# COST

Domain Committee
"Chemistry and Molecular Sciences and Technologies"

## COST Action *D38*

## *Metal-Based Systems for Molecular Imaging Applications*

# MONITORING
# PROGRESS REPORT

***Period:*** *from 25/08/2006 to 31/12/2009*

This Report is presented to the relevant Domain Committee and contains two parts:

***I. Management Report*** *prepared by the COST Office/Grant Holder*
***II. Scientific Report*** *prepared by the Chair of the Management Committee of the Action*

The report is a "cumulative" report, i.e. it is updated annually and covers the entire period of the Action.

Confidentiality: the documents will be made available to the public via the COST Action web page except for chapter *II.C. Self evaluation*.

Based on the monitoring results, the COST Office will decide on the following year's budget allocation.

## *I. Management Report* *prepared by the COST Office*

### *I.A. COST Action Fact Sheet*

**Title**

Metal-Based Systems for Molecular Imaging Applications

**Contacts**

| MC Chair | Science Officer: | Administrative Officer: |
|---|---|---|
| Dr Eva JAKAB TOTH<br>Tel. +33 2 38 25 76 25<br>Fax. +33 2 38 63 15 17<br>eva.jakabtoth@cnrs-orleans.fr | Dr Erwan ARZEL<br>COST Office<br>earzel@cost.esf.org<br>003225333817 | Ms Marie-Eve HASTIR<br>COST Office<br>mehastir@cost.esf.org<br>+32 2 533 38 45 |

**Details**

| | | | |
|---|---|---|---|
| Draft Mou: | 232/06 | Mou: | |
| Start of Action: | 25/08/2006 | Entry into force: | 15/05/2006 |
| End of Action: | 24/08/2011 | CSO approval date: | 29/03/2006 |

**Objectives**

The main objective of the Action is the development of metal-based imaging probes forcellular and molecular imaging applications, based on MRI, PET, SPECT and optical imagingthat will facilitate early diagnosis, assessment of disease progression and treatmentevaluation.The goal of this Action is to further the development of innovative imaging probes through thepursuit of innovations in a number of different areas, ranging from the design of imaging unitsendowed with enhanced sensitivity to the control of the structural and electronic determinantsresponsible for the molecular recognition of the target molecule.At present, in vivo diagnostic systems basically assess the structure and function of humanorgans. Therefore, for important diseases such as cancer and cardiovascular pathologies,and also diseases of the central nervous system, only the late symptoms are detected. It isexpected that the advances in genomics and proteomics will have a tremendous impact onhuman health care of the future. However, advances in molecular biology are alreadyredefining diseases in terms of molecular abnormalities. With this knowledge, newgenerations of diagnostic imaging agents can be defined that aim at the detection of thosemolecular processes in vivo.The molecular imaging approach offers a great potential for earlier detection andcharacterisation of disease, and evaluation of treatment. However, more research isnecessary to bring these ideas to clinical applications and a key aspect relates to thedevelopment of high-specificity, high-sensitivity imaging probes for the different detectionmodalities.Additionally, the Action includes research activities dealing with the exploitation of peculiarnuclear properties of given isotopes for therapeutic effects, thus integrating the diagnosticand the therapeutic stages.Apart from its use in early diagnosis in clinical practice, the molecular imaging approach willhave also a major impact on the development of new pharmaceuticals. The regulatoryagencies indicate that the use of surrogate or bio-markers can accelerate drug approvalprocedures. Molecular imaging can be considered as such a bio-marker, since it has greatpotential to make better predictions on the effectiveness and toxicity of drugs. Therefore, thedevelopment of this field will not only enable early diagnosis, but will also significantlyincrease the availability and speed to market of new drugs.

| Parties | | | | | | | |
|---|---|---|---|---|---|---|---|
| Country | Date | Country | Date | Country | Date | Country | Date |
| Belgium | 11/05/2006 | Cyprus | 28/03/2007 | Czech Republic | 11/05/2006 | Finland | 12/07/2006 |
| France | 03/07/2006 | Germany | 18/05/2006 | Greece | 23/06/2006 | Hungary | 07/07/2006 |
| Italy | 17/05/2006 | Netherlands | 23/05/2006 | Poland | 11/05/2006 | Portugal | 11/05/2006 |
| Spain | 29/05/2006 | Switzerland | 15/05/2006 | Turkey | 21/10/2009 | United Kingdom | 20/06/2006 |

Total: 16

| Intentions to accept the MoU | | | | | | | |
|---|---|---|---|---|---|---|---|
| Country | Date | Country | Date | Country | Date | Country | Date |

Total: 0

| Working Groups |
|---|
| D38-0001-06 Optical Imaging<br>_D38-0002-06 MRI Contrast Agents<br>_D38-0003-06 Nuclear Imaging probes<br>_D38-0004-06 Nanosized probes for Molecular Imaging<br>_D38-0005-06 Targeted Probes<br>_D38-0006-06 Responsive Probes |

| Website |
|---|
| http://www.cost.esf.org/index.php?id=189&action_number=d38 |

## *I.B. Management Committee member list*

| Management Commitee | | |
|---|---|---|
| Chair | Vice Chair | DC Rapporteur |
| Dr Eva JAKAB TOTH<br>Centre de Biophysique Moleculaire, CNRS Rue Charles Sadron 45071 Orleans Cedex 2 France<br>**eva.jakabtoth@cnrs-orleans.fr** | Professor Frank ROSCH<br>FB 04: Chemistry, PharmacyJohannes Gutenberg-Universitat Mainz Fritz-Strassmann Weg 2 55128 Mainz Germany<br>**frank.roesch@uni-mainz.de** | Professor Jan REEDIJK<br>CBACLeiden Institute of ChemistryLeiden University PO Box 9502 2300 RA Leiden Netherlands<br>**reedijk@chem.leidenuniv.nl** |

| Belgium | |
|---|---|
| Professor Robert MULLER<br>**MC Member**<br>Faculty of Medicine and PharmacyUniversity of Mons-Hainaut 24 Av. du champ de Mons 7000 MONS Belgium<br>**Robert.Muller@umh.ac.be** | Professor Jean-Francois DESREUX<br>**MC Member**<br>Chimie Analytique et Nucleaire, B6Universite de Liege Sart Tilman 4000 Liege Belgium<br>**jf.desreux@ulg.ac.be** |
| Professor Alfons VERBRUGGEN<br>**MC Member**<br>Catholic University Leuven Herestraat 49 3000 Leuven Belgium<br>**Alfons.Verbruggen@farm.kuleuven.be** | |

| Cyprus | |
|---|---|
| Dr Constantinos PITRIS<br>**MC Member**<br>Faculty of EngineeringUniversity of Cyprus 75 Kallipoleos St 1678 Nicosia Cyprus<br>**cpitris@ucy.ac.cy** | |

| Czech Republic | |
|---|---|
| Professor Ivan LUKES<br>**MC Member**<br>Faculty of ScienceUniversita Karlova- Charles University Hlavova 2030 Cz 128 40 Prague 2 Czech Republic<br>**lukes@natur.cuni.cz** | |

| Finland | |
|---|---|
| Dr Ilkka HEMMILA<br>**MC Member**<br>PerkinElmer Life and Analytical SciencesWallac Oy(University of Turku / as Docent) Wallac Oy, PO Box 10 FIN 20101 Turku Finland<br>**ilkka.hemmila@perkinelmer.com** | Professor Christer LINDQVIST<br>**MC Member**<br>Faculty of Natural SciencesAbo Akademi University Tykistokatu 6 20520 TURKU Finland<br>**clindqvi@abo.fi** |

| France | |
|---|---|
| Dr Marinella MAZZANTI<br>**MC Member**<br>CEA, Laboratoire de Reconnaissance IoniqueSCIB/DRFMC/DSM N/A - Please update this record 38054 Grenoble France<br>**mazzanti@drfmc.ceng.cea.fr** | |

| Germany | |
|---|---|
| Dr Thomas BRUMBY<br>**MC Member**<br>BayerScheringPharma AG Mllerstr 178 13342 Berlin Germany<br>**thomas.brumby@bayerhealthcare.com** | |

| Greece | |
|---|---|
| Dr Penelope BOUZIOTIS<br>**MC Member**<br>National Center for Scientific Research DemokritosInstitute of Radioisotopes and Radiodiagnostic Products Patriarchou Grigiriou and Neapoleos Street, Aghia Paraskevi 15310 Athens Greece<br>**bouzioti@rrp.demokritos.gr; pennybil@yahoo.gr** | Dr Dimitrios PETRIDIS<br>**MC Member**<br>(pending)<br>**dpetrid@ims.demokritos.gr**<br>PENDING |

| Hungary | |
|---|---|
| Professor Erno BRUCHER<br>**MC Member**<br>Institute of Inorganic and Analytical ChemistryUniversity of Debrecen N/A - Please update this record 4010 Debrecen Hungary<br>**ebrucher@delfin.klte.hu** | |

| Italy | |
|---|---|
| Professor Ulderico MAZZI<br>**MC Member**<br>University of Padova Via F.Marzolo, 5 35131 Padova Italy<br>**ulderico.mazzi@unipd.it** | Professor Silvio AIME<br>**MC Member**<br>Facolta di Scienze M.F.N.Universita degli studi di Torino Via Pietro Giuria 7 10125 Torino Italy<br>**silvio.aime@unito.it** |

| Netherlands | |
|---|---|
| Dr Holger GRUELL<br>**MC Member**<br>Philips ResearchMolecular Imaging Contrast AgentsSector of Molecular Medicine High Tech Campus 4 5656AE Eindhoven Netherlands<br>**holger.gruell@philips.com** | Dr Joop PETERS<br>**MC Member**<br>Biocatalysis and Organic ChemistryApplied SciencesDelft University of Technology Julianalaan 136 2628 BL Delft Netherlands<br>**J.A.Peters@tudelft.nl** |
| Professor Klaas NICOLAY<br>**MC Substitute Member**<br>Eindhoven University of Technology PO Box 513 5600 MD Eindhoven Netherlands<br>**k.nicolay@tue.nl** | |

| Poland | |
|---|---|
| Dr Renata MIKOLAJCZAK<br>**MC Member**<br>IAE Radioisotope Centre POLATOM Swierk 05-400 Otwock Poland<br>**r.mikolajczak@polatom.pl** | Professor Aleksander BILEWICZ<br>**MC Member**<br>Institute of Nuclear Chemistry and Technology Dorodna 16 03-195 Warsaw Poland<br>**abilewic@ichtj.waw.pl** |

| Portugal | |
|---|---|
| Professor Carlos Frederico GERALDES<br>**MC Member**<br>NMR LaboratoryFaculty of Science and TechnologyUniversity of Coimbra Rua dos Estudos, P.O. Box 3126 3001-401 Coimbra Portugal<br>**geraldes@ci.uc.pt** | Dr Isabel REGO DOS SANTOS<br>**MC Member**<br>Instituto Tecnologico e Nuclear N/A - Please update this record Sacavem Portugal<br>**isantos@itn.pt** |

| Spain | |
|---|---|
| Professor Maria Teresa RODRIGUEZ BLAS<br>**MC Member**<br>Facultad de CienciasUniversidade da Coruna Campus da Zapateira s/n 15071 A Coruna Spain<br>**mayter@udc.es** | Professor Enrique GARCIA-ESPANA<br>**MC Member**<br>Universidad de Valencia Apartado de correos 22085 46071 Valencia Spain<br>**enrique.garcia-es@uv.es** |

| Switzerland | |
|---|---|
| Professor Lothar HELM<br>**MC Member**<br>Ecole Polytechnique Federale de Lausanne Local BCH 3108 1015 Lausanne Switzerland<br>**lothar.helm@epfl.ch** | Professor Theo LASSER<br>**MC Member**<br>Ecole Polythnique Federale De Lausanne Station 17 1015 Lausanne Switzerland<br>**theo.lasser@epfl.ch** |
| Professor Helmut MACKE<br>**MC Member**<br>University Hospital Basel Division of Radiological Chemistry, Petersgraben 4 CH-4031 Basel Switzerland<br>**hmaecke@uhbs.ch** | |

| Turkey | |
|---|---|
| Dr Fatih ALGI<br>**MC Member**<br>Canakkale Onsekiz Mart University Canakkale Onsekiz Mart University, Terzioglu Kampusu Fen Edebiyat Fakultesi, Kimya Bolumu Organik Malzeme Lab. TR-17100 Canakkale Turkey<br>**falgi@comu.edu.tr** | |

| United Kingdom | |
|---|---|
| Professor Kevin BRINDLE<br>**MC Member**<br>University of Cambridge 80, Tennis Court Road CB2 1GA Cambridge United Kingdom<br>**kmb@mole.bio.cam.ac.uk** | Professor David PARKER<br>**MC Member**<br>Durham University South Road DH1 3LE Durham United Kingdom<br>**david.parker@dur.ac.uk** |

***IC. Overview activities and expenditure***

## Action D38 - budget from 15-mai-2006 to 31-déc-2009

Generated on 15-déc-2009

**Meetings**

| Meeting Type | Date | Place | Paid part | Cost | Total |
|---|---|---|---|---|---|
| Management Committee | 25-août-2006 | Brussels (BE) | 19 | 9463.7 | |
| Working Group | 23-nov-2006 | Mons (BE) | 3 | 1752 | |
| Working Group | 08-déc-2006 | Basel (CH) | 9 | 4863.21 | |
| Working Group | 08-déc-2006 | Orleans (FR) | 8 | 4741.87 | |
| Working Group | 08-déc-2006 | Basel (CH) | 7 | 4681.76 | |
| Working Group | 14-déc-2006 | Lausanne (CH) | 7 | 2835.72 | |
| Working Group | 10-janv-2007 | Durham (uk) | 4 | 2122.16 | |
| In conjunction with Works | 03-mai-2007 | Eindhoven (NL) | 58 | 31210.16 | |
| Working Group | 29-févr-2008 | Orleans (FR) | 14 | 8164.72 | |
| In conjunction with Works | 27-avr-2008 | Sacavém (PT) | 46 | 32048.6 | |
| Working Group | 16-juin-2008 | Porquerolles (FR) | 4 | 2432.54 | |
| Working Group | 19-sept-2008 | Dublin (IE) | 13 | 7337.09 | |
| Working Group | 03-oct-2008 | Athens (GR) | 18 | 15820.06 | |
| Working Group | 19-févr-2009 | Delft (NL) | 13 | 8316.68 | |
| In conjunction with Works | 25-avr-2009 | Warsaw (PL) | 56 | 41456.23 | |
| Working Group | 25-août-2009 | Cologne (DE) | 18 | 10623.51 | |
| Working Group | 11-sept-2009 | Firenze (IT) | 14 | 9758.35 | |
| | | | | | **197628.4** |

**STSM**

| Beneficiary | Date | From | To | Cost | Total |
|---|---|---|---|---|---|
| Ms Petra Fouskova | 13-nov-2006 | Orleans (FR) | Lausanne (CH) | 1460 | |
| Ms Coralie Thirifays | 19-nov-2006 | Mons (BE) | Torino (IT) | 950 | |
| Ms Kirti Dhingra | 04-déc-2006 | Tuebingen (DE) | 45071 Orleans Cedex 2 ( | 1000 | |
| Ms Malgorzata Norek | 08-janv-2007 | Delft (NL) | Lyon (FR) | 750 | |
| Mr Vojtech Kubicek | 16-janv-2007 | Prague (CZ) | Delft (NL) | 1500 | |
| Ms Aline Nonat | 12-févr-2007 | Grenoble (FR) | Lausanne (CH) | 1130 | |
| Mr Miloslav Polasek | 12-mars-2007 | Prague (CZ) | Coimbra (PT) | 2300 | |
| Dr Esposito Giovanna | 02-mai-2007 | Torino (IT) | Basel (CH) | 2100 | |
| Ms Sara Figueiredo | 10-mai-2007 | Coimbra (PT) | Torino (IT) | 1950 | |
| Dr Jan Plutnar | 01-oct-2007 | Prague (CZ) | Lausanne (CH) | 2000 | |
| Mr Daniel Schühle | 03-nov-2007 | Delft (NL) | Prague (CZ) | 950 | |
| Ms Roberta Napolitano | 12-nov-2007 | Turin (IT) | Cambridge (uk) | 2450 | |
| Mr Frederic Gumy | 16-déc-2007 | Lausanne (CH) | Turku (FI) | 1220 | |
| Ms Stepanka Jankova | 01-nov-2007 | Prague (CZ) | Delft (NL) | 2500 | |
| Dr anne-sophie chauvin | 04-févr-2008 | Lausanne (CH) | Strasbourg (FR) | 640 | |
| Dr Eleni Gourni | 10-mars-2008 | Athens (GR) | Mainz (DE) | 2500 | |
| Ms Anna Hamplova | 01-avr-2008 | Prague (CZ) | Orleans (FR) | 2500 | |
| Dr Petr Hermann | 25-oct-2008 | Prague (CZ) | Orleans (FR) | 1110 | |
| Mr Mihály Purgel | 01-sept-2008 | Debrecen (HU) | La Coruna (ES) | 2500 | |
| Ms EFTYCHIA KOUMAR | 17-nov-2008 | OTWOCK (PL) | Mainz (DE) | 2500 | |
| Ms Agnieszka Majkowska | 01-févr-2009 | Warsaw (PL) | Mainz (DE) | 2400 | |
| Dr Andrea Sabatié | 23-févr-2009 | NANTES (FR) | Prague (CZ) | 1380 | |
| Mr Valery Radchenko | 08-mars-2009 | Mainz (DE) | Athens (GR) | 2060 | |
| Mr André Martins | 23-mars-2009 | COIMBRA (PT) | ORLÉANS (FR) | 1500 | |
| Ms Marianna Fekete | 01-juin-2009 | 4010 Debrecen (HU) | 15100 Alessandria (IT) | 1450 | |
| Dr Yuliy Tyrcho | 08-juin-2009 | Debrecen (HU) | Orleans (FR) | 1450 | |
| Mr Kevin Roland | 06-sept-2009 | Mons (BE) | Torino (IT) | 1100 | |
| Mr João Teixeira Correia | 05-sept-2009 | Coimbra (PT) | Prague (CZ) | 2200 | |
| | | | | | **47 550** |

**Workshops**

| Title | Date | Place | | Cost | Total |
|---|---|---|---|---|---|
| WG D38/04-06 meeting | 23-nov-2006 | Mons (BE) | | 640 | |
| WG D38/03-06 meeting | 08-déc-2006 | Basel (CH) | | 490 | |
| WG D38/05-06 meeting | 08-déc-2006 | Basel (CH) | | 580 | |
| WG 02 meeting MRI Con | 08-déc-2006 | Orleans (FR) | | 800 | |
| WG D38 01-06 meeting | 14-déc-2006 | Lausanne (CH) | | 804 | |
| WG D38/06-06 meeting | 10-janv-2007 | Durham (uk) | | 228 | |
| WG D38/002/06 meeting | 29-févr-2008 | Orleans (FR) | | 1 000 | |
| Action D38 Annual Works | 27-avr-2008 | Sacavém (PT) | | 5 000 | |
| WG D38/04 meeting | 16-juin-2008 | Porquerolles (FR) | | 1 350 | |
| Joint D38 WG1 and WG6 | 19-sept-2008 | Dublin (IE) | | 900 | |
| Joint D38 WG3 and WG5 | 03-oct-2008 | Athens (GR) | | 1 580 | |
| WG D38/02 meeting | 19-févr-2009 | Delft (NL) | | 1 000 | |
| COST D39 Annual Works | 28-avr-2009 | Warsaw (PL) | | 5 513 | |
| COST D38 WG1+WG6 A | 25-août-2009 | Cologne (DE) | | 1 404 | |
| Joint Working Group mee | 11-sept-2009 | Firenze (IT) | | 2 500 | |
| | | | | | **23 789** |

**General Support Grants**

| Title | Date | | | Cost | Total |
|---|---|---|---|---|---|
| | | | | | **0** |

**Schools**

| Type | Date | Place | title | Cost | Total |
|---|---|---|---|---|---|
| | | | | | **0** |

**Honoraria**

| Title | Date | Expert | | Cost | Total |
|---|---|---|---|---|---|
| | | | | | **0** |

**Grant**

| Grant Holder | Date | | | Cost | Total |
|---|---|---|---|---|---|
| | | | | | **0** |

**Dissemination**

| Title | Date | | | Cost | Total |
|---|---|---|---|---|---|
| | | | | | **0** |

**268967.6**

## *II. Scientific Report*

## *II.A. Results achieved up to 31 December 2009*

### *Introduction - Summary of the work performed within each working group*

The main objective of the Action is to increase the knowledge of the chemistry of Metal-Based Systems for Molecular Imaging Applications and to apply this knowledge to the development of novel diagnostic agents and to therapy through an interdisciplinary approach that starting from chemists has involved physicists, biologists and physicians. This Action consists of six complementary Working Groups.

**WORKING GROUP 1, *Optical Imaging.*** Lanthanide luminescent probes suitable for *in cellulo* imaging and visualization of cellular molecular events have been developed. Detection of gene expression, follow-up of cancer treatment, and small animal imaging are the main goals. Important issues involve uptake kinetics, localization, compartmentalization profiles, *in vivo* quenching processes. Targeting specific epitopes is crucial, while fundamental aspects such as luminescence energy transfers or quenching of the probes by metabolites are also receiving attention.

In 2009, multimodal imaging has become a key focus of the working group. Bünzli, Faulkner, Parac-Vogt and Petoud have all published on this topic, and the development of new routes to assemblies and heterometallic arrays continues to prove a productive area of research, both in the area of helicates (where effective imaging in cellulo has been demonstrated conclusively, as can be seen from publications 2,3 and 5 in the list from the working group), and in the area of lanthanide architectures where no less than four new classes of synthetic approach to such systems have been identified during 2009 by working group members (see publications 13-15 and 20 in the list). There will continue to be developments within this field, and WG1 will work in conjunction with other working groups within the action to develop multimodal probes in which the detection limit of the MRI component approaches more closely to that of the luminescent component. Having established a range of complexes that can be used as building blocks for a broad range of applications he working group is also beginning to focus on broadening the clinical utility of luminescent probes for imaging. New approaches to using luminescent complexes to assess transient analyte concentrations (e.g. publications (11,23 and 26) and tag specific cell lines (e.g. are being investigated by all the groups within WG1. Developments in imaging techniques are also being actively investigated, with the focus on optimising two photon excitation processes for imaging with lanthanide complexes, and in developing new detector systems that can finally realise the potential of NIR probes (Strasbourg, and Oxford).

**WORKING GROUP 2, *MRI Contrast Agents.*** Novel Gd(III) and Mn(II) relaxation probes have been designed, synthesized, developed and characterized in depth through a variety of experimental and computational methods. Several innovative ligands for efficient coordination of Gd(III) have been explored: i) a new phosphinic-acid DOTA-like ligand, DO3APBP, containing a geminal bis(phosphonic acid) moiety as a highly effective bone-seeking group; ii) phosphorus acid and Pyridine-N-oxide analogues of $H_4$DOTA; iii) new tripodal picolinate ligands based on TACN; iv) a bifunctional chelator resulting from the attachment of a para-nitrobenzyl substituent to PCTA; v) a new series of chelators suitable for the preparation of $q$=2 Gd-complexes based on the 1,2-HOPO and AAZTA basic structures; vi) a family of bifunctional ditopic chelators by using DOTA-MA derivatives; vi) an easily conjugable, stable GdDOTA-monopropionamide complex with an optimal water exchange rate; vii) tripodal chelates containing hydroxyquinoline-based binding groups; viii) functionalized picolinate Gd-complexes. New macromolecular systems endowed with high relaxivity per particle: micelles and liposomes based on new Gd-conjugated calixa[4]arenes scaffolds; DOTA-phosphonic acid conjugated to $TiO_2$ nanoparticles; GdDOTA-derivatives attached to nanosized mesoporous silicas; gold nanoparticles loaded with ~50 $q$=2 Gd-chelates at the surface. Dual imaging probes based on lanthandes, designed for magnetic resonance and optical applications have

been developed. New advances have been made towards a better understanding of electron spin relaxation in Gd-complexes by combining NMR and EPR data. Density functional theory calculations have been applied to the characterization of MRI contrast agents and Ln(III) luminescent probes with potential application for *in cellulo* imaging. Finally, new important insights have been gained on the effects of small endogenous ligands (citrate, phosphate, hydrogen-carbonate, amino acids) on the rates of decomplexation and on the relaxation properties of Gd-complexes with DTPA and DOTA derivatives.
These activities have been the object of over 45 publications in the current year, ca. 40% of which resulting from collaborative work within the WG or with teams of other WGs. Each group within the WG has an intense research activity both inherent to the Action D38 general objective and to related topics. All these research activities are financially supported by several grants raised by the different teams independently and/or jointly. We expect that all these efforts will result in the availability of more efficient MRI probes suitable for current scanners (high magnetic field strength), for molecular imaging applications (high sensitivity) and safer for *in vivo* use (significant advances in the understanding of the equilibria involving a Gd-based CA in a physiological medium).

**WORKING GROUP 3, *Nuclear Imaging probes*.** New chemistry based on the use of radioactive metal chelates to provide innovative solutions is the main task. Radiopharmaceuticals based on positron emitters like $^{89}$Zr, $^{64}$Cu and $^{68}$Ga are under intense scrutiny. The development of a PET radionuclide generator $^{44}$Ti/$^{44}$Sc, adapted to imaging of long biological processes, is undertaken. Biological evaluation of the radiometal-labelled compounds is an essential part of the activities. The first operational 5 mCi $^{44}$Ti/$^{44}$Sc generator system, suitable for the production of sufficient activity for human PET-studies, has been established in Mainz. Successful labelling of DOTA-conjugated peptidic structures have been performed in cooperation between the group of Mainz and Warsaw. Novel chelators have been synthesized and tested for the complexation of Gallium-68. The in vitro evaluation of arsenic-labeled biomolecules have been performed in binding studies of free arsenic to cancer cells and in internalization studies of Arsenic-74 labeled bevacizumab.
The labeling of DOTATOC with $^{44}$Sc and DOTATATE with $^{46}$Sc were performed. The obtained radiobioconjugates exhibited high stability in saline and PBS buffers. Studies of new chelators for effective binding of $^{89}$Zr to biomolecules were also undertaken. Scandium radioisotopes have become very interesting both for targeted radiotherapy and diagnosis. However, information about behaviour of scandium(III) complexes in aqueous solution are rather scarce. A common project between Subatech and Prague's lab was focused on determination of stability constants of scandium(III) complexes with the ligands commonly considered as suitable for applications in nuclear medicine.

**WORKING GROUP 4, *Nanosized probes.*** The aim of this workgroup is a manifold and comprehensive study of various nanosized systems for molecular imaging. Different kinds of nanoprobes are developed: (i) lanthanide oxide nanoparticles (ii) multifunctional hybrid nanoparticles, (iii) vectorization of iron oxide nanoparticles to target different pathology; (iv) "Lipocest" based on (Chemical Exchange Saturation Transfer); (v) liposomes or lipid-based nanoparticles.
With the aim of non-invasively trace neurons using the magnetic resonance imaging

(MRI) method, iron oxide nanoparticles, acting as negative MRI contrast agents, were covalently linked to Wheat Germ Agglutinin (WGA), a lectin used as a neuronal tracer in histological tracing experiments. Micellar calix[4]arene systems have been investigated that show a very high relaxivity (27.9 $s^{-1}mM^{-1}$ at 20 MHz and 37 °C). Methods to prepare liposomes incorporating this calixarene derivative in the membrane have been exploited. Gold nanoparticles and core/shell nanoparticles with a core of lanthanide oxide and a shell of polysiloxane (possibly luminescent) have been synthesised for the detection by luminescence, magnetic resonance (MRI) or scintigraphy. These techniques are combined with therapeutic applications (radiotherapy, neutrotherapy and curietherapy). Detailed in vitro studies focused on the consequences of contrast agent binding, cellular internalization and compartmentalization for the observed relaxometric properties. The research on nanovesicles-based (liposomes and polymersomes) CEST agents has been continued leading to interesting results and new applications.

**WORKING GROUP 5, *Targeted Probes***. The development and evaluation of new targeted probes in all fields of molecular imaging (optical, MRI, nuclear medicine, ultrasound) is the task of this WG. The development of specific and high affinity targeting agents with optimal pharmacokinetics is instrumental to an early diagnosis. Several targets have been addressed including somatostatin receptors, sialic acid, and hydroxyapatite on tumor bones.

An apoferritin-based nanocarrier (Mn-Apo) has been investitaged as highly efficient targeted MRI probe. By containing up to 300-400 Mn(II) aqua ions encapsulated in the inner cavity, it yielded a remarkable relaxivity value (per apoferritin) of ca. 4000-7000 $mM^{-1}s^{-1}$. Mn-Apo being formed by endogeneously occuring molecules and ions displays a high biocompatibility. It targets hepatocytes with high efficiency and is able to discriminate healthy cells from hepatocarcinoma ones.
Cell penetrating agents have been developed for intracellular MR imaging.
A series of peptide-based probes have been investigated for cancer imaging. These studies included exendin-4-based radiopharmaceuticals for glucagon-like peptide-1 (GLP-1) receptor PET/CT and SPECT/CT imaging, $^{99m}$Tc labeled [$Lys^{40}$(Ahx-HYNIC-$^{99m}$Tc)$NH_2$]Exendin-4 as potential radiopharmaceutical for insulinoma diagnostics, radiolabelled Bicyclic Somatostatin-based Analogues for SPECT/PET Imaging of Neuroendocrine Tumors, a comparative study of a Dota-Bombesin analog labeled with Y-90, Lu-177, Ga-68 and Sc-44, for targeted radiotherapy, radiochemical and preliminary in vitro evaluation and the assessment of the efficacy of radionuclide treatment DOTATATE Y-90 in patients with progressive metastatic gastroenteropancreatic neuroendocrine carcinomas (GEP-NETs) (a phase II study).
Apoptosis imaging agents, like $^{99m}$Tc-labeled HYNIC-cys-annexin A5 have been investigated. Studies on bone targeting were pursued using phosphinic-acid DOTA-like ligands. The in vivo investigation of radiolabeled self –assembled nanoparticles of modified dextrin (dexC16) was also reported.

**WORKING GROUP 6, *Responsive Probes.*** The research activity within this Working Group has led to the design, synthesis, and characterization (*in vitro*, *in cellulo*, and *in vivo*) of a wide array of metal-based systems of interest for MRI and Optical imaging modalities able to generate/modulate a signal/contrast in response to a specific physico-

chemical variable of their environment. The great potential and versatility of NMR/MRI techniques has been fully exploited and probes able to generate a different signal/contrast ($T_1$ relaxation, CEST, heteronuclear detection) have been produced and tested. Similarly, the peculiar emissive properties of lanthanide-based metal complexes have been extensively used in order to improve their responsiveness characteristics.
As one of the relevant issues for responsive probes is represented by the difficulty to get an imaging response independent from the probe concentration, particular attention has been devoted to the development of multimodal agents, either designing systems able to generate different signal/contrast in the same imaging modality (*e.g.* multi-emissive probes in Optical Imaging or CEST agents in MRI) or developing multi-modal probes for hybrid imaging technologies (*e.g.* PET-MR or MRI-OI).
Despite the relatively small number of members, the quality of the research carried out within this WG is witnessed by over 30 publications.
The collaboration within this WG and with the other teams of the Action is constantly growing up and a higher number of joint papers are expected to be published in the next period.
The framework of this collaborative networking is mainly based on the high complementarity level between the expertise and skills of the WG teams that have been also shared with the other partners of the Action (e.g. organization of joint meetings, STSM).
The cooperation among the WG teams is also reinforced thanks to other collaborative ongoing projects and new future proposals are in the pipeline.

In the following the detailed activities of each working group are reported.

## WORKING GROUP 1: Optical Imaging

The **Birmingham** group (**Pikramenou**) has focused on sensor development using luminescence techniques, and has developed effective methods for intercalative recognition of DNA using Pt(II) hairpin complexes in combination with cyclodextrins.

The **Lausanne** group (**Bünzli**, Chauvin) has focused on three main axes. Firstly it demonstrated that the bimetallic luminescent helicates used for *in vitro* cell imaging and DNA analysis during the past two years can be developed into a full class of bioprobes with a wide range of applications: the excitation wavelength has been extended into the visible range, the helicates have been successfully bioconjugated with avidin and several antibodies, the feasibility of multiphoton excitation has been proven and the helicates are presently being introduced into nanoparticles. The second focus dealt with tightening the collaboration with **Dr I. Hemmilä** (**PerkinElmer**) and improving the time-resolved microscope on loan from Wallac Oy by simplifying its design, incorporating a more powerful light source and by introducing the possibility of changing the time delay. Finally, most of the efforts of this team have been devoted to specifically detect biomarkers expressed by cancerous cells and tissues with antibodies conjugated to lanthanide luminescent bioprobes ($Eu^{III}$, $Tb^{III}$), either commercially available, or based on bimetallic helicates. This aspect was conducted in collaboration with Prof. H.-A. Lehr (Pathology Laboratory, Lausanne University Hospital) and Prof. M. Gijs (Microtechnics

Laboratory, EPFL). Lab-on-a-chip devices were produced in which live cells can be cultured and grow rapidly thanks to a specially optimized protocol. When in contact with sections of cancerous tissues, immunohistochemical analyses can be conducted on the chips with a large gain in time and reactant consumption with respect to classical procedures. In addition simultaneous detection of two biomarkers is feasible. In the left part of the figure below, a goat anti-rabbit IgG antibody conjugated to a $Tb^{III}$ helicate detects Her2/*neu* receptors expressed on the membrane of breast cancer cells while a goat anti-mouse IgG antibody conjugated to the $Eu^{III}$ helicate illuminates estrogen receptors expressed by the nucleus membrane. The concentrations of these two receptors determine the therapy to be used. Systematic tests of a large number of tissue sections are presently under way as well as the development of an analytical integrated device for use by medical doctors.Finally, collaboration with Prof. L. Helm (WG2) has resulted in the publication of a joint paper on dual MRI/luminescent probes.

The **Leuven** group (**Parac-Vogt, Binnemans**) have begun to develop multi-modal probes. The initial aim of the Leuven group was the exploitation of Eu-tetracycline (EuTC) complex as an optical probe for the sensing of hydrogen peroxide. The results pointed out that the peroxide enhanced EuTC luminescence is most likely due to partial oxidation of TC which proceeds via radical mechanism, complicating the straightforward application of EuTC. The alternative routes, in which radical pathways do not occur, are currently being explored. An ongoing research activity of the Leuven group which fits in the frame of the COST project, is the development of bi-modular agents with contrasting and luminescent properties. We have synthesized a series of novel ditopic ligands by derivatizing the DTPA moiety, which serves as a ligand for gadolinium(III). The secondary coordination group, such as 8-hydroxychinoline, catechol or phenantroline, was successfully introduced into the DTPA moiety. A series of transition metal ions with luminescent properties were incorporated into the Gd-complex resulting in an agent with contrasting and luminescent properties. We are currently exploring physico-chemical and biological properties of these novel complexes.

The **Oxford** group (**Faulkner**) has continued to develop the use of multi-modal probes as tools in imaging, and has developed a number of new routes to access heterometallic lanthanide complexes. Studies on NIR luminescence and the development of methods for imaging lanthanide containing systems continue- development is now limited by the available technology, and the current focus in this area is on the development of new instrumentation in collaboration with the Lasers for Science Facility at the Rutherford Appleton Laboratory. Possible upconverting systems based on heterometallic complexes are also being explored.
Great advances have been made in synthetic methodology; these include the application of a variety of Click reactions to conjugation of lanthanide complexes, the use of orthogonal protecting groups to mask binding sites in symmetrical arrays, and the development of bimodal tetrametallic probes which incorporate two different kinds of lanthanide ions and an azo dye chromophore.

The **Petoud** group (**CNRS**) has made a significant contribution in their first year as part of the working group. Key breakthroughs include the use of micellar systems to contain

and control chromophores, and the combination of nanotubes with luminescent lanthanides to develop a new approach to oxygen sensing.

The **Strasbourg** groups (**Ziessel** and **Charbonniere**) have developed a modular approach to complex design that enables them to control the luminescence properties of a wide range of systems. In the process, they have begun to develop families of multi-modal imaging agents. Both groups continue to collaborate extensively within the network.

## WORKING GROUP 2: MRI Contrast Agents

The group of ***Joop Peters***, in collaboration with the groups in Mons, Orléans and Prague, has evaluated conjugates of calixa[4]arenes and lanthanide chelates for their potential as MRI contrast agents. The compounds appear to have a strong tendency to form densely packed micelles with a very high relaxivity (p.e., $r_1 = 31.2\ s^{-1}\ mM^{-1}$ at 25 ºC and 20 MHz, with a density of relaxivity of 39.2 $(g\ l^{-1})^{-1}\ s^{-1}$, one of the highest reported up to now). A derivative with two $C_{18}$ aliphatic chains attached to calix[4]arene, was incorporated into the membrane of liposomes (8.8% loading of calixarene) The calixarene facilitates water diffusion through the lipid bilayer resulting in an almost three times enhanced relaxivity ($21.2\ s^{-1}mM^{-1}$ at 20 MHz and 37 ºC) compared to common paramagnetic liposomes. Those vesicles are promising canditates for molecular imaging applications of tumours due to their high relaxivity per particle. In collaboration with the groups in Prague and Mons, the $Gd^{3+}$ complex of a new phosphinic-acid DOTA-like ligand, DO3APBP, containing a geminal bis(phosphonic acid) moiety as a highly effective bone-seeking group, was evaluated. The relaxivity of the Gd-DO3APBP complex ($r_1 = 7.4\ s^{-1}\ mM^{-1}$, 20 MHz/25 ºC/pH=7.5) is unexpectedly high as compared with other monohydrated chelates of similar size thanks to a significant contribution from the second hydration sphere. The $\tau_M^{298}$ is 198 ns. Further increase in the relaxivity was observed in the presence of Zn(II), Mg(II) or Ca(II) ions, due to formation of coordination polymers. Slowing down of the tumbling rate of the Gd-DO3APBP complex upon adsorption on hydroxyapatite also leads to an increase of the relaxivity ($r_1 = 17\ s^{-1}\ mM^{-1}$, 20 MHz/25 ºC/pH= 7.5).

The group of ***Ivan Lukeš*** has focused the activity on three main topics: 1) Investigation of relations between structure of CA and their efficiency; 2) Conjugation of CA with organic or inorganic carriers; 3) CA's based on Mn(II) complexes.
1) In collaboration with Delft, Mons and Coimbra a paper was published on the role of the second hydratation sphere. For this purpose several tetraphosphorus DOTA derivatives were synthesized and studied. In a different study, a new ligand ($DOTAP^{BP}$) was synthesized and its Ln complexes studied. $GdDOTAP^{BP}$ showed a high dependence of relaxivity on ions, such are Mg(II), Ca(II) and Zn(II), in solution. This dependence is explained by formation of oligomeric species that significantly affects the rotational correlation time. 2) the research was mainly oriented to complete the study on the $DO3A^{pyO}$ complexes to the conjugation of the ligand with dendrimers. A comparative study of the Ln complexes of PAMAM conjugates with $DO3A^{pyO}$ and $DO3AP^{BnNH2}$ showed

that the different MRI properties are caused by a different internal rotation of the conjugates. Furthermore, a collaborative work with Delft on conjugates of calixa[4]arenes and lanthanide chelates was carried out. With the same group (and with Mons) $TiO_2$ nanoparticles were tested as inorganic carrier. A new ligand $DOTA^{PhP}$ bearing phosphonic acid in a side arm was synthesized and found to very efficient for anchoring the surface. The project will continue during next year. 3) Initial studies were performed in collaboration with Orléans.

The group of ***Pascal Fries*** has prepared a new cyclodecapeptide incorporating two prolylglycine sequences as β-turn inducers and carrying four side chains with acidic carboxyl groups for cation complexation. The experimental water relaxivities are undoubtedly 30% higher than the expected values for this complex. This leads the team to propose the existence of a large second-sphere (2S) contribution to relaxivity, which is due to the interaction of water molecules with the hydrophilic peptide ligand via hydrogen-bonding.
New tripodal picolinate ligands based on the 1,4,7-triazacyclononane anchor were also prepared and their lanthanide complexes characterized by NMR, fluorescence and potentiometric studies. The resulting water exchange rate is optimized for the future design of high relaxivity macromolecular gadolinium based contrast agents with values measured by $^{17}O$ NMRD falling in the range of optimum values of (30 to 50)$\times 10^6$ $s^{-1}$ predicted by the SBM theory. The high luminescence efficiency is also retained for the terbium complex.

The group of ***Erno Brücher*** studied the effect of the small endogenous ligands (citrate, phosphate, hydrogen-carbonate, amino acids) on the rates of decomplexation and relaxation properties of the Gd(III)-complexes with DTPA and DOTA derivatives. The rates of transmetallation reactions of Gd(DTPA) and Gd(BOPTA) with Zn(II) and Cu(II) slow down, while those of the Gd(DTPA-BMA) increase in the presence of citrate and phosphate. The rates of dissociation of the macrocyclic Gd(DOTA) and Gd(HP-DO3A) are not affected by the presence of the small endogenous ligands. The relaxation properties of the Gd(III)-complexes formed with the heptadentate DOTA derivatives in the presence of different endogenous ligands are mainly determined by the hydrogen-carbonate ions, which form ternary complexes and because of their large concentration, successfully compete with the other ligands. In another study, the attachment of para-nitrobenzyl substituent to PCTA was found to give a bifunctional chelator with fast formation kinetics towards Ln(III), Y(III) and In(III) ions. The fast formation and slow acid catalyzed dissociation of Ln($NO_2$-Bn-PCTA) complexes makes the $NO_2$-Bn-PCTA ligand suitable for labeling biological vectors with radioisotopes for nuclear medicine applications. The attachment of amide substituents to the cyclen macrocycle resulted in tris(amide) type ligands that form complexes with Ln(III) ions that has high hydration number ($q > 1$), fast water exchange rate and reasonable kinetic inertness. The presence of an antenna in these ligands makes the Ln(III)-complexes potentially useful as Optical Imaging probes, while the high relaxivity and fast water exchange are satisfy the most important requirements of the MRI contrast agents. The Debrecen group collaborates with the groups of Torino and Alessandria as well as with the group of Sherry (University of Texas at Dallas).

The main results achieved by the group of ***Carlos Platas-Iglesias*** were: i) the design, synthesis and full physicochemical characterization of new structural entries for the design of new Gd(III)-based contrast agents with potential application in MRI; the new ligands prepared are based on macrocyclic platforms, which is expected to provide an important kinetic stability of the corresponding Gd(III) complexes. This work was carried out in collaboration with the group of Éva Tóth. ii) the use of quantum chemical calculations (density functional theory calculations) for the characterization of potential MRI contrast agents and related systems; iii) the use of quantum chemical calculations for the characterization of lanthanide(III) luminescent probes with potential application for *in cellulo* imaging. This work was carried out in collaboration with Ziessel´s group (WG 1); iv) The structural characterization of Ga(III) and In(III) complexes of ligands based on triazamacrocycles with potential application in positron emission tomography (PET). This research was done in collaboration with the group of Joop Peters.

The main results in the group of ***Mauro Botta*** can be summarized as follows: **a**) A new series of 1,2-HOPO-based complexes of $Gd^{III}$ has been prepared and characterized. The occurrence of $q$=2, high $k_{ex}$ and high stability provide support for their use as precursors for high relaxivity MRI CA for current and future clinical applications. New $Gd^{III}$-HOPO complexes featuring a mesitylene-derived ligand cap have been prepared. Relaxometric characterization reveals that the complexes tend to form large aggregates in solution with slow tumbling rates and unique pH-dependent relaxivities which may prove useful in tissue pH mapping. **b**) Optimization of the paramagnetic probe for conjugation to macromolecular scaffolds. New systems were designed, synthesised and characterized by NMR relaxometry: i) bifunctional agents based on the AAZTA structure with hydroxyl, carboxyl and chloro functional groups; ii) The Ugi four component reaction was exploited to obtain in a single synthetic step bifunctional ditopic chelators by using DOTA monoamide derivatives as amino and acid components; iii) An easily conjugable, stable GdDOTA-monopropionamide complex with an optimal water exchange rate exhibits enhanced relaxivity (> 120 %) upon formation of macromolecular aggregates. **c**) macrocyclic Gd(III) complexes were grafted on mesoporous silicas characterised by different particle size, shape and pore dimensions which influence number, localization and relaxivity of the grafted paramagnetic complexes. **d**) With **Debrecen** a new octadentate chelator comprising a 1,4-diazepane ring and two iminodiacetic groups and showing an unprecedented selectivity in the complexation of heavy Ln(III) cations has been prepared and characterized. With **Aime (WG 5)** and **Parker (WG 6),** the relaxometric properties of various Gd complexes have been investigated.

The group of ***Marinella Mazzanti*** has continued the development of small lanthanide chelates and the study of their relaxivity and photophysical properties. A particular attention has been devoted to the design of ligands that can lead both to mono and bis-aquo gadolinium complexes with high stability and to highly luminescent lanthanide complexes emitting in the Visible and Near-IR range. Particularly high stability in water has been obtained with tripodal chelates containing hydroxyquinoline-based binding groups. Moreover, these podates have shown a strong variation of the relaxivity with the pH. In our continued study of tetrazolate-based complexes we have demonstrated that the luminescence properties (such as excitation wavelength and quantum yields) of tetrazolate-based ligands can be effectively tuned by ligand substitution. We have also

made important progress in the development of synthetic routes for the functionalization of the picolinate complexes of Gd having one or two water molecules coordinated to the metal. The thiol group has appeared as the most versatile for peptide binding and for the grafting of lanthanide complexes on quantum dots. The first MRI experiments have been performed on peptide bound complexes and we have found that these systems are very promising for cellular imaging. We are planning to apply this strategy also to other stable bis-aquo complexes recently developed in our laboratory. We have also shown that highly stable Ln(III) complexes can be easily included in silica nanoparticles for the development of multicolour labels.

The research results of the group of ***Lothar Helm*** published in 2009 can be divided into two branches: first towards a better understanding of electron spin relaxation in Gd-complexes and second the development of high field contrast agents. Two $Gd^{3+}$ complexes with new tripodal picolinate based ligands have been studied (in collaboration with the Grenoble group) and it has been found that the electron spin relaxation properties are surprisingly different. While the electron spin relaxation remains for $[Gd(ebpatcn)(H_2O)]$ at small values which should not affect relaxivity in macromolecular complexes, experimental data indicate a rather fast electron spin relaxation for the phosphonate containing complex $[Gd(pbpatcn)(H_2O)]^-$. An EPR study of glasses and magnetically dilute powders of $[Gd(DTPA)(H_2O)]^{2-}$, $[Gd(DOTA)(H_2O)]^-$ and macromolecular P792 was carried out at the X- and Q-bands and at 240 GHz. The results show that the zero-field splitting parameters for these complexes are quite different in a powder as compared to the frozen aqueous solution. In several complexes, an inversion of the sign of the axial component D of the zero field splitting is observed, indicating a significant structural change. New mid-size complexes with two water molecules in the first sphere of $Gd^{3+}$ have been synthesized and tested. Very high relaxation enhancements (per particle) can be achieved by building gold nanoparticles loaded with ~50 Gd-chelates at the surface. These compounds open a new way for building high relaxivity contrast agents for molecular imaging.

The group of ***Eva Toth*** has been active in the development of enzymatically activated MR imaging agents. We also continued to work on Ln-based probes, including PARACEST agents, in the objective of detecting $Ca^{2+}$ concentration, in collaboration with MPI Tubingen. New lanthanide-based systems have been explored for dual modality imaging applications, combining optical and MRI techniques. We reported prototypes of a versatile scaffold for $Ln^{3+}$ complexation where MRI and luminescence requirements are both satisfied using the same ligand. Recently we proposed an alternative strategy for the rapid development and screening of luminescent lanthanide compounds and sensitizers. Our approach was to incorporate the hydrophobic chromophore in a micelle that consists of amphiphilic chelates of the luminescent lanthanide and to use the energy transfer between the two non-covalently linked moieties. This concept combines several advantages: i) the use of hydrophobic chromophores including large aromatic moieties with high level of conjugation that are poorly soluble in aqueous solution, ii) the use of chromophores that do not possess binding groups for the formation of coordination bonds with the lanthanide cations, iii) the formation of polymetallic lanthanide compounds.

In an effort to gain more insight into the relation between ligand structure and relaxivity, we have investigated $Gd^{3+}$ complexes based on new ligand scaffolds with respect to their hydration state, water exchange rate and rotation (in collaboration with C. Platas-Iglesias, E. Brucher and C. Geraldes). In collaboration with Prague, we have developed new pyridine-containing macrocyclic ligands for $Mn^{2+}$ chelation which ensure relatively high stability and bishydration.

**WORKING GROUP 3: *Nuclear Imaging Probes***

**MAINZ:**

Chemistry

1. *Radioisotope Production* The first operational 5 mCi $^{44}$Ti/$^{44}$Sc generator system, suitable for the production of sufficient activity for human PET-studies, has been established in Mainz. Successful labelling of DOTAconjugated petidic structures have been performed in cooperation between the group of Mainz and Warsaw.

2. *Novel Chelators for Gallium-68*

a. Mono and bifunctional N3S3-type chelators have been synthesised and characterised with respect to labelling conditions, stability of the radio-chelate and octanol/water partition coefficient. Ga-68 chelates derived from these precursors might provide sufficient lipophilic properties for the penetration of the blood brain barrier and thereby facilitate neuro-imaging with a positron emitting metal nuclide.

b. Various azomethin-containing open chain chelators have been synthesised and labelled with Ga

68. The obtained radio-chelates provide affinity to the efflux transporter p-glycoprotein. The compounds were furthermore investigated as imaging agents for the p-glycoprotein status.

c. A convenient and time effective method for the generation of the n.c.a. labelling agent [$^{68}$Ga]Ga(acac)3 has been developed. This second generation solid-phase purified labelling agents facilitates Ga-labelling under mild, anhydrous conditions.

d. Several synthetic porphyrin and chlorine containing natural products have been labelled with Ga68 using microwave enhanced reaction conditions. These new labelled compounds may serve as tumor localising agents and provide a tool for the monitoring of photodynamic therapy. Furthermore, these agents retain affinity to arteriosclerotic plaques.

In vitro and in vivo evaluation of radiolabelled probes

- A set of azomethin containing imaging agents have been screened for their affinity to pglycoprotein in an in vitro cell assay. Chosen highly potent candidates were furthermore evaluated in healthy controls and tumor bearing rats.
- A preliminary human study has been performed with a Ga-68 labelled amino acid derivative. This class of compounds may combine the availability of the positron emitter Ga-68 with the clinical relevance of an amino acid-transporter targeting radioprobe.
- Various polypyrrol structures have been evaluated in vitro to assess the stability of the corresponding Ga-complexes as well as affinity to serum proteins. Furthermore, an in vitro model for active transport of the imaging agent into arteromatous plaques has been conducted.

1. Development of online post-processing of $^{68}Ge/^{68}Ga$ generators providing $^{68}Ga$ synthons for labelling lipohilic compounds under unhydrous conditions
2. Synthesis and evaluation of $^{68}Ga$-porphyrines
3. Development of new bifunctional triaza- and tetraaza-based chelators for $^{68}Ga$ conjugation and labelling chemistry
4. Synthesis and evaluation of $^{68}Ga$-Schiff´base derivatives for imaging p-glycoprotein-related effects in tumor biology
5. Synthesis and evaluation of $^{68}Ga$-DOTA-based amino acids for tumor detection and first human application
6. Synthesis and evaluation of $^{68}Ga$-DOTA-based bisphosphonates for imaging bone deseases and first human application
7. Synthesis and evaluation of $^{177}Lu$-DOTA-based bisphosphonates for palliation of bone diseases and first human application
8. Synthesis and evaluation of $^{68}Ga$-DOTA-based glucosamines for imaging inflammation
9. Design, construction and evaluation of a 5 mCi $^{44}Ti/^{44}Sc$-generator
10. Labelling of DOTA-conjugated octreotides with $^{44}Sc$ and first human application of $^{44}Sc$-DOTA-TOC

**ATHENS**

In vitro evaluation of arsenic-labeled biomolecules *Cell lines used:* The human breast cancer cell lines MGFP, M121 and M165 were used for this work. The cancer cell line MGFP served as the positive control and the cancer cell lines M121 and M165 are transfected with the isoforms VEGF121 and VEGF165, respectively. MDA-MB-231 (human, Caucasian, breast, adenocarcinoma) cells infected with virus expressing either GFP as a control, VEGF 121 or VEGF 165. The virus was made in Pheonix cells using the plasmid pLXRSpBMN-IRES-GFP. The VEGF clone is human.

*1. Binding of free arsenic to cancer cells*

It is known that free arsenic is used as a drug for the treatment of leukemia. It is trapped into the cancer cells and destroys them, playing a therapeutic role. In our case we tested if it is possible to internalize free arsenic into the breast cancer cells MGFP, M121 and M165. Preliminary studies showed that the rate of internalization of free arsenic was found to be time-dependent.

*2. Internalization studies of Arsenic-74 labeled bevacizumab*

Internalization experiments were performed using the cancer cell lines MGFP, M121 and M165. We were unable to measure the surface-bound and internalized activities since the radioactivity of the added Arsenic-labeled antibody was not sufficient. In another experiment which was performed, we were unable to obtain a stable radiochemical product. This in turn could not be used for internalization experiments. Alternative modifications of the antibody have lead to a stable product, which will in turn be used for in vitro evaluation studies, and in vivo assessment in tumor models developed with the above-mentioned cell lines.

Angiogenesis is a vital process in the growth and metastasis of tumors. The development of anticancer multipotential diagnostic and therapeutic tools, such as radio-immuno-magnetic conjugates, that target the angiogenic process is an area of major growth in

oncology. Bevacizumab (Avastin®, Roche) is a humanized monoclonal antibody which binds all VEGF-A isoforms. In recent studies it has been shown that radiolabeled bevacizumab accumulates in VEGF-expressing tumors with high specificity.Our efforts are focused on targeting VEGF-A with Bevacizumab-conjugated Ferromagnetic Nanoparticles (bc-FNs), thus leading to a highly-specific targeted contrast agent that could be utilized in Magnetic Resonance Imaging (MRI) applications. An iron oxide compound, namely magnetite $Fe_3O_4$ was employed as the FN constituent mainly due to its almost ideal biocompatibility. Incubation of varying concentrations of bevacizumab with the $Fe_3O_4$ FNs via a DMSA mediator led to the formation of Bc-FNs. The conjugation between the FNs and Bevacizumab was directly demonstrated by means of UV-vis spectrophotometry in the supernatant samples that were drawn under magnetic retraction of the Bc-FNs. In addition, the modification of the magnetic properties of the formed Bc-FNs was investigated in great detail around human body temperature conditions by means of a Superconducting Quantum Interference Device (SQUID) magnetometer. Finally, the binding efficiency of Bc-FNs was investigated by means of standard immunostaining tests with FITC-goat anti-human IgG in the VEGF-165 transfectants M165 of human breast cancer cells since they overexpress the cell-associated 165 isoform of VEGF.

Our results give strong evidence for the efficient formation of Bc-FNs that meet the three cornerstone prerequisites for medical applications that is (i) efficient magnetic properties, (ii) high binding affinity and, (iii) good specificity to relevant cancer cell lines. Thus, the Bc-FNs examined in this work deserve further investigations in both *in vitro* and *in vivo* applications as contrast agent in MRI.

**PRAHA:**

A method for determination of thermodynamic stability and kinetic parameters of Ga(III) macrocyclic complexes was found. Bifunctional phosphinic acid derivative of triazacyclononane was synthesized and proved to complex Ga(III) fast in stable and inert complexes. Ln(III) complexes of tetraphosphorus acid cyclen derivatives were proved to be kinetically inert and stable in vitro/in vivo. Ln(III) complexes of mono(pyridine-*N*-oxide) derivative of DO3A exhibit similar properties to those of DOTA and can be conjugated to a larger systems (PAMAM). A successive substitution of acetate for methylphosphonate pendant arm in DOTA skeleton leads to a lower kinetic inertness of their Cu(II) complexes.

**PRAHA / MAINZ**

A novel ligand containing three phosphinate groups (for Ga chelation) and three carboxyl groups
(for bioconjugation) was synthesized. Chemical properties were evaluated, including: (i) full
analytical characterization; (ii) crystal structures with Ga(III) and Fe(III); (iii) potentiometric
determination of protonation constants and complex stabilities with Ga(III), Cu(II), Zn(II), Mg(II),
Ca(II), Gd(III) and Ln(III). The ligand combines the following properties: (i) high thermodynamic/kinetic stability and fast formation kinetics for Ga(III) complexes; (ii) extremely facile and efficient synthesis; (iii) possibility of conjugation to biomolecules

without necessity of protecting groups. A simple synthetic protocol for preparation of peptide conjugates was developed and proof of principle delivered by synthesis of four amino acid conjugates as model compounds. $^{68}$Ga labelling properties for the free ligand were investigated in Mainz, showing superior complexation behaviour compared to established ligands DOTA and NOTA. Similar investigation of the conjugates is under way.

**PRAHA collaboration** with J. A. Peters, Delft, NL and C. Platas-Iglesias, A Coruna, ES)

Structural study of complexes of triaza-macrocycle based ligands with N3S3 donor set

Two new ligands 1,4,7-triazacyclodecane based N3S3 ligands have been synthesized by reaction of the free triazacycloalkanes with ethylene sulfide. These are model compounds for novel bifunctional chelators. Purpose of study was investigation of complexation behaviour. Complexes with Ga(III), In(III) and Fe(III) have been characterized by single crystal X-ray diffraction. Solution structures of Ga(III) and In(III) complexes of the new ligands have been investigated employing NMR spectroscopy and DFT calculation.

1. Ga(III) complexes. We continue research on phosphinic acid derivatives of 1,4,7-tirazacyclononane (tacn). In collaboration with the Mainz and TU Munich groups we have finished investigations of the tris(2-carboxyethylphosphinic acid) tacn derivative showing a very fast and efficient complexation of non-carried added $^{68}$Ga even under highly acidic conditions (directly in eluate from $^{68}$Ga/$^{68}$Ge generator) or at pH up to 5. The derivative is very suitable for formation of multimeric peptide conjugates; the conjugation chemistry is under current investigation. The chemistry connected with the work has been submitted for publication.[1] To understand the properties of the ligand, other simpler phosphinic acid derivatives (with H and Ph substituents on phosphorus atom) of tacn have been synthesized and are now investigated. In collaboration with the Mainz group, a utilization of DOTA bis(phosphonate) derivatives for PET imaging of calcified tissues with $^{68}$Ga was investigated. The derivative having one-carbon spacer between DOTA-monoamide moiety and bis($PO_3H_2$) group was shown to be the most suitable. Its $^{68}$Ga complex has been even successfully used in one patient and results were encouraging; the results have been accepted as “Image of Month”.[2] In collaboration with the Orleáns group, a method for determination of stability constants and kinetic inertness of gallium(III) complexes with macrocyclic ligands was developed and tested on Ga(III)-DOTA and Ga(III)-DOTAmonoamide systems; data are ready for publication and has been presented on COST D38 WG3+WG5 Workshop in Florence.[3]

2. Lanthanide(III) complexes. In collaboration with the Delft, Mons and Coimbra groups, lanthanide(III) complexes of DO3A-phosphinic acid derivative with bis(phosphonate) side arms were investigated for a bone-targeting. They show a good affinity for hydroxoapatite and the ligand exhibited a fast complexation.[4] In continuation of search for other ligand types, we investigated properties of differently phosphor-substituted tetrakis(phosphorus acid) derivatives of DOTA. The complexes are thermodynamically much less stable than that of DOTA but they are sufficiently kinetically inert and they are highly hydrophilic as given by the presence of a rich second-hydration sphere.[5] Biodistribution of complexes of monophosphorus acid derivatives of DOTA was investigated in collaboration with the Hradec Králové (Prof. A. Lázničková; member of COST BM607 Action; $^{111}$In and $^{90}$Y complexes) [6] and Sacavém groups ($^{153}$Sm and $^{166}$Ho complexes) [7] and the biodistributions were shown to be fully similar to the profiles of the DOTA complexes. In

collaboration with the Brno group (Dr. Přemysl Lubal) we showed that lanthanide(III) complexes of monopyridine-*N*-oxide DOTA derivatives are stable and are formed somewhat faster than the DOTA complexes; we also uncover a new kind of isomerism in complexes of DOTA-like ligands.[8] In collaboration with the Nantes group, we investigated Sc(III) complexes of the common polyamino-polycarboxylate ligands (DTPA, DOTA, NOTA, EDTA) as there is no information about solution behaviour of even the simplest Sc(III) complexes. We found a method for determination of stability constants and confirmed the speciation by multinuclear NMR measurements. In collaboration with the Sacavém, Coimbra and Orleáns groups, we investigated lanthanide(III) complexes of bis(phosphonate) derivative of DOTA, *trans*-DO2A2P; combine thermodynamic, kinetic and structural (both in the solid state and in solution) showed intermediate properties between complexes of DOTA and DOTP and the data are ready for publication.[9]
3. Copper(II) complexes. Research was focused mainly on cyclam derivatives. We showed (in collaboration with the Brno group) that complex of fully substituted cyclam derivatives (2 methylphosphonate and 2 methyl groups on nitrogen atoms) are not kinetically inert and, so, not suitable for radiochemical applications.[10] Differently phosphonate-substituted cyclam derivatives are under investigation. It was also shown that a higher number of phosphonic acid groups on DOTA skeleton leads to lower kinetic inertness of the copper(II) complexes. Investigations of complexes of monophosphorus acid derivatives of DOTA showed that their properties depend on substituents on phosphorus atom.

**WARSAW**

Chemistry Chelators for scandium-44 In cooperation with Mainz the stability and lipophilicity of scandium complexes with DOTA and NOTA ligands were studied. Also studies of stability and lipophilicity of labelled Sc-DOTATATE and Sc-DOTATOC were performed. Using $^{13}C$ NMR spectroscopy the structure in aqueous solution of obtained Sc-DOTA and Sc-NOTA complexes were investigated. It was found that $Sc^{3+}$ exhibits in DOTA complex coordination number (CN) 8, like $Lu^{3+}$ and $Y^{3+}$, while in Ga-DOTA complex $Ga^{3+}$ has CN=6. In the case NOTA ligands $Sc^{3+}$ like $Ga^{3+}$ forms coordinative saturated complexes with CN=6. Chelators for zirconium-88 The studies of new chelators for effective binding of $^{89}Zr$ to biomolecules were performed. For the studies we have chosen following acyclic ligands, N,N-bis(2-hydroxybenzyl)ethylenediamine-N,Ndiacetic acid (HBED), acidic octadentatate ligand with hydroxypirydinones groups (LICAM) and for comparison desferrioxamine (DFO) ligand. The preliminary results indicate that HBED forms more stable complexes with $^{89}Zr$ than until now used DFO ligand.

WARSAW cooperation with Mainz: In cooperation with Mainz the labeling of DOTATOC with $^{44}Sc$ and DOTATATE with $^{46}Sc$ were performed. It was found that for efficient labeling (>97%) 15 min. at 95$^{o}$C is enough. The obtained radiobioconjugates exhibited high stability in saline and PBS buffers. Moreover, there weren't observed decomplexation reaction in the present of the excess ($10^{-2}$ M) of acyclic ligands like EDTA, DTPA and free cations like $Fe^{3+}$, $Ca^{2+}$, $Cu^{2+}$ and $Mg^{2+}$. It was found by HPLC method, that scandium bioconjugates are hydrophilic and the retention time of $^{46}Sc$-DOTATATE is nearly the same as $^{177}Lu$-DOTATATE.
Chelators for zirconium-89: The studies of new chelators for effective binding of $^{89}Zr$ to biomolecules were performed The stability of desferrioxamine $^{89}Zr$ complex was compared with stability of $^{89}Zr$ complexes with DTPA, HBED and acidic tripodal 3,4

hydroxypyridinone ligand. The best stability in saline and PBS buffers was observed for DTPA and 3,4 hydroxypyridinone ligands.

**Group of Isabel Santos**:
i) Synthesis of a novel tetraazamacrocycle 10-(2-sulfanylethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid ($H_4$DO3ASH) and characterization by multinuclear NMR spectroscopy, 2D NMR techniques and mass spectrometry. **In collaboration with Èva Tóth group**, the protonation constants of $H_4$DO3ASH were determined by potentiometry and the stability constants of the DO3ASH complexes with $Ce^{3+}$, $Sm^{3+}$ and $Ho^{3+}$ were also determined by potentiometry and UV-Vis spectroscopy. UV-Vis spectrophotometric data on $Ce^{3+}$–DO3ASH and relaxivity measurements on the $Gd^{3+}$–DO3ASH complex suggested that the thiol group does not coordinate to the metal, even in its deprotonated form. Using $^{153}$Sm and $^{166}$Ho, the complexes $^{153}$Sm/$^{166}$Ho–DO3ASH were synthesized in quantitative yield (> 98%) and their stability and in vivo behavior evaluated. Complex $^{153}$Sm–DO3ASH has shown a high stability under physiological conditions and in vivo The biodistribution profile presents a rapid total excretion from the whole animal body, mainly *via* the urinary pathway. The conjugation of DO3ASH to biologically relevant molecules is currently underway.
ii) The group has also synthesized and fully characterized a new DOTA-like chelator bearing a quinazoline moiety (H3L) for targeting EGFR´s. In **collaboration with Èva Tóth Group**, the protonation constants of this novel dota-like compound were determined by potentiometry and UV–vis titration and the stability constant of GaL determined by potentiometry and 71Ga NMR. The values found indicated that H3L displays excellent affinity for Ga3+, when compared with other 12-membered as well as 13- or 14-membered tetraazamacrocycles. Certainly due to this, the radioactive complex 67GaL could be obtained in high yield and high radiochemical purity, being very stable towards hydrolysis, and kinetically inert to transchelation in the presence of an excess of DTPA. Cells studies (A431) have shown inability to cross the membrane and absence of any in vitro growth inhibition of the A431 cells, certainly due to the hydrophilic character of the Ga –complex. In vivo, 67GaL is very stable presenting a high rate of total excretion (84.2%, 4 h after injection). Despite the favourable biodistribution profile in healthy animals, in vivo studies in tumor-bearing mice were not performed owing to the low uptake of 67GaL in A431 cells, which led us to expect a low in vivo retention in target tumor tissues. Taking into account the whole set of results, further structural modifications are being done to promote the penetration of the cell membrane and to optimize the biological performance of DOTA-like gallium complexes bearing a quinazoline pharmacophore.
iii) **In collaboration with Lubal group**, multinuclear and kinetic studies of Ln(III) complexes of 2-[4,7,10-tris(phosphonomethyl)-1,4,7,10-tetraazacyclododecane-1-yl] acetic acid ($H_7$DOA3P) were performed. Based on multinuclear NMR studies, seven protonation constants for $H_7$DOA3P were determined and a protonation sequence was proposed. As expected, the overall basicity of $H_7$DOA3P is higher than that of $H_4$DOTA, $H_5$DO3AP and *trans*-$H_6$DO2A2P but lower than that of $H_8$DOTP. The protonation constants of the lanthanide complexes $[Ln(DOA3P)]^{4-}$ (Ln= Ce, Pr, Sm, Eu, Yb) were also determined by multinuclear NMR spectroscopy, without control of ionic strength. The acid-assisted dissociation of $[Ln(DOA3P)]^{4-}$ (Ln = Ce and Eu) and the kinetics of complex formation were studied by UV-vis spectroscopy and by steady-state/time-resolved

luminescence spectroscopy, at different pH and temperatures. The kinetic inertness of the complexes is slightly the same, since they have almost the same tendency to protonation and decomposition. The species $(H_3DOA3P)^{4-}$ and $(H_2DOA3P)^{5-}$ seem to be active for reaction with Ln(III) ions and the difference in reactivity is not remarkable, due to the similar structure of both active species. The reaction intermediate is $[Eu(DOA3P)(H_2O)_4]^*$, and the rate-determining step is the transfer of the Eu(III) ion into macrocyclic cavity when "out-of-cage" Eu(III) complex with bound pendant arms is transformed to the fully coordinated "in-cage" $[Eu(DOA3P)]^{4-}$ complex.

iv) **In collaboration with the Hermann group**, we have studied reactions of do3ap, $do3ap^{PrA}$ and $do3ap^{ABn}$ with $^{153}Sm$ and $^{166}Ho$. According to solution studies, the final radiolabelled species formed under the labeling conditions were considered to be the $[M(L)]^{z-}$ ($z$ = 1 or 2); therefore, the $^{153}Sm/^{166}Ho$-do3ap, $^{153}Sm/^{166}Ho$-$do3ap^{PrA}$ and $^{153}Sm/^{166}Ho$-$do3ap^{ABn}$ complexes prepared have a negative overall charge. They are hydrophilic, exhibit a low protein binding and a good stability *in vitro*. The complexes show a good biological profile, with a fast clearance from most organs and a high total excretion at 2 h post injection. Moreover, it is also possible to conclude that the functionalization of the phosphorus acid pendant arm by propionic acid or *p*-aminobenzyl side chains does not influence the radiochemical properties, the biological profile and the bone uptake of the macrocyclic complexes. Additionally, the results suggest that the use of the phosphinic acid pendant arms to modify the $H_4$dota skeleton might be a suitable alternative to other dota-like ligands for the design of new BFCA valuable for radiolanthanide imaging and/or targeted therapy.

**Coimbra**

1) Studies of lanthanide complexes of phosphonic acid monoester and phosphinic acid analogues of $H_4$DOTA: structure in solution (using NMR), relaxivity of Gd(III) complexes, EPR studies (collaboration with Prague, Mons, Delft, Sherry -Dallas)
2) Studies of a Gd(III) complex of a (*bis*)-Hydroxymethyl-substituted DTTA ligand: relaxivity studies, NMR based structure of Ln(III) complexes, HSA binding, kinetic studies (collaboration with Lausanne)
3) Studies of a Gd(III) complex of DO3A-N-α-aminopropionate: relaxivity studies, NMR, kinetic studies (collaboration with Orleans)
4) Collaboration in a study of PAMAM dendrimers conjugated with an uncharged gadolinium(III) chelate with a fast water exchange (collaboration with Prague and Delft)
5) Review on classification of MRI CAs (collaboration with Mons)

**SUBATECH, Nantes:** Scandium radioisotopes have become very interesting both for targeted radiotherapy and diagnosis. However, information about behaviour of scandium(III) complexes in aqueous solution are rather scarce. Despite extensive investigations of complexes of the ligand with various metal ions, there are completely no data about the scandium(III) complexes. Therefore, a common project between Subatech and Prague's lab was focused on determination of stability constants of scandium(III) complexes with the ligands commonly considered as suitable for applications in nuclear medicine.

A Short Term Scientific Mission has allowed to investigate the solution behaviour of scandium(III) complexes of acyclic ($H_5$DTPA) and cyclic ($H_4$DOTA, $H_3$NOTA ). The chosen ligands (and their derivatives) are the most commonly used in nuclear medicine and are considered as the standard ligands. Originally planned $H_4$TETA was available only as nonstoichiometric hydrochloride, a form not suitable for potentiometric titrations. For stability constant determination, potentiometric titrations together with $^{1}H/^{45}Sc$ NMR measurements were used. As the investigated ligands forms weak complexes with alkali metal ions, tetramethylammonium cation was used in the background electrolyte. Titrations were done in a wide range of pH from ~1 to 12 depending on the system investigated.
We found a useful methodology for evaluation of stability constants in system of the "standard" ligands with trivalent scandium. Independent (NMR) confirmations of equilibrium times and a correctness of calculated distribution diagrams are necessary in any cases. For the first time, reasonable numerical values of stability constants of Sc(III) with $H_3$NOTA, $H_4$DOTA, and $H_5$DTPA were obtained; these two last ones are very suitable exhibiting high stability constants for radiochemical applications. To fully complement the obtained data, other basic ligand such as EDTA and TETA would be studied in addition to, decomplexation kinetics of the complexes should be checked and it will be done in a near future in collaboration between these partners. We hope thus that data obtained during this STSM will be treated by people involved in COST network as well as other people from the international scientific community as "standard" data for the studied Sc(III)-ligand systems.

In addition to this, radiochemical method (Shubert's method) was employed to determine the stability constants at a trace scale, using a cationic exchange resin. For each ligand tested : DTPA, NOTA, DOTA and TETA, a 1:1 complex id formed with high stability constant values (log □ > 15).
Moreover, the reversibility has been monitored by UV/vis in a highly simplified media (in presence of apo-transferrine and carbonates at pH 7). We have evidenced that this equilibrium was reversible at the time scale of our experiments for DOTA and DTPA but a quick transfer of scandium occurred for TETA. Experiments with NOTA are ongoing.

## WG 4: Nanosized Probes

### *University of Mons*

*Characterization of vectorized iron oxide nanoparticles using the immunoprecipitation method combined to the Perls' Prussian blue iron staining*
Wheat Germ Agglutinin (WGA) is a lectin that is used as a neuronal tracer in histological tracing experiments. With the aim of non-invasively trace neurons using the magnetic resonance imaging (MRI) method, iron oxide nanoparticles, acting as negative MRI contrast agents, were covalently linked to WGA. This lectin conjugation was achieved thanks to carboxyl groups borne by the nanoparticle surface. Binding sites remaining free were reacted with polyethylene glycol (PEG), which is known to prevent opsonization by blood proteins. WGA-conjugated USPIO (ultrasmall particles of iron oxide) are thus expected to be captured and transported by neurons. *In vivo* experiments of MRI

neuronal tract tracing in the central nervous system will be performed with these WGA-USPIO-PEG as well as with USPIO-PEG that will be used as control nanoparticles. An important step consists in the verification of the presence of WGA on the USPIO surface. For this, the well-known immunoprecipitation technique was combined to the Perls' Prussian blue iron staining method. Practically, WGA-USPIO-PEG and USPIO-PEG were subsequentely incubated with a rabbit anti-WGA antibody and with protein A-coated sepharose beads, with the aim of forming nanoparticle-antibody-beads complexes that will settle after centrifugation. Indeed, protein A binds to the Fc (Fragment, crystallizable) part of rabbit antibodies, and the beads can be pelleted by spinning down. Uncomplexed USPIO remained in the supernatant and were eliminated by several washings in buffer. Beads-containing pellets were digested with 5M HCl and underwent a treatment with a 5% potassium ferrocyanide solution, giving a blue colloidal complex (ferric ferrocyanide), also called Prussian blue, in presence of $FeCl_3$. Only WGA-USPIO-PEG incubated with the anti-WGA antibody and the sepharose beads showed a blue coloration (Figure 1), demonstrating the presence of the vector molecule WGA at the USPIO surface.This method could also be applied to verify the presence of any vector at the iron oxide nanoparticle surface, with the condition that an antibody specific for the vector molecule exists.

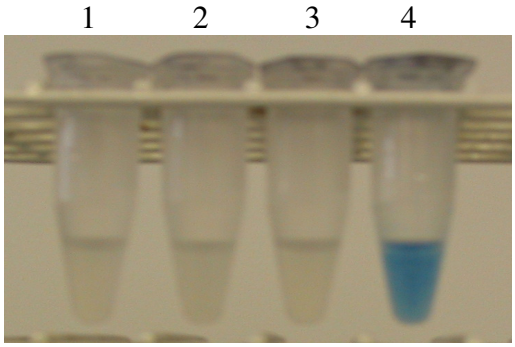



Figure 1

1. USPIO-PEG + beads
2. USPIO-PEG + anti-WGA ab + beads
3. WGA-USPIO-PEG + beads
4. USPIO-WGA-PEG + anti-WGA ab + beads

***Delft University of Technology***

Two $C_{18}$ aliphatic chains were attached to calix[4]arene. This compound forms micelles with a radius of 4.9 nm (cmc < 0.25µM, 37 °C) and a very high relaxivity (27.9 $s^{-1}mM^{-1}$ at 20 MHz and 37 °C). Methods to prepare liposomes incorporating this calixarene derivative in the membrane have been exploited. When the surfactant wass present during the preparation of the lipid film, a significant fraction of bicelles was obtained besides the liposomes. The incorporation into existing liposomes is rather ineffictive whereas the addition of the surfactant prior to the extrusion leads to spherical liposomes and practically quantitative incorporation of 8.8 mol% calixarene. This means that a relatively high Gd loading of the liposomes can be achieved rather easily. The calixarene

facilitates water diffusion through the lipid bilayer resulting in an almost three times enhanced relaxivity (21.2 $s^{-1}mM^{-1}$ at 20 MHz and 37 °C) compared to common paramagnetic liposomes. Those vesicles are promising canditates for molecular imaging applications of tumours due to their high relaxivity per particle.

***University of Lyon :Multimodal nanoparticles from imaging to therapy***

The team developed nanoparticles for applications in the domain of bio-imaging and bio-therapy. The multifunctional character of the nanoparticles derived from the association of different building blocks in the same object. Nanoparticles have been synthesised for the detection by luminescence, magnetic resonance (MRI) or scintigraphy. These techniques are combined with therapeutic applications (radiotherapy, neutrotherapy and curietherapy). Two types of particles have been particularly studied last year: gold nanoparticles and core/shell nanoparticles with a core of lanthanide oxide and a shell of polysiloxane (possibly luminescent). The team has proven that oxide particles can be directly formed at room temperature, this synthesis is very original in comparison with other methods developed in the literature. The developed devices allowed us to control the elaboration parameters to tune the particle size between 1 and 3.5 nm, a size range that is crucial for in-vivo applications (*See Figure 1*).

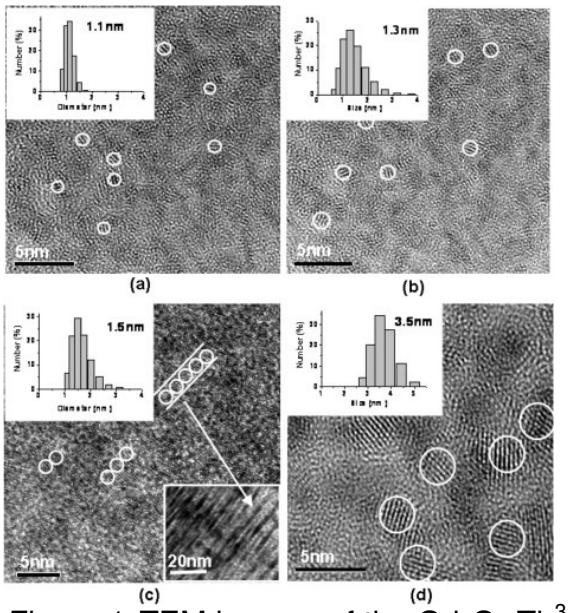



*Figure 1*: TEM images of the $Gd_2O_3$:$Tb^{3+}$ particles obtained by a rapid NaOH addition (a) before and (b) after annealing at 160 °C and by progressive NaOH addition (c) before and (d) after annealing. Insets contain the size distribution of the corresponding particles.

Another important result concerns gold nanoparticles coated by a derivative of diethylenetriaminepentaacetic acid (DTPA), these chelates encapsulate some $Gd^{3+}$ for MRI applications. Thanks to their design, these gold nanoparticles can be followed by X-ray imaging and by MRI (See Figure 2).

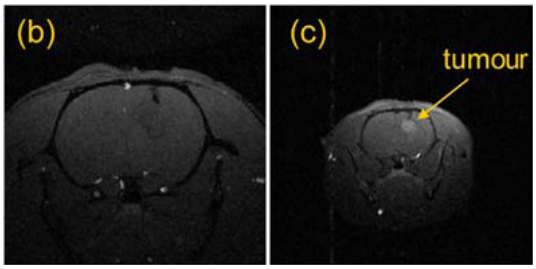



*Figure 2: $T_1$-weighted images of brain of diseased rats before and after intaveinous injection of the gold nanoparticles.*

When injected to healthy animals, these particles freely circulate without undesirable accumulation and are quickly removed by urine. The irradiation of tumour bearing rats with X-Ray microbeam 20 minutes after nanoparticles injection led to a longer survival of the rats. The same manipulation has been performed with core/shell nanoparticles based on a gadolinium oxide core and have similarly demonstrated a longer survey for injected rats (*See Figure 3*).

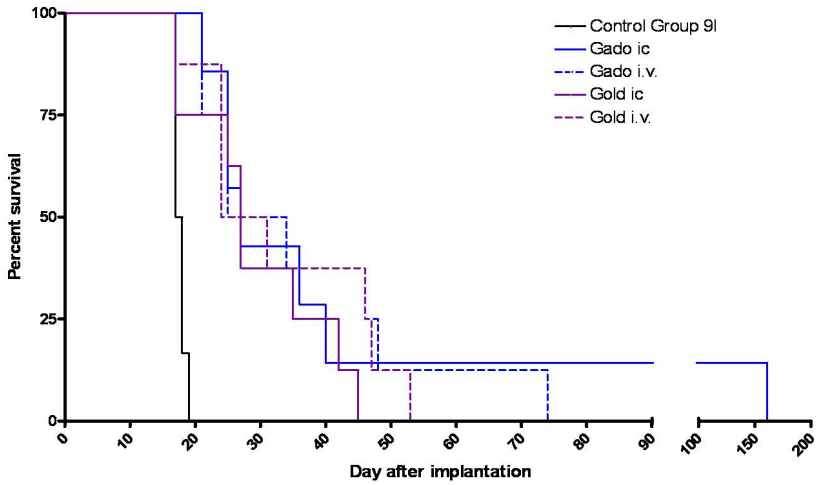



*Figure 3: Comparative survey of mice after irradiation (blue curve: gadolinium by in vivo and in vitro injections; violet curve: gold by in vivo and in vitro injections)*

***Eindhoven University of Technology***

**Lipid-based nanoparticles for molecular imaging**

**General:** In 2009 we have performed detailed in vitro studies focusing on the consequences of contrast agent binding, cellular internalization and compartmentalization for the observed relaxometric properties.

**1. Morphology, binding behavior and MR-properties of paramagnetic collagen-binding liposomes** – Collagen is an important component of the extracellular matrix (ECM) and plays an important role in normal tissue maturation and in pathological processes such as atherosclerosis and myocardial infarction. The diagnostics of the

latter diseases using MRI could strongly benefit from the use of collagen-specific contrast agents. We have developed a bimodal liposomal MR contrast agent that was functionalized with CNA35, a collagen adhesion protein of the Staphylococcus aureus bacterium. The liposomes were characterized in terms of CNA35 protein conjugation and loading. The overall morphology was assessed with DLS and cryo-TEM, while cryo-TEM tomography was used to visualize the protein coverage of the liposomes. The binding properties of the contrast agent were investigated using a fluorescence assay based on the rhodamine content of the liposomes. The bulk relaxivity was determined using regular relaxometry while the MR-properties of liposomes in their bound state were studied using NMR depth profiling. This CNA35 functionalized contrast agent and the set of in vitro experiments we performed indicate the potential of this technology for in vivo molecular imaging of collagen.

**2. Cellular Compartmentalization of Internalized Paramagnetic Liposomes Strongly Influences Both T1- and T2-Relaxivity** – We have investigated the consequences of cellular internalization and compartmentalization of contrast agent for the T1- and T2-shortening efficacy. Cultured endothelial cells were incubated with paramagnetic liposomes that were conjugated with a cyclic RGD-peptide to enable internalization by means of the □v□3-integrin receptor. Non-targeted liposomes served as a control. This study showed that □v□3 targeting dramatically increased the uptake of paramagnetic liposomes. This targeting strategy, however, strongly influenced both the longitudinal and transverse relaxivity of the internalized paramagnetic liposomes. Strong relaxivity "quenching" was observed for □v□3-targeted liposomes.

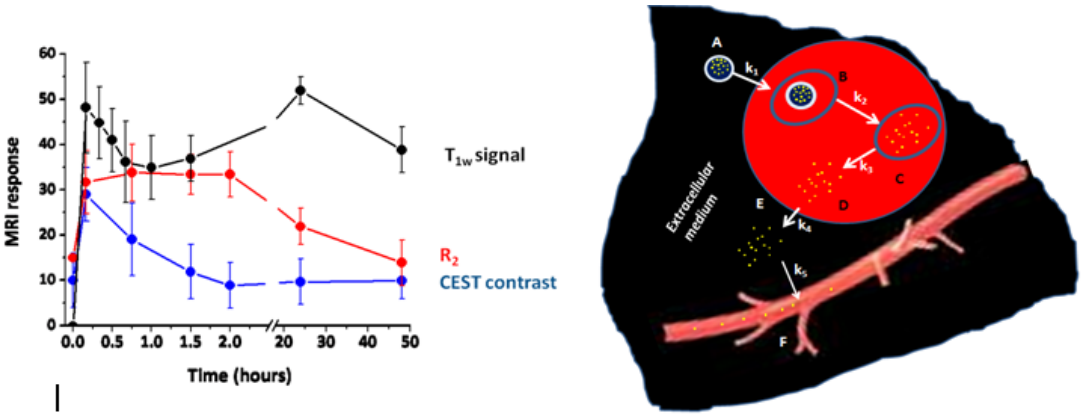
**3. Three-Compartment T1-Relaxation Model for Intracellular Paramagnetic Contrast Agents** – We have developed a model describing the effective longitudinal relaxation rate constant R1 for water in three cellular compartments experiencing possible equilibrium water exchange, and to apply this model to explain the effective R1 dependence on the overall concentration of a cell-internalized Gd-based contrast agent (CA). The model voxel comprises three compartments representing extracellular, cytoplasmic, and vesicular (e.g., endosomal, lysosomal) subcellular spaces. Relaxation parameters were calculated for contrast agent restricted to the cytoplasmic or vesicular compartments. The size or the number of CA-loaded vesicles was varied. The simulated data were then separately fitted with empirical mono- and biexponential inversion recovery expressions. The voxel CA-concentration dependencies of R1 can be used to qualitatively and quantitatively understand a number of different experimental observations reported in the literature. Most important, the simulations reproduced the relaxivity "quenching" for cell-internalized contrast agent that has been observed.

**4. Internalization of annexin A5-functionalized iron oxide particles by apoptotic Jurkat cells** – Apoptosis plays an important role in the etiology of various diseases. Several studies have reported on the use of annexin A5-functionalized iron oxide particles for the detection of apoptosis with MRI, both in vitro and in vivo. The protein annexin A5 binds with high affinity to the phospholipid phosphatidylserine, which is exposed in the outer leaflet of the apoptotic cell membrane. In the present study we have investigated the possible internalization of commercially available annexin A5-functionalized iron oxide particles, and the effects of their spatial distribution on relaxation rates R2*, R2 and R1. Two different incubation procedures were performed, where (1) Jurkat cells were either incubated with the contrast agent after induction of apoptosis or (2) Jurkat cells were simultaneously incubated with the apoptotic stimulus and the

contrast agent. Transmission electron microscopy images and relaxation rates showed that the first incubation strategy mainly resulted in binding of the annexin A5-iron oxide particles to the cell membrane, whereas the second procedure allowed extensive membrane-association as well as a small amount of internalization. Owing to the small extent of internalization, only minor differences were observed between the □R2*/□R2 and □R2/□R1 ratios of cell pellets with membrane-associated or internalized annexin A5 particles. Only the increase in R1 appeared to be diminished by the internalization. Internalization of annexin A5-iron oxide particles is also expected to occur in vivo, where the apoptotic stimulus and the contrast agent are simultaneously present. Where the extent of internalization in vivo is similar to that observed in the present study, both T2*- and T2-weighted MR sequences are considered suitable for the detection of these particles in vivo.

**University of Torino**

The research on nanovesicles-based (liposomes and polymersomes) CEST agents has been continued leading to interesting results and new applications. In particular the ability of paramagnetically loaded vesicles to act as $T_1$, $T_2$ or CEST agents, accordingly with the entrapped Ln-complex, has been exploited to develop an *in vivo* method for the assessment of the vesicles fate after intratumor injection. In particular, a kinetic model has been developed in order to determine the kinetic constant of each of the biological processes involved (Fig. 1).




$$\frac{d[A]}{dt} = -k_1[A] \;;\; \frac{d[B]}{dt} = k_1[A] - k_2[B] \;;\; \frac{d[C]}{dt} = k_2[B] - k_3[C] \;;$$

$$\frac{d[D]}{dt} = k_3[C] - k_4[D] \;;\; \frac{d[E]}{dt} = k_4[D] - k_5[E] \;;\; \frac{d[F]}{dt} = k_5[E]$$

Fig.1. Upper left: Plot of the time evolution of the three contrast modes detected after intratumor injection (murine B16 melanoma) of paramagnetically loaded stealth liposomes. Upper right: schematic description of the kinetic model proposed for analyzing the MRI data. Bottom: Set of differential equations used for describing the kinetic model.

This method has been applied to compare the *in vivo* stability between liposomes and polymersomes. The long lasting CEST effect detected for the polymeric vesicles (Fig. 2) with respect to liposomes is an indication of the higher stability of the former nanosystem towards cellular uptake.

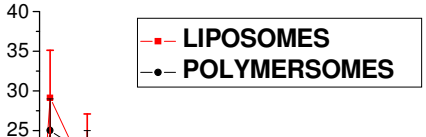


Fig.2: Comparison between the time evolution of CEST contrast in the tumor between polymersomes and liposomes

**WORKING GROUP 5, *Targeted Probes***

**MRI-Targeting Probes**

***S. Aime*** *(*University of Torino, Italy)

1) The limited sensitivity of MRI is the major drawback to the thorough involvement of this modality in Molecular Imaging applications. Some years ago it has been reported on the use of Gd-loaded apoferritin with a relaxivity of ca. 600-800 $mM^{-1}s^{-1}$.The attainable relaxation enhancement of Gd-loaded apoferritin is limited by the number of Gd-HPDO3A (< 10) that is possible to entrap in the inner cavity. In order to increase the number paramagnetic ions entrapped inside the cavity we shifted from $Gd^{3+}$ to $Mn^{2+}$ since it has been reported that manganese ions enter the protein to yield well characterized nanosized crystals of beta-MnOOH. However, to improve $T_1$-relaxation enhancement capabilities it is necessary to solubilize the metal ion payload inside the inner cavity. The dissolution of beta-MnOOH occurs via the reduction of Mn(III) to Mn(II) operated with aminopolycarboxilic acids that also act as coordination ligands for sequestering weakly coordinated manganese ions on the outer surface of the protein. The reductive treatment has allowed to generate an apoferritin-based nanocarrier (Mn-Apo) containing up to 300-400 Mn(II) aqua ions encapsulated in the inner cavity. This yielded to the remarkable relaxivity value (per apoferritin) of ca. 4000-7000 $mM^{-1}s^{-1}$. Mn-Apo being formed by endogeneously occuring molecules and ions, displays a high biocompatibility. It targets hepatocytes with high efficiency and is able to discriminate healthy cells from hepatocarcinoma ones, on the basis of the different expression of ferritin receptors. Mn-loaded apoferritin is a very efficient probe specific for liver imaging. In particular, it could be useful in the diagnosis of a variety of liver diseases involving differences in the hepatic iron storing capabilities. Furthermore, the outer surface of Mn-Apo can be easily

functionalized to endow it with targeting capabilities and to design Mn probes characterized by very high sensitivity.
2) Specific targeting of tumors by combined delivery of drugs and of imaging agents represents an attractive strategy for treatment of cancer. To this purpose we investigated whether neural cell adhesion molecule **(**NCAM)-targeted liposomes may enhance drug delivery and allow magnetic resonance imaging (MRI) in a SCID mice model of NCAM-positive Kaposi's sarcoma. NCAM binding peptide-coated liposomes loaded with both doxorubicin and a lipophilic Gadolinium derivative were generated. NCAM-targeted liposomes induced an enhanced *in vitro* doxorubicin internalization within Kaposi's cells as detected by MRI in respect to untargeted polyethylene glycol-liposomes. Internalization resulted in enhanced apoptosis. *In vivo* weekly administration of NCAM-targeted liposomes containing 5 mg/kg doxorubicin for four consecutive weeks induced a significant reduction of tumor mass and vascularization and an enhanced cell necrosis and apoptosis in respect to untargeted liposomes. These effects were associated with an enhanced concentration of doxorubicin within the tumor and a reduced systemic toxicity of doxorubicin. By electron microscopy, NCAM-targeted liposomes were detected mainly within tumor cells whereas the untargeted were mainly accumulated in the extracellular space. Gd-labeled liposomes allowed the MRI of drug delivery at the tumor region. The intensity of MRI signal was partially hampered by the "quenching" of the attainable relaxation enhancement upon the endosomal entrapment of the Gd-labeled liposomes. In conclusion, targeting NCAM may be a suitable strategy for specific drug delivery and imaging by liposomes in NCAM-expressing tumors. Moreover, treatment with NCAM-targeted liposomes showed enhanced therapeutic effect and reduced toxicity in respect to untargeted liposomes.

**Intracellular Targeting Agents**

***J. Engelmann*** *(*Max-Planck Institute for Biological Cybernetics, Tübingen, Germany)

Aim of this project is the development of novel intracellular targeted contrast agents (CA) for Magnetic Resonance Imaging (MRI). Such probes should show a specific accumulation in targeted cells compared to non-targeted cells by specifically binding to mRNA (via an antisense peptide nucleic acid) or by specific enzymatic cleavage of the vector part. In the recent years several intracellular CA were synthesized in our group using cell penetrating peptides (CPP) to deliver such probes into cells. These CA were efficiently taken up and were able to enhance contrast in MR images in cultured cells even at low micromolar labeling concentrations and, thus, are already usable for ex vivo labeling of cells. However, a lack of targeting specificity was observed. This is most likely due to the predominantly endosomal uptake and entrapment of the CA preventing a sufficient interaction with cellular targets. A novel cysteine rich peptide was developed and extensively tested which is able to deliver cargo molecules into the entire cytosol avoiding at least partially this endosomal entrapment. These studies resulted in an international patent application. Coupling a Gd-loaded DOTA chelate to this peptide led to a highly efficient intracellular contrast agent for MRI. It combined a better internalization compared to known CPP with an unexpected high contrast enhancement of labeled cells. This is likely due to the distribution of this contrast agent complex in the entire cytosol, resulting in the access to a larger pool of water molecules and, thereby,

avoiding “relaxivity quenching” as it is observed for probes which remain entrapped in endosomal vesicles.
In parallel, we tested non-peptide delivery systems (e.g. lipid based systems like coupling of cholesterol) for their ability to enhance cytosolic uptake of targeted imaging probes. The use of cholesterol increased the uptake efficacy further on but did not solve the problem of endosomal entrapment.
**In collaboration with the group of Dr. Goran Angelovski (WG 6)** newly developed Gd-based neuroanatomical tracers were tested in vitro and in vivo for their ability to visualize neuronal connectivity by means of MRI.
In a joint research project, silsesquioxanes bearing Gd-chelates as macromolecular contrast agents were developed and their stability under physiological conditions was evaluated. Whereas the silsesquioxane core is hydrolyzing the Gd-chelates remained stable. The degradability of such systems under physiological conditions may open up new opportunities for even larger and still well defined macromolecular CAs for MRI, whose fragments are then readily excreted *via* the kidneys.
Silica-based nanoparticles functionalized with targeting moieties, CPPs for intracellular delivery and highly stable Gd-chelates are synthesized as well to be used as high relaxivity intracellular targeted probes. The biological evaluation of such nanoparticles is under progress.
In addition, Gd-DOTA derivatives or paramagnetic nanoparticles were coupled to targeted antibodies/antibody fragments (e.g. to specifically visualize beta-cells/islets in the pancreas in vivo). Promising preliminary results were recently obtained.

**“Spin off projects”:**
The MPI for Biological Cybernetics is acting as coordinator in the joint research project “Molecular in vivo imaging of cellular therapeutics (CeTheProbes)” [partners: MPI (Engelmann, Angelovski (WG6)), University of Tübingen and Hannover Medical School, Germany], funded by the German Ministry for Education and Research (BMBF), FKZ 01EZ0813.
Furthermore, the group is participant in a FP7 grant proposal for the large-scale integrating project “A new class of high-performance targeted nanovectors for combined cancer diagnosis and therapy (Nanotheranostics)”.

**Peptide-based probes for cancer imaging**
***H. Maecke*** **(**University Hospital Basel, Basel, Switzerland, University Hospital Freiburg, Freiburg, Germany)
**Exendin-4-based radiopharmaceuticals for glucagon-like peptide-1 (GLP-1) receptor PET/CT and SPECT/CT imaging** (collaboration Basel, Freiburg, London, Marburg and Goeteborg)
Strong over-expression of glucagon-like peptide-1 (GLP-1) receptors in human insulinoma provides an attractive target for imaging. It has been shown previously in patients that GLP-1 receptor SPECT/CT using [Lys$^{40}$(Ahx-DOTA-$^{111}$In)$NH_2$]-exendin-4 localizes hardly detectable insulinomas. However, [Lys$^{40}$(Ahx-DOTA-$^{111}$In)$NH_2$]-exendin-4 imaging has drawbacks related to the use of $^{111}$In, in that it is costly and carries a relatively high radiation burden for the patient. The aim of this study was the preclinical evaluation of [Lys$^{40}$(Ahx-DOTA-$^{68}$Ga)$NH_2$]-exendin-4 for PET/CT and [Lys$^{40}$(Ahx-HYNIC-$^{99m}$Tc)$NH_2$]-exendin-4 for SPECT/CT. **Methods:** Internalization, biodistribution,

dosimetry and imaging studies were performed in the Rip1Tag2 mouse model of pancreatic beta cell carcinogenesis and compared with our gold standard [Lys$^{40}$(Ahx-DOTA-$^{111}$In)$NH_2$]-exendin-4. Poly-glutamic acid and Gelofusine, a gelatin-based plasma expander, were used for renal uptake reduction studies. **Results:** The tumor uptake of [Lys$^{40}$(Ahx-DOTA-$^{68}$Ga)$NH_2$]-exendin-4 was very high, at 205±59 %IA/g (% injected activity per gram tissue). Other GLP-1 receptor positive organs showed > 4.8 times lower dose deposition. There was no significant difference in tumor and organ uptake between [Lys$^{40}$(Ahx-DOTA-$^{111}$In)$NH_2$]-exendin-4 and [Lys$^{40}$(Ahx-DOTA-$^{68}$Ga)$NH_2$]-exendin-4. [Lys$^{40}$(Ahx-HYNIC-$^{99m}$Tc/EDDA)$NH_2$]-exendin-4, however showed significantly less tumor and organ uptake compared with its $^{111}$In and $^{68}$Ga labeled sister compounds. It is to be noted that the significantly lower tumor and organ uptake of [Lys$^{40}$(Ahx-HYNIC-$^{99m}$Tc/EDDA)$NH_2$]-exendin-4 did not result in inferior tumor-to-organ ratios or reduced image quality. All radiopeptides tested showed a high tumor-to-background ratio, resulting in visualization of small tumors (maximum diameter between 1.0 and 3.2 mm) by SPECT and PET imaging. The only exception was the kidneys, which also showed high uptake. This could be reduced by 49-78% using poly-glutamic acid, Gelofusine or a combination of the two. **Conclusion:** These very promising pharmacokinetic and imaging data show that [Lys$^{40}$(Ahx-DOTA-$^{68}$Ga)$NH_2$]-exendin-4 and [Lys$^{40}$(Ahx-HYNIC-$^{99m}$Tc/EDDA)$NH_2$]-exendin-4 are suitable candidates for clinical GLP-1 receptor imaging studies.

**First results of $^{99m}$Tc labeled [Lys$^{40}$(Ahx-HYNIC-$^{99m}$Tc)$NH_2$]Exendin-4 as potential radiopharmaceutical for insulinoma diagnostics** (collaboration Warsaw, Krakow, Basel, Freiburg)

The aim of this project is to develop the formulation of dry kit for $^{99m}$Tc labeling of [Lys$^{40}$(Ahx-HYNIC)$NH_2$]Exendin-4 and initiate the clinical evaluation of this agent. The peptide was custom-synthesized by Peptide Specialty Laboratories. Peptide purity checked by HPLC was 80%. 20 µg [Lys$^{40}$(Ahx-HYNIC)$NH_2$]Exendin-4 was dissolved in 200µl water. After added 50 mg tricine in 500µl water, 5 mg EDDA (ethylenediamine-N,N'-diacetic acid) and 40 µg $SnCl_2$ in 100 µl 0.1N HCl to peptide solution, radiolabelling was carried out by the addition 0.5-1 ml of generator eluate (10-20mCi radioactivity) followed by 30 min incubation at 80$^o$C. Radiochemical purity of [Lys$^{40}$(Ahx-HYNIC-$^{99m}$Tc)NH2]Exendin-4 controlled by TLC and HPLC showed over 90% radiochemical yield and percentage of non-bound $^{99m}$Tc-pertechnetate as well as colloidal forms of $^{99m}$Tc was in the range of 5%. Wet $^{99m}$Tc-labelling of [Lys$^{40}$(Ahx-HYNIC)$NH_2$]Exendin-4 was performed to optimize the amount and concentration of reagents, temperature and reaction time which was then transferred to [Lys$^{40}$(Ahx-HYNIC)$NH_2$]Exendin-4 dry kit formulation. [Lys40(Ahx-HYNIC)$NH_2$]Exendin-4 was successfully labeled with technetium-99m with radiochemical yields over 90%. The main peak in HPLC radiochromatogram indicated two radiolabeled species. Further characterization of these species is planned as well as the in vitro and in vivo evaluation of [Lys40(Ahx-HYNIC-$^{99m}$Tc)NH2]Exendin-4 to confirm its diagnostic potential.

**Radiolabelled Bicyclic Somatostatin-based Analogues: a Novel Class of Potential Radiotracers for SPECT/PET Imaging of Neuroendocrine Tumors** (collaboration Basel, Muttenz, Bubendorf, Berne, Freiburg)

Somatostatin receptors ($sst_1$-$sst_5$) are expressed on many neuroendocrine tumors, with $sst_2$ being the most important one. Several radiolabelled somatostatin analogues have been developed for receptor targeting of sst-positive tumors. Bicyclic somatostatin-based radiopeptides have not been studied yet. Based on the hypothesis that the introduction of conformational constraints may lead to subtype selectivity or may help to delineate structural features determining pansomatostatin potency we developed and evaluated this new class of potential radiotracers for imaging and/or therapy of neuroendocrine tumors. **Methods:** The bicyclic peptides were synthesised by standard fluorenylmethoxycarbonyl (Fmoc) strategy. The macrocyclic chelator 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) was coupled for labelling with the radiometals $^{177}$Lu and $^{68}$Ga. Binding affinity and receptor subtype profile of all the derivatives was evaluated, comparatively to somatostatin-28 (SRIF-28). The radiolabelled analogues were evaluated *in vitro* (serum stability, internalization and efflux studies) and *in vivo* (biodistribution and imaging studies) using HEK-$sst_2$ and HEK-$sst_3$ cell lines. **Results:** The new analogues showed high affinity for the receptors $sst_2$, $sst_3$ and $sst_5$, and moderate affinity for the $sst_1$ and $sst_4$, while exhibited agonistic properties. *In vitro* studies performed with the $^{177}$Lu-labelled analogues demonstrated high serum stability and superiority of the $^{177}$Lu-AM3, compared to the other compounds, as far as internalization rates and cellular retention concerns. The biodistribution profile of $^{177}$Lu-AM3 in nude mice bearing concomitantly $sst_2$- and $sst_3$-tumors showed high and receptor-mediated uptake in the tumors, with a relatively fast washout and very low background. Kidneys were the only other tissue accumulating radioactivity. Tumor-to-kidney ratio exceeded 1 and was improved further by preinjection of lysine. PET/CT imaging studies of $^{68}$Ga-AM3 at 1 h p.i. were characterized by clear localization of the tumor, visualization of the kidneys and negligible background. **Conclusion:** The high rigidity of these new bicyclic somatostatin-based analogues led to high affinity for $sst_2$, $sst_3$ and $sst_5$ and to agonistic properties. Among the DOTA-bicyclic analogues the *in vitro* and pharmacokinetic data of $^{177}$Lu/$^{68}$Ga-AM3 make this peptide an excellent candidate as an imaging and especially as a PET radiotracer.

**Comparative study of a Dota-Bombesin analog labeled with Y-90, Lu-177, Ga-68 and Sc-44**, **for targeted radiotherapy, radiochemical and preliminary in vitro evaluation** *(collaboration Warsaw, Mainz, Athens)*

The specific aim of this study was to compare the labelled compounds of a DOTA-Bombesin analog with $^{90}$Y, $^{177}$Lu, $^{68}$Ga and $^{44}$Sc. The peptidic analog under study is DOTA-BN[2-14]$NH_2$, our initial *in vitro* and *in vivo* evaluation of the $^{90}$Y and $^{177}$Lu labelled DOTA-BN[2-14]$NH_2$ indicated that the binding affinities to PC-3 cells in vitro are metal-mediated. Thus we decided to extend our study for other $M^{+3}$ type radiometals such as $^{68}$Ga and $^{44}$Scand. At the same time we would like to evaluate a potential of this analog for PET imaging. The radioisotopes $^{90}$Y (N.C.A) and $^{177}$Lu(C.A) were produced at the Radioisotope Centre Polatom while $^{68}$Ga and $^{44}$Sc were eluted from semi-automated generators of $^{68}$Ge/$^{68}$Ga and $^{44}$Ti/$^{44}$Sc respectively provided at the Institute of Nuclear Chemistry, University of Mainz. ***Methods:*** The cold complexes of $^{67}$Ga/$^{43}$Sc-DOTA-BN[2-14]$NH_2$ which were synthesized and characterized by HPLC and MS analysis were suitable for their further use in Binding Affinity study using the Human Prostate cancer cell line PC-3. The labeling method for the $^{68}$Ga and $^{44}$Sc derivatives was studied in relation to the incubation time and to the specific activity of the final sample. The

radiochemical evaluation involved ITLC-SG analysis and Solid Phase extraction with concern to final specific activity of the radiolabel species. So far *in vitro* studies included the internalization and the efflux study of the $^{68}$Ga-DOTA-BN[2-14]$NH_2$. ***Results and Conclusions:*** The specific activity achieved for $^{68}$Ga-DOTA-BN[2-14]NH2 was 7.5 GBq $^{68}$Ga/μmol DOTA-BN[2-14]$NH_2$ and kinetic study showed that the 10-15 min incubation is enough for completion of the complexation. The obtained labeling yield was >95% while the sample was purified before its further use. The study on the labeling method for $^{44}$Sc-DOTA-BN[2-14]$NH_2$ did not gave encouraging results probably due to high concentration of impurities in the eluted solution of $^{44}$Sc. The internalization study of the $^{68}$Ga-DOTA-BN[2-14]$NH_2$ as compared with that obtained for the $^{90}$Y and $^{177}$Lu respective analogs showed lower internalization rate but similar percentage of internalization at the end of incubation. The efflux study revealed a quicker externalization rate of $^{68}$Ga-DOTA-BN[2-14]$NH_2$ as compared with $^{90}$Y-DOTA-BN[2-14]$NH_2$ and $^{177}$Lu-DOTA-BN[2-14]$NH_2$. The study on the labeling protocol for $^{44}$Sc-DOTA-BN[2-14]$NH_2$ as well as the *in vitro* Binding Affinity studies of the cold complexes of Ga and Sc are in progress in order to establish the differences between radiolabeled complexes of DOTA- peptidic derivatives.

**Efficacy of radionuclide treatment DOTATATE Y 90 in patients with progressive metastatic gastroenteropancreatic neuroendocrine carcinomas (GEP-NETs): a phase II study.** (collaboration Warsaw, London, Basel, Freiburg)
Background: To evaluate the clinical and radiological effectiveness of [DOTA0, D-Phe1, Tyr3]-octreotate (DOTATATE) Y 90 in patients with extensive progressive gastroenteropancreatic neuroendocrine carcinomas (GEP-NETs). Materials and methods: Sixty patients with histologically proven GEP-NETs were treated with DOTATATE Y 90. Clinical responses were assessed 6 weeks after completing therapy and then after each of the 3- to 6-month intervals. The radiological response was classified according to RECIST criteria. Results: At 6 months after final treatment, radiological partial response (PR) was observed in 13 subjects (23%), and the remaining patients had stable disease (SD) (77%). Clinical PR at 6 months was in 43 patients (72%), nine patients had SD and progressive disease (PD) was noted in eight patients. Median progression-free survival (PFS) was 17 months, while the median overall survival (OS) was 22 months. In eight patients with early PD, the PFS was 4.5 and OS 9.5 months, while in those with SD or PR, PFS and OS were 19.5 and 23.5 months, respectively. After 12 months of follow-up, five patients had World Health Organization (WHO) grade 2 or 3 renal toxicity. Haematological toxicity (WHO grade 3 and 4) was noted during therapy in 10% of patients and persisted in 5%. Conclusions: DOTATATE Y 90 therapy is effective and relatively safe in patients with GEP-NET. Standard doses of DOTATATE Y 90 result in a relatively low risk of myelotoxicity. However, due to ongoing risk of renal toxicity, careful monitoring of the kidney is recommended.

***A. Verbruggen*** **(**KULeuven group, Belgium)
The laboratory of radiopharmacy of the KULeuven (Head: Prof. A. Verbruggen) has been involved in the development of several $^{68}$Ga and $^{99m}$Tc labelled radiopharmaceuticals. Primarily, the KULeuven group is researching the radiolabelling of AnnexinV and AnnexinV fusion proteins with $^{99m}$Tc and $^{68}$Ga to enable in vivo imaging of the probe.

Both isotopes are labelled site-specifically, ensuring that the radioactive label does not interfere with the targeting capacity of the protein. In addition, the biodistribution and pharmacokinetics of the radiolabelled protein are being studied in healthy mice, in a model of hepatic apoptosis and in tumour bearing animals.
Secondly, this group is successfully developing several SPECT and PET tracers for visualization of necrosis. This research involves $^{99m}Tc(CO)_3$ and $^{68}Ga$ labeled bis-DTPA-pamoic acid derivatives and bis-DTPA-bis-indole derivatives and DOTA-analogues. For this purpose, several animal models (including but not limited to repefused hepatic infarction, ethanol induced hepatic necrosis, ethanol induced muscular necrosis and reperfused myocardial infarction models) are being used. Finally, this group optimised the labelling of $^{68}Ga$-Dotatoc in terms of buffer choice during labelling. Also an automated system for the radiosynthesis has been developed, significantly reducing the radiation dose to the operators during routine synthesis while improving the overall labelling yield. A manuscript on this subject is ready for submision.

**Site-specific labeling of 'second generation' annexin V with (99m)Tc(CO)(3) for improved imaging of apoptosis in vivo** (Bioorg Med Chem. 2010 Jan 4)
In this study 'second generation' AnxV was specifically labeled with (99m)Tc in three different ways outside the binding region of the protein to obtain an improved target-to-background activity ratio. The compounds were tested in vitro and in vivo in normal mice and in a model of hepatic apoptosis (anti-Fas mAb). The apoptosis binding was most prominent for the HIS-tagged 'second generation' AnxV labeled with (99m)Tc(CO)(3) in comparison to (99m)Tc-HYNIC-cys-AnxV and (99m)Tc(CO)(3)-DTPA-cys-AnxV.

**Molecular imaging of cell death** (Methods. 2009 Jun;48(2):178-87)
Apoptosis (programmed cell death) and necrosis (uncontrolled cell death) are two distinct processes of cell death that have been described. Non-invasive molecular imaging of these two processes can have several clinical applications and has various approaches in pre-clinical research. Apoptosis imaging enables a specific and early measurement of response in cancer patients. In case of acute myocardial infarction (AMI) and cerebral stroke the degree of both apoptosis and necrosis is abundant. Imaging of both types of cell death is crucial for diagnosis and could differentiate between "real" and "rescuable" cell damage. In a pre-clinical setting cell death imaging offers the possibility for dynamic study protocols and repeated measurements of cell death in the same animal. This review provides an overview of the radiopharmaceutical development and in vivo evaluation of apoptosis and necrosis detecting radioligands that have emerged so far. Some apoptosis radiopharmaceuticals have made it to clinical trials ((99m)Tc-labeled Anx and (18)F-ML-10) while others need further optimization and evaluation (e.g., (18)F-WC-II-89). (99m)Tc-glucarate has been widely used in patients to image necrosis, but this radiopharmaceutical only works early after the onset of necrosis. Other necrosis avid probes like (123)I labeled hypericin and its monocarboxylic acid derivative and (99m)Tc(CO)(3)-bis-hydrazide-bis-DTPA pamoic acid need further evaluation but show already promising results for imaging of necrosis. As a general conclusion molecular imaging of both apoptosis and necrosis is necessary to understand the cell death process in several pathologies.

**Preliminary in vivo evaluation of a novel $^{99m}Tc$-labeled HYNIC-cys-annexin A5 as an apoptosis imaging agent.** (Bioorg Med Chem Lett. 2008 Jul 1;18(13):3794-8)

A novel cys-annexin A5 with a single cysteine-residue at its concave side has been developed by site-directed mutagenesis to allow conjugation through thiol-chemistry without affecting its apoptotic cell binding properties and was derivatized with HYNIC in a 1:1 stoichiometry. Similar to that of the 1st generation $^{99m}$Tc-HYNIC-annexin A5, the novel $^{99m}$Tc-HYNIC-cys-annexin A5 derivative shows in normal mice mainly renal and, to a lesser extent, hepatobiliary excretion. In murine models of hepatic apoptosis there was 257% increase in hepatic uptake of 99mTc-HYNIC-cys-annexin A5 as compared to normal mice. Using the novel tracer agent, acute reperfused myocardial infarction in rabbits was unequivocally delineated at 7h post-injection by muSPECT. The results indicate that the novel $^{99m}$Tc-HYNIC-cys-annexin A5 shows similar apoptosis avidity as the 1st generation $^{99m}$Tc-HYNIC-annexin A5.

***J. Peters*** (Delft University of Technology)
The emphasis of the work in Delft was on bone targeting agents, a project **in collaboration with the Charles University in Prague**. A new phosphinic-acid DOTA-like ligand, DO3APBP, containing a geminal bis(phosphonic acid) moiety as a highly effective bone-seeking group, was synthesized in high yield. Its crystal structure was determined by X-ray analysis. Complexation with lanthanide(III) ions occurs under mild conditions (pH = 8–9, 25 ◦C, 2–3 h). $^{1}$H, $^{31}$P, and $^{17}$O NMR spectroscopy show that DO3APBP forms nine-coordinated lanthanide(III) complexes with one water molecule in the first coordination sphere except for Ln = Er–Lu, which have in addition a species without lanthanide(III)-bound water. Selective formation of only two diastereomers (out of four possible) suggests that the coordinated phosphinate phosphorus atom occurs exclusively in one of the enantiomeric forms. The ratio of the twisted square antiprism (TSA) and square antiprism (SA) diastereomers changes along the lanthanide series; the gadolinium(III) complex has about 35% of the TSA species. The bis(phosphonate) moiety remains free for anchoring to osseous tissue. The $^{1}$H longitudinal relaxivity of the Gd-DO3APBP complex ($r_1$ =7.4 $s^{-1}$ $mM^{-1}$, 20 MHz, 25 ◦C, pH = 7.5) is unexpectedly high compared to that of other monohydrated chelates of similar size thanks to a significant contribution from the second hydration sphere. The water residence time $\tau_M^{298}$ is 198 ns. Further increase in the relaxivity was observed in the presence of Zn(II), Mg(II) or Ca(II) ions, due to formation of coordination polymers. Slowing down of the tumbling rate of the Gd-DO3APBP complex upon adsorption on hydroxyapatite also leads to an increase of the relaxivity ($r_1$ = 17 $s^{-1}$ $mM^{-1}$, 20 MHz, 25 ◦C, pH = 7.5).

***R. Mikolajczak*** (Radioisotope Centre POLATOM, Otwock, POLAND)

**Separation of micro amount of Sc from macro amount of Ti using ion exchange chromatography**
The scandium-47 is a β emitter of moderate radiation energies (max. 439 and 600 keV) with a 3.35 d half life. In addition, $^{47}$Sc emits γ-rays of 159 keV suitable for imaging. Hence, its use may be considered in radiotherapy. The carrier-free $^{47}$Sc can be produced in a nuclear reactor in 2 ways, either from $^{47}$Ti by (n,p) reaction or from $^{46}$Ca by (n,γ) and consecutive β decay of $^{47}$Ca. Several methods of Sc separation from Ti have been described, including solvent extraction, ion exchange or extraction chromatography. We are presenting the preliminary separation results of microgram quantities of Sc from gram quantities of Ti using the commercially available ion exchange resin DGA (N,N,N',N'-tetra-n-octyldiglycolamide ) resin (EiChrom Corp), assuming similar behavior of Sc and

Y. So far, the weight distribution factors for Ti were measured on DGA resin while there are no data for Sc. **Methods:** First the determination of the resin capacity factor (k') for Sc was performed as described by Horwitz. Then experimental separation of Sc from excess of Ti was carried out with $^{46}$Sc as a tracer. The solution containing Ti (20 mg/ml) in 3.5 M $HNO_3$ was spiked with 10 MBq $^{46}$Sc (S.A. of about 2 GBq/mg Sc). The solution was loaded on 0.3 g DGA column bed weight. The flow rates ranging from 1.0 to 5.0 ml/min were used. $^{46}$Sc was eluted from the column with 0.05 M HCl and content of $^{46}$Sc was determined by gamma spectrometry. **Results:** The shape of curve presenting k' versus $HNO_3$ concentration for Sc is very similar to that for Y. The maximal k' value for Sc is around $4x10^5$ at 3.5 M $HNO_3$ and for Y it is around $1.5x10^5$ at 3 M $HNO_3$. The proposed method permitted relatively fast (in the process lasting about 5 h) separation of microgram quantities of Sc from about 5 g $TiO_2$. The total yield of separation was about 80% (73-85%). The $^{46}$Sc break-through from the column during loading of the Ti/Sc mixture did not exceed detection limit for $^{46}$Sc (around 1 kBq/fraction) for the flow between 1.0 and 2.5 ml/min. With the flow increased to 5.0 ml/min a significant increase in the Sc radioactivity detected was observed (10 kBq/fraction). The $^{46}$Sc was recovered from the column in about 20 ml 0.05M HCl. **Conclusions:** The ion exchange DGA resin seems to be a promising bed for separation of Sc from Ti. The preliminary results obtained using mixed Ti and $^{46}$Sc need to be verified using in nuclear reactor irradiated targets.

***P. Hermann*** (Prague)
**Ga(III) complexes**. We continue research on phosphinic acid derivatives of 1,4,7-tirazacyclononane (tacn). **In collaboration with the Mainz and TU Munich groups** we have finished investigations of the tris(2-carboxyethylphosphinic acid) tacn derivative showing a very fast and efficient complexation of non-carried added $^{68}$Ga even under highly acidic conditions (directly in eluate from $^{68}$Ga/$^{68}$Ge generator) or at pH up to 5. The derivative is very suitable for formation of multimeric peptide conjugates; the conjugation chemistry is under current investigation. The chemistry connected with the work has been submitted for publication. To understand the properties of the ligand, other simpler phosphinic acid derivatives (with H and Ph substituents on phosphorus atom) of tacn have been synthesized and are now investigated. **In collaboration with the Mainz group,** a utilization of DOTA bis(phosphonate) derivatives for PET imaging of calcified tissues with $^{68}$Ga was investigated. The derivative having one-carbon spacer between DOTA-monoamide moiety and bis($PO_3H_2$) group was shown to be the most suitable. Its $^{68}$Ga complex has been even successfully used in one patient and results were encouraging; the results have been accepted as "Image of Month". **In collaboration with the Orleans group**, a method for determination of stability constants and kinetic inertness of gallium(III) complexes with macrocyclic ligands was developed and tested on Ga(III)-DOTA and Ga(III)-DOTAmonoamide systems; data are ready for publication and has been presented on COST D38 WG3+WG5 Workshop in Florence.

**Lanthanide(III) complexes** The lanthanide(III) complexes of DO3A-phosphinic acid derivative with bis(phosphonate) side arms were investigated for a bone-targeting **In collaboration with the Delft, Mons and Coimbra groups**. They show a good affinity for hydroxoapatite and the ligand exhibited a fast complexation. In continuation of search for other ligand types, we investigated properties of differently phosphor-substituted

tetrakis(phosphorus acid) derivatives of DOTA. The complexes are thermodynamically much less stable than that of DOTA but they are sufficiently kinetically inert and they are highly hydrophilic as given by the presence of a rich second-hydration sphere. Biodistribution of complexes of monophosphorus acid derivatives of DOTA was investigated **in collaboration with the Hradec Králové** (Prof. A. Lázničková; member of COST BM607 Action; $^{111}$In and $^{90}$Y complexes) and **Sacavém groups** ($^{153}$Sm and $^{166}$Ho complexes) and the biodistributions were shown to be fully similar to the profiles of the DOTA complexes. **In collaboration with the Brno group** (Dr. Přemysl Lubal) we showed that lanthanide(III) complexes of monopyridine-*N*-oxide DOTA derivatives are stable and are formed somewhat faster than the DOTA complexes; we also uncover a new kind of isomerism in complexes of DOTA-like ligands. **In collaboration with the Nantes group**, we investigated Sc(III) complexes of the common polyamino-polycarboxylate ligands (DTPA, DOTA, NOTA, EDTA) as there is no information about solution behaviour of even the simplest Sc(III) complexes. We found a method for determination of stability constants and confirmed the speciation by multinuclear NMR measurements. **In collaboration with the Sacavém, Coimbra and Orleans groups**, we investigated lanthanide(III) complexes of bis(phosphonate) derivative of DOTA, *trans*-DO2A2P; combine thermodynamic, kinetic and structural (both in the solid state and in solution) showed intermediate properties between complexes of DOTA and DOTP and the data are ready for publication.

**Copper(II) complexes**. Research was focused mainly on cyclam derivatives. We showed (**in collaboration with the Brno group**) that complex of fully substituted cyclam derivatives (2 methylphosphonate and 2 methyl groups on nitrogen atoms) are not kinetically inert and, so, not suitable for radiochemical applications. Differently phosphonate-substituted cyclam derivatives are under investigation. It was also shown that a higher number of phosphonic acid groups on DOTA skeleton leads to lower kinetic inertness of the copper(II) complexes. Investigations of complexes of monophosphorus acid derivatives of DOTA showed that their properties depend on substituents on phosphorus atom.

***I. Prata*** (University of Coimbra, Portugal )

**In vivo investigation of radiolabeled self –assembled nanoparticles of modified dextrin (dexC16) (University of Coimbra + University of Minho)**

Amphiphilic molecules, such as surfactants or lipids, spontaneously self-assemble in water, forming self-aggregates, such as micelles, bilayer membranes, tubes and vesicles. Size, density and colloidal stability of nanoparticles can be controlled, by changing the degree of substitution of hydrophobes and its hydrophobicity. This kind of structure is suitable for trapping hydrophobic substances, such as fluorescent probes, proteins, and hydrophobic pharmaceuticals. Solid nanoparticles made from biodegradable polymers have been widely investigated for long-term delivery of drugs. They can potentially provide benefits such as increased therapeutic effect, prolonged bioactivity, controlled release rate, and finally decreased administration frequency, thereby increasing patient compliance.

We have produced and characterized self –assembled nanoparticles of modified dextrin (dexC16) and investigated the in vivo behaviour of their radiolabelled analogues.

**Functionalized Gold Nanoparticles as MRI Contrast Agents**

**(University of Coimbra + University of Minho)**
The new imaging paradigm Molecular Imaging – the in vivo characterization and measurement of biological processes at cellular and molecular level aims at quantifying molecular changes associated with the onset and development of pathologic states, providing therefore early diagnosis and prognosis of disease. The imaging of cells and cellular and sub-cellular structures, requires imaging agents of high relaxivity density endowed with targeting ability to specific cellular receptors. Nanoparticles are ideal platforms for the development of molecular imaging and therapeutic agents as they provide for a high density surface clustering of reports and targeting vectors.
Gold nanoparticles are especially attractive for imaging and therapy due to their surface plasmon resonance (SPR) enhanced light scattering and absortion, which allows efficient convertion of absorbed light into localized heat. Metal chelators of the DO3A monoamide type, known to complex $Ln^{3+}$ ions with high thermodynamic and kinetic stability, were used for complexation of $Gd^{3+}$ ions as reporters for MR detection and gamma imaging and biodistribution ($^{153}Sm^{3+}$) studies. At present the team of the University of Minho (Portugal) is finishing the functionalization of these nanoparticles with peptides bearing the RGD motive for neovasculature targeting and soon we will start their in vitro and vivo evaluation.

## WG6: Responsive Probes

The **Angelovski**'s group concentrates on the synthesis of responsive contrast agents (SCA) sensitive to changes in calcium concentration or pH and their *in vitro* and *in vivo* characterization.
DO3A-based ligands appended with a propylphosphonate side arm and their $Gd^{3+}$ and $Eu^{3+}$ complexes were investigated. The obtained results indicate that the thermodynamic stability and kinetic inertness of these complexes is sufficient for their *in vivo* application. A novel series of complexes containing alkylaminobis(methylenephosphonate) side chains for $Ca^{2+}$-chelation were synthesized and investigated. Unlike previously published examples, the $r_1$ of these complexes decreases upon addition of $Ca^{2+}$. The $r_1$ changes are not only dependent on the chain length, but also on the concentration of the complex. Extensive physicochemical studies demonstrate that $Ca^{2+}$-induced aggregate formation is the primary cause for the unusual behavior observed in these complexes. Employing an alternative type of MRI mechanism to assess the $Ca^{2+}$ concentration, $Eu^{3+}$ and $Yb^{3+}$ complexes of a tetraamide ligand were prepared and their chemical exchange saturation transfer (CEST) response was investigated. The CEST effect upon $Ca^{2+}$ addition decreases in both, $Yb^{3+}$ and $Eu^{3+}$ complexes. Finally, a molecule bearing a macrocyclic DOTA-type chelator and an acyclic chelator based on the 5-aminoisophthalamide diethylenediaminetetraacid was synthesized. This novel ligand system possessing two different chelating moieties can form heterometallic lanthanide complexes, one of which exhibits a MRI and luminescence response. Studies show that structural improvements are required to develop an analogous agent that will be able to incorporate two different lanthanide ions with a sufficient kinetic and thermodynamic stability for the potential for application in vivo as an efficient dual-emissive and dual modal agent.

**Hamacek**'s group has been continuing the development and the characterisation of polymetallic Ln(III) complexes. The thermodynamic and photophysical studies were achieved for dinuclear complexes formed with an unsymmetrical tripodal ligand. Although these complexes appear inappropriate for sensing purposes, the obtained results are valuable for the ligand improvement. In addition to this system, we were prepared trinuclear Eu(III) and Gd(III) complexes with an unusual triangular topology of cations. The Eu(III) complex exhibits good luminescence properties despite the coordination of two water molecules per cation. However, the latter fact is favourable for the water relaxivity of the Gd(III) analogue and promising preliminary results have been obtained in collaboration with **Terreno**'s group.

**Lowe** has been using click chemistry to link DOTA-based Ln-chelates to biomolecules of interest and also to generate dual mode MR-optical or MR-PET agents. The collaboration with **Faulkner** in WG 1 on developing multi-modal agents has now been published. The unexpected reactivity with 'naked' azide of chelates bearing pendant propargyl groups bind to form unsubstituted triazole that exhibit pH responsive luminescent and $^{1}$H NMR behaviour has been further explored and published. This behaviour is not due to reversible ligation of the triazole, but due to deprotonation of the NH-acidic bound triazole, resulting in a switch from 'pyridinic' to triazolide coordination. Luminescence studies on Eu(III) analogues of esterase activated contrast agents were also concluded and published in this period.

In Durham, **Parker** has primarily focused on developing emissive probes responsive to their environment, including mechanistic work to understand their mode of action and physicochemical basis. A critical assessment has been made of the invasiveness of optical probes on cellular homeostasis; examples have been defined in which the presence of the probe not only may perturb the permeability of the cell (enhancing uptake) but also changes the intracellular localisation profile - observed in concentration dependent studies. An example has been reported of a system localising within a cell where use of a Eu complex allows observation while the presence of the Tb analogue allows local cell damage to be inflicted (via creation of singlet oxygen) following irradiation at 355 nm. A proof of concept study has assessed the scope and utility of $^{19}$-F labelled probes for use in 19-F MR spectroscopy and imaging. Cases have been defined in which the observed chemical shift (or shift ratio of two resonances) signals a change in either local pH (allowing chemical shift imaging studies) or local anion concentration.

**Terreno's** group focused the research activity on the development of responsive MRI nanoprobes. Part of the work has been carried out in collaboration with **Geraldes** (WG2), and it dealt with the design of Gd-loaded liposomes properly formulated in order to act as sensors of enzymatic activity. Several approaches have been pursued:
i) the addition of cationic polymers acting as substrates for specific enzymes (e.g. protamine) to a suspension of negatively charged liposomes encapsulating a Gd(III) complex leads to the formation of insoluble aggregates that causes a relaxivity decrease. In the presence of specific proteases the system gets soluble and the relaxivity enhances as a function of the proteolytic activity;

ii) a similar approach has been followed to design cationic paramagnetic liposomes able to respond to the presence of hyaluronidases after addition of the negatively charged substrate hyaluronic acid;
iii) formulation of liposomes incorporating an amphiphilic lipopeptide acting as substrate for Matrix Metallo Proteinases (MMPs). The specific cleavage of the peptide exposed to the liposome surface destabilizes the vesicle membrane, thus inducing a release of the encapsulated imaging probe with the consequent relaxivity enhancement.
In collaboration with **Parker**, the pH responsive properties of 19F-containing Ln(III) complexes have been evaluating in order to test their potential as intracellular pH probes. Work has been carrying out with the aim of developing paramagnetic pH sensitive liposomes, especially for setting up MRI protocols for imaging drug delivery.
The research on CEST agents has been mainly focused on the optimization of the determinants controlling the ability to generate contrast in liposomal agents and on the development of improved procedures for the contrast detection.

## ***II.B. Dissemination of results***

### ***Action related Publications and Reports***

*Those marked with an asterisk are joint papers*

**WG1:**


1. S. V. Eliseeva and J.-C. G. Bünzli.
   Lanthanide Luminescence for Functional Materials and Bio-sciences
   *Chem.Soc.Rev.* **2010,** *39*, 189-227 (Published on the web, September 11, 2009)
*2. Vanesa Fernandez-Moreira, B. Song, V. Sivagnanam, A.-S. Chauvin, C. D. B. Vandevyver, M. A. M. Gijs, I. A. Hemmila, H.-A. Lehr, and J.-C. G. Bünzli.
   Bioconjugated Lanthanide Luminescent Helicates as Multilabels for Lab-on-a-Chip Detection of Cancer Biomarkers
   *Analyst* **2010,** *135*, 42-52 (Published on the web, November 20, 2009)
3. Venkataragavalu Sivagnanam, Bo Song, Caroline Vandevyver, and Martin A. M. Gijs.
   On-Chip Immunoassay Using Electrostatic Assembly of Streptavidin-Coated Bead Micropatterns
   *Anal.Chem.* **2009,** *81*, 6509-6515
4. J.-C. G. Bünzli.
   Lanthanide luminescent bioprobes
   *Chem.Lett.* **2009,** *38*, 104-109
5. E. Deiters, B. Song, A.-S. Chauvin, C. Vandevyver, and J.-C. G. Bünzli.
   Luminescent Bimetallic Lanthanide Bioprobes for Cellular Imaging with Excitation

into the Visible
*Chem.Eur.J.* **2009,** *15*, 885-900

*6. L. Moriggi, A. Aebischer, C. Cannizzo, A. Sour, A. Borel, J.-C. G. Bünzli, and L. Helm.
A ruthenium-based metallostar: synthesis, $^{1}$H relaxivity and sensitized luminescence
*Dalton Trans.* **2009,** 2088-2095

*7. B. Song, V. Sivagnanam, C. D. B. Vandevyver, I. A. Hemmilä, H.-A. Lehr, M. A. M. Gijs, and J.-C. G. Bünzli.
Time-Resolved Lanthanide Luminescence for Lab-on-a-Chip Detection of Biomarkers on Cancerous Tissues
*Analyst* **2009,** *134*, 1991-1993

8. Svetlana Eliseeva, Gerald Auböck, Frank van Mourik, Andrea Cannizo, Bo Song, Emmanuel Deiters, Anne-Sophie Chauvin, Majed Chergui, and Jean-Claude G. Bünzli. Multiphoton-excited luminescent lanthanide bioprobes: two- and three-photon cross sections of dipicolinates derivatives and binuclear helicates. Submitted to *J. Chem. Phys. B*, 2009.

*9. Stephane Diring, Melissa C. Solomons, Jonathan A. Faiz, Zoe Pikramenou, Raymond Ziessel, "Luminescent $Pt^{II}$ intercalators incorporating a β-cyclodextrin host for DNA recognition", manuscript to be submitted

10. L. L. Ruston, G. M. Robertson and Z. Pikramenou “Luminescence Screening Assays for Identification of Sensitisers for Lanthanides based on the Controlled Formation of Ternary Lanthanide Complexes with DTPA-bisamide Ligands” *Chem Asian J.* in press, on the web Dec 2009.

11. J. M. Davidson, Z. Pikramenou, A. Ponce and R. E. P. Winpenny “Measurement of Parts per Million Level Gaseous Concentration of Hydrogen Sulfide by Ultraviolet Spectroscopy using 1,1,1,5,5,5-Hexafluoropentan-2,4-dione as a Derivative by Reaction of Cu(hfac)(1,5-Cyclooctadiene)” *Anal. Chem.* 2009, 81, 3669-3675.

12. Bimetallic Lanthanide Complexes Derived from Macrocycle-Appended m-Xylyl Derivatives: Synthesis and Spectroscopic Properties , Placidi, MP; Natrajan, LS; Sykes, D,, Kenwirght, A.M. Faulkner S. *Helv. Chim. Acta* 2009, 92, 2427-2438

13. Controlled preparation of a heterometallic lanthanide complex containing different lanthanides in symmetrical binding pockets Natrajan, LS; Villaraza, AJL; Kenwright, AM; Faulkner, S, *Chem. Commun*. 2009, 6020-6022

*14. Changing the local coordination environment in mono- and bi- nuclear lanthanide complexes through "click" chemistry Jauregui, M; Perry, WS; Allain, C, Stasiuk, G; Snaith, J.S.; Kenwright, AM, Lowe, MP; Faulkner S. *Dalton Trans*., 2009, 6283-6285.

15. Synthesis and Spectroscopic Studies on Azo-Dye Derivatives of Polymetallic Lanthanide Complexes: Using Diazotization to Link Metal Complexes Together, Placidi, MP; Villaraza, AJL; Natrajan, LS; Kenwright AM, Faulkner S, *J. Am. Chem. Soc*. 2009, 131, 9916-7

16. Sensitised luminescence in lanthanide containing arrays and d-f hybrids, Faulkner, S; Natrajan, LS; Perry, WS; Sykes, D. *Dalton Trans*., 2009, 3890-3899.

17. [Ru(bipy)(3)](2+) and [Os(bipy)3](2+) chromophores as sensitisers for near-infrared luminescence from Yb(III) and Nd(III) in d/f dyads: contributions from Forster,

Dexter, and redox-based energy-transfer mechanisms , Lazarides, T; Tart, NM; Sykes, D, Barbieri, A; Faulkner, S. Ward, MD, *Dalton Trans*, 2009, 3971-3979.
*18. Relaxation and luminescence studies on hydrated bipyridyl- and terpyridyl-based lanthanide complexesKubicek V ; Hamplova A; Maribe L; Mameri S; Ziessel R ; Toth E; Charbonniere L, *Dalton Trans*. 2009, 8466-9474
*19. Solution Structure and Dynamics, Stability, and NIR Emission Properties of Lanthanide Complexes with a Carboxylated Bispyrazolylpyridyl Ligand; Mato-Iglesias M, Rodriguez-Blas MT; Platas-Iglesias C; Starck M; Kadjane P; Ziessel R; Charbonniere L. *Inorg. Chem*. 2009, 48, 1507-1518
*20. Hydrophobic chromophore cargo in micellar structures: a different strategy to sensitize lanthanide cations, Bonnet CS, Pellegatti L, Buron F, Shade CM Villette S, Kubicek V, Guillaumet G, Suzenet F, Petoud S, Toth E, *Chem, Commun*. 2010, 124-126.
21. Novel Antennae for Luminescent Lanthanide Cations Emitting in the Visible and in the Near-infrared: From Small Molecules to Polymetallic Lanthanide Containing Nanocrystals, Petoud, S. *Chimia*, 2009,63, 745-752
22. Synthesis and Solid-State, Solution, and Luminescence Properties of Near-Infrared-Emitting Neodymium(3+) Complexes Formed with Ligands Derived from Salophen, Uh H, Badger, Geib SJ, Petoud S, Helv. Chim. Acta, 2009, 92, 2313-2329.
23. Decorated carbon nanotubes with unique oxygen sensitivity, Kauffman DR Shade CM, Uh H, Petoud S, Star A, *Nature Chemistry*, 2009, 1, 500-506.
24. Mono- and Terfluorene Oligomers as Versatile Sensitizers for the Luminescent $Eu^{3+}$ Cation, Oxley DS, Walters RW, Copenhafer JE, Meyer TY, Petoud S, Edenborn HM, Inorg. Chem. 2009, 48, 6332-6334.
*25. Lanthanide(III) Complexes of Pyridine-N-Oxide Analogues of DOTA in Solution and in the Solid State. A New Kind of Isomerism in Complexes of DOTA-like Ligands, M. Polasek, J. Kotek, P. Hermann, I. Cisarova, K. Binnemans and I. Lukes, *Inorganic Chemistry* 48, 466-475 (2009).
26 Tetracycline)europium(III) Complex as Luminescent Probe for Hydrogen Peroxide Detection, G. Dehaen, G. Absillis, K. Driesen, K. Binnemans, T.N. Parac-Vogt ,Helvetica Chimica Acta 92, 2387-2397 (2009).
*27. Synthesis and Characterization of Dinuclear Heterometallic Lanthanide Complexes Exhibiting MRI and Luminescence Response,I. Mamedov, T. Parac-Vogt, N. K. Logothetis and G. Angelovski, *Submited for publication*

**WG2**

1.* Gadolinium(III) complexes of 1,4,7-triazacyclononane based picolinate ligands: simultaneous optimization of water exchange kinetics and electronic relaxation. A. Nonat, M. Giraud, C. Gateau, P. H. Fries, L. Helm, M. Mazzanti, *Dalton Trans.* **2009**, 8033-8046.
2. Multiple-Frequency and Variable-Temperature EPR Study of Gadolinium(III) Complexes with Polyaminocarboxylates: Analysis and Comparison of the Magnetically Dilute Powder and the Frozen-Solution Spectra. M. Benmelouka, J. Van Tol, A. Borel, S. Nellutla, M. Port, L. Helm, L.-C. Brunel, A. E. Merbach, *Helv. Chim. Acta*, **2009,** *92*, 2173-2185.

3.* Lanthanide chelates of (bis)-hydroxymethyl-substituted DTTA with potential application as contrast agents in magnetic resonance imaging. S. Silvério, S. Torres, A. F. Martins, J. A. Martins, J. P. André, L. Helm, M. I. M. Prata, A. C. Santos, C. F. G. C. Geraldes, *Dalton Trans.*, **2009**, 4656-4670.
4. A ruthenium-based metallostar: synthesis, sensitized luminescence and $^{1}H$ relaxivity. L. Moriggi, A. Aebischer, C. Cannizzo, A. Sour, A. Borel, J.-C. G. Bünzli, L. Helm, *Dalton Trans.*, **2009**, 2088-2095.
5. Gold Nanoparticles Functionalized with Gadolinium Chelates as High-Relaxivity MRI Contrast Agents. L. Moriggi, C. Cannizzo, E. Dumas, C. R. Mayer, A. Ulianov, L. Helm, *J. Am. Chem. Soc.*, **2009,** *131*, 10828–10829.
6. A Gadolinium-Binding Cyclodecapeptide with a Large High-Field Relaxivity Involving Second-Sphere Water. C. S. Bonnet, P. H. Fries, S. Crouzy, O. Seneque, F. Cisnetti, D. Boturyn, P. Dumy, P. Delangle, *Chem. Eur. J.*, **2009**, *15*, 7083-7093.
7. Water Stability and Luminescence of Lanthanide Complexes of Tripodal Ligands Derived from 1,4,7-triazacyclononane: pyridine carboxamide *versus* picolinate donors. G. Nocton, A. Nonat, C. Gateau, M. Mazzanti, *Helv. Chim. Acta*, **2009**, *92*, 2257-2273.
8. Structural and Photophysical Studies of Highly Stable Lanthanide Complexes of Tripodal 8-Hydroxyquinolinate Ligands based on 1, 4,.7-triazacyclononane. A. Nonat, J. Pécaut, D. Imbert, M. Giraud, M. Mazzanti, *Inorg. Chem.*, **2009**, *48*, 4207-4218.
9. Remarkable Tuning of the Coordination and Photophysical Properties of Lanthanide Ions in a Series of New Tetrazole-Based Complexes. E. S. Andreiadis, R. Demadrille, J. Pécaut, D. Imbert, M. Mazzanti, *Chem. Eur. J.*, **2009,** *15*, 9458-9476.
10. Solution Structure and Dynamics, Stability and NIR Emission Properties of Lanthanide Complexes with a Carboxylated bispyrazolylpyridyl Ligand. M. Mato-Iglesias, T. Rodríguez-Blas, C. Platas-Iglesias, M. Starck, P. Kadjane, R. Ziessel, L. Charbonnière, *Inorg. Chem.*, **2009**, *48*, 1507-1518.
11.* Macrocyclic Receptor Exhibiting Unprecedented Selectivity for Light Lanthanides. A. Roca-Sabio, M. Mato-Iglesias, D. Esteban-Gómez, É. Tóth, A. de Blas, C. Platas-Iglesias, T. Rodríguez-Blas, *J. Am. Chem. Soc.* **2009**, *131*, 3331-3341.
12.* Structural Study of Ga(III), In(III), and Fe(III) Complexes of Triaza-macrocycle Based Ligands with $N_3S_3$ Donor Set. J. Notni, K. Pohle, J. A. Peters, H. Görls, C. Platas-Iglesias, *Inorg. Chem.*, **2009**, *48*, 3257-3267.
13. Luminescence Properties of Heterodinuclear Eu-Pt Complexes From Unusual Nonadentate Ligands. P. Kadjane, C. Platas-Iglesias, R. Ziessel, L. Charbonnière, *Dalton Trans.* **2009**, 5688-5700.
14.* Stability, Water Exchange and Anion Binding Studies on Lanthanide(III) Complexes with a Macrocyclic Ligand based on 1,7-diaza-12-crown-4: Extremely Fast Water Exchange on the $Gd^{3+}$ Complex. Z. Pálinkás, A. Roca-Sabio, M. Mato-Iglesias, D. Esteban-Gómez, C. Platas-Iglesias, A. de Blas, T. Rodríguez-Blas, É. Tóth, *Inorg. Chem.*, **2009**, *48*, 8878-8889.
15.* Equilibrium and Kinetic Properties of the Lanthanoids(III) and Various Divalent Metal Complexes of the Heptadentate Ligand AAZTA. Z. Baranyai, F. Uggeri, G. B. Giovenzana, A. Bényei, E. Brücher, S. Aime, *Chem. Eur. J.*, **2009**, *15*, 1696 – 1705.

16. Synthesis of the (S)-2-(p-nitrobenzyl)-PCTA, a New Bifunctional Ligand for Radiopharmachology: Equilibrium, Formation/Dissociation Kinetics of the Ln(III)[(S)-2-(p-Nitrobenzyl)-PCTA] complexes. G. Tircsó, Z. Kovács, E. Tircsóné Benyó, P. Jurek, G. E. Kiefer, A. D. Sherry, *Bioconjugate Chem.*, **2009**, *20*, 565-575.
17. Lanthanide(III) Complexes of Tris(amide) PCTA Derivatives as Potential Bimodal Magnetic Resonance and Optical Imaging Agents. F. A. Rojas-Quijano, E. T. Benyó, G. Tircsó, F. K. Kálmán, Z. Baranyai, S. Aime, A. D. Sherry, Z. Kovács, *Chem. Eur. J.*, **2009**, *15*, 13188-13200.
18. Effect of the Regiochemistry of Butyl Amide Substituents on the Solution-State Structures of Lanthanide(III) DOTA-Tetraamide Complexes. T. Mani, G. Tircso, P. Zhao, A D. Sherry, M. Woods, *Inorg. Chem.,* **2009**, *48,* 10338-10345.
19. Modulation of water exchange in Eu(III) DOTA-tetraamide complexes: considerations for in vivo imaging of PARACEST agents. T. Mani, G. Tircsó, O. Togao, P. Zhao, T. C. Soesbe, M. Takahashi, A. D. Sherry, *Contrast Media Mol. Imaging*, **2009**, *4,* 183-191.
20. Synthesis and biological evaluation of lanthanide ion DOTA-tetraamide complexes bearing peripheral hydroxyl groups. A. Pasha, M. Lin, Gy. Tircsó, M. Woods, G. E. Kiefer, A. D. Sherry, X. Sun, *J. Biol. Inorg. Chem.* **2009**, *14,* 421-438.
21.* Equilibrium studies on the complexation of $Gd^{3+}$, $Cu^{2+}$ and $Zn^{2+}$ with the ligands BOPTA, DTPA and DTPA-BMA. The kinetics of the metal exchange reactions of Gd(BOPTA) with $Cu^{2+}$, $Zn^{2+}$ and $Eu^{3+}$ ions. Z. Baranyai, Z. Pálinkás, F. Uggeri, E. Brücher, *Eur. J. Inorg. Chem.*, submitted
22.* $^{1}$H NMR Relaxivity of Aqueous Suspensions of Titanium Dioxide Nanoparticles coated with a gadolinium(III) chelate of a DOTA-monoamide with a phenylhosphonate pendant arm. I. Řehoř, V. Kubíček, J. Kotek, P. Hermann, I. Lukeš, J. Száková, L. Vander Elst, R.N. Muller, J.A. Peters, *J. Mater. Chem.*, **2009**, *19*, 1494-1500.
23.* Calix[4]arenes as molecular platforms for magnetic resonance imaging (MRI) contrast agents. D.T. Schühle, J. Schatz, S. Laurent, L. Vander Elst, R.N. Muller, M.C.A. Stuart, J.A. Peters, *Chem. Eur. J.,* **2009**, *15*, 3290-3296.
24.* Gd(III) complex of a monophosphinate-bis(phosphonate) DOTA analogue with a high relaxivity. Lanthanide(III) complexes for imaging and radiotherapy of calcified tissues. T. Vitha, V. Kubíček, J. Kotek, P. Hermann, L. Vander Elst, R.N. Muller, I. Lukeš, J.A. Peters, *Dalton Trans.* **2009**, 3204-3214.
25.* PAMAM dendrimers conjugated with an uncharged gadolinium(III) chelate with a fast water exchange: the influence of chelate charge on rotational dynamics. M. Polášek, P. Hermann, J.A. Peters, C.F.G.C. Geraldes, I. Lukeš, *Bioconjugate Chem.*, **2009**, *20*, 2142-2153.
26*. NMR characterization of lanthanide(3+) complexes of tetraazatetrakisphosphinato and tetraazatetrakisphosphonato ligands. G.A. Pereira, L. Ball, A.D. Sherry, J.A. Peters, C.F.G.C. Geraldes, *Helv. Chim. Acta* , **2009**, *92*, 2532-2550.
27.* Densely arranged Gd(III)-chelates with a fast water exchange on a calix[4]arene scaffold: a potential MRI contrast agent. D.T. Schühle, M. Polášek, I. Lukeš, T. Chauvin, É. Tóth, J. Schatz, U. Hanefeld, M.C.A. Stuart, J.A. Peters, *Dalton Trans.*, **2010**, 185-191.
28.* Lanthanide(III) Complexes of Phosphorus Acid Analogues of H4dota as Model Compounds for the Evaluation of the Second-Sphere Hydration. Z. Kotková, G. A.

Pereira, K. Djanashvili, J. Kotek, J. Rudovský, P. Hermann, L. Vander Elst, R. N. Muller, C. F. G. C. Geraldes, I. Lukeš, J. A. Peters, *Eur. J. Inorg. Chem.*, **2009**, 119–136.

29. Pyridine-N-oxide Analogues of DOTA and Their Gadolinium(III) Complexes Endowed with a Fast Water Exchange on the Square-Antiprismatic Isomer. M. Polášek, M. Šedinová, J. Kotek, L. Vander Elst, R. N. Muller, P. Hermann, I. Lukeš, *Inorg. Chem.*, **2009**, *48*, 455–465.
30. Lanthanide(III) Complexes of Pyridine-N-Oxide Analogues of DOTA in Solution and in the Solid State. A New Kind of Isomerism in Complexes of DOTA-like Ligands. M. Polášek, J. Kotek, P. Hermann, I. Císařová, K. Binnemans, I. Lukeš, *Inorg. Chem.*, **2009**, *48*, 466–475.
31.* $Mn^{2+}$ complexes with pyridine-containing 15-membered macrocycles: Thermodynamic, kinetic, crystallographic and $^{1}H/^{17}O$ relaxation studies. B. Drahoš, J. Kotek, P. Hermann, I. Lukeš and É. Tóth, *Inorg. Chem.*, in press.
32.* A solution thermodynamic study of the Cu(II) and Zn(II) complexes of EBTA. X-ray crystal structure of the dimeric complex $[Cu_2(EBTA)(H_2O)_3]_2$. Z. Baranyai, G. Bombieri, F. Meneghetti, L. Tei, M. Botta, *Inorg. Chim. Acta*, **2009**, *362*, 2259–2264.
33. 1,2-Hydroxypyridonate/Terephthalamide Complexes of Gadolinium(III): Synthesis, Stability, Relaxivity, and Water Exchange Properties. E. J. Werner, J. Kozhukh, M. Botta, E. G. Moore, S. Avedano, S. Aime, K. N. Raymond, *Inorg. Chem.*, **2009**, *48*, 277-286.
34. Relaxivity modulation in Gd-functionalised mesoporous silicas. Carniato, L. Tei, W. Dastrù, L. Marchese, M. Botta, *Chem. Commun.*, **2009**, 1246–1248.
35. Fast and easy access to bifunctional chelators for MRI applications. G. Gugliotta, M. Botta, G. B. Giovenzana, L. Tei, *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 3442-3444.
36. Application of the Ugi four component reaction to the synthesis of ditopic bifunctional chelating agents. L. Tei, G. Gugliotta, S. Avedano, G. B. Giovenzana, M. Botta, *Org. Biomol. Chem.*, **2009**, *7*, 4406-4414.
37.* A new bifunctional $Gd^{III}$ complex of enhanced efficacy for MR-Molecular Imaging applications. L. Tei, G. Gugliotta, Z. Baranyai, M. Botta, *Dalton Trans.*, **2009**, 9712–9714.
38. Synthesis and Relaxometric Properties of Gd(III) Complexes of New Triazine-based Polydentate Ligands. L. Tei, M. Benzi, F. Kielar, M. Botta, C. Cavallotti, G. B. Giovenzana, S. Aime, *Helv. Chim. Acta*, **2009**, *92*, 2414-2426.
39. Effect of a Mesitylene-Based Ligand Cap on the Relaxometric Properties of Gd(III) Hydroxypyridonate MRI Contrast Agents. E. J. Werner, M. Botta, S. Aime, K. N. Raymond, *Contrast Media Mol. Imaging*, **2009**, *4*, 220-229.
40.* Dramatic Increase of Selectivity for Heavy Lanthanide(III) Cations by Tuning the Flexibility of Polydentate Chelators. L. Tei, Z. Baranyai, E. Brücher, C. Cassino, F. Demicheli, N. Masciocchi, G. B. Giovenzana, M. Botta, *Inorg. Chem.*, in press; DOI: 10.1021/ic901848p.
41. Design Principles and Theory of Paramagnetic Fluorine-Labelled Lanthanide Complexes as Probes for $^{19}F$ Magnetic Resonance: A Proof-of-Concept Study. K. H. Chalmers, E. De Luca, N. H. M. Hogg, A. M. Kenwright, I. Kuprov, D. Parker, M. Botta, J. I. Wilson, A. M. Blamire, *Chem. Eur. J.*, in press; DOI: 10.1002/chem.200902300.
42.* R. Garcia, P. Fousková, L. Gano, A. Paulo, P. Campello, É. Tóth, I. Santos

A quinazoline-derivative DOTA type gallium(III) complex for targeting Epidermal Growth Factor Receptors: synthesis, characterization and biological studies
*J. Biol. Inorg. Chem.* **2009**, *14,* 261-271.

43.* V. Kubíček and É. Tóth
Design and function of metal complexes as contrast agents in MRI
In "Advances in Inorganic Chemistry", Eds. C. D. Hubbard and R. van Eldik, Vol. 61, "Metal Ion Controlled Reactivity"; **2009**, *61*, 63-129.

44. C. S. Bonnet and É. Tóth
Smart magnetic resonance imaging agents relevant to potential neurological applications
*American Journal of Neuroradiology,* **2010,** *in press*.

45.* M. F. Ferreira, A. F. Martins, J. A. Martins, P. M. Ferreira, É. Tóth and C. F.G.C. Geraldes
Gd(DO3A-N-α-aminopropionate): a versatile and easily available synthon with optimized water exchange for the synthesis of high relaxivity, targeted MRI contrast agents
*Chem. Commun.* **2009**, 6475-6477.

46.* V. Kubíček, A. Hamplová, L. Maribé, S. Mameri, R. Ziessel, É. Tóth, L. Charbonnière
Relaxation and luminescence studies on hydrated bipyridyl- and terpyridyl-based lanthanide complexes
*Dalton Trans*. **2009**, 9466-9474.

47.* C. S. Bonnet, L. Pellegatti, F. Buron, C. M. Shade, S. Villette, V. Kubíček, G. Guillaumet, F. Suzenet, S. Petoud and É. Tóth
Hydrophobic chromophore cargo in micellar structures: a different strategy to sensitize lanthanide cations
*Chem. Commun.* **2010,** *46*, 124–126.

48. C. S. Bonnet and E. Toth
Magnetic resonance imaging probes for sensing biologically relevant metal ions
*Future Medicinal Chemistry, in press.*

**WG3:**

1. Complexes of low energy beta emitters $^{47}$Sc and $^{177}$Lu with zoledronic acid for bone pain therapy. Majkowska, M. Neves, I. Antunes, A. Bilewicz. *Appl. Radiat. Isotop*. 67, 11-13 (**2009**).
2. * Sara Lacerda, M. P. Campello, F. Marques, L. Gano, V. Kubicek, P. Fouskov, Eva Toth and I. Santos, A novel tetraazamacrocycle bearing a thiol pendant arm for labeling biomolecules with radiolanthanides" *Dalton Trans*. 4509–4518 (**2009**).
3. * R. Garcia, P. Fousková, L. Gano, A. Paulo, P. Campello, E. Toth, I. Santos, A quinazoline-derivative DOTA-type gallium(III) complex for targeting epidermal growth factor receptors: synthesis, characterisation and biological studies, J. Biol. Inorg. Chem. 14, 261- 271 (**2009**).
4. * Maria Paula Campello, Marina Balbina, Isabel Santos, Premysl Lubal, Radek Ševcík, Romana Ševcíková, Ln(III) Complexes of 2-[4,7,10-tris(phosphonomethyl)-

1,4,7,10-tetraazacyclododecane-1-yl] acetic acid (H7DOA3P): Multinuclear NMR and Kinetic Studies", *Helvetica Chimica Acta* 92, 2398-2413 (**2009**).
5. * S. Lacerda, F. Marques, P. Campello, L. Gano, V. Kubıcek, P. Hermann, and Isabel Santos, Chemical, radiochemical and biological studies of Sm and Ho complexes of H4dota analogues containing one methylphosphonic/phosphinic acid pendant arm, *J. Labelled Compounds and Radiopharmaceuticals* DOI:10.1002/JLCR.1697 (**2009**).
6. A.Majkowska, M.Neves, I.Antunes, A.Bilewicz, Complexes of low energy beta emitters $^{47}$Sc and $^{177}$Lu with zoledronic acid for bone pain therapy Appl. Radiat.Isotop. 67, 11–13 (**2009**).
7. * J Notni, P. Hermann, J. Havlíčková, J. Kotek, V. Kubíček, J. Plutnar, N. Loktionova, P. Riss, F. Rösch, I. Lukeš, "*A triazacyclononane based bifunctional phosphinate ligand for preparation of multimeric $^{68}$Ga PET tracers*" submitted to *Chem. Eur. J.*
8. * M. Fellner, R. P. Baum, V. Kubíček, P. Hermann, I. Lukeš, V. Prasad, F. Rösch, „*PET/CT imaging of osteoblastic bone metastases with $^{68}$Ga-bisphosponates - first in human study*" *Eur. J. Nucl. Med. Mol. Imag.*, **2010**, *37*, accepted; DOI: 10.1007/s00259-009-1355-y
9. * T. Vitha, V. Kubíček, J. Kotek, P. Hermann, L. V. Elst, R. N. Muller, I. Lukeš, J. A. Peters, „A Gd(III) complex of a monophosphinate-bis(phosphonate) DOTA analogue with a high relaxivity; lanthanide(III) complexes for imaging and radiotherapy of calcified tissues" Dalton Trans., 3204–3214 (**2009**).
10. * Z. Kotková, G. A. Pereira, K. Djanashvili, J. Kotek, J. Rudovský, P. Hermann, L. V. Elst, R. N. Muller, C. F. G. C. Geraldes, I. Lukeš, J. A. Peters, „*Lanthanide(III) complexes of phosphonic acid monoester and phosphinic acid analogues of $H_4$DOTA as model compounds for evaluation of the second-sphere hydration*" *Eur. J. Inorg. Chem.*, 119–136 (**2009**).
11. M. Försterová, M. Petřík, A. Lázničková, M. Lázníček, P. Hermann, I. Lukeš, F. Melichar, „Complexation and biodistribution study of $^{111}$In and $^{90}$Y complexes of bifunctional phosphinic acid analogues of $H_4$dota" Appl. Radiat. Isotop. 67, 21–29 (**2009**).
12. * S. Lacerda, F. Marques, P. Campello, L. Gano, V. Kubíček, P. Hermann, I. Santos, „Chemical, radiochemical and biological studies of Sm and Ho complexes of $H_4$dota analogues containing one methylphosphonic/phosphinic acid pendant arm" J. Labelled Compnd. Radiopharm. **2010**, DOI: 10.1002/jlcr.1697.
13. * M. Polášek, J. Kotek, P. Hermann, I. Císařová, K. Binnemans, I. Lukeš, „Lanthanide(III) complexes of pyridine-N-oxide analogues of DOTA in solution and in the solid state. A new kind of isomerism in complexes of DOTA-like ligands" Inorg. Chem. (**2009**), 48, 466–475.
14. I. Svobodová, J. Havlíčková, J. Plutnar, P. Lubal, J. Kotek, P. Hermann, „Metal complexes of 4,11-dimethyl-1,4,8,11-tetraazacyclotetradecane-1,8-bis(methylphosphonic acid). Thermodynamic and formation/decomplexation kinetic studies"Eur. J. Inorg. Chem. **2009**, 3577–3592.
15. J. Champion, C Alliot, S Huclier, D Deniaud, Z Asfari and G Montavon; Determination of stability constants between complexing agents and At(I) and At(III) species present at ultra-trace concentrations. Inorganica Chimica Acta, 362, 2654-2661 (**2009**)

16. C. CUTLER, S. HUCLIER and S. JURISSON. En préparation, à soumettre à Chem. Review, 2010.
17. S. HUCLIER-MARKAI, A. SABATIE, P. HERMANN, M. PARIS, C. VIDAUD, C. CUTLER. Evalutation of Scandium-polyaminopolycarboxylic complexes as a new generation of PET agent and radiopharmaceutical. En préparation, à soumettre à J. Radiolabelled Comp., **2010**.
18. *Zuzana Kotková, Giovannia A. Pereira, Kristina Djanashvili, Jan Kotek, Jakub Rudovský, Petr Hermann, Luce Vander Elst, Robert N. Muller, Carlos F.G.C. Geraldes, Ivan Lukeš and Joop A. Peters. Lanthanide(III) complexes of phosphonic acid monoester and phosphinic acid analogues of $H_4$DOTA as model compounds for evaluation of the second-sphere hydration. Eur. J. Inorg. Chem*., 119-136 (***2009***).
19. *Carlos F.G.C. Geraldes, Sophie Laurent. Classification and Basic Properties of Contrast Agents for Magnetic Resonance Imaging. *Contrast Media Mol. Imaging*, **4**, 1-23 (**2009**).
20. Sara Silvério, Susana Torres, André F. Martins, José A. Martins, João P. André, Lothar Helm, M. Isabel M. Prata, Ana C. Santos and Carlos F.G.C. Geraldes. (*bis*)-Hydroxymethyl-substituted DTTA skeleton: a new lead for the synthesis of high relaxivity MRI contrast agents? *Dalton Trans*., 4656-4670 (**2009**)
21. *Miguel F. Ferreira, André F. Martins, José A. Martins, Paula M. Ferreira, Éva Tóth and Carlos F.G.C. Geraldes Gd(DO3A-N-α-aminopropionate): a versatile and easily available synthon with optimized water exchange for the synthesis of high relaxivity, targeted MRI contrast agents. ***Chem. Commun*.., 6475-6477 (2009).**
22. *Miloslav Polášek, Petr Hermann, Joop A. Peters, Carlos F. G. C. Geraldes and Ivan Lukeš. PAMAM dendrimers conjugated with an uncharged gadolinium(III) chelate with a fast water exchange: the influence of chelate charge on rotational dynamics. *Bioconj. Chem*., **20**, 2142–2153 (**2009**).
23. *Giovannia A. Pereira, Laura Ball, A. Dean Sherry, Joop A. Peters and Carlos F.G.C. Geraldes. NMR Characterization of $Ln^{3+}$ Complexes of Tetraazaphosphinate and Phosphonate Ligands. *Helv. Chim. Acta*, **92**, 2532-2551 (**2009**).
24. *M. Polášek, M. Šedinová, J. Kotek, L. V. Elst, R. N. Muller, P. Hermann, I. Lukeš, „Pyridine-N-oxide analogues of DOTA and their gadolinium(III) complexes endowed with a fast water exchange on the square-antiprismatic isomer“ Inorg. Chem. **2009**, 48, 455–465.
25. *I. Řehoř, V. Kubíček, J. Kotek, P. Hermann, I. Lukeš, J. Száková, L. V. Elst, R. N. Muller, J. A. Peters, „$^1$H NMR Relaxivity of Aqueous Suspensions of Titanium Dioxide Nanoparticles Coated with a Gadolinium(III) Chelate of a DOTA-monoamide with a Phenylhosphonate Pendant Arm“ J. Mater. Chem. **2009**, 19, 1494–1500.
26. *M. Polášek, P. Hermann, J. A. Peters, C. F. G. C. Geraldes, I. Lukeš, “PAMAM dendrimers conjugated with an uncharged gadolinium(III) chelate with a fast water exchange: the influence of chelate charge on rotational dynamics” Bioconjugate Chem. **2009**, 20, 2142–2153.
27. *V. Kubíček, T. Vitha, J. Kotek, P. Hermann, L. V. Elst, R. N. Muller, I. Lukeš, J. A. Peters, „Highly Effective Responsive Agents for Magnetic Resonance Imaging: Metal-Induced Polymerization of Dota-Bisphosphonate Conjugates“ Contrast Media Mol. Imag. **2010**, submitted.

28. Z. Kotková, J. Kotek, D. Jirák, P. Jendelová, Z. Berková, P. Hermann, I. Lukeš, “Cyclodextrin-based Bimodal Fluorescence/MRI Contrast Agent: an Efficient Way for Cellular Imaging” Chem. Eur. J. **2010**, submitted
29. Pruszyński M, Majakowska A, Loktionova NS, Rösch F. Radiolabeling of DOTATOC with the new generator-derived positron emitter $^{44}$Sc. Bioconj Chem, (**2009**) submitted
30. Zoller F, Riss PJ, Montforts F-P, Rösch F. Efficient post-processing of aqueous generator eluates facilitates $^{68}$Ga-labelling under anhydrous conditions. Radiochim. Acta, accepted (**2009**)
31. Jahn M, Radchenko V, Filosofov D, Hauser H, Eisenhut M, Jennewein M, Rösch F. Separation and purification of no carrier added arsenic from bulk amounts of germanium being adequate to radiopharmaceutical labeling chemistry. Radiochim. Acta, accepted (**2009**)
32. *Fellner M, Baum RP, Peters JA, Lukeš I, Hermann P, Prasad V, Rösch F. PET/CT imaging of osteoblastic bone metastases with $^{68}$Ga-bisphosponates - first in human study. Eur J Mol Imag Biol (**2009**) in press
33. Riss PJ, Nagel V, Rösch F. Studies towards the development of lipophilic bifunctional $N_3S_3$ chelators for $^{68}$Ga. Radiochim. Acta, accepted (**2009**)
34. Riss PJ, Rösch F. Effective microwave-assisted direct radiosynthesis of [$^{18}$F]PR04.MZ, and [$^{18}$F]LBT999: Selective dopamine transporter ligands for qunatitative molecular imaging by means of PET. Bioorg Med Chem. 17(22) (**2009**) 7630-4.
35. *Pruszyński M, Loktionova NS, Filosofov DV, Rösch F. Post-elution processing of $^{44}$Ti/$^{44}$Sc generator-derived $^{44}$Sc for medical application. Appl. Radiat. Isot., (**2009**) accepted
36. Filosofov DV, Loktionova NS, Rösch F. A $^{44}$Ti/$^{44}$Sc radionuclide generator for potential application of $^{44}$Sc-based PET-radiopharmaceuticals. Radiochim. Acta, accepted (**2009**)
37. Burchardt C, Riss PJ, Zoller F, Maschauer S, Prante O, Kuwert T, Roesch F. [$^{68}$Ga]Ga-DO(2)A-(OBu-l-tyr)(2): synthesis, $^{68}$Ga-radiolabeling and in vitro studies of a novel $^{68}$Ga-DO(2)A-tyrosine conjugate as potential tumor tracer for PET. Bioorg Med Chem Lett. 19(13) (**2009**) 3498-501.
38. Riss PJ, Kroll C, Nagel V, Rösch F. NODAPA-OH and NODAPA-(NCS)n: Synthesis, $^{68}$Ga-radiolabelling and in vitro characterisation of novel versatile bifunctional chelators for molecular imaging. Bioorg Med Chem Lett. 18 (**2008**) 5364–5367
39. Asti M, De Pietria G, Fraternalia A, Grassib E, Sghedonib R, Fioronib F, Roesch F, Versaria A, Salvoa D. Validation of $^{68}$Ge/$^{68}$Ga generator processing by chemical purification for routine clinical application of $^{68}$Ga-DOTATOC. Nuclear Medicine Biology 35 (2008) 721-724
40. Jennewein M, Lewis MA, Zhao D, Tsyganov E, Slavine N, He J, Watkins L, Antich PP, Hermanne A, Rösch F, Mason RP, Thorpe PE. Vascular imaging of solid tumors in rats with a radioactive arsenic-labeled antibody that binds exposed phosphatidylserine. Clin. Cancer Res 14/5 (**2008**) 1377-85
41. Roesch F, Filosofov DV. Production, radiochemical processing and quality evaluation of Ge-68 suitable for production of a $^{68}$Ge / $^{68}$Ga generator. IAEA

document "Radionuclide generators using long parent radionuclides for medical applications", **2008**, accepted

**WG4:**


**1.** *« Contrast Agents for MRI: Recent Advances", S. Laurent, L.Vander Elst, R.N. Muller dans "Encyclopedia of Magnetic Resonance", eds R. K. Harris and R. Wasylishen, John Wiley: Chichester. DOI: 10.1002/9780470034590.emrstm1049. Published on 15th March 2009.*

2. "Iron oxide bsed MR contrast agents : from chemistry to cell labeling", S. Laurent, S. Boutry, I. Mahieu, L. Vander Elst, R.N. Muller, *Curr. Med. Chem.* 16(35), 4712-27 (2009)
3. "How to quantify iron in an aqueous or biological matrix: a technical note", S. Boutry, D. Forge, C. Burtea, I. Mahieu, O. Murariu, S. Laurent, L. Vander Elst, R.N. Muller, *Contrast Med. Mol. Imaging*, accepted (2009)

4. "How to perform accurate and reliable measurements of longitudinal and transverse relaxation times of MRI contrast media in aqueous solutions", C. Henoumont, S. Laurent, L. Vander Elst, *Contrast Med. Mol. Imaging*, accepted (2009)

**5.** "Lanthanide Complexes for Magnetic Resonance and Optical Molecular Imaging", S. Laurent, L. Vander Elst, R.N. Muller, *Q. J. Nucl. Med. Mol Imaging*, 53, accepted (2009)
6. « Magnetic iron oxide nanoparticles for biomedical applications », S. Laurent, J.L. Bridot, L. Vander Elst, R. Muller, *Future Medicinal Chemistry*, accepted (2010)
7. * Řehoř, V. Kubíček, J. Kotek, P. Hermann, I. Lukeš, J. Száková, L. Vander Elst, R.N. Muller, J.A. Peters: $^{1}$H NMR Relaxivity of Aqueous Suspensions of Titanium Dioxide Nanoparticles coated with a gadolinium(III) chelate of a DOTA-monoamide with a phenylhosphonate pendant arm, J. Mater. Chem. 19 (2009) 1494-1500.
8. D. Abou, D. Schuhle, J. Peters, H. Wolterbeek : Somatostatin targeted liposomes for MRI / SPECT dual imaging probes, J. Labelled Compd. Radiopharm. 52 (2009) S54.
9. * M. Polášek, P. Hermann, J.A. Peters, C.F.G.C. Geraldes, I. Lukeš :PAMAM dendrimers conjugated with an uncharged gadolinium(III) chelate with a fast water exchange: the influence of chelate charge on rotational dynamics, Bioconj. Chem. 20 (2009) 2142-2153.
10. Mulder et al. Nanoparticulate assemblies of amphiphiles and diagnostically active materials for multimodality imaging. Acc. Chem. Res. (2009) vol. 42 (7) pp. 904-14
11. Hak et al. A high relaxivity Gd(III)DOTA-DSPE-based liposomal contrast agent for magnetic resonance imaging. Eur. J. Pharm. Biopharm. (2009) vol. 72 (2) pp. 397-404
12. Sanders et al. Morphology, binding behavior and MR-properties of paramagnetic collagen-binding liposomes. Contrast Med. Mol. Imaging (2009) vol. 4 (2) pp. 81-8
13. Kok et al. Cellular compartmentalization of internalized paramagnetic liposomes strongly influences both T(1) and T(2) relaxivity. Magn. Reson. Med. (2009) vol. 61 (5) pp. 1022-1032

14. Strijkers et al. Three-compartment T(1) relaxation model for intracellular paramagnetic contrast agents. Magn. Reson. Med. (2009) vol. 61 (5) pp. 1049-1058
15. van Tilborg et al. Internalization of annexin A5-functionalized iron oxide particles by apoptotic Jurkat cells. Contrast Med. Mol. Imaging (2009) vol. 4 (1) pp. 24-32
16. Poly-beta-cyclodextrin based platform for pH mapping via a ratiometric 19F/1H MRI method. E. Gianolio, R. Napolitano, F. Fedeli, F. Arena, S. Aime, *Chem. Commun.* **2009**, 6044-6046.
17. Chemistry of Molecular Imaging. T.J. Meade, S. Aime, *Acc. Chem. Res.* **2009**, *42*, 821.Pushing the sensitivity envelope of lanthanide-based magnetic resonance imaging (MRI) contrast agents for molecular imaging applications. S. Aime, D. Delli Castelli, S. Geninatti Crich, E. Gianolio, E. Terreno, *Acc. Chem. Res.* **2009**, *21*, 822-831.
18. EMIL European network in cancer molecular imagery. I. Carpusca, S. Mergui, A. Jacobs, S. Aime, C. Loewik, *BIOFUTUR* **2009**, 59.

**WG5:**

1. MR "in vivo" preclinical molecular and cellular imaging. Geninatti-Crich, S; Lanzardo, S; Alberti, D, et al *Minerva Biotecnologica*, 2009, 21, 111-121.
2. Towards improved boron neutron capture therapy agents: evaluation of in vitro cellular uptake of a glutamine-functionalized carborane. Crivello, A; Nervi, C; Gobetto, R, et al. *Journal Of Biological Inorganic Chemistry*, 2009, 14, 883-890.
3. High-Relaxivity Gadolinium-Modified High-Density Lipoproteins as Magnetic Resonance Imaging Contrast Agents. Briley-Saebo, KC; Geninatti-Crich, S; Cormode, DP, et al. *Journal Of Physical Chemistry B*, 2009, 113, 6283-6289.
4. Targeting exofacial protein thiols with Gd-III complexes. An efficient procedure for MRI cell labelling. Digilio, G; Catanzaro, V; Fedeli, F, et al. *Chemical Communications*, 2009, 8, 893-895.
5. Tuning Glutamine Binding Modes in Gd-DOTA-Based Probes for an Improved MRI Visualization of Tumor Cells. Stefania, R; Tei, L; Barge, A, et al. *Chemistry-A European Journal*, 2009, 15, 76-85.
6. Cell-Penetrating Peptides and Peptide Nucleic Acid-Coupled MRI Contrast Agents: Evaluation of Cellular Delivery and Target Binding. R. Mishra, W. Su, R. Pohmann, J. Pfeuffer, M. G. Sauer, K. Ugurbil and J. Engelmann. *Bioconjugate Chemistry* (2009), 20(10), 1860-1868.
7. CPP or Cholesterol Conjugated Antisense PNA for Cellular Delivery. R. Joshi, R. Mishra, W. Su and J. Engelmann. *Journal of Peptide Science* (2009), accepted.
8. Evaluation of radiological and clinical efficacy of $^{90}$Y-DOTATATE therapy in patients with progressive metastatic midgut neuroendocrine carcinomas. J.B Cwikla, A. Sankowski, K.G. Jeziorski, A. Nasierowska-Guttmejer, J.R. Buscombe, R. Mikolajczak, D. Pawlak, J. Walecki. *Pol J Radiol, 2009; 74(1):25-32*
9. Efficacy of radionuclide treatment DOTATATE Y-90 in patients with progressive metastatic gastroenteropancreatic neuroendocrine carcinomas (GEP-NETs): a phase II study. J. B. Cwikla, A. Sankowski, N. Seklecka, J. R. Buscombe, A. Nasierowska-Guttmejer, K. G. Jeziorski, R. Mikolajczak, D. Pawlak, K. Stepien and

J. Walecki: Annals of Oncology Advance Access published online on October 15, *2009 Annals of Oncology*, doi:10.1093/annonc/mdp372

10. Kinetics and thermodynamics of adsorption on hydroxyapatite of the [$^{160}$Tb]terbium-complexes of the bone targeting ligands DOTP and BPPED. C. Rill, Z.I. Kolar, G. Kickelbick, H.Th. Wolterbeek, J.A. Peters. *Langmuir* 25 (2009) 2294-2301.
11. Design of targeted contrast agents for molecular imaging. K. Djanashvili, J.A. Peters. Proceedings of The International Seminar on Chemistry 2008 (pp. 10-23), Jatinangor, Indonesia, 30-31 October 2008.
12. * A structural study on Ga(III), In(III), and Fe(III) complexes of triaza-macrocycle based ligands with N3S3 donor set J. Notni, K. Pohle, J.A. Peters, H. Görls, C. Platas-Iglesias. *Inorg. Chem*. 48(2009) 3257-3267.
13. * Gd(III) complex of a monophosphinate-bis(phosphonate) DOTA analogue with a high relaxivity. Lanthanide(III) complexes for imaging and radiotherapy of calcified tissues. T. Vitha, V. Kubíček, J. Kotek, P. Hermann, L. Vander Elst, R.N. Muller, I. Lukeš, J.A. Peters. *Dalton Trans*. 2009, 3204-3214.
14. Somatostatin targeted liposomes for MRI / SPECT dual imaging probes. D. Abou, D. Schuhle, J. Peters, H. Wolterbeek. *J. Labelled Compd. Radiopharm*. 52 (2009) S54.
15. Site-specific labeling of 'second generation' annexin V with (99m)Tc(CO)(3) for improved imaging of apoptosis in vivo. De Saint-Hubert M, Mottaghy FM, Vunckx K, Nuyts J, Fonge H, Prinsen K, Stroobants S, Mortelmans L, Deckers N, Hofstra L, Reutelingsperger CP, Verbruggen A, Rattat D. *Bioorg Med Chem*. **2010** Jan 4.
16. Molecular imaging of cell death. De Saint-Hubert M, Prinsen K, Mortelmans L, Verbruggen A, Mottaghy FM. *Methods*. **2009** Jun;48(2):178-87.
17. Preliminary in vivo evaluation of a novel $^{99m}$Tc-labeled HYNIC-cys-annexin A5 as an apoptosis imaging agent. Fonge H, de Saint Hubert M, Vunckx K, Rattat D, Nuyts J, Bormans G, Ni Y, Reutelingsperger C, Verbruggen A. Bioorg Med Chem Lett. **2008** Jul 1;18(13):3794-8.
18. * PET/CT imaging of osteoblastic bone metastases with $^{68}$Ga-bisphosponates - first in human study. M. Fellner, R. P. Baum, V. Kubíček, P. Hermann, I. Lukeš, V. Prasad, F. Rösch, *Eur. J. Nucl. Med. Mol. Imag.*, **2010**, *37*, accepted; DOI: 10.1007/s00259-009-1355-y
19. * A Gd(III) complex of a monophosphinate-bis(phosphonate) DOTA analogue with a high relaxivity; lanthanide(III) complexes for imaging and radiotherapy of calcified tissues T. Vitha, V. Kubíček, J. Kotek, P. Hermann, L. V. Elst, R. N. Muller, I. Lukeš, J. A. Peters. *Dalton Trans.* **2009**, 3204–3214.
20. * Lanthanide(III) complexes of phosphonic acid monoester and phosphinic acid analogues of $H_4$DOTA as model compounds for evaluation of the second-sphere hydration. Z. Kotková, G. A. Pereira, K. Djanashvili, J. Kotek, J. Rudovský, P. Hermann, L. V. Elst, R. N. Muller, C. F. G. C. Geraldes, I. Lukeš, J. A. Peters. *Eur. J. Inorg. Chem.* **2009**, 119–136.
21. Complexation and biodistribution study of $^{111}$In and $^{90}$Y complexes of bifunctional phosphinic acid analogues of $H_4$dota. M. Försterová, M. Petřík, A. Lázničková, M. Lázníček, P. Hermann, I. Lukeš, F. Melichar. *Appl. Radiat. Isotop.* **2009**, *67*, 21–29.
22. * Chemical, radiochemical and biological studies of Sm and Ho complexes of $H_4$dota analogues containing one methylphosphonic/phosphinic acid pendant arm.

S. Lacerda, F. Marques, P. Campello, L. Gano, V. Kubíček, P. Hermann, I. Santos. *J. Labelled Compnd. Radiopharm.* **2010**, *53*, 36–43.
23. * Lanthanide(III) complexes of pyridine-N-oxide analogues of DOTA in solution and in the solid state. A new kind of isomerism in complexes of DOTA-like ligands. M. Polášek, J. Kotek, P. Hermann, I. Císařová, K. Binnemans, I. Lukeš. *Inorg. Chem.* **2009**, *48*, 466–475.
24. Metal complexes of 4,11-dimethyl-1,4,8,11-tetraazacyclotetradecane-1,8-bis(methylphosphonic acid). Thermodynamic and formation/decomplexation kinetic studies. I. Svobodová, J. Havlíčková, J. Plutnar, P. Lubal, J. Kotek, P. Hermann, " *Eur. J. Inorg. Chem.* **2009**, 3577–3592.

**WG6:**

1. Unsymmetrical Tripodal Ligand for Lanthanide Complexation: Structural, Thermodynamic and Photophysical Studies. B. El Aroussi, N. Dupont, G. Bernardinelli, J. Hamacek, *Inorg. Chem.* **2009**, DOI: 10.1021/ic901757u.
2. Self-Assembly of a Trinuclear Luminescent Europium Complex. S. Zebret, N. Dupont, G. Bernardinelli, J. Hamacek, *Chem. Eur. J.* **2009**, 3355.
3. Click chemistry with lanthanide complexes: a word of caution. G.J. Stasiuk, M.P. Lowe, *Dalton Trans.* **2009**, 9725-9727.
4. Luminescence Study of Eu(III) Analogues of Esterase-Activated Magnetic Resonance Contrast Agents. M. Giardiello, M.P. Lowe, *Inorg. Chem.* **2009**, *48*, 8515–8522.
5. *Changing the local coordination environment in mono- and bi- nuclear lanthanide complexes through click chemistry. M. Jauregui, W.S. Perry, C. Allain, L.R. Vidler, M.C. Willis, A.M. Kenwright, J.S. Snaith, G.J. Stasiuk, M.P. Lowe, S. Faulkner, *Dalton Trans.* **2009**, 6283-6285.
6. * Relaxometric, Thermodynamic and Kinetic Studies of Lanthanide(III) Complexes of DO3A based Propylphosphonates. I. Mamedov, P. Táborský, P. Lubal, S. Laurent, L. Vander Elst, H. A. Mayer, N. K. Logothetis, G. Angelovski, *Eur. J. Inorg. Chem.*, **2009**, 3298-3306.
7. *Variation of hydration state and water exchange dynamics with ligand structure and stereochemistry in lanthanide (III) complexes based on substituted 1,4-diazepine ligands. E. M. Elemento, D. Parker, E. Gianolio, S. Aime and L. Lattuada, *Org. Biomol. Chem.* **2009**, 7, 1120-1131.
8. Cell penetrating metal complexes as optical probes: a mechanistic approach to targeted responsive systems based on lanthanide luminescence. C. P. Montgomery, B. S. Murray, E. J. New, D. Parker, R. Pal. *Acc. Chem. Res.* **2009**, *42*, 925-937.
9. Comparative study of the constitution and chiroptical properties of emissive lanthanide complexes with a common tetraazatriphenylene sensitiser: the nature of the sensitiser determines quenching sensitivity and cellular uptake. E. J. New, D. Parker, R. D. Peacock, *Dalton Trans.* **2009**, 672-679.
10. The mechanism of cell uptake of luminescent lanthanide optical probes: the role of macropinocytosis and the effect of enhanced membrane permeability on compartmentalisation. E. J. New, D. Parker, *Org. Biomol. Chem.* **2009**, *7*, 851-855.
11. A europium luminescence assay of lactate and citrate in biological fluids. R. Pal, D. Parker, L. C. Costello, *Org. Biomol. Chem.* **2009**, *7*, 1525-1528.

12. Lanthanide (III) ion complexing compounds, luminescent lanthanide (III) ion complexes and use thereof as fluorescent labels, L. Lamarque, D. Parker, C. P. Montgomery, *UK Pat. Appl.* **2009**, GB 0900913.5 (priority January 20 2009).
13. Emissive and cell permeable pyridyl and pyrazoyl-1-azaxanthone lanthanide complexes and their behaviour in cellulo. C. P. Montgomery, E. J. New, L. O. Palsson, D. Parker, A. S Batsanov, L. Lamarque, *Helv. Chim. Acta*, **2009**, *92*, 2186-2213.
14. * Paramagnetic fluorine labelled lanthanide complexes as probes for $^{19}$-F magnetic resonance imaging and spectroscopy, K. H. Chalmers, E. De Luca, A. M. Kenwright, N. H. M. Hogg, I. Kuprov, D. Parker, M. Botta, J. I. Wilson and A. M. Blamire, *Chem. Eur. J.* **2009**, DOI: 10.1002/chem.200902300.
15. The mechanism of quenching of the lanthanide excited state for optical probes using sensitised emission. G-L Law, D. Parker, S. L. Richardson and K-L. Wong, *Dalton Trans.* **2009**, 8481-8484.
16. Responsive and reactive terbium complexes with an azaxanthone sensitiser and one naphthyl group: applications in ratiometric oxygen sensing in vitro and in regioselective cell killing. , G-L. Law, R. Pal, L-O. Palsson, D. Parker, K. L. Wong, *Chem. Commun.* **2009**, 7321-7333.
17. Selective imaging of damaged bone structure (microcracks) using a targeting supramolecular Eu(III) complex as a lanthanide luminescent contrast agent, B. McMahon, P. Mauer, C.P. McCoy, T. Clive Lee, T. Gunnlaugsson, *J. Am. Chem. Soc.* **2009**, *131*, 17542-17543.
18. Formation of Novel Di-Nuclear Lanthanide Luminescent Sm(III), Eu(III) and Tb(III) Triple Stranded Helicates from a C2 Symmetrically 2,6-Dicarboxylic Amide based 1,3-Xylene Ligand in CH3CN. S. Comby, F. Stomeo, C.P. McCoy, T. Gunnlaugsson, *Helvetica Chem. Acta* **2009**, *94*, 2461-2473.
19. 4-Amino-1,8-naphthalimide based Tröger's bases as high affinity DNA targeting fluorescent supramolecular scaffolds. E.B. Veale, D.O. Frimannsson, M. Lawler, T. Gunnlaugsson, *Org. Lett.* **2009**, *11*, 4040-4043.
20. Luminescent self-assembly formation on a gold surface observed by reversible 'off-on' switching of Eu(III) emission. N.S. Murray, S.P. Jarvis, T. Gunnlaugsson, *Chem. Commun.* **2009**, 4959-4961.
21. Metal directed synthesis of enatiomerially pure dimetallic lanthanide luminescent triple-stranded helicates. F. Stomeo, C. Lincheneau, J.P. Leonard, J.E. O'Brien, R. Peacock, C.P. McCoy, T. Gunnlaugsson, *J. Am. Chem. Soc.* **2009**, **131**, 9636-9637.
22. Demonstration of bidirectional photoinduced electron transfer (PET) sensing in 4-amino-1,8-naphthalimide based thiourea anion sensors. E.B. Veale, G.M. Tocci, F.M. Pfeffer, P.E. Krugera, T. Gunnlaugsson, *Org. Biomol. Chem.* **2009**, 3447-3454.
23. Mixed f-d coordination complexes as dual visible and near infrared (NIR) emitting probes for targeting DNA. A.M. Nonat, S.J. Quinn, T. Gunnlaugsson, *Inorg. Chem.* **2009**, *48*, 4646-4649.
24. Fluorescence imaging of bone cracks (microdamage) using visibly emitting, 1,8-naphthalimide-based PET sensors. R. Parkesh, T. Clive Lee, T. Gunnlaugsson, *Tetrahedron Lett.* **2009**, *50*, 4114-4116.
25. Lanthanide luminescent gold nanoparticles: pH-driven self-assembly formation between Eu(III)-cyclen conjugated AuNPs and sensitising β-diketonate antenna in water. C.S. Bonnet, J. Massue, S.J. Quinn, T. Gunnlaugsson, *Org. Biomol. Chem.*

**2009**, *7*, 3074-3078.

26. The recognition of anions using delayed lanthanide luminescence: The use of Tb(III) based urea functionalised cyclen complexes. C.M. Gomes dos Santos, T. Gunnlaugsson, *Dalton Trans.* **2009**, 4712–4721.
27. Luminescent sensing and formation of mixed f-d metal ion complexes between a Eu(III)-cyclen-phen conjugate and Cu(II), Fe(II), and Co(II) in buffered aqueous solution. A.M. Nonat, A.J. Harte, K. Sénéchal-David, J.P. Leonard, T. Gunnlaugsson, *Dalton Trans.* **2009**, 4703–4711.
28. Lanthanide macrocyclic quinolyl conjugates as luminescent molecular switches and integrated logic gates using $H^+$ and $O_2$ as inputs. C.S. Bonnet, T. Gunnlaugsson, *New. J. Chem.* **2009**, *33*, 1025-1030.
29. Crystallographic, 1H NMR and CD studies of sterically strained thiourea anion receptors possessing two stereogenic centres. H.D. Paduka Ali, S.J. Quinn, T. McCabe, P.E. Kruger, T. Gunnlaugsson, *New. J. Chem.* **2009**, *33*, 739-800.
30. Exploring the luminescent sensing of anions by the use of an urea functionalized 1,10-phenanthroline (phen) based (3:1) Eu(III) complex. C.M. G. dos Santos, T. Gunnlaugsson, *Supramol. Chem.* **2009**, *21*, 173-180.
31. Methods for an improved detection of the MRI-CEST effect. E. Terreno, J. Stancanello, D. Longo, D. Delli Castelli, L. Milone, H.M. Sanders, M.B. Kok, F. Uggeri, S. Aime, *Contrast Media Mol Imaging* **2009**, *4*, 237-247.
32. Lanthanide-loaded paramagnetic liposomes as switchable magnetically oriented nanovesicles. E. Terreno, D. Delli Castelli, S. Aime, *Methods Enzymol.* **2009**, *464*, 193-210.
33. Osmotically Shrunken LIPOCEST Agents: An Innovative Class of Magnetic Resonance Imaging Contrast Media Based on Chemical Exchange Saturation Transfer. E. Terreno, D. Delli Castelli, E. Violante, H.M. Sanders, N.A. Sommerdijk, S. Aime, *Chem. Eur. J.* **2009**, *15*, 1440-1448.

- ***Conferences and Workshops***

- **Working Group Meeting (WG2, MRI Contrast Agents)**

The 3rd meeting of Working Group 2 “MRI Contrast Agents” of COST D38 Action “Metal-Based Systems for Molecular Imaging Applications” was held at Delft University of Technology, the Netherlands, on 19-20 February, 2009. The organization of this meeting was done by Kristina Djanashvili (Delft University of Technology). In total, 41 participants have attended the meeting.

The scientific program was distributed throughout two half days and included 9 oral presentations by all member teams of the working group (25 min+5 min discussion) and poster sessions where 12 posters were presented.

The meeting was opened with the lecture given by invited speaker Dr. Louise van der Weerd from Leiden University, the Netherlands (45 min + 5 min discussion). This lecture has initiated a lively discussion about the strategy choices in the design of new imaging

agents. Aspects such as effects of the structure of the agents on their blood-brain-barrier crossing ability and the cell uptake were discussed. All the participants agreed that this input by an expert from medical field was very enriching for the working group and should be encouraged in the future meetings.
The next WG meeting was decided to take place in Coimbra, Portugal, in February 2010.

- **Joint WG1/WG6 meeting**

A one-day joint meeting with WG1 was held in Cologne on the 25$^{th}$ of August within the 7-ICfE (International Conference on f-Elements).
Over 30 researchers attended the meeting as representatives of the WGs teams including two invited speakers, Harri Harma (University of Turku, Finland) and Peter Caravan (Harvard University, Charlestown, MA, USA), who opened the two scientific sessions.
In addition to the invited contribution, 10 oral contributions (20 mins each) and 4 flash oral presentations (5 mins each, selected from posters) from WGs members completed the scientific programme. In addition to oral contributions, a significant number of posters illustrating the research carried out within WG1/WG6 were presented and discussed during the ICfE poster session.
The meeting closed with a round table chaired by Stephen Faulkner (University of Oxford) in which the future development of the science underpinning the core aims of the action. Particular attention was paid to the ways in which these aims could be facilitated by liaison with clinical scientists to define key areas which can be use to illustrate the effectiveness to the wider community, and with engineers who can accelerate the development of new equipment for imaging. It was agreed that the working groups' meeting in 2010 should also invite participants for the biomedical sciences and engineering, and suggested that this meeting might be structured to encourage formation of clusters that could work together to achieve these aims.
The Next joint meeting of these working groups will take place in Tuebingen in April 2010.

- **Joint WG3/WG5 meeting in conjunction with COST Action BM0607 "Targeted Radionuclide Therapy"**

A two-day combined COST D38 (working groups 3 and 5) and COST BM0607 (working group 4) meeting took place in Florence, Italy, at the CUBE Polo Biomedico Careggi University of Florence on Friday 11th and Saturday 12th of September 2009. The scientific program involved 21 oral and some poster presentations organized in five sessions: (i) Targeting probes, (ii) $^{68}$Ga-labbeled compounds, (iii) $^{44}$Ti/$^{44}$Sc generators and $^{44}$Sc chemistry, (iv) Targeted radionuclide therapy and (v) Development of in vitro and animal models.

The meeting was judged by the participants as very interesting and rewarding with excellent scientific discussions, leading to plans for further studies, collaborations and networking. The participants agreed to organise a similar meeting in 2010 together with Prof. Mazzis Bressanone meeting TERACHEM-2010.

It should be noted that about 30% of the participants were very young scientists involved in COST with STSM or joint research program.

- **COST D38 annual meeting, Warsaw , April 2009:**

The Annual Workshop of the COST D38 Action *"Metal-Based Systems for Molecular Imaging Applications"* was organized in **Warsaw**, Poland, on the **25-27th April 2009**. The local organizer of the meeting was Renata Mikolajczak, from the IAE Radioisotope Centre POLATOM. The scientific program contained both oral and posters presentations. The 32 oral (20 to 30 mins each) and have been divided into three main topics and in each of these main topics were represented by two Working Groups: Magnetic Resonance Imaging and Nanosized Probes for Molecular Imaging (WG2+WG4), Nuclear Imaging and Targetting Probes (WG3+WG5), Optical Imaging and Responsive Probes (WG1+ WG6). Prior to the scientific program, the Management Committee had its 4$^{th}$ meeting. The Scientific Sessions started on Saturday afternoon (25th April) with an invited lecture given by Prof. Stephane Petoud from the Univ. of Pittsburgh, USA, and ended at Monday midday. In addition to the invited and oral presentations we had 36 poster presentations, which were exposed all along the meeting and discussed during long coffee breaks and lunch time. Very importantly, a one hour round table was included in the scientific program, open to all participants, which was dedicated to a discussion on "D38 Action: what has been done, what will be done till the end of the Action and beyond".

- ***Scientific and Technical Cooperation***

Scientific and Technical Cooperation within the Action has continued to be very relevant as witnessed by the high number of joint publications (***64***) and the exchange of personel among the participating teams (8 STSM in 2009). The tight collaboration in D38 Network granted full access to specialized equipment and methodologies that are available in few laboratories. Each group is more and more developing a specific expertise and a specialized instrumentation that is shared with the other participants in terms of joint activities as well as of training of young researcher (STSM).

Key collaborations within the past year have involved both intra- and inter-working group projects. The collaborative research projects involving 2-3 or in some cases even more teams keep increasing.

Some of them are listed below (the list is not exhaustive):
*Brimingham* collaborates with Prof Ziessel and Dr Angelovski within D38.
*Leuven*- Scientific and technical cooperation continues with Prof. Robert Muller and Prof. Luce Vander Elst from Department of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons-Hainaut, Belgium, which also participates in COST D38 action. The collaborations were successfully established with Dr. Goran Angelovski (Max Planck Institute for Biological Cybernetics, *Tübingen, Germany)* which participates in the WG6, and the group of Prof. Ivan Lukes, from Charles University, Prague.

*Lausanne*- Scientific cooperation encompasses the physical and biological sciences, with key collaborators being Prof. H.-A. Lehr (Pathology Laboratory, Lausanne University Hospital) and Prof. M. Gijs (Microtechnics Laboratory, EPFL), Prof. L. Helm (WG2), and Prof I. Hemmila (WG1)
*Oxford*- Scientific cooperation continues with Prof. Thorfinnur Gunnlaugsson (TCD, WG6), Dr Mark Lowe (Leicester), and a new collaboration has begun with Prof. Mauro Botta (Alessandria). Collaborations related to the clinical development of techniques allied to the action are in progress with Prof. Kieran Clarke (Physiology, Oxford), Prof. Risto Kauppinen (Dartmouth, US), and Dr Keith Brain (Pharmacology, Birmingham)
*Petoud* has extensive collaborations with colleagues at the University of Pittsburgh, and with Prof. Toth.
The Strasbourg groups continue to collaborate extensively within WG1, as evinced by their published output.

i) A collaboration between **Angelovski** (Tuebingen) and **Parker** (Durham) has led to an EC funded Marie Curie Fellowship (to Dr. A. Mishra 2009-2011) for Dr. Mishra to work in Durham on targeted contrast agents.
ii) **Parker** has been working with **Botta** (WG 2) on relaxation analysis of 19-F labelled systems leading to a full paper in Chem Eur J.
iii) **Terreno** has been cooperating with **Parker** on liposomal models for responsive 19-F MR probes.
iv) **Parker** has been cooperating with **Geraldes** (WG2) on NMR studies of the selective interaction of enantiomers of chiral complexes with serum albumin, using difference saturation transfer methods.
v) **Angelovski** has been collaborating with the group of Dr. Eva Toth (WG 2, CNRS Orleans) on the physicochemical characterization of $Ca^{2+}$-sensitive MR probes. Two manuscripts are in preparation.
vi) **Angelovski** has been cooperating with Dr. Sophie Laurent and Prof. Luce Vander Elst (WG 4, University of Mons) on relaxometric studies of pH-sensitive Ln-DO3A-alkylphosphonate complexes. The results are published in Eur. J. Inorg. Chem.
vii) **Angelovski** has been collaborating with Dr. Tatjana Parac-Vogt, (WG 1, KU Leuven) on the physicochemical characterization of heterometallic lanthanide complexes. The manuscript with the obtained results has been submitted for publication.
viii) **Terreno** and **Geraldes** (WG2) have been collaborating on responsive paramagnetic liposomes. A PhD student supervised by Geraldes (Sara Figuereido) joined Terreno's group during this year.
ix) **Terreno** has been collaborating with **Nicolay, Gruell, Strijkers** (WG4) on the design of paramagnetic liposomes acting as CEST agents.

- ***Transfer of results***

Dissemination of the results has been mainly obtained by publishing original papers (191 COST-acknowledged papers in 2009). The central role of the D38 Action in the field of Molecular Imaging is also witnessed by the role of the journal "Contrast Media and Molecular Imaging" (6 issues/year published by Wiley) whose editors and numerous member of the Editorial Boards are from the member teams of the Action.

1. Transfer of scientific results has been efficiently achieved through **COST** action meetings (such as the annual meeting of the overall Action in Warsaw, or WG meetings), poster and oral presentations at major international meetings, as well as through direct discussions between the groups involved, and with interested parties outside the groups.
2. Industrial funding was secured between CISbio (**Mathis**) and **Parker** employing a PhD student in Durham for 39 months till December 2011.
3. Unito (WG 4 and 6) received good support from **Bracco** for the development of targeting agents and responsive (in particular LipoCest) agents.
4. **Serono-Merck** is supporting Unito for a PhD position (2008-2011) in the field of targeting agents.
5. **Parker** has filed two patents; one with **Mathis**/**Lamarque** (CISbio), relating to luminescent probe development and another based on ratiometric analysis of citrate and lactate concentrations of sub-microlitre samples of biological fluids.

***Annex : Case study of Action COST D38, realized by Technopolis in Nov. 2009. in the frame of a Comprehensive Impact Assessment of COST***

technopolis |group|

http://www.technopolis-group.com/site/

**CMST Action D38 Metal-Based Systems for Molecular Imaging Applications**

## *Problem*

Diseases such as cancer and cardiovascular pathologies, and also diseases of the central nervous system, often develop slowly, and can take several years before their first symptoms appear. As a consequence, only the late symptoms of a disease are detected, making treatment difficult and expensive.

Long before the late symptoms develops, however, the body's metabolism begins to change. With a view to detecting illnesses when metabolic changes begin to occur, and thus improving the probability of successful treatment, scientists are working on the *in vivo* visualisation of molecular events occurring at cellular level – called **molecular imaging**. The visualisation of molecules which are the 'signature' of a given disease offers a great potential for earlier detection and characterisation of disease, and evaluation of treatment.

However, more research is necessary to bring these ideas to clinical applications, and a key aspect relates to the development of high-specificity, high-sensitivity **imaging probes** for the different imaging applications. Imaging probes are a special class of pharmaceuticals that are used in conjunction with medical imaging scanners, such as

MRI (magnetic resonance imaging), ultrasound and nuclear imaging, allowing healthcare practitioners to see disease and injuries in a non-invasive way.[1]

## *Goals*

The main objective of Action D38 is to increase the knowledge of the chemistry of metal-based systems for molecular imaging applications and to apply this knowledge to the development of **metal-based imaging probes** for cellular and molecular imaging applications, based on MRI, PET (positron emission tomography), SPECT (single photon computed tomography) and optical imaging. It is thought that metal-based systems will be the preferred choice for a number of molecular imaging probes because, among the 65 elements with metallic properties, it will always be possible to identify the element with peculiar properties required for a desired application.

The Action started in August 2006 and will end in 2011.

## *Activities*

Context

Traditionally, European industry has held the global leadership in the field of *in vivo* diagnostic agents. However, there is now a challenge to this European lead, as the U.S. has undertaken an impressive range of activities, creating national programmes and a dedicated institute.

The establishment of European research programmes on molecular imaging is essential to maintaining the competitiveness of European industries and academic institutions vis-à-vis the U.S. In FP6, two Networks of Excellence NoE (European Molecular Imaging Laboratories for Combating Cancer EMIL, which is more biologically orientated than D38, and Innovative Diagnostics by Molecular Imaging DiMI) and one integrated Project IP were initiated.

Action D38 strengthens these efforts as it acts as a link between chemists already involved in these initiatives and other researchers, whose work complements the work pursued in the two NoE and in the IP. Accordingly, much attention has been devoted to planning joint meetings and exchange of personnel. Furthermore, coordination of joint activities has been sought with other COST Actions which have overlapping interests (for example BMBS Action B21 “Physiological Modelling of MR Image Formation”).

D38 also has close links to big conferences. Both the “European Conference of Biological Inorganic Chemistry” in Thessaloniki in June 2010 as well as the “5th European Molecular Imaging Meeting” in Warsaw in May 2010 will have whole sessions dedicated to D38.

[1] For example: Following an injection with the molecular imaging probe 18F-FDG, which targets active tumours, a patient suspected of having metastasized cancer is scanned using positron emission tomography (PET). The distribution of the probe is detected and appears here as dark spots. The scan shows the presence of multiple tumours and the information will be used to guide the course of therapy.

Participation

COST Action D38 has not brought together a new network. The core of the network of Action D38 has been together since 1993, when Action D1 was running. Then came a whole procession of Actions – D8, D18 and finally D38. Obviously, new members always joined the Actions. Approximately 50 labs are participating in Action D38.

More specifically, Action D38 has its roots in COST Actions D18 (Lanthanide Chemistry for Diagnosis and Therapy) and B12 (Radiotracers for in vivo Assessment of Biological Functions). Both Actions resulted in the development of *in vivo* diagnostics and in exploring new routes for radiotherapeutic drugs. However, molecular imaging implies a qualitative jump by defining a new strand of chemical research that is embedded in biological and medical research.

As a consequence, the Action, though mainly based on chemistry, also integrates expertise in the field of biology, medicine and imaging technologies because optimising the efficacy of radiotracers, MRI and optical imaging probes cannot be pursued without coherent interdisciplinary collaboration.
There are no official participants from non-COST countries. However, the Action is maintaining links with several U.S. scientists. For them it is less burdensome from an administrative point of view to participate in the Action as Invited Speaker rather than through an institutional participation.

Practically all participants are affiliated to universities or research institutes. Only two MC members (Germany, Netherlands) are from industry. However, up to December 2008, Action participants have been able to secure industrial funding for PhD positions in the UK and Italy as well as for a development project. Also, the first annual conference was organised by one of the industrial participants.

Industrial participation is lower than in previous Actions. This may be due to the fact that in molecular imaging development is dispersed, with much research going on in parallel and only a small market to be expected per imaging probe, as imaging probes are very specific. This makes participation less interesting for industry.

A significant number of participants involved in the Action are Early Stage researchers ESR. The Action has specifically sought and encouraged ESR involvement, for example by dedicating plenty of time to poster sessions. Moreover, between 25 August 2006 and 31 December 2008, 21 STSM of ESR (of which 14 women) took place.

## *Activities and Outputs-*

The Action consists of six complementary Working Groups:
WORKING GROUP 1, *Optical Imaging*

WORKING GROUP 2, *MRI Contrast Agents*

WORKING GROUP 3, *Nuclear Imaging probes*

WORKING GROUP 4, *Nanosized probes for molecular imaging*

WORKING GROUP 5, *Targeted Probes*

WORKING GROUP 6, *Responsive Probes*

Apart from the research conducted in the framework of the COST Action, specific networking activities have been taking place. Between the start of the Action in August 2006 and the end of December 2008 took place

- 13 meetings, of which 12 Working Group Meetings (2 in conjunction with a Workshop) and 1 Management Committee meeting.
- 11 Workshops, most of which were Joint Workshops between different Working Groups
- 21 STSM

In 2008 alone

- 145 Action-related journal articles were published and
- 10 research collaborations between COST D38 participants taken up

## *Scientific outcomes and impacts*

The Action is very successful scientifically, as can be seen from the very high number of publications.

Recently, Action participants have submitted a proposal for a Marie Curie Initial Training Network. The Action is also thinking of submitting a new proposal for a COST Action.

The Action is also very successful in terms of networking: By networking the most qualified groups active in the development of high-sensitive imaging probes, it is contributing to the build-up of a European research community in the field of molecular imaging.

## *Socio-economic impact*

Though based on basic research, the fact that there is industry participation shows that the Action is not very far removed from industrial application. There are basically two impacts to be expected:

- The first impact is more short-term: Molecular imaging probes make the drug-discovery process more efficient as they allow to follow the effects of a drug in small animals, thus enabling better predictions on the effectiveness and toxicity of drugs. Imaging probes also have the potential to replace histological examinations, for which the animal has be killed. The development of this field will significantly increase the availability and “time to market” of new drugs.
- The second impact is more long-term: By allowing the visual detection of metabolic changes, molecular imaging probes are expected to facilitate early diagnosis of disease, assessment of disease progression and treatment evaluation.

## *Societal impacts*

Better and faster availability of new drugs, early diagnosis of disease in humans and reduced killings of test animals need not necessarily be looked upon as economic impacts. They are also societal impacts, contributing to the welfare of humans and animals.