# Platform set-up by multifunctional PEG microgels for bead-based assay in immunoglobulin detection

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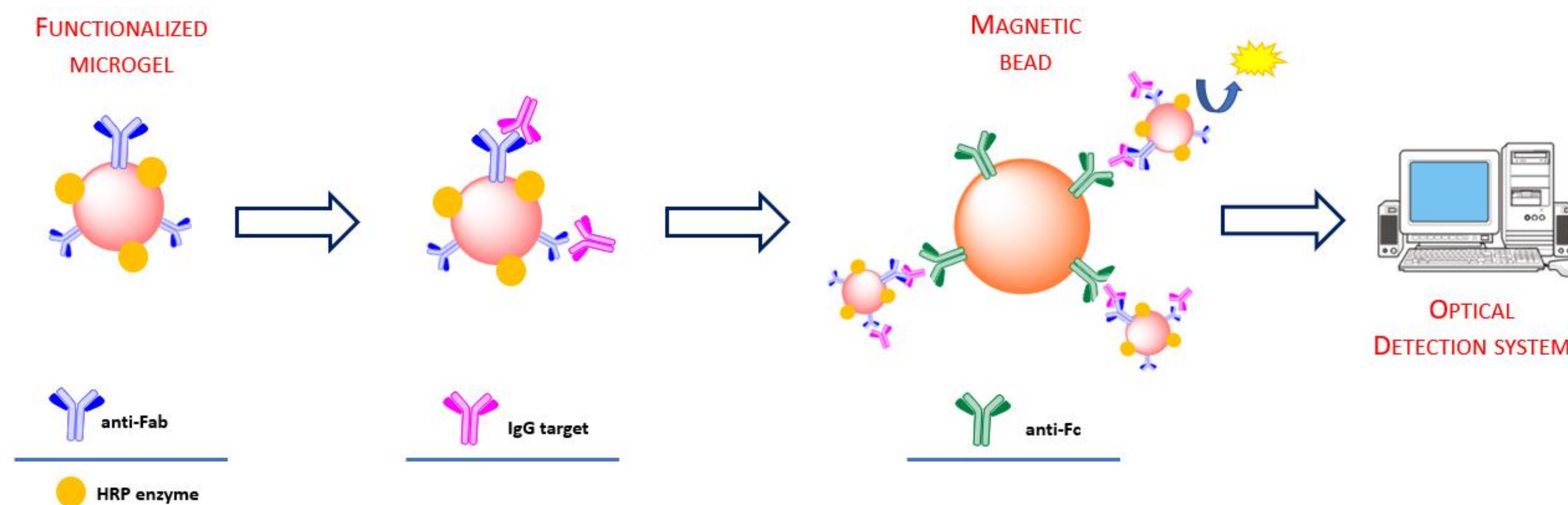
## AIM OF THE WORK

Detection and quantification of protein biomarkers for diagnosis disease are particularly challenging due to their low concentrations in clinical samples.[1] Traditional immunoassay as ELISA suffers from low sensitivity and specificity which greatly limits its potential in highly sensitive detection. In this work, the microgels-based platform[2,3] is applied to design innovative immunoassay biosensors for immunoglobulin IgG detection in the biomedical field. Microgels are multifunctional particles with chemical flexibility and a highly tunable nature. The application of the microgels-based platform will give the possibility to overcome the limited abilities of standard techniques. Microgels act as the carrier of both antibodies and HRP for the sandwich structure formation and to produce a signal amplification.



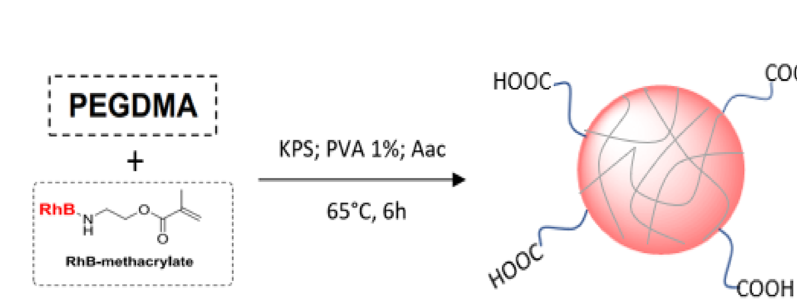

## METHODS

**1. Microgels synthesis and characterization**
Microgels composed of Poly(ethylene glycol) dimethacrylate (PEGDMA, Mn 550), Rhodamine B methacrylate fluorescent dye and polyacrylic acid AAc were synthesized. Microgels size and surface charge determination was conducted using Dynamic light scattering. The optical barcode was imaged by using Confocal laser scanning microscope CLSM.

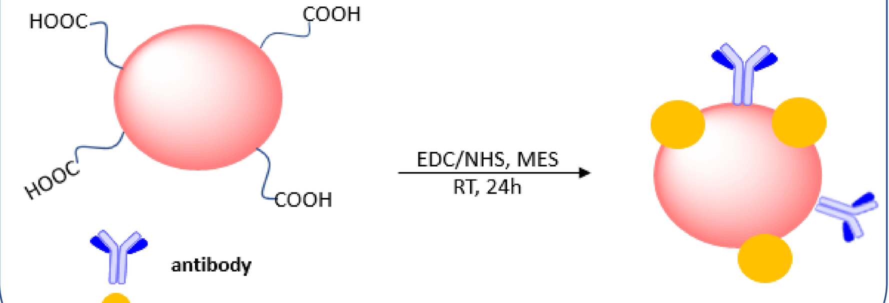
**2. Microgels functionalization**
As a proof of concept, IgG anti-IgG antibody system is used to prove the interaction of the particles. Biomolecules were immobilized onto microgels surface by performing COOH groups activation with Ethyl-N'-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) and N-hydroxy succinimide (NHS).

**3. Particles interaction**
Magnetic beads and microgels were put in contact. The optical signal produced by the enzyme is recovered by using the plate reader EnSight Multimode Microplate Reader.
The clusters formed among particles were imaged by CLSM following the microgels optical barcode.

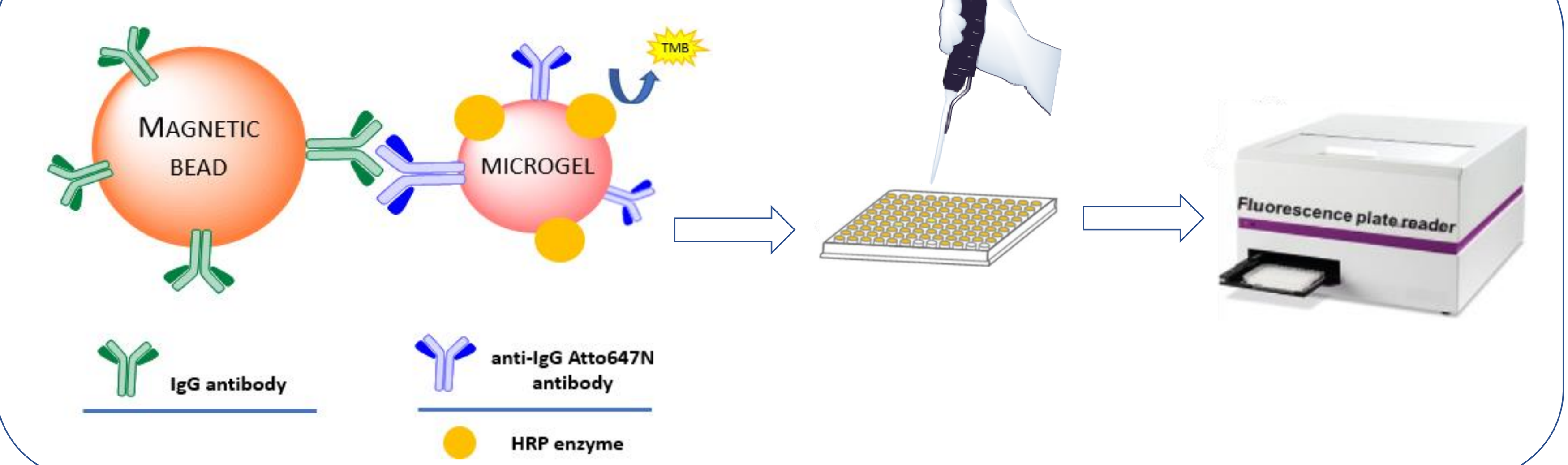
1. MICROGELS SYNTHESIS




2. MICROGELS FUNCTIONALIZATION



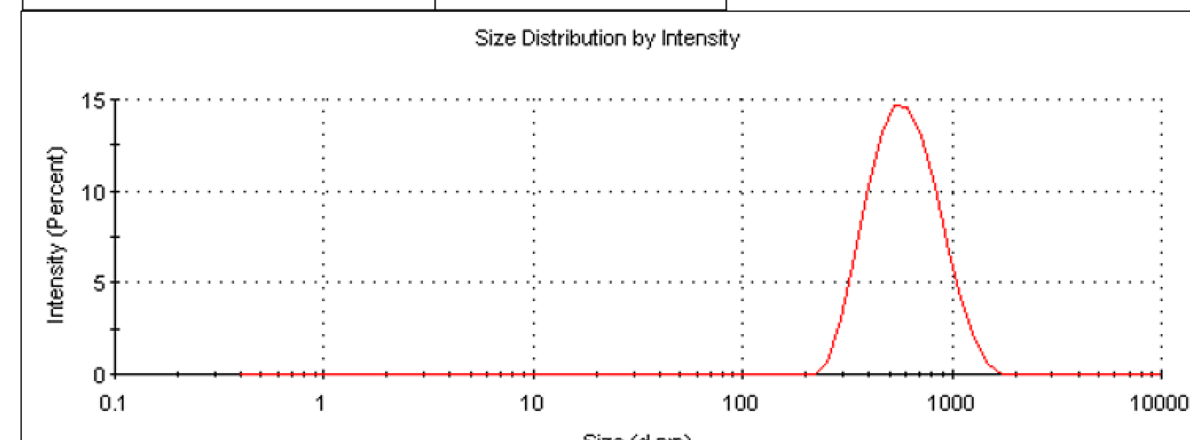

3. PARTICLES INTERACTION




## RESULTS

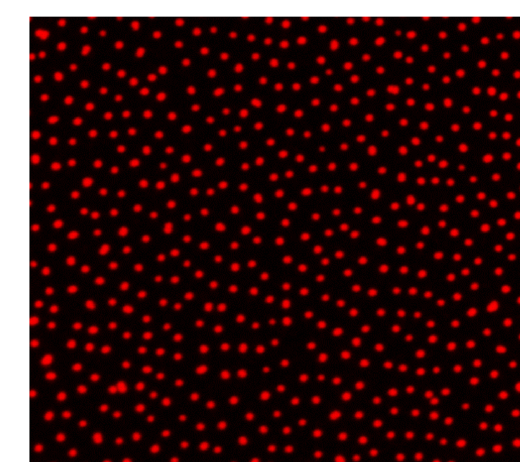
**Microgels characterization**

| Size (nm) | Zeta potential |
|---|---|
| 663.2±17.17 | -26.1±0.1 mV. |




Microgels size determination was conducted using Dynamic light scattering. Core microgels result in uniform in size. The polydispersity index (PDI) recorded was very low 0.046 highlighting that the microgel solution was monodisperse.

MICROGEL OPTICAL BARCODE



The encoded microgels were analyzed by confocal laser scanner microscopy showing the red emission originating from rhodamine B dye.

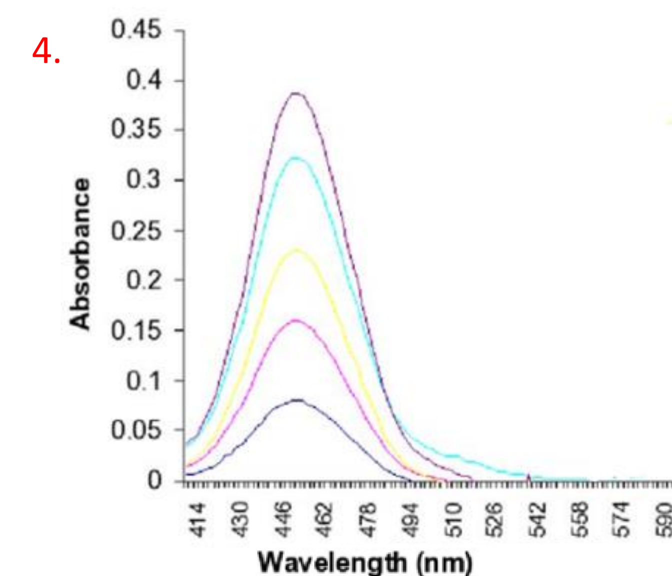
**Microgels functionalization**
Core microgels are involved as detection particles. They were functionalized by a double conjugation of anti-IgG atto647N antibodies and horseradish peroxide HRP enzyme. Magnetic particles are involved as capture particles. They are functionalized with IgG antibodies.

4. After the double coupling, the catalytic activity of HRP enzyme was evaluated. The enzyme was still able to react with its TMB substrate producing a colour signal at 450 nm. Increasing amount of HRP was conjugated to microgels resulting in an enhancement of the colourimetric signal.
5. Microgels coated with anti-IgG atto647N and HRP were imaged by using CLSM. Images showed the fluorescent signal of antibodies onto microgels. The ability of anti-IgG atto647 to bind the IgG after coupling was tested by performing interactions with magnetic beads coated with IgG.

**Particles interactions**

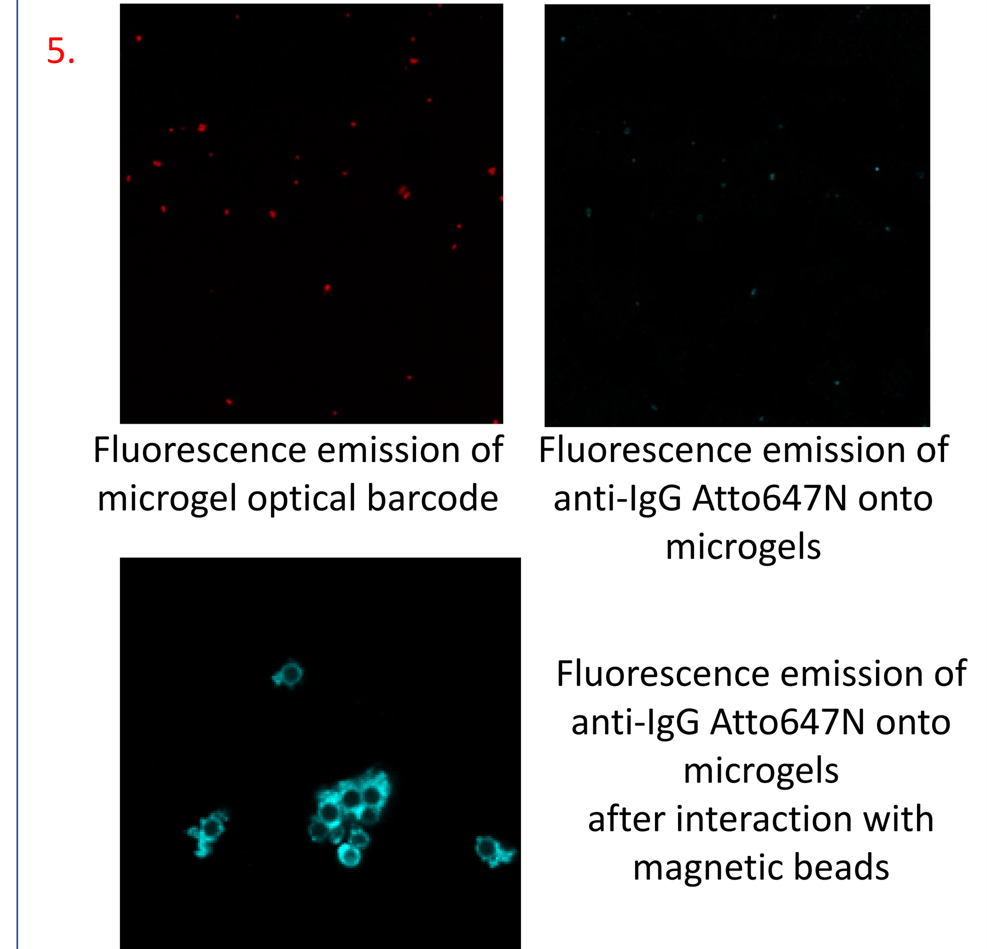
6. To prove the possibility of interaction among particles, magnetic beads and microgels were put in contact in different concentration ratios.
7. The recovered optical signal produced by HRP enzyme increased as microgels number increases.

4.




The signal produced by enzyme reaction was proportional to its concentration.

5.

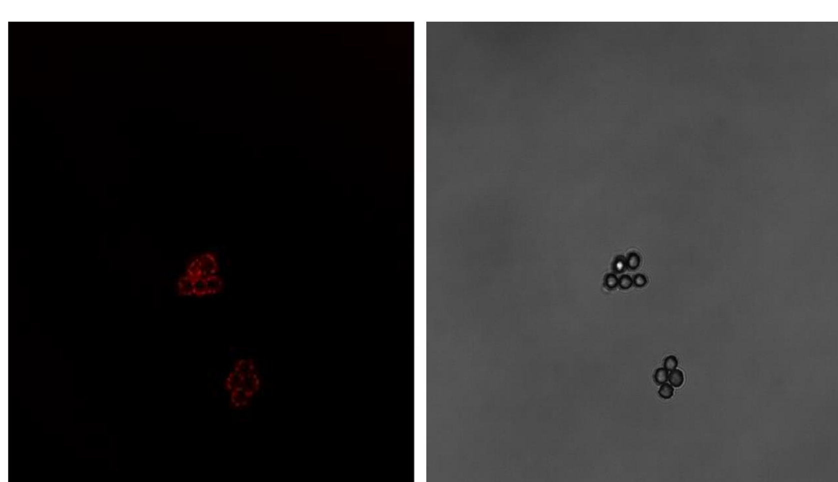


Fluorescence emission of microgel optical barcode

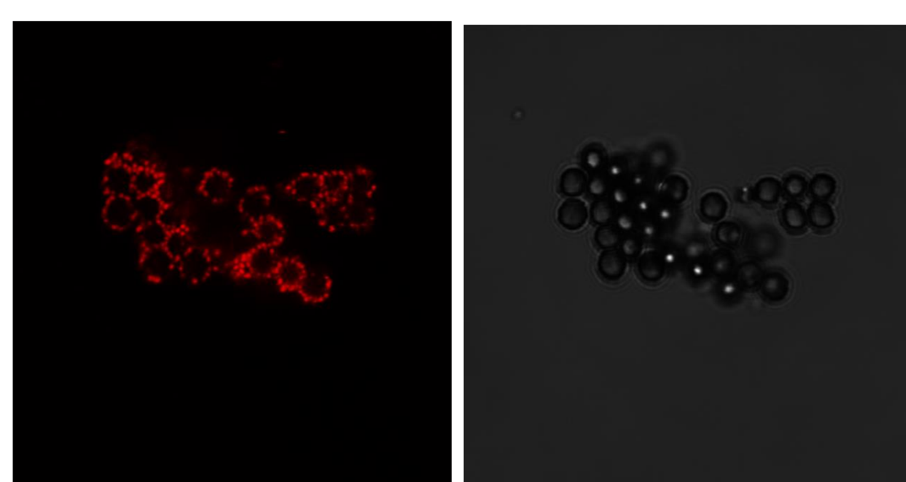
Fluorescence emission of anti-IgG Atto647N onto microgels

Fluorescence emission of anti-IgG Atto647N onto microgels after interaction with magnetic beads

6. LOW AMOUNT OF MICROGELS



HIGH AMOUNT OF MICROGELS



Microgels interacted with magnetic beads resulting in clusters formation. The clusters size increased, as the microgels number increased, too.

7. OPTICAL SIGNAL DETECTION

After the interaction, TMB molecules were introduced to react with the HRP enzyme. The colourimetric signal produced by the enzyme-TMB reaction was recovered. It was proportional to the microgels amount.

## CONCLUSIONS

The microgels-based platform is applied to design innovative immunoassay biosensors. As a proof of concept, IgG anti-IgG antibody system is used to prove the interaction of the particles. After the conjugation of anti-IgG atto647 and HRP onto microgels, biomolecules were still able to exploit their biological activity (Fig. 4,5). The interactions among microgels and magnetic beads produced clusters due to their binding (Fig. 5,6). The next step will involve anti-Fab and anti-Fc antibodies specific for the IgG target binding.

## REFERENCES

[1] Appleyard, D., C.; Chapin, S., C.; Srinivas, R., T.; Doyle, P., S. *Nature Protocols*. **2011**, *6*, 1761-1774.
[2] Causa, F.; Aliberti, A.; Cusano, A., M.; Battista, E.; Netti, P., A. *J. Am. Chem. Soc.* **2015**, *137*, 1758–1761.
[3] Battista, E.; Mazzarotta, A.; Causa, F.; Cusano, A., M.; Netti, P., A. *Polym. Int.* **2016**, *65*, 747–755.