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# SUSNANO SPRING SCHOOL

# DNA-Based Nanosensors for Environmental Applications

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ICN2 Institut Català de Nanociència i Nanotecnologia
EXCELENCIA SEVERO OCHOA

17-18/04/2024
ICN2, UAB Campus, Bellaterra, Spain

NANO Bioelectronics & Biosensors Group

MARIE CURIE ALUMNI

# DNA Nanotechnology


Design and manufacture of artificial nucleic acid structures for technological uses

DNA is used as engineering material
NOT as a carrier of the genetic information!


## Synthetic DNA!

## *Why DNA as a material?*

- Easy to synthesize (low cost)
- Easy to engineer and to attach different molecules
- Biocompatible
- Easy to predict the structure (predictable base-pairing (A-T, G-C))


Srisomwat C.

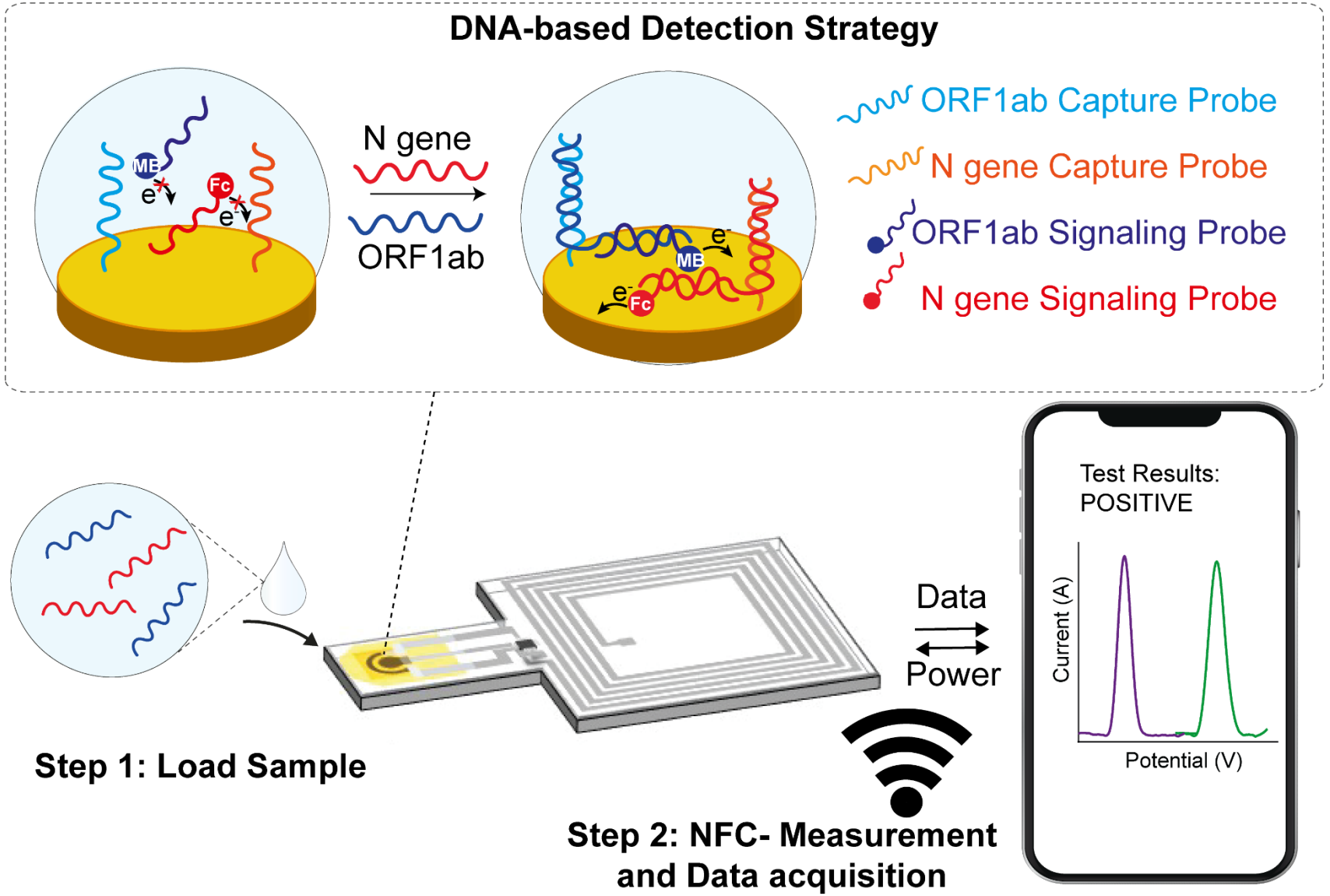

Urban M.


Maroli G.


Rosati G.

# Detection of Pathogens (bacteria, fungi, virus)






Rossetti M. *et al*, (2024) **Biosens Bioelectron.**, 250: 116079 doi.org/10.1016/j.bios.2024.116079

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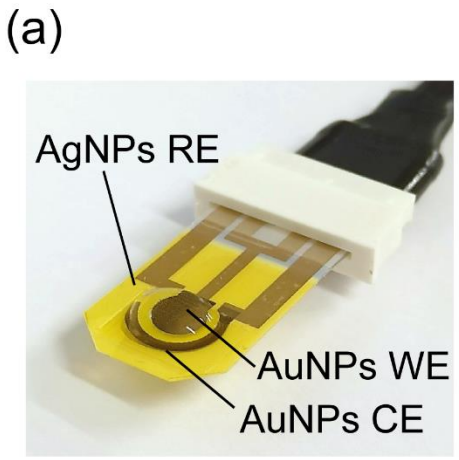
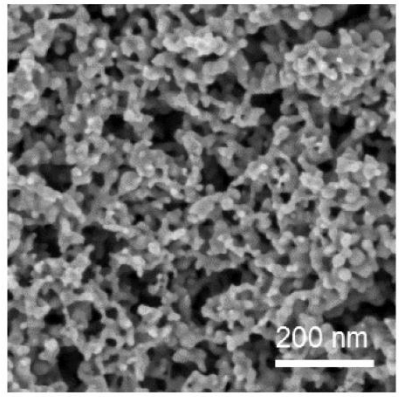
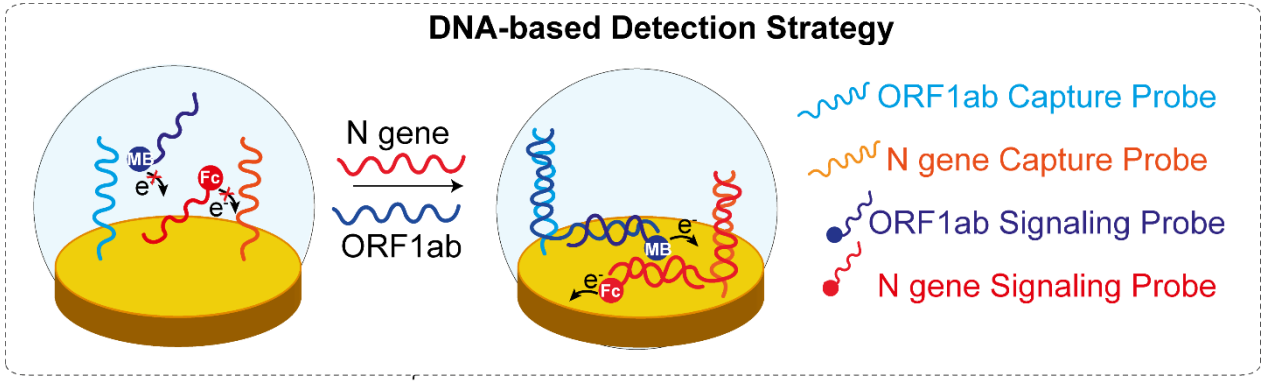
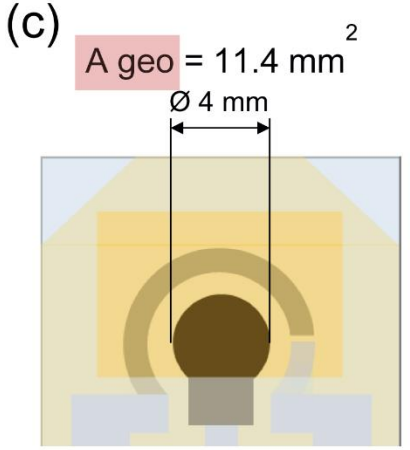
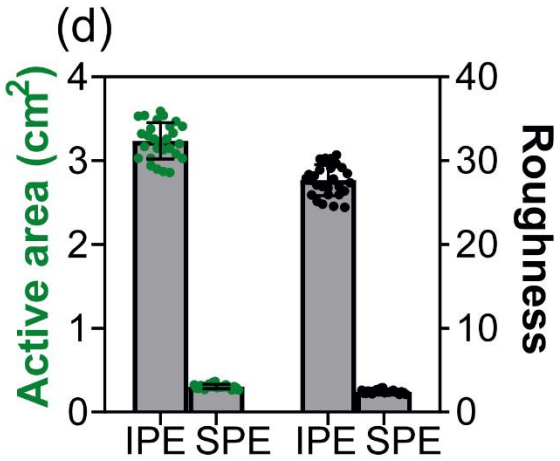
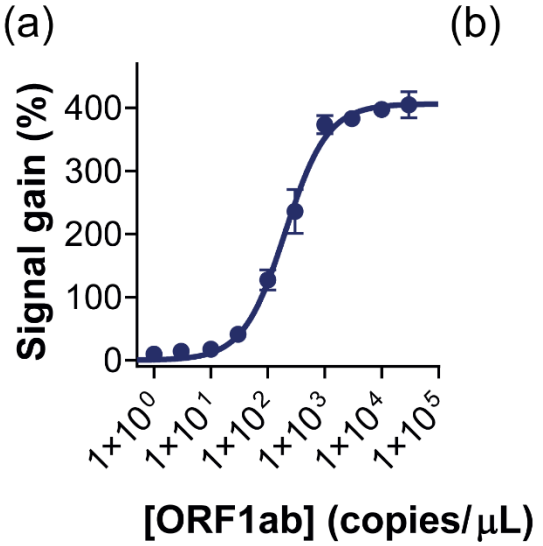


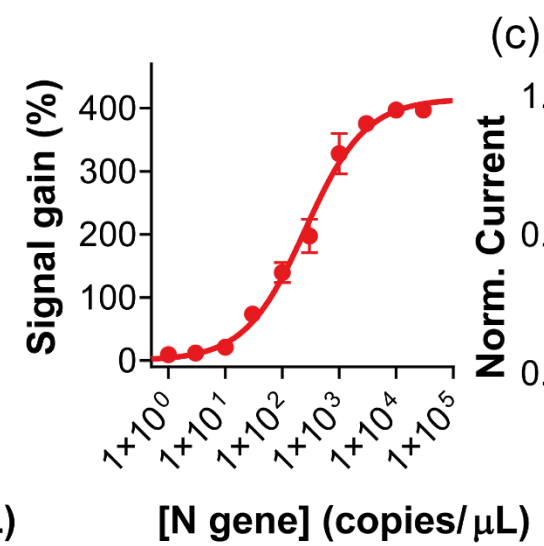



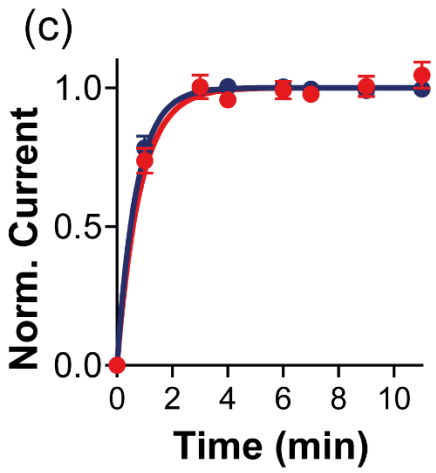



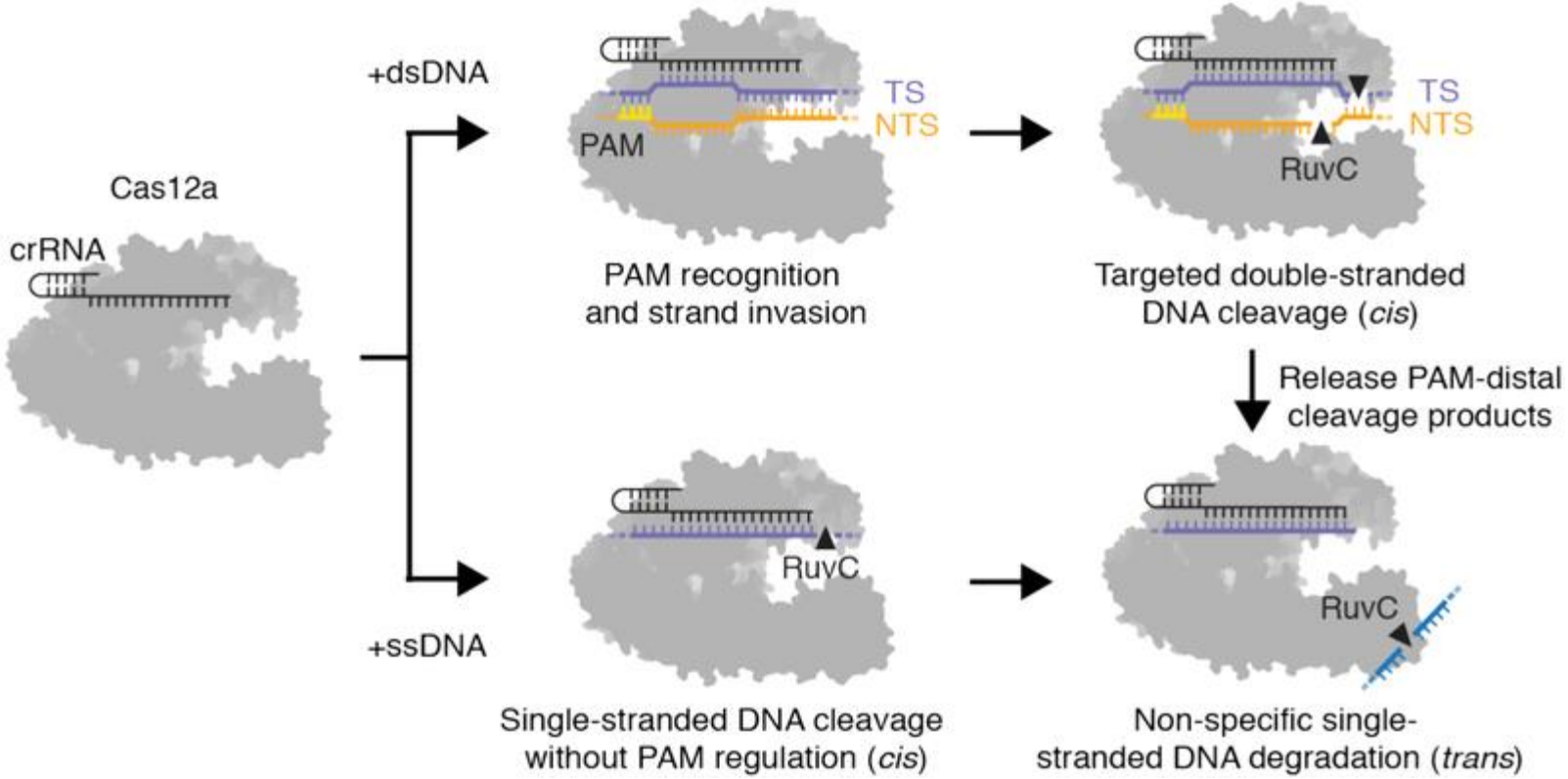



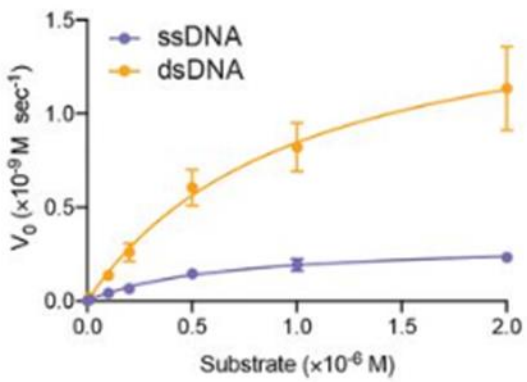

Rossetti M. *et al*, (2024) **Biosens Bioelectron.**, 250: 116079 doi.org/10.1016/j.bios.2024.116079

# Detection of Pathogens (bacteria, fungi, virus)
# CRISPR-Cas technology






| Activator | $k_{cat}/K_m$ (sec$^{-1}$ M$^{-1}$) |
|---|---|
| ssDNA | 5.00 ± 0.57 × 10$^{6}$ |
| dsDNA | 1.71 ± 0.17 × 10$^{7}$ |

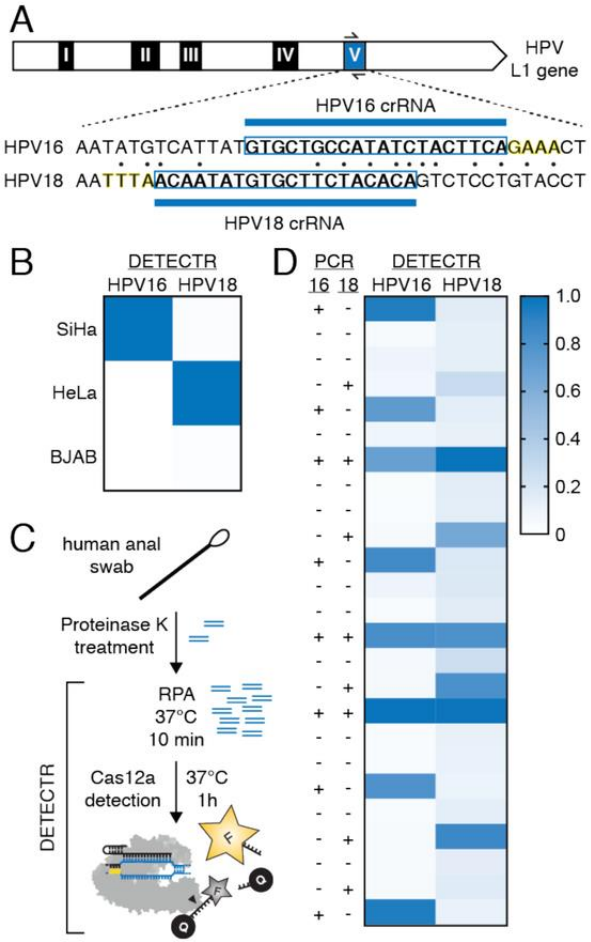



Chen J.S. *et al*, (2018) **Science**, 360, 436

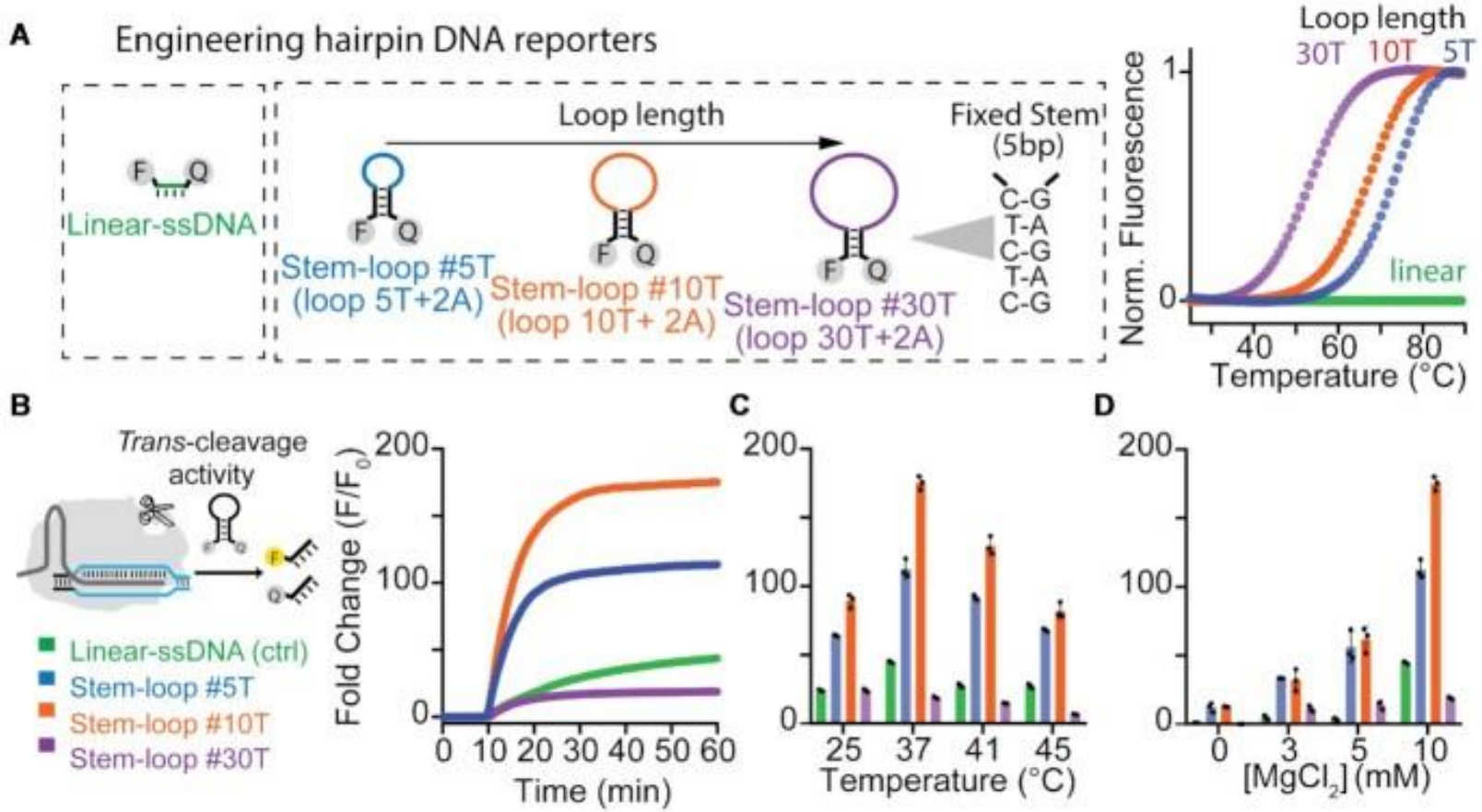
# Detection of Pathogens (bacteria, fungi, virus)
# CRISPR-Cas technology



DNA endonuclease-targeted CRISPR trans reporter




Chen J.S. *et al*, (2018) **Science**, 360, 436




Rossetti M. *et al*, (2022) **Nucleic Acids Res.**, 50, 8377

TOR VERGATA
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LABORATORY OF SENSING AND BIOMIMETIC SYSTEMS

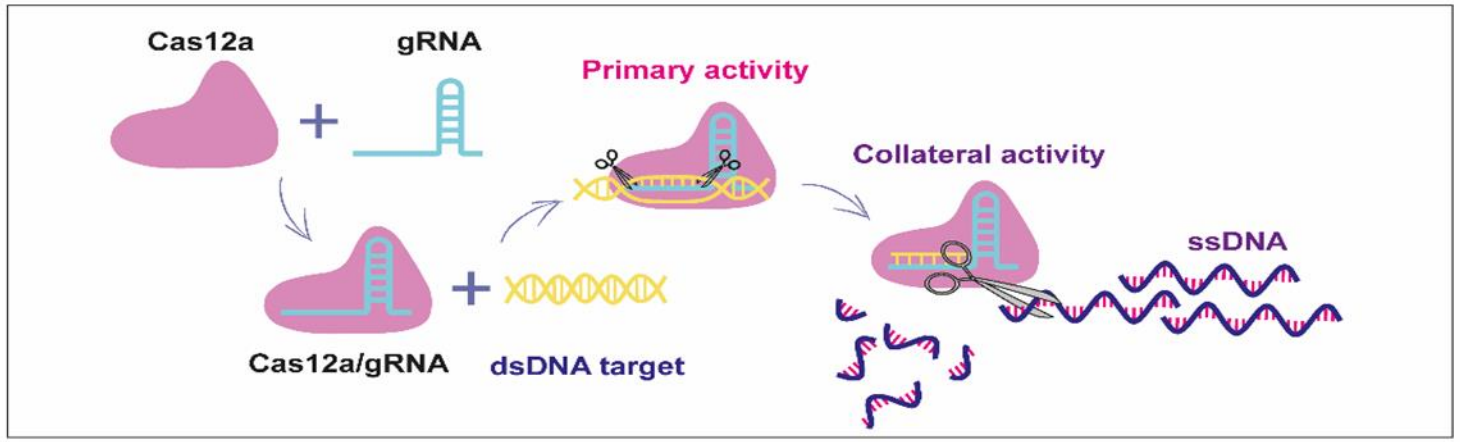
Prof. Porchetta A.

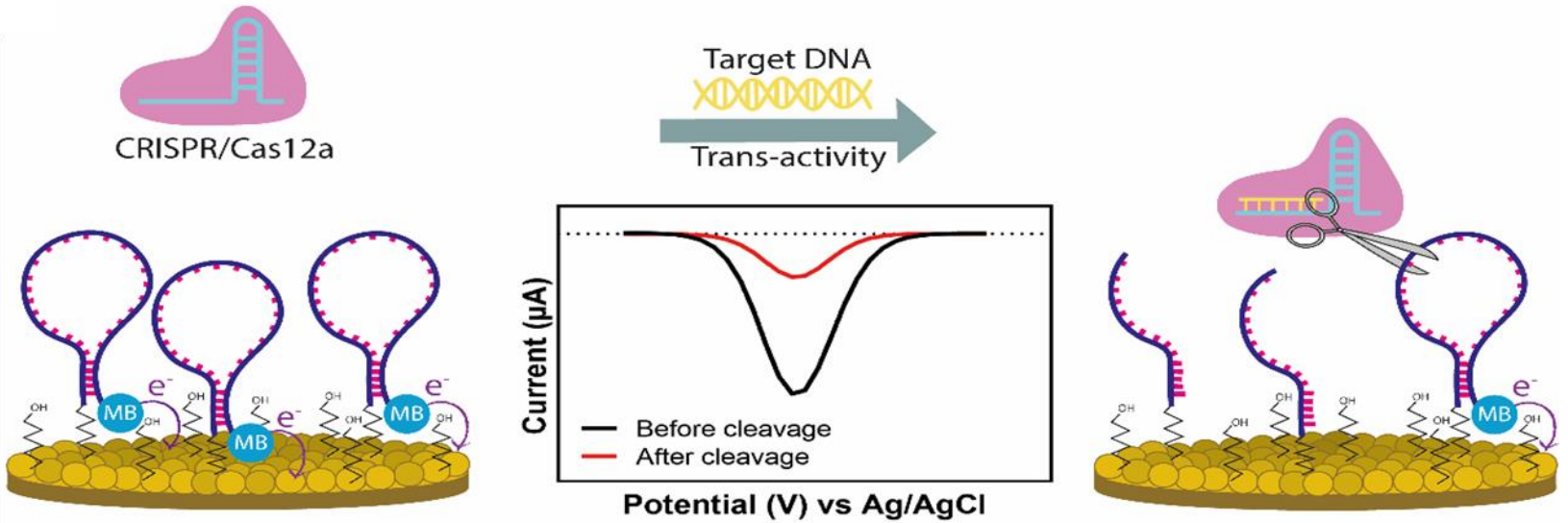
# Detection of Pathogens (bacteria, fungi, virus)
# CRISPR-Cas technology



Angela Gilda

*Escherichia coli*, *Staphylococcus aureus*




Gilda A.C. *et al*, (2024) **Biosens Bioelectron.**, under revision

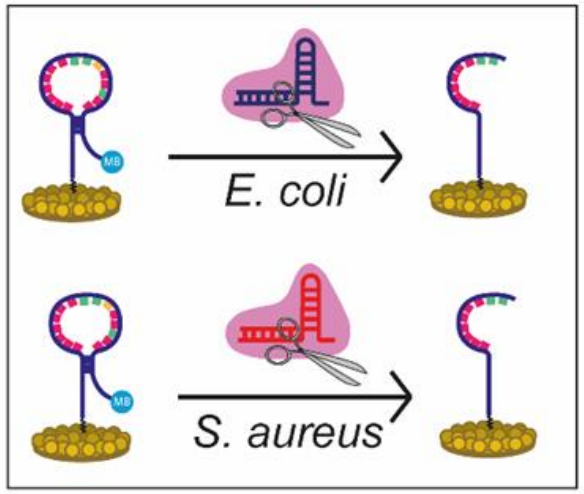
# Detection of Pathogens (bacteria, fungi, virus)
# CRISPR-Cas technology

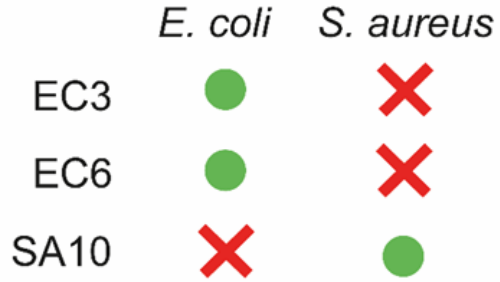
Angela Gilda

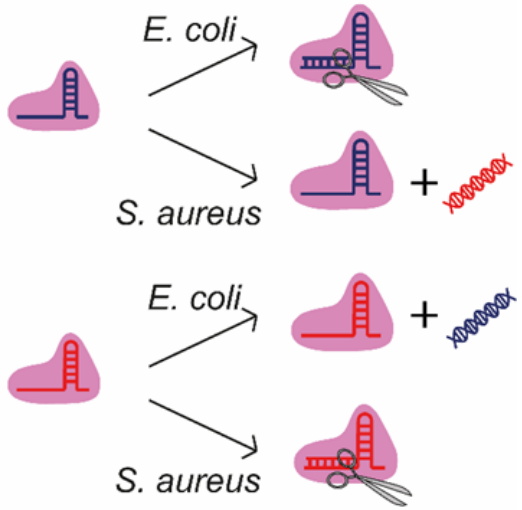
Angela Gilda

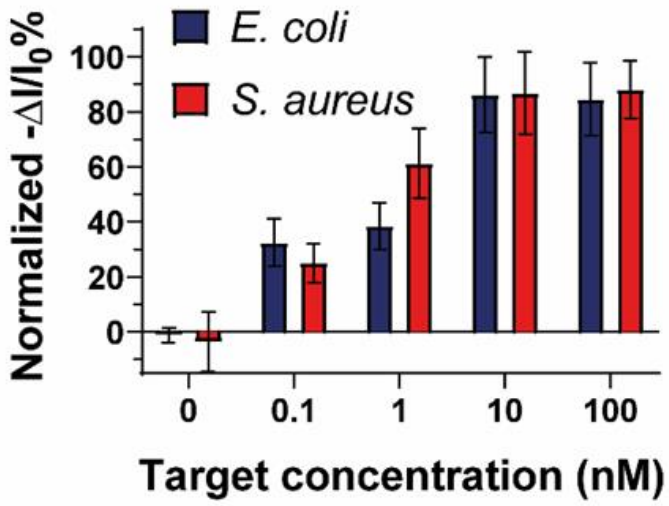


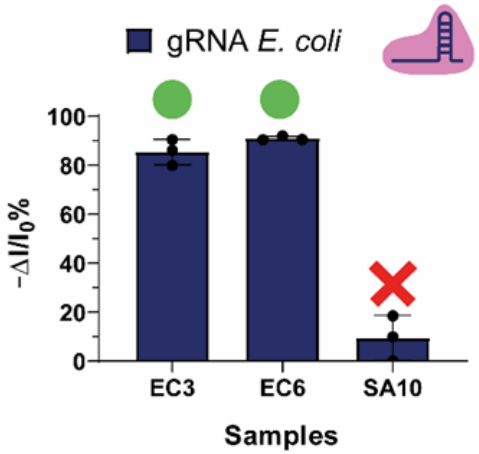
*Escherichia coli*, *Staphylococcus aureus*

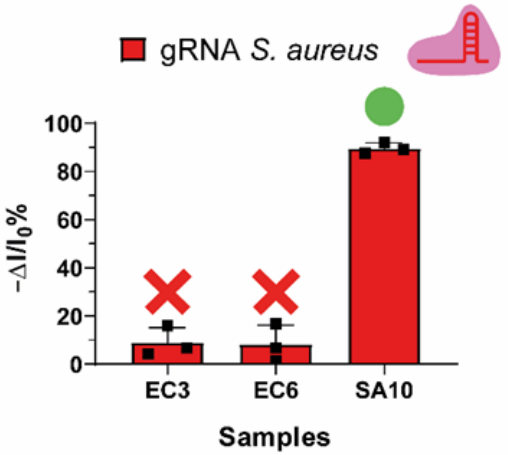


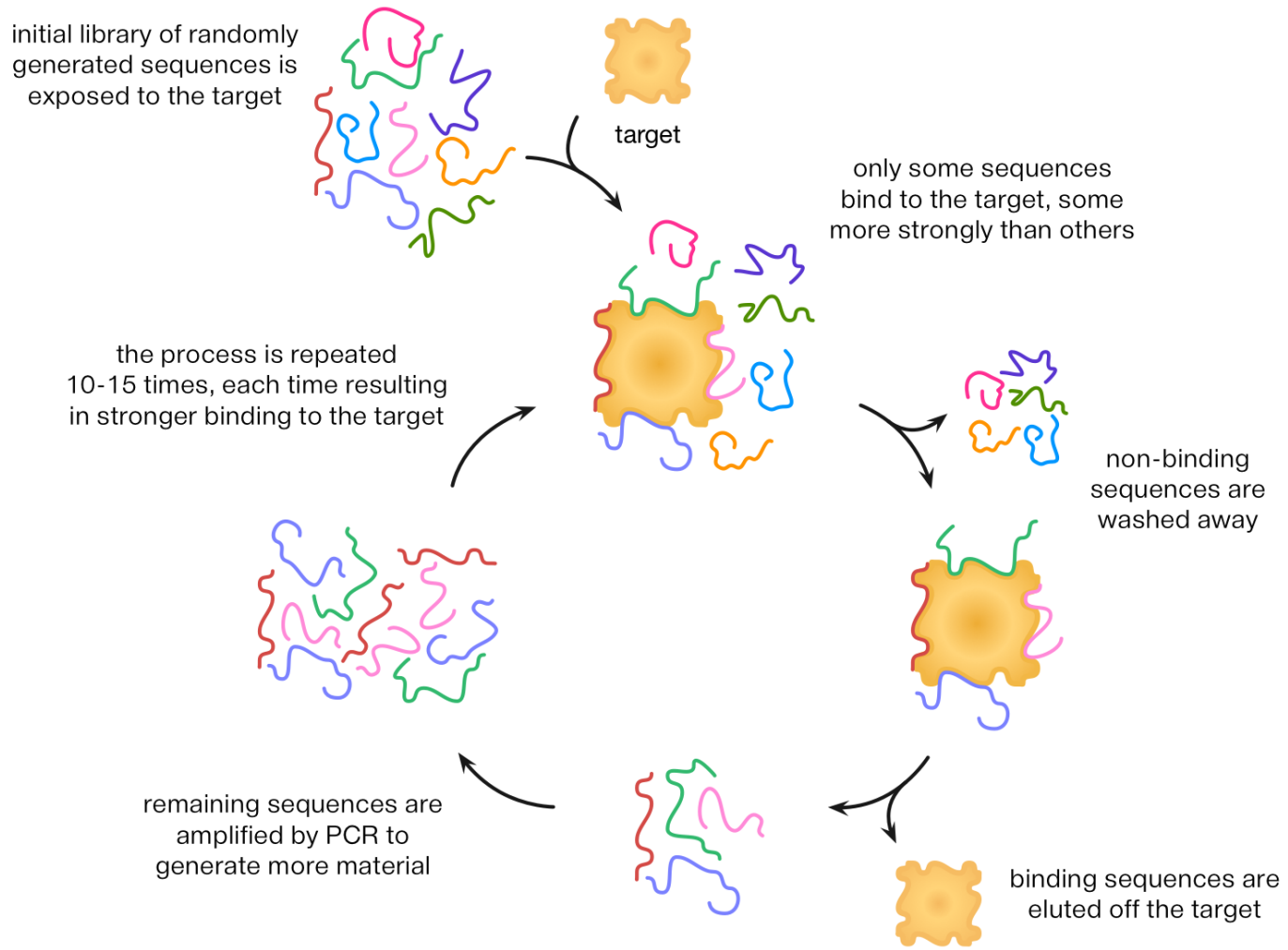




Gilda A.C. *et al*, (2024) **Biosens Bioelectron.**, under revision

# Detection of antibiotics, pesticides, metals



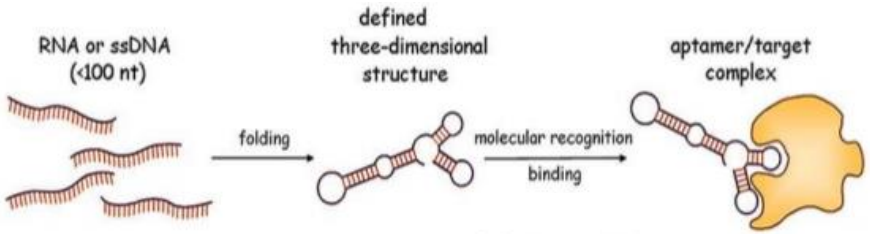
Systematic Evolution of Ligands by Exponential Enrichment (SELEX)



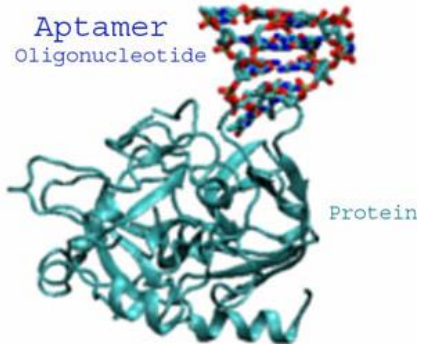

Ellington A.D., Szostak J.W. (1990) **Nature**, 346, 818
Tuerk C., Gold L. (1990) **Science**, 249, 505

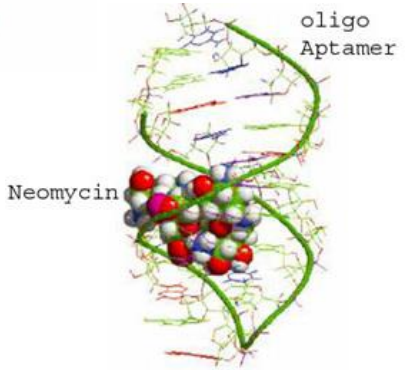
# Aptamers






| | Aptamers | Antibodies |
|---|---|---|
| Molecular weight | Small (12-30 kDa) | Relatively big (150-180 kDa) |
| Production Time | Few hours to months | Several months (6 months) |
| Batches variations | Low | High |
| Targets | Wide range of targets | Immunogenic molecules |
| Shelf life | Long | Short |
| Allowed Chemical modification | Various modifications | Limited modifications |
| Nuclease degradation | Sensitive | Resistant |
| Stability | Very stable | Sensitive to T and pH changes |
| Cost | Lower | Higher |

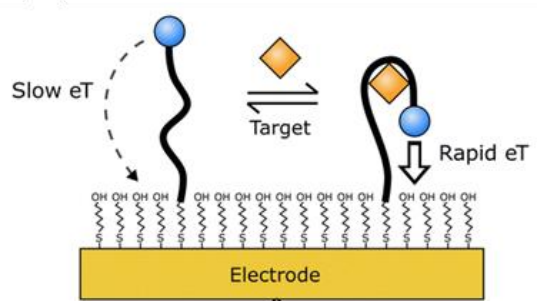


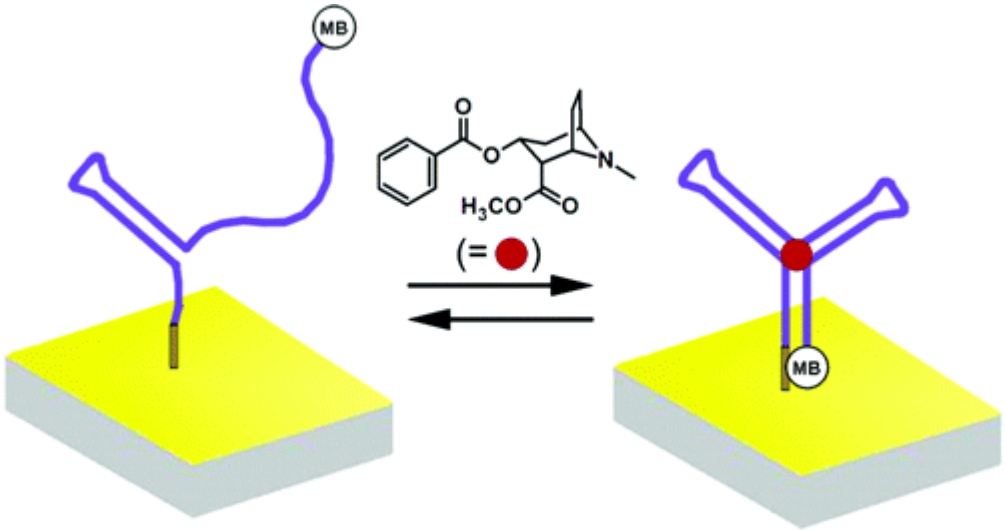

Cho E.J., Lee J.W., and Ellington A.D. (2009) Annual Review of Analytical Chemistry, 2, 241

# Electrochemical Aptamer-based biosensors (E-AB sensors)

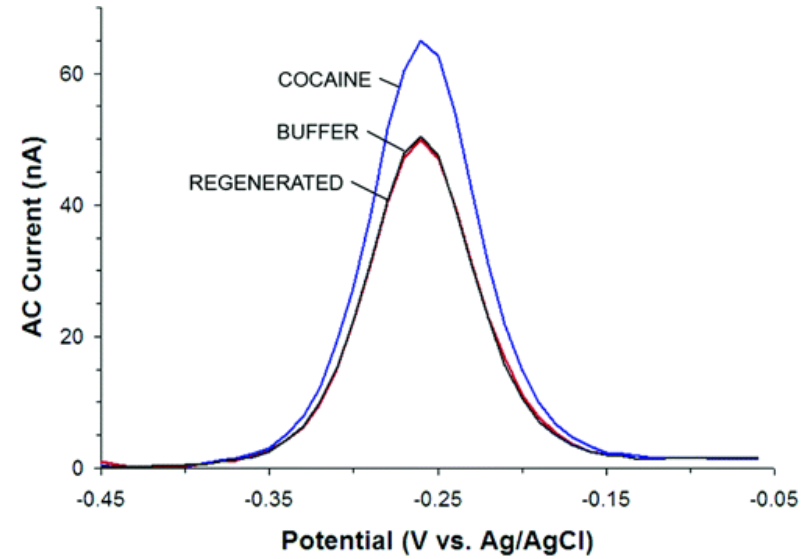


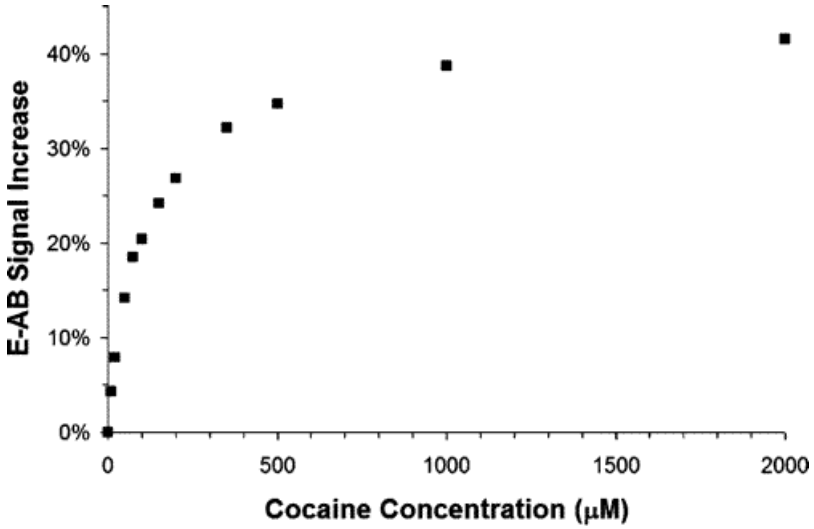



Verrinder E. *et al.* (2024) ***Sens. Diagn.*** 3, 95




Monitoring of the folding into a triple-stem conformation of the cocaine-responsive aptamer upon binding to cocaine

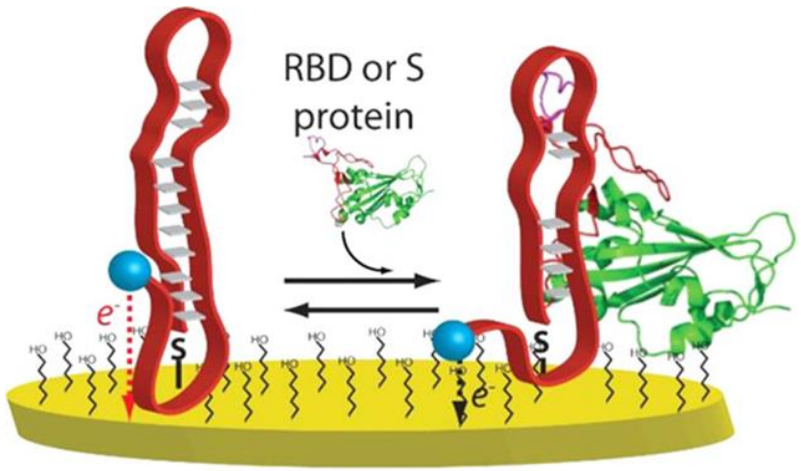


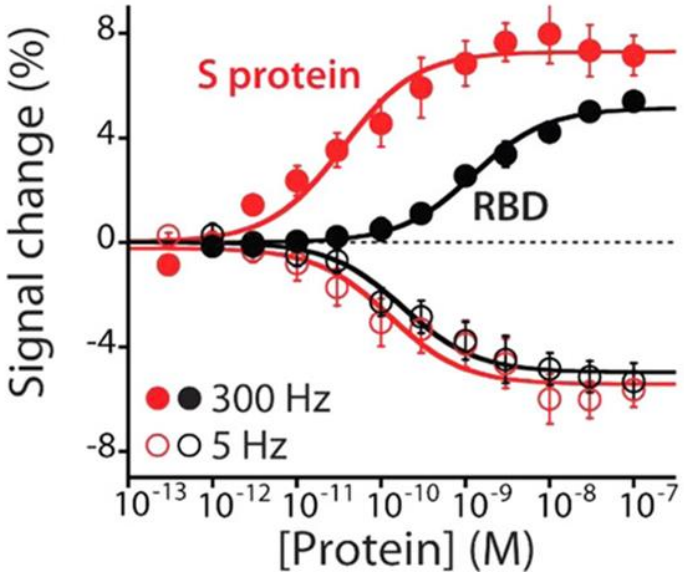



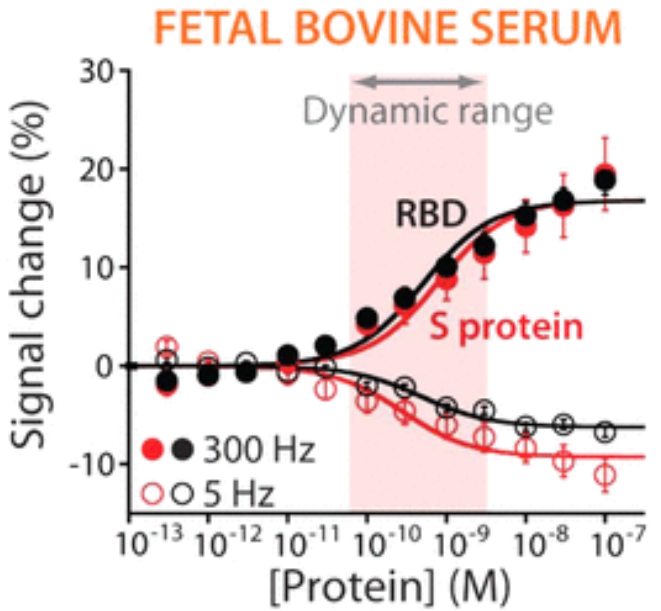

Baker B.R. *et al.* (2006) **J. Am. Chem. Soc.**, 128, 10, 3138–3139

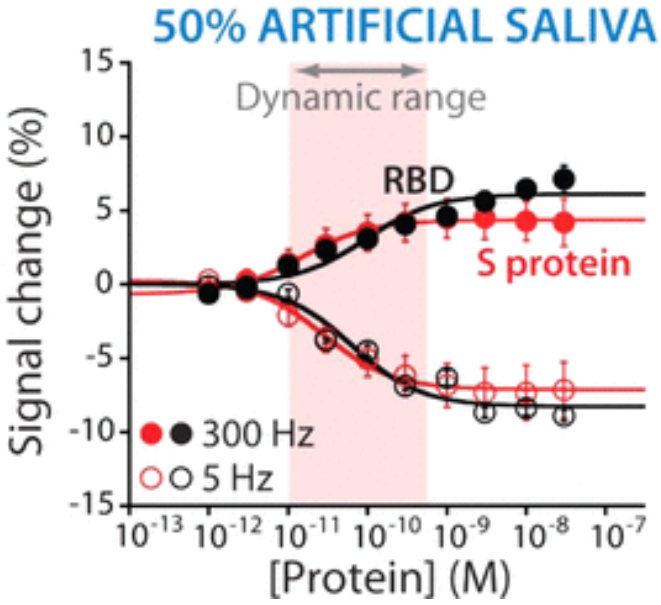
# Electrochemical Aptamer-based biosensors (E-AB sensors)



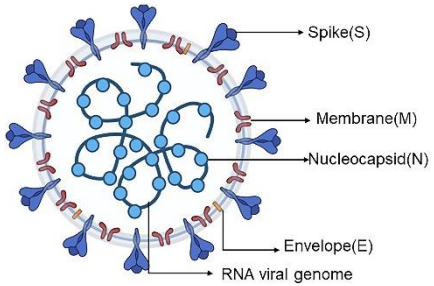


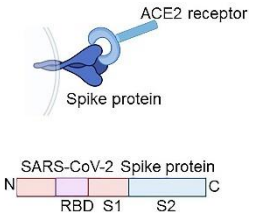




Andrea Idili



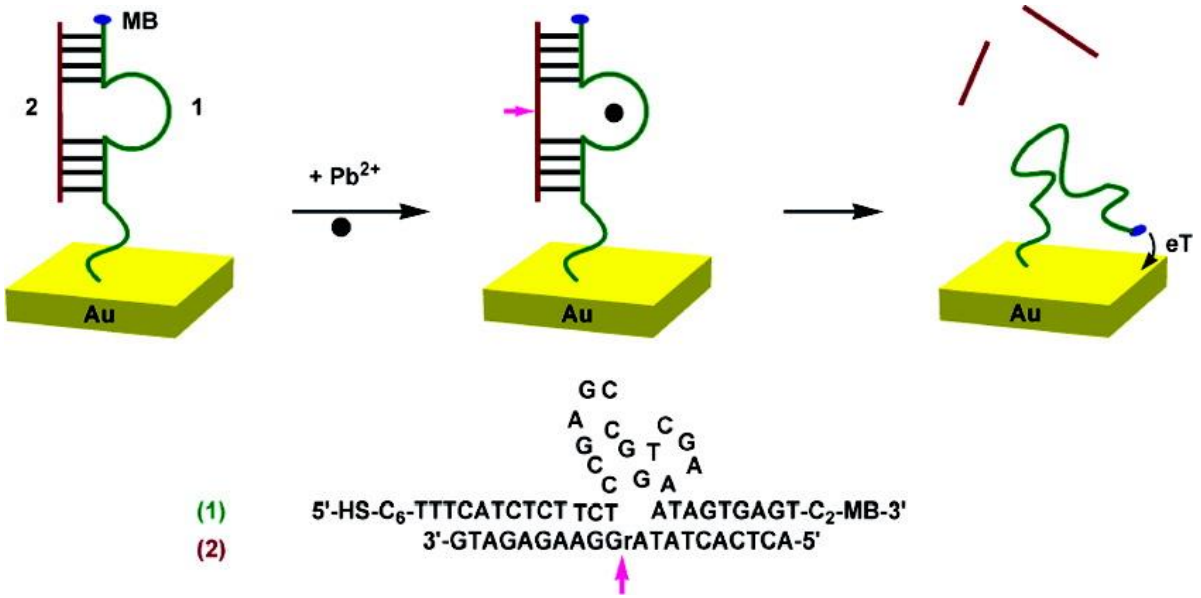



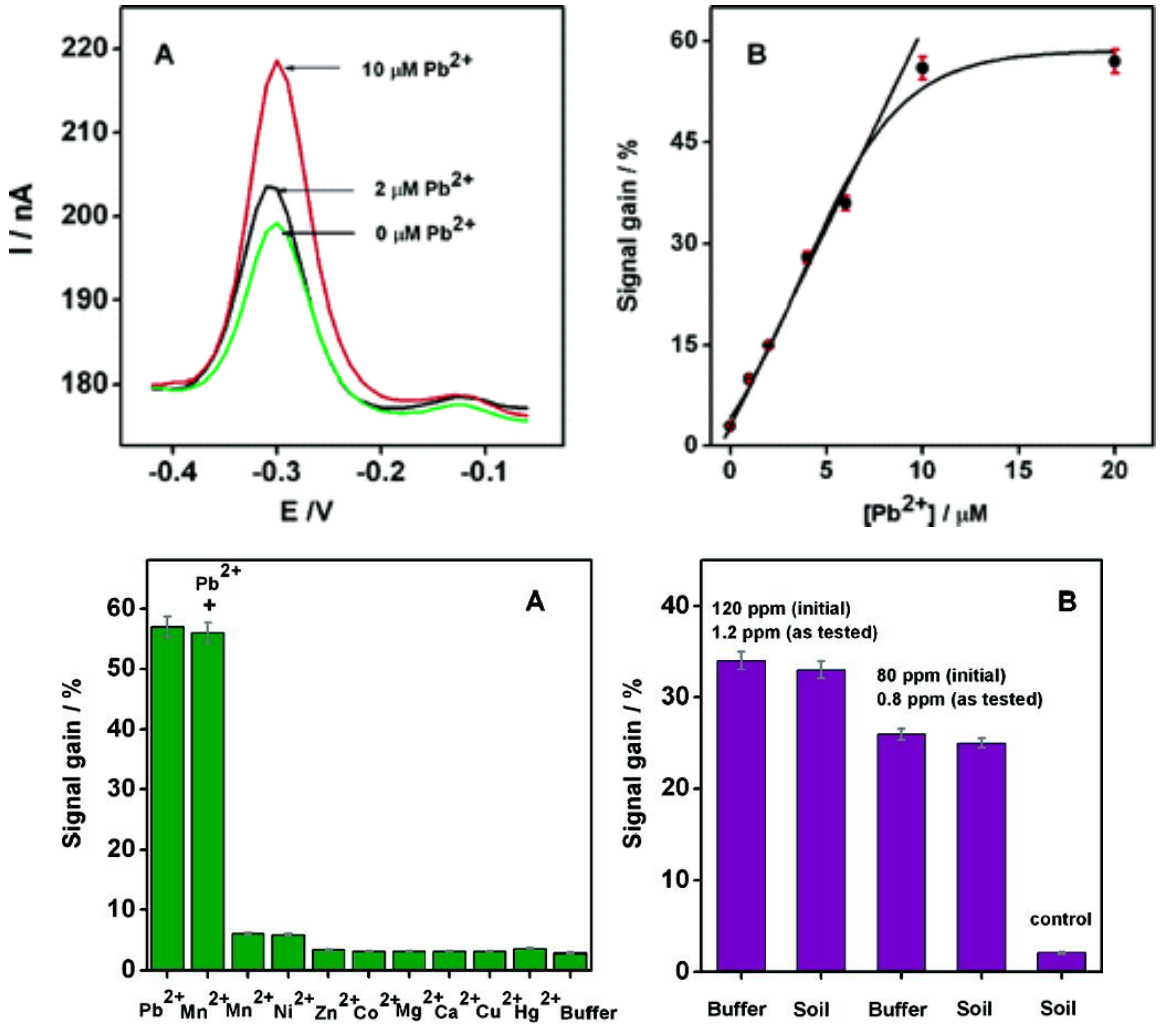

RBD= Receptor Binding Domain

Idili A. *et al.* (2021) **ACS Sens.** 6, 8, 3093

# Electrochemical DNAzyme-based biosensors






Xiao Y. *et al.* (2007) **J. Am. Chem. Soc.** 129, 2, 262

# Conclusions



- We can rationally design DNA-based platforms for the sensitive and selective detection of pathogens, antibiotics, pesticides, metals, etc.
- Different sensing strategies can exploit different output signals.
- We believe that in the near future it will be possible to translate such analytical assays into real and commercially available sensing platforms.

ICN2 Institut Català de Nanociència i Nanotecnologia

NANO Bioelectronics & Biosensors Group

The ICN2 is funded by the CERCA programme / Generalitat de Catalunya.
The ICN2 is supported by the Severo Ochoa Centres of Excellence programme, Grant CEX2021-001214-S, funded by MCIN/AEI/10.13039.501100011033.

Funded by the European Union

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2D BioPAD

My research activity is currently funded by the European Union's Horizon 2020 research and innovation programme grant agreement N. 101120706 ("2D-BioPAD")

# Thank you for your attention!

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