Radiochemistry Research and Training, UC Davis (R2@UCDavis)

1. TITLE PAGE*

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3. ABSTRACT*

The report contains a summary of the accomplishments made during the R2@UCDavis proposal. In brief we proposed to develop new and highly innovative radiotracer methods and to enhance training opportunities to ensure the future availability of human resources for highly specialized fields of radiotracer development chemistry and clinical nuclear medicine research and allied disciplines. The overall scientific objectives of this proposal were to utilize “click” chemistry to facilitate fast and site-specific radiolabeling. Progress was made on all initial goals presented. This funding has to date resulted in publications in high impact journals such as Acta Biomaterialia, Molecular Imaging and Biology, Nuclear Medicine and Biology and most recently Environmental Science and technology, and it is anticipated that through the collaborations established during the time course of this funding that future research will be published in clinically relevant journals such as Science Translational Medicine and the Journal of Nuclear Medicine. Trainees involved in this proposal have gone on to further their careers in both academia, industry and the private sector. The collaborative forums established during the time course of this funding will ensure the future availability of human resources for highly specialized fields of radiotracer development chemistry and clinical nuclear medicine research and allied disciplines.

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*5.*EXECUTIVE SUMMARY*

The R2@UCDavis proposal in response to the FOA DE-FOA-0000646 Integrated Nuclear Medicine Research and Training Projects of Excellence proposed to address the three important goals set forth by DOE. In brief we proposed to develop fast, site-specific and generic radiolabeling approaches, to integrate involvement of postdoctoral fellows (Ph.D. radiochemists) and M.D., or M.D.-Ph.D. fellows and to enhance training opportunities to ensure the future availability of human resources for radiotracer development chemistry and clinical nuclear medicine research and allied disciplines. The overall scientific objective of this proposal was to utilize "click" chemistry to advance radiotracers as molecular imaging agents. We investigated "clickable" ring-strained cyclooctynes, "clickable' cages, 'clickable" anti-$\alpha_v\beta_6$ diabodies and "clickable" nano/biomaterials.

"Clickable" ring-strained cyclooctynes. Radiolabeled peptides were successfully synthesized via strain promoted click reactions between ADIBO and azide-peptides using both a pre-click and post-click approaches i.e. the constructs were clicked and then radiolabeled (pre-click) or radiolabeled and then clicked (post-click) with copper-64. The most successful yields and shorter reaction times were achieved when the construct was fully assembled (pre-click) and then radiolabeled. Radiochemical yields using the pre-click approach were greater than 99%. The peptides radiolabeled with copper-64 were found to be stable at 24 hours in mouse serum and cell binding studies showed specificity towards the $\alpha_v\beta_6$ receptor. Although initial *in vivo* imaging with these constructs looked promising and predominantly renal excretion was observed target specificity was lost at the later imaging time points. This is likely due to the addition of the ADIBO-based fused triazole ring system. Fluorine-18 constructs were also synthesized in high purity, were found to be stable in mouse serum and cell binding studies showed specificity towards the $\alpha_v\beta_6$ receptor. Unfortunately this radiolabeling approach also had undesirable effects on the pharmacokinetics, resulting in compromised tumor uptake. This work was published in Nuclear Medicine and Biology (2013) and Journal of Radioanal Nucl Chem (2014).

"Clickable' cage for the capture of [^{18}F]fluoride. This was the most challenging goal and sadly resulted in the most disappointing results. The cages were based on a cylindrophane based anion host with a goal to build a structure that contained an acetylene function to click to the peptide. After 3 synthetic chemistry postdoctoral fellows spent significant efforts trying various synthetic routes, temperatures, solvents, microwave heating without success this goal was abandoned. An alternative strategy using the NOTA chelator and AlF chemistry was pursued and subsequently translated *in vivo* in a mouse model as well as in non-human primates (paper in preparation for Journal of Nuclear Medicine).

'Clickable" anti-$\alpha_v\beta_6$ diabody to image integrin expression in vivo with PET. Two novel diabodies with high affinity and selectivity for the $\alpha_v\beta_6$ integrin were developed and radiolabeled with fluorine-18 and copper-64. Both radiolabeled diabodies demonstrated excellent specificity and retention of immunoreactivity and small animal imaging successfully visualized $\alpha_v\beta_6$ positive tumors, This work is published in Nuclear Medicine and Biology (2015) and Molecular Imaging and Biology (2017).

"Clickable" biomaterials. An additional approach that was pursued successfully leveraging "click" chemistry was the development of implantable biomaterials that could harness *in vivo* click chemistry to enhance the delivery of small molecule drug. This research involved a team that included radiochemists, organic chemists, orthopaedic surgeons and biomedical engineers. Dr Oneto, MD and Ph.D. in organic chemistry was a trainee in the Sutcliffe laboratory, He developed an alginate polymer that was covalently modified to incorporate the *trans*-cyclooctene moiety and subsequently clicked *in vivo* to an ^{111}In-tetrazine molecule. This paper was published in Acta Biomaterial (2014) and demonstrated that an implantable scaffold could be chemically modulated through "click" chemistry and improve the targeted delivery of a small molecule drug.

"Clickable" nanoparticle. Liposomal and iron oxide nanoparticles were developed. A liposomal scaffold was constructed to contain trifunctionality with a cholesterol containing moiety for insertion into liposomes, a chelator for coordinating copper-64 for positron emission tomography (PET) imaging or lutetium-177 for therapy and ADIBO for clicking to the peptide. This scaffold is assembled and currently under *in vitro* and *in vivo* testing. (Data expected to be published in JNM or Theranostics). Iron oxide nanoparticles both targeted and untargeted

were developed. Azide functionalized NPs were radiolabeled via a "click" reaction with [^{64}Cu]-ADIBO-NOTA yielding radiolabeled [^{64}Cu]-NPs of uniform shape and size with a high radiochemical purity (>99%), high specific activity, and high stability over 24 h across a pH range of 5-9. These non-targeted nanoparticles were used to quantitatively track and visualize NP transport and accumulation *in vivo* in lettuce. Both PET/CT and autoradiography showed that [^{64}Cu]-NPs entered the lettuce seedling roots and were rapidly transported to the cotyledons with the majority of the accumulation inside the roots. Uptake and transport of intact NPs was size-dependent. This data is published in environmental Science and Technology (2017). Addition of integrin specific peptides to the NP surface will facilitate targeted delivery in mouse models and is currently under investigation.

Fostering interdisciplinary interactions and enhancing training opportunities. Trainees had the opportunity to gain hands on experience in the synthesis of a wide variety of tracers and had the opportunity to shadow imaging staff during research projects. Trainees included Boucher (Ph.D.), Davis (Ph.D.), Haunsner (PhD), Hu (PhD), Oneta (MD, Ph.D.), Rippner (Ph.D.), Satpati (PhD) and White (Ph.D.), I have selected one trainee, Ryan Davis Ph.D. to highlight as an example of the training received during the time of this funding. Since joining my lab in 2013 Ryan (Ph.D. in organic synthesis) has become adept in the synthesis, purification, and characterization of peptides, has synthesized various linkers and prosthetic group precursors, for the attachment of fluorine-18 and copper-64, developed the clickable nanoparticles described above, worked tirelessly to develop the cage structures described above, performed *in vitro* testing of peptides to examine affinity and specificity and was trained in radiochemistry using both manual and automated approaches. In addition Ryan was trained in all aspects of cGMP production, helped set up the GMP laboratory, developed the quality assurance standard operating procedures for the analysis of radiolabeled peptides and helped generate the data necessary for the filing of an exploratory investigational new drug application (eIND). Ryan is first author on two papers published to date, is preparing several papers based on the “clickable” nanoparticle scaffolds and will be joint co-author on the first-in-human paper that is currently in preparation for submission to Nature Medicine. It is expected that he will publish at least 5 papers based on the work he performed during this funding and he is currently preparing a K25 award proposal. I believe Ryan is a great example for aspiring radiochemists.

In addition to the standard imaging course offered at UC Davis, weekly group meetings and journals clubs additional collaborative forums were established to facilitate the interaction between basic scientists and clinical scientists. The Nuclear Medicine Research Operations Committee (NMROC) was established by Sutcliffe (PI) and collaborators Cherry (BME) and Badawi (Radiology) in 2015 to provide a forum for interactions between basic scientists, clinicians, technicians and nurses to meet. We meet weekly and a typical agenda includes invited speakers, discussion on research progress, discussion on interesting journal publications and general nuclear medicine business. The Cancer Center’s Clinical Innovation Groups (CCCIGs) were established to promote multidisciplinary collaborations to enhance clinical and translational cancer research. These groups include Brain, Breast, GI, GU, Hematology, Phase I and Thoracic Malignancies and are composed of medical, surgical and radiation oncologists, basic scientists, pathologists, radiologists and group specific subspecialists. New collaborations I have established during the timeline of this funding through interactions at NMROC and the CCCIGs include but are not limited to Chew (MD, oncology), Daly (MD, PhD, radiation oncology), Foster (MD, nuclear medicine), Gholami (MD, Ph.D., surgical oncology), Kim (MD, PhD, hematology and oncology), Matsukuma (MD, pathology) and McPherson (Ph.D., director of basic science cancer center). Both forums will continue to ensure the future availability of human resources for highly specialized fields of radiotracer development chemistry and clinical nuclear medicine research and allied disciplines.

In summary we exploited “click” chemistry to radiolabel small molecules, peptides and diabodies for applications in cancer as well as environmental sciences. The overall conclusion from our work is that there is a fine balance between using fast chemistry and having a high affinity ligand whilst retaining good *in vivo* pharmacokinetics. Without the funding from DOE it would simply not have been possible to investigate innovative approaches for radiochemistry (an area not typical funded by other agencies), streamline those approaches most appropriate for clinical translation and in addition provide the interdisciplinary forum necessary for training for the next generation of radiochemists.