Production and purification of $^{225}$Ra and $^{225}$Ac at TRIUMF’s Isotope Separation On-line (ISOL) facility and subsequent radiolabeling studies with α-emitter $^{225}$Ac

C. F. Ramogida$^{1,2}$, A. K. H. Robertson$^{1,3}$, P. Kunz$^6$, C. Zhang$^4$, U. Jermilova$^1$, J. Lassen$^6$, I. Bratanovic$^1$, V. Brown$^1$, C. Rodriguez-Rodriguez$^5$, L. Southcott$^1$, V. Radchenko$^1$, F. Bénard$^4$, C. Orvig$^7$, P. Schaffer$^1$

$^1$Life Sciences, TRIUMF – Vancouver, Canada; $^2$Chemistry, Simon Fraser University – Burnaby, Canada; $^3$Physics & Astronomy, University of British Columbia – Vancouver, Canada; $^4$Molecular Oncology, BC Cancer Agency – Vancouver, Canada; $^5$Centre for Comparative Medicine – Vancouver, Canada; $^6$Accelerator Division, TRIUMF – Vancouver, Canada; $^7$Chemistry, University of British Columbia – Vancouver, Canada
Nuclear Medicine with Radiometals

Radiometal
- $\gamma$ or $\beta^+$ emitter (imaging)
- $\alpha$, $\beta^-$, or Auger $e^-$ emitter (therapy)

Chelating Ligand

Linker

Targeting vector (e.g.; antibody, peptide)

Cell surface receptor
Cell membrane
Cell cytoplasm
Targeted Radionuclide Therapy

- Targeted, site-specific, and non-invasive

- Alpha (α)
  - Energy: 5–9 MeV
  - Range: < 1 cell

- Beta (β–)
  - Energy: 0.05–2.3 MeV
  - Range: < 10 cells, 50–1000 cells

- Auger electrons

References:
Targeted Alpha-Therapy

α-particles have high LET (~100 keV/µm) and typical range in tissue of 50 – 100 µm (< 10 cell diameters)

LET 0.2 keV/µm

LET 4 – 26 keV/µm

LET 50 - 230 keV/µm
Targeted Alpha-Therapy with Actinium-225 ($^{225}\text{Ac}$)

Actinium-225 ($^{225}\text{Ac}$) has a relatively long half-life ($t_{1/2} = 10\text{ d}$) followed by four fast alpha decays.
Targeted Alpha-Therapy with $^{225}\text{Ac}$

$^{225}\text{Ac}$ labeled prostate specific membrane antigen (PSMA) has shown remarkable therapeutic response in patients – **complete remission**

PET images of the $^{68}\text{Ga}$-labeled analogue

Targeted Alpha-Therapy with $^{225}$Ac: Current Challenges

- Current world-wide production = 1.7 Ci/yr (63 GBq/yr) – enough for < 2000 patients
- No non-radioactive surrogate – chemistry is virtually unexplored
- $^{225}$Ac chelation and retention of daughters in vivo remains a challenge

Daughter isotopes are released from chelating agent due to 100 keV recoil energy associated with $\alpha$ emission

A. K. H. Robertson, C. F. Ramogida, P. Schaffer, V. Radchenko, Current Radiopharmaceuticals, 2018, 11, 156.
TRIUMF – Canada’s Particle Accelerator Centre

Isotope production via spallation of uranium, etc… targets

ISAC = Isotope Separator and Accelerator; RIB = Radioactive Ion Beam

500 MeV H⁺ cyclotron
Medical Isotope Production at TRIUMF’s ISAC ISOL facility

Isotope Separator and Accelerator (ISAC)
Isotope Separation On-line (ISOL)
Implantation Station – Ion Collector

Dr. Peter Kunz
Target Dissolution

$^{225}\text{Ra}/^{225}\text{Ac}$ etched off Al stage using 0.1 M HCl

Activity Produced:
$^{225}\text{Ra}$ (1.1 – 7.5 MBq)
$^{225}\text{Ac}$ (1.4 – 18.0 MBq)

Experiments performed by Dr. Peter Kunz

Efficiency of activity transfer was first studied using low activity (<1 kBq) samples and quantified via alpha spectroscopy.
Target Dissolution

$^{225}$Ra/$^{225}$Ac etched off Al stage using 0.1 M HCl

Activity Produced:
$^{225}$Ra (1.1 – 7.5 MBq)
$^{225}$Ac (1.4 – 18.0 MBq)

> 99% of all implanted $^{225}$Ra/$^{225}$Ac activity* was retrieved from SEM stage, quantified using **HPGe gamma spectroscopy**
Radiochemical Separation

Step 1: Load

Target sol'n
\( ^{225}\text{Ra}/^{225}\text{Ac} \)

\( 4 \text{ M HNO}_3 \)

\( 2 \text{ mL} \)

DGA, Branched (35 - 40 mg)

Radiochemical Separation

Step 1: Load

Target sol'n $^{225}\text{Ra}/^{225}\text{Ac}$

$2\text{ mL}$

$4\text{ M HNO}_3$

DGA, Branched (35 - 40 mg)

$^{225}\text{Ra}$

---

Radiochemical Separation

Step 1: Load

Target soln
$^{225}$Ra/$^{225}$Ac

2 mL

4 M HNO$_3$

DGA, Branched (35 - 40 mg)

Step 2: Wash

2 mL

4 M HNO$_3$

$^{225}$Ra

Radiochemical Separation

Step 1: Load
- Target sol’n $^{225}\text{Ra}^{225}\text{Ac}$
- $2\text{ mL}$ $4\text{ M HNO}_3$
- DGA, Branched (35 - 40 mg)

Step 2: Wash
- $4\text{ M HNO}_3$
- $2\text{ mL}$

Step 3: Elute
- $0.05\text{ M HNO}_3$
- $0.4\text{ mL}$

$^{225}\text{Ra}$ and $^{225}\text{Ac}$ separation diagram.
Radiochemical Separation

**Step 1: Load**
- Target sol'n $^{225}\text{Ra/}^{225}\text{Ac}$
- 2 mL
- 4 M HNO$_3$
- DGA, Branched (35 - 40 mg)

**Step 2: Wash**
- 4 M HNO$_3$
- 2 mL

**Step 3: Elute**
- 0.05 M HNO$_3$
- 0.4 mL

Radiochemical Separation

**Step 1: Load**
- Target sol’n
  - $^{225}$Ra/$^{225}$Ac
  - 2 mL
  - 4 M HNO$_3$
  - DGA, Branched (35 - 40 mg)

**Step 2: Wash**
- 4 M HNO$_3$
- 2 mL
- Wait ~ 17 d for $^{225}$Ac grow-in; repurify

**Step 3: Elute**
- 0.05 M HNO$_3$
- 0.4 mL

Dissolution of Al and separation of $^{225}$Ra & $^{225}$Ac

- $^{225}$Ra
- $^{225}$Ac
- $^{211}$Fr
- $^{213}$Bi

**References**
### Summary of A = 225 Production at ISOL

<table>
<thead>
<tr>
<th>Run</th>
<th>Date</th>
<th>Duration [h]</th>
<th>Implantation</th>
<th>RIB Yields [ions/s]</th>
<th>Activity Produced [MBq]</th>
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<tr>
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<tr>
<td>1</td>
<td>Dec '15</td>
<td>13.3</td>
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<td>$3.8 \times 10^6$</td>
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<td>4</td>
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<td>7.1</td>
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<td>$5.7 \times 10^7$</td>
<td>10.5</td>
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<td>5</td>
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<td>$9.3 \times 10^7$</td>
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<td>$1.3 \times 10^8$</td>
<td>18.0</td>
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<td>6</td>
<td>Apr '17</td>
<td>80.7</td>
<td>Shorted</td>
<td>$9.0 \times 10^7$</td>
<td>7.5</td>
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<td></td>
<td>$2.8 \times 10^6$</td>
<td>1.7</td>
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</tbody>
</table>

*aEE = extraction electrode; bLIS = Laser ionisation source; cquantified by HPGe γ-spec*
Small library of chelating ligands tested against the current "gold standard"

\[ ^{225}\text{Ac}^{3+} + ^{225}\text{Ac} \rightarrow \text{Radiolabeled complex} \]

- DOTA (CN = 8)
- \(\text{H}_4\text{octapa} \quad \text{CN} = 8\)
- \(\text{H}_2\text{CHXoctapa} \quad \text{CN} = 8\)
- \(\text{macropa} \quad \text{CN} = 8\)
- \(\text{H}_2\text{bispa}^2 \quad \text{CN} = 8\)

CN = coordination number

%RCY = percent radiochemical yield

Stability of preformed $^{225}$Ac-complexes against transchelation to serum proteins.
Stability of preformed $^{225}\text{Ac}$-complexes in 5-fold excess La$^{3+}$ at ambient temperature

Time (days)

% intact $^{225}\text{Ac}$-complex

$^{225}\text{Ac}$-macropa
$^{225}\text{Ac}$-DOTA
$^{225}\text{Ac}$-bispa$^2$
$^{225}\text{Ac}$-phospa
$^{225}\text{Ac}$-octapa
$^{225}\text{Ac}$-CHXoctapa

5:1 La$^{3+}$ to ligand ratio
Efforts Towards Targeted Delivery: $^{225}$Ac-DOTA-CycMSH

**Background:** α-Melanoma-stimulating hormone peptide shows high affinity for the melanocortin 1 receptor (MC1R) which is highly expressed in majority of melanomas (skin cancer).

- High receptor binding affinity
- High tumour uptake and tumor to non-target tissue ratios
- Rapid internalization of tracer

**DOTA-CycMSH CCZ01048**

(F. Bénard, BC Cancer)

PET image of $^{68}$Ga-CCZ01048 (S.A. ~200 MBq/nmol) at 2 h p.i. in mice bearing B16F10 tumours

**225**Ac Radiolabeling of DOTA-CycMSH

DOTA-α−MSH + **225**Ac³⁺ → NH₄OAc buffer 85°C, 45 min

pH 6

iTLC-SG developed in 0.05 M citric acid, pH 5.

82% RCY

**225**Ac-citrate

R_f = 1.00

**225**Ac-peptide

R_f = 0.00

Unlabelled **225**Ac³⁺
**225Ac Radiolabeling of DOTA-CycMSH**

\[
\text{DOTA-\(\alpha\)-MSH} + \text{225Ac}^{3+}, \text{NH}_2\text{OAc buffer, 85°C, 45 min, pH 6} \rightarrow \text{225Ac-peptide}, \text{225Ac-citrate}
\]

- **Rf = 0.00** for 225Ac-peptide
- **Rf = 1.00** for 225Ac-citrate

**iTLC-SG developed in 0.05 M citric acid, pH 5.**

**Corrected RCY 32%**

Unlabelled 225Ac³⁺
**225Ac Radiolabeling of DOTA-CycMSH**

\[ \text{DOTA-} \alpha\text{-MSH} + 225\text{Ac}^{3+} \xrightarrow{\text{NH}_4\text{OAc buffer}} 85^\circ\text{C}, 45\text{ min} \xrightarrow{\text{pH 6}} \]

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Molar Activity (kBq/nmol)</th>
<th>Total injected peptide (nmol)</th>
<th>Total injected activity (kBq)</th>
<th>Unlabeled:labeled peptide ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-blocking</td>
<td>&gt; 200</td>
<td>~ 0.1</td>
<td>~ 20</td>
<td>~2,440:1</td>
</tr>
<tr>
<td>Blocking</td>
<td>1.6</td>
<td>~14</td>
<td>~ 22</td>
<td>~305,000:1</td>
</tr>
</tbody>
</table>

**Note:** 225Ac-CCZ01048 was purified via RP-HPLC (for non-blocking study only) to remove excess unlabeled CCZ01048, and C18 sep-pak to remove free 225Ac^{3+}.
In Vivo Biodistribution of $^{225}$Ac-DOTA-CycMSH

Purified radiotracer injected via tail vein into mice bearing melanoma tumours (B16F10 cells); organs harvested at 2 h p.i.

Gamma counter: Window A

Window A = $^{225}$Ac energy (60-120 keV); Window B = $^{221}$Fr energy (180-260 keV); Window C = $^{213}$Bi energy (400-480 keV)
In Vivo Biodistribution of $^{225}$Ac-DOTA-CycMSH

Window A (Ac-225) Window B (Fr-221) Window C (Bi-213)

* $p < 0.01$

Window A = $^{225}$Ac energy (60-120 keV); Window B = $^{221}$Fr energy (180-260 keV); Window C = $^{213}$Bi energy (400-480 keV)
Conclusions & Future Work

- **MBq quantities** of $^{225}$Ra (1.1 – 7.5 MBq) & $^{225}$Ac (1.4 – 18.0 MBq) can be produced via the ISOL technique by irradiation of UC$_x$ targets → isotopically pure $^{225}$Ac product

- Simple, one-step purification of $^{225}$Ac yields product of high radionuclidic purity, while $^{225}$Ra can be stored and used as a generator

- Isolated $^{225}$Ac enables preclinical radiolabeling, in vitro, and in vivo studies with a variety of novel chelating ligands and bioconjugates

- Medical Isotope Production via ISOL has shifted towards production of other exotic and medically relevant isotopes
  - $A = 224 \mid ^{212}$Pb via $^{224}$Ra (10$^8$ ions/s)
  - $A = 165 \mid ^{165}$Er via $^{165}$Tm (10$^{10}$ ions/s)
Future Work

- Designing more effective $^{225}$Ac-radiopharmaceuticals: Elucidating the coordination environment of Ac-complexes using $\beta$-NMR

$\beta$-NMR with liquid samples - Dr. Monika Stachura (TRIUMF)

ISOL will provide access to $^{230}$Ac, $^{234}$Ac $\rightarrow$ suitable for $\beta$NMR measurement
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Prof. Chris Orvig
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Orvig group

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Katharina Rück

10 min documentary highlighting TRIUMF’s efforts to produce $^{225}\text{Ac}$

rarestdrug.com
TRIUMF’s 500 MeV Isotope Production Facility could be a significant $^{225}\text{Ac}$ source

Current production (worldwide)

<table>
<thead>
<tr>
<th>Isotope Production</th>
<th>Facility</th>
<th>Monthly Production [Ci/month]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{229}\text{Th}$ generators</td>
<td>Breeder reactor</td>
<td>$^{226}\text{Ra}(n, 2n)^{225}\text{Ra}$</td>
</tr>
<tr>
<td></td>
<td>$^{226}\text{Ra}(\gamma)^{225}\text{Ra}$</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>e⁻ accelerator</td>
<td>$^{226}\text{Ra}(\gamma)^{225}\text{Ra}$</td>
</tr>
<tr>
<td></td>
<td>low-energy p⁺ cyclotron</td>
<td>$^{226}\text{Ra}(p, 2n)^{225}\text{Ac}$</td>
</tr>
<tr>
<td></td>
<td>high-energy p⁺ accelerator</td>
<td>$^{232}\text{Th}(p, x)^{225}\text{Ac}$</td>
</tr>
</tbody>
</table>

Potential future production

*Theoretical maximum* $^{225}\text{Ac}$ monthly production [Ci/month] for a single facility

\[ ^{225} \text{Ac} \] must be purified from thorium and many other elements.

<table>
<thead>
<tr>
<th>Elements with isotopes in thorium</th>
</tr>
</thead>
<tbody>
<tr>
<td>target 1 week after EOB</td>
</tr>
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</table>

- challenging purification chemistry
- complex radiation hazards
Target Removal
Targeted Alpha-Therapy with $^{225}\text{Ac}$

$^{225}\text{Ac}$ labeled prostate specific membrane antigen (PSMA) has shown remarkable therapeutic response in patients – complete remission

PET images of the $^{68}\text{Ga}$-labeled analogue