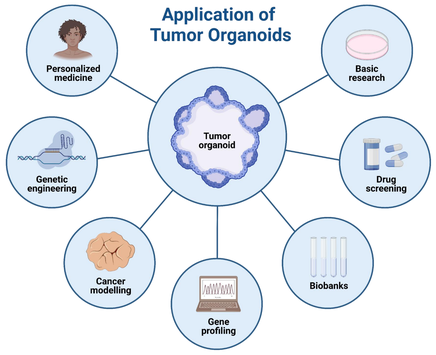
Application of In-Cell Fast Photochemical Oxidation of Proteins for the Study of Organoids

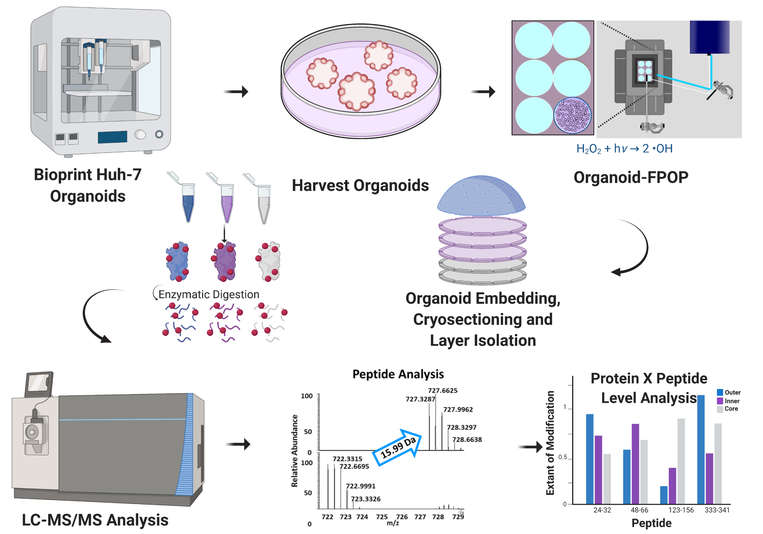
Raquel Shortt,[1] and Lisa M. Jones[2]
[1]Department of Pharmaceutical Sciences, University of Maryland, Baltimore, MD
[2]Department of Chemistry and Biochemistry, University of California, San Diego, CA

1. Organoids as a Model System for Cancer

It has been demonstrated that immortalized cell lines in function differently compared to cells in tissue. The two-dimensional models do not simulate *in vivo* environments and these issues have necessitated the development of new systems that mimic native conditions. Organoids are a multicellular three-dimensional model system that resemble the corresponding organ. The complexity of organoids makes them difficult for structural studies. The complexity of organoids makes them difficult for structural studies. Therefore, we have extended in-cell fast photochemical oxidation of proteins (IC-FPOP) into Huh-7 liver organoids. IC-FPOP is a valuable, mass spectrometry (MS)-based tool to probe protein structures and interactions in -cells[1]. It was recently adapted to a platform incubator with an XY movable stage (PIXY), where thousands of proteins were modified in cells, in a fraction of time compared to the flow system[2]. The organoid model system is the latest application of IC-FPOP further validating its usage for structural proteomics.



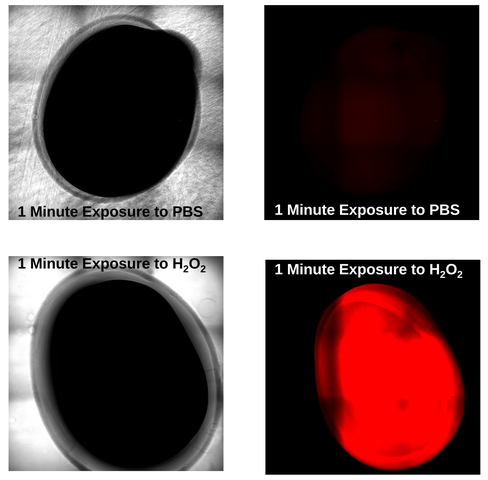
2. IC-FPOP on Huh-7 Organoids



Organoids were cultured for 5 days post printing. After digestion, ~1 ug of peptides from each organoid layer were separated on an EvoSep One LC and detected on an Orbitrap Fusion Lumos MS in DDA mode.

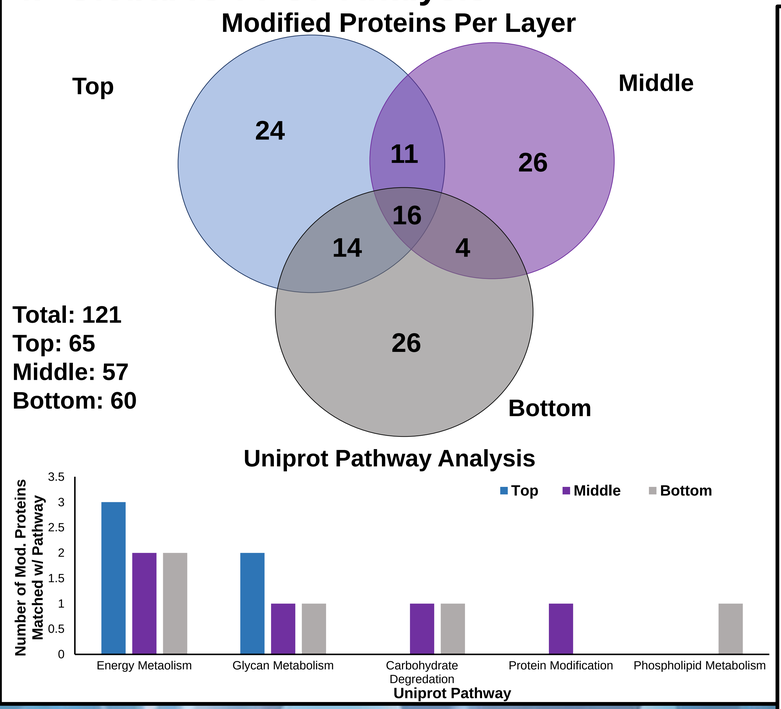
3. PO1 to Detect H_2O_2 Penetration

Five uM of fluorescent peroxide indicator PO1 was incubated with organoids for 50 min, then exposed to PBS or 100 mM H_2O_2 for one minute.

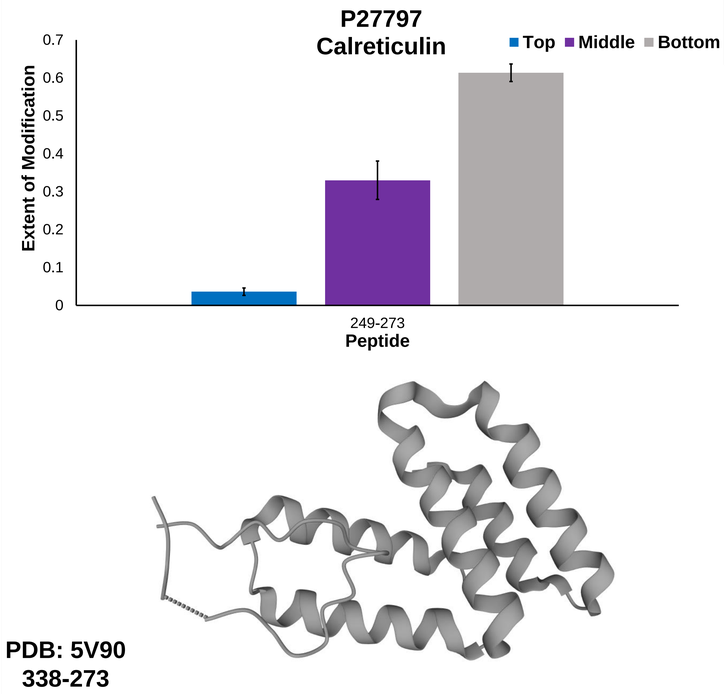


The fluorescence intensity was **3.5x** higher in the samples treated with 100 mM H_2O_2. This supports the use of one minute incubation times for IC-FPOP experimentation.

4. Global IC-FPOP Analysis



5. Differences in Extent of Modification



6. Conclusions

- **Peroxide perfusion throughout the organoids was first confirmed by fluorescence microscopy.**
- **The spatial resolution obtained by sectioning the organoid ensured sufficient peroxide penetration in each layer.**
- **Uniprot pathways analysis revealed IC-FPOP modified proteins involved in processes associated with glycan metabolism & protein modification, demonstrating the method's ability to interrogate native tumorigenic interactions.**
- Further investigation of the proteins modified in organoid layers showed differences in the extent of modification for calreticulin a calcium-binding chaperone that promotes proper protein folding in the ER.
- To improve the number of FPOP modifications, a range of peroxide incubation times will be explored.
- As shown by previous IC-FPOP manuscripts, offline reverse phase (RP) fractionation will be applied to expand proteome coverage.
- **This is the first study where bioprinted 3D organoids were applied to the innovative structural biology method IC-FPOP.**

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References: 1. Kaur, U.; Johnson, D. T.; Jones, L. M., Validation of the Applicability of In-Cell Fast Photochemical Oxidation of Proteins across Multiple Eukaryotic Cell Lines. *J Am Soc Mass Spectrom* **2020,** *31* (7), 1372-1379.
2. Johnson, D. T.; Punshon-Smith, B.; Espino, J. A.; Gershenson, A.; Jones, L. M., Implementing In-Cell Fast Photochemical Oxidation of Proteins in a Platform Incubator with a Movable XY Stage. *Anal Chem* **2020**.
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