Introduction
In the field of molecular imaging nanoparticulate structures are becoming increasingly important, since they can carry high payloads of contrast generating materials while their surface can be functionalized to improve bio-compatibility or to introduce specificity. The pharmacokinetic profile as well as the tissue penetration potential of the nanovehicles is of paramount importance. Hence, there is a great demand for nanostructures of which the final morphology and size can be judiciously controlled.

In the current study we created a variety of well-defined nano-sized supramolecular structures based on two amphiphilic molecules: Gd-DTPA-DSA, a Gd$^{3+}$ chelating lipid which exhibits paramagnetic properties for MRI and P2fA2, a fluorescein labeled apolipoprotein E derived cell penetrating peptide. We demonstrate how we can control the morphology and size of the imaging probes by varying the ratio of the two aforementioned amphiphiles and thereby optimizing the molecular relaxivity of the resulting lipidic aggregates. The two most effective formulations were selected for in vitro studies.

Methods and Results
Aqueous dispersions of mixtures of P2fA2 and Gd-DTPA-DSA were prepared and dynamic light scattering (DLS) revealed that the aggregates had narrow size distributions and that their mean hydrodynamic diameters increased with decreasing P2fA2/Gd-DTPA-DSA ratios (Figure 1). Cryogenic transmission electron microscopy (cryo-TEM) of the aggregates revealed a similar trend (Figure 1). At P2fA2 concentrations ≥ 50 mol% small micellar structures with diameters of 5-8 nm were observed. By decreasing the P2fA2 concentration plate-like morphologies appeared with a constant thickness of 5-8 nm of which the aspect ratios increased, from 10 x 15-25 nm (33 mol% P2fA2) to 10-15 x 150 nm (25 mol% P2fA2) and up to 10-15 x 250 nm (20 mol% P2fA2). In the preparations that contained 10-12.5 mol% P2fA2 fully grown ribbons were present of up to 25 nm in width and with immense lengths.

Nuclear magnetic resonance dispersion profiles (NMRD-profiles) were obtained (Figure 2A) to assess relaxivity. Typical macromolecular profiles with maximum values around 20 MHz were observed. These indicate a diminished mobility of the Gd$^{3+}$ chelates in the lipidic aggregates which causes their tumbling rate to decrease. These results demonstrate that our approach allowed the formation of structures with tunable ionic relaxivities, varying between 13 and 18 mM$^{-1}$s$^{-1}$ at 20 MHz (inset Figure 2A), which is 3 to 5 times higher than commercially available Gd-DTPA at this field strength. Interestingly, the highest ionic relaxivity of 18 mM$^{-1}$s$^{-1}$ was observed for the formulation containing 33% P2fA2, the preparation with predominantly small plate-like aggregates.

Based on these results we set out to test these P2fA2 containing nanostructures for their suitability as MR imaging probes for extravascular targets, such as macrophages. The two formulations with a high relaxivity and small size, i.e. those with 33 mol% (small platelets) and 50 mol% (micelles) P2fA2 were selected for further in vitro experiments with cultured mouse macrophage cells (J774A1). The incorporation of P2fA2 allowed the visualization of nanoparticle uptake using fluorescence confocal laser scanning microscopy (CLSM). The CLSM images (Figure 2), show nuclei in blue (DAPI) and P2fA2 in green, merged with bright field images of the macrophage cells. Control cells revealed no nanoparticle associated fluorescence (Figure 2B), while significant amounts of intracellular fluorescence were observed after 30 min of incubation for cells that were incubated with micellar (Figure 2C) and plate-like (Figure 2D) nanoparticles.

Conclusions
We have demonstrated that by carefully controlling the ratio of two functional amphiphiles Gd-DTPA-DSA and P2fA2, it was possible to create a variety of well-defined supramolecular structures, containing both fluorescein and Gd$^{3+}$ chelates. Moreover, we showed that by changing the morphology we could optimize the relaxivity of the imaging probes. Efficient macrophage uptake and the multifunctional character of this nanoparticulate platform was confirmed by CLSM. We envision the application of this contrast agent platform for pathologies in which macrophage activity plays a key role, such as atherosclerosis, rheumatoid arthritis, or cancer.