

## Kinetics of aggregation and magnetic separation of multicore iron oxide nanoparticles: effect of the grafted layer thickness

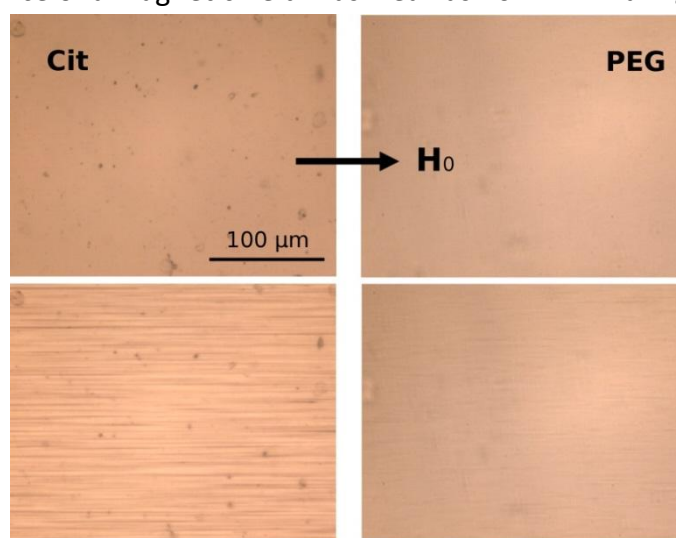
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Magnetic microbeads are commonly used in immunoassays to detect trace levels of antigens. Despite weaker magnetic attraction, we aim at developing efficient magnetic capture of multi-core magnetic nanoparticles (MNP) also called nanoflowers,<sup>[1]</sup> of outer diameter 30-60 nm, by using moderate magnetic field strengths. Recent work showed that magnetic interactions between MNPs of this size can still be strong enough to induce a reversible phase separation in the presence of a magnetic field  $B$  as weak as 10 mT.<sup>[2]</sup> During

phase separation, MNPs are gathered into micron-sized drop-like aggregates whose magnetic interaction with the applied field is much stronger than between individual nanoparticles and larger than thermal agitation  $kT$ . These fluid-like aggregates can be separated much more easily from buffer than single MNPs. Moreover, it is beneficial in continuous filtration to assemble the aggregates well before they are captured by magnetized collectors, by conveying the MNP suspension to the micro-filter across a microchannel submitted to a uniform external magnetic field  $H_0$ . This communication reports the capture



**Figure 1.** Snapshots of aqueous suspensions of citrated (left) and PEGylated (right) MNPs at  $\varphi_0=0.13\%$  submitted to  $H_0=13.5 \text{ kA}\cdot\text{m}^{-1}$  at  $t=0$  (upper row) and 5 min after field application (bottom row).

of multi-core MNP in microfluidic channels under magnetic and flow fields and a phase diagram in terms of Mason number, dipolar coupling constant, and thickness of the organic coating wrapping the multi-core MNPs: short citrate molecules or PEG chains.<sup>[3,4]</sup> The goal of such microfluidic chip with magnetic micro-pillars is to pre-concentrate MNPs in magneto-immunoassays, therefore preliminary results on their coating by an antibody will also be presented.

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Session: Nano-biomaterials

Keywords: Polypeptides, Copolymer Self-Assemblies, pH-Sensitivity, Nanoprecipitation, Drug Delivery

## Self-Assembly Behavior of Amphiphilic PEO-b-Polypeptide Based Nanoparticles as Promising Drug Delivery Carriers

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Engineered nanoparticles such as drug-loaded polymeric micelles or liposomal formulations, possessing a stimuli-responsive behavior can improve the therapeutic efficacy of a drug.<sup>[1]</sup> Stimuli-responsive drug delivery systems are based on a structural or conformational change in response to a cellular or extracellular stimulus of chemical, biochemical, or physical origin, resulting in the release of active species within a specific biological environment.<sup>[2]</sup> Self-assembled polymeric vesicles or polymersomes which membrane is composed of at least one polypeptide block have emerged among the most promising drug delivery platforms, as ascribed to their versatile properties, with well-defined block sizes and functionalities, secondary structure such as  $\alpha$ -helix for biomimicry, biocompatibility and biodegradability.<sup>[3]</sup> In this study, amphiphilic copolymers based on polypeptides possessing particular pH-responsivity (e.g. rod-coil transition) are obtained by ring opening polymerization (ROP) of the corresponding *N*-carboxyanhydride (NCA) monomers from PEG-NH<sub>2</sub> as macro-initiator. PEG incorporation brings stealth properties, enabling longer blood circulation for facilitated drug accumulation at the tumor site through the Enhance Permeability Retention (EPR) effect.<sup>[4]</sup> Different hydrophobic components among polypeptides were also studied to investigate effects on self-assembly that enable designing different kind of nanostructures. Statistical co-polypeptide blocks were composed of poly(*L*-glutamic acid) and poly(*L*-lysine) segments, respectively as a polyanion or a polycation bringing pH dependent charge, and poly(*L*-phenylalanine) segments, whose aromatic units can bring pi-pi stacking interactions and serve as hydrophobic reservoir for drug encapsulation. Several types of self-assembled polypeptide-based nanoparticles were obtained by nanoprecipitation. Then their detailed characterization was investigated by combining different physicochemical techniques. These copolymers combine several properties desirable in drug delivery applications such as fine control of their sizes, biocompatibility and biodegradability, pH-responsiveness and possibility to store molecules, either hydrophobic ones embedded within their membrane (of thickness varying with the hydrophobic block length) or hydrophilic ones (dyes, drugs...) within their aqueous compartment. These nanoparticles are highly promising candidates for the field of controlled drug delivery.

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Session Nanobiology

Keywords: Dendrimers, MRI, Gd-chelates

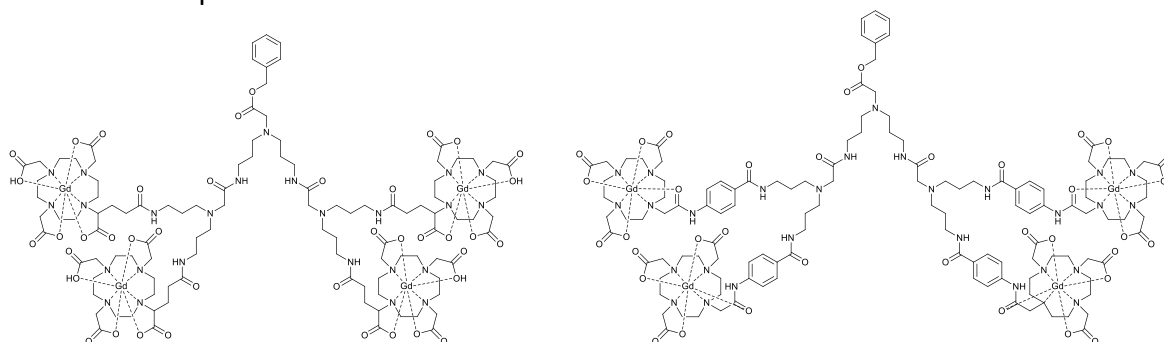
## Functionalizable Dendritic Platform Bearing Four Gd Chelates for Nanometric High Sensitive MRI Probes

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Atherosclerosis and its cardiovascular complications are one of the leading causes of death in developed countries and pose a major public healthcare problem. It consists in the formation of plaques at the wall of the arteries. These plaques are composed of lipid deposits rich in cholesterol (atheroma) enveloped in a fibrous gangue (sclerosis). It is therefore very important to have protocols for the early detection and the analysis of these plaques and their evolution. For this purpose, we have recently started the synthesis of a targeted bimodal organic nanoplatfrom. This nanoplatfrom will carry a biovector for specific targeting, a fluorophore for optical imaging and four Gd<sup>3+</sup> complexes that will constitute the MRI probe.

This communication will present the first results concerning the synthesis of the magnetic part of the nanoplatfrom.



In order to synthesize G1-Gd<sub>4</sub>, a semi-dendritic core was first prepared<sup>1</sup> and this was followed by the grafting of the four macrocyclic gadolinium complexes.<sup>2</sup> The syntheses and the characterizations of the species leading to G1-Gd<sub>4</sub> will be discussed.

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Nanobiology

Keywords: Fluorescent Nanoparticles, Bioimaging, Cell tracking, Biosensing

## Ultrabright Dye-loaded Polymer Nanoparticles for Bioimaging and Biosensing

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Fluorescent polymer nanoparticles encapsulating large quantities of dyes, so-called dye-loaded polymer nanoparticles (NPs), have emerged recently as an attractive alternative to inorganic fluorescent NPs, notably quantum dots.<sup>[1]</sup> These new nanomaterials can combine biodegradability and low toxicity with superior brightness. The latter can drastically improve speed, resolution and sensitivity in fluorescence bioimaging.

One of the major challenges in assembling dye-loaded NPs is to overcome aggregation caused quenching, which strongly limits the achievable brightness. We recently introduced a new approach to avoid dye aggregation through the encapsulation of charged fluorophores with bulky hydrophobic counterions.<sup>[2,3]</sup> This counterion approach leads, on the one hand, to particles up to 100 times brighter than quantum dots. On the other hand, it induces a collective behavior of hundreds of dyes inside the nanoparticles (ON/OFF switching) that could be tuned by controlling dye organization in the polymer matrix.<sup>[4]</sup>

Based on these NPs a technology for long-term fluorescence labeling of living cells with programmed color codes was developed. For this, three nanoparticles with distinct absorption and emission bands but identical size and surface properties that are endocytosed equally well by living cells were developed. Mixing NPs of three colors in different proportions generates cell populations of any desired color code. This technology can be applied to various cell lines and can be used for tracking different exogenous and endogenous cell populations in living zebrafish.<sup>[5]</sup>

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Session (Nanobiology, Biotechnology)

Keywords: 'Zwitterionic polymers, Nanoparticle, Protein Corona, Antifouling'

## Zwitterionic Polymer Ligands: an Ideal Surface Coating to Suppress Protein-Nanoparticle Corona Formation?

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In the last few years, zwitterionic polymers have been developed as antifouling surface coatings. However, their ability to completely suppress protein adsorption at the surface of nanoparticles in complex biological media remains undemonstrated. Here we investigate the formation of hard (irreversible) and soft (reversible) protein corona around model nanoparticles (NPs) coated with sulfobetaine (SB), phosphorylcholine (PC) and carboxybetaine (CB) polymer ligands in model albumin solutions and in whole serum. SB, PC and CB based polymers have been synthesized by RAFT and a terminal vinylimidazole block was added at the end of the polyzwitterion to ensure anchoring at the quantum dots surface. Hard protein corona formation was quantified after ultracentrifugation by fluorescamine assay. Fluorescence Correlation Spectroscopy (FCS) was used to characterize dynamic interactions between proteins and QDs. We show for the first time a complete absence of protein corona around SB-coated NPs, while PC- and CB-coated NPs undergo reversible adsorption or partial aggregation. Single NP tracking in the cytoplasm of live cells corroborate these *in vitro* observations. Finally, we discuss the impact of the modification of globally neutral polyzwitterions by the addition of some negatively or positively charged monomers on protein adsorption and intracellular diffusion. We have also shown that the addition of small neutral targeting moieties preserves antifouling and enable efficient intracellular targeting.

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Session Nanobiology

Keywords: SEIRAS, Surface modification, Membrane Proteins, Sensors

## Surface enhanced difference infrared spectroscopy on membrane proteins

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Membrane proteins act as signaling and trafficking platforms for processes fundamental to life. An estimated third of all proteins are membrane-integral or membrane-associated. Between 80 and 90% of targets of marketed drugs are membrane proteins. However, in stark contrast with the crucial roles that they play in metabolism currently, less than 1% of all available atomic structures in the protein data base (PDB) are integral membrane proteins. The reason for the observed discrepancy between significance and knowledge lies in the difficulties associated with the handling and study of membrane proteins.

A comprehensive understanding of the functioning of membrane proteins requires knowledge of their working parameters, their unique structure and their specific membrane environment and thus the creation of tools that give access to these parameters in a biomimetic environment. Vibrational spectroscopies provide molecular fingerprints of the catalytic reaction of a protein. By combining infrared spectroscopies and solid-state nanostructures of protein monolayers, the proteins can be studied during their reaction, i.e. by monitoring proton translocation. The label free detection of the signature of protonated acidic residues, the protein backbone and the overall hydrogen bonding network in the protein is possible. The approach can also be coupled to electrochemistry. Examples on studies of transport proteins, including glucose transporters, as well as from enzymes from the respiratory chain, will be given.