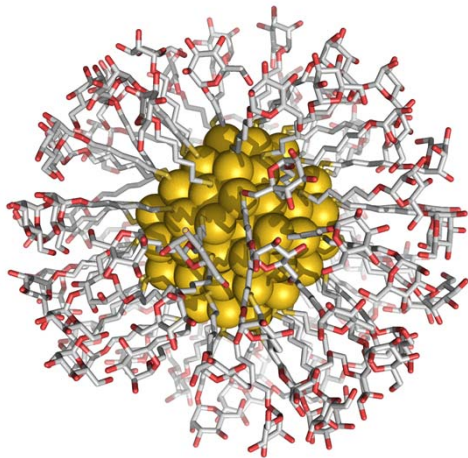




CIC biomagUNE
Biomaterialetako Ikerkuntza Kooperatiboko Zentroa
Centro de Investigación Cooperativa en Biomateriales
<http://www.cicbiomagune.es>

ciber-bbn
Networking Research Centre on Bioengineering,
Biomaterials and Nanomedicine
www.ciber-bbn.es



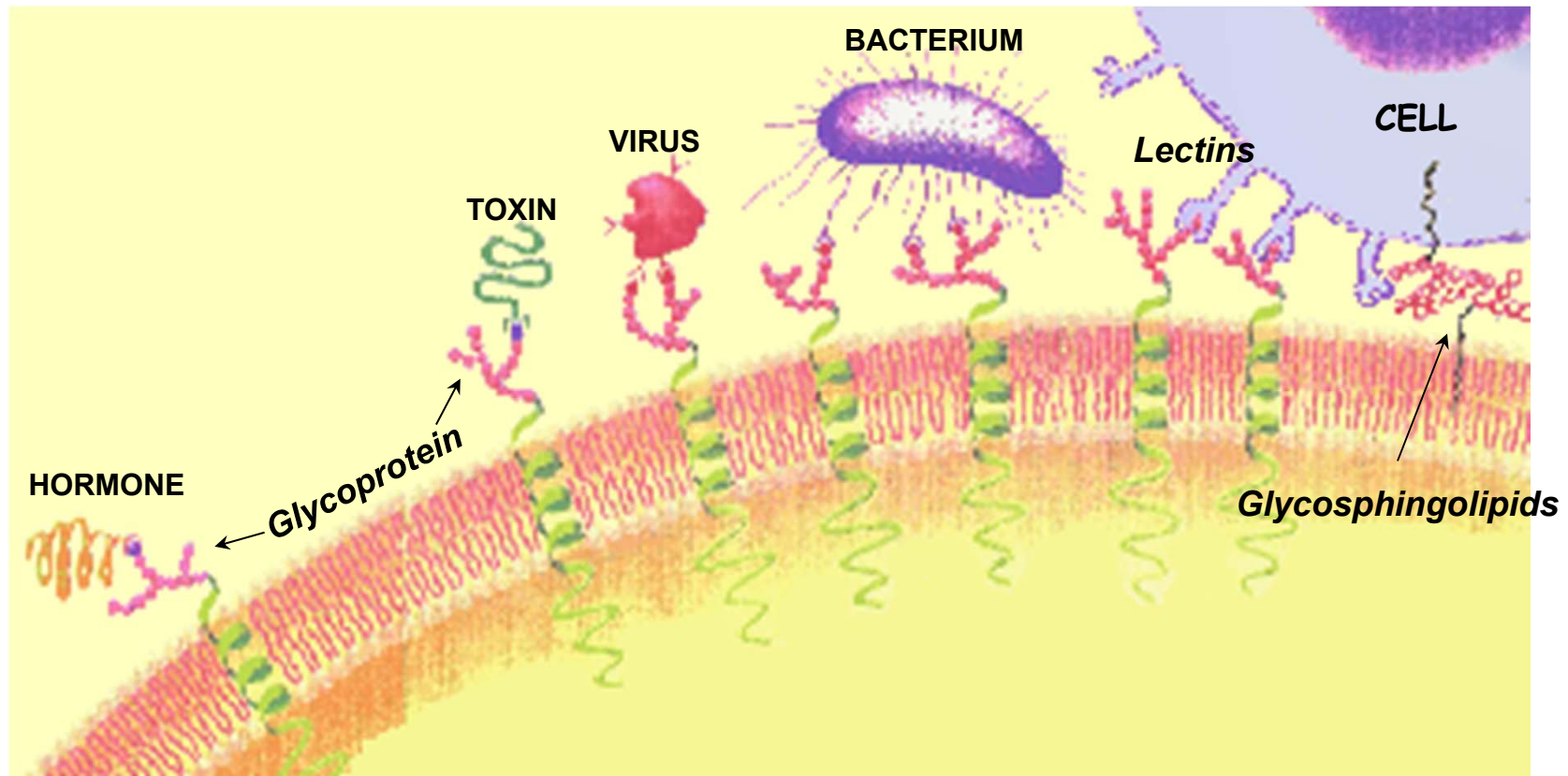
1.6 nm mannose-GNPs

Glyco-NanoParticles (GNPs) and their potential biomedical applications

International Summer School on Fluorescent Nano-Particles in Biomedicine
XIX Escuela Internacional de Verano Nicolás Cabrera
Madrid, Spain, 16th-20th July 2012

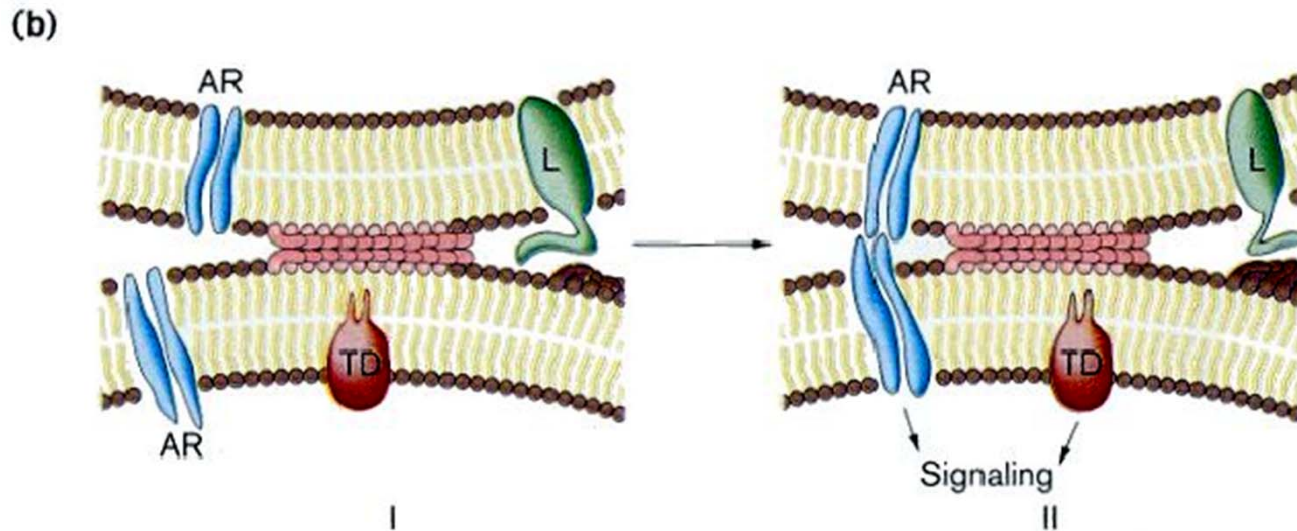
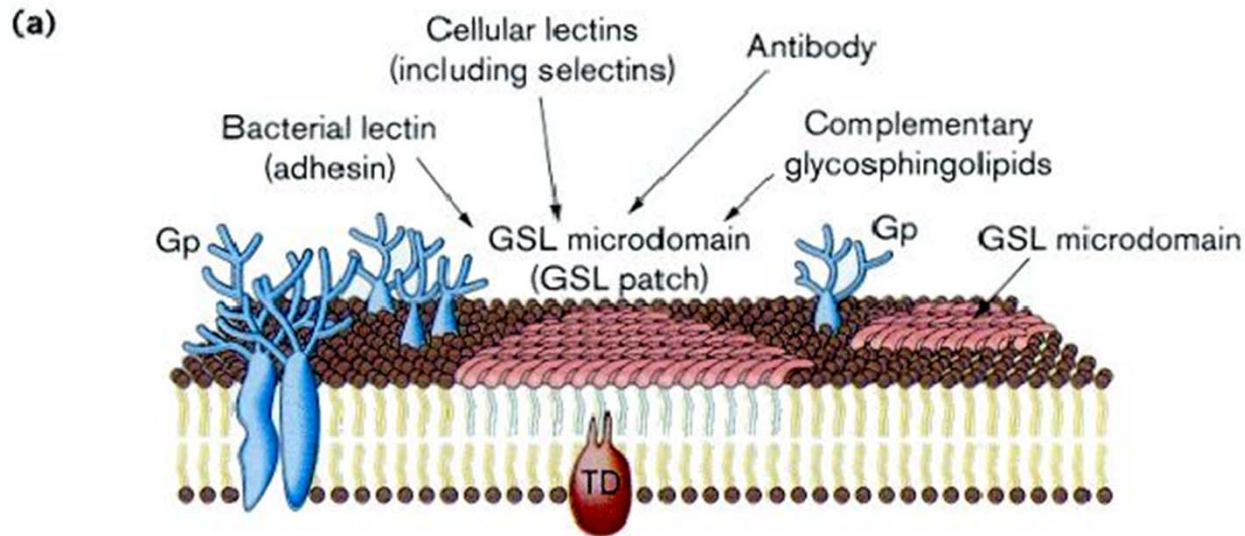
Soledad Penadés
Laboratory of GlycoNanotechnology
Biofunctional Nanomaterials Unit

Carbohydrates in Cell Recognition



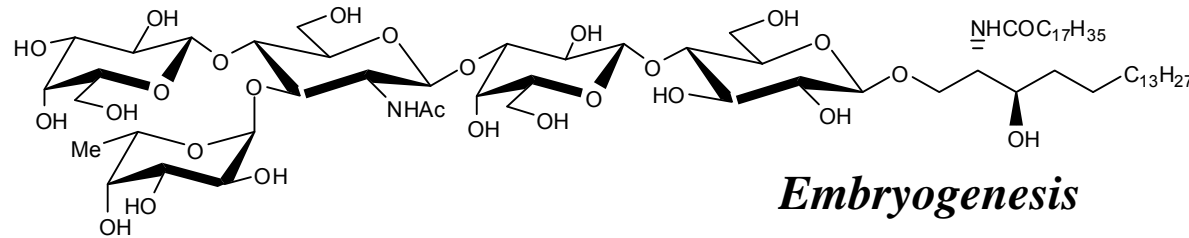
1993, *Scientific American*

Glycolipid-Glycolipid Interaction and Cellular Adhesion

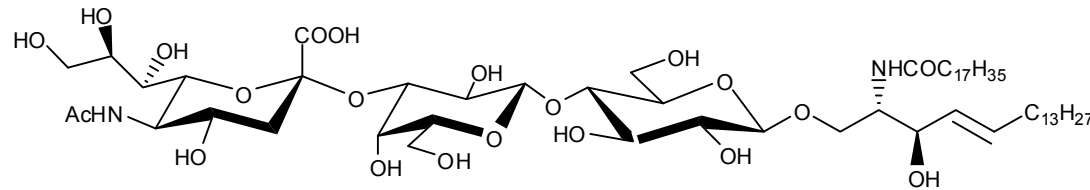


Glycolipids involved in carbohydrate self-recognition

Lewis X
(SSEA-1)

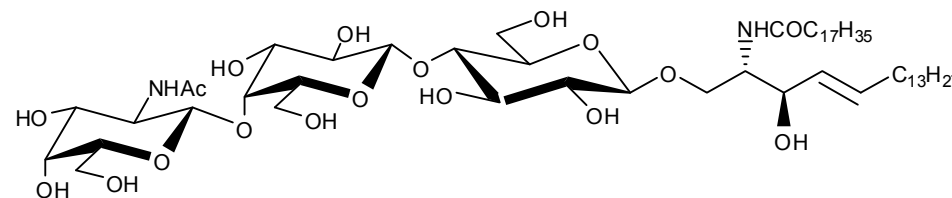


GM3



Metastasis

Gg3



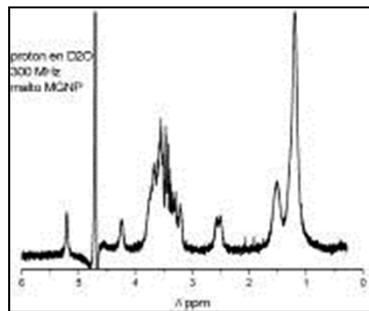
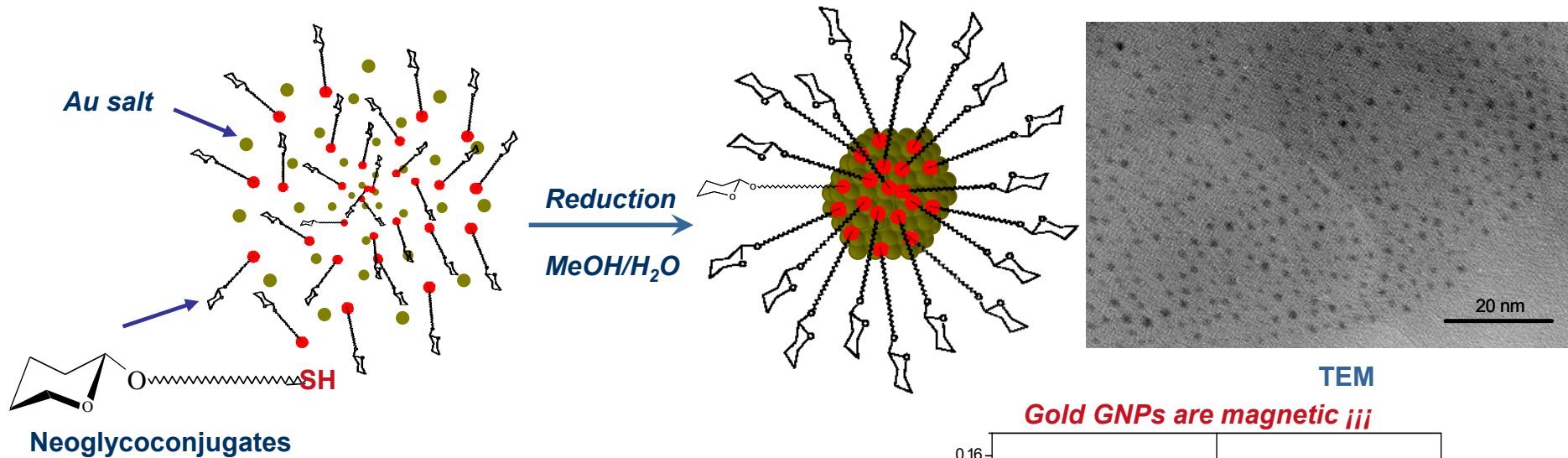
Glyconanotechnology

A chemical strategy at the interface Carbohydrate-Nanotechnology

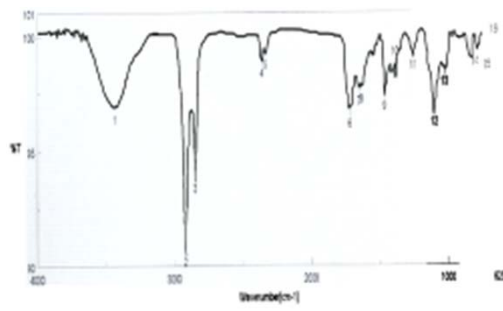
- To create polyvalent tools (SAMs and GNPs),
mimicking carbohydrate presentation at the cell
surface
- To study and intervene in carbohydrate-mediated
biological processes,
by means of biophysical techniques (AFM, TEM, SPR,..)

In situ formation of 1.8 nm glyconanoparticles

Inspired in Brust-Shiffrin method, *Chem Commun* 1994

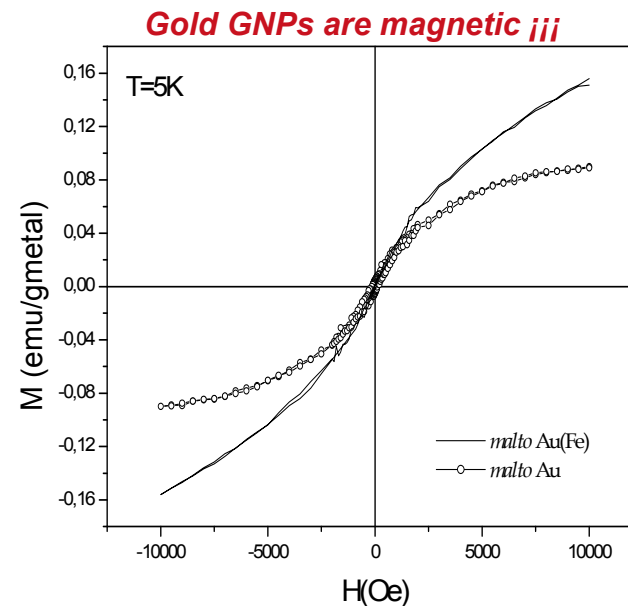


¹H-NMR



FT-IR

UV, ICP-AES, Elemental Analysis, MALDI-TOF

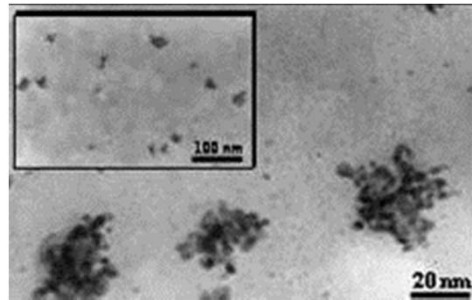


P. Crespo et al., Phys. Rev. Lett., 2004

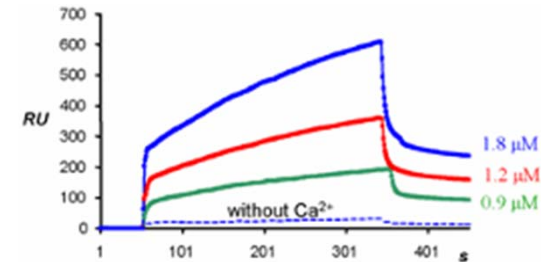
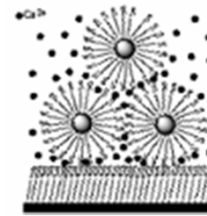
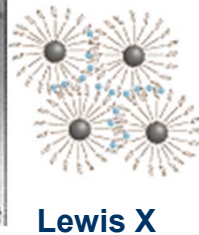
P. Crespo et al., Phys. Rev. Lett., 2006

J. M de la Fuente et al., J. Phys Chem. B 2006

Glyconanotechnology for carbohydrate-carbohydrate interactions



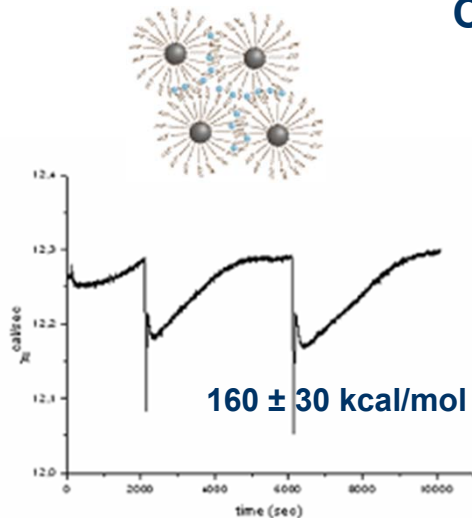
TEM, *Angew. Chem. Int. Ed.* 2001



SPR, *Angew. Chem. Int. Ed.* 2002

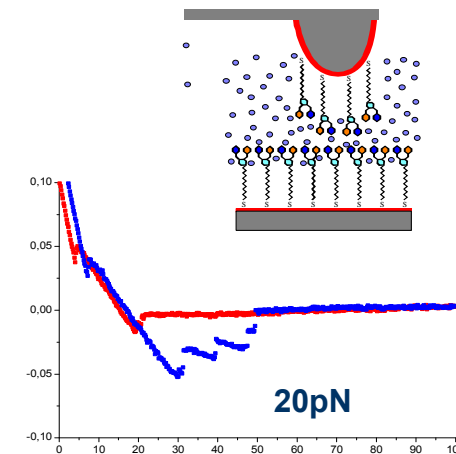
CARB-CARB INTERACTIONS IN WATER

Stabilizing, specific, multivalent and calcium dependent



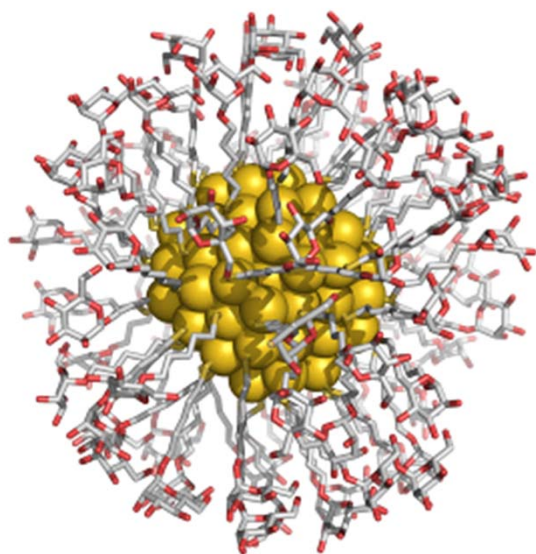
ITC, *J. Am. Chem. Soc.* 2005

**Lewis X self-interaction
A mechanism
for cell-cell adhesion**



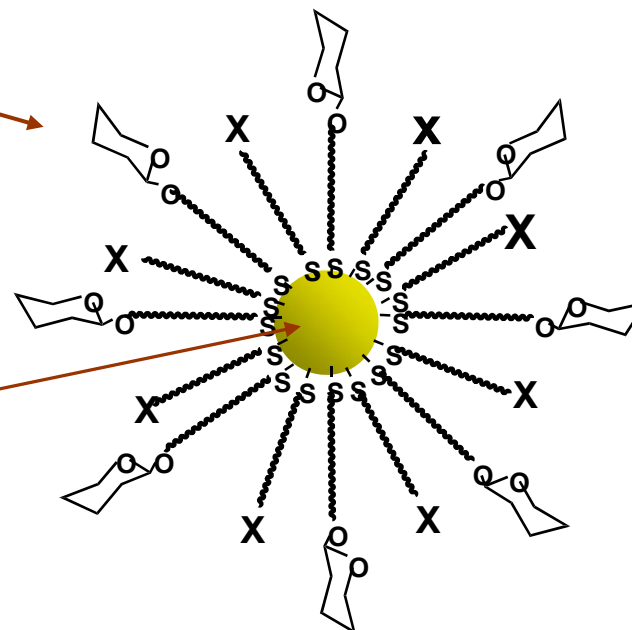
AFM, *Angew. Chem. Int. Ed.* 2001

From glyconanoparticles to nanocells



SAMs of antigenic oligosaccharides

Metal cluster
Au, Ag, AuFe,
AuCu, FeO@Au
CdS, CdSZn, CdSe,
CdTe



Gold Glyconanoparticles

- easy to prepare and purify
- exceptionally small (<2nm)
- water soluble, biocompatible
- highly polyvalent
- resistant to enzyme degradation
- not cytotoxic
- singular physical properties
(*permanent magnetism, fluorescent*)

Differing Density and Ligands

- control over density
- control over number of ligands
- up to four different ligands
- different spacer's length

X= DNA, RNA, lipids, peptides, proteins,
fluorescence probes,
biotin, anti-virales and drugs

Reviews: JM de la Fuente et al., *Glycoconjugate J.* (2004) 21, 149;
BBA-Gen Subjects (2006) 1760, 636;
I García et al., *Nanomedicine* (2011), 5, 777

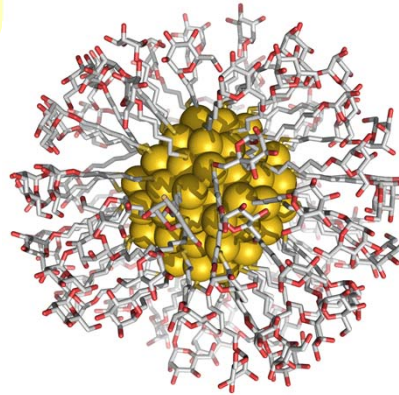
Applications of metallic glyco-nanoparticles

CHEMICAL GLYCOBIOLOGY

- Carb-Carb Interactions
- Carb-Protein Interactions

NANOMATERIALS DESIGN

- Microstructure Manipulation
- Quantum-Dot Bioconjugates
- Magnetic Bioconjugates



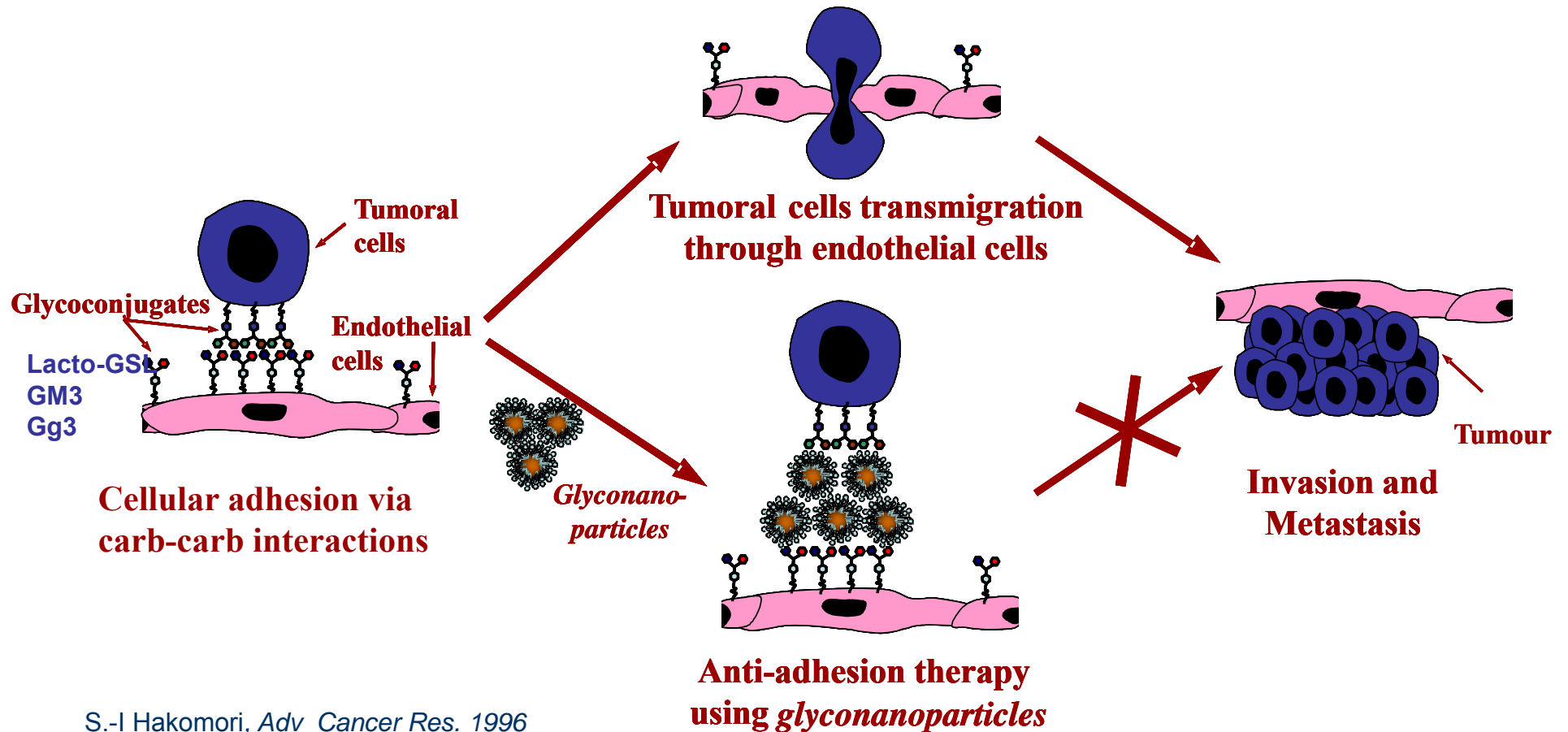
BIOTECHNOLOGY AND BIOMEDICINE

- Anti-Adhesion Agents
 - *Metastasis of melanoma cells*
 - *Microbicides against HIV*
- Synthetic Nanovaccines
- Multimodal Contrast Agents
 - *To image brain tumors in vivo*
 - *To label and track cells*

GlycoNanoParticles (GNPs) for biomedical applications

- Glyconanoparticles to study carbohydrate-mediate interactions
- Glyconanoparticles in biomedical problems
 - ▲ Anti-Adhesion Agents and Vaccines
 - *Metastasis of melanoma cells*
 - *HIV infection*
 - *Synthetic anti-carbohydrate vaccines*
 - ▲ Multimodal Contrast Agents for Molecular Imaging
 - Sugar/Gd-coated gold nanoparticles
 - Magnetic *glyco-ferrites@Au*

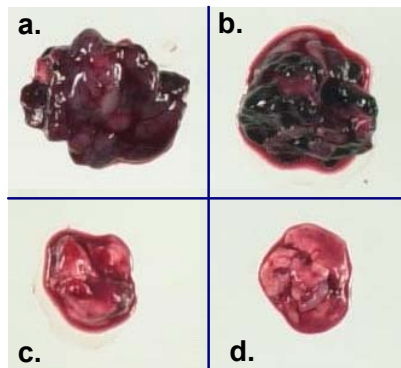
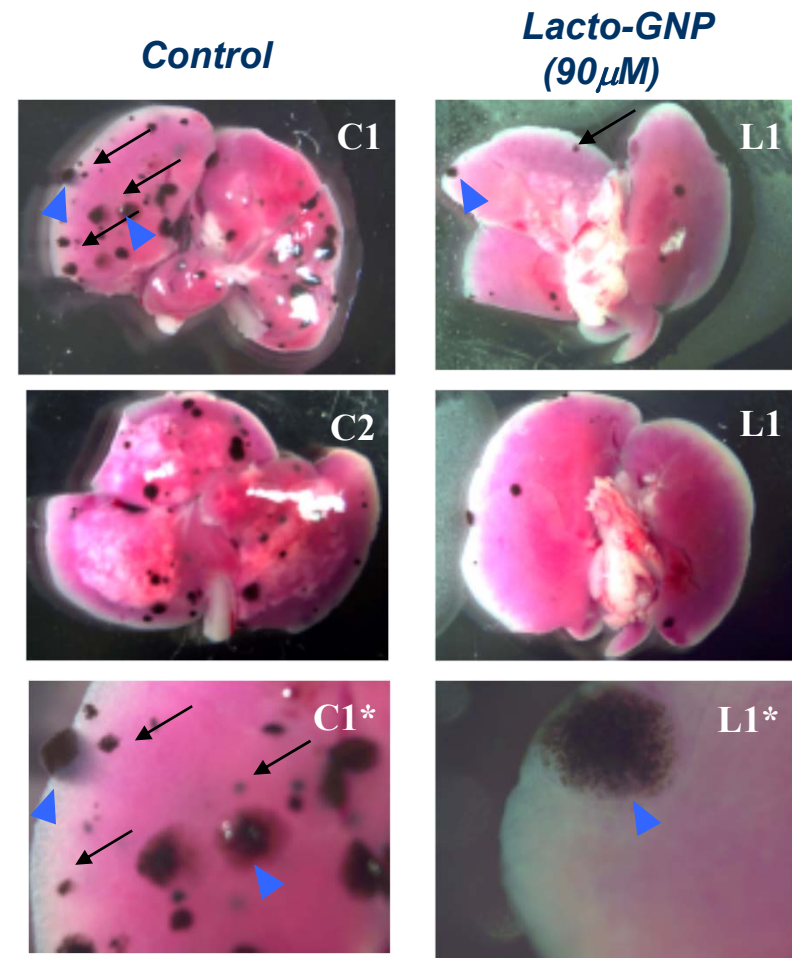
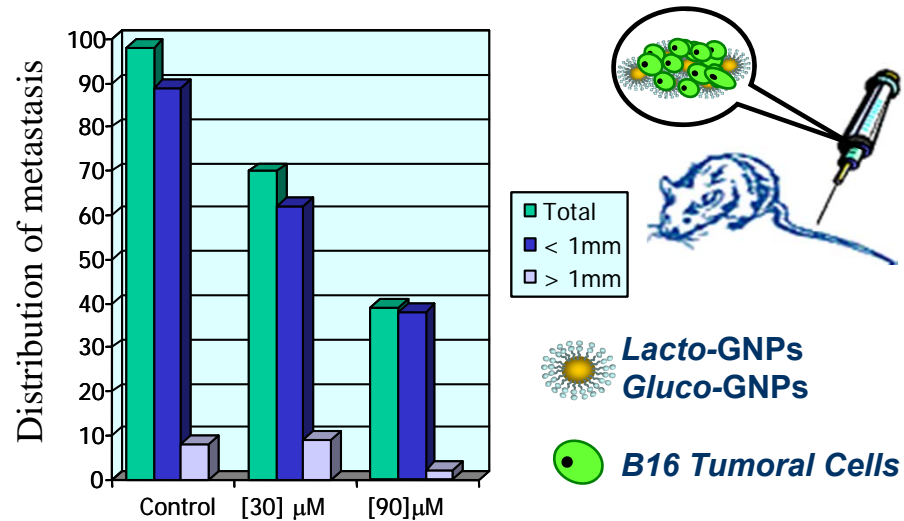
Glyconanoparticles as anti-adhesion agents in metastasis of melanoma



S.-I Hakomori, *Adv Cancer Res.* 1996

A.G. Barrientos, 2002

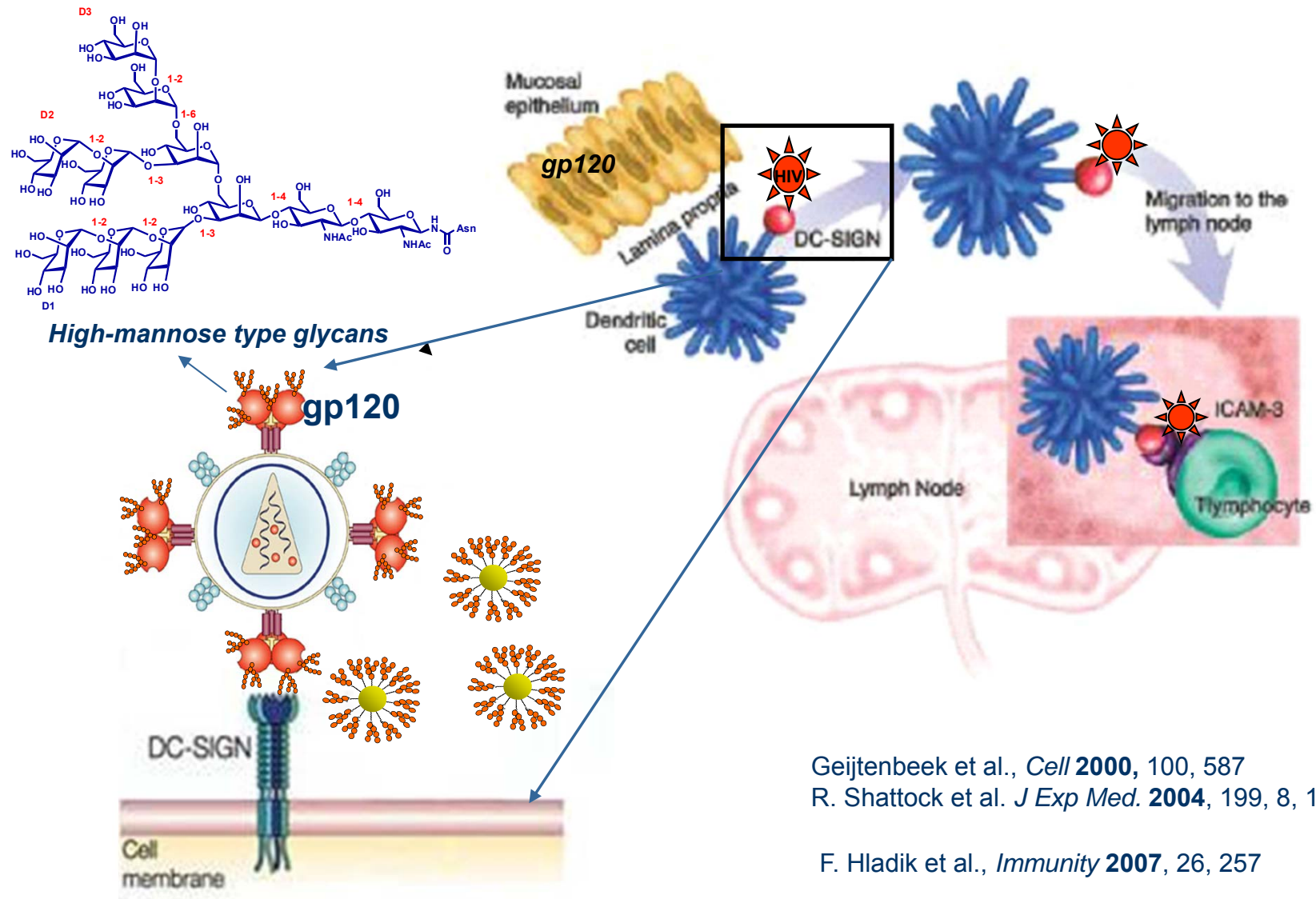
Glyconanoparticles in metastasis of melanoma



Lungs of mice coinjected with:

- B16 Cells
- B16 Cells + Gluco-GNPs
- Negative control
- B16 Cells + Lacto-GNPs

MannoGNPs in HIV trans-Infection of T-cells

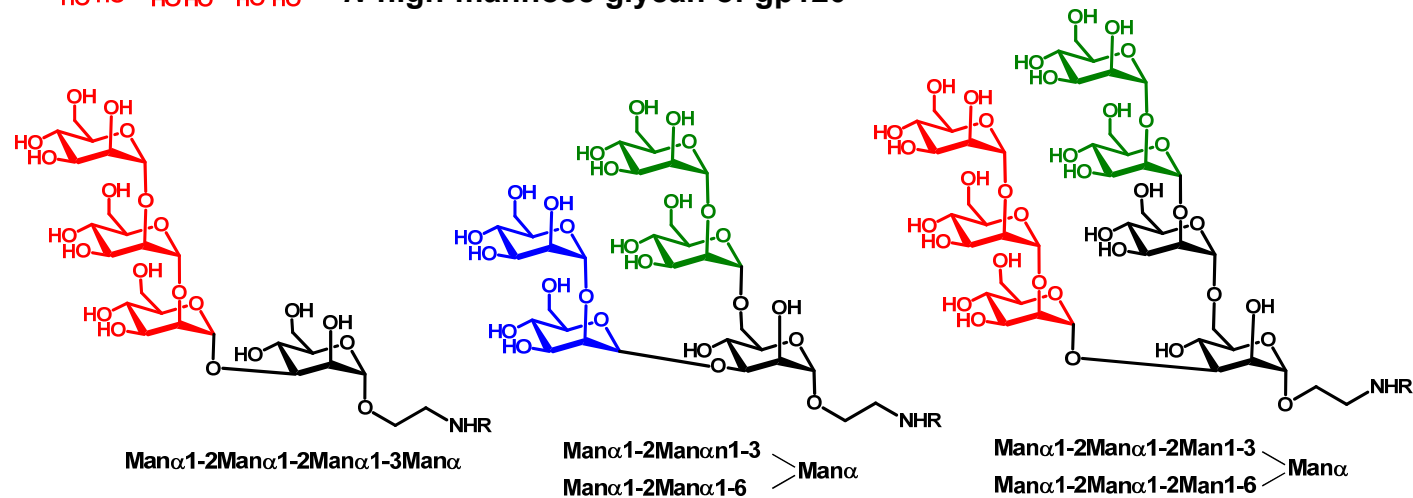
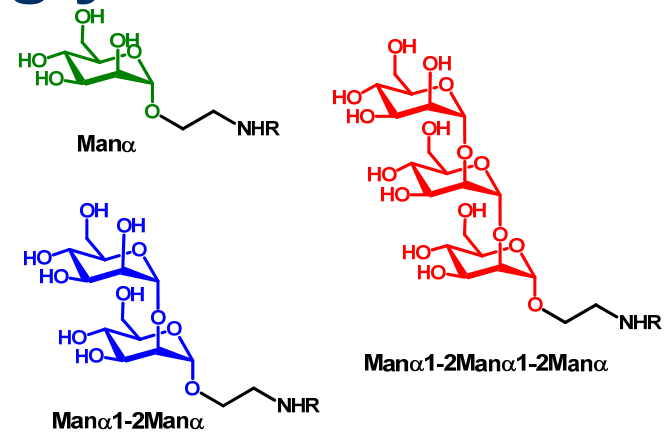
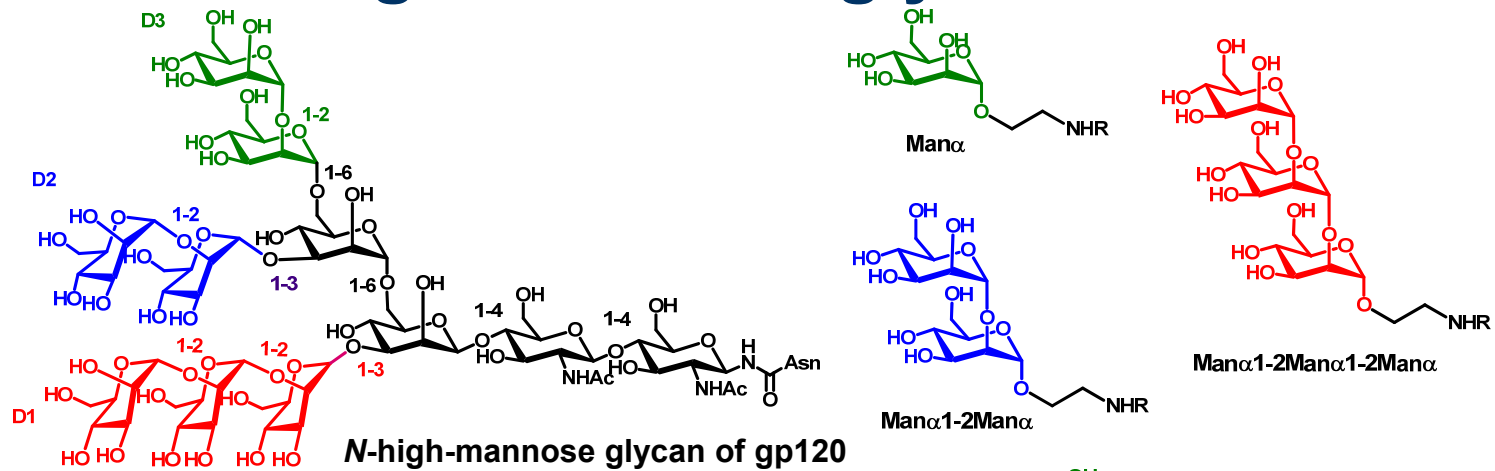


Geijtenbeek et al., *Cell* **2000**, 100, 587
 R. Shattock et al. *J Exp Med.* **2004**, 199, 8, 106

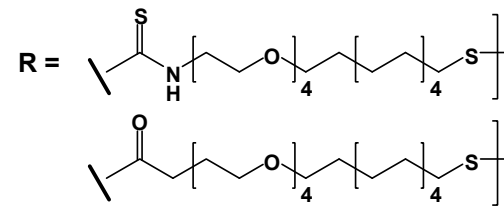
F. Hladik et al., *Immunity* **2007**, 26, 257

Feinberg et al., *Science* 2001
 Mitchell et al., *J Biol Chem* 2001

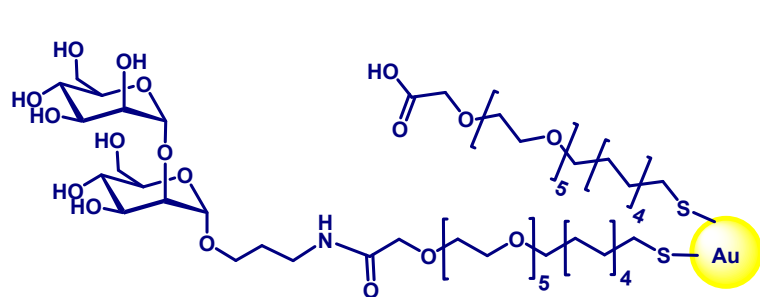
Selected oligomannosides of the HIVgp120 high-mannose glycans



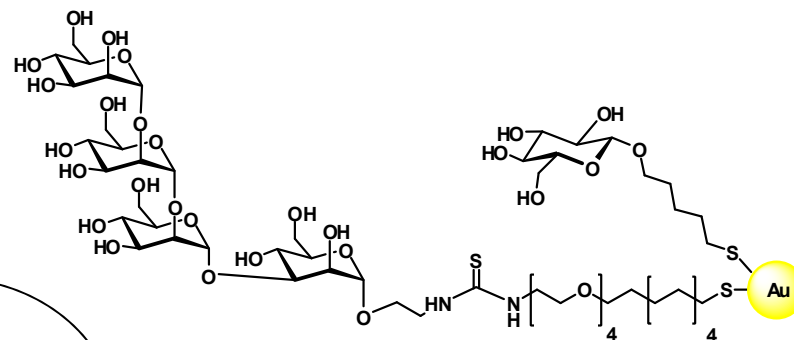
Wong CH et al. *Angew Chem.* 2004, 43, 1000



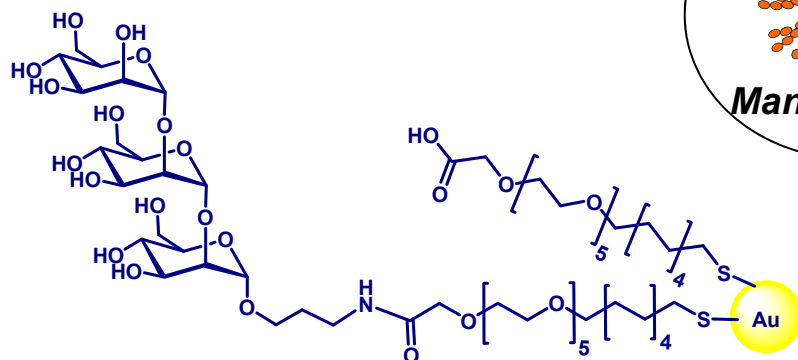
Manno-GNPs with varying density of oligomannosides



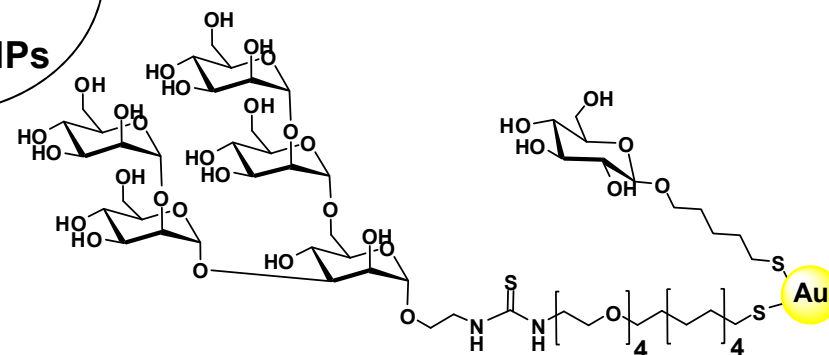
Man2-GNP (D 10, 50, 100%)



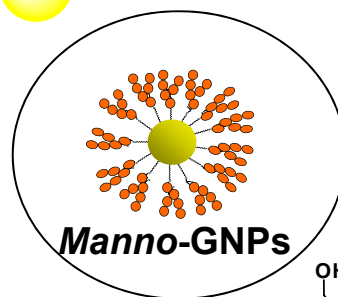
Man4-GNP, (Te 10, 50%)



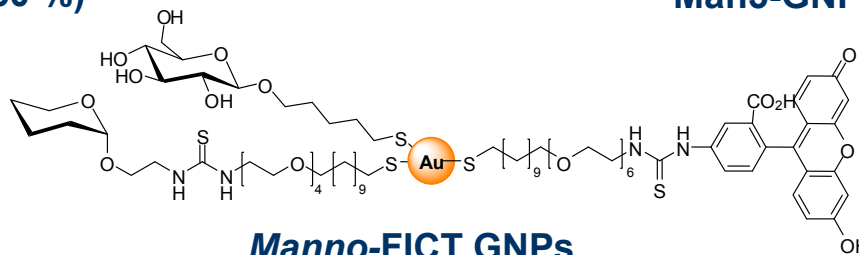
Man3-GNP (T 10, 50%)



Man5-GNP (P 10, 50%)



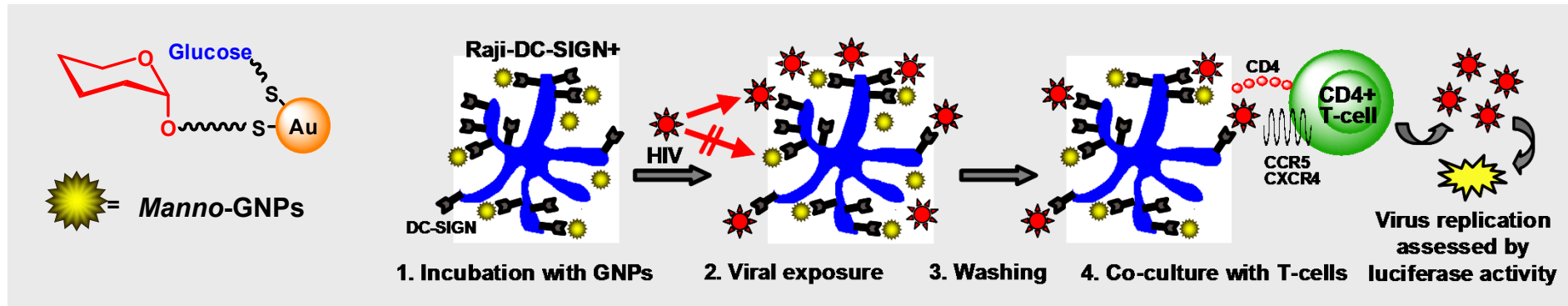
Manno-GNPs



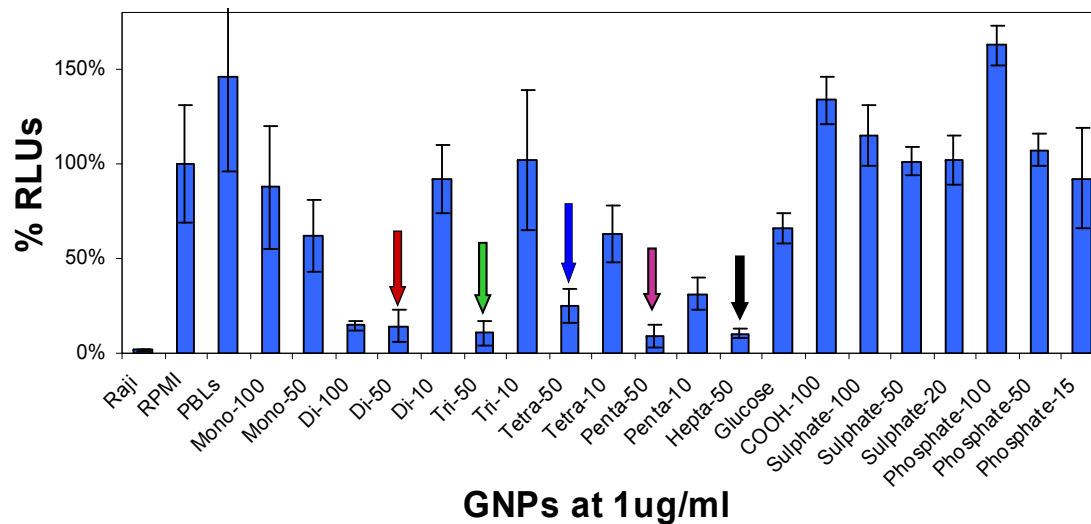
Manno-FICT GNPs

- Biosensor experiments
- NMR techniques
- Cellular assays

Manno-GNPs inhibit DC-SIGN-mediated HIV-1 *trans*-infection of human T cells



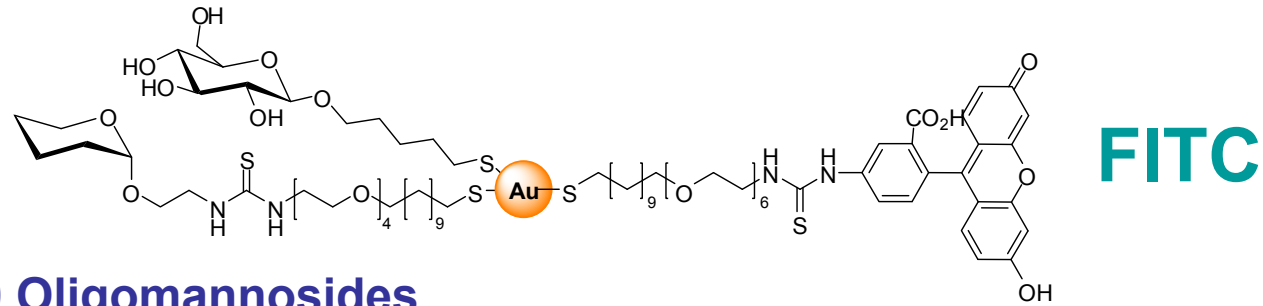
DC-SIGN transfer GNP/Raji DC-SIGN/JR Renilla



GNP	IC50 (ng/mL)
Man ₂ 50% GNP	80 (~2.0 nM)
Man ₃ 50% GNP	166 (~1.6 nM)
Man ₄ 50% GNP	41 (~0.3 nM)
Man ₅ 50% GNP	58 (~0.6 nM)
Man ₇ 50% GNP	79 (~0.5 nM)

O. Martínez-Ávila et al., *ChemBioChem* 2009
(with L. M. Bedoya, J. Alcamí, AIDS Unit, ISCIII)

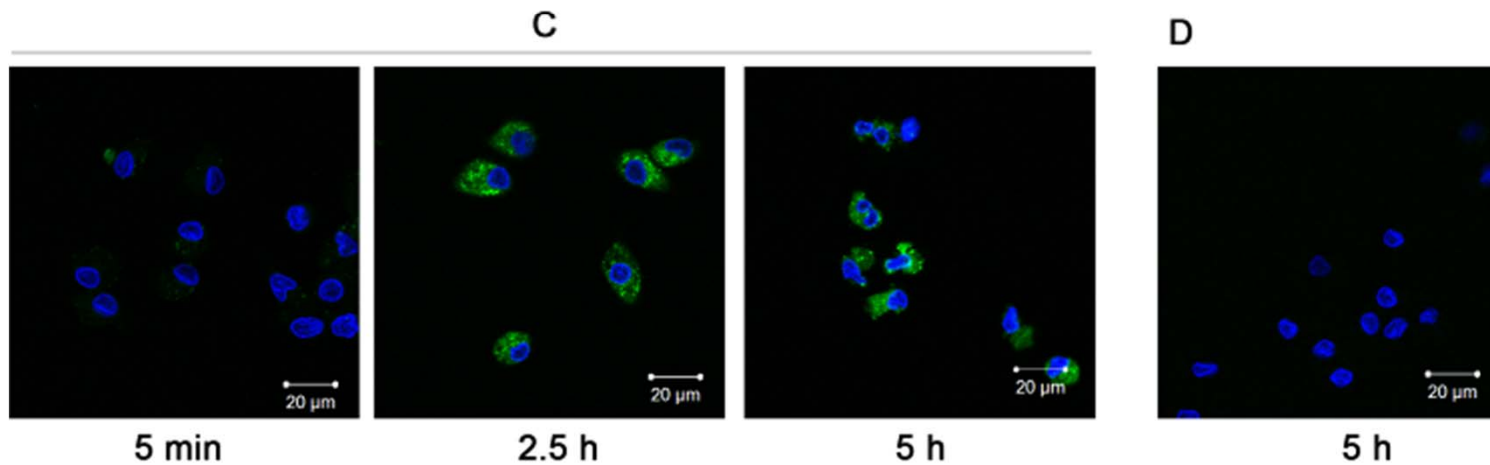
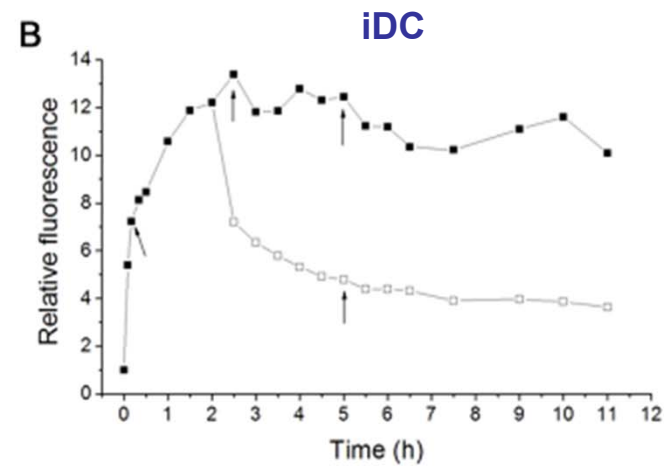
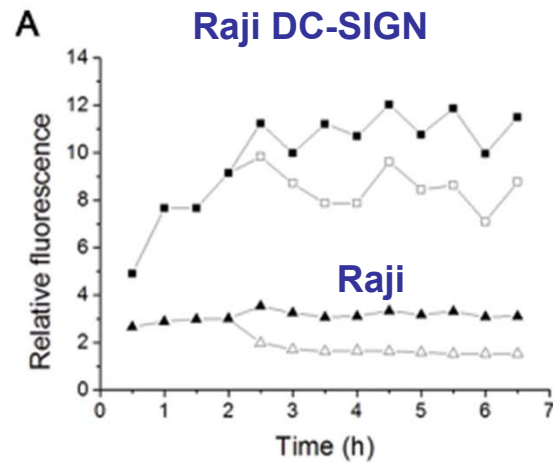
Fluorescent *manno*GNPs for in vitro cellular screening



HIVgp120 Oligomannosides

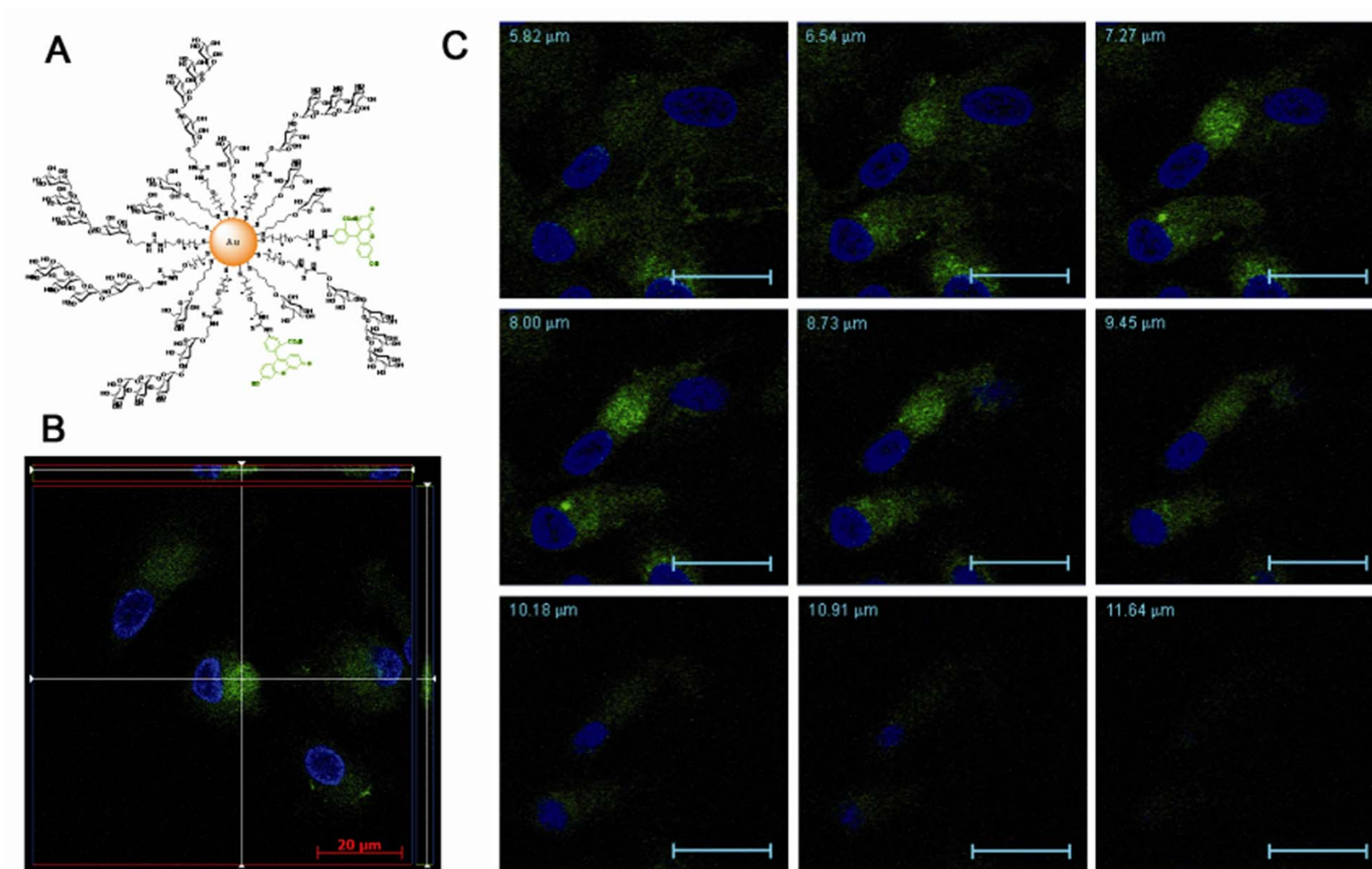
- Uptake and fate of FITC-*manno*GNPs in DCs by Flow Cytometry and Confocal Microscopy

FC analysis of the uptake of FITC-mannoGNPs by Raji DC-SIGN, Raji, and iDCs

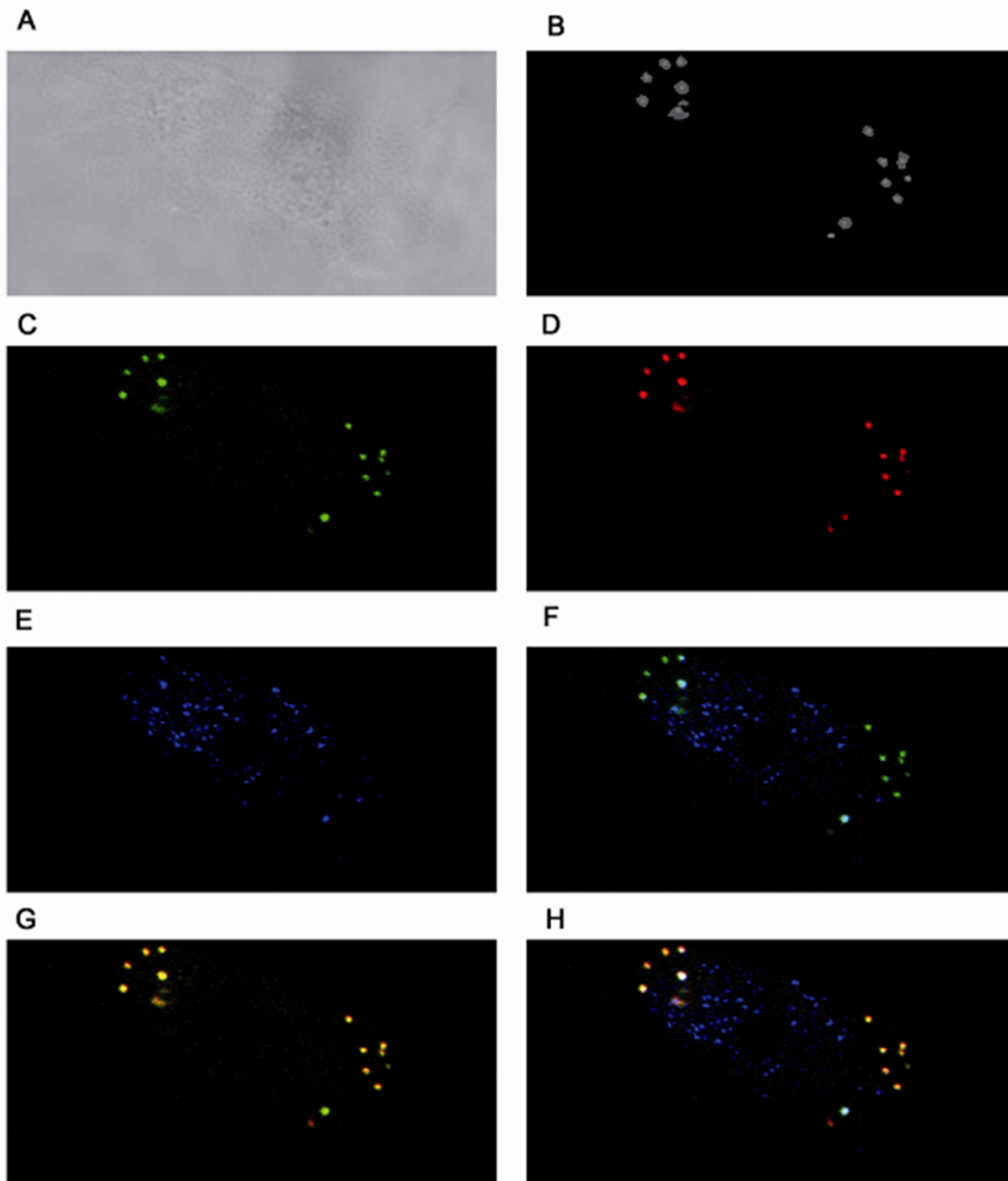


B. Arnáiz et al. *Bioconjugate Chem.*2012

Confocal images of single plane series of a Z-stack of iDCs incubated with FITC-Te50GNP



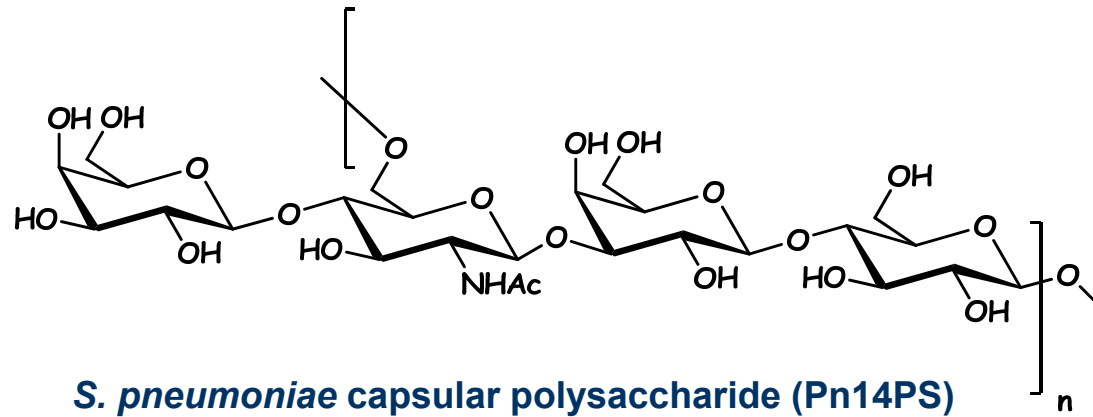
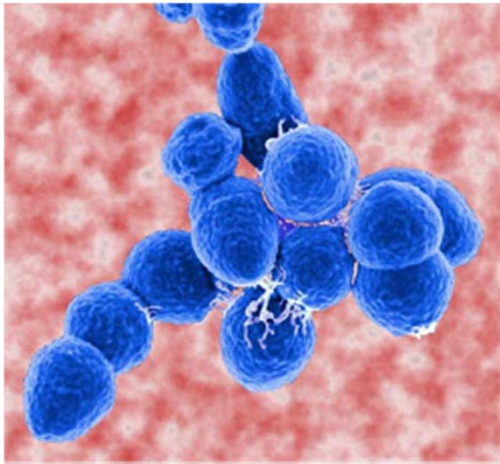
Quantitative colocalization of FITC-Te50GNPs with Early Endosome and DC-SIGN



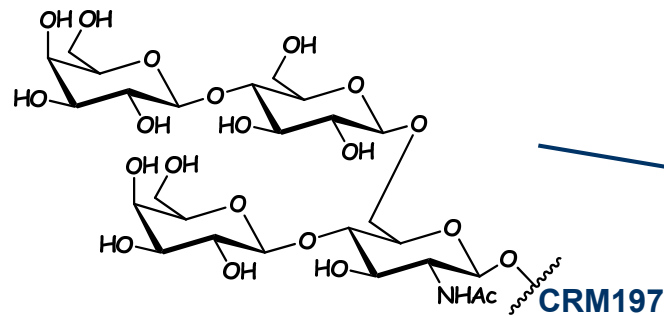
Objects	Nr. Counts	Percentage (%)
FITCTe50GNPs+ organelles (green)	1413	100
EEA1+ organelles (red)	603	43
DC-SIGN+ organelles (blue)	871	62
Triple colocalization	518	36

B. Arnáiz et al. *Bioconjugate Chem.* **2012**

Gold GNPs as carrier for vaccine against *S. pneumoniae*

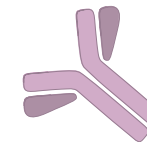
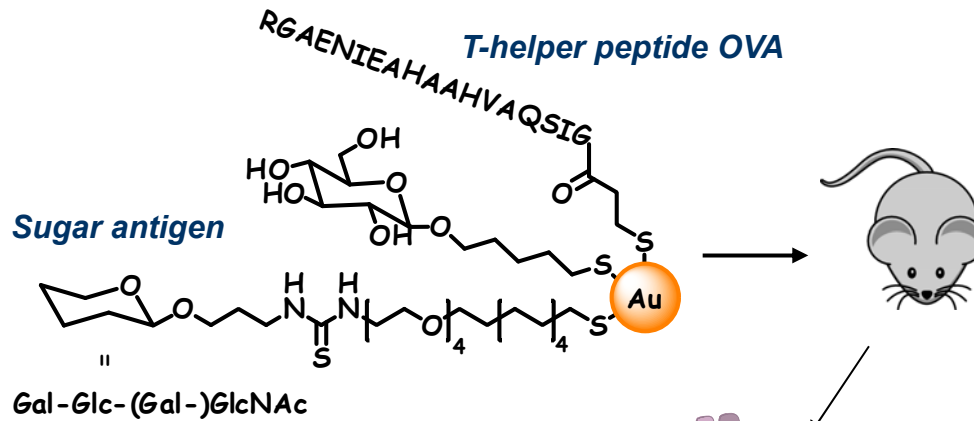


S. pneumoniae: major cause of acute respiratory bacterial infection



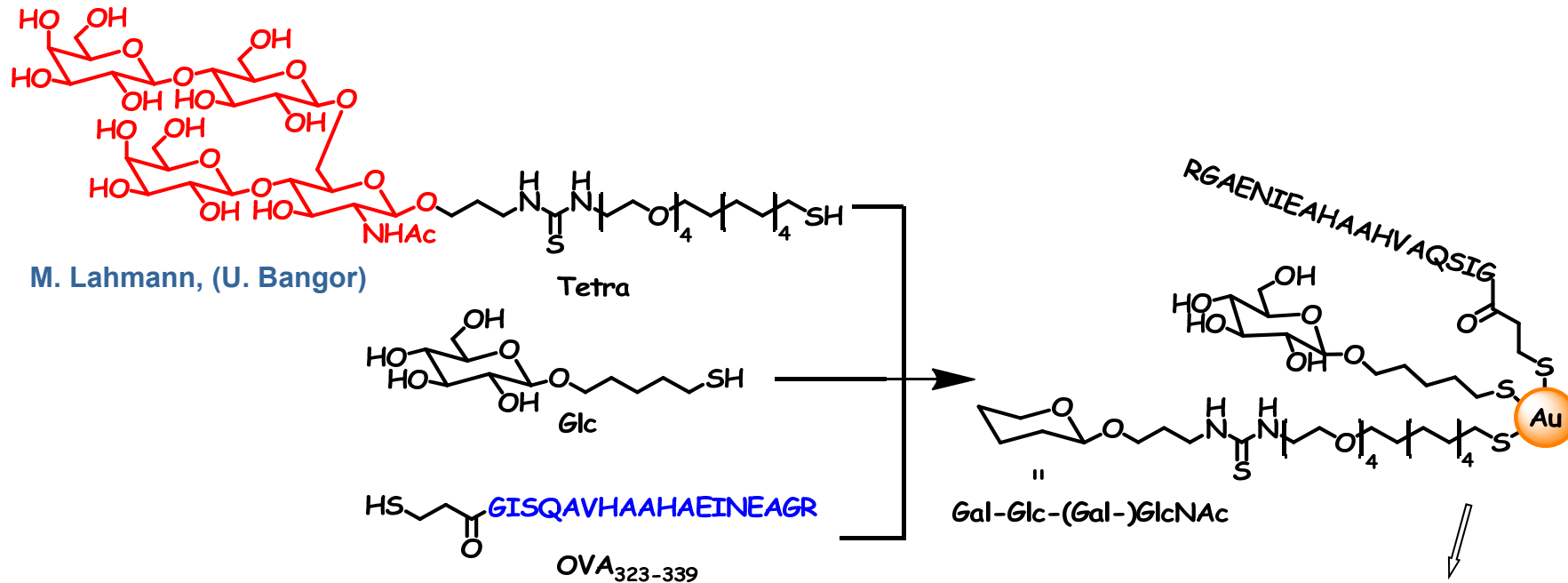
Gal(β1→4)Glc(β1→6)[Gal(β1→4)]GlcNAc

(Safari, D. et al. *Infect. Immun.* 2008, 76, 4615-4623)



Specific IgG anti-*S. pneumoniae* capsular polysaccharide

Gold GNPs coated with a synthetic epitope of Pn14PS



GNPs	Metal core diameter	Tetra:Glc:OVA
GNP-1	1.8 ± 0.5 nm	45 : 50 : 5
GNP-2	1.9 ± 0.3 nm	50 : 50 : 0
GNP-3	1.9 ± 0.5 nm	0 : 90 : 10
GNP-4	2.1 ± 0.4 nm	40 : 50 : 10
GNP-5	1.7 ± 0.6 nm	20 : 70 : 10

Anti-carbohydrate IgG

IgG promote phagocytosis of bacteria

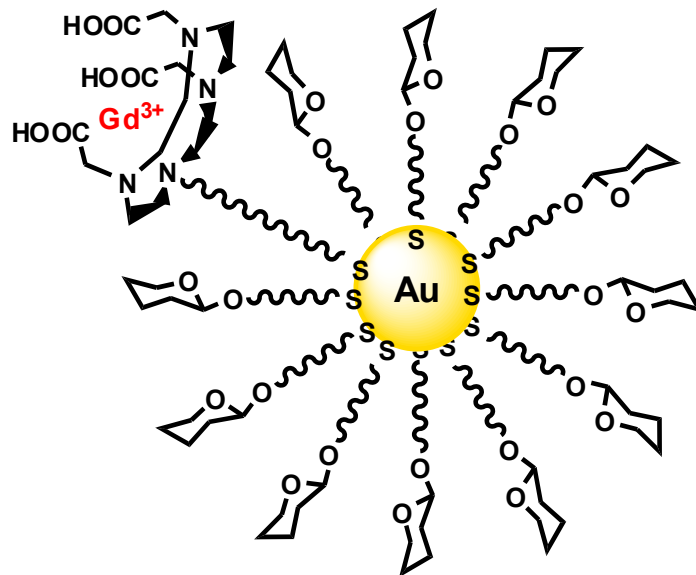
Specific IgG
anti-*S. pneumoniae*
capsular polysaccharide

GlycoNanoParticles (GNPs) for biomedical applications

- Glyconanoparticles to study carbohydrate self-interactions
- Glyconanoparticles as Anti-Adhesion Agents
- Glyconanoparticles as Synthetic Anti-Carbohydrate Vaccines
- **Magnetic Glyconanoparticles as Multimodal Probes for Molecular Imaging**
 - *for in vivo glioma imaging*
 - *for labelling cell surface receptors*
 - *for in vivo labelling and tracking neural stem cells*
 - *for detection the vulnerability of atherosclerotic plaques*

Magnetic glyconanoparticles as MRI probes

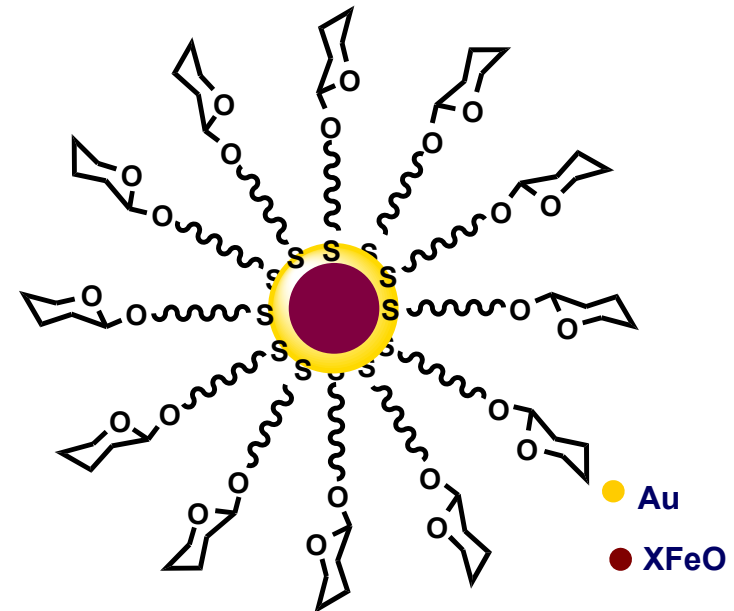
Organic Shell Modification



Paramagnetic GNPs (Agents in T_1)

M. Marradi et al, *Chem Commun* 2009,

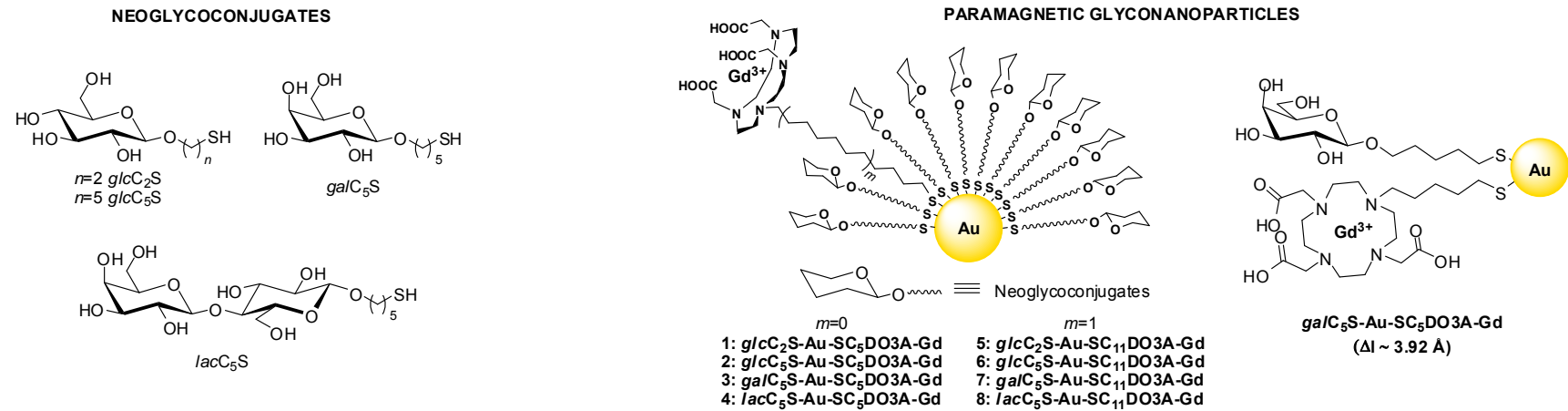
Gold Core Modification



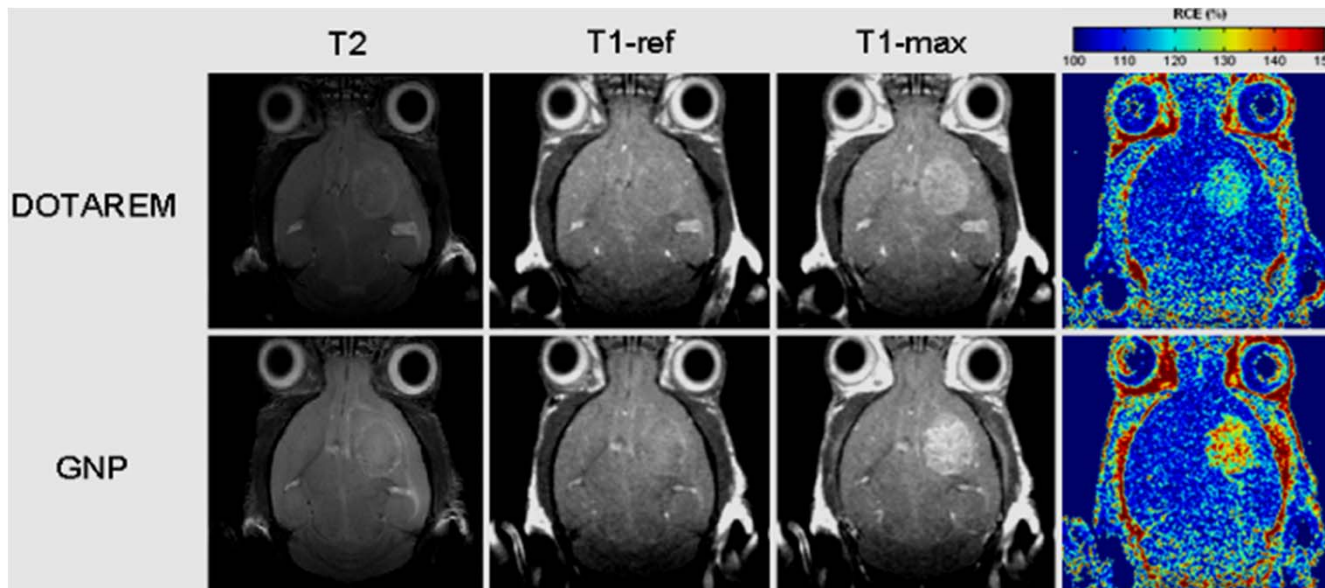
**Superparamagnetic core/shell
XFeO@Au glyco-ferrites (Agents in T_2)**

J. Gallo et al., *J. Mat. Chem* 2010

Gd-Based Paramagnetic Glyconanoparticles in MRI

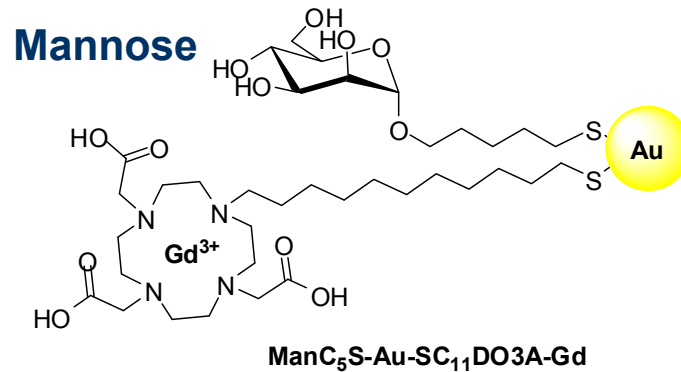


In vivo T1-weighted images of GL261 glioma in mice with GlcC5/DO3AC11-GNP

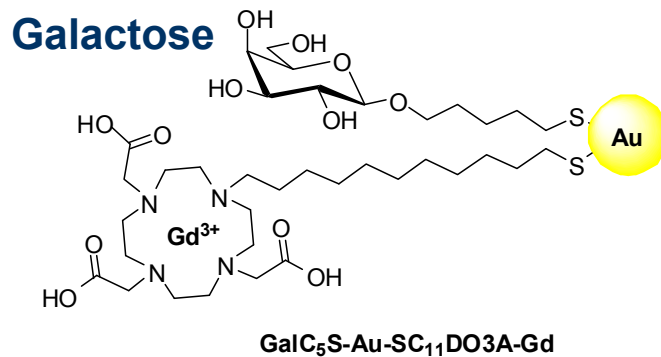


A. Irure, M. Marradi 2009
 (with C. Arus, UAB/CIBER-BBN)

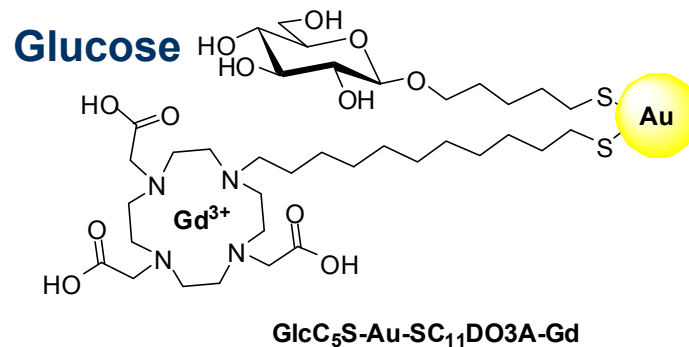
Gd-based paramagnetic glyconanoparticles for labeling cell surface receptors by MRI



**Raji-DC-SIGN/ Raji
DC-SIGN**

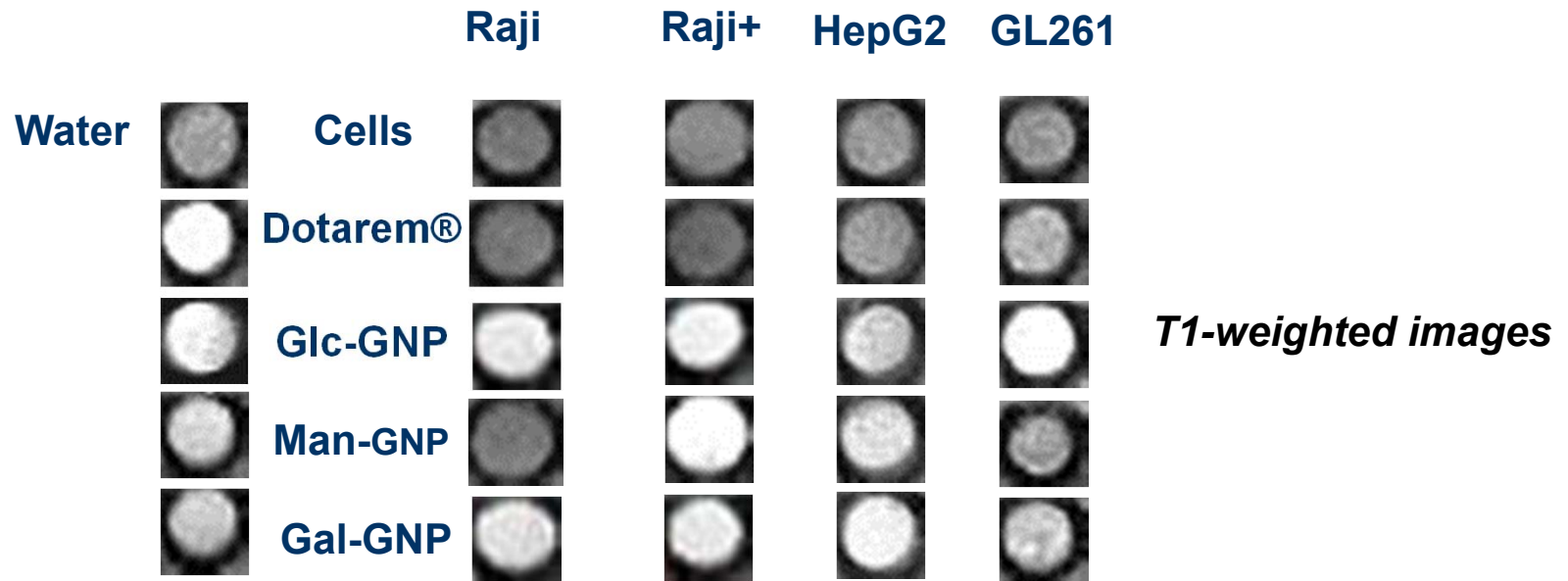


**HepG2
Asialoglycoprotein**



**GL261
Glucose Transporter**

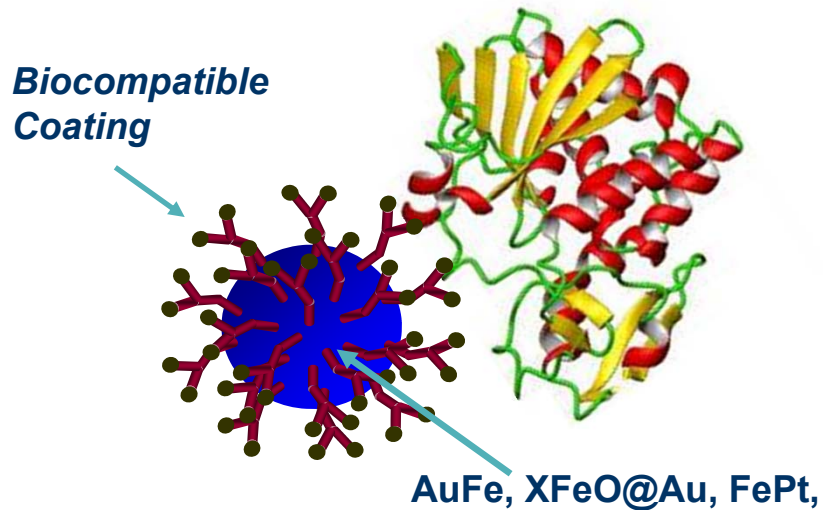
Gd-based paramagnetic GNPs for labeling cell surface receptors by MRI



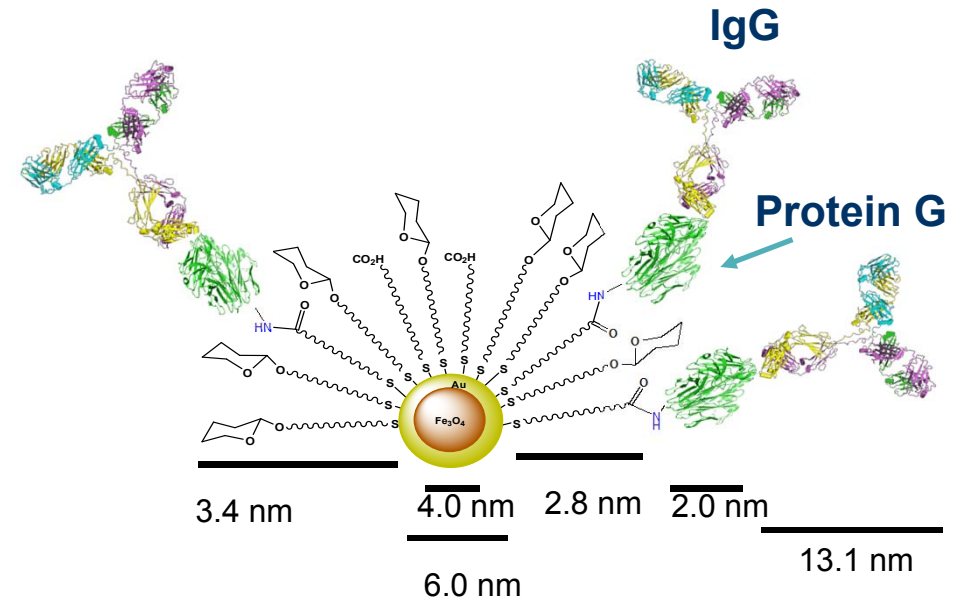
Gd-GNP	Raji	Raji+	HepG2	GL261
Glc ₅ S-Au-SC ₁₁ DO3A-Gd	70%	71%	37%	70%
ManC ₅ S-Au-SC ₁₁ DO3A-Gd	65%	74%	54%	53%
GalC ₅ S-Au-SC ₁₁ DO3A-Gd	57%	66%	66%	57%

%ΔT1

Targeted superparamagnetic *immuno-glyco-ferrites* for labelling in vivo cells, tissue, and organs



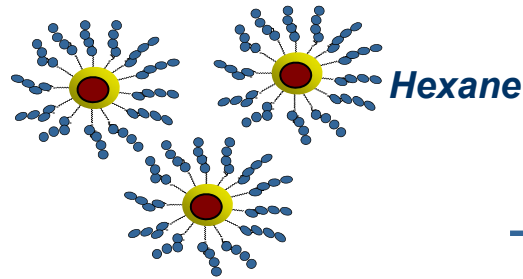
**Superparamagnetic GNPs
(Agents in T_2)**



**Core/shell XFeO@Au
immuno glyco-ferrites**

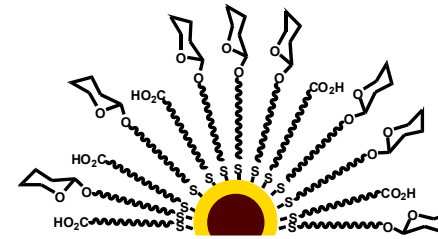
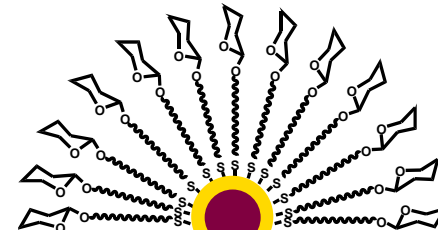
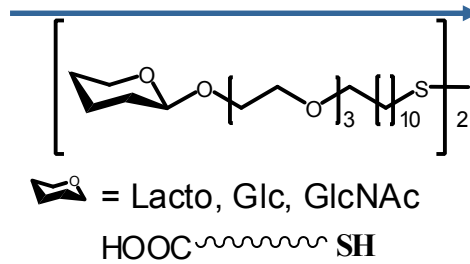
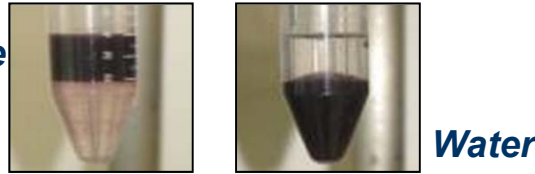
- Cerdan et al *Magn Reson Med* 1989
- Lewin et al. *Nat Biotech* 2000
- Lee JH et al. *Nature Med* 2007
- Veiseh et al. *Cancer Res* 2009
- Van Kastern et al. *Proc Natl Acad Sci USA*, 2009
- Hadjipanayis et al. *Cancer Res*. 2010
- De la Fuente et al., *ACSNano* 2011

Water-soluble superparamagnetic GNPs (glyco-ferrites)

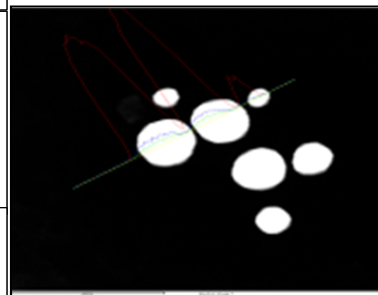
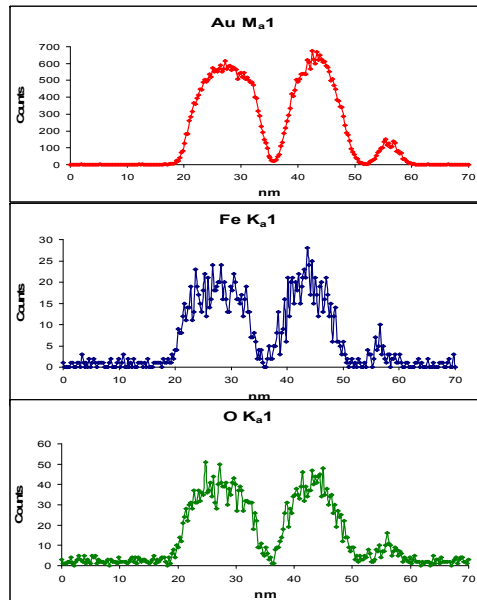


oleic acid, oleyl amine
 $XFe_3O_4@Au$

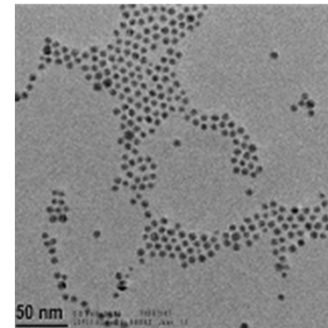
L. Wang et al. *J. Mater. Chem.* 2005



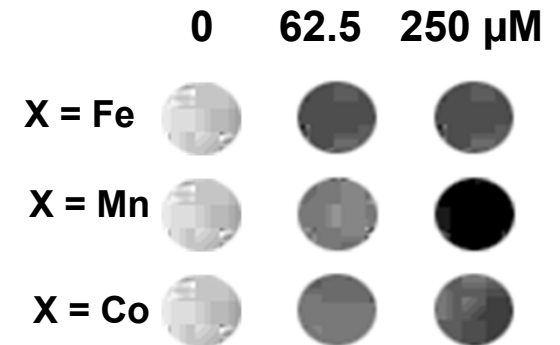
$XFe_2O_3@Au$
 glyco-ferrites



STEM-EDXS

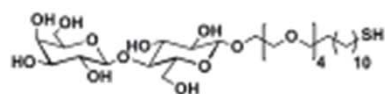


TEM

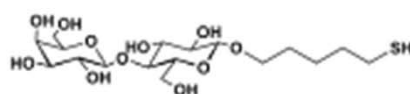


MRI

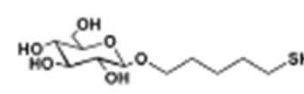
Superparamagnetic $\text{Fe}_3\text{O}_4@Au$ glyco-ferrites



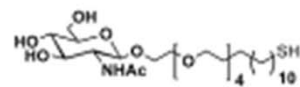
LactC₁₁SH: 1



LactC₅SH: 2



GlcC₅SH: 3

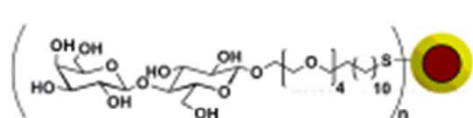


GlcNAcC₁₁SH: 4

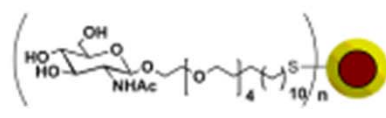


LinkerSH: 5

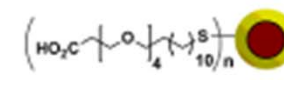
A)



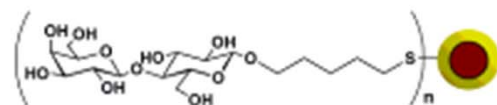
$\text{Fe}_3\text{O}_4@Au@LactC_{11}$



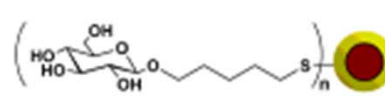
$\text{Fe}_3\text{O}_4@Au@GlcNAcC_{11}$



$\text{Fe}_3\text{O}_4@Au@Linker$

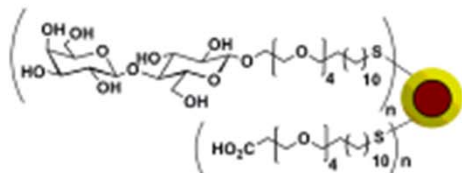


$\text{Fe}_3\text{O}_4@Au@LactC_5$

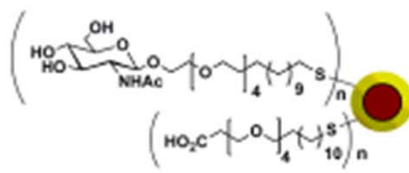


$\text{Fe}_3\text{O}_4@Au@GlcC_5$

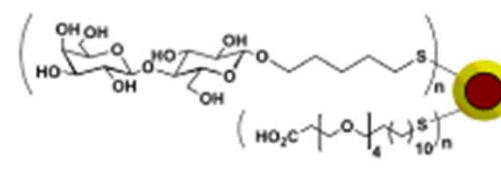
B)



$\text{Fe}_3\text{O}_4@Au@LactC_{11}/Linker$



$\text{Fe}_3\text{O}_4@Au@GlcNAcC_{11}/Linker$

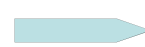


$\text{Fe}_3\text{O}_4@Au@LactC_5/Linker$

**Glyco-ferrites, r2 157 [mM-1s-1]
Resovist..... r2 150 [mM-1s-1]**

Glyco-nanoferrites as a versatile platform to attach antibodies

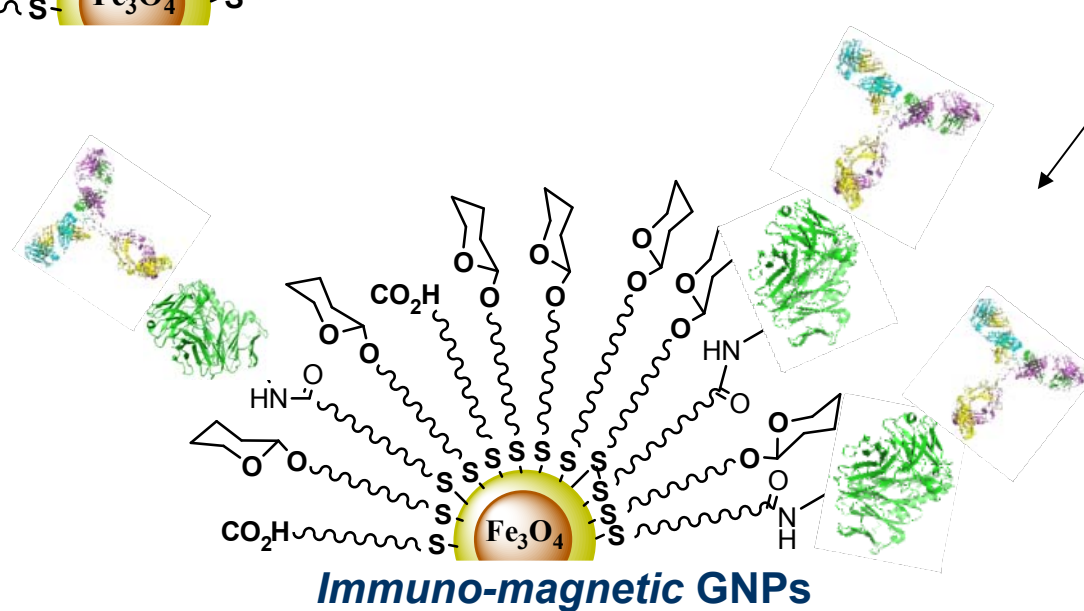
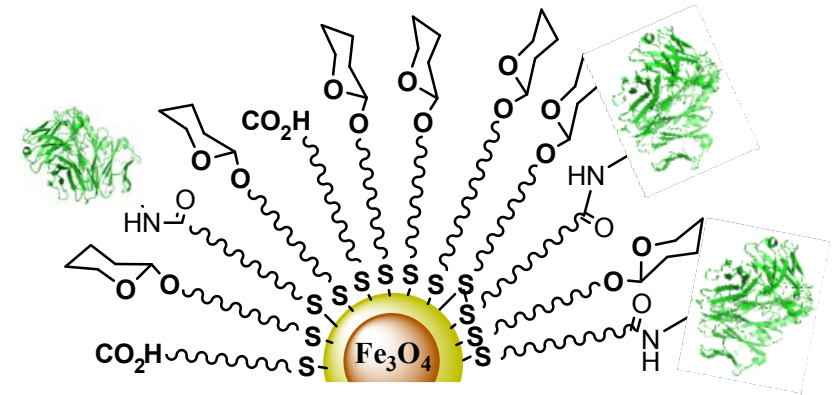
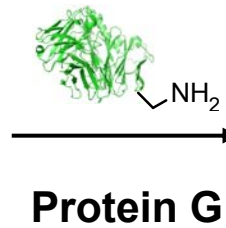
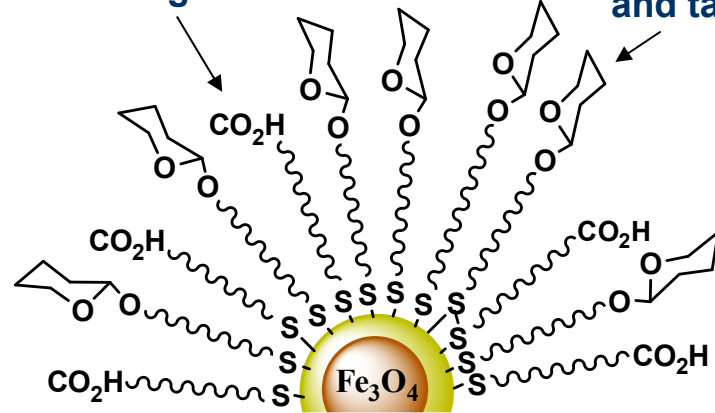
Protein G immobilization



Oriented antibody

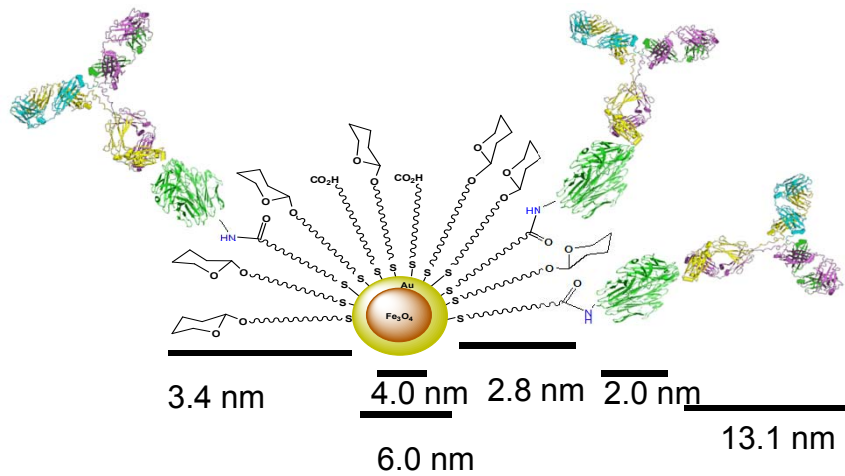
Free -COOH group
for linking

Sugar for biocompatibility
and targeting

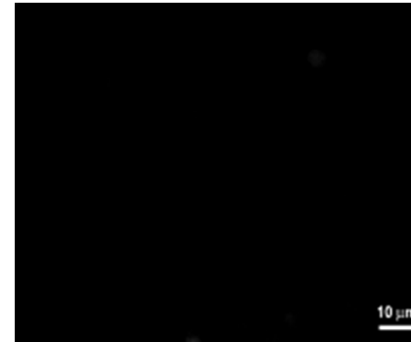


Antibody
Incubation

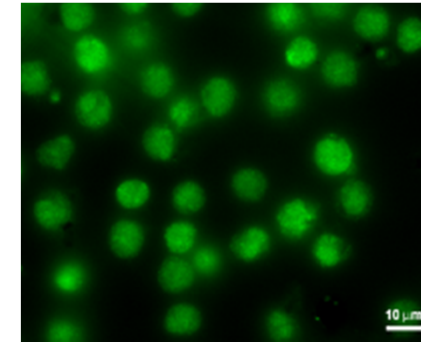
In vitro selective labelling of cells by *immuno-magnetic-GNPs*



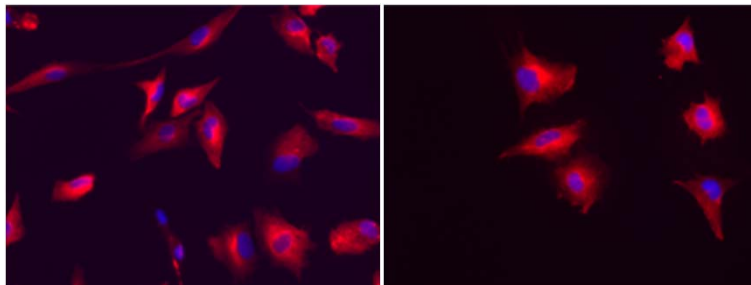
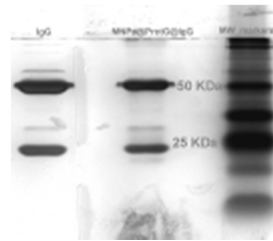
Raji cells



Raji DC-SIGN cells

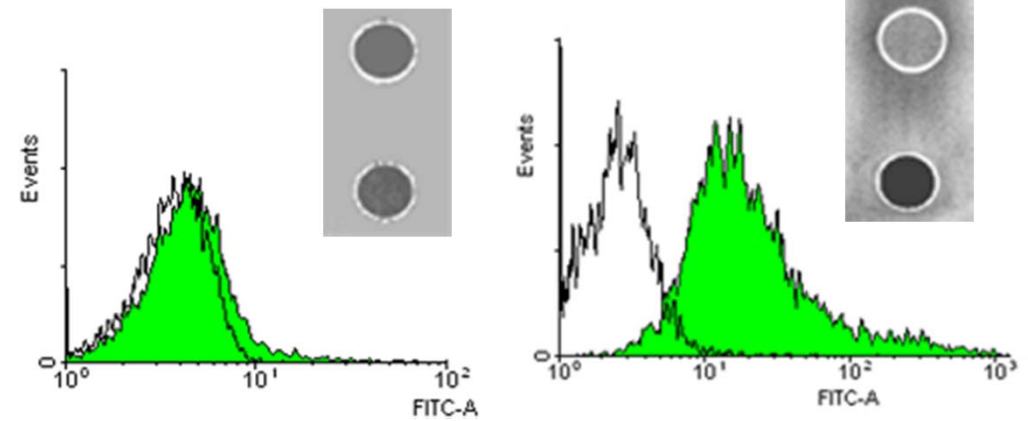


MALDI-TOF
QCM
SDS-PAGE



Stained by free
anti-β tubulin IgG

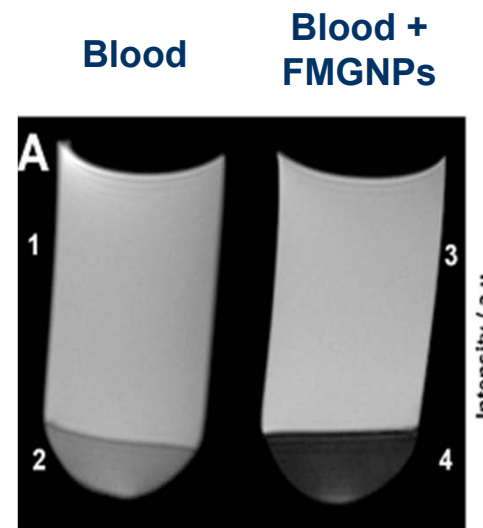
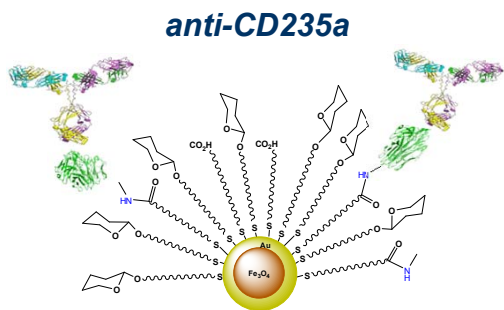
Stained by anti-β
tubulin IgG-GNPs



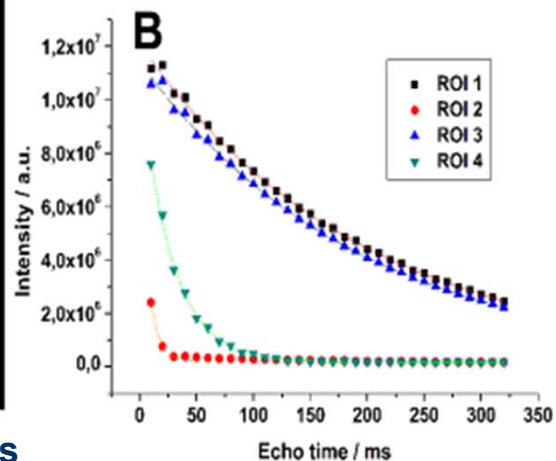
Labelled with anti-DC-SIGN IgG-GNPs

Ex vivo specific labelling of blood cell populations with immuno-fluorescent glyco-ferrites

Erythrocytes



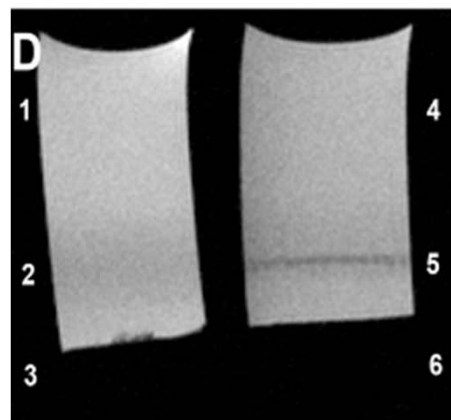
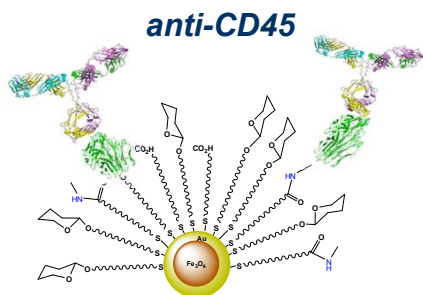
Blood + anti-CD235a GNPs



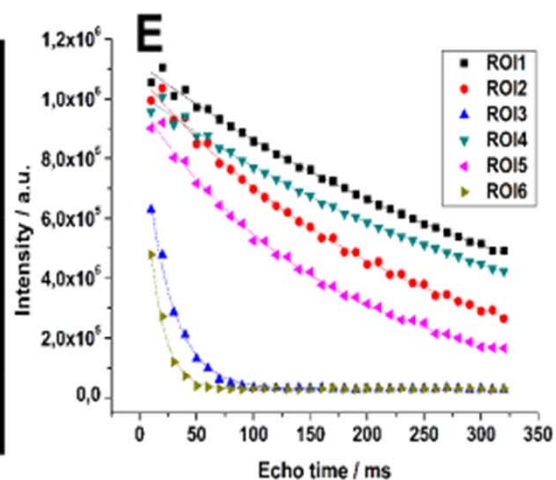
C

ROI	T ₂
1	192 ± 8
2	28 ± 1
3	198 ± 8
4	7 ± 1

Leukocytes



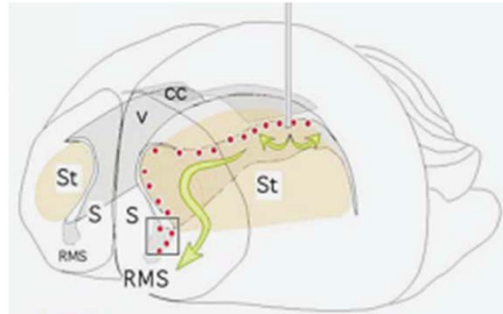
Blood + anti-CD45 GNPs



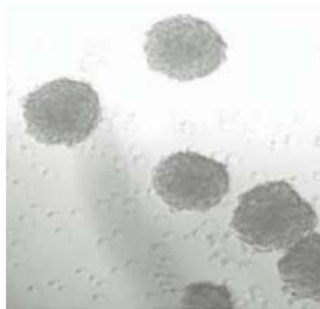
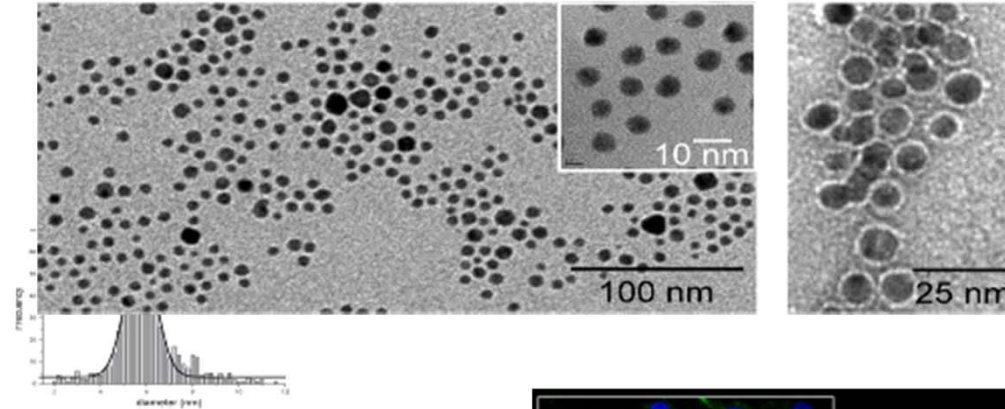
F

ROI	T ₂ /ms
1	388 ± 48
2	238 ± 18
3	24 ± 1
4	364 ± 45
5	164 ± 8
6	14 ± 1

In vivo labeling neural stem cells with Nilo2-GNPs



**Subventricular Zone (SVZ)
Rostral Migratory Stream (RMS)**



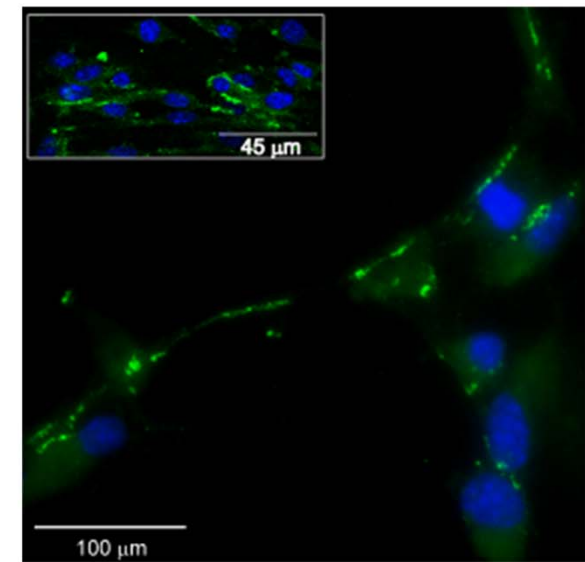
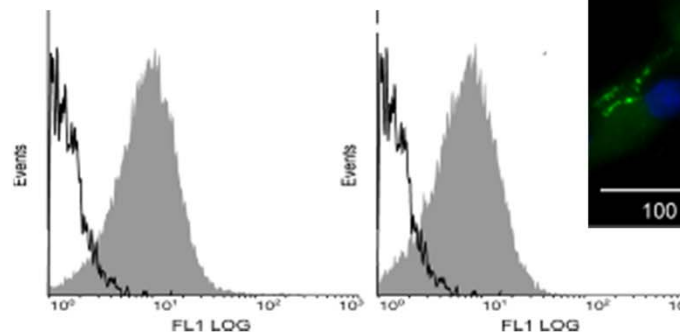
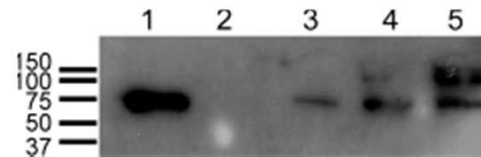
**NSCs expanded
"in vitro" to
neurospheres**

Specific monoclonal antibody against NSC

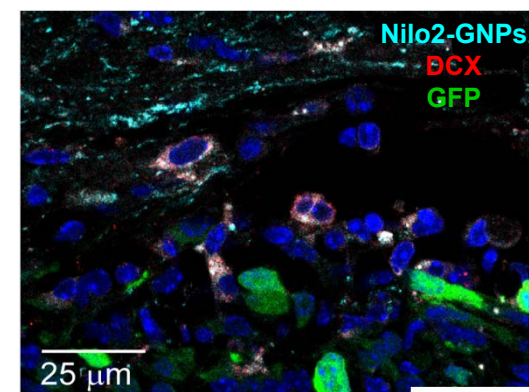
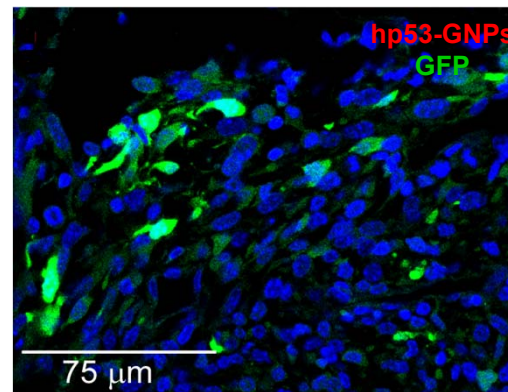
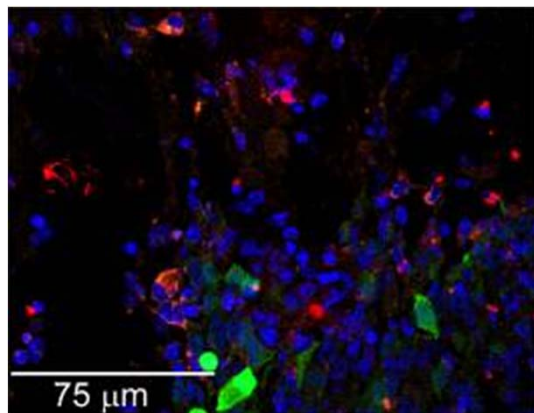
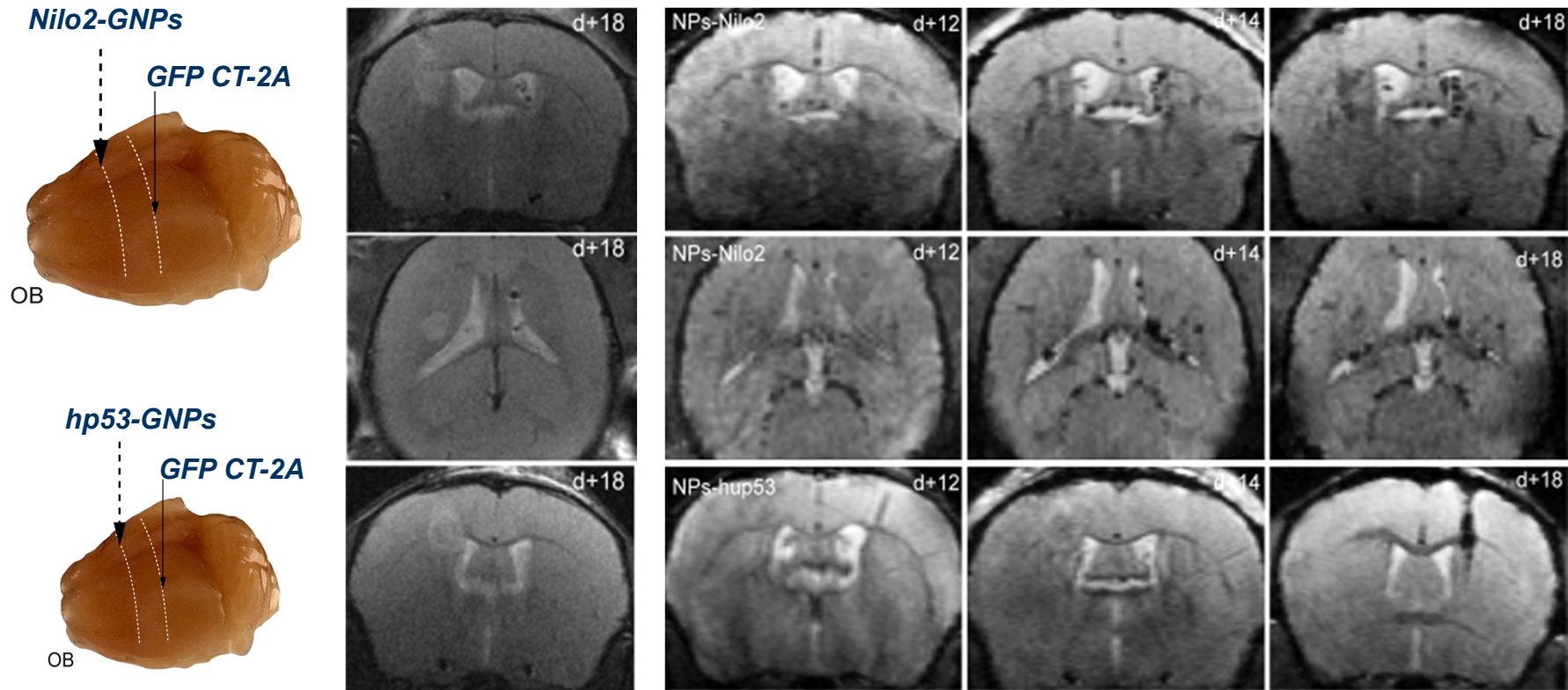


Nilo1, Nilo2

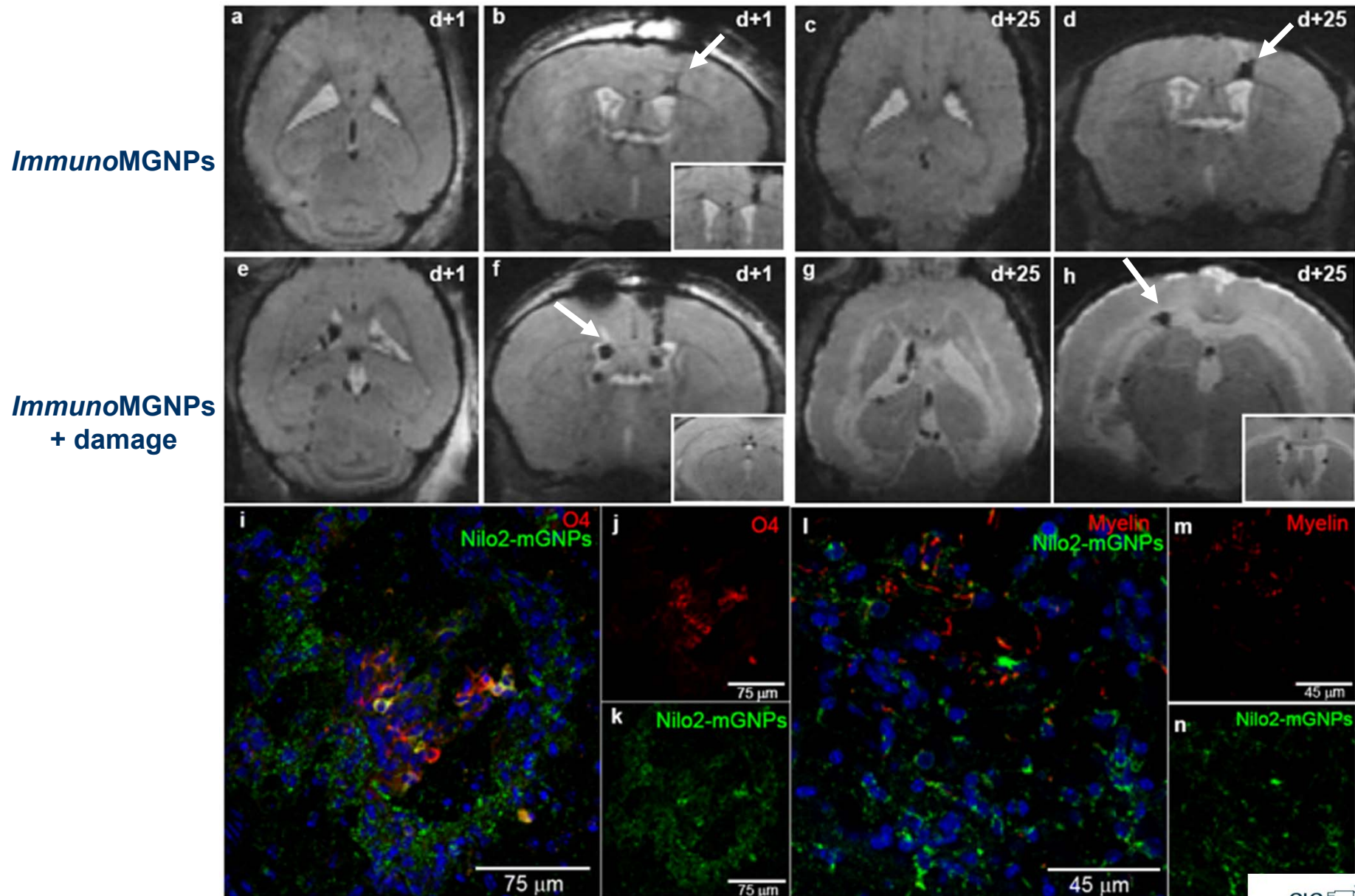
I. del Valle et al., Neuroscience 2010



In vivo tracking of endogenous neural progenitors in response to tumor



In vivo tracking of endogenous neural progenitors in response to chemical demyelination



From Seville to....Donostia-San Sebastián.

Donostia-San Sebastián

2007-

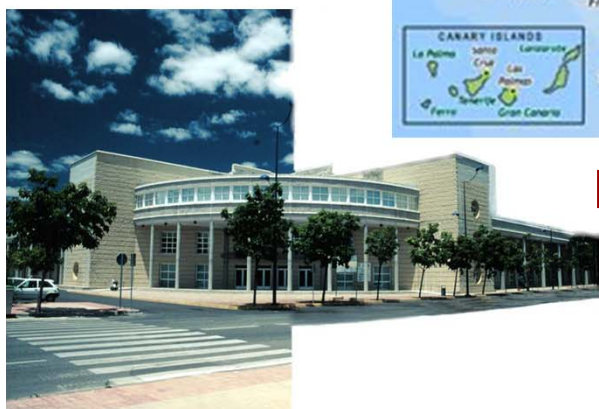


CICbiomaGUNE (Biomaterials Cooperative Research Centre) is a non-profit association with the basic aim of **creating, producing, promoting and applying scientific and technological knowledge** in the **biomaterials** field by carrying out systematic research and experimental development work.

1997-2006



From Sevilla



**Lab of GlycoNanotechnology, CIC biomaGUNE
Donostia-San Sebastián (2011)**



Carbohydrate Group, Seville (2006)



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P.M. Enriquez *J. Gallo* *A. Irure*
I. Garcia
B. Arnaiz
N. Genicio
M. Carril *P. di Gianvincenzo*



**Centro de Investigaciones
Científicas Isla de la Cartuja-CSIC**

Sevilla

2000-2006

Grupo de Carbohidratos

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Isabel Garcia
Caroline Clavel
Andrea Ragusa
Marco Marradi
David Alcántara
Olga Martínez**

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C. Arus (UAB)
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M. Lehman (U. Bangor)
Neural Stem Cells: A. Silva (CIB-CSIC)
M. Desco (H.U. GM)**

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EVA Centre for AIDS Reagents (gp120)**



Donosti-San Sebastian

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