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Chelator Free Radiolabeling of PEGylated Graphene Nanosheet

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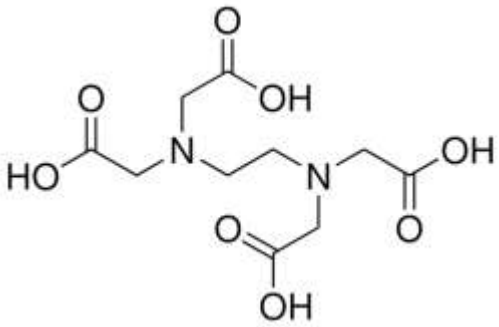
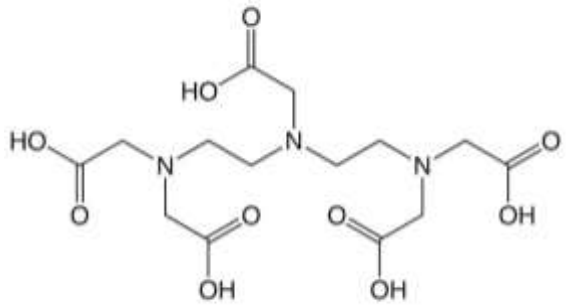
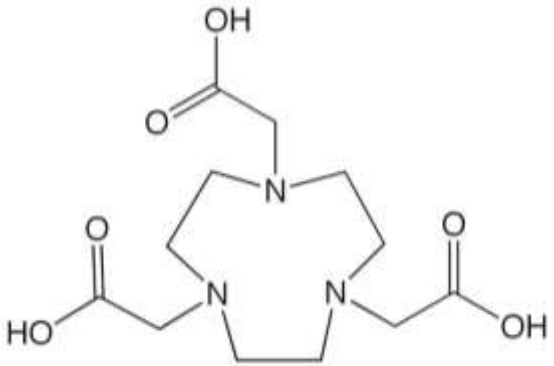
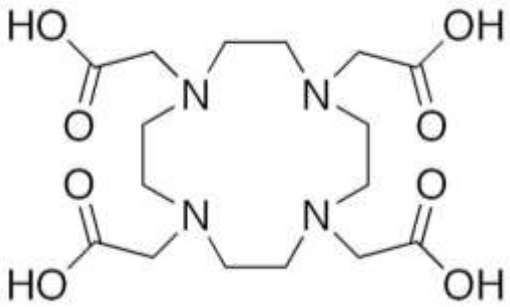
Chelators

- Chelators are molecules that can form multiple bonds with a single metal ion, enhancing stability.
- They are used to capture and transport metal ions in biological and chemical systems.
- Common chelators include EDTA, DTPA, NOTA and DOTA, each with specific applications.
- Although chelators provide a stable linkage between the biomolecules/Nanoparticles and the radioactive isotope, they may also introduce certain limitations.
- Such as potential interference with the biological activity of the molecule, alteration of its pharmacokinetics, or increased immunogenicity.









Chelator Free Radiolabelling

- Chelator-free radiolabelling refers to the process of directly labelling biomolecules/nanoparticles with radioisotopes without the use of a chelating agent.
- Strategies chelator-free radiolabelling, including:

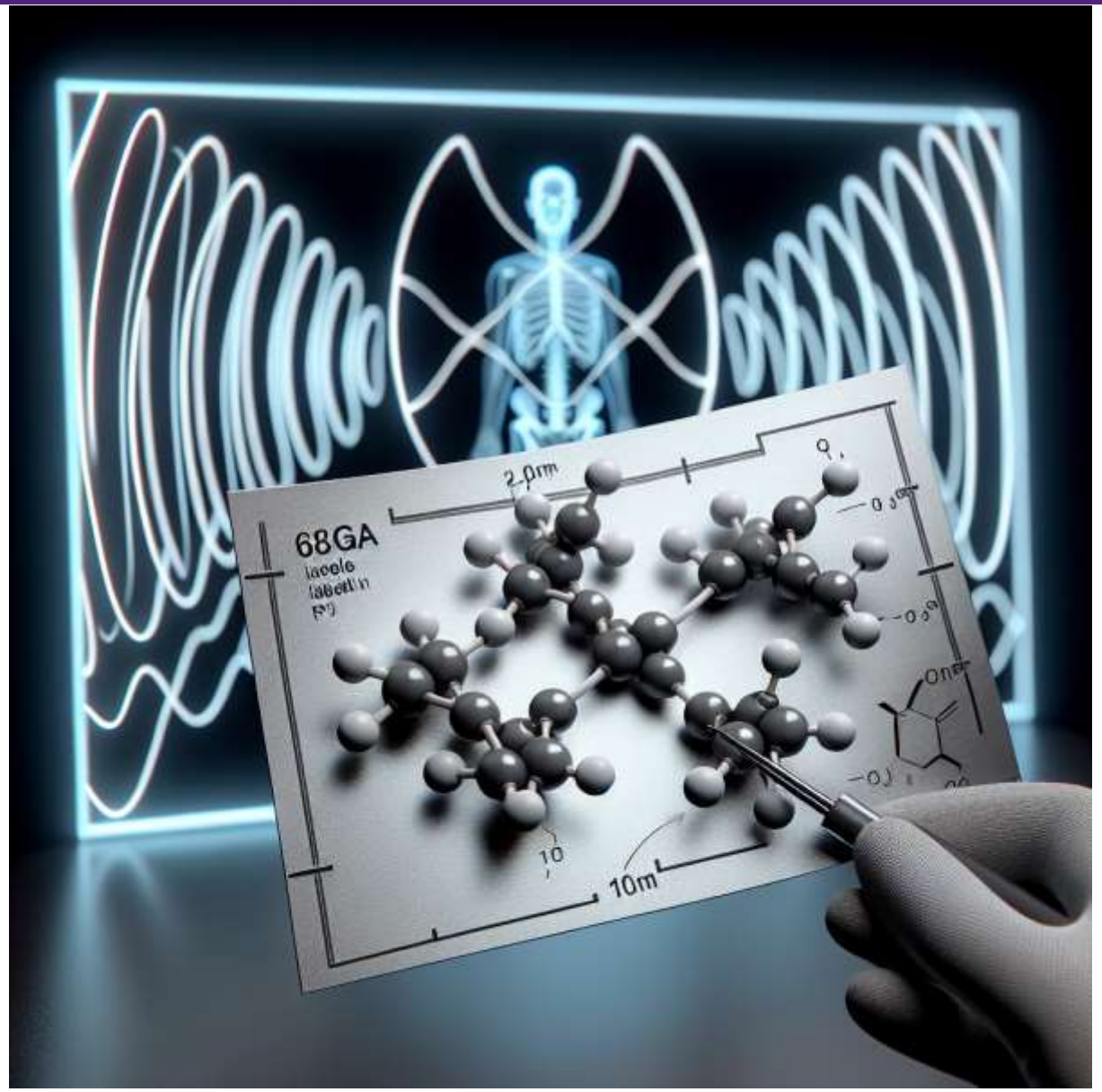
➢ **Direct labelling**

➢ **Biorthogonal chemistry**

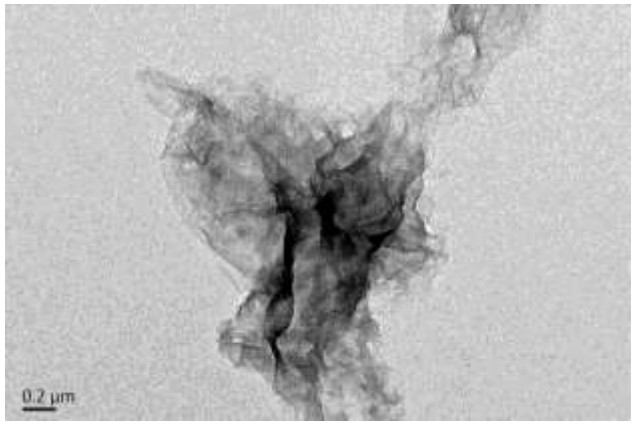
➢ **Click chemistry**

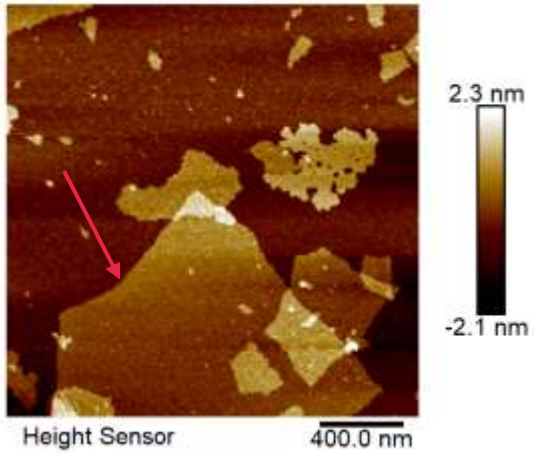
Why Chelator Free?

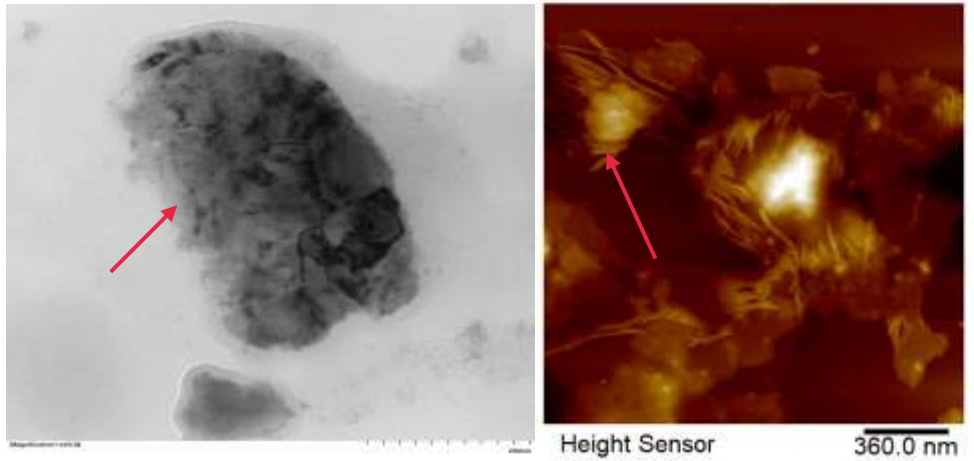
➢ Advantages: Simplified Chemistry, Improved Pharmacokinetics, Decreased Immunogenicity , Cost Effectiveness etc.

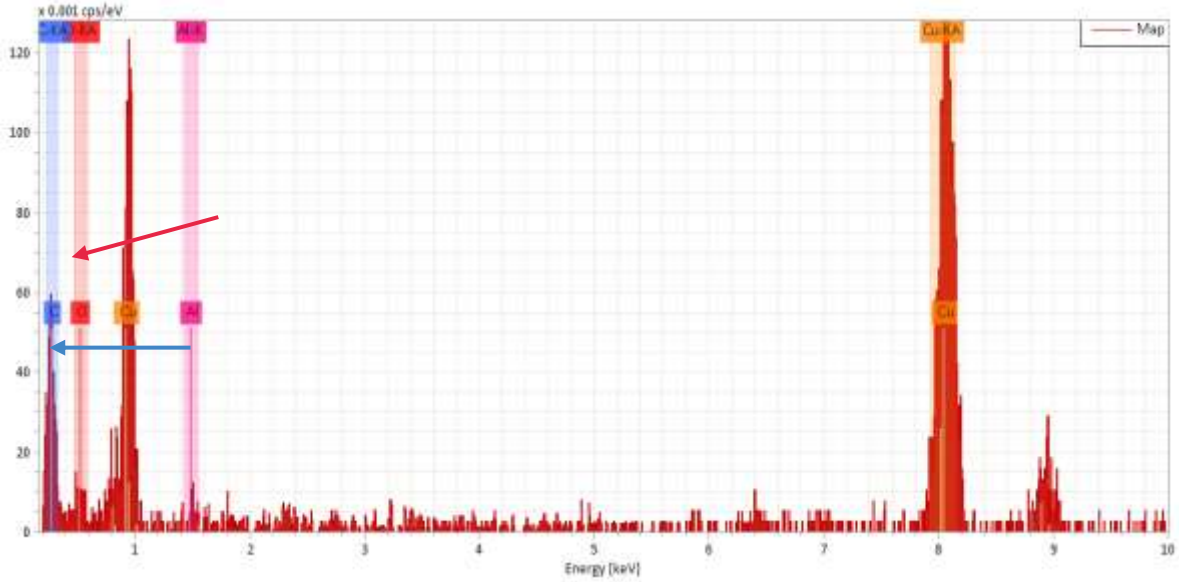


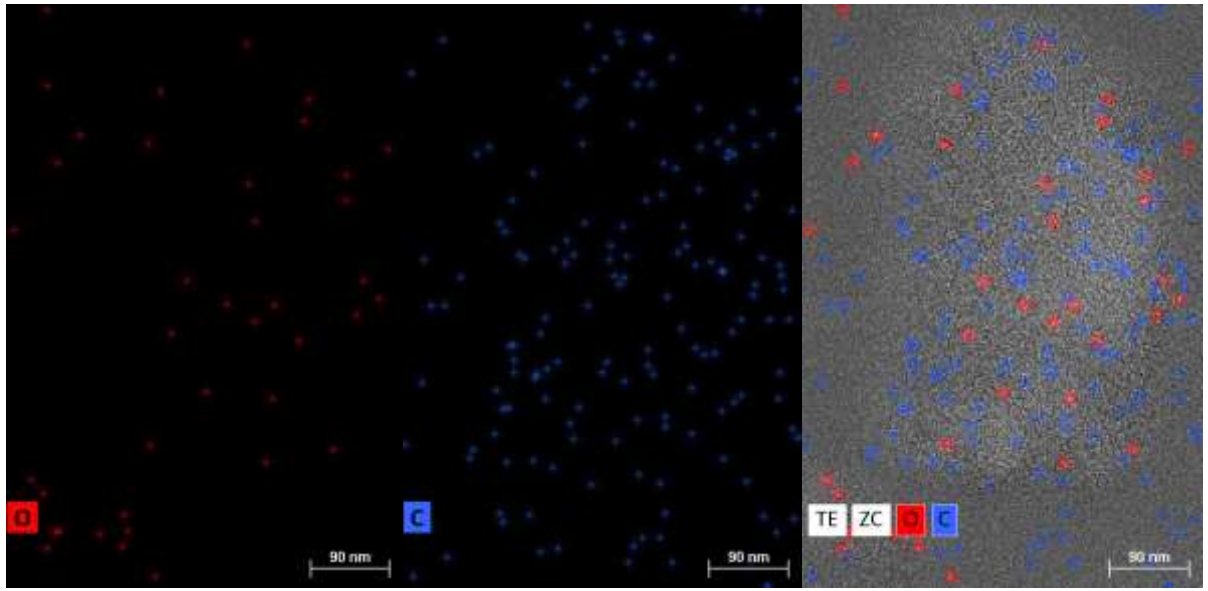
Modification Graphene Oxide Nanosheets





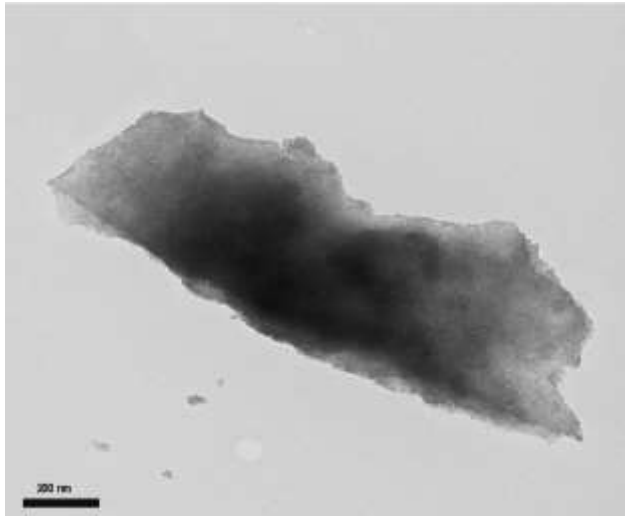


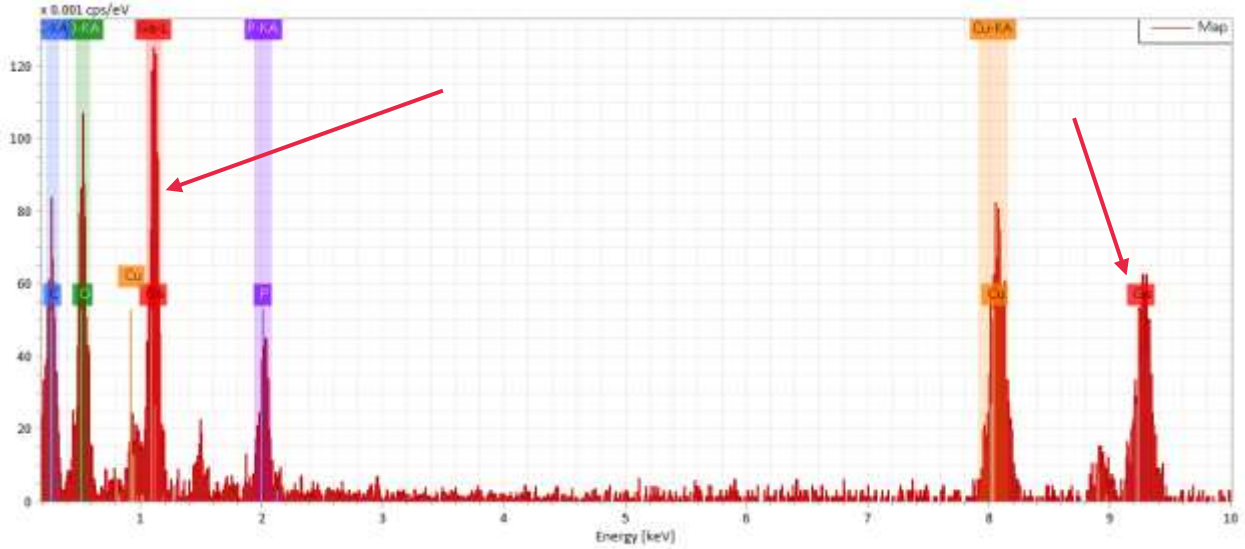




Labelling with Cold Gallium Graphene Oxide Nanosheets

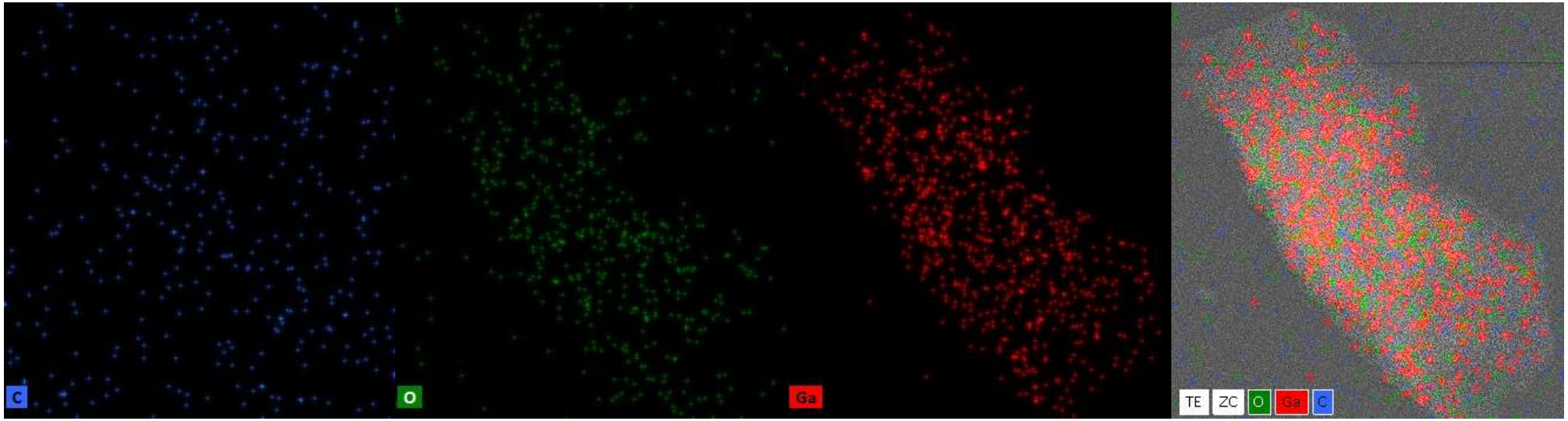
TEM Image of Graphene Oxide Nanosheet Doped with Gallium (Ga)





EDS of GO-PEG-Ga

TEM Elemental images of GO-PEG-Ga



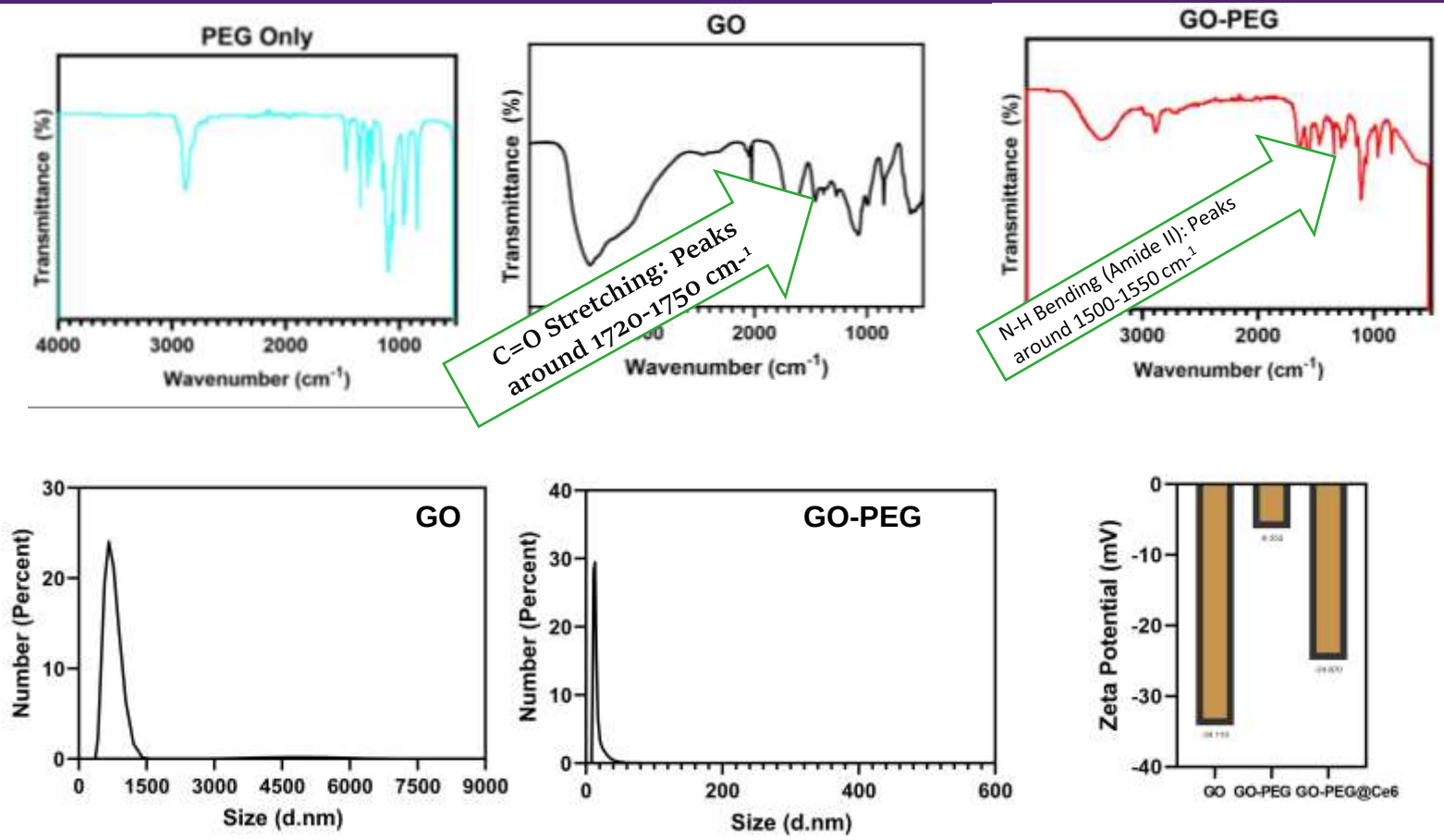
Characterization of Graphene Oxide Nanosheets

C=O Stretching: Peaks around 1720-1750 cm^{-1}

N-H Bending (Amide II): Peaks around 1500-1550 cm^{-1}

PEG Backbone: Additional peaks in the region of 1100-1300 cm^{-1} corresponding to the stretching vibrations of C-O-C bonds in the PEG backbone.

Methoxy Group: Peaks around 2850-3000 cm^{-1} may indicate the stretching vibrations of C-H bonds in the methoxy group (-OCH3) of the methoxyamine PEG



^{68}Ga Radiolabelling of Graphene Oxide Nanosheets

a) **TLC of ^{68}Ga in 50mM EDTA as control.**

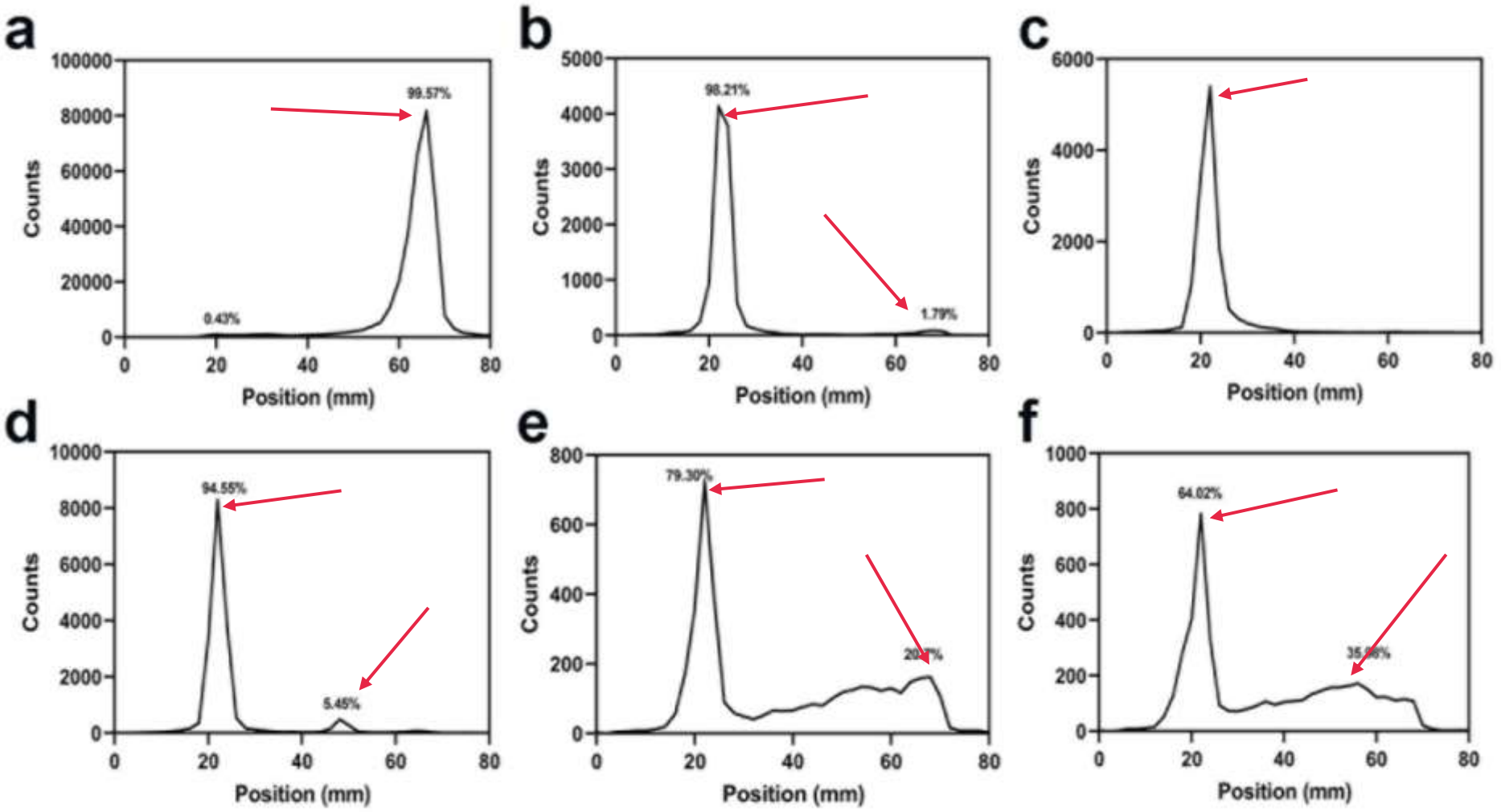
b) **TLC of GO-PEG-Ce6@^{68}Ga in Ammonium acetate buffer**

c) **TCL of GO-PEG-Ce6@^{68}Ga in PBS.**

d) **TLC of GO-PEG-Ce6@^{68}Ga in 50mM EDTA at 0 min.**

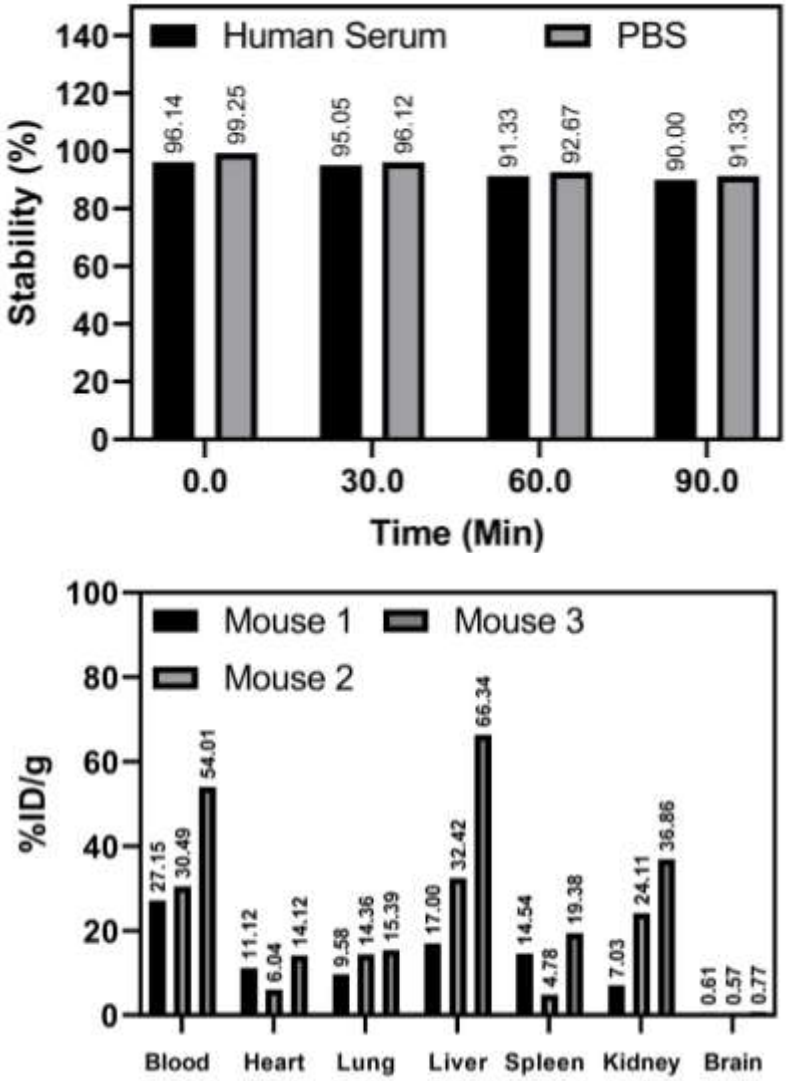
e) **TLC of GO-PEG-Ce6@^{68}Ga in 50mM EDTA at 30 min.**

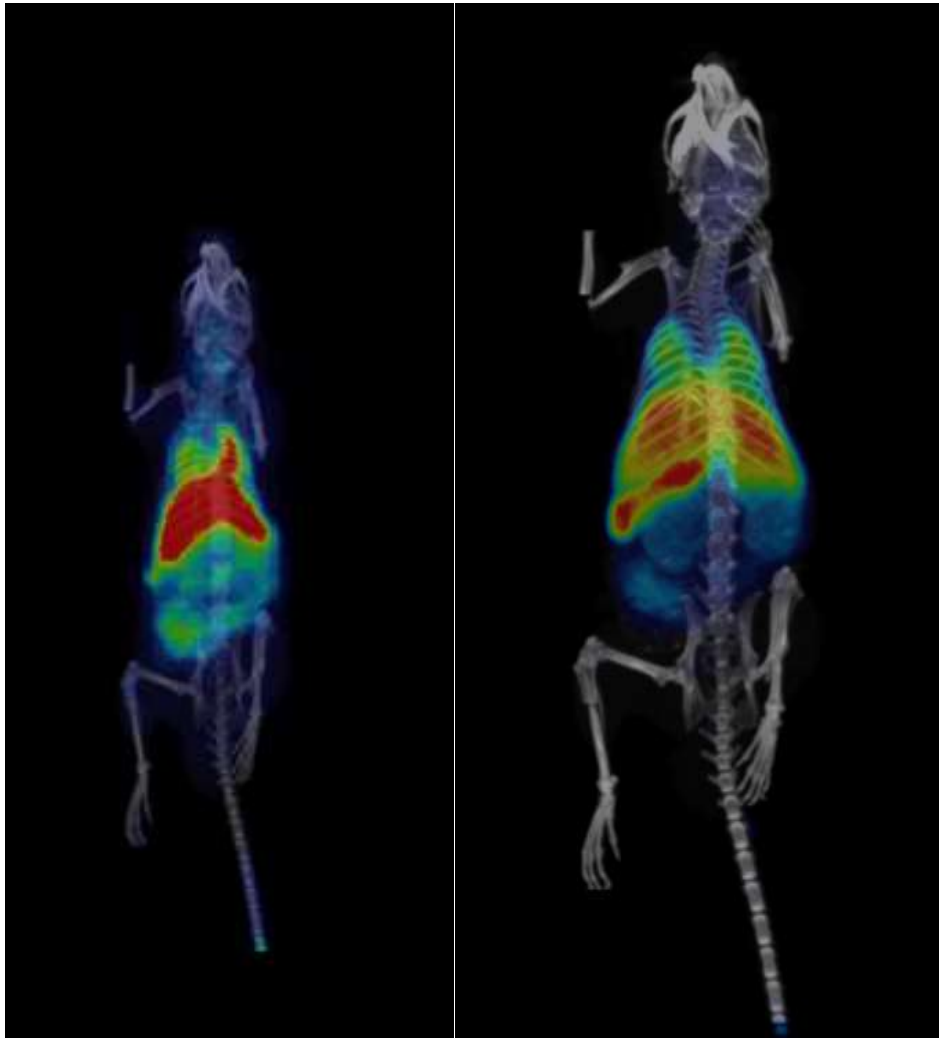
f) **TLC of GO-PEG-Ce6@^{68}Ga in 50mM EDTA at 60 min**



Preliminary Biological Studies

- ✓ The Radiolabelled Nanosheets displayed very good stability in Human Serum and PBS.
- ✓ In vivo PET Imaging showed material accumulation in liver, lungs, pancreases and in kidney.
- ✓ Ex Vivo Gamma counting



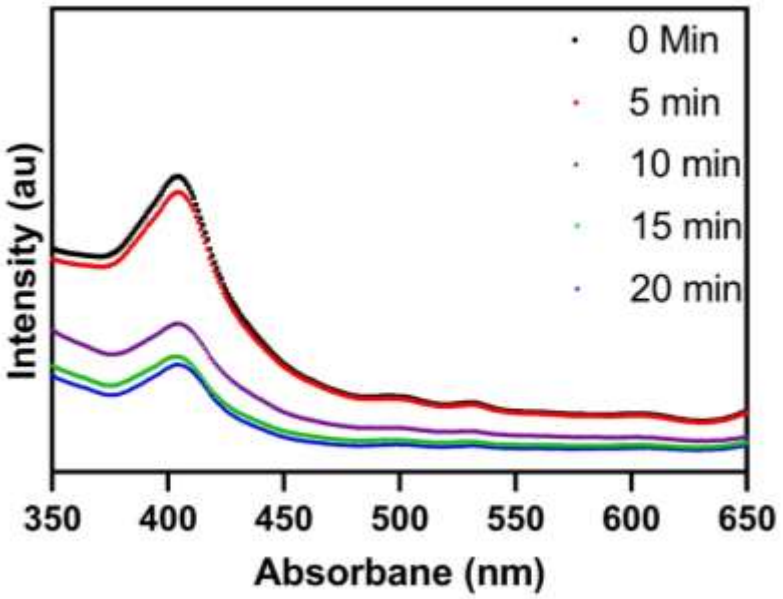


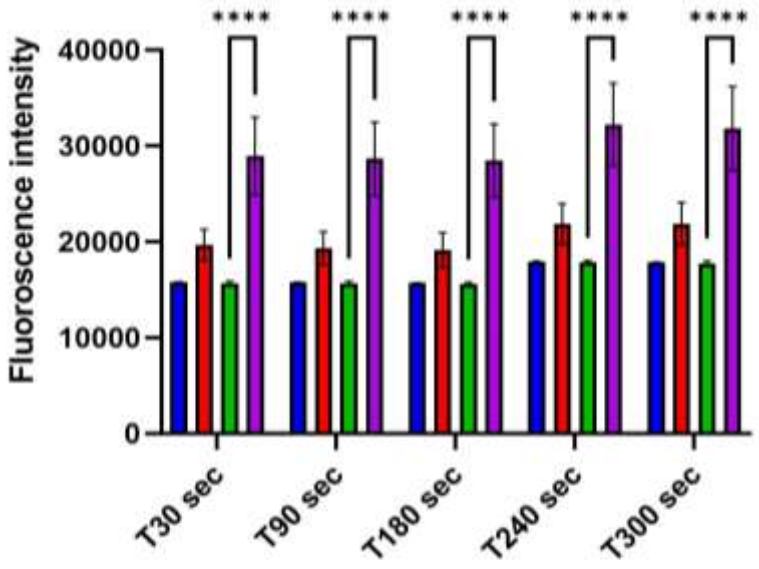
Cherenkov Radiation Induced Energy Transfer (CRIT)

- ✓ Cherenkov radiation-induced energy transfer" likely refers to a process where the energy carried by Cherenkov radiation is transferred to another medium or particle.
- ✓ If a particles travel through a medium at a speed greater than the speed of light in that medium, Cherenkov radiation can be emitted.
- ✓ Radio isotopes induce CRIT when they undergo decay (Alpha , Beta , Gamma)

- ➢ Bernhard, Y., Collin, B. & Decréau, R. Redshifted Cherenkov Radiation for in vivo Imaging: Coupling Cherenkov Radiation Energy Transfer to multiple Förster Resonance Energy Transfers. Sci Rep 7, 45063 (2017). https://doi.org/10.1038/srep45063

CRET induced ROS generation





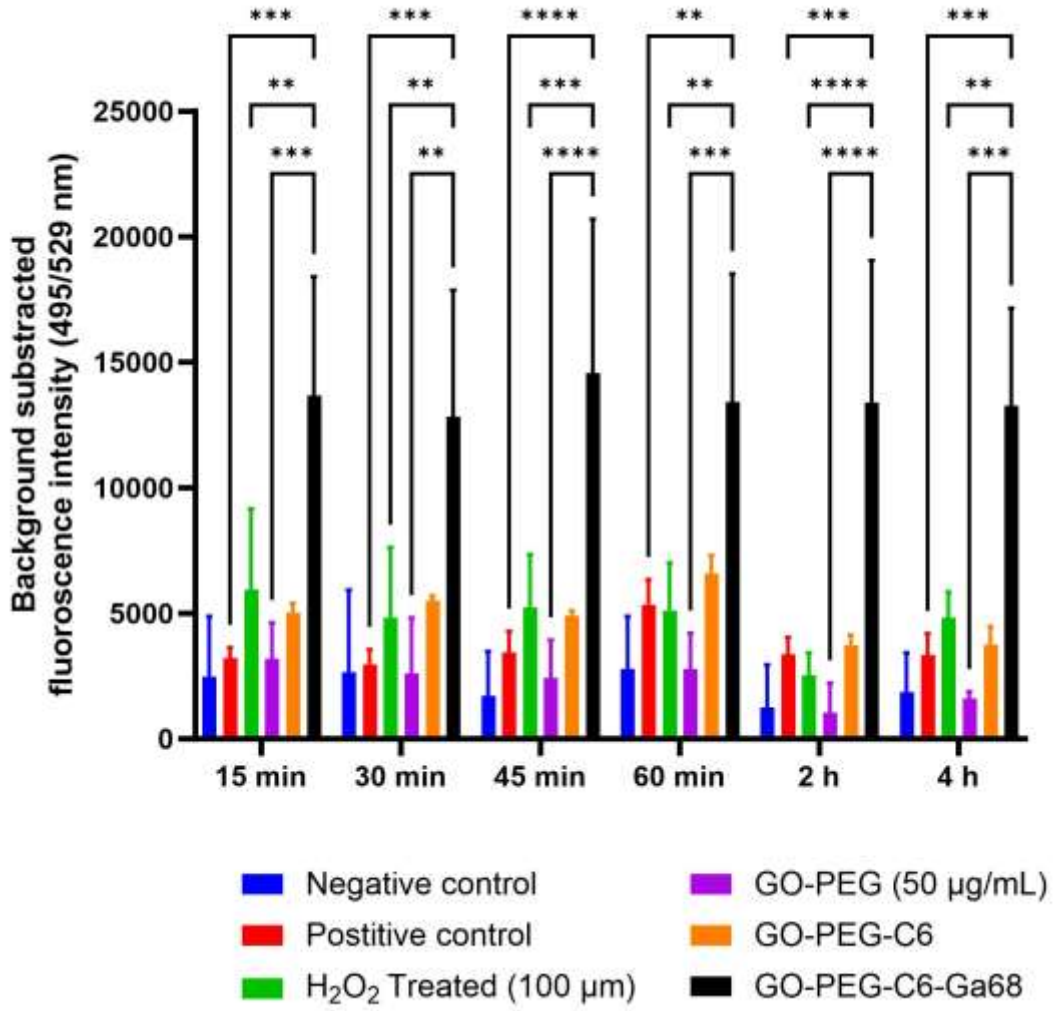
- ❑ **The Singlet Oxygen Sensor Green reagent is highly selective for $^1O_2$.**
- ❑ **In the presence of singlet oxygen, it emits a green fluorescence like that of fluorescein**
- ❑ **(excitation/emission maxima ~504/525 nm).**
- ➢ **As shown in the graph, the $^1O_2$ is generated only in the presence of both Ce6 and ^{68}Ga**

- ➢ **Singlet oxygen ($^1O_2$) is generated, often through photosensitization reactions.($^1O_2$) undergoes a Diels-Alder cycloaddition reaction with the conjugated double bonds of DPBF.**
- ➢ **This reaction forms an endoperoxide product.The formation of the endoperoxide leads to a decrease in DPBF's absorbance, typically around 410 nm.**
- ➢ **Monitoring this decrease serves as a reliable method for detecting and quantifying singlet oxygen in chemical and biological systems.**

Intra Cellular ROS generation

- **H2DCFDA, or 2′,7′-dichlorodihydrofluorescein diacetate, is a non-fluorescence probe that can passively diffuse into cells.**
- **Once inside the cell, the acetate groups of H_2DCFDA are cleaved by intracellular esterases.**
- **Finally, upon oxidation by ROS (hydroxyl, peroxyl, and other species), the nonfluorescent H_2DCFDA is converted to the highly fluorescent 2′,7′-dichlorofluorescein (DCF).**

- **At any time point the ROS levels in cells treated with GO-PEG-C6-^{68}Ga is at least 2.5-fold more than cells treated with ROS inducer (abcam) and 100 µmol H_2O_2.**



Conclusion and Future Planed work

- ✓ **Developing more controls around the design of experiments .**
- ✓ **Cellular Toxicity Studies in different cell lines.**
- ✓ **Evaluating the efficiency of light independent CRIT induced PDT in *Ex vivo* and *in vivo* tumour models.**

Acknowledgement

Professor Kristofer Thurecht
Dr Nicolas Fletcher
Dr Arun Balaji
Thurecht Group Members

National Imaging Facility

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