

**Membrane Proteomic Analysis of Protozoan
Parasite *Trypanosoma cruzi***

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T. cruzi – Chagas' Disease

T. cruzi is endemic to **Latin America**

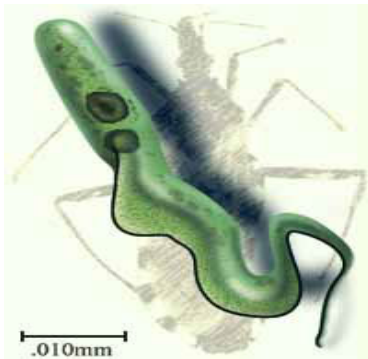
Chagas disease affects 16-18 million people worldwide

100 million are at risk of infection

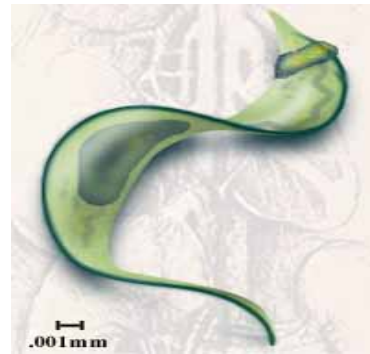


- Leading cause of heart failure
- Current treatment method is highly toxic and exhibits many dangerous side effects
- No effective cure and vaccine

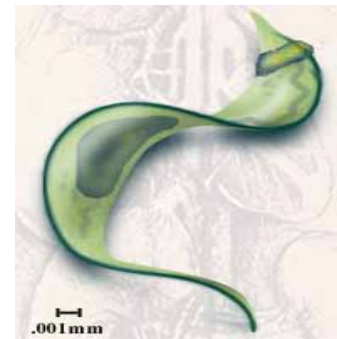
Four Life Cycle Stages of *T. cruzi*



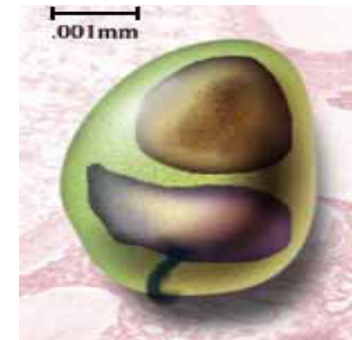
Epimastigote



Trypomastigote



Metacyclic



Amastigote

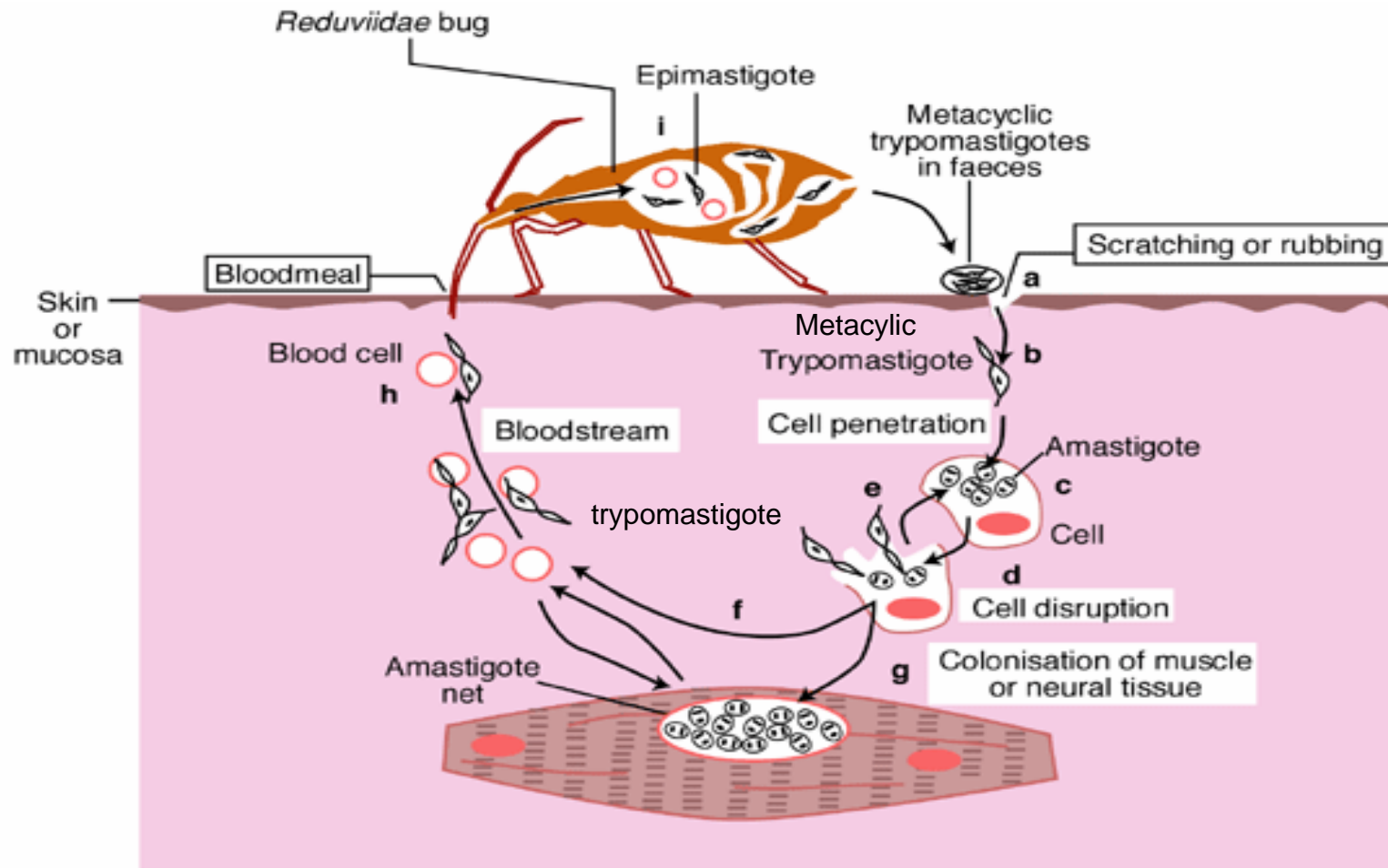
Triatoma infestans



Insect vector for Chagas' disease



Life Cycle of *T. cruzi*



Schematic representation of the life cycle of the flagellate protozoan *Trypanosoma cruzi*

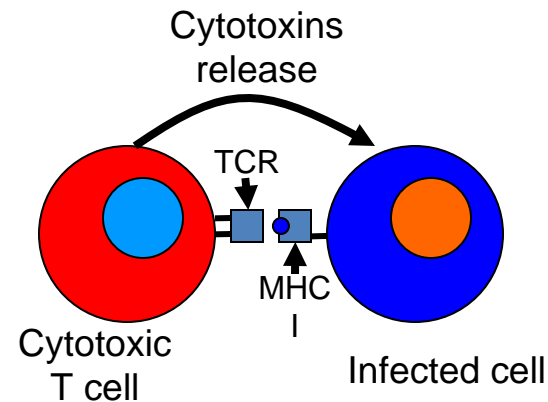
Control of the Infection: Immune Response

- Innate immunity
 - First line of defense (present in all individuals at all times)
 - Immediate (0 – 4 hours)
 - Non-specific
 - Does not generate lasting protective immunity
- Adaptive (acquired) immune response (late: > 96 hours)
 - Is initiated if innate immune response is not adequate (> 4 days)
 - Antigen-specific immunity
 - Generates lasting protective immunity (e.g. Antibodies, memory T-cells)

CD8⁺ T Cell Response

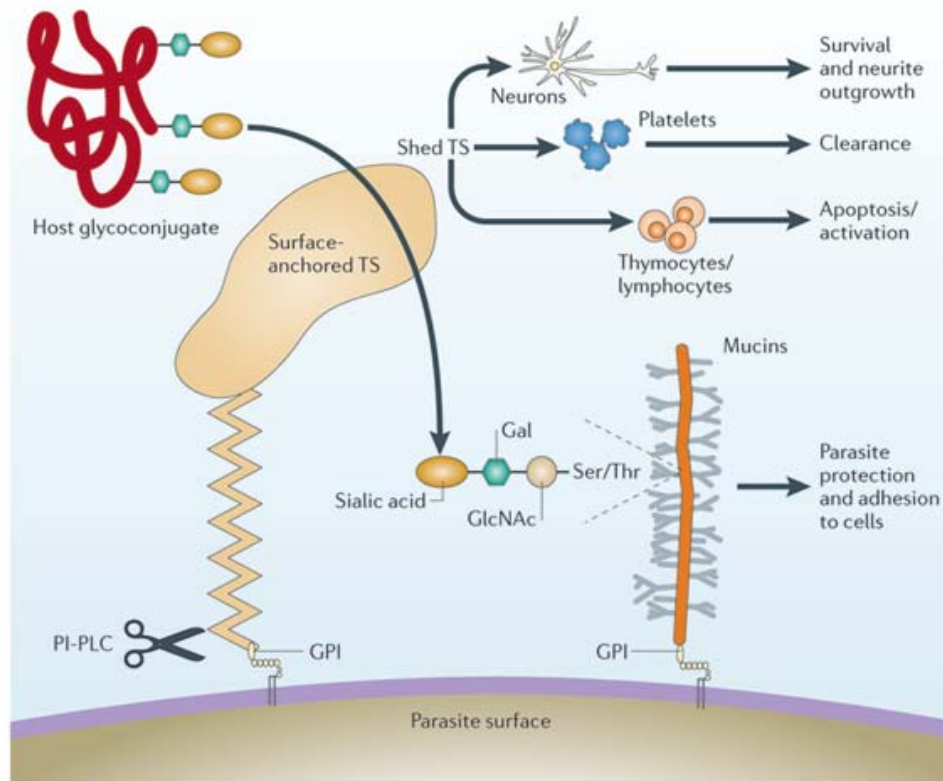
Several parasites including *T. cruzi* all induce strong CD8⁺ T cell responses that are crucial for control of the infections

Cytotoxic T cells (CD8⁺) recognize antigen presented by **MHC I** and kills the cell



Some remained T cells become memory cells and mount strong response if the pathogen is detected again

Membrane Proteomic Analysis of *T. cruzi*



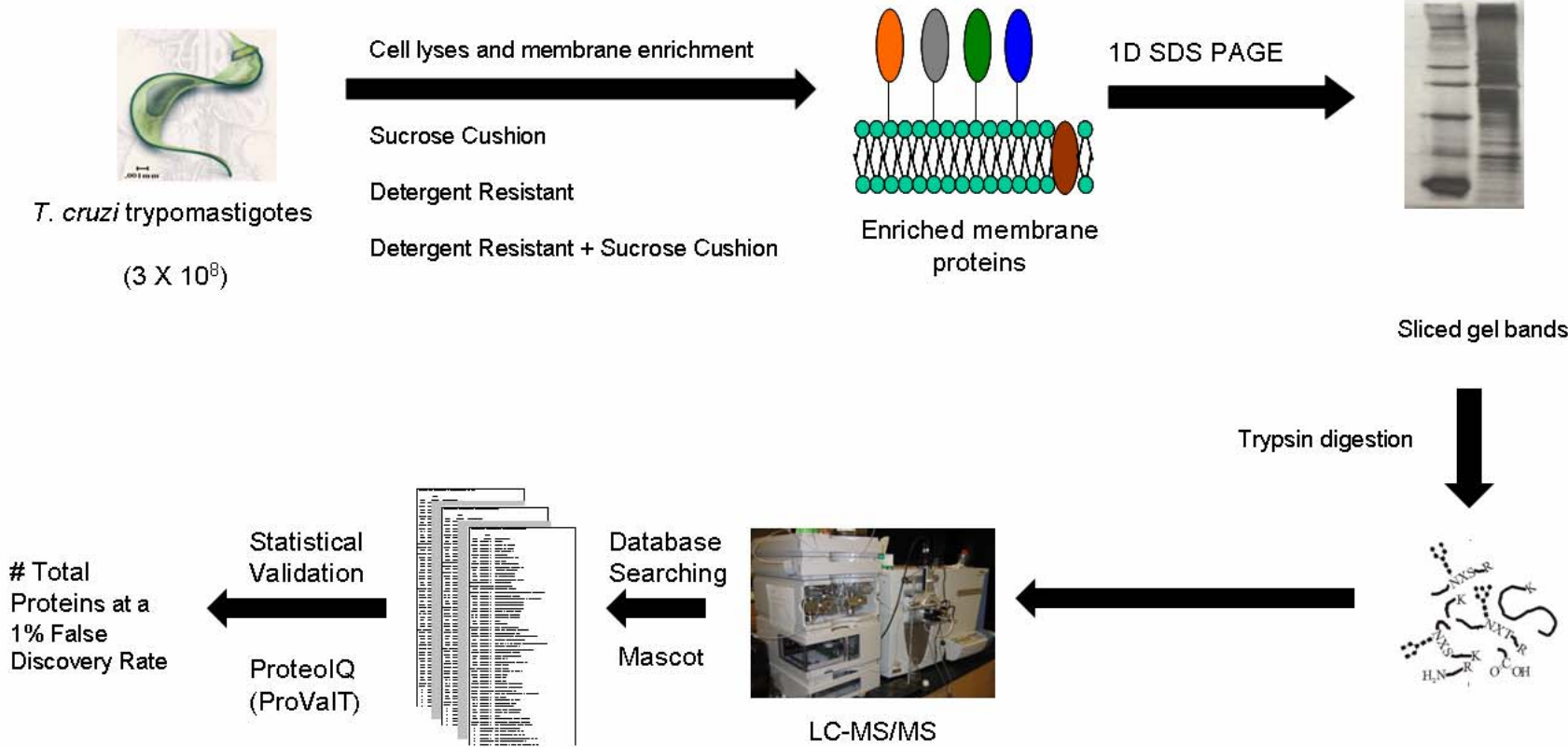
TS and several other membrane proteins are proven to be the predominant targets of CD8⁺ T-cells

Important for parasite survival and represent potentially vaccine target

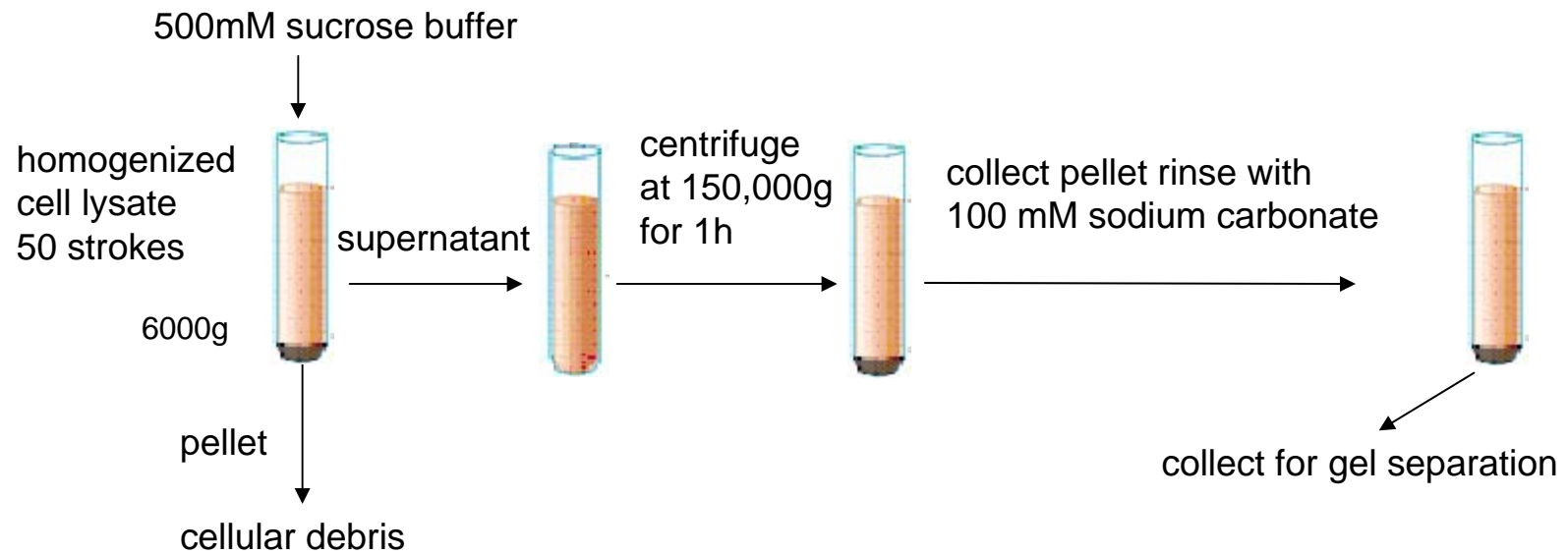
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While these *T. cruzi* cell surface proteins are of critical importance, there have been no proteomic studies specifically targeting them

Methodology for Membrane Proteome Analysis

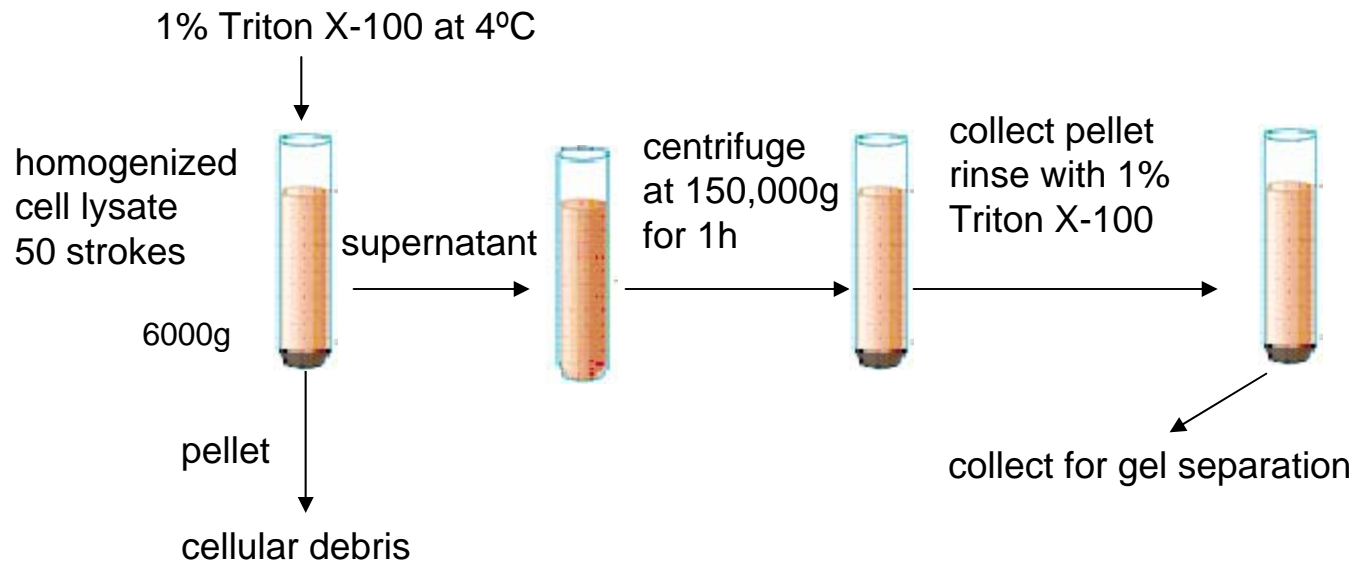


Sucrose Cushion Preparation



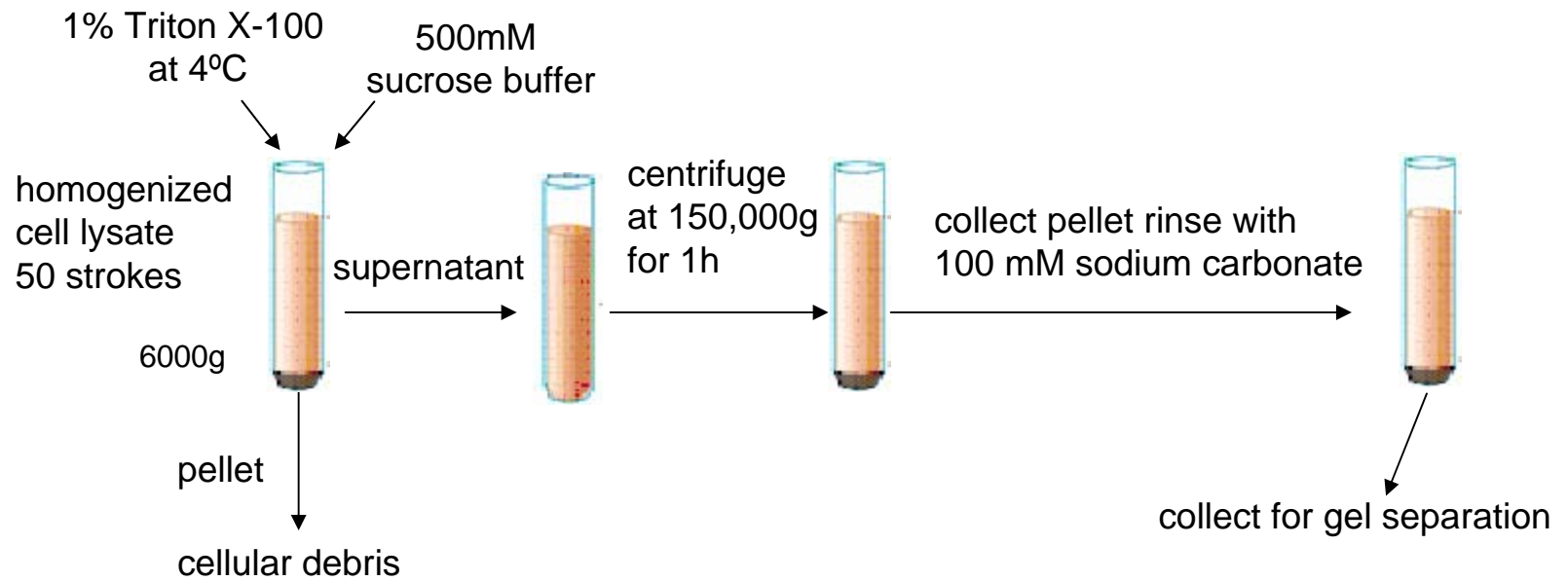
- Points here: 1) highly abundant soluble proteins depleted
2) washing step can remove membrane associated proteins

Detergent Resistant Preparation



- Points here:
- 1) we hope adding triton X-100 can get better lysis
 - 2) enrich the GPI-anchored membrane proteins by removing some other integral membrane protein

Detergent Resistant + Sucrose Cushion



Detergent sucrose combined preparation expected to enrich GPI anchored trans-sialidase

Results and Discussion

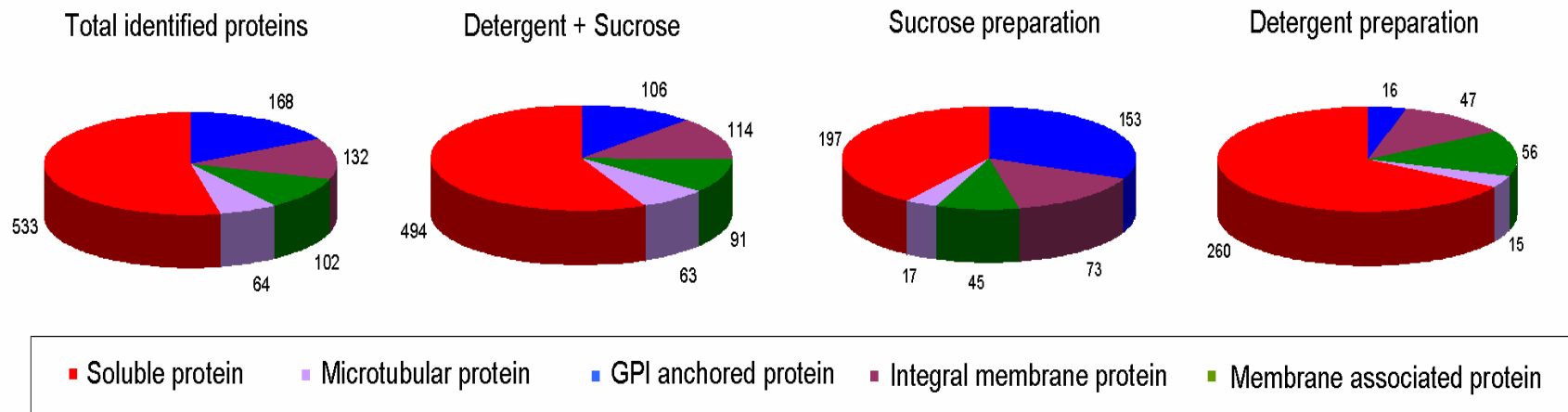
Results	Sucrose Cushion	Detergent Resistant	Detergent + Sucrose
# of spectra	26396	30646	32859
# of unique peptides	2794	3096	4021
# of protein groups	631	609	962
# of proteins	1726	1301	2196

Summary of results from three different membrane preparations. As shown above the detergent + sucrose method generates the highest number of peptides, protein groups, and proteins.

Total 1029 protein groups identified below a protein false discovery rate of 1%.

403 protein groups identified including 145 TS are membrane proteins, almost count for 40% within the whole identification

Categories of All Proteins Identified



Classification of identified protein groups from different preparations, membrane proteins were significantly enriched in sucrose method and detergent + sucrose method.

Major Protein Families

Gene Name	# of proteins identified after membrane enrichment	# of proteins in whole cell analysis	# of proteins in Genome database
→ Trans-sialidase	145	44	1430
→ GP63	6	2	425
→ MASP	13	5	1377
→ Mucins	4	0	758
RHS	39	90	752
→ GlycosylTransferase	9	2	139
ATPase	10	10	99
→ Ribosomal	70	212	525
→ Heat shock/Chaperonins	23	61	94

Major protein group levels in the membrane enrichment preparation compared with a whole cell analysis and the genome annotation. The combined membrane enrichment strategies resulted in a significant enrichment in cell surface and organelle membrane bound protein groups such as trans-sialidases and glycosyl transferases. As shown above the membrane enrichment also reduced the abundance of soluble proteins such as ribosomal and heat shock proteins which were abundant in the whole cell proteome analysis.

Membrane Protein Sub-cellular Localization

GPI anchored plasma membrane proteins

TS

MASP

Mucins

GP 63

Non-GPI anchored plasma membrane proteins

ATPase

cAMP specific phosphodiesterase

receptor-type adenylate cyclase

Mitochondrial

ADP,ATP carrier protein 1, mitochondrial precursor

mitochondrial phosphate transporter

reiske iron-sulfur protein precursor

Prohibitin

ATP synthase

NADH-cytochrome B5 reductase

ER

oligosaccharyl transferase subunit

COP-coated vesicle membrane protein gp25L precursor

reticulon domain protein

endoplasmatic reticulum retrieval protein

Golgi

Golgi SNARE protein-like

Syntaxin

UDP-Gal or UDP-GlcNAc-dependent glycosyltransferase

Nucleus

importin alpha

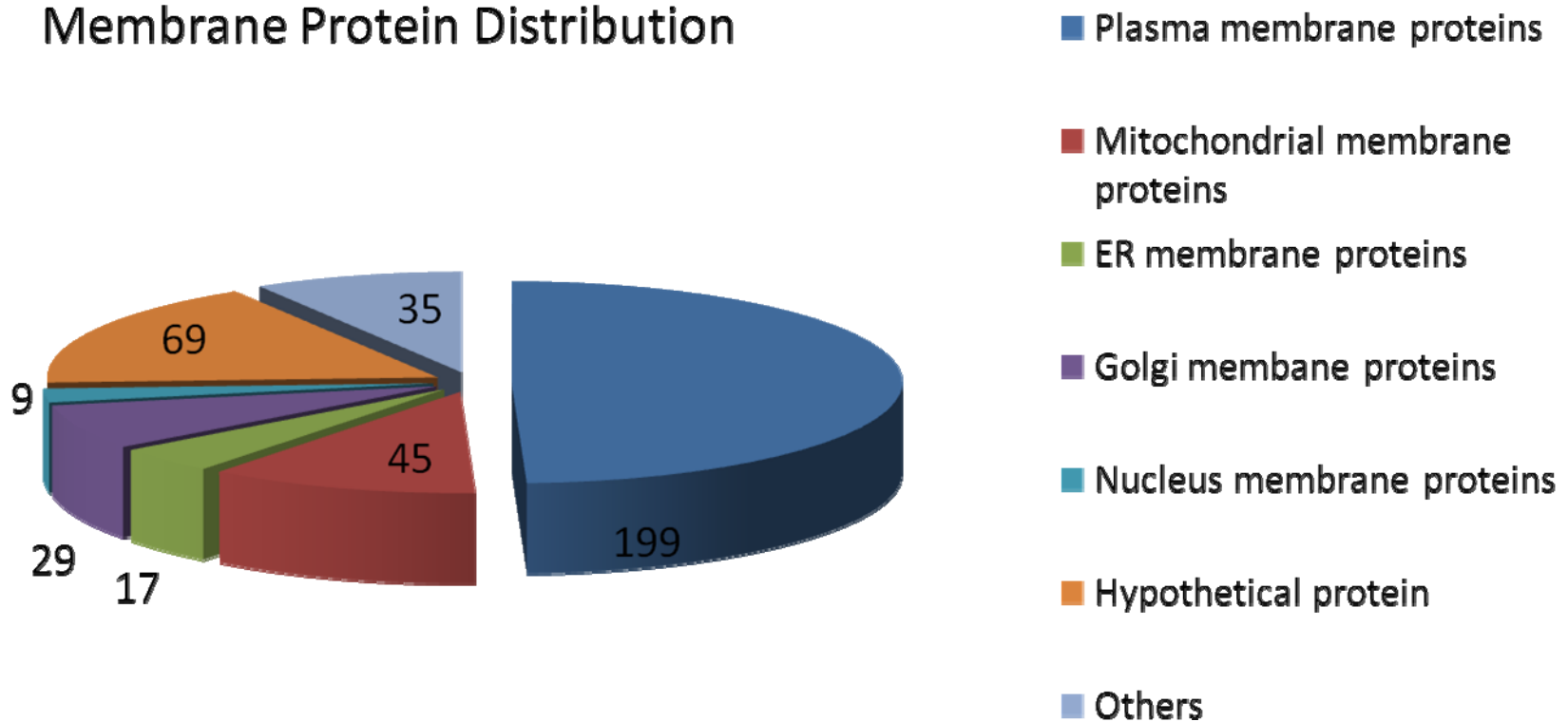
CAS/CSE/importin domain protein

major vault protein

Hypothetical and others

Membrane Protein Distribution Pie Chart

Membrane Protein Distribution



Plasma membrane proteins are most abundant in our preparation

Trans-sialidase Levels at different preparations

Sequence ID	Combined LTQ	Detergent LTQ	Sucrose LTQ
Tc00.1047053508581.10	4	2	3
Tc00.1047053460111.10	2	0	4
Tc00.1047053510483.250	0	0	4
Tc00.1047053508857.30	45	154	28
Tc00.1047053506339.10	4	1	1
Tc00.1047053508903.10	5	2	3
Tc00.1047053511089.80	6	0	2
Tc00.1047053506783.20	1	0	2
Tc00.1047053506001.40	1	0	2
Tc00.1047053508853.20	1	0	4
Tc00.1047053508523.30	14	2	3
Tc00.1047053509333.10	5	1	7
Tc00.1047053507069.160	3	1	3
Tc00.1047053503993.10	22	186	17
Tc00.1047053505025.150	5	0	1
Tc00.1047053508871.10	5	0	1
Tc00.1047053510625.40	3	1	2
Tc00.1047053503907.10	3	0	3
Tc00.1047053509525.20	0	0	3
Tc00.1047053509815.100020	6	1	3
Tc00.1047053505931.30	44	69	29
Tc00.1047053511451.80	0	0	5
Tc00.1047053510137.10	3	0	1
Tc00.1047053508873.330	2	0	2
Tc00.1047053511173.130	1	1	2
Tc00.1047053505949.30	4	0	3
Tc00.1047053506577.80	2	0	2
Tc00.1047053507981.10	3	0	0
Tc00.1047053509979.320	2	0	2
Tc00.1047053510061.30	35	1	4

SPC Range

0	
1 - 76	
77 - 152	
153 - 228	
229 -	

Top 30 trans-sialidase (ts) groups are shown. Cumulative spectral counts display the identified ts proteins among the three preparations. Spectral count abundances are increased in the Detergent + Sucrose method.

Sequence ID	Combined LTQ	Detergent LTQ	Sucrose LTQ
Tc00.1047053511089.80	6	0	0
Tc00.1047053506653.10	0	0	1
Tc00.1047053506961.25	3	0	3
Tc00.1047053511757.100	0	0	1
Tc00.1047053509333.10	5	0	5
Tc00.1047053509821.20	3	1	1
Tc00.1047053506245.100060	116	245	7
Tc00.1047053508523.30	0	0	1
Tc00.1047053507859.100080	0	0	3
Tc00.1047053506841.20	4	0	14
Tc00.1047053509539.10	9	11	2
Tc00.1047053503427.9	1	1	3
Tc00.1047053509419.70	0	0	5
Tc00.1047053401961.10	0	0	2
Tc00.1047053503759.10	0	0	2
Tc00.1047053511875.20	0	0	1
Tc00.1047053508045.120	2	0	2
Tc00.1047053504115.10	3	0	1
Tc00.1047053511855.10	1	0	0
Tc00.1047053503533.30	0	0	2
Tc00.1047053509753.270	0	0	2
Tc00.1047053504333.20	0	0	1
Tc00.1047053506813.140	0	0	1
Tc00.1047053507479.20	0	1	2
Tc00.1047053509817.50	0	2	2
Tc00.1047053510363.60	0	0	1
Tc00.1047053470827.20	5	0	5
Tc00.1047053509959.60	0	0	2
Tc00.1047053508375.20	0	0	1
Tc00.1047053508501.320	0	0	1

SPC Range

0	
1 - 62	
63 - 124	
125 - 186	
187 -	

Top 30 unique trans-sialidase (ts) groups are shown. Cumulative spectra counts display the identified ts proteins among the three preparations. The Sucrose method results in an increased number of unique ts identifications.

Uniquely Identify Large Gene Family Members

Trans-sialidase Tc00.1047053508061.154 was uniquely identified

Tc00.1047053508061.154 Unique Peptide

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MLSRVAAVQAPRTHNRRRCVTGSSGRRREGRESERQRP  
NMSRRVFASAVLLLLLVMMICCTGEAAVAGGSKTRDAINP  
FTGTTR DATWKDVEINTESASLR VPSLVEVQGHFAVSE  
ARCGEKNAGAGSCPRILSKHLDISGY.....STTAIEGREVM  
LITAPVYAKEENGKGR LHLWVTDNAR VYDVGLVSREND  
AAASSLLMRDNNK ELISLYENK SDGAYNLVAVRLTEKLVR  
IKEVVKTWKDL.....KWLAVSEGD*
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Tc00.1047053509539.10

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SLLLEDAHEEGNWD RARIATAHSNGSG  
GTNSTHTRTHNRCGVTGSSGRRMEG  
RESERQRPNMSRRVSTFTVLLLLLVFL  
MICC.....SKITSLRISGLVKVGDDVFAV  
AESQCGEGTEPXSCAGIVSKHLNISDD  
PLDISTSDTSLFDKARVYDVGXRPREN  
DDAAASSLLIKDKNK ELISLYENK KSDG  
SYNLVA.....LYNEKLLSDNL*
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Tc00.1047053506813.140

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MLSRVAAVMAPRTHNRRRV TGSRRRREGR  
ESEPERNM.....VEWGENEGDERPFMMAR  
CAGGYDVYRSTSHGVS WYDLGEPINRVWG  
NSRDRAGDGVKSGE TTAIEGKKVMLITAPVY  
PKDNGKGR LHLWVTDNAR VHDVGPISREDD  
DSAASSLLKSKDEL VLLYEKRNGDGPYSPVA  
MRLTEQLERIKSAWKDESGVTLKGSEAKWPV  
GDMGQTPVYYFANNKFTLSATVS.....LDDRA  
RVRVSCVLLLLLGLCALVAVL*
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Conclusions

- Three different strategies were successfully used to enrich and identify the membrane proteins of *T. cruzi*
- To date this is the most comprehensive membrane proteome reported for *T. cruzi*
- Future study on these membrane proteins especially the trans-sialidases would be important for the development therapeutics for the treatment of Chagas' disease.

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All Our Group Members

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GPI-Anchored Protein

