# Characterization of organic molecules grafted to silica and bismuth nanoparticles by NMR

Céline Henoumont[1], Gauthier Hallot[2], Estelle Lipani[1], Catherine Gomez[2], Robert N. Muller[1,3], Luce Vander Elst[1], Marc Port[2], Sophie Laurent[1,3]

[1]  General, Organic and Biomedical Chemistry Unit, NMR and Molecular Imaging Laboratory, University of Mons, 19 Avenue Maistriau, B-7000 Mons, Belgium

[2]  Laboratoire de Génomique, Bioinformatique et Chimie Moléculaire (EA 7528), Equipe Chimie Moléculaire, Conservatoire National des Arts et Métiers (Cnam), HESAM, Université, 2 rue Conté, 75003 Paris, France

[3]  Center for Microscopy and Molecular Imaging (CMMI), 8 Rue Adrienne Boland, 6041 Gosselies, Belgium

## Abstract

NMR is a powerful characterization tool and we propose to study the surface of silica or bismuth nanoparticles dedicated to medical applications in order to evidence the covalent grafting of organic molecules on their surface. For that aim, DOSY experiments are particularly useful and allow to discriminate molecules interacting strongly with the nanoparticle surface from molecules simply weakly adsorbed at the surface. We were so able to characterize thoroughly the surface of different silica and bismuth nanoparticles.

COSY NMR spectra were recorded on silica NPs grafted with PEG at different pH (figure S-1).
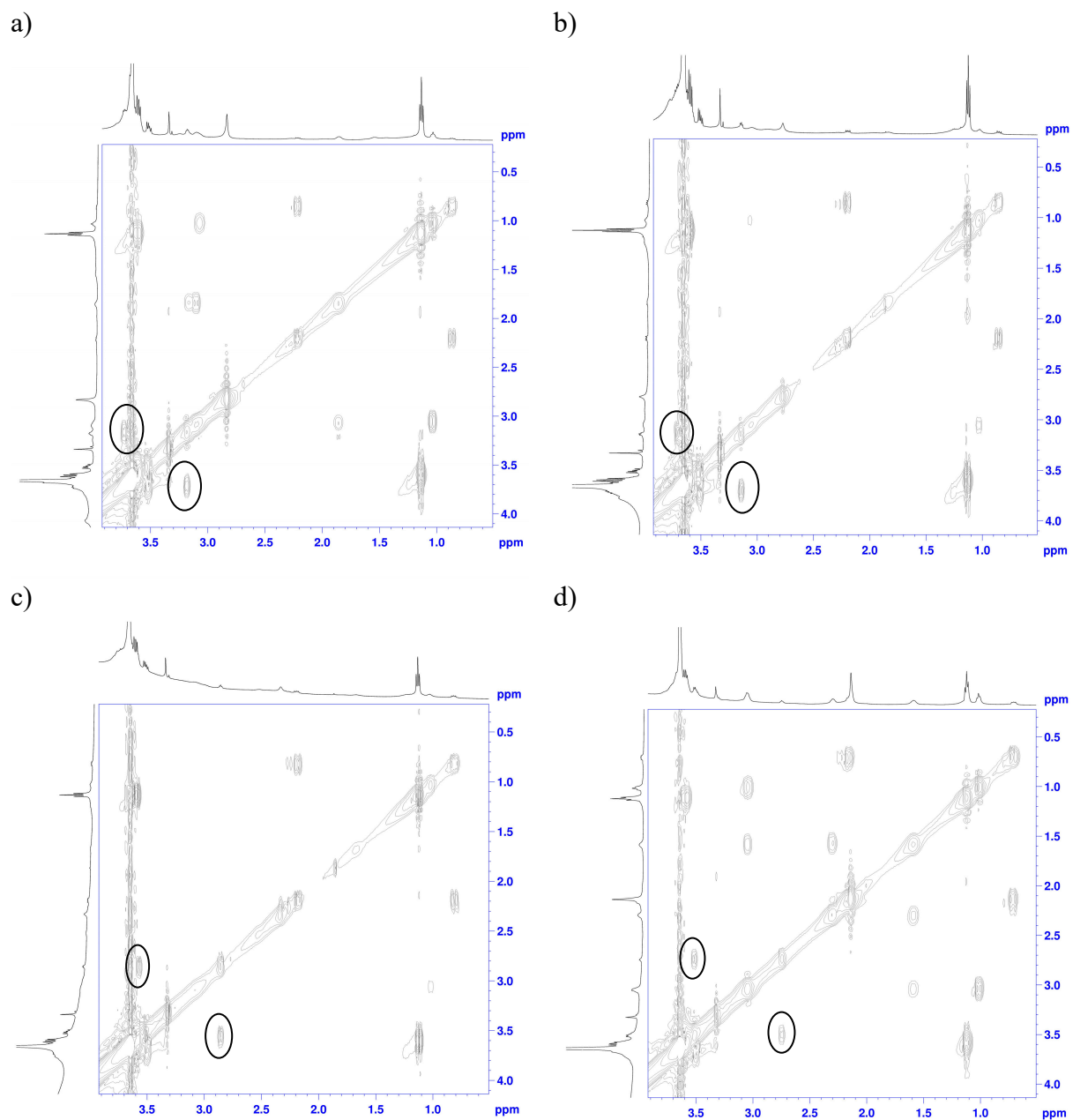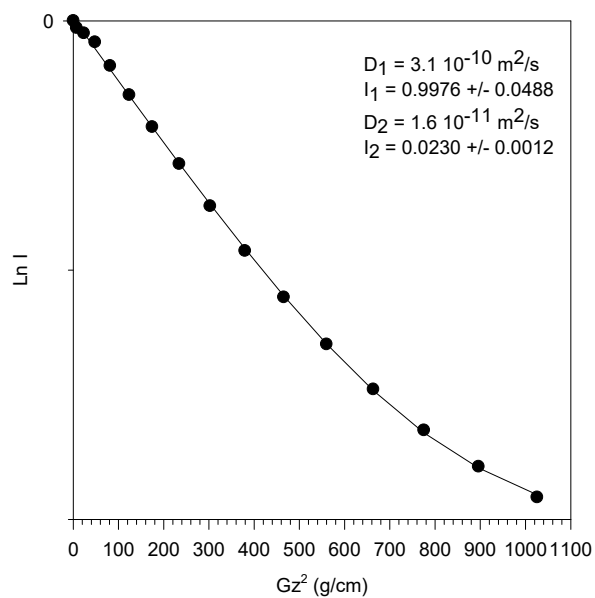
Figure S1: COSY spectra of silica NPs grafted with PEG at different pH : a) pH = 6 ; b) pH = 9 ; c) pH = 10.5 ; d) pH = 12

Diffusion curves extracted from the measurements registered with a diffusion time of 600 ms (figure S2):
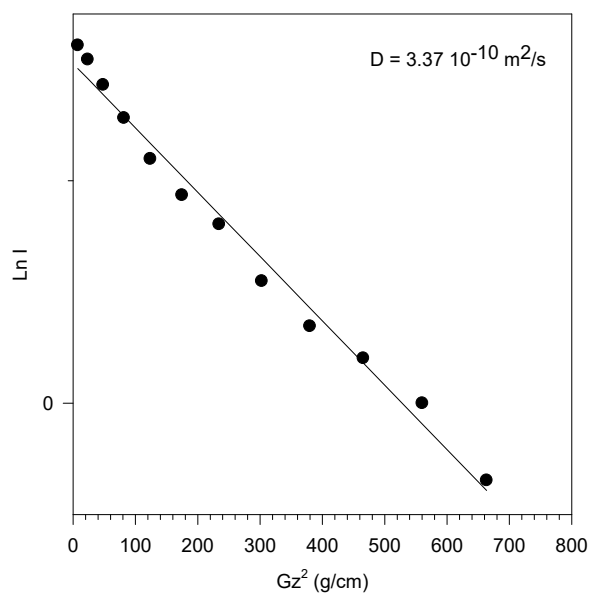
a)

b)



Figure S2: Diffusion curves extracted from DOSY measurements performed on silica NPs grafted with PEG : a) signal at 3.6 ppm  b) signal at 3.1 ppm corresponding to the methylene next to the amine function

NMR spectrum of silica NPs grafted with PEG and the La-complex after a reaction time of 24h (figure S3):
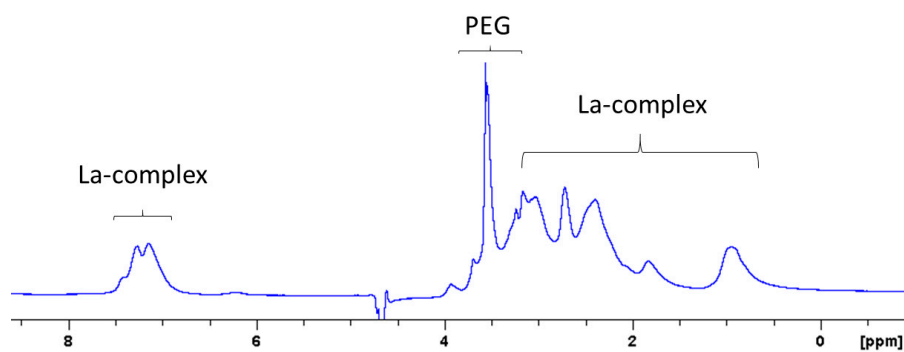


Figure S3: 1D NMR spectrum recorded with the noesypr1d sequence on a water solution prepared with 10% $D_2O$ of the NPs grafted with PEG and the La-complex.

NMR spectrum of silica NPs grafted with PEG and the La-complex, registered with an external reference (TSP) to estimate the proportions of PEG and La-complex at the NP surface:
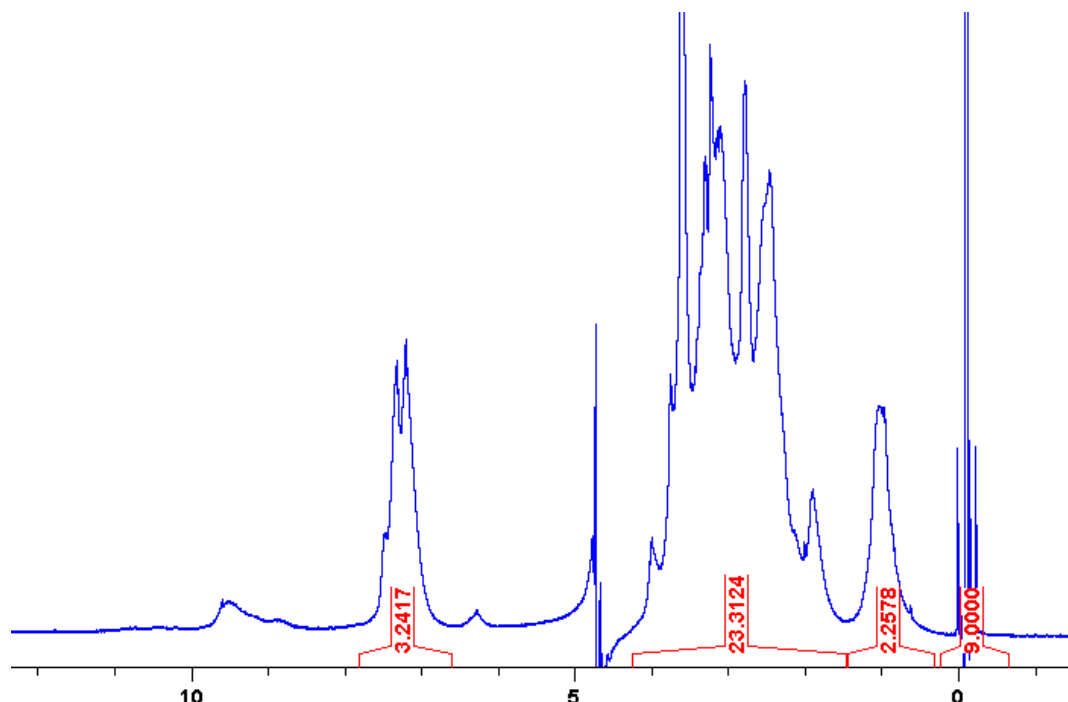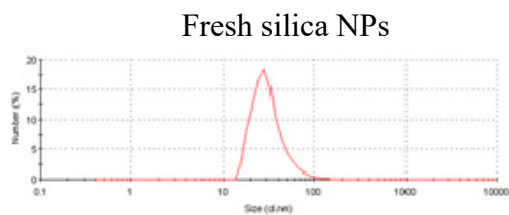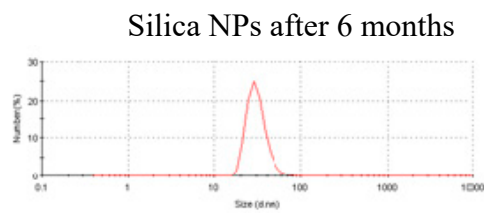


Figure S4: 1D NMR spectrum recorded with the noesypr1d sequence on a water solution prepared with 10% $D_2O$ of the NPs grafted with PEG and the La-complex. An external reference visible at 0 ppm (TSP) was used at a known concentration of 0.8 M.

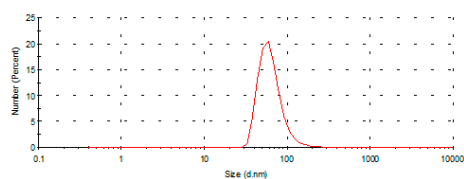DLS measurements performed on fresh silica NPs grafted with PEG and on the same particles after 6 months:

| Fresh silica NPs | Silica NPs after 6 months |
|---|---|
|  |  |
| $d = 32.13 \pm 14.85$ nm | $d = 31.01 \pm 8.27$ |

DLS measurements performed on Bi@LA-PEG$_{750}$-OMe NPs



$d = 65 \pm 29$ nm

Figure S5: DLS measurements, shown as number distributions, performed on fresh silica NPs grafted with PEG and on the same particles after 6 months and on Bi NPs grafted with PEG

Comparison between the spectrum of the bismuth NPs solution stabilized with citrate and that of gluconic acid and isopropanol (figure S6). The peaks of citrate are clearly visible in the NPs solution as well as other peaks : the signals between 3.4 and 4.2 ppm can be attributed to gluconic acid, whereas those at 1.2 ppm and between 3.9 and 4 ppm can be attributed to isopropanol.
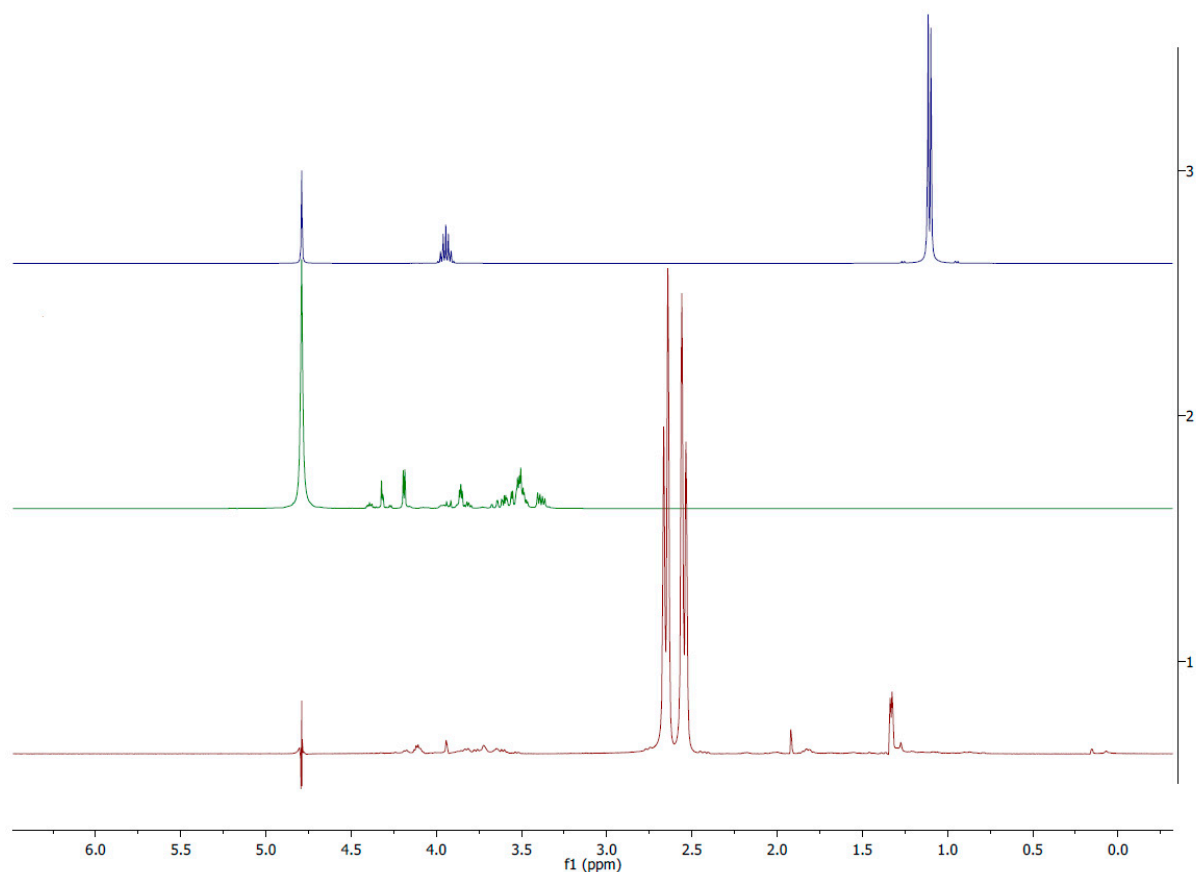


Figure S6: 1D NMR spectra recorded on the bismuth NPs solution stabilized with citrate (bottom spectrum), on gluconic acid (middle spectrum) and on isopropanol (above spectrum).

Comparison between the 1D NMR spectrum of the ligand AL-PEG$_{750}$-OMe and that of the NPs solution stabilized with this ligand (figure S7):
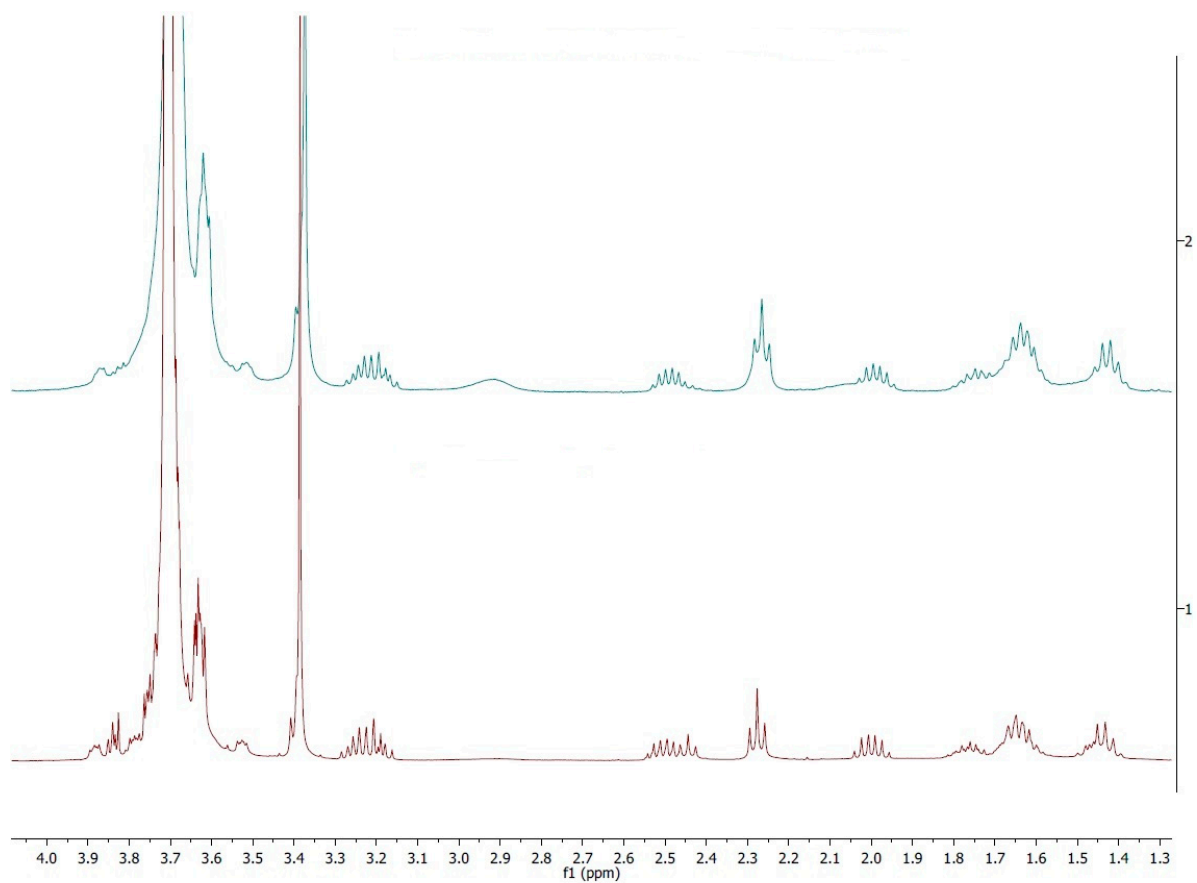


Figure S7: Comparison between 1D NMR spectra recorded with the sequence noesypr1d on water solutions prepared with 10% D$_2$O of the bismuth NPs stabilized with AL-PEG$_{750}$-OMe (above spectrum) and of AL-PEG$_{750}$-OMe (bottom spectrum).