

Synthesis of a Multimodal Imaging Agent for the Visualization of Pancreatic Beta-Cells *in vivo*

Imaani Easthausen^{1,2}, Christian Brand³, Wolfgang A. Weber⁴, Jason S. Lewis³, Thomas Reiner³

¹Gateways to the Laboratory Program, Weill Cornell/Rockefeller/Sloan-Kettering Tri-Institutional MD/PhD Program, New York, NY

²Bard College, Annandale-on-Hudson, NY

³Department of Radiology, Memorial Sloan-Kettering Cancer Center, New York, NY

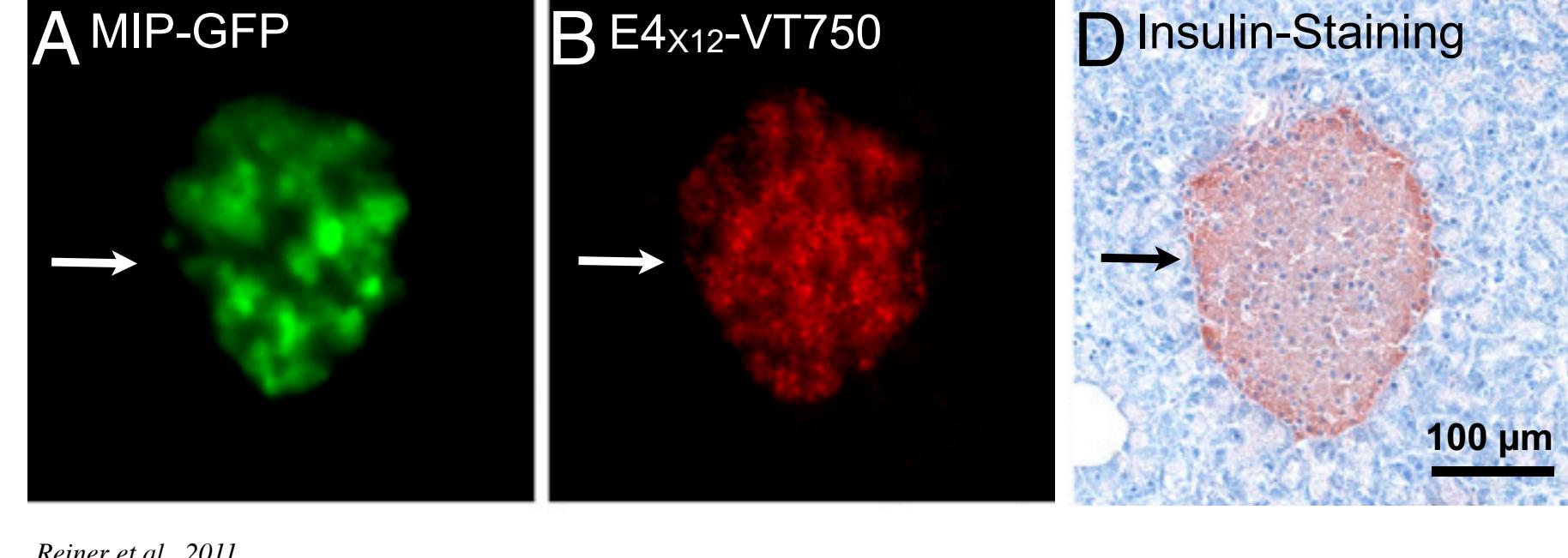
⁴Department of Molecular Imaging and Therapy Services, Memorial Sloan-Kettering Cancer Center, New York, NY

Abstract

The accurate visualization and quantification of **pancreatic β -cell mass** are critical to the improved understanding, diagnosis, and treatment of both **diabetes** and **insulinoma**. The ability to accurately track β -cell mass *in vivo* could allow for earlier detection and more targeted therapies for these diseases. Previous studies have been successful in creating **exendin-4 derivatives** that are capable of binding selectively to the glucagon-like peptide 1 receptor (GLP-1R) expressed on the surface of β -cells in the pancreas. This project reports on the synthesis of a multimodal imaging agent that combines both nuclear (PET) and optical (fluorescence) imaging in a **single-molecule probe**. Using a six-step reaction sequence, we synthesized a novel imaging agent comprising a copper chelator for the incorporation of radioactive ^{64}Cu , a fluorescent dye (sulfo-Cy5), and an exendin-4 analog as a biomarker. Preliminary *in vivo* studies suggest that the multimodal imaging agent has good selectivity and a high binding affinity for GLP-1R. In the clinical setting, the multimodal nature of the probe will allow it to be utilized for **intraoperative imaging** during surgery as well as for **whole body imaging** with the flexibility of a **single-molecule compound**.

The Role of Beta Cells in the Pancreas

- ◆ Pancreatic β -cells are the major **insulin producers** in the pancreas.
- ◆ Both **diabetes** and **insulinoma** are characterized by β -cell mass.
 - ◆ Diabetes is accompanied by the under-production of β -cells.
 - ◆ Insulinoma gives rise to an over-proliferation of β -cells.
- ◆ β -cells are **heavily concentrated** in Islets of Langerhans, and make up around 60-80% of all islet cells.
- ◆ **Islet of Langerhans** comprise about 1-2% of the pancreas.



Synthesis of Cu-E4_{X12}-Sar-Fl

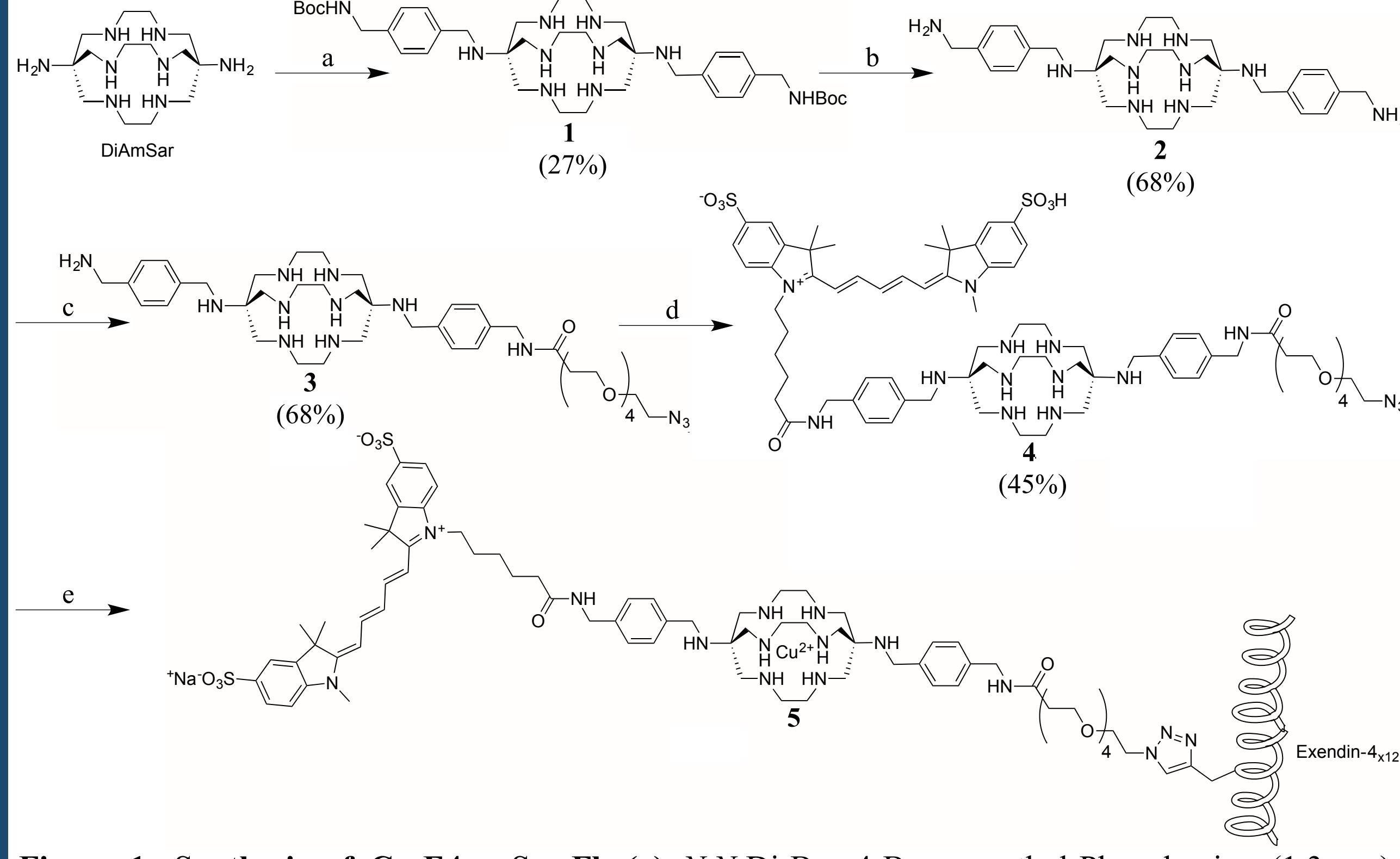


Figure 1: Synthesis of Cu-E4_{X12}-Sar-Fl. (a) *N,N*-Di-Boc-4-Bromomethyl-Phenylamine (1.3 eq.), Na_2CO_3 (2.6 eq.), DMF, 70 °C, 5 hrs. (b) TFA, CH_2Cl_2 , rt, 1 hr. (c) Azide-PEG4-NHS ester (0.5 eq.), Et_3N , DMF, rt, 2 hrs. (d) Sulfo-Cy5 NHS ester (1.1 eq.), Et_3N , DMF, rt, 1.5 hrs. (e) E4_{X12} (1.0 eq.), H_2O , CuSO_4 , NH_4OAc , PBS, rt, 16 hrs.

In vivo Imaging with Cu-E4_{X12}-Fl

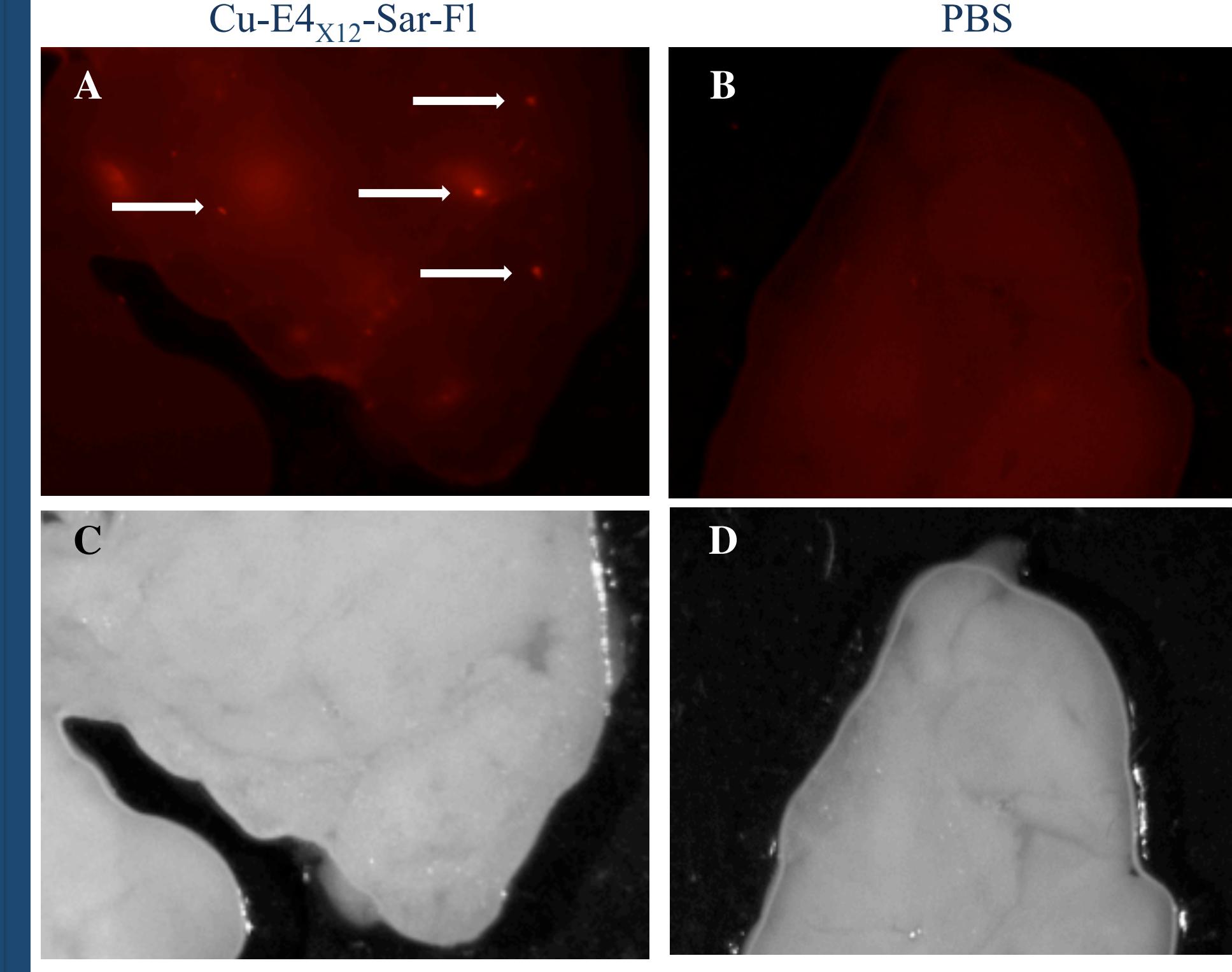


Figure 4: Fluorescence Imaging of Murine Pancreas Tissue. (A) Fluorescence image after injection with Cu-E4_{X12}-Sar-Fl. White arrows pointing to individual islets. (B) Fluorescence image after injection with PBS. (C) White light image after injection with Cu-E4_{X12}-Sar-Fl. (D) White light image after injection with PBS.

HPLC and Mass Spec

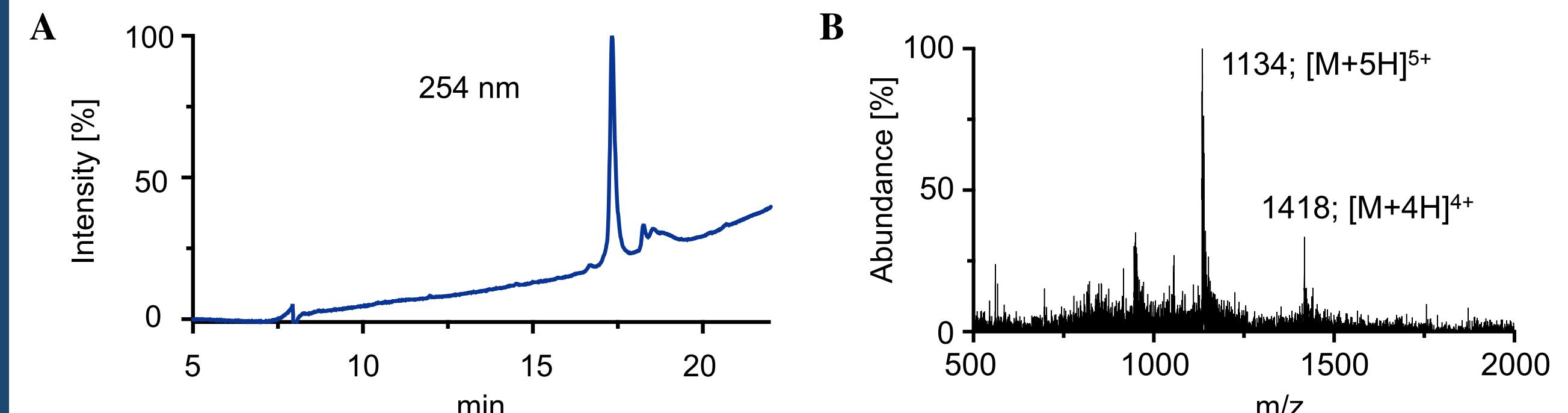


Figure 2: Cu-E4_{X12}-Sar-Fl. (A) HPLC chromatogram of Cu-E4_{X12}-Sar-Fl. (B) ESI-MS trace of Cu-E4_{X12}-Sar-Fl. ESI-MS (m/z) = 1134 [$\text{M}+5\text{H}$]⁵⁺; 1418 [$\text{M}+4\text{H}$]⁴⁺.

Radiolabeling with ^{64}Cu

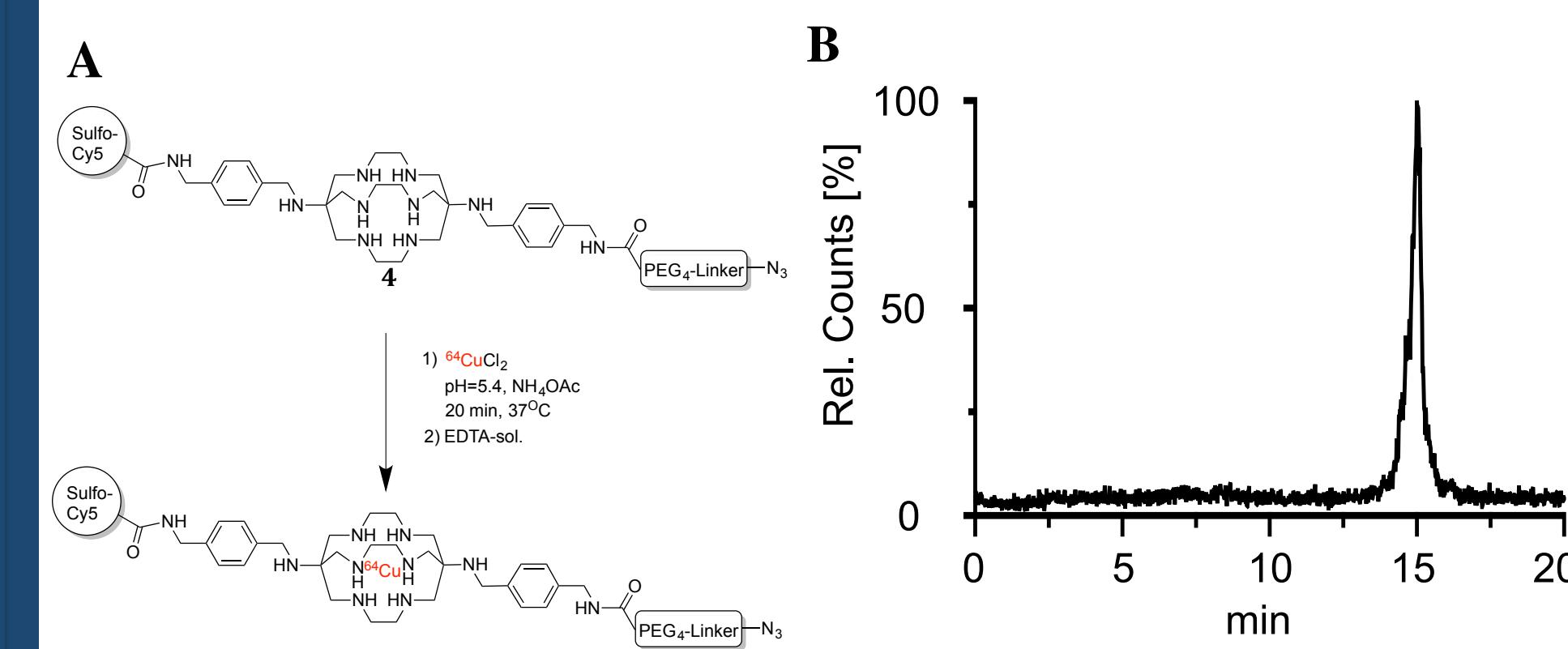


Figure 5: Radiolabeling with ^{64}Cu . (A) $^{64}\text{CuCl}_2$ (2.24 mCi), NH_4OAc , 37 °C, 20 min, pH = 5.7. (B) HPLC chromatogram. Major peak indicates ^{64}Cu -E4_{X12}-Sar-Fl.

Targeting GLP1-R

- ◆ Glucagon-like peptide 1 receptor (GLP1-R) is expressed on the surface of β -cells making it a good target for the selective imaging of β -cells.
- ◆ **Exendin-4** is a peptide extracted from the saliva of the gila monster that has been shown to bind to GLP1-R with a similar affinity to the mammalian glucagon-like peptide 1 (GLP1).
- ◆ Previous studies have shown that a **pentyoic acid** can be substituted with the lysine at the twelfth position without altering the binding affinity of exendin-4. This allows the peptide to be used as a **biomarker** in conjugation with other molecules.

Utilizing Exendin-4

Peptide	Amino acid sequence
GLP1	HAEGTFTSDVSSYLEGQAAKEFIawlVKGR
Exendin-4	HGEGTFTSDLS K QMEEAVRLFIEWLKNGGPSSGAPPPS
E4 _{x12}	HGEGTFTSDLS X QMEEAVRLFIEWLKNGGPSSGAPPPS

Islet of Langerhans Treated with Cu-E4_{X12}-Sar-Fl

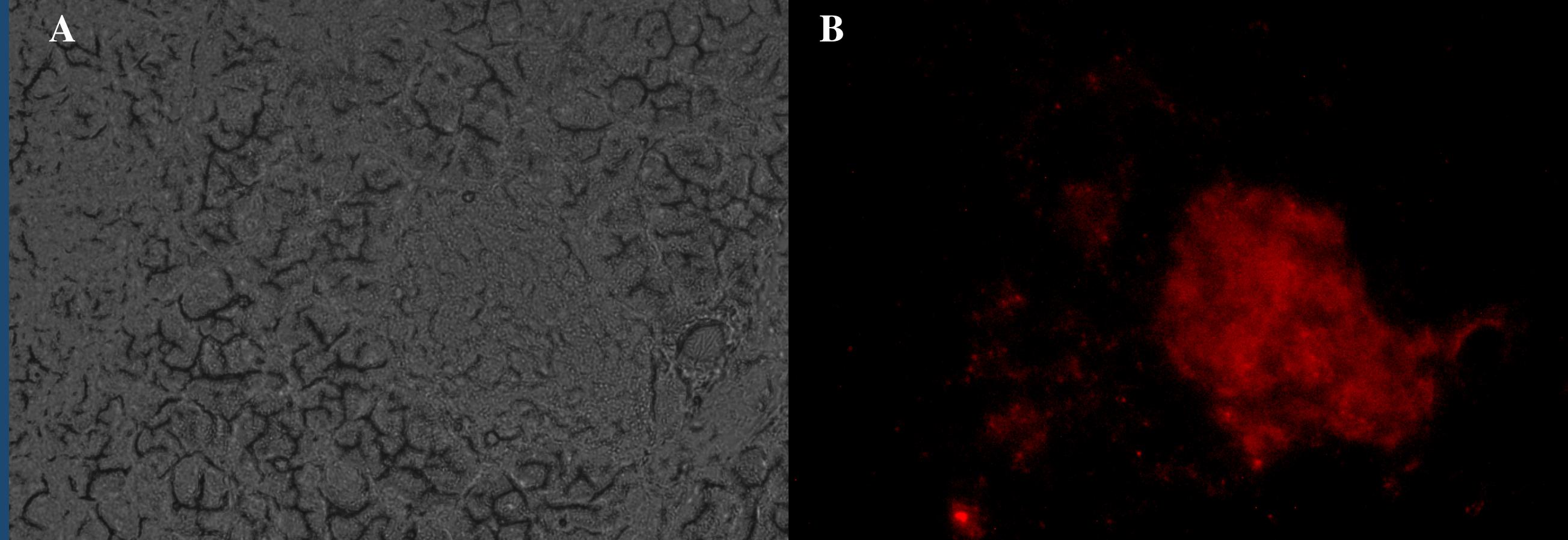


Figure 3: Fluorescence Imaging of Single Islet of Langerhans. Fluorescence microscopy images taken 1 hour after injection of Cu-E4_{X12}-Sar-Fl into nude mice. (A) White light image of single islet. (B) Fluorescence image of single islet.

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Moving Forward

- ◆ Cu-E4_{X12}-Sar-Fl demonstrates **good selectivity** and a **high binding affinity** *in vivo*.
- ◆ Future studies utilizing mice that are expressing GFP in their β -cells will be used to demonstrate co-fluorescence and **validate** current data.
- ◆ Future *in vivo* PET experiments will evaluate the performance of Cu-E4_{X12}-Sar-Fl as a PET imager.