One-step labelling of PSMA PET radiotracers with Gallium-68:

Utilising the THP chelator

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Ga-68: the new Tc-99m?

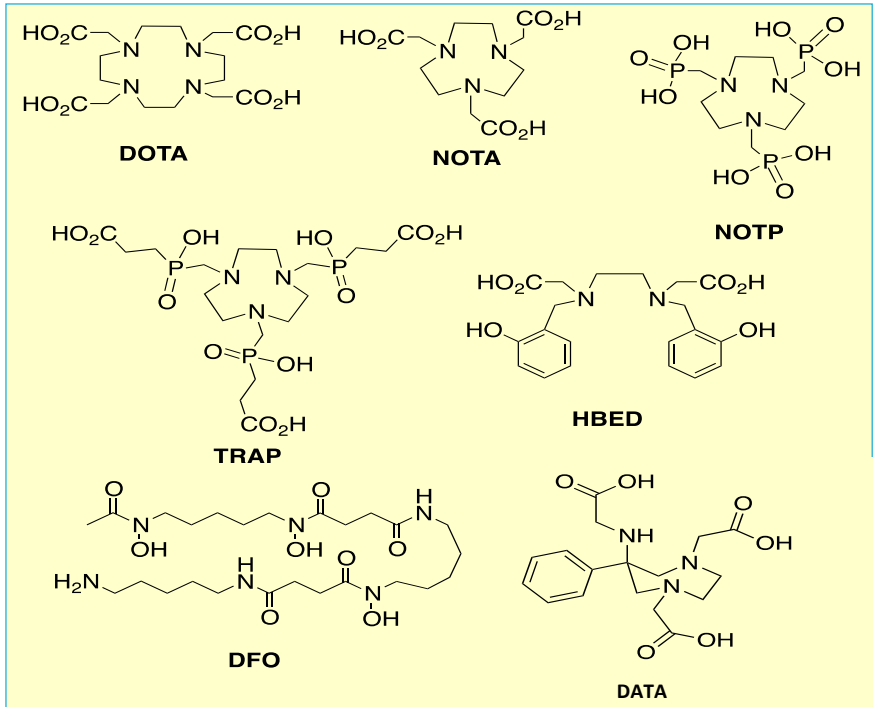
^{68}Ga: short half life (68 min) positron emitter versatile, generator produced….what's not to like?

- Tc-99m generator and *kits* revolutionised nuclear medicine in 1970s - available in all major hospitals
- In most cases, all we need is the generator, syringe, shield and single kit vial
- Can we do this with ^{68}Ga?
  - We have ^{68}Ga generator: E&Z generator: marketing authorisation in 2014, others on track
  - We have chelators, lots of them…
- In theory YES…
- …In practice NO
- Current Ga-68 chelators need one or more of: heat (90°C), time (30 min), acid, purification step, automated cartridge-based system
- A one step "shake and bake" kit - syringe and vial - would make Ga-68 PET accessible to more centres – wide patient impact

no

To paraphrase Alan Jay Lerner:

"Why can't gallium be more like technetium?"



What is a “kit”?

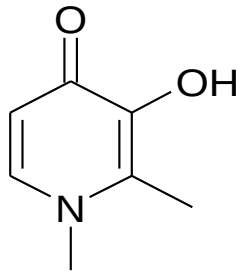
- Cartridge for an automated synthesis module to automate a multi-step series of reactions, perhaps including purification?

- Set of vials and reagents for multistep manual labelling (e.g. including pH adjustment, heat, purification…)?

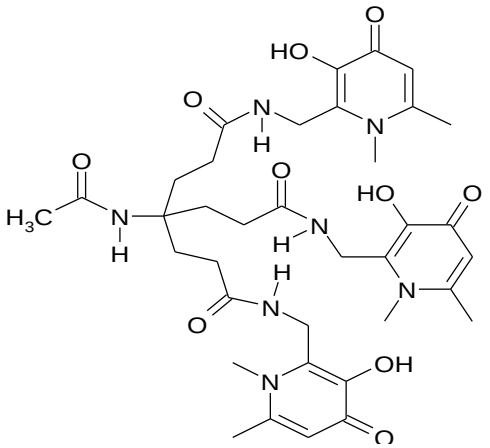
- A single vial into which generator eluate can be injected to produce the final product ready for patient administration in 1 – 2 min at room temperature?
  - Then have a cup of tea
  - Like most Tc-99m labelling since 1970s

Tris(hydroxypyridinone) ligands

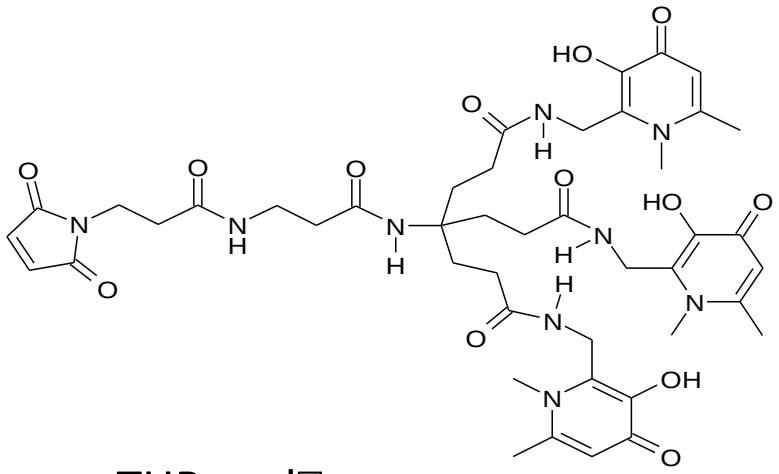
- To achieve rapid room temperature labelling we need a chelator with low barriers to chelation
- But with short half life (68 min) we can sacrifice some in vivo kinetic stability
- Strategy:
  - Abandon macrocyclic chelators
  - Learn from iron chelators (Fe^{3+} and Ga^{3+} have same charge, very similar ionic radius)



Deferiprone



THP-Ac



THP-mal

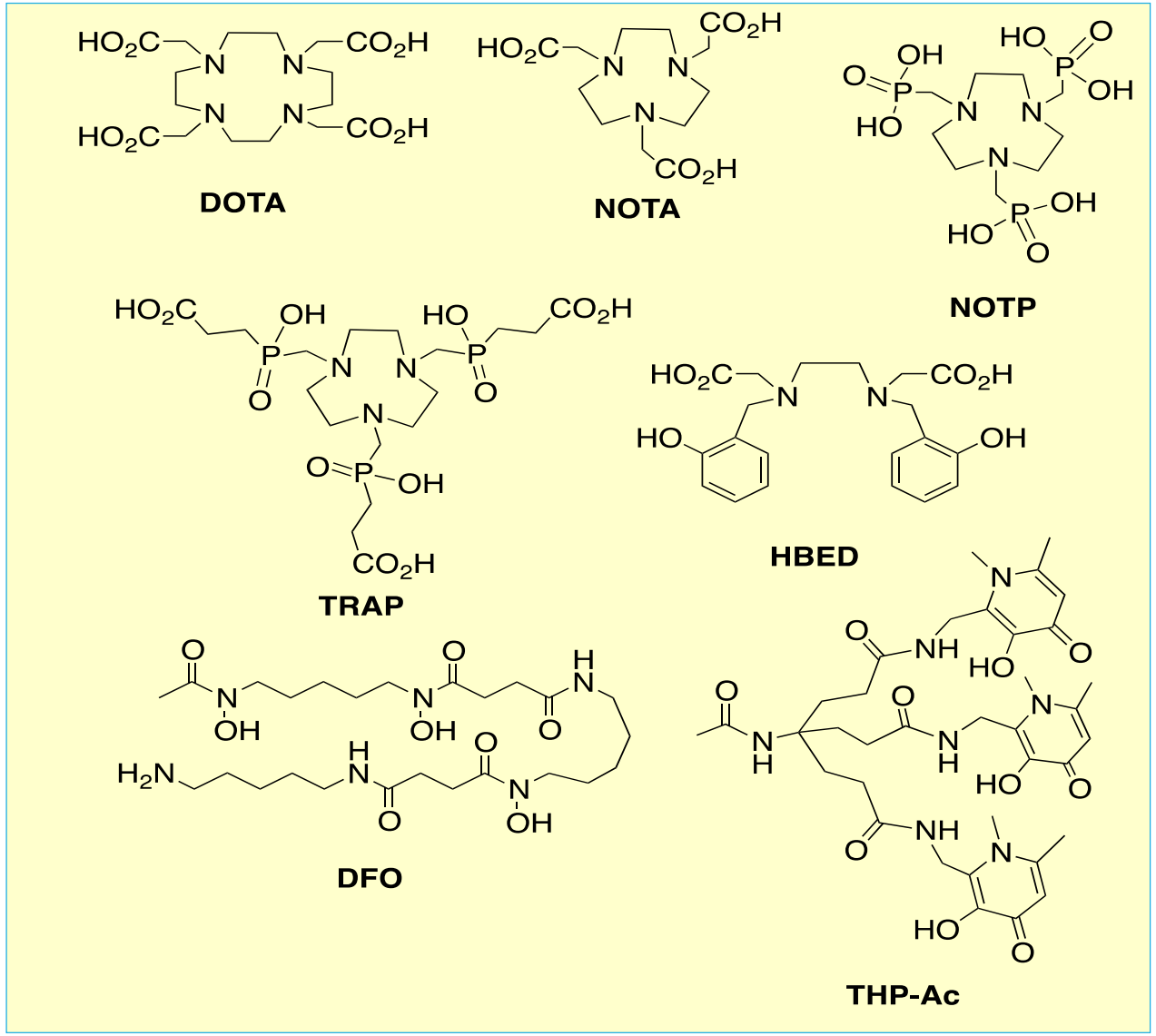
Berry et al. *Chem Commun* 2011;47:7068–7070.

Comparison with accepted chelators: two methods

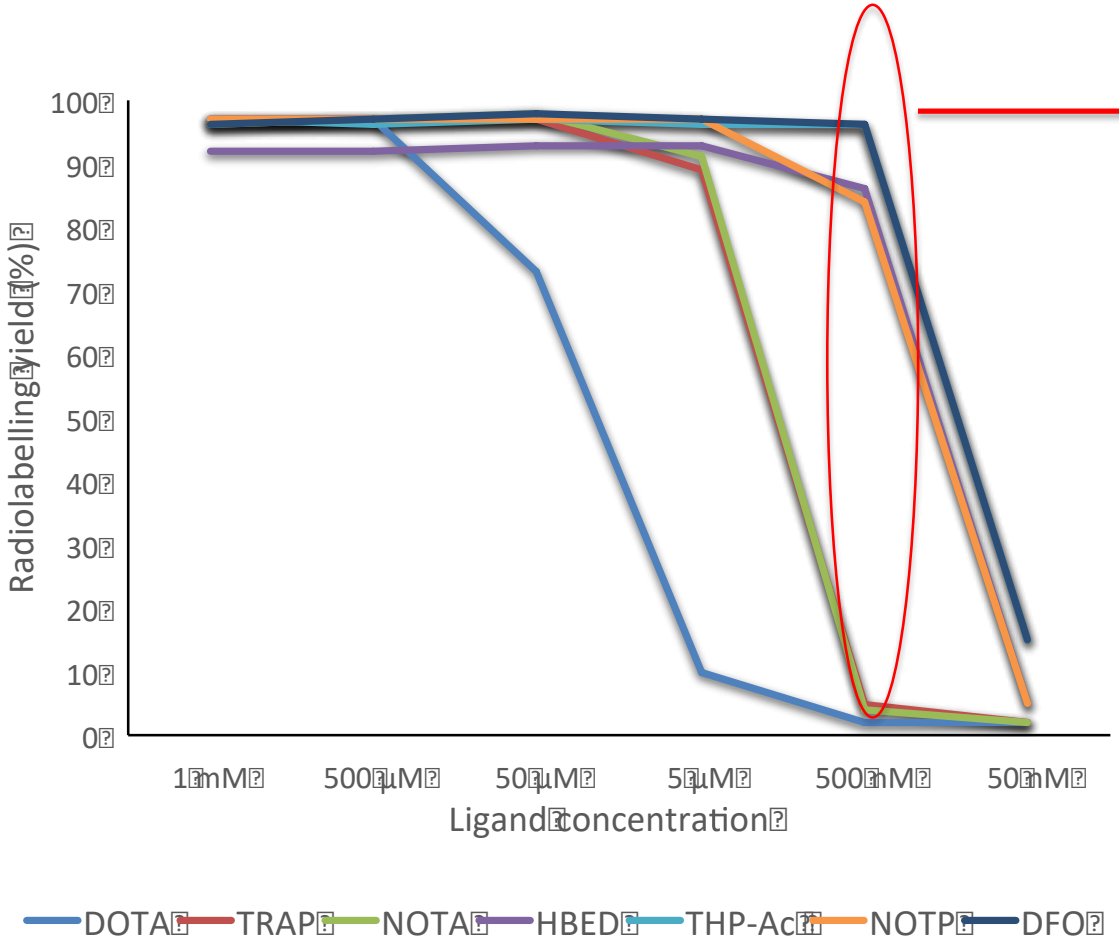
1. Measure labelling efficiency at progressively lower ligand concentration
   (fixed time, temp, pH)

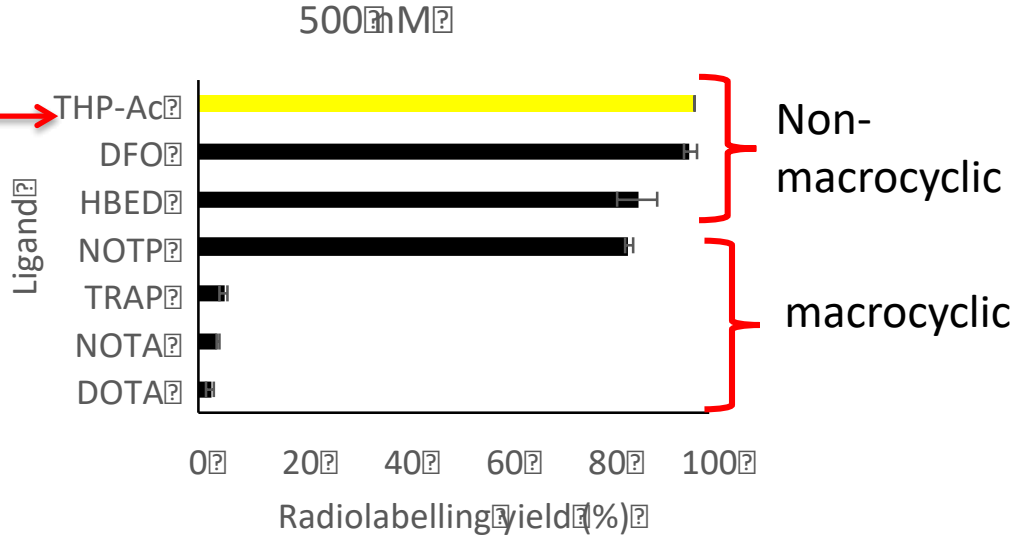
2. Label 1:1 mixture of two chelators, analyse by HPLC
   (fixed time, temp, pH)

   - the “FIGHT!” method



Method 1: reduce concentration



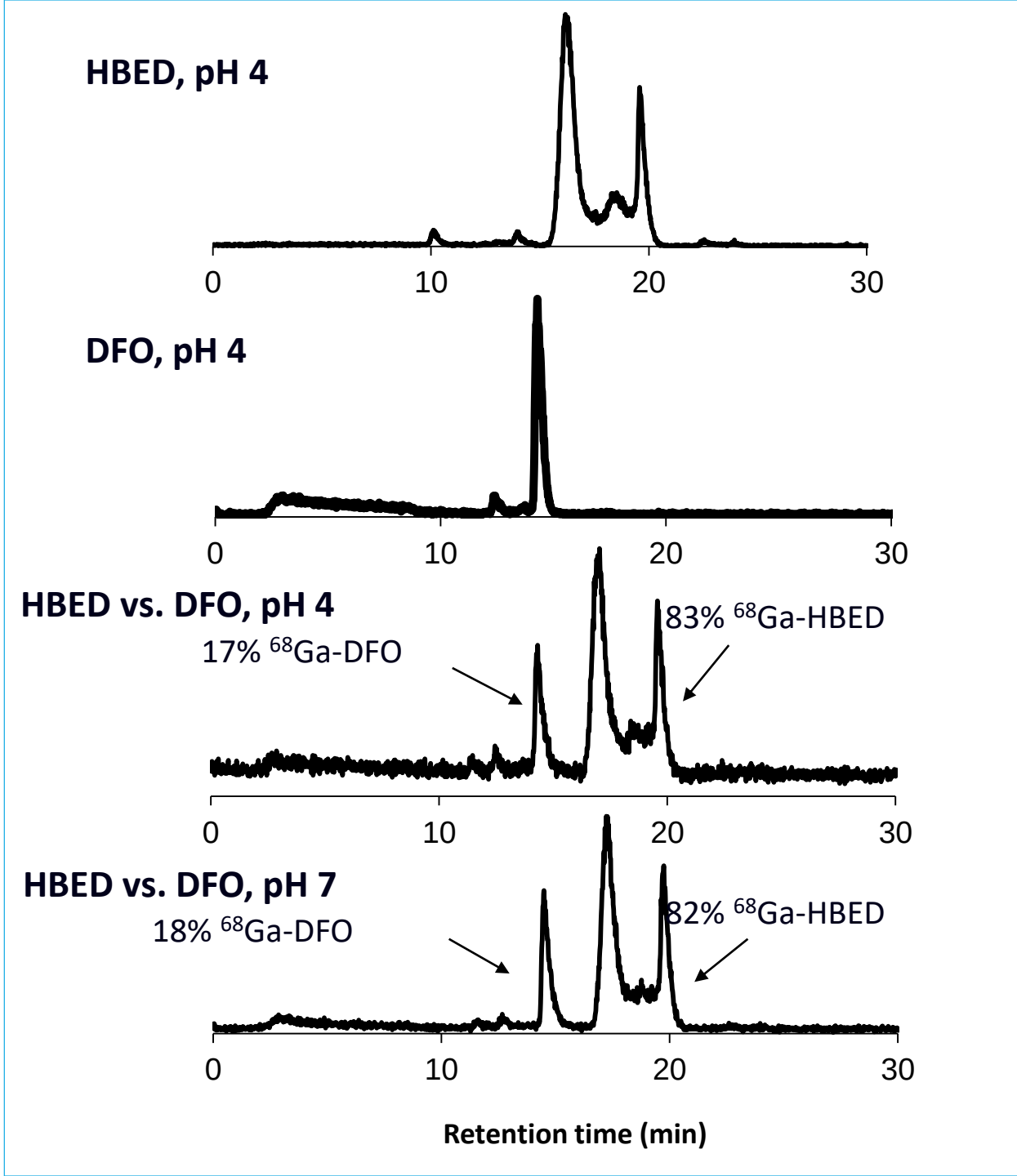


Competition studies

First measure radio-HPLC of ^{68}Ga-complex - characteristic retention time

Then: Chelators mixed in a 1:1 ratio and labelled with ^{68}Ga

…example HBED vs.DFO

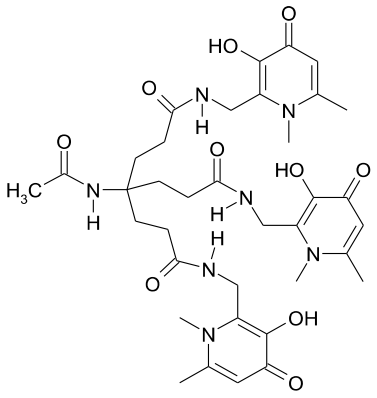


Competition results, room temp, pH 7

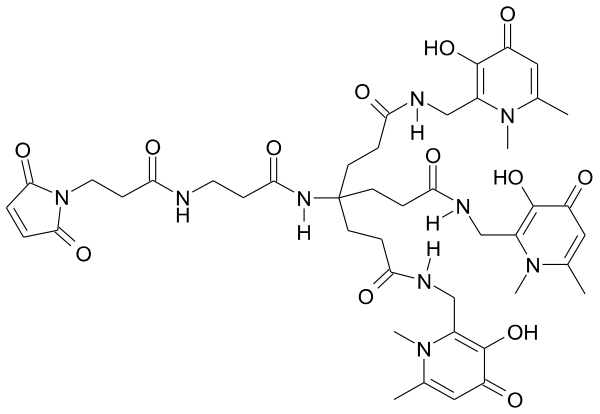
	DOTA	**NOTA**	**NOTP**	**HBED**	**TRAP**	**THP-Ac**	**DFO**
DOTA				98.8% HBED		100% THP-Ac	100% DFO
NOTA				41.4% HBED		99% THP-Ac	32.8% DFO
NOTP				0% HBED		92% THP-Ac	25.6% DFO
HBED	1.2% DOTA	58.6% NOTA	100% NOTP		61.9% TRAP	100% THP-Ac	18.4% DFO
TRAP				38.1% HBED		100% THP-Ac	25% DFO
THP-Ac	0% DOTA	1% NOTA	8% NOTP	0% HBED	0% TRAP		0% DFO
DFO	0% DOTA	67.2% NOTA	74.4% NOTP	81.6% HBED	75% TRAP	100% THP-Ac	

In every 2-way fight, THP-Ac wins by at least an order of magnitude
Similar results at pH 4

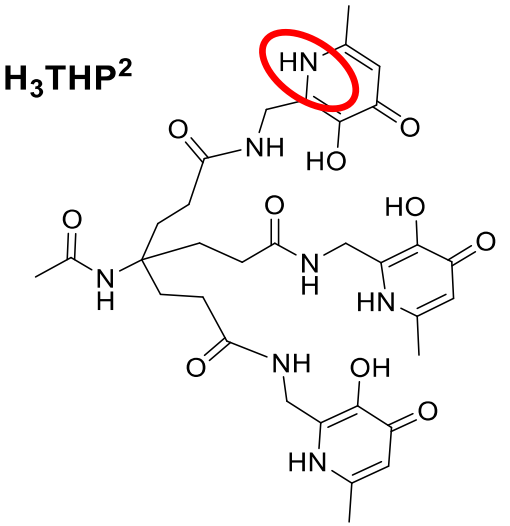
New variants of THP

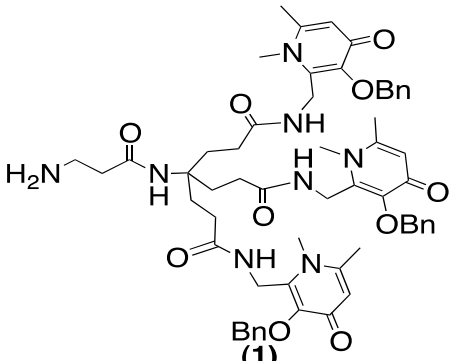


THP-Ac

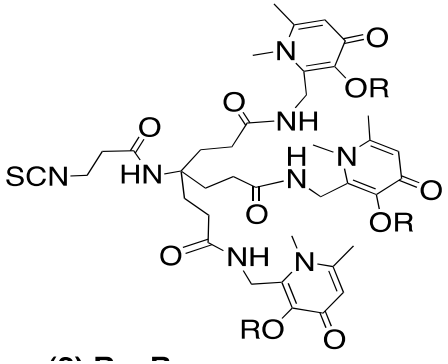


THP-mal

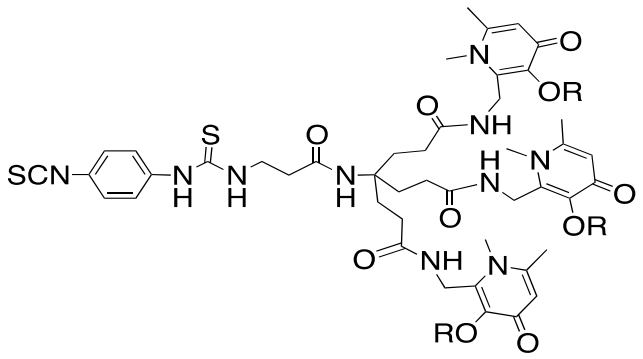




THP-NH$_2$



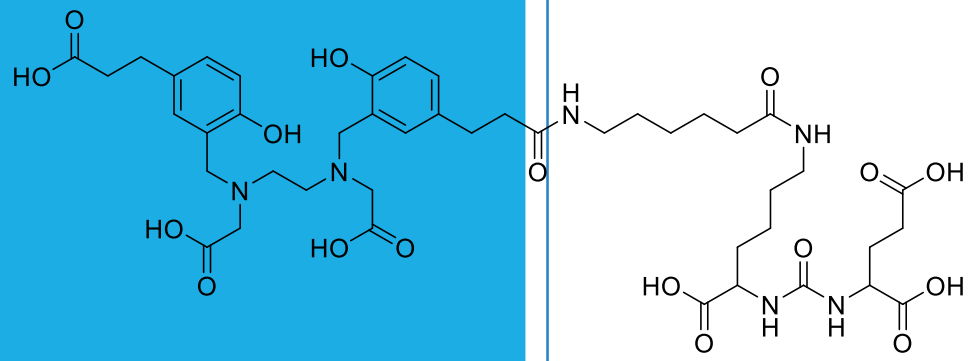
THP-NCS



THP-PhNCS

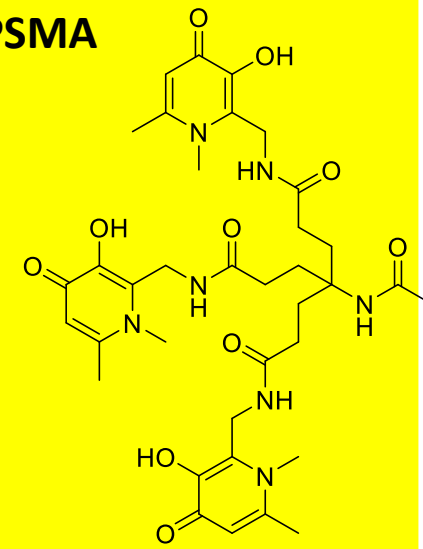
Can this advantage be turned into simple kits?
THP-PSMA Tracer Design

HBED-CC-PSMA

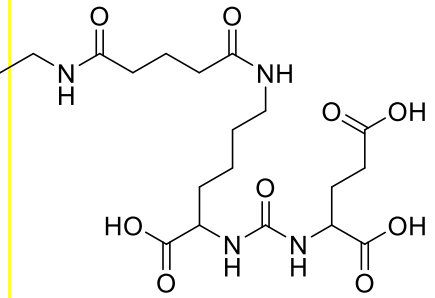


Specific target for prostate cancer

THP-PSMA



Specific target for prostate cancer



Aim:

Deliver a tracer that specifically targets prostate cancer

and

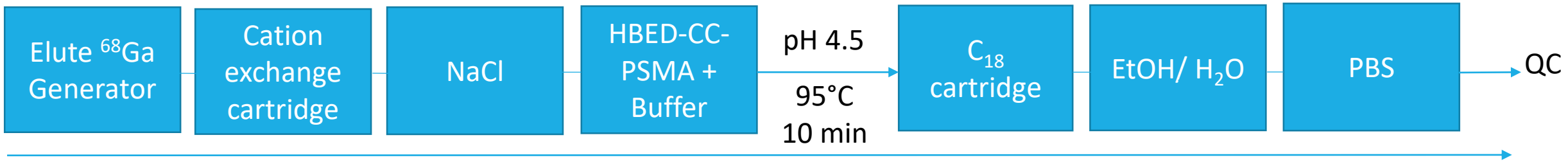
can be labelled quickly and easily

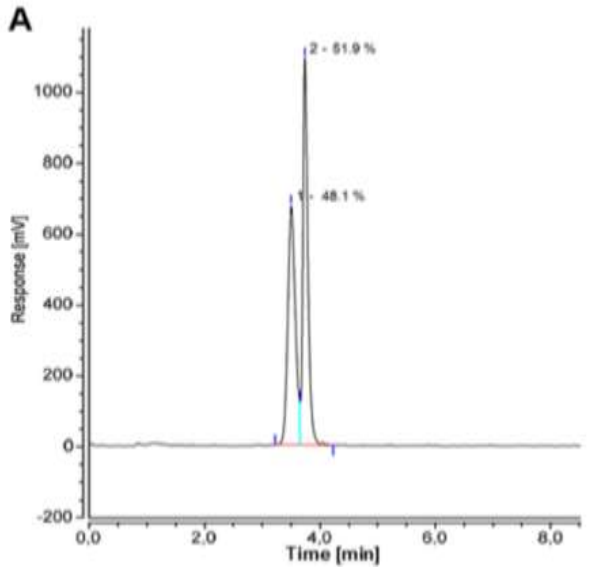
to

widen the adoption of PSMA imaging

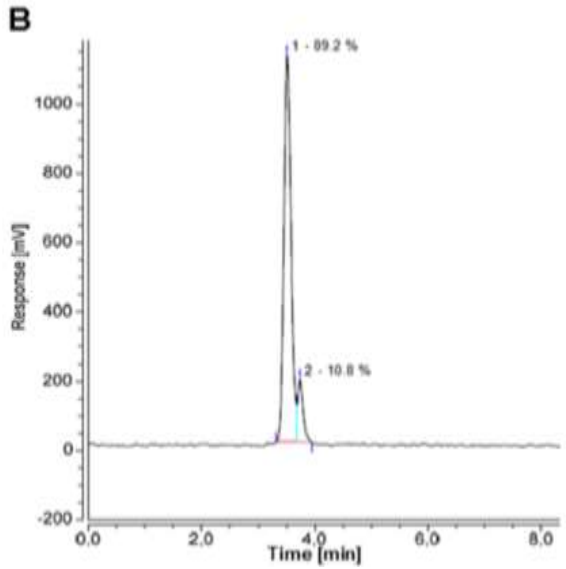
Young JD, Abbate V, Imberti C, et al. 68Ga-THP-PSMA: a PET imaging agent for prostate cancer offering rapid, room temperature, one-step kit-based radiolabeling, *Journal of Nuclear Medicine* 2017

^{68}Ga-HBED-CC-PSMA:
game-changer in prostate cancer…





Room temperature labelling



95°C labelling

…BUT Multiple step synthesis
35 minutes
Requires a synthesis unit/cartridge
Isomers formed

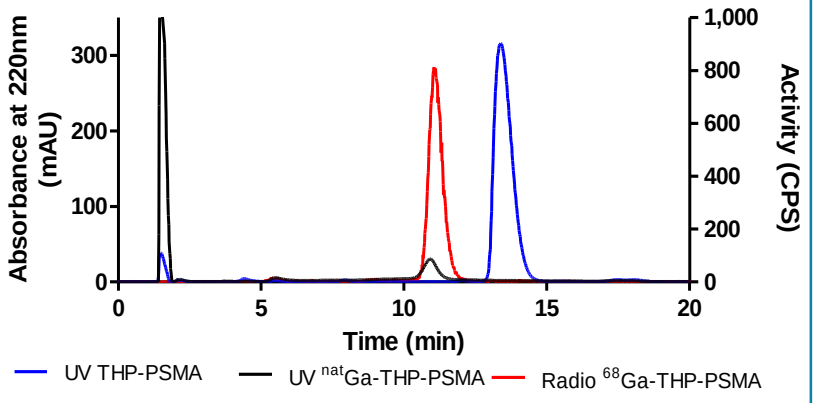
Eder, M. *et al.* Novel Preclinical and Radiopharmaceutical Aspects of [68Ga] Ga-PSMA-HBED-CC: A New PET Tracer for Imaging of Prostate Cancer. *Pharmaceuticals* **7,** 779–796 (2014).

THP-PSMA:
Development of radiolabelling process

Elute E&Z ^{68}Ga generator → 1M Sodium Bicarbonate 27µl, THP-PSMA 3µl (0.01µg- 5µg) → RT, 5 min, pH 6-7 → QC

HPLC

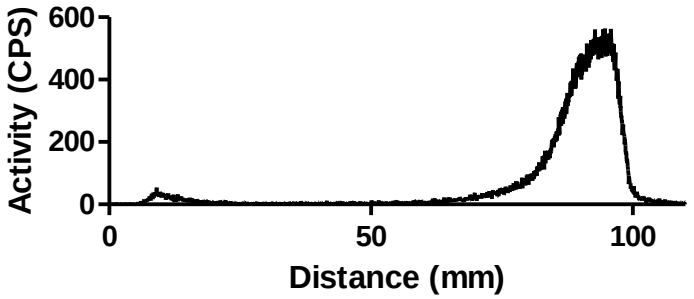
Agilent Eclipse XDB C_{18}
5 micron 4.6 x 150 mm
Isocratic mobile phase:
87.5% H_2O, 12.5% ACN,
0.075% TFA, 0.05% TEA, 1 mL/min



iTLC

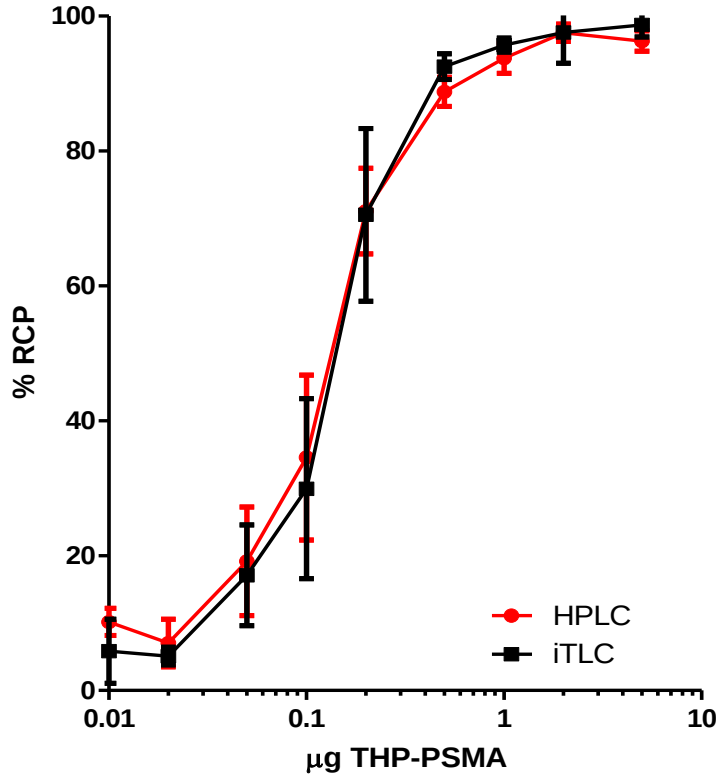
Varian SG I0001 strips (10cm length)
1M ammonium acetate in
water/methanol (1:1)

iTLC trace



Rf = 0; un chelated ^{68}Ga
Rf = 0.8-1; ^{68}Ga-THP-PSMA

Radiochemical Purity



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THP-PSMA: Single vial kit labelling

E & Z generator

5mL, 0.1M HCL

5mL eluate
122-202 MBq

Freeze dried kit

THP-PSMA
(40µg 26nmol)
sodium bicarbonate
(44mg)
sodium phosphate
buffer (157.5mg)

pH 6-7

N=3

IRE generator

1.1mL, 0.1M HCL

1mL eluate
600-660 MBq
+ 4mL 0.1M HCl

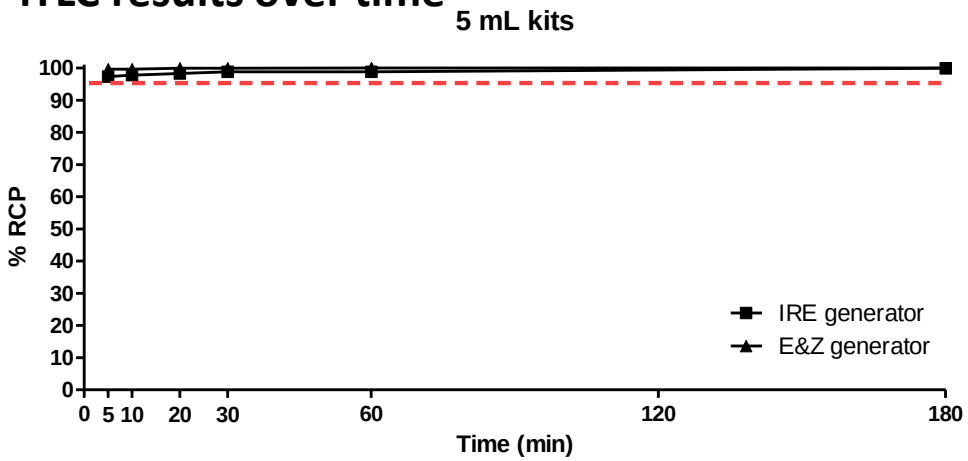
Freeze dried kit

THP-PSMA
(40µg 26nmol)
sodium bicarbonate
(44mg)
sodium phosphate
buffer (157.5mg)

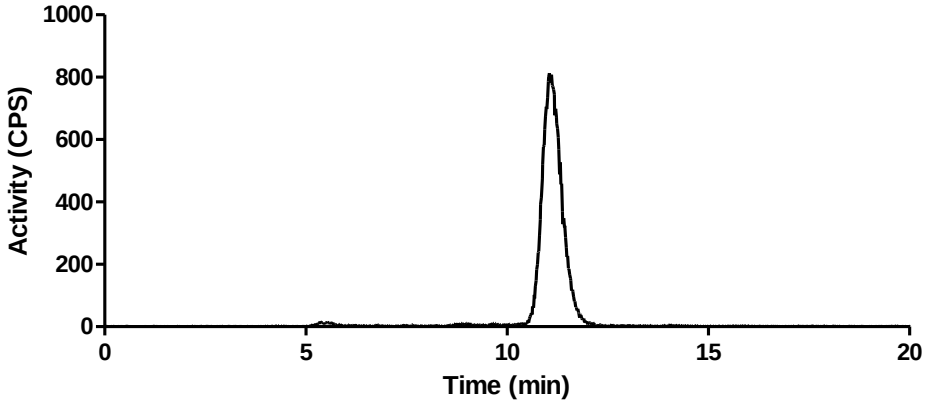
pH 6-7

N=3

iTLC results over time



Example HPLC result: 10 min post reconstitution



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THP-PSMA: Conclusion

Result

Aim: To label a PSMA radiotracer with ^{68}Ga, in a simple and rapid procedure, suitable for translation into a GMP radiopharmaceutical kit

Elute ^{68}Ga generator → Single vial kit → pH 6-7, RT, 2-5 min → QC

Single vial, single step synthesis
2-5 minutes, single isomer

PSMA: Phase 2 multicentre trial ongoing

*Thp is a **platform** for new generation of kit-based Ga-68 imaging agents including proteins (e.g. scFv antibodies)*

Note: ^{68}Ga generators utilised must have ^{68}Ge breakthrough low enough for direct human use < 0.001%. Eckert and Ziegler Radiopharma GmbH (E&Z) and Galli EO IRE ELiT (IRE) generators meet this criteria.

Thank You

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Peter Mac

Disclosure

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