



UNIVERSITÀ DEGLI STUDI DI NAPOLI  
**FEDERICO II**



***Colloidal Solutions of Gold Nanoparticles as Colorimetric Immunosensors for Fast Detection of SARS-CoV-2***

**Raffaele Velotta, Bartolomeo Della Ventura and Antonio Minopoli**

**Dipartimento di Fisica *Ettore Pancini* – Università di Napoli *Federico II***

# Outline

- Tests in the context of Covid-19 pandemics
- Rapid test technology (most spread): Lateral Flow Assay (LFA)
- Alternative technology (for antigenic test): colorimetric biosensor
- Physical modelling
- Perspectives (saliva and other)



Health workers carry out mass screening using rapid antigen tests at a school in France.

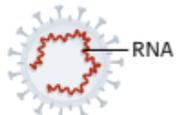
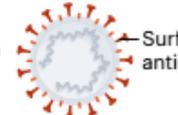
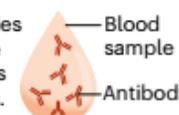
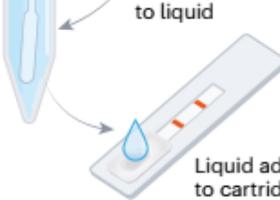
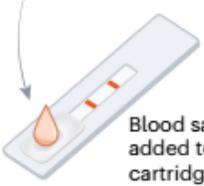
# RAPID CORONAVIRUS TESTS: A GUIDE FOR THE PERPLEXED

Scientists still debate whether millions of cheap, fast diagnostic kits will help control the pandemic. Here's why. **By Giorgia Guglielmi**

TIMOTHY SAUNDERS/INPHOTODISC

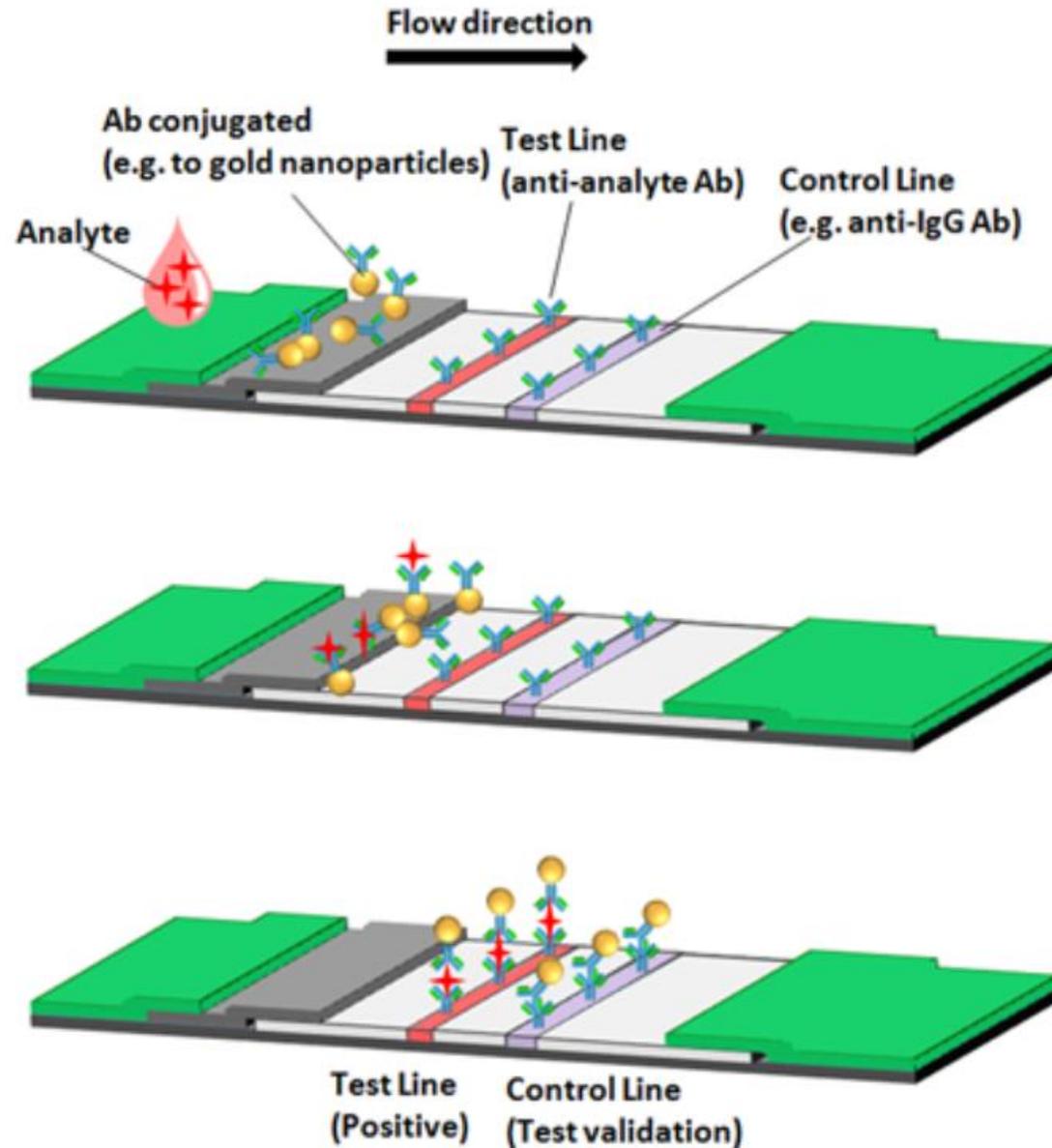
## HOW COVID-19 TESTS WORK

Two kinds of coronavirus test look for viral material. A third examines the immune response to infection.

Nucleic-acid-based test	Antigen test	Antibody test (serological)
<p><b>How it works</b></p>		
<p>Detects viral genetic material.</p>  <p>RNA</p>	<p>Detects proteins on surface of the virus.</p>  <p>Surface antigens</p>	<p>Detects antibodies that the immune system produces against the virus.</p>  <p>Blood sample</p> <p>Antibody</p>
 <p>Nasal or throat swab</p>  <p>RNA extracted and converted to DNA</p>  <p>PCR amplifies DNA using reagents and PCR machines</p> <p>Usually requires a centralized laboratory; some machines can be brought to test sites. Variations include LAMP, CRISPR and sequencing-based tests that amplify and detect DNA in a range of ways.</p>	 <p>Nasal or throat swab</p>  <p>Sample added to liquid</p>  <p>Liquid added to cartridge</p> <p>Point-of-care test that can be done by non-experts.</p>	 <p>Blood sample</p>  <p>Blood sample added to cartridge</p> <p>Point-of-care test that can be done by non-experts.</p>
<p><b>What a test tells you</b></p>		
<p>Whether any viral genetic material is present, even at low levels.</p>	<p>Whether the virus is present in high concentrations. (Whether you are likely to be infectious.)</p>	<p>Whether you are likely to have had the virus. It does not detect an active infection.</p>
<p><b>Time and cost</b></p>		
 <p>Hours/days</p>  <p>\$\$\$</p>	 <p>Minutes</p>  <p>\$</p>	 <p>Minutes</p>  <p>\$</p>
<p><b>General reliability*</b></p>		
<p>Very sensitive and specific.</p>	<p>Misses infections with low virus levels.</p>	<p>Variable, but some tests are very specific.</p>

\*The chance that a test result is a true positive or a true negative depends not only on a test's own reliability, but also on background rates of infection, and on whether a person shows symptoms.

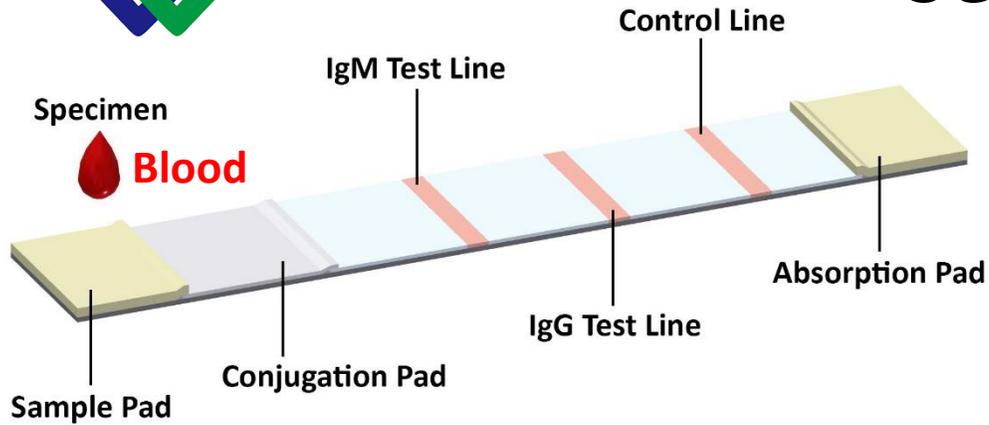
# Lateral Flow Assay



Koczula, K. M. & Gallotta, A.

Lateral flow assays. *Essays Biochem.* **60**, 111–120 (2016).

# LFA for COVID-19 (serological and antigenic)



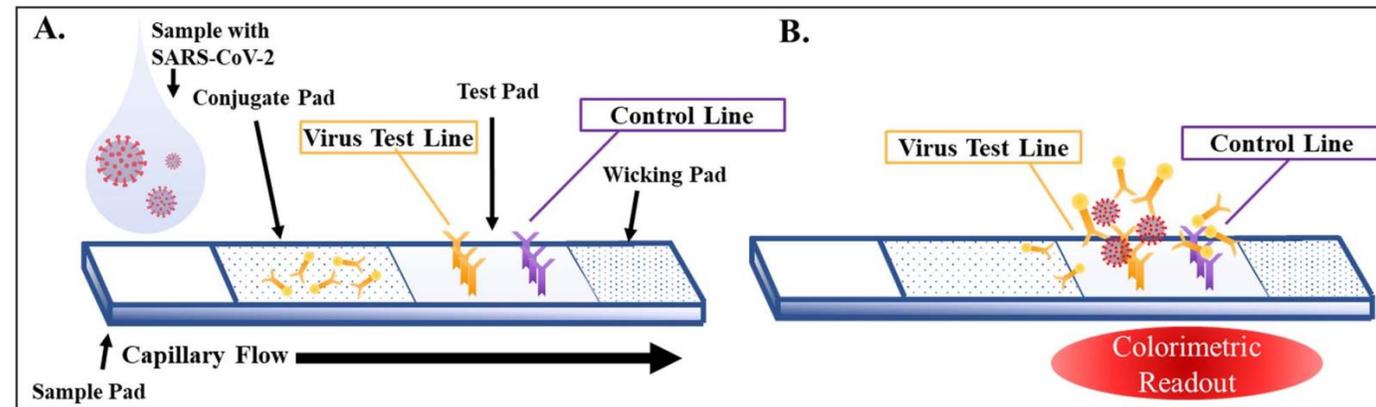
Serological test

**LFA**

😊 *Easiness of use*

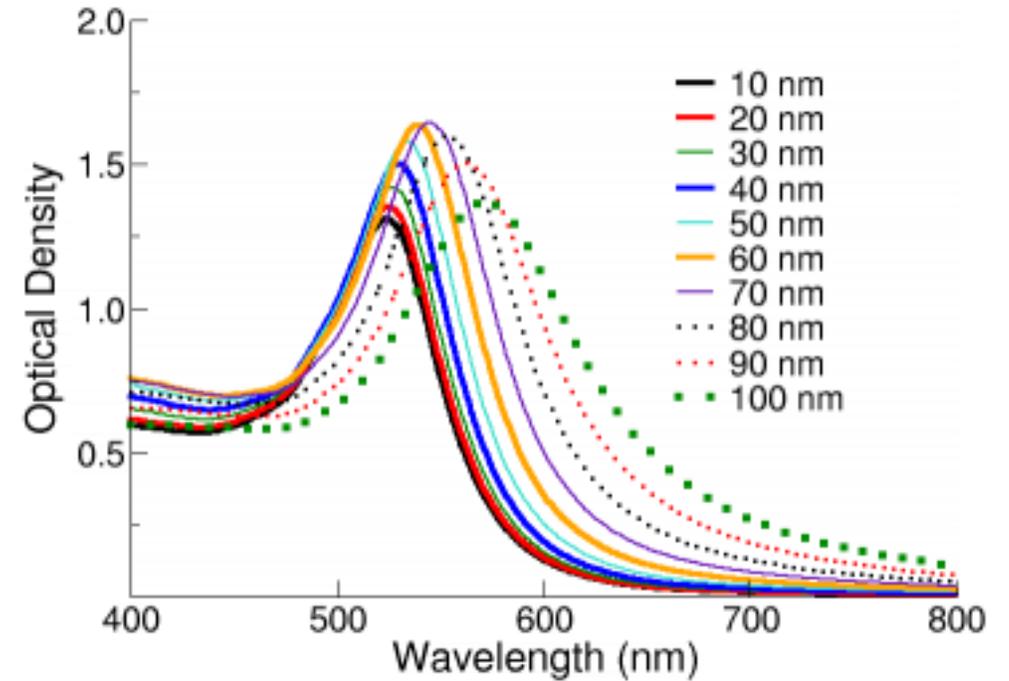
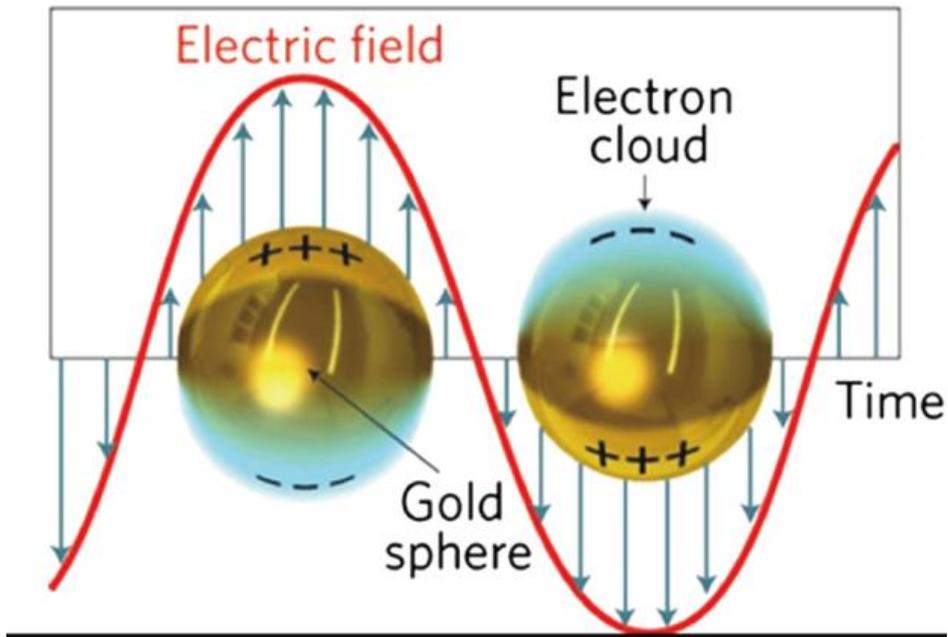
😞 *Sensitivity*

Antigenic test



Mahmoudinobar, F., Britton, D. & Montclare, J. K. Protein-based lateral flow assays for COVID-19 detection. *Protein Eng. Des. Sel.* **34**, 1–10 (2021).

# Localized Surface Plasmon Resonance

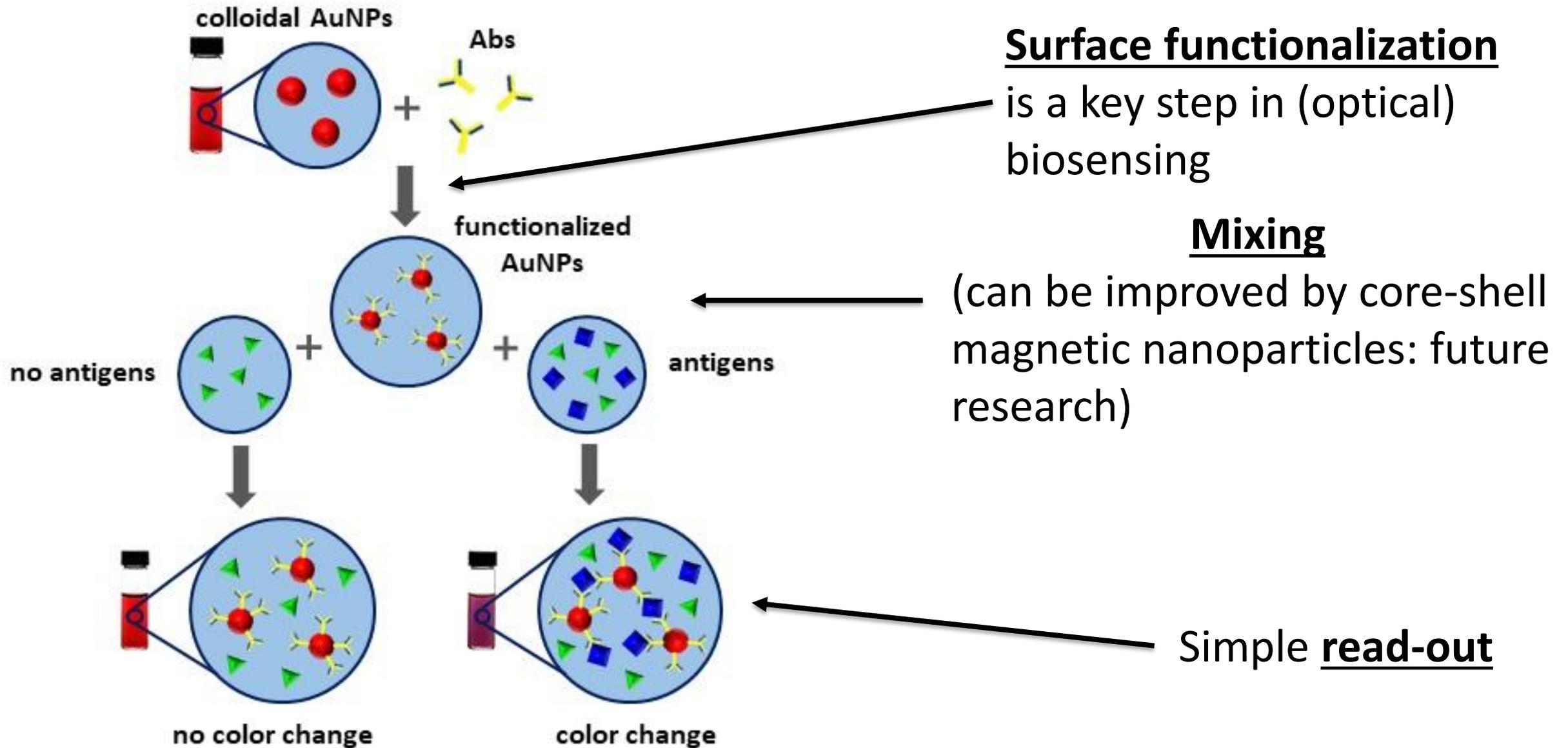


FOR SUB-WAVELENGTH PARTICLES, THE ABSORPTION INTENSITY DOMINATES OVER THE SCATTERING EFFICIENCY.

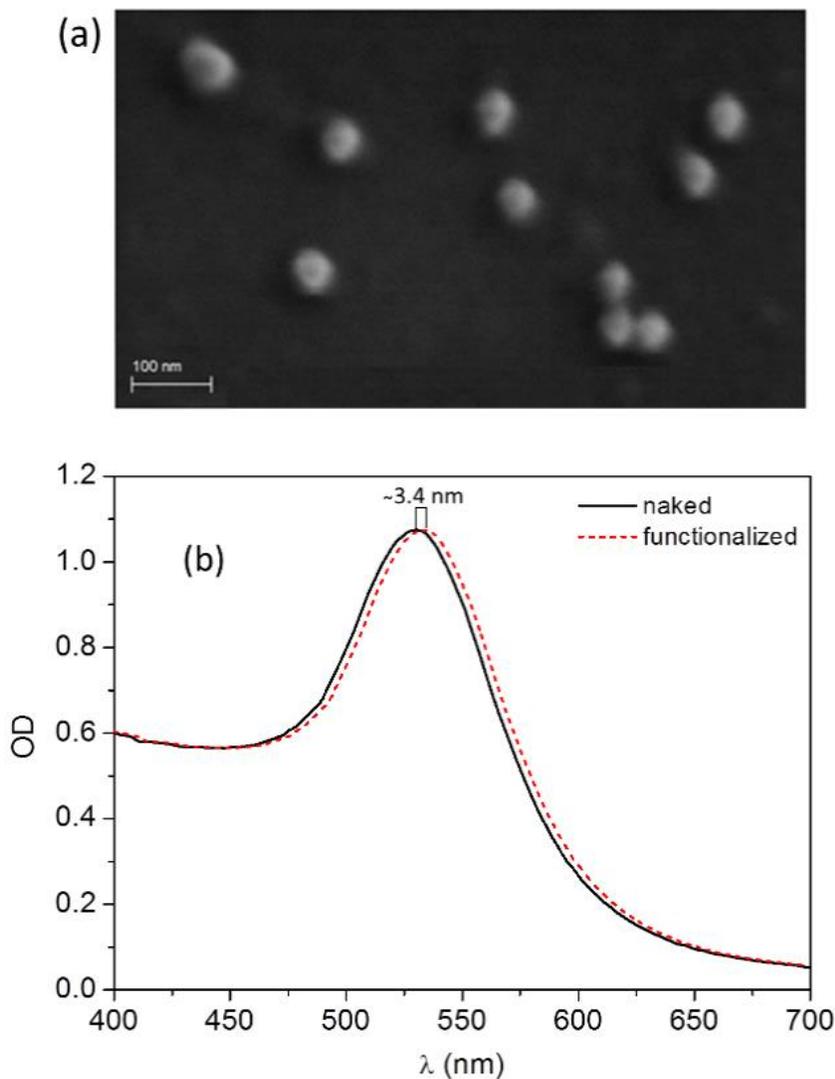


AuNPs size →

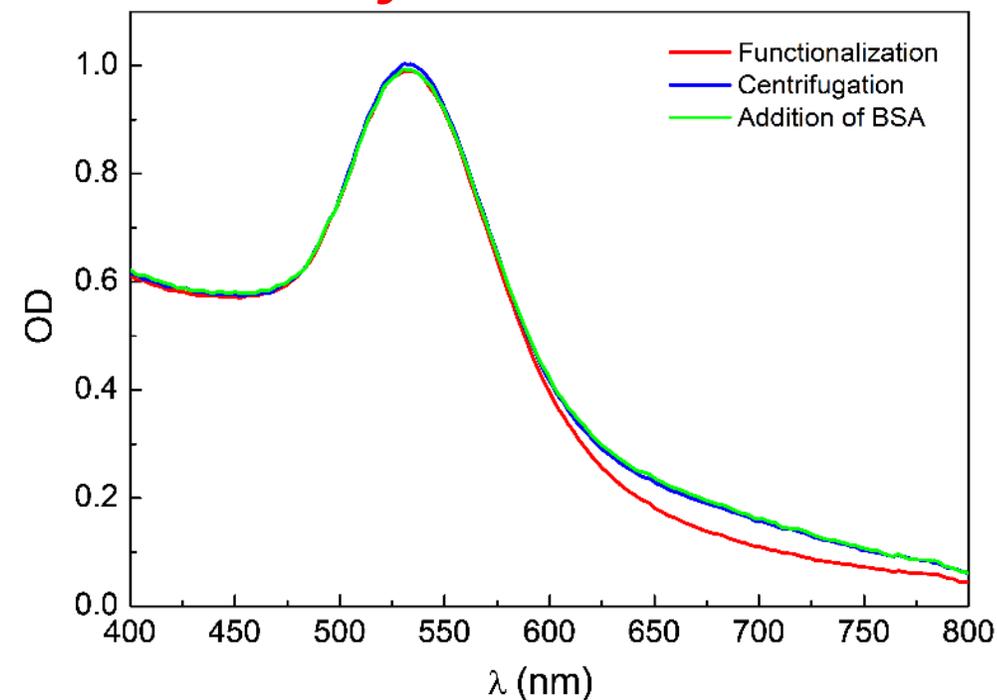
# Colorimetric biosensing by LSPR



# Gold NP functionalization



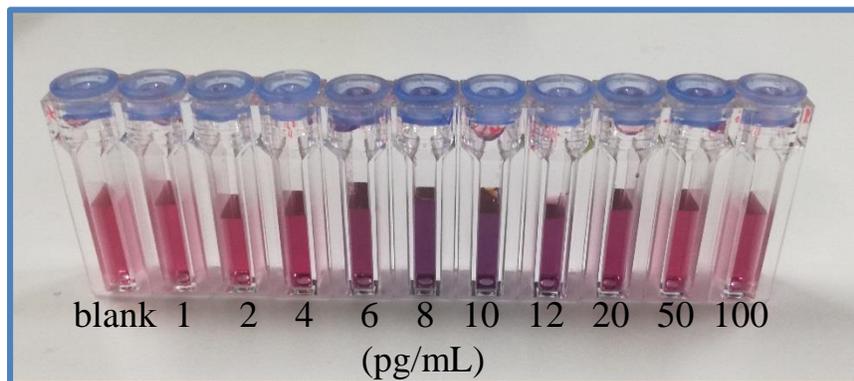
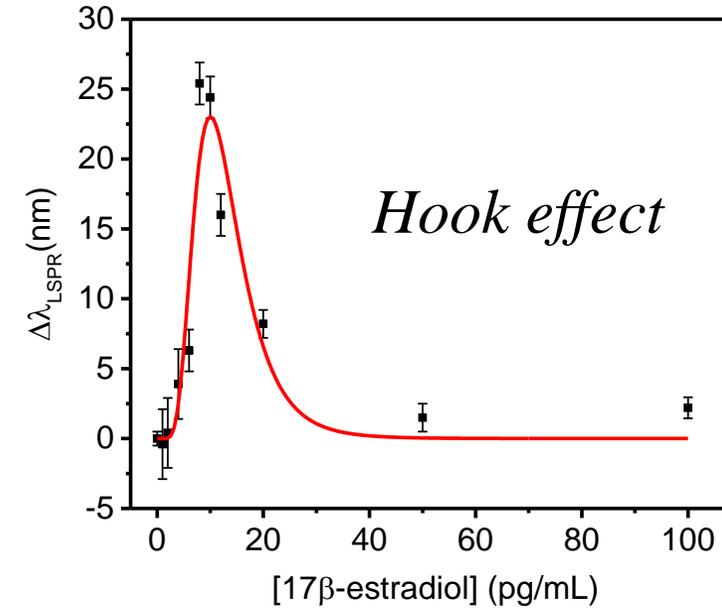
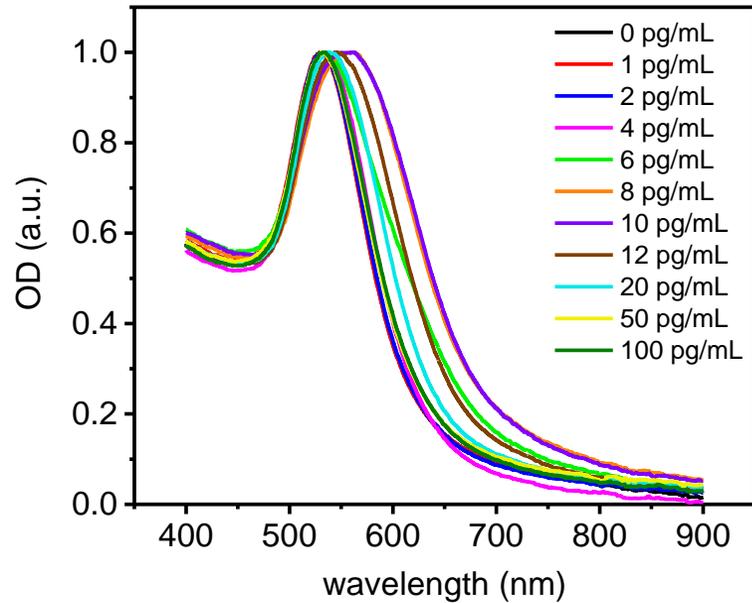
## Robust functionalization



## Colorimetric Immunosensor by Aggregation of Photochemically Functionalized Gold Nanoparticles

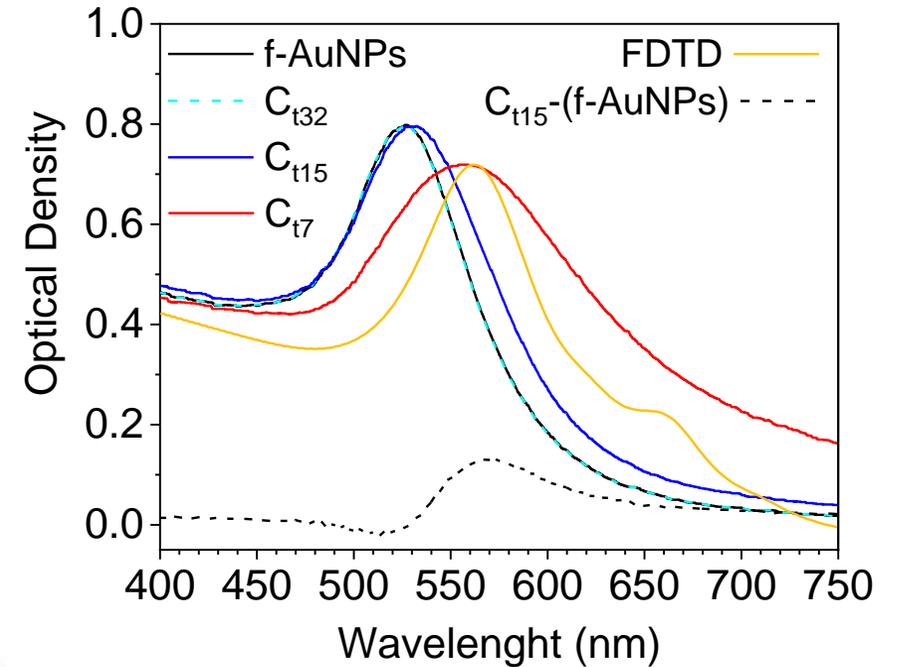
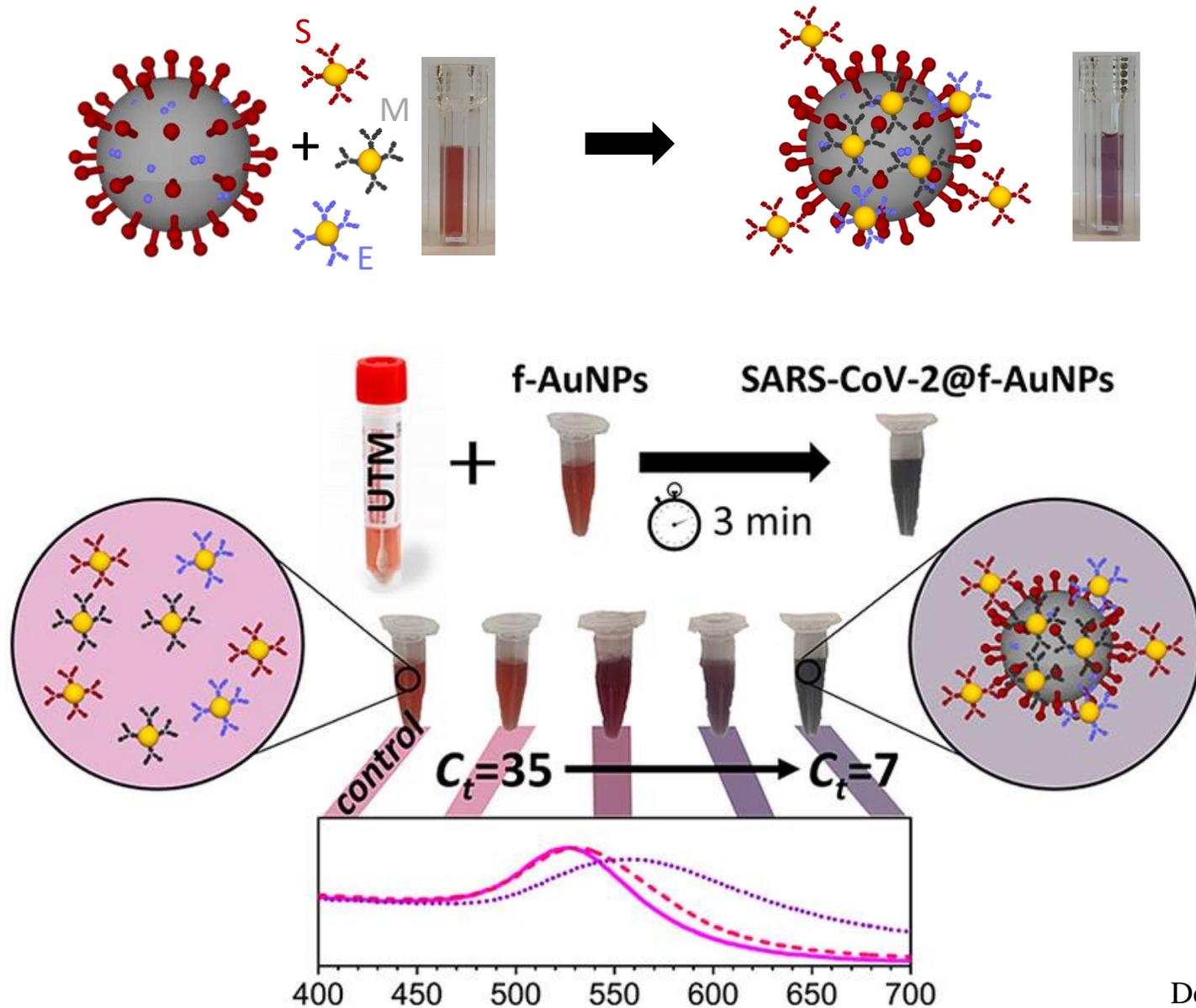
Marzia Iarossi,<sup>†,||</sup> Chiara Schiattarella,<sup>†,‡</sup> Ilaria Rea,<sup>‡</sup> Luca De Stefano,<sup>‡</sup> Rosalba Fittipaldi,<sup>§</sup> Antonio Vecchione,<sup>§</sup> Raffaele Velotta,<sup>\*,†,||</sup> and Bartolomeo Della Ventura<sup>†</sup>

# Detection of 17 $\beta$ -estradiol (E2) in tap water by AuNPs



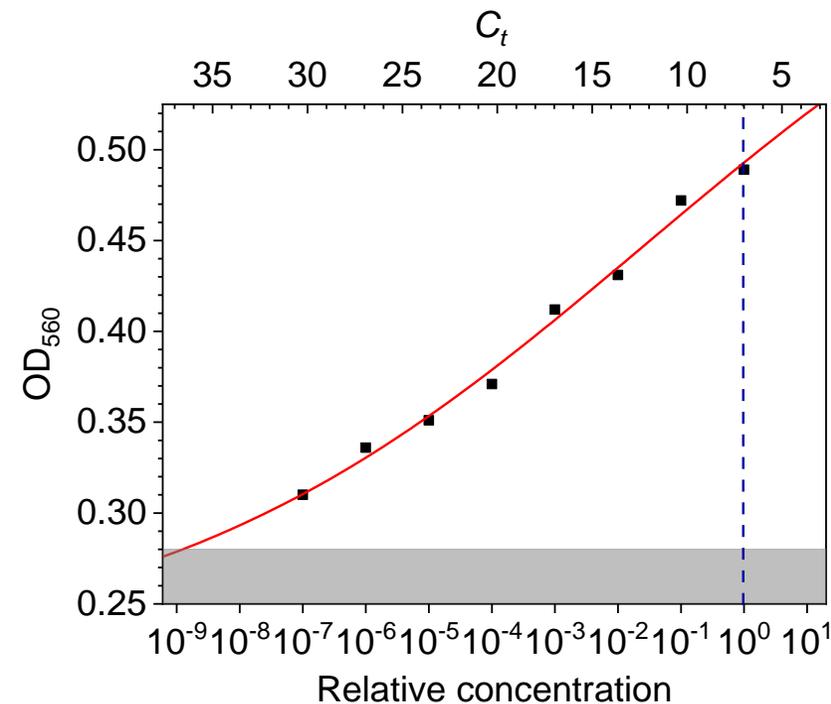
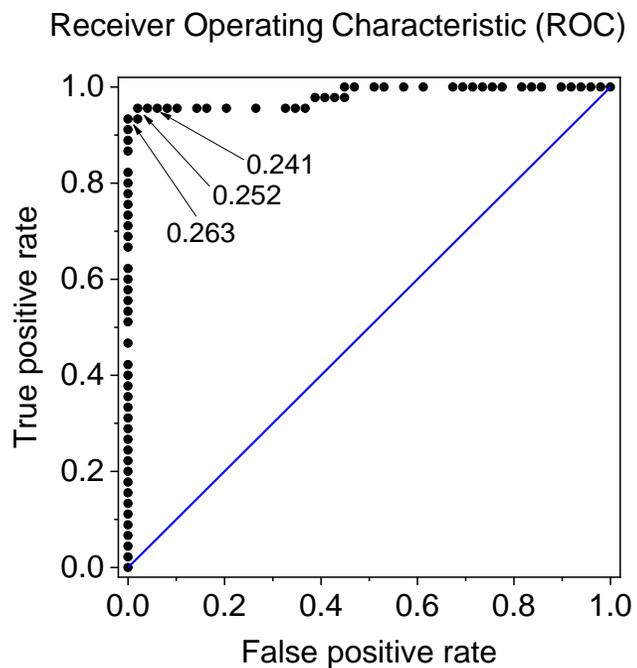
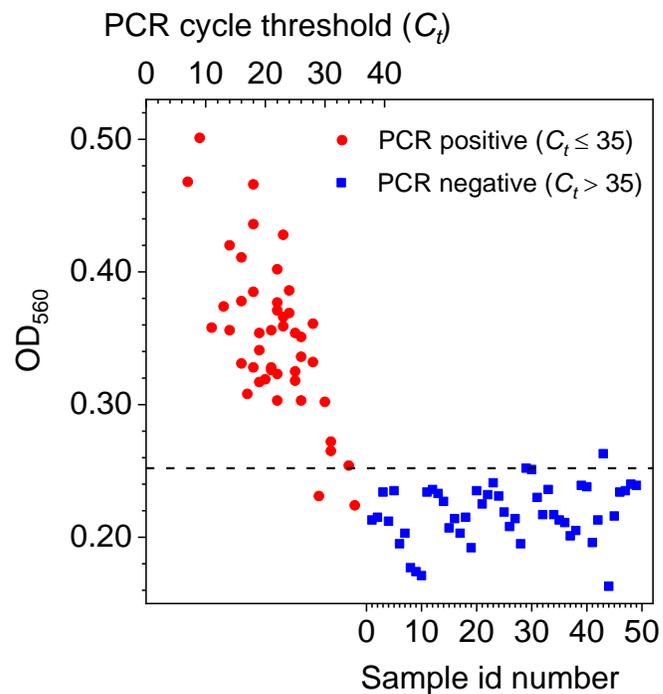
**E2 detected in the range of 1-50 pg/mL.**

# Detection of SARS-CoV-2 in nasal and throat swabs



A visual and quick response is obtained from patients with high viral loads ( $C_t > 15$ ) contributing to identify the so-called superspreaders and to downregulate the pandemic spread

# Detection of SARS-CoV-2 in nasal and throat swabs



Positive samples



Negative samples



$$OD_{560}(C) = \Delta(OD_{560}) \frac{C^n}{K^n + C^n} + OD_{560}(0)$$

**Sensitivity 94%    Specificity 100%**

**Limit of detection  $C_t = 30$**

# Modeling the colorimetric biosensor

Number of f-AuNPs in 1 mL of colloidal solution  $\cong 10^{11}$

Virion:  $\varnothing=100$  nm with 2000 *M*, 20 *E* and 100 *S* (10 nm height)

$\approx 10^8$  virions/mL

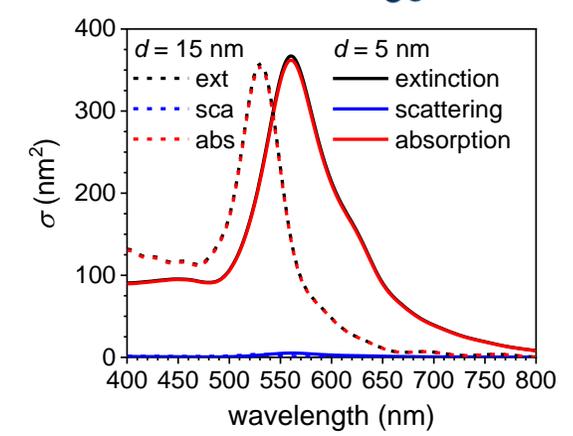
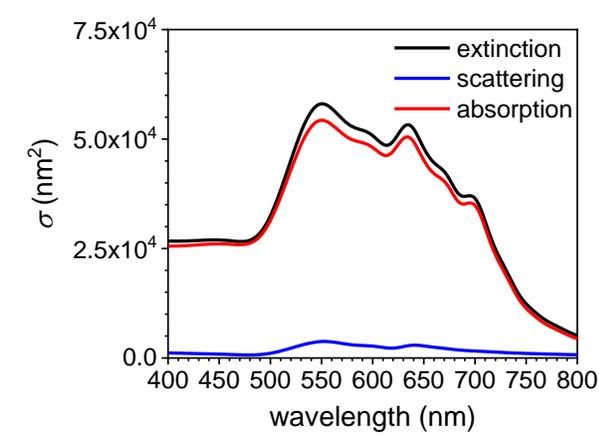
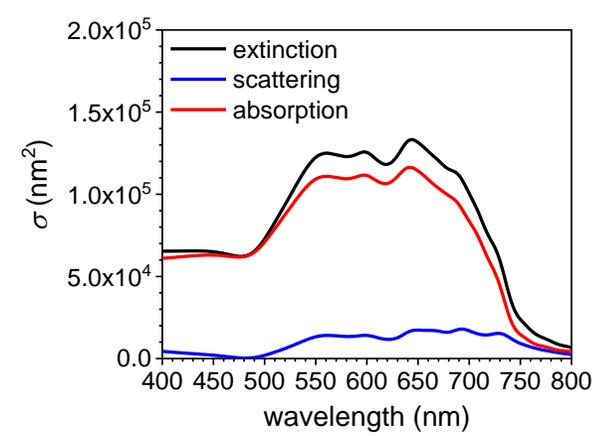
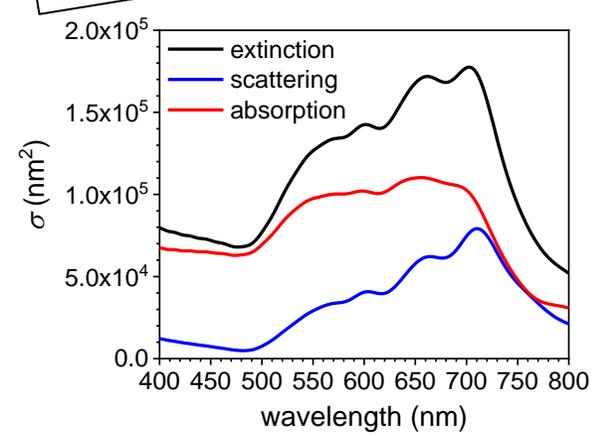
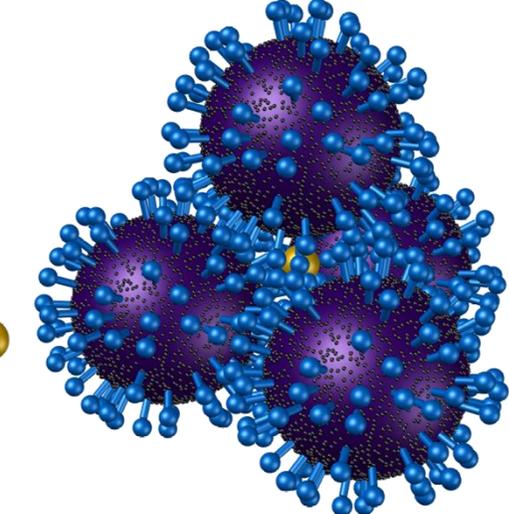
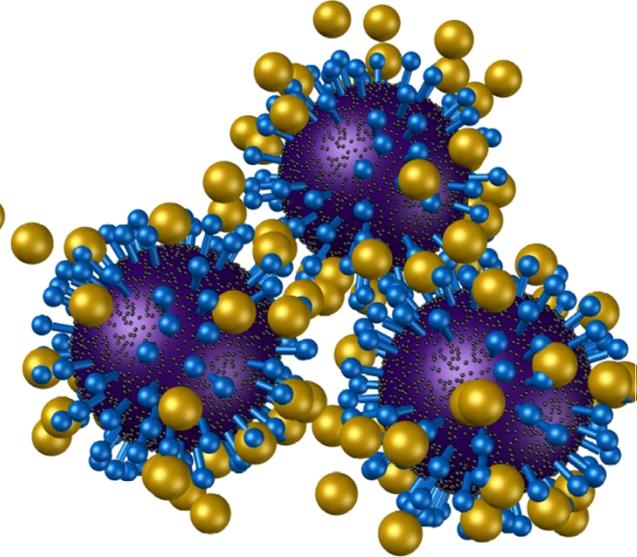
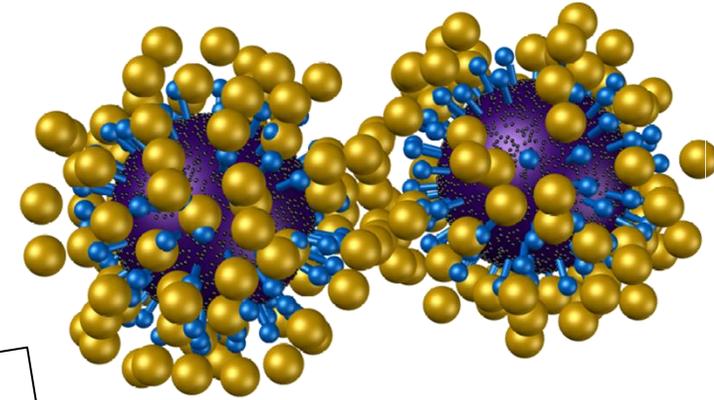
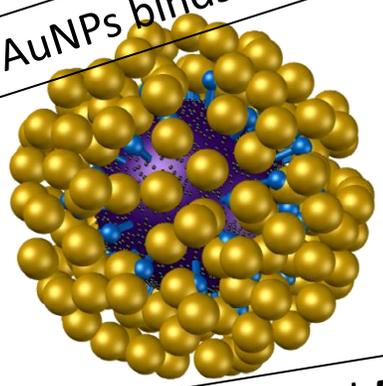
$\sim 10^9$  virions/mL

$\sim 10^{10}$  virions/mL

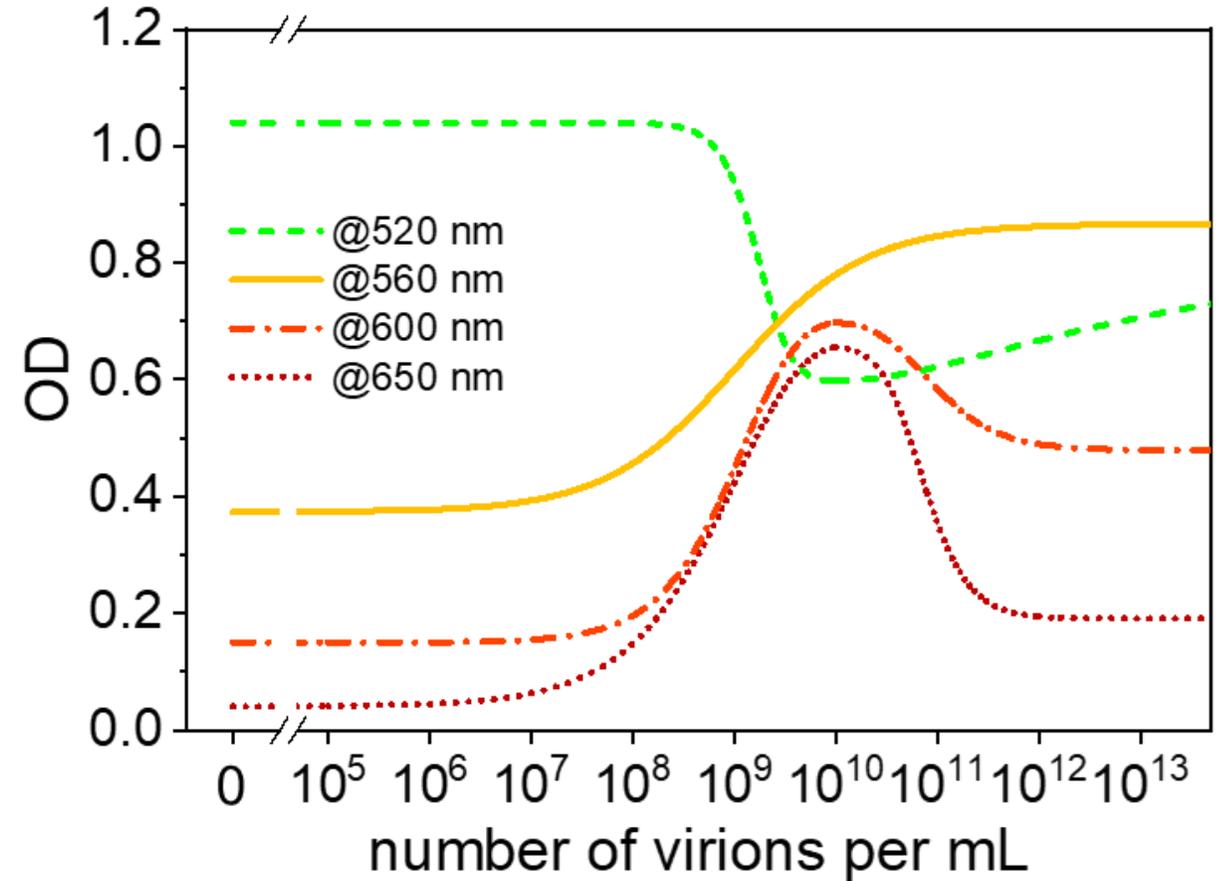
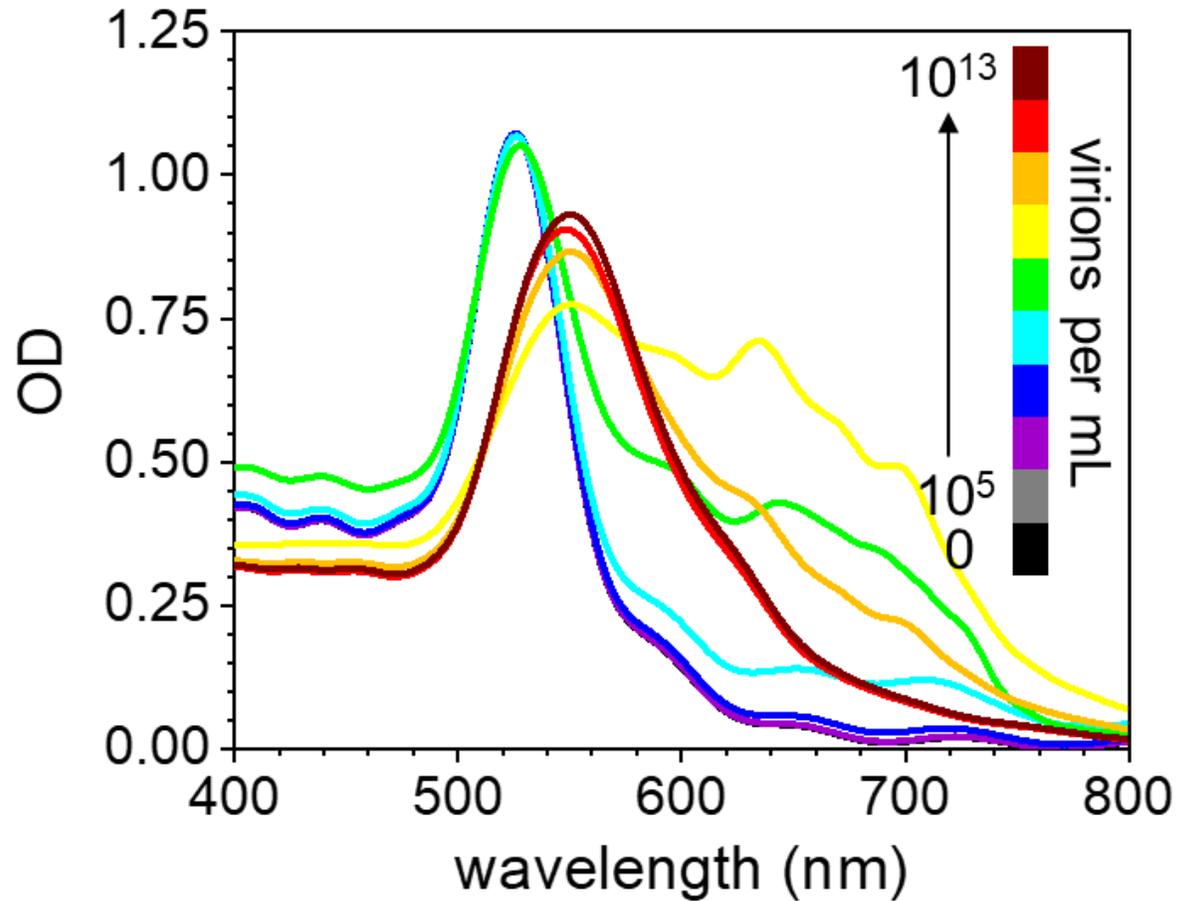
$\approx 10^{11}$  virions/mL

100 f-AuNPs binds *S*

80 f-AuNPs binds *M* and *E*



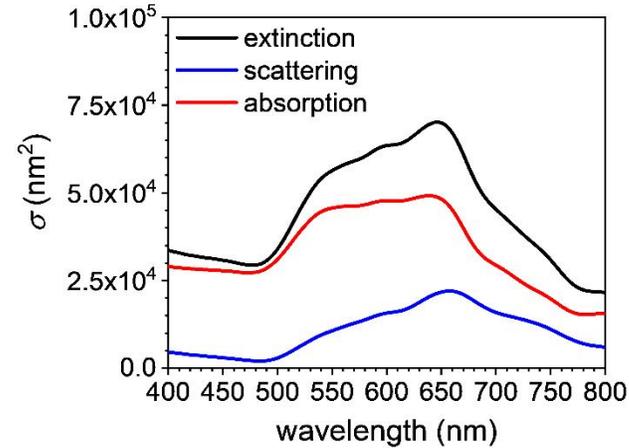
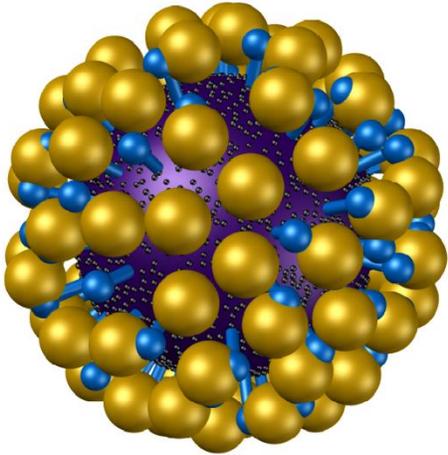
# Simulation: extinction spectra and dose-response curve



***Reading at 560 is optimal,  
in full agreement with the experiment.***

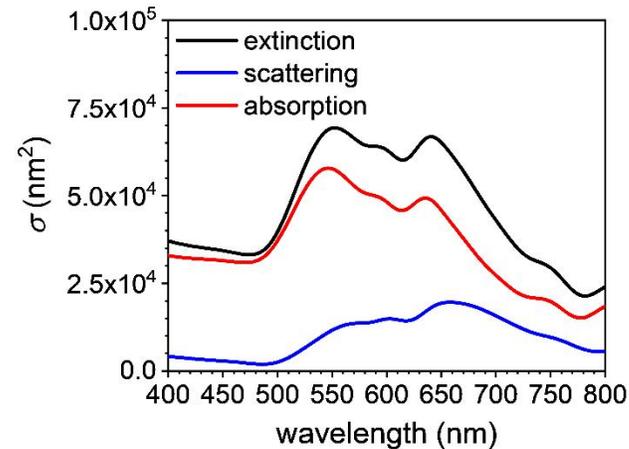
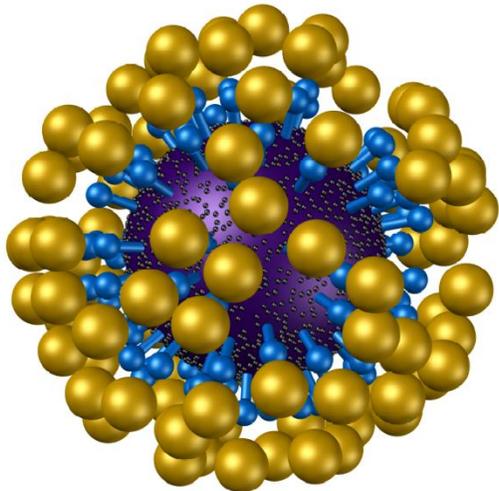
# Complete coating matters

a.



80 f-AuNPs binds **M** and **E**

b.



100 f-AuNPs binds **S**

One order of magnitude is lost in LOD

# Key step in biosensing: functionalization procedure

*Aimed features:*

- *effective*
- *fast*
- *cheap*
- *reliable (reproducible)*

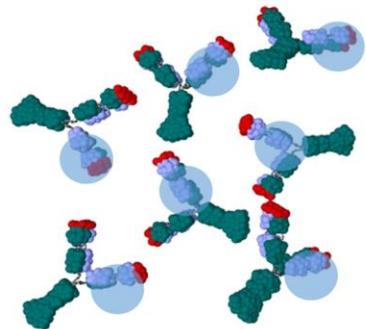
KEY STEP IN BIOSENSING: FUNCTIONALIZATION PROCEDURE



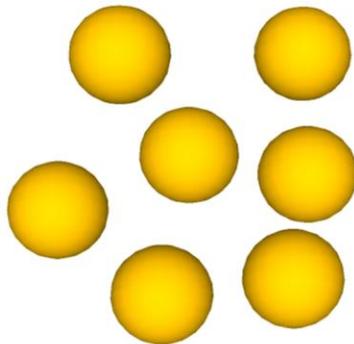
*Photochemical Immobilization Technique*

UV-activated IgGs

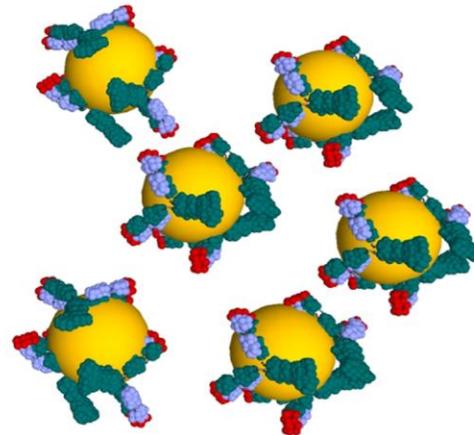
AuNPs



+

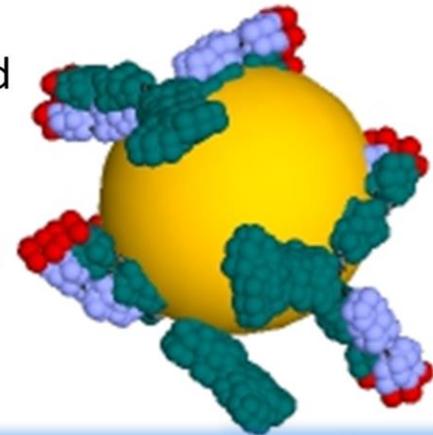


Functionalized AuNPs by IgGs



Fab exposed

Fab exposed



Fab exposed

# SALIVA (patent pending)

## Clinical validation

### First study Napoli, January 2021

- 100 specimen (70 negatives, 30 positives with **Ct ≤ 30**)
- Reference standard RT-PCR
- Specificity 99% (69/70)
- Sensitivity 90% (27/30)

		RT-PCR		
		Positive	Negative	Total
TGS VELOX Ag Saliva	Positive	27	1	28
	Negative	3	69	72
Total		30	70	100

### Second study Rome, March 2021

- 213 specimen (120 negatives, 93 positives with **Ct ≤ 30**)
- Reference standard RT-PCR
- Specificity 99% (119/120)
- Sensitivity 96% (89/93)

		RT-PCR		
		Positive	Negative	Total
TGS VELOX Ag Saliva	Positive	89	4	93
	Negative	1	119	120
Total		90	123	213

# CONCLUSIONS

- Colorimetric immunosensor sensitive to “*virion*”
- Good performances against SARS-CoV-2
- Photochemical Immobilization Technique (PIT) is a powerful tool for (gold) surface functionalization

## ... and PERSPECTIVES

- Rapid test with saliva
- Application to other viruses

# Co-authors



Bartolomeo Della Ventura, *RTDa*



Antonio Minopoli, *Post-doc*