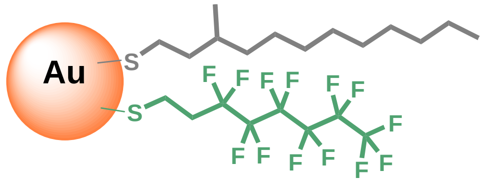
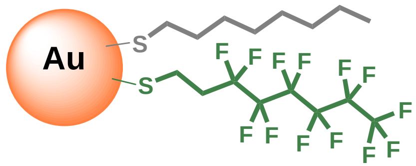
gold nanoparticles protected by H-/F- mixed-monolayers

✓ *branched ligand*

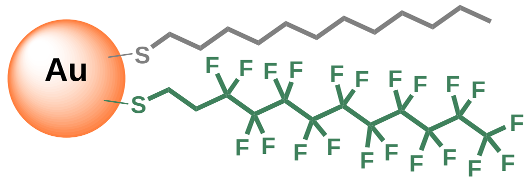


NP-br-C12/F6

✓ **ligands of equal length**

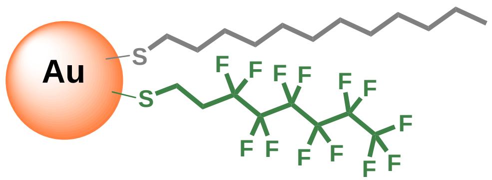


NP-C8/F6

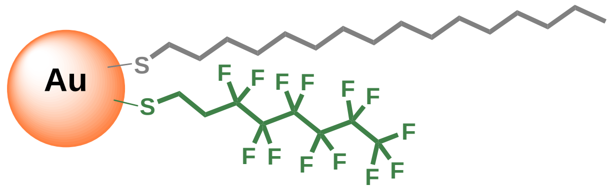


NP-C12/F10

✓ **ligands of different length**

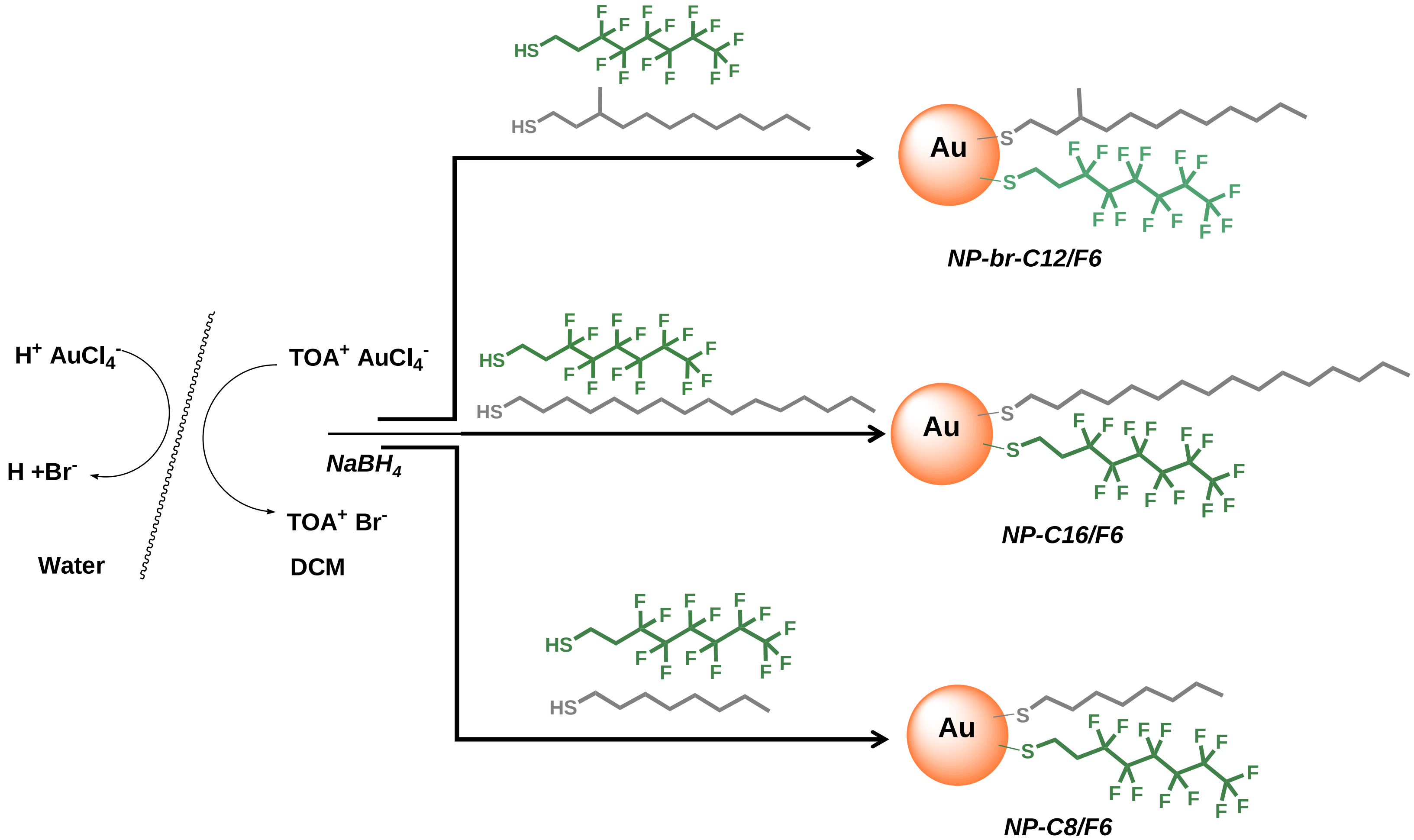


NP-C12/F6

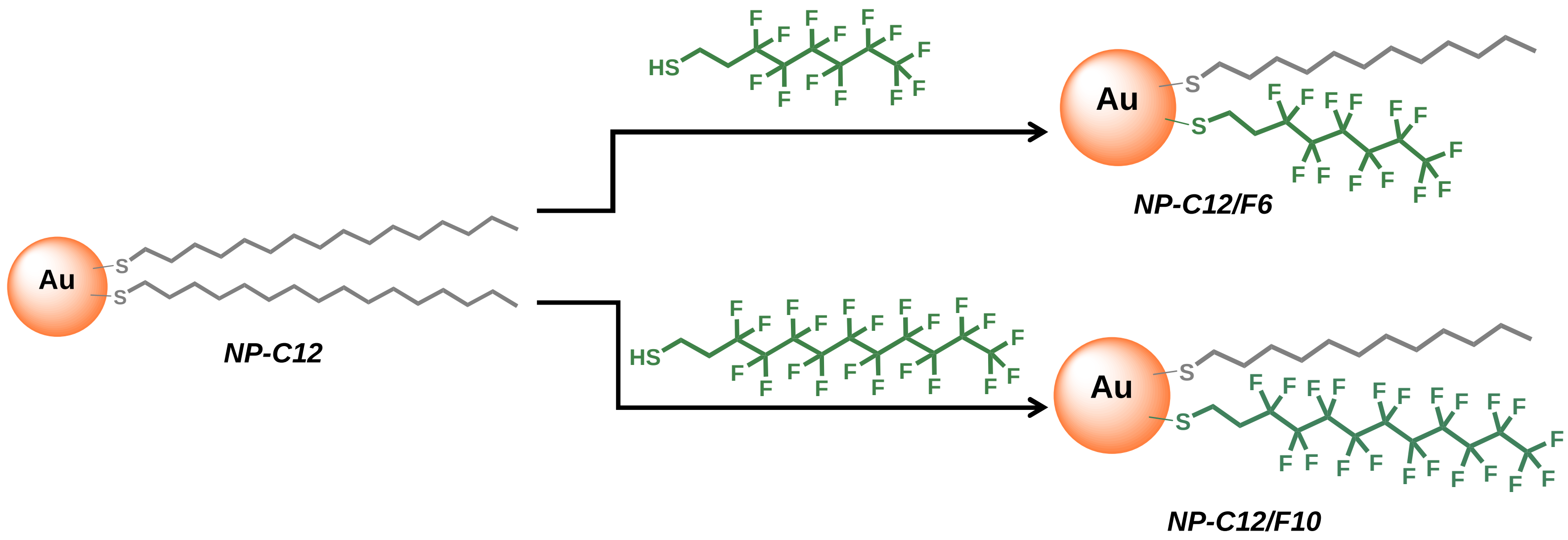


NP-C16/F6

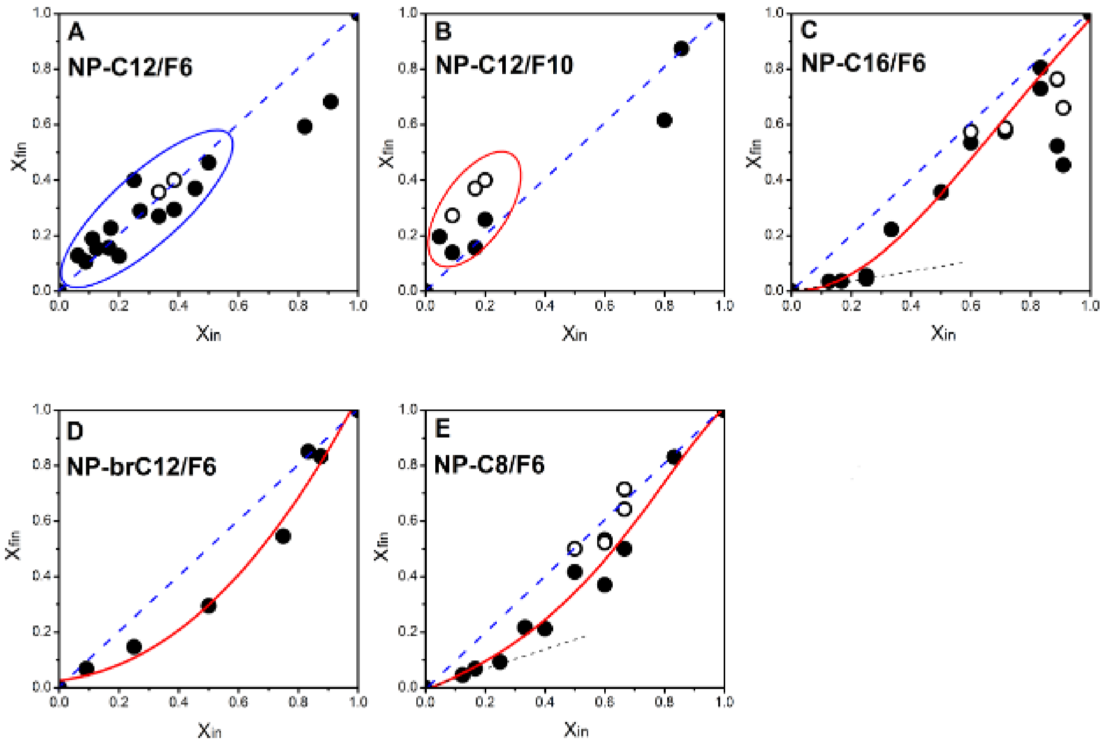
Preparation of MMNPs by direct synthesis



Synthesis of MMNPs by ligand exchange



effect of ligands ratio on monolayer composition

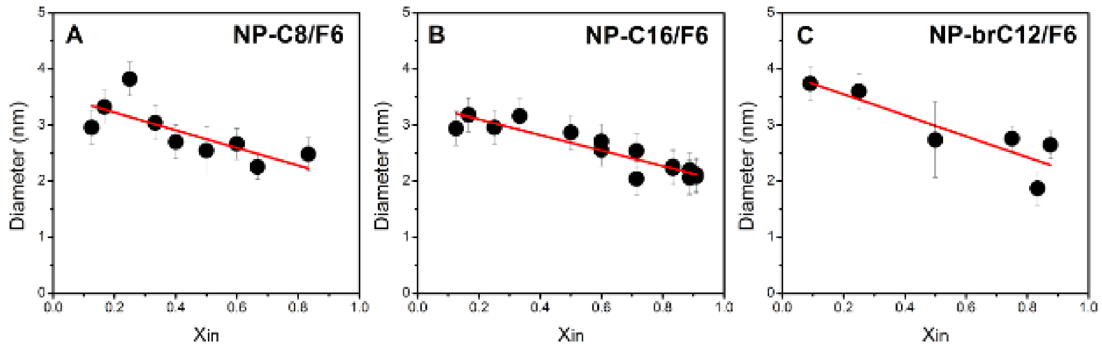


Panel A and B: experimental data of the monolayer compositions for nanoparticles ***NP-C12/F6*** *and* ***NP-C12/F10****, respectively, as a function of composition of the initial mixture in the place exchange reaction.*
Panel C, D, and E: experimental data of the monolayer compositions for nanoparticles ***NP-C16/F6****,* ***NP-brC12/F6****, and* ***NP-C8/F6****, respectively, as a function of the composition of the initial reaction mixture.*
Open circles represent NPs soluble upon addition of fluorinated solvents.

Sologan, M.; Cantarutti, C.; Bidoggia, S.; Polizzi, P.; Pengo, P.; Pasquato, L. *Faraday Discussions* **2016.**

effect of F-ligand amount on NP core size

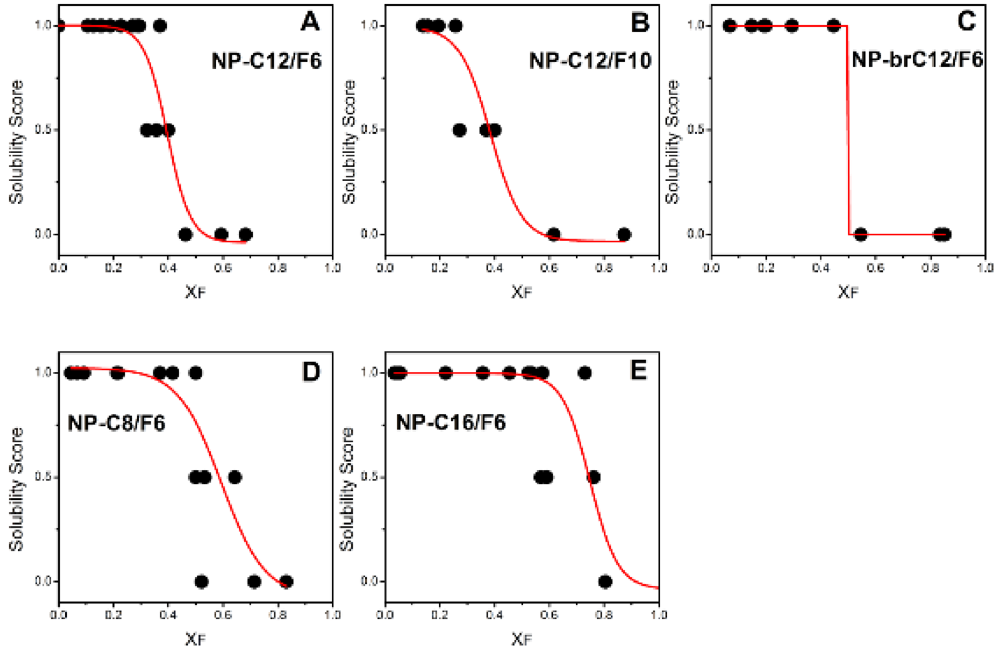
NPs obtained by direct synthesis



Dependence of the nanoparticles core diameter on the initial molar fraction of the fluorinated ligand.
Error bars represent the standard deviation of the average diameter measured from TEM analyses.
In the case of multiple preparations with the same initial loading of the fluorinated component, the experimental points represent the average of the diameters and error bars represent their standard deviation.

Sologan, M.; Cantarutti, C.; Bidoggia, S.; Polizzi, P.; Pengo, P.; Pasquato, L. *Faraday Discussions* **2016.**

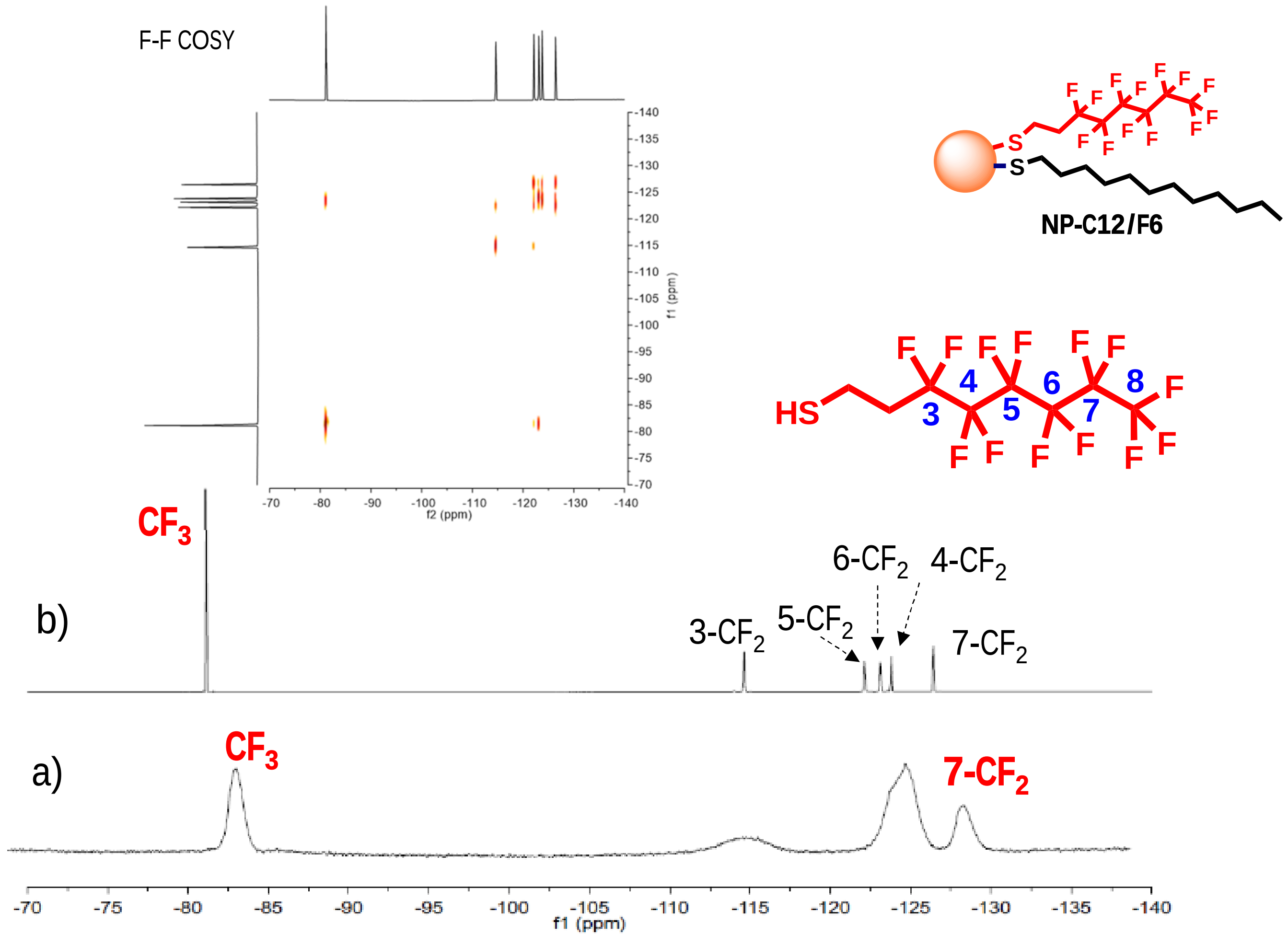
solubility



Comparison of the solubility transitions for the MMNPs as a function of the molar fraction of fluorinated component in the monolayer. The solubility is expressed according to the following score: s core = 1 is assigned to the nanoparticles soluble in chloroform. Score = 0.5 is assigned to the nanoparticles soluble in hexane, score = 0 is assigned to the nanoparticles soluble in hexafluorobenzene.

Sologan, M.; Cantarutti, C.; Bidoggia, S.; Polizzi, P.; Pengo, P.; Pasquato, L. *Faraday Discussions* **2016.**

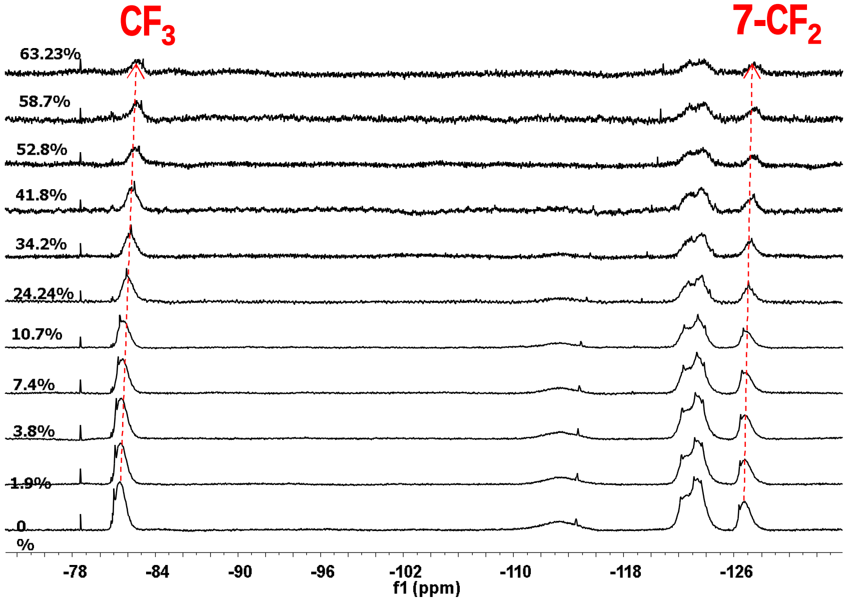
Characterization of gold MMNPs

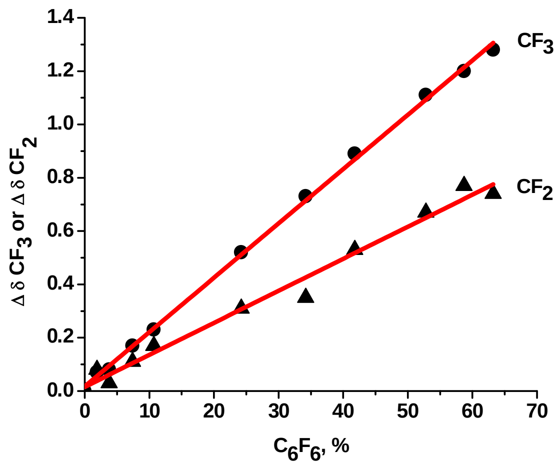


^{19}F-NMR (470.13 MHz, $CDCl_3$) spectrum of : a) NP-C12/F6 (5.4/1) and b) HS-F6.

Characterization of gold MMNPs

Influence of the solvent composition on the chemical shift of MMNPs

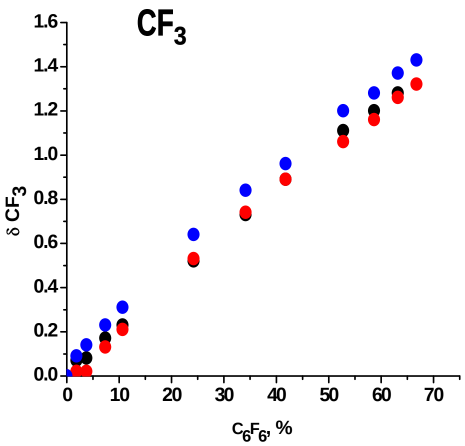


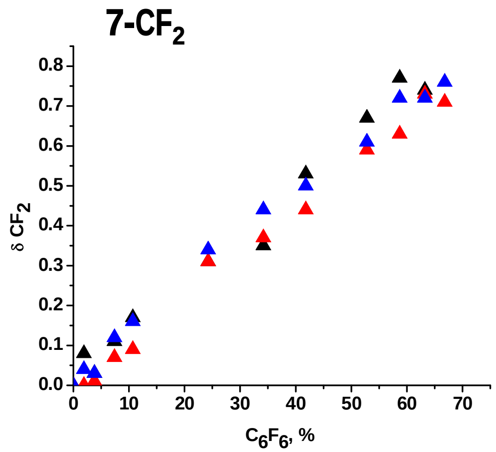


[19]F NMR spectra of **NP-C16/F6, with X_{F6} = 0.357,** at increasing amounts of C_6F_6.

Characterization of gold MMNPs

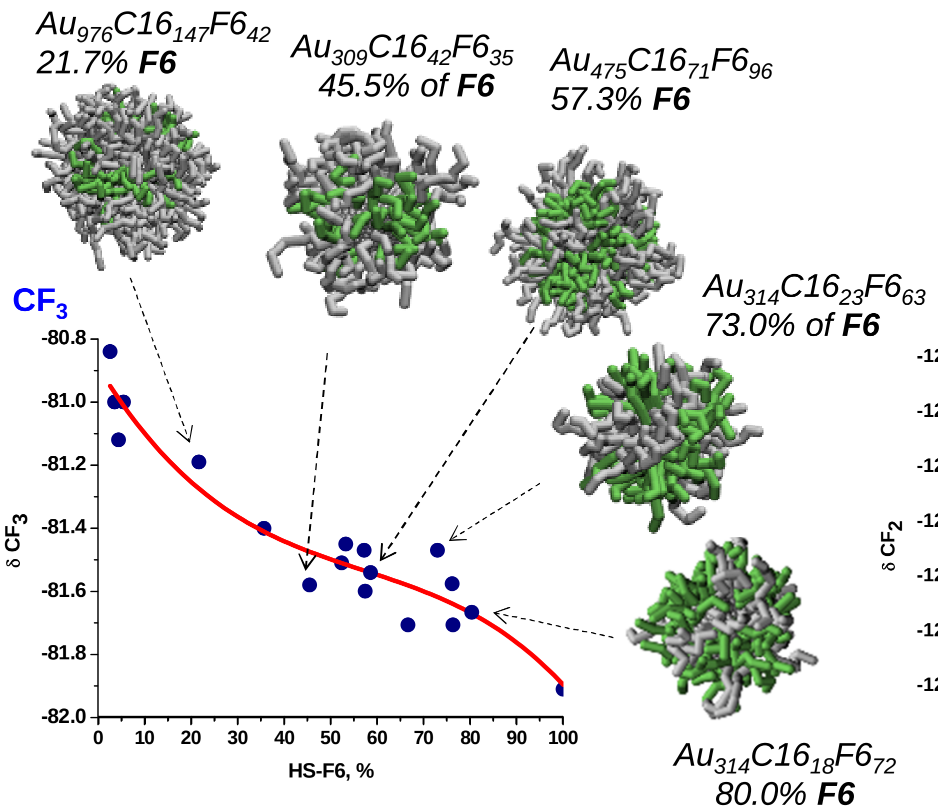
Influence of the solvent composition on the chemical shift of MMNPs



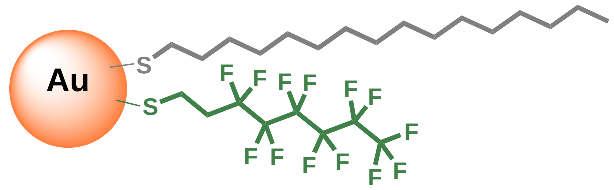


NP-C16/F6, X_{F6} = 0.217
NP-C16/F6, X_{F6} = 0.357
NP-C16/F6, X_{F6} = 0.573

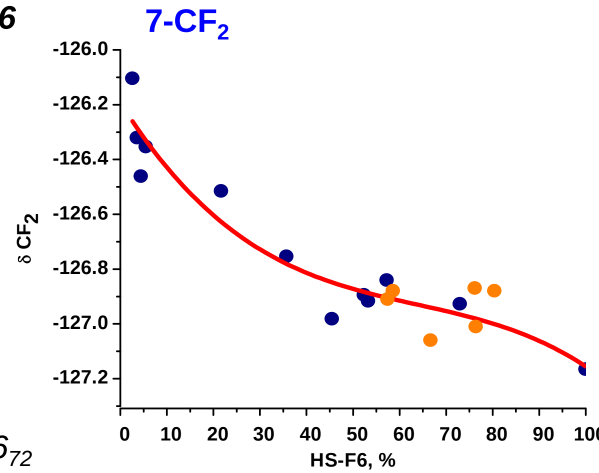
gold NPs protected by ligands of different length



CF_3 *chemical shift vs. monolayer composition*



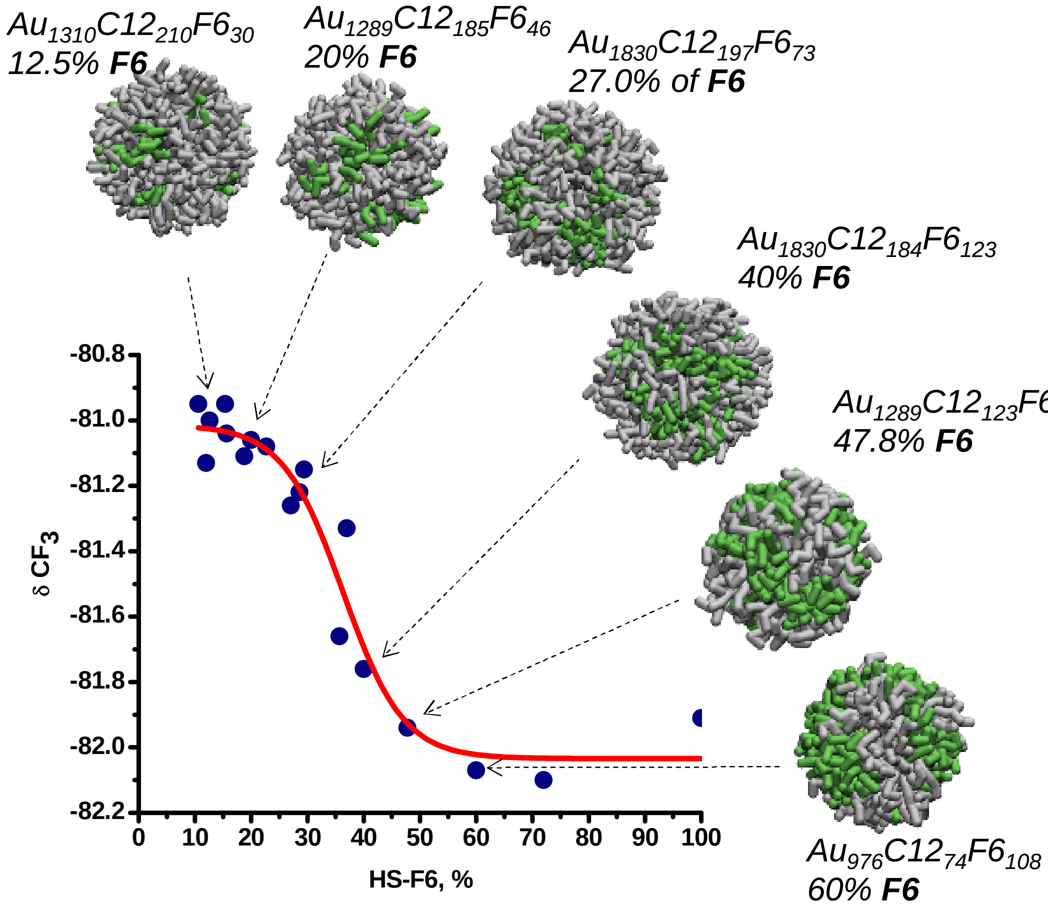
NP-C16/F6



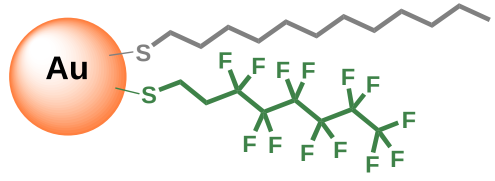
CF_2 *chemical shift vs. monolayer composition*

L. Pasquato *et al.* ACS Nano 2016

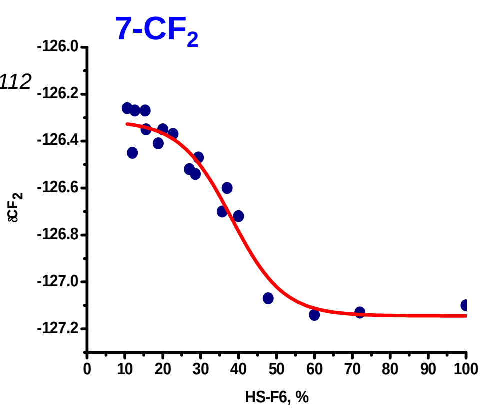
gold NPs protected by ligands of different length



***CF_3* chemical shift vs. monolayer composition**



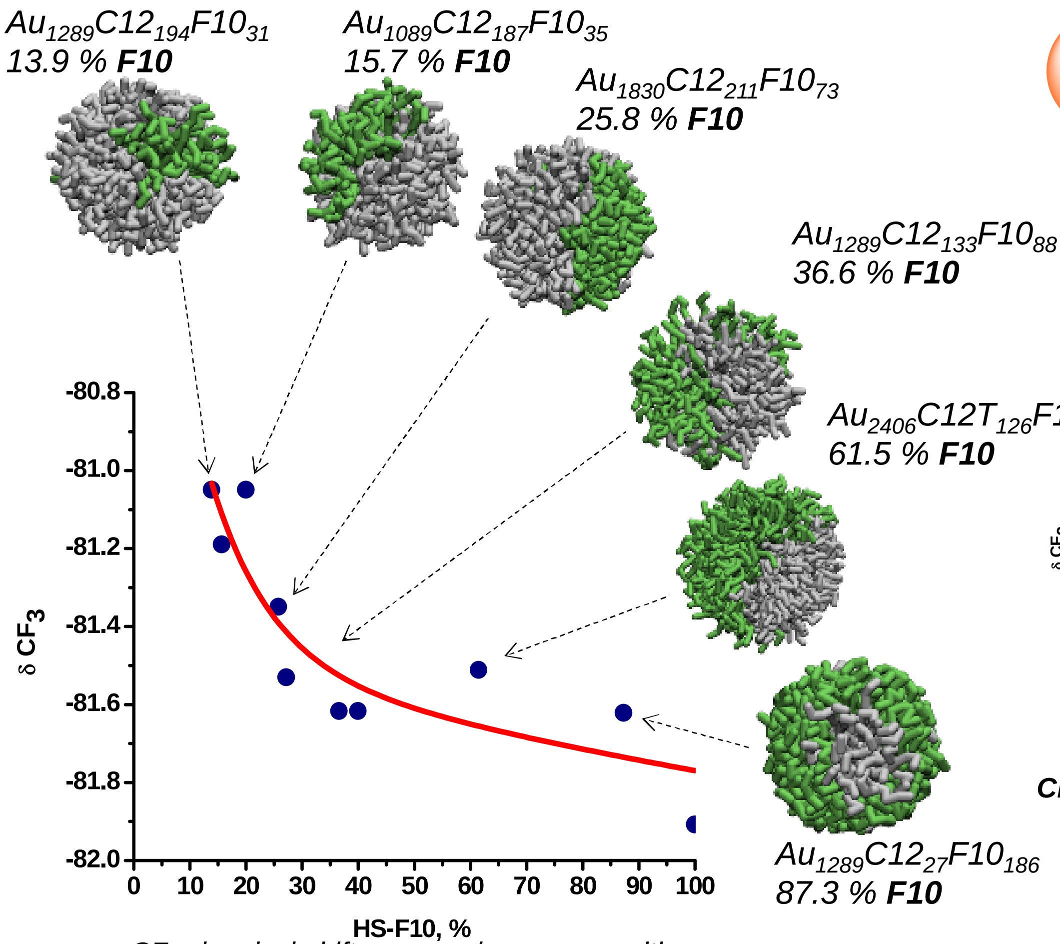
NP-C12/F6



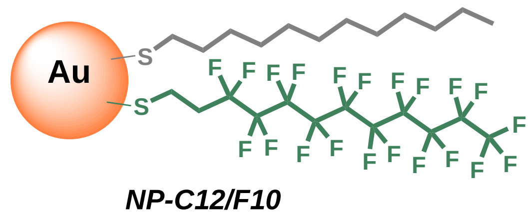
***CF_2* chemical shift vs. monolayer composition**

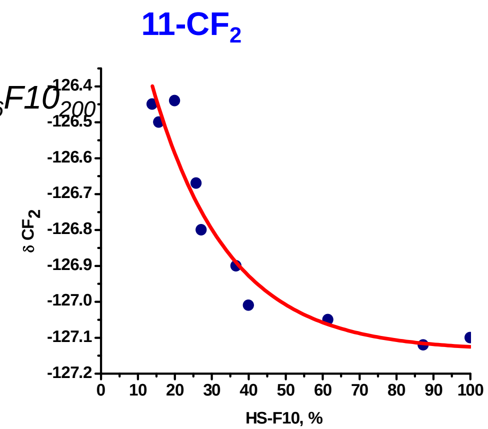
L. Pasquato et al. submitted

gold NPs protected by ligands of equal length



CF_3 chemical shift vs. monolayer composition



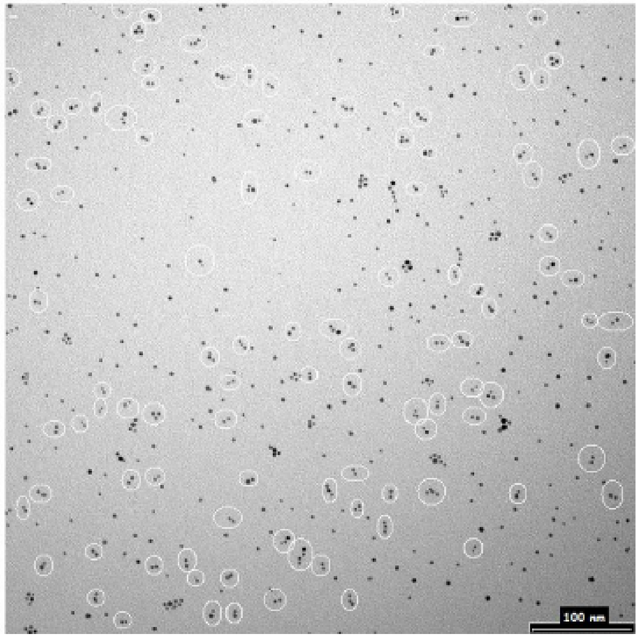


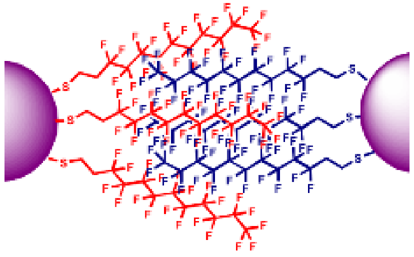
CF_2 chemical shift vs. monolayer composition

L. Pasquato et al. submitted

Au-NPs C12/F10 mixed monolayers

Tendecy to form dimers...

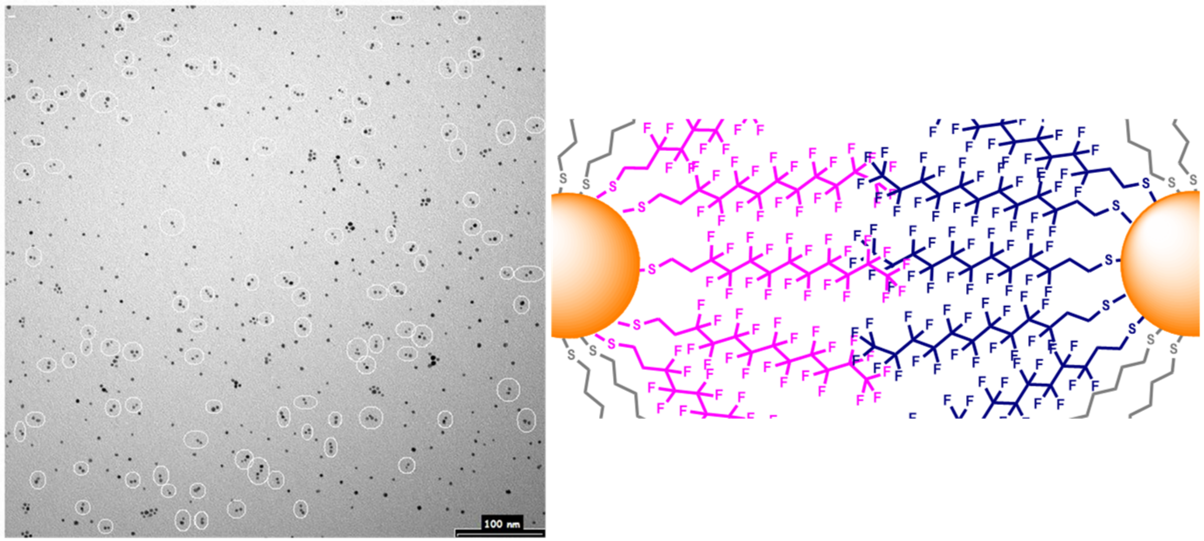




TEM image of NP-C12/F10, ratio 1.5:1, c = 10 ng/mL in $CHCl_3$

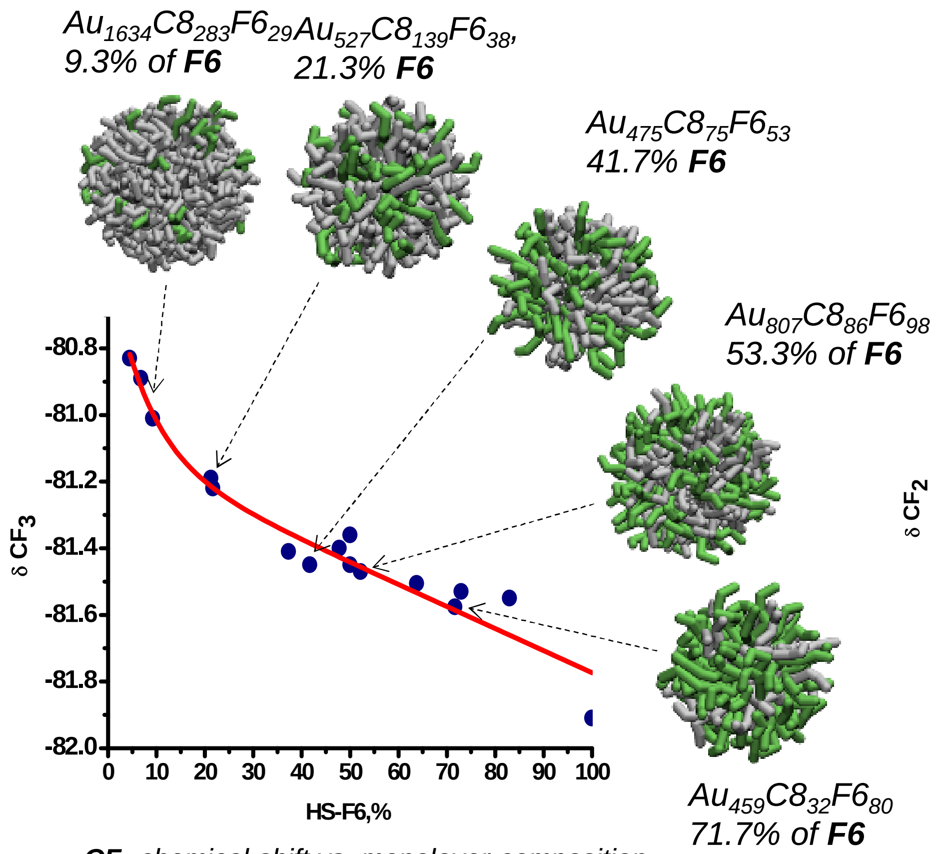
Au-NPs C12/F10 mixed monolayers

Tendecy to form dimers…

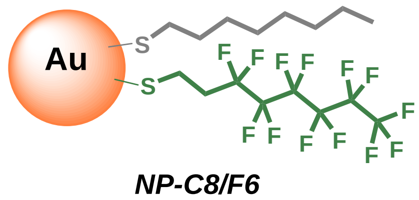


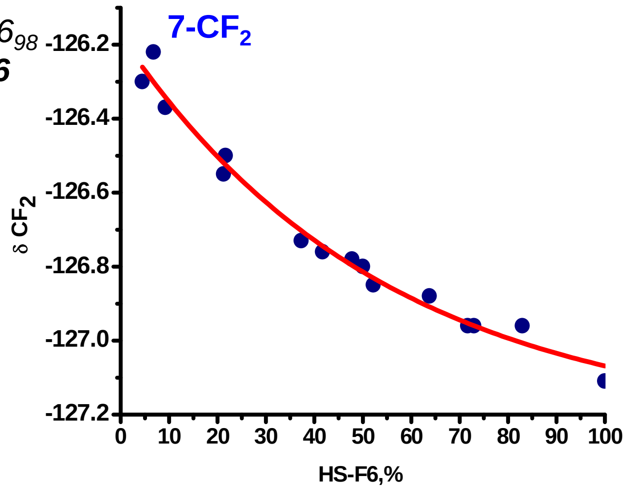
TEM image of NP-C12/F10, ratio 1.5:1, c = 10 ng/mL in $CHCl_3$

gold NPs protected by ligands of equal length



CF_3 chemical shift vs. monolayer composition

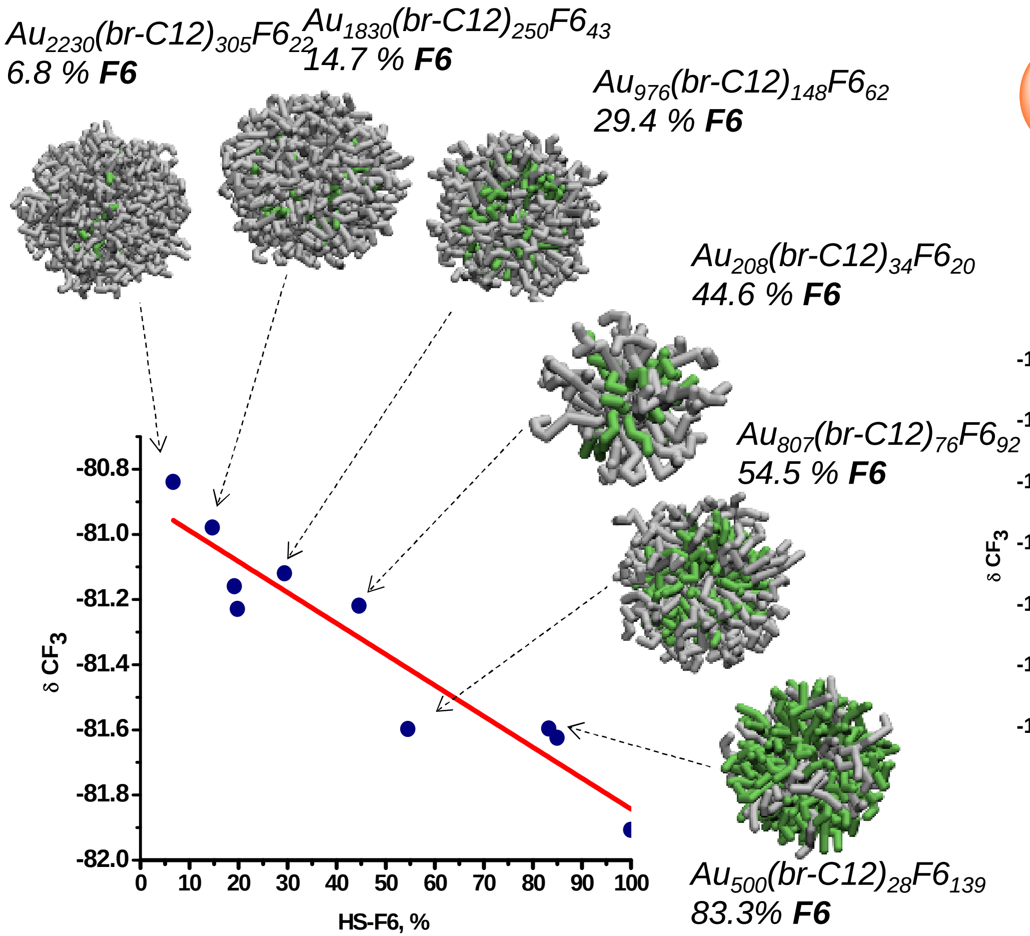




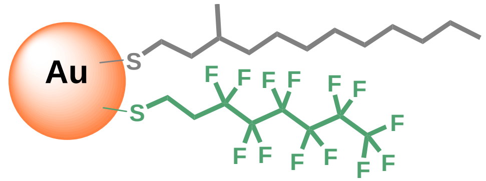
CF_2 chemical shift vs. monolayer composition

L. Pasquato *et al.* ACS Nano 2016

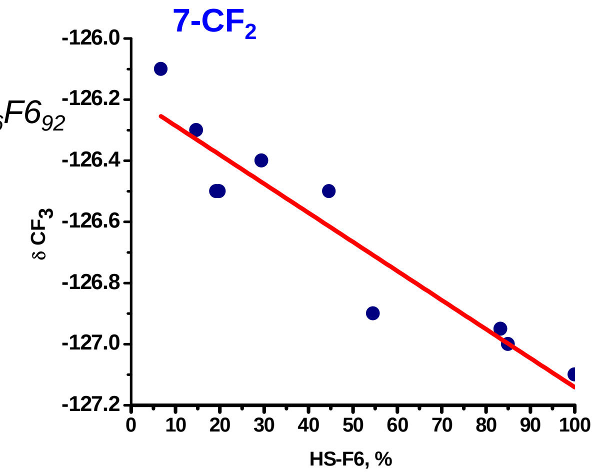
gold NPs protected by branched ligands



***CF_3** chemical shift vs. monolayer composition*



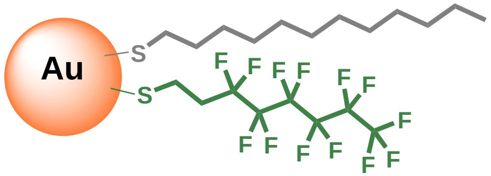
NP-br-C12/F6



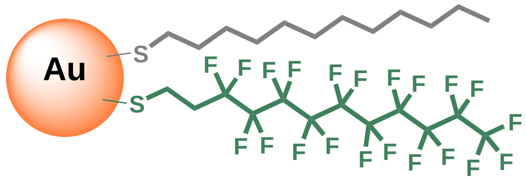
***CF_2** chemical shift vs. monolayer composition*

L. Pasquato *et al.* ACS Nano 2016

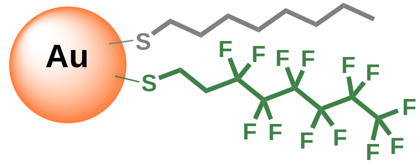
gold NPs protected by H/F ligands of different length and size



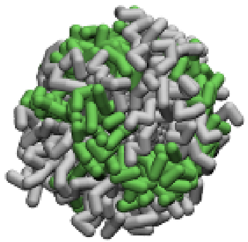
NP-C12/F6



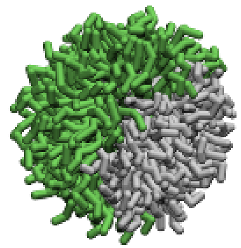
NP-C12/F10



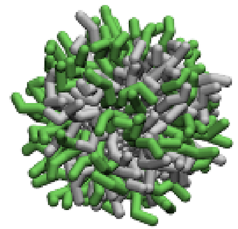
NP-C8/F6



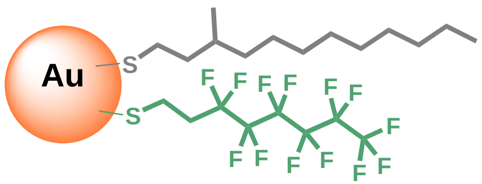
$Au_{1289}C12_{123}F6_{112}$
47.8% **F6**



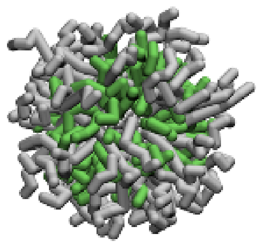
$Au_{2406}C12T_{126}F10_{200}$
61.5 % **F10**



$Au_{807}C8_{86}F6_{98}$
53.3% of **F6**



NP-br-C12/F6



$Au_{807}(br\text{-}C12)_{76}F6_{92}$
54.5 % **F6**

L. Pasquato *et al.* ACS Nano 2016

^{19}F-^{1}H HOESY experiments

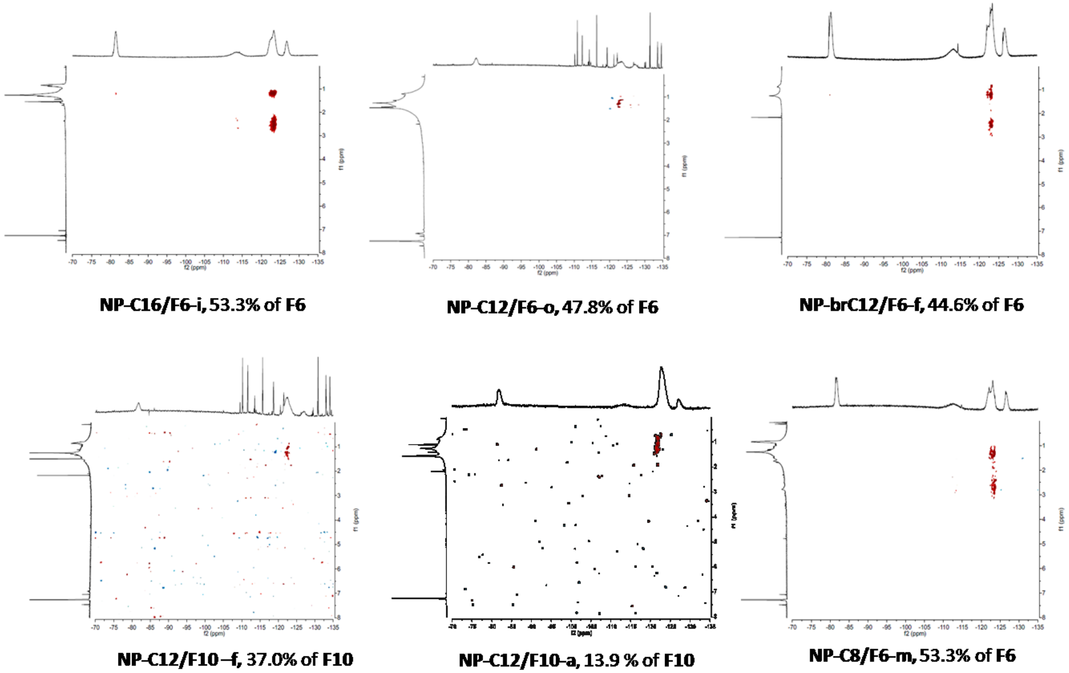
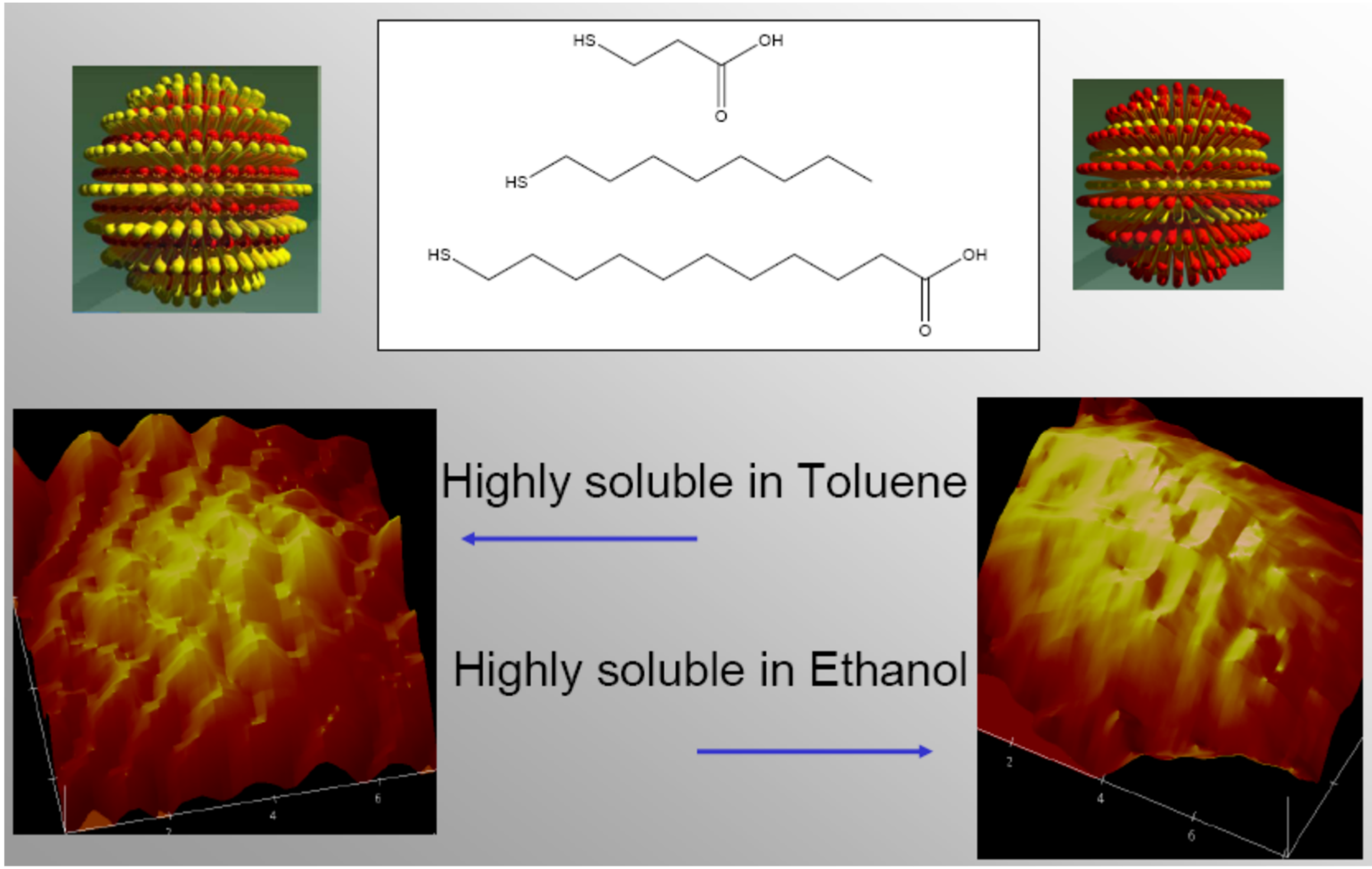


Figure S7. ^{19}F-^{1}H HOESY (470.08 MHz, $CDCl_3$) spectra of **NP-C16/F6-i, NP-C12/F6-o, NP-brC12/F6-f, NP-C12/F10-f, NP-C12/F10-a** and **NP-C8/F6-m.** For the experiment with **NP-C12/F10-f** a mixture of $CDCl_3/C_6F_6$ 4:1 was used as solvent.

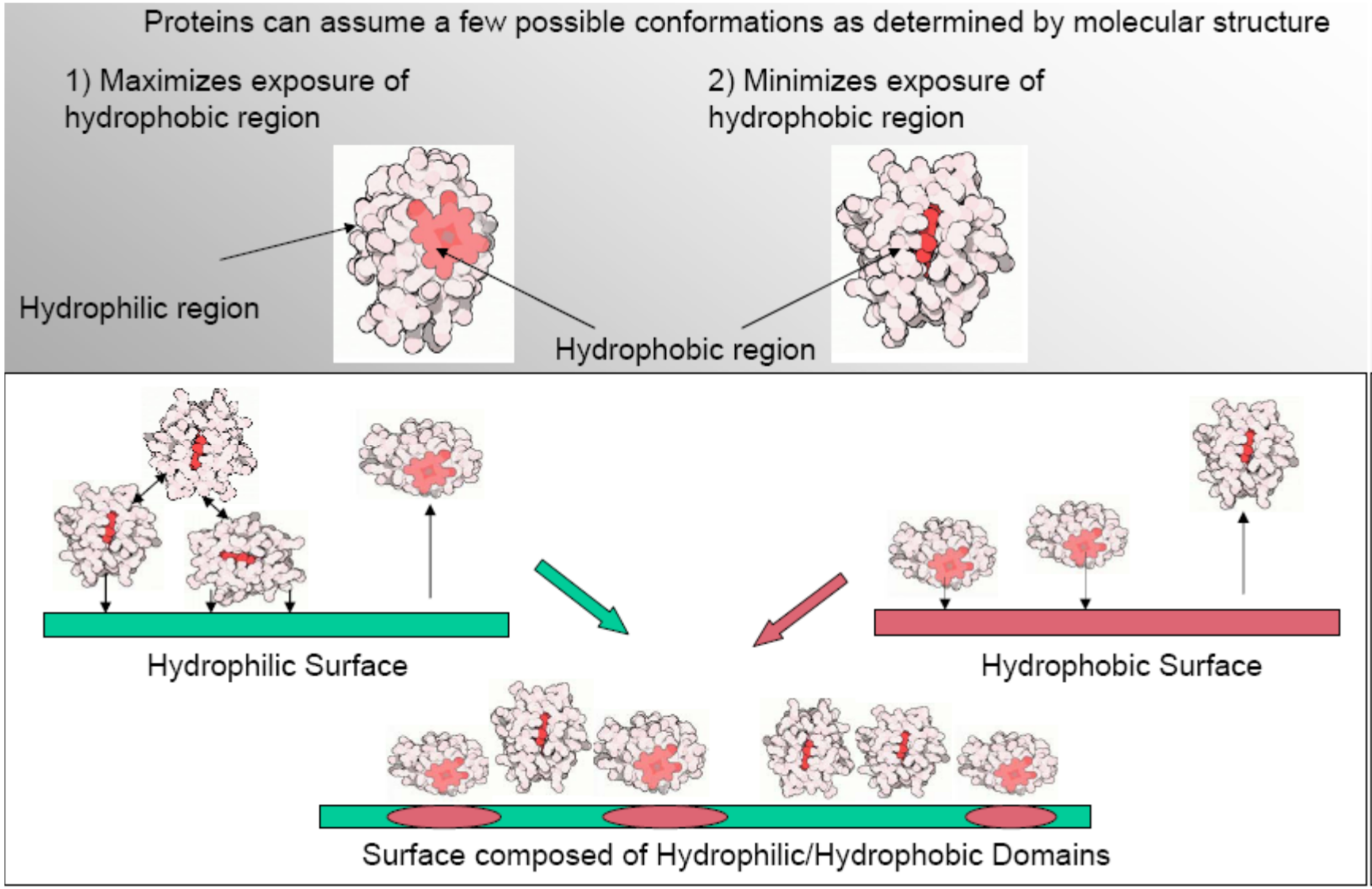
mixed-monolayer properties

tuning the surface chemistry

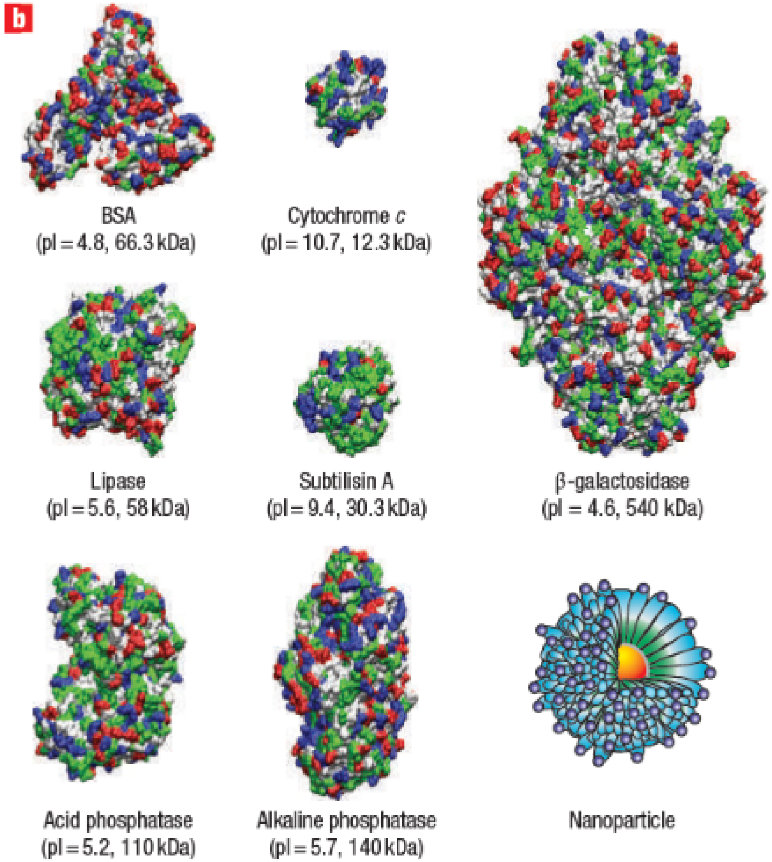


Centrone, A. et al *PNAS*, **2008**, *105*, 9886.

protein nonspecific absorption



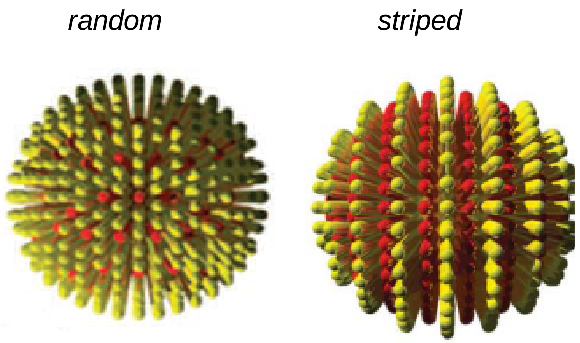
Surface properties of proteins



Colour scheme for the proteins: nonpolar residues (grey), basic residues (blue), acidic residues (red) and polar residues (green).

C.-C. You et al. Nature Nanotech. 2007

the mechanism of membrane penetration and toxicity depend on surface structure



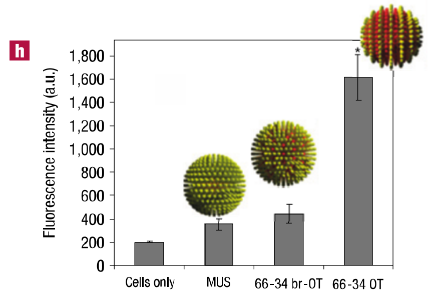
A. Verma et al. Nature Mater. 2008
S. Sabella et al. Nanoscale 2014

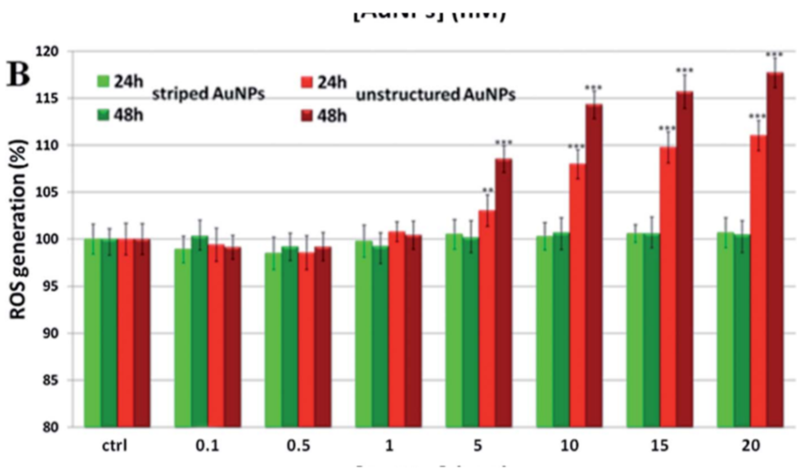
Effect of the NP surface morphology on cellular uptake and toxicity

Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles

AYUSH VERMA[1], OKTAY UZUN[1], YUHUA HU[2], YING HU[1], HEE-SUN HAN[3], NICKI WATSON[4], SUELIN CHEN[1], DARRELL J. IRVINE[1,5]* AND FRANCESCO STELLACCI[1]*

Nature Mater. 2008

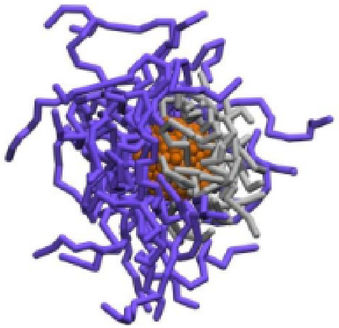




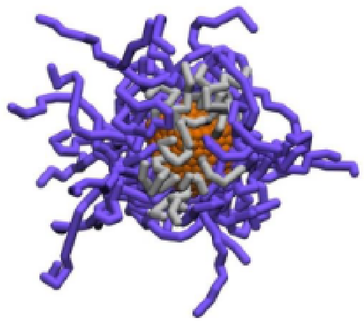
S. Sabella et al. Nanoscale, 2014, 6, 7052

Interaction of Nanoparticles with cells/membranes

project SINFONIA with P. Posocco



NP-C8TEG/F8PEG
Janus
size: 1.6 nm
$Au_{140}(C8TEG)_{24}(F8PEG)_{32}$ ratio 1/1.3



NP-C8TEG/F8PEG
Striped
size: 1.9 nm
$Au_{260}(C8TEG)_{20}(F8PEG)_{36}$ ratio 1:2

biological studies
credits to: Alessandro Tossi
Sabrina Pacor
Milena Guida

computational studies
credits to: Paola Posocco
Domenico Marson
Silvia Boccardo

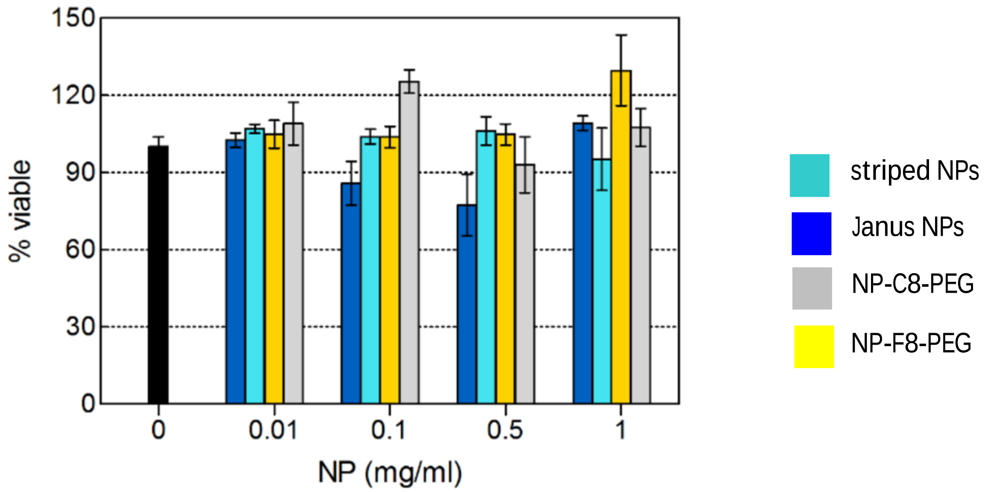
by comparison homoligand NPs: **NP-C8PEG** and **NP-F8PEG**

L. Pasquato, P. Posocco, *Small 2019*.

Citotoxicity: MTT test

credits to: Alessandro Tossi
Sabrina Pacor
Milena Guida

MEC-1 cells, complete medium, 24h

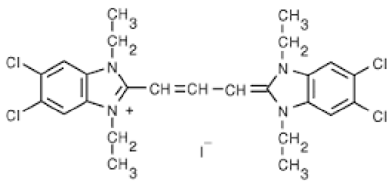


Cytotoxicity of NPs treated cells. MEC-1 cells viability, evaluated by MTT assay, after 24h treatment with the NPs concentrations indicated on x-axes; data are expressed as mean ± SEM of the measured O.D. of experiments rep at least three times and performed at least in triplicate.

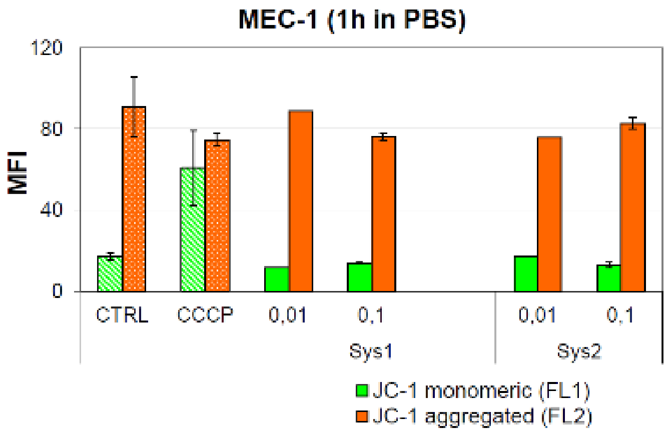
L. Pasquato, P. Posocco, *Small 2019*.

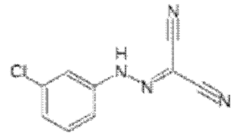
Mitochondrial activity

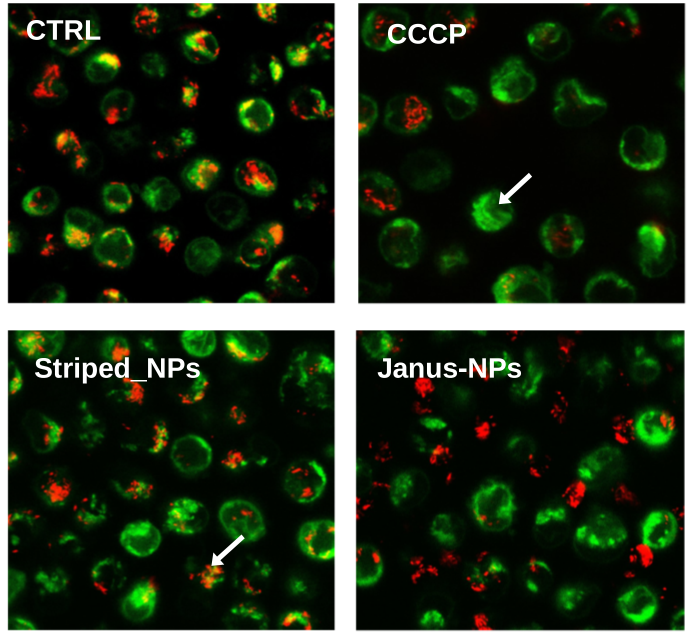
evaluation of apoptotic damage to mitochondrial functionality



JC-1 mitochondrial potential sensor







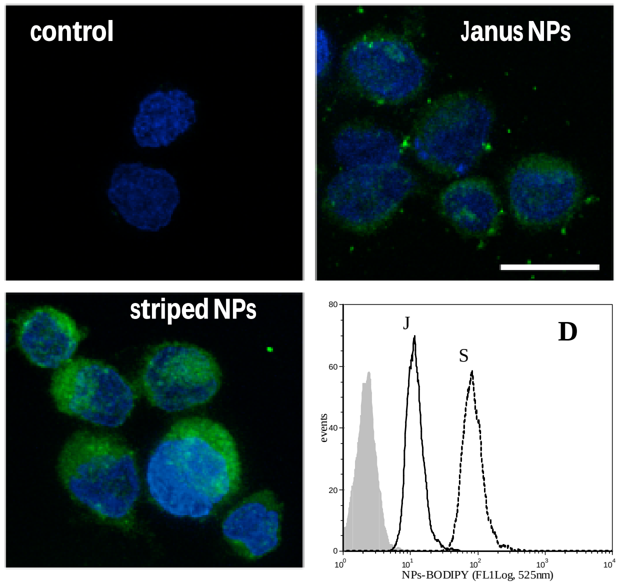
Flow cytometry: Striped- and Janus- GNP did not the orange fluorescence of treated cells with respect to untreated controls.

Confocal microscopy: only the positive control CCCP caused disaggregation, conc. 0.1 mg/ml.

GNPs do not cause mitochondrial damage

L. Pasquato, P. Posocco, *Small 2019*.

cell internalization of NPs



Janus and striped NPs cross the plasma membrane and reach the cytoplasm

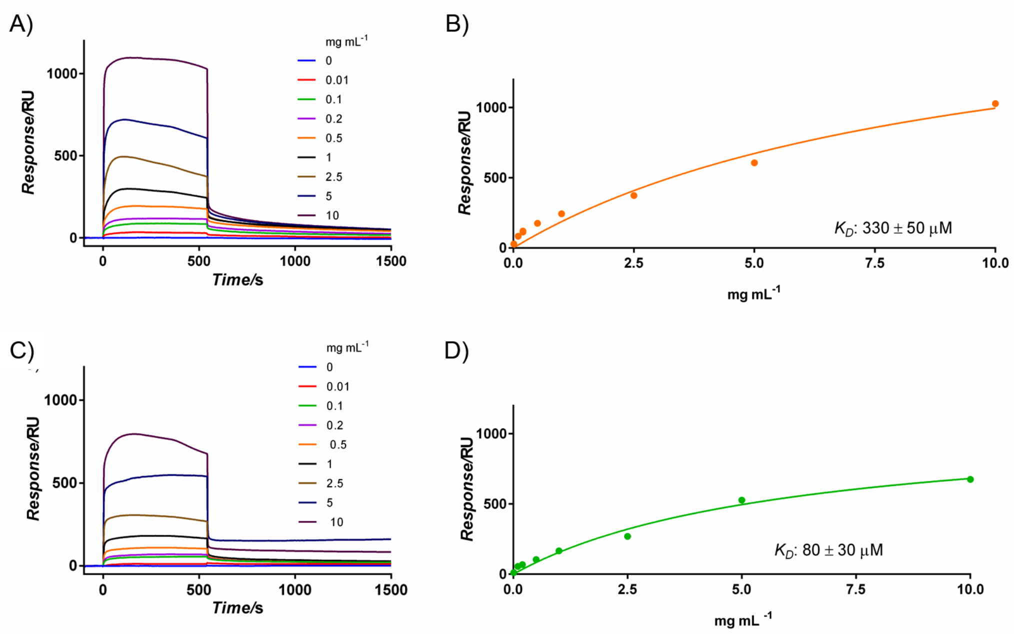
internalization is favoured by the stripe-like morphology of the monolayer.

MEC-1 cells treated with **BODIPY-tagged NPs**. **A**) Confocal images of control cells, **B**) cells treated with 1 mg/r
Janus NP and **C**) cells treated with 0,1 mg/ml striped, for 60 min prior to counterstaining nuclei with Hoecst c
Panel **D** represents the flow cytometric overlay of green fluorescence emitted from untreated (grey peak) and
BODIPY-NP treated cells, 1mg/ml Janus (J) and 0,1 mg/ml striped (S).

L. Pasquato, P. Posocco, *Small 2019*.

SPR Experiments – binding NPs-model membranes

The sensor surface is dextran coated, chip L1
Liposomes of DOPC



Nanoparticles	K_{diss}, μM
NP-striped	80 ± 28
NP-Janus	330 ± 50
NP-F8-PEG	60 ± 50
NP-C8-PEG	118 ± 50

L. Pasquato, P. Posocco, *Small 2019*.

Computational studies of NP-membrane interaction by MARTINI mapping

credits to: Paola Posocco
Domenico Marson

Nanoparticle/ composition	ΔG_{adh} [kcal/mol]	$N_{contacts}$	% contacts non-PEG component	% contacts PEG component
NP-Striped	-38.9 ± 1.0	25 ± 1	37	63
NP-Janus	-28.6 ± 1.5	21 ± 2	41	59
NP-F8-PEG	-51.0± 1.2	32 ± 2	27	73
NP-C8-PEG	-44.1 ± 0.8	31 ± 2	28	72

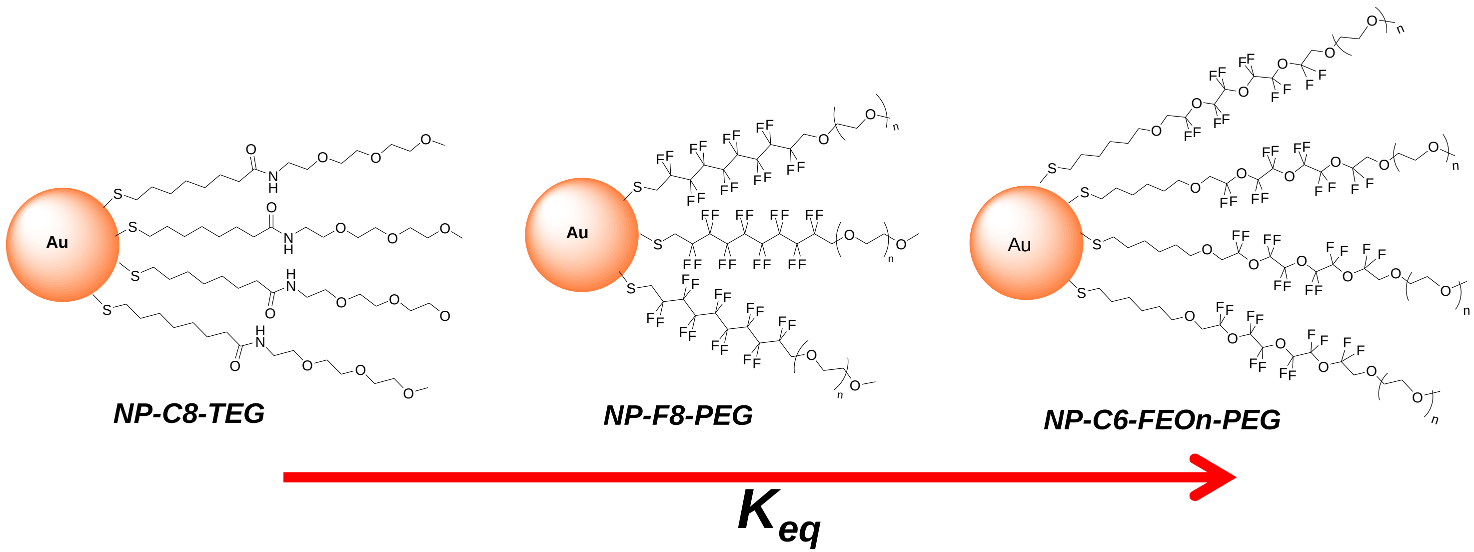
Detachment of NP from the membrane by «umbrella sampling»

L. Pasquato, P. Posocco, *Small 2019*.

gold nanoparticles protected by amphiphilic fluorinated ligands

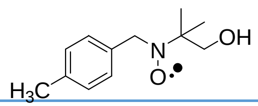
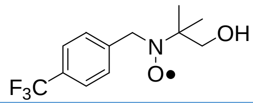
steric effect?

	a(N)/G	a(2H_β)/G	g-factor	d core	K_{eq}/M^{-1}
Water	16.25	10.14	2.0056	-	-
NP-C8-TEG	15.67	8.97	2.0057	1.6 nm	104
NP-F8-PEG	15.46	8.68	2.0057	2.7 nm	176
NP-C6-FEOn-PEG	**15.45**	**8.65**	**2.0057**	**1.4 nm**	**593**



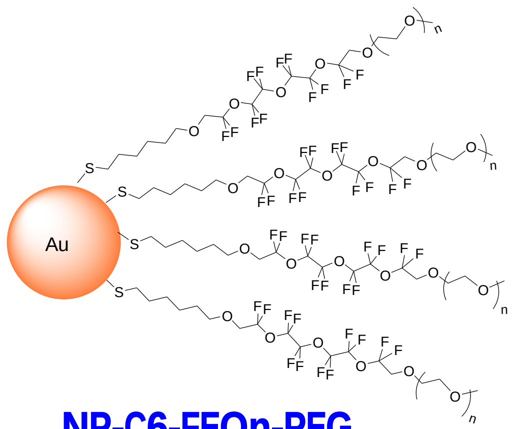
drug loading - *H*- vs. *F*-monolayer

- desolvation energy
- hydrophobic interactions
- halogen bonds

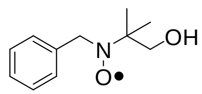
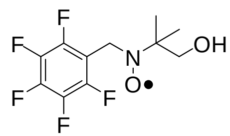
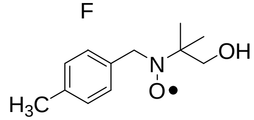
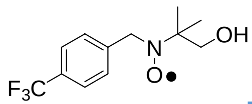
	 K_{eq} / M^{-1}	 K_{eq} / M^{-1}	$K_{eq}(F)/K_{eq}(H)$
NP-C8-TEG	2.2	4	1.8
NP-F8-PEG	5.7	29	5.1
NP-FEO$_n$-PEG	16	100	6.2

NP-C8-TEG → NP-F8-PEG: 2.6 (first column), 7.2 (second column)

NP-F8-PEG → NP-FEO$_n$-PEG: 2.8 (first column), 3.4 (second column)



NP-C6-FEOn-PEG

	K_{eq} / M^{-1}	$K_{eq}(F)/K_{eq}(H)$
	6.2	12.9
	80	
	16	6.25
	100	

L. Pasquato et al. J. Mater. Chem. 2015, 3, 432-439.