NanoSpain Conf 2017

March 07-10, 2017 – San Sebastian (Spain)

IK4 CIDETEC

Research Alliance

Poly(methacrylic acid)-based single-chain polymer nanoparticles for targeting and imaging pancreatic tumors *in vivo*

Marco Marradi

Introduction

Pancreatic cancer

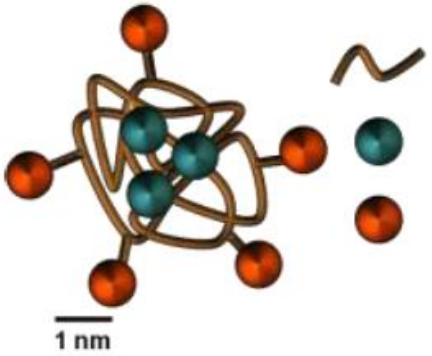
→ 5th cause of death from cancer in Europe in 2012

→ estimated 78,000 deaths (6.2% of total)

→ low 5-year survival rate (around 5%)

→ Late detection and 85% unresectable

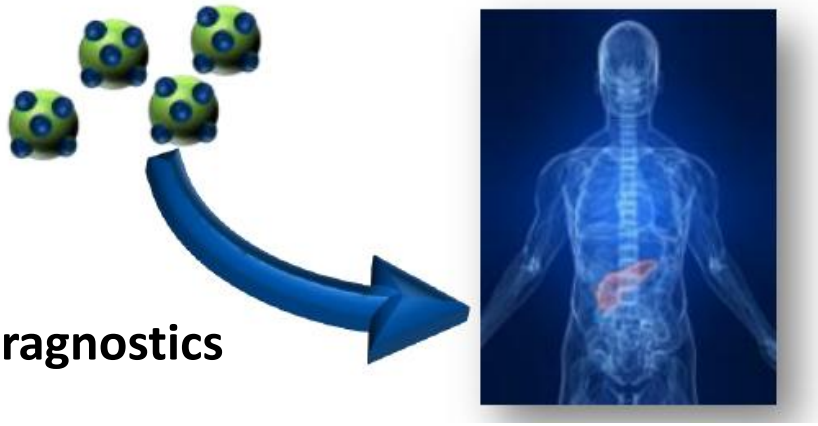
→ Limitation of current imaging systems for early detection, accurate staging and post-therapy monitoring



Polymer chain

Radiotracer

Targeting molecule

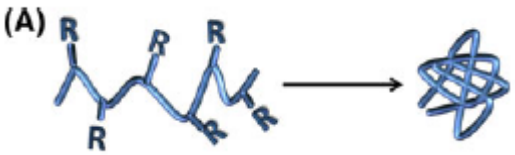


Nanotechnology-based approaches for new theragnostics

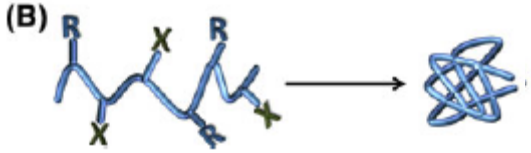
FUNCTIONAL POLYMER NANOPARTICLES

IK4 CIDETEC
Research Alliance

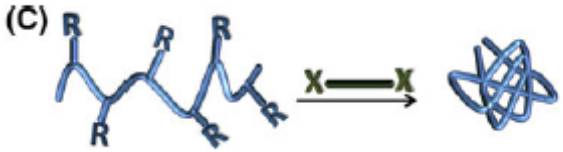
SCPNs: Single-chain polymer NPs (collapse of a unique polymer chain) as novel nanocarriers



Intrachain homocoupling



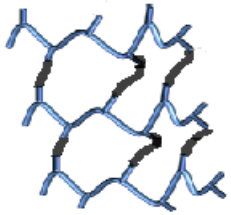
Intrachain heterocoupling



Crosslinker-induced intrachain collapse

Drawbacks:

- **Ultra-dilute** reaction conditions (no viable for **large scale synthesis**) to avoid inter-molecular cross-linking



Inter-crosslinking

- **High temperatures**

- Use of **organic solvents**

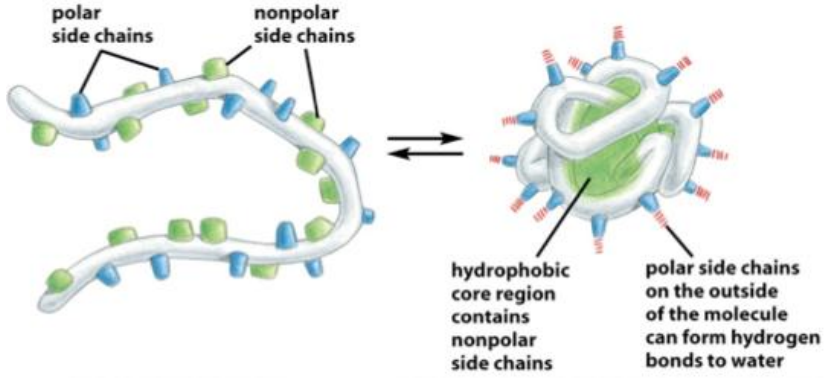
- Use of **metal catalysts**

Advantages:

- Vast choice of **precursors** (biocompatible, polyfunctionalizable, …)

- Control in size (from 200nm down to 5nm)

- Mimicking the protein folding



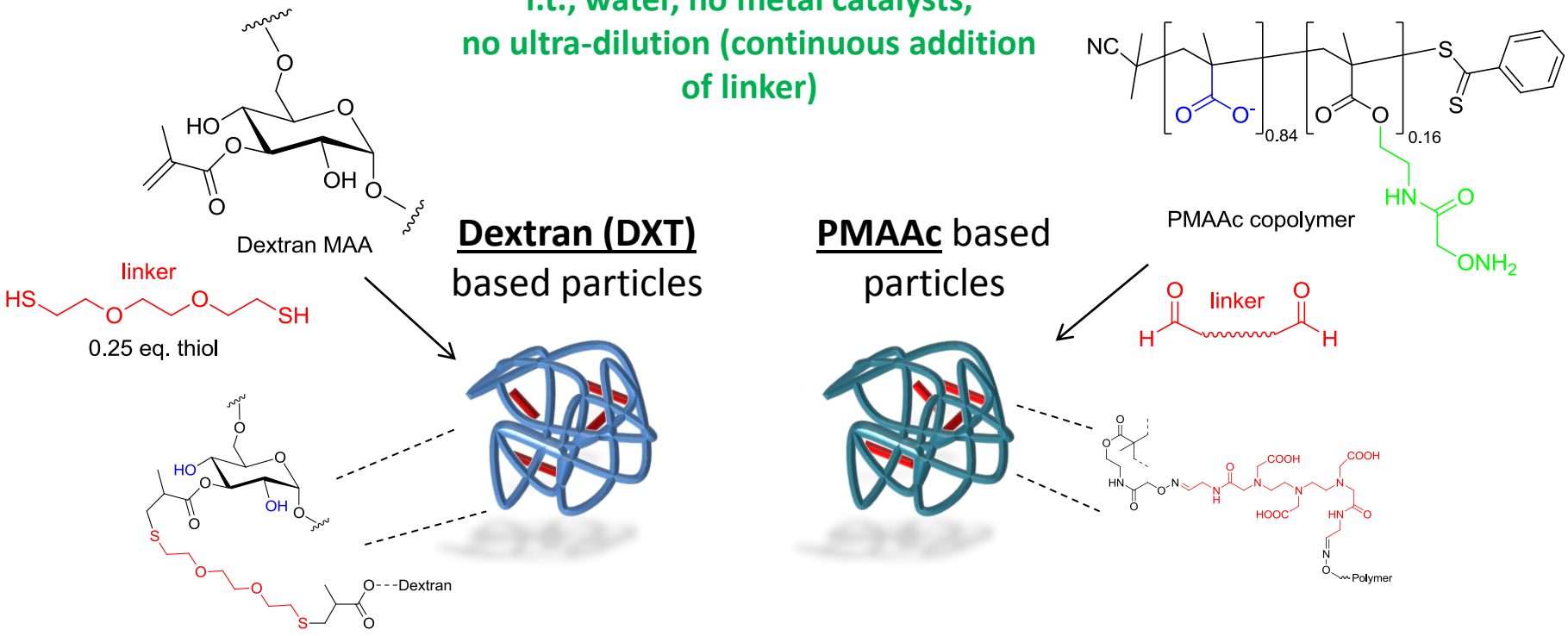
Reviews: *Cell. Mol. Life Sci.* 2012, *69*, 337; *Polym. Chem*. 2015, *6*, 181; *Chem. Soc. Rev*. 2015, *44*, 6122

SCPNs: Patented technology

A process for preparing water-dispersible SCPNs: **versatility**

Wide range of polymer particles have been obtained based on natural and synthetic polymers.

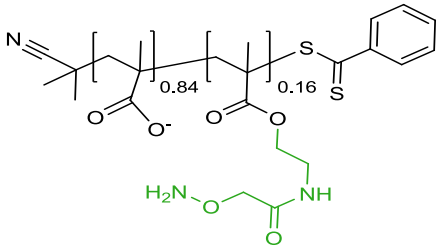
r.t., water, no metal catalysts, no ultra-dilution (continuous addition of linker)



Marradi et al. *J. Mater. Chem. B*, 2017, 5, 1143

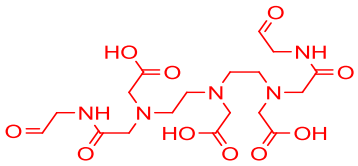
Marradi et al. *Biomacromolecules*, 2016, 17, 3213

Synthesis of PMAAc-SCPNs

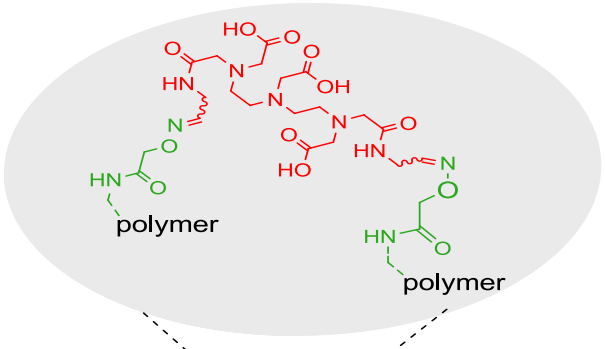


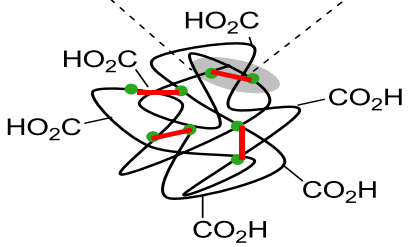
PMAAc copolymer

pH 6, r.t.
H_2O

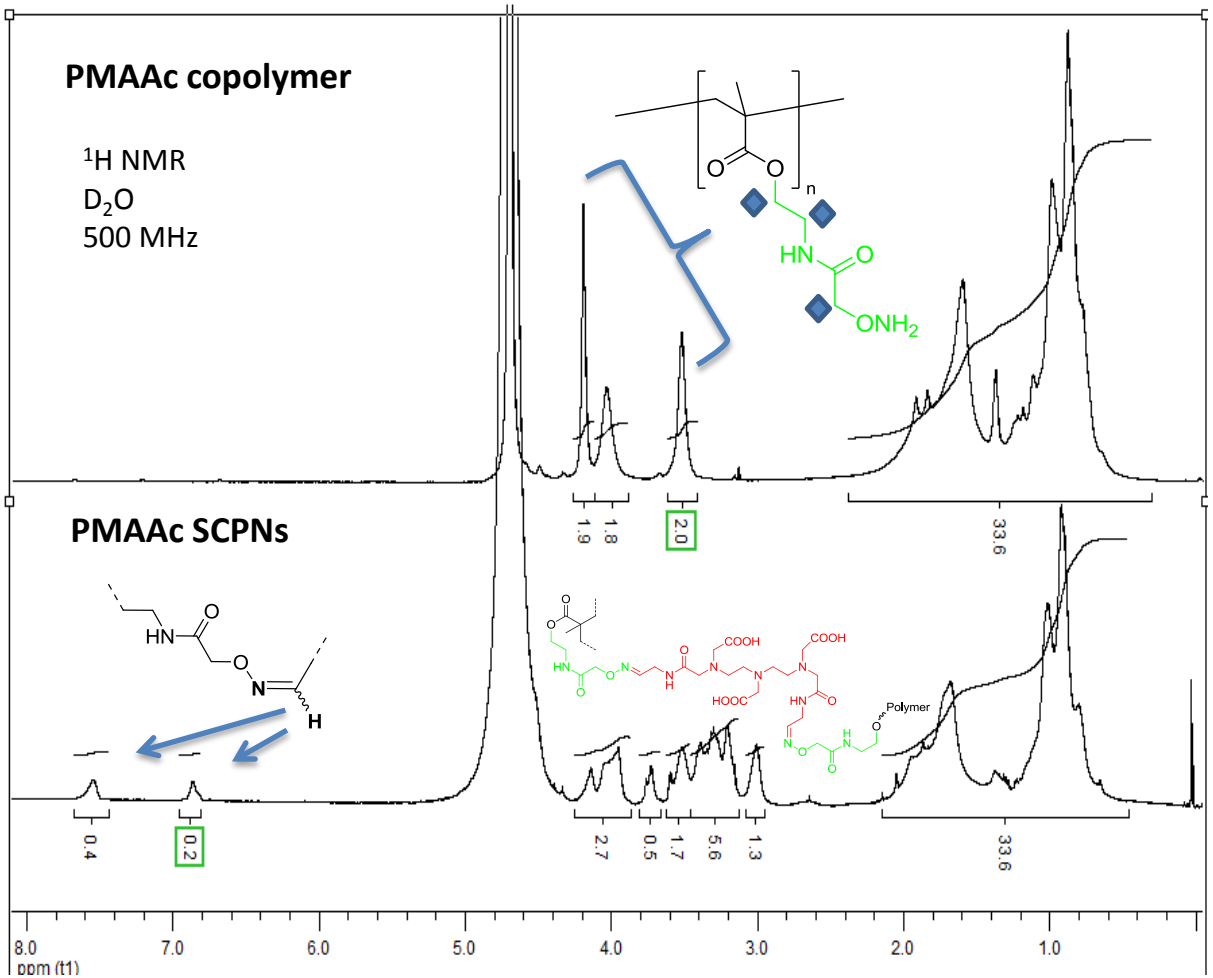


dialdehyde cross-linker



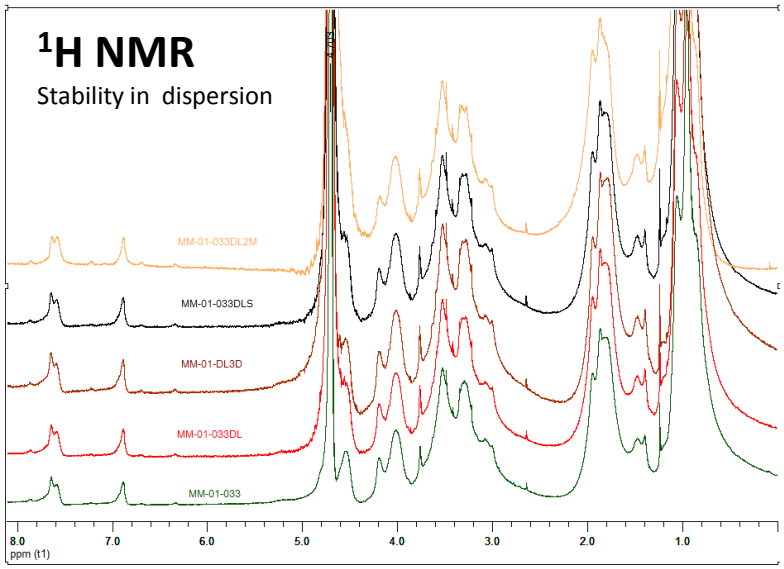


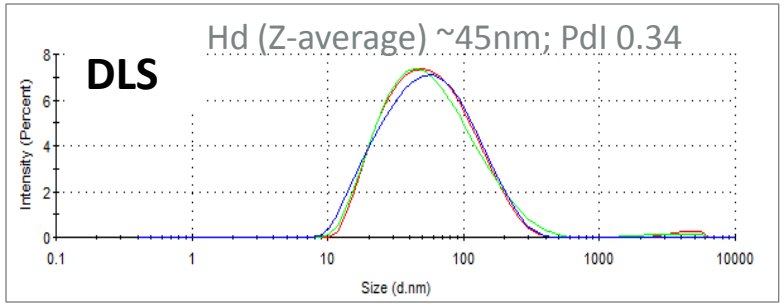
PMAAc SCPN



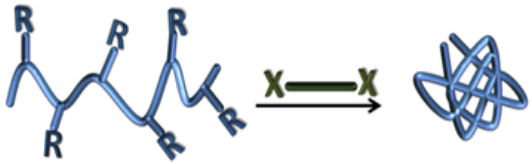
The *O*-alkyloximes are stable to mild reducing agents
2.2 < pH < 3.7 PRECIPITATION

Characterization of PMAAc-SCPNs

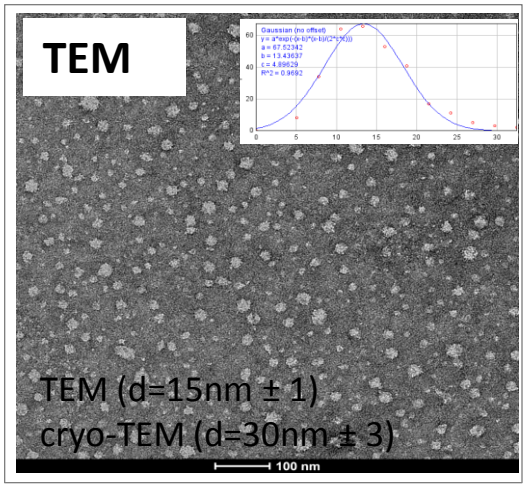


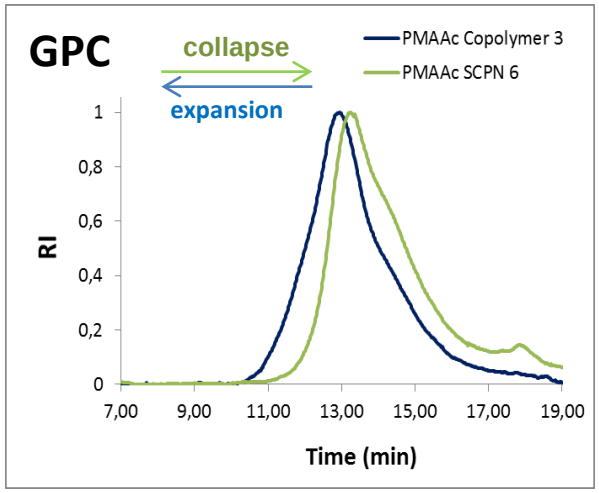


Z-pot (NaCl 1mM, pH 7): -40 mV



53KDa 75KDa





Sample (2 mg/mL, H_2O) filtered (0.2 µm)	Diffusion Coeff. (D)
Precursor-polymer	408 ± 4
Single chain-nanoparticle	474 ± 5

Indication of collapse?

Viscosizer - Proof of collapse

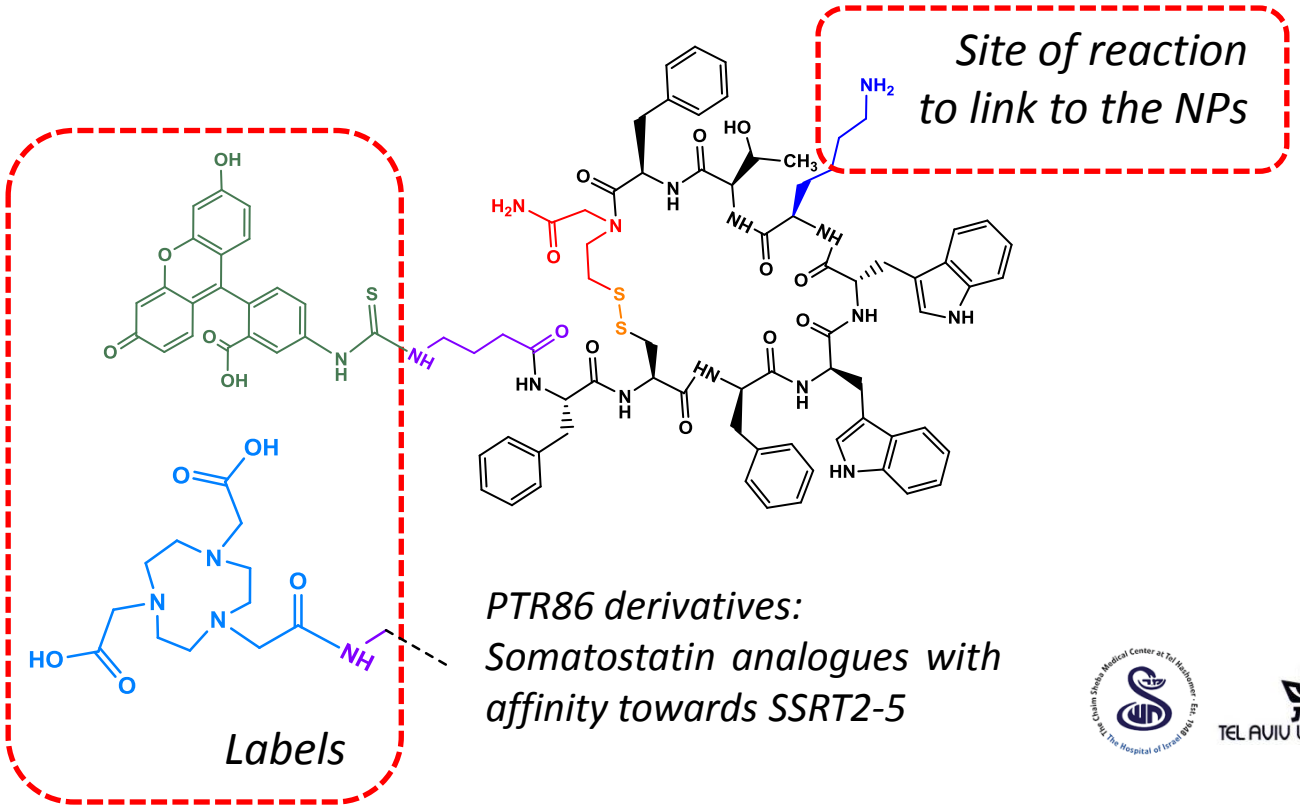
Targeting peptide PTR86

Somatostatin analogue (peptide PTR86)

Somatostatin receptors are over-expressed in a variety of malignant tumors.

FITC-PTR86

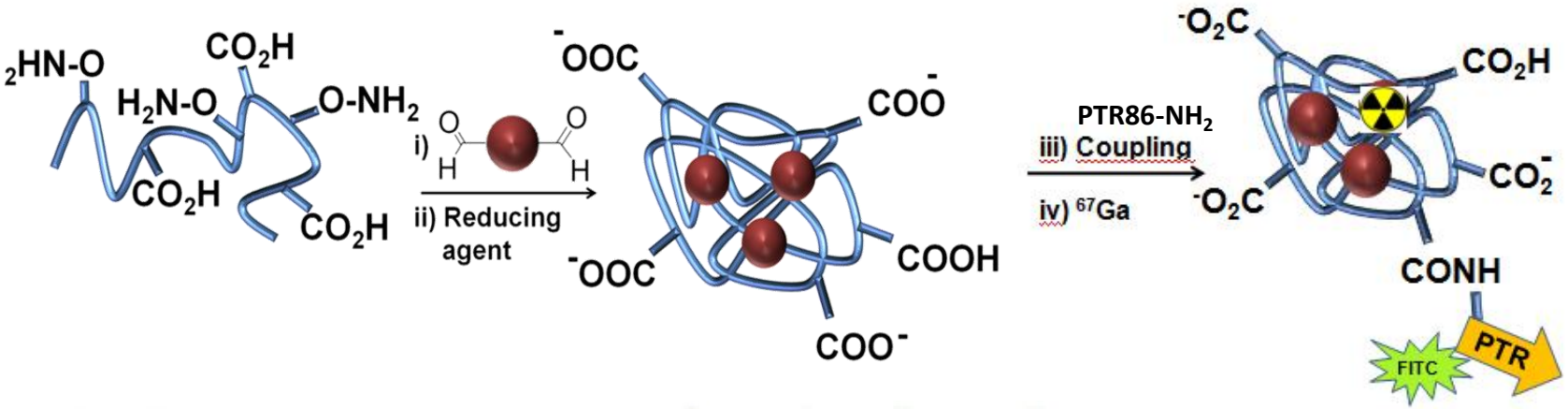
PTR86-NOTA



PTR86 derivatives:
Somatostatin analogues with affinity towards SSRT2-5

TEL AVIV UNIVERSITY

Functionalization: Targeted and radiolabeled PMAAc-SCPNs



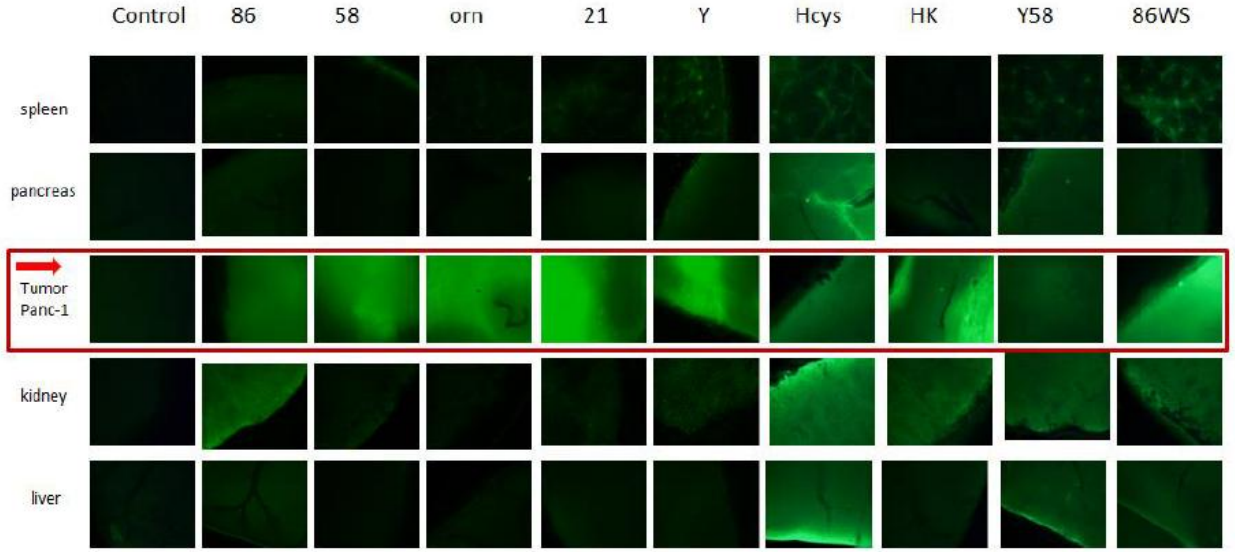


Figure 5.5. Signal intensity in the different organs (namely, spleen, pancreas, tumour, kidneys and liver) for the different SSTR ligands assayed. PTR-58 was selected for subsequent *in vivo* investigations using nuclear imaging.

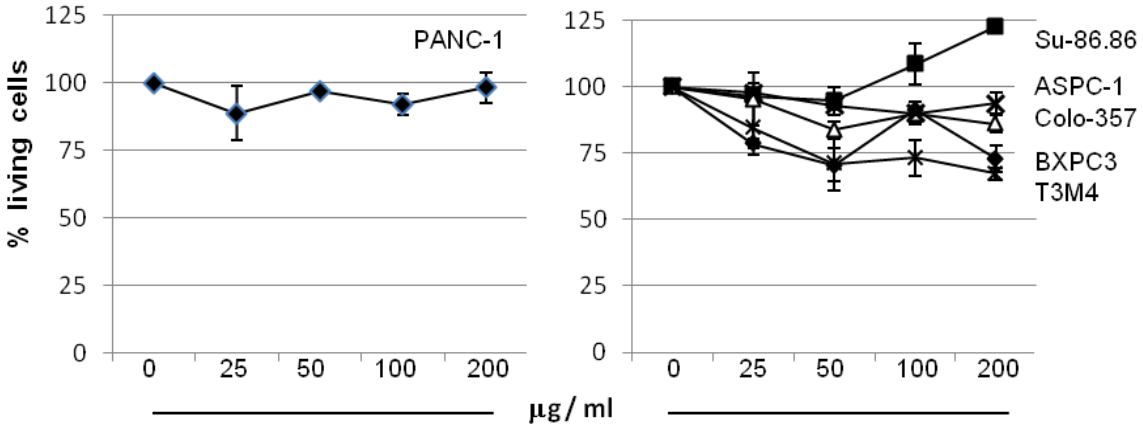
Quantification of PTR86 loading by fluorescence spectroscopy

Fluorescent imaging with targeted peptides

Toxicity studies

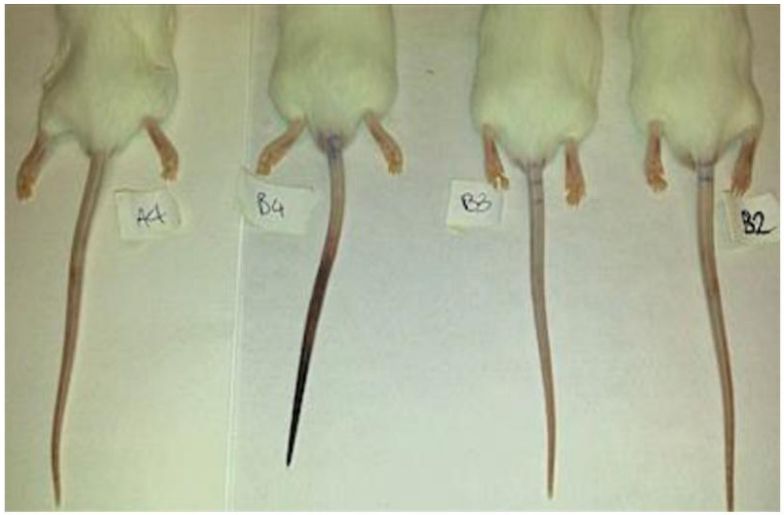
In vitro cytotoxicity
to assess the safety of SCPN

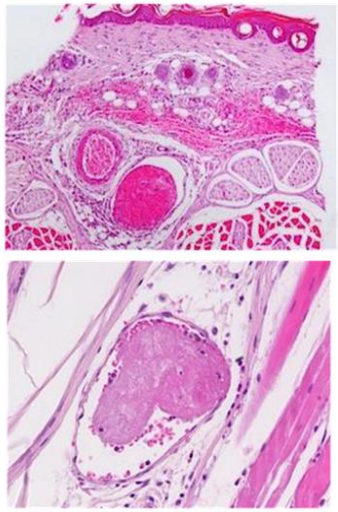
Toxicity was tested in 6 different pancreatic adenocarcinoma cell lines.



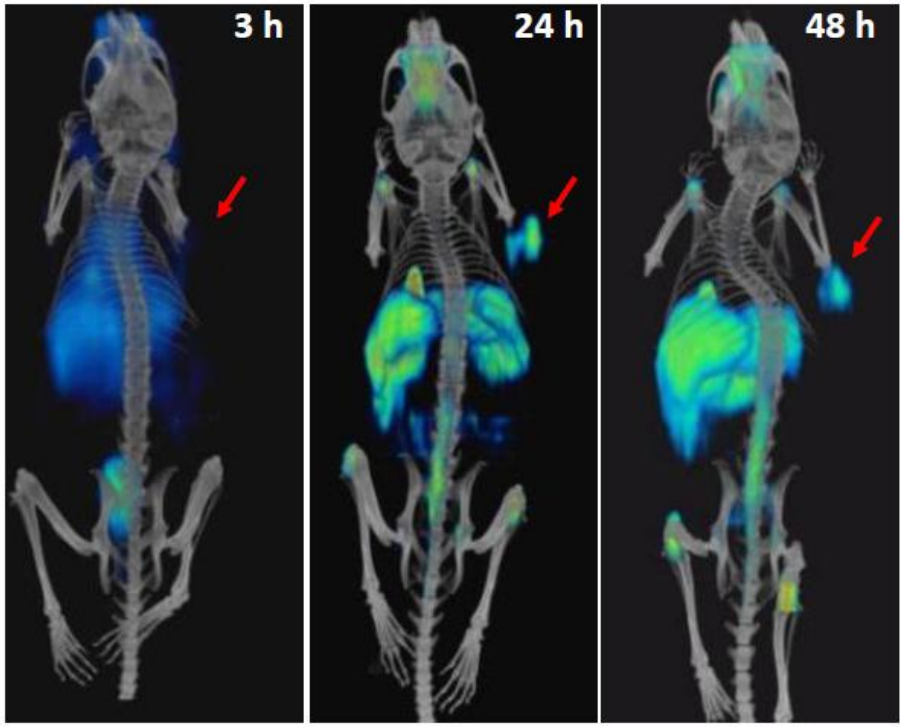
In vivo toxicity studies in animals

Dose: i.v. (10 ml/kg physiological saline) of PMAAc SCPNs (12,5 mg/kg and 100 mg/Kg) and sacrificed 24 hours after treatment. One (B4) out of five mice treated with 12,5 mg/kg had dark and necrotic tail.

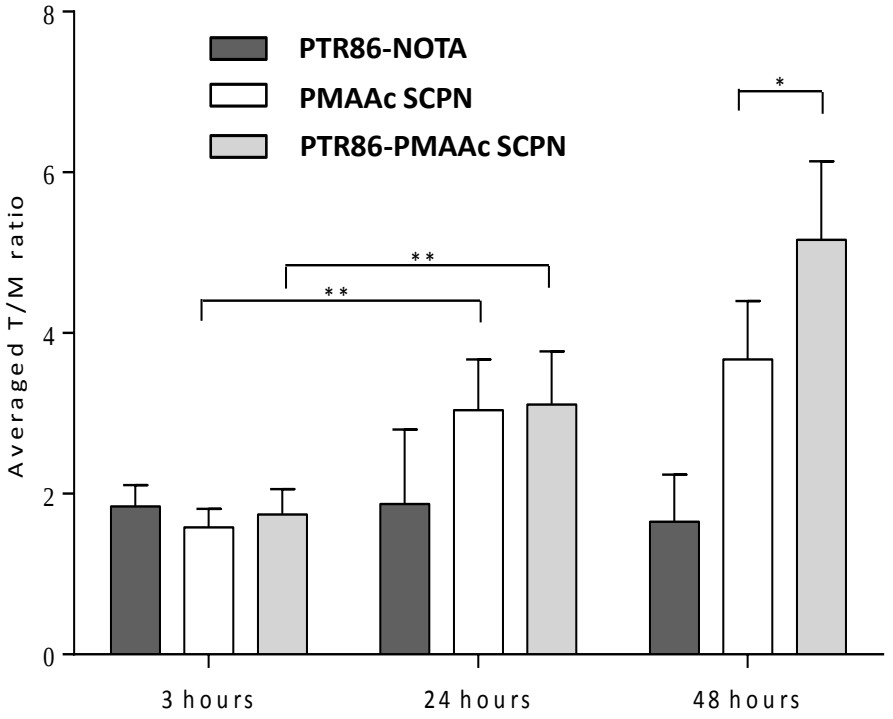




SPECT Imaging of targeted and radiolabeled PMAAc-SCPNs



SPECT-CT images of mice bearing subcutaneous human pancreatic ductal adenocarcinoma (PANC-1)
Dose: **i.v. injection of 1 mg/Kg of SCPNs**



Accumulation of the SCPNs in the tumor expressed as **tumor-to-muscle ratio**

Conclusions

→ A new **synthetic route** has been established for the synthesis of PMAAc-SCPNs in **water**.

→ Simultaneous incorporation of the **targeting** peptide PTR86 (receptor specificity) and the radionuclide ^{67}Ga for SPECT **imaging** was achieved.

→ The results obtained in this study indicate higher retention of the SCPNs in the tumor when these were decorated with the PTR86 peptide, leading to higher tumor-to-muscle ratios as determined from *in vivo* images.

Acknowledgements

Collaborators:

Funding:

ETORTEK
biomaGUNE'15

Contacts: H.-J. Grande (hgrande@cidetec.es)
M. Marradi (mmarradi@cidetec.es)

This project has received funding from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement No 604434 (PneumoNP) and No 263307 (SaveMe).

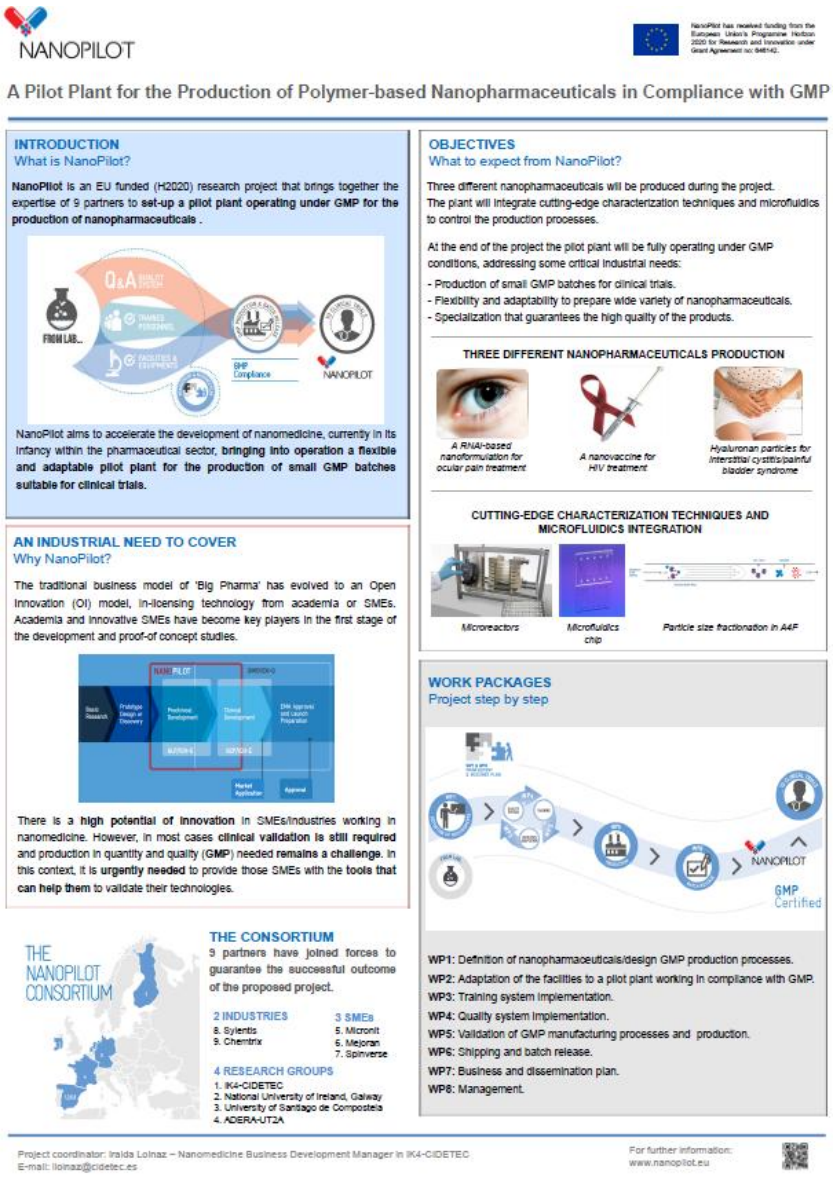
IK4 CIDETEC
Research Alliance

PNEUMONP

POSTER nº7

NanoPilot EU project: A Pilot plant for the production of Polymer based Nanopharmaceuticals in Compliance with GMP

www.nanopilot.eu



ESKERRIK ASKO
MUCHAS GRACIAS
THANK YOU VERY MUCH
MERCI BEAUCOUP
DANKESCHÖN