Characterizations of inorganic NPs

Focus on Bio-functionalization

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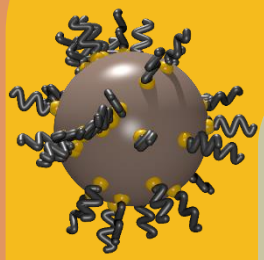
Nanoparticles for biology

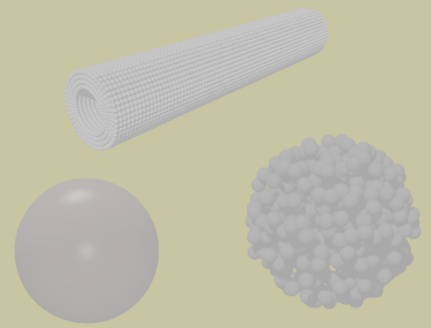
Functionalization for biomedical applications

Therapy

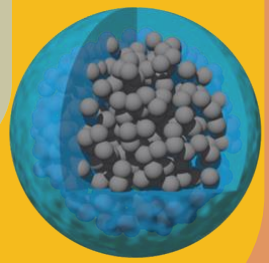
Stabilization in physiological media
Surface modification



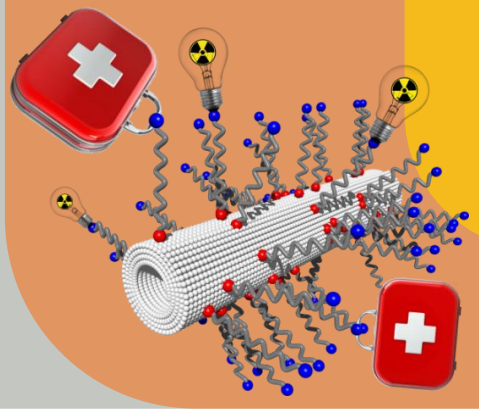
NP core



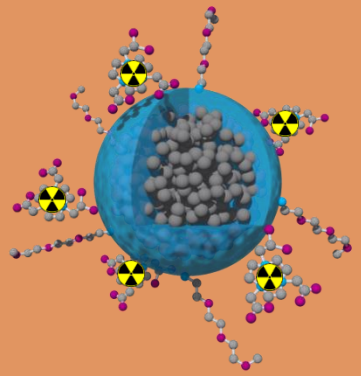
Specific properties



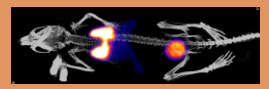
Biocompatible molecules – polymers…



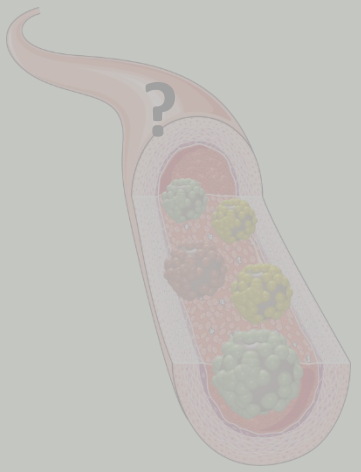
Radioelement – Drug – Antibody – Protein …



Diagnosis

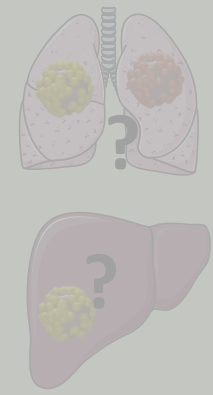


Biocirculation?



Toxicity?

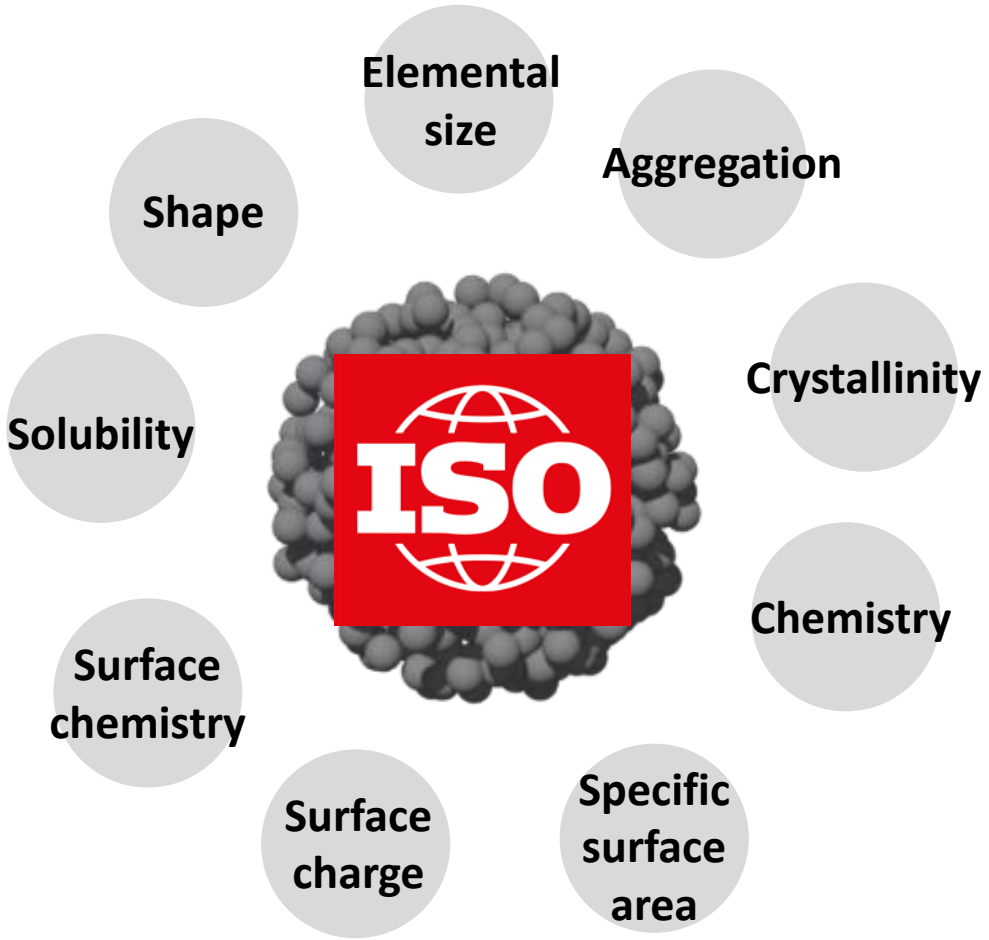
Biodistribution?

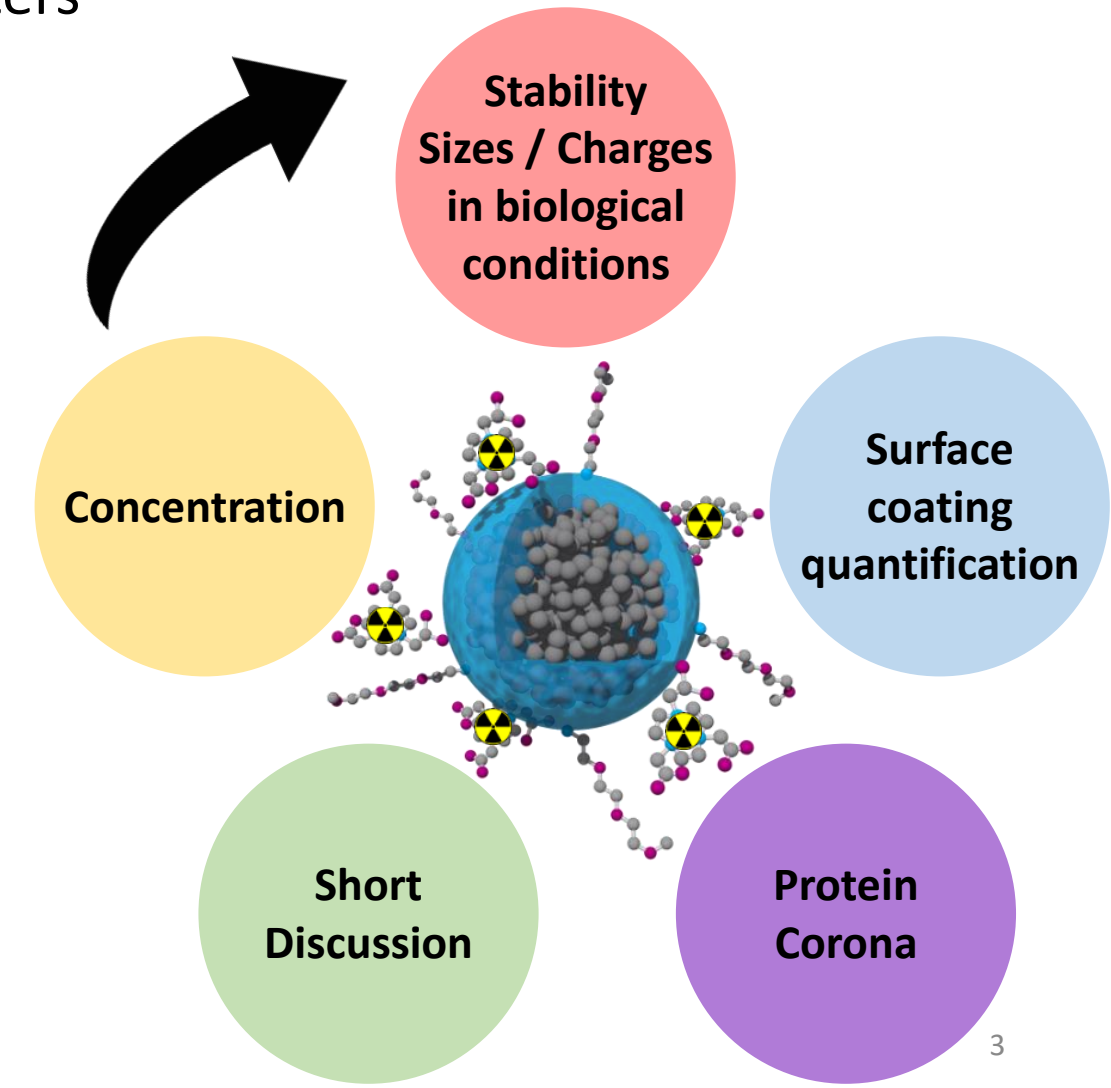


Cellular uptake?

Nanoparticles

- 9 ISO parameters to study
- For biomedical applications: More parameters

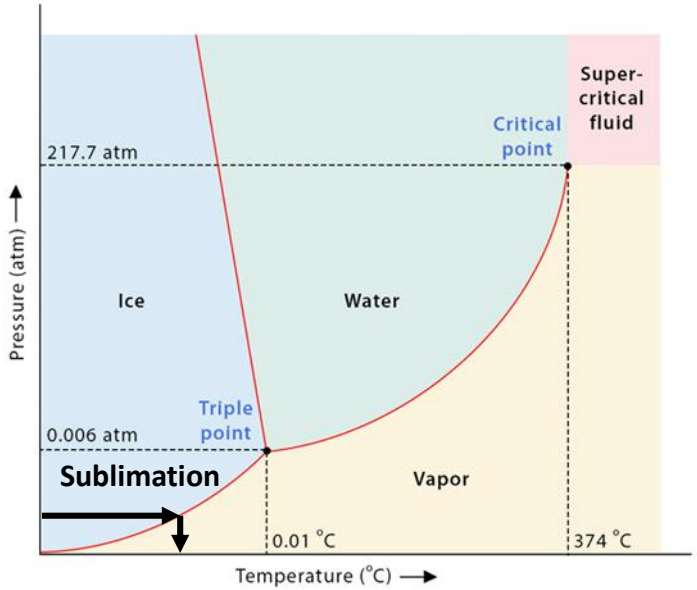




Concentration

Concentration

- (Freeze) Drying
  - NPs powder
  - Freeze Drying at low P and T increase stability of NPs compared to normal drying
  - Will quantify the whole sample (coatings and other element included): mg_{NP}/mL

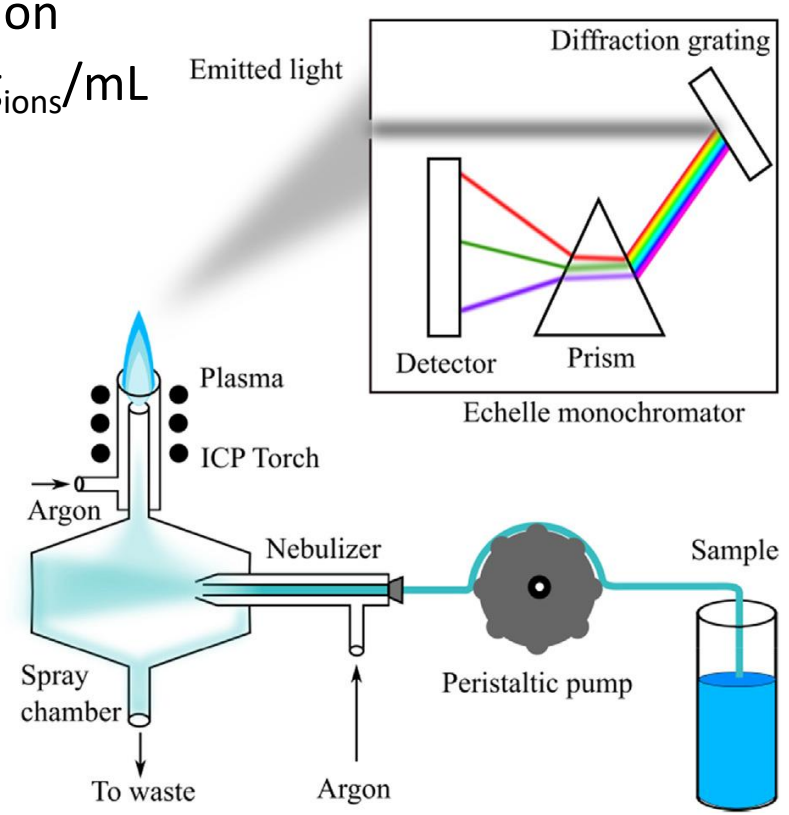


https://doi.org/10.1016/j.ejpb.2021.05.024

Concentration

Concentration

- (Freeze) Drying
- ICP Induced Coupled Plasma
  - Need to dissolve NP's suspension
  - Titrate the inorganic part: : mg_{ions}/mL



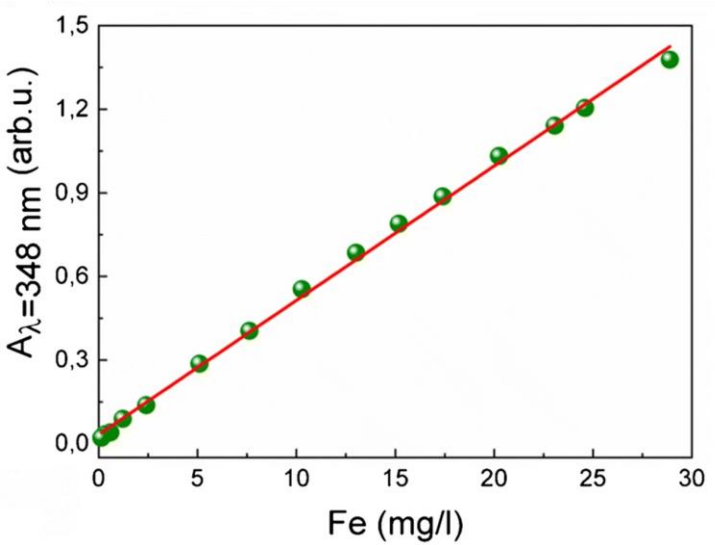
Concentration

Concentration

- (Freeze) Drying
- ICP Induced Coupled Plasma
- UV-Visible
  - Suspension as such or diluted
  - For colored NPs
    - Depend on the NPs size so need reproducible NPs

    or preliminary other quantification (ICP or Freeze drying)

https://doi.org/10.1007/s00604-020-04454-w



Concentration

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- (Freeze) Drying
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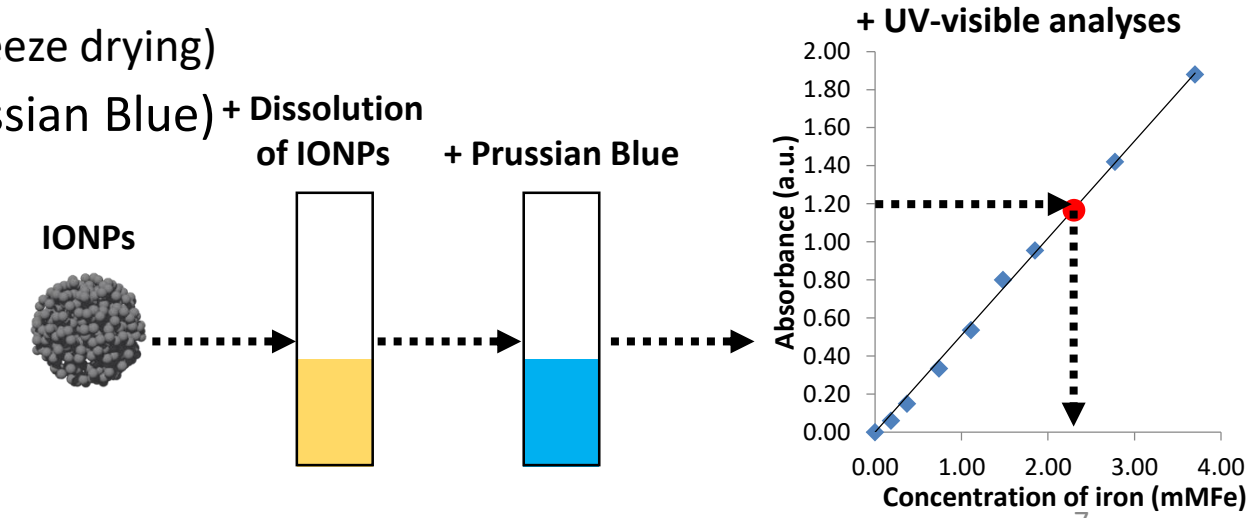
  - Suspension as such or diluted
  - For colored NPs
    - Depend on the NPs size so need reproducible NPs

    or preliminary other quantification (ICP or Freeze drying)
  - With a reactants for titration (example Prussian Blue)

or chemical titration (Redox): mg_{ions}/mL

$HCl + \text{Dissolved } Fe_3O_4 + K_4Fe^{II}(CN)_6, 3\ H_2O \rightarrow [Fe^{III}_4(Fe^{II}(CN)_6]_3$



Concentration

Concentration

- (Freeze) Drying
- ICP Induced Coupled Plasma
- UV-Visible
- Magnetic Susceptibility

Concentration

Concentration

- (Freeze) Drying
- ICP Induced Coupled Plasma
- UV-Visible
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  - Suspension as such or diluted
  - For magnetic NPs
    - Depend on the NPs size so need reproducible NPs

    and preliminary other quantification (ICP or Freeze drying

https://doi.org/10.3390/magnetochemistry8090107

https://doi.org/10.1016/j.heliyon.2023.e16601

	Magnetic NPs		
Me	**Me-O**	**MFe_2O_3**	**Doped**
Fe	Fe_3O_4	Cu	Zn^{+2}, Ni^{+2}, Al^{+3}, Mn^{+2}, Ag^{+}
Co	γ-Fe_2O_3	Co	Y^{+3}, Nd^{+3}, Ti^{+4}, Cd^{+2}, Dy^{+3}
Ni		Mn	Gd^{+3}, Cu^{+2}, Yb^{+3}, Eu^{+3}, Zr^{+4}
CoPt		Zn	In^{+3}, Cr^{+2}, Pr^{+3}, Sm^{+3}, Ho^{+3}
FePt		Ni	Er^{+3}, Mg^{+2}, La^{+3} or Ce^{+3}

Concentration

Concentration

- (Freeze) Drying
- ICP Induced Coupled Plasma
- UV-Visible
- Magnetic Susceptibility
  - Suspension as such or diluted
  - For magnetic NPs
  - History of this method

https://doi.org/10.1016/j.memsci.2009.12.025

Magnetic susceptibilities of $FeCl_3$ solutions.

Concentration (ppm)	Volume magnetic susceptibility k (SI unit, containing tap water)
300	−0.9E−05
3000	−0.7E−05
30,000	2.5E−05
300,000	34.2E−05

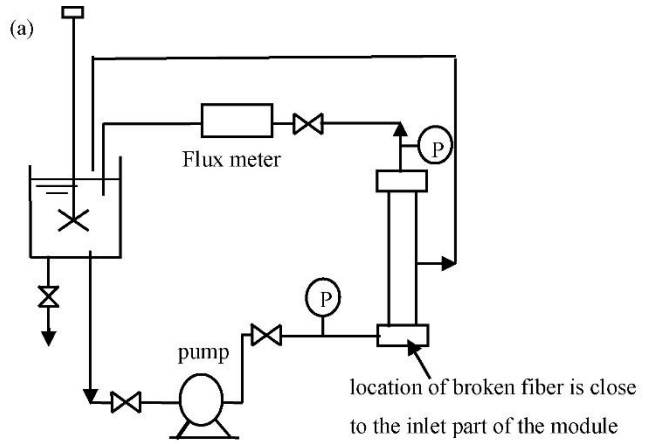
$FeCl_3$ solutions were diluted with tap water, k (tap water) = −0.9E−5.

Up to 10^4 X more sensitive to magnetic NPs than Fe

Magnetic susceptibilities of Fe_3O_4 suspensions (b).

Diluted times	Concentration (ppm)	Volume magnetic susceptibility k (SI unit)			
		Diluted with tap water		Diluted with canal water	
		k (containing tap water)	k	k (containing canal water)	k
100	12.69	0.2E−05	1.1E−05	0.3E−05	1.2E−05
1000	1.269	−0.4E−05	0.5E−05	−0.3E−05	0.6E−05
2000	0.6345	−0.6E+00	0.3E−05	−0.6E−05	0.3E−05
4000	0.3173	−0.7E−05	0.2E−05	−0.7E−05	0.2E−05
6000	0.2115	−0.7E−05	0.2E−05	−0.7E−05	0.2E−05
7000	0.1813	−0.8E−05	0.1E−05	−0.8E−05	0.1E−05
8000	0.1586	−0.9E−05	0.0E+00	−0.9E−05	0.0E+00

Initial concentration is 1.269 g L^{-1}, k (tap water) = −0.9E−5 SI unit, k (canal water) = −0.9E−5 SI unit.

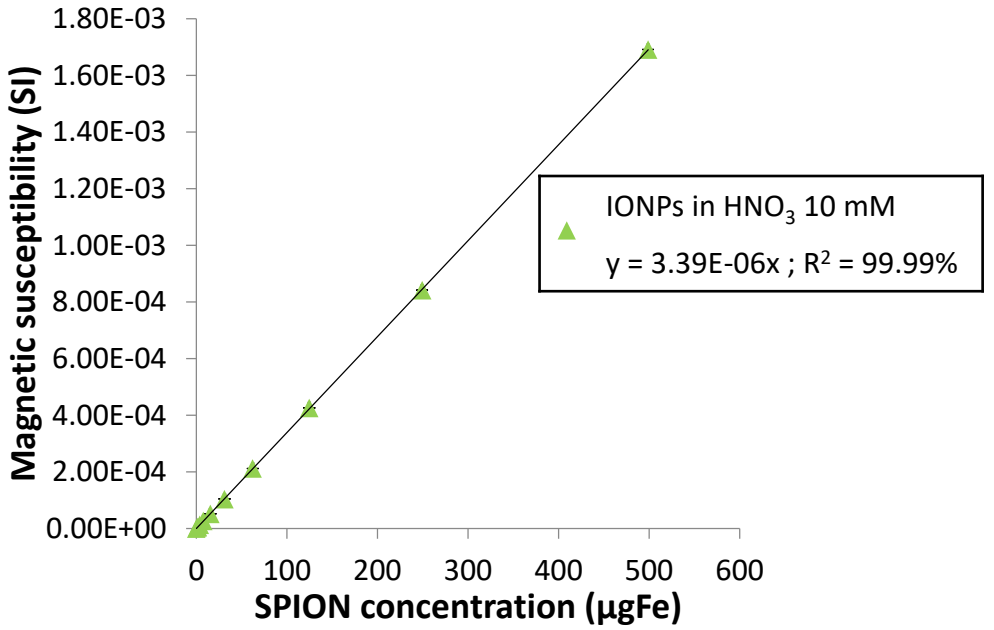


Concentration

Concentration

https://doi.org/10.1039/C3AN02153J

- (Freeze) Drying
- ICP Induced Coupled Plasma
- UV-Visible
- Magnetic Susceptibility
  - Suspension as such or diluted
  - For magnetic NPs
  - History of this method
  - Not destructive, fast (< 5 s) and reproducible



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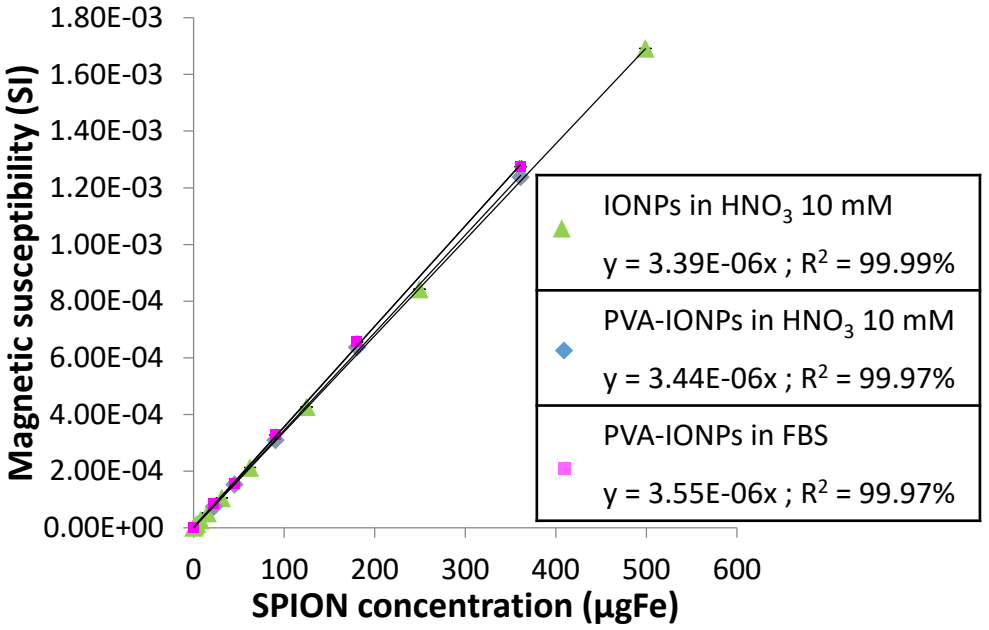
  - Not influenced by environment

  (coatings and solvent)



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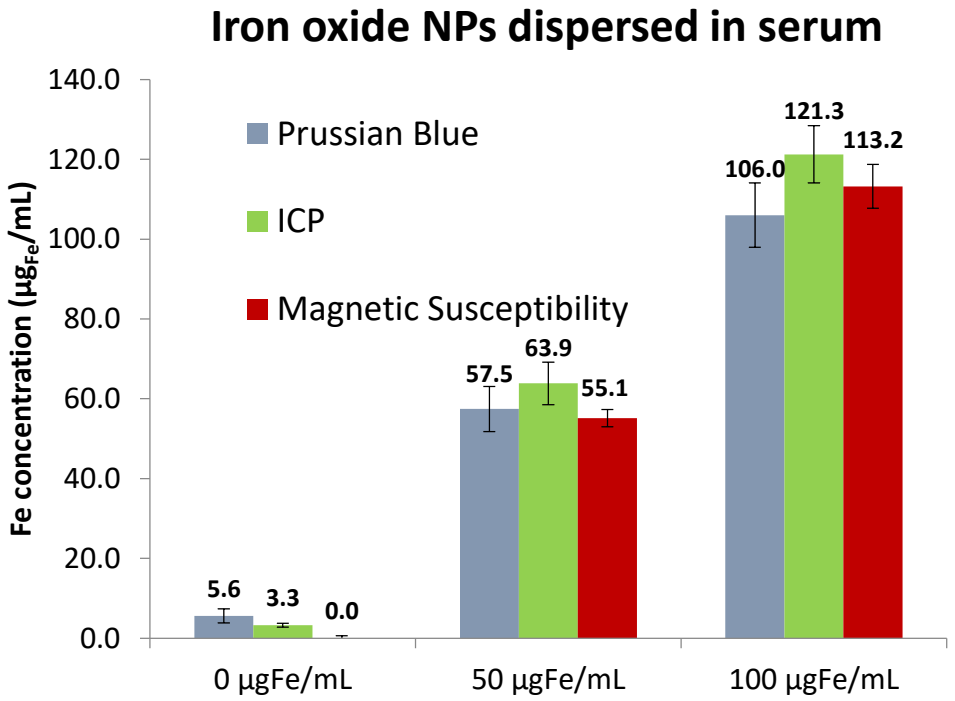
  - Not destructive, fast (< 5 s) and reproducible
  - Not influenced by environment

  (coatings and solvent)
  - Not sensitive to endogenous iron

  (for biological samples)



Concentration

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- ICP Induced Coupled Plasma
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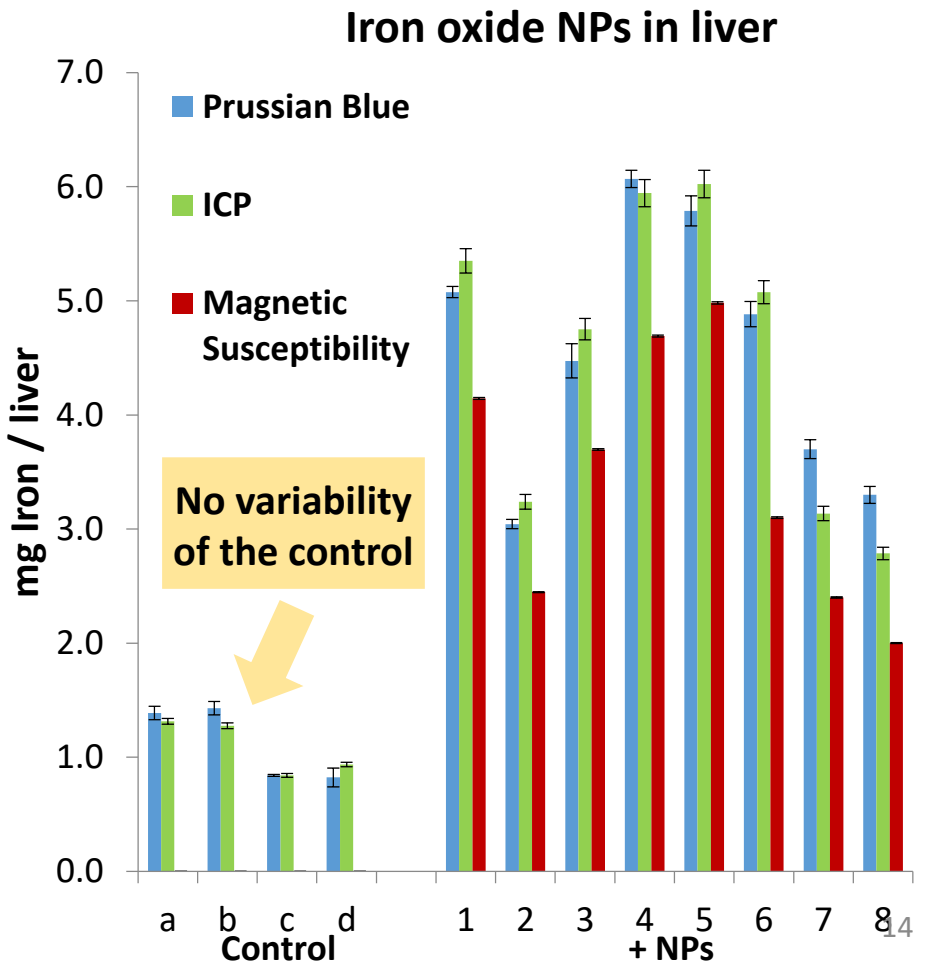


Concentration

Concentration

- (Freeze) Drying
- ICP Induced Coupled Plasma
- UV-Visible
- Magnetic Susceptibility

Concentration		
	Pros	**Cons**
(Freeze) Drying	Easy to use	Sensitive to whole sample
	Good to store sample?	Need powder form
ICP	Accurate (ppb)	Destructive
	Chemistry possible (see previous lecture)	Not for organic
UV-visible	Fast	Destructive
	0.1 ppm	Complicated for complex colored system
Magnetic susceptibility	Very fast and non destructive	Only for magnetic NPs + need preliminary quantification
	Not influenced by endogenous ions and environment	2 ppm

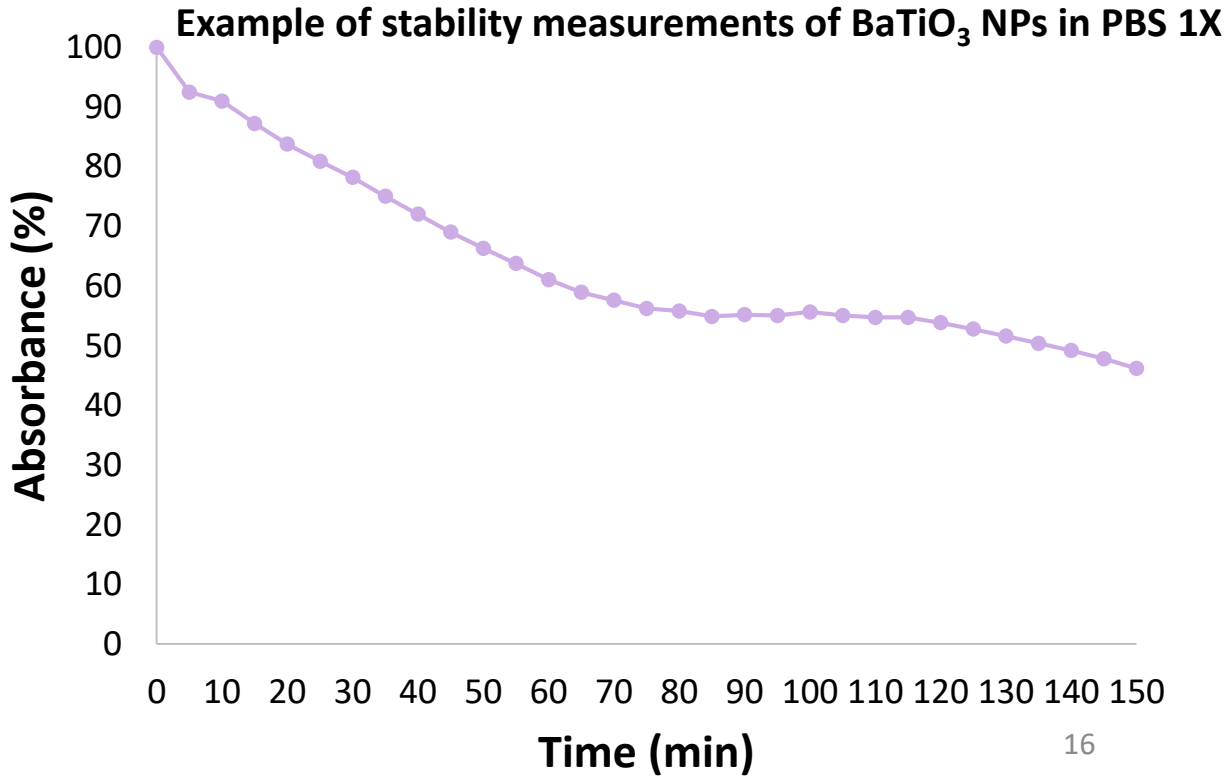
Methods for biological samples (cells, organs…)

Stability / Sizes / Charges in biological environment

Concentration | **Stability / Sizes / Charges**

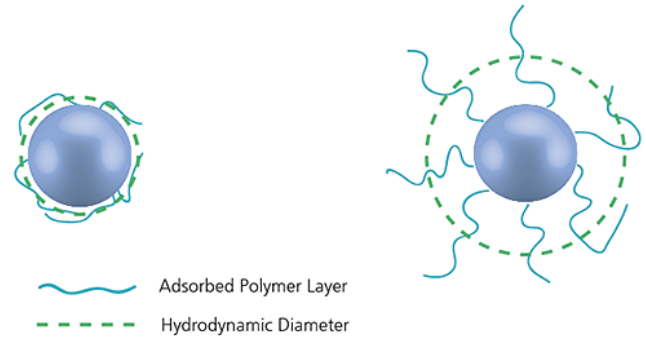
- UV-Visible
  - Evolution of absorbance vs time

Quantification of sedimentation



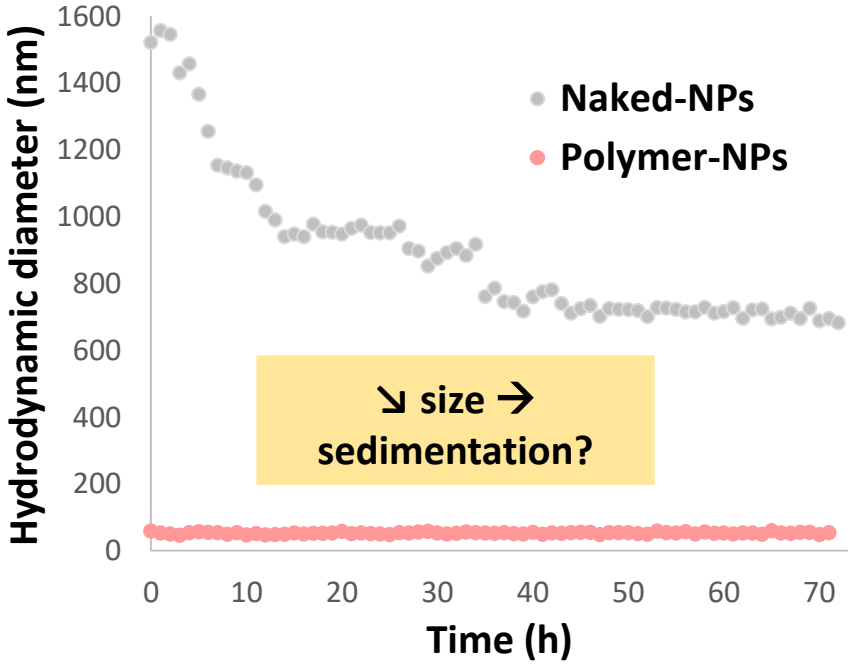
Stability / Sizes / Charges in biological environment

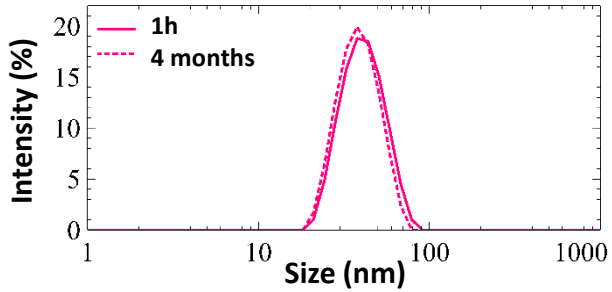
Concentration → **Stability / Sizes / Charges**



- UV-Visible
- DLS Dynamic Light Scattering or NTA Nanoparticle Tracking Analysis
  - For NPs in suspension
    - Evolution of size over time (hours or several days/weeks)

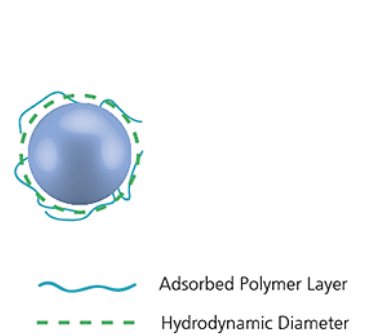
Be careful with conclusions on DLS data



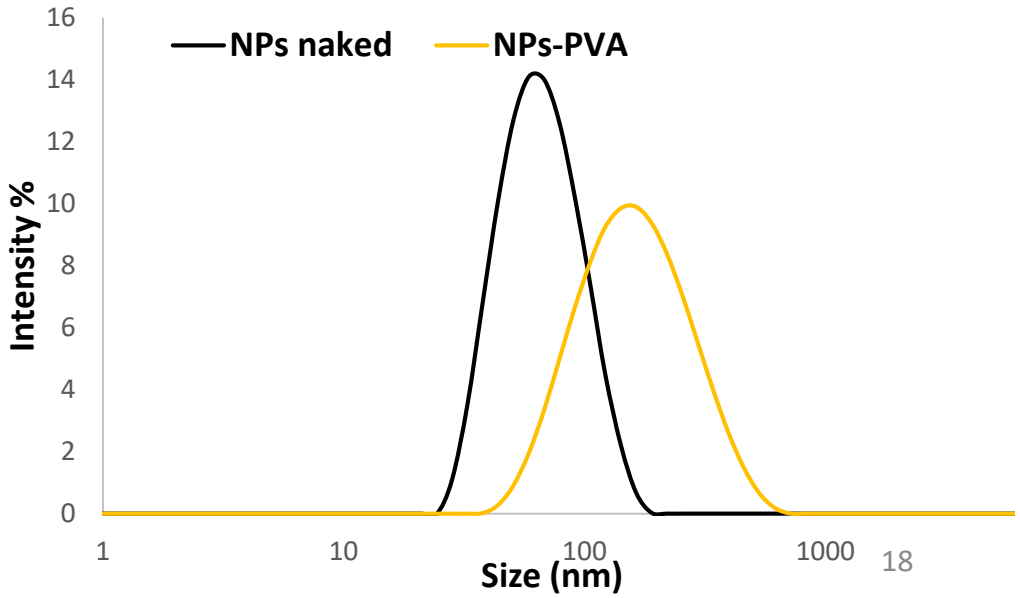


Stability / Sizes / Charges in biological environment

Concentration → **Stability / Sizes / Charges**

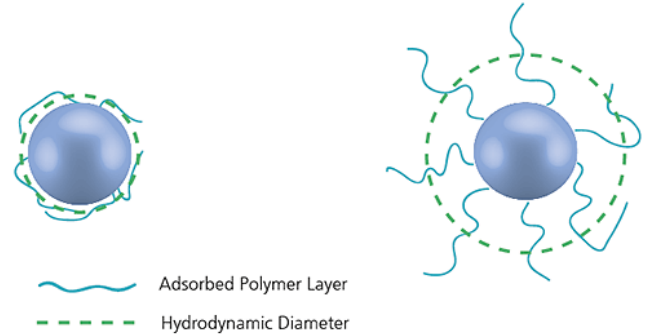


- UV-Visible
- DLS Dynamic Light Scattering or NTA Nanoparticle Tracking Analysis
  - For NPs in suspension
    - Evolution of size over time (hours or several days/weeks)
    - Polydispersity influenced by:
      - Coatings



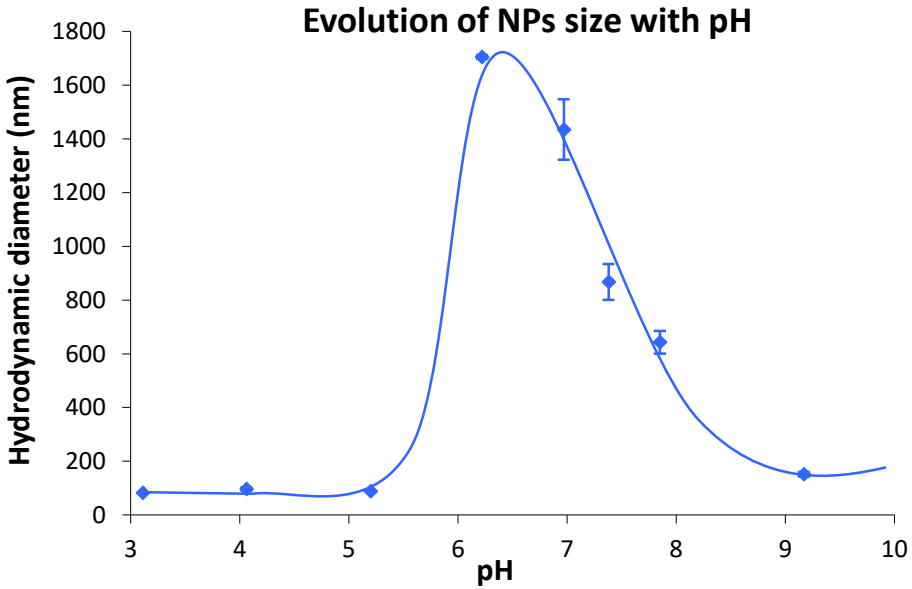
Stability / Sizes / Charges in biological environment

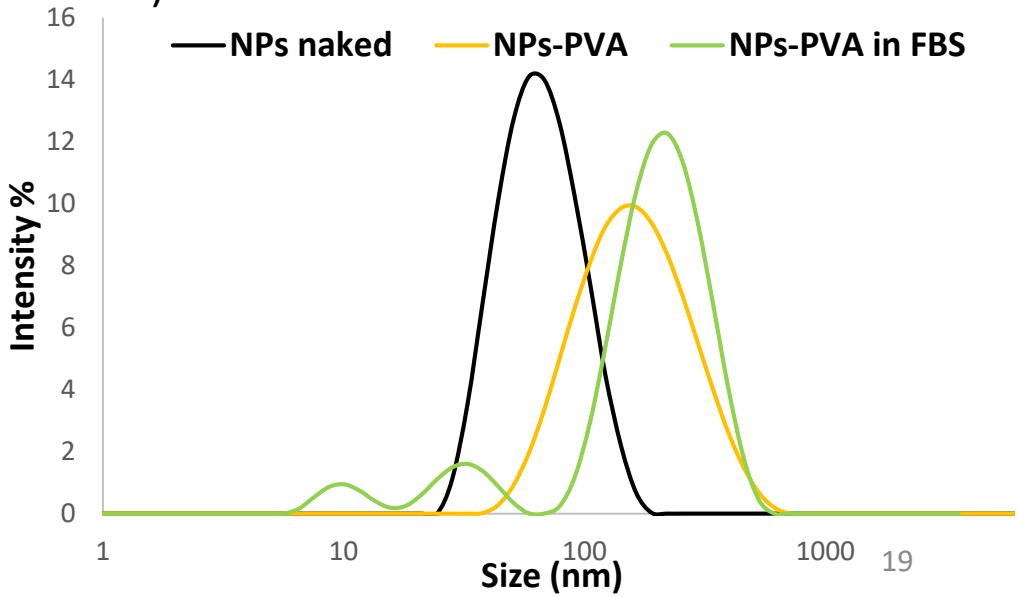
Concentration | **Stability / Sizes / Charges**



- UV-Visible
- DLS Dynamic Light Scattering or NTA Nanoparticle Tracking Analysis
  - For NPs in suspension
    - Evolution of size over time (hours or several days/weeks)
    - Polydispersity influenced by:
      - Coatings or Environment (pH, ionic strength, medium…)

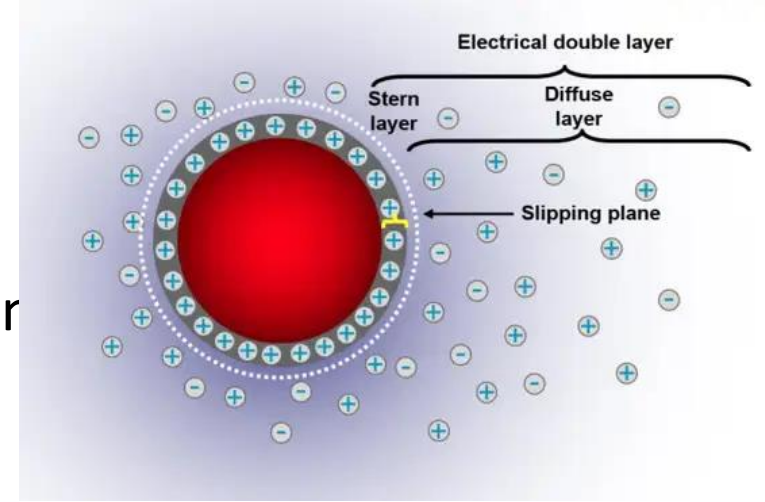
→ Medium in size measurements



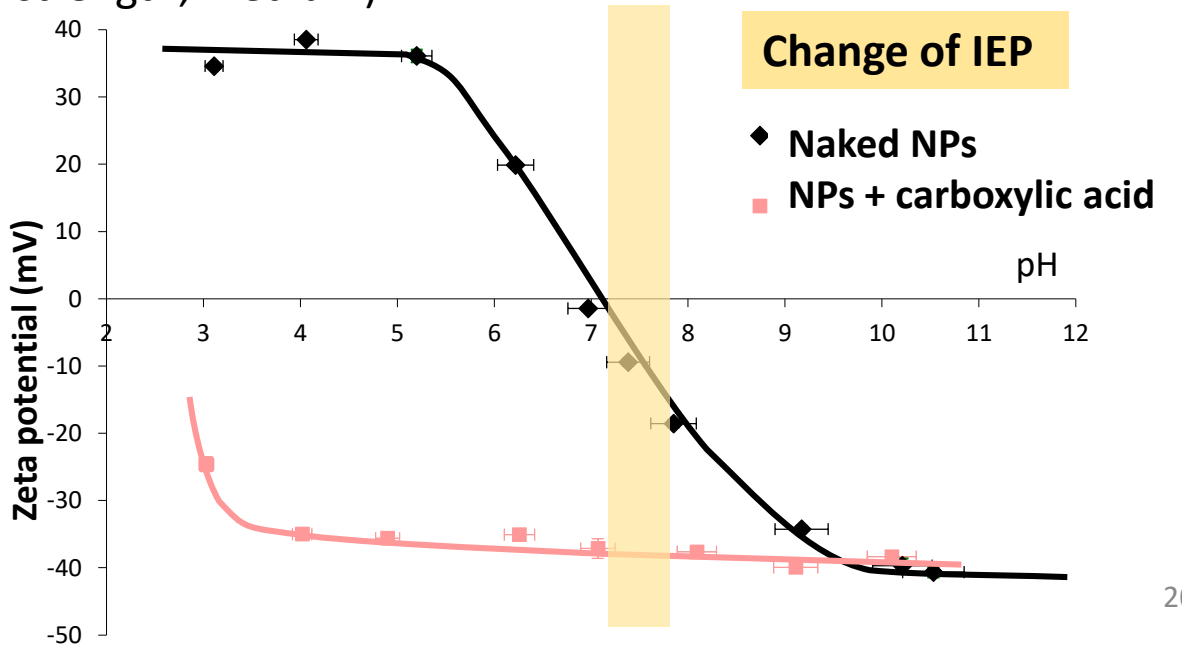


Stability / Sizes / Charges in biological environment

Concentration | **Stability / Sizes / Charges**

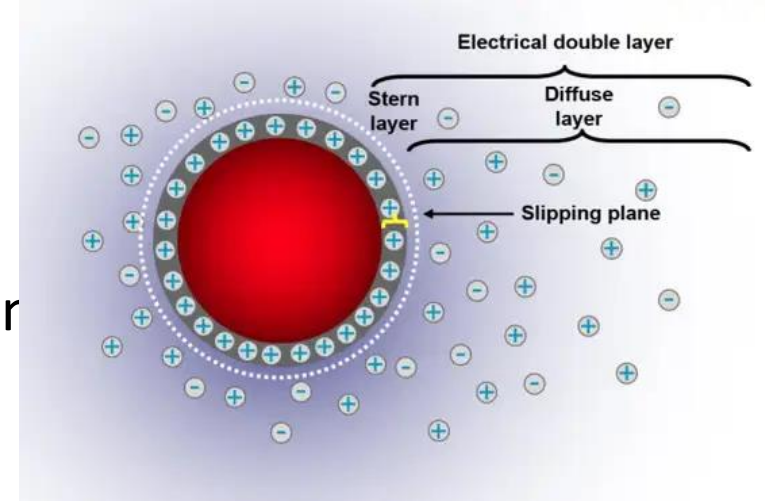


- UV-Visible
- DLS Dynamic Light Scattering or NTA Nanoparticle Trackir
- Zeta potential
  - For NPs in suspension
    - Dependent on environment (pH, ionic strength, medium)
    - Can prove correct functionalization



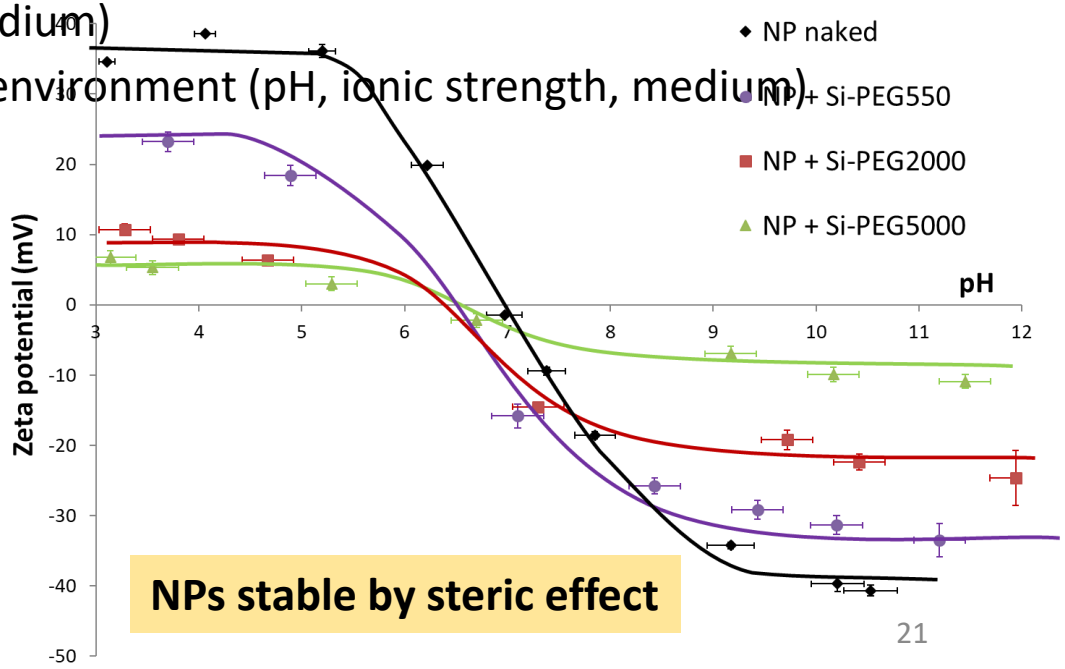
Stability / Sizes / Charges in biological environment

Concentration | **Stability / Sizes / Charges**



- UV-Visible
- DLS Dynamic Light Scattering or NTA Nanoparticle Trackir
- Zeta potential
  - For NPs in suspension
    - Dependent on environment (pH, ionic strength, medium)
    - Can prove correct functionalization Dependent on environment (pH, ionic strength, medium)
  - ”Stability” = f(Zeta) not totally correct

Zeta Potential (mV)	bility
0 to ± 10	ghly stable - rapid coa ulation/flocculation
± 10 to 20	imited stabi y
± 20 to 30	oderately stable
> ± 30	High table



NPs stable by steric effect

Stability / Sizes / Charges in biological environment

Concentration | **Stability / Sizes / Charges**

- UV-Visible
- DLS or NTA
- Zeta potential

Stability / Sizes / Charges		
	Pros	**Cons**
UV-visible	Easy to use and quantification possible	Complicated for long stability quantification
	Not destructive	
DLS	For short and long stability measurements	Biased observation possible due to technique ~ d^6
	Not destructive + Can prove coatings	Sensitive to environment
Zeta	For short and long stability measurements	Complicated in very saline media
	Not destructive + Can prove coatings	Sensitive to environment

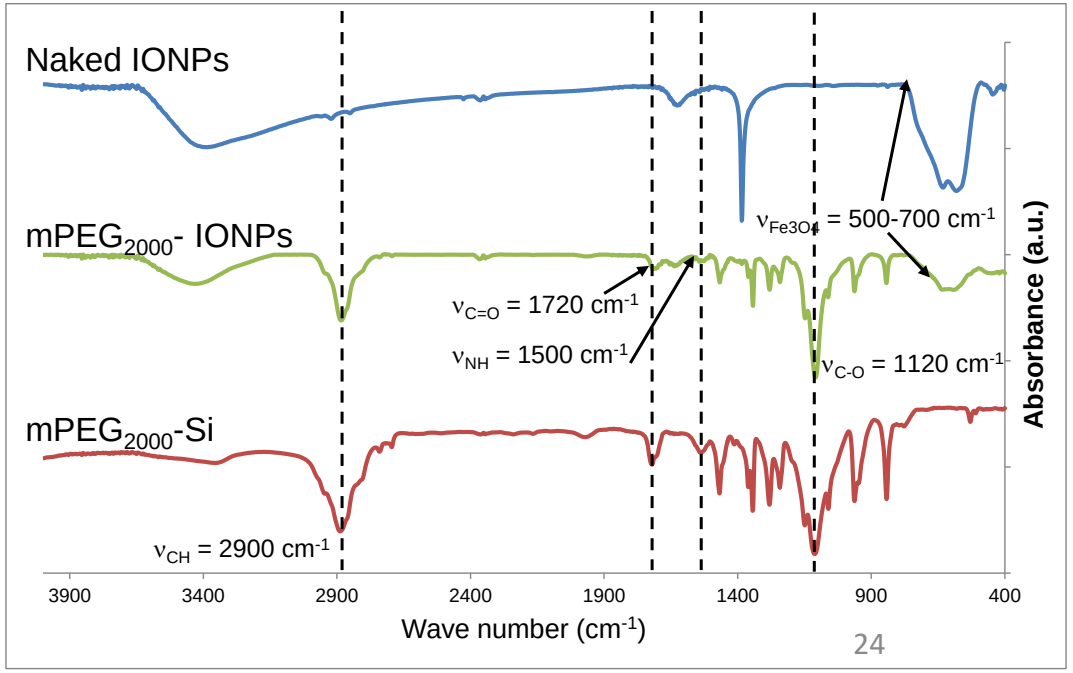
Coating quantification

- Coatings can be proven by stability and/or size and charge measurements
- However, quantification of surface coatings is crucial for bio-applications
  - In this part, coatings are assumed to be well washed out
- For this quantification, it is important to have SSA of naked NPs before coatings:
  - Via BET (see first lecture)
  - If not possible, a calculation can be done from sizes of the naked NPs

Coating quantification

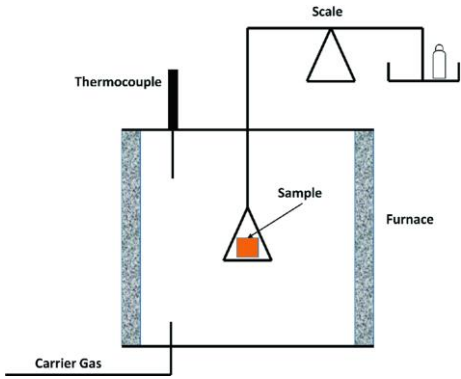
- FTIR Fourier transform infrared spectroscopy
  - 2-5 mg of powder in KBr pellet or droplet (vibration of inorganic NP not present)
  - Show vibration of chemical bonds and so molecules on surface of NPs
    - Not really quantitative + difficult for complex system with no specific bond
    - From my point of view does not prove grafting

FTIR spectra of Fe_3O_4 NPs naked and grafted with Si-PEG_{2000}

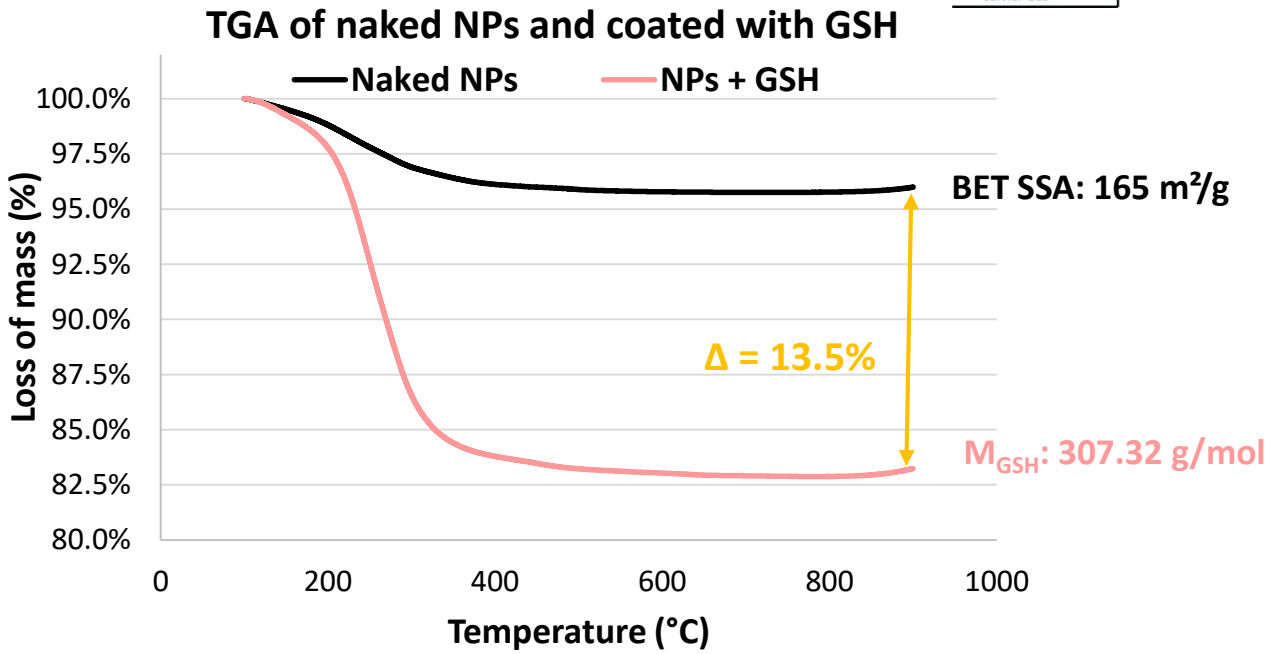


Coating quantification

Concentration | Stability / Sizes / Charges | **Coating quantification**



- FTIR Fourier transform infrared spectroscopy
- TGA Thermogravimetric analysis
  - 1-5 mg of powder
    - Mass of molecules on NPs surface
    - Quantitative for one type of grafting



Equation linking TGA to organic molecules on the surface of NPs:

$$n_{molecules} = \frac{\frac{\Delta}{M_{molecule}} \times N_A}{(100 - \Delta) * S_{BET} \times 10^{18}}$$

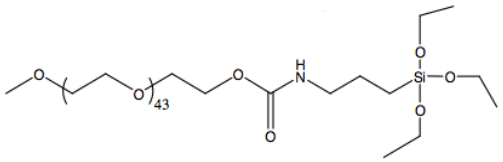
$n_{molecules}$: molecule per nm² NPs
Δm : loss of mass (%)
$M_{molecule}$: Molecular weight of organic molecule g/mol
S_{BET}: SSA of naked NPs in m²/g
N_A: Avogadro number

$$n_{GSH} = \frac{\frac{\Delta}{M_{molecule}} \times N_A}{(100 - \Delta) * S_{BET} \times 10^{18}} = 1.85\ {}^{GSH}/_{nm^2}$$

Coating quantification

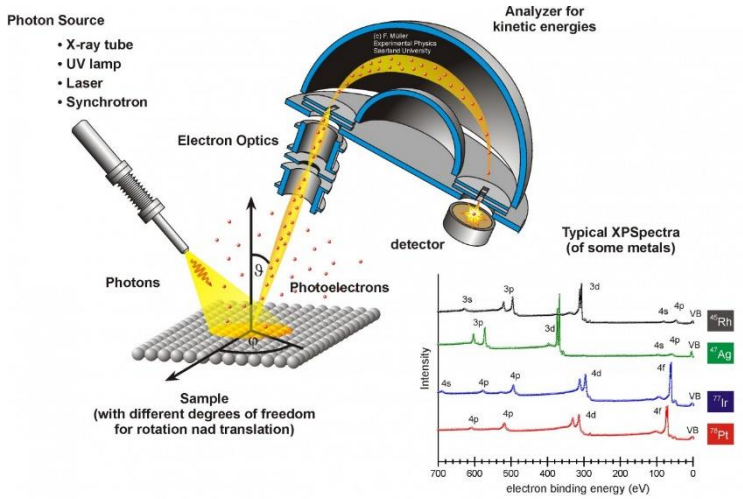
Concentration | Stability / Sizes / Charges | **Coating quantification**

- FTIR Fourier transform infrared spectroscopy
- TGA Thermogravimetric analysis
- XPS X-Ray photoelectron spectroscopy
  - Quantitative for element on surface



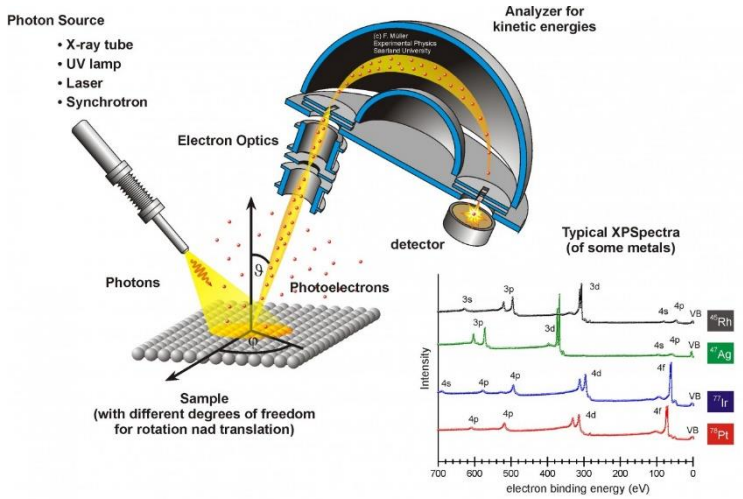
m-PEG$_{2000}$-Si

	Area ratio	C	O	N	Si
mPEG$_{2000}$-Si	Theoretical	65.5%	33%	0.75%	0.75%
	XPS measured	66%	32%	1%	1%

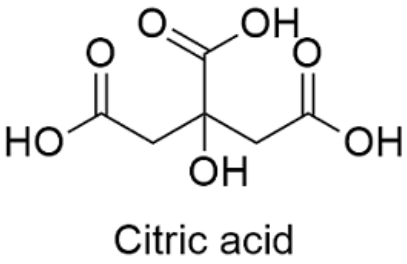


Coating quantification

Concentration | Stability / Sizes / Charges | **Coating quantification**

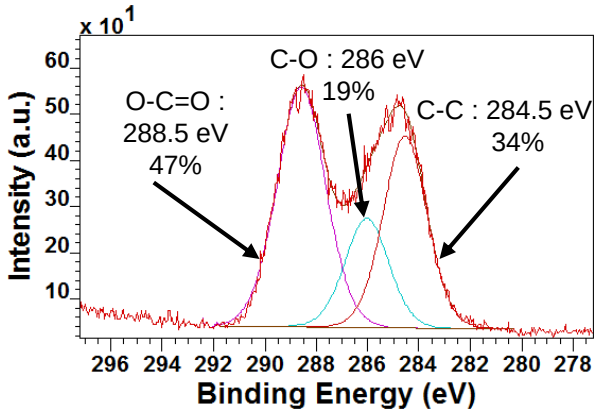


- FTIR Fourier transform infrared spectroscopy
- TGA Thermogravimetric analysis
- XPS X-Ray photoelectron spectroscopy
  - > 10 mg of powder
    - Quantitative for element on surface
    - Peaks deconvolution for different chemical bonds



Citric acid

Deconvolution of Carbon peaks of citric acid



Area ratio	C-H	C-O	O-C=O
Theoretical	33.3%	16.7%	50%
XPS measured	34%	19%	47%

Coating quantification

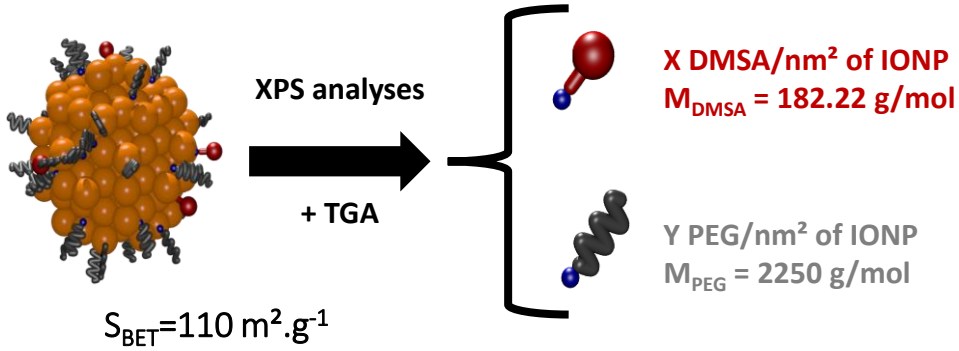
Concentration | Stability / Sizes / Charges | **Coating quantification**

https://doi.org/10.1166/jnn.2019.16796

- FTIR Fourier transform infrared spectroscopy
- TGA Thermogravimetric analysis
- XPS X-Ray photoelectron spectroscopy
  - > 10 mg of powder
    - Quantitative for element on surface
    - Peaks deconvolution for different chemical bonds
    - Coupled with TGA, quantification of 2 molecules on NPs' surface

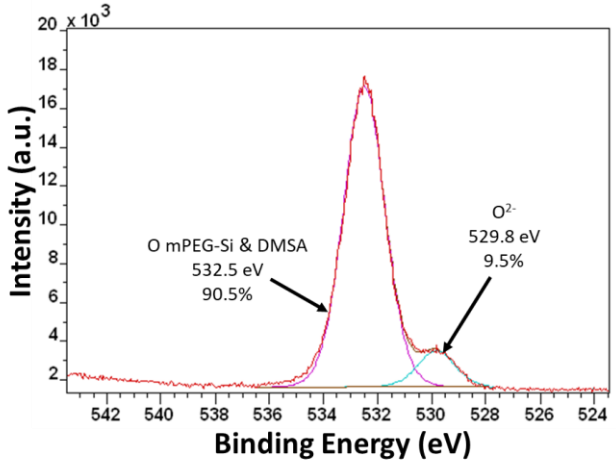
Example of co-functionalization of SPION with DMSA & PEG

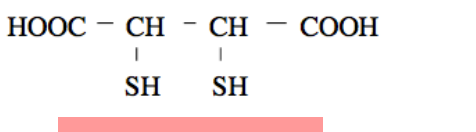
Quantification



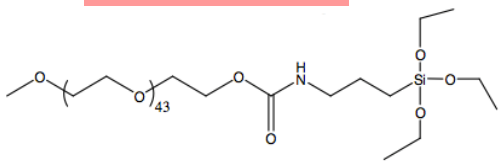
S_{BET}=110 $m^2.g^{-1}$

Deconvolution of O1s peak in XPS





DMSA



m-PEG_{2000}-Si

Sample \ Element	O1s (calculated) IONP	O1s (calculated) PEG	O1s (calculated) DMSA	Fe2p	C1s (calculated IONP	C1s (calculated PEG	C1s (calculated DMSA	S1s	N1s	Si2p
$mPEG_{2000}$-Si-IONPs-DMSA	3.1	29.1	0.3	1.45	5.3	58.2	0.3	0.15	1.1	1.1

- XPS is quantitative for surface molecules
  - N and Si from PEG & S from DMSA
  - O from IONPs from deconvolution of O1s peak
  - Thus O1s and C from DMSA calculated = 2 * S
  - O from DMSA and PEG calculated
  - C for PEG calculated from O from PEG
  - Rest of C pollution

$$\frac{n_{DMSA}}{n_{PEG}} = \frac{n_S/2}{n_{Si}} = \frac{0.15\%/2}{1.1\%} \Rightarrow n_{DMSA} = 0.0682 * n_{PEG}$$

Coating quantification

Concentration | Stability / Sizes / Charges | **Coating quantification**

https://doi.org/10.1166/jnn.2019.16796

- FTIR Fourier transform infrared spectroscopy
- TGA Thermogravimetric analysis
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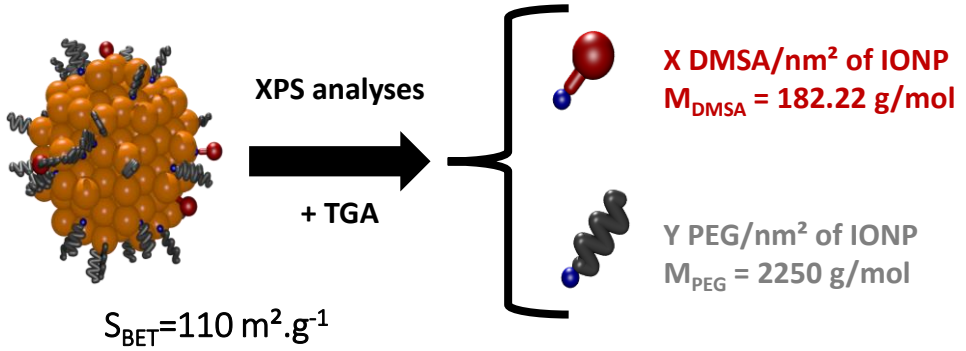
    - Coupled with TGA, quantification of 2 molecules on NPs' surface

Example of co-functionalization of SPION with DMSA & PEG



$$\frac{n_{DMSA}}{n_{PEG}} = \frac{n_{S}/2}{n_{Si}} = \frac{0.15\%/2}{1.1\%} \Rightarrow n_{DMSA} = 0.0682 * n_{PEG}$$

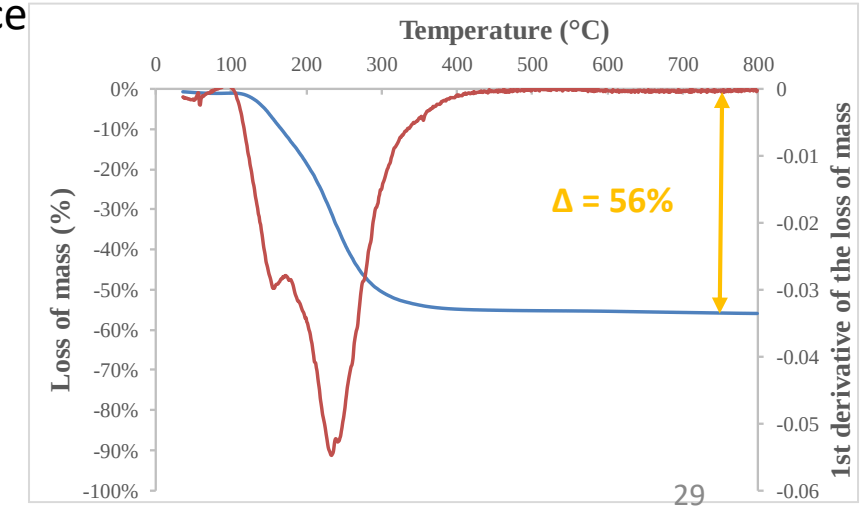
- TGA loss of mas is 56%
  - Loss of mass due to naked IONP neglected
  - Approximation the whole molecules burned

$$m_{molecules}(g) = n_{PEG_{2000}} \times (M_{PEG_{2000}}) + n_{DMSA} \times (M_{DMSA}) = 56\% \times 100g$$

$$m_{molecules}(g) = n_{PEG_{2000}} \times [(M_{PEG_{2000}}) + 0.0682 \times n_{PEG_{2000}} \times (M_{DMSA})]$$

$$n_{PEG_{2000}} = \frac{56\ g}{[(2250 \times 98\%) + 0.0682 \times 182.22 \times 98\%]} = 0.02526\ mol\ \&\ n_{DMSA} = 2.1\ 10^{-3} mol$$

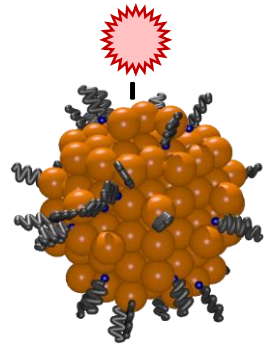
3.1 PEG/nm²
0.2 DMSA/nm²



Coating quantification

Concentration | Stability / Sizes / Charges | **Coating quantification**

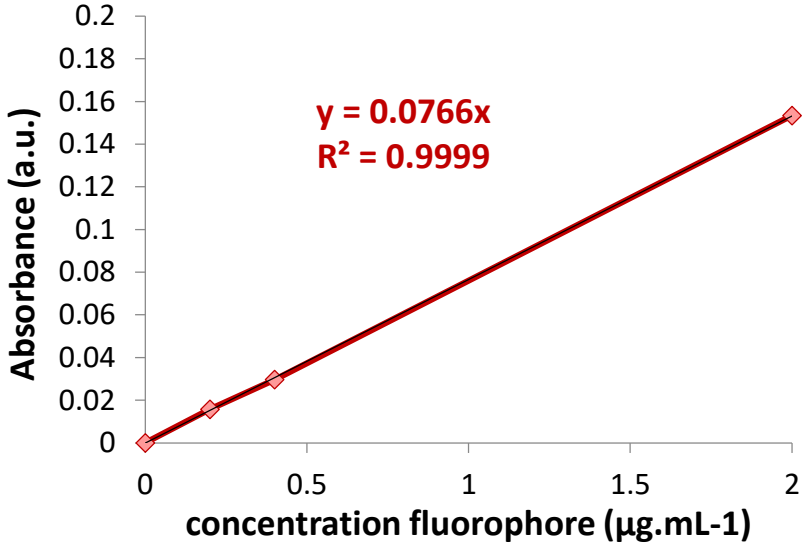
- FTIR Fourier transform infrared spectroscopy
- TGA Thermogravimetric analysis
- XPS X-Ray photoelectron spectroscopy
- UV-visible
  - Direct detection of colored or fluorescent molecules



Equation linking UV-visible to florescent molecules on the surface of NPs:

$$n_{fluorophore} = \frac{c_{fluorophore} * V_{suspension} * \frac{Na}{M_{fluorophore}}}{c_{NP} * V_{suspension} * S_{BET} \times 10^{18}}$$

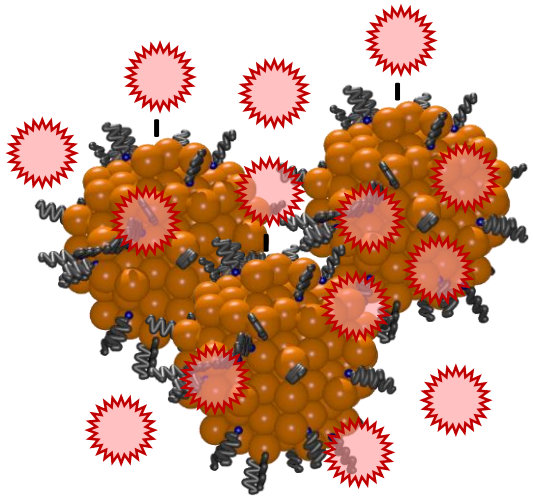
$n_{fluorophore}$: molecules of fluorophore per nm² of NPs
$c_{fluorophore}$ or c_{NP} : concentration of fluorophore and NPs in g/L
$V_{suspesnsion}$: Volume of suspension in L
$M_{fluorophore}$: Molecular weight of the fluorophore in g/mol
S_{BET}: SSA of naked NPs in m²/g
N_A: Avogadro number



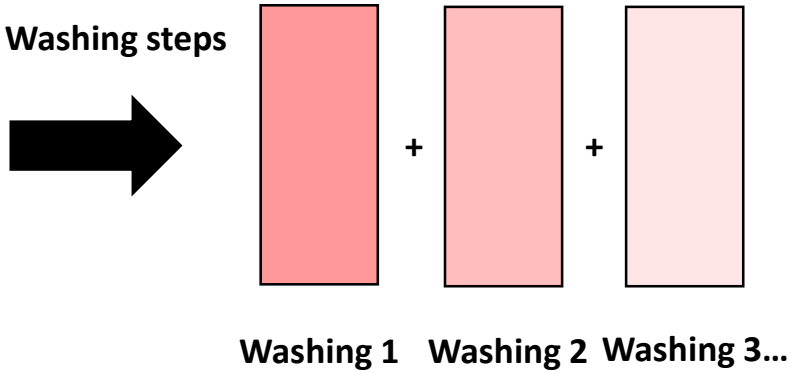
Coating quantification

Concentration | Stability / Sizes / Charges | **Coating quantification**

- FTIR Fourier transform infrared spectroscopy
- TGA Thermogravimetric analysis
- XPS X-Ray photoelectron spectroscopy
- UV-visible
  - Direct detection of colored or fluorescent molecules
  - Indirect detection of colored or fluorescent molecules



https://doi.org/10.1038/s41598-021-86762-6

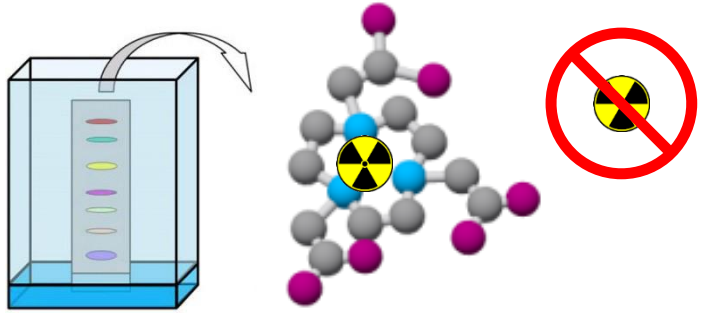


$$n_{grafted} = n_{initial} - n_{washings}$$

Coating quantification

- FTIR Fourier transform infrared spectroscopy
- TGA Thermogravimetric analysis
- XPS X-Ray photoelectron spectroscopy
- UV-visible
- Other method to detect coatings
  - ICP if molecule has a different element but destructive
  - Radiodetection for radioactive molecule
    - NPs in suspension
    - Instant thin layer chromatography to separate radioactive molecule and check it is still in its macrocycle
    - AR-2000 radiochromatograph to quantify fluorescent

https://doi.org/10.2967/jnumed.108.051243
https://doi.org/10.3390/cancers11121962



Coating quantification

Concentration | Stability / Sizes / Charges | **Coating quantification**

- FTIR Fourier transform infrared spectroscopy
- TGA Thermogravimetric analysis
- XPS X-Ray photoelectron spectroscopy
- UV-visible
- Other method to detect coatings
  - ICP if molecule has a different element but destructive
  - Radiodetection for radioactive molecule

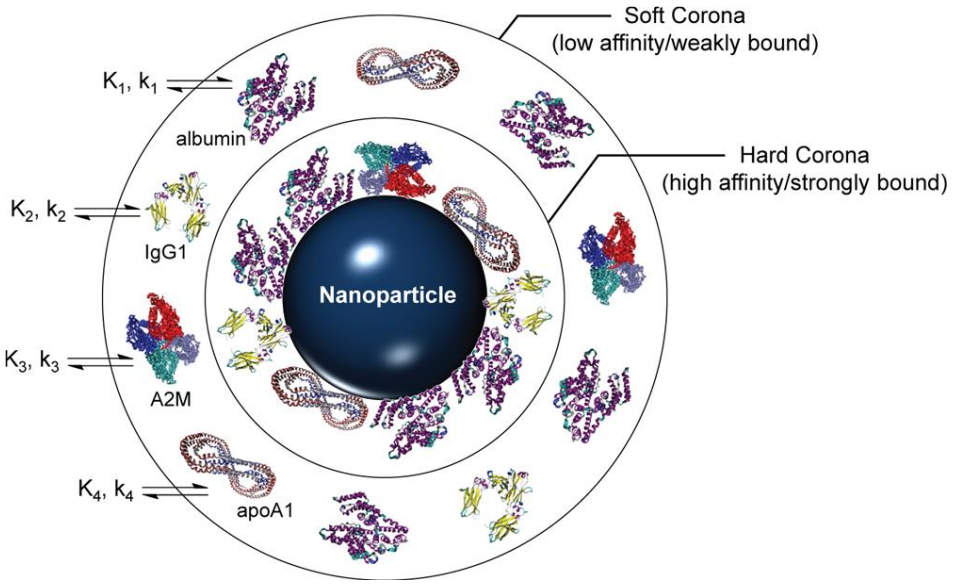
Complementary methods

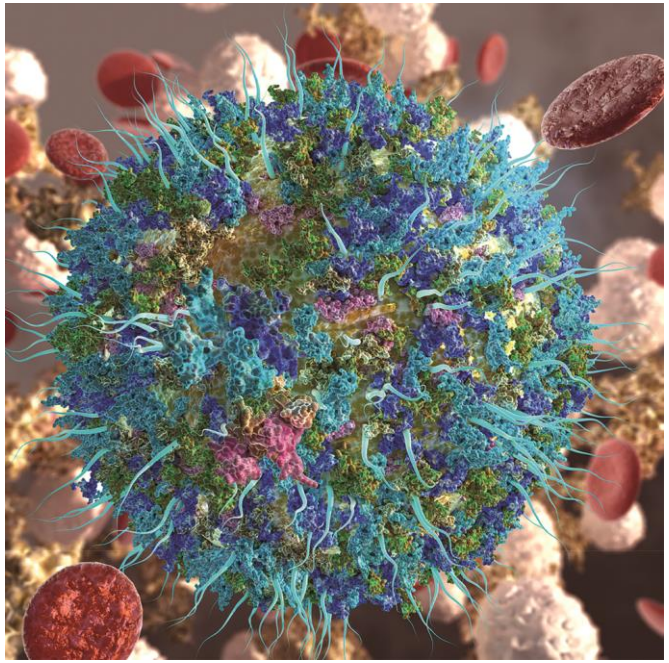
Coating quantification		
	Pros	**Cons**
FTIR	Fast first answer	Powder / destructive
		Grafting difficult to prove
TGA	Quantification possible	Powder / destructive
XPS	Quantitative	Powder
	Deconvolution possible for multi molecules	Important mass
UV-visible	Fast	Color / fluorescence
	Indirect or direct detection	Careful of interference
ICP + Radio	Direct or indirect	ICP destructive
	Fast and not destructive	Only for radioelement

Protein Corona

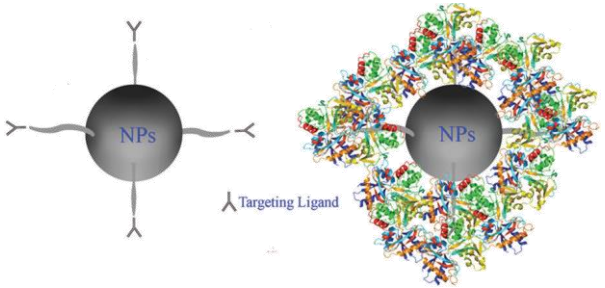
Concentration | Stability / Sizes / Charges | Coating quantification | **Protein Corona**

- PC Protein Corona
  - Nanoparticles (NPs) used for biomedical purposes will be coated by proteins immediately upon contact with a physiological environment leading to different biological behaviors.
  - PC is influenced by surface chemistry of NPs and influences their biological behaviors





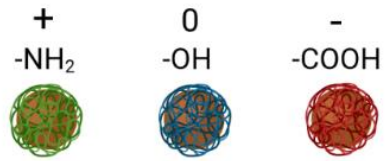
https://doi.org/10.1021/ar500190q
http://dx.doi.org/10.1021/acsnano.7b08008
https://doi.org/10.1039/C3CC37307J



Protein Corona

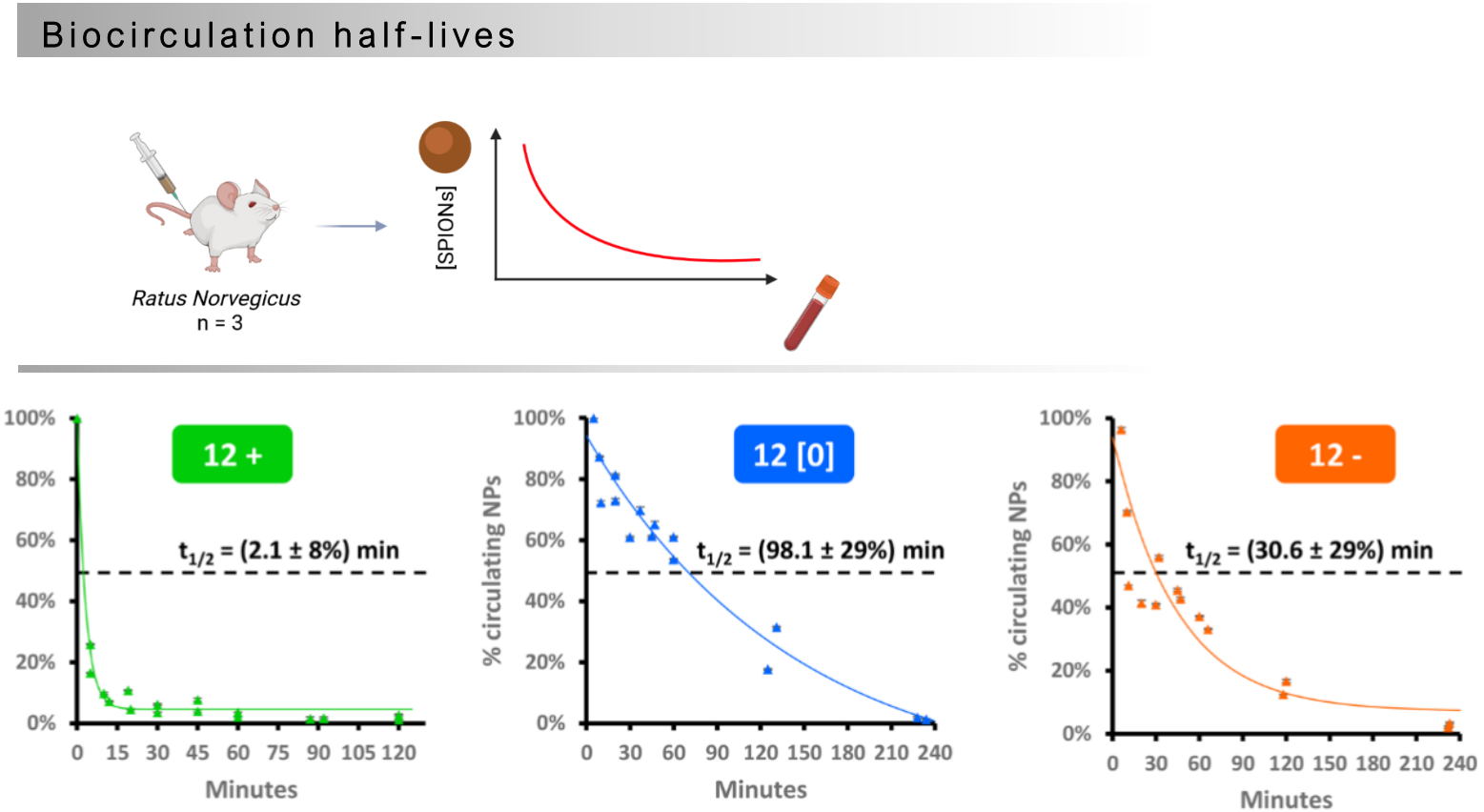
- PC Protein Corona
- Example of role of PC

https://doi.org/10.1021/acsnano.3c02041



NPs functionalized with PVA
Different sizes: 12 & 31 kDa
Different charges

	Size (nm)	Charge pH 7 (mV)
PVA	90 ± 31	+8 ± 2
	95 ± 18	1 ± 3
	91 ± 22	-7 ± 2

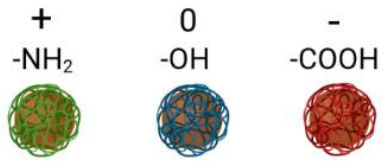


Protein Corona

Concentration | Stability / Sizes / Charges | Coating quantification | **Protein Corona**

- PC Protein Corona
- Example of role of PC

https://doi.org/10.1021/acsnano.3c02041



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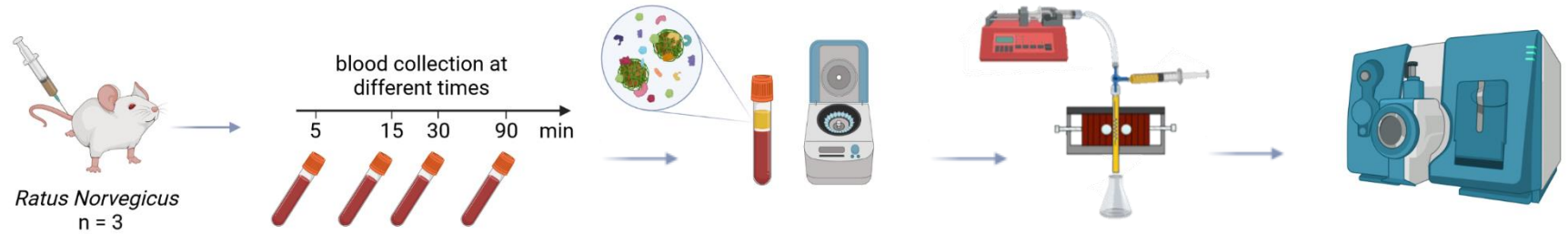
	Size (nm)	Charge pH 7 (mV)
PVA	90 ± 31	+8 ± 2
	95 ± 18	1 ± 3
	91 ± 22	-7 ± 2

Identification of proteins adsorbed on PVA-SPIONs at different times



Alpha-2-HS-glycoprotein	Fibrinogen alpha chain	Ig kappa chain C region, B allele
Apolipoprotein A-I	Fibrinogen beta chain	Lipoprotein lipase
Apolipoprotein A-II	Fibrinogen gamma chain	Matrix Gla protein
Apolipoprotein C-I	Fibronectin	Metalloproteinase inhibitor 3
Apolipoprotein E	Ficolin-1	Murinoglobulin-1
Coagulation factor VII	Hemoglobin subunit alpha-1/2	Osteopontin
Complement C3	Hemoglobin subunit beta-1	Secreted phosphoprotein 24
Extracellular matrix protein 1	Hemoglobin subunit beta-2	Serine protease inhibitor A3K

↘ circulation time

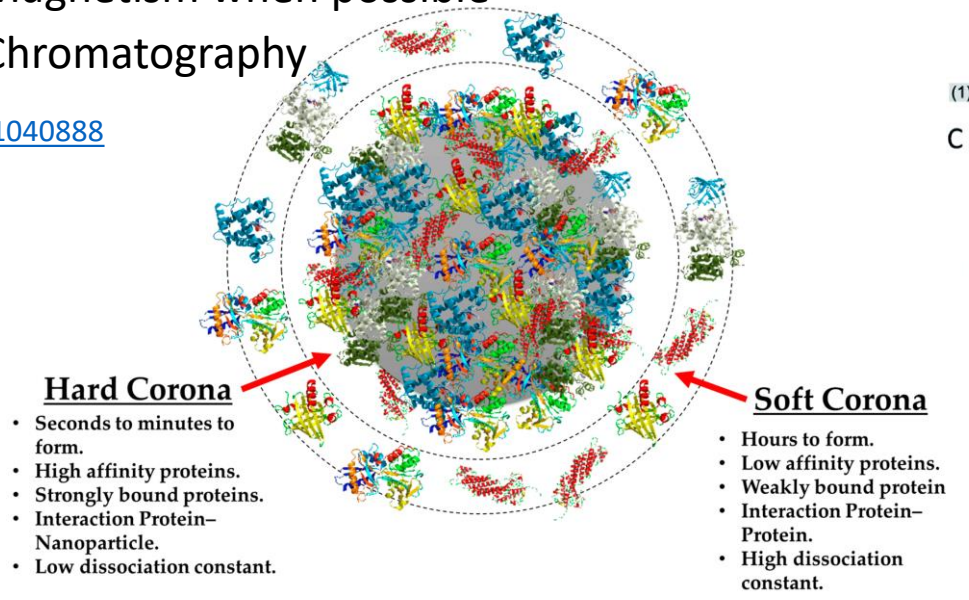
↗ circulation time

Effect on biocirculation:
✓ 24 proteins with 8 essential
Protein corona influenced:
× Polymer size
✓ Surface charge

Protein Corona

- PC Protein Corona
- Example of role of PC
- Methods to separate protein hard corona
  - In suspension and destructive
    - Mainly centrifugation
    - Magnetism when possible
    - Chromatography

https://doi.org/10.3390/nano11040888



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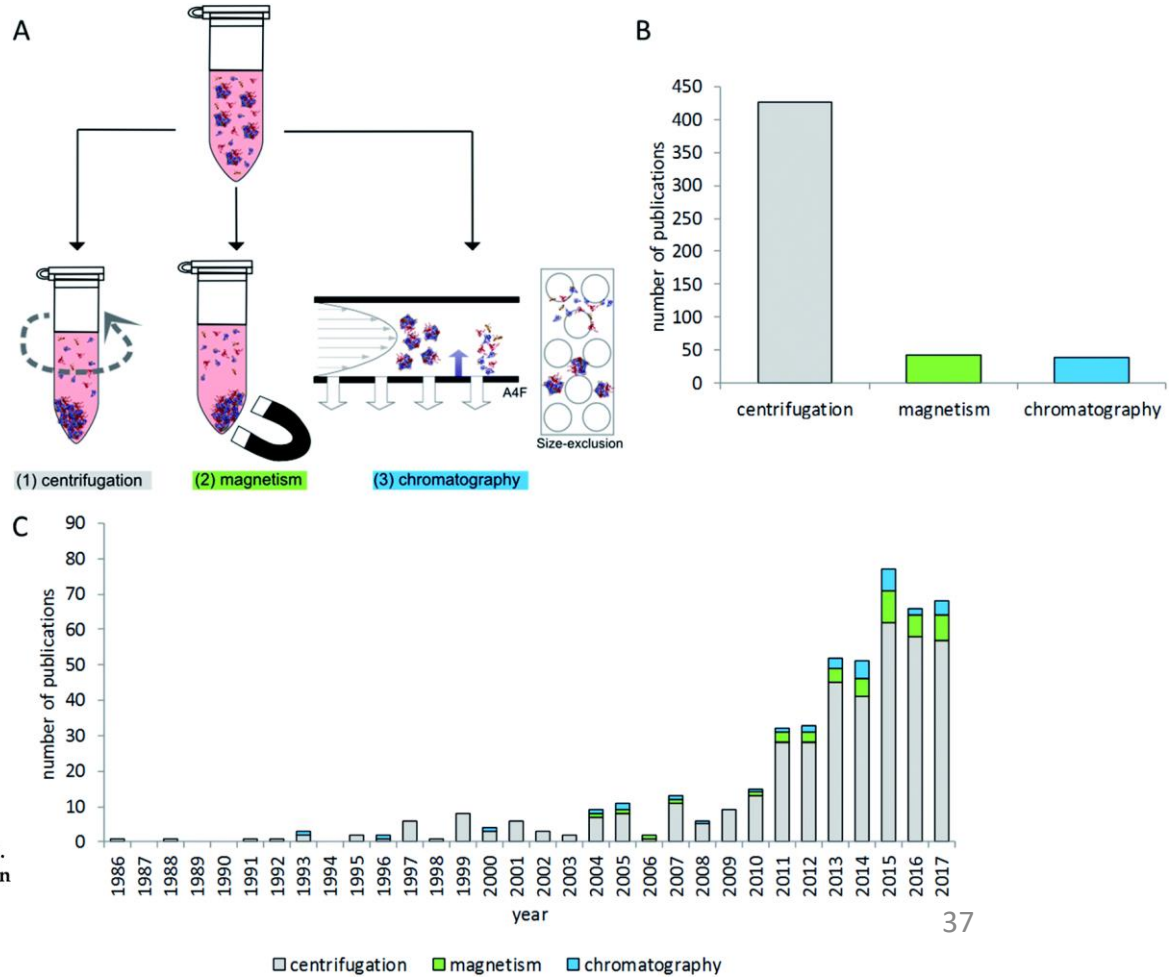
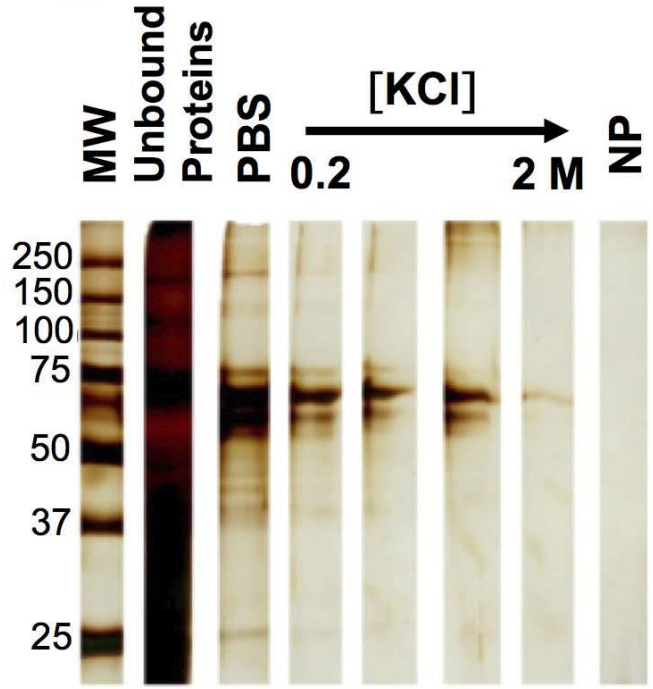


Protein Corona

Concentration | Stability / Sizes / Charges | Coating quantification | **Protein Corona**

- PC Protein Corona
- Example of role of PC
- Methods to separate protein hard corona
- Methods to quantify protein corona
  - NPs in suspension or powder and destructive
  - PC could be indirectly observed via methods presented before
  - Protein assays like ICP for cysteine or UV-visible titration
  - Sodium dodecyl sulfate–polyacrylamide gel electrophoresis SDS page
    - Separate by size and / or charge (1D vs 2D): not really quantitative with many proteins
  - Liquid Chromatography coupled to Mass Spectroscopy
    - Separation by size then semi-quantitative analysis

https://doi.org/10.1038/srep05020



More information → https://doi.org/10.1016/j.copbio.2017.02.009

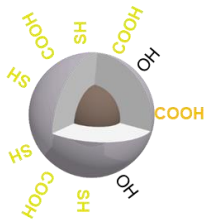
Protein Corona

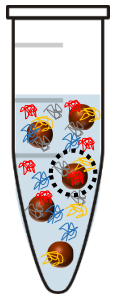
Concentration | Stability / Sizes / Charges | Coating quantification | **Protein Corona**

https://doi.org/10.1021/acs.bioconjchem.8b00554

- PC Protein Corona
- Example of role of PC
- Methods to separate protein hard corona
- Methods to quantify protein corona
- Needs of robust methods
  - Importance of replicates & controls

	Surface	Size (nm)	Z-Potential pH 7 (mV)
Silica	COOH	93 ± 17	-29 ± 3
	COOH/SH	104 ± 20	-29 ± 3
	OH	88 ± 18	-25 ± 3

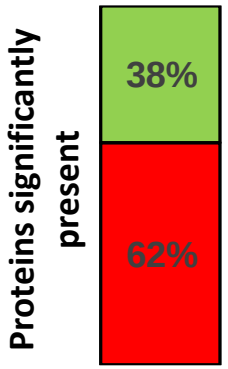


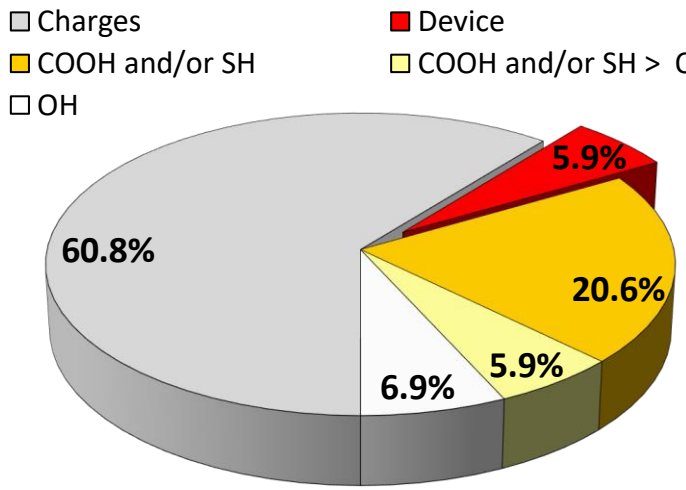


NPs in serum
2.8 mL / m² NPs

+ control

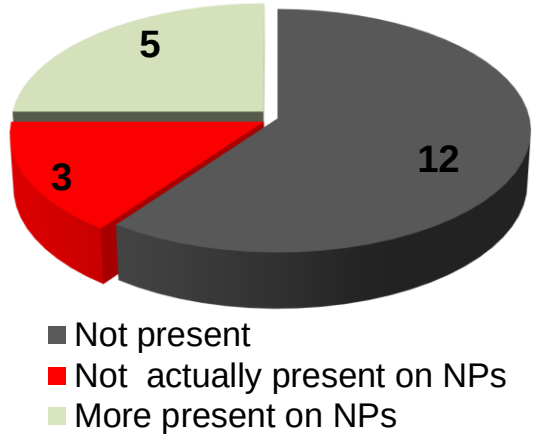
+ triplicate





Proteins with affinities to the different NPs

>60% not present significantly
Charges > COOH > OH > SH

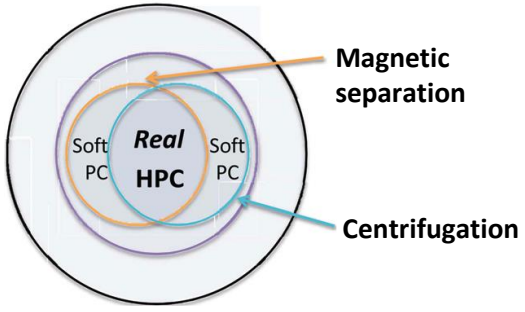


Out of 20 most abundant 8 present and 3 only on device

Protein Corona

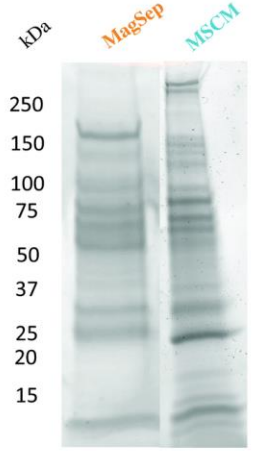
Concentration | Stability / Sizes / Charges | Coating quantification | **Protein Corona**

- PC Protein Corona
- Example of role of PC
- Methods to separate protein hard corona
- Methods to quantify protein corona
- Needs of robust methods
  - Importance of replicates & controls
  - Importance of separation methods

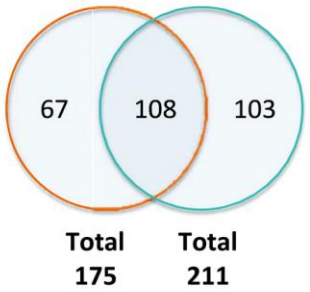


https://doi.org/10.1039/C7AN00646B

a)



b)



c)

	MagSep	MSCM
1	Serum albumin	Apolipoprotein B-100
2	Complement C3	Complement C3
3	Fibrinogen gamma chain	Serum albumin
4	Fibrinogen beta chain	Complement C4-A
5	Complement factor H	Complement C4-B
6	Complement C4-A	Apolipoprotein A-I
7	Complement C4-B	Apolipoprotein E
8	Apolipoprotein B-100	Antithrombin-III
9	Apolipoprotein A-I	Alpha-2-macroglobulin
10	Ig gamma-1 chain C region	Histidine-rich glycoprotein
11	Complement component C9	Plasminogen
12	Ceruloplasmin	Isoform 3 of Plasma protease C1 inhibitor
13	Fibrinogen alpha chain	Complement factor H
14	Fibronectin	Complement C1q subcomponent subunit C
15	C4b-binding protein alpha chain	Kininogen-1
16	Ig kappa chain C region	Plasma kallikrein {Fragment}
17	Complement C1r subcomponent	Alpha-1-antitrypsin
18	Ig gamma-3 chain C region	Coagulation factor V
19	Complement C1s subcomponent	Inter-alpha-trypsin inhibitor heavy chain H2
20	Beta-2-glycoprotein 1	Gelsolin

Different PC after centrifugation and magnetic separation

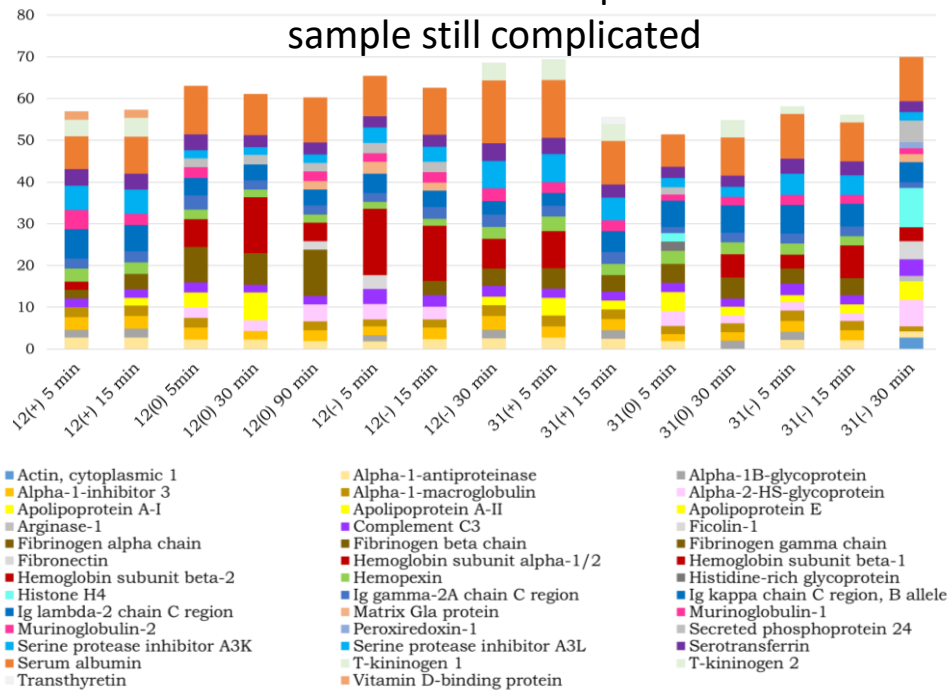
Protein Corona

Concentration | Stability / Sizes / Charges | Coating quantification | **Protein Corona**

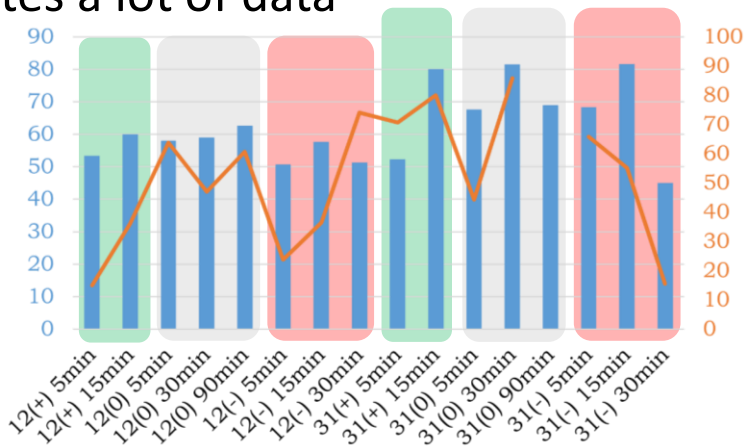
- PC Protein Corona
- Example of role of PC
- Methods to separate protein hard corona
- Methods to quantify protein corona
- Needs of robust methods
- Data management
  - PC generates a lot of data

6 NPs x 3 replicates x 3 time points x 70 proteins ≈ **4000 variables**

Focus on 20 most abundant proteins of each sample still complicated



Number of different proteins found on various NPs and percentage of common proteins per replicate



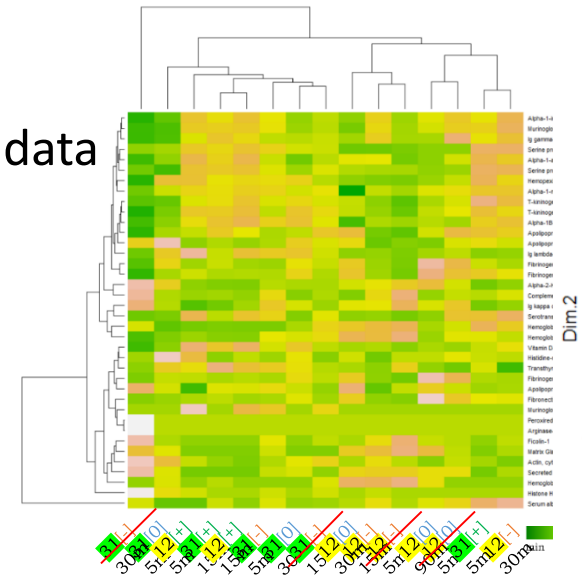
https://theses.hal.science/tel-04427957

Protein Corona

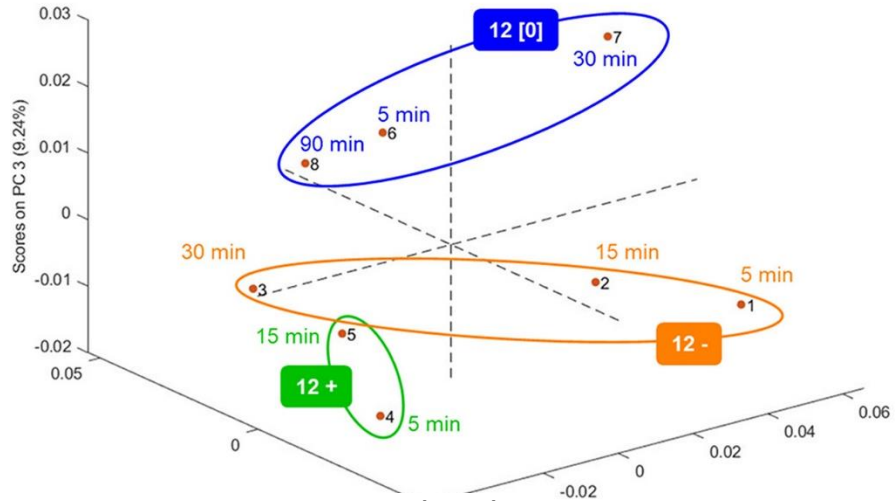
Concentration | Stability / Sizes / Charges | Coating quantification | **Protein Corona**

- PC Protein Corona
- Example of role of PC
- Methods to separate protein hard corona
- Methods to quantify protein corona
- Needs of robust methods
- Data management
  - PC generates a lot of data
  - Presentation of data

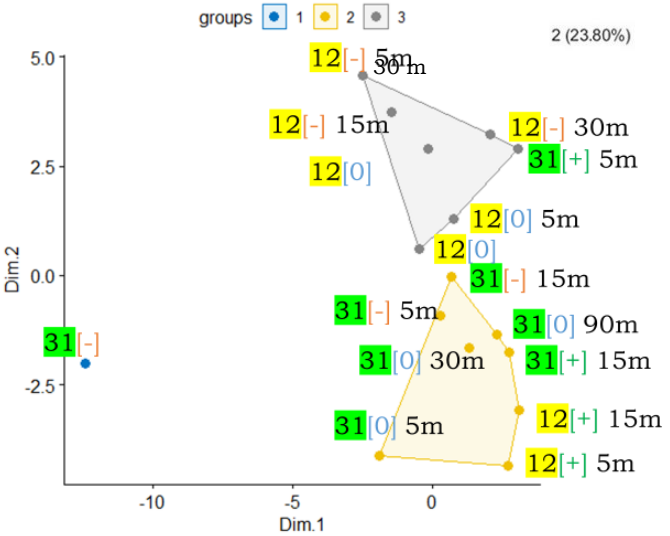
Heat map to improve readability



Principal Component Analysis



Multidimensional Scaling with k-means method (k = 3)



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Short discussion

- Interactions of NPs with bio-characterizations (stability, color, quenching…)
- Optimization of synthesis methods: Biocompatibility / Reproducibility / Scale-up

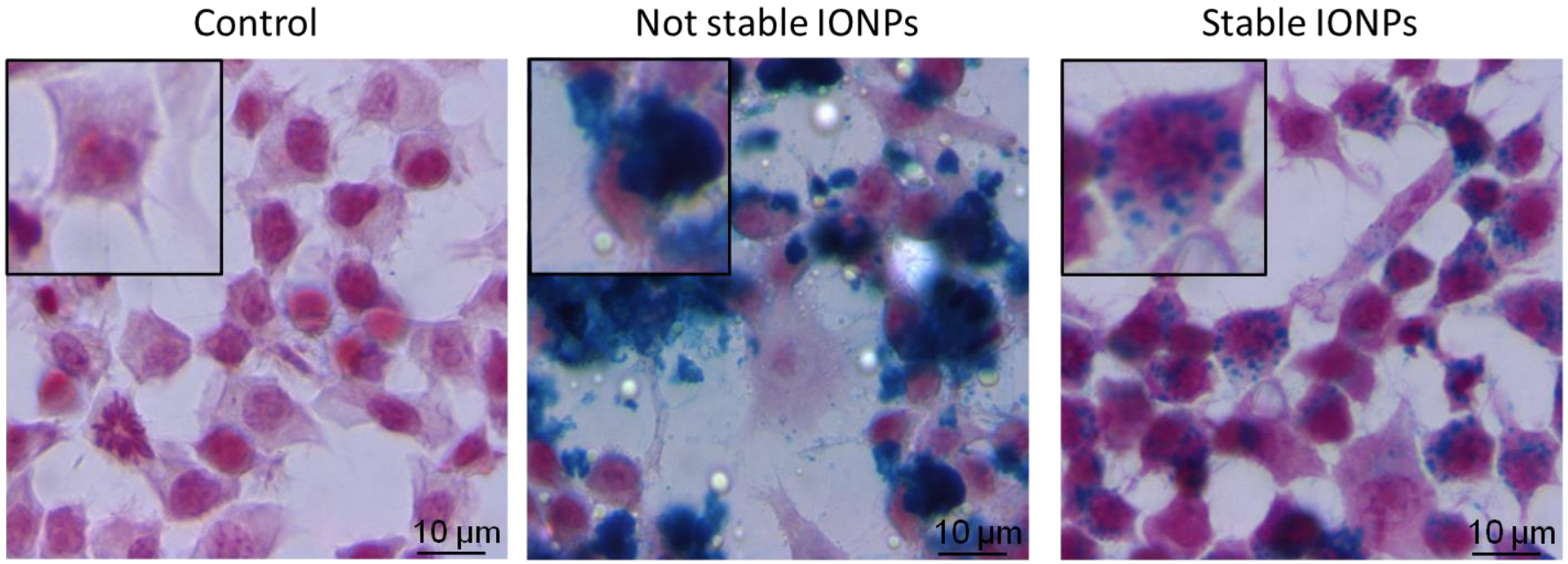
Short discussion

Concentration | Stability / Sizes / Charges | Coating quantification | Protein Corona | **Short Discussion**

- Interactions of NPs with bio-characterizations
  - Stability of NPs influence bio-characterizations

https://doi.org/10.1166/jbn.2015.1996

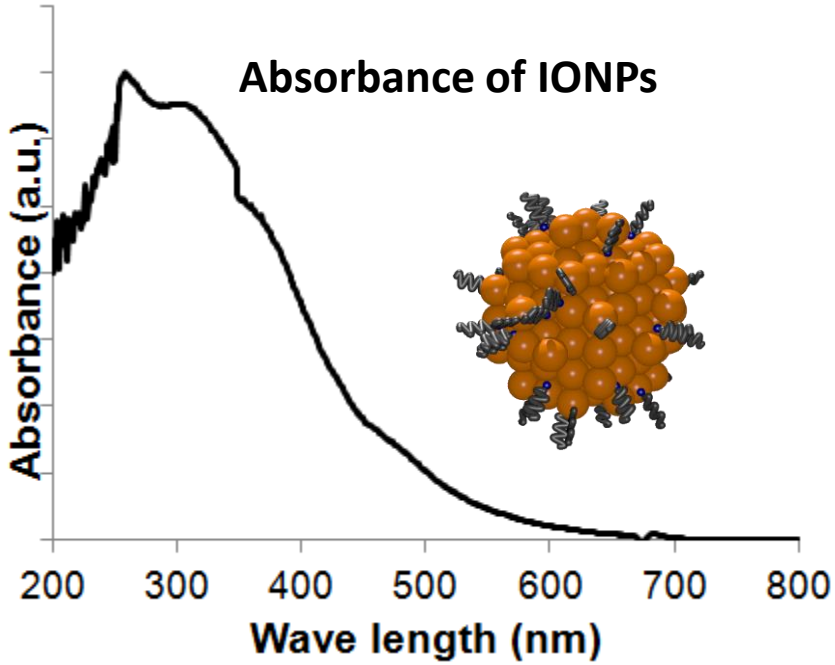
IONPs internalization macrophages revealed in Prussian Blue

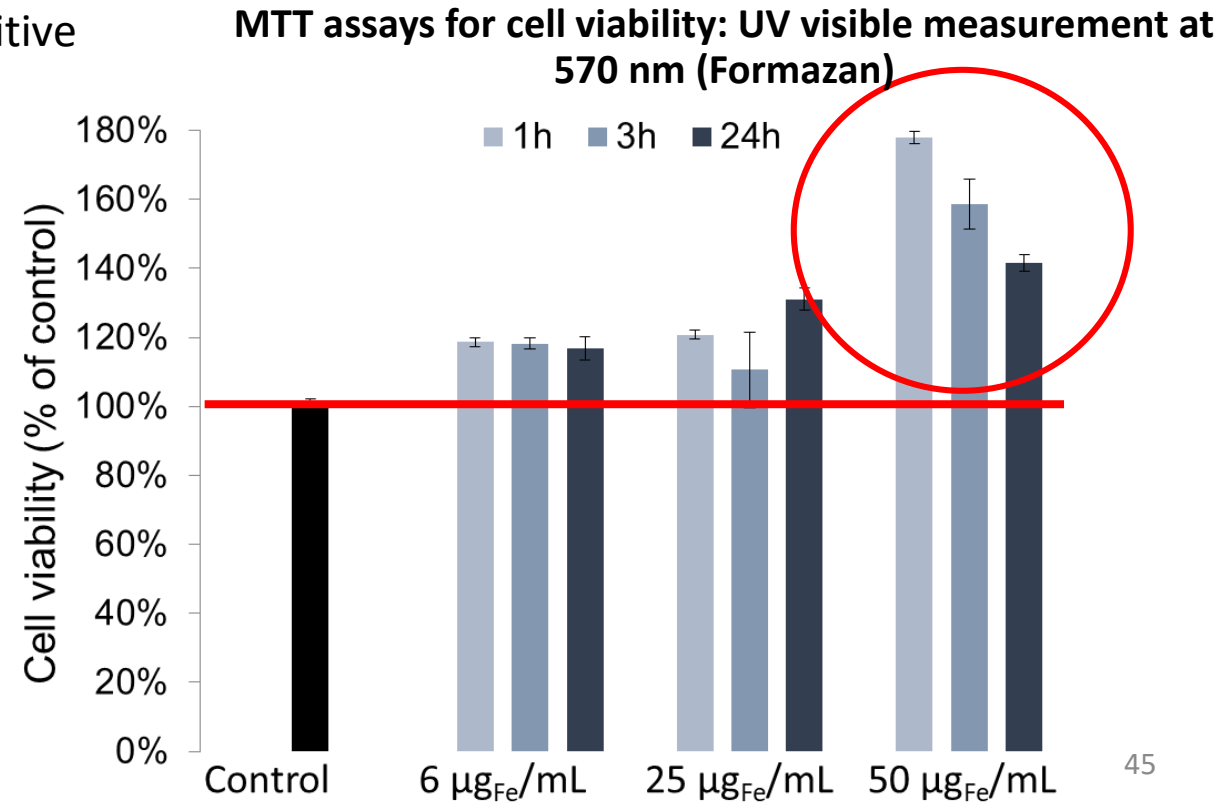


Short discussion

- Interactions of NPs with bio-characterizations
  - Stability of NPs influence bio-characterizations
  - NPs have UV-visible absorbance influencing biological measurements
    - Color and instability generate false positive

https://doi.org/10.1166/jbn.2015.1996

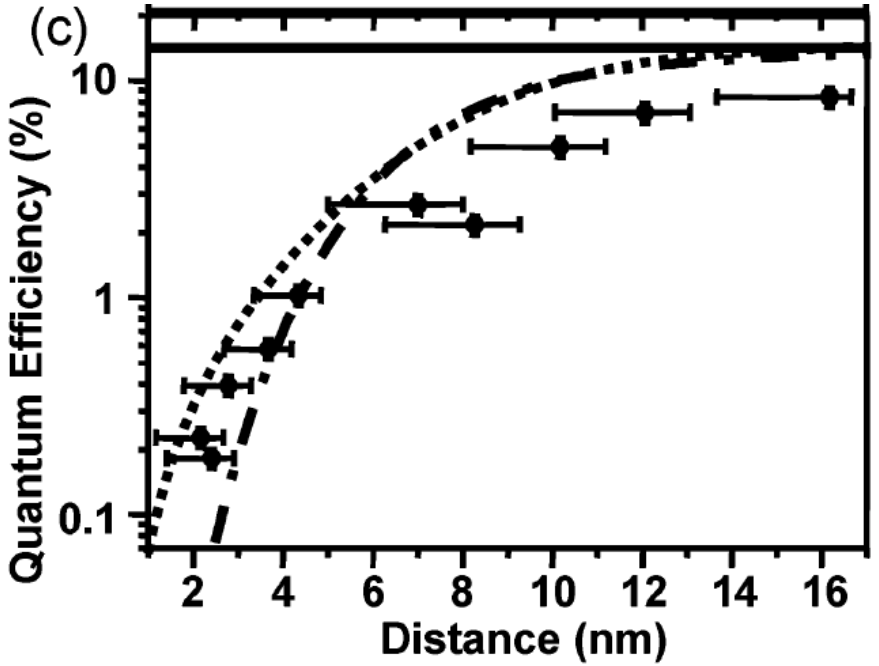




Short discussion

- Interactions of NPs with bio-characterizations
  - Stability of NPs influence bio-characterizations
  - NPs have UV-visible absorbance influencing biological measurements
  - NPs generates quenching

https://doi.org/10.1021/nl0480969

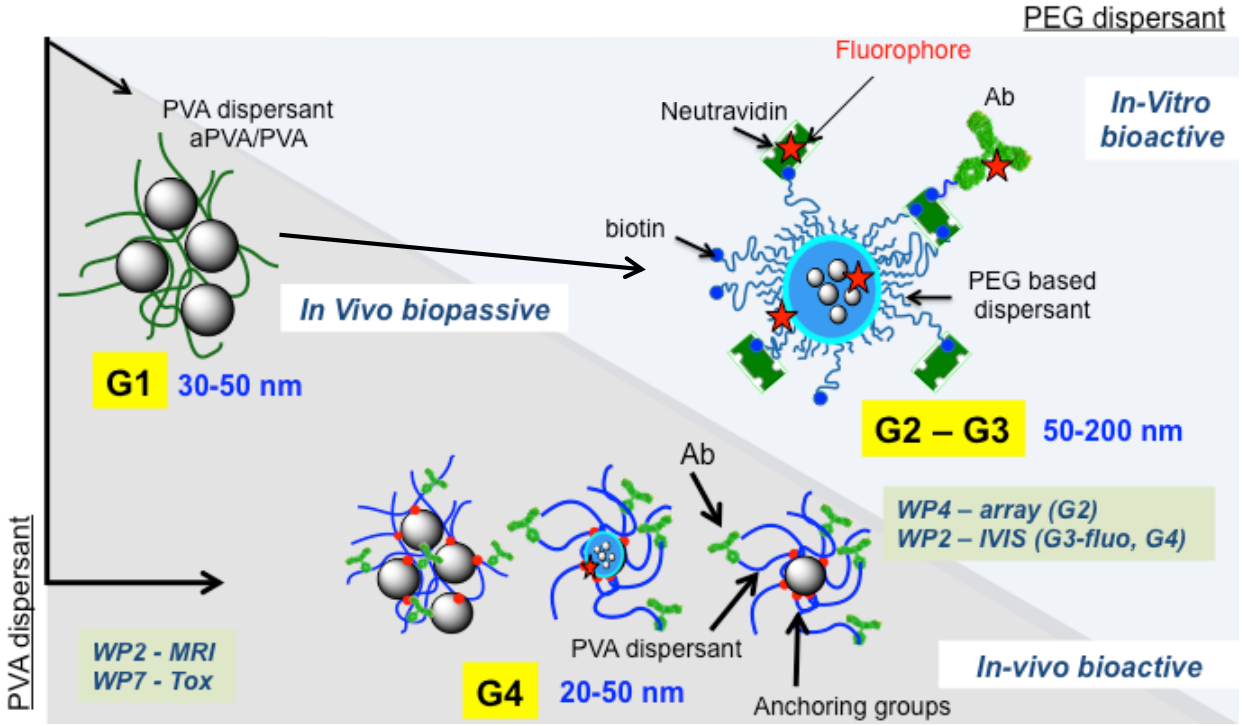


Quantum efficiency of Cy5 fluorescent molecule as a function of their distance to the gold nanoparticle surface

Short discussion

- Interactions of NPs with bio-characterizations
- Optimization of synthesis methods
  - Less toxic chemical used: easier it will be for bio-applications
  - Working with well characterized Master Batch

Example of NPs developed during a European project involving many biological partners



Short discussion

Concentration | Stability / Sizes / Charges | Coating quantification | Protein Corona | **Short Discussion**

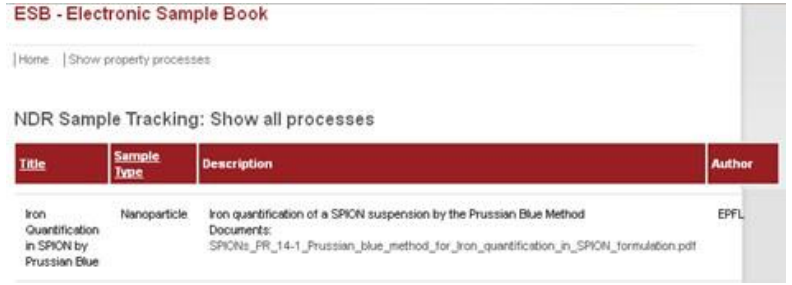
- Interactions of NPs with bio-characterizations
- Optimization of synthesis methods
  - Less toxic chemical used: easier it will be for bio-applications
  - Working with well characterized Master Batch
  - Reproducibility
    - Electronic Lab-book
    - SOPs (Standard Operating Procedures)
    - CofA (certificate of analysis)

SEVENTH FRAMEWORK PROGRAMME

NanoDiaRA

Particles, Molecules & Cells · Diagnosis in-vitro & in-vivo · Rheumatoid Arthritis & Osteoarthritis

Example of production follow-up during the European project Nanodiara


ESB - Electronic Sample Book

Home | Show property processes

NDR Sample Tracking: Show all processes

Title	Sample Type	Description	Author
Iron Quantification in SPION by Prussian Blue	Nanoparticle	Iron quantification of a SPION suspension by the Prussian Blue Method Documents: SPIONs_PR_14-1_Prussian_blue_method_for_Iron_quantification_in_SPION_formulation.pdf	EPFL

Certificate of Analysis

	Acceptance criteria	Results
Colloid characterization- Ferrofluid in water		
Raw Materials	SPION MB4 PVA-OH Merck 4.88 Lot 1.41350.1000 AminoPropyl Triethoxy Silane (99%) from SigmaAldrich (440140)	
Appearance	Dark brown liquid	Dark brown liquid
pH	6-8	7.6
Iron [mg/mL] (Mag Susc.)	4-6	4.0
Particle size [nm] (TEM)	7-8	7.74 ± 2.05 (counting 478 particles) (MB4 SPION)
Mean hydrodynamic diameter [nm] (PCS)	30-100	50 ± 10 (obtained for 10µL in 1.5mL DI water)
PVA content	17%-30%	23.5%
Charge [mV] (ZetaPALS)	20-50 mV better < 25mV	+21.3 ± 2.0 (obtained for 10µL in 1.5mL DI water)

Short discussion

Concentration | Stability / Sizes / Charges | Coating quantification | Protein Corona | **Short Discussion**

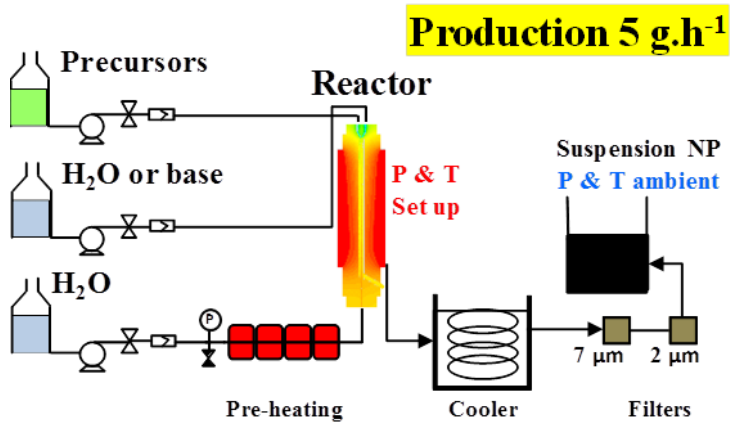
- Interactions of NPs with bio-characterizations
- Optimization of synthesis methods
  - Less toxic chemical used: easier it will be for bio-applications
  - Working with well characterized Master Batch
  - Reproducibility
  - Scale-up?
    - Robust protocols?
    - New methods to synthesize NPs?

SEVENTH FRAMEWORK PROGRAMME

NanoDiaRA

Particles, Molecules & Cells · Diagnosis in-vitro & in-vivo · Rheumatoid Arthritis & Osteoarthritis

https://doi.org/10.1039/C1CC15470B



Production of NPs before and after synthesis optimization for Merck Serono pharmacokinetic studies during the Nanodiara project

	SPIONs	PVA-SPION	Silica SPIONs
Before scale-up (per day)	1-3 g	1-3 g	0.08 g
After scale-up (per day)	**30-100 g**	**30-100 g**	**2-10 g**

Short discussion

- Interactions of NPs with bio-characterizations
- Optimization of synthesis methods
  - Less toxic chemical used: easier it will be for bio-applications
  - Working with well characterized Master Batch
  - Reproducibility
  - Scale-up
  - Pharmaceutical formulation?
    - Think earlier to avoid re-characterizing again all the samples?

  Discussion with other speakers?

Summary

Concentration → Stability / Sizes / Charges → Coating quantification → Protein Corona → Short Discussion

Parameters	Techniques presented
Concentration	Freeze Drying, ICP, UV-Visible, Magnetic Susceptibility
Stability / Sizes / Charges	UV-Visible, DLS or NTA, DCS[a], Zeta potential
Coating quantification	FTIR, TGA, XPS, UV-visible, ICP, Radiodetection
Protein Corona	PC influences biological behaviors of NPs Separation of protein hard corona: • Centrifugation, Magnetism, Chromatography Methods to quantify protein corona: • SDS page, LC-MS/MS, Protein assays Needs of robust methods Data management
Short Discussion	Interactions of NPs with bio-characterizations • Stability, UV-visible absorbance, quenching Optimization of synthesis methods • Less toxic chemical, less steps, reproducibility, Scale-up, Pharmaceutical formulation?

Techniques	Sample preparation / quantity[1]	Destructive?[2]
DCS	Suspension as such or diluted (100 µL)	Yes
DLS	Suspension as such or diluted (40 µL)	No
Freeze drying	Powder (few mg)	Yes
FTIR	Powder (2-5 mg) in KBr or Droplet	Yes or No
ICP	Suspension dissolved (ppm)	Yes
Mag. Susc.	Suspension as such or diluted (200-800µL)	No
NTA	Suspension as such or diluted (100 µL)	No
Protein Corona	Separation in suspension (few µL) Quantification in suspension of powder	Yes Yes
Radiodetection	10 µL of suspension	Yes
TGA	1-5 mg of powder	Yes
UV-Visible	Suspension as such or diluted 1mL	No
XPS	100 mg of powder	Yes
ZETA	Suspension as such or diluted 1mL	No

Thank you!

[a] Not presented but also possible

[1] Quantity is sometimes difficult to estimate as it will depend on NPs parameters (size, color …)

[2] I assumed the technique is destructive if you have to dry powder or you change a parameter (T, vacuum, chemical…)

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