

RBC HYPOXIC METABOLIC REPROGRAMMING

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01

RBC oxygen transport
and beyond

WHO DID THE WORK?



Travis Nemkov
Hansen Lab



Julie A. Reisz Haines, PhD
Metabolomics Core



Monika Dzieciatkowska, PhD
Proteomics Core



Matthew J Wither, PhD
Metabolomics Core



Ryan C. Hill, PhD
Hansen Lab &
Proteomics Core



Kirk C. Hansen, PhD
Proteomics



Kaiqi Sun, PhD
Univ. Texas, Houston

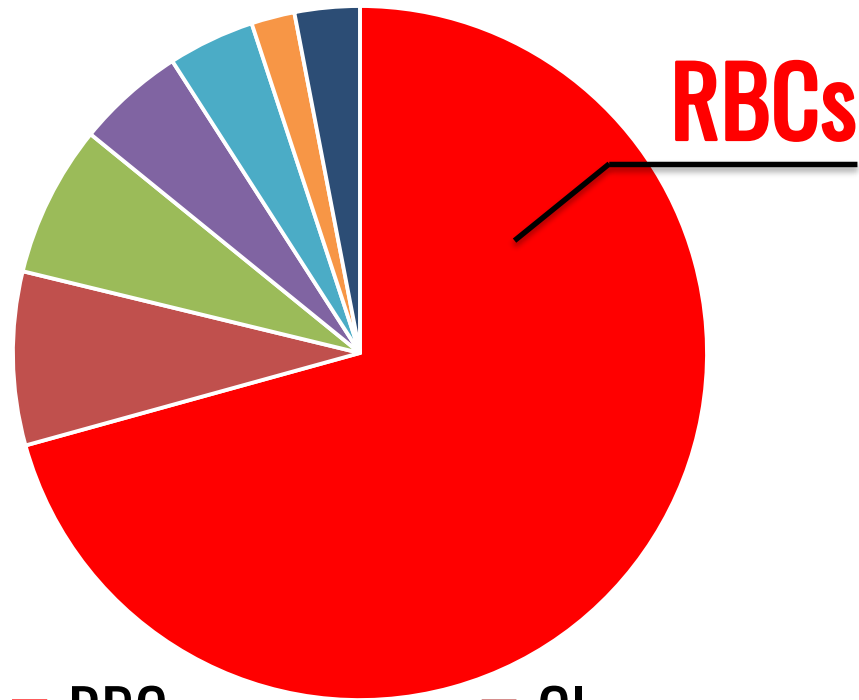


Anren Song, PhD
Univ. Texas, Houston



Liu Hong, PhD
Univ. Texas, Houston

Most abundant host cell in human body? **RBCs, by far**



- RBCs
- Glya
- Endothelium
- Derm
- PLTs
- Bone Marrow
- Other

- 25 Trillion RBCs out of 30 Trillion host cell in the human body;
- Lifespan of ~120 days
- ~0.2 Trillion RBCs made everyday;
- Significant daily energy expenditure in embryonic and adult life;

SIMPLE

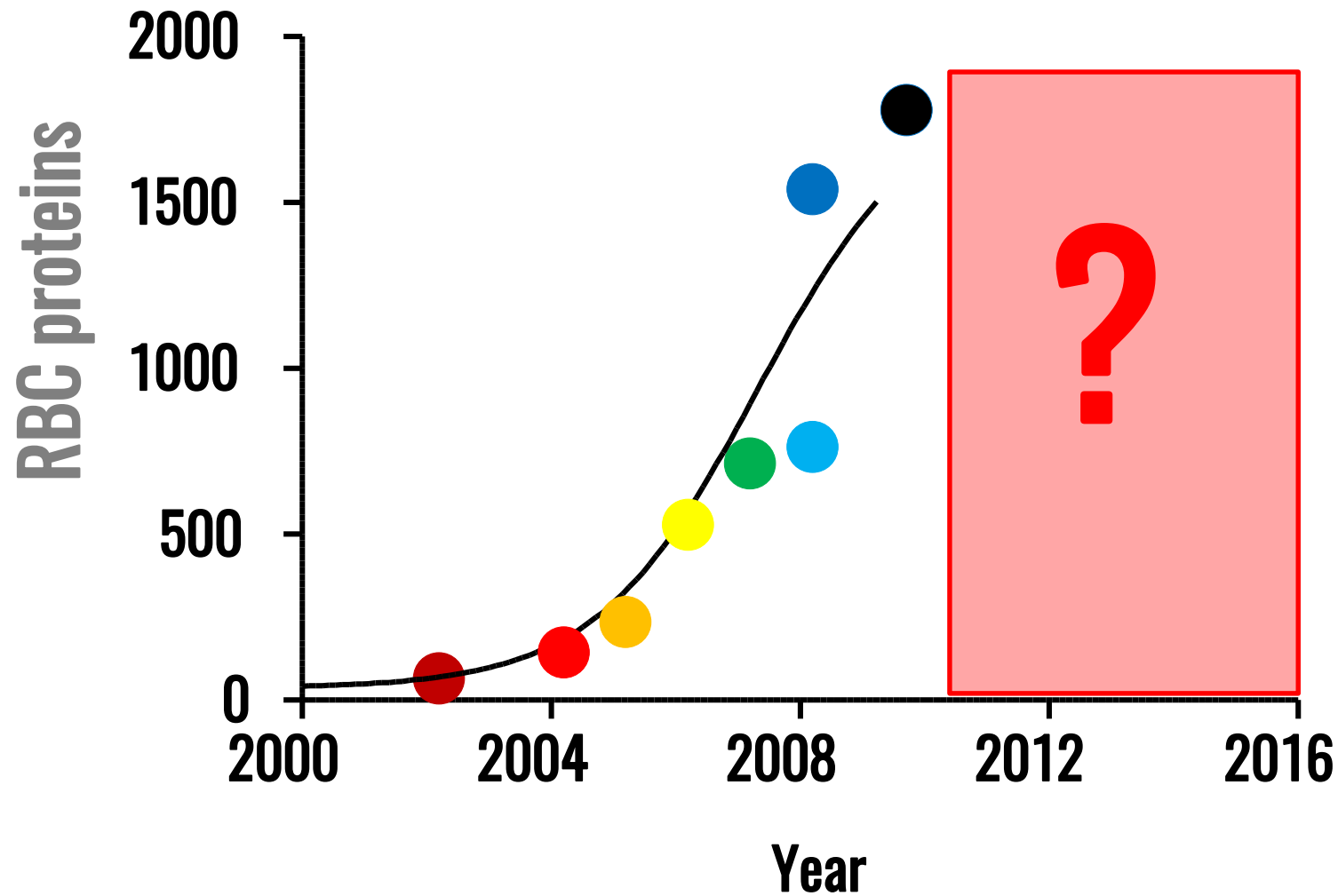


- Lose nuclei and organelles during maturation;
- No ribosomes: devoid of *de novo* protein synthesis capacity;
- 98% of cytosol is hemoglobin!

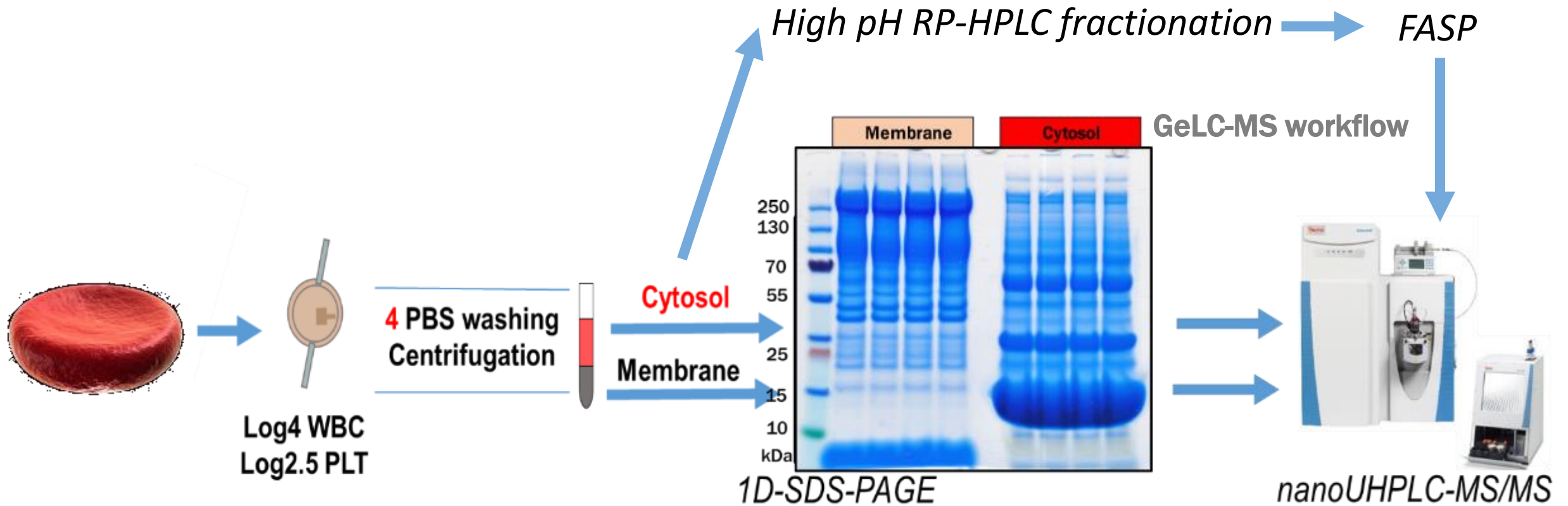
YET COMPLEX

- They still preserve the proteasome;
- The 2% of the proteome that is not Hb includes more than 1500 proteins (as of now)

RBC Proteomics: Unanticipated complexity

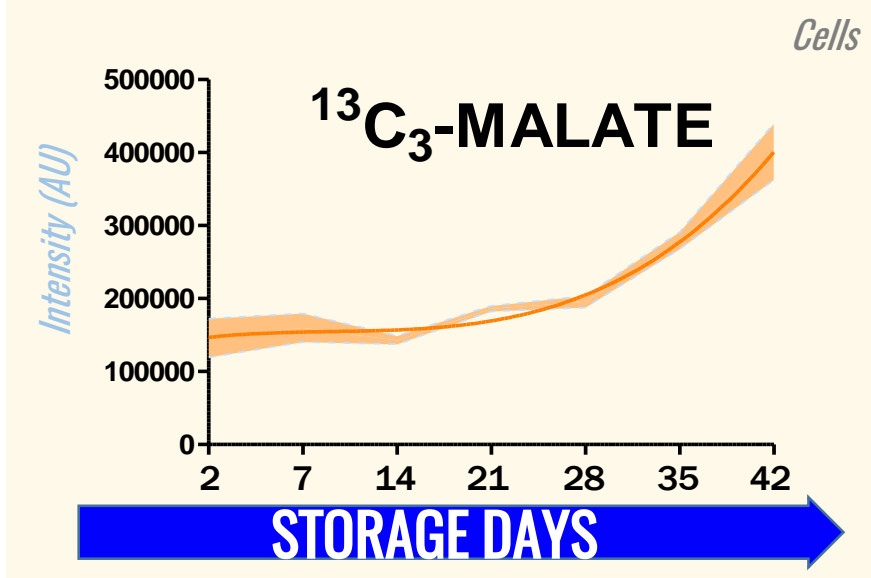
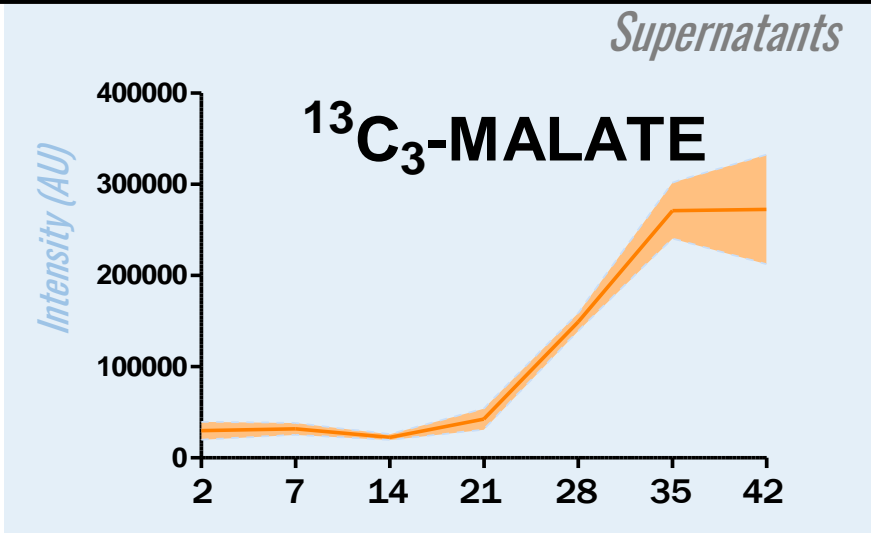
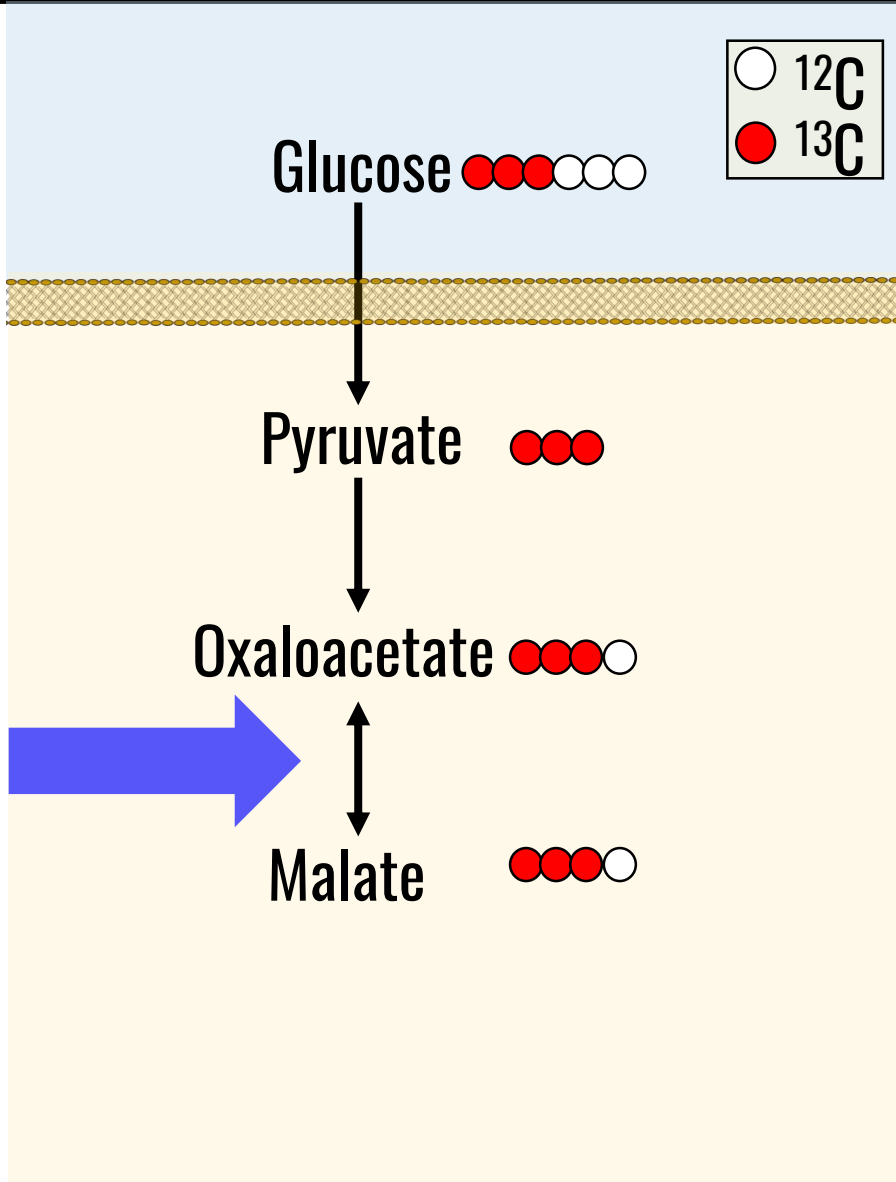


Project DEEP RED

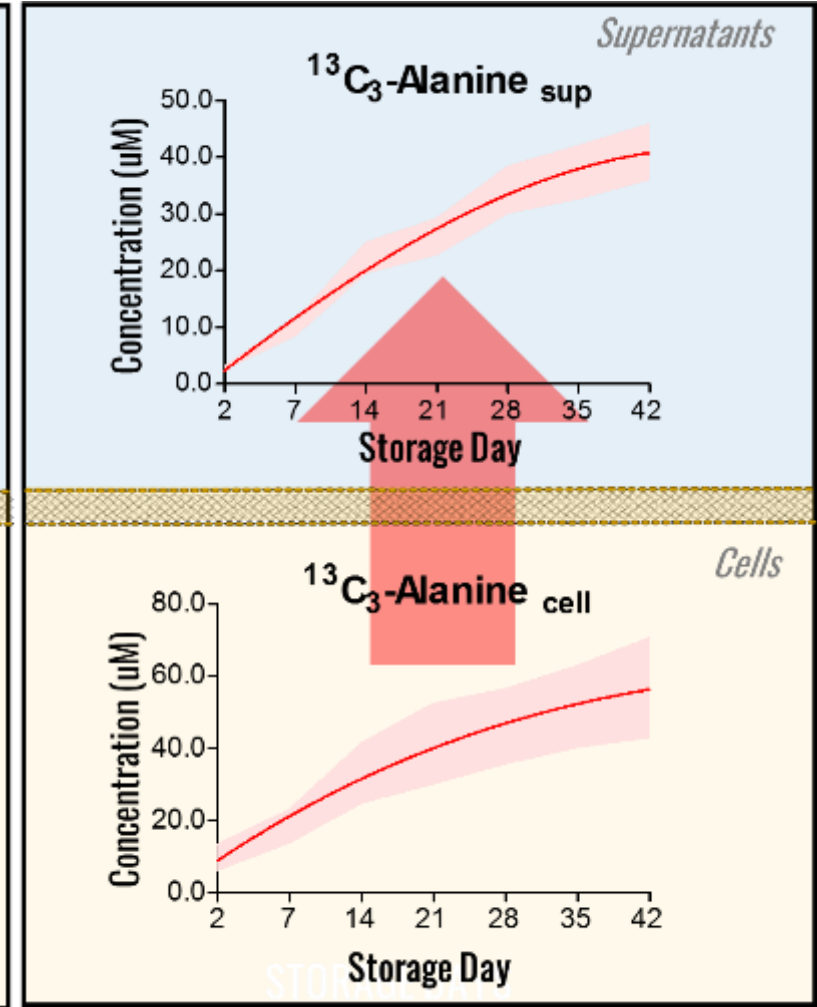
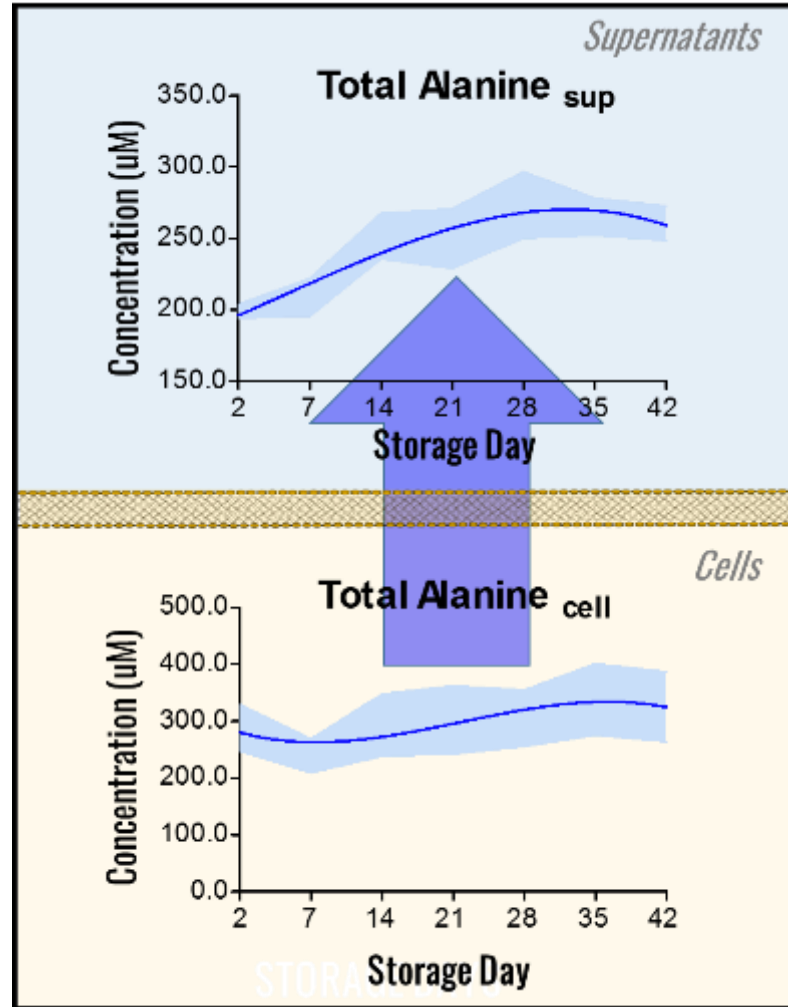
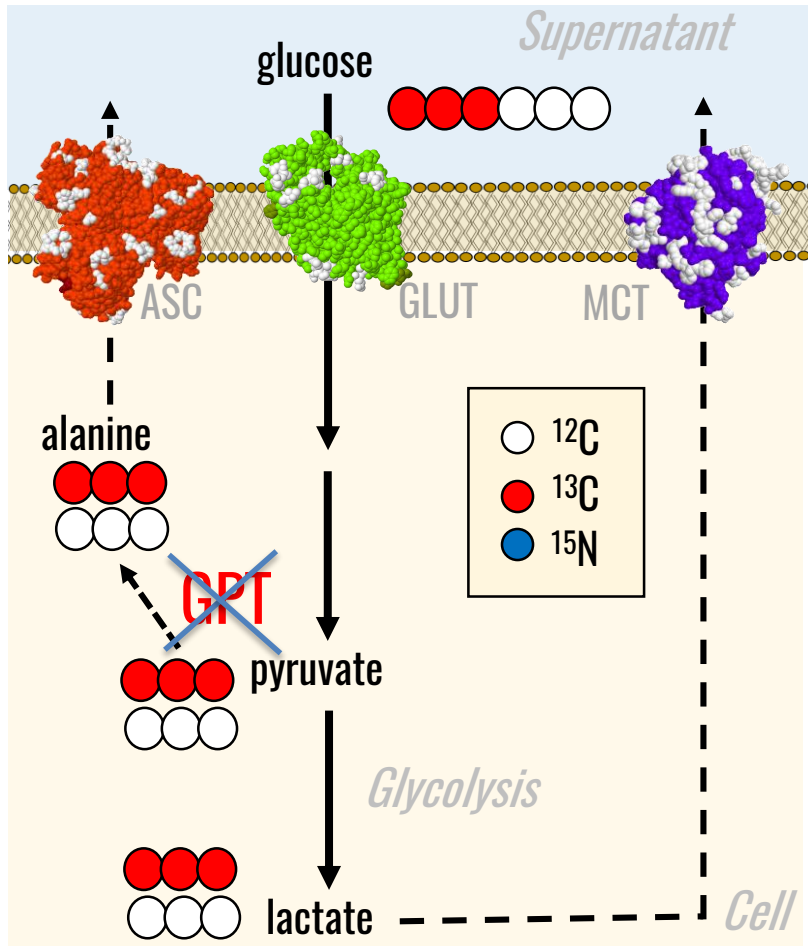


RBCs: Cytosolic isoforms of TCA cycle enzymes

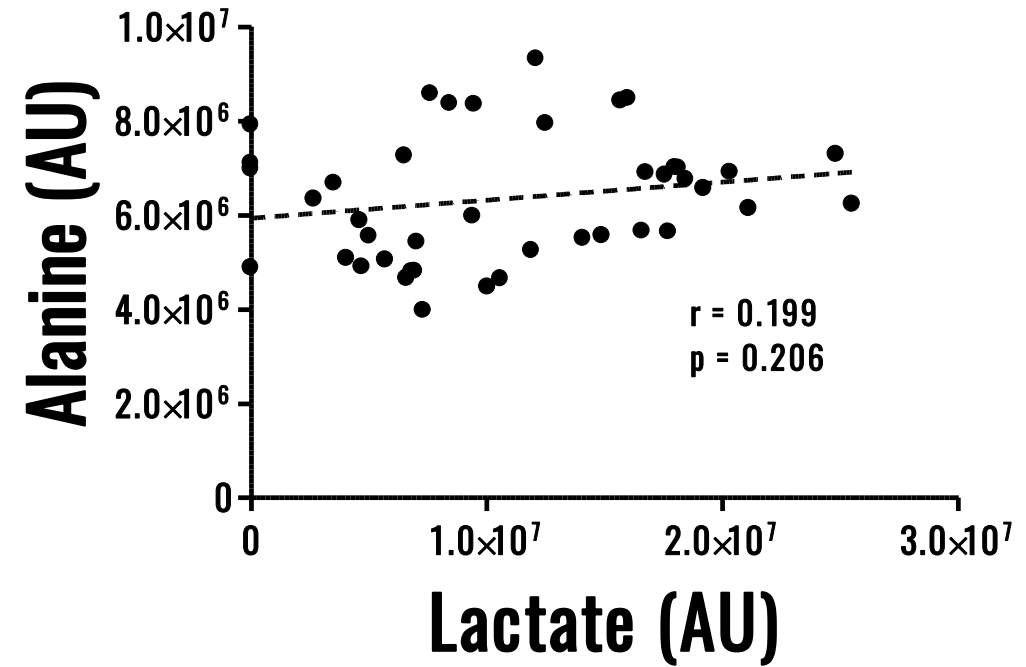
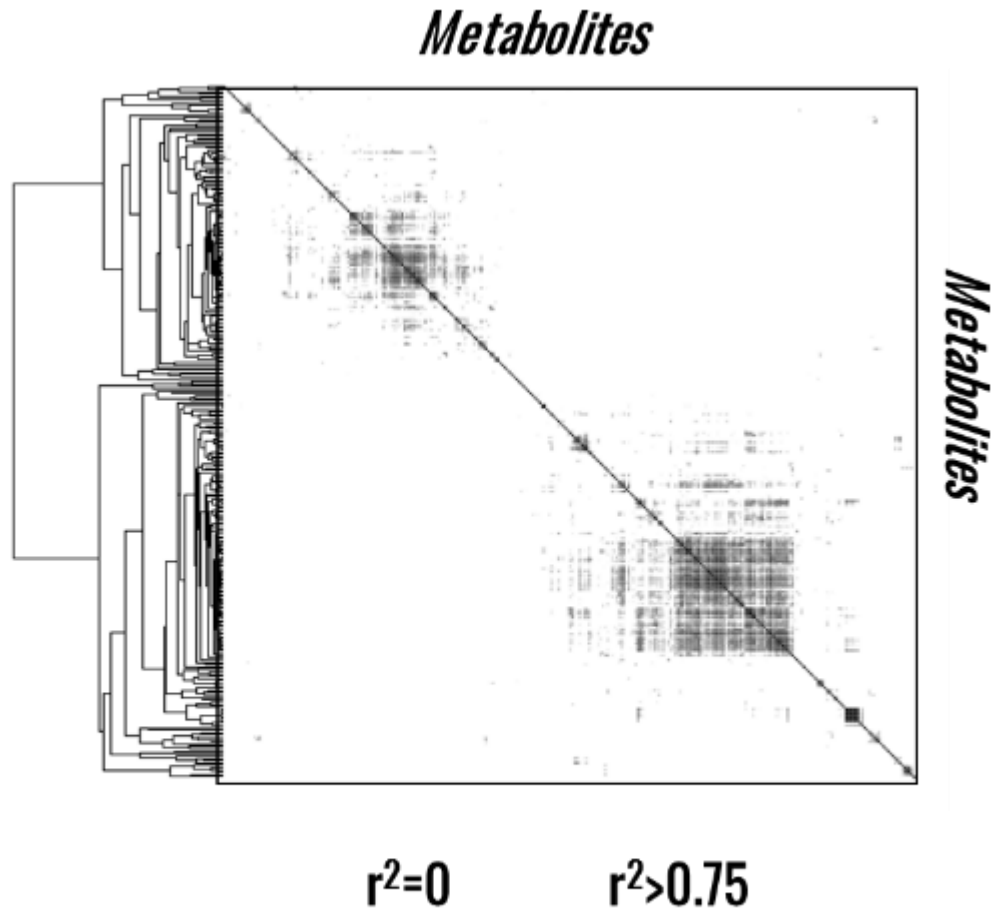
Malate dehydrogenase 1, cytoplasmic (P04925)



RBCs: ... 2806 proteins and counting... (Introduction to Quantitative Metabolic Flux Analysis)

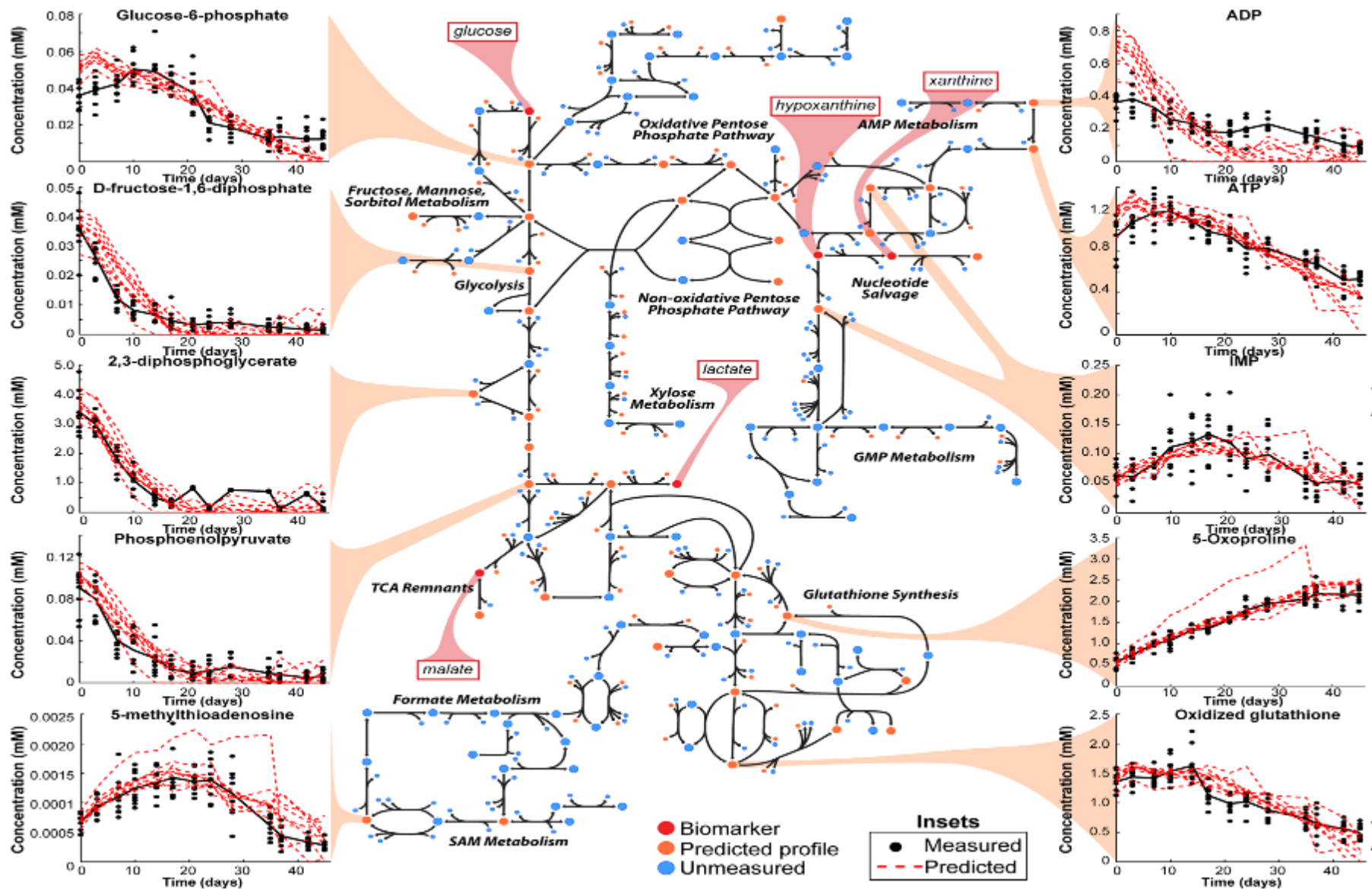


META-INFORMATION: METABOLIC LINKAGE



G6PDH deficient donors (<10% residual activity)

Systems Biology: Predicting RBC metabolic fluxes from steady state data



Aarash Bordbar
Sinopia Biosciences



James Yurkovich
UCSD, Palsson's Lab



Bernard Palsson
UCSD, San Diego, CA



Neema Jamshidi
UCLA, Los Angeles, CA

RBCs: CLINICAL METABOLOMICS

- **Clinical biochemistry of RBCs is routine practice**
 - Easy to collect;
 - Extremely abundant
 - Only 4-12 metabolic parameters monitored;
- **High Throughput metabolomics platform**
(+300 parameters in 3 minute)
- **Blood testing in HEALTH and DISEASE**
 - Hypoxia;
 - Sickle cell disease;
 - Inflammation;
 - Ageing;
 - Down Syndrome;



Prof. Andrew Monte (R35)

PERSONALIZED MEDICINE INITIATIVE

If you still prefer Metabolon over us... maybe bad news



NIH National Metabolomics Resource Map

02

RBC metabolic reprogramming
in high altitude hypoxia

High Altitude hypoxia: ALTITUDEOMICS

- **21 subjects**

(12 males and nine females, age 19-23 yo)

- **5 time points**

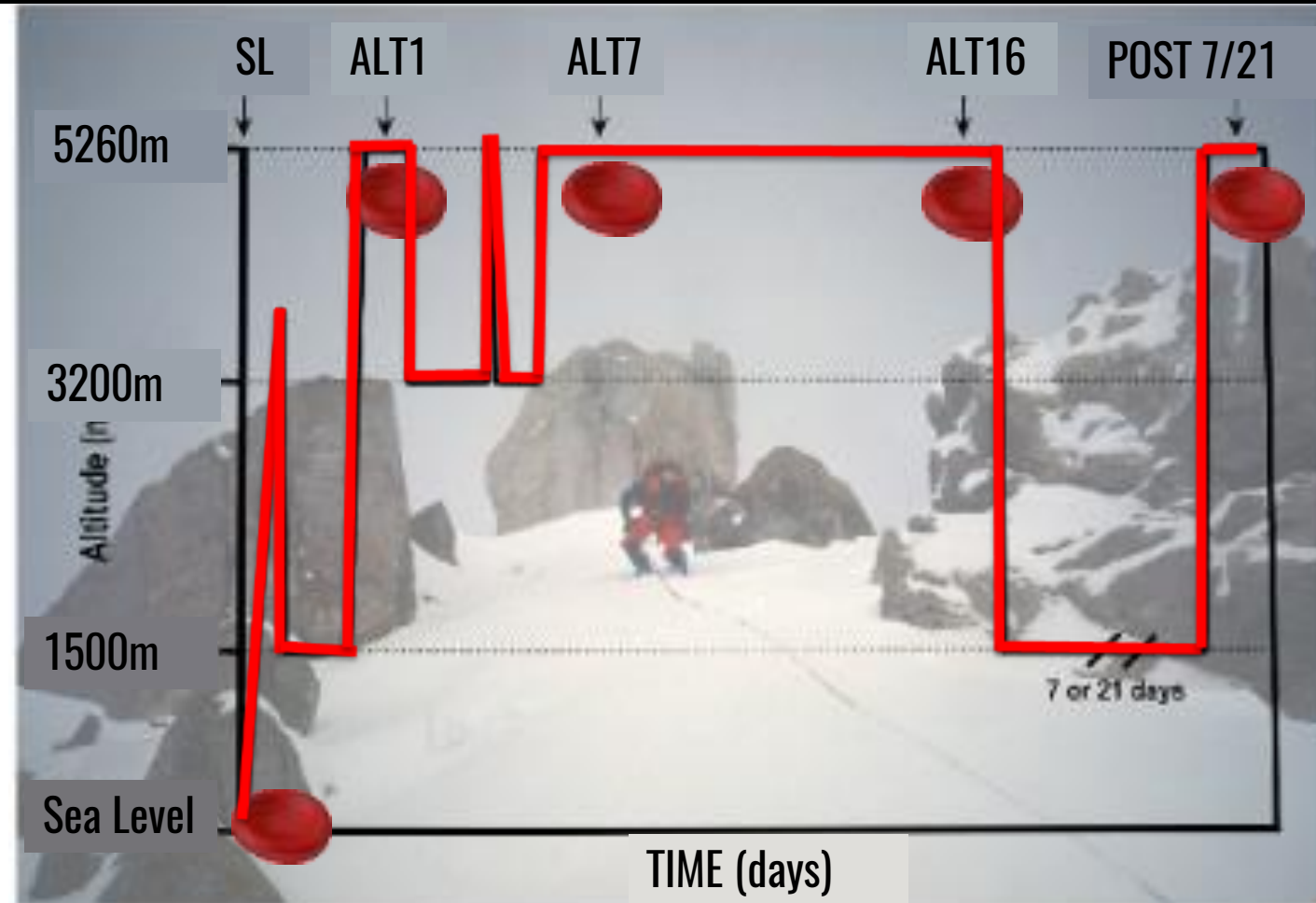
(Sea Level, Day 1, 7, 16 at high Altitude,
Reascending either at Day 7 or 21 from descending)

First day at high altitude: noon and 8 pm blood draws

Robert C Roach – Altitude Research Center – Denver

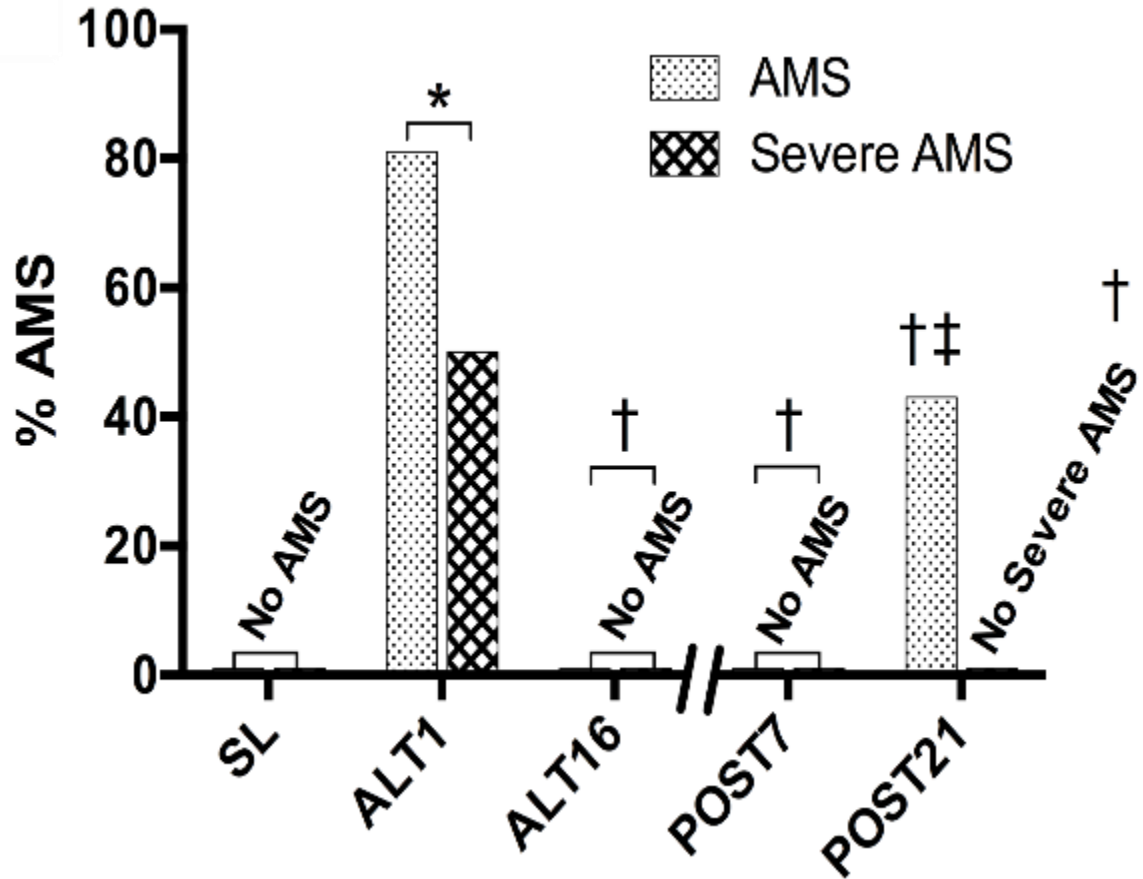
Yang Xia – University of Texas Houston

Kirk C Hansen – University of Colorado Denver

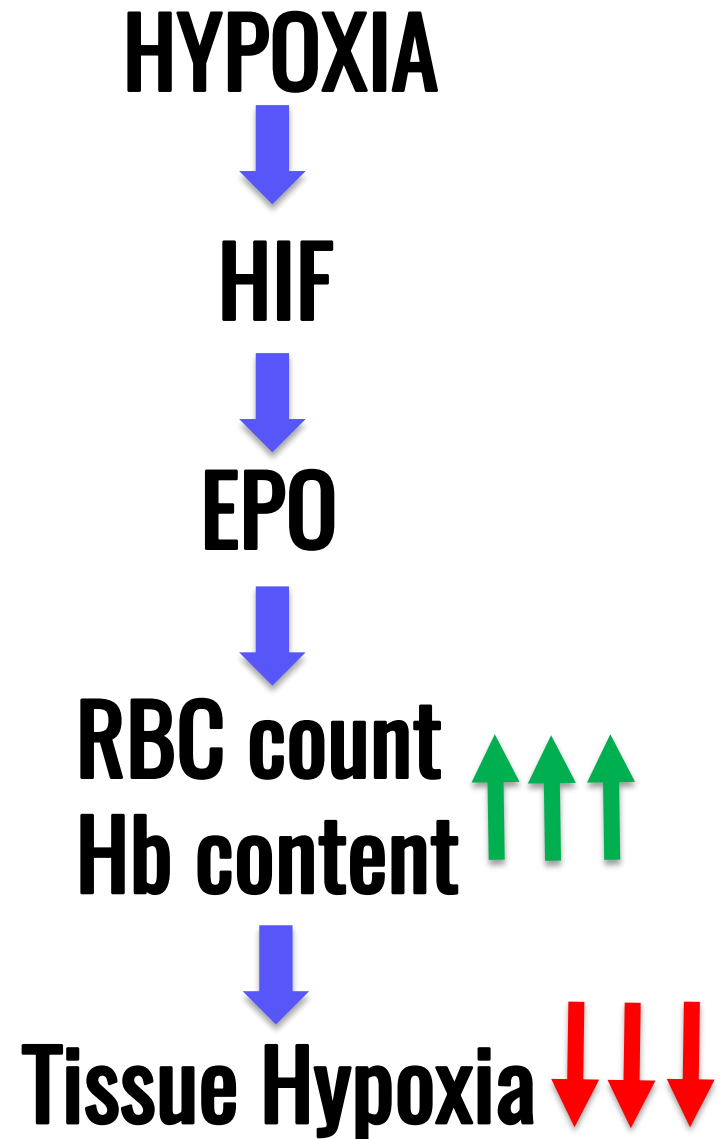


Altitude Research Center

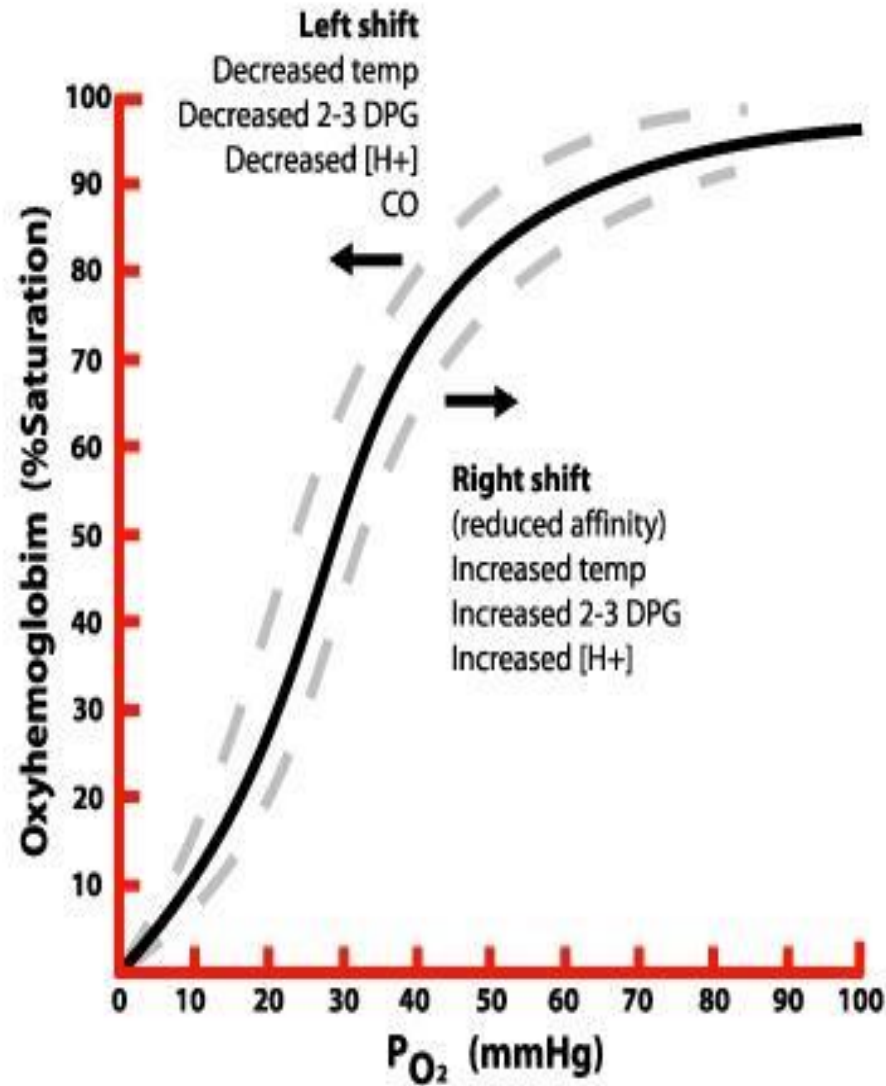
Acclimatization: HB concentration increases only after 7 days – but acclimatization is faster!



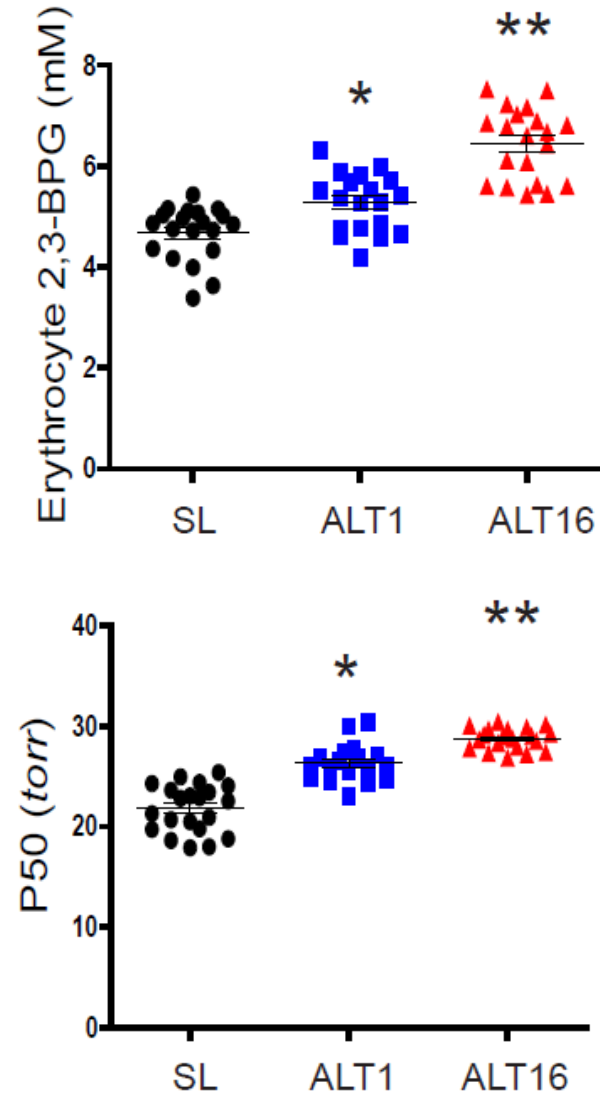
*AMS = Acute mountain sickness



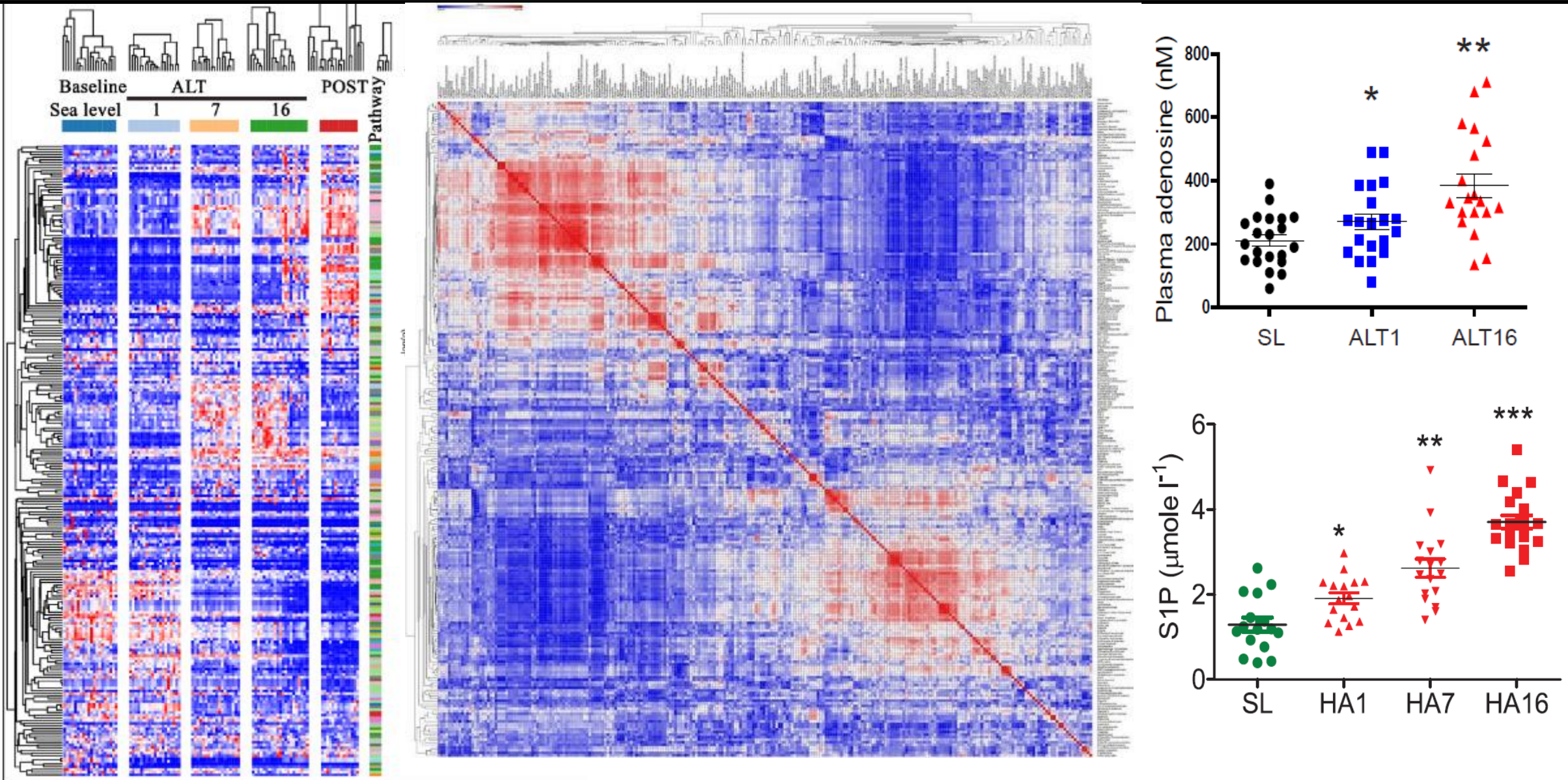
Acclimatization: DPG increase promotes O₂ off-loading and tissue oxygenation



TISSUE OXYGENATION POTENTIAL



High Altitude hypoxia: metabolic reprogramming in RBCs as early as hours from ascent





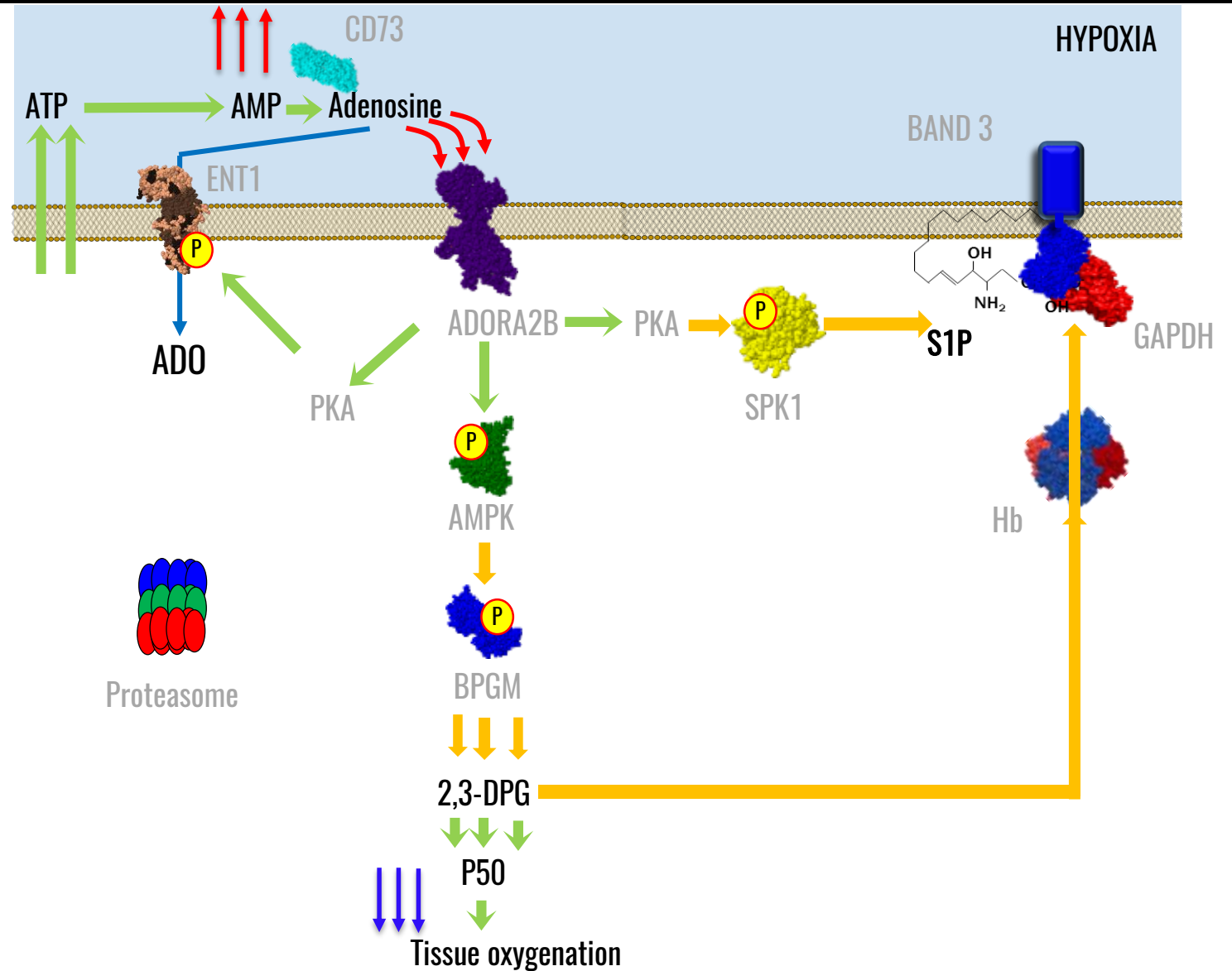
Liu et al. *Circulation* 2016; Sun*, Zhang*, D'Alessandro* et al. *Nature Comm* 2016; Haines et al. *Blood* 2016; *minor revisions*; Song et al *Nat Comm* 2017

Model: Adenosine and Sphingosine 1 phosphate stabilize deoxyHb and promote glycolysis under hypoxia

HUMAN

MOUSE

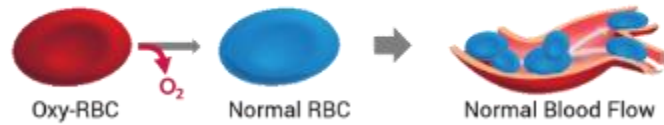
Altitudeomics cohort, Ex vivo experiments
 Untargeted Metabolomics
¹³C_{1,2,3}-glucose experiments
 Quantitative targeted metabolomics
 CD73, ADORA (all receptors) and SPK1 kd mice
 Immunofluorescence
 Activity assay
 Competitive activity assay
 Competitive binding
 Crystallography
 Pharmacological activation of AMPK
 Pharmacological inhibition of AMPK
 Proteasome inhibition
 Redox proteomics
 Ubiquitylation assay
 PKA activation/inhibition
 BALF protein content, neutrophil infiltration
 Hydroxyprobe, P50 and DPG measurement



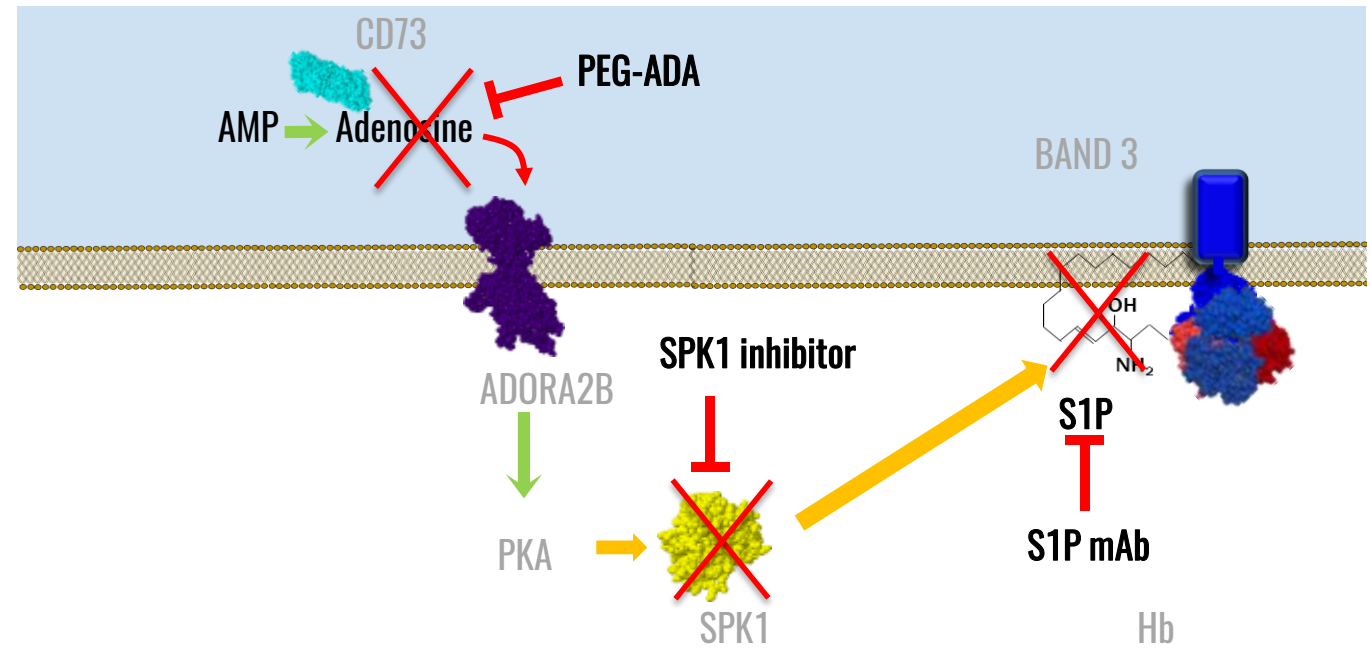
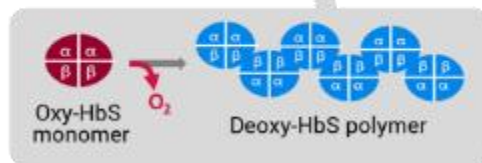
SCD: Sickling is induced by HbS polymerization at the membrane. Does Sphk1 play a role?



Normal RBC



HbSS RBC



03

RED BLOOD CELL STORAGE
and INTEGRATED OMICS

Sales April 2014-March 2015 - x1,000,000

108

MOST CONSUMED PRESCRIPTION DRUG IN THE US/WORLD

21

7

Viagra

Ventolin

Crestor

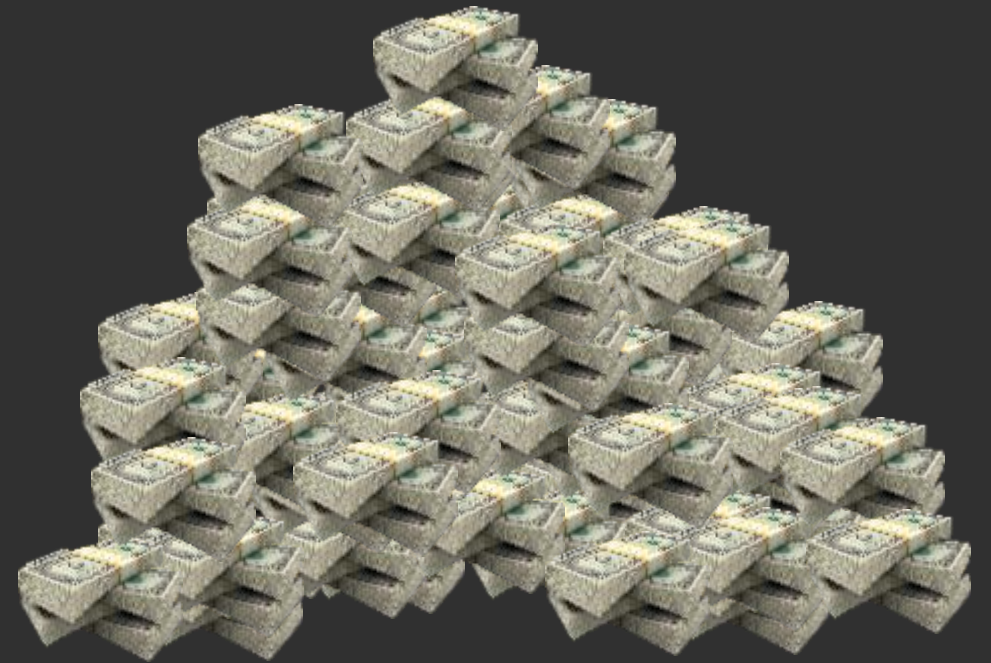
Synthroid

RBC
Transfusion

ECONOMIC BURDEN (year/US \$)

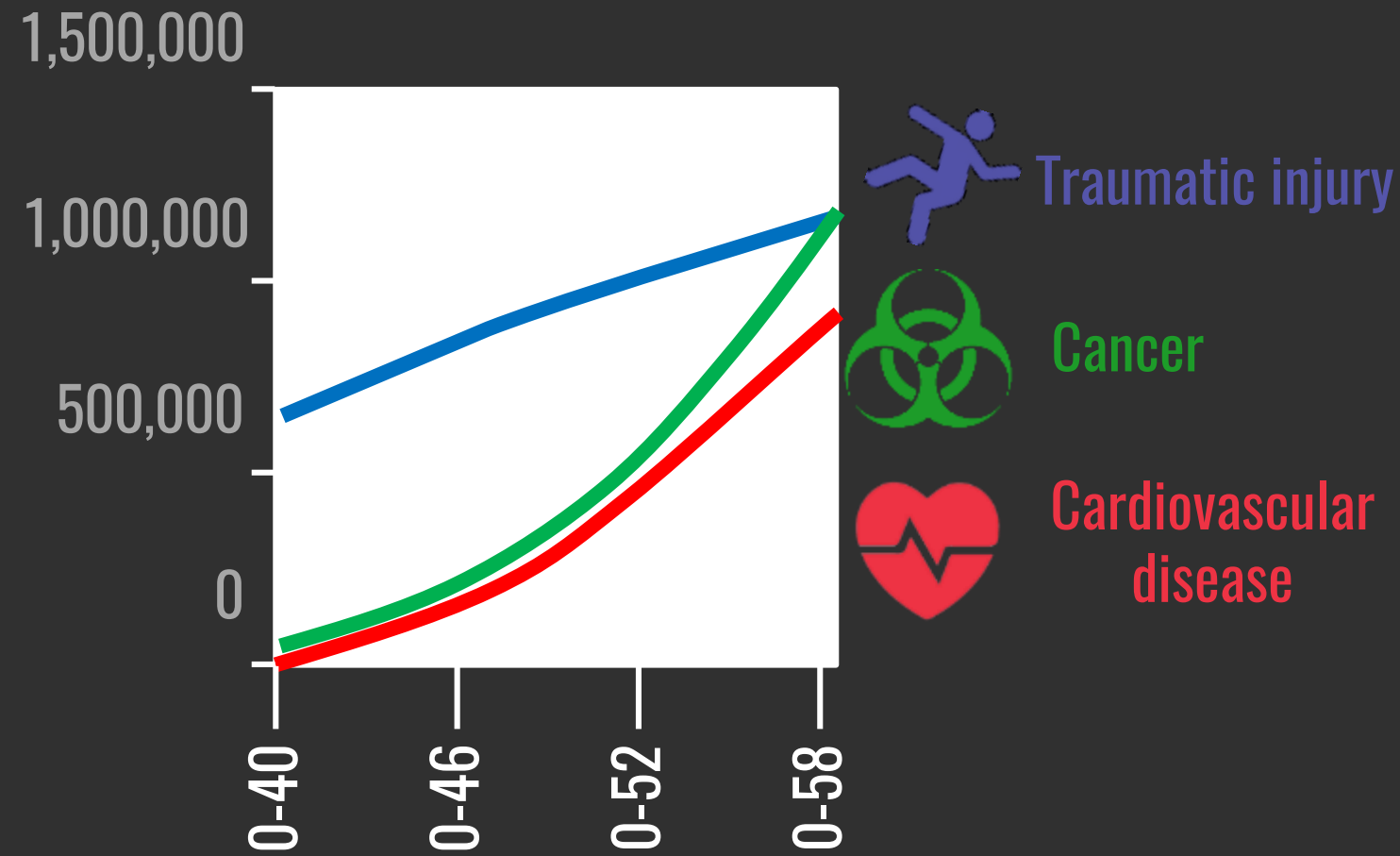


\$220-300/unit

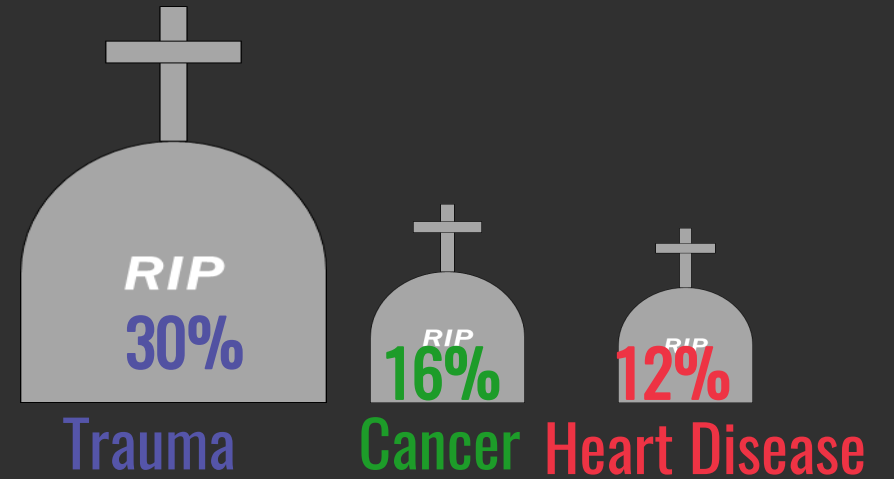


\$3.5-4.5 billion/year in the US alone
6th in rank of all drugs by sales!!!

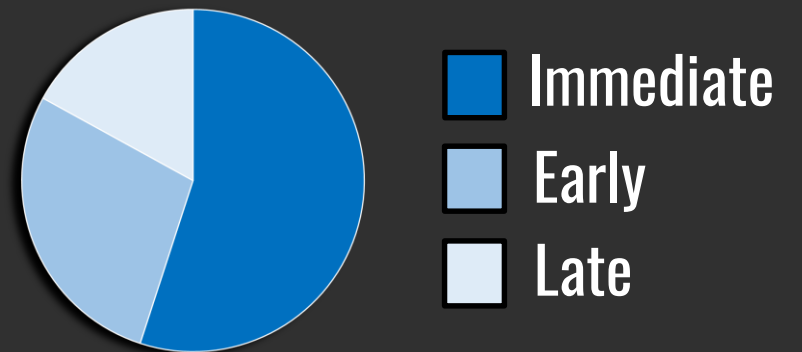
CAUSES of DEATH (<59 age)

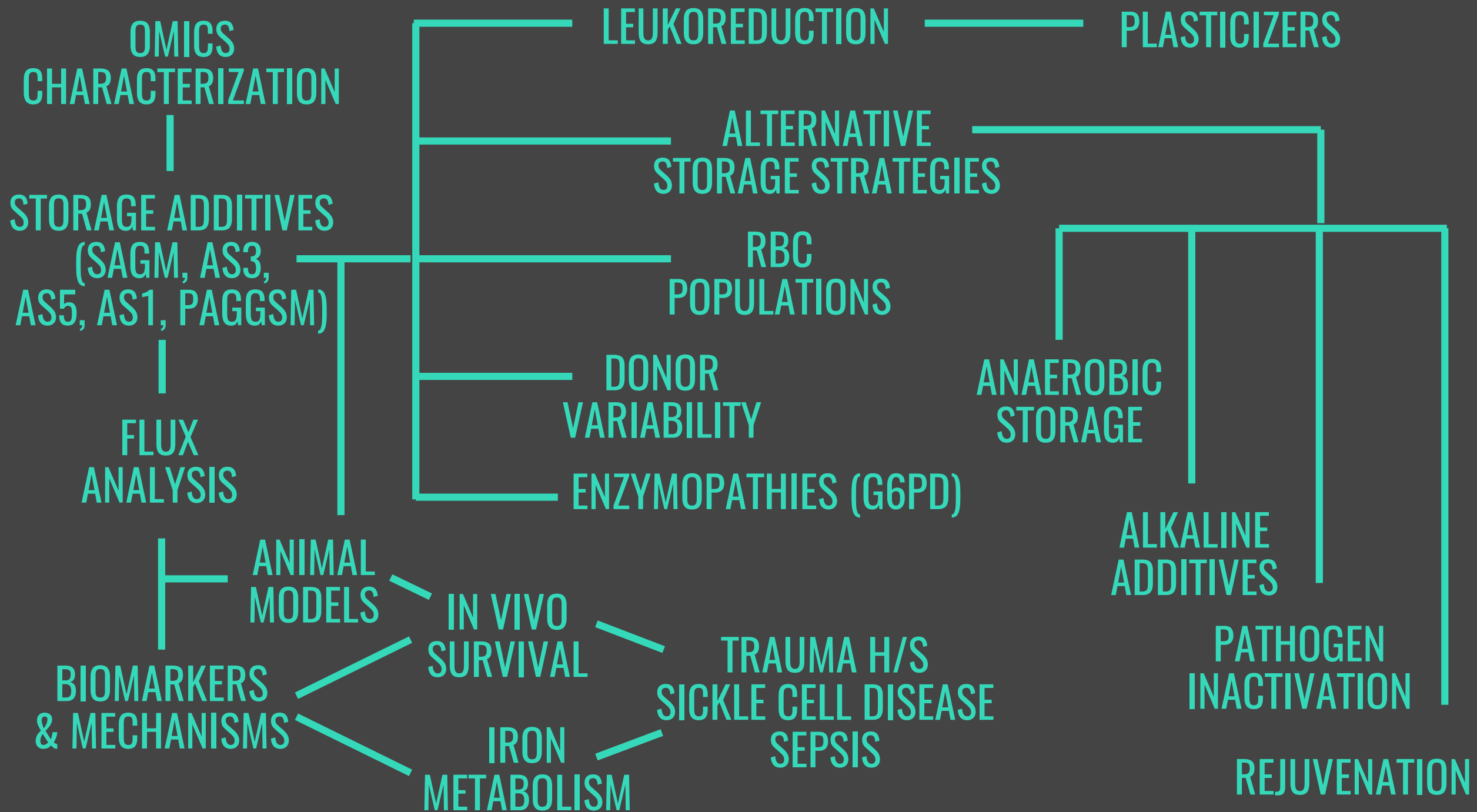


LIFE YEARS LOST



40% PREVENTABLE





RBC storage: Can we make it better?

CONFERENCE REPORT

2015 proceedings of the National Heart, Lung, and Blood Institute's State of the Science in Transfusion Medicine symposium

*Steven L. Spitalnik,¹ Darrell Triulzi,² Dana V. Devine,³ Walter H. Dzik,⁴ Anne F. Eder,⁵ Terry Gernsheimer,⁶ Cassandra D. Josephson,⁷ Daryl J. Kor,⁸ Naomi L. C. Luban,⁹ Nareg H. Roubinian,¹⁰ Traci Mondoro,¹¹ Lisbeth A. Welniak,¹¹ Shimian Zou,¹¹ and Simone Glynn,¹¹ for the State of the Science in Transfusion Medicine Working Groups**

4. “How can we make better products?” Develop improved methods for preparing classical products and determine whether there are alternative ways of preparing analogues (e.g., synthetic, bioengineered, “biopharmed”).

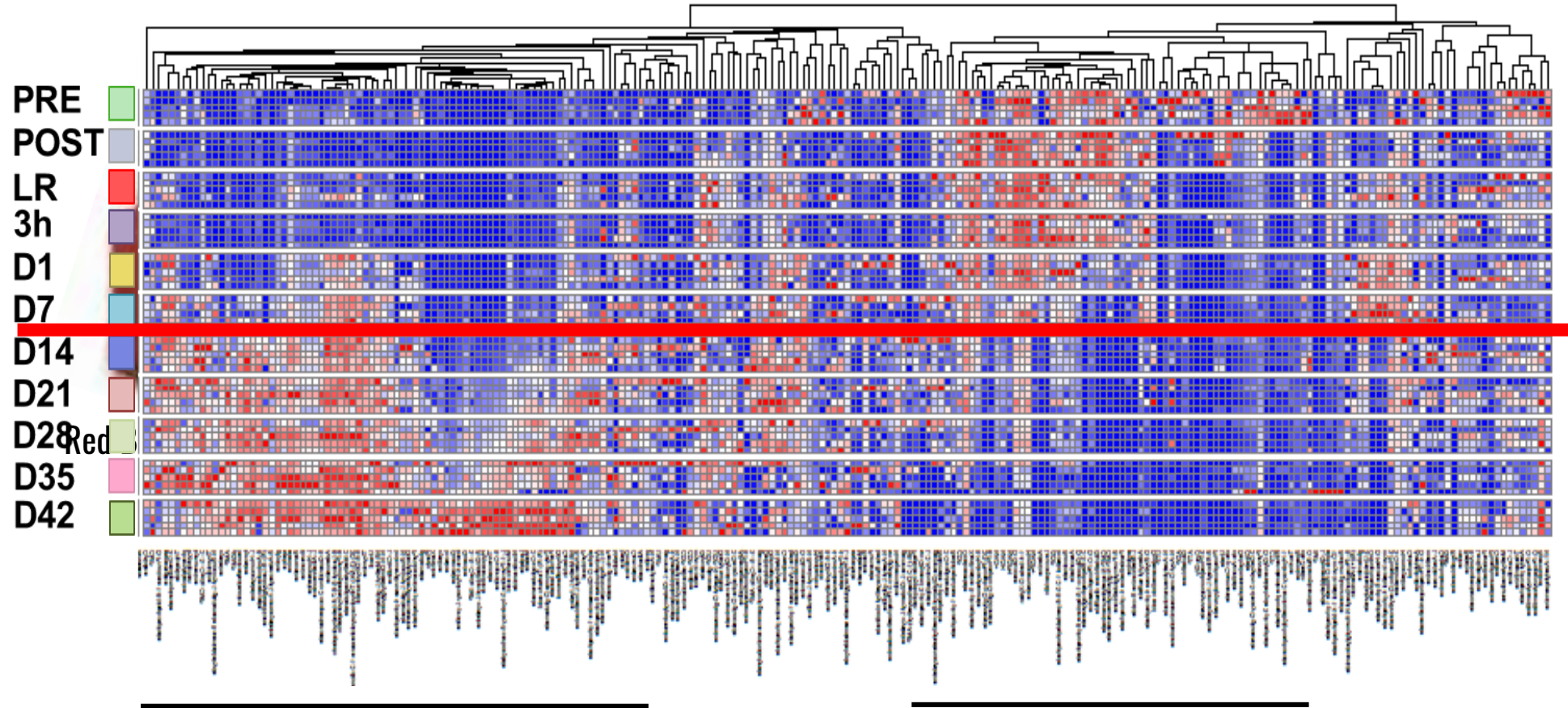
**BLOOD BANK
STORAGE QUALITY**
We can make it better

RBC storage: *how long is too long?*



RBC Extracts

AS3: Significant metabolic lesions tend to accumulate by **storage day 14**

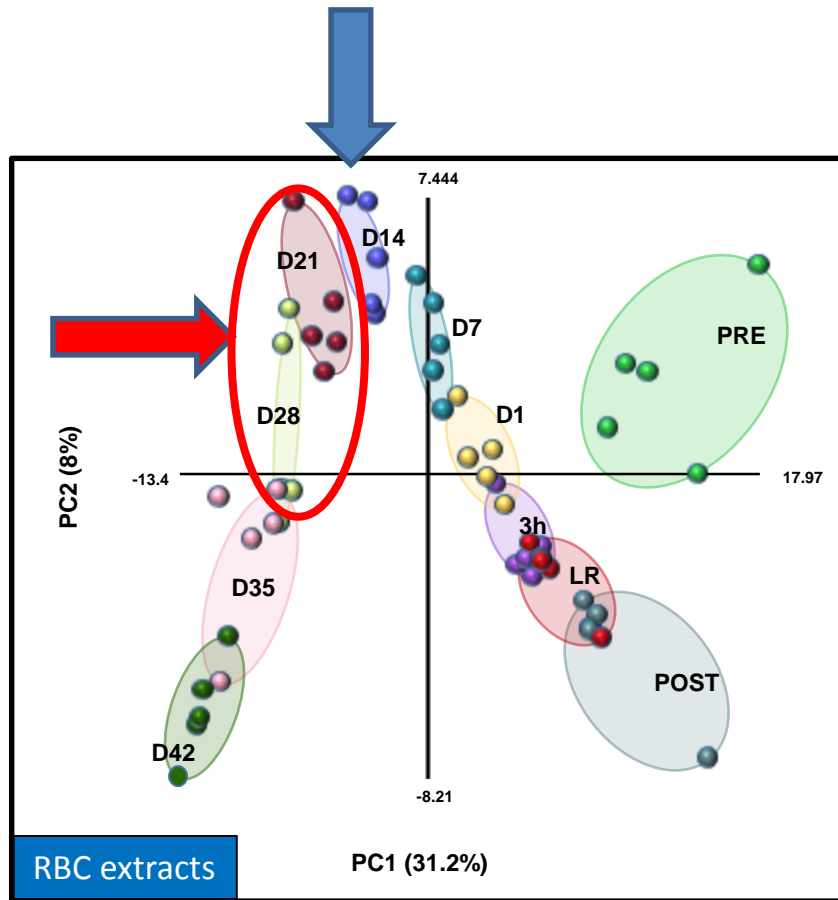


Metabolites increasing by storage day 14

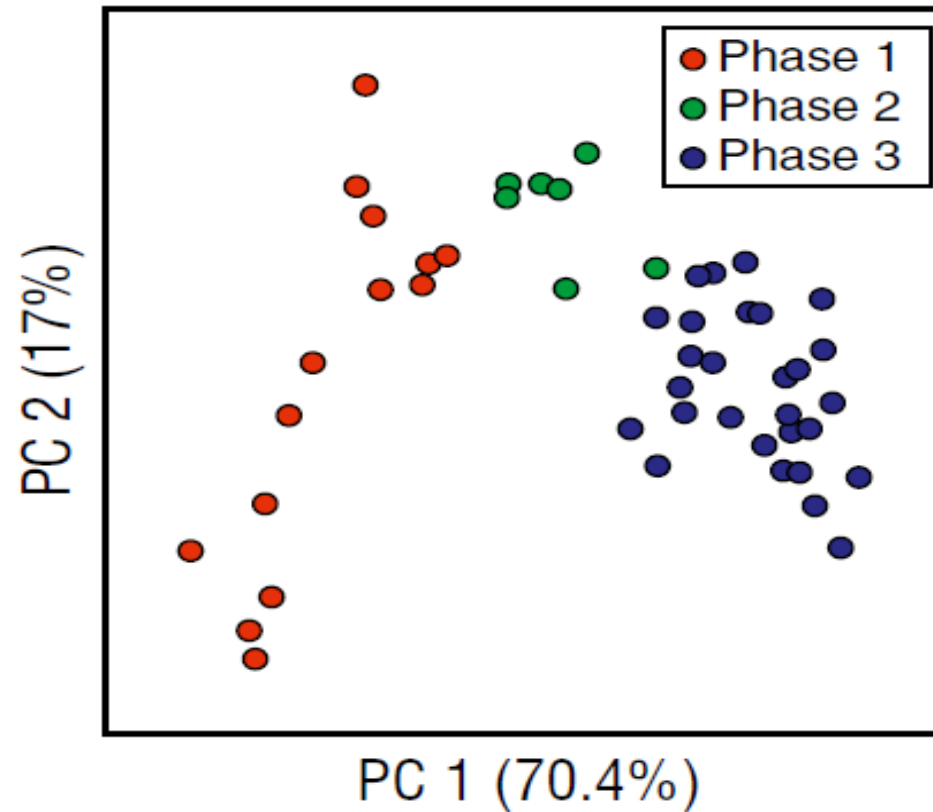
Metabolites decreasing within storage day 14

Statistics: *significant changes occur between storage week 2 and 3*

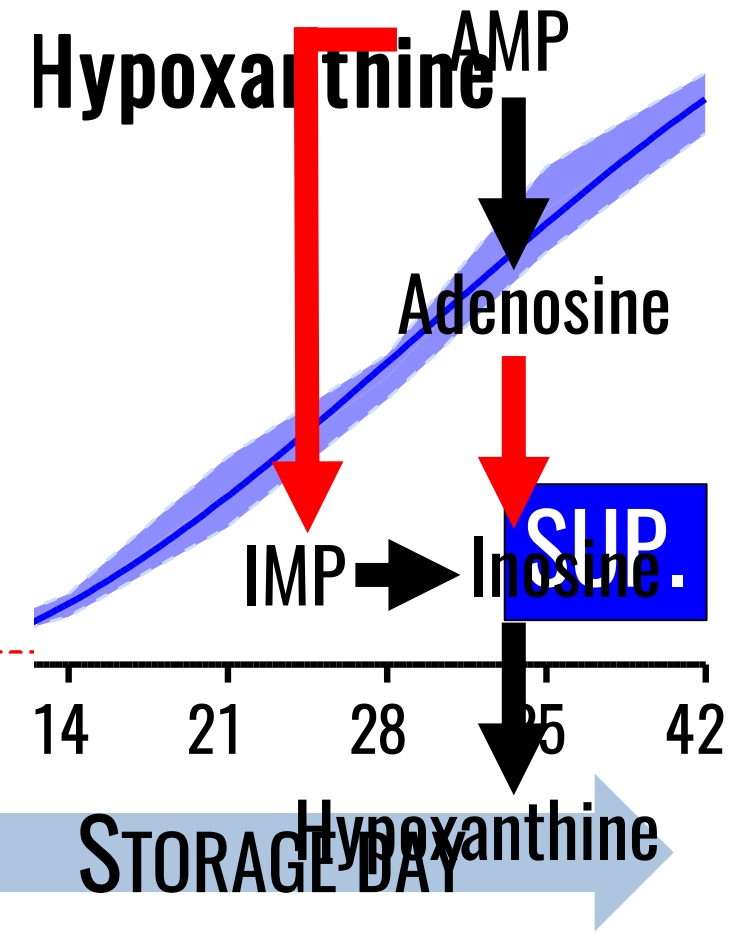
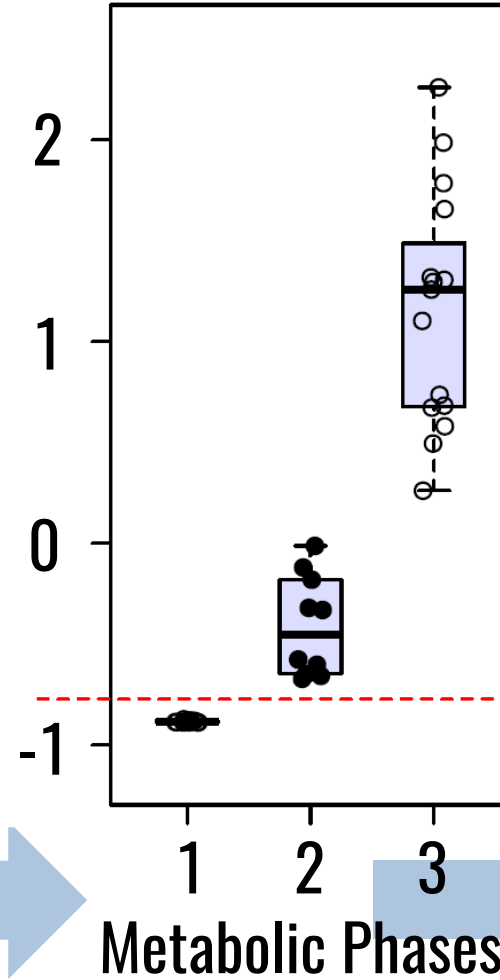
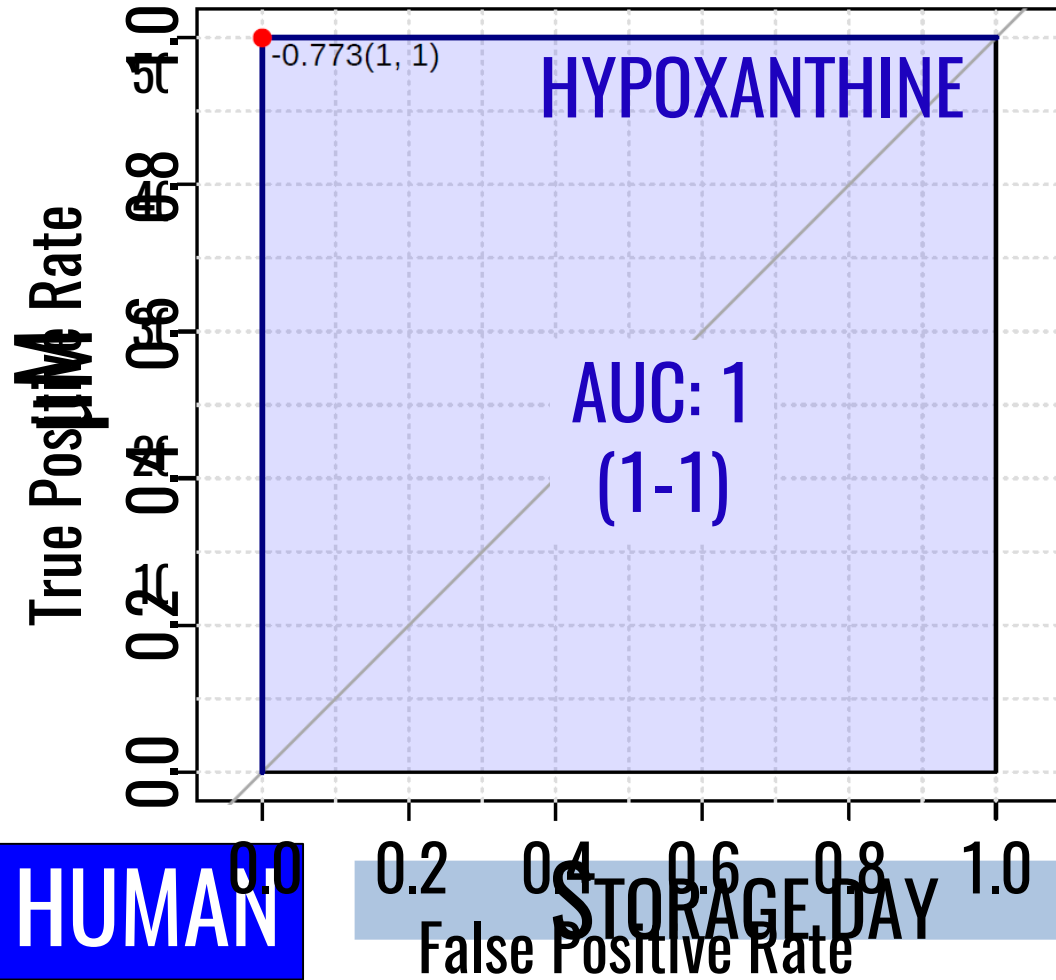
AS3: Significant metabolic lesions tend to accumulate by storage day 14



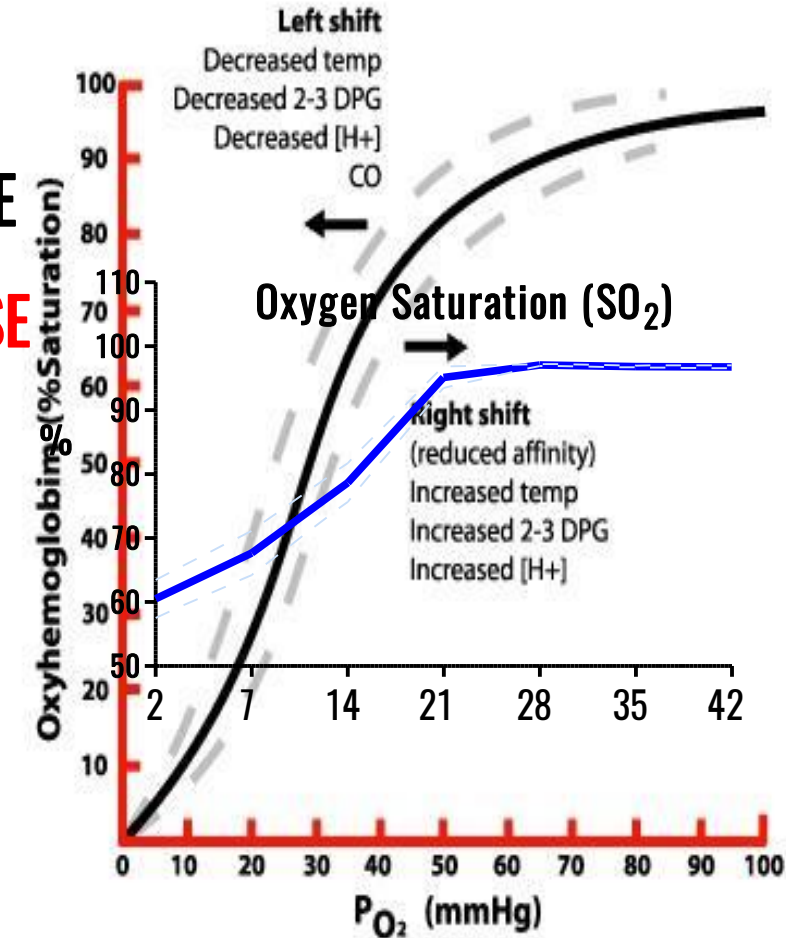
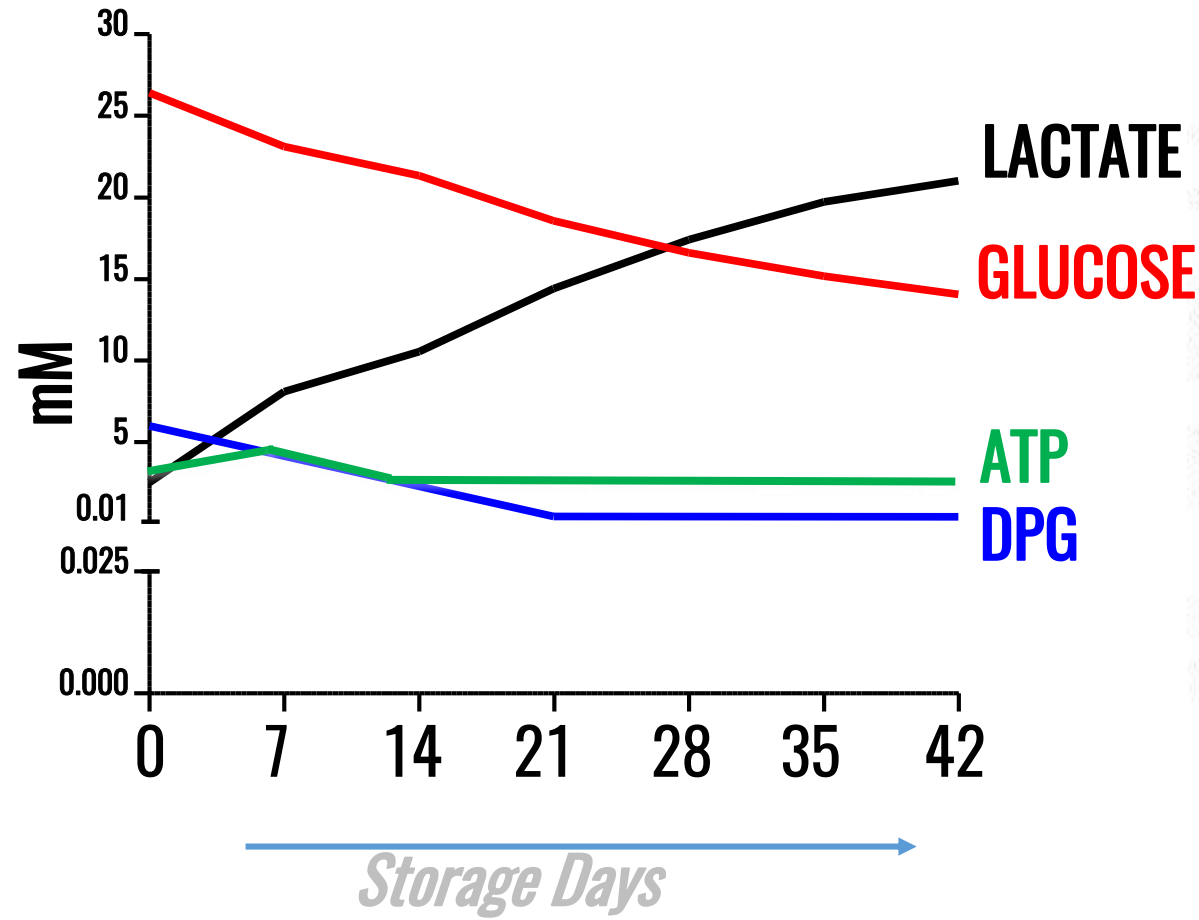
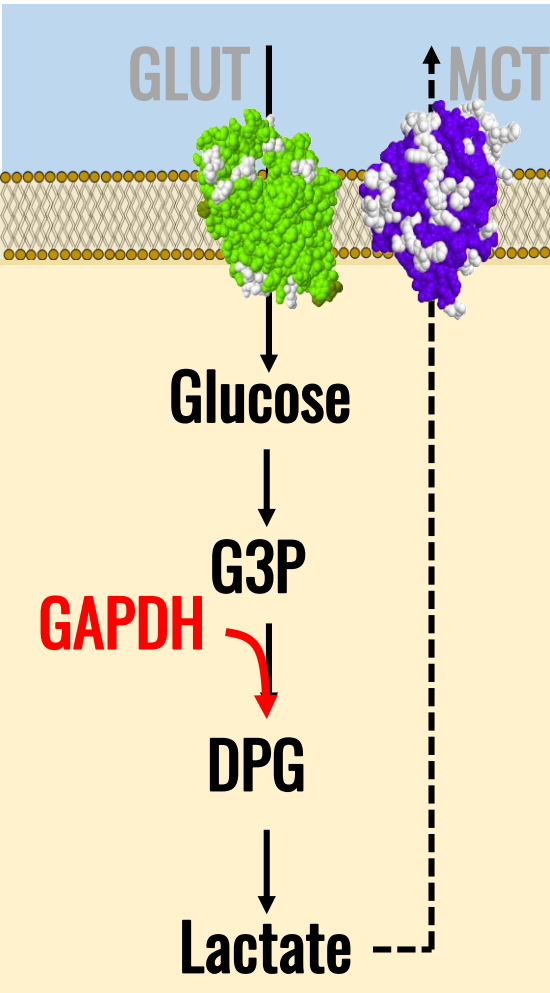
PCA SAGM Validation Dataset Whole Sample Biomarkers



Hypoxanthine: *keep it in mind*

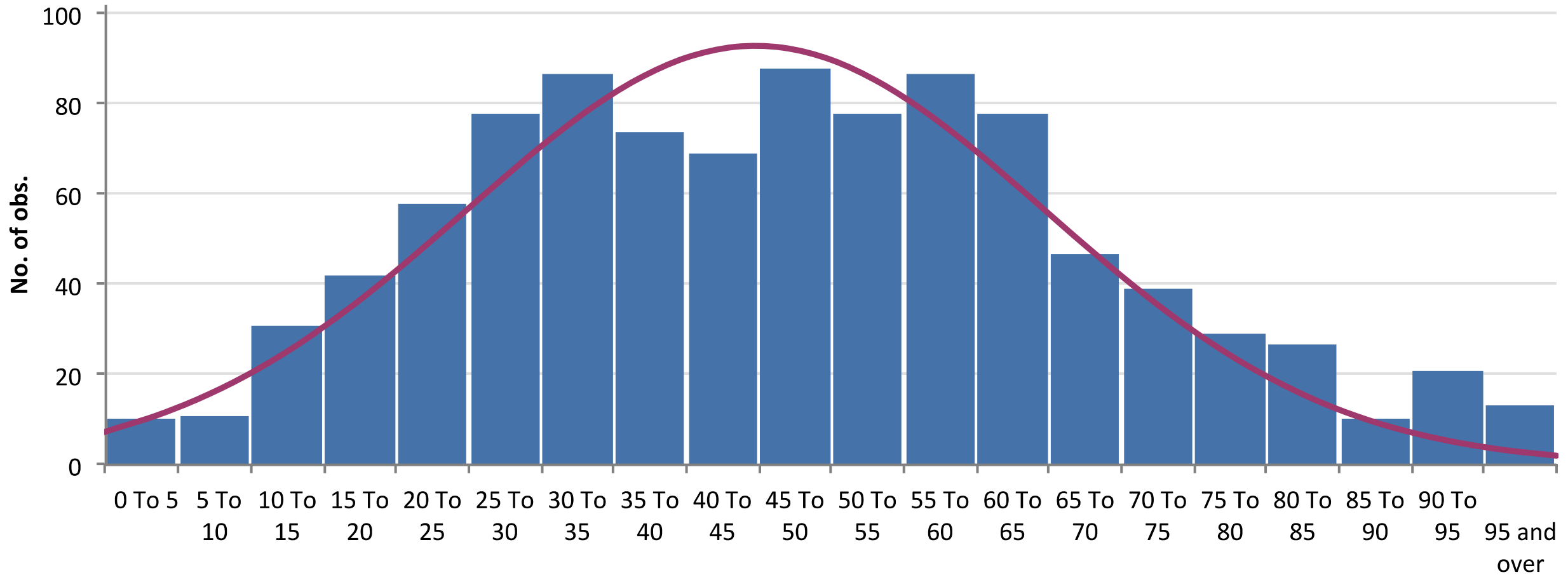


RBC storage: ...and GAPDH oxidation, affecting activity and potentially band 3 binding



From energy to redox: stored RBCs are challenged with oxidative stress

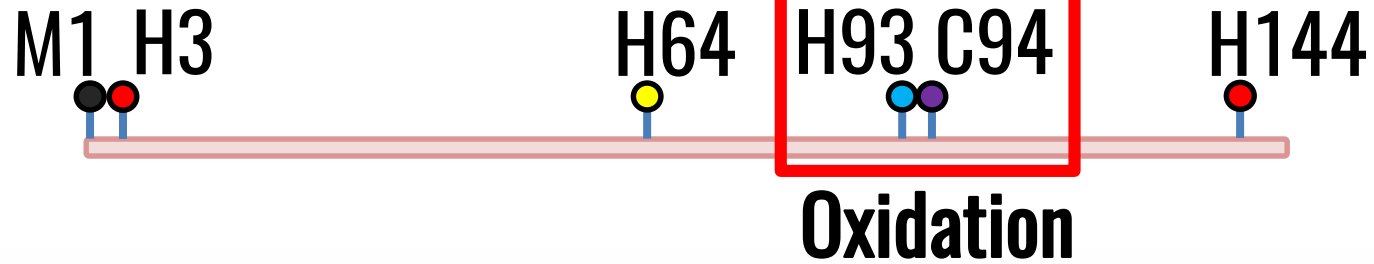
Histogram for "SO2 corrected"



N=997 - Data kindly provided by Dr. Tatsuro Yoshida, *New Health Sciences inc*

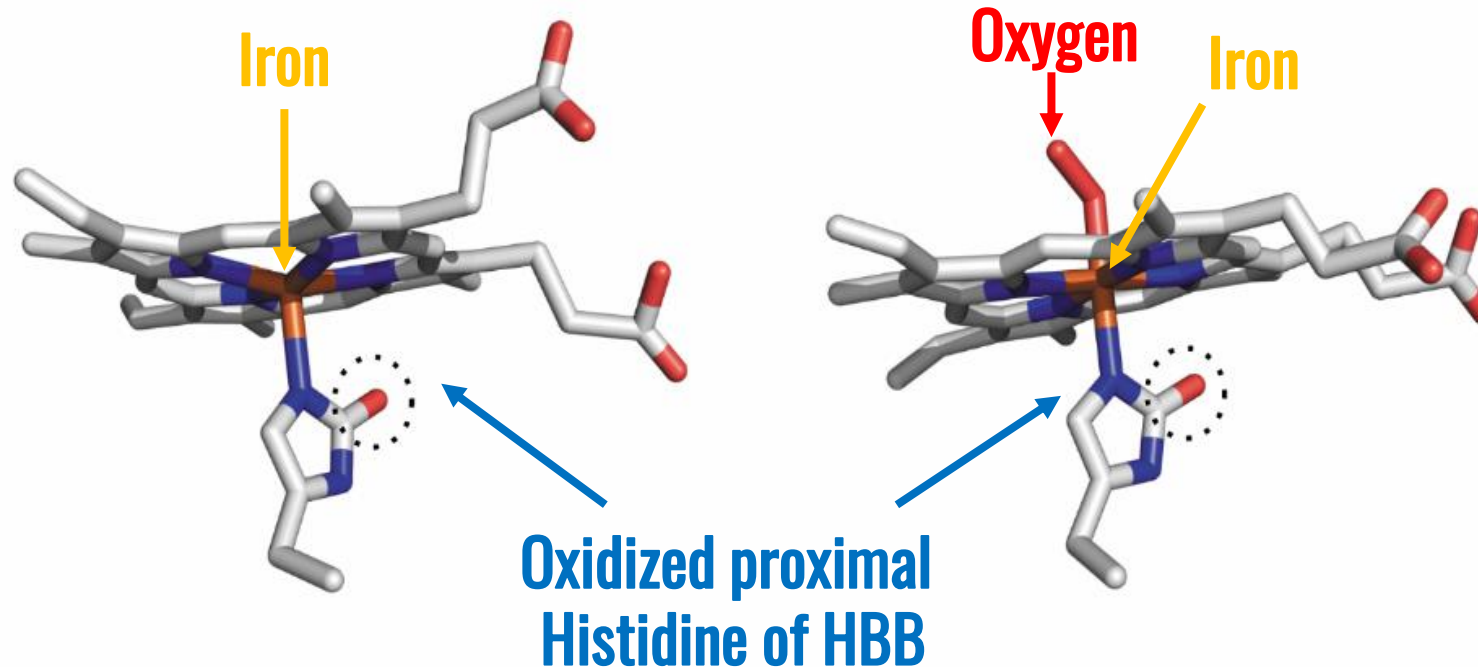
Oxidative stress targets: ROS target the most abundant RBC proteins

Hemoglobin Beta
(HBB)

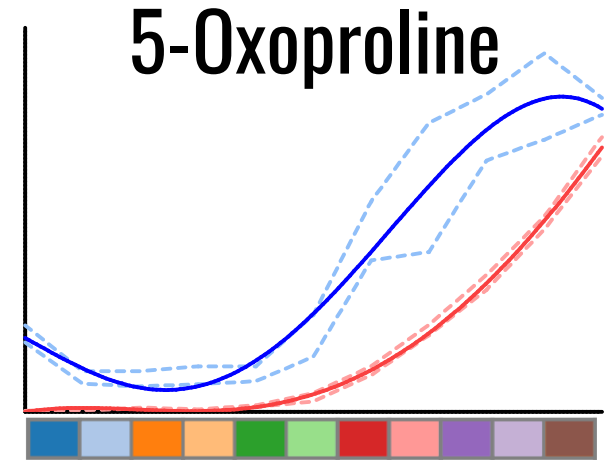
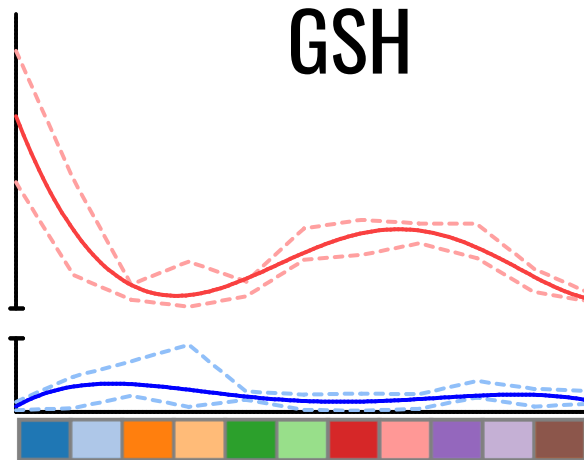
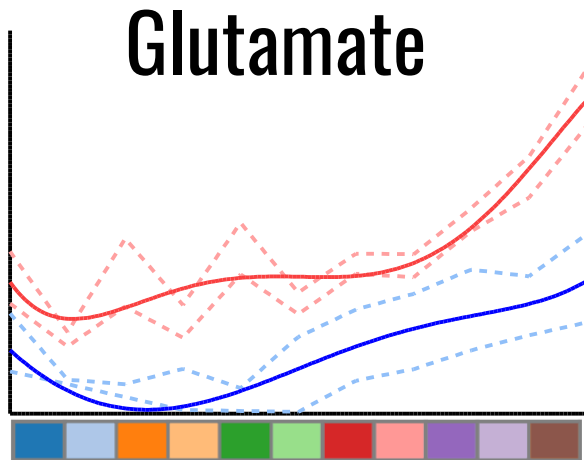
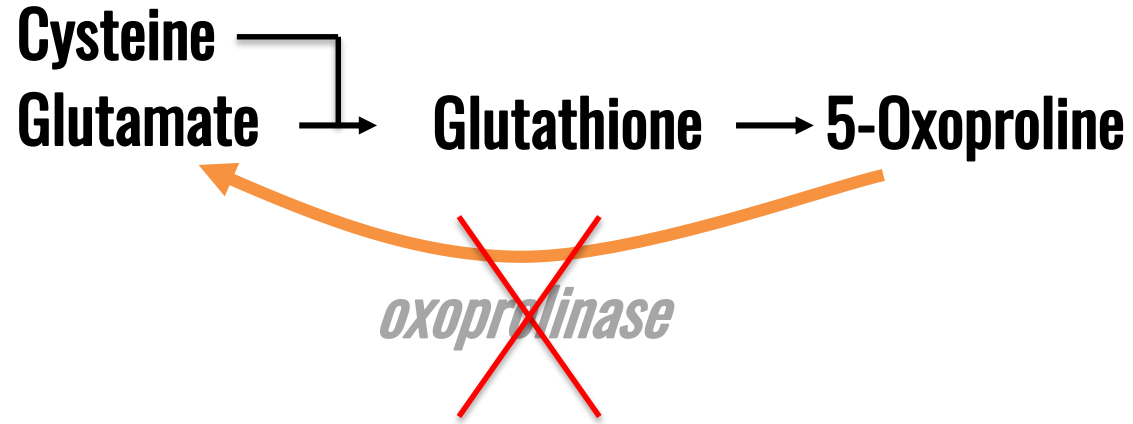


Deoxygenated Hemoglobin

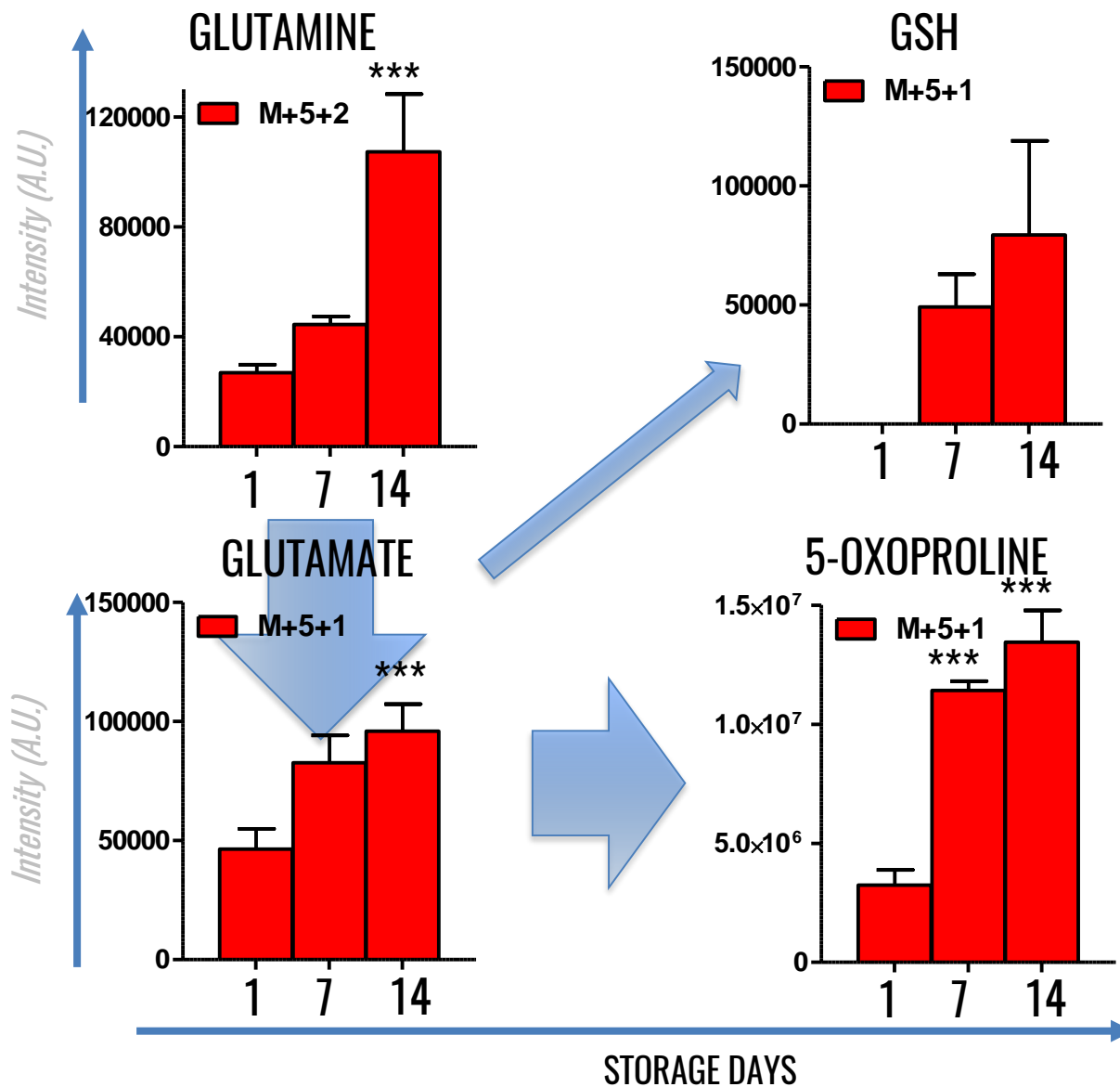
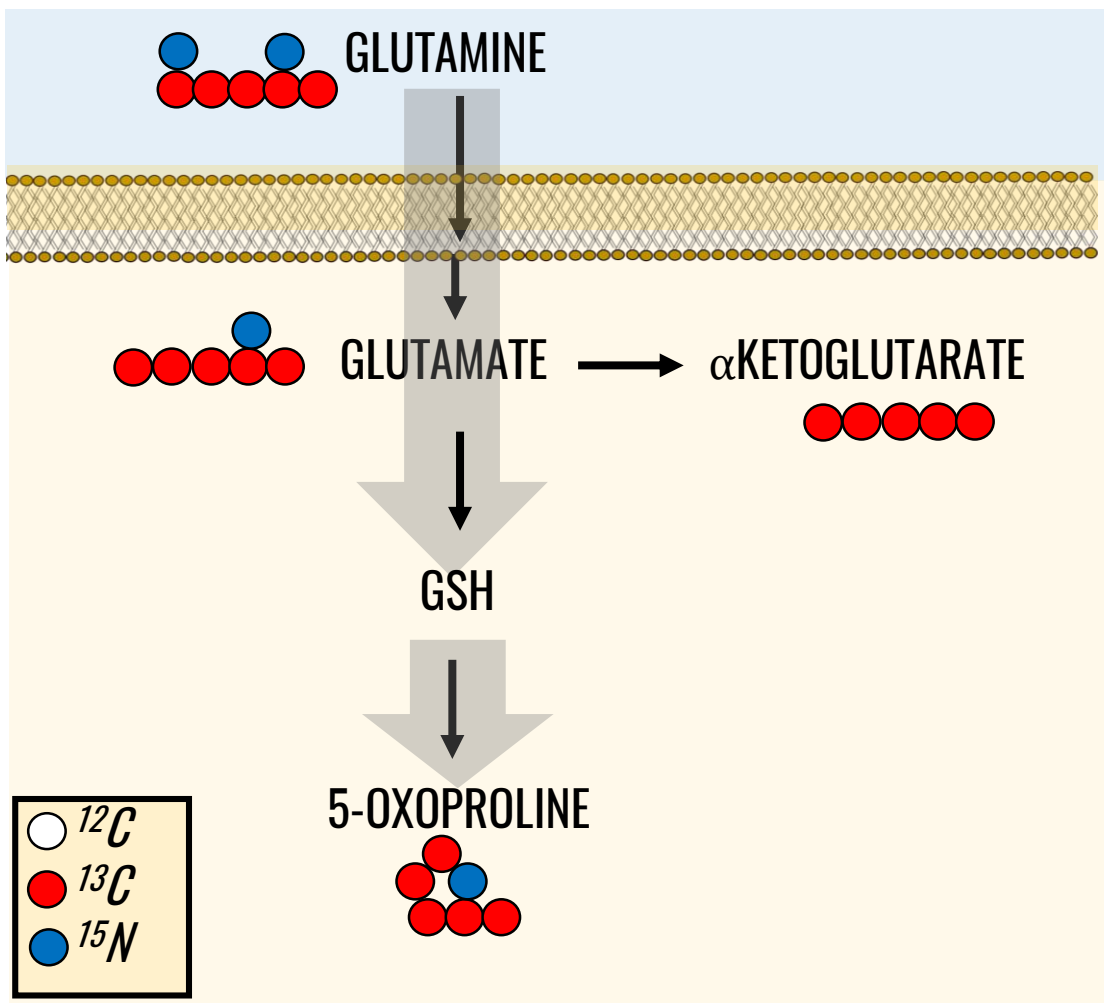
Oxygenated Hemoglobin



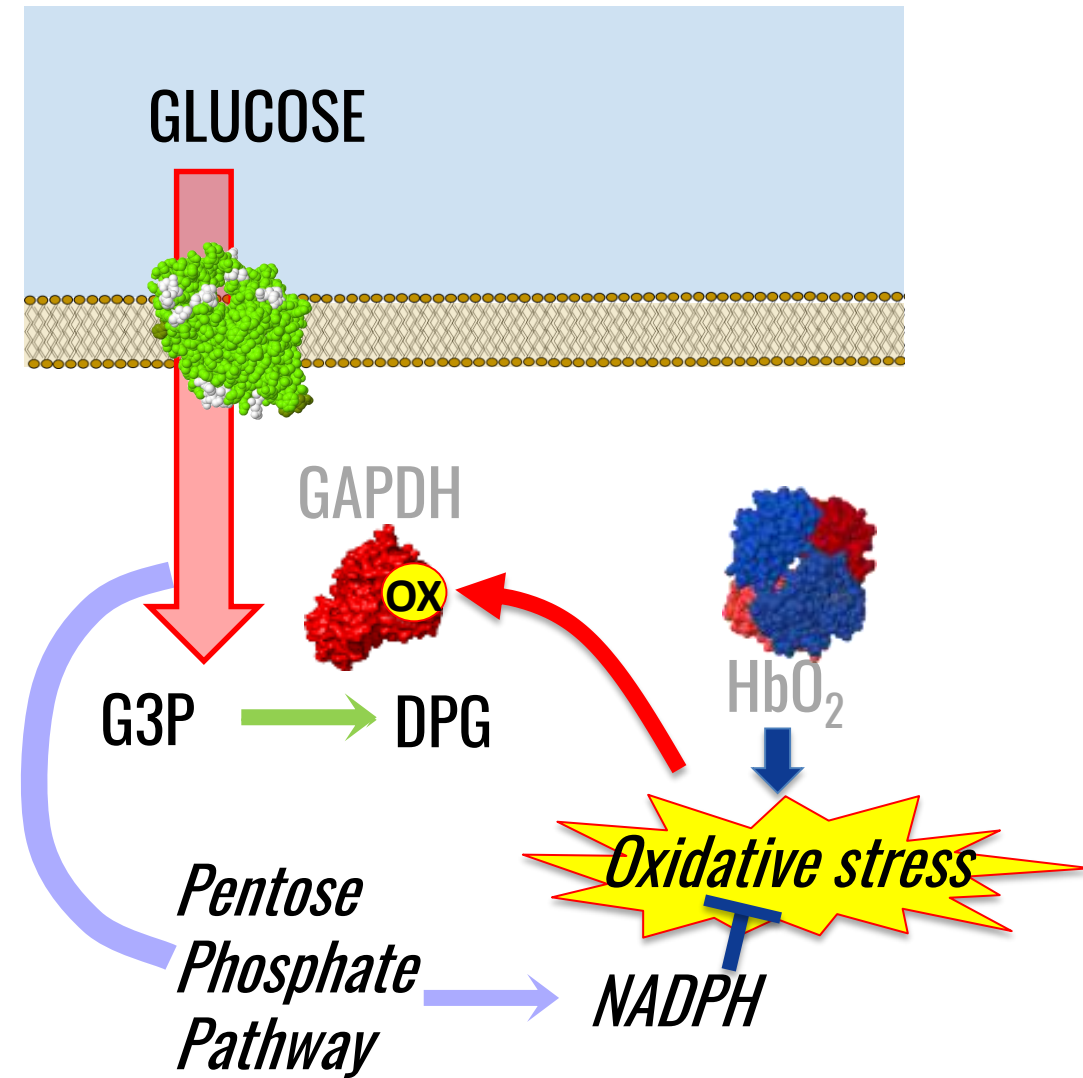
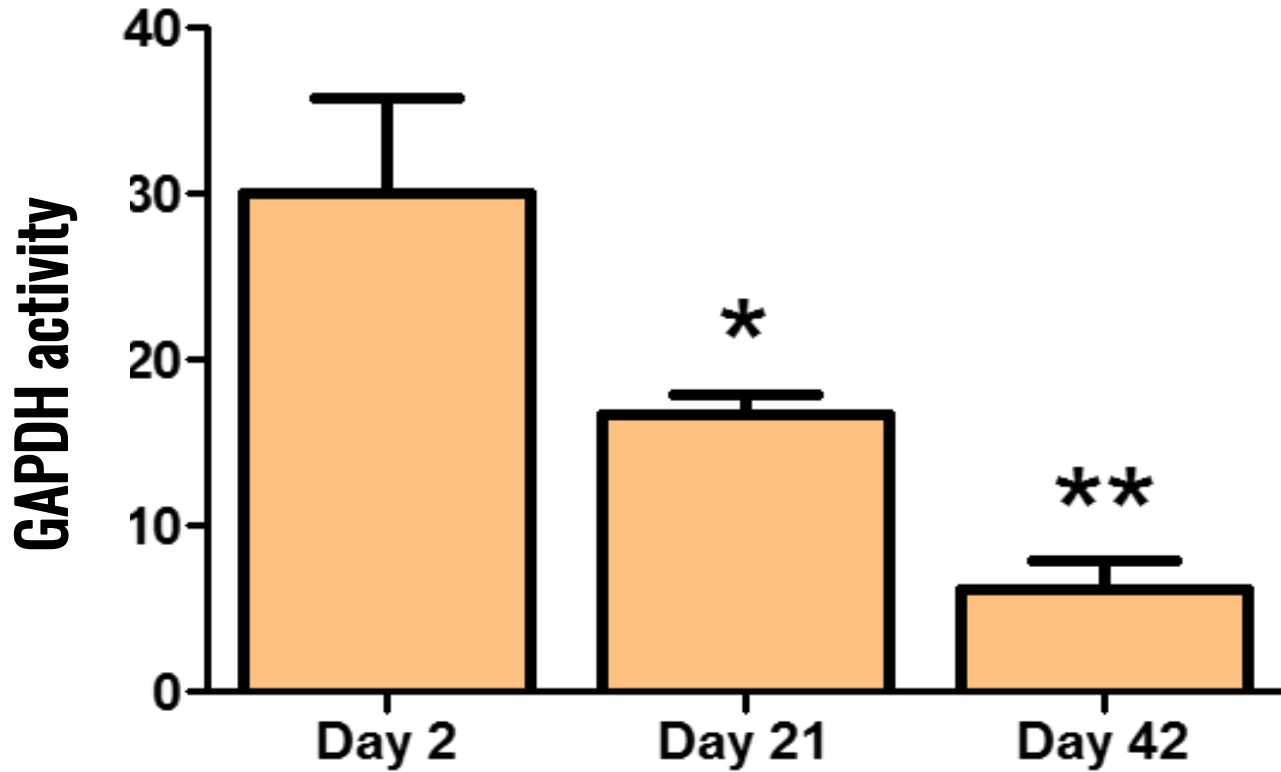
RBC antioxidant capacity: GSH pools plummet



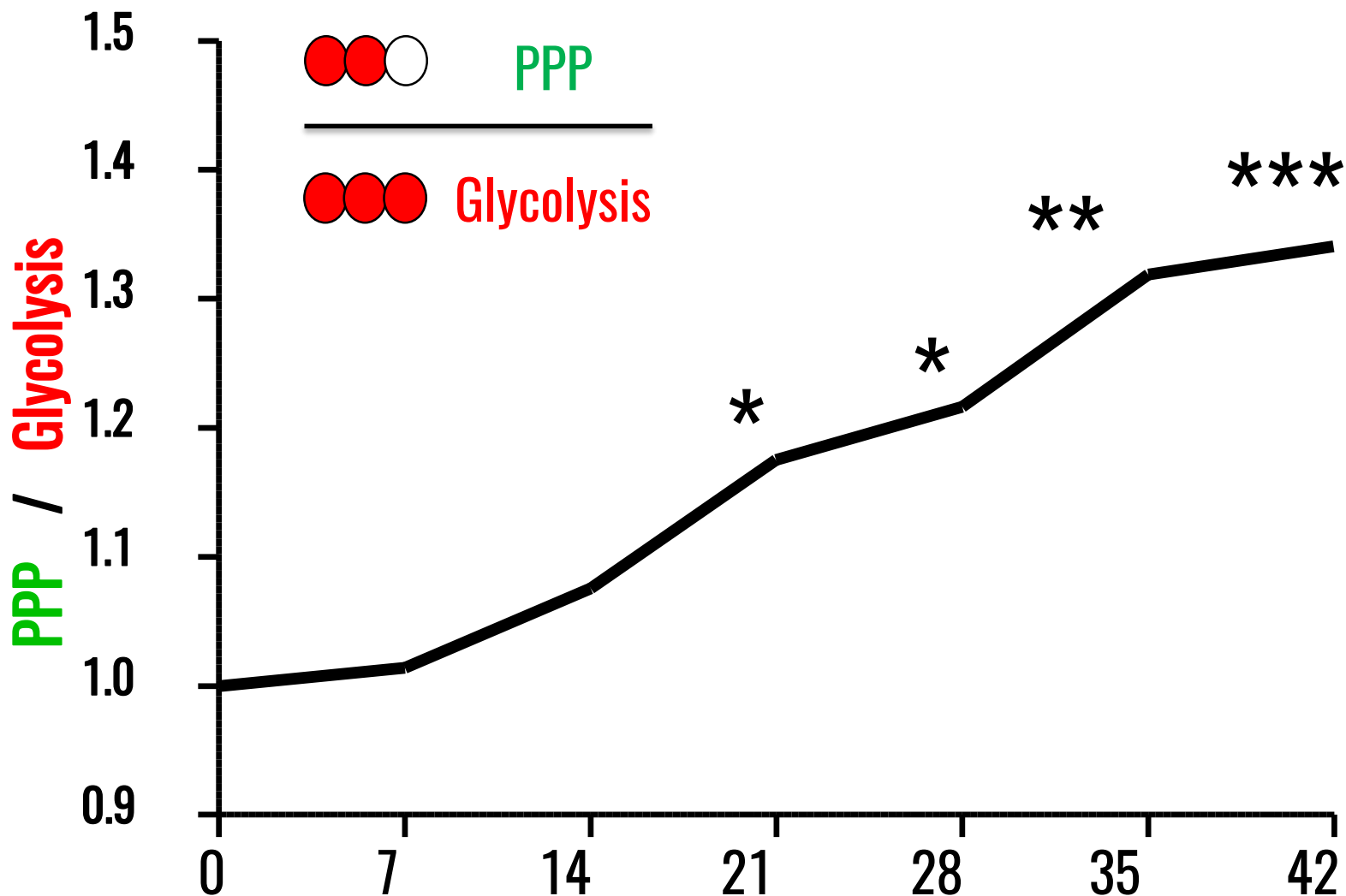
RBC antioxidant capacity: GSH synthesis is compromised during RBC storage



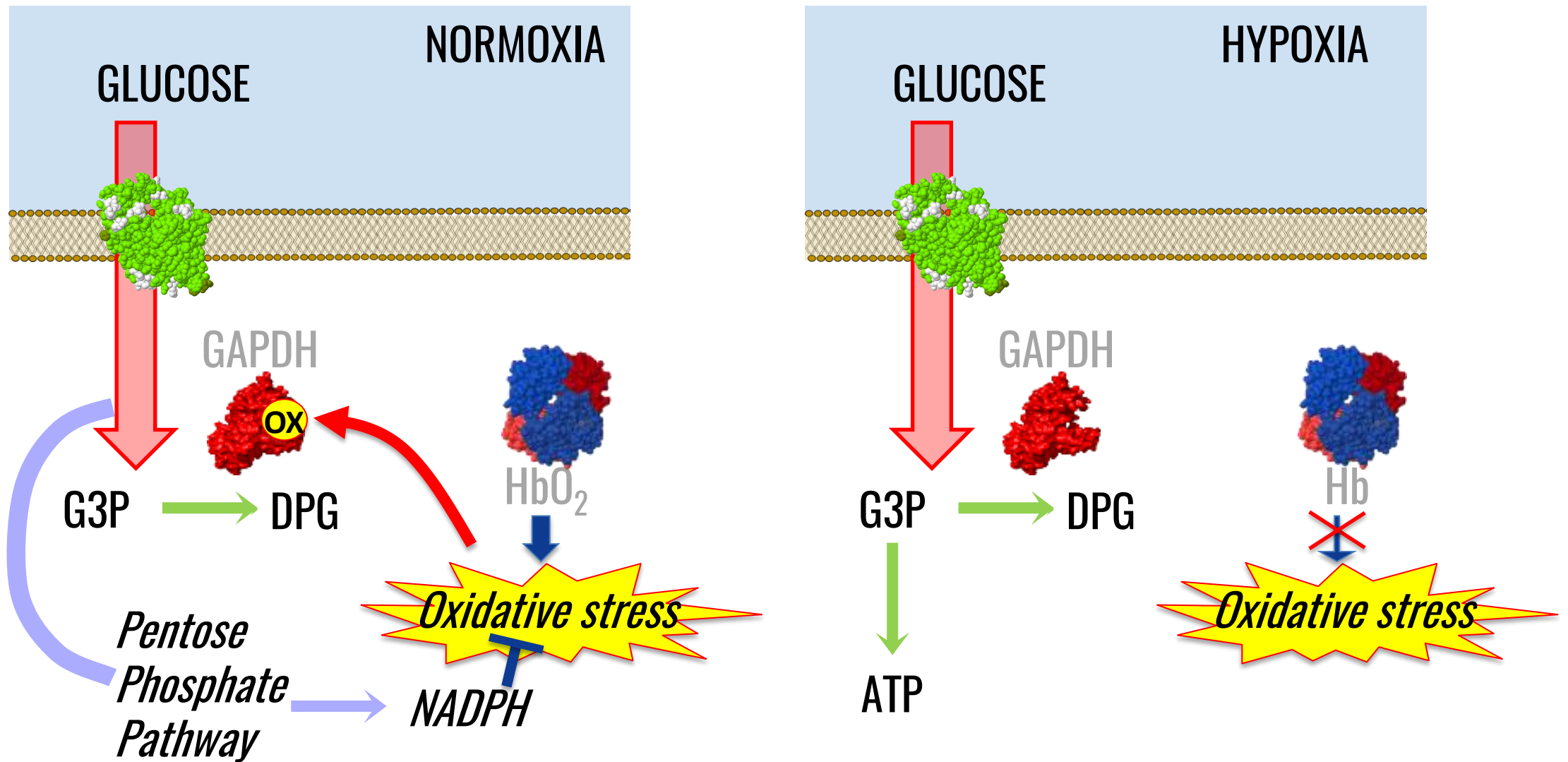
GAPDH: redox sensitive thiols at the interface between energy and redox metabolism



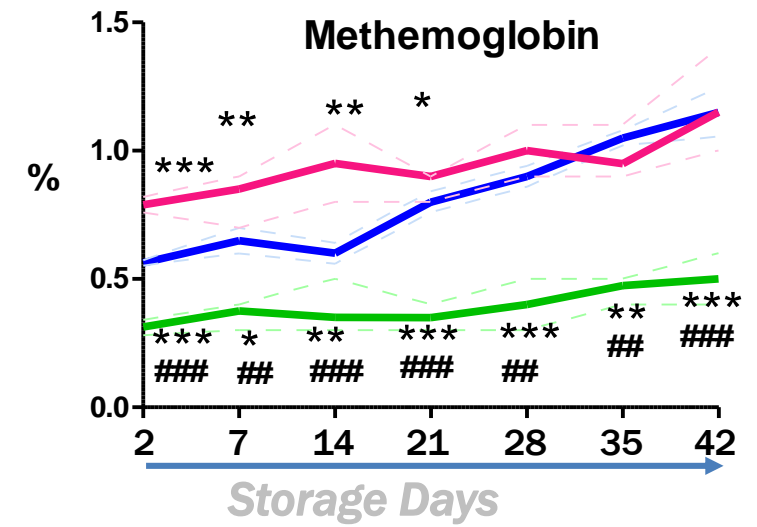
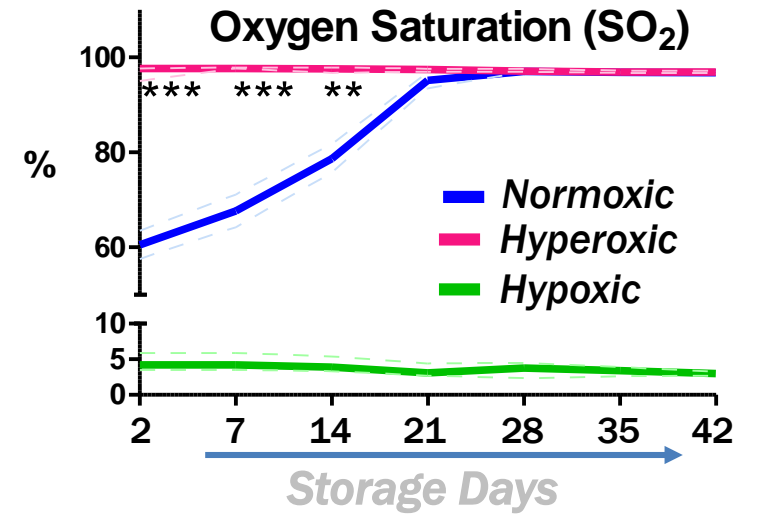
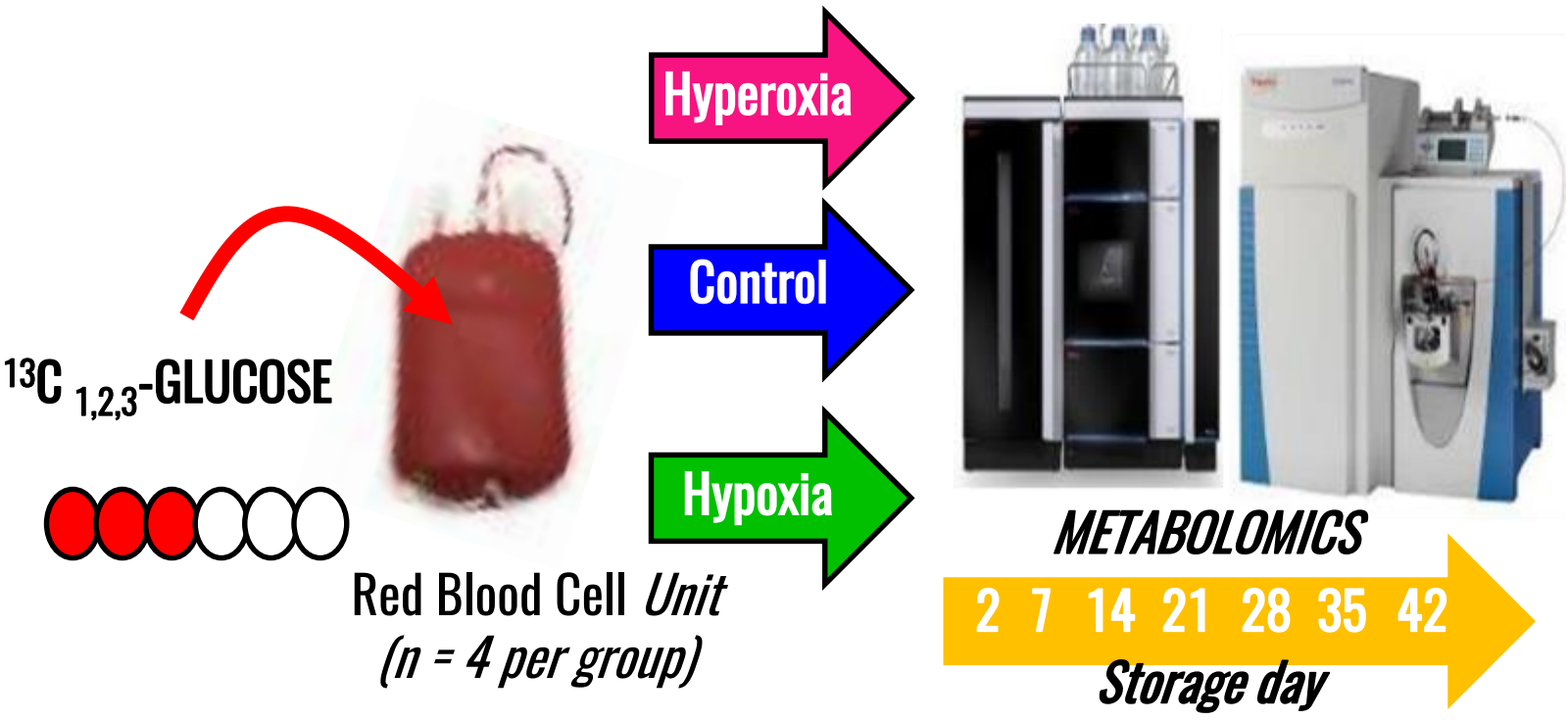
RBC storage: steady state of PPP are depressed, not fluxes!



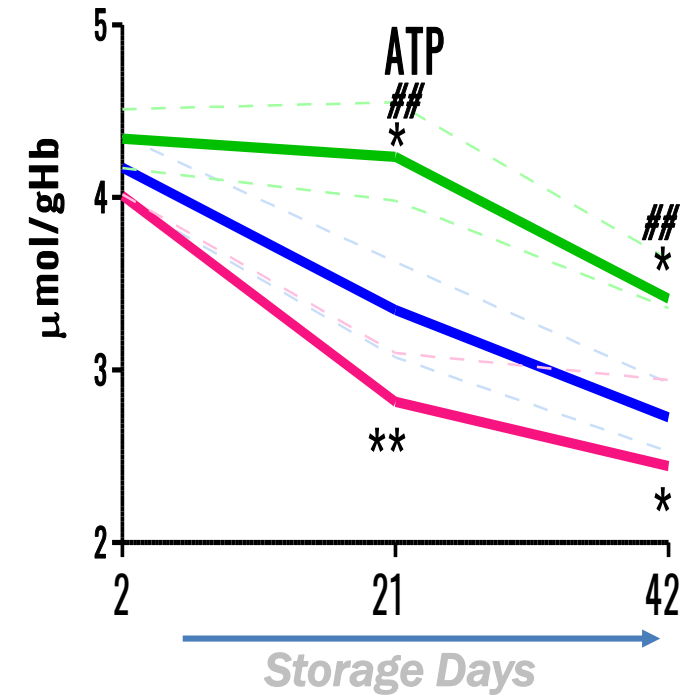
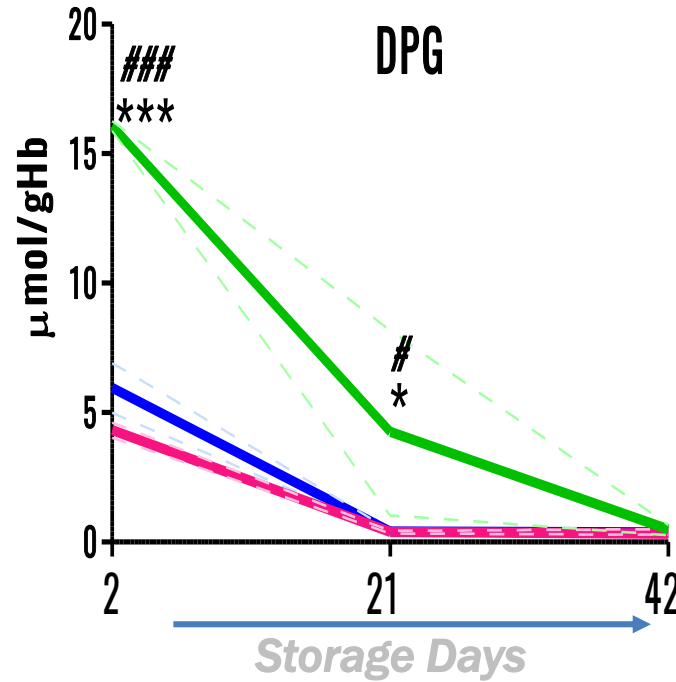
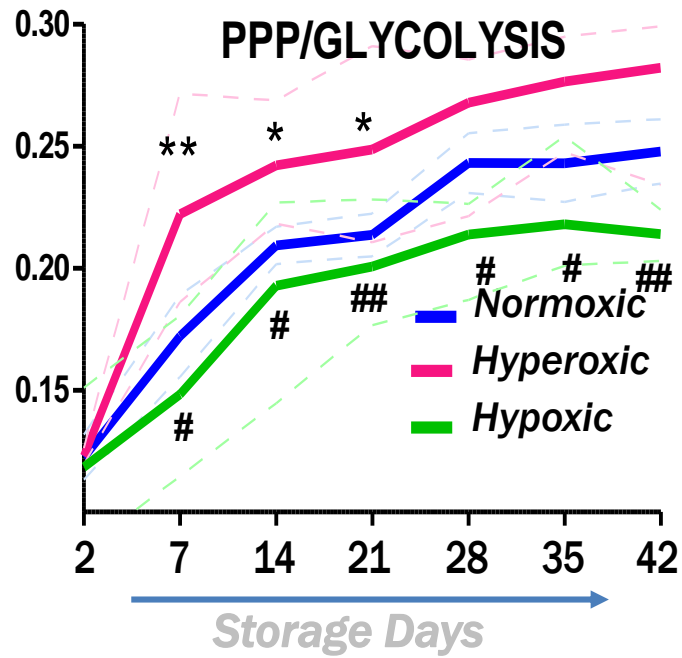
Hypothesis: anaerobic storage should promote glycolysis by decreasing oxidative stress



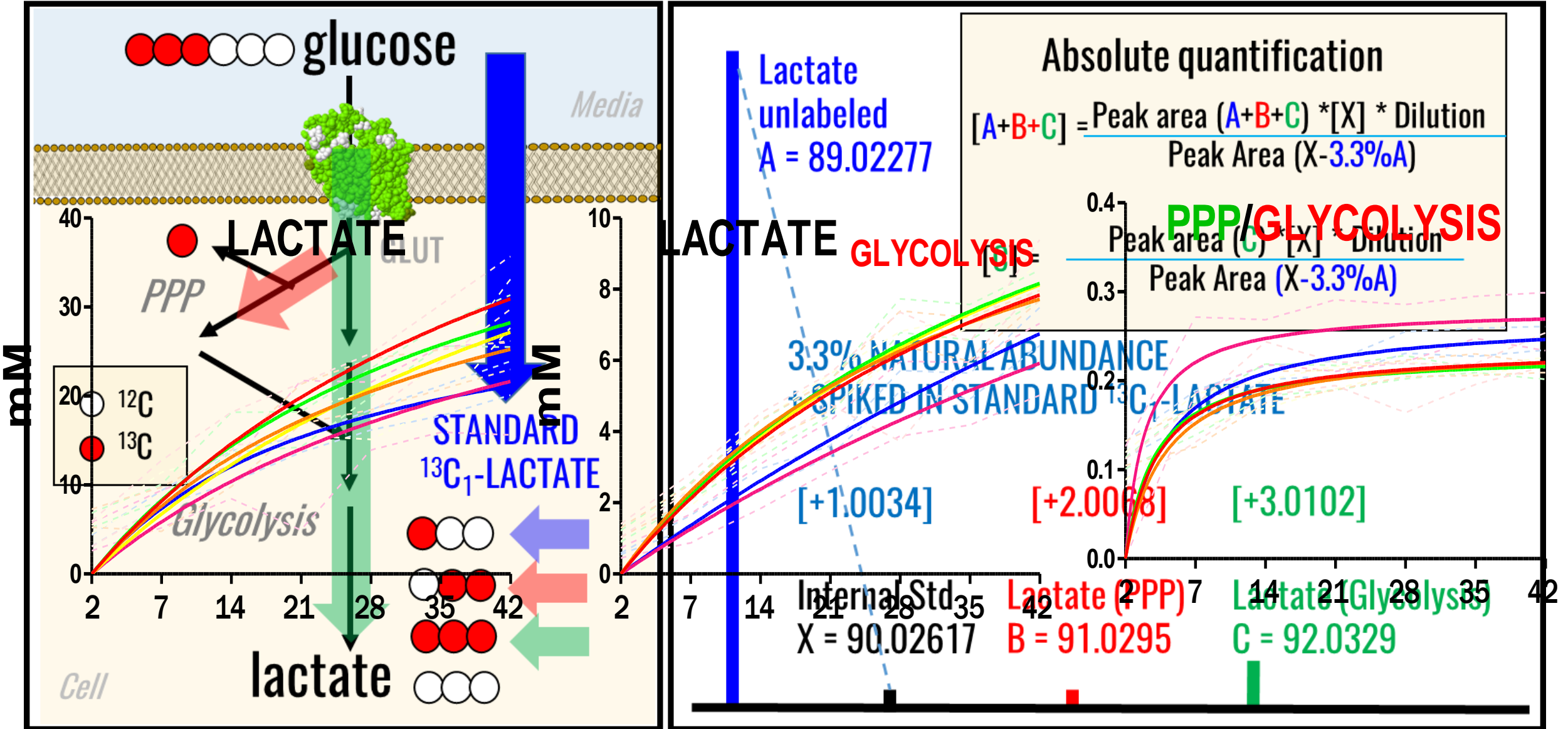
RBC storage: Hyperoxia promotes the mechanisms



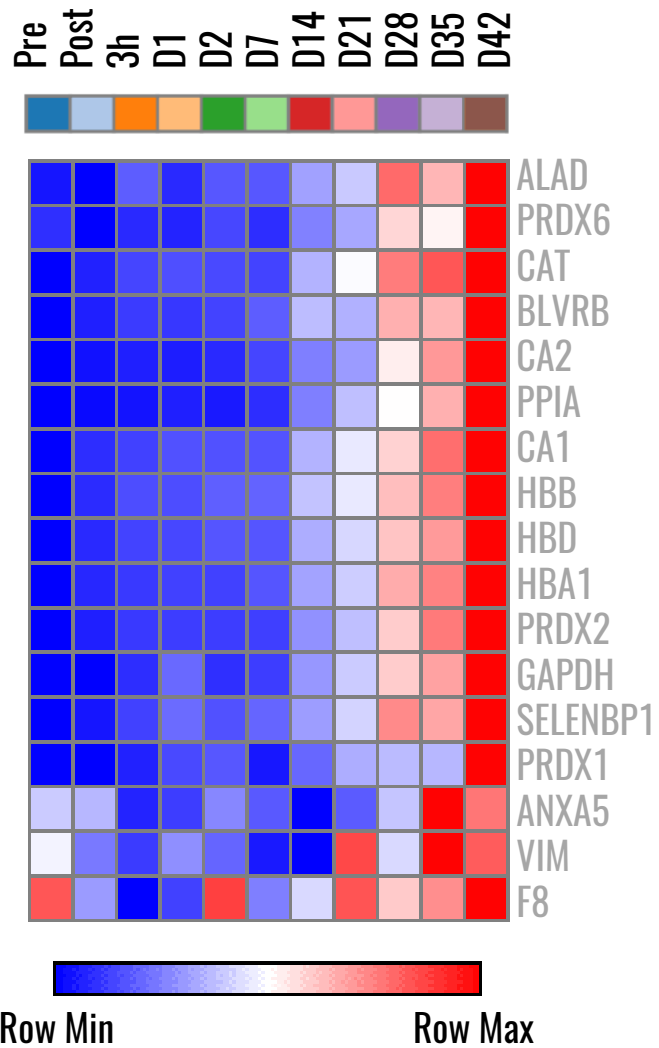
Anaerobic storage : Improved energy metabolism



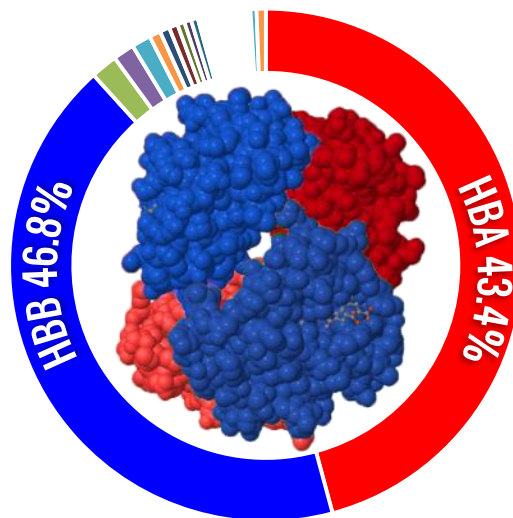
Quantitative metabolic fluxes



RBC supernatant: markers of STORAGE QUALITY

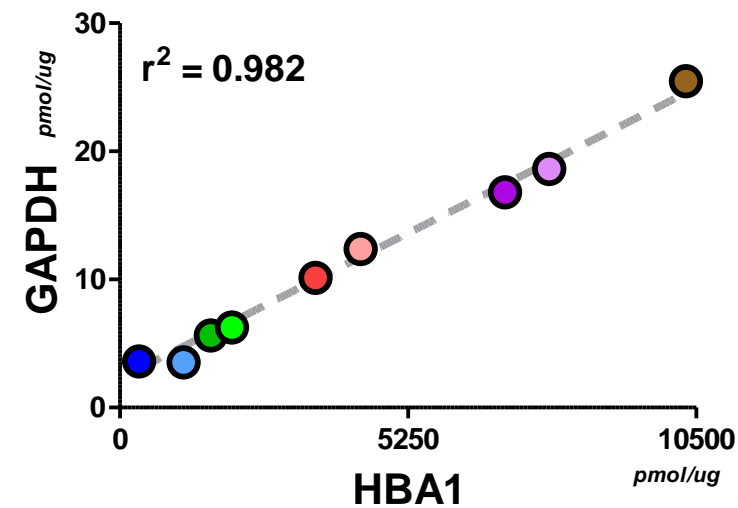
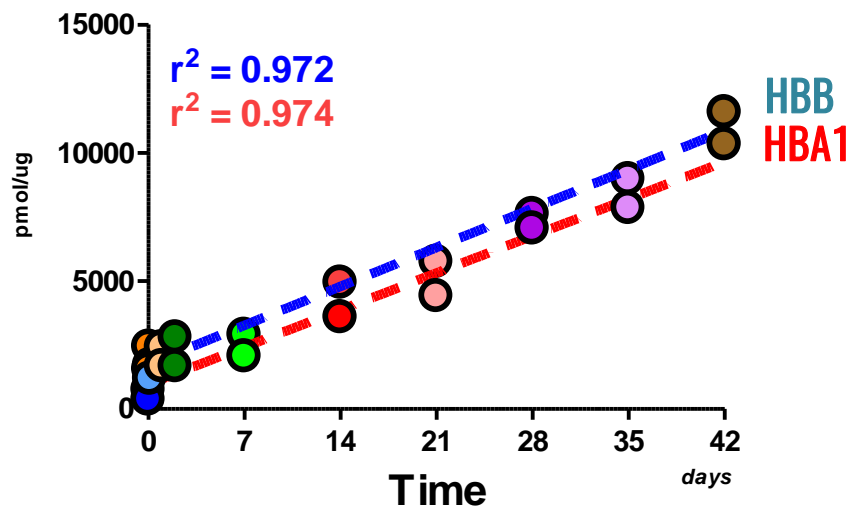
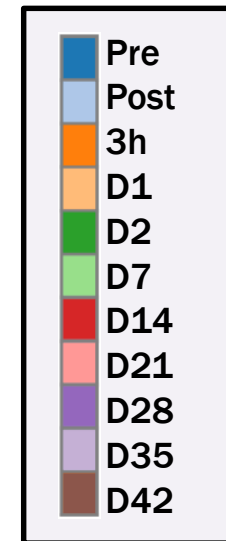


Day 42 top proteins by abundance— pmol/ug

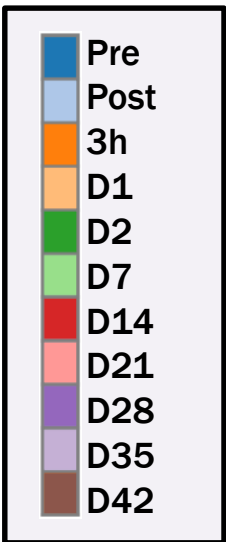
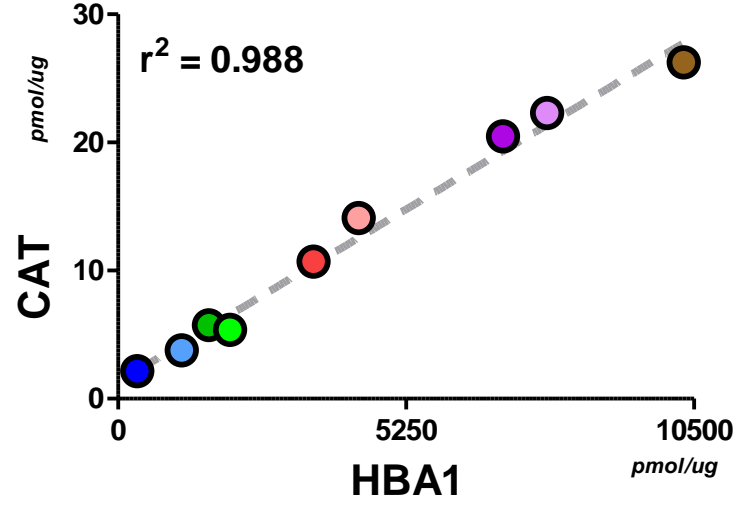
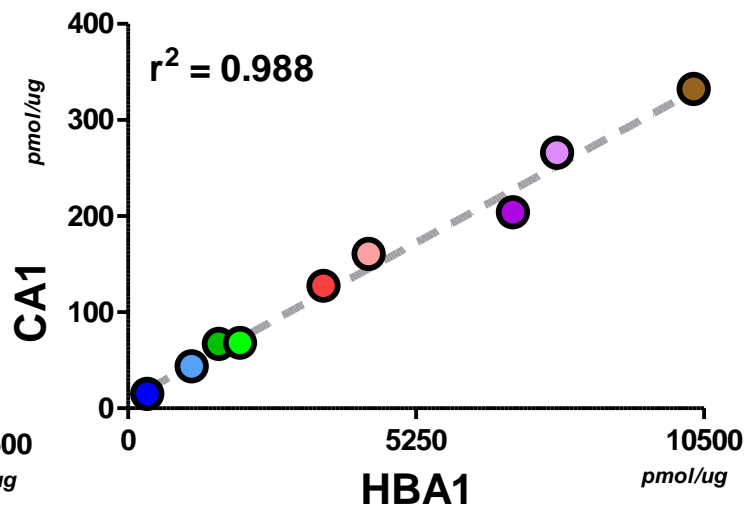
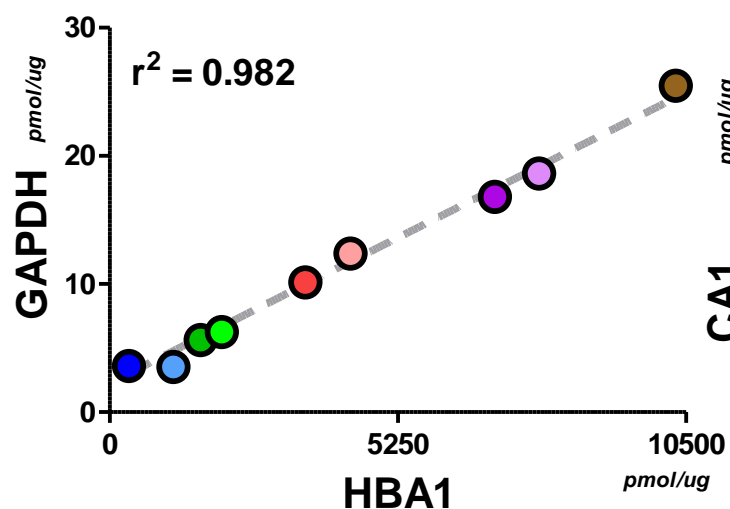
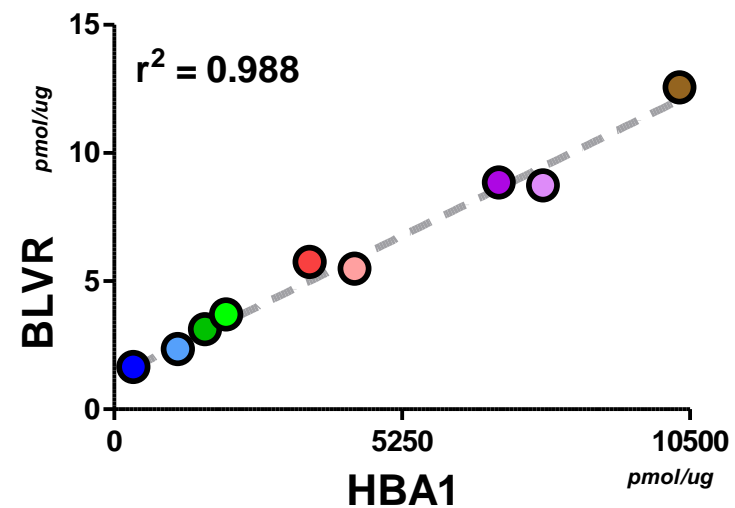
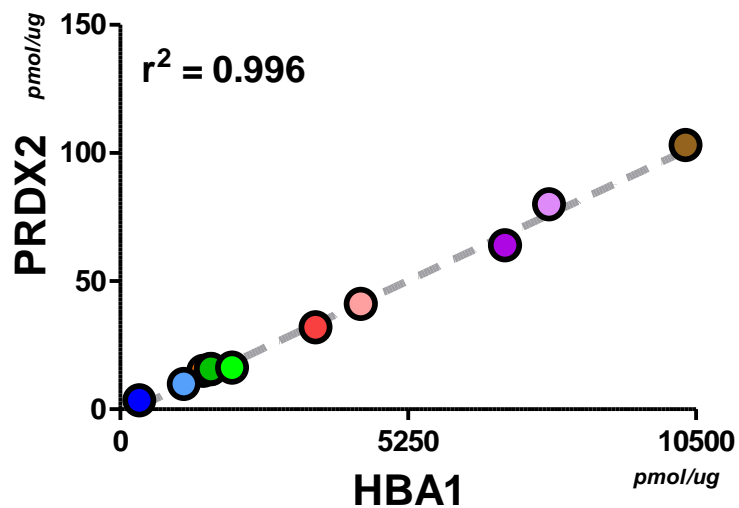
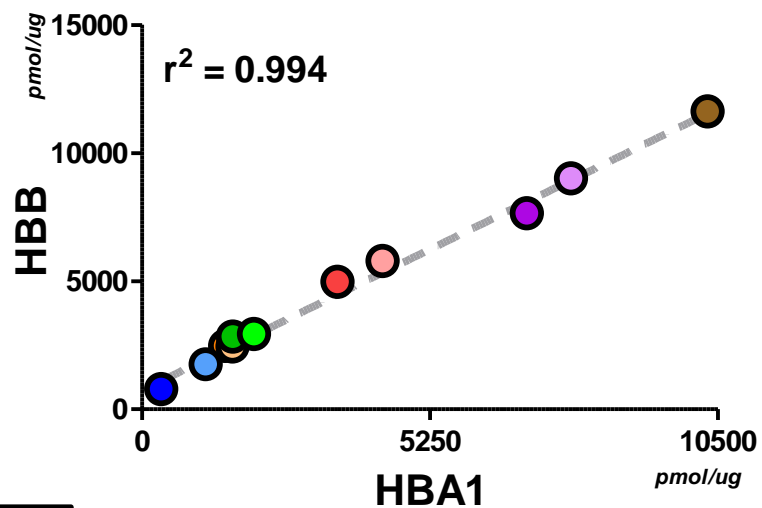


HBA1
 $Y = 206.62 X + 970.29$
 $R^2 = 0.974$

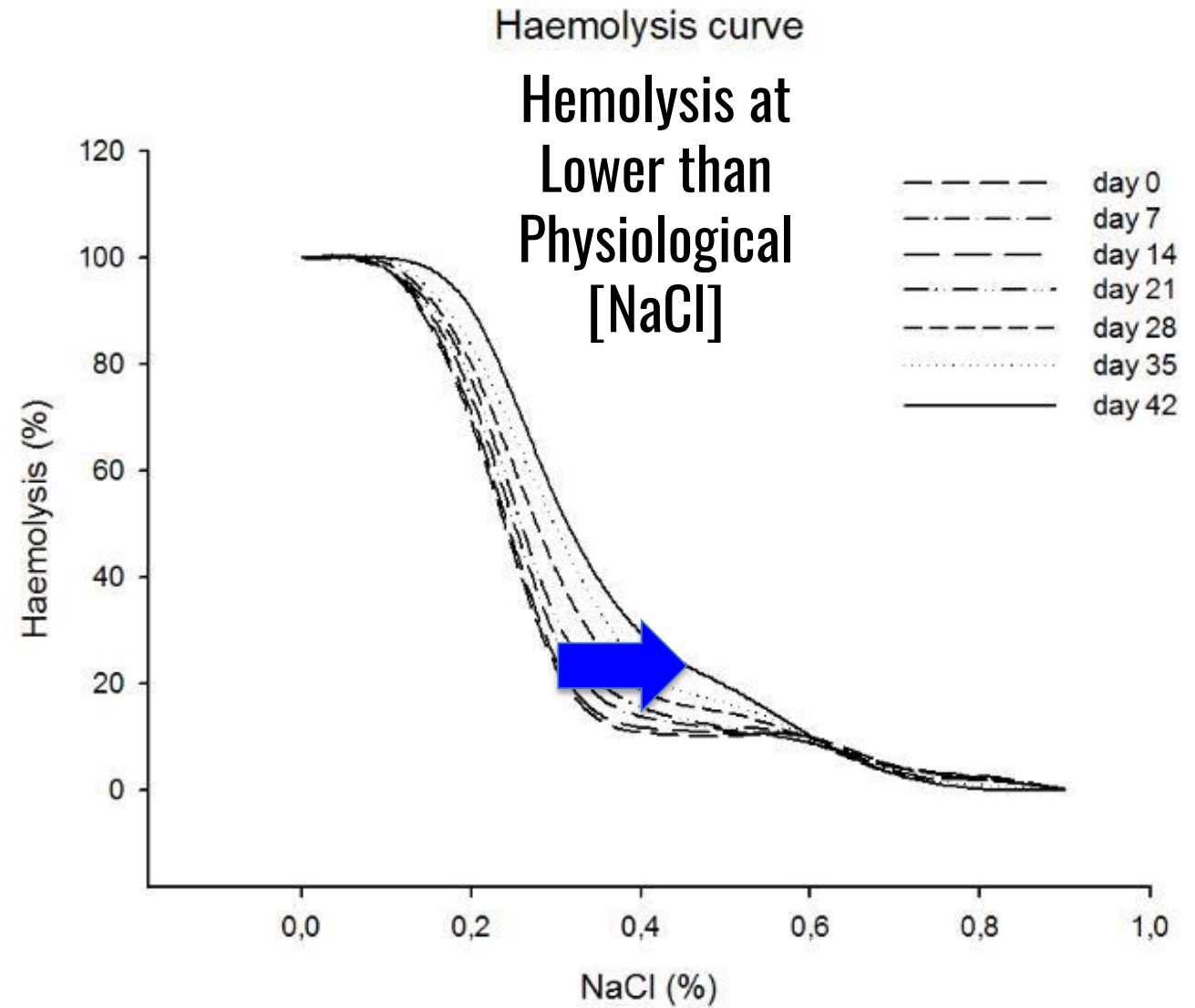
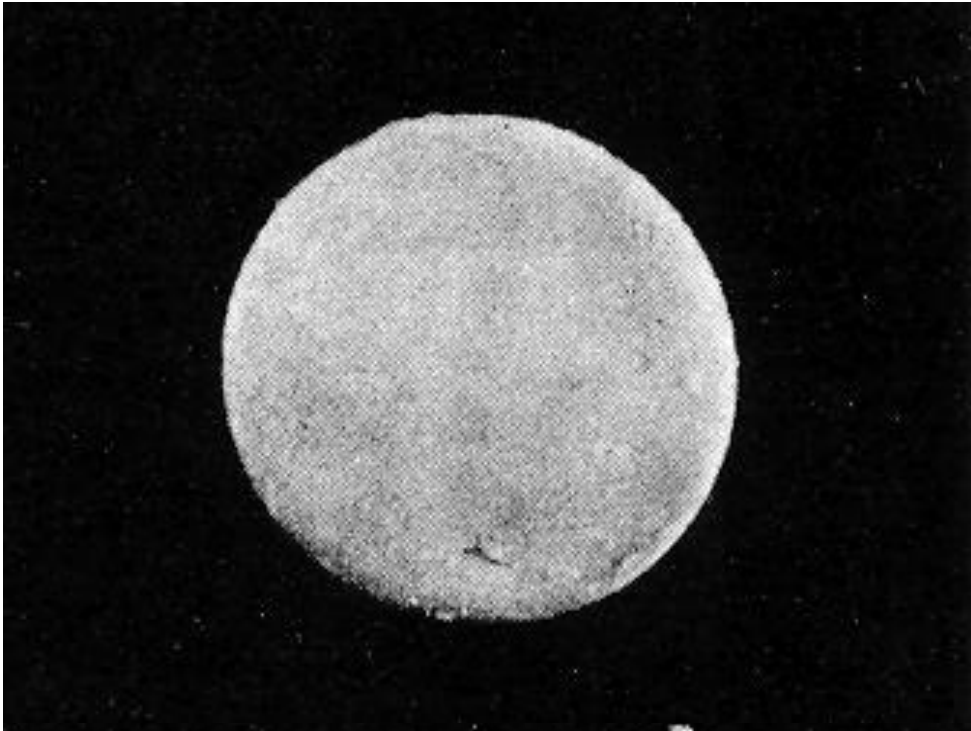
HBB
 $Y = 217.22 X + 1742.89$
 $R^2 = 0.972$



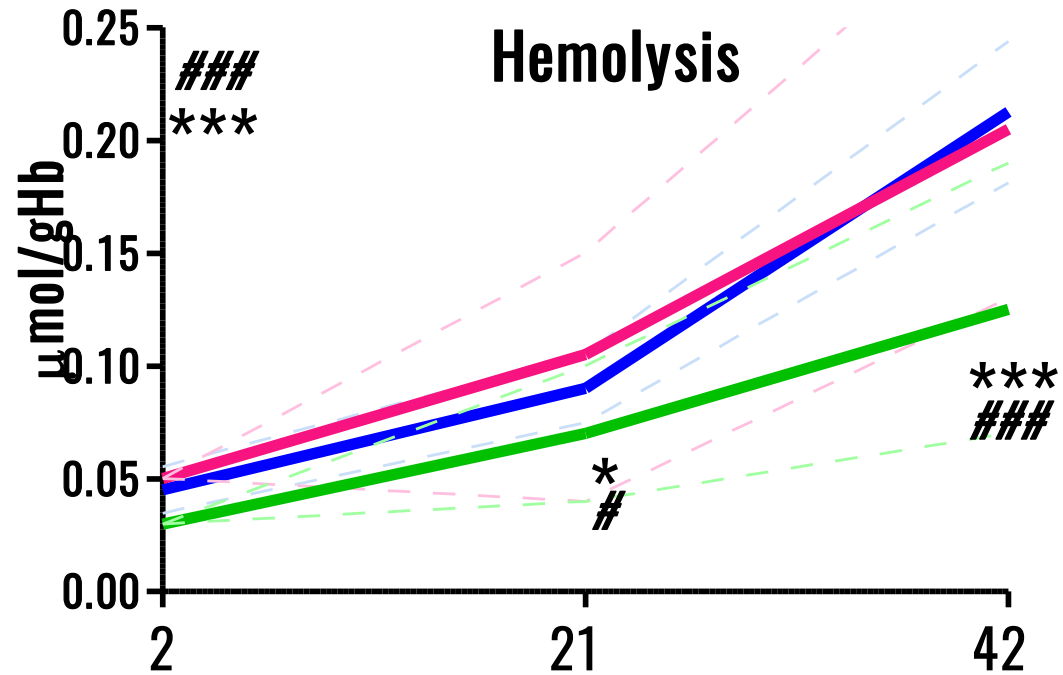
RBC storage: Quality control markers of RBC storage correlating with hemolysis and Hb



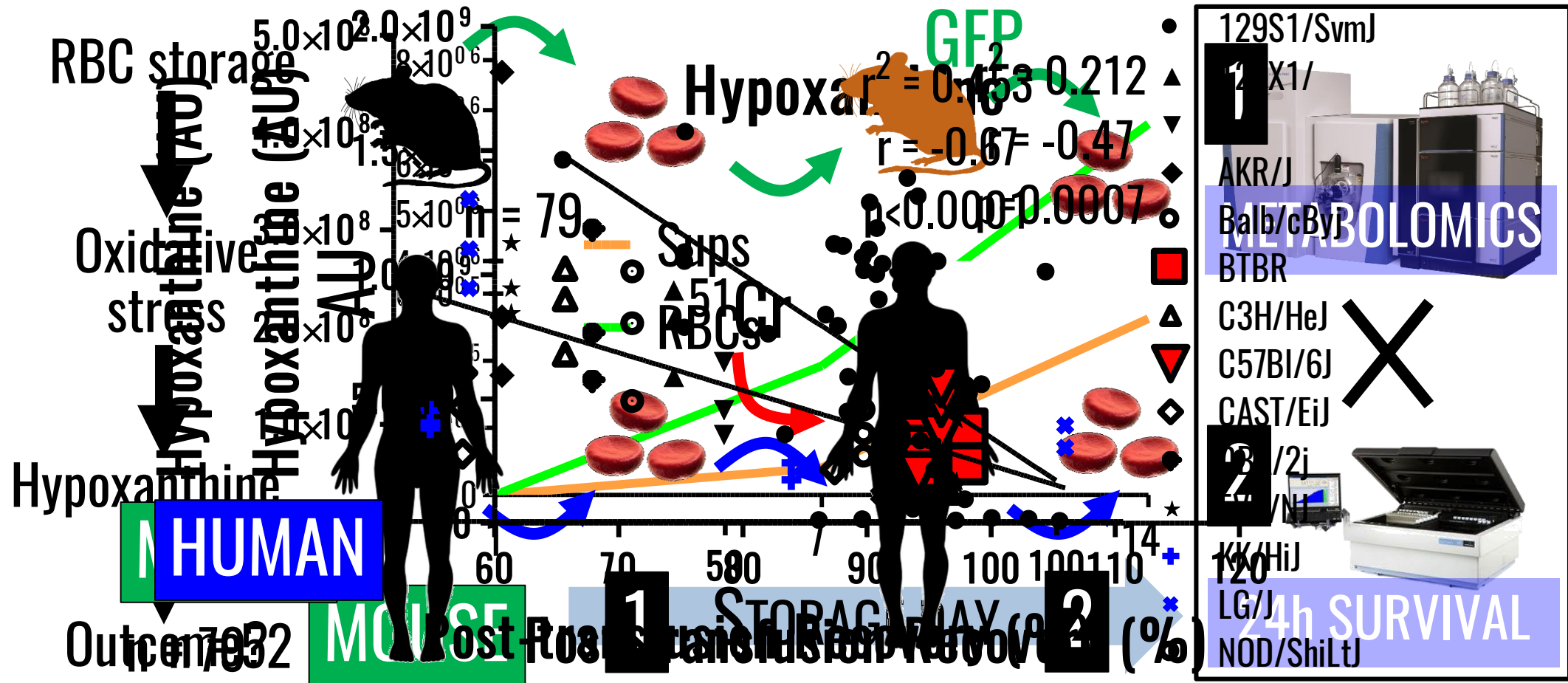
RBC storage: Vesiculation impairs RBC morphology and promotes osmotic fragility



Anaerobic storage : preserves RBC morphology

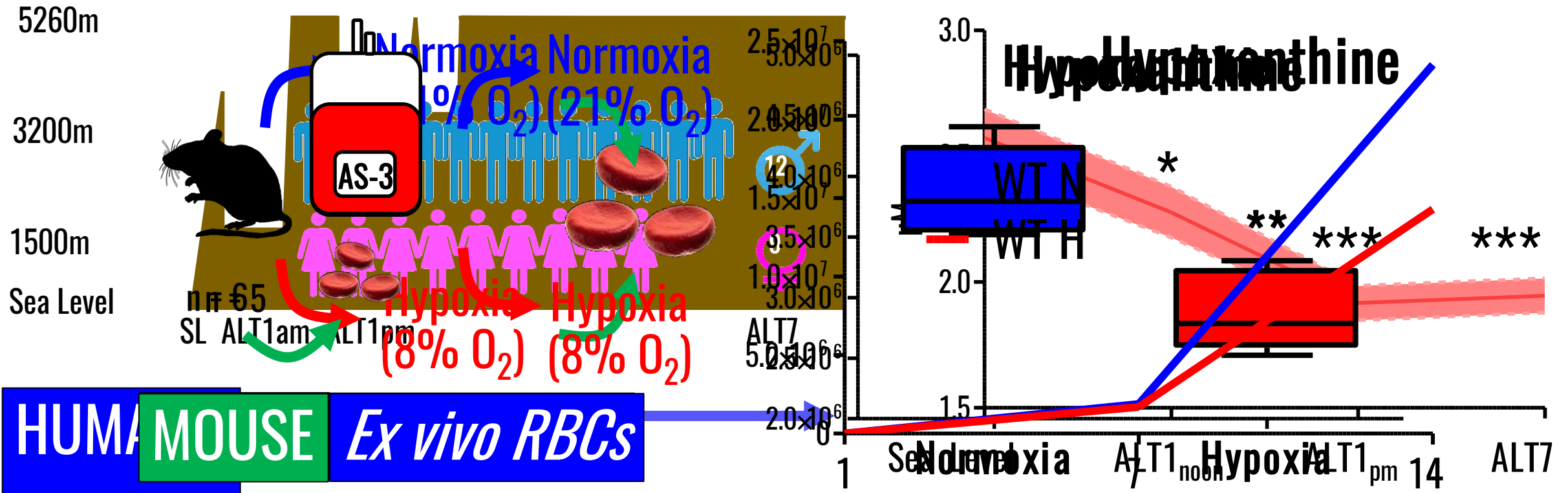


Purine Oxidation markers: Predictors of post-transfusion survival in mice and humans

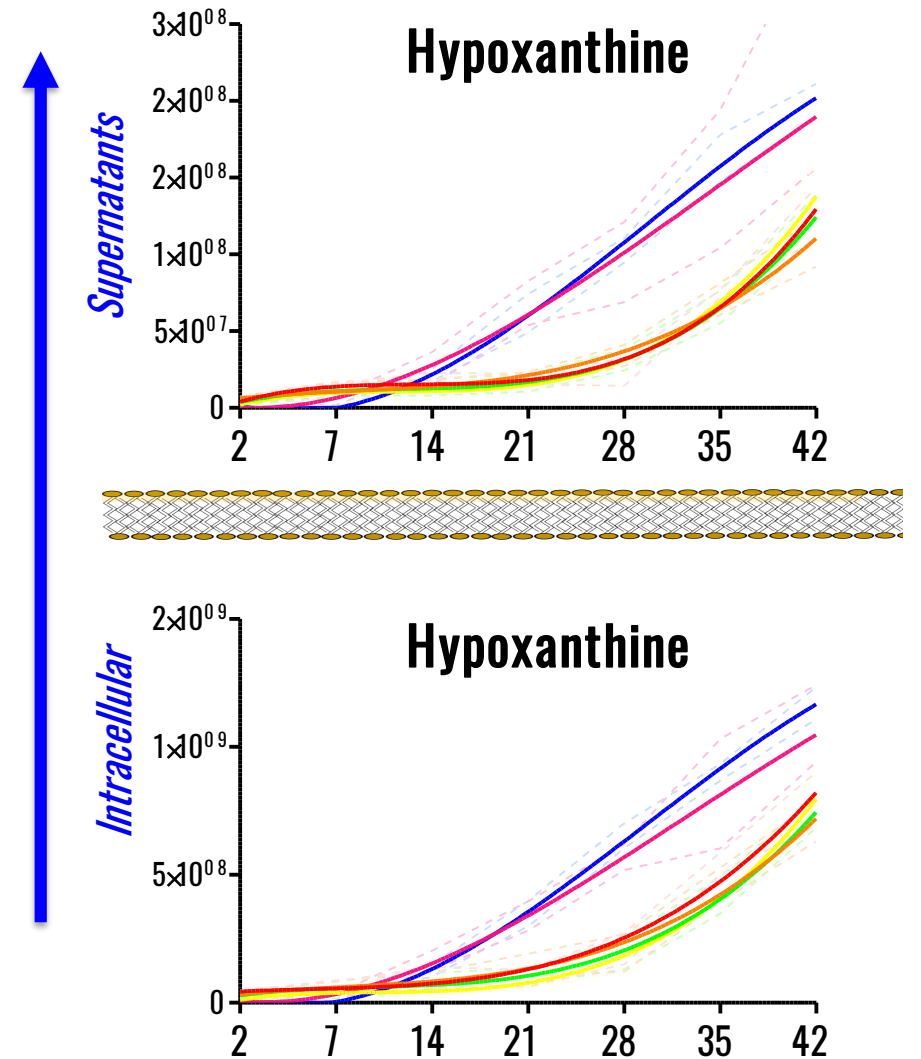
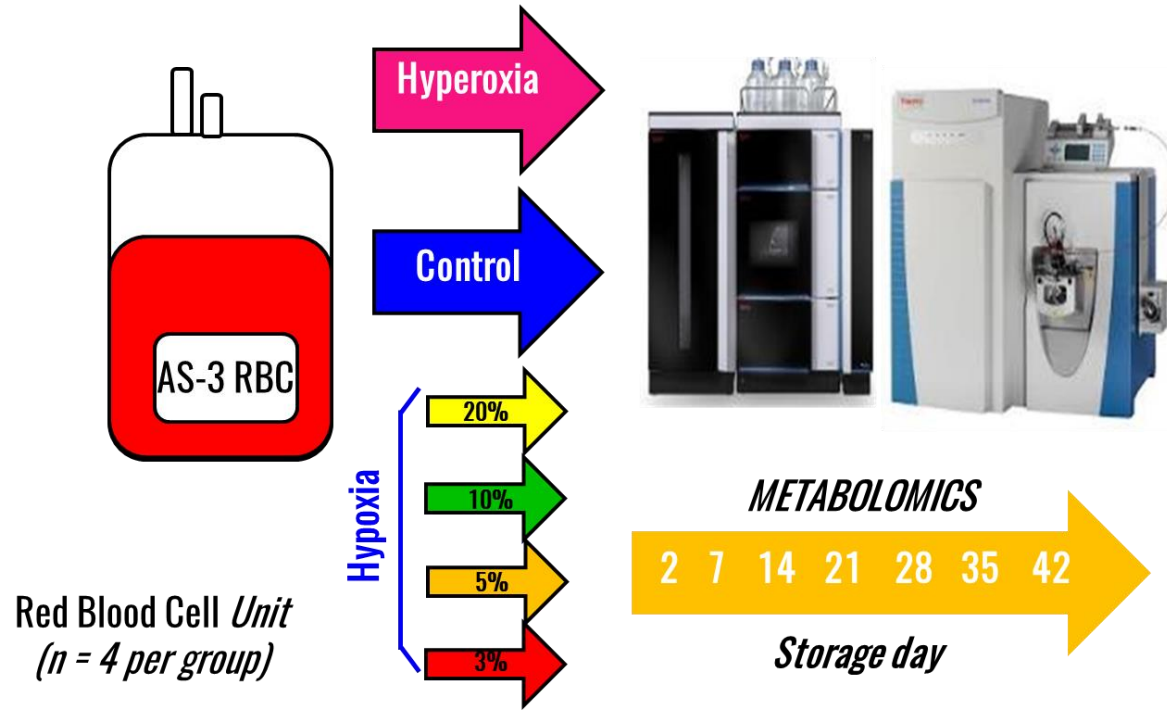


Courtesy of Drs. Eldad A. Hod, Columbia University and James C. Zimring, Bloodworks NW

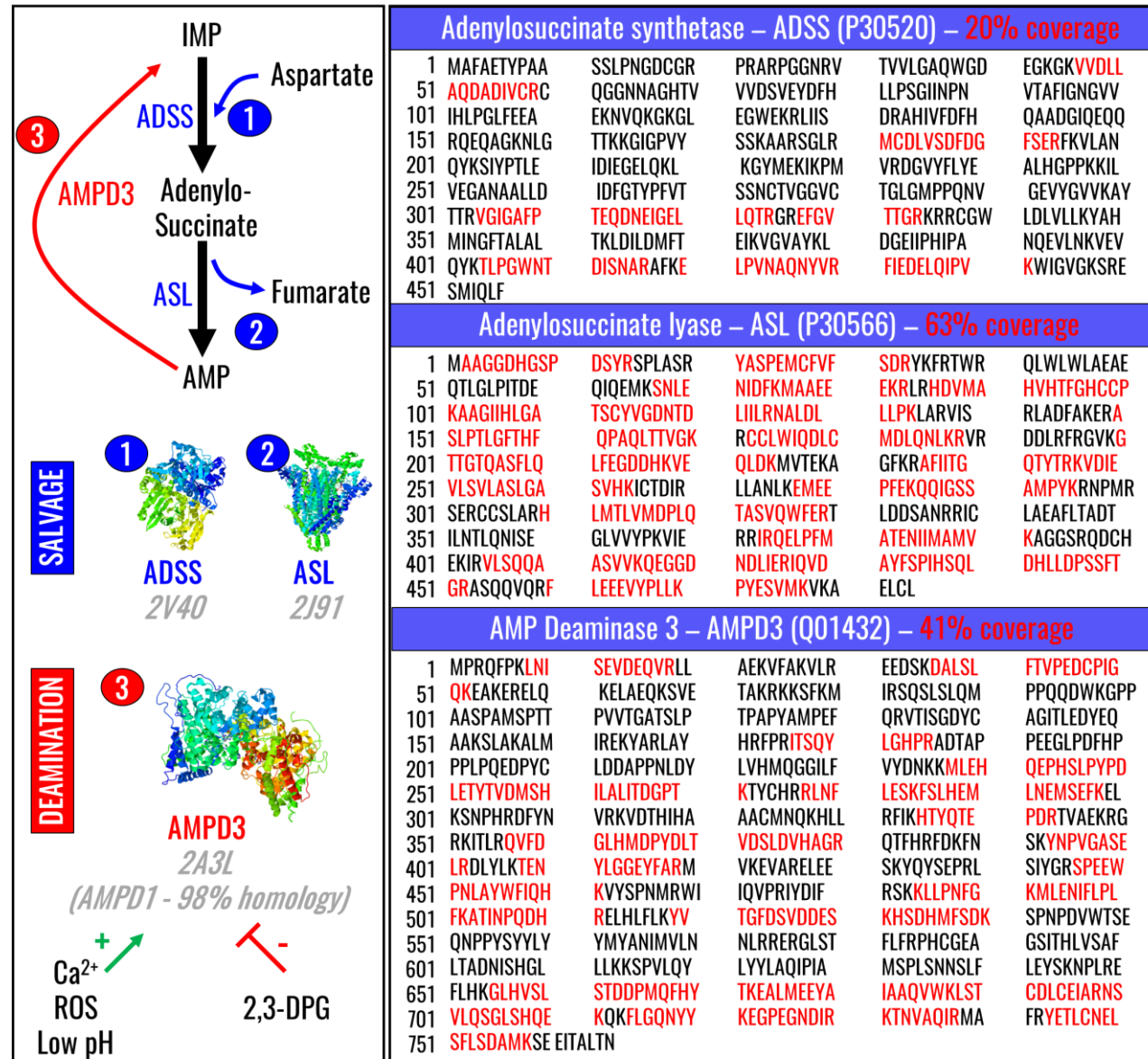
Hypoxia prevents purine deamination



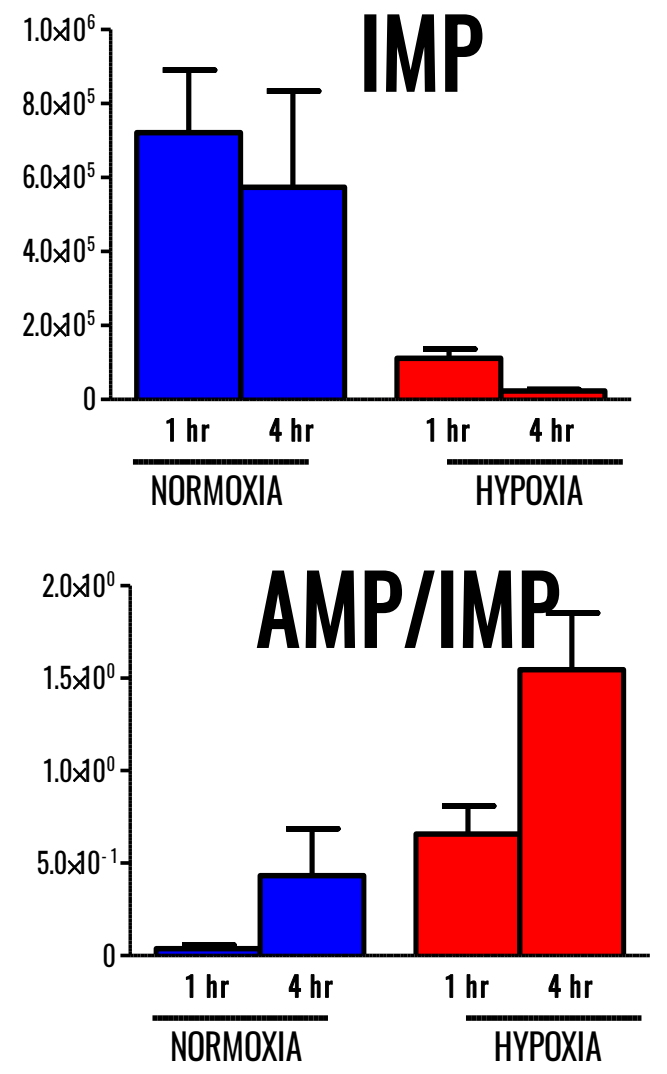
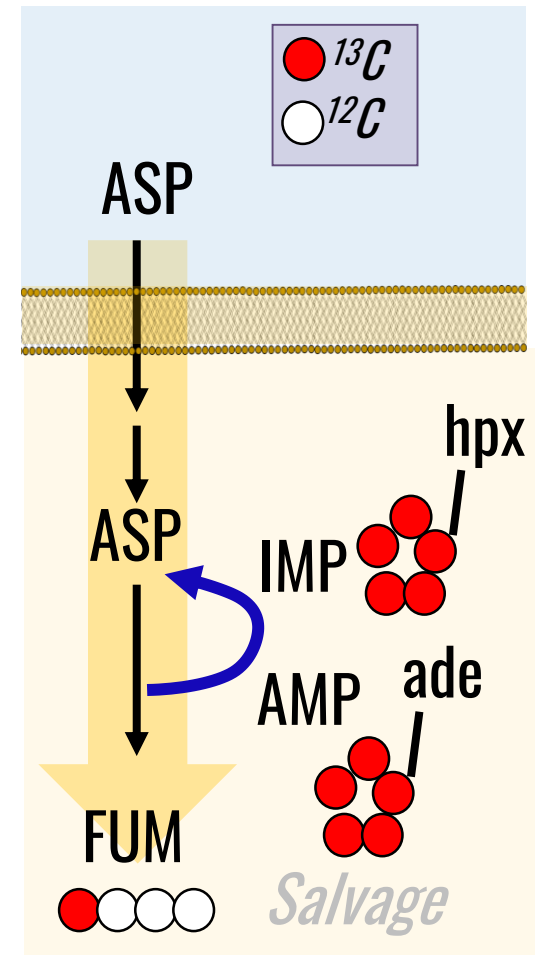
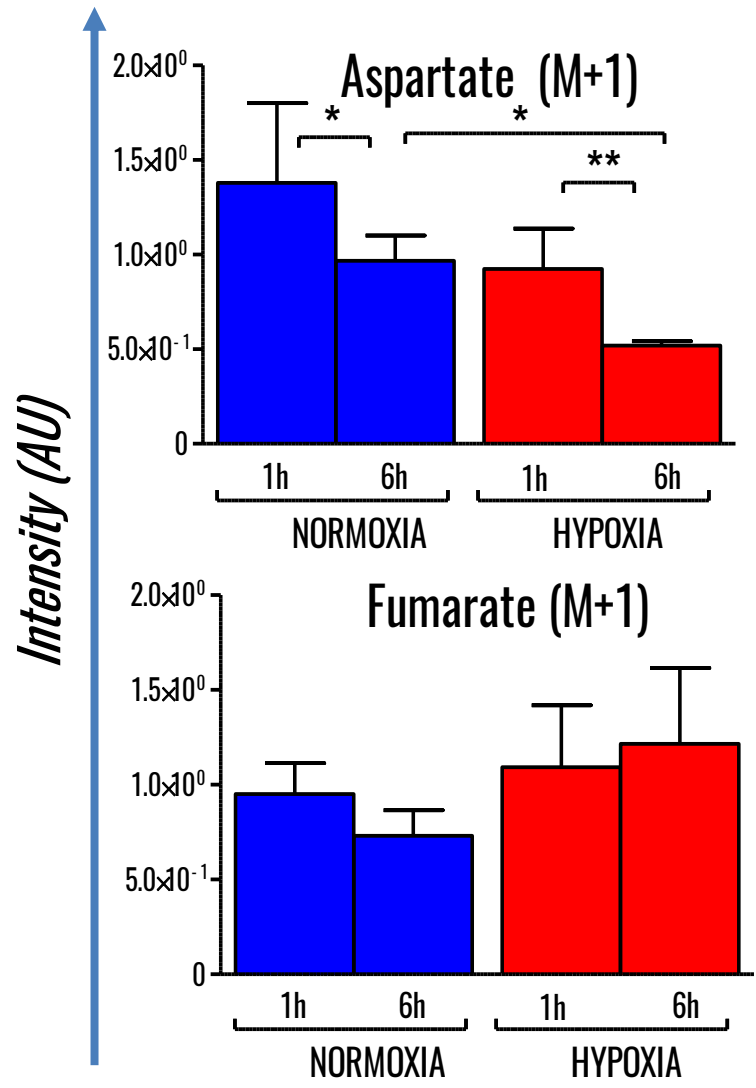
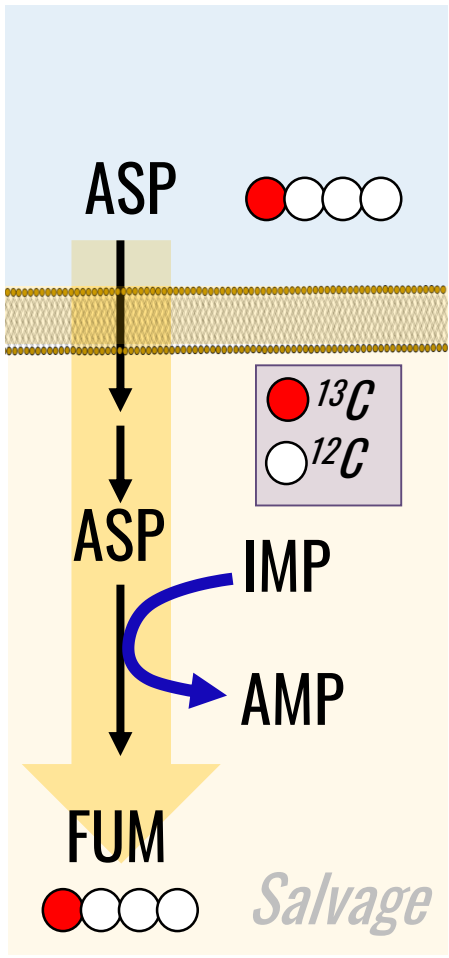
Hypoxia limits purine oxidation and Improves salvage: survival?



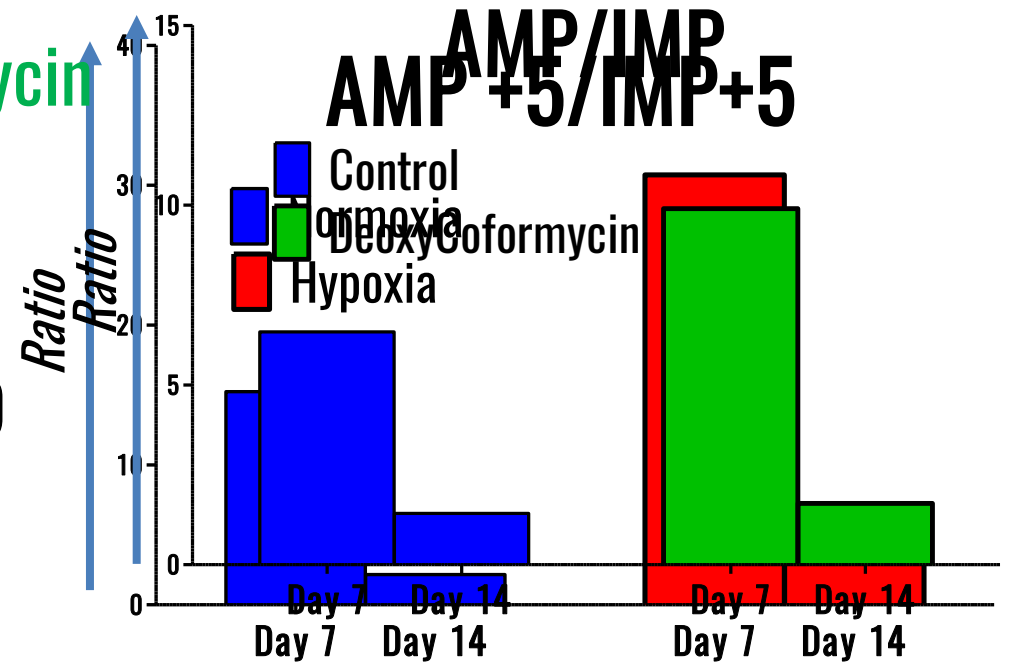
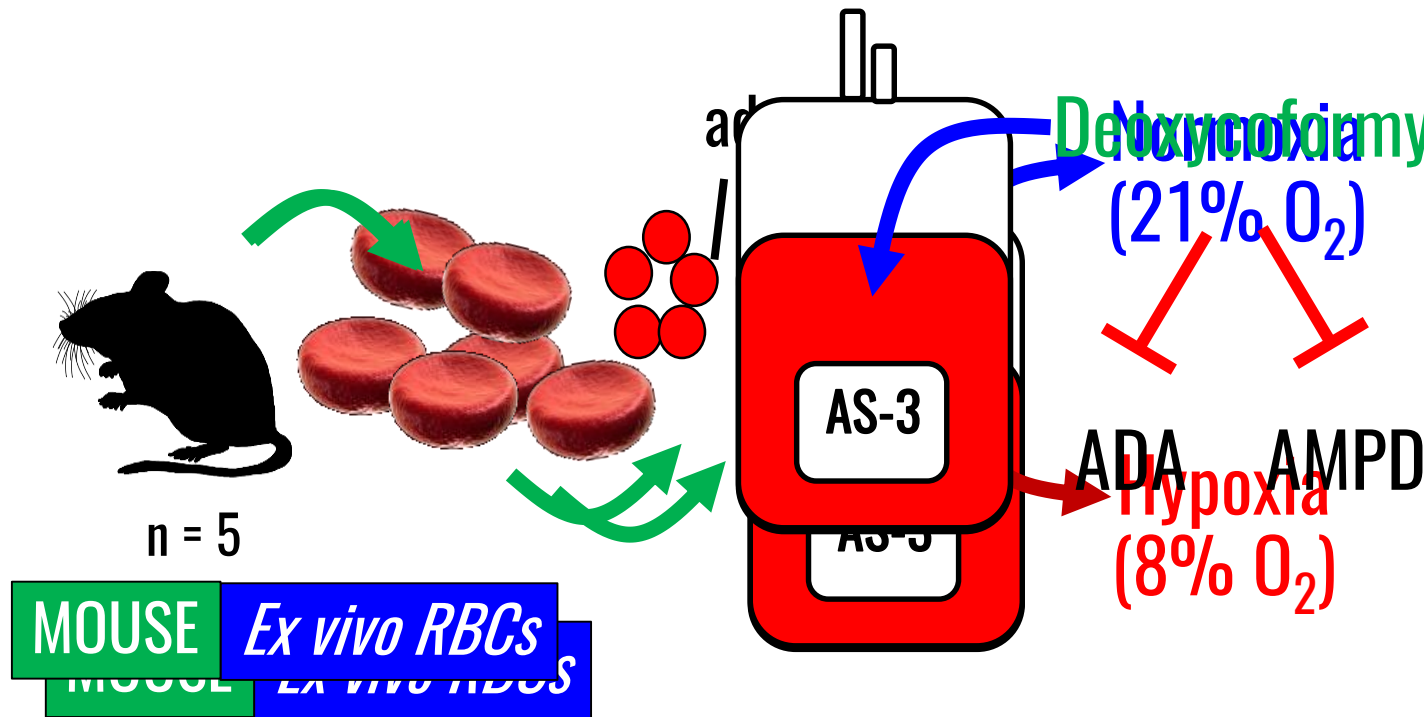
Hypoxia limits purine oxidation and Improves salvage: survival?



Hypoxia limits purine oxidation or Improves salvage?

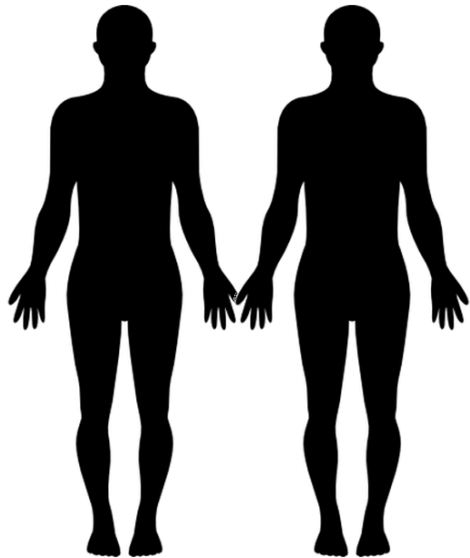


Hypoxia limits purine deamination



Where do we go from here

Heritability of
Good vs Poor
Storage Traits



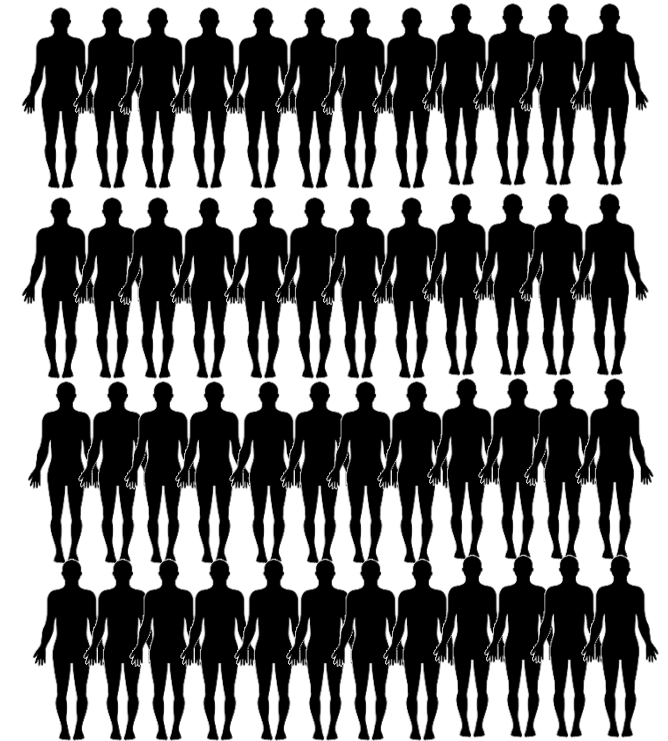
TWINS

Underwater mammals



ZOO-OMICS

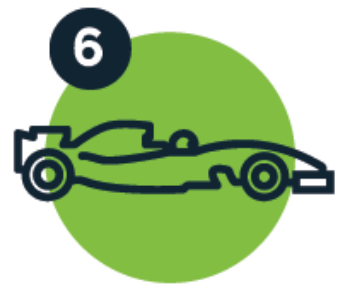
Donor effect on Storability



REDS III Omics

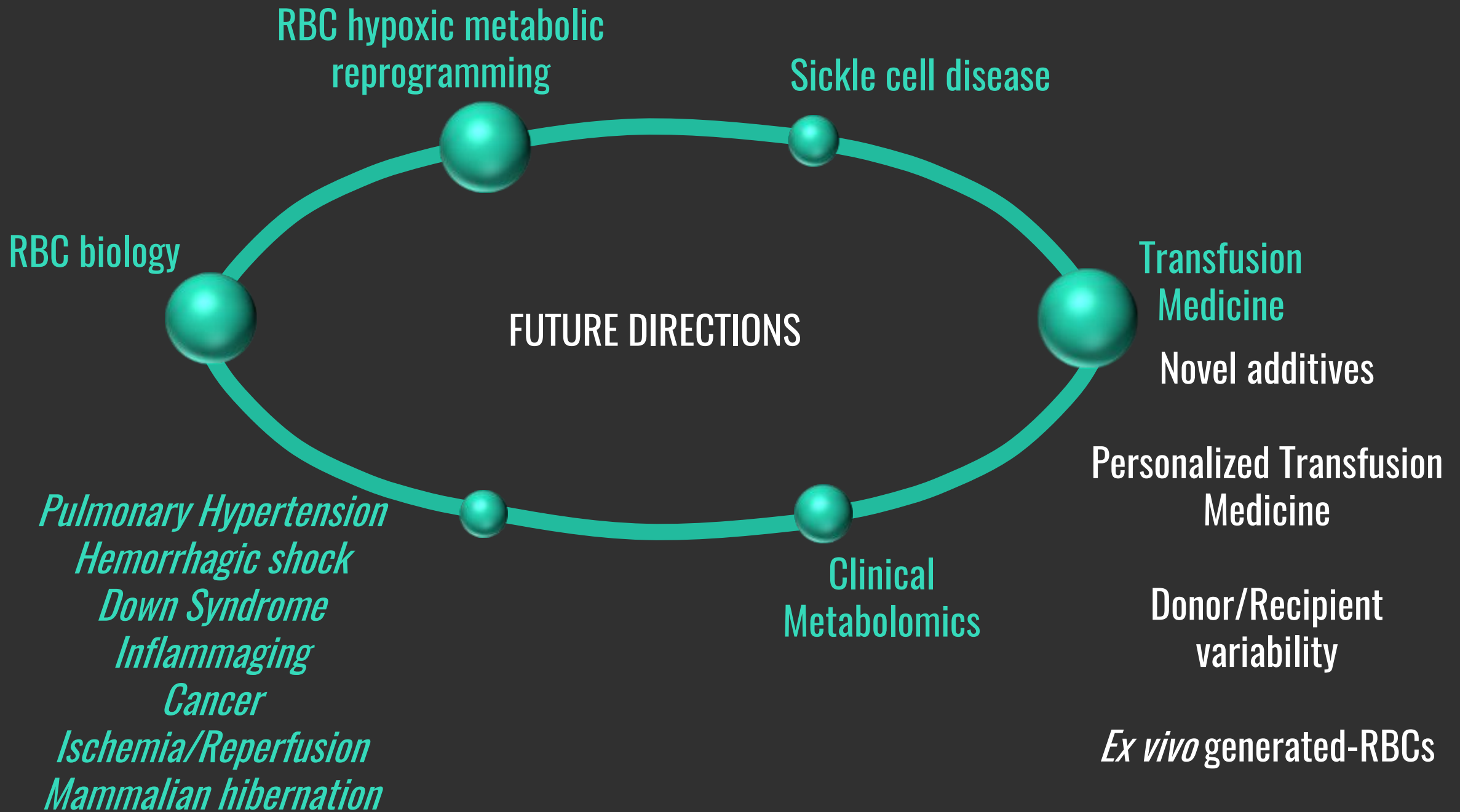


6 Milestones in Metabolomics: Driving our understanding of the metabolome



High-throughput metabolomics

In April 2017, Kirk Hansen and his team described a 3-min method that exploits recent technical advancements in UHPLC and fast scanning high-resolution MS technologies¹⁴. They describe this as combining the advantages of rapid flow-injection TOF-MS with the selectivity of conventional chromatography-based metabolomics. While not applicable for the measurement of all compounds, the robustness of this approach makes it useful for the analysis of a wide range of biological matrices relevant to basic science and clinical routine practice, including biofluids, cell and tissue extracts.



THANK YOU for your attention

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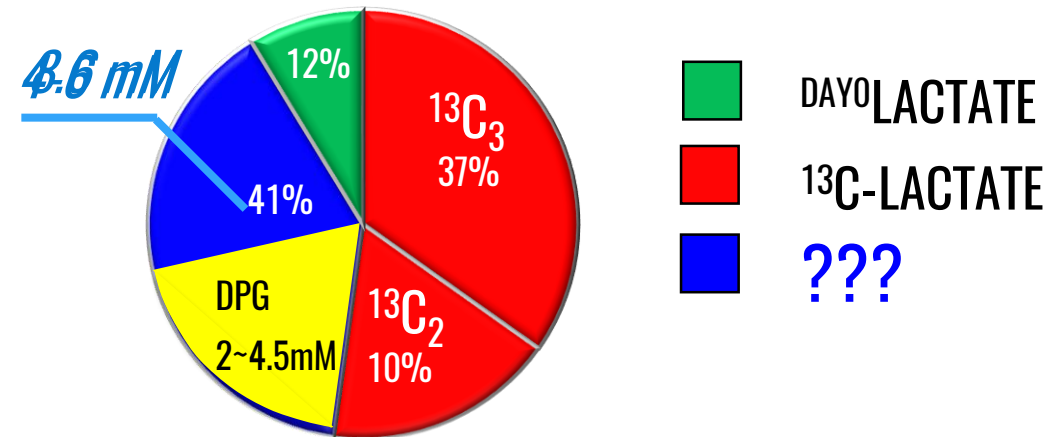
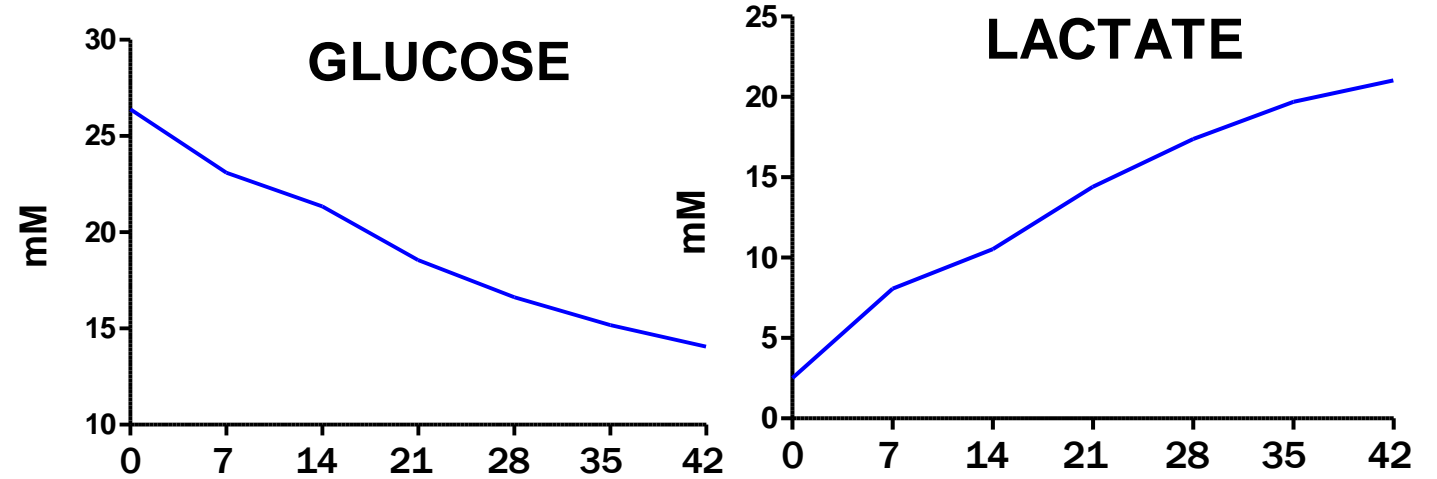
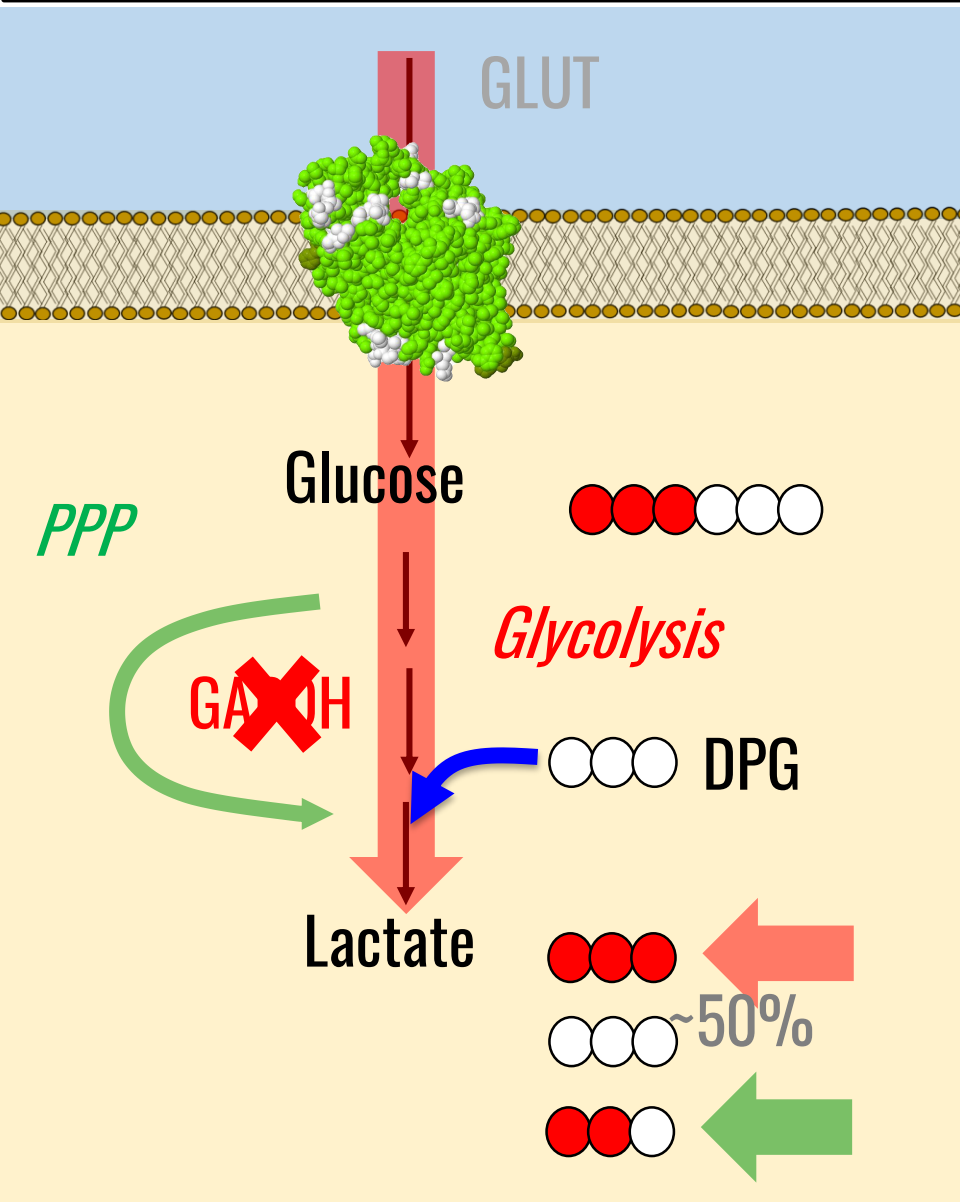
Twins

Thomas Raife

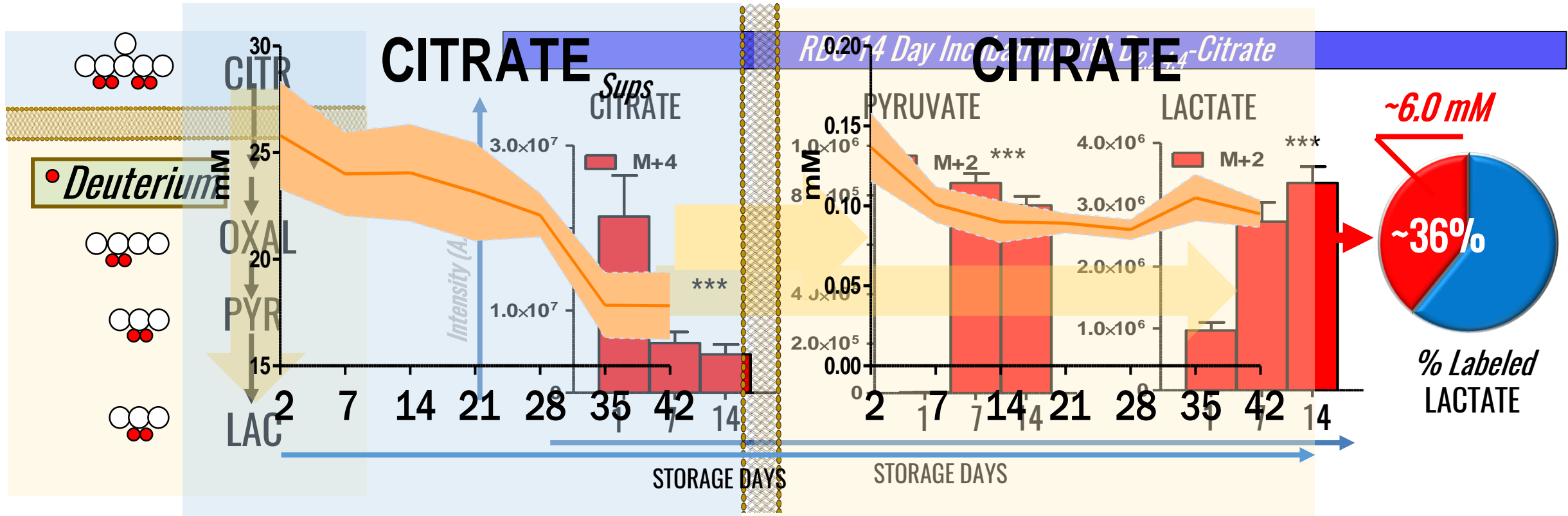
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Lactate dilemma: Metabolomics suggests activation of unexpected pathways in RBCs



Lactate dilemma: only AS3 additives contain citrate!



RBC storage: Redox signaling promotes PPP, but antioxidant defenses are overwhelmed by D21

