

11<sup>th</sup> International Conference on Isotopes, Saskatoon



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# Iodine-Radiolabeled Mesenchymal Stem Cell (MSC)- Exosomes and Their CD73 Enzymatic Activities

Chang-Tong Yang <sup>a,b\*</sup>, Ruenn Chai Lai<sup>c</sup>, Sai Kiang Lim<sup>c</sup>, David Chee Eng Ng <sup>a,b</sup>

<sup>a</sup> *Department of Nuclear Medicine and Molecular Imaging, Singapore General Hospital, 169608 Singapore;*

<sup>b</sup> *Duke-NUS Medical School, 8 College Road, 169857 Singapore;*

<sup>c</sup> *Institute of Molecular & Cell Biology, 8A Biomedical Grove #05-16 Immunos, 138648, Singapore;*

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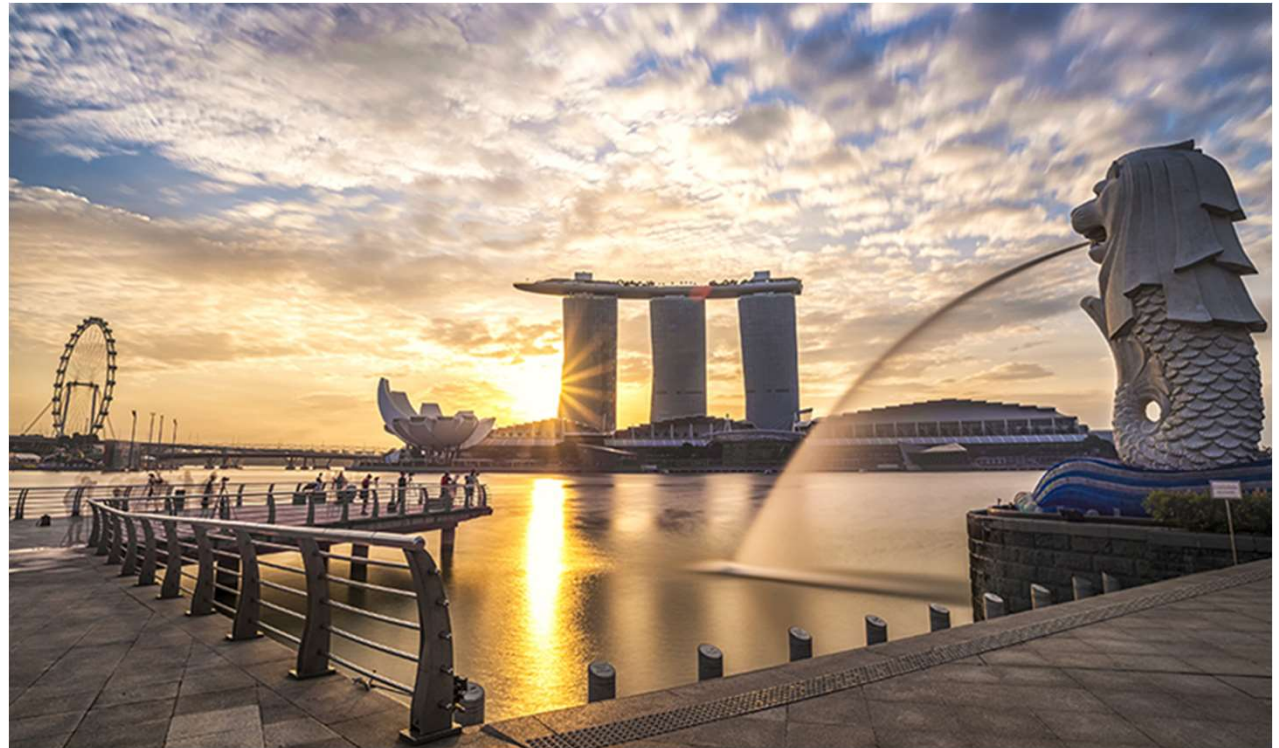
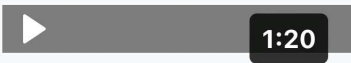
Flag



Coat of arms

**Motto:** *Majulah Singapura* (Malay)  
"Onward Singapore"

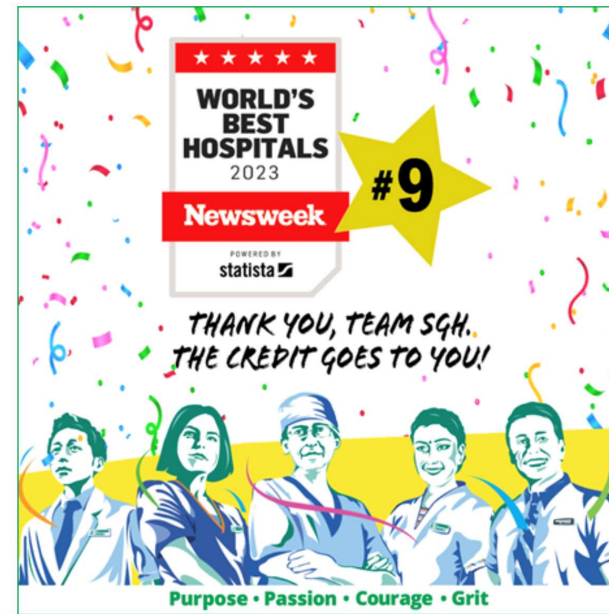
**Anthem:** *Majulah Singapura* (Malay)  
"Onward Singapore"



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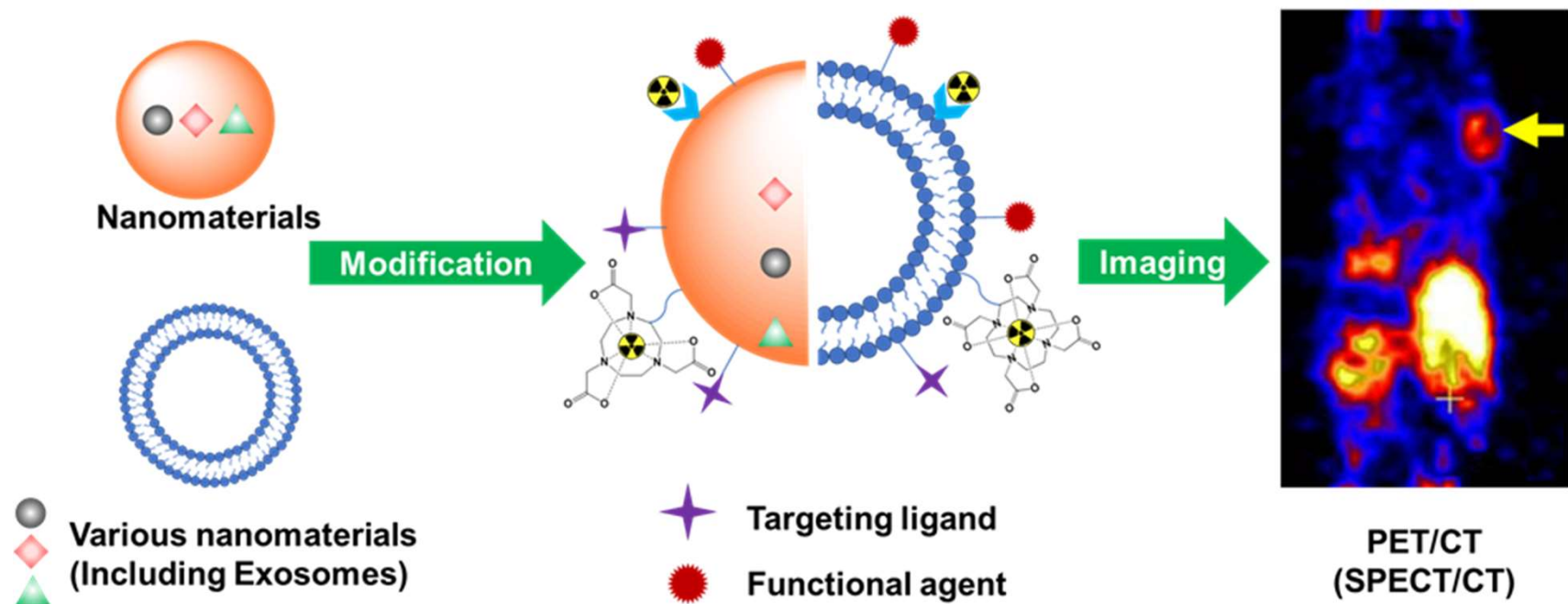
# Introduction

- “Translating MSC exosomes into pharmaceuticals” (TEx2Pharm)” is to develop potency assays that will transform MSC exosomes into a drug platform for multiple diseases.
- In elucidating a mechanism of action as any proposed activity must be compatible with the spatiotemporal distribution of exosomes.

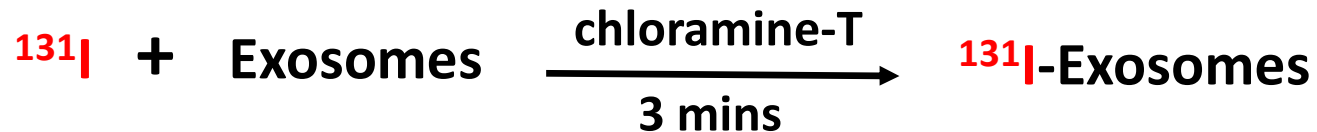
# Introduction

- MSC-derived exosomes have shown therapeutic potential in the areas of cardiovascular, orthopaedic, ophthalmologic, immune, dermatologic diseases and radiation sickness.
- Efficient radioisotope-labeling of exosomes remains as a challenging process.
- We demonstrate iodine-131 radiolabeled exosomes using both chloramine-T and Pierce Iodination methods, and characterized I-labeled exosomes via their CD73 enzymatic activities.

# Various Nanomaterials through Modifications for Nuclear Imaging PET/CT (SPECT/CT) Probes

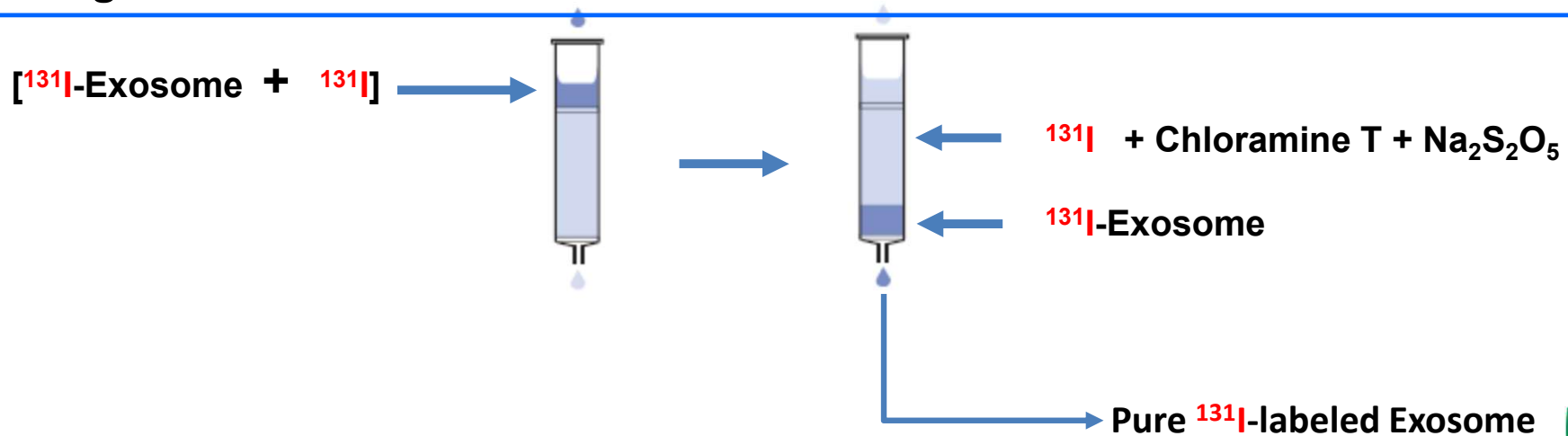


# <sup>131</sup>I-Radiolabeled Exosomes by Chloramine-T

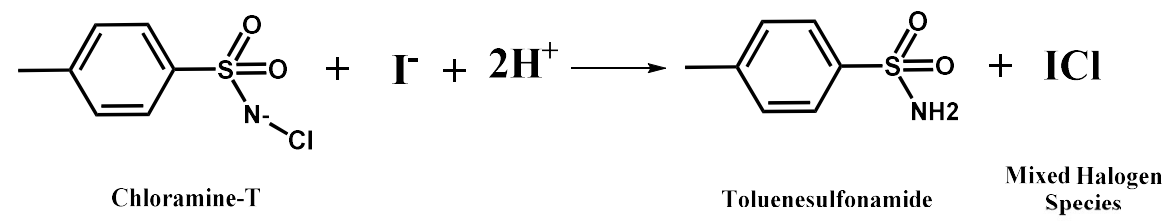


Reductant sodium metabisulfite  $\text{Na}_2\text{S}_2\text{O}_5$  is used to terminate the labeling

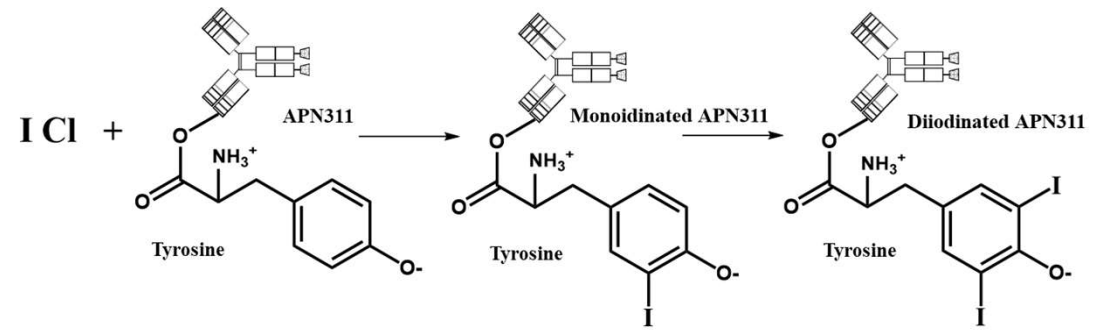
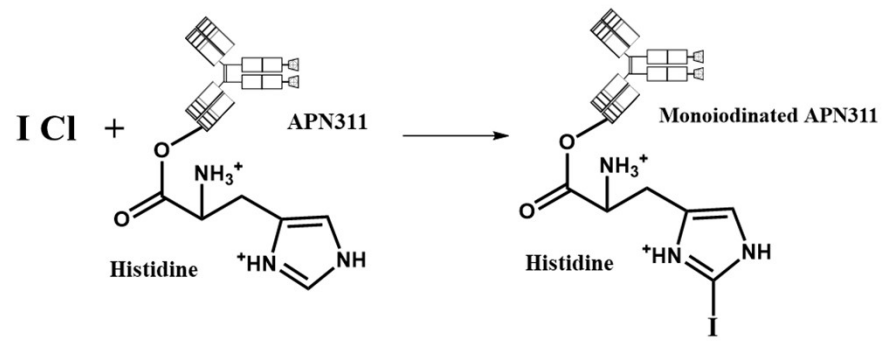
Purification: Excess free iodine will be removed from the labeled MSC exosomes using size exclusion PD10 column filtration.



# Mechanism for I-Labeling of Exosome

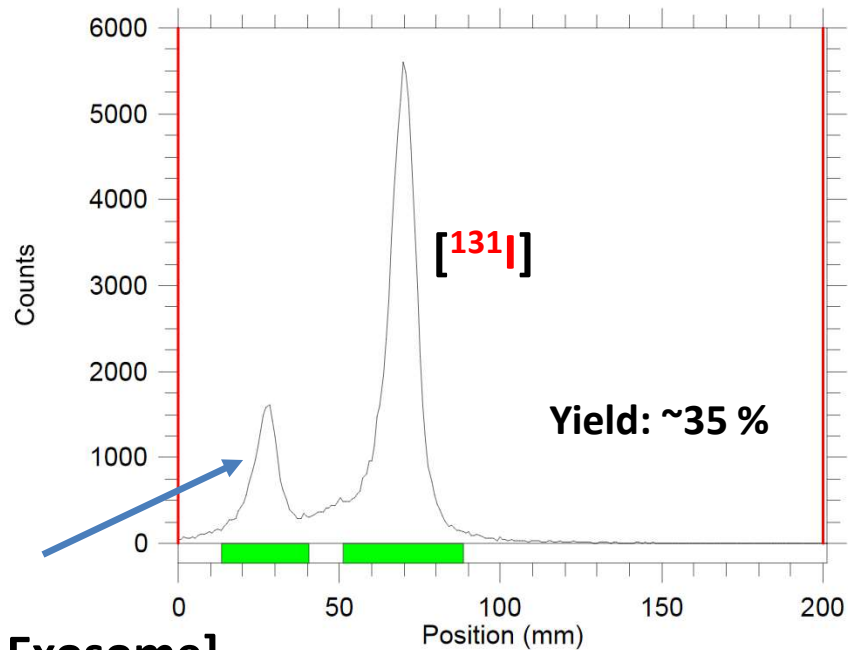


**I = <sup>131</sup>I**

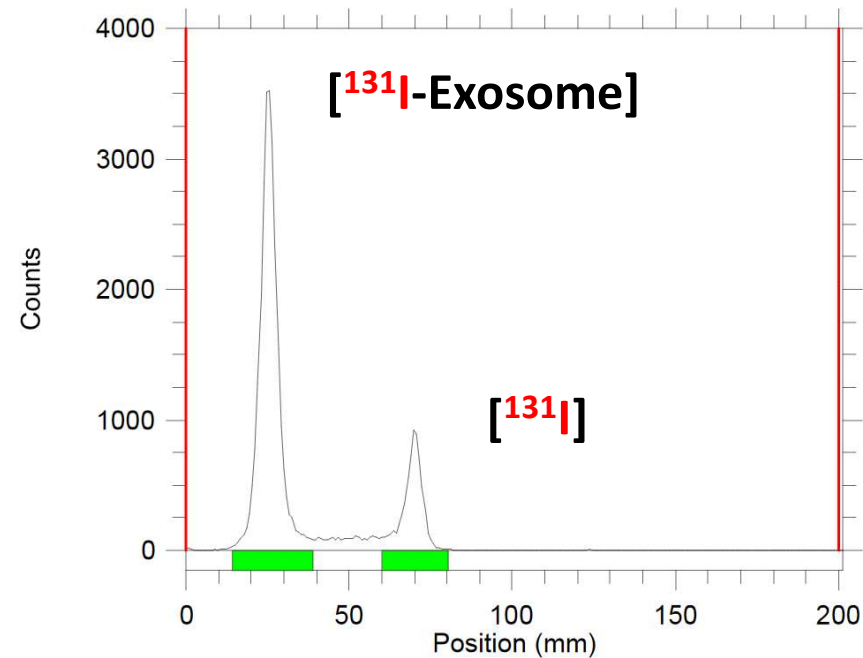




# <sup>131</sup>I-Radiolabeled Exosomes by Chloramine-T



Before Purification



After Purification

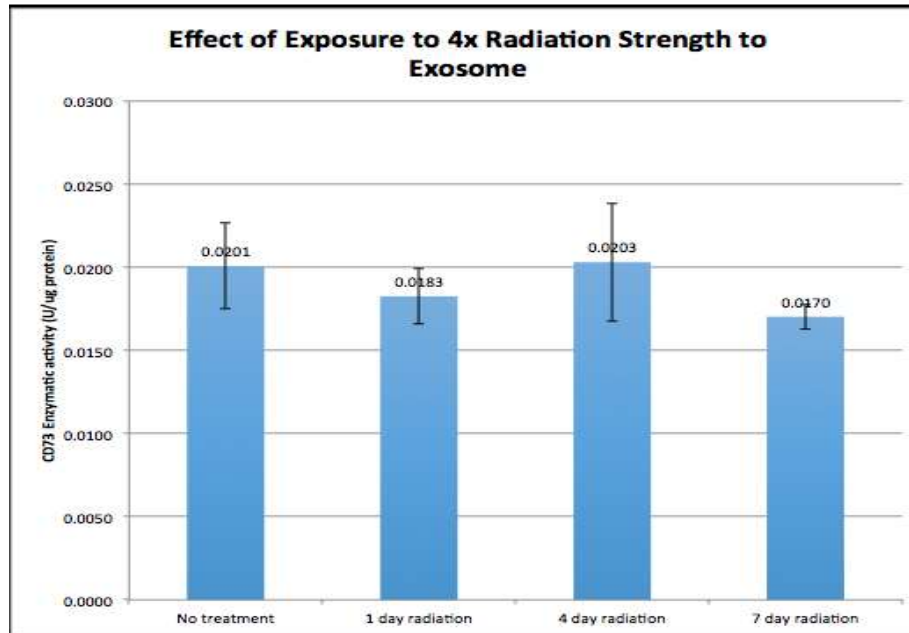
Solvent: MeOH:H<sub>2</sub>O = 85%:15%

## Why CD73 enzymatic activity

- CD73, a surface ecto-50-nucleotidase (NT5E), is recognized as an anti-inflammatory molecule, capable of converting adenosine monophosphate (AMP) to adenosine.
- MSC exosomes have been shown to alleviate immune dysfunction and inflammation in preclinical animal models. This therapeutic effect is attributed, in part, to their ability to promote the polarization of anti-inflammatory M2-like macrophages.
- MSC exosomes mediate M2-like macrophage polarization through exosomal CD73 activity

Ref: Lai RC, Lim SK, Toh WS. Mesenchymal Stromal Cell Exosomes Mediate M2-like Macrophage Polarization through CD73/Ecto-50-Nucleotidase Activity. *Pharmaceutics* 2023, 15, 1489.

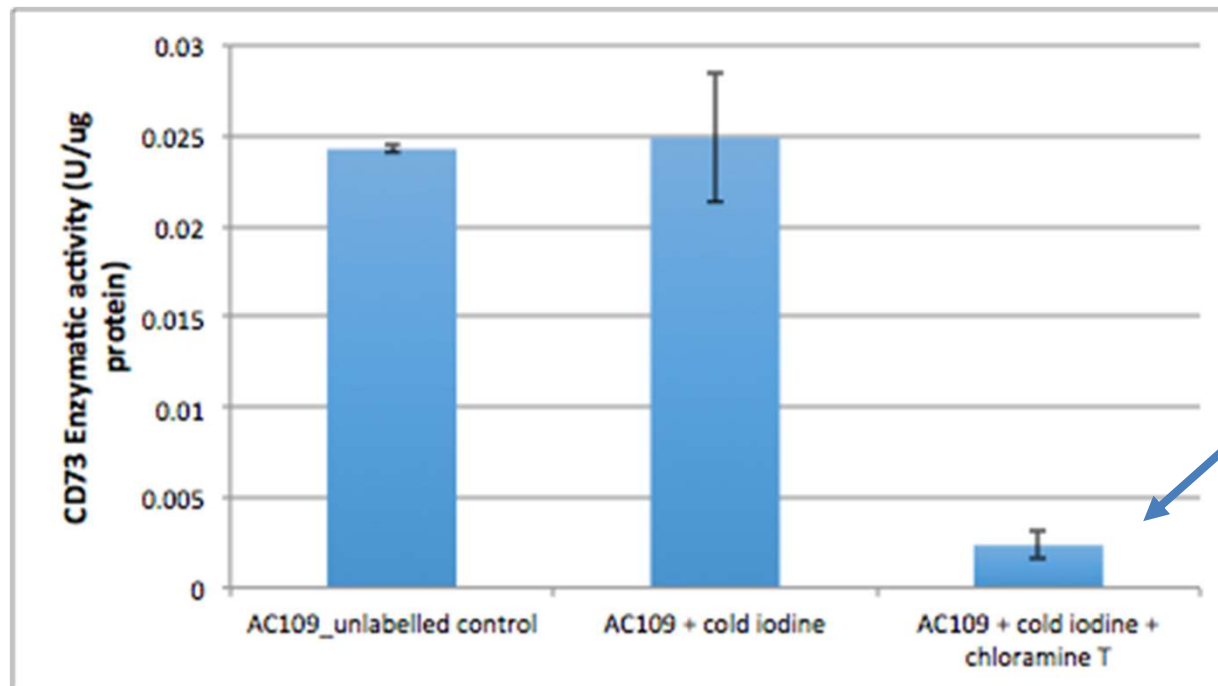
# CD73 Enzymatic Activity Measurements (1)



- Put exosome under the radiation materials (in separate tubes) in -20 °C for 1, 4, and 7 days.
- No significant difference of all-time point compared with no treatment, suggest that the radiation has no effect on exosome

# CD73 Enzymatic Activity Measurements (2)

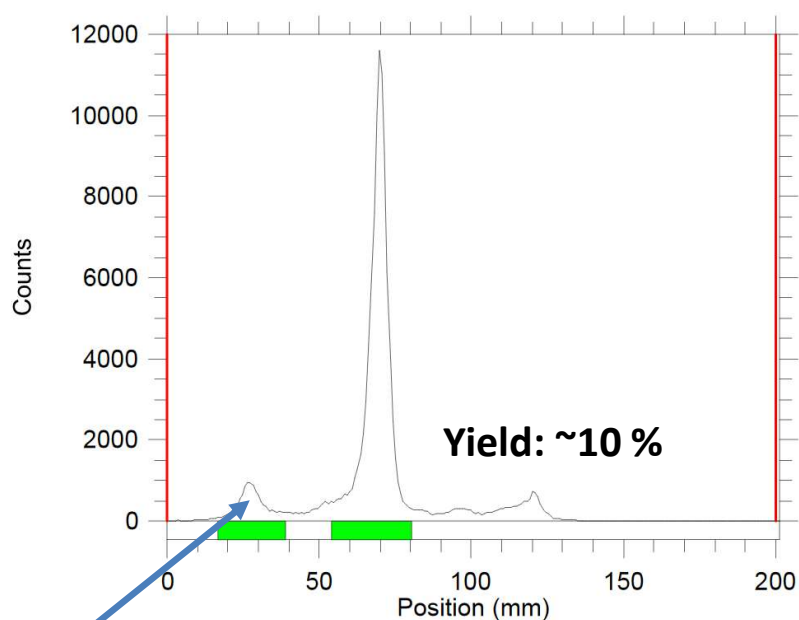
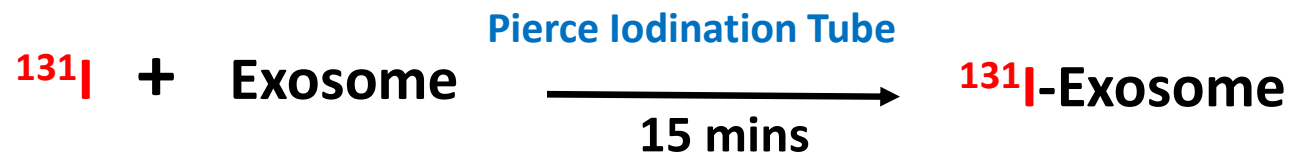
--Effect of cold chloramine I-labeling on exosomes



P: I-labeled exosomes  
Chloramine T  
 $\text{Na}_2\text{S}_2\text{O}_5$   
Unreacted NaI,  
Unreacted exosome

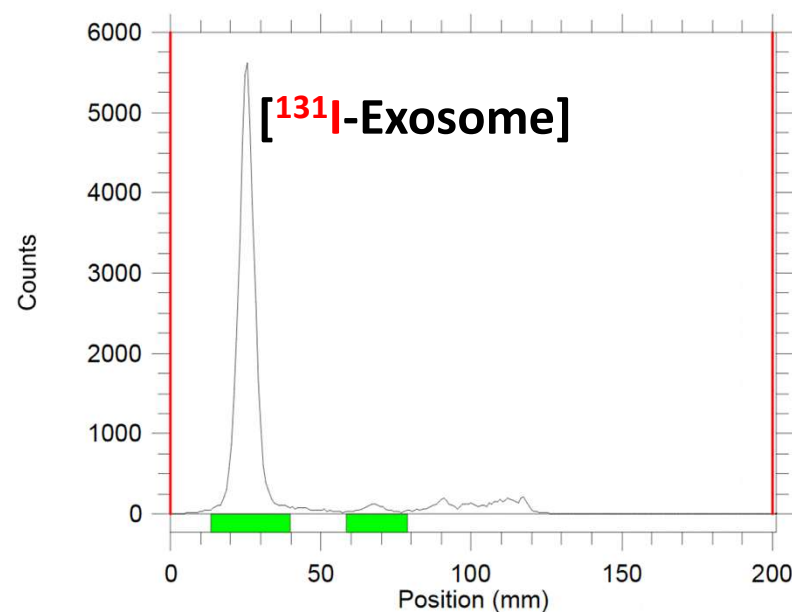
- The CD73 enzymatic activity was wiped out in labeled sample, suggesting the labeling process damaged the exosome.

# Pierce Iodination Reagent Labeling Method (Tubes)



Before Purification

$^{131}\text{I}$ -Exosome

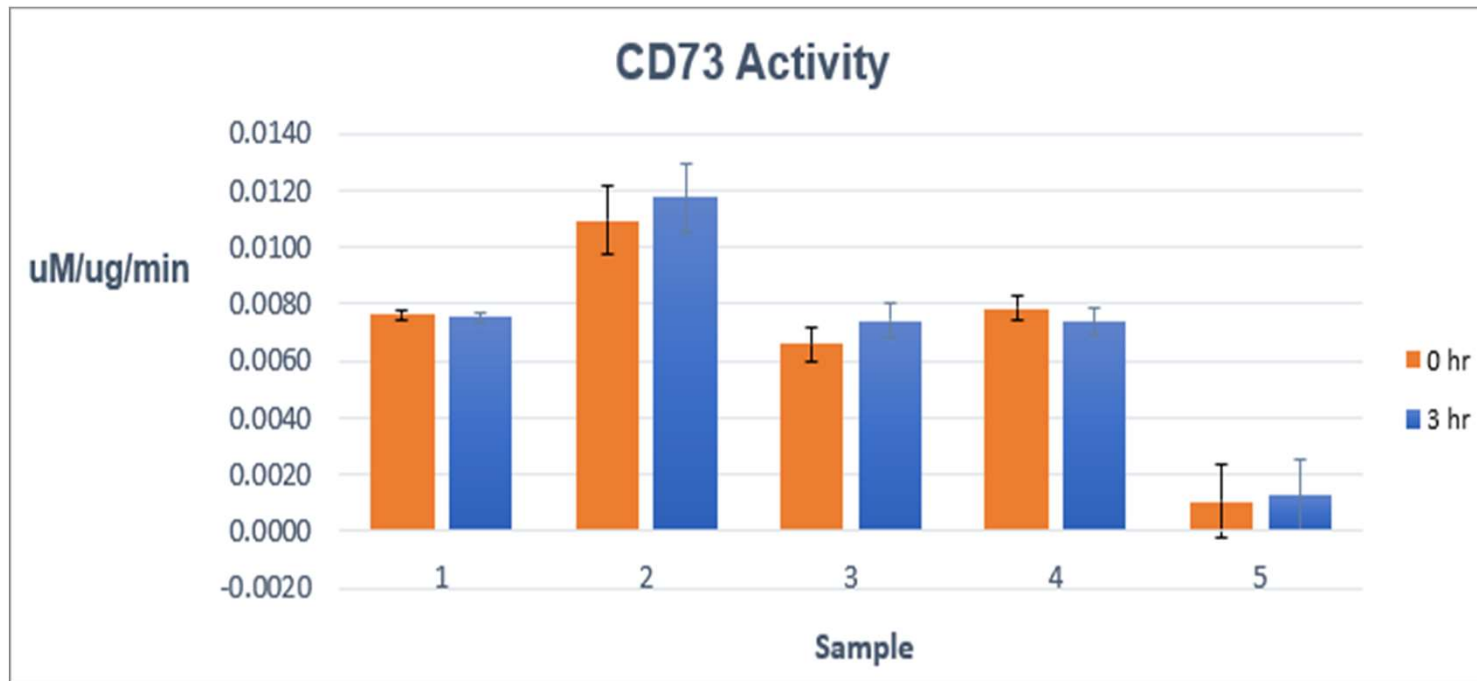


After Purification

Purification: Using PD10 column

# CD73 Enzymatic Activity Measurements (3)

--Effect of cold Pierce Iodination I-labeling on exosome



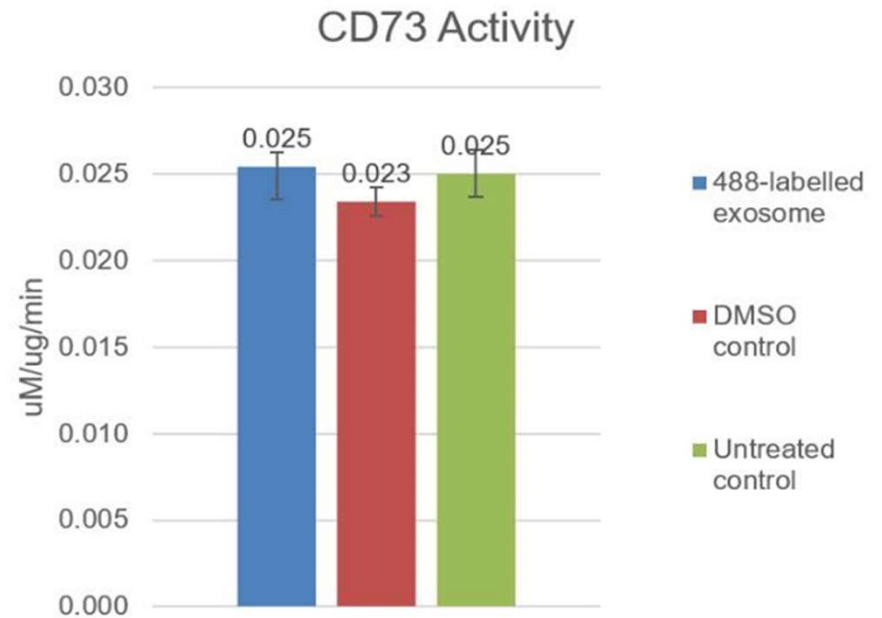
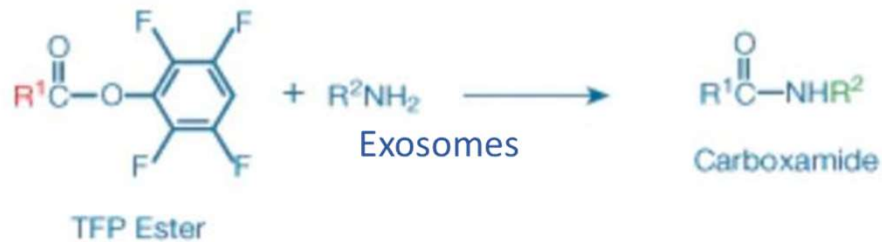
1. Exosomes in H<sub>2</sub>O (control)
2. Exosomes in PBS
3. I-Exosomes in PBS + PI tube
4. I-Exosomes in PBS + PI beads
- 5 --4 after run Biorad P30 column

- While with Pierce iodination (PI) method, the CD73 enzymatic activity drops by 50 % (both tube and beads), when compared to that of the unlabeled exosomes, and the particles kept the same size.

**Table 1: Iodine-labeled of exosomes with radiolabeling yield, CD73 activity and particle size of post-labeling**

	Radio-labeling yield (%)	CD73 activity	Particle size
<b>Chloramine T</b>	35-40	No	increased
<b>Pierce Iodination</b>	10	50%	same

# Fluorescence-Labeled Exosomes

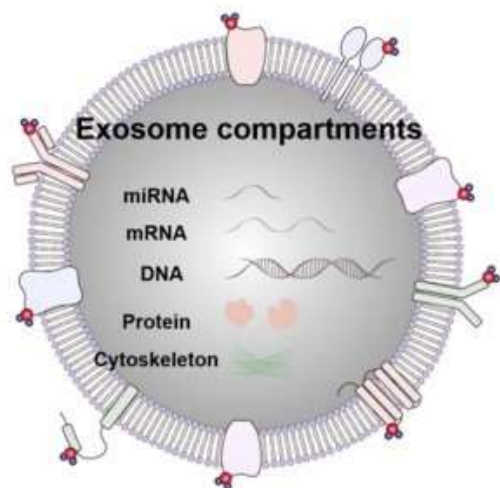


**intact CD73 enzymatic activity of fluorescence-labeled exosomes**

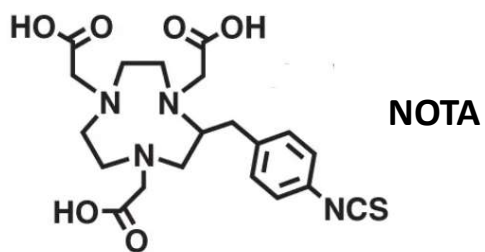
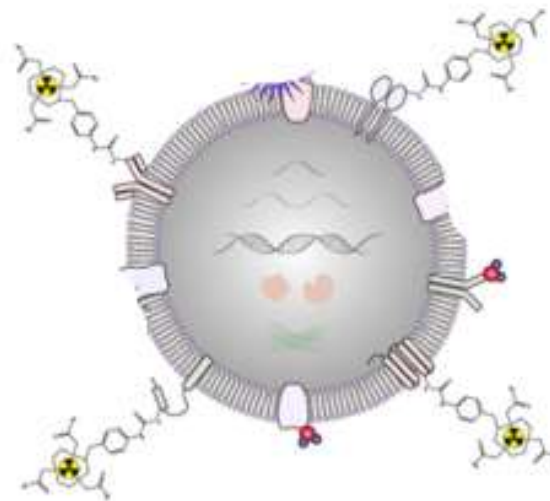
Ref: Lai RC, Lim SK, Toh WS. *Pharmaceutics* 2023, 15, 1489.



# <sup>68</sup>Ga Radiolabeled Exosomes



Route 1: (1) NOTA + Ga-68 labeling : 0.5 h, 95 °C  
(2) Exo + NOTA-Ga-68 : pH > 8, flux 4 h, 4 °C



Ga-68

Route 2: (1) Exo + NOTA : pH > 8, flux 4 h, 4 °C  
(2) Exo-NOTA + Ga-68 labeling: 1 hour, RT

## Conclusion

- Using chloramine T method showed that the CD73 enzymatic activity of I-labeled exosomes was destroyed, suggesting the labeling process damaged the structure of exosomes.
- By comparison, using Pierce Iodination method preserved the CD73 enzymatic activity, indicating that exosomes can be radiolabeled using Pierce Iodination for in vitro and in vivo tracking and pharmacokinetic studies.

# Acknowledgments



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**Thank you!**