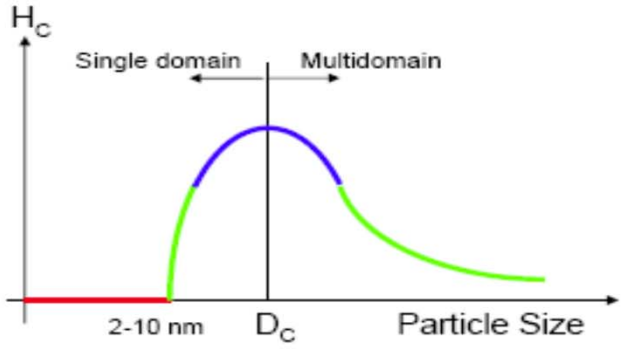
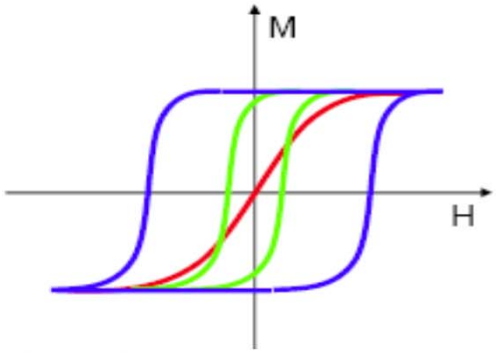
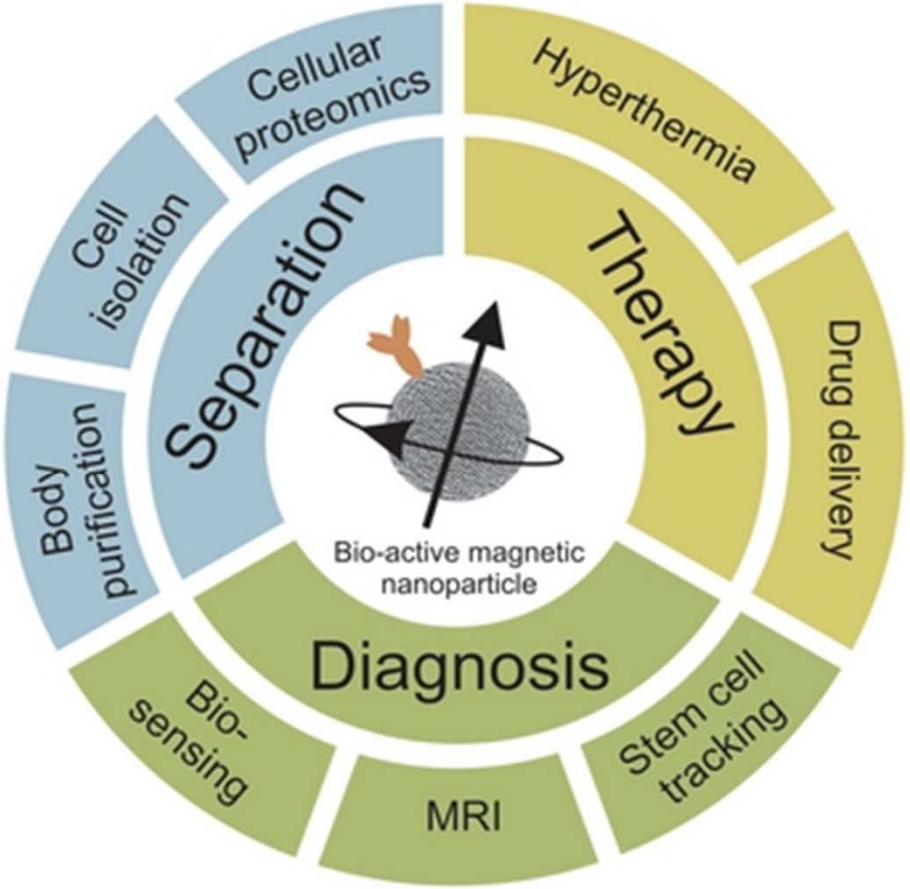
SPION as Multifunctional Contrast Agent for Molecular Imaging

H.Hofmann, Powder Technology Laboratory

Superparamagnetic Iron Oxide Nanoparticles (γ-Fe_2O_3) for medical application

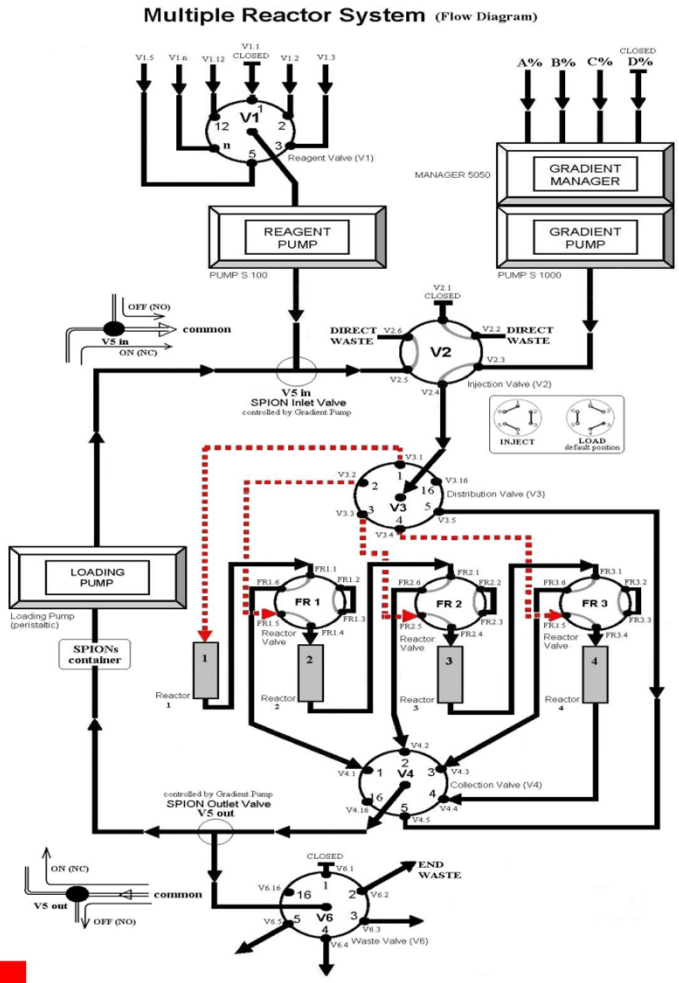






Device development for functionalisation (Lab scale and scale-up)

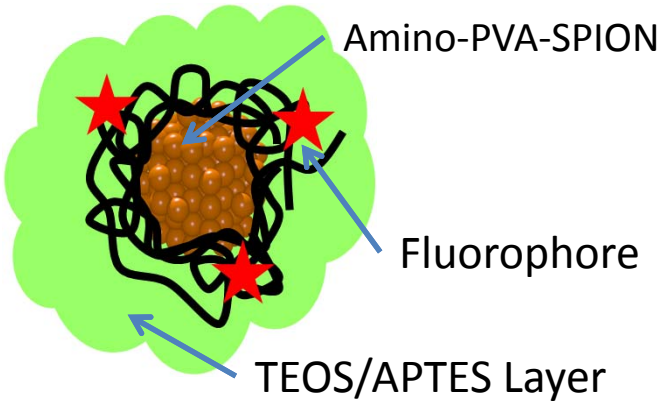
- All important unit operations are defined and integrated into the Labview programme for automated coating procedures
- Transfer of knowledge (i.e. magnetic reactor development and process) to industry

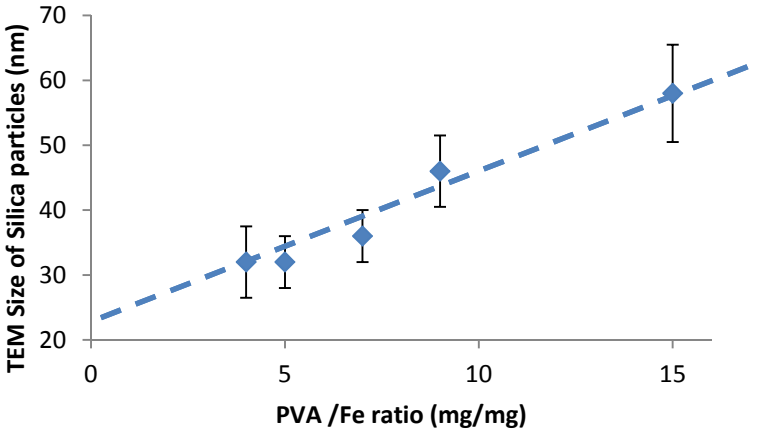


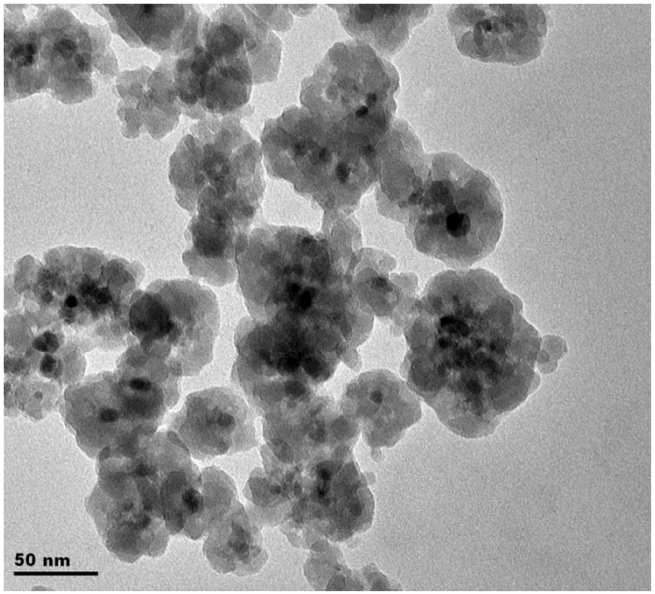
CSEM Reactor inegrated into the EPFL set-up

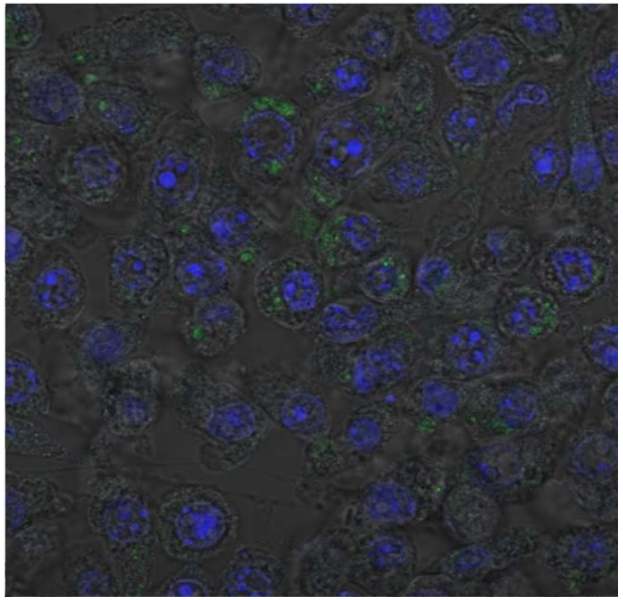
Multifunctional particles with controlled size

New method for the synthesis of SPION beads, which could include also fluorophore, coated with amino-silane and PEG-biotin were developed for in vitro investigation



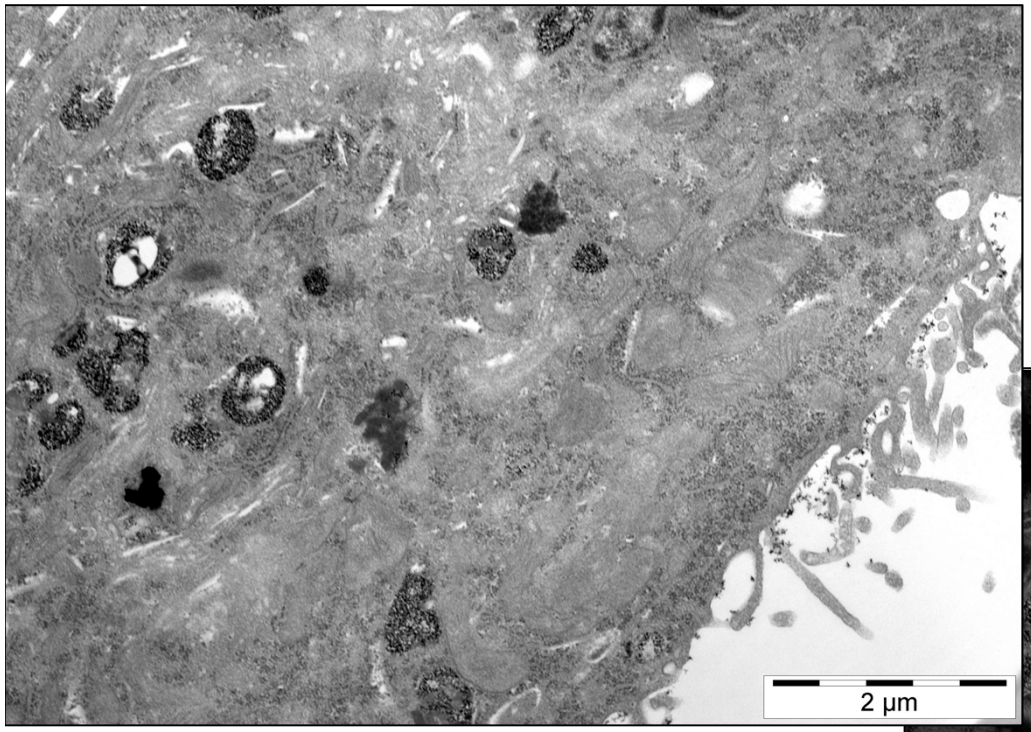




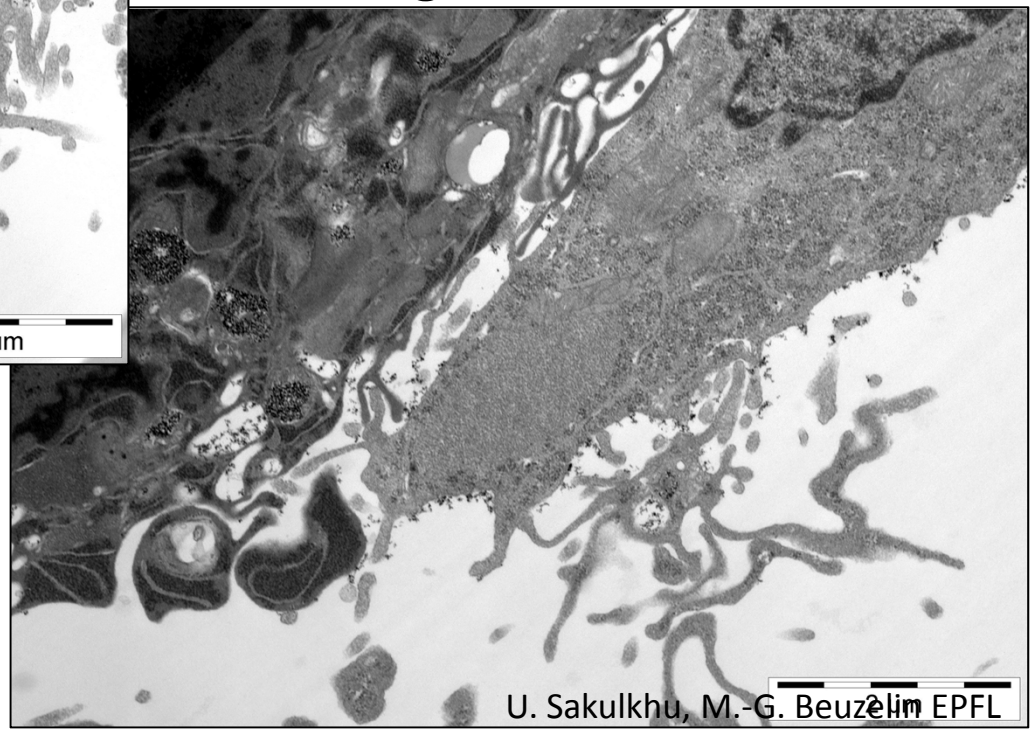


Particles developed by Lionel Maurizi

Cellular up-take I
HeLa +PVA/Amino-PVA coated SPiON



- 0.2mg/mL → pics
- 0.4mg/mL → less cells, flatten
- 0.8mg/mL few cells, debris.



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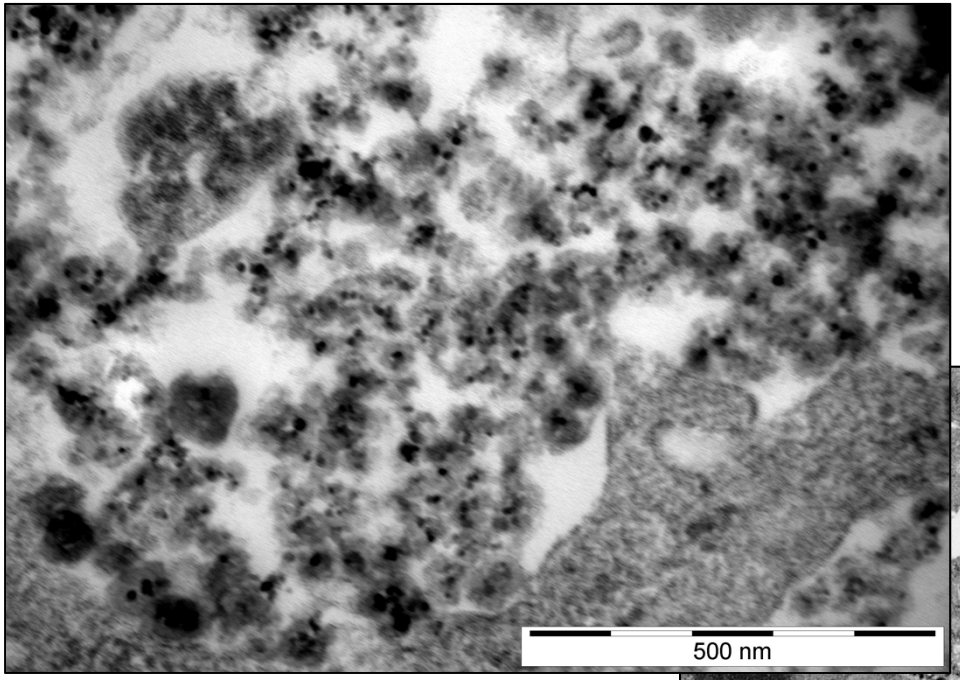
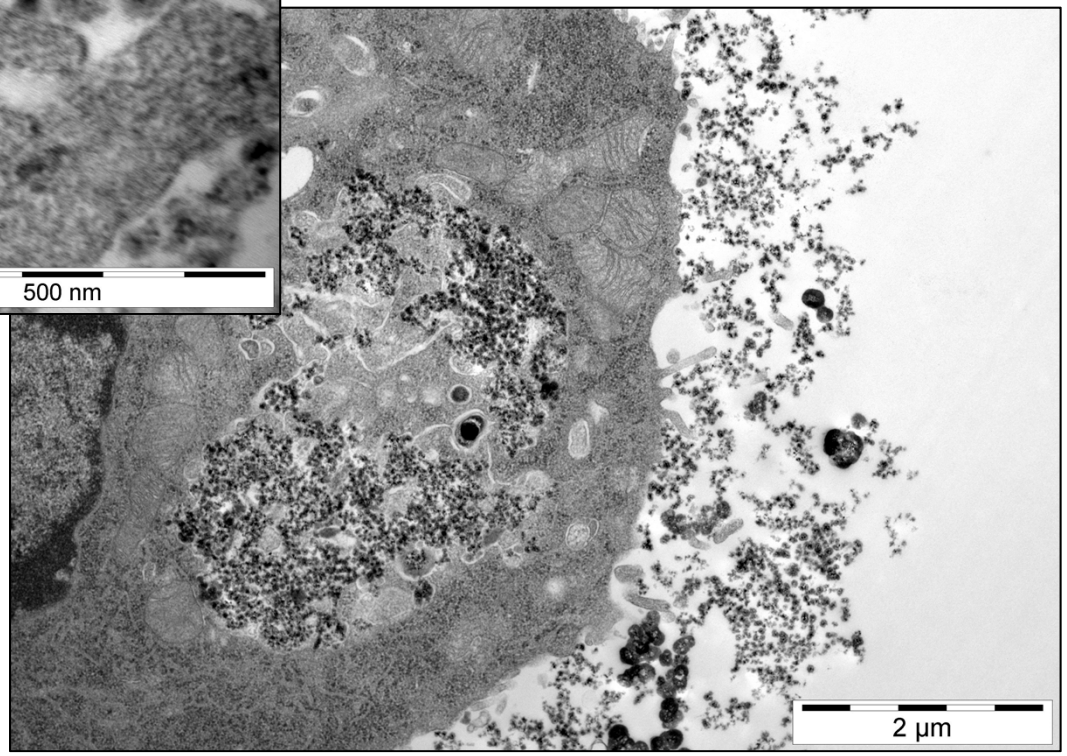
Cellular up-take II
RAW 264.7 + A-PVA-10%FITC-SPIONs-silica (0.4mg/mL)





Superparamagnetic Iron Oxide Particles

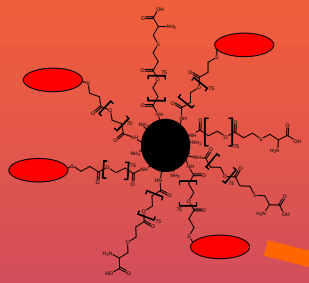
Research

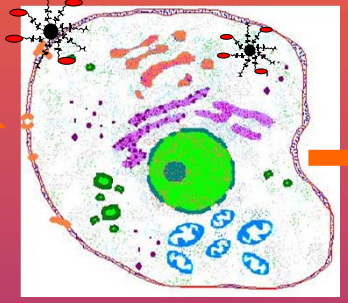
Protein identification
nanoESI-MS/MS

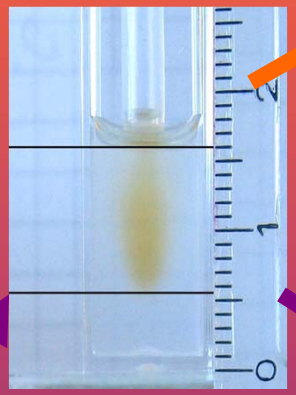
Particle library

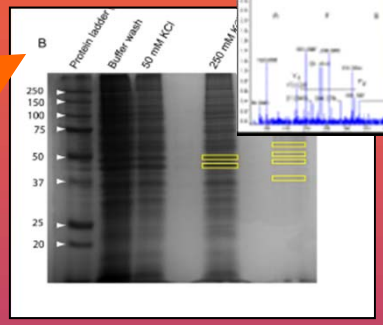
Spec. adsorption
at cell surfaces
organelles, ECM proteins

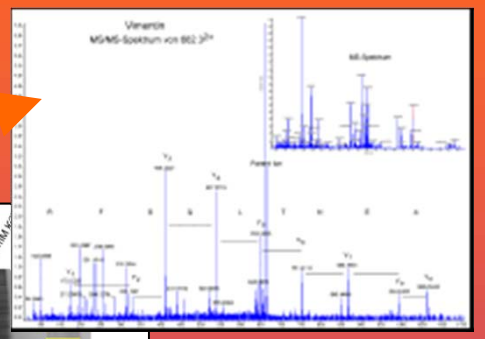
SDS-PAGE

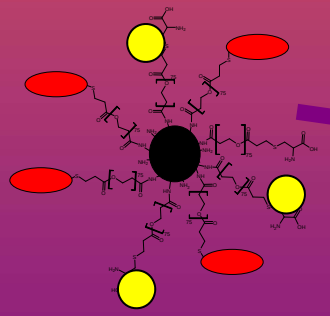


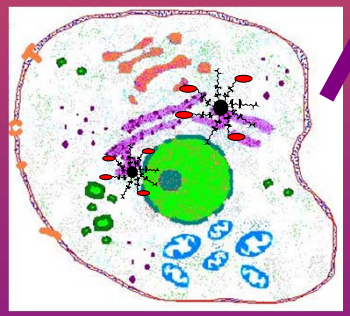




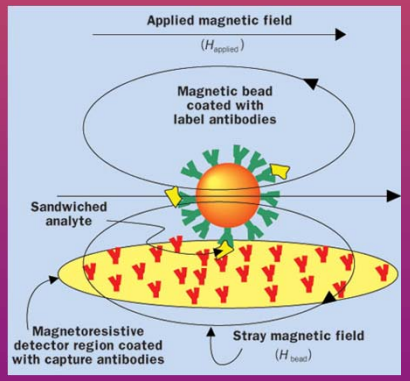








Mag separation
and concentration



Particle derivatized with specific antibodies

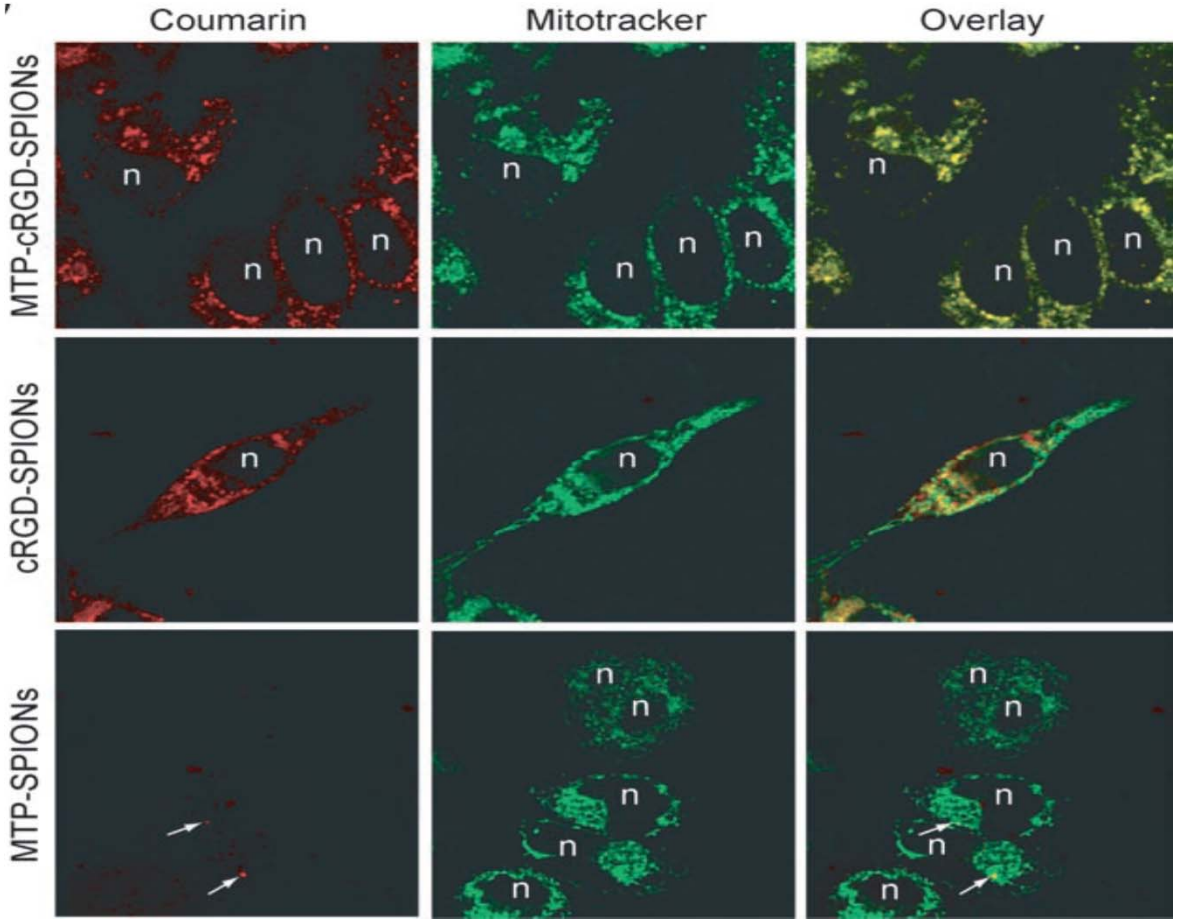
Diagnosis

Quantitative detection
Magnetic, ELISA

Targeting of organelles

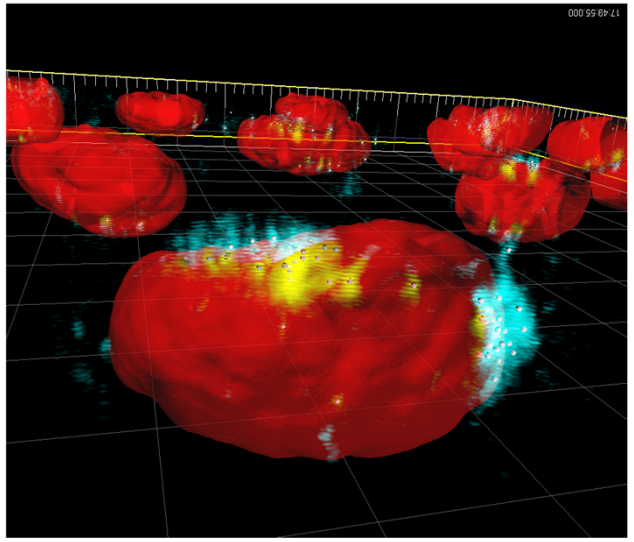
Mitochondria Targeting

SPION with Coumarin and Mitochondria targeting peptide



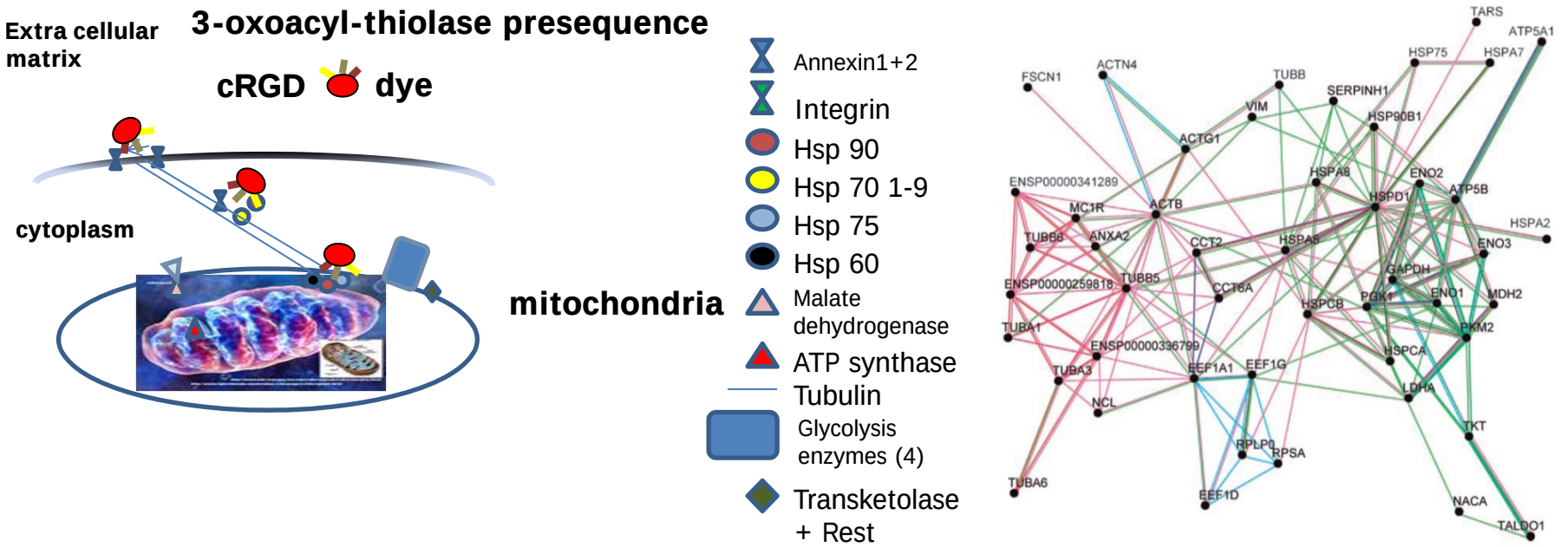
Nucleus Targeting

SPION with ALEXA and NTP QPSPSPTGC



Picture prepared from J.Salaklang

Protein Fishing



48 out of 58 proteins could be related to: Up-take mechanism, transport to mitochondria, mitochondria membrane, including energy related processes. Evidence view of the protein interaction network in STRING (J. Salaklang, B. Steitz, A. Fink-Petri and H.Hofmann)

Protein	Acession	Protein molecular weight (Da)	Theoritical Protein IEP	Core	organic shell (PVA)				Inorganic shell					Metalic shell	Protein bound NP
				Positive	Highly positive	Positive	Neutral	Negative	Negative		Positive		Negative	Negative	
				SPION	-NH2	-NH2/OH	OH	COOH	PVA/TEOS	TEOS	PVA/APTES	APTES	Ti	Au	
Alpha-2-HS-glycoprotein	P12763|FETUA_BOVIN	38419	5.26												11
Complement C3	Q2UVX4|CO3_BOVIN	187252	6.41												10
Apolipoprotein A-I	P15497|APOA1_BOVIN	30276	5.71												10
Fibrinogen alpha chain	P02672|FIBA_BOVIN	67012	6.73												9
Alpha-1-antiproteinase	P34955|A1AT_BOVIN	46104	6.05												9
Actin, cytoplasmic 2	P63258|ACTG_BOVIN	41793	5.31												9
Actin, cytoplasmic 1	P60712|ACTB_BOVIN	41737	5.29												9
Apolipoprotein E	Q03247|APOE_BOVIN	35980	5.55												9
Serum albumin	P02769|ALBU_BOVIN	69294	5.82												8
Pigment epithelium-derived factor	Q95121|PEDF_BOVIN	46229	6.57												8
Hemoglobin subunit alpha	P01966|HBA_BOVIN	15184	8.07												8
Complement factor B	P81187|CFAB_BOVIN	85366	7.87												8
Alpha-2-macroglobulin	Q7SIH1|A2MG_BOVIN	167575	5.71												7
Prothrombin	P00735|THRB_BOVIN	70506	5.97												7
Kininogen-2	P01045|KNG2_BOVIN	68710	6.09												7
Alpha-2-antiplasmin	P28800|A2AP_BOVIN	54711	5.45												7
Hemoglobin fetal subunit beta	P02081|HBBF_BOVIN	15859	6.51												7
Clusterin	P17697|CLUS_BOVIN	51114	5.73												6
Hemoglobin subunit beta	P02070|HBB_BOVIN	15954	7.02												6
Thrombospondin-1	Q28178|TSP1_BOVIN	129534	4.74												5
Inter-alpha-trypsin inhibitor heavy chain H4	Q3T052|ITIH4_BOVIN	101513	6.22												5
Inter-alpha-trypsin inhibitor heavy chain H3	P56652|ITIH3_BOVIN	99551	5.59												5
Gelsolin	Q3SX14|GELS_BOVIN	80731	5.54												5
Kininogen-1	P01044|KNG1_BOVIN	68890	6.14												5
Apolipoprotein A-IV	Q32PJ2|APOA4_BOVIN	43018	5.3												5
Actin, alpha skeletal muscle	P68138|ACTS_BOVIN	42051	5.23												5
Actin, alpha cardiac muscle 1	Q3ZC07|ACTC_BOVIN	42019	5.23												5
Actin, aortic smooth muscle	P62739|ACTA_BOVIN	42009	5.24												5
Actin, gamma-enteric smooth muscle	Q5E9B5|ACTH_BOVIN	41877	5.31												5
Myosin-10	Q27991|MYH10_BOVIN	229097	5.43												4
Plasminogen	P06868|PLMN_BOVIN	91216	7.68												4
Heat shock protein HSP 90-alpha	Q76LV2|HS90A_BOVIN	84731	4.92												4
Apolipoprotein A-II	P81644|APOA2_BOVIN	11202	7.8												4
Coagulation factor V	Q28107|FA5_BOVIN	248981	5.53												3
Heat shock protein HSP 90-beta	Q76LV1|HS90B_BOVIN	83253	4.96												3
78 kDa glucose-regulated protein	Q0VCX2|GRP78_BOVIN	72400	5.07												3
Tetranectin	Q2KIS7|TETN_BOVIN	22144	5.47												3

High
Middle
Low
None

U. Sakulkhu, EPFL

In vitro tox : Results summary (Charité)

- **Whole blood survival assay**

no short-term general toxicity of PVA SPIONs at concentrations less than 1000µg/ml on the different white blood cell populations BUT dose-dependent cell activation in terms of pro-inflammatory cytokine secretion

- **Survival and activation of isolated CD14+ Monocytes**

no significant effects of PVA SPIONs at concentrations less than 1000µg/ml on survival

- **Apoptosis of isolated CD4+ T cells**

no significant effects of PVA SPIONs at concentrations less than 1000µg/ml on caspase-3/7-activity

- **Proliferation and Activation (CD25) of isolated CD4+ T cells**

no significant effects of PVA SPIONs at concentrations less than 1000µg/ml on T cell proliferation and activation

- **ATP levels of isolated CD4+ T cells**

no significant effects of PVA SPIONs at concentrations less than 1000µg/ml on T cell ATP levels

- **SPION uptake and survival of MSCs**

PVA-SPIONs are stored by hMSCs in intracellular vesicles and do not significantly affect proliferation and metabolic activity of hMSCs *in vitro.*

EPFL

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

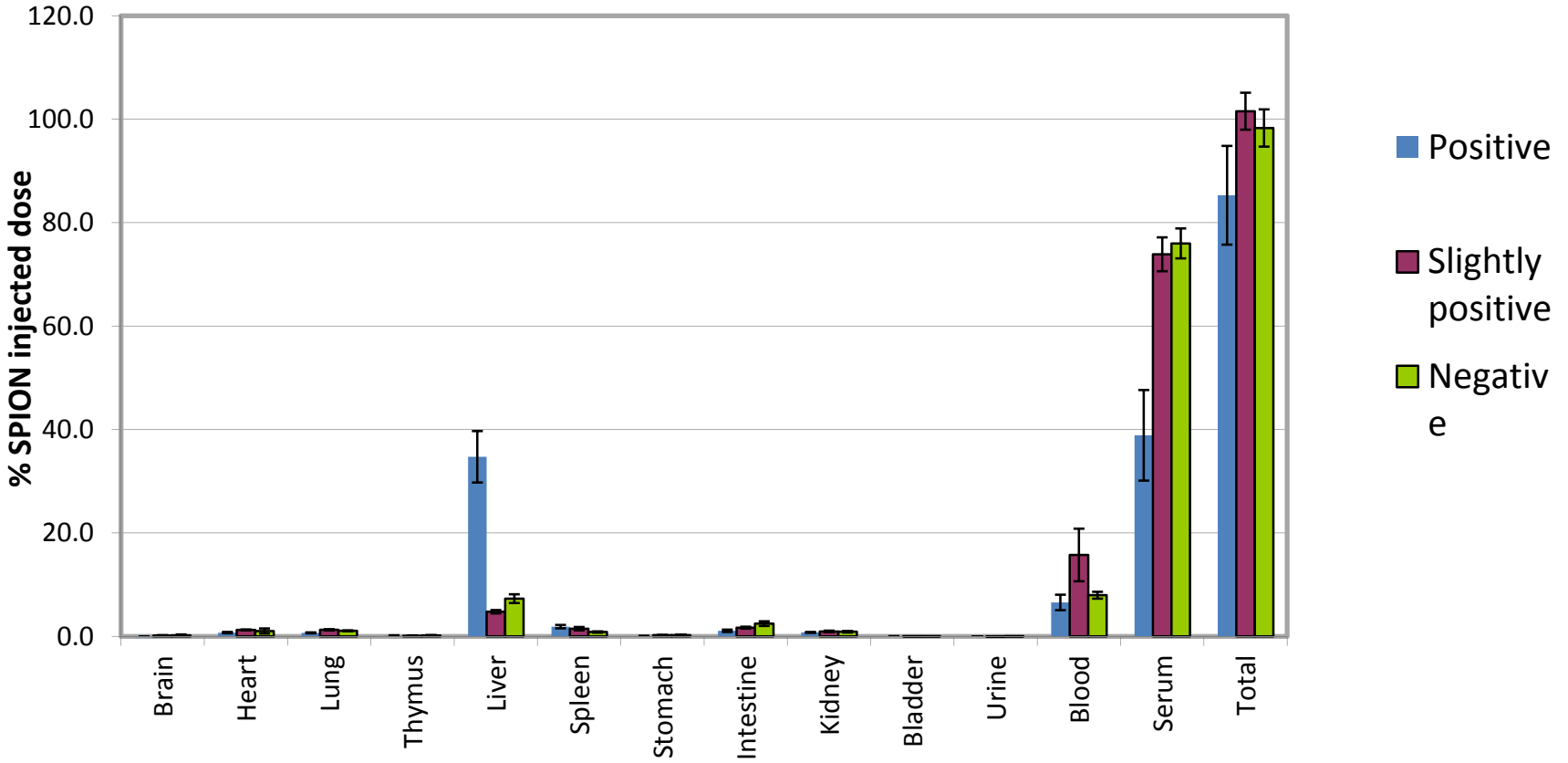
NanoDiaRA
Particles, Molecules & Cells · Diagnosis in-vitro & in-vivo · Rheumatoid Arthritis & Osteoarthritis

Extended Acute Toxicity: Results

- No mortality, no clinical signs
- No treatment-related effects on bw, bw-gain, food consumption
- aPVA: effects on clinical pathology
- aPVA-SPION
  - No mutagenic in Ames test and in vitro MNT (not shown)
  - No relevant toxicity in rats after a single i.v. application
  - Clear detection of high iron levels in liver and spleen of rats treated with SPION (main kill and recovery)

Biodistribution (rat)

3 different charged nanoparticles at 15 min post-injection

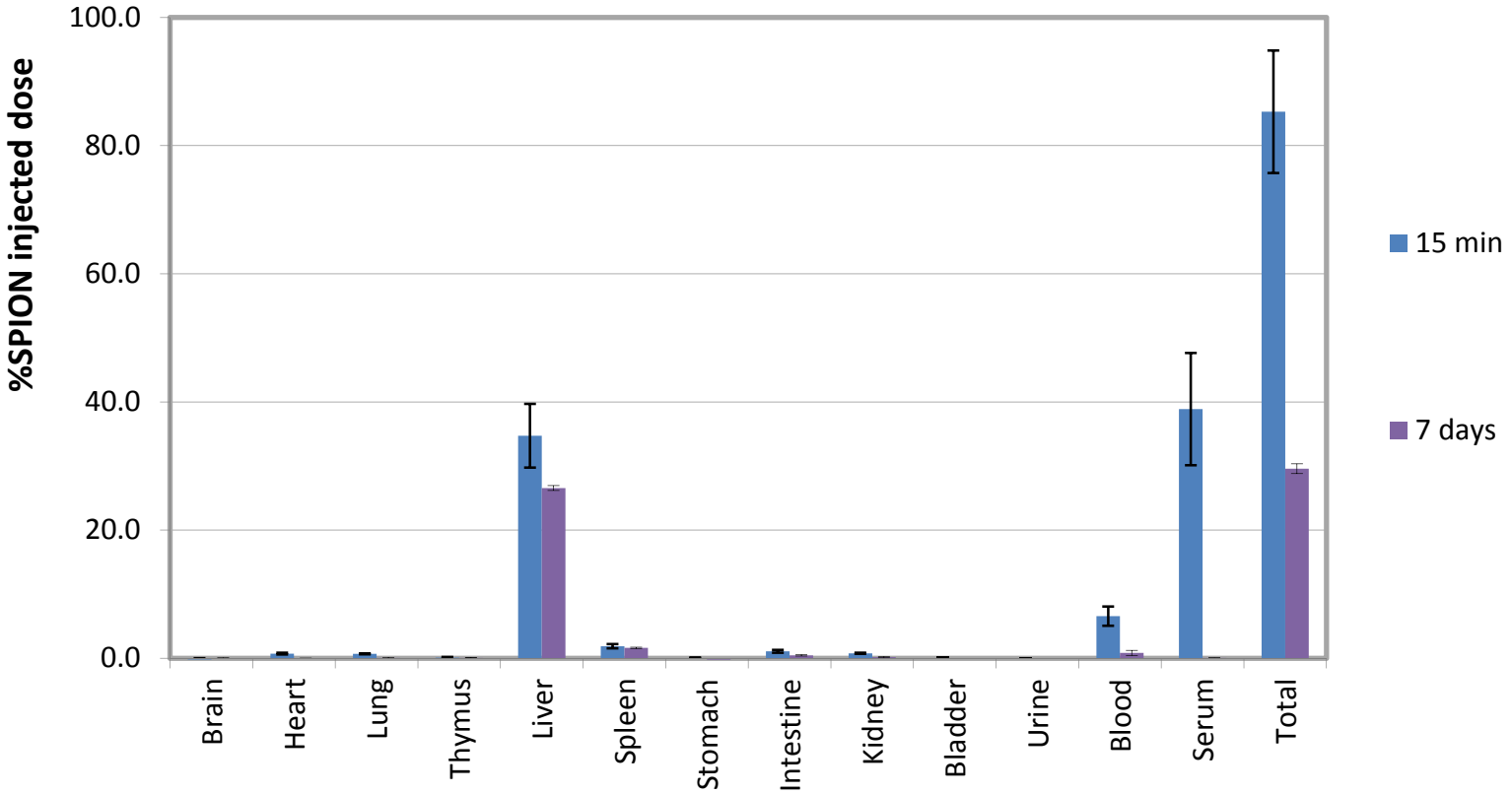


- Almost 100% of SPION injected dose were recovered.
- Nanoparticles are mainly found in Liver and Serum.
- Neutral and negative have similar behaviour.

Merck-Serono
L.Maurizi, EPFL

Biodistribution II

Positively charged nanoparticle at 15 min and 7 days post-injection

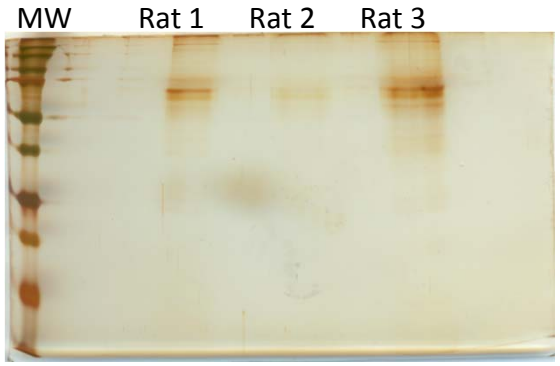


- At 7 days post-injection
  - 30% of SPION injected dose was detected
  - 90% of detected SPION located in Liver and SPION was eliminated from blood.

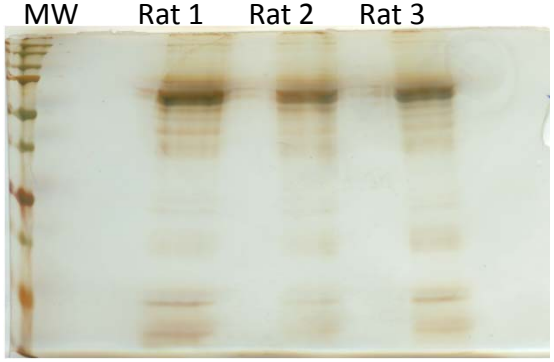
Merck-Serono
L.Maurizi, EPFL

Protein corona
SPION in rat

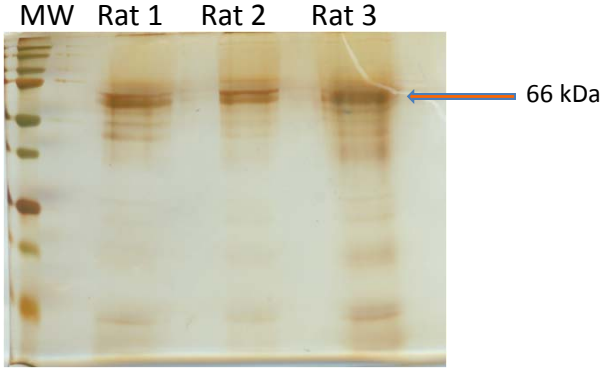
Positively charge NP at 15 min



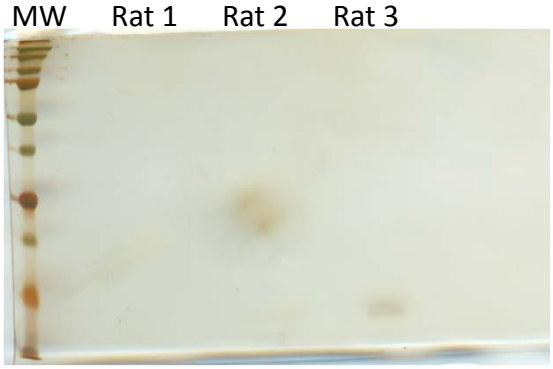
Neutral NP at 15 min



Negatively charged NP at 15 min



Positively charged NP at 7 days



- Common protein at 66 kDa
- Neutral and Negatively charged nanoparticles share the similar pattern of protein adsorption.
- From the intensity of the bands, detected proteins are correlated to SPION amount in serum.

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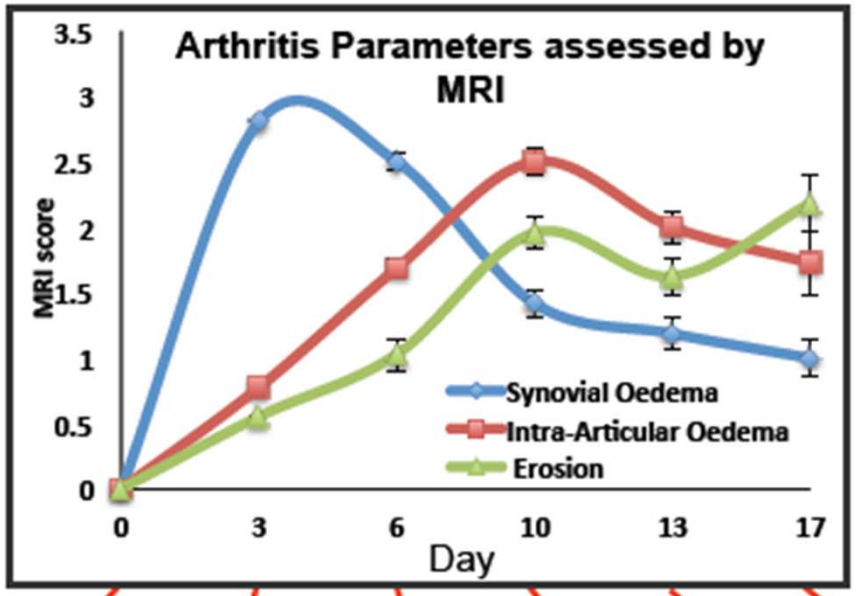
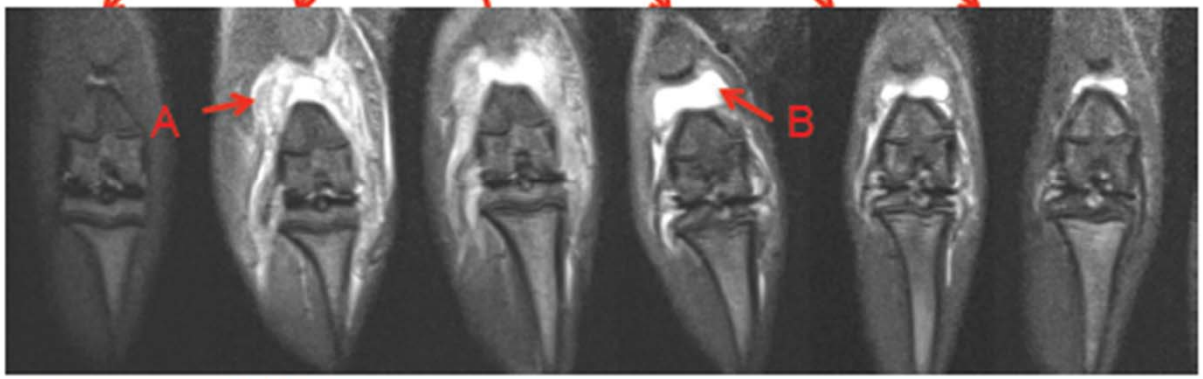
Characterization of Rat AIA Model using MRI

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

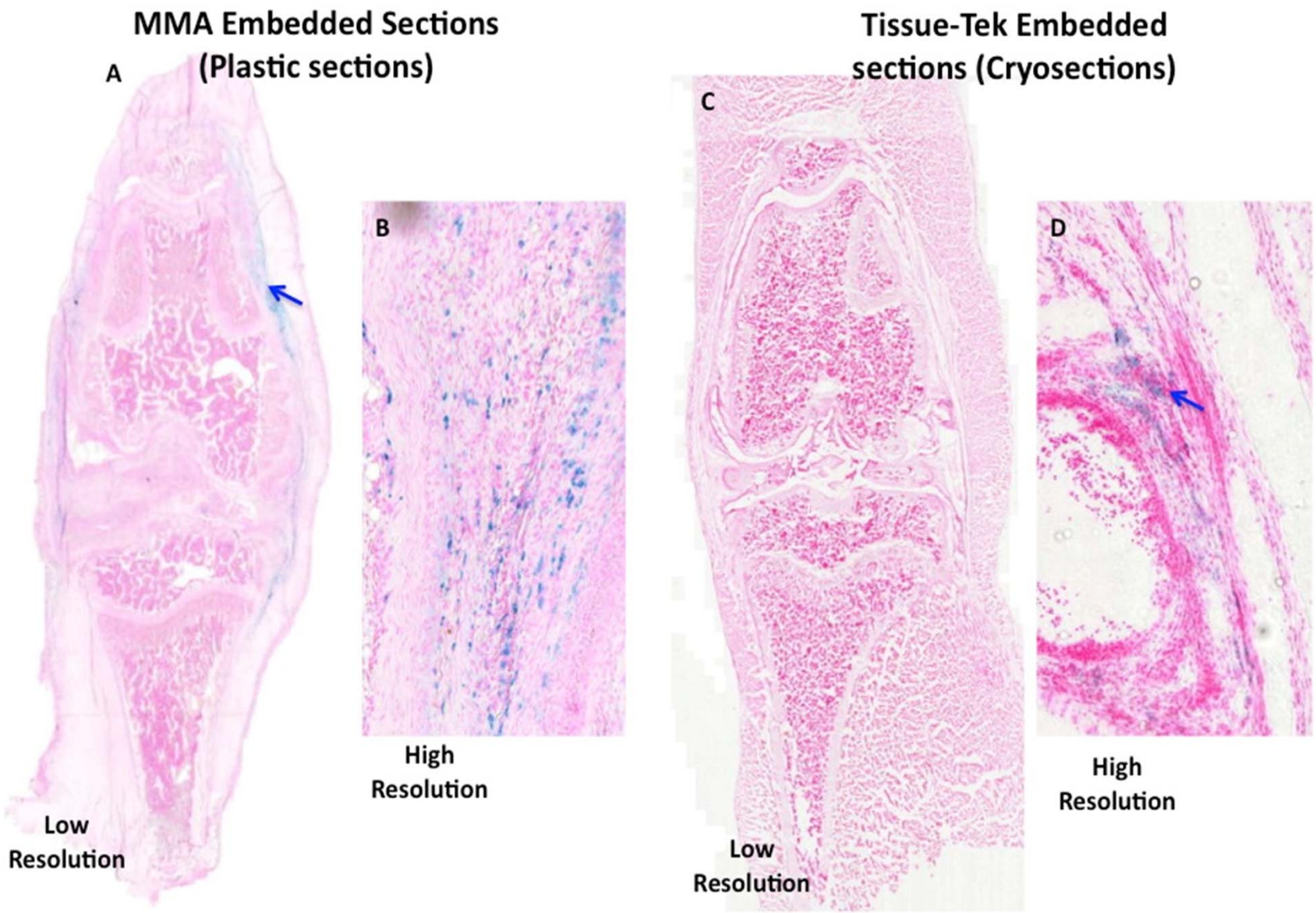


- Inflammation has two components: synovial oedema and intra-articular oedema (effusion) peaking at day 3 and 6 respectively and regressing after 2 weeks



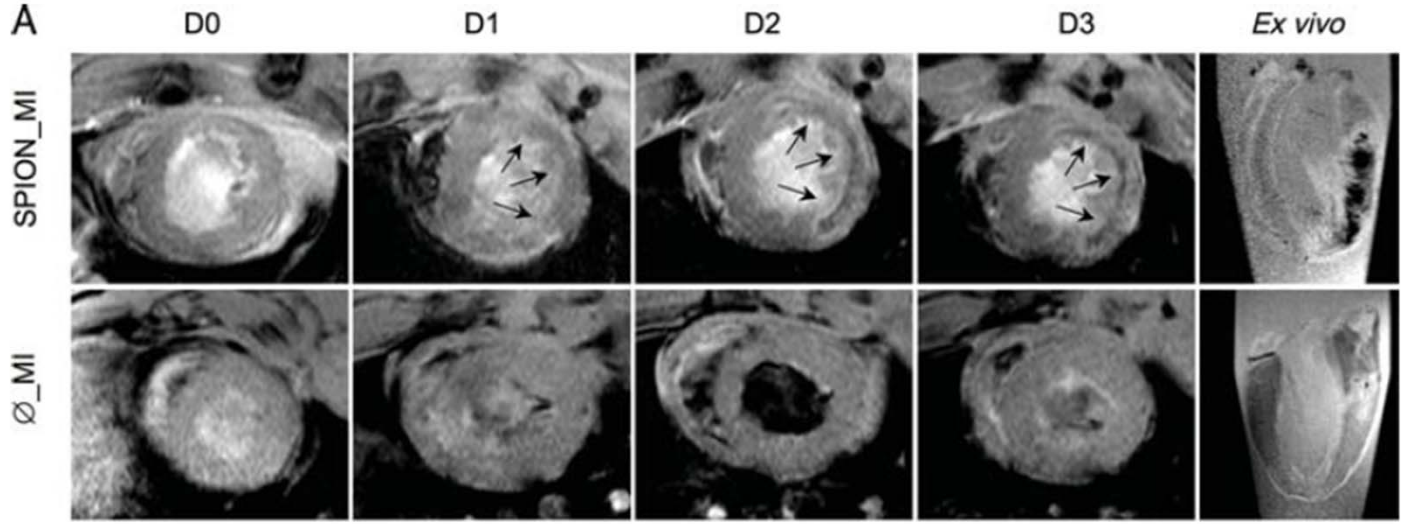
MR T2 STIR images showing: (A) synovial and (B) intra-articular oedema

Histology


MMA Embedded Sections
(Plastic sections)
A
B
Low
Resolution
High
Resolution
Tissue-Tek Embedded
sections (Cryosections)
C
D
Low
Resolution
High
Resolution

In-vivo Monocyte Targeting

injection of fluorescent SPION (10 mg/kg) 3 days before the ischaemia–reperfusion.



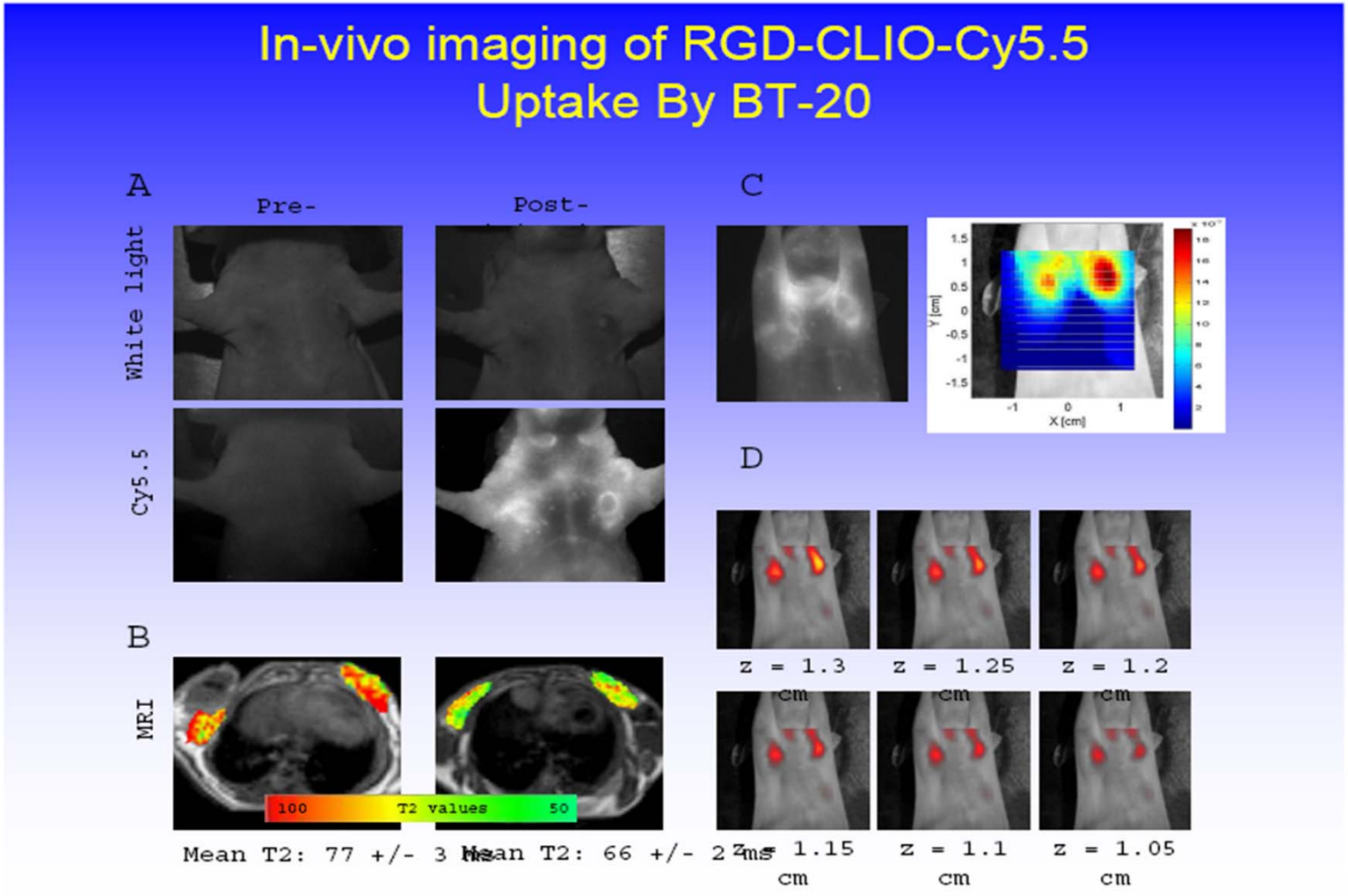
With SPION

Without SPION

In vivo magnetic resonance imaging of the infarcted groups. The first line corresponds to a representative rat of the SPION_MI group and clearly shows the appearance over time [Day (D) 0 to D3] of a hypointense (black) signal in the myocardial infarction area (arrows). The second line corresponds to a representative rat of the Ø_MI group and does not show any hypointense signal.

X. Montet et al, European Heart Journal, 2009

In-vivo Molecular Imaging of Cancer Cells



Collaboration with X. Montet, HUG

Pros and Cons

- MRI application for liver diagnostic is FDA approved and in clinical use
- Applications in imaging, drug delivery, hyperthermia are in pre-clinical and clinical tests
- Biocompatibility approved
- Multifunctional particles allowing active diagnosis and therapeutic applications
- Methods for synthesis, surface modification established in industrial scale
- Toolbox for Theranostics
- Acceptance of nanotechnology based treatments by patients

- Behaviour of particles in the different organs not known in detail
- Particle-protein interaction still under investigation
- Clearing mechanism?
- Combination of diagnosis and therapy useful (**ELSI**)?
- Added value? Risk-Benefit balance not yet established.
- Economics, market, health assurances?

Next steps

1. More detailed understanding of the protein adsorption on nanoparticles and their influence on the behavior in blood and tissue (*in vivo*)

2. Combine methods and results with results fromtoxicity research with engineered nanoparticle

3. GMP conform fabrication of particles for research (high reproducibility, particle library, standardized particles and coatings)

4. Bring the “Nanoregulation” forward to facilitate translational research

5. Pre-clinical development and tests

6. Combine diagnosis with therapy

Thank you for your attention

EPFL-LTP:
BERNAU Vianney , BEUZELIN OLLIVIER Marie-Gabrielle,
COULLEREZ Géraldine , MAURIZI Lionel,
SAKULKHU Usawadee

Nanodiara FP7project EU Framework 7 Programme,
contract no NMP4-LA-2009-228929