Development of a Novel Immuno-PET Probe to Detect Osteomyelitis Infection

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Introduction

What is Osteomyelitis?

- Osteomyelitis is a rare bacterial infection of the bone.
- Commonly caused by the *Staphylococcus aureus* bacterium.
- Affects <1% of global population.
- Affects ~10% of diabetic patients due to prevalence of foot ulcerations in this population.
- Critical to examine this issue due to the increasing rates of diabetes in Hawaii especially among Native Hawaiian and Pacific Islander populations[1].
- Early and accurate diagnosis is essential to establish effective treatment and prevent surgical amputation[2].

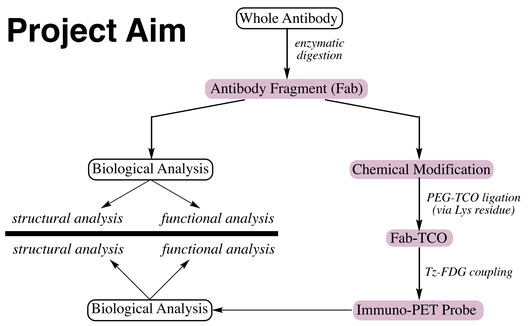
Challenges with Diagnosing Osteomyelitis

- Patients with osteomyelitis can be asymptomatic.
- Infection is visually similar to aseptic foot inflammation.

PET Imaging as a Potential Tool for Diagnosing Osteomyelitis

- Positron emission tomography is a non-invasive technique that uses radioactive tracers to show images of organ and tissue function[3].
- The concept of an Immuno-PET probe exploits the natural relationship between antibody and antigen to develop a tracer that is specific for the *S. aureus* antigen.

Project Aim



Materials and Methods

[Fab-TCO]: To a solution of the ZIK-F(ab')$_2$ antibody fragment (Fab; 3.4 mg / mL) in PBS buffer was added a DMSO solution of TCO-PEG$_4$-NHS. The mixture was incubated for 1 hour at room temperature and then quenched with Tris-HCl. The **Fab-TCO** product was purified through a desalt spin column.

[Tz]: To a solution of **1** and **2** in DMF was added Hunig's Base followed by $MgSO_4$ and EDC. After stirring for 25 minutes, **4** was added and the reaction was stirred overnight. The crude mixture was filtered, concentrated *in vacuo* and redissolved in 1:1 TFA:CH_2Cl_2. After stirring overnight the reaction was quenched with saturated $NaHCO_3$. The organic layer was extracted with $NaHCO_3$ (2x) and the combined organic layers were washed with saturated NaCl (1x). The reaction was then dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give **Tz** as a pink solid.

[Tz-FDG]: To a solution of **Tz** and **FDG** in DMF was added acetic acid. The reaction was incubated at 75 ° C for 20 minutes. Upon completion the solvent was removed *in vacuo* and the crude material was recrystallized from hot EtOAc to give **Tz-FDG** as a pink solid. Its structure was verified by ^{1}H NMR.

[Immuno-PET Probe]: To a solution of **Fab-TCO** in PBS was added a DMSO solution of **Tz-FDG**. After stirring at room temperature for 20 minutes, the product Immuno-PET probe was purified through a desalt spin column.

Results

Compound Selection

Tz-FDG

•Linker containing the radioactive glucose tag.

^{18}FDG

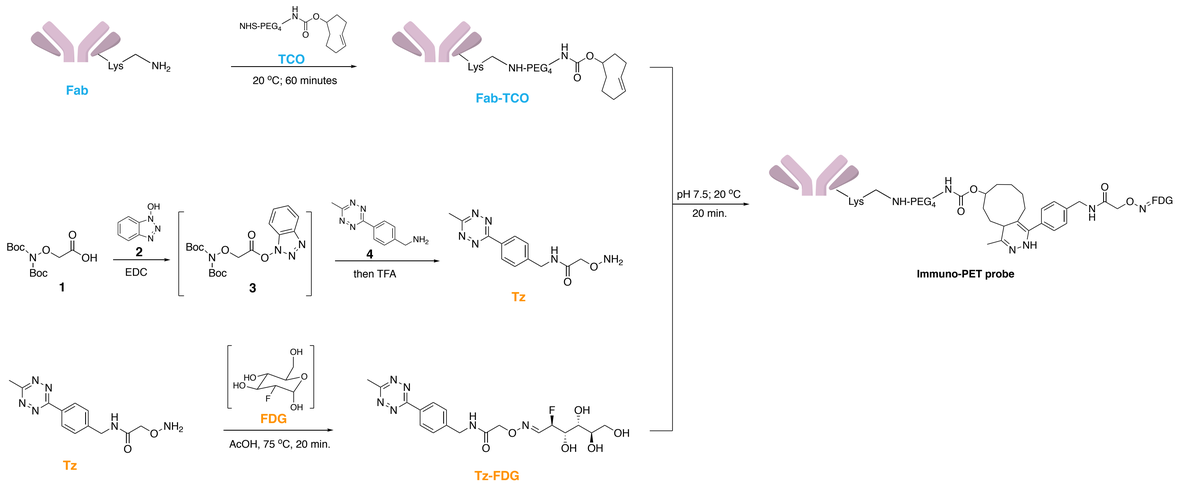
- Fluorine-18-fluorodeoxyglucose (^{18}FDG) is typically used as a glucose analog to investigate intracellular metabolism.
- Fluorine-18 acts as the radioactive tracer for imaging.

Fab-TCO

•The biologically active antibody fragment that binds to the antigen.

•In this preliminary experiment a ZIK-F(ab')$_2$ was used due to the full characterization of the ZIK-E antibody fragment.

Chemical Synthesis of Tz-FDG and Fab-TCO



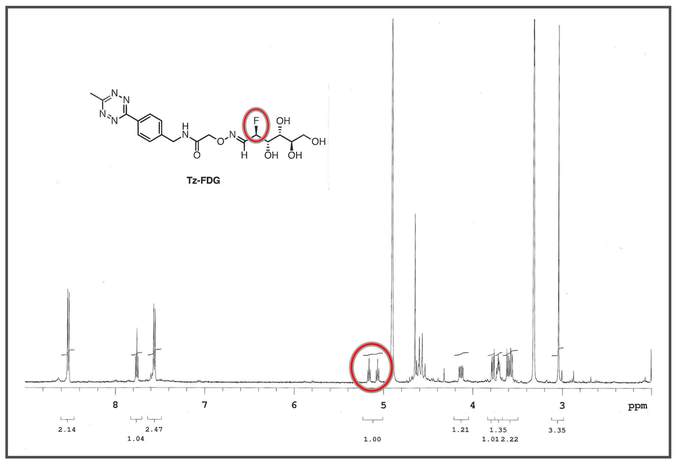


Figure 1. ^{1}H NMR of Tz-FDG. Spectrometer: Agilent INOVA 500 MHz. Solvent: Methanol-d_4; number of scans: 32.

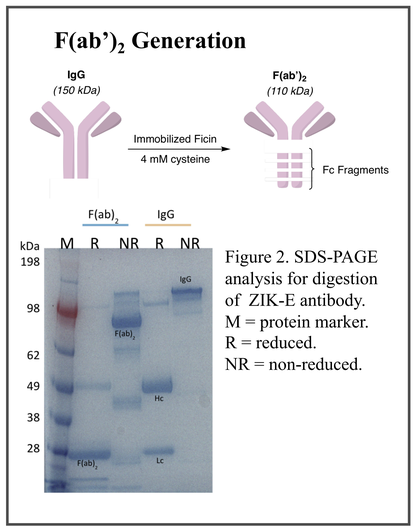


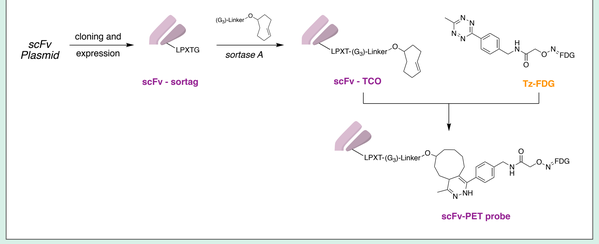
Figure 2. SDS-PAGE analysis for digestion of ZIK-E antibody. M = protein marker. R = reduced. NR = non-reduced.

Discussion

- The two triplets at 5.0-5.2 ppm on the ^{1}H NMR indicate that Tz-FDG was created.
- Loss of pink color during synthesis of the Immuno-PET probe suggests that the reaction went to completion.
- Protein mass analysis results are pending.
- Characterization of the Immuno-PET probe for affinity and specificity is dependent on verification of final compound.

Conclusion and Future Directions

- Final product is not verified however ^{1}H NMR and observations suggest that the compound has been created.
- This preliminary study used the ZIK-E antibody, future studies would entail use of *S. aureus* antibody.
- The *S. aureus* antibody would be sequenced and genetically modified so that a single TCO group attaches to the antibody.
- Following an analogous synthetic approach radioactive ^{18}FDG would be used.
- The Immuno-PET tracer can serve as an unambiguous diagnostic tool for detecting osteomyelitis infection and could be part of a screening process for diabetic patients.



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For Further Information

Please contact *acullen@queens.org*. For more information on this and related projects can be obtained at *www.qbcr.jabsom.hawaii.edu.*