

Proteomic approaches for the study of protozoa parasites

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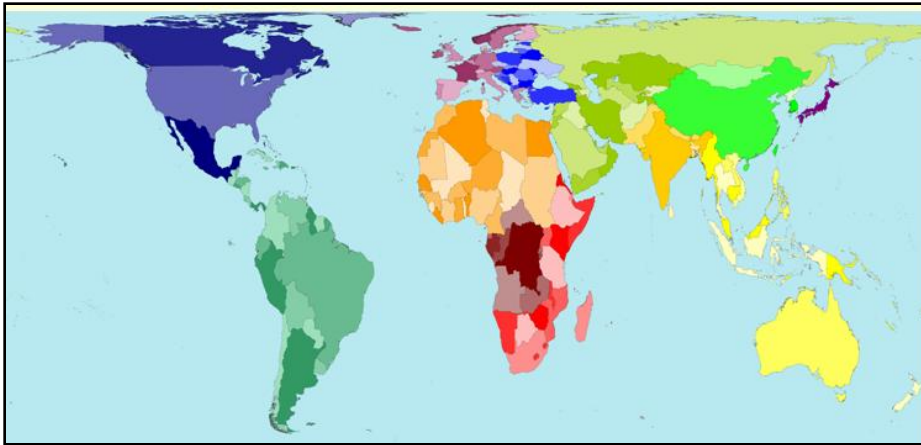


ICCC-12 Conference 2010

Florianópolis, SC, 26 September-01 October, 2010

Annex Table 2 Deaths by cause, sex and mortality stratum in WHO regions,^a estimates for 2002Figures computed by WHO to assure comparability;^b they are not necessarily the official statistics of Member States, which may use alternative rigorous methods.

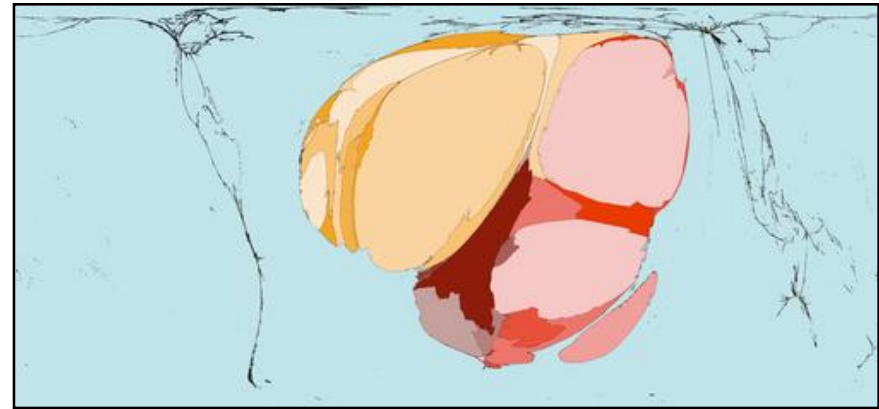
Cause ^d	SEX ^c						AFRICA		THE AMERICAS		
	Both sexes		Males		Females		Mortality stratum		Mortality stratum		
	High child, high adult	High child, very high adult	Very low child, very low adult	Low child, low adult	High child, high adult						
<i>Population (000)</i>	<i>6 224 985</i>	<i>3 131 052</i>	<i>3 093 933</i>	<i>311 273</i>	<i>360 965</i>	<i>333 580</i>	<i>445 161</i>	<i>73 810</i>			
	(000)	% total	(000)	% total	(000)	% total	(000)	(000)	(000)	(000)	(000)
TOTAL Deaths	57 029	100	29 891	100	27 138	100	4 657	6 007	2 720	2 701	541
I. Communicable diseases, maternal and perinatal conditions and nutritional deficiencies	18 324	32.1	9 365	31.3	8 959	33	3 245	4 426	167	482	227
Infectious and parasitic diseases	10 904	19.1	5 795	19.4	5 109	18.8	2 211	3 414	69	195	133
Tuberculosis	1 566	2.7	1 030	3.4	536	2.0	143	205	1	26	19
STIs excluding HIV	180	0.3	91	0.3	89	0.3	41	52	0	1	1
Syphilis	157	0.3	84	0.3	72	0.3	39	50	0	1	0
Chlamydia	9	0.0	0	0.0	9	0.0	1	0	0	0	0
Gonorrhoea	1	0.0	0	0.0	1	0.0	0	0	0	0	0
HIV/AIDS	2 777	4.9	1 447	4.8	1 330	4.9	479	1 616	14	50	39
Diarrhoeal diseases	1 798	3.2	939	3.1	859	3.2	351	356	2	34	21
Childhood diseases	1 124	2.0	563	1.9	562	2.1	308	219	0	1	4
Pertussis	294	0.5	147	0.5	147	0.5	78	53	0	0	3
Poliomyelitis ^e	1	0.0	0	0.0	0	0.0	0	0	0	0	0
Diphtheria	5	0.0	3	0.0	3	0.0	1	1	0	0	0
Measles	611	1.1	306	1.0	305	1.1	180	131	0	0	0
Tetanus	214	0.4	107	0.4	107	0.4	49	35	0	0	0
Meningitis	173	0.3	90	0.3	83	0.3	8	12	1	8	9
Hepatitis B ^f	103	0.2	71	0.2	32	0.1	10	10	1	3	2
Hepatitis C ^f	54	0.1	35	0.1	18	0.1	4	4	5	2	0
Malaria	1 272	2.2	607	2.0	665	2.5	557	579	0	1	0
Tropical diseases	129	0.2	79	0.3	50	0.2	28	28	0	12	4
Trypanosomiasis	48	0.1	31	0.1	17	0.1	24	23	0	0	0
Chagas disease	14	0.0	8	0.0	7	0.0	0	0	0	11	4
Schistosomiasis	15	0.0	10	0.0	5	0.0	1	1	0	1	0
Leishmaniasis	51	0.1	30	0.1	21	0.1	4	4	0	0	0
Lymphatic filariasis	0	0.0	0	0.0	0	0.0	0	0	0	0	0
Onchocerciasis	0	0.0	0	0.0	0	0.0	0	0	0	0	0



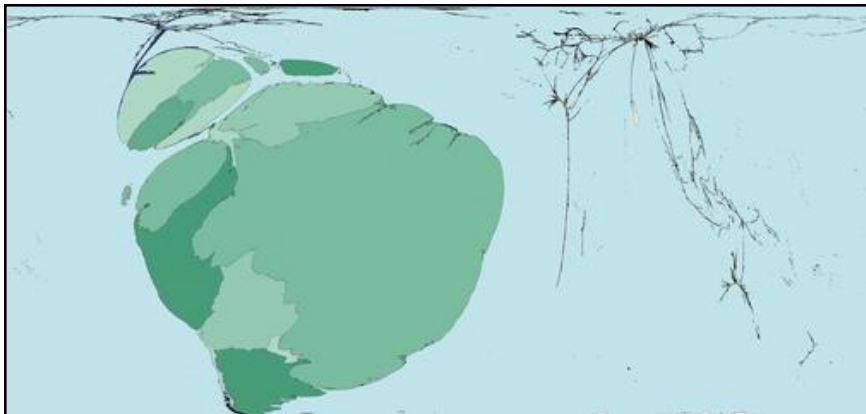
Territories are sized in proportion to the absolute number of people who died from each disease in one year.

<http://www.worldmapper.org/>

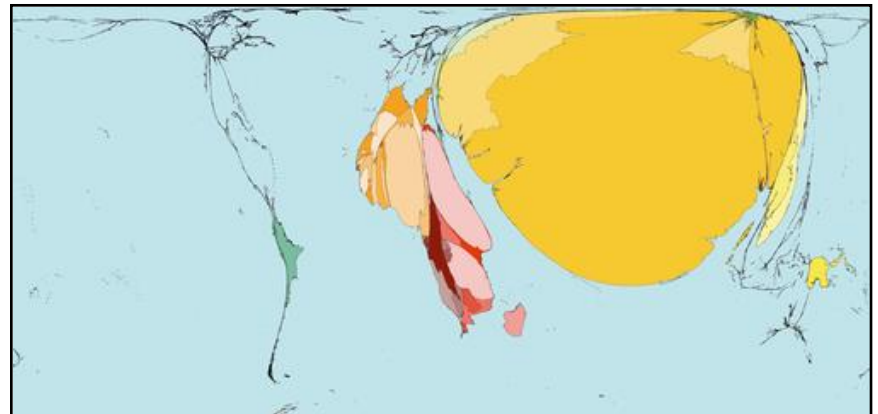
Sleeping sickness



Chagas disease




Leishmaniasis



Annex Table 3 Burden of disease in DALYs by cause, sex and mortality stratum in WHO regions,^a estimates for 2002Figures computed by WHO to assure comparability;^b they are not necessarily the official statistics of Member States, which may use alternative rigorous methods.

Cause ^d	SEX ^c						AFRICA		THE AMERICAS		
	Both sexes		Males		Females		Mortality stratum		Mortality stratum		
							High child, high adult	High child, very high adult	Very low child, very low adult	Low child, low adult	High child, high adult
<i>Population (000)</i>	<i>6 224 985</i>		<i>3 131 052</i>		<i>3 093 933</i>		<i>311 273</i>	<i>360 965</i>	<i>333 580</i>	<i>445 161</i>	<i>73 810</i>
	(000)	% total	(000)	% total	(000)	% total	(000)	(000)	(000)	(000)	(000)
TOTAL DALYs	1 490 126	100	772 912	100	717 213	100	160 415	200 961	46 868	81 589	17 130
I. Communicable diseases, maternal and perinatal conditions and nutritional deficiencies	610 319	41.0	296 796	38.4	313 523	43.7	115 317	150 405	3 106	16 334	7 210
Infectious and parasitic diseases	350 333	23.5	179 307	23.2	171 025	23.8	75 966	111 483	1 228	6 719	3 944
Tuberculosis	34 736	2.3	21 905	2.8	12 831	1.8	3 786	5 480	12	506	410
STIs excluding HIV	11 347	0.8	3 855	0.5	7 492	1.0	1 930	2 444	75	487	69
Syphilis	4 200	0.3	1 970	0.3	2 230	0.3	1 028	1 417	2	56	20
Chlamydia	3 571	0.2	302	0.0	3 269	0.5	364	428	55	241	15
Gonorrhoea	3 365	0.2	1 473	0.2	1 892	0.3	520	573	16	183	31
HIV/AIDS	84 458	5.7	42 663	5.5	41 795	5.8	14 620	49 343	454	1 594	1 163
Diarrhoeal diseases	61 966	4.2	32 353	4.2	29 614	4.1	11 548	11 689	106	1 494	750
Childhood diseases	41 480	2.8	20 713	2.7	20 767	2.9	11 061	7 934	54	177	162
Pertussis	12 595	0.8	6 283	0.8	6 312	0.9	3 078	2 165	52	163	146
Poliomyelitis ^b	151	0.0	76	0.0	74	0.0	11	4	3	6	1
Diphtheria	185	0.0	96	0.0	89	0.0	24	24	0	2	7
Measles	21 475	1.4	10 727	1.4	10 748	1.5	6 328	4 587	0	0	0
Tetanus	7 074	0.5	3 530	0.5	3 543	0.5	1 620	1 155	0	6	8
Meningitis	6 192	0.4	3 082	0.4	3 110	0.4	394	497	43	356	280
Hepatitis B ^f	2 170	0.1	1 459	0.2	711	0.1	302	280	20	56	38
Hepatitis C ^f	1 004	0.1	668	0.1	336	0.0	120	122	77	29	1
Malaria	46 486	3.1	22 243	2.9	24 242	3.4	20 070	20 785	0	86	25
Tropical diseases	12 245	0.8	8 273	1.1	3 973	0.6	2 939	2 743	9	604	178
Trypanosomiasis	1 525	0.1	966	0.1	559	0.1	744	740	0	0	0
Chagas disease	667	0.0	343	0.0	324	0.0	0	0	8	483	171
Schistosomiasis	1 702	0.1	1 020	0.1	681	0.1	621	713	0	74	0
Leishmaniasis	2 090	0.1	1 249	0.2	840	0.1	208	175	1	38	5
Lymphatic filariasis	5 777	0.4	4 413	0.6	1 364	0.2	976	1 035	0	9	1
Onchocerciasis	484	0.0	280	0.0	204	0.0	390	80	0	1	1



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a quick guide to... SEQUENCED GENOMES

The genomes of more than 180 organisms have been sequenced since 1995. The Quick Guide includes descriptions of these organisms and has links to sequencing centers and scientific abstracts.

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Written by Kate Ruder and Edward R. Winstead
Created for GNN by Mary S. Gibbs

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Aeropyrum pernix (Archaea) [back to top](#)

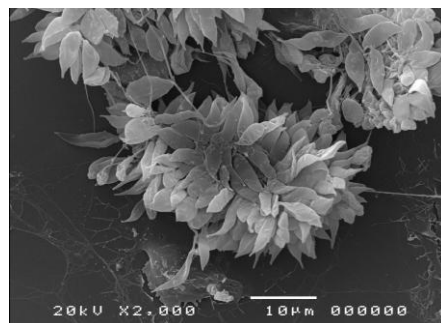
This microbe was isolated from a hydrothermal vent on the ocean floor near Kodakara-Jima Island, Japan. It grows at temperatures up to 100°C (212°F) and is able to live in the presence of oxygen.

» Sequenced by: [National Institute of Technology and Evaluation](#) *A. pernix* K1 [Abstract](#)

» Image: © Y. Sako, Kyoto University.

http://www.genomenewsnetwork.org/resources/sequenced_genomes/genome_guide_p1a.shtml

180 !! Since 1995





Human
31 000



Thale cress
26 000



Nematode worm
18 000



Fruit fly
13 000



Yeast
6000



Tuberculosis microbe
4000

Caption: Comparison of the number of genes in different organisms.

Image for use with the Wellcome Trust genome announcement only, no archiving, no sales, no web use. For further clearance contact j.whiting@wellcome.ac.uk or Tel. +44 (0)20 7611 8347.
©Wellcome Trust 2001

Illustration: Dominic Li



We've got the sequence, and now what...?

DUSZYN
TORONTO STAR 00

Trypanosomatid Genomes



El-Sayed et al. 2005; Ivens et al. 2005; Berriman et al., 2005

Among ~8,000 coding genes in *Leishmania*
~6,200 are common to the tritryp (*Leishmania major*,
Trypanosoma cruzi, *Trypanosoma brucei*)
~1,000 are *Leishmania*-specific

only ~200 genes (including pseudogenes) are
species-specific (*L. major*, *L. braziliensis*, *L. infantum*)

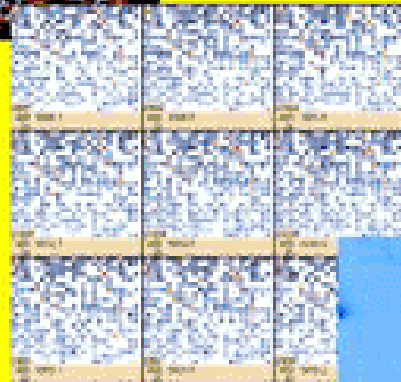
Ivens et al. 2005; Peacock et al. 2007; Smith et al., 2007



Genome, Transcriptome, Proteome

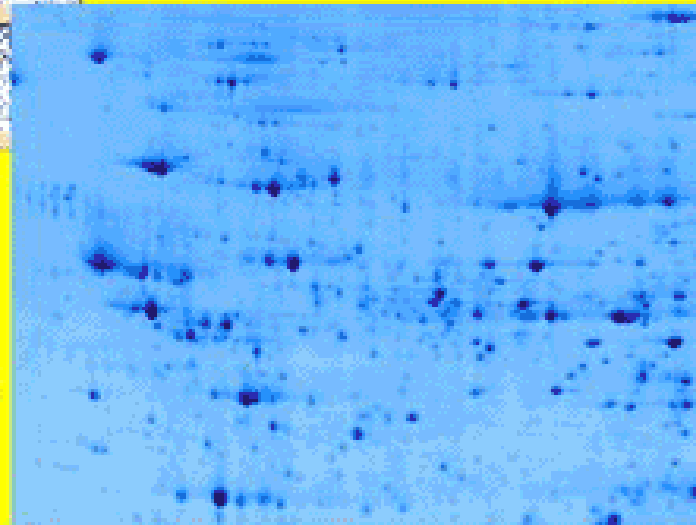


← Genome (all genes): What *could* happen



Transcriptome (all mRNA's):
What *might be* happening ←

Proteome (all proteins):
What *is* happening →



One genome

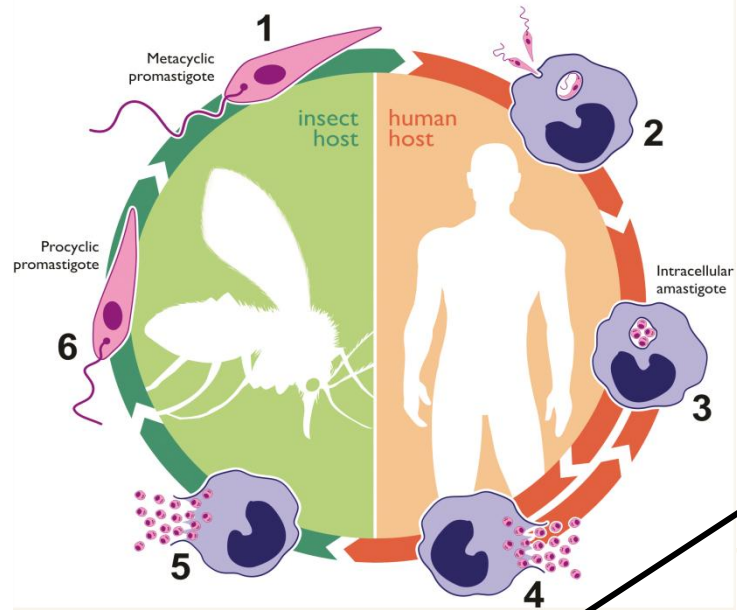


Two proteomes

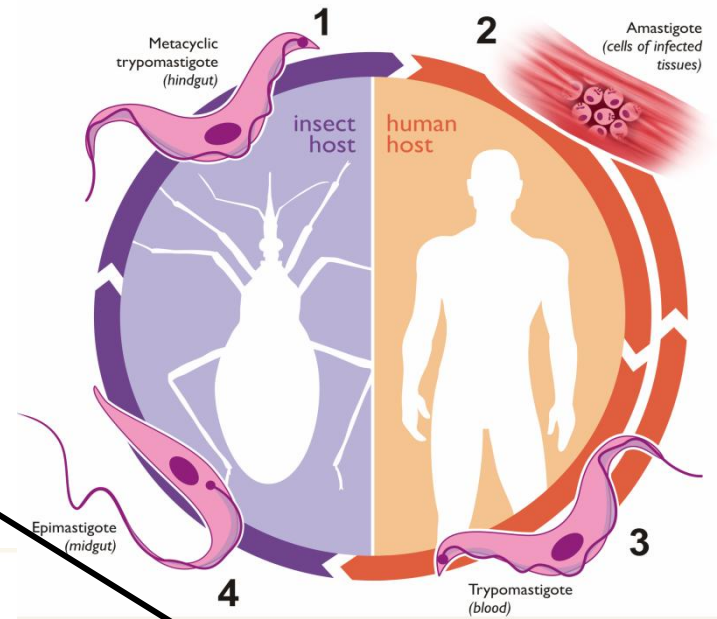
The complexity of an organism is mainly based on the complexity of its proteome rather than on the complexity of its genome.

The protein expression in a cell is very dynamic. Different proteins are expressed at different times including different isoforms and modifications.

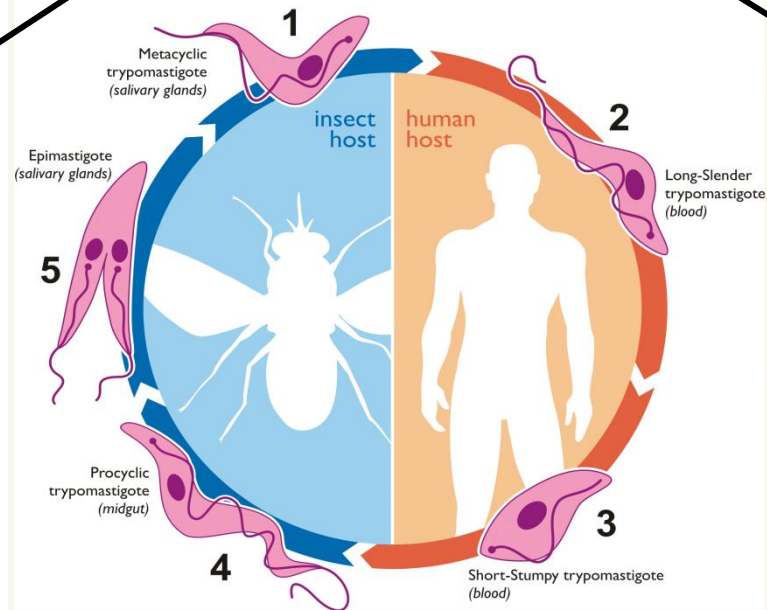
Leishmania life cycle




T. cruzi life cycle



T. brucei life cycle





All Sequencing

Human (HGP)

Pathogens

Blast

Protozoan Genomes

The Sanger Institute protozoan sequencing effort is concentrated on pathogens and model organisms. Below is a list of projects funded, underway or completed; all data from these projects are immediately and freely available.

Data are accessible in a number of ways; for each organism there is a BLAST server, allowing you to search the sequences with your own query. Sequences can also be downloaded directly by FTP. In addition, for those organisms being sequenced using a cosmid approach, finished and annotated cosmids are submitted to EMBL and other public databases (these continue to be accessible by BLAST and FTP from here). Enquiries about sequencing or analysis should be directed to [Matt Berriman](#).

<i>Babesia bigemina</i>			
Whole Genome Shotgun	10 Mb	Shotgun Complete	Wellcor

<i>Babesia bovis</i>			
EST Sequencing	10,000 reads	~1600 reads	NWO WOTRC

<i>Bodo saltans</i>			
Whole Genome Sequencing	~35 Mb	Sequencing	Wellcor

<i>Crithidia sp.</i>			
Whole Genome Shotgun	~35 Mb	Sequencing	Wellcor

<i>Dictyostelium discoideum</i>			
Eichinger et al. Nature 435: 43-57 (2005)			
chromosome 6	1.5 fragment	Draft sequence	Europe
chromosome 5	5.5	Draft sequence	Medical

<i>Eimeria tenella</i>			
partial genome shotgun	~ 60	draft assembly	BBSRC

<i>Entamoeba dispar</i>			
Genome Survey Sequencing	20,000 reads	Shotgun complete	BBSRC

<i>Entamoeba histolytica</i>			
Loftus et al. Nature 433: 865-8 (2005)			
Whole Genome Shotgun	20 Mb	Draft sequence	Wellcor

<i>Entamoeba moshkovskii</i>			
Genome Survey Sequencing	20,000 reads	Shotgun complete	BBSRC

<i>Entamoeba terrapinae</i>			
Genome Survey Sequences	20,000 reads	Shotgun complete	BBSRC

<i>Leishmania braziliensis</i>			
Peacock et al. Nat. Genet. 39: 839-47 (2007)			
Whole genome shotgun (5x)	~33.5	High quality draft sequence	Wellcome Trust

<i>Leishmania infantum</i>			
Peacock et al. Nat. Genet. 39: 839-47 (2007)			
Whole genome shotgun (5x)	~33.5	High quality draft sequence	Wellcome Trust

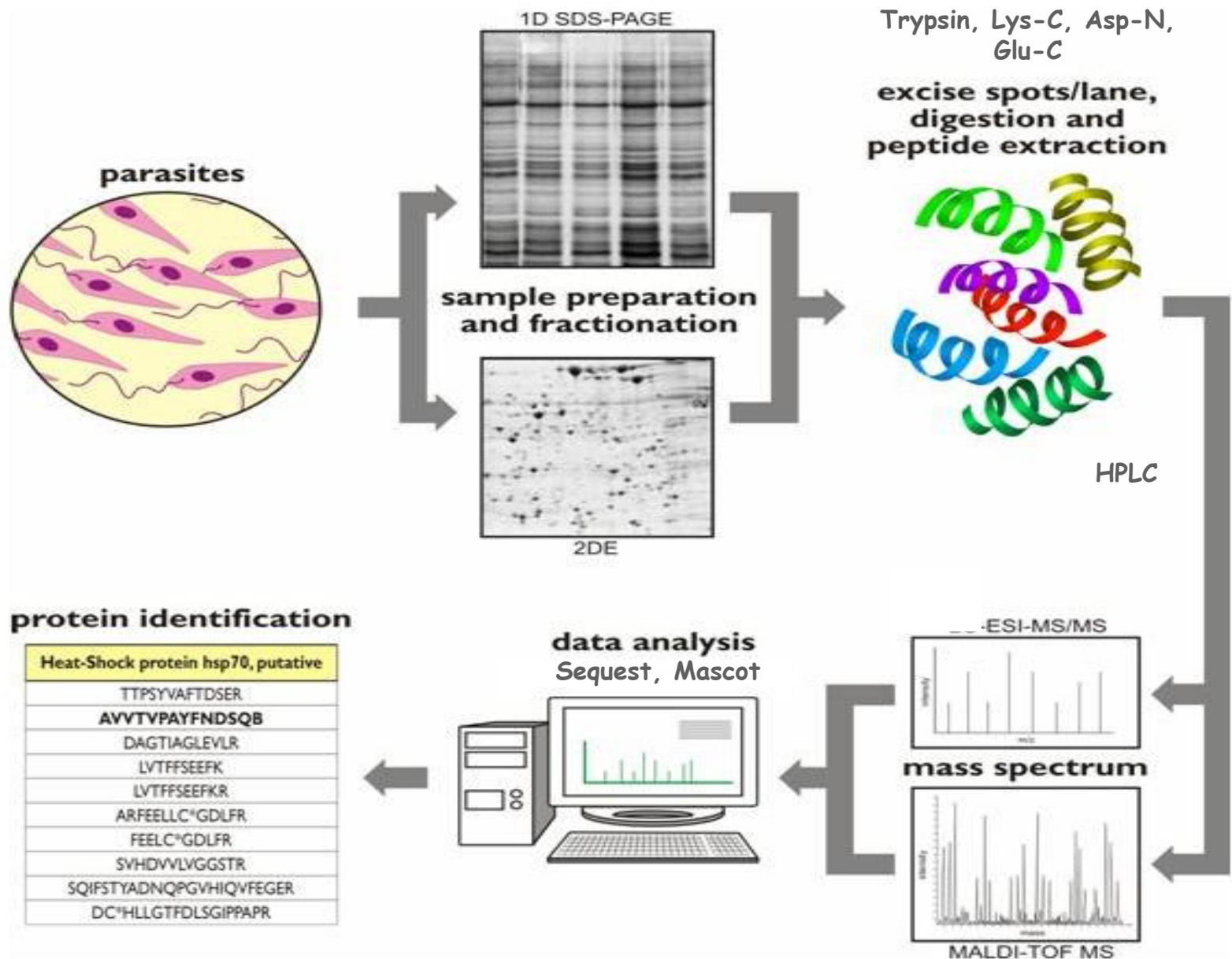
<i>Leishmania major</i>			
Ivens et al. Science 309: 438-42 (2005)			
Chromosomes 4-26,28,30-34,36	27.8	Finished (barring 2 gaps)	Beowulf Genomics/Wellcome Trust European Commission

<i>Leishmania mexicana</i>			
Whole genome shotgun (5x)	~33.5	sequencing	Wellcome Trust

<i>Leptomonas seymouri</i>			
4x coverage	~35	sequencing	Wellcome Trust

<i>Neospora caninum</i>			
whole genome shotgun	~55	Sequencing	BBSRC

Typical experiment of proteomic analysis



Proteomic studies on protozoan parasites

- ✧ Global proteome profiling
- ✧ Comparative proteomic analysis of developmental stages
- ✧ Subcellular proteome, secretome and post-translational modifications
- ✧ Drug resistance mechanisms and identification of new therapeutic targets
- ✧ Host-parasite interactions, virulence markers

Proteomic studies on protozoan parasites

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Leishmania spp.

*Trypanosoma
cruzi*

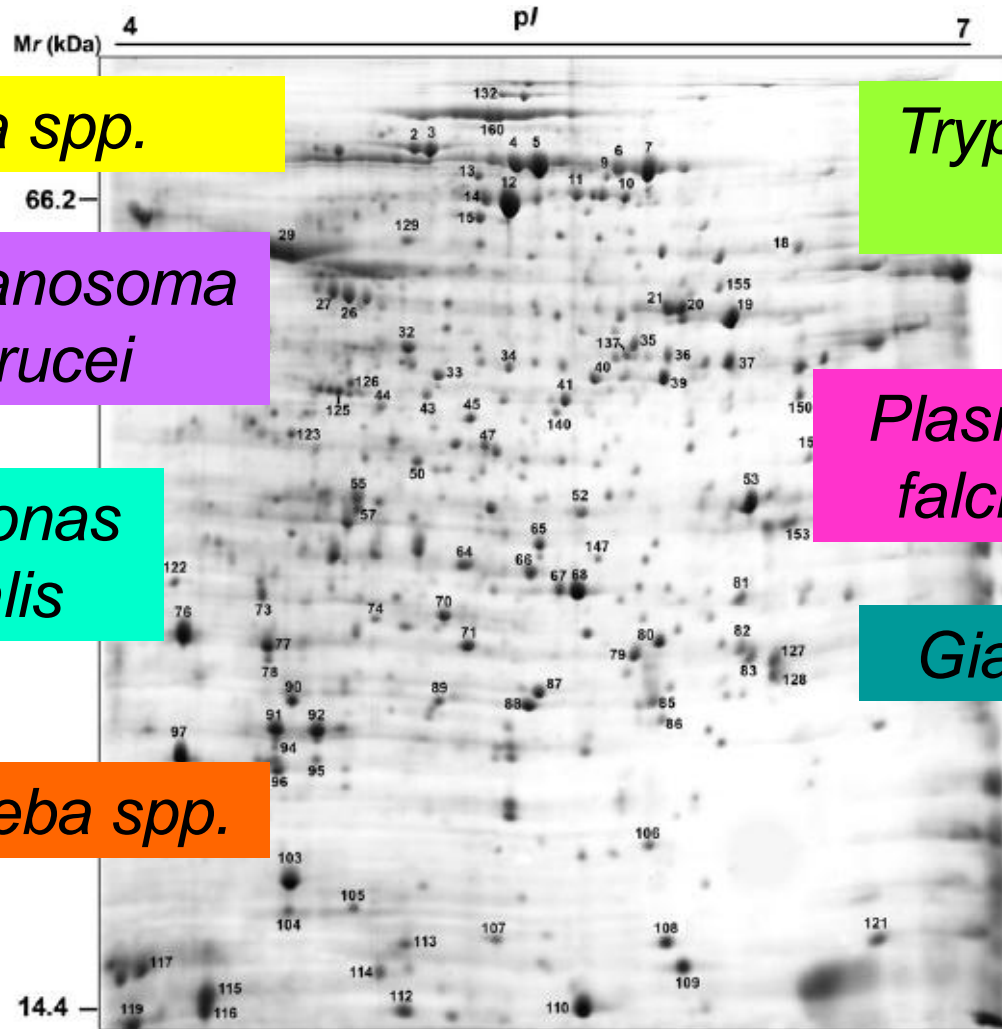
*Trypanosoma
brucei*

*Trichomonas
vaginalis*

*Plasmodium
falciparum*

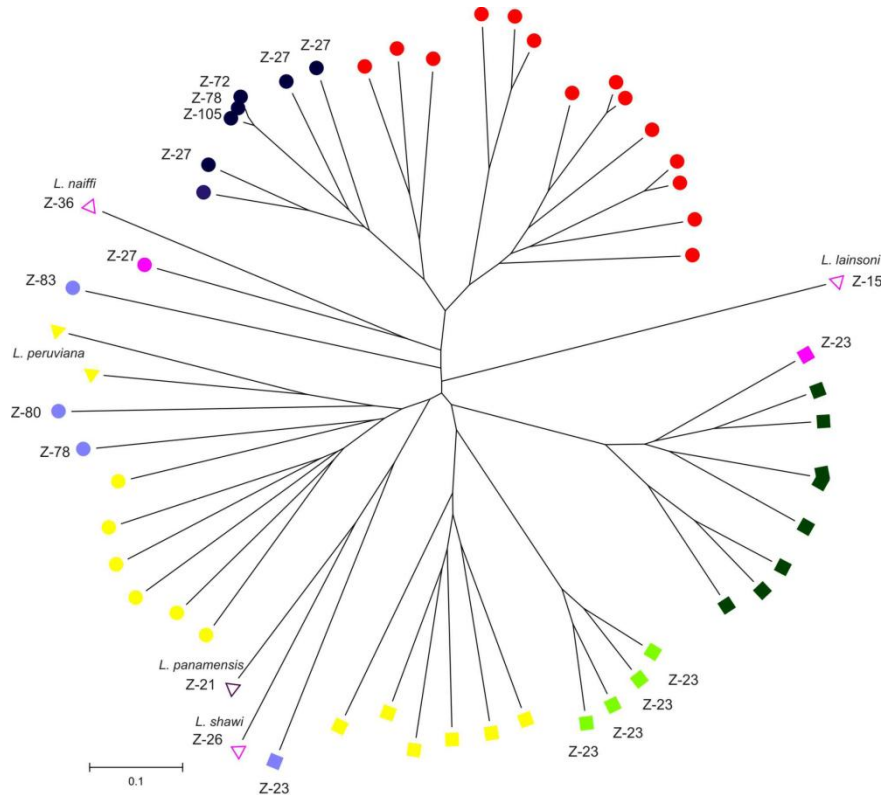
Entamoeba spp.

Giardia spp.



Leishmania (Viannia) braziliensis

Molecular polymorphism



MICROSATELLITES

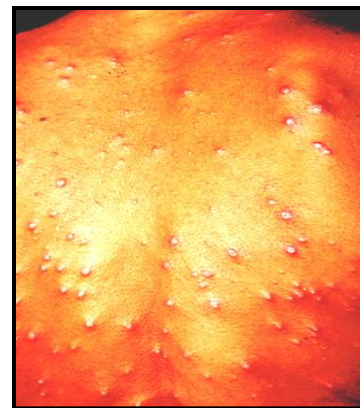
Clinical pleomorphism

Cutaneous lesion



de Oliveira-Neto *et al.* 2000

Disseminated form



Turetz *et al.* 2002



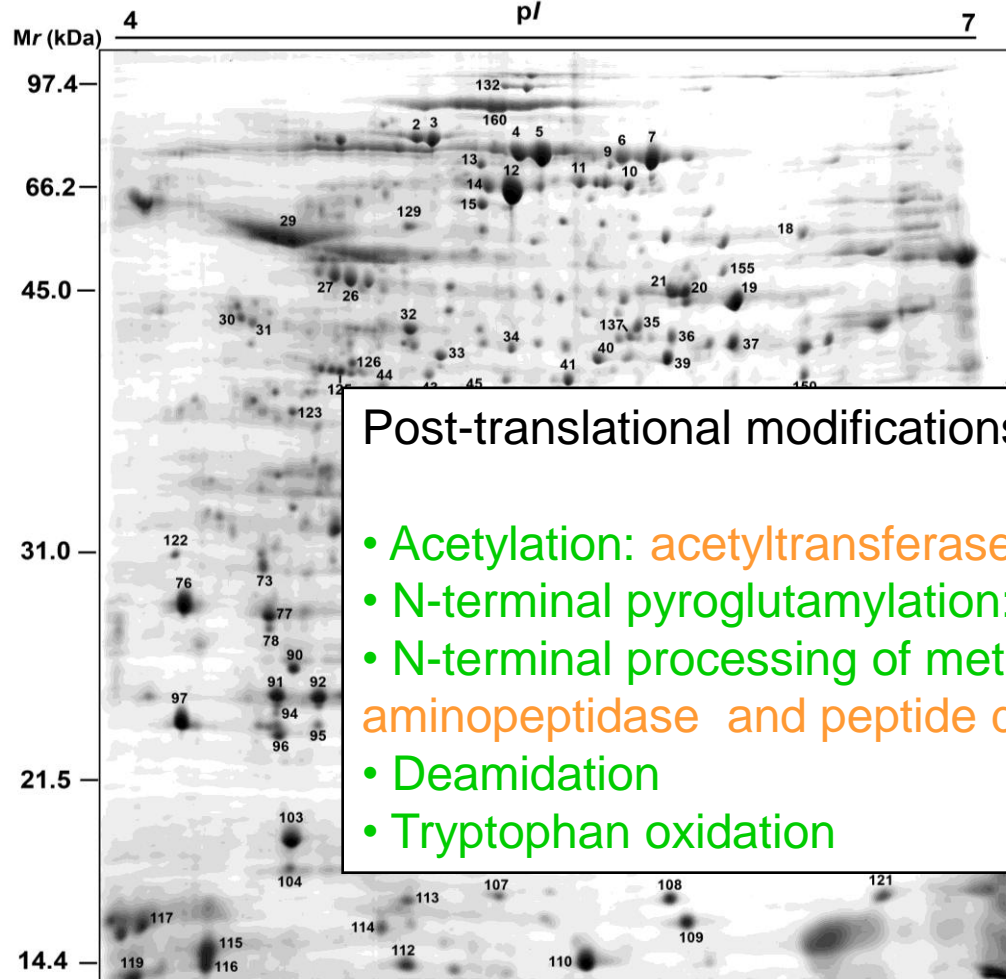
Chappuis *et al.* 2007

Mucosal lesion

Proteome analysis of *Leishmania (Viannia) braziliensis* by two-dimensional gel electrophoresis and mass spectrometry

Patricia Cuervo^{a,b,c}, Jose Batista de Jesus^d, Magno Junqueira^e, Leila Mendonça-Lima^d,
Luis Javier González^f, Lázaro Betancourt^f, Gabriel Grimaldi Jr.^a,
Gilberto Barbosa Domont^e, Octavio Fernandes^b, Elisa Cupolillo^{a,*}

Molecular & Biochemical Parasitology 154 (2007) 6–21

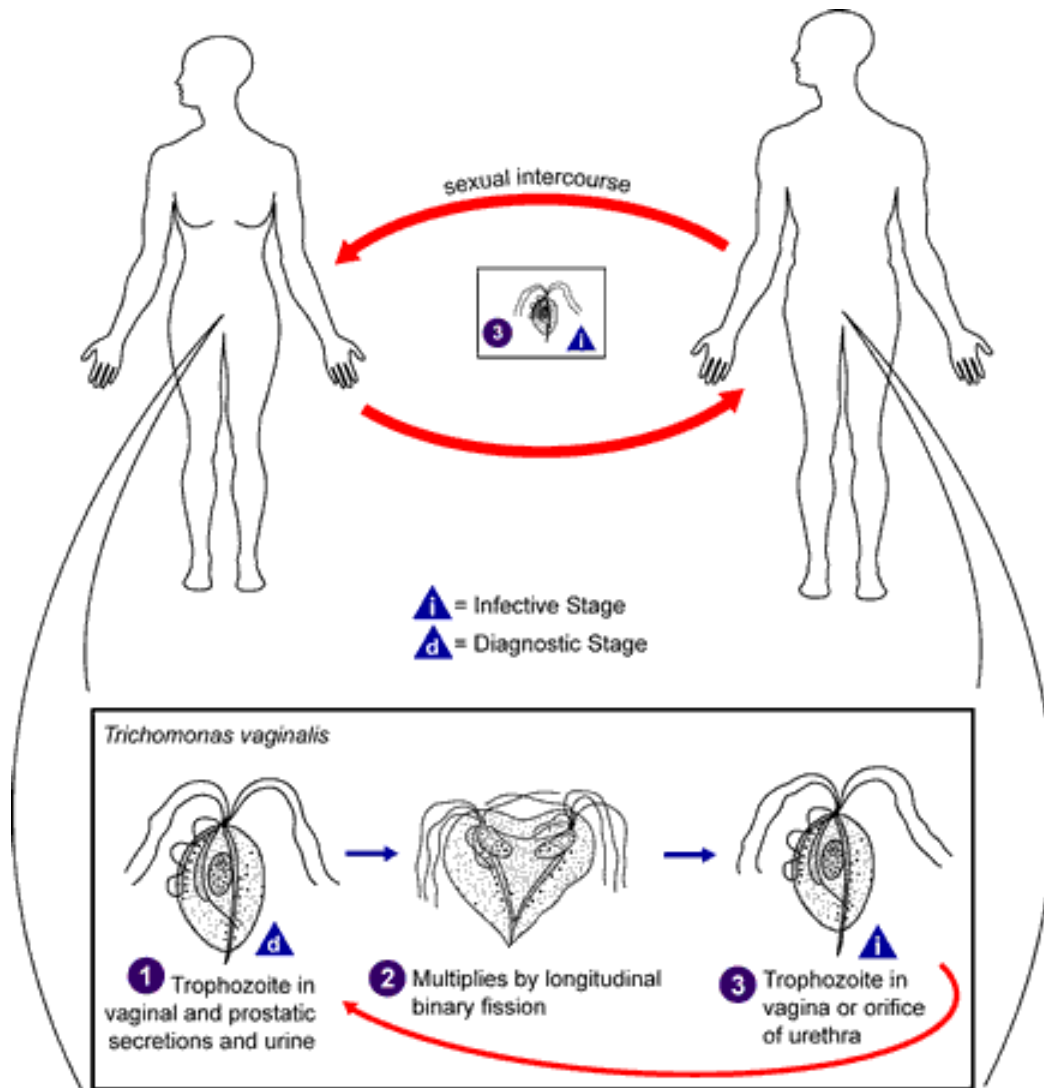


~20% of identified proteins
contain modified peptides

Post-translational modifications:

- Acetylation: acetyltransferases
- N-terminal pyroglutamylation: glutaminyl cyclase
- N-terminal processing of methionine: methionine aminopeptidase and peptide deformylase
- Deamidation
- Tryptophan oxidation

Trichomonas vaginalis



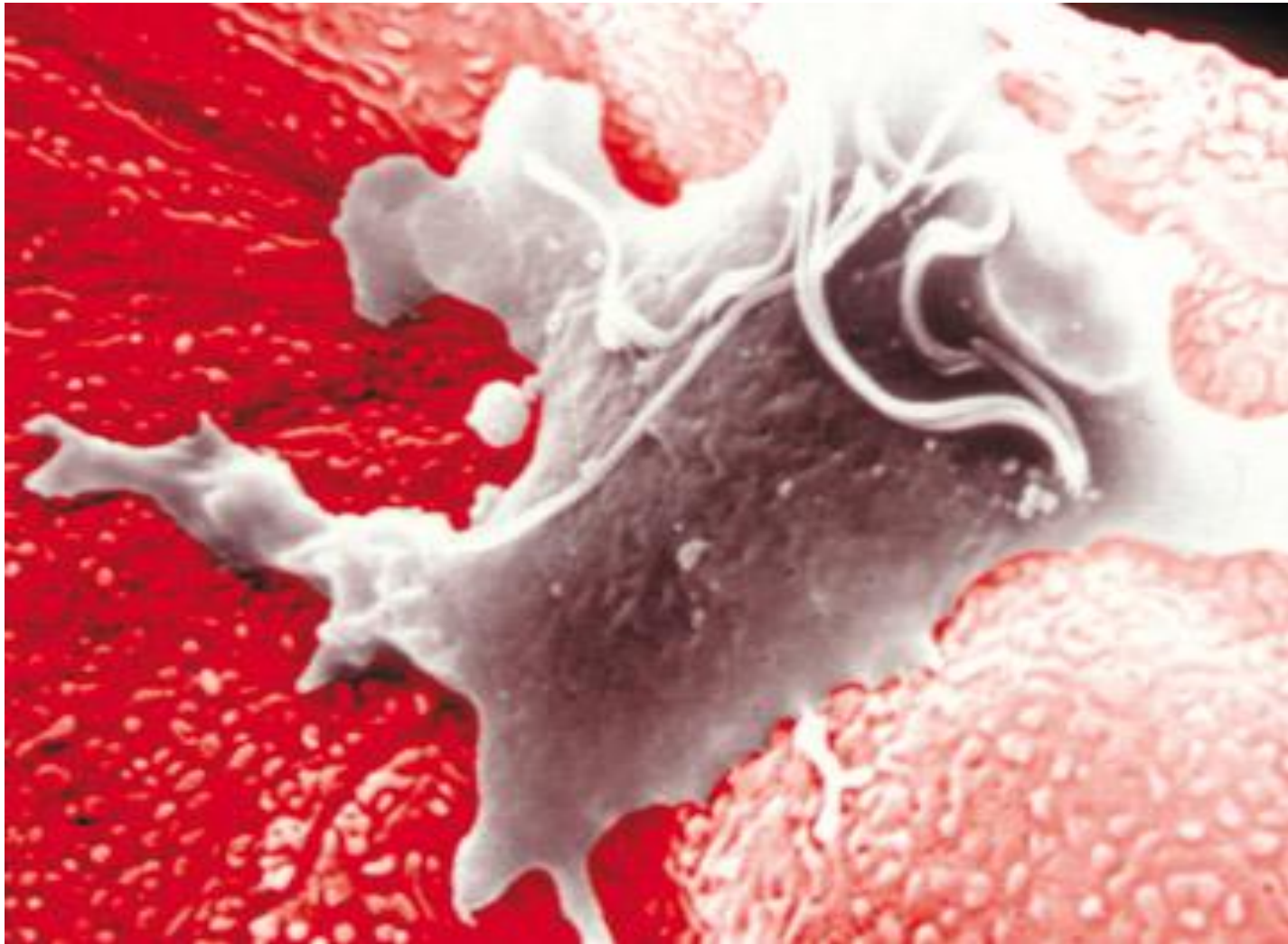
More than 180 million cases/year around the world

Symptomatic women

- Important cofactor in amplification of HIV transmission
- May induce rupture of placental membranes
- Pre-term delivery
- Low-birth-weight resulting in an increase in infant mortality

Human Urogenital Trichomoniasis

Trichomonas vaginalis

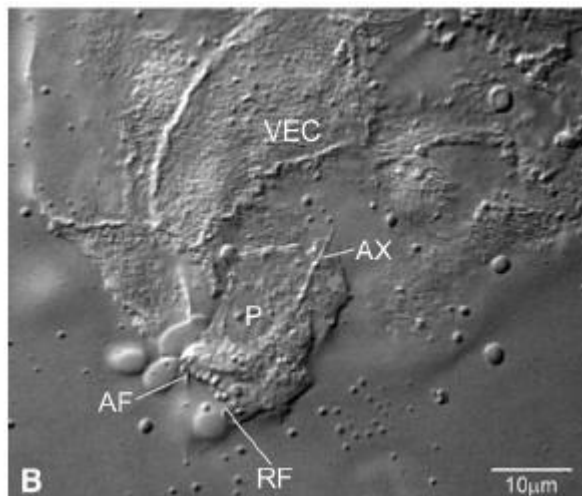
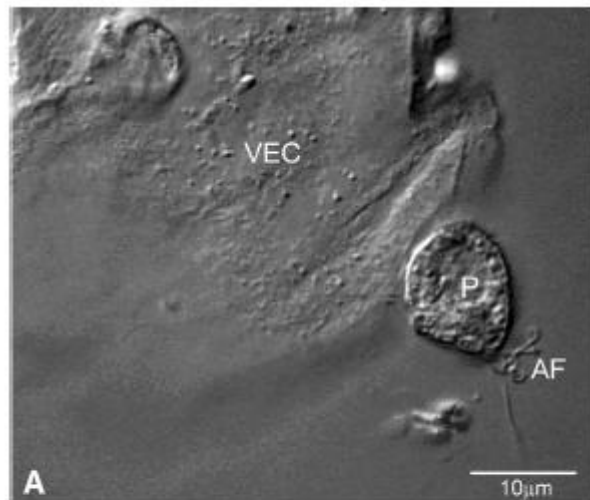


In contact with epithelial cells: ellipsoid → amoeboid

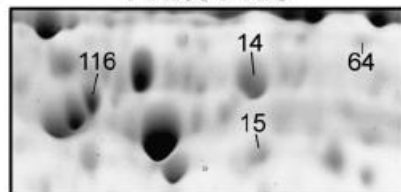
Differential soluble protein expression between *Trichomonas vaginalis* isolates exhibiting low and high virulence phenotypes

Patrícia Cuervo^a, Elisa Cupolillo^a, Constança Britto^b, Luis Javier González^c,
Fernando Costa e Silva-Filho^d, Letícia Coutinho Lopes^e,
Gilberto Barbosa Domont^{f,*}, Jose Batista De Jesus^{b,9,*}

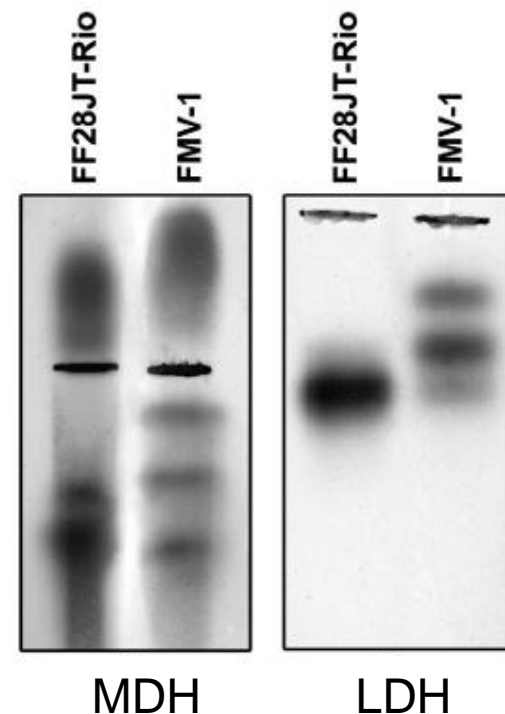
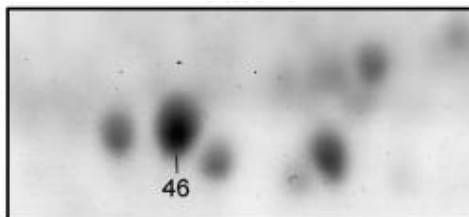
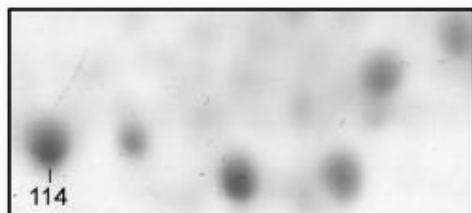
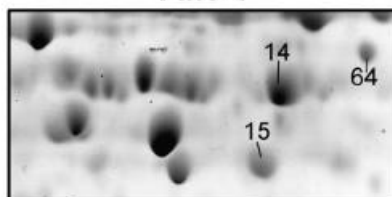
JOURNAL OF PROTEOMICS 71 (2008) 109–122



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FMV-1



Proteomic studies on protozoan parasites

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- ✧ Comparative proteomic analysis of developmental stages
- ✧ Subcellular proteome, secretome and post-translational modifications
- ✧ Drug resistance mechanisms and identification of new therapeutic targets
- ✧ Host-parasite interactions, virulence markers

A proteomic view of the *Plasmodium falciparum* life cycle

NATURE | VOL 419 | 3 OCTOBER 2002 |

Laurence Florens*, Michael P. Washburn†, J. Dale Raine‡, Robert M. Anthony§, Munira Grainger||, J. David Haynes§¶, J. Kathleen Moch§, Nemone Muster*, John B. Sacci§#, David L. Tabb*☆, Adam A. Witney§#, Dirk Wolters†#, Yimin Wu**, Malcolm J. Gardner††, Anthony A. Holder||, Robert E. Sinden‡, John R. Yates*† & Daniel J. Carucci§

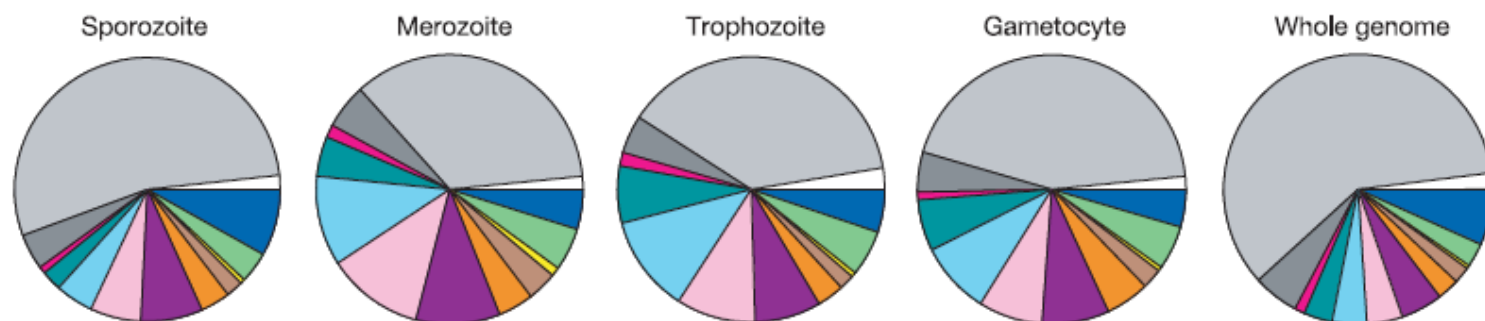


Table 2 Examples on enzymes in stage-specific metabolic pathways

Locus	Stage				Enzyme	EC number†	Reaction catalysed
	Spz*	Mrz*	Tpz*	Gmt*			
End of glycolysis							
PF10_0363	1.2	–	2.4	–	Pyruvate kinase	2.7.1.40	<i>P</i> -enolpyruvate to pyruvate
MAL6P1.160	8.6	66.9	18.8	14.7	Pyruvate kinase		
PF13_0141	46.2	83.9	70.9	78.8	L-lactate dehydrogenase	1.1.1.27	Pyruvate to lactate
TCA cycle and oxidative phosphorylation							
PF10_0218	12.3	–	–	–	Citrate synthase	4.1.3.7	Acetyl coA + oxaloacetate to citrate
PF13_0242	3.2	–	16.9	8.8	Isocitrate dehydrogenase (NADP)	1.1.1.41	Isocitrate to 2-oxoglutarate + CO ₂
PF08_0045	2.9	–	2.2	23.1	2-Oxoglutarate dehydrogenase e1 component	1.2.4.2	2-Oxoglutarate to succinyl CoA
PF10_0334	–	–	3.5	27.7	Flavoprotein subunit of succinate dehydrogenase	1.3.5.1	Succinate to fumarate
PFL0630w	3.7	–	–	12.1	Iron-sulphur subunit of succinate dehydrogenase		
PF14_0373	–	–	–	12.7	Ubiquinol cytochrome oxidoreductase	1.10.2.2.	Ubiquinol to cytochrome c reductase in electron transport
PFB0795w	–	–	–	14.2	ATP synthase F1, α -subunit		
PFI1365w	–	–	–	8.8	Cytochrome c oxidase subunit	1.9.3.1	
PFI1340w	–	–	–	8.8	Fumarate hydratase	4.2.1.2	Fumarate to malate
MAL6P1.242	30.4	–	–	40.9	Malate dehydrogenase	1.1.1.37	Malate to oxaloacetate

Plasmodium metabolic pathways can be found at <http://www.sites.huji.ac.il/malaria/>. Spz, sporozoite; mrz, merozoite; tpz, trophozoite; gmt, gametocyte.

*The sequence coverage (that is, the percentage of the protein sequence covered by identified peptides) measured in each stage is reported.

†Enzyme Commission (EC) numbers are reported for each protein.

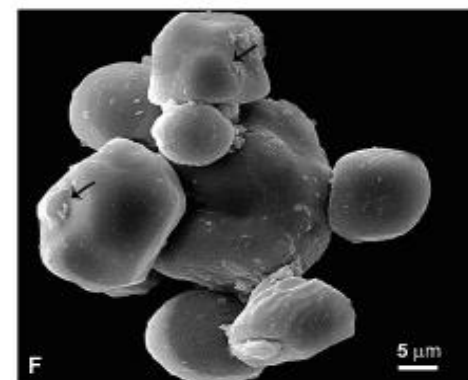
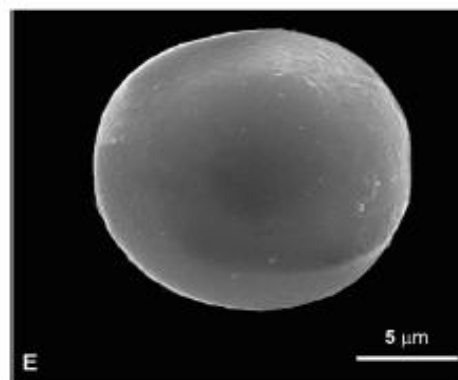
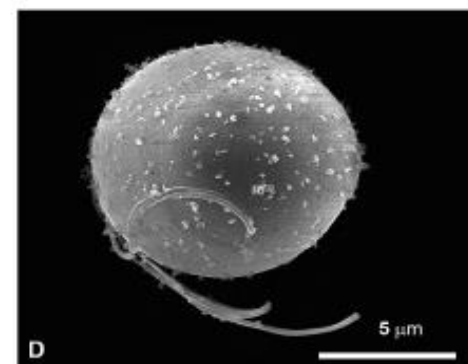
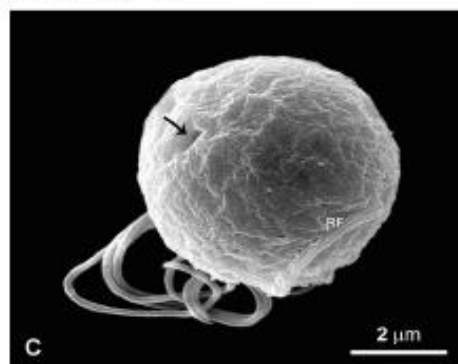
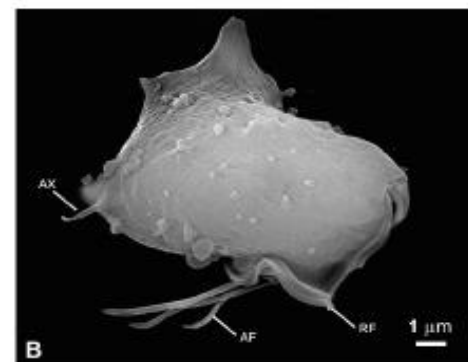
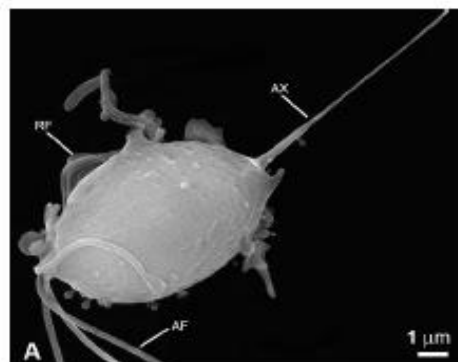
A further proteomic study on the effect of iron in the human pathogen *Trichomonas vaginalis*

Jose Batista De Jesus¹, Patricia Cuervo^{2,3}, Magno Junqueira⁴, Constança Britto¹,
Fernando Costa e Silva-Filho⁵, Maurílio Jose Soares⁶, Elisa Cupolillo²,
Octavio Fernandes³ and Gilberto Barbosa Domont⁴

Proteomics 2007, 7, 1961–1972

Figure 2. Scanning electron microscopy of *T. vaginalis* FF28Jt-Rio strain cultured by 48 h at 37°C. (A-B) Amoeboid and oval trophozoites commonly found in axenic iron-rich medium. (C-F) Morphological transformation to spherical shapes observed after culture in axenic iron-depleted medium. (C-D) Micrographs show internalization process of anterior and recurrent flagella. (C) The axostyle is not seen in the rounded cells, and an arrow shows the possible location of axostyle retraction. (F) Some irregular forms with internalized flagella, without external visible axostyle and presenting protuberances (arrows), are also observed. AX, axostyle; AF, anterior flagella; RF, recurrent flagellum.

Trophozoites

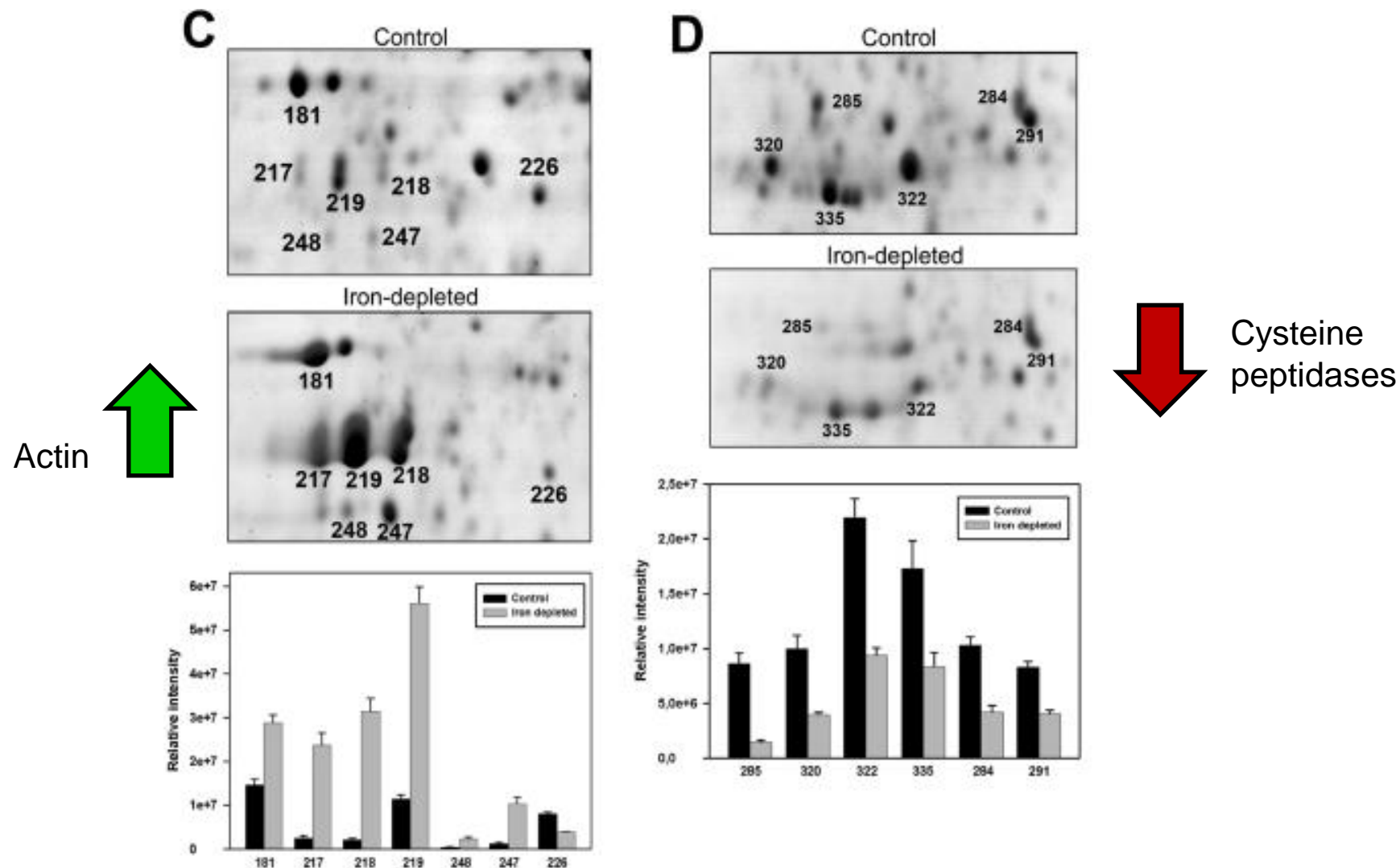


Pseudocysts

A further proteomic study on the effect of iron in the human pathogen *Trichomonas vaginalis*

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Proteomics 2007, 7, 1961–1972



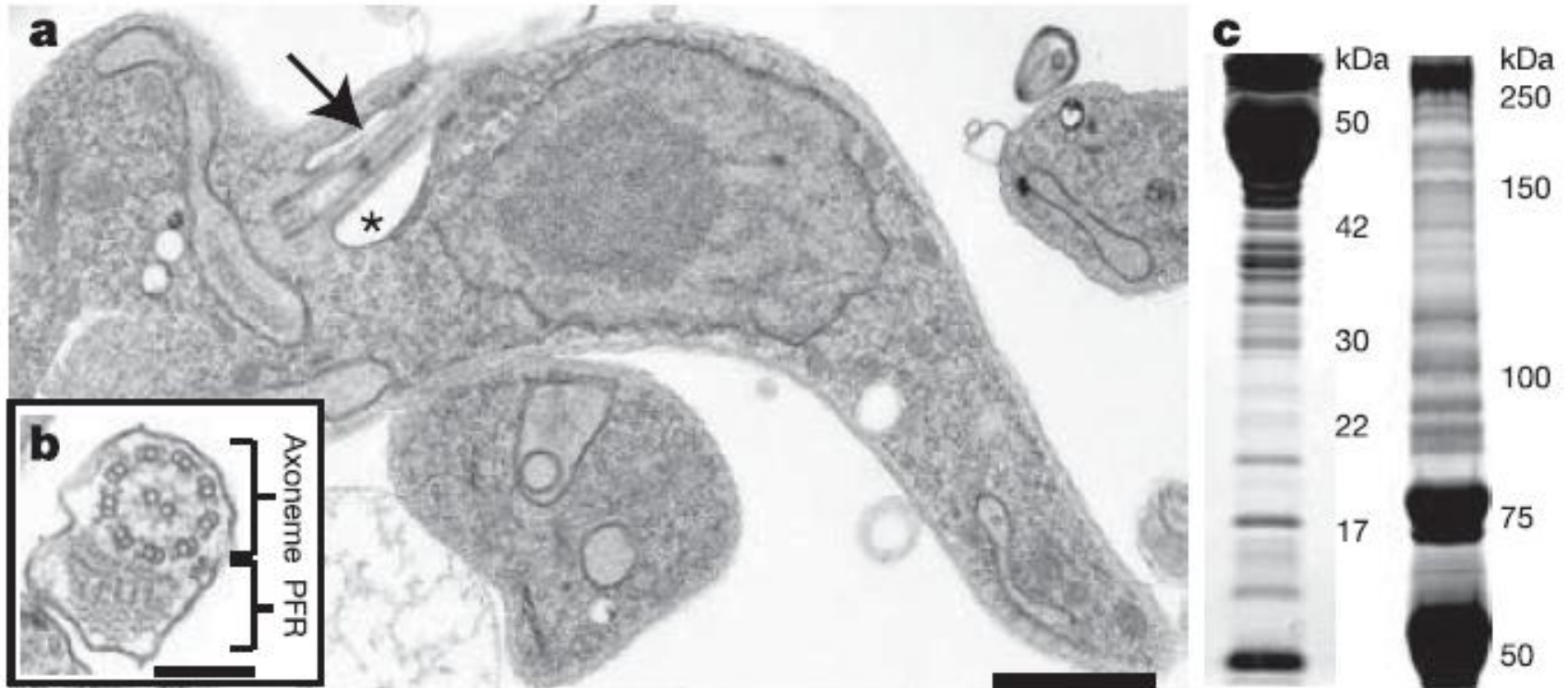
Proteomic studies on protozoan parasites

- ★ Global proteome profiling
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Flagellar motility is required for the viability of the bloodstream trypanosome

Richard Broadhead^{1*}, Helen R. Dawe^{2*}, Helen Farr^{2*}, Samantha Griffiths^{2*}, Sarah R. Hart^{3*}, Neil Portman^{2*}, Michael K. Shaw², Michael L. Ginger², Simon J. Gaskell³, Paul G. McKean¹ & Keith Gull²

Nature 2006; 440(7081):224-7.

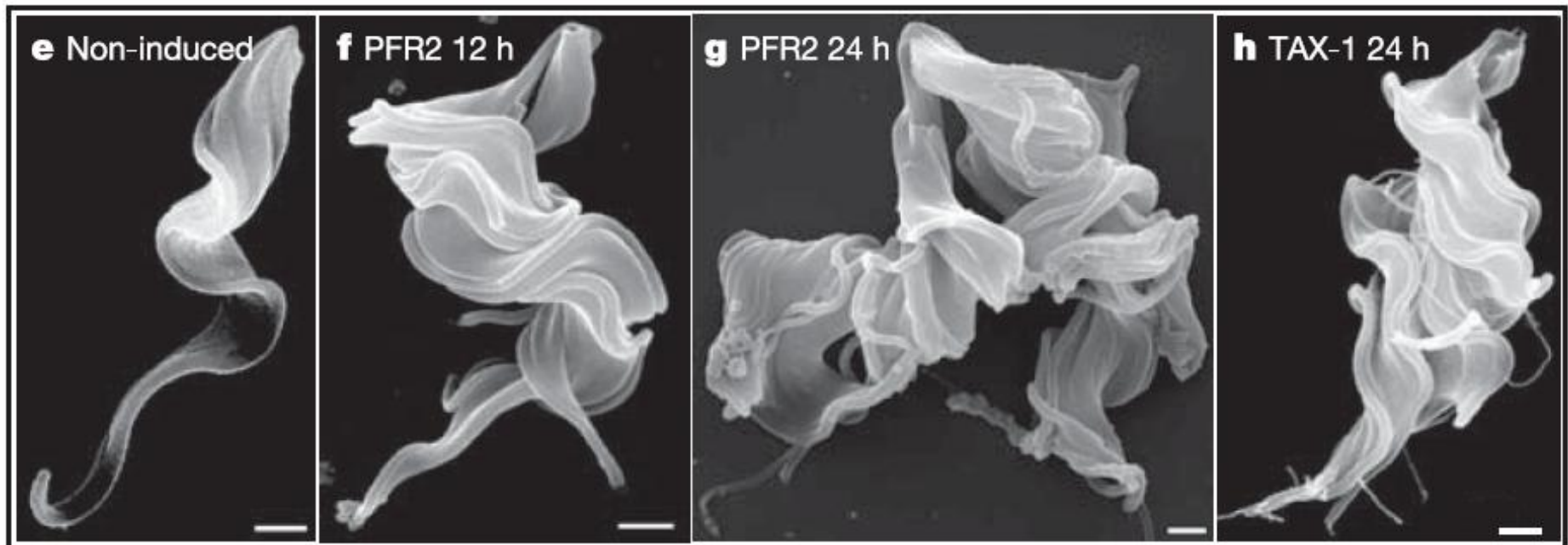


Proteomic analysis

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Lethal failure of cytokinesis

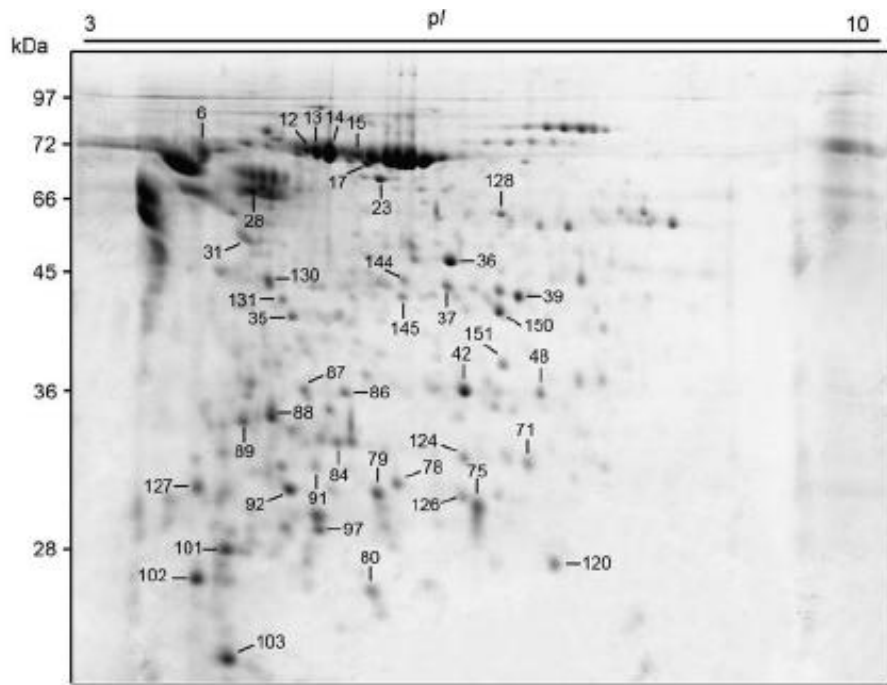
RNAi

Proteomic characterization of the released/secreted proteins of *Leishmania (Viannia) braziliensis* promastigotes

Patricia Cuervo^{a,*}, Jose B. De Jesus^{b,c,f}, Leonardo Saboia-Vahia^{b,e}, Leila Mendonça-Lima^e,
Gilberto B. Domont^d, Elisa Cupolillo^{a,*}

JOURNAL OF PROTEOMICS 73 (2009) 79–92

Putative virulence factors detected in the extracellular medium collected from promastigotes of *L. (V.) braziliensis*



Immunomodulating proteins

Cyclophilin a	XP_001561947
IgE-dependent histamine-releasing factor, putative	XP_001565400

Signal Transduction proteins

Histidine secretory acid phosphatase	XP_001569291
Activated protein kinase C receptor LACK	XP_001566321
Pyruvate kinase	XP_843114

Intracellular survival

Proteasome activator protein pa26, putative	XP_001568187
Heat shock protein hsp70	XP_001566325
Proteasome alpha 1 subunit, putative	XP_001568579
Enolase	XP_001563419
Proteasome alpha 5 subunit, putative	XP_001567859
Putative elongation factor 1 beta	XP_001564363
Heat shock protein hsp70 – related protein.	XP_001566867
Peroxiredoxin	XP_001562236
Tryparedoxin peroxidase 2	AAV31765

Proteomic characterization of the released/secreted proteins of *Leishmania (Viannia) braziliensis* promastigotes

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JOURNAL OF PROTEOMICS 73 (2009) 79–92

1 – Peptides automatically identified in the Mascot® database by using the GPS Explorer™ Software, and confirmed by manual sequencing.

Protein name	NCBI accession no.	Mr theor. da (Mr exp. kDa)	pI theor. (pI exp.)	Pep. identified by MS/MS	Pep. sequence■	Error± da	Ion Score	Protein Score	Signal P prediction ^c	Secretome P prediction ^d	Target P prediction ^e
Histidine secretory acid phosphatase, putative	XP_001569291	48438 (70.8)	5.19 (4.29)	3	YNDPSLVESPLFPSTR	0.10	69	104	No	Yes (0.710)	NSS
					ADC*VLNLYDVAAAFEASGR	0.11	23				
					TIADNEPVPLR	0.07	13				
Heat-shock protein hsp70, putative	XP_001566325	71232 (71.9)	5.40 (5.09)	4	LVTFFSEEFKR	0.05	17	188	No	Yes G– (0.526)	NSS
					FEELC*GDLFR	0.05	25				
					<u>SQIFSTYADNQPGVHIQVFEGER</u>	0.11	55				
					DC*HLLGTFDLSGIPPAPR	0.08	93				
Protein disulfide isomerase	XP_001569341	52357 (53.5)	5.02 (4.41)	4	GFPTLYVFR	0.06	34	101	Yes (0.999)	No	S (0.76:
					SQVLM*TYIDGDQYRPVSR	0.10	11				
					QLGIPEGAEFFAFVIDHDR	0.09	40				
					<u>QLGIPEGAEFFAFVIDHDR</u>	0.11	18				
Pyruvate dehydrogenase E1 beta subunit, putative	XP_001565729	36269 (40.9)	5.23 (5.02)	4	DAIQSALDEELAR	0.08	60	181	No	Yes (0.789)	NSS
					<u>VFVIGEEVAQYQGAYK</u>	0.10	65				
					<u>DITLIGFSR</u>	0.06	20				
					LAAEGVOAEVINLR	0.08	36				
Activated protein kinase c receptor (LACK)	XP_001566321	34424 (35.3)	5.94 (5.46)	8	GWVTSLAC*PQQAGSYIK	0.08	46	350	No	Yes G–/G+ (0.872/0.665)	NSS
					HSVDSDYGLPNHR	0.07	32				
					DVLAVAFSPDDR	0.05	59				

5% secreted by classic pathway

Unconventional secretion mechanisms

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A Proteomics Screen Implicates HSP83 and a Small Kinetoplastid Calpain-related Protein in Drug Resistance in *Leishmania donovani* Clinical Field Isolates by Modulating Drug-induced Programmed Cell Death*

Baptiste Vergnes†§, Benjamin Gourbal†¶, Isabelle Girard‡, Shyam Sundar||, Jolyne Drummelsmith†**, and Marc Ouellette‡ ††

HSP83 interferes with mitochondrial membrane potential increasing antimonials resistance and reducing drug-mediated programmed cell death.

SKCRP14.1 promotes antimonial-induced programmed cell death, but protects against miltefosine-mediated PCD.

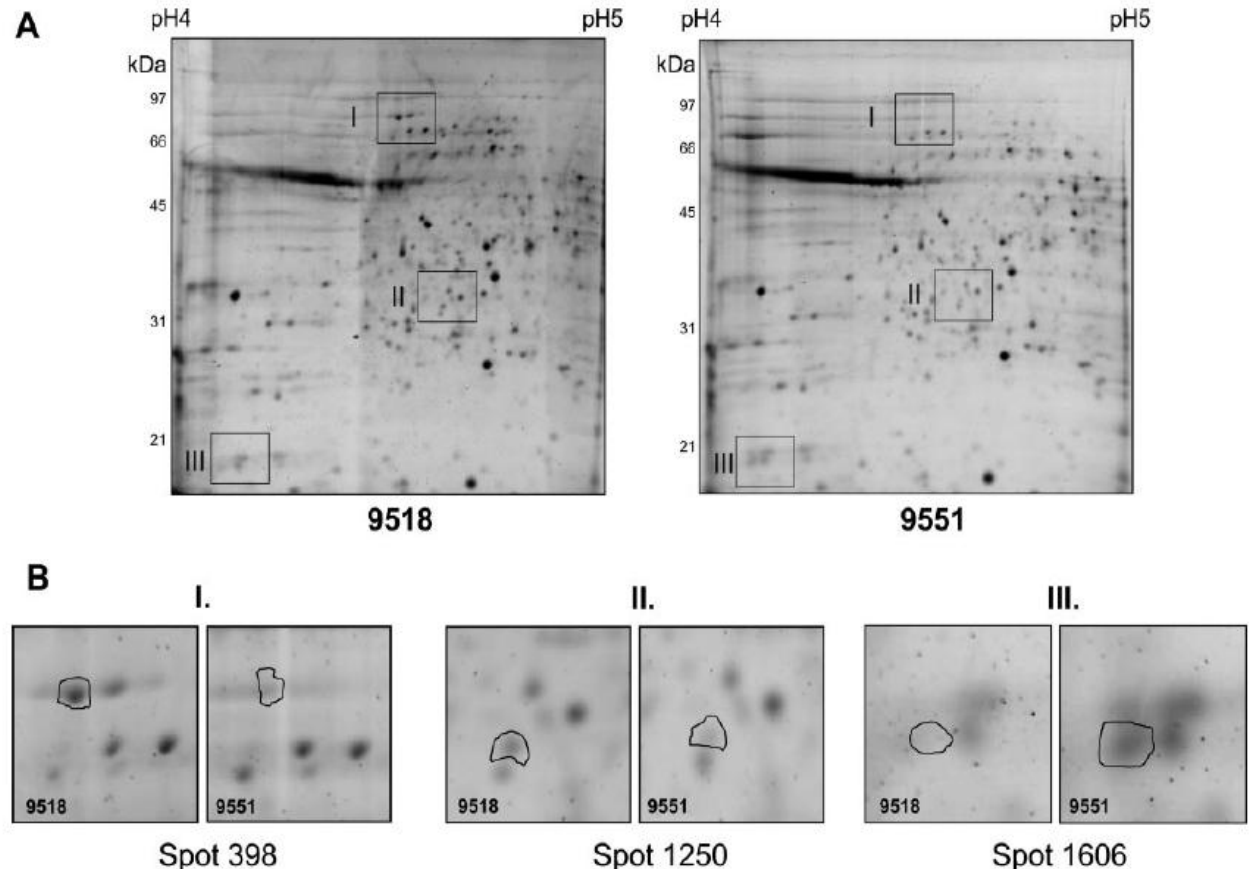


FIG. 2. Proteomics analyses of resistance in *L. donovani*. A, representative 2D gels (pH 4–5) comparing *L. donovani* 9518 resistant and *L. donovani* 9551 sensitive strains. B, insets (I–III) corresponding to zoomed gel areas with spots showing a difference in protein expression between the two strains.

Proteomic studies on protozoan parasites

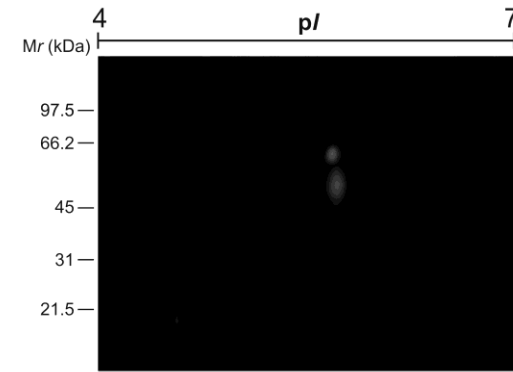
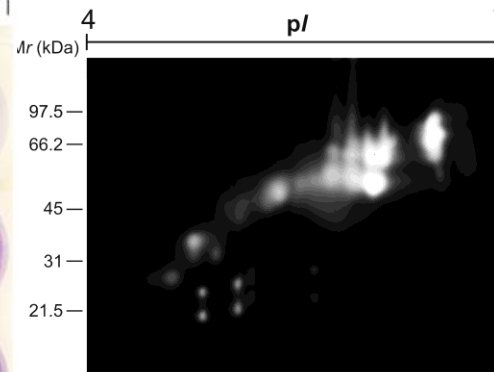
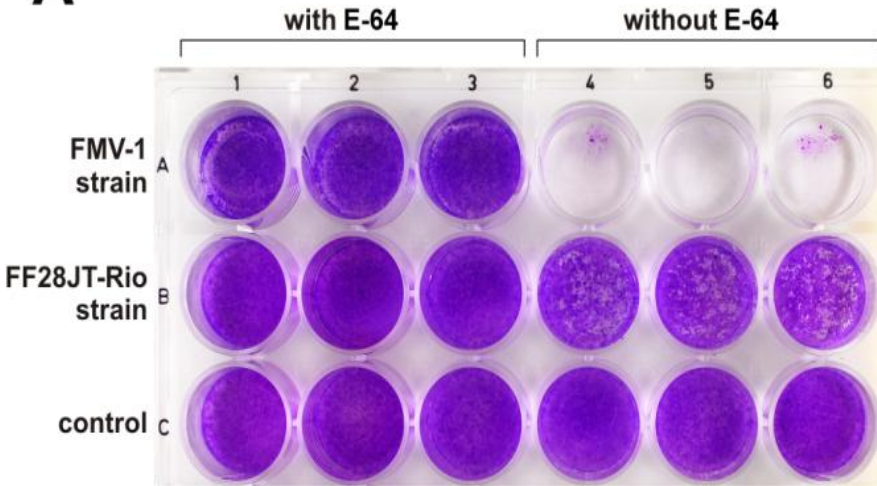
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- ✧ Host-parasite interactions, virulence markers

Cysteine Peptidase Expression in *Trichomonas vaginalis* Isolates Displaying High- and Low-Virulence Phenotypes

J. Proteome Res., 2009, 8 (3), 1555-1564•

Jose Batista De Jesus, Patrícia Cuervo, Constança Britto, Leonardo Sabóia-Vahia, Fernando Costa e Silva-Filho, Andre Borges-Veloso, Débora Barreiros Petrópolis, Elisa Cupolillo, and Gilberto Barbosa Domont.

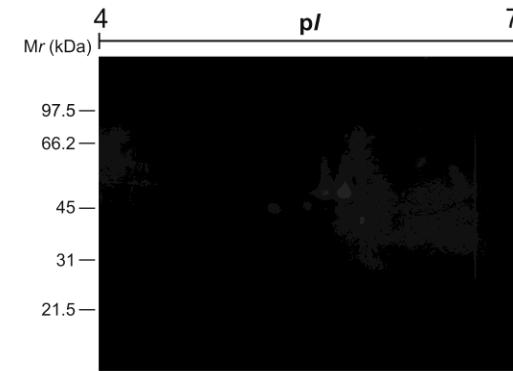
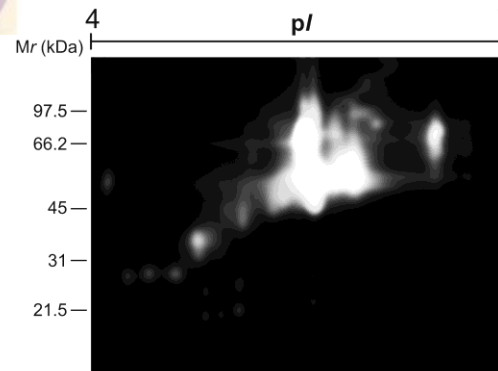
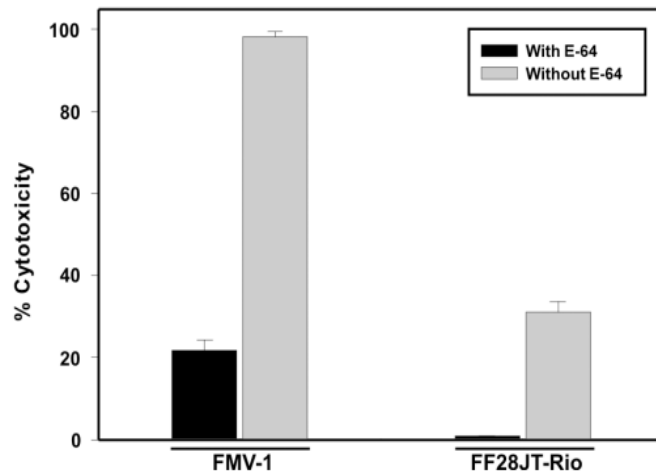
A



A

B

B



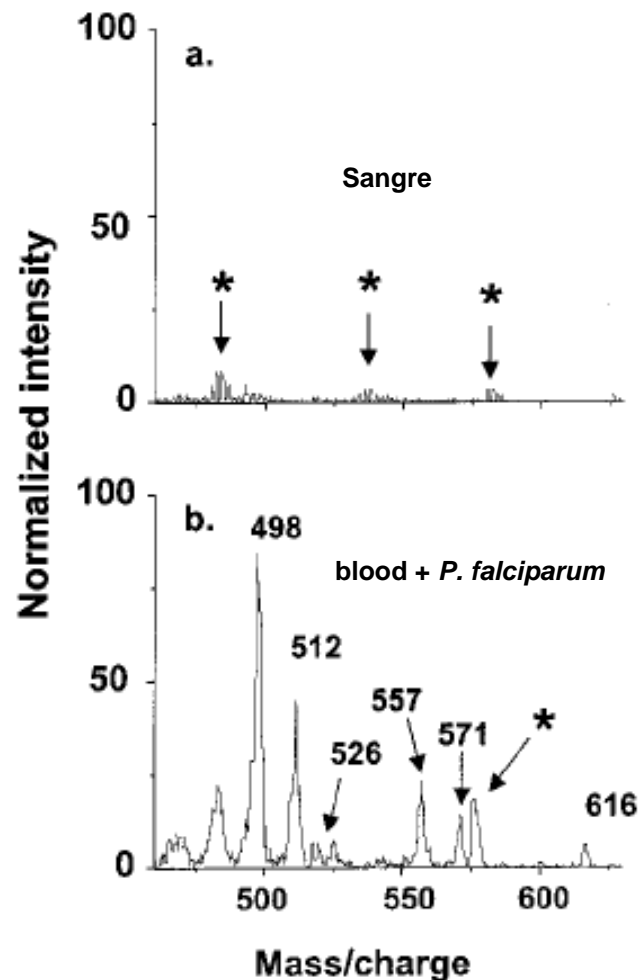
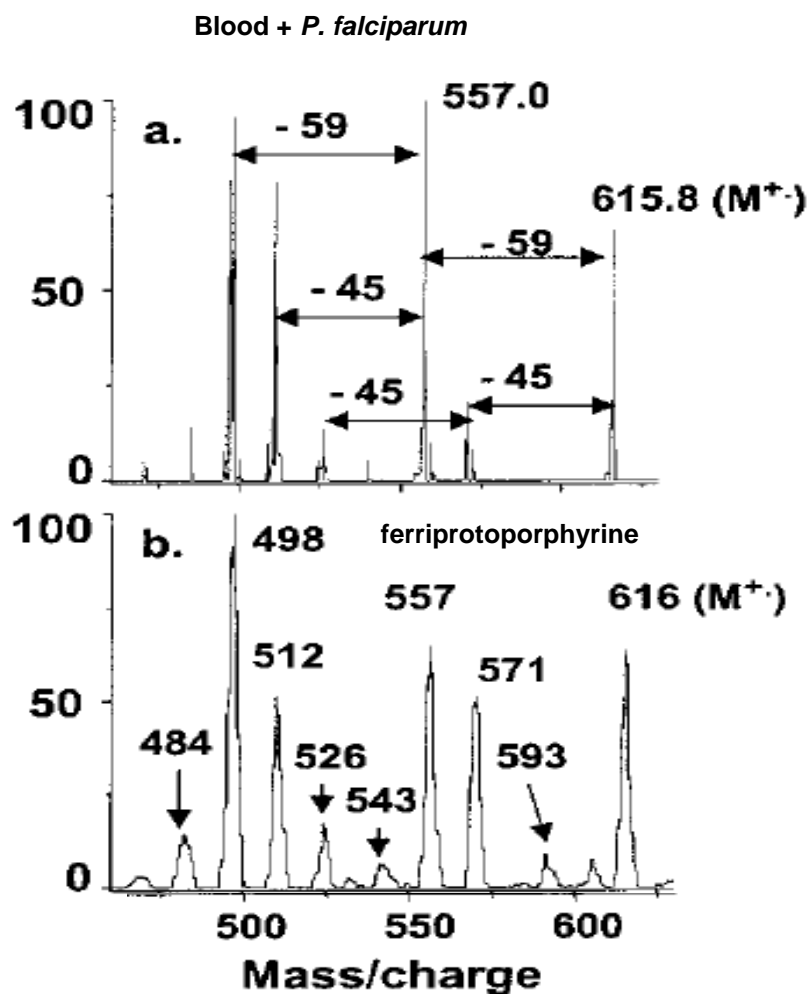
C

D

T. vaginalis papain-like CPs are the main factors involved in the cellular damage. CP4 isoform is a potential virulence marker

Detection of Malaria Parasites in Blood by Laser Desorption Mass Spectrometry

P. A. Demirev,^{*,†} A. B. Feldman,[†] D. Kongkasuriyachai,[‡] P. Scholl,[†] D. Sullivan, Jr.,[‡] and N. Kumar[‡]



RAPID DETECTION OF MALARIA INFECTION *IN VIVO* BY LASER DESORPTION MASS SPECTROMETRY

PETER F. SCHOLL,* DARIN KONGKASURIYACHAI,* PLAMEN A. DEMIREV, ANDREW B. FELDMAN, JEFFREY S. LIN, DAVID J. SULLIVAN, JR., AND NIRBHAY KUMAR

Am. J. Trop. Med. Hyg., 71(5), 2004, pp. 546–551

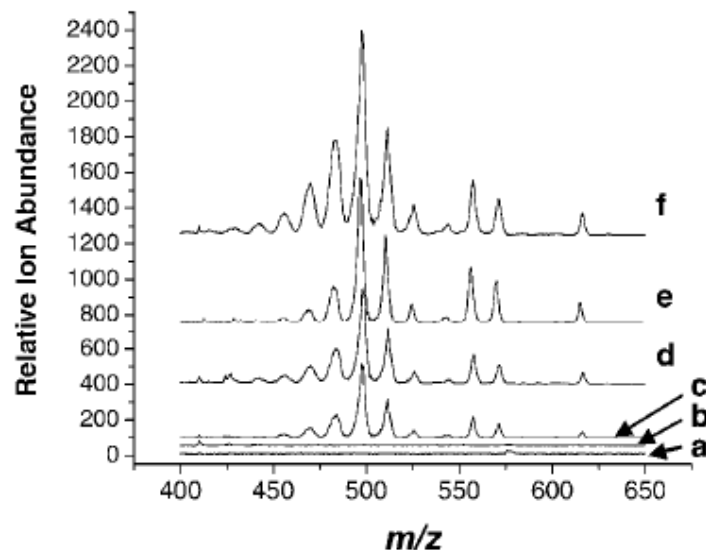


FIGURE 1. Laser desorption mass spectra of a heme standard and blood samples from a mouse inoculated with 10^6 parasite-infected erythrocytes. The heme molecular ion (m/z 616, M^+) fragments via consecutive cleavages of the two propionic acid side chains to yield m/z 571 (M-COOH), 557 (M-CH₂COOH), 512 (M-CH₂COOH, -COOH), and 498 (M-CH₂CH₂COOH, -COOH). a, Day 0, preinfection; b, Day 1; c, Day 2; d, Day 4; e, Day 6; f, Heme standard, 1.25 pmol. Each spectrum represents the sum of 300 individual laser shot spectra.

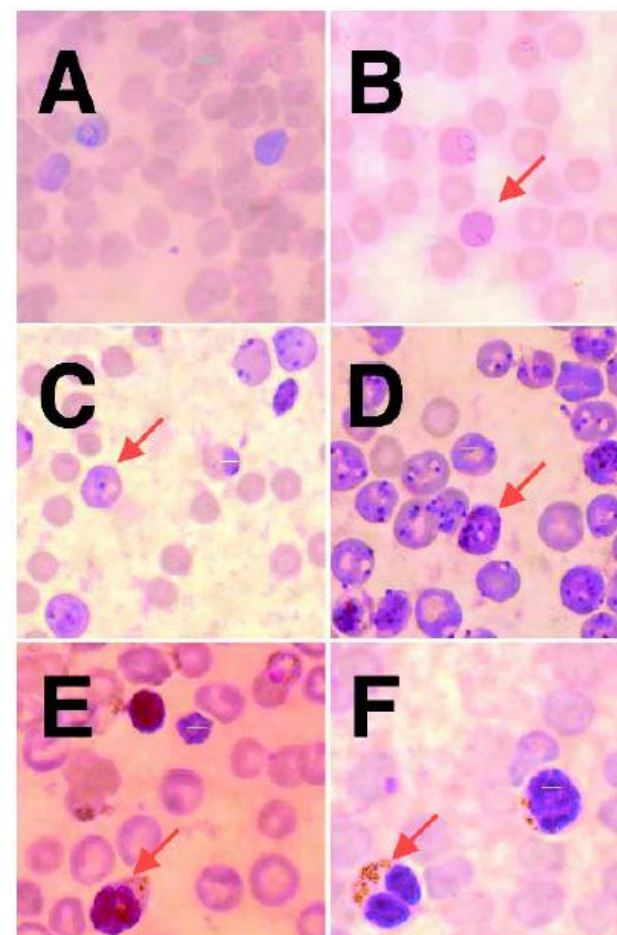
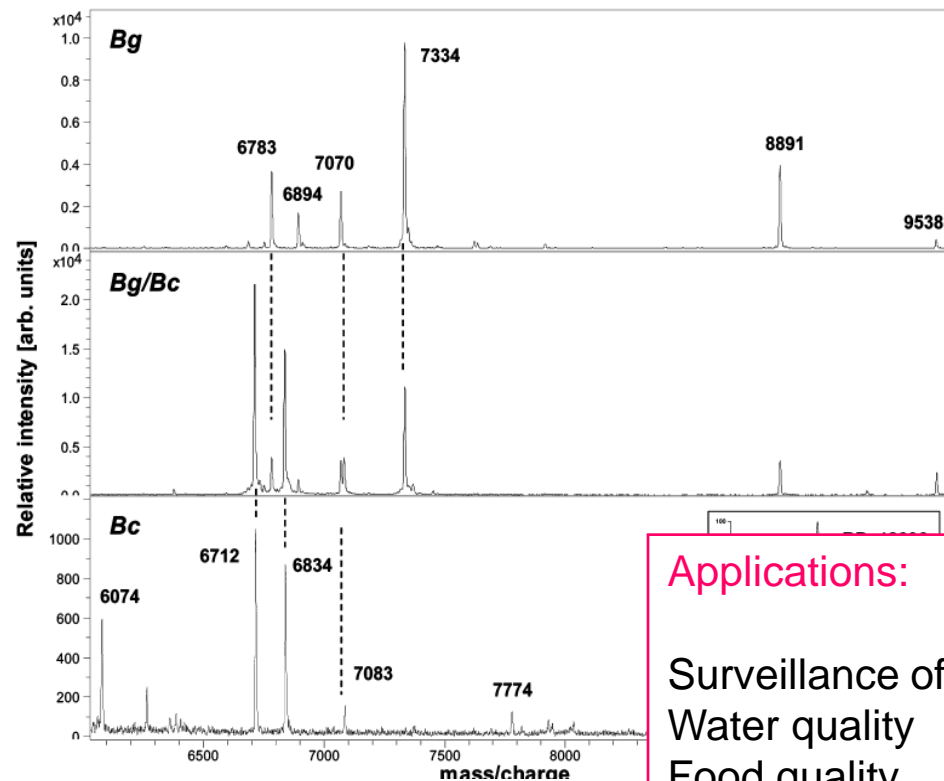
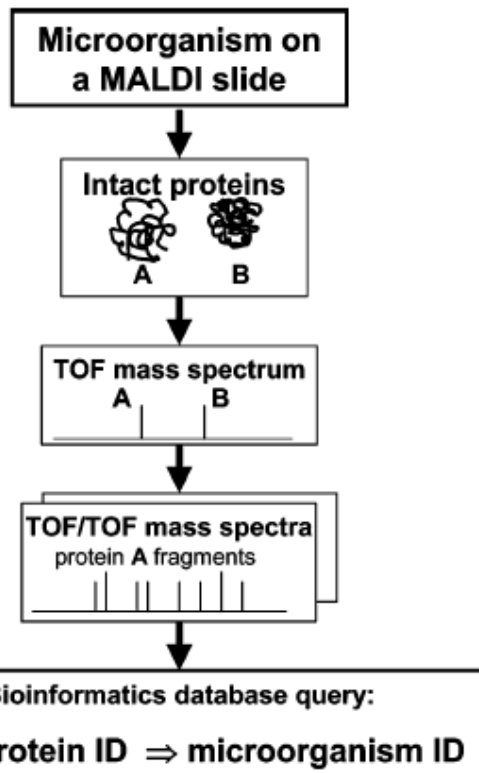


FIGURE 3. Light microscopy of Giemsa-stained thin blood smears from the mouse in Figure 1 and Figure 2a during the time course of the asynchronous *Plasmodium yoelii* infection. The day 24 sample (F) was microscopically scored as parasite negative but the laser desorption mass spectrometry heme signal persisted due to the presence of hemozoin crystal-filled leukocytes. Arrows indicate hemozoin crystals. A, Day 2. An erythrocyte bearing a single-ring stage parasite is visible in the upper left quadrant. B, Day 4. C, Day 10. D, Day 18. E, Day 20. F, Day 24. Mouse erythrocytes are 3–5 μ m in diameter.

Intact microorganisms identification using MALDI-TOF (matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry) - BIOMARKERS

- Holland et al. Rapid Commun Mass Spectrom. 1996;10(10):1227-32.
- Claydon et al. Nat Biotechnol. 1996 Nov;14(11):1584-6.



Applications:

Surveillance of air quality
Water quality
Food quality
Medical diagnosis
Taxonomy
Biological reserve quality

```
graph TD; A([Basic research, biomarker description and characterization]) --> C([Biomarker validation]); C --> B([Development, Entry in a chain of rational design]);
```

Basic research,
biomarker description
and characterization

Development,
Entry in a chain of
rational design

Biomarker validation



FIOCRUZ-Br

UFRJ-Br

Rede Proteômica de
Rio de Janeiro - Br

CIGB - Cuba

