Cover Page

Title: Positron-Emitting Analogues of Alpha-Emitting Therapy Radionuclides

Institution: **Los Alamos National Laboratory Isotope Production Program**

Address: **Los Alamos National Laboratory, P.O. Box 1663, Los Alamos, New Mexico 87545**

Administrative Point of Contact: **Chris Paul Trujillo**
Budget Officer
LANL CFO-STE PADSTE
PO Box 1663, MS C331
Los Alamos, NM 87544
Ph. 505-667-8442, chrispt@lanl.gov

Lead PI: **Dr. Jonathan W. Engle**
LANL Chemistry Division
Inorganic, Isotope, and Actinide Group
DOE Isotope Program
PO Box 1663, MS J975
Los Alamos, NM 87544
Ph. 505-695-6203, jwengle@lanl.gov

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DOE/Office of Science Program Office Technical Contact: Dr. Dennis Phillips

Supplement to Cover Page for Collaborative Proposals

Lead Institution: Los Alamos National Laboratory (LANL); Dr. Jonathan Engle (PI)
Collaborating Institution: Lawrence Berkeley National Laboratory (LBNL); Dr. Rebecca Abergel (PI)

Lead PI for Project (Point of Contact and Research Activity Coordinator): Dr. Jonathan Engle, LANL

This team is uniquely qualified to develop Ce based PET imaging methods to characterize the biological fate and transport of two alpha emitting actinides that have therapeutic potential, namely ^{225}Ac and ^{227}Th. To achieve this goal, the LANL and LBNL teams will be lead by Jonathan Engle and Rebecca Abergel, respectively. Primary responsibility for nuclear data measurement and radionuclide production (Sections 3.1 – 3.3 below) will be led by Jonathan Engle (LANL cross section measurements, IPF target design), Lee Bernstein (LBNL cross section measurements), and Stosh Kozimor (LANL radiochemical isolation of n.c.a ^{134}Ce). James O'Neil will oversee radiochemical separation of LBNL irradiated targets. Evaluations of the chemical stability and binding kinetics of proposed ligand systems will be led by Rebecca Abergel (LBNL). Engle and Abergel will coordinate the use of LANL and LBNL-produced material. Rebecca Abergel will also lead biodistribution experiments designed to demonstrate the equivalence of $^{134}Ce/^{134}La$ with alpha emitters of interest using μPET (μPET experiments will be led by James O'Neil).

This proposed project will make use of the Los Alamos Isotope Production Facility, Chemistry Division Counting Facilities, and TA-48 Hot Cell Facilities. LBNL's 88" Cyclotron and Heavy Element Research Laboratory's extensive array of radioanalytical and biological equipment and μPET scanning suite. Detailed descriptions of these facilities are given in Appendix 4/5.

Collaborative Proposal Information					
	Names	Institution	Year 1 Budget	Year 2 Budget	Total Budget
Lead	Jonathan Engle	LANL	$200,000	$200,000	$400,000
Co-PI	Rebecca Abergel	LBNL	$245,040	$165,285	$410,325
		Total	$445,040	$365,285	$810,325

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Project Narrative: Positron-Emitting Analogues of Alpha-Emitting Therapy Radionuclides

1. Project Summary

Rapid-throughput, kinetic biodistribution assays are essential to the development of novel radiotherapeutics, especially those containing alpha-emitting nuclides. These nuclides are often actinides (e.g., ^{225}Ac, ^{227}Th) with lengthy decay chains. Their chemical and pharmacokinetic behavior is challenging to emulate and their handling often poses logistical challenges to many laboratories. For this reason we propose to develop novel analogous systems using radioisotopes of cerium and lanthanum, whose ionic radius and coordination geometries are known to be similar to those of actinium and thorium. The $^{134}Ce/^{134}La$ parent/daughter pair emits positrons, enabling its use in positron emission tomography imaging. Theoretical codes predict that the parent, ^{134}Ce, is best produced using protons with energies above 50 MeV; we propose to measure these reactions to resolve ambiguity in the predictions and establish optimal irradiation parameters. With these optimal parameters, we propose a rapid chemical isolation of the desired ^{134}Ce using thenoyltrifluoroacetone (TTA) extraction, making use of the differences in redox accessibility of Ce^{III} and Ce^{IV}. With ligand selection, we hypothesize that the oxidation state of the parent, ^{134}Ce, can be "tuned" to +3 (to mimic ^{225}Ac) or +4 (to mimic ^{227}Th). We will demonstrate this hypothesis by comparing traditional biodistribution experiments (with ^{225}Ac and ^{227}Th) with μPET imaging of injected $^{134}Ce^{III}$ and $^{134}Ce^{IV}$. Success will open the door to efficient evaluation of novel targeting mechanisms with rapid, time-dependent, and non-invasive assays using positron-emitting analogues of costly and scarce alpha-emitting radionuclides.

2. Background/Introduction

2.1 The Need for Positron-Emitting Analogues of Alpha-Emitting Therapeutic Radionuclides

Novel production methods for alpha-emitting radionuclides proposed for radiotherapeutic treatment of human disease have stimulated intense interest in the clinical application of these nuclides in the past decade. In these applications, alpha-emitters are chemically coupled to biological targeting vectors to selectively deliver the radioactive payload to the disease, sparing nearby healthy tissue. Relatively few radionuclides possess physical decay characteristics that make them desirable for this application, so exploratory research is heavily focused on a small number of candidates, all of which are identified in recent recommendations by the Nuclear Science Advisory Committee [1], e.g., ^{225}Ac ($t_{1/2}$ = 9.92 d, 5.7 MeV α), ^{227}Th ($t_{1/2}$ = 18.72 d, 5.8 MeV α), and ^{212}Pb ($t_{1/2}$ = 10.64 h, 0.3 MeV β^-).

However, developing new drugs to target these alpha-emitters to physiological systems of interest is challenging. Raw materials often have limited availability, making even scoping studies with the radionuclide of interest difficult and costly to arrange. Furthermore, the dosimetric potency of the radionuclides (and their daughters) listed above makes biodistribution studies in human subjects impossible to contemplate. This is especially true for ^{225}Ac and ^{227}Th, each of whose decay chains emit 4 alpha particles.

The past few decades of pharmaceutical development offer a paradigm for the efficient development of new drugs, employing non-invasive imaging of model, living systems to rapidly assay the biodistribution of candidate molecules *in vivo*. Positron Emission Tomography (PET) imaging is the unquestioned standard for these assays, with established quantitative capability at scales even relevant to sensitive neurological systems, where receptors exist in nanomolar concentrations. Three-dimensional, tomographic imaging devices are now routinely coupled with multiple anatomical imaging modalities (CT, MRI) to generate registered images of the time-dependent pharmacokinetics of injected, radiolabeled

compounds. No other tool offers such a utilitarian window into the early phases of a new drug's investigation, which can be conducted without perturbing the living system.

However, none of the alpha emitters' decay chains emit sufficient positrons to produce a signal that can be detected by PET. In order to employ PET to explore new uses of alpha emitters, positron-emitting, surrogate radionuclides will have to be developed. Because the choice of element affects the chemistry and biochemistry of the final molecule, it is highly desirable to utilize positron-emitters of elements whose chemical properties are most similar to those of the alpha emitter in question. In general, f-block metal ions are considered to be hard Lewis acids, and, as such, they are commonly stabilized by hard-donor oxygen-based ligands known to strongly bind transition metals. However, important differences arise along the 4f and 5 series, owing to changes in metal ionic radii, orbital mixing, and oxido-reduction potentials. Arguably, actinium's most distinguishing feature is its large ionic radius, which is mimicked most closely by the early lanthanides (Table I). Despite an impressive lack of available solution thermodynamic data for this element, trivalent Ac^{3+} speciation is expected to be similar to that of ions such as La^{3+} and Ce^{3+} under physiological conditions [2]. In contrast, the metal ions Zr^{4+}, Ce^{4+}, and Th^{4+} have often been hypothesized to behave in a similar manner and are even commonly proposed for use as surrogates of highly radioactive transuranic tetravalent ions [3,4]. Solution thermodynamics have highlighted the shared stability properties of chelated Zr^{4+}, Ce^{4+}, and Th^{4+}, with Ce^{4+} and Th^{4+} displaying the highest equivalence due to their close ionic radii (Table I) [5].

Among the 4f-elements, the redox active cerium ion is uniquely positioned to serve as a PET imaging mimic for two alpha emitting actinides that have therapeutic potential, namely ^{225}Ac and ^{227}Th. For example, because the cerium oxidation state can be stabilized as either +3 or +4 in biological systems with suitable complexing agents, the fate and transport of either Ac^{3+} or Th^{4+} can be characterized using a PET active cerium surrogate. In this scenario, Ce^{3+} would follow Ac^{3+}, meanwhile Ce^{4+} would track Th^{4+} (Figure 1). Hence, we propose to establish ^{134}Ce/^{134}La ($t_{1/2}$ = 75.9 h / 6.67 m, 2.7 MeV β^+) as a positron-emitting analogue of ^{225}Ac and ^{227}Th. The short half-life of the daughter, ^{134}La, enables the pair to be used as an "*in vivo* generator", such as has been proposed previously for ^{140}Nd/^{140}Pm [6]. Development of a production strategy is proposed, as is work to synthesize and verify that common ligands can stably bind actinium and thorium for *in-vivo* experiments using LBNL's unique actinide biodistribution assay capabilities to demonstrate the equivalence of ^{134}Ce/^{134}La with promising alpha-emitting radionuclides.

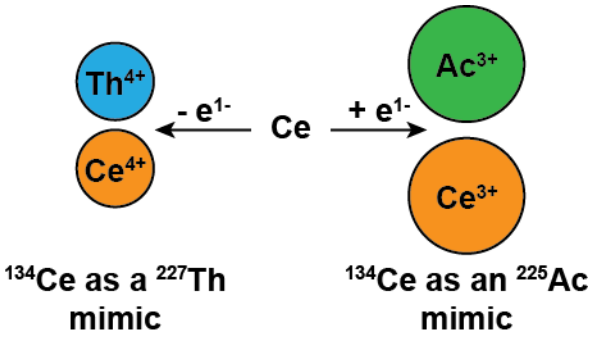

FIG 1. Cartoon showing how the small Ce^{4+} cation mimics Th^{4+}, while the large Ce^{3+} cation mimics Ac^{3+}.

^{134}Ce is an excellent candidate for production in the LANL IPF "B-slot" via the ^{nat}La(p,5n)^{134}Ce reaction and is accessible via several heavy ion imitated reaction channels as well, and the isolation of no-carrier-added Ce in the La target can be accomplished via TTA extraction. We next propose to utilize differences in the accessibility of Ce^{III} and Ce^{IV} redox to remove the majority of the ^{139}La target material via TTA extraction. Following this extraction and after a small ion exchange column that will finely purify the ^{134}Ce sample we will generate oxidation state pure Ce^{III} or Ce^{IV} stock solutions used in the subsequent chelation/*in-vivo* studies. Upon completion of ^{134}Ce production, the ^{134}La daughter produce will re-emerge as the ^{134}Ce decays. The *in vivo* equivalence between the biodistributions of chelated ^{135}Ce/^{135}La with chelated ^{227}Th and ^{225}Ac will be demonstrated using small animal µPET and traditional biodistribution experiments.

The proposed work is necessarily collaborative. The Isotope Production Facility (IPF) at Los Alamos National Laboratory is one of only two facilities in the country with sufficient proton energy to form ^{134}Ce and has the beam intensity to contemplate production on a large scale. IPF is also capable of acquiring the nuclear data necessary to understand the yields and achievable radionuclidic and radioisotopic purity of these unmeasured reactions. The 88" cyclotron at Lawrence Berkeley National Laboratory is one of only 4 machines in the US capable of measuring proton-induced excitation functions up to 60 MeV in a way that complements and energetically overlaps those we will propose at IPF. To establish the *in vivo* relevance of the ^{134}Ce/^{134}La we are confident we can produce at IPF, LBNL's co-located small animal PET and biodistribution facilities are essential and uniquely capable of handling actinides and gamma emitters. Both institutions value student and postdoc engagement, which will occur at all stages of this project.

2.2 Controlling Solution Thermodynamics and Redox Properties of Radiometals in Vivo

As they belong to the same denomination in the periodic table, both actinide elements Ac and Th display high coordination numbers and are best chelated by high denticity ligands that contain hard donor atoms, such as polyaminocarboxylates and hydroxamates [7,8]. However, Ac(III) and Th(IV) are the main oxidation states present under physiological conditions, when bound to these common small organic ligands, resulting in complexes that bear different charges and exhibit different solution stabilities, depending on the protonation state, acidity and denticity of the chelating molecule. In turn, these differences in coordination chemistries and redox properties will affect the biological behavior and distribution of the unchelated or chelated elements.

Despite a recent tremendous resurgence in the interest of employing metallic radioisotopes such as ^{225}Ac and ^{227}Th for targeted therapy, developing chelating ligands with optimized complex stabilities and kinetics remains a significant biomedical challenge. To date, only a handful of exist that can be linked to targeting vectors in bioconjugate constructs. The linear diethylenetriaminepentaacetic acid (DTPA), and macrocyclic 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) archetype ligands have been used in a large majority of ^{225}Ac studies (Figure 2) [9,10]. However, thorough solution thermodynamic studies with the corresponding complexes are lacking and stability constants may only be estimated by comparisons with other (non radioactive) trivalent 4f-elements. Current available chelators do not simultaneously have high thermodynamic stability and fast association. In general, macrocycles such as DOTA have both slow off and on rates, while acyclic ligands like DTPA display faster formation kinetics but form complexes of lower stability. For example, DOTA has a greater stability with lanthanides than DTPA by about 2 orders of magnitude, but the latter displays faster kinetics by 4 orders of magnitude [11–14]. Moreover, the stabilities of DTPA and DOTA complexes formed with tetravalent f-block metals such as Th(IV) are 10 to 20 orders of magnitude higher than those of the corresponding trivalent metal complexes [15,16], implying large differences in *in vivo* stabilities. A summary of available and relevant thermodynamic stability constants is provided in Table I. The higher intrinsic stability of Th(IV) complexes has also motivated the development of new chelators for ^{227}Th in several research and development groups [17–19].Hydroxypyridinone- (HOPO-) and terephthalamide- (TAM-) containing ligands have been the focus of these efforts, resulting in extremely stable complexes and promising ligands for antibody conjugation to tetravalent radiometals. Noteworthy examples of these new classes of ligands include the linear octadentate ligand 3,4,3-LI(1,2-HOPO), as well as the macrocyclic structures BPDETLysH22-3,2-HOPO and ΦTAM (Figure 2) [5,17,19]. Also notable, modifications of the hexadentate natural siderophore Desferrioxamine B (DFO) have afforded an octadentate version, DFO* (Figure 2), that displays high *in vivo* stability when complexed to ^{89}Zr(IV) [18] and could therefore be envisaged as a ligand for ^{227}Th(IV) chelation.

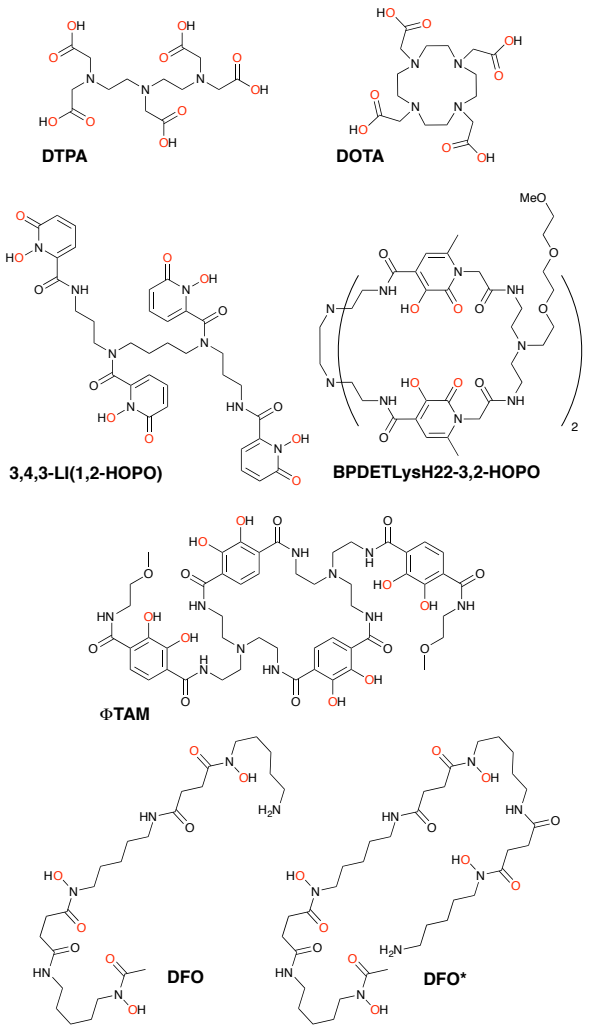

FIG 2. Common (DTPA, DOTA, DFO) and new investigational ligands (3,4,3-LI(1,2-HOPO), BPDETLysH22-3,2-HOPO, ΦTAM, DFO*) currently used for the chelation of ^{225}Ac, ^{227}Th, or ^{89}Zr radiometals in therapy and imaging bioconjugate constructs. Metal-binding oxygen donors are highlighted in red.

The biggest challenge in deriving surrogate PET imaging complexes for ^{225}Ac and ^{227}Th is to control the metal oxidation state and resulting thermodynamic and kinetic complex stabilities with specific ligands. Several studies have highlighted ^{89}Zr(IV) as an adequate imaging surrogate for ^{227}Th(IV) [5,20,21], and the SPECT radiometal ^{177}Lu(III) has been used for ^{225}Ac(III). While the use of these PET and SPECT agents have provided significant insight in the biological fate and transport of ^{227}Th and ^{225}Ac, significant chemical differences between the Zr^{4+}/Th^{4+} and Lu^{3+}/Ac^{3+} pairs – largely associated with the size miss-matches and corresponding differences in Lewis acidity – limit the utility of these isotopes as PET/SPECT imaging surrogates. Limitations associated with Lu^{3+} as a suitable chemical mimic of Ac^{3+} are compounded by ^{177}Lu inability to rival established quantitative, kinetic power of PET imaging. This mismatch between solution chemistry and physical decay characteristics is the basis for the proposed development of ^{134}Ce/^{134}La as a positron-emitting analogue of both ^{225}Ac and ^{227}Th. The energetic barrier (124 kJ.mol^{-1}) imposed by the redox potential of Ce(IV)/Ce(III) can only be compensated by strong stabilization of the Ce(IV) ions. The Ce(IV)DTPA/Ce(III)DTPA potential was recently determined as 780 mV *vs* NHE, still a large positive value [15]. Polyaminocarboxylate ligands such as DOTA and DTPA will therefore form stable Ce(III) complexes in aqueous solutions under physiological conditions in the presence of Ce(III) starting reagents, whereas they can also form strong Ce(IV) complexes when mixed with Ce(IV) salts but may not be able to drive the equilibrium either way [22–24]. In contrast HOPO-based ligands will drive the equilibrium towards the formation of stable Ce(IV) complexes, independent of the oxidation state of the starting Ce material, as illustrated by the Ce(IV)3,4,3-LI(1,2-HOPO)/Ce(III)3,4,3-LI(1,2-HOPO) system that exhibits a negative redox potential of -140 mV [25]. Controlling the oxidation state of Ce complexes can therefore be achieved by using different starting Ce(III) or Ce(IV) solutions as well as through the selection of adequate ligands. Based on existing thermodynamic and stability data, we anticipate that DOTA, currently the most sought out ligand for ^{225}Ac chelation in bioconjugates, will provide a perfect example of the Ce(IV)/Ce(III) solution versatility. In addition, the new HOPO-based ligands, which are now the best leads for ^{227}Th bioconjugation, should also display extremely high stabilities for Ce(IV), reinforcing the dual promise of ^{134}Ce/^{134}La as an imaging companion for both ^{225}Ac and ^{227}Th.

Table I: Summary of known Thermodynamic Stability Constants for Complexes of Interest.

Cation	Ionic Radius (CN = 8, pm)	Ionic Radius (CN = 6, pm)	Log β_{110}			
			DTPA	DOTA	3,4,3-LI(1,2-HOPO)	ΦTAM
La^{3+}	116	103	19.5	22.0	16.4	-
Ce^{3+}	114	101	20.4	23.0	17.4	-
Ce^{4+}	97	87	34.0	-	41.5	-
Th^{4+}	105	94	28.7	-	40.1	53.7
Zr^{4+}	84	72	35.8	-	43.1	-
Ac^{3+}	-	112	-	-	-	-

3. Research Plan and Methods

The research team has been chosen for its unique ability to address the complex, multi-disciplinary issues tackled in this proposal. Primary responsibility for nuclear data measurement and radionuclide production (Sections 3.1 – 3.3 below) will fall to Jonathan Engle (LANL cross section measurements, IPF target design), Lee Bernstein (LBNL cross section measurements), and Stosh Kozimor (LANL radiochemical isolation of n.c.a ^{134}Ce). James O'Neil (LBNL) will lead radiochemical separation of LBNL irradiated targets. Evaluations of the chemical stability and binding kinetics of proposed ligand systems will be led by Rebecca Abergel (LBNL), Engle and Abergel will coordinate the use of LANL and LBNL-produced material. Rebecca Abergel will also lead biodistribution experiments designed to demonstrate the equivalence of ^{134}Ce/^{134}La with alpha emitters of interest using μPET (μPET experiments will be led by James O'Neil).

3.1 Nuclear Data Measurement

Any optimal design of a production target for ^{134}Ce requires good knowledge of the production cross sections over the energy range being considered. No available nuclear data presently describes the formation of the ^{134}Ce parent radionuclide. The most readily accessible reactions that lend themselves to scale up entail proton- and deuteron-bombardments of lanthanum targets. Of these two, only high-energy proton beams are available from domestic accelerators with intestines suited to large-scale radionuclide production. Alpha-induced channels require irradiation of isotopically enriched ^{134}Ba, which is a much more expensive and logistically troublesome target. Heavy-ion induced reactions are also plausible, but these capabilities are limited to very few facilities worldwide and achievable beam intensities are always at least 1-2 orders of magnitude lower than those available for proton beams at the DOE's flagship proton accelerators in Los Alamos and Brookhaven. Two measurements of proton-initiated production from lanthanum targets have been reported, placing the rate at 73.2 MBq/μAh (2 mCi/μAh) between 72.1 and 62.1 MeV [26], and a thick target experiment reported the production rate below 61 MeV to be approximately 150 MBq/μAh (4 mCi/μAh) [27] (See Figure 3 below). Replacing the "B slot", or medium energy target in the routine production stack at IPF should yield approximately 150 GBq (4 Ci) of ^{134}Ce in a 4-day irradiation. However, the principle radioisotopic impurity coproduced is ^{135}Ce, which ideally would be minimized to reduce the dose from the proposed generator system. Proposed nuclear data measurements are needed to inform target design and irradiation plans, both to accurately predict yields and to minimize the formation of ^{135}Ce.

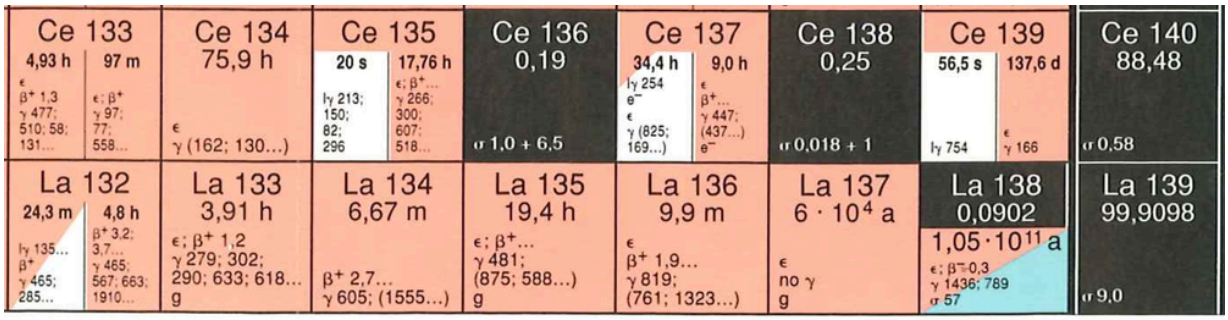

FIG. 3. Table of Isotopes showing mass region of interest.

3.1.1 ^{nat}La(p,6n)^{134}Ce

The calculated threshold for the ^{nat}La(p,6n)^{134}Ce reaction is 49.8 MeV. Available theoretical models predict discrepant energy maxima for this excitation function. Calculation of these cross sections using the TALYS-1.8 [28,29] and EMPIRE-3.2 [30,31] reaction codes differ significantly over the energy range of interest to this proposal. This discrepancy is due in large part to the differences in the pre-compound reaction model being used (see Figure 4). To experimentally characterize the energy range of interest, we propose two sets of measurements, one at the LBNL cyclotron (<60 MeV) and the other at the LANL IPF (100 – 50 MeV). Both facilities have extensive experience making similar measurements [32,33], which offer an ideal opportunity to engage students and postdocs in the fundamental description of nuclear reactions through direct measurement.

The discrepancies in predictions from the best modern codes have profound implications for the design of targets for not only the production of ^{134}Ce, but also for the production of other radionuclides at the Isotope Production Facility at LANL and the Brookhaven Linear Isotope Production facility (BLIP) utilizing energetic proton-induced reactions. The standard approach for calculating the production of radionuclides using (p,xn) reactions is to use the semi-classical ALICE model. However, the reliability of ALICE is shown to be suspect for beam energies above 50 MeV [34]. Figure 5 shows the ratio of the calculated cross sections for the ^{139}La(p,xn) reaction for x≤2 using EMPIRE where the hybrid monte-carlo simulation pre-equilibrium particle emission model is "toggled" via the HMS parameter. The differences are profound.

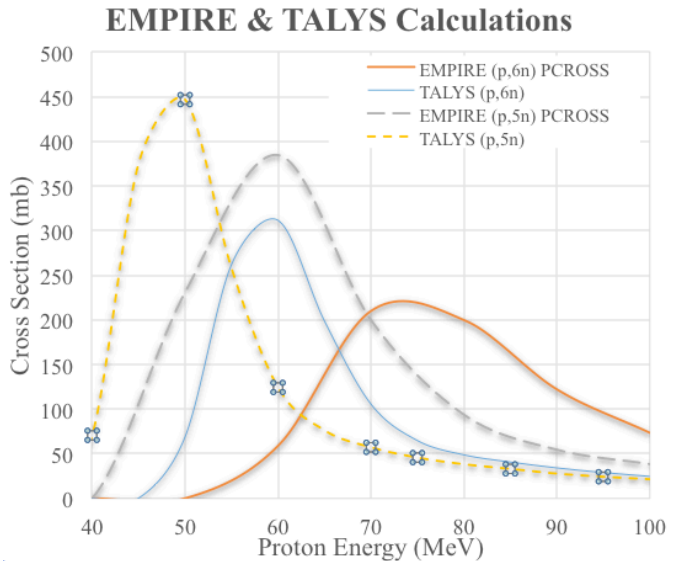

FIG. 4. Plot of TALYS / EMPIRE excitation functions for ^{nat}La(p,6n)^{134}Ce, ^{nat}La(p,5n)^{134}Ce.

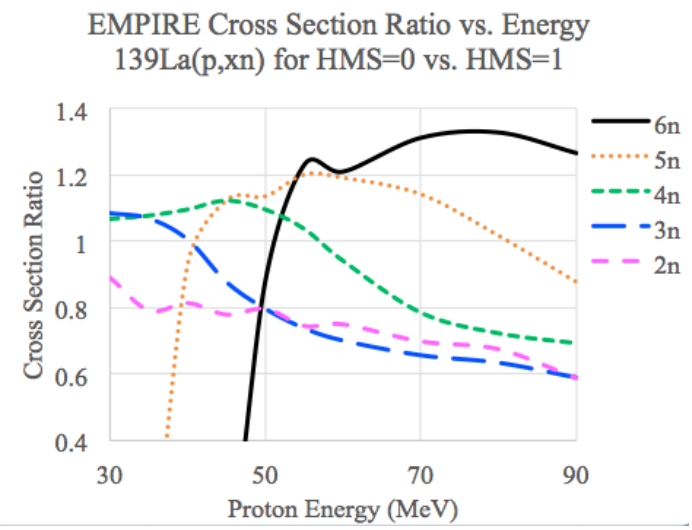

FIG 5. EMPIRE calculated cross section ratios between results toggling the binary states of the HMS parameter.

To resolve these discrepancies, standard stacked foil techniques [33,35,36] will be employed at IPF and the LBNL cyclotron facilities, acquiring approximately 20 energy points for all proton-induced reactions on effectively monoisotopic targets of lanthanum. In this experiment the beam will be made incident on a "foil pack" of La+Cu+Ti+Nb targets interleaved with Al degraders in order to perform cross section measurements via activation over a range of incident particle energies simultaneously.

Table II below describes a potential, non-optimized experimental set-up at LBNL. The thickness of the "foil pack" and aluminum beam degraders will be chosen so as to ensure that the energy straggle of the beam does not exceed 150 keV over the entire range of the experiment. The LANL-LBNL-BNL-ORNL collaboration carried out the first of these experiments at the 88-Inch cyclotron in April of 2016 using a stack of Cu+Ti fluence monitor foils interleaved with Al degraders. The foils were counted using the HPGe detector at the 88-Inch cyclotron which has an approximate photopeak efficiency @ 1.33 MeV in excess of 5% at the front surface. Data collected in this experiment is currently being analyzed by a graduate student who will be involved in this project (Andrew Voyles).

Table II: Stack Design for Example LBNL 60 MeV Irradiation.

Foil Number	Foils (w/thickness)	Energy In (MeV)	Energy Out (MeV)	Straggle (keV)
1	Cu+Ti+La+Nb (~20μm)	60	59.65	14
2	Al (2 mm)	59.65	54.89	50
3	Cu+Ti+La+Nb (~20μm)	54.89	54.52	53
4	Al (2 mm)	54.52	49.57	73
5	Cu+Ti+La+Nb (~20μm)	49.57	49.17	75
6	Al (2 mm)	49.17	43.57	93
7	Cu+Ti+La+Nb (~20μm)	43.57	43.13	95
8	Al (2 mm)	43.13	37.09	146
9	Cu+Ti+La+Nb (~20μm)	37.09	36.6	147

At both facilities proton irradiations of approximately 100 nAh (integrated current) will produce small amounts of several radionuclides in thin cerium films. The activated area of these foils will be carefully matched to calibrated source geometries for HPGe gamma spectrometry, which is an established capability at both facilities. The foil packs will be removed post-shot and their activities determined through high-resolution gamma-ray spectroscopy using the aforementioned HPGe at the 88-Inch cyclotron. A minimum of 30 minutes will be required to transfer the foils to the counting facility, making it impossible to measure activities with lifetimes shorter than 3 minutes. The foils will be placed at least 10 cm from the front face of the HPGe to minimize summing corrections for coincident gamma-rays.

Table III below lists the three strongest decay γ-rays for the activities of interest produced in the irradiation ($^{137\text{-}134}Ce$ and $^{134\text{-}136}La$). The last column lists the initial decay rates for a 25 μm thick La foil that has been irradiated for 1 hour with a beam current of 1 μA assuming a 100 mb production cross section. The relatively low gamma-ray production rate of ^{134}Ce relative to ^{135}Ce may necessitate that the targets be counted far from the HPGe for the first several days prior to being moved in closer to obtain good statistics for the ^{134}Ce.

Table III: Nuclear decay data including principle gamma emissions which will be used for residuals' quantification following proposed proton irradiations of lanthanum.

Nuclide	Half Life	Formation Pathway	Gamma-ray (keV)	Intensity (%)	Initial Decay rate/100 mb (kBq)
^{134}Ce	3.16(4) d	(p,6n)	162.306 (10)	0.230 (16)	0.953
			130.414 (15)	0.209 (15)	0.866
			323.59 (5)	0.0156 (16)	0.647
^{135}Ce	17.7(3) h	(p,5n)	265.56 (2)	41.8 (14)	742
			300.07 (2)	23.5 (5)	417
			606.76 (2)	18.8 (6)	334
^{136}Ce	Stable	(p,4n)			
^{137}Ce	9.0(3) h	(p,3n)	447.15 (8)	1.68 (*)	58.7
			436.59 (3)	0.250 (12)	8.73
			915.80 (13)	0.0486 (24)[1]	1.7
^{137m}Ce	34.4(3) h	(p,3n)	254.29 (5)	11.1 (4)	101
^{138}Ce	Stable	(p,2n)			
^{139}Ce	137.641(20) d	(p,n)	165.8575 (11)	80 (8)	7.61
^{134}La	6.45(16) m (sec. equilibrium w/ ^{134}Ce)	^{134}Ce β^-	604.721 (2)	5.04 (20)	20.9
			563.246 (5)	0.362 (14)	1.5
			1483.52 (3)	0.145 (6)	0.145
^{135}La	19. (2) h (sec. equilibrium w/ ^{135}Ce)	^{135}Ce β^-	480.51 (2)	1.52 (*)	27
			874.51 (2)	0.16 (3)	2.84
			587.83 (2)	0.11 (18)	1.95
^{136}La (likely unseen)	9.87(3) m	(p,p3n)	818.51 (4)	2.30 (*)	219
			1322.99 (4)	0.265 (12)	25.2
			1310.41 (7)	0.099 (5)	0.00942

The suite of residuals quantified in each foil will be used to calculate the absolute, energy-dependent probability of the relevant nuclear reactions for arbitrary proton-irradiation scenarios. Investigators for this project are experienced in these measurements and in the problems that typically arise when they are undertaken. In addition to generating absolute cross sections to facilitate production of ^{135}Ce, the measurements will also help improve reaction modeling in this mass region by providing insight into the pre-equilibrium reaction mechanisms that play such an important role in this energy region. The results

will be analyzed and published in the appropriate peer-reviewed nuclear science and/or radiochemistry journal.

3.2 ^{134}Ce/^{134}La Large Scale Production

Using data gathered in the experiments above, 2 IPF targets will be designed with optimal incident and exit energies, maximizing yield while minimizing the formation of ^{135}Ce and target mass to simplify subsequent radiochemical processing. Previous experience at large facilities has shown that lanthanide targets typically perform well in Inconel-encapsulated environments, where integrated beam currents of over 5000 μAhrs have been accumulated on dysprosium and promethium targets (see, e.g., [37]). Following proposed nuclear data measurements, we will design an energy-optimized ("A" or "B" slot) IPF target for irradiation to test integrated yields in the energy region designated by our cross section measurements and to assist in the development of radiochemical separations techniques that can be applied at large scale.

In year 2 of this proposal we will produce ^{134}Ce locally at LBNL to facilitate the bio-uptake work being performed by the PI (Abergel). Additional thick lanthanum foils have been included in the procurement budget for this purpose. The foils will be transported to the Berkeley hot cells located in the lab run by James O'Neil at the LBNL site. Following dissolution and separation the Ce samples will be delivered to Rebecca Abergel's lab. We envision at least three production runs at the 88-Inch cyclotron for this purpose.

3.3 Production Radiochemistry

Advancing chemical methodologies and capabilities in lanthanide separations represents a longstanding challenge associated within the DOE Isotope Program's missions in isotope production. For instance, developing high-through-put production schemes for lanthanides that are compatible with processing restrictions associated with hot-cell facilities offers widespread opportunity to access valuable lanthanide isotopes, in general. Some areas identified in *The 2015 Long Range Plan for the DOE-NP Isotope program* include production of neutron-deficient lanthanide tracers (^{148}Eu, ^{149}Eu) and purification of multi-milligram to multi-gram quantities of long-lived lanthanide fission (i.e. ^{139}La, ^{151}Sm) present in MK-42 targets. As described herein, another emerging production area that would benefit from new methods in lanthanide processing techniques is establishing methods in PET that support preclinical and clinical evaluations of alpha emitting nuclides.

As described above, among the few candidates that could serve as PET surrogates for the valuable ^{225}Ac and ^{227}Th alpha emitting isotopes, the ^{134}Ce/^{134}La ($t_{1/2}$ = 75.9 h / 6.67 m, 2.7 MeV β^+) pair is particularly attractive. Unfortunately, purification of the ^{134}Ce and ^{134}La isotopes from the ^{139}La target material represents one of the most difficult separations known in inorganic chemistry. The challenge lies in separating adjacent lanthanides, as these 4*f*-elements share many chemical properties. For example, the trivalent oxidation state is common across the lanthanide series and 4*f*-element reactivity is dominated by their hard oxophilic properties [38–40]. The most common approach used in lanthanide separations involves combining cation exchange chromatography with an appropriate chelating agent (*e.g.* α-hydroxyisobutyric acid; HIB) in the mobile phase [41]. For many reasons this ion exchange approach is not compatible with ^{134}Ce/^{134}La production. For instance, the large mass of ^{139}La target would require an enormous column and require days to complete the HPLC based separation. This strategy would also produce prohibitively large quantities of mixed waste in the form of spent mobile phase used during the separation.

To overcome these challenges, we present here a three-step alternative (Figure 6). Step 1 exploits the

accessibility of the Ce^{III}/Ce^{IV} redox properties to remove the majority of the ^{139}La target material. Step 2 involves a small (gravity feed) ion exchange column designed for finely purifying the ^{134}Ce sample from any residual ^{139}La. Finally, step 3 involves Ce^{III}/Ce^{IV} valence adjustments to generate oxidation state pure Ce^{III} or Ce^{IV} stock solutions used in the subsequent chelation/*in-vivo* studies. Upon completion of ^{134}Ce production, the ^{134}La daughter produce will re-emerge as the ^{134}Ce decays.

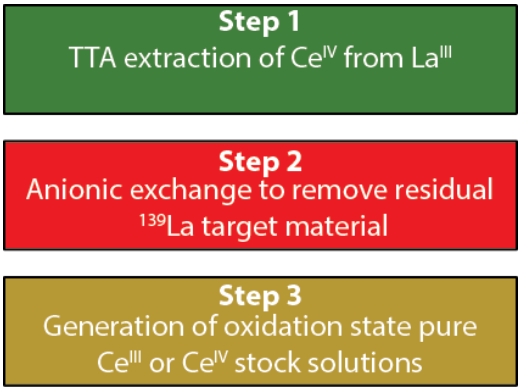

FIG 6. Schematic showing the proposed strategy to purify $^{134}Ce/^{134}La$ from the ^{nat}La target material.

To remove ^{134}Ce from the majority of the ^{139}La target material, we will exploit well-known cerium oxidation chemistry. It is well known that Ce^{IV} can be generated in aqueous media using simple oxidizing agents (i.e. sodium bromate, $NaBrO_3$). After generating the $Ce^{IV}_{(aq)}$ it is established that Ce^{IV} can be separated from lanthanides via liquid/liquid extraction into a variety of common solvents. Among the many possible Ce^{IV} extraction procedures – which range from using ethereal solvents [42], tributyl phosphate (TBP) [43], butyl acetate [44], and methyl isobutyl ketone [45] – that which involves thenoyltrifluoroacetone (TTA) [46] seems especially attractive. In the original report, Smith and Moore demonstrated that Ce^{IV} was effectively separated from fission products (including trivalent lanthanides, i.e. ^{140}La). The Ce^{IV} was first extracted into xylene solutions containing TTA (0.5 M) from sulfuric acid (1 M) solutions of sodium bromate. Subsequently, the Ce^{IV} was back extracted into nitric acid (10 M). Based on recent coordination chemistry studies carried out using macroscopic quantities of stable cerium, we propose that the extraction procedure involves generating the neutral $Ce^{IV}(TTA)_4$ complex, which we have recently characterized by single crystal X-ray diffraction (Figure 7). The neutral complex passes into the organic phase. Subsequent addition of HNO_3 converts $Ce^{IV}(TTA)_4$ to the anionic $Ce(NO_3)_6^{2-}$ anionic complex, well-established in the solid state [47] and soluble in aqueous media. This $Ce(NO_3)_6^{2-}$ is back extracted into nitric acid. At this stage in the purification process, the solution matric is poised for further purification by anion exchange chromatography (Figure 6, Step 2). For example, it is well established that Ce^{IV} can be retained on an anion exchange resin (K_d = 220; 8 M), while persistent +3 lanthanides will have no absorption. Hence, passing the solution through a small (gravity fed) anion exchange resin will allow the $^{139}La^{III}$ target material to be easily removed. Alternatively, there are numerous accounts that suggest Ce^{IV} can be separated from lanthanides using the commercially available DGA resin (see, e.g., [48] and references therein). Elution of the $Ce^{IV}(NO_3)_6^{2-}$ from either the anion or DGA column using dilute nitric acid will provide the Ce^{IV} stock solution needed in Step 3. Alternatively, addition of hydroxylamine hydrochloride (which is well-established for reducing aqueous solutions of Ce^{IV} to Ce^{III}) can be added to force Ce^{III} to elute from the columns, thereby generating a +3 cerium stock solution.

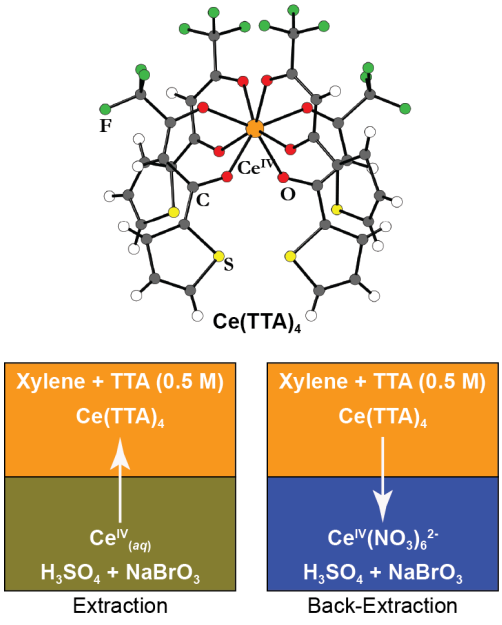

FIG 7. Top: "Ball and stick" representation of the $Ce(TTA)_4$ coordination complex. Bottom: schematic showing Ce^{IV} extraction/back-extraction chemistry.

3.4 Stability and Kinetics of Ce^{III}/Ce^{IV} Complexation

Metal complexes can be prepared *in situ* or isolated by combining the free ligands and metal salts in defined pH conditions before filtering and crystalizing the resulting compounds. Chemical and structural characterization of these complexes will be performed as thoroughly as possible (NMR, UV-Visible, FT-IR, mass spectrometry, X-ray crystallography, X-ray absorption fine structure spectroscopy). The formation of the desired Ce(III) or Ce(IV) complexes in situ when using Ce(III) or Ce(IV) stock solutions will be confirmed spectroscopically.

An important desired characteristic of the proposed surrogate imaging pair is its ability to perfectly mimic the kinetic and thermodynamic stabilities of complexes formed between the therapeutic ^{225}Ac(III) or ^{227}Th(IV) and ligands currently used for bioconjugation (DTPA, DOTA, HOPO). To establish a complete data set enabling comparisons between the different metal/ligand combinations, thermodynamic stability constants will be determined in aqueous solutions for the Ac complexes of all ligands as well as for the Ce(IV) and Th(IV) DOTA complexes (the constants for other metal/ligand combinations have already been reported, see Table I). The longer-lived ^{227}Ac and ^{232}Th isotopes as well as cold Ce will be used in this case, to allow for equilibrium formation. The use of the Bjerrum method for metal complex stability measurement (pH titrations of ligand and metal + ligand) continues to be the standard procedure but it has limitations, particularly for complicated systems with more than one complex species in solution, very stable complexes, or very radioactive samples. Since most metal-ligand complexation reactions give rise to changes in the UV-Vis and luminescence spectra relative to the parent metal and ligand species, spectrophotometric and fluorometric techniques can be used to monitor these reactions at reasonably low concentrations. Identification of the species in solution during the titrations as well as determination of the correlated individual spectra are then achieved through factor analysis, and least squares refinements provide the corresponding thermodynamic constants. To minimize the amount of ^{227}Ac used in these measurements, metal competition titrations will be used, as previously described for other f-elements [5,15,25].

Finally, in addition to thermodynamic stability, the ligands should display fast kinetics of metal binding, for improved targeting construct preparation, and slow kinetics of dissociation, for enhanced *in vivo* stability and minimal bleeding of nuclide from the conjugate. The kinetics of Ce(III) or Ce(IV) coordination with the three common ligands and metal exchange in aqueous solutions will be followed spectroscopically, as well as their pH and temperature behaviors. Comparisons will be established between cold Ce solutions and ^{134}Ce, which should rapidly decay into ^{134}La(III) (independent of the starting ^{134}Ce oxidation state) and give rise to differences in behavior we can exploit to understand an utilize the similarities with thorium and actinium alpha-emitters.

3.5 Biodistribution and PET Studies

There is no direct method to image the biodistribution of alpha-emitting isotopes ^{225}Ac and ^{227}Th in order to accurately evaluate the in vivo equivalence of imaging surrogates such as ^{89}Zr or the proposed $^{134}Ce/^{134}La$ pair. Ex-vivo biodistribution studies relying on radioactive detection are the most precise mean of comparing the respective burdens of therapeutic radiometals with those of imaging radioisotopes. To probe the biological behaviors of ^{89}Zr chelates and bioconjugates, direct comparisons have recently been made between PET images and radioactive counts of ^{89}Zr-Ligand complexes (with Desferrioxamine B or 3,4,3-LI(1,2-HOPO) as the ligand (Figure 2) and bifunctional ^{89}Zr-Ligand-trastuzumab derivatives [20,21]. These studies demonstrated the relevant use of radioanalysis to compare the uptake and clearance patterns of the various chelate entities and to support the PET images in mice. To investigate potential differences attributable to the varied radiometal oxidation states, we propose to perform biodistribution

studies using standard counting methods in parallel with microPET imaging studies similarly to these ^{89}Zr published studies.
We will examine the biodistribution of ^{225}Ac(III) complexed to those most standard ligands currently envisioned for targeted therapy, DTPA and DOTA. The ^{227}Th(IV) biodistribution study will be carried out with DOTA as well (to mirror initial preclinical uses) but also to the emerging ligand 3,4,3-LI(1,2-HOPO), which is a member of the HOPO family of ligands that is currently under investigation by several pharmaceutical companies and academic groups [5,18–21]. To parallel the biodistribution studies, PET images will be acquired with the presumed equivalent complexes formed with Ce(III) in the case of ^{225}Ac(III) or with ^{134}Ce(IV) in the case of ^{227}Th(IV). We will also use ^{89}Zr(IV) as a control imaging isotope surrogate to evaluate any differences between ^{134}Ce(IV) and ^{89}Zr(IV) when employed as ^{227}Th(IV) imaging companions.
Those studies will be performed with the metal chelates (not the bioconjugates) as they constitute the primary building block for alpha-therapy constructs. It is likely that linkage to targeting vectors further affects the radiometal distribution, however our focus here is on metal complex stability and equivalence between therapeutic and imaging isotopes. Our primary goal is to ensure that the proposed Ce/La pair is an adequate PET imaging surrogate for the therapeutic isotopes, which will be revealed through these preliminary complex stability and biodistribution experiments. Further studies with selected bioconjugates for clinical applications will then be necessary but are beyond the scope of this work. The proposed biodistribution and microPET imaging study designs are detailed below.

3.5.1 ^{225}Ac and ^{227}Th complex biodistribution studies

The *in vivo* stability and biodistribution of those selected ^{225}Ac and ^{227}Th complex systems will be determined in healthy mice by liquid scintillation counting and/or gamma counting, using standard methods developed in the LBNL BioActinide laboratory [49,50]. Briefly, groups of mice (n = 4) will be exposed intravenously (tail vein injection) with one of four selected ligand-radionuclide complexes. Mice will be housed per groups of 4 in disposable stock cages and excreta will be collected daily post intervention until scheduled necropsy (30 min, 1, 4, 24, 48 h, 4 and 8 d). Full necropsies will be conducted and urine, feces, blood, and organs collected for analysis by gamma counting. All samples will then be processed to heat and chemical treatments, and subsequently re-analyzed by liquid scintillation and gamma counting. These methods will enable full metabolic balance on the injected radiometals, taking into account decay products. The general design for these studies is presented in Table IV. Particular attention will be paid to any tissue uptake of the radionuclides, which would indicate release of the free metal cation, revealing instability of the complexes tested and will be important in the comparisons with imaging companion isotopes.
For each radiometal (^{225}Ac or ^{227}Th), 20 groups (14 female and 6 male) will be formed (2 different ligands, 7 necropsy time points for female groups and 3 necropsy time points for male groups). We plan to compare groups pairwise following a one-way ANOVA statistical model. Considering a maximum of 190 pairwise comparisons and a sampling ratio of 1, a power analysis was performed at the one-sided 0.05 level of significance. Based on previously published studies investigating the in vivo stability of several radiometal complexes in the proposed mouse model, the standard deviation for our dependent variable (% injected isotope dose per gram of tissue, as assessed by radioanalysis) is 0.5 units. We would be interested in any differences between samples greater than 0.3 units (effect of 0.6). Assuming equal variability and sample size in the different complexes, an alpha of .05, and power of .80, we determined that we would need 4 animals per group to detect an effect of 0.6.

Table IV. Study Design for Biodistribution Evaluation of Ac and Th Complexes in Normal Mice[a, b]

Group[d]	Intervention	Challenge Dose (nCi)[c]	#Animals/ group	Route	Scheduled Necropsy
A1 – A7	^{225}Ac(III)-DTPA	50	4F	iv	30 min, 1, 4, 24, 48 h, 4 and 8 d
A8 – A10	^{225}Ac(III)-DTPA	50	4M	iv	1 h, 24 h, and 4 d
B1 – B7	^{225}Ac(III)-DOTA	50	4F	iv	30 min, 1, 4, 24, 48 h, 4 and 8 d
B8 – B10	^{225}Ac(III)-DOTA	50	4M	iv	1 h, 24 h, and 4 d
C1 – C7	^{227}Th(IV)-DOTA	50	4F	iv	30 min, 1, 4, 24, 48 h, 4 and 8 d
C8 – C10	^{227}Th(IV)-DOTA	50	4M	iv	1 h, 24 h, and 4 d
D1 – D7	^{227}Th(IV)-HOPO	50	4F	iv	30 min, 1, 4, 24, 48 h, 4 and 8 d
D8 – D10	^{227}Th(IV)-HOPO	50	4M	iv	1 h, 24 h, and 4 d
Total: 28F groups/84F mice and 12M groups/36M mice					

[a]Intervention is achieved by intravenous injection in a warmed lateral tail vein of 0.2 mL of the challenge radiometal complex in MES buffer, pH 6.8.
[b]Whole animal, blood, and tissue challenge isotope content are determined at scheduled necropsy time points. Excreta are collected daily until necropsy.
[c]Based on published studies using these or similar isotopes.
[d]Groups 1 to 7 and 8 to 10 correspond to respective necropsy time points (7 time points and 3 time points for female and male groups, respectively). A similar nomenclature is applied to each intervention.

3.5.2 $^{134}Ce/^{134}La$ microPET equivalence studies

The *in vivo* stability and biodistribution of those selected ^{134}Ce and ^{89}Zr complex systems will be determined in healthy mice by microPET imaging. Briefly, groups of mice (n = 5) will be exposed intravenously (tail vein injection) with one of six selected ligand-radionuclide complexes. Mice will be housed per groups of 5 in disposable stock cages and microPET imaging experiments will follow the pharmacokinetics of the injected complexes. Excreta will be collected daily and until scheduled necropsy 8 days after the intervention. Mice will be anesthetized during the acquisition of PET images and data will be recorded via static scans at seven scheduled time points between 30 min and 8 d after the intervention. Full necropsies will be conducted and urine, feces, blood, and organs collected for analysis by gamma counting. All samples will then be processed to heat and chemical treatments, and subsequently re-analyzed by liquid scintillation and gamma counting. The general design for these studies is presented in Table V.

Table V. Study Design for Biodistribution Evaluation of Zr and Ce Complexes in Normal Mice[a, b]

Group[d]	Intervention	Challenge Dose (μCi)[c]	#Animals/ group	Route	microPET Imaging Time Points	Scheduled Necropsy
E1 – E2	^{134}Ce(III)-DTPA	250	5F/5M	iv	30 min, 1, 4, 24, 48 h, 4 and 8 d	8 d
F1 – F2	^{134}Ce(III)-DOTA	250	5F/5M	iv	30 min, 1, 4, 24, 48 h, 4 and 8 d	8 d
G1 – G2	^{134}Ce(IV)-DOTA	250	5F/5M	iv	30 min, 1, 4, 24, 48 h, 4 and 8 d	8 d
H1 – H2	^{134}Ce(IV)-HOPO	250	5F/5M	iv	30 min, 1, 4, 24, 48 h, 4 and 8 d	8 d
J1 – J2	^{89}Zr(IV)-DOTA	250	5F/5M	iv	30 min, 1, 4, 24, 48 h, 4 and 8 d	8 d
K1 – K2	^{89}Zr(IV)-HOPO	250	5F/5M	iv	30 min, 1, 4, 24, 48 h, 4 and 8 d	8 d
Total: 6F groups/30F mice and 6M groups/30M mice						

[a]Intervention is achieved by intravenous injection in a warmed lateral tail vein of 0.2 mL of the challenge radiometal complex in MES buffer, pH 6.8.
[b]Whole animal, blood, and tissue challenge isotope content are determined at one unique time point: 8 days post metal challenge. Excreta are collected daily post intervention until necropsy.
[c]Based on Zr-89 injection levels used in published mouse PET imaging studies.
[d]Groups 1 and 2 correspond to female and male groups.

In this study, 12 groups (6 female and 6 male) will be formed (3 different radiometals or oxidation states, and 2 ligand per metal condition). We plan to compare groups pairwise following a one-way ANOVA statistical model. Considering a maximum of 66 pairwise comparisons and a sampling ratio of 1, a power analysis was performed at the one-sided 0.05 level of significance. Based on previously published studies investigating the biodistribution of ^{89}Zr complexes through microPET imaging in mice, the standard deviation for our dependent variable (% injected isotope dose per gram of tissue) is 0.5 units. We would be interested in any differences between samples greater than 0.3 units (effect of 0.6). Assuming equal variability and sample size in the different complexes, an alpha of .05, and power of .80, we determined that we would need 5 animals per group to detect an effect of 0.6.

3.6 Training

The Isotope Program at LANL and the Heavy Element Chemistry Group at LBNL play important roles in educating young scientists in nuclear physics, radiochemistry and f-element coordination chemistry. The goal of training graduate and undergraduate students and postdoctoral scholars is not just to expose them to the intellectual issues associated with research in these areas but also to provide them with a working laboratory environment. The proposed project will add a unique collaborative component to existing programs, combining expertise and skills in nuclear data measurement, radionuclide production and accelerator targetry, radiochemistry and pharmacokinetics. It will provide the fundamental basis to prepare early-stage scientists for future leadership roles in academic, governmental, and industrial positions, particularly in areas related to isotope production, nuclear medicine, nuclear energy and security. Regular weekly subgroup meetings are held within the respective research groups led by each principal investigator, in addition to monthly seminar series in the LBNL Heavy Element Chemistry group, the LANL Radiochemistry Division, the LBNL Nuclear Physics Division, and the UCB Nuclear Engineering Department. Weekly research meetings are held in the Kozimor and Engle research groups, as well as weekly and intermittent research gatherings in the Isotope Program at LANL, LANSCE, and LANL Chemistry and Physics Divisions.

A new LANL postdoc will be hired to work on this effort with a focus on radiochemical separation, and will be jointly mentored by Engle and Kozimor. The postdoc will have meetings with both advisors, in addition to regularly updating the Isotope Team on their progress and having access to significant additional expertise in the Isotope Team and LANL Chemistry Division. This postdoc will travel to LBNL approximately twice per year during the project to transfer knowledge on radiochemical separations and to participate in experiments.

The student who will work on this effort at LBNL attends weekly meeting with his thesis advisor (Bernstein). He is also a member of the Joint LBNL/UCB Nuclear Data Group and has regular access to three scientists with a combined 50+ years of experience in nuclear physics, gamma-ray spectroscopy and nuclear data evaluation. Student and postdoctoral trainees will undertake the preponderance of effort proposed for this work. They will do so with access to established scientists and successful mentors at LANL and LBNL.

4. Timetable

	FY 2018				FY 2019			
Nuclear Excitation Function Measurements								
Procurement and Experiment Planning	■	■						
LBNL Irradiation (<60 MeV)		■						
Spectrometry/Analysis of LBNL foils			■	■				
LANL Irradiation (<100 MeV)			■					
Spectrometry/Analysis of LANL foils				■	■			
Publish Excitation Functions						●		
Large Scale Production and Radiochemical Isolation								
Design IPF Target					■			
Irradiate IPF targets, test radiochemistry						■	■	
Procurement and Planning	■							
Tracer-scale Radiochemistry Development		■	■	■	■			
Publish Radiochemistry of Irradiated IPF Target								●
Stability and Kinetics of Ce/La Complexes and Relevance to Alpha-Emitter Biology								
Metal Complex Characterization	■	■						
Solution Thermodynamic Stability Studies	■	■	■					
Kinetic Stability Studies			■	■	■	■		
Publish In Vitro Thermodynamic and Kinetic Stability Results							●	
Ac and Th Biodistribution Studies			■	■	■			
Zr and Ce Biodistribution Studies (microPET)						■	■	■
Publish Biodistribution Equivalence Results								●

5. Project Management Plan

LANL and LBNL teams will be led by Jonathan Engle and Rebecca Abergel, respectively. Primary responsibility for nuclear data measurement and radionuclide production (Sections 3.1 – 3.3 below) will fall to Jonathan Engle (LANL cross section measurements, IPF target design), Lee Bernstein (LBNL cross section measurements), and Stosh Kozimor (LANL radiochemical isolation of n.c.a ^{134}Ce). Radiochemical separation of LBNL irradiated targets will be led by James O'Neil (LBNL). Evaluations of the chemical stability and binding kinetics of proposed ligand systems will be led by Rebecca Abergel (LBNL), coordinating the use of LANL and LBNL-produced material. Rebecca Abergel will also lead biodistribution experiments designed to demonstrate the equivalence of ^{134}Ce/^{134}La with alpha emitters of interest using µPET (µPET experiments will be led by James O'Neil).

We plan monthly conference calls involving all project participants, as well as smaller meetings with interested parties especially to plan the logistical details of LBNL and IPF irradiations, meet radiochemical processing milestones, and optimize the design and utility of biodistribution and µPET experiments.

All research groups meet at least weekly with students and postdocs to ensure that students receive needed mentorship and make consistent progress towards research goals. The project will provide an excellent opportunity for students to enjoy collaborative interaction with colleagues at involved institutions, and transfer of radiochemical separation processes and experimental data will necessarily occur routinely through already established collaborative channels.

6. Project Objectives

6.1 Cross section measurements describing proton-induced reactions on ^{nat}La

6.2 Development of radiochemical isolation technique for recovery of n.c.a ^{134}Ce from irradiated ^{nat}La

6.3 Irradiation and radiochemical processing of IPF target

6.4 Development of complexing schemes for $^{134}Ce/^{134}La$ using established chelation ligands

6.5 Demonstration of $^{134}Ce/^{134}La$ *in-vivo* equivalence to alpha emitters ^{227}Th and ^{225}Ac via biodistribution and μPET experiments.

Appendix 1. Biographical Sketches

Jonathan W Engle

Education and Training:

Degree Earned	Institution	Major Area	Degree and Year
Undergraduate	Colorado College	Religion: Islamic Pol. Sci.	B.A. 2002
	University of Utah	Physics	B.S. 2007
Graduate	University of Utah	Science Education	M.Ed. 2006
	University of Wisconsin	Medical Physics	M.S. 2009
	University of Wisconsin	Medical Physics	Ph.D. 2011
PostDoc	Los Alamos National Laboratory	Radioisotope Production	2012-2014

Research and Professional Experience:

Dates	Position and Location
2014 – present	Scientist, Isotope Production Program, LANL
2012 – 2014	Frederick Reines Postdoctoral Fellow, Isotope Production Program, LANL
2011 – present	Associate, Cyclomedical Applications Group, Knoxville, Tennessee
2007 – 2011	Cyclotron Jockey & Graduate Researcher, University of Wisconsin, Madison, Wisconsin
2005 – 2007	Laboratory Technician, Utah Center for Advanced Imaging Research, Salt Lake City

Selected Publications (of 58):

Engle J W, Mashnik S G, Parker L A, Jackman K R, Bitteker L B, Ullmann J L, Gulley M S, Pillai C, John K D, Birnbaum E R, and Nortier F M (**2015**). Nuclear excitation functions from 40-200 MeV proton irradiation of terbium. *Nuclear Instruments and Methods in Physics Research, Section B*. Accepted, October 2015.

Engle J W, James M R, Mashnik S G, Kelsey C T IV, Wolfsberg L E, Reass D A, Connors M A, Bach H T, Fassbender M E, John K D, Birnbaum E R, Nortier F M (**2014**). MCNPX characterization of the secondary neutron flux at the Los Alamos Isotope Production Facility. *Nuclear Instruments and Methods in Physics Research, Section A*. 754, 71-82.

Engle J W, Weidner J W, Ballard B D, Fassbender M E, Hudston L, Jackman K, Dry D, Wolfsberg L, Bitteker L J, Ullmann J L, Gulley M S, Pillai C, Birnbaum E R, John K D, Mashnik S G, Nortier F M (**2014**). Ac, La and Ce radioimpurities in ^{225}Ac produced in 40-200 MeV proton irradiations of thorium. *Radiochimica Acta*, 102(4), 325-332.

Engle J W, Mashnik S G, Weidner J W, Fassbender M E, Bach H T, Ullmann J L, Couture A J, Bitteker L J, Gulley M S, John K D, Birnbaum E R, Nortier F M (**2013**). Fission fragments produced from proton irradiation of thorium between 40 and 200 MeV. *Transactions of the American Nuclear Society* 109, 14-17.

Engle J W, Mashnik S G, Weidner J W, Wolfsberg L E, Fassbender M E, Jackman K, Couture A, Bitteker L J, Ullmann J L, Gulley M S, Pillai C, John K D, Birnbaum E R, and Nortier F M (**2013**). Cross sections from proton irradiation of thorium at 800 MeV. *Physical Review C* 88, 014604.

Engle J W, Birnbaum E R, Nortier F M, Rau J A, Trellue H R (**2013**). Purification of ^{242}Pu by irradiation with thermal neutrons. *Nuclear Instrumentation Methods in Physics Research B*, 298, 70-75.

Engle J W, Mashnik S G, Bach H, Couture A, Jackman K, Gritzo R, Ballard B D, Fassbender M, Smith D M, Bitteker J L, Gulley M S, Pillai C, John K D, Birnbaum E R, Nortier F M (**2012**). Cross sections from 800 MeV proton irradiation of terbium, LA-UR-12-22703. *Nuclear Physics A*, 893, 87-100.

Engle J W, Hong H, Zhang Y, Valdovinos H F, Yang Y, Barnhart T E, Theuer C P, Nickles R J, Cai W (**2012**). Positron emission tomography imaging of tumor angiogenesis with a ^{66}Ga-labeled monoclonal antibody, *Molecular Pharmaceutics* 9, 1441-1448.

Engle J W, Lopez-Rodriguez V, Gaspar-Carcamo R E, Valdovinos H F, Valle-Gonzalez M, Trejo-Ballado F, Severin G W, Barnhart T E, Nickles R J (**2012**). Very high specific activity $^{66/68}Ga$ from zinc targets for PET. *Applied Radiation and Isotopes* 70(8), 1792-1796.

Synergistic Activities:

Special International Industrial Partnerships:

- Since 2011, I have been an Associate of the Cyclomedical Applications Group, which has contracted my services with regards to the installation and operation of several global radionuclide production facilities, including sites in the US, Peru, Switzerland, Brazil, Algeria.
- I am the sole United States representative for Scansys®, a Danish company specializing in automated radiochemistry systems custom-fabricated for research use.

Professional Society and Scientific Committee Memberships:

- International Advisory Committee Member for the International Workshop on Targetry and Target Chemistry (2014 – present); Chairman of the 2016 meeting in Santa Fe, New Mexico
- Los Alamos Neutron Science Center, Users Group Executive Committee (2014 – present)
- Scientific Organizing Committee, 15th International Workshop on Targetry and Target Chemistry
- American Nuclear Society (2014 – present)
- Society for Nuclear Medicine (2008 – present)
- International Society of Radiopharmaceutical Science (2009 – present)

Awards:

- Frederick Reines Postdoctoral Fellowship in Experimental Sciences, 2013-2014
- EJNMMI 2012 Best Basic Science Paper (*EJNMMI 2012, 39(1): 138-148*).
- Bursary to the 14th Workshop on Targetry and Target Chemistry, Playa Del Carmen, 2012
- US DOE Travel Award to Int Symposium on Radiopharmaceutical Sciences, Amsterdam, 2011
- General Electric PETtrace Cyclotron Level I Service Engineer Training
- NIH Institutional Training Grant, Univ. of Wisc. Radiological Sciences Training Program, 2010-2011.
- US DOE Bursary to the International Symposium on Radiochemical Sciences, Edmonton, 2009
- Bursary to the Practicum of the 12th International Targetry Workshop, Seattle, 2008
- University of Utah College of Education Shizuko/Nakagawa Scholarship, 2005, 2006
- Colorado College Grant 1998-2002
- Lockheed Martin/National Merit Scholarship 1998-2002
- Colorado College Trustee Scholarship 1998-2002

Reviewing:

Biomaterials (2015 – present), Theragnostics (2014 – present), Bioconjugate Chemistry (2014 – present), Nuclear Instruments and Methods in Physics Research B (2013 – present), DOE Small Business Innovation Research Grants (2012 – present), Medical Physics (2010 – present), American Journal of Nuclear Medicine and Molecular Imaging (2011 – present) and Associate Editorial Board Member (2014 – present), Applied Radiation Isotopes (2011 – present), Nuclear Medicine & Biology (2012 – present), Colloids and Surfaces B: Biointerfaces (2013 – present). Molecular Pharmaceutics (2013 – present)

International Invited Talks:

Engle J W, Nortier F M, Jensen, M (**2013**). Accelerator production of radionuclides. Workshop on Fast Neutron Applications at Spallation Sources, Abingdon, United Kingdom, September 30 – October 1.

Engle J W, Taylor W A, Bene B J, Nortier F M, Trellue H R, Rabin M R (**2013**). ^{163}Ho production in electron capture mass spectroscopy. NuMass 2013: The Future of Neutrino Mass Measurements. Milan, Italy, February 4 – 7.

Engle J W, Gagnon K, Ellison P A, Barnhart T E, Murali D, DeJesus O T, Nickles R J (**2012**). Production of electrophilic ^{34m}Cl for use in PET imaging. *7th International Symposium on*

Radiohalogens. Whistler, Canada, September 15-19.

Domestic Invited Talks:

Engle J W. Emerging Radionuclides (**2016**). Society for Nuclear Medicine and Molecular Imaging Annual Meeting, San Diego, California, June 11.

Engle J W. A Department of Energy palette of radionuclides, a new set of brushes (**2014**). University of Wisconsin Department of Medical Physics PET Tea Colloquium, Madison, Wisconsin, May 15.

Engle J W and Nortier F M. Cross section measurements for medium energy radioisotope production (**2014**). Colloquium on the Application of Nuclear Physics in Medicine, Industry, and Research. University of California Davis Physics Department, Davis, California, USA, September 22.

Identification of Potential Conflicts of Interest or Bias in Selection of Reviewers:

Collaborators and Co-authors:

Ahlers, Elisabeth, University of Denver; Bach, Hong, LANL; Ballard, Beau, Bechtel Marine Propulsion Corporation; Barnhart, Todd, University of Wisconsin; Birnbaum, Eva R, LANL; Bitteker, Leo J, LANL; Cai, Weibo, University of Wisconsin; Connors, Michael, LANL; Converse, Alex, University of Wisconsin; Couture, Aaron , LANL; Cutler, Cathy, University of Missouri; Ellison, Paul, University of Wisconsin; Emborg, Marina, University of Wisconsin; Fassbender, Michael, LANL; Gott, Matthew, University of Missouri; Gulley, Mark , LANL; Hong, Hao, University of Wisconsin; Hudston, Lisa, LANL; Jackman, Kevin, LANL; James, Michael, LANL; Joers, Valery, University of Wisconsin; John, Kevin, LANL; Jurisson, Sylvia, University of Missouri; Kelsey, Charles, LANL; Kettring, Alan, University of Missouri; Leigh, Brian, University of Wisconsin; Lenz, John, Michigan State University; Liu, Brian, University of Wisconsin; Mashnik, Stepan, LANL; Moddrell, Charles, Moddrell Mfg. Inc.; Nathanson, Alex, University of Wisconsin; Nickles, Robert, University of Wisconsin; Nortier, Francois, LANL; Olivas, Eric, LANL; Onofre, DeJesus, University of Wisconsin; Pillai, Chandra, LANL; Rabin, Michael, LANL; Rau, John, LANL; Reass, David, LANL; Redman, Lindsay, New Mexico State University; Runde, Wolfgang, LANL; Severin, Greg, Danish Technical University; Smith, Donna, LANL; Theuer, Charles, University of Wisconsin; Trellue, Holly, LANL; Ullmann, John, LANL; Valdovinos, Hector, University of Wisconsin; Weidner, John, NNSA; Wilbur, D Scott, University of Washington; Wolfsberg, Laura, LANL; Zhang, Yin, University of Wisconsin; Bach, Hong, LANL;

Graduate and Postdoctoral Advisors:

Advisor Name	**Organizational Affiliation**
Robert J Nickles	University of Wisconsin
Francois M Nortier	LANL
Eva R Birnbaum	LANL

Graduate and Postdoctoral (Co-)Advisees:

Advisee Name	**Organizational Affiliation**
Keriann A DeLorme	Air Force Institute of Technology, M.S.
Lauren A Marus	University of New Mexico, Ph.D. student
Valery Radchenko	LANL, Postdoctoral Researcher
Maryline Ferrier	LANL, Seaborg Postdoctoral Fellow
Michelle Mosby	LANL, Postdoctoral Researcher
Kevin Bennett	LANL, Postdoctoral Researcher

Rebecca J. Abergel

Staff Scientist
Chemical Sciences Division
Lawrence Berkeley National Laboratory
MS 70A-1105, Berkeley, California 94720
Phone: (510) 486-5249
Email: rjabergel@lbl.gov

Education and Training

2006, University of California, Berkeley, CA, Chemistry, Ph.D.
2002, École Normale Supérieure, Paris, France, Chemistry, B.S.

Research and Professional Experience

1. Professional Appointments

2015-present	Deputy Director, Institute for Resilient Communities, Lawrence Berkeley National Laboratory
2010-present	Staff Scientist, Lawrence Berkeley National Laboratory
2009-2010	Project Scientist, Lawrence Berkeley National Laboratory
2008-2009	Research Specialist, University of California, Berkeley
2007-2008	Postdoctoral Research Fellow, University of California, Berkeley and Fred Hutchinson Cancer Research Center, Seattle (joint appointment)

2. Selected Awards

Early Career Award, DOE (2014-2019); Early Career Investigator Annual Radiation Research Society Meeting Award (2016); MIT Technology Review Innovators Under 35 – France (2014); Director's Award for Exceptional Scientific Achievement, LBNL (2013); STEM Women at the Lab Award (2013); Junior Faculty NCRP Award, Radiation Research Society (2013); Young Investigator Award, Cooley's Anemia Foundation (2009-2010); European Commission Marie Curie Actions Scholarship, European School of Haematology (2004)

Related Publications

1. Sturzbecher-Hoehne, M., Yang, P., D'Aleo, A., Abergel, R.J. "Intramolecular Sensitization of Americium Luminescence in Solution: Shining Light on Short-Lived Forbidden 5f Transitions" *Dalton Trans*. **2016**, *45*, 9912-9919.
2. An, D.D., Kullgren, B., Jarvis, E.E., Abergel, R.J. "From early prophylaxis to delayed treatment: Establishing the plutonium decorporation activity window of hydroxypyridinonate chelating agents" *Chem. Biol. Int.* **2016***, In Press.*
3. Allred, B.E., Rupert, P.B., Gauny, S.S., An, D.D., Ralston, C.Y., Sturzbecher-Hoehne, M., Strong, R.K., Abergel, R.J. "Siderocalin-Mediated Recognition, Sensitization, and Cellular Uptake of Actinides" *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 10342-10347.
4. Sturzbecher-Hoehne, M., Choi, T.A., Abergel, R.J. "Hydroxypyridinonate Complex Stability of Group (IV) Metals and Tetravalent f-Elements: The Key to the Next Generation of Chelating Agents for Radiopharmaceuticals" *Inorg. Chem.* **2015**, *54*, 3462-3468.
5. Choi, T.A., Endsley, A.N., Bunin, D.I., An, D.A., Morales-Rivera, J.A., Villalobos, J.A., Shinn, W.M., Dabbs, J.E., Chang, P.Y., Abergel, R.J. "Biodistribution of the Multidentate Hydroxypyridinonate Ligand [14C]-3,4,3-LI(1,2-HOPO), a Potent Actinide Decorporation Agent" *Drug. Dev. Res.* **2015**, *76*, 107-122.

6. An, D.A., Villalobos, J.A., Morales-Rivera, J.A., Rosen, C.J., Bjornstad, K.A., Gauny, S.S., Choi, T.A., Sturzbecher-Hoehne, M., Abergel, R.J. "^{238}Pu Elimination Profiles after Delayed Treatment with 3,4,3 LI(1,2 HOPO) in Female and Male Swiss-Webster Mice" *Int. J. Rad. Biol.* **2014**, *90*, 1055-1061.
7. Sturzbecher-Hoehne, M., Kullgren, B., Jarvis, E.E., An, D.D., Abergel, R.J., "Highly Luminescent and Stable Hydroxypyridinonate Complexes: A Step Towards New Curium Decontamination Strategies" *Chem. Eur. J.*, **2014**, *20*, 9962.
8. Deblonde, G.J.P., Sturzbecher-Hoehne, M., Abergel, R.J., "Solution Thermodynamic Stability of Complexes Formed with the Octadentate Hydroxypyridinonate Ligand 3,4,3-LI(1,2-HOPO): A Critical Feature for Efficient Chelation of Lanthanide(IV) and Actinide(IV) Ions" *Inorg. Chem.* **2013**, *52*, 8805.
9. Sturzbecher-Hoehne, M., Deblonde, G.J.P., Abergel, R.J., "Solution Thermodynamic Evaluation of Hydroxypyridinonate Chelators 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) for UO_2(VI) and Th(IV) Decorporation" *Radiochim. Acta* **2013**, *101*, 359.
10. Bunin, D.I., Chang, P.Y., Doppalapudi, R.S., Riccio, E.S., An, D.D., Jarvis, E.E., Kullgren, B., Abergel, R.J.. "Dose-Dependent Efficacy and Safety Toxicology of Hydroxypyridinonate Actinide Decorporation Agents in Rodents: Towards a Safe and Effective Human Dosing Regimen" *Rad. Res.* **2013**, *179*, 171.

Synergistic Activities

- Associate Editor for the *International Journal of Radiation Biology*
- Corresponding Member (USA) for *Radioprotection*
- Organizer of the 2nd Intl. Symposium on Radiological Resilience & Beyond (Japan, 2016)
- Organizer of the 1st Intl. Symposium on Radiological Resilience & Beyond (USA, 2015)
- Organizer of the 11th Intl. Conf. on Health Effects of Incorporated Radionuclides (USA, 2013)
- LBNL Public Outreach Activities: Berkeley High School Science Mentor (2015), Science at the Theater (2013, 2014), Open House Chemistry Stands (2012, 2011)
- LBNL Radioactive Drug Research Committee Member (2009-) and Chair (2011-present)
- LBNL Chemical Sciences Divisional Staff Committee Member (2014-Present)
- LBNL Chemical Sciences Divisional Council Member (2011-Present)
- LBNL Chemical Sciences Division Diversity & Inclusion Officer (2016-Present)

Collaborators and Co-Editors outside of Own Institution LBNL

Michelle Agarande (French Institute for Nuclear Safety and Radioprotection, IRSN); Eric Ansoborlo (French Atomic Energy Commission, CEA); Jean Aupiais (CEA); Jonathan Barasch (Columbia U); Shannon Biros (Grand Valley State U); Polly Chang (SRI International); Anthony D'Aléo (French National Center for Scientific Research, CNRS); Christophe Den Auwer (U of Nice); Lynn Francesconi (Hunter College CUNY); Raymond Guilmette (Lovelace Respiratory Research Institute, LRRI); Ga-Lai Law (Hong Kong Polytechnic U); Jason Lewis (Memorial Sloan Kettering Cancer Center); Olivier Maury (CNRS); Anne Mason (U Vermont); Marinella Mazzanti (CEA); Dunstana Melo (LRRI); Gilles Muller (San Jose State U); Guillaume Phan (IRSN); Thomas Sorensen (U of Copenhagen); Roland Strong (Fred Hutchinson Cancer Research Center); Kai Vetter (UC Berkeley); Christopher Vulpe (UC Berkeley); Ping Yang (PNNL)

Graduate and Postdoctoral Advisors

Kenneth N. Raymond, Ph.D., Graduate Advisor, University of California, Berkeley
Roland K. Strong, Ph.D., Postdoctoral Supervisor, Fred Hutchinson Cancer Research Center

Graduate Students and Postdoctoral Scholars Supervised During the Past Five Years

1. Graduate Students (total advised: 10), last 5 years:
Emeline Rostan (current), Marie-Claire Illy (2015, CEA), Annaig Bertho (2015, Université Paris-Descartes), Marion Losno (2014, CEA), Florian Brulfert (2013, Université Paris-Sud); Solène Hébert (2013, Saint-Gobain); Christophe Goujon (2011, CEA)

2. Postgraduate Scholars (total advised: 9), last 5 years:
Gauthier Deblonde (current); Peter Agbo (current); Ilya Yakovlev (Albert-Ludwigs-University of Freiburg); Xin Liu (Applied Materials); Benjamin Allred (Stanford University); Nagender Panyala (Georgia Institute of Technology); Taylor Choi (Achaogen, Inc.); Manuel Sturzbecher-Hoehne (Picoquant); Cindy Wu (California Department of Public Health)

James P. O'Neil
Staff Scientist, Radiochemist
Lawrence Berkeley National Laboratory
1 Cyclotron Road, MS_55_121, Berkeley, CA 94720
jponeil@lbl.gov, 510-486-5276

Education and Training:

Institution and Location	Degree	Year	Field of Study
California Polytechnic State University, San Luis Obispo, CA	B.S.	1985	Chemistry
University of Utah, Salt Lake City, UT	Ph.D.	1991	Synthetic Organic Chemistry
University of Illinois, Urbana-Champaign, IL	Postdoc	1991–1993	Organic and Radiochemistry
Lawrence Berkeley National Laboratory, Berkeley, CA	Postdoc	1994	Radiopharmaceutical Chemistry

Research and Professional Experience:

1995–2005 Staff Scientist, Supervisor Biomedical Isotope Facility, LBNL. Supervise the operation and maintenance of the medical cyclotron and provide radiochemistry support and radiopharmaceutical preparations for department and division staff and researchers.

2005–present Staff Scientist, Head of Chemistry and Radiochemistry group, Department of Radiotracer Development and Imaging Technologies, LBNL. Oversee the operation of the Biomedical Isotope Facility and Chemistry group. Providing support for department and division staff and researchers.

Synergistic Activities:

- Workshops on Targetry and Target Chemistry, member International Organizing Committee, Scientific Advisory Committee (3 conferences). (1995-date)
- International Nuclear Target Design Society, member since 1999
- Mentor and CoPI for DOE sponsored Integrated Radiochemistry Research Projects of Excellence: CARE (California Alliance for Radiochemistry Education) training program (2009-2014).
- Mentor for various DOE/LBNL sponsored Summer Undergraduate Training Programs (1996-2014).
- LBNL Radiation Safety Committee Member
- Department Safety Committee Representative/Liasion

Selected Publications (10)

O'Neil, JP; VanBrocklin, HF. Preparation of fluorine-18 gas from a 10 MeV cyclotron: A target system for the CTI RDS 111 cyclotron. Nucl Instr Meth in Physics Research, Section A, 438(1), 166-172, 1999.

O'Neil, JP; VanBrocklin, HF. New Solid and Gas Phase Targets for the CTI RDS-111 Cyclotron. in "Proceedings of the 15th International Conference on the Applications of Accelerators in Research and Industry" Duggan, JL; Morgan, IL. ed. AIP Press, 936-939, 1999.

Powell, J and O'Neil, JP. Production of [15O]water at low-energy proton cyclotrons. Applied Radiation and Isotopes 64(7), 755-759, 2006.

Powell J and O'Neil JP. A simple Low-Cost Photodiode Radiation Detector for Monitoring In Process PET Radiochemistry, AIP Conference Proceedings, 1509, pp249-253, 2012.

VanBrocklin, HF; O'Neil, JP. Cyclotron production of [18F]fluoride ion and [18F]fluorine gas and their medical applications. in "Applications of Accelerators in Research and Industry" Duggan, JL; Morgan, IL. ed. AIP Press, New York, 1329-1332, 1997.

Powell, J; O'Neil, JP; Cerny, J. Production of an Accelerated Oxygen-14 Beam. Nuclear Instruments and Methods in Nuclear Research B 204, 440-443, 2003.

Vandehey, NT; O'Neil, JP; Slowey, AJ; Boutchko, R; Druhan, JL; Moses, WW; Nico, PS. Monitoring Tc Dynamics in a Bioreduced Sediment: An Investigation with Gamma Camera Imaging of Tc-99m-Pertechnetate and Tc-99m-DTPA. Environmental Science & Technology, 46 (22), 12583-12590, 2012.

Vandehey, NT; O'Neil, JP. Capturing [11C]CO2 for use in aqueous applications. Applied Radiation and Isotopes. 90, 74-8, 2014.

Slowey, AJ; Vandehey, NT; O'Neil, JP; Boutchko, B; Moses, WW; Nico, PS. Chemical stability of 99mTc–DTPA under aerobic and microbially mediated Fe(III)-reducing conditions in porous media, Applied Radiation and Isotopes, 94, 175-181, 2014.

Hooker, JM; O'Neil, JP; Romanini, DW; Taylor, SE; Francis, MB. Genome-free Viral Capsids as Carriers for Positron Emission Tomography Radiolabels. *Molecular Imaging and Biology*. 10(4),182-91, 2008.

Identification of Potential Conflicts of Interest or Bias in Selection of Reviewers:

Boutchko, Rostyslav, Lawrence Berkeley National Laboratory
Budinger, Thomas, Lawrence Berkeley National Laboratory
Druhan, Jennifer, Stanford University
Francis, Mathew, University of California, Berkeley
Gullberg, Grant, Lawrence Berkeley National Laboratory
Jagust, William, Lawrence Berkeley National Laboratory
Janabi, Mustafa, Lawrence Berkeley National Laboratory
Moses, William, Lawrence Berkeley National Laboratory
Nico, Peter, Lawrence Berkeley National Laboratory
Powell, James; Consultant, Cambridge, England
Rabinovici, Gil, University of California, San Francisco
Rayz, Vitaly, University of California, San Francisco
Saloner, David, University of California, San Francisco
Taylor, Scott, Lawrence Berkeley National Laboratory

Graduate and Postdoctoral Advisors and Advisees:

Keck, Gary, University of Utah; Graduate Advisor
Katzenellenbogen, John, University of Illinois; Postdoctoral Sponsor
VanBrocklin, Henry, University of California, San Francisco; Postdoctoral Sponsor
Aanei, Ioana; University of California, Berkeley
Behrens, Christopher; University of California, San Francisco
Farkas, Michelle; University of Massachusetts, Amherst
Hooker, Jacob; Harvard University
Leggett, Christina; Lawrence Berkeley National Laboratory
Romanini, Dante; Amgen Corporation
Slowey, Aaron; Lawrence Berkeley National Laboratory
Vandehey, Nicholas; Lawrence Berkeley National Laboratory
Wang, Ross; Anaspec

Lee Bernstein

Title: Nuclear Data Group Leader, LBNL.
Professor of Practice, U.C. Berkeley Dept. of Nuclear Engineering.
Contact Information:
Email: bernstein2@llnl.gov
Phone: (510)-486-4951

(a) Professional Preparation

Rutgers University	Physics	B.A. – 1988
University of Maryland	Physics	M.S. - 1990
Rutgers University	Physics	Ph.D. – 1994
Post-Doc: LLNL	N-division	1994-1996

(b) Appointments

- Nuclear Data Group Leader, Lawrence Berkeley National Lab – June 2016-present
- Professor of Practice, U.C. Berkeley Dept. of Nuclear Eng. – January 2015-present
- NIF Nuclear Diagnostics Deputy Group Leader, LLNL – 2009-2013
- Staff Scientist - LLNL – 1996-present
- Postdoctoral Researcher – LLNL – 1994-1998

(c) Recent Awards

- Fellow of the American Physical Society (Nuclear Physics) – September 2015
- Three LLNL Physics and Life Sciences Directorate Awards for the Outstanding Mentoring, Post-doctoral supervision and External Leadership – 2015
- LLNL Directors Science and Technology Award for the Development of the RAGS diagnostic system at the National Ignition Facility– 2014

(d) Publications (Selected 10)

1. Dipole Strength Distribution of ^{74}Ge. R. Massarczyk, R. Schwengner, L. A. Bernstein, M. Anders, D. Bemmerer, R. Beyer, Z. Elekes, R.Hannaske, A. R. Junghans, T. Kogler, M. Roder,1 K. Schmidt, A. Wagner, and L. Wagner. Phys. Rev. C92, 044309 (2015).
2. Statistical properties of ^{243}Pu, and $^{242}Pu(n, \gamma)$ cross section calculation. T.A.Laplace, F.Zeiser, M.Guttormsen, A.C.Larsen, D.L.Bleuel, L.A.Bernstein, B.L.Goldblum, S.Siem, F.L.Bello Garotte, J.A.Brown *et al.*. Phys.Rev. C 93, 014323 (2016).
3. g-ray decay from neutron-bound and unbound states in ^{95}Mo and a novel technique for spin determination. M.Wiedeking, M.Krticka, L.A.Bernstein, J.M.Allmond, M.S.Basunia, D.L.Bleuel, J.T.Burke, B.H.Daub, P.Fallon, R.B.Firestone, *et al*. Phys.Rev. C 93, 024303 (2016).
4. γ decay from the quasicontinuum of $^{197,198}Au$. F.Giacoppo, F.L.Bello Garrote, L.A.Bernstein, D.L.Bleuel, R.B.Firestone, A.Gorgen, M.Guttormsen, T.W.Hagen, M.Klintefjord, P.E.Koehler, A.C.Larsen, H.T.Nyhus, T.Renstrom, E.Sahin, S.Siem, T.Tornyi. Phys. Rev. C91, 054327 (2015).
5. EGAF: Measurement and Analysis of Gamma-ray Cross Sections. R.B.Firestone, K.Abusaleem, M.S.Basunia, F.Becvar, T.Belgya, L.A.Bernstein, H.D.Choi, J.E.Escher, C.Genreith, A.M.Hurst, M.Krticka, P.R.Renne, Zs.Revay, A.M.Rogers, M.Rossbach, S.Siem, B.Sleaford, N.C.Summers, L.Szentmiklosi, K.van Bibber, M.Wiedeking, Nucl.Data Sheets 119, 79 (2014).
6. Photon Strength Function at Low Energies in ^{95}Mo, M. Wiedeking, L.A. Bernstein, J.M. Allmond, M.S. Basunia, D.L. Bleuel, J.T. Burke, P. Fallon, R.B. Firestone, B.L. Goldblum, R. Hatarik, M. Krtička, P.T. Lake, A.C. Larsen, I-Y. Lee, S.R. Lesher, S. Paschalis, M. Petri, L. Phair, N.D. Scielzo, Nucl. Data Sheets 119, p. 258 (2014).

7. Relative light yield and temporal response of a stilbene-doped bibenzyl organic scintillator for neutron detection. J.A. Brown, B.L. Goldblum, L.A. Bernstein, D.L. Bleuel, N.M. Brickner, J.A. Caggiano, B.H. Daub, G.S. Kaufman, R. Hatarik, T.W. Phillips, S.A. Wender, K. van Bibber, J. Vujic, N.P. Zaitseva. Journal of Applied Physics 115 #19, 193504 (2014).
8. States in ^{196}Pt observed with the (n, n'g) reaction, E. Tavukcu, L.A. Bernstein, K. Hauschild, J.A. Becker, P.E. Garrett, C.A. McGrath, D.P. McNabb, W. Younes, P. Navratil, R.O. Nelson. Physical Review C65, #6, 64309, (2002).
9. Probing reaction dynamics with the ^{196}Pt (n, xng) reactions for $x \leq 15$, L.A. Bernstein, J.A. Becker, W. Younes, D.E. Archer, K. Hauschild, G.D. Johns, R.O. Nelson, W.S. Wilburn, D.M. Drake. Physical Review C57, #6, 2799-2803, (1998).
10. Studying the role of nuclear structure effects in neutron-induced reactions using GEANIE at LANSCE, L.A. Bernstein, D.E. Archer, J.A. Becker, P.E. Garrett, K. Hauschild, C.A. McGrath, D.P. McNabb, W. Younes, M. Devlin, D.M. Drake. Nuclear physics. A, Nuclear and hadronic physics, 682, 404-414, (2001).

(e) Current Collaborators/Persons with CoI:

- LBNL: L.W. Phair, P. Fallon, R.M. Clark, M.S. Basunia.
- LLNL: D.H.G. Schneider, N.D. Scielzo, J.T. Burke, D.L. Bleuel, J.A. Caggiano
- University of California: K.A. Van Bibber, J. Vujic, R. Slaybaugh, B.L. Goldblum. A.M. Hurst, J.C. Batchelder, R.B. Firestone.
- University of Oslo: M. Guttormsen, S.Siem, A.C. Larsen, G.M. Tveten, S. Rose. F. Giacoppo,
- LANL: M. Devlin.
- iThemba Labs: M. Wiedeking
- Charles University: M. Krticka, F. Becvar.
- ORNL: C.D. Nesaraja.
- BNL: M.W. Herman, E.A. McCutchan.
- Other Institutions: F. Gunsing, J.N. Wilson.

(e) Graduate and Postdoctoral Advisors and Advisees:

- Thesis Advisor: Prof. Jolie Cizewski, Rutgers University
- Post-doctoral Advisors: Drs. Eugene Henry and John Becker (LLNL ret.)
- Former students and Post-docs: Dr. Bethany Goldblum (nee Lyles) (UCB), D.P. McNabb (LLNL), C.A. McGrath (INL), P.E. Garrett (Guelph), E. Algin (nee Tavukcu), A. Schiller, J.A. Church (LLNL), J. R. Cooper, E. Rodroguez-Vieitez, S.R. Lesher (Univ. of Wisconsin), J.T. Burke (LLNL), D.L. Bleuel (LLNL), N.D. Scielzo (LLNL), A. McEvoy (LANL), R. Hatarik (LLNL), J. M. Allmond (ORNL), M. Wiedeking (iThemba labs), A. Kritcher (LLNL), L. Crespo (University of Oslo). B. Daub (LLNL), P.F. Davis.

STOSH A. KOZIMOR, Ph.D.
Chemistry Division-IIAC; Mail Stop J514; Los Alamos National Laboratory
Los Alamos, New Mexico 87544

Education and Training

2006 – 2009 Distinguished Reines Postdoctoral Fellow, Los Alamos National Laboratory
Mentors: Dr. Carol J. Burns and Dr. David L. Clark.
Research: Quantifying Covalency in Thorium, Uranium, and the Trans-Uranic Elements by Ligand K-edge X-ray Absorption Spectroscopy and non-Resonant Inelstic X-ray Scattering

2005 – 2006 Post-Doctoral Scholar, University of California, Berkeley, CA
Advisor: Professor Jeffrey R. Long
Research: Exploring the Synthesis and Magnetic Properties of Metal Clusters Containing Uranium and Transition Metals

2000 – 2005 Doctor of Philosophy in Chemistry, University of California, Irvine, CA
Advisor: Professor William J. Evans
Thesis: Exploring the Effects of Steric Crowding on the Chemistry of Lanthanide and Actinide Complexes

1994 – 1999 Bachelor of Science in Chemistry and Minor in Mathematics Fort Lewis College, Durango CO. *Summa Cum Laude*
Advisor: Professor Joel W. Gohdes
Research: *Progress Toward A Molecular Imprint Polymer Based Stereoselective Epoxide Catalyst*

Research and Professional Experience

2014 – present Los Alamos National Laboratory C-IIAC Isotope Production Team
Technical Focus: Production of radiopharmaceuticals.

2014 – present Visiting Scientist at the Institute for Transuranium Science (ITU)

2013 – present Principal Investigator for the Los Alamos National Laboratory
Office of Basic Energy Science Heavy Element Chemistry Program

2013 – present Principal Investigator for the Nuclear Forensic University Summer School
http://pearl1.lanl.gov/external/nuclear-forensics/

2008 – 2014 Member of the Los Alamos National Laboratory C-NR Radiochemistry Team
Technical Focus: Activation Products, Lanthanides, and Dissolution

2009 – present Technical Staff Member, Los Alamos National Laboratory
Chemistry Division: Inorganic, Isotope, and Actinide Chemistry Group

Publications (Most closely related to the proposed project, out of a total of 57)

"Influence of Pyrazolate vs. N-Heterocyclic Carbene Ligands on the Slow Magnetic relaxation of Homoleptic Trischelate Lanthanide (III) and Uranium (III) Complexes" Meihaus, K. R.; Minasian, S. G.; Lukens, W. W. Jr.; Kozimor, S. A.; Shuh, D. K.; Tyliszczak, T.; Long, J. R. *J. Am. Chem. Soc.* **2014** *136*, 6056-6068.

"Electron Localization in a Mixed-Valence Niniobium Benzene Complex" Gianetti, T.; Nocton, G.; Minasian S. G.; Kaltsoyannis, N.; Kilcoyne, A. L. D.; Kozimor S. A.; Shuh, D. K.; Tyliszczak, T.; Bergman, R.; Arnold, J. *Chem. Sci.* **2015**, *6*, 993-1003. **DOI:** 10.1039/C4SC02705A, ASAP.

"Covalency in Lanthanides. An X-ray Absorption Spectroscopy and Density Functional Theory Study of $LnCl_6^{x-}$ (x = 3, 2)" Löble, M. W.; Keith, J. M.; Altman, A. B.; S. Chantal E. Stieber, Batista, E. R.; Boland, K. S.; Conradson, S. D.; Clark, D. L.; Pacheco, J. L.; Kozimor, S. A.; Martin, R. L.; Minasian, S. G.; Olson, A. C.; Scott, B. L.; Shuh, D. K.; Tyliszczak, T.; Wilkerson, M. P.; Zehnder, R. A. *J. Am. Chem. Soc.* **2015**, *137*, 2506-2523.

"Transformation in U_3O_8 Materials Following Controlled Exposure to Temperature and Humidy." Tamasi, A. L.; Boland, K. S.; Czerwinski, K.; Ellis, J. K.; Kozimor, S. A.; Martin, R. L.; Pugmire, A. L.; Reilly, D.; Scott, B. L.; Sutton, A. D.; Wagner G. L.; Walensky, J. R.; Wilkerson, M. P. *Anal. Chem.,* **2015**, *87*, 410-4217.

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Synergistic Activities

Honors

- Scientific Participant in providing "Analytical Support for the WIPP Project," which was awarded as the LANL Large Team Distinguished Performance Award, **2015**.
- Los Alamos Award Program in recognition of outstanding contributions to the WIPP INVESTIGATION PROJECT. **2014.**
- Scientific Participant on "Operation Tomadachi," which was awarded as the LANL Large Team Distinguished Performance Award, **2011**.
- Los Alamos Distinguished Postdoctoral Award, **2009**.
- Poster Award Winner **2009** Inorganic Gordon Research Conference, Biddeford ME.
- Distinguished Frederick Reines Fellowship from the Nuclear Management Technology Division (NMT-DO) with the classification of Technical Staff Member and title of Reines Fellow, **2006**.
- Director's Fellowship from Los Alamos National Laboratory, **2005**.
- The Joan Rowland Award for 2005 for meritorious performance in graduate studies in chemistry at the University of California, Irvine.
- University of California Regents Dissertation Fellowship, 2003.

Professional

- Elected Vice Chair for the Inorganic Gordon Conference, **2016**, and Chair for the **2018** meeting.
- Organizer for the SSRL User meeting workshop on Advances in Actinide Science from Synchrotron Spectroscopy, **2014**.
- Elected Vice Chair for the Users' Executive Committee for SSRL, **2013**
- Member of the editorial committee for the publication series Inorganic Synthesis.
- Member of the SSRL "User Elected Committee," **2012** to present.
- Member of the Seaborg Institute "Strategy Team," **2012**.

Collaborators From Other Institutions

Juan S. Lezama (Stanford); Gerald T. Seidler (University of Washington); David K. Shuh (LBNL); Stefan Minasian (LBNL); John Klaehn (INL); Dean Peterman (INL); Eric Schelter (University of Pennsylvania); Suzanne C. Bart (Purdue University); Dennis Nordlund (SSRL), Dimosthenis Sokaras (SSRL), John M. Berg (LANL), Melissa Denecke (Manchester), Tonya Vitova (INE), Michael Neidig (Rochester), Nik Kaltsoyannis (University of College London), Thomas Albrecht-Schmitt (FSU), Jonas Peters (Caltech), Trevor Hayton (UCSB), William Evans (UCI).

Appendix 2: Current and Pending Support

Jonathan Engle

Current PI is supported by Department of Energy Programmatic funds through the Office of Science.

Investigator: Jonathan W Engle	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Current, DOE-EC-LAB 15-1386	
Project/Proposal Title: Nuclear Data for Spallation Neutron Radioisotope Production	
Source of Support: DOE	Location of Project: LANL
Annual Award Amount: $500,000	Award Period: July 2016 – July 2021
Annual Award Amount to PI's Research: $500,000	
Person-Months Per Year Committed to Project: 6.5 Pers. Months: Cal.	
Research Description: The goal of this project is direct measurement of neutron-induced reactions of interest to the production of ^{32}Si and therapeutic alpha emitters, and confirmation of these energy discretized values with white spectrum activations using spallation neutrons at DOE accelerator facilities. Synergies and/or overlaps with this Proposal/Award: This program is focused on gathering nuclear data needed to describe spallation neutron radionuclide production at intense charged particle irradiation facilities. There is no direct overlap.	

Investigator: Jonathan W Engle	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Current, LANL LDRD 20160601ECR	
Project/Proposal Title: Spallation Neutrons for Radionuclide Production: Capability Development	
Source of Support: LANL	Location of Project: LANL
Annual Award Amount: $168,000	Award Period: January 2016 – January 2018
Annual Award Amount to PI's Research: $168,000	
Person-Months Per Year Committed to Project: 2.5 Pers. Months: Cal.	
Research Description: The goal of this project is the development of a national capability using >20 MeV quasimonoenergetic neutron beams at Lawrence Berkeley National Laboratory for direct measurement of neutron-induced reactions by the activation method. Synergies and/or overlaps with this Proposal/Award: This program is focused on building a new experimental capability at LBNL's 88" cyclotron employing accelerator-produced neutron beams. There is no direct overlap.	

Investigators: Eva R Birnbaum / Jonathan W Engle	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Current, LANL LDRD 20150575ER	
Project/Proposal Title: Fundamental Actinium Science in Search of Radiopharmaceuticals	
Source of Support: LANL	Location of Project: LANL
Annual Award Amount: $350,000	Award Period: October 2015 – September 2017
Annual Award Amount to PI's Research: $15,000	
Person-Months Per Year Committed to Project: 0.5 Pers. Months: Cal.	
Research Description: The goal of this project is first elemental spectroscopic characterization of actinium with the goal of informing first steps towards *de novo* design of ligand environments useful	

for Ac-225 as a targeted radionuclide in alpha radiotherapy. Synergies and/or overlaps with this Proposal/Award: There is no direct overlap.

Investigators: Jonathan W Engle	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Current, DOE FOA LAB-14-1099	
Project/Proposal Title: Auger-Emitting $^{119}Te/^{119}Sb$ Generators for Radiotherapy	
Source of Support: LANL	Location of Project: LANL
Annual Award Amount: $470,000	Award Period: November 2015 – November 2017
Annual Award Amount to PI's Research: $470,000	
Person-Months Per Year Committed to Project: 1 Pers. Months: Cal.	
Research Description: The goal of this project is to establish the production of Auger-emitting $^{119}Te/^{119}Sb$ generators through the characterization of proton-initiated production routes, radiochemical isolation of no-carrier-added ^{119}Te from bulk Sb targets, design of a generator system, and investigation of useful chelators for ^{119}Sb. Synergies and/or overlaps with this Proposal/Award: There is no direct overlap.	

Investigators: Michael E Fassbender	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Current, DOE FOA LAB-14-1099	
Project/Proposal Title: Design of a U-230/Th-226 Generator System and Evaluation of Th-226 Chelation Chemistry	
Source of Support: DOE	Location of Project: LANL
Annual Award Amount: $200,000	Award Period: April 2016 – April 2018
Annual Award Amount to PI's Research: $15,000	
Person-Months Per Year Committed to Project: 0.25 Pers. Months: Cal.	
Research Description: The goal of this project is to study the production of alpha-emitting generators of $^{230}U/^{230}Pa$ by investigation of the $^{232}Th(p,3n)^{230}Pa$ nuclear reaction. Synergies and/or overlaps with this Proposal/Award: There is no direct overlap.	

Investigators: Michael E Fassbender	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Pending	
Project/Proposal Title: $^{238}U(p,xn)\ ^{237,236}Np$ Nuclear Reaction Cross Section Acquisition and Target Design for the Production of ^{236g}Np and ^{236}Pu from Uranium Targets	
Source of Support: DOE	Location of Project: LANL
Annual Award Amount: $435,000	Award Period: TBD
Annual Award Amount to PI's Research: $120,000	
Person-Months Per Year Committed to Project: 1 Pers. Months: Cal.	
Research Description: The goal of this project is to study the production of alpha-emitting generators of $^{230}U/^{230}Pa$ by investigation of the $^{232}Th(p,3n)^{230}Pa$ nuclear reaction. Synergies and/or overlaps with this Proposal/Award: There is no direct overlap.	

Rebecca Abergel

Current and Pending Support

Investigator: Rebecca J. Abergel	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Current	
Project/Proposal Title: Harnessing f-Orbital Bonding Through Precision Antenna Ligand Design for Actinide Complexation	
Source of Support: DOE	Location of Project: LBNL
Annual Award Amount: $500,000	Award Period: 08/01/2014–07/31/2019
Annual Award Amount to PI's Research: $500,000	
Person-Months Per Year Committed to Project: 6.0 Pers. Months: Cal.	
Research Description: The goal of this project is to develop a new library of synthetic ligands that can act as luminescence sensitizing chelating agents for trivalent actinides. Correlations between photophysical, thermodynamic, kinetic, and structural properties of the resulting complexes with the electronic structure of the designed ligands and of the different actinide ions are studied. Synergies and/or overlaps with this Proposal/Award: This program is focused on the optimization of luminescence sensitization pathways through the design of synthetic organic molecules. It leverages the solution characterization techniques developed in the proposed project, as well as the overall experience with actinide coordination systems. There is no direct overlap.	

Investigator: Rebecca J. Abergel (David K. Shuh)	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Current	
Project/Proposal Title: Heavy Element Chemistry - Actinide Chemistry Group	
Source of Support: DOE	Location of Project: LBNL
Annual Award Amount: $3M	Award Period: 10/01/2013–Ongoing
Annual Award Amount to PI's Research: $150,000	
Person-Months Per Year Committed to Project: 1.2 Pers. Months: Cal.	
Research Description: This project studies the coordination and solution chemistry of heavy elements with natural ligands such as siderophores. Synergies and/or overlaps with this Proposal/Award: This program leverages coordination chemistry characterization techniques to be used in the proposed project. There is no direct overlap.	

Investigator: Rebecca J. Abergel (Kai Vetter)	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Current	
Project/Proposal Title: Multi-disciplinary research to enhance understanding of transport, risks, and mitigation of radioisotopes for improved radiological resilience	
Source of Support: LBNL LDRD	Location of Project: LBNL
Annual Award Amount: $600,000	Award Period: 10/01/2014–09/30/2016
Annual Award Amount to PI's Research: $145,000	
Person-Months Per Year Committed to Project: 1.2 Pers. Months: Cal.	

Research Description: The goal of this project is to collaborate with Japanese counterparts to design and perform measurements in a specific area in the restricted area in the vicinity of the Fukushima-Daiichi Nuclear Power Plant, to better understand the contamination and transport of cesium in the environment including the biosphere in general and humans specifically. Synergies and/or overlaps with this Proposal/Award: This program is focused on the coordination of cesium isotopes in biological systems. It leverages the solution characterization techniques developed in the proposed project, as well as the expertise in handling radioactive materials. There is no direct overlap.

Investigator: Rebecca J. Abergel	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Current	
Project/Proposal Title: Coordination Chemistry Characterization of Conjugated Th-Chelators for Alpha-Therapy Applications	
Source of Support: Bayer Healthcare Pharmaceuticals	Location of Project: LBNL
Annual Award Amount: $155,000	Award Period: 10/01/2015–09/30/2016
Annual Award Amount to PI's Research: $155,000	
Person-Months Per Year Committed to Project: 0.6 Pers. Months: Cal.	
Research Description: This project studies the coordination and solution chemistry of complexes formed between medically relevant heavy elements (Th, Zr, Gd) and proprietary targeted bioconjugated chelators from Bayer Healthcare Pharmaceuticals. Synergies and/or overlaps with this Proposal/Award: This program leverages the solution characterization techniques developed in the proposed project, as well as the expertise in handling radioactive materials. There is no direct overlap.	

Investigator: Rebecca J. Abergel	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Current	
Project/Proposal Title: Biodistribution and Toxicity Evaluation of Accelerator-Produced Actinium-225	
Source of Support: DOE-NP	Location of Project: LBNL
Annual Award Amount: $50,000	Award Period: 01/01/2016–09/30/2016
Annual Award Amount to PI's Research: $50,000	
Person-Months Per Year Committed to Project: 0.6 Pers. Months: Cal.	
Research Description: The major goal of this project is to determine the biokinetic profiles of Ac-225 and Ac-227 in mice. Synergies and/or overlaps with this Proposal/Award: This program leverages the expertise in handling radioactive materials and performing in vivo biodistribution studies. There is no direct overlap.	

Pending

Investigator: Rebecca J. Abergel (Shannon Biros)	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Pending	
Project/Proposal Title: Development of Multipodal Ligands for Selective f-Element Chelation	
Source of Support: DOE	Location of Project: GVSU, UT, LBNL
Annual Award Amount: $200,000	Award Period: 10/01/2016–09/30/2019
Annual Award Amount to PI's Research: $80,000	
Person-Months Per Year Committed to Project: 0.2 Pers. Months: Cal.	

Research Description: This program seeks to support fundamental research in ligand design and in understanding extractant-target interactions, with a major goal aimed at obtaining "a predictive understanding, at molecular- and nano-scale dimensions, of the basic chemical and physical principles involved in chemical separations systems".
Synergies and/or overlaps with this Proposal/Award: This program is focused on the actinide extraction characterization for new ligands. There is no direct overlap with the proposed project.

Investigator: Rebecca J. Abergel	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Pending	
Project/Proposal Title: Exploring the use of a hydroxypyridinone decorporation agent for the removal of toxic residual gadolinium from MRI contrast agent administration	
Source of Support: NIH	Location of Project: LBNL
Annual Award Amount: $250,000	Award Period: 09/01/2016–08/31/2018
Annual Award Amount to PI's Research: $250,000	
Person-Months Per Year Committed to Project: 0.6 Pers. Months: Cal.	
Research Description: The major goal of this project is to determine an in vivo efficacy dosing regimen of the therapeutic chelating agent 3,4,3-LI(1,2-HOPO) for the removal of gadolinium from internal deposits after gadolinium-based contrast agent injection in clinical magnetic resonance imaging procedures. Synergies and/or overlaps with this Proposal/Award: This program is focused on the preclinical development of a decorporation agent. There is no direct overlap with the proposed project.	

Investigator: Rebecca J. Abergel	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Pending	
Project/Proposal Title: Formulation of 3,4,3-LI(1,2-HOPO)	
Source of Support: NIH	Location of Project: LBNL
Annual Award Amount: $52,000	Award Period: 07/01/2016–01/31/2017
Annual Award Amount to PI's Research: $52,000	
Person-Months Per Year Committed to Project: 1.05 Pers. Months: Cal.	
Research Description: The goal of this project is to supervise the manufacturing of clinical trial material for the first-in-human evaluation of drug product HOPO 14-1 as an actinide decorporation agent. Synergies and/or overlaps with this Proposal/Award: This program is focused on the manufacturing of a decorporation product to be used in a clinical trial. There is no direct overlap with the proposed project.	

Investigator: Rebecca J. Abergel	Other Agencies to which this proposal has been/will be submitted: N/A
Support: Pending	
Project/Proposal Title: Evaluating the use of 3,4,3-LI(1,2-HOPO) for removal of toxic gadolinium and lead	
Source of Support: LBNL-Innovation	Location of Project: LBNL
Annual Award Amount: $150,000	Award Period: 10/01/2016–09/30/2017
Annual Award Amount to PI's Research: $150,000	
Person-Months Per Year Committed to Project: 0.6 Pers. Months: Cal.	
Research Description: The major goal of this project is to evaluate the in vivo efficacy of the therapeutic chelating agent 3,4,3-LI(1,2-HOPO) at removing gadolinium and lead from internal deposits. Synergies and/or overlaps with this Proposal/Award: This program is focused on the preclinical development of a decorporation agent. There is no direct overlap with the proposed project.	

Appendix 3: Literature Cited

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Appendix 4/5: Description of Facilities, Resources, and Equipment

The LANL Isotope Production Facility (IPF) and TA-48 Hot Cell Facilies

LANL IPF is a dedicated target irradiation facility located at the Los Alamos Neutron Science Center (LANSCE), which uses up to 100 MeV protons at beam currents up to 450 μA to produce isotopes via our 800-MeV accelerator. Three target slots allow target irradiation to be optimized by energy range for a particular isotope. Available beam time is estimated to be ~3000 hours / year for the main accelerator facilities.

The LANL Count Room capability occupies more than 7000 square feet of LANL Building RC-1 at TA-48, and is dedicated to performing qualitative and quantitative assay of gamma, beta, and alpha-emitting radionuclides in a variety of matrices and over a wide range of activity levels. Founded in support of the US Testing Program, this facility is currently funded ~70% by a range of national security programs, and the balance in support of other internal and external customers. The Countroom's more than 65 systems include High Purity Germanium (HPGe) gamma- and X-ray spectrometers, alpha spectrometers and counters, and beta counters, operate 24x7x365, and perform more than 70,000 measurements annually.

The Los Alamos Hot Cell Radiological Facility is a cGMP compliant facility consisting of 13 hot cells with a sample load shielding capacity of 1 kCi of 1 MeV gamma rays per cell for the remote handling of highly activated samples. The Hot Cells are equipped for separation, purification and wet chemistry activities with standard laboratory equipment, and the ability to perform radioassay of materials within the cells. The facility also contains fume hoods for radiological chemistry and reagent preparation. Available instrumentation includes counting capabilities described above, ICP-OES, HPLC, balances, centrifuges, materials diagnostics and characterization.

LBNL 88" Cyclotron

The Lawrence Berkeley National Laboratory 88-inch Cyclotron is a K=140 sector-focused cyclotron with both light- and heavy-ion capabilities. Protons and other light-ions are available at high intensities (10-20 pμA) up to maximum energies of 55 MeV (protons), 65 MeV (deuterons), 170 MeV (3He) and 140 MeV (4He). Most heavy ions through uranium can be accelerated to maximum energies which vary with the mass and charge state. The 88-Inch Cyclotron supports ongoing research programs in nuclear structure, astrophysics, heavy element studies, fundamental interactions, symmetries, and technology R&D by LBNL and U.C. Berkeley. Major instrumentation at the 88-Inch Cyclotron include the Berkeley Gas-filled Separator (BGS), and VENUS, the most powerful superconducting ECR ion source in the world. The BASE Facility provides well-characterized beams of protons, heavy ions, and other medium energy particles which simulate the space environment. The National Security Space (NSS) community and researchers from other government, university, commercial, and international institutions use these beams to understand the effect of radiation on microelectronics, optics, materials, and cells.

LBNL Facilities and Equipment

LBNL is a member of the national laboratory system supported by the U.S. Department of Energy through its Office of Science. It is managed by the University of California (UC) and is charged with conducting unclassified research across a wide range of scientific disciplines. The Principal Investigator Rebecca Abergel is a member of the Heavy Element Chemistry Group in the Chemical Sciences Division of LBNL and all of the Heavy Element Chemistry Group laboratories will be available for this project. The Heavy Element Chemistry Group has laboratory facilities for performing radionuclide experiments in a suite of laboratories located in one building on the main LBNL site. The Heavy Element Research Laboratory (HERL) is the main location for transuranic chemistry at LBNL. The HERL is operated and managed by the Heavy Element Chemistry Group, which has a long history of safely handling metals and radioactive materials. Operations in the HERL are supported by a 0.5 FTE Ph.D. scientific staff member.

The HERL consists of three interconnecting laboratories with dedicated radiochemical glove boxes, fume hoods, and standard wet chemical laboratory apparatus. The nuclear counting room shares internal access with these main laboratories. Surrounding the main radiochemistry laboratories are specialized, satellite laboratories designed to accommodate experimental work with radionuclides:

- Suite A (2 laboratories, 1000 square feet) is dedicated to spectroscopic work with metals/radionuclides
- Suite B (2 laboratories, 1000 square feet, four fume hoods three glove boxes) is dedicated to synthetic organic and inorganic chemistry with non-transuranic materials.

Abergel Group Laboratories:

Four suites of laboratories are exclusively assigned to the research group led by Dr. Abergel.

- Suite C (two laboratories, 1,700 square feet, two fume hoods, renovated in 2012) includes a preparatory area with walk-in cold and warm rooms, a small clean room with controlled temperature, lighting and humidity for minimal animal housing, a dedicated area for animal procedures, a separate room for sterile molecular biology work.
- Suite D (two laboratories, 600 square feet, two fume hoods) includes high temperature furnaces and liquid scintillation counting equipment; it is dedicated to the processing and analysis of radiological and biological samples.
- Suite E (one laboratory, 250 square feet, two fume hoods, renovated in 2010) houses two liquid-chromatography/ mass spectrometry systems and is dedicated to analytical characterization, thermodynamic and kinetic evaluations of radionuclide complexing species.
- Suite F (two laboratories, 1,500 square feet, renovated in 2013) includes a tissue culture area, one fume hood and two laminar flow hoods, for the simultaneous handling of biological and radiological materials.

All laboratories have card-key controlled access with internal gowning areas and hand- and-foot monitoring equipment placed in all exit areas. They are fully equipped for chemical, biochemical, and radiological studies including HEPA-filtered chemical fume hoods and routine laboratory equipment.

Laboratories of the Heavy Element Chemistry Group at LBNL are fully equipped for chemical, biochemical, radiological, and BSL2 molecular biology studies including HEPA-filtered chemical fume hoods, laminar flow hoods, walk-in cold and warm rooms, water baths, table-top centrifuges, cell counters, microscopes, water distillers, survey meters and routine laboratory equipment such as pH meters, balances, ice machine, conventional gel electrophoresis equipment, autoclaves, incubators, gel dryer, and refrigerators/freezers (-20°C, -80°C). Study animals are housed in a separate clean room with controlled lighting, temperature and humidity that is interconnected to the molecular biology actinide laboratory suite. Cages and animal supplies are cleaned and sterilized in the central LBNL Animal Facility.

Major equipment available for this project include the following:

- Two PCR workstations, a nanodrop spectrophotometer, a Spectramax UV-Vis microplate reader, an Invitrogen iBlot dry blotting system, an Invitrogen BenchPro™ 4100 western processing system, an alphaimager HP imaging system, a thermal cycler, a Roche LightCycler 480 real-time PCR system, an Agilent bioanalyzer, a Tri-Carb 2910 liquid scintillation analyzer, three high temperature furnaces, an Agilent LC-MS system coupled with electrospray, UV-vis, fluorescence detections and radioanalysis, a Waters UPLC-QTofMS/MS coupled with radioanalysis are dedicated to radiological and biochemical work in satellite laboratories of the ACG. A Packard A5530 gamma spectrometer and a Siemens MicroPET scanner are available for the proposed studies at the LBNL Biomedical Imaging Facility.
- An EPR spectrometer, a Cary 5G UV/Vis/NIR spectrometer, three Ocean Optics fiber optic spectrometers, an FT-IR spectrometer, a Jobin Yvon Horiba (JY-H) Fluorolog-3 fluorometer coupled to a diode-array Nd:YAG-dye laser system, a Quantum Designs SQUID MPMS magnetometer a 300

MHz superconducting NMR spectrometer, and an automated potentiometric titration apparatus under computer control incorporating Ocean Optics fiber optic spectrometer and fluorometer are maintained in the satellite laboratories of the ACG.

- A Cary 6000i UV/Vis/NIR spectrometer, an X-ray powder diffractometer, an ESI/MALDI mass spectrometer, an automated potentiometric titrator connected to an Ocean Optics UV-Vis are located in the HERL; a solid state Ge γ-spectrometer, an α spectrometer, and a liquid scintillation counter are available in the nuclear counting room.

This is a list of the facilities and other resources of part of the Department of Cellular and Tissue Imaging (DCTI) at Lawrence Berkeley National Laboratory (LBNL) in Berkeley, California. This part of the DCTI is located in Buildings 55, 55A, 56 and 56 at LBNL. The major chemical laboratory facilities at DCTI include four chemical synthesis laboratories (300 sq. ft. each) in Building 55 that are equipped with all the necessary organic synthesis apparatus. Radiochemical facilities include the DCTI-run Biomedical Isotope Facility (BIF) in Building 56 housing LBNL's medical cyclotron and an associated radiopharmaceutical laboratory (1600 sq. ft.), and two radiochemistry laboratories in Building 55.

Radiochemistry and organic synthesis laboratories: In addition to the medical cyclotron (see the Equipment section), the Biomedical Isotope Facility (BIF) contains an 800 sq. ft. radiolabeling laboratory equipped with four hot cells (three with remote handling manipulators), two fume hoods and a laminar flow hood. Additional equipment includes multiple HPLC systems with in-line UV/Vis, conductivity and/or radioactivity detectors equipped with data collection systems, a Bioscan Radio-TLC scanner, a PE Cyclone Plus Phosphorimager system and an Agilent 5890 gas chromatograph. A variety of automated radiosynthesis devices are available, both built in-house and three commercial devices; a Resonance Instruments Microwave Cavity; and additional radio-organic synthesis equipment and supplies to handle all labeling experiments. In addition, Building 55 contains two other laboratories dedicated for radiochemical synthesis, one as a radiometals isotope synthesis lab with a shielded fume hood and a second room with three shielded and ventilated hot cells. In addition to standard analytical equipment, Building 55 includes radio-TLC, radio-GC and radio-HPLC equipment; a Perkin-Elmer Wizard 1480 gamma counter; and a Packard A5530 gamma well-counter for counting tissue samples, dose calibrators and an EG&G Ortec germanium detector with multichannel analyzer software (Gammavision).
The major organic chemical laboratory facilities include four chemical synthesis laboratories (300 sq. ft. each) in Building 55 that are equipped with all the necessary organic synthesis apparatus for precursor preparation and analysis. A ThermoQuest (Thermo Separations Products) HPLC (equipped with an autosampler, a diode array UV detector and a fluorescence detector) coupled to a ThermoQuest (Finnigan) LCQ duo mass spectrometer, and various HPLC and data collection systems are available for analytical and preparative purification and analysis.

Biochemistry facilities in DCTI include three 200 sq. ft. labs equipped with a Packard 2500 liquid scintillation counter, two Waters HPLC systems with UV/Vis and ESA Colouchem II electrochemical detectors, a Brandell cell harvester, a Sorvall RC28S and a Beckman J6B centrifuge, a Turner 450 fluorometer, a YSI 2700 biochemistry analyzer, a Branson 2200 sonicator and a Branson 250 microprobe cell disrupter, a Potter-Elvehjem teflon/glass homogenizer, a Microm HM505E microtome, a Lipshaw refrigerated cryostat, a Baker Laminar Flow Hood, Hoefer electrophoresis equipment and power supply, a VHR 2310 Incubator, a Molecular Dynamics Phosphor Imager, an isovolumic red blood cell heart perfusion apparatus, a Diametrics IRMA Blood Analysis System, a Cameron Instruments Blood Oxygen Content. Other support equipment such as dry boxes and vacuum lines are available, along with LBNL support service facilities such as machine shops, electronic shops, and microanalytical laboratories.

Animal Facilities: The animal care facility at LBNL will house animals involved in this research. These animals are obtained from an LBNL-approved source and are delivered to LBNL Building 86. LBNL is

accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC), and the animal laboratory staff at Building 86 is devoted to full-time care of housed animals.
The major LBNL laboratory facilities include electronic shops, machine shops, welding shops, shops for the fabrication of specialty equipment, and instrument shops for repair and calibration.

Equipment: 11 MeV proton-only RDS Cyclotron: Building 56 (1600 sq. ft.) houses the Biomedical Isotope Facility (BIF) containing a dedicated 11 MeV CTI RDS-111 medical cyclotron. The cyclotron is designed for up to 50 μA simultaeous extraction on dual ports for the production of ^{18}F, ^{11}C, ^{13}N and ^{15}O short-lived positron-emitting isotopes as well as many other positron-emitting isotopes of interest for medical, environmental and industrial concerns. The facility has a full complement of shielded hot cells and radiochemical synthesis, purification and testing equipment.
Small Bore multi-layer animal PET: A microPET R4 (rodent) system (Concorde Microsystems Inc.), a commercially available animal PET scanner with an animal port of 120 mm diameter and an axial field of view (FOV) of 78 mm, allows whole-body PET scans of rodents with a few bed positions was recently acquired and installed in B55. The scanner supports spatial resolution of 1.85 mm full-width at half-maximum (FWHM) in the axial direction and 1.66 mm FWHM in the transaxial direction.
The detector material is LSO (CTI, Knoxville, Tenn.), owing to its high stopping power, high light output, and fast decay time [8]. A scintillator block of 19×19×10 mm^3 is sawed into an 8×8 crystal array with 9-mm depth cuts, such that the block is still held together by a 1-mm- thick LSO layer at the bottom. The cuts are filled with a reflective material after chemical polishing of the crystal surfaces [9] to improve light collection. The resulting crystal size of 2.1×2.1×10 mm^3 and a center-to-center distance of 2.4 mm pro- vide high spatial resolution of around 2 mm at the center of the to- mograph. The 64 crystals of a detector block are coupled to a position-sensitive photomultiplier (PS-PMT, Hamamatsu R5900-C8) via a 100-mm-long multi-clad fiber optic bundle. Together with the read-out boards, located at the backside of the PMT, these components form a detector (Fig. 1).
Four of these detectors are enclosed next to each other in a module. For the microPET R4 system, 24 modules are arranged in a ring providing 32 full crystal rings with an axial length of 78 mm and a ring diameter of 148 mm while the electronic FOV is restricted to a diameter of 100 mm in the transaxial direction.
Single photon emission computed tomography (SPECT): A General Electric Millennium VG3 dual-headed gamma camera with the Hawkeye CT system is available at DCTI in Building 55. The system has a large (54 × 40 cm) field of view and variable geometry that allow for whole-body as well as planar and brain SPECT imaging. The Hawkeye module provides anatomical information and attenuation compensation. The intrinsic resolution of each 3/8″ crystal is 3.8 mm FWHM. For whole-body imaging, the LEHR parallel-hole collimators give a resolution of 7.4 mm FWHM. Fan-beam collimators are available, providing a resolution of 6.5 mm FWHM over most of the field of view. Two pinhole collimators with either 0.5 mm or 1.5 mm apertures are also available for small animal imaging. Additional state-of-the-art clinical SPECT scanners are available to this program at the University of California at San Francisco.

Appendix 6: Data Management Plan

Data types and sources
Nuclear data generated by the proposed work will be shared with the public through the submission of peer-reviewed manuscripts, conference presentations (NuDat, CSEWG), and by interaction with the DOE's Nuclear Data program.

Content and format
The repository chosen by the isotope production arm of the nuclear data community is EXFOR, jointly maintained by the National Nuclear Data Center (NNDC) in Brookhaven and the IAEA. Interaction with these bodies will be accomplished through the CSEWG meeting, previously mentioned and by publication in appropriate forums where data are conscientiously selected for inclusion in the repository.

Sharing and preservation
The proposed work includes milestones for the communication of measured data to the public via the publications, conferences, and internationally maintained repositories outlined previously. No access restrictions are anticipated, and the data must be communicated in the specified formats to be acceptible for publication or sharing in these accepted scientific forums. The data are expected to be available indefinitely as the repositories depend on international funding for their continued operation, and any delay in their availability will only result from the time devoted to careful analysis of experimental results.

Protection
No HSR will be conducted by the proposed work and no communication of PII is anticipated for any reason.

Rationale
The plan of the proposed work is to make all measured data available to the scientific community as expeditiously as possible.

Software & Codes
No software will be created as a result of the proposed work.

Data types and sources
Nuclear data generated by the proposed work will be shared with the public through the submission of peer-reviewed manuscripts, conference presentations (NuDat, CSEWG), and by interaction with the DOE's Nuclear Data program.

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Rationale

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Software & Codes

No software will be created as a result of the proposed work.

Appendix 7: Other Attachments: Proposed Use of Vertebrate Animals at the Lawrence Berkeley National Laboratory (LBNL)

Animals used in these studies are young female mice (12 to 14 weeks old for biodistribution studies). The maximum animal use is 500 per year.

A7.1 Animal Welfare – General Statement of Assurance

The Lawrence Berkeley National Laboratory Animal Welfare Research Committee (AWRC) serves as the formal Institutional Animal Care and Use Committee (IACUC) and holds an Assurance of Compliance #A3054-01 from the Public Health Service's NIH Office of Laboratory Animal Welfare; it is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International accreditation number 512). The AWRC meets monthly to review and approve protocols involving the use of vertebrate animals. Approval is granted for three years and includes annual reviews.
No animal will be used in more than one major operative procedure from which it is allowed to recover unless scientifically justified or required as a veterinary procedure. Paralytics will not be used without appropriate anesthesia. Medical care for animals will not be withheld and will be available and provided or supervised as necessary by a qualified veterinarian. The animals' living conditions, including housing, feeding, and non-medical care, will be appropriate for the species, contribute to their health and comfort, and will not deviate from the standards set forth in the USDA Animal Welfare Act and in the current Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Academy of Sciences.
Animals that would otherwise experience severe or chronic pain/distress and cannot be relieved by standard methods will be euthanized immediately and/or as indicated in LBNL IACUC protocol. When an animal loses up to 20% of their total body weight, they will be euthanized. Typically, when animals lose a significant percentage of their total body weight, they may also exhibit the following signs and symptoms of loss of motility (lethargy), loss of appetite, changes in physical behavior and mannerisms and these can be configured by the IACUC for euthanasia compliance. The euthanasia criteria should not include any parameter that could confound the study endpoint (i.e., euthanasia based on any specific hematopoietic parameters).
Personnel conducting animal procedures will have read the pertaining IACUC protocols and be appropriately qualified and trained in those procedures. The training and qualification of such personnel will be appropriately documented. The proposed study protocols do not unnecessarily duplicate previous experiments.

A7.2 Animal Use Justification

Live animals must be used in these studies, because the phenomena investigated (biodistribution of new radionuclide complex agents) depend on the pharmacokinetics of engineered platforms and on kinetic transfers of ligands and metals between and among the several fluid and cellular compartments of the intact animal in the presence of its homeostatically controlled fluid medium. Only small amounts of radionuclides are needed to obtain accurate counting statistics in tissue and excreta samples, avoiding large radionuclide inventories and reducing the amount of handled radioactive materials. The small body size allows conduct of many experiments with statistically useful numbers of animals.

There will be some individual variability in the rates of circulatory mixing and excretion of the test systems, which, combined, will be reflected as variability in efficacy and toxicity. Groups of four mice are used in the biodistribution tests with radioanalysis and groups of 5 mice are used in PET imaging studies. All animals assigned to the studies will undergo terminal necropsy as part of the study design.

Spare animals will be euthanized or made available to other investigators with LBNL IACUC-approved protocols. All animals will be euthanized by humane, approved means. The anticipated numbers of animals to be used, based on the proposed experimental designs and including control animals, are presented in the tables below.

Study	**Species, Strain**	**Age Range**	**Sex**	**Total # of Animals**
Stability and Biodistribution Evaluation in Normal Mice - Radioanalysis				
- 2 radionuclides (^{225}Ac, ^{227}Th) - 2 ligand conditions - 7 time points 30 min, 1, 4, 24, 48 h, 4 and 8 d - n = 4	Mice, Athymic Nude	12-14 weeks	Female	112 ± 11
- 2 radionuclides (^{225}Ac, ^{227}Th) - 2 ligand conditions - 3 time points 1 h, 24 h, and 4 d - n = 4	Mice, Athymic Nude	12-14 weeks	Male	48 ± 5
Stability and Biodistribution Evaluation in Normal Mice – microPET Imaging				
- 6 metal/ligand combinations - 1 necropsy time point - n = 5	Mice, Athymic Nude	12-14 weeks	Female	30 ± 3
- 6 metal/ligand combinations - 1 necropsy time point - n = 5	Mice, Athymic Nude	12-14 weeks	Male	30 ± 3
TOTAL MICE				**F: 142 ± 14** **M: 78 ± 8**

*Please note that ~10% additional animals are included in the estimate to account for extra animals that may be excluded from studies due to potential health concerns or weight/age ranges that falls out of the acceptable range for recruitment into each study.

A7.3 Experimental Procedures

Animals and Husbandry

Animals used will be female athymic nude mice from Simonsen Laboratories. Animals will be received at the LBNL Animal Facility and will be transferred to the experimental laboratories a week prior to the first study event. Each animal will be weighed and identified with an ink tattoo on the tail, and will then be placed into cages for an acclimation period of one week. Observations will be made to ensure that animals are aware of food and water locations, as well as excreting normal amounts of urine and feces upon placement in cages. Animals will be randomized at least one day prior to the first procedure and identified with color-coded cage tags to identify dose and necropsy group. All experimental and control groups will contain four to eight mice. While evaluating the responses of the animals and conducting the analyses, the technical staff will be aware of the treatment history of each animal and sample. Based on the relatively objective endpoints to be examined, however, bias is not expected to influence the results of the study.

Food and water will be provided ad libitum. The targeted conditions for animal room environment will include 18-22 °C controlled temperature, 30-70% controlled relative humidity, and a 12-hour light cycle. Animals will be observed a minimum of twice daily for morbidity and mortality. If found moribund, animals will be euthanized, under Isoflurane anesthesia. Only healthy animals will be chosen for the studies; therefore, a Laboratory attending animal veterinarian or designee will visually examine the animals before release from quarantine. In consultation with the veterinary staff palliative and/or prophylactic treatments may be approved under emergency situations. If needed, the animal may be removed from the study.

Radiometal Complex Solution Preparation

The isotopes will be reconstituted in 0.1 M HNO_3 stock solutions. Challenge materials will be stored under lock and key in a controlled area. Complex solutions will be prepared by mixing and aliquot of the stock solution with an excess of ligand and adjusting the pH to near 5.0 by dropwise addition of 1.0 M NH_4OAc. The mixture will be kept at 50°C for 60 min and purified on a Chelex column, using NH_4OAc as the eluent. The complex solutions will then be diluted in MES buffer. The solutions will all be filter-sterilized (0.2 μm) prior to injection. The injection solutions will be prepared freshly for each study. These procedures may be adjusted for $^{134}Ce/^{134}La$ based on the radiochemistry and solution thermodynamic and kinetic stability results

Isotope Delivery

The isotope (0.20 mL of radiometal-complex solution) will be administered by intravenous injection (iv, 27 gauge needle), under Isoflurane anesthesia into a warmed lateral tail vein. The puncture will then be sealed with ferric subsulfate solution. Three dosing standards will be prepared on each day of dosing, by injecting the isotope solution in vials capped with septum. These standards will be processed similarly to experimental samples to serve as references.

In-Life Assessments, Euthanasia and Necropsy

Animals will be observed twice daily for clinical signs of adverse events, gross motor and behavioral activity, and observable changes in appearance and food consumption. Abnormal clinical signs include, but are not limited to, severely ruffled coat, hunched posture, labored breathing and severe lethargy. A veterinarian or designee will make decisions regarding the euthanasia of weak or moribund animals. All of the animals will be weighed daily from contamination (Day-0) until the end of the study. Cages will be changed daily to facilitate excreta collection. Approximately 5 min prior to the acquisition of PET images, mice will be anesthetized by inhalation of 2% isoflurane and placed on the scanner bed. PET data for each mouse will be recorded via static scans. At the scheduled necropsy time points, blood will be drawn by heart puncture under Isoflurane anesthesia, and mice will be killed by cervical dislocation; urine expelled from the bladder will be added to the last urine collection. Dead mice will be dissected immediately. Tissues to be harvested for radiochemical analysis are: liver, kidneys, spleen, heart, lungs, thymus, and all other collected organs in the abdominal cavity. The skeleton will be defleshed, and all bone samples and remaining soft tissues collected for analysis. All samples harvested will be placed individually directly into appropriately labeled specimen containers for processing. Blood samples will be aliquoted for both radioanalysis and total WBC counts.

Sample Processing and Disposition of Samples

All study animal carcasses and tissues will be held for analysis post necropsy. All samples except liquid blood samples will be dried at 100°C, followed by controlled high-temperature burning at 575°C. The resulting ashes will be chemically treated with concentrated nitric acid. A defined aliquot of these acidified solutions or of the blood solutions will then be transferred (at a minimum volume) and homogenized with 1N nitric acid and scintillation cocktail into a 20-mL scintillation vial for metal content analysis. Isotope content will be determined bi- in each sample by gamma counting (with windows set around the different energies of the isotopes of interest and daughters) and alpha-liquid scintillation

counting (PerkinElmer TRI-CARB 2910TR Liquid Scintillation Counter, with α/β discrimination). Until completion of each study, all test articles, vehicle articles, and processed samples will be stored. Each study will be considered completed once a report has been issued by LBNL. After completion of each study, samples will be discarded according to LBNL standard operating procedures for waste disposition.

Data Analysis

Bioburden changes (target tissue and elimination organ relative changes to controls) will be the primary endpoints of the biodistribution studies. The experimental results will be expressed as normalized metal content relative to the challenge dose (i.e. % of administered dose). The time-course for each metal (with or without treatment) will be analyzed using non-compartmental methods. For each treatment group results will be expressed as mean ± standard deviation and compared statistically across treatment groups by one-way analysis of variance (ANOVA) followed by adequate multiple-comparison tests for post-hoc analysis, using commercial software such as Microsoft Excel and GraphPad Prism.

A7.4 LBNL Veterinary and Animal Care Facilities

The appropriate treatment and care of all research animals is requisite for the conduct of quality research. Our laboratory recognizes and accepts the responsibility for humane treatment of all research subjects, according to the Guide for the Care and Use of Laboratory Animals. The LBNL animal colony is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International AAALAC (accreditation number 512). Veterinary care is supplied as needed. Mice are ordered as needed; after receipt from the supplier, they usually remain in quarantine in the main colony no longer than two weeks before use. A clinical veterinarian evaluates the health status of the animals before they are released from quarantine. Mice are fed pelleted or powdered animal chow (Purina, St. Louis, Mo.). Our facilities maintain temperature and humidity conditions within ranges appropriate for humane housing and cages are checked daily for adequacy of food and water supply. Before use, mice are housed individually or three per cage in metabolism cages, and four per cage in approved plastic stock cages. Animals are removed to clean sterilized cages weekly. All of the procedures used in these studies, including anesthesia and euthanasia, are subject to approval by the LBNL Institutional Animal Care and Use Committee and are reviewed annually.

Procedures for Ensuring Minimum of Distress, Discomfort, Pain, and Injury

Each study is evaluated to determine in advance any possible sources of discomfort or pain to the animals. This is part of the formal Institutional Animal Care and Use Committee (IACUC) approval process. The study animals will be observed at least twice daily for any physical or behavioral changes which might indicate that the animal is experiencing distress, discomfort, pain, or injury, and any such observations will be promptly reported to the responsible veterinary staff and Study Director/PI. Some clinical observations (e.g., hypoactivity, rough fur, hunched posture) are typically expected in preclinical safety and efficacy studies. The studies are designed to minimize these effects by selecting dose levels for studies that produce only mild clinical signs; however, the reality of pre-clinical testing is that severe clinical signs, moribundity, and death sometimes occur. When animals appear to be suffering or are in moribund condition the decision is made to euthanize the animal immediately. Isoflurane anesthesia is used for all procedures except ip injections and euthanasia.

Methods of Euthanasia

Euthanasia is by cervical dislocation, which is approved by the American Veterinary Medicine Association (AVMA). It is our practice to euthanize the animals if they become seriously injured or develop other unexpected painful conditions that do not respond to treatment. All of the procedures used in these studies, including anesthesia and euthanasia, have been approved by the IACUC Committee at LBNL and are reviewed annually.