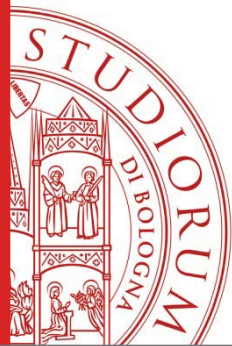


When size matters

Ramspec @Modena, 2/10/2014



**Pierluigi
Reschiglian**
Full professor



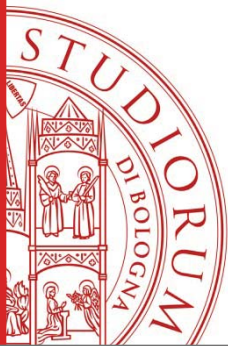
**Barbara
Roda**
Researcher



**Andrea
Zattoni**
Researcher



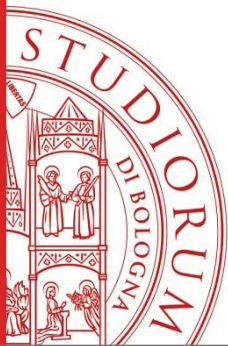
**Valentina
Marassi**
PhD student



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.

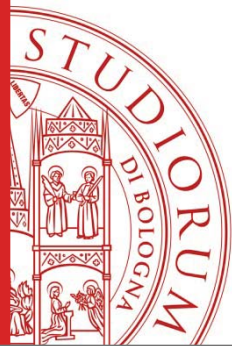
Methods development and customised analyses for clients which manufacture nano/biotech materials.



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

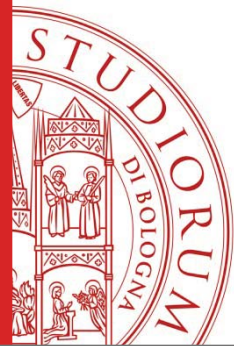
Separation and characterization
FFF/SEC/HPLC-UV/DAD-FLD-MALS, and DLS



Eclipse Dualtech, Wyatt Technology Europe GmbH,
Germany

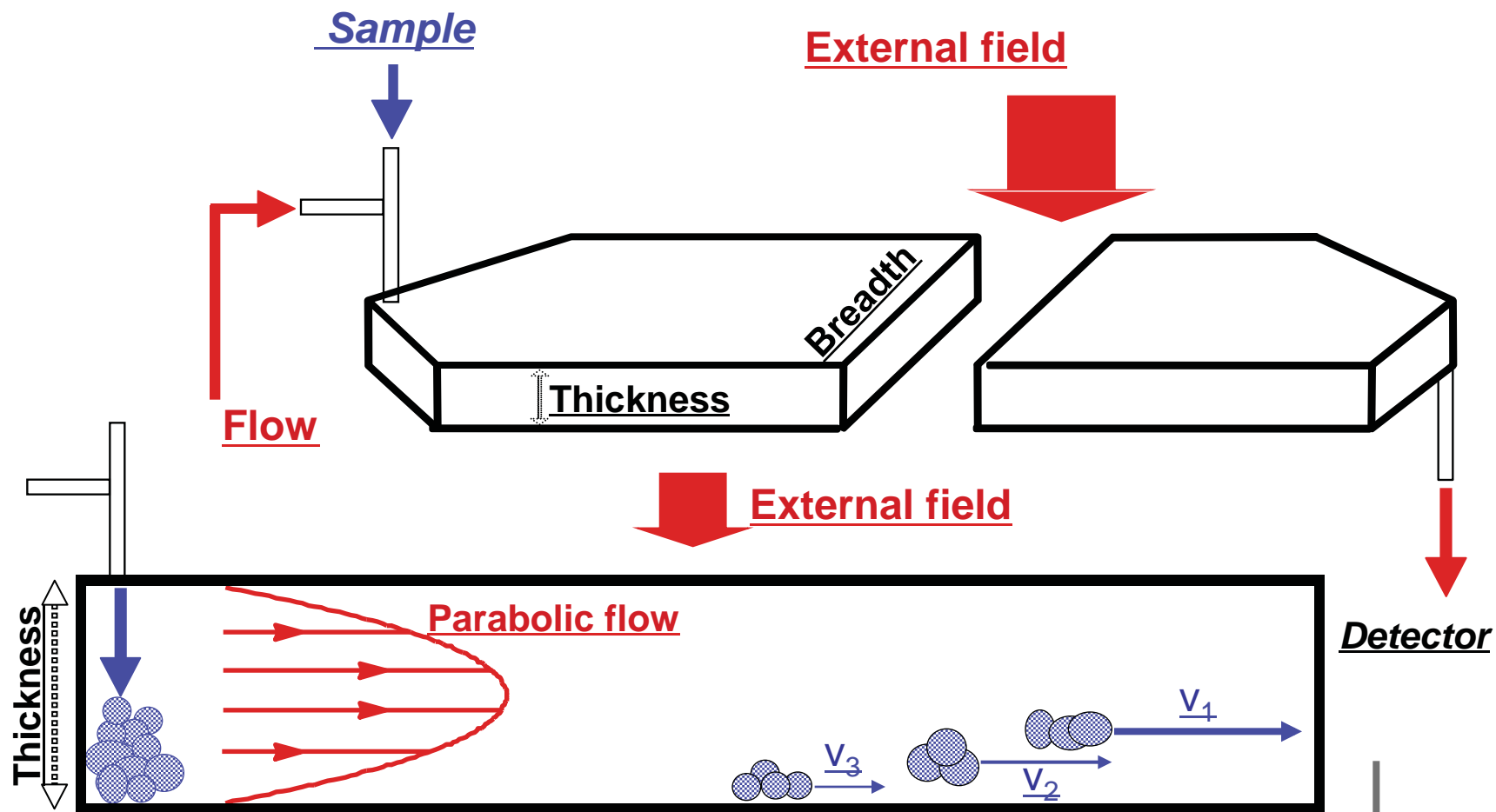
ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA

IL PRESENTE MATERIALE È RISERVATO AL PERSONALE DELL'UNIVERSITÀ DI BOLOGNA E NON PUÒ ESSERE UTILIZZATO AI TERMINI DI LEGGE DA ALTRE PERSONE O PER FINI NON ISTITUZIONALI

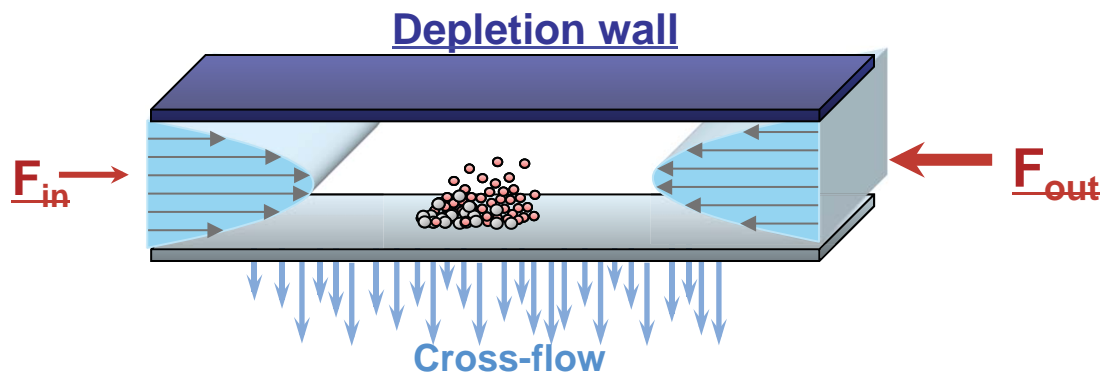


Flow field flow fractionation

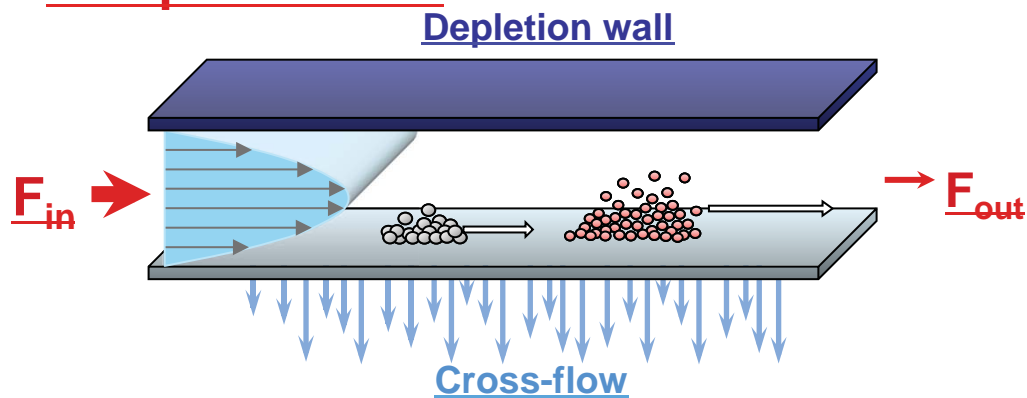
Separation and characterization

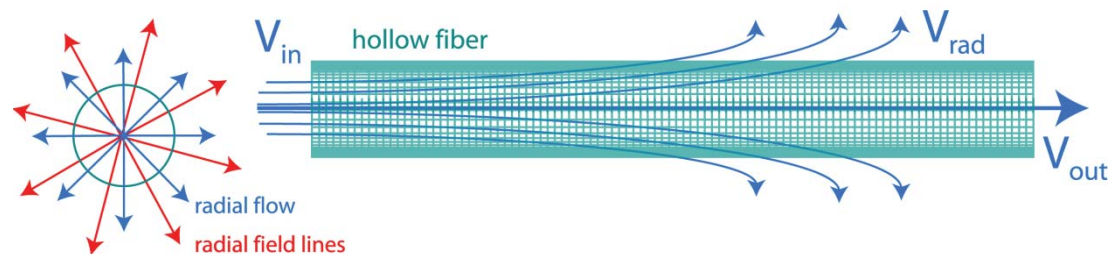


Sample focusing:



Sample elution:

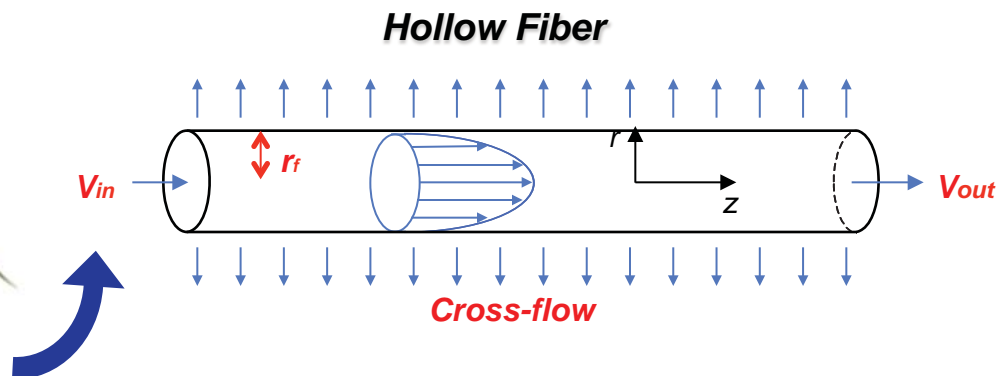
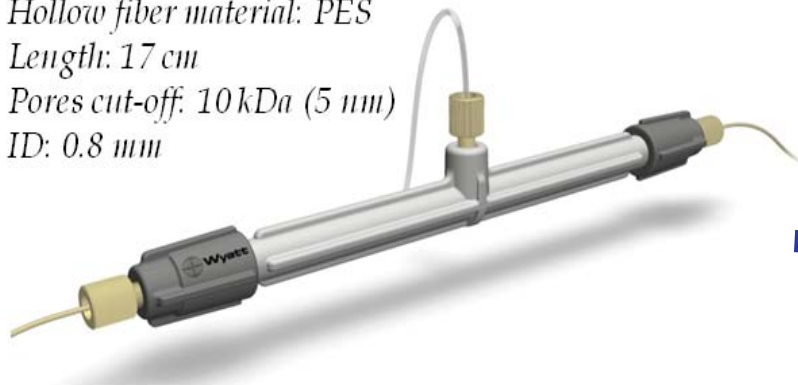




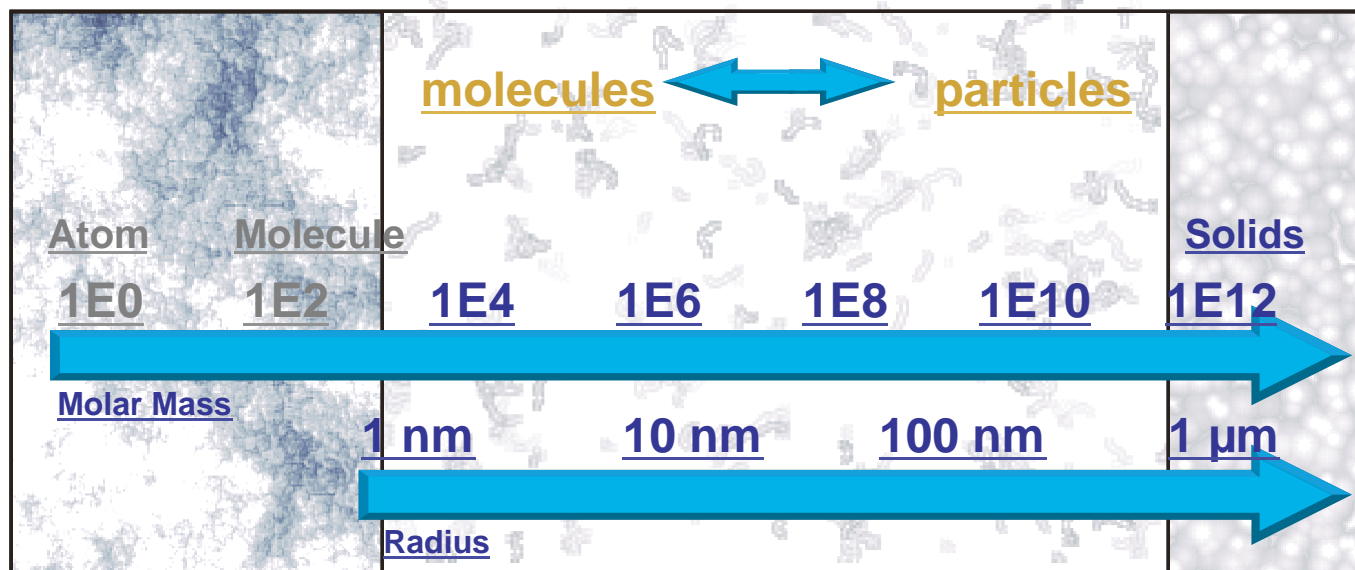
The cross-flow is generated by the elution flow, which splits into a **longitudinal** and a **radial** direction

Cross-flow
outlet

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

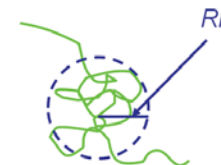
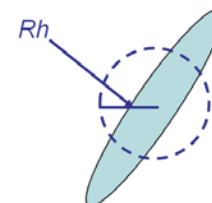
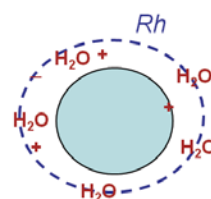


FIFFF: working range



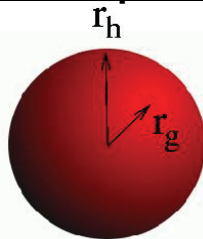
- Macromolecules: proteins, protein complexes, nucleic acids.
- Nanoparticles: polymers, metal nanoparticles, viruses, virus-like particles liposomes, lipoproteins, protein aggregates, subcellular components.
- Microparticles: large protein aggregates, whole cells.

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.

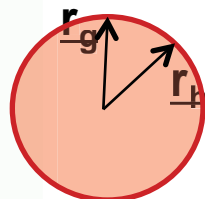


MALS detection, on the other hand, allows for the calculation of

Solid sphere **Empty sphere**



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

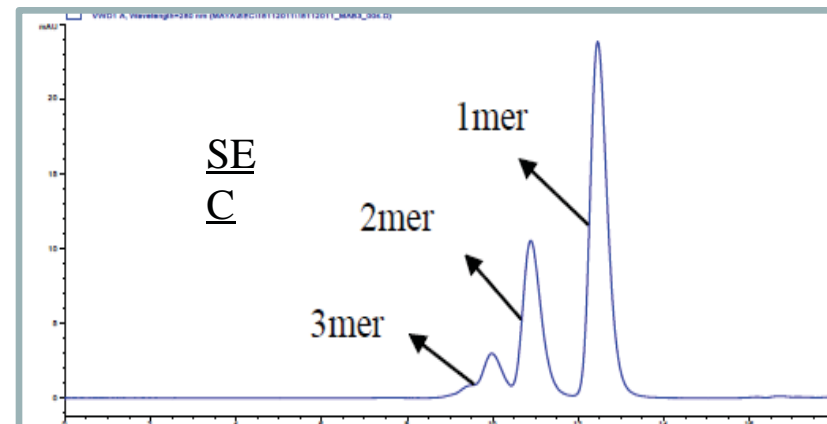
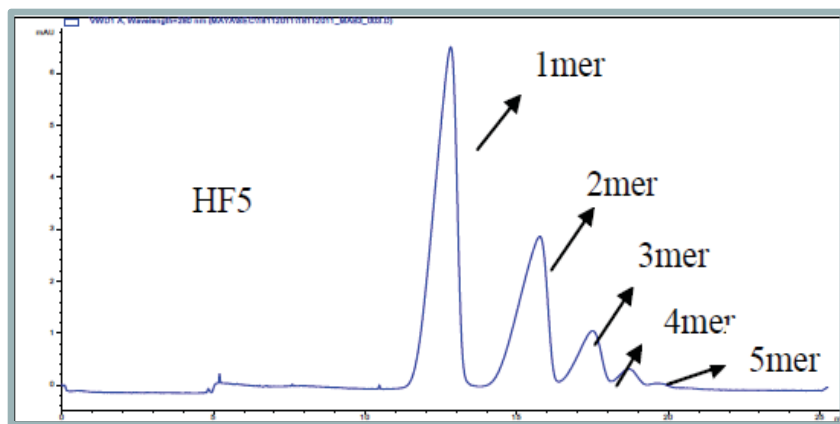
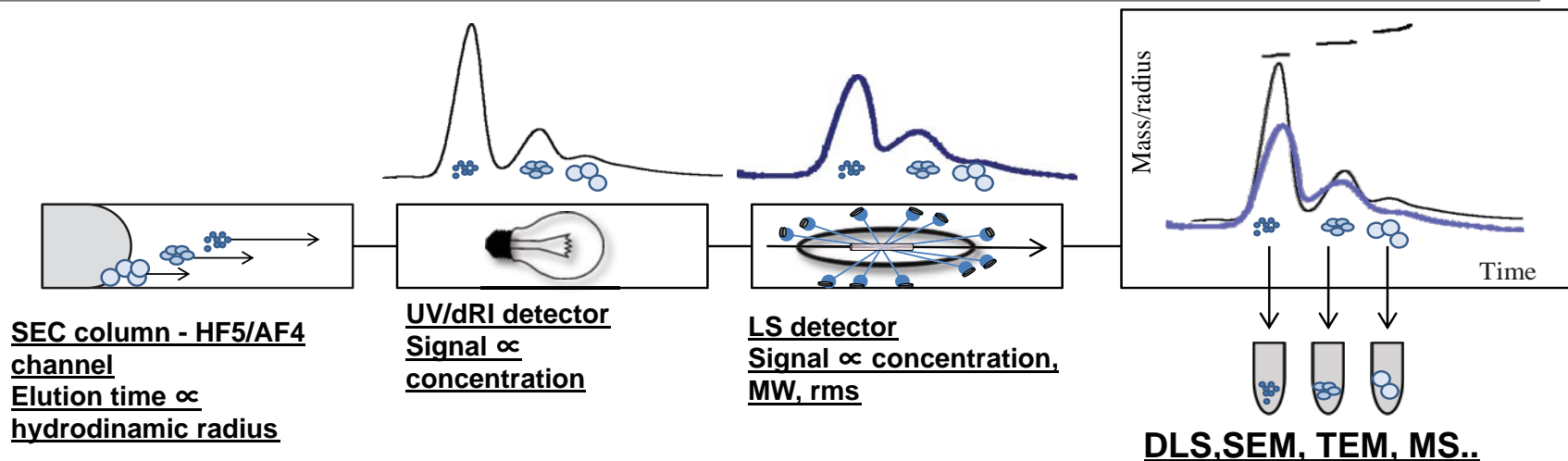


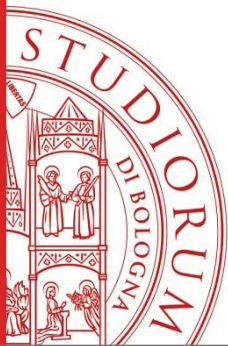
$$\rho = \frac{r_g}{r_h} = 1$$

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

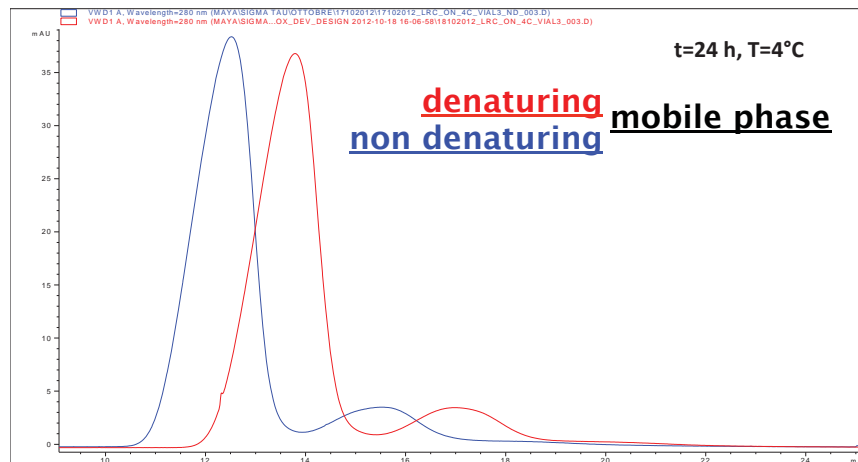
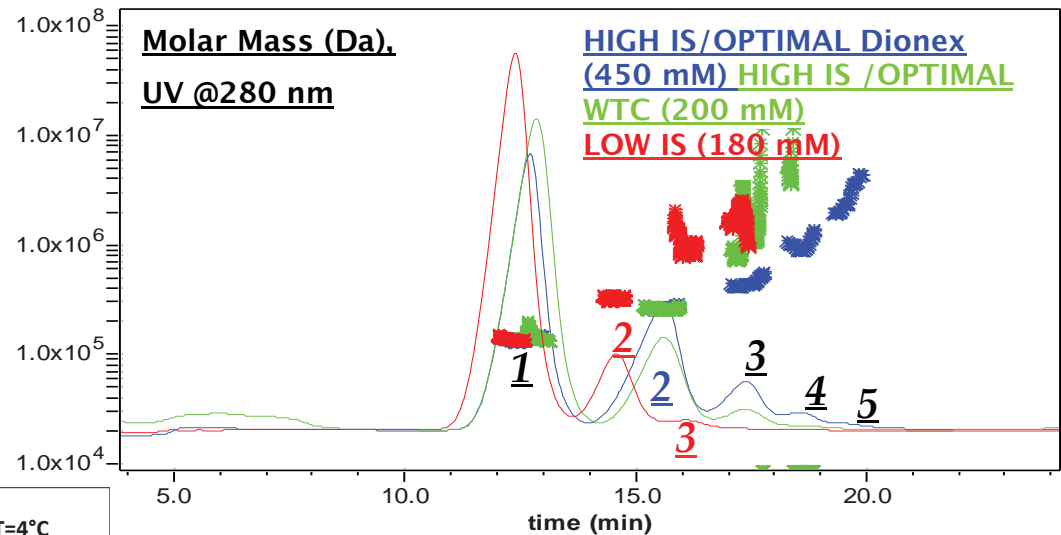




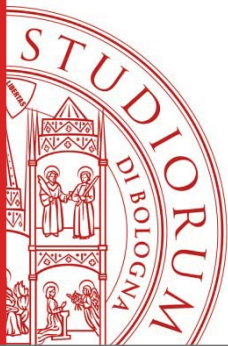
Problem solving

Flexibility of separation conditions

Aggregation of monoclonal antibodies*



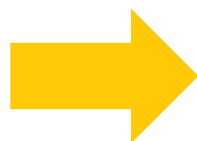
Reversible/
irreversible
aggregation of
therapeutic proteins
(Avidinox®)



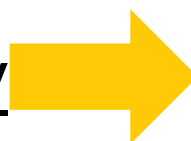
Problem solving

Formulation study for commercialisation

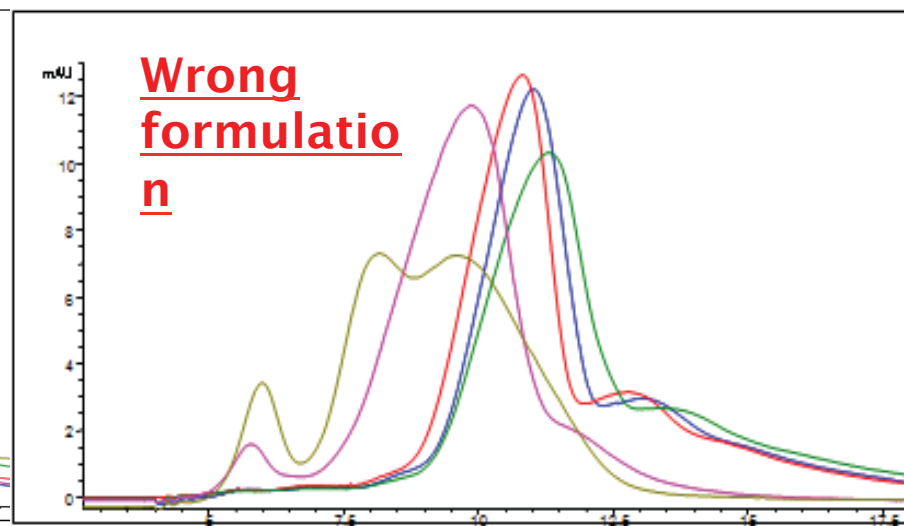
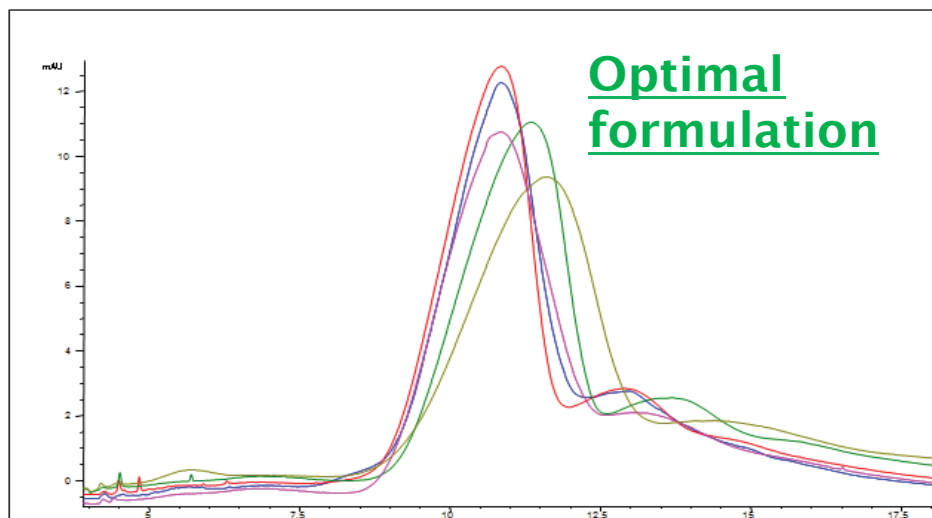
Active principle*

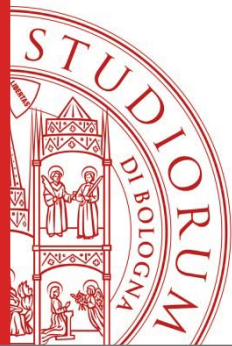


Stability



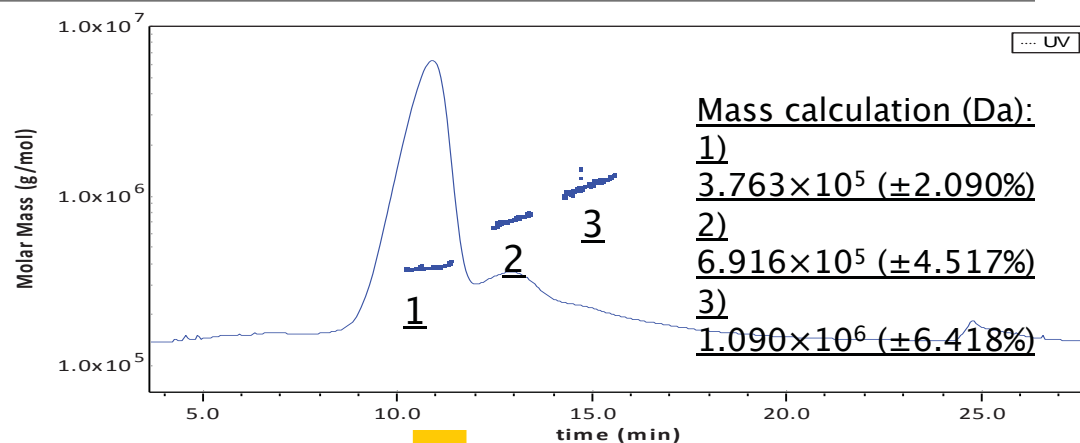
Formulation screening



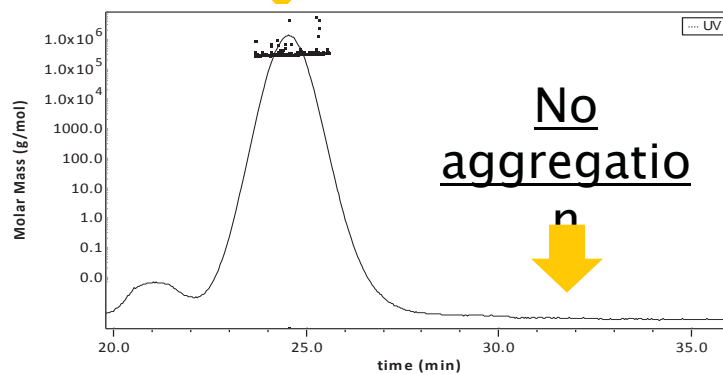


Problem solving

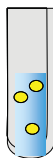
Characterization of aggregates – safety control

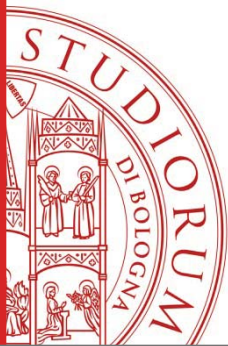


isolation



Stable over time
Isolated (no aggregates)

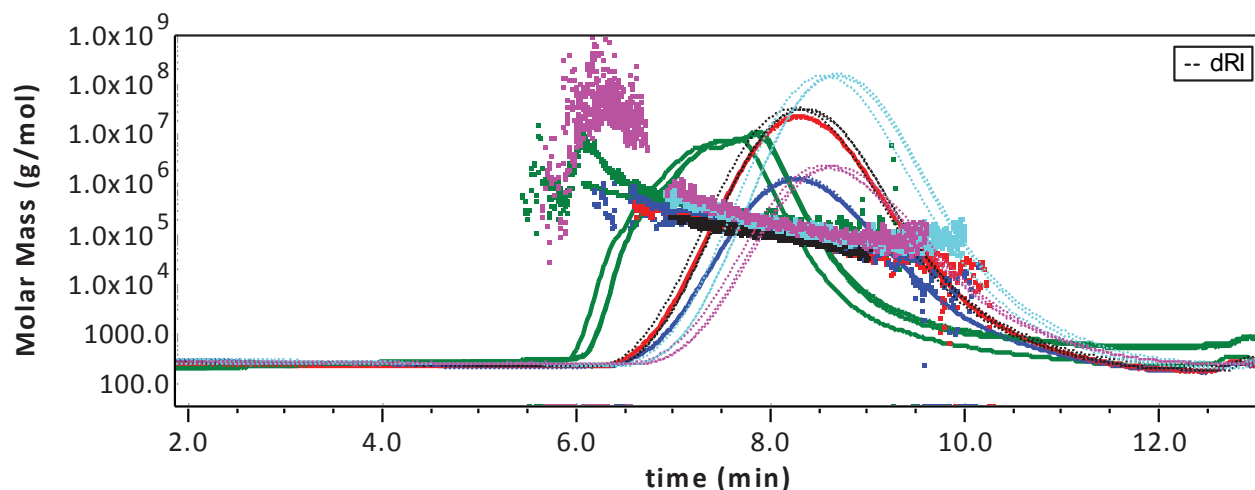




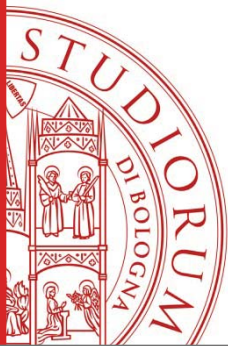
The analytical services

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.

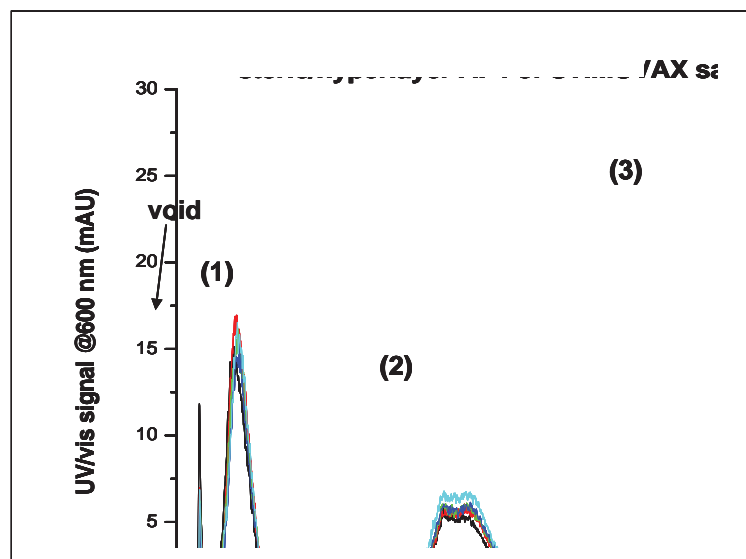


Irradiated/non irradiated alginates can be distinguished and characterized to verify their molar mass and polydispersity

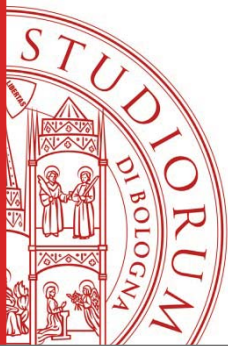


The analytical services

Stability study and dimensional study



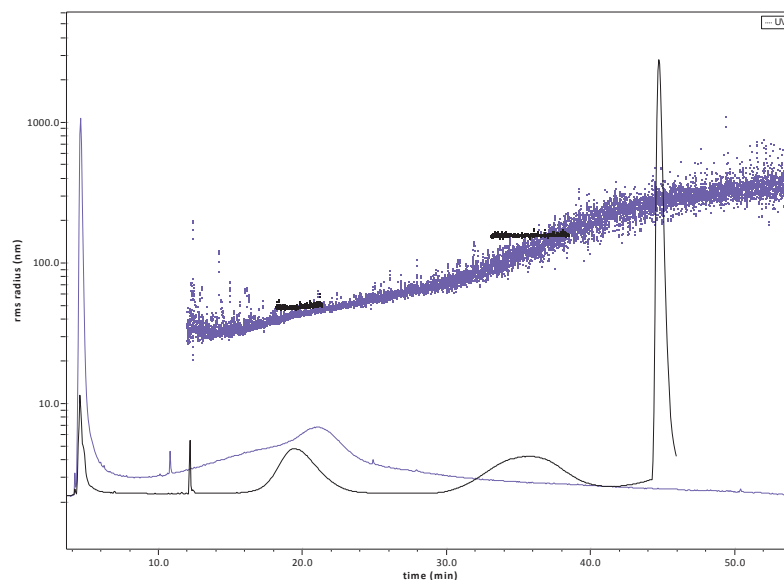
- Due to its high repeatability, steric/hyperlayer AF4 could be used for quality control of the supermicrometer-sized population of [OMITTED]. Fractions with a narrow size distribution can be collected and reused for further characterization and applications.
- From the tentative, preliminary DLS analysis 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.

The analytical services

Environmental particles

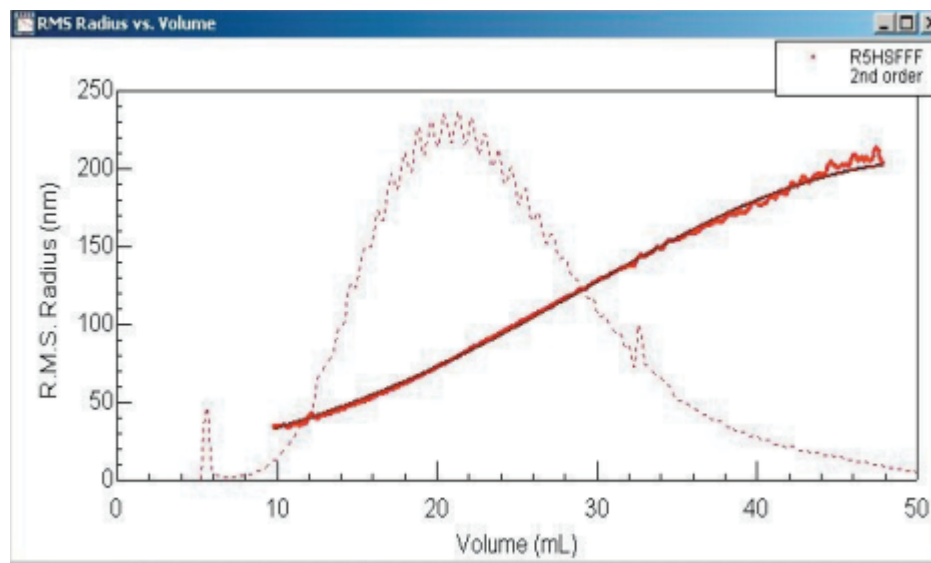
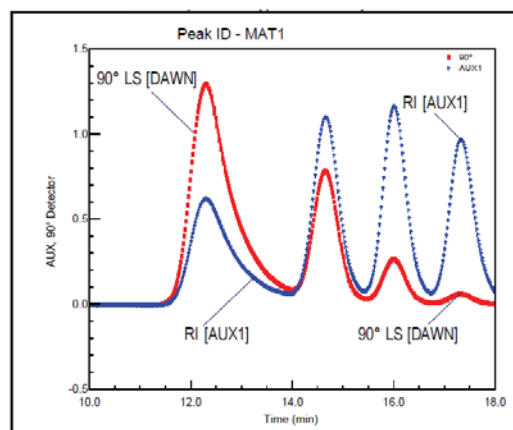
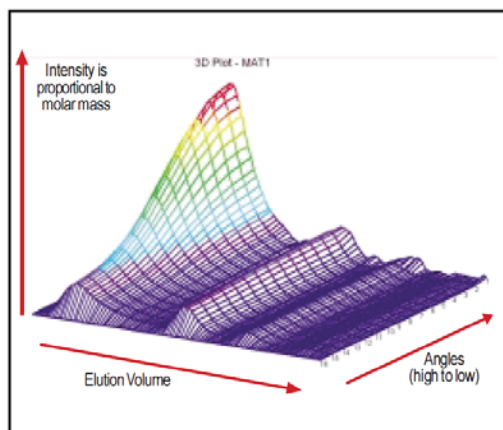


Figure 1. FlowFFF-MALLS analysis of soil colloids. FlowFFF constant field run was applied and Astra particles mode used with a linear Zimm fitting. Nearly linear increase of RMS radius with elution volume and good linear Zimm fitting of LS data even with larger particles.

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).

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)

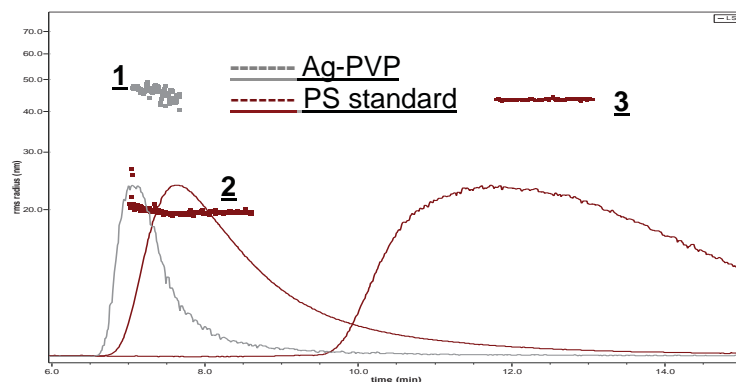
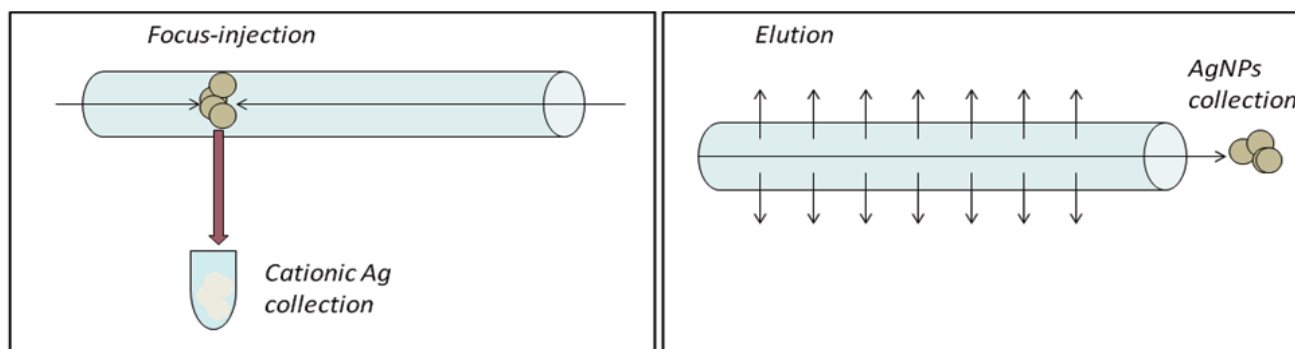
Polystyrene Nanocomposites

Branching of PMMA

The analytical services

Metal nanoparticles

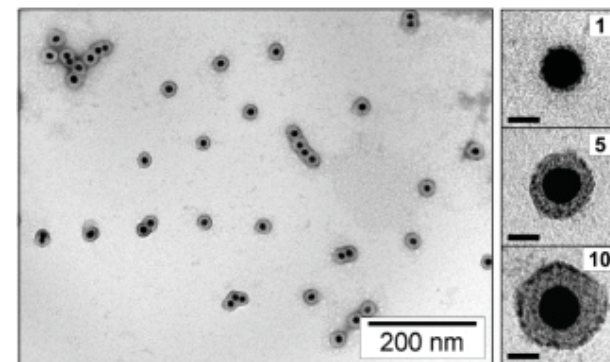
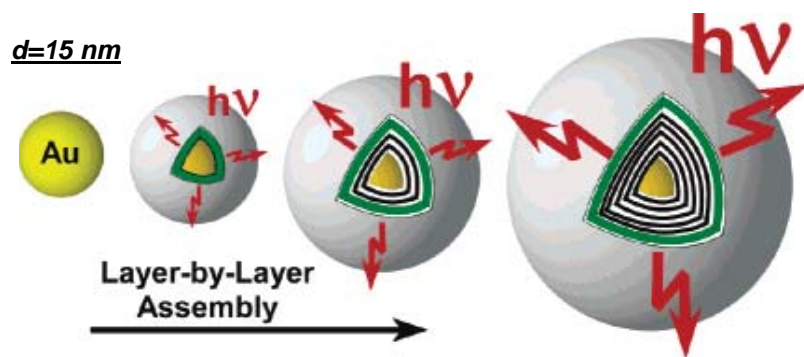
A method able to size characterize AgPVP nanoparticles has been developed and demonstrated to be able to study both behaviour of particles over time and Ag⁺ release from particles themselves.



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

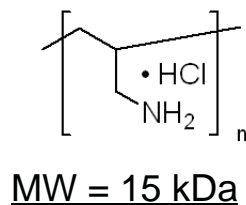
The analytical services

LBL-coated metal nanoparticles

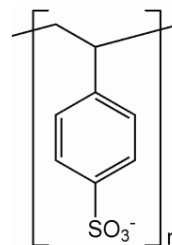


G Schneider, G Decher Nano Letters (2006), 6, 530-536

Poly Allylamine
Hydrochloride (PAH)

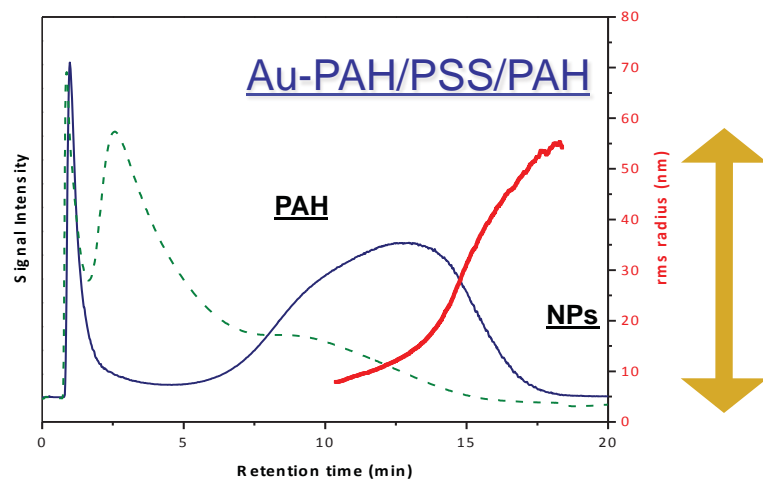


Sodium Polystyrene
Sulfonate (PSS)

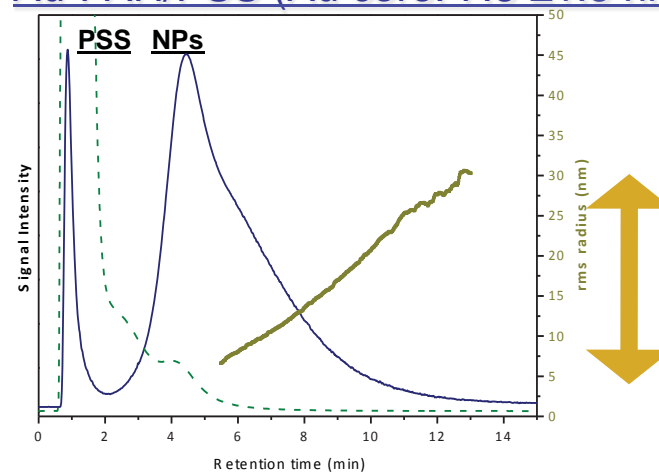


MW = 4.3 kDa

Free polymer separated from the NPs



Au-PAH/PSS (Au core: 7.5 ± 1.5 nm)



UV signal @ 230 nm - MALS signal @ 90°

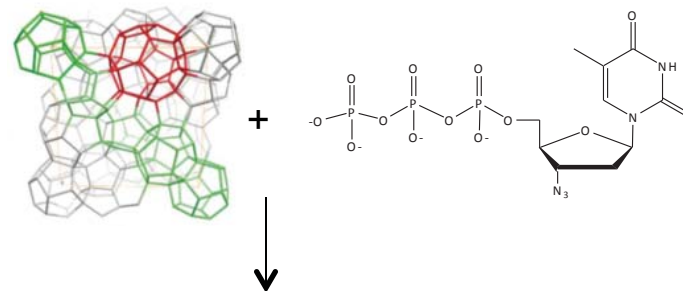
Higher state aggregation for triple-layer NPs

MOFs as nanovectors

Iron clusters

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

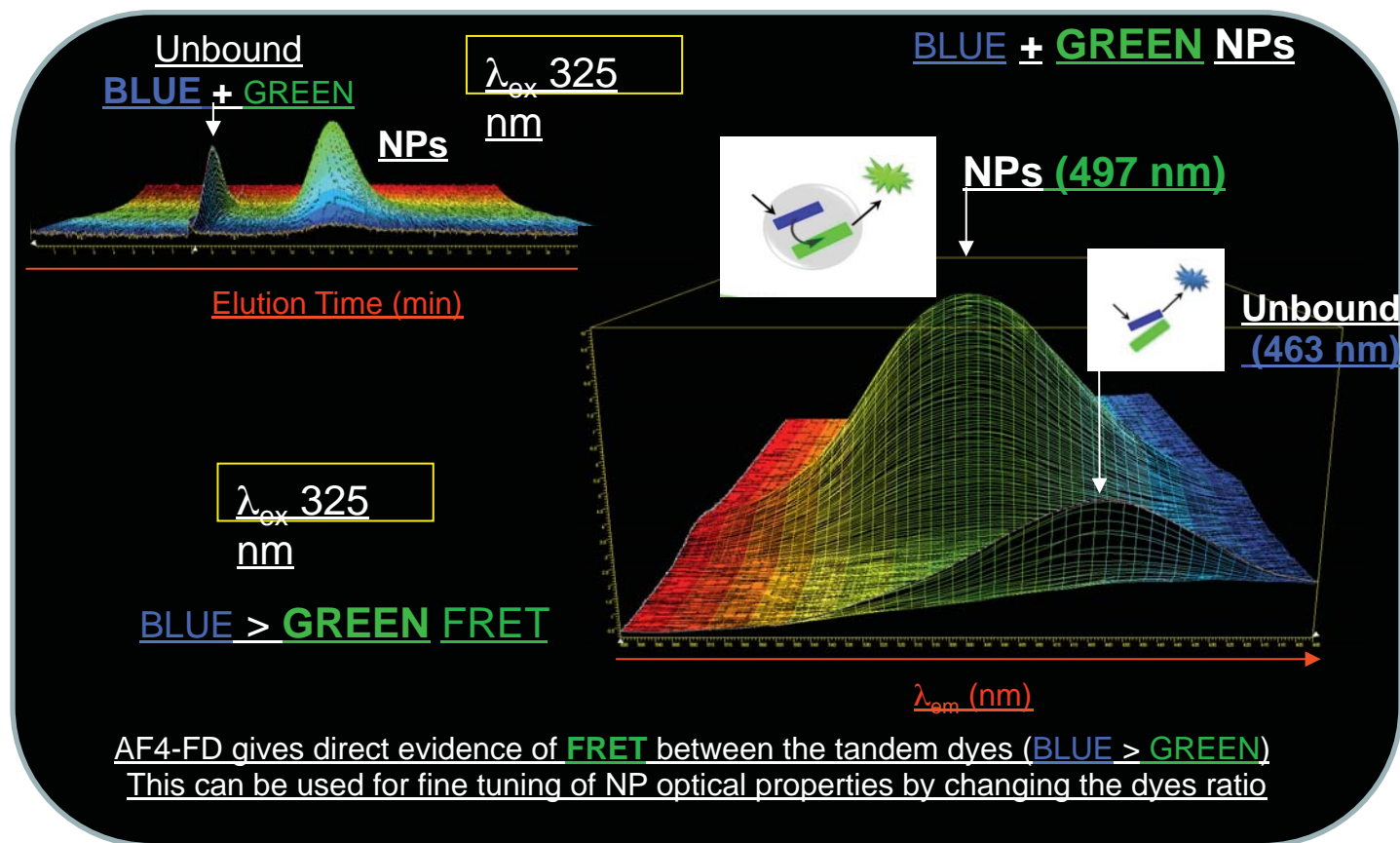
- ▶ Regular Porosity
- ▶ Convenient synthesis (mild conditions)
- ▶ Biodegradability
- ▶ Biocompatibility



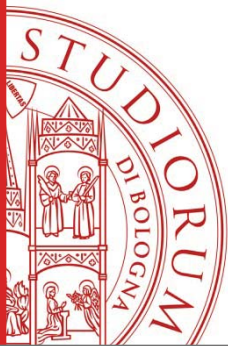
When used as nanovectors

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

Available **CUS**
(**Coordinatively Unsaturated Sites**) for
host-guest interaction with drug molecules



Fluorescence-resonance energy transfer



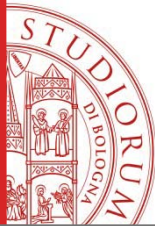
What more we offer Consulting and more

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..



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UNIVERSITÀ DI BOLOGNA

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