

# Practical Sessions/Introduction

**MEDICIS-Promed Summer School**  
**“Development and Pre-clinical Evaluation of**  
**Radiopharmaceuticals”**

**ANTÓNIO PAULO**

**([apaulo@ctn.tecnico.ulisboa.pt](mailto:apaulo@ctn.tecnico.ulisboa.pt))**

**Grupo de Ciências Radiofarmacêuticas**

**Centro de Ciências e Tecnologias Nucleares, IST-UL**

**Bobadela, 07/06/2018**

---

**Session 1:** Synthesis, purification and characterization of a DOTA-conjugated peptide/João Correia

**Session 2:** Radiolabelling and radioanalytical control of a DOTA-conjugated peptide /Célia Fernandes

**Session 3:** Biodistribution studies and cell-based assays/ Lurdes Gano and Paula Raposinho

---

09:45:-11:15*	Group1/ Session 1; Group 2/Session 2; Group 3/Session3
11:30-13:00	Group1/Session 2; Group 2/Session 3; Group 3/Session 1
13:00-14:30	Lunch
14:30-16:00	Group1/Session 3; Group 2/Session 1; Group 3/Session 2

---

**Group 1**

Annie Ringvall Moberg  
Nhat-Tan Vuong  
Johanna Pitters  
Marina Nazarova  
Vadim Gadelshin  
Roberto Formento Cavaier  
SANJIB CHOWDHURY

**Group 2**

Maddalena Maietta  
Simon Thomas Stegemann  
Alexandra Litvinenko  
Francesco Cicone  
IOANNA PRIONISTI  
Grigory Karateev  
Daniel Thibaut

**Group 3**

Andrew Burgoyne  
Kristof Dockx  
Telma Marques  
Mattia Mazza  
Filip Puicea  
Alina Raicu

# **Session 1: *Synthesis, purification and characterization of a DOTA-conjugated peptide***

(João Correia/Alice D'Onofrio)

- Brief introduction to solid phase peptide synthesis
- Manual *versus* automated peptide synthesis
- Automated peptide synthesis: hands-on simulation of a peptide synthesis run
- Cleavage and isolation of crude peptide
- Purification by HPLC, analytical control and characterization by MS
- Conjugation reactions on solid support or in solution

## ***Session 2: Radiolabelling and radioanalytical control of a DOTA-conjugated peptide***

(Célia Fernandes)

- Radiolabelling of a DOTA-conjugated peptide (ER3) with  $^{111}\text{In}$ .
- Radioanalytical control of the radiolabelled  $^{111}\text{In}$ -ER3 by HPLC and ITLC-SG and determination of the radiochemical yield (RCY) and purity.
- If necessary, purification of  $^{111}\text{In}$ -ER3 by solid phase extraction (using Sep-Pak C18 cartridges) and determination of RCP after purification

## **Session 3: *Biodistribution studies and cell-based assays***

(Lurdes Gano and Paula Raposinho)

### **Animal studies / Biodistribution of $^{111}\text{In}$ -ER3 in mice/Lurdes Gano:**

- Evaluation of tissue distribution in CD1 female mice at different time points
- In vivo Stability Analysis
- Tumor Targeting in Balb/c nude mice MCF-7 Xenografts (Tumor induction/ Tumor uptake/ Target:non-target ratios and blockade studies)

# Session 3: *Biodistribution studies and cell-based assays*

(Lurdes Gano and Paula Raposinho)

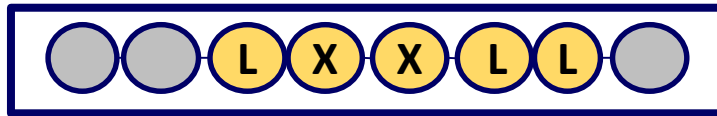
## Cell-based assays/Paula Raposinho

- Cell culture room: Equipment and organization of a cell culture laboratory.
- Cell culture mediums: Properties and special requirements of media.
- Types of cell cultures and typical adherence cell morphology.
- Applications, advantages and limitations of cell-based assays.
- MCF-7 cells culture: Morphological analysis of adherent MCF-7 living-cells in culture cell flasks, under an inverted phase microscope.
- Subculturing and cryopreservation of cells culture.
- Counting MCF-7 cells and preparation of a 24-well plate with cells for internalization assay.
- Internalization of  $^{111}\text{In-ER3}$  in MCF-7 cells:
  - Preparation of radiopeptide solution
  - Incubation of  $^{111}\text{In-ER3}$  with cells
  - Removing membrane associated fraction and recovering internalized fraction
  - Analysis of counts and results

# ER ligand

## Peptides targeting the ER

- Coactivators present NR boxes with LXXLL sequences (L- Leucine; X – any aminoacid) that bind to the LBD of ER
- Peptidomimetics with high affinity and selectivity towards ER  $\alpha$



ER3 = Lys-Lys-Ile-Leu-His-Arg-Leu-Leu-Gln

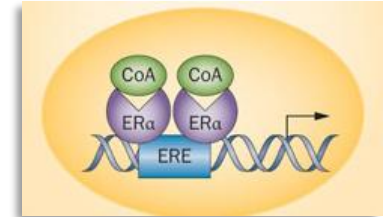
IC50 ( $\mu\text{M}$ )

0.157  $\pm$  0.008

### Synthesis

Automated Fmoc-based solid-phase synthesis

### Nuclear Compartment



Nat. Rev. Endocrinol (2013) doi:10.1038/nrendo.2013.179

CoA: Coactivators

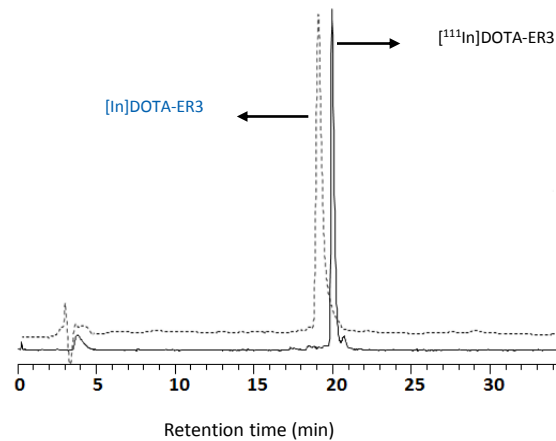
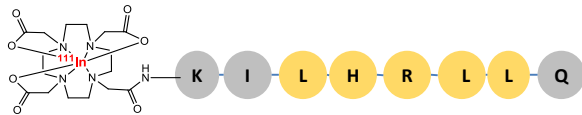
ERE: Estrogen Response Element



Automated Microwave Peptide Synthesizer

Rink  
Amide  
Resin

# $^{111}\text{In}$ -ER3 peptide



➤ High radiochemical yield and purity

➤ High *in vitro* stability (>95%) in solution, blood serum and apotransferrin excess