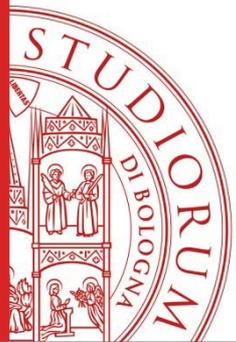


# *When size matters*

*Ramspec @Modena, 2/10/2014*



**Pierluigi  
Reschiglian**  
Full professor



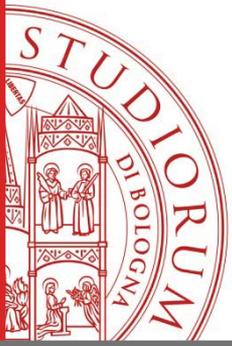
**Barbara  
Roda**  
Researcher



**Andrea  
Zattoni**  
Researcher



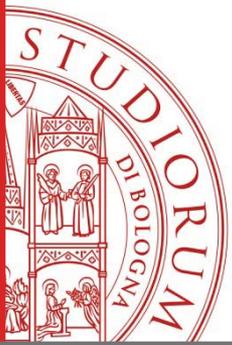
**Valentina  
Marassi**  
PhD student



Our services can be summarized as:

Development of custom-fit methodologies for quality control (QC) of nano/biotech materials to be outsourced to QC labs which employ the same instrumentation.

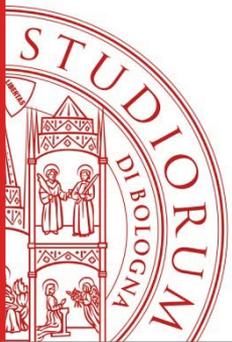
Methods development and customised analyses for clients which manufacture nano/biotech materials.



# Reference techniques for multifunctional NPs characterization

- ✓ Electron microscopy (SEM, TEM)  
↓ no native (dispersion) conditions, time-expensive
- ✓ Light scattering (DLS, PCS, QELS)
- ✓ Spectroscopic technique (UV/Vis, Fluorescence)  
↓ no multimodal data distribution, sample modifications, limits with aggregates

## FFF: a separation method for NPs



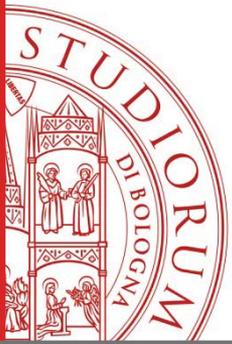
# Our technologies

Separation and characterization  
FFF/SEC/HPLC-UV/DAD-FLD-MALS, and DLS



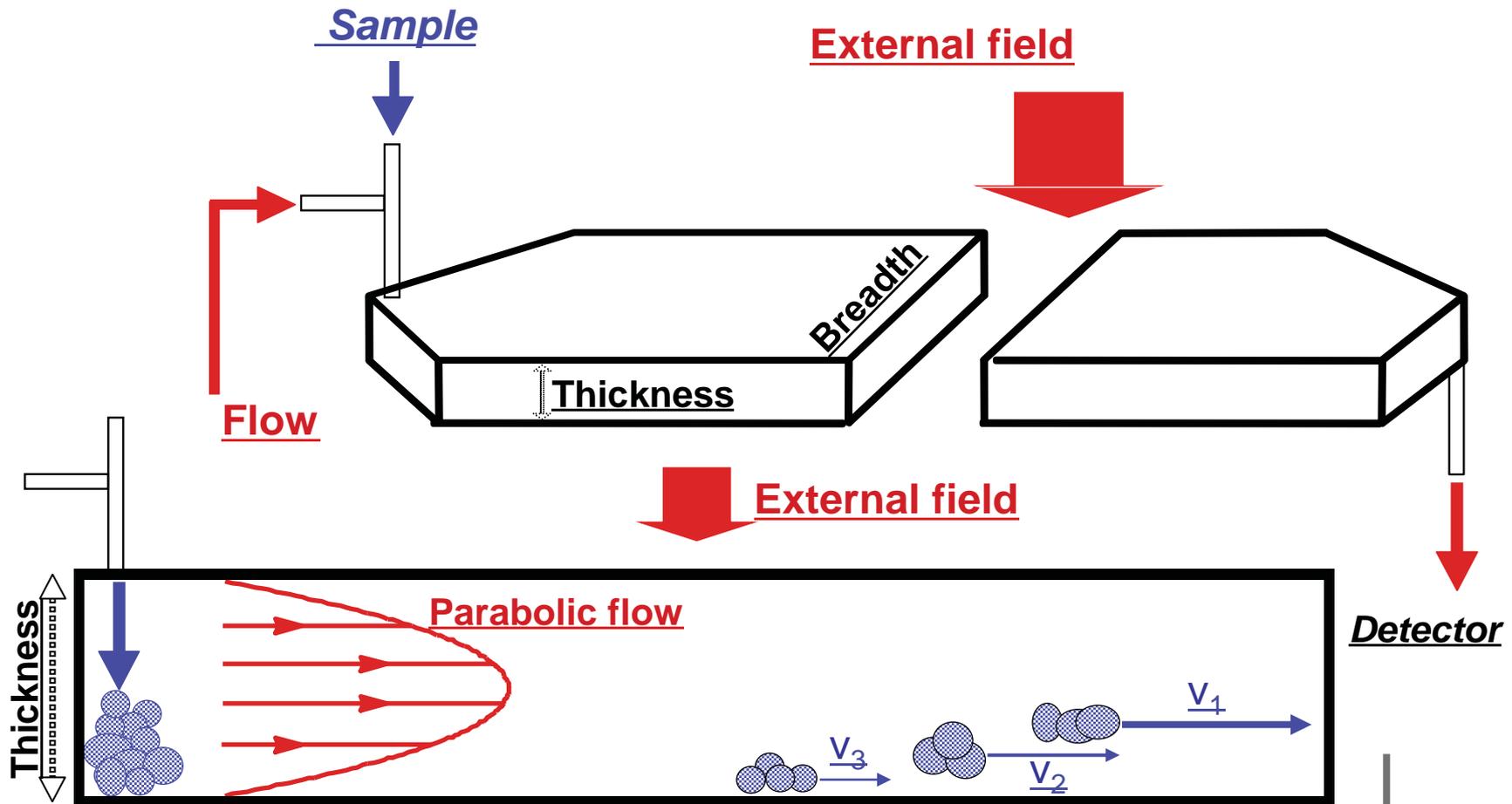
**Eclipse Dualtech, Wyatt Technology Europe GmbH,**  
**Germany**

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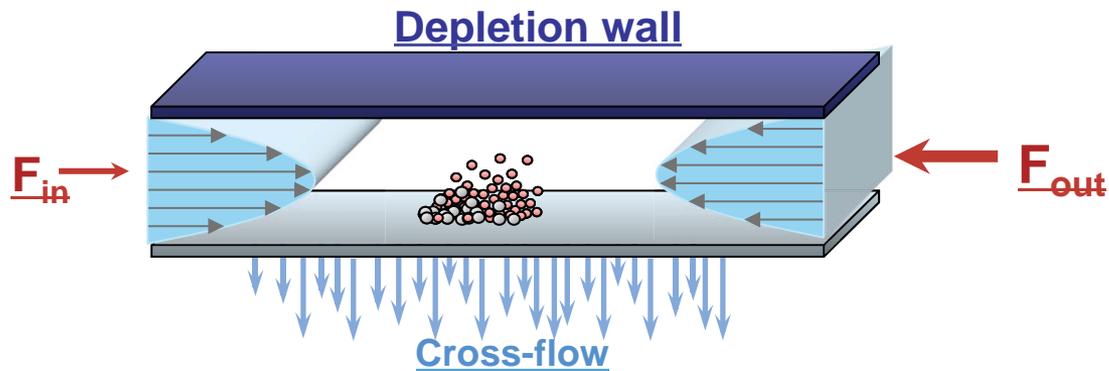


# Flow field flow fractionation

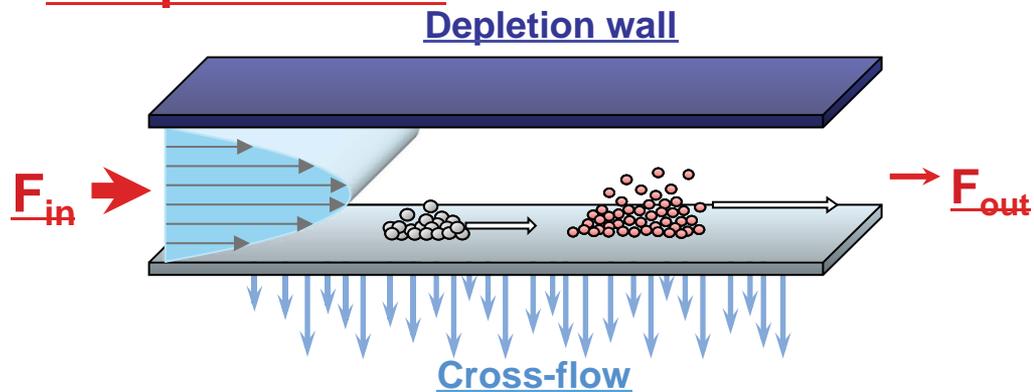
## Separation and characterization

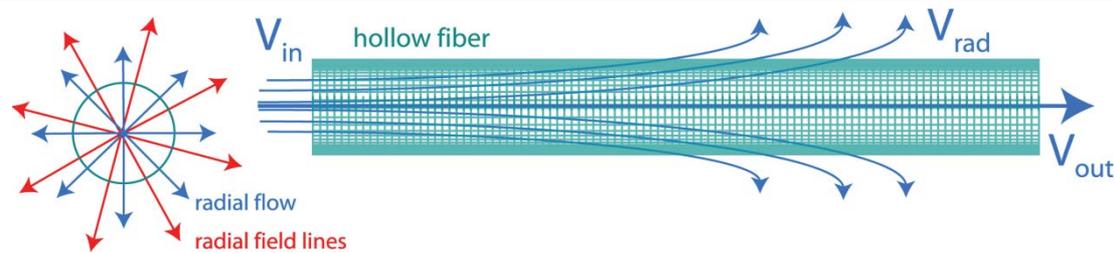


### Sample focusing:



### Sample elution:

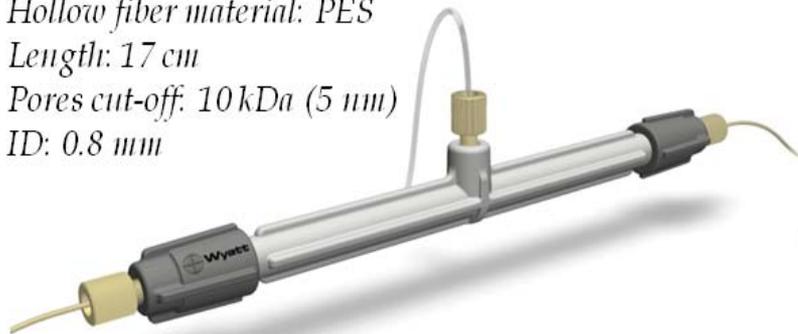




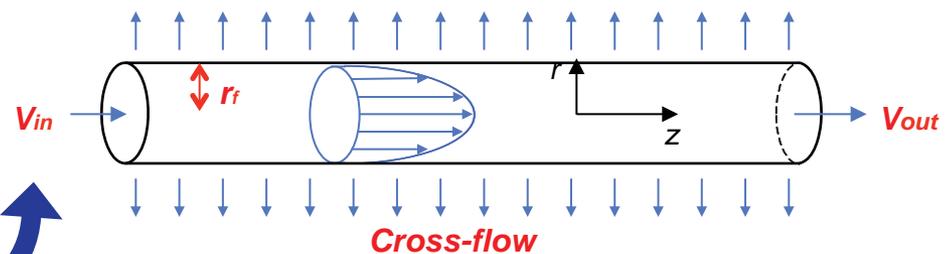
The cross-flow is generated by the elution flow, which splits into a **longitudinal** and a **radial** direction

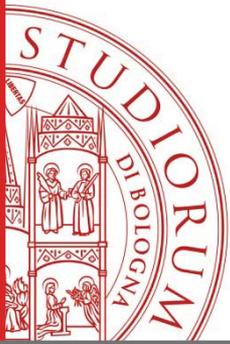
**Cross-flow outlet**

Hollow fiber material: PES  
 Length: 17 cm  
 Pores cut-off: 10 kDa (5 nm)  
 ID: 0.8 mm

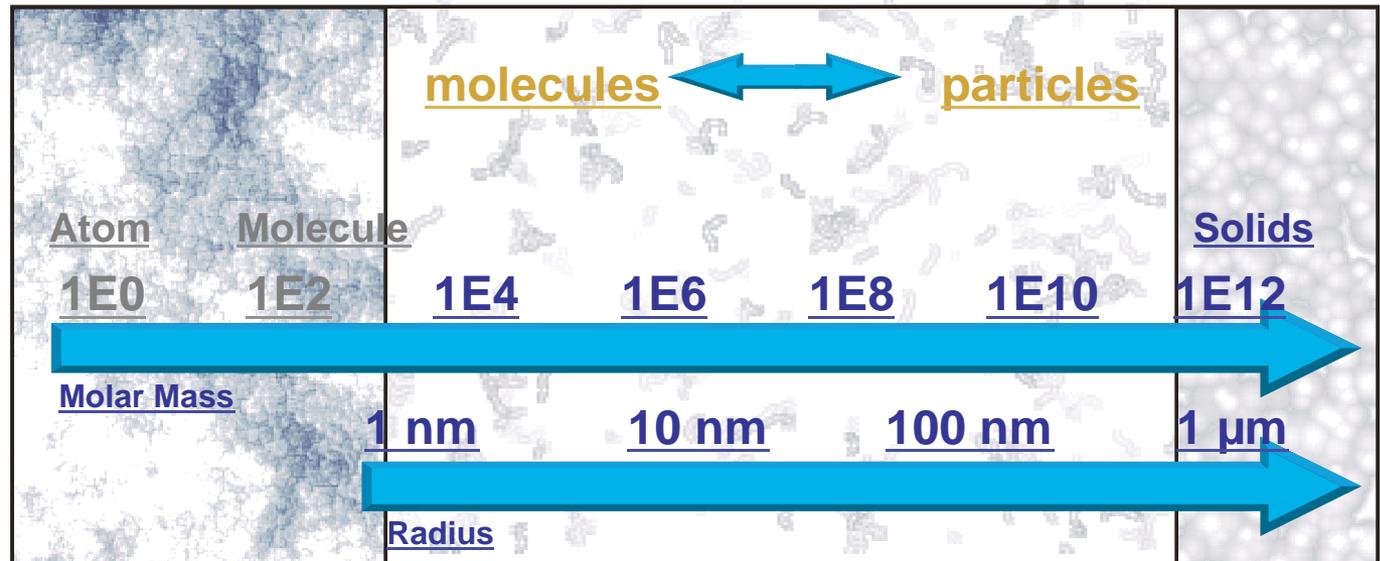


**Hollow Fiber**

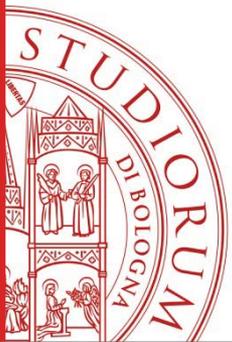




# FIFFF: working range



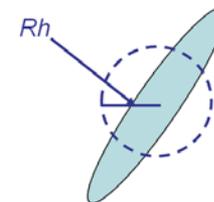
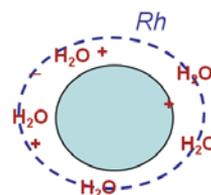
- Macromolecules: proteins, protein complexes, nucleic acids.
- Nanoparticles: polymers, metal nanoparticles, viruses, virus-like particles liposomes, lipoproteins, protein aggregates, subcellular components.
- Microparticles: large protein aggregates, whole cells.



# MALS detection

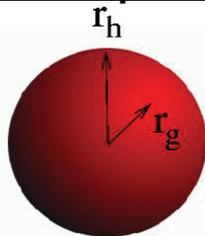
## Conformational study

In **FFF**, a carrier liquid flow generates a second radial/transversal flow (*cross-flow*), allowing for the separation between species presenting different diffusion coefficients. Being the diffusion coefficient of a particle directly linked to its hydrodynamic radius  $r_h$ , a first dimensional information is obtained.

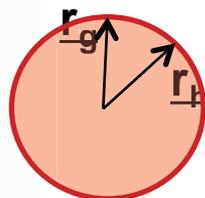


**MALS** detection, on the other hand, allows for the calculation of

**Solid sphere** **Empty sphere**

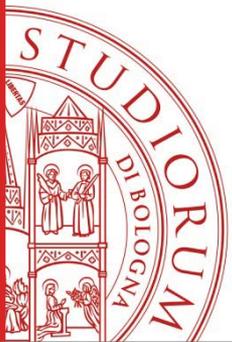


$$\rho = \frac{r_g}{r_h} = 0.77$$



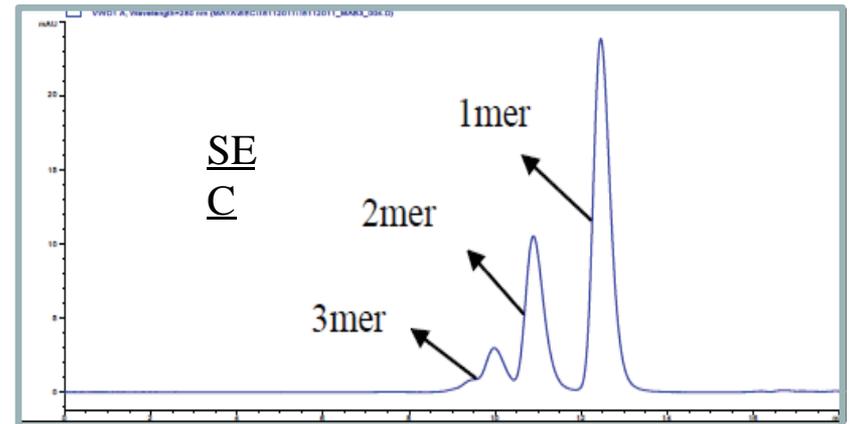
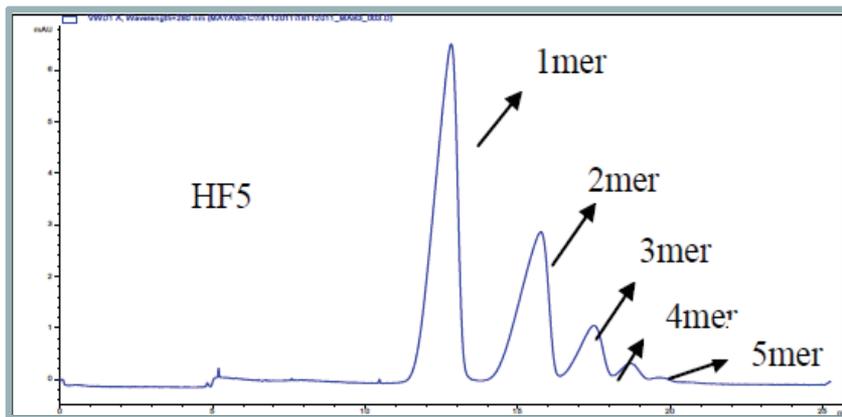
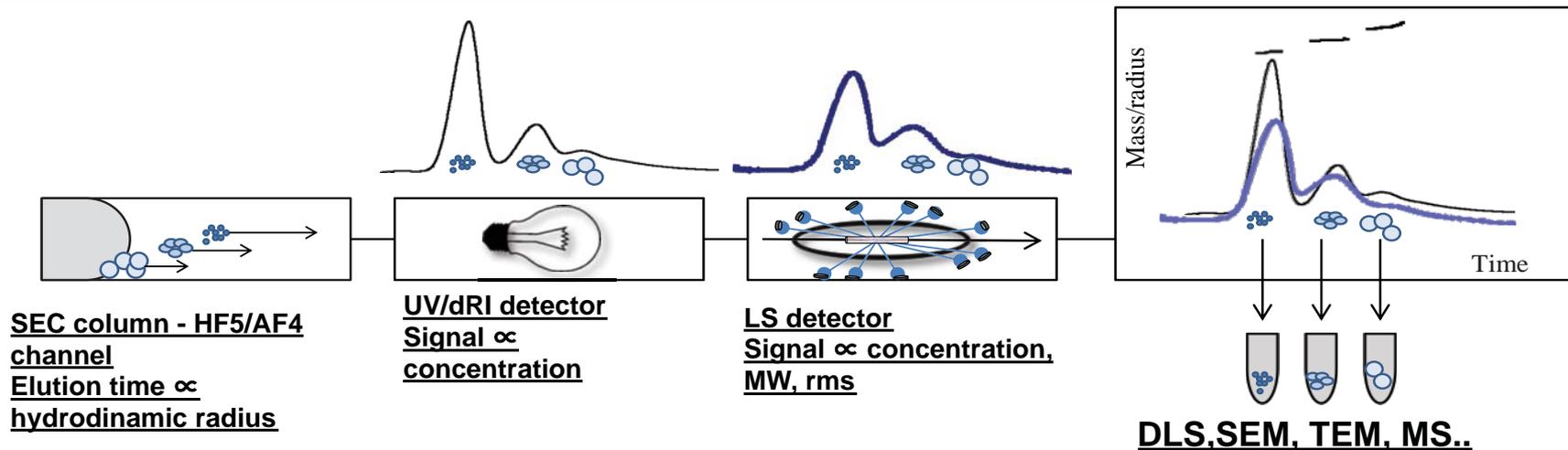
$$\rho = \frac{r_g}{r_h} = 1$$

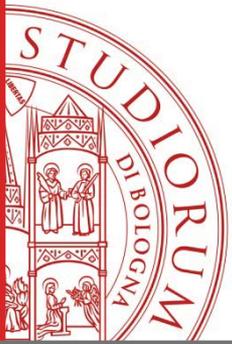
particles' average mean square radius  $r_g$ , which depends on particle shape and compactness. By correlating  $r_g$  and  $r_h$  it is possible to determine particles shape; more in detail, a  $r_g/r_h$  ratio of 1.7 is typical for rod structures.



# The analytical services

## *Setup and potential*

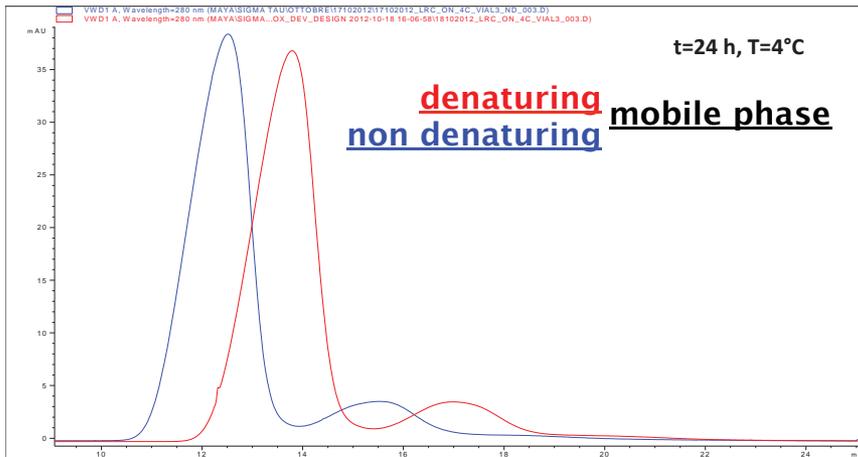
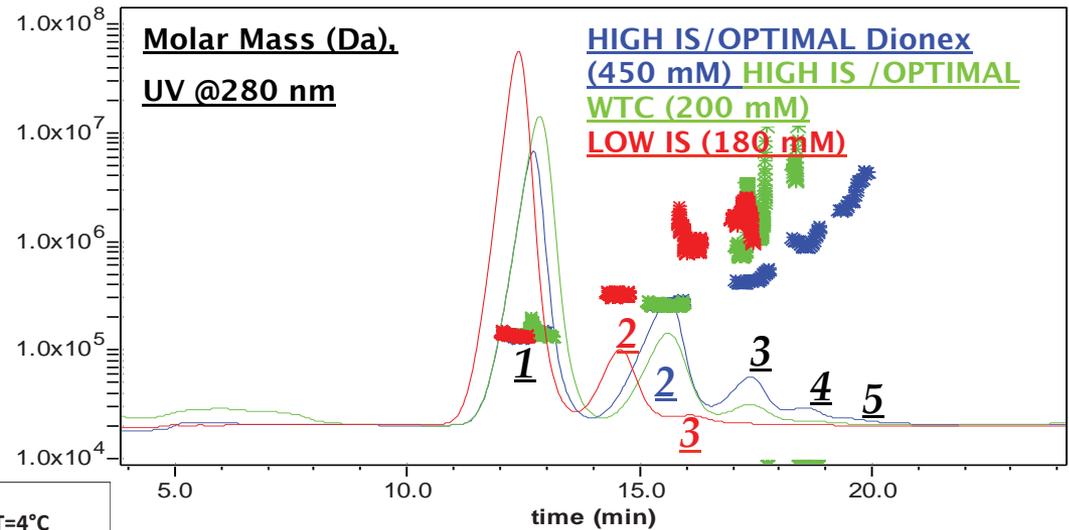




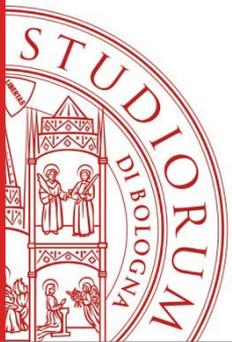
# Problem solving

## Flexibility of separation conditions

### Aggregation of monoclonal antibodies\*



Reversible/  
irreversible  
aggregation of  
therapeutic proteins  
(Avidinox®)



# Problem solving

*Formulation study for commercialisation*

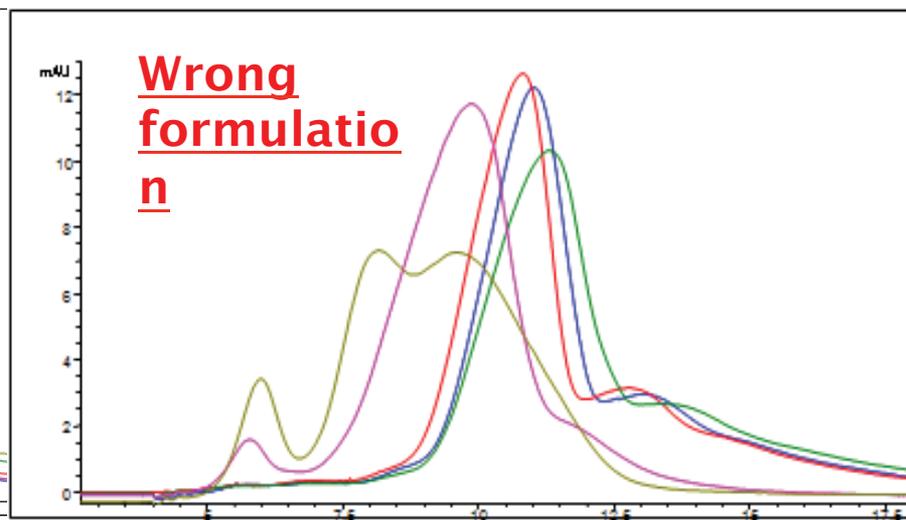
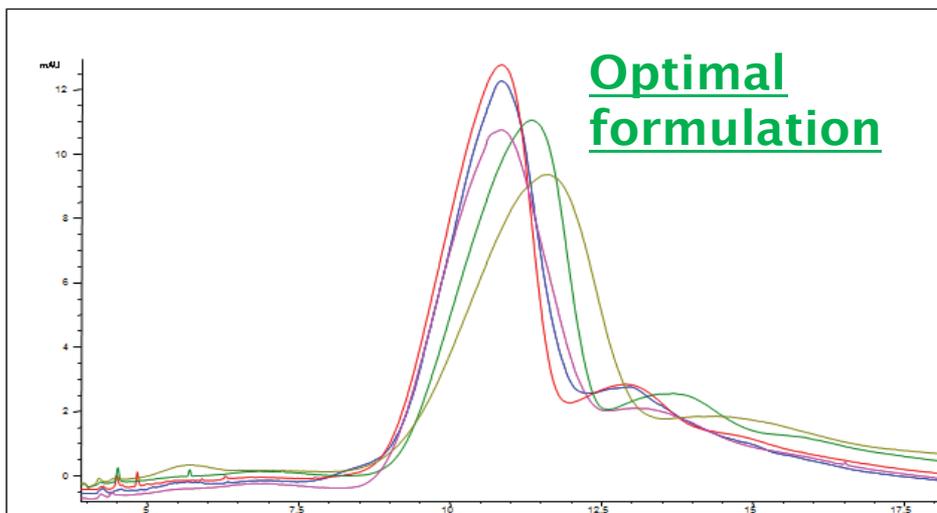
Active principle\*

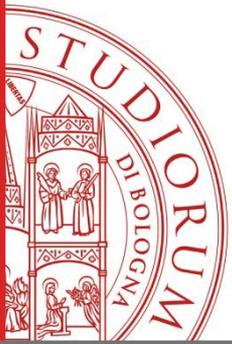


Stability



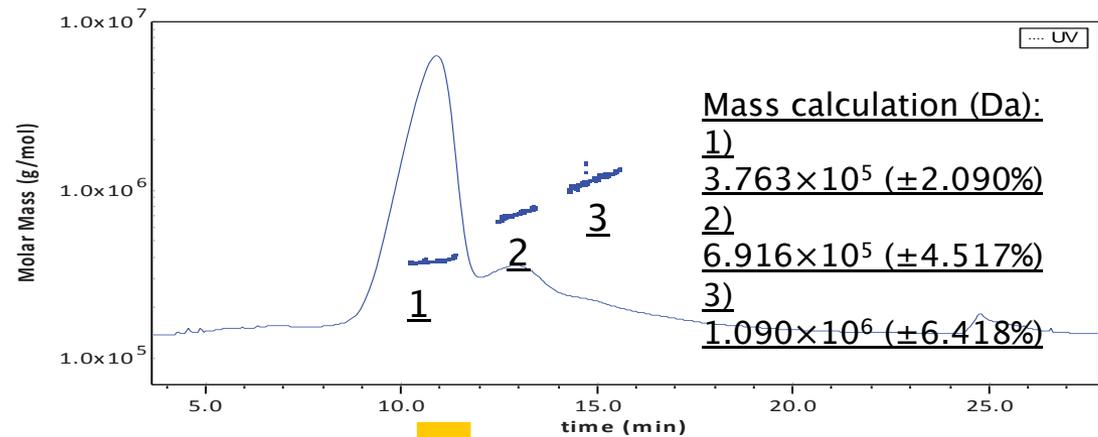
Formulation screening



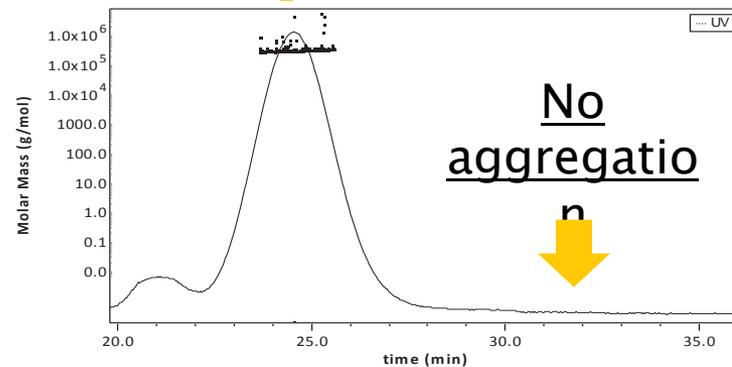


# Problem solving

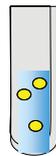
## Characterization of aggregates – safety control

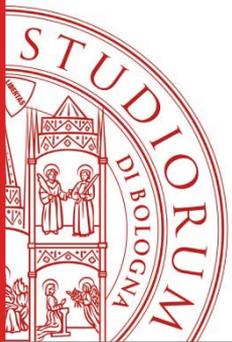


isolation



Stable over time  
Isolated (no aggregates)

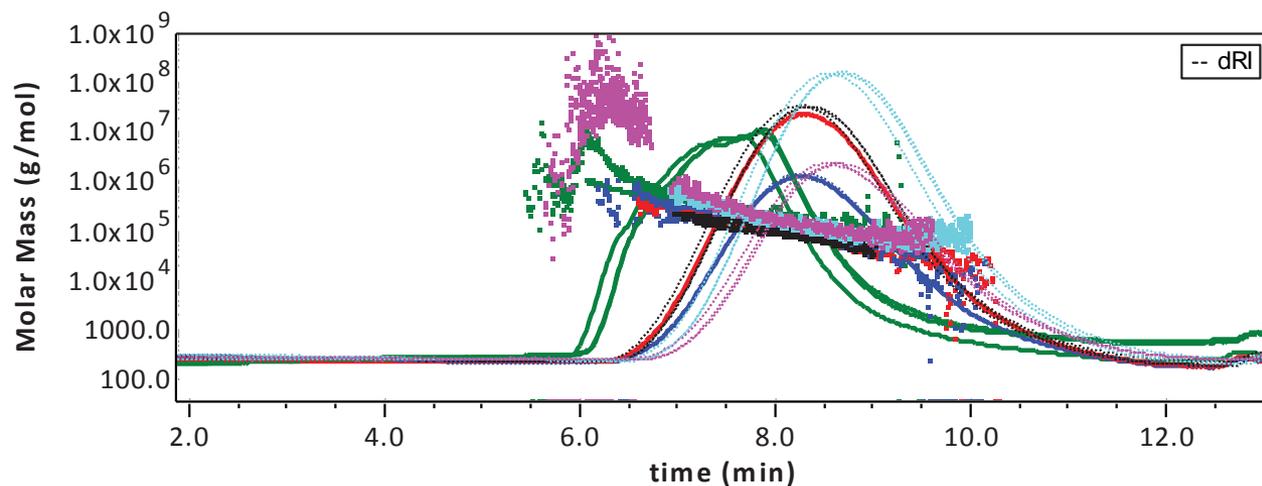




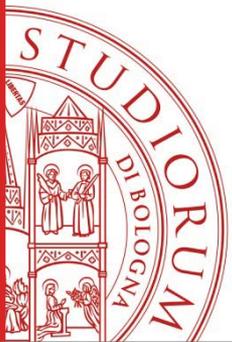
# The analytical services

## Characterization of food thickening agents

Sodium alginates are polysaccharides widely used as a food additive or as a sterile wound dressing. The molecular weight properties of the alginate contribute directly to the end-use performance of these products. When used as a thickening agent, the higher the molecular weight, the better the gel properties will be.

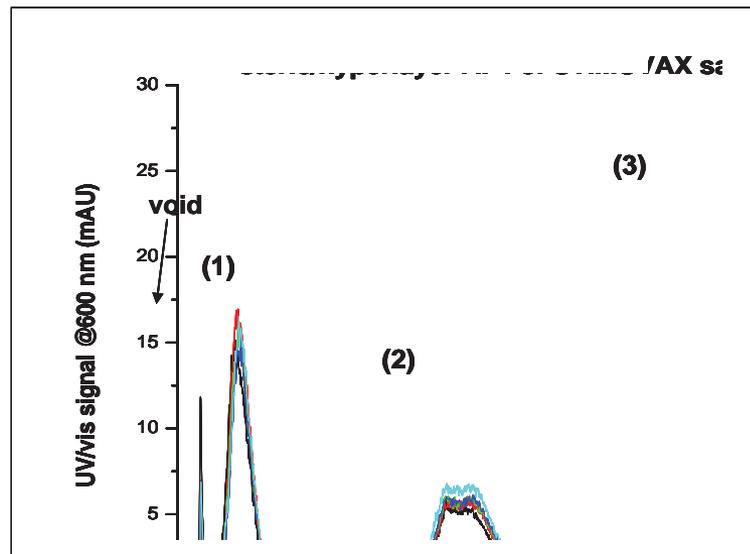


Irradiated/non irradiated alginates can be distinguished and characterized to verify their molar mass and polydispersity

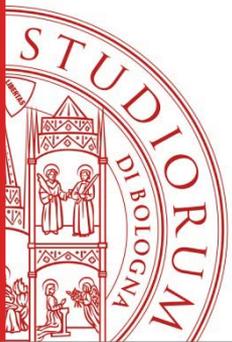


# The analytical services

## Stability study and dimensional study



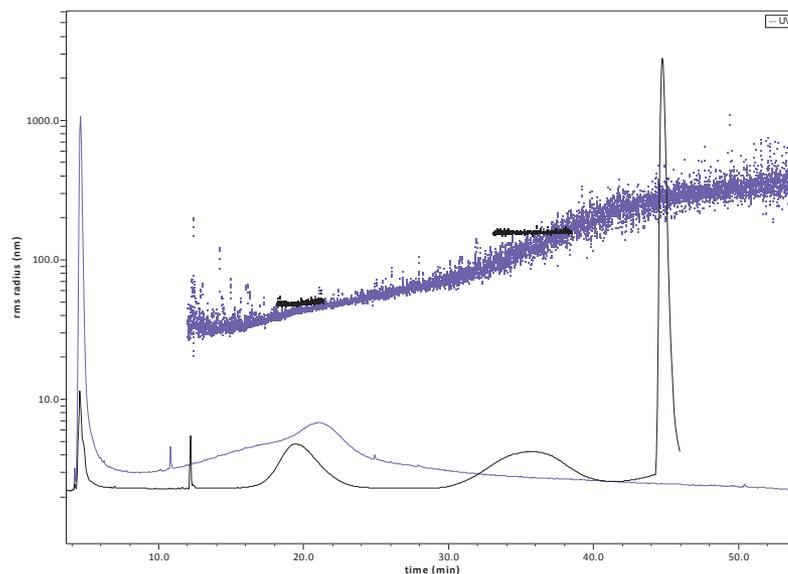
- Due to its high repeatability, steric/hyperlayer AF4 could be used for quality control of the supermicrometer-sized population of [OMITTED]. Fractions with a narrow size distribution can be collected and reused for further characterization and applications.
- From the tentative, preliminary DLS analysis a substantial amount (about one third) of submicrometer-sized particles was found in [OMITTED]. Online AF4-DLS could be then used to obtain additional information on the [OMITTED] size distribution in a very broad size range. Correlation with different levels of bioassay response could be then sought.



# The analytical services

## Tattoo ink

Feasibility study and rapid method development for the fractionation and subsequent characterization of tattoo inks.



AF4(HF5)-UV-MALS allowed to characterise tattoo inks of different colours.  
The use of standards allow for a further validation of the method.

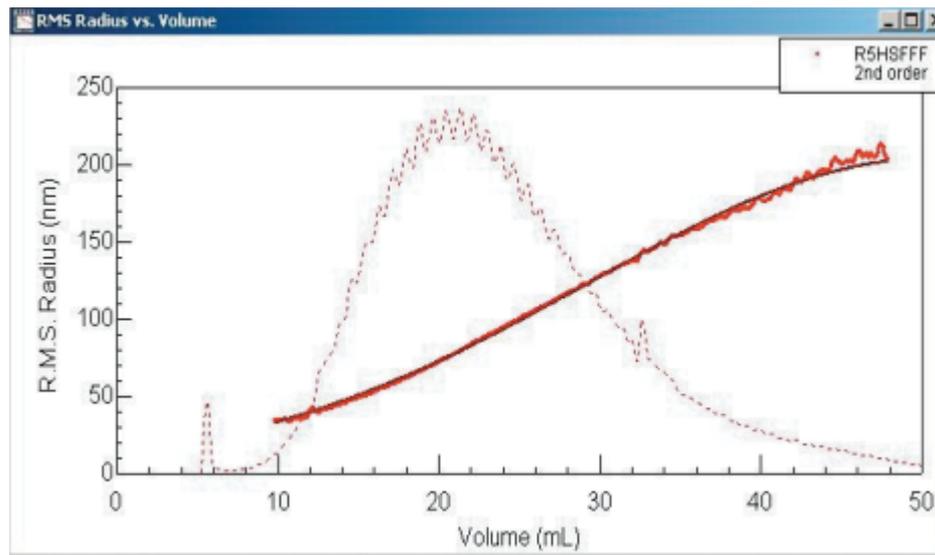
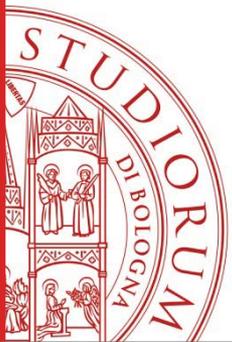


Figure 1. FlowFFF-MALLS analysis of soil colloids. FlowFFF constant field run was applied and Astra particles mode used with a linear Zimm fitting. Nearly linear increase of RMS radius with elution volume and good linear Zimm fitting of LS data even with larger particles.

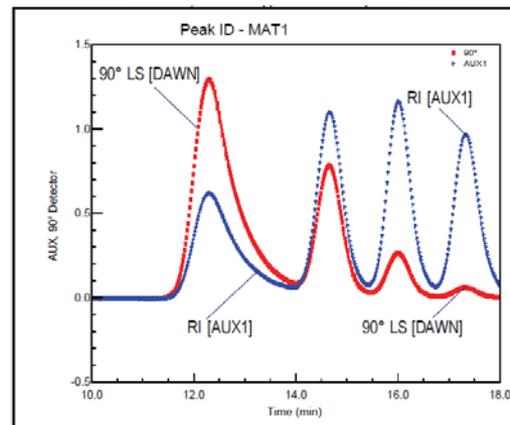
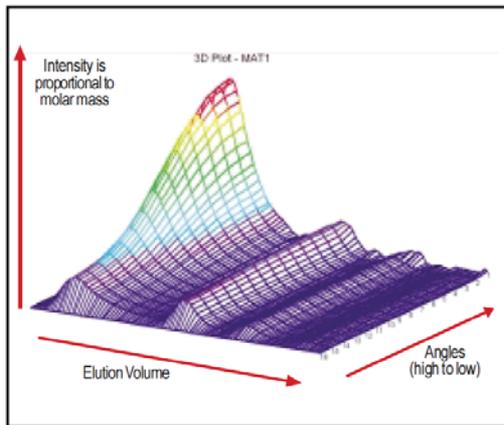
Synthetic iron oxides showed ratios  $R_g/R_h$  around 1 (thick rods, aspect ratio  $< 5$ ) while soil extracts showed values up to 1.6 (indicative of thin plates).



# The analytical services

## Polymers

By connecting a laser scattering detector to any chromatography system, the absolute molar mass and size distributions of a sample can be determined directly, without resorting to calibration or making assumptions about a sample's conformation.



**Polydimethylsiloxane**  
**(SEC-MALS, toluene)**  
**A simple calibration with**  
**standards would have been**  
**erroneous!**

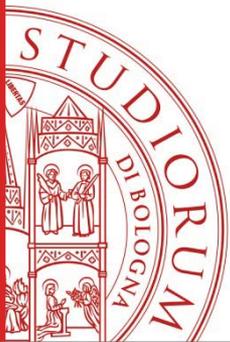
**Polychloroprene**  
**(SEC-MALS, in THF).**  
**Samples could not be**  
**distinguished by the**  
**response from a**  
**viscosimeter !**

**Polyurethane**

**Polyethylene oxide (PEO)**

**Polystyrene Nanocomposites**

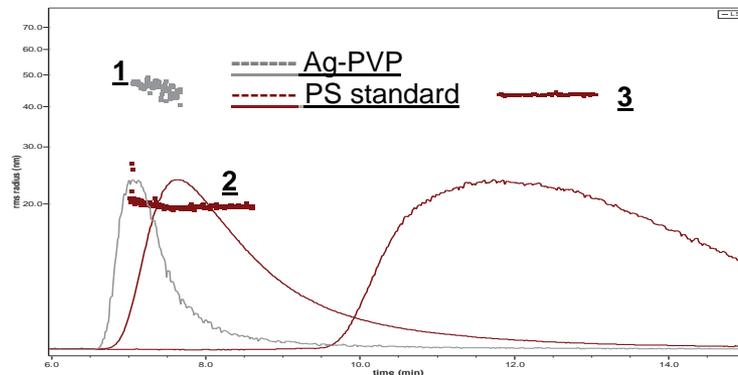
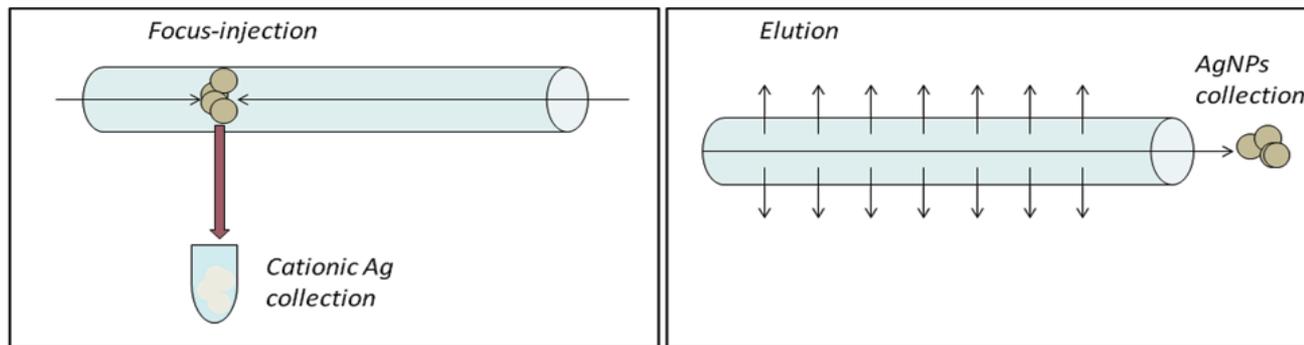
**Branching of PMMA**



# The analytical services

## Metal nanoparticles

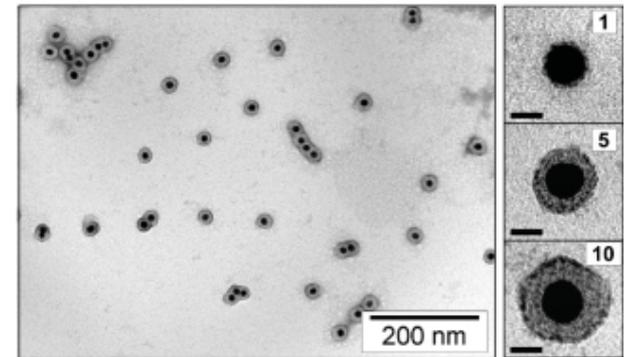
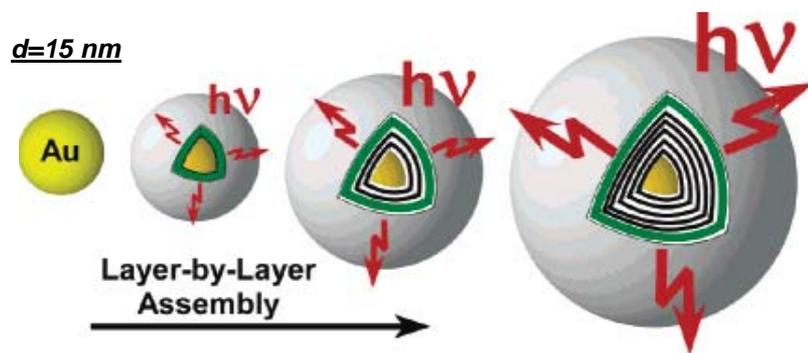
A method able to size characterize AgPVP nanoparticles has been developed and demonstrated to be able to study both behaviour of particles over time and Ag<sup>+</sup> release from particles themselves.



1. AgPVP nanoparticles; rh=25nm, rg=45nm
2. Polystyrene standard; rh=25.5nm, rg=20nm
3. Polystyrene standard; rh=51.5nm, rg=40nm

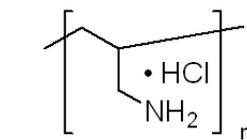
# The analytical services

## LBL-coated metal nanoparticles



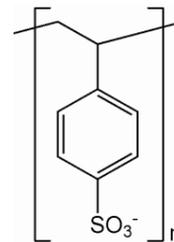
G Schneider, G Decher Nano Letters (2006), 6, 530-536

Poly Allylamine  
Hydrochloride (PAH)

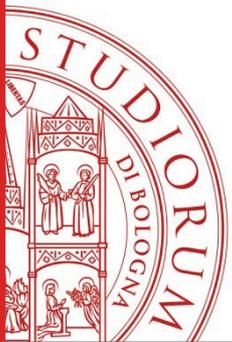


MW = 15 kDa

Sodium Polystyrene  
Sulfonate (PSS)

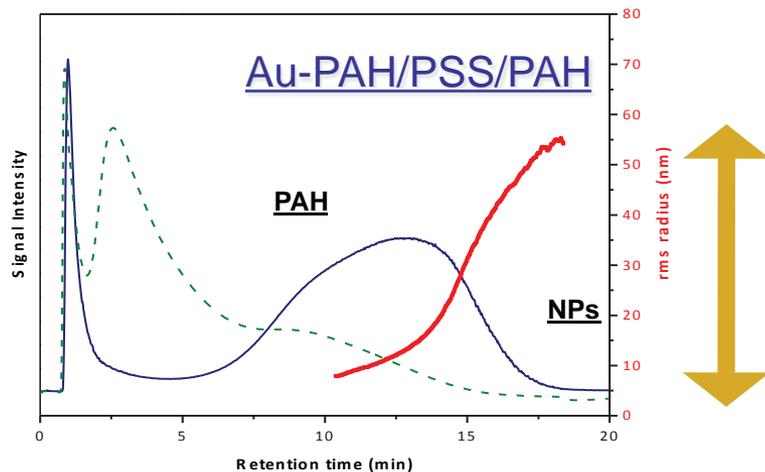


MW = 4.3 kDa

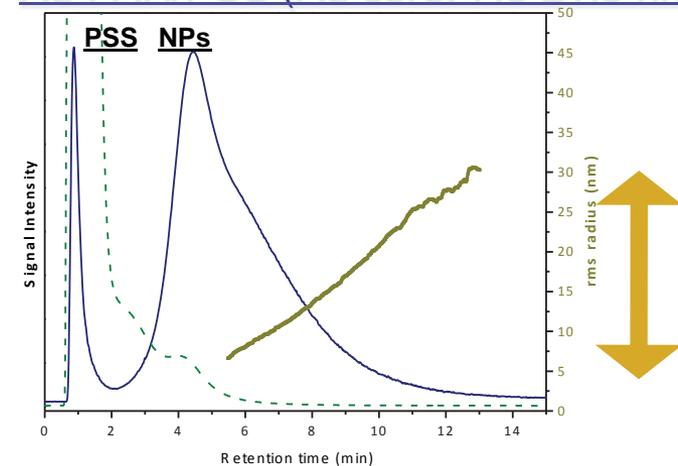


# AF4-MALS of multilayered gold NPs

Free polymer separated from the NPs

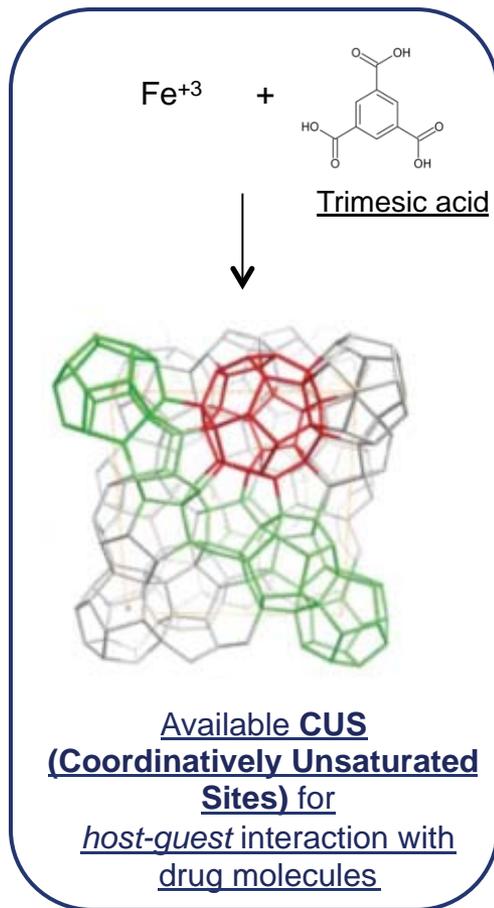


**Au-PAH/PSS (Au core:  $7.5 \pm 1.5$  nm)**



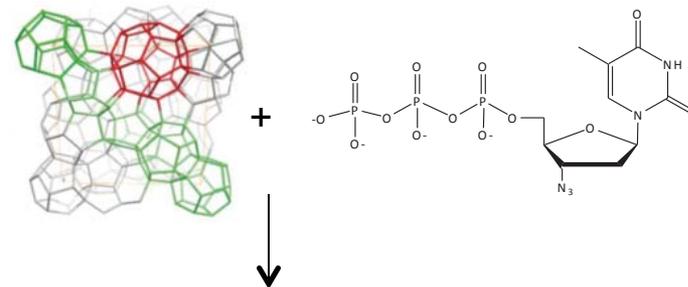
UV signal @ 230 nm - MALS signal @ 90°

Higher state aggregation for triple-layer NPs



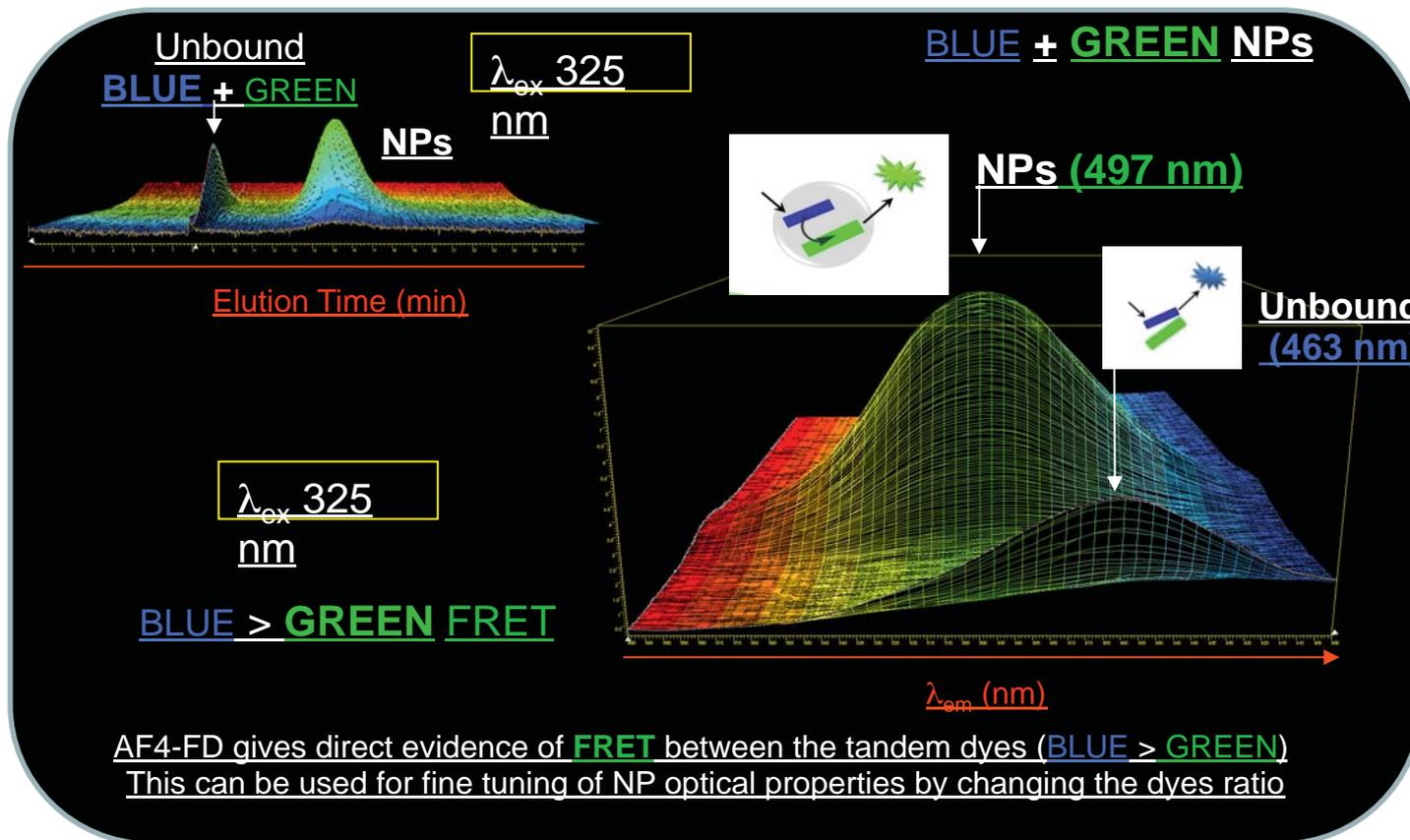
Crystalline NPs made of metal ions / clusters coordinated to organic molecules

- ▶ Regular Porosity
- ▶ Convenient synthesis (mild conditions)
- ▶ Biodegradability
- ▶ Biocompatibility

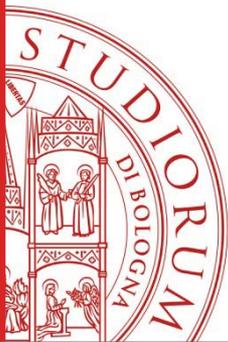


When used as nanovectors

- Do they interact with the drug?
- Do they aggregate?
- Size distribution?



## Fluorescence-resonance energy transfer



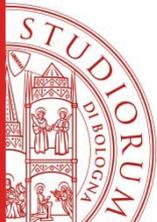
## What more we offer Consulting and more

Method development, synthesis support, expert advice and consulting from the highest level professional and researchers) with the comfort of having a single reference.

- HPLC- UV -FLD
- DLS
- UV-Vis and DAD spectrophotometers (well plate option)
- Atomic absorption (flame and graphite furnace)
- GC-MS, LC-MS
- IR
- SEM, TEM
- Scientific databank access

### Some examples:

- CTU technical reports
- Analyses of Calcium residues in bone matrix (IOR, Bologna)
- Asbestos detection in water samples..



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

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Dipartimento di Chimica G. Ciamician  
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*www.unibo.it*