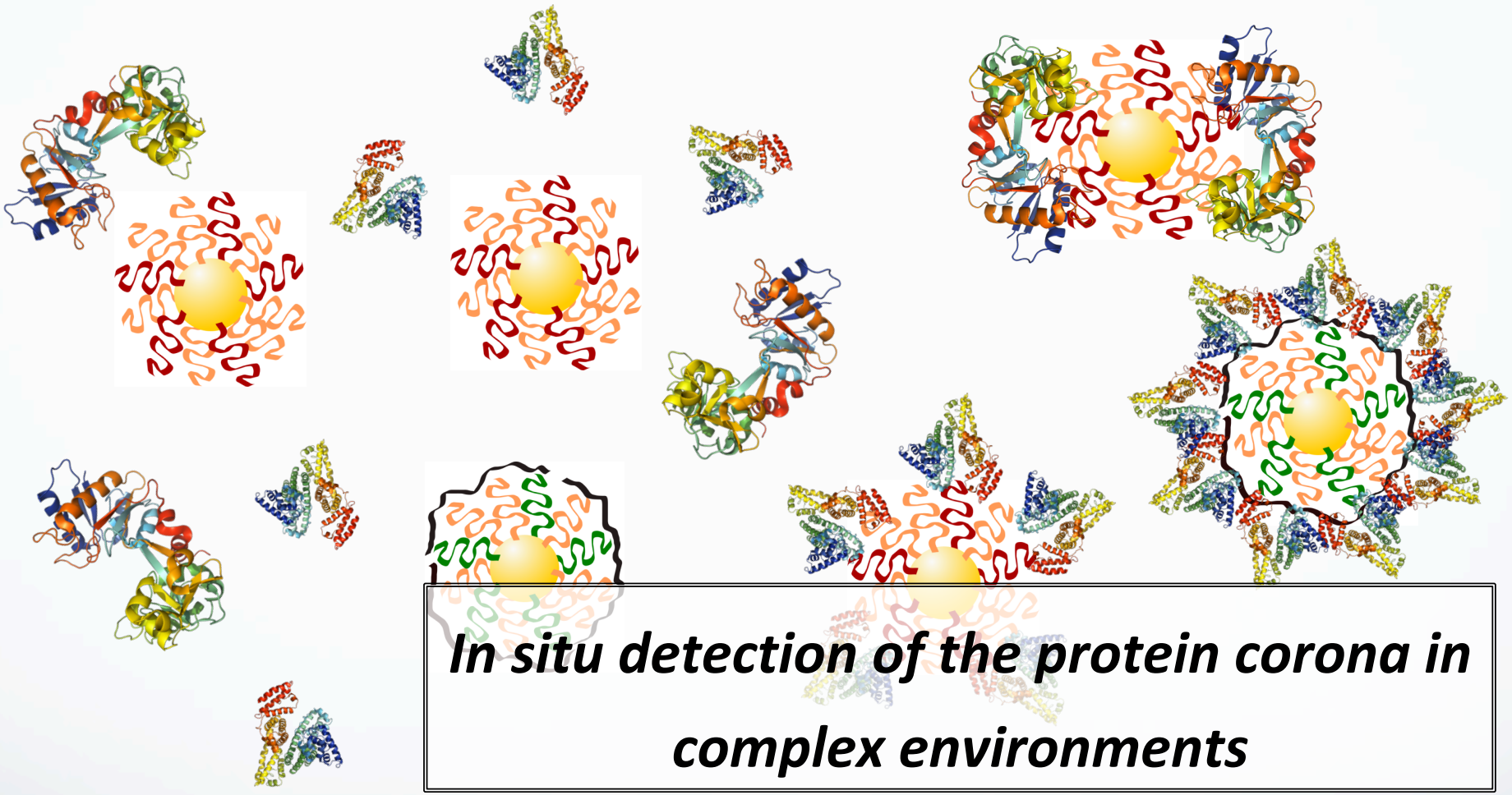


In situ detection of the protein corona in complex environments

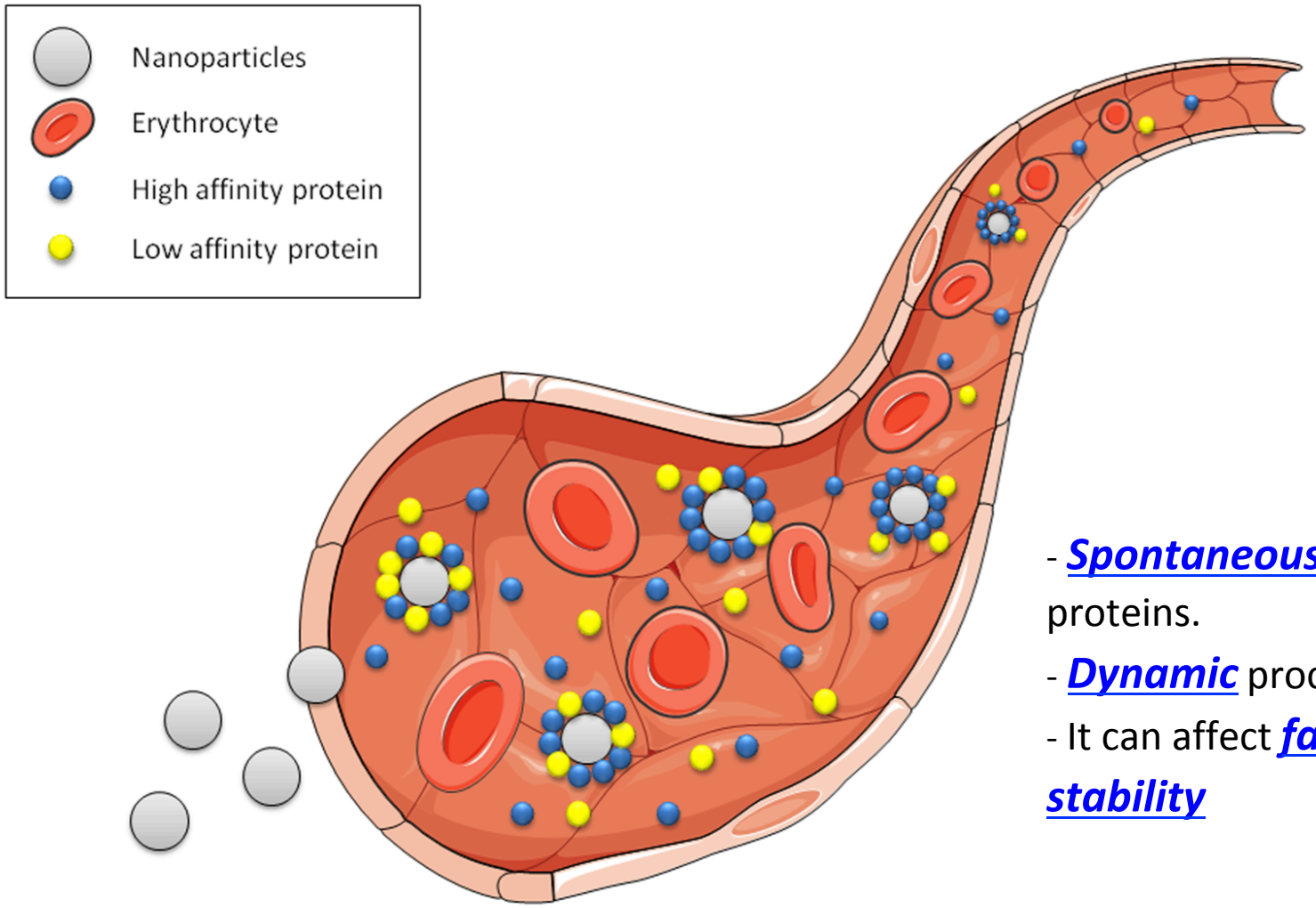
Mónica Carril, *D. Padro, P. del Pino, C. Carrillo-Carrion, M. Gallego & W.J. Parak*

ikerbasque
Basque Foundation for Science

CIC biomaGUNE
Biomaterialetako Ikerkuntza Kooperatiboko Zentroa
Centro de Investigación Cooperativa en Biomateriales

mcarril@cicbiomagune.es

Protein Corona



- ***Spontaneous*** adsorption of proteins.
- ***Dynamic*** process (equilibrium)
- It can affect ***fate***, ***uptake*** and ***stability***

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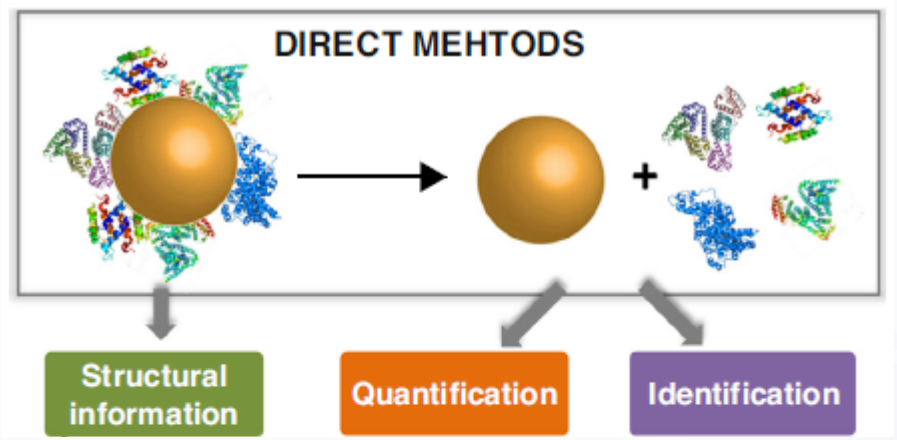
Protein Corona

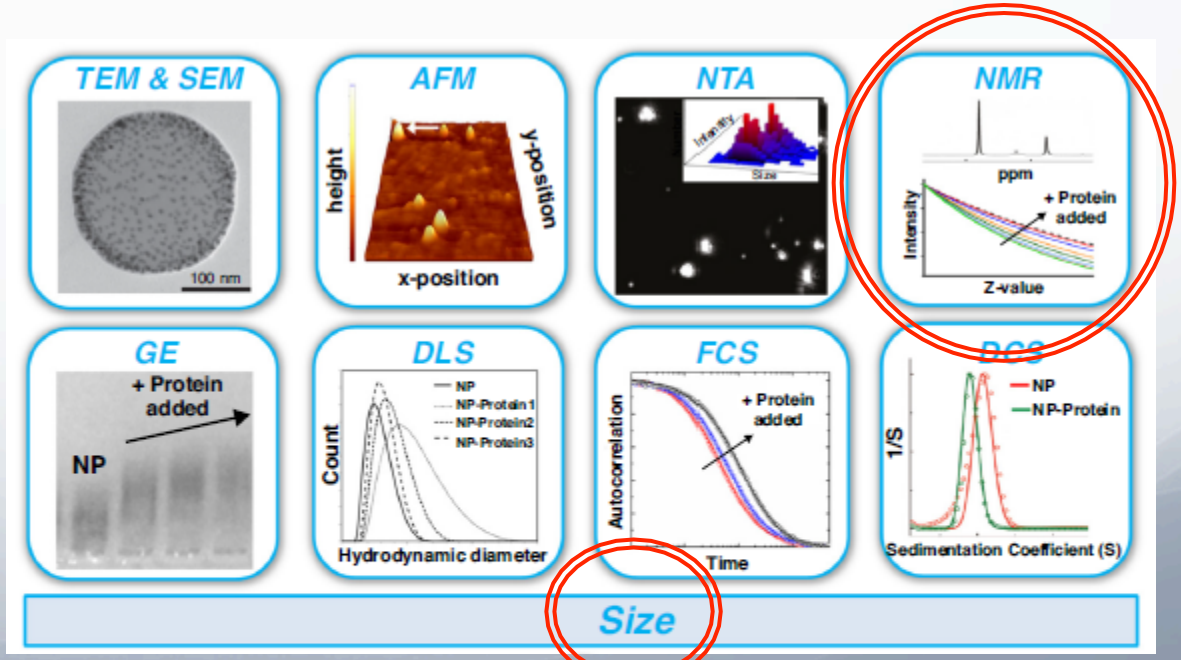
Direct methods: directly analyze the proteins that are adsorbed on the NP surface.
Sample digestion or isolation.
Non-equilibrium situation.



Indirect methods: analyze the changes in the properties of the underlying NPs due to the presence of a protein corona.

size, charge, mass, absorbance, and fluorescence…

^{19}F



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Why ^{19}F-NMR

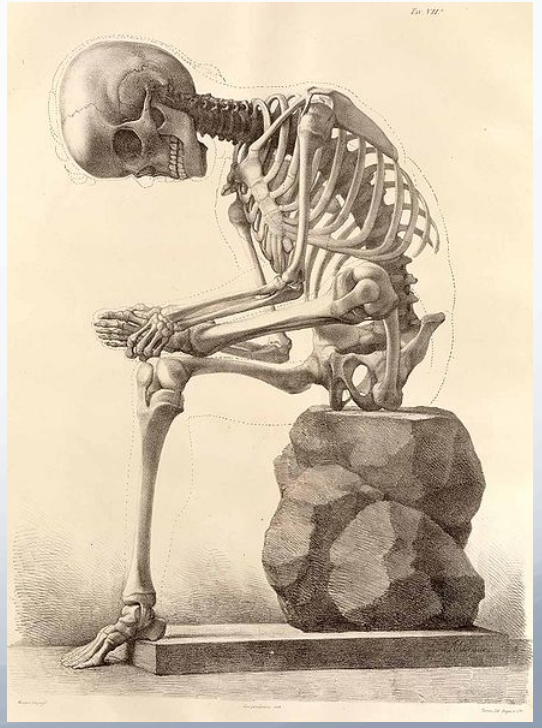
100% Natural Abundance.

^{19}F is not present in physiological media.

No background signal or interference.

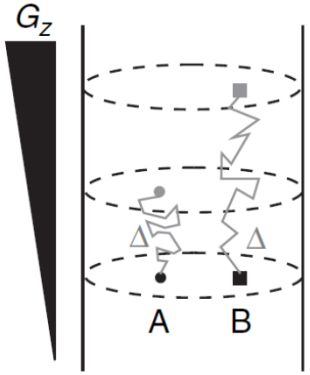
} **NO** sample isolation

MR is technique that can be translated to *in vivo* studies.

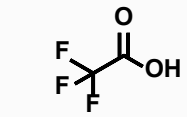


Spatial mapping of each molecule (gradients)

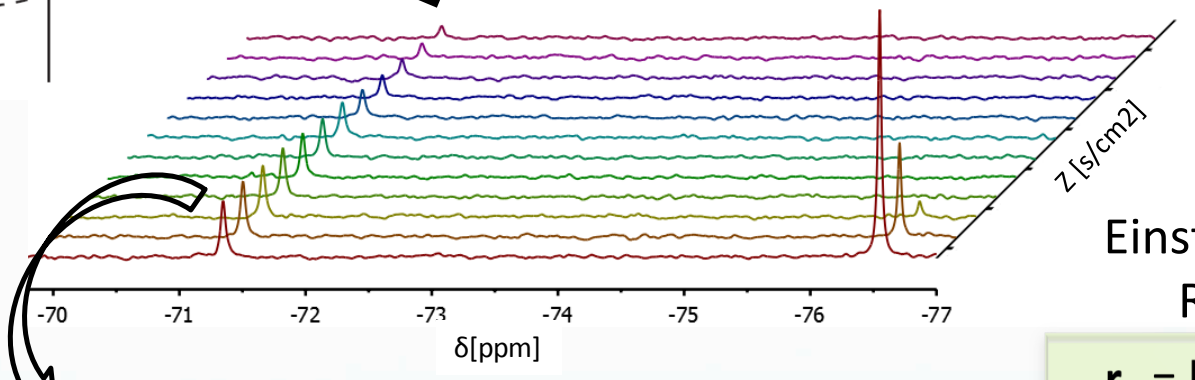
^{19}F-NMR diffusion measurement



NP-F/COOH



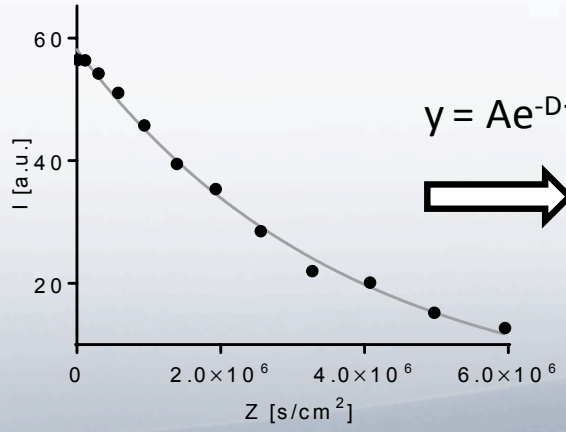
TFA



Einstein-Stokes Relation

$$r_h = k_B T/(6\pi\eta D)$$

Z	I (Z)
16498.19	56.441
114620.00	56.341
301093.10	54.21
575822.90	51.051
938948.80	45.735
1390287.00	39.456
1930065.00	35.37
2558011.00	28.525
3274443.00	22.009
4078996.00	20.135
4972081.00	15.226
5953503.00	12.696

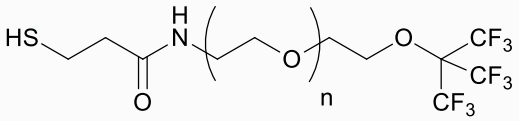


$y = Ae^{-D \cdot Z}$

D value

^{19}F-labelled Au NPs

HS-PEG-F

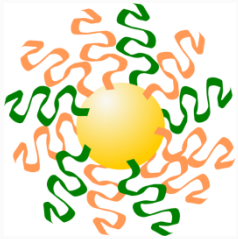


NP-F

r_C = 1.9 ± 0.5 nm

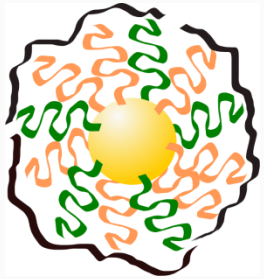
NP-F/COOH

r_C = 1.5 ± 0.7 nm



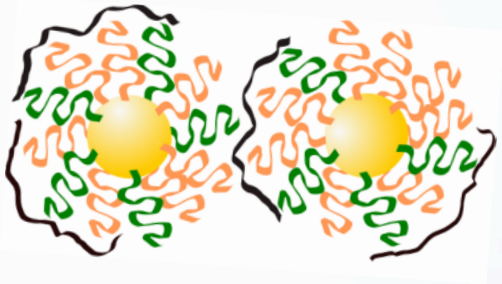
NP-F/NH_2

r_C = 1.7 ± 0.6 nm



NP-F/NH_2@PMA

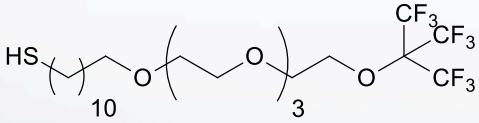
r_C = 1.7 ± 0.6 nm

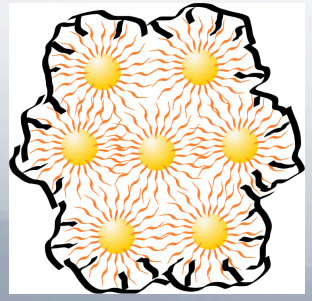


[NP-F/NH_2@PMA]*2

r_C = 1.7 ± 0.6 nm

$HSC_{11}TEG$-F

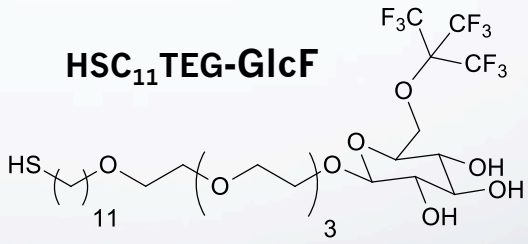


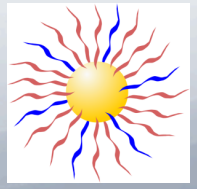


NP-TEGF@PMA

r_C = 12.6 ± 3.8 nm

$HSC_{11}TEG$-GlcF



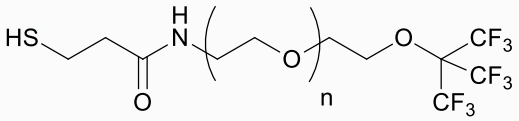


NP-GlcF/OH

r_C = 0.9 ± 0.6 nm

^{19}F-labelled Au NPs

HS-PEG-F



NP-F

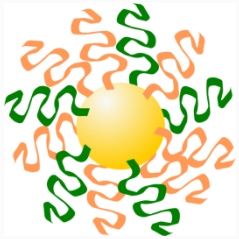
r_C = 1.9 ± 0.5 nm

r_h = 8.7 ± 0.3 nm

NP-F/COOH

r_C = 1.5 ± 0.7 nm

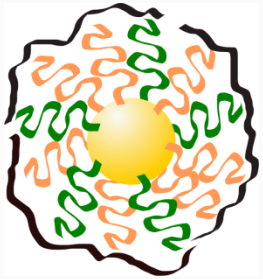
r_h = 9.0 ± 0.1 nm



NP-F/NH_2

r_C = 1.7 ± 0.6 nm

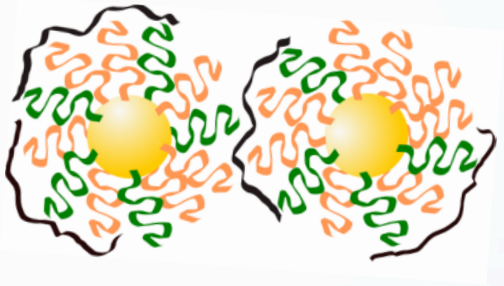
r_h = 8.6 ± 0.1 nm



NP-F/NH_2@PMA

r_C = 1.7 ± 0.6 nm

r_h = 7.3 ± 0.6 nm

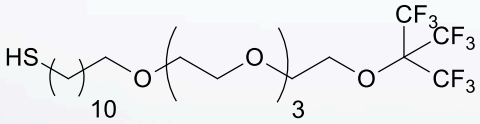


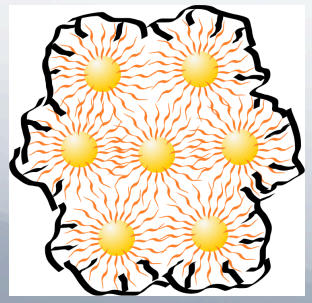
[NP-F/NH_2@PMA]*2

r_C = 1.7 ± 0.6 nm

r_h = 20.2 ± 1.0 nm

$HSC_{11}TEG$-F



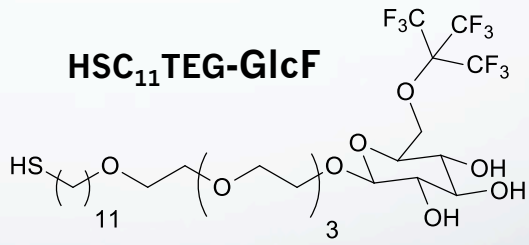


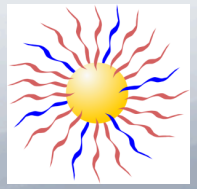
NP-TEGF@PMA

r_C = 12.6 ± 3.8 nm

r_h = 12.6 ± 1.3 nm

$HSC_{11}TEG$-GlcF





NP-GlcF/OH

r_C = 0.9 ± 0.6 nm

r_h = 3.0 ± 0.1 nm

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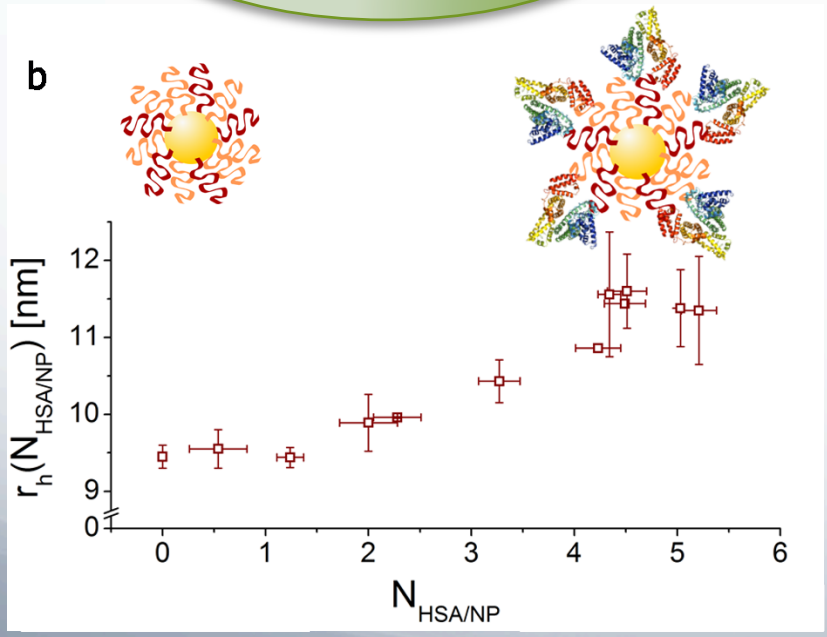
NP-F/COOH

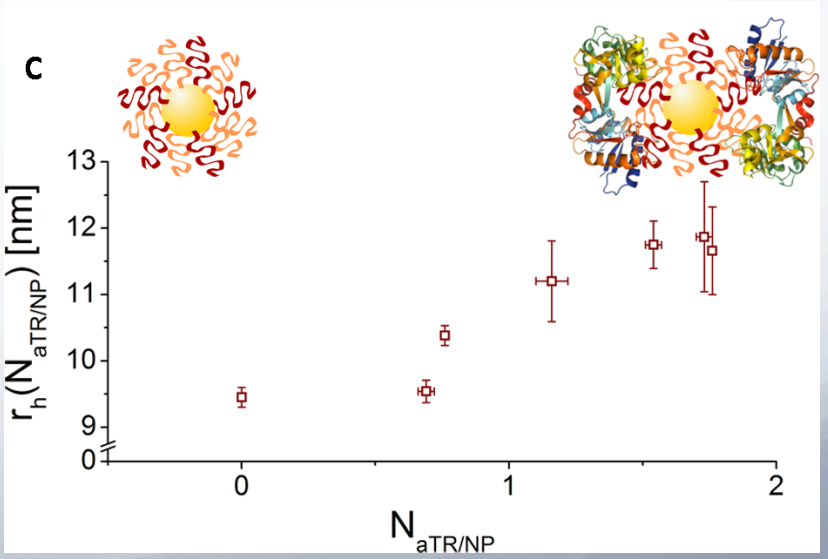
1. EDC/NHS
2. HSA or aTR

Covalently bound corona

HSA
$N_{HSA/NP}$ max ≈ 5
$\Delta r_h = 1.9 \pm 0.2$

aTR
$N_{aTR/NP}$ max ≈ 2
$\Delta r_h = 2.4 \pm 0.2$





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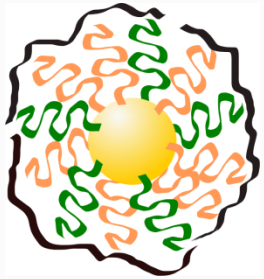
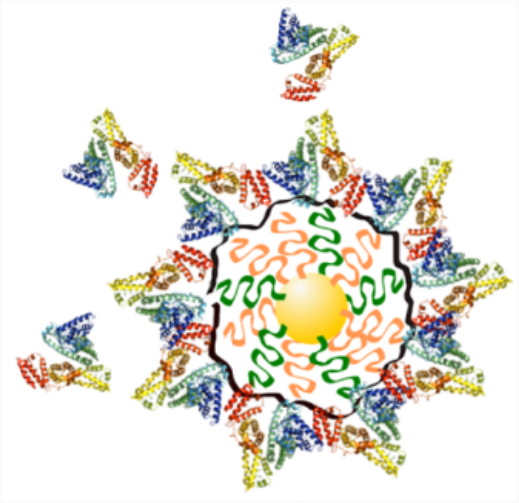
Spontaneous corona



NP-F/NH_2@PMA

HSA or TR



HSA

$N_{HSA/NP}$ max ≈ 65.6 ± 2.6

Δr_h = 4.9 ± 0.1

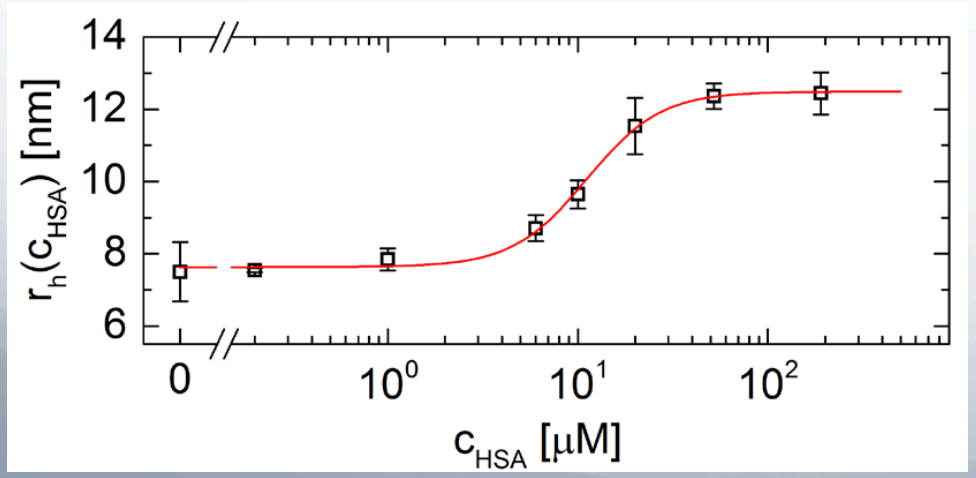
K'_D = 13.6 ± 0.9

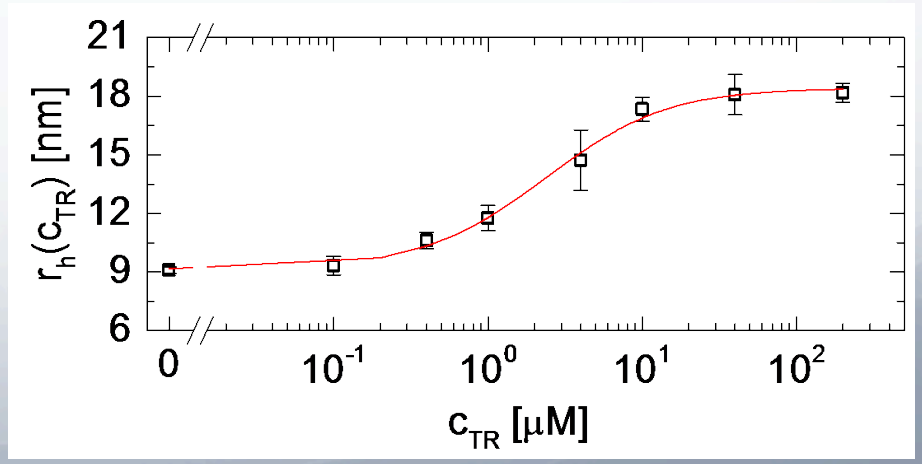
TR

$N_{TR/NP}$ max ≈ 191± 11.4

Δr_h = 9.2 ± 0.3

K'_D = 4.1 ± 0.7





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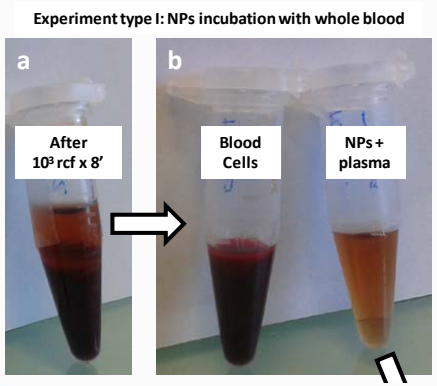
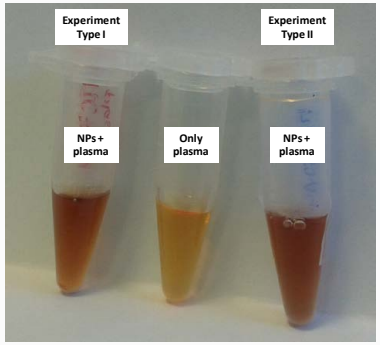
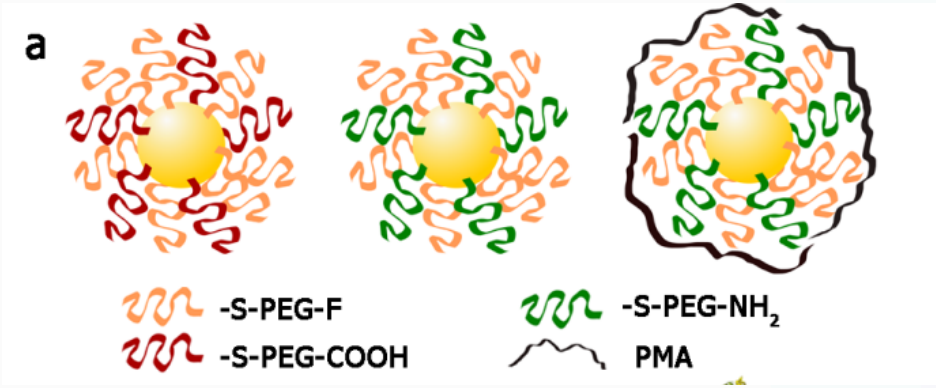
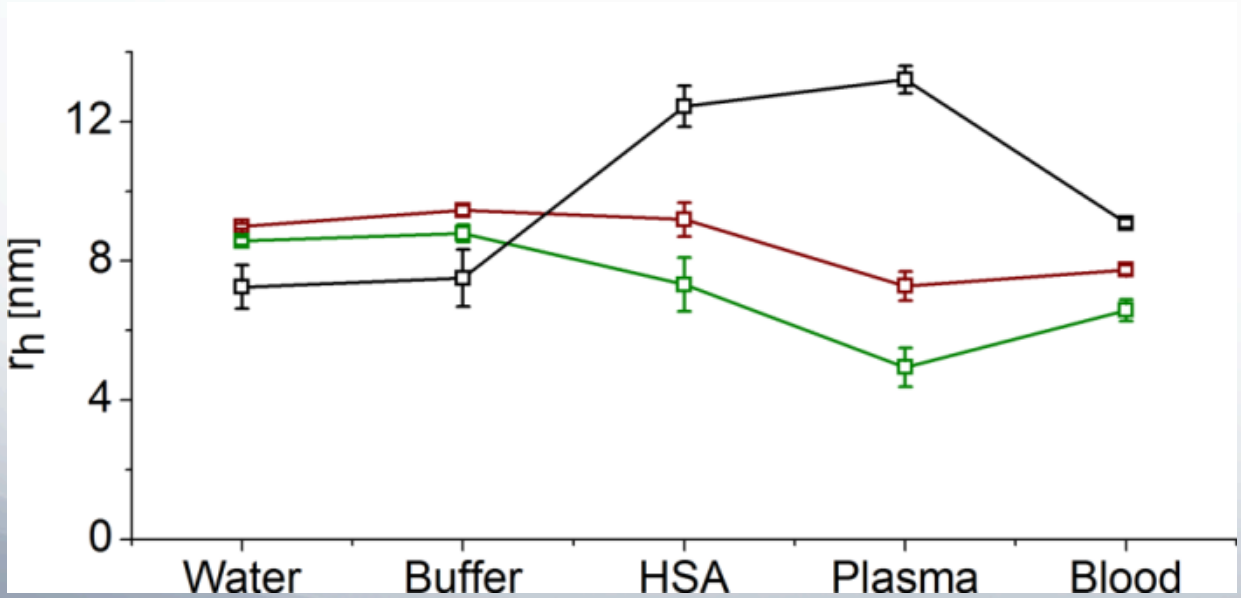
Measurements in blood/plasma









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Summary

- It is possible to measure ***diffusion*** of fluorinated NPs in ***complex media*** without isolation by ***^{19}F NMR***.

- This technique can be used to study ***size changes*** due to ***protein corona*** formation.

- Expand the ***scope*** of proteins studied.

- Evaluate protein corona ***in vivo*** by ^{19}F MRS.

Thank you
for your attention

Wolfgang J Parak
Pablo del Pino
Daniel Padró
Carolina Carrillo-Carrión
Marta Gallego
Jorge Blanco

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