
RADIOPHARMACEUTICAL SCIENCES

Annual Activity Report 2013

UNIT: Chemical and Radiopharmaceutical Sciences / C²TN

TEAM

Name	Category	R&D (%)
Isabel Rego dos Santos	Coordinator Researcher	100
António Manuel Rocha Paulo	Principal Researcher	100
João Domingos Galamba Correia	Principal Researcher	100
Célia Maria da Cruz Fernandes	Auxiliary Researcher	100
Fernanda Marujo Marques	Auxiliary Researcher	100
Filipa Fernandes Mendes	Auxiliary Researcher (Ciência 2007), until August; Principal Researcher (FCT Investigator), since Sept.	100
Goreti Jesus Ribeiro Morais	Auxiliary Researcher (Ciência 2008)	100
Maria Cristina das Neves Oliveira	Auxiliary Researcher	100
Maria de Lurdes Barreira Patricio Gano	Auxiliary Researcher	100
Maria Paula Cordeiro Crespo Cabral Campello Aboim de Barros	Auxiliary Researcher	100
Paula Dolores Galhofas Raposinho	Auxiliary Researcher	100
Amadeu Rodrigues	Técnico Profissional Especialista Principal	90% RSG+ 10% UCQR
Elisabete Correia	Técnica Profissional Principal	100
Sofia Gama	Post-doctoral Researcher	100
Elisa Palma	Post-doctoral Researcher	50
Filipe Vultos	PhD Student	100
Maurício Morais	PhD Student	100
Francisco Silva	PhD Student	100
Susana Cunha	PhD student	100
Raid Mansour	IAEA training	25
Eser Ucar,	ERASMUS student	25
Annica de Barros Rosa	MSc Student	25
Y. Chen	PhD Student	10
Vanessa Santos	MSc Student	10
Nuno Lemos,	MSc Student	10
Inês Rodrigues	MSc Student + BI	100
Vera Ferreira	MSc Student + BI	100
Letícia Quental	MSc Student + BI	100
Mariana Pinto	MSc Student	25
Sofia Monteiro	BI	100
Maria Belo	BI	25
Elisabete Ribeiro	BI	30
Patrique Nunes	BI	50
Ana Filipa Silva	BSc Student	25
Pedro Miguel Dias,	BSc Student	30%
Maria Fateixa Palmeiro	BSc student	25%
Inês Matreno	BSc Student	25%

OBJECTIVES

The main goal of the Radiopharmaceutical Sciences (RS) group is to carry on basic/oriented research on molecular or nanosized halogen- (^{18}F and ^{125}I) or metal-based (e.g. $^{99\text{m}}\text{Tc}$, ^{188}Re , ^{67}Ga , ^{111}In , ^{153}Sm) compounds towards the development of diagnostic (PET or SPECT) and therapeutic radioactive tools. Within this area of research, the most relevant topics that have been addressed during 2013 were the following:

- Bone-seeking Platforms for Cancer Theranostics
- DNA-binding Multifunctional Complexes for Auger Therapy
- Organometallic Complexes for *in vivo* Probing of NOS
- Copper Complexes for *in vivo* Targeting of Telomerase
- Nanoconstructs for Targeted Delivery of Radionuclides
- New Molecular Imaging tools for Cystic Fibrosis

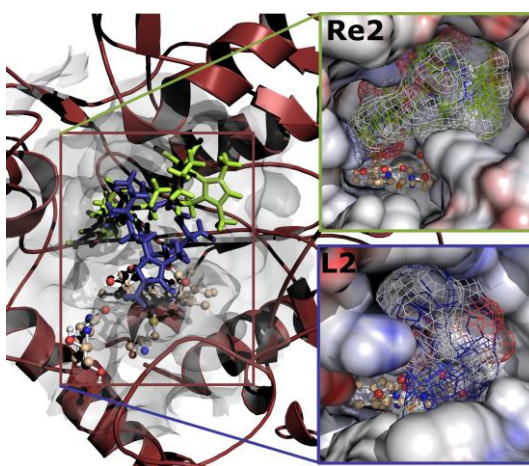
In collaboration with academia and industry partners, the RS group aims also to apply its facilities and expertise in the evaluation of the therapeutic potential and optimization of pharmacokinetics of non-radioactive drugs.

Based on all these activities, the RS group contributes for the advance training of undergraduate, graduate and post-graduate students in the field of modern Radiopharmaceutical Sciences, both at national and international level.

MAIN ACHIEVEMENTS

Targeting of Inducible Nitric Oxide Synthase with $\text{M}(\text{CO})_3$ -Complexes ($\text{M} = ^{99\text{m}}\text{Tc}/\text{Re}$)

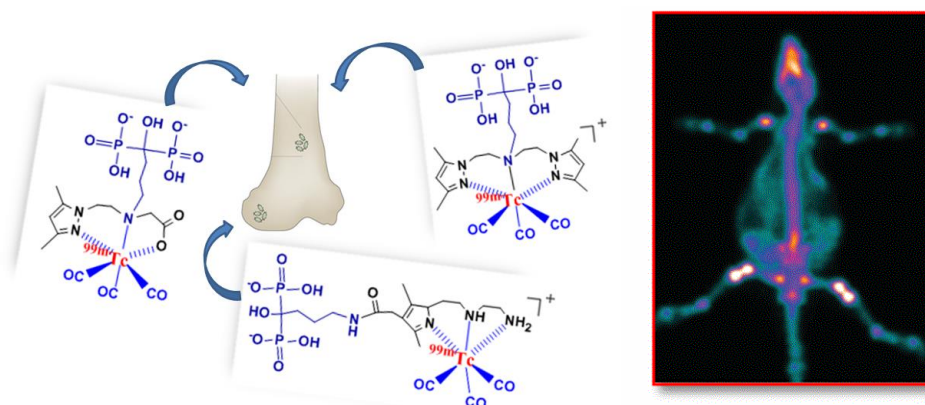
Aiming to probe the Nitric Oxide Synthase (NOS) enzymes, whose expression is related to diseases such as cancer and neurodegenerative disorders, we are exploring $^{99\text{m}}\text{Tc}(\text{CO})_3$ -complexes bearing NOS-recognizing units for SPECT imaging. Enzymatic activity studies demonstrated that some of the Re congener complexes inhibit purified inducible NOS as well as the cytosolic enzyme. To shed light on the specific iNOS/Re-complexes interactions and to establish a structure-activity relationship, computational studies were performed in combination with NMR spectroscopy and X-ray crystallography. The computational studies, including molecular docking, MD simulations and FEP calculations, allowed the identification of the interactions responsible for the iNOS-recognizing ability of the complexes, proving that electrostatic interactions between the $\text{Re}(\text{CO})_3$ core and R260/R382 are a key issue. These findings will allow a more rationale design of $\text{M}(\text{CO})_3$ -complexes ($\text{M} = ^{99\text{m}}\text{Tc}/\text{Re}$) for *in vivo* NOS targeting, offering better opportunities to obtain best performing compounds.



Molecular surfaces of the active site of the complexes L2:iNOS and Re2:iNOS colored according to electrostatic potential. This figure was generated using the VASCo PyMOL plug-in (B. L. Oliveira, I. S. Moreira, P. A. Fernandes, M. J. Ramos, I. Santos, J. D.G. Correia, Theoretical studies on the binding of rhenium(I) complexes to inducible nitric oxide synthase, *J. Mol. Graph. Model.* **2013**, 45, 13-25).

Bisphosphonate-containing $^{99m}\text{Tc}(\text{I})/\text{Re}(\text{I})$ -complexes for theranostic of MBD

Radiolabeled bisphosphonates (BPs) bind to bone matrix in areas of increased bone turnover, characteristic of metastization sites. In the particular case of the matched pair $^{99m}\text{Tc}/^{188}\text{Re}$, radiolabeled BPs might offer the possibility of exploring a theranostic approach for metastatic bone disease (MBD). Having this in mind, we synthesized complexes of the type *fac*- $[\text{M}(\text{CO})_3(\text{k}^3\text{-L})]^+$ ($\text{M} = ^{99m}\text{Tc}, \text{Re}$) stabilized by azolyl-containing bifunctional chelators with different molecular weight, overall charge, (lipo)hydrophilic nature and different positions of BP attachment. Biodistribution studies in mice have shown that the complex bearing the BP unit at position 4 of the azolyl ring (**Tc1**) presents the most favourable pharmacokinetics. Cell uptake studies have shown that this complex presents also the highest accumulation in the cytosol. In comparison with **Tc1**, the ^{188}Re congener (**Re1**) showed a similar bone-seeking ability in healthy mice and a similar accumulation in specific cell lines. Altogether, these results point out that this family of complexes has potential for the design of new theranostic tools for MBD.



Schematic drawing of selected examples of BP-containing *fac*- $[\text{M}(\text{CO})_3(\text{k}^3\text{-L})]^+$ complexes and planar whole-body gamma camera image of a rat injected radioactive complex. (C. Fernandes, S. Monteiro, P. Mendes, L. Gano, F. Marques, S. Casimiro, L. Costa, J. D.G. Correia, I. Santos, Biological assessment of novel bisphosphonate-containing $^{99m}\text{Tc}/\text{Re}$ organometallic complexes, *J Organomet Chem* (2013), <http://dx.doi.org/10.1016/j.jorganchem.2013.10.038>).

EDUCATION AND TRAINING

- **High School/University Visits:** The group has been visited by 500 students from High School and 25 from Universities
- **Graduation:** i) Radiopharmacy in Nuclear Medicine Course, ESTeSL.
- **Post-graduation:** i) Coordination and teaching of Radiopharmaceutical Chemistry in the MSc Pharmaceutical and Therapeutic Chemistry/FFUL; ii) Teaching of Chemical Systems and Reactivity in the 2nd Cycle of Chemistry, FCUL; iii) Teaching at the Master in Pharmaceutical Sciences, Lusófona University; iv) Radiopharmacy in the Integrated MSc Pharmaceutical Sciences, FFUL; v) Teaching of Genetic Engineering in the Integrated MSc in Biological Engineering and in Biomedical Engineering, Bioengineering Department, IST; vi) Teaching of Molecular Biotechnology in the MSc in Biotechnology and Microbiology, Bioengineering Department, IST.
- **Young scientists:** Several young scientists got MSc or PhD degrees in the group, playing a major role in our projects.

OTHERS

Expertise Provided: Public Nuclear Medicine Centers, Nuclear Medicine Champalimaud Foundation, INFARMED, IAEA, National and International Science Foundations (Portuguese, USA, Argentina, Uruguay and Chile) International Conferences and International Journals.

Publications: International Journals: 25; Proceedings: 5; Invited Lectures: 13; Posters: 24; PhD thesis: 1; MSc thesis: 4; BSc thesis: 4; Participation in Juries: 20.

RELEVANT PAPERS

- Influence of the bifunctional chelator on the pharmacokinetic properties of $^{99m}\text{Tc}(\text{CO})_3$ -abeled cyclic α -melanocyte stimulating hormone analog, Morais M, Oliveira BL, Correia JD, Oliveira MC, Jiménez MA, Santos I, Raposinho PD., *J Med Chem.* 2013 Mar 14;56(5):1961-73. doi: 10.1021/jm301647t.
- Emerging protein targets for metal-based pharmaceutical agents: An update, A. de Almeida, B. L. Oliveira, J. D. G. Correia, G. Soveral, A. Casini, *Coordination Chemistry Reviews*, 257, 2689-2704 (2013), doi: 10.1016/j.ccr.2013.01.031.
- Insights into the structural determinants for selective inhibition of nitric oxide synthase isoforms, Oliveira, B.L., Moreira, I.S., Fernandes, P.A., Ramos, M.J., Santos, I., & Correia, J.D.G. (2013). *Journal of Molecular Modeling*, 19(4), 1537-1551. DOI: 10.1007/s00894-012-1677-8.
- Novel Heterobimetallic Radiotheranostic: Preparation, Activity and Biodistribution” L. Adriaenssens, Q. Liu, F. Chauv-Picquet, S. Tasan, M. Picquet, F. Denat, P. Le Gendre, F. Marques, C. Fernandes, F. Mendes, L. Gano, M. P. C. Campello, E. Bodio, *ChemMedChem* 2013. <http://onlinelibrary.wiley.com/doi/10.1002/cmdc.201300494/pdf>
- Carbohydrate-based molecules suitable for nuclear molecular imaging, Ribeiro Morais G, Falconer R A; Santos I, *Eur J Org Chem*, 1401-1414 (2013) (Cover Picture) DOI: 10.1002/ejoc.201201457.

FUNDS

- Molecular and Nano Tools for Cancer Theranostics, **EXCL/QEQ-MED/0233/2012**, Leading Institution: IST/CTN. PI- Isabel Santos.
- Synthesis, Characterization and Biological Assessment of Multi-Functional Bone-Seeking Agents, **PTDC/QUI-QUI/115712/2009**. Leading Institution: IST/CTN.P.I.- Isabel Santos.
- Synthesis and pre-clinical evaluation of novel estradiol-based indium complexes for targeted radiotherapy of tumors, **PTDC/QUI-QUI/111891/2009**. Leading Institution: IST/CTN, P I - Lurdes Gano.
- Radiolabeled benzazole derivatives for in vivo imaging of amyloid aggregation, **PTDC/QUI-QUI/102049/2008**. Leading Institution: IST/CTN, PI - António Paulo.
- Albumin binding-domain fusions to improve protein pharmacokinetics, **PTDC/SAU-FAR/115846/2009**. Leading Institution: Associação para o Desenvolvimento do Ensino e Investigação da Microbiologia (ADEIM), Faculdade de Farmácia, Universidade de Lisboa. CTN P. I.: João Galamba Correia.
- Preclinical evaluation of ruthenium potential drugs for cancer therapy, **PTDC/QUI-QUI/118077/2010**. Leading Institution: Fundação da Faculdade de Ciências, Universidade de Lisboa. CTN P. I. Fernanda Marques.
- Targeting telomerase inhibition with new anti-tumoral Cu(II) complexes, **PTDC/QUI-QUI/114139/2009**, Leading Institution: IST/CTN. P.I. – António Paulo, Sofia Gama.
- A Molecular Imaging Approach to Cystic Fibrosis, **EXPL/BIM-MEC/0115/2012**, Leading Institution: IST/CTN. P. I. – Filipa Mendes.
- Nitric Oxide Synthase targeting with $\text{Re}(\text{I})/^{99m}\text{Tc}(\text{I})$ -complexes containing L-Arg derivatives: A structure-activity study, FCT - **PTDC/QUI-QUI/121752/2010**, Leading Institution IST/CTN. P.I. – João Galamba Correia.
- Dual Targeting Strategy for EGFR Positive Tumors, **EXPL/QEQ-MED/1950/2013**,
- IST-ID, Investigador Responsável: Célia Fernandes

INTERNATIONALIZATION

- Synthetic Probes for Chemical Proteomics and Elucidation of Biosynthetic Pathways, COST Action CM1004.
- Theragnostics Imaging and Therapy: An Action to Develop Novel Nanosized Systems for Imaging-Guided Drug Delivery, COST Action TD1004.
- Functional metal complexes that bind to biomolecules, COST Action CM1105.

- Bimodal PET-MRI molecular imaging technologies and applications for in vivo monitoring of disease and biological processes, COST Action TD1007.
- Target-specific and Heterobimetallic Platinum Complexes: Synthesis, Characterization and Mechanistic Studies, Bilateral Portugal-Spain.

RESEARCHERS TEAM

NAME: Isabel Rego dos Santos

CATEGORY: Coordinator Researcher

IST-ID: 25348

ACTIVITIES

Nº	Activity Description	R&D (%)
1	Synthesis, Characterization and Biological Assessment of Multi-Functional Bone-Seeking Agents, PTDC/QUI-QUI/115712/2009 (P.I.: I. Santos, C ² TN-IST).	20
2	Molecular and Nano Tools for Cancer Theranostic, EXCL/QEQMED/0233/2012 -2013-16 (P.I.: I. Santos, C ² TN-IST).	20
3	PI of an International PhD program presented to FCT last year – Radiopharmaceutical Sciences in Imaging and Cancer Therapy – RadITer.	15
4	Coordinator of the Thematic Strand: Radiopharmaceutical Sciences and Health Physics. PI of the C ² TN Strategic Program presented to FCT for the Unit Evaluation.	15
5	Scientific Coordinator of the Radiopharmaceutical Sciences Group.	15
6	President of C ² TN.	15
Total		100

WORK SUMMARY

Nº	Work Summary and Main Achievements
1	The synthesis and evaluation of metal complexes bearing BP and/or a cytotoxic agente and/or a beta emitter has continued. Biodistribution studies in mice have shown that the complex bearing the BP unit at position 4 of the azolyl ring were the most favourable, being better than the gold standard ^{99m} Tc-MDP. Cell fragmentation studies have shown that this complex presents also the highest accumulation in the cytosol. The biological properties of this Tc complex prompted the synthesis of the analogue with ¹⁸⁸ Re, a β- emitter. The radiosynthesis, purification and analytical control of the precursor <i>fac</i> -[¹⁸⁸ Re(CO) ₃ (H ₂ O) ₃] ⁺ was optimized and using this starting material the best performing ^{99m} Tc complexes were prepared with ¹⁸⁸ Re. These beta emitter complexes were evaluated in specific cell lines and its bone-seeking ability assessed in healthy mice. I have been involved on the coordination of the work.
2	I have been involved on the coordination of the project <i>Molecular and Nano Tools for Cancer Theranostics</i> (EXCL/QEQ-MED/0233/2012). The main activities during 2013 were: Synthesis and characterization of micelles for drug and radioactivity delivery; Synthesis and evaluation of compounds for DNA targeting.
3	I have been PI of an International PhD program entitled – Radiopharmaceutical Sciences in Imaging and Cancer Therapy –RadITer The program, coordinated by ITN/IST, involved University of Zurich, Free University of Brussels, CQE/IST, IMM/FMUL and Champalimaud Foundation. The evaluation was positive, and the non-acceptance was based on administrative issues.
4	As president of C ² TN, I have been writing and coordinating the Strategic Program of C2TN, submitted to FCT for evaluation. This program is based on three thematic strands, which were coordinated by myself (Radiopharmaceutical Sciences and Health Physics), by Manuel Almeida (Advanced Materials) and by Isabel Prudêncio (Earth Systems, Radioactivity and

	Cultural Heritage).
5	President of C2TN – After the election in September 2013, I have been coordinating this Research Unit.

PUBLICATIONS

Book Chapter

- Paulo A, Ribeiro Morais G, Santos I, “Organometallic Chemistry of Rhenium and Technetium Fuelled by Biomedical Applications”, *Organometallic Chemistry: Recent Advances*, Chapter 44, p 589-604 John Wiley & Sons (2013)

Journals

- Biological assessment of novel bisphosphonate-containing ^{99m}Tc/Re organometallic complexes, C. Fernandes, S. Monteiro, P. Mendes, L. Gano, F. Marques, S. Casimiro, L. Costa, J. D.G. Correia, I. Santos, *J Organomet Chem* (2013), <http://dx.doi.org/10.1016/j.jorganchem.2013.10.038>.
- New polydentate Ru(III)-Salan complexes: Synthesis, characterization, anti-tumour activity and interaction with human serum proteins, C. P. Matos, A. Valente, F. Marques, P. Adão, M. P. Robalo, R. F. M. Almeida, J. C. Pessoa, I. Santos, M. H. Garcia, A. I. Tomaz, *Inorganica Chimica Acta* 394 (2013) 616–626. <http://dx.doi.org/10.1016/j.ica.2012.09.026>.
- Screening organometallic binuclear thiosemicarbazone ruthenium complexes as potential antitumour agents: cytotoxic activity and human serum albumin binding mechanism, B. Demoro, R. F. de Almeida, F. Marques, C. P. Matos, L. Otero, J. C. Pessoa, I. Santos, A. Rodríguez, V. Moreno, J. Lorenzo, D. Gambino, A. I. Tomaz. *Dalton Trans* 42 (2013) 7131-7146. <http://pubs.rsc.org/en/content/articlepdf/2013/dt/c3dt00028a>.
- Important cytotoxicity of novel iron(II) cyclopentadienyl complexes with imidazole based ligands, C. Gonçalves, T. S. Morais, M. P. Robalo, F. Marques, F. Avecilla, C. P. Matos, I. Santos, A. I. Tomaz, M.H.Garcia, *J. Inorg Biochem* 129 (2013) 1-8. <http://dx.doi.org/10.1016/j.jinorgbio.2013.07.033>.
- Cellular uptake mechanisms of an antitumor ruthenium compound: the endosomal/lysosomal system as a target for anticancer metal-based drugs, L. Côte-Real, A. P. Matos, I. Alho, T. S. Morais, A. I. Tomaz, M. H. Garcia, I. Santos, M. P. Bicho, F. Marques. *Microsc Microanal* 19 (2013) 1122-1130. <http://dx.doi.org/10.1017/S143192761300175X>.
- New bifunctional metalloproteinase inhibitors: an integrated approach towards biological improvements and cancer therapy, S. M. Marques, C. C. Abate, S. Chaves, F Marques, I Santos, E Nuti, A Rossello, M. A. Santos, *J Inorg Biochem.* 127 (2013) 188-202. <http://dx.doi.org/10.1016/j.jinorgbio.2013.03.003>.
- Biological activity and cellular uptake of -C5H5)(PPh3)(Me2bpy)] [CF3SO3] complex, Morais, T.S., Santos, F., Corte-Real, L., Marques, F., Robalo, M.P., Madeira, P.J.A., & Garcia, M.H. (2013). *Journal of Inorganic Biochemistry*, 122, 8-17. <http://dx.doi.org/10.1016/j.jinorgbio.013.01.011>.
- Influence of the bifunctional chelator on the pharmacokinetic properties of ^{99m}Tc(CO)₃-abeled cyclic α -melanocyte stimulating hormone analog, Morais M, Oliveira BL, Correia JD, Oliveira MC, Jiménez MA, Santos I, Raposinho PD., *J Med Chem.* 2013 Mar 14;56(5):1961-73. doi: 10.1021/jm301647t.
- Target-specific Tc(CO)₃-complexes for in vivo imaging, Morais, M., Paulo, A., Gano, L., Santos, I., & Correia, J.D.G. (2013). *Journal of Organometallic Chemistry*, 744, 125-139. DOI: 10.1016/j.jorganchem.2013.05.050.
- Mono- and dicationic Re(I)/^{99m}Tc(I) tricarbonyl complexes for the targeting of energized mitochondria, Moura, C., Mendes, F., Gano, L., Santos, I., & Paulo, A. (2013), *Journal of Inorganic Biochemistry*, 123, 34-45. DOI: 10.1016/j.jinorgbio.2013.02.008.
- Insights into the structural determinants for selective inhibition of nitric oxide synthase isoforms, Oliveira, B.L., Moreira, I.S., Fernandes, P.A., Ramos, M.J., Santos, I., & Correia, J.D.G. (2013). *Journal of Molecular Modeling*, 19(4), 1537-1551. DOI: 10.1007/s00894-012-1677-8.

- Radiosynthesis and *in vivo* Evaluation of a ^{18}F -Labelled Styryl-Benzoxazole Derivative for beta-Amyloid Targeting, Ribeiro Morais G, Knies T, Bergmann R, Gano L, Abrunhosa A, Santos I, Paulo A, *Appl Rad Isotopes*, 82, 100-104 (2013) doi: 10.1016/j.apradiso.2013.07.003.
- Carbohydrate-based molecules suitable for nuclear molecular imaging, Ribeiro Morais G, Falconer R A; Santos I, *Eur J Org Chem*, 1401-1414 (2013) (Cover Picture) DOI: 10.1002/ejoc.201201457.
- Re(I) and $^{99\text{m}}\text{Tc}$ (I) tricarbonyl complexes with ether-containing pyrazolyl-based chelators: Chemistry, Biodistribution and Metabolism, C. Fernandes, L. Maria, L. Gano, I. C. Santos, I. Santos, Paulo, *Journal of Organometallic Chemistry* (2013), <http://dx.doi.org/10.1016/j.jorganchem.2013.11.013>.
- Kinetic study of formation/dissociation of Cu(II) and Zn(II) complexes of cyclen macrocyclic ligand with pendant thiol group, Sevcikova, R; Lubal, P; Campello, MPC; Santos, I., *Polyhedron* 62 (2013) 268–273. doi.org/10.1016/j.poly.2013.06.052.
- Subramanian S₁, Pandey U, Morais M, Correia JD, Santos I, Samuel G., Comparative biological evaluation of two [$^{99\text{m}}\text{Tc}(\text{CO})_3$]-dextran pyrazolyl mannose conjugates developed for use in sentinel lymph node detection, *Q J Nucl Med Mol Imaging*. 2013 Oct 30. [Epub ahead of print]

Other publications

- Letícia Quental, Goreti Ribeiro Morais, Isabel Santos, António Paulo, Molecular imaging agentes for detection of β -amyloid plaques in Alzheimer's disease, *Revista Saúde & Tecnologia*, Novembro (10), 5-9 (2013), ISSN: 1646-9704.
- F, Mendes, L. Gano, C. Fernandes, A. Paulo, I. Santos, Perfusion and specific radioprobes for cardiac imaging, *CARDIOTECHNIX 2013 - Proceedings of the International Congress on Cardiovascular Technologies*, 79-83 (2013).
- B. Martins, A. Canudo, D. Dantas, S. Chaves, V. Santos, B. Freitas, C. Oliveira, D. C. Costa, I. Santos, C. Fernandes, Evaluation of protein-losing gastroenteropathy in pediatrics with $^{99\text{m}}\text{Tc}$ -Human Serum Albumin - technical and methodological developments, *European Journal of Nuclear Medicine and Molecular imaging*, vol:40 pg:S147 -S147, (2013) (Abstract).

COMMUNICATIONS

Oral/invited talks

- *Radiopharmaceuticals for Inflammation/Infection: Chemical and Labelling Aspects*, I. Santos, *Technical Meeting on Infection Imaging with Radiopharmaceuticals, IAEA Headquarters, Vienna, Austria*, May 13-17th (2013).
- *Metal Building Blocks for Targeted –Specific Delivery of Radiation*, I. Santos, *Radiometals 2013, Santa Rosa, USA*, June 13-16th (2013).
- *Radiopharmaceutical Sciences Group: Research, Highlights and Facilities*, G. Ribeiro Morais, I. Santos, *Fundação Champalimaud, Human Brain Project Workshop*, June 26th (2013).
- *Perfusion and specific radioprobes for cardiac imaging*, F. Mendes, L. Gano, C. Fernandes, Paulo, I. Santos, *Cardiotechix 2013 International Congress on Cardiovascular Technologies – Symposium VisualCardio*, Vilamoura, 21th September (2013).
- *Mitochondria-targeted Re(I)/ $^{99\text{m}}\text{Tc}$ (I) Tricarbonyl Complexes*, A. Paulo, C. Moura, F. Mendes, L. Gano, I. Santos, *WG3 meeting – COST CM1105, Barcelona*, 10th September (2013).
- *Small Core Gold Nanoparticles Stabilized by Thiolated DOTA Derivatives*, Francisco Silva, Maria P. C. Campello, L. Gano, Isabel Santos, A. Paulo, *Nanoparticles for early Diagnostics of Inflammatory diseases*, November 20/21st, 2013, Lisbon.
- *Avaliação biológica de novos complexos de $^{99\text{m}}\text{Tc}$ (I) no diagnóstico de metástases ósseas*, C. Fernandes, S. Monteiro, E. Palma, L. Gano, F. Marques, J. D. G. Correia, I. Santos, *XIV Congresso Nacional de Medicina Nuclear*, Porto, Portugal, 5-7th December (2013).
- *Complexos de ^{111}In derivados de estradiol: Avaliação pré-clínica para o diagnóstico do cancro da mama*, S. Cunha, F. Vultos, C. Fernandes, F. Marques, M.C. Oliveira, I. Santos, L. Gano, *XIV Congresso Nacional Medicina Nuclear*, Porto, Portugal, 5-7th December (2013).
- *Evaluation of protein-losing gastroenteropathy in pediatrics with $^{99\text{m}}\text{Tc}$ -Human Serum Albumin - technical and methodological developments*, B. Martins, A. Canudo, D. Dantas, S. Chaves, V.

- Santos, B. Freitas, C. Oliveira, D. C. Costa, I. Santos, C. Fernandes, *Annual Congress of the European-Association-of-Nuclear-Medicine (EANM)*, Lyon, France, Oct 19-23th, (2013).
- *Pharmacokinetic properties of a ⁶⁷Ga-labeled anti-TNF VHH single domain antibody containing a bacterial albumin-binding domain (Zag)*, J. D. G. Correia, M. Morais, L. Gano, I. Santos, C. Cantante, S Lourenço, C. Santos, C. Fontes, F. Silva, J. Gonçalves, *2nd International Workshop on Innovative Personalized Radioimmunotherapy, WIPR 2013*, Nantes, France, July (2013).
 - *Indium-111 Radiolabelled Peptide Towards the Estrogen Receptor*, F. Vultos, C. Fernandes, I. Santos, J. D. G. Correia, L. Gano, *The Future of radiation oncology: Imaging, Dosimetry, Biology & Therapy - Seventh Berder Workshop*, France, September (2013).
 - *Bimodal Imaging of Sentinel Lymph Node with ^{99m}Tc(CO)₃-labeled Mannosylated Nanoparticles bearing a NIR-fluorophore*, M. Morais, C. Xavier, S. Hernot, T. Lahoutte, V. Caveliers, J. D. G. Correia, I. Santos, *Annual Meeting of Cost Action 1004*, Athens, Greece, September (2013).

Poster presentations

- *Organometallic Tc-99m/Re Complexes for in vivo targeting of beta-amyloid plaques*, Nunes P, Morais G, Palma E, Gano L, Santos I, Paulo A, *Drug Discovery Therapy World Congress 2013*, 3-6th June 2013, Boston, USA.
- *New benzimidazole derivatives for the targeting of beta-amyloid aggregates*, Ribeiro Morais G, Quental L, Oliveira C, Palma E, Gano L, Miranda H, Outeiro T, Abrunhosa A, Santos I, Paulo A, *EMIM 2013*, 26-27th May 2013, Turin, Italy. Abstract 47
- *Peptides targeting alpha-synuclein*, Quental L A, Raposinho P, Santos I, Paulo A, Ribeiro Morais G, *1st Symposium on Medicinal Chemistry*, 17 May 2013, Braga, Portugal. Abstract PC66-
- *Evaluation of ^{99m}Tc-TMEOP as Probe for Functional Monitoring of Multidrug Resistance*, F. Mendes, L Gano, C. Fernandes, A. Paulo, I. Santos “”, *Molecular Biology in Portugal and EMBL (and EMBL Alumni)*, 18th July (2013), FCUL, Lisbon.
- *⁶⁷Ga-Labelled Gold Nanoparticles Carrying Bombesin as a Target-Specific Vector: Synthesis, Characterization and Biological Evaluation*, Francisco Silva, A. Zambre, Lurdes Gano, António Paulo, R. Kannan, Isabel Santos, *1st Symposium on Medical Chemistry of University of Minho*, Braga - Campus de Gualtar, 17th May 2013.
- *Anticancer Copper(II) and Platinum(II) Complexes with Anthracene-Containing Terpyridine Ligands*, S. Gama, I. Rodrigues, F. Mendes, I. Santos, M. Ravera, A. Paulo, *1st International Symposium on Functional Metal Complexes that Bind to Biomolecules*, Second Whole Action Meeting of the COST Action CM1105, Barcelona, Spain – September, 2013.
- *MC1R-Targeted Metallopeptides: NMR Structural Analysis*, M. Morais, P. D. Raposinho, M. C. Oliveira, M. A. Jiménez, D. Pantoja-Uceda, J.D. G. Correia, I. Santos, *Chemistry of Metals in Biological Systems Summer School, COST Action CM 1105, Lisbon, Portugal, May 12-19 (2013)*.
- *Novel ^{99m}Tc(I)-labeled bone-seeking molecules for bone imaging*, C. Fernandes, S. Monteiro, P. Mendes, L. Gano, F. Marques, S. Casimiro, L. Costa, I. Santos, *13th International Conference on Cancer-Induced Bone Disease (CIBD)*, Miami, Florida, USA, November 6th-9th, (2013).
- *Indium-111 radiolabelled peptide for theranostics of ER positive tumours*, F. Vultos, C. Fernandes, I. Santos, J. Correia, L. Gano, *NanoFar, Autumn School*, Santiago de Compostela, Spain, October 21-25th, (2013), poster.
- *Bisphosphonate-Containing M(CO)₃-Complexes (M = ^{99m}Tc/Re) as Bone-Seeking Agents*, C. Fernandes, S. Monteiro, P. Mendes, L. Gano, F. Marques, J. D. G. Correia, L. Costa, S. Casimiro, I. Santos, *The 3rd Global Cancer Genomics Consortium Symposium (GCGCS)*, Lisbon, Portugal, IMM, September 18-20th (2013).
- *¹¹¹In-DOTAGA-Estradiol based complexes with binding affinity for the estrogen receptor*, S. Cunha, C. Fernandes, C. Bernhard, F. Marques, F. Denat, I. Santos, L. Gano, *1st Symposium on Medicinal Chemistry of University of Minho*, Braga, Portugal, 17th of May, (2013), poster.
- *Targeted-Receptor Bimodal Probe for Sentinel Lymph Node Detection*, J. D. G. Correia, M. Morais, M. P. C. Campello, C. Xavier, S. Hernot, T. Lahoutte, V. Caveliers, I. Santos, *Cost Action 1004 Annual Meeting, Annual Meeting of Cost Action 1004*, Athens, Greece, September (2013).
- *Bimodal Nanoprobe for Sentinel Lymph Node Detection by Positron Emission Tomography and Optical Imaging*, M. P. C. Campello, M. Morais, C. Xavier, S. Hernot, T. Lahoutte, J. D. G. Correia, V. Caveliers, I. Santos, *The 3rd Global Cancer Genomics Consortium Symposium (GCGCS)*, IMM, Lisbon, Portugal, September (2013), poster.

- *Detection of Sentinel Lymph Node by Single Photon Emission Computed Tomography and Optical Imaging with a Bimodal Probe*, M. Morais, C. Xavier, S. Hernot, T. Lahoutte, V. Caveliers, J.D. G. Correia, I. Santos, *The 3rd Global Cancer Genomics Consortium Symposium (GCGCS), IMM*, Lisbon, Portugal, September (2013) poster.
- *Albumin Binding domains Zag and ProtH improve pharmacokinetics and affects biodistribution of an anti-TNF VHH*, C. Cantante, S. Lourenço, J. Leandro, M. Morais, S. Oliveira, L. Gano, C. Fontes, P. Leandro, J. D. G. Correia, F. Silva, J. Gonçalves, *PEGS Summit Europe 2013*, Lisbon, Portugal, November (2013).
- *Bisphosphonate-Containing M(CO)₃-Complexes (M = ^{99m}Tc/Re) as Bone-Seeking Agents*, C. Fernandes, S. Monteiro, P. Mendes, L. Gano, F. Marques, J. D. G. Correia, L. Costa, S. Casimiro, I. Santos, *The 3rd Global Cancer Genomics Consortium Symposium (GCGCS), IMM*, Lisbon, Portugal, September (2013).

EDUCATION

- Invited Professor at Faculdade de Ciências da Universidade de Lisboa: Chemical Systems and Reactivity.
- Coordinator of the discipline Radiopharmaceutical Chemistry, Faculdade de Farmácia da Universidade de Lisboa, Master Course on Pharmaceutical and Therapeutic Chemistry.
- Supervision of Eser Ucar, ERASMUS student under supervision (3 months).
- Co-Supervision of the BI: Sofia Monteiro (PTDC/QUI-QUI/115712/2009).
- Co-Supervision of the BI: Elisabete Ribeiro, Letícia Quental e Maria Belo no âmbito do projeto em curso EXCL/QEQ-MED/0233/2012.
- Co-supervision of Doctor Raid Mansour, fellowship supported by IAEA (3 months).
- Co-supervision of Ph. D. Thesis by Maurício da Silva Morais, Faculdade de Ciências, Universidade de Lisboa.

PROJECTS

- Molecular and Nano Tools for Cancer Theranostics, EXCL/QEQ-MED/0233/2012, Leading Institution: IST/ITN. PI.
- Target-specific and Heterobimetallic Platinum Complexes: Synthesis, Characterization and Mechanistic Studies” (Acções Integradas Luso-Espanholas/2012), PI.
- Synthesis, Characterization and Biological Assessment of Multi-Functional Bone-Seeking Agents, PTDC/QUI-QUI/115712/2009. P.I.
- COST Action CM1105: Functional metal complexes that bind to biomolecule. MC/Participant.
- COST Action TD1004 Theragnostics Imaging and Therapy: An Action to Develop Novel Nanosized Systems for Imaging-Guided Drug Delivery. MC/Participant.

COLLABORATIONS

- Maria Helena Garcia, Faculdade Ciências, Universidade de Lisboa
 - João Costa Pessoa, CQE, IST
 - Luis Costa, IMM, FMLisboa
 - Durval Costa, Fundação Champalimaud
 - Antero Abrunhosa, ICNAS, Universidade Coimbra
 - Filomena Botelho, IBILI, Universidade Coimbra
 - Roger Alberto, Zurich University
 - Adoracion Quiroga, Universidad Autónoma de Madrid, Espanha
 - Olga Iranzo, CNRS, Marseille, França.
 - Tony Lahoutte, Free University of Brussels, Belgium
 - Vicky Cavaliers, Free University of Brussels, Belgium
-

NOME: António Manuel Rocha Paulo

CATEGORY: Principal Researcher

IST-ID: 5355

ACTIVITIES

Nº	Activities Description	R&D (%)
1	Scientific coordination of the project "Radiolabeled Benzazole Derivatives for In Vivo Imaging of Amyloid Aggregation" (PTDC/QUI/QUI/102049/2008)	15
2	Collaboration in the scientific coordination of the project "Targeting telomerase inhibition with new anti-tumoral Cu(II) complexes" (PTDC/QUI-QUI/114139/2009)	10
3	Collaboration in the coordination of the task "Telomerase Inhibitors Based on Cold or Radioactive Cu(II) Complexes (⁶⁴ Cu) Bearing Bioactive Peptides" within the project "Molecular and Nano Tools for Cancer Theranostics" (EXCL/QEQ-MED/0233/2012)	15
4	Coordination of the task "Multifunctional Tc-based Tools for Cell-Specific Targeting" within the project "Molecular and Nano Tools for Cancer Theranostics" (EXCL/QEQ-MED/0233/2012)	15
5	Collaboration in the scientific coordination of the bilateral project "Target-specific and Heterobimetallic Platinum Complexes: Synthesis, Characterization and Mechanistic Studies" (Acções Integradas Luso-Espanholas/2012)	10
6	Supervision of the PhD work of Francisco Silva (SFRH/BD/47308/2008) entitled "Targeted Nanoradiopharmaceuticals for Cancer Diagnosis and/or Therapy: Synthesis, Characterization and Biological Evaluation"	15
7	Supervision of the MSc Thesis of Vanessa Santos entitled "Fractionation of HMDP kits for labelling with ^{99m} Tc: Influence of Temperature and Age of Fractions"	5
8	Supervision of the MSc Thesis of Nuno Lemos entitled "Phytochemical agents as sensitizers in Radio-Therapy in Nuclear Medicine"	3
9	Collaboration in the supervision of the research work of Raid Mansour (AIEA fellowship)	5
10	Collaboration in the elaboration of a "Doctoral Program" and on the "Strategic Project" of C ² TN	5
11	Responsible by the NMR spectrometer of the UCQR	2
Total		100

WORK ACTIVITIES

Nº	Work Activities and Main Achievements
1	<p>During the last months of this project, a new family of ^{99m}Tc(I) and Re(I) tricarbonyl complexes with (S,N,O)-tridentate ligands of the cysteamine type containing a benzothiazole pharmacophore for amyloid binding were synthesized, characterized and biologically evaluated. This work has been performed by Patrique Nunes (Research Assistant (BI)) under my supervision. All tested ^{99m}Tc complexes were unable to cross the BBB in mice and did not emerge as promising probes for in vivo imaging of the deposition of amyloid aggregates in the brain.</p> <p>Benzothiazole derivatives are an interesting class of compounds to design anticancer drugs. Hence, we have evaluated the potential relevance of the synthesized ^{99m}Tc(I) and Re(I) tricarbonyl complexes for cancer theranostics, by combining the potential cytotoxicity of the cold (non-radioactive) Re complexes with the imaging capabilities of the ^{99m}Tc counterparts. The evaluation of the cytotoxicity of the Re(I) complexes in PC-3 and MCF-7 human tumor cells has proved that the complexes have a moderate cytotoxicity against these cell lines. Cell uptake studies with the ^{99m}Tc congeners confirmed that the compounds display a</p>

	<p>significant accumulation in the same cell lines. Biodistribution studies in adequate tumor bearing mice models are foreseen for the more promising compounds.</p>
2	<p>This project is focused on the synthesis and <i>in vitro</i> evaluation of Cu(II) complexes that are expected to interact with telomeric DNA and, therefore, useful in the design of telomerase inhibitors. The PI of this FCT-funded project is Sofia Gama, a Post-doctoral Researcher who is working under my supervision. During 2013, I've collaborated in the scientific coordination of the project and in the supervision of Inês Rodrigues (Research Assistant (BI)).</p> <p>New mixed bipyridine-terpyridine Cu(II) complexes were synthesized and evaluated <i>in vitro</i>. FRET assays have shown that the complexes do not bind to G-quadruplex DNA. However, depending on the cyclic amine substituents of the bipy ligands, some complexes showed an impressive plasmid DNA cleaving ability in the absence of any exogenous oxidizing or reducing agents. Therefore, these compounds emerged as a new class of very efficient artificial DNA nucleases.</p> <p>Complexes of the type [(Ant-tpy)CuCl₂], containing a terpyridine ligand functionalized with an anthracenyl fragment, have been also synthesized. For purposes of comparison, the related [(Ant-tpy)PtCl] and [(Ant-py)RuCl₃] complexes were also prepared and evaluated. Preliminary results have shown that one of the Pt(II) complexes can stabilize G-quadruplex DNA, which indicates that this family of complexes might have potential for the design of telomerase inhibitors.</p>
3	<p>The work performed in this activity is related with the one described above in Activity 2 but involves the synthesis of Cu(II) complexes with N₂S₂-tetradentate ligands of the bis(thiosemicarbazone) type, comprising or not an extended aromatic system and terminal cyclic amines to improve the selectivity for G-quadruplex. By exploring this type of ligands, we expected to have a better ability of synthesizing the corresponding complexes with radioactive copper (⁶⁴Cu). In this way, we anticipate synergistic effects between the cold and radioactive Cu(II) complexes. So far, we succeeded in the synthesis of several bis(thiosemicarbazone) ligands containing terminal piperidine and morpholine groups, as well as in the synthesis of the corresponding Cu(II) complexes. The biological evaluation of the ligands and respective Cu(II) complexes will start soon and will comprise cytotoxicity assays, inhibition of telomerase activity and interaction with ds-DNA and G-quadruplex DNA.</p> <p>The work performed in this activity involved the effort of the researchers Paula Campello and Goretí Morais, as well as of Inês Rodrigues (Research Assistant (BI)).</p>
4	<p>The main objective of this project is to introduce molecular and nanosized ^{99m}Tc based compounds relevant to design Auger-emitting radiopharmaceuticals. Following the previous work of our group on pyrazolyl-diamine M(I) (M = Re, ^{99m}Tc) tricarbonyl complexes as multifunctional structures for the design of Auger-emitting radiopharmaceuticals, we have embarked in the evaluation of the effect of using different spacers to link DNA intercalators (anthracenyl or acridine orange fragments (AO) to the framework of pyrazolyl-diamine ^{99m}Tc(I) tricarbonyl complexes. We have hypothesized that the use of linkers of different length might affect the cell killing ability of the complexes, as it would influence the distance of ^{99m}Tc to the target DNA. So far, we have synthesized the anthracenyl-containing compounds and we will start with their <i>in vitro</i> biological evaluation (cleavage of plasmid DNA, cell uptake and radiotoxicity assays). The synthesis of the AO-containing congeners is under way. The ^{99m}Tc compounds with a better ability to induce cell death will be then used for further functionalization with NLS and/or bioactive peptides and to be grafted to suitable AuNPs.</p> <p>This research work has been performed by Annica Rosa (MSc student, FCT-UNL) and Letícia do Quental ((Research Assistant (BI)), under my supervision.</p>
5	<p>My contribution to this bilateral project has been mainly the scientific coordination of the work related with heterobimetallic complexes combining Re(I) or ^{99m}Tc(I) cores with <i>trans</i>-Pt(II) cores. Within this topic, we were able to synthesize a heterobimetallic Pt/Re complex by conjugating a Pt(II) complex containing a picolinic acid ligand with a pyrazolyl-diamine Re(I) tricarbonyl complex through formation of an amide bond between the two complexes. We have also investigating the functionalization of the same Pt(II) complex with the corresponding pyrazolyl-diamine chelator for further coordination of the <i>fac</i>-[^{99m}Tc(CO)₃]⁺</p>

	<p>core. The functionalization of the Pt(II) complex with a pyrazolyl-diamine framework having a BOC-protected terminal amine group was successfully performed. However, the removal of the BOC-protecting group was accompanied by formation of Pt(II) complexes of the Magnus' green salt type. To overcome these difficulties, related N-donor ligands of the bis(pyridyl)amine type ($\text{H}_2\text{N}(\text{CH}_2)_2\text{N}(\text{py}^*)_2$) were also evaluated. The Re(I) tricarbonyl complex $[\{\text{H}_2\text{N}(\text{CH}_2)_2\text{N}(\text{py}^*)_2\}\text{Re}(\text{CO})_3]^+$ was synthesized and its coupling to Pt(II) and Pt(IV) complexes was studied. The purification and characterization of the resulting heterobimetallic complexes is underway, as well as the coupling of $(\text{H}_2\text{N}(\text{CH}_2)_2\text{N}(\text{py}^*)_2$ to the same starting Pt(II) and Pt(IV) complexes.</p> <p>This work has been done by Leticia do Quental ((Research Assistant (BI)), under my supervision.</p>
6	<p>The main goal of the PhD work of Francisco Silva was the synthesis, characterization and biological evaluation of ^{67}Ga-labelled AuNPs compounds aiming at the design of target specific (nano)radiopharmaceuticals. During 2013, the most important achievements were the following:</p> <p>i) AuNPs (BBN-AuNP-DOTA) containing a coordinating DOTA derivative and bombesin (BBN) analogues were prepared, characterized and labelled with ^{67}Ga. The resulting ^{67}Ga-BBN-AuNP-DOTA showed a high <i>in vitro</i> stability, e.g. in the presence of human serum and against transchelation with transferrin. ^{67}Ga-BBN-AuNP-DOTA showed a significant uptake in human prostate PC-3 cells, contrarily to ^{67}Ga-AuNP-DOTA without the targeting BBN analogue. Intraperitoneal injection of ^{67}Ga-BBN-AuNP-DOTA in PC-3 tumor xenografts led to a moderate tumor uptake and a high pancreatic uptake, pointing out for a receptor-mediated uptake in the target tissues.</p> <p>ii) AuNPs (AuNP-DTPA) nanoparticles stabilized with a DTPA derivative were functionalized with a trifunctional DOTA chelator containing a GE-11 peptide and a thioctic acid derivative. These AuNPs (AuNP-DTPA-DOTA-GE-11) were labeled with ^{67}Ga using direct and pre-labelling approaches, and their <i>in vitro</i> and <i>in vivo</i> behavior was evaluated. Cell uptake studies in a EGFR-positive cancer cell line have shown a remarkable uptake of the radiolabeled AuNP-DTPA-DOTA-GE-11 if compared with the ^{67}Ga-labeled trifunctional DOTA chelator.</p>
7	<p>The MSc thesis of Vanessa Santos (Master Course "Mestrado em Medicina Nuclear", ESTeSL/IPL) dealt with the fractionation of HMDP (hydroxymethylene diphosphonate - <i>Osteocis</i>[®]) in order to maximize the use of $^{99\text{m}}\text{Tc}$-labeled diphosphonates. Being a practice poorly supported by literature, the aim of the work was to assess its influence on the quality of the final product, because it may be advantageous in the management of resources by the services. This comprised the study of the influence of storage temperature and age of the fractions in the radiochemical purity and biodistribution profile of the radiopharmaceutical obtained by labelling of the fractionated kits.</p> <p>Vanessa Moura successfully defended her MSc thesis in March 2013.</p>
8	<p>The MSc thesis of Nuno Lemos (Master Course "Mestrado em Medicina Nuclear", ESTeSL/IPL) consisted on the elaboration of a project aiming at the design of polymeric nanoparticles containing a therapeutic radionuclide, curcumin as a radiosensitizer and antibodies with affinity for antigens present on cells of non-Hodgkin lymphoma. The major goal of the project was to assess the influence of curcumin on the radiotoxic effects of radiolabelled nanoparticles.</p> <p>Nuno Lemos successfully defended her MSc thesis in March 2013.</p>
9	<p>Together with João Galamba Correia, I have been in charge of the supervision of the research work of Raid Mansour, who has been involved in a 2 month training in the RS group that has been sponsored by the AIEA. During this period, Raid Mansour has synthesized a series of melanocortin analogues using SPPS methodologies, and has performed its labeling with $^{99\text{m}}\text{Tc}$ based on $^{99\text{m}}\text{Tc}(\text{I})$ tricarbonyl and $^{99\text{m}}\text{Tc}(\text{V})$ oxocomplexes. All the obtained radiometallated peptides were evaluated <i>in vitro</i> using murine melanoma cells.</p>
10	<p>Active participation in the elaboration of the Doctoral Programme "Radiopharmaceutical Sciences in Molecular Imaging and Cancer Therapy" (RadITER) that has been submitted by the RS group of C²TN to the call launched by the FCT in 2013. Collaboration in the preparation of the Strategic Project of C²TN, particularly in the part relative to the</p>

	Radiopharmaceutical Sciences and Health Physics Thematic Strand.
11	C ² TN is equipped with a Unity Inova Varian 300 MHz multinuclear spectrometer with pulsed field gradient (PFG) probes. This NMR machine is used by researchers of the Groups of Radiopharmaceuticals Sciences, Solid State and f-Element Chemistry to study the molecular structures in solution of organic, inorganic and organometallic compounds, based on a variety of multinuclear and/or 2D techniques. Whenever required, I've provided technical and scientific support to the users of the spectrometer, particularly in the case of young students and researchers.

PUBLICATIONS

- C. Moura, F. Mendes, L. Gano, I. Santos, A. Paulo, Mono- and dicationic Re(I)/Tc-99m(I) tricarbonyl complexes for the targeting of energized mitochondria, *J. Bioinorg. Chem.*, 123, 34-45 (2013), doi: 10.1016/j.jinorgbio.2013.02.008.
- G. Ribeiro Morais, L. Gano, T. Kniess, R. Bergmann, A. Abrunhosa, I. Santos, A. Paulo, "Radiosynthesis and in vivo Evaluation of a 18F-Labelled Styryl-Benzoxazole Derivative for beta-Amyloid Targeting", *Appl. Rad. Isot.*, 82, 100–104 (2013), doi: 10.1016/j.apradiso.2013.07.003.
- M. Morais, A. Paulo, L. Gano, I. Santos, J. D. G. Correia, Target-specific Tc(CO)₃-complexes for in vivo imaging, *J. Organomet. Chem.*, 744, 125-139 (2013), doi: 10.1016/j.jorganchem.2013.05.050.
- Letícia Quental, Goreti Ribeiro Morais, Isabel Santos, António Paulo, Molecular imaging agents for detection of β-amyloid plaques in Alzheimer's disease, *Revista Saúde & Tecnologia*, Novembro (10), 5-9 (2013), ISSN: 1646-9704.
- F. Mendes, L. Gano, C. Fernandes, A. Paulo, I. Santos, Perfusion and specific radioprobes for cardiac imaging, *CARDIOTECHNIX 2013 - Proceedings of the International Congress on Cardiovascular Technologies*, 79-83 (2013).
- A. Paulo, Design of Radioprobes for PET and SPECT imaging, *CARDIOTECHNIX 2013 - Proceedings of the International Congress on Cardiovascular Technologies*, 5-78 (2013).

COMMUNICATIONS

- *Organometallic Tc-99m/Re Complexes for In Vivo Targeting of Beta-Amyloid Plaques*, P. Nunes, G. Morais, E. Palma, L. Gano, I. Santos, A. Paulo, *Drug Discovery & Therapy World Congress 2013, Boston, USA, June 3-6 (2013)*, Poster.
- *Perfusion and specific radioprobes for cardiac imaging*, F. Mendes, L. Gano, C. Fernandes, A. Paulo, I. Santos, *Cardiotech2013 International Congress on Cardiovascular Technologies – Symposium VisualCardio, Vilamoura, 21th September (2013)*, Invited Talk.
- Design of Radioprobes for PET and SPECT imaging, A. Paulo, *Cardiotech2013 International Congress on Cardiovascular Technologies – Symposium VisualCardio, Vilamoura, 21th September (2013)*, Invited Talk.
- *Evaluation of ^{99m}Tc-TMEOP as Probe for Functional Monitoring of Multidrug Resistance*, F. Mendes, L. Gano, C. Fernandes, A. Paulo, I. Santos, Molecular Biology in Portugal and EMBL (and EMBL Alumni), 18 July (2013), FCUL, Lisboa, Poster.
- *⁶⁷Ga-Labelled Gold Nanoparticles Carrying Bombesin as a Target-Specific Vector: Synthesis, Characterization and Biological Evaluation*, Francisco Silva, A. Zambre, Lurdes Gano, António Paulo, R. Kannan, Isabel Santos, *1st Symposium on Medical Chemistry of University of Minho, Braga - Campus de Gualtar, 17 de Maio de 2013*, Poster.
- *Mitochondria-targeted Re(I)/^{99m}Tc(I) Tricarbonyl Complexes*, A. Paulo, C. Moura, F. Mendes, L. Gano, I. Santos, *WG3 meeting – COST CM1105, Barcelona, 10th September 2013*, Invited Talk.
- *Anticancer Copper(II) and Platinum(II) Complexes with Anthracene-Containing Terpyridine Ligands*, S. Gama, I. Rodrigues, F. Mendes, I. Santos, M. Ravera, A. Paulo, 1st International Symposium on Functional Metal Complexes that Bind to Biomolecules, Second Whole Action Meeting of the COST Action CM1105, Barcelona, Spain – September, 2013, **Invited Talk**.
- *Small Core Gold Nanoparticles Stabilized by Thiolated DOTA Derivatives*, Francisco Silva, Maria P. C. Campello, L. Gano, Isabel Santos, A. Paulo, *Nanoparticles for early Diagnostics of inflammatory diseases, November 20/21 2013, Lisbon*, Invited Talk.

EDUCATION

- Invited Coordinator Professor at ESTeSL, Disciplines: Radiopharmacy I (2nd year, 2nd Semester), Radiopharmacy II (3rd year, 1st Semester).
- A. Paulo, Invited Professor, Faculdade de Farmácia da Universidade de Lisboa, Master Course on Pharmaceutical and Therapeutic Chemistry: lectures on Fundamentals of Radioactivity, Production of Radionuclides and Basics Aspects of Coordination Chemistry, discipline Radiopharmaceutical Chemistry.
- Supervision of the Master thesis "Phytochemical agents as sensitizers in Radiotherapy in Nuclear Medicine" within the Master Course "Mestrado em Medicina Nuclear", Nuno Lemos, ESTeSL/IPL, Discussed and approved in March 2013.
- Supervision of the Master thesis "Fracionamento de kits de HMDP para marcação com ^{99m}Tc – Influência da temperatura e da idade das fracções", Vanessa Santos, ESTeSL/IPL, Discussed and approved in March 2013.
- Arguing Member of the jury of the Msc of F. Toscano, Biological Evaluation of cationic ^{99m}Tc(I) complexes as probes for tumoral detection and functional monitoring of multidrug resistance", Tese de Master Course in Biomedical Inorganic Chemistry, FCUL, 13th December 2013.
- Member of the jury for the recruitment of an auxiliary professor in the area of Nuclear Medicine at ESTeSL.

PROJECTS

- Radiolabeled Benzazole Derivatives for In Vivo Imaging of Amyloid Aggregation, PTDC/QUI/QUI/102049/2008. Leading Institution: IST/ITN. IST/ITN Coordinator: A. Paulo (25%).
- Targeting telomerase inhibition with new anti-tumoral Cu(II) complexes, PTDC/QUI-QUI/114139/2009. Leading Institution: IST/ITN. Member of the research team (15 %).
- Synthesis and Pre-clinical Evaluation of Novel Estradiol-Based Indium Complexes for Targeted Radiotherapy of Tumors, PTDC/QUI-QUI/111891/2009. Leading Institution: IST/ITN. Member of the research team (5%).
- Molecular and Nano Tools for Cancer Theranostics, EXCL/QEQ-MED/0233/2012. Leading Institution: IST/ITN. Member of the research team (25%)
- Target-specific and Heterobimetallic Platinum Complexes: Synthesis, Characterization and Mechanistic Studies" (Acções Integradas Luso-Espanholas/2012). Member of the IST/ITN team.
- COST Action TD1007 "Bimodal PET-MRI molecular imaging technologies and applications for in vivo monitoring of disease and biological processes". National MC substitute member.
- COST Action CM1105: Functional metal complexes that bind to biomolecule, participant.
- COST Action TD1004 Theragnostics Imaging and Therapy: An Action to Develop Novel Nanosized Systems for Imaging-Guided Drug Delivery. Participant.

COLLABORATIONS

- Mauro Ravera (Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale "Amedeo Avogadro", Alessandria, Italy): Synthesis, characterization and biological evaluation of Pt compounds.
- Tiago Outeiro (Cell and Molecular Neuroscience Unit, Instituto de Medicina Molecular): In vitro evaluation of the interaction of organic and organometallic compounds with amyloid aggregates.
- Raghuraman Kannan (Department of Radiology from the University of Missouri): collaboration in the supervision of the PhD work of Francisco Silva.
- João Costa Pessoa (IST/CQE): Collaboration in the supervision of the Post-doctoral researcher Elisa Palma.
- Adoración Quiroga (Departamento de Química Inorgánica, Univ. Autónoma de Madrid): Synthesis, characterization of heterobimetallic complexes of Pt/Re and Pt/^{99m}Tc.
- Ramon Vilar (Imperial College, UK): Evaluation of the interaction of metallic complexes with G-quadruplex DNA.
- Fernanda Carvalho (IST/CQE): Electrochemical studies of metallic complexes.

- José Ascenso (IST/CQE): NMR studies of metallic complexes and AuNPs.

NAME: João Domingos Galamba Correia

CATEGORY: Principal Researcher

IST-ID: IST25450

ACTIVITIES

Nº	Activity Description	R&D (%)
1	<i>Nitric oxide synthase targeting with Re(I)/^{99m}Tc(I)-complexes containing L-Arg derivatives: a structure-activity study – PTDC/QUI-QUI/121752/2010. <u>Principal Investigator</u></i> In cooperation with Prof. M. J. Romão and Prof. M. L. Macedo (REQUIMTE/FCT - Universidade Nova de Lisboa), and Prof. M. J. Ramos (REQUIMTE/Depart. Chem. Biochem. FC-UP, Universidade do Porto).	35
2	<i>Albumin binding-domain fusion to improve protein pharmacokinetics – PTDC/SAU-FAR/115846/2009. <u>IST coordinator</u></i> In collaboration with Prof. J. Gonçalves (PI) from the Unit of Retrovirus and Associated Infections, Faculty of Pharmacy, University of Lisbon.	15
3	<i>Novel radiolabeled peptide conjugates for transporting antibody fragments through the Blood Brain Barrier (BBB). <u>IST coordinator</u>.</i> In collaboration with Prof. Miguel Castanho from the physical biochemistry unit of IMM, Faculty of Medicine, University of Lisbon and Technophage S.A.	5
4	<i>ICTP and NTx peptides as markers of bone resorption. <u>IST coordinator</u>.</i> In collaboration with Prof. L. Costa and Dr ^a S. Casimiro from the Unit of Clinical and Translational Oncology Research Unit of IMM, Faculty of Medicine, University of Lisbon.	5
5	<i>Synthesis, characterization and biological assessment of multi-functional bone-seeking agents – PTDC/QUI-QUI/115712/2009. <u>Team member</u></i> In collaboration with Prof. L. Costa and Dr ^a S. Casimiro from the Unit of Clinical and Translational Oncology Research Unit of IMM, Faculty of Medicine, University of Lisbon.	15
6	<i>Molecular Imaging Approach to Cystic Fibrosis, FCT – EXPL/BIM-MEC/0115/2012, 2013-14. <u>Team member</u></i>	5
7	<i>Molecular and Nano Tools for Cancer Theranostics, FCT – EXCL/QEQ-MED/0233/2012. <u>Team member</u></i>	5
8	<i>Bimodal dextran-based probes for Sentinel Lymph Node Detection by SPECT and Optical Imaging. <u>Team member</u>.</i> In collaboration with Prof. T. Lahoutte and Prof. V. Caveliers from the In Vivo Cellular and Molecular Imaging Laboratory, Vrije Universiteit Brussel and the Nuclear Medicine Department, UZ Brussel, Brussels, Belgium. The project was partially supported by the IAEA.	5
9	Teaching activities	2.5
10	Participation in the elaboration of the: - strategic plan of the thematic strand <i>Radiopharmaceutical Sciences and Health Physics / Diagnostic, Therapies and Public Health</i> , C ² TN - Doctoral Program entitled <i>Radiopharmaceutical Sciences in Molecular Imaging and Cancer Therapy</i> (RadITer) submitted to FCT.	2.5

11	Management of Laboratory infrastructure – Solid Phase Peptide Synthesis Laboratory.	5
Total		100

WORK SUMMARY

N°	Work Summary and Main Achievemnts
1	Aiming to probe the Nitric Oxide Synthase (NOS) enzymes, whose expression is related to diseases such as cancer and neurodegenerative disorders, we are exploring $^{99m}\text{Tc}(\text{CO})_3$ -complexes bearing NOS-recognizing units for SPECT imaging. Enzymatic activity studies demonstrated that some $\text{Re}(\text{CO})_3$ -complexes inhibit purified inducible NOS as well as the cytosolic enzyme. Aiming to shed light on the specific iNOS/Re-complexes interactions and to establish a structure-activity relationship we are combining computational studies with NMR spectroscopy and X-ray crystallography. Regarding computational chemistry, we have studied the general structural determinants for selective inhibition of nitric oxide synthase isoforms as well as the binding of $\text{Re}(\text{CO})_3$ -complexes to inducible nitric oxide synthase. The results obtained have been published in peer-reviewed journals (B. L. Oliveira et al, <i>J. Mol. Model.</i> 2013 , <i>19</i> , 1537; B. L. Oliveira et al <i>J. Mol. Graph. Model.</i> 2013 , <i>45</i> , 13). Moreover, the oxigenase domains of iNOS, eNOS and nNOS have been cloned, expressed and purified. The three isoforms have shown to be soluble and active, and protein production can now be established for functional and structural studies. The experimental work has been partially performed by B. L. Oliveira and M. Morais under my supervision as well as by Dr. Filipa Mendes (IST) and the team of Prof. M. J. Romão, FCT-UNL.
2	Small domain antibodies (sdAbs) present high potential for both molecular in vivo imaging and therapy. They are rapidly cleared from blood circulation, and new strategies to extend their half-lives are needed for therapeutic applications. We have selected a bacterial albumin-binding domain (ABD) from protein Zag to be fused to an anti-tumor necrosis factor (TNF) single variable-domain heavy-chain region antibody (VHH) to delay blood clearance. The anti-TNF VHH and the fusion protein VHH-Zag were conjugated to p-SCN-Bn-NOTA. The immunoreactivity of the VHH-based proteins was preserved upon conjugation to the NOTA chelator. The radioconjugates ^{67}Ga -NOTA-VHH and ^{67}Ga -NOTA-VHH-Zag (> 95% radiochemical purity after gel filtration). The biodistribution studies in healthy female CD-1 mice showed that the Zag domain affected the pharmacokinetic properties of VHH, with impressive differences in blood clearance (0.028 ± 0.004 vs 1.7 ± 0.8 % I.A./g) and total excretion (97.8 ± 0.6 vs 25.5 ± 2.1 % I.A.) for ^{67}Ga -NOTA-VHH and ^{67}Ga -NOTA-VHH-Zag, respectively, at 24 h p.i. This work, performed under my coordination, has been done by M. Morais as well as by Dr. L. Gano (IST), and by the Team of Prof. J. Gonçalves, Faculty of Pharmacy, University of Lisbon.
3	We have synthesized and characterized a set of peptide conjugates that contain chelators for radiometallation. Preliminary biological results with the resulting radiopetides, both in cell and animal models, have shown that some of the peptides present high potential for transporting antibody fragments through the Blood Brain Barrier (BBB) This collaborative work between the radiopharmaceutical sciences group and the IMM team, performed under my coordination, has been done by M. Morais as well as by Dr. L. Gano (IST).
4	One of the goals of this research project is the study the potential effects of ICTP and CTX peptides on breast cancer cells invasive phenotype, namely in the regulation of proliferation, apoptosis, migration and adhesion. To achieve this goal we have prepared and characterized the peptides SP4 (ICTP) and CTX, which have been evaluated in the breast cancer cell line MDA-MB-231. The data obtained revealed a discrete increase in cell proliferation, a stimulation of cell migration as well a significant effect on cell adhesion in the presence of CTX ($p < 0,05$ – $p < 0,01$). These preliminary results suggest a possible effect of these peptides on breast cancer cells, contributing for an invasive phenotype. Synthesis of novel peptide derivatives and the study of the underlying mechanism of action are currently underway. This work has been performed by the BSc student P. M. Dias from FCT, UNL.
5	Radiolabeled bisphosphonates (BPs) bind to bone matrix in areas of increased bone turnover, characteristic of metastization sites. Nitrogen-containing BPs may have also a

	<p>direct anti-tumour activity. We synthesized complexes of the type $fac-[M(CO)_3(k^3-L)]^+$ ($M = {}^{99m}Tc, Re$) stabilized by bifunctional chelators with different molecular weight, overall charge, (lipo)hydrophilic nature and different positions of BP attachment. Biodistribution studies in mice have shown that the complex bearing the BP unit at position 4 of the azolyl ring (Tc1) presents the most favourable pharmacokinetics, which is better than the gold standard ${}^{99m}Tc$-MDP. Cell fragmentation studies have shown that this complex presents also the highest accumulation in the cytosol. The biological properties of Tc1 prompted us to prepare the analogue complex with ${}^{188}Re$, which is a β^- emitter. Firstly, we optimized the radiosynthesis, purification and analytical control of the precursor $fac-[{}^{188}Re(CO)_3(H_2O)_3]^+$. Reaction of the latter with the appropriate chelator under optimized conditions gave complex ${}^{188}Re1$ with high radiochemical purity (> 95%) after purification by solid phase extraction. The complex was evaluated in specific cell lines and its bone-seeking ability assessed in healthy mice. I have been mainly involved in the supervision of the synthetic procedures, namely of the chelators and non-radioactive rhenium complexes, and respective characterization.</p>
6	<p>The main goal of this project is to develop radioactive probes for detecting the expression of normal and rescued CFTR trafficking mutants at the membrane of epithelial pulmonary cells in Cystic Fibrosis.</p> <p>The anti-CFTR antibody ECL1 was purified and radiolabeled directly with ${}^{99m}Tc(I)$. Furthermore, the bioconjugate CFTRinh-172a-Pz, which contains a pyrazolyl-diamine chelator (Pz), reacted with $fac-[M(CO)_3(H_2O)_3]^+$ ($M = {}^{99m}Tc, Re$), yielding complexes of the type $fac-[M(CO)_3(k^3-CFTRinh-172a-Pz)_3]^+$. Cell studies with the rhenium complex are currently underway.</p> <p>The synthetic and characterization work, as well as the radiolabeling studies have been performed by the students V. Ferreira and B. L. Oliveira under my direct supervision. Dr. Filipa Mendes is the PI of this project, being responsible for its overall coordination.</p>
7	<p>Apart from the work already described in the previous items 1 (NOS project) and 3 (multi-functional bone seeking agents project), in which my contribution has been included, I have been also involved in tasks 1 and 6 of the project <i>Molecular and Nano Tools for Cancer Theranostics</i> (EXCL/QEQ-MED/0233/2012), as described below:</p> <p>Task 1 - Multifunctional and Nano Bone Seeking agents – member of the team which coordinates the work related to the synthesis of the polymeric precursors MePEG-b-PCL and NH_2-PEG-b-PCL as well as the conjugate Pz-CONH-PEG-b-PCL. After purification and characterization of all polymers, we have prepared model Block Copolymer Micelles (BCMs) with MePEG-b-PCL and evaluated their physical characteristics, namely hydrodynamic diameter, size distribution and zeta potential.</p> <p>Task 6 - Design, synthesis and characterization of peptides – responsible for the design, synthesis and characterization of the peptides that will be used in other tasks as targeting molecules. The latter include DNA-binding peptides, GE11 and FRHR peptides, amongst others.</p>
8	<p>We have evaluated the biological properties of the receptor-targeted bimodal probes Dex-Man-Tc-IR775 and Dex-Man-${}^{68}Ga$-IR775 for sentinel lymph node (SLN) mapping by nuclear and optical imaging techniques. Firstly, we have prepared the probes with high radiochemical yield and specific activities, and characterized them by chromatographic comparison of the γ-trace of the radioactive probe with the UV-vis trace of the respective “cold surrogate”. Biodistribution studies in Wistar rats have shown that both probes presented significant accumulation in the popliteal node and enhanced extraction (> 90 %) at 1 h p.i. Both probes enabled SLN mapping by nuclear and NIR optical imaging techniques as well as NIR imaged-guided excision.</p>
9	<ul style="list-style-type: none"> - Invited Lecturer at the Faculdade de Medicina da Universidade de Lisboa, <i>Master Course in Oncobiology 2013</i>, Radiopharmaceutical Science and Cancer Therapy - Drug Discovery and Development in Oncology, March 2013 - Invited Lecturer at the Faculdade de Farmácia da Universidade de Lisboa, <i>Master Course in Pharmaceutical and Therapeutic Chemistry 2013/2014</i>, Radiopharmaceutical Chemistry, December 2013
10	<p>Participation in the preparation of the:</p> <ul style="list-style-type: none"> - Strategic plan of the thematic strand <i>Radiopharmaceutical Sciences and Health Physics /</i>

	<p><i>Diagnostic, Therapies and Public Health, C²TN.</i></p> <ul style="list-style-type: none"> - Doctoral Program entitled <i>Radiopharmaceutical Sciences in Molecular Imaging and Cancer Therapy</i> submitted to FCT.
11	<p>Management of Laboratory infrastructure – Solid Phase Peptide Synthesis Laboratory:</p> <ul style="list-style-type: none"> - Responsible for the MW-assisted Solid Phase Peptide Synthesizer – CEM Liberty. - Training of the new users.

PUBLICATIONS

- M. Morais, A. Paulo, L. Gano, I. Santos, J. D. G. Correia, Target-specific Tc(CO)₃-complexes for in vivo imaging, *Journal of Organometallic Chemistry*, 744, 125-139 (2013), doi: 10.1016/j.jorganchem.2013.05.050.
- A. de Almeida, B. L. Oliveira, J. D. G. Correia, G. Soveral, A. Casini, Emerging protein targets for metal-based pharmaceutical agents: An update, *Coordination Chemistry Reviews*, 257, 2689-2704 (2013), doi: 10.1016/j.ccr.2013.01.031.
- B.L. Oliveira, I. S. Moreira, P. A. Fernandes, M. J. Ramos, I. Santos, J. D. G. Correia, Theoretical studies on the binding of rhenium(I) complexes to inducible nitric oxide synthase, *Journal of Molecular Graphics & Modelling*, 45, 13-25 (2013), doi: 10.1016/j.jmngm.2013.07.007.
- B.L. Oliveira, I. S. Moreira, P. A. Fernandes, M. J. Ramos, I. Santos, J. D. G. Correia, Insights into the structural determinants for selective inhibition of nitric oxide synthase isoforms, *Journal of Molecular Modelling*, 19, 1537-1551 (2013), doi: 10.1007/s00894-012-1677-8.
- M. Morais, B. L. Oliveira, J. D. G. Correia, M. C. Oliveira, M. A. Jimenez, I. Santos, P. D. Raposinho, Influence of the bifunctional chelator on the pharmacokinetic properties of ^{99m}Tc(CO)₃-Labeled Cyclic alpha-Melanocyte Stimulating Hormone Analog, *Journal of Medicinal Chemistry*, 56, 1961-1973 (2013), doi: 10.1021/jm301647t.

COMMUNICATIONS

- *Pharmacokinetic properties of a ⁶⁷Ga-labeled anti-TNF VHH single domain antibody containing a bacterial albumin-binding domain (Zag)*, J. D. G. Correia, M. Morais, L. Gano, I. Santos, C. Cantante, S Lourenço, C. Santos, C. Fontes, F. Silva, J. Gonçalves, *2nd International Workshop on Innovative Personalized Radioimmunotherapy, WIPR 2013, Nantes, France, July (2013)*, oral.
- *Targeted-Receptor Bimodal Probe for Sentinel Lymph Node Detection*, J. D. G. Correia, M. Morais, M. P. C. Campello, C. Xavier, S. Hernot, T. Lahoutte, V. Caveliers, I. Santos, *Cost Action 1004 Annual Meeting, Annual Meeting of Cost Action 1004, Athens, Greece, September (2013)*, poster.
- *Bimodal Nanoprobe for Sentinel Lymph Node Detection by Positron Emission Tomography and Optical Imaging*, M. P. C. Campello, M. Morais, C. Xavier, S. Hernot, T. Lahoutte, J. D. G. Correia, V. Caveliers, I. Santos, *The 3rd Global Cancer Genomics Consortium Symposium (GCGCS), IMM, Lisbon, Portugal, September (2013)*, poster.
- *MC1R-Targeted Metallopeptides: NMR Structural Analysis*, M. Morais, P. D. Raposinho, M. C. Oliveira, M. A. Jiménez, D. Pantoja-Uceda, J. D. G. Correia, I. Santos, *Summer School on Chemistry of Metals in Biological Systems, COST Action CM 1105, Louvain-la-Neuve, Belgium, May (2013)*, poster.
- *Bimodal Imaging of Sentinel Lymph Node with ^{99m}Tc(CO)₃-labeled Mannosylated Nanoparticles bearing a NIR-fluorophore*, M. Morais, C. Xavier, S. Hernot, T. Lahoutte, V. Caveliers, J. D. G. Correia, I. Santos, *Annual Meeting of Cost Action 1004, Athens, Greece, September (2013)*, oral.
- *Detection of Sentinel Lymph Node by Single Photon Emission Computed Tomography and Optical Imaging with a Bimodal Probe*, M. Morais, C. Xavier, S. Hernot, T. Lahoutte, V. Caveliers, J. D. G. Correia, I. Santos, *The 3rd Global Cancer Genomics Consortium Symposium (GCGCS), IMM, Lisbon, Portugal, September (2013)* poster.
- *Albumin Binding domains Zag and ProtH improve pharmacokinetics and affects biodistribution of an anti-TNF VHH*, C. Cantante, S. Lourenço, J. Leandro, M. Morais, S. Oliveira, L. Gano, C. Fontes, P. Leandro, J. D. G. Correia, F. Silva, J. Gonçalves, *PEGS Summit Europe 2013, Lisbon, Portugal, November (2013)*, poster.

- *Indium-111 Radiolabelled Peptide Towards the Estrogen Receptor*, F. Vultos, C. Fernandes, I. Santos, J. D. G. Correia, L. Gano, *The Future of radiation oncology: Imaging, Dosimetry, Biology & Therapy - Seventh Berder Workshop, France, September (2013)*, Invited talk.
- *Avaliação biológica de novos complexos de ^{99m}Tc(I) no diagnóstico de metástases ósseas*, C. Fernandes, S. Monteiro, E. Palma, L. Gano, F. Marques, J. D. G. Correia, I. Santos, *XIV Congresso Nacional Medicina Nuclear, Porto, Portugal, December (2013)*, oral.
- *Bisphosphonate-Containing M(CO)₃-Complexes (M = ^{99m}Tc/Re) as Bone-Seeking Agents*, C. Fernandes, S. Monteiro, P. Mendes, L. Gano, F. Marques, J. D. G. Correia, L. Costa, S. Casimiro, I. Santos, *The 3rd Global Cancer Genomics Consortium Symposium (GCGCS), IMM, Lisbon, Portugal, September (2013)*, poster.
- *Indium-111 radiolabelled peptide for theranostics of ER positive tumours*, F. Vultos, C. Fernandes, I. Santos, J. Correia, L. Gano, *NanoFar, Autumn School, Santiago de Compostela, Spain, October (2013)*, poster.

EDUCATION

- Supervisor and jury member, Ph. D. Thesis, *Re and ^{99m}Tc Tricarbonyl probes for target-specific detection of melanoma and sentinel lymph node*, by Maurício da Silva Morais, Faculdade de Ciências, Universidade de Lisboa, 2nd December 2013.
- Co-supervisor, Bsc. Thesis Biochemistry, *Fragmentos de colagénio do tipo I, ICTP e CTX, como mediadores celulares em cancro da mama*, by Pedro Miguel Dias, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 6th June 2013.
- Participation as a jury member, MSc. Thesis, *A molecular imaging approach to cystic fibrosis*, by Vera Filipa Cerqueira Ferreira, Faculdade de Ciências, Universidade de Lisboa, 24th July 2013.
- Attendance of the *Training school on Chemical probes in chemical proteomics and biosynthesis studies*, organized within the framework of COST Action CM 1004 – Synthetic Probes for Chemical proteomics & Elucidation of Biosynthetic Pathways, Universität Duisburg-Essen, Campus Essen, Germany, November 11-15, 2013.
- Short-Term Scientific Mission (STSM) to the Group of Dr. Khuloud T. Al-Jamal, Institute of Pharmaceutical Science, King's College London - *Design of novel target-specific nanocapsules as carriers of drugs and radioactivity for cancer theranostic*. Within the framework of COST Action TD1004 Theragnostics Imaging and Therapy: An Action to Develop Novel Nanosized Systems for Imaging-Guided Drug Delivery. London, December 3-13, 2013.

CONTRACTS

- Proposta de Prestação de Serviços n° JDG1/2013 - *Estudos de biodistribuição de pequenos péptidos radioactivos (Tecnécio-99m e Gálio-68) em modelo animal de ratinhos*, 15th December 2013, Instituto de Medicina Molecular, October – December 2013, 1350 €.

NAME: Célia Maria da Cruz Fernandes

CATEGORY: Auxiliary Researcher

IST-ID: 5452

ACTIVITIES

N°	Activity description	R&D (%)
1	Synthesis, Characterization and Biological Assessment of Multi-Functional Bone-Seeking Agents (PTDC/QUI-QUI/115712/2009). In collaboration with Prof. Luis Costa and Dr ^a Sandra Casimiro from the Unit of Clinical and Translational Oncology Research Unit of IMM, Faculty of Medicine, University of Lisbon.	20
2	Synthesis and Pre-clinical Evaluation of Novel Estradiol-Based Indium Complexes for Targeted Radiotherapy of Tumors – PTDC/QUI-QUI/111891/2009. <i>Team member</i> .	15

	In collaboration with Doutora Lurdes Gano (PI) from Radiopharmaceutical Sciences Group, IST/ITN.	
3	Molecular and Nano Tools for Cancer Theranostics- EXCL/QEQ-MED/0233/2012. <i>Team member</i> . Co-coordinator of task 1. Multifunctional and Nano Bone Seeking...	20
4	Supervision of the PhD work of Filipe Vultos (SFRH/BD/84509/2012) entitled “ ¹¹¹ In complexes bearing specific peptides towards the oestrogen receptor for cancer theranostic: Synthesis and biological evaluation”.	15
5	^{99m} Tc-HSA (“in house” formulation) adequate for clinical application.	5
6	Biological evaluation of radiolabeled glucose derivatives	5
7	Mass Spectrometry Studies	10
8	Management of the HPLC lab/equipments	5
9	Management of a chemical synthesis laboratory (SQII)	5
Total		100

WORK SUMMARY

Nº	Work Summary and Main Achievements
1	<p>Radiolabeled bisphosphonates (BPs) bind to bone matrix in areas of increased bone turnover, characteristic of metastization sites. Nitrogen-containing BPs may have also a direct anti-tumour activity. We synthesized complexes of the type <i>fac</i>-[M(CO)₃(k³-L)]⁺ (M = ^{99m}Tc, Re) stabilized by bifunctional chelators with different molecular weight, overall charge, (lipo)hydrophilic nature and different positions of BP attachment. Biodistribution studies in mice have shown that the complex bearing the BP unit at position 4 of the azolyl ring (Tc1) presents the most favourable pharmacokinetics. Cell fragmentation studies have shown that this complex presents also the highest accumulation in the cytosol. The biological properties of Tc1 prompted us to prepare the analogue complex with ¹⁸⁸Re, which is a β⁻ emitter. Firstly, we optimized the radiosynthesis, purification and analytical control of the precursor <i>fac</i>-[¹⁸⁸Re(CO)₃(H₂O)₃]⁺. Reaction of the latter with the appropriate chelator under optimized conditions gave complex ¹⁸⁸Re1 with high radiochemical purity (> 95%) after purification by solid-phase extraction. The complex was evaluated in specific cell lines and its bone-seeking ability assessed in healthy mice.</p> <p>I have been deeply involved in the synthesis of the functionalized chelators, as well as, their radiolabelling with ¹⁸⁸Re(I) and ^{99m}Tc(I), discussing results and supervising students involved in this task.</p>
2	<p>The oestrogen receptor (ERα) plays an important role in the clinical care of breast cancer patients. Aiming to contribute for the development of radionuclide-targeted therapeutic agents of cancer as safer alternatives to conventional therapies this strategy uses estradiol-based radiometal complexes as specific delivery vectors to insert Auger electron-emitter radionuclides (<i>e.g.</i> ¹¹¹In) within ER expressing-tumor cells.</p> <p>We have successfully synthesized novel 16α-substituted estradiols with different spacer chains and coupled to different bifunctional chelating agents (DTPA, DOTA, DOTAGA). Among them, estradiol-DOTAGA-like chelators presented adequate ERα binding affinity and selectivity. The ¹¹¹In-estradiol based complexes were prepared in high specific activity and radiochemical purity and are stable <i>in vitro</i> in human blood serum and in the presence of apo-transferrin. Animal studies indicate high <i>in vivo</i> stability and rapid clearance from main organs. The moderate cell uptake found is probably due to the low lipophilicity of the complexes. However, the relative ERα binding affinity of the ¹¹¹In-DOTAGA-estradiol complexes and their evaluation in ERα-expressing cell lines suggest that uptake may occur via an ER-mediated process. My contribution in this project has been fundamental in the proposal of new estradiol derivatives synthetic strategies, radiolabelling with ¹¹¹In, together with the co-supervision of the synthetic work performed by Susana Cunha and Filipe Vultos (BI).</p>
3	<p>In addition to the work already described in the previous items 1 (multi-functional bone seeking agents project) and 2 (Estradiol-Based Indium Complexes for Targeted Radiotherapy), in which my contribution has been included, I have been also involved in the tasks 1, 3 and 6 of the project <i>Molecular and Nano Tools for Cancer Theranostics</i></p>

	<p>(EXCL/QEQ-MED/0233/2012), as described below:</p> <p>Task 1 - Multifunctional and Nano Bone Seeking agents – member of the team coordinating the synthesis of the polymeric precursors MePEG-b-PCL and NH₂-PEG-b-PCL as well as the conjugate Pz-CONH-PEG-b-PCL. After purification and characterization we have prepared model Block Copolymer Micelles (BCMs) with MePEG-b-PCL and evaluated their physical characteristics, namely hydrodynamic diameter, size distribution and zeta potential.</p> <p>Task 3 - Multifunctional Indium Complexes for Cell-Specific Nuclear Targeting – member of the team involved in the design of ¹¹¹In-based theranostic agents bearing GE11 analogs or estradiol, to target EGFR and ER, respectively, in different malignant tumours. GE11, a novel and almost unexplored peptide for EGFR, has been synthesized, characterized and successfully coupled to the bifunctional chelator DOTAGA.</p> <p>Task 6 - Design, synthesis and characterization of peptides – participation in the design of the bioactive peptides that will be used in other tasks of the project.</p>
4	<p>The oestrogen receptor (ER) is overexpressed in nearly 60% of breast cancers and is a relevant target for cancer imaging and radionuclide therapy. As peptides are considered good vectors for the development of target specific radiopharmaceuticals, the main goal of the PhD work of Filipe Vultos was the design, synthesis and biological evaluation of ¹¹¹In complexes bearing peptides that target ER expressing tumour cells as delivery systems of Auger electrons to ER(+) tumours.</p> <p>This PhD work started in July and a family of small peptides with known ER targeting ability have been synthesized by solid phase and conjugated to DOTA and DTPA based chelating agents. The conjugates were radiolabelled with ¹¹¹In and the peptidic constructs are currently being studied as potential theranostic agents for ER positive tumors. To increase the potential for Auger therapy, the synthesis of ¹¹¹In coordinating bifunctional chelating agents for peptidic conjugation bearing DNA intercalators is also underway.</p> <p>My contribution has been fundamental in the proposal of the synthetic strategies for the preparation of the DOTA and DTPA based compounds as well as for the <i>in vitro</i> evaluation of the ¹¹¹In labeled conjugates, together with the co-supervision of the PhD work of Filipe Vultos.</p>
5	<p>Protein-losing gastroenteropathy (PLGE) is characterized by an excessive loss of proteins into the bowel lumen due to abnormal mucosal permeability. The clinical presentation is variable, depending on the underlying cause. ^{99m}Tc labeled Human Serum Albumin (HSA) has been used to confirm and localize PLGE. Nevertheless, HSA supply is limited, since it is produced from human plasma, involving potential immunological and infectious risks. Therefore, it was impossible to obtain pre-prepared cold kits of HSA to label with ^{99m}Tc for clinical application. So, taking into account our wide experience in the preparation of kits for labeling with ^{99m}Tc, Prof. Durval Costa (Champalimaud Foundation, Nuclear Medicine) requested my collaboration for training Dr. Bruno Martins in the preparation of ^{99m}Tc-HSA (“in house” formulation) and implementation of quality control practices required for clinical application.</p> <p>So, this collaboration enabled the preparation of ^{99m}Tc-HSA “in house” (in the Nuclear Medicine Center of Champalimaud Foundation) adequate for “in vivo” administration allowing detection, localization and follow-up of cases with occult or intermittent PLGE, with impact on therapeutic strategies.</p>
6	<p>The clinical relevance of [¹⁸F]-2-fluorodesoxiglucose (FDG) in tumor diagnosis, that is taken up by tumor cells mainly by facile diffusion through the glucose transport protein Glut1, prompted the group of Roger Alberto (Switzerland) to develop ^{99m}Tc(I) labeled glucose analogues.</p> <p>Under this project the PhD student Yunjun Shen (Prof. Roger Alberto student, Zurich University) visited our laboratory (3 weeks) in order to assess the biological activity of several ^{99m}Tc(I)-glucose analogues under the supervision of Dra P. Raposinho. In the scope of this work I have been involved in the preparation of the different ^{99m}Tc(I)-glucose analogues.</p>
7	<p>Since May 2013, I am responsible for the Electrospray ionization quadrupole ion trap mass spectrometry (ESI/QITMS) analysis of bioactive peptides, metal complexes and organic compounds synthesized by the Radiopharmaceutical Sciences Group. I am grateful to Dr</p>

	Joaquim Marçalo for his collaboration on my training and support on this task.
8	High performance liquid chromatography is a crucial technique in the development of radioprobes for in vivo imaging or therapy. This technique is also important in the purification, identification and evaluation of the chemical purity of bioactive peptides and others biomolecules. - Responsible for the management/maintenance of four HPLC equipments (three analytical and a semi-preparative one) - Training of new users.
9	Responsible for the management/maintenance of chemical synthesis laboratory (SQII) namely chemicals and equipment. Responsible for providing information and training to the laboratory workers about chemicals handling and chemistry synthesis techniques and the safe use of apparatus available on the laboratory namely on vacuum line techniques for handling air-sensitive compounds. Organize disposal of hazardous chemicals and solvents.

PUBLICATIONS

- C. Fernandes, L. Maria, L. Gano, I. C. Santos, I. Santos, A. Paulo, Re(I) and ^{99m}Tc (I) tricarbonyl complexes with ether-containing pyrazolyl-based chelators: Chemistry, Biodistribution and Metabolism, *Journal of Organometallic Chemistry* (2013), <http://dx.doi.org/10.1016/j.jorganchem.2013.11.013>
- C. Fernandes, S. Monteiro, P. Mendes, L. Gano, F. Marques, S. Casimiro, L. Costa, J. D. G. Correia, Isabel Santos, Biological assessment of novel bisphosphonate-containing ^{99m}Tc /Re-organometallic complexes, *Journal of Organometallic Chemistry* (2013), <http://dx.doi.org/10.1016/j.jorganchem.2013.10.038>.
- B. Martins, A. Canudo, D. Dantas, S. Chaves, V. Santos, B. Freitas, C. Oliveira, D. C. Costa, I. Santos, C. Fernandes, Evaluation of protein-losing gastroenteropathy in pediatrics with ^{99m}Tc -Human Serum Albumin - technical and methodological developments, *European Journal of Nuclear Medicine and Molecular imaging*, vol:40 pg:S147 -S147, (2013).
- F. Mendes, L. Gano, C. Fernandes, A. Paulo, I. Santos, Perfusion and specific radioprobes for cardiac imaging. CARDIOTECHNIX 2013 - Proceedings of the International Congress on Cardiovascular Technologies, 79-83, (2013).

COMMUNICATIONS

Invited talks

- *Perfusion and specific radioprobes for cardiac imaging*, F. Mendes, L. Gano, C. Fernandes, A. Paulo, I. Santos, Cardiotecnix2013 International Congress on Cardiovascular Technologies – Symposium VisualCardio, Vilamoura, 21 Setembro, (2013). Invited Talk.

Oral presentations

- *Avaliação biológica de novos complexos de ^{99m}Tc (I) no diagnóstico de metástases ósseas*, C. Fernandes, S. Monteiro, E. Palma, L. Gano, F. Marques, J. D. G. Correia, I. Santos, XIV Congresso Nacional de Medicina Nuclear, Porto, Portugal, 5-7 de Dezembro (2013), Oral.
- *Complexos de ^{111}In derivados de estradiol: Avaliação pré-clínica para o diagnóstico do cancro da mama*, S. Cunha, F. Vultos, C. Fernandes, F. Marques, M.C. Oliveira, I. Santos, L. Gano, XIV Congresso Nacional Medicina Nuclear, Porto, Portugal, 5-7 de Dezembro (2013), Oral.
- *Indium-111 Radiolabelled Peptide Towards the Estrogen Receptor*, F. Vultos, C. Fernandes, I. Santos, J. Correia, L. Gano. *The Future of radiation oncology: Imaging, Dosimetry, Biology & Therapy - Seventh Berder Workshop, September 25-28, (2013) (Cancerpole - grand ouest)*, Oral.
- *Evaluation of protein-losing gastroenteropathy in pediatrics with ^{99m}Tc -Human Serum Albumin - technical and methodological developments*, B. Martins, A. Canudo, D. Dantas, S. Chaves, V. Santos, B. Freitas, C. Oliveira, D. C. Costa, I. Santos, C. Fernandes, *Annual Congress of the European-Association-of-Nuclear-Medicine (EANM), Lyon, FRANCE, Oct 19-23, (2013)*, Oral.

Poster presentations

- *Novel ^{99m}Tc(I)-labeled bone-seeking molecules for bone imaging*, C. Fernandes, S. Monteiro, P. Mendes, L. Gano, F. Marques, S. Casimiro, L. Costa, I. Santos, *13th International Conference on Cancer-Induced Bone Disease (CIBD)*, Miami, Florida, USA November 6th-9th, (2013), Poster.
- *Indium-111 radiolabelled peptide for theranostics of ER positive tumours*, F. Vultos, C. Fernandes, I. Santos, J. Correia, L. Gano, *NanoFar, Autumn School, Santiago de Compostela, Spain, October 21-25, (2013)*, Poster.
- *Bisphosphonate-Containing M(CO)₃-Complexes (M = ^{99m}Tc/Re) as Bone-Seeking Agents*, C. Fernandes, S. Monteiro, P. Mendes, L. Gano, F. Marques, J. D. G. Correia, L. Costa, S. Casimiro, I. Santos, *The 3rd Global Cancer Genomics Consortium Symposium (GCGCS), Lisbon, Portugal, IMM, September 18-20, (2013)*, Poster.
- *Evaluation of ^{99m}Tc-TMEOP as Probe for Functional Monitoring of Multidrug Resistance*, Mendes F, Gano L, Fernandes C, Paulo A, Santos I, P27, *Molecular Biology in Portugal and EMBL (and EMBL Alumni), FCUL, Lisboa, 18 Julho, (2013)*, Poster.
- *[¹⁵³Sm]-DOTA-phosphine-ruthenium bimetallic complex as new radiotheranostic*, L. Adriaenssens, Q. Liu, F. Picquet, S. Tasan, M. Picquet, F. Denat, P. le Gendre, MPC. Campello, F. Marques, C. Fernandes, F. Mendes, Gano L, Bodio E, P11, *WIPR 2013: International Workshop on Personalized Radio-immunotherapy, Nantes, França, 9-12 Julho, (2013)*, Poster.
- *¹¹¹In-DOTAGA-Estradiol based complexes with binding affinity for the estrogen receptor*, S. Cunha, C. Fernandes, C. Bernhard, F. Marques, F. Denat, I. Santos, L. Gano, *1st Symposium on Medicinal Chemistry of University of Minho, Braga, Portugal, 17th of May, (2013)*, Poster.

EDUCATION

Theses supervision

- Supervisor of Elisabete Ribeiro, BI Project EXCL/QEQ-MED/0233/2012.
- Co-supervisor of Sofia Monteiro, BI Project PTDC/QUI-QUI/115712/2009.
- Co-supervision of Eser Ucar, ERASMUS student under supervision of Prof. I. Santos (3 months). Preparation and characterization of solid lipid nanoparticles for the delivery of paclitaxel towards the folate receptor.

Jury membership

- Main Jury member (arguente): M. Sc. Thesis, *Fracionamento de kits de HMDP para marcação com ^{99m}Tc – Influência da temperatura e da idade das fracções*", Vanessa Santos, ESTeSL/IPL, Discussed and approved in March 2013.

PROJECTS

Running

Team Member

- (25%) *Synthesis and Pre-clinical Evaluation of Novel Estradiol-Based Indium Complexes for Targeted Radiotherapy of Tumors* (PTDC/QUI-QUI/111891/2009). Leading Institution: IST/ITN, Lisbon, Portugal. Principal Investigator: Maria de Lurdes Barreira Patrício Gano.
- (15%) *Albumin binding-domain fusions to improve protein pharmacokinetics*, PTDC/SAU-FAR/115846/2009. Leading Institution: Associação para o Desenvolvimento do Ensino e Investigação da Microbiologia (ADEIM), Faculdade de Farmácia, Universidade de Lisboa. Principal Investigator: João Manuel Braz Gonçalves.
- (35%) *Molecular and Nano Tools for Cancer Theranostics*, EXCL/QEQ-MED/0233/2012, Leading Institution: Associação do Instituto Superior Técnico para a Investigação e o Desenvolvimento (IST-ID), Lisbon, Portugal. IST/ITN Principal Investigator: I. Santos.

Submitted

Principal Researcher

- (50%) *Dual Targeting Strategy for EGFR Positive Tumors* (EXPL/QEQ-MED/1950/2013). Leading Institution: Associação do Instituto Superior Técnico para a Investigação e o

Desenvolvimento (IST-ID), Lisbon, Portugal. Principal Investigator: Célia Fernandes (50%).
Recommended for funding.

COLLABORATIONS

- Prof. Luis Costa, Clinical and Translational Oncology Research Unit, IMM, FML, Portugal – Evaluation of bisphosphonates/Re/citotoxic complexes in animal models with induced bone metastasis.
- Prof. Durval C. Costa, Champalimaud Foundation, Lisbon, Portugal – Training of Dr. Bruno Martins in the preparation and quality control of ^{99m}Tc-HSA (“in house” formulation) for clinical application.
- Prof. Filomena Botelho, Instituto de Biofísica/Biomatemática, IBILI, Faculdade de Medicina Universidade de Coimbra.
- Prof. Roger Alberto, Zurich University, Switzerland- In vitro and in vivo biological evaluation of metal complexes.

NAME: Fernanda Marujo Marques

CATEGORY: Auxiliary Researcher

IST-ID: 5359

ACTIVITIES

Nº	Activity Description	R&D (%)
1	Synthesis and Pre-clinical Evaluation of Novel Estradiol-Based Indium Complexes for Targeted Radiotherapy of Tumors, PTDC/QUI-QUI/111891/2009-... (Scientific Coordinator: L. Gano, IST/CTN)	20
2	Synthesis, Characterization and Biological Assessment of Multi-Functional Bone-Seeking Agents, PTDC/QUI-QUI/115712/2009-... (Scientific Coordinator: I. Santos, IST/CTN)	20
3	Molecular and Nano Tools for Cancer Theranostic, EXCL/QEQ-MED/0233/2012 -... (Scientific Coordinator: I. Santos, IST/CTN)	10
4	Preclinical evaluation of ruthenium potential drugs for cancer therapy, PTDC/QUI-QUI/118077/2010-... (Scientific Coordinator: M. H. Garcia, FCUL)	30
5	Synthesis, Characterization and Biological Evaluation of Novel Agents for Boron Neutron Capture Therapy (collaboration with Departamento de Química-QOPNA, Universidade de Aveiro)	15
6	Chemical characterization and biological properties of novel heteronuclear lanthanide ruthenium complexes (collaboration with Institut de Chimie Moléculaire de l'Université de Bourgogne)	5
Total		100

WORK SUMMARY

Nº	Work Summary and Main Achievements
1	Synthesis of estradiol-based complexes of In-111 containing different bifunctional chelators and characterization as specific vehicles for inserting the Auger electron-emitter In-111 into ER expressing breast cancer cells. In the scope of this project my contribution has been the cellular uptake studies by confocal microscopy and the evaluation of the relative binding affinity of the compounds with the estrogen receptors subtypes ER α and ER β .
2	Synthesis of multi-functional specific agents which combines a bisphosphonate group (zoledronic acid mimetic) a cytostatic unit (docetaxel) and a gamma or beta emitter metal fragment for imaging or therapy. Chemical/Radiochemical characterization of the compounds has been done with the ^{99m} Tc congeners to evaluate the cellular uptake on

	<p>metastatic MDAMB231 human breast cells and the pharmacokinetic profile in healthy mice and breast tumor bearing mice model.</p> <p>In the scope of this project my contribution has been the evaluation of the antiproliferative properties of the inactive compounds and studies of the cellular uptake and cellular distribution of the ^{99m}Tc congeners. The cellular effects by the bisphosphonates labeled with beta emitters are underway.</p>
4	<p>Synthesis and characterization of metal complexes and the evaluation of the mechanisms of action. Pre-clinical evaluation and in vivo performance of selected complexes in an orthotopic breast cancer model is underway (IPATIMUP) to evaluate the antitumor efficacy and side effects of the prospective metallo drugs.</p> <p>In the scope of this project my contribution has been the evaluation of mechanisms of action and the identification of the cellular targets involved in cell death. In addition, my contribution is also the coordination of all the biological studies conducted at the IST/CTN and the others participating institutions.</p>
5	<p>Synthesis, characterization and biological evaluation of carboranyl methylbenzo[<i>b</i>]acridones as novel agents for boron neutron capture therapy.</p> <p>In the scope of this work my contribution has been the biological studies and the coordination of all the work. So far some boron compounds have been synthesized (Aveiro University) and were evaluated as promising agents for BNCT. Studies of the effects of neutron irradiation (IST/CTN, Reactor Group) at cellular level are underway.</p>
6	<p>Synthesis, characterization and biological evaluation of heterobimetallic radiotheranostic agents based on DOTA macrocyclic ligands.</p> <p>In the scope of this work my contribution was the radiochemical labeling studies with Sm-153.</p>

PUBLICATIONS

- “Biological assessment of novel bisphosphonate-containing $^{99m}\text{Tc}/\text{Re}$ organometallic complexes” C. Fernandes, S. Monteiro, P. Mendes, L. Gano, F. Marques, S. Casimiro, L. Costa, J. D.G. Correia, I. Santos, J. Organomet Chem (2013), <http://dx.doi.org/10.1016/j.jorgchem.2013.10.038>.
- “New polydentate Ru(III)-Salan complexes: Synthesis, characterization, anti-tumour activity and interaction with human serum proteins” C. P. Matos, A. Valente, F. Marques, P. Adão, M. P. Robalo, R. F. M. Almeida, J. C. Pessoa, I. Santos, M. H. Garcia, A. I. Tomaz, *Inorganica Chimica Acta* 394 (2013) 616–626. <http://dx.doi.org/10.1016/j.ica.2012.09.026>
- “Biological Activity and Cellular Uptake of $[\text{Ru}(\eta^5\text{-C}_5\text{H}_5)(\text{PPh}_3)(\text{Me}_2\text{bpy})][\text{CF}_3\text{SO}_3]$ Complex” T. S. Morais, I. Santos, L. Côte-Real, F. Marques, M. P. Robalo, P. J. A. Madeira, M. H. Garcia, *J Inorg Biochem* 122 (2013) 8-17. <http://dx.doi.org/10.1016/j.jinorgbio.2013.01.011>
- “Screening organometallic binuclear thiosemicarbazone ruthenium complexes as potential anti-tumour agents: cytotoxic activity and human serum albumin binding mechanism” B. Demoro, R. F. de Almeida, F. Marques, C. P. Matos, L. Otero, J. C. Pessoa, I. Santos, A. Rodríguez, V. Moreno, J. Lorenzo, D. Gambino, A. I. Tomaz. *Dalton Trans* 42 (2013) 7131-7146. <http://pubs.rsc.org/en/content/articlepdf/2013/dt/c3dt00028a>
- “Important cytotoxicity of novel iron(II) cyclopentadienyl complexes with imidazole based ligands”, C. Gonçalves, T. S. Morais, M. P. Robalo, F. Marques, F. Avecilla, C. P. Matos, I. Santos, A. I. Tomaz, M.H.Garcia, *J Inorg Biochem* 129 (2013) 1-8. <http://dx.doi.org/10.1016/j.jinorgbio.2013.07.033>
- “Cellular uptake mechanisms of an antitumor ruthenium compound: the endosomal/lysosomal system as a target for anticancer metal-based drugs” L. Côte-Real, A. P. Matos, I. Alho, T. S. Morais, A. I. Tomaz, M. H. Garcia, I. Santos, M. P. Bicho, F. Marques. *Microsc Microanal* 19 (2013) 1122-1130. <http://dx.doi.org/10.1017/S143192761300175X>
- “New polydentate Ru(III)-Salan complexes: Synthesis, characterization, anti-tumour activity and interaction with human serum proteins” C. P. Matos, A. Valente, F. Marques, P. Adão, M. P. Robalo, R. F.M. de Almeida, J. C. Pessoa, I. Santos, M. H. Garcia, A. I. Tomaz, *Inorg Chim Acta* 394 (2013) 616–626. <http://dx.doi.org/10.1016/j.ica.2012.09.026>

- First polymer ruthenium-cyclopentadienyl complex as potential anticancer agent” A. Valente, M. H. Garcia, F. Marques, Y. Miao, C. Rousseau, P. Zinck, *J Inorg Biochem* 127 (2013) 79–81. <http://dx.doi.org/10.1016/j.jinorgbio.2013.07.002>
- “New bifunctional metalloproteinase inhibitors: an integrated approach towards biological improvements and cancer therapy” S. M. Marques, C. C. Abate, S. Chaves, F Marques, I Santos, E Nuti, A Rossello, M. A. Santos *J Inorg Biochem.* 127 (2013) 188-202. <http://dx.doi.org/10.1016/j.jinorgbio.2013.03.003>
- “Novel Heterobimetallic Radiotheranostic: Preparation, Activity and Biodistribution” L. Adriaenssens, Q. Liu, F. Chauv-Picquet, S. Tazan, M. Picquet, F. Denat, P. Le Gendre, F. Marques, C. Fernandes, F. Mendes, L. Gano, M. P. C. Campello, E. Bodio, *ChemMedChem* 2013. <http://onlinelibrary.wiley.com/doi/10.1002/cmdc.201300494/pdf>

COMMUNICATIONS

- “Searching for gallium bioactive compounds: a new gallium (III) complex as prospective antitumoral and anti-trypanosoma cruzi compound”. I. Machado, M. Fernández, L. Becco, R. F. Brissos, N. Zabarska, P. Gomes, F. Marques, B. Garat, D. Gambino, 12th International Symposium on metal ions in Biology and Medicine, Punta del Este, Uruguay, 11-13 March 2013.
- “Cyclopentadienyl ruthenium (macro)metalloodrugs: large spectrum antitumor agents”. M. H. Garcia, T. S. Morais, A. I. Tomaz, A. Valente, F. Marques, Drug Discovery and Therapy, World Congress, Boston, USA, 3-6 June 2013.
- “Novel Pathways for Anticancer Therapy: the Role of Golgi network in the mechanism of cell death of organometallic complexes”. F. Marques, A. P. Matos, L. Côte-Real, T. S. Morais, A. I. Tomaz, M. H. Garcia, M. P. Bicho, Microscopy at the Frontiers of Science Tarragona, Spain 17-20 September 2013.
- “Complexos de 111-In derivados de estradiol: avaliação pré-clínica para o diagnóstico do cancro da mama” XIV Congresso Nacional Medicina Nuclear, Porto 5-7 December 2013.
- “Novel Pathways for Anticancer Therapy: the Role of Golgi network in the mechanism of cell death of organometallic Ru complexes”. F. Marques, A. P. Matos, L. Côte-Real, T. S. Morais, M. H. Garcia, Congresso de microscopia: Microscopy and Research, , Egas Moniz - Cooperativa de Ensino Superior CRL, Monte da Caparica 9-10 December 2013.

COLLABORATIONS

- Cellular ultrastructural analysis, Centro de Investigação Interdisciplinar Egas Moniz and CESAM-Lisboa, Faculdade de Ciências de Lisboa (António Matos, President of Portuguese Society of Microscopy)
 - Confocal microscopy, Instituto Medicina Molecular, Faculdade Medicina de Lisboa (José Rino, Head of BioImaging Unit)
 - Anatomical pathology - Histology and Immunohistochemistry, Hospital de Santa Maria (Francisco Tortosa, Medical Doctor in Anatomical pathology)
 - Evaluation of Ru compounds in human tumor animal models, IPATIMUP and Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (ICBAS-UP) (Nuno Mendes, MSc Animal House Technical Coordinator/ Head of IPATIMUP Cell Line Bank and Fátima Gartner, Vice-President of the Scientific Council - ICBAS, Full Professor of Veterinary Pathology).
 - Synthesis of boro compounds for BNCT, Universidade de Aveiro, Departamento de Química Química Orgânica, Produtos Naturais e Agroalimentares (Artur Silva, President of Chemistry Department, Full Professor of Chemistry).
 - Mechanisms of genetic lesion, Faculdade de Ciências Médicas da Universidade de Lisboa, Laboratório de Genética (Jorge Gaspar, Professor of Faculdade de Ciências Médicas Universidade Nova de Lisboa).
 - Novel heteronuclear lanthanide ruthenium complexes, collaboration with Institut de Chimie Moléculaire de l'Université de Bourgogne (Pierre Le Gendre, Professor of Institute of Molecular Chemistry).
-

NAME: Filipa Fernandes Mendes

CATEGORY: Auxiliary researcher (Ciência 2007) until August; Principal Researcher (FCT), since September.

IST-ID: 5493

ACTIVITIES

Nº	Activity Description	R&D (%)
1	Scientific coordination of the project A Molecular Imaging Approach to Cystic Fibrosis - EXPL/BIM-MEC/0115/2012 In collaboration with Prof. Carlos Farinha – DQB, FCUL	35
2	Targeting telomerase with new anti-tumoral Cu(II) Complexes PTDC/QUI-QUI/114139/2009 - <i>Coordination of Tasks 2 and 5</i> In collaboration with Dr. Sofia Gama (PI) and Dr. António Paulo	10
3	Radiolabeled Benzazole Derivatives for In Vivo Imaging of Amyloid Aggregation - PTDC/QUI-QUI/102049/2008 - team member In collaboration with Dr. António Paulo (PI)	5
4	Molecular and Nano Tools for Cancer Theranostics EXCL/QEQ-MED/0233/2012 - <i>Coordination of Task 2</i> In collaboration with Dr. João Galamba and Dr Isabel Santos (PI)	5
5	Target-specific and Heterobimetallic Platinum Complexes: Synthesis, Characterization and Mechanistic Studies <i>Acções Integradas Luso Espanholas E-23/12 - team member</i> In collaboration with Prof. Adoracion Quiroga, Universidad Autónoma de Madrid, Spain	5
6	Preclinical Evaluation of Ruthenium Potential Drugs for Cancer Therapy - PTDC/QUI-QUI/118077/2010- <i>team member</i> In collaboration with Prof Helena Garcia, DQB, FCUL (PI)	5
7	Novel Heterobimetallic Radiotheranostic Agents In collaboration with Prof. Michel Picquet, Université de Bourgogne, France	5
8	Evaluation of new Copper complexes as anti-tumoral agents In collaboration with Prof Fernanda Carvalho (IST) and Dr Olga Iranzo, CNRS, France	10
9	Teaching	7,5
10	Supervision of student's projects/ theses preparation	5
11	Preparation of projects/proposals	5
12	Management of laboratory infrastructure	2,5
Total		100

WORK SUMMARY

Nº	Work Summary and Main Achievements
1	A Molecular Imaging Approach to Cystic Fibrosis The FCT funded project initiated in July 2013 has as main goal to develop radioactive probes to detect the expression of rescued mutants of the protein CFTR at the membrane of epithelial pulmonary cells of Cystic Fibrosis. New radiolabelled molecules based on <i>small organic inhibitors</i> and <i>antibodies (Abs)</i> were prepared. The CFTR inhibitor 172a was radiolabelled with the $^{99m}\text{Tc}(\text{CO})_3^+$ core, using the bifunctional chelator approach. Furthermore, to assess if the metal complex still maintained its ability to interact with CFTR, a non-radioactive Re surrogate was synthesized and its inhibitory efficacy assessed through the iodide efflux assay. At 50 μM the compound inhibited approximately 56% of CFTR activity in cells expressing the wt protein. Regarding the antibodies, we started with the optimization of the ^{99m}Tc -labelling of the anti-CFTR Ab ECL1. The ECL1 antibody was first purified from rabbit serum, reduced to generate

	<p>sulfhydryl groups and then radiolabelled with $^{99m}\text{Tc(I)}$, with a yield of 25%. Further optimization is underway.</p> <p>The synthetic and characterization work, as well as the radiolabeling studies have been performed by the BI V. Ferreira under of my supervision and of Dr. João Correia. I am responsible for the overall coordination of the project.</p>
2	<p><i>Targeting telomerase inhibition with new anti-tumoral Cu(II) Complexes</i></p> <p>This project is focused on the synthesis and in vitro evaluation of Cu(II) complexes that are expected to interact with telomeric DNA. During 2013, I was responsible for the coordination of Tasks 2 and 5, with focused on the biological evaluation of the complexes synthesized by the other team members.</p> <p>In particular, new mixed bipyridine-terpyridine Cu(II) complexes were synthesized and evaluated in vitro, in terms of their cytotoxic activity and double helix DNA interaction. Depending on the cyclic amine substituents of the bipy ligands, some complexes showed an impressive plasmid DNA cleaving ability in the absence of any exogenous oxidizing or reducing agents. Therefore, these compounds emerged as a new class of very efficient artificial DNA nucleases.</p> <p>Additionally, complexes of the type [(Ant-tpy)CuCl₂], containing a terpyridine ligand functionalized with an anthracenyl fragment, have been also synthesized. For purposes of comparison, the related [(Ant-tpy)PtCl] and [(Ant-py)RuCl₃] complexes were also prepared and evaluated. Preliminary results have shown that these complexes present moderate cytotoxicity.</p> <p>These studies were performed under my supervision by I Rodrigues (BI). The PI of this project is Sofia Gama and, together with Dr A. Paulo, the overall coordinator.</p>
3	<p><i>Radiolabeled Benzazole Derivatives for In Vivo Imaging of Amyloid Aggregation</i></p> <p>In the last year of this project I was involved in the evaluation of a new family of benzothiazole-containing $^{99m}\text{Tc/Re}$ tricarbonyl complexes, in order to assess their relevance for imaging beta-amyloid aggregates. The pre-clinical evaluation comprised ex vivo autoradiographic studies with transgenic animal models of AD with the ^{99m}Tc-labeled complexes. Furthermore, the Re complexes were used in fluorescence microscopy studies to assess their ability to detect amyloid plaques.</p> <p>In addition, as benzothiazole derivatives are an interesting class of potential anticancer drugs, we have evaluated the potential of the synthesized complexes for cancer theranostics, by combining the cytotoxicity of the cold (non-radioactive) Re complexes with the imaging capabilities of the ^{99m}Tc counterparts. I was responsible for evaluation of the cytotoxicity of the Re(I) complexes in PC-3 and MCF-7 human tumor cells. Overall, the complexes have a moderate cytotoxicity against these cell lines.</p> <p>These studies were performed in collaboration with other team members – P. Nunes and L. Gano. Dr A. Paulo was responsible for the overall coordination.</p>
4	<p><i>Molecular and Nano Tools for Cancer Theranostics</i></p> <p>Within this project which started in June, I was involved in the coordination of Task 2 - <i>Metal complexes bearing L-arginine derivatives ($^{99m}\text{Tc}/^{188}\text{Re}$) for detection and /or treatment of NO/iNOS-related tumors</i> - in collaboration with Dr. João Galamba.</p> <p>Aiming to probe the Nitric Oxide Synthase (NOS) enzymes, whose expression is related to diseases such as cancer and neurodegenerative disorders, we are exploring $^{99m}\text{Tc}(\text{CO})_3$-complexes bearing NOS-recognizing units for SPECT imaging. Previous, enzymatic activity studies demonstrated that some Re(CO)₃-complexes inhibit purified inducible NOS. Therefore, we are pursuing the evaluation of the cell uptake kinetics of $^{99m}\text{Tc(I)}$-complexes bearing iNOS targeting moieties, namely L-arginine derivatives, in iNOS-expressing human breast cancer and human melanoma cell lines.</p> <p>Additionally, the oxygenase domains of iNOS, eNOS and nNOS have been cloned, expressed and purified. The three isoforms have shown to be soluble and active, and protein production can now be established for functional and structural studies.</p> <p>The experimental work has been partially performed by M. Morais and the team of Prof. M. J. Romão, FCT-UNL, under my supervision as well as of Dr. João Galamba (IST). Dr Isabel Santos is the overall coordinator of this project.</p>

5	<p><i>Target-specific and Heterobimetallic Platinum Complexes: Synthesis, Characterization and Mechanistic Studies</i></p> <p>This bilateral collaborative project that started in 2012 has as main goal the development of novel target-specific and heterobimetallic platinum complexes, to assess their interest as 1 anticancer drugs. During 2013, I was involved in the study of the biological properties of 2 sets of complexes:</p> <p>First I was responsible for the study of target-specific Pt complexes bearing bioactive peptides of the RGD type. The resulting complexes were evaluated for their antitumoral activity in a panel of human cell lines expressing different levels of integrin receptors. Unexpectedly, no selectivity was presented by the “targeted” complexes. Secondly, we concluded the work regarding the characterization of novel Pt complexes containing ligands with photophysical and DNA-intercalating properties. I performed cytotoxicity assays against a panel of human cells and DNA interaction studies. Altogether the results show that we have improved the cytotoxicity of the ligands using a non-conventional platinum structure core and that the distance of the DNA-binding group to the metal is determinant for the cytotoxic activity. The work was included in an oral presentation in an international meeting, a poster in a Spanish national meeting and a manuscript is also currently submitted.</p>
6	<p><i>Preclinical Evaluation of Ruthenium Potential Drugs for Cancer Therapy</i></p> <p>In the framework of this FCT project, we have previously found that a new organometallic complex of Ru-Cp, TM34 is very active against all tumorigenic cell lines, its efficiency largely surpassing that of cisplatin.</p> <p>Further studies have shown that apoptosis is the major mechanism of cell death exerted by TM34 and, in this context we focused on the identification of which specific molecular mechanisms were involved. Studies were carried out in breast cancer cells exposed to 2 Ru complexes: the above mentioned TM34 and a new related polymeric complex PMC1. In parallel cisplatin was also studied. We used a Apoptosis Array, which is a fast and sensitive tool that allows to simultaneously detect the relative levels of expression of 35 apoptosis-related proteins. The results showed increased expression of the proteins cytochrome C, catalase and Hsp70 in cells incubated with both complexes and Hsp60 just for PMC1. These studies suggested that apoptosis is induced by the two Ru complexes through the intrinsic mitochondrial pathway. Additionally, the complexes also seem to induce high levels of oxidative stress in tumor cells. The work was included in one paper in preparation.</p>
7	<p><i>Novel Heterobimetallic Radiotheranostic Agents</i></p> <p>In the continuation of the collaborative work initiated in 2012, novel heteronuclear lanthanide ruthenium complexes previously prepared at the Institut de Chimie Moléculaire de l'Université de Bourgogne were explored at CTN for their potential as future imaging probes. In 2013 we finalized the evaluation of the biological properties of the complexes (and respective ligands). I have been primarily involved in the cytotoxicity experiments and in the preparation of progress reports and of the resulting manuscript. The work resulted in one poster communication in an International Conference and one paper already accepted.</p>
8	<p><i>Evaluation of new Copper complexes as anti-tumoral agents</i></p> <p>The search for antitumoral agents through the coordination of selective ligands to metal centers constitutes a promising field of research. In this line of research, 2 types of Copper complexes were evaluated as potential therapeutic agents. First, two novel Cu(II) complexes with tetradentate ligands of the phenantroline type were evaluated for their antiproliferative properties and DNA interaction. Overall, the complexes presented low to moderate cytotoxicity. As for the studies of DNA interaction, both Cu(II) complexes by themselves are poorly active as DNA nucleases, however, the addition of activators enhanced the activity of the complexes, indicating the involvement of ROS in the process.</p> <p>A different family of Cu complexes was also studied. Here, polynuclear Cu(I) camphor complexes, either of dimeric or polymeric structure, were assessed in terms of their antiproliferative properties. All the Cu(I) camphor complexes studied display cytotoxic activity against human colon cancer cell line HT29, ranging from high to moderate depending on the characteristics of the camphor ligand.</p> <p>These studies were performed in the framework of the Group active collaborations with academic partners working in Bioinorganic Chemistry, particularly in this case with Prof</p>

	Fernanda Carvalho, IST and Dr Olga Iranzo, CNRS, France.
9	Teaching Since Sept 2013 I am an Invited Assistant Professor at the Departamento de Bioengenharia, Instituto Superior Técnico, ULisboa (average 3h/week). I gave lectures and laboratory classes to students of the <i>Mestrados Integrados in Biological Eng</i> and in Biomedical Eng and of the MSc in Biotechnology and in Microbiology
10	Supervision of students projects/ thesis preparation During 2013 Vera Ferreira finished the experimental that conducted to the preparation of the thesis entitled “A Molecular Imaging approach to Cystic Fibrosis”. She successfully defended her MSc thesis in Biochemistry, FCUL in July 2013. Another Master Student, Fernando Toscano, had already finished the experimental work in late 1012, but during 2013 I supervised the data analysis and interpretation and thesis preparation. He successfully defended his MSc thesis in <i>Química Inorgânica Biomédica</i> entitled "Avaliação biológica de complexos catiónicos de $^{99m}\text{Tc(I)}$ como sondas para detecção tumoral e monitorização funcional de resistência a múltiplos fármacos” in December 2013. During 2013 I was also responsible for the supervision of an Undergraduate Student from the Degree in Nuclear Medicine, ESTeSL/IPL. She performed laboratory work and prepared the monography “Medicina Nuclear Convencional e o seu contributo diagnóstico no Carcinoma da Próstata localizado e metastizado”, which she defended in early 2014.
11	Preparation of projects/proposals Collaboration in the preparation of the Strategic Project of C ² TN, particularly in the Thematic Strand – Health and Life Sciences: Radiopharmaceutical Sciences and Health Physics. Participation in the elaboration of the Doctoral Programme “Radiopharmaceutical Sciences in Molecular Imaging and Cancer Therapy”, submitted by the RS group of C ² TN to the 2013 FCT call.
12	Management of the Laboratory of Biochemistry and Molecular Biology I am responsible for the DNA and protein analysis equipment of this laboratory and for the training and technical and scientific support of new users.

PUBLICATIONS

Journals

- Gabano E, Gama S, Mendes F, Gariboldi MB, Monti E, Bombard S, Ravera M (2013) "Study of the synthesis, antiproliferative properties, and interaction with DNA and polynucleotides of cisplatin-like Pt(II) complexes containing carcinogenic polyaromatic amines" J Biol Inorg Chem, 7:791-801
- Moura C, Mendes F, Gano L, Santos I, Paulo A (2013) "Mono- and dicationic Re(I)/ $^{99m}\text{Tc(I)}$ tricarbonyl complexes for the targeting of energized mitochondria" J Inorg Biochem, 123:34-45

Books of proceedings

- Mendes F, Gano L, Fernandes C, Paulo A, Santos I (2013) “Perfusion and specific radioprobes for cardiac imaging” CARDIOTECHNIX 2013 - Proceedings of the International Congress on Cardiovascular Technologies, 79-83.

COMMUNICATIONS

Oral presentations

- “Perfusion and specific radioprobes for cardiac imaging” (2013). Cardiotecnix2013 International Congress on Cardiovascular Technologies – Symposium VisualCardio, Vilamoura, 21st September, invited talk.
- “New Platinum (II) complexes with DNA-targeting ability” (2013). 1st International Symposium on Functional Metal Complexes that Bind to Biomolecules, Second Whole Action Meeting of the COST Action CM1105, 10th September, Barcelona, Spain.

Poster presentations

- Cabrera S, Herrera JM, Mendes F, Gama S, Santos IR, Ranninger CN, Quiroga AG (2013) “Trans-Pt(II) complexes as covalent and intercalator binders: a double DNA-targeting approach” XVII National Meeting of the Spanish Society of Medicinal Chemistry "Advances in Drug Discovery: Successes, Trends and Future Challenges", 24th October, Madrid, Spain.
- Paulo A, Moura C, Mendes F, Gano L, Santos I (2013) “Mitochondria-targeted Re(I)/^{99m}Tc(I) Tricarbonyl Complexes” 1st International Symposium on Functional Metal Complexes that Bind to Biomolecules, Second Whole Action Meeting of the COST Action CM1105, 10th September, Barcelona, Spain, oral presentation.
- Gama S, Rodrigues I, Mendes F, Santos I, Ravera M, Paulo A (2013) “Anticancer Copper(II) and Platinum(II) Complexes with Anthracene-Containing Terpyridine Ligands”, 1st International Symposium on Functional Metal Complexes that Bind to Biomolecules, Second Whole Action Meeting of the COST Action CM1105, 9 September, Barcelona, Spain, oral presentation.
- Mendes F, Gano L, Fernandes C, Paulo A, Santos I (2013) “Evaluation of ^{99m}Tc-TMEOP as Probe for Functional Monitoring of Multidrug Resistance”, P27, *Molecular Biology in Portugal and EMBL (and EMBL Alumni)*, 18th July, FCUL, Lisbon.
- Adriaenssens L, Liu Q, Picquet F, Tasan S, Picquet M, Denat F, le Gendre P, Campello MPC, Marques F, Fernandes C, Mendes F, Gano L, Bodio E (2013) “ [¹⁵³Sm]-DOTA-phosphine-ruthenium bimetallic complex as new radiotheranostic” P11, WIPR 2013: International Workshop on Personalized Radio-immunotherapy, 9-12th July, Nantes, France.

EDUCATION

Theses Supervision

- MSc Thesis in Biomedical Inorganic Chemistry - *Avaliação biológica de complexos catiónicos de ^{99m}Tc(I) como sondas para deteção tumoral e monitorização funcional de resistência a múltiplos fármacos*, Fernando Toscano, Faculdade de Ciências, Universidade de Lisboa – 13th December 2013
- MSc Thesis in Biochemistry - *A Molecular Imaging approach to Cystic Fibrosis*, Vera Ferreira, Faculdade de Ciências, Universidade de Lisboa – 24th July 2013
- Research Project/Monograph, Degree in Nuclear Medicine, *Medicina Nuclear Convencional e o seu contributo diagnóstico no Carcinoma da Próstata localizado e metastizado*, Inês Matreno, Escola Superior de Tecnologia da Saúde de Lisboa – 17th January 2014

Teaching

- Equiv. Invited Auxiliary Professor, Departamento de Bioengenharia, Instituto Superior Técnico, Universidade de Lisboa (3h/week – theoretical and laboratory classes) – 1^o Semester 2013/2014. Courses: Genetic Engineering – Integrated Master Biological Engineering and Integrated Master Biomedical Engineering; Molecular Biotechnology - MSc Biotechnology and MSc Microbiology.

PROJECTS

Principal Researcher - 50%

- *A Molecular Imaging Approach to Cystic Fibrosis*, FCT - EXPL/BIM-MEC/0115/2012, Associação do Instituto Superior Técnico para a Investigação e o Desenvolvimento (IST-ID).

Team Member

- 30% - *Molecular and Nano Tools for Cancer Theranostics*, FCT - EXCL/QEQ-MED/0233/2012. IST-ID, Principal Researcher: I. Santos.
- 15% - *Nitric Oxide Synthase targeting with Re(I)/^{99m}Tc(I)-complexes containing L-Arg derivatives: A structure-activity study*, FCT - PTDC/QUI-QUI/121752/2010. IST-ID, Principal Researcher: J. D. G. Correia.
- 15% - *Targeting telomerase inhibition with new anti-tumoral Cu(II) Complexes*, FCT-PTDC/QUI-QUI/114139/2009. Instituto Superior Técnico, Principal Researcher: Sofia Gama.

- 15% - *Preclinical evaluation of ruthenium potential drugs for cancer therapy*, FCT - PTDC/QUI-QUI/118077/2010. Fundação da Faculdade de Ciências da Universidade de Lisboa, Principal Researcher: Maria Helena Garcia.
- 8% - *Radiolabeled Benzazole Derivatives for In Vivo Imaging of Amyloid Aggregation*, FCT-PTDC/QUI-QUI/102049/2008. Instituto Superior Técnico, Principal Researcher: António Paulo.

Submitted

- 15% - *Dual Targeting Strategy for EGFR Positive Tumors*, FCT- EXPL/QEQ-MED/1950/2013, 2014-15 – *recommend for funding*, IST-ID, Principal Researcher: Célia Fernandes.

Internationals

- *Functional metal complexes that bind to biomolecules*, COST Action CM1105 – EU. Principal Researcher: I. Santos.
- *Target-specific and Heterobimetallic Platinum Complexes: Synthesis, Characterization and Mechanistic Studies*, Acções Integradas Luso-Espanholas E-23/12. Principal Researcher: I. Santos.

COLLABORATIONS

Nationals

- Prof. Margarida Amaral e Prof Carlos Farinha, BioFIG – Faculdade de Ciências, ULisboa.
- Prof. Helena Garcia, CCM, Faculdade de Ciências, ULisboa.
- Prof. Fernanda Carvalho, IST, ULisboa.

Internationals

- Prof. Adoracion Quiroga, Universidad Autónoma de Madrid, Spain. Visited CTN on October 17-18th.
- Prof Mauro Ravera, Universita del Piemonte Orientale “Amedeo Avogadro”, Italy.
- Dr. Olga Imnazo, CNRS, Marseille, France.

NAME: Goreti Jesus Ribeiro Morais

CATEGORY: Auxiliary Researcher (CIÊNCIA 2008)

IST-ID: 430

ACTIVITIES

Nº	Activity Description	R&D (%)
1	Participation in the project “Molecular and Nano tools for Cancer Theranostics”-EXCL/QEQ-MED/0233/2012 (Scientific Coordinator: I. Santos C ² TN)	15
2	Participation in the project “Targeting telomerase inhibition with new anti-tumoral Cu(II) complexes” – PTDC/QUI-QUI-114139/2009 (Scientific Coordinator: S Gama C ² TN)	20
3	Participation in the project “Benzazole derivatives with fluorine-18 and technetium-99m for in vivo imaging of amyloid deposits”– PTDC/QUI/102049/2008 (Scientific Coordinator: A. Paulo C ² TN)	15
4	Coordination of the project “Radiofluorinated compounds targeting fibrillar alpha-synuclein”	15
5	Seeking funding	10
6	Supervision of M. Sc. Students/Teaching	20
7	Management of the Organic Chemistry Laboratory III	5
Total		100

WORK SUMMARY

N°	Work Summary and Main Achievements
1	<p>The work performed in this activity is related with the one described below in activity 2, and involves the synthesis of Cu(II) complexes with N₂S₂-tetradentate ligands of the bis(thiosemicarbazone) type, comprising or not an extended aromatic system and terminal cyclic amines to improve the selectivity for G-quadruplex. By exploring this type of ligands, we expected to have a better ability of synthesizing the corresponding complexes with radioactive copper (⁶⁴Cu). I have participated in the synthesis of the ligands.</p> <p>Moreover, in this task, I have designed the synthetic strategy tailored to the development of doxorubicin- and estradiol-containing bioconjugates for EGFR/ER targeting.</p>
2	<p>This project is focused on the synthesis and <i>in vitro</i> evaluation of Cu(II) complexes that are expected to interact with telomeric DNA and, therefore, useful in the design of telomerase inhibitors.</p> <p>I have established and optimized the synthesis of several substituted thiosemicarbazides and of novel bis(thiosemicarbazones) comprising or not an extended aromatic system containing terminal piperidine and morpholine groups. The synthesis of the corresponding new complexes of Zn(II) e Cu(II) is been underway.</p>
3	<p>This multidisciplinary project involves the IST/ITN, ICNAS and IMM. The main goal of this project is the design of radiolabeled amyloid-avid probes for the early diagnosis of neurodegenerative diseases by Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT).</p> <p>I have optimized the synthesis of ¹⁸F-benzimidazole derivatives at the ICNAS, using the cyclotron-produced ¹⁸F. To perform the radiofluorinations, I have carried out one short-term at the ICNAS.</p> <p>I have also synthesized the ¹²⁵I-labeled IMPY, a known beta-amyloid ligand used for the <i>in vitro</i> competitive binding assays to the beta-amyloid aggregates.</p> <p>Within this project I have collaborated in the supervision of Patrique Nunes in the synthesis of (S,N,O)-tridentate ligands of the cysteamine type containing a benzothiazole pharmacophore for amyloid binding.</p>
4	<p>Deposition of alpha-synuclein aggregates is the neuropathological feature that defines Parkinson disease (PD) and Dementia with Lewy Bodies (DLB). These neurodegenerative diseases affect millions of persons worldwide and pose a significant impact in public health. The <i>in vivo</i> detection of alpha-synuclein by molecular imaging may allow an early diagnosis of the synucleinopathies (PD and DLB), increasing the potential of their successful early treatment and would be useful in the therapeutic follow-up and on the high-throughput screening of new therapies. So far, the development of radiotracers targeting fibrillar alpha-synuclein is an unmet goal. This work aims to find new useful tools to visualize alpha-synuclein aggregates, using optical and/or PET imaging techniques.</p> <p>In the scope of this project I have designed ASI peptide-based analogues, bearing in their structure a fluorescein, a glucose residue and a prosthetic group to introduce fluorine (¹⁹F/¹⁸F). I have also been involved in the synthesis, purification and characterization of the ASI peptides analogues. Within this project I have supervised the M. Sc Student Leticia Quental</p>
5	<p>In this activity I have written and submitted several projects to national and international funding schemes. Moreover, I have dedicated time to write a new project and to submit it to the FCT-researcher call.</p>
6	<p><i>Supervision of Students</i></p> <p>In this activity I have supervised the M.Sc Student Leticia Quental and collaborated with Prof A. Paulo in the supervision of Patrique Nunes.</p> <p><i>Teaching</i></p> <p>In this activity I have organized and lectured tutorials in Organic Chemistry to MSc, PhD students at the RSG.</p>
7	<ul style="list-style-type: none"> - Train new team members at the Organic Chemistry Lab III - Maintenance of the equipment - Maintain a safe and clean laboratory environment - Enforce all safety regulations

PUBLICATIONS

Book chapter

- Paulo A, Ribeiro Morais G, Santos I, "Organometallic Chemistry of Rhenium and Technetium Fuelled by Biomedical Applications", *Organometallic Chemistry: Recent Advances*, Chapter 44, p 589-604 John Wiley & Sons (2013)

Peer-reviewed articles

- Al-Sarairoh Y M J, Sutherland M, Springett B R, Ribeiro Morais G, Loadman P M, Errington T J, Smith P J, Patterson L H, Shnyder S D, Falconer R A "Pharmacological inhibition of polysialyltransferase ST8SiaII modulates tumour cell migration" *Plos One*, 8(8):e73366 (2013)
- Ribeiro Morais G, Kniess T, Bergmann R, Gano L, Abrunhosa A, Santos I, Paulo A "Radiosynthesis and *in vivo* Evaluation of a ¹⁸F-Labelled Styryl-Benzoxazole Derivative for beta-Amyloid Targeting" *Appl Rad Isotopes*, 82, 100-104 (2013)
- Cunha S, Gano L, Ribeiro Morais G, Thiemann T, Oliveira MC "Progesterone receptor targeting with radiolabelled steroids: an approach in predicting Breast Cancer Response to Therapy" *J Steroid Biochem Mol Biol*, 137, 223-241 (2013)
- Ribeiro Morais G, Falconer R A, Santos I "Carbohydrate-based molecules suitable for nuclear molecular imaging" *Eur J Org Chem*, 1401-1414 (2013) (Cover Picture)
- Oliveira M C, Neto C, Ribeiro Morais G, Thiemann T "Steroid receptor ligands for breast cancer targeting: an insight into their potential role as PET imaging agents" *Curr Med Chem*, 20 (2), 222-245 (2013)

Non-peer reviewed articles

- Oliveira MC, Ribeiro Morais G, Thiemann T, "Steroid receptor ligands as PET imaging agents" *Diagnostic Imaging Europe March*, 70-72 (2013)
- Quental L, Ribeiro Morais G, Santos I, Paulo A, "Molecular imaging agents for detection of β -amyloid plaques in Alzheimer's disease" *Saúde e Tecnologia*, Nov, 5-9 (2013)

COMMUNICATIONS

Oral presentations

- *Radiopharmaceutical Sciences Group: Research, Highlights and Facilities*, G. Ribeiro Morais, I. Santos, *Fundação Champalimaud, Human Brain Project Workshop*, June 26th 2013 (invited talk)

Poster presentations

- *Organometallic Tc-99m/Re Complexes for in vivo targeting of beta-amyloid plaques*, Nunes P, Morais G, Palma E, Gano L, Santos I, Paulo A, *Drug Discovery Therapy World Congress 2013*, 3-6 June 2013, Boston, USA
- *New benzimidazole derivatives for the targeting of beta-amyloid aggregates*, Ribeiro Morais G, Quental L, Oliveira C, Palma E, Gano L, Miranda H, Outeiro T, Abrunhosa A, Santos I, Paulo A, *EMIM 2013*, 26-27 May 2013, Turin, Italy. Abstract 47
- *Peptides targeting alpha-synuclein*, Quental L A, Raposinho P, Santos I, Paulo A, Ribeiro Morais G, *1st Symposium on Medicinal Chemistry*, 17 May 2013, Braga, Portugal. Abstract PC66
- *Design and synthesis of membrane type matrix metalloproteinase (MT-MMP) targeted anti-tumor agents*, Falconer R A, Youssef A M, Warren J G, Ribeiro Morais G, Mitchell A, Shnyder S D, Gill J H, Patterson L H, Loadman P M, *AACR Annual Meeting 2013*, 6-10 April 2013, Washington, DC, USA. Abstract P5669

EDUCATION

- Supervisor, M. Sc. Thesis, *Compostos peptídicos para visualização de agregados de alfa-sínucleína*, by Letícia Quental, Faculdade de Farmácia, Universidade de Lisboa, 16th July 2013
- Organizer and lecturer tutorials in Organic Chemistry to graduated students of the RSG.

PROJECTS

Running

Team Member

- *Benzazole derivatives with fluorine-18 and technetium-99m for in vivo imaging of amyloid deposits*, Fundação para a Ciência e Tecnologia (FCT), PTDC/QUI/102049/2008 (team member, 15%)
- *Bimodal PET-MRI molecular imaging technologies and applications for in vivo monitoring of diseases and biological processes*, COST Action TD1007
- *Molecular and Nano tools for Cancer Theranostics*, Fundação para a Ciência e Tecnologia (FCT) EXCL/QEQ-MED/0233/2012 (team member, 15%)
- *Inhibition of telomerase by new complexes of Cu(II)*, Fundação para a Ciência e Tecnologia, Portugal – PTDC/QUI-QUI-114139/2009 (team member, 15%)

Submitted

Principal Researcher

- *Radioiodinated Plectin-1-targeting peptides for in-vivo detection of pancreatic cancer*, FCT-EXPL/QEQ-MED/0902/2013. Leading Institution: IST-ID
- *Bioconjugates targeting alpha-synuclein aggregates*, Innovation Grant Parkinsons's UK INN-13B-12, Leading Institution: IST-ID

FCT-Researcher

- *Nuclear tools for diagnosis and therapy of pancreatic cancer*, FCT-IF-01079/2013. Leading institution: C²TN

CONTRACTS

- Trabalho de consultoria; Sociedade de advogados, NDR-Neville de Rougemont; para um gabinete de Advogados, 28-29 Maio 2013, 1000 euros.

COLLABORATIONS

- Torsten Knies, PET-center, Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Dresden, Germany – Training on the radiochemistry of the cyclotron-produced ¹⁸F.
 - Antero Abrunhosa, ICNAS, Coimbra – Production of ¹⁸F.
 - Alfonso Fernández-Mayorales, Bioorganic Chemistry Group, “Instituto de Química Orgánica General” (IQOG), Madrid, Spain - Synthesis of biologically active compounds for the radiolabeling with ¹⁸F.
 - Tiago Outeiro, Unidade de Neurociência Celular e Molecular, IMM, Lisboa/ Department of NeuroDegeneration and Neurorestoration, University of Göttingen, Germany – Preparation of beta-amyloid, alpha-synuclein and insulin aggregates.
 - Cristina Pereira, Centro Neurociências (CNC), Coimbra – Triple Transgenic animal models of Alzheimer's disease.
 - Miguel Castanho, Unidade Bioquímica física, IMM, Lisboa – Cellular model of the brain-blood barrier (BBB).
 - Amélia Pilar, Faculdade de Ciências, Universidade de Lisboa – Synthesis of biologically active nucleosides for the radiolabeling with ¹²⁵I.
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NAME: Maria Cristina das Neves Oliveira

CATEGORY: Auxiliary Researcher

IST-ID: 5371

ACTIVITIES

Nº	Activity Description	R&D (%)
1	Molecular and Nano Tools for Cancer Theranostics- EXCL/QEQ-MED/0233/2012. <i>Team member.</i> Co-coordinator of task 3. Multifunctional Indium Complexes for Cell-Specific Nuclear Targeting: Synthesis and Pre-Clinical Evaluation	15
2	Synthesis and Pre-clinical Evaluation of Novel Estradiol-Based Indium Complexes for Targeted Radiotherapy of Tumors – PTDC/QUI-QUI/111891/2009. <i>Team member.</i> In collaboration with Doutora Lurdes Gano (PI) from Radiopharmaceutical Sciences Group, IST/ C ² TN.	25
3	Albumin binding-domain fusion to improve protein pharmacokinetics – PTDC/SAU-FAR/115846/2009. <i>Team member.</i> In collaboration with Prof. João Gonçalves (PI) from the Unit of Retrovirus and Associated Infections, Faculty of Pharmacy, University of Lisbon.	15
4	Radioiodination of thioflavin derivatives with high binding affinity for beta-amyloid plaques In collaboration with Doutor António Paulo from Radiopharmaceutical Sciences Group, IST/C ² TN	20
5	Radioiodination and biological evaluation of butyrylcholinesterase inhibitors In collaboration with Prof Amélia P. Rauter from CQB-FCUL	15
6	Management/maintenance of the radioiodination facilities	5
7	Training of new research students	5
Total		100

WORK SUMMARY

Nº	Work Summary and Main Achievements
1	The therapeutic potential of In-111 has been demonstrated in a few clinical and pre-clinical studies with labeled peptides or mAbs, including hEGF. Although ¹¹¹ In complexes are being evaluated for targeted radiotherapy of EGFR-expressing cancers, to our knowledge, such approach has never been studied for tumors expressing estradiol receptors (ER). Novel ¹¹¹ In-based theranostic agents bearing GE11 analogs or estradiol, to target EGFR and ER, respectively, in different malignant tumors are being designed in our group. GE11, a novel and almost unexplored peptide for EGFR, has been synthesized, characterized and successfully coupled to the bifunctional chelator DOTAGA. The labeling of the novel bioconjugate GE11-DOTAGA with ¹¹¹ InCl ₃ /InCl ₃ is currently underway. The promising biological results obtained with the ¹¹¹ In-DOTAGA-estradiol complexes, within the current project PTDC/QUI-QUI/111891/2009, led us to explore the 17 α -position of estradiol for coupling different bifunctional chelators (BFC). To this end, the synthesis and characterization of novel 17 α -substituted estradiols and their coupling to BFCs through different spacer chains is currently underway.
2	The oestrogen receptor (ER α) plays an important role in the clinical care of breast cancer patients. Aiming to contribute for the development of radionuclide-targeted therapeutic agents of cancer as safer alternatives to conventional therapies this strategy uses estradiol-based radiometal complexes as specific delivery vectors to insert Auger electron-emitter radionuclides (e.g. ¹¹¹ In) within ER expressing-tumor cells. We have successfully synthesized novel 16 α -substituted estradiols with different spacer chains and coupled to different bifunctional chelating agents (DTPA, DOTA, DOTAGA). Among them, estradiol-DOTAGA-like chelators presented adequate ER α binding affinity and selectivity. The ¹¹¹ In-estradiol based complexes were prepared in high radiochemical yield and purity and are

	<p>stable <i>in vitro</i> in human blood serum and in the presence of apo-transferrin. Animal studies indicate high <i>in vivo</i> stability and rapid clearance from main organs. The moderate cell uptake found is probably due to the low lipophilicity of the complexes. However, the relative ERα binding affinity of the ^{111}In-DOTAGA-estradiol complexes and their evaluation in ERα-expressing cell lines suggest that uptake may occur via an ER-mediated process</p> <p>As team member of this project my contribution has been delineating alternative synthetic strategies, discussing results and supervising students involved in this task.</p>
3	<p>Small domain antibodies present high potential for molecular <i>in vivo</i> imaging and therapy. Owing to the low molecular weight they are rapidly cleared from blood circulation, and new strategies to extend their half-lives are needed for therapeutic applications. Plasma protein binding can be an effective approach to improve pharmacokinetics properties of short-life molecules. Thus, a bacterial albumin-binding domain (Zag) derived from <i>Streptococcus zooepidemicus</i> fused to an anti-TNF VHH small domain antibody was proposed as a strategy to improve the pharmacokinetic properties of therapeutic proteins. Both antibodies were conjugated to S-2-(4 isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid and labeled with ^{67}Ga. The anti-TNF and albumin-binding properties of both conjugates were preserved upon conjugation. To confirm the longest half-life of the VHH-ZAG antibody comparative biodistribution studies of both ^{67}Ga labeled antibodies were carried out in healthy female CD-1 mice up to 24 h. Biodistribution of ^{67}Ga-NOTA-VHH and ^{67}Ga-NOTA-VHH-Zag demonstrated that the Zag domain affected the pharmacokinetic profile of VHH, with impressive differences in blood clearance and total excretion at 24 h. In this way, the anti-TNF VHH in fusion with the Zag domain presents a higher therapeutic potential than the unmodified VHH. The ^{125}I-labeling of biomolecules within this project is on my responsibility.</p>
4	<p>Alzheimer's disease (AD) is a brain disorder showing progressive memory loss and decrease of cognitive function. Excessive amounts of β-amyloid (Aβ) plaques are commonly detected in the postmortem brains of AD patients. These Aβ plaques are believed to play an important role in the pathogenesis of the disease. Aβ plaque-specific imaging agents for detecting and monitoring the changes of Aβ plaque deposition in living brains that may serve as potential biomarkers for the disease are being developed in our group. Several fluorinated derivatives based on variety of core structures are labeled with radiofluorine as positron emission tomography (PET) imaging agents. To be effective as brain imaging agents these derivatives should display high binding affinity for Aβ aggregates. To assess the binding affinity by competitive binding assays it was necessary to synthesize radioligands with recognized binding characteristics, such as the radioiodinated thioflavin derivatives ^{125}I-TZDM and ^{125}I-IMPY. ^{125}I-TZDM and ^{125}I-IMPY were prepared from the corresponding tributyltin derivatives by an iododestannylation reaction, which resulted in tracers with high specific activity and high radiochemical purity. The determination of the binding affinity of the novel compounds for Aβ aggregates is currently underway.</p>
5	<p>Acetylcholinesterase (AChE) is the enzyme responsible for the breakdown of the neurotransmitter acetylcholine, leading to termination of cholinergic neurotransmission in the brain of Alzheimer (AD) patients. The loss of cholinergic neurotransmission is generally associated to a reduced AChE concentration. One of the most promising approaches to treat the disease involves the design of drugs with an AChE inhibition profile. However, in advanced AD, AChE levels in the brain have already decreased, while the activity of the related enzyme butyrylcholinesterase (BChE) is still quite high suggesting that AChE hydrolysis may also occur via BChE catalysis. In fact, it has been reported that the specific inhibition of BChE is important in raising AChE levels and improving cognition. Thus, novel inhibitors to selectively target each cholinesterase have been synthesized at CQB-FCUL. To assess the ability of these inhibitors to image BChE in AD, a purine nucleoside with high inhibition potency was successfully radioiodinated with ^{125}I. The radioiodinated nucleoside, prepared from its brominated precursor by radiohalogen exchange, was obtained, after HPLC purification, in high specific activity and radiochemical purity. The ability of the tracer to cross the BBB was investigated by biodistribution studies in an animal model. Cell uptake studies in BChE expressing cell lines are still ongoing.</p>
6	<p>Responsible for the management/maintenance of radioiodination facilities, namely</p>

	radioactive chemicals, equipment and disposal of radioiodine contaminated waste. I am also responsible for the management/maintenance of the HPLC equipment dedicated to the analysis of ¹²⁵ I-labelled compounds and for the training of the new users.
7	Responsible for the training of new research students on radioiodination techniques and safe handling of radioiodinated compounds.

PUBLICATIONS

- S. Cunha, L. Gano, G. Ribeiro Morais, T. Thiemann, M.C. Oliveira, Progesterone receptor targeting with radiolabelled steroids: An approach in predicting breast cancer response to therapy, *Journal of Steroid Biochemistry and Molecular Biology*, 137, 223-241(2013), doi: 10.1016/j.jsbmb.2013.04.003
- M. Morais, B.L. Oliveira, J.D.G. Correia, M.C. Oliveira, M.A. Jiménez, I. Santos, P.D. Raposinho, Influence of the bifunctional chelator on the pharmacokinetic properties of ^{99m}Tc(CO)₃-labeled cyclic α -melanocyte stimulating hormone analog, *Journal of Medicinal Chemistry*, 56, 1961-1973 (2013), doi: 10.1021/jm301647t
- M.C. Oliveira, C. Neto, G.R. Morais, T. Thiemann, Steroid receptor ligands for breast cancer targeting: An insight into their potential role as pet imaging agents, *Current Medicinal Chemistry*, 20, 222-245 (2013) PMID: 23231090
- M.C Oliveira, G. Ribeiro Morais, T. Thiemann, Steroid receptor ligands as PET imaging agents, *Diagnostic Imaging Europe*, March, 70-72 (2013). Non-peer reviewed article.

COMMUNICATIONS

- *Complexos de ¹¹¹In derivados de estradiol: avaliação pré-clínica para o diagnóstico do cancro da mama*, S. Cunha, F. Vultos, C. Fernandes, F. Marques, M.C. Oliveira, I. Santos, L. Gano, XIV Congresso Nacional Medicina Nuclear, Porto, Portugal, December (2013), Oral.
- *New benzimidazole derivatives for the targeting of beta-amyloid aggregates*, G. Ribeiro Morais, L. Quental, M. C. Oliveira, E. Palma, L. Gano, H. Miranda, T. Outeiro, A. Abrunhosa, I. Santos, A. Paulo, EMIM 2013, Turin, Italy, May 26-27 (2013), Abstract 47, Poster.
- *MC1R-Targeted Metallopeptides: NMR Structural Analysis*, M. Morais, P. D. Raposinho, M. C. Oliveira, M. A. Jiménez, D. Pantoja-Uceda, J.D. G. Correia, I. Santos, *Chemistry of Metals in Biological Systems Summer School, COST Action CM 1105, Lisbon, Portugal, May 12-19 (2013)*, Poster.

EDUCATION

- Co-supervisor of undergraduated student, Monography, *Ligandos com afinidade para as placas β -Amilóide: marcação com ¹²⁵I*, by Ana Filipa Silva, Escola Superior de Tecnologia da Saúde de Lisboa, June 2013

PROJECTS

Team Member

- Molecular and Nano Tools for Cancer Theranostics- EXCL/QEQ-MED/0233/2012.
- Synthesis and Pre-clinical Evaluation of Novel Estradiol-Based Indium Complexes for Targeted Radiotherapy of Tumors – PTDC/QUI-QUI/111891/2009.
- Albumin binding-domain fusion to improve protein pharmacokinetics – PTDC/SAU-FAR/115846/2009.

COLLABORATIONS

- Prof Amélia P. Rauter, Carbohydrate Chemistry Group, FCUL. Collaboration on radiosynthesis and biological evaluation of butyrylcholinesterase inhibitors.
- Prof. Filomena Botelho, Instituto de Biofísica/ Biomatemática, IBILI, FMUC, Coimbra. Collaboration within the project PTDC/QUI-QUI/111891/2009

- Prof Thies Thiemann, Faculty of Science, United Arab Emirates University, United Arab Emirates, Collaboration on synthesis/radiosynthesis and biological evaluation of steroids.

NAME: Maria de Lurdes Barrela Patricio Gano

CATEGORY: Auxiliary Researcher

IST-ID: 5373

ACTIVITIES

Nº	Activity Description	R&D (%)
1	Coordination of the project, Synthesis and Pre-clinical Evaluation of Novel Estradiol-Based Indium Complexes for Targeted Radiotherapy of Tumors, PTDC/QUI-QUI/111891/2009. In collaboration with Prof. Filomena Botelho from IBILI, Faculty of Medicine, University of Coimbra.	30
2	Albumin binding-domain fusion to improve protein pharmacokinetics, PTDC/SAU-FAR/115846/2009. <i>Team member.</i> In collaboration with Prof. João Gonçalves (PI) from the Unit of Retrovirus and Associated Infections, Faculty of Pharmacy, University of Lisbon.	10
3	Biological evaluation of ⁶⁷ Ga-labelled gold nanoparticles carrying bombesin as a target-specific vector	10
4	Synthesis, characterization and biological assessment of multi-functional bone-seeking agents, PTDC/QUI-QUI/115712/2009. <i>Team member.</i> In collaboration with Prof. Luis Costa from the Unit of Clinical and Translational Oncology Research Unit of IMM, Faculty of Medicine, University of Lisbon.	10
5	Preclinical evaluation of ruthenium potential drugs for cancer therapy, PTDC/QUI-QUI/118077/2010. <i>Team member.</i> In collaboration with Prof. M. H. Garcia (PI) from Faculty of Sciences, University of Lisbon.	5
6	Molecular and Nano Tools for Cancer Theranostics- EXCL/QEQ-MED/0233/2012. <i>Team member.</i> Co-coordinator of task 3. Multifunctional Indium Complexes for Cell-Specific Nuclear Targeting: Synthesis and Pre-Clinical Evaluation.	10
7	Supervision of the PhD work of Filipe Vultos (SFRH/BD/84509/2012) entitled “ ¹¹¹ In complexes bearing specific peptides towards the oestrogen receptor for cancer theranostic: Synthesis and biological evaluation”.	10
8	Radiolabeled Benzazole Derivatives for In vivo Imaging of Amyloid Aggregation, PTDC/QUI-QUI/102049/2008. <i>Team member.</i>	5
9	Radioiodination and biological evaluation of butyrylcholinesterase inhibitors In collaboration with Prof Amélia P. Rauter from CQB-FCUL	5
10	Management of Animal Facilities; Management of Radiation Protection Program in the Radiopharmaceutical Sciences Group	5
Total		100

WORK SUMMARY

Nº	Work Summary and Main Achievements
1	The ultimate goal of this project is the development of radionuclide targeted therapeutic agents of cancer as safer alternatives to conventional cancer therapies. The oestrogen receptor (ER) is an important tumour target due to its overexpression in many malignant cells as compared to normal cells. Thus, our strategy uses estradiol-based complexes as specific delivery vectors to insert Auger electron-emitter radionuclides (<i>e.g.</i> ¹¹¹ In) within tumor cells over-expressing ER.

	<p>A set of novel 16α-substituted estradiols with different spacer chains and coupled to different bifunctional chelating agents (DTPA, DOTA, DOTAGA) have been successfully synthesized and biologically evaluated. Among them, estradiol-DOTAGA-like chelators presented adequate ERα binding affinity and selectivity. The ¹¹¹In-estradiol complexes were prepared in high radiochemical yield and purity and are stable <i>in vitro</i> in human blood serum and in the presence of apo-transferrin. Animal studies indicate high <i>in vivo</i> stability and rapid clearance from main organs. Cell studies point to a moderate uptake. However, the relative ERα binding affinity of the ¹¹¹In-DOTAGA-estradiol complexes and their evaluation in ERα-expressing cell lines suggest that uptake may occur via an ER-mediated process.</p> <p>Most of the experimental work within this project has been done by the PhD students under my supervision (PI of the project) - S. Cunha and F. Vultos.</p>
2	<p>Small domain antibodies present high potential for molecular <i>in vivo</i> imaging and therapy. Owing to the low molecular weight they are rapidly cleared from blood circulation, and new strategies to extend their half-lives are needed for therapeutic applications. Plasma protein binding can be an effective approach to improve pharmacokinetics properties of short-life molecules. Thus, a bacterial albumin-binding domain (Zag) derived from <i>Streptococcus zooepidemicus</i> fused to an anti-TNF VHH small domain antibody was proposed as a strategy to improve the pharmacokinetic properties of therapeutic proteins. Both antibodies were conjugated to S-2-(4 isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid and labeled with ⁶⁷Ga. The anti-TNF and albumin-binding properties of both conjugates were preserved upon conjugation. To confirm the longest half-life of the VHH-ZAG antibody comparative biodistribution studies of both ⁶⁷Ga labeled antibodies were carried out in healthy female CD-1 mice up to 24 h. Biodistribution of ⁶⁷Ga-NOTA-VHH and ⁶⁷Ga-NOTA-VHH-Zag demonstrated that the Zag domain affected the pharmacokinetic profile of VHH, with impressive differences in blood clearance and total excretion at 24 h. In this way, the anti-TNF VHH in fusion with the Zag domain presents a higher therapeutic potential than the unmodified VHH.</p> <p>Animal experimentation (biodistribution and pharmacokinetics) are at my responsibility.</p>
3	<p>The suitable biological properties of the gold nanoparticles (AuNP) as drug-carrier systems have been explored in the Radiopharmaceutical Sciences Group for the development of multifunctional AuNP for specific delivery of radiometals (<i>e.g.</i> ⁶⁷Ga) into tumour cells for <i>in vivo</i> imaging. Most of the work (synthesis and characterization of AuNP, biomolecules coupling and radiolabelling) has been done by the PhD student F. Silva under the supervision of A. Paulo and by P. Campello.</p> <p>My contribution for this work has been the biological evaluation of ⁶⁷Ga-AuNP carrying bombesin (BBN) for target-specific delivery into BBN expressing tumours. To achieve that goal the studies included:</p> <ul style="list-style-type: none"> - Citotoxicity in human prostate PC3 tumour cells; - Receptor binding affinity of the multifunctional AuNP; - Uptake and internalization of ⁶⁷Ga-AuNP by PC3 cells; - Study of ⁶⁷Ga-AuNP uptake mechanism by tumour cells; - Establishment of a suitable animal model (human PC3 xenograft Balb/c nude mice) for biodistribution and <i>in vivo</i> stability studies. <p>These studies indicated that ⁶⁷Ga-BBN-AuNP have higher uptake in PC-3 cells than the ⁶⁷Ga-AuNP without BBN. Administration of ⁶⁷Ga-BBN-AuNP in PC-3 tumour xenografts led to a moderate tumour uptake and a high pancreatic uptake, pointing out for a receptor-mediated uptake.</p>
4	<p>Radiolabeled bisphosphonates (BPs) bind to bone matrix in areas of increased bone turnover, characteristic of metastization sites. Nitrogen-containing BPs may have also a direct anti-tumour activity. We synthesized complexes of the type <i>fac</i>-[M(CO)₃(k³-L)]⁺ (M = ^{99m}Tc, Re) stabilized by bifunctional chelators with different molecular weight, overall charge, (lipo)hydrophilic nature and different positions of BP attachment. Biodistribution studies in mice have shown that the complex bearing the BP unit at position 4 of the azolyl ring (Tc1) presents a more favourable pharmacokinetics profile than the gold standard ^{99m}Tc-MDP. Cell fragmentation studies have shown that this complex presents also the</p>

	<p>highest accumulation in the cytosol. The biological properties of Tc1 prompted us to prepare the analogue complex with ^{188}Re, which is a β^- emitter. Firstly, we optimized the radiosynthesis, purification and analytical control of the precursor $\text{fac-}[^{188}\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$. Reaction of the latter with the appropriate chelator under optimized conditions gave complex $^{188}\text{Re1}$ with high radiochemical purity (> 95%) after purification by solid phase extraction. The complex was evaluated in specific cell lines and its bone-seeking ability assessed in healthy mice.</p> <p>My participation in this project has been related to the animal studies namely biodistribution, in vivo stability and mice imaging of the $^{99\text{m}}\text{Tc}/^{188}\text{Re}$.</p>
5	<p>Chemotherapy remains an important therapy in many malignant tumours. Cisplatin drugs are valuable agents for this purpose. However the undesirable side-effects of and the resistance to these chemotherapeutic drugs agents is a major obstacle in the successful treatment of cancer patients. Thus, preclinical evaluation of ruthenium potential drugs for cancer therapy aiming to contribute for the search of alternative more selective and efficient chemotherapeutic drugs than the existing ones is a very demanding issue. Several Ru/Fe/Ga complexes has been screened to evaluate their cytotoxicity against cancer cell lines, the uptake and selectivity by tumor and health cells and also the mechanisms of cell death. From those studies two Ru complexes have been selected for animal studies in human xenograft tumour mice to evaluate their therapeutic efficacy.</p> <p>My contribution for this project has been the evaluation of:</p> <ul style="list-style-type: none"> -Toxicity of the Ru complexes in mice; - Establishment of a human PC3 xenograft Balb/c nude mice to assess the therapeutic efficacy, the metal biodistribution and the in vivo stability of the Ru complexes versus cisplatin. Tumours collected before and after drug treatment were analyzed by histology immunohistochemistry. The Ru content of tumors and main organs was evaluated by ICP-MS. Biological samples (blood and liver homogenates) were analyzed by LC-MS.
6	<p>The main goal of this project is the consolidation and extension of our research activities within the scope of ongoing projects. Thus, apart from the work already described in the previous items 1 (Estradiol-based indium complexes project), 4 (multi-functional bone seeking agents project), in which my contribution has already been described, I am also involved in the tasks 1,2,3 and 6 of the project <i>Molecular and Nano Tools for Cancer Theranostics</i> (EXCL/QEQ-MED/0233/2012):</p> <p>Within Task 1 (<i>Multifunctional and Nano Bone Seeking agents</i>) and Task 2 (<i>Metal complexes bearing iNOS</i>) my contribution will be on biological evaluation of the radioactive compounds in animals.</p> <p>Co-coordination of Task 3 (<i>Multifunctional Indium Complexes for Cell-Specific Nuclear Targeting: Synthesis and Pre-Clinical Evaluation</i>) in which GE11, a peptide for EGFR has been synthesized, characterized and successfully coupled to the bifunctional chelator DOTAGA for labelling with $^{111}\text{InCl}_3/\text{InCl}_3$. Additionally the synthesis and characterization of novel 17α-substituted estradiols and their coupling to BFCs through different spacer chains is currently underway.</p> <p>In Task 6 - <i>Design, synthesis and characterization of peptides</i> my contribution has been discussing the ER-binding and DNA-binding peptides to be synthesized along the project.</p>
7	<p>The oestrogen receptor (ER) is overexpressed in nearly 60% of breast cancers and is a relevant target for cancer imaging and radionuclide therapy. As peptides are considered good vectors for the development of target specific radiopharmaceuticals, the main goal of the PhD work of Filipe Vultos was the design, synthesis and biological evaluation of ^{111}In complexes bearing peptides that target ER expressing tumour cells as delivery systems of Auger electrons to ER(+) tumours.</p> <p>This PhD work started in July and during 2013 a family of small peptides with known ER targeting ability have been synthesized by solid phase and conjugated to DOTA and DTPA based chelating agents. The conjugates were radiolabelled with ^{111}In and the peptidic constructs are currently being studied as potential theranostic agents for ER positive tumors. To increase the potential for Auger therapy, the synthesis of ^{111}In coordinating bifunctional chelating agents for peptidic conjugation bearing DNA intercalators is also underway.</p>
8	<p>During the last months of this project, a new family of $^{99\text{m}}\text{Tc}(\text{I})$ and $\text{Re}(\text{I})$ tricarbonyl</p>

	<p>complexes with (S,N,O)-tridentate ligands of the cysteamine type containing a benzothiazole pharmacophore for amyloid binding were synthesized, characterized and biologically evaluated. This work has been performed by Patrique Nunes (Research Assistant (BI)) under my supervision. All tested ^{99m}Tc complexes were unable to cross the BBB in mice and did not emerge as promising probes for in vivo imaging of the deposition of amyloid aggregates in the brain.</p> <p>Benzothiazole derivatives are an interesting class of compounds to design anticancer drugs. Hence, we have evaluated the potential relevance of the synthesized $^{99m}\text{Tc}(\text{I})$ and $\text{Re}(\text{I})$ tricarbonyl complexes for cancer theranostics, by combining the potential cytotoxicity of the cold (non-radioactive) Re complexes with the imaging capabilities of the ^{99m}Tc counterparts. The evaluation of the cytotoxicity of the $\text{Re}(\text{I})$ complexes in PC-3 and MCF-7 human tumor cells has proved that the complexes have a moderate cytotoxicity against these cell lines. Cell uptake studies with the ^{99m}Tc congeners confirmed that the compounds display high accumulation into the cells. Biodistribution studies in adequate tumor bearing mice models are foreseen for the most promising compounds. I have been involved in the cell and animal studies of these compounds.</p>
9	<p>Acetylcholinesterase (AChE) is the enzyme responsible for the breakdown of the neurotransmitter acetylcholine, leading to termination of cholinergic neurotransmission in the brain of Alzheimer (AD) patients. The loss of cholinergic neurotransmission is generally associated to a reduced AChE concentration. One of the most promising approaches to treat the disease involves the design of drugs with an AChE inhibition profile. However, in advanced AD, AChE levels in the brain have already decreased, while the activity of the related enzyme butyrylcholinesterase (BChE) is still quite high suggesting that AChE hydrolysis may also occur via BChE catalysis. In fact, it has been reported that the specific inhibition of BChE is important in raising AChE levels and improving cognition.</p> <p>Thus, novel inhibitors to selectively target each cholinesterase have been synthesized at CQB-FCUL. To assess the ability of these inhibitors to image BChE in AD, a purine nucleoside with high inhibition potency was successfully radioiodinated with ^{125}I. The radioiodinated nucleoside with high specific activity and radiochemical purity was then investigated by biodistribution studies in mice to evaluate its ability to cross the blood brain barrier. Cell uptake studies in BChE expressing cell lines are still ongoing.</p>
10	<p>Responsible to assure that the laboratory animal facilities (maintenance of the housing, feeding and care requirements of small rodents) and team members involved in the animal experimentation respect the principles of laboratory animal science on animal care, protection and welfare and are properly accredited by the National Authority, Direcção Geral de Alimentação e Veterinária according to DL 129/92; Portaria 1005/92, DL 113/2013 and EU Directives. All the research projects are also approved by the national authorities.</p> <p>The Group of Radiopharmaceutical Sciences is committed to a Radiation Protection Program (RPP) according to the license from DGS nº 431/13, proc nº 1650. This RPP is designed to control operations with radioactive compounds conducted within the research laboratories of the group that may result in the potential exposure to ionizing radiation. It is my duty to supervise the maintenance of this RPR as well as the training of personnel on its implementation.</p>

PUBLICATIONS

- G. R. Morais, L. Gano, T. Kniess, R. Bergmann, A. Abrunhosa, I. Santos, A. Paulo, Radiosynthesis and in vivo evaluation of a ^{18}F -labelled styryl-benzoxazole derivative for b-amyloid targeting, *Appl Radiat Isotop*, 82:100-104 (2013).
- M. Morais, A. Paulo, L. Gano, I. Santos, J. D. G. Correia, Target-specific $\text{Tc}(\text{CO})_3$ -complexes for in vivo imaging, *J. Organ Chem* 744:125-139 (2013).
- S. Cunha, L. Gano, G. Morais, T. Thiemann, M.C. Oliveira, Progesterone receptor targeting with radiolabelled steroids: An approach in predicting breast cancer response to therapy, *Steroid Biochem. Mol. Biol* 113:223-241 (2013).

- C. Moura, F. Mendes, L. Gano, I. Santos, A. Paulo, Mono- and dicationic Re(I)/^{99m}Tc(I) tricarbonyl complexes for the targeting of energized mitochondria, *J. Inorg. Biochem* 123:34–45 (2013).
- S. Chaves, A. Capelo, L. Areias, S. M. Marques, L. Gano, M. A. Esteves, M. A. Santos, A novel tripodal tris-hydroxypyrimidinone sequestering agent for trivalent hard metal ions: synthesis, complexation and in vivo studies, *Dalton Trans.* 42(17):6033–6045 (2013).
- M. A. Santos, A. Capelo, L. Areias, S. M. Marques, L. Gano, M. A. Esteves, S. Chaves, A Bifunctional Tripodal Hydroxypyrimidinone-Based Chelator for Metal Decorporation, *Acta of the International Symposium on Metal Complexes-ISMEC 2013*, Volume 3, p. 57-58, (2013).
- F. Mendes, L. Gano, C. Fernandes, A. Paulo, I. Santos, Perfusion and specific radioprobes for cardiac imaging, *Proceedings of the 1st International Congress on Cardiovascular Technologies – CARDIOTECHNIX 2013*, p. 79-83, (2013).

COMMUNICATIONS

Oral presentations

- *A Bifunctional Tripodal Hydroxypyrimidinone-Based Chelator for Metal Decorporation*, M. A. Santos, A. Capelo, L. Areias, S. M. Marques, L. Gano, M. A. Esteves, S. Chaves, *XXIV International Symposium on Metal Complexes, ISMEC 2013*, Burgos, Spain, June (2013), oral.
- *Pharmacokinetic properties of a ⁶⁷Ga-labeled anti-TNF VHH single domain antibody containing a bacterial albumin-binding domain (Zag)*, J. D. G. Correia, M. Morais, L. Gano, I. Santos, C. Cantante, S. Lourenço, C. Santos, C. Fontes, F. Silva, J. Gonçalves, *2nd International Workshop on Innovative Personalized Radioimmunotherapy, WIPR 2013*, Nantes, France, July (2013), oral.
- *Indium-111 Radiolabelled Peptide Towards the Estrogen Receptor*, F. Vultos, C. Fernandes, I. Santos, J. D. G. Correia, L. Gano, *The Future of radiation oncology: Imaging, Dosimetry, Biology & Therapy - Seventh Berder Workshop*, France, September (2013), Invited talk.
- *Perfusion and specific radioprobes for cardiac imaging*, F. Mendes, L. Gano, C. Fernandes, A. Paulo, I. Santos, *1st International Congress on Cardiovascular Technologies – CARDIOTECHNIX 2013*, Vilamoura, Portugal, September (2013), oral.
- *Mitochondria-targeted Re(I)/^{99m}Tc(I) Tricarbonyl Complexes*, A. Paulo, C. Moura, F. Mendes, L. Gano, I. Santos, *WG3 meeting – COST CM1105*, Barcelona, September (2013), Invited Talk.
- *Small Core Gold Nanoparticles Stabilized by Thiolated DOTA Derivatives*, Francisco Silva, Maria P. C. Campello, L. Gano, Isabel Santos, A. Paulo, *Nanoparticles for early Diagnostics of inflammatory diseases*, November 2013, Lisbon, invited talk.
- *Complexos de ¹¹¹In derivados de estradiol: avaliação pré-clínica para o diagnóstico do cancro da mama*, S. Cunha, F. Vultos, C. Fernandes, F. Marques, M. C. Oliveira, I. Santos, L. Gano *XIV Congresso Nacional Medicina Nuclear*, Porto, December (2013), oral.
- *Avaliação biológica de novos complexos de ^{99m}Tc(I) no diagnóstico de metástases ósseas*, C. Fernandes, S. Monteiro, E. Palma, L. Gano, F. Marques, J. D. G. Correia, I. Santos, *XIV Congresso Nacional Medicina Nuclear*, Porto, December (2013), oral.

Poster presentations

- *¹¹¹In-DOTAGA-Estradiol based complexes with binding affinity for the estrogen receptor*, S. Cunha, C. Fernandes, C. Bernhard, F. Marques, F. Denat, I. Santos, L. Gano, *1st Symposium on Medicinal Chemistry of University of Minho*, Braga, Portugal, May (2013), poster.
- *⁶⁷Ga-Labelled gold nanoparticles carrying bombesin as a target-specific vector: Synthesis, characterization and biological evaluation*, F. Silva, A. Zambre, L. Gano, A. Paulo, R. Kannan, I. Santos, *1st Symposium on Medicinal Chemistry of University of Minho*, Braga, Portugal, May (2013), poster.
- *New benzimidazole derivatives for the targeting of beta-amyloid aggregates*, G. Ribeiro Morais, L. Quental, C. Oliveira, E. Palma, L. Gano, H. Miranda, T. Outeiro, A. Abrunhosa, I. Santos, A. Paulo, *EMIM 2013*, Turin, Italy, May (2013), poster.
- *Organometallic Tc-^{99m}/Re Complexes for in vivo targeting of beta-amyloid plaques*, P. Nunes, G. Morais, E. Palma, L. Gano, I. Santos, A. Paulo, *Drug Discovery Therapy World Congress 2013*, Boston, USA, June (2013), poster.

- *Evaluation of ^{99m}Tc -TMEOP as Probe for Functional Monitoring of Multidrug Resistance*, F. Mendes, L. Gano, C. Fernandes, A. Paulo, I. Santos, *Molecular Biology in Portugal and EMBL (and EMBL Alumni)*, Lisbon, July (2013), poster.
- *[^{153}Sm]-DOTA-phosphine-ruthenium bimetallic complex as new radiotheranostic*, L. Adriaenssens, Q. Liu, F. Picquet, S. Tasan, M. Picquet, F. Denat, P. le Gendre, M. P. C. Campello, F. Marques, C. Fernandes, F. Mendes, L. Gano, E. Bodio, *2nd International Workshop on Innovative Personalized Radioimmunotherapy, WIPR 2013*, Nantes, France, July (2013), poster.
- *Bisphosphonate-Containing $M(\text{CO})_3$ -Complexes ($M = ^{99m}\text{Tc}/\text{Re}$) as Bone-Seeking Agents*, C. Fernandes, S. Monteiro, P. Mendes, L. Gano, F. Marques, J. D. G. Correia, L. Costa, S. Casimiro, I. Santos, *The 3rd Global Cancer Genomics Consortium Symposium (GCGCS), IMM*, Lisbon, Portugal, September (2013), poster.
- *Indium-111 radiolabelled peptide for theranostics of ER positive tumours*, F. Vultos, C. Fernandes, I. Santos, J. Correia, L. Gano, *NanoFar, Autumn School*, Santiago de Compostela, Spain, October (2013), poster.
- *Albumin Binding domains Zag and ProTH improve pharmacokinetics and affects biodistribution of an anti-TNF VHH*, C. Cantante, S. Lourenço, J. Leandro, M. Morais, S. Oliveira, L. Gano, C. Fontes, P. Leandro, J. Correia, F. Silva, J. Gonçalves, *PEGS Summit Europe 2013*, Lisbon, November (2013), poster.
- *Novel ^{99m}Tc (I)-labeled bone-seeking molecules for bone imaging*, C. Fernandes, S. Monteiro, P. Mendes, L. Gano, F. Marques, S. Casimiro, L. Costa, I. Santos, *13th International Conference on Cancer-Induced Bone Disease (CIBD)*, Miami, Florida, USA, November (2013), poster.

EDUCATION

- Invited Lecturer at the Faculdade de Farmácia da Universidade de Lisboa, Master Course on Clinical Analysis.
- Invited Lecturer at the Faculdade de Farmácia da Universidade de Lisboa, Master Course in Pharmaceutical Sciences: Discipline of Radiopharmacy.
- Supervisor of undergraduated student, Monography, *A utilização de antagonistas da bombesina em medicina nuclear*, by Maria Fateixa Palmeiro, Escola Superior de Tecnologia da Saúde de Lisboa, 20 December 2013.

PROJECTS

Principal Investigator

- *Synthesis and pre-clinical evaluation of novel estradiol-based indium complexes for targeted radiotherapy of tumors* (PTDC/QUI-QUI/111891/2009). Leading Institution: IST/ICTN, Bobadela, Portugal. IST/CTN Principal Investigator: L. Gano.

Team Member

- *Radiolabeled benzazole derivatives for in vivo imaging of amyloid aggregation*, PTDC/QUI-QUI/102049/2008. Leading Institution: IST/CTN, Bobadela, Portugal. IST/CTN Principal Investigator: A. Paulo.
 - *Synthesis, characterization and biological assessment of multi-functional bone-seeking agents*, PTDC/QUI-QUI/115712/2009. Leading Institution: IST/CTN, Bobadela, Portugal. IST/CTN Principal Investigator: I. Santos.
 - *Albumin binding-domain fusions to improve protein pharmacokinetics*, PTDC/SAU-FAR/115846/2009. Leading Institution: Associação para o Desenvolvimento do Ensino e Investigação da Microbiologia (ADEIM), Faculdade de Farmácia, Universidade de Lisboa. Principal Investigator: J. Gonçalves.
 - *Preclinical evaluation of ruthenium potential drugs for cancer therapy*, PTDC/QUI-QUI/118077/2010. Leading Institution: Fundação da Faculdade de Ciências, Universidade de Lisboa. Principal Investigator: H. Garcia.
 - *Molecular and nano tools for cancer theranostics*, EXCL/QEQ-MED/0233/2012. Leading Institution: IST-ID, Bobadela, Portugal. IST/CTN Principal Investigator: I. Santos.
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NAME: Maria Paula Cordeiro Crespo Cabral Campello Aboim de Barros

CATEGORY: Auxiliary Researcher

IST-ID: 5375

ACTIVITIES

N°	Activity Description	R&D (%)
1	Collaboration in the scientific coordination of the task “Telomerase Inhibitors Based on Cold or Radioactive Cu(II) Complexes (^{64}Cu) Bearing Bioactive Peptides” within the project “Molecular and Nano Tools for Cancer Theranostics” (EXCL/QEQ-MED/0233/2012).	10
2	Collaboration in the task “Multifunctional In/ ^{111}In complexes for Cell-Specific Nuclear Targeting: Synthesis and Precinical Evaluation” within the project “Molecular and Nano Tools for Cancer Theranostics” (EXCL/QEQ-MED/0233/2012).	10
3	Nanocarriers as Versatile Platforms for Targeted Delivery of Radionuclides to Tumors.	20
4	Biological Evaluation of Novel Heteronuclear Lanthanide-Ruthenium Complexes.	15
5	Kinetic Study of Formation/Dissociation of Cu(II) and Zn(II) Complexes of Cyclen Macrocyclic Ligand with Pendant Thiol Group.	15
6	Radiolabeled Mannosylated Dextran Derivatives bearing a NIR-fluorophore for Sentinel Lymph Node Imaging. This project was partially supported by the IAEA.	15
7	Supervision of the MSc Thesis of Mariana Nogueira Pinto “Complexos Organometálicos de Tc(I) e Re(I) para Radiometalação de péptidos Biologicamente Activos”	10
8	Management of Laboratory infrastructure – Chemistry Laboratory.	5
Total		100

WORK SUMMARY

N°	Work Summary and Main Achievements
6	<p>Following the work initiated in 2012, the biological properties of the receptor-targeted bimodal probes Dex-Man-Tc-IR775 and Dex-Man-^{68}Ga-IR775 for sentinel lymph node (SLN) were assessed by nuclear and optical imaging techniques.</p> <p>It was found that the $^{99\text{m}}\text{Tc}$-dextran derivative has eight pirazoyl-diamine (Pz) chelating units and thirteen mannose units per mol of dextran, whereas the ^{68}Ga derivative presents five DOTA chelators and fifteen mannose units per mol of dextran. The “cold surrogates” were prepared and fully characterized from the chemical and physical point of view. The pharmacokinetics and SLN mapping of both probes was evaluated in Wistar rats.</p> <p>The probes were prepared with high specific activities. Biodistribution studies have shown that both probes presented significant accumulation in the popliteal node and enhanced extraction. The new bimodal probes exhibit suitable biological properties for SLN mapping by nuclear and optical imaging techniques as well as for NIR image-guided excision.</p> <p>The work performed in this activity involved also the effort of the researchers João Galamba and the graduate student Mauricio Morais. Dr. I. Santos is responsible for the overall coordination of this research project.</p> <p>The work resulted in three poster communications in an International Conference and in the submission of one paper.</p>

7	<p>The main goal of the Msc work of Mariana Pinto is the synthesis, characterization and biological evaluation of ^{99m}Tc labelled compounds aiming the development of specific radiopharmaceuticals able to detect and/ or treat neoplasms. The use of biologically active peptides to direct the radiopharmaceuticals for tumour tissues has been a strategy exploited, since peptides receptors are over expressed on a wide variety of tumors. In this project, it is proposed to study the usefulness of different tridentate bifunctional chelators (BF) in the radiometallation of peptides. These BFs should lead the stabilization of hydrophilic complexes of Re (I)/Tc (I) with adequate pharmacokinetics for the peptide labelling. As proof of concept, this study will focus on bombesin analogue (BBN).</p> <p>The experimental work of this project began in October 2013 and so far it has already been possible to synthesize and characterize two new BFs, as well as</p>
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PUBLICATIONS

- Romana Ševčíková, Přemysl Lubal, Maria Paula Cabral Campello, Isabel Santos, Kinetic study of formation/dissociation of Cu(II) and Zn(II) complexes of cyclen macrocyclic ligand with pendant thiol group, *Polyhedron* 62 (2013) 268–273. doi.org/10.1016/j.poly.2013.06.052

COMMUNICATIONS

Oral presentations

- *Kinetic study of metal complexes with tetraazamacrocyclic ligands with carboxylate/phosphonate pendant arms*, Přemysl Lubal, Petr Hermann, Jan Kotek, M. Paula C. Campello, Isabel Santos, *International Symposium, Lisbon, June 18-22 (2012)*, oral presentation.

Poster presentations

- *Targeted-Receptor Bimodal Probe for Sentinel Lymph Node Detection*, João D. G. Correia, M. Morais, C. Xavier, S. Hernot, T. Lahoutte, V. Caveliers, M. P. Campello, I. Santos, 3rd Global Cancer Genomics Consortium Symposium, Lisbon, September 18-20th, 2013. (Poster)
- *Targeted-Receptor Bimodal Probe for Sentinel Lymph Node Detection*, João D. G. Correia, M. Morais, C. Xavier, S. Hernot, T. Lahoutte, V. Caveliers, M. P. Campello, I. Santos, Cost Action 1004 Annual Meeting, Athens, Greece, September 1-3rd, 2013. (Poster)
- *^{153}Sm -DOTA-phosphine-ruthenium bimetallic complex as new radiotheranostic*, Louis Adriaenssens, Qiang Liu, Fanny Picquet, Franck Denat, Pierre le Gendre, Maria Paula Cabral Campello, Fernanda Marques, Filipa Mendes, Célia Fernandes, Lurdes Gano, Ewen Bodio, International Workshop on Innovative Personalized Radioimmunotherapy, WIPR: latest progress in the field of RadioImmunoTherapy, Nantes, France, July 9-12, 2013. (Poster)
- *Small-core gold nanoparticles stabilized with thiolated DOTA-based ligand*, F Silva, M.P.Campello, L. Gano, A. Paulo, I. Santos, Cost action 1004, Annual Meeting, Athens, Greece, September 1-3rd 2013. (Poster)
- *Kinetic study of formation / dissociation of Cu(II) and Zn(II) complexes of cyclen macrocyclic ligand with pendant thiol group*, Romana Ševčíková, Přemysl Lubal, Maria Paula Cabral Campello, Isabel Santos, Medicinal Redox Inorganic Chemistry, Redox Modulation of Health and Disease: From Inorganic Chemistry to Translational Medicine, 20 - 22 July 2013, Erlangen-Nürnberg, Germany. (Poster)

EDUCATION

- Supervisor, M. Sc. Thesis, *Complexos Organometálicos de Tc(I) e Re(I) para Radiometalação de Péptidos Biologicamente Activos*, Faculdade de Ciências da universidade Técnica de Lisboa, Departamento de Química e Bioquímica, started on 2nd October 2013.

PROJECTS

Running

- *Molecular and Nano Tools for Cancer Theranostics*, FCT- EXCL/QEQ-MED/0233/2012. Total Funding 496.065.00€. Prime Contractor: IST-ID, Coordinator: I. Santos. Partners: Instituto de Medicina Molecular (IMM/FM/UL); Instituto de Tecnologia Química e Biológica (ITQB/UNL); Universidade de Aveiro (UA); Universidade de Coimbra. M. P. C Campello: member of the research team (25%).
- *From drug design to new materials: structural approach in emergent fields*, FCT-RECI/QEQ-QIN/0189/2012 (2013-2016). Total Funding: 498.869.00€. Prime contractor: IST-ID, Coordinator: M Teresa Duarte, Konstantin Luzyanin, M. T. Nunes, Partners: IBB, ICEMS Lisboa/IST/UTL, IST/ITN/UTL. M. P. C Campello: member of the research team (15%).

Submitted

- *Heterobimetallic Complexes for Cancer Theranostic*. FCT, Cooperação Transnacional, Acordos Bilaterais Portugal – França. Coordinator: IST/ITN/UTL. M. P. C Campello; ICMUB (Chimie Moléculaire de l'Université de Bourgogne) Ewen Bodio, (Still waiting the evaluation).

COLLABORATIONS

- Prof. RNDr. Petr Hermann; Charles University in Prague, Department of Inorganic Chemistry, Faculty of Science, Hlavova, Prague, Czech Republic.-Evaluation of the thermodynamic and kinetic properties of lanthanide and copper complexes, in solution.
- Prof. Premysl Lubal; Department of Chemistry, Faculty of Science, Masaryk University, Czech Republic. Evaluation of the thermodynamic and kinetic properties of lanthanide and copper complexes, in solution.
- Dr. Michel Picquet, Institut de Chimie Moléculaire de l'Université de Bourgogne (ICMUB), CNRS Equipe "Architecture, Réactivité, Electrochimie et Catalyse Organométallique" (ARECO), DIJON - Synthesis and biological evaluation of radioactive heteronuclear lanthanide ruthenium complexes.
- Dr. Ewen Bodio, Institut de Chimie Moléculaire de l'Université de Bourgogne (ICMUB), CNRS, Equipe "Architecture, Réactivité, Electrochimie et Catalyse Organométallique" (ARECO), DIJON, France - Synthesis and biological evaluation of radioactive heteronuclear lanthanide ruthenium complexes.
- VicKy Caveliers, Vrije Universiteit Brussel, Department BEFY, Brussels, Belgium. – Biological evaluation of the ⁶⁸Ga- mannosylated dextran derivative.

NAME: Paula Dolores Galhofas Raposinho


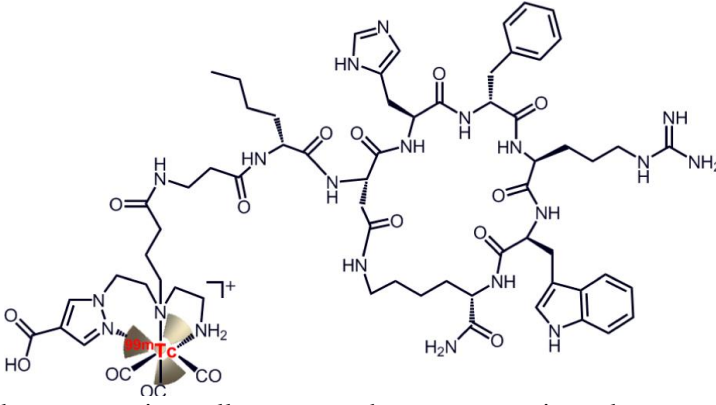
CATEGORY: Auxiliary Researcher

IST-ID: 5382

ACTIVITIES

Nº	Activity Description	R&D (%)
1	Pre-clinical evaluation of lactam-based cyclic α -MSH analogs for lung metastasis imaging through Melanocortin receptor 1 (MC1R)-targeting	20
2	Pre-clinical evaluation of lactam-based cyclic α -MSH analogs for human melanoma imaging through MC1R-targeting	20
3	Development of novel ^{99m} Tc-labeled lactam-based cyclized α -MSH analogs, for melanoma imaging	20
4	Evaluation of MC1R-binding affinity of α -MSH analogs cyclized via alkylamine-bridge	10
5	Biological evaluation of radiolabeled glucose derivatives: Cellular uptake mediated by glucose transporter Glut1	30

WORK SUMMARY

N°	Work Summary and Main Achievements
1	<p>Following our interest to design radioactive probes for melanoma imaging, namely through specific MC1R-targeting, this project, initially funded by Covidean, led to the development of several radiolabeled linear and cyclic α-MSH derivatives. I previously demonstrated the advantages of $^{99m}\text{Tc}(\text{CO})_3$-labeled lactam bridge-cyclized α-MSH analogs on melanoma uptake and the improvement of the pharmacokinetic profile of the cyclic radiopeptide by structural (charge) modifications on the pyrazolyl-diamine chelator. In particular, I demonstrated that the introduction of carboxylate groups shifted the excretion pathway of a radiopeptide from mainly hepatobiliar toward mainly renal and, simultaneously, improved notably tumor-to-non target-organs ratios. Indeed, the relevant biological properties achieved with $^{99m}\text{Tc}(\text{CO})_3$-Pz₃-$\beta$AlaNleCycMSH_{hex} and $^{99m}\text{Tc}(\text{CO})_3$-Pz₄-$\beta$AlaNleCycMSH_{hex} highlighted the potential usefulness of both compounds as melanoma imaging agents (Morais M, Oliveira BL, Correia JDG, Oliveira MC, Jiménez MA, Santos I, Raposinho PD, 2013, Influence of the bifunctional chelator on the pharmacokinetic properties of $^{99m}\text{Tc}(\text{CO})_3$-labeled cyclic α-MSH analog, <i>J. Med. Chem.</i>, 56(5),1961-73).</p>  <p>$^{99m}\text{Tc}(\text{CO})_3$-Pz₃-$\beta$AlaNleCycMSH_{hex} as metastatic melanoma imaging probe</p> <p>High mortality of malignant melanoma is associated with the occurrence of metastatic melanoma due to its aggressiveness and resistance to current chemotherapy and immunotherapy regimens. The purpose of this study was to examine whether the lactam bridge-cyclized radiopeptide $^{99m}\text{Tc}(\text{CO})_3$-Pz₃-$\beta$AlaNleCycMSH_{hex} could be also an effective imaging probe for metastatic melanoma detection.</p>  <p>The B16F10 melanoma murine cells were used as a metastatic melanoma model as they are very aggressive tumor cells, inducing pulmonary metastasis when intravenously injected in C57BL6 mice. Cellular internalization and retention of $^{99m}\text{Tc}(\text{CO})_3$-Pz₃-$\beta$AlaNleCycMSH_{hex} were evaluated in B16F10 melanoma cells. The pharmacokinetics and tumor targeting properties of $^{99m}\text{Tc}(\text{CO})_3$-Pz₃-$\beta$AlaNleCycMSH_{hex} were determined in B16F10 pulmonary metastatic melanoma-bearing C57BL6 mice and compared with normal mice at 1 and 4 h post-injection. $^{99m}\text{Tc}(\text{CO})_3$-Pz₃-$\beta$AlaNleCycMSH_{hex} exhibited significantly higher uptake value in metastatic melanoma-bearing lung than that in normal lung. Pulmonary metastatic melanoma imaging was performed by SPECT.</p>
2	<p>$^{99m}\text{Tc}(\text{CO})_3$-Pz₃-$\beta$AlaNleCycMSH_{hex} as human melanoma imaging probe</p> <p>It was found that MC1 receptor was overexpressed on most murine and human melanoma, making it a promising molecular target for melanoma imaging and therapy. However, for different human and murine melanoma cell lines, the MC1R expression ranges from several hundred to around 10000 receptors per cell.</p> <p>The goal of this study was to evaluate the lactam-based cyclic alpha-MSH analog $^{99m}\text{Tc}(\text{CO})_3$-Pz₃-$\beta$AlaNleCycMSH_{hex}, a successful imaging probe for tumor murine model with very high</p>

MC1R expression, as a potential molecular probe for melanoma imaging in melanoma xenografted mouse models. The human A375 melanoma cells, known to have relatively low number of MC1R and human C32 melanoma cells (MC1R density unknown) were used as human melanoma models. Cellular internalization and retention of $^{99m}\text{Tc}(\text{CO}_3)\text{-Pz}_3\text{-}\beta\text{AlaNleCycMSH}_{\text{hex}}$ were examined in both human melanoma cells but low internalization levels were observed. The pharmacokinetics and tumor targeting properties of $^{99m}\text{Tc}(\text{CO}_3)\text{-Pz}_3\text{-}\beta\text{AlaNleCycMSH}_{\text{hex}}$ were determined in A375 and C32 melanoma-bearing nude mice at 1h post-injection. However, $^{99m}\text{Tc}(\text{CO}_3)\text{-Pz}_3\text{-}\beta\text{AlaNleCycMSH}_{\text{hex}}$ exhibited low tumor uptake in both xenografts which can be explained by a low number of MC1R receptors by cell.

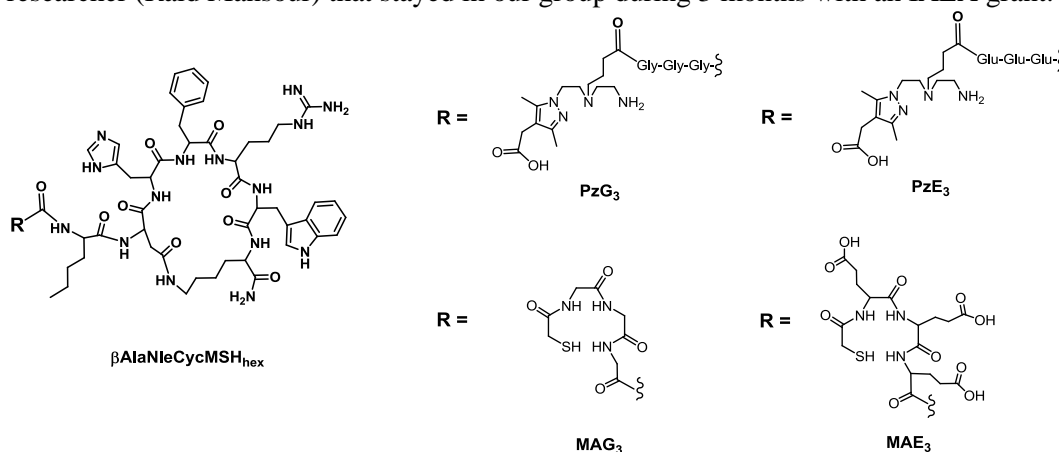
Evaluation of MC1R expression in human and murine melanoma cell lines by Western Blot (WB)

While B16F1 and B16F10 melanoma cell lines are known to have very MC1R capacity (more than 10000 sites/cell), A375 human cell line has low receptor density (about 400sites/cell) and there is no data about the receptor expression in C32 cells.

Thus, the aim of this work was the evaluation of MC1R expression in human melanoma cells used in the uptake studies and in xenografts inoculation as well as in the tumor samples obtained in biodistribution studies by western blot assays. To perform the WB assays I used a rabbit monoclonal primary antibody to MC1R from Abcam (ab125031) that should reacts with human, but not with mouse and rat, synthetic peptide corresponding to residues in human MC1R. Goat polyclonal anti-rabbit HRP (conjugated to Horse Radish Peroxidase) was used as a secondary antibody. The predicted band should have a MW of 35KDa.

Some preliminary assays were performed using different conditions (primary antibody dilution, secondary antibody, incubation time with primary antibody, chemiluminescent substrate).

3 In our continued effort to design radioactive probes for specific melanoma imaging, through MC1R-targeting, with higher tumor uptakes and improved pharmacokinetics, herein our goal was to further improve the overall biological profile of $^{99m}\text{Tc}(\text{CO})_3$ -labeled $\beta\text{AlaNleCycMSH}_{\text{hex}}$ through structural/charge modification on the spacer between the chelator and the peptide $\beta\text{AlaNleCycMSH}_{\text{hex}}$. Linkers comprising three glycines (Gly, G: neutral aminoacids) or three glutamic acid (Glu, E: negatively charge) were used and conjugated to two different chelators. The synthesis and the chemical characterization of the four resulting conjugates as well as its radiolabeling with ^{99m}Tc were performed by a Syrian researcher (Raid Mansour) that stayed in our group during 3 months with an IAEA grant.



I assessed the MC1R-binding affinity of cold conjugates by competitive binding assays in B16F1 melanoma cells and using ^{125}I -NDP as radioligand. However, comparatively to the peptide alone ($\beta\text{AlaNleCycMSH}_{\text{hex}}$) that shows IC_{50} in nanomolar range (2,8 nM), an important loss of affinity was observed for conjugates $\text{MAG}_3\text{-}\beta\text{AlaNleCycMSH}_{\text{hex}}$ and $\text{Pz-G}_3\text{-}\beta\text{AlaNleCycMSH}_{\text{hex}}$ (IC_{50} : 239 and 558 nM), and even more pronounced for the ones containing the three glutamic acids $\text{MAE}_3\text{-}\beta\text{AlaNleCycMSH}_{\text{hex}}$ and $\text{Pz-E}_3\text{-}\beta\text{AlaNleCycMSH}_{\text{hex}}$, which presented extremely high IC_{50} values (in the micromolar range). Accordingly this result, the cellular uptake of ^{99m}Tc -labeled conjugates in MC1R-expressing melanoma B16F1 cells was very low and almost negligible for the ones where the glutamic acids were introduced. These results predicted a very low tumor uptake in B16F1

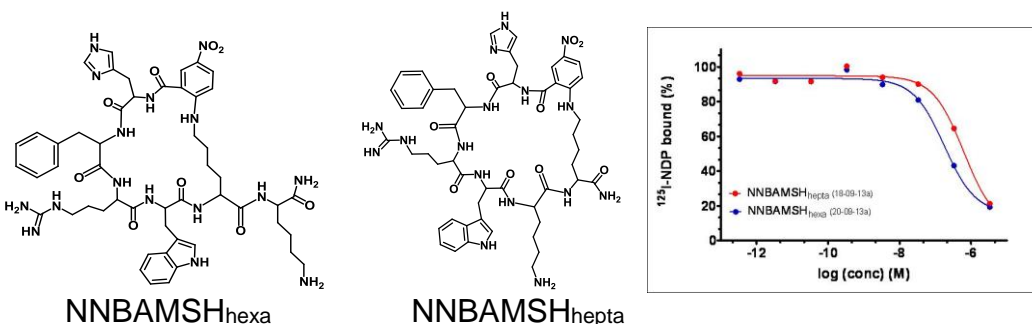
melanoma-bearing mice.

The improvement of pharmacokinetics, without compromising the *in vitro/vivo* MC1R-targeting, previously achieved with the introduction of carboxylate groups on the chelator, was not observed in this study indicating that the three carboxylate groups introduced in the linker and the linker size may interfere significantly with the binding of the peptide to the MC1 receptor.

Further studies with shorter linkers and the introduction of only one glutamic acid were planned for 2014.

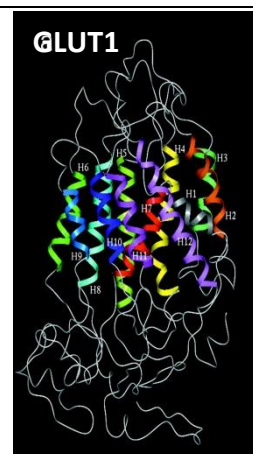
- 4 Among the cyclic α -MSH analogs already described by our group, and besides the promising ones cyclized via a lactam-bridge, an alkylthioaryl- and an alkylamine-bridged peptide derivatives, (c[S-NO₂-C₆H₃-CO-His-DPhe-Arg-Trp-Cys]-Lys(pz)-NH₂ and c[NH-NO₂-C₆H₃-CO-His-DPhe-Arg-Trp-Lys]-Lys(pz)-NH₂), were previously designed, synthesized, ^{99m}Tc-labeled and reported (Morais M, [Raposinho PD](#), Oliveira MC, Santos I, Pantoja-Uceda D, Jiménez MA, Correia JDG, **2012**, MC1R-Targeting Properties of ^{99m}Tc(I)-Labeled Cyclic α -MSH analogs, *Organometallics*, 31 (16), 5929-39).

Based on this work, and trying to understand how the ring's structure and size affects the affinity of the modified peptide to the MC1R, the affinity of the analogs c[NH-NO₂-C₆H₃-CO-His-DPhe-Arg-Trp-Lys]-Lys-NH₂ and c[NH-NO₂-C₆H₃-CO-His-DPhe-Arg-Trp-Lys-Lys]-NH₂ was evaluated by competitive binding assays using ¹²⁵I-NDP as radioligand and B16F1 melanoma cells expressing the MC1R.



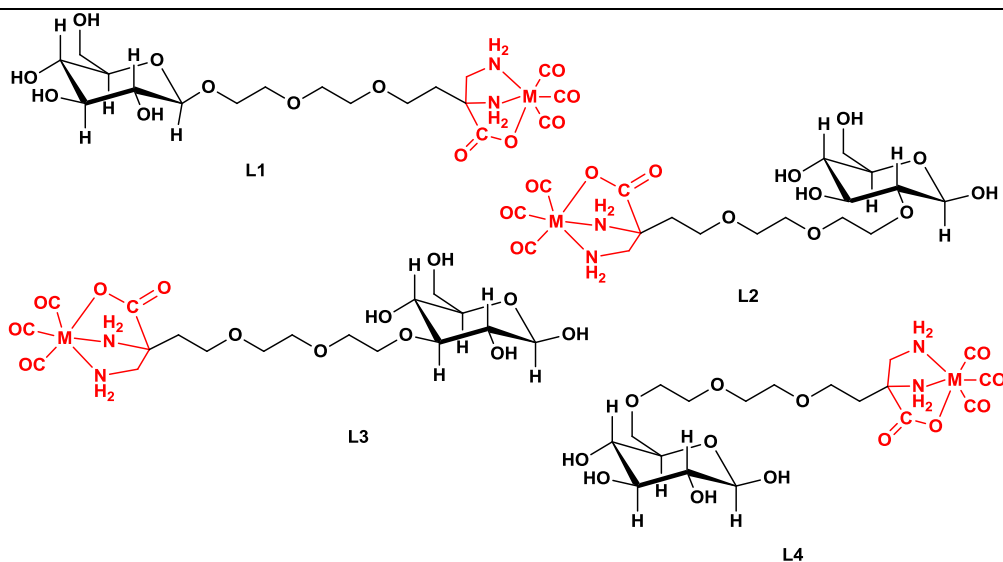
The analog NNBAMSH_{hepta} with an additional Lys residue inside the ring of the peptide presented about 3 times less affinity when compared with the analog NNBAMSH_{hexa} (IC₅₀=495±101 vs 155±17 nM).

- 5 Several research projects have been explored in collaboration with the group of Prof. Roger Alberto of University of Zurich (Switzerland), in which my contribution is always the biological evaluation of their compounds, using *in vitro* or *in vivo* tumoral models. Among them, and due to the clinical relevance of [¹⁸F]-2-fluorodesoxiglucose (FDG) in tumor diagnosis, which is taken up by tumor cells mainly by facile diffusion through the glucose transport protein Glut1, the group of Roger Alberto developed inexpensive and readily available ^{99m}Tc-labeled glucose analogues. Apart from the low costs of Tc-99m, a Re-186 or Re-188 labeled "FDG" analogue would open the opportunity for radiotherapy of various tumors with a simple and unspecific tracer.



a) Cellular uptake of ^{99m}Tc-labeled glucose derivatives

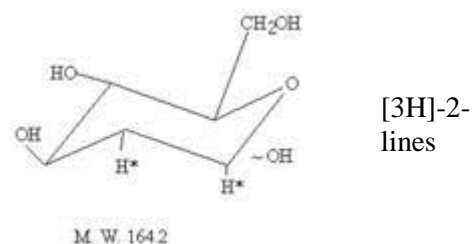
During this collaboration project, we received in our group a PhD student of Professor Roger Alberto Group, Yunjun Shen, in a 2 weeks (11-22 March 2013) Short-Term Scientific Mission (STSM) under the COST Action CM1105: Functional metal complexes that bind to biomolecules. This mission aimed the *in vitro* evaluation of several ^{99m}Tc complexes, bearing glucose as a biomolecule, which have been designed and synthesized at the Zurich University. I biologically characterized the four organometallic complexes of glucose functionalized with the chelator at positions C-1, C-2, C-3, and C-6 of the hexose (L1-L4).



For the labeling, the organometallic precursor $[^{99m}\text{Tc}(\text{H}_2\text{O})_3(\text{CO})_3]^+$ was chosen. First, the four ^{99m}Tc -labeled complexes were tested for transport through the cell membrane via Glut1 and indirectly also for Glut1 interaction/inhibition. For these experiments, several carcinoma cell lines (colon carcinoma HT29, breast cancer MCF-7 and MDA-MB-231, ovarian carcinoma A2780 and vulva carcinoma A431) and different incubation conditions (including different mediums with or without glucose) have been used. Low to moderate cellular uptake was observed. The uptake of complexes was then challenged in the presence of cytochalasin B (0-100 μM), a potent Glut1 inhibitor, to verify specific transport via Glut1 or binding to Glut1. However, no significant uptake inhibition was observed. Glut1 exists in two conformations and offers an extracellular as well as an intracellular binding site for glucose. Since cytochalasin B is binding intracellularly, it still could be speculated that the complexes are interacting at the extracellular binding site of glucose. Therefore, the uptake of radiolabeled compounds was also been evaluated in the presence of increasing concentrations of 2-Deoxy-D-glucose (0-20 mM). However, the fact that a large excess of 2-Deoxy-D-glucose did not reduce internalization of the complexes gives evidence that they do not compete for a common binding site. These complexes appeared to be transported into the cells by an unspecific uptake mechanism (e.g. passive diffusion) rather than by active transport via the sodium-independent Glut1 transporter.

b) Cellular uptake of [3H]-2-deoxyglucose

In order to validate the cell assays and to make a comparison with the data obtained for ^{99m}Tc -complexes it was performed similar assays with the deoxyglucose (from PerkinElmer). Several cell and experimental conditions were tested.



c) Evaluation of GLUT1 and GLUT4 expression by western blot

Finally, by Western Blot (WB) analysis, some preliminary studies were performed in order to prove that the tumor cells used in the uptake assays expressed the transporter GLUT1. I also pretended to have the information of which cell lines express more GLUT1 to eventually use such cells in an in vivo model (tumors-bearing nude mice) to evaluate the ^{99m}Tc -labeled derivatives. The expression of GLUT1 in the developed tumors will be check. However, in that preliminary WB studies, and despite several experimental conditions already tested, I was not able to detect a specific band correspondent to GLUT1.

PUBLICATIONS

- M. Morais, B.L. Oliveira, J.D.G. Correia, M.C. Oliveira, M.A. Jiménez, I. Santos, P.D. Raposinho, Influence of the bifunctional chelator on the pharmacokinetic properties of

^{99m}Tc(CO)₃-labeled cyclic α-MSH analog, *J. Med. Chem.*, 56(5),1961-73 (2013), doi.org/10.1021/jm301647t

COMMUNICATIONS

- *MC1R-Targeted Metallopeptides: NMR Structural Analysis*. M. Morais, P. D. Raposinho, M. C. Oliveira, M. A. Jiménez, D. Pantoja-Uceda, J. D. G. Correia, I. Santos, *Chemistry of Metals in Biological Systems Summer School, COST Action CM 1105, Lisbon, Portugal, May 12-19 (2013)*, Poster.

PROJECTS

- *Molecular and Nano Tools for Cancer Theranostics*, EXCL/QEQ-MED/0233/2012. Leading Institution: Associação do Instituto Superior Técnico para a Investigação e o Desenvolvimento (IST-ID), Lisbon, Portugal. IST/ITN Principal Investigator: I. Santos *Recommended for funding, Team Member (15%)*.

COLLABORATIONS

- Y. Chen, University of Zurich, Switzerland, 11-22 March 2013, STSM on COST Action CM1105: Functional metal complexes that bind to biomolecules, Training on ^{99m}Tc radiolabeling and in vitro characterization of glucose derivatives.
- R. Mansour, Syria, September-December 2013, IAEA grant, Training on Synthesis and ^{99m}Tc-labeling of cyclic α-MSH analogs.