

# Mapping of Merozoite Surface Protein 1-19 Binding Regions in Band 3 Peptide of Red Blood Cell Membrane

*Heather Mentzer<sup>1</sup>, Lixiao Zeng<sup>2</sup>, Christos G. Takoudis<sup>2</sup>, Athar H. Chishti<sup>3</sup>, University of South Carolina<sup>1</sup>, Chemical Engineering Department<sup>2</sup>, Pharmacology Department<sup>3</sup>, University of Illinois at Chicago*

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# Acknowledgements



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Professor Athar H. Chishti and Lixiao Zeng  
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# Outline

- Overall project description
- Information on Malaria
- Description of Merozoite surface protein 1-19 in plasmodium falciparum parasite
- Description of the Band 3 peptide in red blood cell (RBC) membranes
- Biacore optical sensor
  - Surface plasmon resonance
- Results
- Conclusions

# Overall Purpose and Project

- Understand the mechanism of Band 3 as a crucial invasion receptor for the malaria parasite.
- From this research new vaccines can be discovered
- Project: Evaluate and analyze the binding sites for \*MSP1-19 in Band 3 peptide region.
- Experiments: concentration dependence
  - \*GST-\*5ABC, GST-6A, GST-5, GST-6, GST-5ABC6A, GST-6A5ABC with \*Trx-19, \*Trx-19A, and Trx-19B

\*MSP1-19 is Merozoite Surface Protein 1, 19 is the molecular weight in kDa

\*glutathione S-transferase (GST), thioredoxin (Trx)- these are fusion components

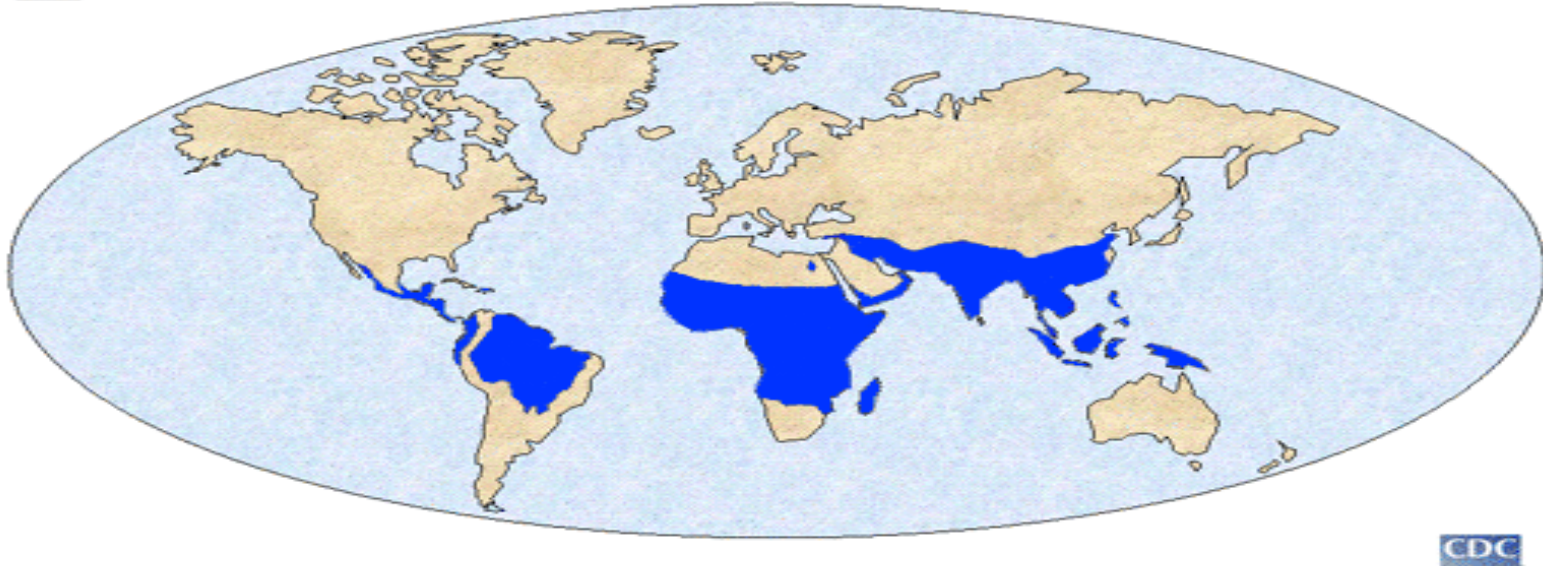
\*5ABC, 6A, 5, 6, 5ABC6A, 6A5ABC are segments in band 3 peptide.

\*Trx-19, Trx-19A, Trx-19B are the MSP1-19, MSP1-19A, MSP1-19B protein

# Malaria Statistics

## Geographic Distribution of Malaria

 Distribution of Malaria



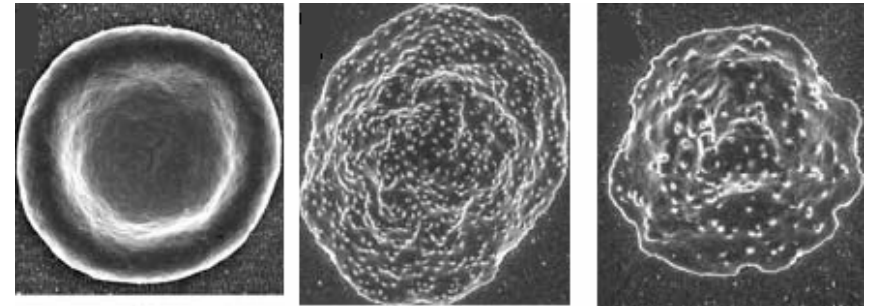
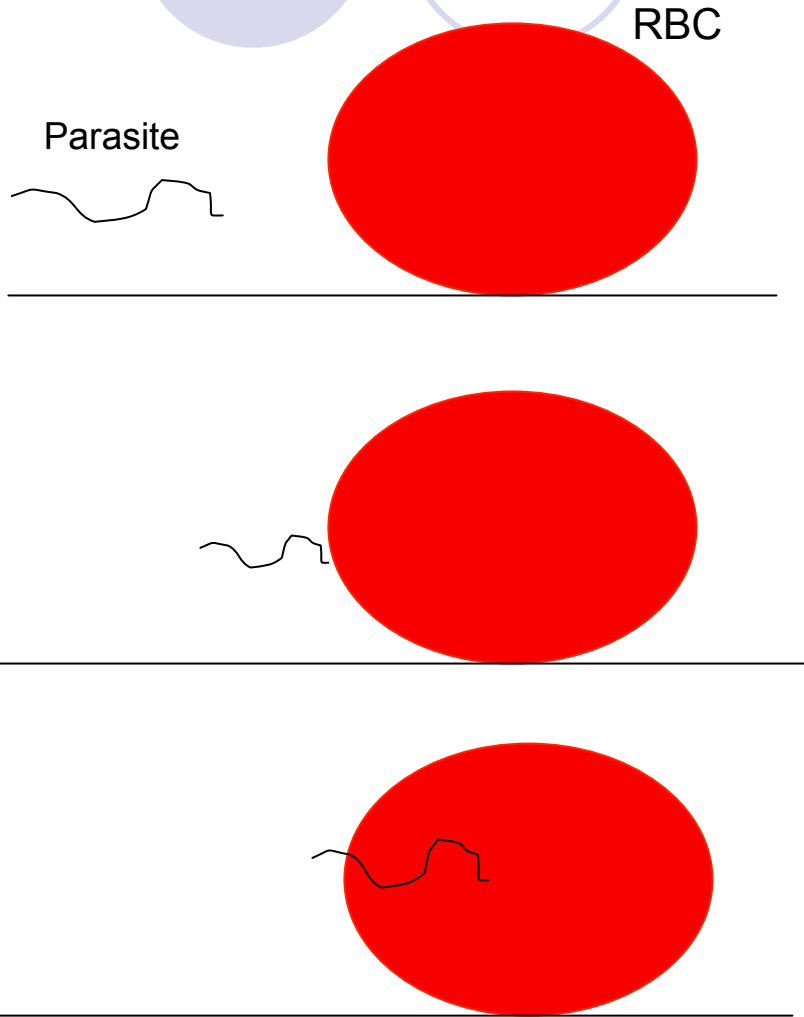
- Figure depicts the distribution of malaria. This doesn't mean that areas not designated doesn't get the disease.
- 1 million deaths are attributed to malaria yearly
- Over 40% of the world's population is affected by this disease
- Over 300 million cases that report severe symptoms yearly
- Plasmodium falciparum
  - One of the four strands of malaria
  - This is the most widespread and dangerous of the strands

# Mechanism of the invasion of *p. falciparum*

- \**P. falciparum* invasion is possible by the erythrocyte inside the RBC
- Two regions in the band 3 peptide may be the host receptors in the RBC membrane
  - 5ABC, 6A
- *P. falciparum* Merozoite Surface Protein 1 (MSP1) is a major membrane protein
  - Attaches to the merozoite surface from its C-terminus\*\*
  - MSP1 is essential in the invasion of *p. falciparum*
    - We use MSP1-19 to see if the protein plays a role in the invasion of RBCs.

\*\*C-terminus is the carboxyl group at the end of the MSP1-19 strand

# Mechanism of the invasion of *p. falciparum*



A

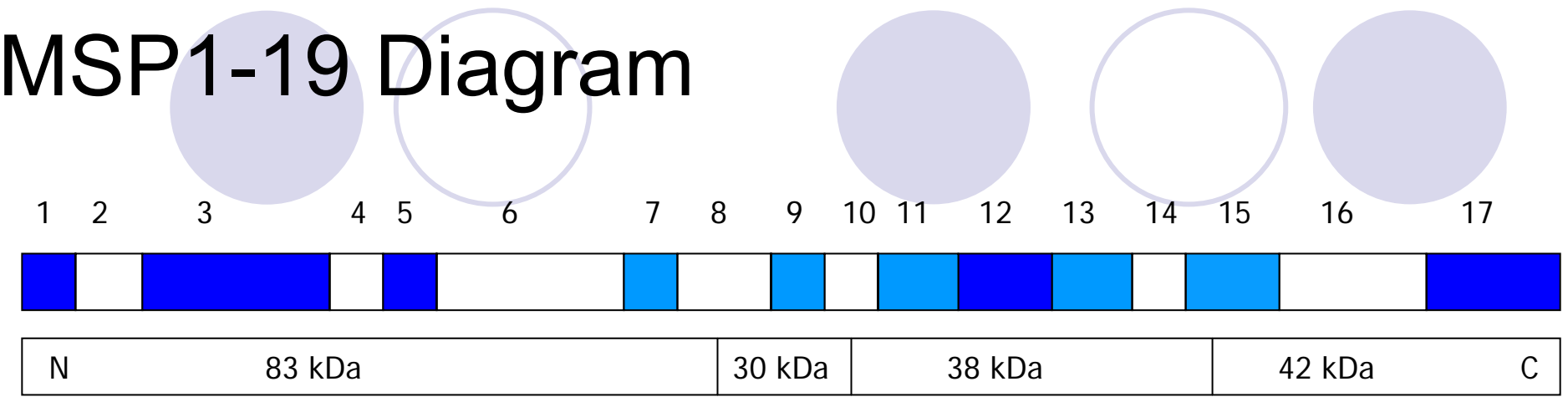
B

C

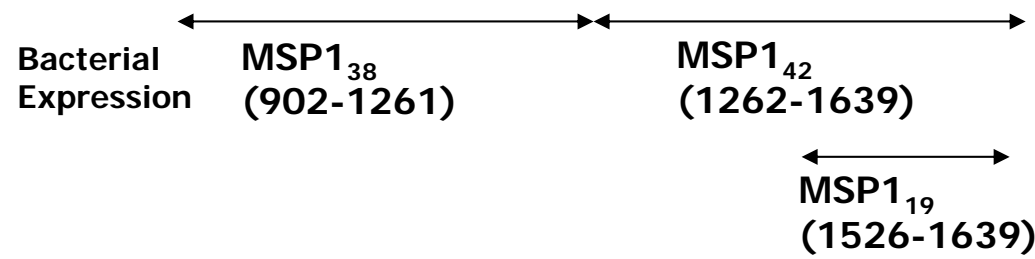
- A. Normal red blood cell\*
- B. Parasite infected RBC-knobs
- C. Close up view of affected RBC

\* Fairhurst, Rick M.; Baruch, Dror I; al ect. *Abnormal display of PfEMP-1 on erythrocytes carrying haemoglobin C may protect against malaria.* Nature, vol 435, issue7045, Pg 1117- 1121. Jun 2005.

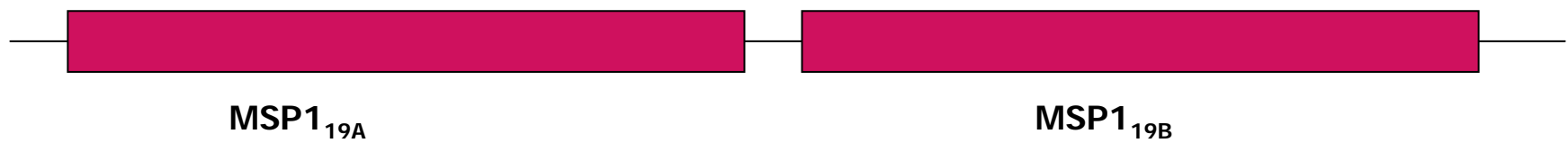
# MSP1-19 Diagram



- Conserved Region
- Susceptible Binding sites
- Not considered



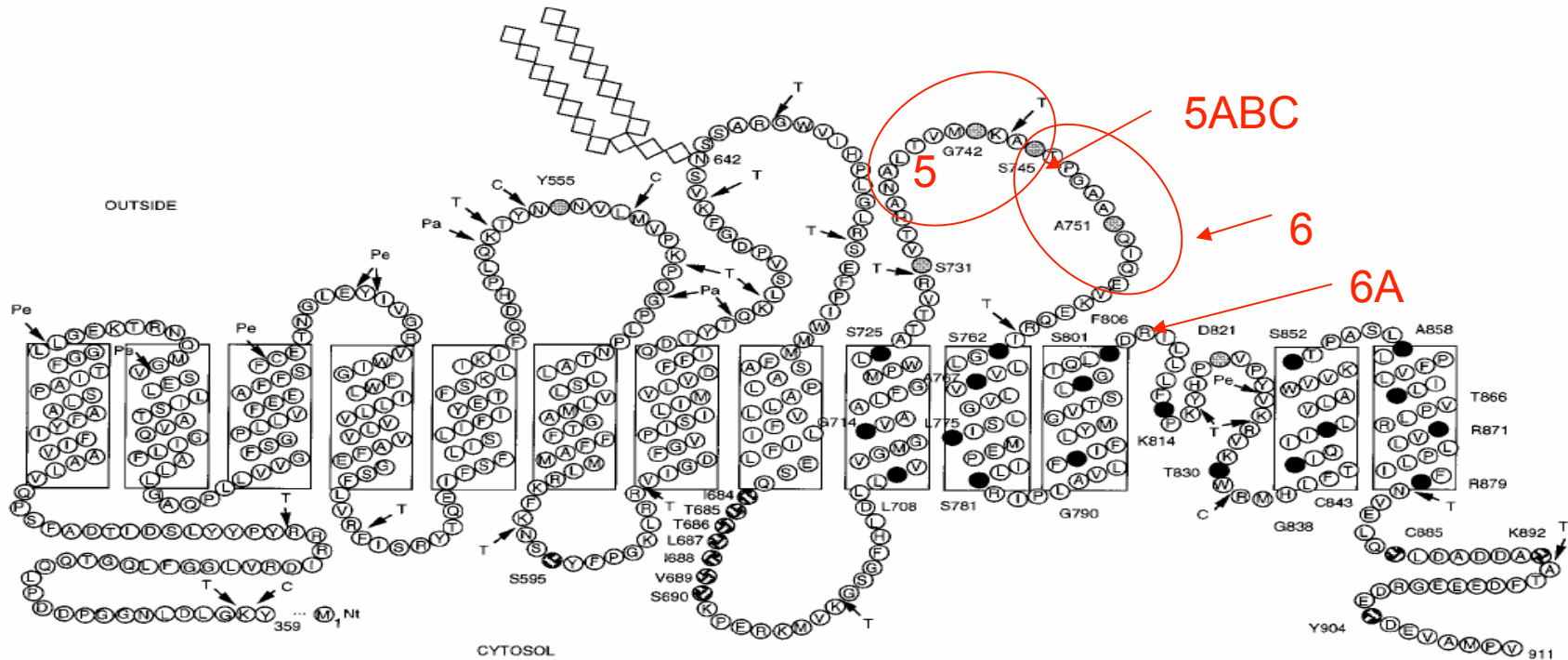
# -the sequence of the MSP1 strand



- MSP1-19 is used to see whether this protein plays a role in the invasion of RBCs.
- Truncated MSP1-19A, MSP1-19B will be used in the mapping of the binding sites of MSP1-19

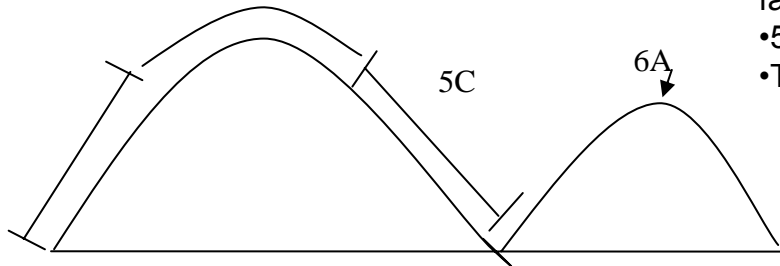


# Band 3 Peptide diagram



5B

5A



- Band 3 peptides are more susceptible to invasion by plasmodium falciparum
- 5ABC is a major segment in the band 3 peptide
- Test 6A to see how it contributes to the binding

# Biacore Optical Sensor



\*Biacore 1000-an early model

- BIA- biospecific interaction analysis
  - Utilizes surface plasmon resonance to perform protein interaction analysis
- \*Biosensor technology
  - Information that can be obtained, specificity, affinity, concentration and kinetic parameters
- \*Sensor chip:
  - CM5
    - Carboxy methyl dextran
    - Consists of a glass support with a thin layer of gold

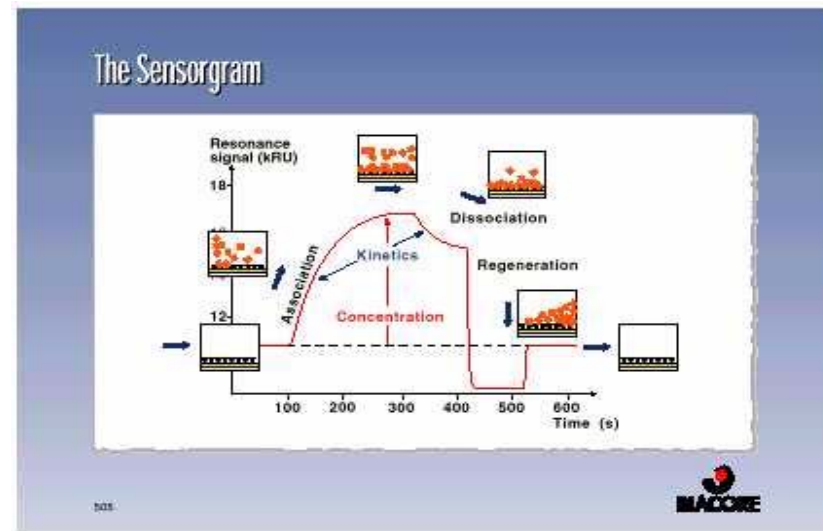
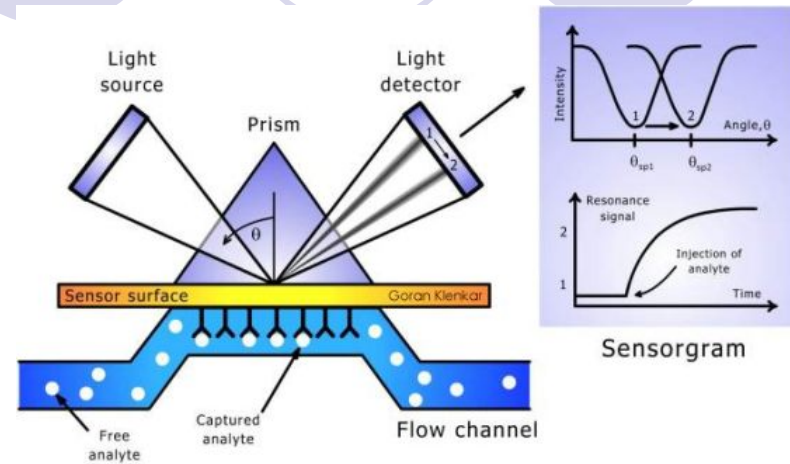
\*[giga.sct.ub.es:800/ImatgesHQ/biacore.jpg](http://giga.sct.ub.es:800/ImatgesHQ/biacore.jpg)

\*[www.biacore.com](http://www.biacore.com)

\*Jonsson, U.; Fagerstam, L.; al ect. *Real-Time Biospecific Interaction Analysis using Surface Plasmon Resonance and a Sensor Chip Technology*. BioTechniques, Vol 11, issue 5. Nov. 1991 pg. 620-627

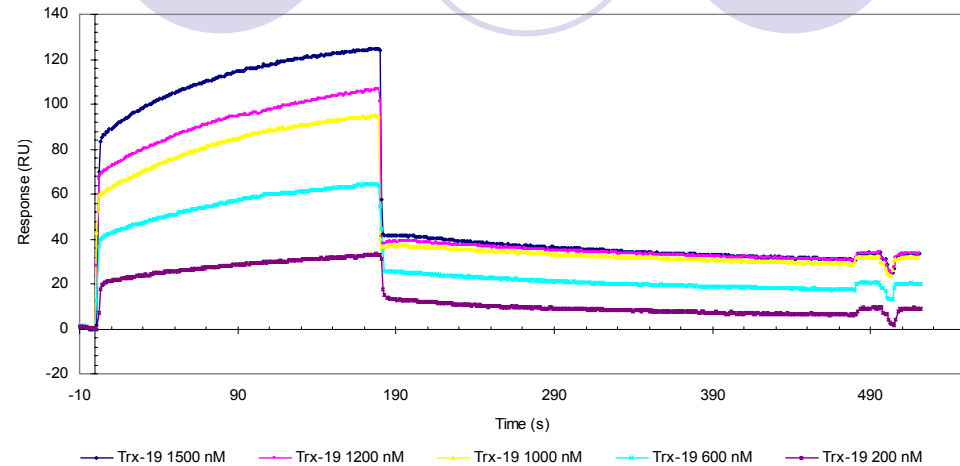
# Surface Plasmon Resonance

- **\* Polarized light hits the layer of glass between two indices on the sensor chip**
  - Higher- glass surface
  - Lower-running buffer in microchannel.
- **Light reflects from higher indices and evanescent wave is formed \***
  - Penetrates the lower index
- **An intensity dip appears-which is the SPR angle.**
  - Angle can change as the response changes
  - Response changes due to the accumulation of mass.



# Results for GST-5ABC and GST6A

Binding of GST5ABC-Trx-19



Parameters for Binding

Bindings Kinetic Constants	KA (1/M)	KD (M)	$\sigma^2$ Chi2 value
GST-5ABC-Trx-19	6.45E+06	1.55E-07	1.67
GST-5ABC-Trx-19A	1.31E+07	7.64E-08	6.07
GST-5ABC-Trx-19B	8.52E+06	1.17E-07	1.77
GST-6A-Trx-19	8.01E+06	1.25E-07	9.34
GST-6A-Trx-19A	4.08E+06	2.45E-07	2.17
GST-6A-Trx-19B	1.24E+07	8.07E-08	7.81

- Experiments:

- GST-5ABC & GST-6A with Trx-19, 19A, 19B

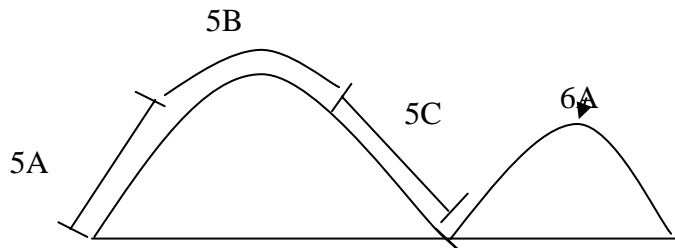
- Strong Bindings seemed to occur

- $K_D$  value of Trx-19A is slightly lower than Trx-19B

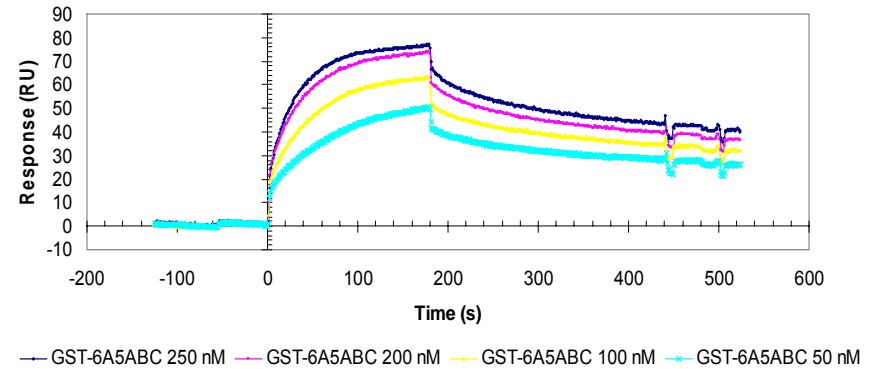
- 19A & 19B help contribute to the overall binding of Trx-19.

# Results for GST-5ABC6A and GST-6A5ABC

- Chimeric proteins- composed of parts
- When chimeric proteins-were used as ligand and Trx19 was the analyte, no binding occurred
- When Trx19A was used as ligand and chimeric proteins were the analyte, strong binding occurred
- Kinetic values for the experiments are close to values from the GST-5ABC experiments



GST-6A5ABC with Trx-19A



Parameters for Binding

Bindings Kinetic Constants	$K_A$ (1/M)	$K_D$ (M)	$\sigma^2$ Chi2 value
GST-5ABC-Trx-19	6.45E+06	1.55E-07	1.67
GST-5ABC-Trx-19A	1.31E+07	7.64E-08	6.07
GST-5ABC-Trx-19B	8.52E+06	1.17E-07	1.77
GST-6A-Trx-19	8.01E+06	1.25E-07	9.34
GST-6A-Trx-19A	4.08E+06	2.45E-07	2.17
GST-6A-Trx-19B	1.24E+07	8.07E-08	7.81
GST-5ABC6A-Trx-19	1.86E+04	5.39E-05	12.0
GST-6A-5ABC-Trx-19	0.828	1.21	12.3
GST-6A5ABC-Trx-19A	9.49E+07	1.05E-08	2.88

# Conclusions



- Binding strengths between the interaction of band 3 peptide and MSP1-19 were found.
- 19A, 19B contributes to the overall binding of Trx-19. MSP1-19 has important binding sites for Band 3 peptide
- GST-6A has similar affinity binding as GST5ABC and the kinetic constants show the 6A is also an important receptor
- Combined 5ABC and 6A region works as receptors in the invasion of *P. falciparum*