

## <sup>18</sup>F[AlF]-radiolabelled Peptides on the Automated Synthesis Platform: Translating the Laboratory Bench Work to Bedside

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### Abstract

Using radiolabelled peptides that bind, with high affinity and specificity, to receptors on tumour cells is one of the most promising fields in modern molecular imaging and targeted radionuclide therapy (1). In the emergence of molecular imaging and nuclear medicine diagnosis and therapy, albeit theranostic, radiolabelled peptides have become vital tools for in vivo visualisation and monitoring physiological and biochemical processes on molecular and cellular levels (2). This approach may benefit patients in the era of personalised medicine.

**Keywords:** <sup>18</sup>F[AlF] method, radiolabelled peptide, molecular imaging, automated platform

### Radiolabelled Peptides in Molecular Imaging

The overexpression of numerous peptide-binding receptors in various tumours and inflammatory tissues have led to the use of radiolabelled peptides for imaging and therapy (2, 3). The cell surface enzyme prostate specific membrane antigen (PSMA), the somatostatin receptor (SST), the bombesin receptor 2 (GRP) and the chemokine receptor (CXCR4) are prominent examples of receptors that are overexpressed as ‘tumour markers’ and linked to tumour development, progression and, often, prognosis, hence inviting researchers to investigate these targets by developing innovative radiopharmaceuticals. Being small, compared to larger targeting compounds like antibodies, makes the peptide an interesting

targeting compound because it allows rapid clearance from the blood pool and non-target tissues (2). Additionally, peptides, which are usually non-immunogenic, possess strong tissue penetration properties and high tumour uptake leading to favourable tumour-to-background ratios for excellent image quality and tumour targeting therapy (2, 4).

The radiolabelled peptide debuted in 1989 when Krenning et al. used an <sup>123</sup>I-radioiodinated somatostatin analogue ([<sup>123</sup>I]204-090) in patients with neuroendocrine tumours (2, 5). Since then, peptides have been labelled with indium-111 (In-111) and technetium-99m (Tc-99m) for single-photon emission computed tomography (SPECT) imaging and with gallium-68 (Ga-68), copper-64 (Cu-64), yttrium-86 (Yt-86) and fluorine-18 (F-18) for positron emission tomography (PET)

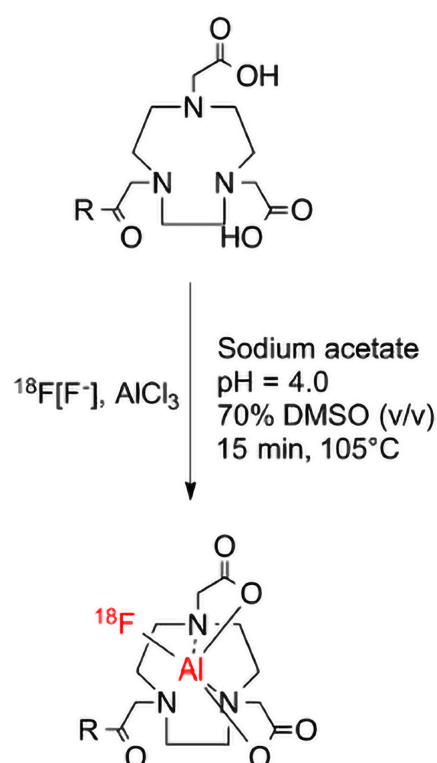
imaging. For PET imaging modality, F-18 is the most extensively used radioisotope due to its favourable decay properties, highest probability of positron decay, low positron penetration depth and suitable half-life, which allows it to carry out even multistep syntheses and be transported to remote hospitals without an onsite cyclotron (6). On the other hand, the half-life is short enough to avoid extended irradiation for patients (7, 8).

Despite its favourable characteristics, the conventional labelling technique for  $^{18}\text{F}$ -fluoride often requires harsh reaction conditions, such as high temperatures and the use of polar aprotic organic solvents under basic conditions, which are unsuitable for the labelling of peptides and small proteins (9, 10). Since 2010, continuous research has overcome this limitation by developing attractive alternatives. One such is the introduction of the bifunctional chelator (BFC) suitable for complexation of  $^{18}\text{F}$ -fluoride bound to a metal and a functional group that allows for bioconjugation to the peptides of interest (1, 6). McBride et al. indicated that fluorine can act as a complexation ligand for  $\text{Al}^{3+}$ , which has been claimed to be stronger than 60 other metal-fluoride bonds (10). The aluminium-fluoride bond (AlF), when in a suitable chemical environment, can be highly stable in vivo and compatible with biological systems (10–15).

Thus, selecting suitable chelators that could stably hold the  $^{18}\text{F}[\text{AlF}]$  complex in such a chemical environment for several hours under physiological, in vivo conditions is a highly attractive alternative to classical F-18 labelling of peptides. Since aluminium ( $\text{Al}^{3+}$ ) forms octahedral complexes, the conjugated peptides from triazacyclononane derivatives, such as 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), seem to be ideal candidates for such an approach (1, 10, 16). As a result, direct radiolabelling of peptides with F-18 in a one-step strategy comprising the chelation of the aluminium-fluoride-18 complex ( $^{18}\text{F}[\text{AlF}]$ ) by the macrocyclic ligand, NOTA, coupled to a peptide, has been investigated and established by several groups (9, 10, 17).

With this available methodology, investigation has already begun to find innovative F-18 labelled peptides, which were previously labelled with the suboptimal radioisotope gallium-68 (Ga-68), such as  $^{18}\text{F}[\text{AlF}]$ -NOTA-octreotides for imaging neuroendocrine tumours,  $^{18}\text{F}[\text{AlF}]$ -NOTA-pentixafor for imaging lymphoproliferative disease and  $^{18}\text{F}$ -PSMA for imaging prostate

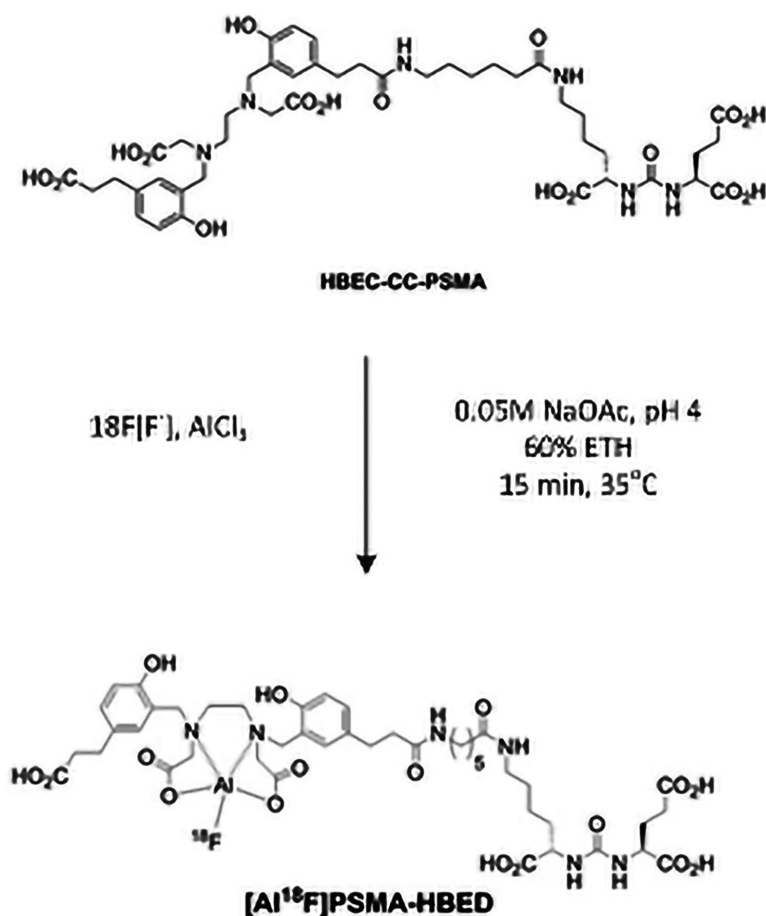
cancer (6, 18). Ga-68, with a half-life of only 68 min, is produced from generators that provide limited activity per synthesis, and, depending on the age of the generator, only one to four patient doses per elution can be produced (19). As the radioisotope properties of F-18 are superior to those of Ga-68, and its availability is high, it can be delivered to all PET centres worldwide, together with FDG shipped daily. Therefore, the motivation towards developing  $^{18}\text{F}$ -labelled peptides is obvious. It allows for producing  $^{18}\text{F}$ -labelled peptides via simple processes, in high yields, without necessary investments in expensive Ga-68 generators (20).



**Figure 1.** Radiolabelling of pentixafor with F-18 (21)

### The Dilemma: Translating the Laboratory Bench Work to the Bedside

Unfortunately, despite  $^{18}\text{F}$ -labelled peptides showing remarkable advantages for receptor imaging and targeted therapy, none of the dedicated, semi-manual or even automated labelling strategies for these peptides, or the NOTA-peptide conjugates themselves, are readily available. Thus, studies on  $^{18}\text{F}[\text{AlF}]$ -



**Figure 2.** Radiolabelling of PSMA with F-18 (22)

NOTA-octreotides,  $^{18}\text{F}[\text{AlF}]-\text{NOTA-pentixafor}$  and even  $^{18}\text{F}$ -PSMA are rare. Most of the required peptide conjugates are only available at dedicated research institutions or centres that can be counted in number and are being backed by strong personnel and research teams. The syntheses are also normally carried out manually by well-trained radiochemists or radiopharmacists.

Thus, a bold move should be considered to close the gap between such high-profile research institutions and ‘normal’ research institutions or university hospitals that provide routine service to patients and are usually operated by nuclear medicine technologists, who prepare the radiopharmaceuticals. This move would include introducing pre-validated synthesis kits and cassettes for radiolabelling peptides on an automated platform or kit base, which could be made accessible to others and made ready to be exploited by other centres with less extensive radiopharmaceutical infrastructures.

## Conclusion

As radiolabelled peptides show remarkable potential in the molecular imaging field, this could promise a new chapter in the era of personalised medicine. Hence, collaborations between stakeholders, researchers and industry players are paramount to making ways for new discoveries and the innovation of pre-validated synthesis kits for radiolabelling peptides on a commercially available, automated platform or kit base, which could be easily handled by technologists.

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None.

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## Authors' Contributions

Drafting of the article: HH

Critical revision of the article for important intellectual content: HRAR, FFAS, VK

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## References

1. Charron CL, Farnsworth AL, Roselt PD, Hicks RJ, Hutton CA. Recent developments in radiolabelled peptides for PET imaging of cancer. *Tetrahedron Lett.* 2016;**57(37)**:4119–4127. <https://doi.org/10.1016/j.tetlet.2016.07.083>
2. Richter S, Wuest F. <sup>18</sup>F-labeled peptides: the future is bright. *Molecules.* 2014;**19(12)**:20536–20556. <https://doi.org/10.3390/molecules191220536>
3. Reubi JC. Peptide receptors as molecular targets for cancer diagnosis and therapy. *Endocr Rev.* 2003;**24(4)**:389–427. <https://doi.org/10.1210/er.2002-0007>
4. Olberg DE, Hjelstuen OK. Labeling strategies of peptides with <sup>18</sup>F for positron emission tomography. *Curr Top Med Chem.* 2010;**10(16)**:1669–1679. <https://doi.org/10.2174/156802610793176747>
5. Krenning EP, Bakker WH, Breeman WA, Koper JW, Kooij PP, Ausema L, et al. Localisation of endocrine-related tumours with radioiodinated analogue of somatostatin. *Lancet.* 1989;**333(8632)**:242–244. [https://doi.org/10.1016/S0140-6736\(89\)91258-0](https://doi.org/10.1016/S0140-6736(89)91258-0)
6. Drude N, Tienken L, Mottaghy FM. Theranostic and nanotheranostic probes in nuclear medicine. *Methods.* 2017;**1(130)**:14–22. <https://doi.org/10.1016/j.ymeth.2017.07.004>
7. Cleeren F, Lecina J, Billaud EMF, Ahamed M, Verbruggen A, Bormans GM. New chelators for low temperature Al<sup>18</sup>F-labeling of biomolecules. *Bioconjug Chem.* 2016;**27(3)**:790–798. <https://doi.org/10.1021/acs.bioconjchem.6b00012>
8. Serdons K, Verbruggen A, Bormans GM. Developing new molecular imaging probes for PET. *Methods.* 2009;**48(2)**:104–111. <https://doi.org/10.1016/j.ymeth.2009.03.010>
9. Allott L, D Pieve C, Turton DR, Smith G. A general [<sup>18</sup>F]AlF radiochemistry procedure on two automated synthesis platforms. *React Chem Eng.* 2017;**2(1)**:68–74. <https://doi.org/10.1039/C6RE00204H>
10. McBride W J, Sharkey RM, Goldenberg DM. Radiofluorination using aluminum-fluoride (Al<sup>18</sup>F). *EJNMMI Res.* 2013;**3(1)**:36. <https://doi.org/10.1186/2191-219X-3-36>
11. Laverman P, McBride WJ, Sharkey RM, Eek A, Joosten L, Oyen WJ, Goldenberg DM, Boerman OC. A novel facile method of labeling octreotide with <sup>18</sup>F-fluorine. *J Nucl Med.* 2010;**51(3)**:454–461. <https://doi.org/10.2967/jnumed.109.066902>
12. McBride WJ, D'Souza CA, Sharkey RM, Goldenberg DM. The radiolabeling of proteins by the [<sup>18</sup>F]AlF method. *Appl Radiat Isot.* 2012;**70(1)**:200–204. <https://doi.org/10.1016/j.apradiso.2011.08.013>
13. Malik N, Zlatopolskiy B, Machulla HJ, Rske SN, Solbach C. One pot radiofluorination of a new potential PSMA ligand [Al<sup>18</sup>F]NOTA-DUPA-Pep. *J Label Compd Radiopharm.* 2012;**55(9)**:320–325. <https://doi.org/10.1002/jlcr.2944>
14. Li L. The biochemistry and physiology of metallic fluoride: action, mechanism, and implications. *Crit Rev Oral Biol Med.* 2003;**14(2)**:100–114. <https://doi.org/10.1177/154411130301400204>
15. Antonny B, Chabre M. Characterization of the aluminum and beryllium fluoride species which activate transducin: analysis of the binding and dissociation kinetics. *J Biol Chem.* 1992;**267(10)**:6710–6718.

16. André JP, Mäcke H, Kaspar A, Künnecke B, Zehnder M, Macko L. In vivo and in vitro  $^{27}\text{Al}$  NMR studies of aluminum(III) chelates of triazacyclononane polycarboxylate ligands. *J Inorg Biochem.* 2002;**88**(1):1–6. [https://doi.org/10.1016/S0162-0134\(01\)00340-3](https://doi.org/10.1016/S0162-0134(01)00340-3)
17. Laverman P, McBride WJ, Sharkey RM, Goldenberg DM, Boerman OC.  $\text{Al}^{18}\text{F}$  labeling of peptides and proteins. *J Label Compd Radiopharm.* 2014;**57**(4):219–223. <https://doi.org/10.1002/jlcr.3161>
18. Lapa C, Lückerrath K, Kleinlein I, Monoranu CM, Linsenmann T, Kessler AF, et al.  $^{68}\text{Ga}$ -pentixafor-PET/CT for imaging of chemokine receptor 4 expression in glioblastoma. *Theranostics.* 2016;**6**(3):428–434. <https://doi.org/10.7150/thno.13986>
19. Boshi S, Lee JT, Beykan S, Slavik R, Wei L, Spick C, et al. Synthesis and preclinical evaluation of an  $\text{Al}^{18}\text{F}$  radiofluorinated GLU-UREA-LYS(AHX)-HBED-CC PSMA ligand. *Eur J Nucl Med Mol Imaging.* 2016;**43**(12):2122–2130. <https://doi.org/10.1007/s00259-016-3437-y>
20. Poschenrieder A, Schottelius M, Schwaiger M, Kessler H, Wester HJ. The influence of different metal-chelate conjugates of pentixafor on the CXCR4 affinity. *EJNMMI Res.* 2016;**6**(1):36. <https://doi.org/10.1186/s13550-016-0193-8>
21. Poschenrieder A, Osl T, Schottelius S, Hoffmann F, Wirtz M, Schwaiger M, et al. First  $^{18}\text{F}$ -labeled pentixafor-based imaging agent for PET imaging of CXCR4 expression in vivo. *Tomography.* 2016;**2**(2):85–93. <https://doi.org/10.18383/j.tom.2016.00130>
22. Malik N, Baur B, Winter G, Reske SN, Beer AJ, Solbach C. Radiofluorination of PSMA-HBED via  $\text{Al}^{18}\text{F}^{2+}$  chelation and biological evaluations in vitro. *Mol Imaging Biol.* 2015;**17**(6):777–785. <https://doi.org/10.1007/s11307-015-0844-6>