Radiopharmaceutical Sciences

Isabel Rego Santos

The *Radiopharmaceutical Sciences Group* (RSG) developed and implemented expertise and facilities to carry on basic/applied oriented research and technology transfer *on specific halogen and metal-based nuclear tools for SPECT and PET molecular imaging and targeted radiotherapy.* The group is multidisciplinary with knowledge on synthetic chemistry, bioconjugation, radiochemistry, animal and cell studies, and molecular biology. Such background enables the RSG to deal with problems of modern Radiopharmaceutical Sciences, to provide education and training at different levels and to act as key partner in several research projects.

The main achievements during 2011 were:

Financial Support:

In collaboration with other Institutions, five new projects were funded by FCT: three coordinated by RSG /ITN and two by FFUL and FCUL.

Research:

For targeted-specific imaging and/or therapy, we pursued the design of molecular or nanoparticles-based multifunctional radio-platforms to target biomarkers related with cancer and neurodegenerative diseases. The radiolabeled targeting agents included peptides, antibody fragments or small molecules.

1 – For SLND, the first specific nanocompound labeled with fac-$[^{99m}Tc(CO)_3]^+$ was isolated. As a result of the superior i*n vitro* and *in vivo* pre-clinical data, human evaluation is underway, coordinated by IAEA.

2- From the designed peptide-based complexes, an excellent *in vivo* targeting of MC1R, for melanoma detection, was achieved using a cyclic melanocortin analog.

3 - In close cooperation with the University of Porto and Institute Rocasolano/Madrid, Molecular Modelling and NMR studies have been performed. A clear understanding of the effect of the metal–core on the targeting vector was accomplished.

5 - Within the framework of running projects, between RSG and different Health-related Research Centres, we continued our efforts to design novel bone- and amyloid aggregates-targeting compounds.

Education and Training

1-Graduation:

Radiopharmacy teaching at ESTeSL and at Faculty of Pharmacy/University of Lisbon.

2-Post-graduation:

a) Coordination of the Master Course Biomedical Inorganic Chemistry: Diagnostic and Therapeutical Applications (ITN/UL). Coordination and teaching of Radiochemistry and Biomedical Inorganic Chemistry in the same M.Sc. course.

b) Coordination and teaching of Radiopharmaceutical Chemistry in the Master Course Pharmaceutical and Therapeutical Chemistry/FFUL.

c) Teaching of Chemical Systems and Reactivity in the 2nd Cycle of Chemistry, FCUL.

d) Teaching at the Master in Pharmaceutical Sciences, Lusófona University.

e) Teaching at the Master in Human Molecular Biology, Faculty of Sciences/UL

e) Teaching at the Master Course in Nuclear Medicine, ESTeSL.

High School/Universities Visits: 14

Expertise Provided:

Nuclear Medicine Centers, INFARMED, IAEA, Science Foundations (Portuguese, Canadian, USA, South Africa, Argentina, Uruguay and Chile), International Conferences and International Journals.

Publications:

International Journals–17; Proceedings–1; Communications–20; Invited Lectures and Seminars: 5.; Ph.D. thesis: 3; BSc thesis: 2; Reports – 2.

Research Team

Researchers

I. SANTOS, Princ., (Agreg.), Group Leader
A. PAULO, Princ.
J.D.G. CORREIA, Princ.
C.FERNANDES, Aux.
F. MARQUES, Aux.
F. MENDES, Aux. contract
G. MORAIS, Aux. contract
L.GANO, Aux.
M.C. OLIVEIRA, Aux.
M.P.C. CAMPELLO, Aux.
P. RAPOSINHO, Aux.

Students

M. CORREIA, Post-Doc, FCT grant
P.S.ANTUNES, Post-Doc, FCT grant (until July)
S. GAMA, Post-Doc, FCT grant
B. OLIVEIRA, Ph.D. student, FCT grant
C. MOURA, Ph.D. student, FCT grant
C. NETO, Ph.D. student, FCT grant
E. PALMA, Ph.D. student, FCT grant
F. SILVA, Ph.D. student, FCT grant
M. MORAIS, Ph.D. student, FCT grant
S. CUNHA, Ph.D. student, FCT grant
T. ESTEVES, Ph.D. student, FCT grant
F. TOSCANO, M.Sc. student
I. RODRIGUES, M.Sc. student
L. CORTE-REAL, M.Sc. student
P. MENDES, M.Sc. student
F. VULTOS,
S. MONTEIRO,
J. CASTRO, Undergraduate student
R. GOMES, Undergraduate student
S. BARROS, Undergraduate student
S. BRITES, Undergraduate student

Technical Personnel

A.RODRIGUES
E. CORREIA

Multifunctional Re(I)/^{99m}Tc(I) Tricarbonyl Complexes for Cell-Specific Nuclear Targeting

Teresa Esteves, Fernanda Marques, António Paulo, José Rino[1], Prasant Nanda[2], C. Jeffrey Smith[2], Isabel Santos

Objectives

The demand for more selective therapeutic approaches makes Auger emitters, such as ^{99m}Tc, an important resource in radionuclide therapy. In this field, we are studying new multifunctional ^{99m}Tc(I)–based frameworks for receptor-specific nuclear targeting.

Results

The multifunctional structures designed comprise: i) a pyrazolyl - diamine chelator to stabilize the metal fragment ii) a DNA intercalating moiety to ensure a close proximity of the radionuclide to DNA iii) a bombesin (BBN) analogue to provide specificity towards cells expressing the gastrin releasing peptide receptor (GRPr) (Fig. 1).

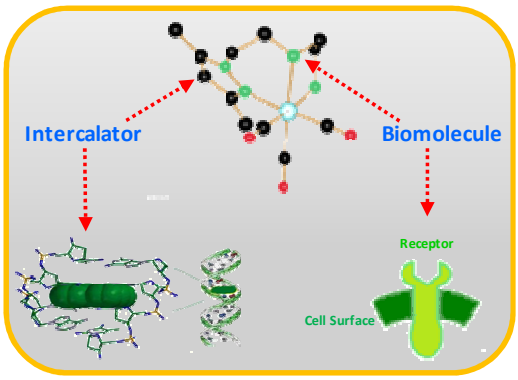


Fig.1. Trifunctional tricabonyl metal complexes

The new trifunctional Re and ^{99m}Tc tricarbonyl complexes were prepared, characterized and studied with GRPr-positive PC3 human prostate tumor cells. Taking advantage of the fluorescence properties of the intercalator, the live-cell uptake of the Re-BBN conjugates was evaluated by time-lapse confocal microscopy imaging, and quantified using the ^{99m}Tc-congeners. From the evaluated complexes the ones containing an intercalator of the acridine orange type and the BBN analogue GGG-BBN[7-14] (Fig. 2) have shown the highest cellular internalization and a remarkably high nuclear uptake.

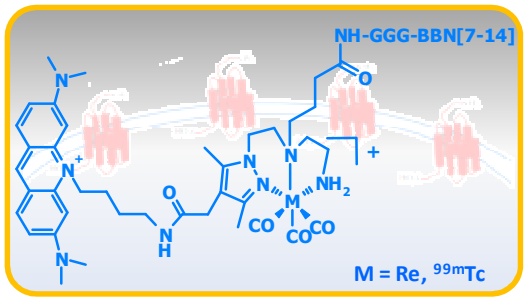


Fig. 2. Chemical structure of a tricarbonyl complex bearing GGG-BBN[7-14] and acridine orange.

Figure 3 shows the significant nuclear uptake of the cell-specific Re complex shown in Figure 2, which has been quantified as 10% using the ^{99m}Tc congener.

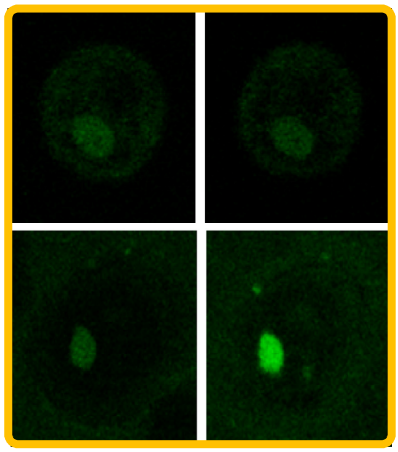


Fig.3. Live-cell uptake of the Re complex, visualized by time-lapse confocal microscopy imaging. Images acquired at 5, 15, 30 and 60 min after incubation with PC3 cells (single cell fluorescence distribution)

Altogether, our data show that the AO intercalator and the metal fragment are co-localized in the nucleus, indicating that they remain connected despite the eventual lysosomal degradation of the metallated peptides.
These complexes are the first examples of ^{99m}Tc bioconjugates that combine specific cell targeting with nuclear internalization, a crucial issue in the potential usefulness of ^{99m}Tc in Auger therapy.

Published work:

T. Esteves, C. Xavier, S. Gama, F. Mendes, P. D. Raposinho, F. Marques, A. Paulo, J. C. Pessoa, J. Rino, G. Viola and I. Santos, Tricarbonyl M(I) (M = Re, ^{99m}Tc) complexes bearing acridine fluorophores: synthesis, characterization, DNA interaction studies and nuclear targeting, *Org. Biomol. Chem.*, 2010, **8**, 4104–4116.

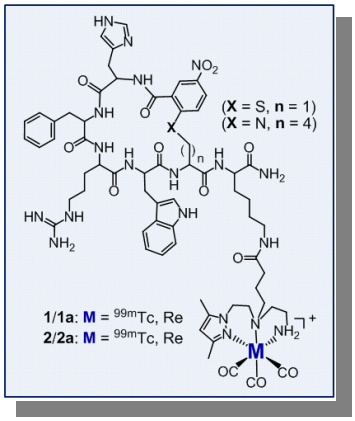
T. Esteves, F. Marques, A. Paulo, J. Rino, P. Nanda, C. J. Smith, I. Santos, Nuclear targeting with cell-specific multifunctional tricarbonyl M(I) (M is Re, 99mTc) omplexes: synthesis, characterization, and cell studies, *J. Biol Inorg Chem*, 2011, **16**, 1141- 1153.

T. Esteves, Complexos de Elementos Emissores de Radiação γ e de Electrões Auger para Diagnóstico e/ou Terapia do Cancro, Tese de Doutoramento, Faculdade de Ciências, Universidade Lisboa, 2011.

[1] IMM, Fac. de Medicina da Univ. de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal.
[2] Univ. of Missouri, School of Medicine, USA

MC1R-Targeting Properties of ^{99m}Tc(I)-Labeled Cyclic α-MSH analogs

M. Morais, P. D. Raposinho, M. C. Oliveira, I. Santos, M. A. Jiménez,[1] D. Pantoja-Uceda,[1] J. D. G. Correia



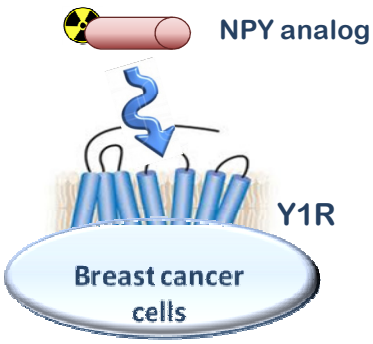
Aimed at the *in vivo* targeting of the Melanocortin 1 Receptor (MC1R) for melanoma imaging, we have synthesized novel cyclic α-MSH analogs containing a thioether or amine bridge within the main ring, as well as their respective metallated (^{99m}Tc/Re) derivatives **1/1a** and **2/2a**. The receptor binding affinity of the peptide conjugates was not significantly affected by metallation with the organometallic core *fac*-$[Re(CO)_3]^+$. The cellular uptake of **1** and **2** are comparable to those previously described for other $^{99m}Tc(CO)_3$-labeled α-MSH analogues.

These encouraging results prompted us to evaluate the *in vivo* MC1R-targeting properties of **1** and **2** in a B16F1 melanoma-bearing mouse model. Additionally, the conformational preferences of the cyclic peptides in solution were also investigated by nuclear magnetic resonance spectroscopy.

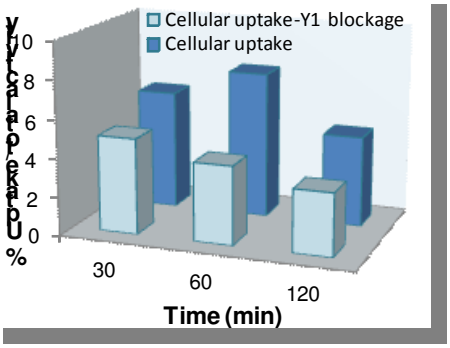
[1] Inst. Química-Física Rocasolano, Consejo Superior de Investigaciones Científicas, Serrano 119, 28006 Madrid, Spain.

Radiolabelled neuropeptide Y analogues for Y1 receptor-targeting in breast cancer

P. Antunes, P. Raposinho, C. Fernandes, I. Rodrigues, I. Santos



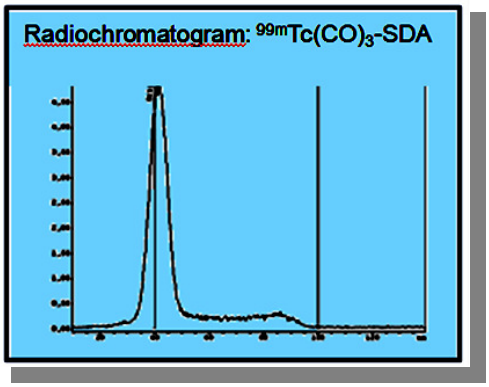
The use of radiolabeled analogs of the Neuropeptide Y (NPY) has recently emerged as a promising approach for *in-vivo* targeting of the highly expressed Y1 receptors in breast cancer. Based on the smallest and selective Y1R agonist [Pro^{30}, Nle^{31}, Bpa^{32}, Leu^{34}]NPY(28-36) (NPY1), several short NPY1 derivatives conjugated to DOTA or to a pyrazolyl-based bifunctional chelator (pzNN) were labeled with ^{67}Ga and ^{99m}Tc, respectively. The corresponding cold Ga/Re congeners were also synthesized and characterized. The *in vivo* stability, as well as the pharmacokinetic profile of these radiopeptides was determined in healthy mice. Cellular uptake studies, using MCF-7 human breast cancer cells expressing Y1R, were also performed. Among ^{99m}Tc-labeled complexes, the most promising one, ^{99m}Tc-pzNN-GluNPY1, presented good cellular uptake (7.6 ± 0.2 % at 1h p.i.) that was inhibited (46%) by Y1R-saturation with the endogenous NPY, indicating a receptor-mediated cellular uptake.



Albumin binding-domain fusions to improve protein pharmacokinetics

J. D. G. Correia, M. Morais, L. Gano, I. Santos, C. S. C. Cantante [1] J. M. B. Gonçalves[1]

The main goal of this research project is to assess whether the fusion of an albumin-binding domain of protein H of Streptococcus pyogenes to a Small Domain Antibody (SDA) anti-TNF will increase serum half life of the resulting fusion protein. Thereby, improving its pharmacokinetic properties and, consequently, enhancing the therapeutic potential. We have labelled the SDA alone and the fusion protein with *fac*-$[^{99m}Tc(CO)_3]^+$ and, after purification by gel-filtration chromatography, we have evaluated the biodistribution profile of $^{99m}Tc(CO)_3$-SDA in mice. Preliminary results indicated that the radiolabeled SDA alone cleared rapidly from blood without accumulation in any particular organ except the kidneys. Evaluation of the biological profile of the radiolabeled fusion protein is underway.

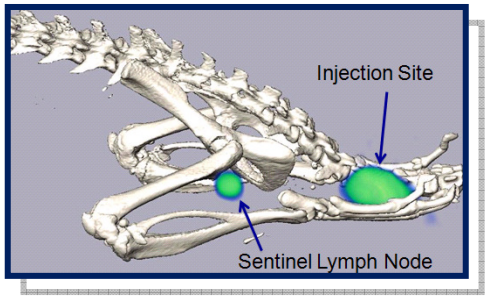


[1] Unidade dos Retrovírus e Infecções Associadas, Fac. de Farmácia da Univ. de Lisboa, Portugal.

$^{99m}Tc(CO)_3$-Mannosylated Dextran Derivatives for Sentinel Lymph Node Detection

M. Morais, Y. Arano,[1] J. D. G. Correia, M. Martins,[2] S. Pereira,[2] I. Santos

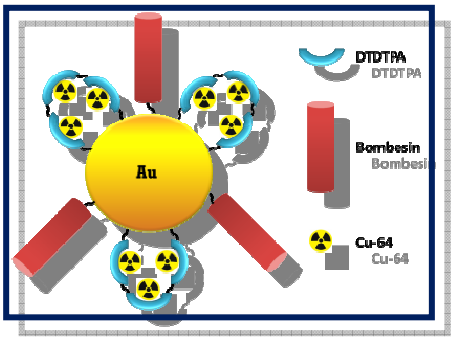
Sentinel lymph nodes (SLN) are the first lymph nodes to receive lymphatic flow as well as metastatic cells from the primary tumor sites. In cases of breast cancer or melanoma, sentinel lymph node detection (SLND) is followed by excision and biopsy of the SLN to detect the presence of metastasis. Accurate SLND is a key issue for tumor staging, evaluation of the extension of surgery, and establishment of the most adequate therapy. Recent studies have shown that receptor-binding mannosylated nanocarriers provide selectivity for mannose receptors on lymph node macrophages. Therefore, we have synthesized and fully characterized the first class of $^{99m}Tc(CO)_3$-mannosylated dextran derivatives with superior biological features for SLN detection. The SPECT/CT studies in mice confirmed that those radiolabeled polymeric nanoparticles are retained in the first lymph node allowing its clear visualization.



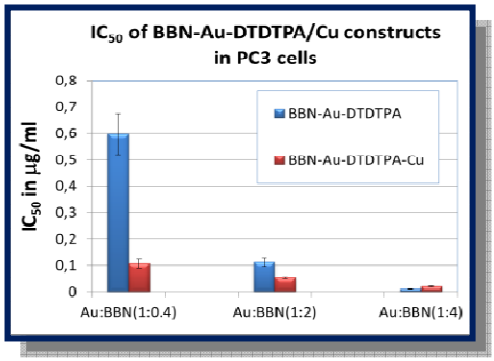
[1] Graduate School of Pharmaceutical Sciences, Chiba Univ., Chiba, Japan.
[2] CICECO, Univ. de Aveiro, Portugal

Multifunctional gold nanoparticles (AuNPs) for theranostic applications

F.Silva, A. Zambre[1], A. Paulo, R. Kannan[1], I. Santos



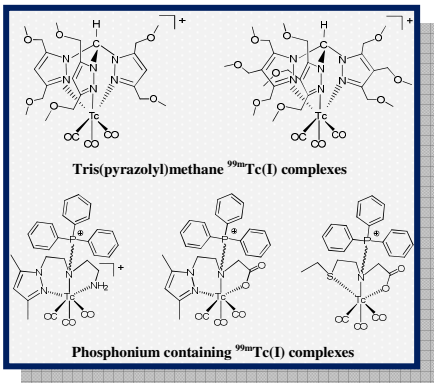
Nanoscience is poised to make significant contributions to both molecular imaging and molecular medicine. Among different nanoparticles, AuNPs play pivotal roles in the design and development of nanoscale tumor specific imaging agents. Searching for multifunctional AuNPs suitable for PET imaging, we have synthesized AuNPs coated with a Di-Thiolated-DTPA (DTDTPA) chelator and a Bombesin (BBN) peptide. BBN-Au-DTDTPA constructs bearing different amounts of BBN have been prepared. All the conjugates showed excellent stability under physiological conditions, displaying also Gastrin Releasing Peptide receptor (GRPr) specificity. Radiolabelling of BBN-Au-DTDTPA with ^{64}Cu, as well as biodistribution studies in PC-3 xenographs bearing mice are currently in progress.



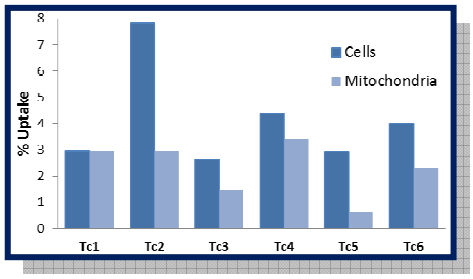
[1] Dep. of Radiology, Univ. of Missouri, USA.

Cationic organometallic complexes as radioactive probes for tumoral detection

F. Mendes, L. Gano, C. Moura, C. Fernandes, A. Paulo, I. Santos

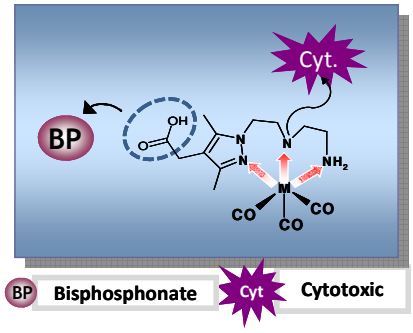


^{99m}Tc-DMEOP and ^{99m}Tc-TMEOP are promising radiotracers for myocardial perfusion imaging. We have shown that these ether-containing tris(pyrazolyl)methane $^{99m}Tc(I)$ complexes are able to accumulate in a variety of human cancer cell lines, but there is a significant reduction of their tumor uptake due to multidrug resistance (MDR). To overcome this drawback, we have synthesized and evaluated ^{99m}Tc-tricarbonyl complexes (**Tc1**-**Tc6**) containing triphenylphosphonium derivatives and stabilized by different bifunctional chelators. In general, these phosphonium-containing ^{99m}Tc complexes showed moderate cellular and mitochondrial uptake, which are dependent on the mitochondrial membrane potential. These features indicate that these complexes are promising for the design of radioactive probes to target the mitochondria of neoplastic tissues.

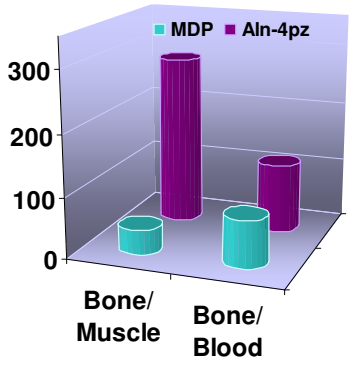


Novel ^{99m}Tc(I)-labeled multi-functional bone-seeking molecules for bone imaging and targeted therapy

C. Fernandes, E. Palma, S. Monteiro, P. Mendes, J. D. G. Correia, L. Gano, L. Costa[1], S. Casimiro[1], I. Santos



Active targeting of therapeutic agents to bone metastasis may improve drug bioavailability at the desired site, reducing their toxicity. This fact encouraged the design of new complexes bearing a bisphosphonate (BP) to deliver radiation and a cytotoxic agent for the treatment of bone metastasis. Toward this goal we started the synthesis and evaluation of Re and ^{99m}Tc tricarbonyl complexes bearing bisphosphonates in different positions of the bifunctional chelator. So far, the best biological data were obtained with the ^{99m}Tc tricarbonyl complex bearing a BP in the 4-position of the pyrazolyl ring. This complex presents a fast blood clearance and high bone uptake. Notably, the target to non target ratios are considerably higher than the ones obtained for ^{99m}Tc-MDP, the gold standard for bone imaging in nuclear medicine.

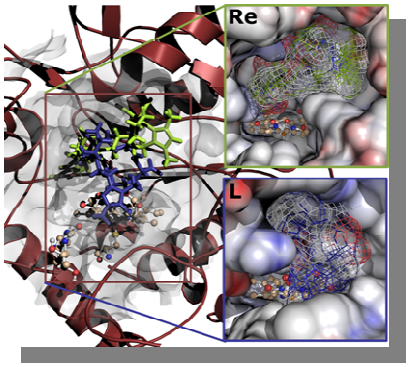


[1]Unidade de investigação Aplicada em Oncologia Clínica, IMM, Fac. de Medicina da Univ. de Lisboa, Lisboa, Portugal.

Targeting of Nitric Oxide Synthase with M(I)-complexes (M = ^{99m}Tc, Re): A structure-activity study

B. L. Oliveira, F. Mendes, P. D. Raposinho, I. Santos, J. D. G. Correia, M. Correia,[1] T. Santos-Silva,[1] M. J. Romão,[1] A. Ferreira,[2] C. Cordeiro,[2] A. P. Freire,[2] I. S. Moreira,[3] P. A. Fernandes,[3] M. J. Ramos[3]

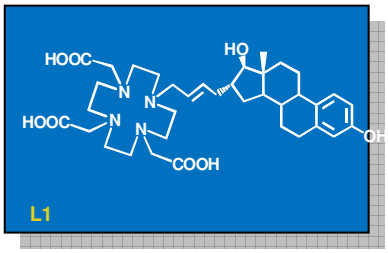
Aiming to probe NOS *in vivo* we have introduced Re/^{99m}Tc(CO)$_3$-complexes containing L-Arg derivatives with high *in vitro* and *in vivo* affinity for the enzyme. Towards a structural understanding of the affinity of the complexes to the enzyme we combined experimental (X-ray, protein:ligand) and computational approaches (Docking and Molecular Dynamics simulations) to achieve that goal. We have cloned, expressed, and purified the iNOS oxygenase domain, and assayed its enzymatic activity. Currently, co-crystallization, pre-incubation and soaking techniques are being explored to obtain single, well diffracting crystals of the desired Re(I)-complexes:iNOS constructs. The MD simulations showed that metal center plays a key role in the organization/orientation of the complexes inside the active pocket of iNOS. The increased complementarity of shape and charge of the rhenium complexes justify their better affinity when compared with the corresponding free ligands.



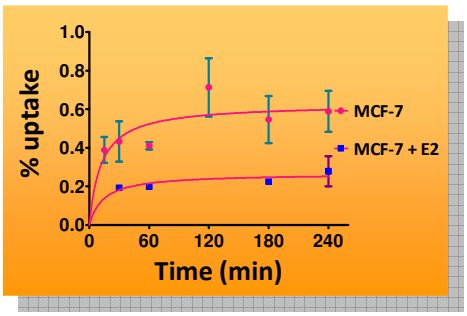
[1] REQUIMTE/CQFB, Dep. de Química, Fac. de Ciências e Tecnologia, Portugal
[2] Centro de Química e Bioquímica, Dep. de Química e Bioquímica, Fac. de Ciências da Univ. de Lisboa, Portugal.
[3] REQUIMTE, Dep. de Química, Fac. de Ciências da Univ. do Porto, Portugal.

Radiolabeling of biomolecules with DOTA like chelators for tumor targeting

S. Cunha, F. Vultos, C. Fernandes, C. Oliveira, M. P.C. Campello, L. Gano, I. Santos

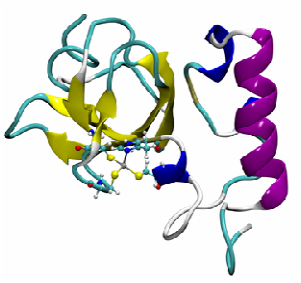


Seeking for new probes for *in vivo* targeting of tumours we have pursued our research efforts on the synthesis of macrocyclic complexes of trivalent radiometals, using different strategies to couple selected biomolecules (e.g. small peptides and estradiol derivatives). In the case of estradiol derivatives, two ^{67}Ga/^{111}In-complexes with a 16α-DOTA-estradiol chelator (**L1**) have been evaluated. Both complexes have a neutral charge and exhibit high stability under physiological concentrations of apotransferrine and in human blood serum. Preliminary biological studies indicate high *in vivo* stability, rapid clearance from main organs and fast overall excretion. Cellular uptake studies in MCF-7 cells suggest a moderate uptake via an estrogen receptor-mediated process.



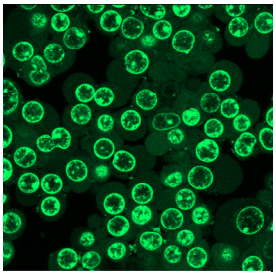
Metal-based anti-cancer agents – new mechanisms of action

S. Gama, F. Mendes, F. Marques, A Casini,[1] J. Rino,[2] J. Coimbra,[3] A. Paulo, T.S. Morais,[4] A.I. Tomáz,[4] M.H. Garcia,[4] I. Santos



Zinc Finger protein- target of Au complexes

The search for anti–cancer metal-based drugs constitute an interesting and emerging research field. In this area, Pt-, Cu-, Ru- and Au-based complexes are being investigated as therapeutic agents. In order to get a better insight on the mechanism of action of several promising complexes, we have studied their uptake on cancer cell lines, and DNA or target proteins interactions by confocal microscopy, ICP-MS, gel electrophoresis and functional assays. Our studies have shown that a set of Pt and Ru-based complexes are potent cytotoxic agents against a range of human tumour cell lines. Moreover, we have demonstrated that interaction with DNA and/or inhibition of novel protein targets are involved in their mechanism of action.

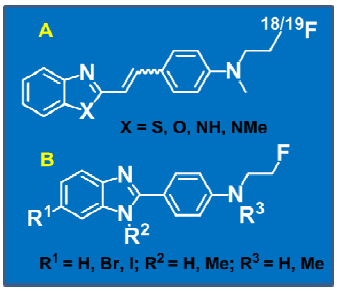


Live imaging of cancer cells after incubation with a Pt(II) complex

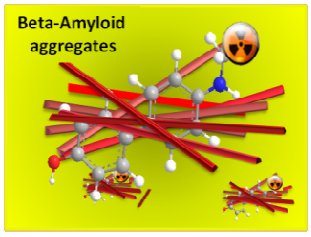
[1]Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne, Suiça; [2]IMM, Fac. de Medicina da Univ. de Lisboa, Portugal; [3]Lab. Central de Análises, Univ. de Aveiro, Portugal; [4] Dep. de Química e Bioquímica, Fac. de Ciências, Univ. de Lisboa, Portugal

Radiofluorinated benzazole derivatives for *in vivo* imaging of amyloid aggregation

G. Morais, A. Paulo, E. Palma, C. Oliveira, I. Santos, T. Kniess,[1] R. Bergman,[1] A. Abrunhosa,[2] H. Miranda,[3] T. Outeiro[3]C. Oliveira,[4] C. Pereira[4]



In the course of our efforts to identify small, planar and highly conjugated compounds to interact with the β-sheet structure of β-amyloid aggregates, we have introduced new fluorinated/radiofluorinated styryl benzazole (**A**) and aryl benzimidazole (**B**) derivatives. These two families of compounds started to be evaluated as radioprobes (^{18}F) for PET imaging of protein aggregates, implied in neurodegenerative disorders such as Alzheimer's disease (AD). So far, such evaluation involved the measurement of the binding affinity towards different types of amyloid aggregates, as well as biodistribution studies in healthy animal models. *In vivo* and *ex vivo* studies in animal models of AD are underway for the more promising ^{18}F-labeled compounds.



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Iodination, radioiodination and biological evaluation of a Kyotorphin derivative

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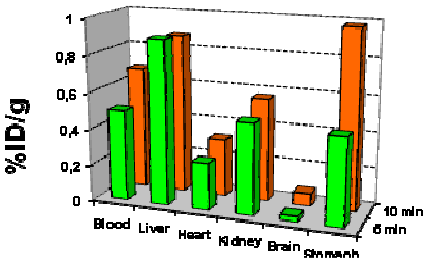
The neuropeptide Kyotorphin (KTP) is potently analgesic when delivered directly to the central nervous system. However, its inability to cross the blood–brain barrier (BBB) precludes its possible clinical use. A new KTP-derivative (KTP-amide or KTP-NH_2) with enhanced lipophilicity and good analgesic ability after systemic delivery was mono-radioiodinated with I-125 (MIK-^{125}I). MIK-^{125}I was obtained in high radiochemical purity after RP-HPLC purification. *In vivo* stability data suggested fast de-iodination in rat blood, kidney and liver homogenate. *In vitro* radiochemical studies also indicate fast degradation in human serum. Biodistribution studies in healthy rats indicate predominant hepatobiliary excretion. The relatively high level of radioactivity in stomach probably reflects enzymatic degradation. The low brain uptake suggests that only a small fraction of MIK-^{125}I was able to cross the BBB.



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