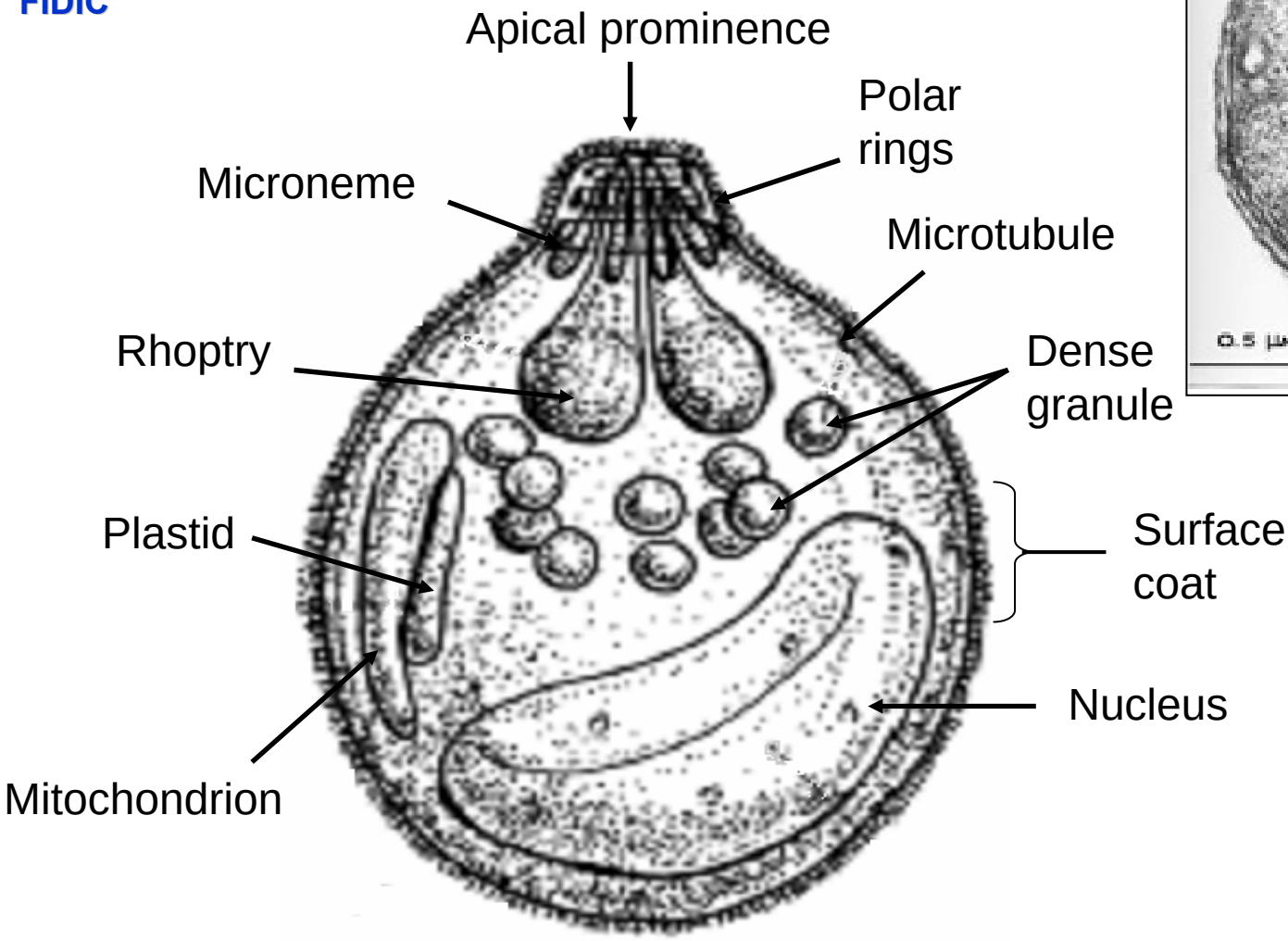

Plasmodium merozoite
invasion of erythrocytes

FIDIC

Merozoite cell structure



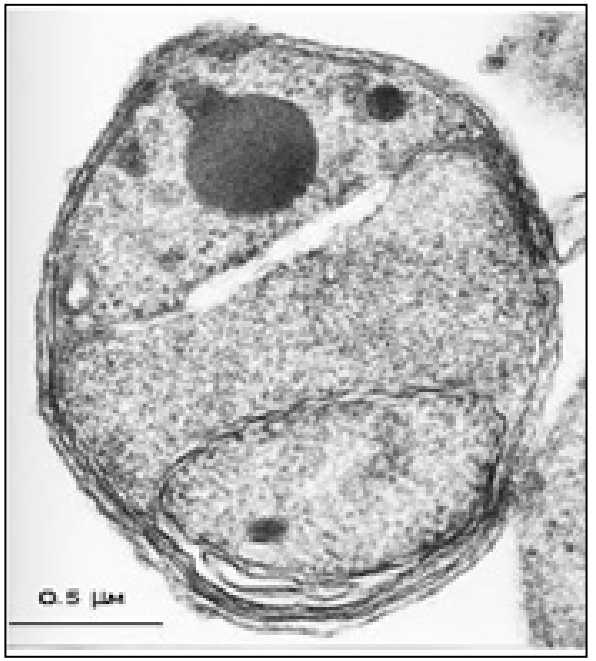
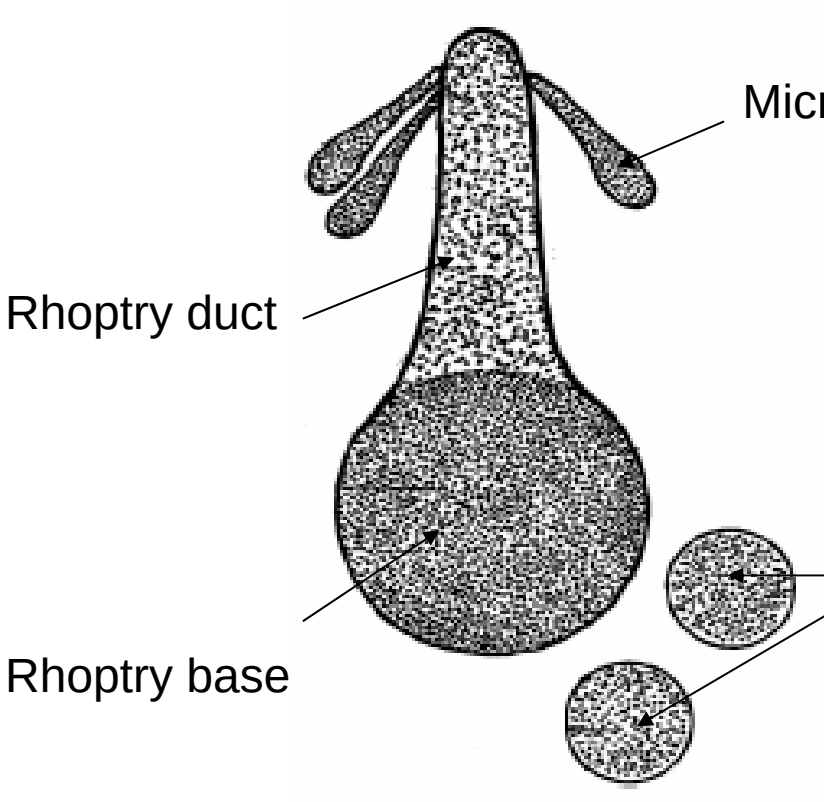


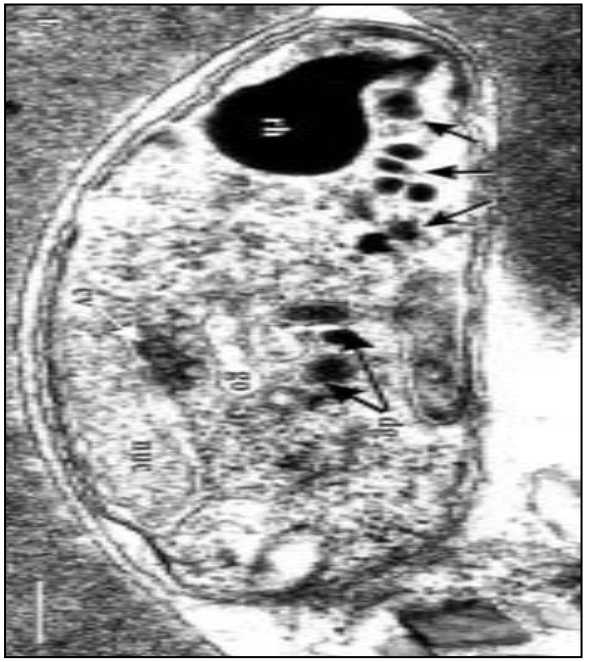
Diagram showing the general structure of a *Plasmodium falciparum* merozoite

Preiser P. *et al.*, 2000

FIDIC

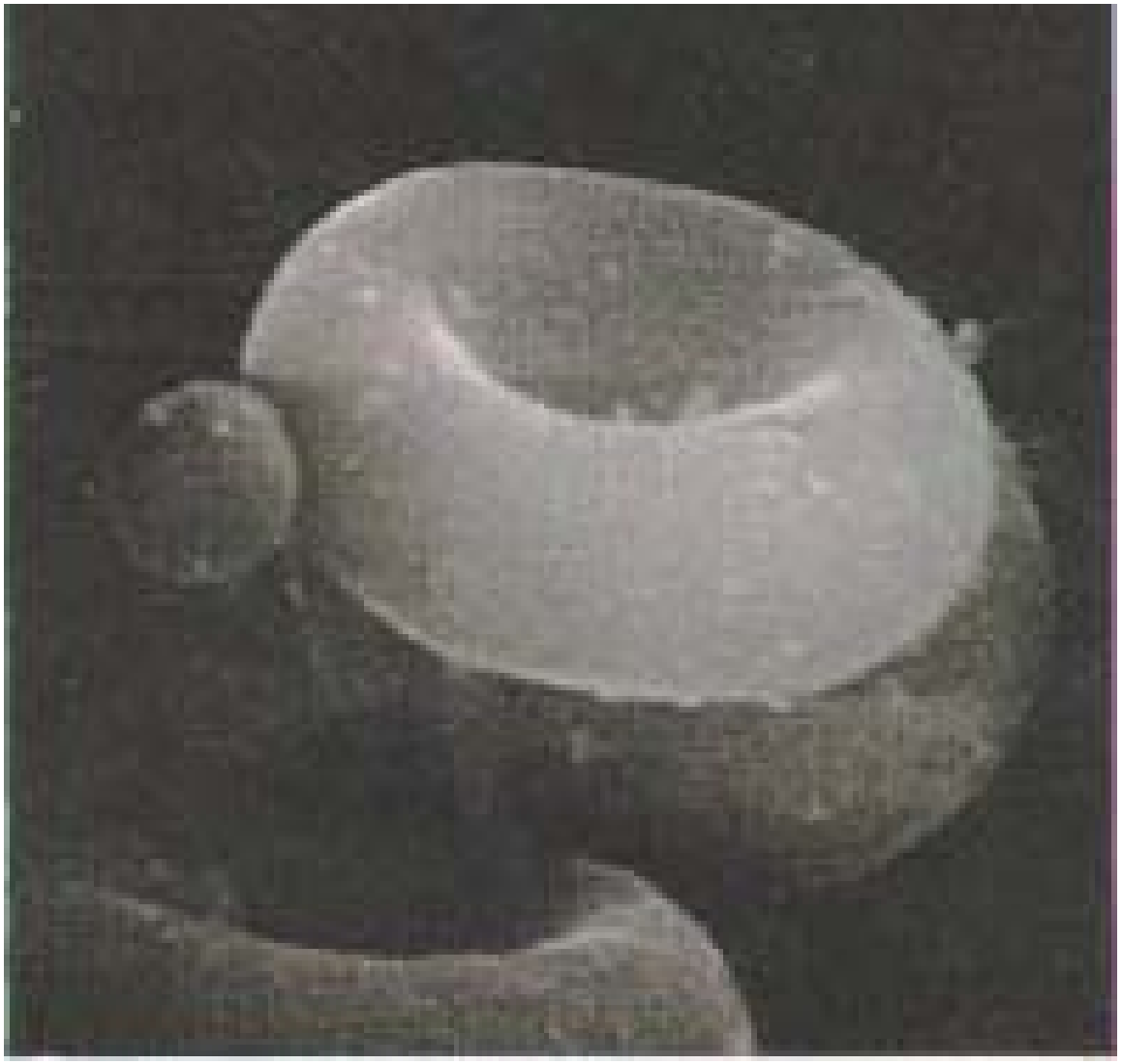
Plasmodium falciparum apical orgenelles





Preiser P. *et al.*, 2000

FIDIC



A merozoite caught invading an erythrocyte, seen by electron microscopy.
Malaria by A. J. Knell

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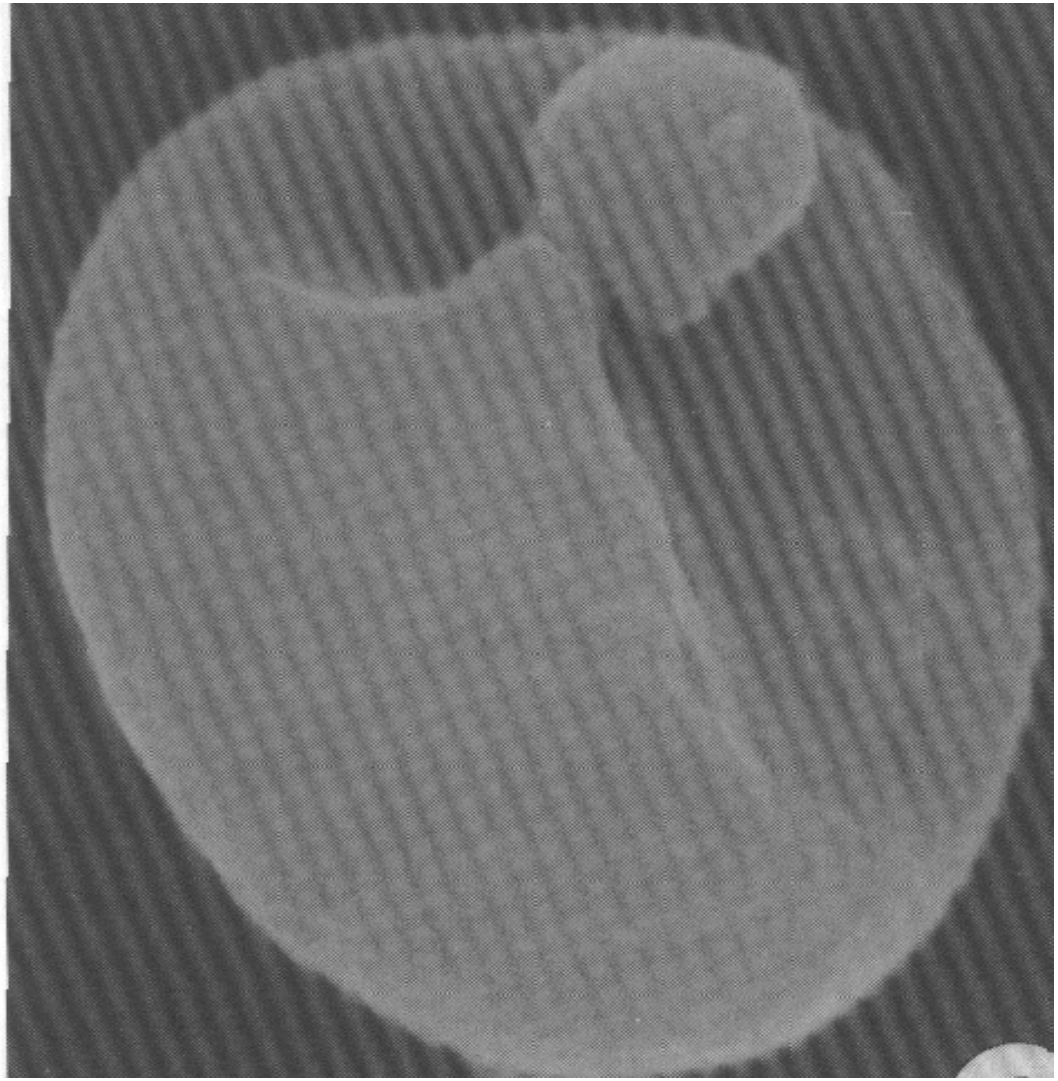

Scanning electron micrograph of a merozoite attached to a red blood cell. The parasite appears to have distorted the red blood cell surface. Magnification x 10.000.
Malaria by A. J. Knell

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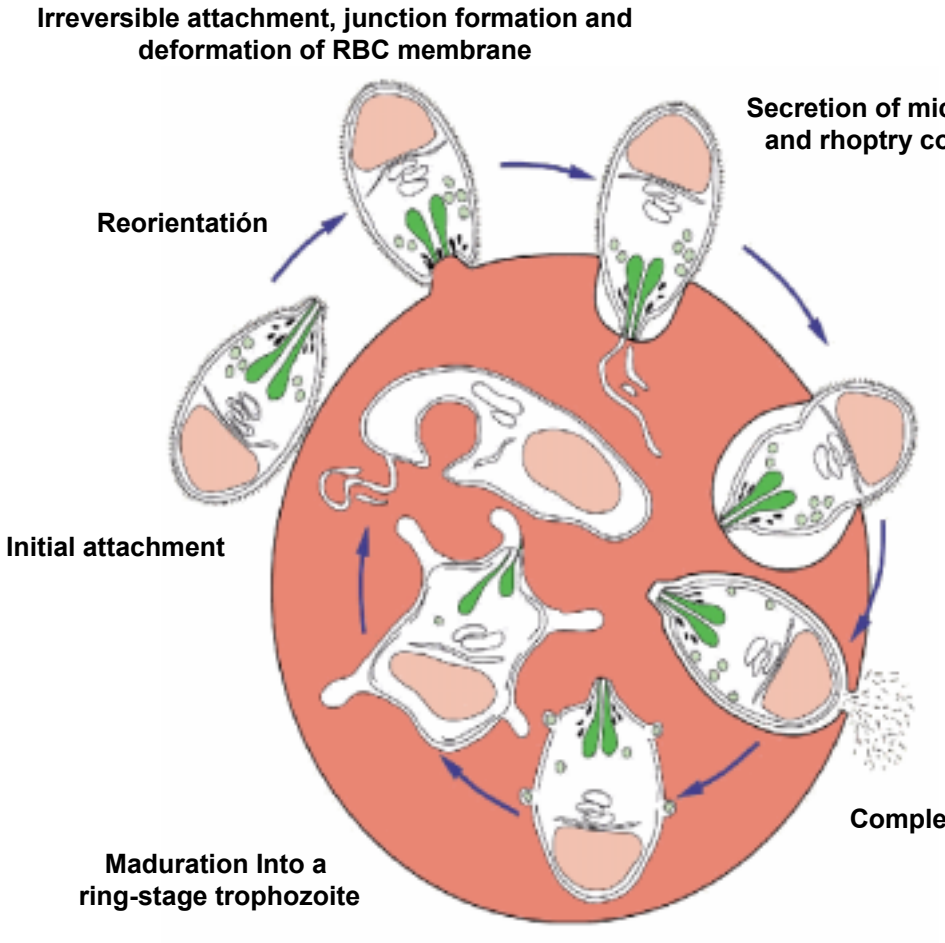
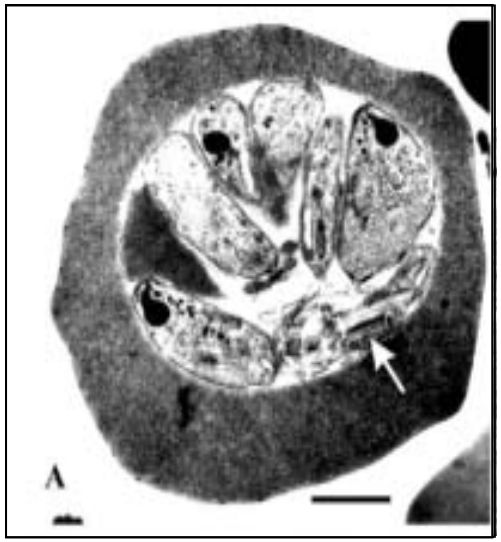
Phases of merozoite invasion





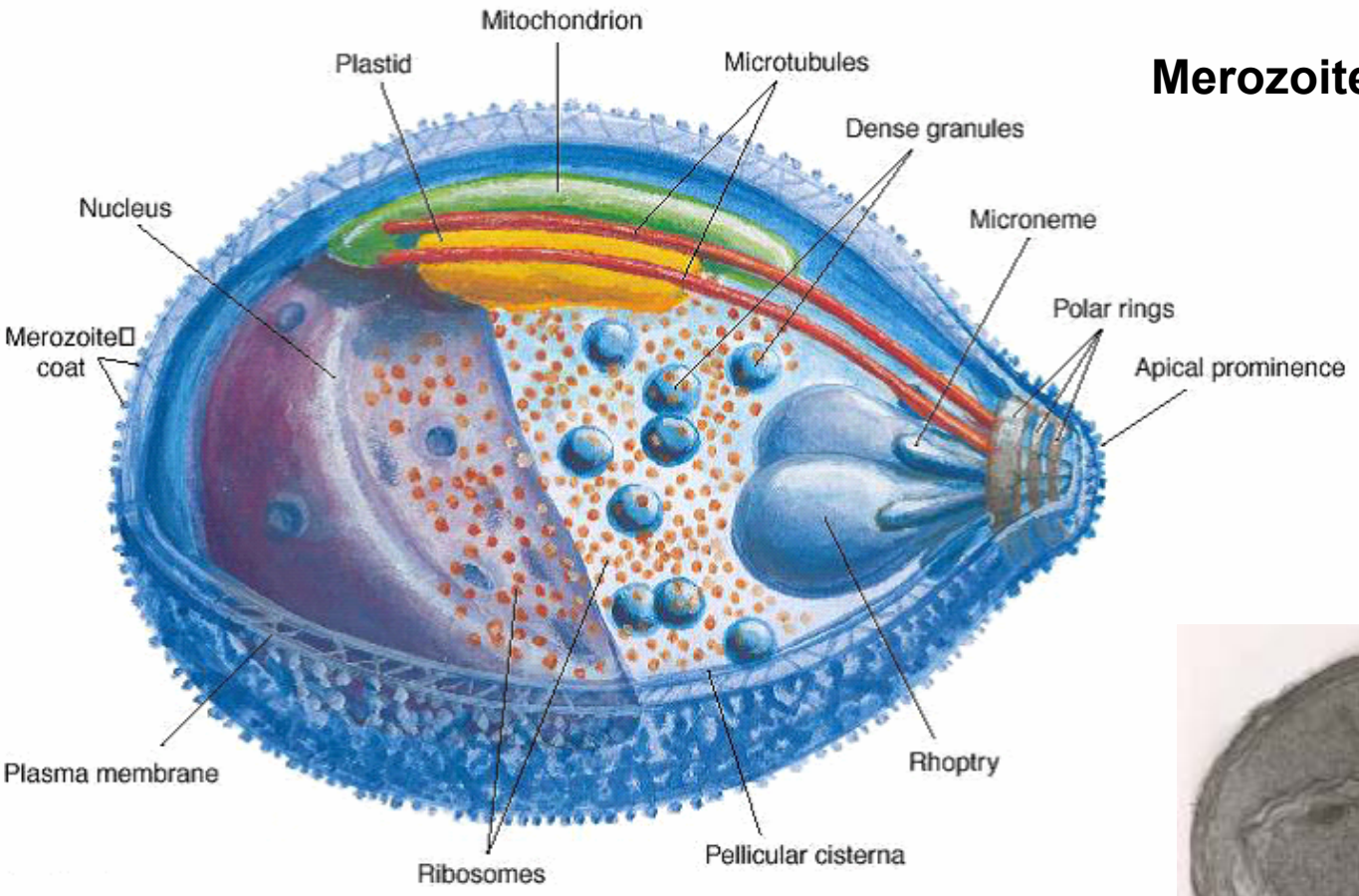
RESA
RIMA
SUB-1
SUB-2
AMA-1

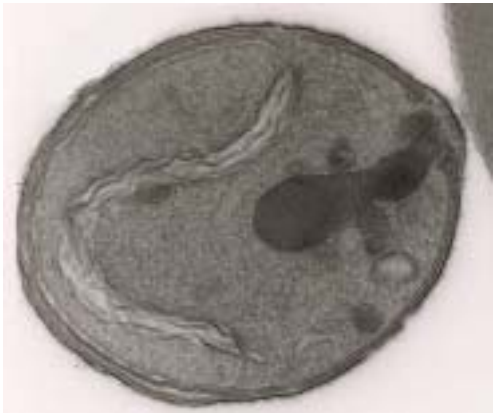
Bannister L. H., *et al*, 1977, Cowman A. F., *et al*, 2000
Sherman I. W., 1998

Morphological development of blood forms

FIDIC

Merozoite



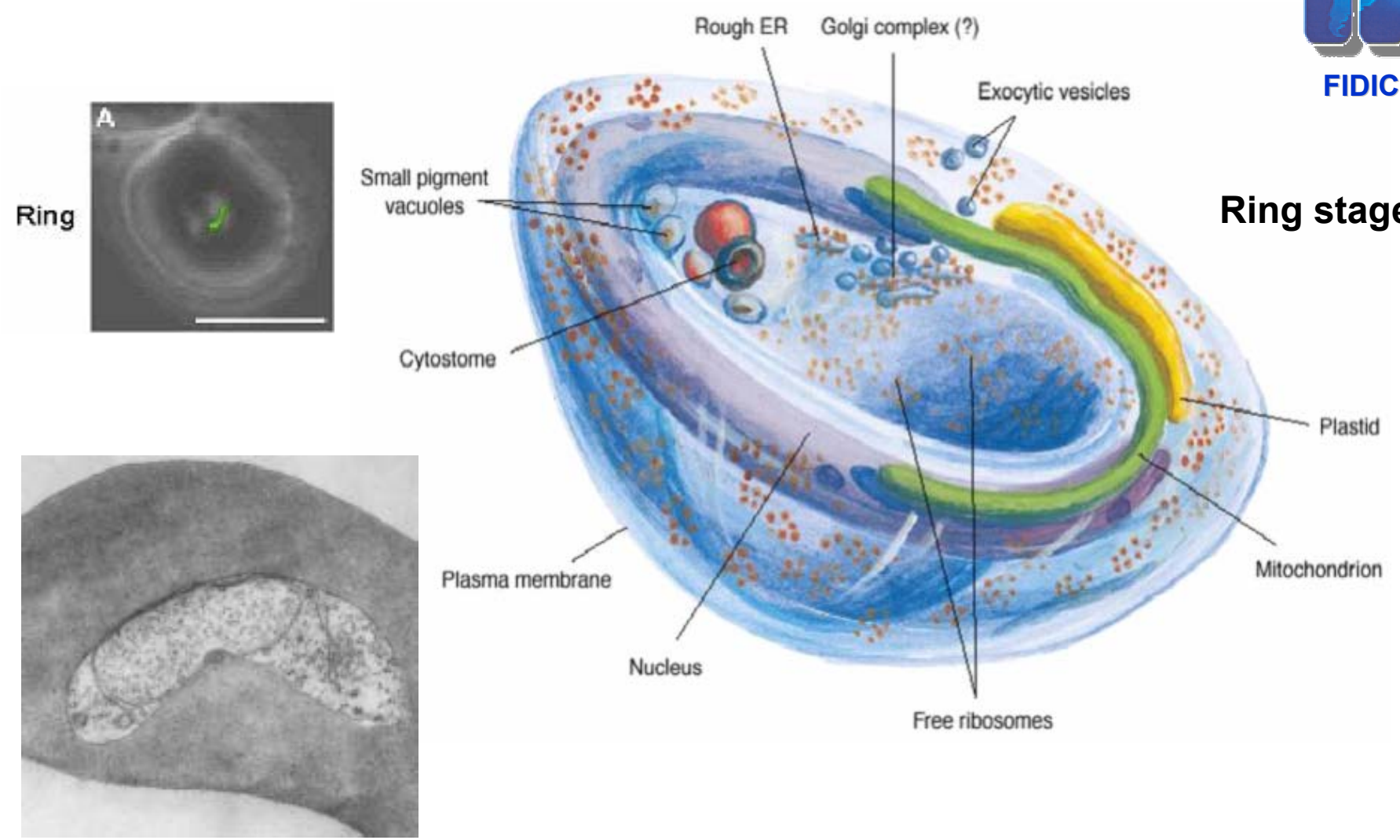


A merozoite, showing the apical prominence with a rhoptry, dense granules, and a very indented nucleus.

Bannister L. H., *et al.* 2000, Sherman I. W., 1998
Anders R. F., *et al.* 1991

Morphological development of blood forms

FIDIC

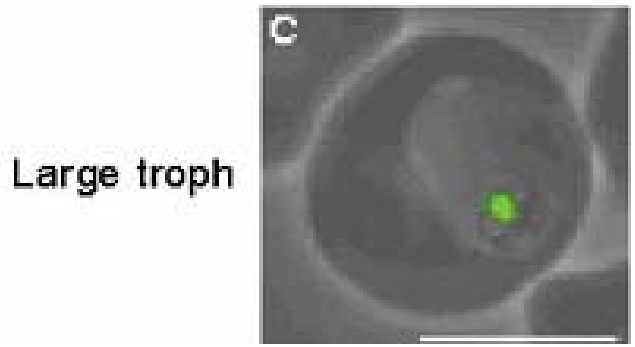


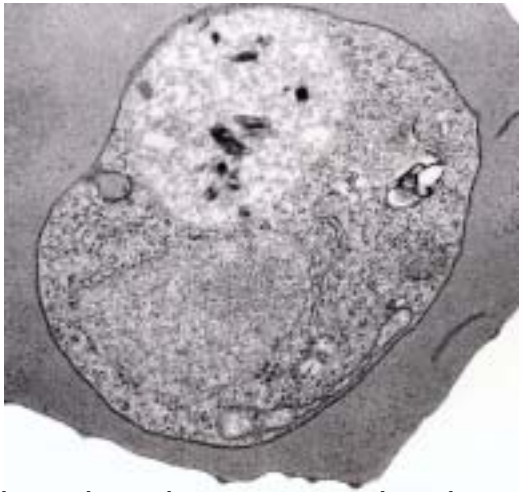
Ring stage

A ring stage of the cup-like form, showing the nucleus surrounded by ribosomes and some endoplasmic reticulum.

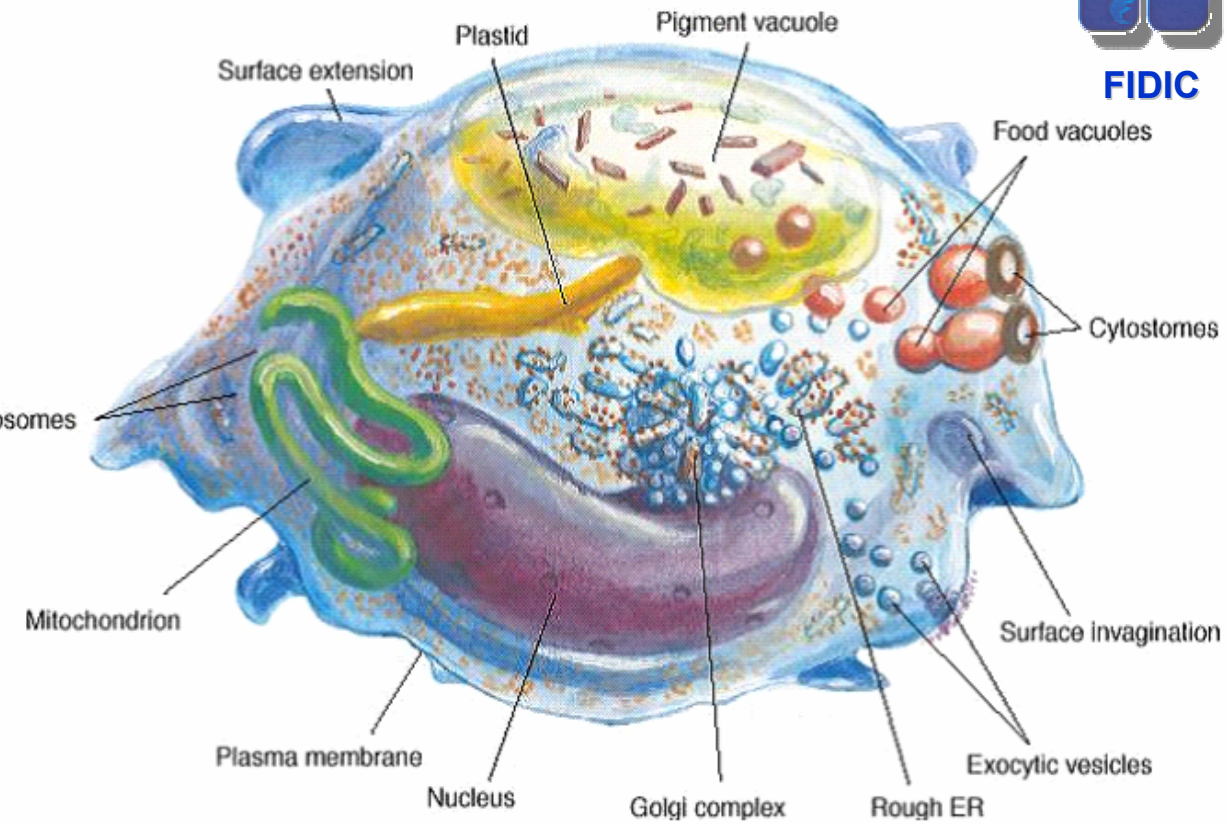
Bannister L. H., *et al.* 2000, Sherman I. W., 1998
Anders R. F., *et al.* 1991

Morphological development of blood forms





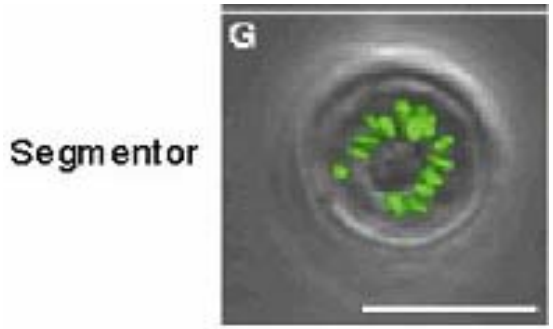
Mid-trophozoite stage, showing the nucleus, the pigment vacuole and a cytostome with a forming food vacuole. "Trophozoite" means the maturing stage. Greek "tropho" means "to nurture".

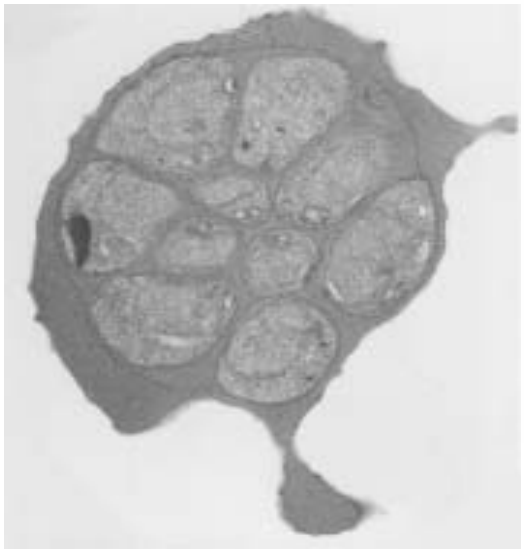


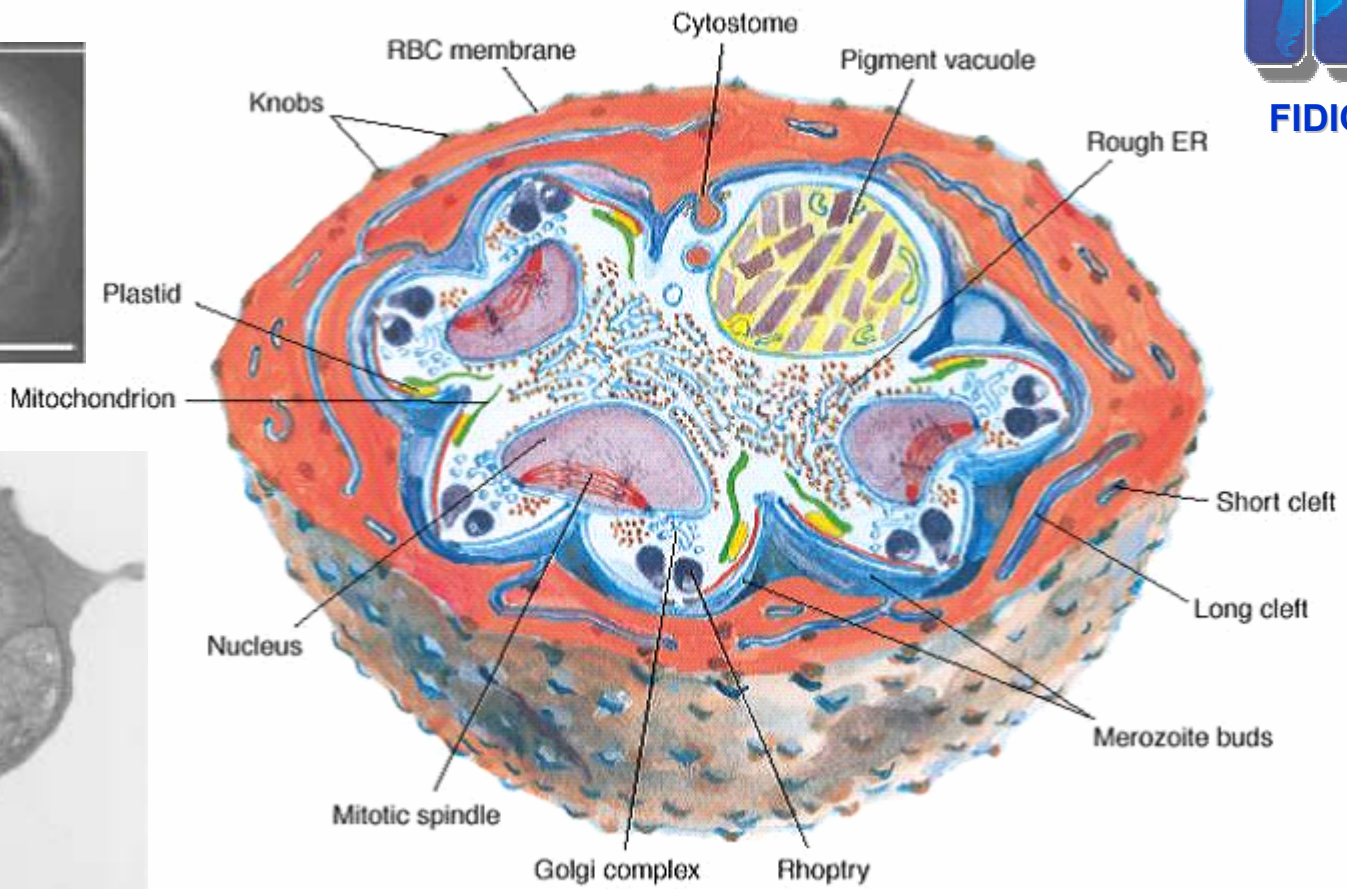
Trophozoite

Bannister L. H., *et al.* 2000, Sherman I. W., 1998
Anders R. F., *et al.* 1991

Morphological development of blood forms





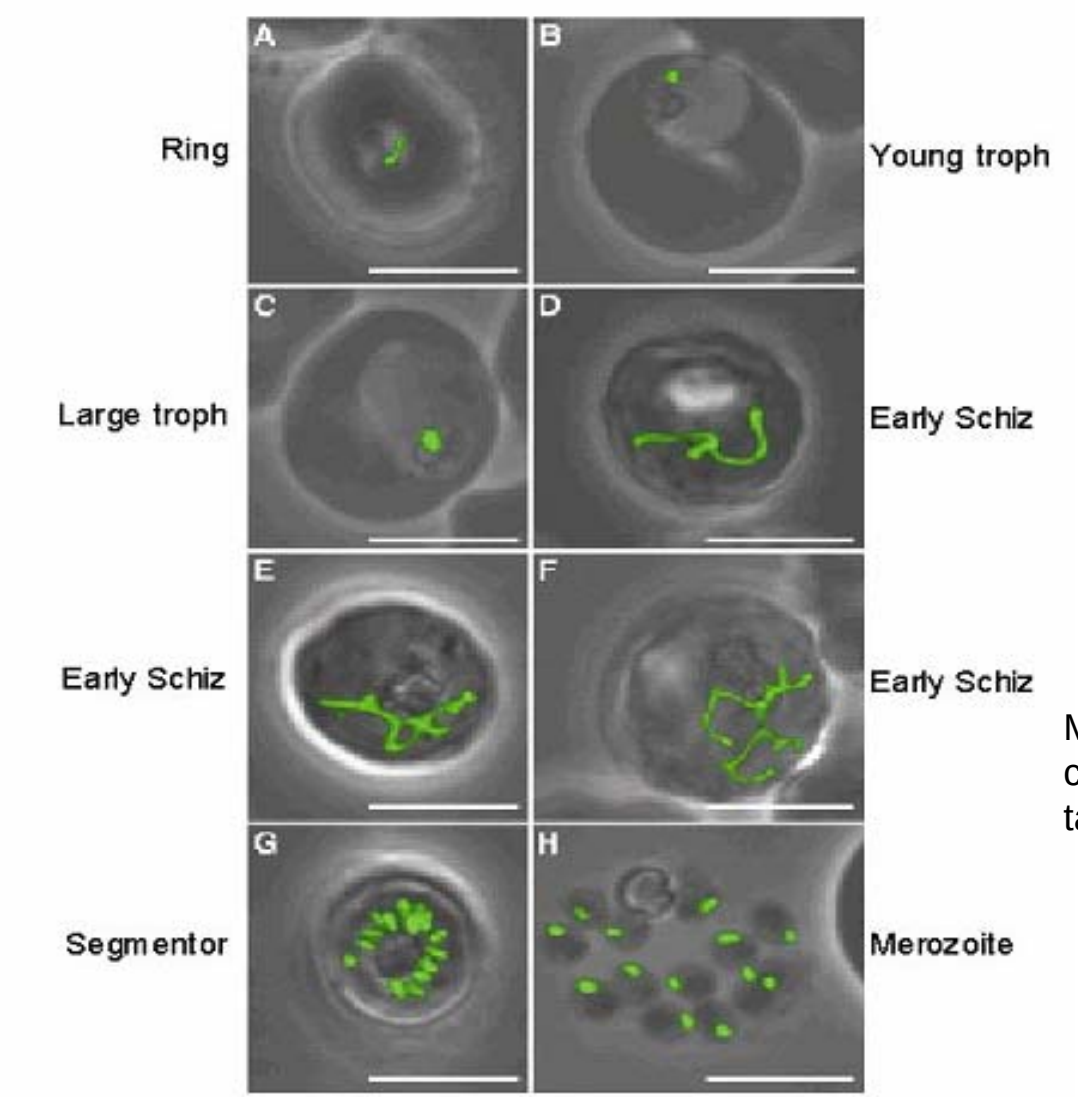


Schizont

A schizont, showing a series of nuclei and developing merozoites containing rounded early rhoptries around their perimeters. Note the irregular appearance of the red blood cell (RBC) surface and the presence of knobs. The name "schizont" comes from the Greek "schizo", meaning "to tear apart".

Bannister L. H., *et al.* 2000, Sherman I. W., 1998
Anders R. F., *et al.* 1991

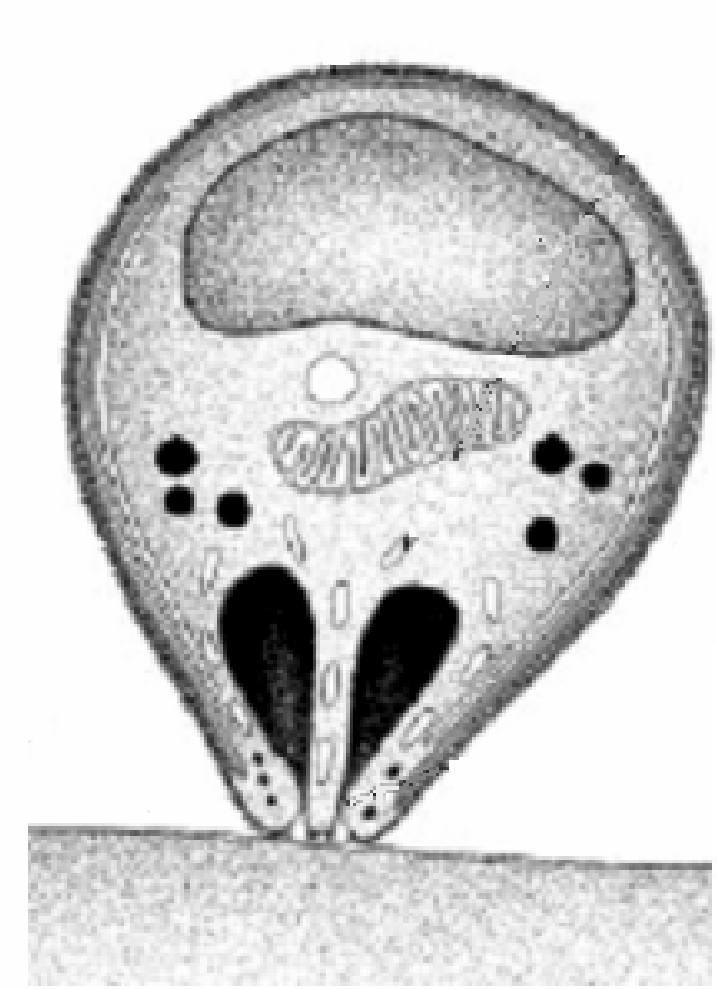
Plasmodium falciparum morphology



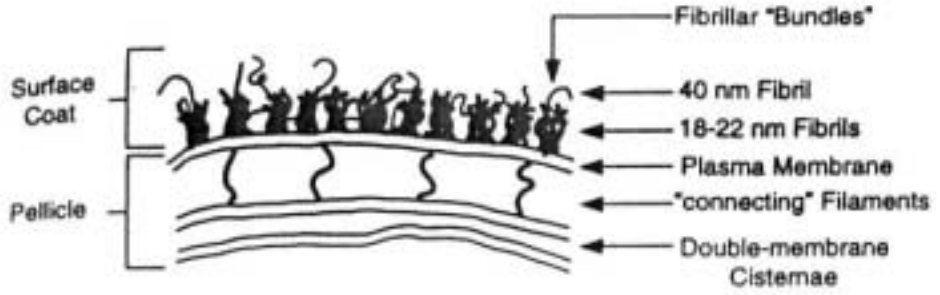
Morphology throughout the asexual life cycle of blood stage parasite expressing targeted Green Fluorescent Protein (GFP)

http://sites.huji.ac.il/malaria/FramInteractions.html

Merozoite surface proteins



- MSP1
- MSP2
- MSP3
- MSP4
- MSP5
- MSP6
- MSP8
- AMA1
- MAEBL
- ABRA
- S-antigen



Merozoite pellicle and surface coat

Bannister L. H., *et al*, 1977, Cowman A. F., *et al*, 2000
Sherman I. W., 1998, Cowman and Crabb, 2002
Chitnis and Blackman, 2000

Fundación Instituto de Inmunología de Colombia

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Receptor-ligand interactions

$$R + L \rightleftharpoons RL$$

$$(r-b) \quad (l-b) \quad b$$

$$b = \frac{(k + r + l) - ((k + r + l)^2 - 4rl)^{1/2}}{2}$$

r > 250 binding sites per cell

l < 200 nM

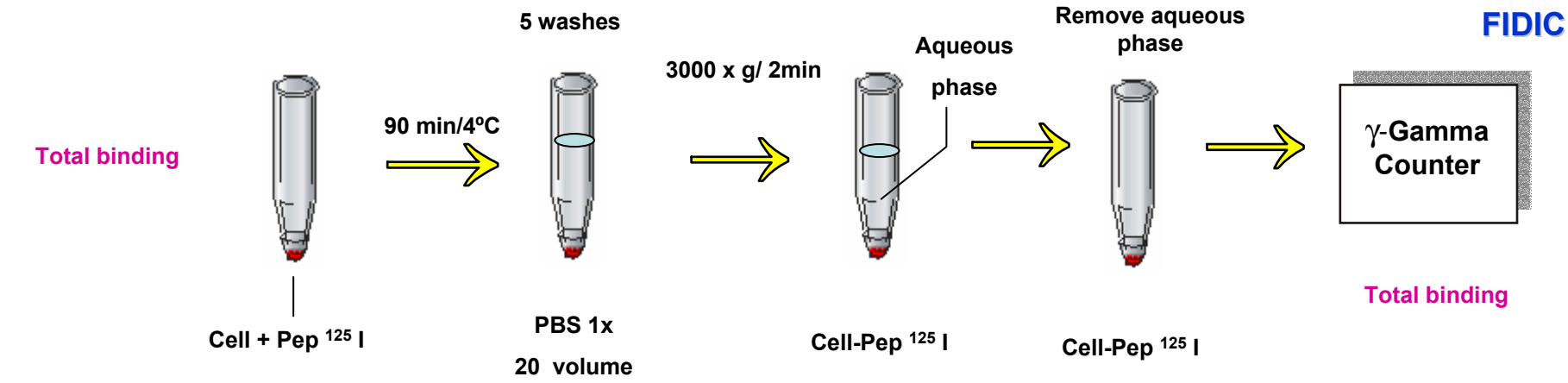
b/l is the binding slope, if $b/l \geq 0.02$

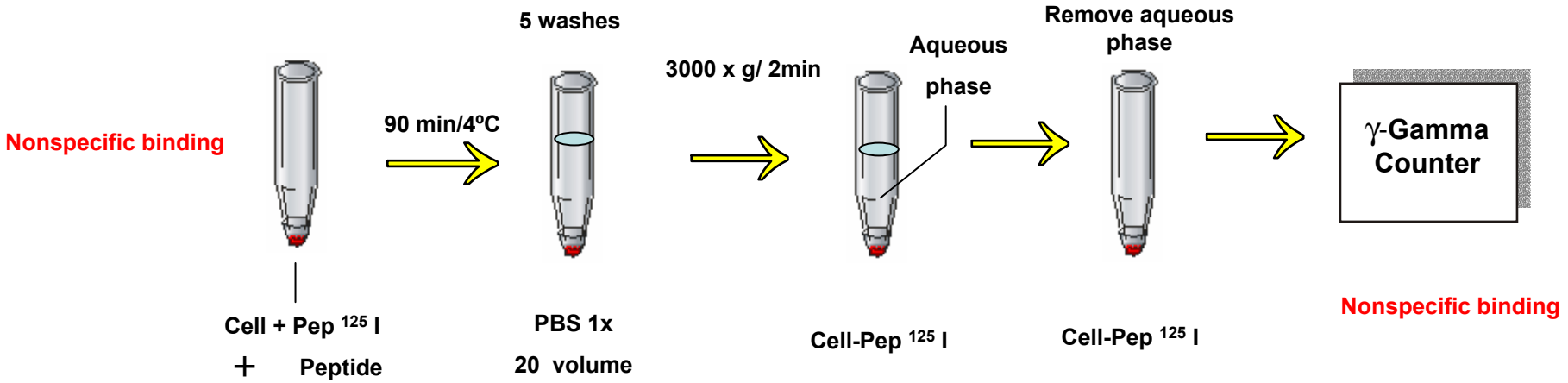
Behr M., *et al*., 2001, Attle A. D. and Raines R. T., 1995,
Hulme E. C., *et al*., 1993, Yamamura H. I., *et al*., 1978

Receptor-ligand binding assay

Urquiza M., *et al.*, 1996, **Parasite Immunology**, 18: 515

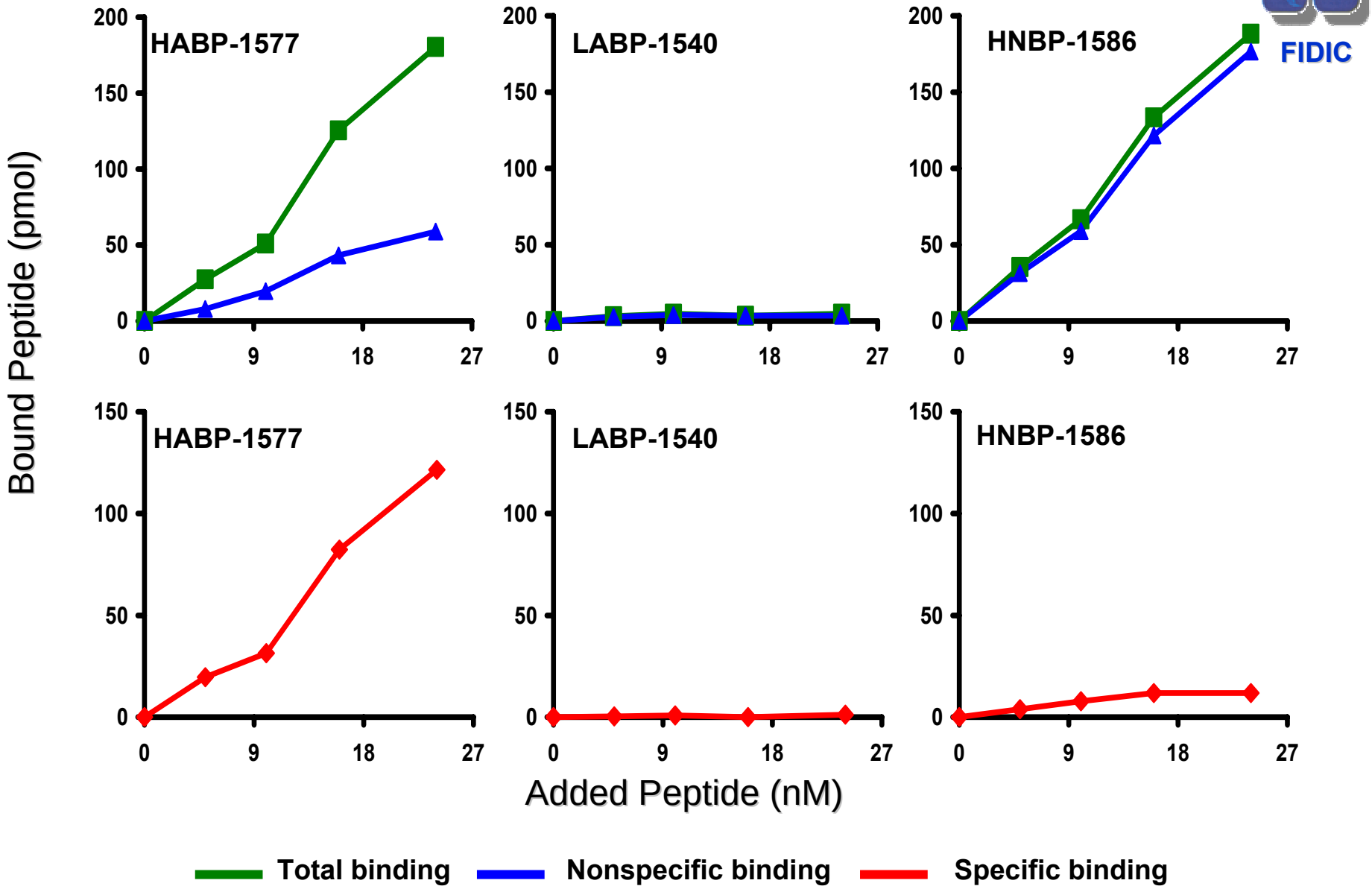
FIDIC





Peptide RBC binding behaviour

Urquiza M., *et al.*, 1996, **Parasite Immunology**, 18: 515



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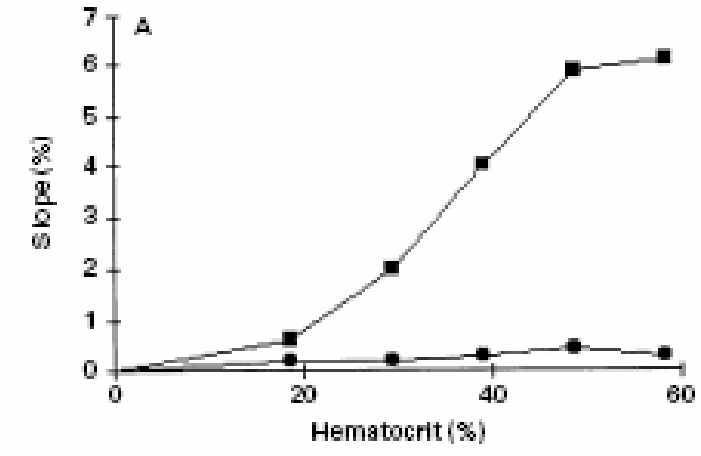
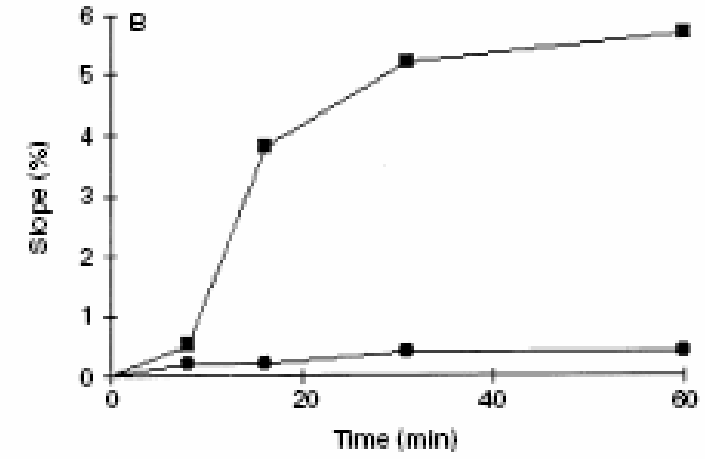
Factors influencing the binding assay

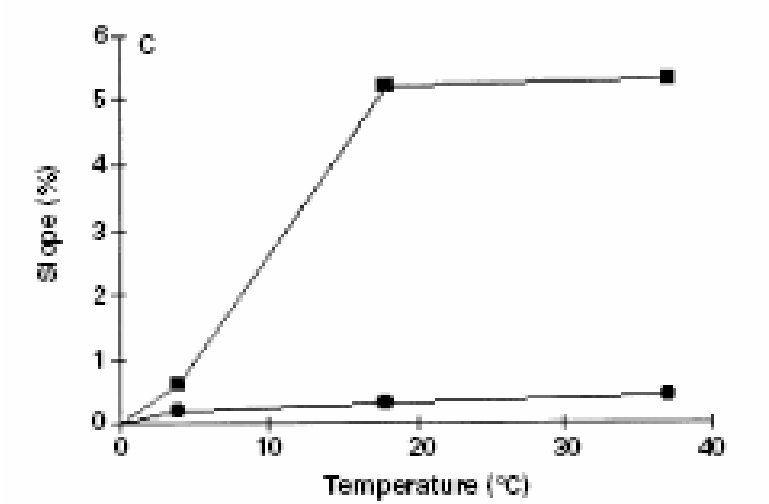
Rodríguez L.E., *et al*., 2000, **Parasitology**, 120: 225



Binding assay at 20% to 60% haematocrit T 18 °C, 90 min).



Effects of reaction time on the slope values of the specific binding curve (haematocrit 50% T 18 °C).

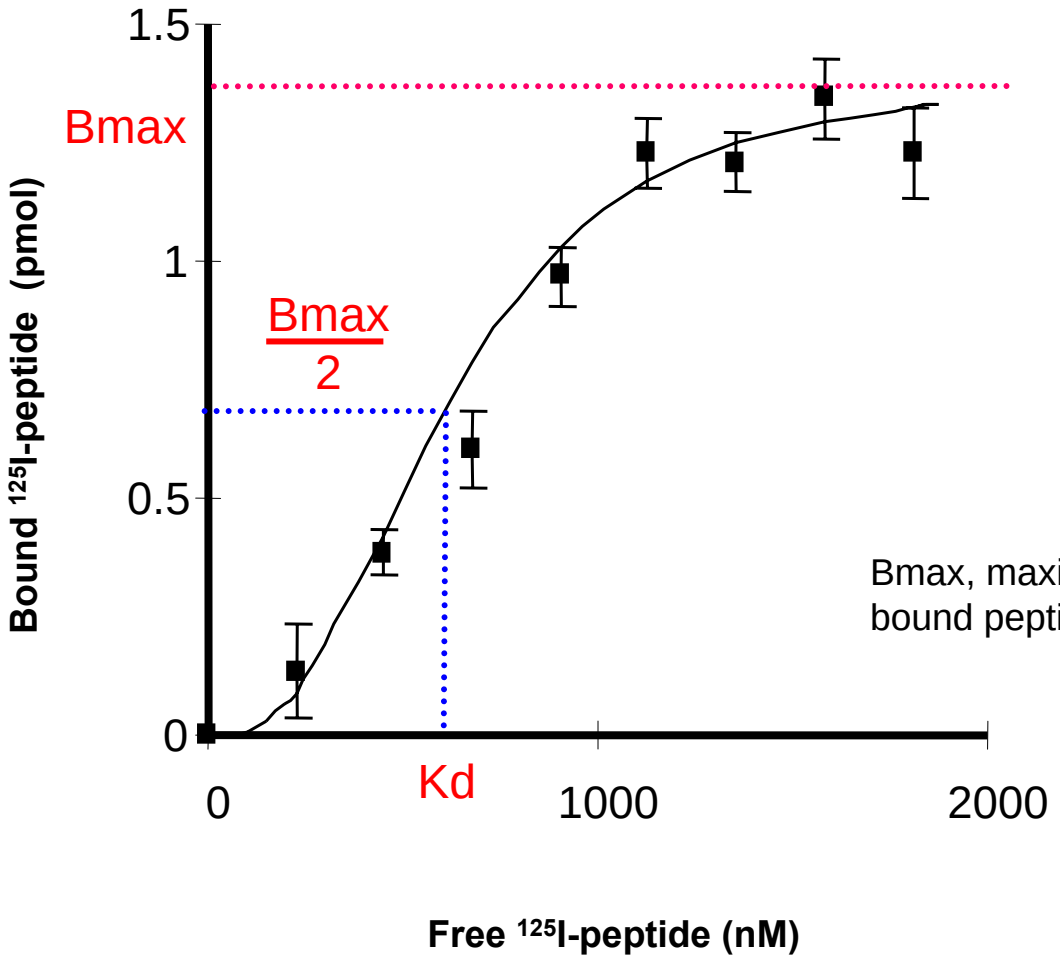


Effects on slope values of specific binding curve at temperatures between 4ºC and 37°C (haematocrit 60% 60 min).

HABP saturation curve

Urquiza M., *et al.*, 1996, **Parasite Immunology**, 18: 515

FIDIC

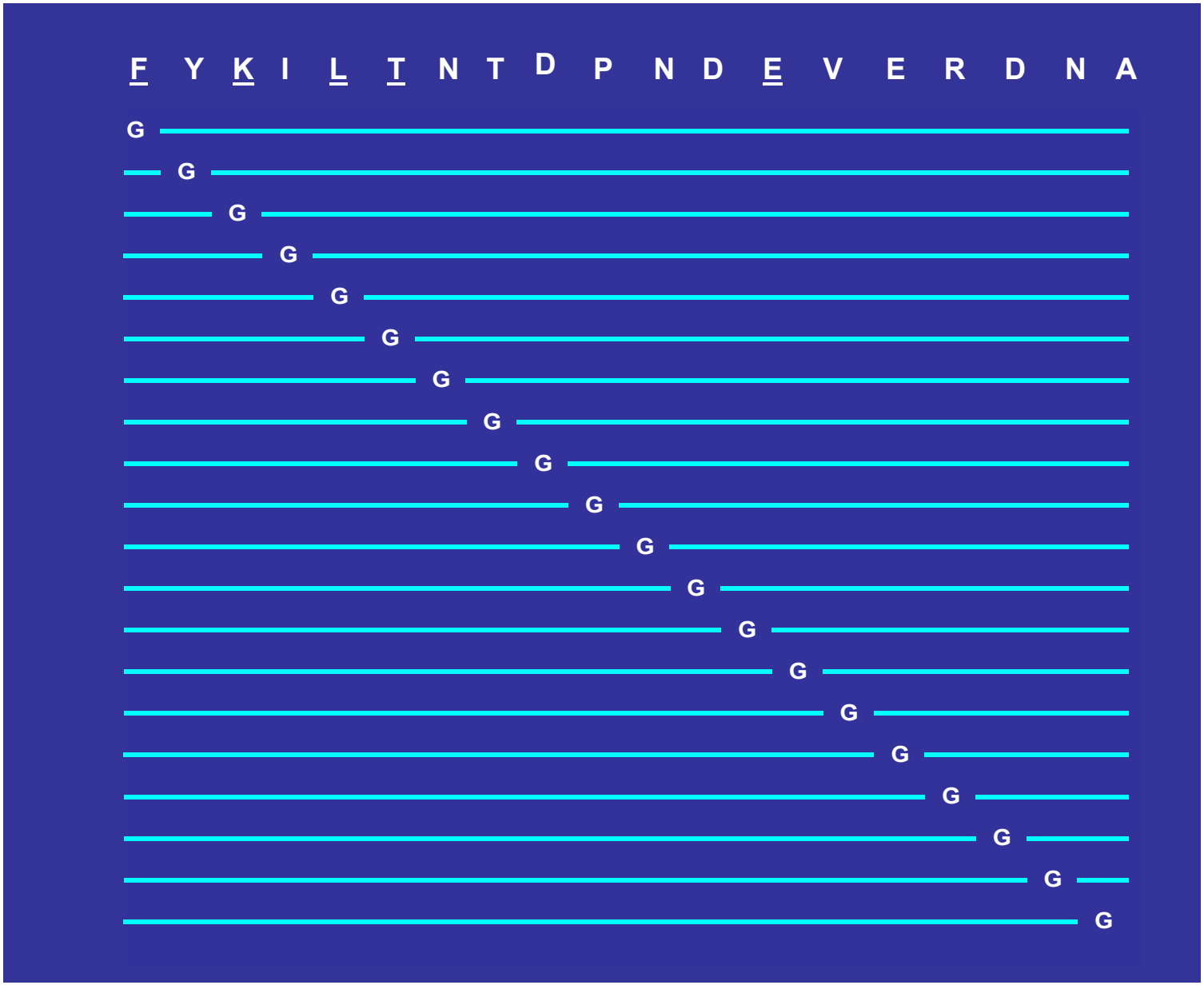


Bmax, maximum amount of bound peptide

High binding glycine analogue peptides

Suárez J. E., *et al*., 2000, **Mem Inst Oswaldo Cruz**, 95: 495

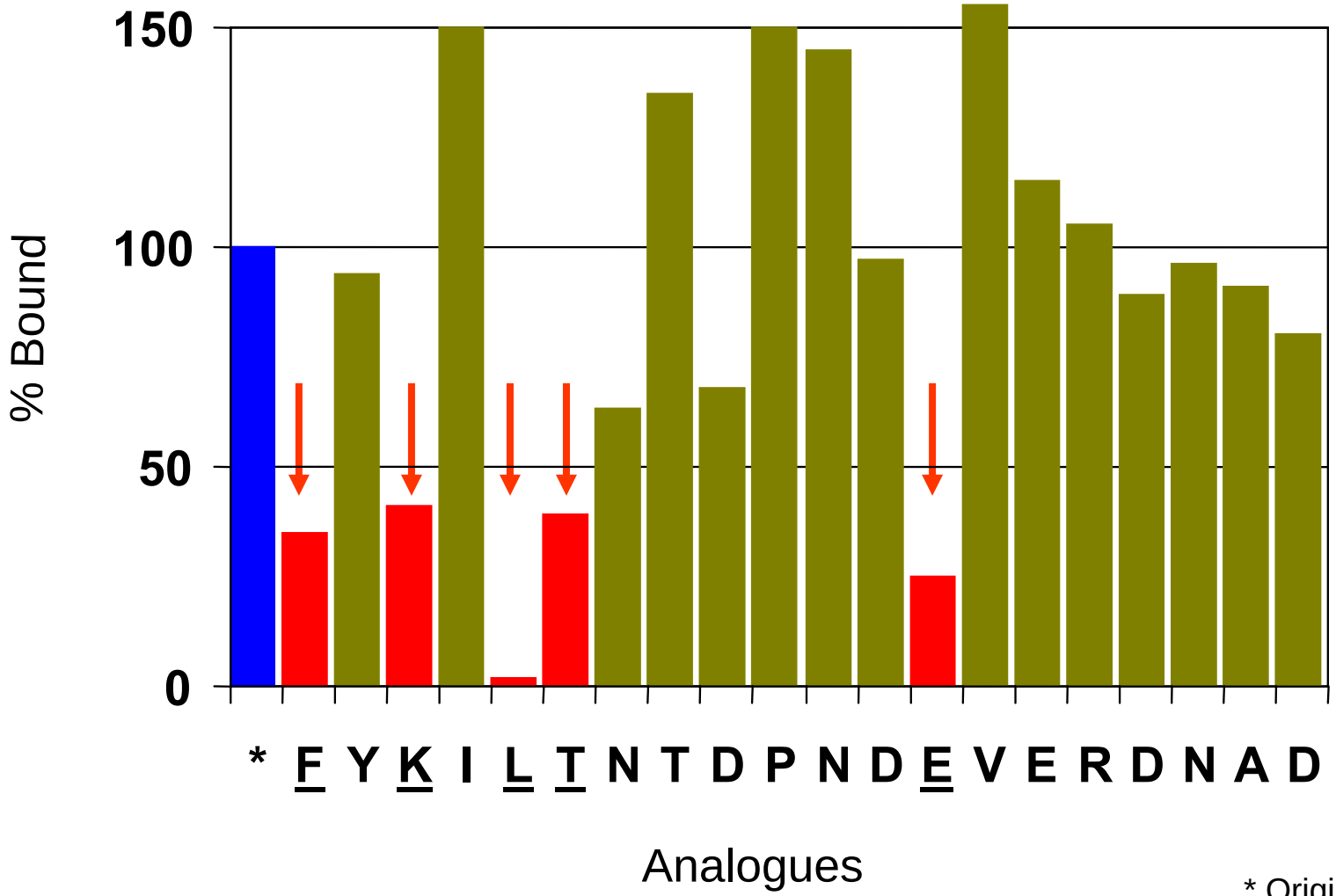
FIDIC



Critical residues for peptide-erythrocyte interaction

Suárez J. E., *et al.*, 2000, **Mem Inst Oswaldo Cruz**, 95: 495

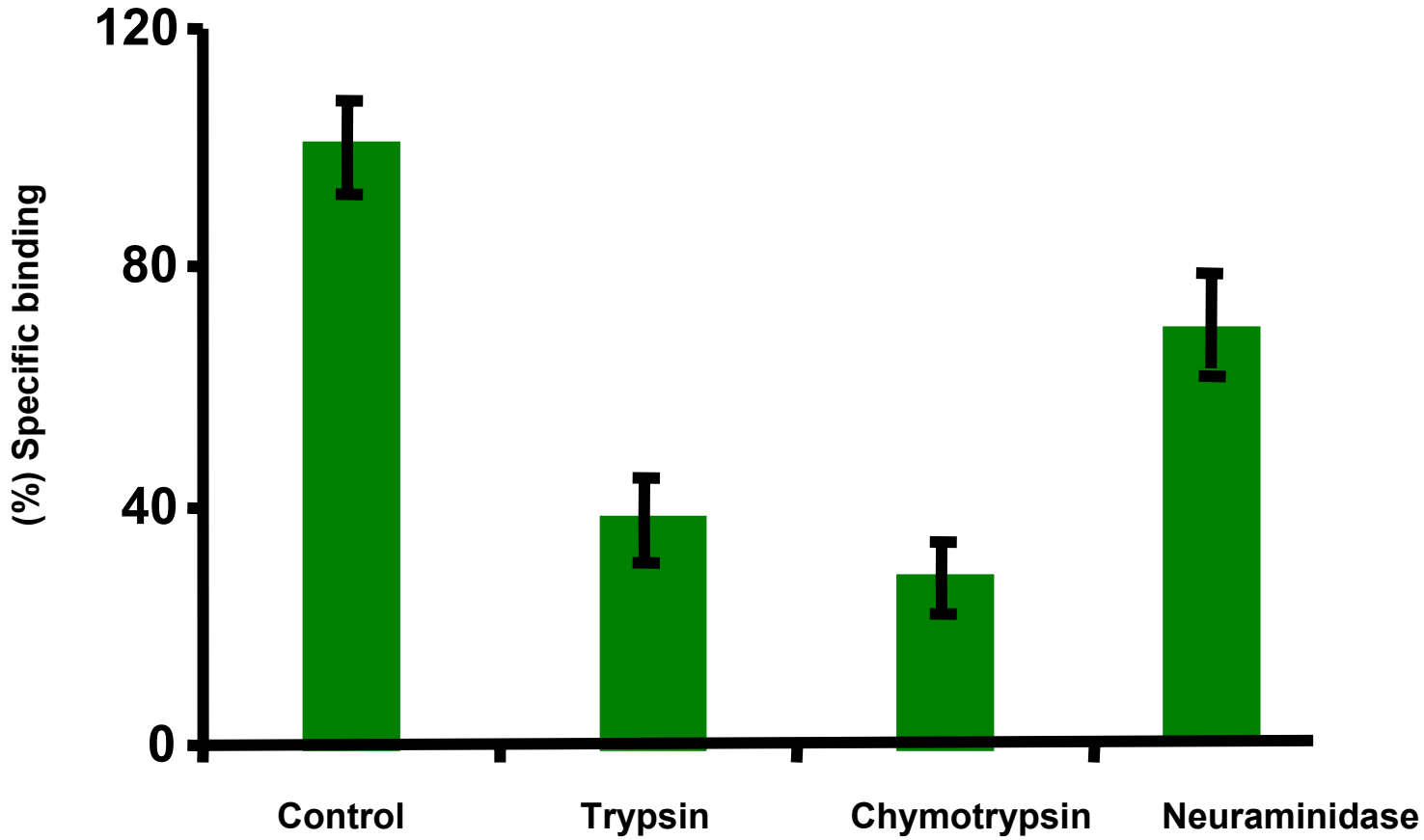
FIDIC



* Original peptide

Receptors for peptide binding on RBC surface

FIDIC



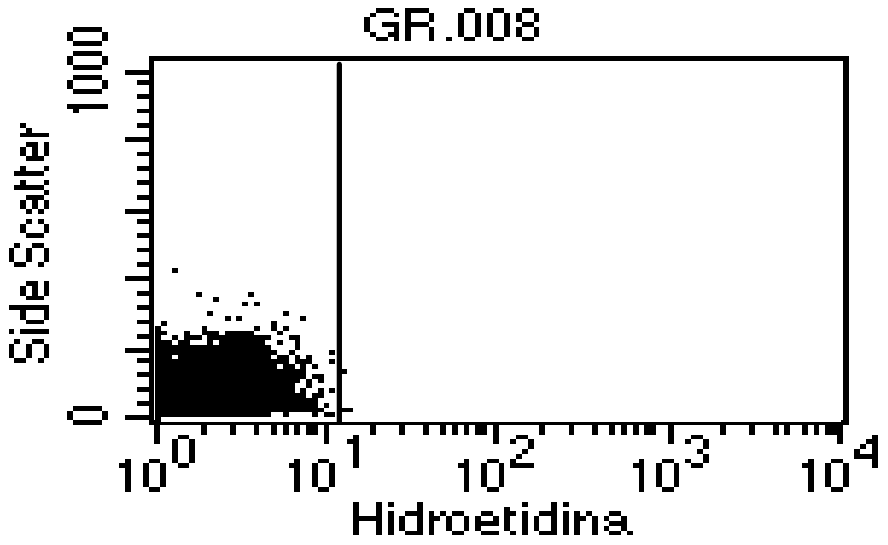
HABP binding to human and non-human erythrocytes

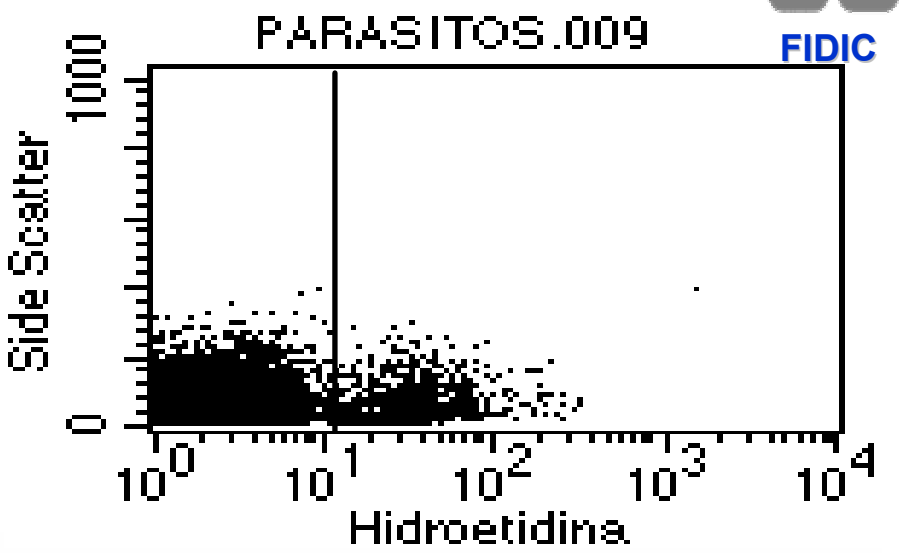
FIDIC

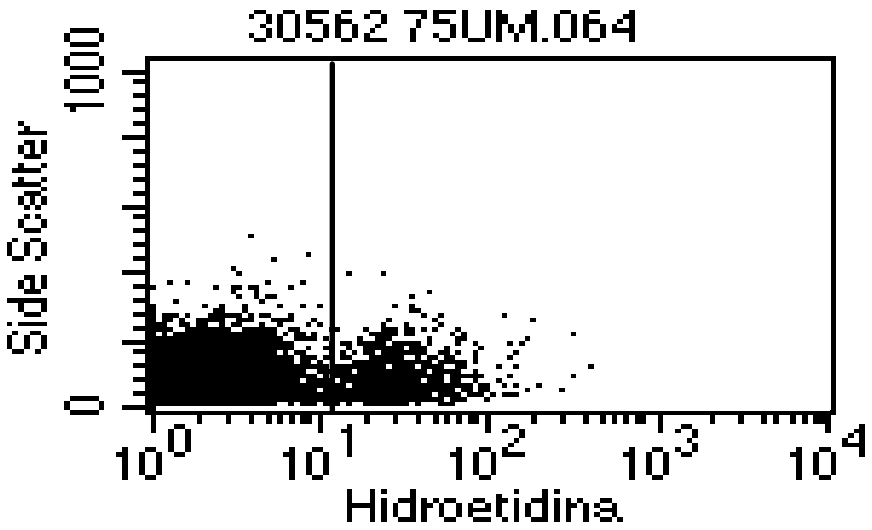
Suárez J. E., *et al*., 2000, **Mem Inst Oswaldo Cruz**, 95: 495

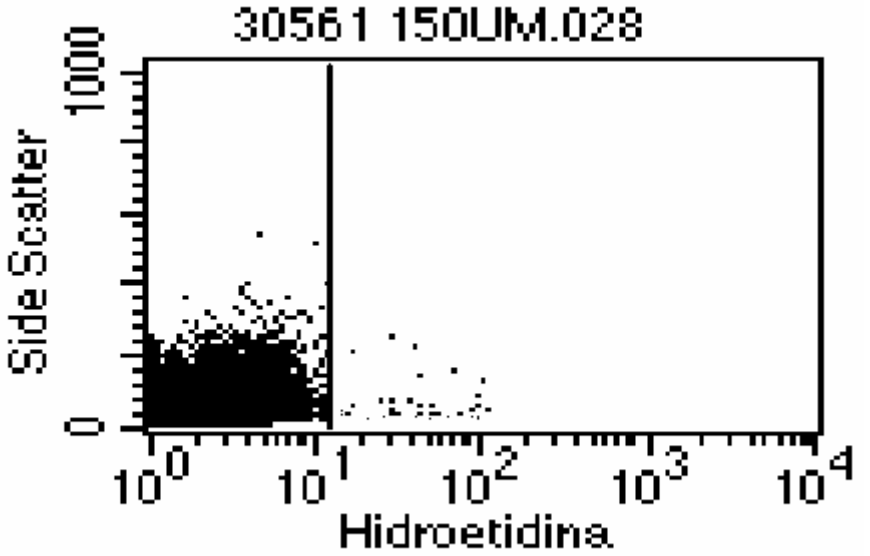
Erythrocytes	Specific binding (%)
Humans	100 ± 8
Aotus	59 ± 6
Rabbits	44 ± 7
Goats	0 ± 4
Horses	0 ± 2
Chickens	8 ± 4

Invasion inhibition assay









Merozoite surface proteins

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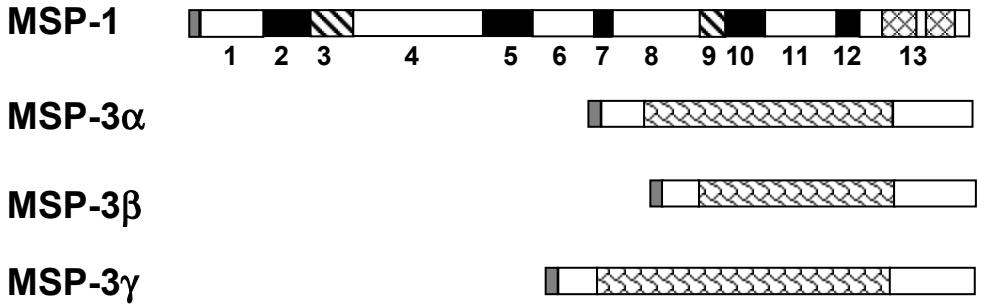
Plasmodium falciparum

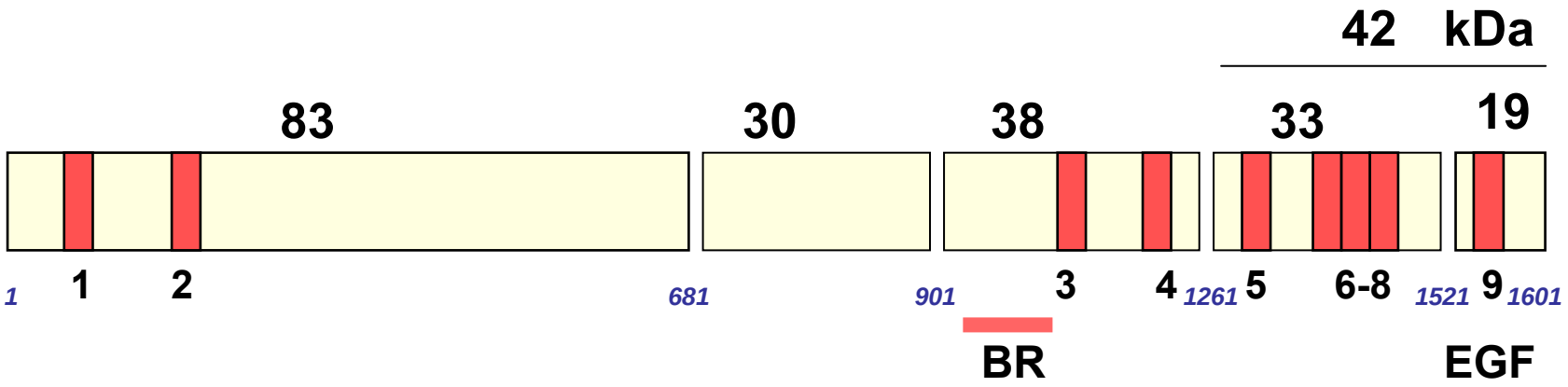
Plasmodium vivax



Conserved
Semi-conserved
Variable
Coiled coil
Amino-acid repeats
EGF-like domain
Signal peptide
GPI addition
200 amino-acids

Sherman I. W., 1998

MSP-1

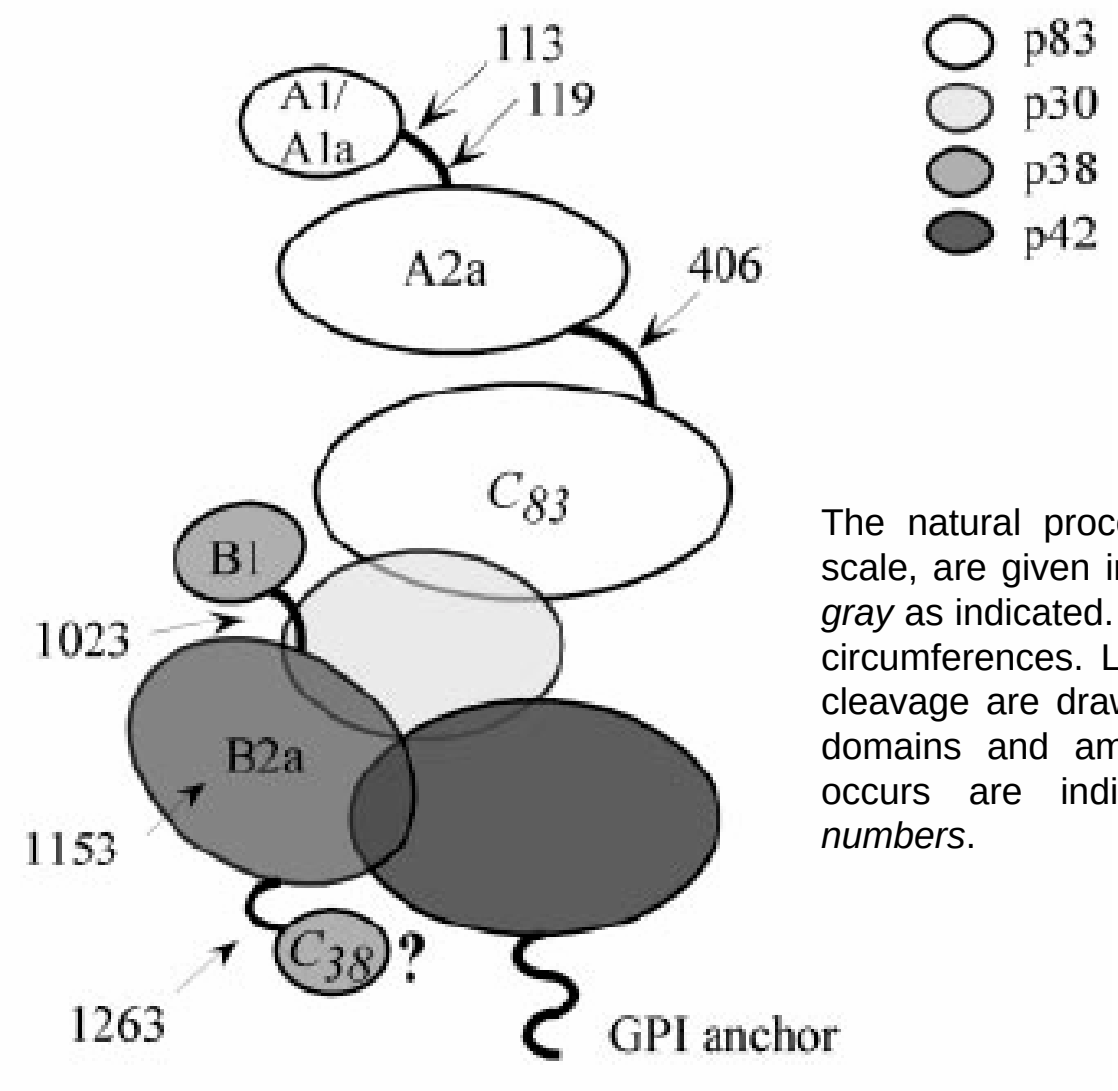


1. GYSLFQKEKMVLNEGTSGTA
2. QIPYNLKIRANELDVLKKLV
3. FKVLSKLEGKLKDNLNLEKK
4. AESNTITTSQNVDDEVDDVY
5. EVLYLKPLAGVYRSLKKQLE
6. LSSYNYIKDSIDTDINFAND
7. VLGYYKILSEKYKSDLDSIK
8. KYINDKQGENEKYLPFLNNI
9. QGMLNISQHQCVKKQCPQNS

- The MSP-I protein is a 195 kDa polymorphic surface membrane protein of the merozoite.
- MSP-1 is synthesized in schizogony and contain variable, semiconserved and constant sequences.
- Extensive proteolytic processing of 83, 30, 38 and 42 (33, 19) kDa fragments.
- 19 kDa contain two cysteine-rich EGF-like regions.
- The SPf66 synthetic malaria vaccine, contains a sequence from the 83 kDa fragment: **YSLFQKEKMVL**

Holder A. A. and Blackman 1994, Nikodem D. P. and Davidson E. A. 2000, Urquiza M. *et al*., 1996, Patarroyo M. E. *et al*., 1988, Lozano J. M. *et al*., 1998

Model of the overall structure of processed MSP-1.

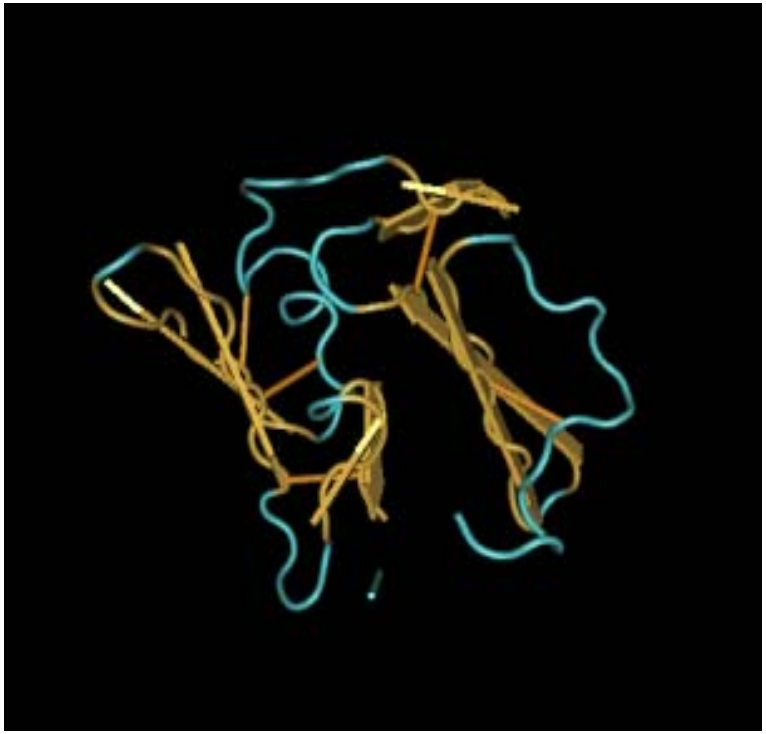


The natural processing fragments, roughly drawn to scale, are given in *white* (p83) or in differently *shaded gray* as indicated. Interacting subunits overlap with their circumferences. Linker regions accessible to thrombin cleavage are drawn as *black lines* connecting various domains and amino acid positions where cleavage occurs are indicated by *arrows* and respective *numbers*.

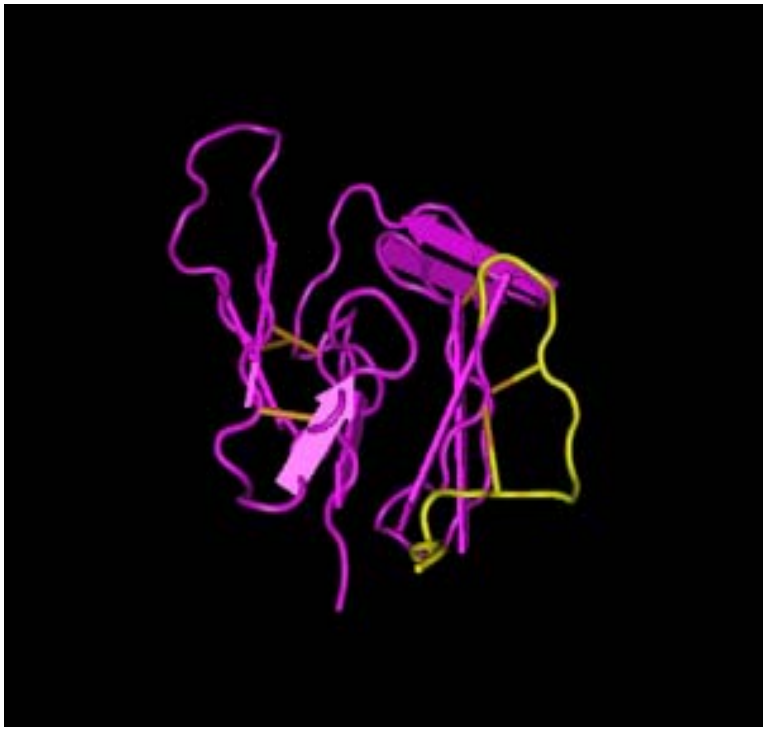
Kauth C. W., *et al*., 2003

MSP-1_{19}

FIDIC



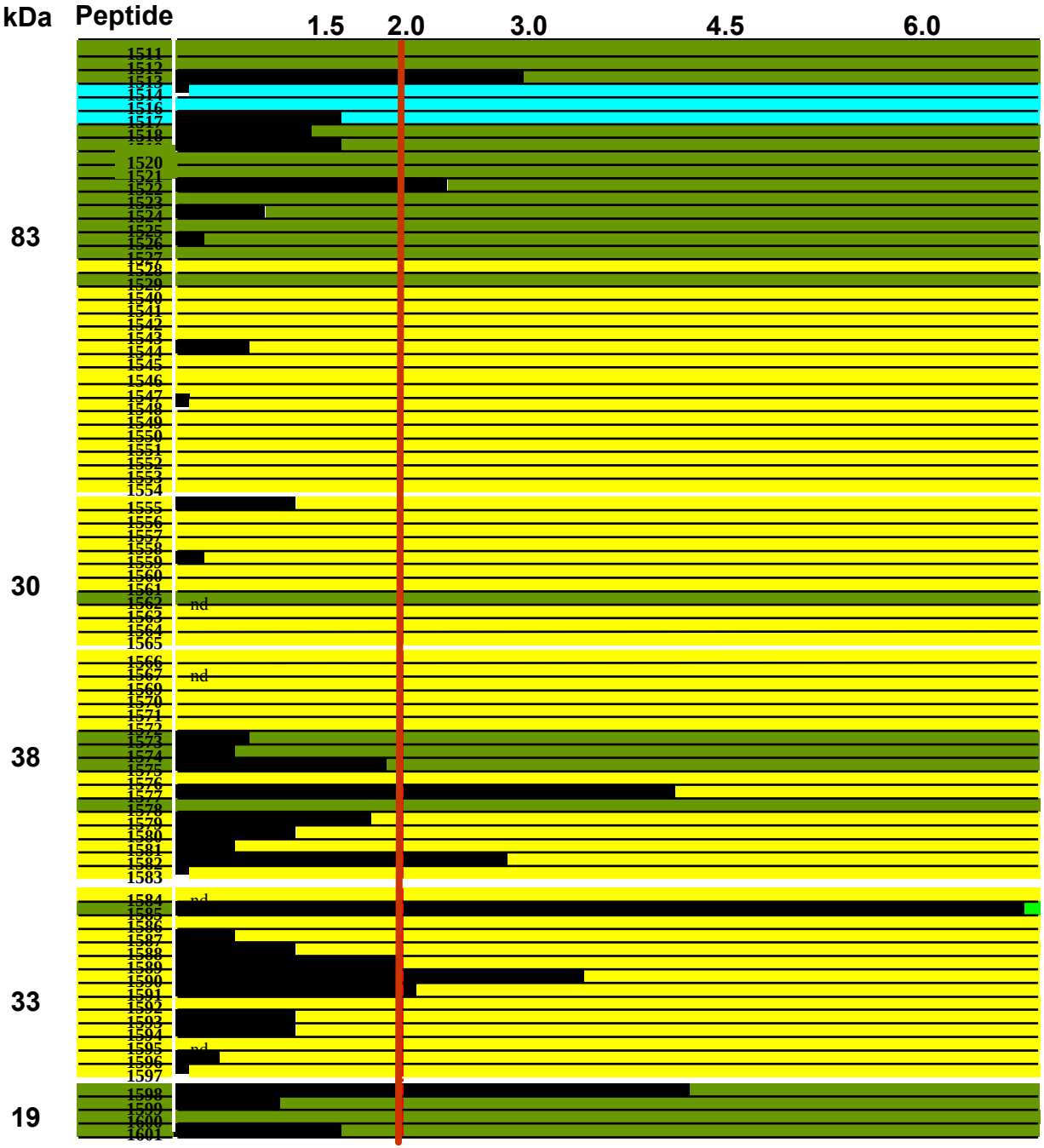
The 3D structure of PcMSP-1_{19}



The 3D structure of PfMSP-1_{19}

Chitarra V. *et al*., 1999, Morgan W.D. *et al*., 1999

Specific MSP-I peptide binding to erythrocytes.



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Urquiza M., *et al.*, 1996, **Parasite Immunology**, 18: 515

Peptide	Kd (nM)
1513	200 ± 23
1522	150 ± 13
1577	180 ± 23
1582	140 ± 14
1585	180 ± 20
1589	250 ± 25
1590	180 ± 18
1591	190 ± 27
1598	240 ± 36
5501	230 ± 45

The binding affinity (black bars) is the slope of the specific binding graph. The red line separates peptides having binding capacity greater than or equal to 0.020 pmol bound/pmol added peptide

Invasion and development inhibition assays

Urquiza M., *et al.*, 1996, **Parasite Immunology**, 18: 515

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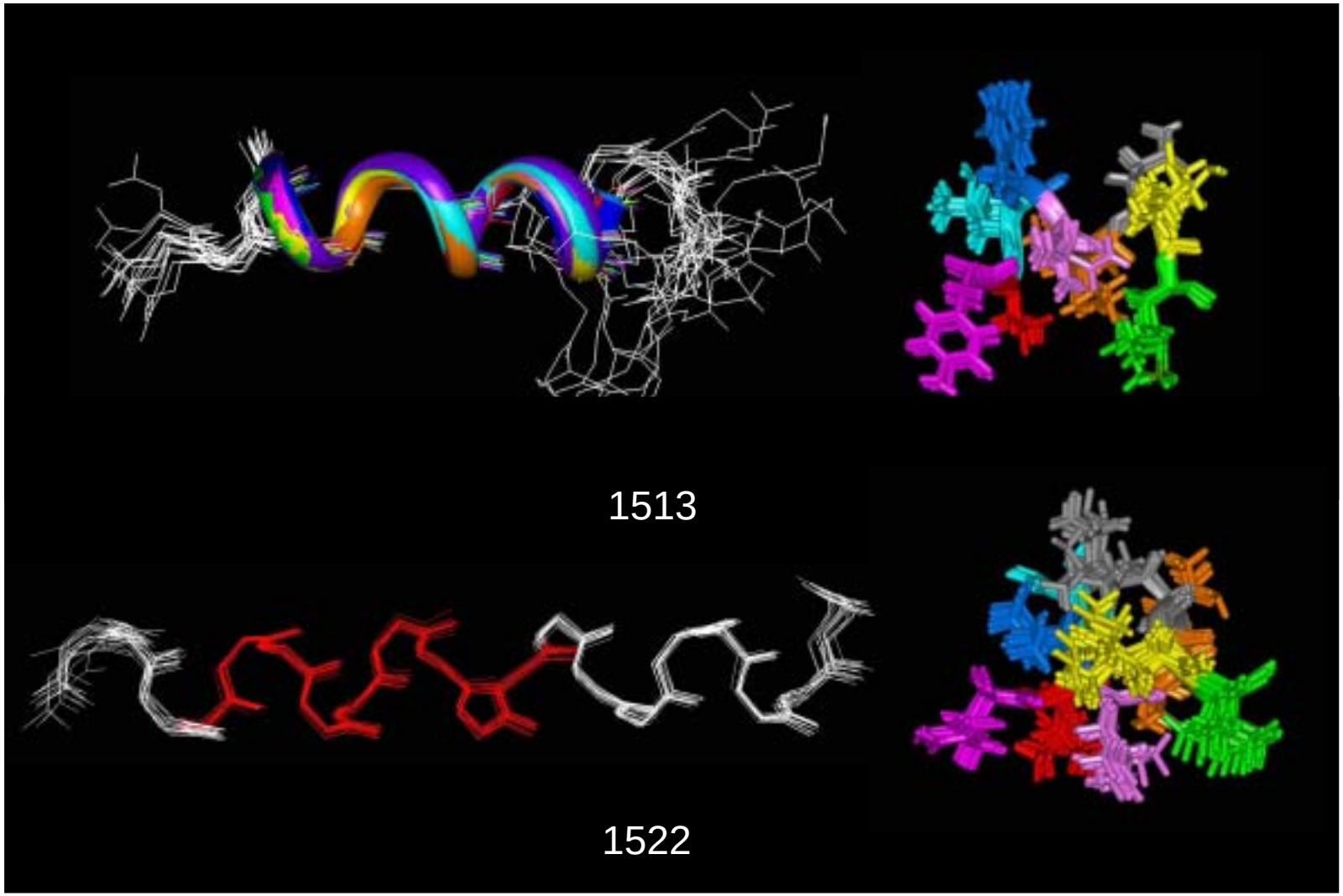
		Peptide	Invasion inhibition	Development inhibition
			% ± SD	% ± SD
High binding activity	200 μM each	1513	60 ± 10	4 ± 8
		1522	60 ± 14	62 ± 21
		1577	43 ± 3	21 ± 3
		1582	50 ± 2	N.D.
		1585	3 ± 8	17 ± 5
		1589	29 ± 1	12 ± 3
		1590	10 ± 5	8 ± 4
		1591	0 ± 7	25 ± 15
		1598	30 ± 4	0 ± 9
Mixture high binding	20 μM each	All	42 ± 11	0 ± 5
	2 μM each	All	32 ± 12	0 ± 7
	0·2 μM each	All	18 ± 3	0 ± 4
Low binding activity	200 μM each	1517	0 ± 6	12 ± 1
		1551	5 ± 1	14 ± 2
		1560	7 ± 8	21 ± 4
		1581	0 ± 3	N.D.
		1596	0 ± 6	N.D.
		Chloroquine	98 ± 19	99 ± 4

Structural features for HABPs MSP-1

Espejo F., *et al.*, 2004, **BBRC**, 315: 418
Cubillos M., *et al*., 2003, **Proteins**, 50: 400

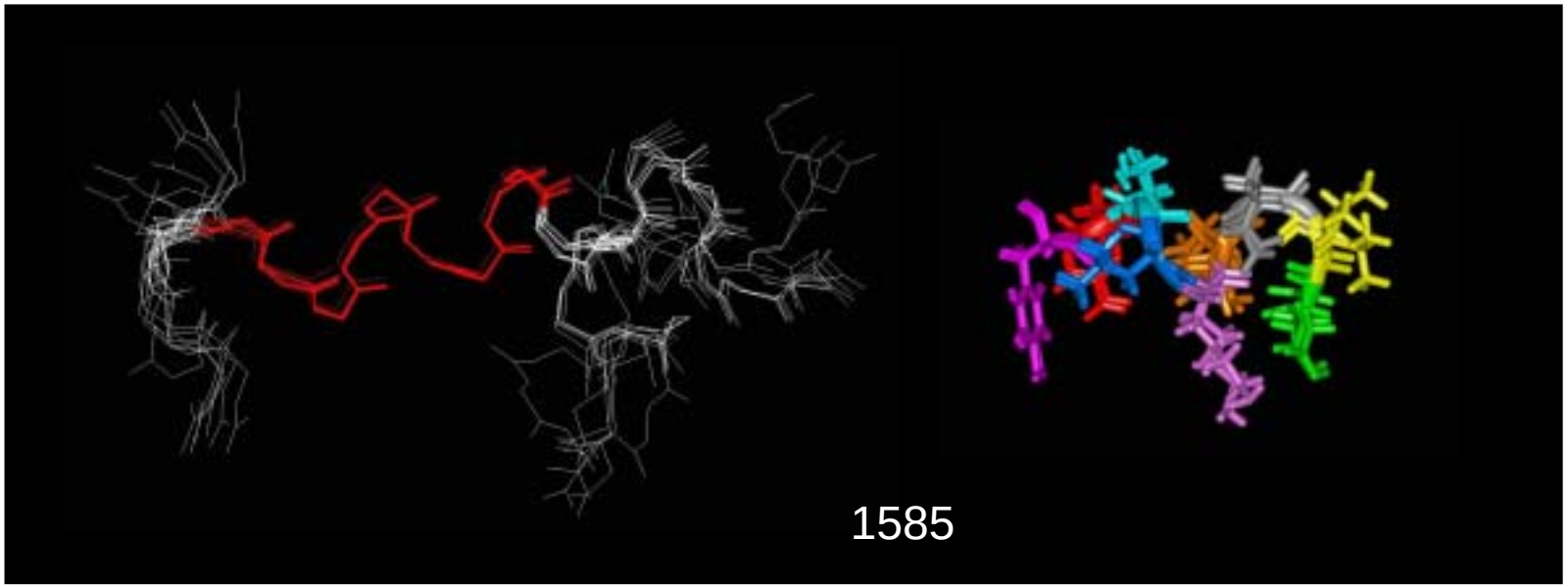
FIDIC



Structural features for HABPs MSP-1

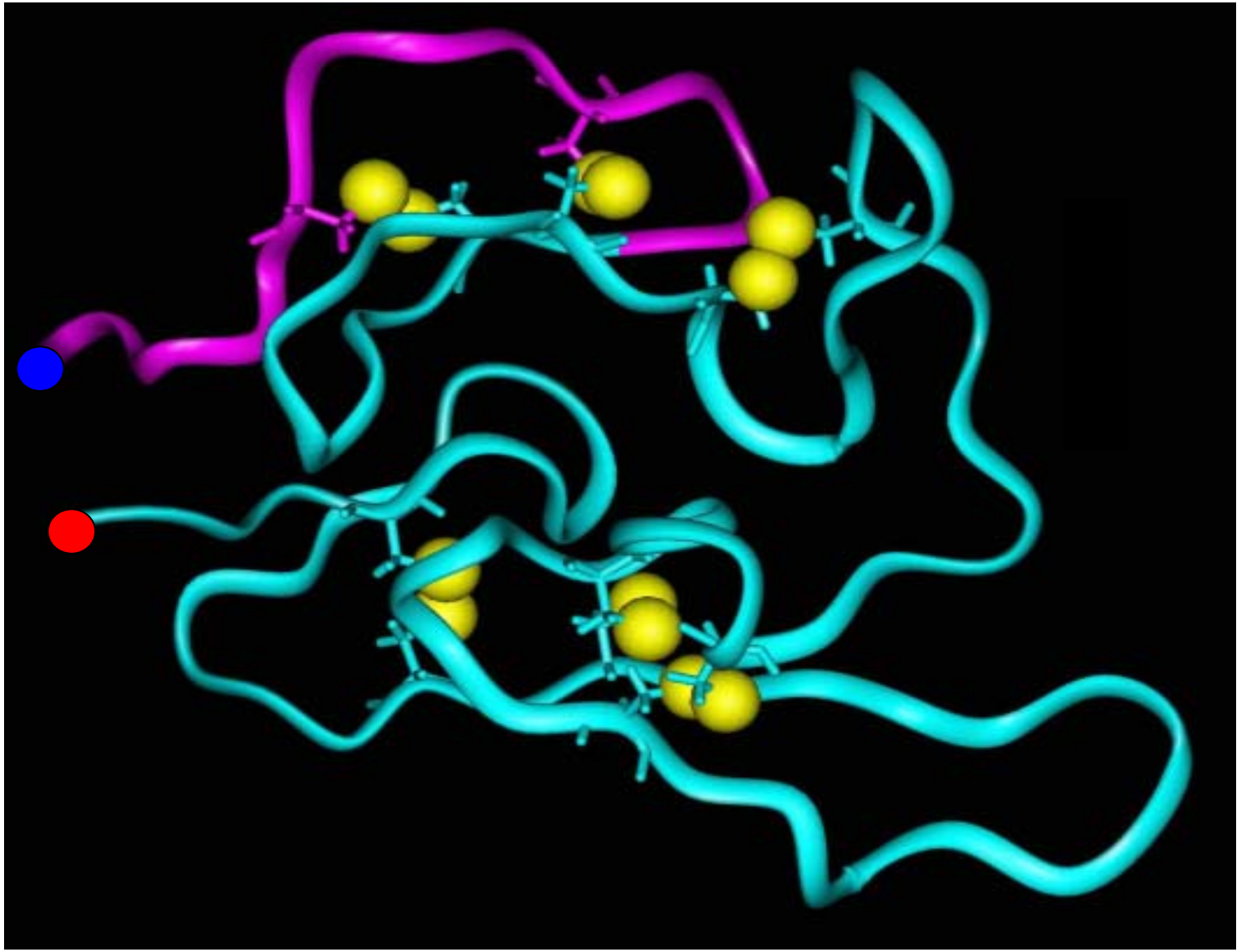
FIDIC

Espejo F., *et al.*, 2001, **Angew Chem Int Ed Engl**., 40: 4654



PfMSP-1_{19} and peptide 5501

Morgan W.D. *et al*., 1999



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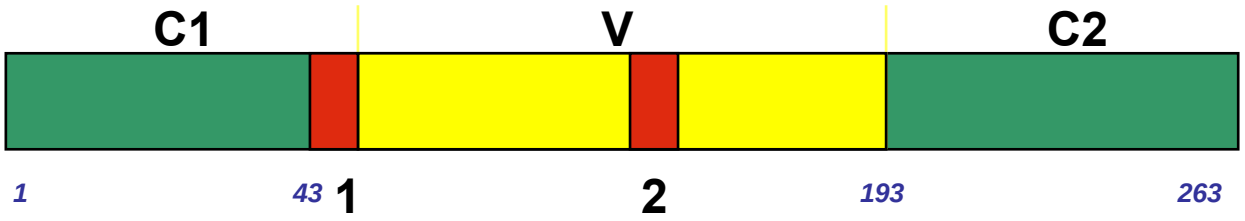
MSP-2



1. KNESKYSNTFINNAYNMSIR
2. INNAYNMSIRRSMAESKPPT
3. NPNHKNAETNPKGKGEVQKP

TT-SNTFINNA (*P. berghei*)

SNTFINNA AHA KNESKYSNTFINNAYNMSIRRSM (*P. y. yoeli*)

SNTFINNA CDC/NIIMALVAC-1[4]

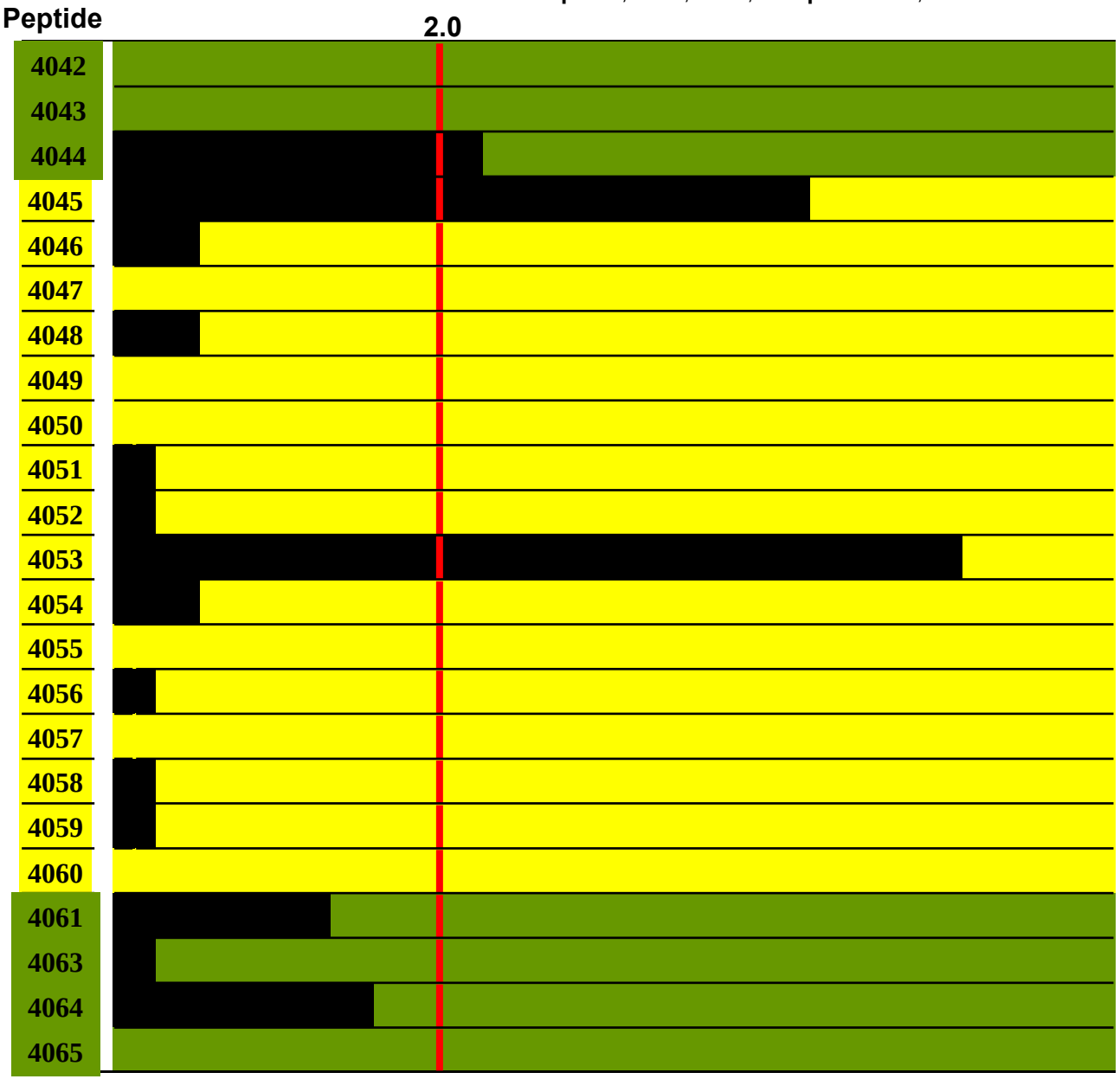
Pf MSP-2 has 45 kDa to 56 kDa.
•It has a highly polymorphic central region flanked by conserved regions.

•**PvMSP-2** (185 kDa) and **PkMSP-2** (105 kDa) are only related in terms of structure.
•Its function has not yet been determined.

Clark *et al*.,1989, Smythe *et al.,* 1988, Ocampo M., *et al*. 2000, Saul A. *et al.,* 1992, Pye D. *et al.,* 1997, Lougouskoi A. A.., *et al.,* 2000, Shi Y. P. *et al.,* 1999

MSP-2 peptide specific binding to erythrocytes.

Ocampo M., *et al.*, 2000, **J Peptide Res.**, 55: 216



Peptide	Kd (nM)
4044	140
4045	67
4053	230

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Invasion and development inhibition assays

Ocampo M., *et al.*, 2000, **J Peptide Res.**, 55: 216

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			Inhibition of invasion		Inhibition of development
		Peptide	Hypoxanthine % ± SD	Giemsa % ± SD	Hypoxanthine % ± SD
		4044	96 ± 12	n.d.	97 ± 20
High binding activity	200 μM each	4045	31 ± 1	n.d.	5 ± 33
		4053	95 ± 5	n.d.	90 ± 15
Mixture	66 μM each	ALL	67 ± 1	42 ± 1	n.d.
	7 μM each	ALL	31 ± 14	26 ± 1	n.d.
	1.3 μM each	ALL	0 ± 6	9 ± 1	n.d.
Low binding activity	200 μM each	4048	4 ± 14	n.d.	5 ± 33
		Chloroquine	100	100	100

The percentage of inhibition was calculated as being the difference between erythrocytes invaded in the control culture without peptide and erythrocytes invaded in the culture to which the peptide mixture had been added.

Structural features for HABPs MSA-2

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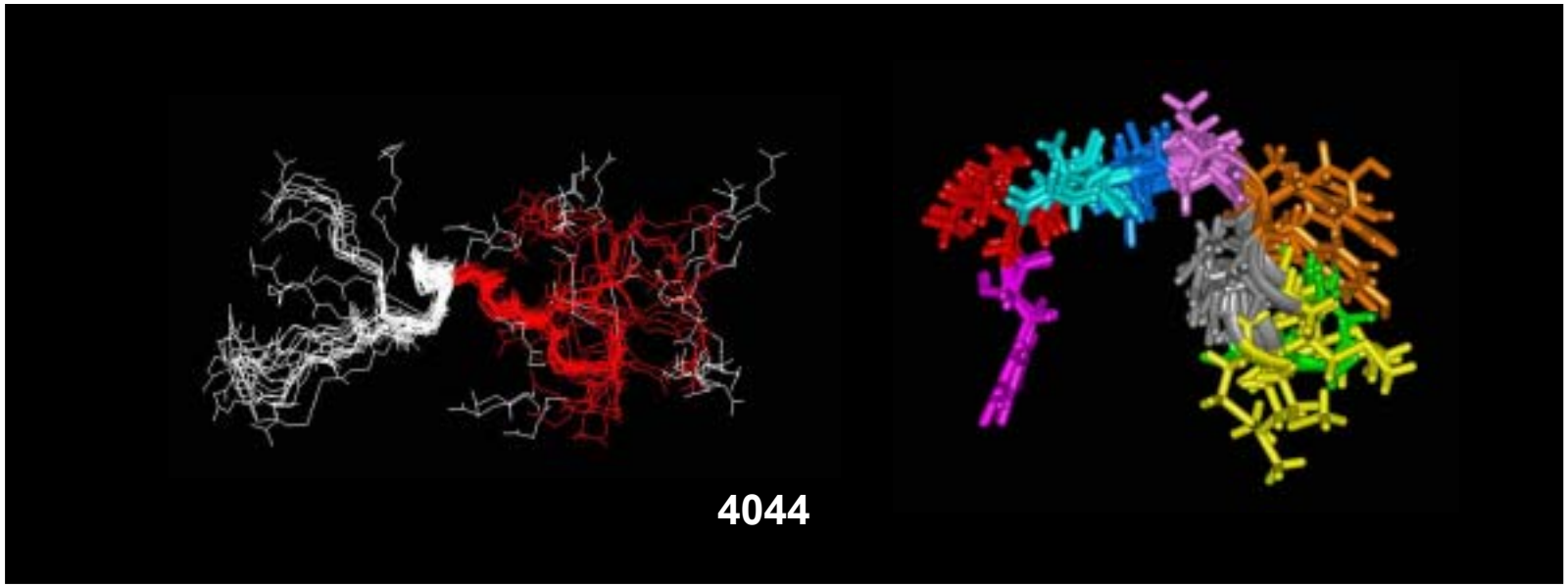
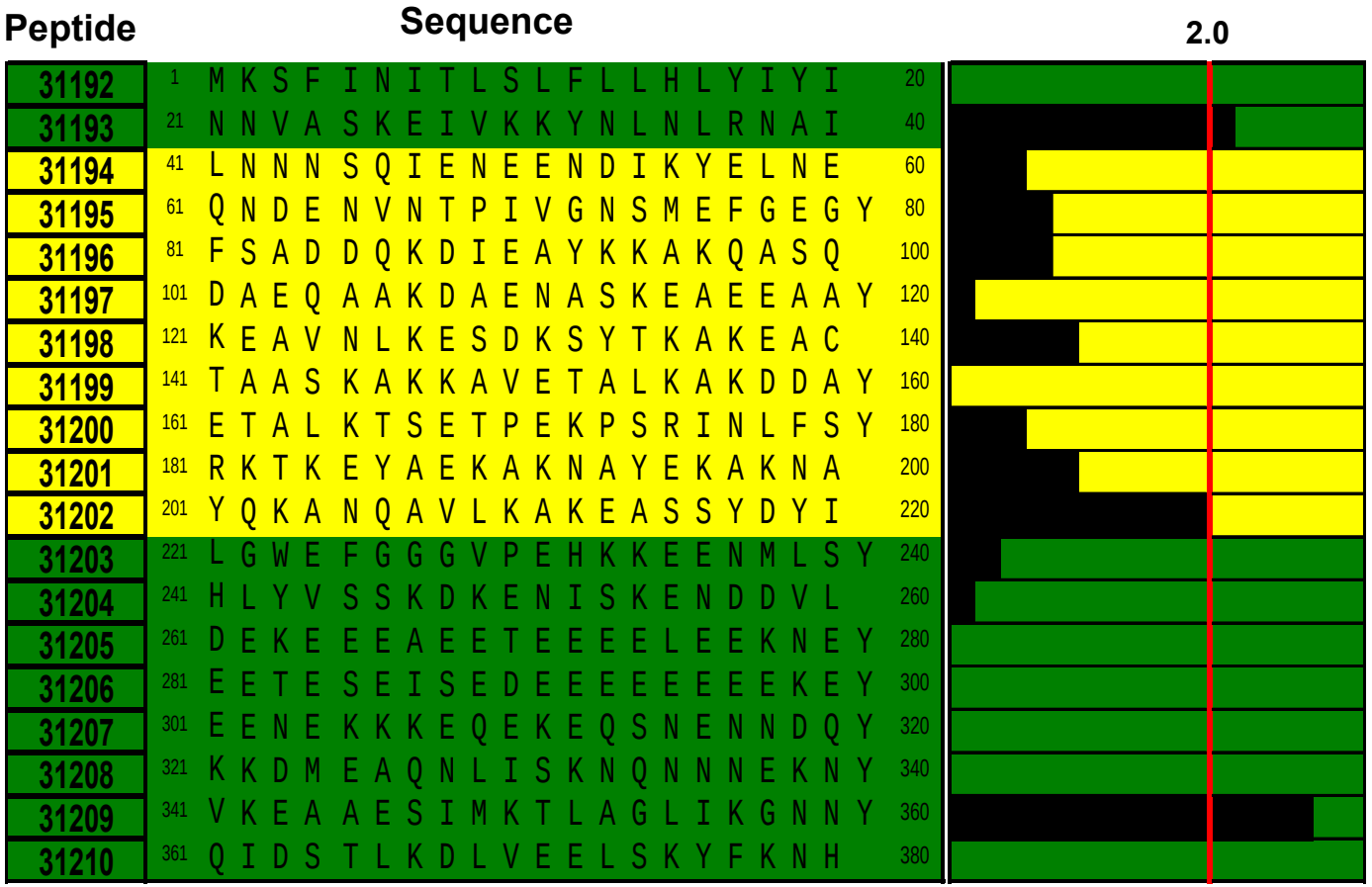
Cifuentes G., *et al*., 2003, **J Med Chem**., 46: 2250



MSP-3

Rodríguez L. E., *et al*., 2004, **submitted**



Peptide	Kd (nM)
31193	140
31202	260
31209	215

- **PfMSP-3** (48 kDa) is very polymorphic (it does not anchor to the membrane).
- It has 3 contiguous regions having alanine-rich repeat amino-acid motifs.
- It possibly includes α helices forming coiled-coils in its structure.
- **PvMSP-3s** shares its general structure but its sequence only shares 30% homology.
- Leucine ziper

Inhibition of parasite invasion to erythrocytes by MSP-3 peptides

Rodríguez L. E., *et al.*, **submitted**

Peptide	% Inhibition Invasion 200 µM [a]	100 µM [a]
31193	85 ± 2	8 ± 6
31202	59 ± 4	38 ± 1
31209	55 ± 1	2 ± 1
Control chloroquine	100 ± 1	
Control EGTA	100 ± 1	

[a] Mean ± standard deviation of three experiments.

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MSP-4

•This is a 40 kDa protein.
•It has an EGF-like domain towards the C-terminal.
•It has a low degree of diversity.

MSP-5

•This is a protein which is closely related to MSP4
•It is very conserved.
•It is present on the merozoite surface

MSP-7

•This is a 22 kDa precursor.
•It presents α helix and β sheet structural elements.
•Its proteolytic processing produces a 19 kDa polypeptide.

McColl & Anders 1994, Oeuvray *et al.,* 1994, Marshall *et al.,* 1997, Gardner *et al.,* 1998, Pearce *et al.,* 2004, Trucco C *et al*., 2001, Wu T. *et al*., 199 Goschnick *et al*., 2004, Przyborski J. M. *et al.*, 2003

MSP-6

Lopéz R., *et al.*, 2004, **submitted**

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Peptide		Sequence		2.0
31173	1	MNKIYNITFLFILLNLYINE	20	
31174	21	NNFIRNELINEKNHNLRNGSY	40	
31175	41	MYNNDKILSKNEVDTNIESN	60	
31176	61	ENSIHESGHKIDGEEVLKANY	80	
31177	81	VDDITYKKKNVDDSEIPFSG	100	
31178	101	YDIQATYQFPSTSGGNNVIP	120	
31179	121	LPIKQSGENQYTVTSISGIQ	140	
31180	141	KGANGLTGATENITQVVQANY	160	
31181	161	SETNKNPTSHSNSTTTSLNNY	180	
31182	181	NILGWEFGGGAPQNGAAEDKY	200	
31183	201	KTEYLLEQIKIPSWDRNNIP	220	
31184	221	DENEQVIEDPQEDNKDEDEDY	240	
31185	241	EETETENLETEDDNNEEIEEY	260	
31186	261	NEEDDIDEESVEEKEEEEEKY	280	
31187	281	KEEEEKKEEKKEEKKPDNEIY	300	
31188	301	TNEVKEEQKYSSPSDINAQN	320	
31189	321	LISNKNKKNDETKKTAENIVY	340	
31190	341	KTLVGLFNEKNEIDSTINNLY	360	
31191	361	EIDSTINNLVQEMIHLFSNNY	380	

Peptide	Kd (nM)
31175	230
31178	200
31191	150

- This is a 36 kDa protein.
- It forms a complex with MSP1 and MSP-7
- It has 85% similarity with MSP3 in the C-terminal region.

MSP-8

Puentes A., *et al.*, 2003, **Peptides**, 24: 1015

FIDIC

Peptide		Sequence		2.0
26348	1	M V F K S S D I F F F L F L V I L Y F N	20	
26349	21	N V V E G E N G T T N I E N N P G N N G Y	40	
26350	41	N M G P S G P K D K D K N I E K D V N H Y	60	
26351	61	N M S M N N N N N N N N N N D N N N N I N Y	80	
26352	81	N N N N N N I N N N N N N N N N N N G N G Y	100	
26353	101	F S N F F N K L F G K K K D N K K E G E Y	120	
26354	121	E K N E E D L N S N K N I E S N K G S A Y	140	
26355	141	V T S N V G D T N N D A K A R D N N N N N Y	160	
26356	161	D D N D D N D E N D D N D D N D D I D E Y	180	
26357	181	I D E R D D N D D N G D D N D D N G D D Y	200	
26358	201	D D D N D D D D D N N D N N N K N N S N Y	220	
26359	221	N L T D T K K E G E K I D L G V Q N K K Y	240	
26360	241	Q N I F S T N N K G L N K Y N I D N E L	260	
26361	261	K E V D A L L K N D N Y I L N K Y H V S	280	
26362	281	F F N N F E E D T Y N K K K F I R P Y D	300	
26363	301	L S L L K S I L I Y R Q R V T R N C V N	320	
26364	321	V F Q D L N A V F G K C Y N K D D T K L	340	
26365	341	S I T R D K V K K E L S R K N R N F V E Y	360	
26366	361	Y L I E M L E N T L N S M N D D F I N K	380	
26367	381	D N F D L N N Y V K E F E L I N Y L L I	400	
26368	401	H E D S D I F L E T Y N L I S G L N S N	420	
26369	421	I E E T S I E K L K Y A I L Q G K Q I N	440	
26370	441	Y K I K D D I Y Y I L K N A Y A K Y F K	460	
26371	461	I D V Y K K G K L L Y P T L Y Y H R N A	480	
26372	481	F I K S F V V E F F N N N K V C E N T K Y	500	
26373	501	C P L N S N C Y V I D D E E T C R C L P	520	
26374	521	G F N N I K I D D E M N C V R D D T L D Y	540	
26375	541	C S R N N G G C D I H A K C S F I N K Q Y	560	
26376	561	I V C E C K D K F E G D G I Y C S Y S F	580	
26377	581	F S S I H N F I F F F I L C L F I F I L Y	600	

Peptide	Kd (nM)
26360	800
26361	500
26368	550
26369	650
26373	450

- This is a 70 kDa protein.
- It has 2 EGF-like domains towards the C-terminal.
- It has a low degree of diversity.
- It forms a complex with MSP1, MSP4 and MSP5

Inhibition of merozoite invasion of human erythrocytes by MSP-8 HABPs

FIDIC

Puentes A., *et al.*, 2003, **Peptides**, 24: 1015

Activity binding	Peptide	Invasion Inhibition (%)[a]	
		200 μM	100 μM
High	26360	33 ± 7	11 ± 2
	26361	98 ± 1	20 ± 5
	26368	93 ± 1	9 ± 3
	26369	32 ± 2	10 ± 1
	26373	23 ± 3	10 ± 4
Low	26348	14 ± 2	4 ± 2
Control	2220	83 ± 7	13 ± 1
Chloroquine (0.5 mg/ml)		96 ± 1	
EGTA (50 mM)		100 ± 7	

[a] Mean ± S.D. from three experiments.

MSP-10

Puentes A., *et al.*, 2004, **Biochimie**, In Press

FIDIC

Peptide	Sequence			2.0
31111	1	M F F K C N Q V F T L V F L L L L Y F N	20	
31112	21	N I V Y T H V D D I K N T S Q K K I T Y	40	
31113	41	D K Y N K N K E N M N N E K N D N K D N	60	
31114	61	K D N I Y N D N I N N D N I N N D N I N	80	
31115	81	N E D E Y K F L S M K H Y K D S L S N K	100	
31116	101	L N N E N D H M N Y L I R K R K D N T Q	120	
31117	121	G S Q H F N E N I E N N E N V E N N E N Y	140	
31118	141	I E N N E N N E N I E N I E N N E N N E Y	160	
31119	161	N N E N I E N N E N N E N N E N S S I M Y	180	
31120	181	N S E S Y N N I I N S N E H N E E Q I K	200	
31121	201	K K E E D L I E A F F P F I L K K L D N Y	220	
31122	221	E S L S L D N K Y D D Y Y N L P N D H N	240	
31123	241	D T H K E N S S D H N L L G Y K L G N N	260	
31124	261	L K S Y L I E E N D V S Q K K T D D I N	280	
31125	281	E S A S S D S E N I Q E I L S T D S N T Y	300	
31126	301	S H L K E R K N Q K A P P G E H K P E V Y	320	
31127	321	K N A L L N S Q V A S P K G E D E K K S Y	340	
31128	341	Q P Q H P L V N S G D Q L Q H P K E I D Y	360	
31129	361	E N A E K I R R T L L K E S R D I K N T Y	380	
31130	381	T A I I D E T V Y K F E Q L I M K G R Y	400	
31131	401	Y A T A V R N F V I F K V N Y I C E Y S	420	
31132	421	K C G P N S R C Y I V E K D K E Q C R C	440	
31133	441	R P N Y I V D M S V N Y F K C I P M K D	460	
31134	461	M N C S K N N G G C D V N A E C T I V E Y	480	
31135	481	G A V K C Q C S H L Y F G D G V F C V K	500	
31136	501	N S Q T K Q T L Y I L F I V I L L V F Q	520	
31137	505	K Q T L Y I L F I V I L L V F Q N F F I	524	

Peptide	Kd (nM)
31121	250
31122	130
31132	600

- This is a 61.2 kDa protein.
- Its proteolytic processing produces a 36 kDa polypeptide.
- It presents an ASN-rich region close to the N-terminal.

Inhibition of merozoite invasion of human erythrocytes by MSP-10 HABPs

FIDIC

Puentes A., *et al.*, 2004, **Biochimie**, In Press

Activity binding	Peptide	Invasion Inhibition (%) [a]	
		(200 µM)	(100 µM)
High	31121	70 ± 1	3 ± 1
	31122	45 ± 2	5 ± 1
	31132	65 ± 2	16 ± 2
Low	31120	19 ± 1	7 ± 1
Chloroquine (0.3 µg/ml)		100 ± 1	
EGTA (12 mM)		100 ± 3	

[a] Mean ± SD from three experiments.

Superposition of domains EGF-like and HABP of proteins MSP-10, MSP-8 and MSP-1.

Puentes A., *et al.*, 2004, **Biochimie**, In Press

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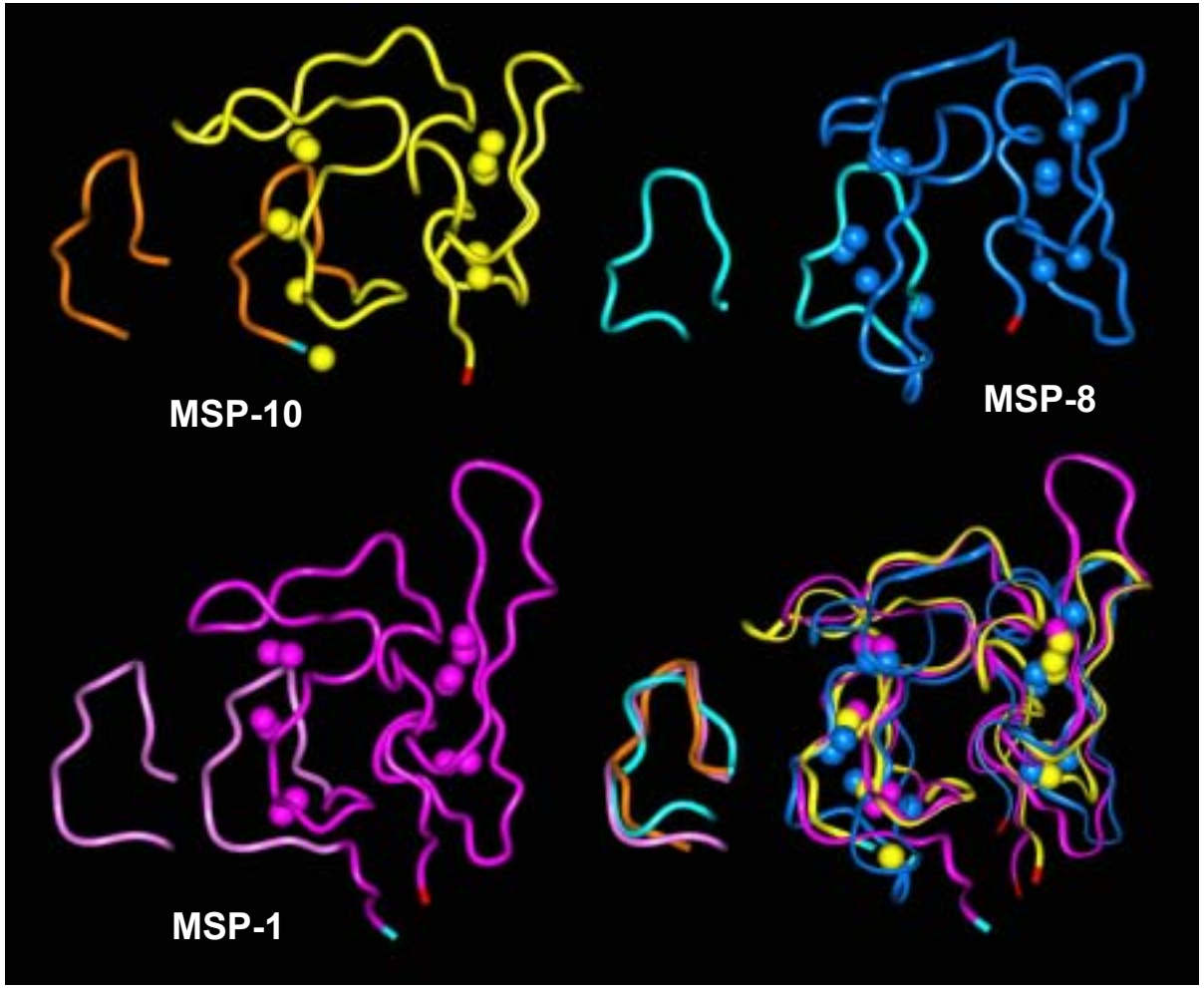
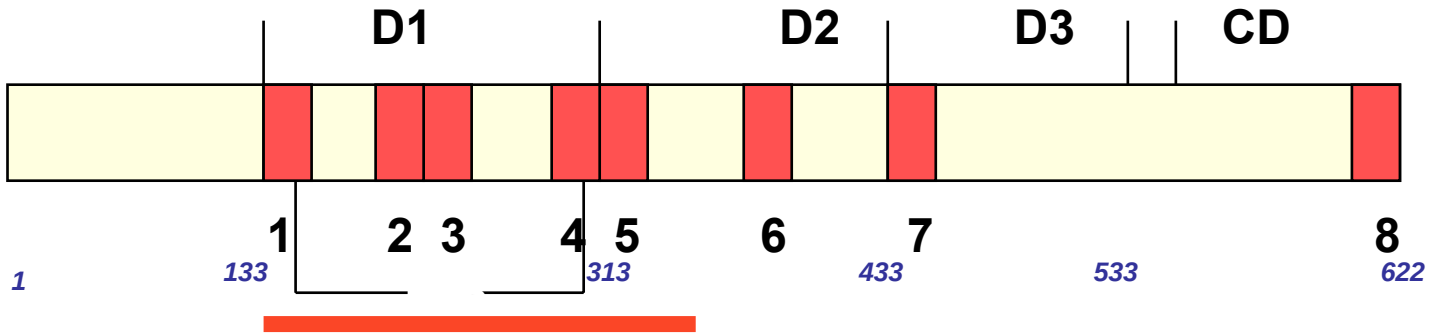
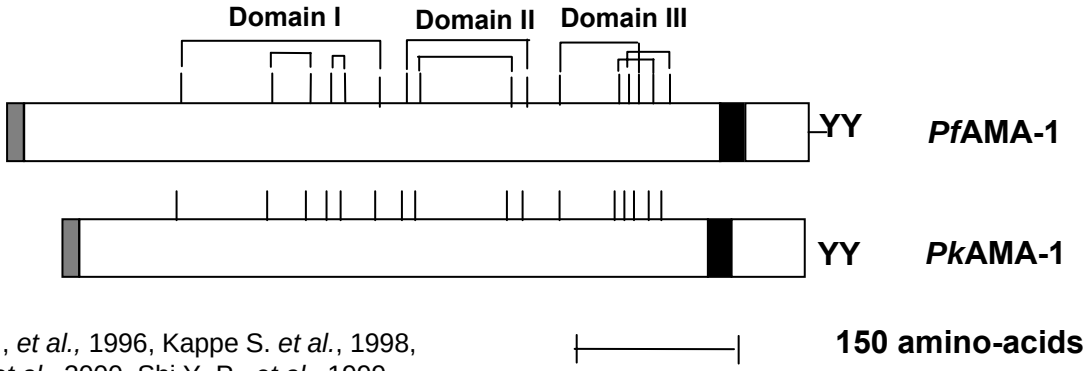


AMA-1



1. DAEVAGTQYRLPSGKCPVFG[3]
2. QYLKDGGFAFPPTEPPLMSPM
3. TLDEMRHFYKDNKVVKNLD
4. VVDNWEKVCPRKNLQNAKF
5. LWVDGNCEDIPHVNETSAID
6. MIKSAFLPTGAFKADRYKSH
7. PIEVEHNFPCSLYKNEIMKE
8. WGEEKRASHTTPVLMEKPYY

LWV DGNCEDIPHVNETSAID L



Signal peptide

Transmembrane domain

Cystein residues & bonds

Hodder A. N., *et al.,* 1996, Kappe S. *et al.*, 1998, Urquiza M., *et al*., 2000, Shi Y. P., *et al.,* 1999.

Apical Membrane Antigen-1 family

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•Anti AMA-1 Mab Fab fragments inhibit *P. knowlesi in vitro* invasion.

•The gene has been cloned and characterised in:
-*P. falciparum* 83/62 kDa

•It is initially localised in the neck of the rhoptries but becomes re-localised on merozoite liberation.

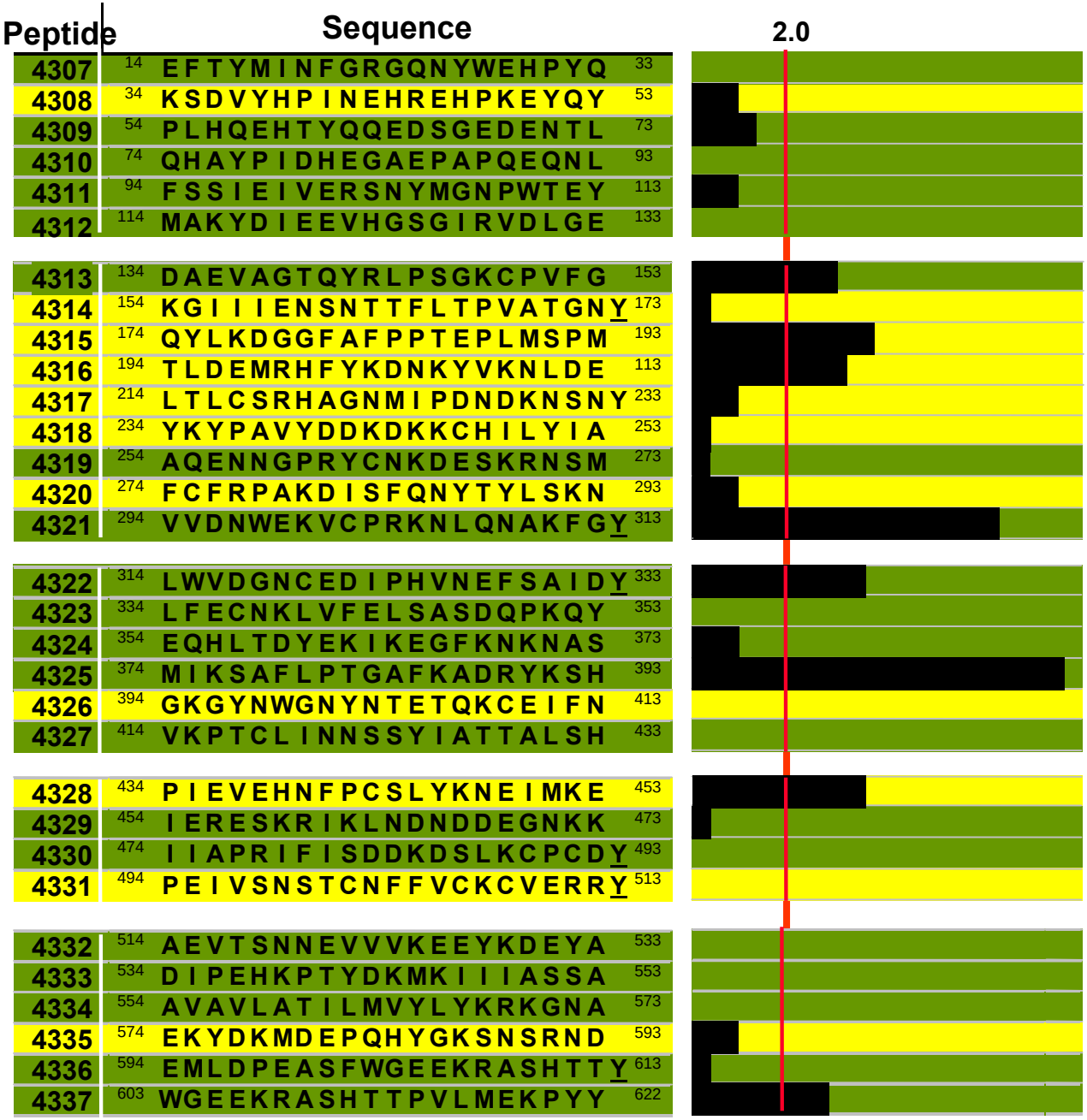
•It has 16 conserved extra-cellular cysteins and 3 domains linked by disulphur bridges.

•It has been detected in advanced ring stages.

Anders R. F., *et al*., 1998, Thomas A. W., *et al*., 1984

AMA-1

Urquiza M., *et al.*, 2001, **Vaccine**, 19: 508



Peptide	Kd (nM)
4313	120 ± 12
4315	120 ± 10
4316	150 ± 14
4322	700 ± 21
4325	100 ± 09
4328	140 ± 11

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Structural features for HABP 4325 AMA-1

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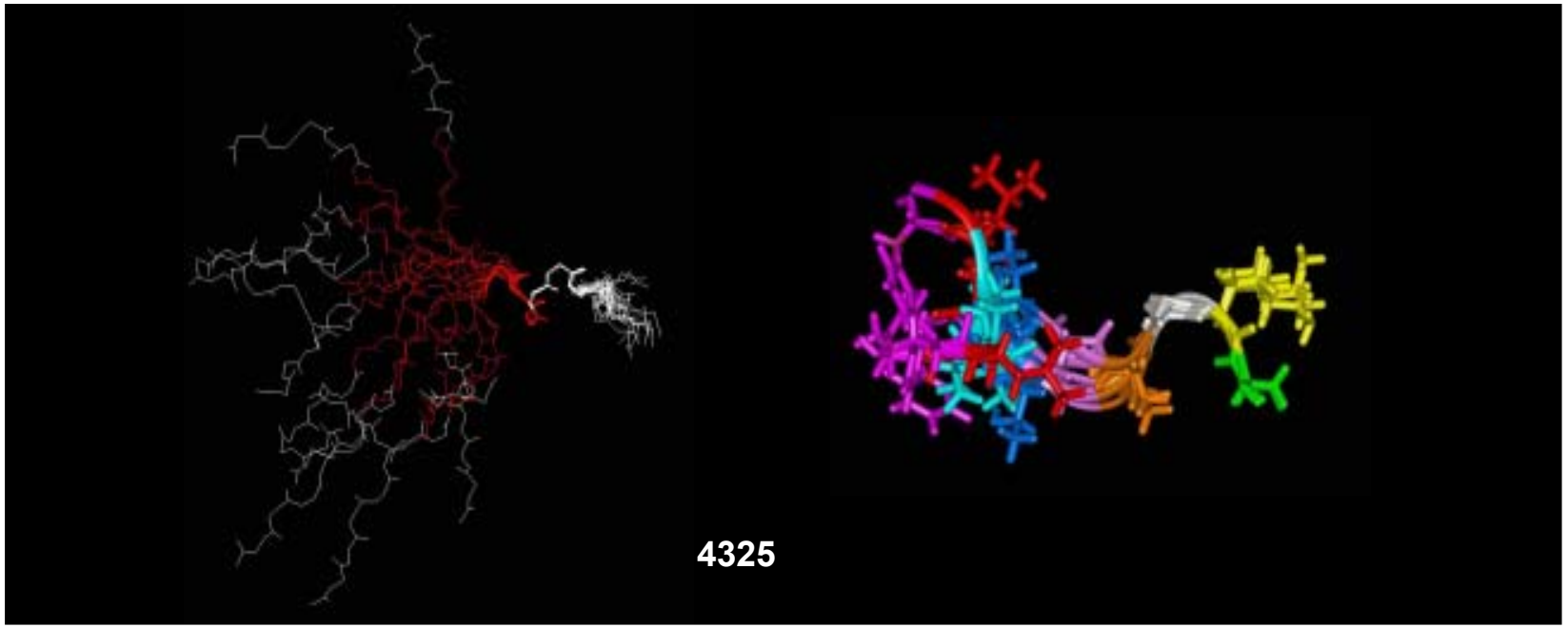
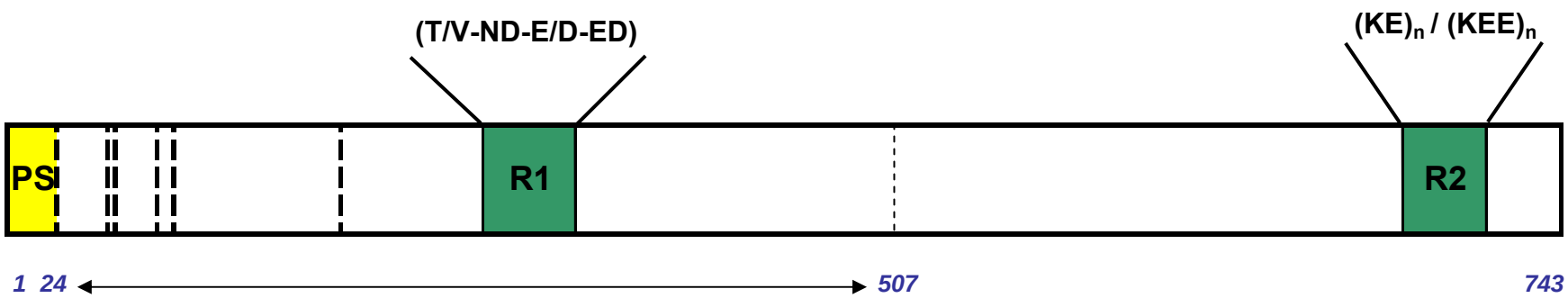
Cubillos M., *et al*., 2002, **Biochimie**, 84: 1181



ABRA



Chymotrypsin protease activity

Localisation:
- Immunes Merozoite Clusters (IMC)
- Schizonts
- Schizont surface in vacuole
- Merozoite-free surfaces
- Culture medium

ABRA is a conserved protein

-Its theoretical weight is 87 kDa
-SDS-PAGE: 101 kDa

Lyon *et al.*, 1986, Stahl *et al*., 1986,
Chulay *et a*l., 1987, Weber *et al*., 1988

ABRA

Curtidor H., *et al*., 2001, **Vaccine**, 19: 4496

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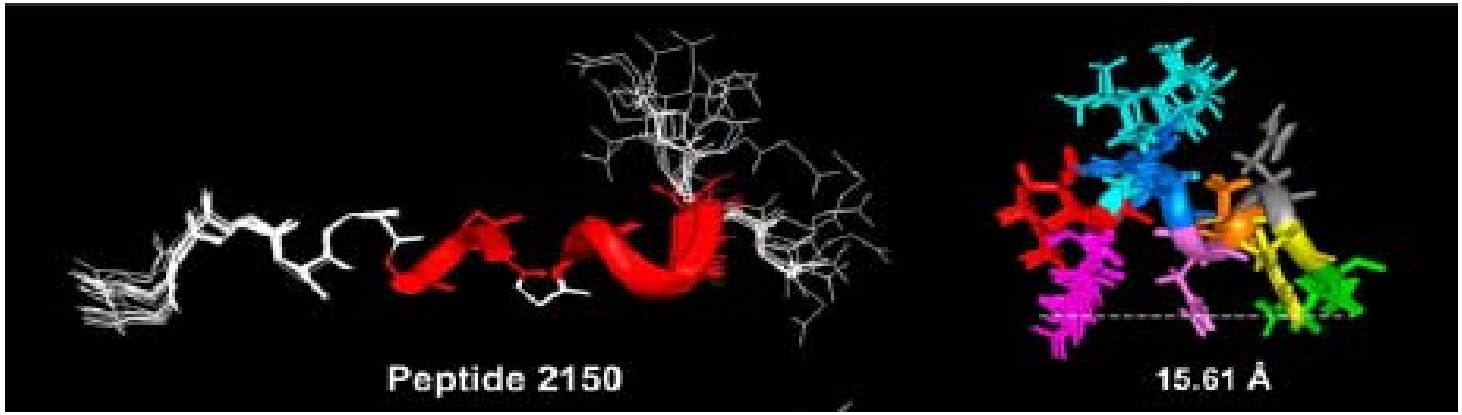
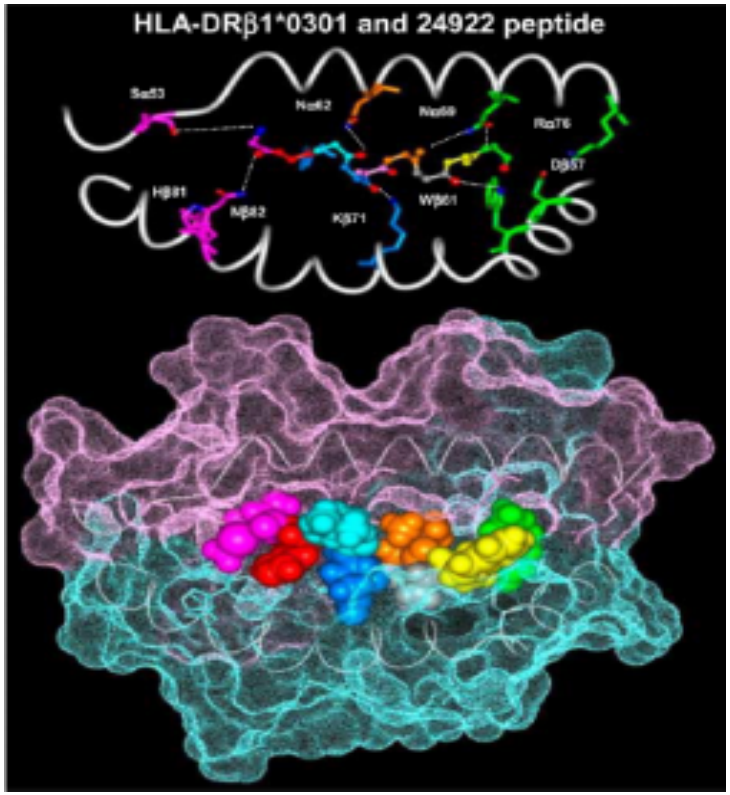
Peptide	Kd (nM)
2148	175
2149	73
2150	80

Structural features for 2150 HABP ABRA

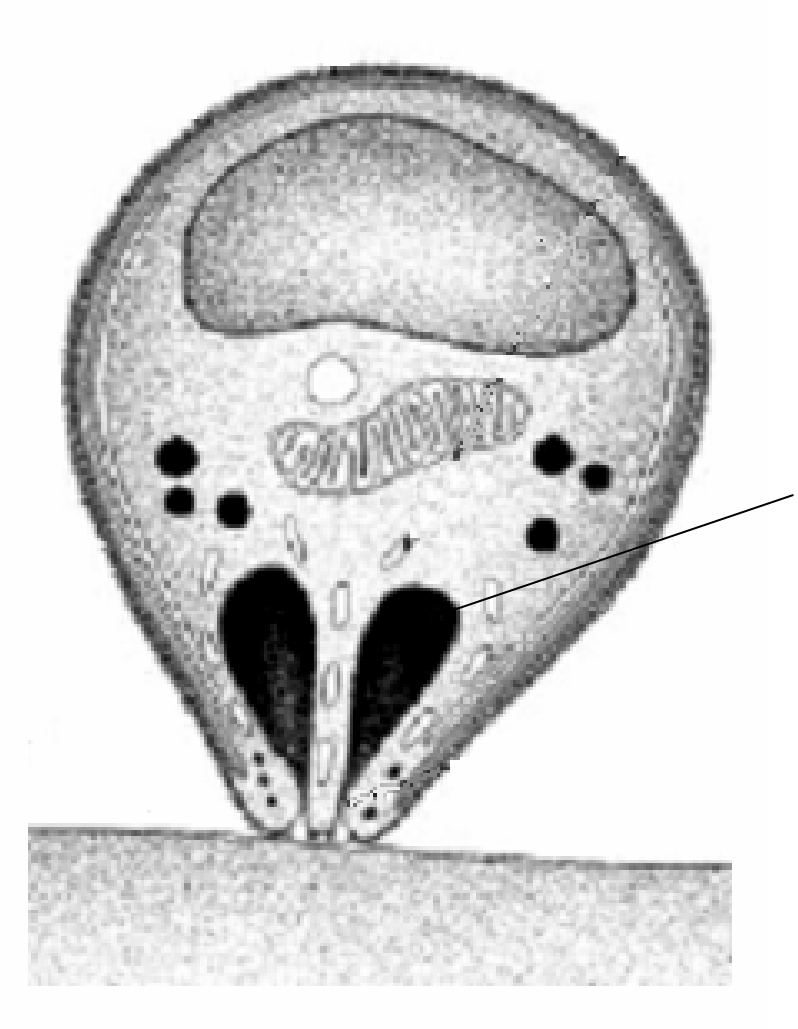
Salazar L. M., *et al*., 2004, **BBRC**, 322: 119

FIDIC





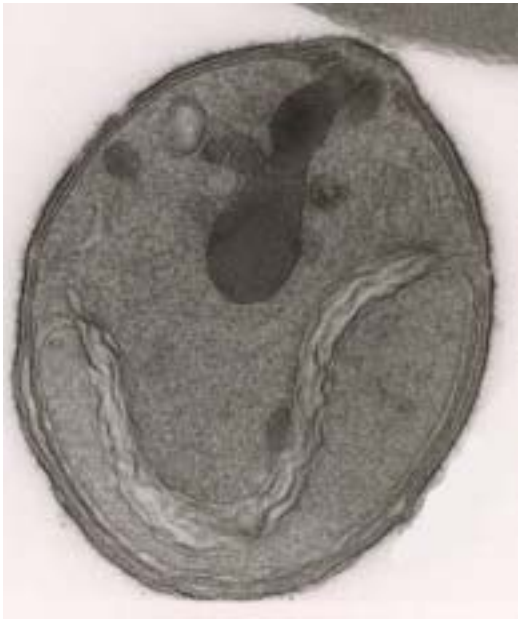
Rhoptry proteins



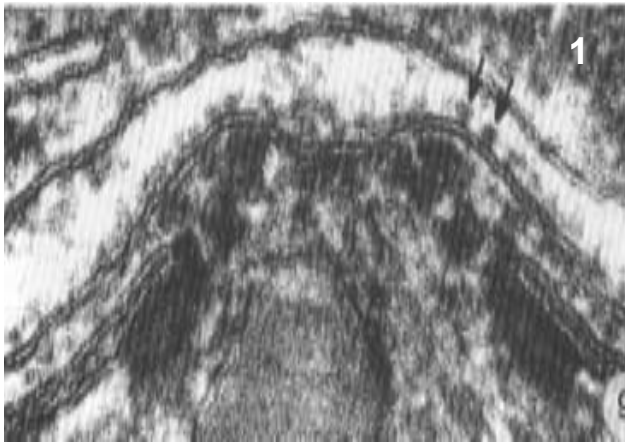
- Rhop-1
- Rhop-2
- Rhop-3
- RAP-1
- RAP_2
- RAP-3
- RAMA
- P225
- Serine protease
- AMA-1
- Pf60.1
- P52
- MCP-1
- MAEBL

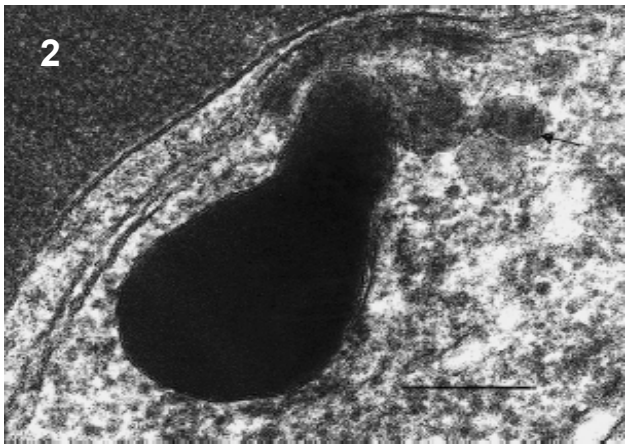
Bannister L. H., *et al*, 1977, Cowman A. F., *et al*, 2000
Sherman I. W., 1998, Cowman and Crabb, 2002
Chitnis and Blackman, 2000

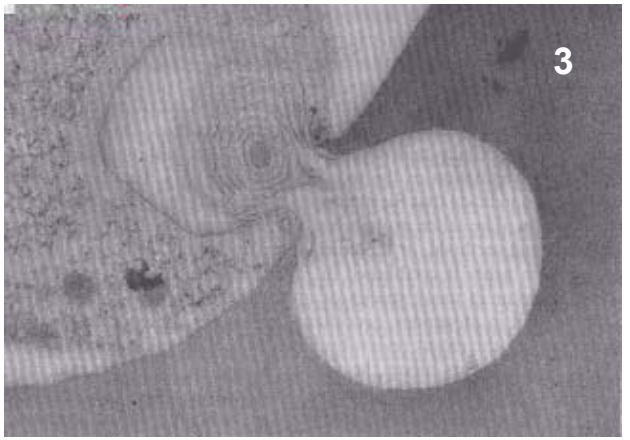
FIDIC



- Apical prominence of a merozoite interacting with red blood cell membrane.
- Rhoptry in a developing *P falciparum* merozoite, showing the basal bulb and the duct placed within the apical prominence.
- Merozoites incubated with red cells in the presence of cytochalasin, showing laminar material in rhoptries.







Preiser P. *et al*., 2000

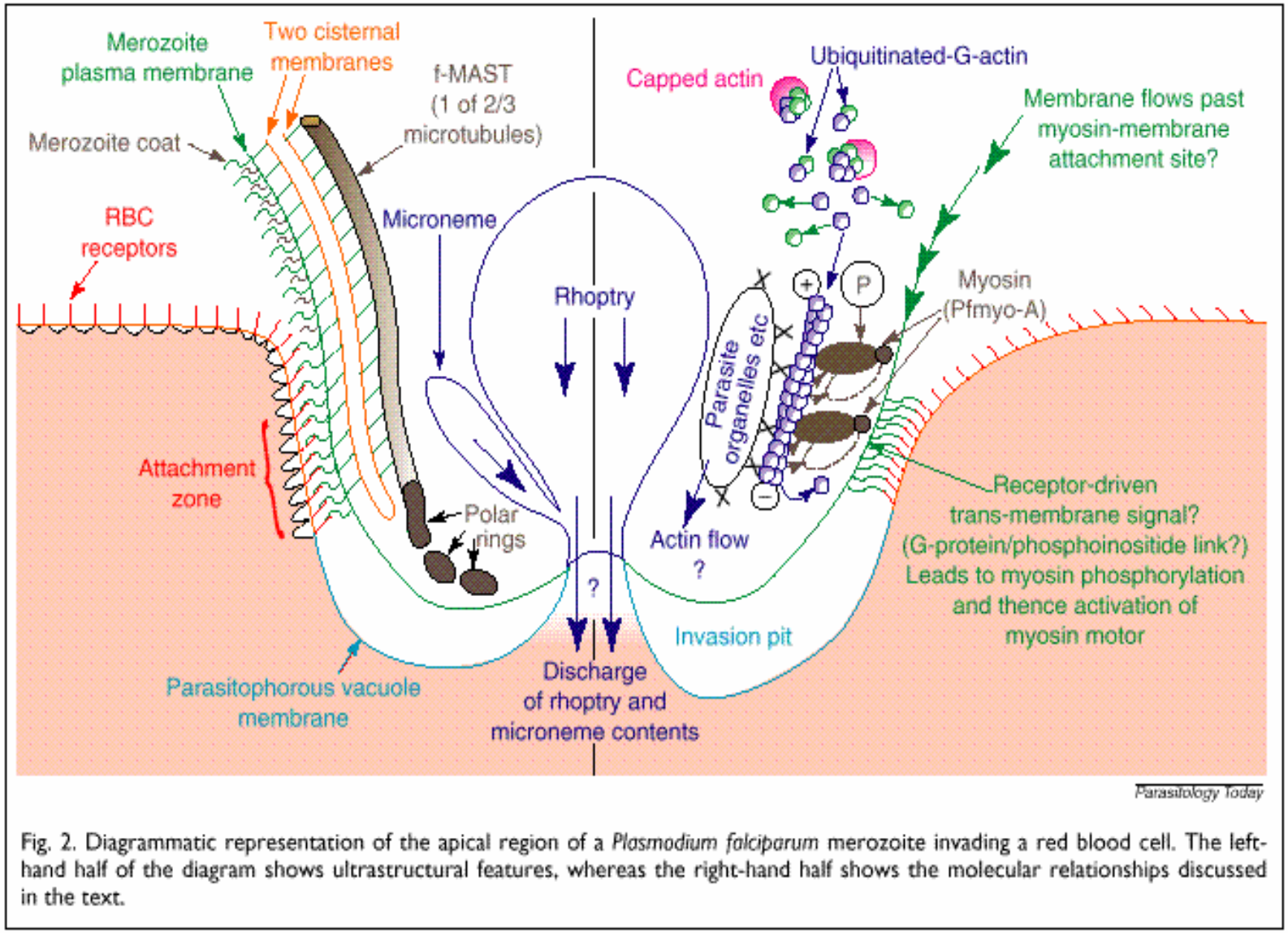
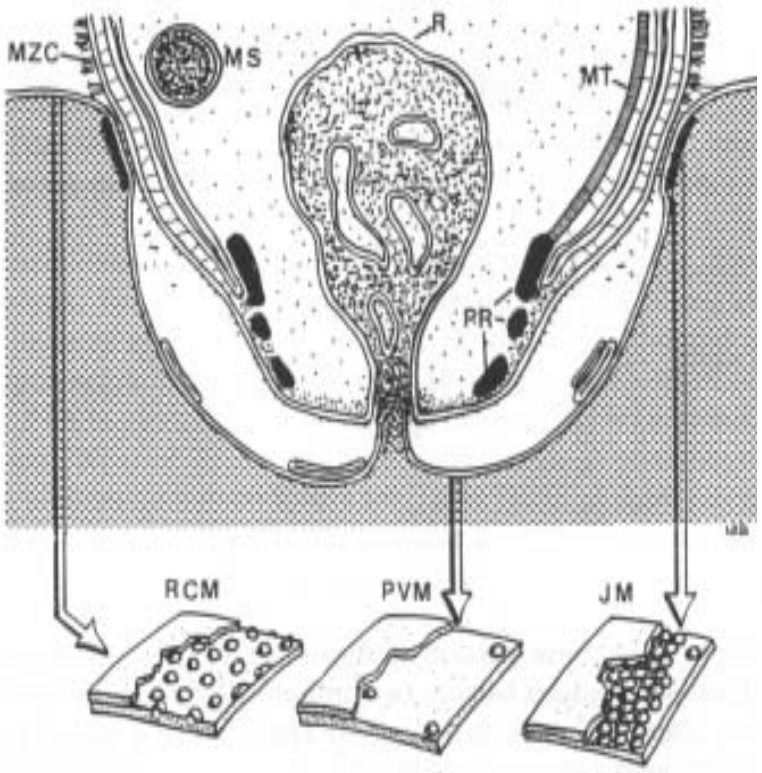
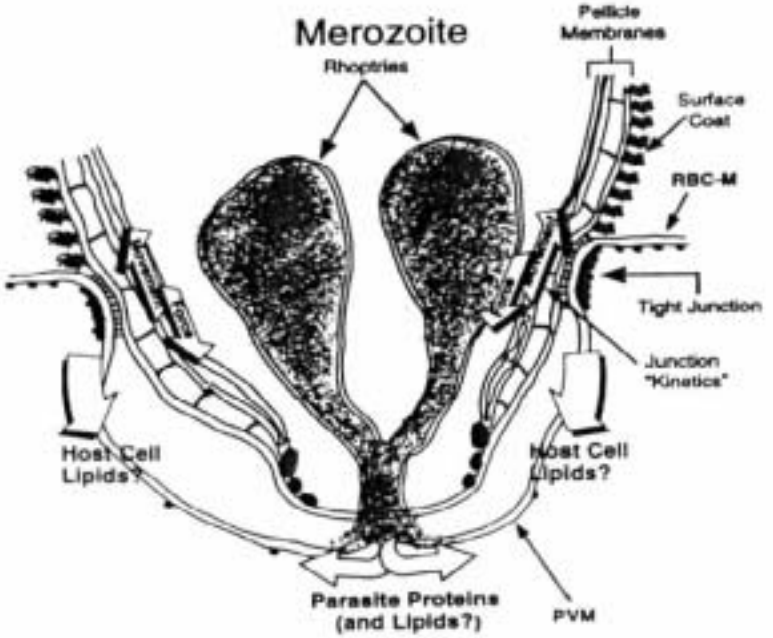


Fig. 2. Diagrammatic representation of the apical region of a *Plasmodium falciparum* merozoite invading a red blood cell. The left-hand half of the diagram shows ultrastructural features, whereas the right-hand half shows the molecular relationships discussed in the text.

Pinder J. *et al*., 2000





FIDIC

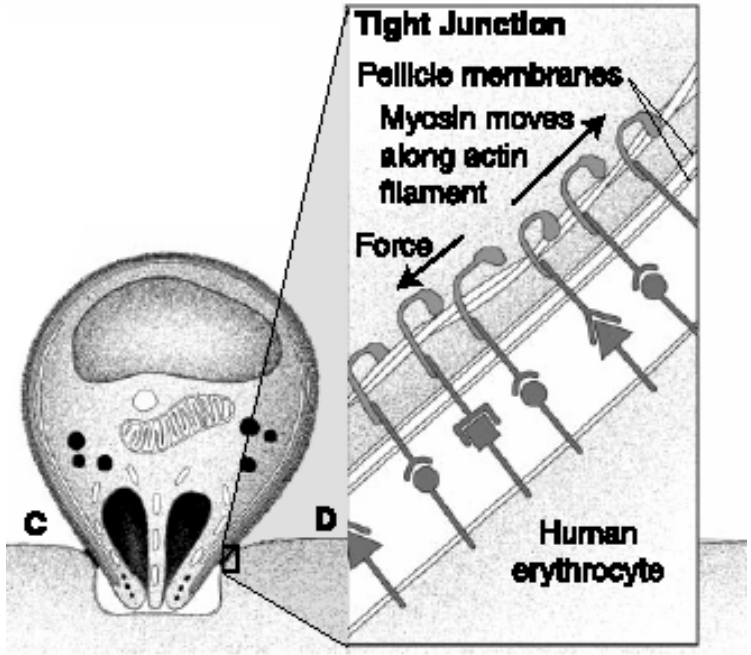


Diagram depicting the structures involved in the formation of the invasion pit and parasitophorous vacuole membrane. PVM; parasitophorous vacuole membrane, JM; close junctional membrane, RCM; external red cell membrane.

Bannister L. H., *et al*, 1977, Cowman A. F., *et al*, 200
Sherman I. W., 1998, Cowman and Crabb, 2002
Chitnis and Blackman, 2000, A. J. Knell, 1991

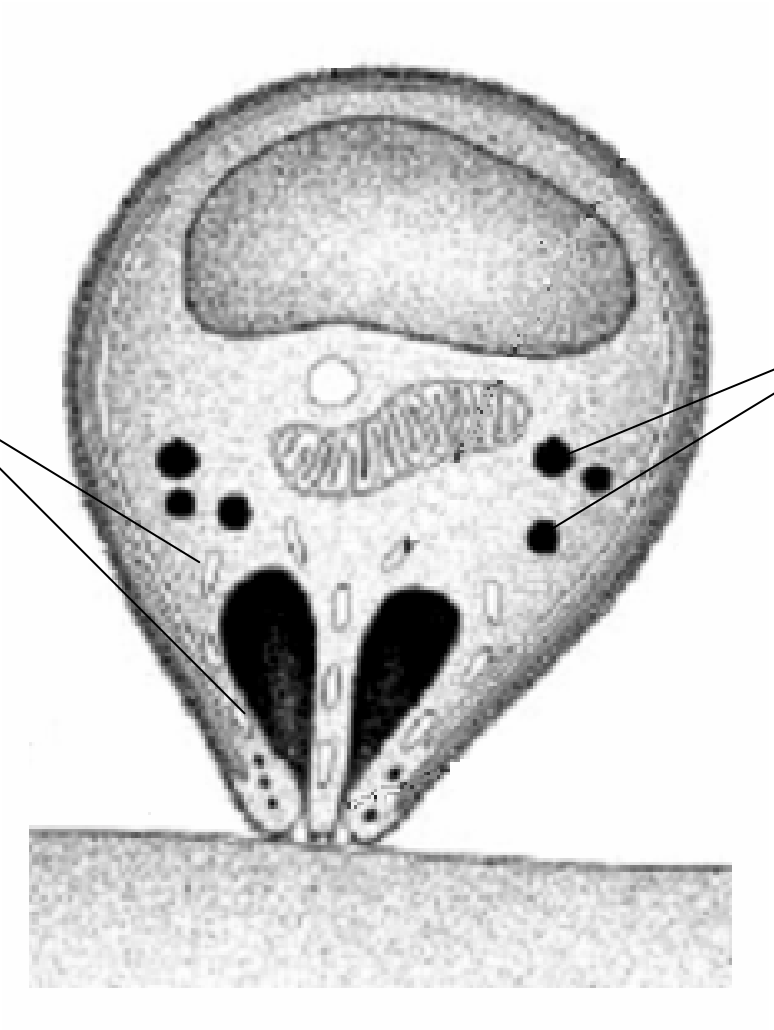
Rhoptry proteins

FIDIC

Protein		Size	Characteristics
Rhop complex	Ag 225	240/225	Integral membrane protein?
	Rhop 1	140(150)	RBC binding protein
	Rhop 2	130(140)	RBC binding protein
	Rhop 3	110/100(105)	RBC binding protein
RAP-I / QF3 / p82		86pr	Associated with RBC membrane
		82/80	-
		65/60	-
RAP-2		40 (37/39)	-
RAMA		170	RBC binding protein
Serine protease		80/76	Serine protease / anchored by GPI
Pf83 / AMA-I		80/66	Integral membrane protein
Pf60.1		60	RBC binding protein?
MCP-I		60	Oxydoreductase domain
		55	Integral membrane protein

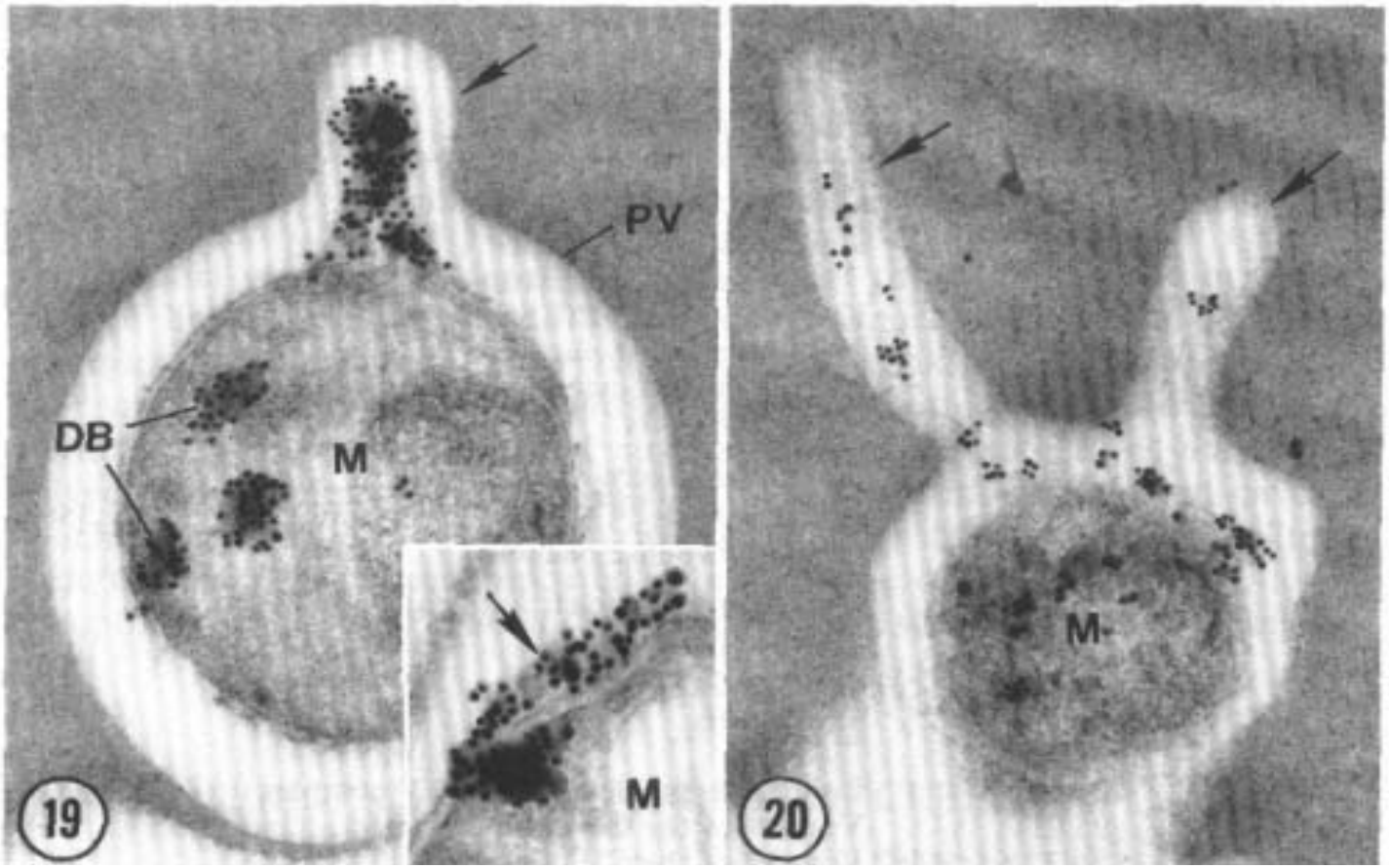
Cooper *et al.*, 1988, Holder *et al.*,1985, Bushell *et al.*,1988

Apical organelle proteins



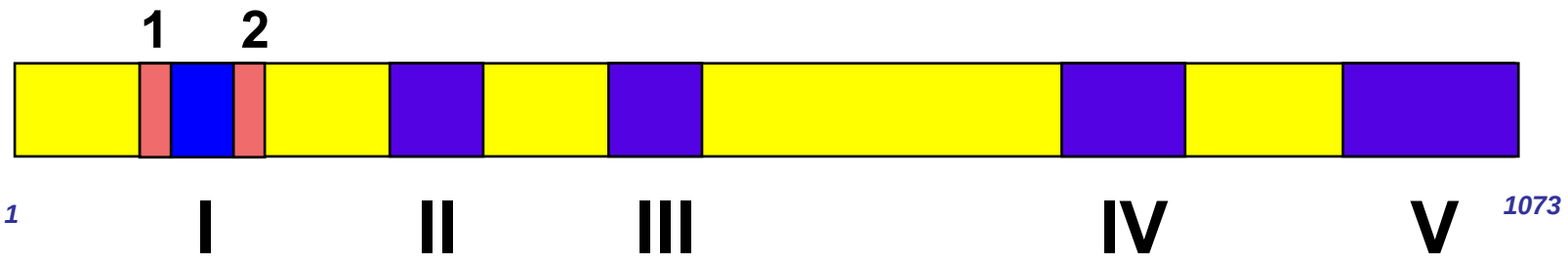
Bannister L. H., *et al*, 1977, Cowman A. F., *et al*, 2000
Sherman I. W., 1998, Cowman and Crabb, 2002
Chitnis and Blackman, 2000

Dense bodies



White-embedded erythrocyte with a recently invaded merozoite (M). Section was labelled with a rabbit antibody specific for the contents of dense bodies (DB) and immunoglobuli-gold. PV; parasitophorous vacuole membrane. Inset: higher magnification of the merozoite showing release of dense body contents (arrow) through the merozoite pellicle.

RESA



I- erythrocyte binding sequences
II and V- repeat sequence regions
III- thermal stabiliser regions
IV- spectrin binding region

Peptide	Sequence
1. 6671	MTDVNRYRYSNNYEAIPHIS
2. 6673	LGRSGGDIIKKMQTLWDEIM

LGRSGGIIKKM QTLWDEIM DINKRK

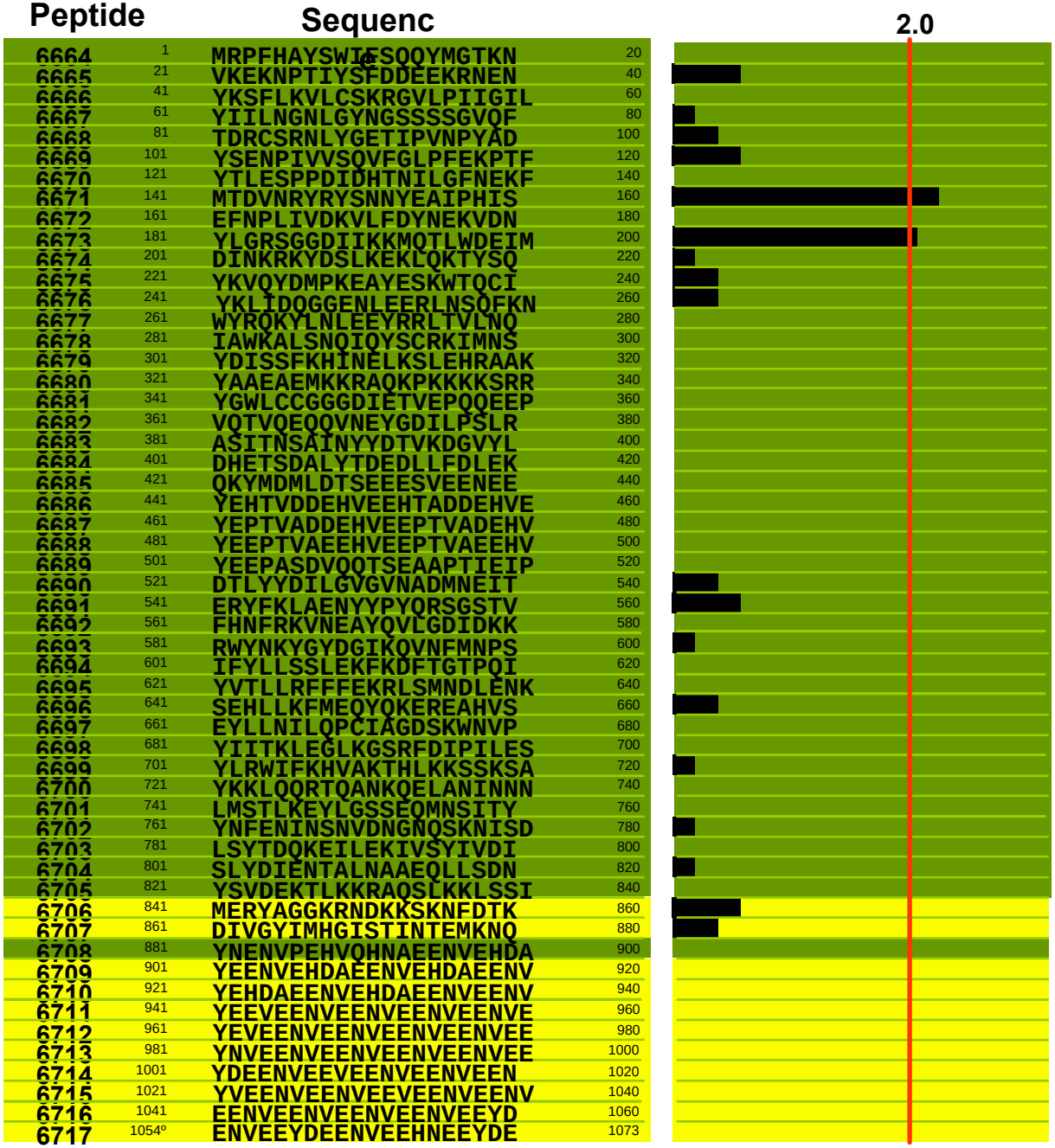
RESA is associated with erythrocyte cytoskeleton after being liberated.

RIMA (14 kD) localised on early trophozoite membrane.

Aikawa *et al*, 1990, Culvenor *et al*, 1991,
Trager W *et al*, 1992, Kulane A. *et al.,* 1997

Pf155/RESA

Vera R., *et al.*, 2000, **Vaccine**, 18: 1289



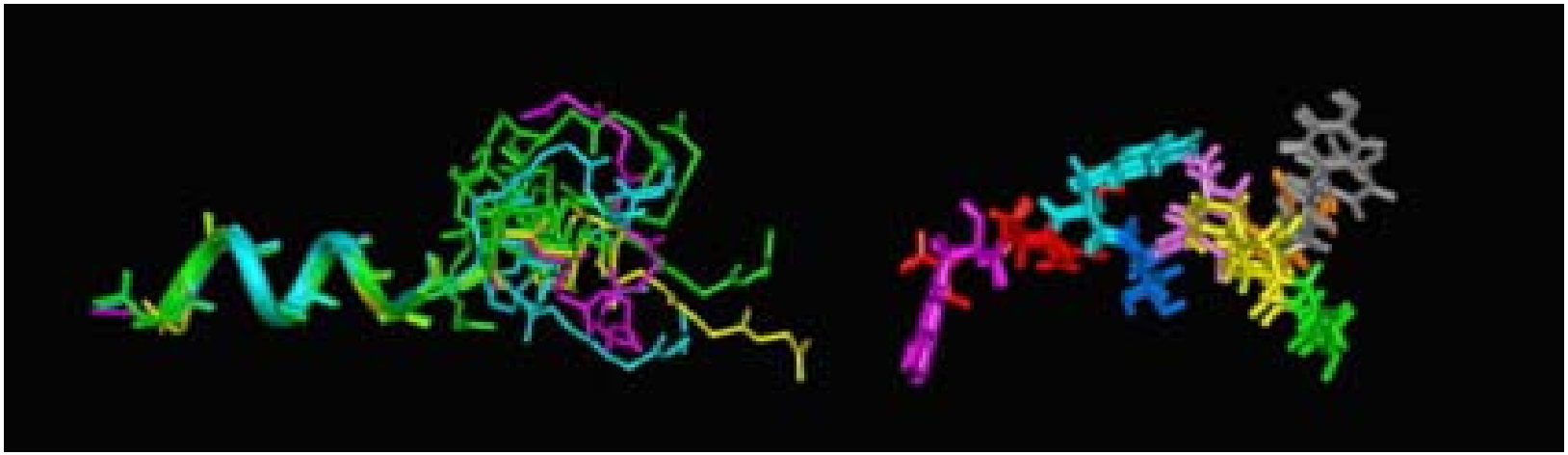
FIDIC

Peptide	Kd (nM)
6671	190
6673	105

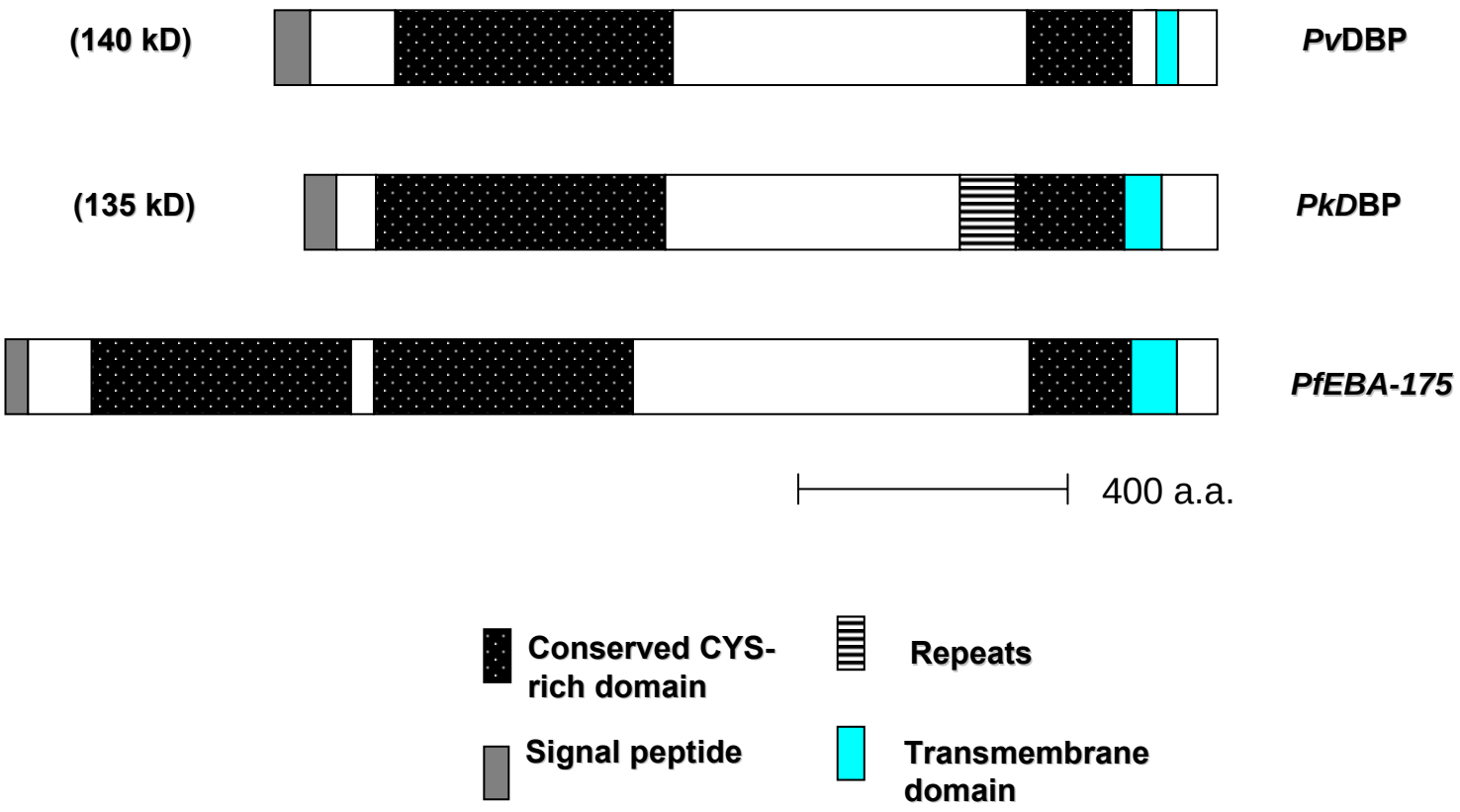
Structural features for HABP 6671 RESA

FIDIC

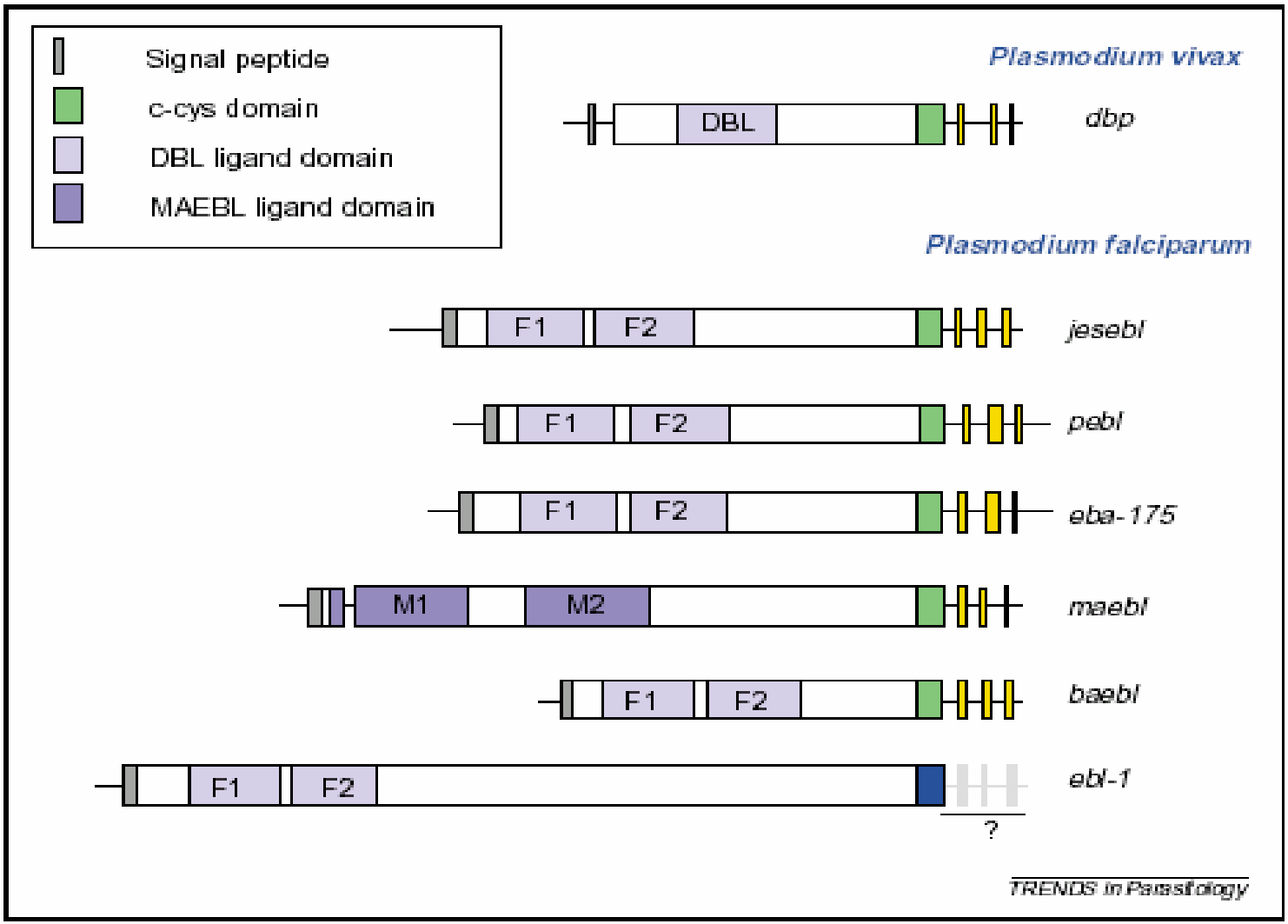
Alba M. P., *et al*., 2004, **BBRC**, 315: 1154



Duffy Binding Protein/EBA-175 Adhesion Family



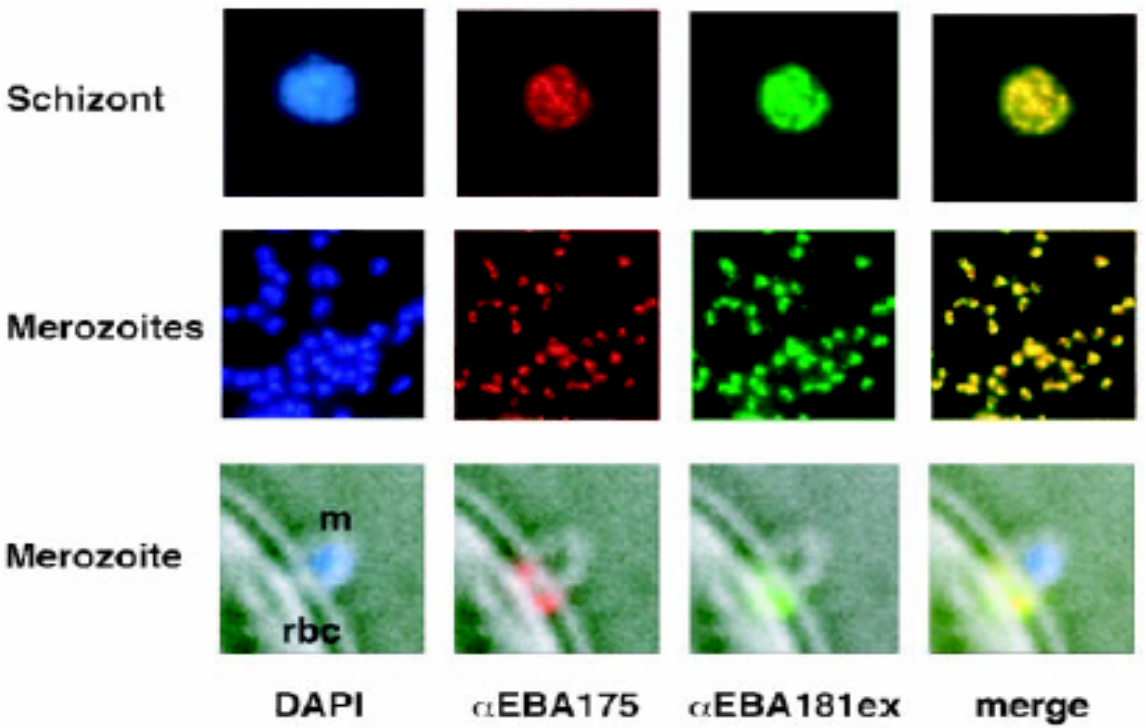
Bannister L. H., *et al*, 1977, Cowman A. F., *et al*, 2000
Sherman I. W., 1998, Cowman and Crabb, 2002
Chitnis and Blackman, 2000



Representation of *P. falciparum* and *P. vivax ebl* multi-exon structure. DBL, duffy binding-like, c-cys, carboxyl cys-rich.

Adams J. H. *et al*., 2001

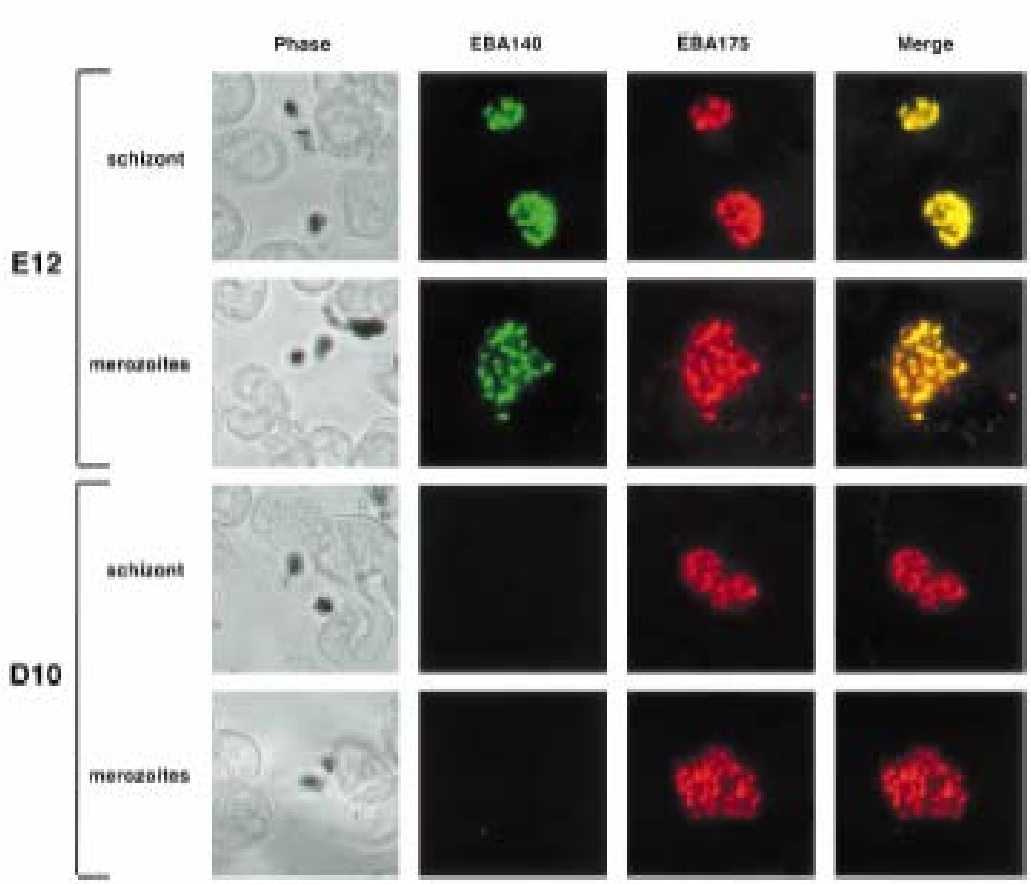
EBA-181 and EBA-175 co-localisation in schizonts and free merozoites



In the *first column* the parasites are stained with 4,6-diamidino-2-phenylindole (*blue*). In the *second column* the parasites have been labelled with anti-EBA-175 antibodies (*red*). In the *third column* the parasites have been labelled with anti-EBA181ex antibodies (*green*). The *fourth column shows* co-localisation of EBA-175 and EBA-181 (*yellow*) shown by an overlay of anti-EBA175 and anti-EBA181ex micrographs. *m*, merozoite; *rbc*, red blood cell.

Gilberger T. W. *et al.*, 2001

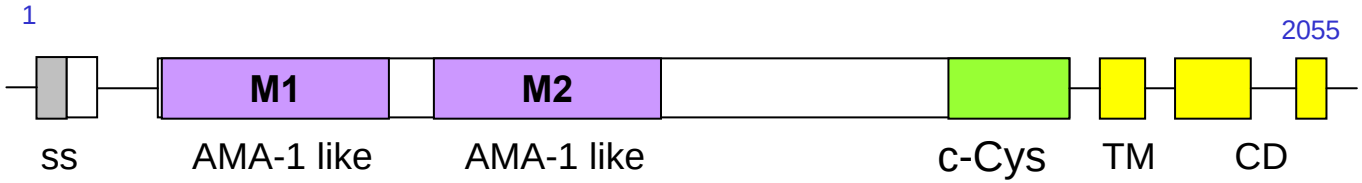
EBA140 and EBA175 proteins co-localise in merozoites

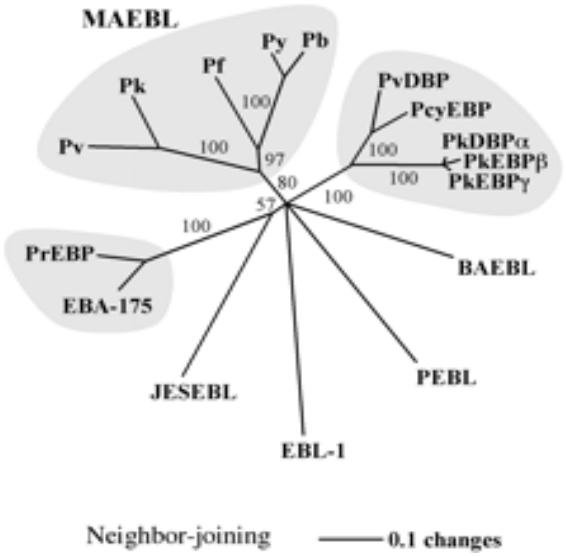


D10 and E12 parasite free merozoite or schizont smears are shown. Parasites were made to react with a mixture of anti-EBA140 and anti-EBA175 antibodies, followed by a mixture of FITC-labelled anti-mouse and rhodamine-labelled anti-rabbit antibodies. 'Phase' shows the brightfield image, whereas 'merge' shows the red and green images overlaid.

Jennifer T. *et al*., 2001

MAEBL





MAEBL is a ~250 kDa protein, located in the rhoptries and on the surface of mature merozoites.

The amino cysteine-rich domain of MAEBL has no similarity to DBL, but instead is similar to the 44-kDa fragment of the AMA-1 rhoptry protein.

The origins of *maebl, ebls,* and *ama-1* predate speciation of *Plasmodium*, *maebl* and other *ebls* obviously have a common ancestor gene but represent distinct lineages.

The *maebl* evolved as a single locus, including its unique structure, in each *Plasmodium* species.

The ancient origen of the maebl, its highly conserved nature among all *Plasmodium* species, its different time of expression and the distinct localization in merozoites, suggest that MAEBL has a distinct role from the DBL-EBP products.

Kappe S. H. I.. *et al*., 1998, Blair P. l., *et al*., 2002, Michon P., *et al*., 2002

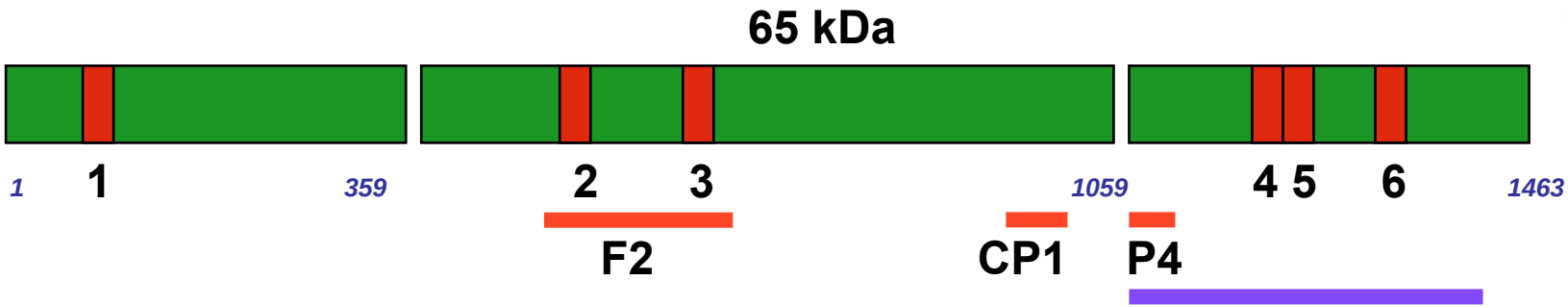
MAEBL

FIDIC

Ocampo M., *et al*., 2004, **BBRC**, 315: 319

Peptide		Sequence		2.0
30181	121	KYKLPIEIPLNKSGLSMYQG	140	
30195	401	TGSCYFLKKKPTCVLKKENH	420	
30198	461	QTNKRVLYENNKKSKRNVRT	480	
30209	681	LNFLNEIRTGYLNKYFKKDV	700	
30212	741	KSKIFSNRFTMKEYDPKTRL	760	
30213	761	FMYYGLYGLGGRLGANIKRD	780	
30219	881	YVSSFIRPDYETKCPPRYPL	900	
30220	901	KSKVFGTFDQKTGKCKSLMDY	920	
30253	1561	RAEILKQIEKKRIEEVMKLY	1580	

EBA-175



1. KSYGTPDNIDKNMSLINKHN
2. NIDRIYDKNLLMIKEHILAI
3. HRNKKNDKLFRDEWWKVIK
4. DRNSNTLHLKDIRNEENERH
5. LTNQNINISQERDLQKHGFH
6. NNNFNNIPSRYNLYDKKLDL

Erythrocyte-binding antigen-175 (EBA-175) interacts directly with the erythrocyte surface by a sialic acid-dependent epitope on glycophorin A. However, the *P. falciparum* can use alternative receptors when the prime receptor (e.g. glycophorin A) is absent or blocked.

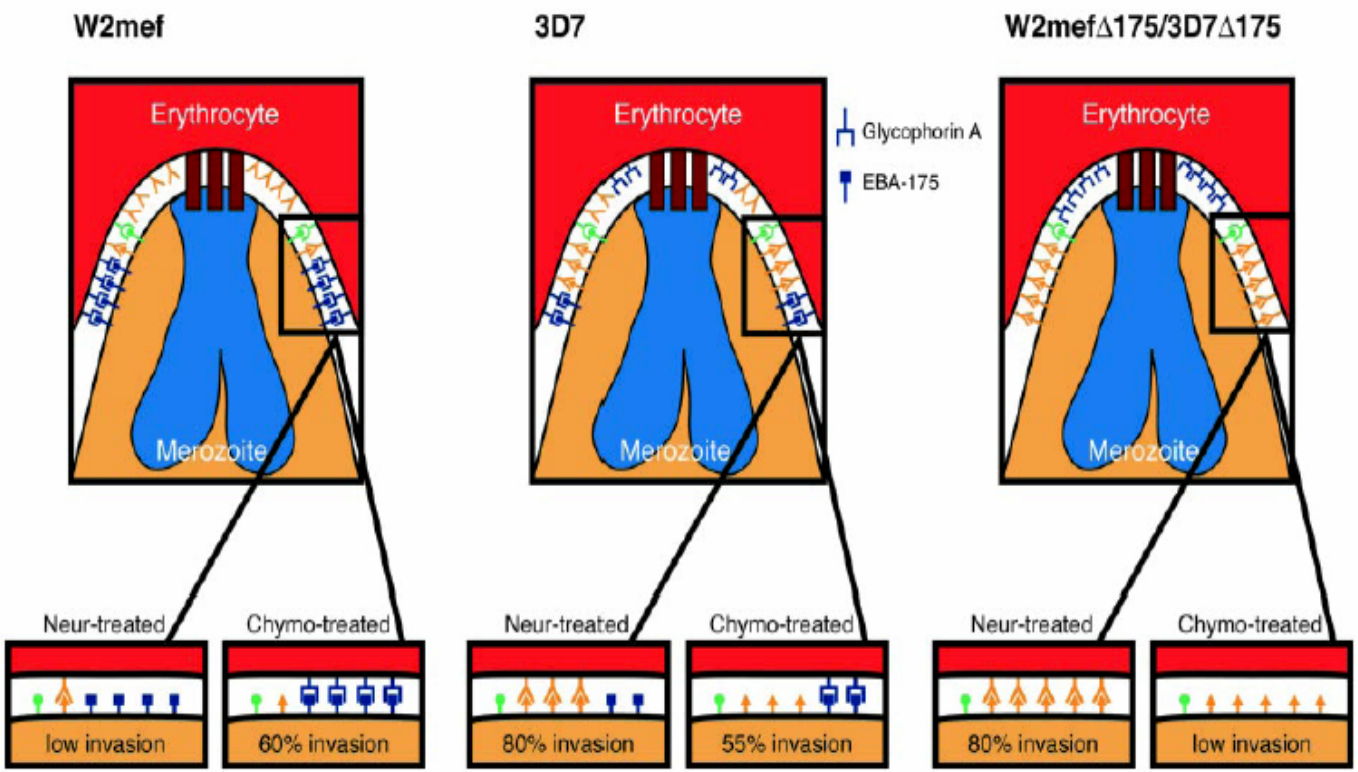
Sim B. K. *et al.,* 1994, Kain K. C. *et al.,* 1993, Orlandi P. A. *et al.,* 1990, Sim B. K., 1998, Rodriguez L. E., *et al.*, 2000

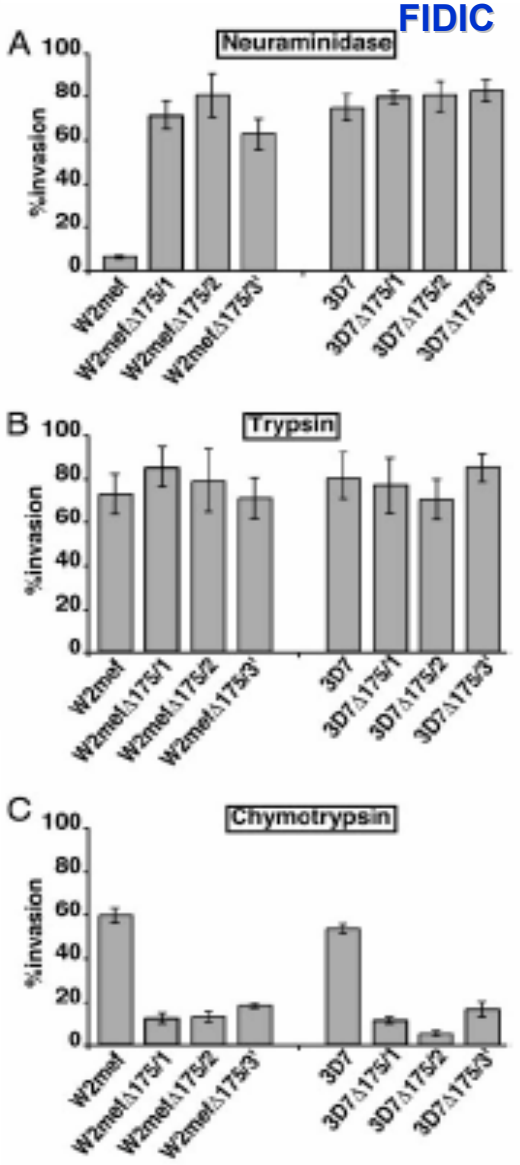
EBA-175

sialic acid-dependent

sialic acid-independent

Truncated forms of EBA-175





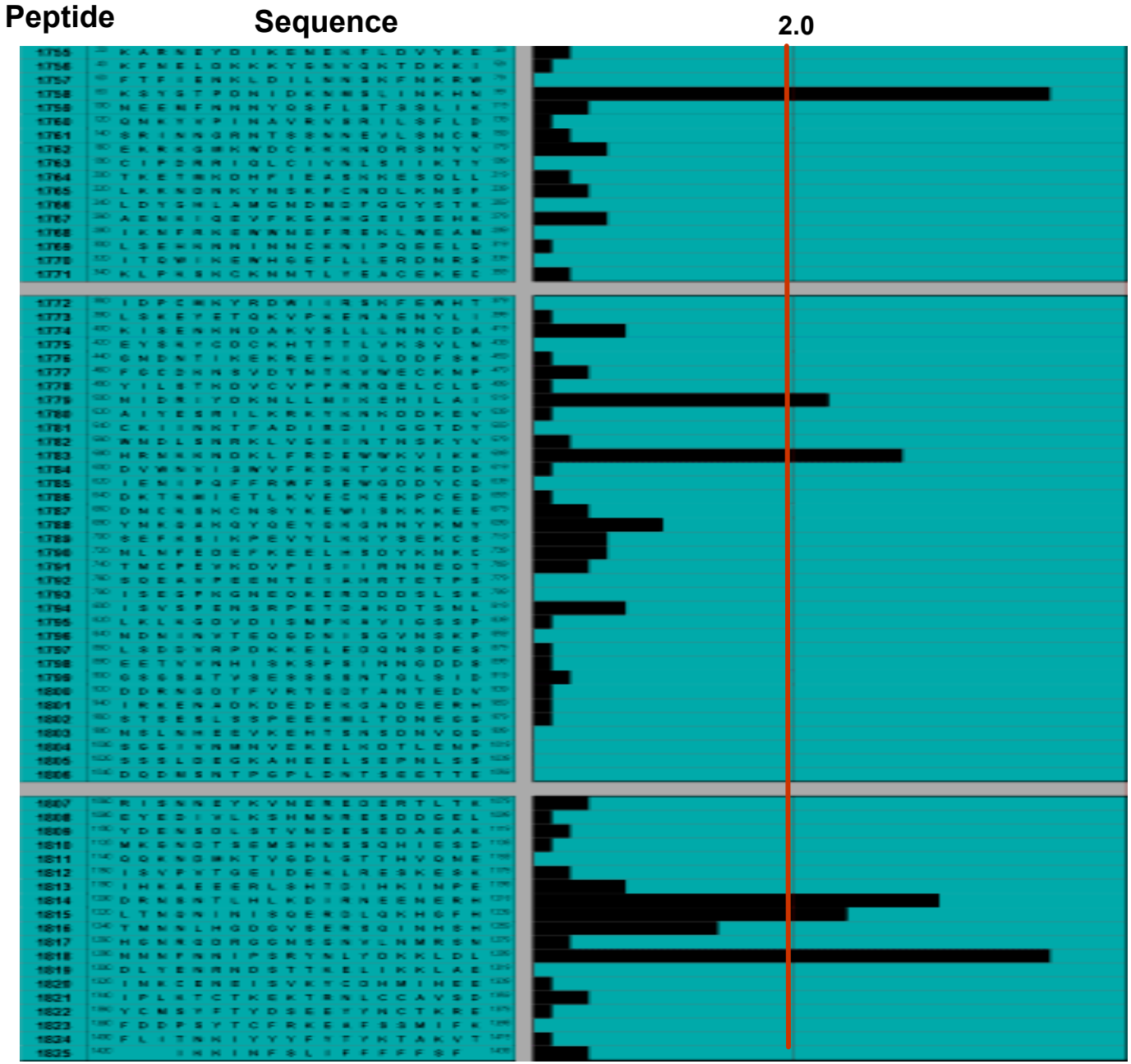
A model for invasion via sialic acid-dependent and -independent pathways in *P. falciparum*.

The EBA-175 ligand is functional in erythrocyte invasion by merozoites that utilize either sialic acid-dependent or -independent invasion pathways.

Duraisingh M. T., et al., 2003

EBA-175

Rodríguez L. E., *et al*., 2000, **Parasitology**, 120: 225



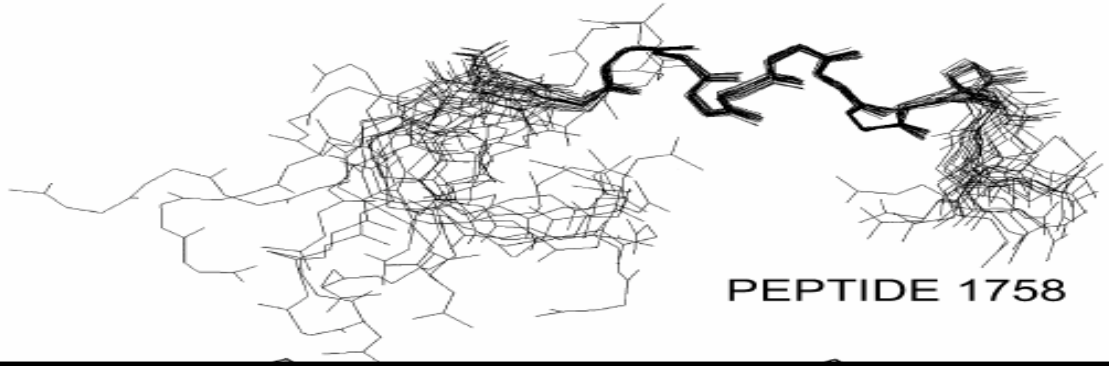
FIDIC

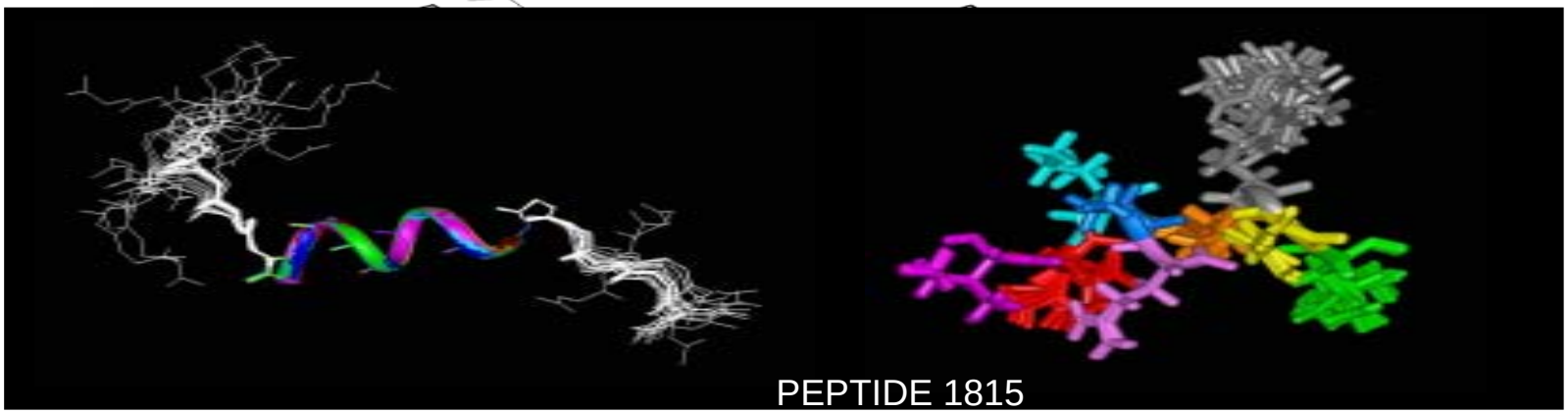
Peptide	Kd (nM)
1758	112
1779	175
1783	139
1814	64
1815	106
1818	146

Structural features for HABPs EBA-175

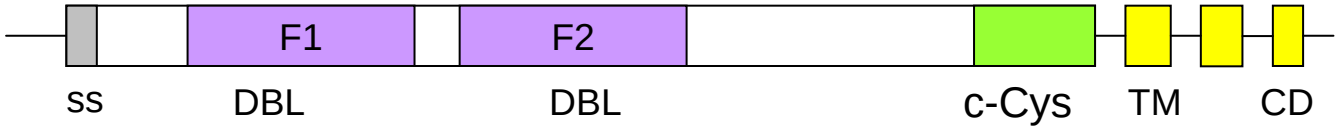
Cifuentes G., *et al*., 2003, **J Struct Biol**., 141: 115
Guzmán F., *et al*. 2002, **Life Sci**., 71: 2779,
Cifuentes G., *et al*., 2004, **Vaccine**, In Press

FIDIC





EBA-140



EBA-140 (pfEBP-2/BAEBL) is located in the micronemes (the same location As EBA-175 and EBA-181) these proteins bind to sialoglycoproteins on the red blood cells surface.

Glycophorin C (GPC) is receptor for EBP-140 and show that the binding on GPC is limited to amino acids (aa's) 14-22 in exon 2.

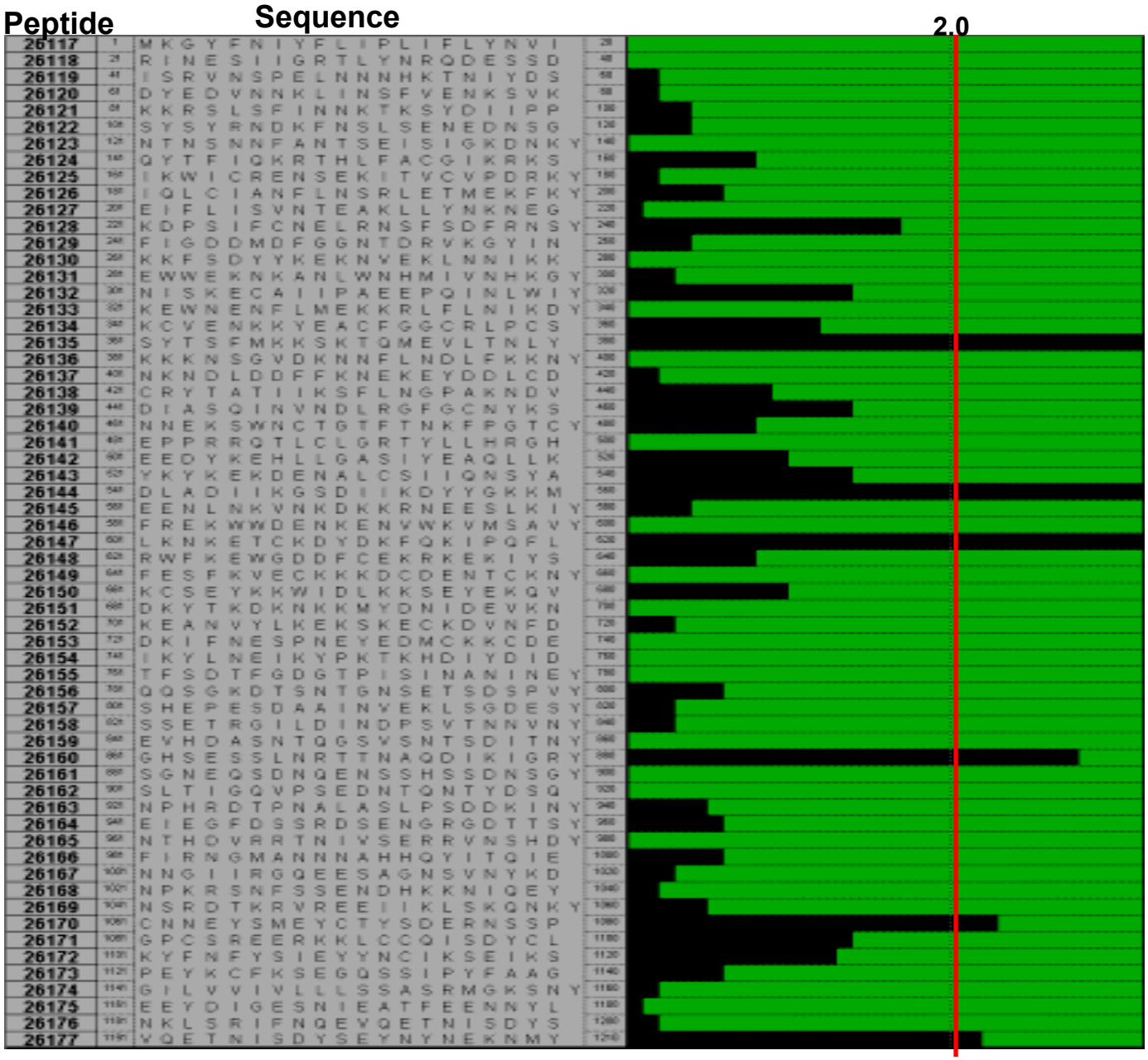
Each of the polymorphisms form in the parasite ligand, BAEBL, bound to a different receptor on erythrocytes.

Why has the parasite evolved to have such diversity? What is the advantage to the parasite to have multiple niches with different erythrocyte receptors?

Thompson J. K., *et al*., 2001, Lobo C. A., et al., 2003, Mayer D. c. G., et al ., 2002, Duraisingh M. T., *et al*., 2003, Miller H. L., 2004

EBA-140

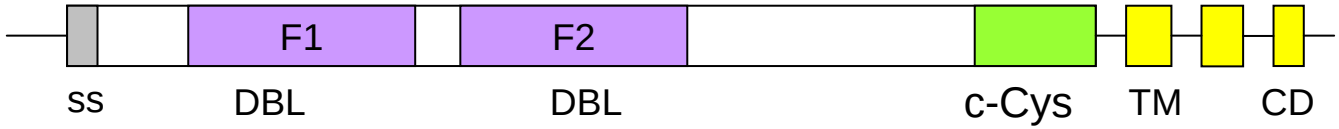
Rodríguez L. E., *et al.*, 2003, **J Peptide Res**, 62: 175



FIDIC

Peptide	Kd (nM)
26135	350
26144	350
26147	500
26160	590
26170	600
26177	750

EBA-181



FIDIC

This ligand is expressed at the same time as EBA-175 and is located within the micronemes.

The EBA-181/JESEBL protein was identified as a ligand interacting in a sialic acid dependent manner with the erythrocyte, such binding being sensitive to erythrocyte treatment with chymotrypsin and resistant to trypsin.

The level of expression of EBA-181differs among parasite lines, and the importance of this ligand for invasion appears to be strain-dependent.

These polymorphisms did not change the erythrocyte-binding specificity. In contrast, each point mutation in JESEBL led to the recognition of different receptors on the erythrocyte.

Why has *P. falciparum* evolved so many pathways for invasion?

Gilberger T. W., *et al*., 2003, Mayer D. C., *et al*., 2004, Lobo C. A., *et al*., 2003

JESEBL / EBA181

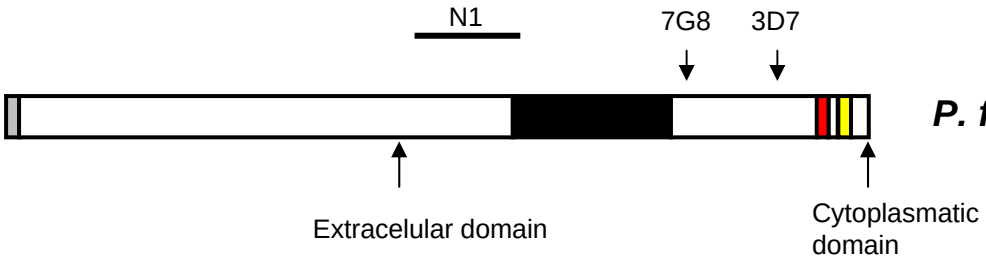
Vera R., *et al.*, 2004, **Biochimie**, In Press

FIDIC

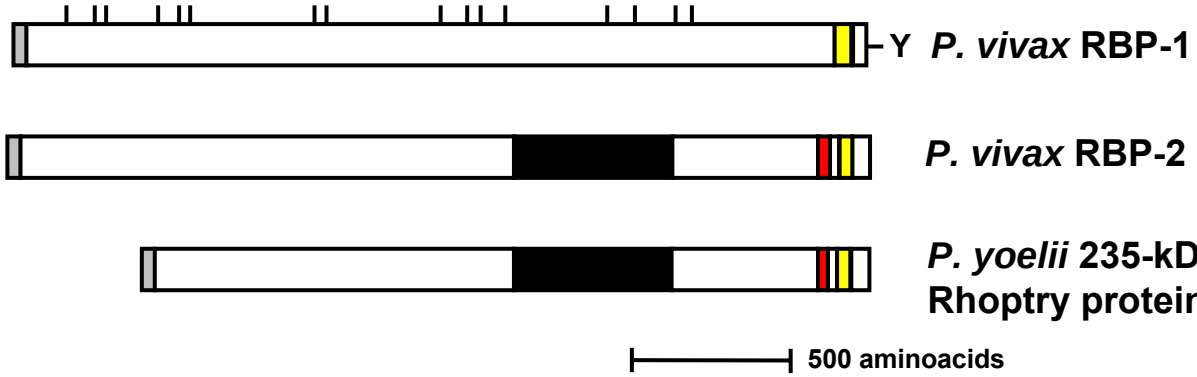
Peptide		Sequence		2.0
30026	1	MKGKMNMCLFFFYSILYVVL	20	
30027	21	CTYVLGISEEYLKERPQGLN	40	
30028	41	VETNNNNNNNNNNNSNSNDAY	60	
30029	61	MSFVNEVIRFIENEKDDKEDY	80	
30030	81	KKVKIISRPVENTLHRYPVS	100	
30031	101	SFLNIKKYGRKGEYLNRNSF	120	
30032	121	VQRSYIRGCKGKRSTHTWIC	140	
30033	141	ENKGNNNICIPDRRVQLCITY	160	
30034	161	ALQDLKNSGSETTDRKLLRDY	180	
30035	181	KVFDSAMYETDLLWNKYGFR	200	
30036	201	GFDDFCDDVKNSYLDYKDVI	220	
30037	221	FGTDLDKNNISKLVEESLKRY	240	
30038	241	FFKKDSSVLNPTAWWRRYGT	260	
30039	261	RLWKTMIQPYAHLGCRKPDE	280	
30040	281	NEPQINRWILEWGKYNCRLM	300	
30041	301	KEKEKLLTGECSVNRKKSDCY	320	
30042	321	STGCNNECYTYRSLINRQRY	340	
30043	341	EVSILGKKYIKVVRYTIFRR	360	
30044	361	KIVQPDNALDFLKLNCSECKY	380	
30045	381	DIDFKPFFEFEYGKYEEKCM	400	
30046	401	CQSYIDLKIQFKNNDICSFN	420	
30047	421	AQTDTVSSDKRFCLEKKEFKY	440	
30048	441	PWKCDKNSFETVHHKGVCVSY	460	
30049	461	PRRQGFCLGNLNYLLNDDIY	480	
30050	481	NVHNSQLLIEIIMASKQEGKY	500	
30051	501	LLWKKHGTILDNQNACKYIN	520	
30052	521	DSYVDYKDIVIGNDLWNDNN	540	
30053	541	SIKVQNNLNLIFERNFGYKV	560	
30054	561	GRNKLFKTIKELKNVWWILNY	580	
30055	581	RNKVWESMRCGIDEVDQRRKY	600	
30056	601	TCERIDELENMPQFFRWFSQY	620	
30057	621	WAHFFCKEKEYWELKLNDKC	640	
30058	641	TGNNGKSLCQDKTCQNVCTNY	660	
30059	661	MNYWTYTRKLAYEIQSVKYD	680	
30060	681	KDRKLFSLAKDKNVTTFLKEY	700	
30061	701	NAKNCSNIDFTKIFDQLDKLY	720	
30062	721	FKERCSCMDTQVLEVKNKEMY	740	
30063	741	LSIDSNSEDATDISEKNGEEY	760	
30064	761	ELYVNHNSVSVASGNKEIEK	780	
30065	781	SKDEKQPEKEAKQTNGTLTVY	800	
30066	801	RTDKDSDRNKGKDTATDTKNY	820	
30067	821	SPENLKVQEHGTNGETIKEEY	840	
30068	841	PPKLPESSETLQSQEQLEAEY	860	
30069	861	AQKQKQEEEPKKKQEEEPKKY	880	
30070	881	KQEEEQKREQEQKQEQEEEEY	900	
30071	901	QKQEEEQQIQDQSQSGLDQSY	920	
30072	921	SKVGVASEQNEISSGQEQNVY	940	
30073	941	KSSSPEVVPQETTSENGSSQY	960	
30074	961	DTKISSTEPNENSVVDRATDY	980	
30075	981	SMNLDPEKVHNENMSDPNTNY	1000	
30076	1001	TEPDASLKDDKKEVDDAKKEY	1020	
30077	1021	LQSTVSRIESNEQDVQSTPPY	1040	
30078	1041	EDTPTVEGKVGDKAEMLTSPY	1060	
30079	1061	HATDNSESESGLNPTDDIKTY	1080	
30080	1081	TDGVVKEQEILGGGESATETY	1100	
30081	1101	SKSNLEKPKDVEPSHEISEPY	1120	
30082	1121	VLSGTTGKEESELLKSKSIEY	1140	
30083	1141	TKGETDPRSNDQEDATDDVVY	1160	
30084	1161	ENSRDDNNSLSNSVDNQSNVY	1180	
30085	1181	LNREDPIASETEVVSEPEDSY	1200	
30086	1201	SRIITTEVPSTTVKPPDEKRY	1220	
30087	1221	SEEVGEKEAKEIKVEPVVPRY	1240	
30088	1241	AIGEPMENSVSVQSPPNVEDY	1260	
30089	1261	VEKETLISENNGLHNDTHRGY	1280	
30090	1281	NISEKDLIDIHLLRNEAGSTY	1300	
30091	1301	ILDDSRRNGEMTEGSESDVGY	1320	
30092	1321	ELQEHNFSTQQKDEKDFDQIY	1340	
30093	1341	ASDREKEEIQKLLNIGHEEDY	1360	
30094	1361	EDVLKMDRTEDSMSDGVNSHY	1380	
30095	1381	LYYNNLSSEEKMEQYNNRDA	1400	
30096	1401	SKDREEILNRSNTNTCSNEHY	1420	
30097	1421	SLKYCQYMERNKDLLETCSE	1440	
30098	1441	DKRLHLCCEISDYCLKFFNP	1460	
30099	1461	KSIEYFDCTQKEFDDPTYNC	1480	
30100	1481	FRKQRFTSMHYIAGGGIIAL	1500	
30101	1501	LLFILGSASYRKNLDDEKGF	1520	
30102	1521	YDSNLNDSAFEYNNNKYNKL	1540	
30103	1541	PYMFDQQINVVNSDLYSEGI	1560	
30104	1561	INVVNSDLYSEGIYDDTTTF	1580	

Peptide	Kd (nM)
30030	394 ± 91
30031	215 ± 37
30045	348 ± 52
30051	595 ± 117
30060	178 ± 42

Reticulocyte binding protein RBP/NBP Adhesion Family



***P. falciparum* NBP-1**



***P. vivax* RBP-1**

***P. vivax* RBP-2**

***P. yoelii* 235-kDa Rhoptry protein**

Plasmodium falciparum
PfRh/NBP gene family

NBP-1
NBP-2a
NBP-2b
NBP-4

NBP-3

HOMOLOGY

REPEATED AMINOACIDS

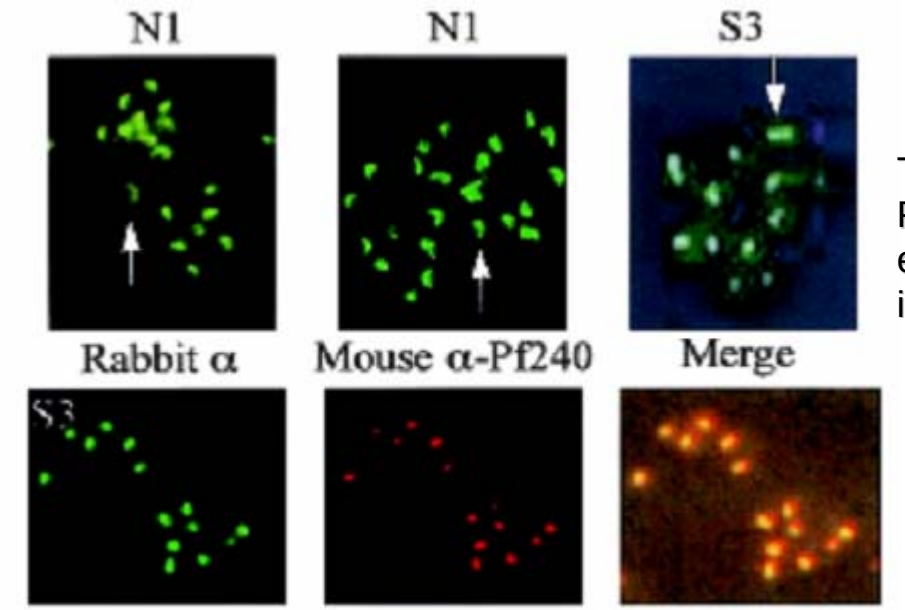
CYSTEINE RESIDUES & BONDS

SIGNAL PEPTIDE

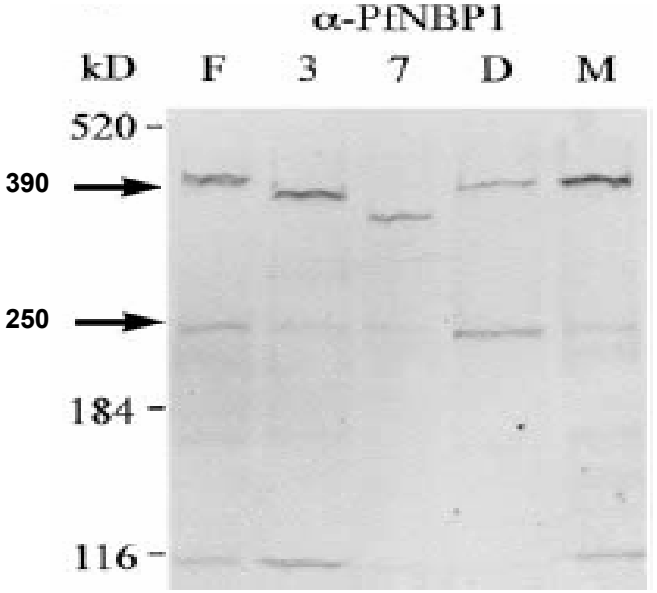
TRANSMEMBRANE DOMAIN

Rayner J. C., *et al.,* 2001
Sherman I.W., *et al*., 1998

PfNBPs are expressed at merozoites' apical end

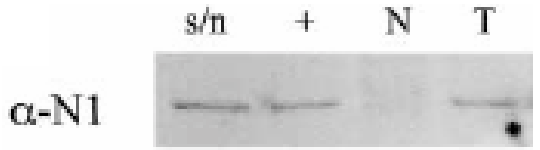


The PfNBP-1 and PfNBP-2a and 2b are ortholog of PvRBP-1 and PvRBP-2. They are located at the apical end of merozoites and play a role in the recognition and invasion of erythrocytes by merozoites.



PfNBP1 forms a complex with PfNBP2b, which is consistent with the known in *P. vivax*. The observed size of the PfNBP-1 in strain differents, shown expressing of a truncated PfNBP-1. By contrast, there were no size differences in PfNBP-2a or PfNBP-2b in these strains.

PfNBP1 binds to a sialic acid dependent trypsin resistant receptor on the erythrocyte surface that appears to be distinct from known invasion receptors (receptor Y).



Taylor *et al*., 2001, Florens *et al*., 2002, Rayner *et al*., 2000, Rayner *et al*., 2001, Triglia *et al*., 2001, Kaneko *et al*., 2002, Triglia *et al*., 2001, Kaneko *et al*., 2002

PfNBP-1

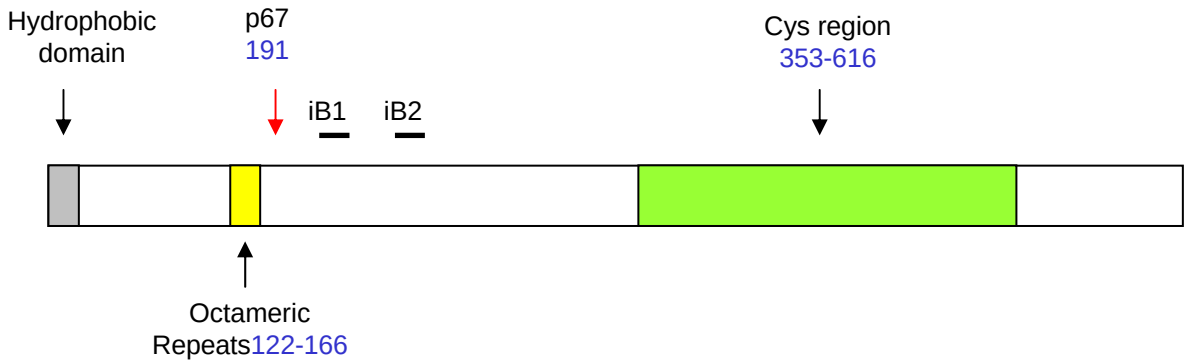
FIDIC

Valbuena J. J., *et al.*, 2003, **Peptides**, 24: 1007

Peptide		Sequence		1.0 2.0
26327	1	LINIETEMKHKQKQLINKMNY	20	
26328	21	DIEKDNITDQYMHDVQQNIFY	40	
26329	41	EPITLKMNEYNTLLNDNHNN	60	
26330	61	NINNEHQFNHLNSLHTKIFSY	80	
26331	81	HNYNKEQQQEYITNIMQRID	100	
26332	101	VFINDLDTYQYEYYFYEVVNQ	120	
26333	121	EYKQIDKNKINQHINNIKNN	140	
26334	141	LIHVKKQFEHTLENIKNNENY	160	
26335	161	IFDNIQLKKKDIDDIIININY	180	
26336	181	NTKETYLKELNKKKMLQNKK	200	
26337	201	KVDEKSEINNHHTLQHDNQNY	220	
26338	221	VEQKNKIKDHNLITKPNNNSY	240	
26339	241	SEESHQNEQMKEQNKNILEKY	260	
26340	261	QTRNIKPHHVHNHNHNHNHNY	280	
26341	281	HNQNQNQKDSTKLQEQDISTY	300	
26342	301	HKLHNTIHEQQSKDNHQGNRY	320	
26343	321	EKKQKNGNHERMYFASGIVV	340	
26344	341	SILFLSSLGFVINSKNNKQEY	360	
26345	361	YDKEQEKQQQNDFVCDNNKM	380	
26346	381	DDKSTQKYGRNQEEVMEISF	400	
26347	386	QKYGRNQEEVMEISFDNDYI	405	

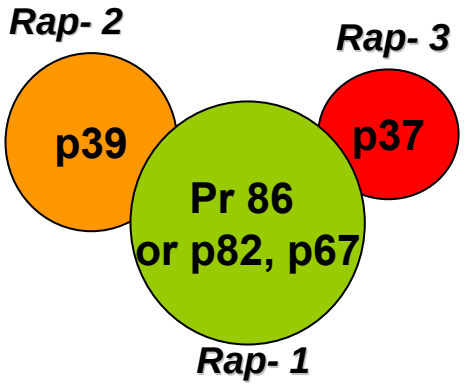
Peptide	Kd (nM)
26332	650
26336	480

Rap-1



Plasmodium falciparum
RAP-1 p82

The genes coding for RAP-1 and RAP-2 have been cloned, revealing no homology to other known genes. Further, RAP-1 and RAP-2 show minimal sequence polymorphisms between *P. falciparum* isolates. This protein of 84 kD, with 782 residues that is present in the rhoptries.

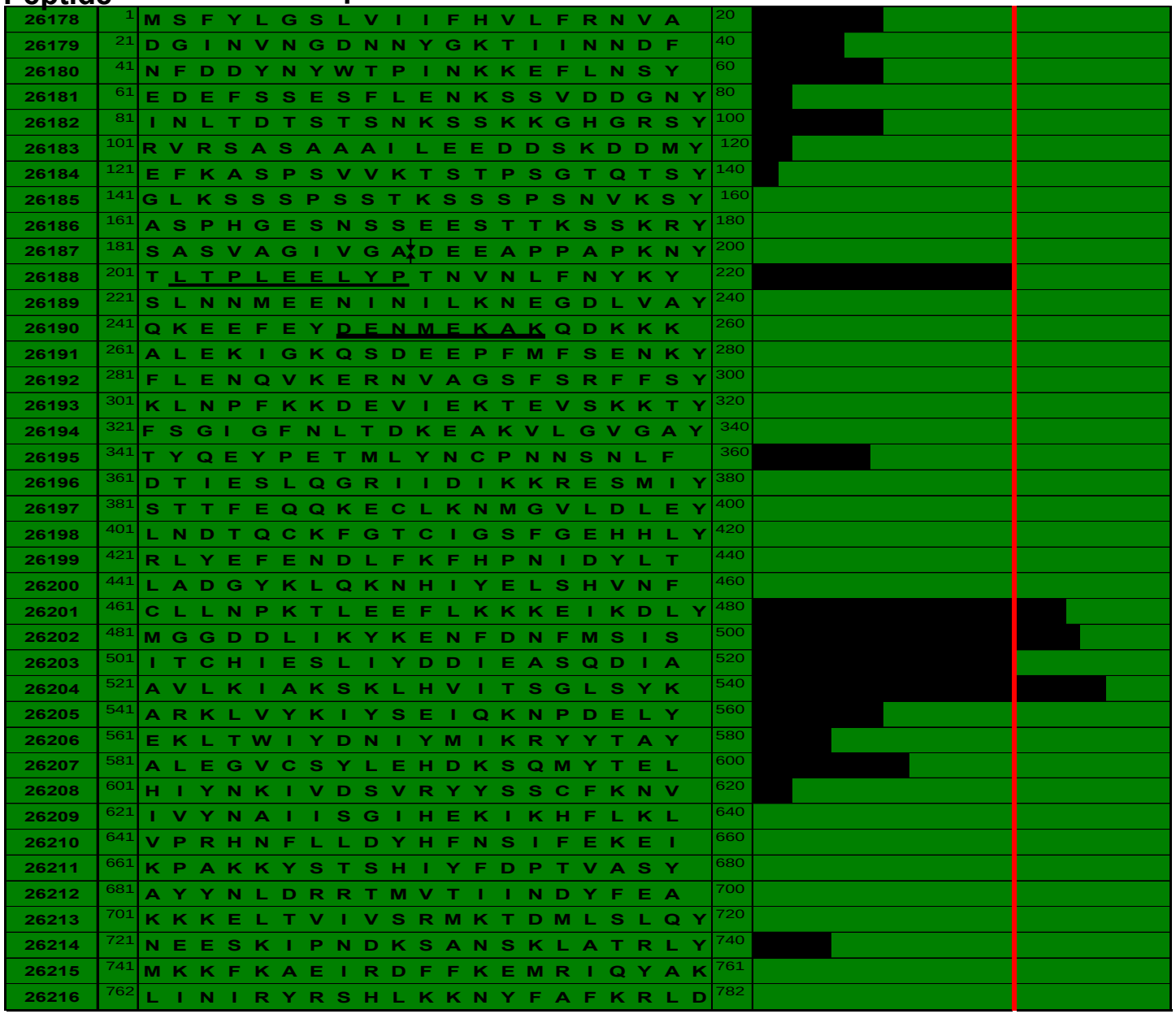


Rhoptry-associated protein 1 (RAP-1), which is processed into molecules of 86, 82, 70, and 67 kDa, forms heterooligomeric protein complexes with the rhoptry proteins RAP-2 and RAP-3.

p67 is relatively abundant in purified free merozoites but is not observed in ring-stage parasites, indicating that p67 is secreted or degraded prior to ring formation and there is a narrow time during which this protein may play a role in merozoite invasion of erythrocytes.

RAP1

Curtidor H., *et al*., 2004, **Vaccine**, 22: 1054



FIDIC

Peptide	Kd (nM)
26188	805
26201	700
26202	900
26204	760

RAP2

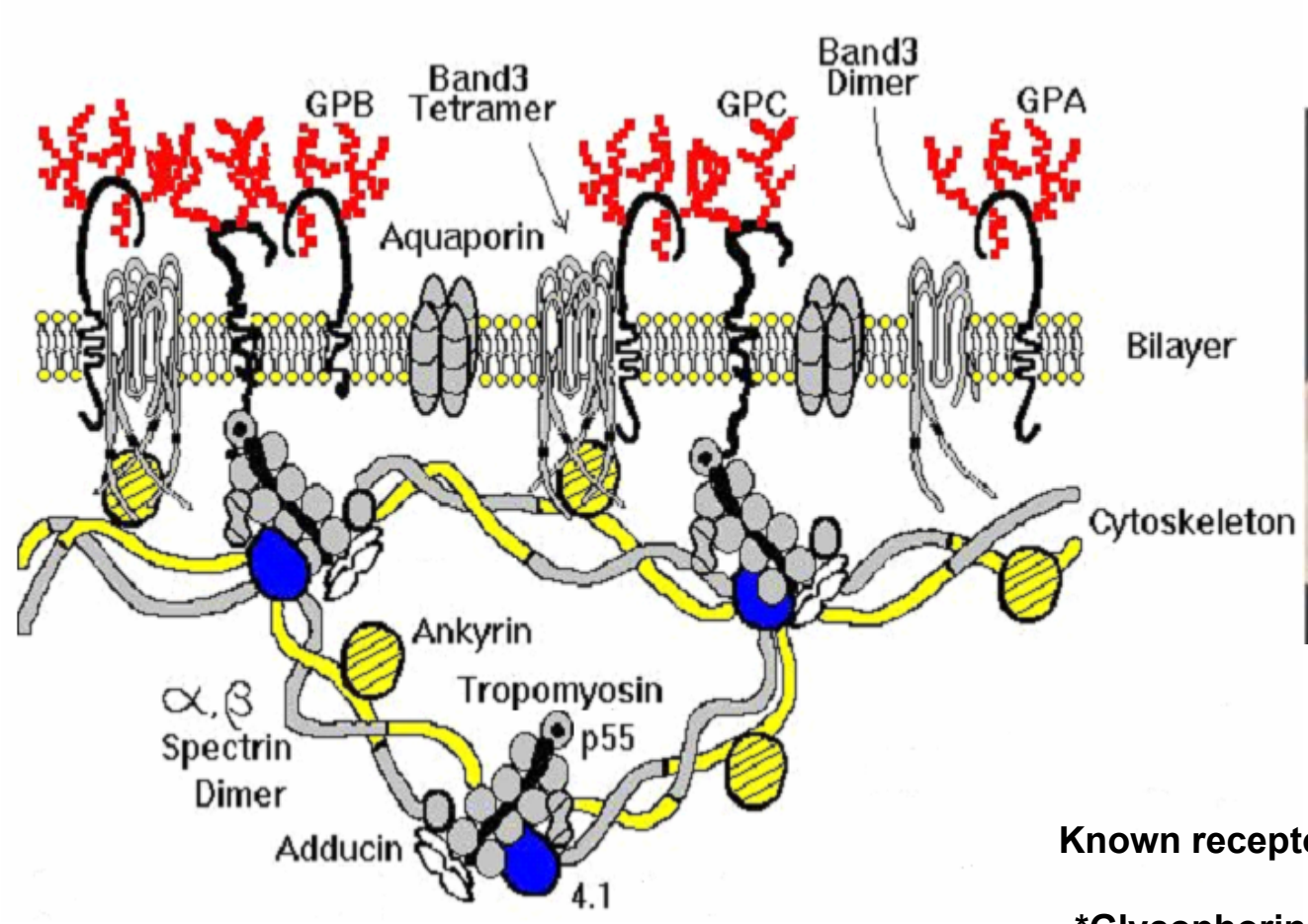
López R., *et al*., 2004, **Biochimie**, 86: 1

FIDIC

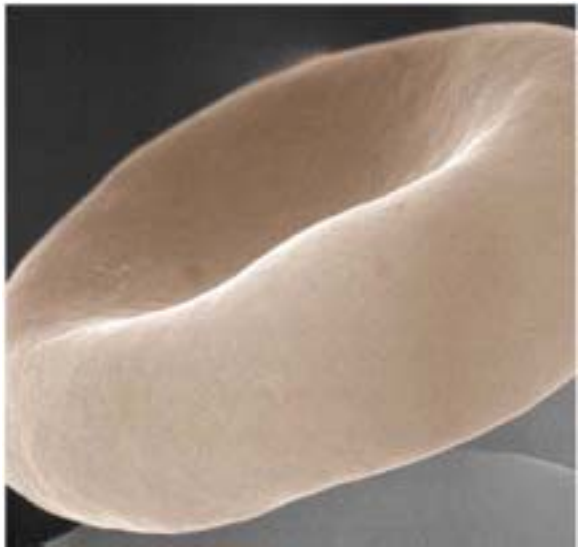
Peptide		Sequence		2.0
26217	1	MGLKFYVLVFLILCLKNVVK	20	
26218	21	GDKCETEFSKLYPESNSLTG	40	
26219	41	LIYAHTAHVHKLSMWVYFIY	60	
26220	61	NHFSSADELIKYLEKTNINT	80	
26221	81	LENSDHTCFARAVTLYLFYY	100	
26222	101	YLKDIKSMLSTDDYQSFFKN	120	
26223	121	KFKDINPLFINDFILILNDKY	140	
26224	141	KFMENLDLYIMKESEREHLV	160	
26225	161	IKKNPFLRVLNKASTTTHATY	180	
26226	181	YKSNPYFIVGSRVHTPYKDY	200	
26227	201	LGDFNKYTEISVLNYVRDYN	220	
26228	221	FLIYAGSRENYYNSDIAGPA	240	
26229	241	RSVNNVISKNKTLGLRKRSSY	260	
26230	261	SLALVGTNNNDPIFAYCEKD	280	
26231	281	NKSEYYGTPDDLITSFFSII	300	
26232	301	KTKMLNSHKTFLRQFDYALF	320	
26233	321	HKTYSIPNLKGFRFLKHLFQ	340	
26234	341	KKNLVNFVGMYENHVSTEIN	360	
26235	361	FLAEDFVELFDVTMDCYSRQ	380	
26236	381	RQYSNRAAENFKAIRELNVL	400	
6800	control	YNNSAFNNNLCSKNAKGLNLN		

Peptide	Kd (nM)
26220	950
26225	700
26229	700
26235	533

Plasmodium falciparum receptors on erythrocyte membrane surface



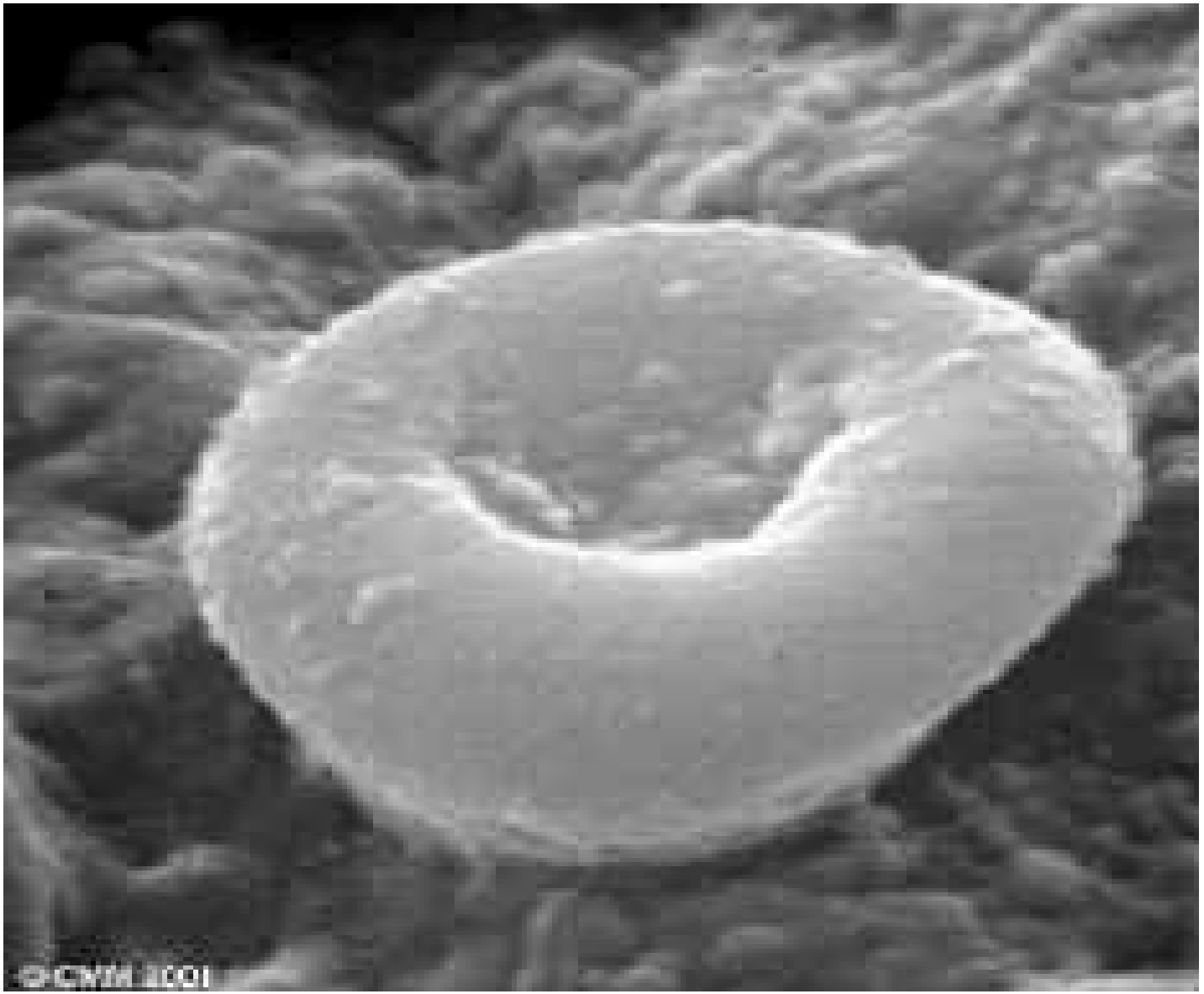
FIDIC



Known receptors	Unknown receptors
*Glycophorin A	*Receptor X
*Glycophorin B	*Receptor Y
*Glycophorin C	*Receptor Z
*Band 3	*Receptor E

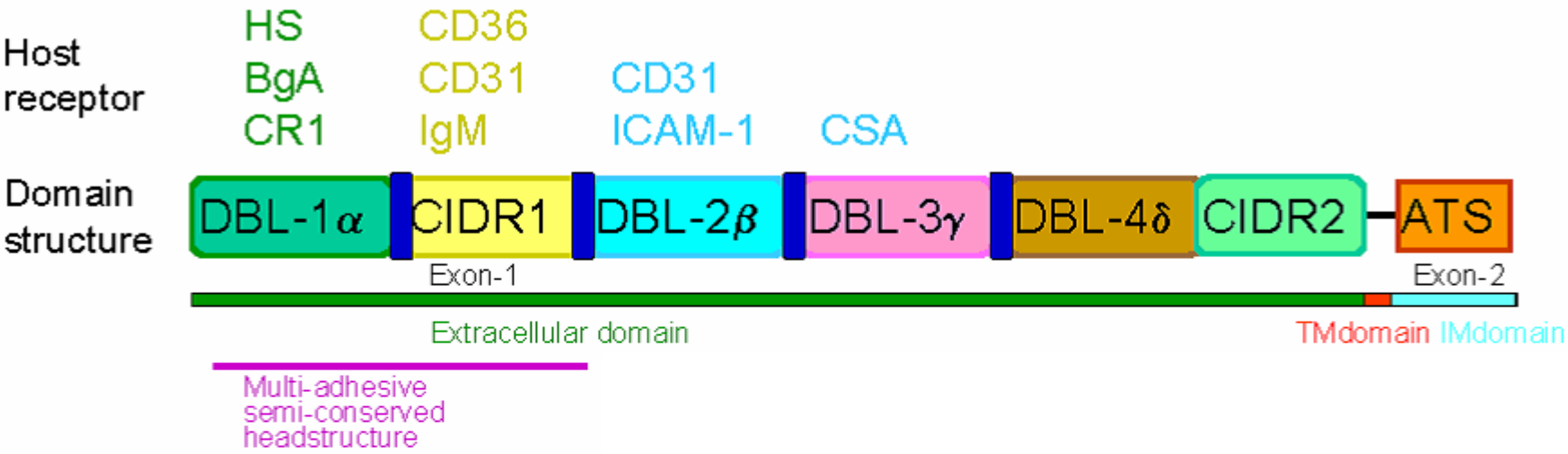
http://dwknowles.lbl.gov/membrane/membrane.html, Dolan *et al*., 1990, Thopmson *et al*., 2001, Rayner *et al*., 2001, Triglia *et al.,* 2001, Baldi *et al*., 2000

FIDIC



PfEMP1

FIDIC



DBL ≡ Duffy Binding Ligand domain - polymorphic
CIDR ≡ Cysteine Rich Inter domain Region - polymorphic
ATS ≡ Acidic Terminal Segment

Highly polymorphic interdomain region

Conserved cytoplasmic domain

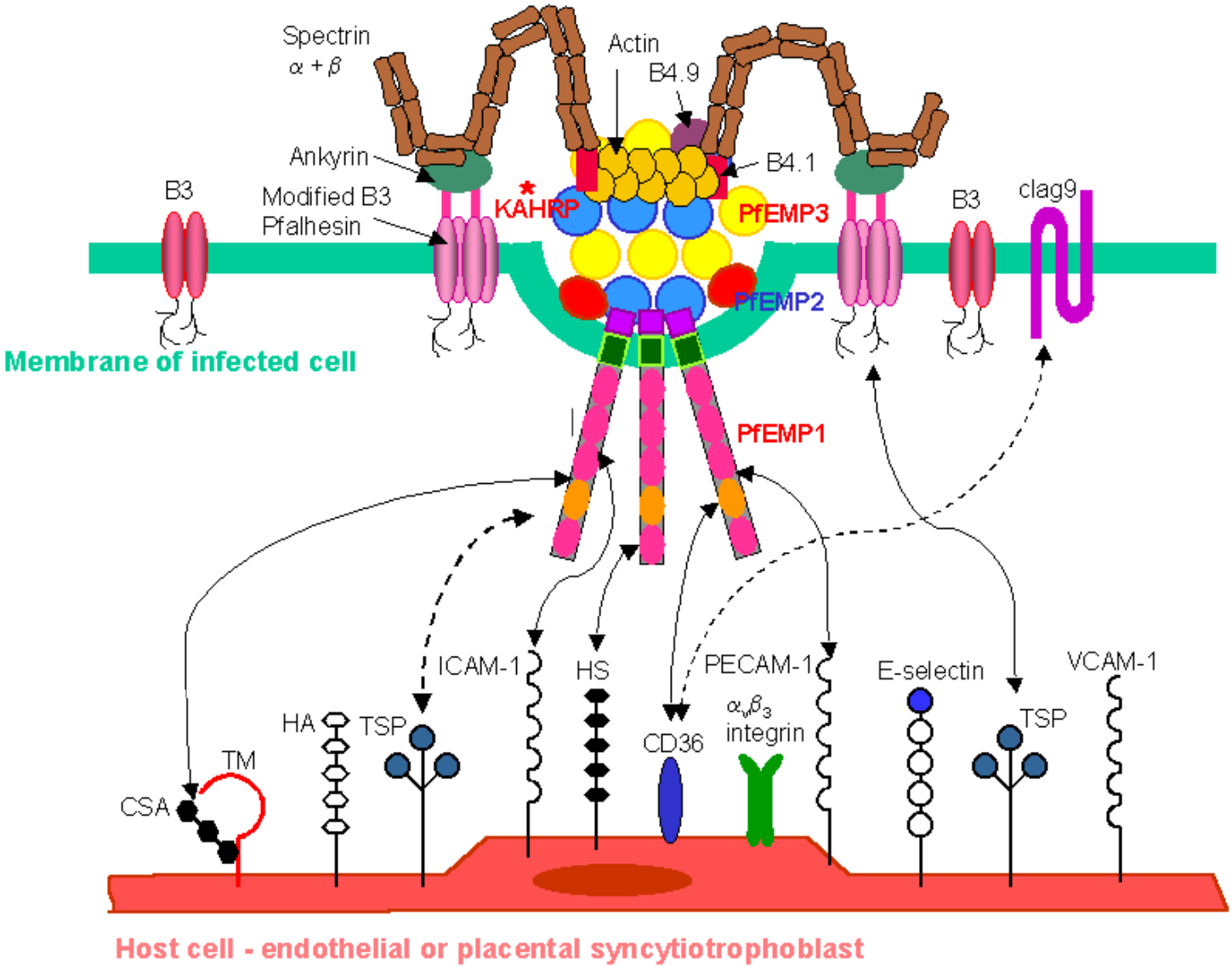
Other molecules that could mediate adherence:

- Sequestrin
- Modified RBC band 3
- Rosettins
- Pf332
- Clag9

Chen B. Q. *et al.*, 1998
http://sites.huji.ac.il/malaria/FramInteractions.html

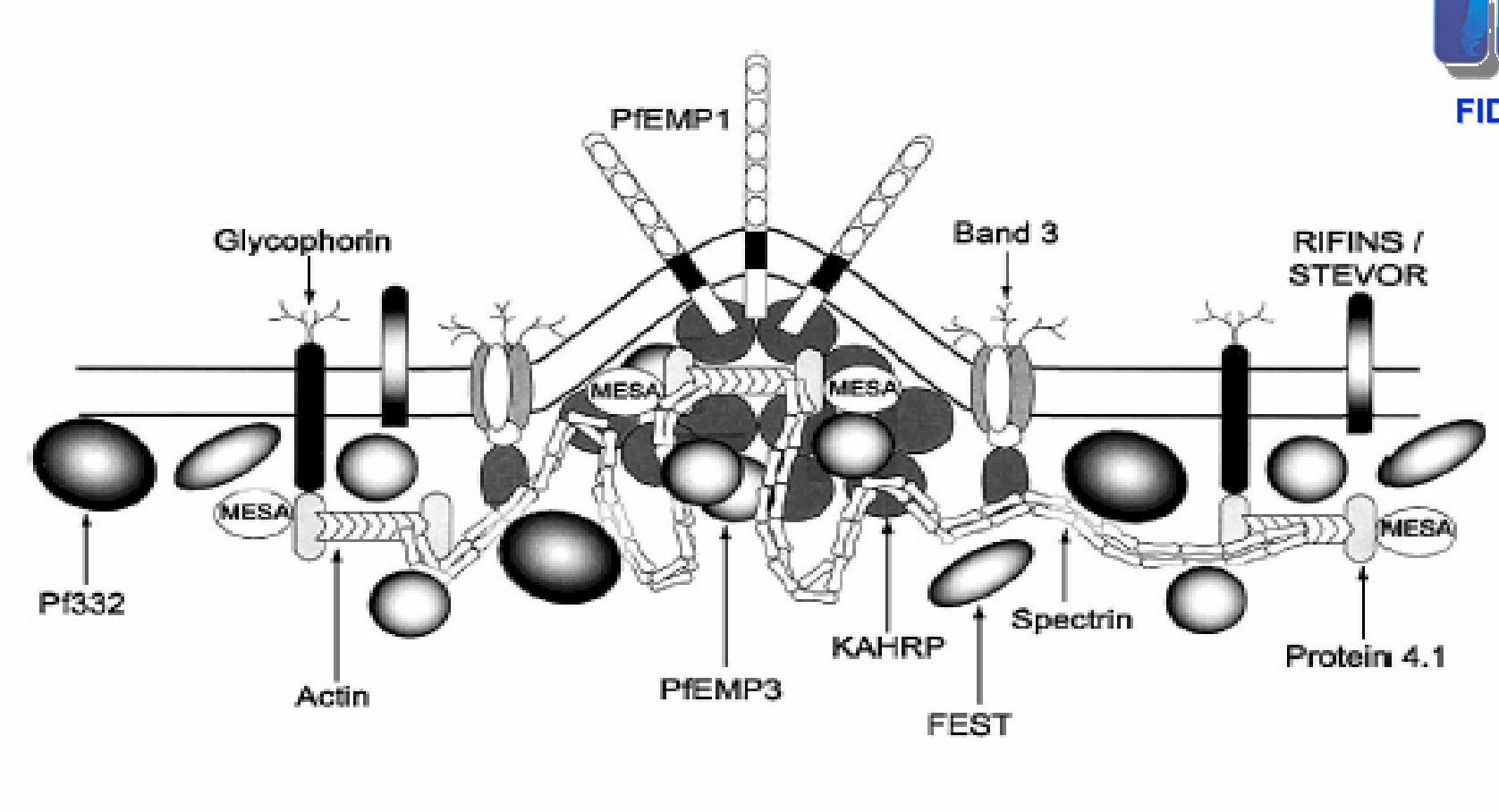
Interaction between modified host cell membrane and endothelial cell

FIDIC



Cooke B and Tilley L, 1999, Cooke B and Coppel R, 1995

The membrane skeleton of a malaria-infected RBC

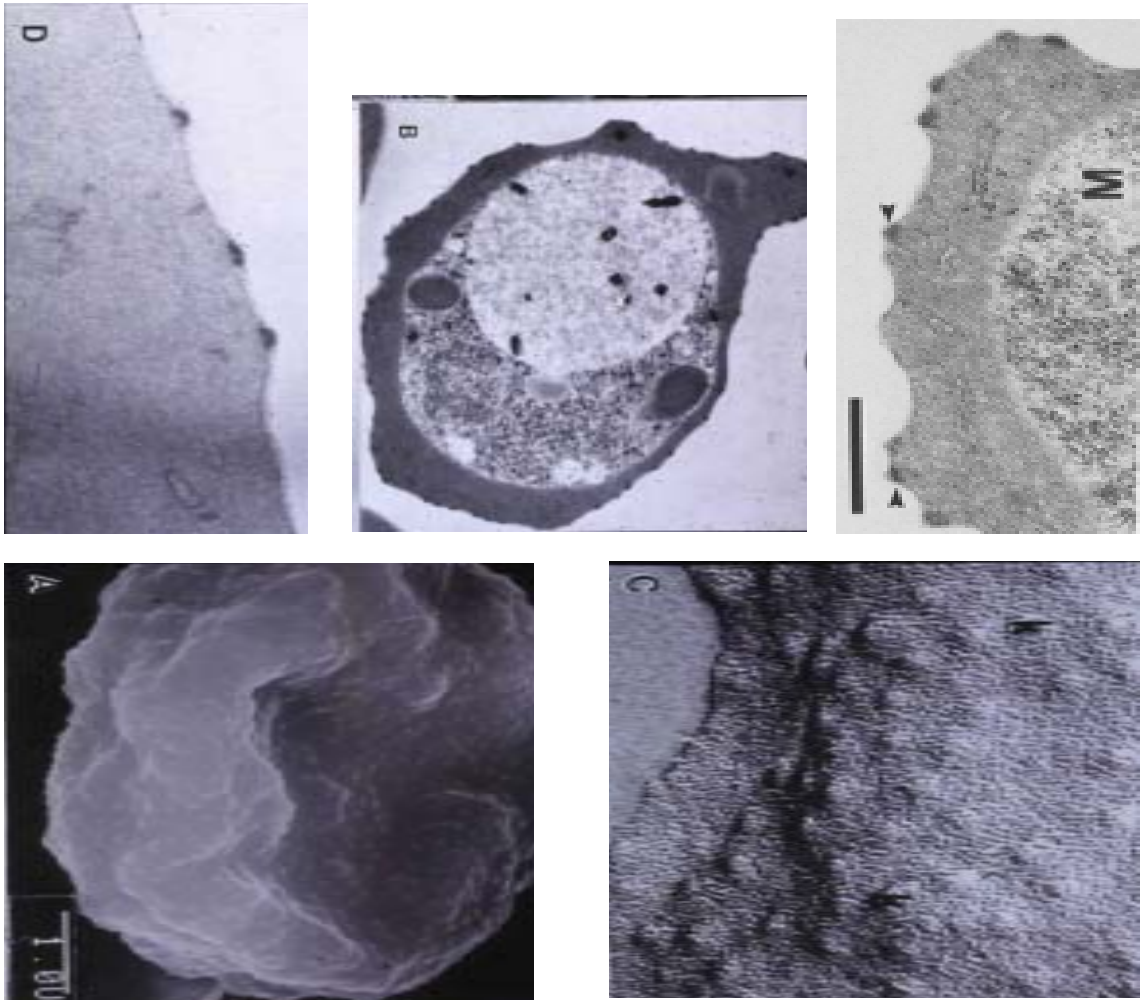


PfEMP-3, RESA, MESA FEST y FIRA (playa), KAHRP y PfEMP-1 (Centro)

Numerous proteins exported from the parasite interact with the red blood cell membrane skeleton proteins and dramatically alter their structure and function.

Cooke B. M., *et al*., 2004

Alteration of infected erythrocyte membrane

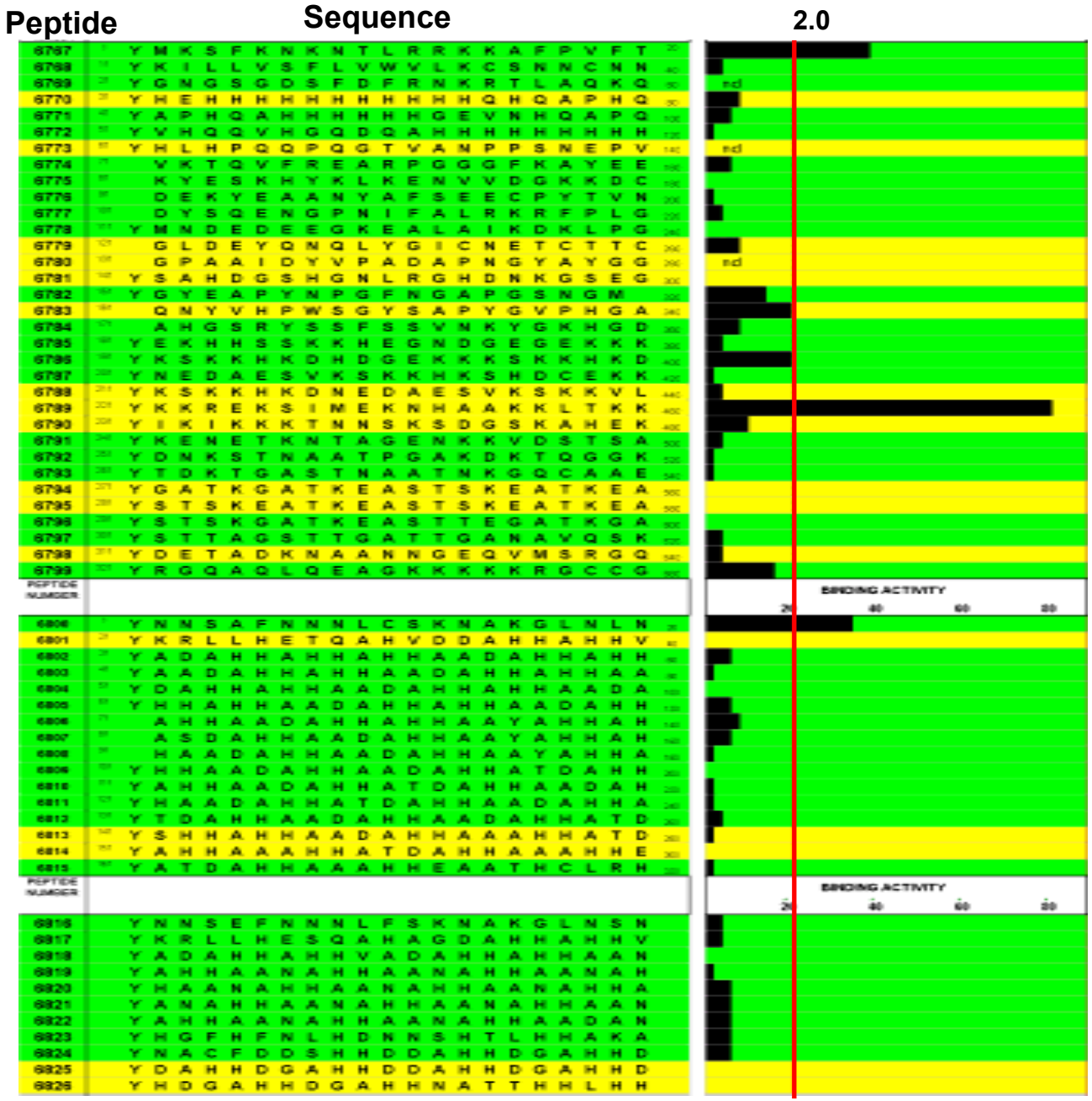


Knobs by A SEM, B TEM, C Freeze-fracture and D. TEM. Knobs are 40nm in height and 90-100nm in width, with an underlying electron-dense material containing several parasite proteins, including histidine –rich protein. PfEMP 1 (*Plasmodium falciparum* erythrocyte membrane protein 1) as well as a modified form of the intrinsic red cell membrane protein band 3 associated with the knob.

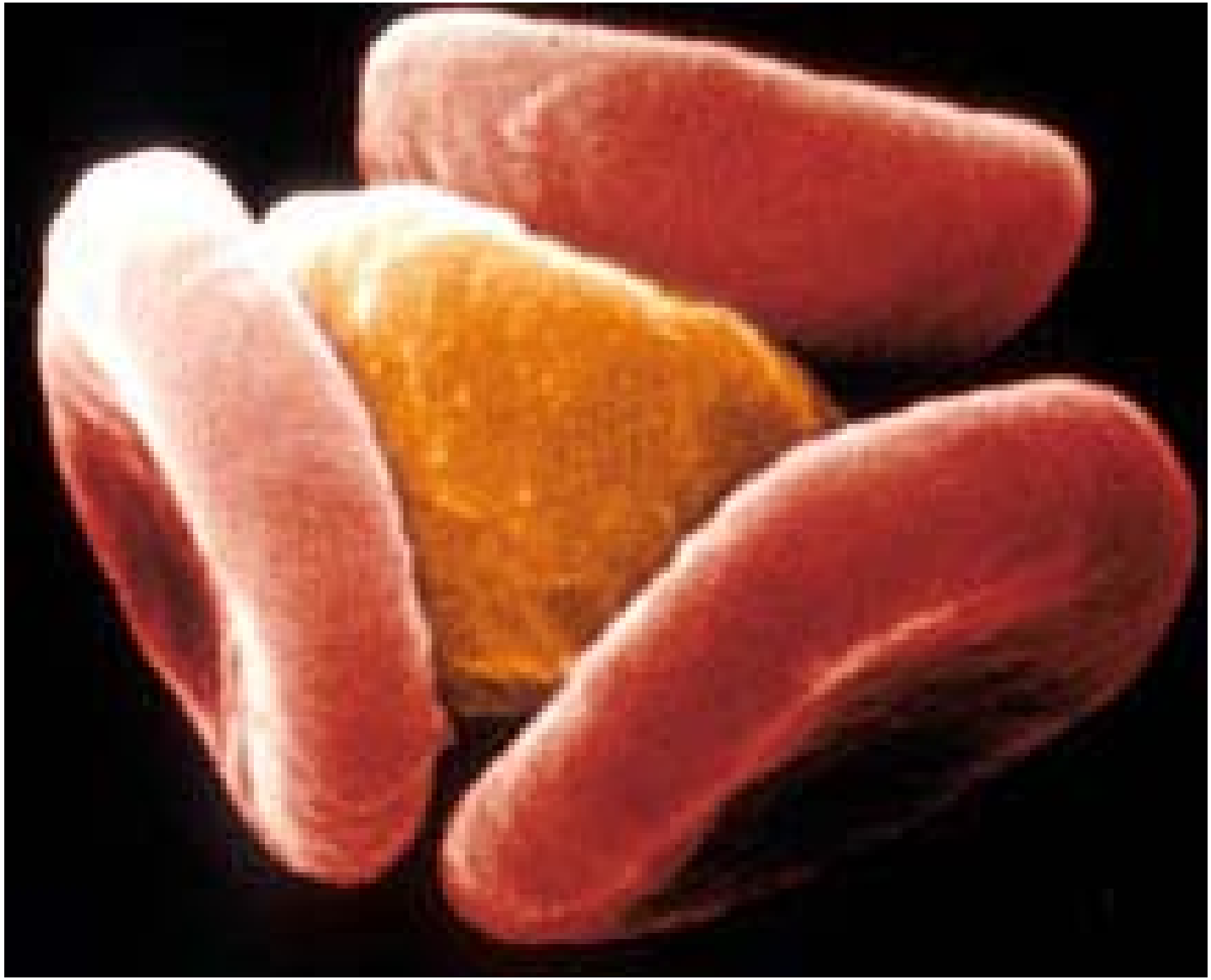
Sherman I. W., 1998, Anders R. F., *et al.* 1991

KAHRP I, HRPII and HRPIII

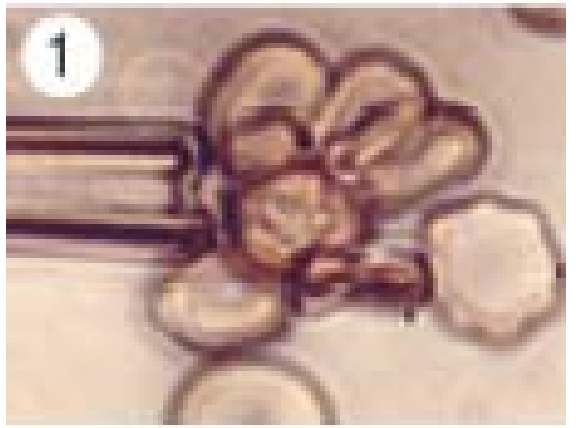
López R., *et al*., 2004, **Acta Tropica**, 75: 349

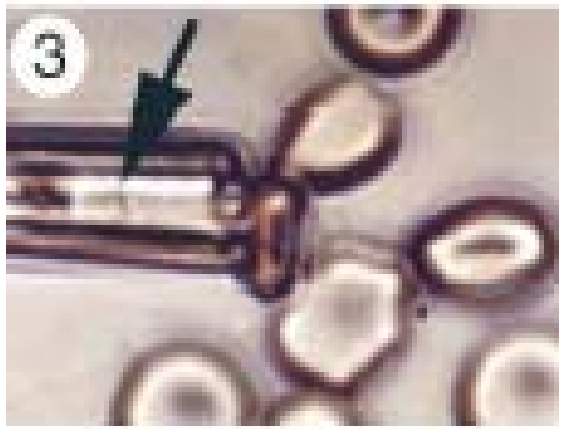


Peptide	Kd (nM)
6786	190
6800	210



Rosetting



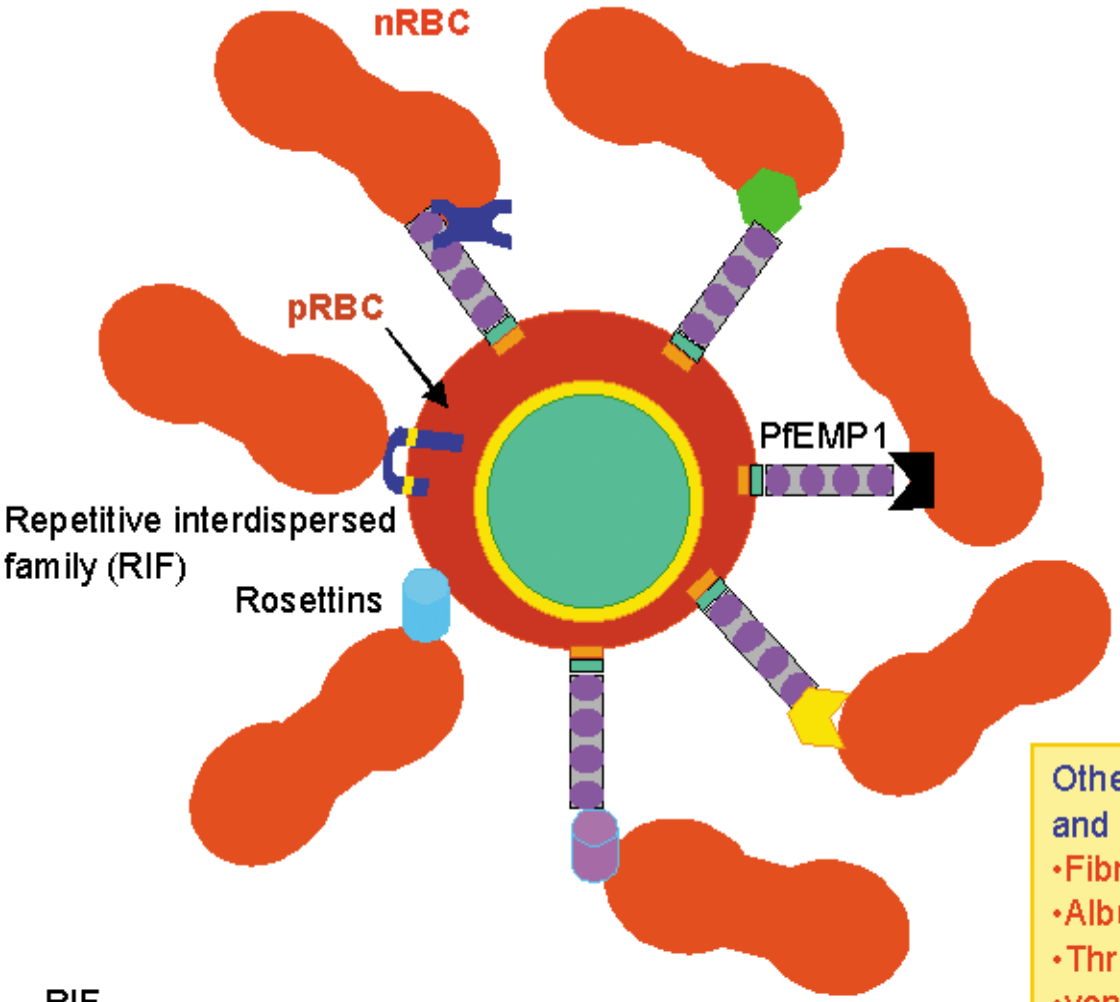


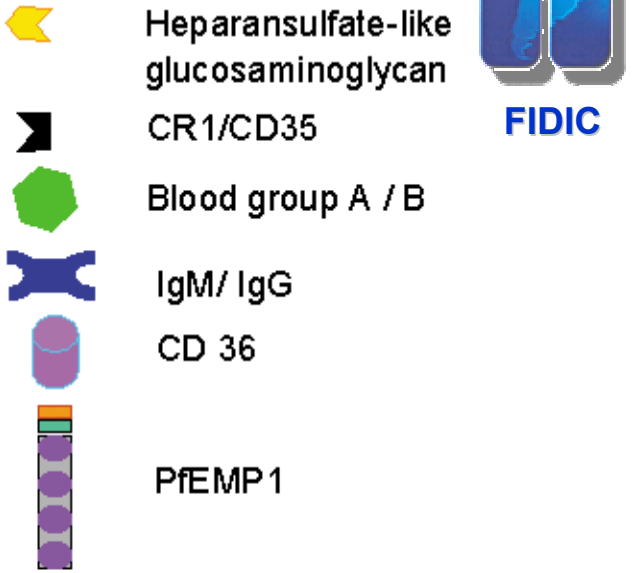
Rosetting, single *P. falciparum*–infected erythrocyte is seen by light microscopy held by a 5-*m*m micropipette. Uninfected erythrocytes are stripped off the infected cell and careful examination confirms that they are indeed infected by a single parasite.

Chen B. Q. *et al*., 1998

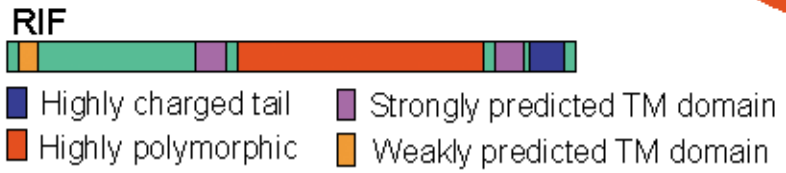
FIDIC

Rosette formation between normal and infected RBC





FIDIC



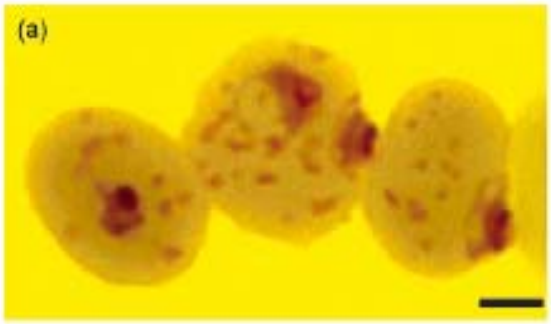
Other serum proteins that bind IRBC and may mediate rosetting
- Fibrinogen*
- Albumin*
- Thrombospondin
- von Willebrand factor

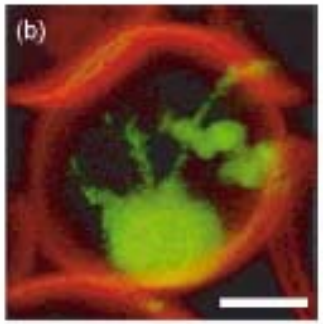
*also mediate rouleaux formation

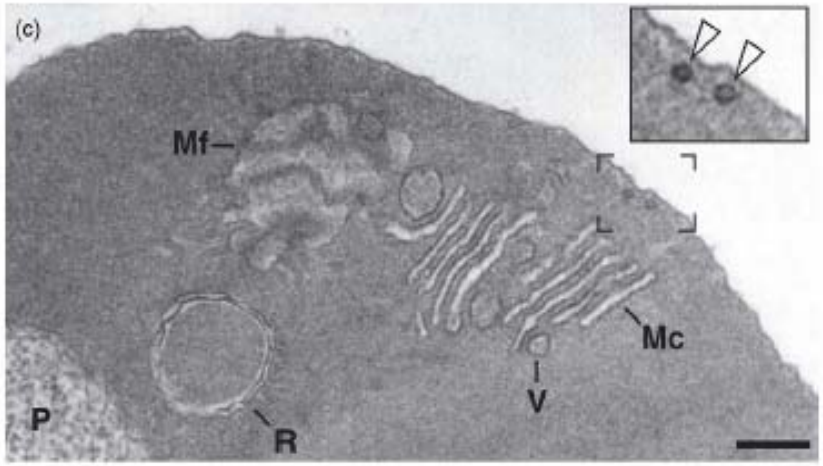
Vogt A. M. *et al.*, 2003

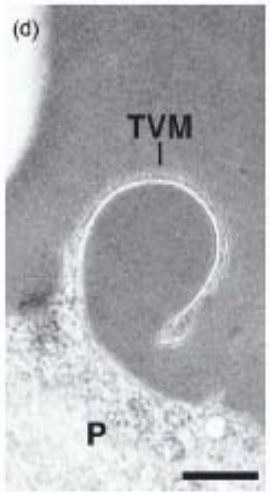
Membrane profiles in *P. falciparum*-infected erythrocytes

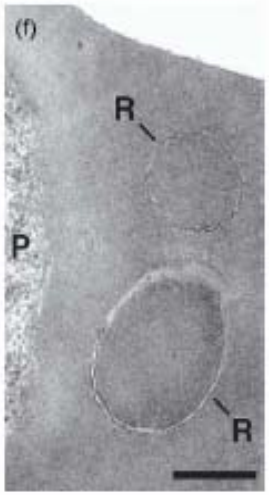
FIDIC





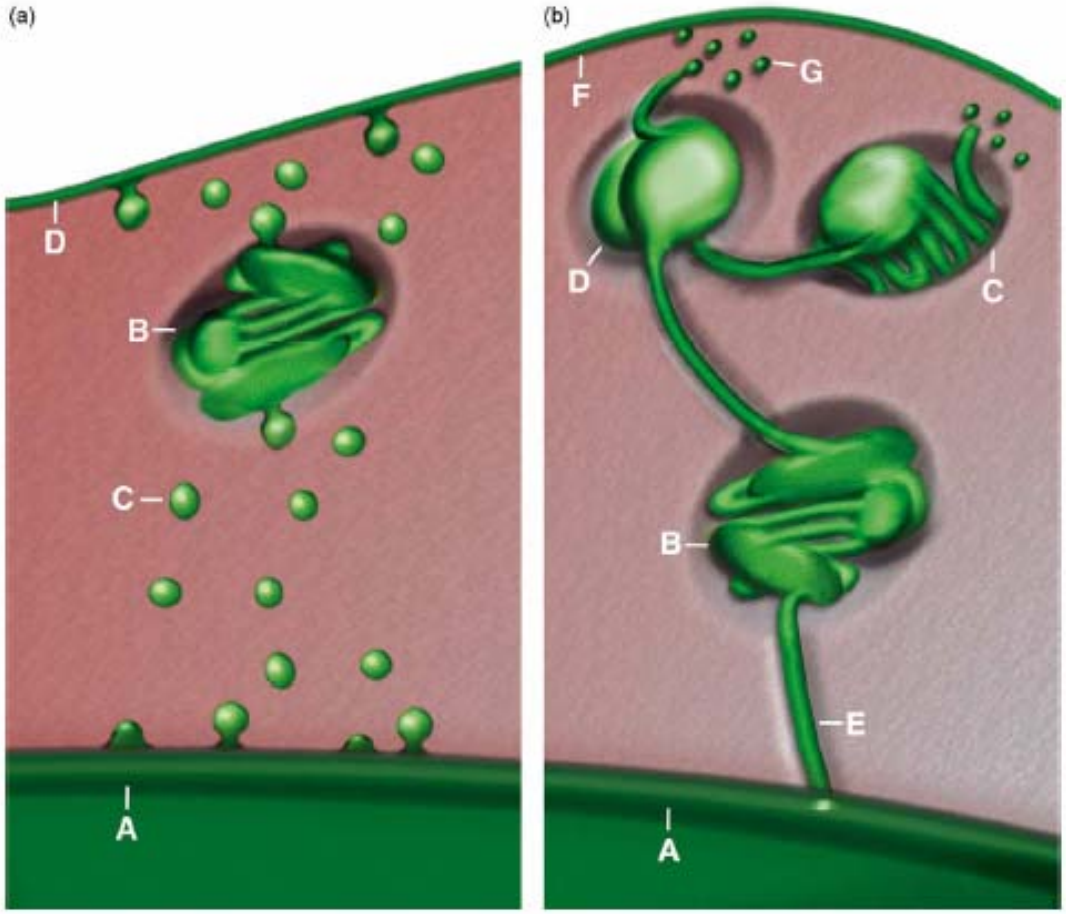






(a) Field-stained *P. falciparum*. This staining method identifies the parasite and reddish stained dot-like structures, corresponding to Maurer's clefts, within the erythrocyte cytosol. (b) Membrane visualisation by live cell fluorometry. Live infected erythrocyte were stained with the fluorescent dyes RH237 (red channel) and LysoSensor Green DND153 (green channel). (c) showing various membrane profiles in the cytoplasm of infected erythrocytes. Inset in (c) shows small vesicles of 15–25 nm in size, between the erythrocyte plasma membrane and Maurer's clefts. Mc, Maurer's clefts in cross section; Mf, Maurer's clefts in flat section; R, ring; V, vesicle; TVM, tubovesicular membrane network; W, whorls.

Przyborski J. M. *et al.*, 2003



Proposed models for protein transport to the plasma membrane of *P. falciparum*-infected erythrocytes. (a) Vesicular transport model and (b) Lateral diffusion model.

Przyborski J. M. *et al.*, 2003

Compilation of Maurer's cleft-associated *P. falciparum* proteins

FIDIC

Protein	Relationship to Maurer's clefts	Suggested function
KAHRP	Transiently associated with cytosolic face	Knob formation/presentation of PfEMP1
MAHRP	Resident/trans-membrane protein	Protection against oxidative stress/protein trafficking
Pf50/43[a]	Integral membrane proteins	Macromolecule transport?
Pf130	Peripheral membrane protein?	Unknown
Pf20, Pf29, Pf45[a]	Unknown	Protein transport?
Pf332	Transiently associated with cytosolic face	Unknown
Pf41-2	Membrane anchored?	Unknown
Pf45[a]	Unknown	Unknown
Pf46[a]	Integral membrane protein	Protein transport?
Pf50	Unknown	Unknown
Pf50, Pf41	Resident/peripheral membrane proteins	Underlying membrane skeletal network
PfEMP1	Transiently associated/trans-membrane protein	Cytoadherance/immune invasion
PfEMP3	Transiently associated with cytosolic face	Protein transport?
PfSar1p	Transiently associated with cytosolic face	Putative COPII
PfSBP1[a]	Resident/integral membrane protein	Anchoring of Maurer's clefts to erythrocyte cytoskeleton
PfSec23p	Transiently associated with cytosolic face	Putative COPI
PfSec31p	Transiently associated with cytosolic face	Putative COPII
PfSEP	Integral membrane protein	Parasite invasion?
STEVOR	Resident/trans-membrane protein	Immune evasion?

[a]It is possible that these studies may have independently identified the same protein.

Przyborski J. M. *et al.*, 2003

SERA/SERP

FIDIC

P. falciparum (126kD).

•This is secreted in the parasitophorous vacuole during trophozoite maturation.

•The gene encoding a protein having a signal sequence, with no transmembrane domain.

•It is processed into 3 fragments: 50, 43 and 18 kDa. The last 2 are bound by cysteines.

•SERA is implicated in merozoite liberation.

•PfSERP-H.

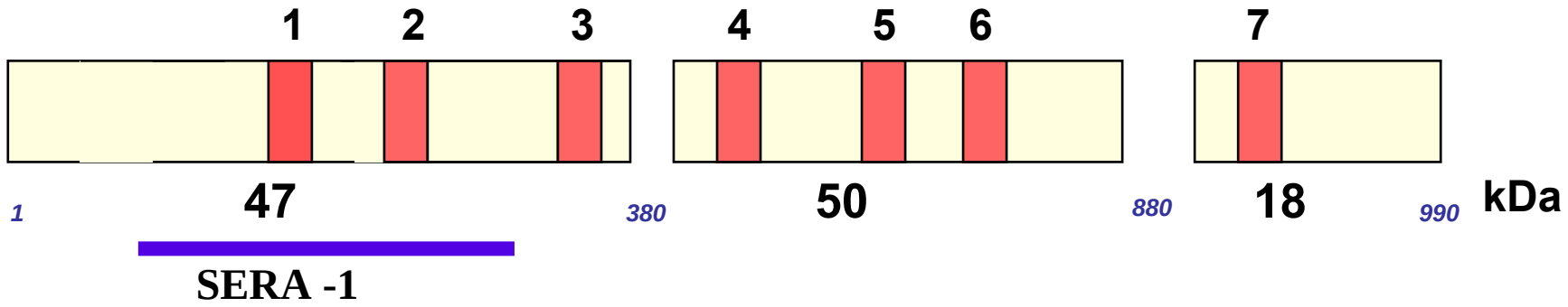
P.vivax

•5 genes have been identified in tandem encoding homologous sequences with PfSERA/SERP.

•Its real function may be quite different to that of having proteolytic activity.

Delplace *et al.*, 1988, Kiefer *et al.*, 1996

SERA/SERP



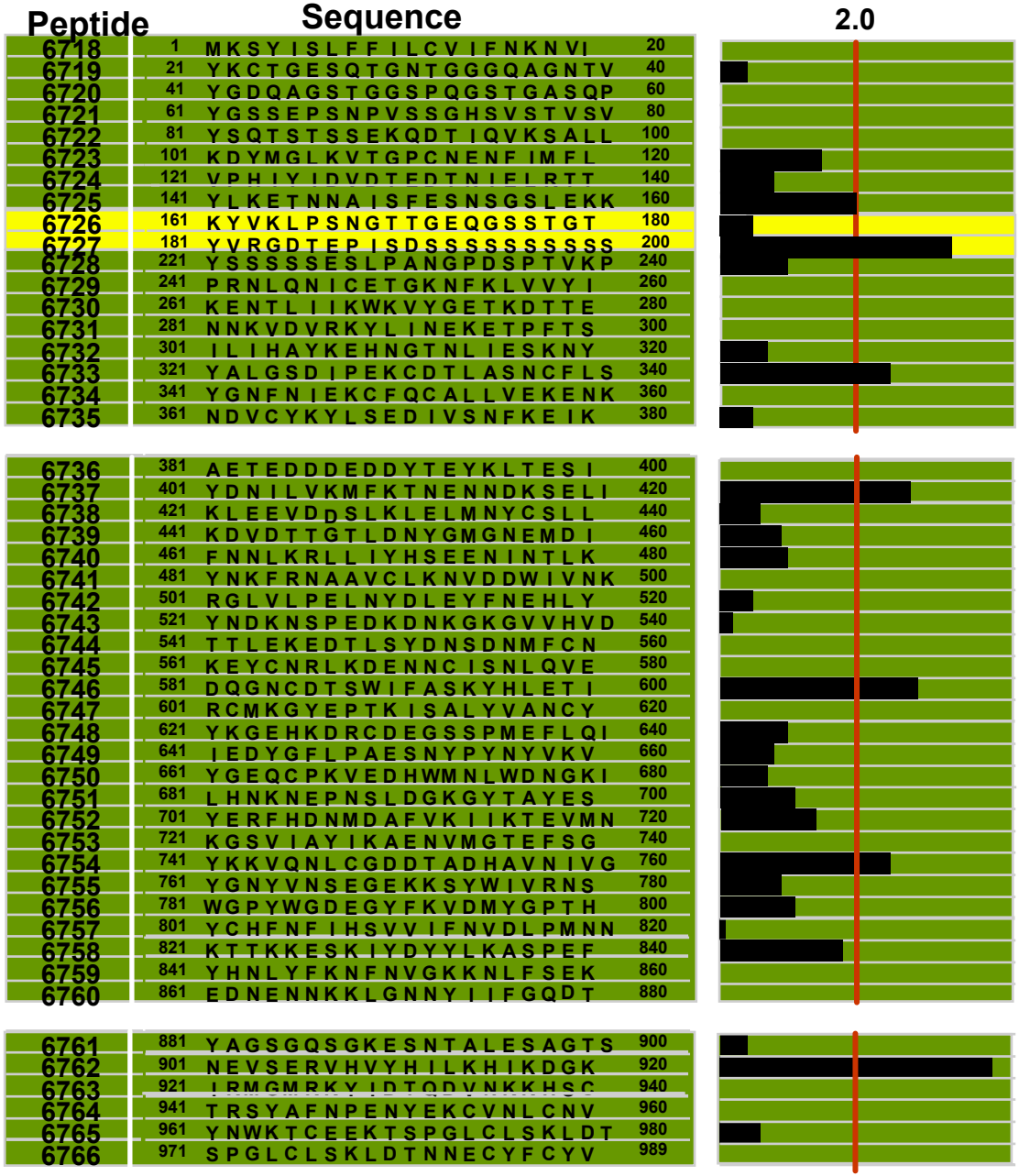
1. LKETNNAISFESNSGSLEKK
2. vRGDTEIPSDSSSSSSSS
3 ALGSDIPEKCDTLASNCFLS
4. DNILVKMFKTNENNDKSELI
5. DQGNCDTSWIFASKYHLETI
6. KKVQNLCGDDTADHAVNIVG
7. NEVSERVHVYHILKHIKDGK

LKETNNAISFESNSGSLEKK KYVKLPSNG

Inselburg, 1991, Puentes A. *et al.,* 2000, Weber J. L. *et al.,* 1997

SERA

Puentes A., *et al.*, 2000, **Parasitol International**, 49: 105



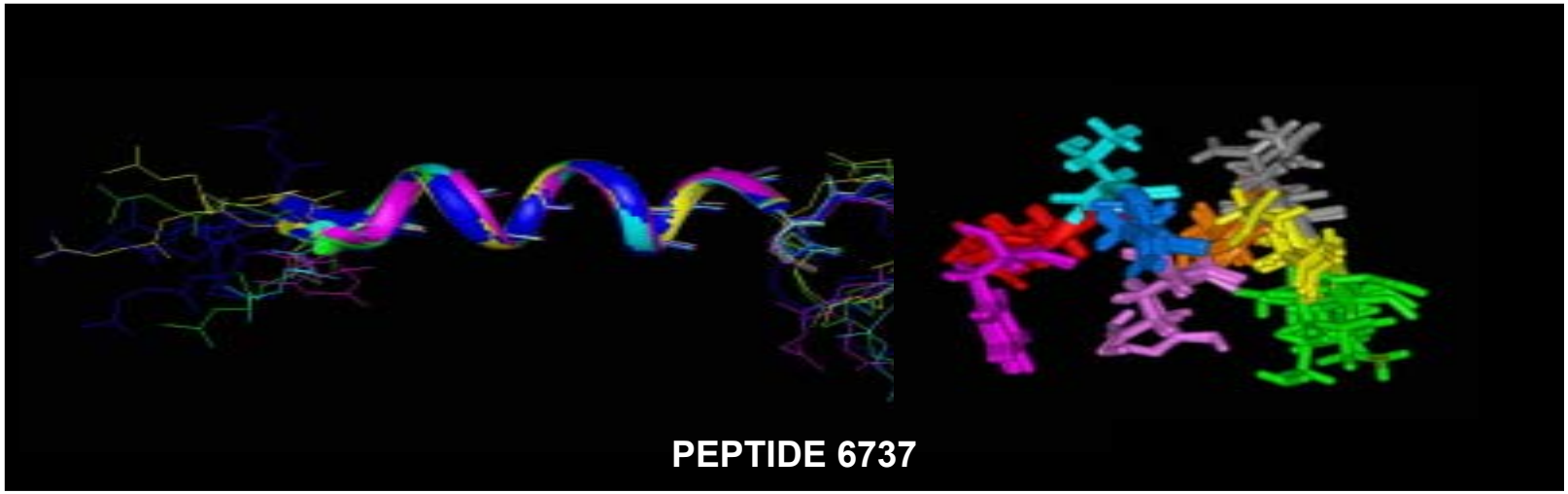
Peptide	Kd (nM)
6725	750
6733	550
6737	500
6746	150
6754	1100
6762	350

FIDIC

Structural features for HABPs SERA

Cubillos M., *et al*., 2003, **Biochimie**, 85: 651

FIDIC



Other proteases

gp76 *P. falciparum*

•This is a 83/76 kDa protease localised in the rhoptries.

•It becomes activated on being liberated from GPI anchoring on the membrane.

•It has a 68 kDa homologue in *P.chabaudi* which is activated in the same way.

•Its specificity is classified as chymotrypsin neutral serine proteases.

•A external fragment from Band 3 can be cleaved from intact erythrocytes; this seems to be required for *P. falciparum* and *P. chabaudi* invasion.

P. falciparum cysteine protease

•This is an 68 kDa neutral protease.

•It is inhibited by leupeptin and antipain.

•It is localised in the apical part.

•The loss of its activity inhibits *P. falciparum* merozoite *in vitro* invasion.

Braun-Breton et al,1988, Roggwiller *et al*., 1996,
Braun-Breton *et al.*,1992, Grellier *et al*, 1989

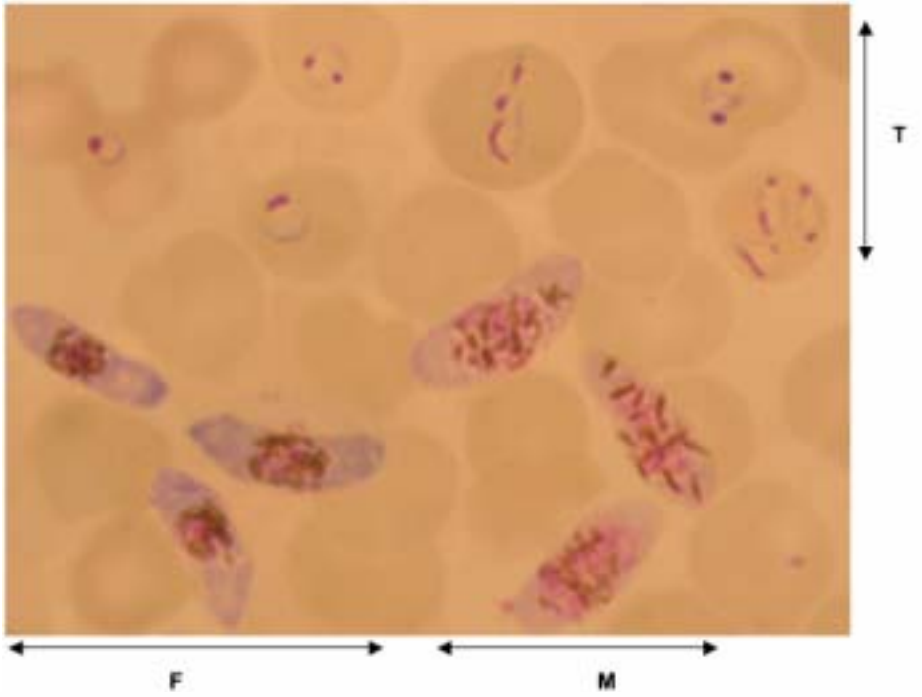


Figure 1
Mature female (F) and male (M) gametocytes and trophozoites (T) of *Plasmodium falciparum* in the blood of malaria-infecte patient. This picture is a composite of several pictures originating from the same Giemsa-stained thin smear.

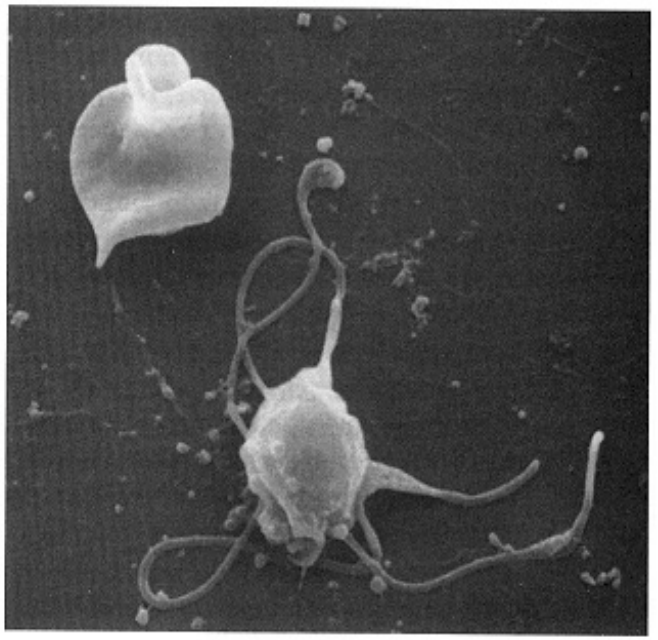
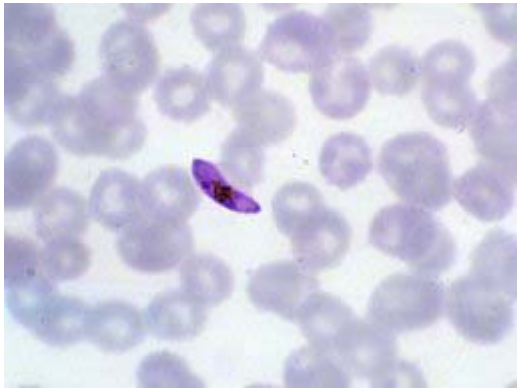
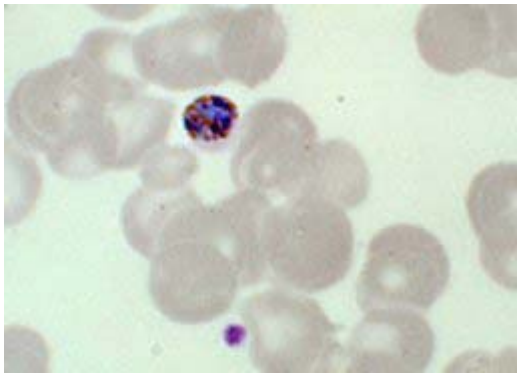


Figure 10. Scanning electron micrograph of exflagellating male gametocyte of *P. yoelii*.

Contreras-Ochoa C., *et al*., 2004,
http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Malaria_il.asp?body=M-R/Malaria/falciparum/body_Malaria_falciparum_il4.htm



Plasmodium falciparum gametocytes: *P. falciparum* gametocytes have a crescent or banana shape.



Plasmodium malariae gametocytes: *P. malariae* gametocytes have a round shape about the size of red blood cells. They have a fine granular appearance.

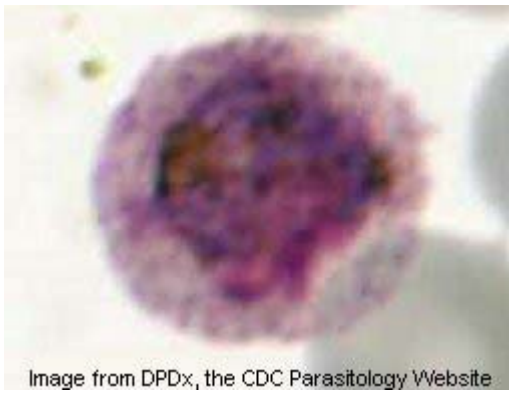


Image from DPDx, the CDC Parasitology Website

Plasmodium ovale gametocytes: these are round gametocytes which are larger than normal red blood cells. They have a granular appearance as well as Schuffner's dots.

http://www.msu.edu/course/zol/316/psppscope.htm

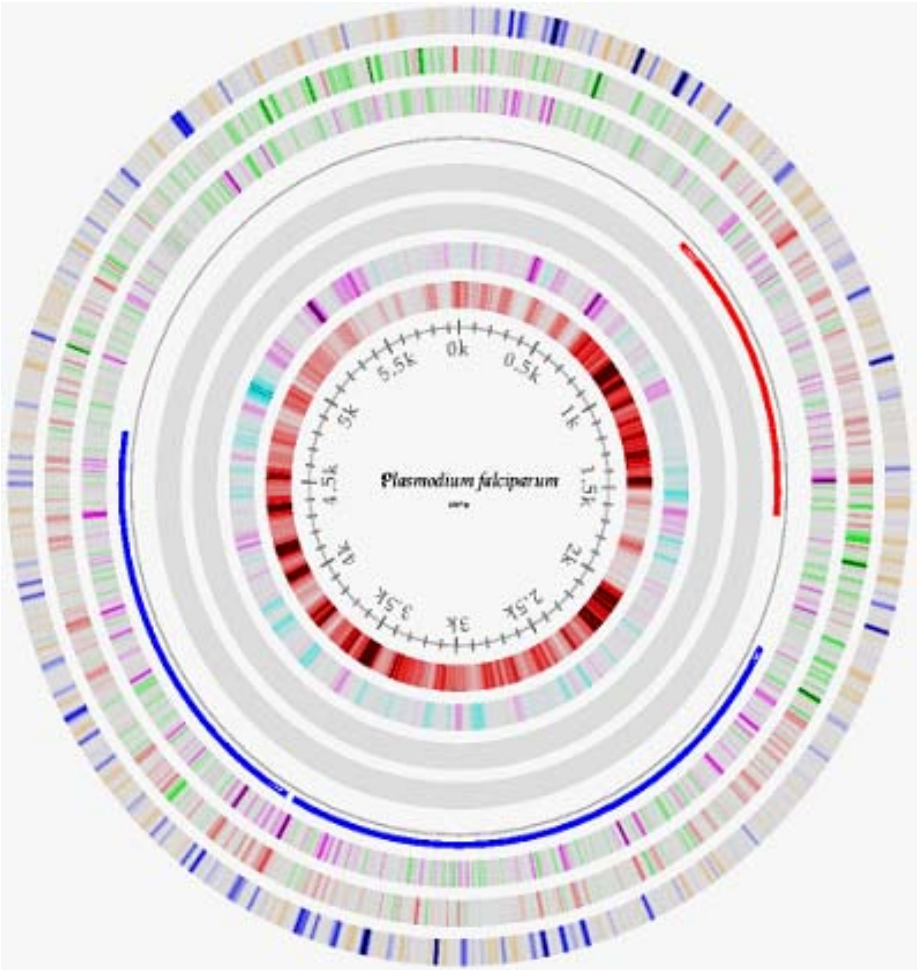
Cuadro I

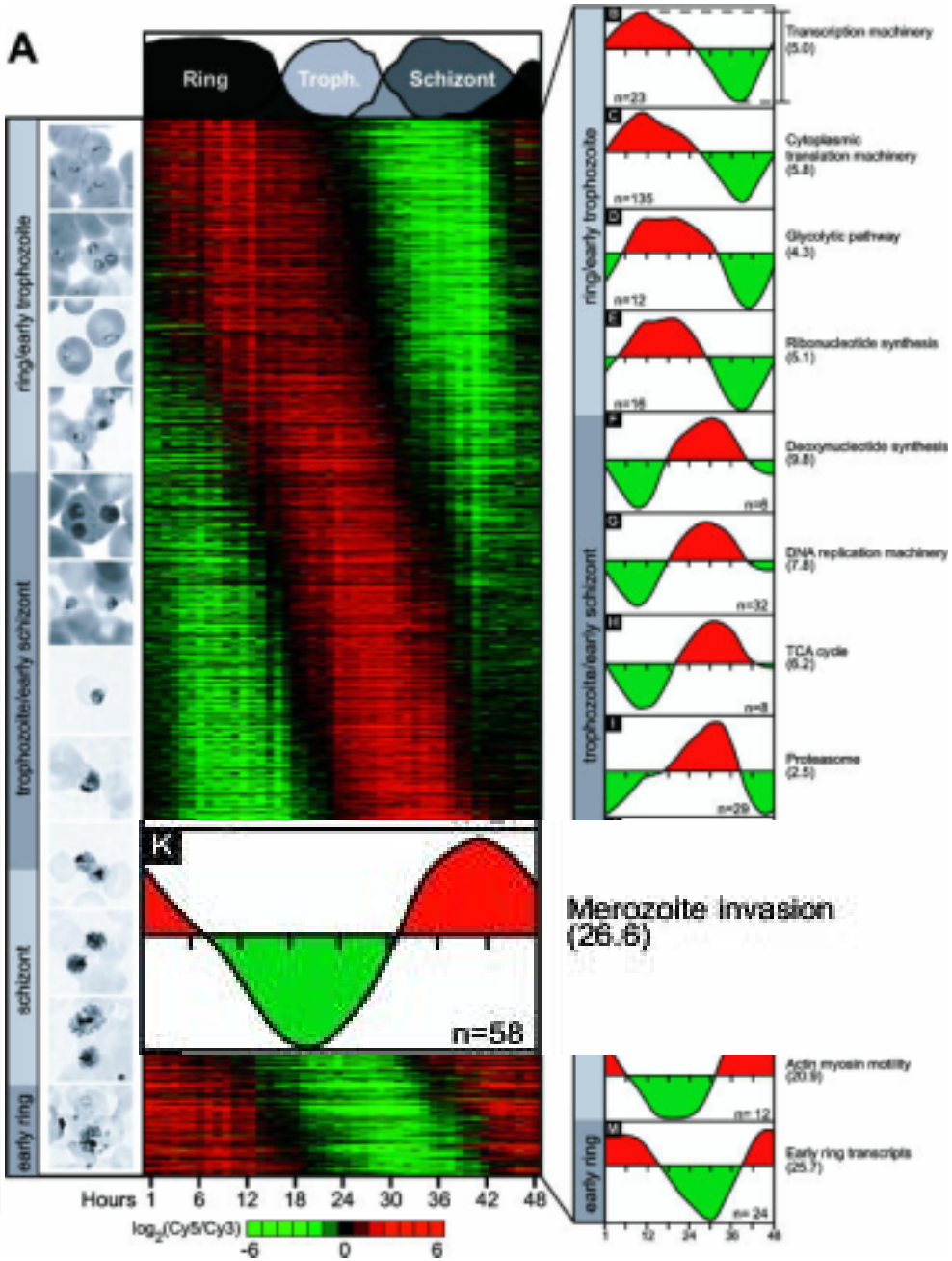
PROTEÍNAS DE GAMETOCITOS DE *PLASMODIUM VIVAX* Y *P FALCIPARUM*

Proteína	*Especie*	*Ubicación*	*Comentario*
Pf11-1	Pf	Vacuola parasitófora de gametocitos	Contribuye a la ruptura del eritrocito durante la gametogénesis[52]
Pf16	Pf	Proteína integral de membrana de gametocitos	Contribuye al bloqueo de la transmisión, candidato a vacuna[53]
Pvs20	Pv	Gametocitos	Función desconocida[54]
Pvs24	Pv	Gametocitos	Función desconocida[54]
Pf25	Pf	Citoplásmica, se expresa en la superficie de gametos, zigoto y ooquineto	Contribuye al bloqueo de la transmisión[55]
Pvs25	Pv	Gametocitos	Contribuye al bloqueo de la transmisión[56]
Pfg27	Pf	En gametocitos desde etapas tempranas	Contribuye al bloqueo de la transmisión[3]
Pvs28	Pv	Gametocitos	Contribuye al bloqueo de la transmisión[56]
Pv42/37	Pv	Citoplasma de macrogametocitos	Función desconocida[54]
Pfs48/45	Pv	Citoplasma de gametocitos y en la superficie de gametos	Contribuye al bloqueo de la transmisión[3]
Alfa tubulina 50 kDa	Pv	Axonema de microgametocitos	Participa en los cambios morfológicos durante la exflagelación y en la motilidad del parásito[57]
Pvs57	Pv	Gametocitos	Función desconocida[54]
Pfs230	Pf	Superficie de gametocitos y gametos	Contribuye al bloqueo de la transmisión[58]
PfEMP-1	Pf	Gametocitos desde etapas tempranas	Contribuye a la producción de gametocitos, regulando su maduración[59]
Pfg377	Pf	Macrogametocitos maduros	Función desconocida[60]
PSLAP	Pf	Gametocitos maduros	Participa en la modulación y protección contra el sistema inmune del mosquito[61]

Contreras-Ochoa C., *et al*., 2004,

Plasmodium falciparum 3D7 genome





Hall N. *et a*l 2002, Gardner M. J., *et al* 2002, Florents L. *et al* ., 2002.

Receptors involved in merozoite invasion

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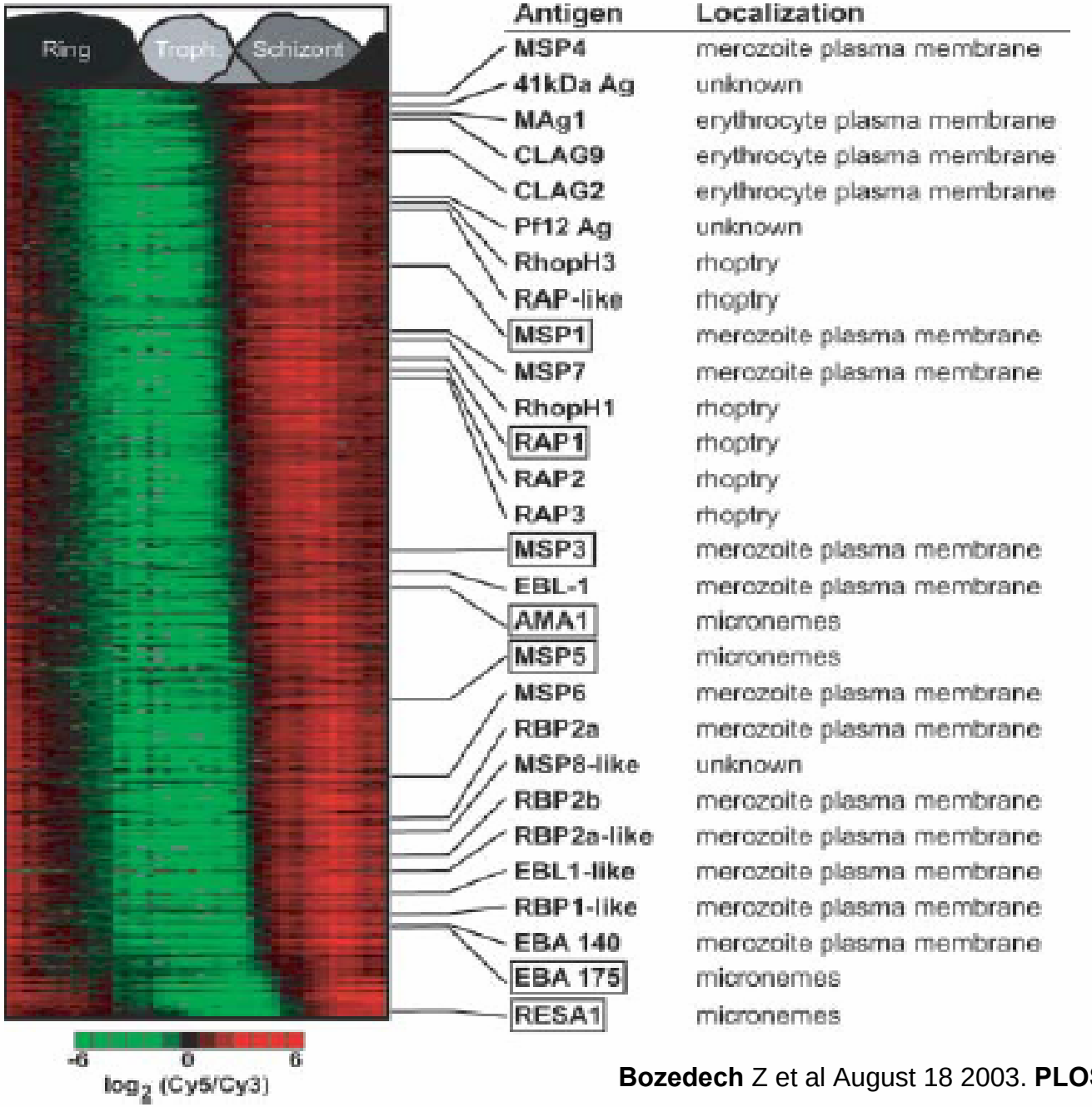
Receptors on RBCs for invasion.

*Glycophorin A
*Glycophorin B
*Glycophorin C
*Band 3
*Receptor X
*Receptor Y
*Receptor Z
*Receptor E

Receptors on endothelial cells for cytoadherence

*ICAM1
*ICAM2
*PECAM1
*VCAM1
*E-selectin
*TSP
*CD31
*CD36
*$\alpha_V\beta_3$Integrin
*HS
*BgA
*CR1
*IgM
*CSA
* Hyaluronate



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Bozedech Z et al August 18 2003. **PLOS Biology** 1, 001

High RBC binding activity peptides

PROTEIN	C	V	Reference
MSP1	3	6	Urquiza M. *et. al* . 1996. *Parasite Immunology* . 18: 515-526
RESA	2	–	Vera R. *et al* . 2000. *Vaccine.* 18: 1289-1293
SERA	6	1	Puentes A. *et. al* . 2000. *Parasitology International.* 49: 105-117
MSA2	1	2	Ocampo M. *et. al* . *J. Peptide Research.* 55: 216-233
EBA175	6	–	Rodríguez L.E *et. al.* 2000. *Parasitology.* 120: 225-235
GBP130	1	–	Suárez J. *et. al* . 2000. *Mem. Inst. Oswaldo Cruz* . 95: 495-501
HRP I, II, II	3	1	López R. *et a* l. 2000. *Acta Tropica.* 75: 349-359
ABRA	5	–	Curtidor H. *et. al* . 2001. *Vaccine.* 19: 4496-4504
AMA1	4	3	Urquiza M. *et. al* . 2001. *Vaccine.* 19: 508-513
EBA140	6	–	Rodríguez L.E.. *et. al* . 2003. *J. Peptide Research.* 62: 175-184
NBP1	2	–	Valbuena J. *et al* . 2003. *Peptides* . 24: 1007-1014
MSP8	5	–	Puentes A. *et. al.* 2003. *Peptides* .24: 1015-1023
MAEBL	9	–	Ocampo M. *et al* . 2004. *Biochem. Biophys. Res. Comm. 315: 319-329*
RAP2	4	–	López R. *et al.* 2004. *Biochemie* . 86: 1-6
RBP2 Ha/Hb	9	3	Ocampo M. *et. al* . 2004. *Parasitol. Int.* 53: 77-88
RAP1	4	–	Curtidor H. *et. al* . 2004. *Vaccine* . 22: 1054-1062
EBA160	5	–	Valbuena J. et al. 2004. *Biochem. Biophys. Res. Comm* . 321: 835-844
CLAG3	5	–	Ocampo M. et. al. 2005. *Proteins Science* . 14: 504-513
EBL1	5	–	Curtidor H. *et. al.* 2005. *Protein Science* . 14: 464-473
JESEBL	5	–	Vera R. *et al* . 2004. *Biochemie. In press*
STEVOR	–	3	García J. *et al.* 2004. *Peptides. In press*
MSP10	3	–	Puentes A. *et al* . 2004. *Biochemie. In press*
MSP3	3	–	Rodríguez L.E. *et. al.* 2004. *Submitted to Protein Science*
SORTILIN	6	–	Vera R. *et al.* 2004. *Submitted to Biochemistry*
RESA-LIKE	3	–	Rodríguez L.E. *et. al.* 2004. *Submitted to J. Int. Parasitol.*
TryThrA	4	–	Curtidor H. *et. al.* 2004. *Submitted to Chembiochem*
HAP	2	–	Valbuena J. *et. al* . 2004. *Submitted to Biol. Chem.*
MSP6	2	1	López R. *et al.* 2004. *Submitted to Peptides*

C : CONSERVED V: VARIABLE

FIDIC's receptor-ligand group

Ricardo Vera, Marisol Ocampo, John J. Valbuena, Ramsés López — front row
Álvaro Puentes, Hernando Curtidor, Javier E. García, Luis E. Rodríguez — back row

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