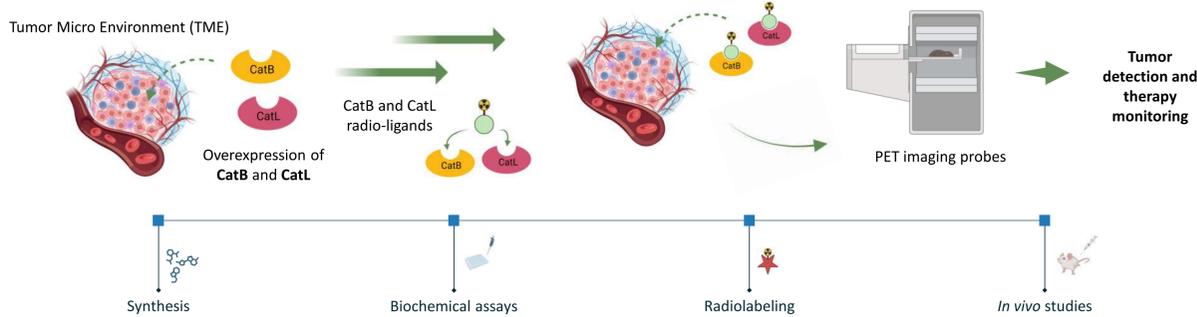


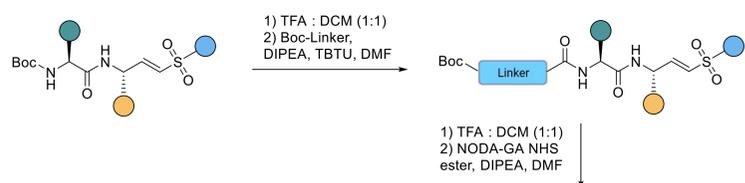
Introduction

Cathepsins, a family of lysosomal cysteine proteases, are involved in critical biological processes, including protein turnover, extracellular matrix remodeling, and antigen processing.

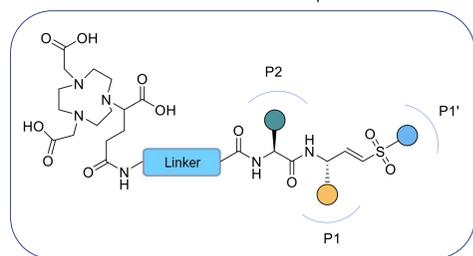


In cancer, the **overexpression of cathepsins**, particularly Cathepsin B (CatB) and Cathepsin L (CatL), is associated with tumor progression, metastasis, and immune modulation. These enzymes contribute to tumorigenesis, invasion, and angiogenesis, making them highly relevant **biomarkers for cancer diagnosis**. The aim of this project is to develop **selective inhibitors of CatB and CatL**, which can be used to create **innovative imaging probes for Positron Emission Tomography (PET) imaging** and further enhance the management of cancer patients.^{1,2}

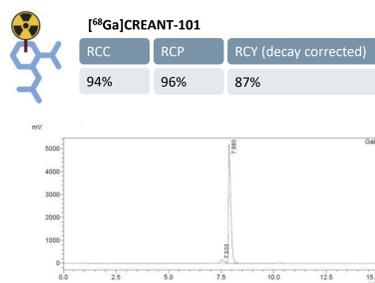
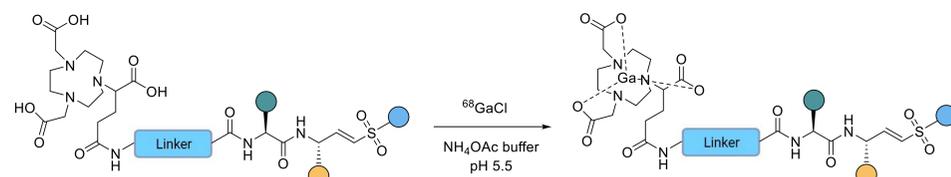
Synthesis of probe precursors



A series of **probe precursors** were synthesized and evaluated against cysteine cathepsins (B, L and S), assessing **affinity, selectivity and off-target profile**.



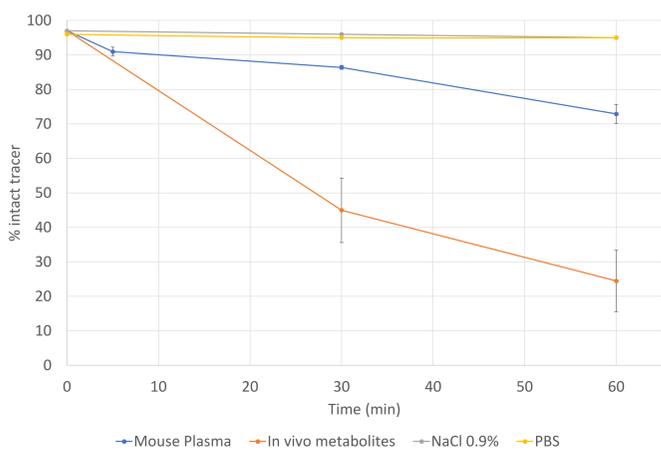
⁶⁸Gallium radiolabeling



Based on **biological evaluation**, selected probe candidates were advanced to **radiolabeling**.

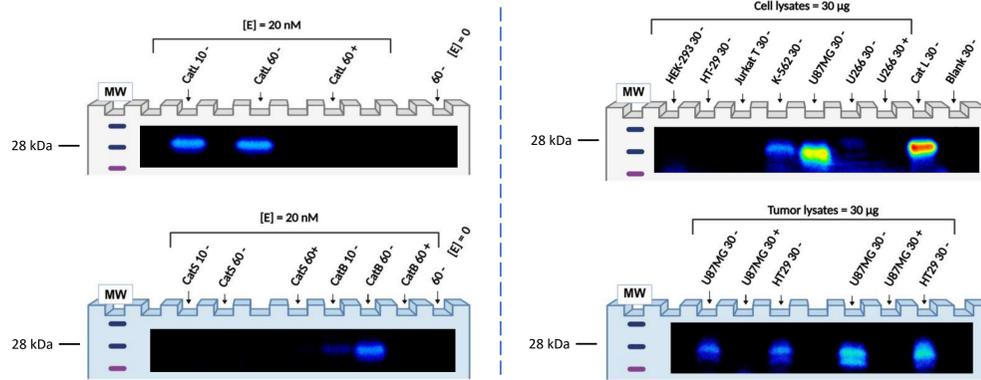
Among them, the probe [⁶⁸Ga]CREANT-101 showed the most favorable **radiochemical yield, conversion and purity**, constituting one of the most promising candidates for further development.

Stability



Probe **stability** was evaluated in **mouse plasma, in vivo, PBS and physiological saline**.

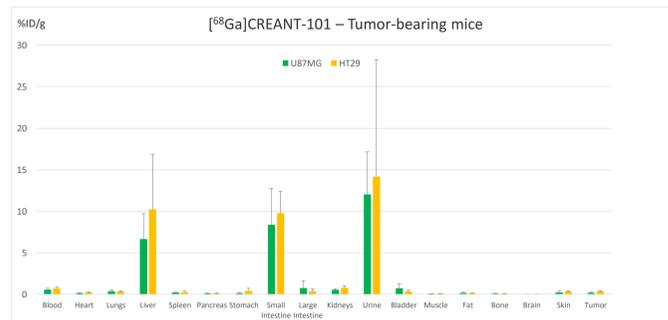
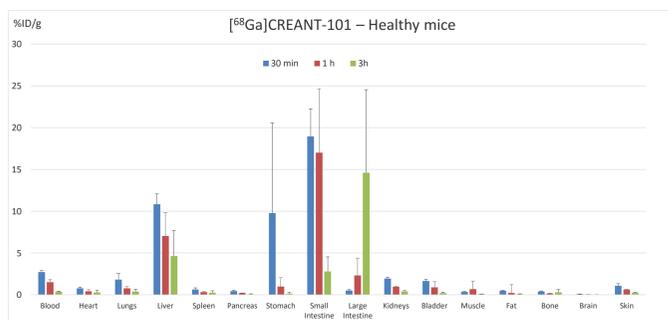
SDS-PAGE autoradiography



SDS-PAGE analyses of the probe incubated with **cathepsins, cell lysates and tumor lysates**.

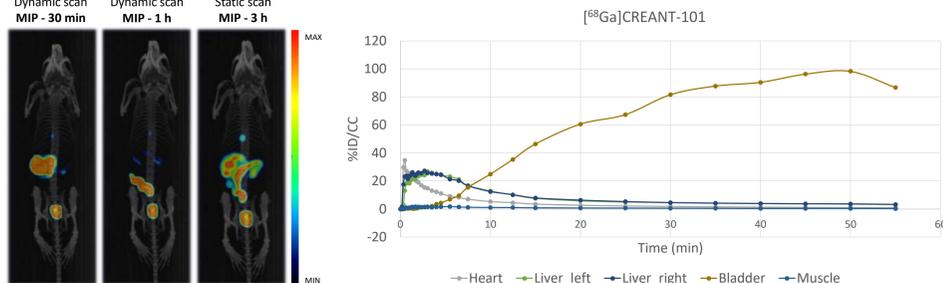
Reference: (+) preincubated with K11777 for 30 minutes at 37 °C; (-) no preincubation; 10 - incubated for 10 minutes at 37 °C; 30 - incubated for 30 minutes at 37 °C; 60 - incubated for 60 minutes at 37 °C. Molecular Weight = CatB 30 kDa, CatL 28 kDa, Cats 24 kDa.

Biodistribution studies

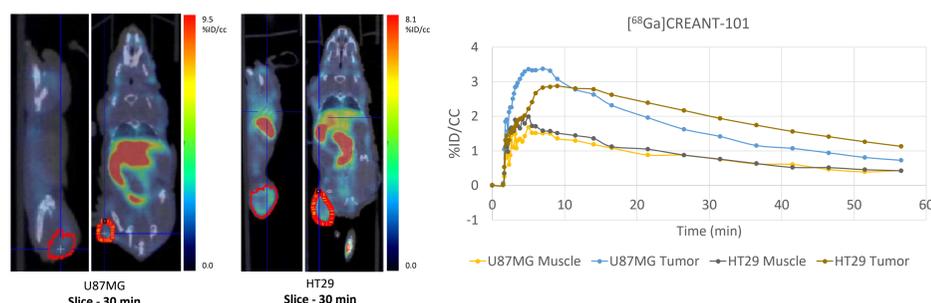


Ex-vivo biodistribution in major organs and tissues.

PET/CT – Healthy mice



PET/CT – Tumor-bearing mice



PET/CT imaging and time-activity curve analysis of the radiolabeled probe.

Conclusion and Outcomes

The developed vinyl sulfone-based probes demonstrated **high selectivity for CatL**, with minimal off-target binding, as confirmed by enzymatic assays and SDS-PAGE. *In vivo* evaluation revealed **reduced nonspecific background** and only limited tumor uptake in tumor-bearing models. Nevertheless, these findings provide a valuable **proof of concept** and serve as a starting point for further optimization of probe design to improve *in vivo* tumor targeting.