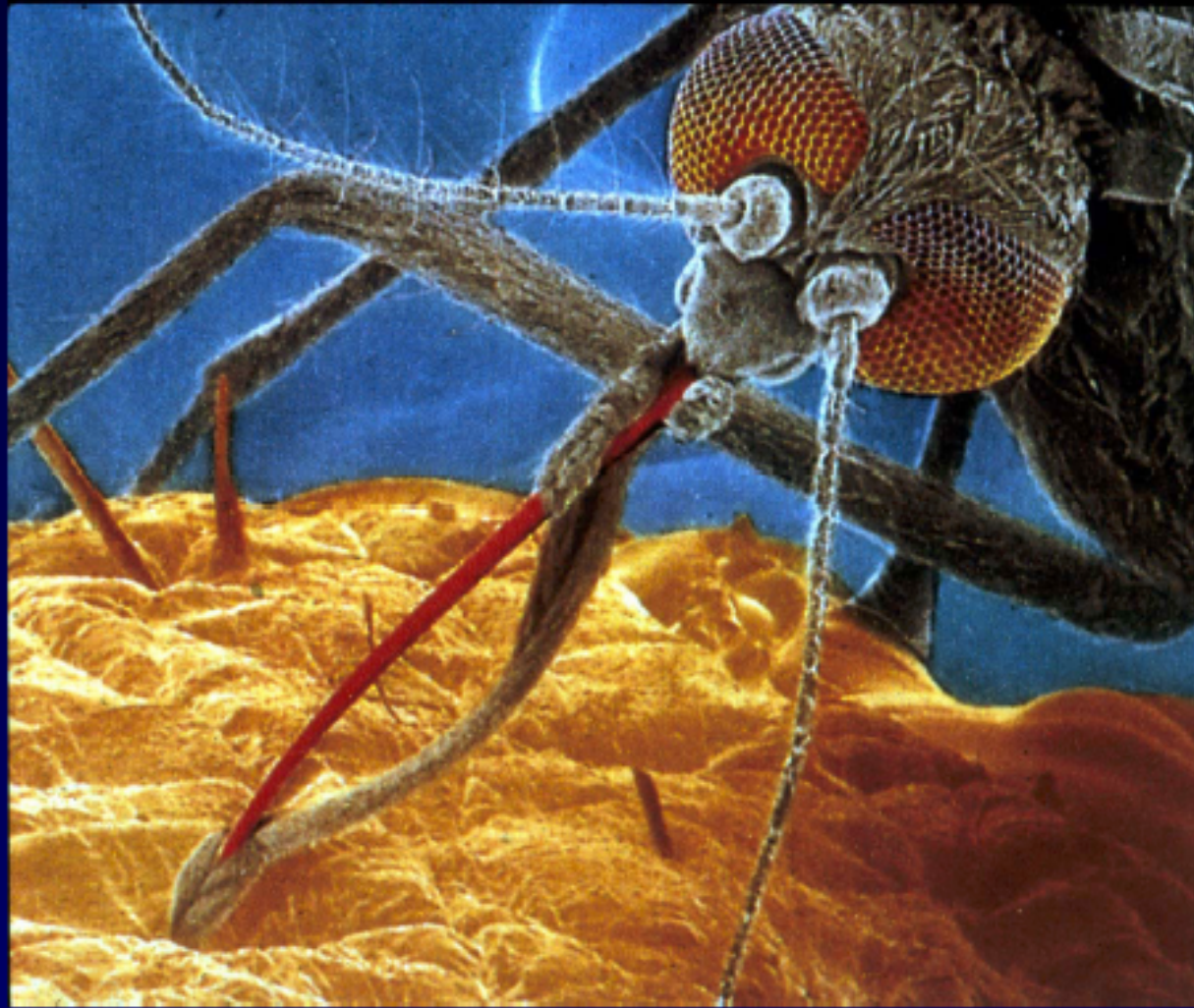
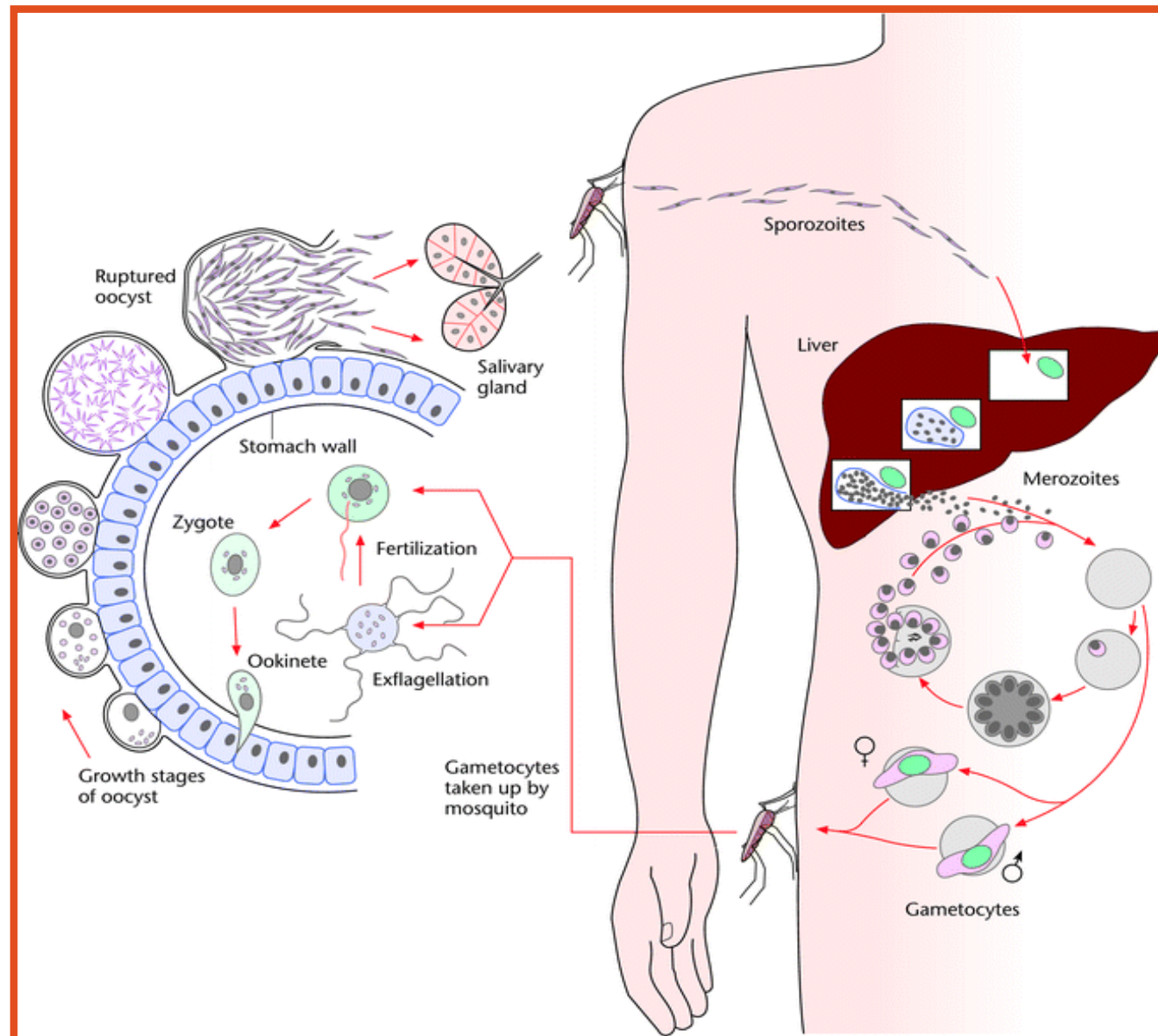


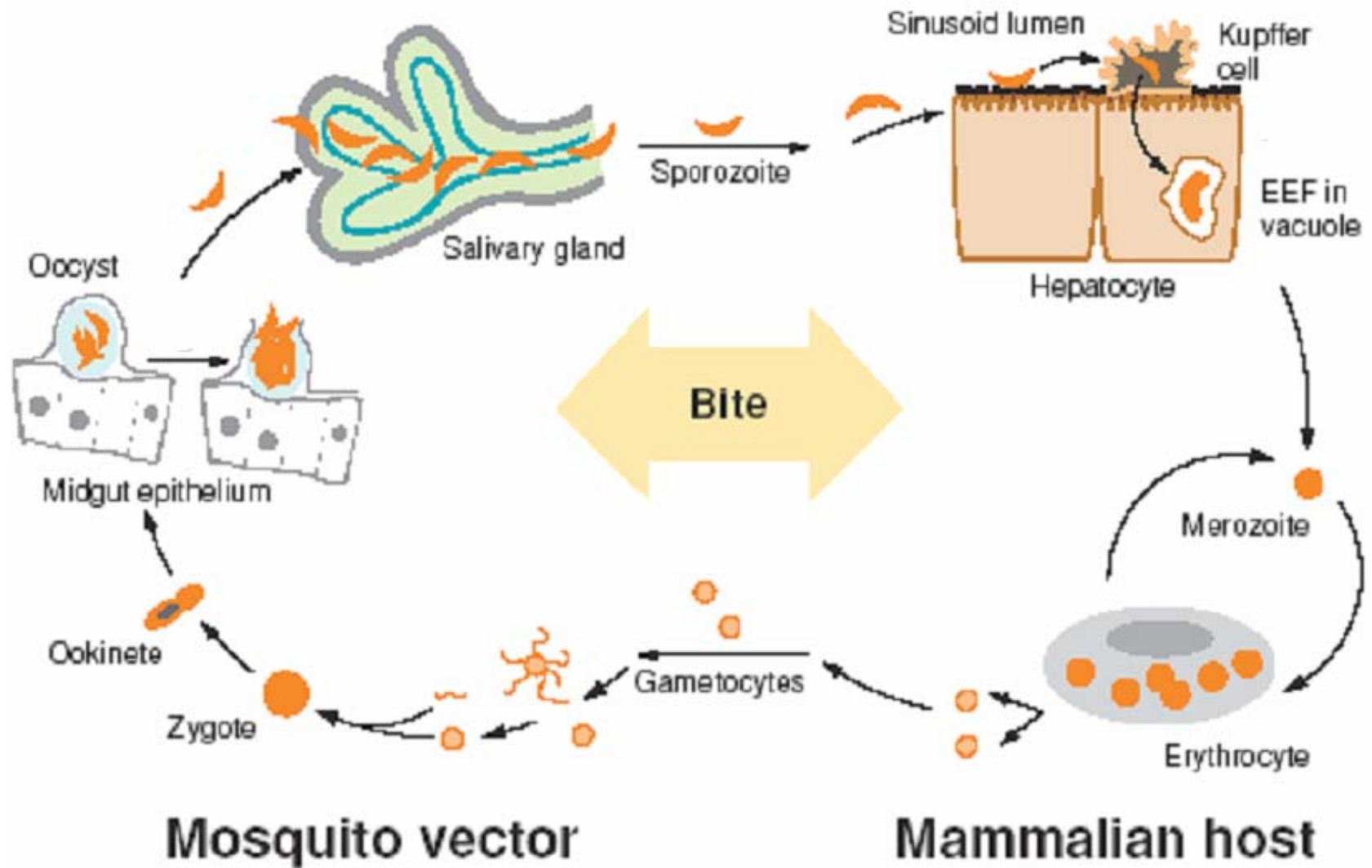
Sporozoite - host interactions



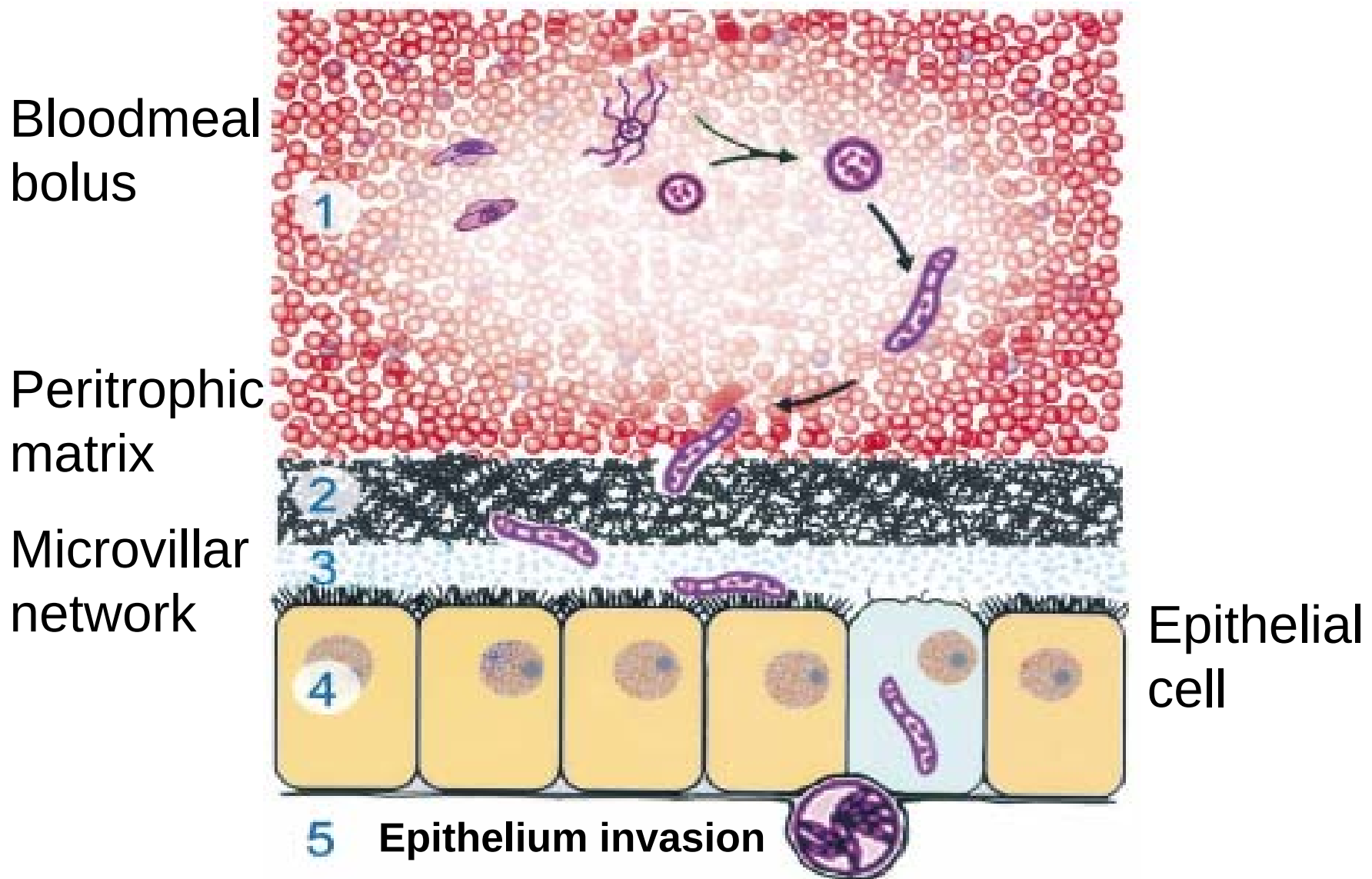
Plasmodium falciparum life cycle



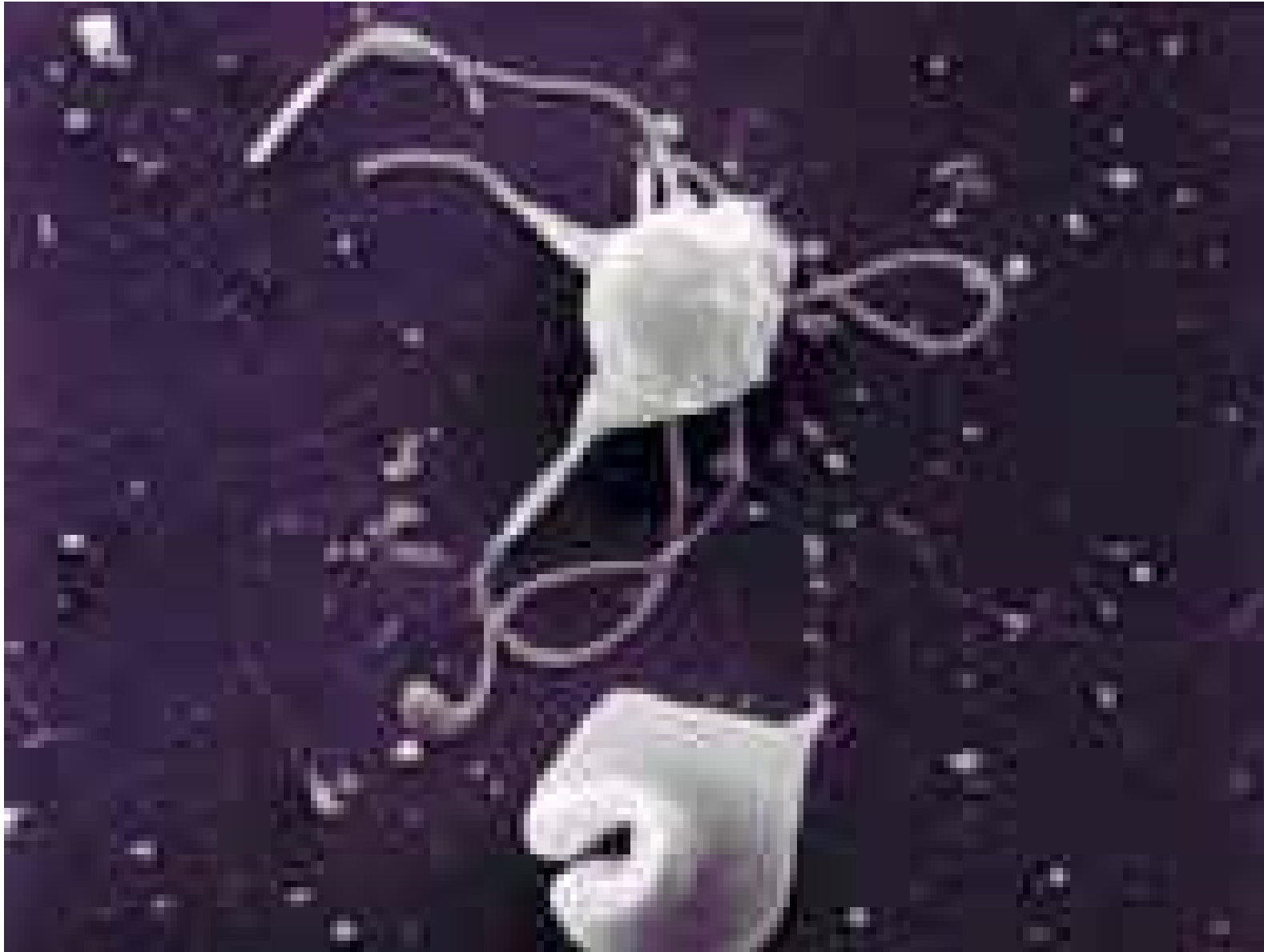
Plasmodium falciparum life cycle



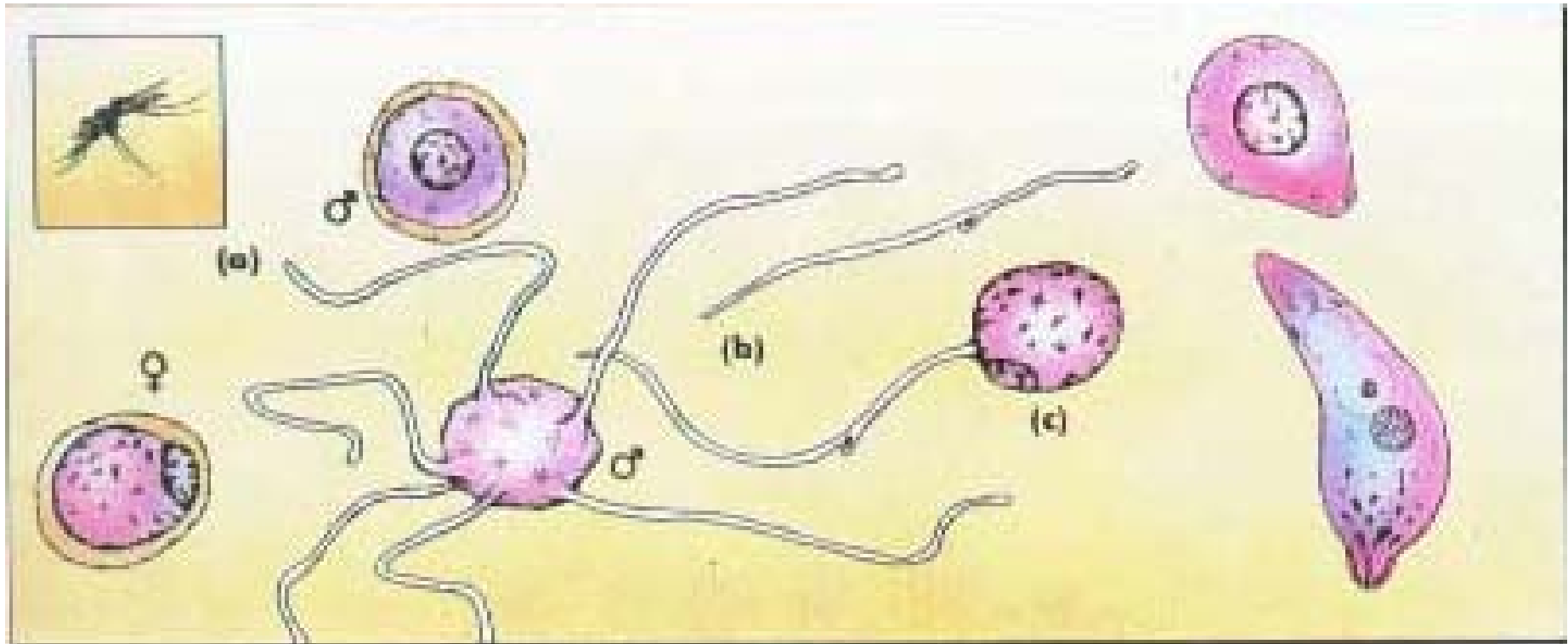
Gametocyte, zygote, ookinete and oocyst



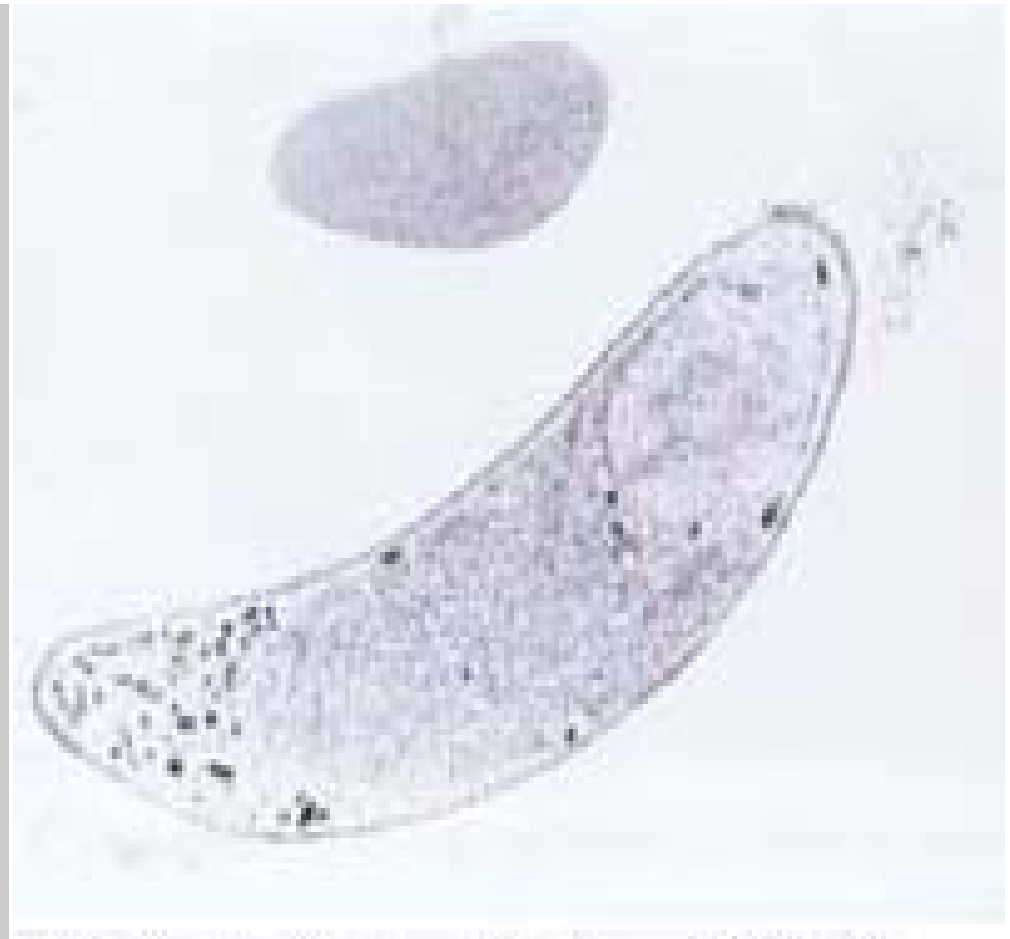
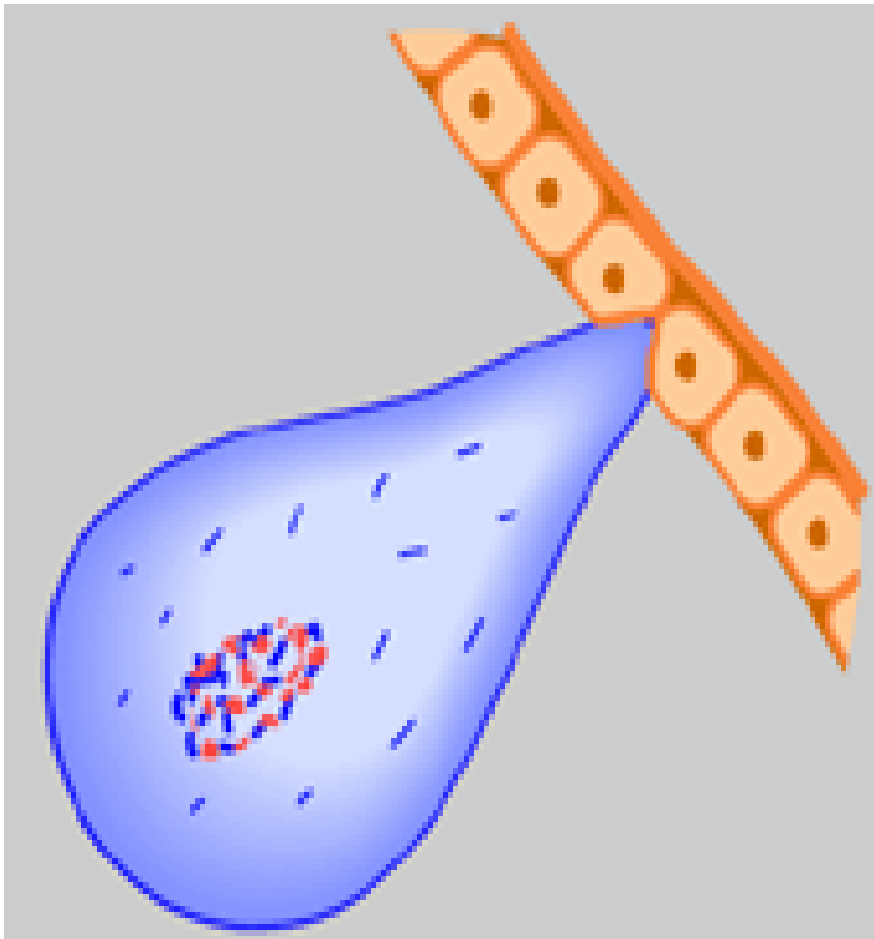
Plasmodium gametocyte



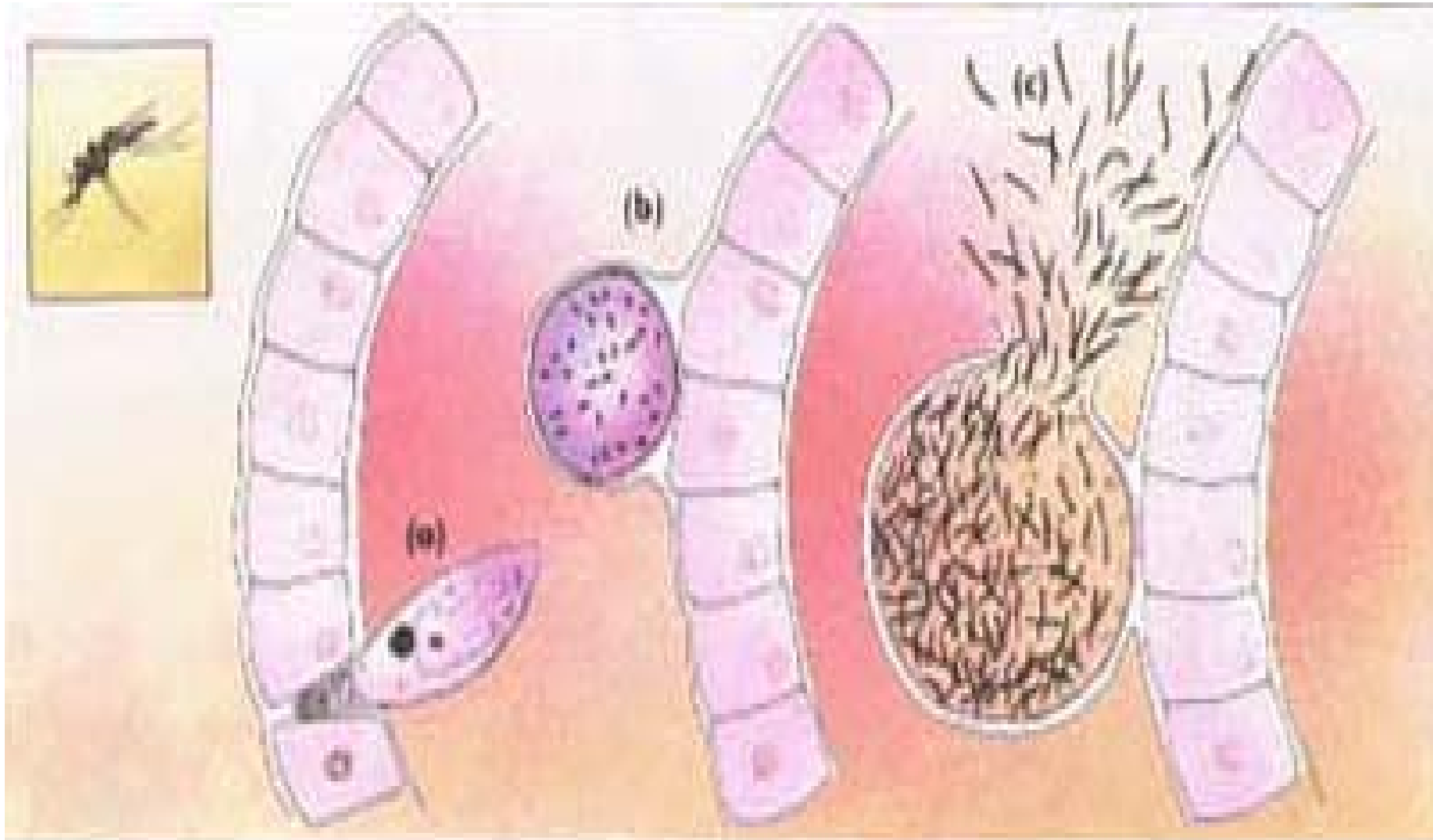
Zygote formation



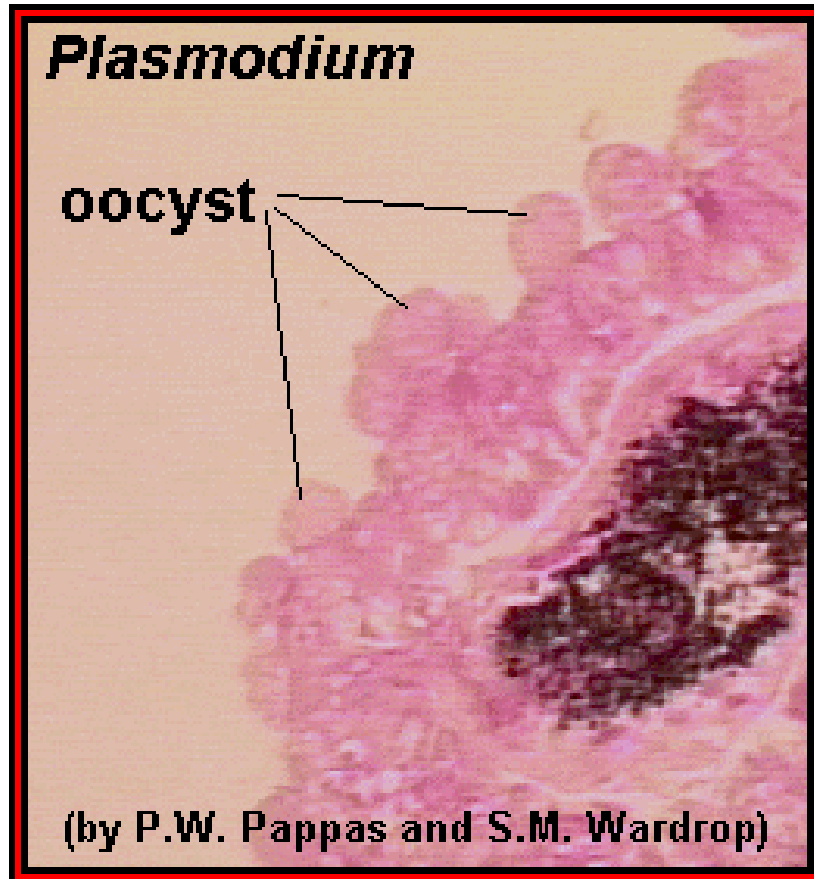
Ookinete



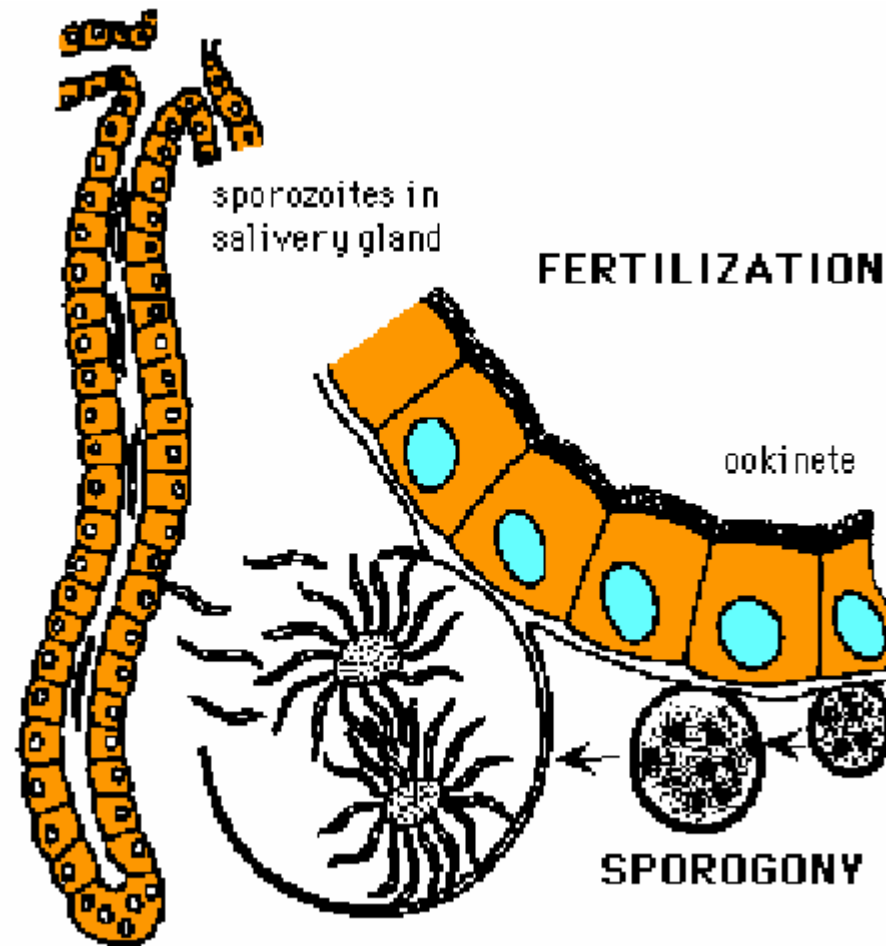
Multiplication in the mosquito



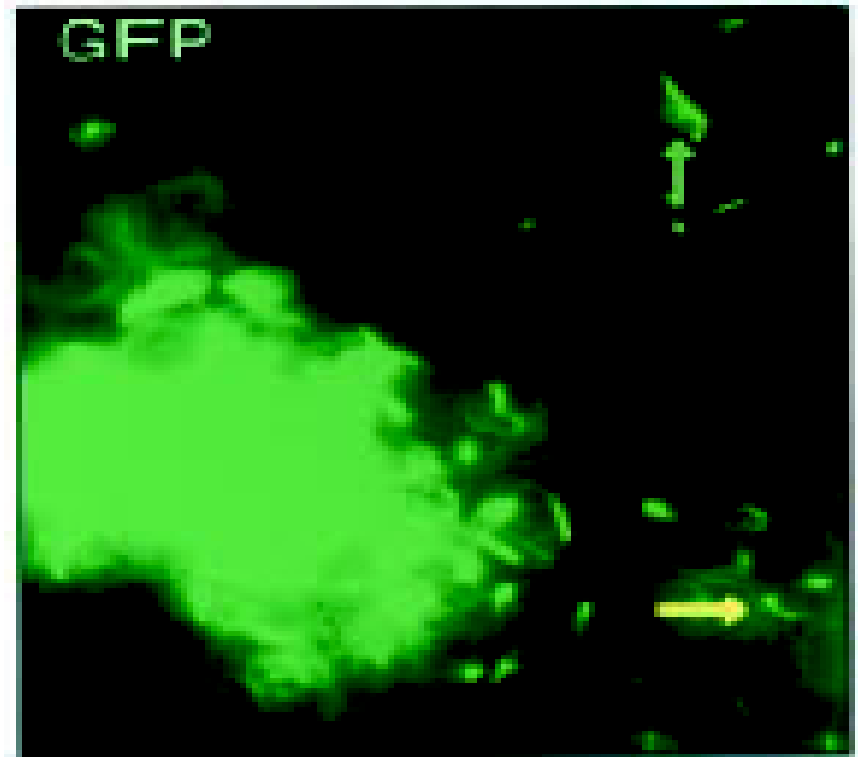
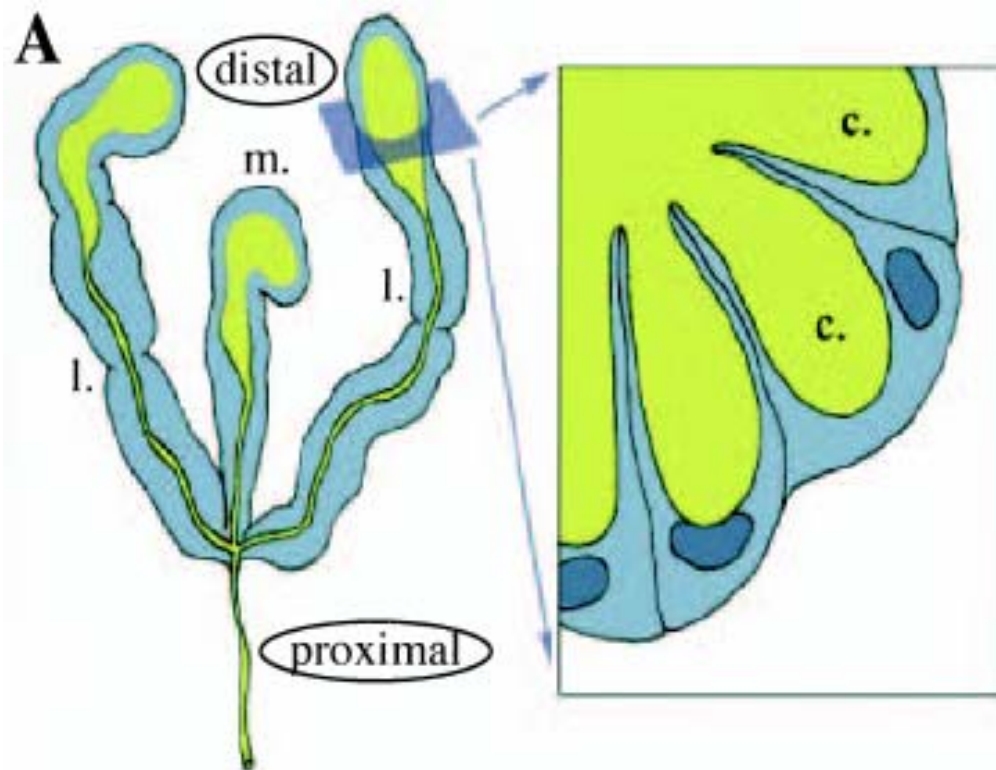
Plasmodium oocysts on the surface of a mosquito gut



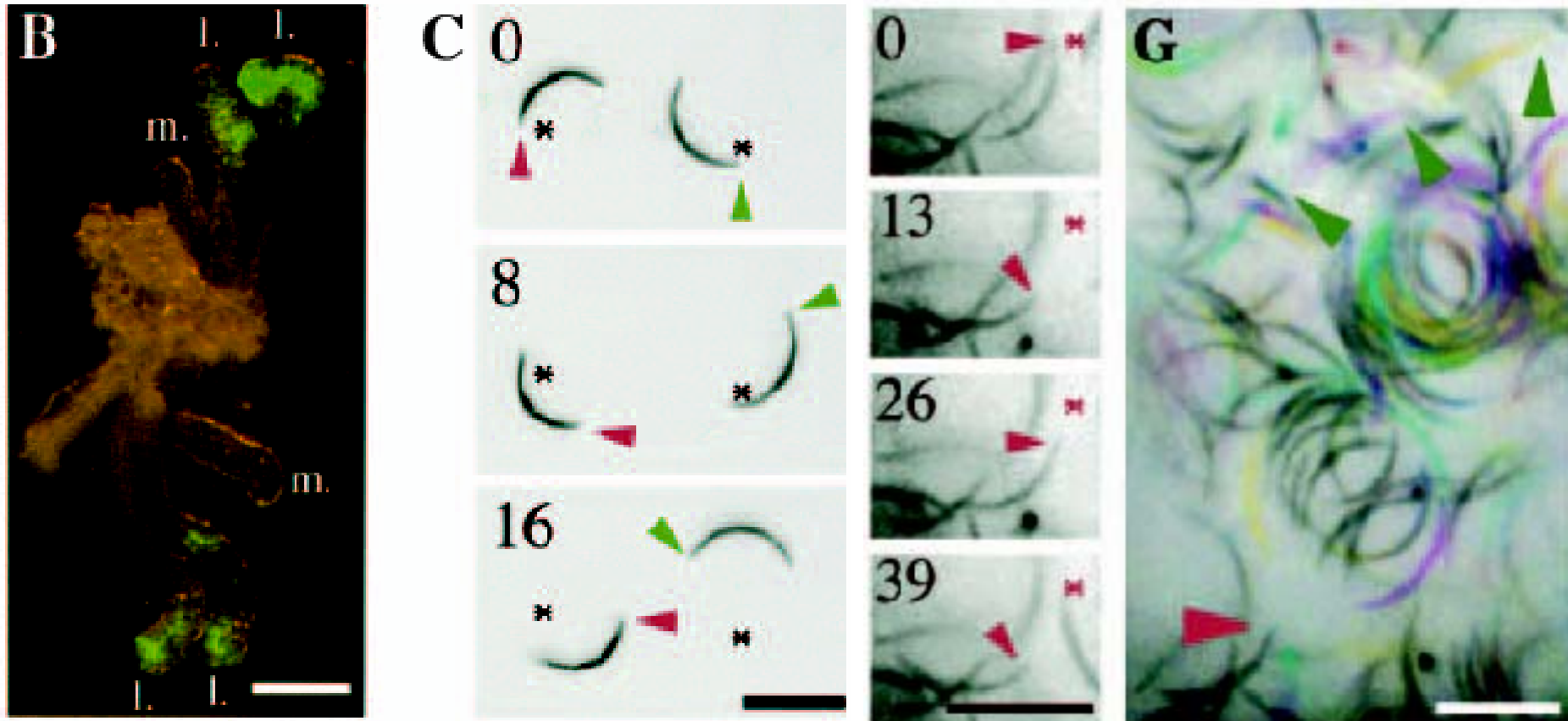
Oocyst ruptures to liberate sporozoites which penetrate the salivary gland



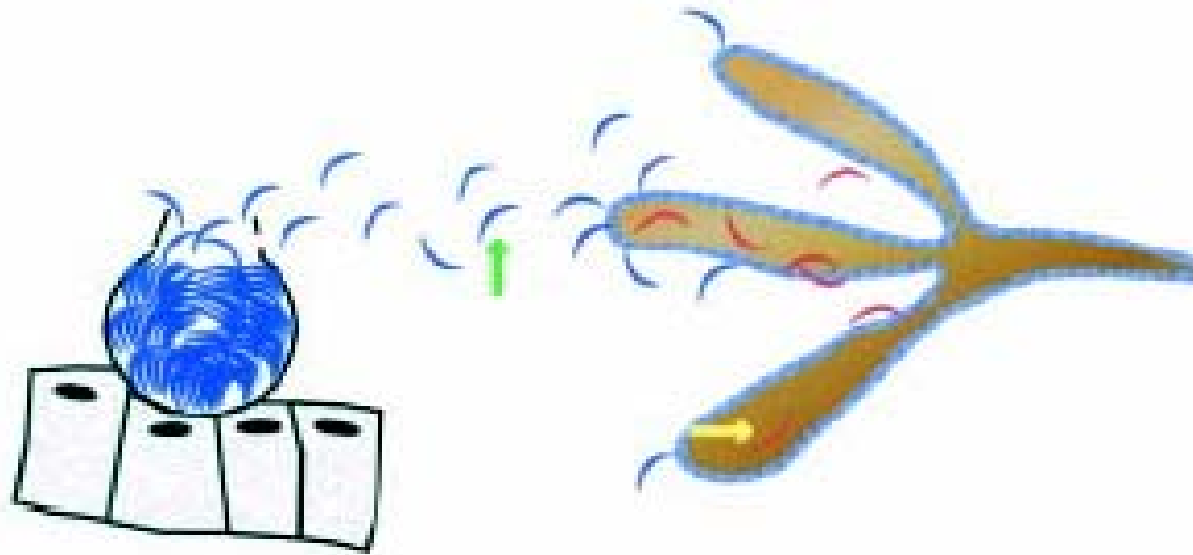
Sporozoites within the salivary gland



Sporozoite motility within the salivary gland



Phenotypic differences of oocyst sporozites and salivary gland sporozoites



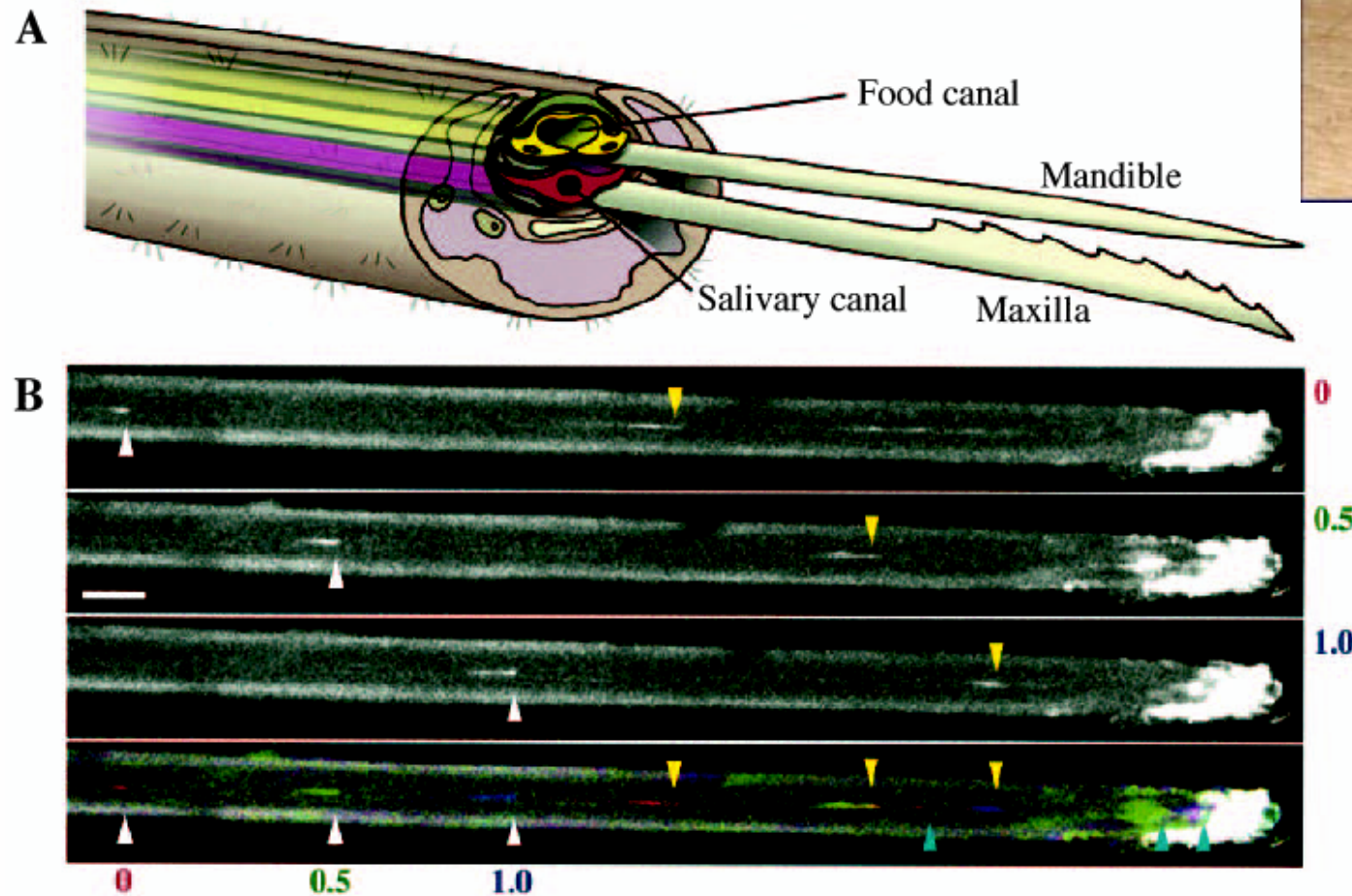
midgut-oocyst
oocyst sporozoites

non-virulent
do not elicit protection
discontinuous gliding

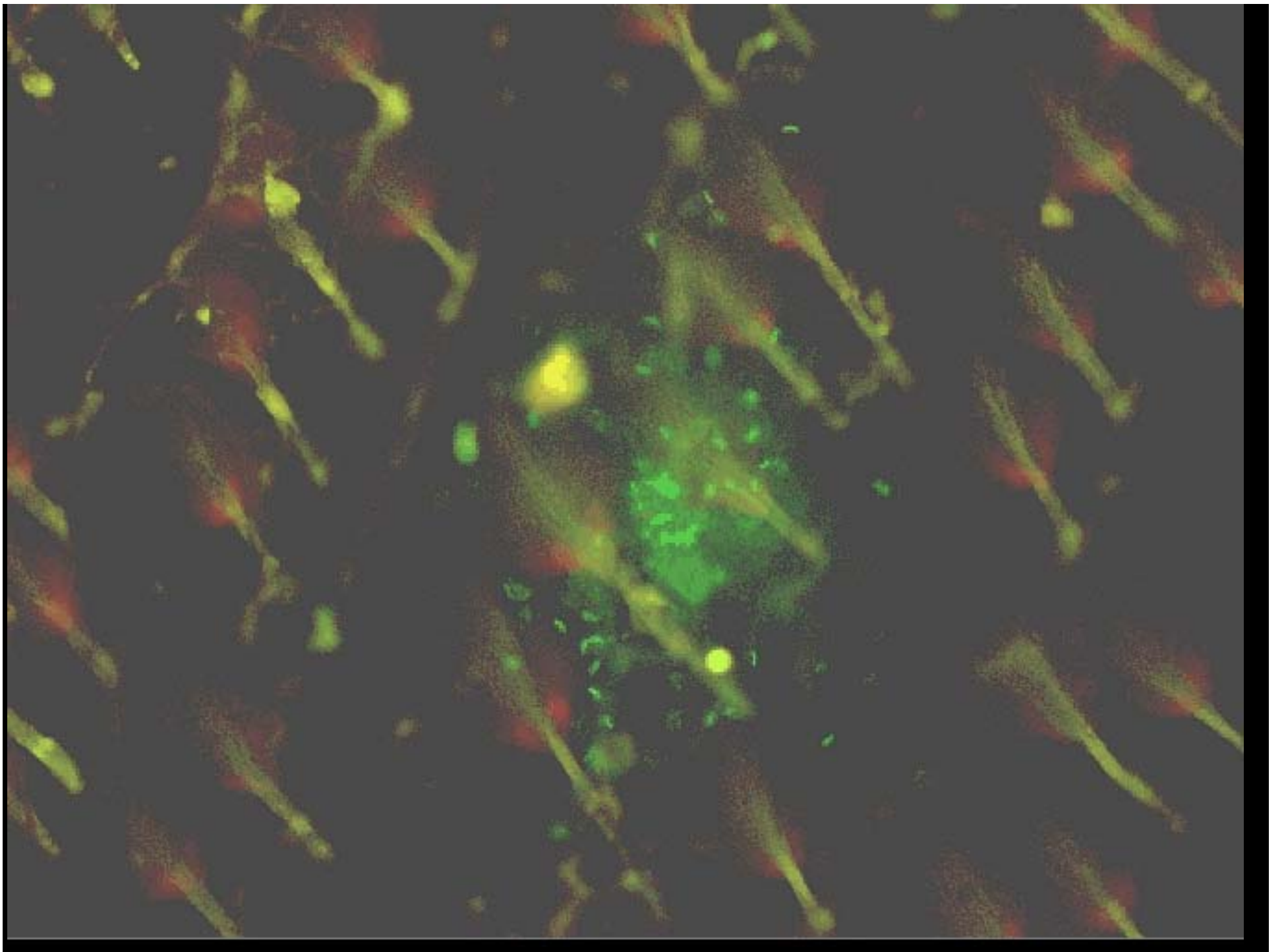
salivary gland
sporozoites

virulent
elicit protection
continuous gliding

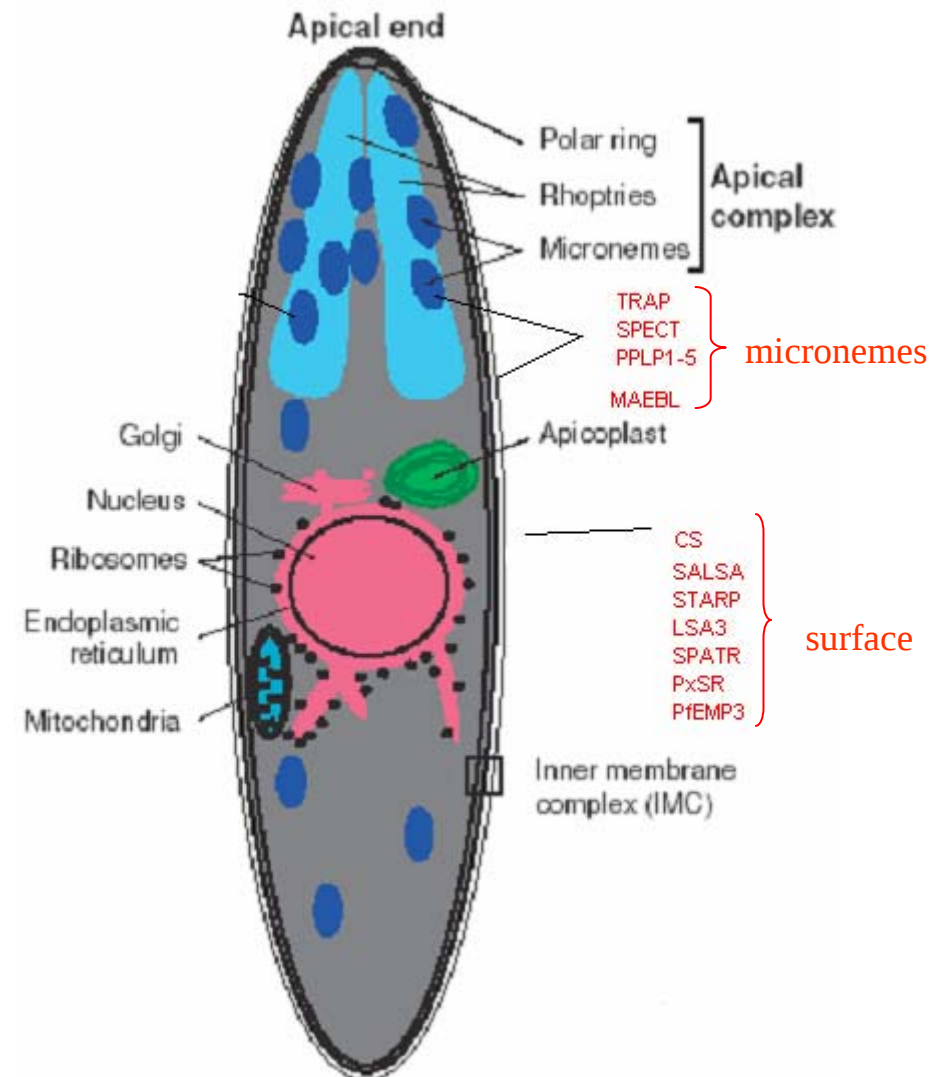
Sporozoite ejection through the salivary canal of a live mosquito's proboscis



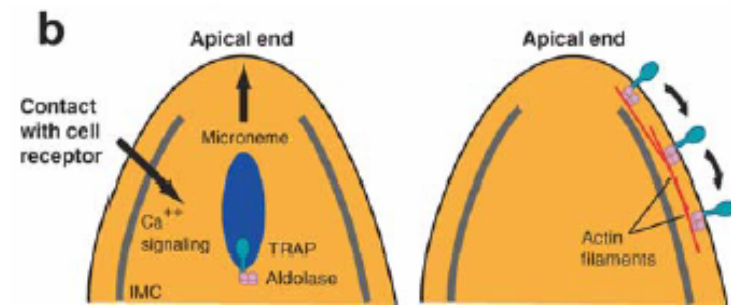
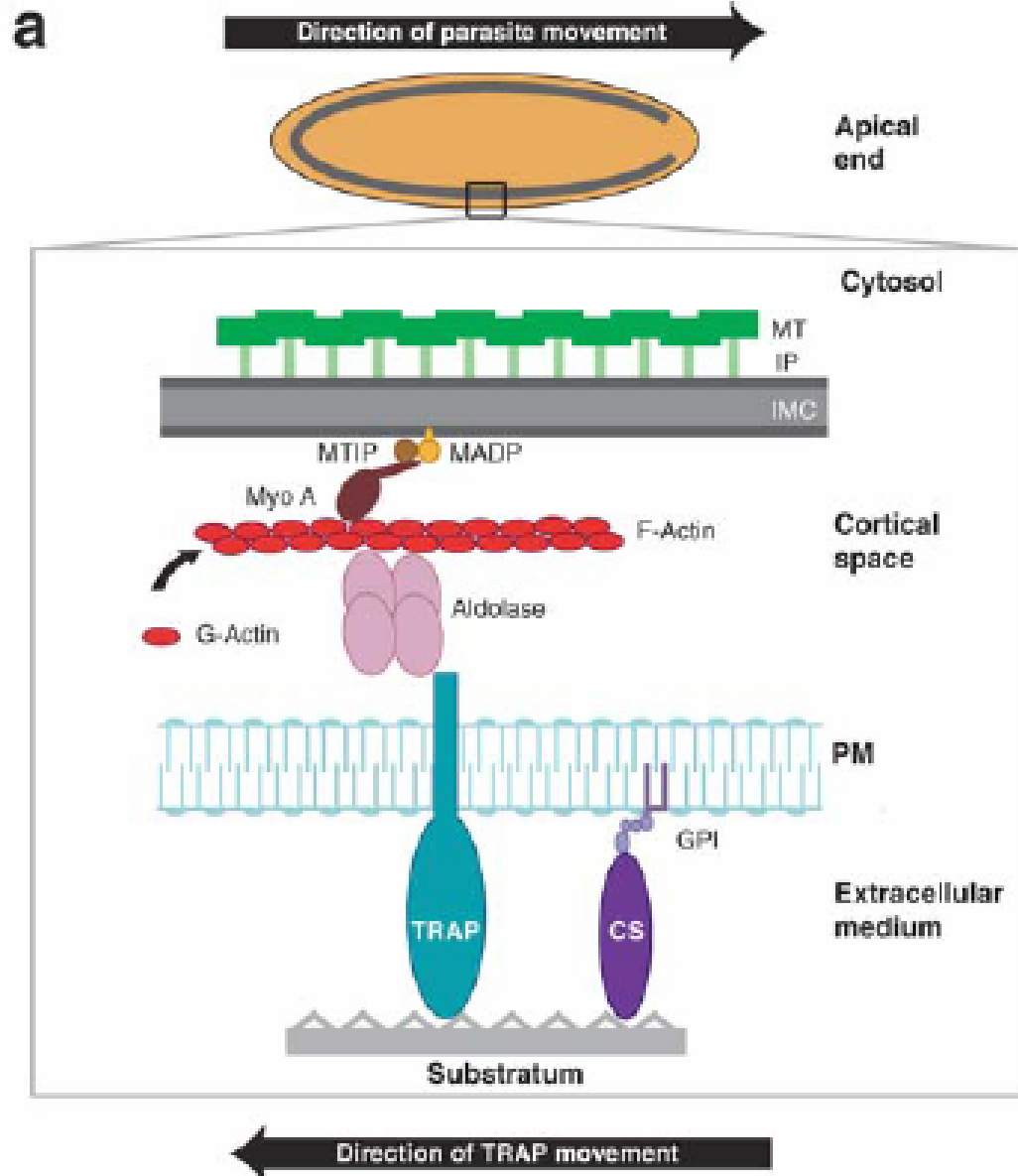




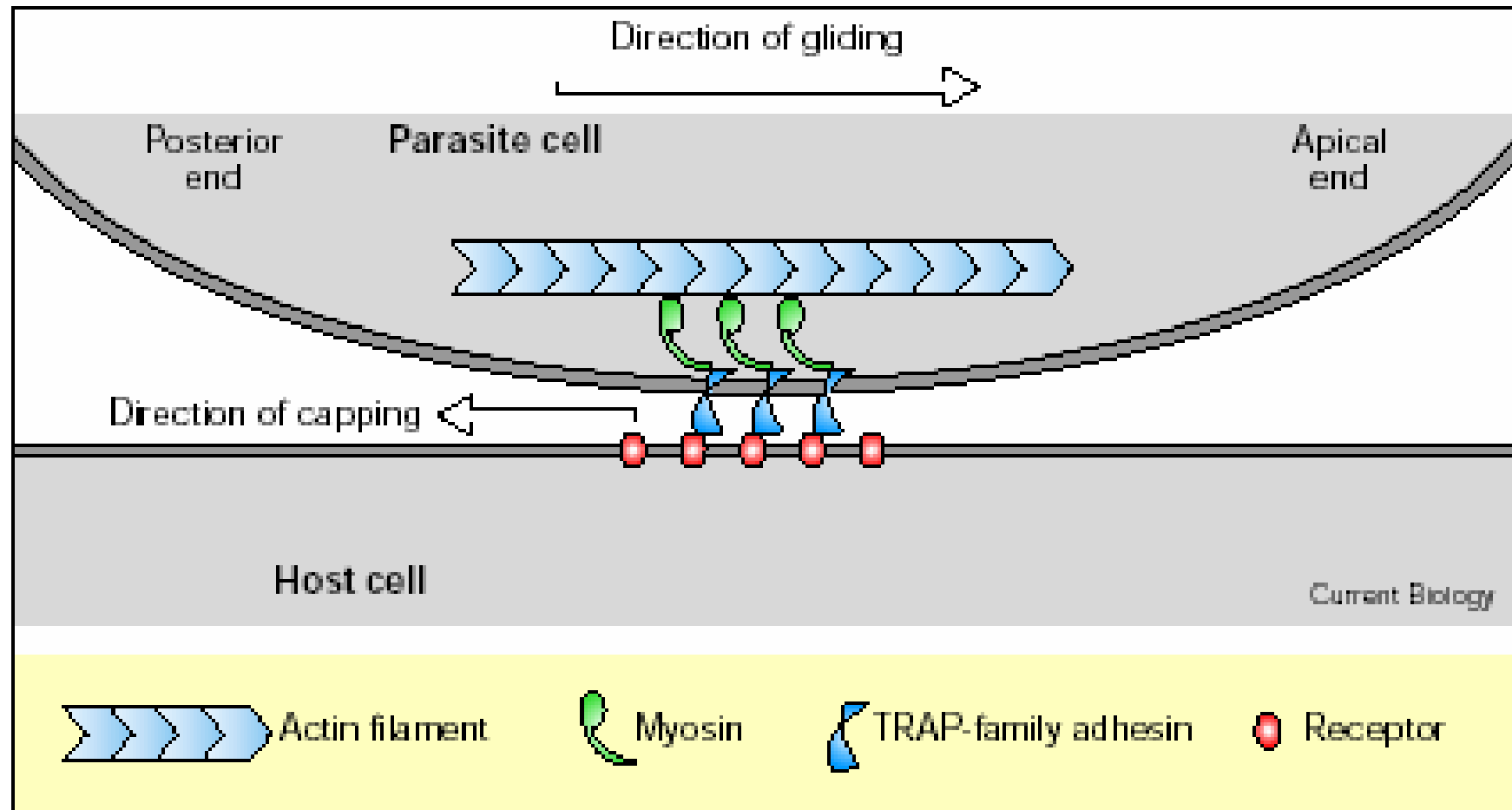
Schematic representation of a *Plasmodium*



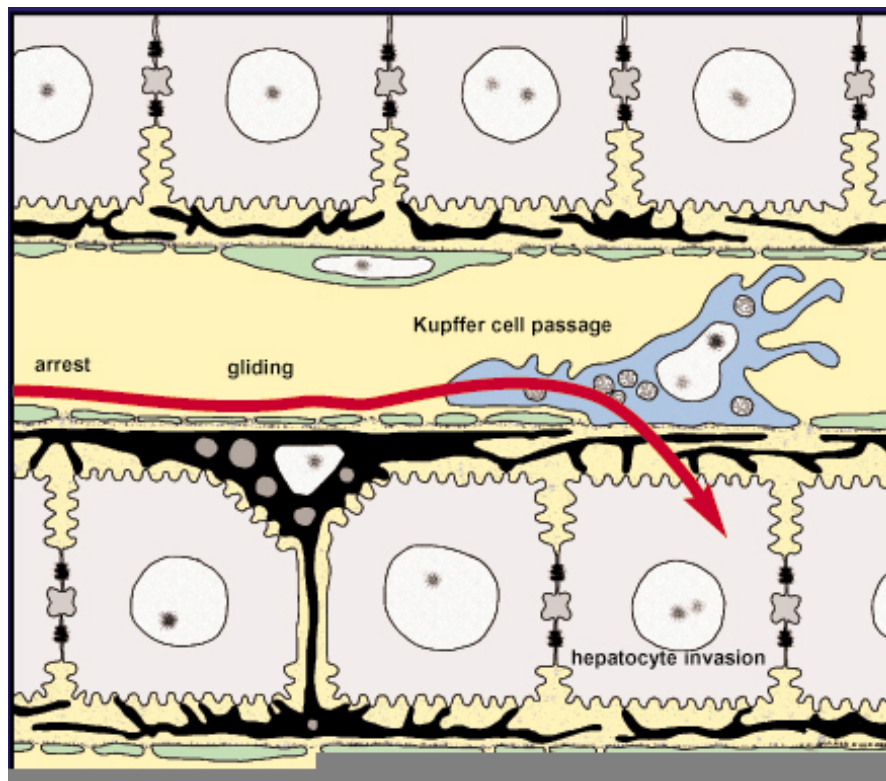
Model of molecular motility machinery in apicomplexa



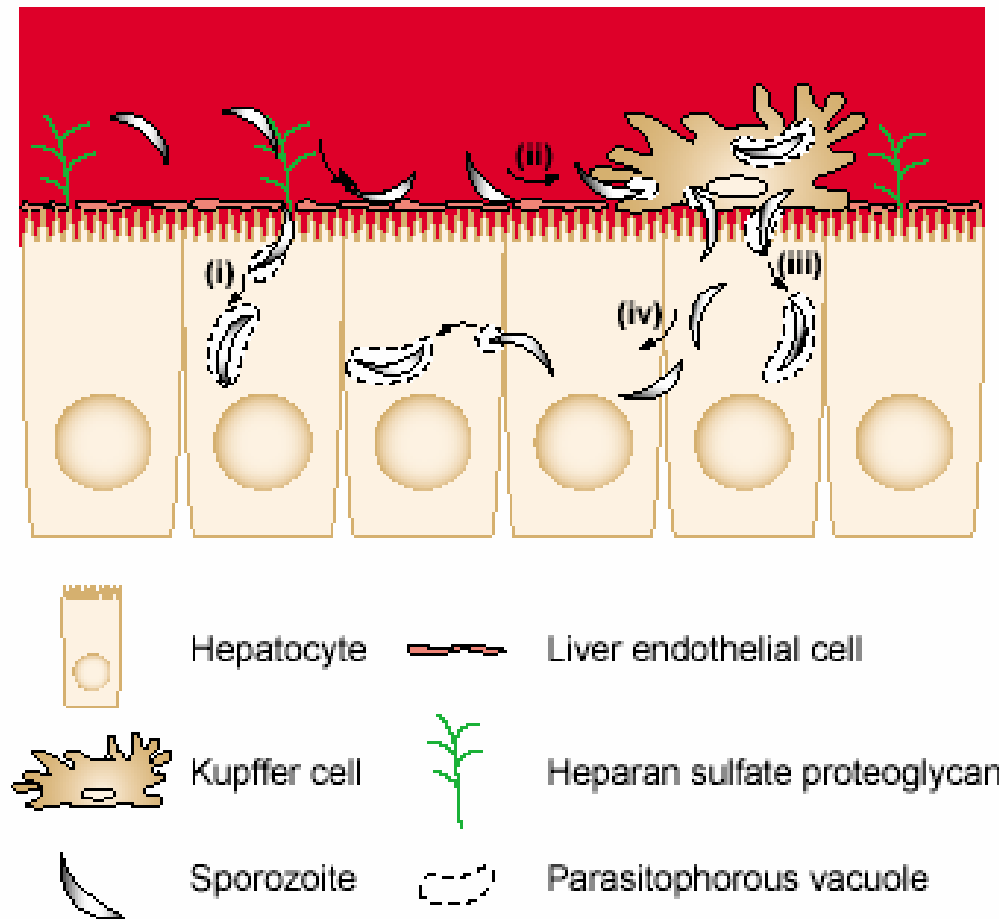
Model of molecular motility machinery in apicomplexa



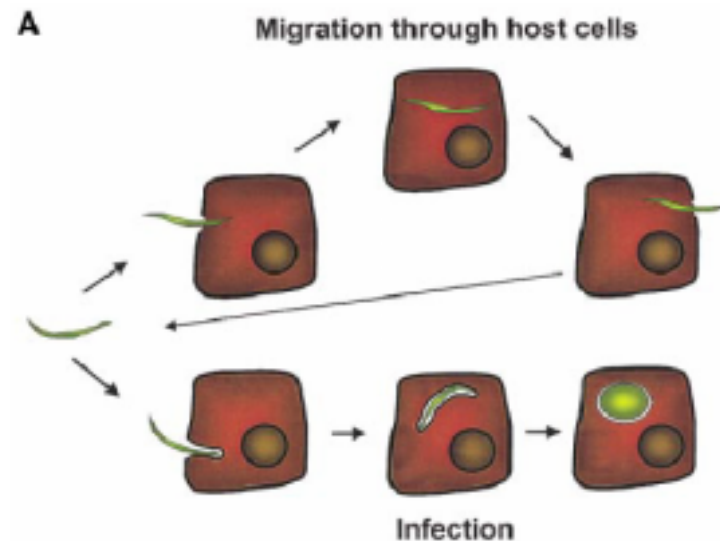
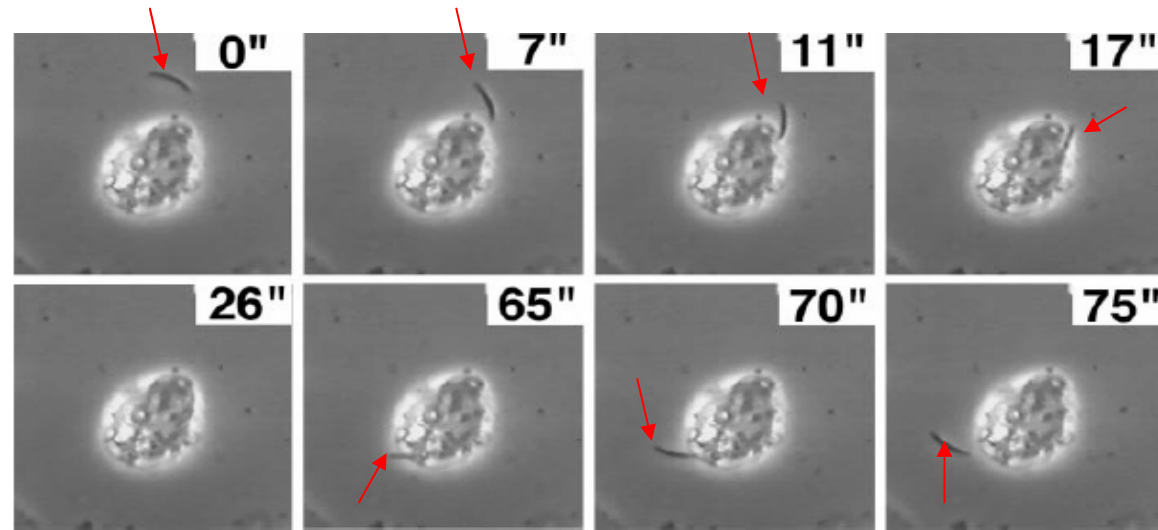
Malaria sporozoites actively enter and pass through Kupffer cells prior to hepatocyte invasion



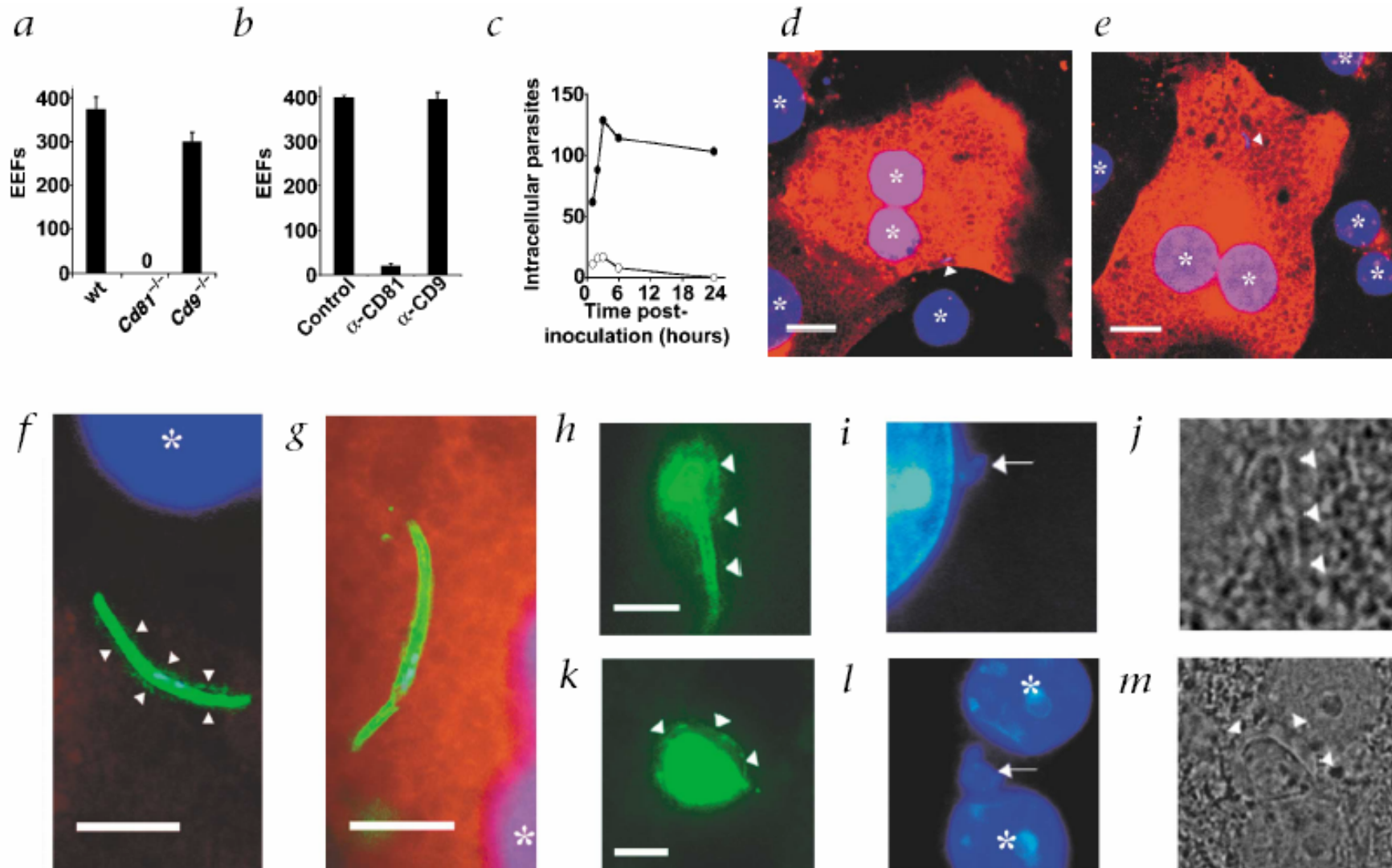
The complexity of sporozoite liver infection



Plasmodium sporozoites migrate through hepatocytes before infection



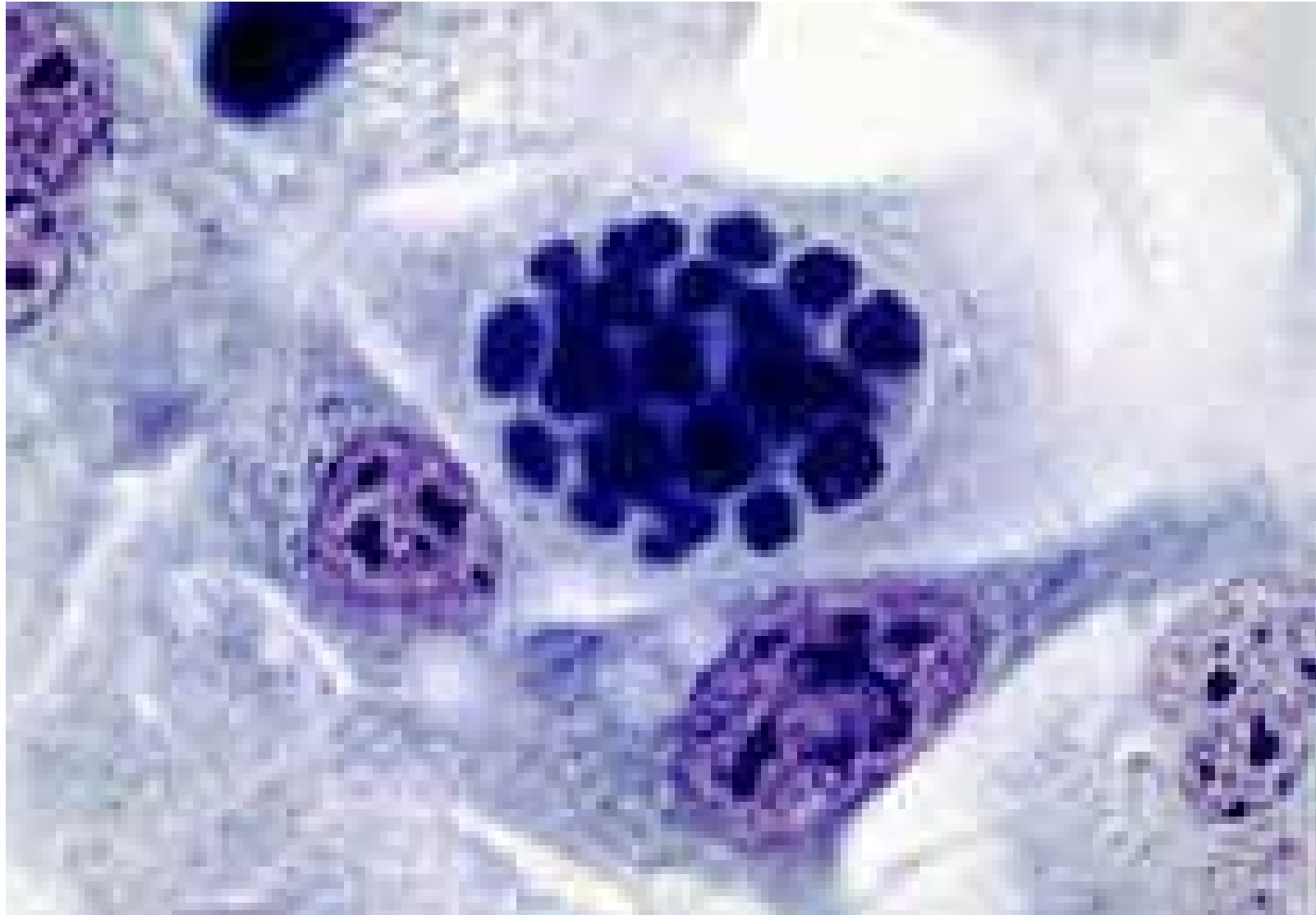
CD81 is required by *P. yoelli* sporozoites for hepatic infection



Electron micrograph of an early hepatic schizont

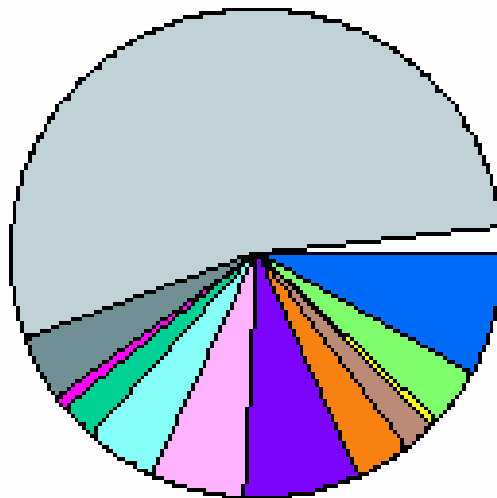


***Plasmodium berghei* exo-erythrocyte schizont in HepG2 cells**

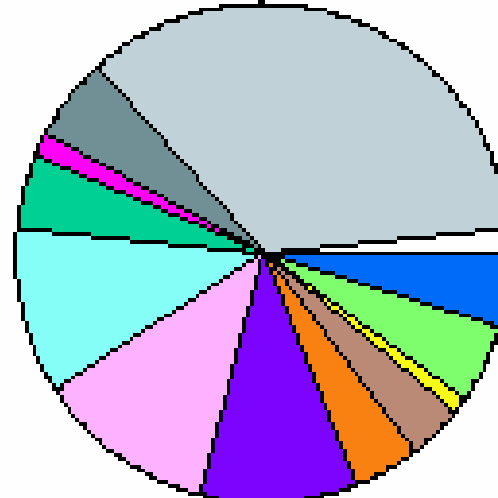


Functional profile of proteins expressed in different *P. falciparum* life-cycle stages

Sporozoite



Merozoite



- Cell surface (apical organelles)
- Cell cycle (DNA processing)
- Cell rescue defence (virulence)
- Cellular communication (signal transduction)
- Cellular transport (transport mechanism)

- Metabolism (energy)
- Protein fate
- Protein synthesis
- Transcription

- Transport facilitation
- Conserved hypothetical
- Hypothetical
- Unclassified

Table 1. Comparative summary of the protein lists for each stage

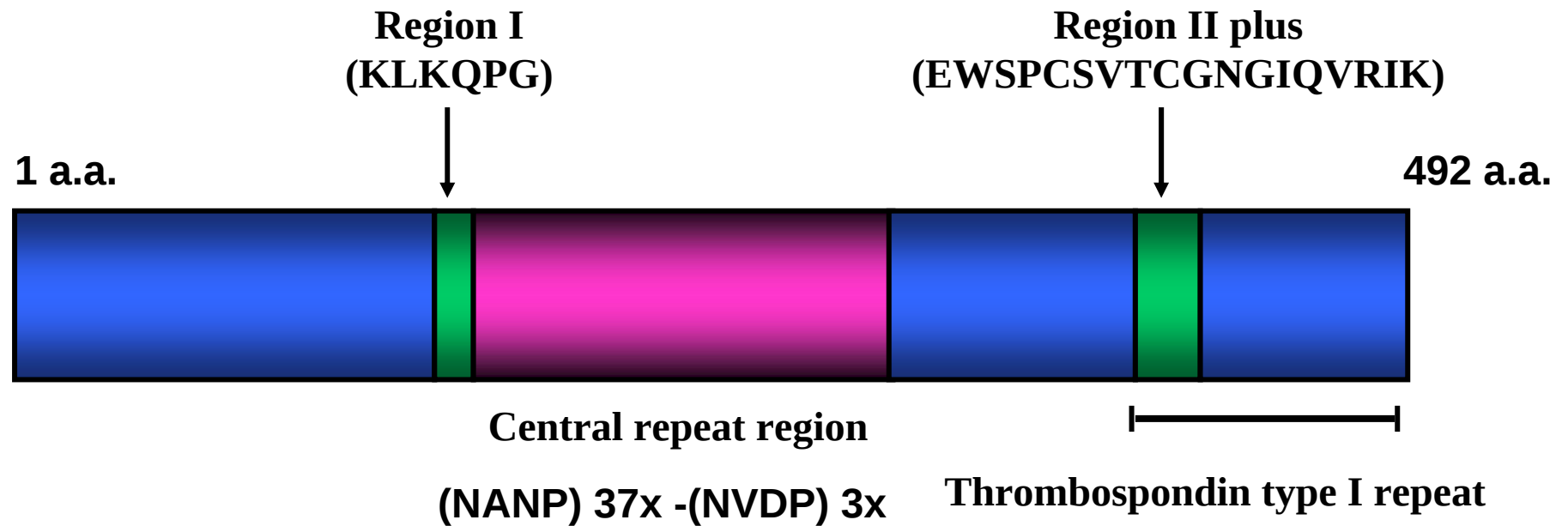
Protein count	Sporozoites	Merozoites	Trophozoites	Gametocytes
152	X	X	X	X
197	-	X	X	X
53	X	-	X	X
28	X	X	-	X
36	X	X	X	-
148	-	-	X	X
73	-	X	-	X
120	X	-	-	X
84	-	X	X	-
80	X	-	X	-
65	X	X	-	-
376	-	-	-	X
286	-	-	X	-
204	-	X	-	-
513	X	-	-	-
2,415	1,049	839	1,036	1,147

Whole-cell protein lysates were obtained from, on average, 17×10^6 , 4.5×10^9 trophozoites, 2.75×10^9 merozoites, and 6.5×10^9 gametocytes.

Plasmodium sporozoite surface and/or apical organelle proteome

Protein	Other stages ^c	Subcellular localization
CS	–	Surface
TRAP	–	Surface, micronemes
MAEBL	–	Surface, micronemes
SPECT	–	Micronemes
EBA175	Mz	Surface, micronemes
P235 ^a	Mz	Rhoptries
AMA1	Mz	Surface, micronemes
STARP	RBC, LS	Surface
SALSA	RBC, LS	Surface
PEMP3	RBC, LS	Surface
LSA3	LS	Surface
P52	–	?
MCP1	Mz	?
SPATR	RBC	Surface
PPLP1	–	Micronemes
PfEMP1 ^{a,b}	iRBC	?
STEVAR ^{a,b}	iRBC	?
RIFINS ^{a,b}	iRBC	?

Circumsporozoite protein (Cs) (42 kDa)



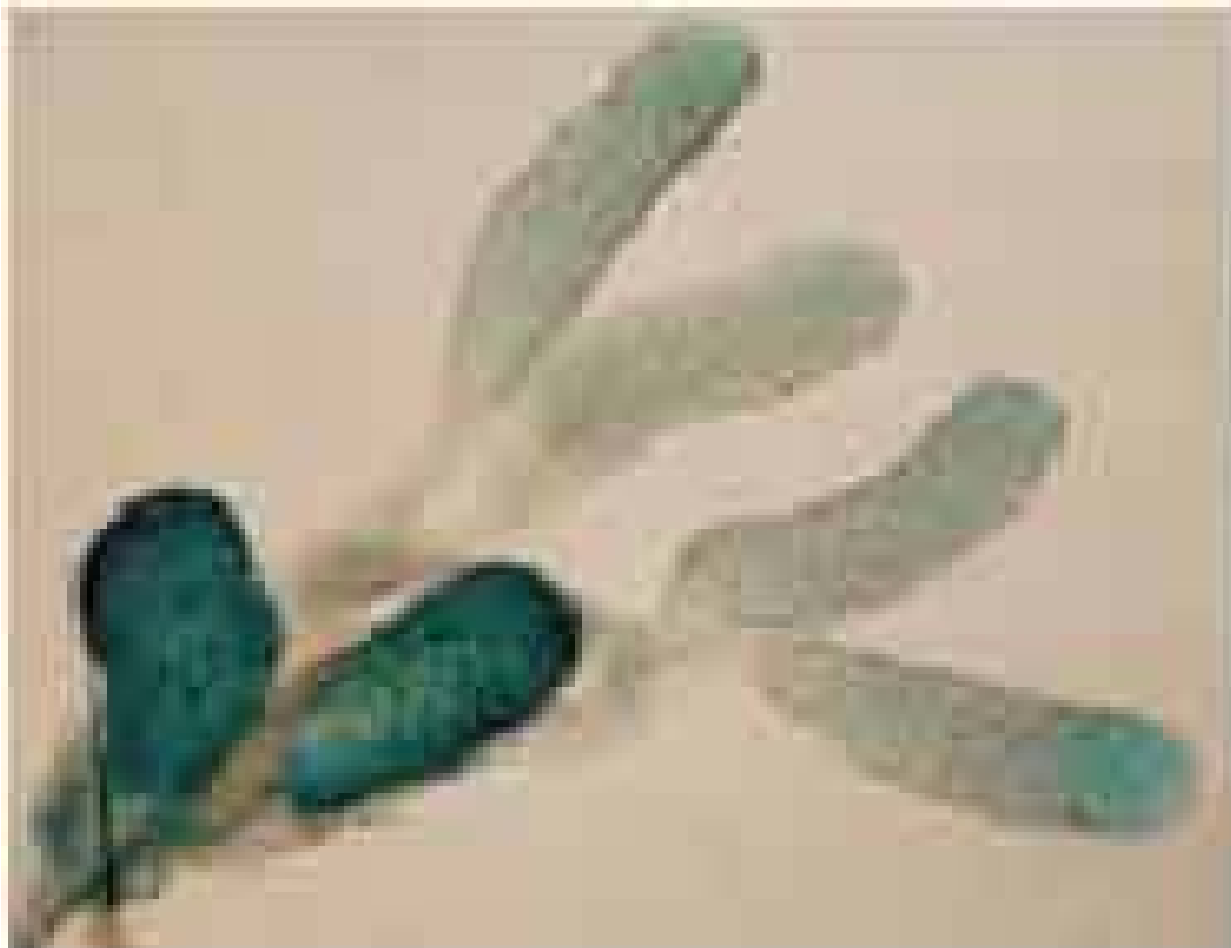
Localisation:

Surface Spz, EEFs, Liver Stage

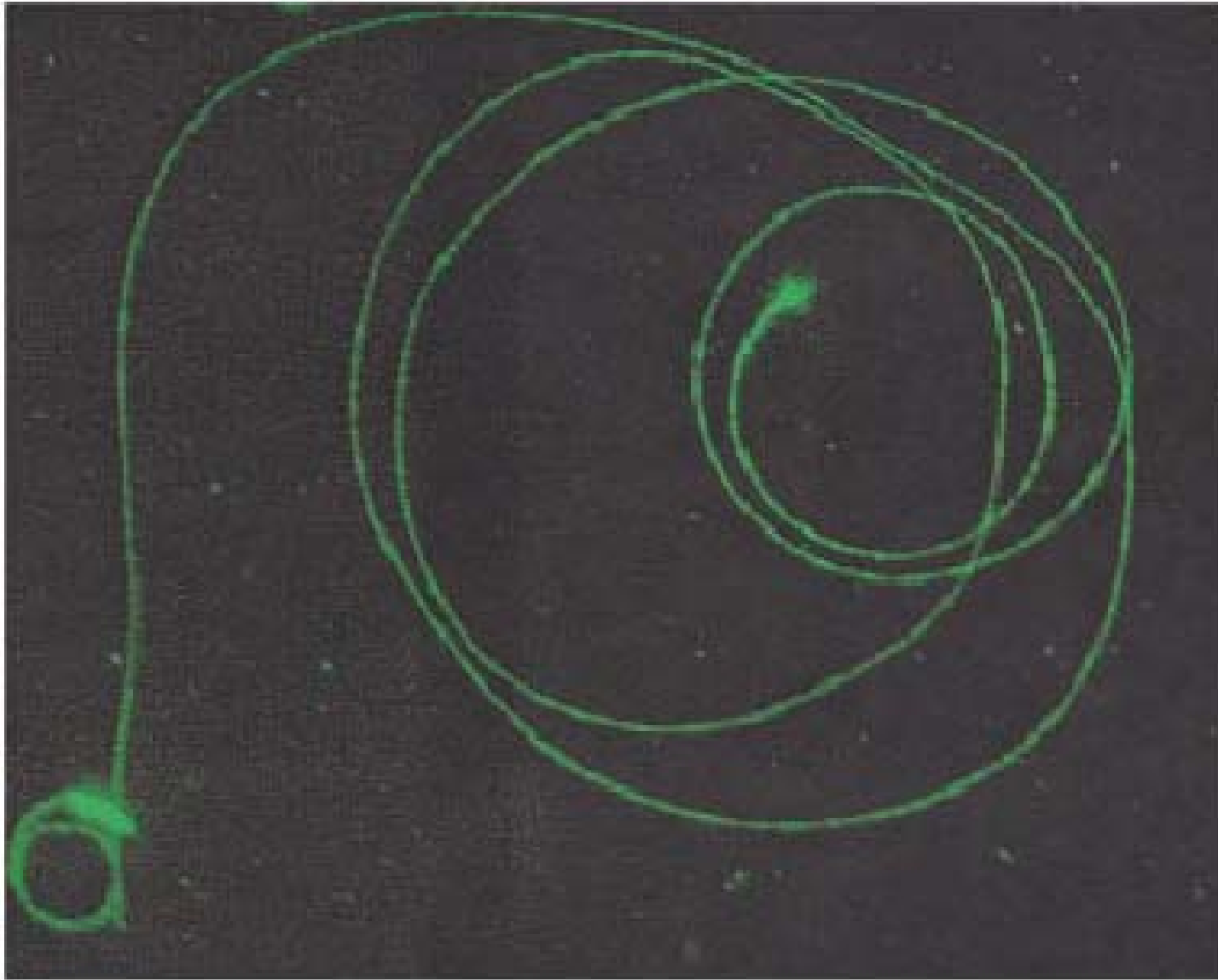
Possible function:

Adhesion, Invasion, gliding motility

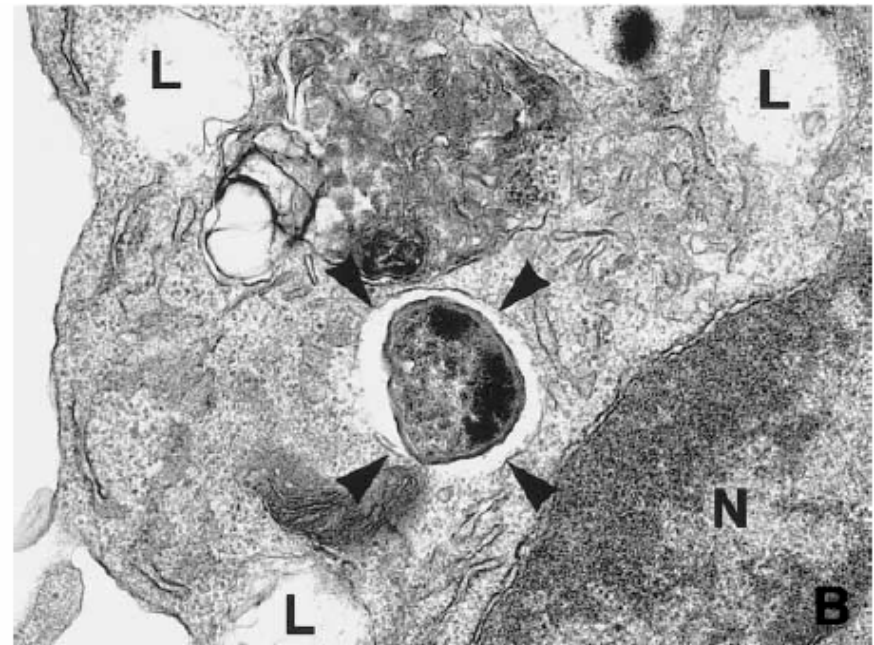
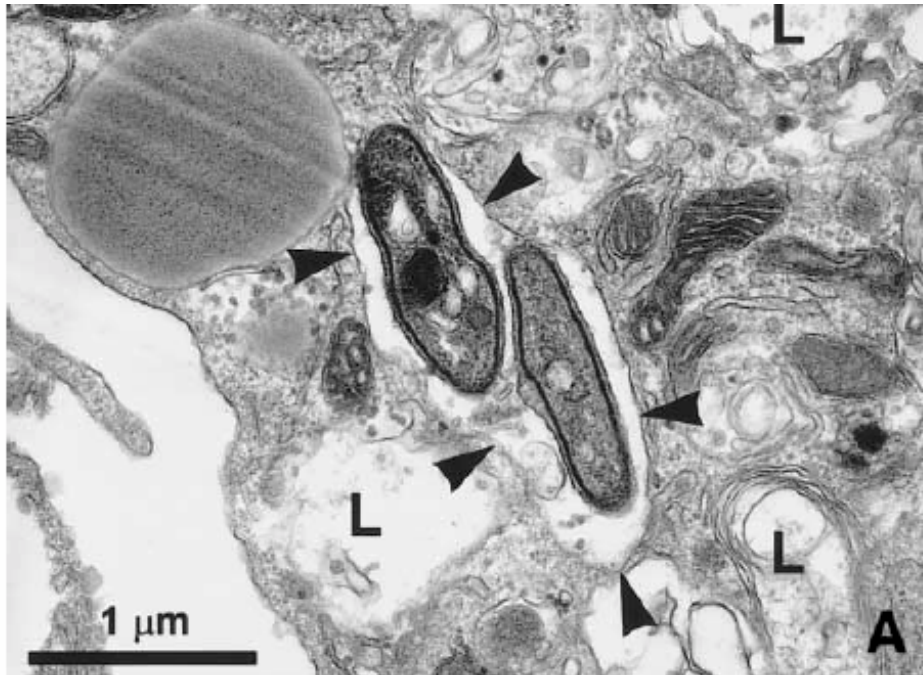
Salivary gland tinction with CS protein



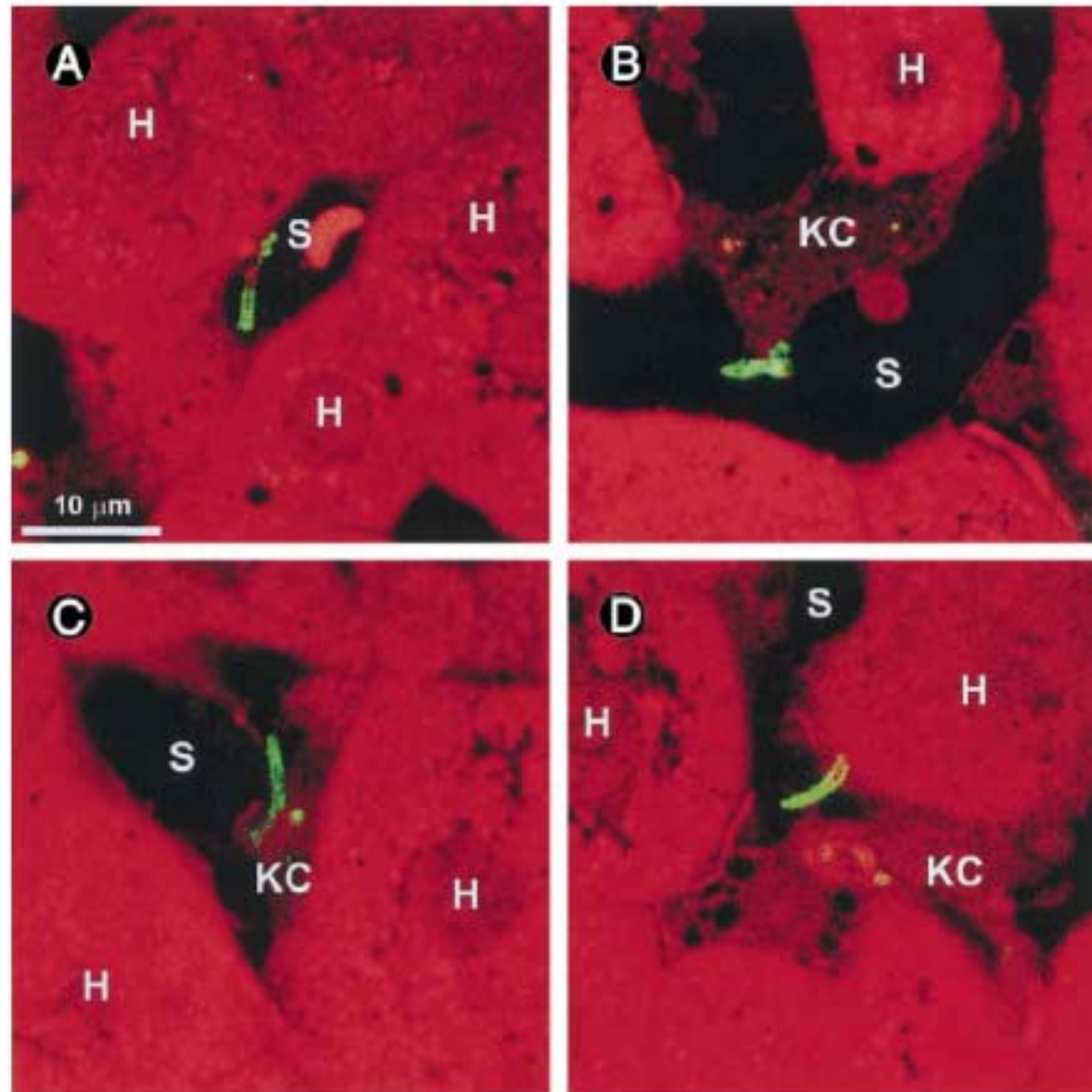
Sporozoite displacement in an assay with CS protein



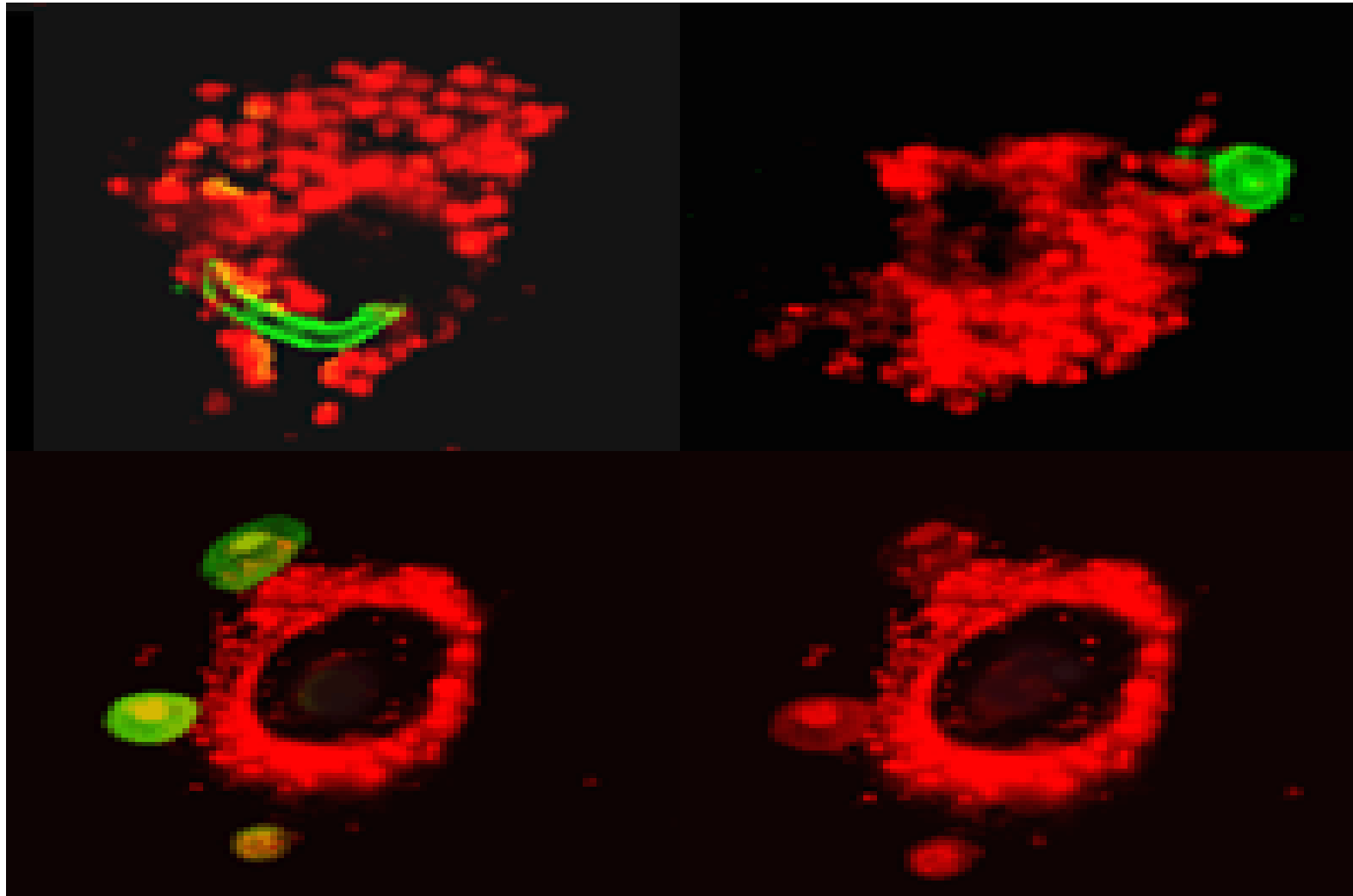
Sporozoites are enclosed in a vacuole in Kupffer cells



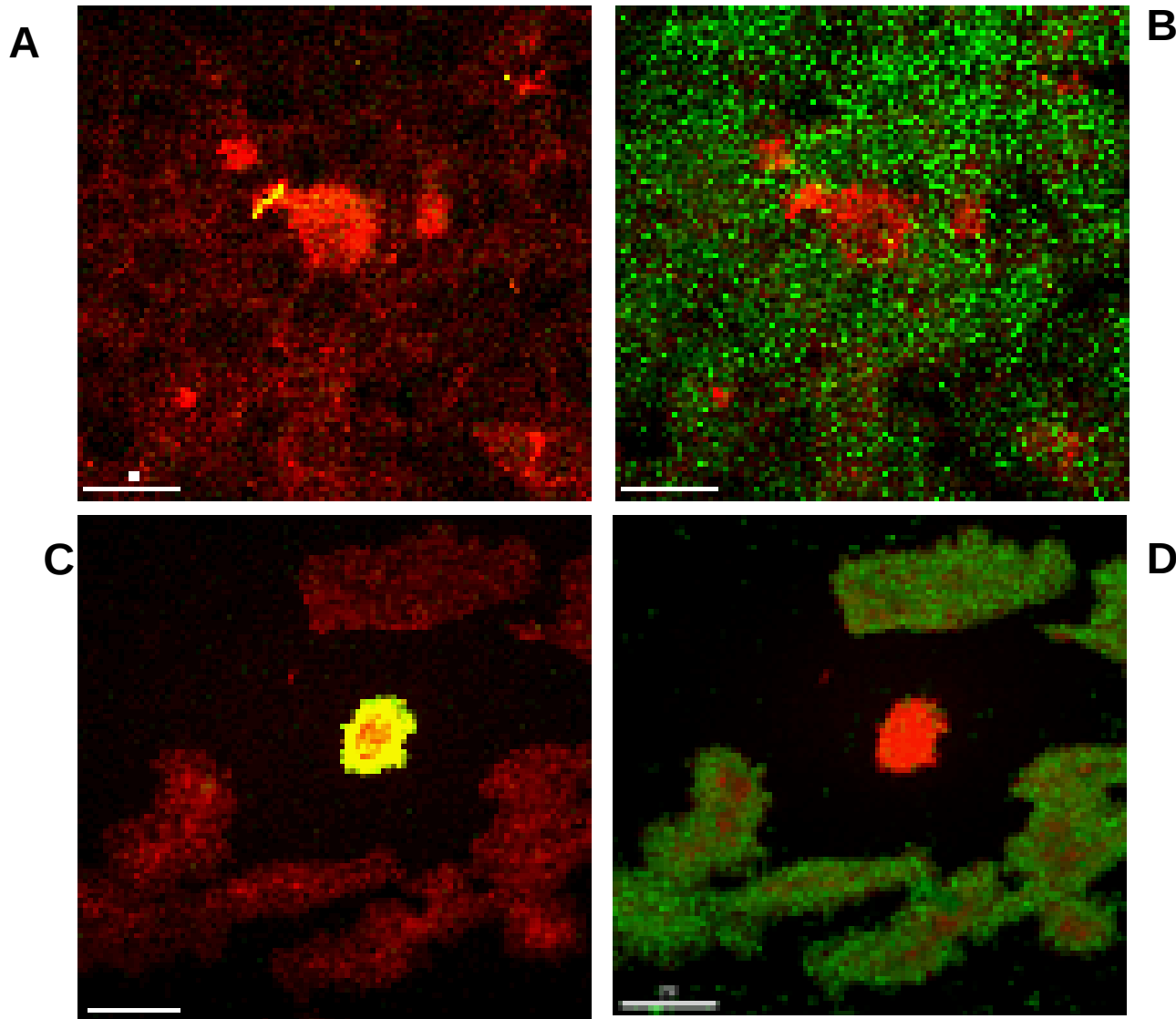
P. bergei* sporozoites pass through Kupffer cells to hepatocytes *in vivo



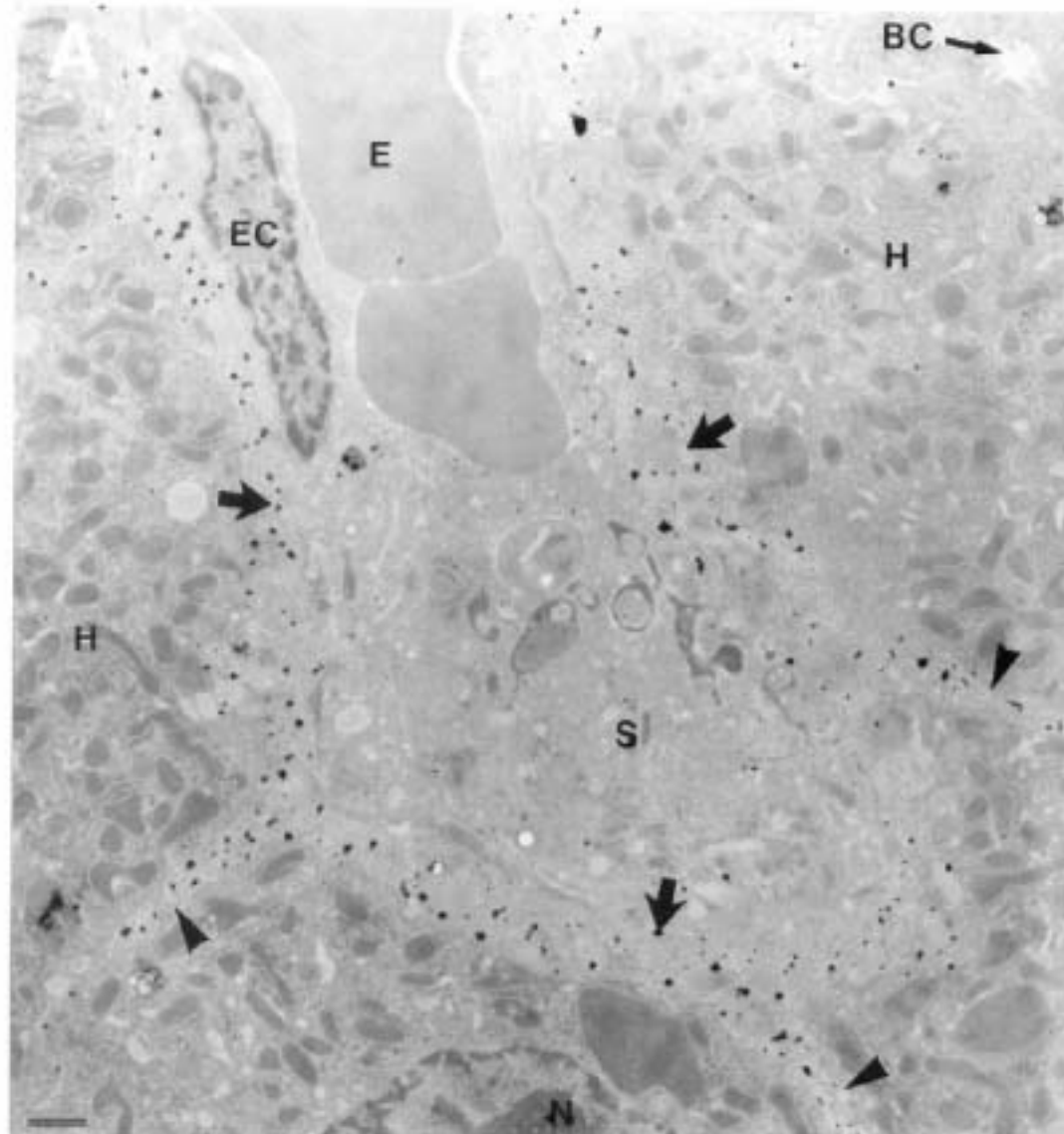
The parasitophorous vacuole enveloping the sporozoite in Kupffer cells there is no binding to lysosomes



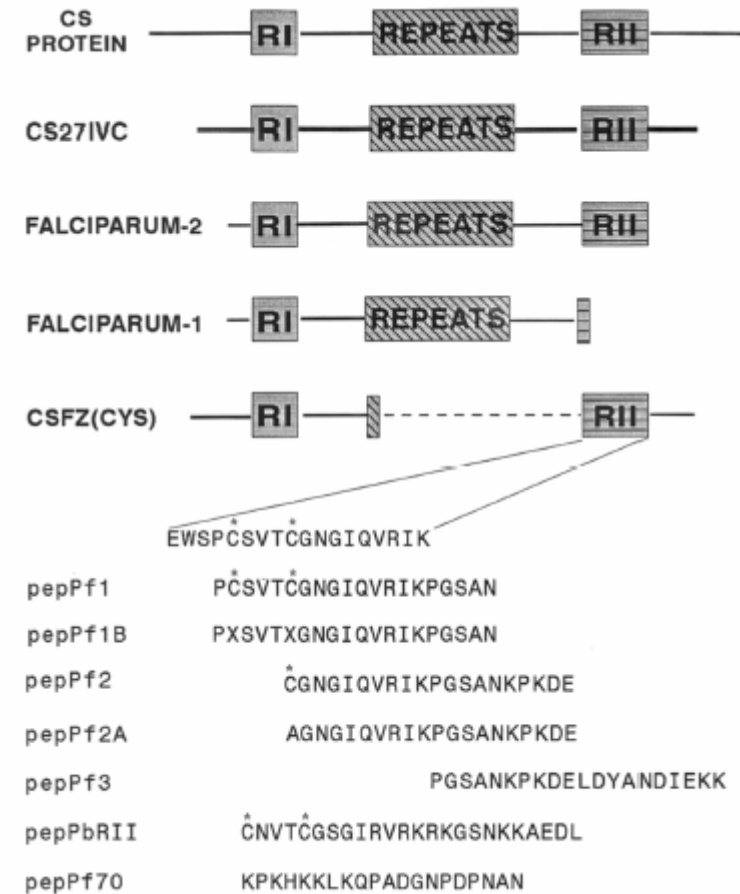
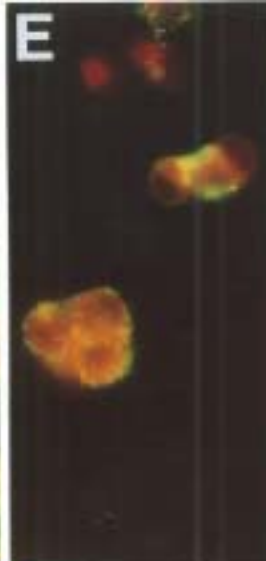
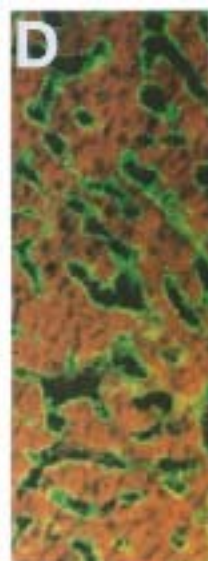
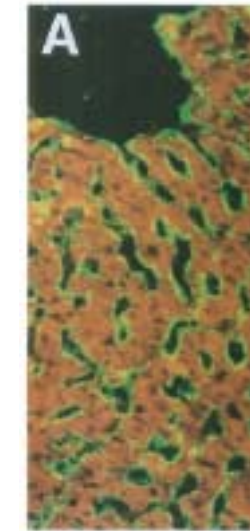
CS protein inhibition of protein synthesis in mammal cells



CS protein binding to human hepatocyte microvilli in the Disse space

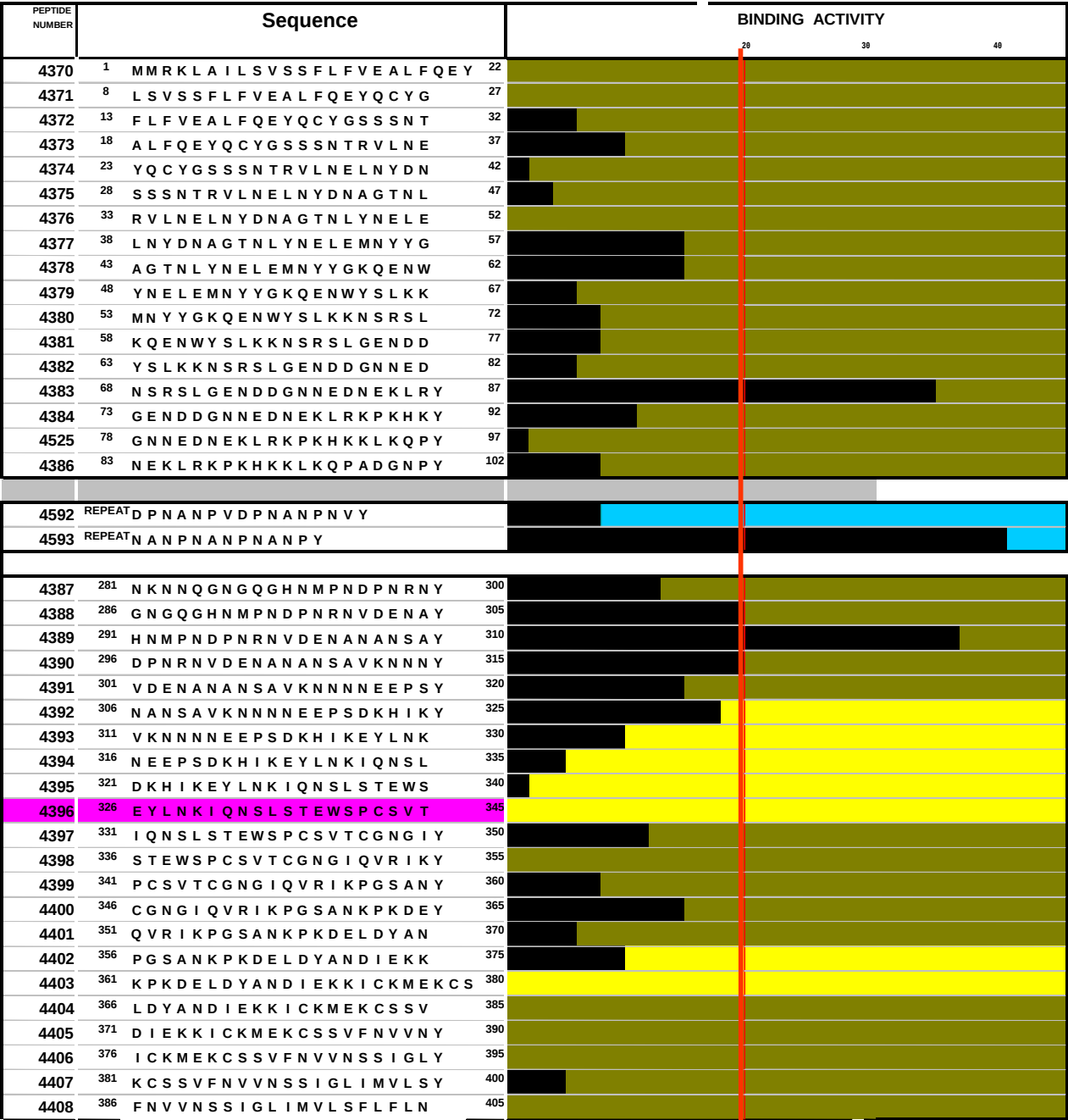


CS protein binding in the liver depends on region II



Plasmodium falciparum circumsporozoite (CS) protein peptides
specifically bind to HepG2 cells

Suarez J, et al, 2001, Vaccine,19:4487



83



383

RTS - S

CS peptides specifically binding to HepG2 cells

Suarez J, et al, 2001, *Vaccine*,19:4487

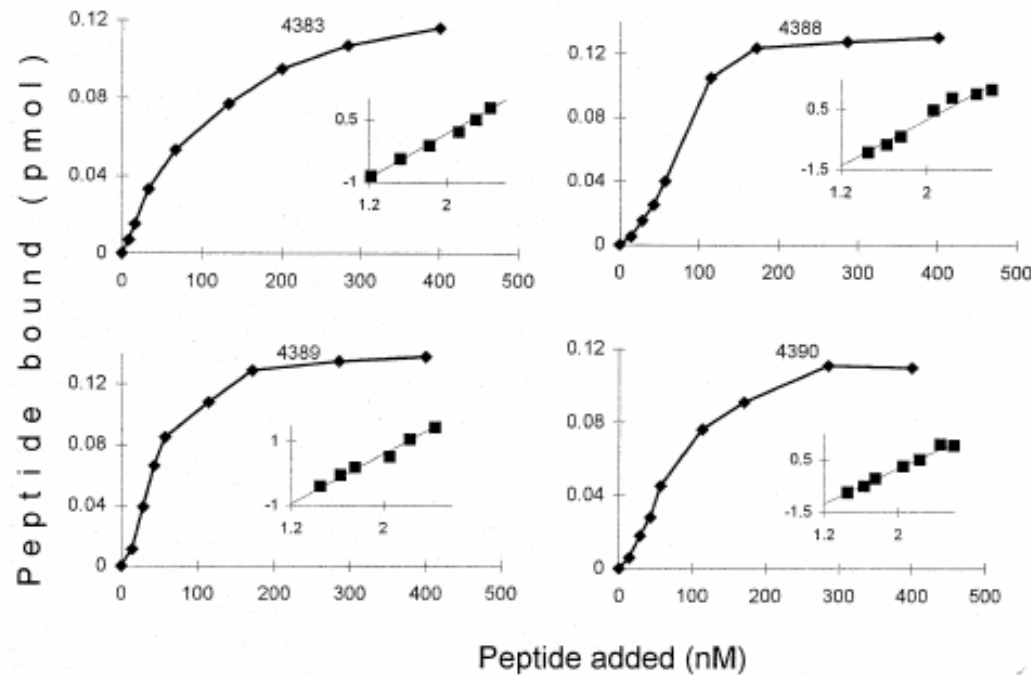
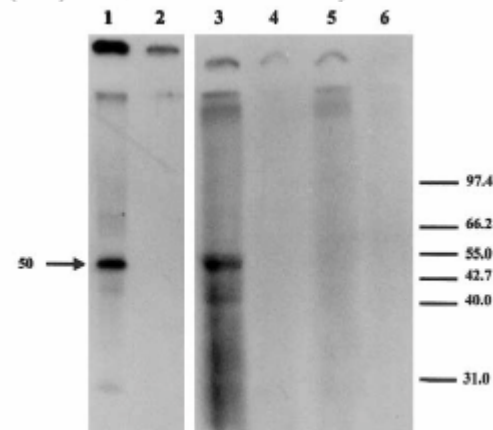


Table 1
Binding constants of CS peptides to HepG2 cells

Peptide	Kd (nM)	n	Binding sites per cell
4383	80 ± 8	1.0 0.1	120 000 ± 11 500
4388	80 ± 11	2.0 0.2	140 000 ± 12 000
4389	50 ± 6	2.0 0.2	140 000 ± 12 000
4390	80 ± 9	2.0 0.2	120 000 ± 10 000



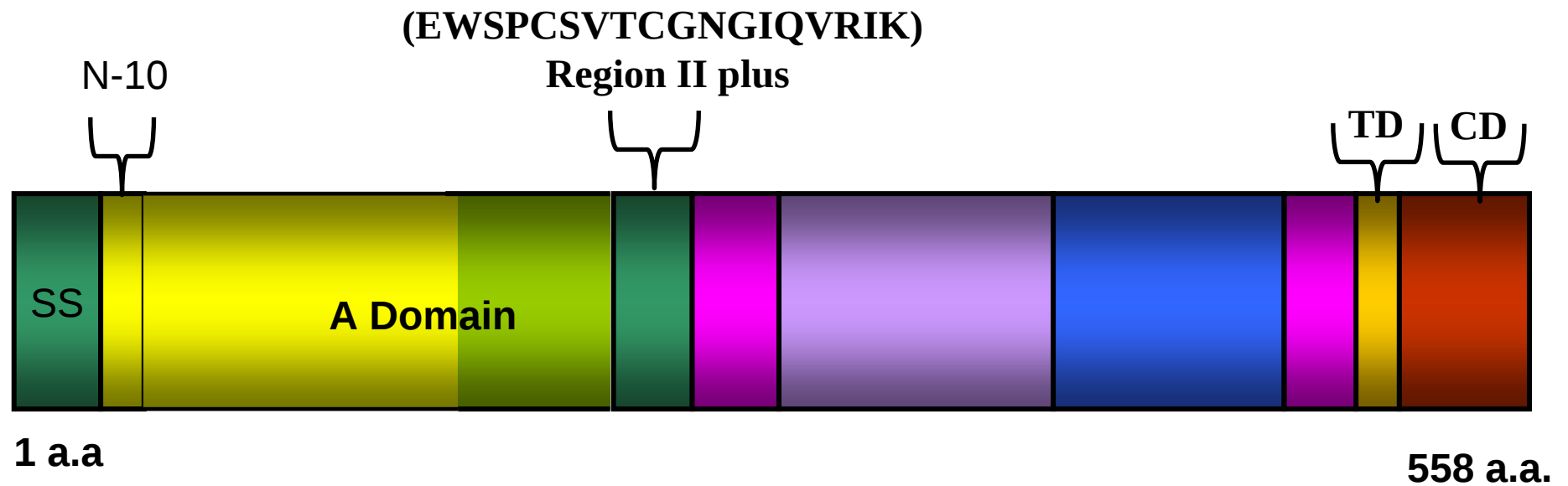
Polimorphic sequence found in different *P. falciparum* isolates
Corresponding to amino acids 326 to 345
Of the NF54 *P. falciparum*

Moreno A. et al. 1993 *J Immunol.* 151:489.

Isolates	Origin	Sequence													
NF54,XP _{8,9} , X _{10,11} ,427 _{1-4,6-10} 3D7	Wets Africa, Honduras, The Netherlands	<u>E</u>	Y	L	<u>N</u>	<u>K</u>	I	<u>Q</u>	N	S	<u>L</u>	S	T	E	W
ItG2G1	Brazil	K			K										
MS2,SO6	Thailand, Brazil	K			K	R									
LE5, 366 ₉	Liberia, Gambia	K			K	T									
366 _t , 399 ₁₋₁₀ , 406 ₁₋₉ , 419 ₁₀	Gambia	K			K	T	K								
366 _{8,10}	Gambia	K			Q										
406 ₁₀ , 419 ₁₋₉	Gambia	K			Q		K								
366 ₂₋₇	Gambia	K			Q		R								
Xs,XP _{12,13} HB3, D10, T4, T9-101,	Honduras, The Netherlands, Papua New Guinea,	Q			K										
WEL, B11, PA, FCR3	Thailand, West Africa, Uganda, Gambia														
S24, T9-98	Angola, Thailand	Q			K	T	K								
7G8	Brazil	Q			K		K		I						

^aAmino acids numbering is based on the CS protein of the NF54 strain of *P. falciparum*. The polimorphic residues are underline, and only amino acids that differ from the NF54 sequence are shown for each strain or isolate. The subscript numbers correspond to the isolate name described in materials and methods

Thrombospondin-Related Anonymous Protein (TRAP)/SSP2 (63 kDa)



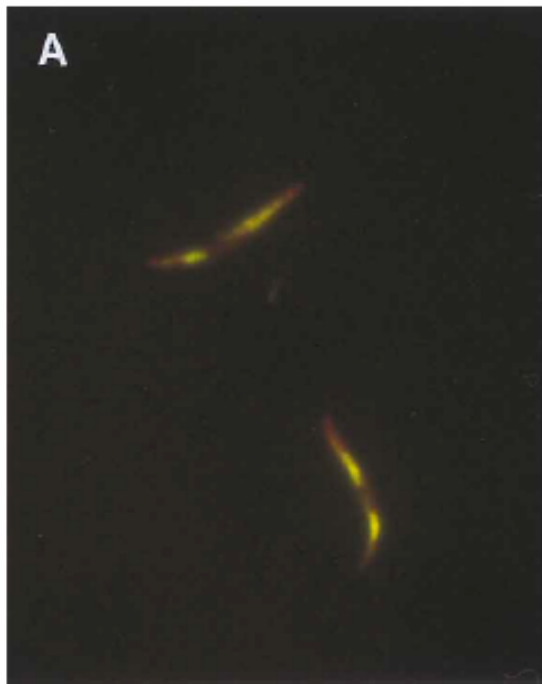
 Sequence similarity Py SSP₂

 Divergent region

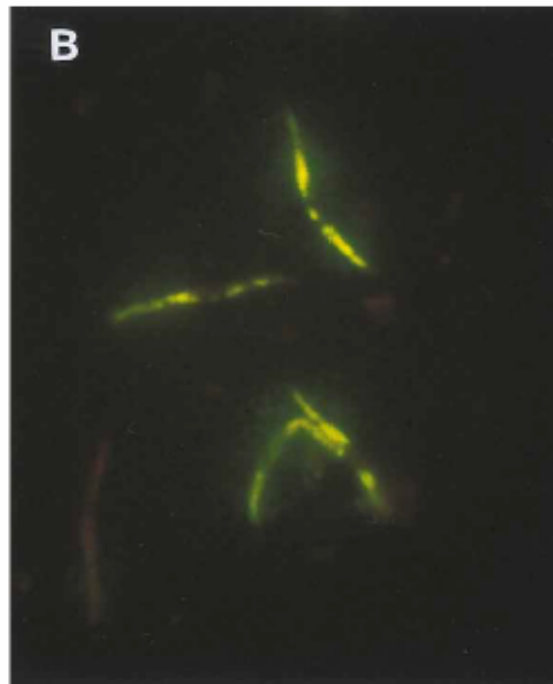
 Binding region

 Conserved region

IFAs of *P. falciparum* SSP2 monoclonal and polyclonal antibodies against sporozoites



mAb SSP2



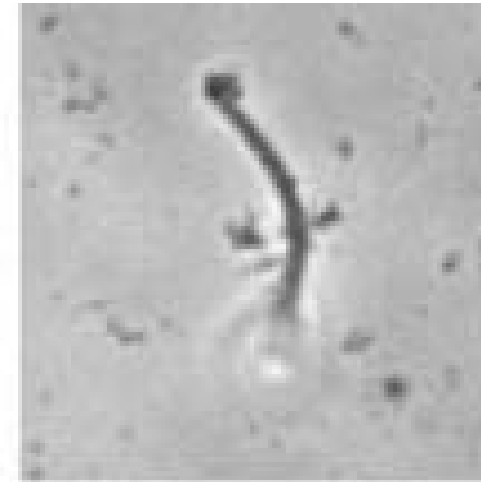
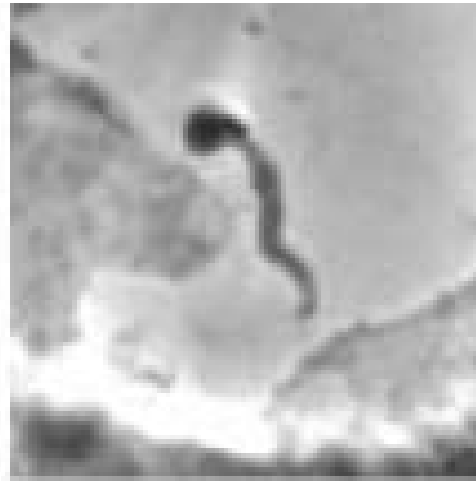
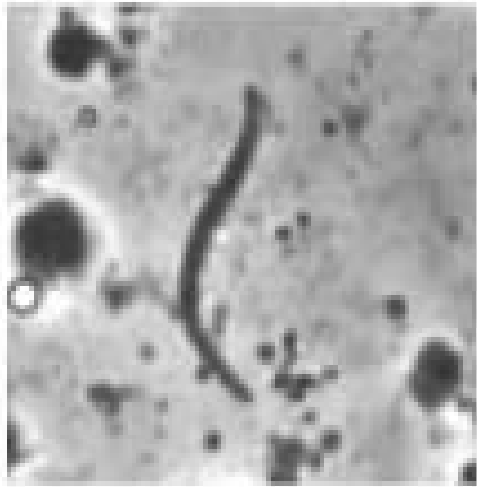
α SSP2



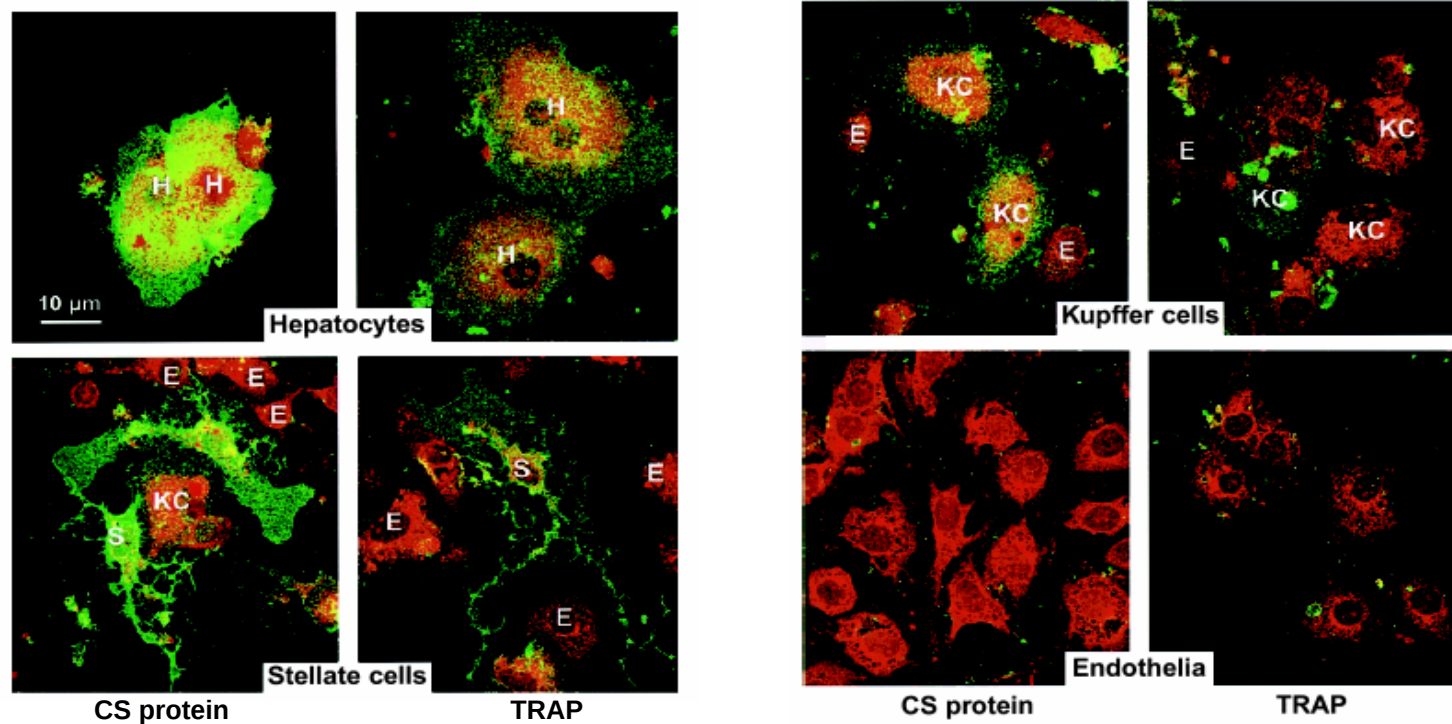
α CSP

Regulated apical exocytosis is observed as a “cap” in the sporozoite extreme

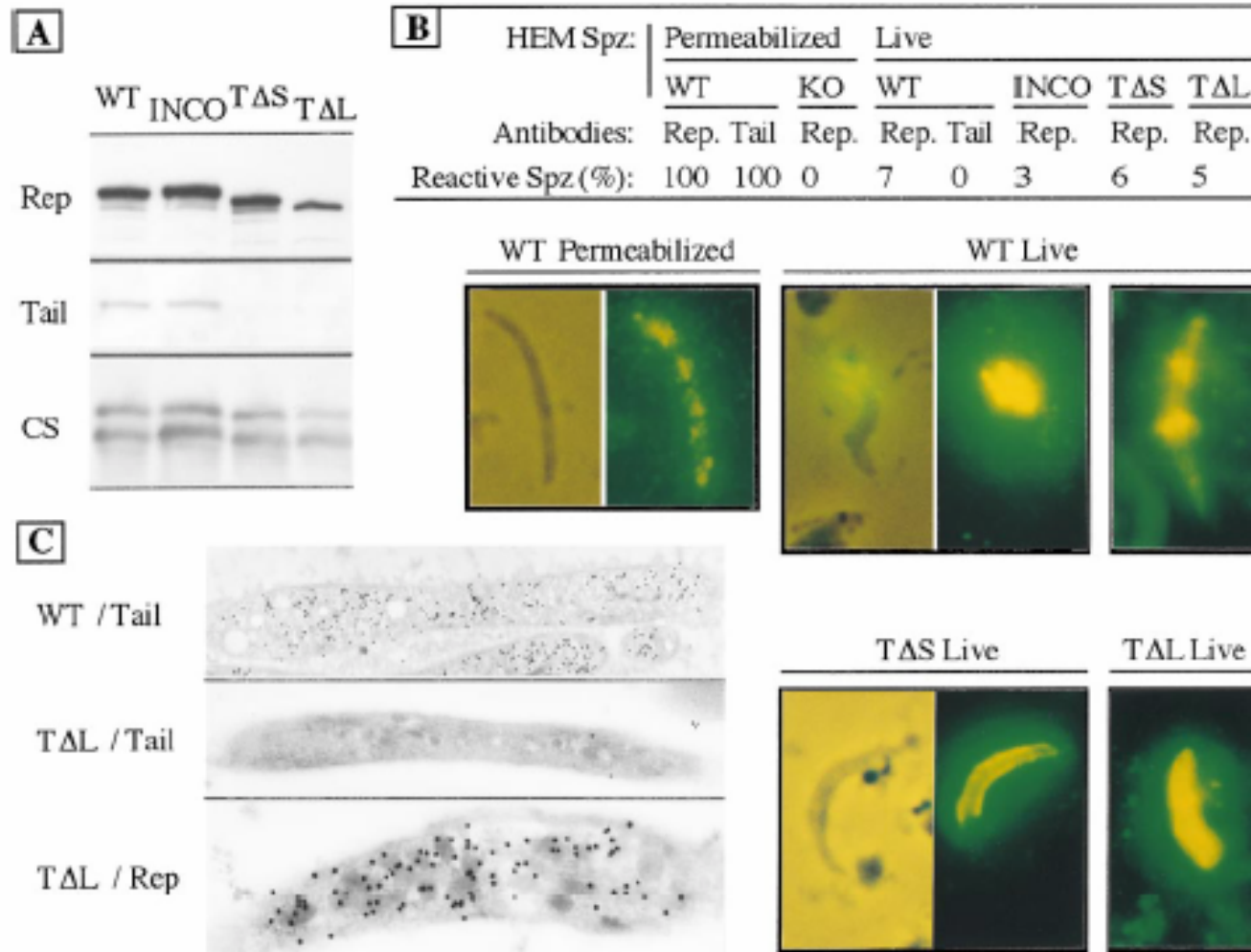
HepG2



CS and TRAP proteins recognise stellate cells, Kupffer cells and hepatocytes but not endothelium cells' sinuosity



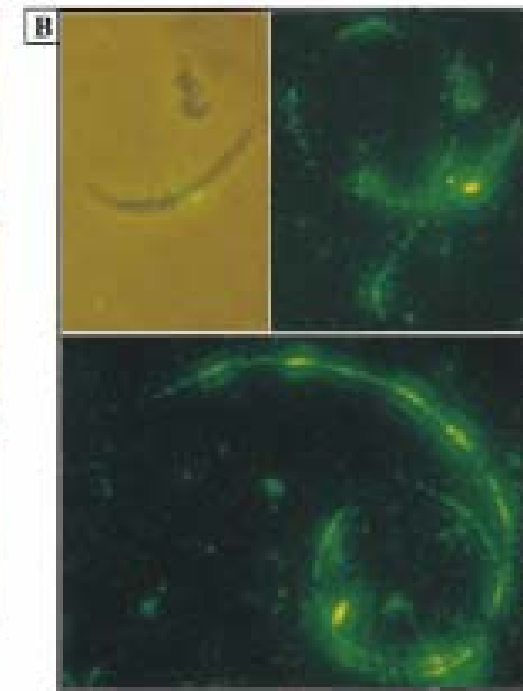
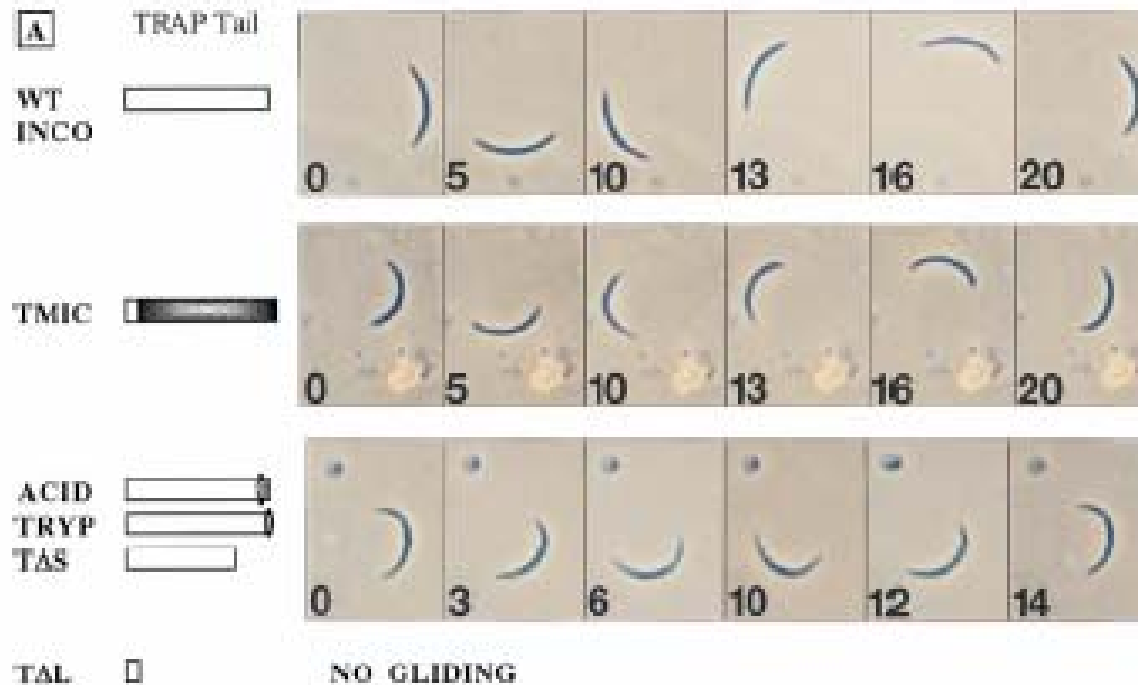
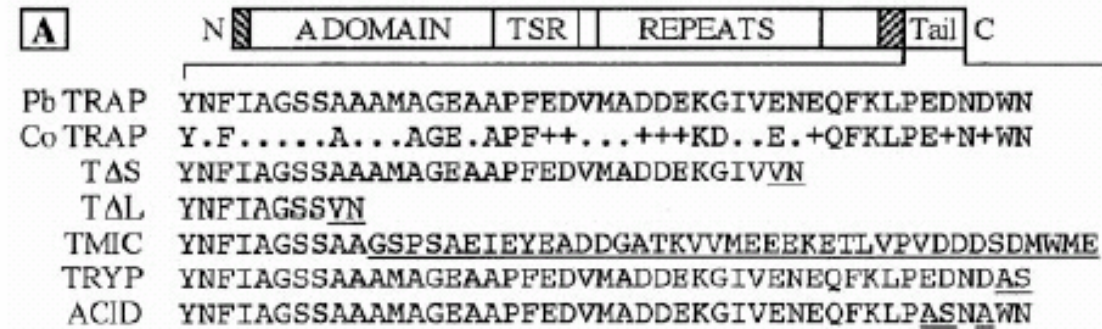
Truncated TRAPs are correctly expressed and found on the sporozoite surface



TRAP(-) sporozoite does not express *in vitro* displacement and mobility

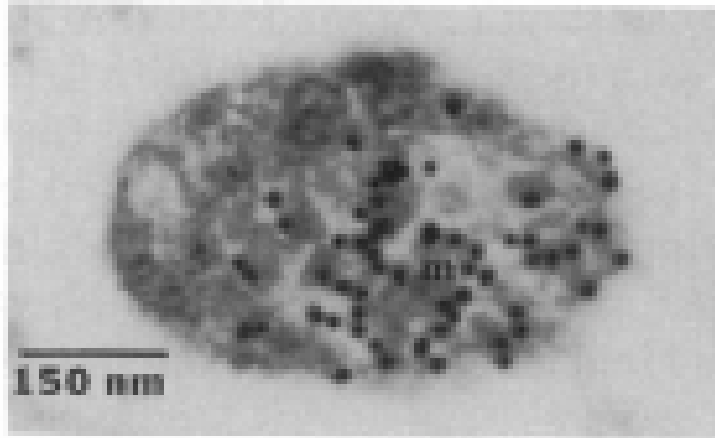


Phenotypical displacement of wild and mutated sporozoites

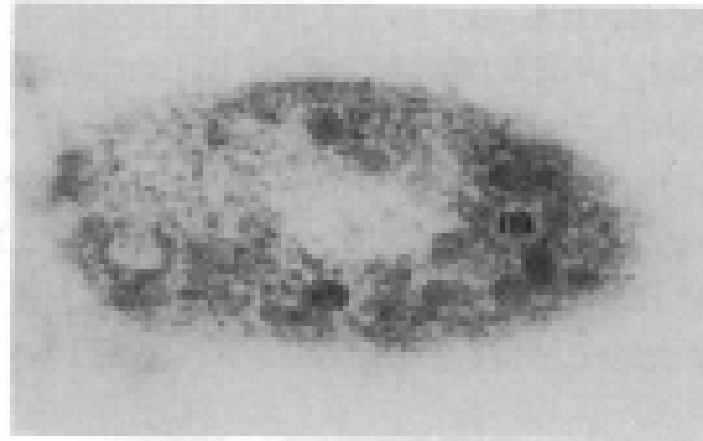


Immuno-electron microscopy of *P. falciparum* SSP2

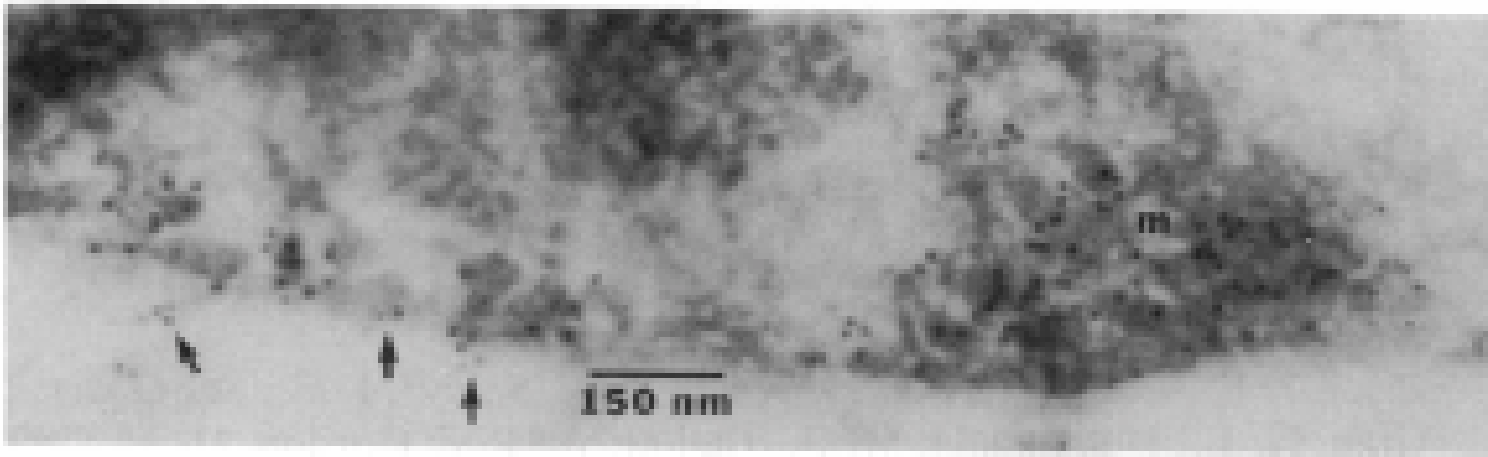
a



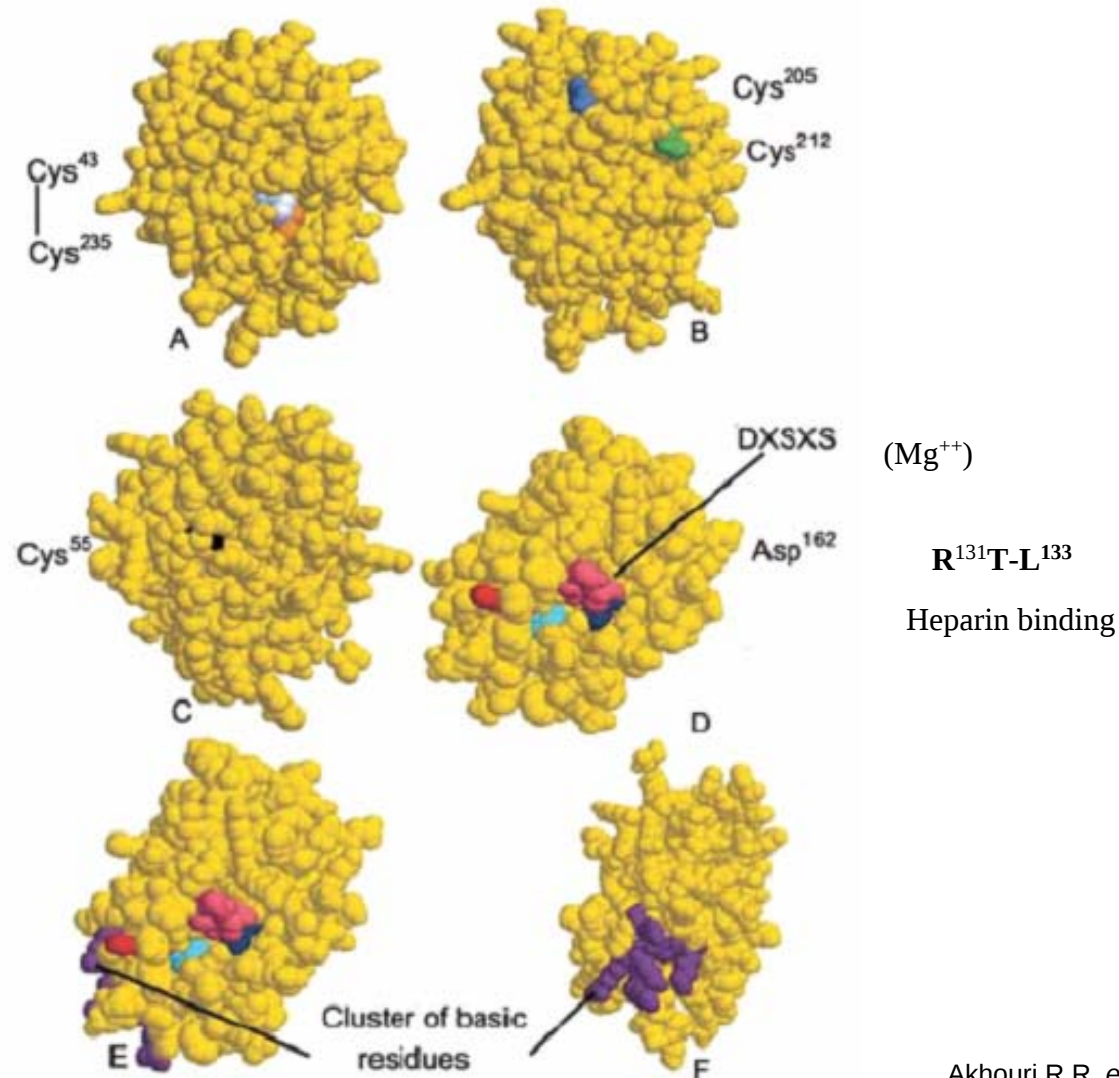
b



c



Homology modelling based von Willenbrand factor and TRAP A domain



Plasmodium sporozoite invasion of insect and mammalian cells is directed by the same dual binding system

A

A-domain

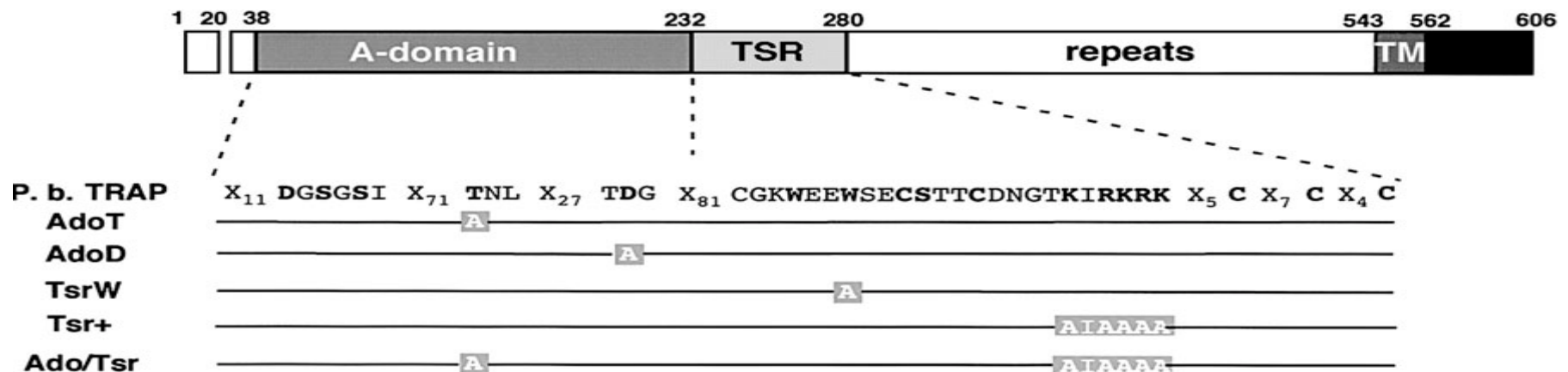
TRAP	X ₁₁	DGSGSI	X ₇₁	TNL	X ₂₇	TDGI	X ₇₂
CD11b	X ₈	DGSGSI	X ₆₃	THT	X ₂₉	TDGE	X ₅₉
coll. α-2 (VI)	X _{7/18}	D-SES-	X _{62/75}	T--	X ₂₆₋₃₈	TDG-	X _{51/55}
matrilin-1	X _{6/17}	D-S-S-	X ₆₅	TMT	X _{29/32}	TDGR	X _{60/62}
factor C2	X ₈	DCSQSV	X ₇₀	TNT	X ₃₅	TDGK	X ₇₄
T20G5.3	X ₇	DGSGSI	X ₆₆	TRT	X ₃₂	TDGR	X ₄₉

B

TSR

TRAP	WEEWSECSTTCD	X ₃	KIRKRRK
thrombosp.	WS-W--CS--CG	X _{4/5}	R-R
properdin	W--W X ₅₋₁₂ C-	X ₃₋₈	R-RR/K
UNC-5	WS-W--C---C-	X _{1/2}	R-R
MIC2	w--Ws-cs-sCG	X ₁₋₅	R-R-R
CS	STEWSPCSVTCG	X ₅	RIK

C



Plasmodium falciparum:
binding studies of peptide
derived from the sporozoite
surface protein 2 to
Hep G2 cells

2001, J. Pept. Res, 58: 285

R. López
H. Curtidor
M. Urquiza
J. Garcia
A. Puentes
J. Suarez
M. Ocampo
R. Vera
L.E. Rodriguez
F. Castillo
G. Cifuentes
M.E. Patarroyo

PEPTIDE NUMBER		SEQUENCE		BINDING ACTIVITY																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
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3239	1	M N H L G N V K Y L V I V F L	35																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				

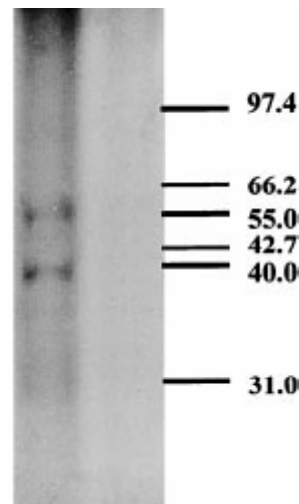
P. falciparum high binding SSP2 peptides containing B epitopes in their sequences

López R. *et al.* 2001, J. Pept. Res, 58: 285

Table 2. Pf SSP2/TRAP peptide affinity constants and critical residue binding to cell Hep G2 cells

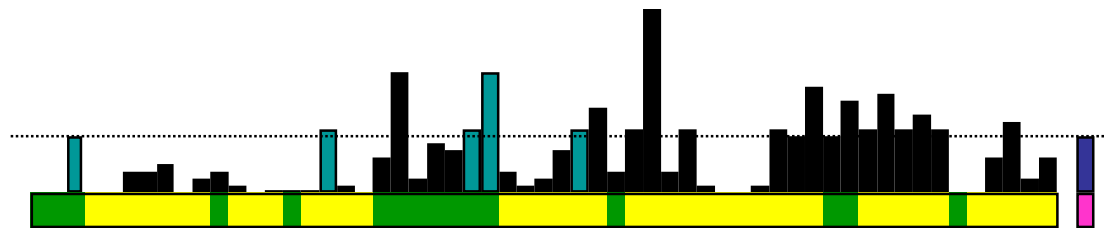
Peptide	Amino acids	Sequence	Kd (nM)	NrSC ^a	Hill
5873	19–41	DLFLVNGRDVQNNIVDEIKYREE	658 ± 10	340 000 ± 2500	1.7
5877	336–355	KGENP <u>NG</u> F <u>DL</u> DE <u>NP</u> EN <u>PP</u> NP	301 ± 4	46 000 ± 1000	1.2
5885	461–485	SE <u>D</u> RE <u>T</u> RPHGR <u>N</u> NENRSYNRKHNNT	585 ± 5	490 000 ± 7000	1.3
6481	478–498	YN <u>R</u> KHNNT <u>P</u> KHPER <u>E</u> EHEKPD	543 ± 7	570 000 ± 4000	1.5
5897	490–510	<u>R</u> EEHEKPDNNKKAGSDNKY	457 ± 8	220 000 ± 8000	1.3
5891	535–555	GAAT <u>P</u> Y <u>A</u> GEPAP <u>F</u> DE <u>T</u> LGEE	685 ± 3	770 000 ± 1000	1.1

a. NrSC, number of binding sites per cell. b. Mean ± SD of three experiments. The critical residue is underlined.



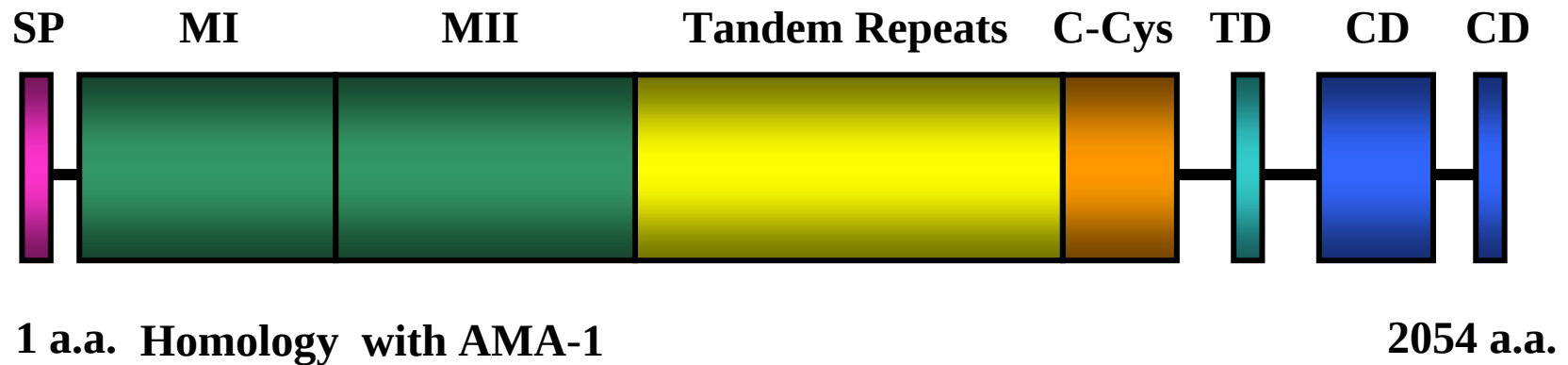
P. falciparum high binding SSP2 peptides containing B epitopes in their sequences

López R. *et al.* 2001, J. Pept. Res, 58: 285

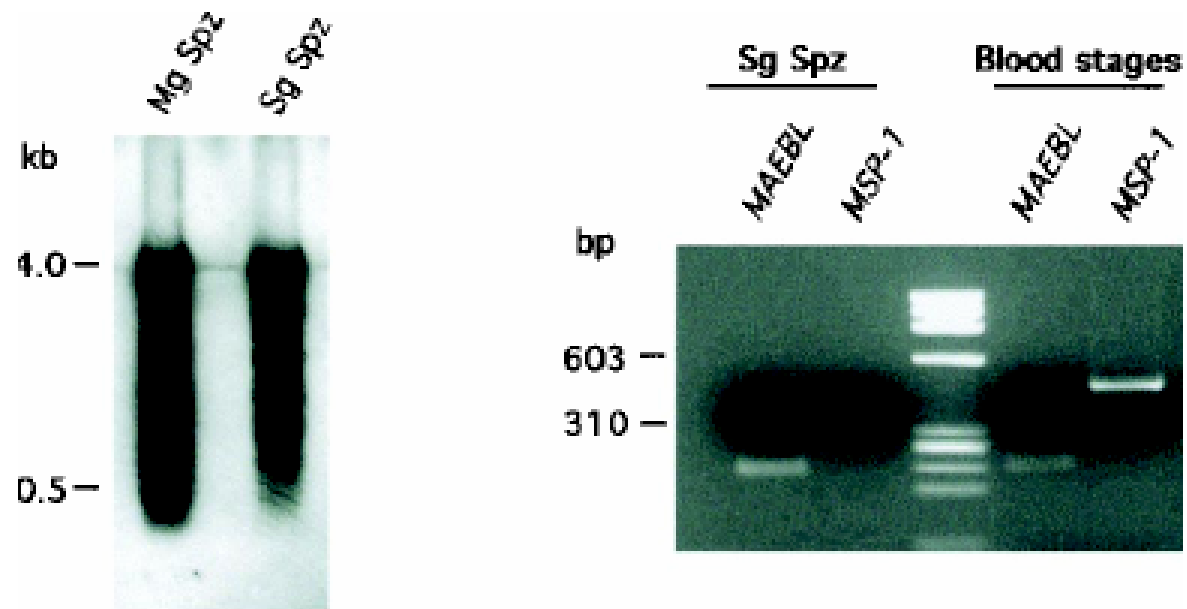


Peptide	amino acids	sequence
3243	(21-35)	FLVNGRDVQNNIVDE
5873	(19-41)	DLFLVNGRDVQNNIVDEIKYREEYCND
		S-S
3287	(241-265)	TASCGVWDEWSPCSVTCGKGTRSR
3279	(201-215)	FLVGCHPSDGKCPLY
3299	(298-313)	DDQPRPRGDNFAVEKP
3323	(417-431)	NDKSDRYIPYSPPLSP
5885	(456-485)	SEDRETRPHGRNNENRSYNRKHNNT

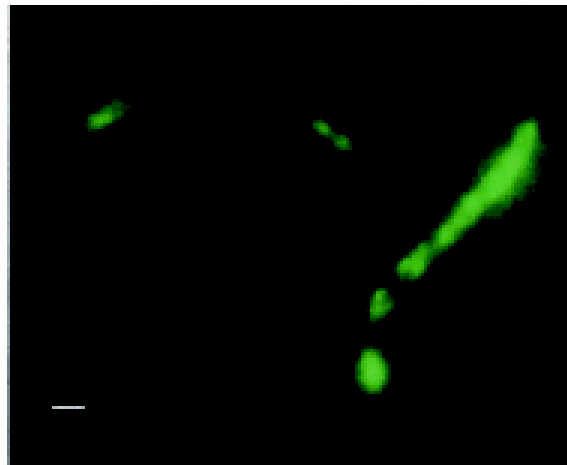
Apical Membrane Antigen/Erythrocyte Binding-Like Protein (MAEBL)(243 kDa)



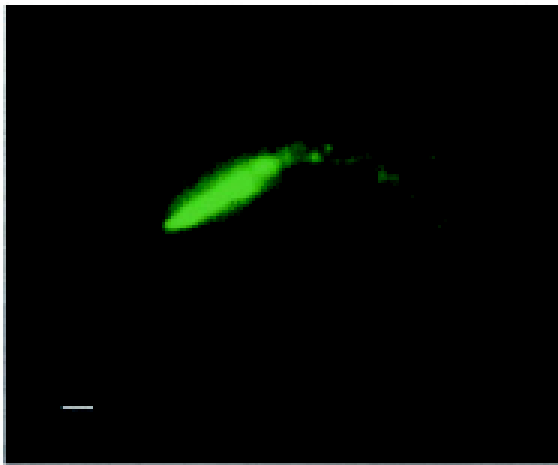
MAEBL sporozoite expression



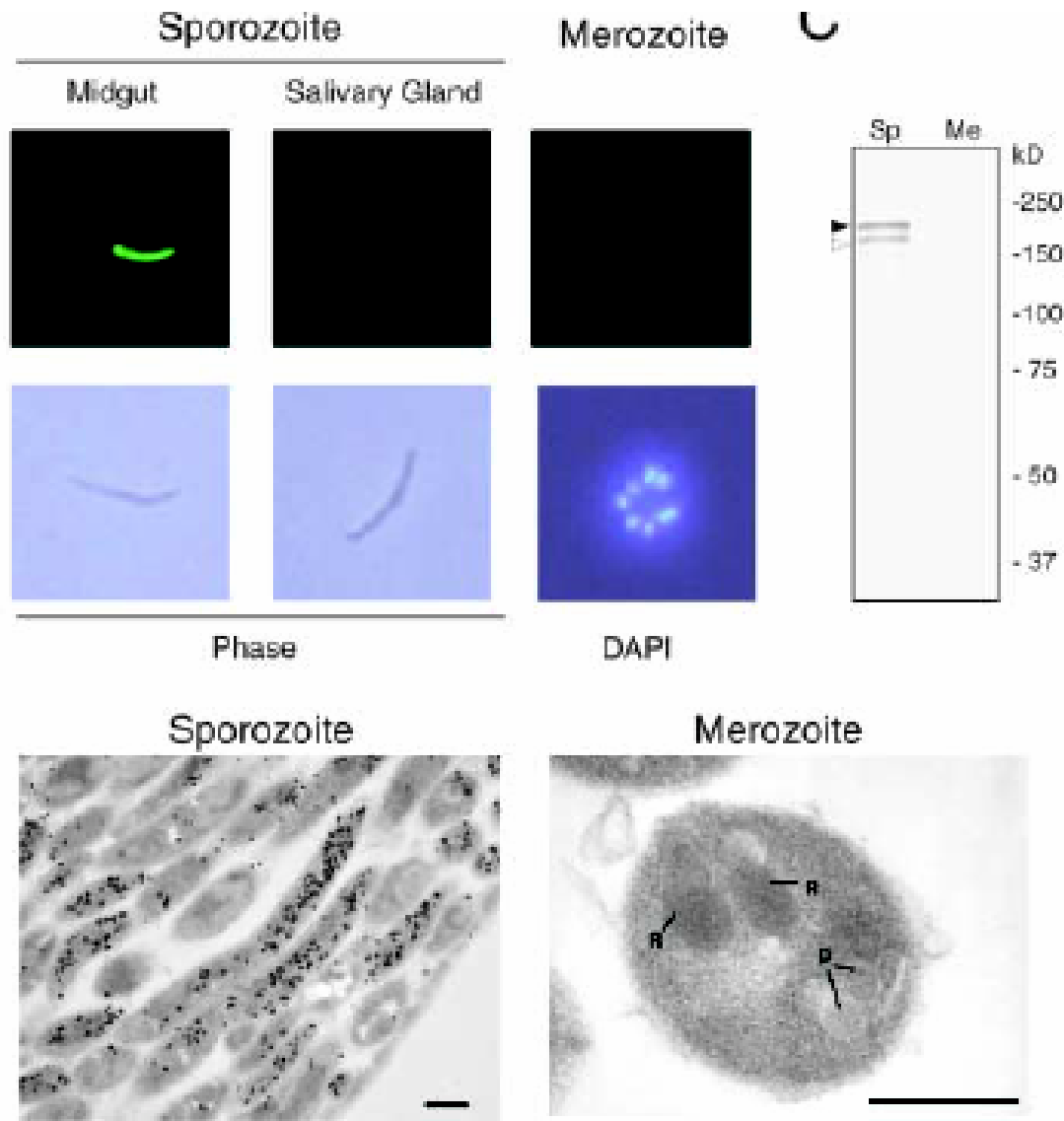
C



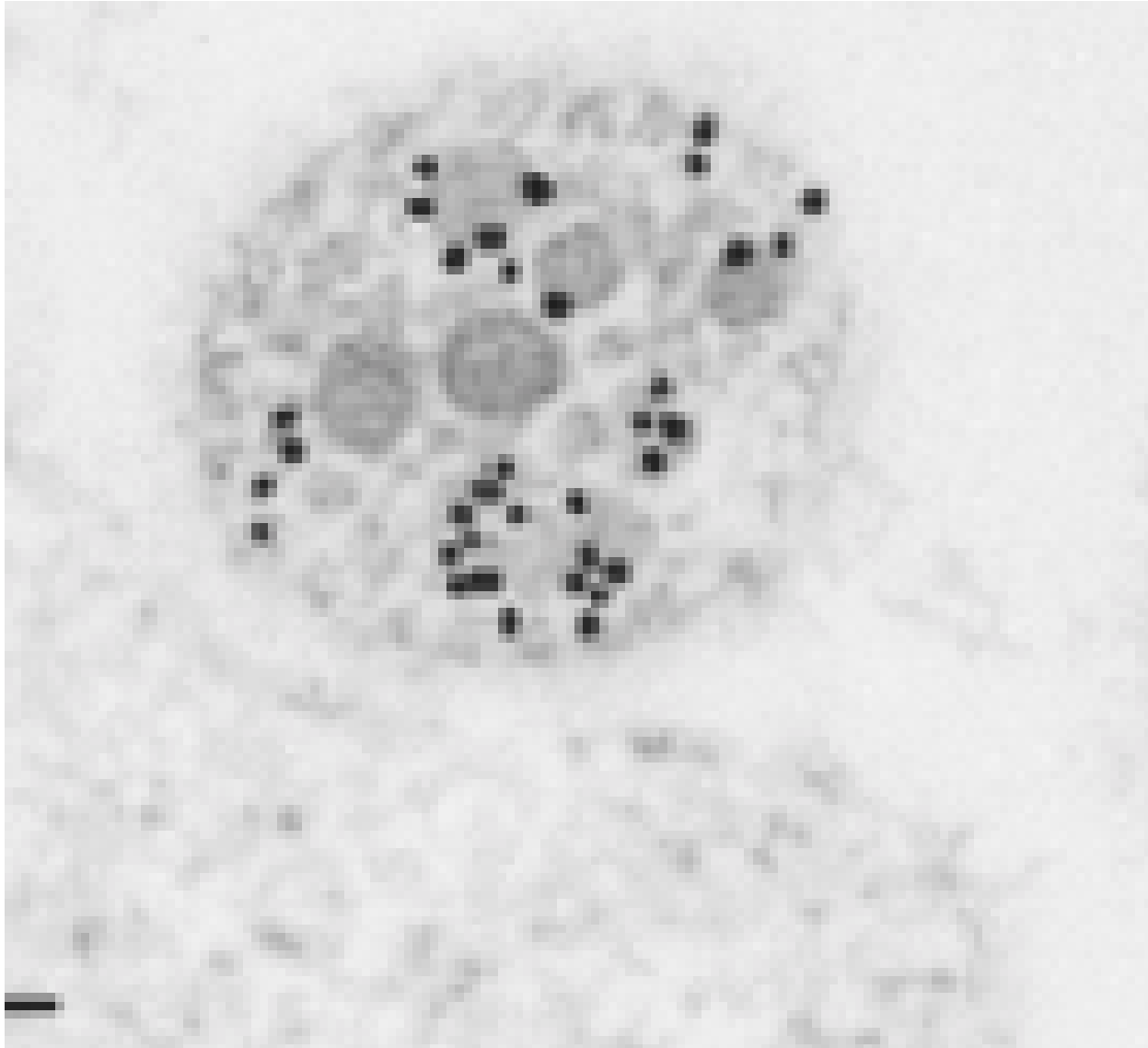
D



PbMAEBL stage-specific production and localisation in micronemes

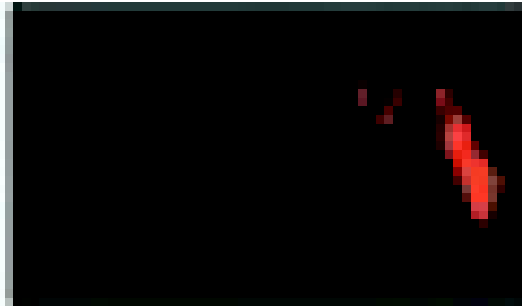


Labelling an oocyst sporozoite section with anti-MAEBL antibodies reveals micronemal localisation

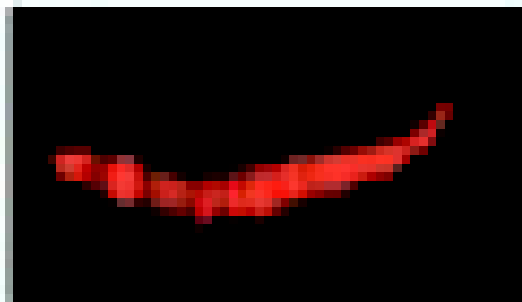


Differential localisation of *P. Berghei* MAEBL salivary gland sporozoites

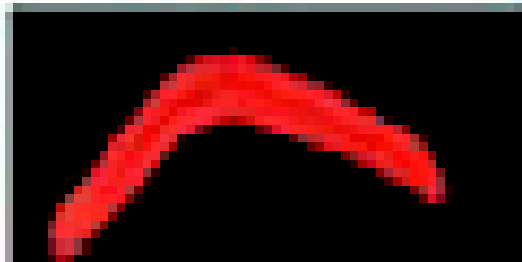
MG D15
sporozoite



MG D22
sporozoite

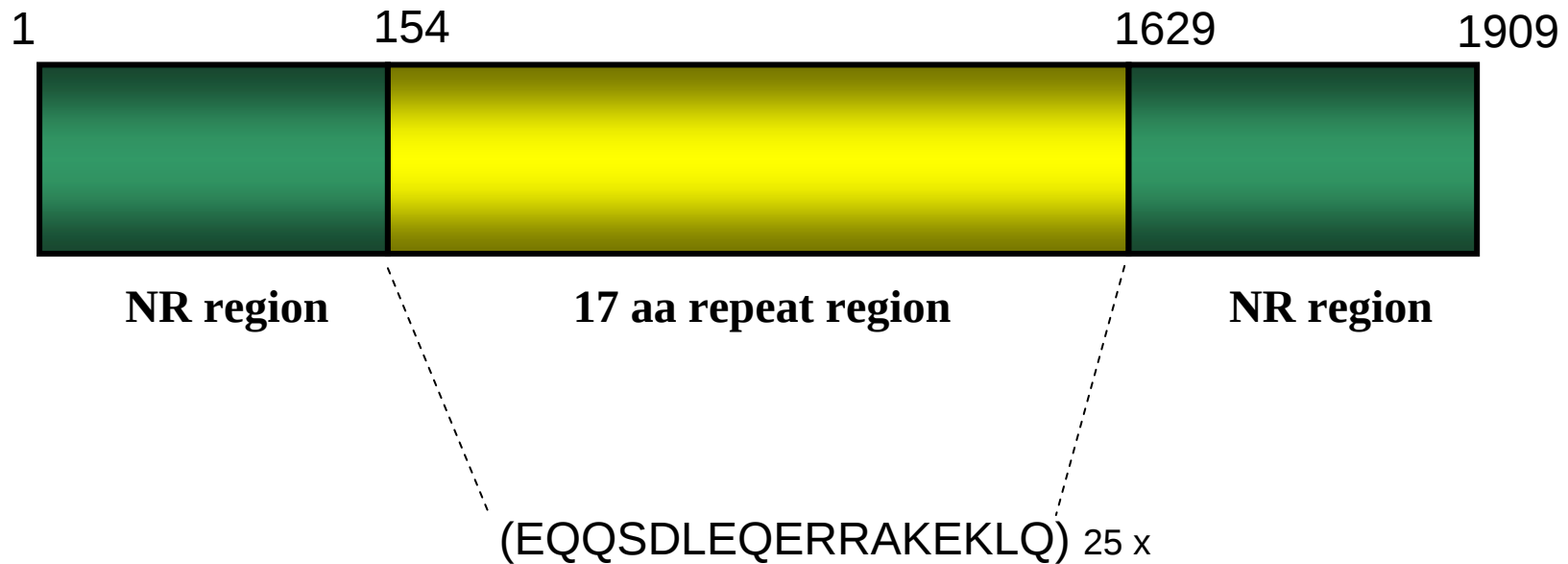


MG D25
sporozoite



Liver Stage Antigen 1 (LSA-1)

(240 kDa)



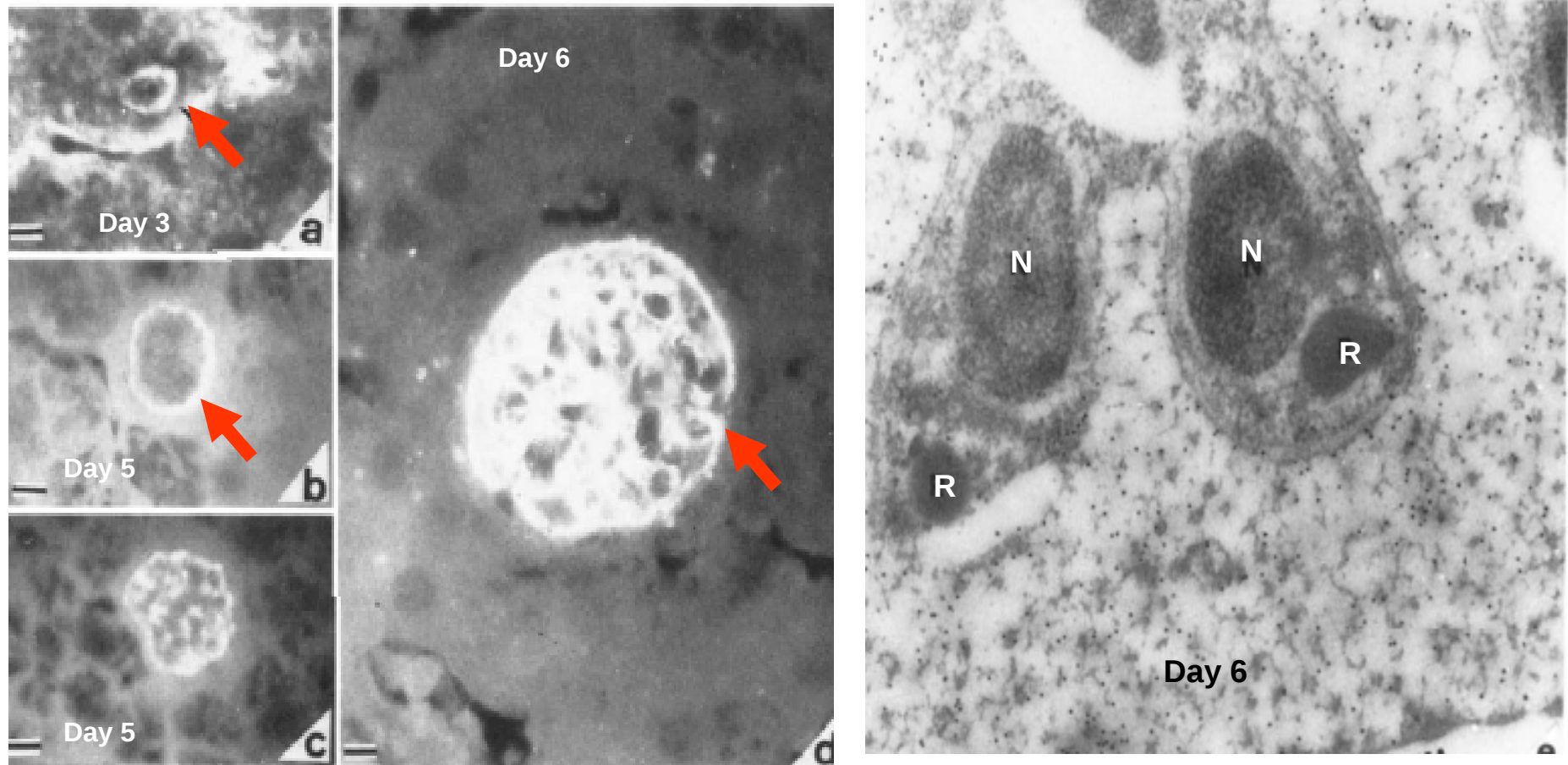
Localisation:

Tpz, PVM, Liver Schz-Mz

Possible function:

Adhesion, Invasion

Localisation of LSA-1 during liver stage schizogony



Peptide of the liver stage antigen-I (LSA-1) of *Plasmodium falciparum* bind to human hepatocytes

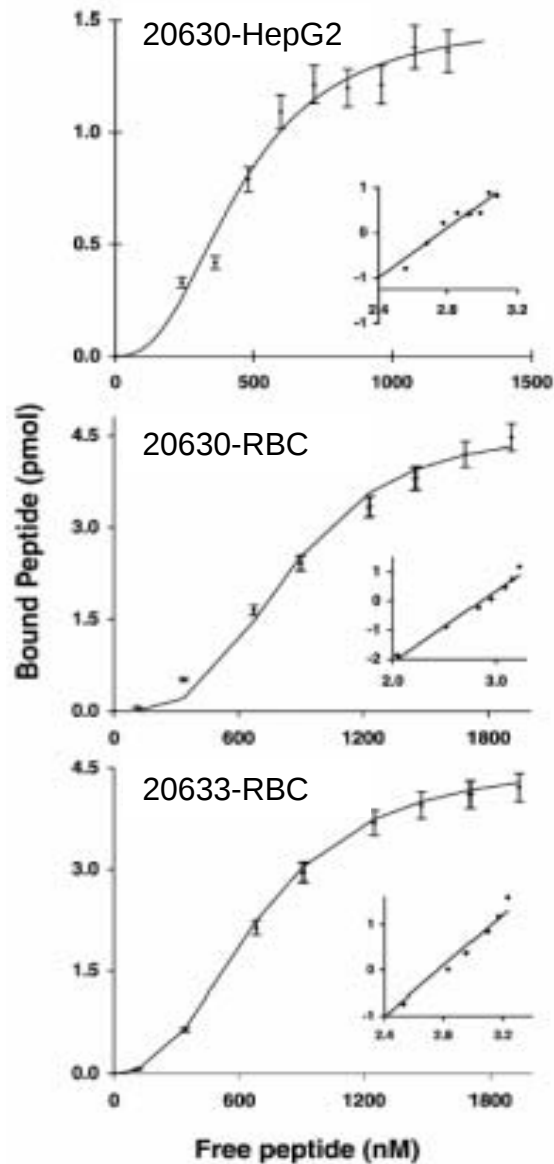
García J. *et al*, 2003, **Peptides**, 24:647

PEPTIDE NUMBER	SEQUENCE			% BINDING ACTIVITY		Erythrocytes	
				HepG2 cells		% Binding Activity	
				20		1	2
20629	1	M K H I L Y I S F Y F I L V N L L I F H	20				
20630	21	I N G K I I K N S E K D E I I K S N L R Y	40				
20631	41	S G S S N S R N R I N E E N H E K K H V Y	60				
20632	61	L S H N S Y E K T K N N E N N K F F D K	80				
20633	81	D K E L T M S N V K N V S Q T N F K S L Y	100				
20634	101	L R N L G V S E N I F L K E N K L N K E Y	120				
20635	121	G K L I E H I I N D D D D K K K Y I K G	140				
20636	141	Q D E N R Q E D L E E K A A K E K L Q G Y	160				
20637	157	K E K L Q G Q Q S D S E Q E R R A Y	173				
20638	174	K E K L Q E Q Q S D L E Q E R L A Y	190				
20639	191	K E K L Q E Q Q S D L E Q E R R A Y	207				
20641	1434	Q E Q Q S D L E Q D R L A K E K L Y	1450				
20640	1604	Q E Q Q S D L E Q E R R A K E K L Y	1620				
20642	1621	Q E Q Q R D L E Q R K A D T K K N L E R Y	1640				
20643	1641	K K E H G D I L A E D L Y G R L E I P A	1660				
20644	1661	I E L P S E N E R G Y Y I P H Q S S L P	1680				
20645	1681	Q D N R G N S R D S K E I S I I E K T N Y	1700				
20646	1701	R E S I T T N V E G R R D I H K G H L E Y	1720				
20647	1721	E K K D G S I K P E Q K E D K S A D I Q Y	1740				
20648	1741	N H T L E T V N I S D V N D F Q I S K Y	1760				
20649	1761	E D I S A E Y D D S L I D E E E E D D E	1780				
20650	1781	D L D E F K P I V Q Y D N F Q D E E N I	1800				
20651	1801	G I Y K E L E D L I E K N E N L D D L D	1820				
20652	1821	E G I E K S S E E L S E E K I K K G K K Y	1840				
20653	1841	Y E K T K D N N F K P N D K S L Y D E H	1860				
20654	1861	I K K Y K N D K Q V N K E K E K F I K S	1880				
20655	1881	L F H I F D G D N E I L Q I V D E L S E Y	1900				
20656	1890	E I L Q I V D E L S E D I T K Y F M K L	1909	ND			

LSA-1 peptides bind to HepG2 and RBC

García J. *et al*, 2003, **Peptides**, 24:647

Saturation assays



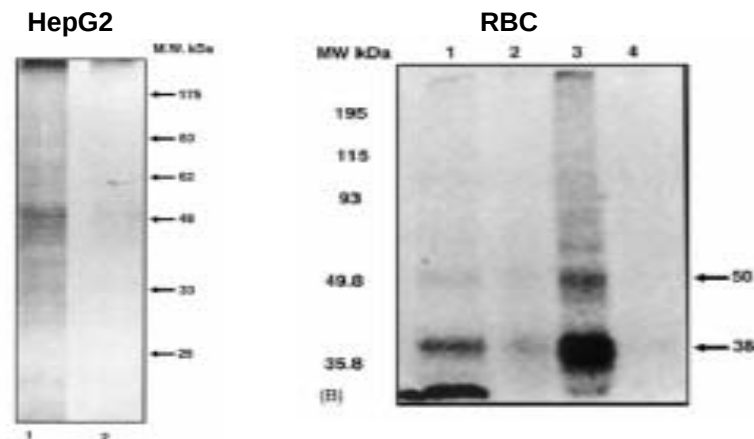
Peptide	K_d (nM)	Sites per cell $\times 10^6$	Hill coefficient
20630 ^a	450	0.7	2.0
20630 ^b	850	8.3	3.3
20633 ^b	700	8.4	2.5

Invasion inhibition

LSA-1 protein high binding peptides and sera analogues invasion and development inhibition assays

Peptide	% Invasion inhibition		% Development inhibition	
	100 μ M	200 μ M	100 μ M	200 μ M
20630	10 $\pm$ 4	34 $\pm$ 2	0 $\pm$ 3	0 $\pm$ 3
20633	48 $\pm$ 1	88 $\pm$ 1	15 $\pm$ 4	85 $\pm$ 1
6762	13 $\pm$ 2	100 $\pm$ 2	0 $\pm$ 5	57 $\pm$ 2
Chloroquine	100 $\pm$ 2	100 $\pm$ 2	100 $\pm$ 2	100 $\pm$ 2

Cell membrane assay

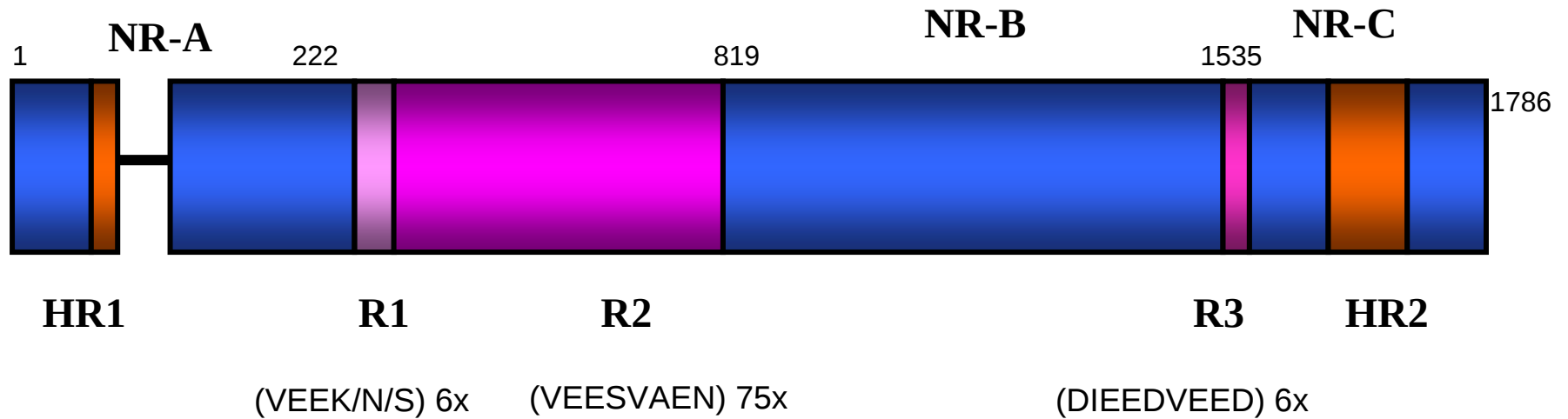


Liver Stage Antigen 3 (LSA-3)

(200 kDa)

Exon 1

Exon 2



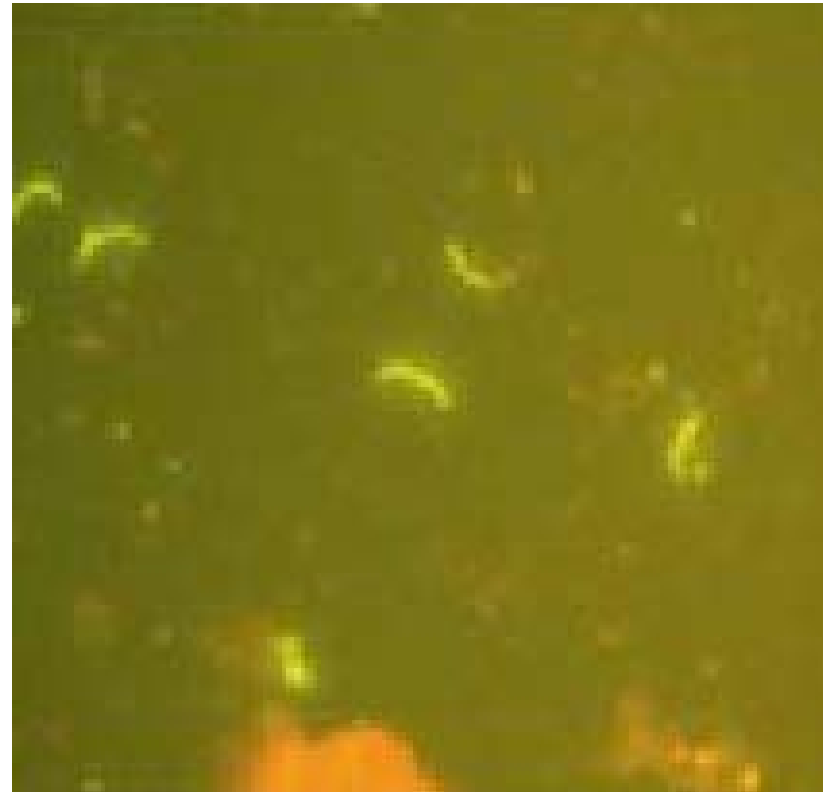
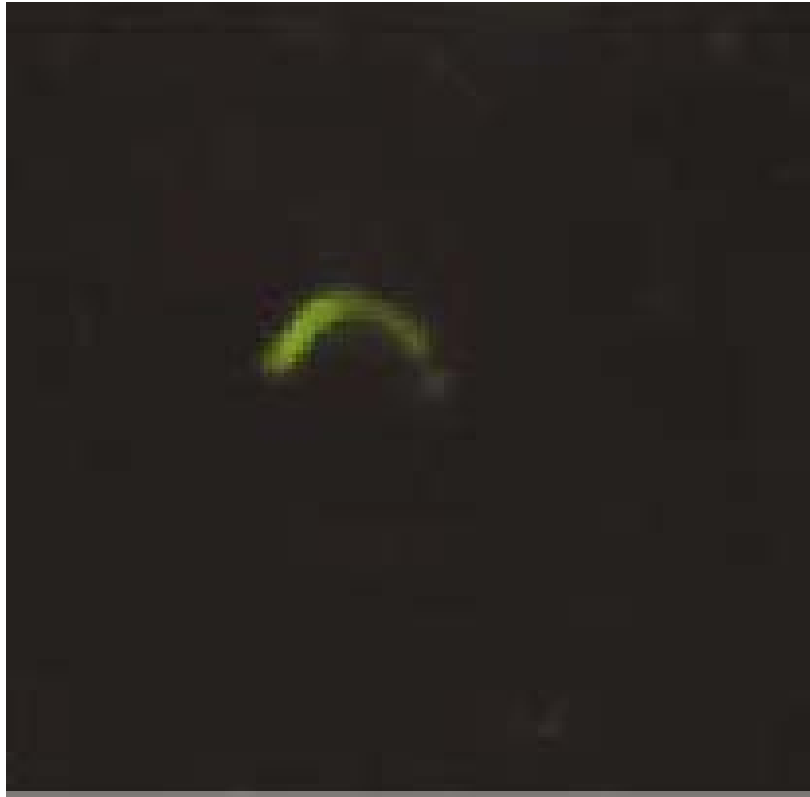
Localisation:

Surface Spz, PV, Liver Stage

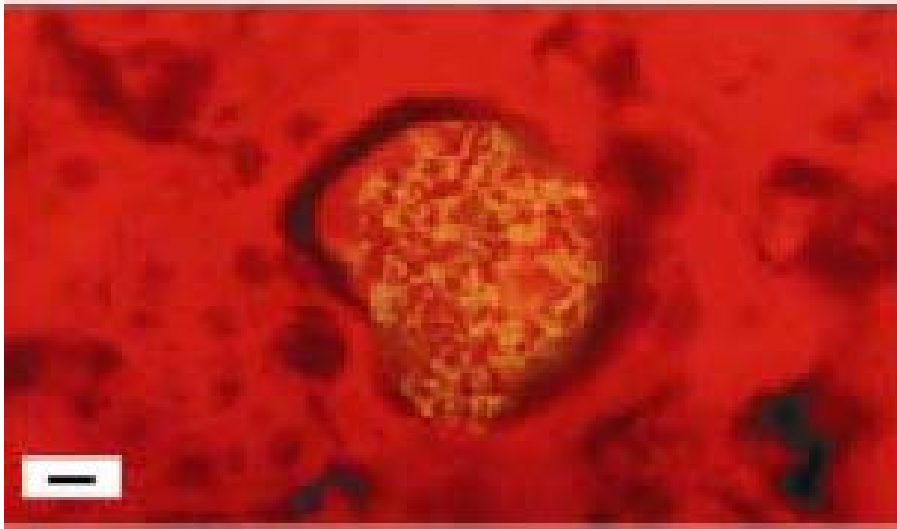
Possible function:

Adhesion, Invasion

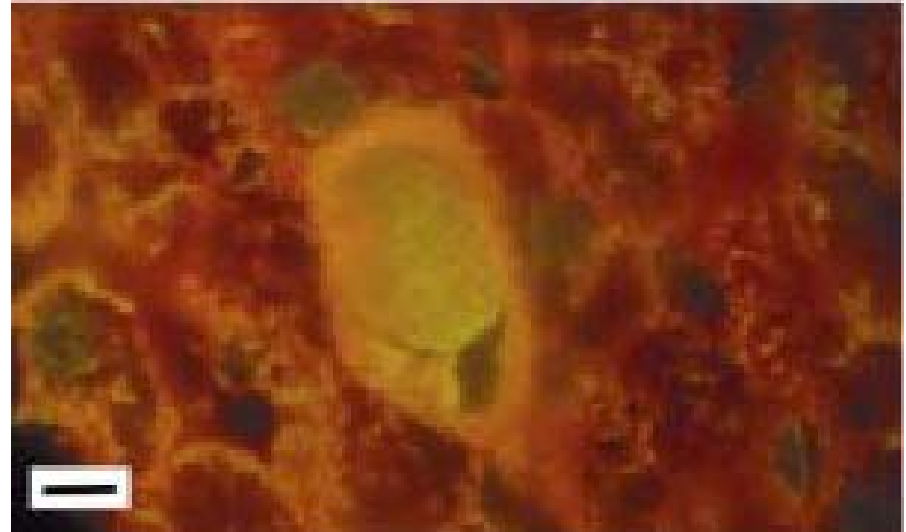
Localisation of LSA-3 in *P. falciparum* sporozoite cytoplasm and membrane



LSA-3 appears to be located in liver schizont parasitophorous vacuole



Anti-NRA Schizont (mouse)



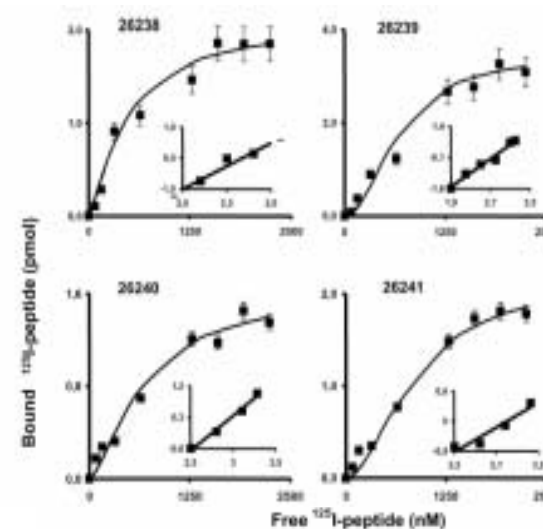
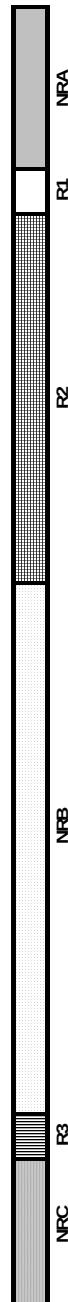
Anti-NRA Schizont (chimpanzee)

Liver Stage Antigen 3 *Plasmodium falciparum* peptides specifically interacting with HepG2 cells

Garcia J. *et al*, 2004, J Mol Med, 82:600

Number Peptide	SEQUENCE	SPECIFIC BINDING ACTIVITY To HepG2 CELLS (%)			
		2	4	6	
26237	1 MTNSNYKSNKTYNENNNEQ	20			
26238	21 ITTIFNRTNMNP IKKCHMREY	40			
26239	41 KINKYFFLIKILTCTILIWA	60			
26240	61 VQYDNNSDINKSWKKNTYVD	80			
26241	81 KKLKLLFNRLSGESQVNGELY	100			
26242	101 ASEEVKEKILDLLEEGNTLT	120			
26243	121 ESVDNKNLEEAEDIKENILY	140			
26244	141 LSNIEEPKENIDNLLNNIGY	160			
26245	161 QNSEKQESVSENVQVSEDFY	180			
26246	181 NELLNSVDVNGEVKENILEEY	200			
26247	201 SQVNDIDFNSLVKSVQEQQY	220			
26248	221 HNVEEKVEESVEEEDSEVEY	240			
26249	241 ENVEEENVEEENDDGSVASSVEY	260			
26250	261 ESIASSVDESIDSSIEENVAY	280			
26251	281 PTVEEIVAPSVVESVAPSVEY	300			
26252	301 ESVEEENVEESVAENVEESVAY	320			
26253	321 ENVEESVAENVEESVAENVEY	340			
26254	341 EIVAPTVEEIVAPTVEEIVAY	360			
26255	361 PSVVESVAPSVEESVEEENVEY	380			
26256	381 ESVAENVEESVAENVEESVAY	400			
26257	401 ENVEESVAENVEESVAENVEY	420			
26258	441 PSVVESVAPSVEESVEEENVEY	460			
26259	461 ENVEESVAENVEESVAENVEY	480			
26260	481 ENVEESVAENVEESVAENVEY	500			
26261	481 ENVEESVAENVEESVAENVEY	500			
26262	501 ESVAENVEESVAENVEEIVAY	520			
26263	521 PTVEEIVAPTVEEIVAPSVVY	540			
26264	541 ESVAESVVEESVEEENVEESVAY	560			
26265	561 ENVEESVAENVEESVAENVEY	580			
26266	581 ESVAENVEEIVAPTVEEIVAY	600			
26268	621 ESVEEENVEESVAENVEESVAY	640			
26269	641 ENVEESVAENVEEIVAPTVEY	660			
26270	661 EIVAPTVEEIVAPSVVESVAY	680			
26271	681 PSVEESVEEENVEESVAENVEY	700			
26272	701 ESVAENVEESVAENVEESVAY	720			
26273	721 ENVEEIVAPTVEEIVAPTVEY	740			
26274	741 EIVAPSVVESVAPSVEESVEY	760			
26275	761 ENVEESVAENVEESVAENVEY	780			
26276	781 ESVAENVEESVAPTVEEIVAY	800			
26277	801 PSVEESVAPSVEESVAENVAY	820			
26278	821 TNLSDNLLSNLLGGIETEEIY	840	nd		
26279	841 KDSILNEIEEVKENVVTITLY	860	nd		
26280	861 ENVEETTAESVTTFSNILEEY	880	nd		
26281	881 IQENTITNDTIEEKLLEELHEY	900	nd		
26282	901 NVLSAALENTQSEEEKKEVIY	920			
26283	921 DVEIEEVKEEVATTIETVEQY	940			
26284	941 AEEKSANTITEIFENLEENAY	960			
26285	961 VESNENVAENLEKLNTEVFNY	980			
26286	981 TVLDKVEETVEISGESLENNY	1000			
26287	1001 EMDKAFSEIFDNVKG IQENY	1020			
26288	1021 LLTGMRFSIETSIIVIQSEK Y	1040			
26289	1041 VDLNENVSSILDNIE NMKE Y	1060			
26290	1061 GLLNKLENISSTEGVQETVY	1080			
26291	1081 EHVEQNYYVDVDPAMKDDY	1100			
26292	1101 LGILNEAGGLKEMFFNLEDVY	1120			
26293	1121 FKSESDVITVEEIKDEPVQKY	1140			
26294	1141 EVEKETVSIIIEEMEENIVDVY	1160			
26295	1161 LEEKEDLTDKMDAVEESIY	1180			
26296	1181 EISSDSKEETESIKDKKEDVY	1200			
26297	1201 SLVVEEVQDNDMDSEVKVLY	1220			
26298	1221 ELKNMEELMKDAVEINDITY	1240			
26299	1241 SKLIEETQELNEVEADLIKDY	1260			
26300	1261 MEKKLEKALKSEDSKEIIDY	1280			
26301	1281 AKDDTLEKVIIEEHDTITTL Y	1300			
26302	1301 DEVVELKDVEEDKIEKVS DLY	1320			
26303	1321 KLEEDLLEKVEKEIKELSE Y	1340			
26304	1341 ILIDYKELKTIEDLLEKK Y	1360			
26305	1361 EIEKDHFEKFEAEAEIKDLY	1380			
26306	1381 EADILKEVSSLEVEEKKLEY	1400			
26307	1401 EVHELKEEVEHIISGDAHIKY	1420			
26308	1421 GLEEDDLLEEDDLKGSILDMY	1440			
26309	1441 LKGDMLGDMDKESLEDVTTY	1460			
26310	1461 KLGERVE SLKDVLSSALGMDY	1480			
26311	1481 EEQMKTRKKAQRPKLEEVL Y	1500			
26312	1501 KEEVKKEPKKKITKKKVRFDY	1520			
26313	1521 IKDKPEPKDEIVEVEMKDED Y	1540			
26314	1541 EEDVEEDIEEDIEEDKVEDIY	1560			
26315	1561 DEDIDEDIGEDKDEVIDLIY	1580			
26316	1581 QKEKRIEKVKAKKKKLEKKV Y	1600			
26317	1601 EGVSGLKKHVDVEMKYYQK Y	1620			
26318	1621 IDKEVDKEVSKALESKNDV Y	1640			
26319	1641 NVLKQNQDFFSKVKNFVKKY	1660			
26320	1661 KVFAAPFISAVAAAFASYVVG Y	1680			
26321	1681 FFTFSLFSSCVTIASSTYLL Y	1700			
26322	1701 SKVDKTKINKNKERPFFYSFV Y	1720			
26323	1721 DIFKNLKHLYLQMKKEKFSKE Y	1740			
26324	1741 KNNNVIEVTNKAEEKGNVQVY	1760			
26325	1761 TNKTEKTKVDKNNKVPKKRY	1780			
26326	1781 TKVDKNNKVPKKRRTQKSKY	1786			

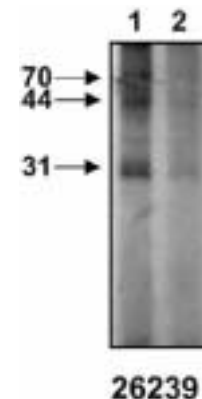
Peptide reported for Neurath *et al*.



Saturation assays

K_d = 480-880 nM

Sites/cell = 0.5-1.5x10⁶

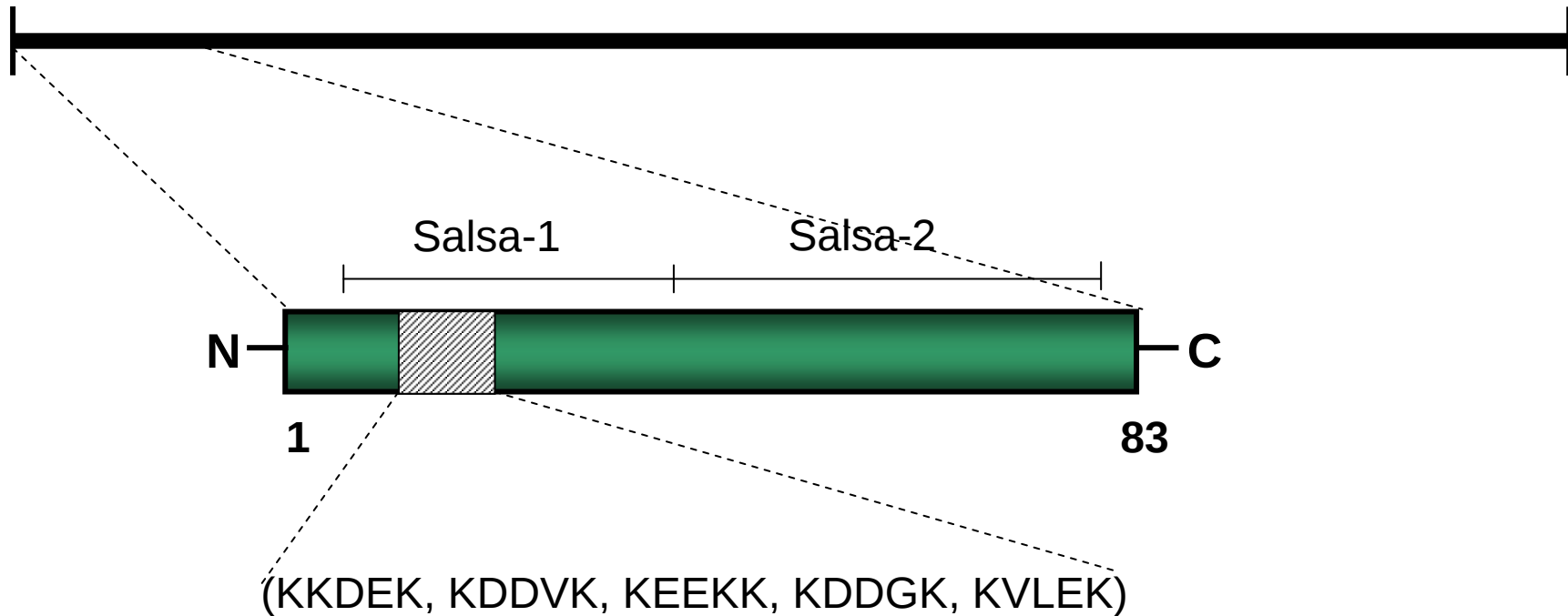


HepG2 Cell membrane assay

26239

Sporozoite And Liver Stage Antigen (SALSA)

(70 kDa)



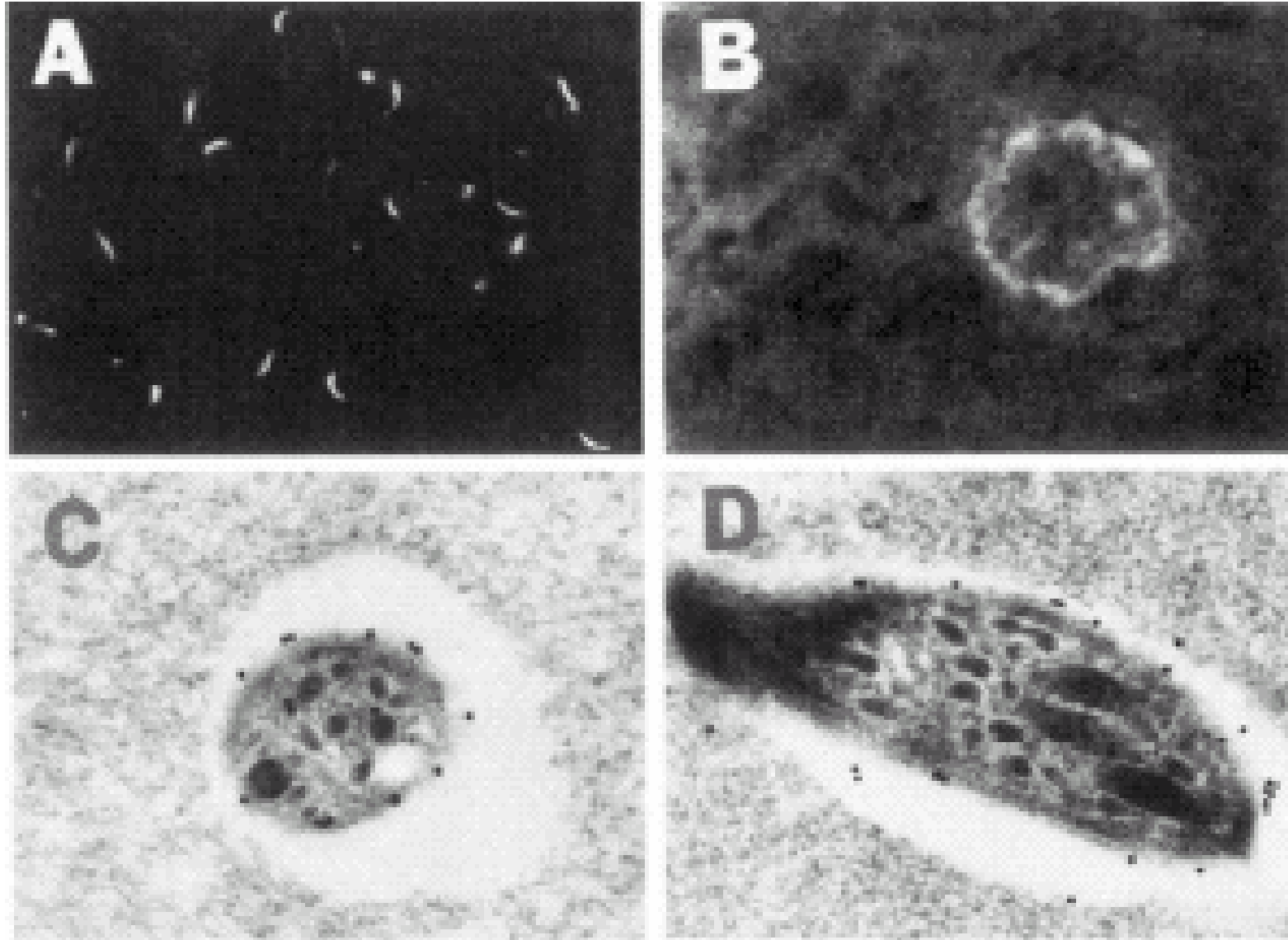
Localisation:

Surface Spz, PV, Liver Stage

Possible function:

Adhesion, Invasion

Localisation of SALSA in *P. falcarum* sporozoites (A, C and D) and liver stage (B)

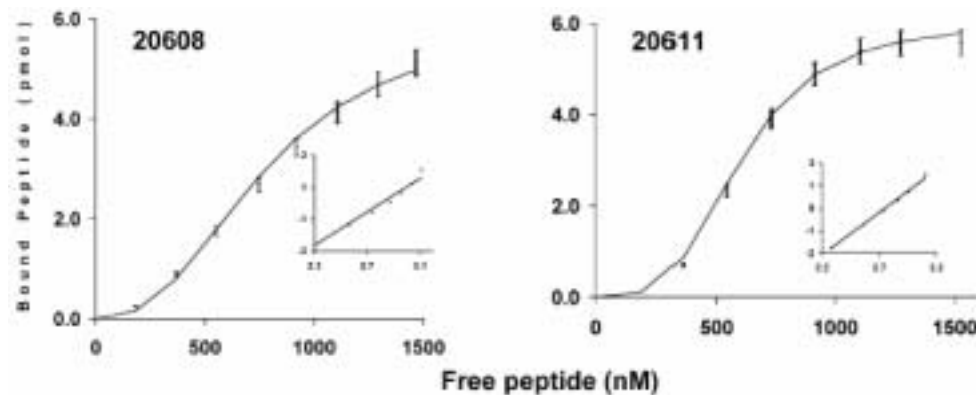


Sporozoite and Liver Stage Antigen *Plasmodium falciparum* peptides bind specifically to human hepatocytes

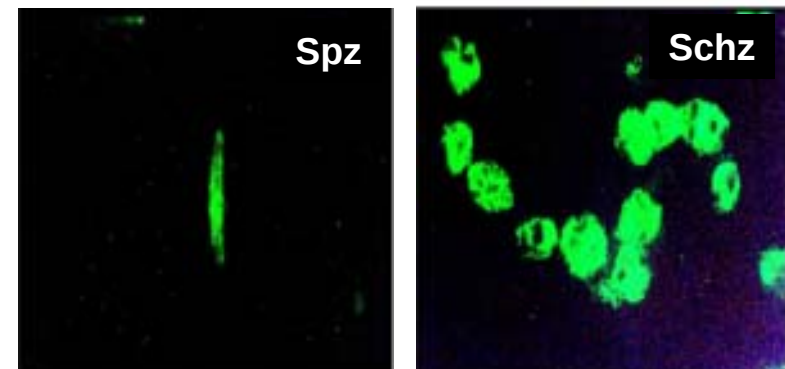
Puentes A, et al, 2004 *Vaccine*, 22:1150

				SLOPE	
PEPTIDE		SEQUENCE		1%	2%
20607	1	RVSTSDTPGGNESSAFPQFY	20		
20608	21	IWSAEKKDEKEASEQGEESHY	40		
20609	41	KKENSQESANGKDDVKEEKY	60		
20610	61	TNEKKDDGKTDKVQEKVLEKY	80		
20611	64	KKDDGTDKVQEKVLEKSPKY	83		

Saturation assay

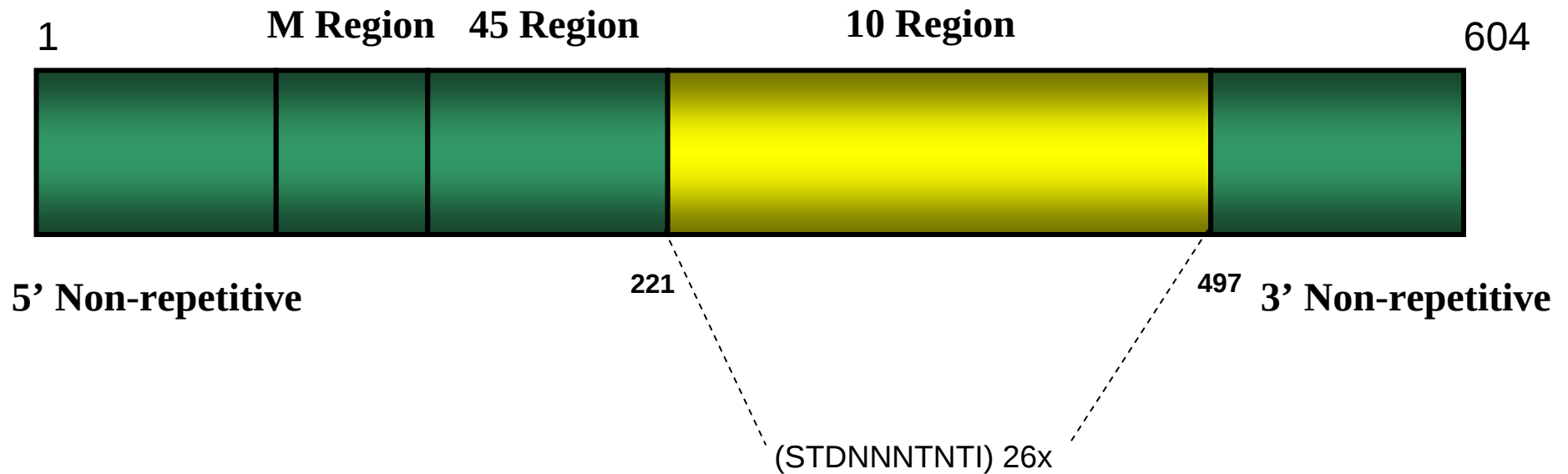


IFA assays



Sporozoite-threonine-asparagine-rich protein (STARP)

(67 kDa)



Localisation:

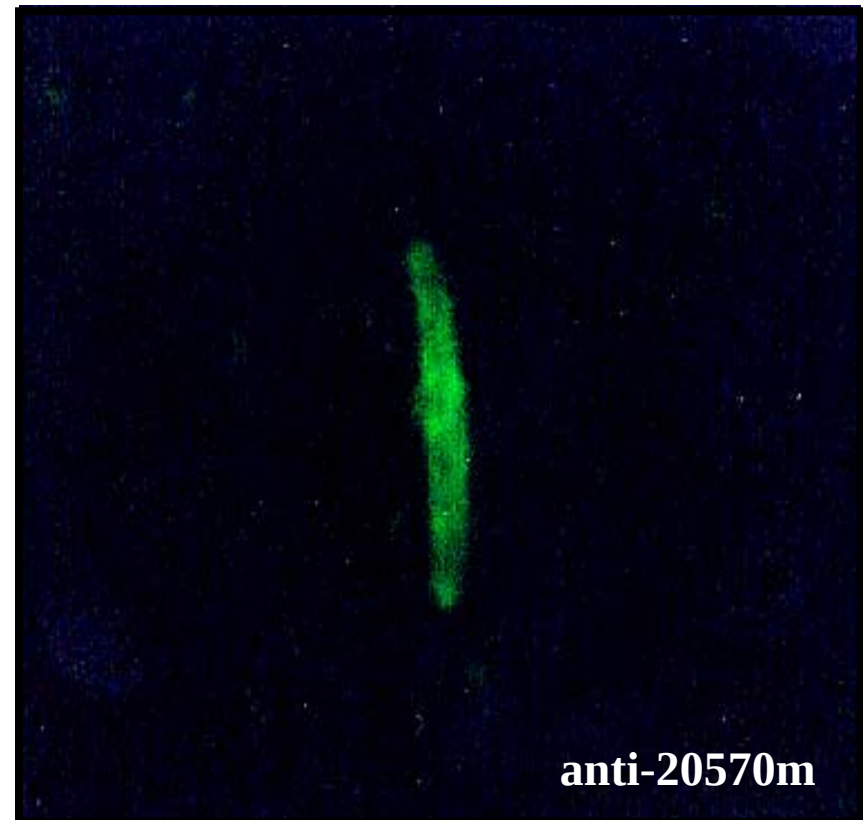
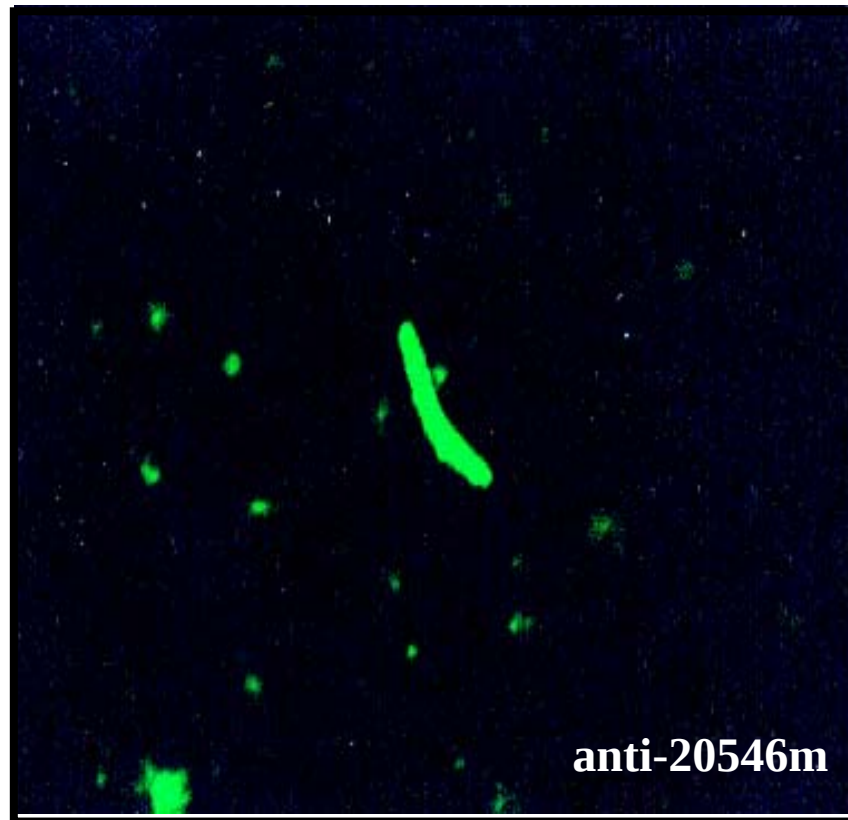
Surface Spz, Liver Stage, RBC

Possible function:

Sporozoite invasion

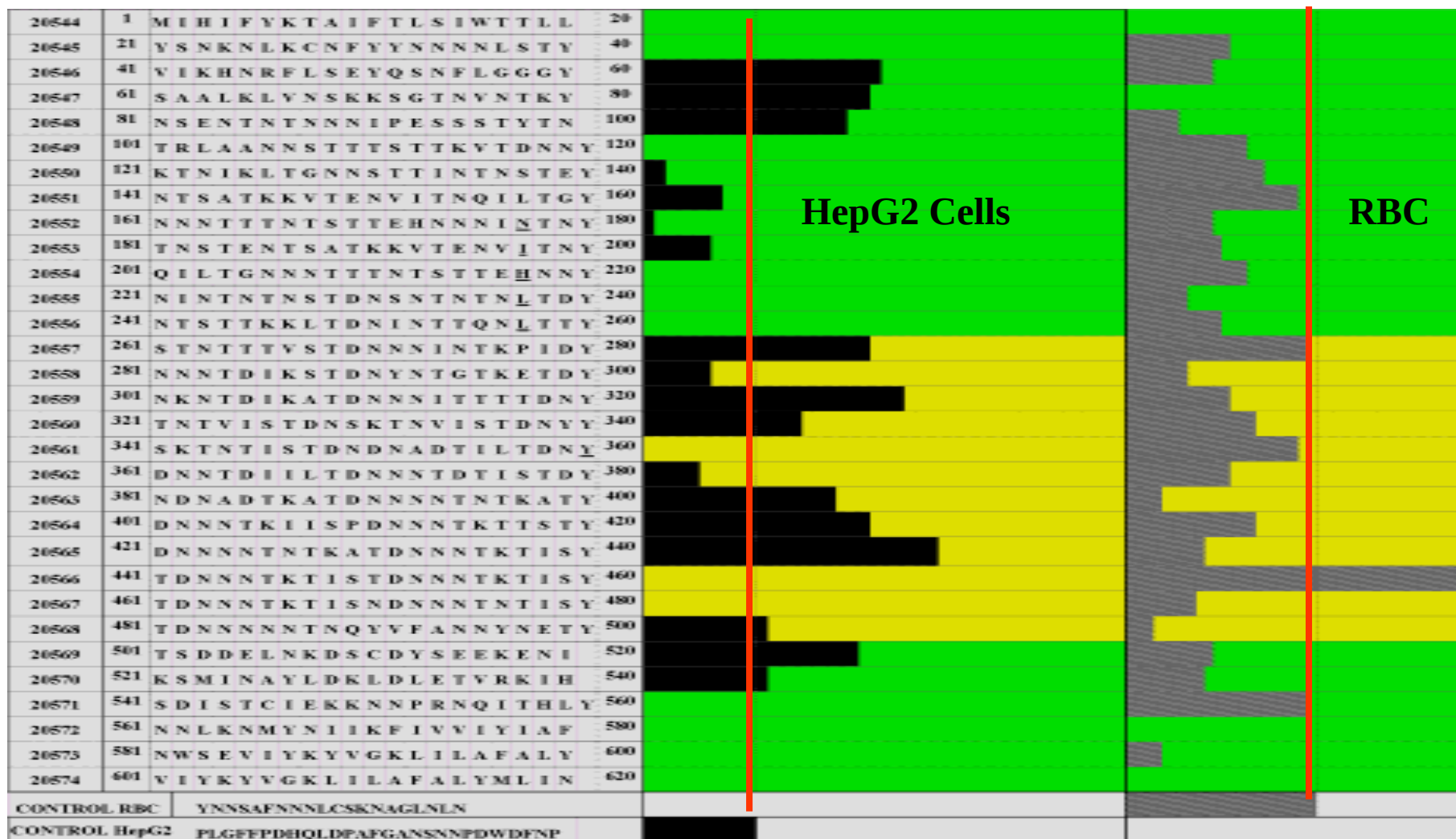
Anti-sporozoite indirect immunofluorescence with *Aotus* monkey sera immunised STARP protein peptide analogues

López R. et al, 2003. Vaccine 21:2404



Identification of specific HepG2 cell binding regions in *Plasmodium falciparum* sporozoite-threonine-asparagine-rich protein (STARP)

Lopez, et al, 2003 Vaccine. 21:2404



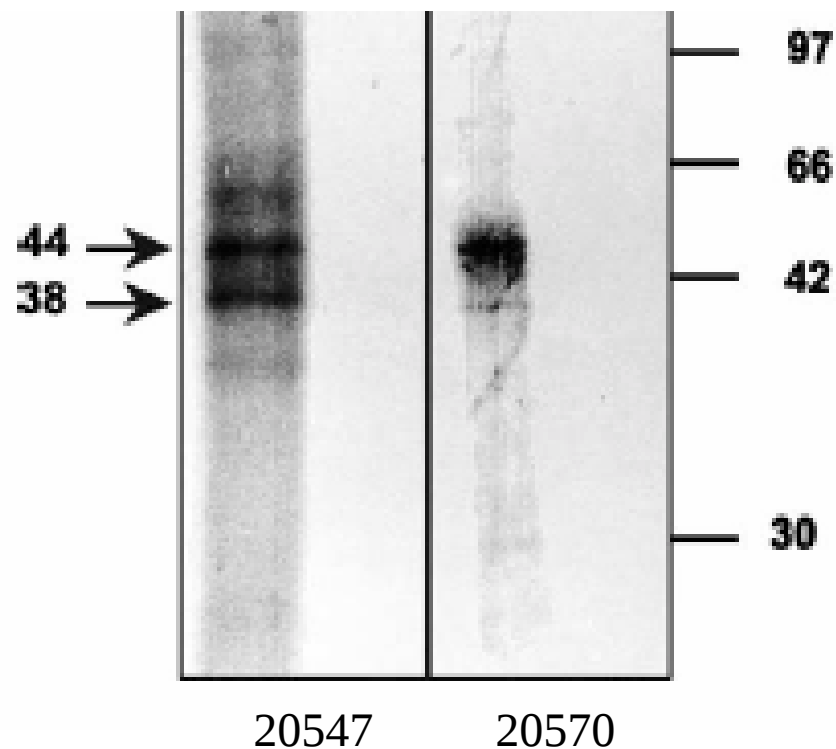
Saturation assays

Lopez R, *et al*, 2003 Vaccine. 21:2404

*Pf*STARP peptide critical residues for Hep G2 cell affinity constants and number of Hep G2 cell binding sites

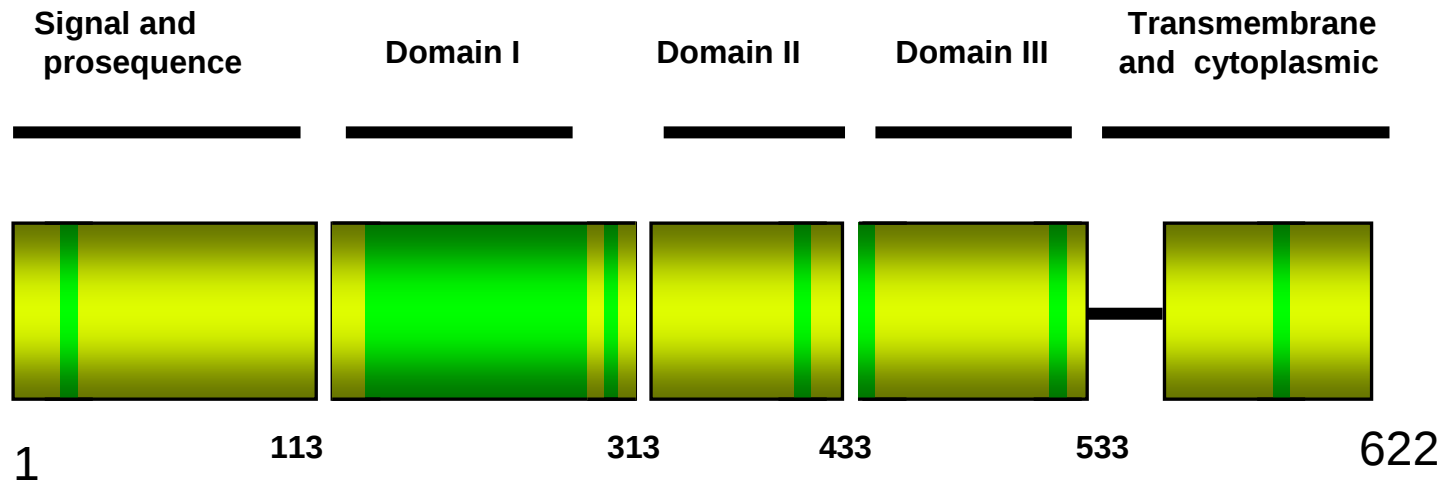
Peptide	Amino acids	Sequence	$K_d \pm S.D.^a$ (nM)	$NrSC^b \pm S.D.^a (\times 10^3)$
20546	41-60	<u>V</u> IKHNRE <u>L</u> SEYQSNFLGGGY	70 ± 5	90 ± 5
20547	61-80	SAALKLVNSKKSGT <u>N</u> VNTKY	219 ± 10	370 ± 10
20548	81-100	NSENT <u>N</u> T <u>N</u> N <u>N</u> PE <u>S</u> S <u>S</u> TYTN	110 ± 8	140 ± 8
20569	501-520	TSD <u>D</u> ELNKDSSDYSEEKENI	90 ± 6	80 ± 6
20570	521-540	K <u>S</u> MINAYLDKLDLETVRKIH	200 ± 9	360 ± 9

HepG2 cell membrane assay



Apical membrane antigen-1 (AMA-1)

(83 kDa)



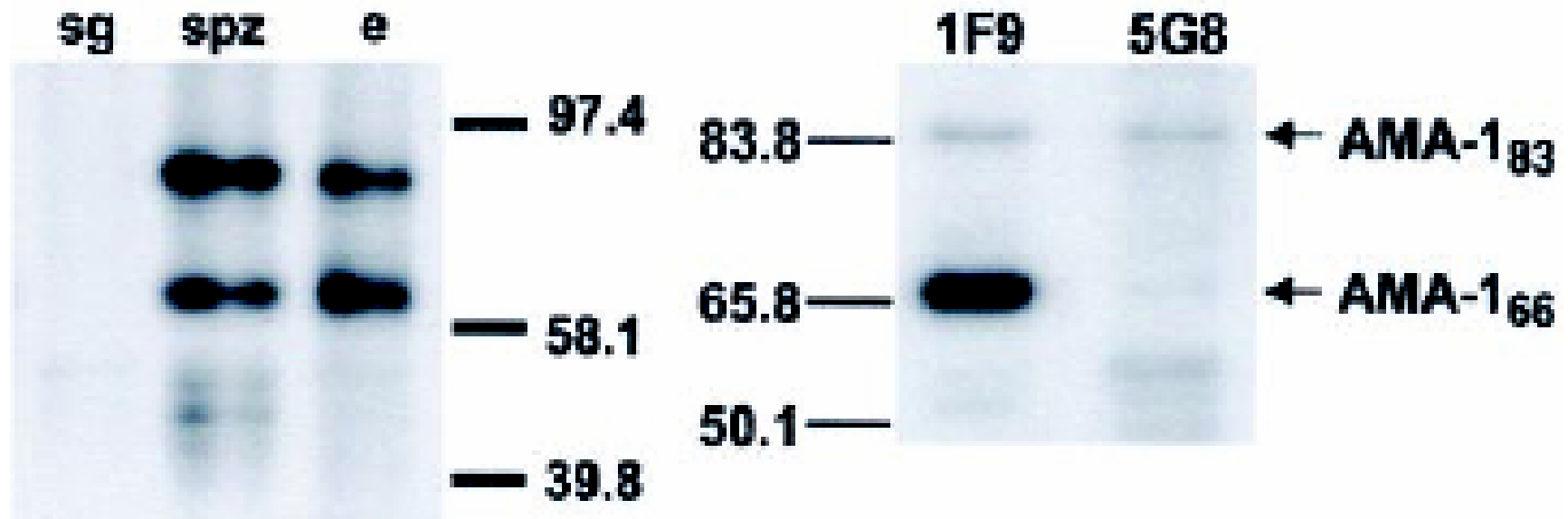
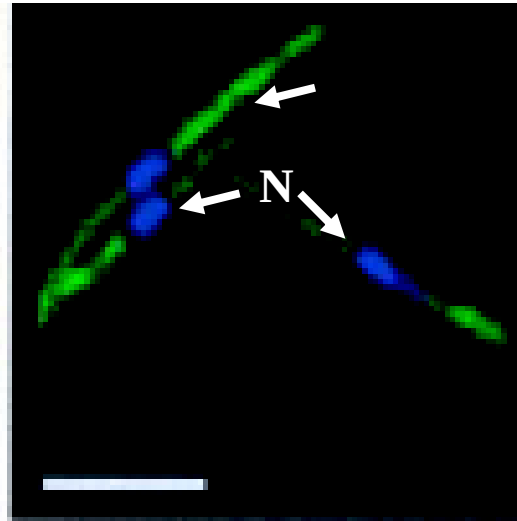
Localisation:

Surface Spz-Mz, micronemes

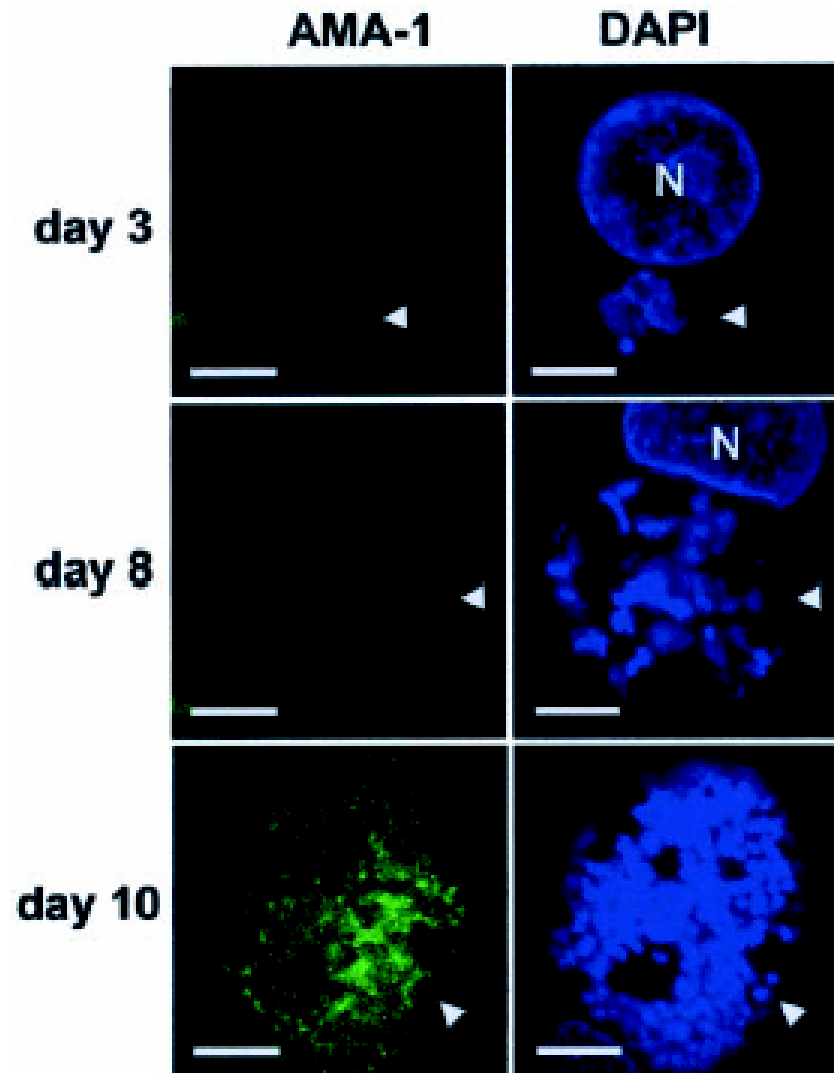
Possible function:

Adhesion, invasion

AMA-1 is expressed in *P. falciparum* sporozoites

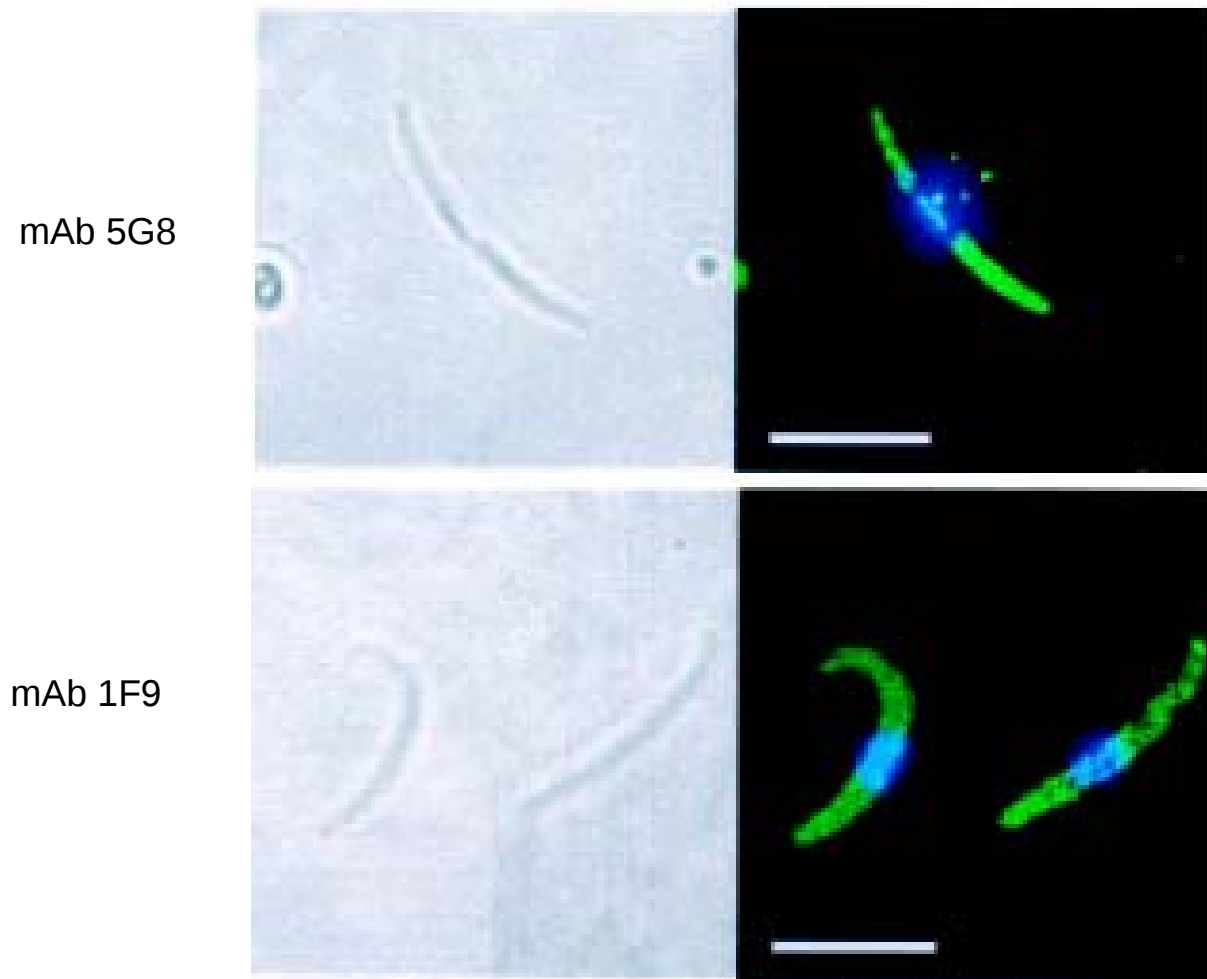


AMA-1 is lost after hepatocyte invasion and re-expressed in liver merozoites



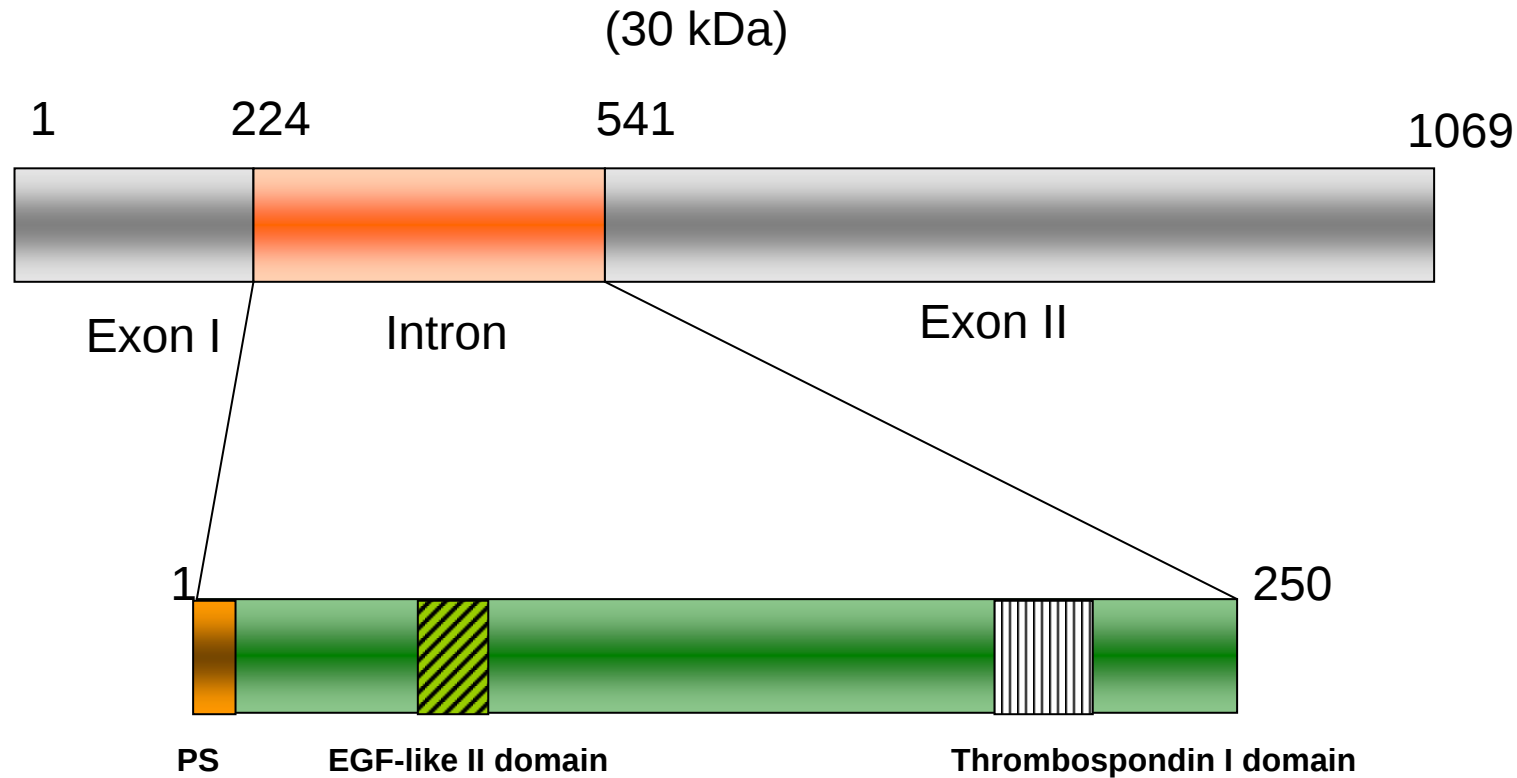
Silvie O. *et al.* 2004 **J Biol Chem.** 279:9490.

Only mature AMA-1 protein translocates to the sporozoite surface



Silvie O. *et al.* 2004 **J Biol Chem.** 279:9490.

Secreted Protein With Altered Thrombospondin Type I Repeat Domain (SPATR)



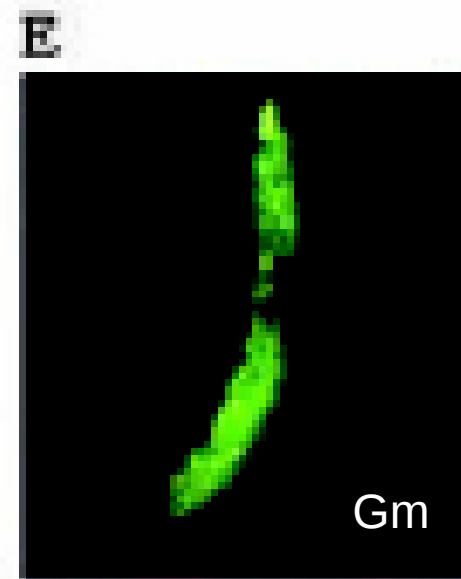
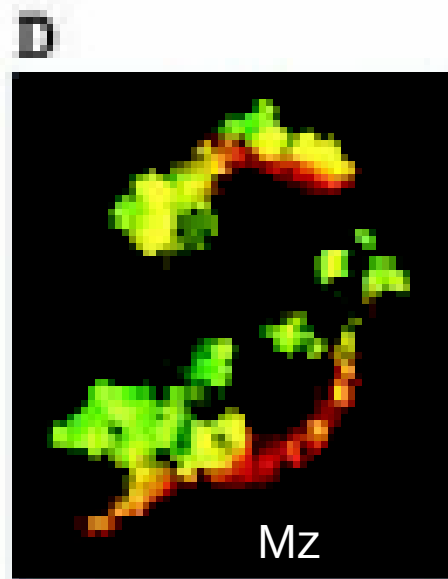
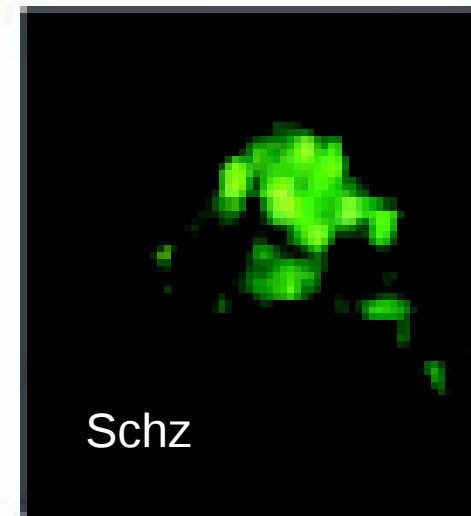
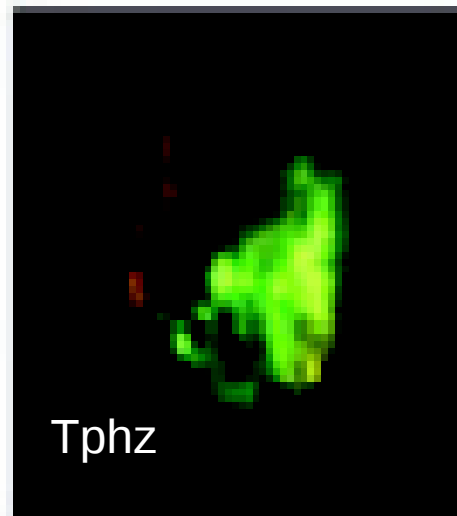
Localisation:

Surface Spz, Liver-Stage, RBC

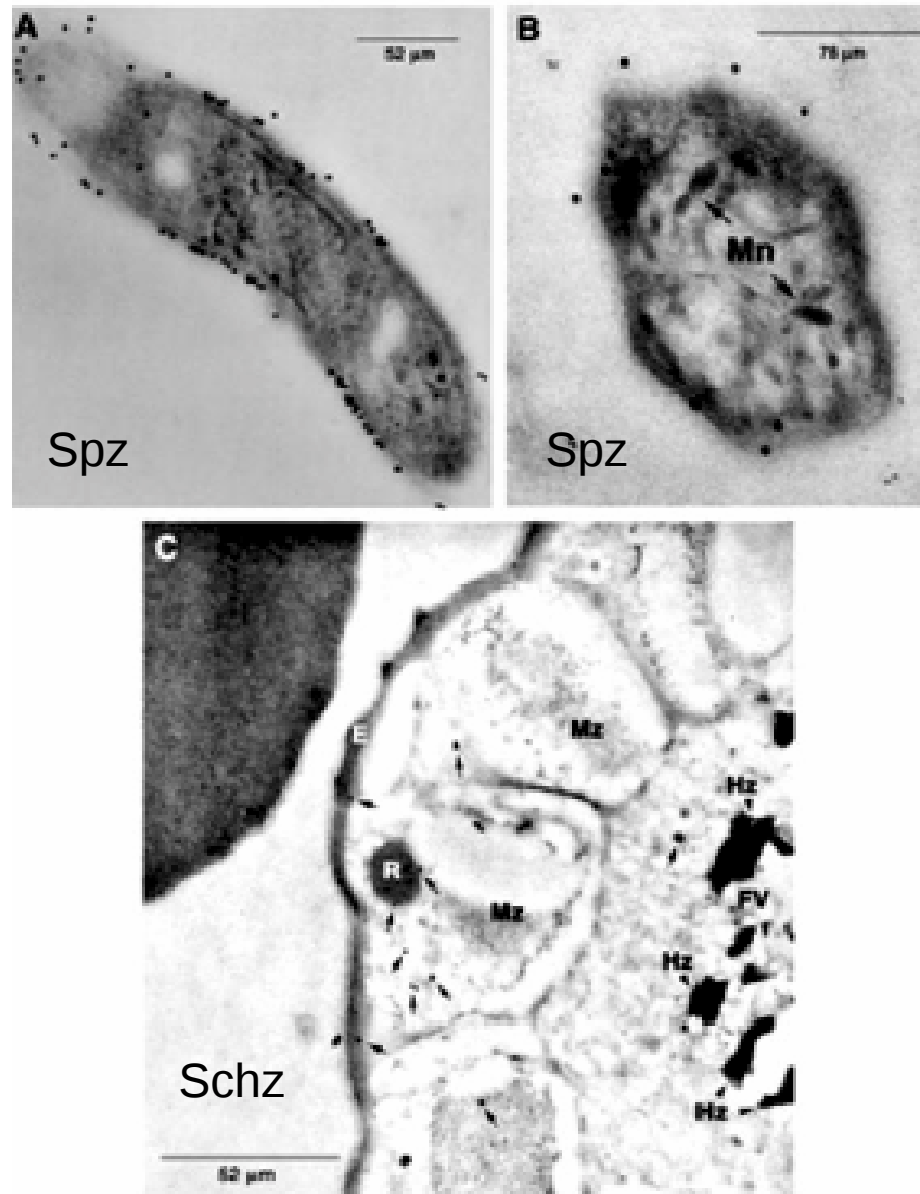
Possible function:

Adhesion, invasion

PfSPATR expression in different *P. falciparum* stages

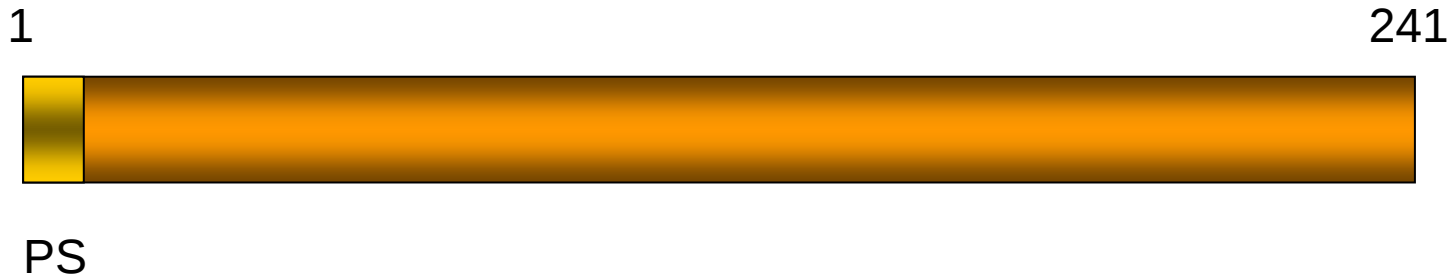


PfSPATR localisation in *P. falciparum* by electron microscopy



Sporozoite microneme Protein Essential for Cell Traversal (SPECT)

(25 kDa)



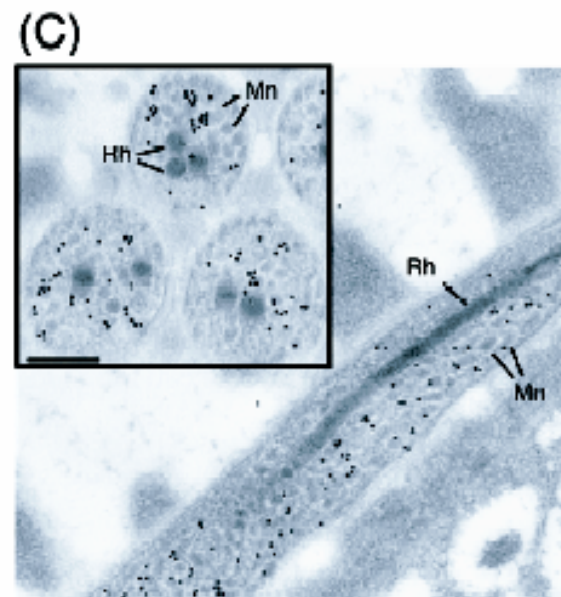
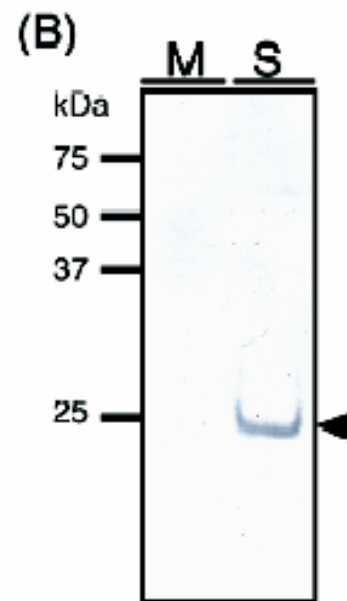
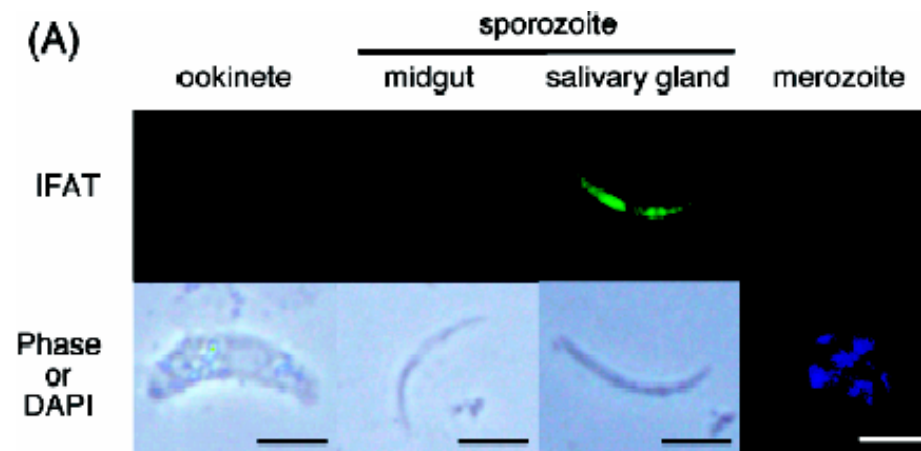
Localisation:

Surface Spz, micronemes

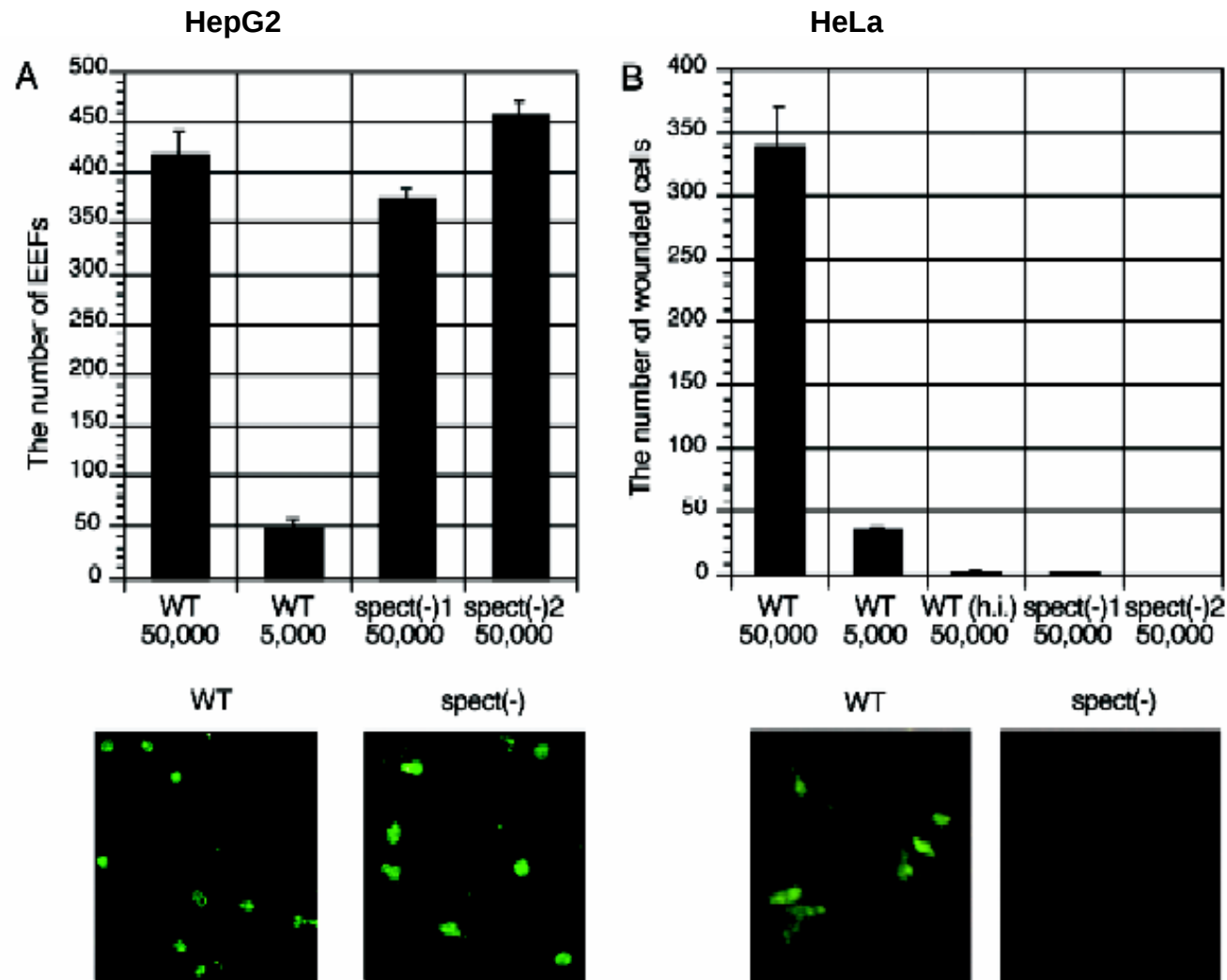
Possible function:

Kupffer cell invasion

SPECT protein specifically produced in infected liver sporozoite stage

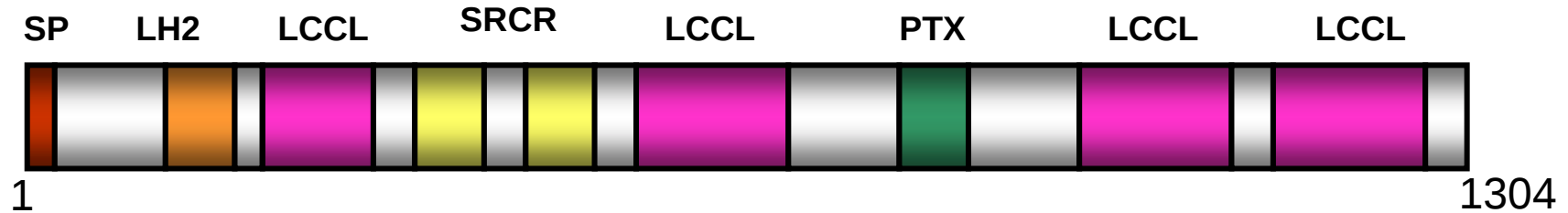


SPECT: sporozoite cell-passage activity



Scavenger Receptor Protein (PxSR)

(148 kDa)



SP: Signal peptide. 1x

LH2: Lipoxygenase homology 2 domain. 1x

LCCL: Limulus factor C-like-Coch-5b2-Lf1 clotting domain. 4x

SRCR: Scavenger receptor cysteine-rich. 2x **PTX:** PenTraXin domain. 1x

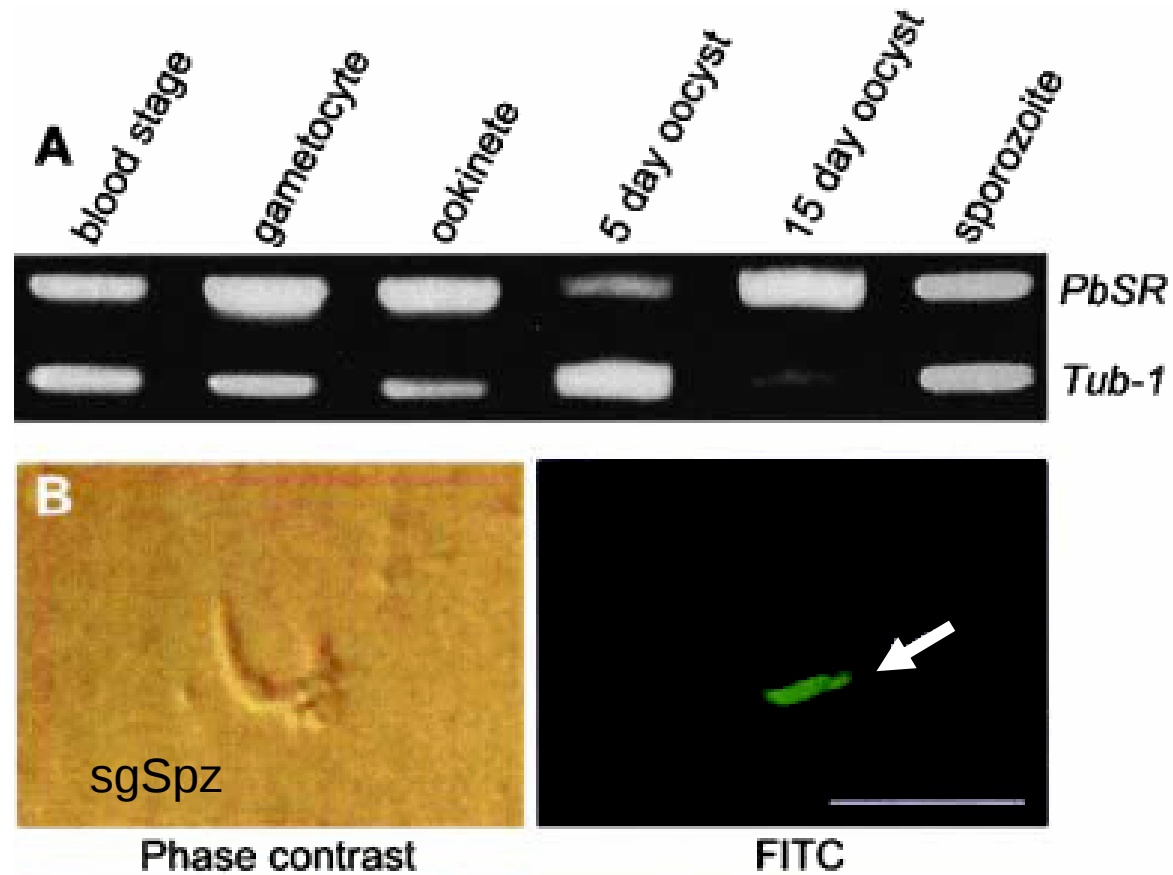
Localisation:

Blood stage, gametocytes, Spz,

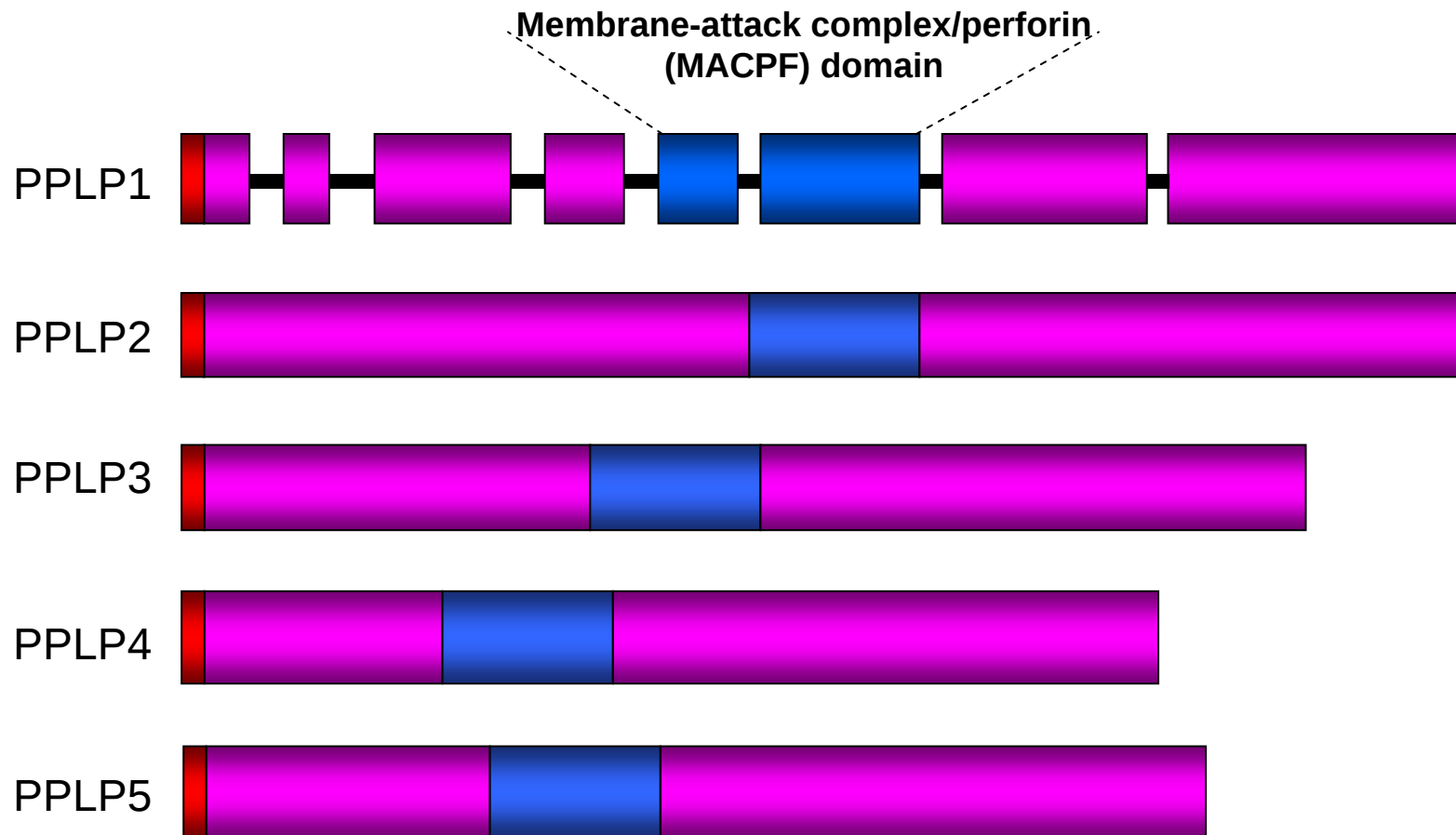
Possible function:

Immune system evasion

PxSR expression



Plasmodium Perforin-Like Protein Family (PPLP)



Overview of the *Plasmodium* perforin-like protein (PPLP) family in *P. falciparum* (Pf) and *P. yoelii* (Py)

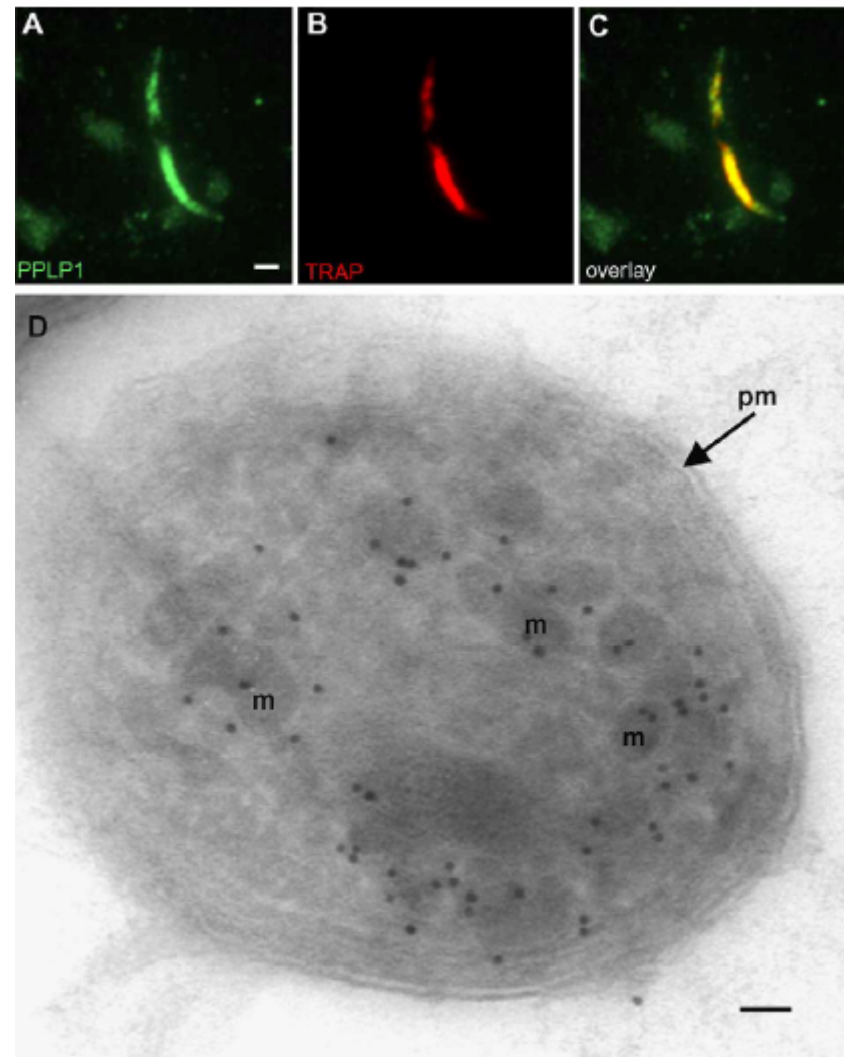
	Gene accession number	Protein accession number	Chromosome localization	Predicted mass ^a (kDa)	Amino acid identity (%)
PPLP1					
Pf	PF0430C	NP_702743	Chro 4	94	57
Py	PY 00454	EAA 16049 ^b	na	91	
PPLP2					
Pf	PFL 0805W	NP_701526	Chro 12	125	49
Py	PY 00181	EAA 21179	na	114	
PPLP3					
Pf	PFI 1145W	NP_704772	Chro 9	93	66
Py	PY05180	EAA 17167 ^b	na	92	
PPLP4					
Pf	PF08_0050	NP_704361	Chro 8	76	52
Py	PY03076	EAA 22606 ^b	na	79	
PPLP5					
Pf	PF08_0052	NP_704363	Chro 8	79	47
Py	PY03943	EAA 15731	na	80	

na: not available.

^a With signal peptide.

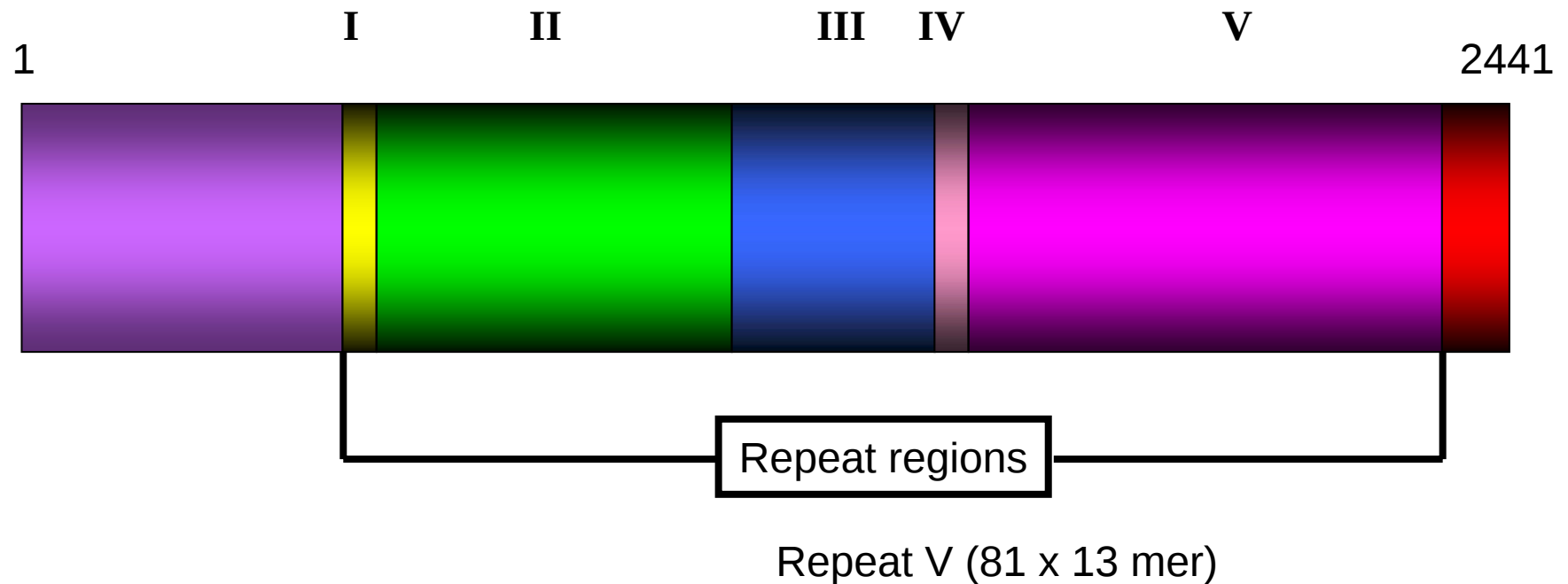
^b Predicted protein was incorrectly annotated.

PyPLP1 localises to sporozoite salivary gland micronemes



PfEMP3 *Plasmodium falciparum* erythrocyte membrane protein 3

(280 kDa)



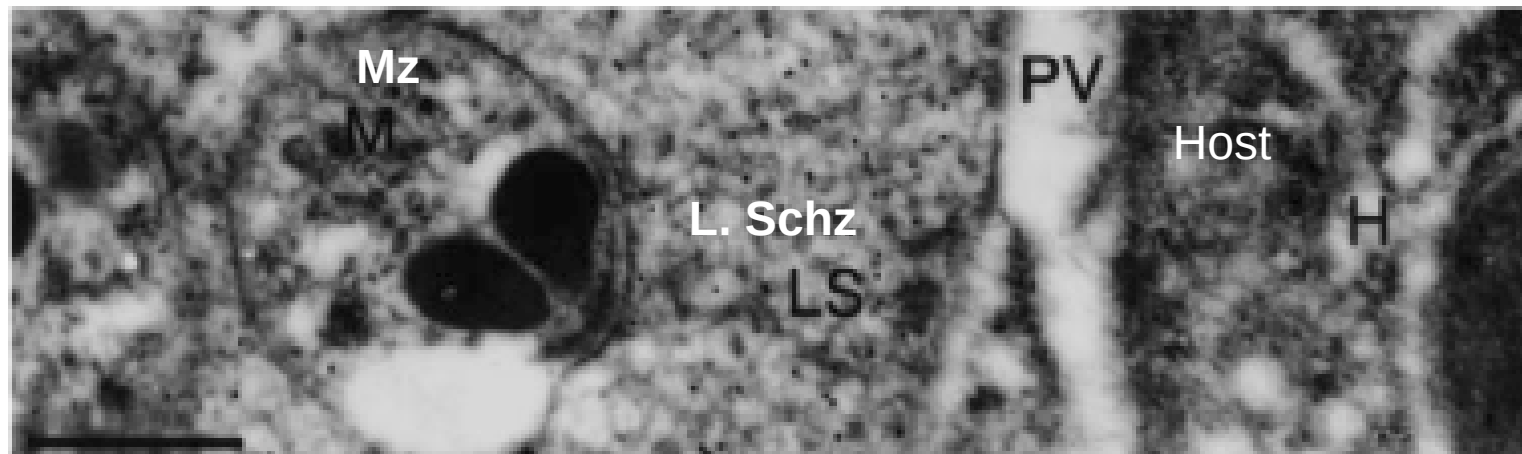
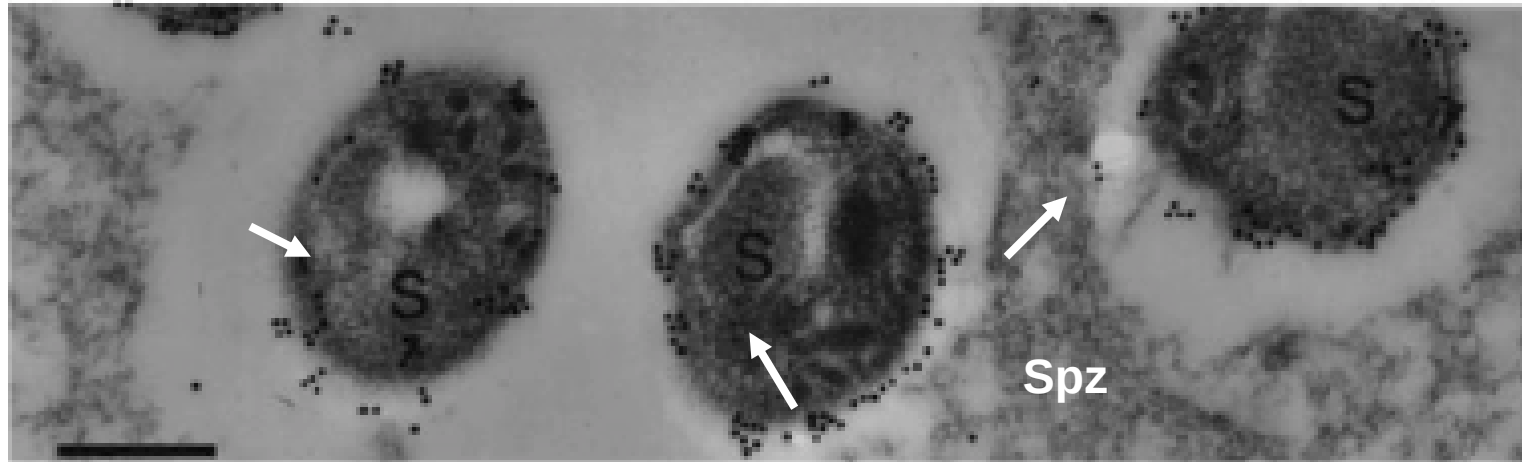
Localisation:

Blood stage, LS, Spz surface

Possible function:

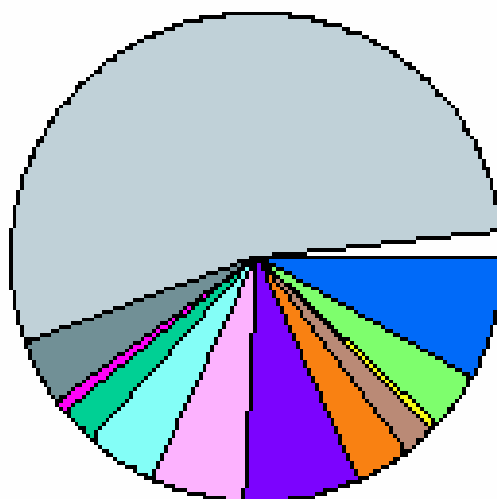
Adhesion, invasion

PfEMP3 localises to merozoite, sporozoite and liver schizont of *P. falciparum*

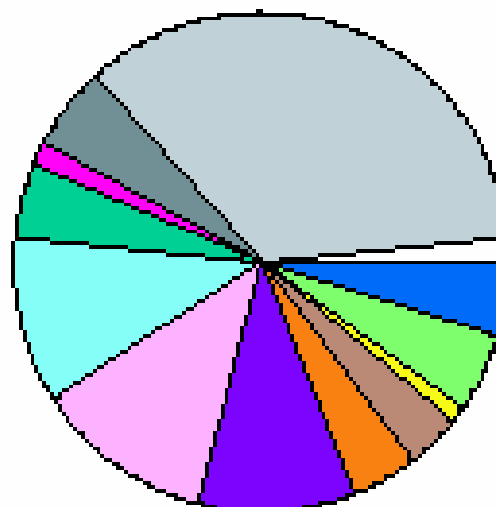


Functional profile of proteins expressed in different *P. falciparum* life-cycle stages

Sporozoite



Merozoite



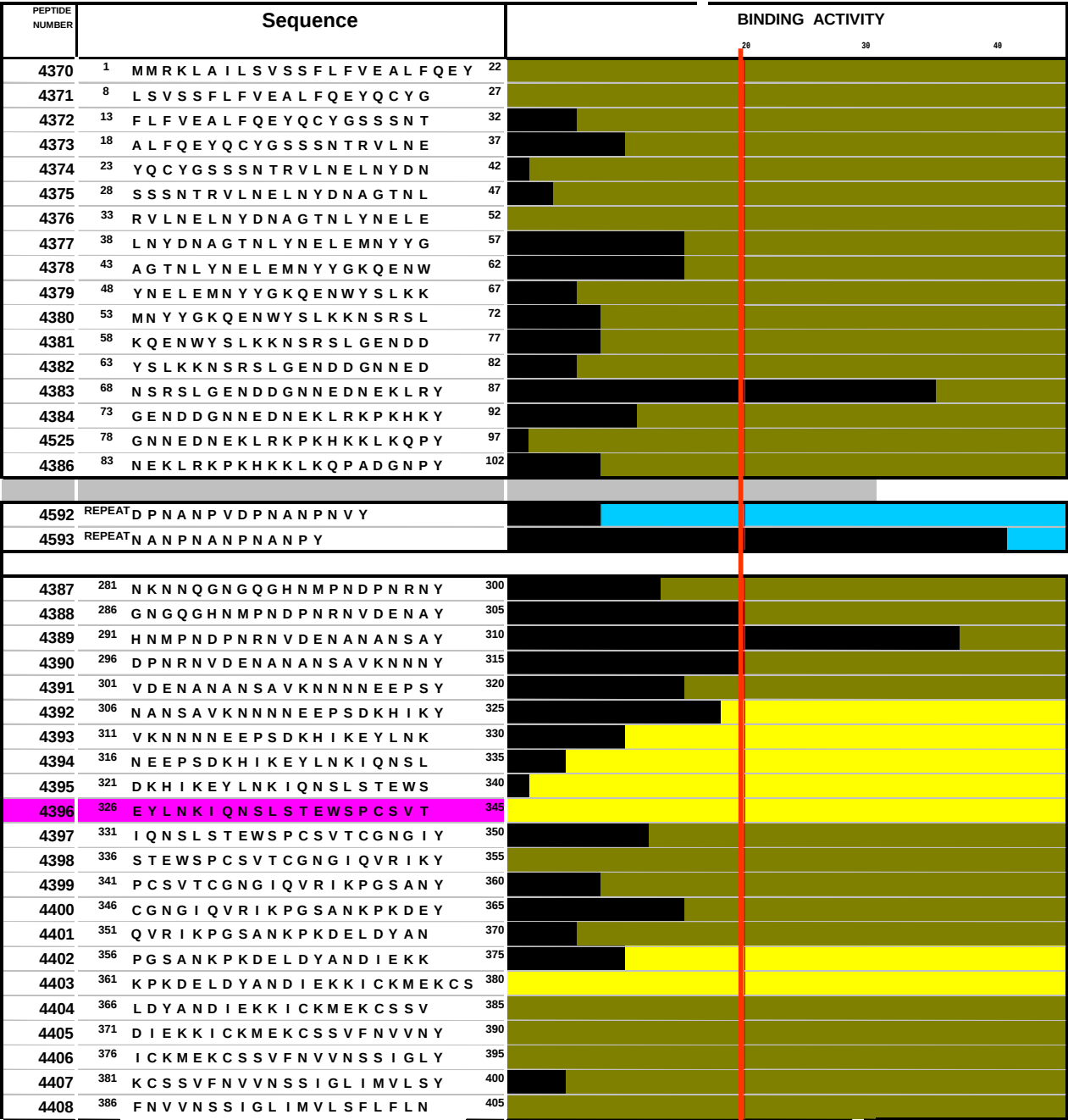
- Cell surface (apical organelles)
- Cell cycle (DNA processing)
- Cell rescue defence (virulence)
- Cellular communication (signal transduction)
- Cellular transport (transport mechanism)

- Metabolism (energy)
- Protein fate
- Protein synthesis
- Transcription

- Transport facilitation
- Conserved hypothetical
- Hypothetical
- Unclassified

Plasmodium falciparum circumsporozoite (CS) protein peptides
specifically bind to HepG2 cells

Suarez J, et al, 2001, Vaccine,19:4487



83



383

RTS-S

Plasmodium sporozoite surface and/or apical organelle proteome

Protein	Other stages	Subcellular Localization	Conserved pep
Cs	-	Surface	2
TRAP	-	Surface, micronemes	7
MAEBL	-	Surface, micronemes	
SPECT	-	Micronemes	
EBA175	Mz	Surface, micronemes	
P235	Mz		
AMA1	Mz	Surface, micronemes	
STARP	RBC, LS	Surface	5
SALSA	RBC, LS	Surface	2
PEMP3	RBC, LS	Surface	
LSA3	LS	Surface	11
LSA1	LS		1
P52	-	?	
MCP1	MZ	?	
SPATR	RBC	Surface	
PPLP1	-	Micronemes	
PfEMP1	ïRBC		
STEVOR	ïRBC		
RIFINS	ïRBC		

