PET AND SPECT RADIOCHEMISTRY

SELECTED EXAMPLES OF LABELLING OF MACROMOLECULES

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MBC, Turino, Italy

PET AND SPECT RADIOCHEMISTRY

WHAT ARE PET AND SPECT ?

PET (Positron Emission Tomography) and SPECT (Single Photon Emission Computed Tomography) are two highly-sensitive, but relatively low-resolution functional imaging technique.

PET and SPECT permit repeated, non-invasive assessment and quantification of specific biological and pharmacological processes in living animals, humans included.

PET and SPECT are probably the most advanced technology currently available for studying in vivo molecular interactions, and represent the method of choice to assess in vivo the distribution, pharmacokinetics and -dynamics of a compound labelled with a radioactive atom.
HOW DOES SPECT WORK?

\[ {_{43}^{99m} \text{Tc}} \rightarrow \gamma \rightarrow 6.0 \text{ h} \]

\[ \gamma : 89\% \]

\[ E_{\text{max}} : 141 \text{ keV} \]

\[ {_{43}^{99m} \text{Tc}} \]

HOW DOES PET WORK?

\[ {_{9}^{18} \text{F}} \rightarrow \beta^+ \rightarrow 109.8 \text{ min} \]

\[ \beta^+ : 96.9\% \]

\[ E_{\text{max}} : 635 \text{ keV} \]

\[ {_{8}^{18} \text{O}} \]

\[ \gamma : 511 \text{ keV} \]

\[ 180^\circ \text{ photon} \]

\[ \gamma : 511 \text{ keV} \]

\[ 180^\circ \text{ photon} \]
**PET AND SPECT RADIOCHEMISTRY**

**HOW DOES PET WORK?**

1. An MRI machine for comparison

2. **SIEMENS EXACT HR+**
Molecular imaging with PET or SPECT requires the preparation of a positron-emitting / single-photon-emitting radio labelled probe (molecule) or radiotracer.

Why Radiochemistry?
PET AND SPECT RADIOCHEMISTRY

PET (SPECT) RADIOCHEMISTRY ... HOW DIFFERENT IS IT FROM CHEMISTRY?

❤ STRESS 1 : RADIOPROTECTION
❤ STRESS 2 : TIME!
❤ STRESS 3 : AUTOMATION (PET ESPECIALLY)

A RADIOPHARMACEUTICAL PREPARATION IMPOSES AN EXHAUSTIVE AND ADVANCED AUTOMATION.

THE GAMMA RAYS FACILITATE IN THIS CASE THE PROCESS MONITORING BY EASY AND SENSITIVE RADIOACTIVITY DETECTION.

THE SUSCEPTIBILITY OF THE CHEMICAL REACTION TO AUTOMATION HAS TO BE TAKEN INTO ACCOUNT AT THE EARLY DESIGN STAGE OF THE RADIOCHEMICAL PATHWAY. CERTAIN MANIPULATIONS OF CLASSICAL CHEMISTRY, SUCH AS LIQUID-LIQUID EXTRACTION OR PRECIPITATION, CANNOT BE ENVISAGED.

AUTOMATION MAY USE BOTH ROBOTIC SYSTEMS AND MODULES ...
THE MOST COMMONLY USED PET RADIOPHARMACEUTICAL?

\[
\begin{align*}
&\text{[\text{\textsuperscript{18}F}]FDG} \\
&\begin{array}{c}
\text{HO} \\
\text{HO} \\
\text{OH}
\end{array}
\end{align*}
\]

PET AND SPECT RADIOCHEMISTRY

2-\text{[\textsuperscript{18}F]}\text{Fluoro-2-deoxy-D-glucose ([\textsuperscript{18}F]FDG)}

\text{RADIOSYNTHESIS: 2 STEPS ONLY}

\begin{align*}
a) \text{fluorine-18 incorporation} & \quad \text{b) deprotection} \\
\text{AcO} & \quad \text{HCl (1mM)} \\
\text{AcO} & \quad 130^\circ \text{C, 5 min} \\
\text{AcO} & \quad \text{K[\textsuperscript{18}F]F-K}_{222} \\
\text{OT} & \quad \text{MeCN} \\
85^\circ \text{C, 5 min} & \quad \text{MeCN}
\end{align*}

PET AND SPECT RADIOCHEMISTRY


1. RADIOSYNTHESES: 2 STEPS ONLY

\[
\begin{align*}
\text{AcO} & \quad \text{AcO} \quad \text{OTf} \\
\text{AcO} & \quad \text{AcO} \quad \text{K}[^{18}\text{F}]\text{F} \quad \text{K}_{2}\text{CO}_{3} \\
\text{MeCN} & \quad 85^\circ\text{C}, 5\text{ min} \\
\text{AcO} & \quad \text{AcO} \quad \text{[}^{18}\text{F}]\text{FDG} \\
\end{align*}
\]

2. PURIFICATION: On CARTRIDGE (No HPLC needed)

3. AUTOMATION

PET AND SPECT RADIOCHEMISTRY

CTI-GPCU (prototype) TRACERlab FX\(_{\text{FDG}}\) TRACERlab MX\(_{\text{FDG}}\) SYNCHROM

FDG - PLUS (prototype) EXPLORA-FDG\(_{\text{SIEMENS medical}}\) SYNTHERA FASTlab

GE Healthcare GE Healthcare
WHAT IS COMMONLY TERMED « MACROMOLECULES »?

MACROMOLECULES =
High-molecular-weight bioactive chemical structures,
- Oligonucleotides single/double-stranded, siRNA ...
- Aptamers
- Peptide Nucleic Acids,
- Peptides
- Proteins
- Antibodies (diabodies …)
- Oligosaccharides
- Nano-objects
PET AND SPECT RADIOCHEMISTRY

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PET AND SPECT RADIOCHEMISTRY

CHEMICAL STRUCTURE OF OLIGONUCLEOTIDES
Partial structures of DNA and RNA presenting all possible nucleobases:
thymine (T), adenine (A), cytosine (C), guanine (G) and uracil (U)
WHAT IS AN OLIGONUCLEOTIDE?
WITH WHICH RADIOISOTOPE?

PET AND SPECT RADIOCHEMISTRY

RADIOISOTOPES: Half-life, Decay mode and Production

<table>
<thead>
<tr>
<th>Radioisotope</th>
<th>Half-life</th>
<th>Decay mode (%)</th>
<th>$E_{y}$ or $E_{y}$ (keV)</th>
<th>Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon-11</td>
<td>20.4 min</td>
<td>$\beta^+$ (99.8) - EC (0.2)</td>
<td>$\beta^+$ 960</td>
<td>$^{18}$O(p,α)$^{14}$C</td>
</tr>
<tr>
<td>Gallium-68</td>
<td>68.3 min</td>
<td>$\beta^+$ (89) - EC (11)</td>
<td>$\beta^+$ 1900</td>
<td>$^{68}$Ge / $^{68}$Ga generator (271 d)</td>
</tr>
<tr>
<td>Fluorine-18</td>
<td>109.8 min</td>
<td>$\beta^+$ (97) - EC (3)</td>
<td>$\beta^+$ 635</td>
<td>$^{18}$O(p,α)$^{18}$F</td>
</tr>
<tr>
<td>Copper-64</td>
<td>12.7 h</td>
<td>$\beta^+$ (19) - EC (41) - $\beta$ (40)</td>
<td>$\beta^+$ 666 / $\beta$ 573</td>
<td>$^{64}$Ni(p,n)$^{64}$Cu</td>
</tr>
<tr>
<td>Yttrium-86</td>
<td>14.7 h</td>
<td>$\beta^+$ (34) - EC (66)</td>
<td>$\beta^+$ 3150</td>
<td>$^{89}$Sr(p,n)$^{89}$Y</td>
</tr>
<tr>
<td>Bromine-76</td>
<td>16.1 h</td>
<td>$\beta^+$ (57) - EC (43)</td>
<td>$\beta^+$ 3900</td>
<td>$^{72}$As($^{3}$He,2n)$^{75}$Br $^{76}$Se(p,n)$^{76}$Br</td>
</tr>
<tr>
<td>Technetium-99m</td>
<td>6.0 h</td>
<td>IT (&gt;99)</td>
<td>$\gamma$ 140</td>
<td>$^{99m}$Mo / $^{99}$Tc generator (67 h)</td>
</tr>
<tr>
<td>Iodine-123</td>
<td>13.2 h</td>
<td>EC (100)</td>
<td>$\gamma$ 159</td>
<td>$^{123}$Te(p,2n)$^{127}$I $^{123}$I</td>
</tr>
<tr>
<td>Indium-111</td>
<td>2.8 d</td>
<td>EC (100)</td>
<td>$\gamma$ 173, 247</td>
<td>$^{111}$Cd(p,n)$^{111}$In $^{112}$Cd(p,2n)$^{112}$In</td>
</tr>
</tbody>
</table>

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PET

SPECT

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**RADIOISOTOPES: Half-life, Decay mode and Production**

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<td>$\beta^-$ 656 / $\beta^-$ 573</td>
</tr>
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**CHEMISTRY**

**NON-METALS**
- covalent-bond making

**METALS**
- prosthetic chelator

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**PET AND SPECT RADIOCHEMISTRY**

✔ WHAT IS AN OLGONUCLEOTIDE?
✔ WITH WHICH RADIOISOTOPE?

**WHICH STRATEGIE FOR LABELLING?**

- The So-Called “True” Labelling Approach
- Simple Addition of a Radioactive Atom
- The Prosthetic Conjugation Approach
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LABELLING STRATEGIES – THE “PROSTHETIC CONJUGATION” APPROACHES

**Approach 1**

1. **CONJUGATION**
2. **RADIOLABELLING**

**Approach 2**

1. **RADIOLABELLING**
2. **CONJUGATION**

**LABELLING STRATEGIES – THE “PROSTHETIC CONJUGATION” APPROACHES**

**Metal Radioisotopes**

**Non-Metal Radioisotopes**

(preferred approach)
LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS

- **Metal Radioisotopes**

LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS

- **Amino Function** (often)
- **Alkyl Linker (if any)**
- **Spacer (if any)**
- **Cheleator**

**Example Structures:**

- Oligo-HN
- Oligo (alkyl-link)-HN
- Oligo-[alkyl-link]-H

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LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS

activated ester or carboxylic acid

\[ \text{CHELATOR} \]

\[ \text{Oligo-NH}_2 \text{ or Oligo\{ALKYL-LINK\}-NH}_2 \]

+ isothiocyanate

\[ \text{CHELATOR} \]

(Thio)UREAS

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LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS

\[ \text{CHELATOR} \]

\[ \text{ALKYL LINKER (IF ANY)} \]

\[ \text{AMINO FUNCTION (OFTEN)} \]

\[ \text{CARBOXYLIC ACID or derivative} \]

\[ \text{spacer (IF ANY)} \]
A range of chelating agents have been described, tailored to the complexation properties of each radiometal. They all need to meet the following requirements: they should:

- co-ordinate the radiometal ion rapidly and quantitatively at micromolar to nanomolar concentrations,
- preferentially bind the radioisotope in the presence of contaminating metal ions,
- form discrete metal complex species to prevent lengthy purification procedures and need for excess ligand,
- co-ordinate the desired radiometal ion in the pH range of 4 to 9,
- co-ordinate the radiometal ion at mild temperatures,
- not release the radiometal ion to adventitious natural ligands in the biological fluid,
- not readily exchange with cations in vivo.

Below are some examples…
**PET AND SPECT RADIOCHEMISTRY**

**LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS: INDIUM-111**

DTPA activated as a bis-(cyclic anhydride) and conjugation with an aminohexyl-modified oligonucleotide followed by indium-111 labelling.

\[
\text{DTPA dianhydride} \xrightarrow{\text{Buffer pH 5}} \text{Oligo-(CH}_2\text{)}_6\text{-HN}^+ \xrightarrow{\left[^{111}\text{In}\right]^{3+}} \text{Oligo-(CH}_2\text{)}_6\text{-HN}^+ \\
\]


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**LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS: TECHNETIUM-99m**

MAG3 activation and conjugation with an aminohexyl-modified oligonucleotide followed by technetium-99m labelling.

\[
\text{S-Acetyl-MAG3} \xrightarrow{\text{DCC, DMF}} \text{S-Acetyl-MAG3-NHS} \xrightarrow{\left[^{99m}\text{Tc}\right]^{4+}} \text{Oligo-(CH}_2\text{)}_6\text{-HN}^+ \\
\]


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LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS: GALLIUM-68

DOTA activation and coupling to an oligonucleotide followed by complexation with gallium-68.

\[
\text{Oligo-(CH}_2\text{}_6\text{-NH}_2 \xrightarrow{\text{Buffer pH 9, RT, 10 h}} \text{EDC/H}_2\text{O, 0°C, 30 min}} \xrightarrow{\text{NaO}_2\text{S, CO}_2\text{H}} \text{Oligo-(CH}_2\text{}_6\text{-NH}_2 \xrightarrow{\left[{^{68}\text{Ga}}\right]\text{Ga}^{3+}} \text{DOTA activation and coupling to an oligonucleotide followed by complexation with gallium-68.}}
\]


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LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS

Preferred approach, except with iodine radioisotopes

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LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS

Preferred approach, except with iodine radioisotopes

Approach 2

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**LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS**

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<tr>
<th>Radioisotope</th>
<th>Half-life</th>
<th>Decay mode (%)</th>
<th>E(_{β} or E(_γ) (keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carbon-11</strong></td>
<td>20.4 min</td>
<td>(β^-) (99.8) - EC (0.2)</td>
<td>960</td>
</tr>
<tr>
<td><strong>Iodine-123</strong></td>
<td>13.2 h</td>
<td>(β^-) (99.9) - EC (0.1)</td>
<td>137</td>
</tr>
<tr>
<td><strong>Indium-111</strong></td>
<td>2.8 h</td>
<td>(β^-) (99.3) - EC (0.7)</td>
<td>117</td>
</tr>
</tbody>
</table>

**NON-METALS**

covalent-bond making

**METALS**

prosthetic chelator

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**LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : CARBON-11**

Oligonucleotide labelling \([^{11}C]methyl\) iodide.

\[
\begin{align*}
\text{\(^{[11]C}\)methyl iodide} & \quad \xrightarrow{\text{LAIH}_4/\text{THF low temperature then H}_2\text{O}} \quad \text{\(^{[11]C}\)CH}_3\text{OH} & \quad \xrightarrow{\text{aq. HI, } \Delta} \quad \text{\(^{[11]C}\)CH}_3\text{I} \\
\text{Oligo} \quad \text{\(^{[11]C}\)CH}_3\text{I} & \quad \xrightarrow{\text{DMF / Buffer pH 8.2, 90°C, 15 min}} \quad \text{\(^{[11]C}\)CH}_3\text{OH} & \quad \xrightarrow{\text{aq. HI, } \Delta} \quad \text{\(^{[11]C}\)CH}_3\text{I} \\
\text{Oligo} \quad \text{\(^{[11]C}\)methyl iodide} & \quad \xrightarrow{\text{DMF / Buffer pH 8.2, 90°C, 15 min}} \quad \text{\(^{[11]C}\)CH}_3\text{OH} & \quad \xrightarrow{\text{aq. HI, } \Delta} \quad \text{\(^{[11]C}\)CH}_3\text{I} \\
\end{align*}
\]

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PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS: CARBON-11

Oligonucleotide labelling using [carbonyl-11C]ethylketene.

**[11C]ethylketene**

\[ ^{11}C \text{CO}_2 \xrightarrow{\text{LiO}_2 \text{H}} \xrightarrow{\text{H}_2\text{O}} \xrightarrow{\text{purification}} {\text{Oligo}}-(\text{CH}_2\text{CH}_2\text{NH}_2)_6-\text{OH} \]

**[11C]CO**

\[ \xrightarrow{\text{Chloroform, RT}} \xrightarrow{\text{HCl/He gas phase}} \xrightarrow{\text{530°C}} \xrightarrow{\text{Hybridized Immobilized}} {\text{Oligo}}^{11}\text{C}-(\text{CH}_2\text{CH}_2\text{HNN})_6 \]


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# PET AND SPECT RADIOCHEMISTRY

## LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS

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</tr>
</thead>
<tbody>
<tr>
<td>Carbon-11</td>
<td>20.4 min</td>
<td>( E_\beta ) (99.6) - EC (0.008)</td>
<td>( E_\gamma ) 1500</td>
</tr>
<tr>
<td>Fluorine-18</td>
<td>109.8 min</td>
<td>( E_\beta ) (97) - EC (3)</td>
<td>( E_\beta ) 635</td>
</tr>
<tr>
<td>Iodine-123</td>
<td>13.2 h</td>
<td>( E_\gamma ) (100)</td>
<td>( E_\beta )</td>
</tr>
<tr>
<td>Bromine-76</td>
<td>16.1 h</td>
<td>( E_\beta ) (57) - EC (43)</td>
<td>( E_\beta ) 3900</td>
</tr>
<tr>
<td>Gallium-68</td>
<td>80.6 min</td>
<td>( E_\beta ) (99) - EC (1)</td>
<td>( E_\beta ) 1900</td>
</tr>
<tr>
<td>Technetium-99</td>
<td>6.0 h</td>
<td>( E_\gamma ) (99.9)</td>
<td>( E_\beta ) 140</td>
</tr>
<tr>
<td>Copper-64</td>
<td>12.7 h</td>
<td>( E_\gamma ) (99) - EC (1) - EC (0.009)</td>
<td>( E_\gamma ) 560 / ( E_\beta ) 275</td>
</tr>
<tr>
<td>Yttrium-89</td>
<td>14.2 h</td>
<td>( E_\gamma ) (99) - EC (1)</td>
<td>( E_\gamma ) 99.9</td>
</tr>
<tr>
<td>Indium-111</td>
<td>2.8 h</td>
<td>( E_\gamma ) (99.9)</td>
<td>( E_\gamma ) 185 / ( E_\beta ) 247</td>
</tr>
</tbody>
</table>

**CHEMISTRY**

**NON-METALS**

covalent-bond making

**METALS**

prosthetic chelator
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<td>$\beta^+$ (100)</td>
<td>$\gamma$ 139</td>
<td></td>
</tr>
<tr>
<td><strong>METALS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallium-68</td>
<td>68.3 min</td>
<td>$\beta^+$ 173, 247 - EC (100)</td>
<td>$\beta^+$ 3980</td>
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LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS: BROMINE-76

Synthesis of N-succinimidyl 4-[76Br]bromobenzoate ([76Br]SBrB) and its use in oligonucleotide labelling.


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<td>$\beta^+$ (100)</td>
<td>$E_x$ 560</td>
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<td>$\beta^+$ (63) - EC (43)</td>
<td>$E_x$ 3000</td>
<td></td>
</tr>
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<td>Gallium-68</td>
<td>68.1 h</td>
<td>$\beta^+$ (69) - EC (11)</td>
<td>$E_x$ 1092</td>
<td></td>
</tr>
<tr>
<td>Tc-99m</td>
<td>6.0 h</td>
<td>$\beta^+$ (69) - EC (11)</td>
<td>$E_x$ 1403</td>
<td></td>
</tr>
<tr>
<td>Copper-64</td>
<td>2.67 h</td>
<td>$\beta^+$ (36) - EC (80)</td>
<td>$E_x$ 1875</td>
<td></td>
</tr>
<tr>
<td>Indium-111</td>
<td>2.8 d</td>
<td>$\beta^+$ (100)</td>
<td>$E_x$ 1170</td>
<td></td>
</tr>
<tr>
<td>Tm-177</td>
<td>52.2 d</td>
<td>$\beta^+$ (71) - EC (29)</td>
<td>$E_x$ 2133</td>
<td></td>
</tr>
<tr>
<td>Yttrium-89</td>
<td>64.8 h</td>
<td>$\beta^+$ (70) - EC (30)</td>
<td>$E_x$ 2375</td>
<td></td>
</tr>
<tr>
<td>Y-89</td>
<td>2.6 d</td>
<td>$\beta^+$ (99.8) - EC (0.2)</td>
<td>$E_x$ 20.4 min</td>
<td></td>
</tr>
<tr>
<td>Ga-67</td>
<td>68.1 h</td>
<td>$\beta^+$ (69) - EC (11)</td>
<td>$E_x$ 1403</td>
<td></td>
</tr>
<tr>
<td>Cu-64</td>
<td>2.67 h</td>
<td>$\beta^+$ (36) - EC (80)</td>
<td>$E_x$ 1875</td>
<td></td>
</tr>
<tr>
<td>Tc-99m</td>
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<td>Indium-111</td>
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<td>$\beta^+$ (100)</td>
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</tr>
<tr>
<td>Y-89</td>
<td>2.6 d</td>
<td>$\beta^+$ (99.8) - EC (0.2)</td>
<td>$E_x$ 20.4 min</td>
<td></td>
</tr>
</tbody>
</table>

NON-METALS: covalent-bond making

METALS: prosthetic chelator
### PET AND SPECT RADIOCHEMISTRY

#### LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS: IODINE-125/123

Two routes to radiiodinated oligonucleotide using p-methoxyphenyl isothiocyanate:

- Direct labelling of an activated benzene ring.

**Diagram:**

- **Route 1:**
  - SCN-\(\text{OCH}_3\) → Scanning, Buffer pH 7 ultrasound RT, 2 h → Oligo-(CH\(_2\)_3-NH\(_2\)) → SCN-\(\text{OCH}_3\) → Na\(^{[125]}\text{I}\) Chloramine-T → Buffer pH 7 45°C, 24 h → Oligo-(CH\(_2\)_3-NH\(_2\)) → SCN-\(\text{OCH}_3\)

- **Route 2:**
  - Buffer pH 7 45°C, 24 h → Oligo-(CH\(_2\)_3-NH\(_2\)) → SCN-\(\text{OCH}_3\) → Na\(^{[125]}\text{I}\) Ox * → Buffer pH 8.5 45°C, 2 h → SCN-\(\text{OCH}_3\)

* Chloramine-T or iodogen


---

#### PET AND SPECT RADIOCHEMISTRY

#### LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS

<table>
<thead>
<tr>
<th>Radioisotope</th>
<th>Half-life</th>
<th>Decay mode (%)</th>
<th>(E_β) or (E_γ) (keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon-11</td>
<td>52.4 min</td>
<td>(β^-) (99.6) - EC (0.4)</td>
<td>(β^-) 1863</td>
</tr>
<tr>
<td>Fluorine-18</td>
<td>109.8 min</td>
<td>(β^-) (97) - EC (3)</td>
<td>(β^-) 635</td>
</tr>
<tr>
<td>Iodine-123</td>
<td>13.2 h</td>
<td>(EC) (100)</td>
<td>(γ) 1399</td>
</tr>
<tr>
<td>Bromine-76</td>
<td>16.1 h</td>
<td>(β^-) (57) - EC (43)</td>
<td>(β^-) 3900</td>
</tr>
<tr>
<td>Gallium-68</td>
<td>68.5 min</td>
<td>(β^-) (93) - EC (11)</td>
<td>(β^-) 1220</td>
</tr>
<tr>
<td>Yttrium-89</td>
<td>64.2 s</td>
<td>(β^-) (19) - EC (81)</td>
<td>(β^-) 820 / (β^-) 673</td>
</tr>
<tr>
<td>Copper-64</td>
<td>12.7 h</td>
<td>(β^-) (96) - EC (4)</td>
<td>(β^-) 1920</td>
</tr>
<tr>
<td>Indium-111</td>
<td>184 min</td>
<td>(β^-) (99.6) - EC (0.4)</td>
<td>(β^-) 118, 207</td>
</tr>
</tbody>
</table>

**CHEMISTRY**

<table>
<thead>
<tr>
<th>NON-METALS</th>
<th>covalent-bond making</th>
</tr>
</thead>
<tbody>
<tr>
<td>METALS</td>
<td>prosthetic chelator</td>
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</tbody>
</table>

---

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F. Dollé  
Service Hospitalier Frédéric Joliot – PB/CEA
PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS: FLUORINE-18

Radiosynthesis of N-succinimidyl 4-[^18F]fluorobenzoate ([^18F]SFB).


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Service Hospitalier Frédéric Joliot – FBM/CEA

PET AND SPECT RADIOCHEMISTRY

OLIGONUCLEOTIDE CONJUGATION WITH NON-RADIOMETALS: FLUORINE-18

Oligonucleotide conjugation with N-succinimidyl 4-[^18F]fluorobenzoate ([^18F]SFB).


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Service Hospitalier Frédéric Joliot – FBM/CEA
**LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS: FLUORINE-18**

Oligonucleotide conjugation with \( N-(4-[{^{18}F}]\text{fluorobenzyl})-2\)-bromoacetamide (\([^{18}F]FBnBrA\)).

\[
\text{Me}_3\text{N}^+\text{CF}_3\text{SO}_3^- \quad \text{or} \quad \text{K}^{[^{18}F]F-K_{222}}
\]

1. **Conventional heating (heating block)**
   - 180°C, 20 min
2. **Microwave activation**
   - 100W, 1 min

\[
\text{DMSO} \quad \text{then} \quad \text{LIAH}_4, \text{THF}
\]

\[
120^\circ \text{C}, 2 \text{ min}
\]

\[
\text{BrCOH}_2\text{Br} \quad \text{H}_2\text{O} / \text{CH}_2\text{Cl}_2
\]

\[
25^\circ \text{C}, 2 \text{ min}
\]

\[
\text{Microwave activation}
\]

\[
100W, 1 \text{ min}
\]

\[
\text{Dollé F et al. - J Label Compds Radiopharm 1997, 39: 319-30.}
\]

\[
\text{Kühnast B et al. - J Label Compds Radiopharm 2000, 43: 837-48.}
\]

\[
\text{Kühnast B et al - Bioconj Chem 2000, 11: 627-36.}
\]

\[
\text{Kühnast B et al - J Label Compds Radiopharm 2003, 46: 1093-103.}
\]

**MACROMOLECULE LABELING: OLIGONUCLEOTIDE and \([^{18}F]FBnBrA\)**

**PET AND SPECT RADIOCHEMISTRY**

**LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS: FLUORINE-18**


**EMMI – Intensive Programme**

F. Dollé  Service Hospitalier Frédéric Joliot – FBM/CEA

---

**LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS: FLUORINE-18**


**EMMI – Intensive Programme**

F. Dollé  Service Hospitalier Frédéric Joliot – FBM/CEA

---

<table>
<thead>
<tr>
<th>Name / C-SC Issue</th>
<th>Length</th>
<th>Sequences</th>
<th>HPLC Rt (min)</th>
<th>MS found (calc)</th>
<th>δ</th>
<th>31P NMR (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[^18]F)c-OL1</td>
<td>9 mer</td>
<td>ACA GAT CCG</td>
<td>14.0 min</td>
<td>2989.0 (2990.0)</td>
<td>14.44</td>
<td></td>
</tr>
<tr>
<td>[^18]F)c-OL2a</td>
<td>18 mer</td>
<td>AG A T A C G G T C C A A T</td>
<td>14.1 min</td>
<td>5847.8 (5846.9)</td>
<td>14.53</td>
<td></td>
</tr>
<tr>
<td>[^18]F)c-OL2b</td>
<td>18 mer</td>
<td>AGAA U ACA CGGU CCA A A U</td>
<td>15.4 min</td>
<td>6346.7 (6345.3)</td>
<td>13.94</td>
<td></td>
</tr>
<tr>
<td>[^18]F)c-OL3</td>
<td>18 mer</td>
<td>A U U G G A C C C U G U A U</td>
<td>15.0 min</td>
<td>5989.7 (5991.0)</td>
<td>13.94</td>
<td></td>
</tr>
</tbody>
</table>

(a) HPLC column and conditions: C18 µBondapak® Waters (300 x 3.9 mm, particle 10 μm); triethylammonium acetate, 50 mM, pH 7 (TEAA) and acetonitrile, gradient elution: linear 5 min from 95/5 to 90/10 (TEAA/acet onitrile) then linear 15 min from 90/10 to 60/40 and wash-out 10 min at 50/50, flow rate: 1.5 mL/min. (b) MALDITOF Spectrometer (SGS, Karlsruhe, Germany). (c) NMR Bruker AMX (300 MHz) apparatus; TMP as internal standard, spectra recorded in water at 298K.

* Analytical data (HPLC, MS, 31P NMR) belong only to the conjugated oligonucleotides.

**Results**

40-60 mCi of ([^18]F)c-OLx, ready-to-use (25-40 mCi/mL in aq. 0.9% NaCl), starting from 1 Ci of [^18]Ffluoride, in 140-160 minutes of radiosynthesis. Automation in place on a Zymate XP (Zymark) robotic system.
**PET AND SPECT RADIOCHEMISTRY**

**LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : FLUORINE-18**

**[18F]Small Interfering RNAs ([18F]-c-SiRNAs)**

<table>
<thead>
<tr>
<th>Code</th>
<th>Combination of Sequence</th>
<th>HPLC Rt (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DUPLEX-1</td>
<td>ON-1: UCG AAG UAU UCC CGG UAC GGU</td>
<td>9.60</td>
</tr>
<tr>
<td></td>
<td>ON-2: TUC AGG CGU CUA AAG GCC CGC AUC GC</td>
<td>10.70</td>
</tr>
<tr>
<td>DUPLEX-2</td>
<td>ON-1: TUC AGG CGU CUA AAG GCC CGC AUC GC</td>
<td>9.80</td>
</tr>
<tr>
<td></td>
<td>ON-2: UCG AAG UAU UCC CGG UAC GGU</td>
<td>9.60</td>
</tr>
<tr>
<td></td>
<td>ON-4: TUC AGG CGU CUA AAG GCC CGC AUC GC</td>
<td>10.70</td>
</tr>
<tr>
<td></td>
<td>ON-5: TUC AGG CGU CUA AAG GCC CGC AUC GC</td>
<td>9.80</td>
</tr>
<tr>
<td>DUPLEX-3</td>
<td>ON-1: UCG AAG UAU UCC CGG UAC GGU</td>
<td>9.60</td>
</tr>
<tr>
<td></td>
<td>ON-2: TUC AGG CGU CUA AAG GCC CGC AUC GC</td>
<td>10.70</td>
</tr>
<tr>
<td></td>
<td>ON-4: TUC AGG CGU CUA AAG GCC CGC AUC GC</td>
<td>9.80</td>
</tr>
</tbody>
</table>

(a) HPLC column and conditions: C18Bondapak®Waters (300 x 7.8 mm, porosity 10 µm); triethylammonium acetate, 50 mM, pH 7.4 (TEAA) and acetonitrile; gradient elution: linear 5 min from 95/5 to 90/10 (TEAA/acetonitrile) then linear 15 min from 90/10 to 75/25 and wash-out 5 min at 50/50, flow rate: 6.0 mL/min.

Results:

15-30 mCi of [18F]-c-SiRNAs, ready-to-use (15-30 mCi/mL in aq. 0.9% NaCl), starting from 1 Ci of [18F]fluoride, in 165 minutes of radiosynthesis.

Automation in place on a Zymate XP (Zymark) robotic system.


**PET AND SPECT RADIOCHEMISTRY**

**MACROMOLECULE LABELING : OLIGONUCLEOTIDE and [18F]FBnBrA**

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS: FLUORINE-18

Oligonucleotide conjugation with 1-[3-(2-[18F]fluoropyridin-3-yl-oxy)propyl]pyrrole-2,5-dione ([18F]FPyME).

\[
\text{BocHN(CH}_2\text{)}_3\text{O} - \xrightarrow{\text{K[18F]F-K222}} \xrightarrow{\text{DMSO, RT, 2-5 min}} \text{H}_2\text{N(CH}_2\text{)}_3\text{O} + \xrightarrow{\text{aq. sat. NaHCO}_3 \text{ dioxane, Vortex, RT, 10 min}} \text{[18F]FPyME}
\]

\[
\text{Oligo-SH} \xrightarrow{\text{DMSO / Buffer pH 7.5 \text{ RT, 15 min}}} \text{Oligo} \xrightarrow{\text{TFA, CH}_2\text{Cl}_2 \text{ DMSO / Buffer pH 7.5 \text{ RT, 2-5 min}}} \text{[18F]FPyME}
\]


PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS: FLUORINE-18

[18F]D4-36 Aptamer ([18F]-c-D4-36)

A series of aptamers targeting the transmembrane receptor tyrosine kinase (RTK) RET (REarranged during Transfection) have been isolated from whole-living cell SELEX protocols.

D4 (98-mer-, chemically stabilized (2'-fluoropyrimidinyl modified) RNA-aptamer) specifically binds to RET with a Kd of 35 ± 3 nM and blocks RET dimerization-dependent signalling pathways induced either by GDNF or by the C634Y activating mutation.

For in vivo 3D-imaging purposes, a shortened version (36-mer only), showing similar binding and selectivity properties, was designed (D4-36).

Results:
24-30 mCi of [18F]-c-D4-36, ready-to-use (20-30 mCi/mL in aq. 0.9% NaCl), starting from 1.0-1.5 Ci of [18F]fluoride, in 150-155 minutes of radiosynthesis.

Automation in place on a Zymate XP (Zymark) robotic system.


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WHAT IS AN OLIGONUCLEOTIDE?

- The So-Called “True” Labelling Approach
- Simple Addition of a Radioactive Atom
- The Prosthetic conjugation approach

WHAT ABOUT OTHER MACROMOLECULES?

Results:
30-100 mCi of [18F]c-TTA-01, ready-to-use (20-65 mCi/mL in aq. 0.9% NaCl), starting from 1 Ci of [18F]fluoride, in 140 minutes of radiosynthesis. Automation in place on a Zymate XP (Zymark) robotic system.
Results:

25-85 mCi of $[^{18}\text{F}]c$-AFIM, ready-to-use (5-15 mCi/mL in aq. 0.9% NaCl), starting from 1 Ci of $[^{18}\text{F}]$fluoride, in 145 minutes of radiosynthesis. Automation in place on a Zymate XP (Zymark) robotic system.
Results:
65-115 mCi of $^{18}$F-c-STxB, ready-to-use (50-75 mCi/mL in aq. 0.9% NaCl), starting from 1 Ci of $^{18}$F-fluoride, in 145 minutes of radiosynthesis. Automation in place on a Zymate XP (Zymark) robotic system.

Results:
15-25 mCi of $^{[18}F]$c-QD (SRA: 8-20 Ci/µmole @ EOB), ready-to-use (10-15 mCi/mL in aq. 0.9% NaCl), starting from 1 Ci of $^{[18}F]$fluoride, in 145 minutes of radiosynthesis.
Automation in place on a Zymate XP (Zymark) robotic system.


WHAT IS AN OLIGONUCLEOTIDE ?
WHAT ABOUT OTHER MACROMOLECULES ?
WHAT ABOUT NANO-OBJECTS ?

WHICH STRATEGIE FOR LABELLING ?
- The So-Called “True” Labelling Approach
- Simple Addition of a Radioactive Atom
- The Prosthetic conjugation approach

ANY NOVEL APPROCHES ?
Of the reactions comprising the ‘Click universe’, the perfect example is the so-called Cu(I)-catalyzed variant of the Huisgen 1,3-dipolar cycloaddition of terminal alkynes to organoazides to form 1,4-disubstituted-1,2,3-triazoles ***. The triazole has also similarities to the ubiquitous amide moiety found in nature, but unlike amides, is not susceptible to cleavage and is nearly impossible to oxidize or reduce.

Hartmuth C, Kolb MG, Finn K and Barry Sharpless
Click Chemistry: Diverse Chemical Function from a Few Good Reactions
**PET AND SPECT RADIOCHEMISTRY**

**MACROMOLECULE LABELLING – ‘CLICK CHEMISTRY’**


**MACROMOLECULE LABELLING – ‘CLICK CHEMISTRY’**

- WHAT IS AN OLIGONUCLEOTIDE?
- WITH WHICH RADIOISOTOPE?
- WHICH STRATEGIE FOR LABELLING?
  - The So-Called “True” Labelling Approach
  - Simple Addition of a Radioactive Atom
  - The Prosthetic conjugation approach
- WHAT ABOUT OTHER MACROMOLECULES?
- WHAT ABOUT NANO-OBJECTS?
- CLICK-CHEMISTRY: A NOVEL APPROACH
- OTHER ALTERNATIVES?
PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS: FLUORINE-18

Toward the fluorine-18-labelling of oligonucleotides …

Fluorine-19

\[ \text{KF-K}_{222} \]

\[ \text{CH}_3\text{CN} \]

100°C, 15 min

then conc. \text{NH}_4\text{OH}

100°C, 15 min

and reverse-phase HPLC

240 minutes, 2.5-5.0% yield


F. Dollé Service Hospitalier Frédéric Joliot – FBM/CEA

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS: FLUORINE-18

Toward the fluorine-18-labelling of oligonucleotides …

SYNTHESIS OF [\( ^{18} \text{F} \)]FLUORINATED ANTISENSE ODN AS WELL AS IN VITRO AND IN VIVO APPLICATIONS WILL BE REPORTED ELSEWHERE.

\[ \text{KF-K}_{222} \]

\[ \text{CH}_3\text{CN} \]

100°C, 15 min

then conc. \text{NH}_4\text{OH}

100°C, 15 min

and reverse-phase HPLC

240 minutes, 2.5-5.0% yield

Radiochemical yield: 0.55-1.10% !!!


F. Dollé Service Hospitalier Frédéric Joliot – FBM/CEA
Fluorine-18 Chemistry for Molecular Imaging with Positron Emission Tomography,
Fluorine and Health: Molecular Imaging, Biomedical Materials and Pharmaceuticals,

Oligonucleotides with radioactive tags for in vivo imaging with SPECT and PET,
Recent Advances of Bioconjugate Chemistry in Molecular Imaging,

In vivo imaging of oligonucleotidic aptamers,
Nucleic Acid and Peptide Aptamers: Methods and Protocols,

The challenge of labeling macromolecules with fluorine-18: Three decades of research,