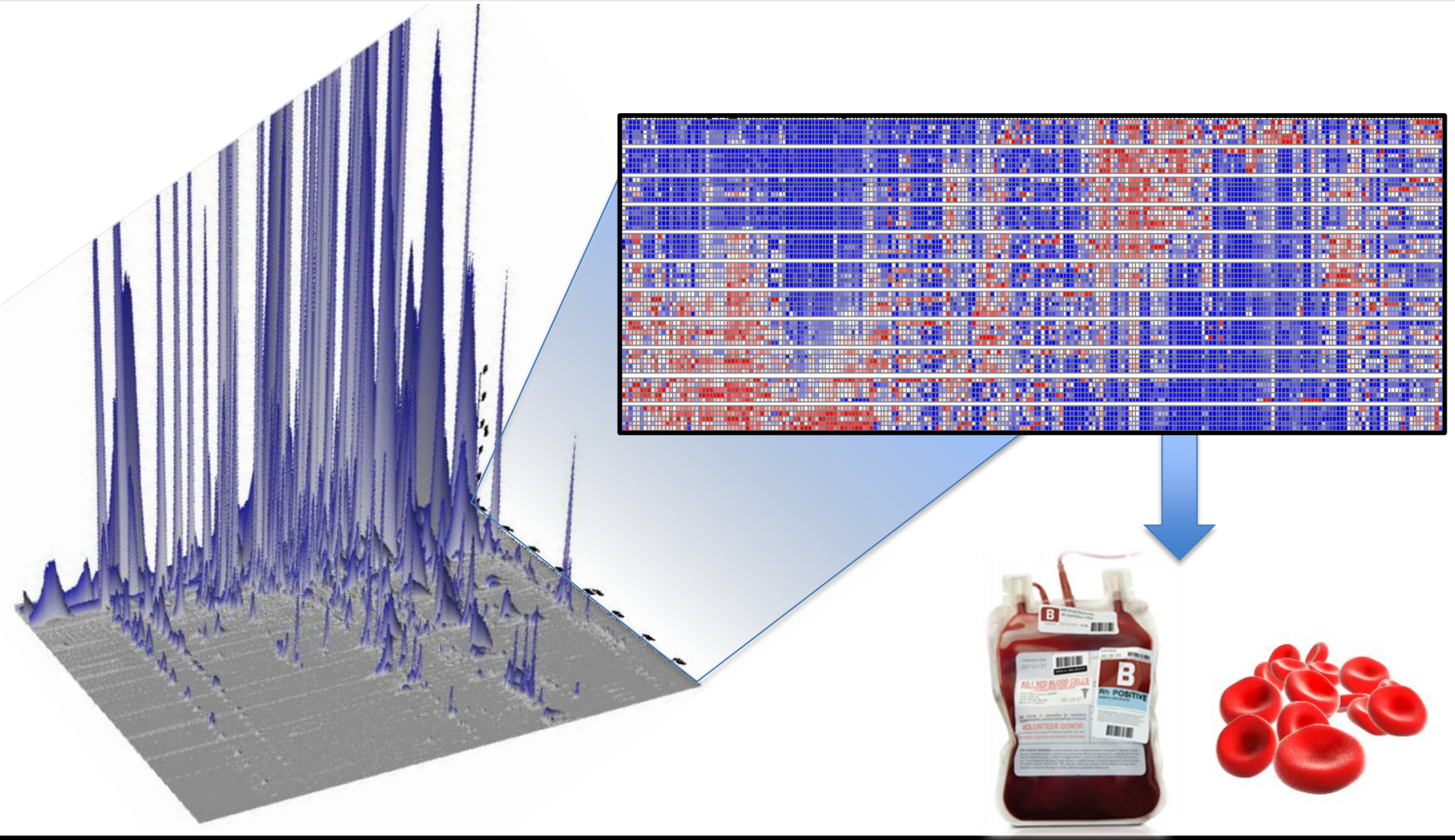
Metabolomics of stored Red Blood Cells

Hansen lab – Department of Biochemistry and Molecular Genetics – University of Colorado Denver – Anschutz Medical Campus



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BEST 2015, Scott Murphy Lecture –
Long Beach, CA - USA 10/22/2015

Angelo D'Alessandro, PhD

Acknowledgments and Disclosures

Endura Transfusion Omics

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BONFILS BLOOD CENTER

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Trauma/HS

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SAGM, Anaerobic, Antioxidants

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Camilla F. L'Acqua

Metabolism and outcomes

GEISEL SCHOOL OF MEDICINE AT DARTMOUTH
NEW HEALTH SCIENCES

Prof. Larry J. Dumont
Tatsuro Yoshida
Zbigniew Szcepiorkowski

AS3 vs AS7, Anaerobic storage +CO2

UNIVERSITY of ROCHESTER MEDICAL CENTER

Prof. Jim Palis

Metabolic regulation in erythropoiesis

HELLENIC REPUBLIC
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SAGM, G6PDH-def

Altitude Research Center
UTHealth The University of Texas

Prof. Robert C Roach

Prof. Yang Xia

AltitudeOmics

My real reason: my father is a non-reumunerated volunteer donor in Italy (>50 donations)

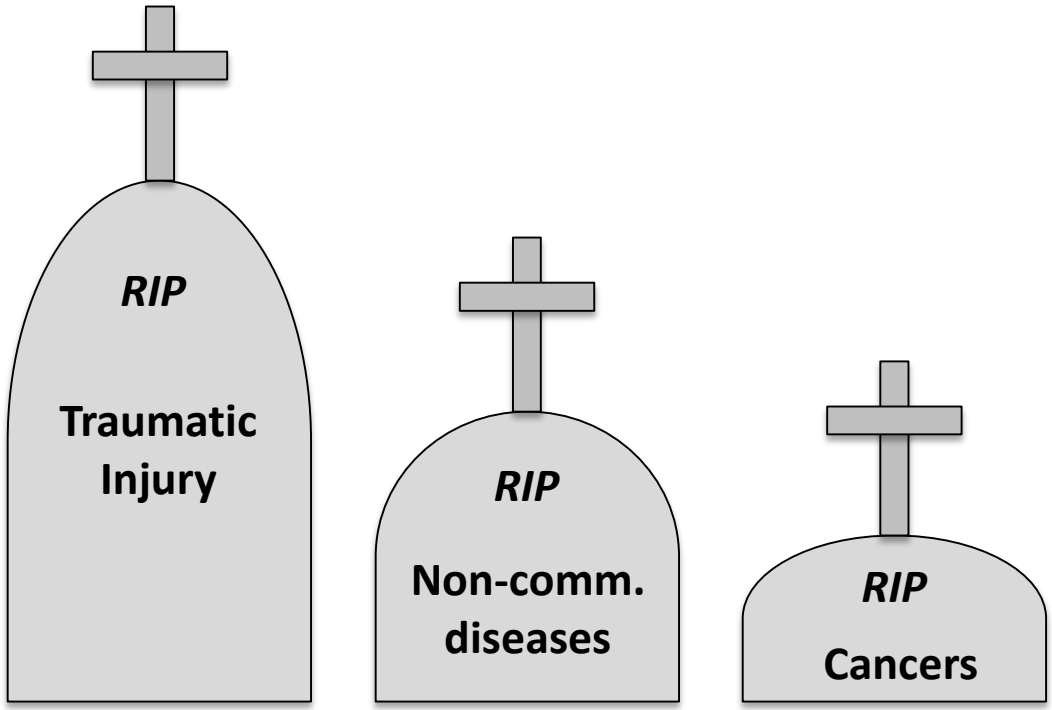
Aldo D'Alessandro

Why does it matter? Leading causes of death under age of 15-59

1.6 deaths/1000 adults

1.0 deaths/1000 adults

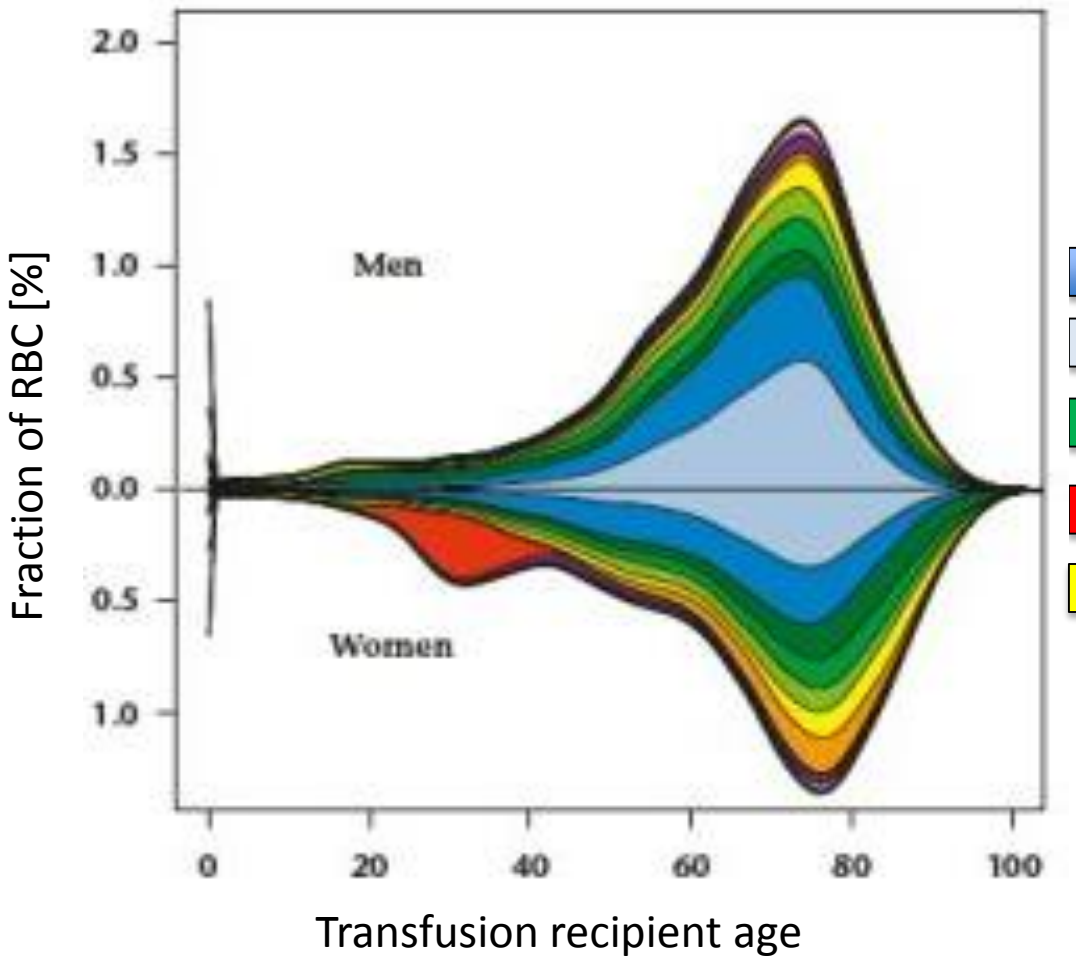
0.5 deaths/1000 adults



What do they have in common?

WHO, 2013

Leading causes of death require High % Transfusion



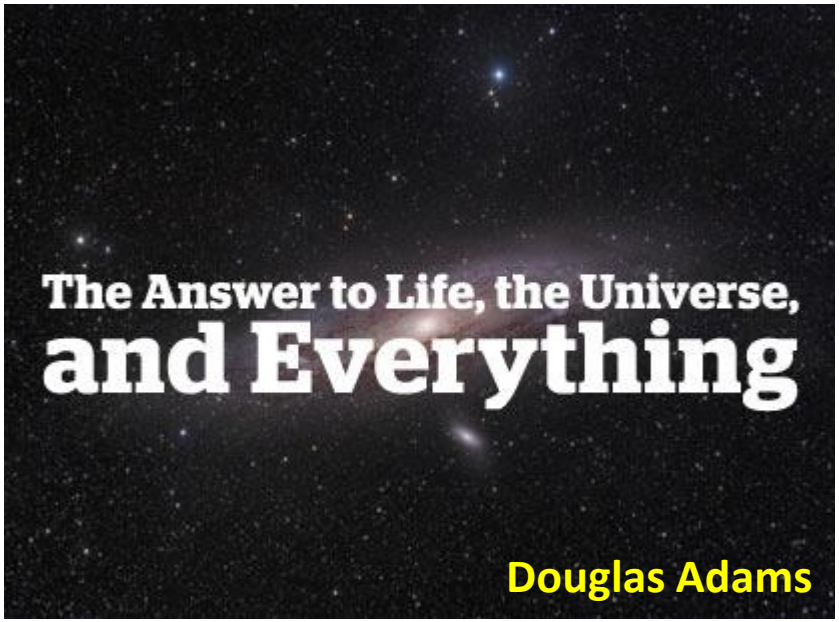
- Neoplasms (Hematological malignancies)
- Circulatory system
- Injury/Trauma
- Pregnancy/Childbirth
- Beta thalassemia/sickle cell disease

Center for Disease Control, 2014

Is blood the answer to...?

LIFE-SAVING

- **~ 108 million** units donated per year (2014 data)
- 4.5 million patients (**15 million units/year**) in the US alone
- Life saving therapy for **leading causes of death** under age of 50:
  - **Massive recipients** (trauma patients, military forces, surgical patients);
  - **Chronic recipients** (blood cancers requiring bone marrow irradiation, genetic diseases – beta-thalassemia, sickle cell anemia)



In vitro storage

Europe

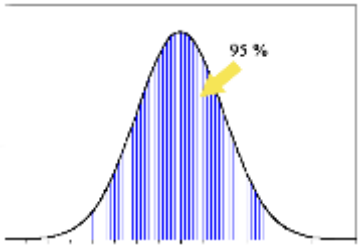
European Council Guidelines

U.S.A.

Standards for storage solution licensure

4°C

42 days



Haemolysis below 0.8 % (95/95 rule)

Haemolysis below 0.8% guarantees that there is a 95% of probability that the 95% of the units meet the 1% haemolysis maximum previously-accepted threshold

***Cr*^{51}**

In vivo survival at 24 hrs from transfusion over 75% threshold

European Council Guidelines, 2012

Long-stored blood: is it safe?

Blood Transfusion
2009

EDITORIAL

Red cell storage: When is better not good enough?

John R. Hess

Blood Bank, University of Maryland Medical Center, Baltimore, Maryland, USA

"Blood for transfusion must be safe, effective, available and cheap," the late John Collins said in a meeting of the U.S. National Academy of Sciences' Institute of Medicine in 1973 during efforts to license the 5-week CPDA-1 blood storage solution[1]. These objectives seem clear individually, but it is usually in their interactions that controversy arises and hard decisions must be made. Most of us are familiar with the interactions of blood safety and the cost of new tests or of new restrictions on the donor population and the availability of components. We all struggle to find new voluntary donors with healthy lifestyles and to justify and pay for increasingly sensitive testing.

However, the interactions between blood's effectiveness and its availability or its cost are less well known. In part this is because the whole concept of blood effectiveness is poorly defined. To the extent that red cell effectiveness has measurable meaning, red cells must be intact, circulate, and survive to be effective, so measures of their hemolysis, *in vivo* recovery, and survival have been gold standards for a technique to collect and manufacture blood components more efficiently[3].

Conventional thinking suggests that blood should be separated into components as quickly as possible[4]. This thinking has been incorporated into regulations saying that blood must be separated into components within 8 hours or cooled to refrigerator temperatures within that time. As most blood, 70% in some countries, is collected on mobile blood drives away from component manufacturing facilities, this has led to most mobile-blood-drive-collected blood being stored on ice with the resulting loss of platelet function. Additional platelets must then be collected by apheresis to make up for this loss.

Two decades ago, Dutch investigators noted that platelets derived from units of whole blood held warm overnight for processing the next morning actually had better platelet yields and better platelet function than those processed immediately after collection. Holding blood warm overnight is also attractive because it allows all the component manufacturing to

Kock et al, 2008; Lelubre et al, 2009; Hess JR, 2009

OLD BLOOD, NEW BLOOD, NO BLOOD? NEED FOR ULTRA HIGH THROUGHPUT

90 MILLION UNITS/YEAR

Randomized Clinical Trials suggest

no major association between storage duration and adverse outcomes

"Restrictive transfusion strategies are safe in most clinical settings, liberal transfusion strategies have not been shown to confer any benefit to patients but have the potential for harm"

Stein et al, 2015; Holst et al., 2015

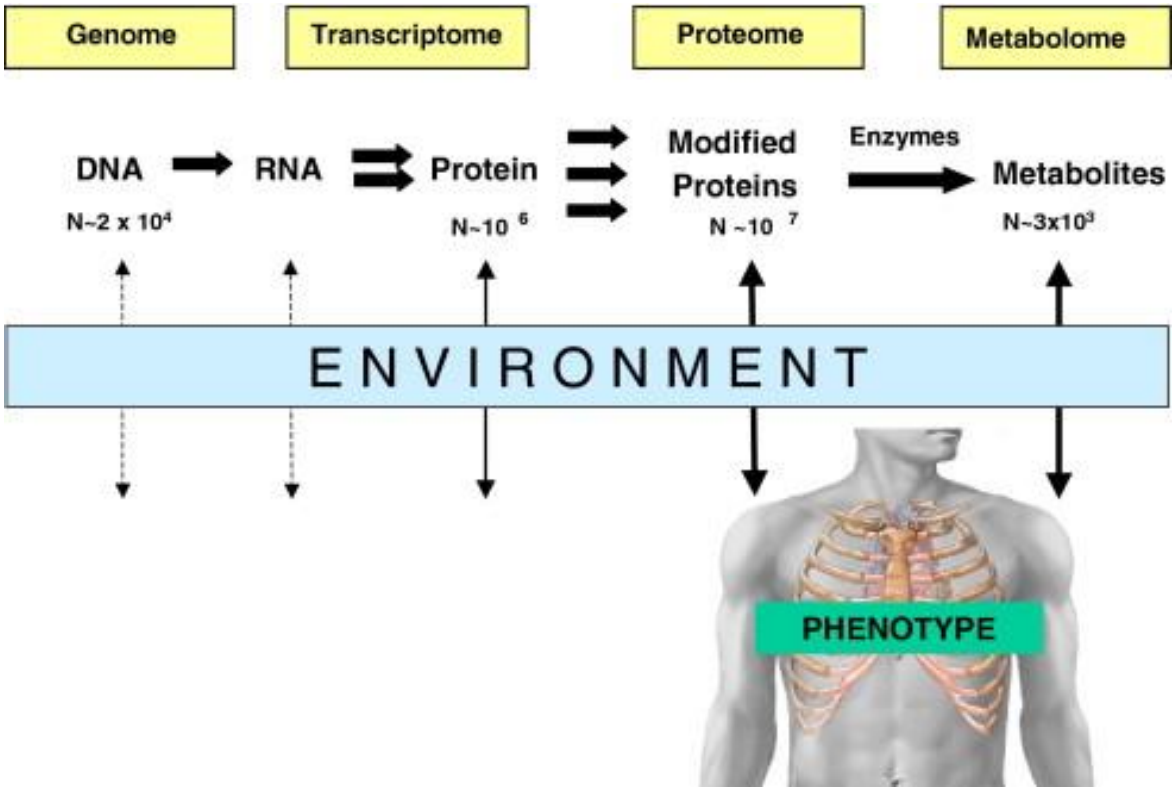
NHLBI awaits advances for the next 5 years

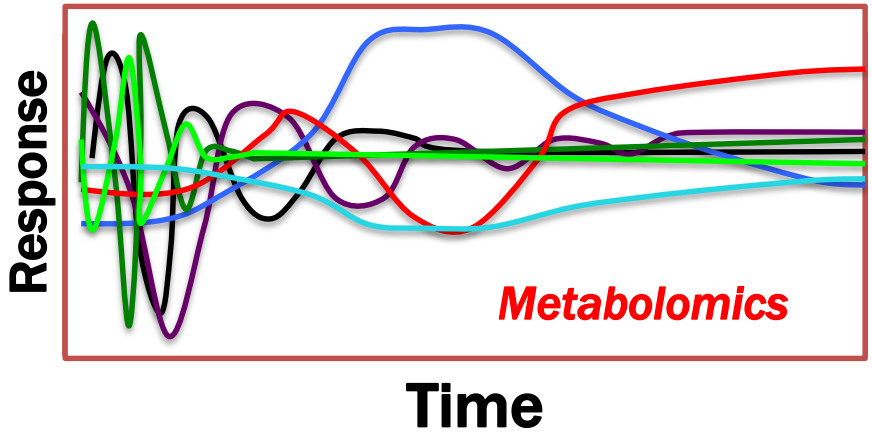
1. Identifying and quantifying the components of each transfusion product (i.e., red blood cells, platelets, plasma, plasma fractions, etc.) "What's in the bag?" OBSERVATIONAL

2. Identifying the appropriate, physiologically-relevant markers to determine transfusion effectiveness "How do we know if it works?"
CORRELATIVE (with gold standards)

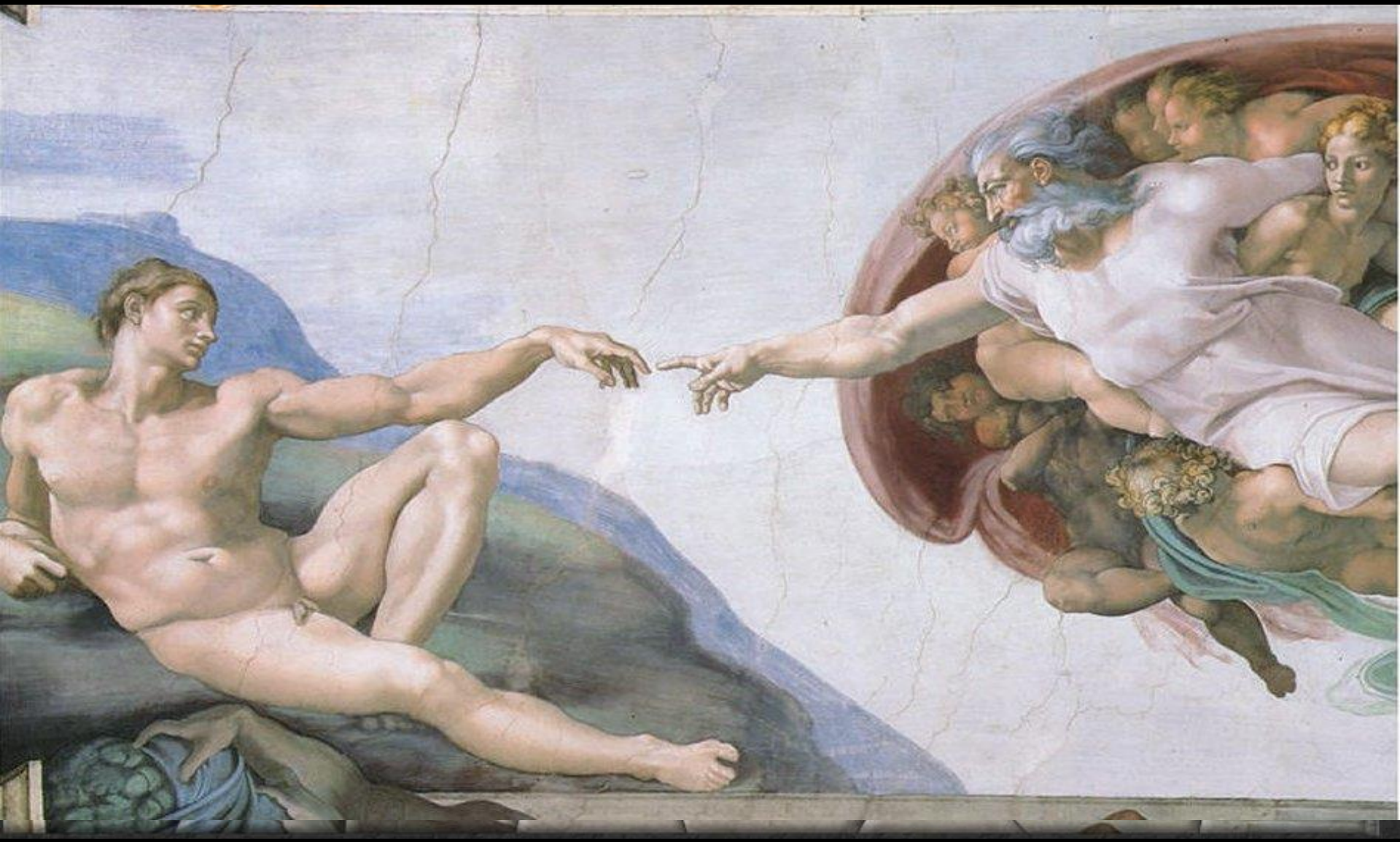
3. Identifying improved methods for preparing classical products."How can we make better products?" **High Throughput and modeling** *Spitalnik et al. 2015*

Metabolomics: closer to the phenotype

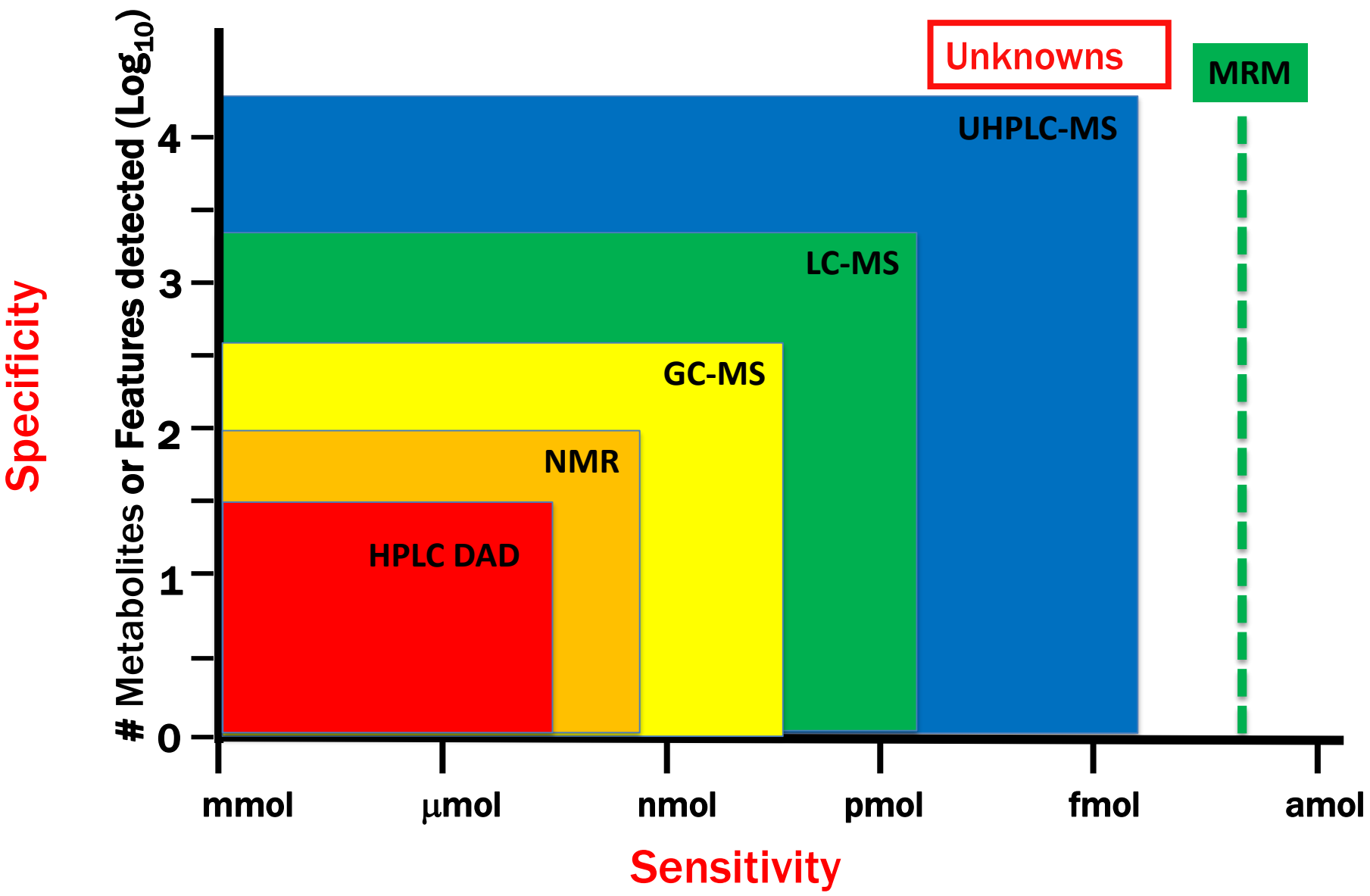




Integrated Omics: putting the pieces together



Metabolomics: from NMR to UPLC-MS



Instrumentation

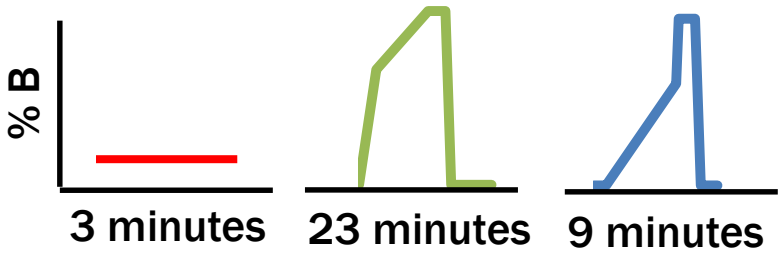
Performance Characteristics	
Resolving power	240,000 @ *m/z* 200
Mass range	50 to 6,000 *m/z*
Scan rate*	Up to 18 Hz at resolution setting of 15,000 @ *m/z* 200
Mass accuracy *	Internal: <1 ppm RMS External: <3 ppm RMS
Sensitivity	Full MS: 500 fg buspirone on column S/N 100:1 SIM: 30 fg buspirone on column S/N 100:1
Dynamic range	>5000:1
Polarity switching	One full cycle in <1 sec (one full positive mode scan and one full negative mode scan at a resolution setting of 60,000)
Multiplexity	Up to 10 precursors/scan
Analog inputs	One (1) analog input (0–1 V) One (1) analog (0–10 V)

*Under defined conditions

Dionex Ultimate 3000 UHPLC

High Pressure Tolerance: Up to 15,000 psi

High Reproducibility/Efficiency

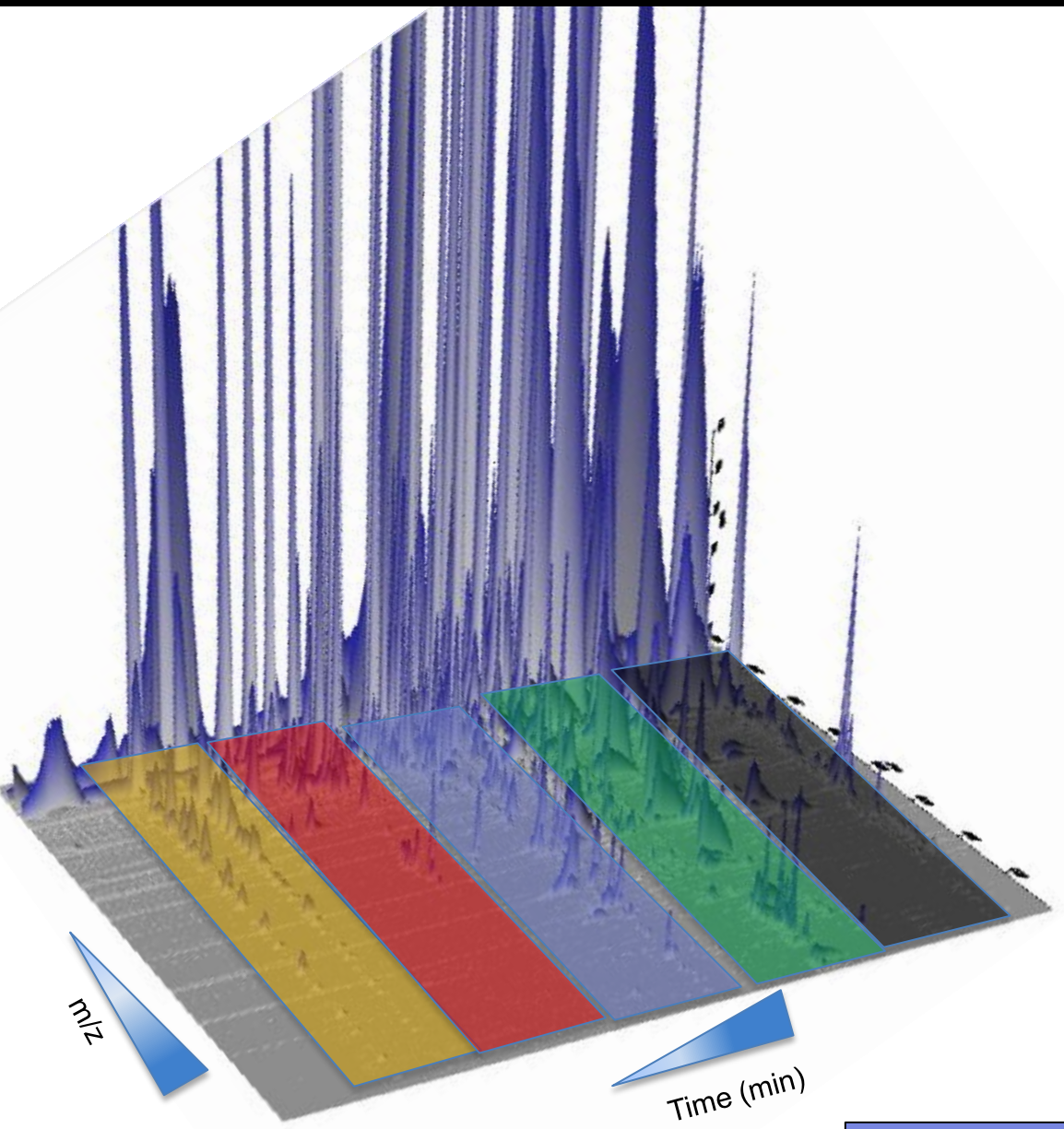


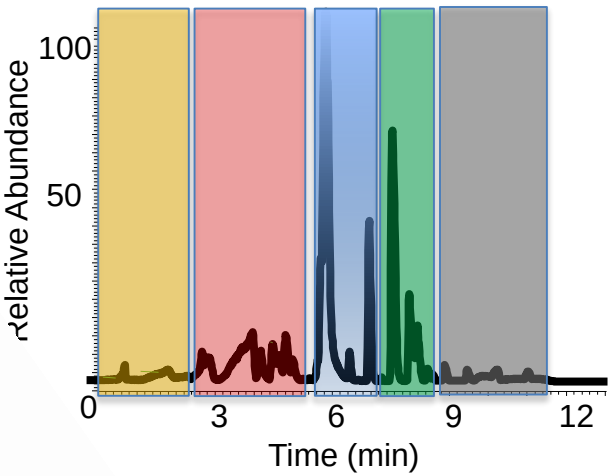
2X

	M³ - C18	M¹⁵ - HILIC
Run Time	3 minutes	15 minutes
Separation	++	+++
Efficiency	+++	++
Coverage	Up to 3,500 features	Up to 15,000 features

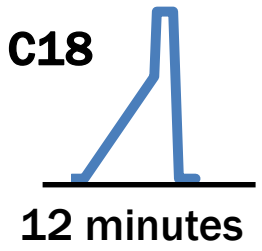
2X

Metabolome coverage



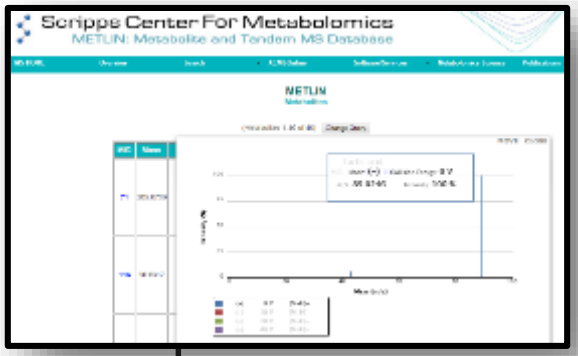


- Polyamines and basic AAs
- Sugars and Carboxylic acids
- Acid and Aromatic AAs and Small Peptides
- Phosphate and Nucleosides
- Fatty acids

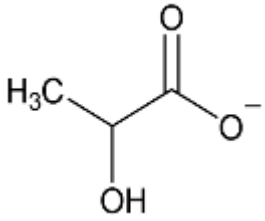


RBC Metabolome UPLC-QExactive run (D'Alessandro et al. unpublished)

Metabolite assignment:
golden rules and databases



* Number of elements x MW
* LEWIS and SENIOR rules
* Isotopic patterns
* H/C ratios
* Element ratio of N, O, P, and S vs C
* Element ratio probabilities



Lactate = 89.023

1.0034 Da

^{13}C – ^{12}C mass difference

1.1%

Natural abundance of ^{13}C

1. What’s in the bag?

Study Design: RBC Storage in AS-3

AS-3 (Nutricel)	
NaCl	70 mM
NaH_2PO_4	23 mM
Citric acid	2 mM
Na-citrate	23 mM
Adenine	2 mM
Glucose	55 mM
pH	5.8

Supernatants: 20 µl (for up to 100 runs)

Packed RBCs: 0.1 ml (for up to 100 runs)

5 donors

11 time points

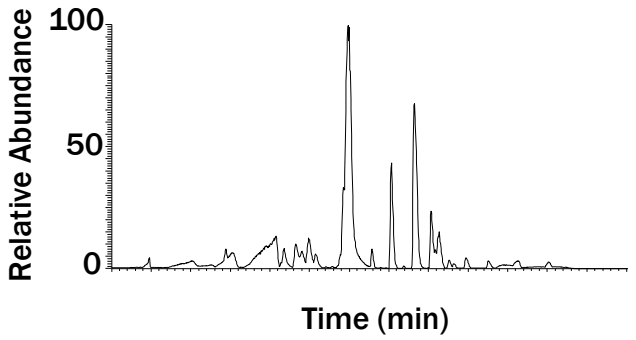
2 fractions

Supernatant

RBC

+ and – ion mode

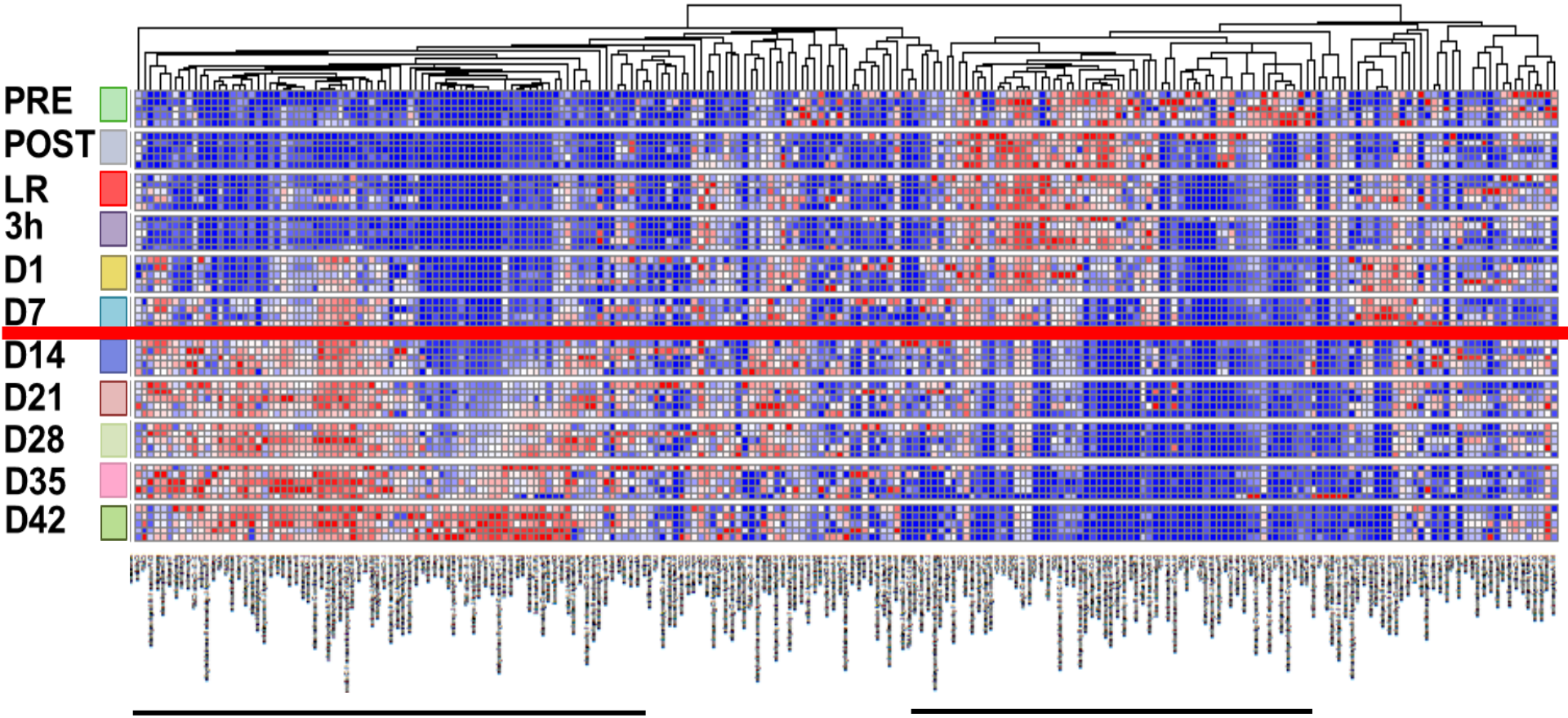
220 analytical runs



How long is too long? Cells

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

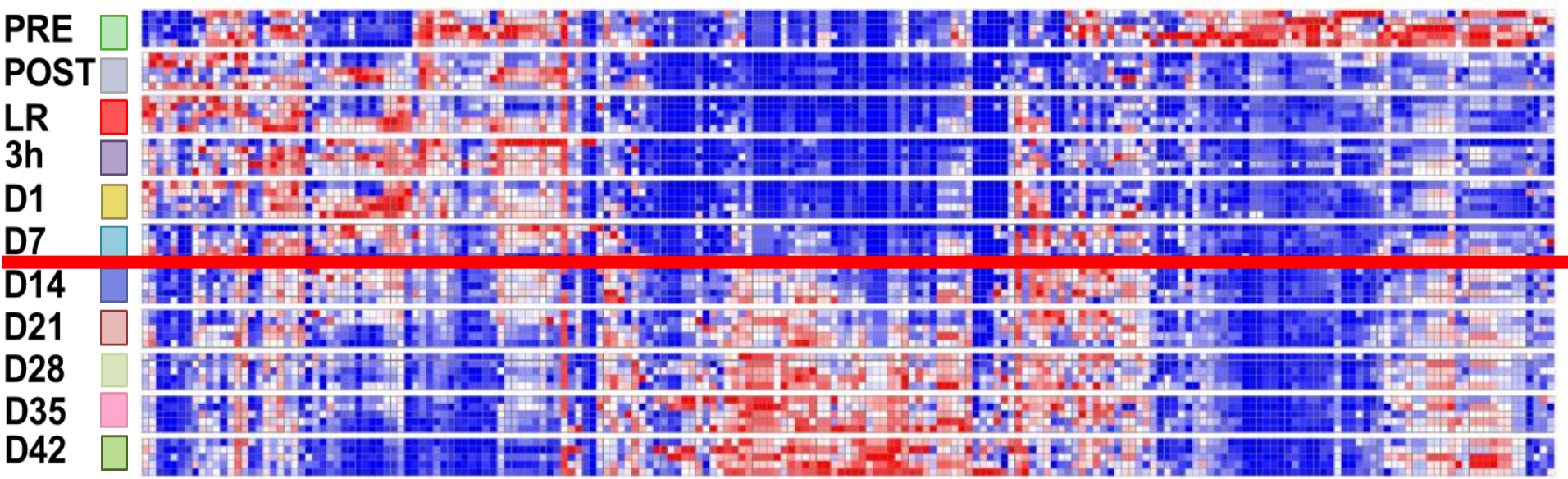
D'Alessandro et al, Transfusion 2015

How long is too long? Supernatants

Min — Max

Supernatants

Significant metabolic lesions tend to accumulate by storage day 14



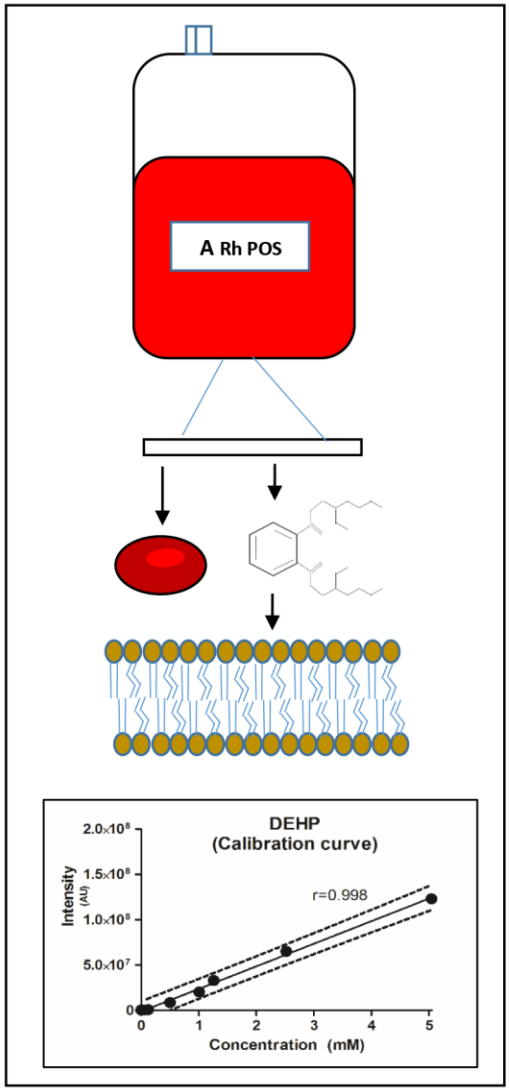
Metabolites decreasing by storage day 14

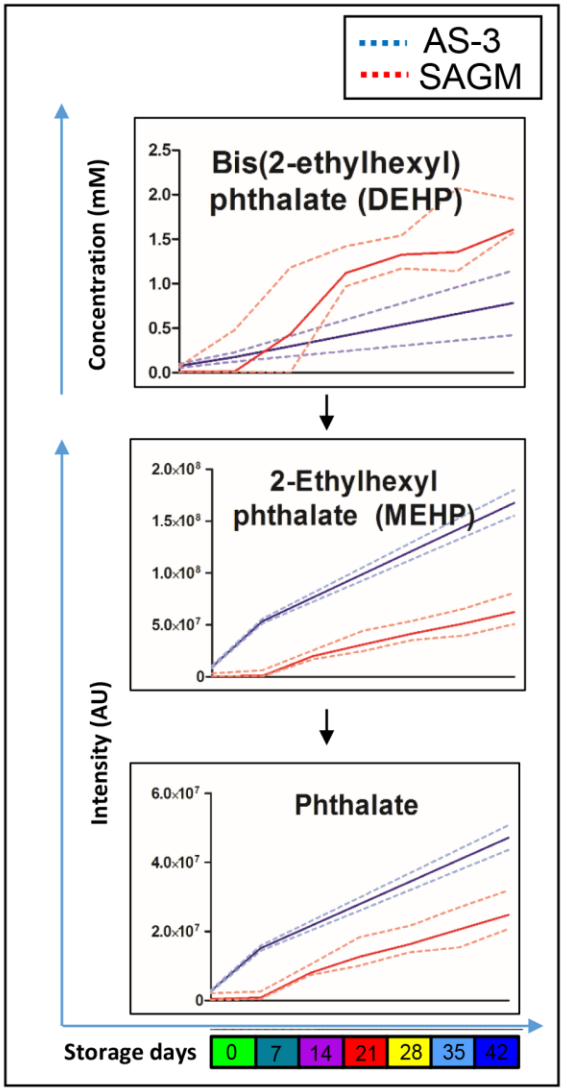
Metabolites increasing within storage day 14

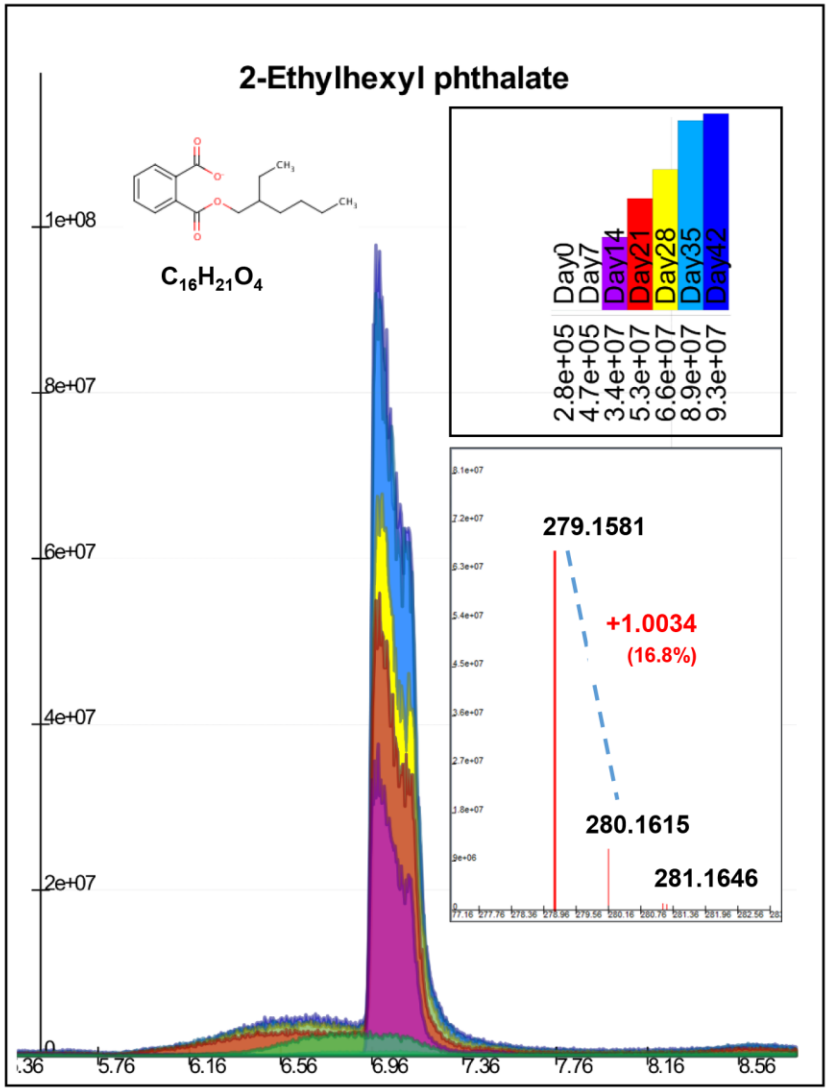
Metabolites washed out be leukoreduction processing

D'Alessandro et al, Transfusion 2015

The analysis also covers plasticizers such as DEHP and other phthalates





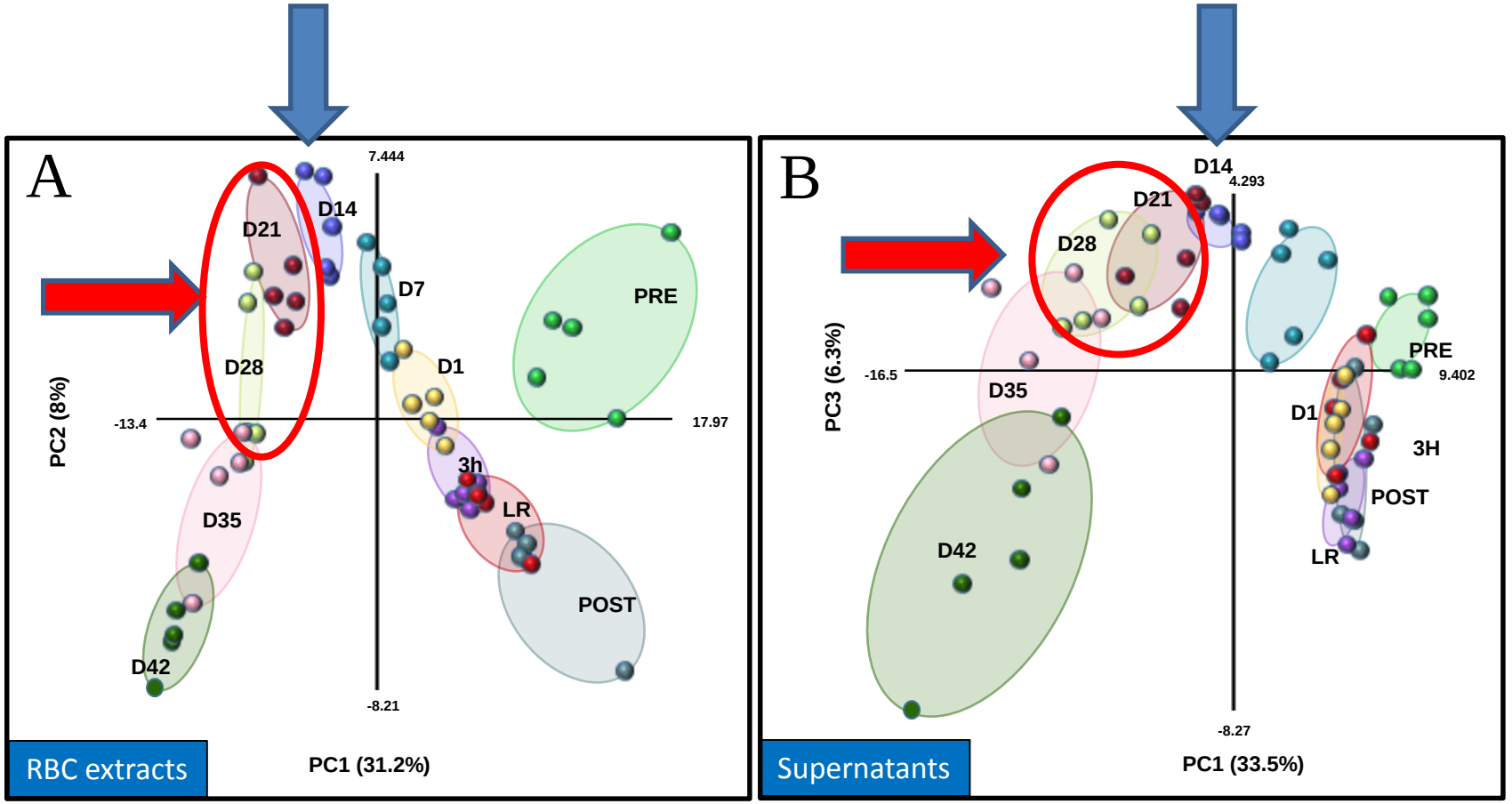


4 min method!

D'Alessandro et al, under review

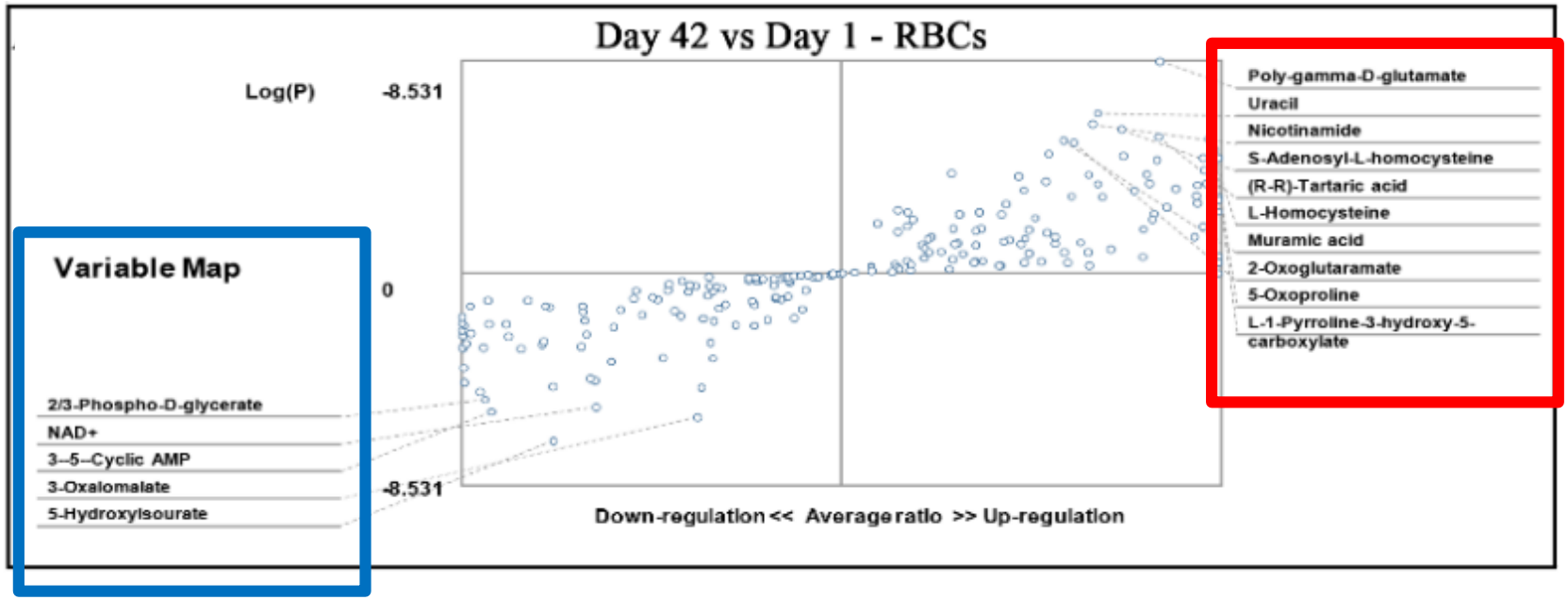
How long is too long? PLS-DA answer

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



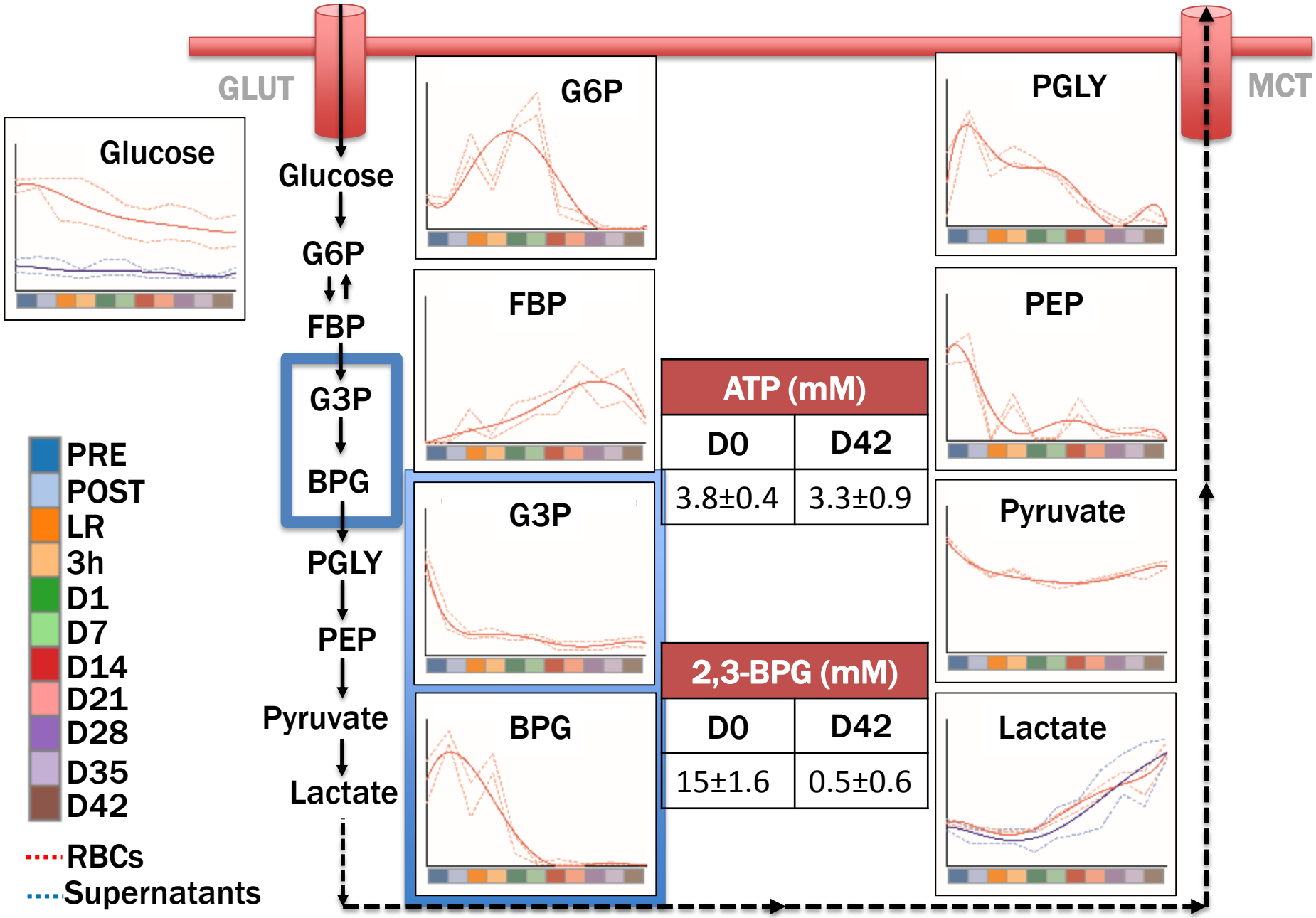
D'Alessandro et al, Transfusion 2015

Energy and Redox metabolism: main contributors to PLS-DA



D'Alessandro et al, Transfusion 2015

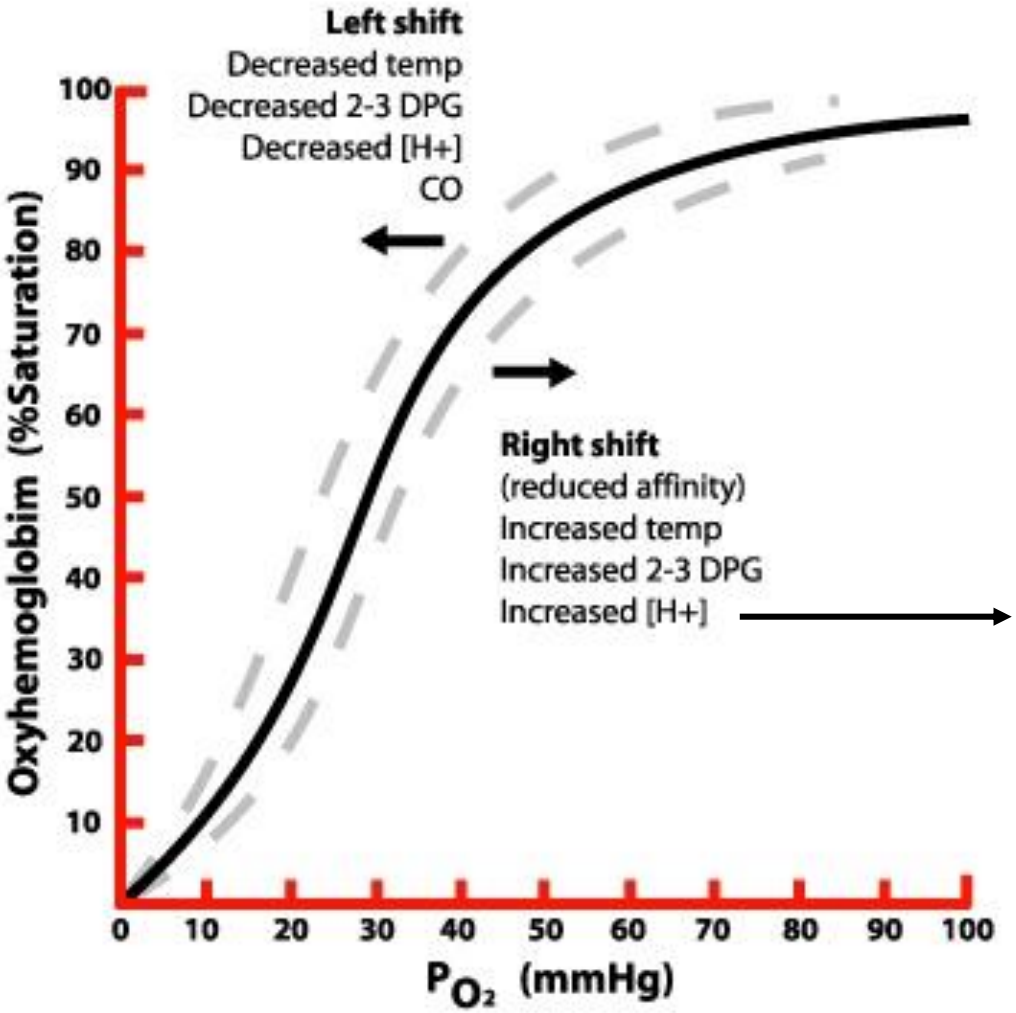
AS3 preserves energy metabolism but not DPG



ATP (mM)	
D0	D42
3.8±0.4	3.3±0.9

2,3-BPG (mM)	
D0	D42
15±1.6	0.5±0.6

Pitfalls of metabolic alterations on cell physiology in aged RBCs: impaired oxygen delivery



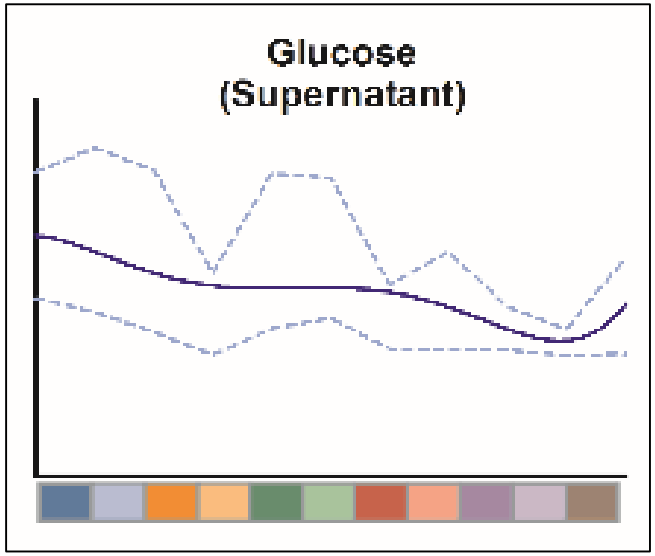
Lower pH: Bohr effect

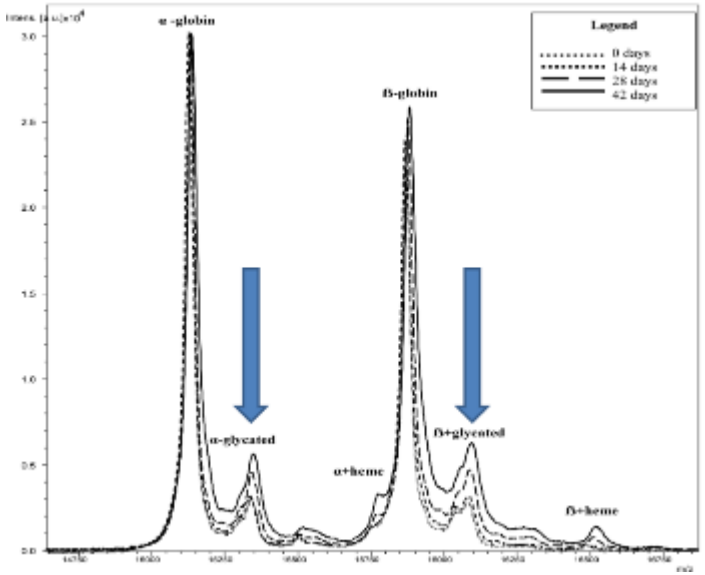
Involving N-terminal amino groups of the α-subunits and the C-terminal histidine of the β-subunits

Samaja et al, 1990; Romero and Romero, 2004

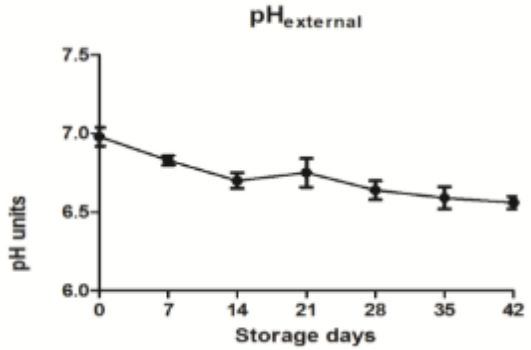
Impaired Energy & Redox Metabolism During Storage

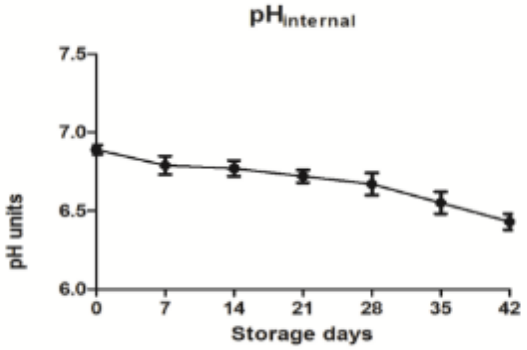
Still ~50% glucose at the end of the storage: and Hb glycation increases (diabetes marker)





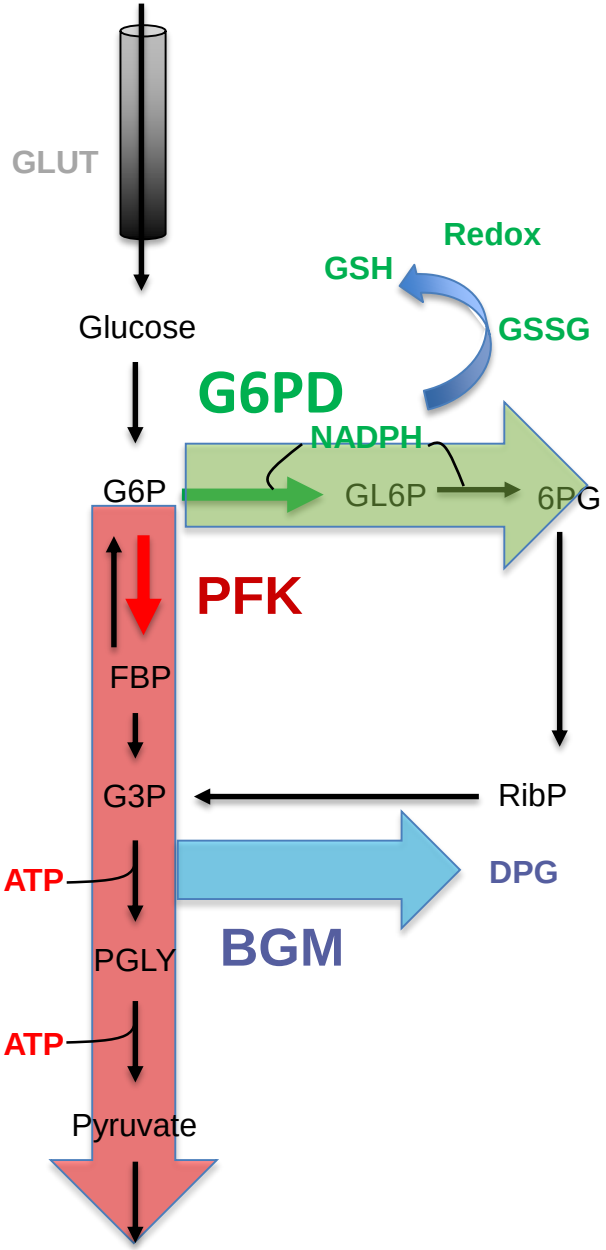
pH lowers and glycolysis slows down

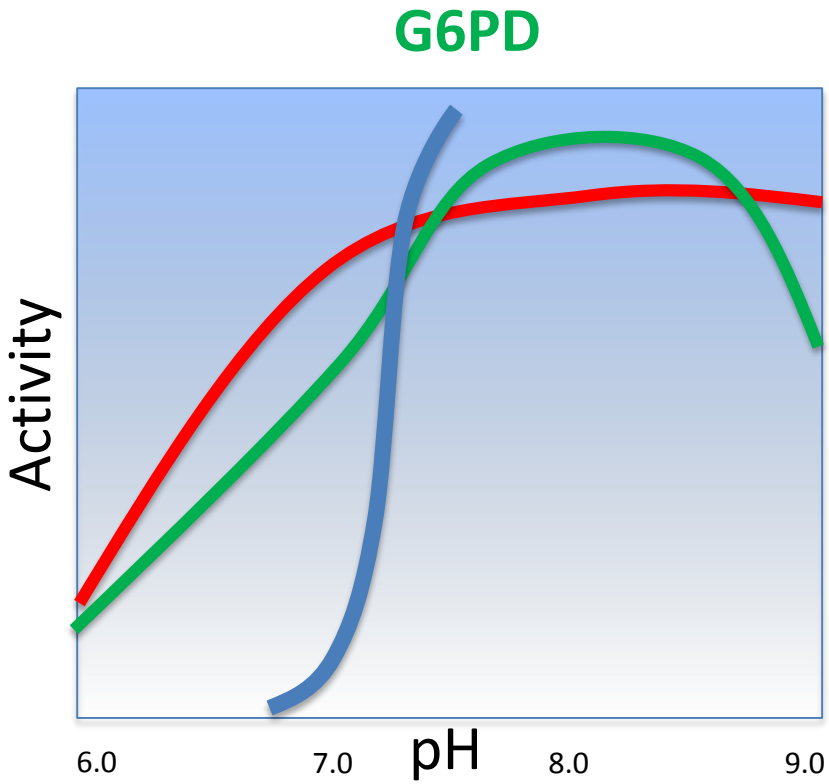


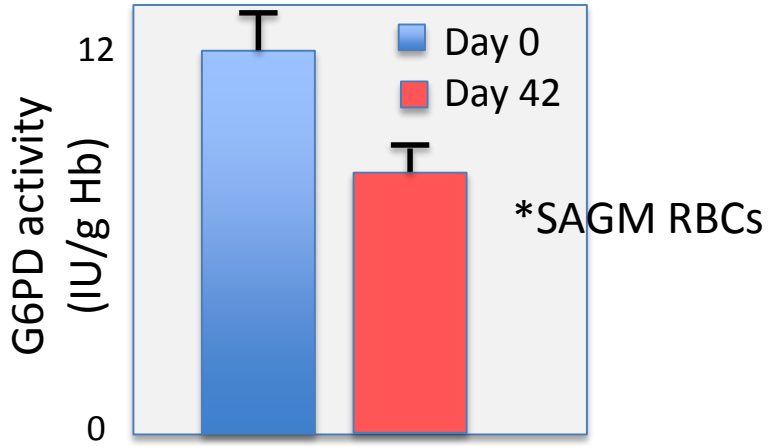


D'Alessandro et al., Haematologica 2012; Vox Sanguinis 2013; Transfusion 2015

Acidification slows down glycolysis and PPP

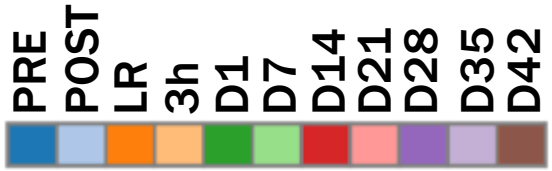






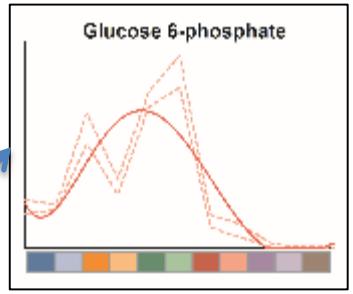
Cohen and Rosemeyer et al., EJB 1969; Erecinska et al., J Neurochem 1995; Rapoport et al, EJB 1977

Pentose Phosphate Pathway: transient activation



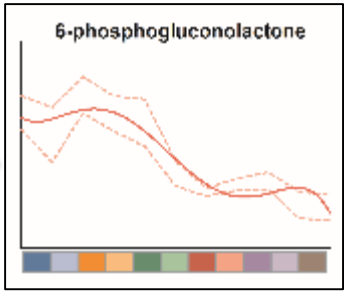
glucose

glucose 6-phosphate

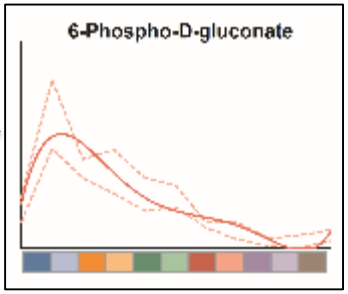


NADPH

6-phosphogluconolactone

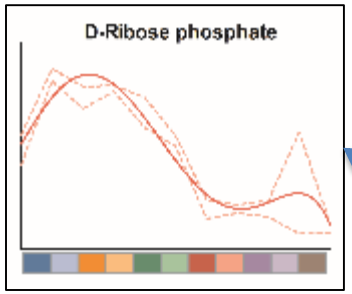


6-phosphogluconate



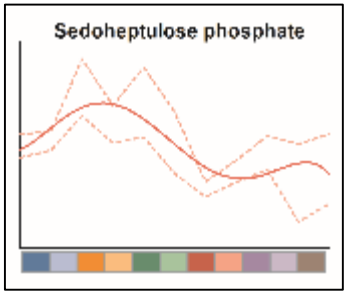
NADPH

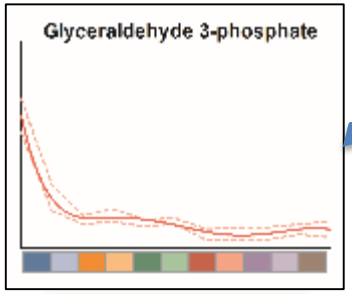
ribulose 5-phosphate



ribose
5-phosphate

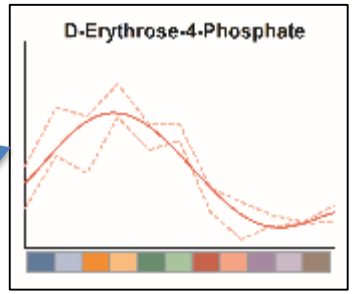
xylulose
5-phosphate





glyceraldehyde
3-phosphate

sedoheptulose
7-phosphate

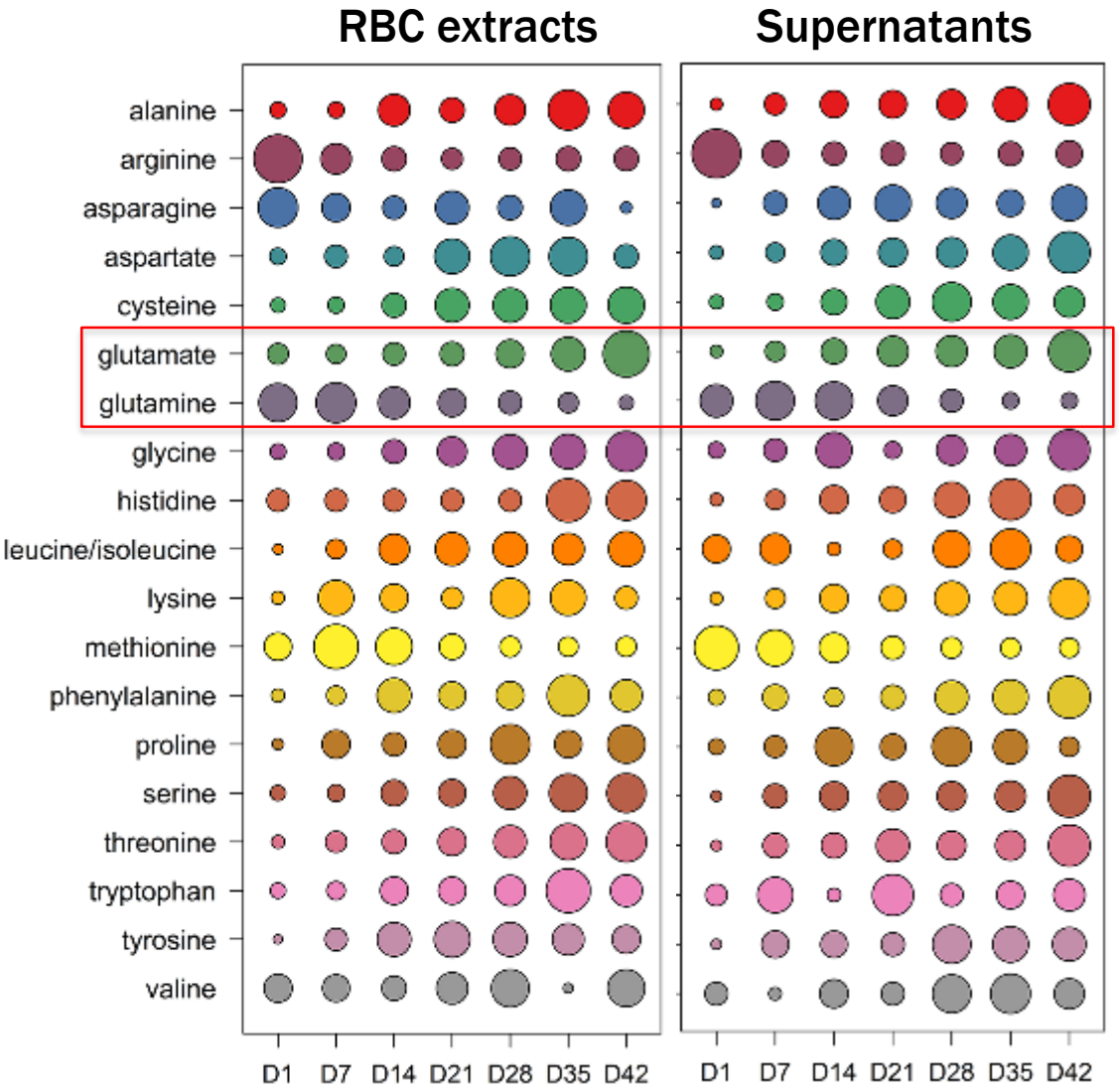


fructose
6-phosphate

erythrose
4-phosphate

glycolysis

Amino Acid Homeostasis is impaired

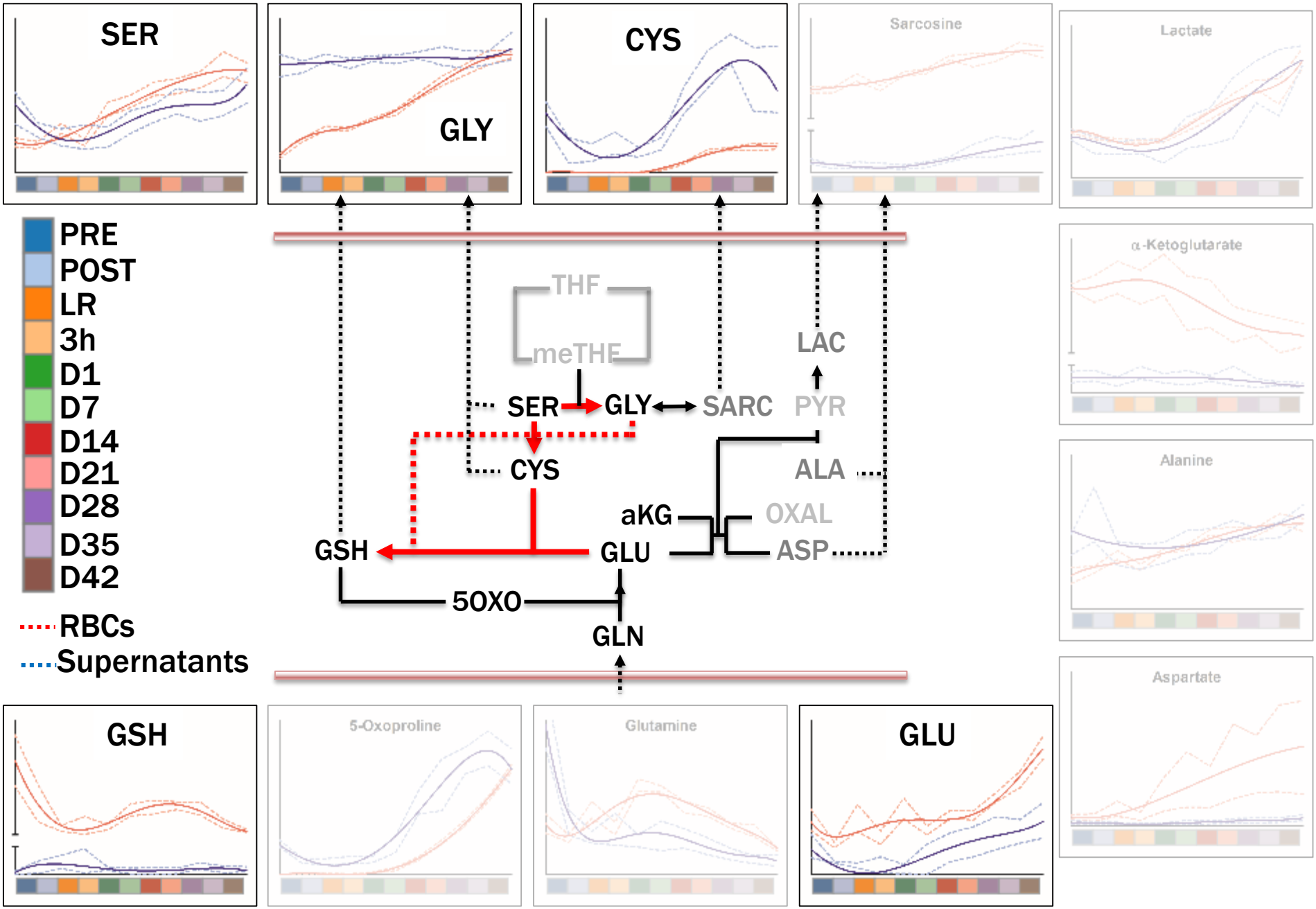


Glutaminase

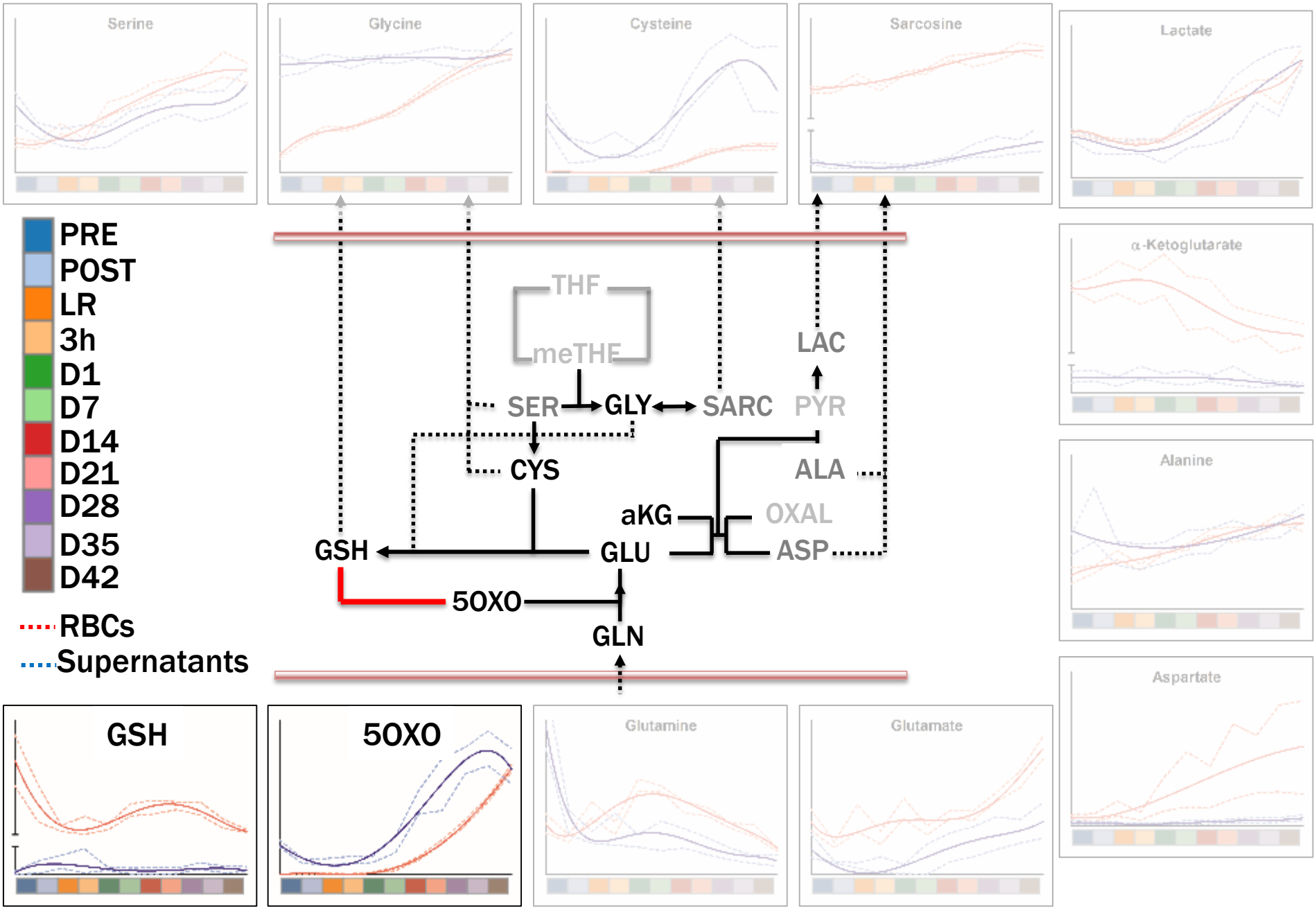
Gln ⟶ Glu

D'Alessandro et al, 2015

Glutathione Homeostasis: synthesis

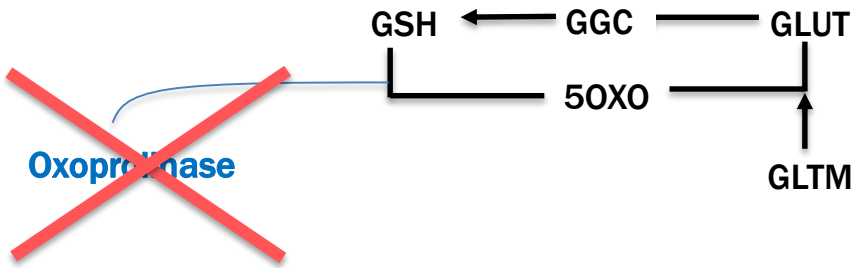


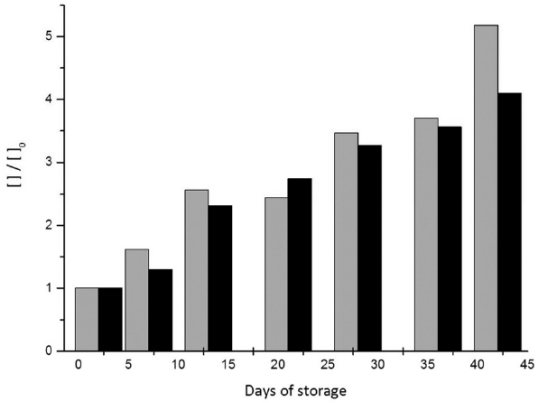
Glutathione Homeostasis: turn-over



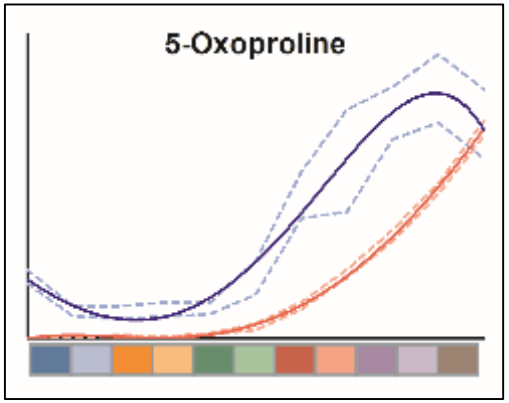
Increased GSH turn-over: in AS3, AS5 and SAGM supernatants

5-Oxoproline, a marker of GSH turnover

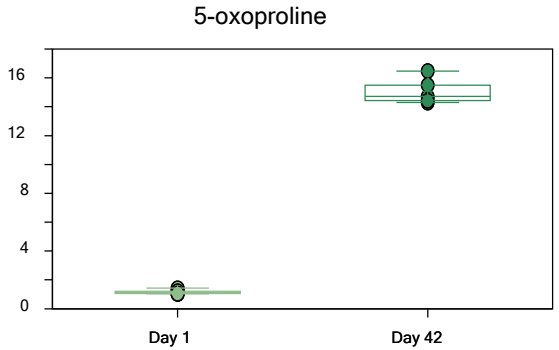




SAGM



AS3

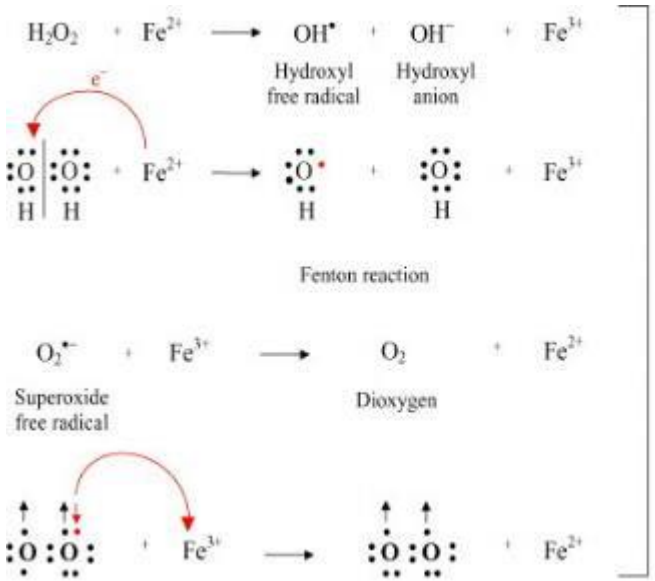


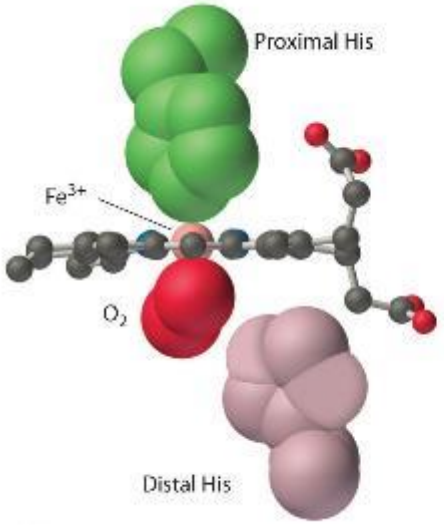
AS5

D'Alessandro et al, Vox Sanguinis and Transfusion 2015; Pertinhez et al, Blood Transfusion 2014

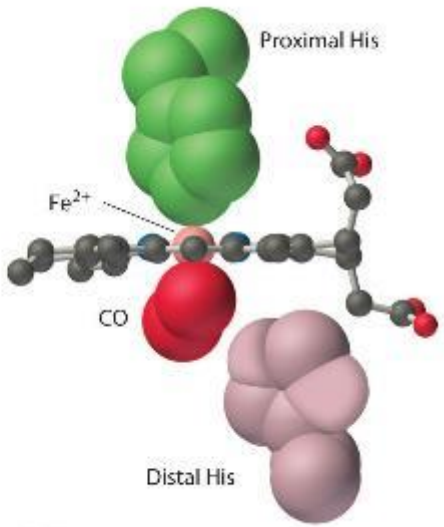
Where does oxidative stress come from?

Oxygen jumps from one Hb molecule to another, a process that promotes formation of oxygen radicals and triggers Haber Weiss and Fenton's reactions





(a) Oxymyoglobin

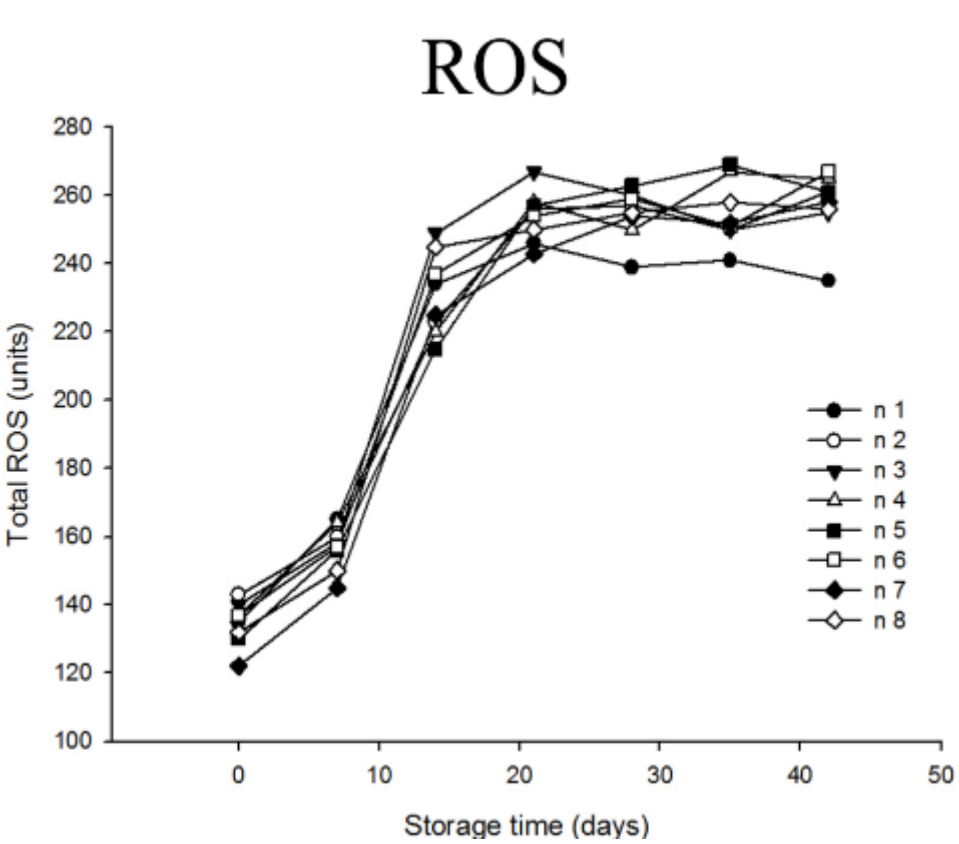


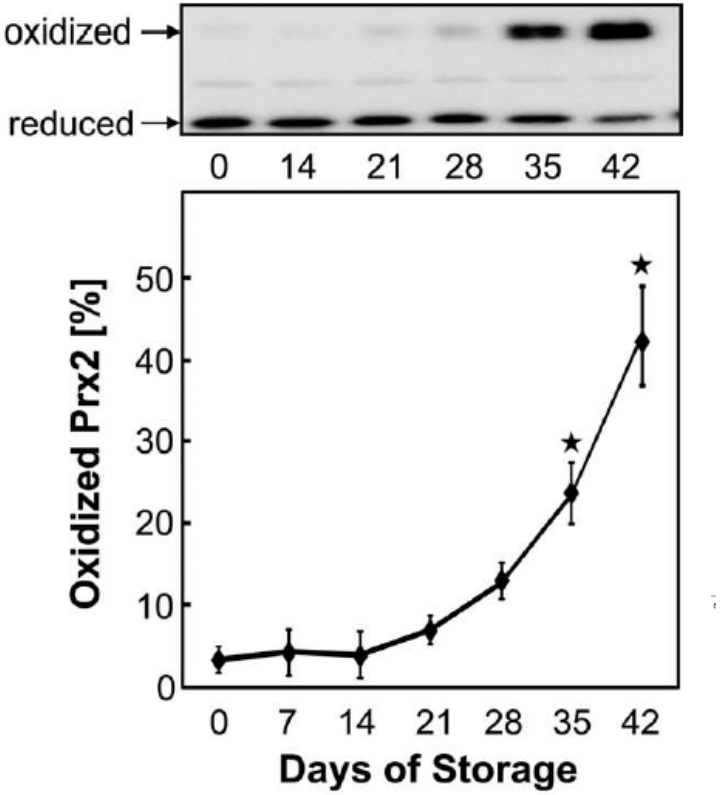
(b) Carbonmonoxymyoglobin

D'Alessandro et al, 2015; Spitalnik and Francis, 2015

Oxygen-induced production of ROS

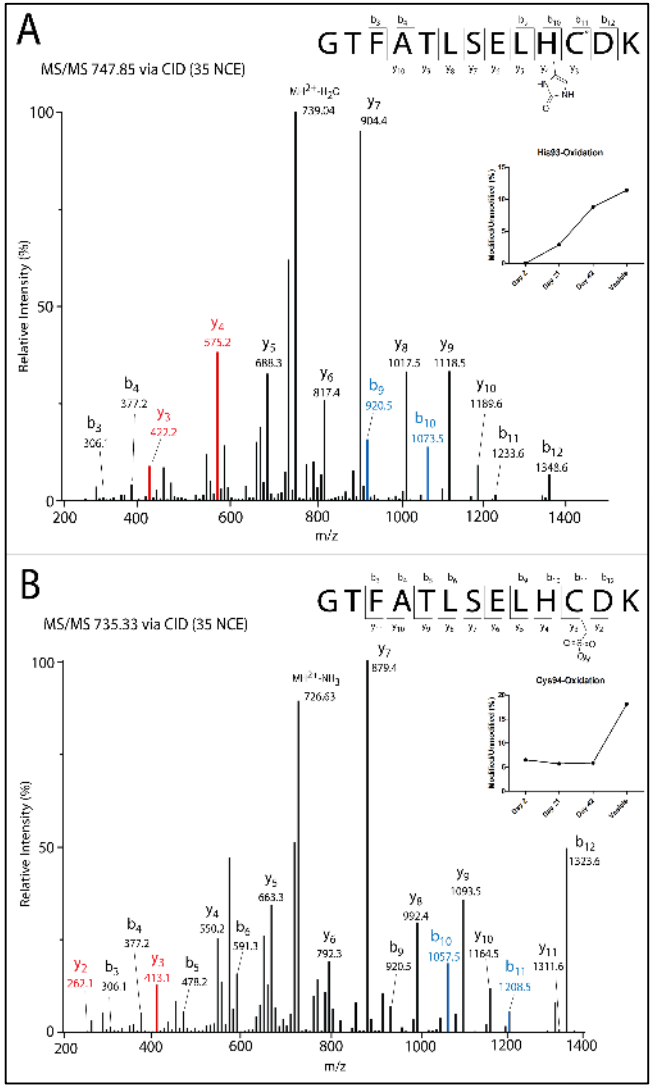
Oxygen jumps from one Hb molecule to another, a process that promotes formation of oxygen radicals and triggers Haber Weiss and Fenton's reactions

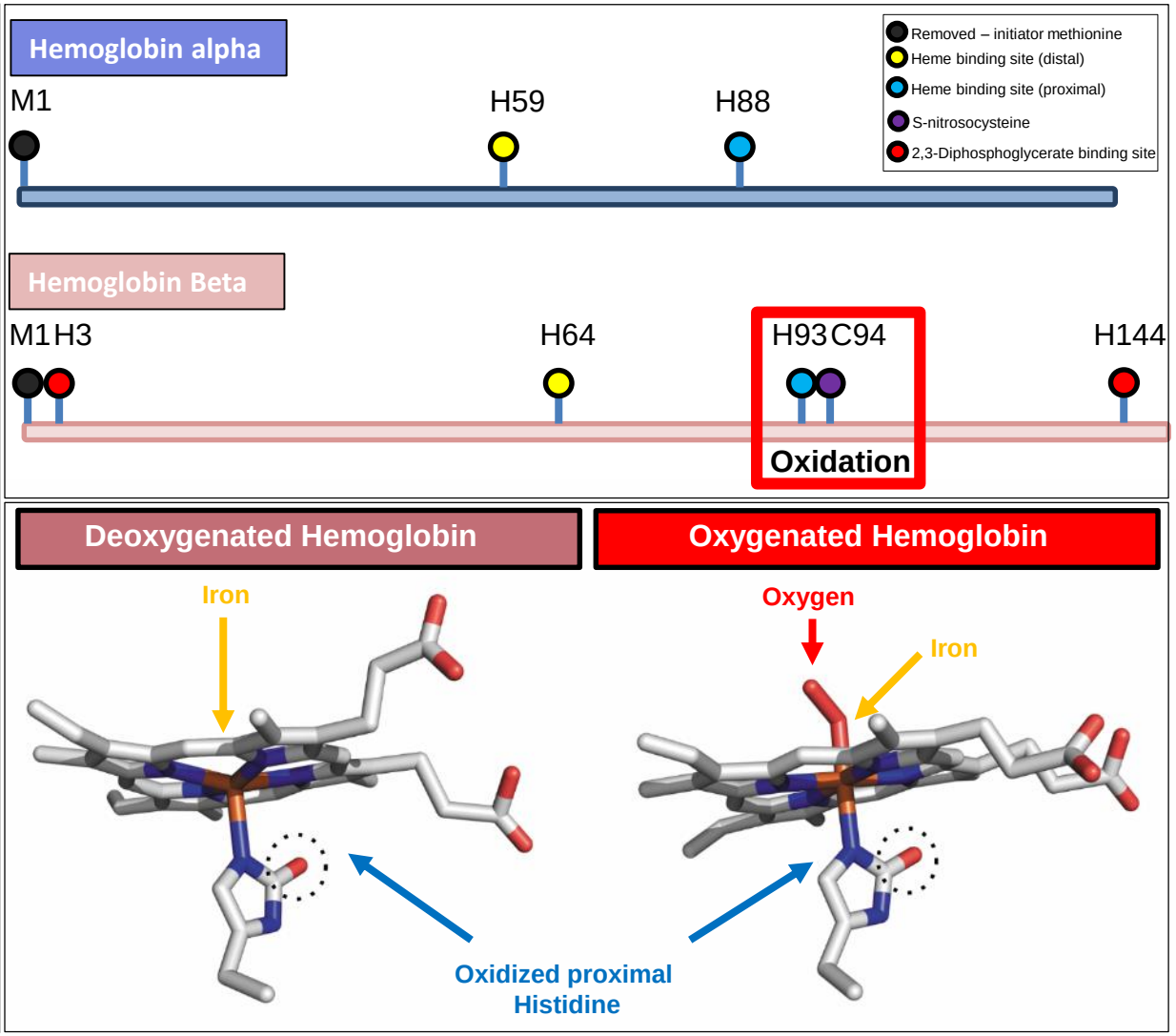




D'Alessandro et al Haematologica 2012

ROS Target the most abundant cytosolic protein…

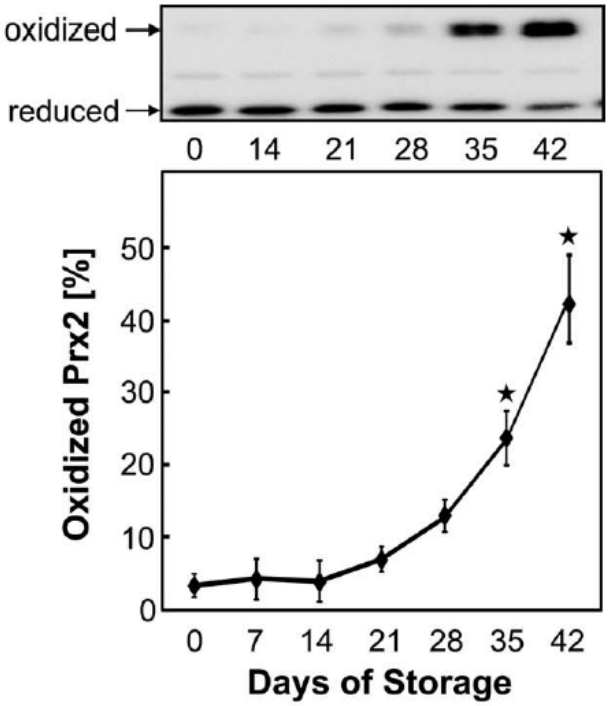


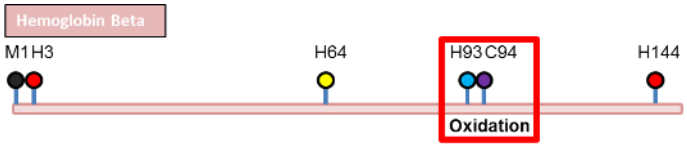


Wither, Dzieciatkowska et al., 2015

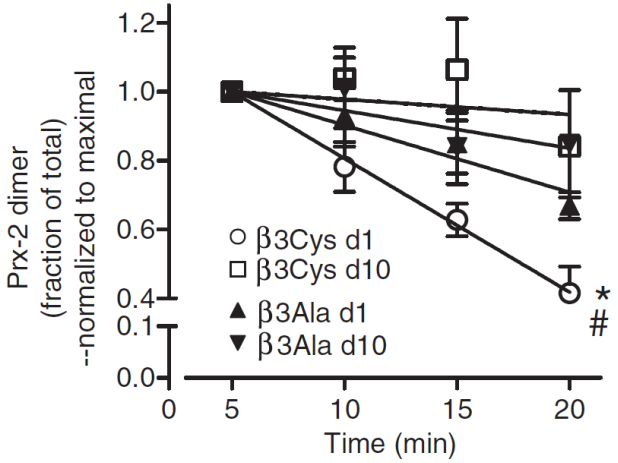
Hb autoxidation keeps in check antioxidant defenses during storage

Storage oxidizes Prdx2





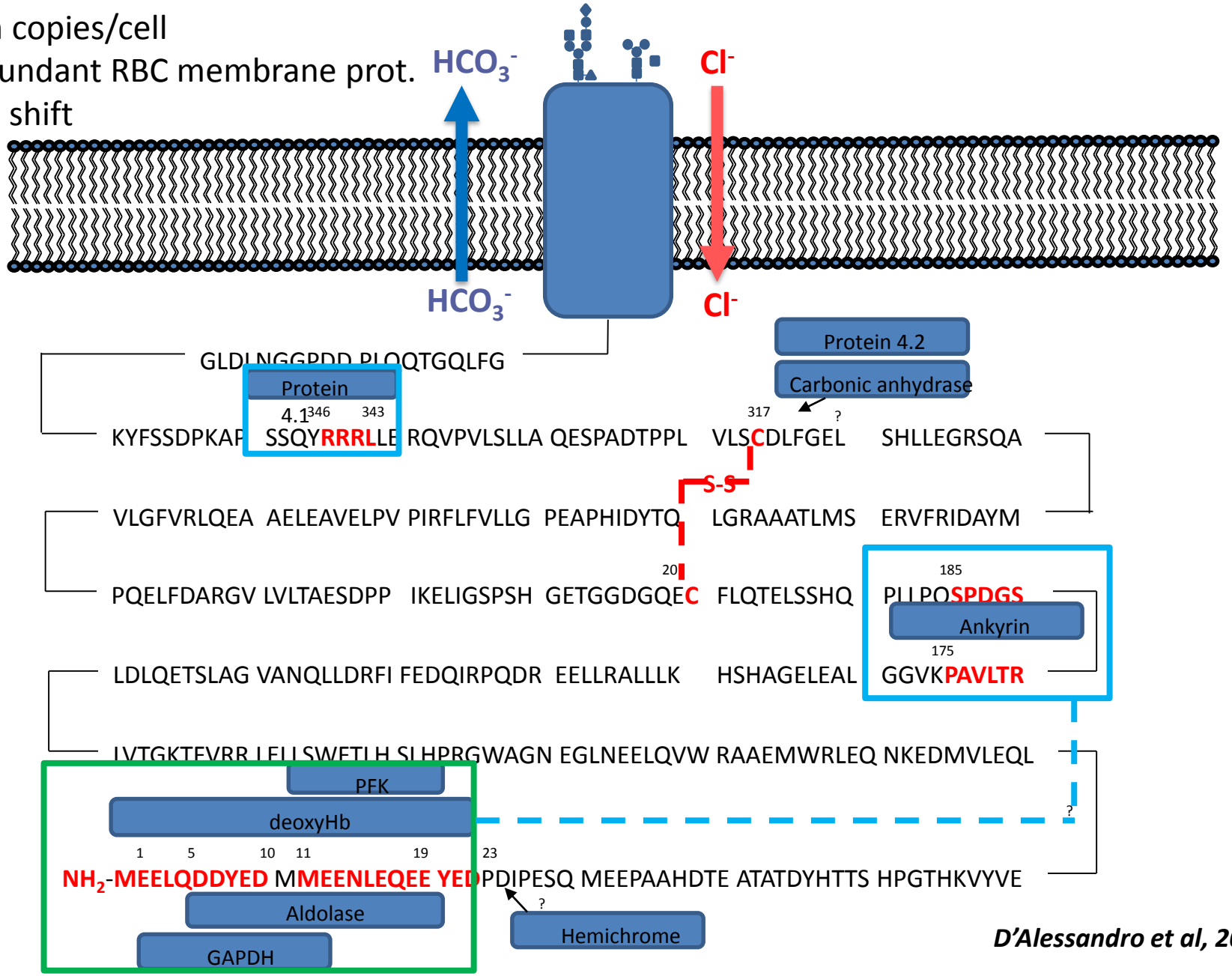
Substitution of Cys β93 → Alanine (mice)
Or Storage-induced oxidation to DHAlanine reduces Prdx2 dimer recycling



Bayer et al. Transfusion 2015; Harper et al. Antiox Redox Signal 2015

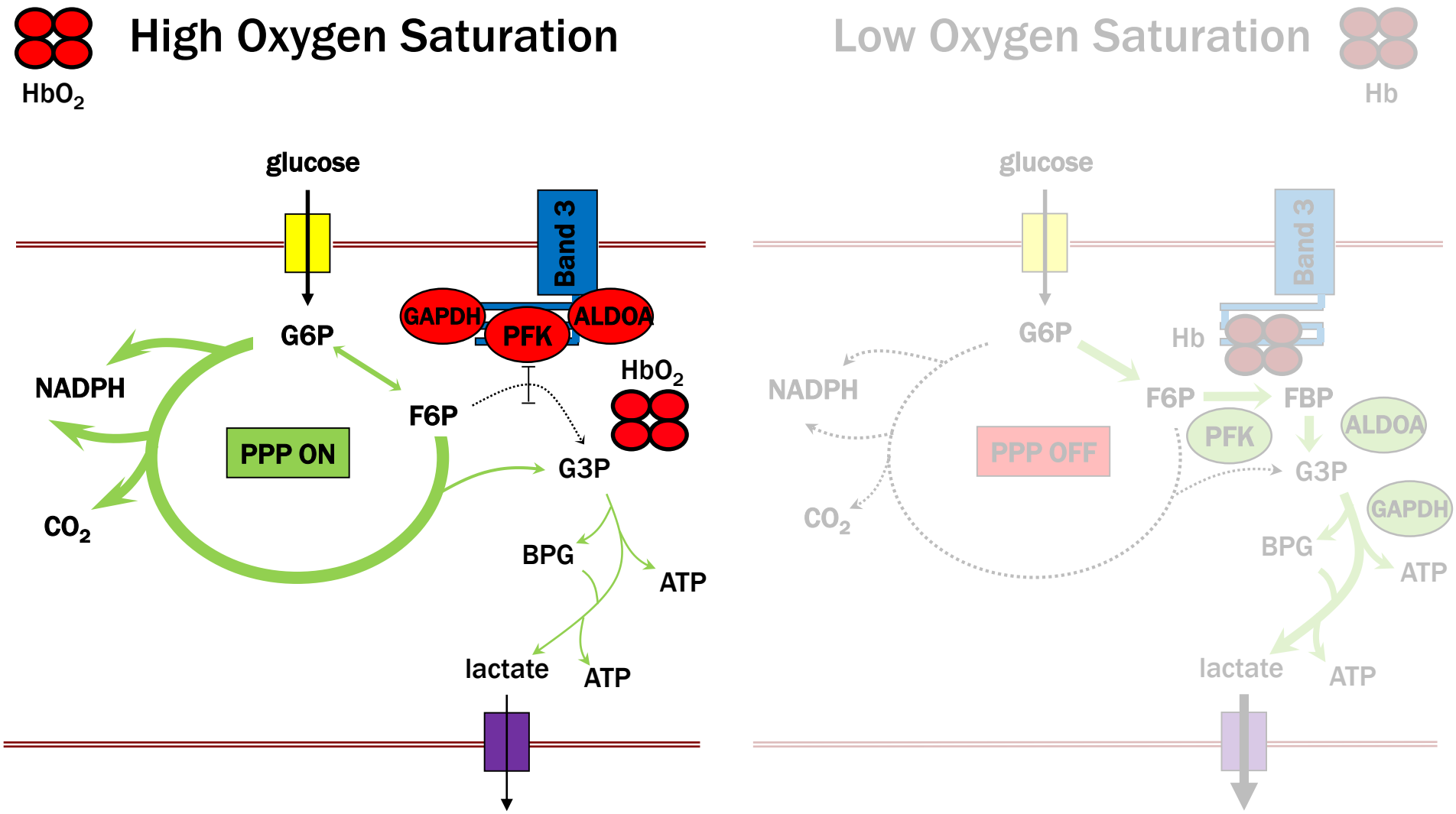
...and membrane protein: Band 3

1 million copies/cell
Most abundant RBC membrane prot.
Chloride shift



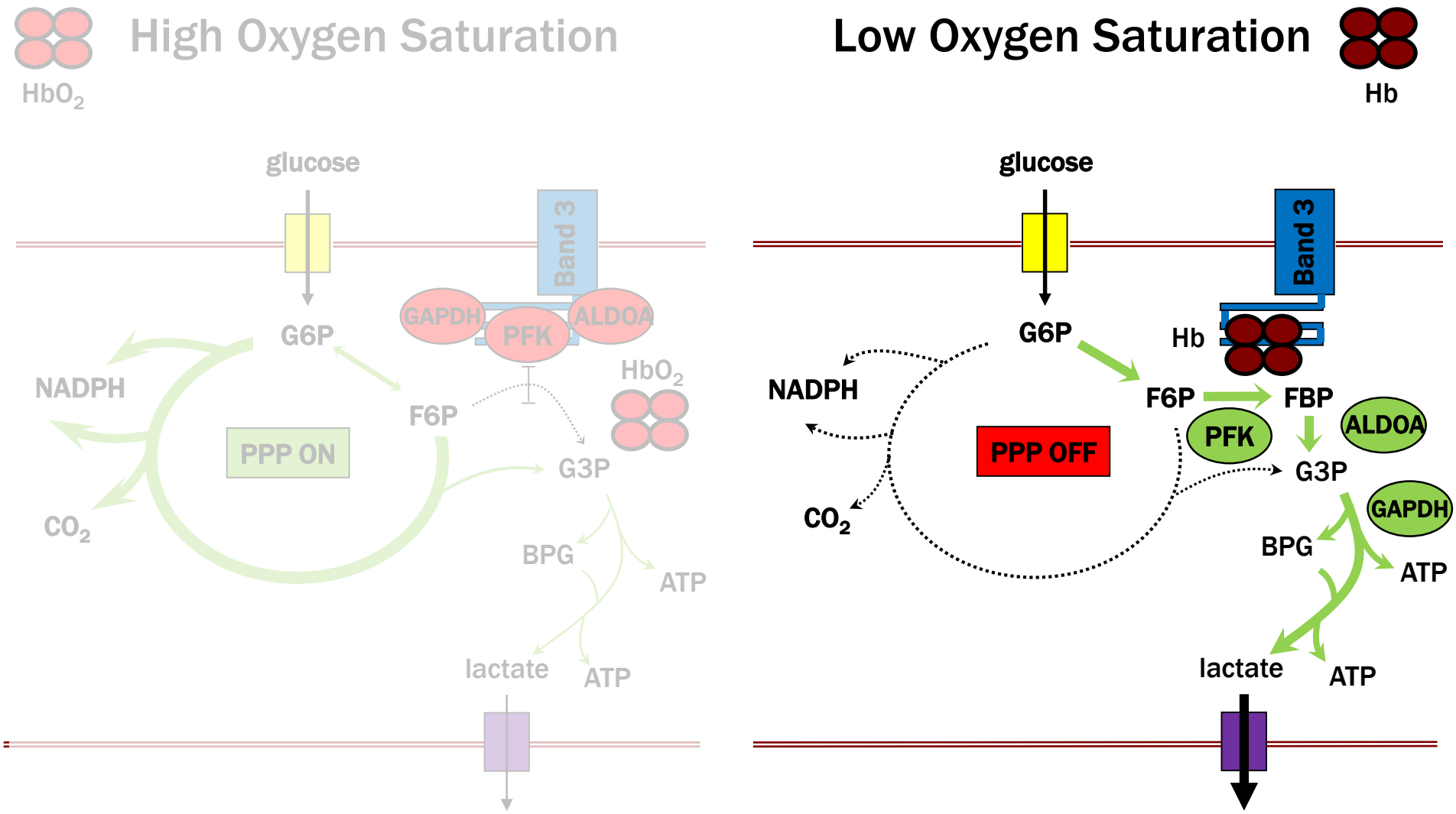
D'Alessandro et al, 2015

Anaerobic Storage of RBCs



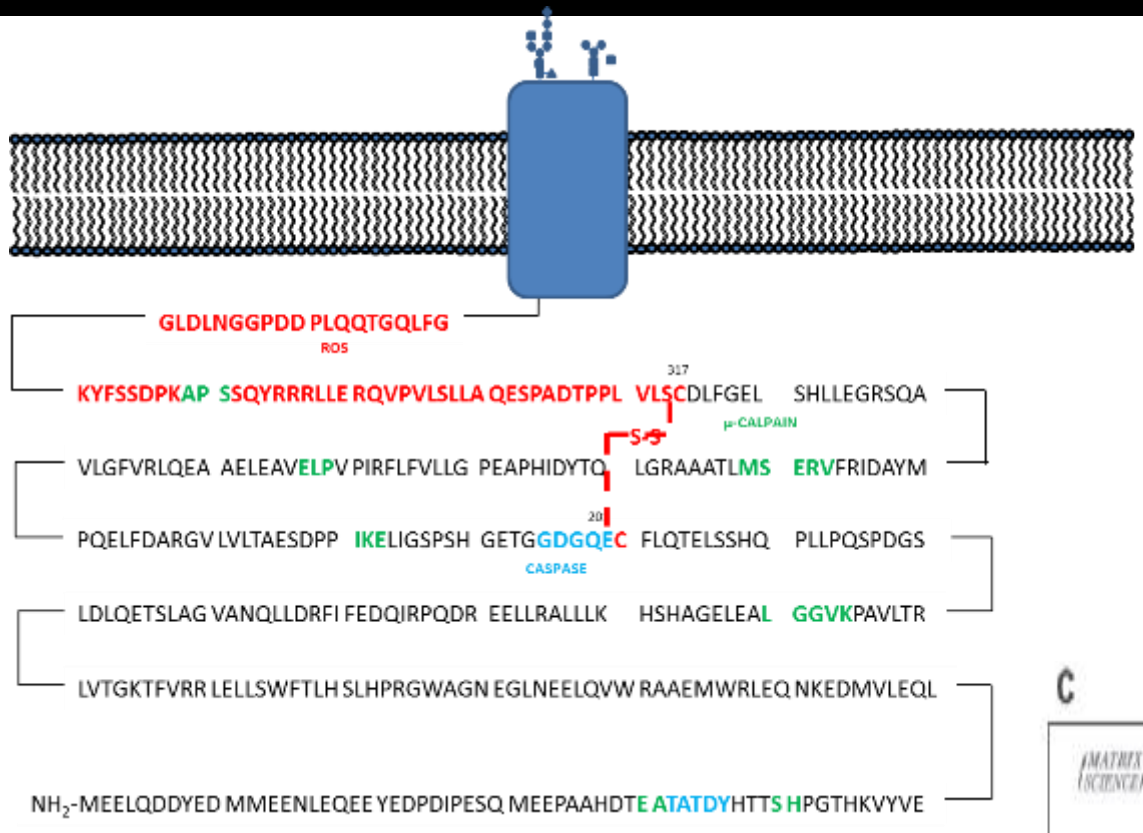
Adapted from Castagnola et al., 2010

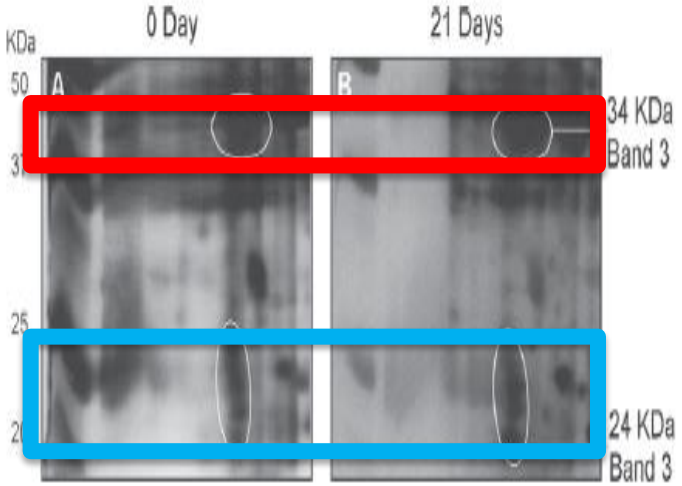
Anaerobic Storage of RBCs

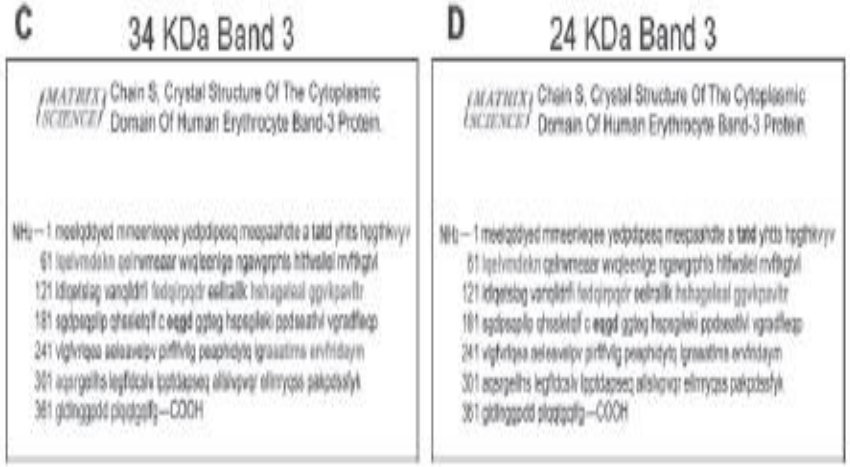


Adapted from Castagnola et al, 2010

Normal storage results in band 3 fragmentation

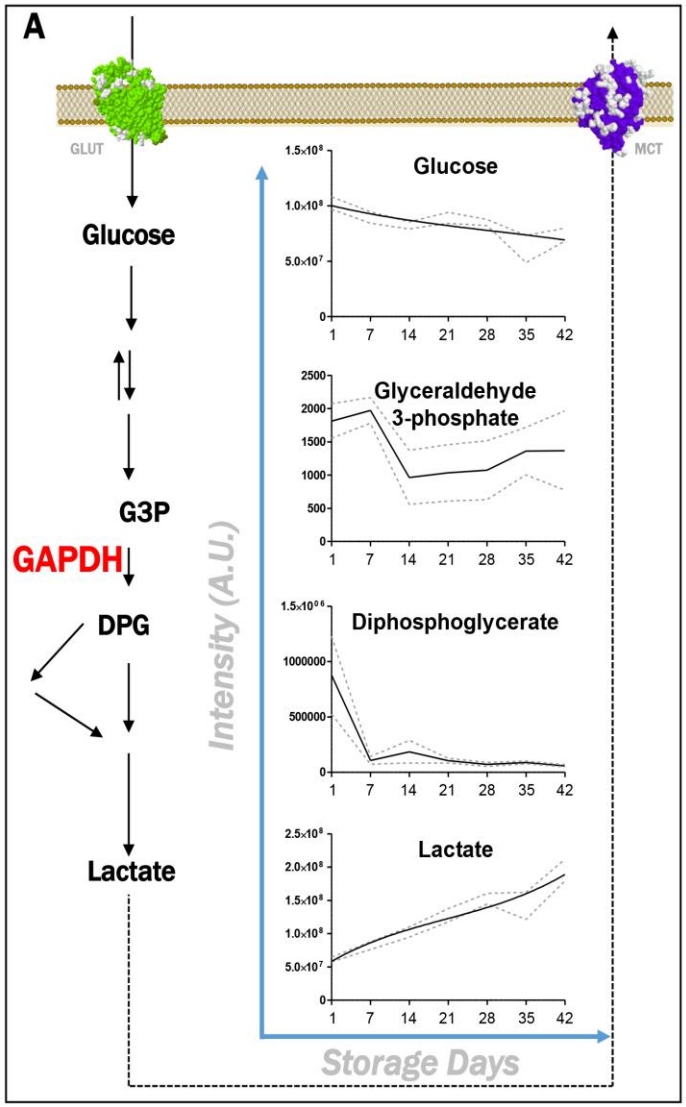


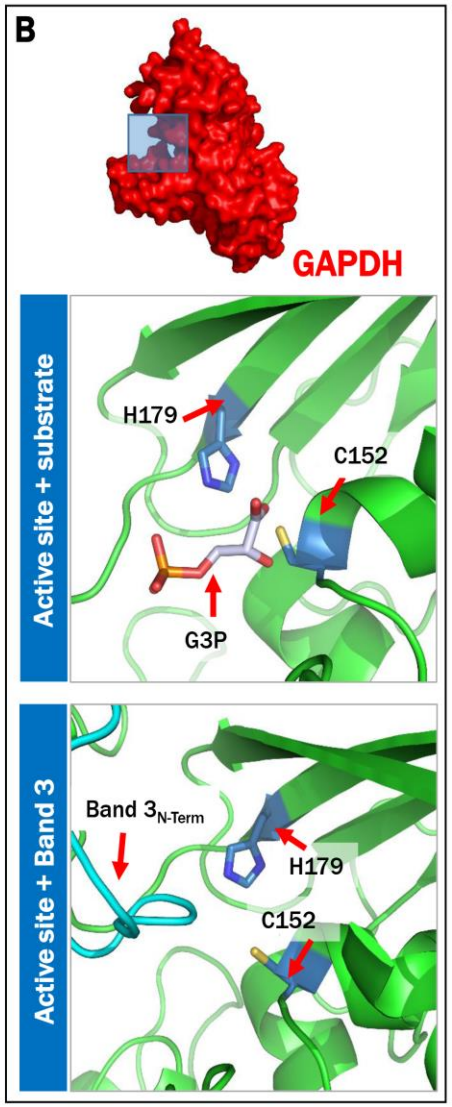


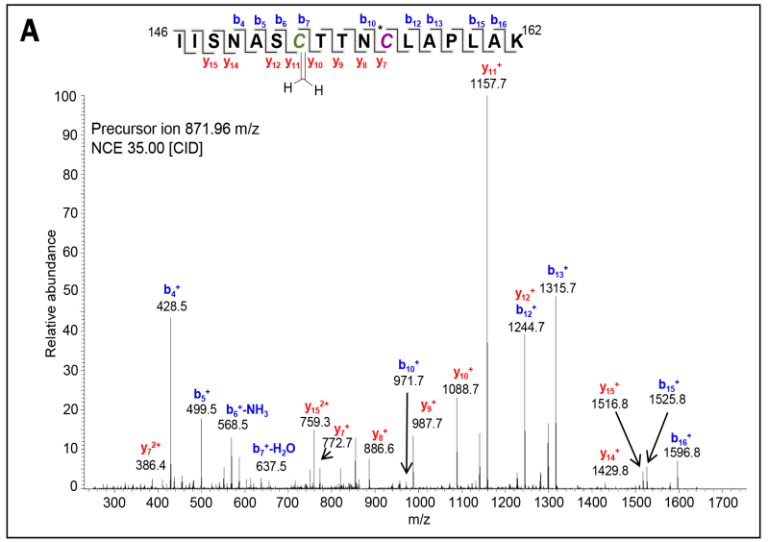


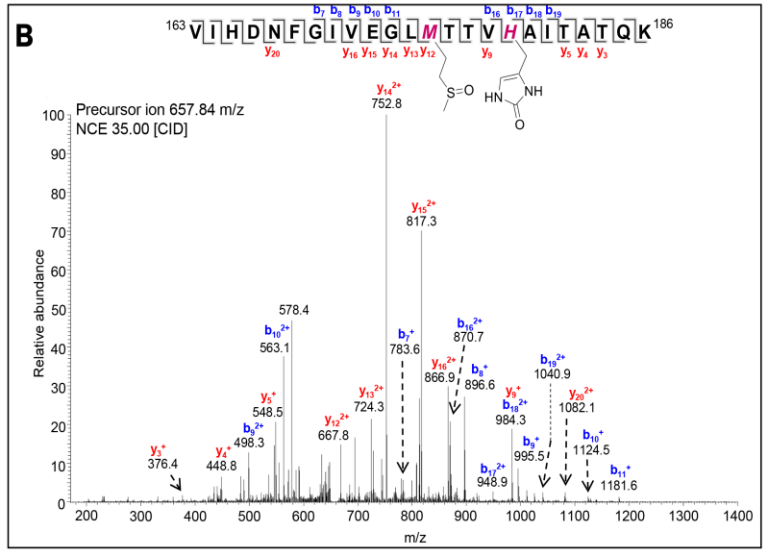
D'Alessandro et al. 2012; Rinalducci et al, 2012

...and GAPDH oxidation, affecting activity and potentially band 3 binding



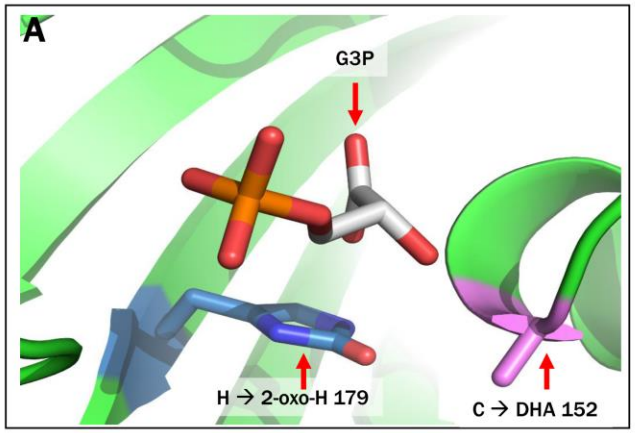


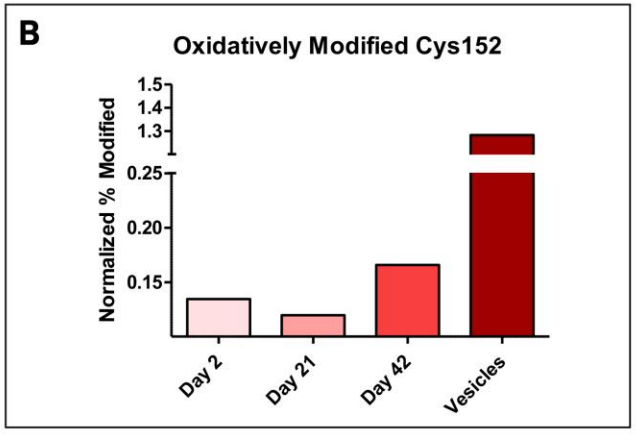


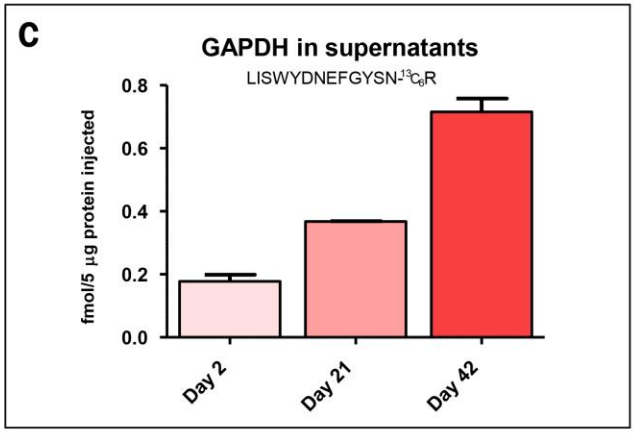


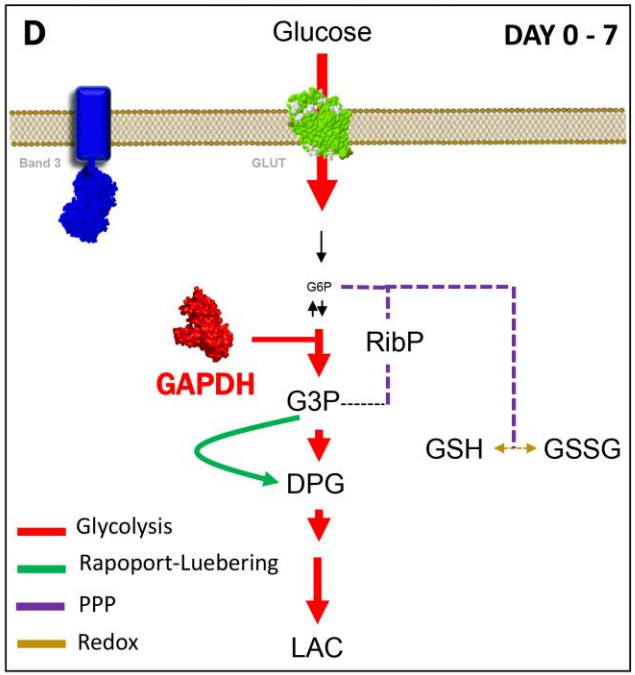
Haines et al, submitted

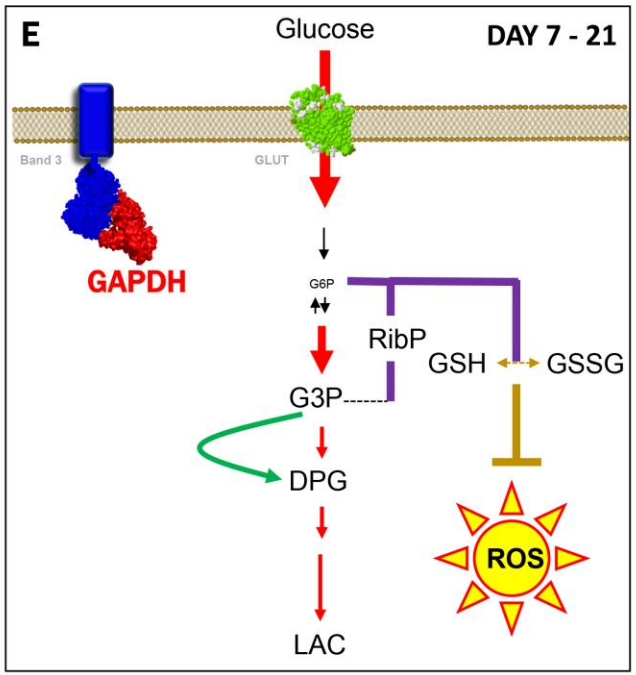
...and GAPDH oxidation, affecting activity and potentially band 3 binding

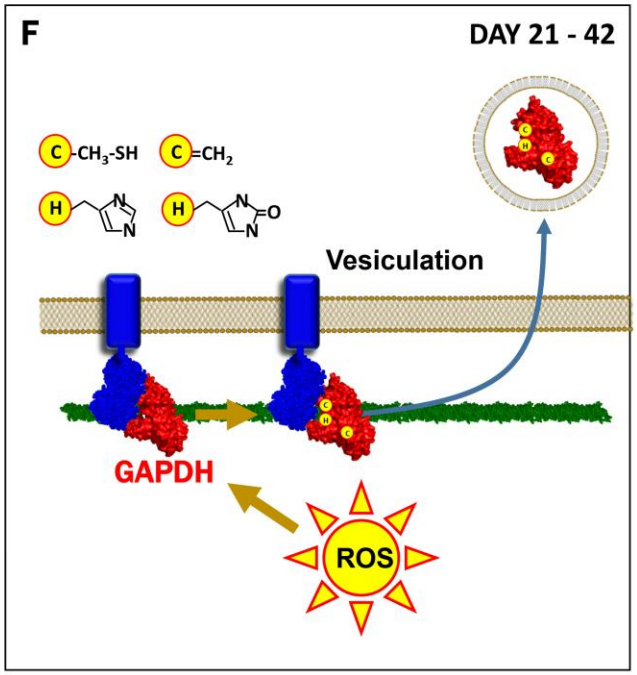






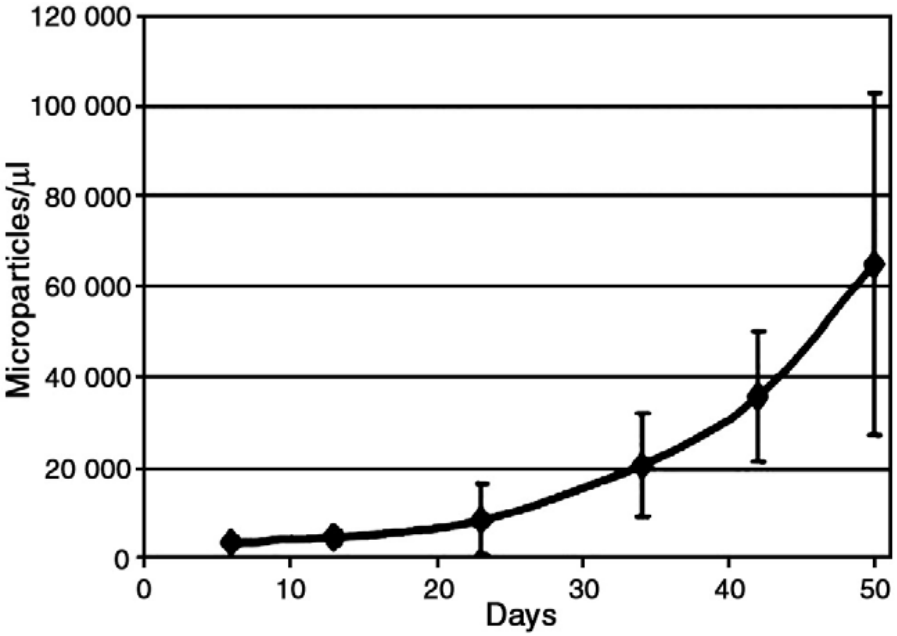


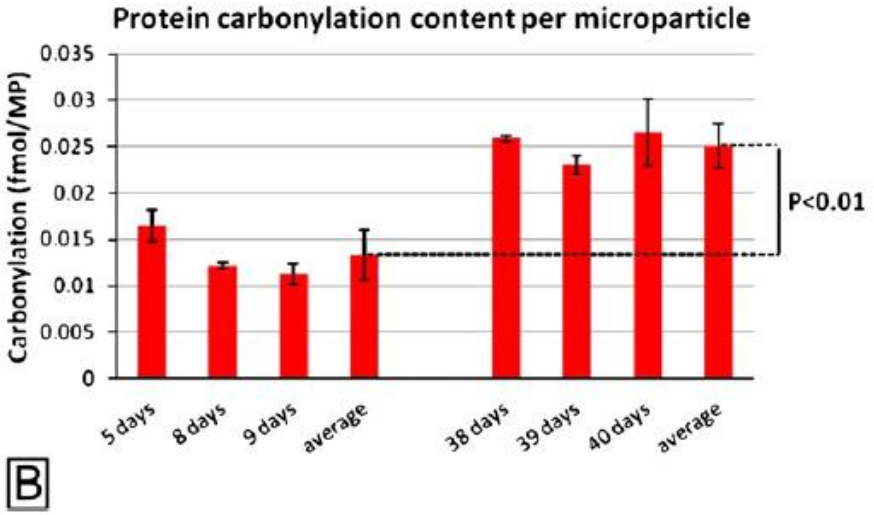




Haines et al, submitted

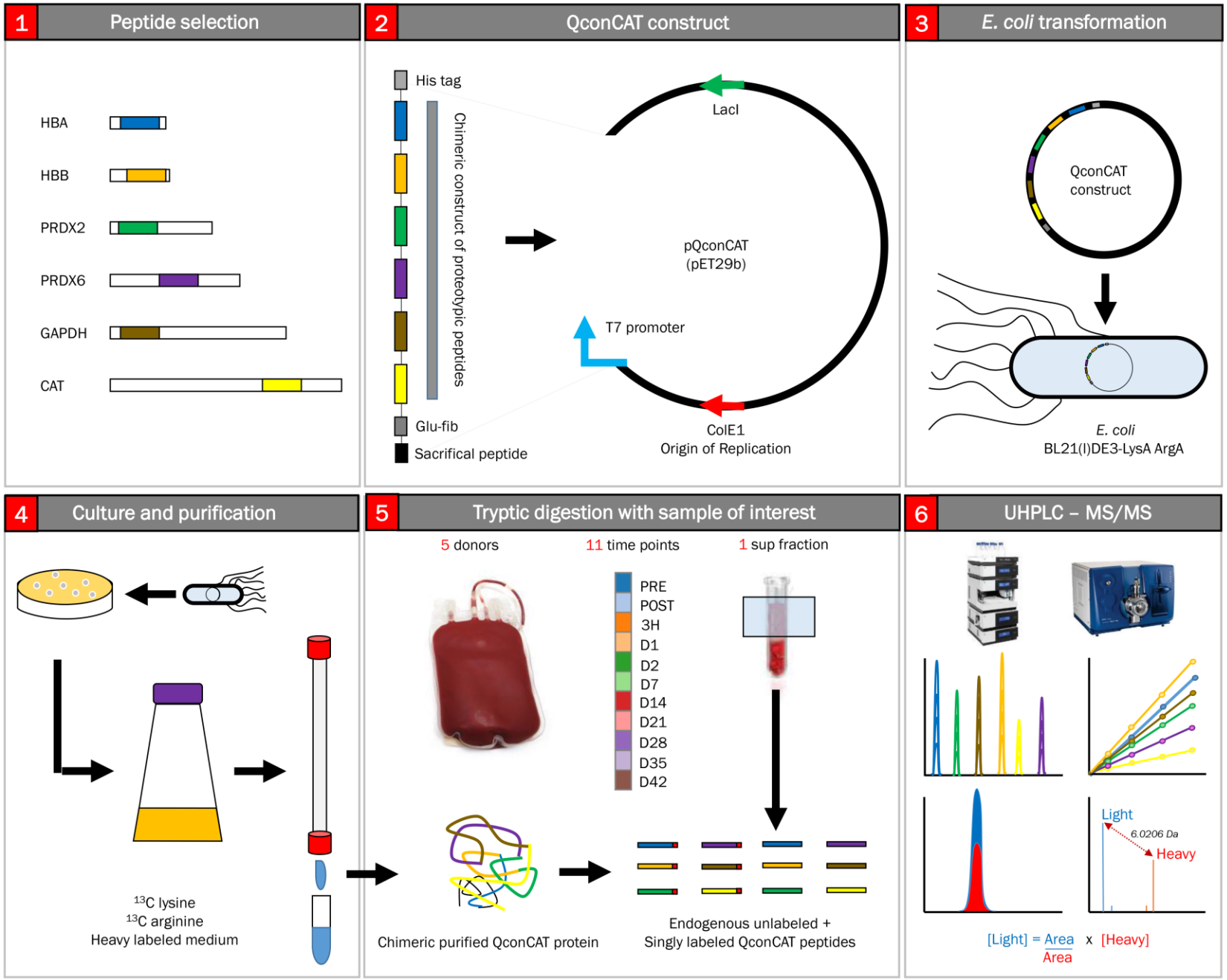
RBC vesiculation increases with storage to get rid of oxidized proteins



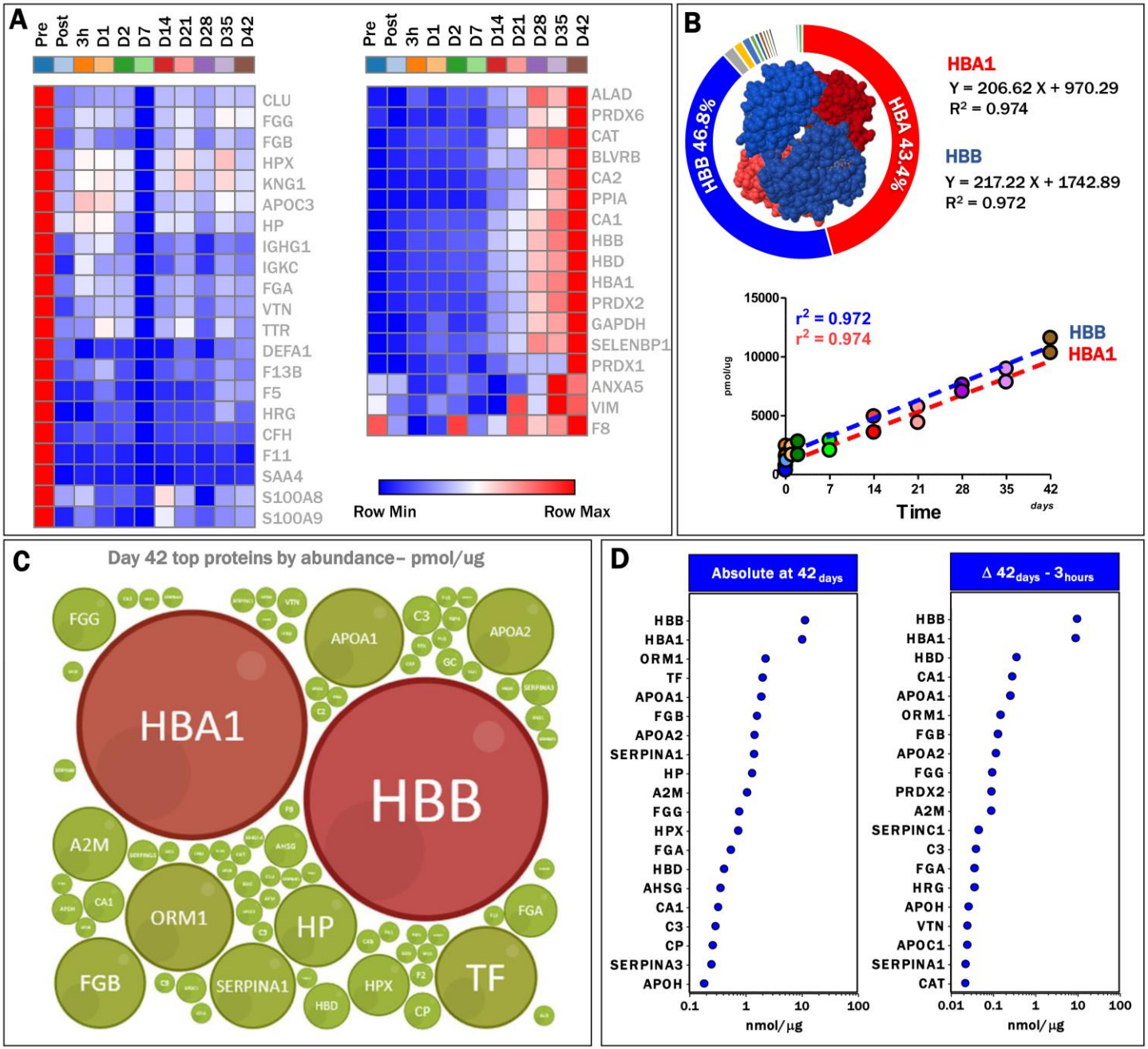


Delobel et al, 2012; Kriebardis et al, 2010

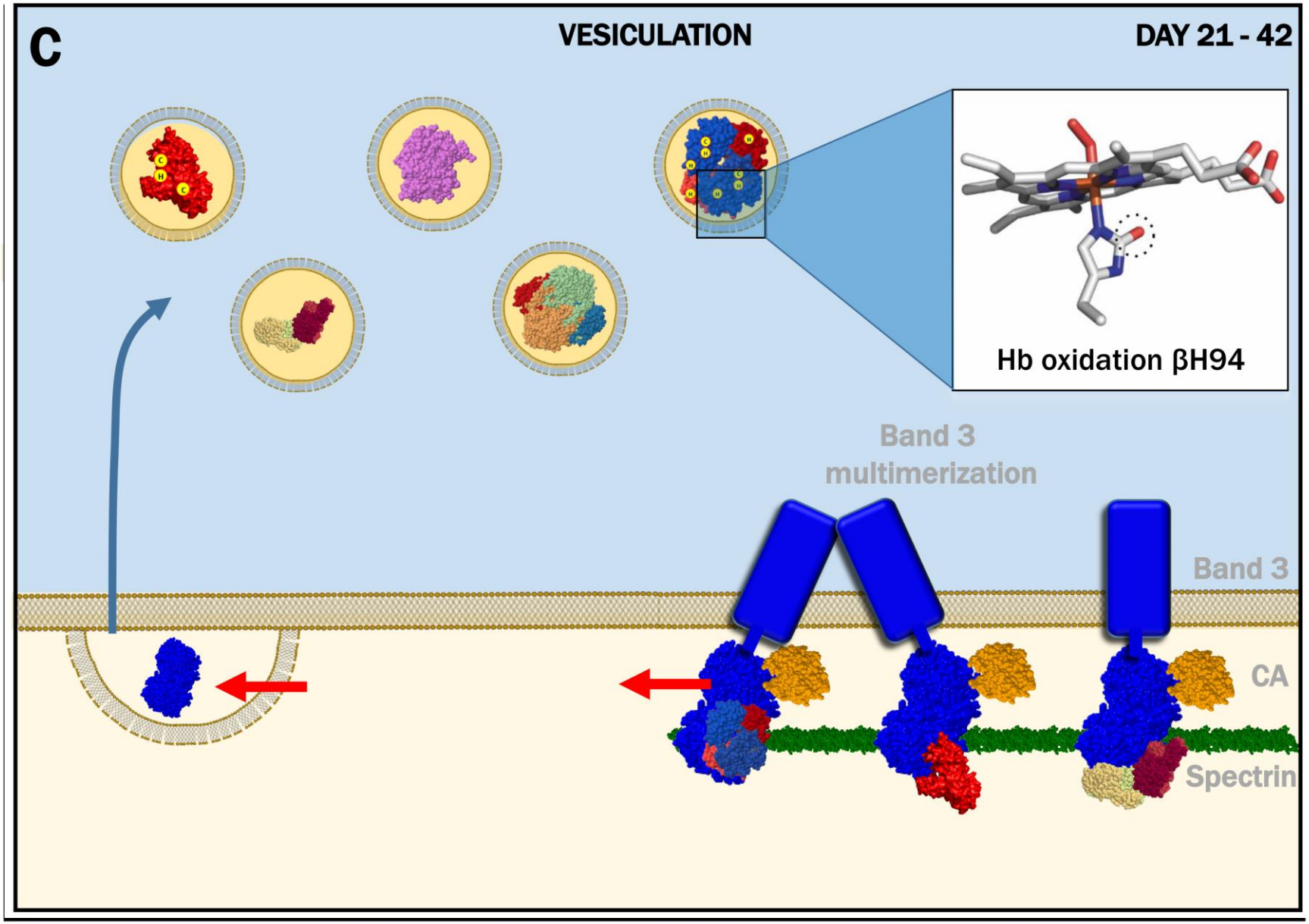
Can we quantify the proteome?



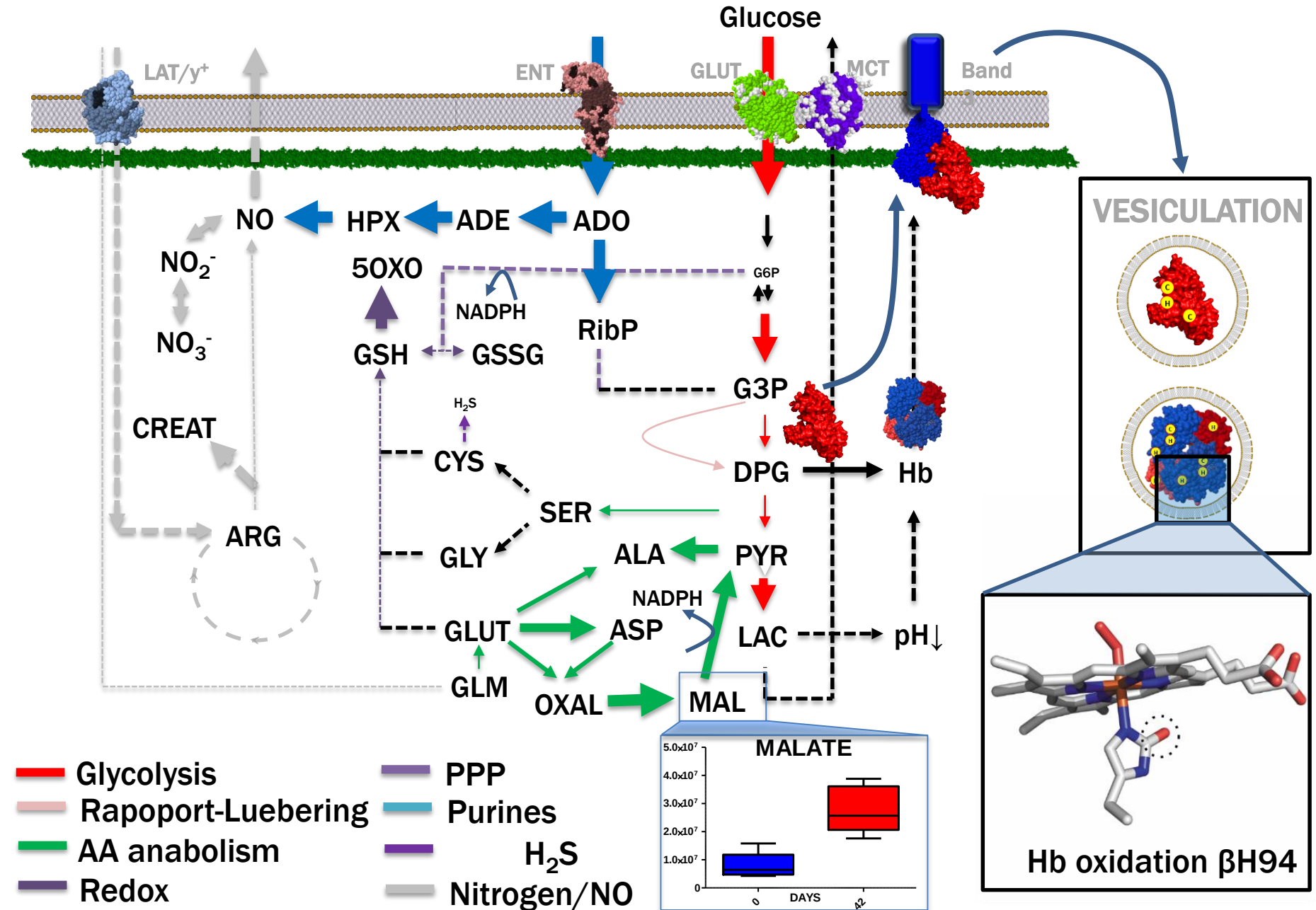
Can we quantify the proteome?



Protein markers in RBC sups correlate with storage duration



Summary: Metabolic adaptation during storage

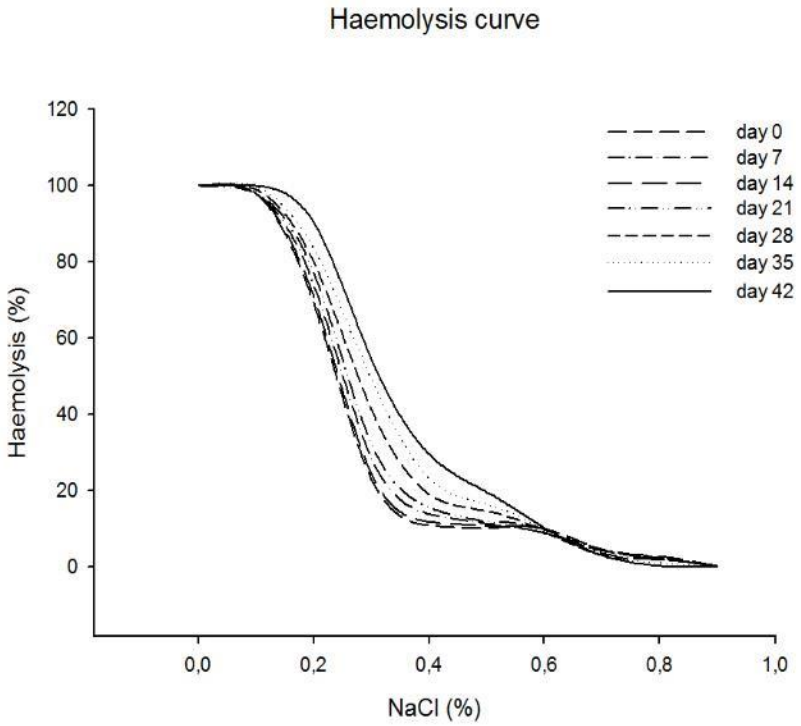


Vesiculation ends up affecting morphology, surface to volume ratios and ultimately osmotic fragility

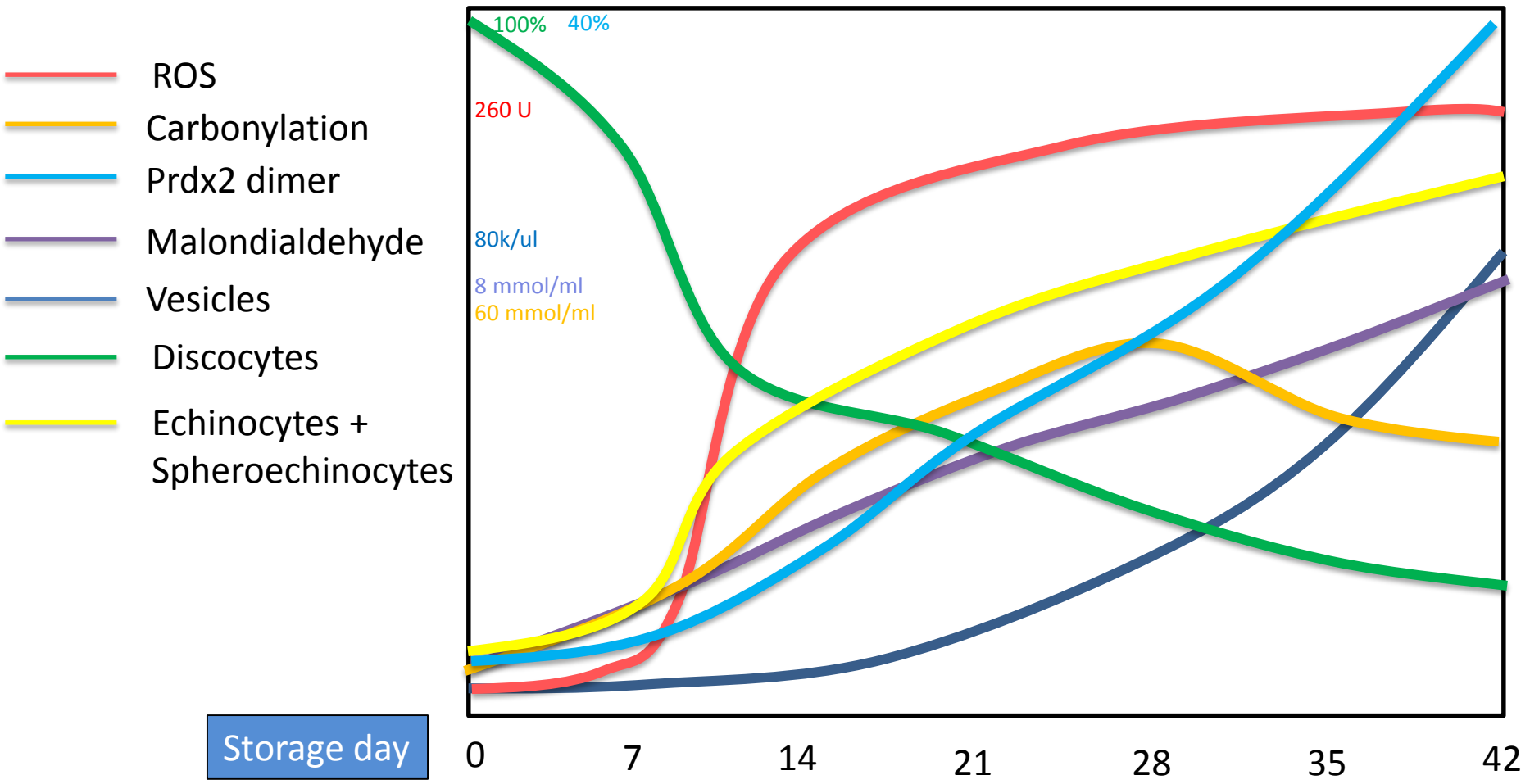
Table 2. SEM erythrocyte shape classification.

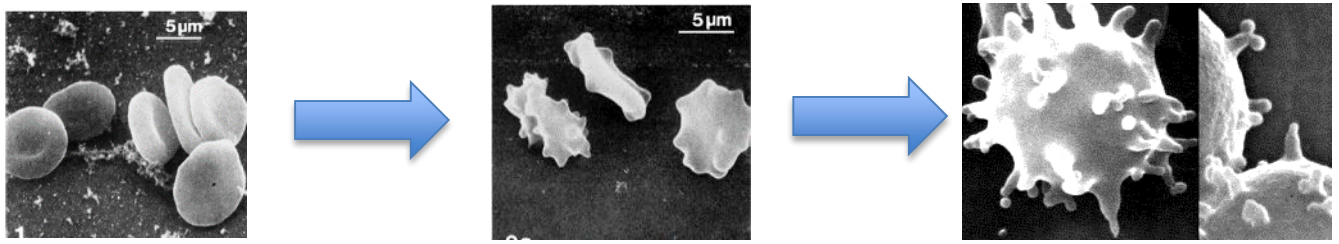
Day	Discocyte (%)	Reversibly* changed RBC (%) (echinocyte and stomatocyte shape)	Irreversibly* changed RBC (%) (spheroechinocyte, spherostomatocyte, spherocyte, ovalocyte, and degenerated shapes)
7	75.3±4.1	15.5±1.9	9.2±3.5
14	55.8±2.7	29.1±2.4	15.1±0.9
21	51.0±4.0	32.6±2.6	16.4±1.4
28	45.6±3.3	35.6±1.7	18.8±1.6
35	35.2±1.9	42.3±2.2	22.5±3.1
42	23.7±2.5	45.3±3.8	31.0 2.9

**Reversible and irreversible changes were classified based on classification by Berezina et al.[23] However, Bessis'[22] shape classification details are provided as well (bold).*



Summary: Oxidation of RBCs promotes storage lesions

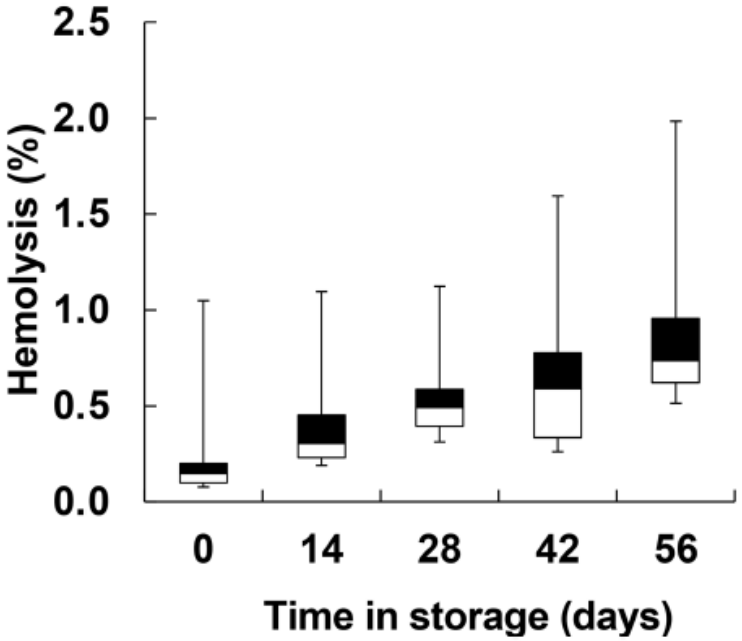


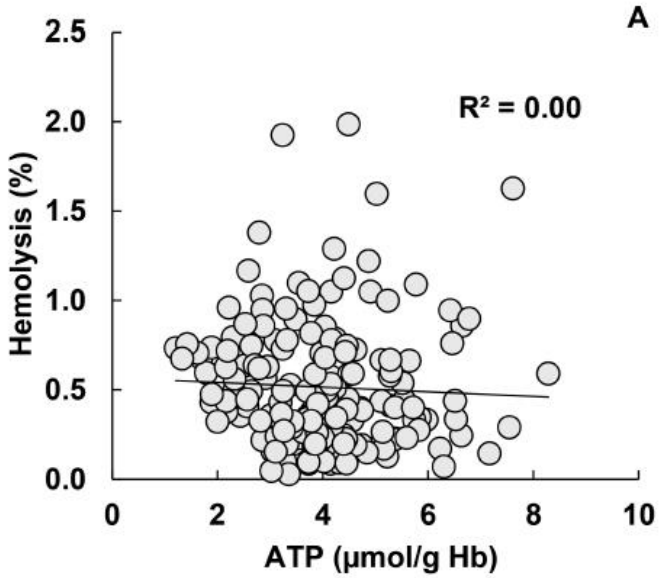


D'Alessandro et al. Transfusion 2015

2. How do we know if it works?

Correlative analysis with golden standards of transfusion medicine

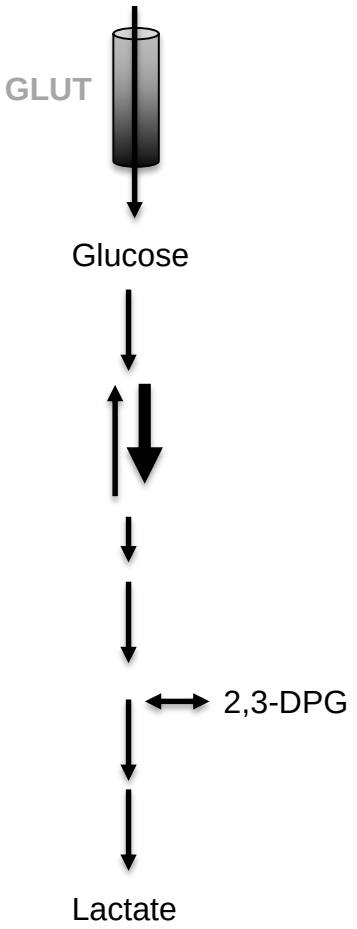


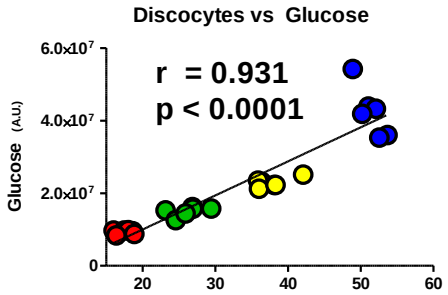


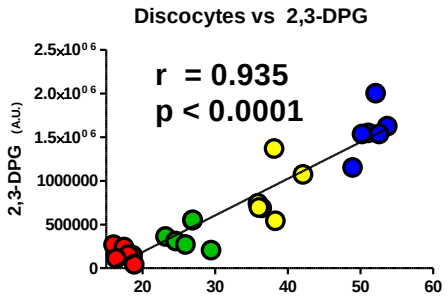
Metabolite	R value*
G6P	−0.41‡
2-Oleoylglycero phosphocholine	0.36‡
ADP	0.51‡

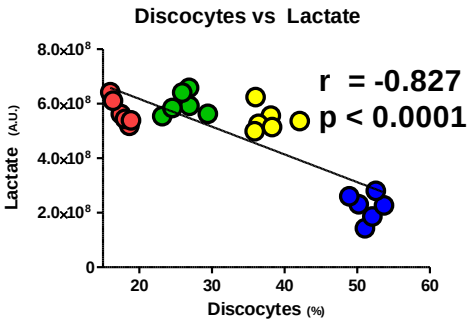
Van 'T Erve et al., 2014/2015

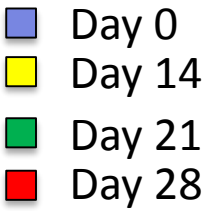
Energy metabolism indeed correlates to morphology







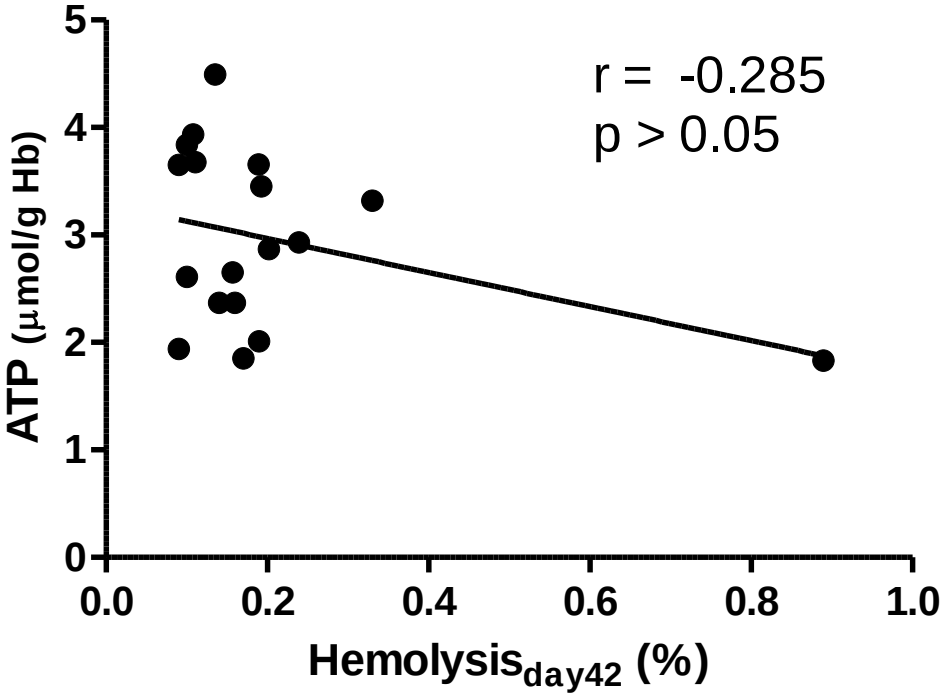


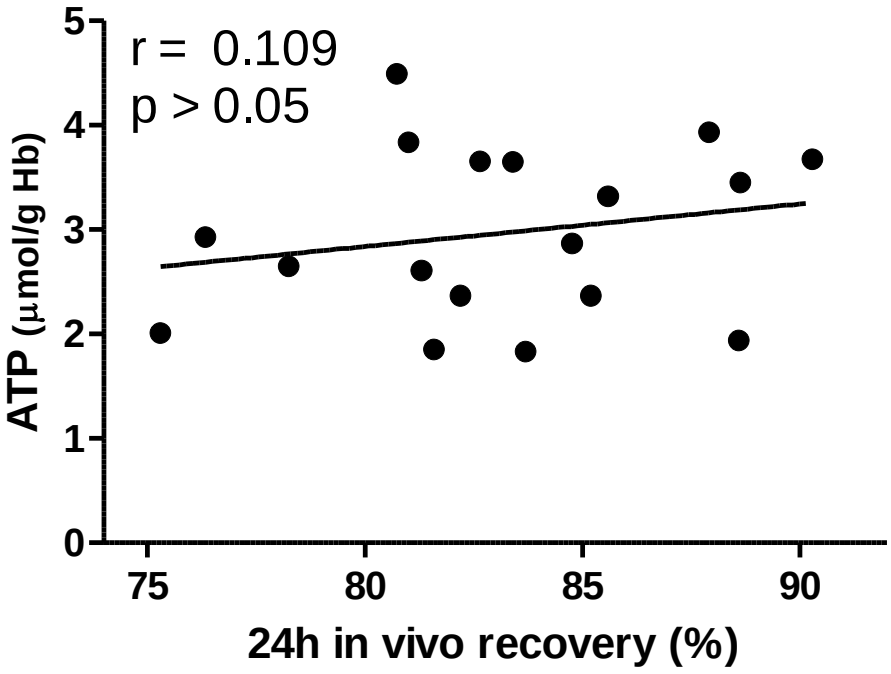


D'Alessandro et al, in preparation

ATP poorly correlates with hemolysis and 24h in vivo recovery

Humans

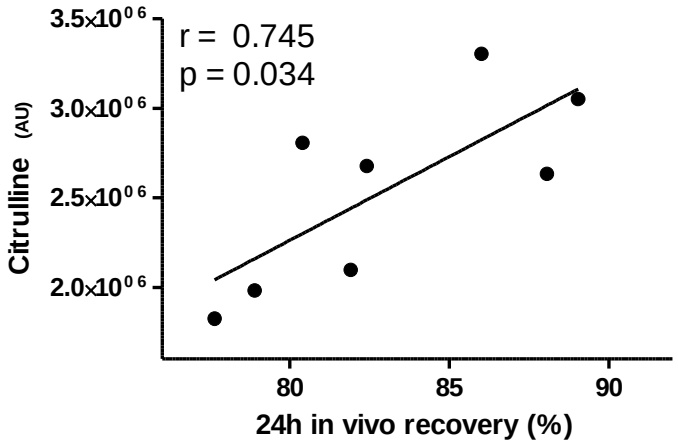


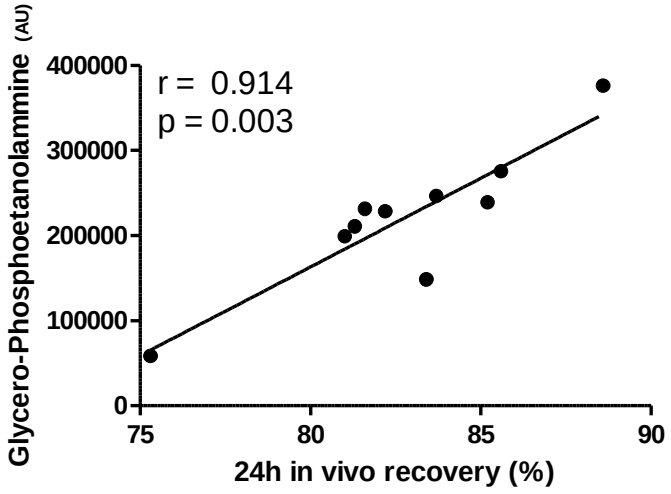


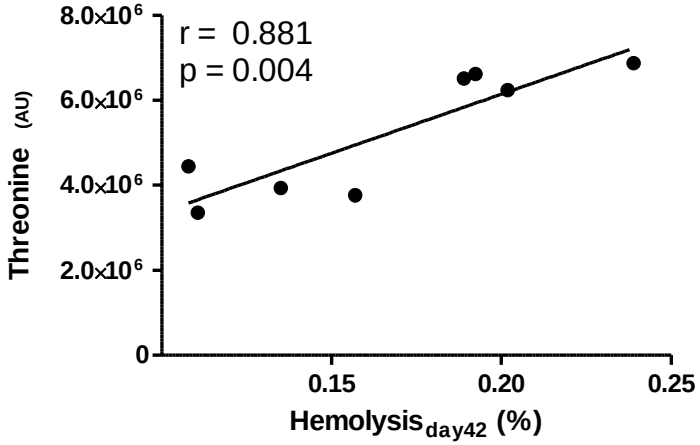
Van 't Erve et al. (Hess JR), 2014 D'Alessandro/Dumont et al. In preparation

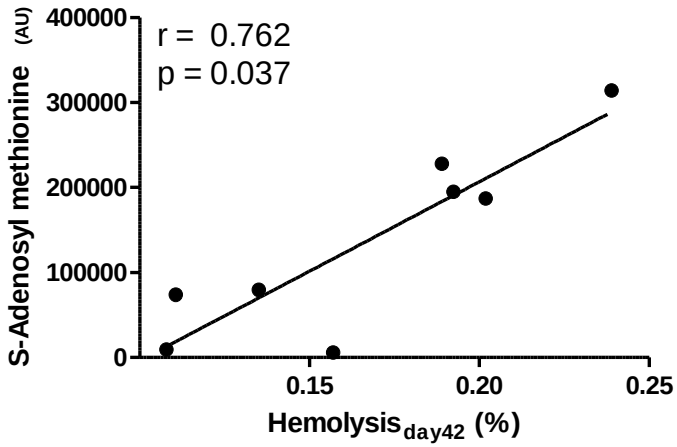
Correlative analysis with 24h in vivo survival and hemolysis

Humans



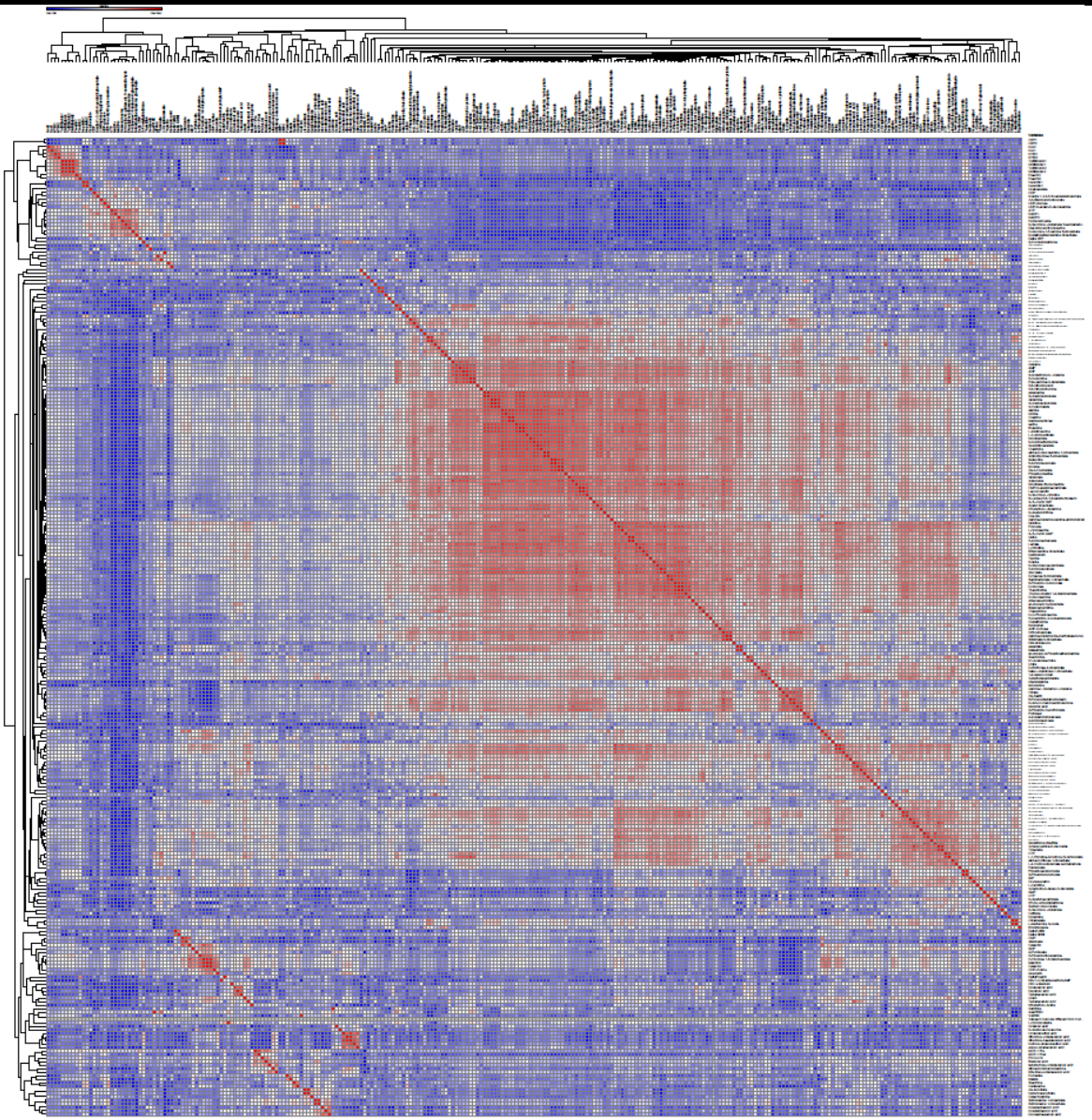






D'Alessandro/Dumont et al. In preparation

Metabolite/Metabolite correlations might reveal unexplored connections of RBC metabolic pathways

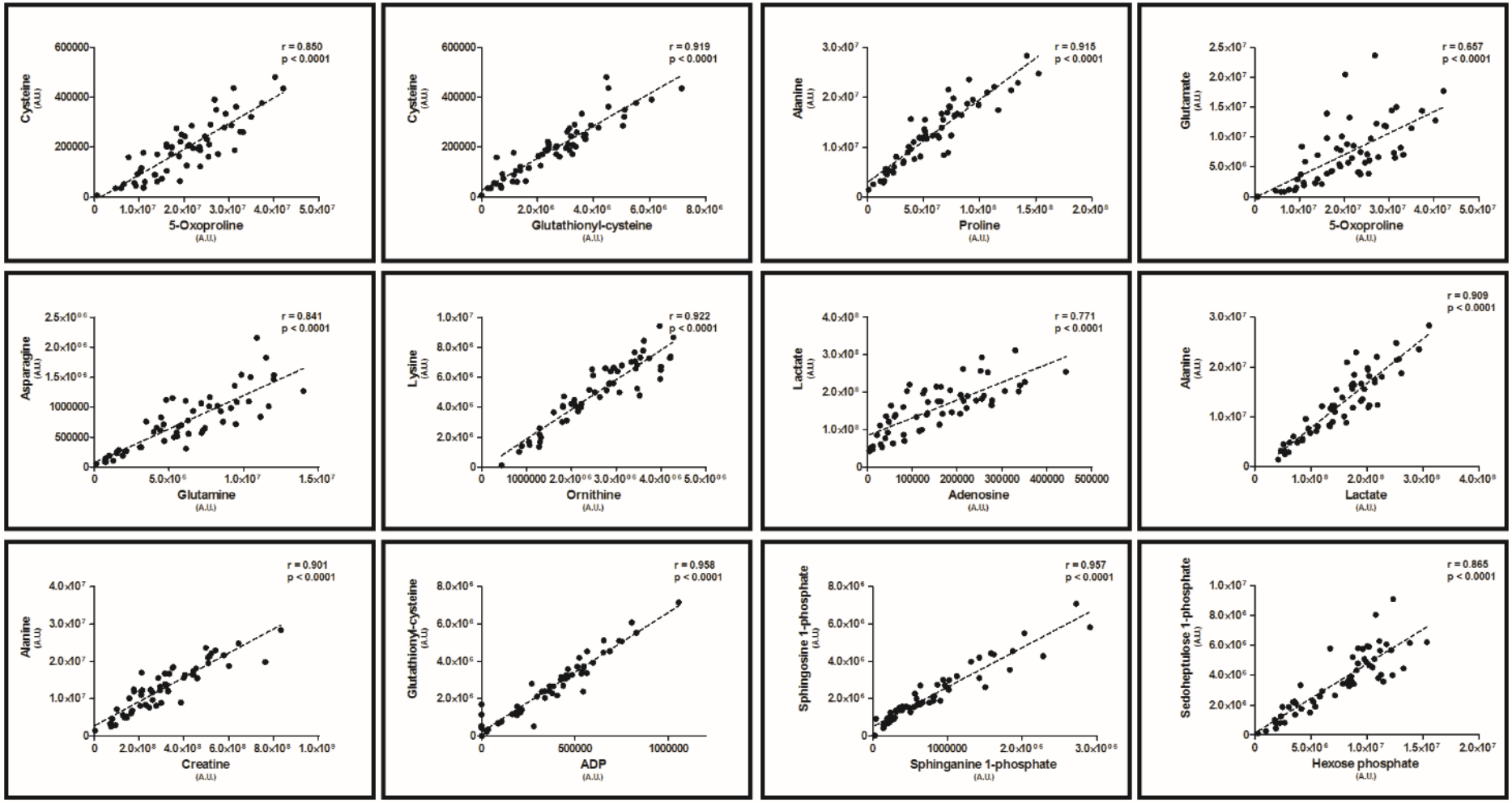


Linear Correlation Coefficients

-1.0 0 1.0

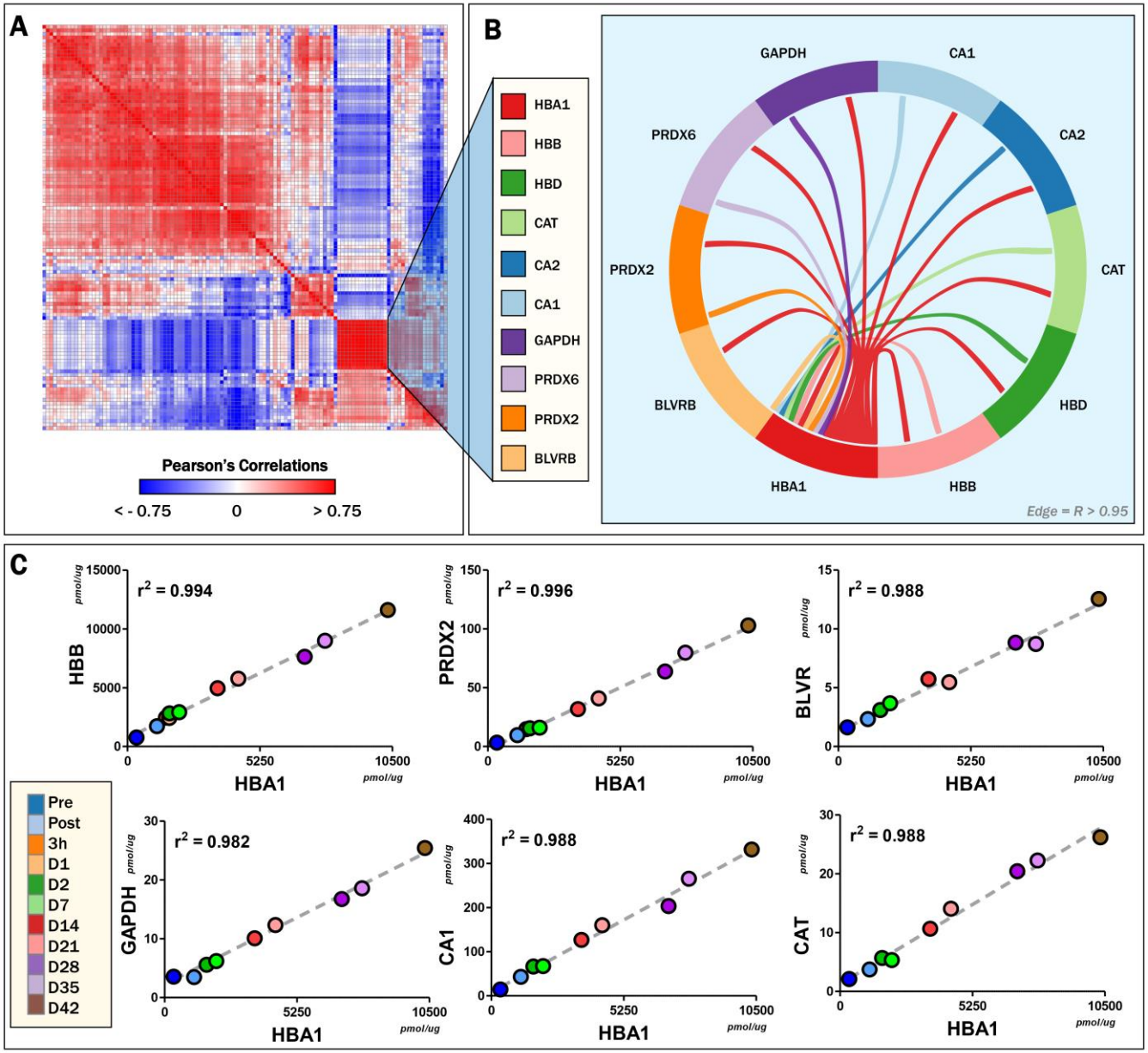
Papers in preparation – Collaboration with Hod E. – Columbia University and Yang X. – Houston, Texas

Metabolite/Metabolite correlations might reveal unexplored connections of RBC metabolic pathways

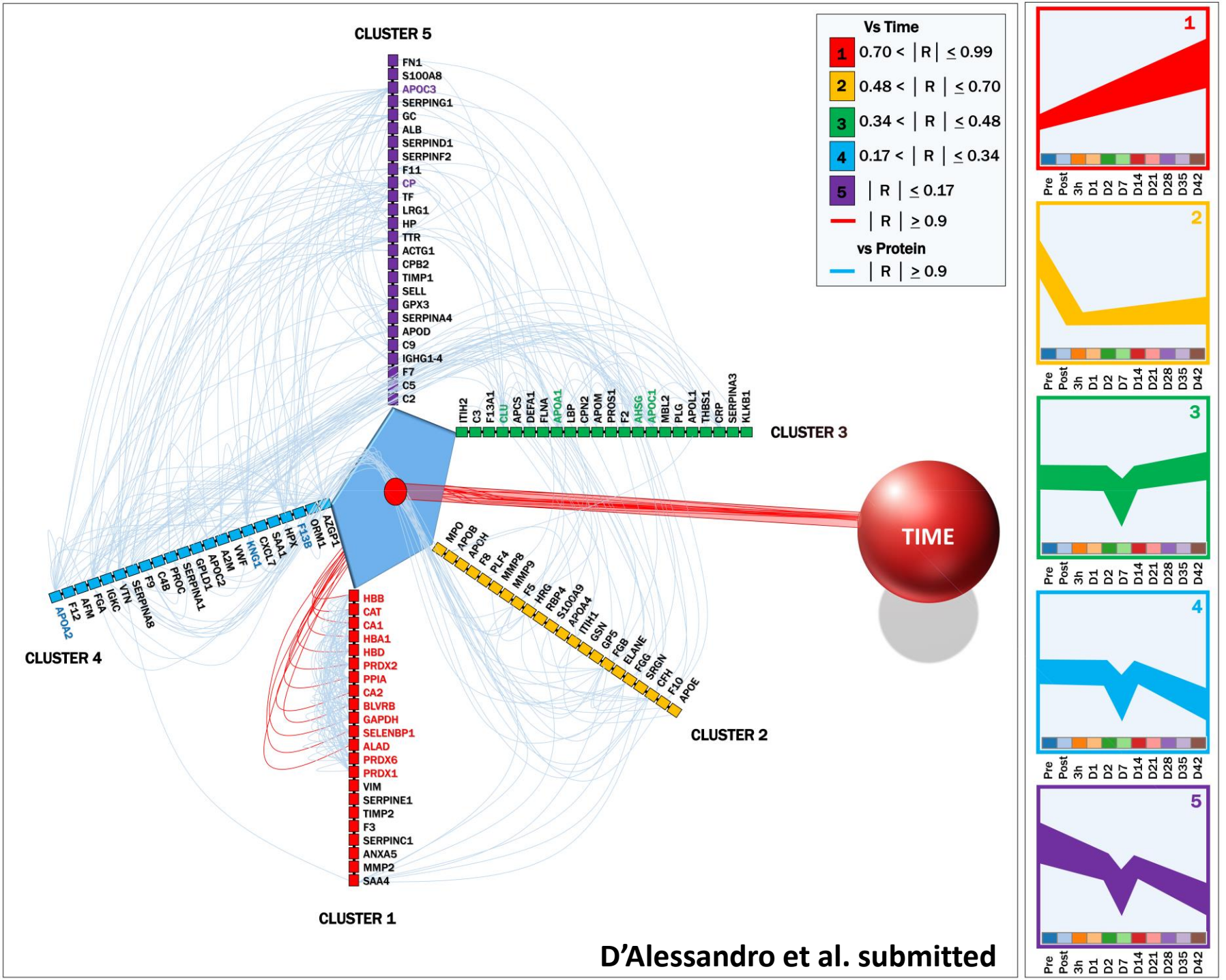


L'Acqua et al., in preparation – Collaboration with Hod E. – Columbia University

Protein markers in RBC sups correlate with hemolysis



D'Alessandro et al. submitted


CLUSTER 5
FN1
S100A8
APOC3
SERPING1
GC
ALB
SERPIND1
SERPINF2
F11
CP
TF
LRG1
HP
TTR
ACTG1
CPB2
TIMP1
SELL
GPX3
SERPINA4
APOD
C9
IGHG1-4
F7
C5
C2
ITIH2
C3
F13A1
CLU
APCS
DEFA1
FLNA
APOA1
LBP
CPN2
APOM
PROS1
F2
AHSG
APOC1
MBL2
PLG
APOL1
THBS1
CRP
SERPINA3
KLKB1
CLUSTER 3
TIME
AZGP1
ORM1
F13B
HPX
SAA1
CXCL7
HNG1
VWF
A2M
APOC2
GPLD1
SERPINA1
PROC
C4B
F9
SERPINA8
VTN
IGKC
FGA
AFM
F12
APOA2
CLUSTER 4
MPO
APOB
APOH
F8
PLF4
MMP8
MMP9
F5
HRG
RBP4
S100A9
APOA4
ITIH1
GSN
GP5
FGB
ELANE
FGG
SRGN
CFH
F10
APOE
CLUSTER 2
HBB
CAT
CA1
HBA1
HBD
PRDX2
PPIA
CA2
BLVRB
GAPDH
SELENBP1
ALAD
PRDX6
PRDX1
VIM
SERPINE1
TIMP2
F3
SERPINC1
ANXA5
MMP2
SAA4
CLUSTER 1
Vs Time
1 0.70 < | R | ≤ 0.99
2 0.48 < | R | ≤ 0.70
3 0.34 < | R | ≤ 0.48
4 0.17 < | R | ≤ 0.34
5 | R | ≤ 0.17
| R | ≥ 0.9
vs Protein
| R | ≥ 0.9
1
2
3
4
5
Pre Post 3h D1 D2 D7 D14 D21 D28 D35 D42
D'Alessandro et al. submitted

Take home messages

- We can use **Omics technologies to describe** "what's in the bag" (AS, cell processing, pathogen inactivation, inter-donor variability)
- Omics data can be used **to correlate** to transfusion outcomes and 24h in vivo survival, hemolysis, morphology
- **Alternative additive solutions** (e.g. AS-7, anti-oxidants) or **storage strategies** (anaerobic storage), as well as alternatives to transfusion of donated RBCs (***ex vivo* generation of RBCs**) can be tested through omics technologies
- Design and testing of novel storage strategies/solutions will be sped up by the joined efforts of transfusion experts, omics investigators and bioinformatics groups: **systems biology** will create ***in silico* predictive models that will be refined on the basis of experimental results**

Thank you for your attention!

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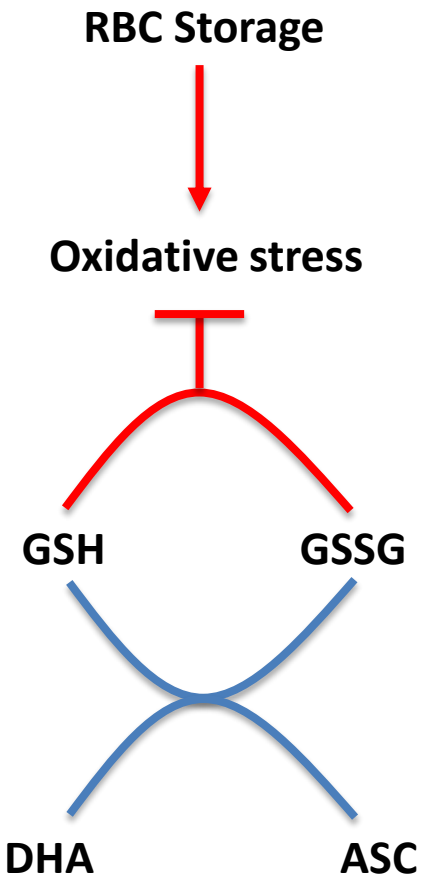
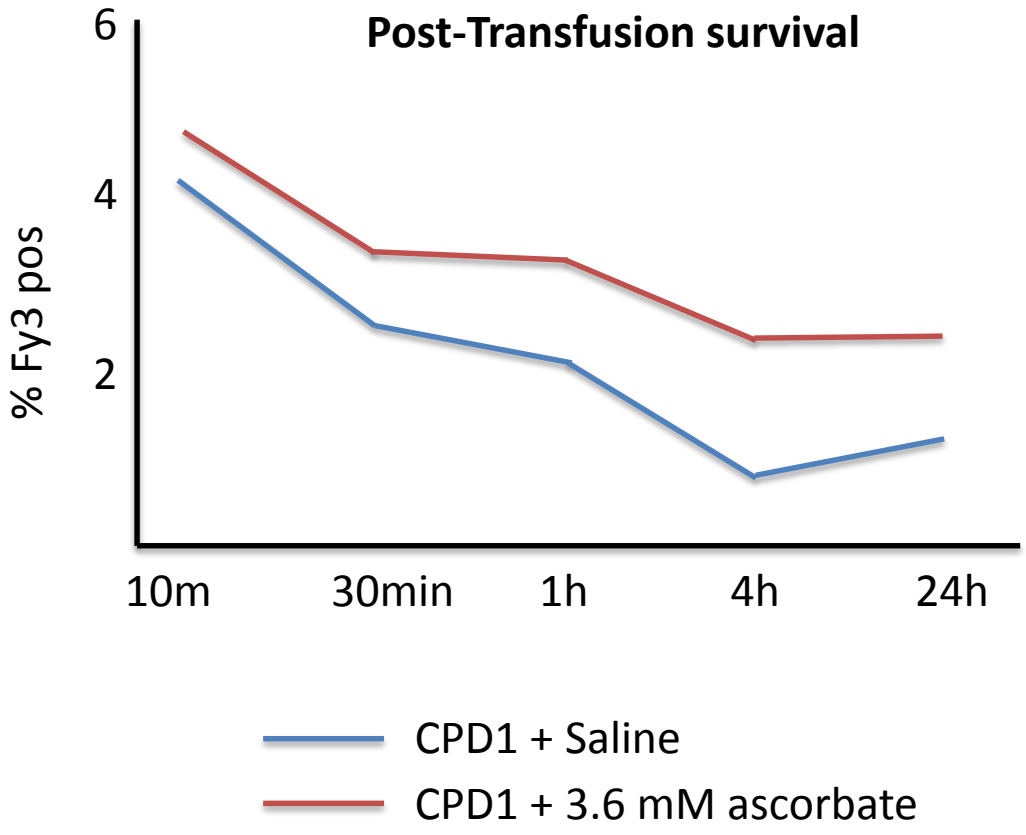
3. How can we make better products?

Supplementation of antioxidants

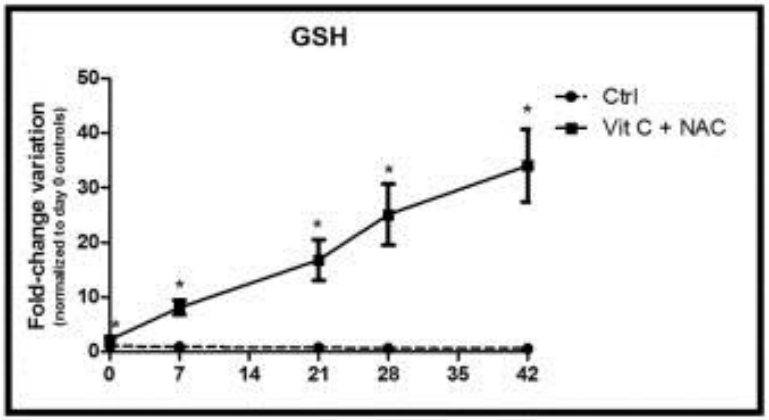
24h recovery, microparticles and alloimmunization parameters are improved by vitamin C in mice

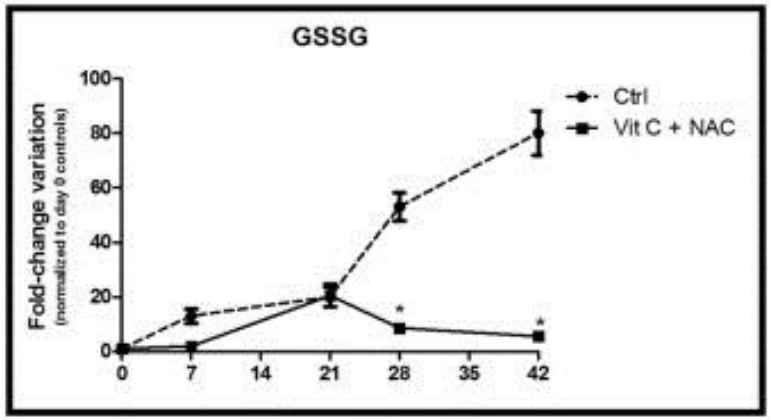


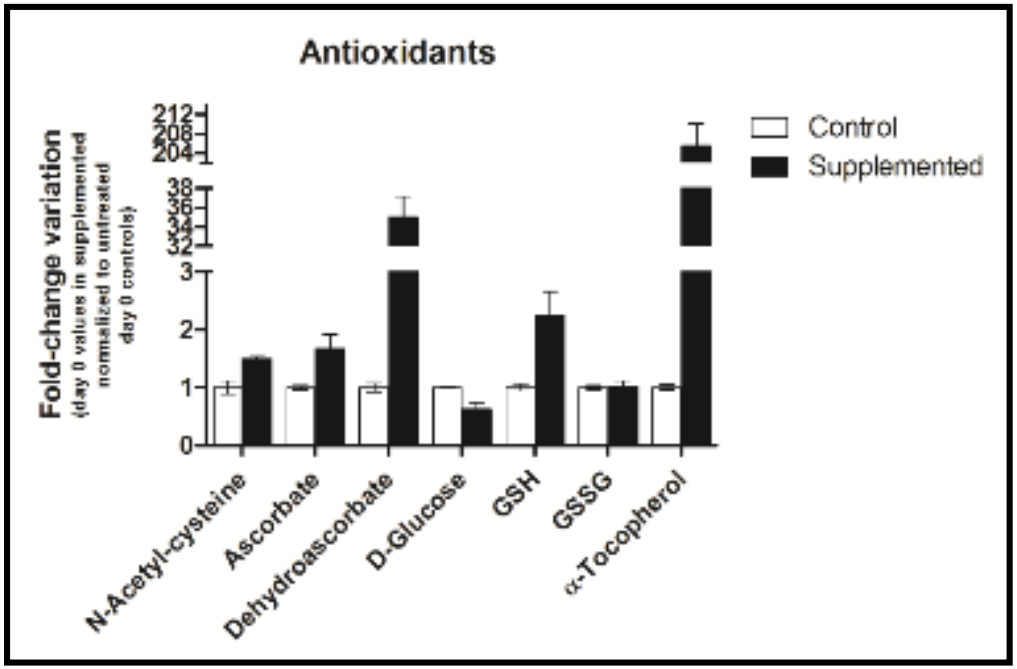


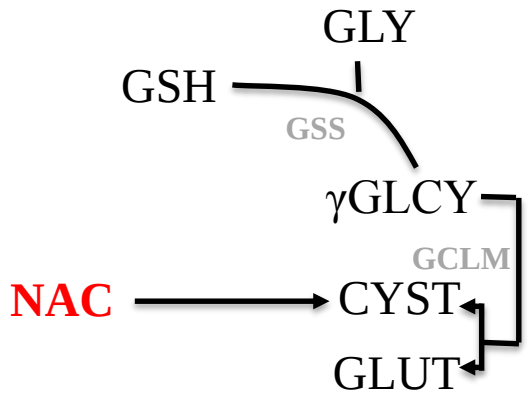
Stowell et al, Transfusion 2013

GSH homesostasis was improved by vitamin C and N-Acetylcysteine supplementation



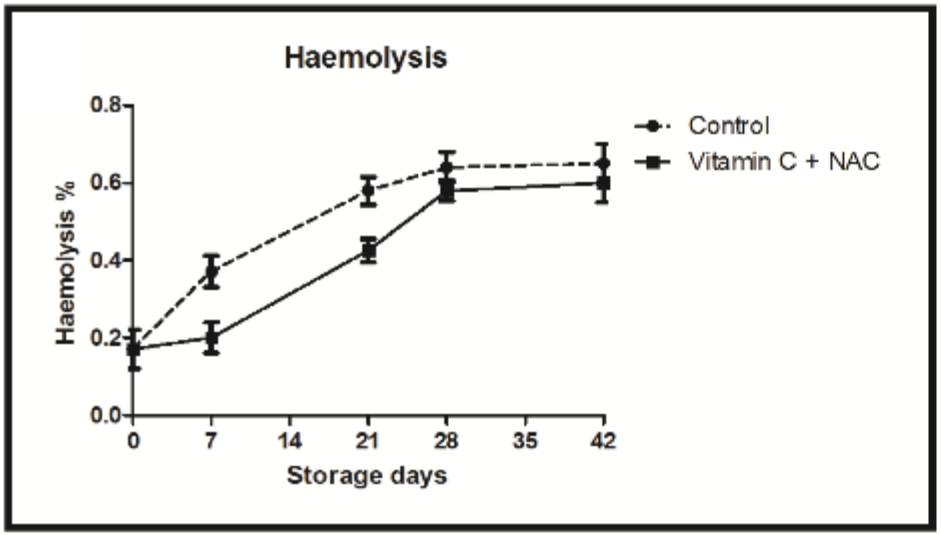


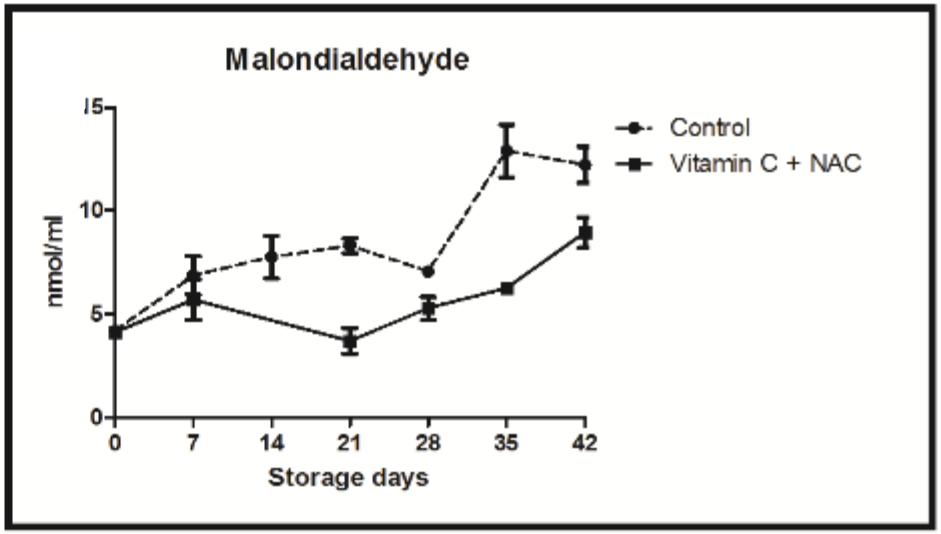




Pallotta et al, Blood Transfusion 2014a

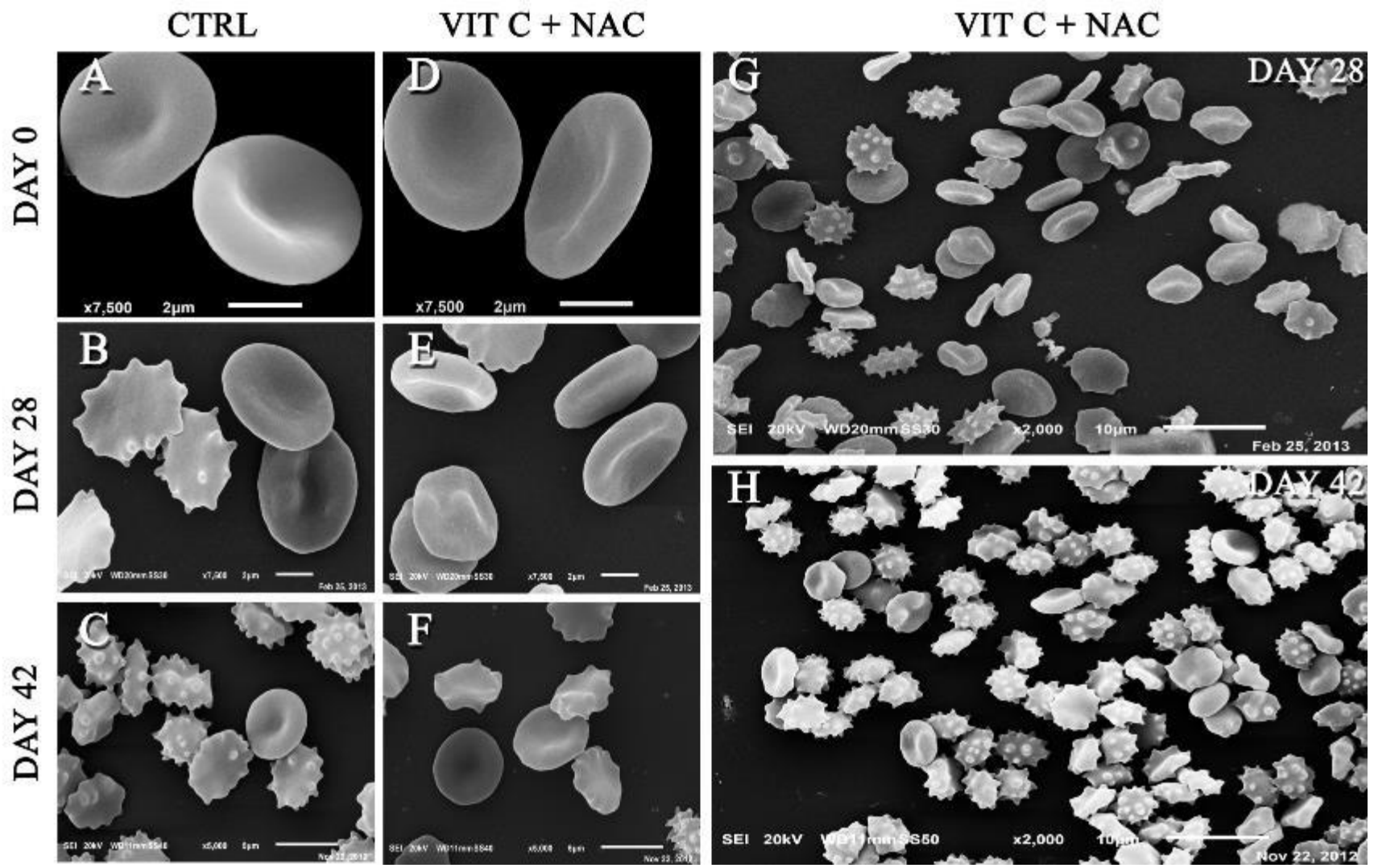
Supplementation of antioxidants (vitamin C and NAC) relieved oxidative stress albeit depressed metabolism





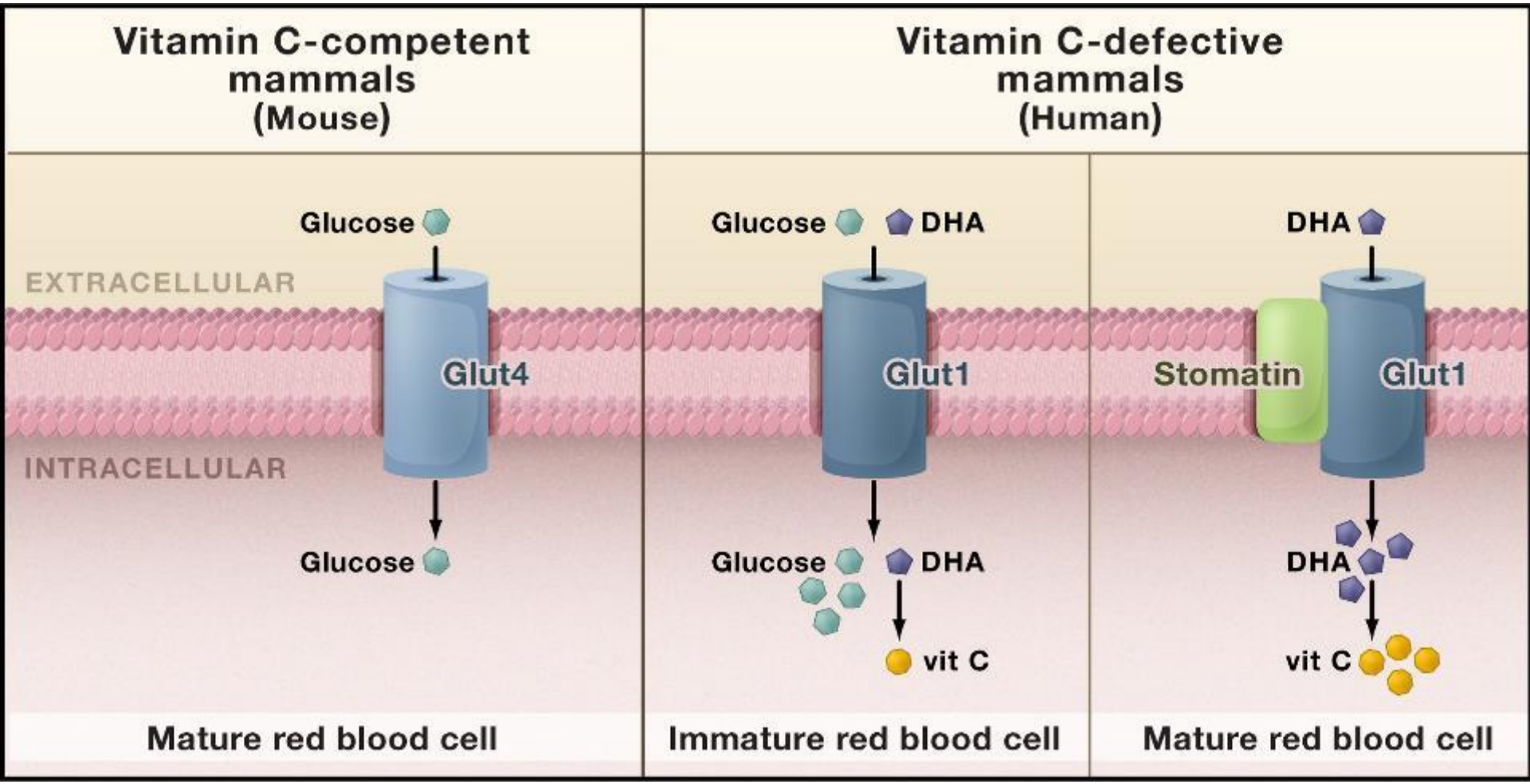
Pallotta et al, Blood Transfusion 2014a

Supplementation of antioxidants (vitamin C and NAC) relieved oxidative stress albeit not morphology after 28 days



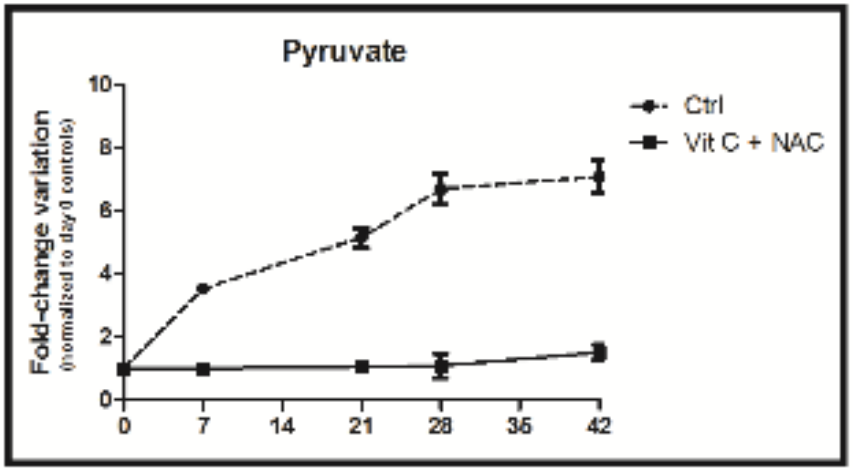
Pallotta et al, Blood Transfusion 2014b

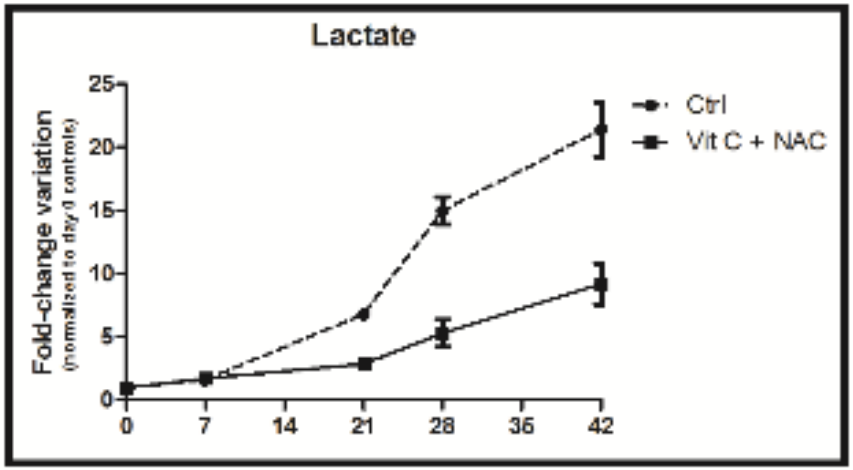
DHA and Glucose share the same transporter in mature RBCs



Troadec et al, 2008

Ascorbate (vitamin C) and glucose compete for the same transporter: metabolism is depressed



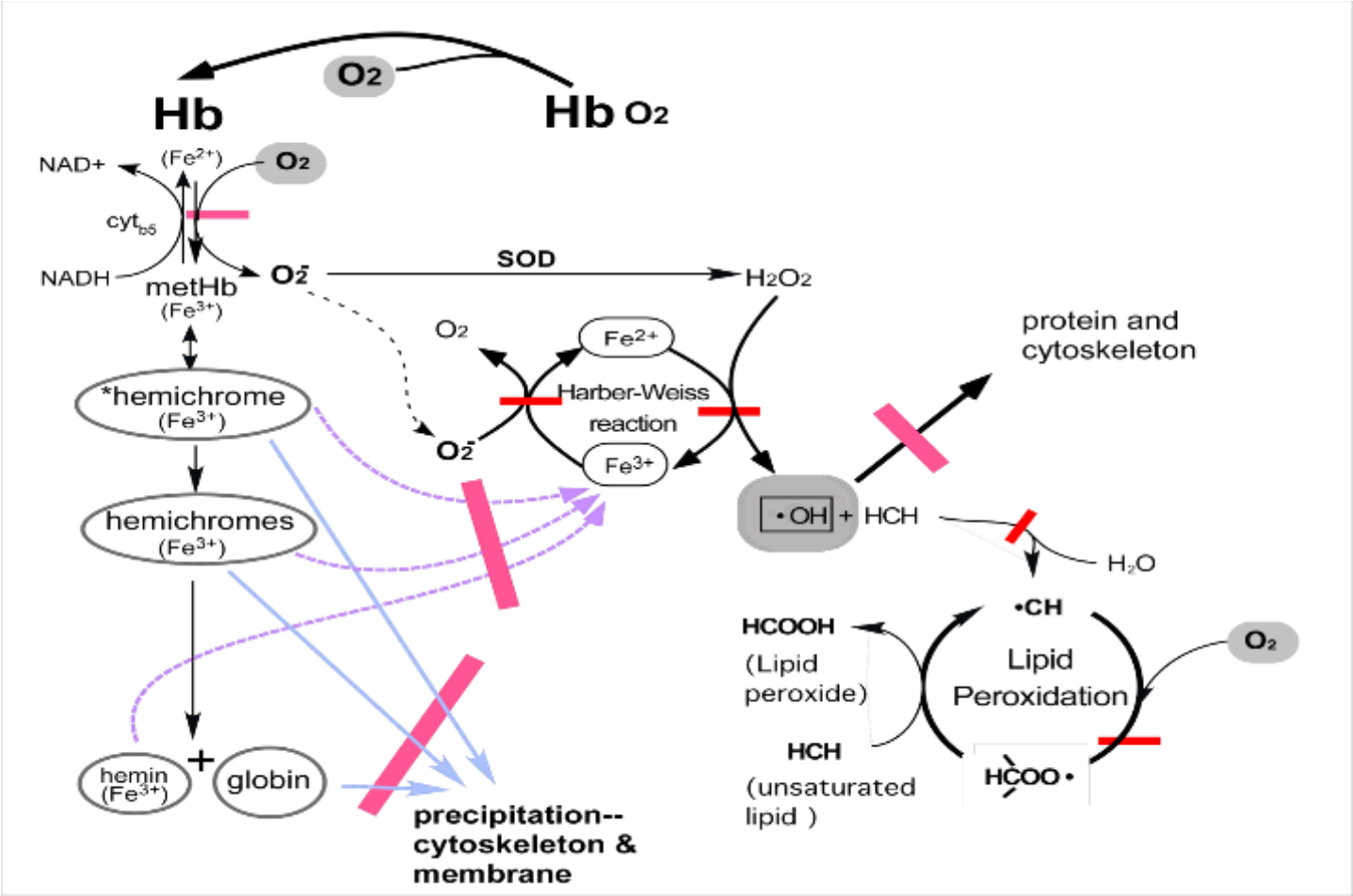


Pallotta et al, 2014

Anaerobic storage

Yoshida et al, 2007, 2008 and 2010; Dumont et al, 2008; Zolla and D'Alessandro, 2013; Longo et al, 2014; D'Alessandro et al, 2013

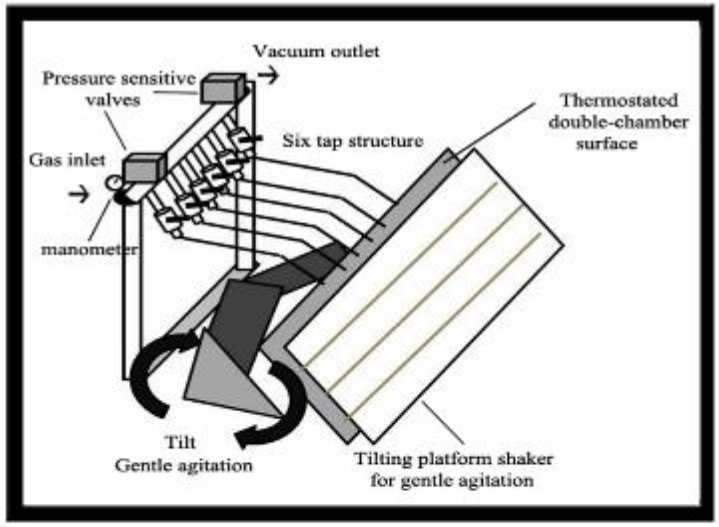
Anaerobic storage: metabolic modulation via oxygen removal

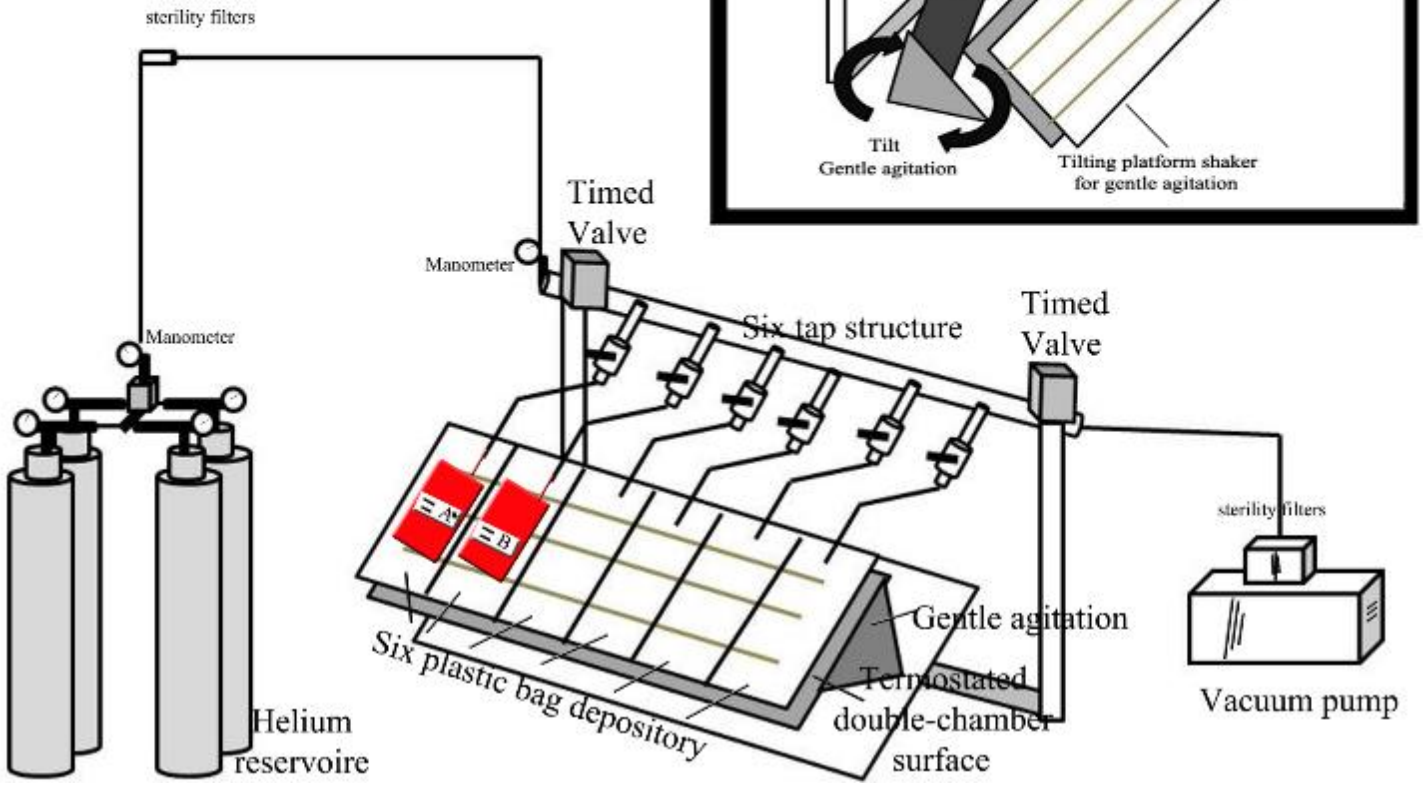


Yoshida T – New Health Sciences Inc

Deoxygenation apparatus for anaerobic storage

No control on CO2 levels!!!





Zolla and D'Alessandro, 2013

Metabolomics changes during anaerobic storage: Reduced hemolysis, vesiculation and improved morphology

Table 1 – RBC-shed microparticles	
Storage day	Microparticles (counted events in the arbitrary time window inside the gated area)
42 (control)	5234 ± 125
42 (deoxygenated)	1865 ± 78

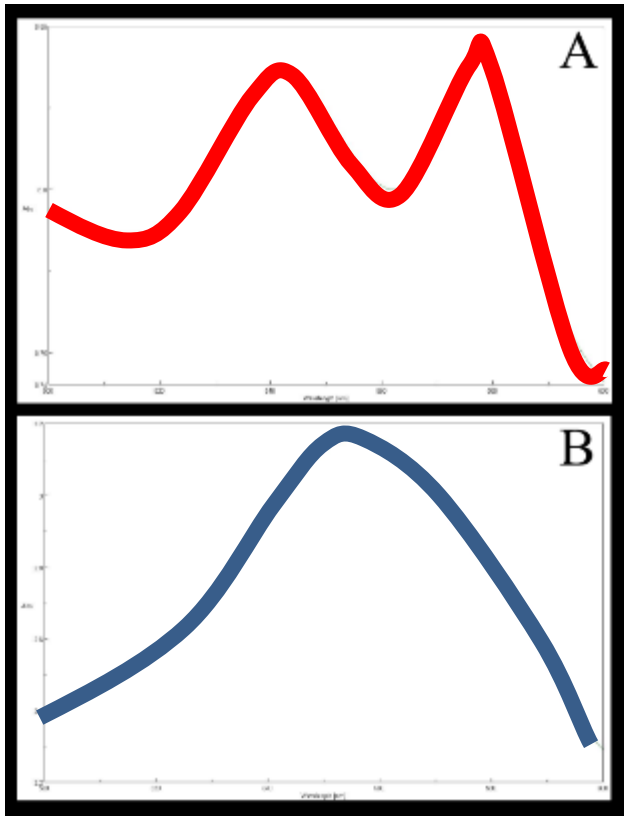
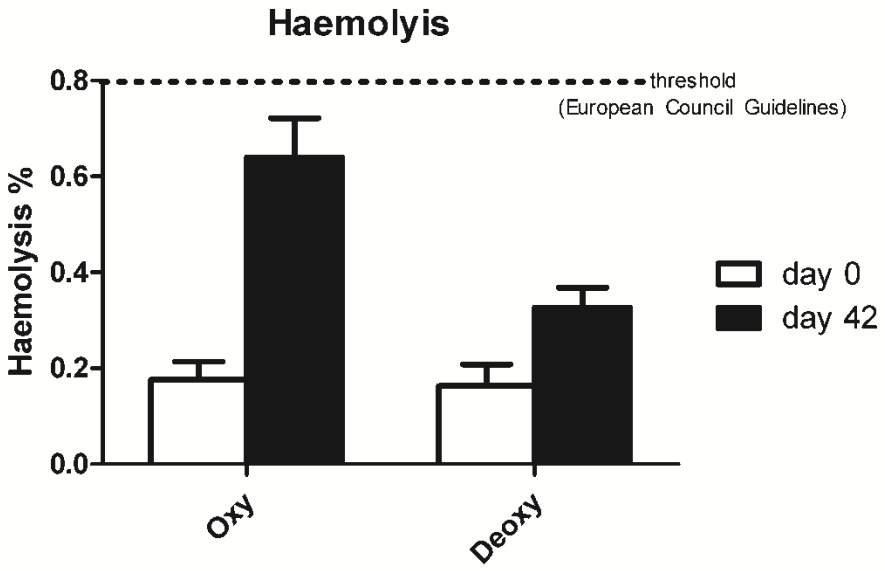
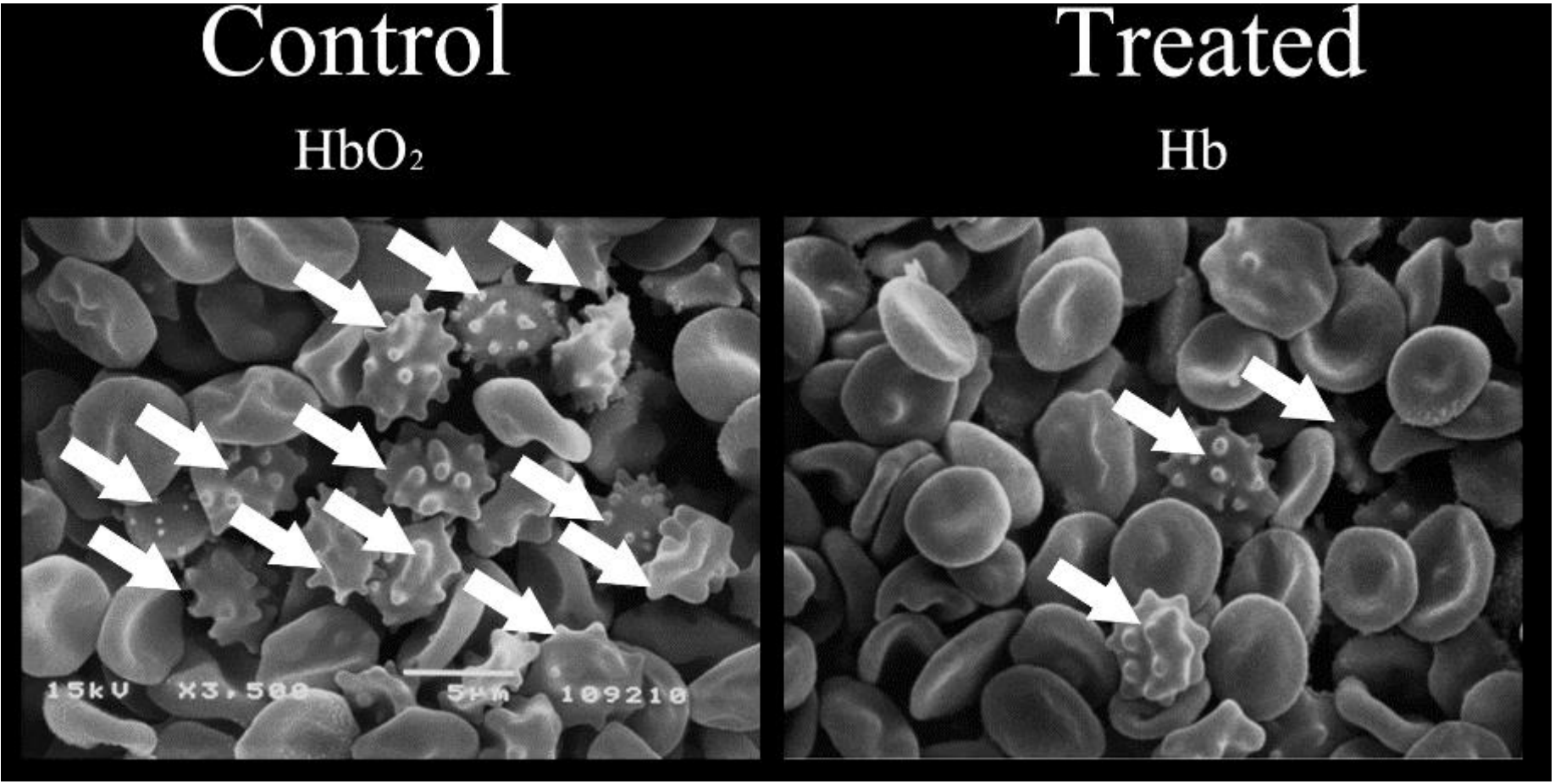


Table 2 – SEM erythrocyte shape classification			
Storage Day	Discocyte (%)	Reversibly* changed RBC (%) (echinocyte and stomatocyte shape)	Irreversibly* changed RBC (%) (spheroechinocyte, spherostomatocyte, spherocyte, ovalocyte, and degenerated shapes)
0	76.5+3.1	19.2 ± 5.7	4.3 ± 2.6
42 Control	20.6 ± 2.5	43.2 ± 3.8	36.2 ± 2.9
42 Deoxygenated	32.1 ± 1.9	45.4 ± 2.2	22.5 ± 3.1
* Reversible and irreversible changes were classified based on classification criteria, as previously reported D'Alessandro et al. [12]			



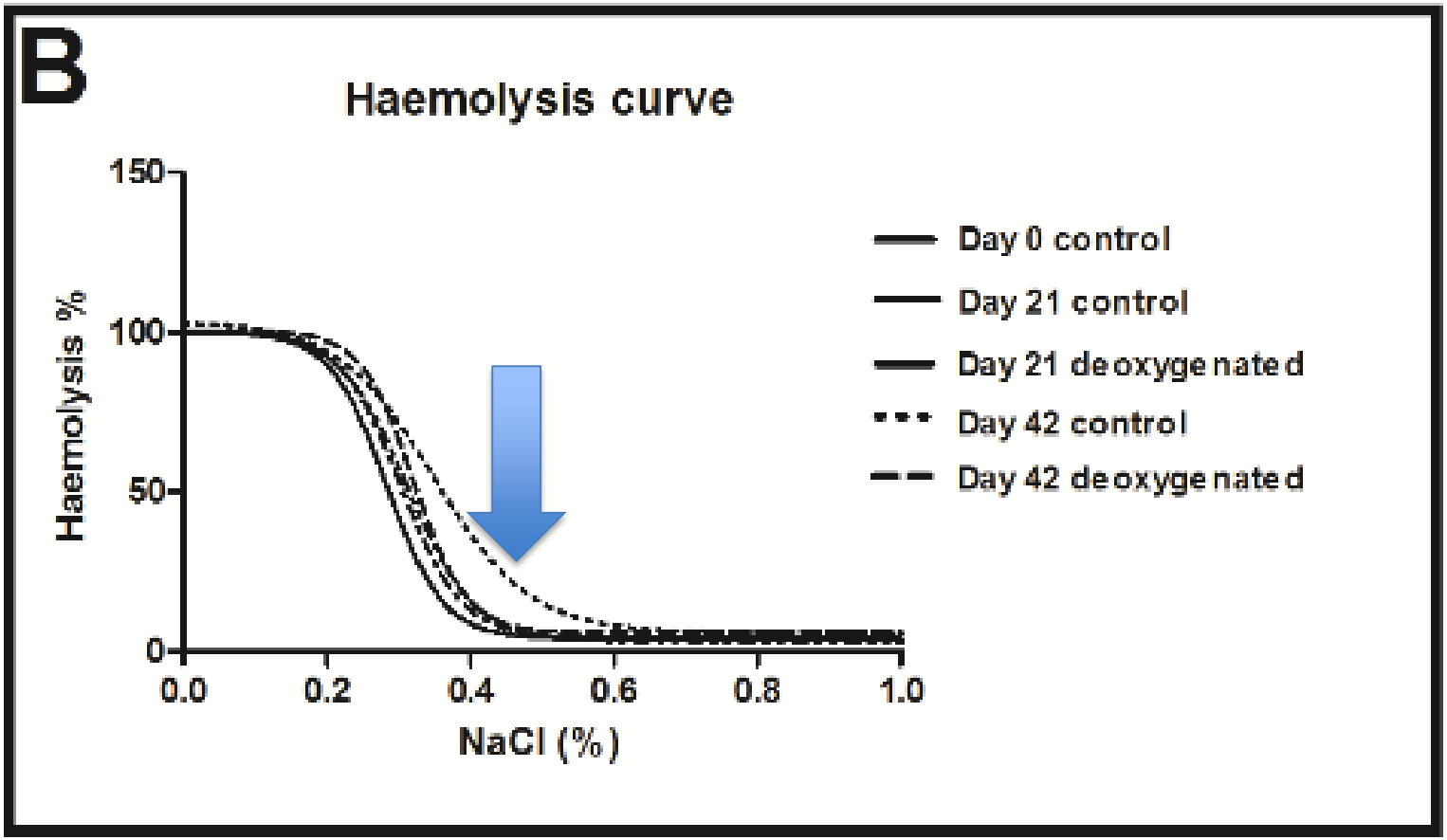
Zolla and D'Alessandro, 2013

Morphology score improved during anaerobic storage



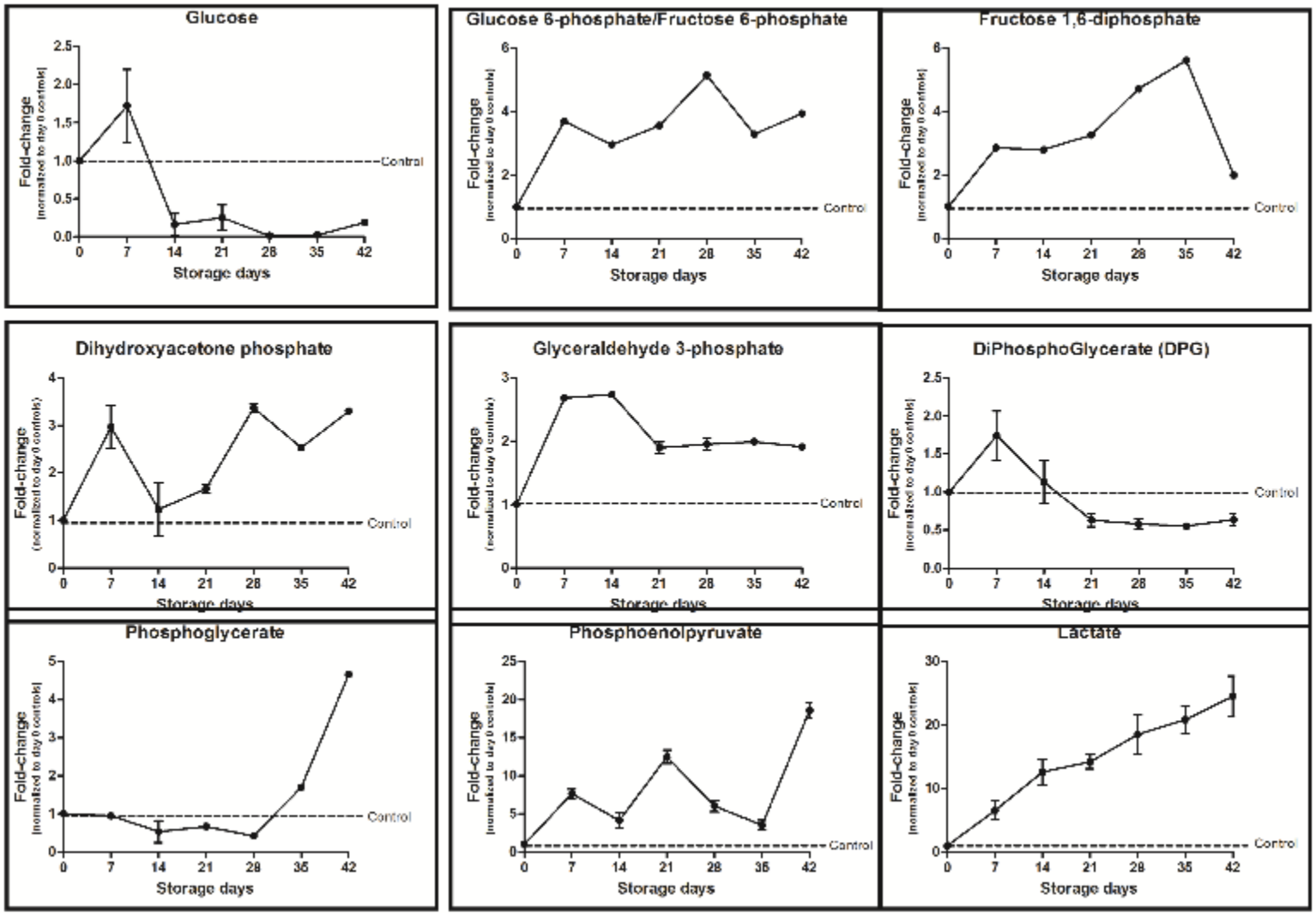
Zolla and D'Alessandro, 2013

Lower Haemolysis, higher Osmotic resistance



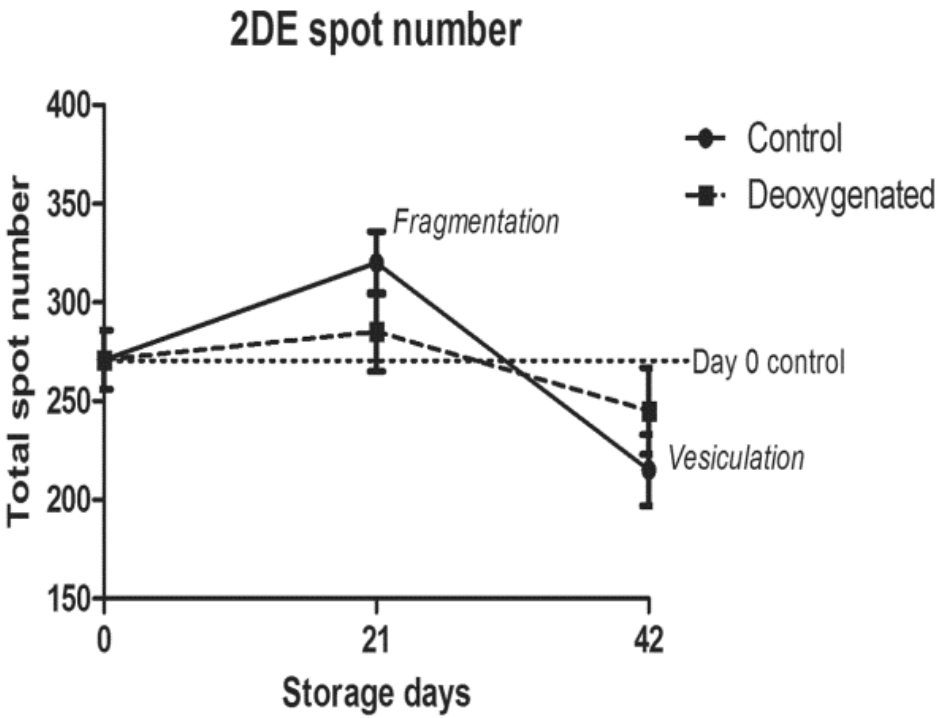
Longo et al, 2014

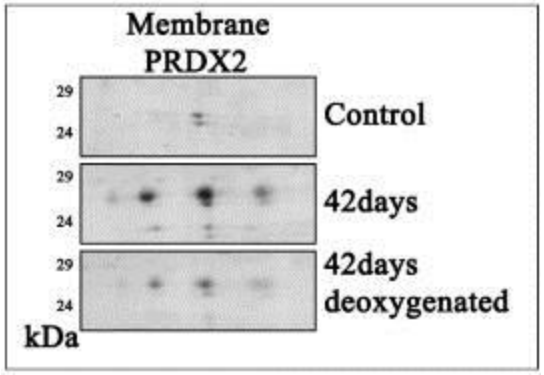
Metabolomics changes during anaerobic storage: Enhanced glycolysis

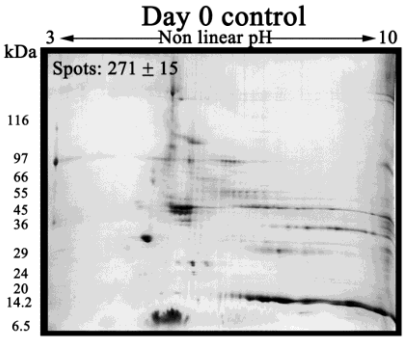


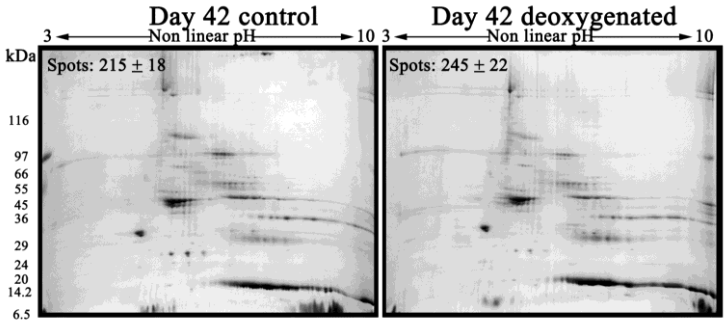
D'Alessandro, Gevi et al, 2013

Anaerobic storage: membrane proteomics profiles of leukoreduced units moderately improved



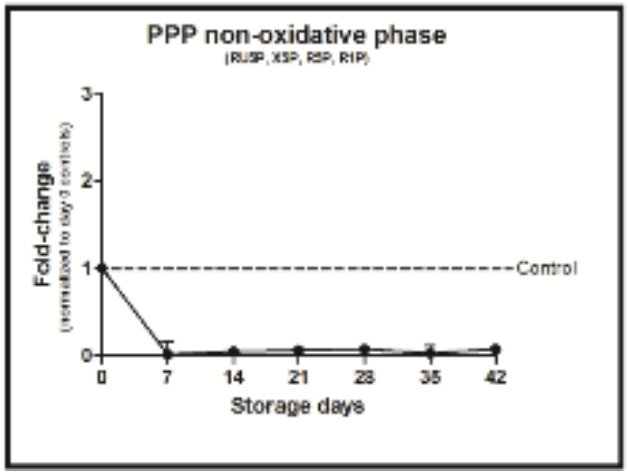


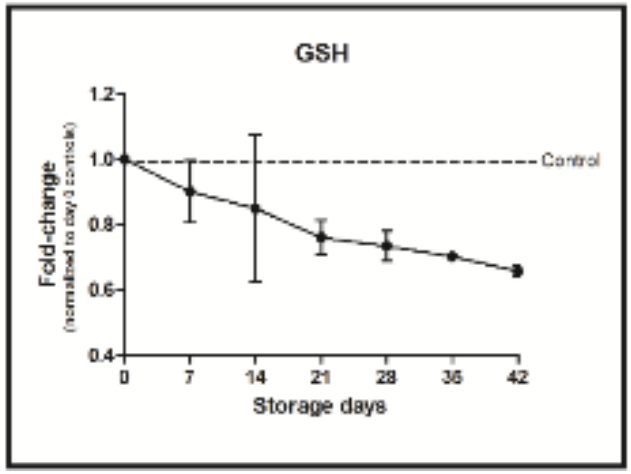


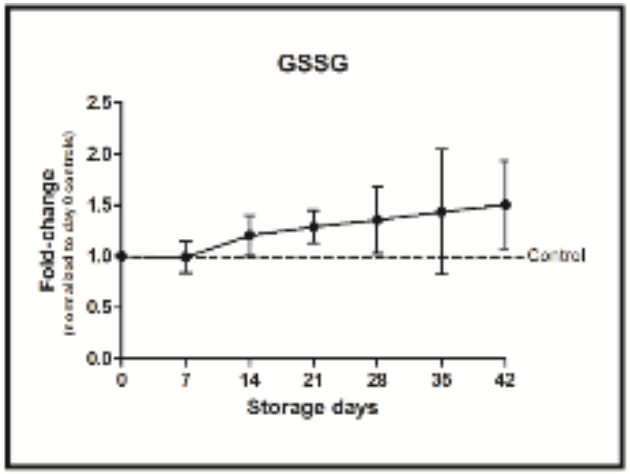


Longo et al, 2014

Metabolomics changes during our anaerobic storage
No shift towards the PPP albeit elevated oxidative stress







D'Alessandro, Gevi et al, 2013

Energy metabolism > Redox poise?
Is down-regulation of PPP an issue?

Storage of RBCs from
G6PDH-deficient donors

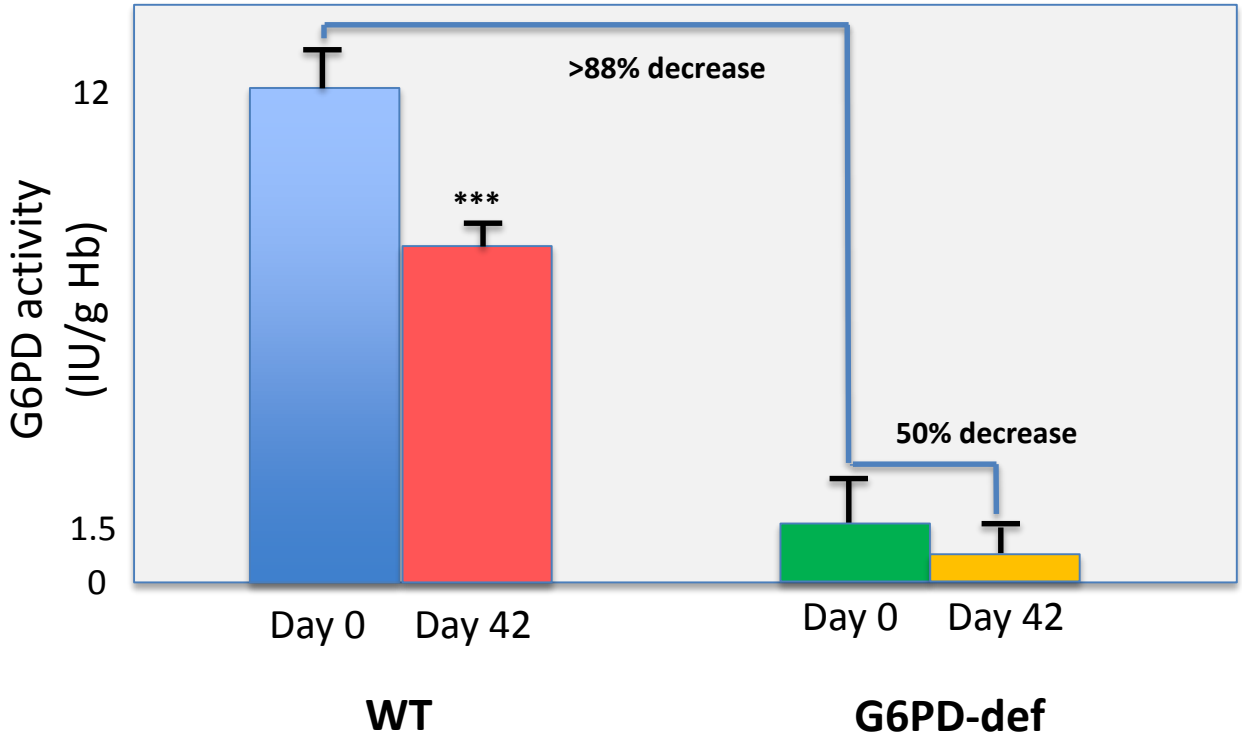
G6PD activity in enzyme deficient donors (Greek area) during routine storage in the blood bank (leukofiltered, SAGM)

G6PD-def (n=6; Mediterranean variant vs control pool)
From 5 Class II and one Class III mutants
(high to mild deficiency 10-25%, no clinical symptoms unless stressed)

	G6PDH activity*	
	NS	Day 42
1	3.2	0.7
2C	12	8.2
3	1.4	0.8
4	0.7	0.3
5	0.8	0.2
6		2.1
7	1.1	0.3

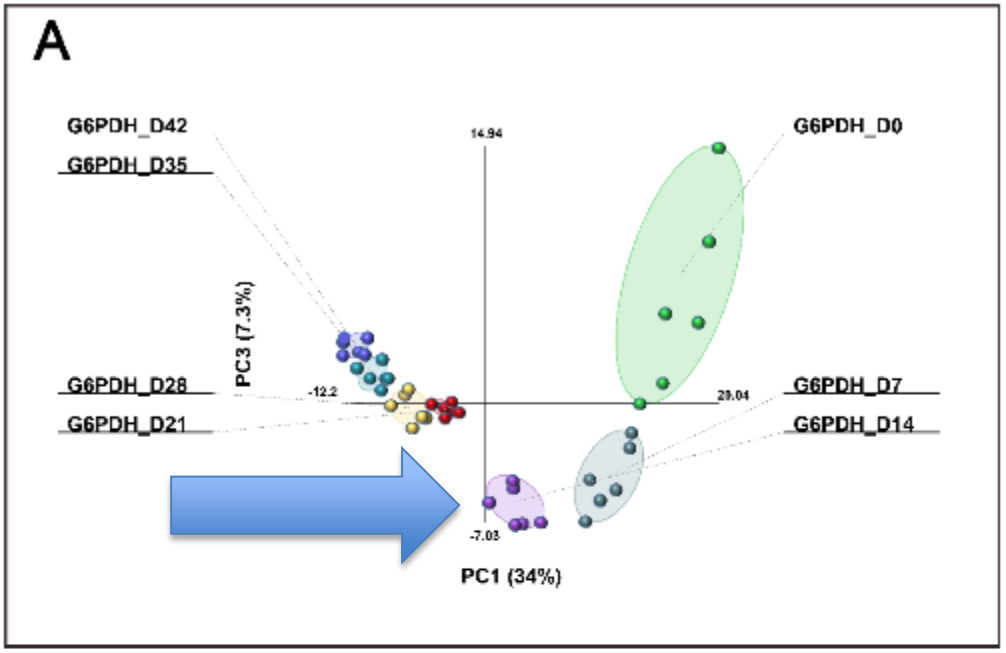
Normal	7,1-13,1 IU/g Hb
Border	**2.1-3.9**
Deficient	**0.3-1.3**

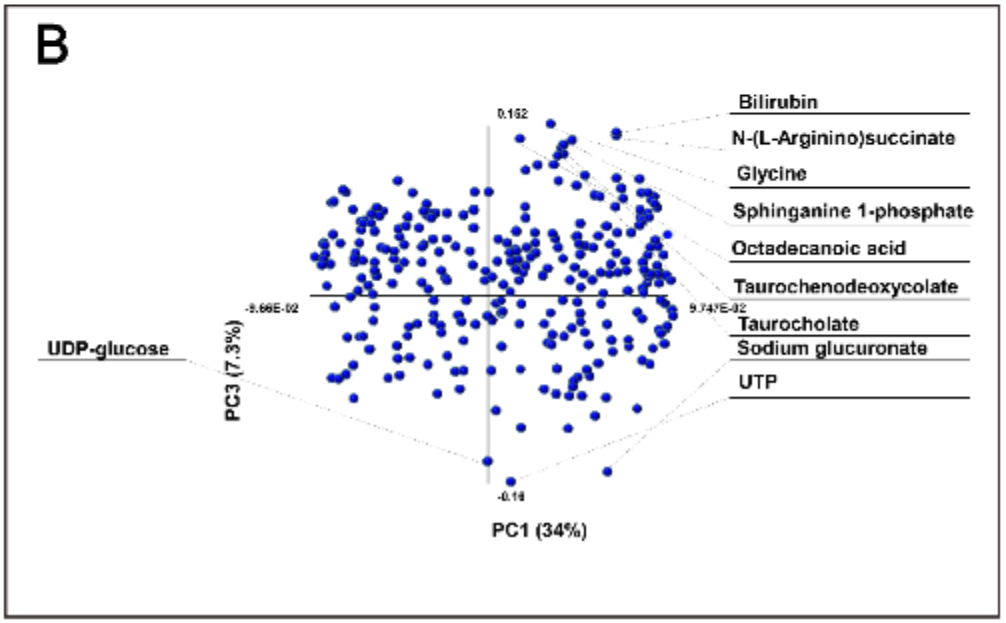
***NS = non-stored = Day 0**

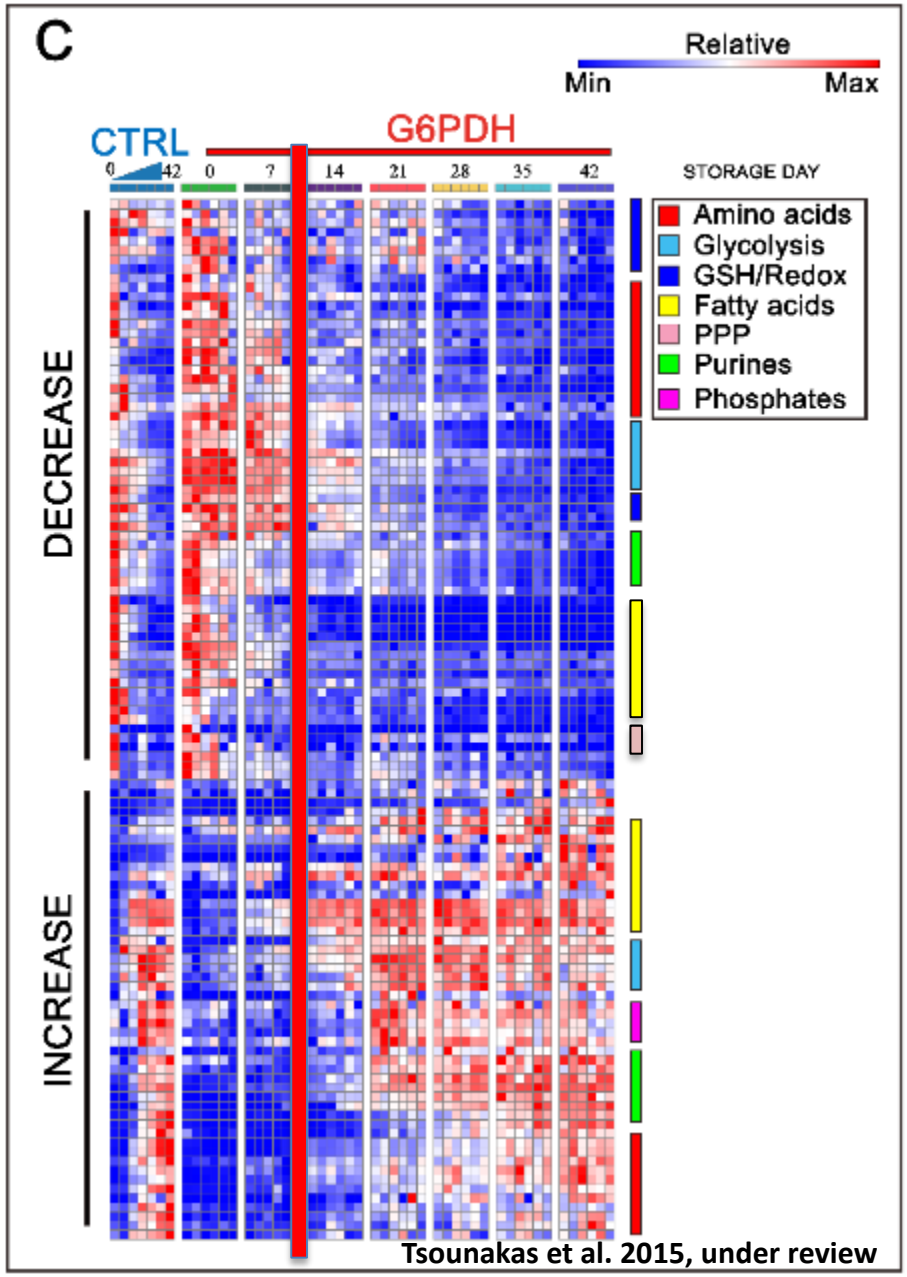


Tsounakas et al. 2015, under review

Metabolic profiles are consistent with what observed in matched controls (SAGM, leukofiltered), but anticipated at day 7

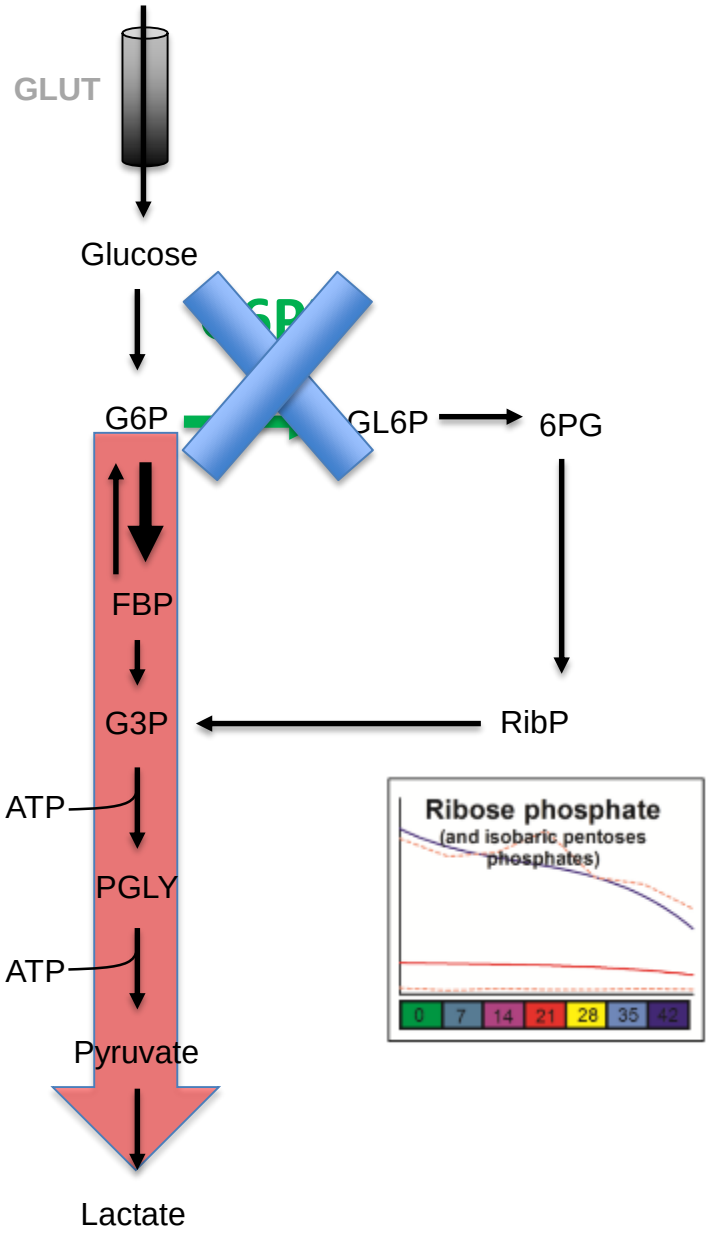


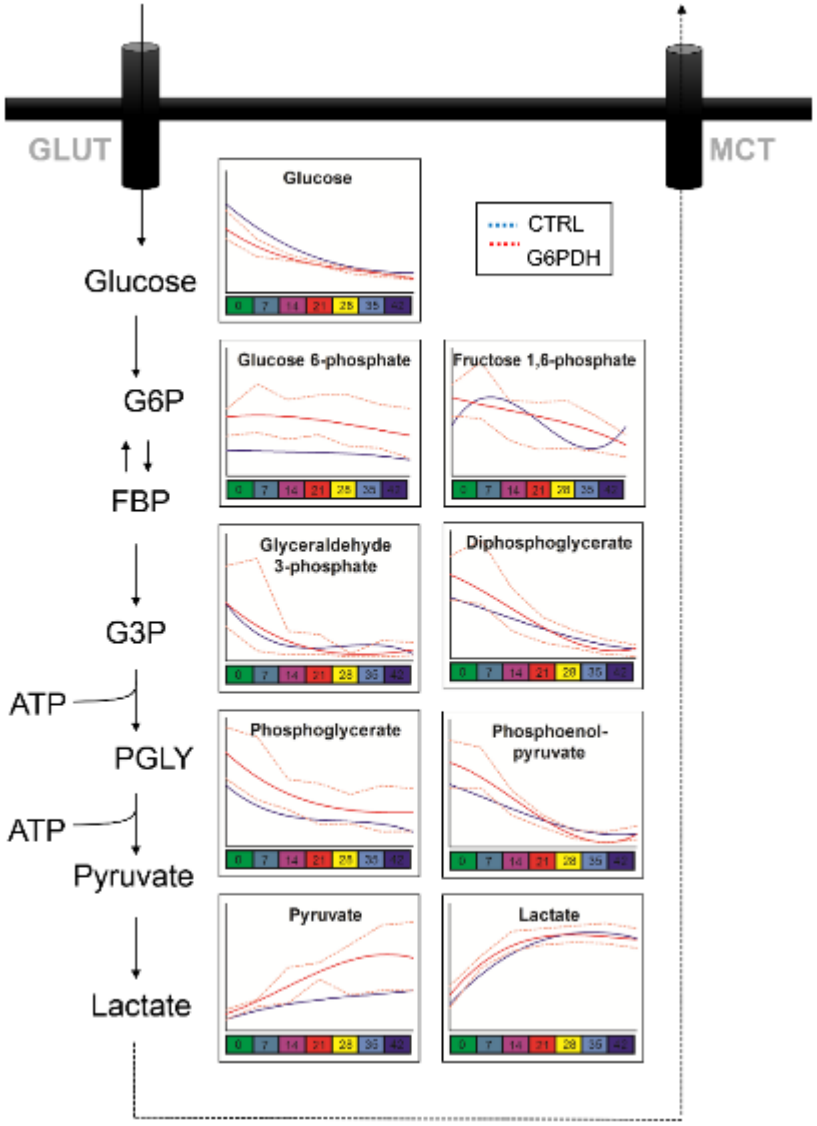




Tsounakas et al. 2015, under review

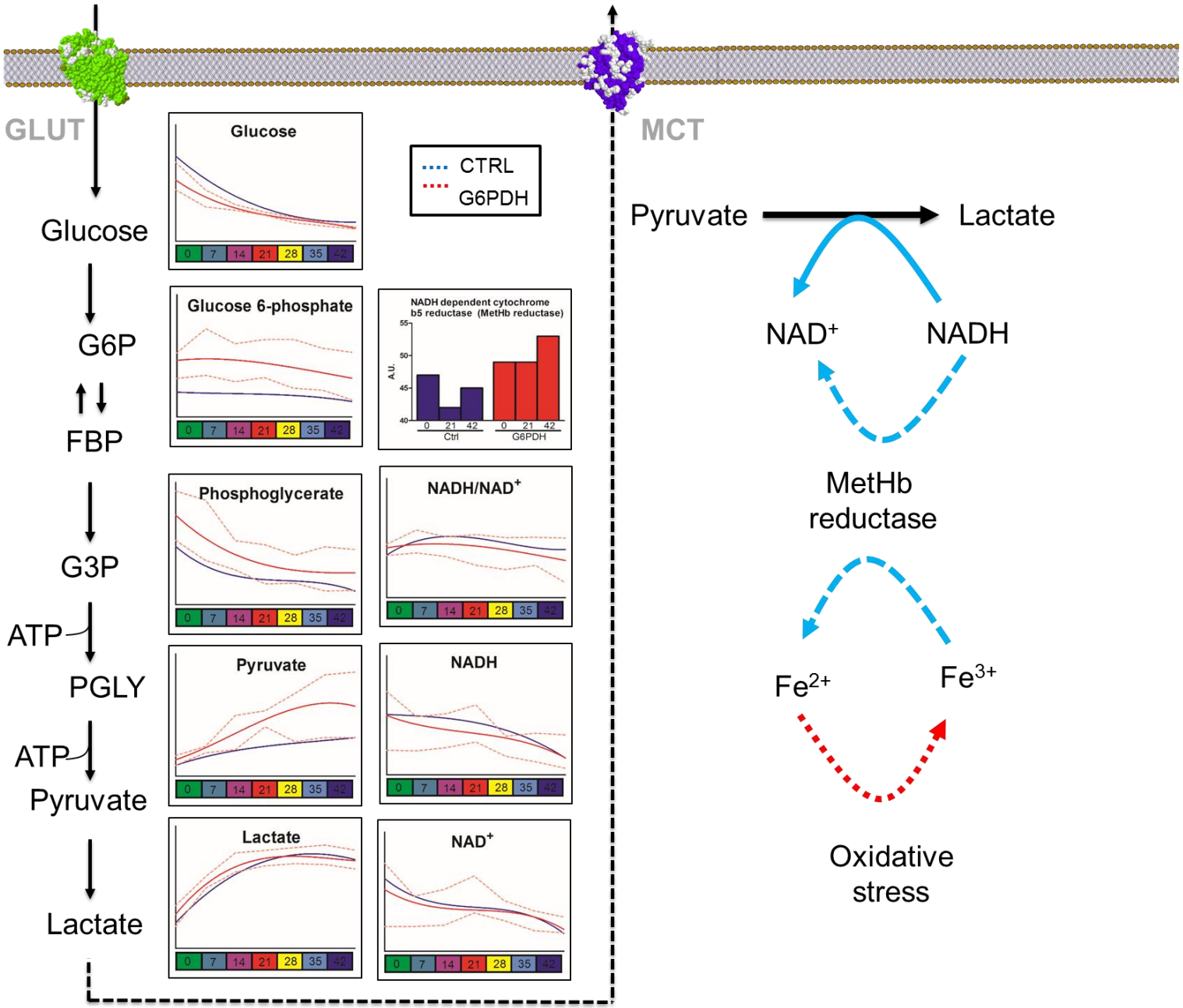
Metabolic adaptations result in increased glycolysis: If cells can't shift to PPP, enhance Embden Meyerhoff



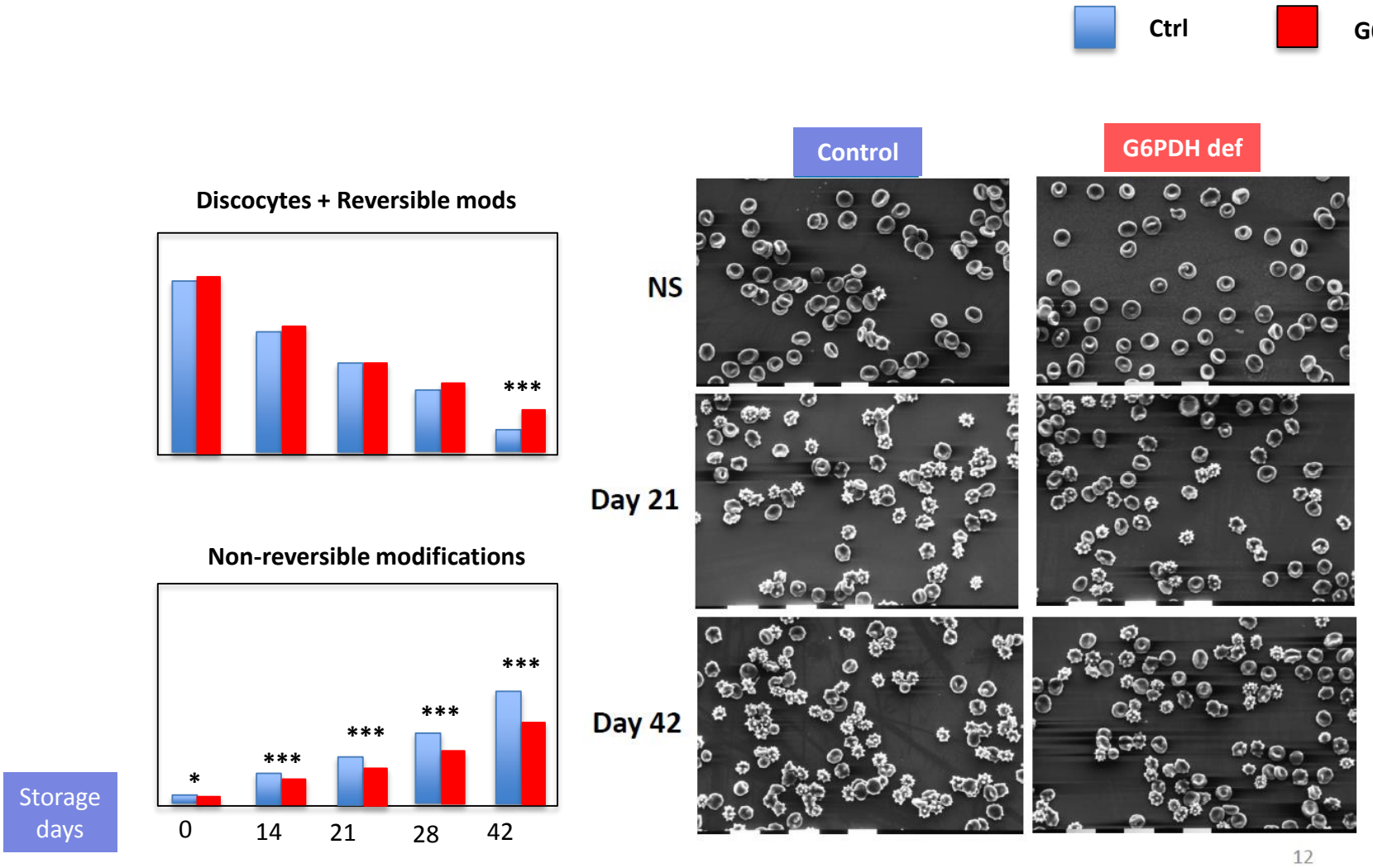


Tsounakas et al. 2015, under review

MetHb reductase uses NADH to counteract Hb iron oxidation

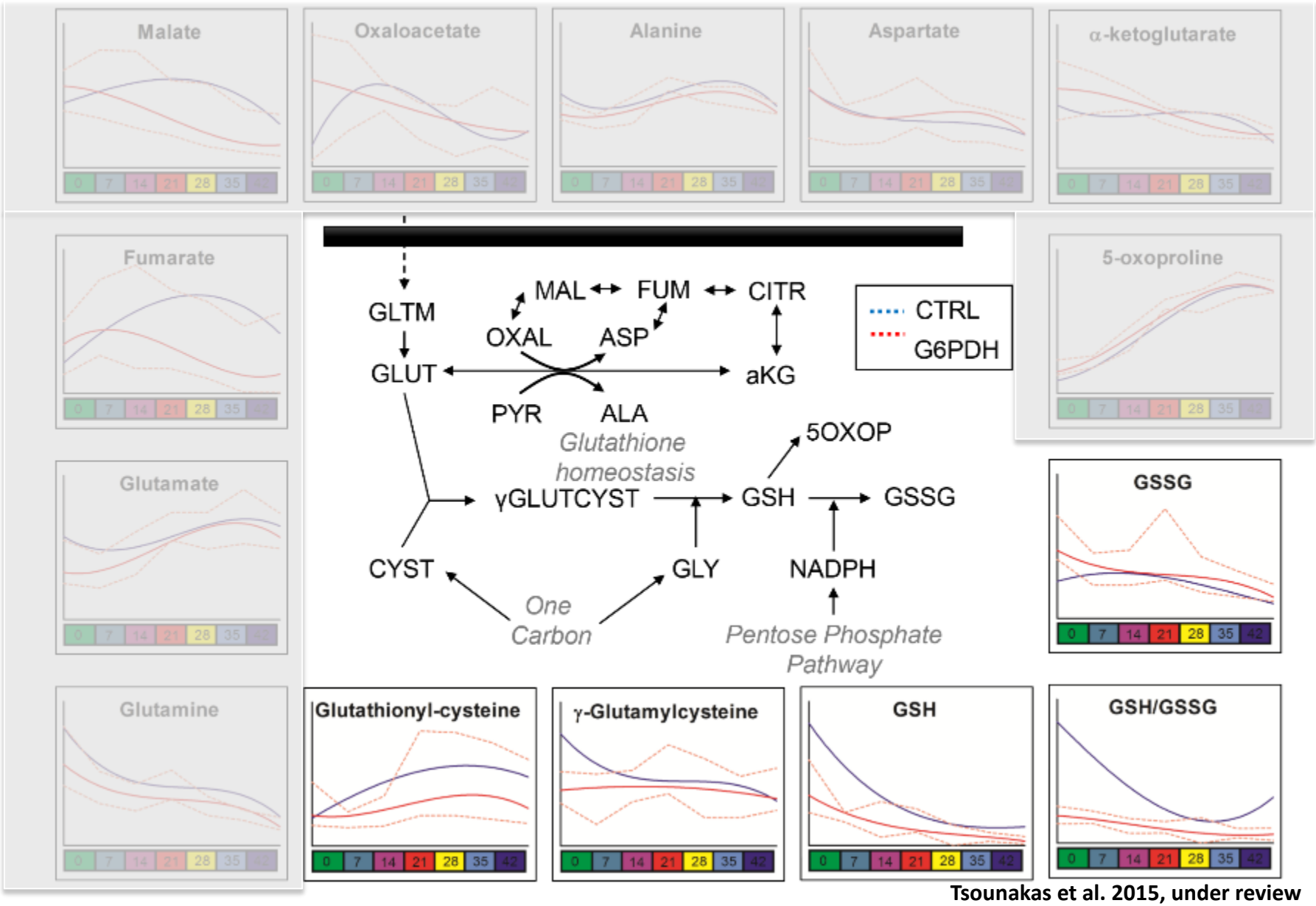


...units from G6PD-def donors have better morphology



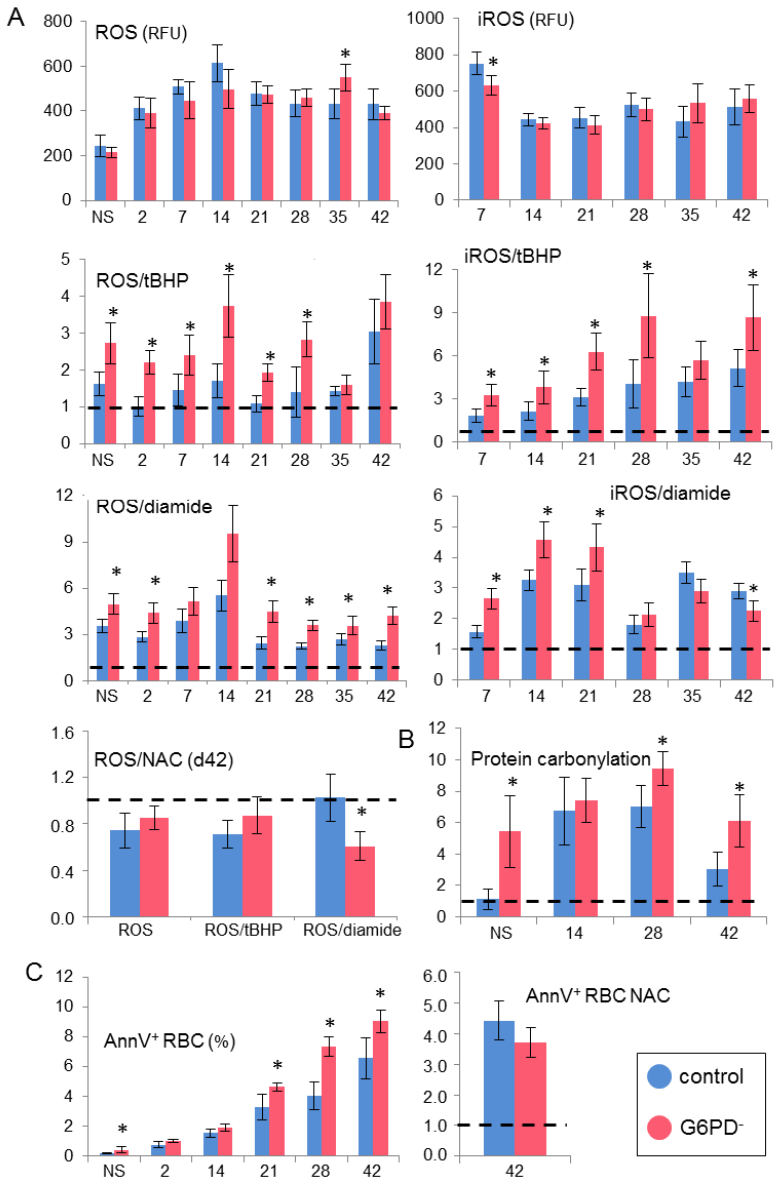
Tsounakas et al. 2015, under review

However, metabolic adaptations result in impaired glutathione homeostasis…

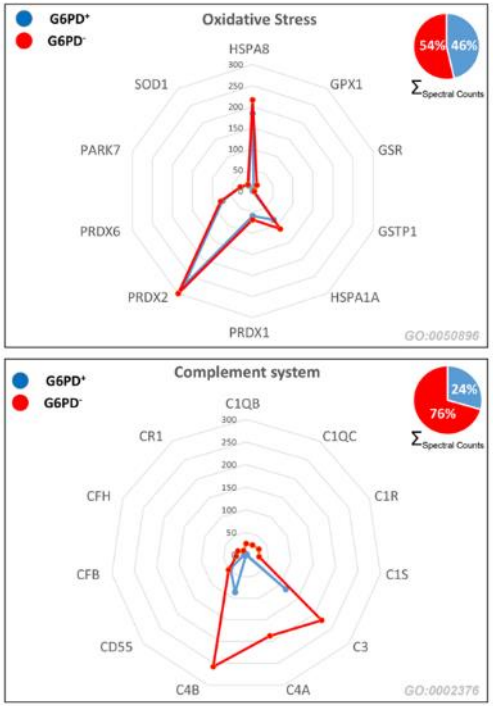


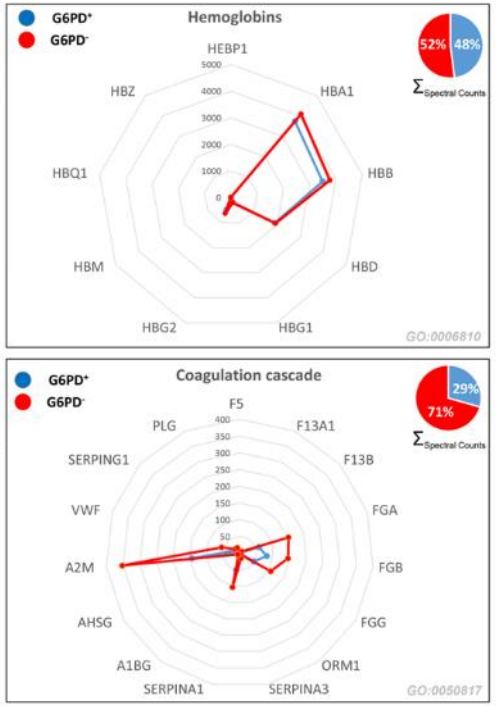
Tsounakas et al. 2015, under review

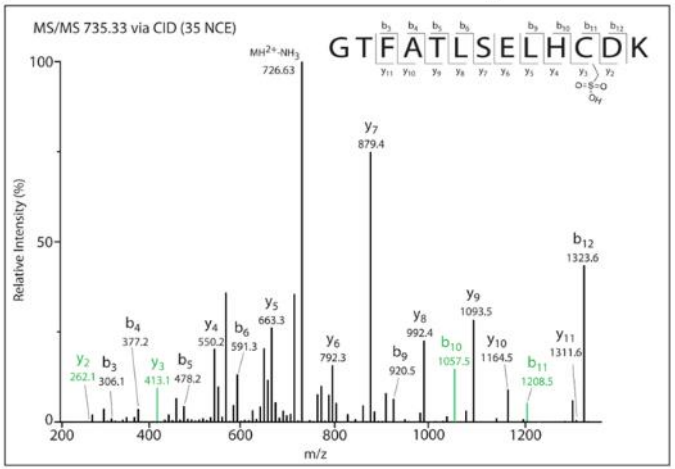
… more oxidative stress sensitive and vesicles are loaded with oxidized Hb and complement components…

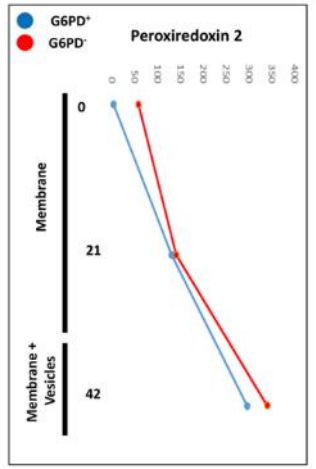


Vesicles





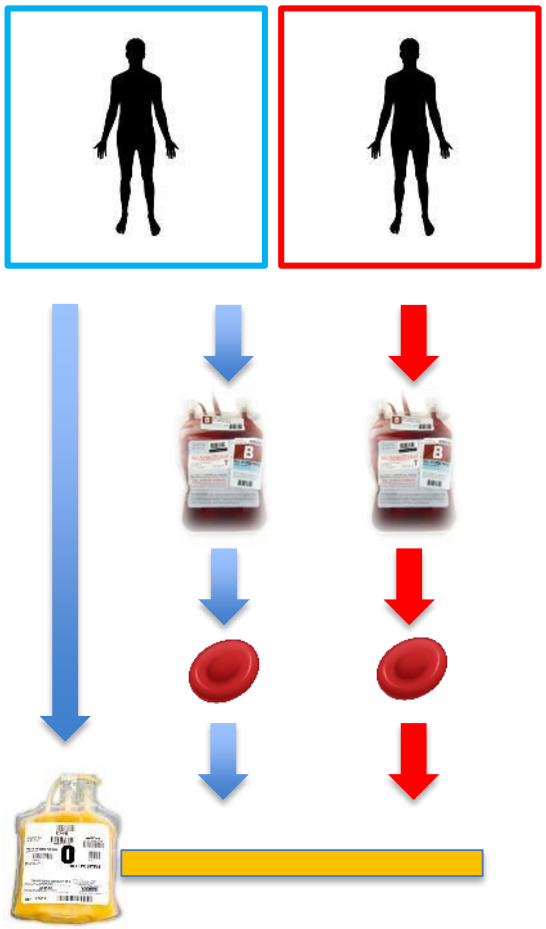


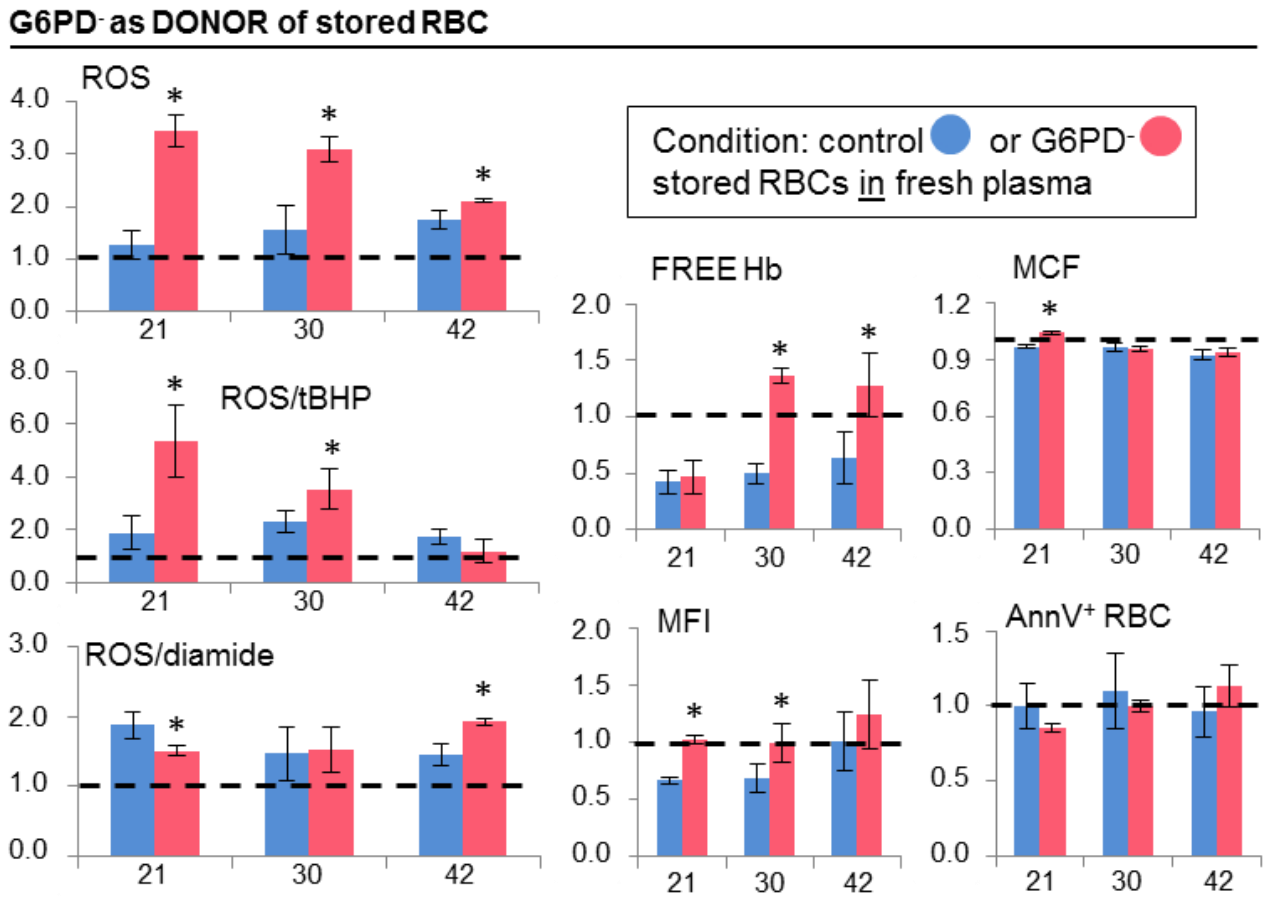


Tsounakas et al. 2015, under review

But G6PDH-def RBCs lyse faster when exposed to control plasma (simulation of transfusion associated stress in trauma recipients)

Ctrl G6PDH def.

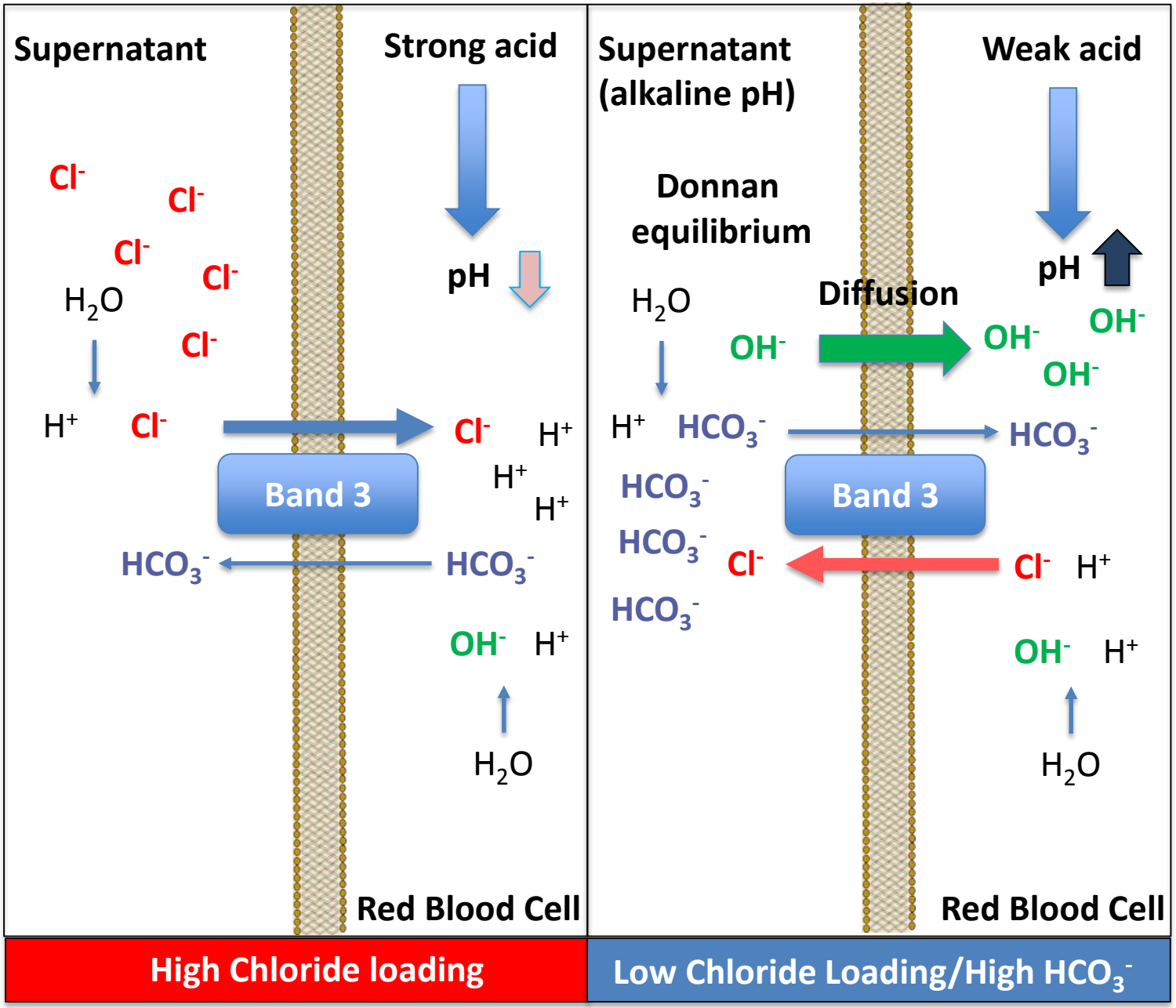




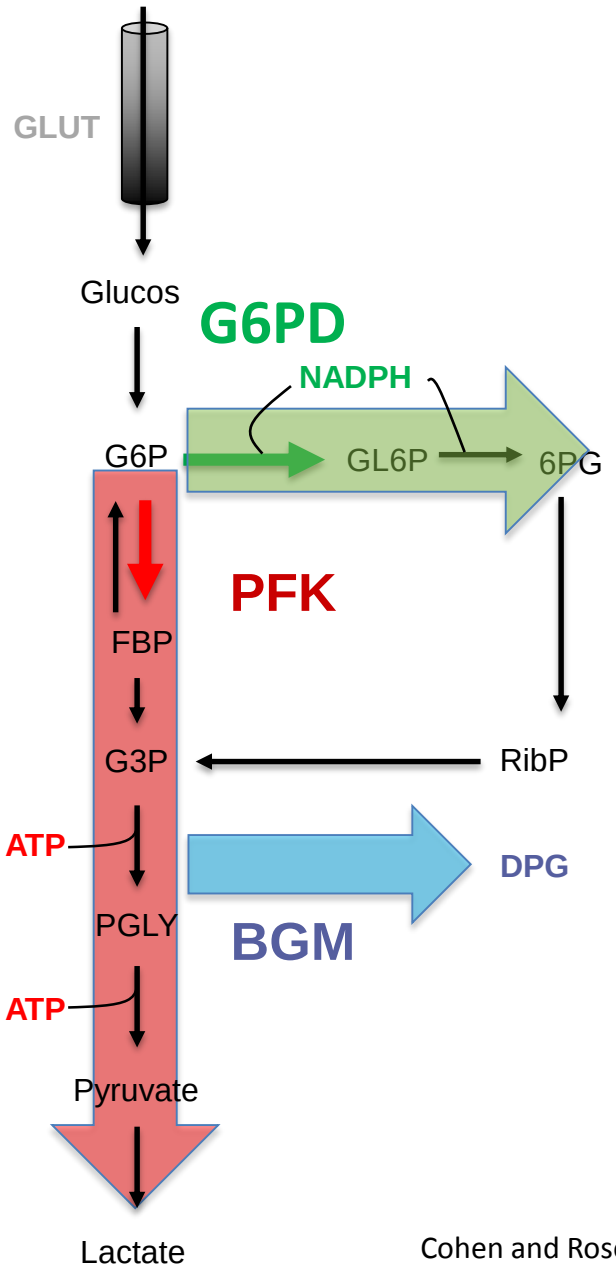
Tsounakas et al. 2015, under review

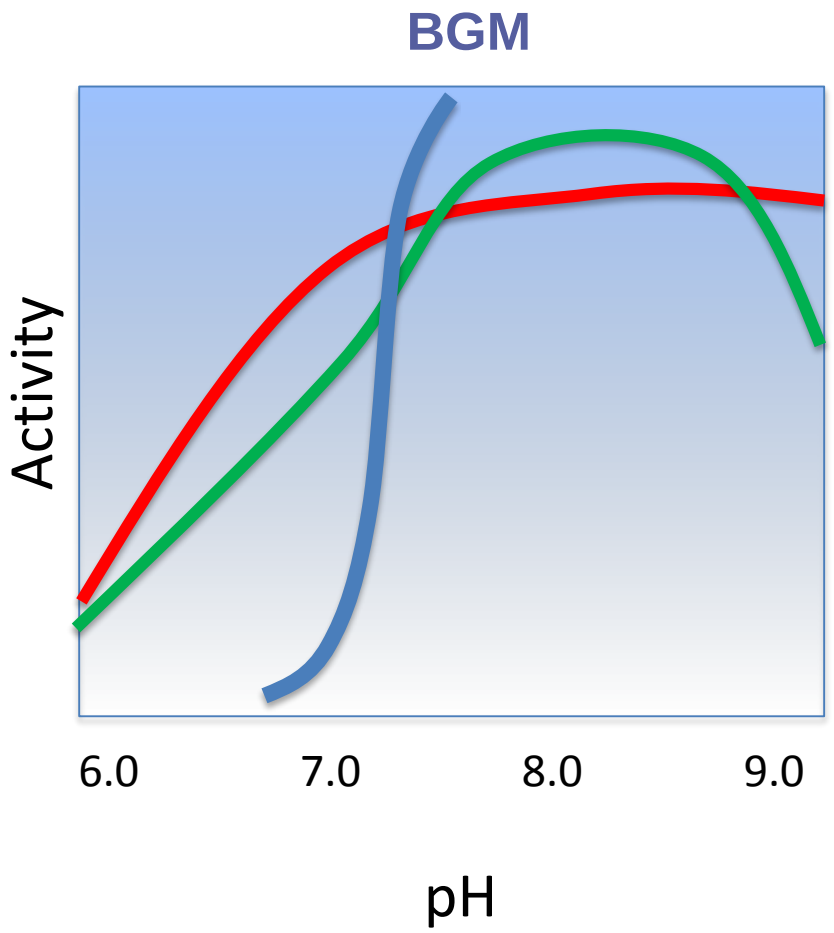
CO_2 levels and alkalinization are important: teachings from Alkaline additive solutions

Chloride shift and Chloride free Additive Solutions



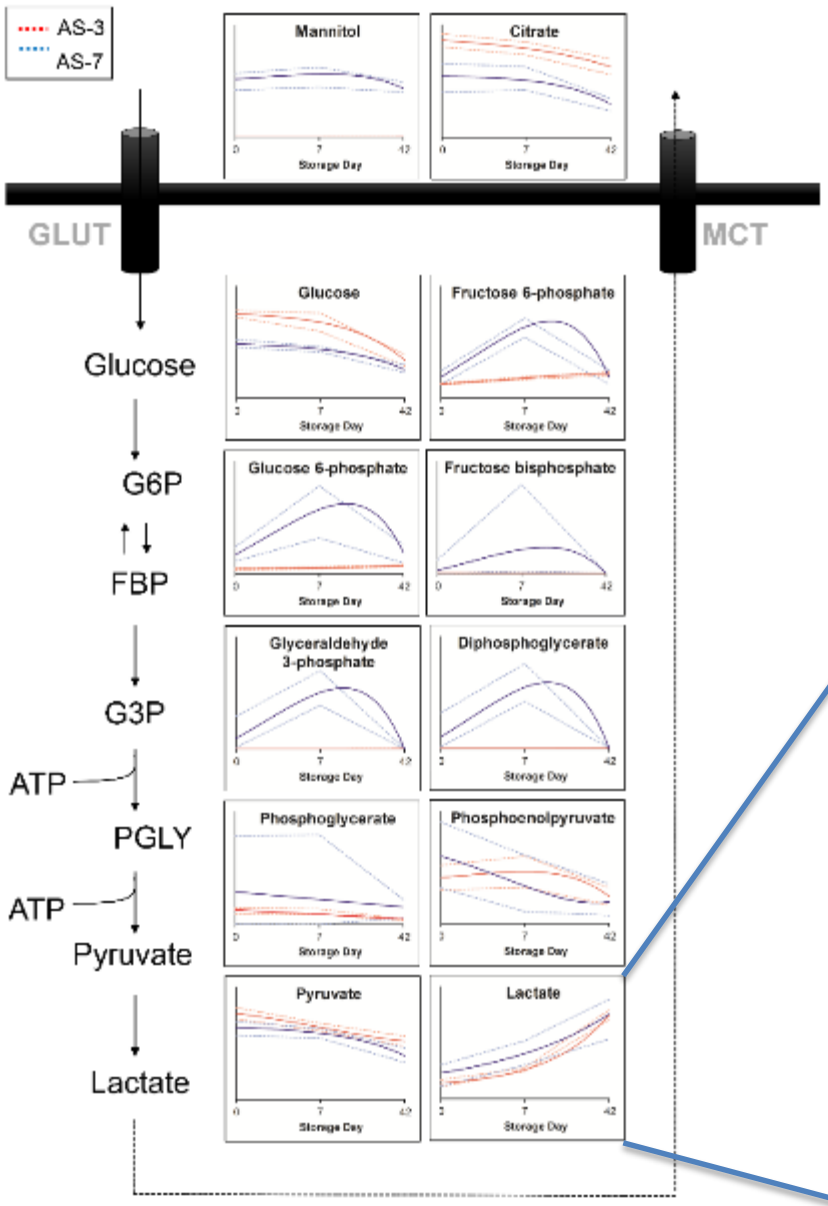
Rationale for the beneficial metabolic effect of intracellular alkalinization

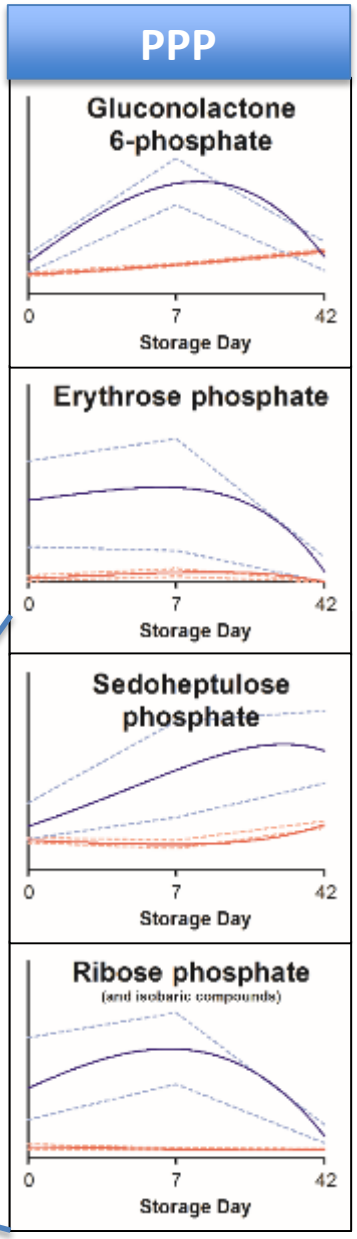


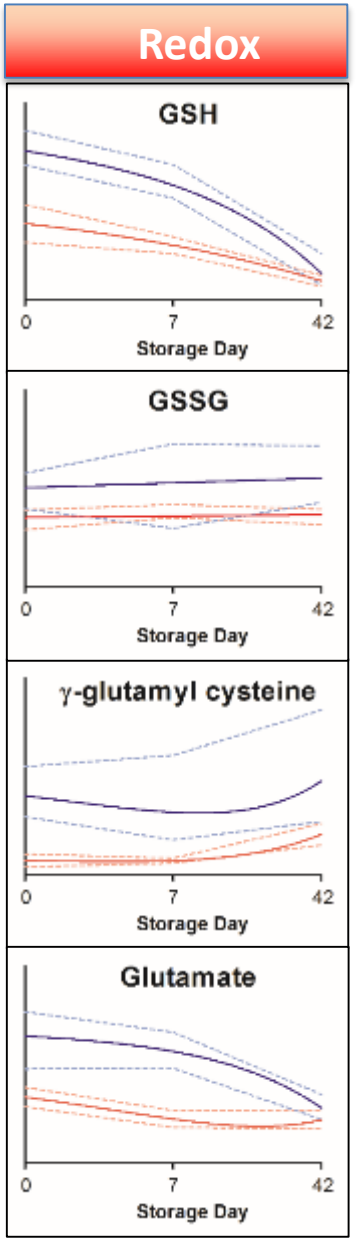


Cohen and Rosemeyer et al., EJB 1969; Erecinska et al., J Neurochem 1995; Rapoport et al, EJB 1977

Alkaline additive solution modulate the redox poise and energy metabolism







Cancelas et al, 2015; D'Alessandro et al., under review

Is beneficial effect of anaerobiosis pH dependent? Anaerobic storage + 5 CO_2 %

pH

7.3

6.9

Ctrl AN AN+CO_2

Alkalinization

pH

Diffusion

OH^- OH^-

Bohr effect

H_2O

Band 3

H^+ OH^-

DeoxyHb

Diffusion

CO_2 CO_2 HCO_3^-

Full Anaerobic

Supernatant (low Chloride)

Balanced pH

Donnan equilibrium

H_2O

Diffusion

OH^- OH^-

Hamberger's (chloride) shift

H^+ HCO_3^- HCO_3^- HCO_3^- HCO_3^- HCO_3^-

Band 3

Cl^- Cl^- Cl^- Cl^- H^+ Cl^- Cl^-

Diffusion

CO_2 CO_2 CO_2 CO_2 CO_2 CO_2 CO_2 CO_2 CO_2

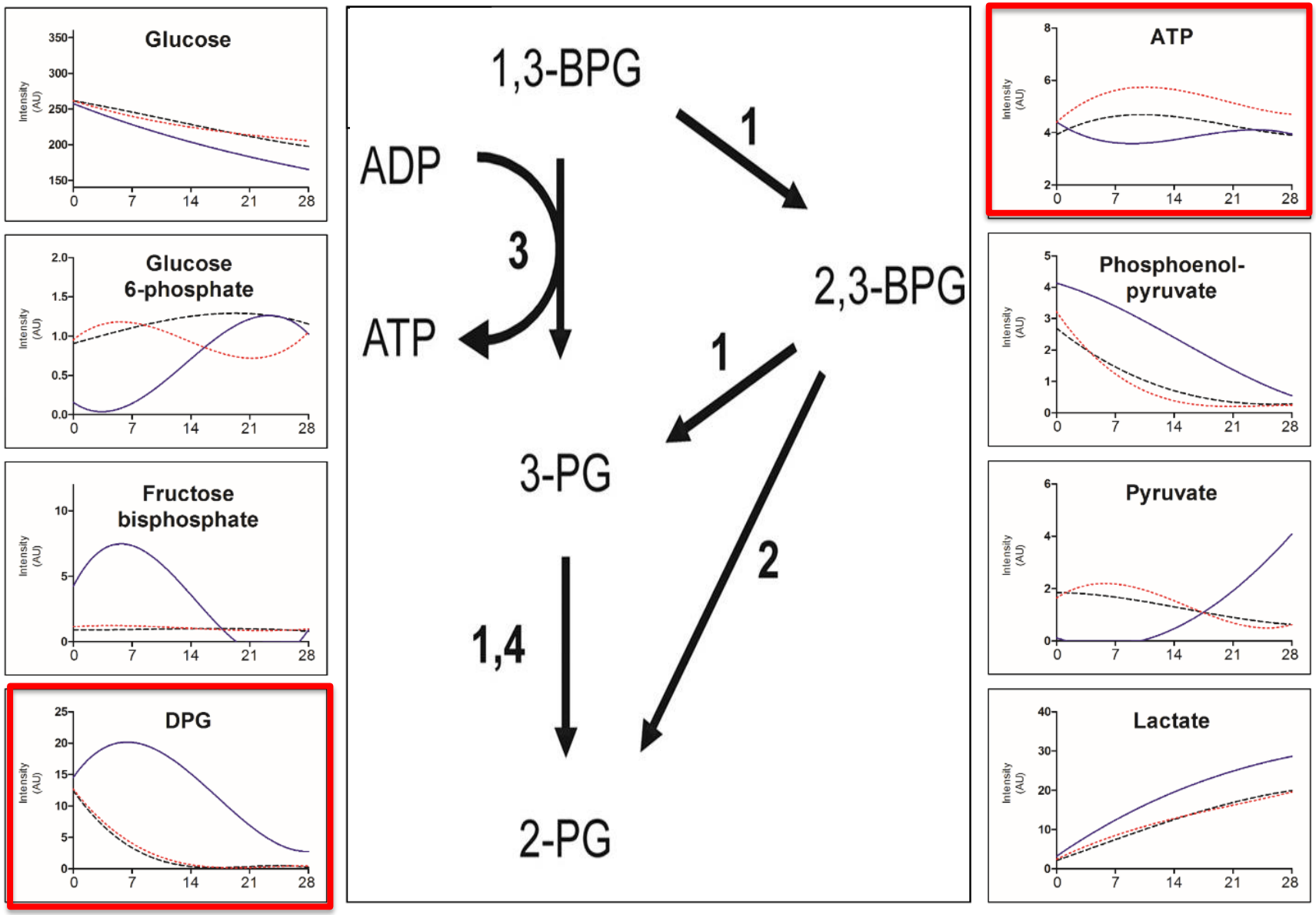
Anaerobic With High CO_2

Red Blood Cell

Anaerobic + CO_2

Yoshida et al., Transfusion 2007 and 2008; Sparrow, Blood Transfusion 2012; D'Alessandro et al, Mol Biosyst 2012

Carbon dioxide masks pH effects on anaerobic storage promoting ATP generation at the expenses of DPG



Solid line=AN-CO_2, Dotted line=AN+CO_2, Dashed line=Control

Dumont et al, 2015