Automated Spatially Targeted Optical Micro Proteomics (autoSTOMP): a tool to study immune regulation of the parasitophorous vacuole membrane (PVM) proteome

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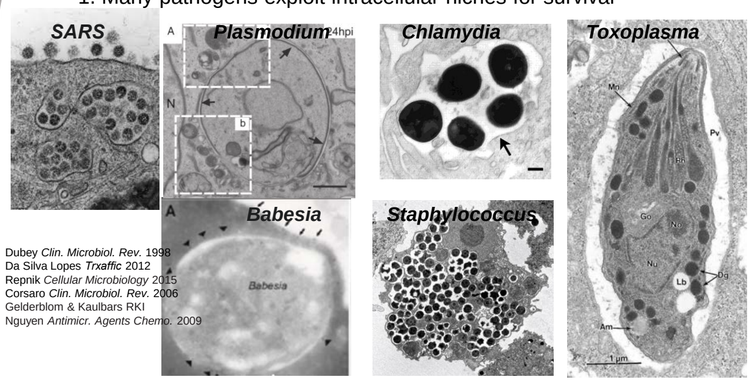
Abstract

The parasitophorous vacuole membrane (PVM) is the battlefield composed of secreted Toxoplasma components that recruit host nutrients and subvert an arsenal of host innate immune sensing components. Within PVM, the parasite proliferates while prevent severe inflammatory response causing host damage. This commensalism should maintain long enough to allow Toxoplasma entering a stage forming cyst that can survive to infect a next host. To identify components of the PVM involved in innate immune sensing, we perform a novel discovery proteomics technique called automated Spatially Targeted Optical Micro Proteomics (autoSTOMP) designed to identify the components of subcellular structures. In autoSTOMP immunofluorescence microscopy identifies structures of interest (SOI) and tag the SOI proteins with biotin tag. Proteins tagged in this way are then precipitated and identified by mass spectrometry. We validated autoSTOMP can selectively identify the distinct proteomic profiles from PVM vicinity regions of Toxoplasma infected primary mouse bone marrow-derived dendritic cells (mBMDCs). Next, we identify innate immune sensing components near PVM in mBMDCs altered at various priming conditions with toll-like receptor ligands that can activate the inflammasome or/with interferon-γ (IFN-γ) that recruits GTPases to PVM). Some known PVM components found in our data suggest the capability of autoSTOMP in spatial resolution of PVM proteome. Now we are at the early stage of analyzing the enriched proteins near PVM. it is hopeful that we can find novel functions of known or under-studied PVM identifies by combining our autoSTOMP data with other experimental assays and contribute to a better understanding of parasite restriction in conjunction with host cell death.

Introduction

1. Many pathogens exploit intracellular niches for survival

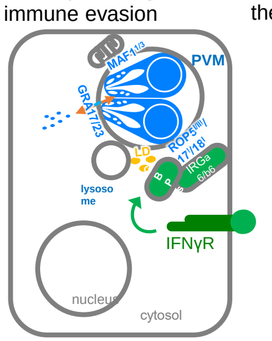


Dubey *Clin. Microbiol. Rev.* 1998
Da Silva Lopes *Trxaffic* 2012
Repnik *Cellular Microbiology* 2015
Corsaro *Clin. Microbiol. Rev.* 2006
Gelderblom & Kaulbars RKI
Nguyen *Antimicr. Agents Chemo.* 2009

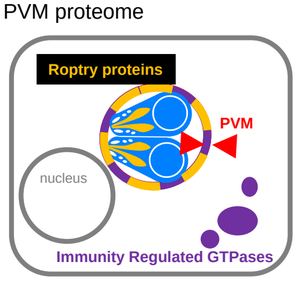
2.The parasite vacuole membrane (PVM) is the interface for *Toxoplasma* growth, persistence and immune evasion

-A complete set of parasite effectors recruited to the PVM is not known

-How cell type, metabolic or inflammatory state influences PVM protein identity is poorly understood



3.There have been technological barriers to isolating and identifying the PVM proteome

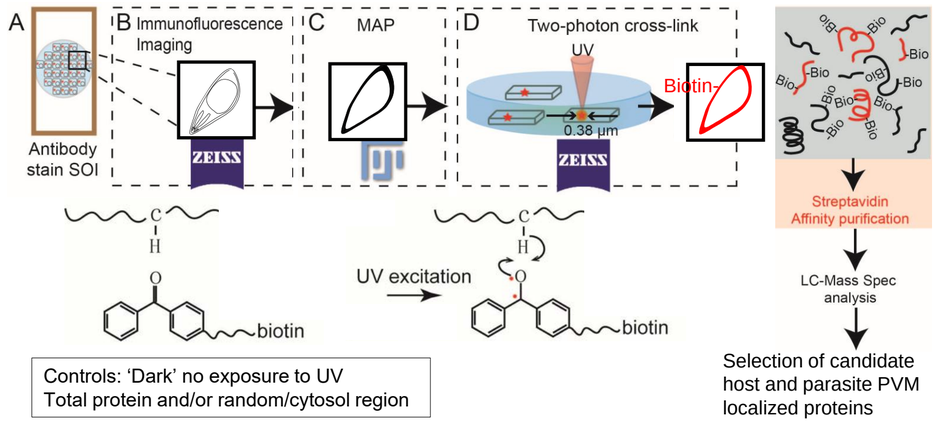


- We have not identified any host or parasite proteins that *uniquely* localized to the PVM.

MBio 7, e02231-15 (2016).
PLoS Pathog 13, e1006362 (2017).
Exp. Mol. Med. 51, 1–10 (2019).
Front. Immunol. 11, 1–18 (2020).
Proc. Natl. Acad. Sci. U. S. A. 111, 1126–1131 (2014).
Frontier. Trends Parasitol. 33, 473–488 (2017).

Method

Automated **S**patially **T**argeted **O**ptical **M**icro **P**roteomics (**autoSTOMP**) uses the two-photon to attach UV-activatable biotin tags to regional proteins for identification by LC-MS

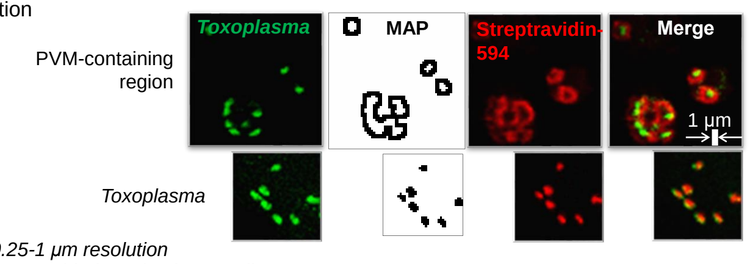


Controls: 'Dark' no exposure to UV
Total protein and/or random/cytosol region

Yin et al., Anal. Chem. 2020, 92, 2005−2010

Validation

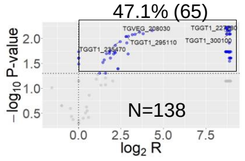
1. AutoSTOMP is a versatile tool to perform proteomics on subcellular structures at micron scale resolution



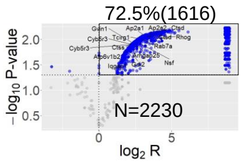
- *~0.25-1 µm resolution*
- *Image-guided tagging is versatile:*
  - *-nearly any structure that can be labeled by an antibody or stain can be targeted*
  - *-subsets of structures (based on size or co-localization) can be targeted*
- *autoSTOMP is performed on fixed samples, providing a snapshot of biology with rapid kinetics*

2. PVM-localized proteins are enriched by autoSTOMP:

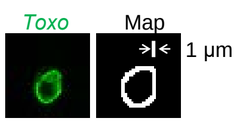
Toxoplasma proteins
PVM VS. BG
47.1% (65)



Mouse proteins
PVM VS. BG
72.5%(1616)



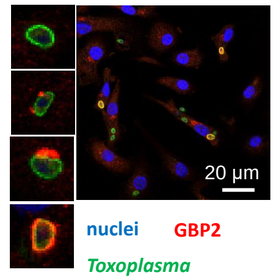
Tg protein	Tg gene
rhoptry protein ROP7	TGME49_295110
alveolin domain containing intermediate filament IMC3	TGME49_216000
IMC12	TGME49_248700
rhoptry protein ROP8	TGME49_215775
IMC7	TGME49_222220
rhoptry protein ROP4	TGME49_295125
rhoptry protein ROP5	TGME49_308090



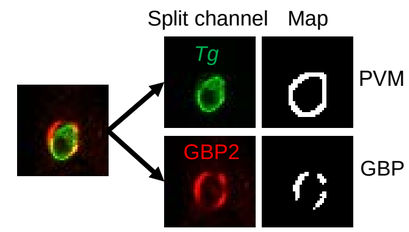
Mouse Gene	Mouse proteins
Iqgap1	Ras GTPase-activating-like protein IQGAP1
Gdi2	Rab GDP dissociation inhibitor beta
Atp6v1b2	V-type proton ATPase subunit B, brain isoform
Arhgap25	Rho GTPase-activating protein 25
Rhog	Rho-related GTP-binding protein RhoG
Gvin1	Interferon-induced very large GTPase 1
Rab7a	Ras-related protein Rab-7a
Ctsd	Cathepsin D
Tcirg1	V-type proton ATPase subunit a
Ap2a2	AP-2 complex subunit alpha-2
Ap2a1	AP-2 complex subunit alpha-1
Ctss	Cathepsin S
Nsf	Vesicle-fusing ATPase
Cyb5r3	NADH-cytochrome b5 reductase 3

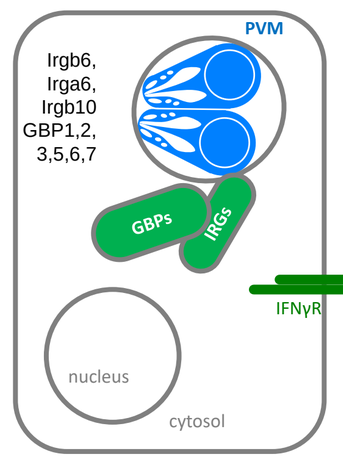
Yin et al., Anal. Chem. 2020, 92, 2005−2010

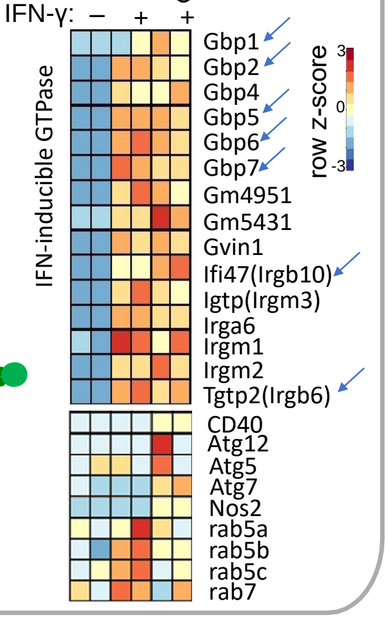
priming changes PVM proteome



~ 50% of PVMs are GBP2+





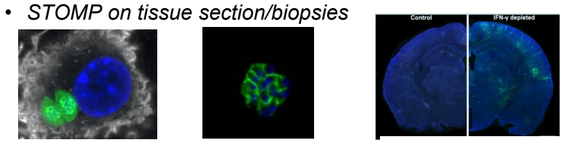


unpublished data

Miscellaneous

Future direction

- *Check the PVM identity alternation in a toxoplasma strain dependent manner (use different strains, rop5/17/18 ko)*
- *Study other parasites*
- *STOMP on tissue section/biopsies*



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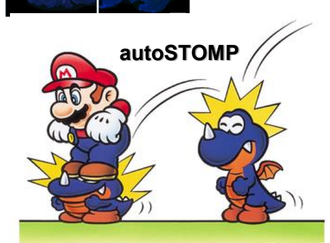

https://tvtropes.org/pmwiki/pmwiki.php/Main/GoombaStomp