of a proof-of-concept biosensing platform for a preliminary analyte gives a useful base for eventual adaptation to another analyte belonging to the same class, new optimizations will be required, with a different amount of effort depending on the developed technology type.

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