

NANOTECHNOLOGIES POUR L'IMAGERIE

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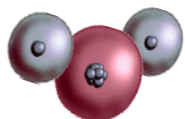
Chaire d'innovation
Technologique 2009-2010

IMAGERIE PAR RESONANCE MAGNETIQUE

Principes de base

L'IMAGERIE PAR RESONANCE MAGNETIQUE (IRM) : PRINCIPE DE BASE

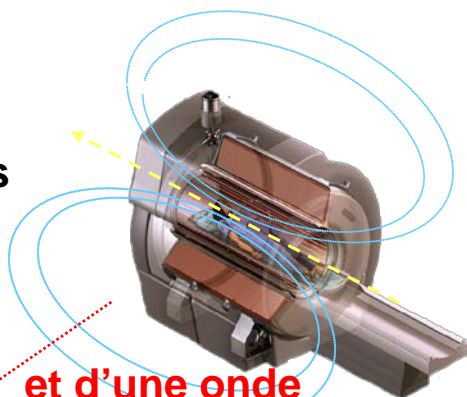
IRM basée sur le signal RMN (Résonance Magnétique Nucléaire) du ^1H



H_2O

60 à 80 % en poids des tissus

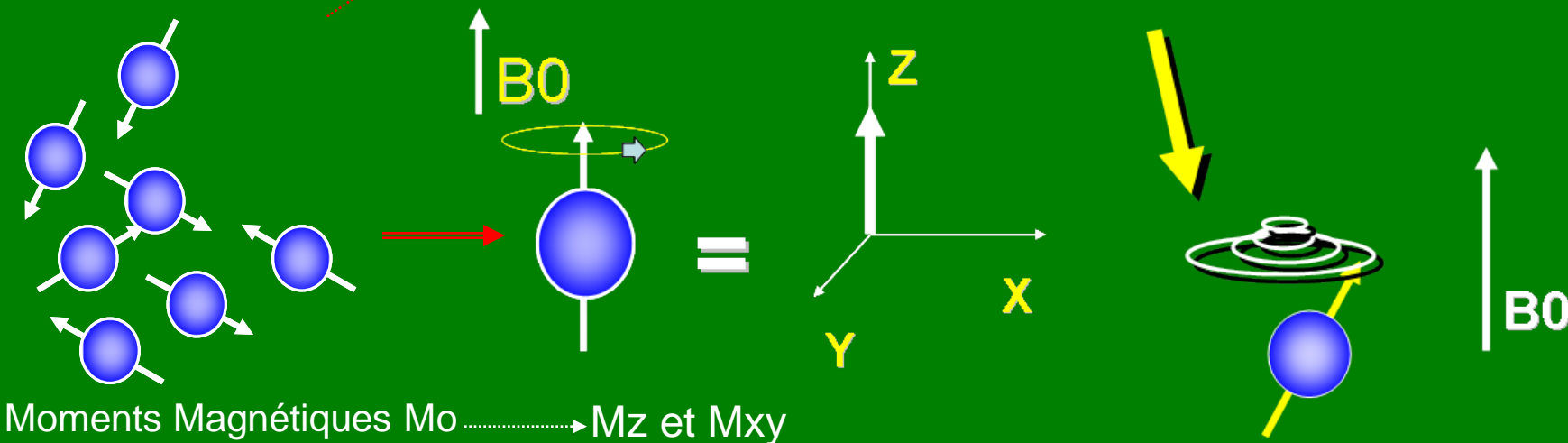
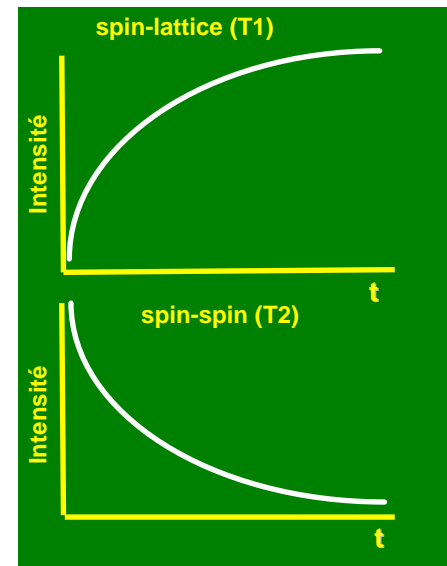
Effet combiné d'un champ magnétique fort B_0 (1,5 ou 2 T)



et d'une onde de radiofréquence (rf)

Relaxation Longitudinale (retour de M_z à M_0)

Relaxation Transversale (retour de M_{xy} à M_0)



L'IMAGERIE PAR RESONANCE MAGNETIQUE (IRM) : PRINCIPE DE BASE

T1 et T2 seront d'autant plus courts que les atomes d'hydrogène de H₂O seront liés à de grosses molécules

Image Pondérée en T1

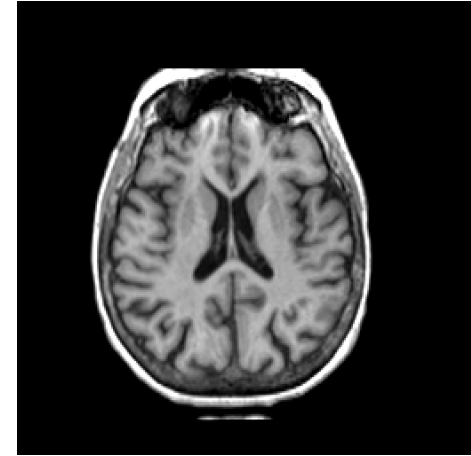
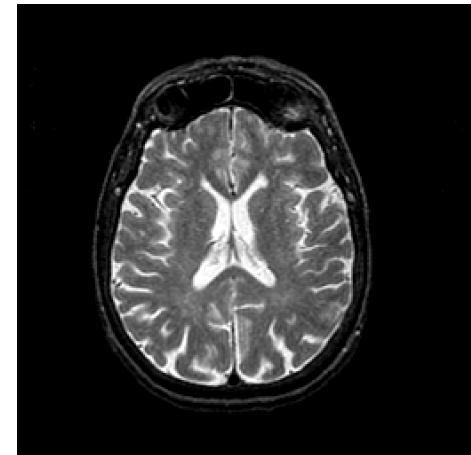
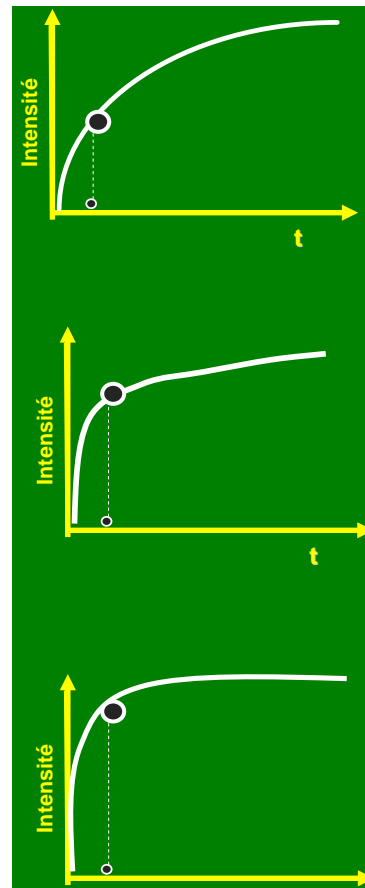
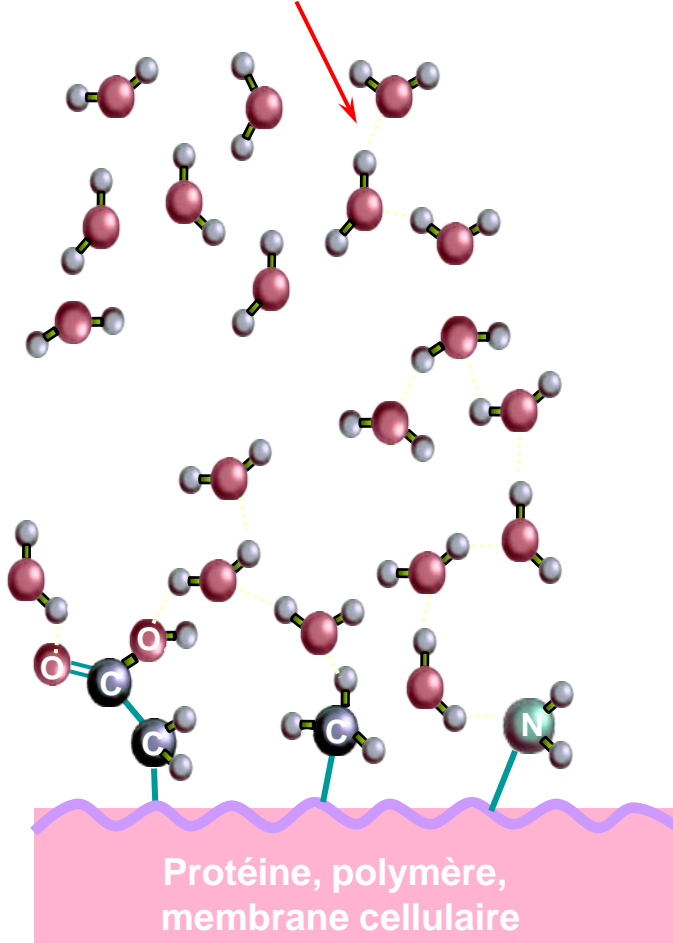


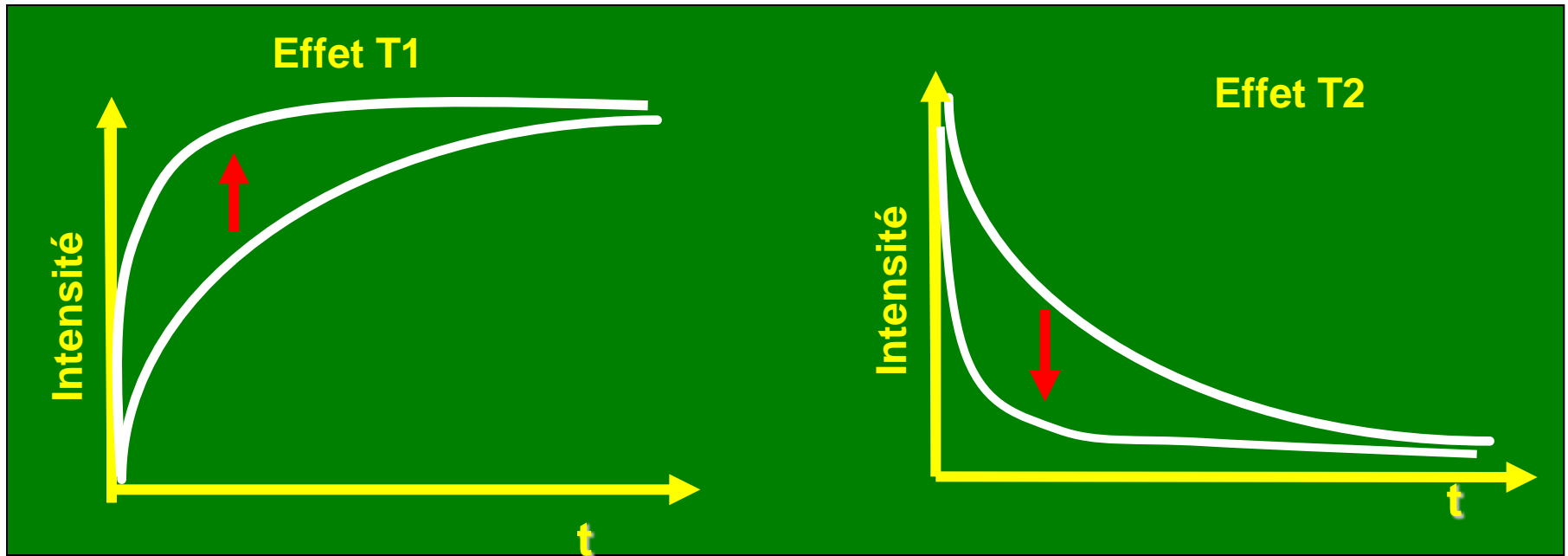
Image Pondérée en T2



Liaison Hydrogène

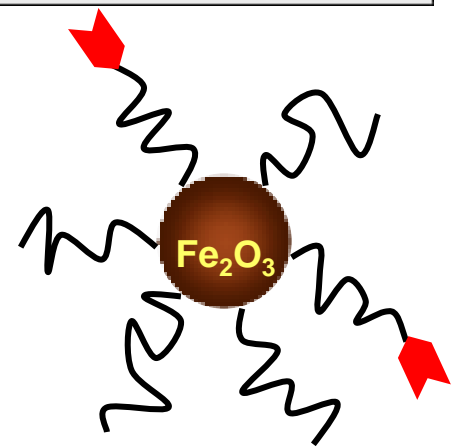
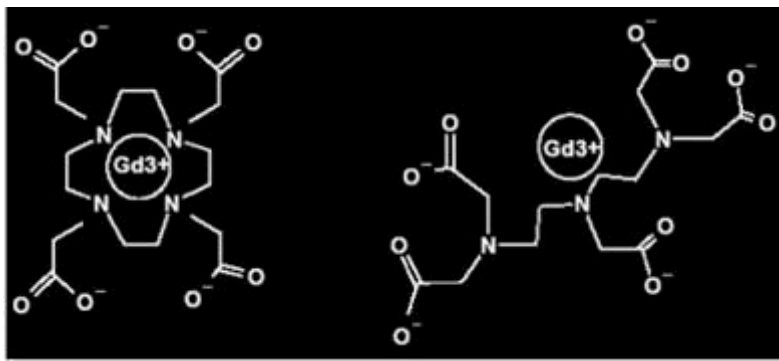


ACTION DES AGENTS DE CONTRASTE



noir → gris → blanc

blanc → gris → noir



PROPRIETES SUPERPARAMAGNETIQUES

- Paramagnétisme: aimantation dirigée sous l'influence d'un champ magnétique (dipôles magnétiques orientés dans le sens du champ)
- A de petites tailles (de l'ordre de 10nm) les nanoparticules magnétiques peuvent être assimilées à un domaine magnétique unique avec un moment magnétique important. La particule monodomaine possède un macrospin (somme des moments magnétiques des atomes qui la constituent), qui, à l'instar d'un moment paramagnétique interagit avec le champ externe et fluctue sous l'agitation thermique.
- A une température suffisamment haute T_B l'énergie thermique est suffisante pour induire la rotation libre de la particule
- En l'absence de champ → perte de magnétisation → permet de maintenir la stabilité colloïdale = SUPERPARAMAGNETISME

SUPERPARAMAGNETISME

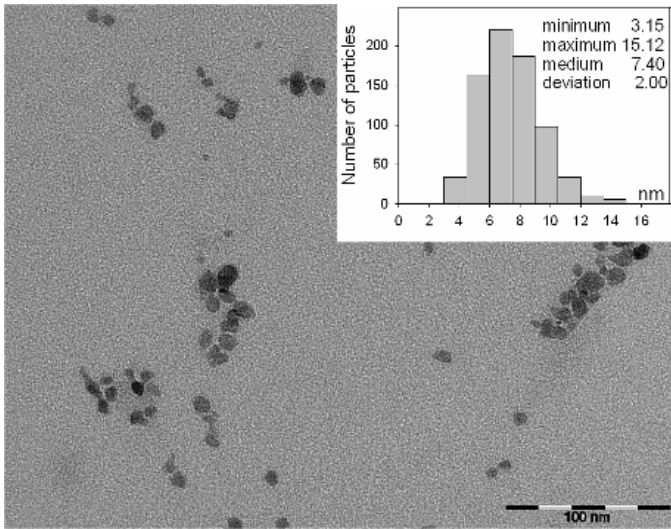
- Si la taille de la particule diminue, le rapport surface/volume augmente → des effets de surface
- Les valeurs de saturation de la magnétisation (= alignement complet de tous les moments individuels d'un échantillon) sont plus petites que pour les phases « en bloc », en raison de la structure cristalline distordue résultant du fort rayon de courbure
- Donc des propriétés magnétiques différentes peuvent être observées avec des nanoparticules de taille légèrement différentes obtenues par des procédés de préparation différents
- Nécessité de développer des études structurales approfondies

NANOPARTICULES D'OXYDES DE FER

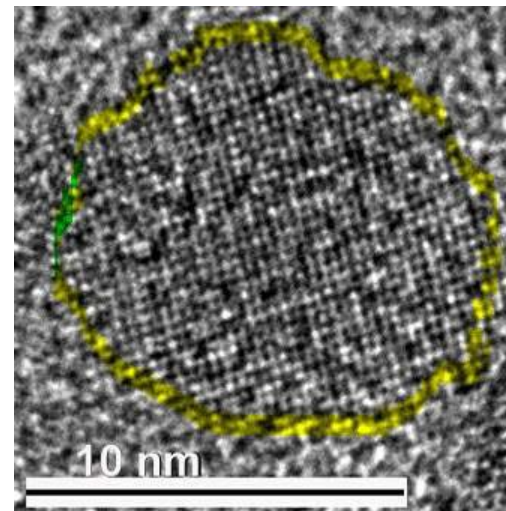
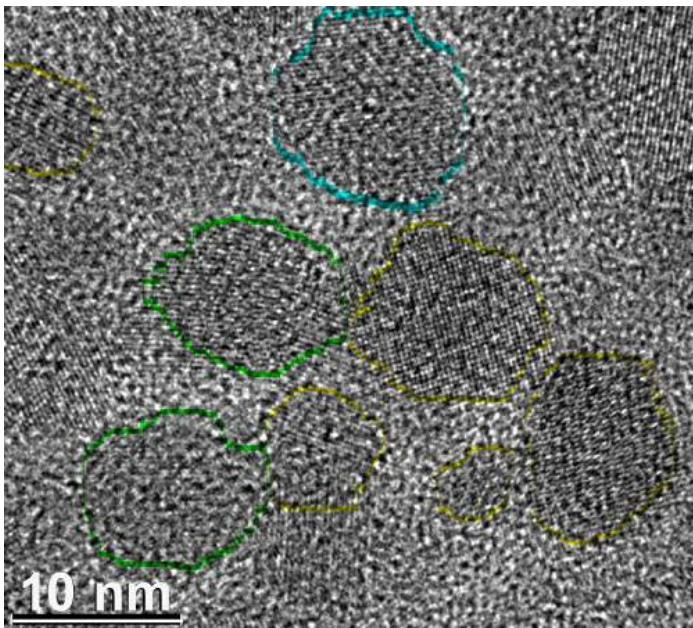
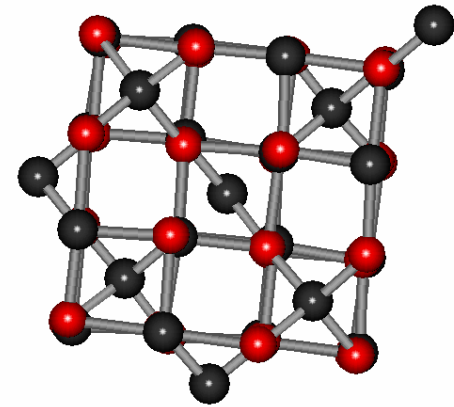
Analyses structurales

TRANSMISSION ELECTRON MICROSCOPY AND HIGH RESOLUTION TRANSMISSION ELECTRON MICROSCOPY OF Maghemite (γ -Fe₂O₃) USPIOs

Di Marco MG et al, Journal of Materials Chemistry, 35, 6354-6360, 2009

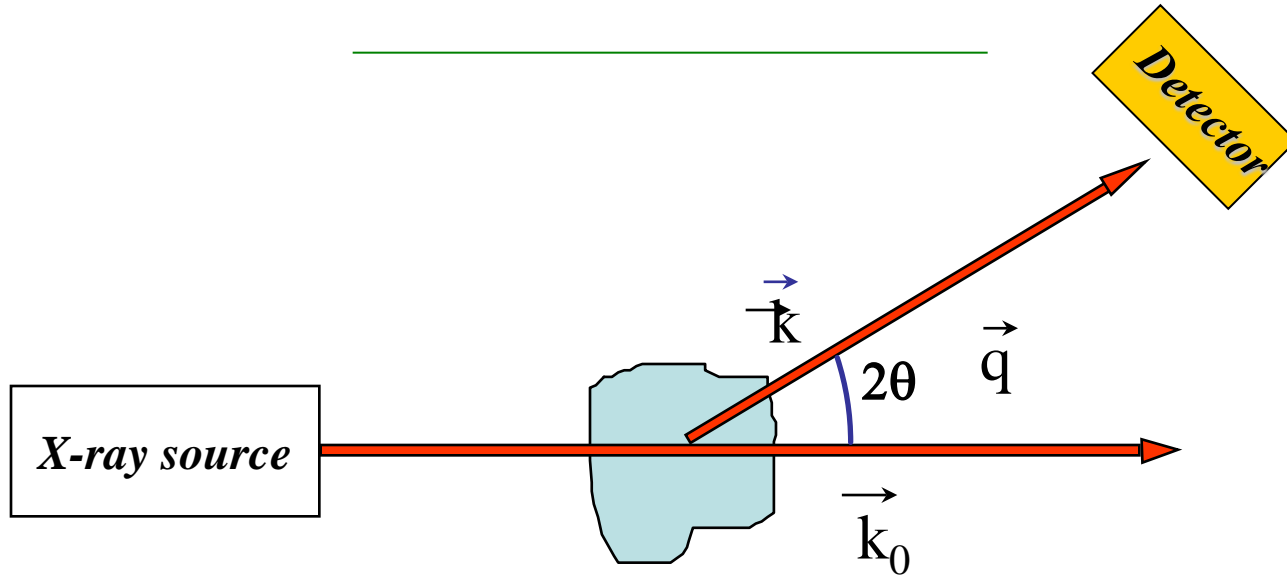


TEM



HRTEM with visible lattice planes

TECHNIQUES DE X ADXD et EDXD



$$q(E, \theta) = 4\pi \sin\theta / \lambda = KE \sin\theta$$

ADXD (Angular Dispersive)

- Energy constant
- $I = I(q) = I(\theta)$

Cu-K α line (8.05 keV) $\rightarrow q_{\max} = 8 \text{ \AA}^{-1}$

EDXD (Energy Dispersive)

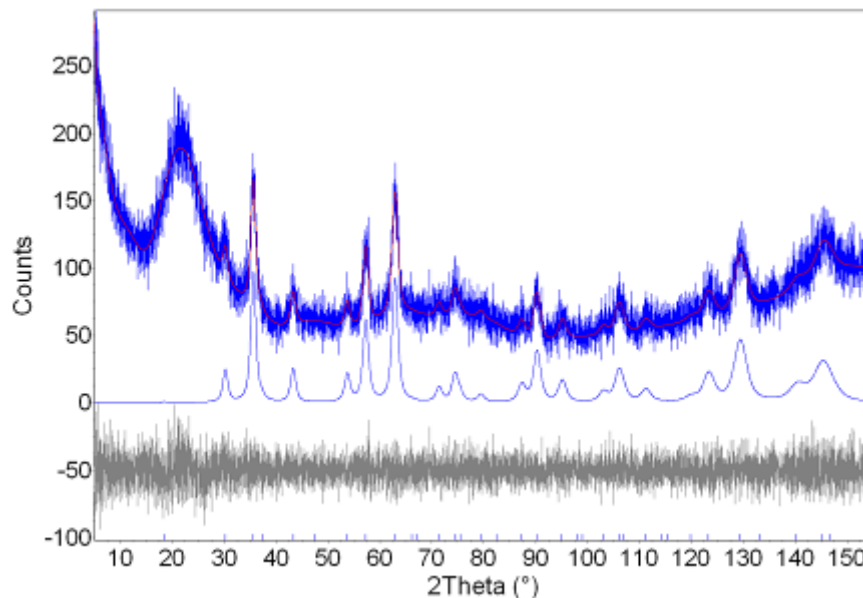
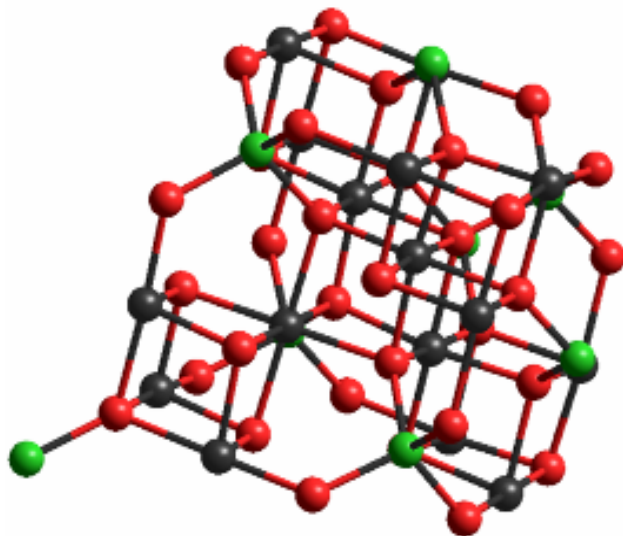
- Angle of diffraction constant
- $I = I(q) = I(E)$

$12 < E < 50 \text{ keV}$ $0.05^\circ < \theta < 40^\circ$
 $0.01 \text{ \AA}^{-1} < q < 32 \text{ \AA}^{-1}$

ADXD (Angular Dispersive X-ray Diffraction)

Di Marco M et al, JACS, 128, 10054-10059 (2006)

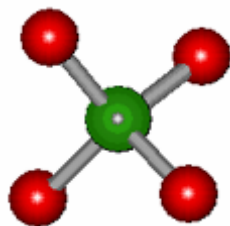
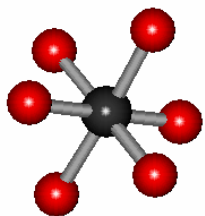
Maghemite ($\gamma\text{-Fe}_2\text{O}_3$)



56 ATOMS:

32 O^{2-} 24 Fe^{3+} dont

16 octaédriques et 8 tétraédriques

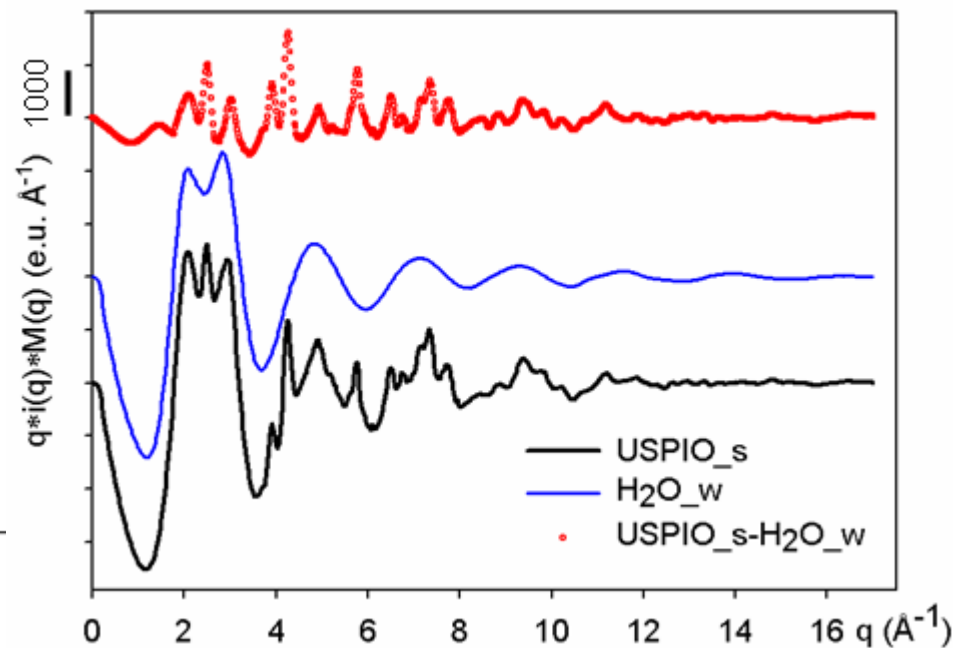
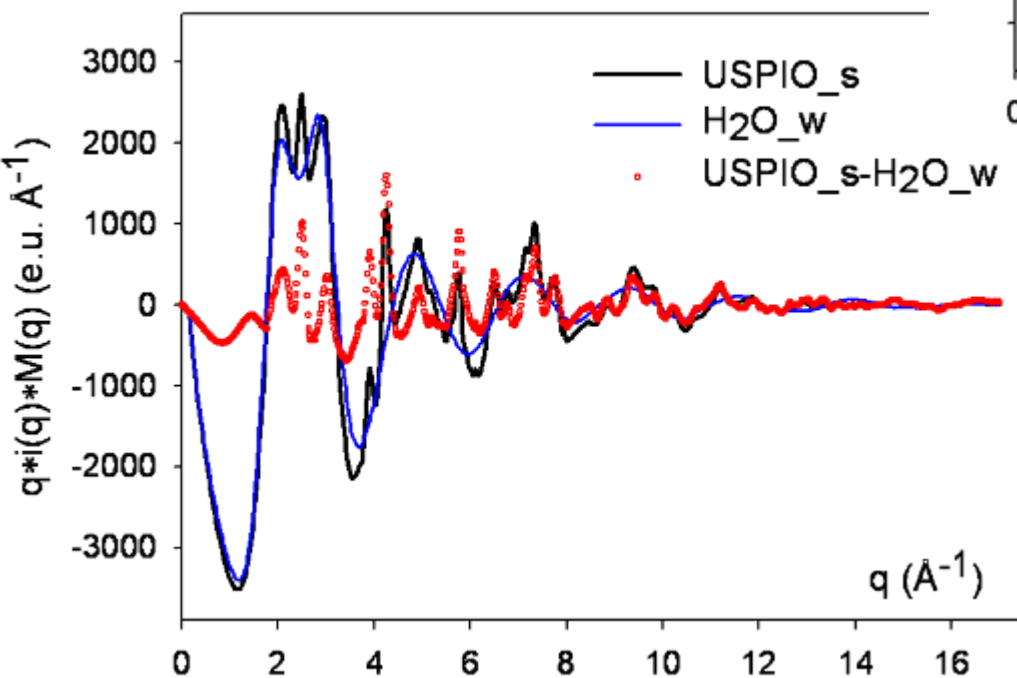
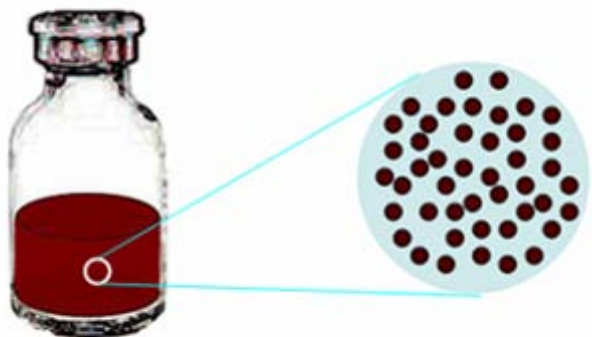


Taille 7.6 nm comparable à celle
trouvée en TEM (7.4 nm)

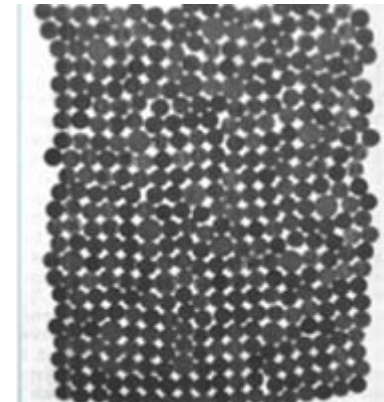
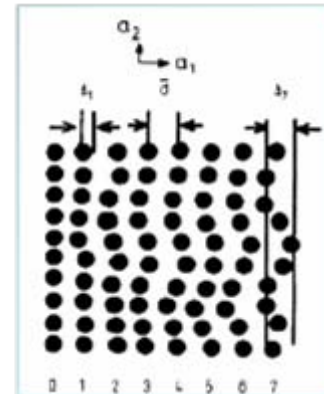
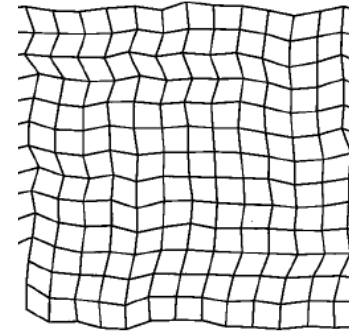
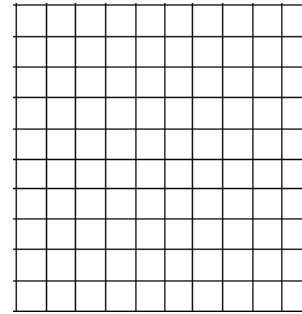


EDXD (Energy Dispersive X-ray Diffraction)

Di Marco M et al, JACS, 128, 10054-10059 (2006)



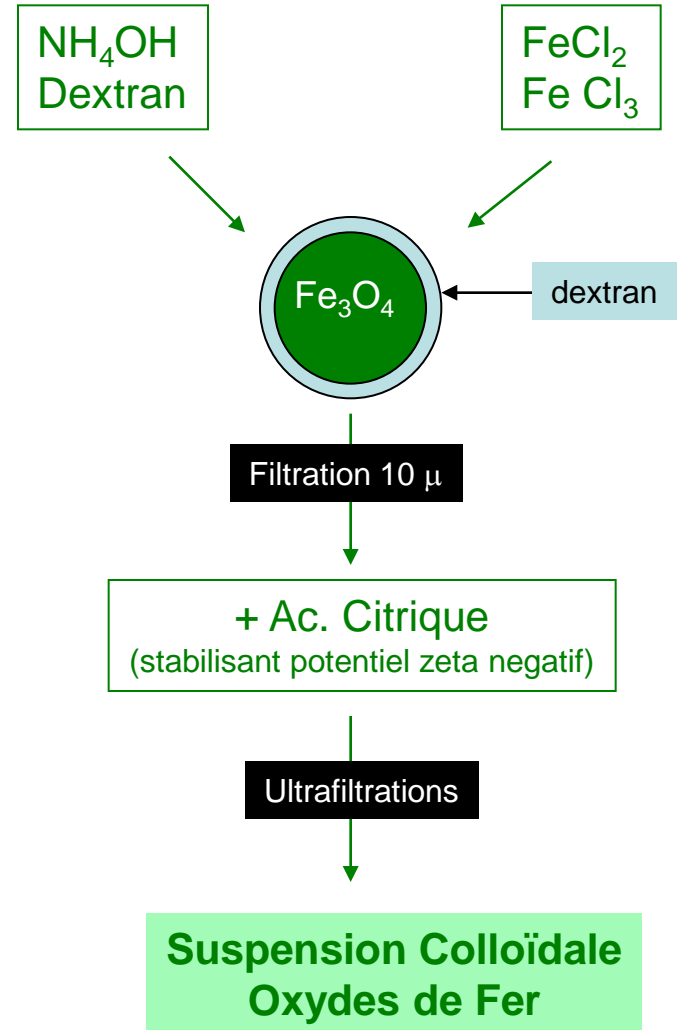
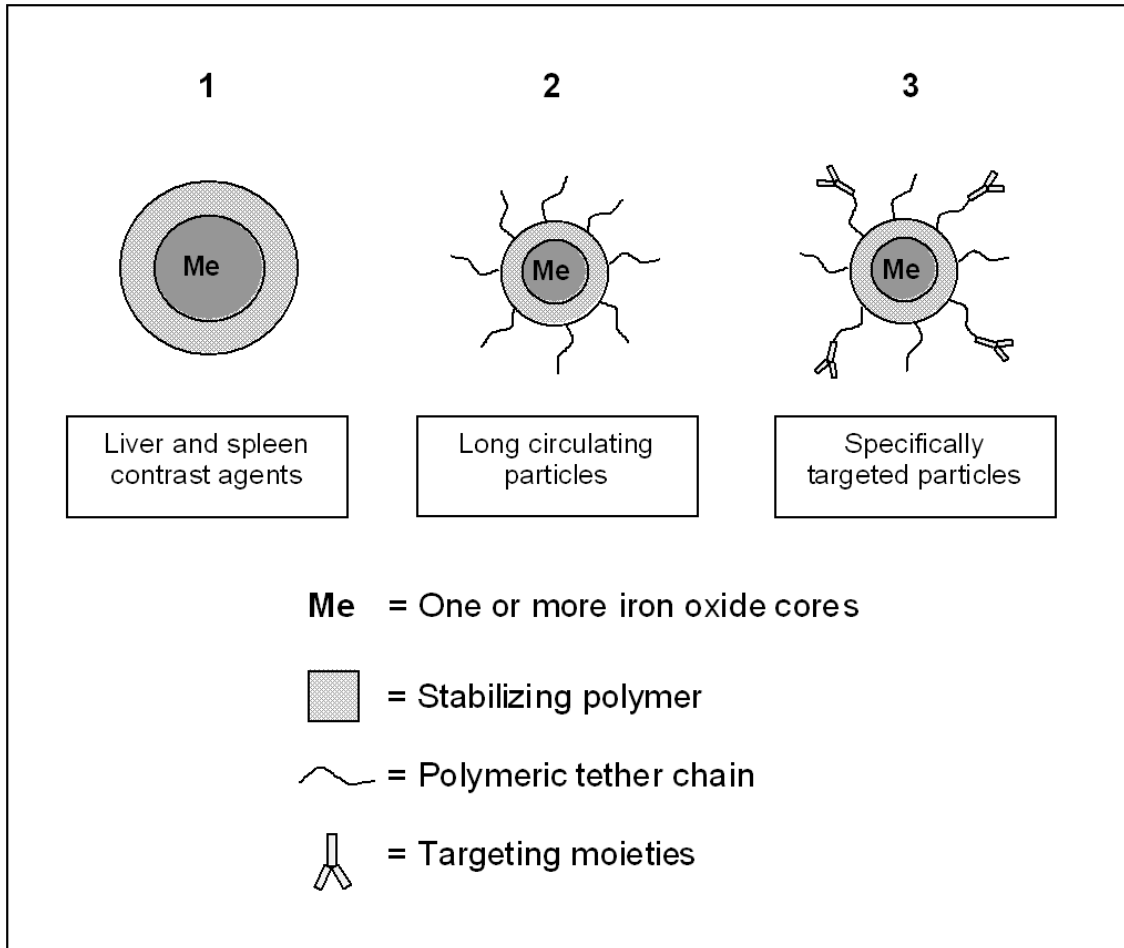
- TEM et AXD show an average crystallite size of ca.7.5 nm whereas EDXD indicates a shorter structural coherence of ca. 4.0 nm similarly to what happens in liquids and other short-range ordered materials
- Within this range, the nanoparticles are characterized by a perfect crystalline coherence
- There is no abrupt breakdown in atomic order but it is present a three dimensional lattice distortion which consists in displacements of the sites away from those of an ordered periodic lattice
- The atoms of the particle show a columnar periodicity but, in the three-dimensional lattice, the lattice periodicity is faster lost when the distance increases radially
- The existence of a structural coherence smaller than the entire volume of the particle could be related to magnetic properties



NANOPARTICULES D'OXYDES DE FER

Préparation

NANOPARTICULES D'OXYDE DE FER

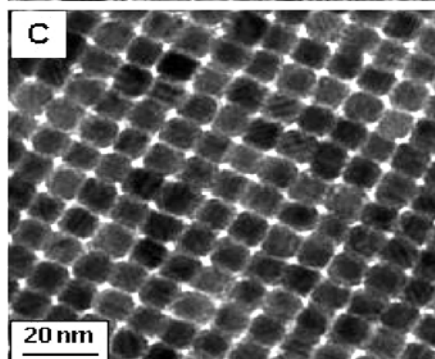
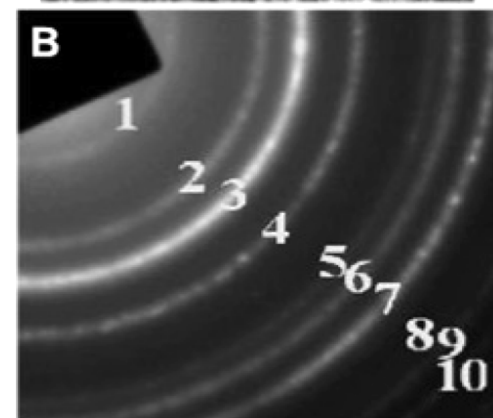
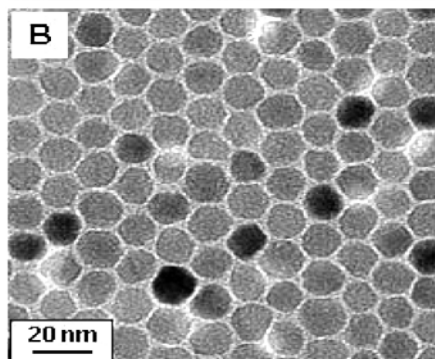
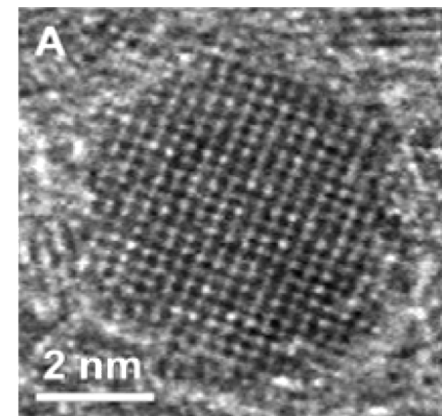
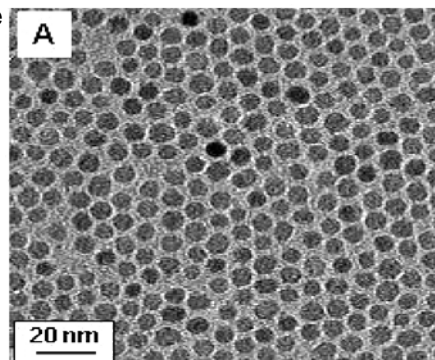
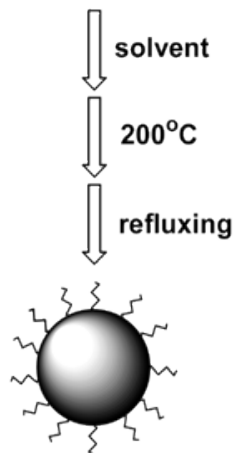
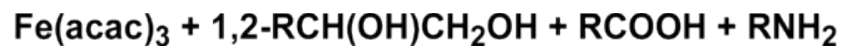


Stabilizing agents are: surfactants and polymers, as dextran, starch, siloxane and poly(ethylene glycol)

PREPARATION OF USPIO BY HIGH TEMPERATURE DECOMPOSITION OF ORGANO METALLIC PRECURSORS

Sun S et al, JACS, 126, 273-279 (2004)

Fe Acetylacetonate Hexadecane diol Oleic ac. oleylamine



TEM bright field images of (A) 6 nm and (B) 12 nm Fe₃O₄ nanoparticles deposited from their hexane dispersion on an amorphous carbon-coated copper grid and dried at room temperature, and (C) a 3D superlattice of 10 nm Fe₃O₄ nanoparticles

(A) High-Resolution TEM image of a 6 nm Fe₃O₄ USPIO (B) electron diffraction (SAED) pattern → single crystal

NANOPARTICULES D'OXYDES DE FER

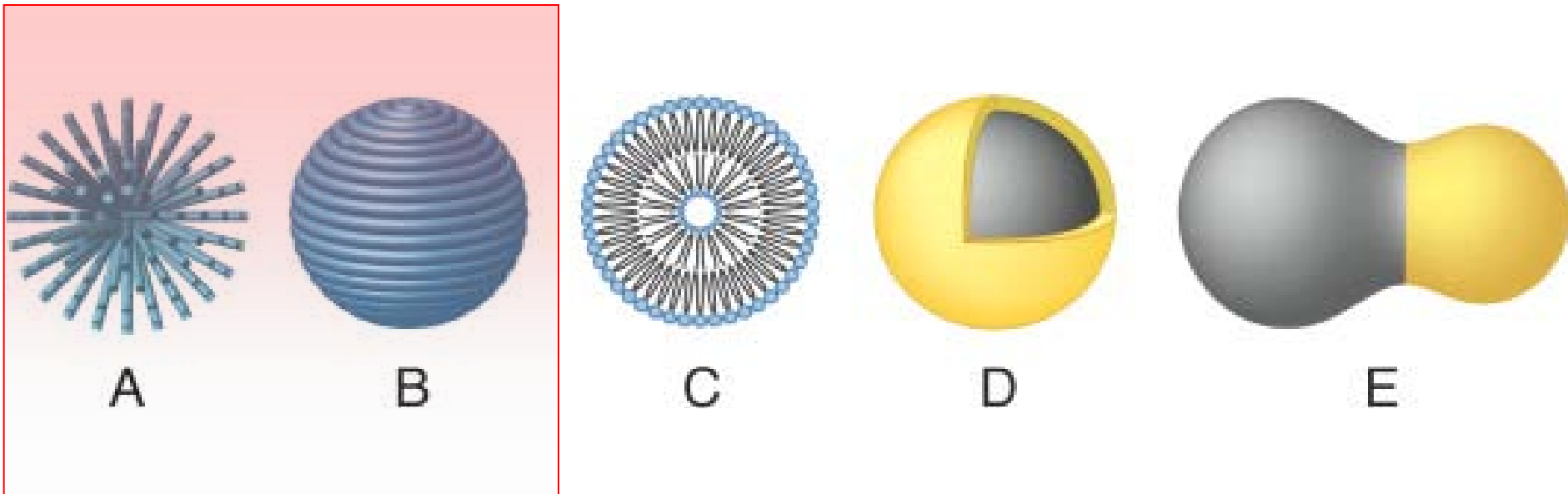
Nature du recouvrement

STABILISATION COLLOIDALE PAR RECOUVREMENT DE POLYMERES

- Stabilisation électrostatique → peu adéquat pour prévenir l'agrégation en milieu biologique
- CAR les sels, électrolytes et l'adsorption de protéines → neutralisation des charges
- Nécessité de recouvrir la surface des nanoparticules par des polymères
- La performance va dépendre de la nature du polymère, de son poids moléculaire, de sa conformation (ie. « boucle » ou « brosse »), du degré de recouvrement et de la manière dont il est fixé à la particule
- Principaux polymères de recouvrement: Dextran, polysilanes et PEG

IRON OXIDE NANOPARTICLES STRUCTURES AND COATING SCHEMES

Sun C. et al., *Advanced drug delivery reviews*, 60, 1252-1265, 2008

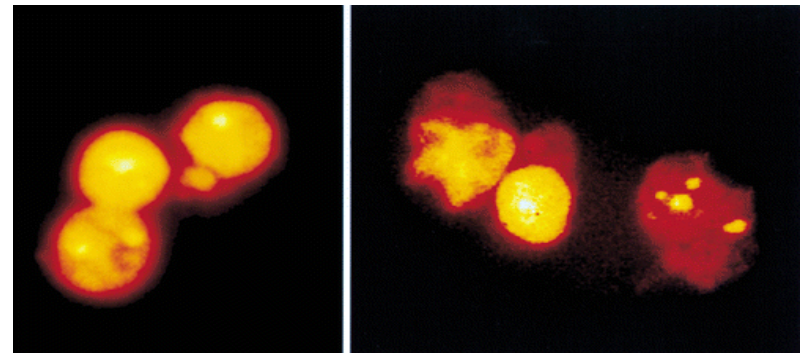
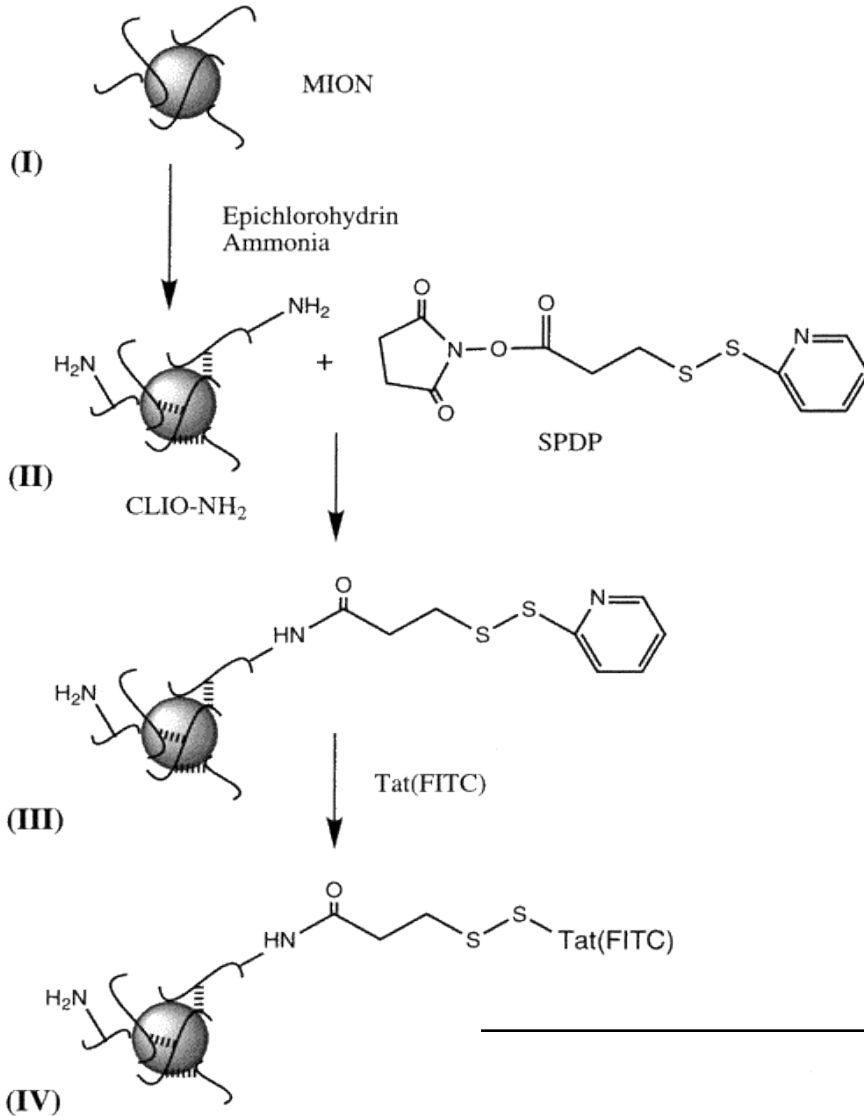
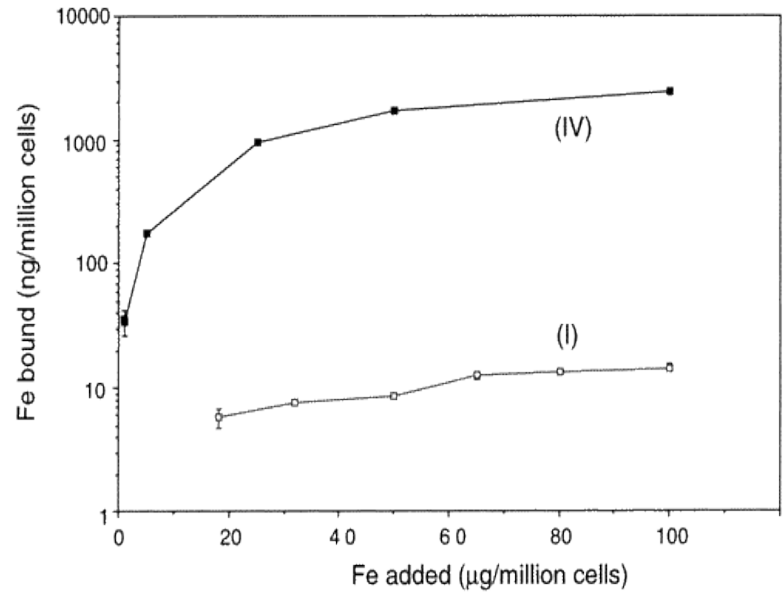


(A) End-grafted polymer coated MNP. (B) MNP fully encapsulated in polymer coating. (C) Liposome encapsulated MNP. (D) Core-shell MNP. (E) Heterodimer MNP.

SYNTHETIC SCHEME OF CROSS LINKED DEXTRAN COATED IRON OXIDE NANOPARTICLES (CLIO) AND TAT FUNCTIONALIZATION

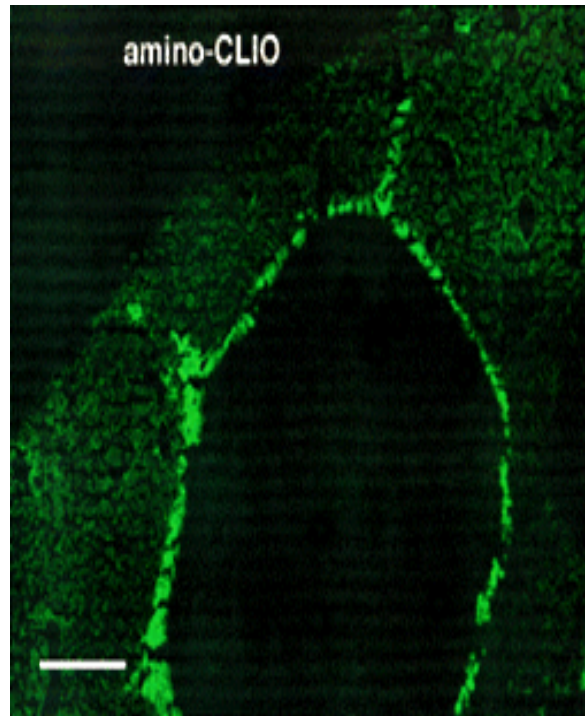
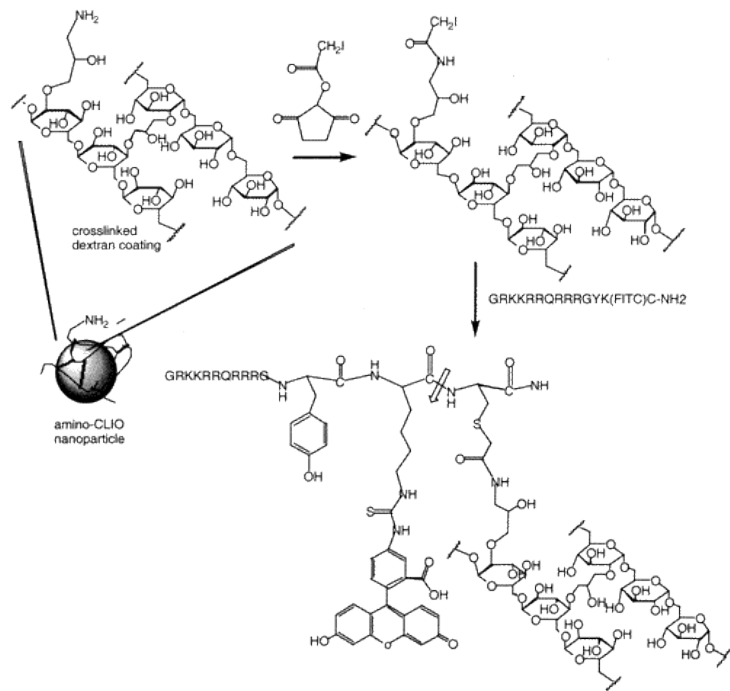
Josephson L. et al., Bioconjugate Chem. 10, 2, 186-191, 1999

Uptake of IV and I by mouse lymphocytes

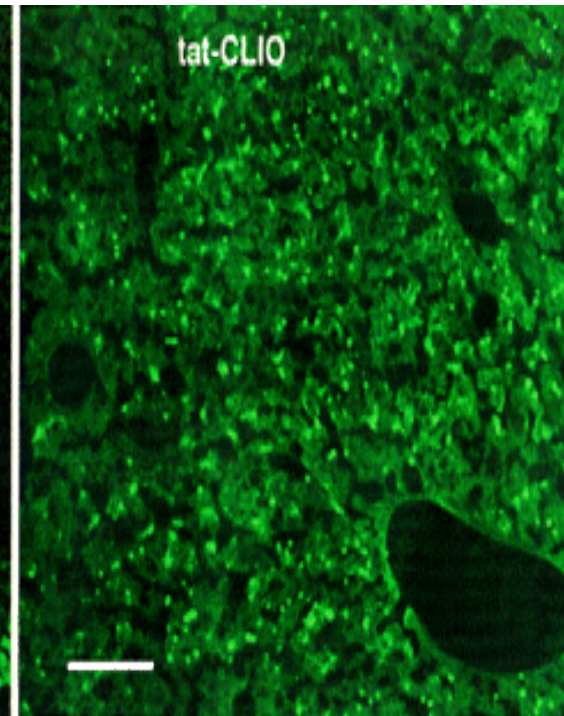


LIVER DISTRIBUTION OF TAT-CLIO

Wunderbaldinger P et al, Bioconjugate Chem., 13 264-268, 2002



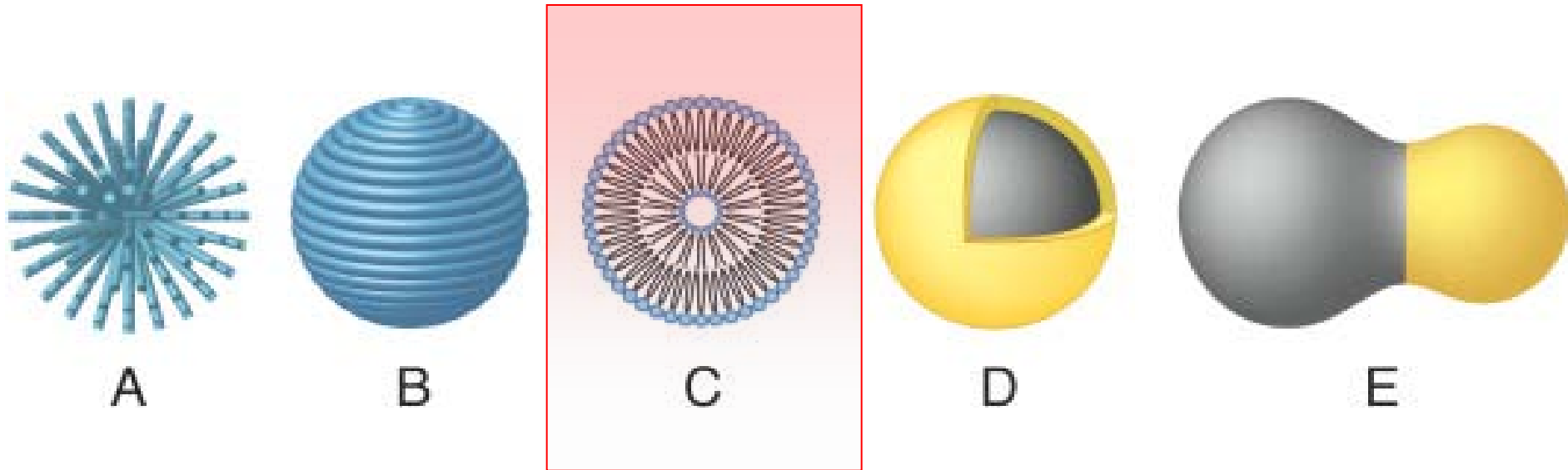
Hepatic vessels (ie. endothelial/Kupffer cells) are fluorescent



Parenchymal distribution of the fluorescence

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Sun C. et al., *Advanced drug delivery reviews*, 60, 1252-1265, 2008



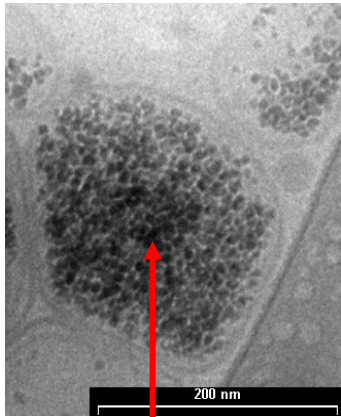
(A) End-grafted polymer coated MNP. (B) MNP fully encapsulated in polymer coating. (C) Liposome encapsulated MNP. (D) Core-shell MNP. (E) Heterodimer MNP.

LIPOSOMES

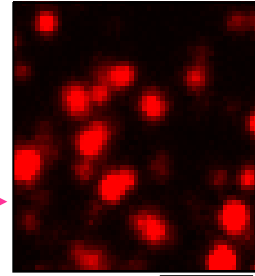
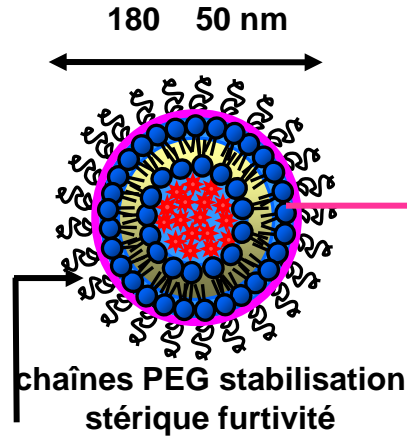
- Permet la fonctionnalisation de surface via les lipides/phospholipides de la bicouche
- Permet l'obtention de doubles fonctionnalités par co-encapsulation de (i) médicaments (« théragnostics ») ou de (ii) colorants fluorescents
- Bonne biocompatibilité

LIPOSOMES SUPERPARAMAGNETIQUES

Martina MS, J. Am. Chem. Soc. 127, 10676-10685, 2005
 Fortin J, Radiology 238,415-424, 2006

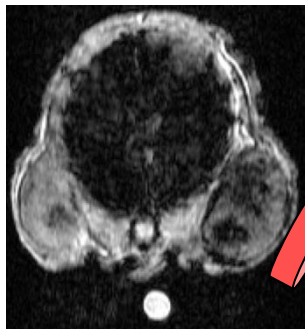


ferrofluide: nanocristaux de
 maghémite $\gamma\text{Fe}_2\text{O}_3$



500 nm
 marqueur fluorescent suivi
in vitro et *in vivo*

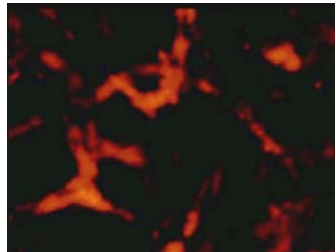
**guidage magnétique *in vivo*
 voie vasculaire chez la souris**



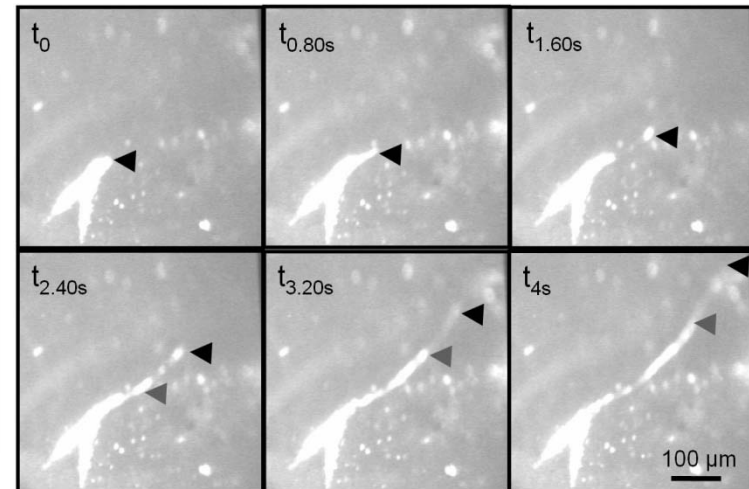
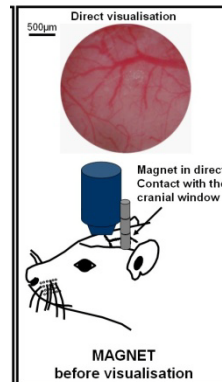
tumeur solide
 adénome prostatique
 humain



aimant



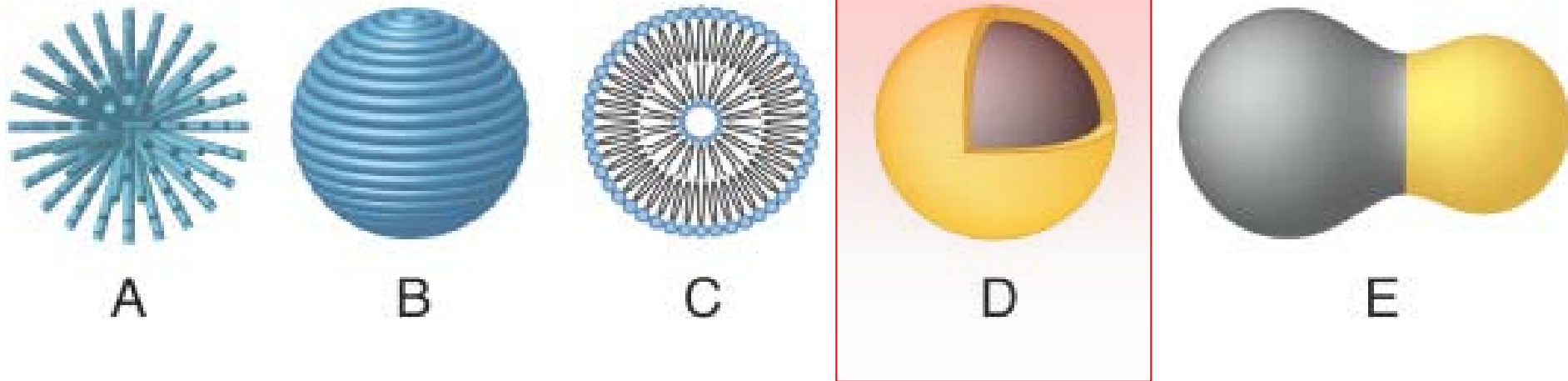
fibroscopie



IRM

IRON OXIDE NANOPARTICLES STRUCTURES AND COATING SCHEMES

Sun C. et al., *Advanced drug delivery reviews*, 60, 1252-1265, 2008



(A) End-grafted polymer coated MNP. (B) MNP fully encapsulated in polymer coating. (C) Liposome encapsulated MNP. (D) Core-shell MNP. (E) Heterodimer MNP.

STRUCTURATION « CŒUR/COURONNE »

- Utilisation d'or ou de siloxane pour la confection de la couronne
- L'utilisation de tétraéthoxysilane (TEOS) permet d'obtenir une « couronne » d'épaisseur contrôlée
- L'utilisation d'alkoxysilanes fonctionnels (aminopropyltriéthoxysilane) permet d'envisager des réactions de couplage ultérieures
- Permet de protéger le « cœur » magnétique de la dégradation chimique
- Préviend la libération de composés potentiellement toxiques

USPIO COATING BY SOL-GEL APPROACH

Lu Y. et al, Nano Letters 2, 183-186, 2002

Tetraethylorthosilicate (TEOS)

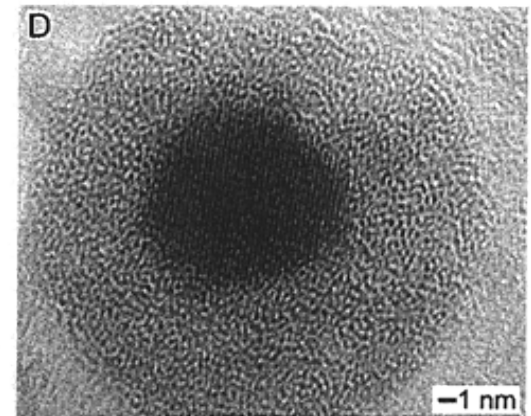
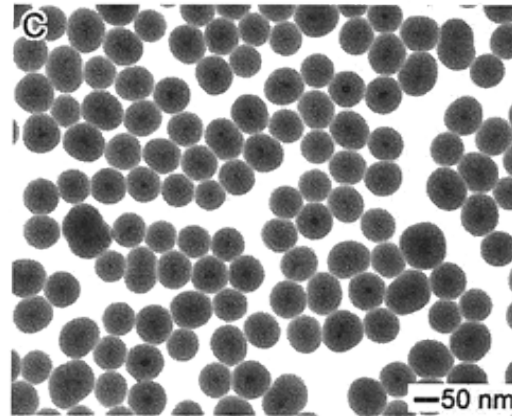
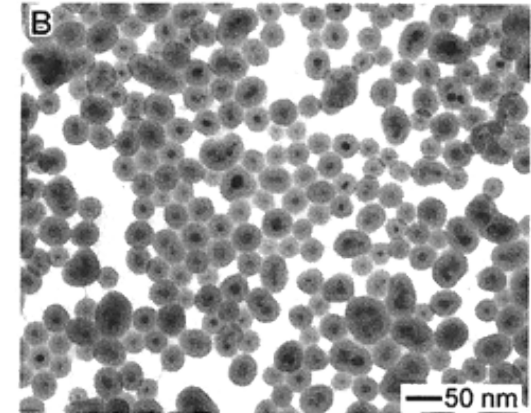
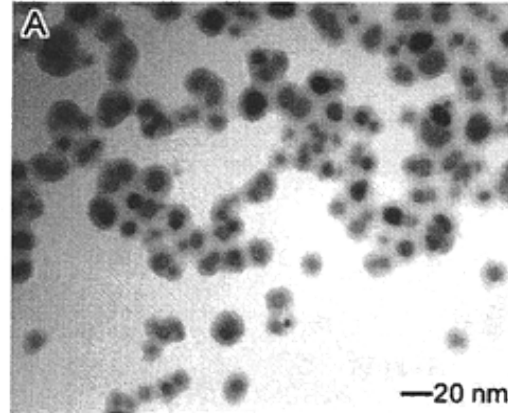
+
USPIO
(water/propanol)



+ NH₃
(hydrolysis of the sol-gel precursor)



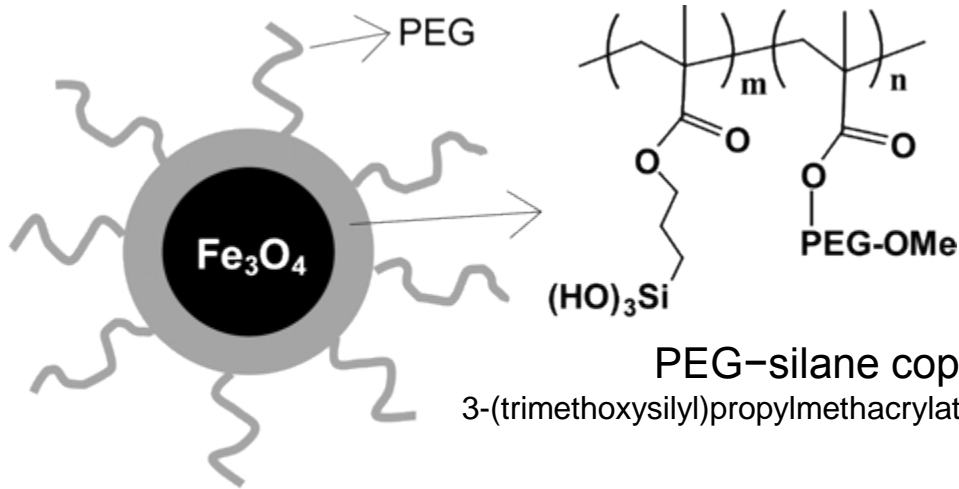
Deposition of the silica
at USPIO's surface



TEM images of USPIO whose surfaces have been coated with silica shells of various thicknesses. Thickness of silica coating is adjusted by controlling the amount of precursor added to the solution: (A) 10, (B) 60, and (C) 1000 mg of Tetraethylorthosilicate (TEOS) to 20 mL of 2-propanol. (D) HRTEM image

USPIO PEGylation BY SOL-GEL APPROACH

Lee H. et al, JACS, 128, 7383-7389, 2006



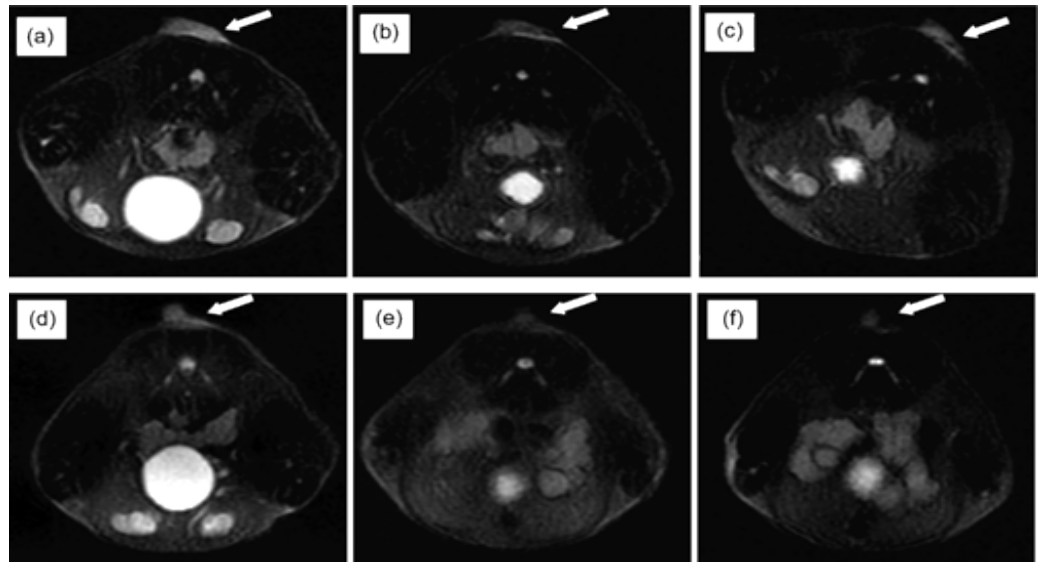
PEG-silane copolymer

3-(trimethoxysilyl)propylmethacrylate-co-polyethyleneglycol

IS-SPION = Polymer coating takes place simultaneously when the iron oxide core is formed
SW-SPION = Polymer coating is done afterwards the iron oxide core is formed

poly(TMSMA-*r*-PEGMA)@SPION

T2-images taken at 0, 1, and 4 h postinjection of 15 mg/kg of IS-SPION (a, b, and c) or SW-SPION (d, e, and f) at the level of the tumor. T2-weighted images (b and e) at 1 h postinjection of SPION show a marked signal drop at the tumor areas; at 4h SW-SPION remains longer in the tumor than IS-SPION. The arrows denote xenograft tumors.

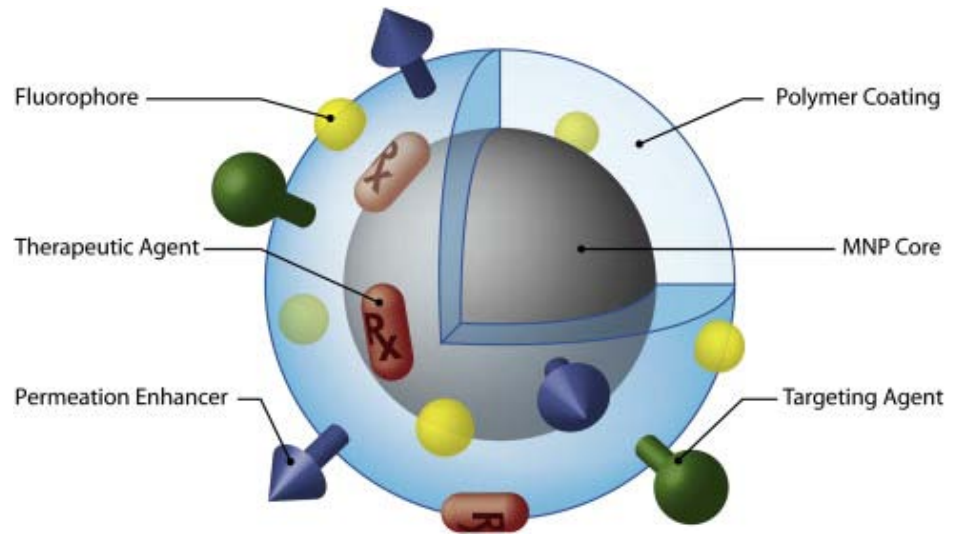


NANOPARTICULES D'OXYDES DE FER

Fonctionnalisation de surface

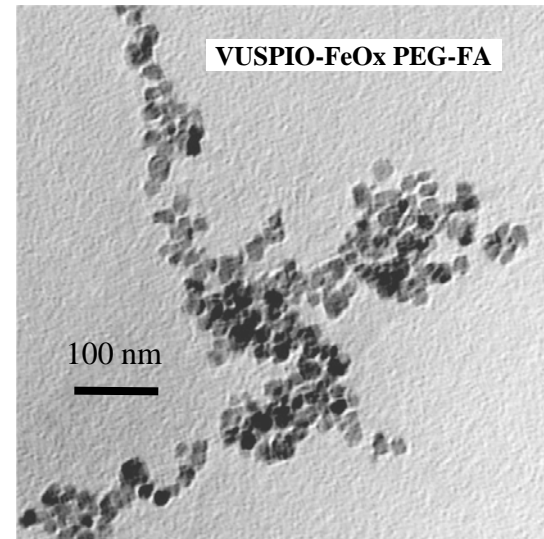
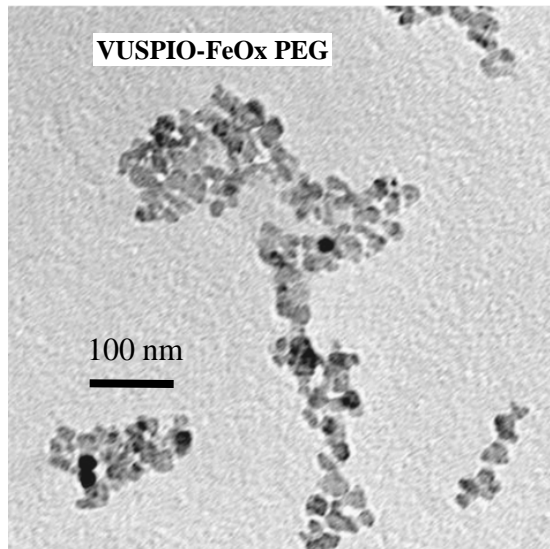
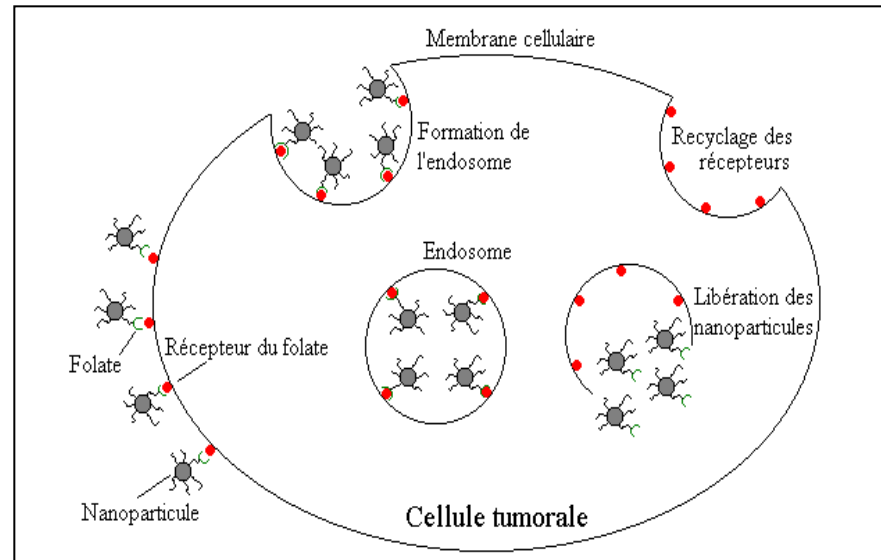
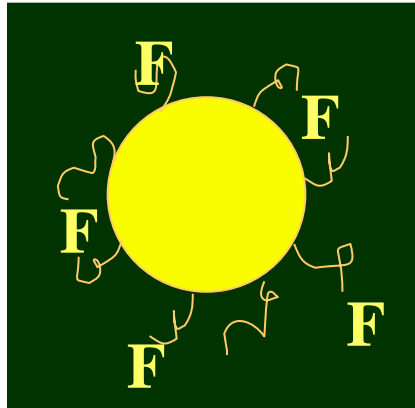
FONCTIONNALISATION PAR DES LIGANDS

- Permet le ciblage des organes/tissus et/ou cellules malades avec une reconnaissance à l'échelle moléculaire
- Les ligands peuvent être des petites molécules (ie. acide folique, hormones etc.) ou des macromolécules (anticorps, protéines etc.)



IRON OXIDE NANOPARTICLES DECORATED WITH FOLIC ACID

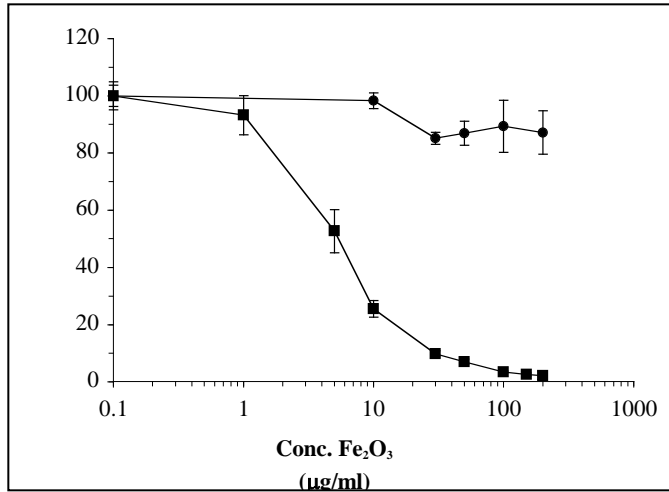
F. Sonvico et al., Bioconj. Chemistry, 2005



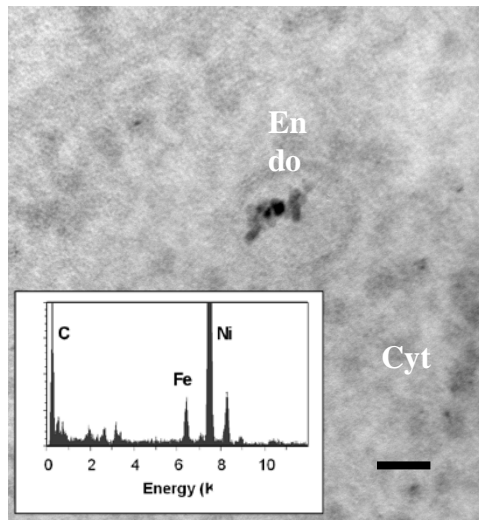
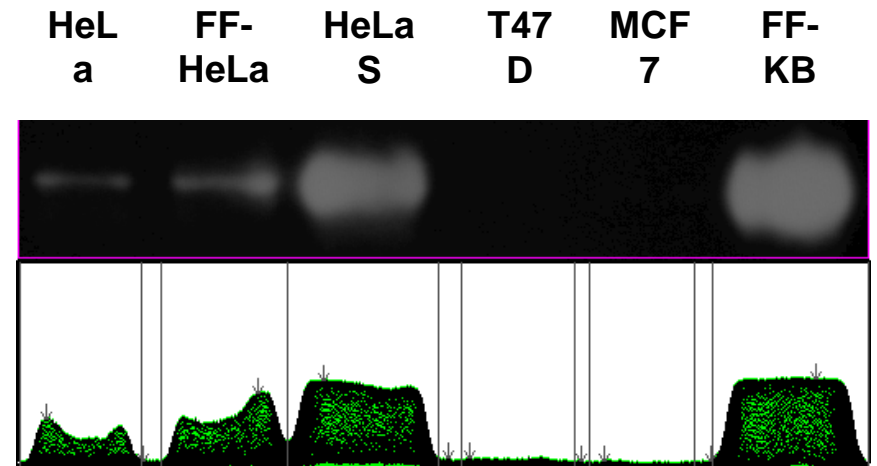
CELLULAR TARGETING OF FOLIC ACID DECORATED USPIO NANOPARTICLES

F. Sonvico et al., Bioconj. Chemistry, 2005

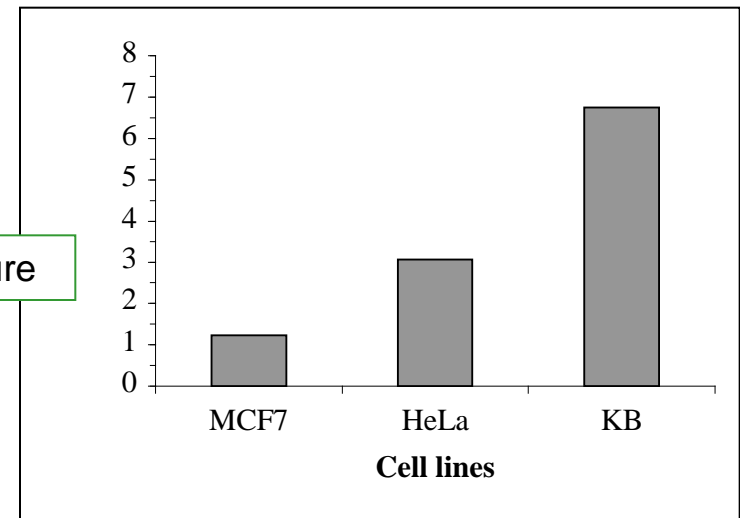
Competitive capture of 3H-folic acid by KB cells



Western blot Folic acid receptor



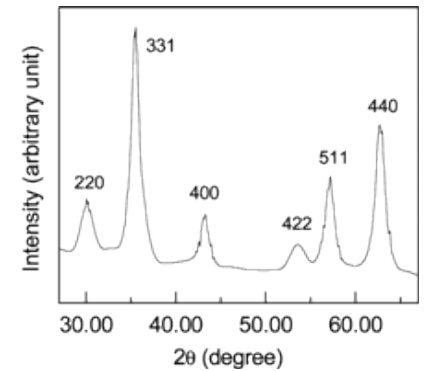
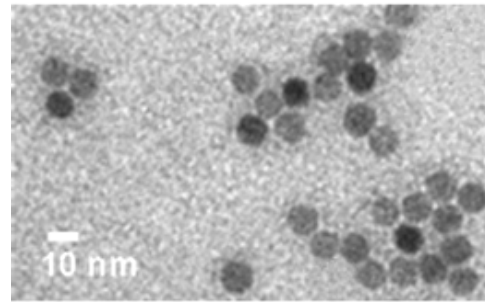
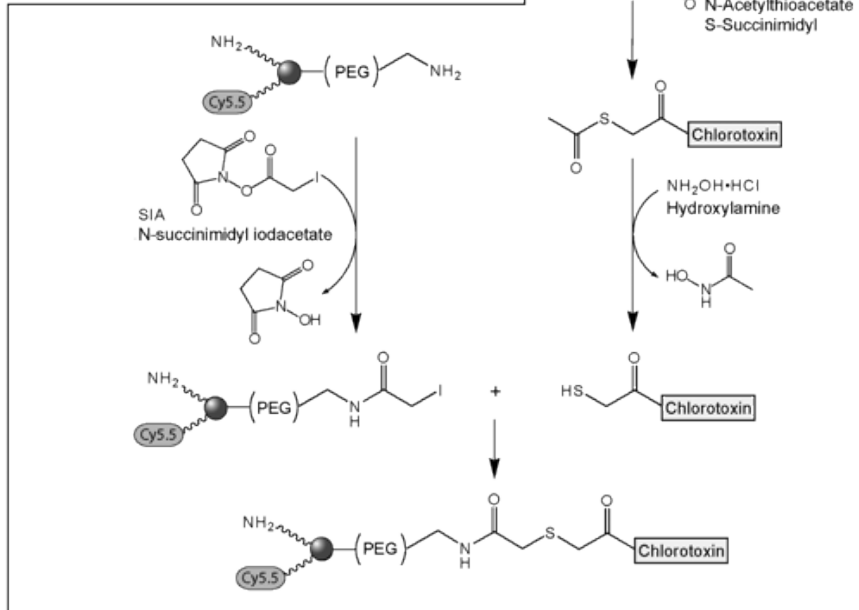
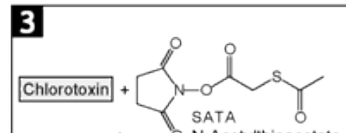
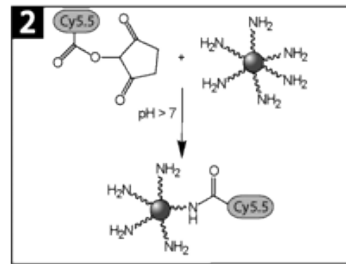
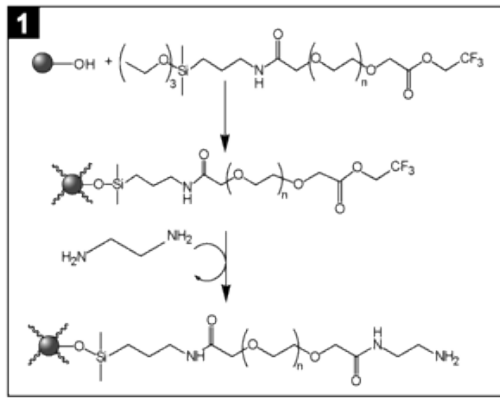
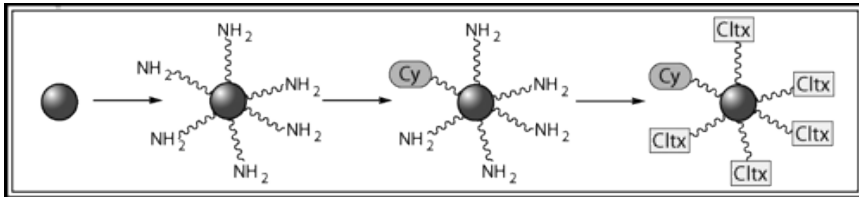
FA-USPIO cell capture



PEG-SILANE DECORATED IRON OXIDE NANOPARTICLES FUNCTIONALIZED WITH CHLOROTOXINE

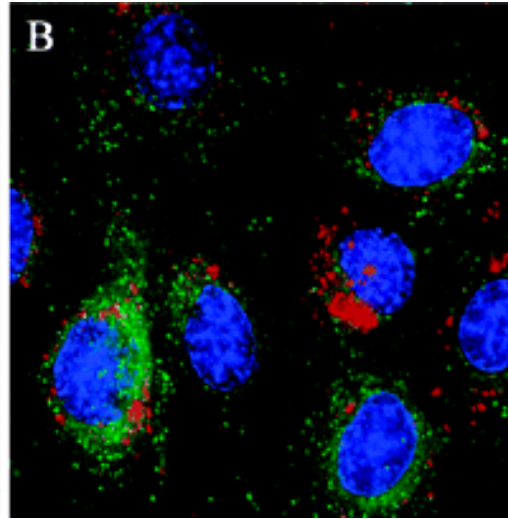
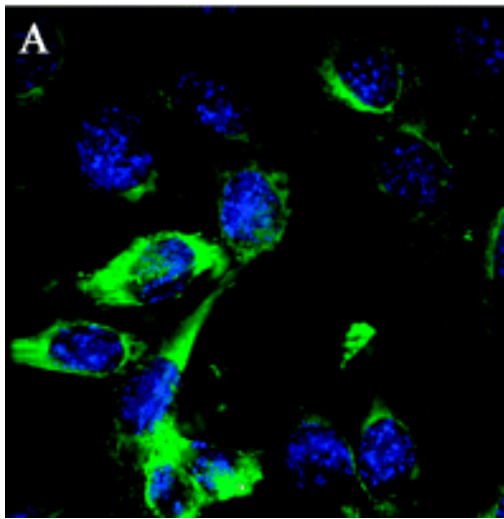
Veiseh O. et al, Nano Letters 5, 1003-1008, 2005

Chlorotoxine = glioma targeting molecule

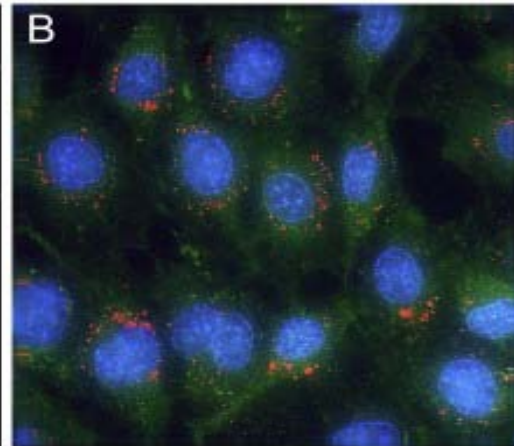
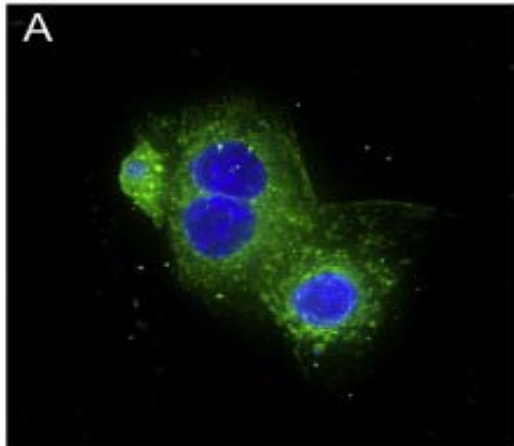


PEG-SILANE DECORATED IRON OXIDE NANOPARTICLES FUNCTIONALIZED WITH CHLOROTOXINE

Veisheh O. et al, Nano Letters 5, 1003-1008, 2005



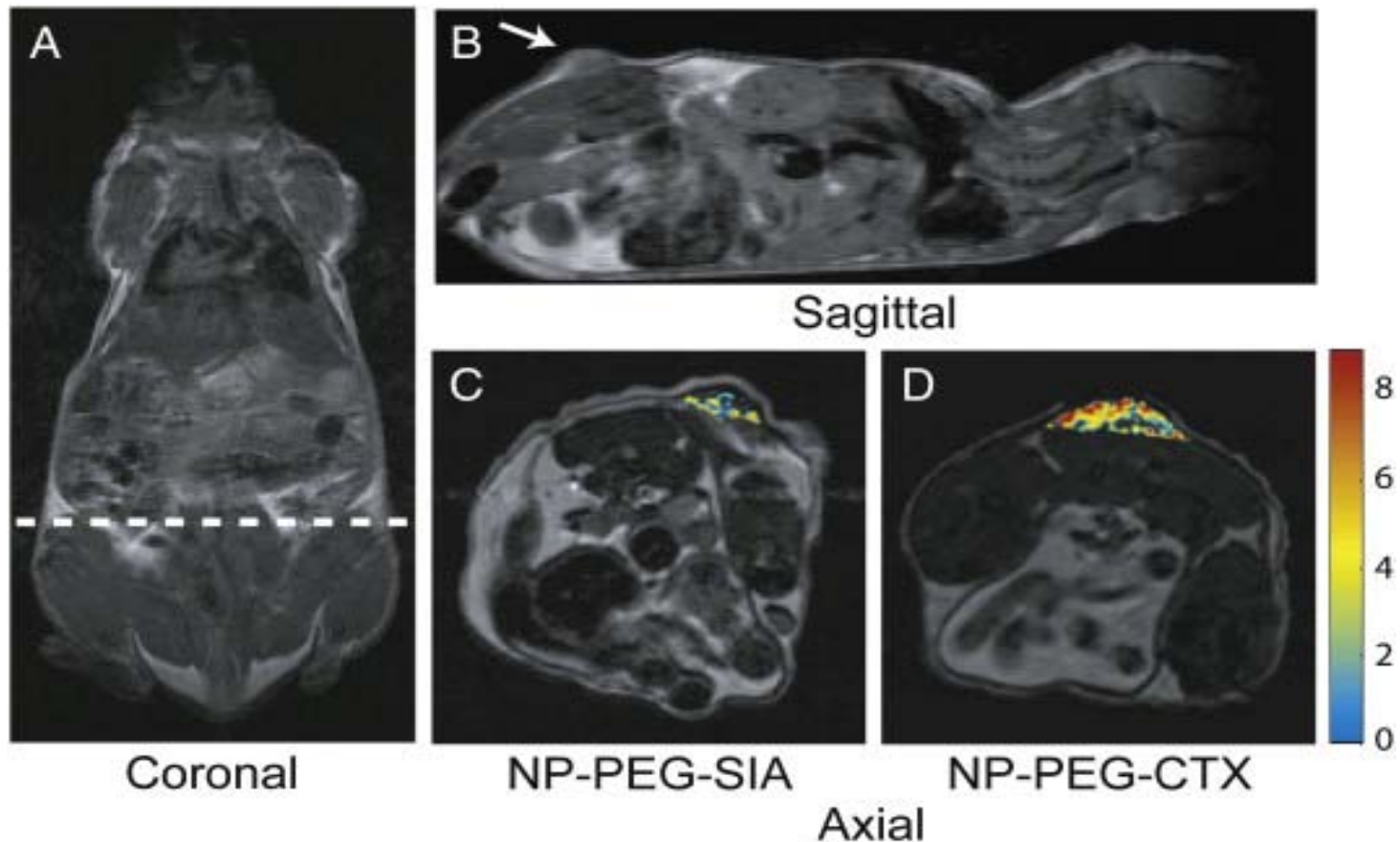
9L cells cultured with control NP-Cy5.5 (A) and (B) NPC-Cy5.5 (**red fluorescence**) (C) MR phantom image of 9L cells cultured with NPCs and control NPs and embedded in agarose



Confocal fluorescent images of cells incubated with chlorotoxin-targeted iron oxide nanoparticles conjugated to Cy5.5. (A) Rat cardiomyocytes (rCM) representing normal cells. (B) 9L glioma cells (**red**) (C) MR phantom image of 9L (top) and rCM (bottom) cells cultured with the chlorotoxin-targeted nanoparticles

MR SIGNAL IN TUMOR WITH CHLOROTOXIN FUNCTIONALIZED IRON OXIDE NANOPARTICLES

Sun C. et al., *Advanced Drug Delivery Reviews*, 60, 1252-1265, 2008



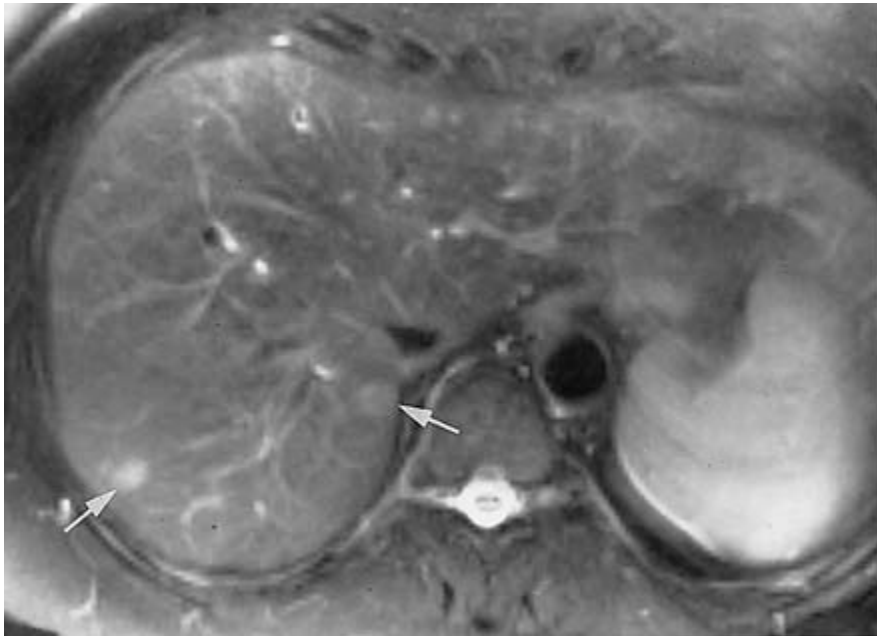
(A) coronal plane with the dotted line displaying the approximate location of the axial cross sections displayed in (C) and (D). Anatomical image in the (B) sagittal plane displaying the location of the 9L xenograft tumor. Change in R2 relaxation for mouse receiving (C) non-targeting PEG-coated iron oxide nanoparticles and (D) CTX-targeted PEG-coated iron oxide nanoparticles (3 h post injection)

NANOPARTICULES D'OXYDES DE FER

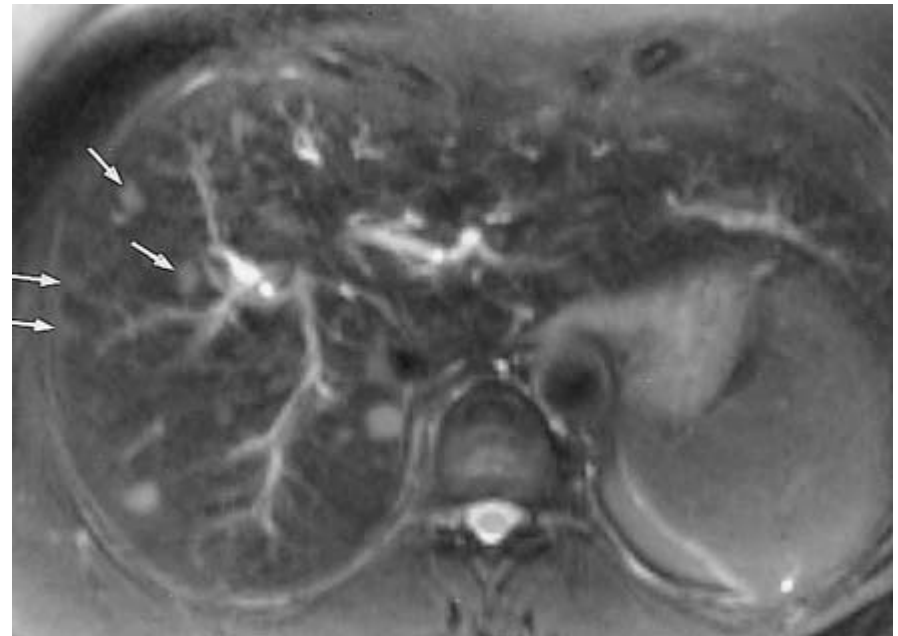
Applications en
radiologie/imagerie

LIVER MR IMAGING WITH IRON OXIDE NANOPARTICLES

Semelka RC. Et al, Radiology, 218, 27-38, 2001



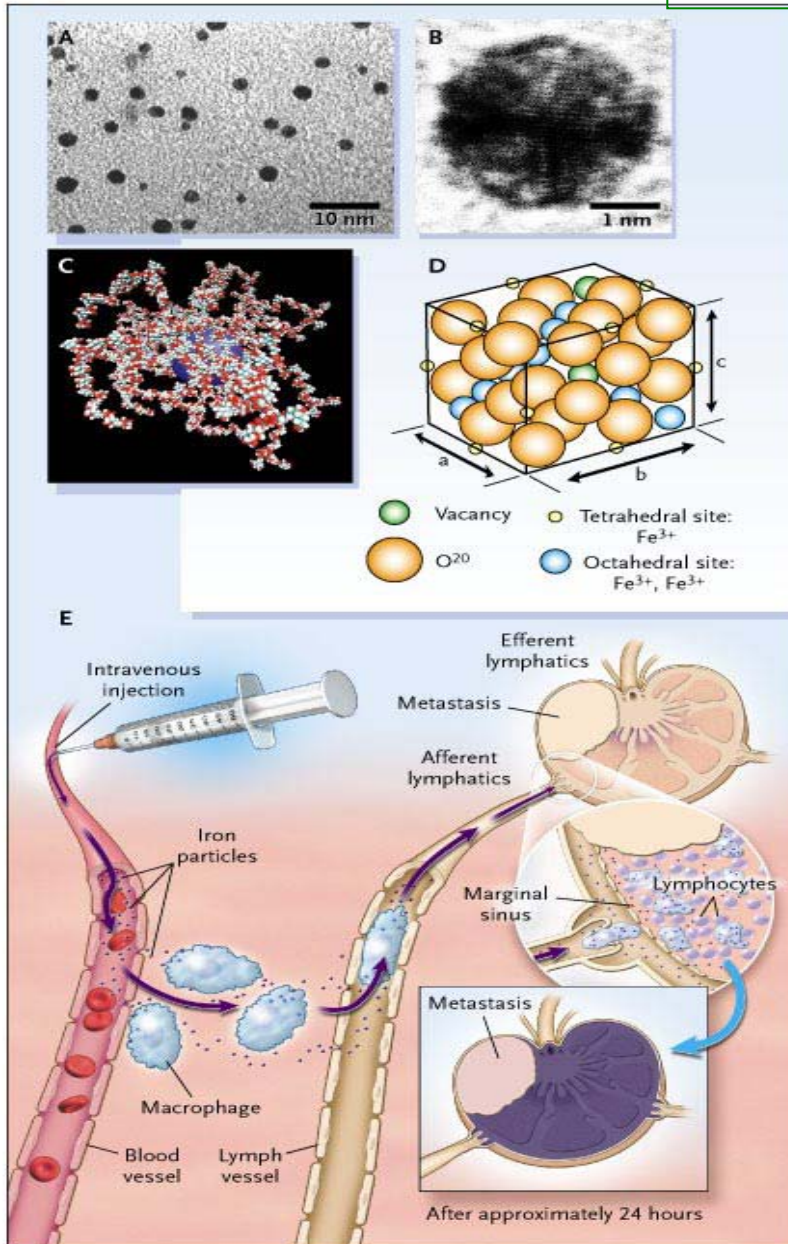
Conventional MRI



Iron oxide MRI

USPIO FOR LYMPHATIC IMAGING

Harisinghani MG et al., New England Journal of Medicine, 348, 2491-2500; 2003



The systemically injected long-circulating particles gain access to the interstitium and are drained through lymphatic vessels. Disturbances in lymph flow or in nodal architecture caused by metastases lead to abnormal patterns of accumulation of lymphotropic superparamagnetic nanoparticles, which are detectable by MRI.

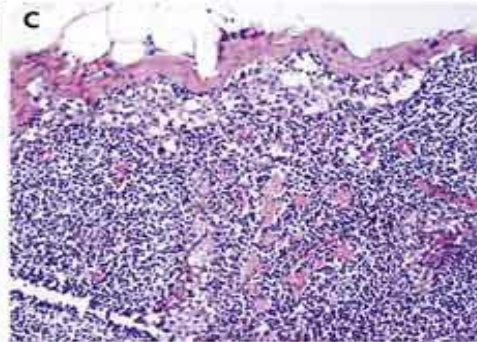
MRI NODAL ABNORMALITIES IN PATIENTS WITH PROSTATE CANCER

Harisinghani MG et al., New England Journal of Medicine, 348, 2491-2500; 2003

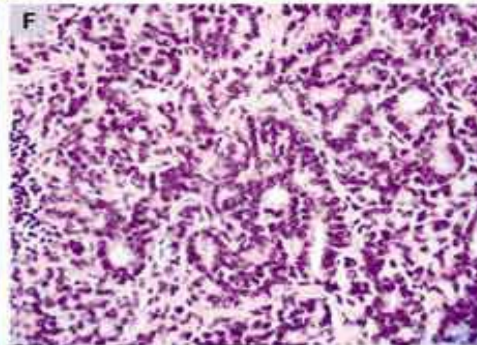
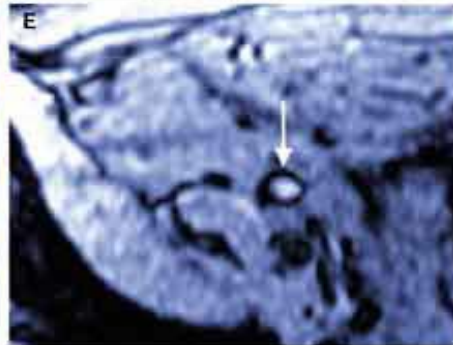
Conventional MRI

MRI with USPIO

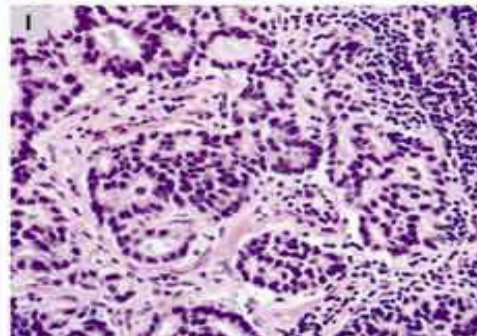
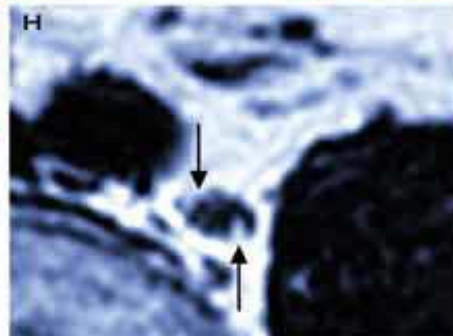
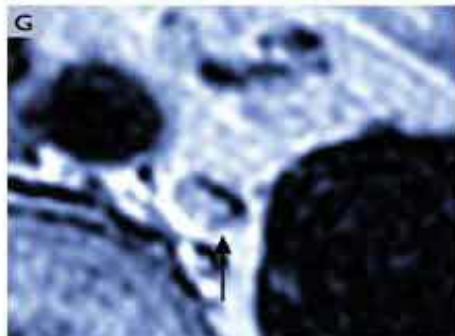
histology



Normal
(USPIO are taken up by healthy Lymph nodes)



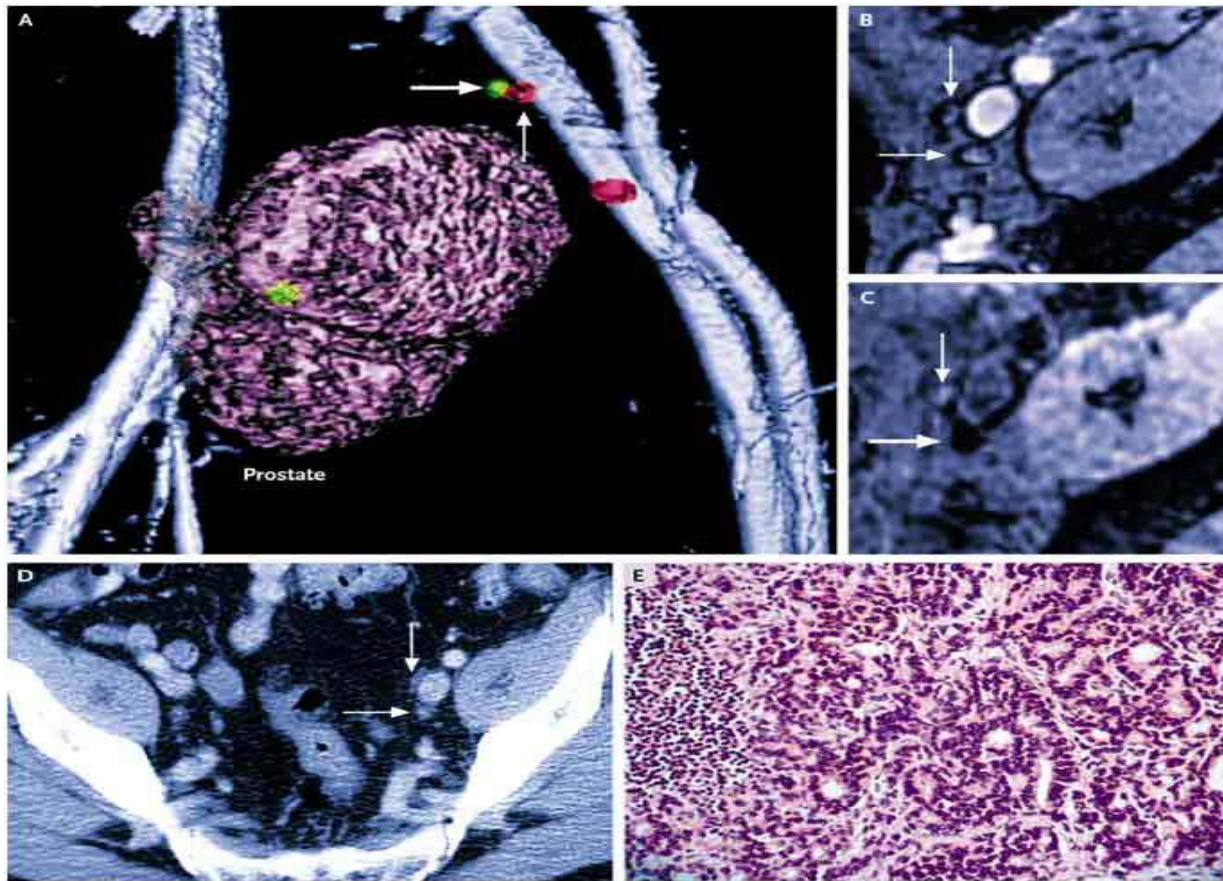
Tumoral
(USPIO are not taken up by Lymph nodes)



Small metastasis
(two hyperintense foci are observed)

THREE DIMENSIONAL RECONSTITUTION OF PROSTATE WITH 1 HEALTHY AND 1 ABNORMAL METASTATIC NODE

Harisinghani MG et al., New England Journal of Medicine, 348, 2491-2500; 2003



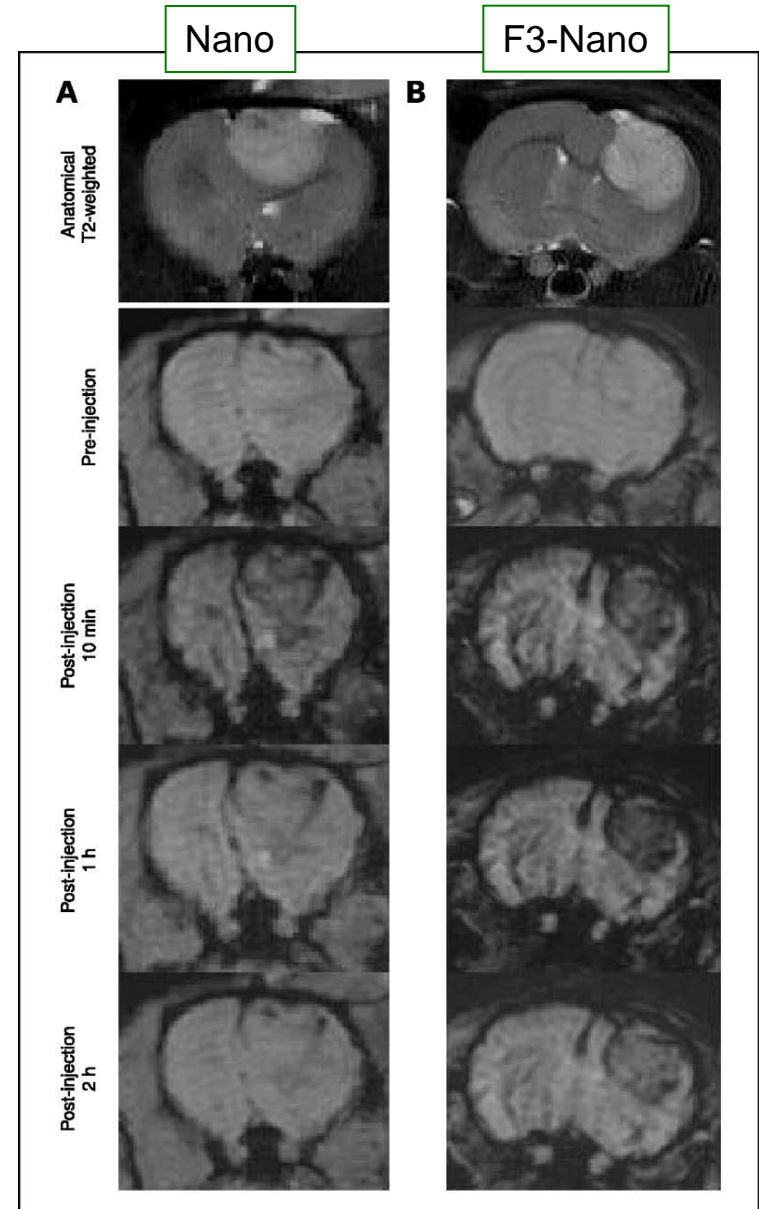
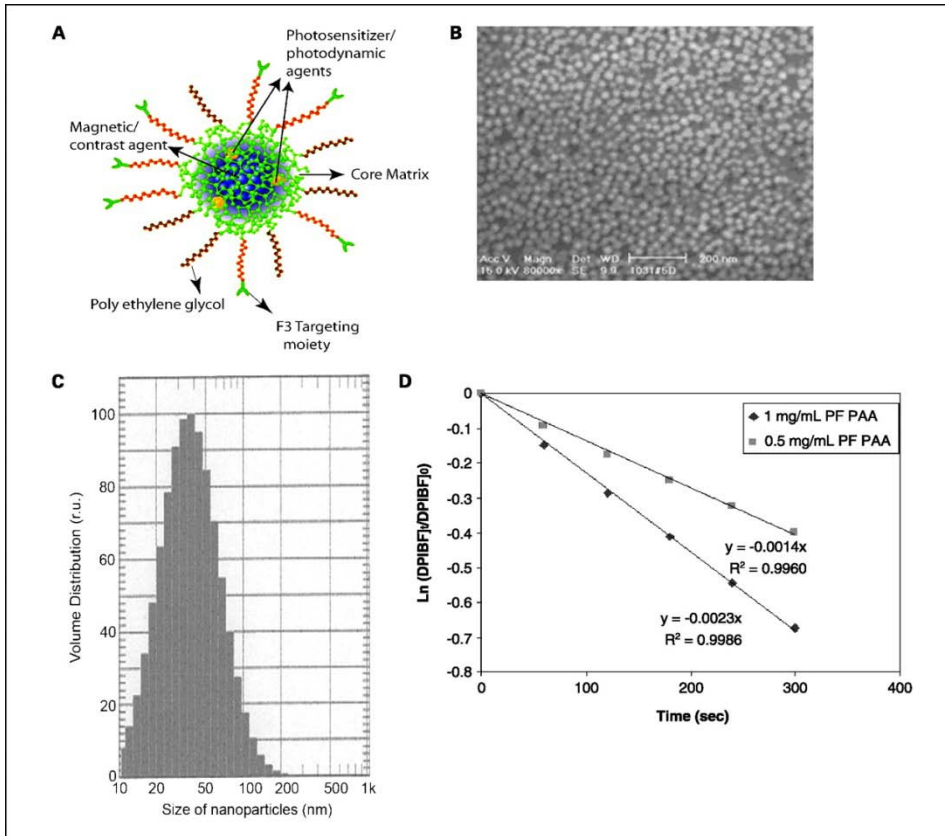
CONVENTIONAL MRI

USPIO MRI

Panel A shows a three-dimensional reconstruction of the prostate, iliac vessels, and metastatic (red) and nonmetastatic (green) lymph nodes, to assist in the planning of surgery and radiotherapy. There is a malignant node (thick arrow) immediately adjacent to the normal node (thin arrow) posteromedial to the iliac vessels. In Panel B, conventional MRI shows that the signal intensity is identical in the two nodes (arrows). In Panel C, MRI with lymphotropic superparamagnetic nanoparticles shows that the signal in the normal node is decreased (thick arrow) but that it is high in the metastatic node (thin arrow). In Panel D, abdominal CT fails to differentiate between the two lymph nodes (arrows). In Panel E, histopathological examination of the malignant lymph node reveals sheaths of carcinoma cells (hematoxylin and eosin, x200).

BRAIN TUMOR IMAGING WITH MULTIFUNCTIONAL NANOPARTICLES

Reddy R. et al., Clin Cancer Research, 22, 6677-6686, 2006

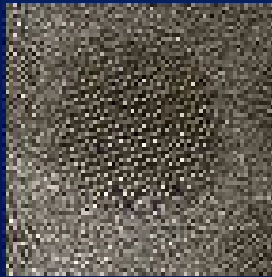


QUANTUM DOTS

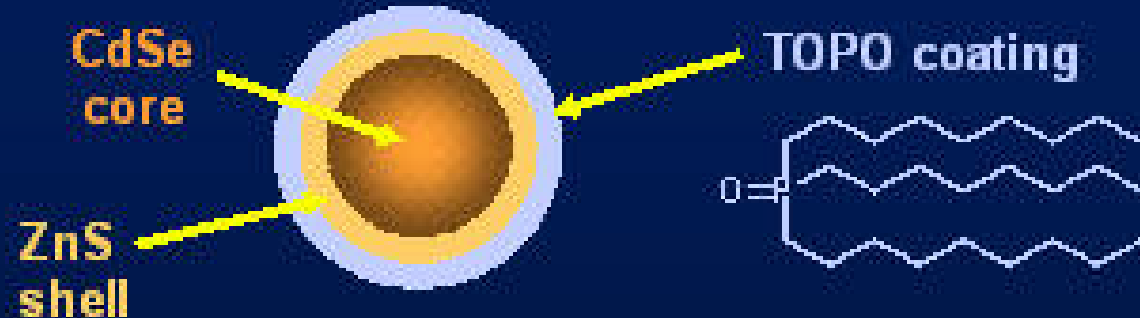
QUANTUM DOTS

- Cristaux semi-conducteurs de dimension nanométrique
- Qui présentent des propriétés de fluorescence ajustables par le contrôle de leur diamètre
- Sous excitation lumineuse dans l'UV, ils ré-émettent des photons dans le spectre du visible avec une longueur d'onde qui va du rouge au bleu quand la taille diminue

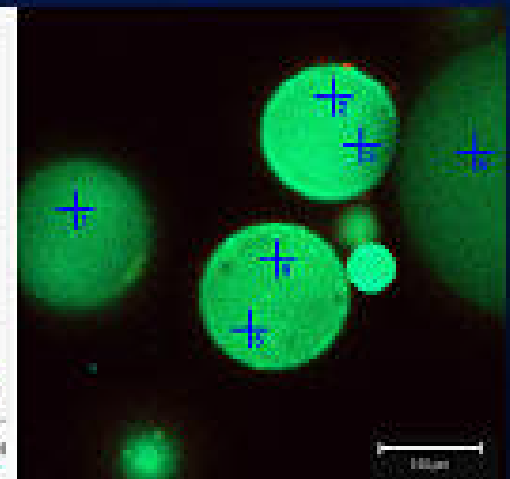
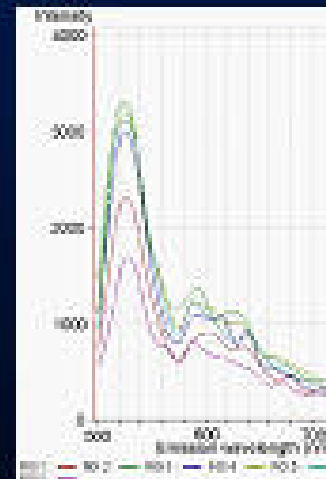
Quantum Dots



TEM of a 4 nm CdSe quantum dot

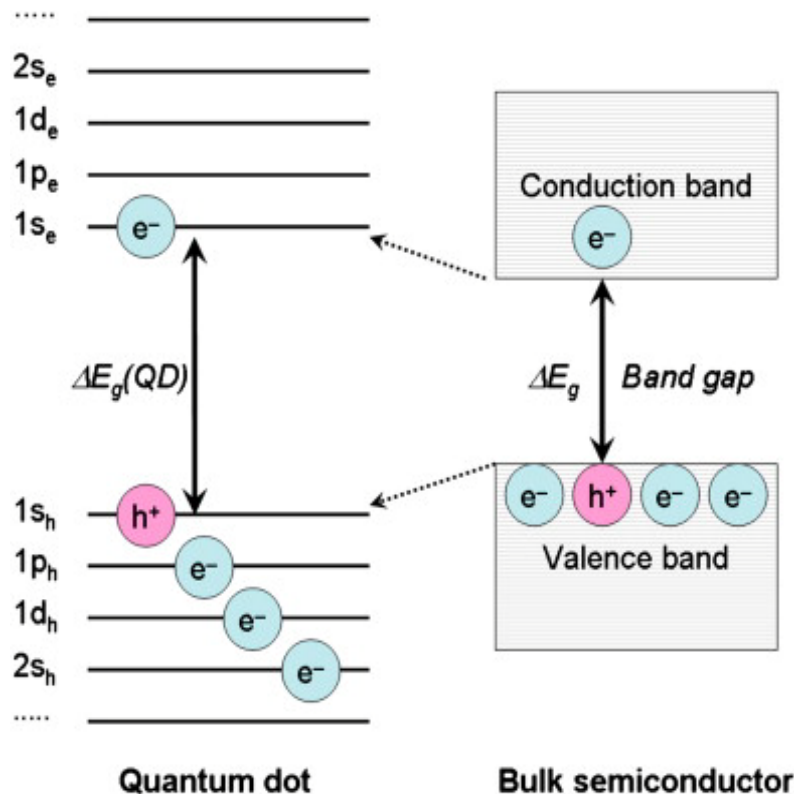


Solutions of differently sized CdSe quantum dots



Fluorescent emission fingerprinting of polymer beads that contain CdSe quantum dots

QD = CONFINEMENT QUANTIQUE

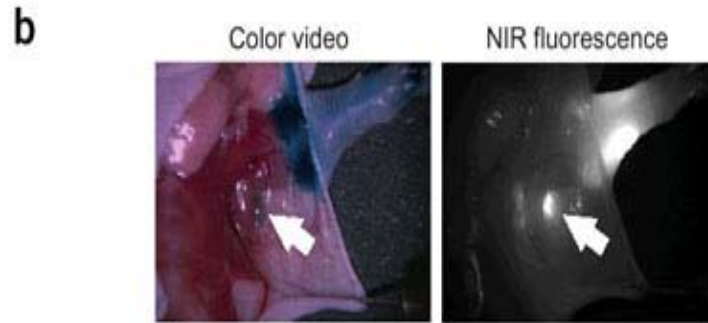
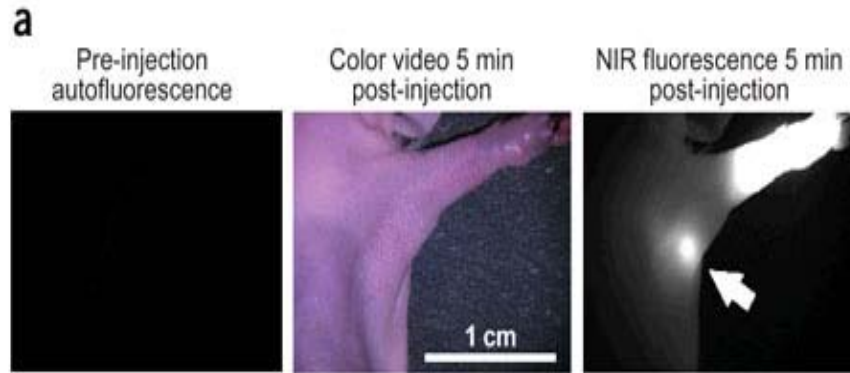


Semi-conducteur en bloc: le niveau énergétique des électrons non excités est si proche qu'il est défini comme « continu » dans la bande de valence. Le « band gap » est interdit aux électrons. Lors d'un phénomène excitatoire, l'électron va être amené dans la bande de conduction et laisser un « trou » dans la bande de valence. La distance entre h^+ et e^- est le rayon de Bohr de l'« excitation ».

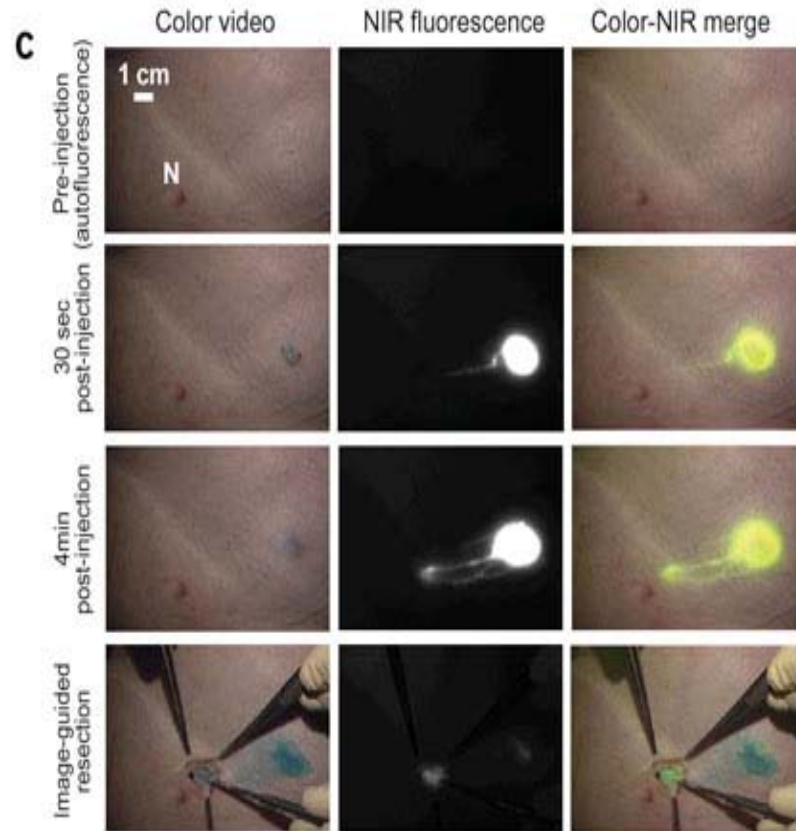
Dans le QD: la taille du semi-conducteur devient plus petite que celle du rayon de Bohr de l'« exciton » et le niveau énergétique des e^- devient discontinu. Ce « confinement quantique » a des répercussions sur les phénomènes d'absorption et d'émission car la soustraction de quelques atomes (quand la taille diminue) modifie fortement les limites de la « band gap »

NIR QD SENTINEL LYMPHE NODE MAPPING IN MOUSE AND PIG

Kim S et al, Nature Biotechnol, 22, 93-97, 2004



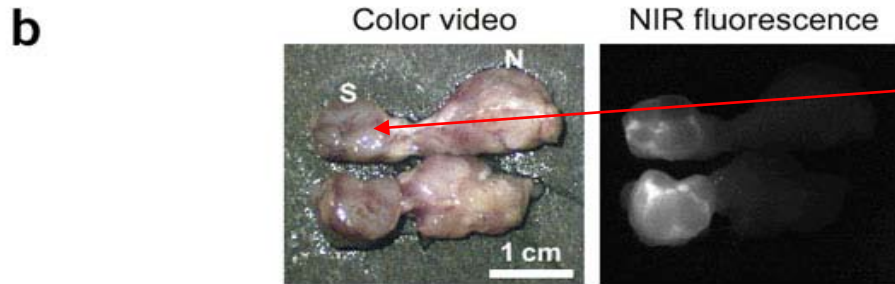
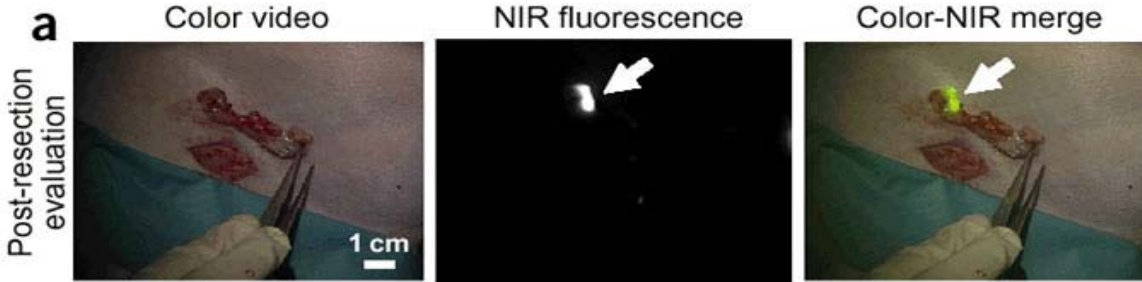
Intradermal injection with NIR QDs in the left paw (a) + blue dye (b) Arrow indicates the putative axillary sentinel lymph node.



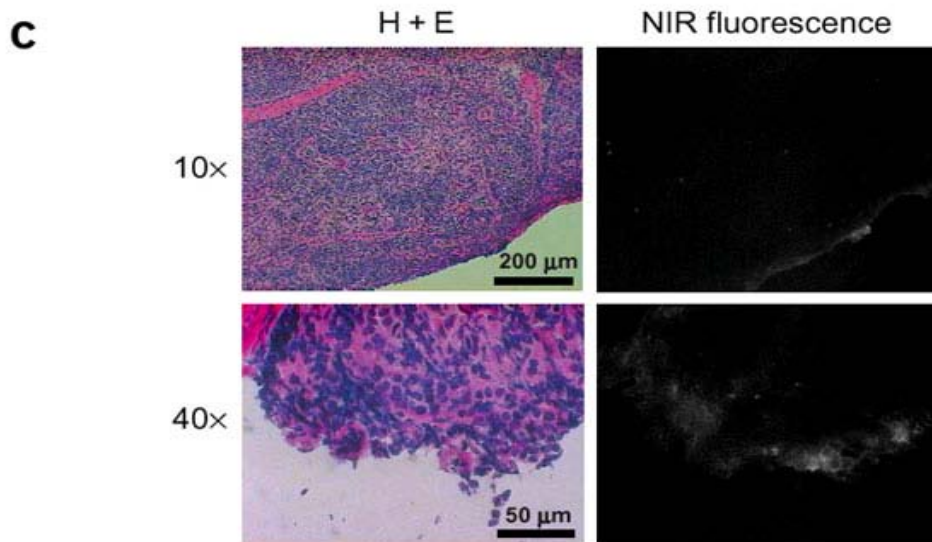
Images of the surgical field in a pig injected intradermally NIR QDs in the right groin. color video (left), NIR fluorescence (middle) and color-NIR merge (right) images are shown. Fluorescence images have identical exposure times and normalization.

POST-RESECTION INSPECTION OF LYMPH NODES MAPPING WITH QD

Kim S et al, Nature Biotechnol, 22, 93-97, 2004



Sentinel lymph node



- Post-resection evaluation of the surgical field. Shown are color video (left), NIR fluorescence (middle) and color-NIR merge (right) images. Arrows indicate the resected sentinel lymph node.
- (b) NIR QD retention by the resected SLN (S) and the next lymph node (N) in the chain is shown in this bisected specimen.
- (c) Histologic analysis of frozen sections of the SLN in b

CONCLUSIONS

Le « **confinement** » de propriétés physico-chimiques comme:

- le paramagnétisme (nanoparticules ultrafines d'oxyde de fer)
- le confinement quantique (quantum dots)

permettent la conception de nanotechnologies capables de visualiser des structures biologiques à l'échelle du tissu ou de la cellule



- Détection précoce du cancer
- Thérapeutiques ciblées
- « Théragnostique »