Membrane Protein Comparison Between Cell Membranes and Extracellular Vesicle Membranes of S. pneumoniae Provide Insights into Extracellular Vesicle Formation and Shedding

Stephanie Biedka[1], Rory Eutsey[2], Luisa Hiller[2], Jonathan S Minden[1,2]

[1]Impact Proteomics, Pittsburgh, PA: [2]Carnegie Mellon University, Pittsburgh, PA

Abstract

Extracellular vesicles (EVs) are complex, cell-derived nanoparticles generated by all cell types. EVs are composed of lipid bilayer membranes and their associated membrane proteins, nucleic acids, and luminal proteins. The mechanism by which Gram-positive bacteria shed EVs is still unknown. EVs from the Gram-positive human pathogen S. pneumoniae, which is a major cause of otitis medi and pneumonia, are of particular interest because of how they EVs modulate the host immune response. To uncover possible mechanisms for EV production and shedding in S. pneumoniae, we have performed a comparative proteomics analysis of EV membrane proteins versus whole-cell membrane proteins.

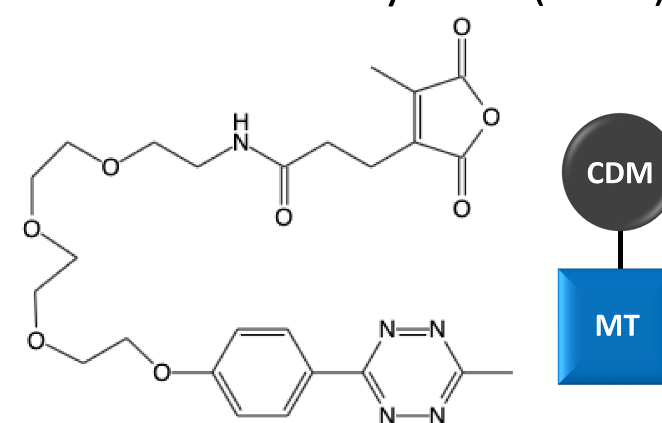
Membrane proteins were enriched from intact S. pneumoniae cells or their EVs using a ProMTag labeling and capture workflow. ProMTag is a bifunctional protein tag where one moiety of the tag is able to form a reversible, covalent link to primary amines on proteins. The other moiety is methyltetrazine, which can form an irreversible, covalent bond with trans-Cyclooctene (TCO) on the surface of beads to capture ProMTagged proteins for cleanup and elution. Using this workflow plasma membrane proteins can be tagged, captured, washed to remove non-plasma membrane proteins, and then eluted in their original, unmodified state.

In this study, intact cells and EVs from S. pneumoniae cultures were separated and the extracellular domains of membrane proteins in these two fractions were labeled with ProMTag. The membrane proteins were then enriched, washed, and eluted using the ProMTag workflow. These membrane protein populations were then TMT labeled and analyzed using mass spectrometry. Comparative analysis revealed membrane proteins that are concentrated or absent in EV membranes relative to bulk plasma membrane from whole cells, indicating a selective process for EV formation in S. pneumoniae. With this information, we present a new model for EV formation and shedding in S. pneumoniae.

ProMTag Workflow Key Components

ProMTag
Protein tagging

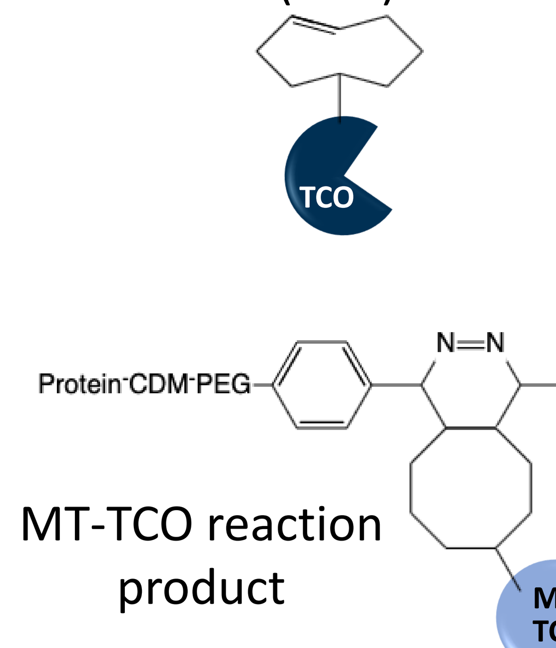
Reversible Protein Binding Reagent
Carboxyethyl-dialkyl-maleic Anhydride (CDM)



Irreversible Bead Binding Reagent
Methyltetrazine (MT)

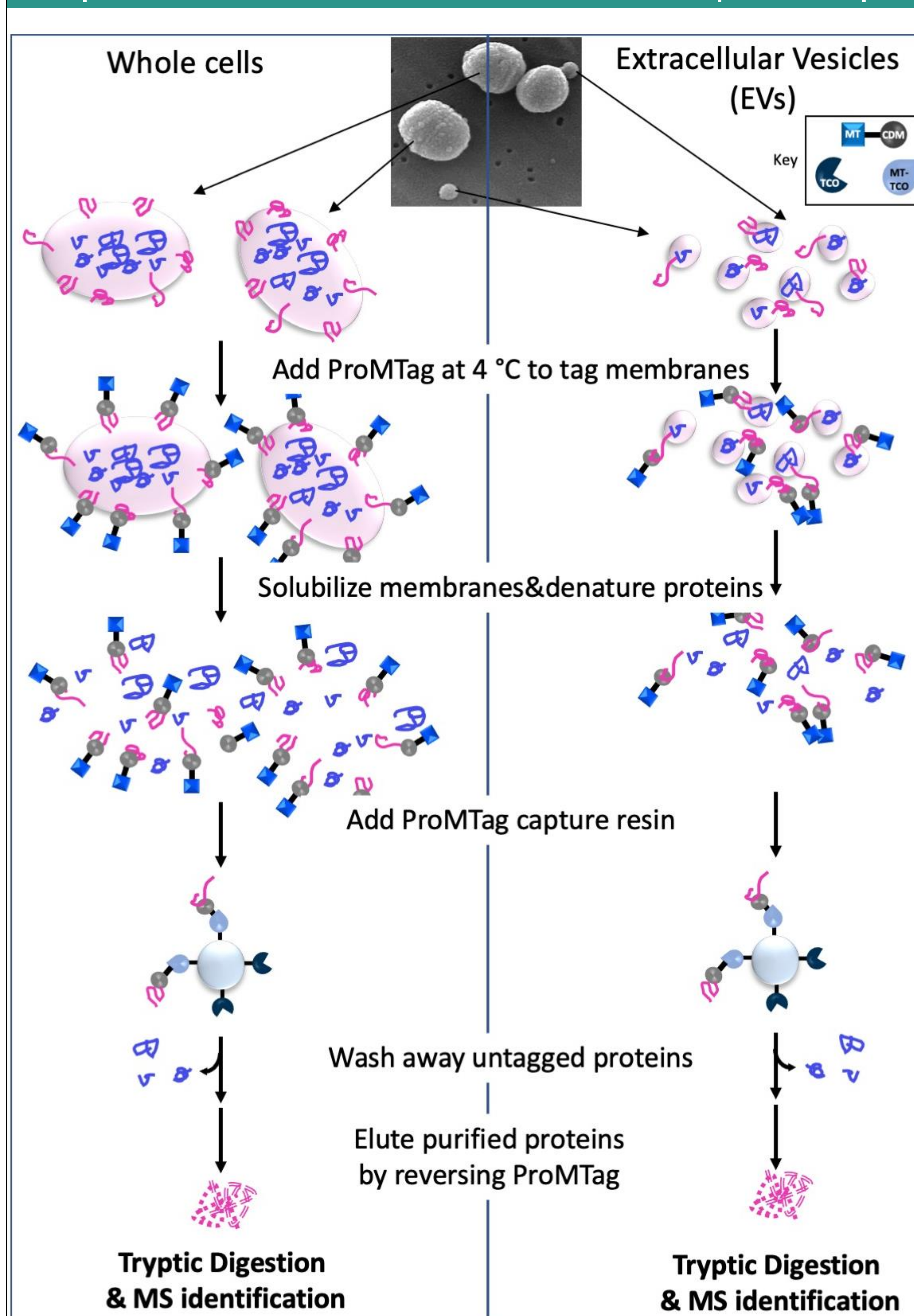
ProMTag Capture
TCO-bead

Trans-cyclooctene (TCO)

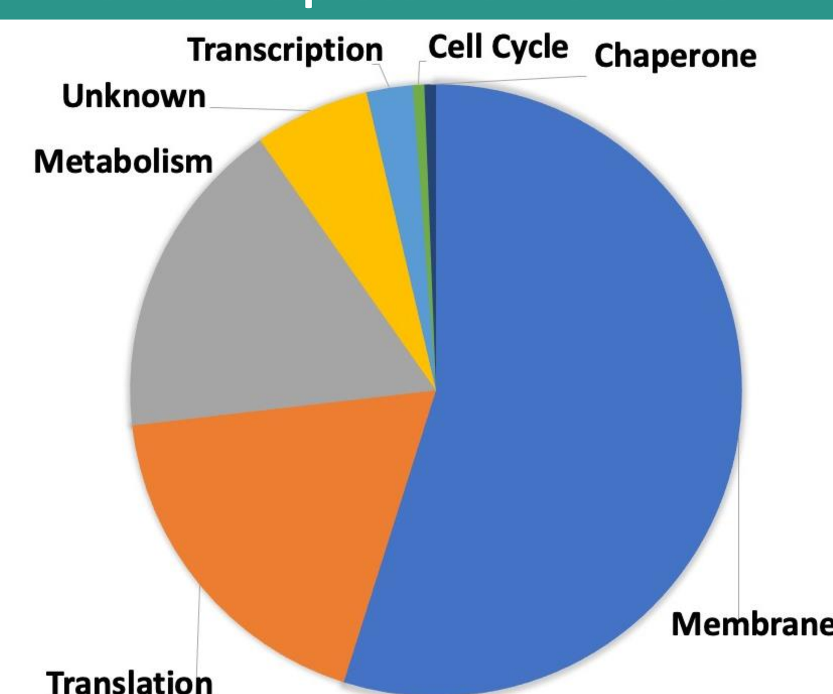


MT-TCO reaction product

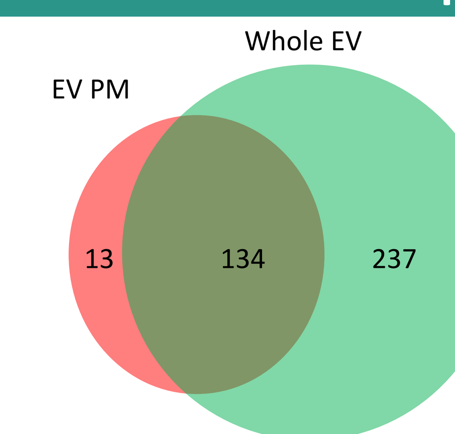
Preparation of whole cell membrane and EV membrane protein samples



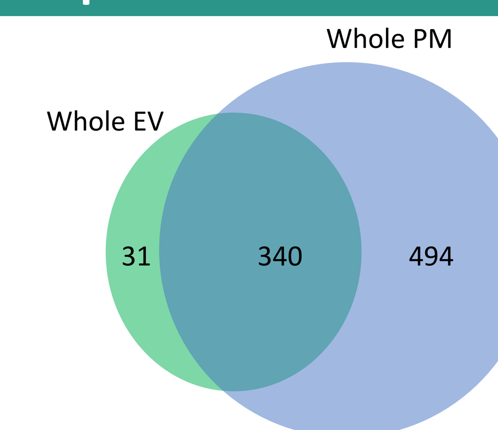
Membrane protein enrichment in EVs



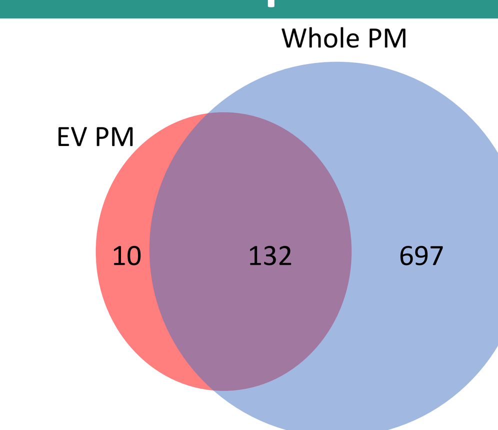
Distribution of EV membrane-enriched proteins and whole-EVs



Distribution of whole-EV proteins and whole-cell membrane proteins



Distribution of membrane-enriched proteins between EVs and whole-cells



Membrane proteins that are highly enriched in extracellular vesicles

Description	|Log Prob|	Total Intensity	Unique peptides	Coverage %
Membrane proteins only detected in extracellular vesicle membranes				
Teichoic acid phosphorylcholine esterase	264.49	4.2E+08	43	51.20
Uncharacterized protein	85.76	6.6E+07	10	80.60
Choline-binding protein	59.97	1.9E+08	11	45.92
Competence protein	42.58	9.1E+07	8	44.44
Putative endo-beta-N-acetylglucosaminidase	15.37	3.8E+07	15	27.35
Pneumococcal vaccine antigen A	7.63	3.1E+07	4	35.29
Choline binding protein D	4.64	2.7E+07	8	25.00
UPF0154 protein spr1697	4.26	1.4E+07	3	47.56
Membrane proteins greatly enriched in extracellular vesicle membranes				
Zinc metalloprotease ZmpB	1589.99	3.4E+09	237	76.28
1,4-beta-N-acetylmuramidase	955.49	5.4E+09	180	80.24
Excalibur domain-containing protein	187.81	3.5E+08	28	69.30

Conclusions

The ProMTag membrane protein capture method is capable of enriching membrane proteins. Further optimization of the washing steps will lead to greater enrichment levels.

The proteins found exclusively on EV membranes and those highly enriched over whole-cell membrane proteins are primarily cell wall modifying enzymes that may be involved in EV biogenesis or influence the binding and release of EV surface-bound molecules, such as DNA, with consequences for EV mobility within biofilms and for host-cell interactions.

Acknowledgements

This work was supported by NSF SBIR Phase II Award 2036199. This work was also supported in part by NIH P30 CA124435 utilizing the Stanford Cancer Institute Proteomics/Mass Spectrometry Shared Resource at the Vincent Coates Foundation Mass Spectrometry Laboratory, Stanford University Mass Spectrometry (RRID:SCR_017801). We thank Ryan Leib and the Stanford University Mass Spectrometry Facility for their help with mass spectrometry analysis, data analysis, and statistical analysis.