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Abstract 26

Chelating Agents for Biomolecular Labelling with Long-Lived PET Radionuclide, Manganese-52

Madeleine Iafrate, W. D. Shingleton, J. Fonslet, G. O. Fruhwirth, P. J. Blower

2nd April, 2019

Properties of Radiomanganese-54, 52, 52m, 51, 51m

Isotope	Emission details	Half-life $t_{1/2}$	Other emissions?	Other considerations?
Manganese-54	γ (100%), 0.84 MeV	313 days	No	Not for human use
Manganese-52	β^+ (30%), 0.24 MeV	5.6 days	γ (100%), 0.74, 0.94, 1.43 MeV	Good image quality but high energy gammas[1]
Manganese-52m	β^+ (97%), 1.17 MeV	21 min	γ (100%), 1.43 MeV	Generator production but poor image quality, high E gamma
Manganese-51	β^+ (97%), 0.96 MeV	46 min	γ (1%); ^{51}Cr γ (10%), 0.32 MeV [2]	Dosimetry from radioactive daughter ^{51}Cr γ, $t_{1/2}$ = 28 days [3]
	2.13 MeV		1.69 MeV	gammas

- Radioisotopes with long physical half-lives are matched for use with processes that have long biological half-lives
- Long-lived isotopes have applications in processes with long biological time frames – for example, cell tracking, antibody labelling

1. Wooten, A. *et al, Appl Radiat Isotopes,* 2015, **96**, 154
2. Graves, SA., *et al, Sci Rep,* 2017, **7**, 3033
3. Chu, SYF., *et al, The Lund/LNBL Nuclear Data Search*, 1999, accessed 20/03/19.

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Copper-64	β^+ (18%), 0.28 MeV	12.7 hrs	γ (43%), 1.35 MeV	
Zirconium-89	β^+ (23%), 0.40 MeV	3.3 days	γ (99%), 0.91 MeV	
Iodine-124	β^+ (23%), 2.13 MeV	4.2 days	γ (85%), 0.60, 0.72, 1.69 MeV	Poor image quality, high energy gammas

1. Wooten, A. *et al*, *Appl Radiat Isotopes*, 2015, **96**, 154
2. Graves, SA., *et al*, *Sci Rep*, 2017, **7**, 3033
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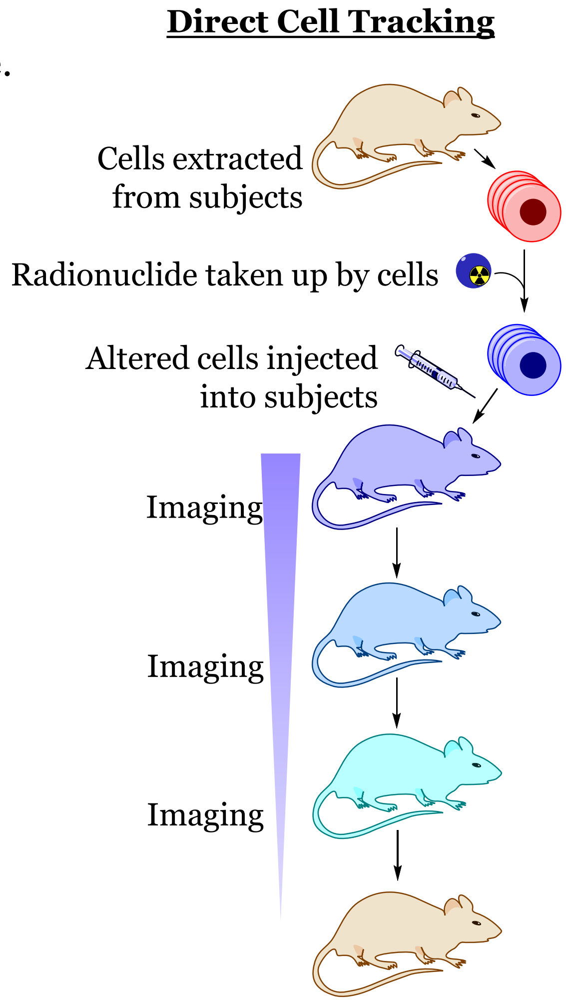
Cell Tracking

- Cell therapies ideally **need to be tracked for as long as possible**.
  - Location
  - Survival
  - Safety/Efficacy



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  - Location
  - Survival
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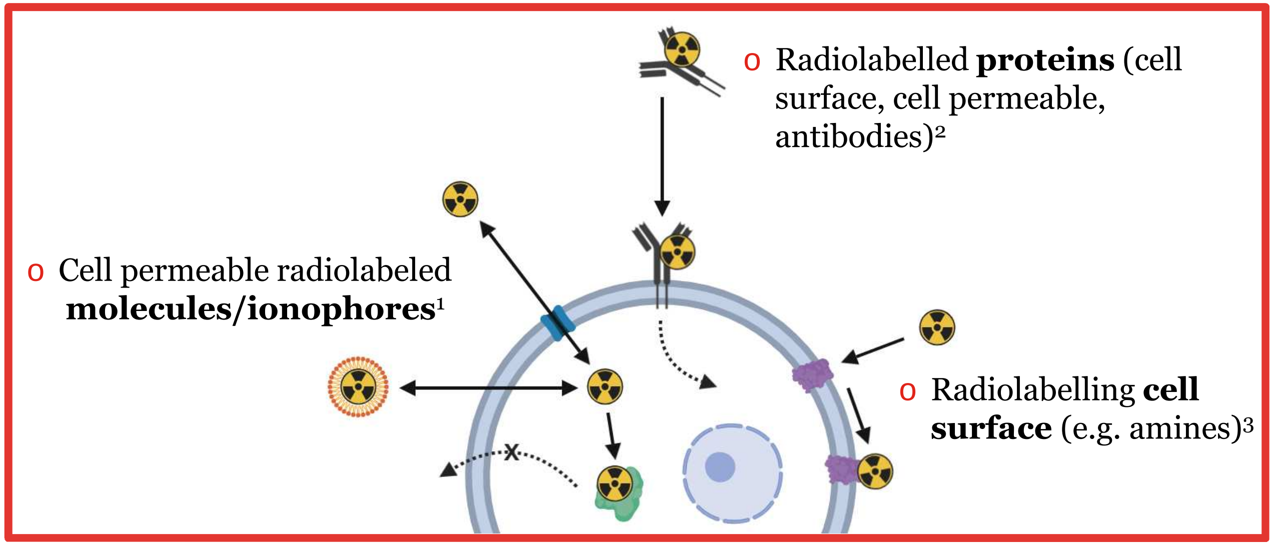
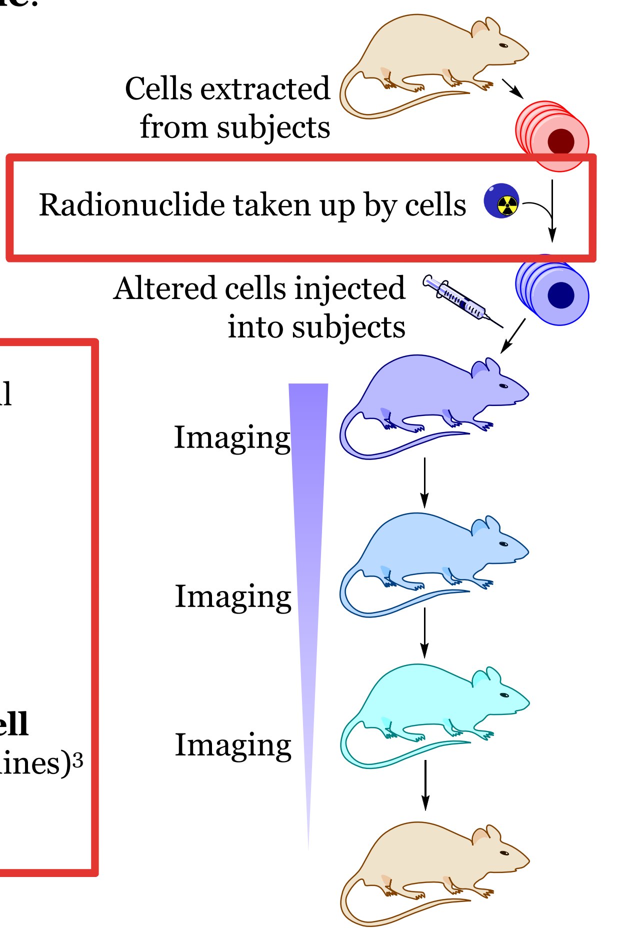
- The physical properties of manganese-52 are ideally suited to direct cell tracking:



- All need a **suitable chelator to stop the radiometal** going free!

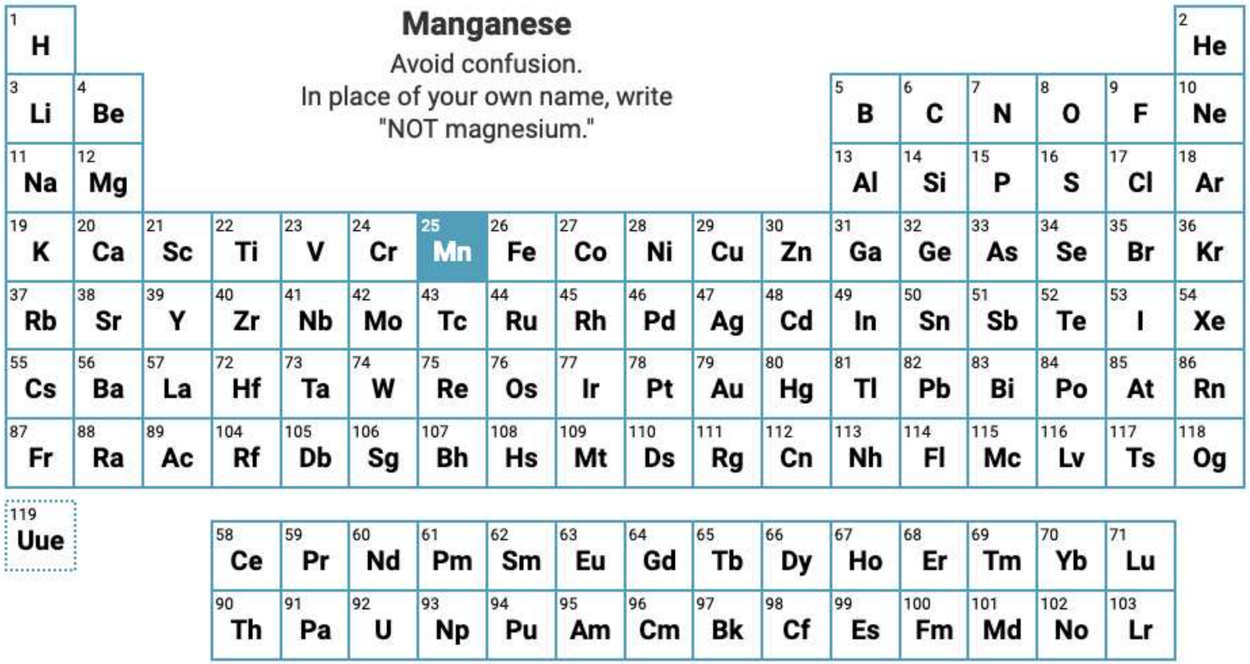
Direct Cell Tracking



1. Gawne, PG., *et al, Dalton Trans.*, 2018, **47**(28), 9283
2. Graves SA., *et al, Bioconj. Chem.*, 2015 **26**(10), 2008
3. Bansal A., *et al, EJNMMI Res.*, 2015, **51**(1), 19

Chemistry of Manganese

- Manganese can exist in a range of different oxidation states, as can its second and third row transition metal congeners, technetium and rhenium.



1. Soon Lee, M., *Elemental Haiku*, http://vis.sciencemag.org/chemhaiku/ accessed 19/03/19

Chemistry of Manganese

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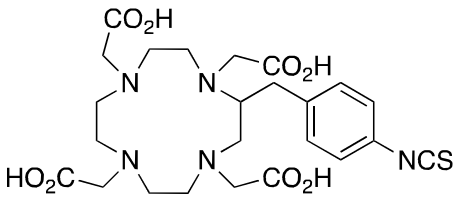
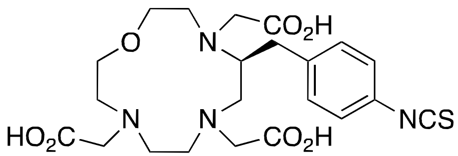
- Chemical comparisons can be drawn between the three metals, which have similar chemistry (due to their periodic relation). However, **manganese is extremely stable in the +2 oxidation state.**

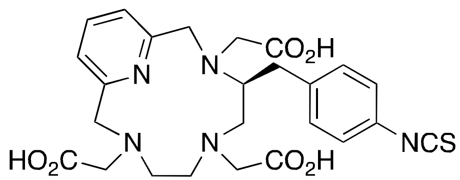
- Commercially available chelators:



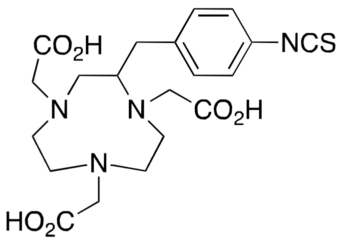
p-SCN-Bn-DOTA



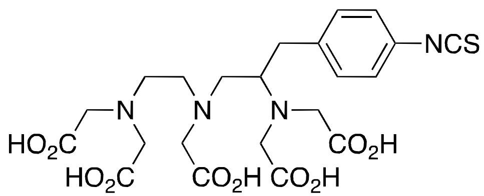
p-SCN-Bn-oxo-DOTA



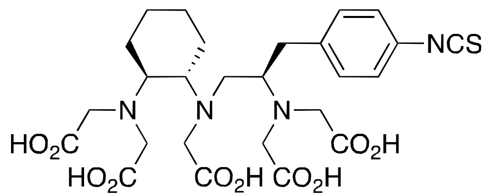
p-SCN-Bn-PCTA



p-SCN-Bn-NOTA



p-SCN-Bn-DTPA

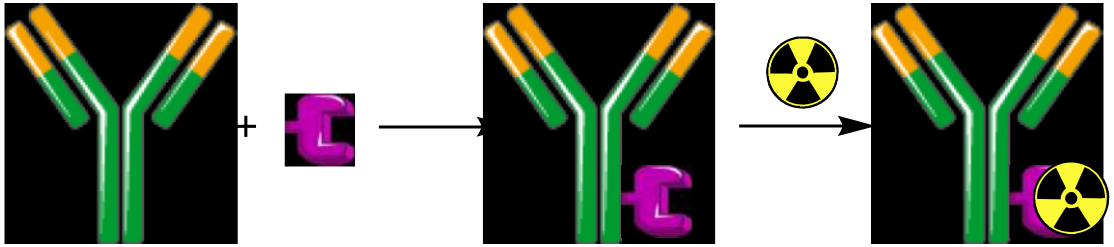


p-SCN-Bn-CHX-A”-DTPA

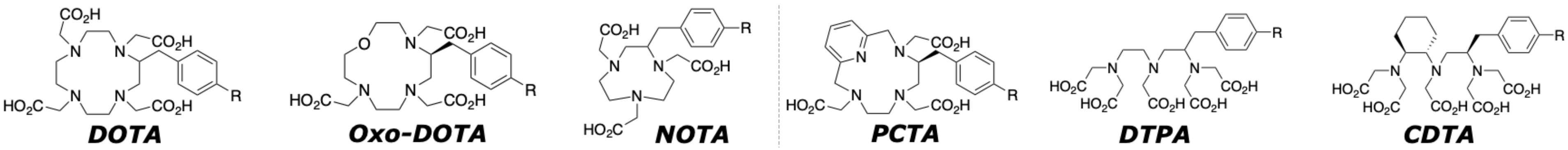
Goal: perform a preliminary survey to determine which if any of these chelators would be optimal for ^{52}Mn biomolecule labelling

Experimental

AIM: Fast, efficient labelling at low concentration under mild conditions.

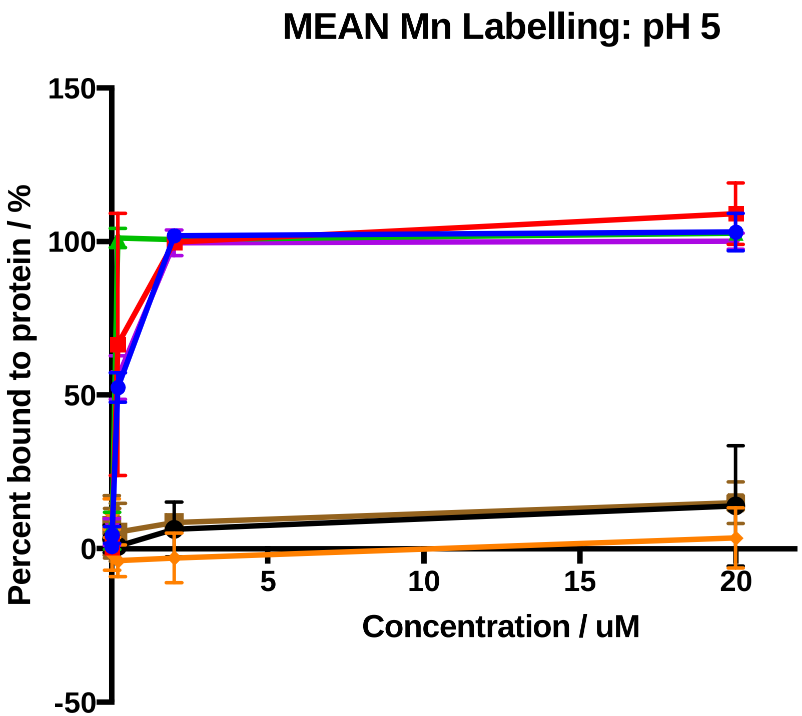


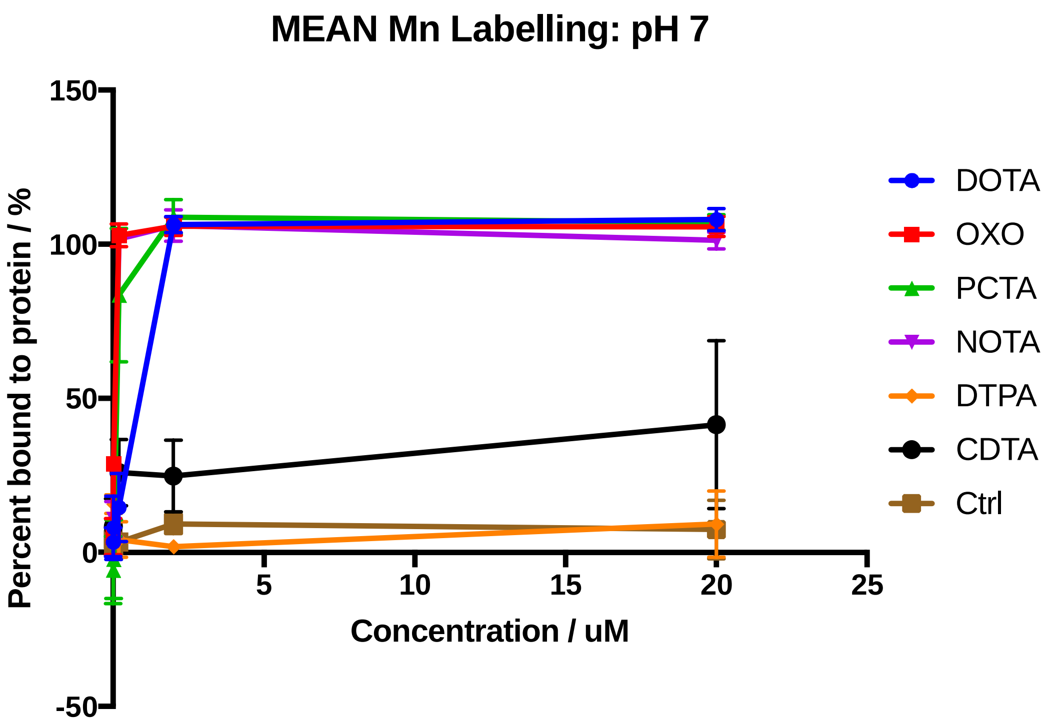
Scheme 1. *The antibody was conjugated with the bifunctional chelators and the conjugates were incubated with ^{52}Mn at a range of concentrations to compare the labelling efficiency of the chelators.*

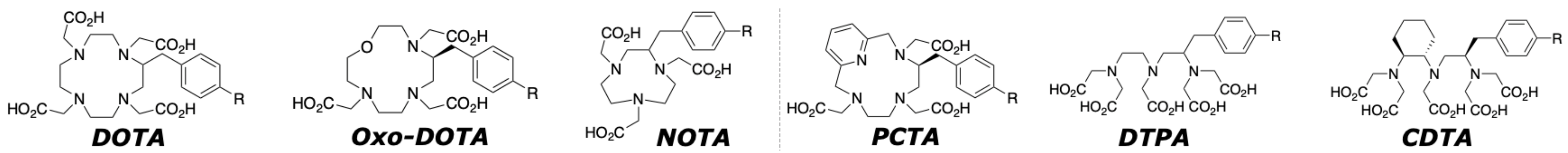


- Several trastuzumab immunoconjugates of bifunctional chelators (DOTA, oxo-DOTA, NOTA, PCTA, DTPA, CDTA, see below panel) were incubated at room temperature with $^{52}MnCl_2$ at a range of antibody concentrations at pH 5 and 7.[2]
- Reactions were quenched after 45 minutes with EDTA (50 mM, 2 μL). Labelling efficiencies from each dilution assay were analysed by iTLC.

Labelling Efficiency: Overview

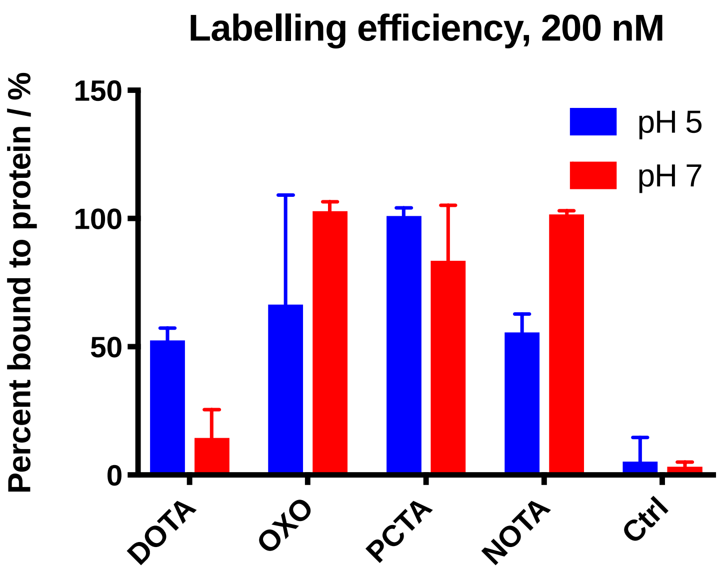


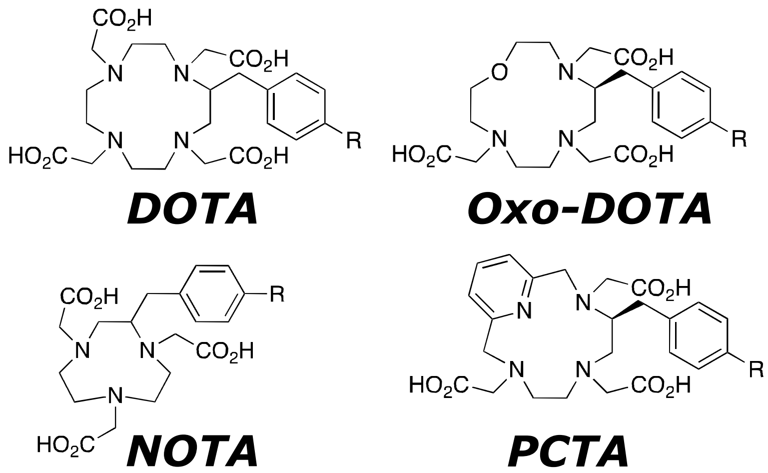




Reaction time: 40 minutes, → EDTA quench
pH 5 = NH_4OAc / pH 7 = PBS
n = 2, errors = σ

Macrocyclic Chelators for Mn(II)





- Of the commercially available macrocyclic chelators tested, oxo-DOTA and NOTA were efficient chelators of Mn(II) at pH 7, and PCTA at pH 5.

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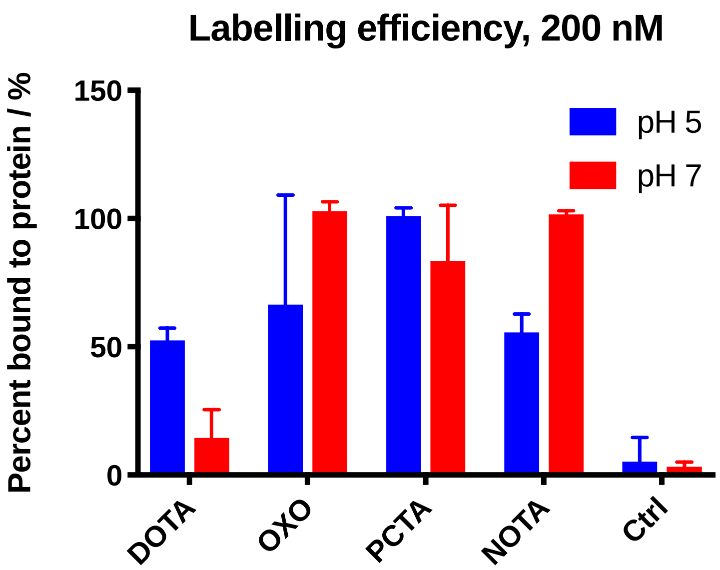
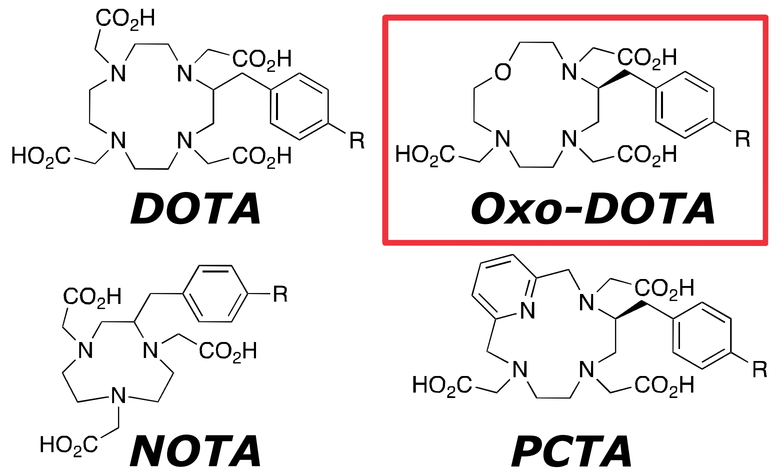
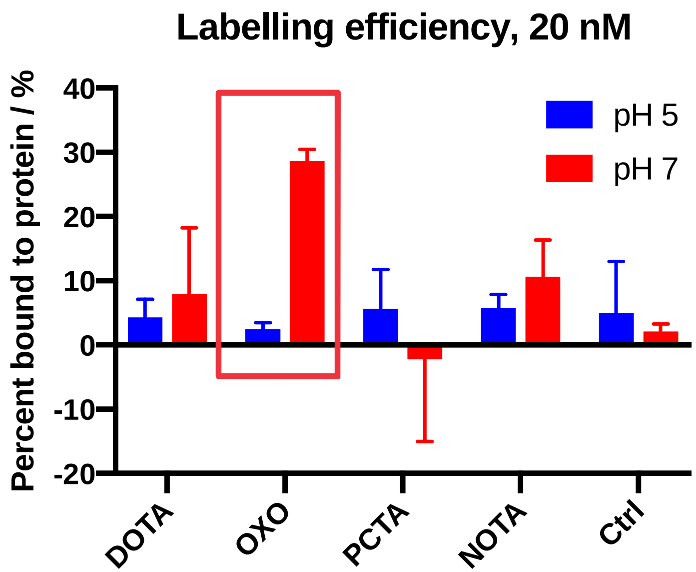


- Of the commercially available macrocyclic chelators tested, oxo-DOTA and NOTA were efficient chelators of Mn(II) at pH 7, and PCTA at pH 5.

- Additionally at even lower concentrations, it is clear that there is a significant result regarding the labelling efficiency of **oxo-DOTA**.





Conclusions

- **Manganese-52 direct cell tracking agents** could monitor cells *in vivo* noninvasively by PET for longer than we've ever been able to before.
- **Macrocyclic chelators** are shown to chelate $^{52}Mn(II)$ at radiopharmaceutically relevant concentrations.
- **Oxo-DOTA** has emerged as a lead compound for future radiopharmaceutical development due to ease of labelling, without the need of heating.
- **pH** changes inside a cell could make a significant difference to the stability of complexes formed:
  - Further studies needed to ascertain whether this is thermodynamic or kinetic instability.
- Future work will investigate **serum stability** of the bifunctional conjugates used.
- This work is transferable to **other manganese isotopes** because they have identical chemical properties.

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Thanks: Phil Blower, Gilbert Fruhwirth, Rafa T. M. de Rosales, Bill Shingleton, and Peter Gawne.

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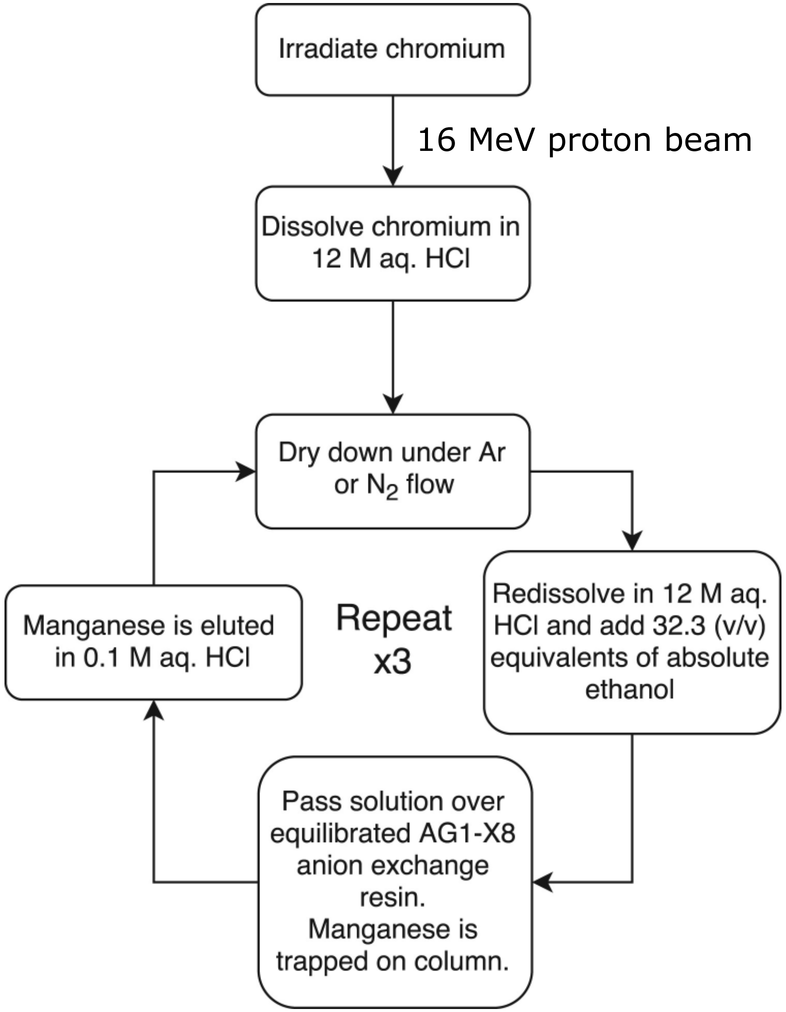
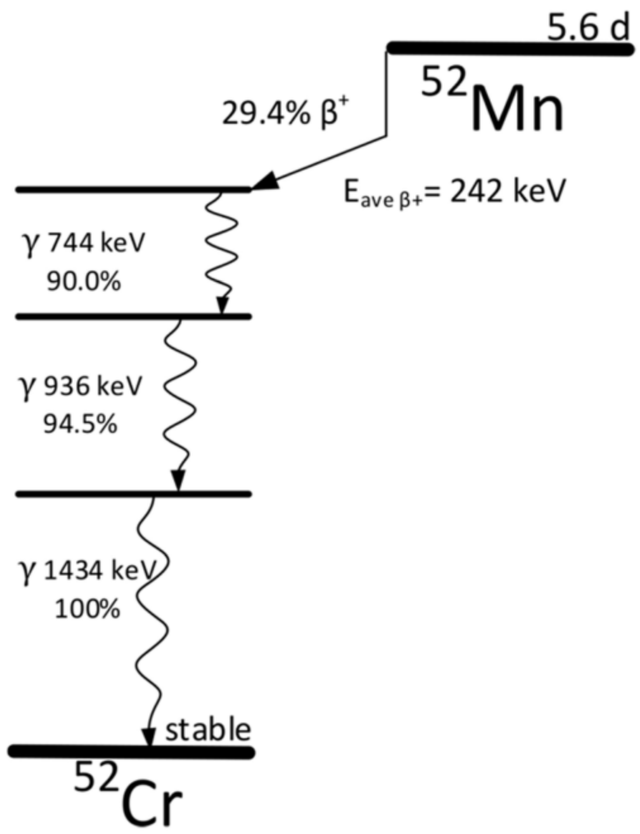
2nd April, 2019

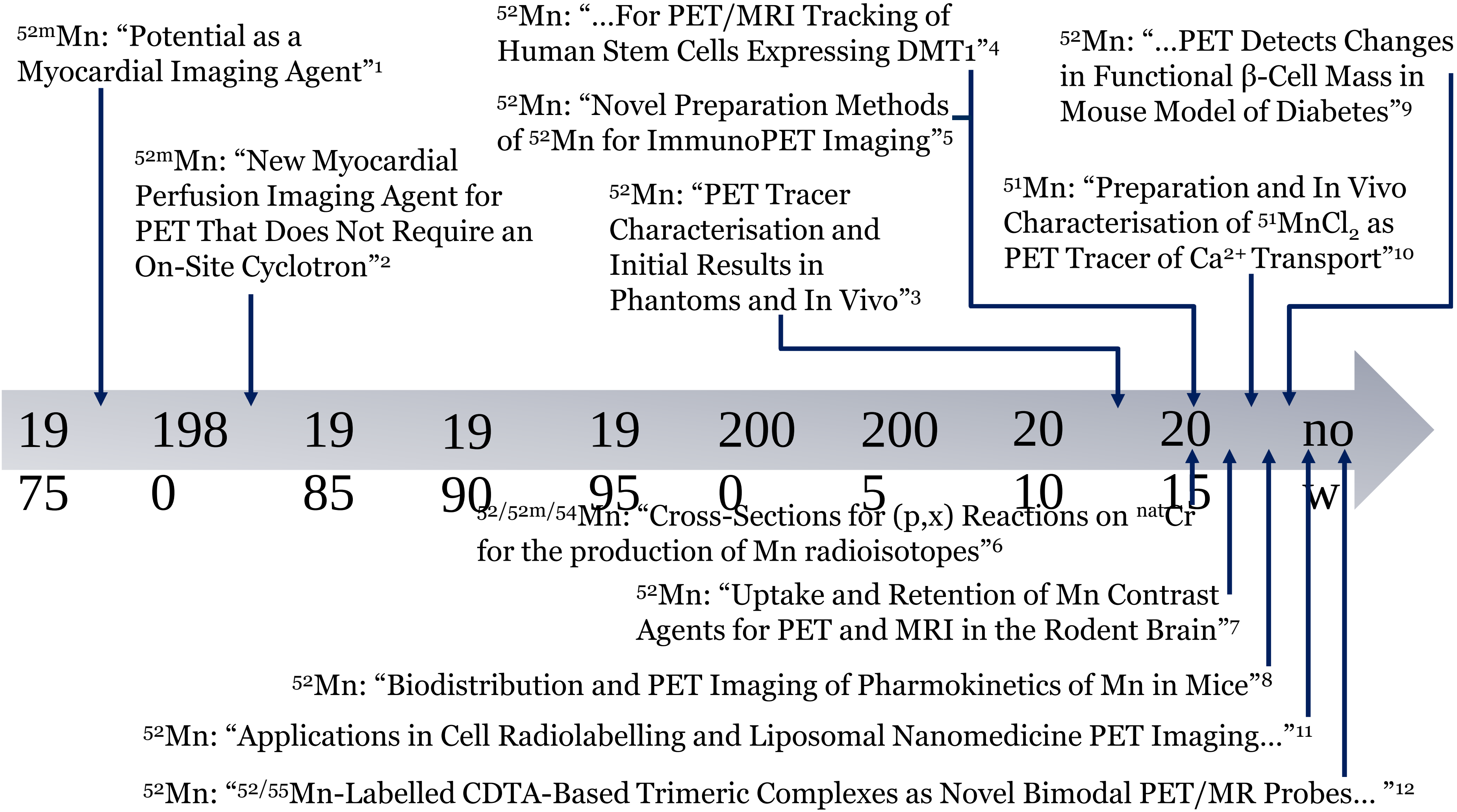
Production of Manganese-52, by J. Fonslet (Hevesy Lab) et al.





1. Folslet, J., *et al, Appl. Rad. Isot..,* 2017, **121**, 18-41

Applications of Radiomanganese in Nuclear Medicine



1. Chauncey, D. *et al, J Nuc Med*, 1977, **18**(9), 933
2. Urquhart, J. *et al, Am J Cardiol*, 1982, **49**(4), 979
3. Topping, GJ. *et al, Med Phys*, 2013, **40**(4), 042502
4. Lewis, CM. *et al, Theransotics*, 2015, **5**(3), 227
5. Graves SA. *et al, Bioconj Chem*, 2015, **26**(10), 2118
6. Wooten, A. *et al, Appl Radiat Isotopes*, 2015, **96**, 154
7. Brunnquell, CL. *et al, Contrast Media Mol Imaging*, 2016, **11**(5), 371
8. Wooten, A. *et al, PLoS One*, 2017, **12**(3), e0174351
9. Hernandez, R. *et al, Diabetes*, 2017, **66**(8), 2163
10. Graves, SA. *et al, Sci Rep*, 2017, **7**(1), 3033
11. Gawne, PG. *et al, Dalton Trans*, 2018, **47**(28), 9283
12. Brandt, MR. *et al, Dalton Trans*, 2019, **48**, 3003