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Defining complex drug mechanisms with metabolomics and multi-omics

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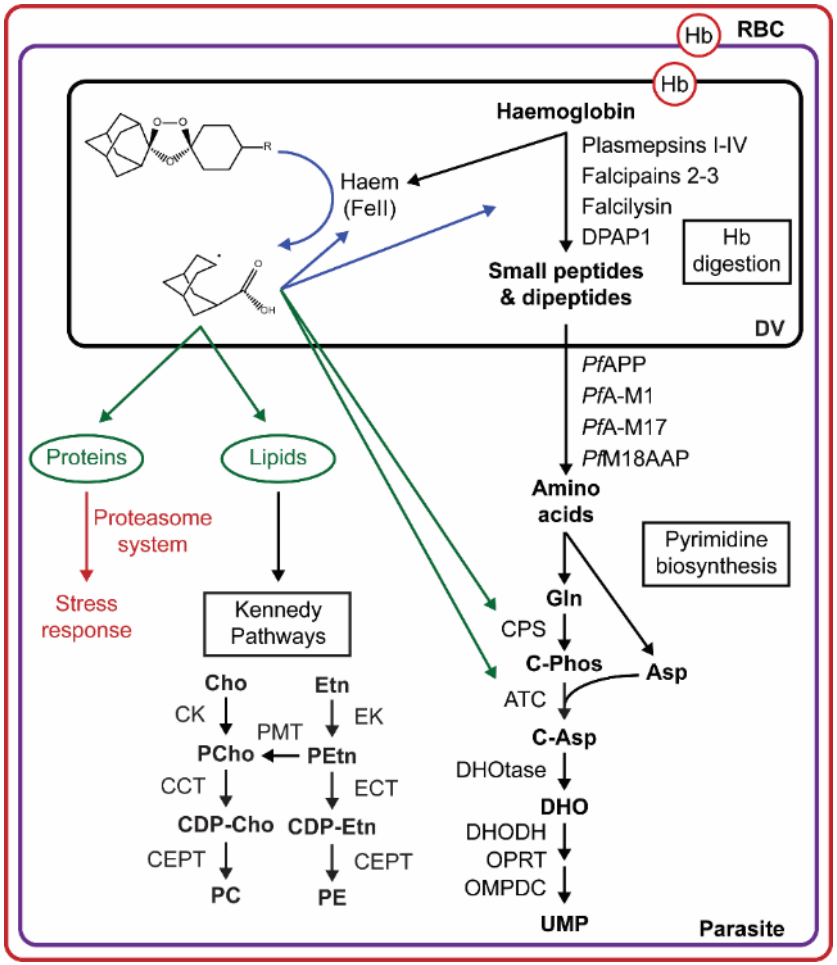
Defining complex drug mechanisms with metabolomics and multi-omics

Graphical Abstract



Abstract: Malaria threatens approximately 40% of the world population, causing 429 000 deaths annually. New ozonide antimalarials (OZs) are now in clinical trials and early clinical usage, but their mechanism of action remains poorly defined. Metabolomics technology offers the opportunity to measure the impact of drug action on cellular metabolism at a system-wide level, allowing unbiased assessment of the key pathways involved in the mechanism of action. The aim of this study was to use metabolomics to reveal the mechanisms of action of OZ antimalarials.

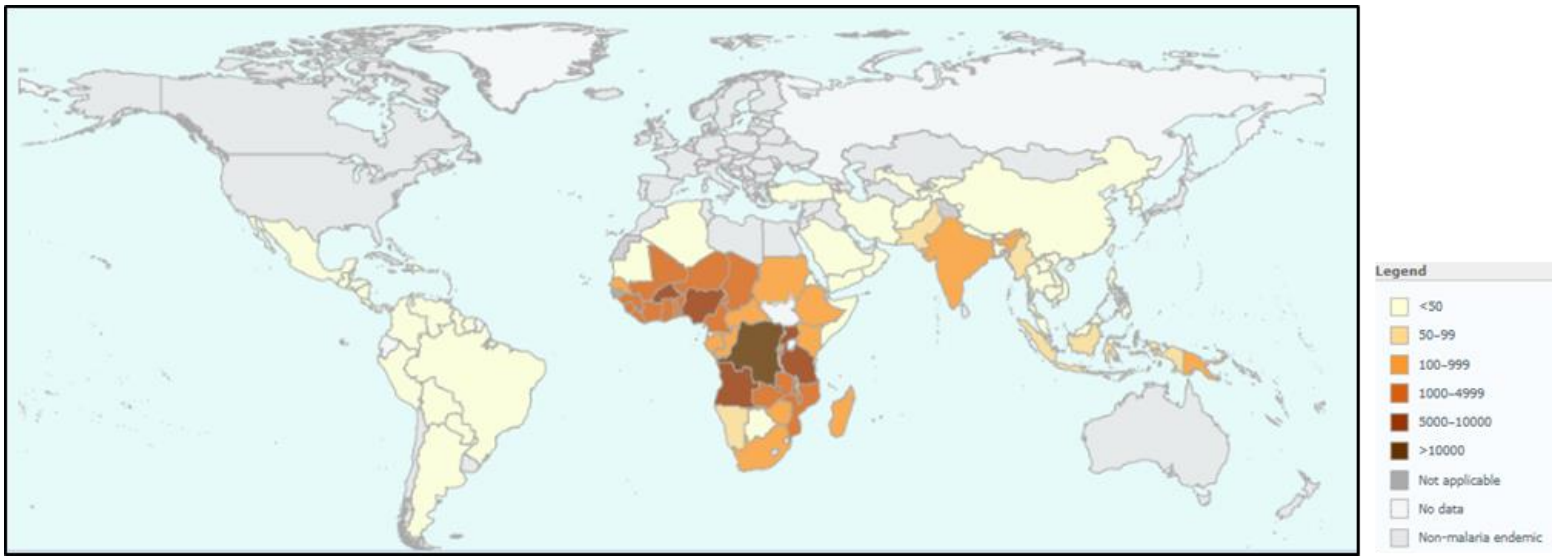
P. falciparum parasites were cultured and treated with OZ antimalarials, followed by metabolomics analysis using LC-MS with high resolution accurate mass spectrometry, revealing depletion of specific small peptides. A dedicated peptidomics method was developed, which revealed drug-induced perturbation to haemoglobin digestion in agreement with the proposal that OZs are activated in the digestive vacuole of the parasite. Additional pathways involved in lipid and nucleotide synthesis were also perturbed with prolonged OZ exposure, and comparative proteomics analysis confirmed the dysregulation of these pathways. This unbiased multi-omics approach revealed an initial impact of OZ antimalarials on haemoglobin digestion, followed by secondary inhibition of additional pathways that are essential for parasite survival and replication.

Keywords: Metabolomics, Proteomics, Peptidomics, Malaria, Drug Resistance

Introduction

Malaria

Each year there are over 200 million cases of malaria worldwide and over 445 000 deaths

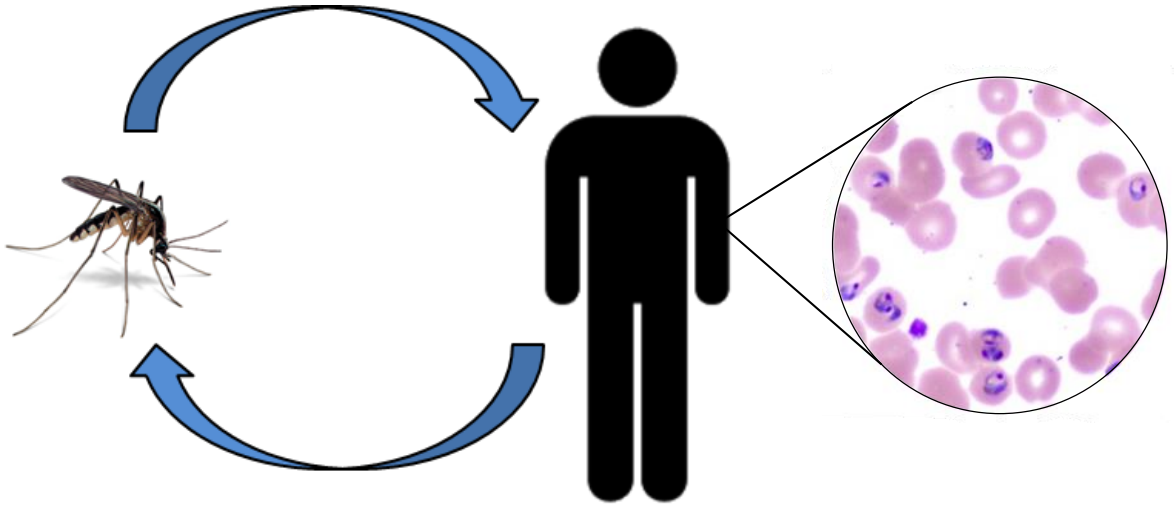


World Malaria Map. WHO, 2015

Introduction

Malaria

- Infectious disease caused by the *Plasmodium* parasite
- Transmitted by the *Anopheles* mosquito

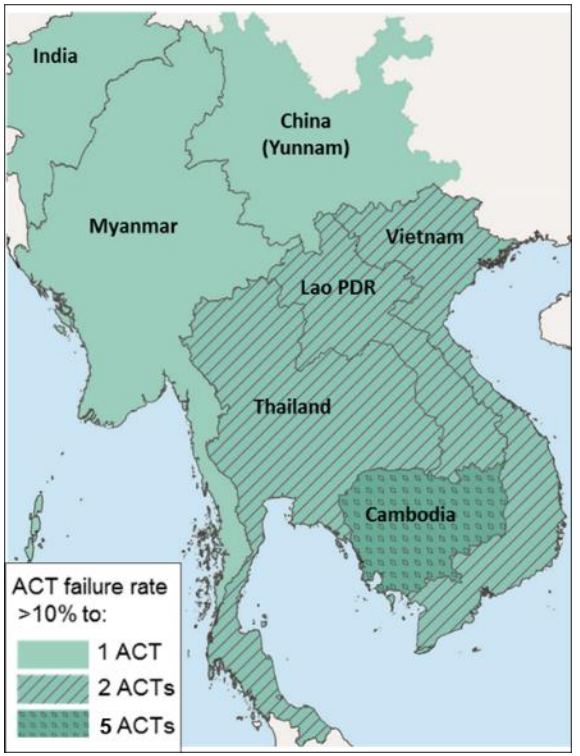


Introduction

Artemisinins

- Artemisinin combination therapies are the first-line treatment for malaria
- ACT treatment is failing in Southeast Asia due to the emergence and spread of artemisinin resistant *Plasmodium falciparum* parasites
- Artemisinin-resistance has recently been reported in Africa[1]
- Severely threatens global malaria control and eradication efforts

New antimalarials are urgently needed

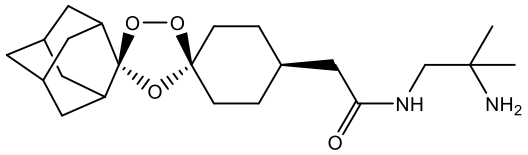


Update on artemisinin and ACT resistance, WHO, 2016

[1] Lu *et al*., 2017

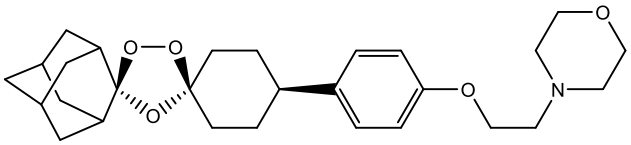
Introduction

OZ antimalarials



OZ277 (arterolane)

Approved for use in India and parts of Africa in combination with piperaquine (Synriam™)

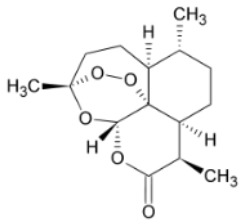


OZ439 (artefenomel)

Undergoing phase IIb clinical trials in combination with ferroquine

Development based on the artemisinin antimalarials

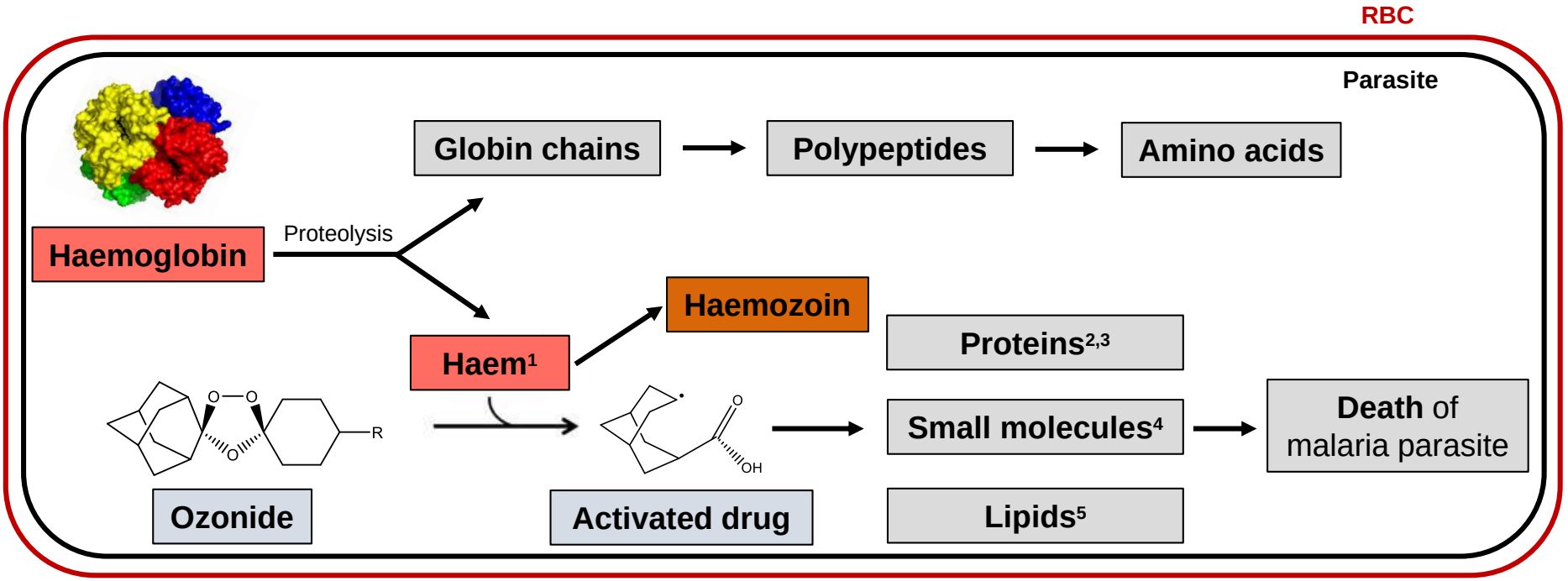
Mode of action is not completely understood



Artemisinin

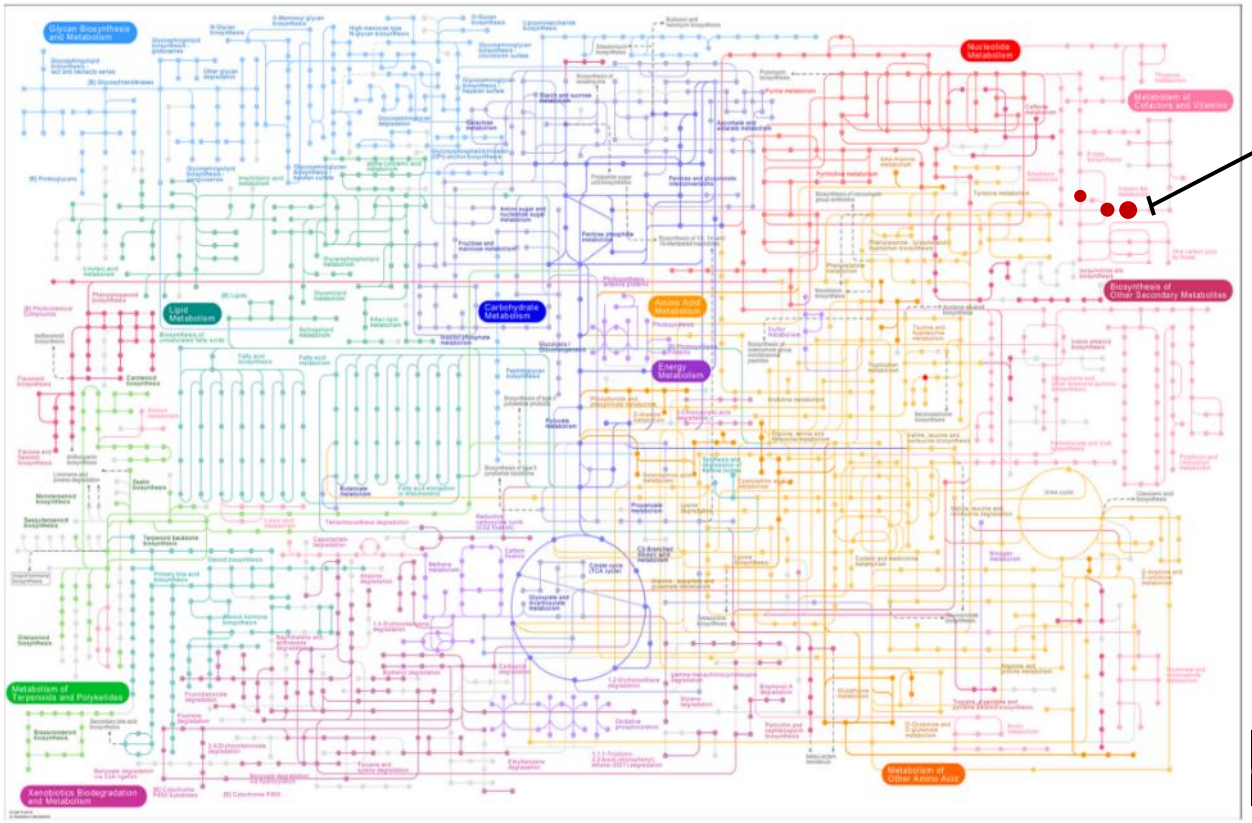
Introduction

OZ mechanism of action



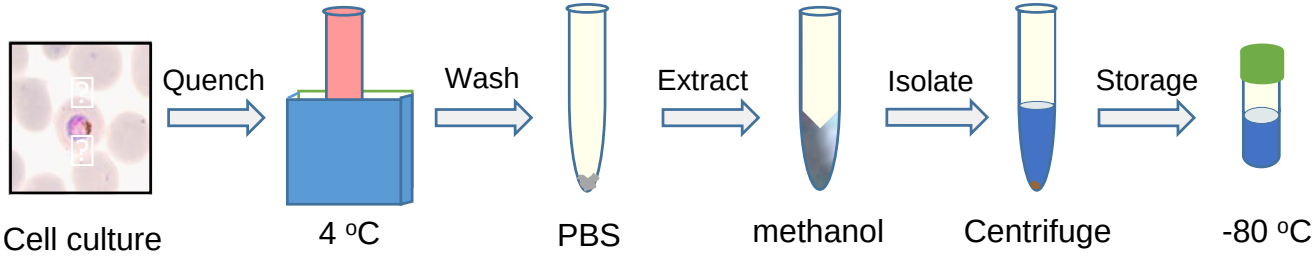
What parasite biochemical pathways are perturbed by ozonide antimalarial treatment?

Methodology



Methodology

Incubation/
Extraction



LC-MS: HILIC-Orbitrap
(Untargeted)

Data Analysis: IDEOM

- Noise filters
- Metabolite identification
- Data visualisation

Creek *et al.* Bioinformatics, 2012

Methodology

Metabolite Identification:

Exact mass + retention time

~300 authentic standard RT's

Exact mass & predicted RT

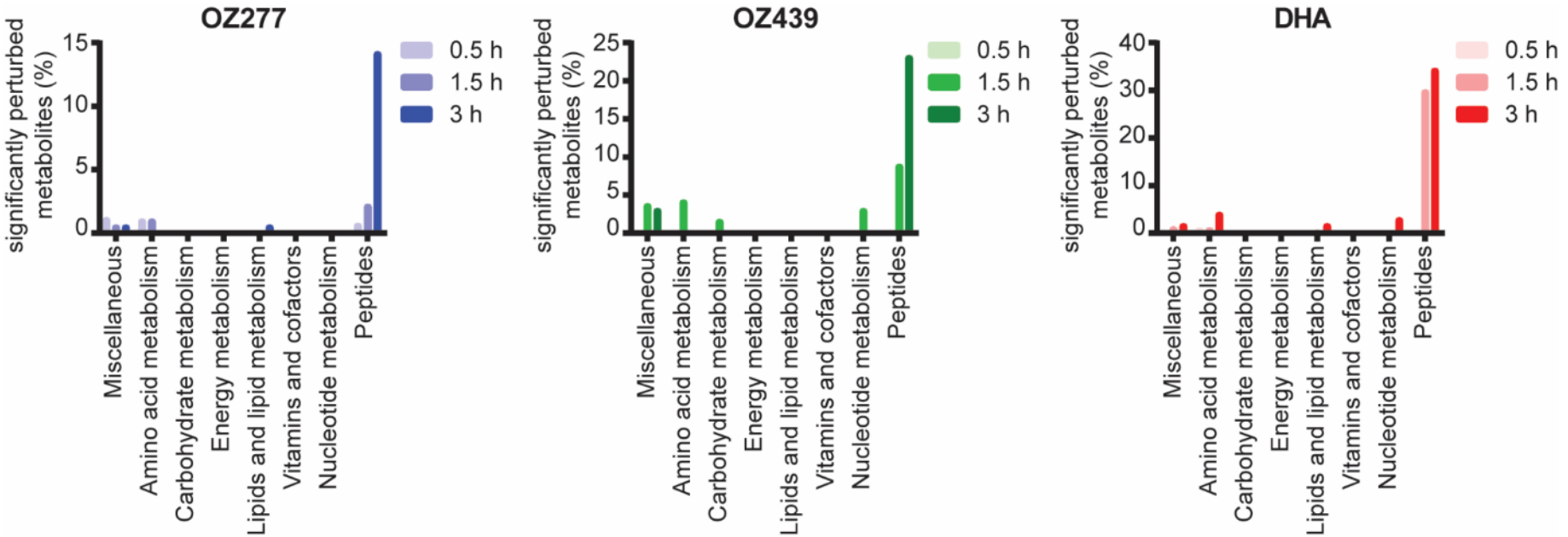
Online metabolite databases

KEGG BioCyc Database Collection hmdb The Human Metabolome Database

Distribution of the 656 putatively identified metabolites

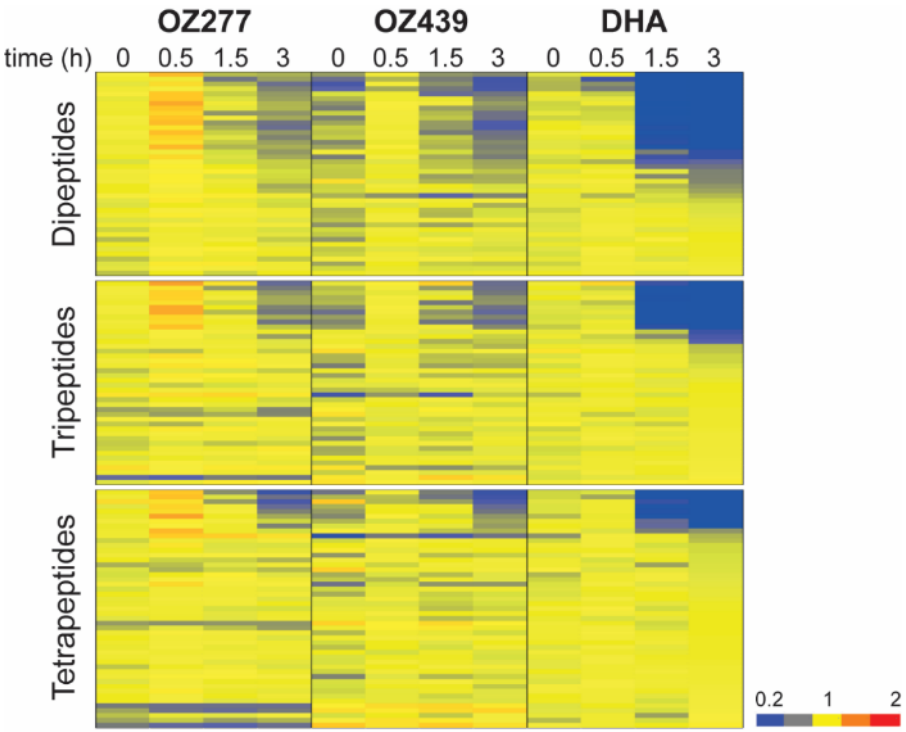
Results and Discussion

Pathway enrichment analysis of significantly perturbed metabolites ($p<0.05$) after treatment with OZ or artemisinin

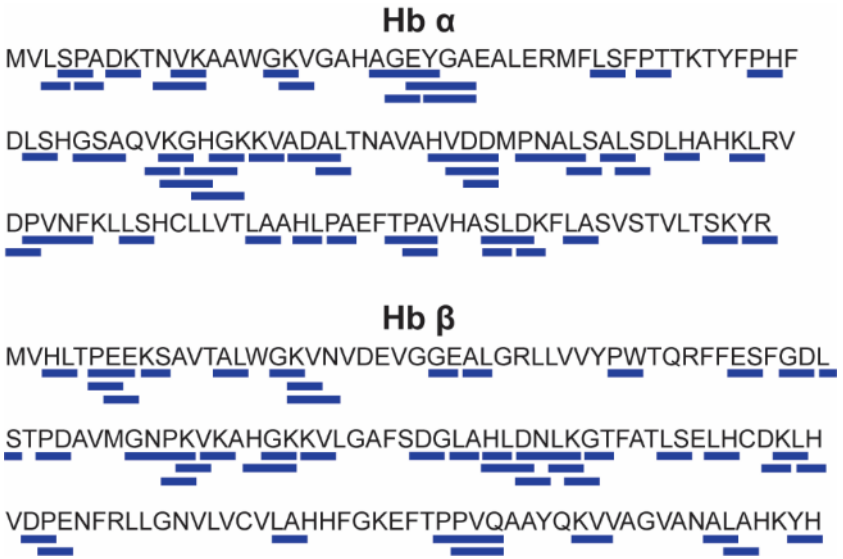


Overview of ozonide-induced peptide perturbations

Heatmap of all putative peptides

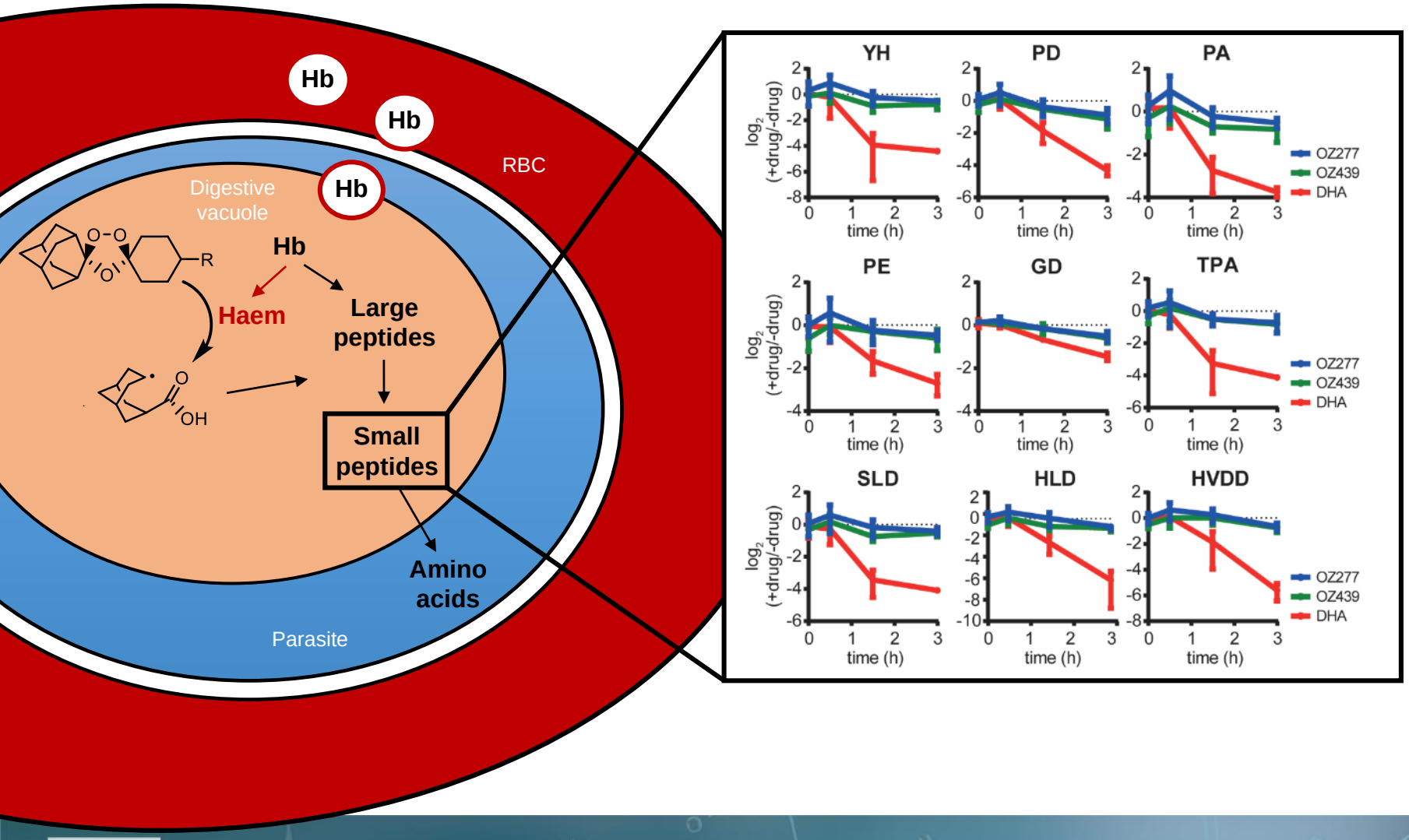


Haemoglobin (Hb) α and β sequence coverage



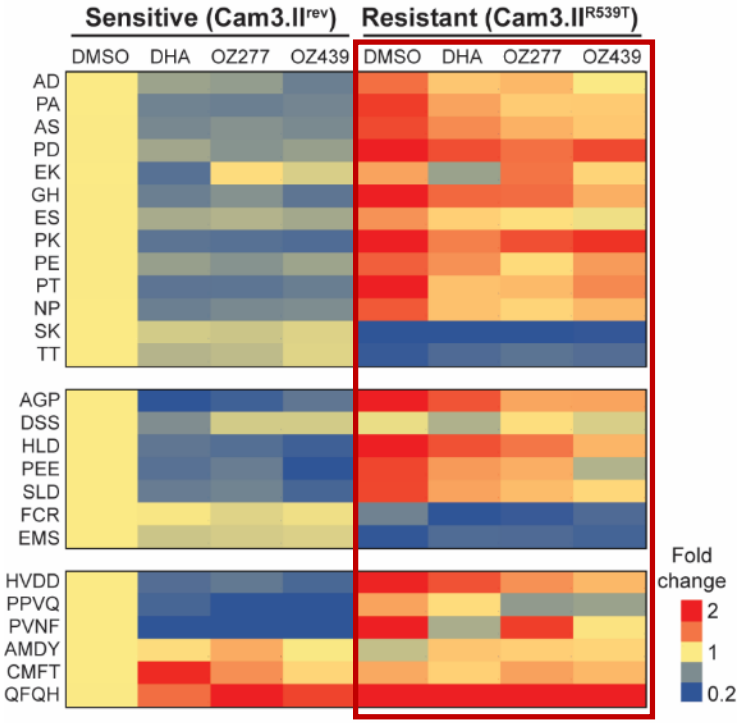
(≥ 1.5 fold-change vs control)

Ozonide-dependent disruption of haemoglobin catabolism

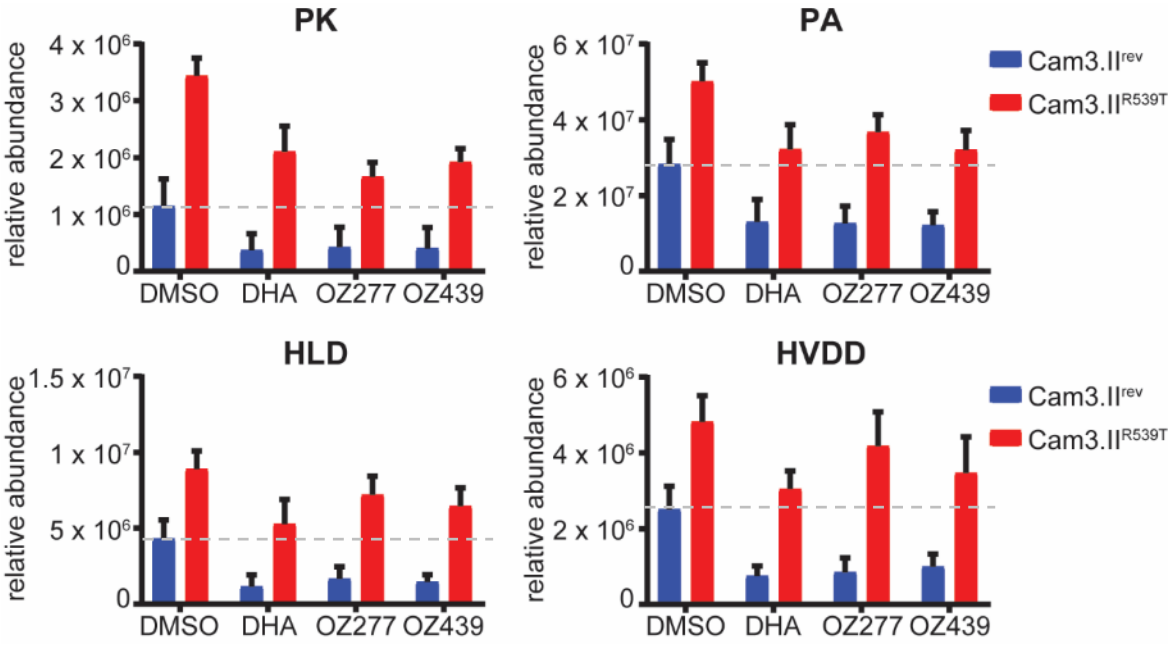


Disruption of haemoglobin catabolism in artemisinin resistant parasites

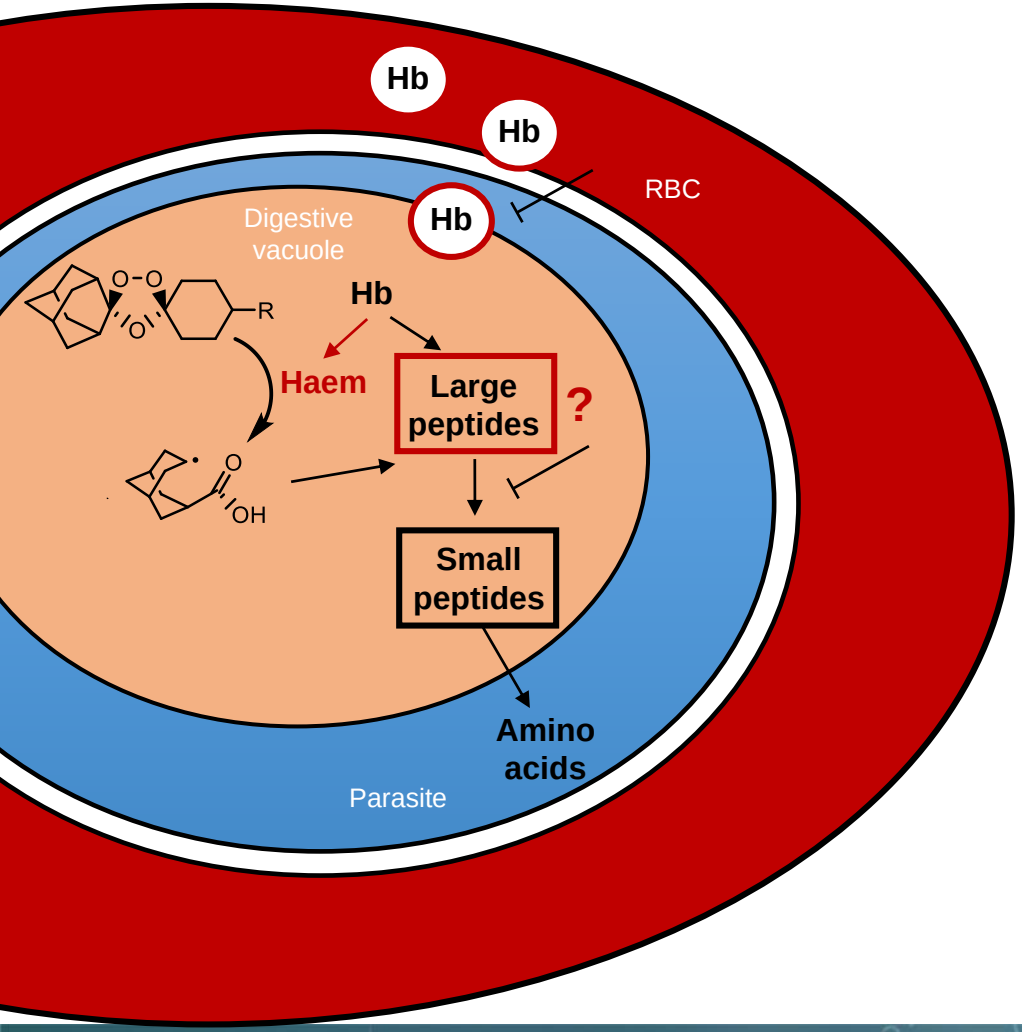
Differentially abundant peptides (fold-change ≥1.5)



Examples of differentially abundant peptides



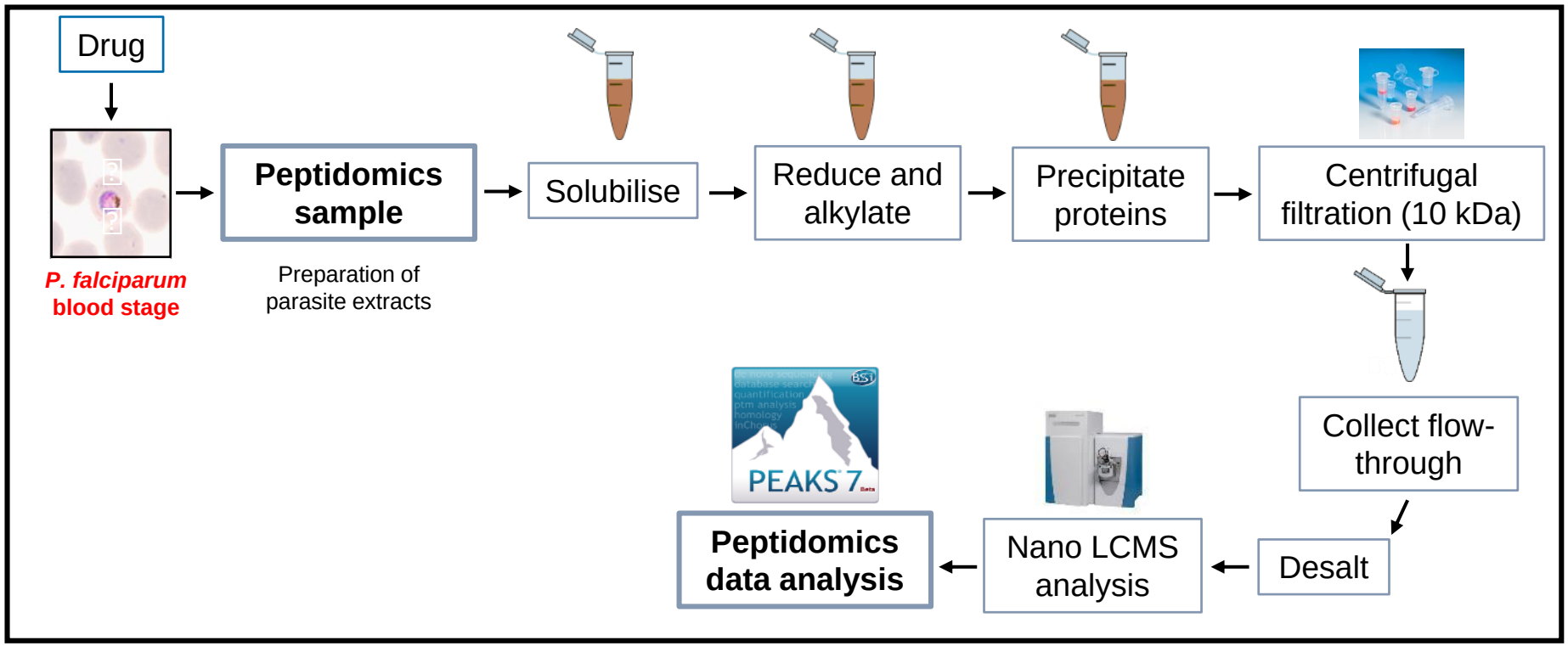
Ozonide-dependent disruption of haemoglobin catabolism



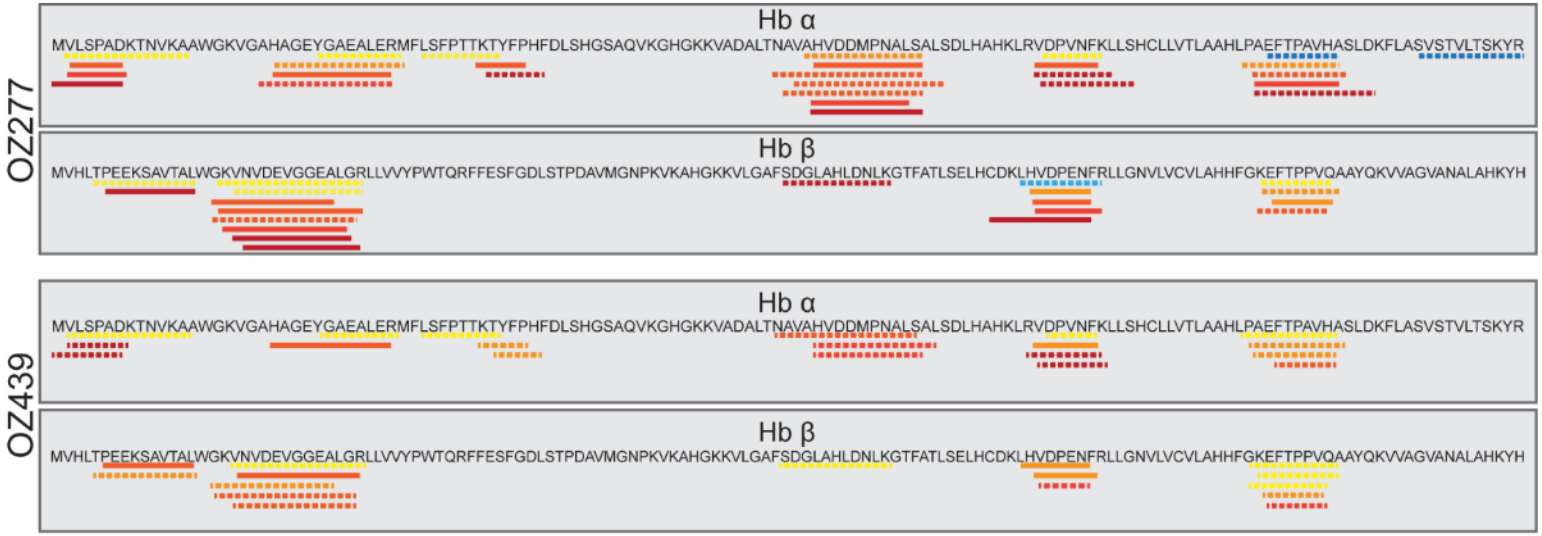
- Disruption of haemoglobin catabolism is involved in ozonide activity
- Depletion of short chain haemoglobin-derived peptides
  - Impaired haemoglobin uptake
  - Inhibition in the degradation pathway

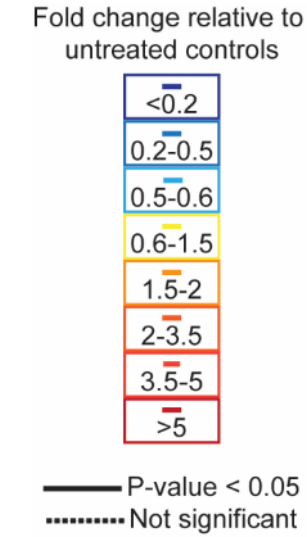
What happens to longer chain haemoglobin peptides?

Peptidomics analysis of ozonide-induced peptide perturbations

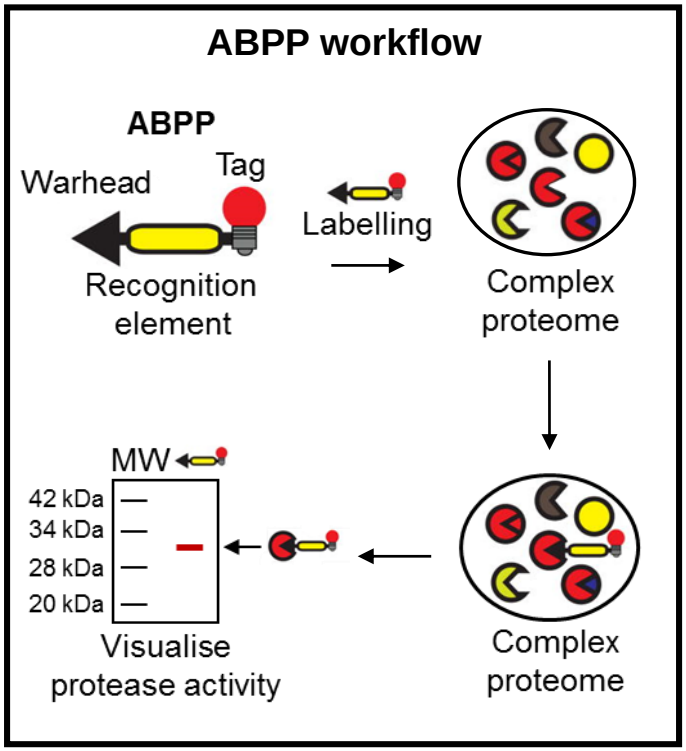


Sequence coverage and relative abundance of all haemoglobin-derived peptides

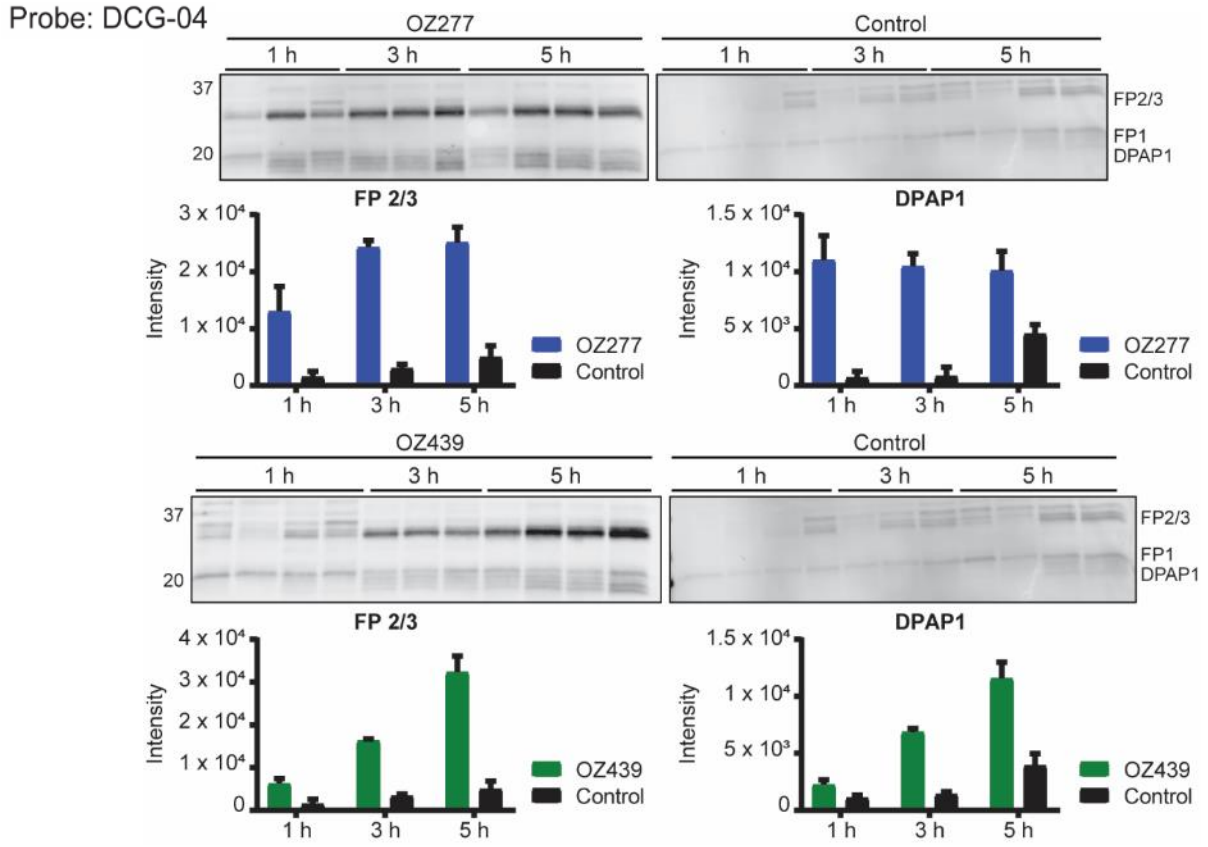




Protease activity in ozonide treated *P. falciparum* parasites

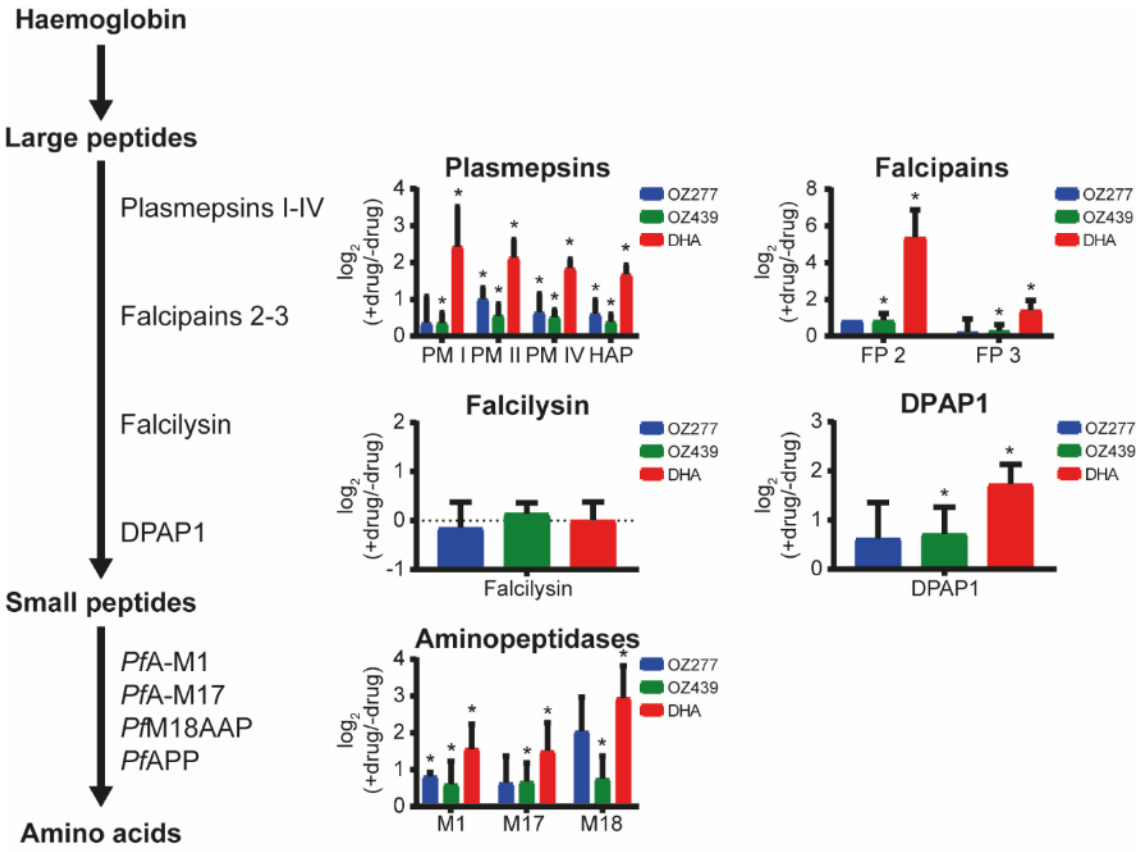


Adapted from Deu *et al*., 2012



Proteomics analysis of ozonide treated *P. falciparum*

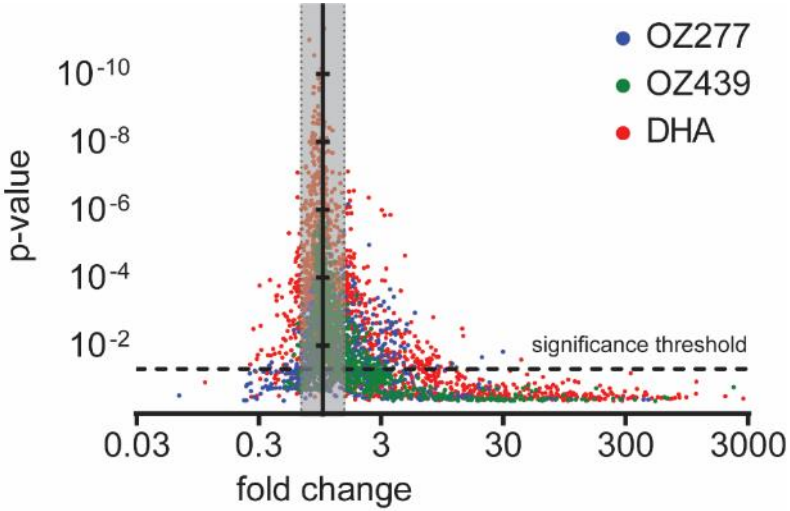
- Targeted analysis of proteases in the Hb digestion pathway
- Most Hb proteases are increased in abundance after treatment
- Elevated protease levels may be a response to impaired Hb digestion



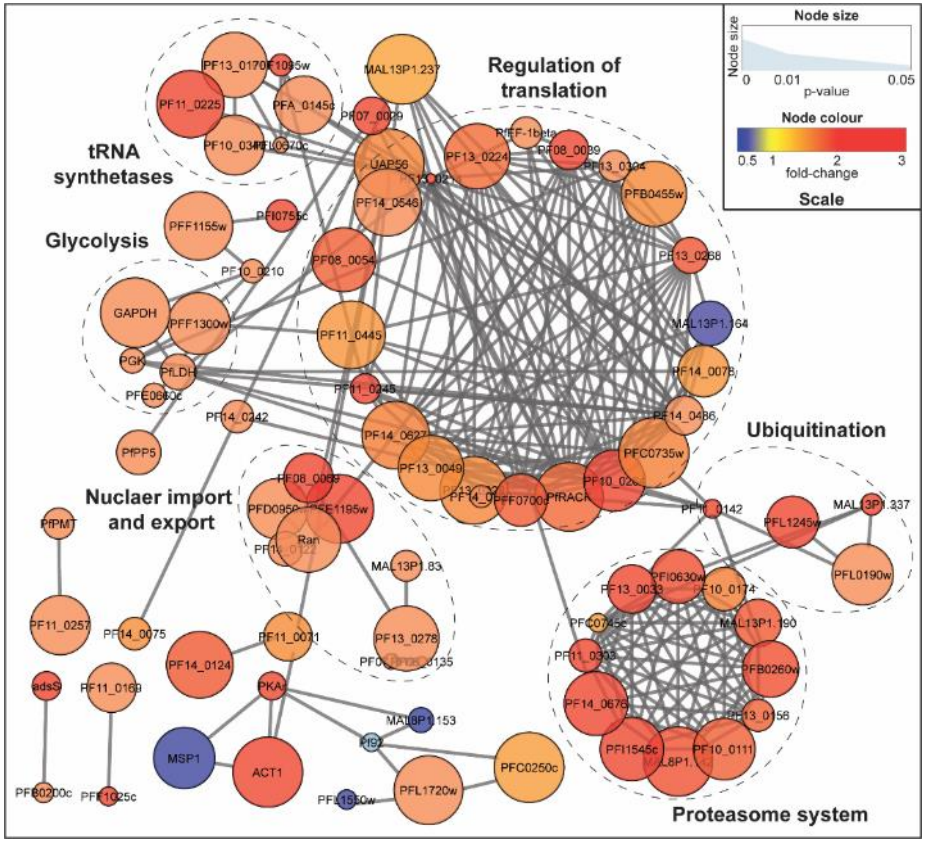
Proteomics analysis of ozonide treated *P. falciparum*

- OZ277 – 1294 proteins identified: ~10% upregulated <1% downregulated
- OZ439 – 1284 proteins identified: ~10% upregulated <1% downregulated
- DHA – 1613 proteins identified: ~20% upregulated ~5% downregulated

Volcano plot of peroxide-induced disruption to the *P. falciparum* proteome



Clustering analysis of parasite proteins perturbed following OZ277 treatment

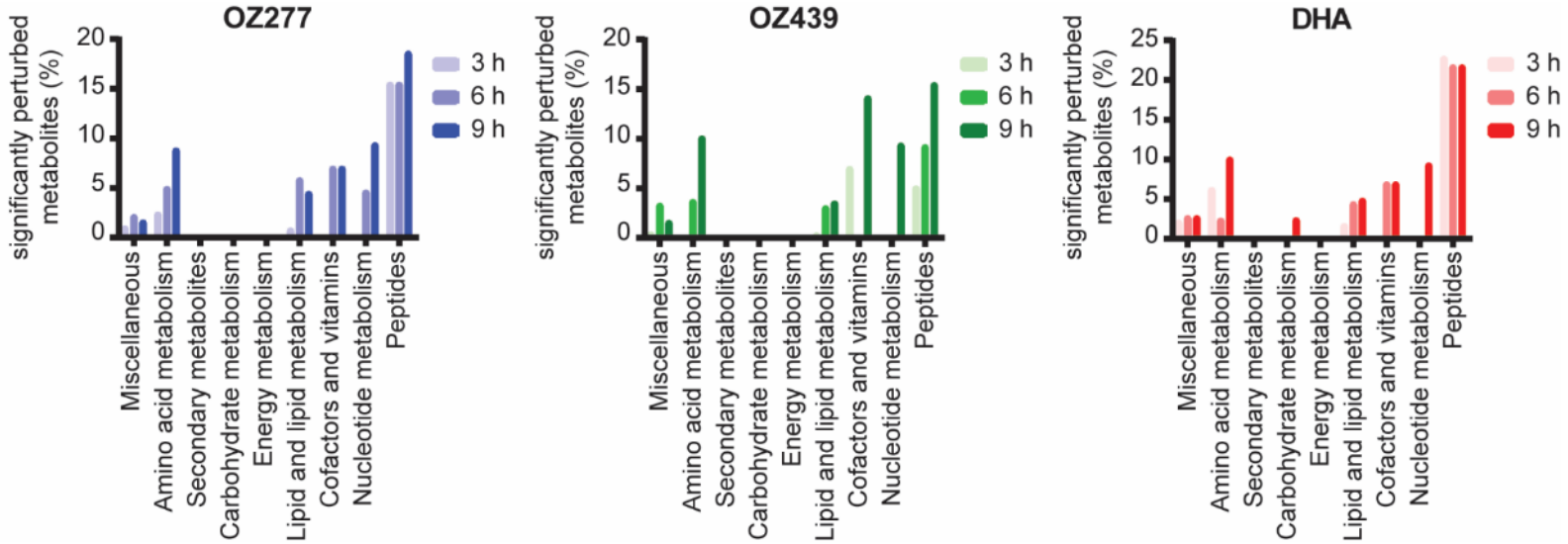


- Two major protein networks upregulated by OZ277
  - Translation regulation (p-value = 5.49E-9)
  - Proteasome system (p-value = 3.44E-6)
- Similar clustering for OZ439 and DHA

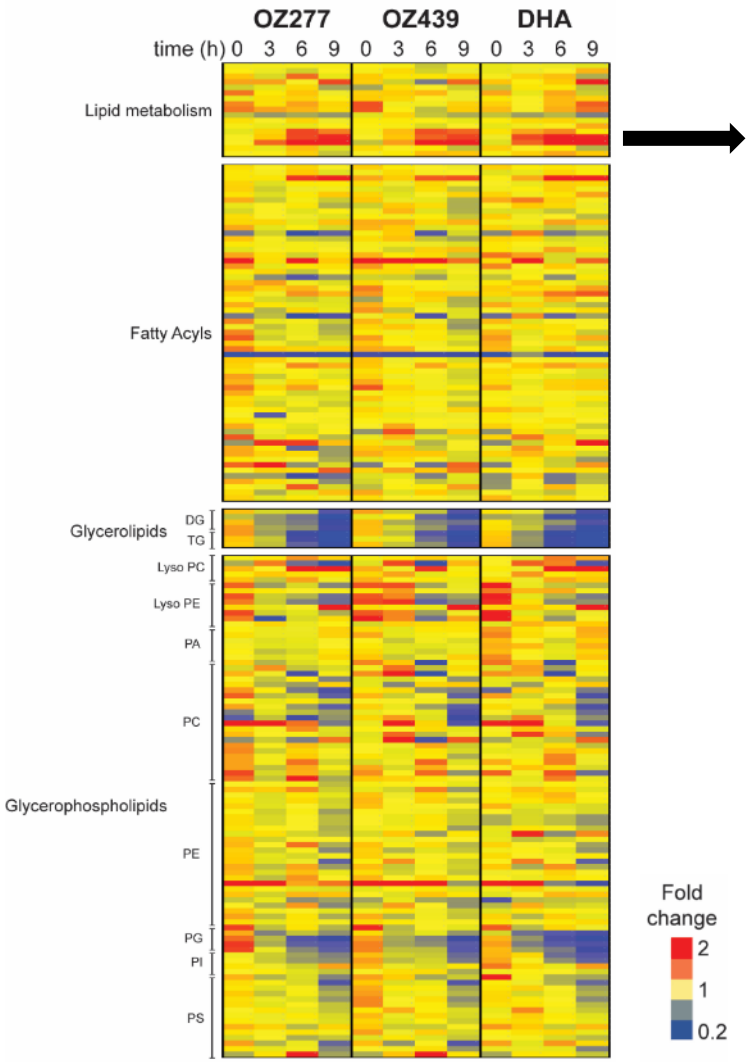
Metabolomics analysis of *P. falciparum* exposed to prolonged OZ treatment

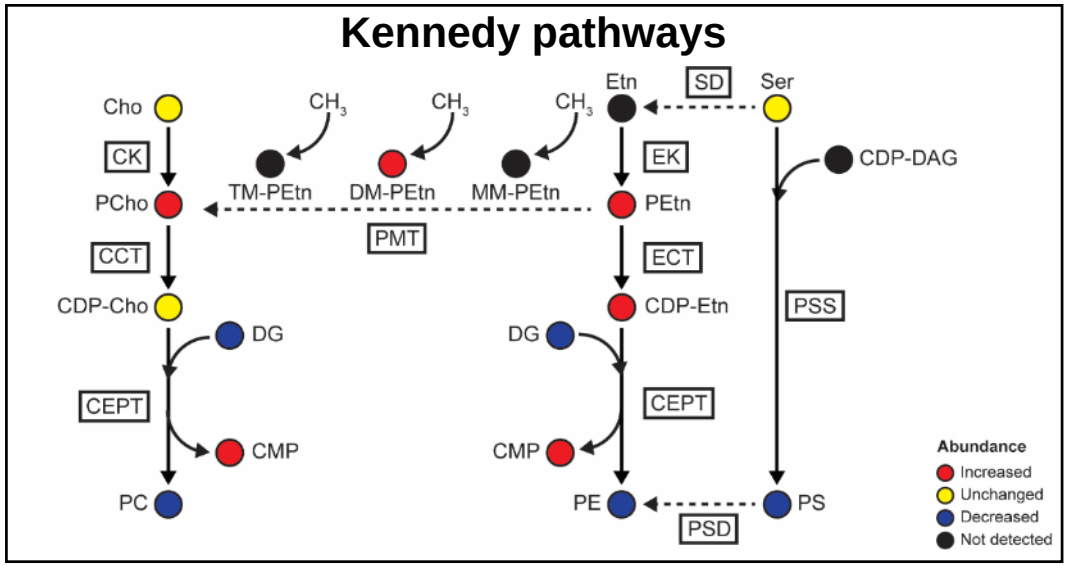
- Up to 9 h of drug exposure

Pathway enrichment analysis of significantly perturbed metabolites ($p<0.05$)

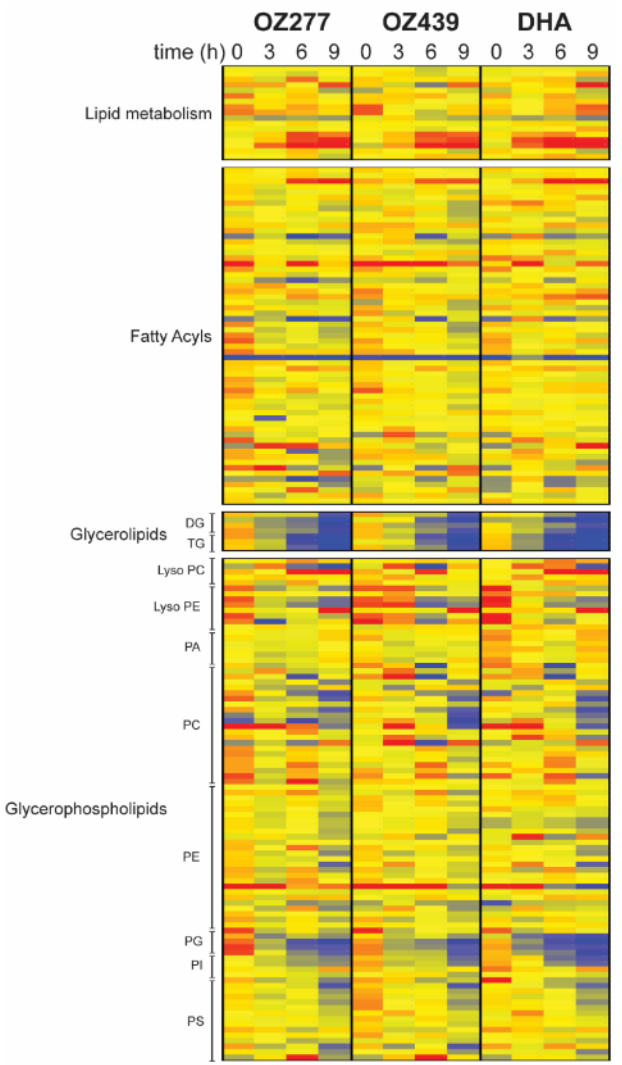


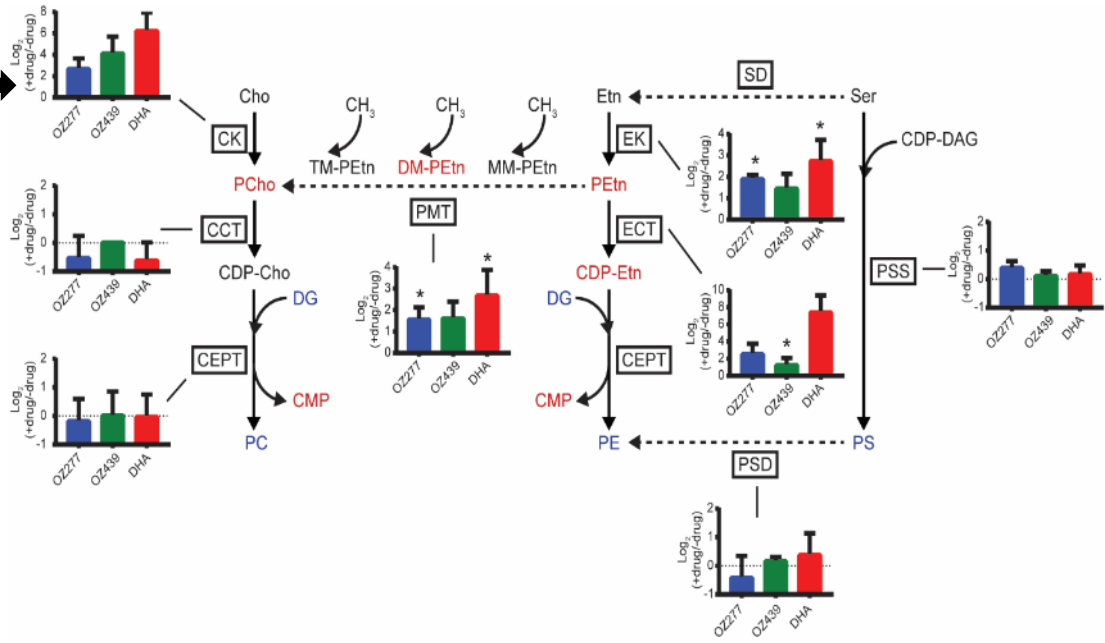
Impact of prolonged OZ exposure on lipid metabolism





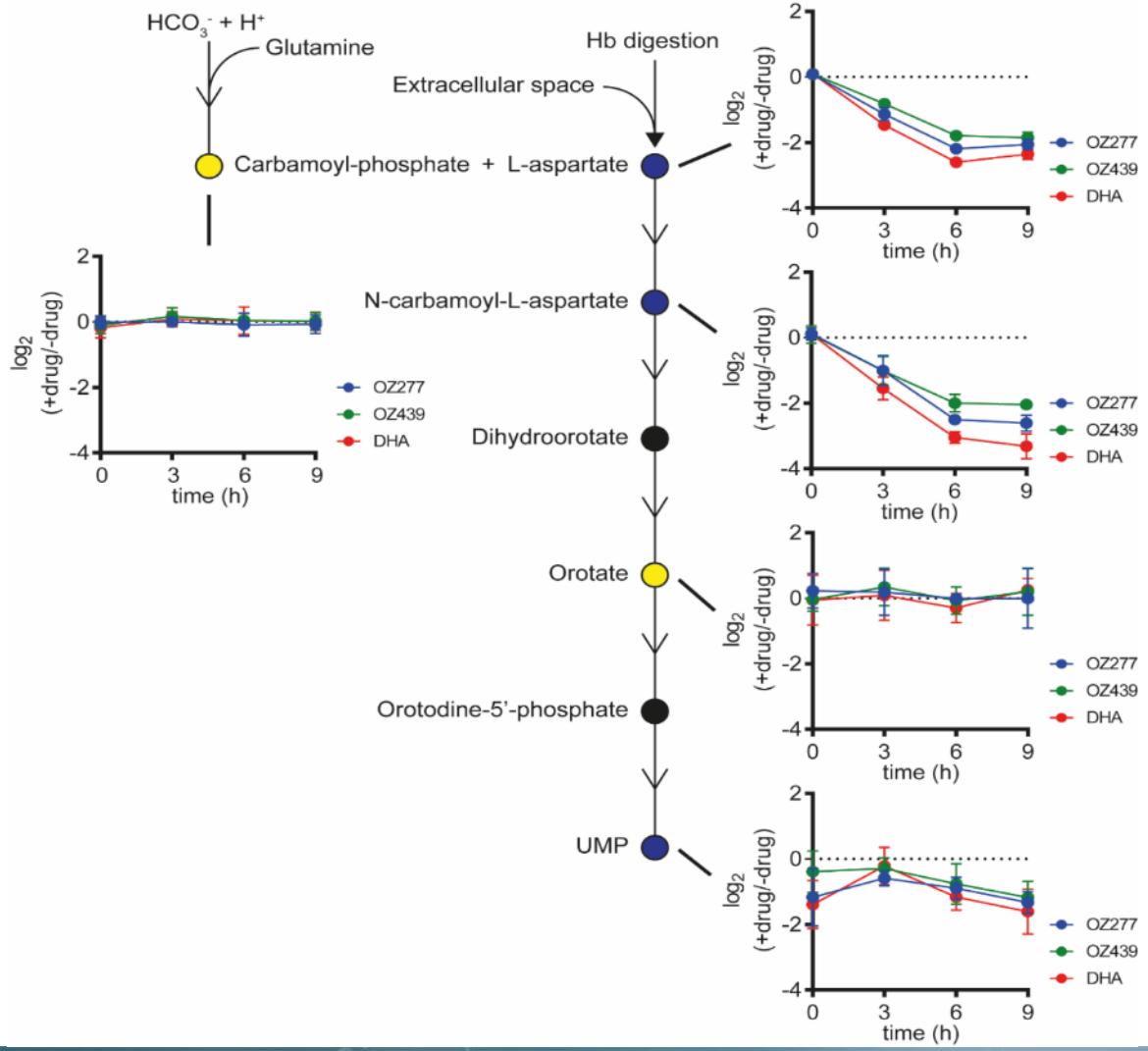
Impact of prolonged OZ exposure on lipid metabolism





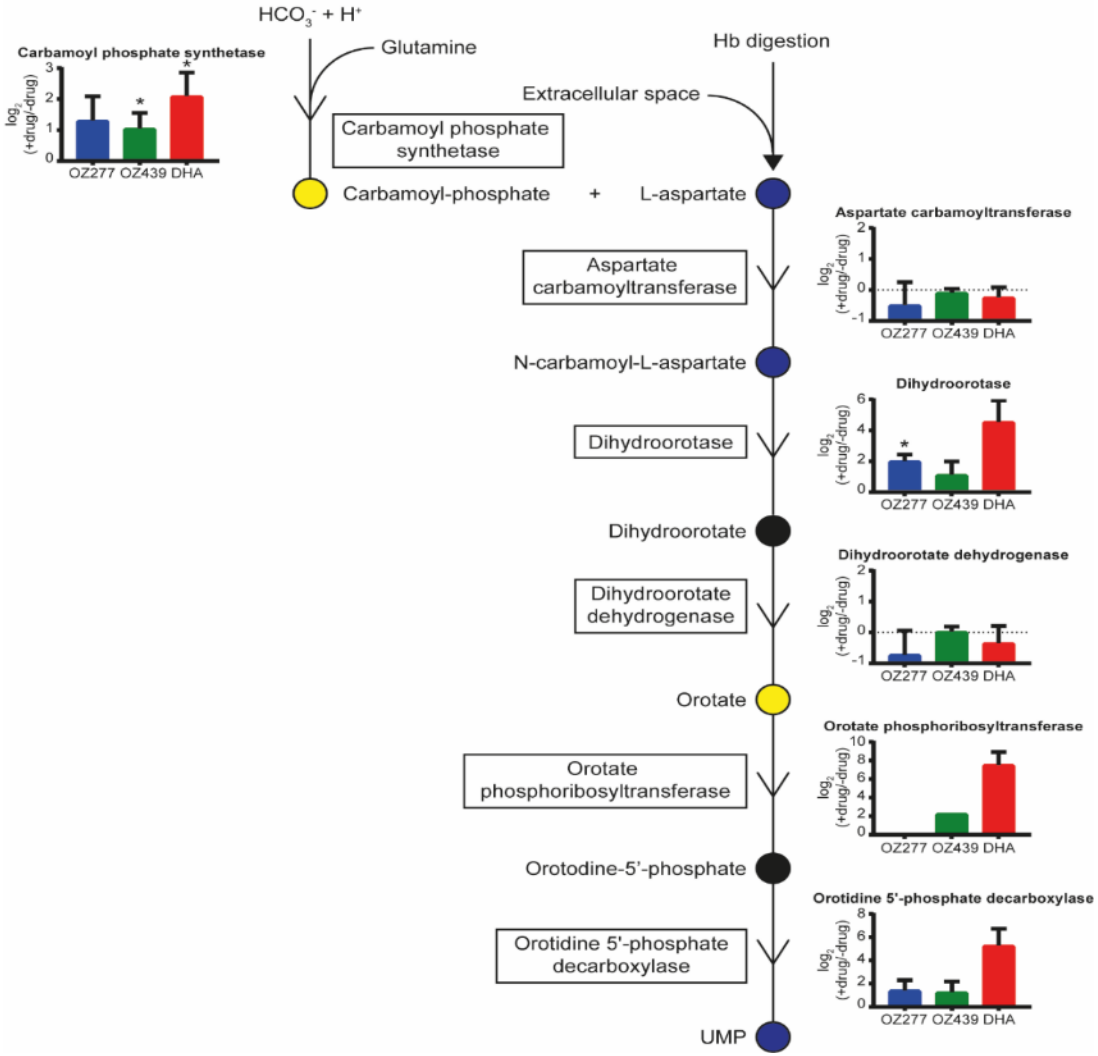
Impact of prolonged OZ exposure on nucleotide metabolism

Metabolites:

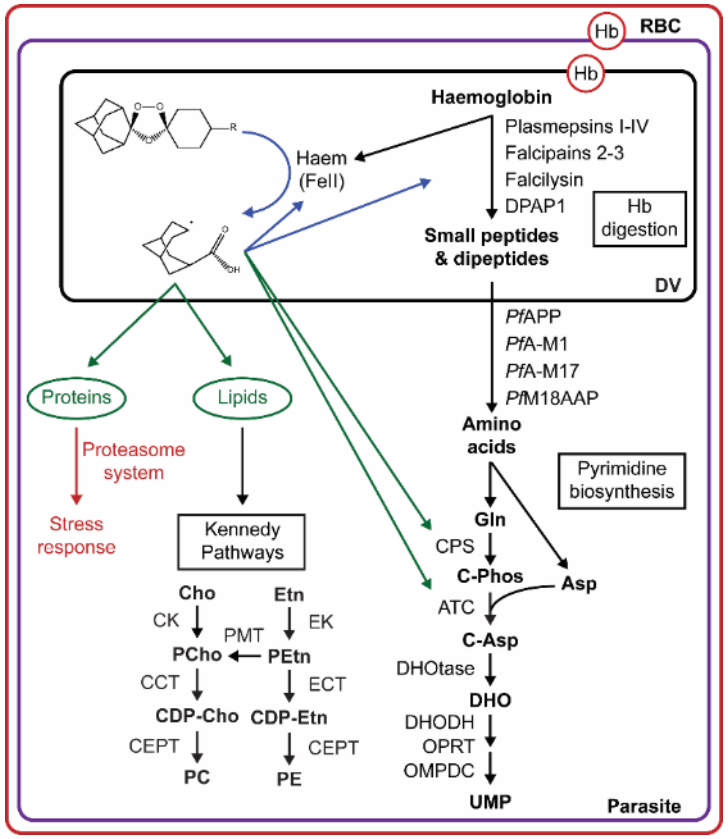


Impact of prolonged OZ exposure on nucleotide metabolism

Proteins:



Conclusions



- Ozonides initially disrupt Hb catabolism
  - Rapid depletion of short-chain Hb peptides (< 3 h)
  - Accumulation of long-chain Hb peptides
- Parasites correct impaired Hb digestion by increasing the abundance and activity of Hb proteases
- Prolonged ozonide exposure induces further damage
  - Kennedy pathways
  - Pyrimidine biosynthesis
- To mitigate ozonide-mediated cellular damage parasites engage a stress response
  - Translational regulation
  - Proteasome system

Acknowledgments

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