



Functional mapping of the *Trypanosoma cruzi* serinome by fluorophosphonate activity-based protein profiling[☆]

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ABSTRACT

Serine hydrolases (SHs) constitute one of the largest enzyme superfamilies in eukaryotes, yet their roles in *Trypanosoma cruzi*, the causative agent of Chagas disease, remain largely uncharacterized. Here, we report an activity-based chemoproteomic map of the *T. cruzi* epimastigote serinome by combining genome-informed *in silico* curation with whole-cell activity-based protein profiling (ABPP) using a panel of cell-permeable fluorophosphonate (FP)-alkyne probes. Whole-cell labelling followed by label-free quantitative proteomics (LFQ-MS) identified 37 enriched SH-like proteins, including 35 with conserved or partially conserved catalytic triad/dyad features, spanning lipases, peptidases, esterases, and previously uncharacterized hydrolases. The 35 SHs represent approximately 63% of the 56 predicted SHs retained after catalytic-site curation. Domain architecture analysis revealed broad structural diversity, while orthologue-based localization data suggested association with multiple subcellular compartments, including glycosomal, mitochondrial, and endosomal localizations. Gene Ontology enrichment highlighted lipid metabolic and catabolic processes as dominant functional themes, and protein-protein interaction network analysis supported functional connectivity among the captured enzymes. Several identified SHs, including oligopeptidase B, prolyl oligopeptidase Tc80, serine carboxypeptidase CPB1, and phospholipase A1 (PLA1) have previously been characterized in trypanosomatids, with roles linked to parasite virulence or host-pathogen interactions. Together, these findings establish a fluorophosphonate-based chemoproteomic resource for the kinetoplastid community and prioritize probe-accessible active *T. cruzi* SHs for future functional validation and antiparasitic inhibitor discovery.

1. Introduction

Chagas disease, caused by the protozoan parasite *T. cruzi*, is a life-threatening neglected tropical disease (NTD) endemic to Latin America and increasingly recognized as a global health concern due to migration and climate change. It is estimated that between 6 and 8 million people are currently infected, with more than 75 million people considered at risk of infection across 21 endemic countries.^{1,2} Transmission occurs primarily through contact with the faeces of triatomine insects, but can also occur congenitally, via blood transfusion, organ transplantation, or orally through contaminated food or drink.^{2,3} The

disease progresses from an often-asymptomatic acute phase to a chronic phase, which may lead to irreversible cardiac, gastrointestinal, and neurological complications. If left untreated, chronic Chagas disease can result in severe disability and death, as evidenced by approximately 10,000 deaths attributed to the disease each year.^{1,4}

Despite its significant disease burden, Chagas disease remains severely underdiagnosed and undertreated. Current therapeutic options are limited to two nitro-heterocyclic drugs, benznidazole and nifurtimox, both developed over 50 years ago. These drugs are primarily effective during the acute phase, show limited efficacy in chronic infections, and are associated with substantial side effects and treatment

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discontinuation rates.^{5,6} Moreover, no vaccine is currently available. These limitations underscore the urgent need to identify new drug targets and therapeutic strategies, in line with the World Health Organization's 2021–2030 roadmap for NTDs.⁷

The serine hydrolase (SH) superfamily constitutes one of the largest and most functionally diverse classes of enzymes, encompassing proteases, esterases, and lipases. In *T. cruzi*, proteases represent key virulence factors involved in immune evasion, nutrient acquisition, and host tissue invasion.^{8,9} The cysteine protease family has been extensively studied in *T. cruzi*, identifying vital proteases such as cruzipain, involved in cell differentiation, metabolism, invasion of the host cell and evasion of the immune response.^{10–14} In contrast, comparatively little is known about the role of SHs in *T. cruzi* biology. In other protozoan parasites such as *Leishmania* spp., SHs have been shown to play pivotal roles in virulence, stage differentiation and host-pathogen interactions.^{15–20} These findings suggest that SHs may also be critical to the *T. cruzi* life cycle and pathogenicity and could offer underexplored opportunities for therapeutic intervention.

Although omics approaches provide valuable information on transcript or protein abundance, abundance does not necessarily correlate with functional activity. Functional annotation of enzyme families such as SHs therefore requires approaches that give insights into enzyme activity. In this context, activity-based protein profiling (ABPP) has emerged as a powerful chemoproteomics technique for interrogating enzyme function in complex biological systems. ABPP utilizes mechanism-based probes that covalently bind to active-site residues, enabling the selective detection of active enzymes within native proteomes and distinguishing them from inactive or zymogen forms where applicable.^{21–23} This technology has been particularly transformative for the functional annotation of serine hydrolases, with fluorophosphonate (FP) probes providing a robust platform to interrogate catalytically active members of this enzyme family in complex proteomes.^{17,24,25} Structural modifications to the FP scaffold can further enhance probe selectivity and cell permeability, providing valuable tools for enzyme profiling in both host and pathogen contexts.

We have recently described the development and application of a series of cell-permeable fluorophosphonate (FP)-based probes to characterize the active serinome of *Leishmania* spp. These proved effective in comparing serinomes across different *Leishmania* species and life-cycle stages, including the disease-critical intramacrophage amastigote, providing insights into the host–pathogen interactome. Here, we further demonstrate their effectiveness for functional profiling kinetoplastid parasites,¹⁷ applying the probes to profile the active serinome of *T. cruzi*.

To our knowledge, the active serine hydrolases of *T. cruzi* have not previously been profiled directly. Here we provide a functional analysis of SHs in *T. cruzi*, combining ABPP with genome-wide *in silico* curation to establish a valuable activity-based chemoproteomic resource for the *T. cruzi* serinome that identifies a prioritized set of catalytically active SHs for future functional validation and inhibitor discovery.

2. Results

2.1. *In silico* prediction of the *T. cruzi* serinome

To establish a comprehensive reference framework for the *T. cruzi* serinome, we first performed a genome-wide *in silico* survey of predicted SHs using the Dm28c 2018 genome reported by Berná et al.²⁶ (Fig. 1). A total of 135 proteins were identified as putative SHs, characterized by the presence of serine hydrolase domains annotated in Pfam, InterPro, CDD, or Panther, or by alignment to the peptidase database MEROPS. Following clustering to remove redundancy and discarding protein fragments, 73 unique proteins remained as putative SHs (Table 1 and Supplementary Data 1). These were classified as esterases, serine peptidases, amidases, or unclassified SHs according to the identified domains or additional information shown in Supplementary Data 1.

To refine this candidate set, we assessed whether each protein possessed catalytic features consistent with serine hydrolase activity. Sequence alignments against reference proteins with known catalytic residues were combined with inspection of AlphaFold-predicted structures to determine whether catalytic residues were positioned appropriately in three-dimensional space (Supplementary Fig. S1). For canonical SHs, we assessed the presence of an S-H-D/E catalytic triad, with the nucleophilic serine typically located within a conserved GxSxG motif. Catalytic geometry was scored as conserved when the Ser O_γ–His N_{ε2} and His–Asp/Glu interatomic distances were within 5 Å in the AlphaFold model and the participating residues had an average pLDDT ≥80, with selected borderline cases accepted when pLDDT values were between 60 and 79. For amidase-signature enzymes a Ser–Ser–Lys constellation was required, and for rhomboids a Ser–His dyad. Borderline cases were classified as partially conserved, with per-protein metrics reported in Supplementary Data 1. Representative active-site structures are shown in Fig. 1b. Ultimately, of the 73 putative SHs, 56 gene products were retained as SH candidates based on conserved or partially conserved catalytic triad/dyad features in *T. cruzi* strain Dm28c (Supplementary Fig. S1).

Of the 56 total genes identified, the majority encoded esterases (29

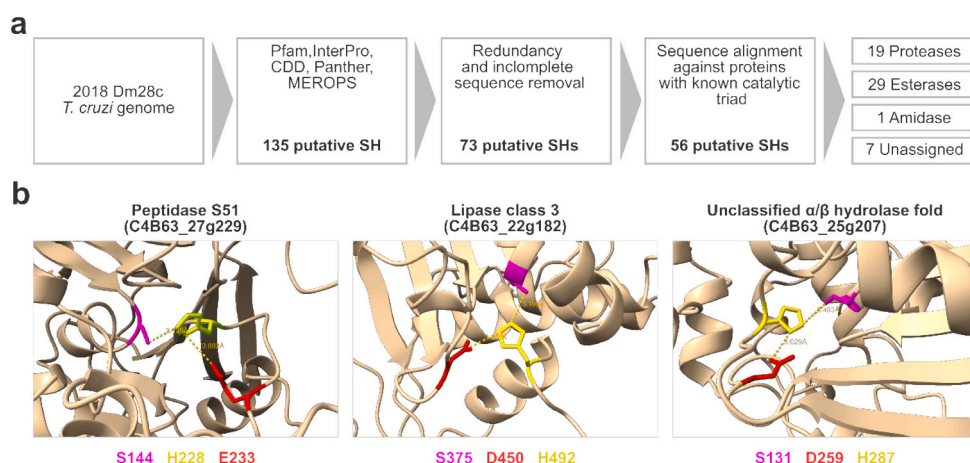


Fig. 1. (a) *In silico* serinome workflow. (b) Close-up view of the active site showing the spatial arrangement of the catalytic S–H–D/E triad in representative proteins from the full dataset (Peptidase S51, Lipase class 3, and an unclassified hydrolase). The pLDDT values are 98.25 (S144), 97.88 (H228), and 89.19 (E233) for Peptidase S51; 86.44 (S375), 63.22 (H492), and 69.44 (D450) for Lipase class 3; and 91.12 (S131), 89.88 (H287), and 89.88 (D259) for the unclassified hydrolase. Residue numbers are indicated in the colour-coded legend, and dashed lines denote interatomic distances (Å). AlphaFold-predicted structures (AF-A0A2V2VFP5-F1-model_v6, AF-A0A2V2UKM7-F1-model_v6, and AF-A0A2V2VHL2-F1-model_v6) were obtained from the AlphaFold Protein Structure Database.

Table 1

Serine Hydrolases identified *in silico* in the *T. cruzi* genome. We identified 73 accession numbers that correspond to putative SHs and assessed each sequence for the presence and conservation of catalytic triad or dyad with the nucleophilic serine. The table shows the list of accession numbers classified as esterase, serine peptidase, amidases, or unclassified SHs according to Pfam, product description, MEROPS classification, other identified domains, or catalytic triad (Supplementary Data 1). The number of copies is shown in parentheses after the accession number for any value greater than one. A single representative accession number is reported for multi-copy genes. 56 out of the 73 genes were selected based on the presence of conserved or partially conserved catalytic triad.

Activity Classification		Catalytic Triad/Dyad	
<i>Fold</i>	Family or Classification	Conserved	Not conserved
Serine peptidases			
<i>β-barrel core fold</i>	S1	C4B63_23g231 C4B63_9g401	C4B63_17g44 C4B63_75g55 C4B63_48g166
	S26	C4B63_31g105 C4B63_13g150	
<i>Subtilisin-type 3-layer α/β sandwich fold</i>	S8	C4B63_10g296 C4B63_95g13	
<i>β-propeller fold and α/β hydrolase fold</i>	S9	C4B63_101g15 C4B63_63g37 C4B63_6g564 C4B63_122g16	
	S10 S15	C4B63_168g38 (12) C4B63_6g235 (3)	
<i>α/β hydrolase fold</i>	S28	C4B63_57g112 C4B63_17g176*	
	S33	C4B63_2g814 C4B63_84g83 C4B63_2g162	
<i>trpG-type fold</i>	S51	C4B63_27g229	
<i>rhomboid fold</i>	S54	C4B63_34g300	C4B63_38g78
<i>GAIN fold</i>	S59		C4B63_19g195
Amidases			
<i>Amidase signature fold</i>	Fatty-acid amide hydrolase (FAAH)	C4B63_55g66	
Esterases			
<i>Patatin fold</i>	Patatin-like Lipase	C4B63_18g341 C4B63_7g246	C4B63_95g29 C4B63_7g337
<i>SGNH hydrolase fold</i>	GDSL Lipase	C4B63_184g21 (3) C4B63_13g128 C4B63_25g256 C4B63_22g182 (2) C4B63_104g97 (17) C4B63_10g143 C4B63_171g8 (2) C4B63_10g145 C4B63_20g182 C4B63_4g367 C4B63_20g60 C4B63_10g276 (2)*	
	Lipase class 3		C4B63_2g44
<i>α/β hydrolase fold</i>	Phospholipid: diacylglycerol acyltransferase (PDAT)	C4B63_5g420 (2)	
	Phospholipase A	C4B63_2g791	C4B63_2g790
	GPI inositol-deacylase PGAP1	C4B63_23g274 C4B63_2g218*	
	LID Hydrolase	C4B63_8g548	
	Phospholipase/Carboxylesterase	C4B63_32g268 C4B63_14g203	
	Bifunctional carbohydrate esterase (CE)	C4B63_83g76	

Table 1 (continued)

Activity Classification	Catalytic Triad/Dyad	
Unclassified	C4B63_44g168 (3)	
	C4B63_232g4 (2)	
	C4B63_46g85	
	C4B63_17g73	
	C4B63_6g260 C4B63_35g372*	
Unclassified	TCDM_11513(5)	
	C4B63_20g152	C4B63_25g205
α/β-Hydrolase fold	C4B63_42g215	C4B63_14g216
	C4B63_60g130	C4B63_7g399
	C4B63_12g197	C4B63_14g209
	C4B63_25g206	C4B63_44g234
	C4B63_25g207	C4B63_20g186
	C4B63_48g32 (2)	C4B63_24g264 (2)
		C4B63_25g137 (2)

* The catalytic triad is partially conserved.

genes), followed by serine peptidases (19 genes), unclassified SHs (7 genes), and one amidase. Most serine peptidase families (S1, S8, S9, S26, S28, S33, S51, and S54) contained one to four candidate genes, with each gene generally present as a single copy in the genome. Notably, peptidase S15 and S10 showed copy-number expansion, with three and twelve predicted copies respectively. Among the esterases, most classes were represented by only 1–2 genes or copies. In contrast, GDSL lipases and lipase class 3 showed notably higher representation. For example, GDSL lipase C4B63_184g21 had 3 copies, while lipase class 3 comprised 10 unique genes, three of which had 2 copies each, and C4B63_104g97 alone accounted for 17 copies (Table 1 and Supplementary Data 1).

2.2. Chemoproteomic profiling of serine hydrolases

Bioinformatics-driven approaches have become indispensable for predicting protein function, pathway integration, and druggability potential. Comparative genomics, sequence-based classification, structural modelling, and machine learning algorithms enable the identification of conserved domains, essentiality, and active site features that help prioritize enzymes for further biological characterization and therapeutic exploration. Databases such as TDR Targets and TriTrypDB further integrate functional genomics with chemical biology information, facilitating target prioritization based on genetic essentiality, lack of human homologues, and druggability indices.^{27,28} For enzyme families such as the SHs, this computational framework is invaluable for distinguishing catalytically active members, identifying non-canonical variants, and informing probe or inhibitor design. However, these methods do not report on activity, a particular limitation for SHs, as many proteases are initially produced as zymogens and only activated in response to specific signals. This provides a strong rationale for ABPP, which can identify catalytically active enzymes directly in a biological context.

We therefore used a panel of cell-permeable FP probes to experimentally profile the active SH landscape of *T. cruzi*. In previous work we had reported the synthesis of a set of alkyl-, benzyl- and aryl fluorophosphonate activity-based probes (ABPs) (Fig. 2) and their application to examine SHs in *Leishmania*.^{17,29} Initial studies explored the use of these probes with *T. cruzi* cell lysate. These extracts were prone to agglutination, likely associated with the complex *T. cruzi* surface glycocalyx,^{30–34} which manifested as poorly resolved, aggregated material on SDS-PAGE. Although this could be partially mitigated, poor labelling was observed, suggesting that agglutination was not the sole barrier and that access of the ABPs to their targets in the lysate may also have been limited (Supplementary Fig. S2). Consequently, exploiting the cell-permeable nature of the probes, a whole-cell labelling approach was adopted. Importantly, as demonstrated in our previous studies,¹⁷ this minimizes target loss associated with lysis.

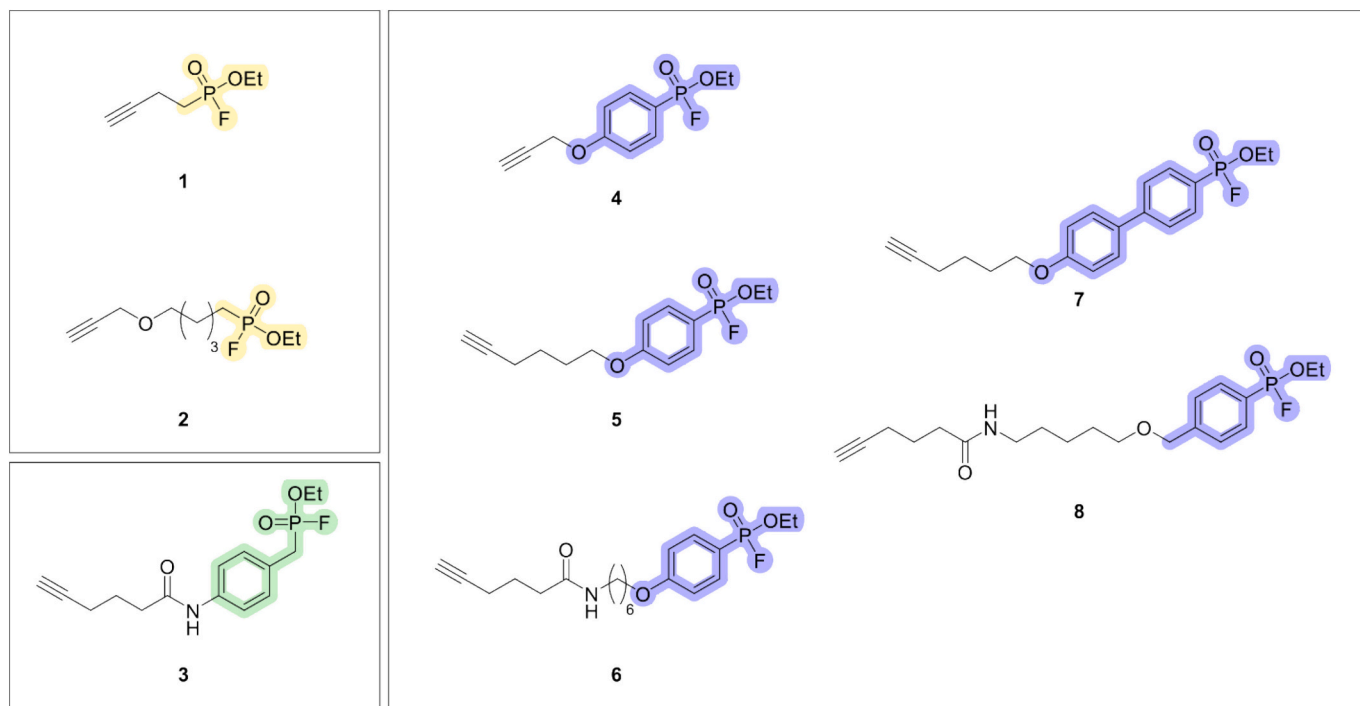


Fig. 2. Structures of the cell-permeable activity-based fluorophosphonate (FP-alkyne) probes used in this study: alkyl-FPs (yellow), benzyl-FP (green), and aryl-FPs (purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

To identify probe-labelled SHs in *T. cruzi*, we employed a label-free quantitative proteomics workflow. After optimisation of the lysis conditions, live parasites were incubated with FP-alkyne probes, followed by cell lysis, click chemistry with biotin-N₃, streptavidin enrichment, reduction, alkylation, digestion, and LC-MS/MS analysis. Three biological replicates were analysed per condition. Differential protein abundance between probe-treated and control samples was assessed using empirical Bayes moderated *t*-tests implemented in the limma package.³⁵ Proteins with a log₂ fold-change >1 and Benjamini-Hochberg-adjusted *p*-values <0.05 were considered significantly enriched (Supplementary Data 2–3).

2.3. Genome assembly influences serine hydrolase identification

Accurate identification of the complete repertoire of enriched serine hydrolases in *T. cruzi* depends on the quality and completeness of the reference genome used for protein annotation. However, the availability of high-quality whole-genome assemblies for *T. cruzi* remains limited due to intrinsic features of the parasite genome that complicate assembly, particularly when using short-read sequencing technologies. These include extensive repetitive sequences, a high abundance of transposable elements, large multigene families such as mucins and trans-sialidases,³⁶ and substantial variability in genome size and chromosome number across strains.³⁷ In addition, frequent aneuploidy and polyploidy, limited synteny, and hybridization events result in highly heterozygous genomes, with early short-read assemblies often failing to fully capture the multiplicity of gene copies present.³⁸ Consequently, analyses based on a single genome assembly may overlook or misrepresent members of expanded serine hydrolase families.

To maximize genome coverage, analyses were performed against both the short-read 2014 Dm28c genome³⁸ (Supplementary Data 2) and the improved long-read-based Dm28c genome assembly reported by Berná et al.²⁶ (Supplementary Data 3). The combined analysis yielded a final set of 36 non-redundant enriched SH-like proteins. To avoid discarding genuine hydrolases in this discovery-oriented workflow, we also checked for SHs without applying a BH *p*-adj threshold, but instead, a

traditional *p*-value <0.05 cut off. With this setup, an additional SH, serine aminopeptidase C4B63.2g814 was identified. Given that this protein was enriched with 2 different ABPs (2 and 7), it was included in the final list. Overall, the 37 identified enzymes comprised 20 predicted esterases (lipases, phospholipases, deacetylases, acyltransferases), 10 serine peptidases, 1 amidase, and 6 hydrolases of unknown function (Table 2). All enriched proteins were supported by *in silico* annotation analysis.

The long-read assembly additionally revealed multiple copies of several enzymes. This is consistent with the known expansion of multigene families in *T. cruzi* such as trans-sialidases, mucins, and mucin-associated surface proteins (MASPs).^{26,39,40} For example, lipase C4B63.104g97 showed the highest copy number with 17 copies, followed by carboxypeptidase CPB1 (C4B63.168g38) with 12 copies. Annotation differences were also observed between assembly versions; for instance, prolyl endopeptidase BCY84.07469 is represented as a full-length sequence in the 2017 assembly but is split into multiple fragments in the 2018 version.

2.4. Probe selectivity and serinome coverage

Having established the ABPP-enriched serinome, we next evaluated the performance of the FP-alkyne probe panel. With the exception of alkyl probe 1, which enriched only 3 SHs, all probes displayed broad labelling profiles, enriching more than ten SHs under the conditions tested. The lower labelling efficiency observed with probe 1 is consistent with our previous observations in *L. mexicana*,¹⁷ and may be attributable to its shorter linker, which can reduce the accessibility and solvent exposure of the alkyne handle by promoting its collapse into hydrophobic protein pockets, thereby limiting the efficiency of the subsequent click reaction. Consistently high log₂ fold changes (≈1–7.5; mean ≈ 3.6), together with elevated -log₁₀ *p*-values in the volcano plot analysis, indicate efficient probe-dependent enrichment (Fig. 3a–b and Supplementary Fig. S3). Probe 7 enriched the highest number of SHs, with 30 targets detected. Probe specificity, defined as the proportion of enriched SHs relative to the total enriched proteins per condition, ranged from

Table 2

Serine hydrolases identified in *T. cruzi*. Using Dm28c 2018 and 2014 genome assemblies, 35 and 33 SHs were identified respectively, for a total of 37 non-redundant proteins. Proteins identified in only 1 assembly are depicted in green and proteins not enriched or absent from a given assembly are shown in orange. The number of copies is shown in parentheses after the accession number, if the copy number is higher than one. Multiple accession numbers per entry indicate that the same gene has been annotated as fragments.

Dm28c 2018	Dm28c 2014	Dm28c 2017	Description	MW (kDa)
C4B63_63g37		BCY84_07469	Prolyl endopeptidase	51.279
C4B63_63g36	TCDM_05965			18.525
C4B63_25g206	TCDM_05110	BCY84_16826	Putative hydrolase, alpha/beta fold family	34.589
C4B63_168g38 (12)	TCDM_09013	BCY84_11480 (11)	Carboxypeptidase (CBP1)	51.760
C4B63_17g73	TCDM_04400	BCY84_18424	Protein phosphatase methylesterase 1 (PPM1)	36.410
C4B63_55g66	TCDM_03869	BCY84_15993	Fatty-acid amide hydrolase 2	65.844
C4B63_12g197	TCDM_00679	BCY84_13025	AB hydrolase-1 domain-containing protein	53.086
C4B63_2g791	TCDM_00198	BCY84_14016	Phospholipase A1 (1-alkyl-2-acetyl-glycerophosphocholine esterase)	46.005
C4B63_32g268	TCDM_07218	BCY84_05056	Lysophospholipase	30.224
C4B63_232g4 (2)	TCDM_05180	BCY84_16779	AB hydrolase-1 domain-containing protein	46.862
C4B63_4g367	TCDM_09444	BCY84_18691	sn-1-specific diacylglycerol lipase	80.454
C4B63_101g15	TCDM_08288	BCY84_22829	Dipeptidyl-peptidase 8-like serine peptidase (DPP-8), Clan SC, Family S9B	93.116
C4B63_44g168 (3)	TCDM_04835	BCY84_18136 (8)	Monoglyceride lipase	35.031
C4B63_84g83	TCDM_06771	BCY84_10959	Serine peptidase	31.122
C4B63_104g97 (17)	TCDM_10538	BCY84_00133 (18)	Lipase	38.792
C4B63_2g162	TCDM_00084	BCY84_14_177	Bem46-like serine peptidase	40.253
C4B63_122g16	TCDM_08245	BCY84_04721	Prolyl Oligopeptidase B	81.145
C4B63_5g420 (2)	TCDM_05768	BCY84_06811	Phospholipid:diacylglycerol acyltransferase	73.593
C4B63_25g207	TCDM_05114	BCY84_16825	Hydrolase-like protein	47.574
C4B63_20g182	TCDM_02695	BCY84_01525	sn-1-specific diacylglycerol lipase (Lipase Class 3)	57.693
C4B63_14g203	TCDM_02873	BCY84_11164	Feruloyl esterase B (CDC16)	42.750
C4B63_171g8 (2)	TCDM_11162	BCY84_03684 (2)	Triglyceride lipase	66.137
C4B63_2g218				38.319
C4B63_2g219	TCDM_00038 TCDM_00039	BCY84_14391	PGAP1-like protein/Alpha/beta hydrolase family	83.144
C4B63_2g217				20.818
C4B63_6g564	TCDM_09281	BCY84_02649	Oligopeptidase B-like protein	104.325
C4B63_10g142* C4B63_10g143	TCDM_04298	BCY84_15097	Lipase	90.583
C4B63_34g300	TCDM_06028	BCY84_16596	Peptidase S54 rhomboid domain-containing protein	30.729
C4B63_25g255* C4B63_25g256	TCDM_05175 TCDM_05176	BCY84_16783	Fungal lipase-like domain-containing protein	63.337
C4B63_10g276 (2)	TCDM_06491	BCY84_15181	Fungal lipase-like domain-containing protein	50.574
C4B63_60g130	TCDM_09808	BCY84_10535	AB hydrolase-1 domain-containing protein	54.133
C4B63_48g32 (2)	TCDM_04898	BCY84_10876	Alpha/beta hydrolase family	40.008
C4B63_20g186 C4B63_20g187*	TCDM_02700	BCY84_01521	Abhydrolase_1 Alpha/beta hydrolase fold-1	30.408
C4B63_57g112	TCDM_04809	BCY84_06130	serine carboxypeptidase S28	53.882
C4B63_2g814	TCDM_00223	BCY84_13992	Serine aminopeptidase S33 domain-containing protein (**)	44.714
C4B63_95g29	TCDM_06899	BCY84_17620	Lipase - Patatin-like phospholipase (PNPLA like)	36.338
C4B63_184g21 (3)	Not present	BCY84_02952 (2)	Esterase	28.379
C4B63_7g246	TCDM_02784	BCY84_20648	Patatin-like phospholipase domain-containing protein 3	47.165
Not present	TCDM_11513	BCY84_22614 (6)	Monoglyceride lipase (MAGL)	41.278
C4B63_46g85	TCDM_08684	BCY84_13698	AB hydrolase-1 domain-containing protein	47.452

* These accession numbers encode part of the gene but they were not detected by chemoproteomics assay.

** This SH was enriched with $\log_2FC > 1.3$, p -value of 0.03 and BH p -adj value of 0.09.

10% (probe 1) to 42% (probe 7), indicating variable but substantial SH enrichment across the probe series (Fig. 3 and Supplementary Fig. S3).

To determine whether probe enrichment was biased toward highly abundant proteins, intensity-based absolute quantification (iBAQ) values of enriched proteins were compared with genome-wide abundance distributions in *T. cruzi*. Enriched proteins spanned a broad abundance range, including highly abundant enzymes such as serine carboxypeptidase CPB1 (C4B63_168g38/TCDM_09013) as well as lower-abundance proteins such as prolyl oligopeptidase (C4B63_6g564/TCDM_09281). These results suggest that probe enrichment is not restricted to the most abundant proteins and support the ability of FP-alkyne probes to capture SHs across a wide dynamic range of protein expression (Fig. 3b).

2.5. Pfam domain architecture analysis reveals a diverse repertoire of serine hydrolases in *T. cruzi*

To characterize the enriched SHs, we performed a systematic analysis of predicted protein domain architectures using Pfam annotations, MEROPS classification, conservation across trypanosomatids, and orthologue-based localization data from TrypTagDB (Fig. 4 and Supplementary Data 4). Among the serine peptidase families, members of the S9 family contained either Peptidase_S9_N or DPPIV_N N-terminal

domains paired with a C-terminal Peptidase_S9 catalytic domain, consistent with prolyl oligopeptidases (POP) and dipeptidyl peptidases IV (DPPIV). Orthologue-based localization data suggest predominantly cytoplasmic localization for C4B63_63g37, C4B63_122g16, and C4B63_101g15, whereas C4B63_6g564 showed partial mitochondrial localization (50%), suggesting the possibility of dual targeting. The S10 family member C4B63_168g38, annotated as a serine carboxypeptidase, showed a striking expansion, with 12 predicted gene copies sharing a conserved domain architecture, consistent with the broader multi-gene family amplification observed in *T. cruzi* for trans-sialidases, mucins, and MASPs.^{26,39,40}

2.6. Gene ontology enrichment and protein interaction network analysis support lipid metabolism as a dominant theme in the ABPP-enriched serinome

To further assess the chemoproteomics dataset and explore the collective biological relevance of the ABPP-enriched SHs, we performed Gene Ontology (GO) enrichment and protein-protein interaction (PPI) network analyses using the STRING database, applying the *T. cruzi* CL Brenner proteome as the reference background. The CL Brenner strain proteome was used because it is the only *T. cruzi* assembly available in STRING; enriched Dm28c proteins were therefore mapped to their CL

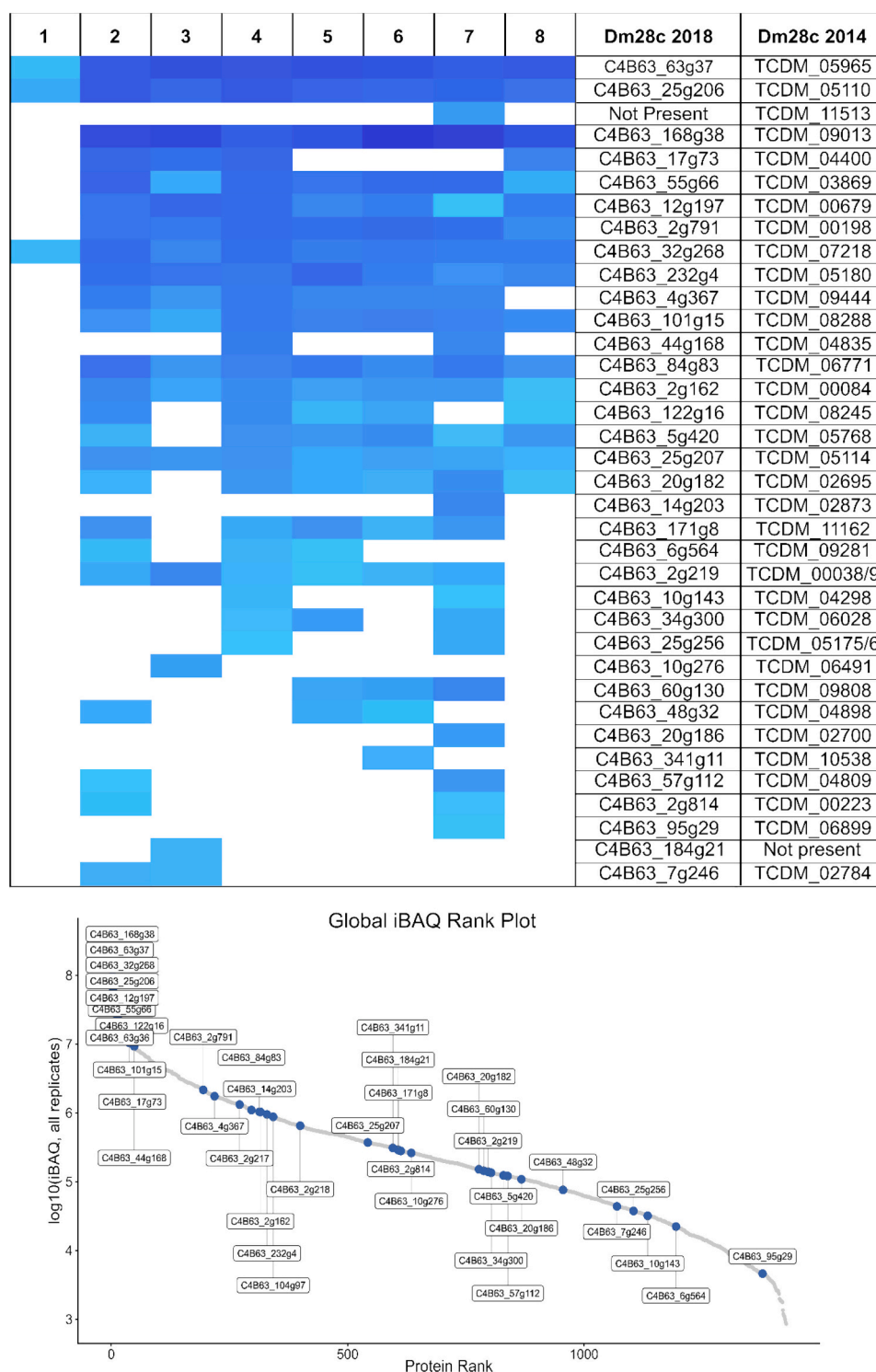


Fig. 3. (a) Log₂ fold-change (Log₂ FC) heatmap showing the enrichment profiles of SHs across the different synthesized probes. Log₂ FC values, reflecting the relative protein abundance between probe-treated and untreated samples, range from 1.2 (light blue) to 7.7 (blue). In brief, *T. cruzi* cultures were treated with the indicated FP-alkyne ABPs, lysed, and subjected to click conjugation with biotin-N₃. Enriched proteins were subsequently analysed by LFQ LC-MS/MS. (b) iBAQ abundance distribution of the *T. cruzi* proteome: iBAQ values for all detected proteins were ranked and plotted as log₁₀-transformed abundances. SHs enriched by the activity-based probes are highlighted within the overall proteome distribution. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Brener homologues for the GO and PPI analyses.

GO enrichment revealed that the most significantly enriched molecular functions included “hydrolase activity”, “carboxylic ester hydrolase activity”, and “serine-type peptidase activity”, supporting the high selectivity of the ABPs toward SHs (Fig. 5a and Supplementary Data

5). Among biological processes, “lipid metabolic process”, “lipid catabolic process”, and “acylglycerol catabolic process” were strongly overrepresented, indicating that many of the enriched enzymes are associated with lipid turnover and energy mobilization. This is consistent with the structural diversity of the lipase families captured and with

Class	Gene Acc.N°	Pfam Structure and TM	N° of copies	Length (aa)	homologue in <i>Leish.</i>	Homologue in <i>T. brucei</i>	Localisation
Ser Peptidase							
S9	C4B63_63g37		1	697	●	●	Cytoplasm
S9	C4B63_6g564		1	903	●	●	Endocytic, MITO (50%)*
S9	C4B63_122g16		1	715	●	●	Cytoplasm
S9	C4B63_101g15		1	839	●	●	Cytoplasm
S10	C4B63_168g38		12	466	●	●	Cytoplasm*
S28	C4B63_57g112		1	483	●	●	ND
S33	C4B63_2g814		1	407	●	●	Endocytic, Cytoplasm
S33	C4B63_84g83		1	280	●	●	ND
S33	C4B63_2g162		1	361	●	●	Endocytic, ER
S54	C4B63_34g300		1	278	●	●	Endocytic, Cytoplasm*
Amidase							
FAAH	C4B63_55g66		1	599	●	●	Nuclear envelope (50%)
Esterase							
Patatin	C4B63_7g246		1	417	●	●	Lipid droplet
Patatin	C4B63_95g29		1	320	●	●	MITO
GDSL	C4B63_184g21		3	252	●	●	Cytoplasm
Lipase3	C4B63_25g256		1	792	●	●	ND
Lipase3	C4B63_104g97		17	348	●	●	Endocytic, Cytoplasm*
Lipase3	C4B63_10g143		1	1243	●	●	Cytoplasm, Endocytic*
Lipase3	C4B63_171g8		2	579	●	●	ER
Lipase3	C4B63_20g182		1	517	●	●	ND
Lipase3	C4B63_4g367		1	686	●	●	MITO, Glycosome (25%)
Lipase3	C4B63_10g276		2	445	●	●	Glycosomes
PDAT	C4B63_5g420		2	647	●	●	Cytoplasm
PLA	C4B63_2g791		1	438	●	●	ER, Nuclear pore
PGAP1	C4B63_2g218		1	1257	●	●	Cytoplasm, Nuclear lumen*
PL/CE	C4B63_32g268		1	281	●	●	Cytosol, Glycosomes
PL/CE	C4B63_14g203		1	390	●	●	Cytoplasm, Endocytic*
	C4B63_44g168		3	312	●	●	Endocytic, PM
	C4B63_232g4		2	414	●	●	ER, Nuclear envelope
	C4B63_46g85		1	388	●	●	MITO, kinetoplast
	C4B63_17g73		1	331	●	●	Cytoplasm, Nuclear lumen
	TCDM_11513		5	373	●	●	Endocytic, Cytoplasm*
Unclassified							
	C4B63_48g32		2	354	●	●	Cytoplasm*
	C4B63_20g186		1	587	●	●	ND
	C4B63_60g130		1	473	●	●	ER, Nuclear envelope
	C4B63_12g197		1	427	●	●	ND
	C4B63_25g206		1	312	●	●	MITO
	C4B63_25g207		1	429	●	●	ND

Transmembrane regions

● Present

● Absent

* low signal/background

ER: endoplasmic reticulum

MITO: Mitochondrion

Fig. 4. Pfam analysis of enriched SHs. The figures are not drawn to scale; only the relative positions of the domains and TM regions are shown. The data for this figure are presented in Supplementary Data S4. Localization was obtained from experimental data in TrypTagDB.⁴¹ FAAH: Fatty-acid amide hydrolase, Patatin: Patatin-like Lipase, GDSL: GDSL Lipase, Lipase3: Lipase class 3, PDAT: Phospholipid:diacylglycerol acyltransferase, PLA: Phospholipase A, PGAP1: GPI inositol-deacylase PGAP1, PL/CE: Phospholipase/Carboxylesterase, ER: endoplasmic reticulum, MITO: mitochondrion, and PM: plasma membrane.

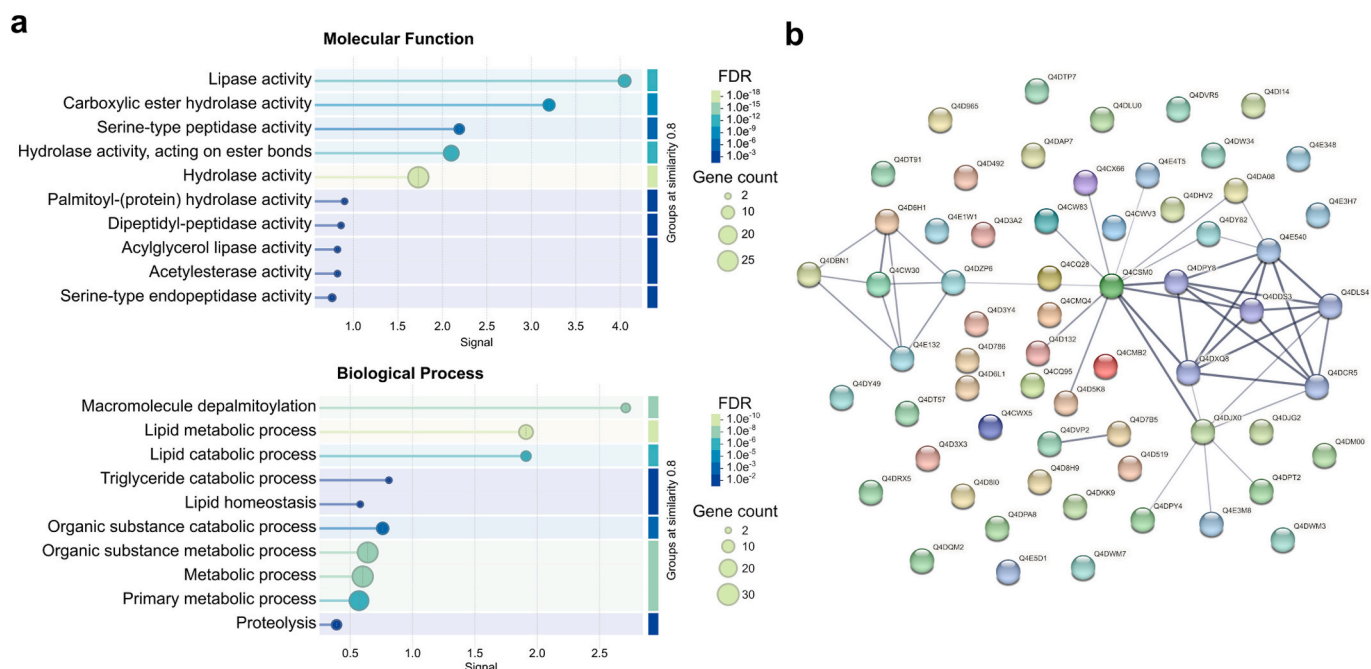


Fig. 5. (a) Gene ontology (GO) analysis for the proteins enriched by the ABPs. The enrichment analysis was performed using the STRING database v12.0,⁴⁸ applying a one-sided Fisher's exact test against the organism-specific background proteome. Resulting *p*-values were corrected for multiple testing using the Benjamini–Hochberg FDR, and enriched terms were ranked according to their FDR values. (b) Protein–protein interaction (PPI) network of enriched SHs generated with STRING database v11.5.⁴⁸

the prevalence of predicted transmembrane regions and inferred organelle-associated architectures described above. Cellular component annotations further indicated that a substantial subset of targets is associated with membranes, supporting the prevalence of membrane-embedded and organelle-associated SHs.

PPI analysis revealed a tightly interconnected network among the enriched proteins (Fig. 5b), with strong associations involving nodes annotated as monoglyceride lipase, lysophospholipase, and prolyl oligopeptidase. These enzymes are also associated with lipid metabolism, membrane remodelling, and peptide processing. The overall network exhibited highly significant enrichment (PPI enrichment *p*-value $< 1 \times 10^{-16}$), indicating that the identified proteins are biologically connected rather than representing a random sampling of the proteome. Together, the GO and PPI analyses underscore the functional coherence of the captured dataset and indicate that lipid metabolism is a dominant biological theme within the active SH landscape of *T. cruzi* epimastigotes.

These functional themes are consistent with the known lipid auxotrophy of trypanosomatids. Unable to synthesize certain lipids *de novo*, *T. cruzi* and related parasites rely heavily on host lipid acquisition and remodelling, with lipid metabolism linked to parasite differentiation, intracellular survival, and virulence.^{42,43} The enrichment of phospholipase A, lysophospholipase, patatin-like phospholipases, and multiple lipase 3 family members suggests that SHs may contribute broadly to these lipid-handling processes. The inferred glycosomal or mitochondrial association of multiple lipases is also noteworthy, as glycosomes are vital trypanosomatid-specific organelles that compartmentalize key metabolic pathways, including glycolysis and aspects of lipid metabolism.^{44–47}

3. Discussion

Combining live-cell ABPP with genome-informed curation, we generated the first activity-based map of the *T. cruzi* epimastigote serinome, identifying 37 enriched SH-like proteins, of which 35 retain a conserved or partially conserved catalytic triad or dyad. Whole-cell labelling with cell-permeable FP-alkyne probes was a key enabling

step, as lysate-based labelling performed poorly due to extract agglutination and restricted probe access. Profiling intact parasites circumvented this barrier while capturing enzymes in their native activity state, minimizing the artifactual activation or inactivation of labile hydrolases that can accompany lysis. However, the same limitations of lysate-based labelling prevented reliable and reproducible gel-based ABPP validation of individual targets. The dataset should therefore be regarded as a discovery-level chemoproteomic resource, with target-level confirmation requiring future competitive ABPP, recombinant enzyme assays, and/or genetic perturbation. Overall, these findings demonstrate the utility of live-cell FP-ABPP for kinetoplastid parasites in which conventional lysate-based approaches are challenging and suggest that this strategy may be broadly applicable to chemoproteomic profiling in such systems.

The 35 catalytically supported SHs recovered correspond to approximately 63% of the 56 SHs predicted *in silico*, representing substantial coverage for an activity-based experiment and consistent with FP probes reporting selectively on the active, probe-accessible fraction of the family. The identity of the uncaptured 37% is biologically informative in itself. Several of these enzymes might be absent in the epimastigote stage, which is the insect-adapted proliferative form and is metabolically and proteomically distinct from the clinically relevant trypomastigote and intracellular amastigote stages. This possibility is consistent with our previous activity-based profiling studies in *Leishmania*, where enzyme activity landscapes varied across life cycle contexts.^{17,29} Others may exist as catalytically inactive zymogens awaiting specific activating signals, or may target substrates not accessible to FP-based probes, such as enzymes with occluded active sites. This interpretation reframes the uncaptured fraction not as a limitation of the approach, but as a biological signal highlighting the depth of SH regulation across the *T. cruzi* life cycle. Future ABPP profiling of trypomastigotes and amastigotes, particularly the disease-critical intracellular amastigote, would be expected to reveal additional active SHs and provide a stage-resolved view of serinome dynamics.

Although the biochemical roles of many of the SHs detected remain to be established, several identified enzymes have well-characterized

functions in parasite virulence or host-pathogen interactions. Among the serine peptidases, three prolyl endopeptidases, C4B63_63g36–7 (TCDM_05965), C4B63_122g16 (TCDM_08245), and C4B63_6g564 (TCDM_09281), have been previously characterized as virulence factors in both *T. cruzi* and *Leishmania* spp., with orthologs implicated in parasite-host processes.^{9,18–20,49–52} More specifically, C4B63_122g16 (TCDM_08245), known as oligopeptidase B (OPB), belongs to the prolyl oligopeptidase family and has been associated with trypomastigote penetration into mammalian cells.^{9,53,54} The carboxypeptidase CPB1 (C4B63_168g38/TCDM_09013), also known as TcSCP, is a lysosomal glycoprotein serine carboxypeptidase identified as a minor antigen recognized by sera from patients with chronic Chagas disease.^{9,55,56} Together with Tc80, these enzymes have been proposed or investigated as candidate therapeutic targets in Chagas disease,⁴⁹ and their enrichment here supports the ability of the workflow to recover biologically relevant SHs. Notably, these enzymes were detected as catalytically active in the epimastigote stage profiled here, whereas their characterized roles in mammalian-cell invasion and host interaction have been established in infective *T. cruzi* stages or in orthologs from related trypanosomatids.

The domain architecture analysis also provided insight into the cellular context in which these enzymes may act. The prevalence of predicted integral membrane topologies, particularly among lipase 3, S33 peptidase, and PGAP1-like family members, suggests that a substantial fraction of the *T. cruzi* serinome may act at membrane interfaces, potentially processing lipidic or membrane-anchored substrates. Notably, a substantial proportion of the enriched SHs are associated with lipid metabolism (54%). This is significant as trypanosomatids, including *T. cruzi*, are unable to synthesize certain lipids and therefore rely on lipid uptake from their hosts and consequently these lipases may represent potential targets for therapeutic intervention.^{42,43} For example, lysophospholipase C4B63_32g268 (TCDM_07218) has been implicated in virulence and pathogenicity across diverse organisms, including bacteria and fungi.^{57,58} This enzyme has homologues identified in *T. brucei*⁵⁹ and *L. mexicana*. Inhibition of a related enzyme class has also been shown to block *Plasmodium falciparum* replication *in vitro*.⁵⁹ Among the other lipases identified here, a phospholipase A1 (C4B63_2g791/TCDM_00198; also known as PLA1) was enriched. Phospholipases A1 have been described as virulence-associated enzymes that participate in the early stages of parasite–host interactions preceding invasion.^{57,58,60–62} Their activity can alter the host-cell lipid profile by generating second messengers, including diacylglycerol, free fatty acids, and lysophosphatidylcholine, which activate the protein kinase C signalling pathway and promote parasite invasion.^{60–62} However, the phospholipase A1 identified in this study does not appear to correspond directly to the phospholipase homologues previously characterized in *Trypanosoma* spp., suggesting that it may represent a distinct family member with a potentially different biological role.

Two findings are particularly noteworthy. Firstly, C4B63_171g8 combines a FYVE zinc finger domain with a class 3 lipase (Lipase_3) catalytic domain, suggesting recruitment to PI3P-enriched endosomal membranes and spatial regulation of hydrolytic activity. This architecture is uncommon but it has been previously predicted in *Arabidopsis thaliana*.^{63,64} To our knowledge, this combination of domains has not been previously reported in trypanosomatids. Second, the enrichment of a catalytically supported rhomboid intramembrane serine protease (C4B63_34g300/TCDM_06028) is notable given the essential role of rhomboid proteases in invasion in apicomplexan parasites.^{65–67} These architectural features underscore that the *T. cruzi* serinome includes not only canonical soluble hydrolases but also membrane-associated and mechanistically unusual enzymes that warrant further functional and chemical-biology investigation.

Finally, we examined conservation of the enriched proteins across the related trypanosomatids *T. brucei* and *Leishmania*. Of the 37 enriched proteins, 29 were present in both related trypanosomatid lineages, one was absent from *Leishmania*, and five were absent in *T. brucei*. Two

proteins, S28 peptidase C4B63_57g112 and the lipase 3 enzyme C4B63_25g256, were absent from both *T. brucei* and *Leishmania* (Fig. 4), suggesting that they may represent candidates for studies of *T. cruzi*-restricted SH function. To place these observations in a broader evolutionary context, we analysed co-occurrence of the identified SHs across representative genomes from the three domains of life (Eukaryota, Archaea, and Bacteria) using the STRING database. As shown in Supplementary Fig. S4, most enriched SHs were broadly conserved across trypanosomatid genomes and eukaryotes more generally, with reduced representation in bacteria and near-absence in archaea. This pattern is consistent with the eukaryotic origin of most SH families identified here. From a target prioritization perspective, SHs that are conserved across trypanosomatids but divergent from human orthologs, for example oligopeptidase B, may be of particular interest.

4. Conclusions

Overall, this study reinforces the role of ABPP in early-stage drug discovery and provides the first activity-based chemoproteomic functional map of the *T. cruzi* epimastigote serinome, identifying 37 enriched SH-like proteins, including 35 with conserved or partially conserved catalytic triad/dyad features, through a live-cell ABPP-LFQ workflow. The dataset highlights lipid-metabolism-associated hydrolases, virulence-associated peptidases, and previously uncharacterized enzymes as candidates for future functional validation. By integrating active-site-directed chemical probes with genome-informed annotation, this work establishes a reference resource for stage-resolved *T. cruzi* serinome biology. Beyond its value as a biological annotation resource, this dataset has direct relevance for chemical biology and antiparasitic target discovery. Serine hydrolases are among the most chemically tractable enzyme classes, and FP-based probes report active-site accessibility in a cellular context. In alignment with the WHO NTD 2021–2030 roadmap objectives for new molecular targets in Chagas disease,⁷ the enriched enzymes identified here therefore represent a prioritized set of probe-accessible and catalytically competent targets for future inhibitor screening,⁶⁸ competitive ABPP, and structure-guided ligand discovery. Questions remain as to the role of SHs in trypanosomatids and intracellular amastigotes and the specific function, essentiality, and therapeutic relevance of the identified enzymes. Work addressing these important questions is currently underway and will be reported in due course.

Code availability statement

The R scripts used in this study are available without restrictions via Zenodo: <https://doi.org/10.5281/zenodo.20626073>.

R scripts were adapted by the authors from open source scripts.⁷⁰ ChatGPT (OpenAI) was used to assist with code optimization and troubleshooting. All final scripts were manually reviewed, tested, and validated by the authors.

CRediT authorship contribution statement

Jaime A. Isern: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **María Gabriela Mediavilla:** Writing – review & editing, Investigation, Formal analysis. **Exequiel O.J. Porta:** Writing – review & editing, Methodology, Conceptualization. **Marcelo L. Merli:** Writing – review & editing, Writing – original draft, Formal analysis. **María Sol Ballari:** Writing – review & editing, Investigation. **Julia A. Cricco:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Guillermo R. Labadie:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Patrick G. Steel:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bmc.2026.118793>.

Data availability

The data that support the findings of this study are available in the supplementary material of this article.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE⁶⁹ partner repository with the dataset identifier PXD080813.

References

- 70% of people with Chagas don't know they're infected - PAHO/WHO | Pan American Health Organization. April 13, 2021. Accessed June 16, 2025. <https://www.paho.org/en/news/13-4-2021-70-people-chagas-dont-know-theyre-infected>.
- Chagas disease. Accessed June 16, 2025. [https://www.who.int/news-room/fact-sheets/detail/chagas-disease-\(american-trypanosomiasis\)](https://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis)).
- Bern C, Montgomery SP, Herwaldt BL, et al. Evaluation and treatment of chagas disease in the United States: a systematic review. *JAMA*. 2007;298(18):2171–2181. <https://doi.org/10.1001/jama.298.18.2171>.
- Lidani KCF, Andrade FA, Bavia L, et al. Chagas disease: from discovery to a worldwide health problem. *Front Public Health*. 2019;7. <https://doi.org/10.3389/fpubh.2019.00166>.
- Hochberg NS, Montgomery SP. Chagas disease. *Ann Intern Med*. 2023;176(2):ITC17–ITC32. <https://doi.org/10.7326/AITC202302210>.
- Abbott A. Characteristics and adverse events of patients for whom Nifurtimox was released through CDC-sponsored investigational new drug program for treatment of Chagas disease — United States, 2001–2021. *MMWR Morb Mortal Wkly Rep*. 2022; 71. <https://doi.org/10.15585/mmwr.mm7110a2>.
- Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021–2030. Accessed June 16, 2025. <https://www.who.int/publications/i/item/9789240010352>.
- Chain CY, Pires Souto DE, Sbaraglini ML, et al. Trypanosoma cruzi virulence factors for the diagnosis of Chagas' disease. *ACS Infect Dis*. 2019;5(11):1813–1819. <https://doi.org/10.1021/acsinfecdis.9b00269>.
- Alvarez VE, Niemirowicz GT, Cazzulo JJ. The peptidases of Trypanosoma cruzi: digestive enzymes, virulence factors, and mediators of autophagy and programmed cell death. *Biochim Biophys Acta*. 2012;1824(1):195–206. <https://doi.org/10.1016/j.bbapap.2011.05.011>.
- Scharfstein J, Schechter M, Senna M, Peralta JM, Mendonça-Previato L, Miles MA. Trypanosoma cruzi: characterization and isolation of a 57/51,000 m.w. surface glycoprotein (GP57/51) expressed by epimastigotes and bloodstream trypomastigotes. *J Immunol*. 1986;137(4):1336–1341.
- Cazzulo JJ, Stoka V, Turk V. Cruzipain, the major cysteine proteinase from the protozoan parasite Trypanosoma cruzi. *Biol Chem*. 1997;378(1):1–10. <https://doi.org/10.1515/bchm.1997.378.1.1>.
- Duschak VG, Couto AS. Cruzipain, the major cysteine protease of Trypanosoma cruzi: a sulfated glycoprotein antigen as relevant candidate for vaccine development and drug target. A review. *Curr Med Chem*. 2009;16(24):3174–3202. <https://doi.org/10.2174/092986709788802971>.
- Franke de Cazzulo BM, Martínez J, North MJ, Coombs GH, Cazzulo JJ. Effects of proteinase inhibitors on the growth and differentiation of Trypanosoma cruzi. *FEMS Microbiol Lett*. 1994;124(1):81–86. <https://doi.org/10.1111/j.1574-6968.1994.tb07265.x>.
- Siqueira-Neto JL, Debnath A, McCall LI, et al. Cysteine proteases in protozoan parasites. *PLoS Negl Trop Dis*. 2018;12(8), e0006512. <https://doi.org/10.1371/journal.pntd.0006512>.
- Alves CR, Souza RS, Charret KDS, et al. Understanding serine proteases implications on Leishmania spp lifecycle. *Exp Parasitol*. 2018;184:67–81. <https://doi.org/10.1016/j.exppara.2017.11.008>.
- Silva-Almeida M, Souza-Silva F, Pereira BAS, Ribeiro-Guimarães ML, Alves CR. Overview of the organization of protease genes in the genome of Leishmania spp. *Parasit Vectors*. 2014;7:387. <https://doi.org/10.1186/1756-3305-7-387>.
- Isern JA, Porta EOJ, Kalesh K, et al. Profiling serine hydrolases in the Leishmania host-pathogen interactome using cell-permeable activity-based fluorophosphonate probes. *Chembiochem*. 2025;26(10), e202500160. <https://doi.org/10.1002/cbic.202500160>.
- Lasse C, Azevedo CS, de Araújo CN, et al. Prolyl oligopeptidase from Leishmania infantum: biochemical characterization and involvement in macrophage infection. *Front Microbiol*. 2020;11, 1060. <https://doi.org/10.3389/fmicb.2020.01060>.
- Motta FN, Azevedo CDS, Neves BP, et al. Oligopeptidase B, a missing enzyme in mammals and a potential drug target for trypanosomatid diseases. *Biochimica*. 2019; 167:207–216. <https://doi.org/10.1016/j.biochi.2019.10.006>.
- Barbosa GR, Marana SR, Stolf BS. Characterization of Leishmania (L.) amazonensis oligopeptidase B and its role in macrophage infection. *Parasitology*. 2022;149(11): 1411–1418. <https://doi.org/10.1017/S0031182022000816>.
- Kidd D, Liu Y, Cravatt BF. Profiling serine hydrolase activities in complex proteomes. *Biochemistry*. 2001;40(13):4005–4015. <https://doi.org/10.1021/bi002579j>.
- Porta EOJ. Mapping the evolution of activity-based protein profiling: a bibliometric review. *Adv Pharm Bull*. 2023;13(4):639–645. <https://doi.org/10.34172/apb.2023.082>.
- Porta EOJ, Steel PG. Activity-based protein profiling: a graphical review. *Curr Res Pharmacol Drug Discov*. 2023;5, 100164. <https://doi.org/10.1016/j.crphar.2023.100164>.
- Simon GM, Cravatt BF. Activity-based proteomics of enzyme superfamilies: serine hydrolases as a case study. *J Biol Chem*. 2010;285(15):11051–11055. <https://doi.org/10.1074/jbc.R109.097600>.
- Barglow KT, Cravatt BF. Activity-based protein profiling for the functional annotation of enzymes. *Nat Methods*. 2007;4(10):822–827. <https://doi.org/10.1038/nmeth1092>.
- Berná L, Rodríguez M, Chiribao ML, et al. Expanding an expanded genome: long-read sequencing of Trypanosoma cruzi. *Microb Genom*. 2018;4(5), e000177. <https://doi.org/10.1099/mgen.0.000177>.
- TDR Targets. Accessed June 16, 2025. <https://tdrtargets.org/>.
- TriTrypDB. Accessed June 16, 2025. <https://tritrypdb.org/tritrypdb/app/>.
- Porta EOJ, Isern JA, Kalesh K, Steel PG. Discovery of Leishmania druggable serine proteases by activity-based protein profiling. *Front Pharmacol*. 2022;13. <https://doi.org/10.3389/fphar.2022.929493>.
- Cardoso MS, Junqueira C, Trigueiro RC, et al. Identification and functional analysis of Trypanosoma cruzi genes that encode proteins of the glycosylphosphatidylinositol biosynthetic pathway. *PLoS Negl Trop Dis*. 2013;7(8), e2369. <https://doi.org/10.1371/journal.pntd.0002369>.
- Fonseca LM, da Costa KM, Chaves V de S, et al. Theft and reception of host cell's sialic acid: dynamics of Trypanosoma cruzi trans-sialidases and mucin-like molecules on Chagas' disease immunomodulation. *Front Immunol*. 2019;10. <https://doi.org/10.3389/fimmu.2019.00164>.
- Flávia Nardy A, Freire-de-Lima CG, Morrot A. Immune evasion strategies of Trypanosoma cruzi. *J Immunol Res*. 2015;2015, 178947. <https://doi.org/10.1155/2015/178947>.
- Balouz V, Mesias AC, Camean CC, et al. Homologous expression of glycosylphosphatidylinositol-anchored glycoproteins in Trypanosoma cruzi. *Methods Mol Biol*. 2019;1955:119–134. https://doi.org/10.1007/978-1-4939-9148-8_9.
- Cardoso MS, Reis-Cunha JL, Bartholomeu DC. Evasion of the immune response by Trypanosoma cruzi during acute infection. *Front Immunol*. 2016;6. <https://doi.org/10.3389/fimmu.2015.00659>.
- Ritchie ME, Phipson B, Wu D, et al. Limma powers differential expression analyses for RNA-seq and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47. <https://doi.org/10.1093/nar/gkv007>.
- El-Sayed NM, Myler PJ, Bartholomeu DC, et al. The genome sequence of Trypanosoma cruzi, etiologic agent of Chagas disease. *Science*. 2005;309(5733): 409–415. <https://doi.org/10.1126/science.1112631>.
- Hakim JMC, Gutierrez Guarnizo SA, Málaga Machaca E, Gilman RH, Mugnier MR. Whole-genome assembly of a hybrid Trypanosoma cruzi strain assembled with Nanopore sequencing alone. *G3 Genes/Genomes/Genetics*. 2024;14(6), jkae076. <https://doi.org/10.1093/g3journal/jkae076>.
- Grisard EC, Teixeira SMR, de Almeida LGP, et al. Trypanosoma cruzi clone Dm28c draft genome sequence. *Genome Announc*. 2014;2(1), e01114–13. <https://doi.org/10.1128/genomeA.01114-13>.
- De Pablos LM, Osuna A. Multigene families in Trypanosoma cruzi and their role in infectivity. *Infect Immun*. 2012;80(7):2258–2264. <https://doi.org/10.1128/IAI.06225-11>.
- Herrerros-Cabello A, Callejas-Hernández F, Gironès N, Fresno M. Trypanosoma Cruzii genome: organization, multi-gene families, transcription, and biological implications. *Genes*. 2020;11(10):1196. <https://doi.org/10.3390/genes11101196>.

41. Dean S, Sunter JD, Wheeler RJ. TrypTag.org: a trypanosome genome-wide protein localisation resource. *Trends Parasitol.* 2017;33(2):80–82. <https://doi.org/10.1016/j.pt.2016.10.009>.
42. Booth LA, Smith TK. Lipid metabolism in *Trypanosoma cruzi*: a review. *Mol Biochem Parasitol.* 2020;240, 111324. <https://doi.org/10.1016/j.molbiopara.2020.111324>.
43. Atella T, Bittencourt-Cunha PR, Araujo MFC, Silva-Cardoso L, Maya-Monteiro CM, Atella GC. *Trypanosoma cruzi* modulates lipid metabolism and highjacks phospholipids from the midgut of *Rhodnius prolixus*. *Acta Trop.* 2022;233, 106552. <https://doi.org/10.1016/j.actatropica.2022.106552>.
44. Kael VC, Emmanouilidis L, Dawidowski M, et al. Inhibitors of glycosomal protein import provide new leads against trypanosomiasis. *Microb Cell.* 2017;4(7):229–232. <https://doi.org/10.15698/mic2017.07.581>.
45. Furuya T, Kessler P, Jardim A, Schnauffer A, Crudder C, Parsons M. Glucose is toxic to glycosome-deficient trypanosomes. *Proc Natl Acad Sci USA.* 2002;99(22):14177–14182. <https://doi.org/10.1073/pnas.222454899>.
46. Parreira de Aquino G, Mendes Gomes MA, Köpke Salinas R, Laranjeira-Silva MF. Lipid and fatty acid metabolism in trypanosomatids. *Microb Cell.* 2021;8(11):262–275. <https://doi.org/10.15698/mic2021.11.764>.
47. Michels PAM, Bringaud F, Herman M, Hannaert V. Metabolic functions of glycosomes in trypanosomatids. *Biochim Biophys Acta.* 2006;1763(12):1463–1477. <https://doi.org/10.1016/j.bbamcr.2006.08.019>.
48. Szklarczyk D, Kirsch R, Koutrouli M, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res.* 2023;51(D1):D638–D646. <https://doi.org/10.1093/nar/gkac1000>.
49. Cazzulo JJ. Proteinases of *Trypanosoma cruzi*: potential targets for the chemotherapy of Chagas disease. *Curr Top Med Chem.* 2002;2(11):1261–1271. <https://doi.org/10.2174/1568026023392995>.
50. Bastos IMD, Motta FN, Grellier P, Santana JM. Parasite prolyl oligopeptidases and the challenge of designing chemotherapeutics for Chagas disease, leishmaniasis and African trypanosomiasis. *Curr Med Chem.* 2013;20(25):3103–3115. <https://doi.org/10.2174/0929867311320250006>.
51. Munday JC, McLuskey K, Brown E, Coombs GH, Mottram JC. Oligopeptidase B deficient mutants of *Leishmania major*. *Mol Biochem Parasitol.* 2011;175(1):49–57. <https://doi.org/10.1016/j.molbiopara.2010.09.003>.
52. Swenerton RK, Zhang S, Sajid M, et al. The oligopeptidase B of *Leishmania* regulates parasite enolase and immune evasion. *J Biol Chem.* 2011;286(1):429–440. <https://doi.org/10.1074/jbc.M110.138313>.
53. Motta FN, Bastos IMD, Faudry E, et al. The *Trypanosoma cruzi* virulence factor oligopeptidase B (OPBTc) assembles into an active and stable dimer. *PLoS ONE.* 2012;7(1), e30431. <https://doi.org/10.1371/journal.pone.0030431>.
54. Burleigh BA, Caler EV, Webster P, Andrews NW. A cytosolic serine endopeptidase from *Trypanosoma cruzi* is required for the generation of Ca²⁺ signaling in mammalian cells. *J Cell Biol.* 1997;136(3):609–620. <https://doi.org/10.1083/jcb.136.3.609>.
55. Soprano LL, Parente JE, Landoni M, Couto AS, Duschak VG. *Trypanosoma cruzi* serinecarboxipeptidase is a sulfated glycoprotein and a minor antigen in human Chagas disease infection. *Med Microbiol Immunol.* 2018;207(2):117–128. <https://doi.org/10.1007/s00430-017-0529-7>.
56. Parussini F, García M, Mucci J, et al. Characterization of a lysosomal serine carboxypeptidase from *Trypanosoma cruzi*. *Mol Biochem Parasitol.* 2003;131(1):11–23. [https://doi.org/10.1016/S0166-6851\(03\)00175-0](https://doi.org/10.1016/S0166-6851(03)00175-0).
57. Flores-Díaz M, Monturiol-Gross L, Naylor C, Alape-Girón A, Flieger A. Bacterial sphingomyelinases and phospholipases as virulence factors. *Microbiol Mol Biol Rev.* 2016;80(3):597–628. <https://doi.org/10.1128/MMBR.00082-15>.
58. Köhler GA, Brenot A, Haas-Stapleton E, Agabian N, Deva R, Nigam S. Phospholipase A2 and phospholipase B activities in fungi. *Biochim Biophys Acta.* 2006;1761(11):1391–1399. <https://doi.org/10.1016/j.bbaliip.2006.09.011>.
59. Davison D, Howell S, Snijders AP, Deu E. Activity-based protein profiling of human and plasmodium serine hydrolases and interrogation of potential antimalarial targets. *iScience.* 2022;25(9), 104996. <https://doi.org/10.1016/j.isci.2022.104996>.
60. Belaunzarán ML, Wilkowsky SE, Lammel EM, et al. Phospholipase A1: a novel virulence factor in *Trypanosoma cruzi*. *Mol Biochem Parasitol.* 2013;187(2):77–86. <https://doi.org/10.1016/j.molbiopara.2012.12.004>.
61. Belaunzarán ML, Wainszelbaum MJ, Lammel EM, et al. Phospholipase A1 from *Trypanosoma cruzi* infective stages generates lipid messengers that activate host cell protein kinase c. *Parasitology.* 2007;134(Pt 4):491–502. <https://doi.org/10.1017/S0031182006001740>.
62. Belaunzarán ML, Lammel EM, de Isola ELD. Phospholipases A in trypanosomatids. *Enzyme Res.* 2011;2011, 392082. <https://doi.org/10.4061/2011/392082>.
63. Jensen RB, La Cour T, Albrethsen J, Nielsen M, Skriver K. FYVE zinc-finger proteins in the plant model *Arabidopsis thaliana*: identification of PtdIns3P-binding residues by comparison of classic and variant FYVE domains. *Biochem J.* 2001;359(Pt 1):165–173. <https://doi.org/10.1042/0264-6021.3590165>.
64. Upton C, Buckley JT. A new family of lipolytic enzymes? *Trends Biochem Sci.* 1995;20(5):178–179. [https://doi.org/10.1016/S0968-0004\(00\)89002-7](https://doi.org/10.1016/S0968-0004(00)89002-7).
65. Gandhi S, Baker RP, Cho S, Stanchev S, Strisovsky K, Urban S. Designed parasite-selective rhomboid inhibitors block invasion and clear blood-stage malaria. *Cell Chem Biol.* 2020;27(11):1410–1424.e6. <https://doi.org/10.1016/j.chembiol.2020.08.011>.
66. Brossier F, Jewett TJ, Sibley LD, Urban S. A spatially localized rhomboid protease cleaves cell surface adhesins essential for invasion by toxoplasma. *Proc Natl Acad Sci.* 2005;102(11):4146–4151. <https://doi.org/10.1073/pnas.0407918102>.
67. Shen B, Buguliskis JS, Lee TD, Sibley LD. Functional analysis of rhomboid proteases during *Toxoplasma* invasion. *mBio.* 2014;5(5), 10.1128/mbio.01795-14. <https://doi.org/10.1128/mbio.01795-14>.
68. Porta EOJ, Kalesh K, Isern JA, Steel PG. Microplate-based enzymatic activity assay protocol powered by activity-based probes. *Methods Mol Biol.* 2025;2921:119–137. https://doi.org/10.1007/978-1-0716-4502-4_6.
69. Perez-Riverol Y, Bandla C, Kundu DJ, et al. The PRIDE database at 20 years: 2025 update. *Nucleic Acids Res.* 2025;53(D1):D543–D553. <https://doi.org/10.1093/nar/gkae1011>.
70. Richter A, Isern JA, Ruwolt M, et al. Proteome-wide quantification of inositol pyrophosphate-protein interactions. *Nat Commun.* 2026;17(1), 4967. <https://doi.org/10.1038/s41467-026-73804-8>.