Nanoparticle-based mobile biosensors for the rapid detection of sepsis biomarkers in whole blood

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Introduction

- **Rapid detection of sepsis biomarkers** :

- Procalcitonin (PCT)

- C-reactive protein (CRP)

- Interleukin 6 (IL-6)

Conventional methods

- ELISA procedurese → too lengthy for early diagnosis schemes
- Automated immunoassay systems → Elecsys® IL-6

- a lateral flow test has recently been proposed that can detect IL-6 in unprocessed blood with surface-enhanced Raman spectroscopy (SERS).

→ requires a microscope for detecting the biomarker at clinically relevant concentrations, which would be cumbersome to implement in bedside diagnosis

- A commercial lateral flow immunoassay from Milenia Biotec can detect IL-6 in 20 min, but only at concentrations higher than 50 pg mL

Biosensors

- **Biosensors** ➡ promising candidates for detecting IL-6 in the context of sepsis
- **Limitations of biosensors** :

- many of these biosensors still require assay times longer than 30 min

- the majority of these devices have only been tested in purifired serum samples

Advantages of plasmonic mobile biosensor

1. Rapid IL-6 detection

2. Reduce the assay time

3. Plasmonic mobile biosensors ➡ detect IL-6 with a limit of detection of 0.1 pg ml and total assay time within 17 min

4. Detect IL-6 spiked into unprocessed blood with the same assay time at concentrations well below the cutoff values indicating sepsis

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5. Excellent sensitivity in blood matrices

6. high portability of the mobile detection scheme make

7. The rapid turnaround time

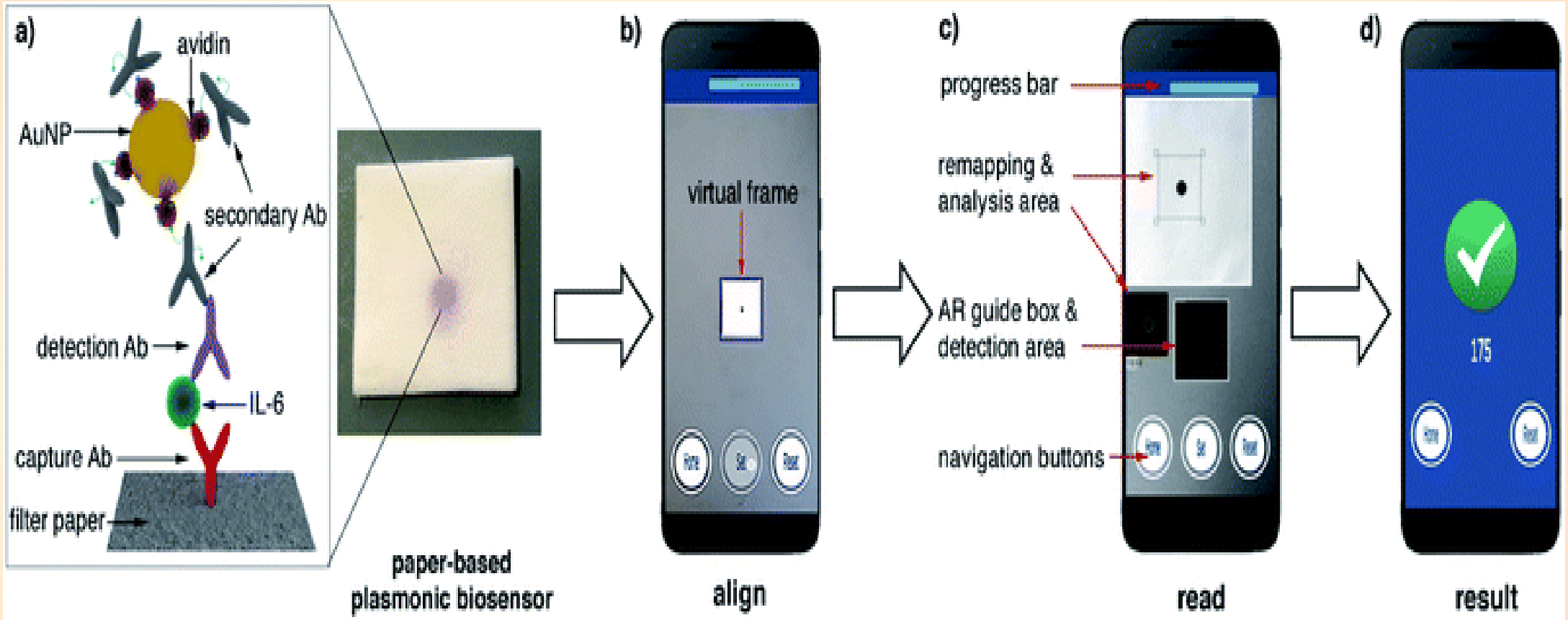
- Cut-off values indicating sepsis risk are not much higher (25 pg mL in newborns and 40–61 pg mL in adults)

Metod

- **The device setup**
- paper-based biosensor paired with a smartphone app for colorimetric signal detection.

- Colorimetric signals are generated by gold nanoprobes modified with avidin and biotinylated antibodies.

plasmonic mobile biosensors Fig. 1



Augmented reality (AR) guidance system

- White balance calibration
- Light artifact detection
- Selection of a region of interest
- measurement of pixel intensity
- Data validation are automatically performed with real-time image processing and data processing.
-

User only needs to hover the phone over the assay to obtain reliable densitometry readings within seconds.

Biosensors were prepared with 3 different methods

- **first method**

spotting biotin–BSA (5 mL, 0.1 mg mL in PBS) on the top paper layer at different concentrations for 5 min (direct adsorption)

- **second method**

spotting PAH (1% (w/v), 10 mL) and letting it dry (10 min) followed by spotting the protein at different concentrations

- **third method**

involved an additional PSS treatment (1%) after adding the protein followed by washing with water and letting the paper dry (10 min)

- Next the paper substrates were blocked with PBS–BSA (1 mL). Immediately afterwards avidin-decorated nanoparticles were spotted onto the substrate (2 mL, 4 min) followed by washing with PBST (1 mL, 3 times).

Nanoprobe fabrication and performance

- Citrate-capped nanoparticles → with 0.1 mM SH-PEG-COOH overnight to obtain carboxylate-coated nanoparticles

- PAH-covered nanoparticles → with 0.1 mM SH-PEG-NH2 overnight in order to obtain amine-coated nanoparticles.

- PVP-coated nanoparticles → were resuspended in water and used without further purification.

- The performance of the avidin-decorated nanoprobes was tested with a model biosensor using paper substrates

Avidin attachment to carboxylate-coated nanoparticles proceeded as follows

- Carboxylate-coated nanoparticles (250 mL) ➡ were concentrated to a final volume of 1 mL and washed 5 times with water with ➡ centrifuge (9000 rpm, 8 min)

- carboxylate moieties ➡ transformed into sulfo-NHS esters via addition of EDC and sulfo-NHS to the nanoparticle suspension in 0.5 M MES buffer of pH 6

- After 30 min ➡ the nanoparticles were pelleted with a centrifuge and the supernatant was substituted with a solution containing avidin

- The pH of the avidin solution was varied from 5.5 to 7.4 with phosphate buffer to test the impact of this parameter on the reaction between the sulfo-NHS esters and amine groups in proteins

- . Then 400 mL of a blocking solution containing glycine) and BSA in phosphate buffer (0.1 M, pH 7) was added for at least 30 min followed by washing 5 times with PBST

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- Avidin was attached to amine-coated or PVP-coated nanoparticles using glutaraldehyde as a crosslinker:
- First glutaraldehyde in 0.1 M bicarbonate buffer of pH 9 was added to the nanoparticle dispersion for different times.
- Then avidin was added to the nanoparticles in the presence of NaCNBH3 reduces the pH-sensitive imine bonds generated by the reaction between amines and aldehydes and yields stable amine bonds.
- After overnight incubation unreacted aldehydes were blocked with glutamic acid (0.1 M) in sodium bicarbonate (0.1 M, 100 mL) for 1 h
- The resulting avidin-decorated nanoparticles were washed by centrifugation 5 times with PBST.

Detection of IL-6 with plasmonic paper-based biosensors

- **Biosensors for the detection of IL-6 were fabricated as follows:**
- Avidin-nanoparticles (100 mL, [Au] 100 mM) were modified with biotinylated anti-rabbit IgG (10 mL) for 1 h
- Washing 3 times with PBST with the aid of a centrifuge (7000 rpm, 5 min, final volume 25 mL)
- Capture antibodies (mouse monoclonal anti-IL-6) were immobilized by adding a drop on the paper substrate (2.5 mL, 10 mg mL)
- letting it dry for at least 10 min
- Biosensors were blocked with PBS–BSA (1 mL, let dry at room temperature for at least 10 min)

- To detect IL-6 the paper biosensors were rehydrated with 1 mL of PBS–BSA followed by the addition of IL-6 spiked at different concentrations in PBST, human serum or whole blood

- the composition of the matrix was not noticeably altered by the spiking procedure, an IL-6 solution with a high concentration of 1 mg mL in PBS was serially diluted more than 104 times with whole serum or blood to yield samples in the concentration range between 50 and 3 pg mL .

-

- Blood samples were stored in tubes containing EDTA as the anticoagulant After 5 min the biosensor was washed up to 4 times with PBST (1 mL).

- Then the detection antibody was added (2.5 mL, 10 mg mL) for 5 min and the biosensors were washed once with PBST.

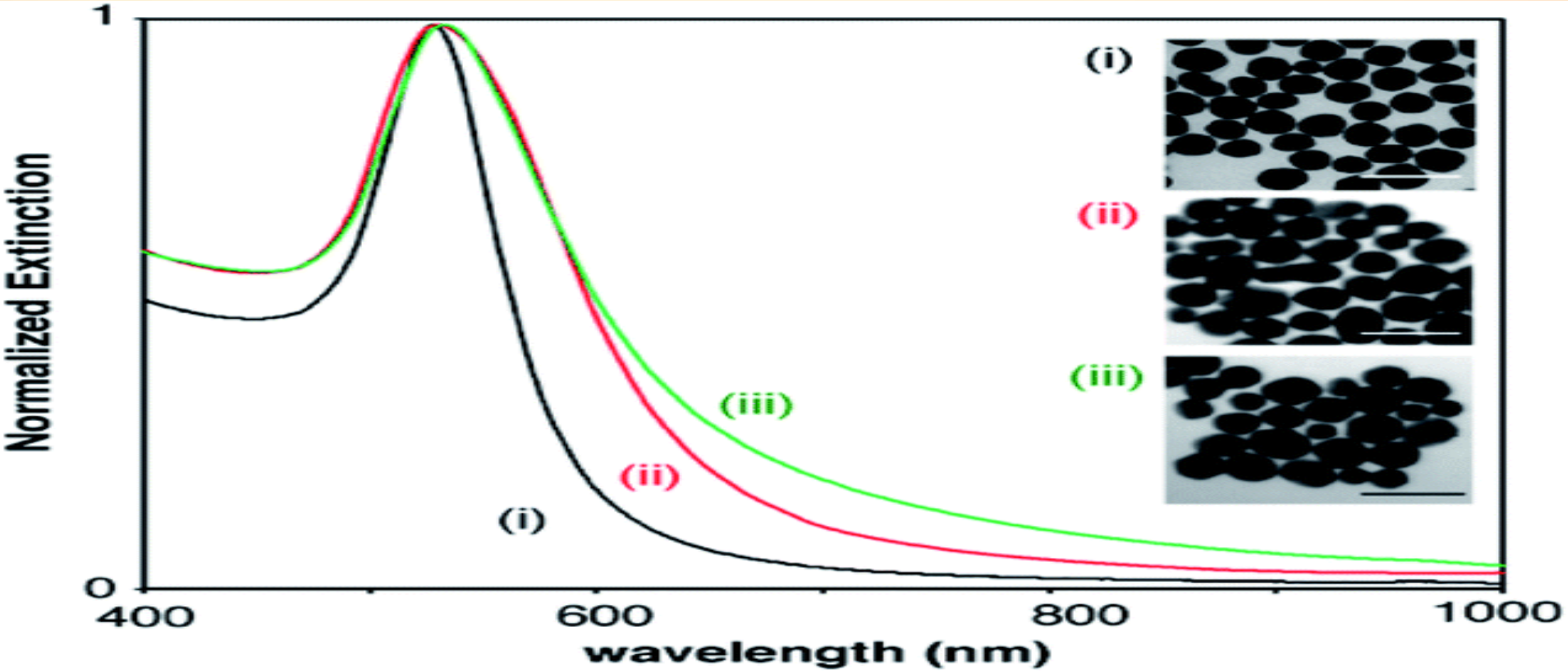
- Finally, avidin-decorated nanoparticles modified with biotinylated anti-rabbit IgG were added (2 mL, [Au] 400 mM) for 5 min. Then the biosensors were washed 4 times with PBST (1 mL) and the colorimetric signals were evaluated with the mobile densitometry app.

Results and discussion

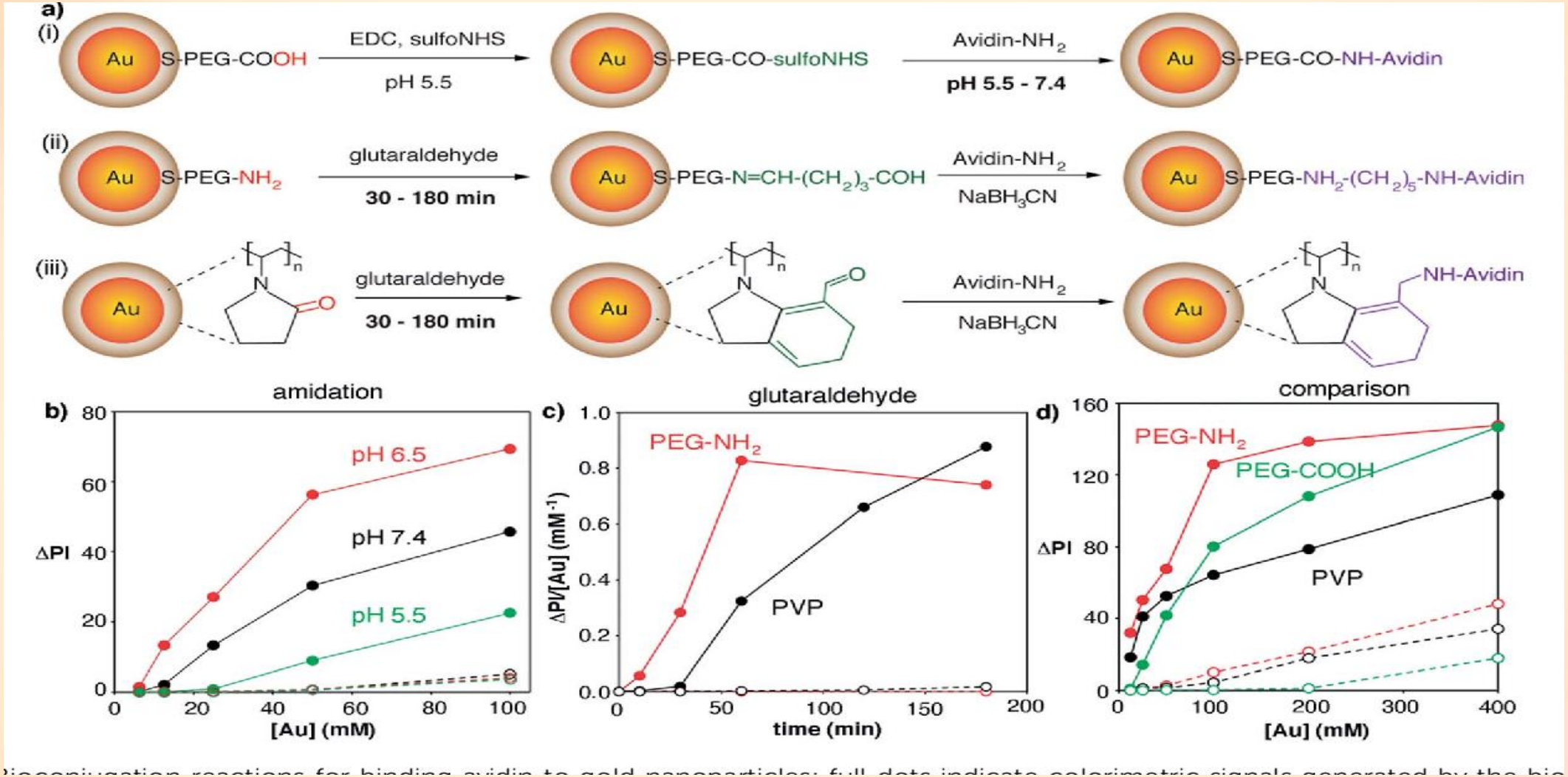
- The gold nanoparticles ➡ starting materials for nanoprobe fabrication the reduction of gold ions with either citrate, PAH or PVP molecules

- Citrate- and PAH-covered nanoparticles ➡ modified with SH-PEG-COOH and SH-PEG-NH2 ➡ in order to obtain stable carboxylate- and amine-coated nanoparticles,

Extinction spectra and representative TEM images (i) PVP-coated, (ii) carboxylate-coated, and (iii) amine-coated nanoparticles Fig. 2



Bioconjugation reactions for binding avidin to gold nanoparticles Fig. 3



Bioconjugation reactions for binding avidin to gold nanoparticles; full dots indicate colorimetric signals generated by the bio

- To test this idea,

→ the signals generated via direct adsorption of biotinylated BSA on paper with those obtained with biosensors fabricated with a modified cellulose matrix.

The paper modifications entailed adding a positively charged polymer PAH, either alone or followed by the addition of negatively charged PSS.

These treatments were chosen to evaluate the role of the paper surface charge in both attaching capture biomolecules and establishing non-specific interactions with the nanoparticles

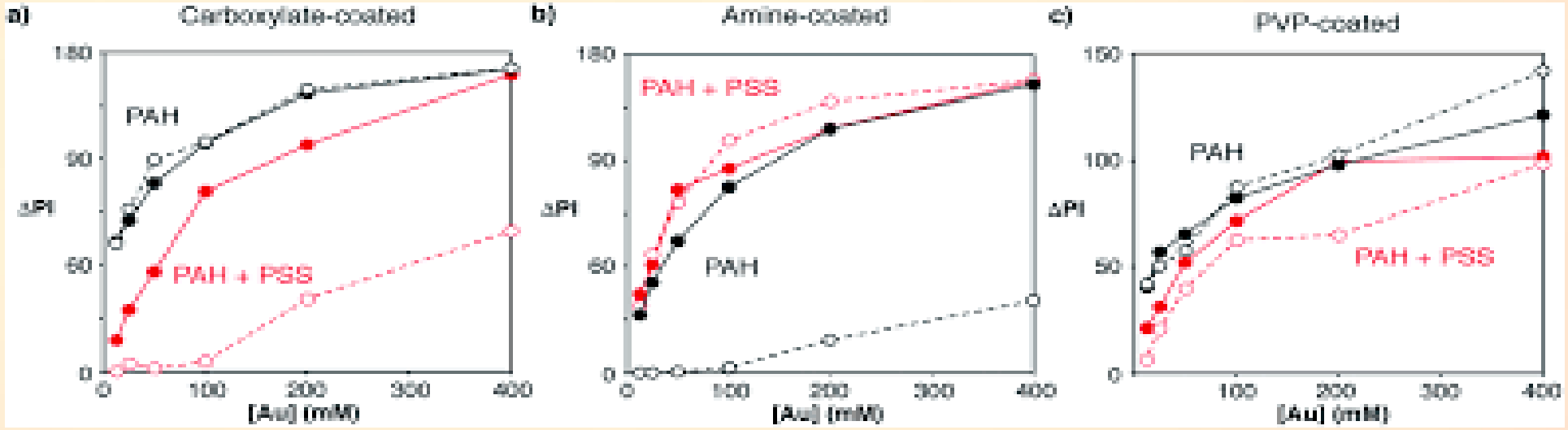
which have different surface charges according to their coatings (zeta potential-2.7, 1.7 and -1.5 mV for carboxylate-, amine-, and PVP-coated nanoparticles modified with avidin.

Con,t

- positively charged polymer (chitosan) were also attempted, but they were discarded because it blocked the pores of the paper, making it difficult to perform washing steps.

- modifying PAH-modified paper substrates with glutaraldehyde for covalent attachment of proteins were rejected because they resulted in yellow coloration of the filter paper that interfered

Colorimetric signals generated by different concentrations of gold nanoprobes modified with avidin Fig. 4



- **After optimizing the fabrication of the biosensor:**

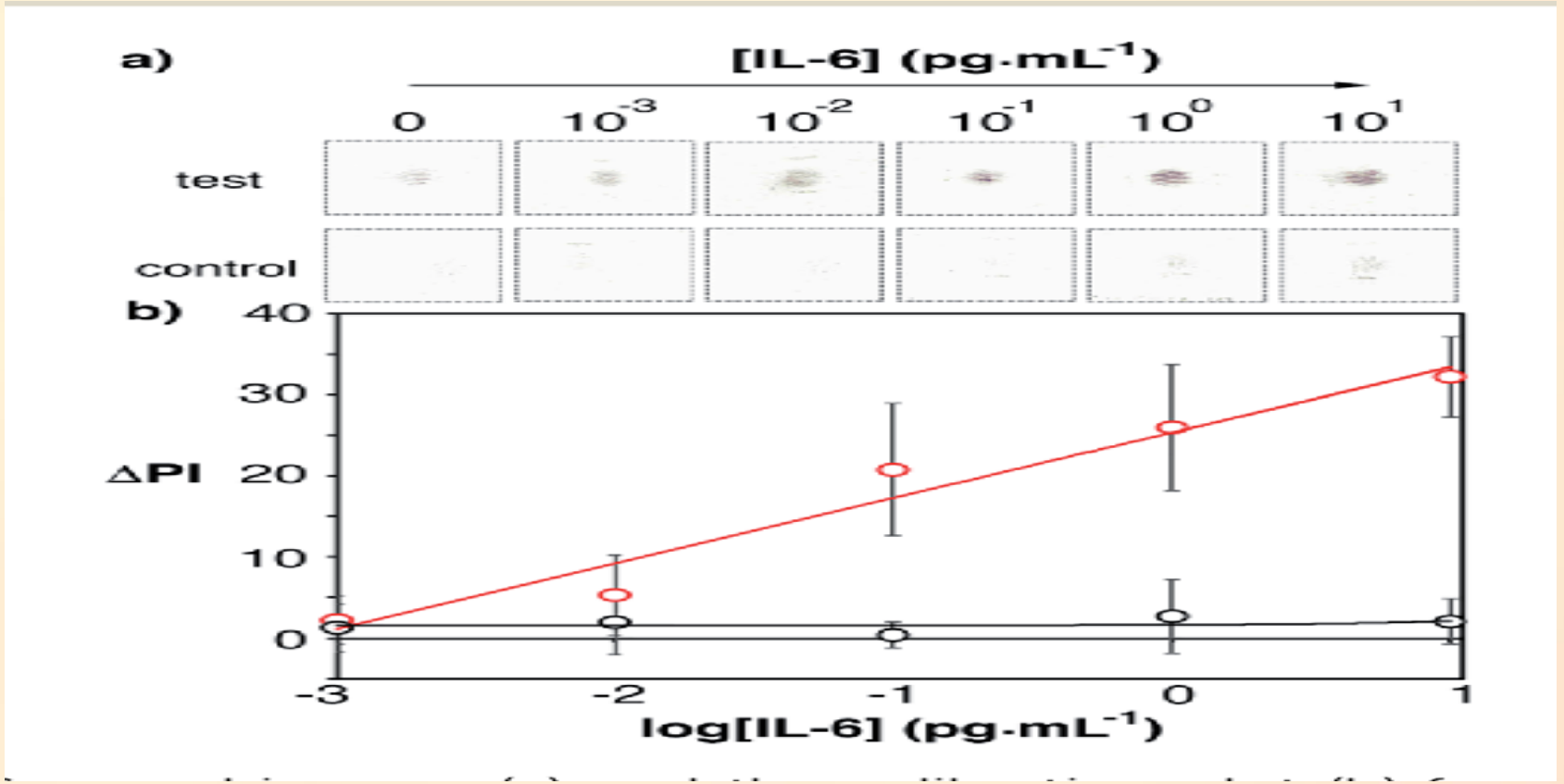
→ to determine the best conditions for detecting IL-6 ↓

- optimizing the concentration of capture and detection antibodies
- as well as the time required to capture IL-6

- Antibody-decorated nanoparticles were obtained by adding biotinylated antibodies to the avidin-modified nanoparticles.

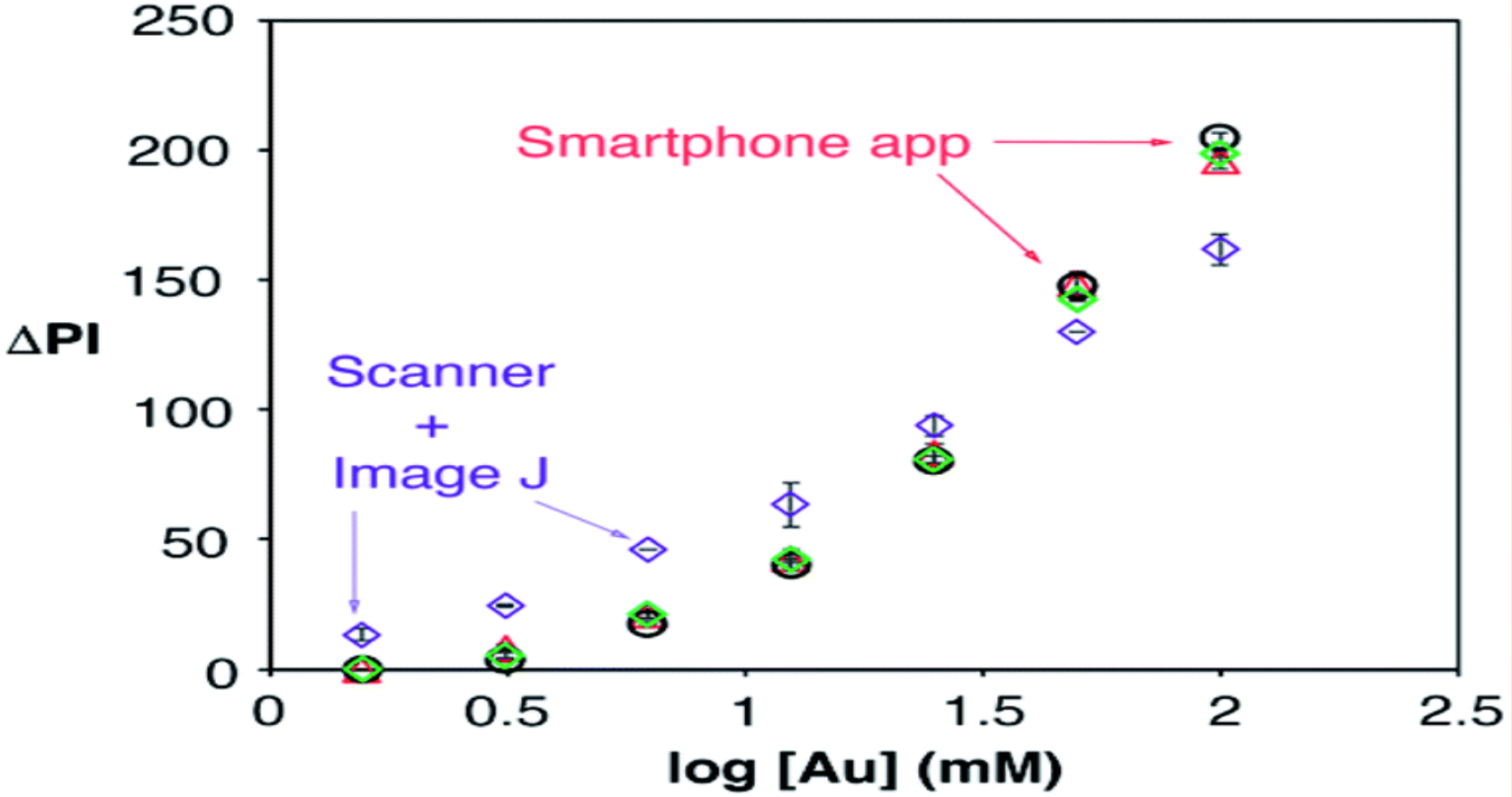
- optimization experiments → the best conditions for detecting IL-6 were to spot the capture antibody on the paper substrate at a concentration of 10 mg mL , and to add the detection antibody at a concentration of 10 mg mL for 5 min

Scanned images (a) and the calibration plot (b) for detecting recombinant IL-6 in PBS with the proposed immunosensors (red dots) and control biosensors without the capture antibody (black dots) Fig. 5



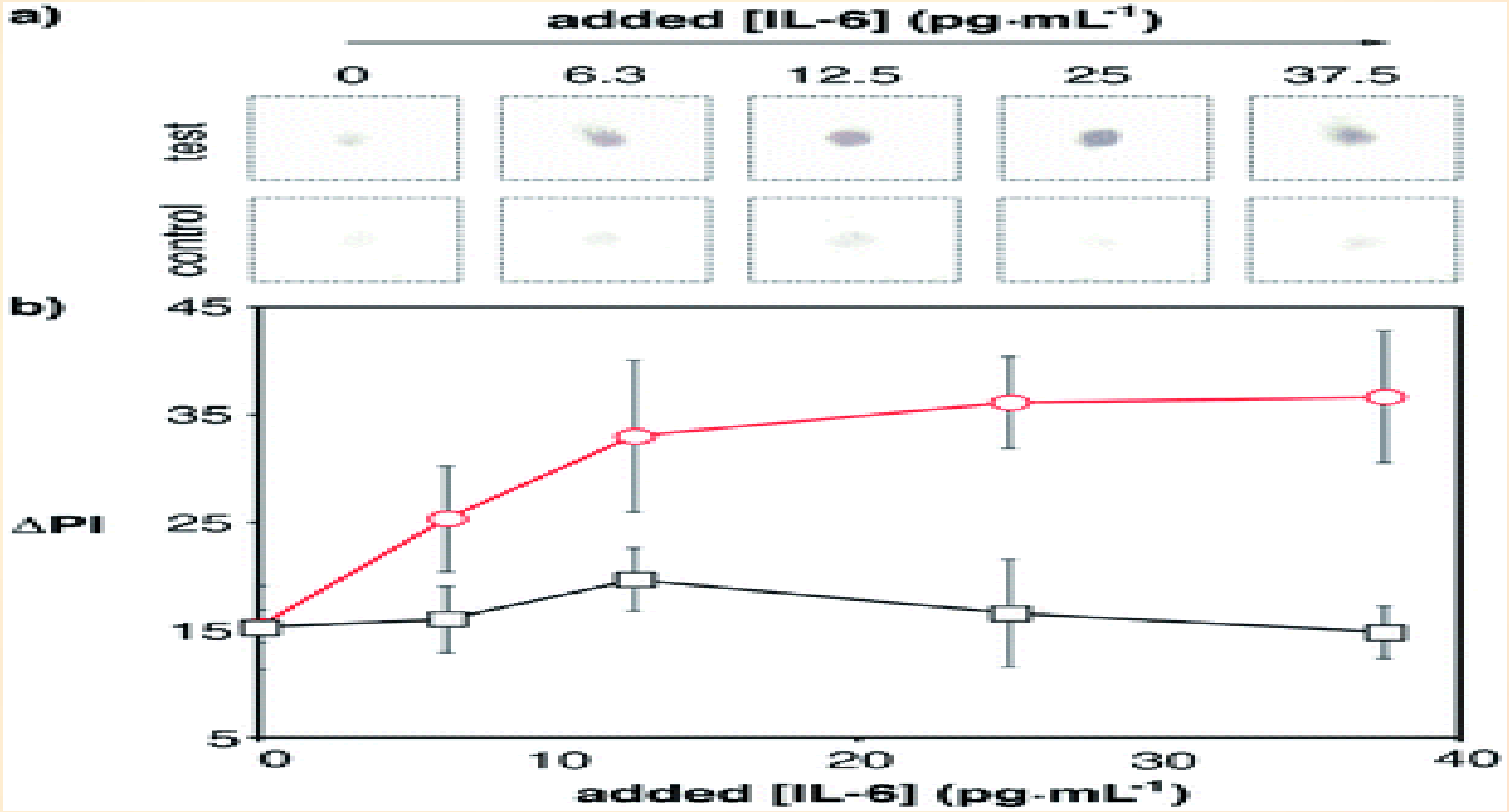
- Detecting IL-6 in the context of sepsis :
- the use of unprocessed blood
- portable readout instrument capable of yielding robust results in different locations with varying environmental conditions.
- mobile app for performing reliable densitometry at the point of care that only requires hovering a smartphone over the assay in order to quantify colorimetric signals .
- The app was calibrated using pieces of paper modified with nanoparticle drops at different concentrations, and therefore with different pixel intensities.
- The calibration was repeated under 3 different room illuminance conditions (200, 570 and 1070 lux) and compared with the same measurements obtained using a desktop scanner and manual image processing with Image .

Calibration plot obtained by evaluating the colorimetric signal generated by gold nanoparticles at different concentrations with the densitometry app when the room illuminance was 200 (black dots), 570 (green diamonds) and 1070 lux (red triangles), and with a desktop scanner (purple diamonds) Fig. 6



- **Next we tested** whether we could detect elevated levels of IL-6 by combining the app with the optimized paper biosensors.

Detection of IL-6 spiked into human blood with plasmonic mobile biosensors Fig. 7



Conclusions

→ reported the fabrication and performance of a plasmonic immunosensor for the rapid detection of IL-6 in the context of sepsis

- **Key aspects of the immunosensor fabrication:** ↓
- including the manufacture of plasmonic nanoprobes decorated with proteins and strategies for attaching capture molecules to the paper substrate were optimized

→ companion app that quantifies the colorimetric signal generated by the plasmonic probe in real time

- The optimized paper biosensors → to detect IL-6 under ideal conditions with a low limit of detection of 0.1 pg mLwithin 17 min
- biomarker was spiked into serum or blood, the biosensors → to detect variations in the basal concentration of IL-6 as small as 12.5 pg mL with 99% confidence

Advantage

- In the context of it would enable the measurement of biomarkers during triage, when prioritizing high-risk patients is essential for improving sepsis outcomes.

- Moreover, the biosensors only require a tiny sample volume (2.5 mL), which makes them suitable for sepsis screening in neonates as well.

- the same strategy could be used to detect other biomarkers and pathogens by using specific antibodies against them.

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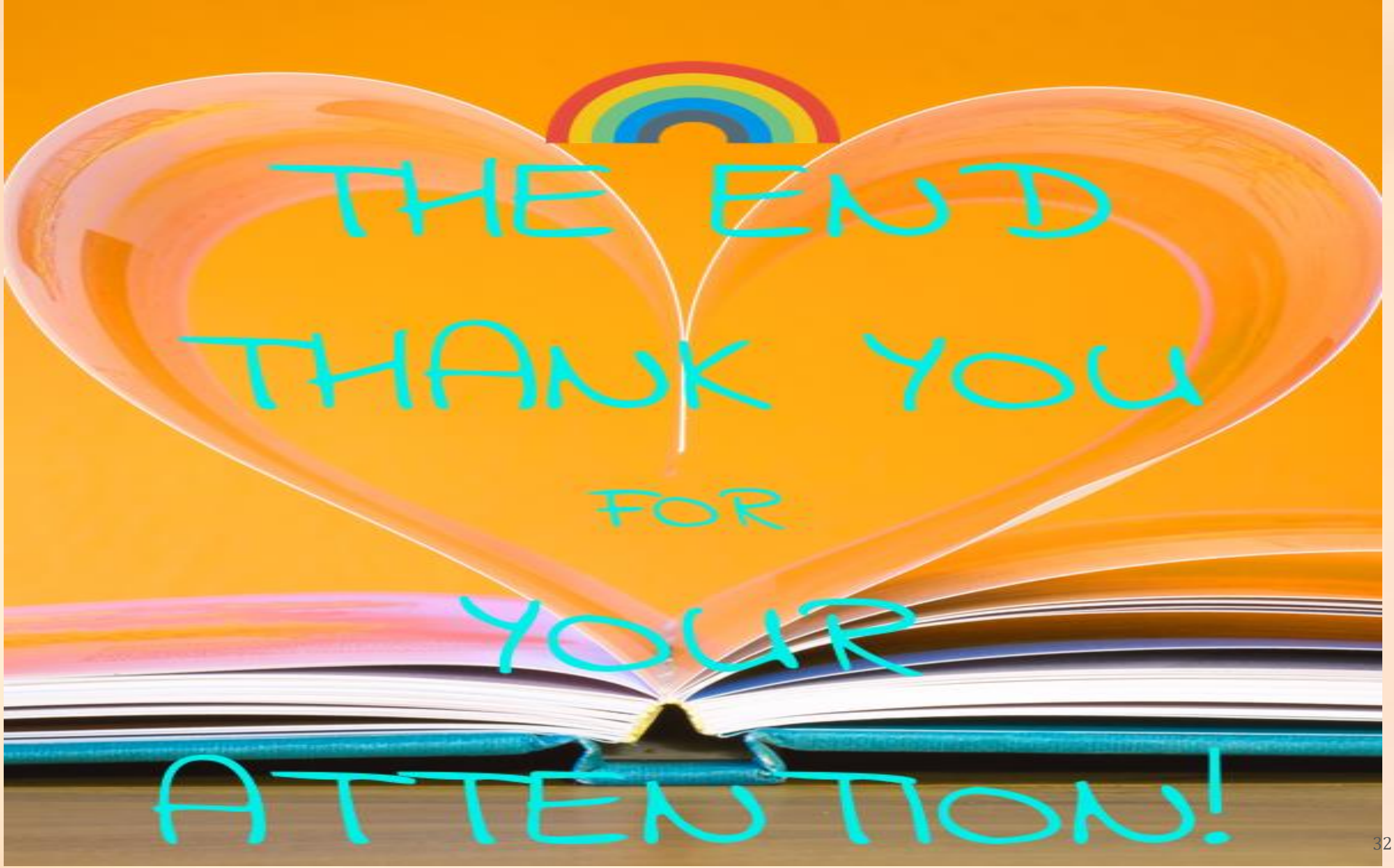
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