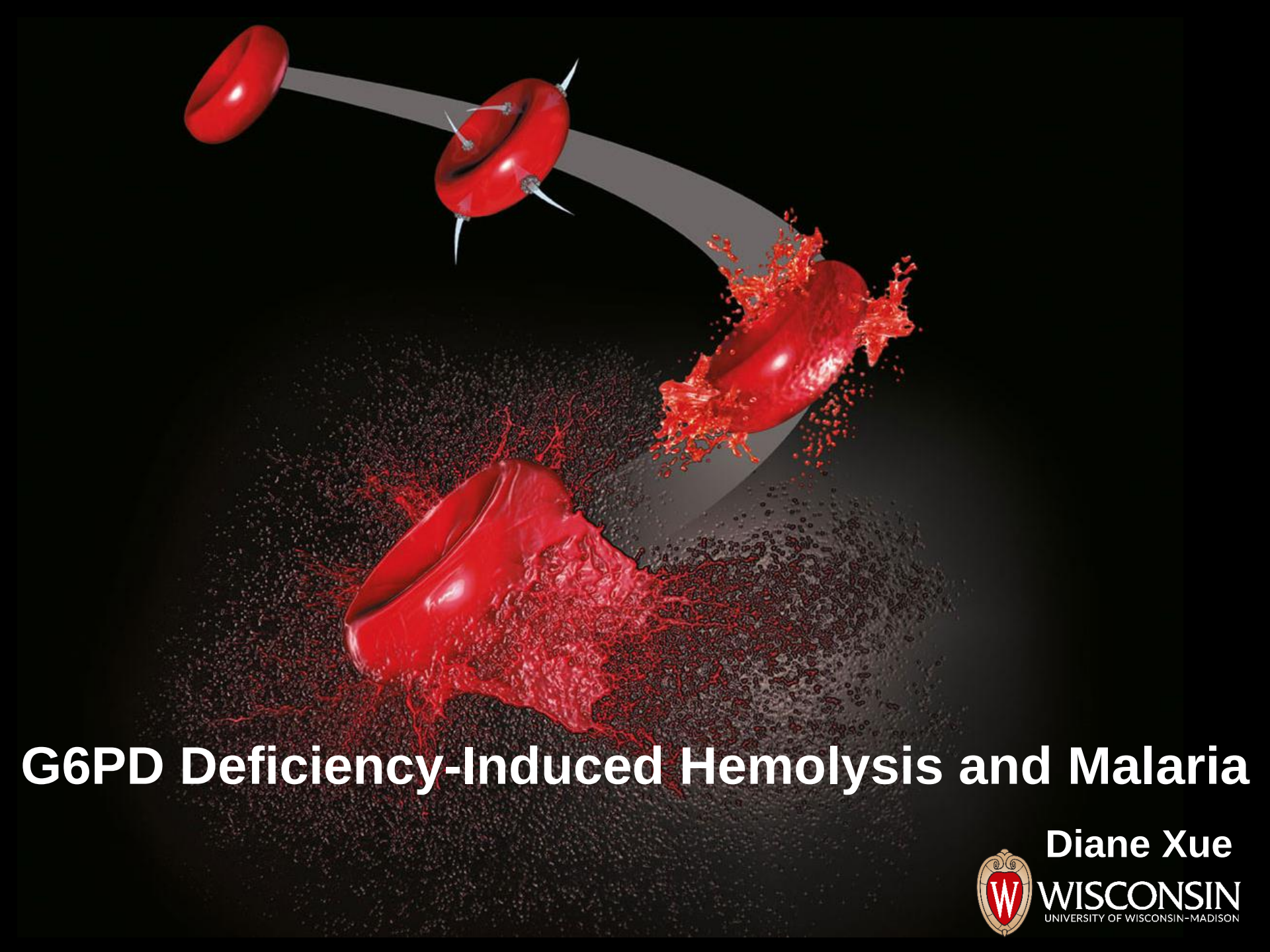
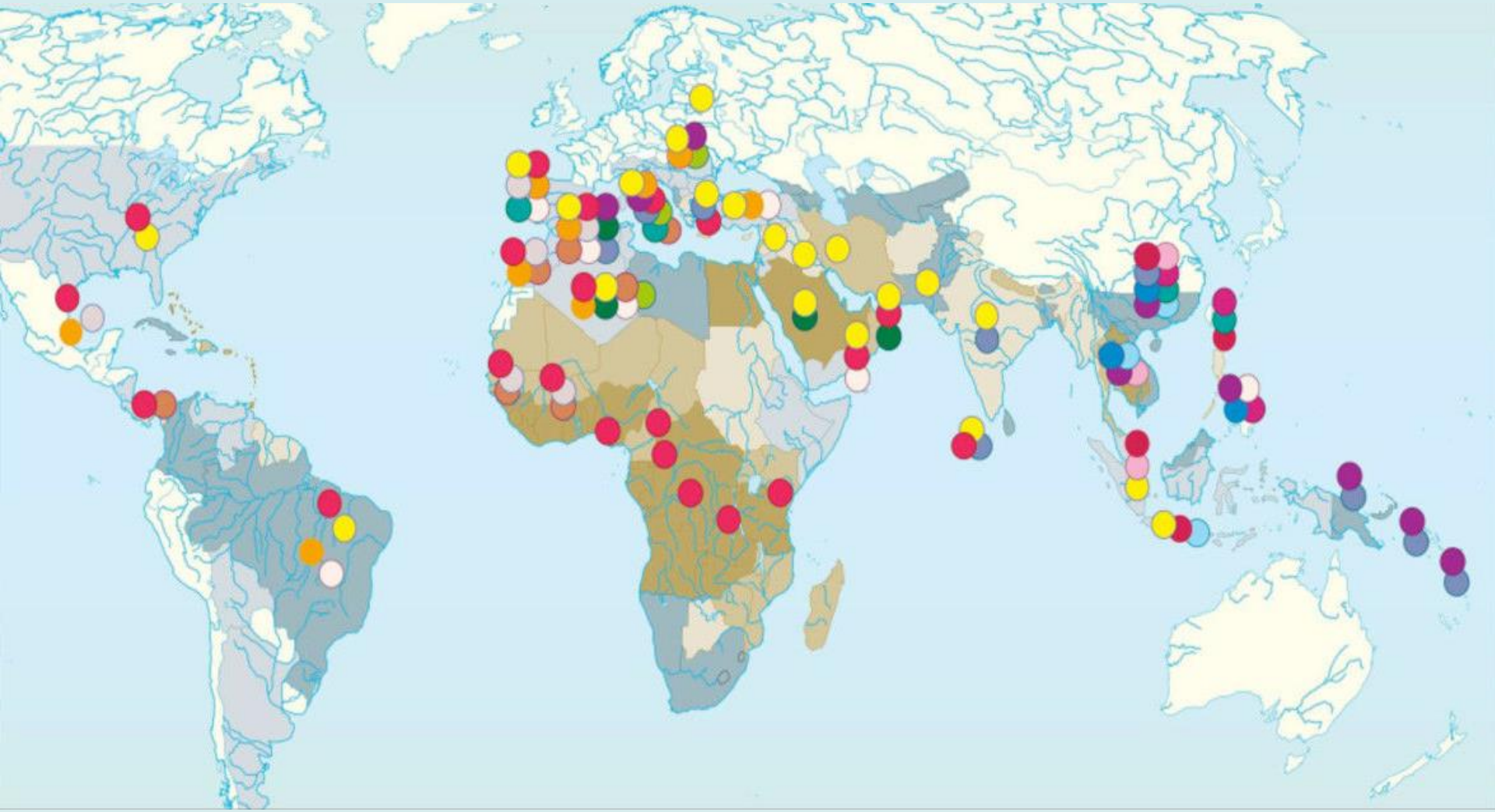


G6PD Deficiency-Induced Hemolysis and Malaria

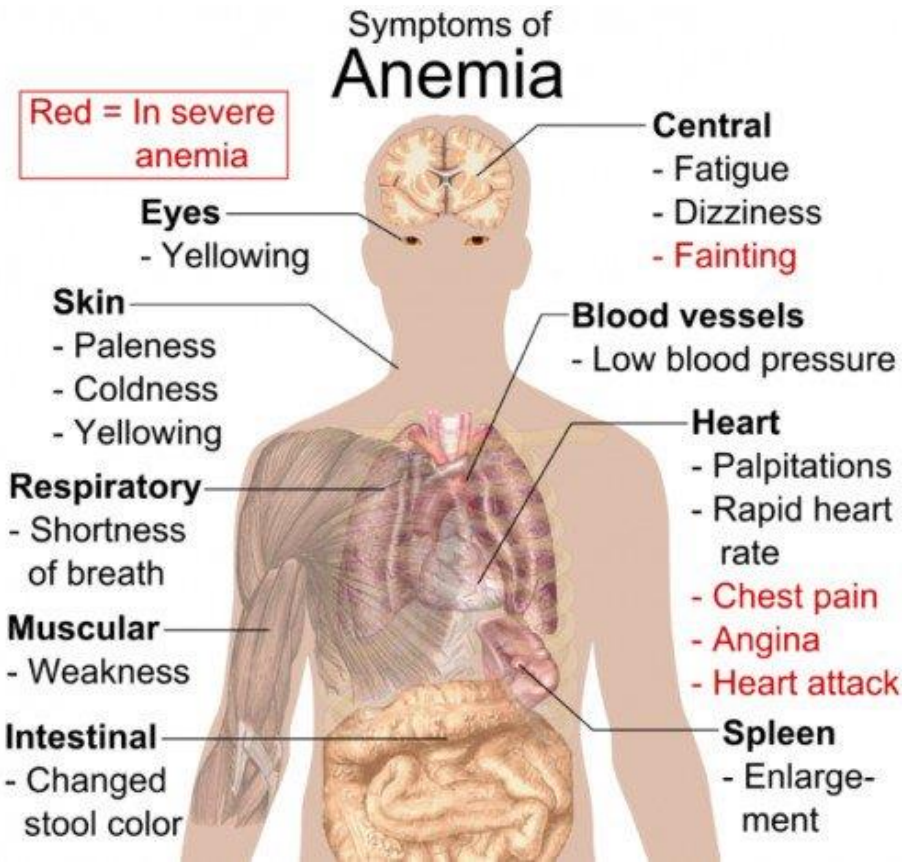
Diane Xue

WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

G6PD Deficiency is the most common Enzymopathy in the World



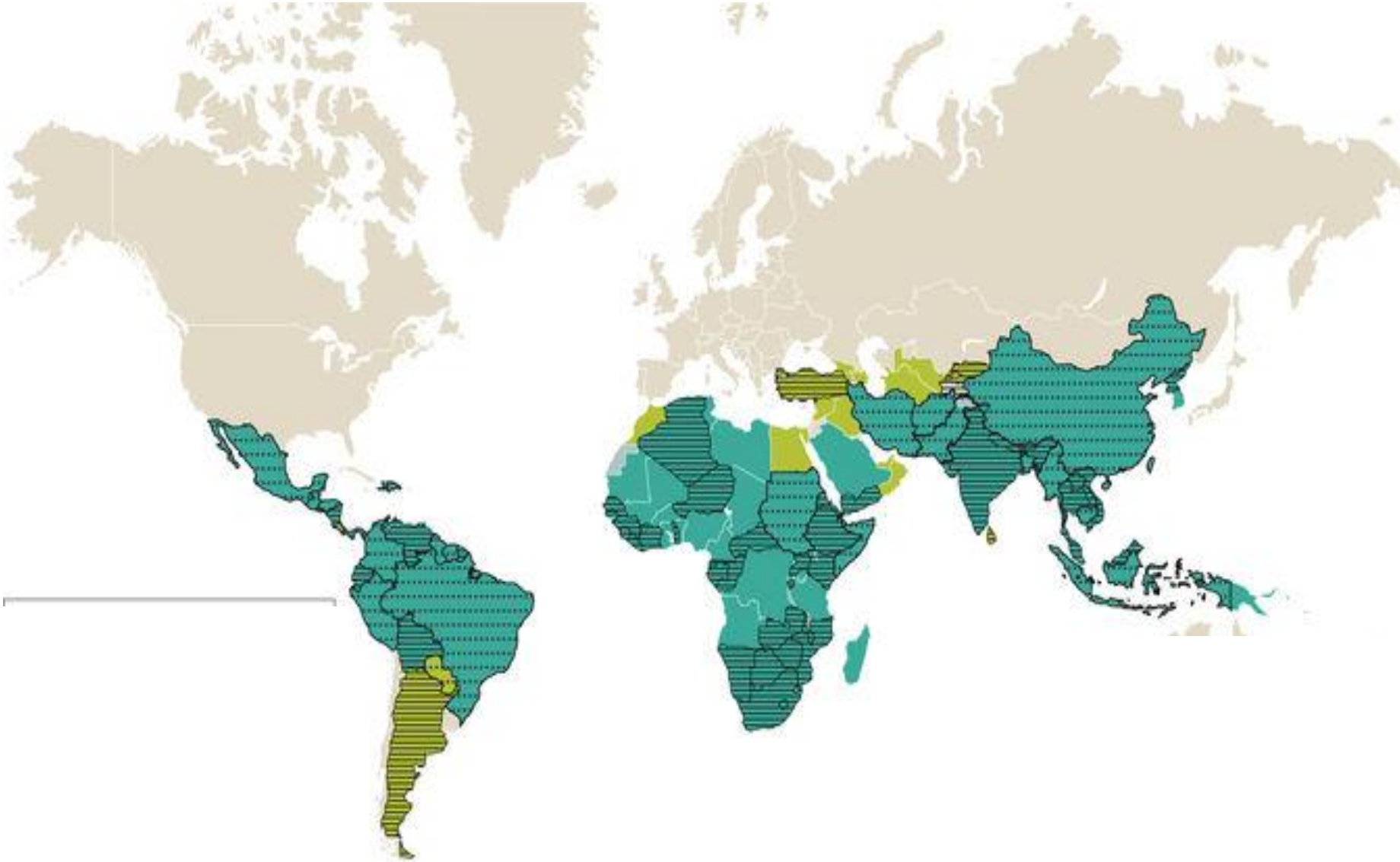
G6PD Deficiency Phenotypes



Hemolytic Anemia
11/100 deaths

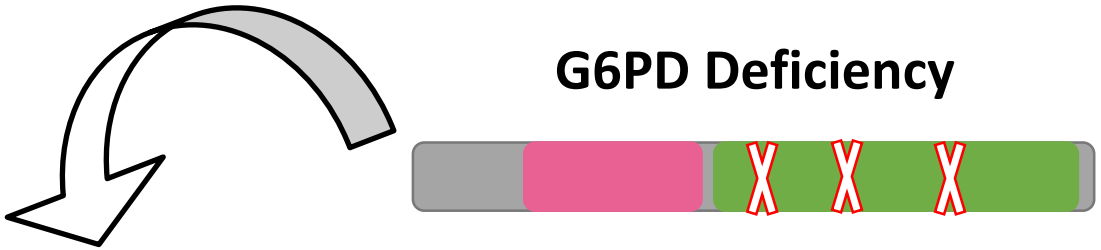
Neonatal Jaundice
7.3/1000

G6PD Deficiency's Indirect Consequence

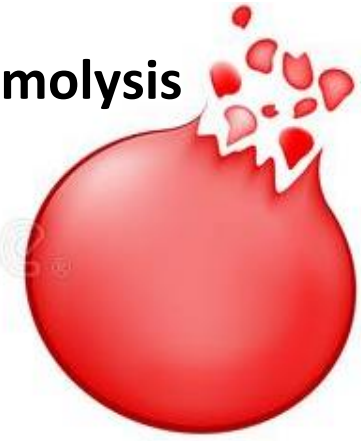


Malaria = 720,000 deaths per year

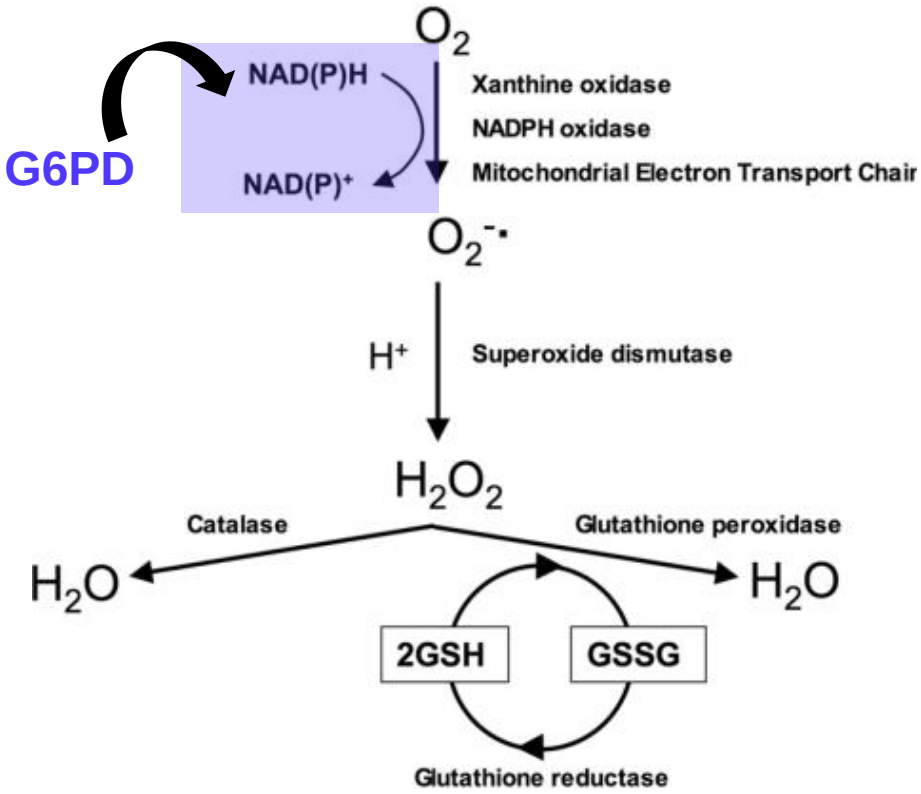
How are G6PD deficiency, Hemolysis, and Malaria related?



Hemolysis



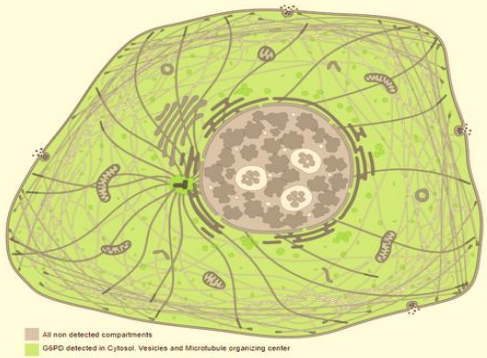
How does G6PD cause Susceptibility for Hemolysis?



No Build-up of Reactive Oxygen Species

What is the function of normal G6PD?

Cellular Component

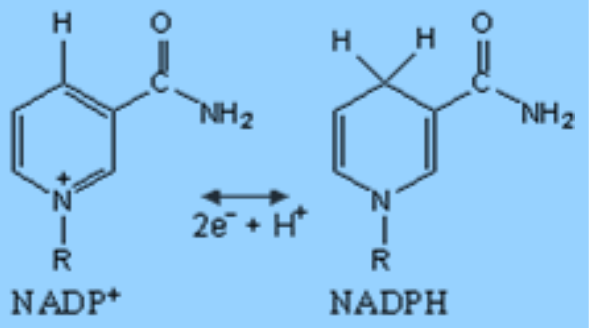


Cytoplasm

Molecular Functions

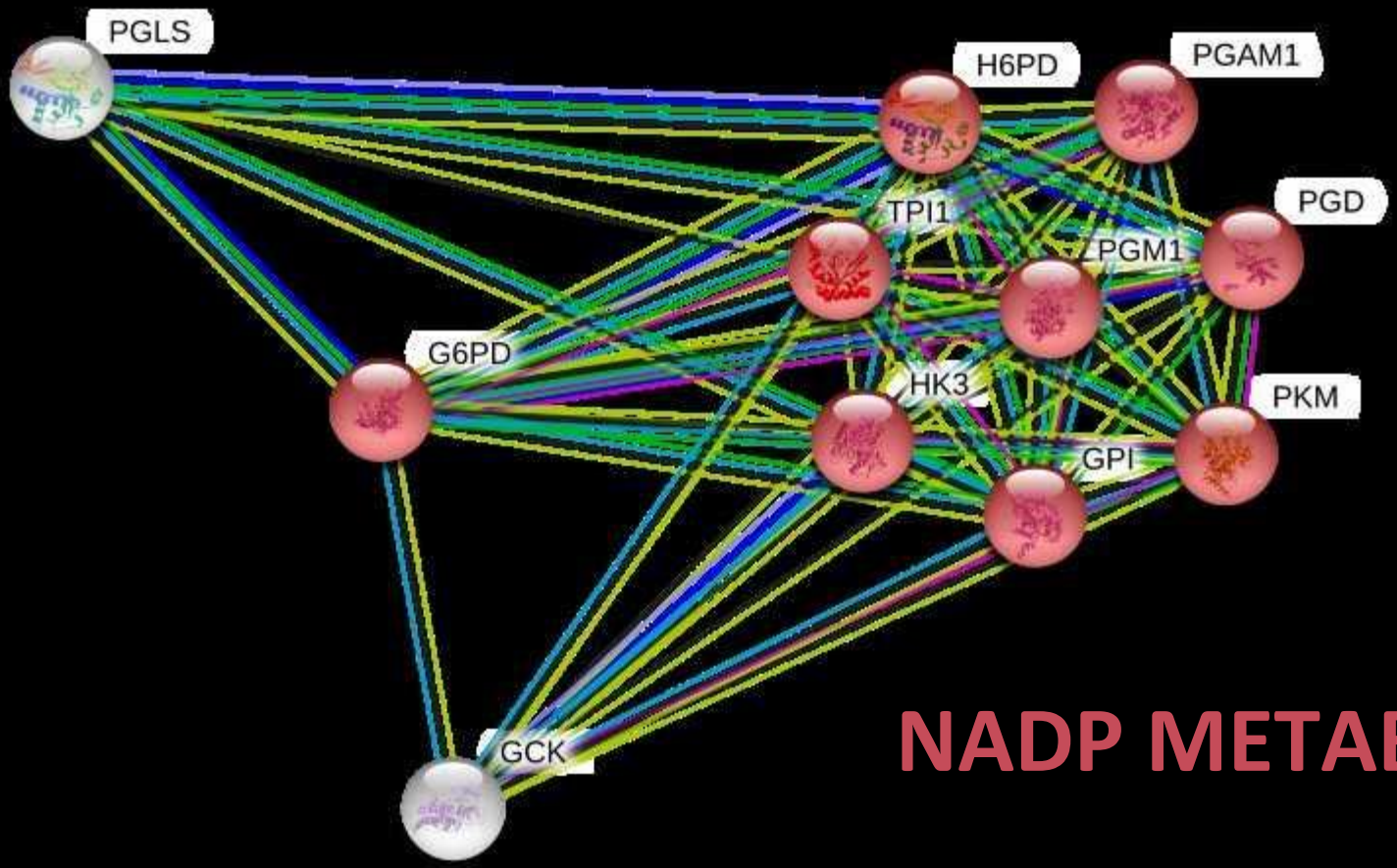
NADP Binding

Biological Process



NADP Metabolism

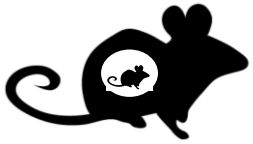
What Proteins Interact with G6PD?



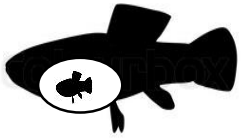
NADP METABOLISM

How well conserved is *G6PD*?

NAD binding domain | C-terminal domain

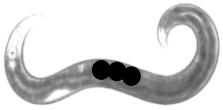


NAD binding domain | C-terminal domain



NAD binding domain | C-terminal domain

NAD binding domain | C-terminal domain



NAD binding domain | C-terminal domain

NAD binding domain | C-terminal domain

NAD binding domain | C-terminal domain

Can G6PD be upregulated?

Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 1465–1469, March 1985
Genetics

Tissue-specific levels of human glucose-6-phosphate dehydrogenase correlate with methylation of specific sites at the 3′ end of the gene

(DNA methylation/housekeeping genes/transcriptional regulation)

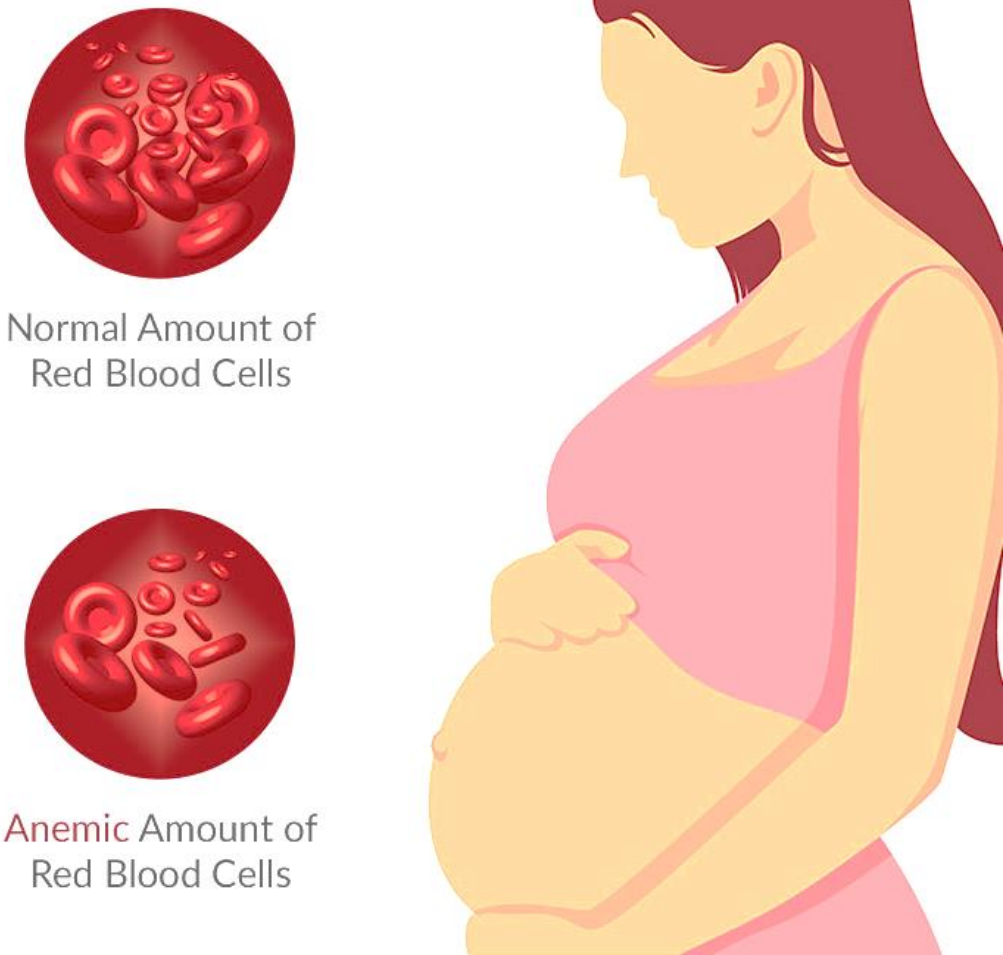
GIORGIO BATTISTUZZI*†, MICHELE D'URSO*‡, DANIELA TONIOLO‡, G. M. PERSICO‡, AND LUCIO LUZZATTO*

*Department of Haematology, Royal Postgraduate Medical School, Ducane Road, London W12 OHS, England; and ‡International Institute of Genetics and Biophysics, Consiglio Nazionale delle Ricerche, Via Marconi 10, 80125 Naples, Italy

Communicated by Paul A. Marks, October 24, 1984

** THIS STUDY WAS ONLY DONE ON MALES

Goal: **How does *G6PD* Methylation affect G6PD levels in Pregnant Females?**



How does *G6PD* Methylation affect G6PD levels (NADP Production) in Pregnant Females?

Aim 1:

Knockout conserved methylation sites of G6PD to identify methylation sites associated with normal levels of NADPH production in pregnant females.

- Bisulfite DNA-Seq
- ClustalOmega
- CRISPR
- Fluorescent Spot Test

Aim 2:

Identify a small molecule that affects methylation of G6PD and can be taken with Primaquine in pregnant females without hemolysis

- Bisulfite DNA-Seq
- Chemical Screen
- Fluorescent Spot Test
- Bisulfite DNA-Seq

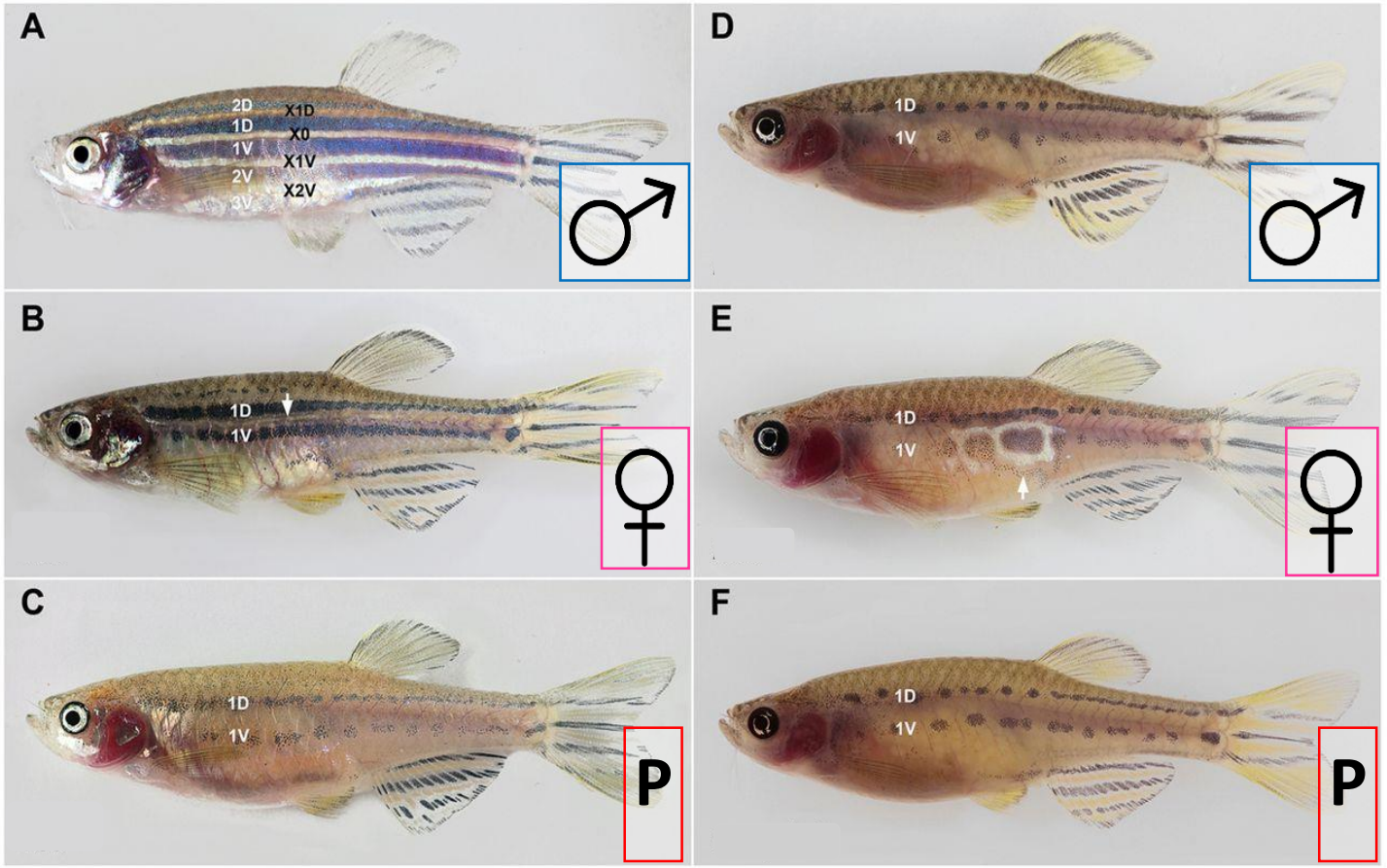
Aim 3

Determine differences in protein interaction complexes required for NADP metabolism between methylated and unmethylated G6PD deficient pregnant zebrafish

- CRISPR
- TAP-MS
- Fluorescent Spot Test

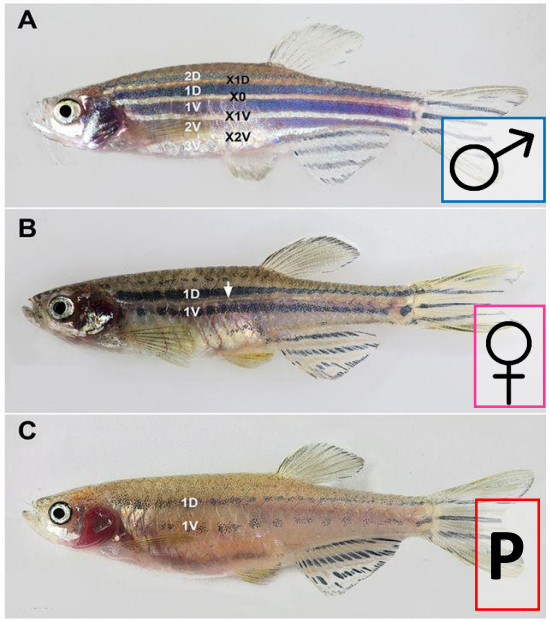
What Model Organism Will I Use?

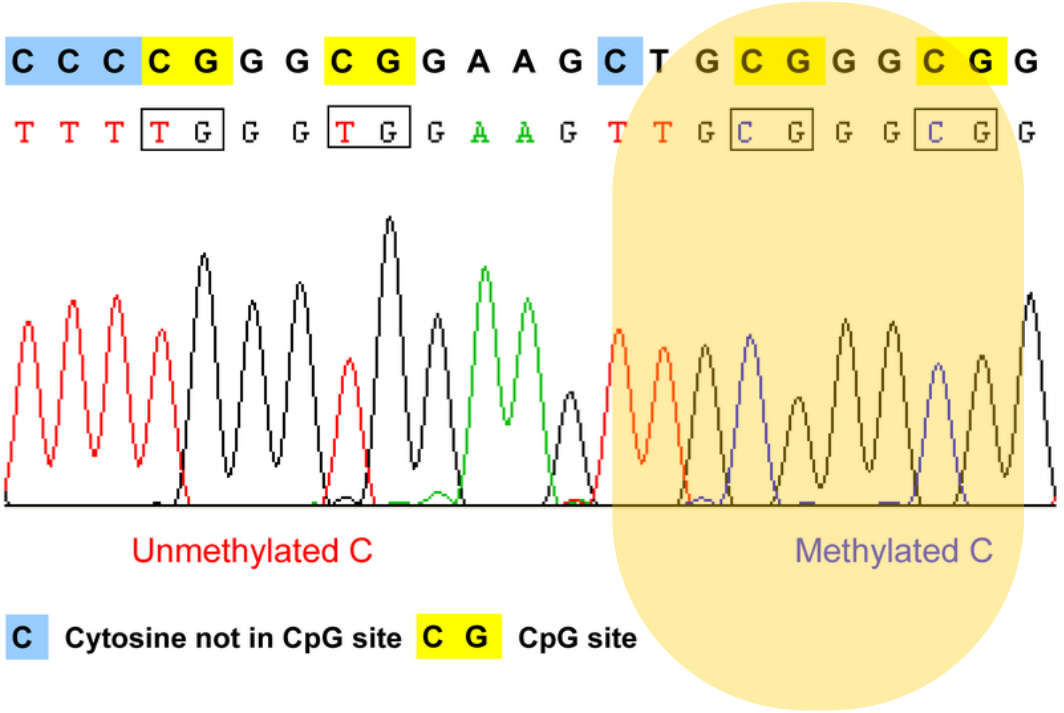
WT (Normal NADPH) **G6PDd (Low NADPH)**

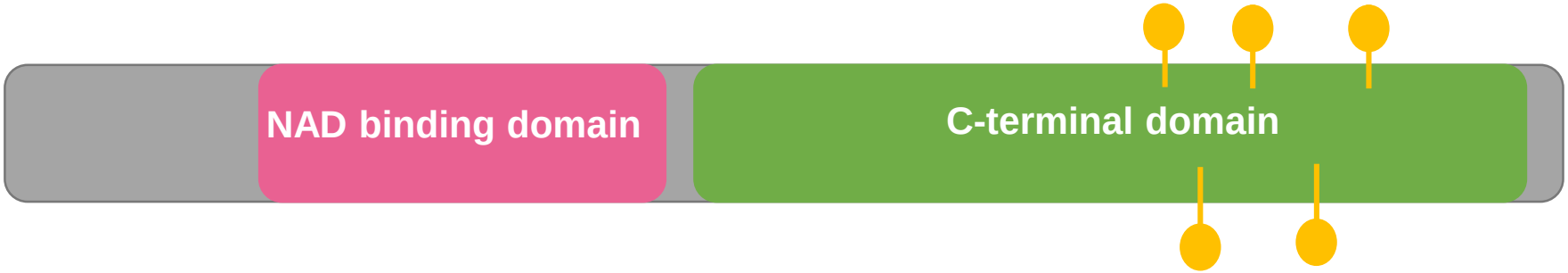


Aim 1: Identify conserved methylation sites in G6PD normal pregnant zebrafish using Bisulfite Sequencing

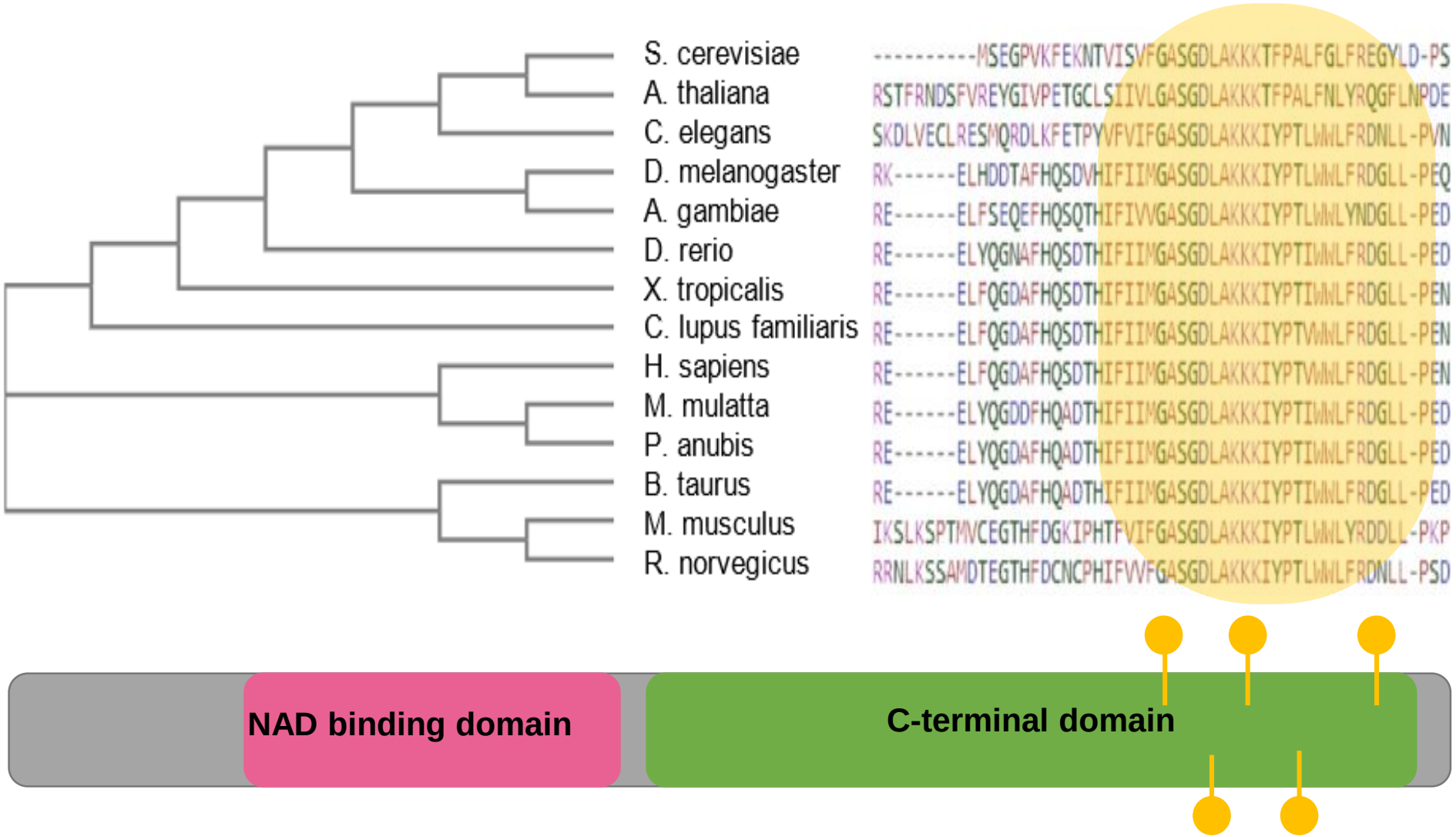
WT (Normal NADPH)



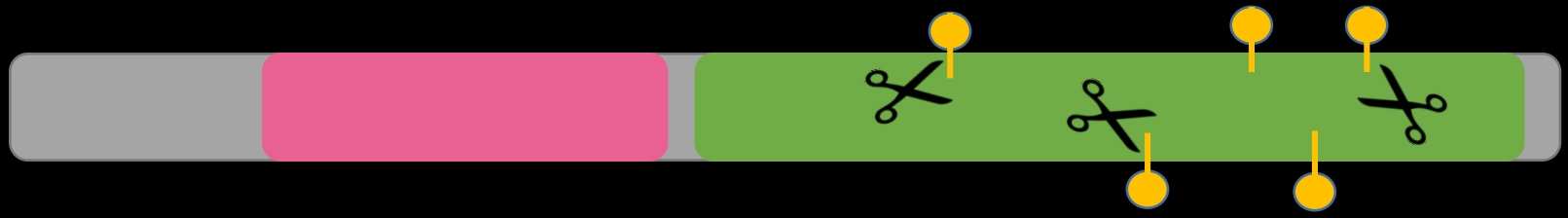




Aim 1: Identify conserved methylation sites in G6PD normal pregnant zebrafish using Phylogenomics and ClustalOmega

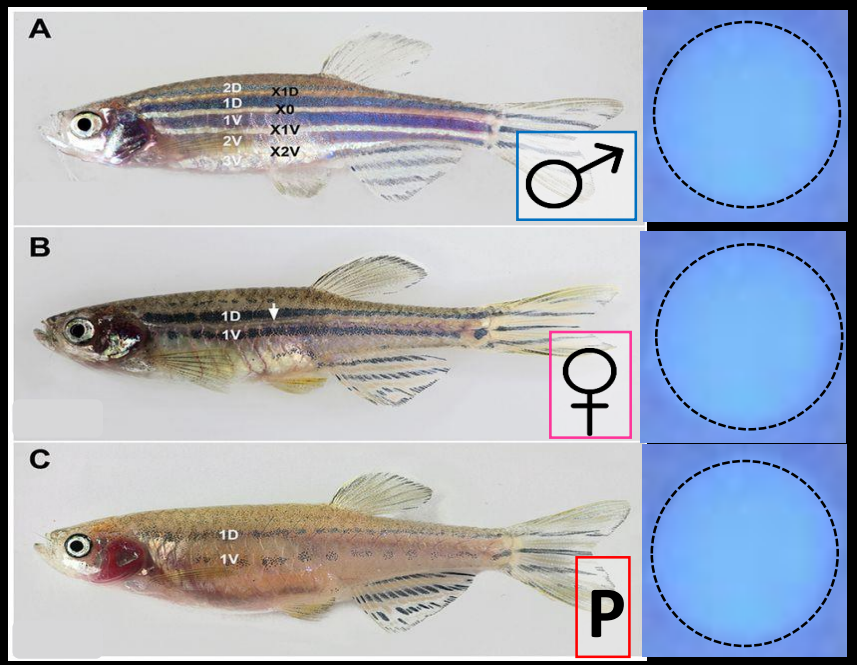


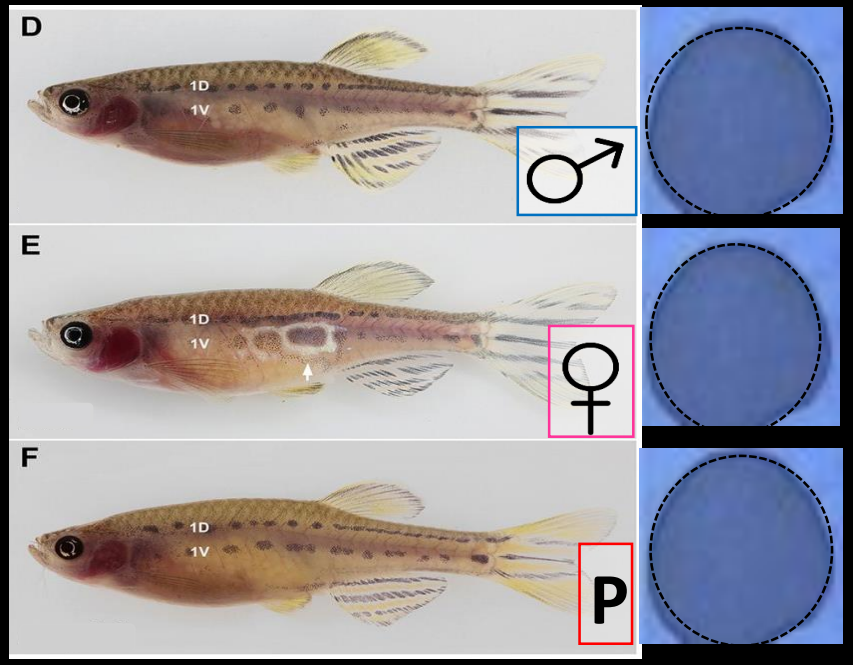
Aim 1: Identify conserved methylation sites in G6PD normal pregnant zebrafish using CRISPR/Cas9 and Fluorescent Spot Test



WT (Normal NADPH)

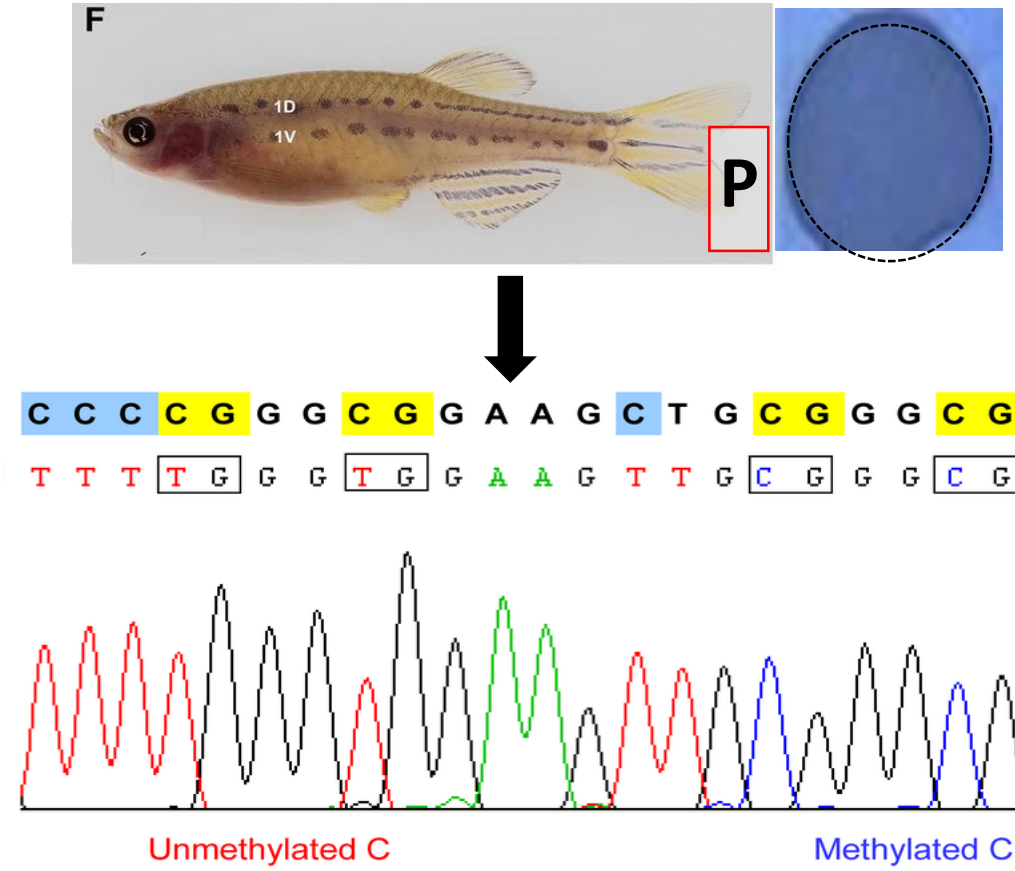
G6PDd (Low NADPH)



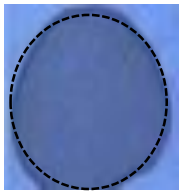


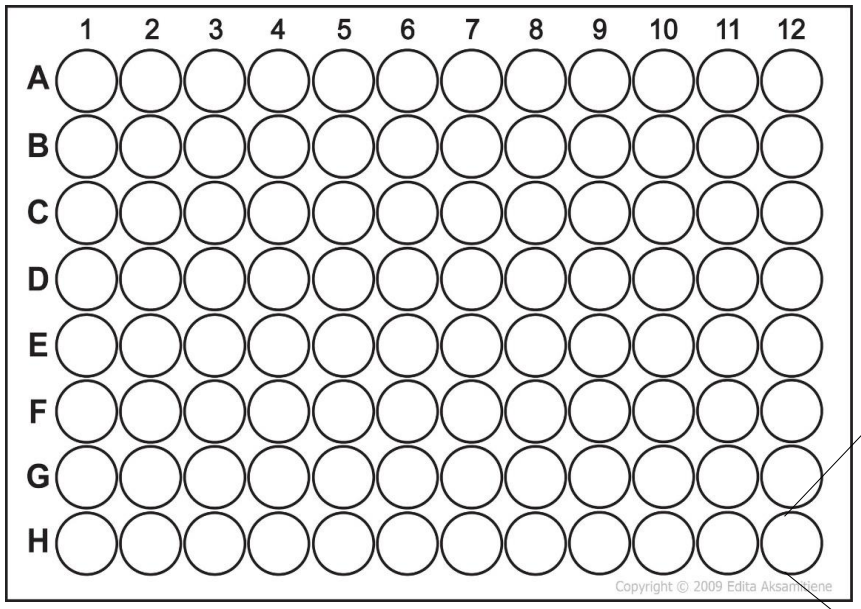
NADPH is naturally fluorescent and represents G6PD level

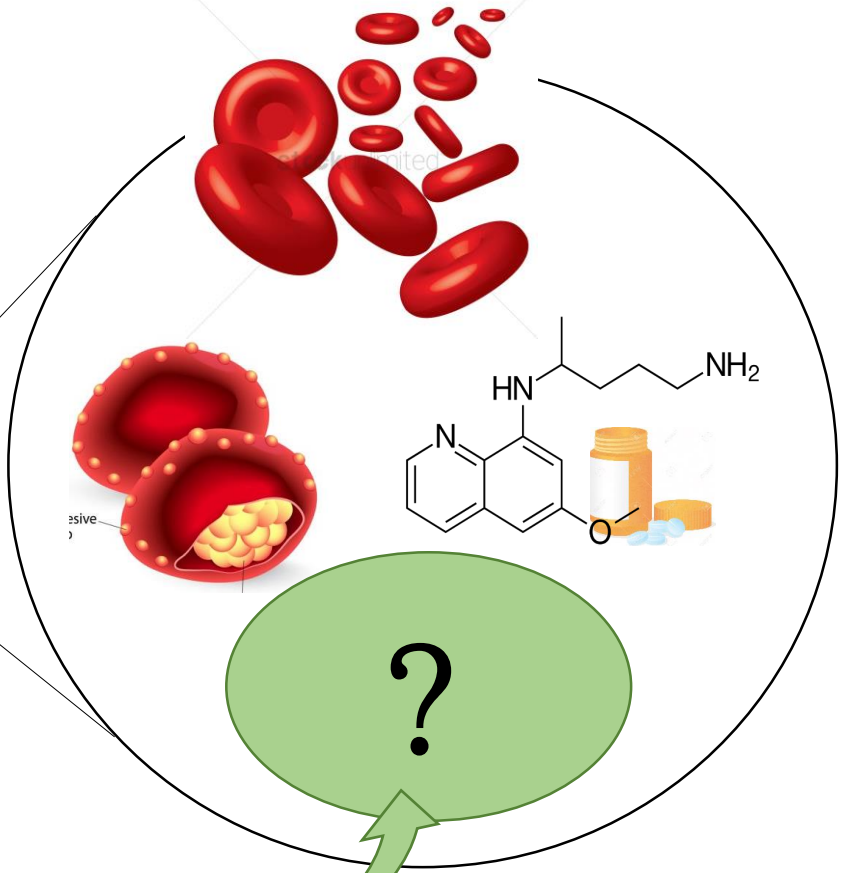
Aim 2: Identify a small molecule that affects G6PD methylation pattern of Pregnant Zebrafish



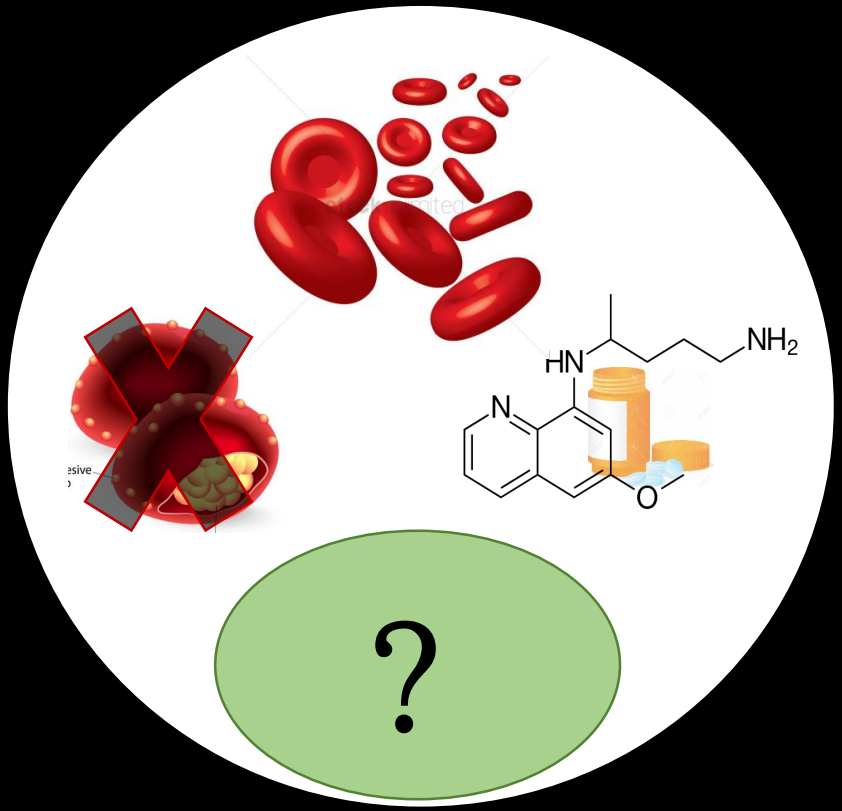
Aim 2: Identify a small molecule that affects methylation, destroys malaria, and can be taken with Primaquine without hemolysis





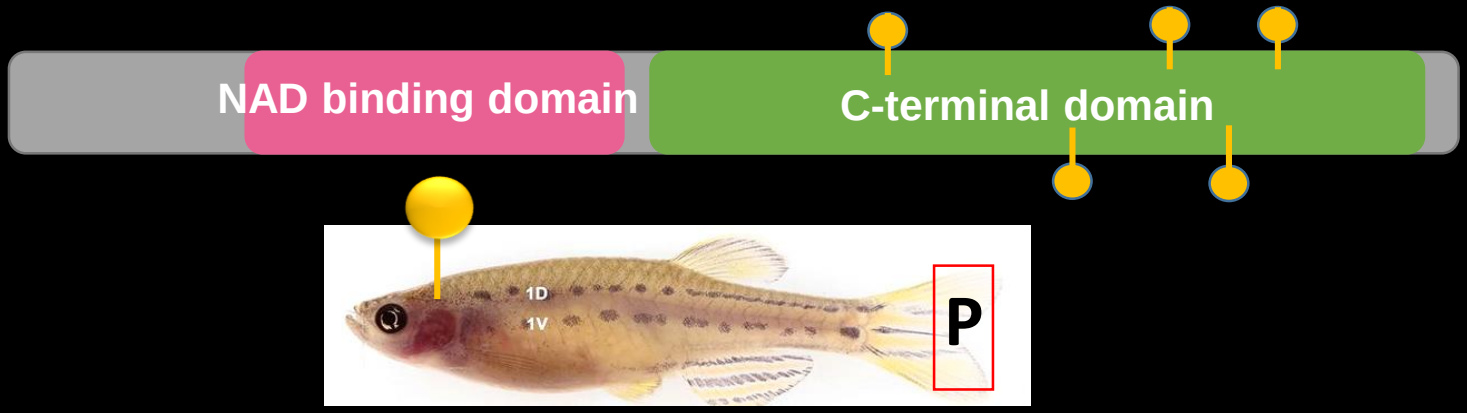


Aim 2: **Identify a small molecule that affects G6PD methylation destroys malaria, and can be taken with Primaquine without hemolysis**

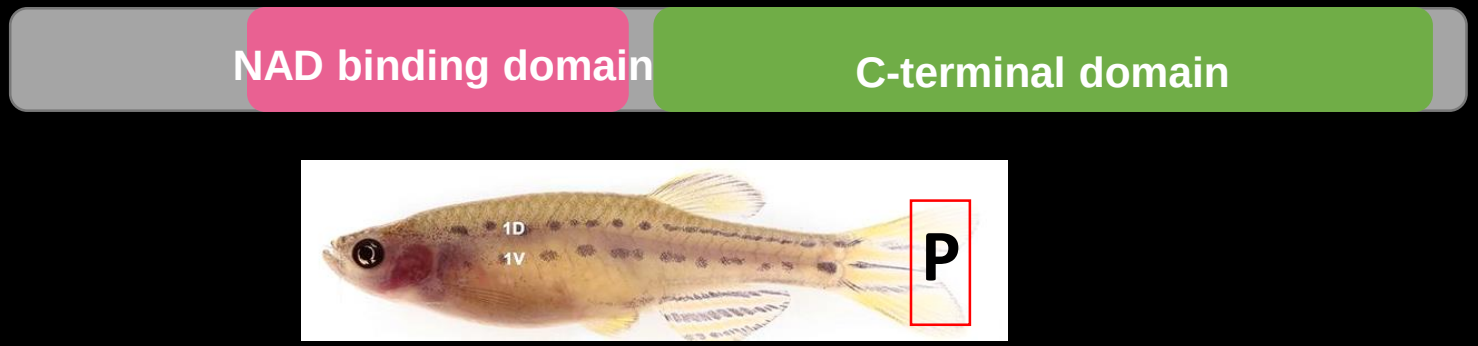


Aim 3: Determine differences in Protein-complexes associated with methylation changes in Pregnant Zebrafish

Control

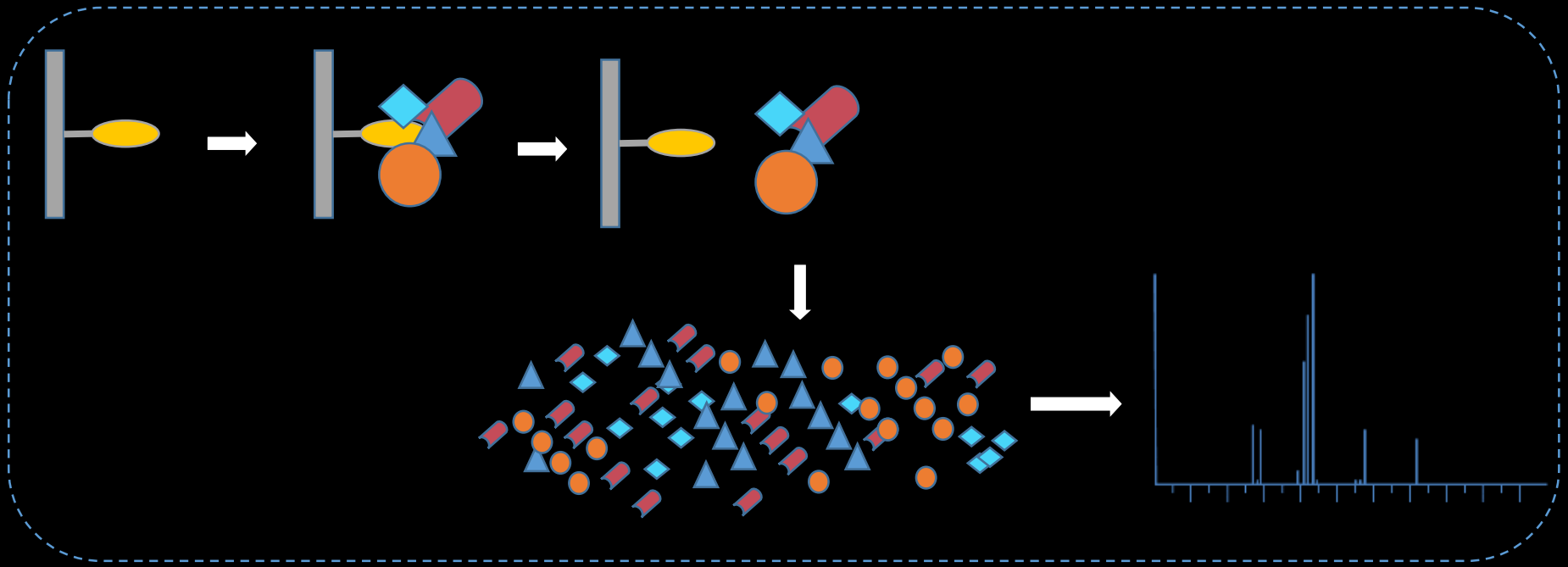


Treatment (KO)

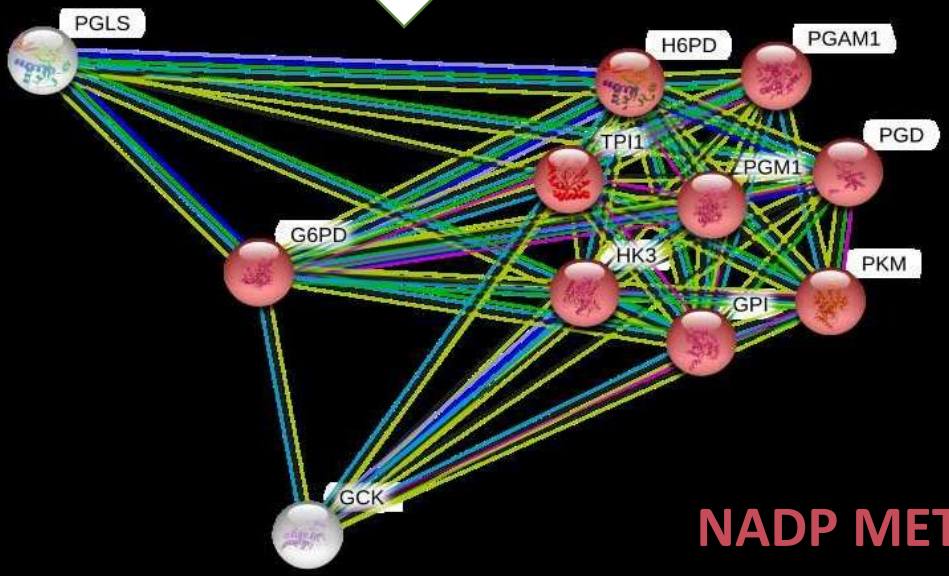


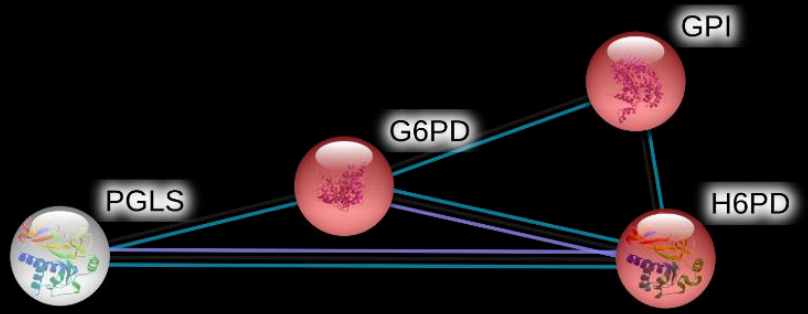
Use CRISPR/Cas9 to create treatment group with no methylation

Aim 3: Determine differences in Protein-complexes associated with methylation changes

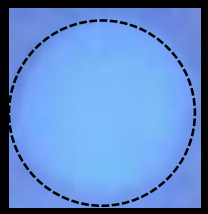


Aim 3: Determine differences in Protein-complexes associated with methylation changes

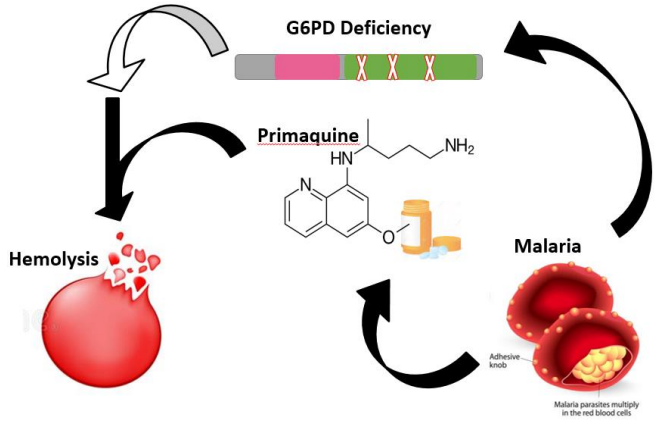


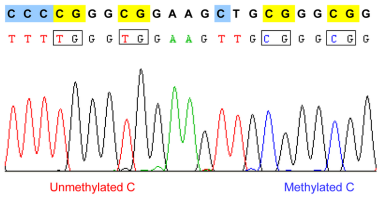


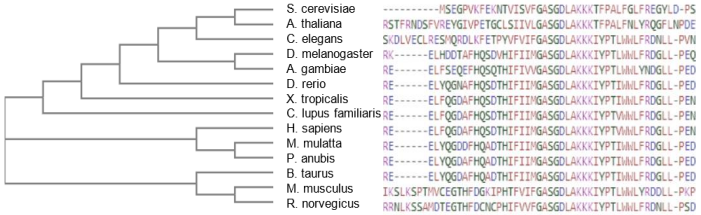
NADP METABOLISM

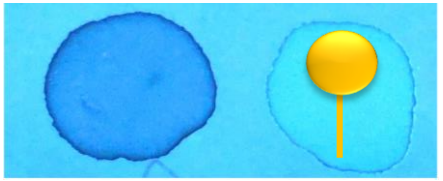


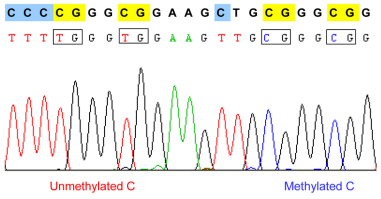
Conclusions

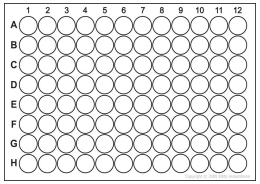


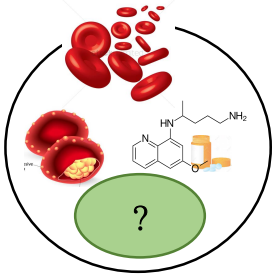


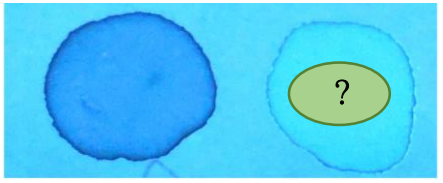


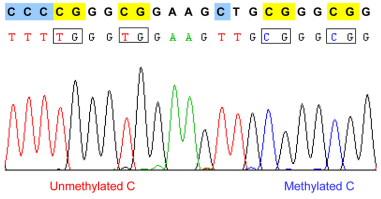


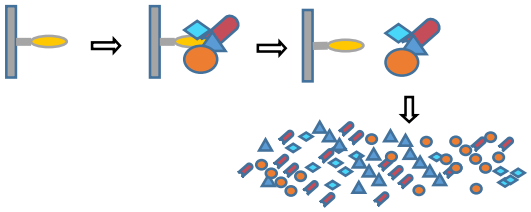


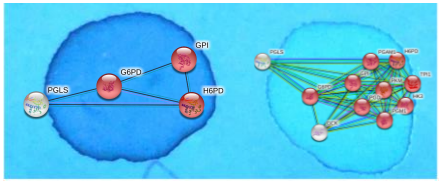












Future Directions

Pharmacogenomics – tailoring drug treatment to one's genes

Determine if methylation effects are similar in humans and other organisms

Discover how to rescue the functional G6PD gene

References

http://soliris.net/resources/images/consq-hemolysis-bg.jpg

https://openi.nlm.nih.gov/detailedresult.php?img=PMC2715191_omcl0101_0015_fig001&req=4

http://intranet.tdmu.edu.ua/data/kafedra/internal/chemistry/classes_stud/en/stomat/ptn/2/03.%20introduction%20to%20%20metabolism.%20investigation%20of%20aerobic%20and%20anaerobic%20oxidation%20of%20glucose.htm

http://geneticsandpublichealth.com/2016/06/05/glucose-6-phosphate-dehydrogenase-deficiency/

https://www.thediabetescouncil.com/diabetes-and-anemia-are-they-related/

https://www.researchgate.net/publication/236088554_Review_of_key_knowledge_gaps_in_glucose-6-phosphate_dehydrogenase_deficiency_detection_with_regard_to_the_safe_clinical_deployment_of_8-aminoquinoline_treatment_regimens_A_workshop_report

https://media.springernature.com/full/springer-static/image/art%3A10.1186%2F1475-2875-11-418/MediaObjects/12936_2012_Article_2605_Fig6_HTML.jpg

https://www.translationalres.com/article/S1931-5244(14)00336-3/fulltext

https://string-db.org/cgi/network.pl?taskId=9ofOGJV7JRtJ

https://ourworldindata.org/malaria

https://www.ncbi.nlm.nih.gov/books/NBK65119/

https://www.nhlbi.nih.gov/health-topics/hemolytic-anemia