



## Abstract

PET imaging agents are often employed in the field of nuclear oncology for the prediction of biodistribution and dosimetry of targeted radionuclide therapy agents (TRT). Radionuclides such as Lutetium-177, Actinium-225, and Bismuth-213 are promising radionuclides for TRT of cancer but don't have a PET imaging surrogate.

Our lab has previously demonstrated the significant impact of the radionuclide selection in the biodistribution of small-molecule PET agents. The aim of this study is to determine whether *in vivo* behavior of tumor targeting alkylphosphocholine (APC) agents radiolabeled with various positron emitting radionuclides can be explained by physicochemical properties like hydrophilicity.

To this end, we will study NM600 (DOTA) with  $^{64}\text{Cu}$  and  $^{86}\text{Y}$ , NM620 (DFO) with  $^{89}\text{Zr}$ , and NM630 (NOTA) with  $^{64}\text{Cu}$ . Log P/D and HPLC will be carried out with each radiolabeled compound to assess the differences in polarity and radio stability respectively. The potential to be gathered from this study is the prediction of *in vivo* behavior of PET imaging analogs radiolabeled with various radiometals.

## Cu-64 NOTA and DOTA Labeling

Turn on the shaker (37°C) and heater (70°C) and add 300  $\mu\text{L}$  of 1M NaOAc buffer(pH of 5.5) to a 1.5mL microcentrifuge tube and add 1M of PBS Buffer.

The pH measured should be around 5.5. Adding to the compound should be a NM-600 concentration of 5ug/ $\mu\text{L}$  that will then be 10ug of NM600 per mCi of activity.

This is then incubated at 95°C at 400rpm for 30 minutes. With a Luer lock syringe, obtain 2mL of ultrapure water to add to the tube and shake, then extract with a 22G long needle syringe.

Pass the content with a HLB filter which the waste would be discarded in a waste vial, and it must be passed with a rate of 1 drop per second. Obtain 2mL of ethanol with a glass syringe. Collect into a 3mL glass vial from HLB filter.

We then place the vial with two vent needles into the heater at 70°C and dry the ethanol under a low flux of nitrogen or argon gas. Add 3mL of excipient(20 mL sterile with 80uL of tween-20) to the dried vial for resuspension.

## Zr-89 DFO Labeling

First, the buffer will be prepared to ensure that there is 1M of HEPES (238 mg/mL) and that there is trace metal water and trace metal ethanol.

Label Eppendorf tube for reaction and glass vial with septum cap for final reaction, and the heating blocks will be started at 37°C for shaker, 70°C for heat block, and 2 needles for venting shall be obtained.

Aliquot 5uL of Zr-89 solution into labeled Eppendorf tube(of course checking the activity on the capintec screen). Add volume for desired activity based on aliquot and record total activity. Add 400uL 1M HEPES buffer, avoiding chelex in the bottom.

The pH measured should be around 6.8 to 7.2. Add 10ug DFO/1mCi activity, pipetting up and down. This will be then shaken for 30 minutes at 37°C 500rpm. Fill a 3mL syringe with around 1.5mL water, put on the needle, and draw up the reaction.

With a rate of (1 drop/sec) run through a small column into waste and check the record activity in the column. Fill the glass syringe with 1.5 mL ethanol and 1mL air, which would elute into the glass vial(1 drop/sec). Evaporate with the heating block at 70°C and nitrogen which would be resuspended in PBS.

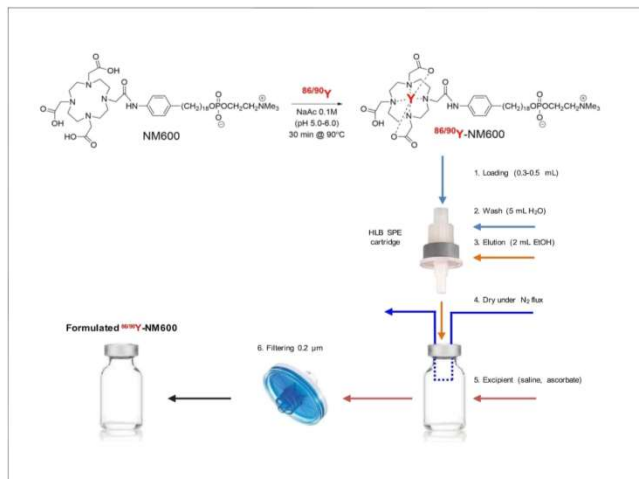


Figure 1: The radiolabeling process as shown of M:  $^{86}\text{Y}$ ,  $^{64}\text{Cu}$ ,  $^{177}\text{Lu}$  (Diluted HCl) with the NM600 compound and the DOTA Chelator (Tetranan). The compound is formulated through process of HLB Cartridge, elution and filtering

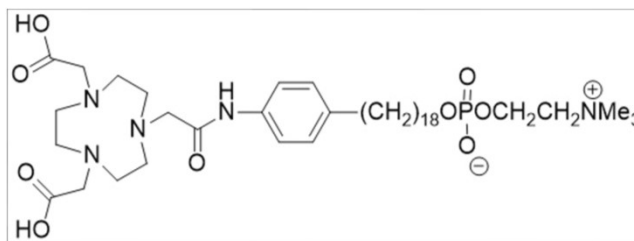


Figure 2: The structure of the NM630 (NOTA) (a hexadentate chelator) is to be radiolabeled with  $\text{Cu}^{64}$

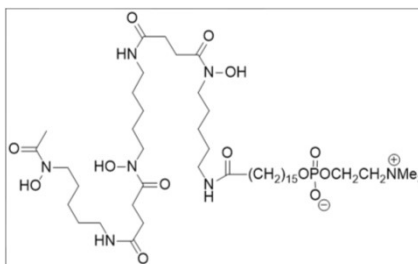


Figure 3: The NM620 with DFO(Deferoxamine chelator) that pairs with the  $\text{Zr}^{89}$

## Literature references

R Hernandez et al. *Choice of radiometal influences the tumor targeting and biodistribution properties of theranostic alkylphosphocholine chelates in vivo* Society of Nuclear Medicine and Molecular Imaging Annual Meeting, 2018.

## Results Graphs Log P and Log D

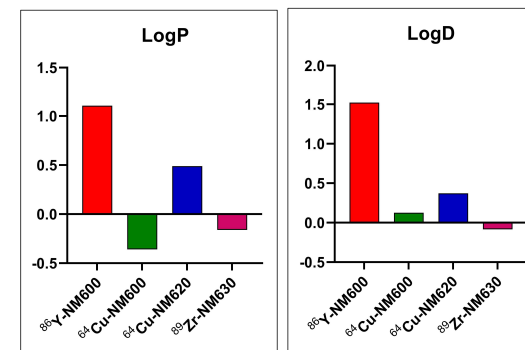


Figure 4: Log P and Log D of the Octanol and Water partitioning for  $\text{Y}^{86}$ ,  $\text{Zr}^{89}$ , and  $\text{Cu}^{64}$

## Biodistribution of murine model

Figure 5: Murine model and biodistribution

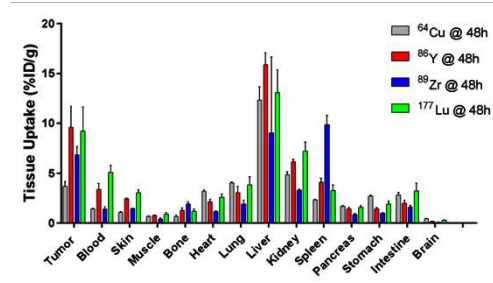


Figure 5: The biodistribution results of the murine model with 48 hr half-lives of  $\text{Lu}^{177}$ ,  $\text{Y}^{86}$ ,  $\text{Cu}^{64}$ , and  $\text{Zr}^{89}$

## Results and Conclusion

**DOTA Cu-64:** Based on the respective Log P analysis (From which we obtained to be: -0.3587), it can be determined that the DOTA chelator with the Cu-64 is hydrophilic. Moreover, The Log D Analysis(Which obtained: 0.1252) details that the respective compound resides within the aqueous phase.

**NOTA Cu-64:** Based on the respective Log P Analysis(0.488496806) we determine that the NOTA chelator with the Cu-64 is hydrophilic, and the obtained Log D(0.3728) determines that the compound resides within the aqueous phase.

**Zr-86 radiolabeled for Zr-86 compound with the DOTA chelator,** we had concluded based on the findings of the Log D (having a partition coefficient of -0.08467) and the Log P(having a partition coefficient of -0.1602) that the compound presents itself to be soluble in the aqueous phase and it is hydrophilic.

## Acknowledgments

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