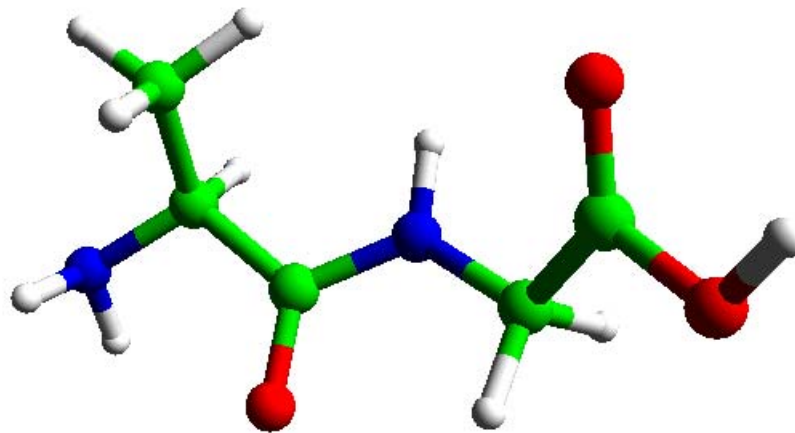


Binding Analysis of a Novel Peptide to Malaria Knob Protein



[http://www.nuigalway.ie/cryst/
oscaill_tutorial/moilin/
biomolecules/ala_gly.png](http://www.nuigalway.ie/cryst/oscaill_tutorial/moilin/biomolecules/ala_gly.png)

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Overview

- Malaria Challenges
- Project Description
 - BIAcore
 - ELISA
- Results and Conclusion
- Future Work

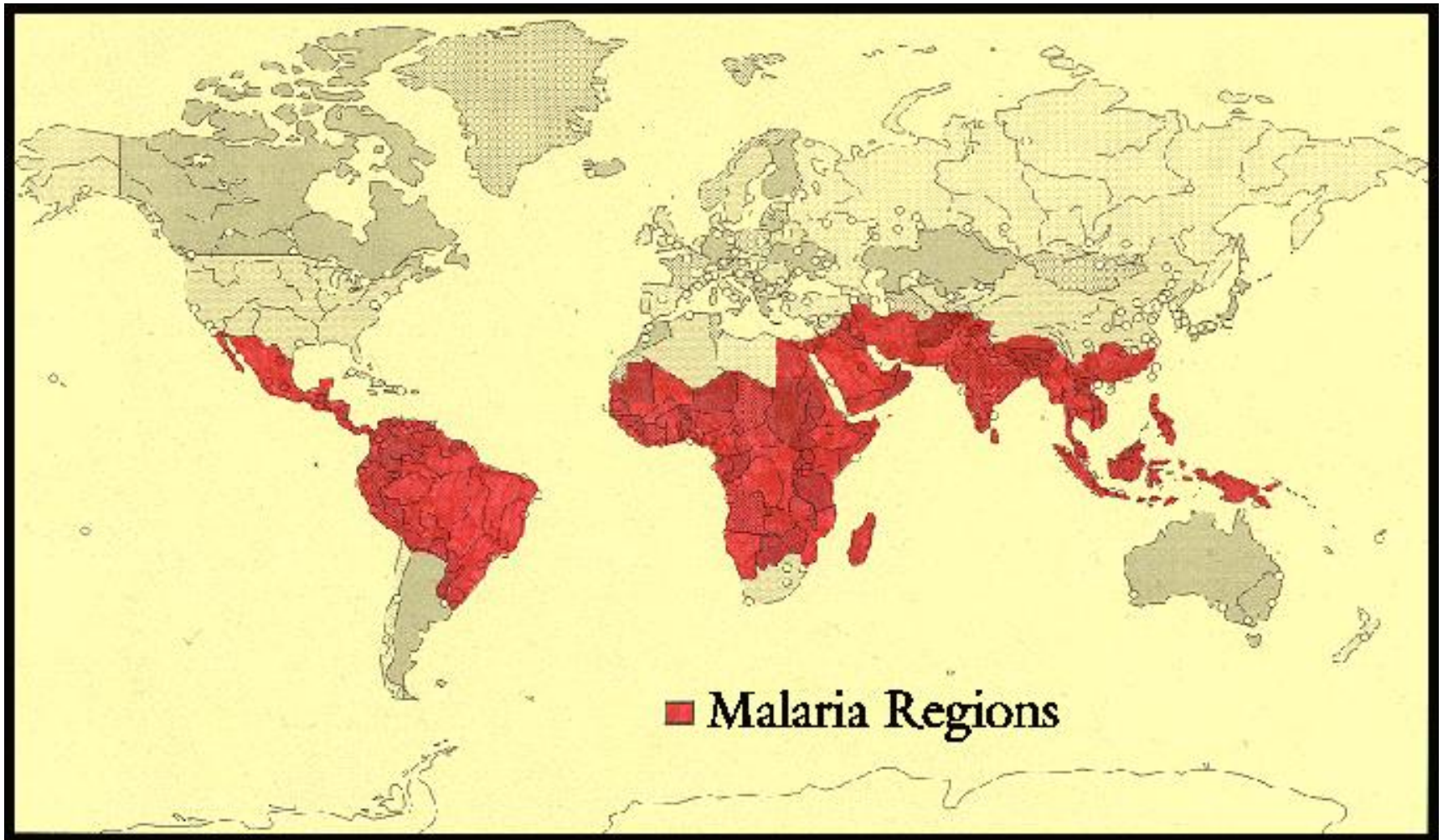
Malaria



- ❑ **Over 5,000 Years Old**
- ❑ **300-500 Mil. Infections /Year**
- ❑ **3 Mil. Deaths /Year**
- ❑ **In Africa: 5,000 Deaths /Day,
1 Child /3 Sec.**

http://www.scienceblogs.com/aetiology/2006/01/mechanism_of_malaria_hide_and.php

Malaria Endemic Regions



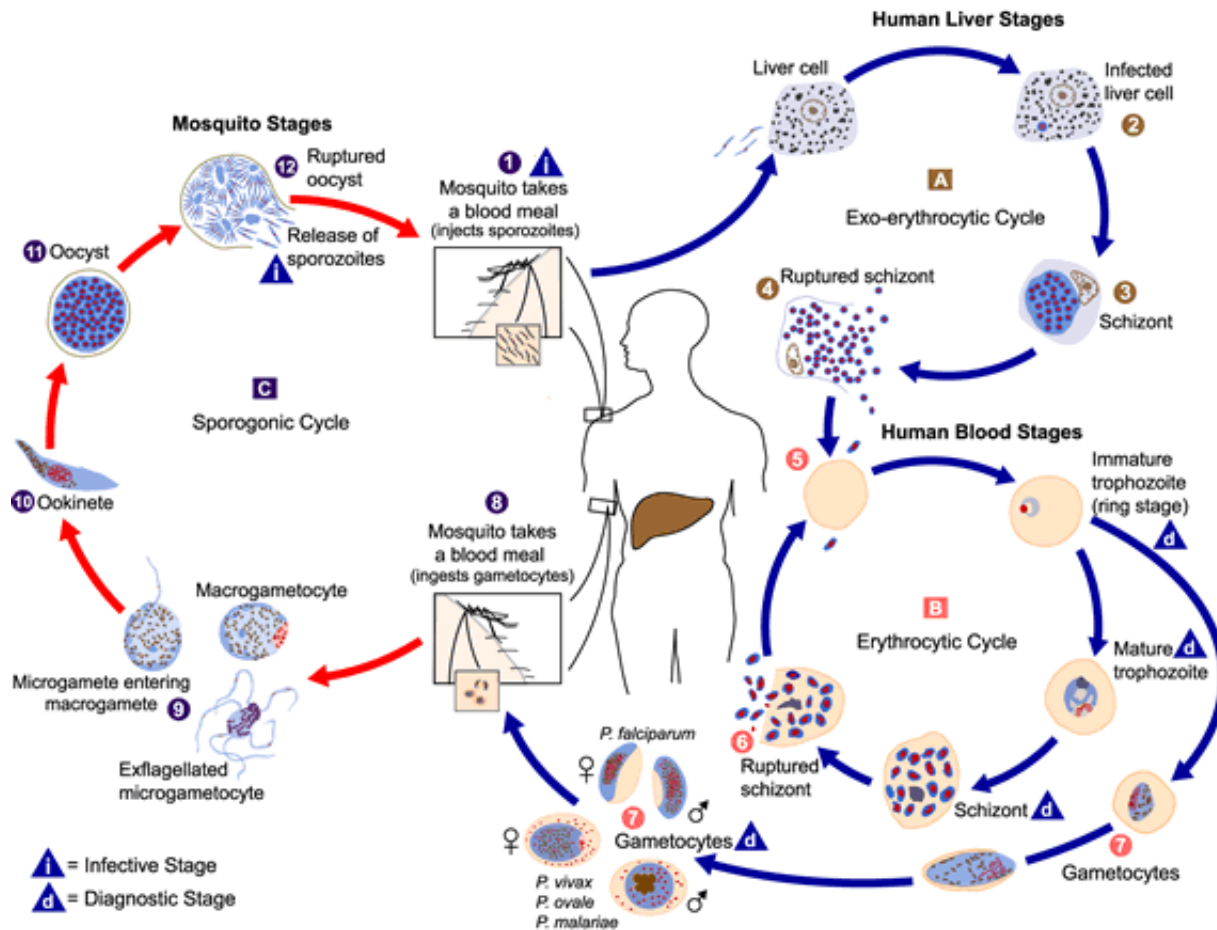
Malaria Challenges



<http://www.jhsph.edu/bin/n/v/22malaria.jpg>

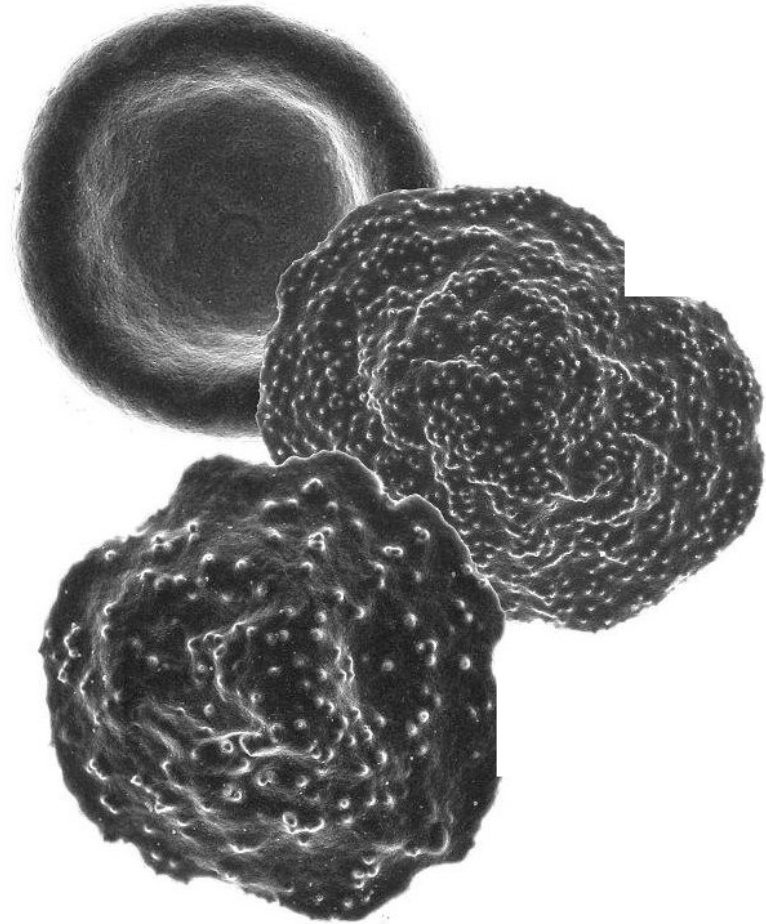
- ❑ Drug Resistance
- ❑ Genetic Complexity
- ❑ Thousands of Antigens Produced
- ❑ Several Life Stages
- ❑ Several Strategies to Confuse Immune System
- ❑ Different Species and Strains
- ❑ Drugs and Mosquito Nets Inaccessible to Many

Malaria Life Cycle

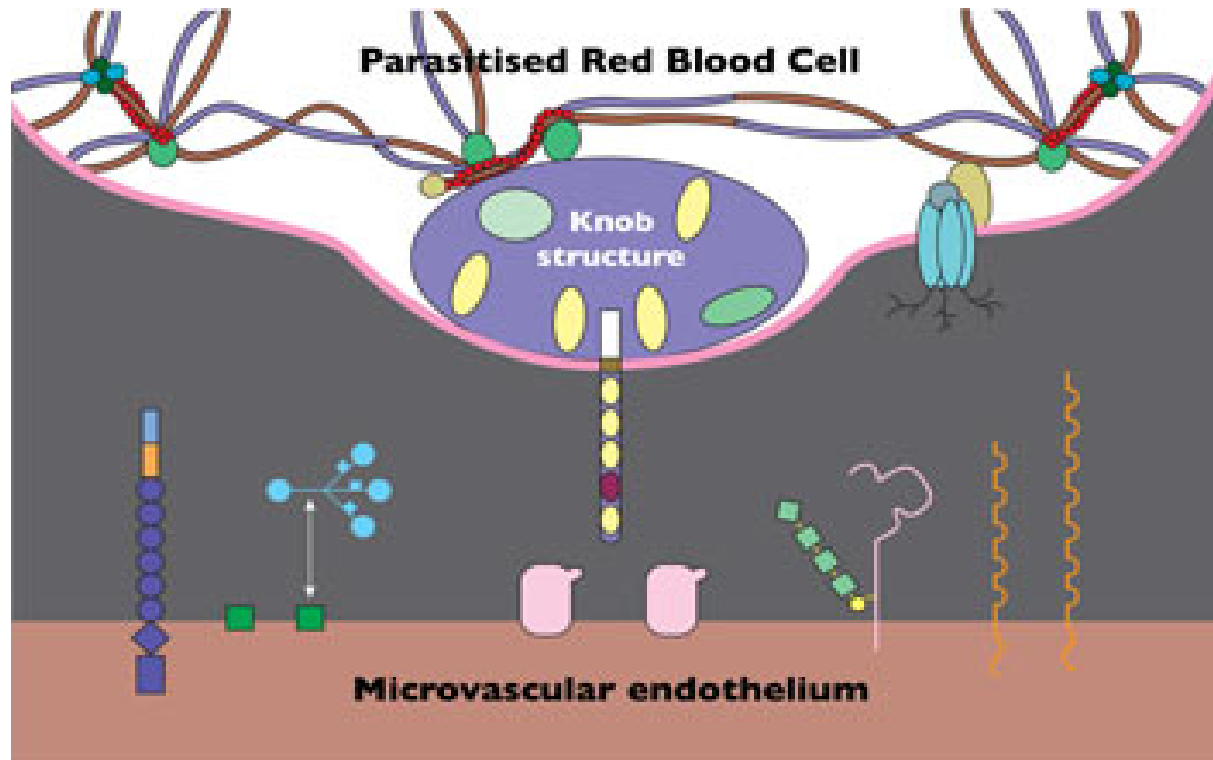


Cerebral Malaria

- ❑ Caused by *Plasmodium falciparum*
- ❑ Red blood cells become covered with knobs
 - Infected cells avoid destruction in spleen
 - Occludes blood vessels



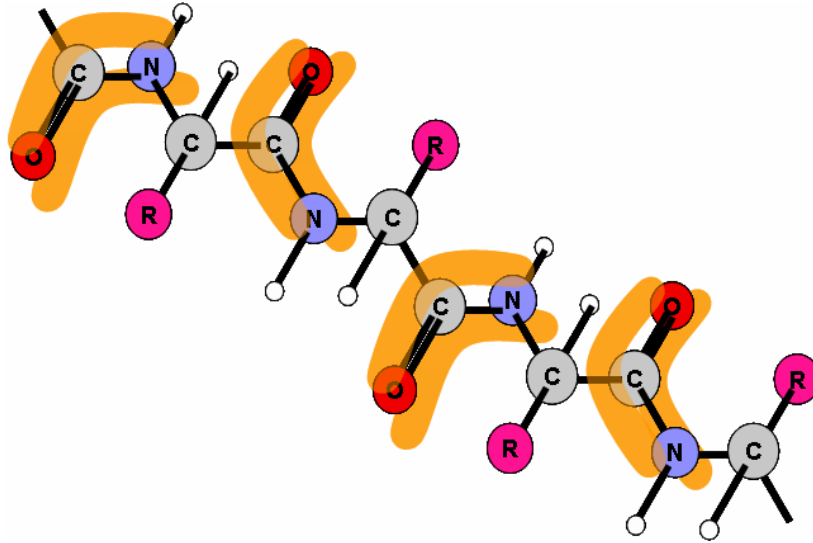
Malaria Knob Protein (KP)



http://www.wehi.edu.au/facweb/faculty/alan_cowman/images/060084-2.jpg

Knobs provide elevation for adhesive ligands, allowing parasitized red blood cells to adhere to blood vessels in the brain.

Peptide Binding Project



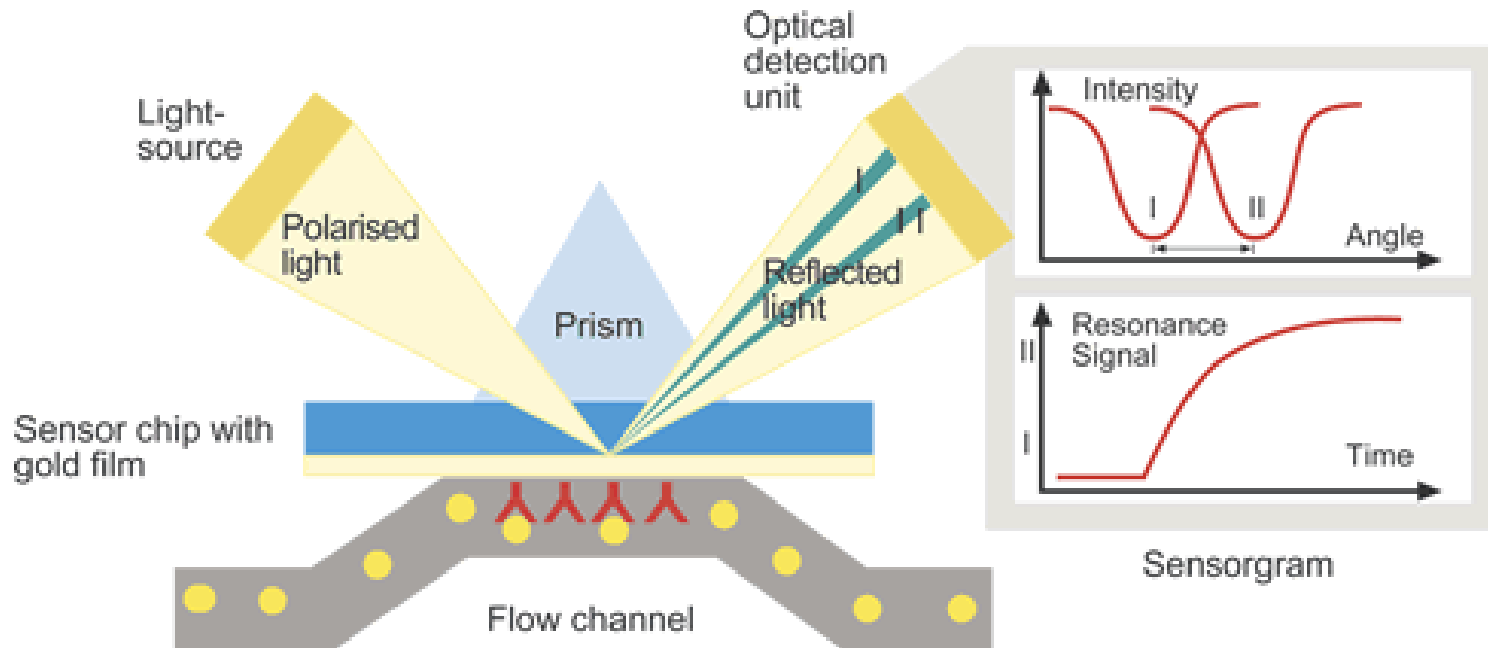
http://www.steve.gb.com/images/science/peptide_backbone.png

A 10 amino acid biotinylated peptide (Biotin-AP) has been synthesized which is expected to bind KP and interrupt knob formation.

BIACore Analysis



BIAcore Operation



<http://www.rci.rutgers.edu/~longhu/Biacore/pic/spr.gif>

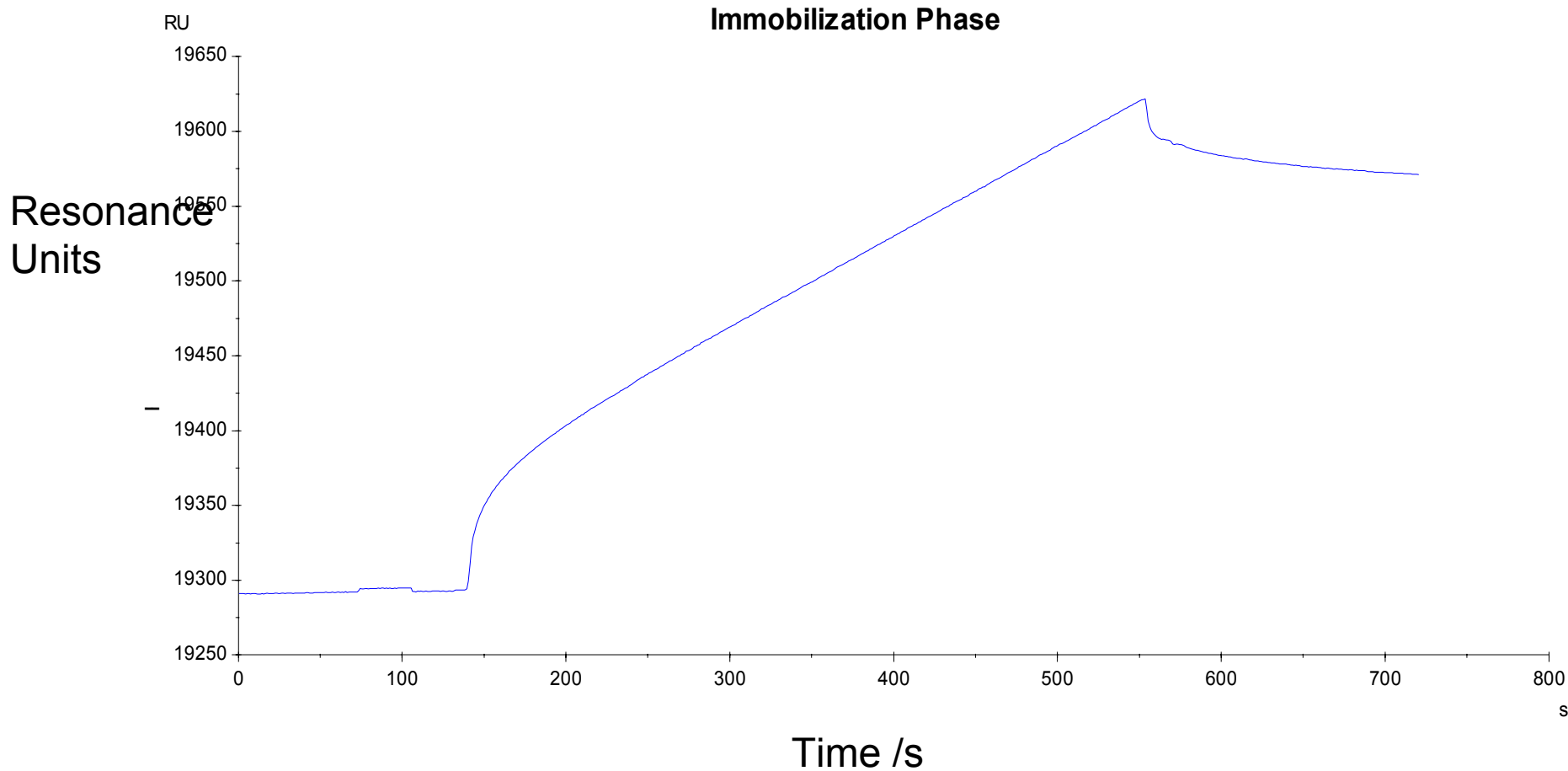
The binding of an analyte to an immobilized ligand changes the surface plasmon resonance (SPR) response angle.

BIAcore Materials

- Sensor Chip:
 - Steptavidin (SA) coated sensor chip
- Ligand:
 - 300 nM Biotin-AP immobilized on sensor chip
- Analyte:
 - 3 types of 2000 nM Knob Protein:
 - Thioredoxin (TRX)
 - Glutathione-S-Transferase (GST)
 - Histidine (His)
- Buffer:
 - HEPES Buffered Saline (HBS) pH 7.4
 - HEPES= N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid

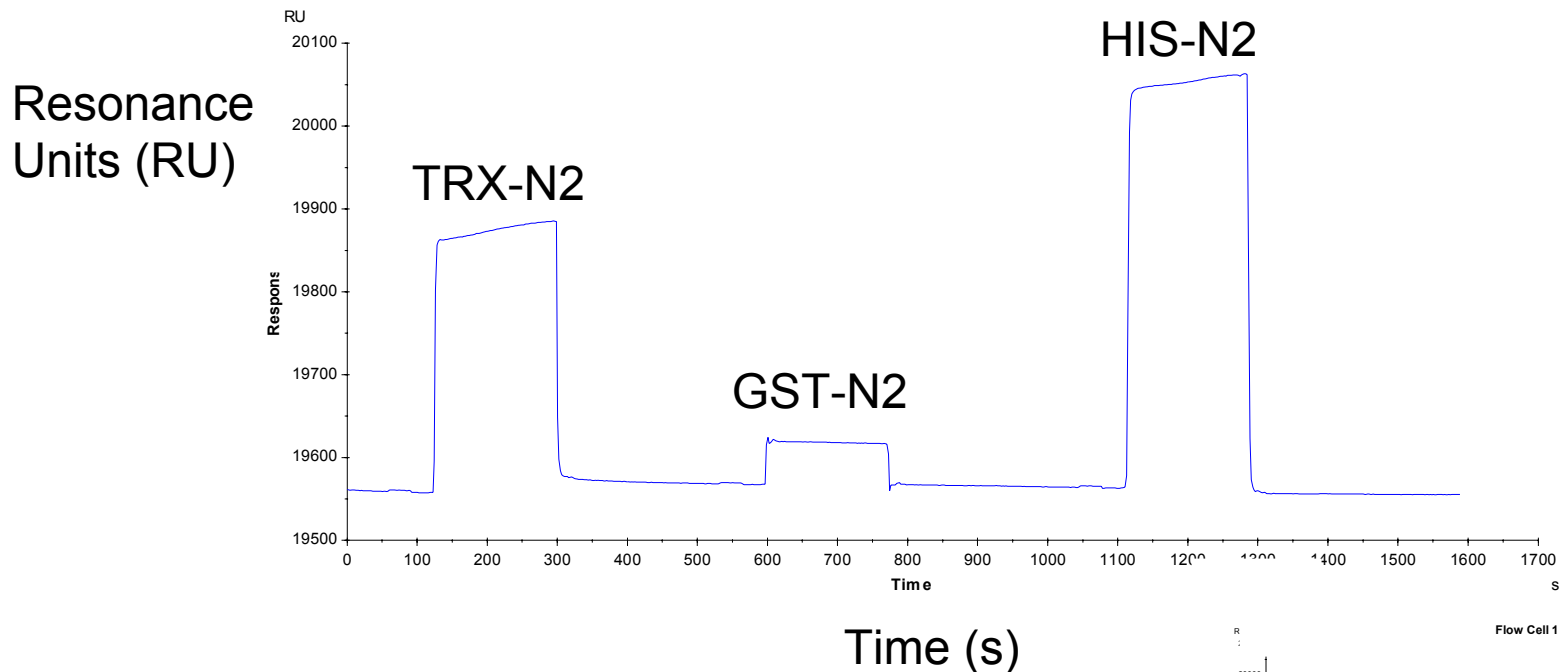
Biotin-AP Immobilization

Injection of 200 nM Biotin-AP

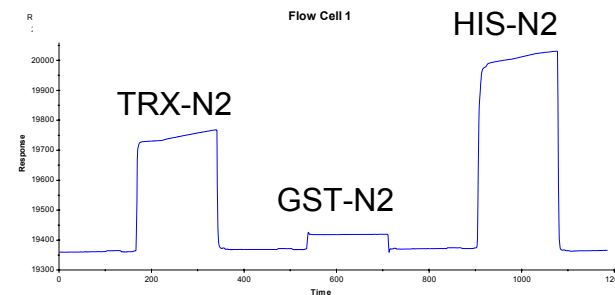


BIAcore Results

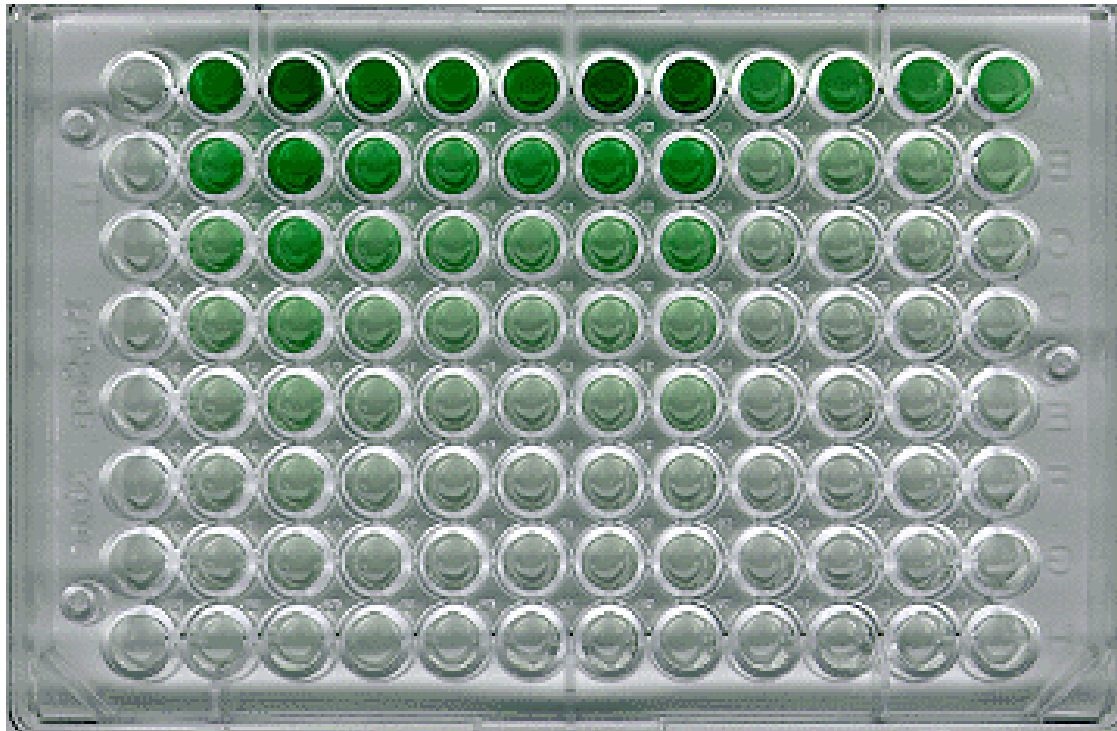
Injection of 2000 nM Knob Proteins



Flow Cell 1 (Negative control) →

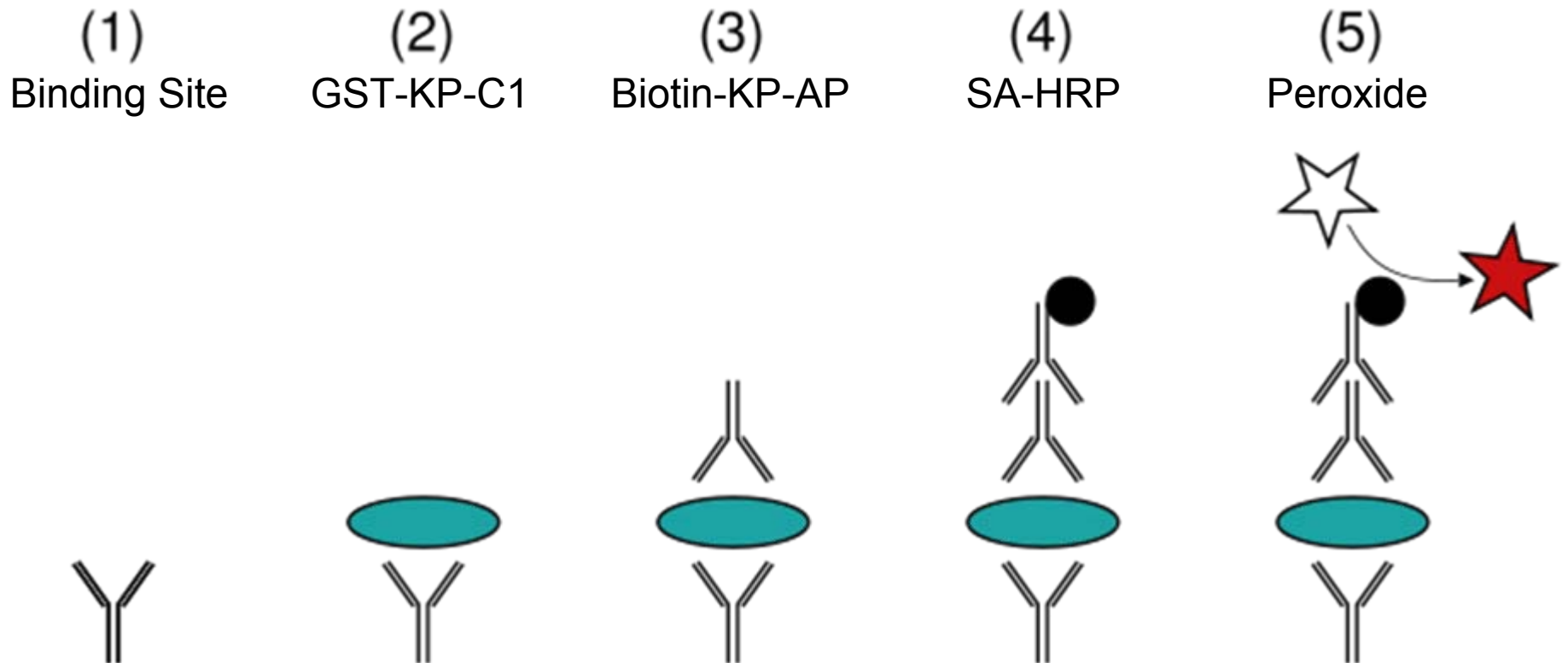


The Enzyme-Linked ImmunoSorbent Assay (ELISA)



Allows the visualization of a protein binding interaction

ELISA



<http://en.wikipedia.org/wiki/ELISA>

KP-C1 = an important segment of KP

KP-AP = synthesized peptide

SA-HRP = streptavidin + horseradish peroxidase

ELISA Materials

Binding Analysis:

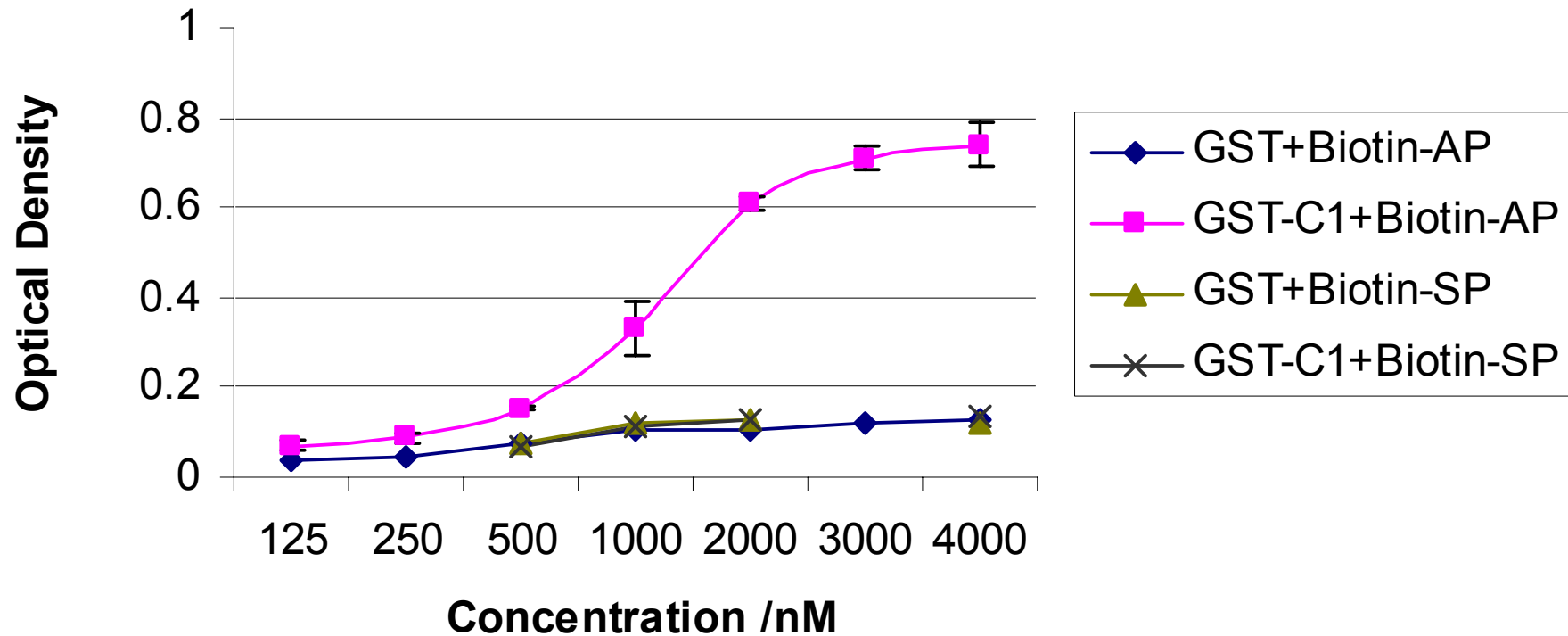
- 10 μg GST-KP-C1 + various conc.
Biotin-KP-AP

Negative controls:

- GST-KP-C1 + Biotin-KP-SP
- GST + Biotin-KP-AP
- GST + Biotin-KP-SP

ELISA Results

KAHRP and KP-AP Binding Interaction



Estimation of Dissociation Constant

$$K_D = \frac{[\text{GST-KP}][\text{KP-AP}]}{[\text{complex}]}$$

When half of GST-KP is complexed with
KP-AP,

$$[\text{GST-KP}] = [\text{complex}]$$

$$K_D = \frac{[\text{complex}][\text{KP-AP}]}{[\text{complex}]}$$

$$K_D = [\text{KP-AP}]$$

$$K_D \approx 1.2 \mu\text{M}$$

Summary

- ELISA results indicate that binding between KP and KP-AP is specific
- Binding of KAHRP to Biotin-KP-AP is moderately strong ($K_D \approx 1.2 \mu\text{M}$)
- Binding was not detected with BIAcore, most likely due to restricted flexibility and low diffusion rates

Future Work

- Alanine Knockout Scan
 - Replace a single amino acid with alanine and compare this peptide's binding affinity with KP-AP
 - Can help determine binding mechanism by identifying important amino acid residues

References

- Melanie Rug, et al. "The Role of KAHRP in knob formation and cytoadherence of *P. falciparum*-infected human erythrocytes." BLOOD July 2006 108 (1): 370-378
- Kirchgatter, K., Del Portillo, HA. "Clinical and molecular aspects of severe malaria", *Anais Da Academia Brasileira De Ciencias* September 2005 77 (3): 455-475

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Questions?