About OMICS Group

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OMICS International has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

METABOLOMIC STUDY OF THE ACUTE INFLAMMATORY RESPONSE IN A PORCINE MODEL OF COMBAT TRAUMA INJURY

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Trauma Injury

- One of the leading causes of morbidity and mortality worldwide[1]

- Leading cause of death for people under 45[2]

- Has significant personal, social & economic impact

1. R. Lozano, M. Naghavi, K. Foreman, S. Lim, K. Shibuya, et al., *The Lancet*, 2012, 380, 2095-2128.
2. E. G. Krug, G. K. Sharma and R. Lozano, *American Journal of Public Health*, 2000, 90, 523-526.
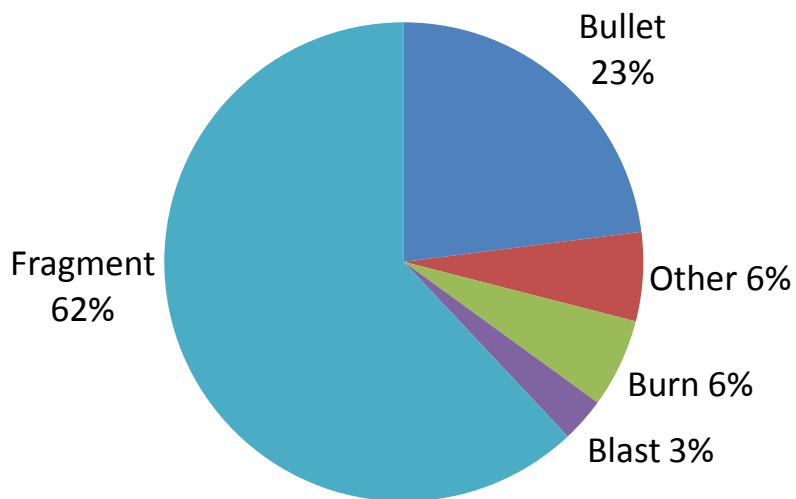
Profile of Combat Trauma

Higher risk of mortality[3]

- Nature of wounding agents
- Multiple wounding
- Persistence of threat
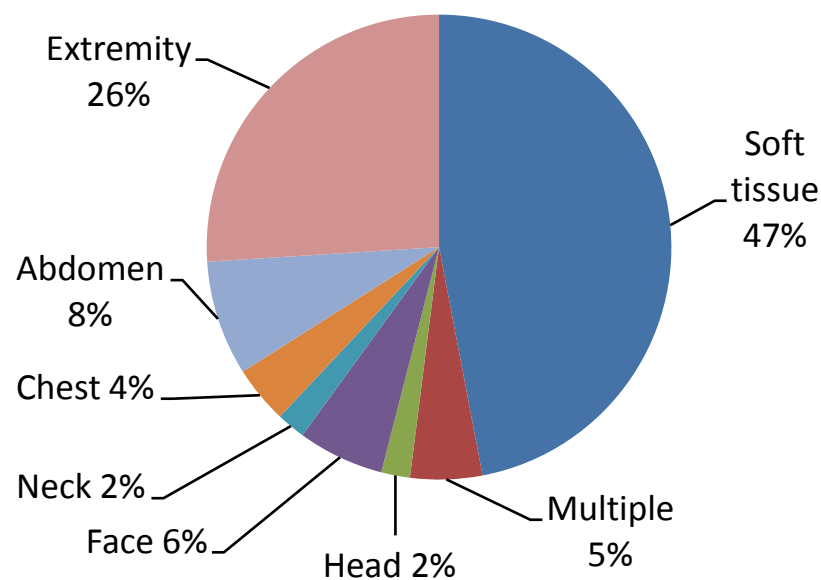- Limited resources
- Delayed access to definitive care

3. Champion, H. R.; Bellamy, R. F.; Roberts, C. P.; Leppaniemi, A., *J Trauma Acute Care* Surg, 2003, 54 (5), S13-S19.

Profile of Combat Trauma

Mechanism of wounding

- Bullet 23%
- Fragment 62%
- Other 6%
- Burn 6%
- Blast 3%

Site of primary injury

- Extremity 26%
- Soft tissue 47%
- Abdomen 8%
- Chest 4%
- Neck 2%
- Face 6%
- Head 2%
- Multiple 5%

Prevalence of penetrating trauma in combat injuries

Secondary Complications After Injury

- Complex network of changes[4]

- Can affect organs far from site of injury

- They can be:
  - Normal physiological changes that proceeds uncontrolled[5]
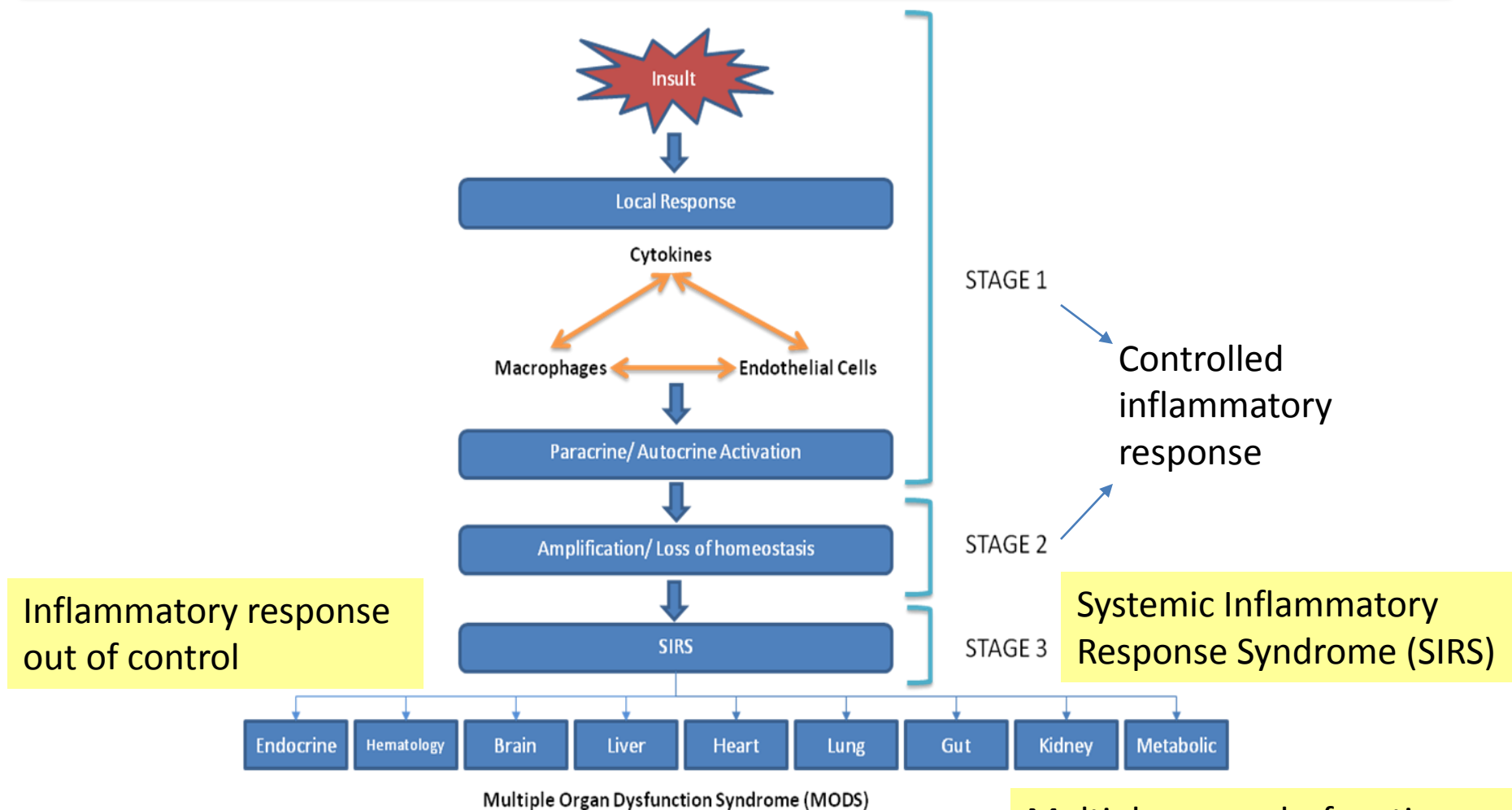 - Pathological changes

4. S. Sevitt, *Injury*, 1972, 4, 151-156.
5. S. Sevitt, *The Lancet*, 1966, 288, 1203-1210.

Traumatic Hemorrhagic Shock (THS)

- Loss of blood - Deficiency of O_2 reaching the organs leads to shock

- Triggers severe metabolic derangements & the complex inflammatory response

- Can lead to the development of Systemic Inflammatory Response Syndrome (SIRS)

Inflammatory Response

Multiple Organ Dysfunction Syndrome (MODS)

Controlled inflammatory response

Systemic Inflammatory Response Syndrome (SIRS)

Inflammatory response out of control

Multiple organ dysfunction syndrome (MODS).

Davies, M. G.; Hagen, P. O., *British Journal of Surgery,* 1997**,** *84* (7), 920-935.

Metabolomics

GENOMICS → TRANSCRIPTOMICS → PROTEOMICS → METABOLOMICS →→→ PHENOTYPE

Genes	mRNAs	Proteins	Metabolites
~ 20,000	$> 10^6$	$> 10^7$	~7,800

Metabolomics

- Comprehensive analysis of all metabolites[5]
- Closer to the phenotype as compared to the other "Omics" levels

5. Fiehn, O., *Plant Mol. Biol.* **2002,** *48* (1-2), 155-171.

Common Analytical Platforms

MULTI-PLATFORM APPROACH

- No single instrument to cover the whole metabolome
- Use of complementary platforms to maximize metabolite coverage

```
                    Metabolomics
                    /          \
              MS-based          NMR
         /     |      |      \
Flow injection  Coupled to   Coupled to gas   Coupled to capillary
or Direct       liquid       chromatography   electrophoresis
injection MS    chromatography  (GC-MS)        (CE-MS)
                (LC-MS)
```
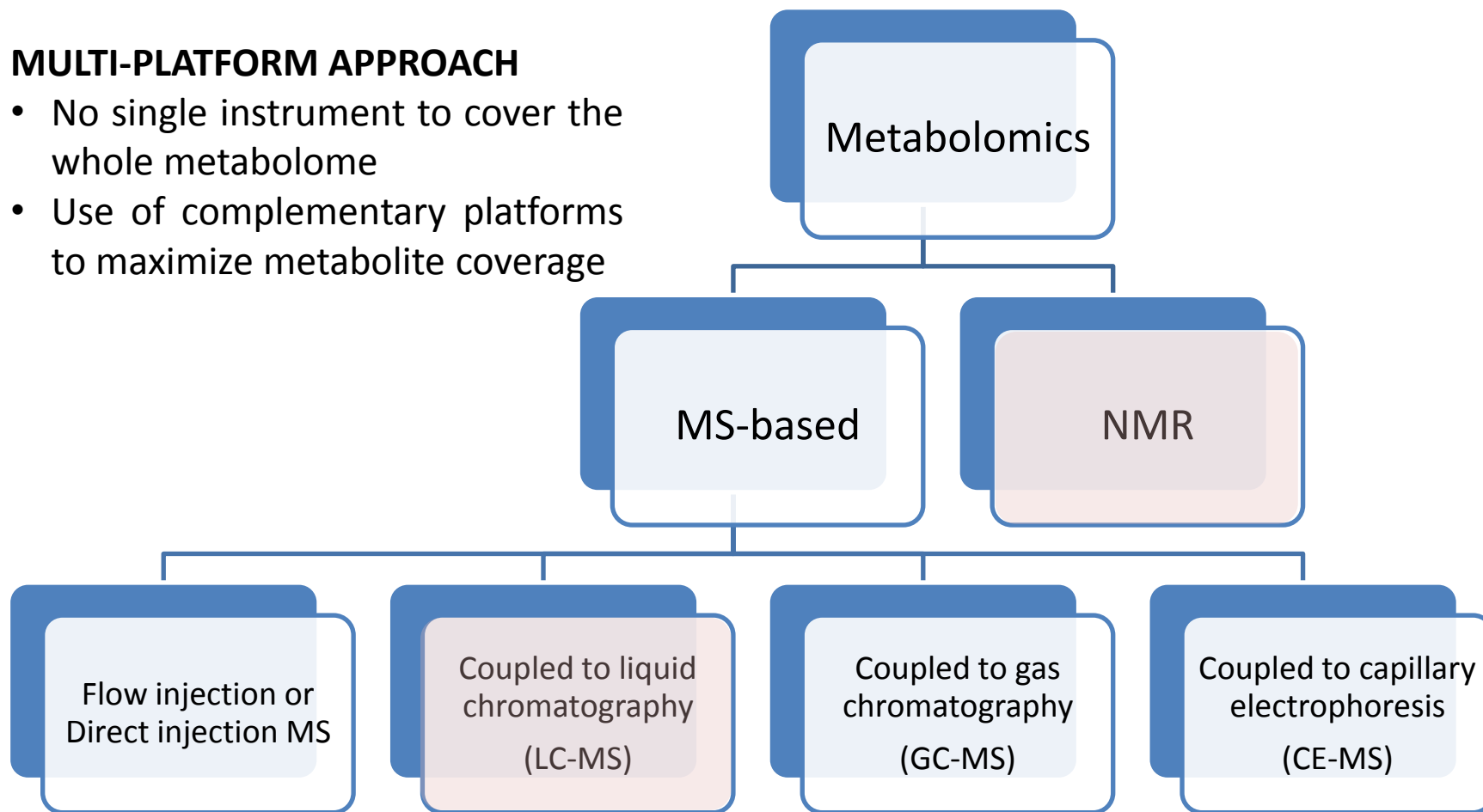
LC-MS and NMR in Metabolomics

NMR

Advantages

- Minimal sample preparation
- Simple analysis
- Specificity

Disadvantages

- Low sensitivity
- Overlapping signals for complex mixtures

LC-MS

Advantages

- High sensitivity
- High selectivity

Disadvantages

- Some metabolites may not be ionized effectively into MS vacuum

Objectives

1) Use NMR and LC-MS to obtain a comprehensive profile of the metabolic changes in a simulation of combat trauma injury in a porcine model

2) Correlate metabolite changes with cytokines and reported protein markers of organ-specific injury

   ➢ Identify potential biomarkers of Systemic Inflammatory Response Syndrome (SIRS) and organ-specific injury

   ➢ Identify metabolites that can modulate inflammatory response

Injury Protocol
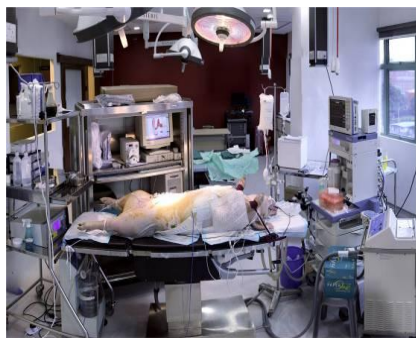
Induce anaesthesia, intubation & instrumentation

Blood Sampling
(Before Trauma/ Sham)
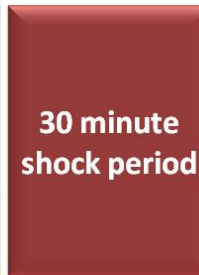
Combat Injury Simulation Phase

Blood Sampling
(After Trauma/ Sham)

Before Trauma   After Trauma

Before Sham   After Sham

Trauma   **Sham**

6. Cho, S. D.; Holcomb, J. B.; Tieu, B. H.; et al., *Shock* **2009**, 31, 87.
7. Wong, Y. C.; Lai, Y. Y.; Tan, et al., *Shock* **2015,** 43 (2).

Instrumental Analysis
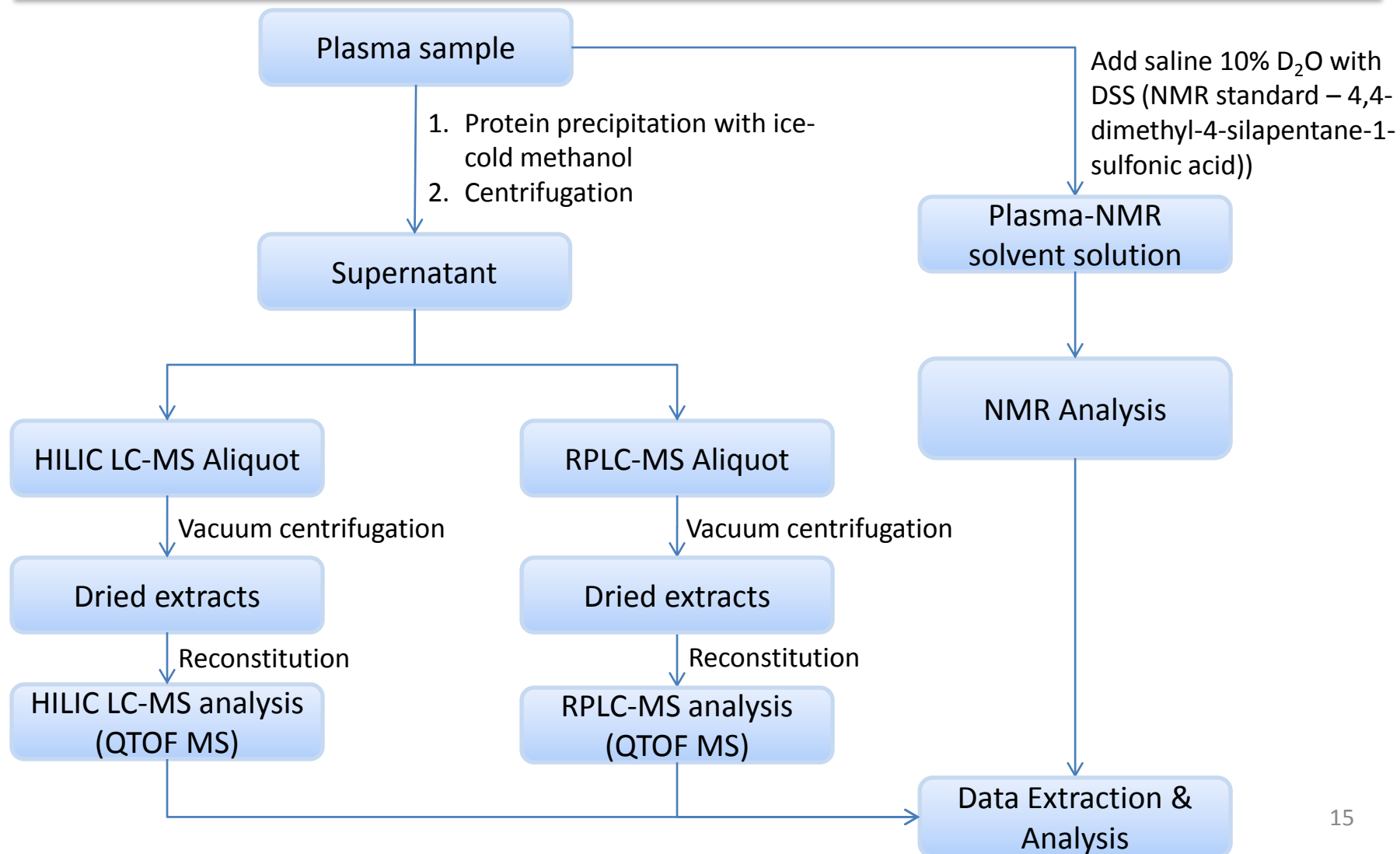
Data Processing & Analysis

Data Pre-processing & Extraction

- XCMS Online for LC-MS data
- Chenomx for NMR-Binning & Profiling

Multivariate Analysis

- Principal Component Analysis (PCA)
- Orthogonal Projection to Latent Structures-Discriminant Analysis (OPLS-DA)

Univariate Statistical Analysis

- Wilcoxon-Mann-Whitney Test using Metaboanalyst on fold change (FC) values

Correlation Analysis & Correlation Network Analysis

- Spearman correlation
- Cytoscape

Metabolic Pathway Analysis

- Metaboanalyst

Metabolite Identification

- LC-MS Data- search of Metlin, Lipidmaps, HMDB, MassBank databases
- NMR Data- Chenomx & literature search
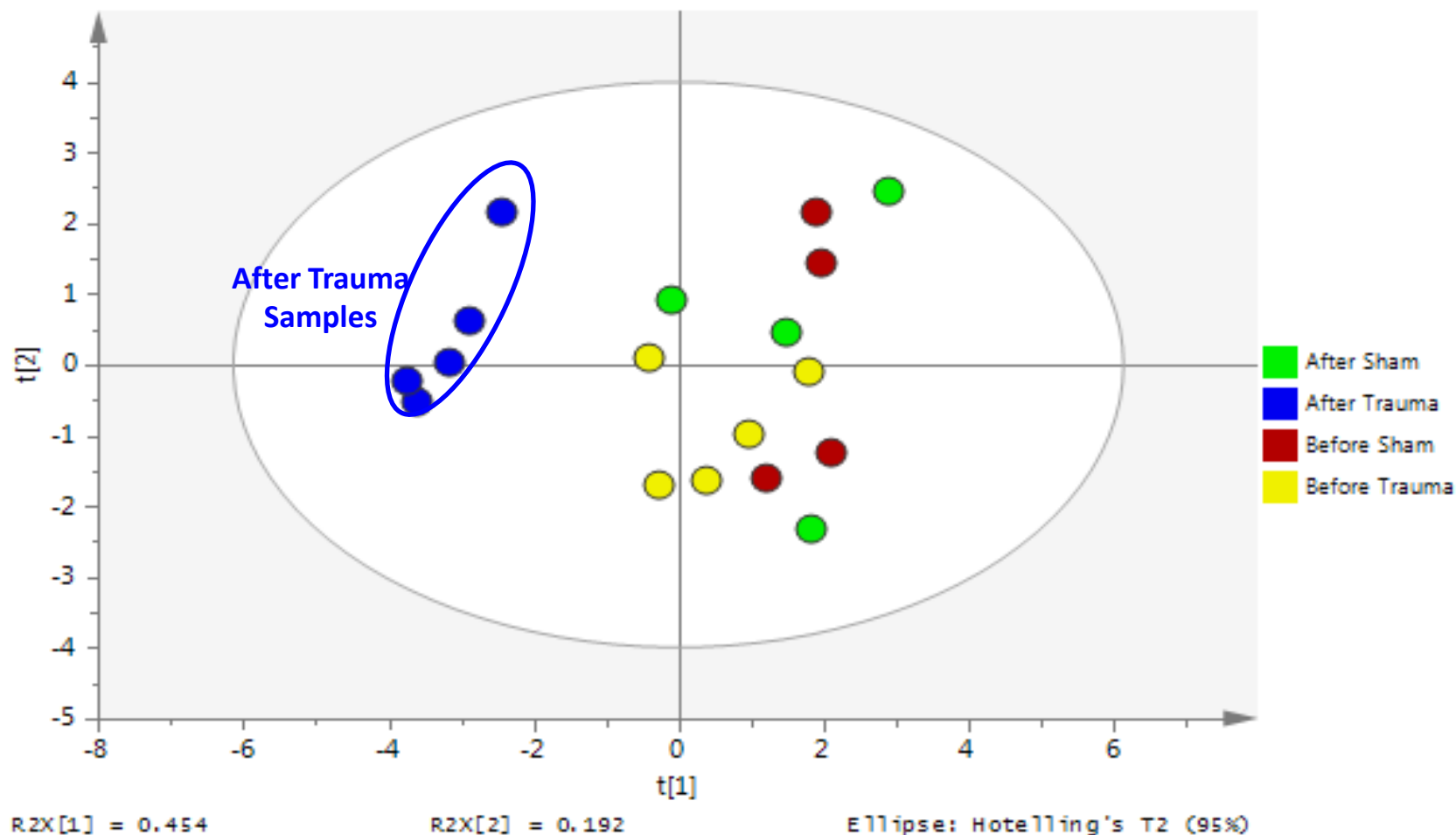
16

After Sham – No significant changes.

Before Sham

After Sham
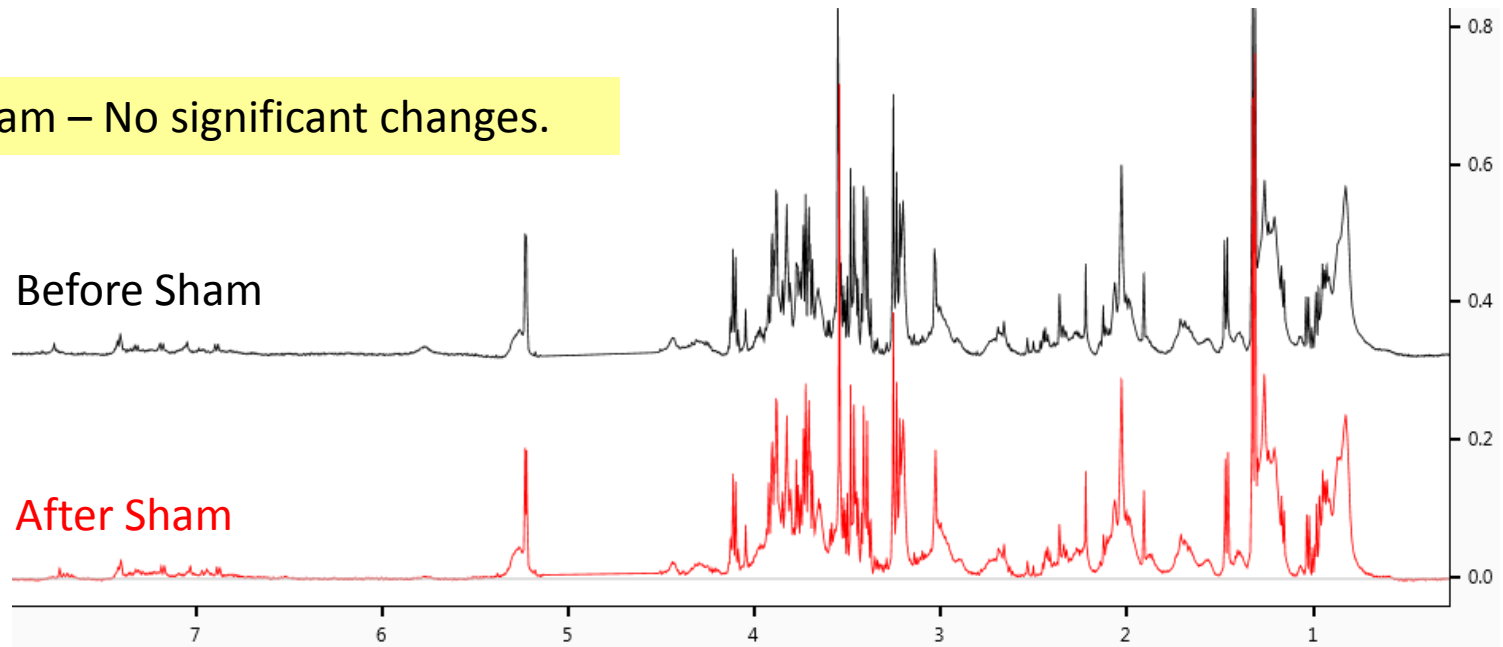
After Truama – Significant changes, e.g. decreases in lipoproteins and glycoproteins.

Before Trauma
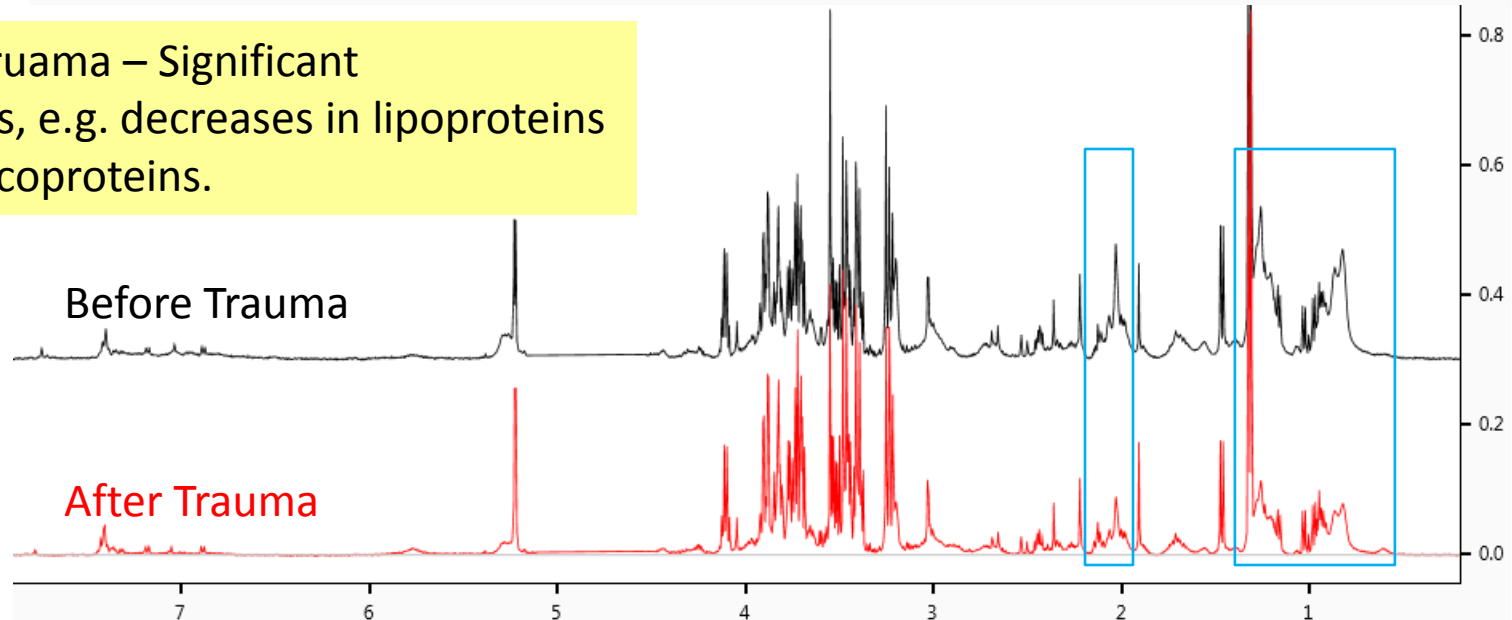
After Trauma

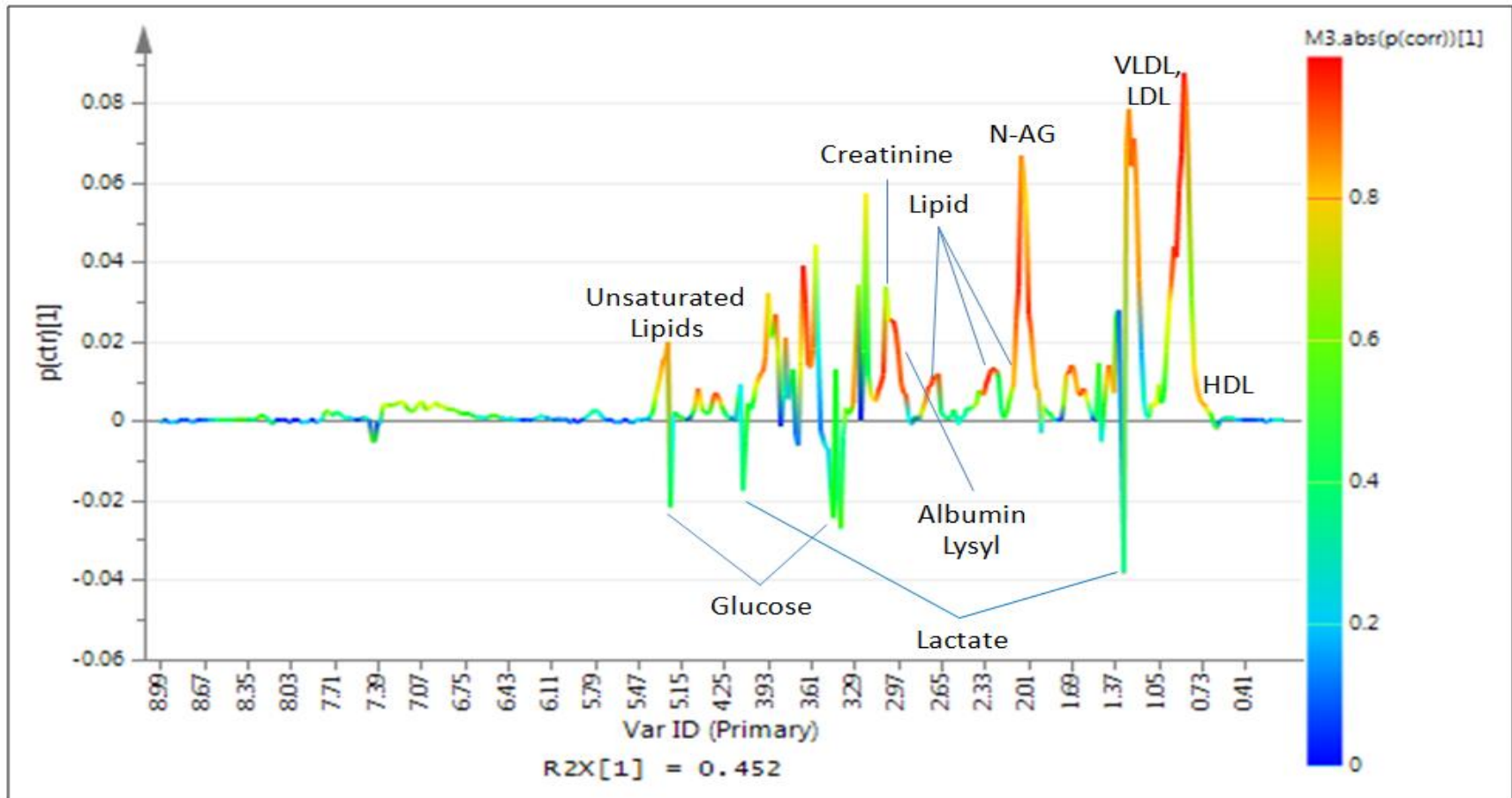**NMR spectra of the metabolomic changes after trauma injury**

NMR: PCA Analysis

R2X[1] = 0.454   R2X[2] = 0.192   Ellipse: Hotelling's T2 (95%)

NMR: OPLS-DA Analysis

N-acetylglycoproteins (N-AG)

LC-MS Chromatograms
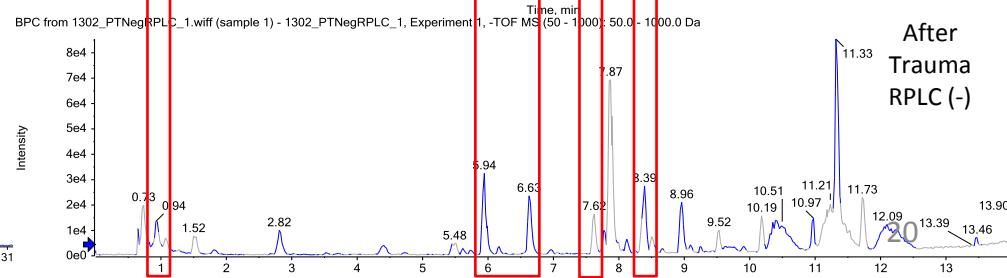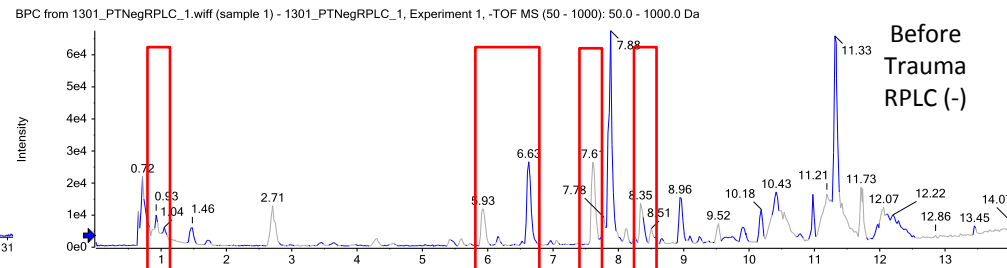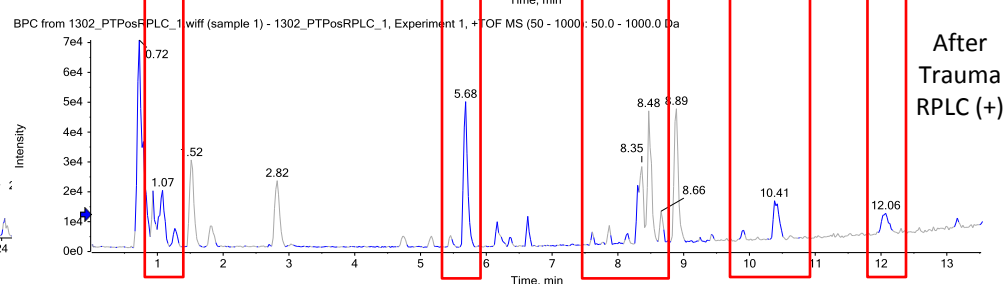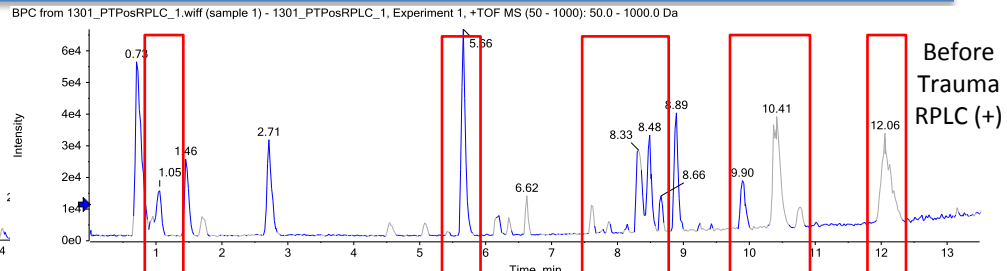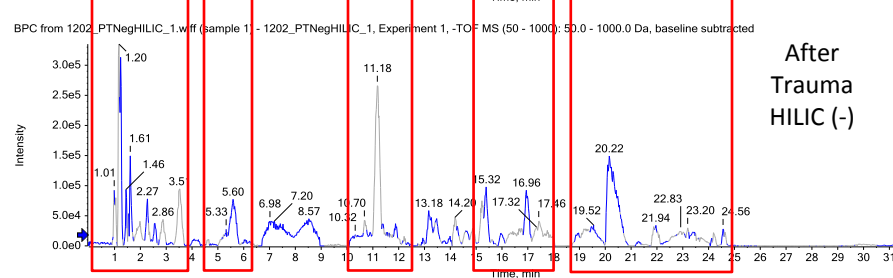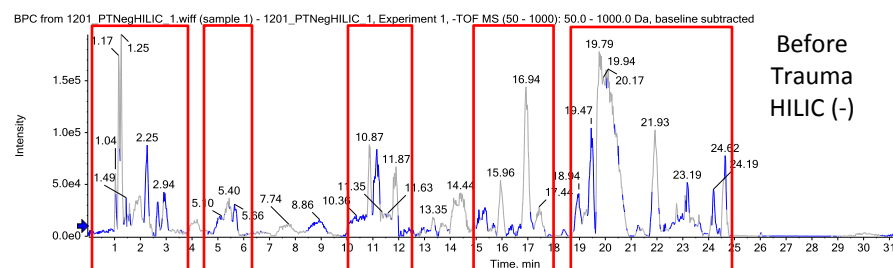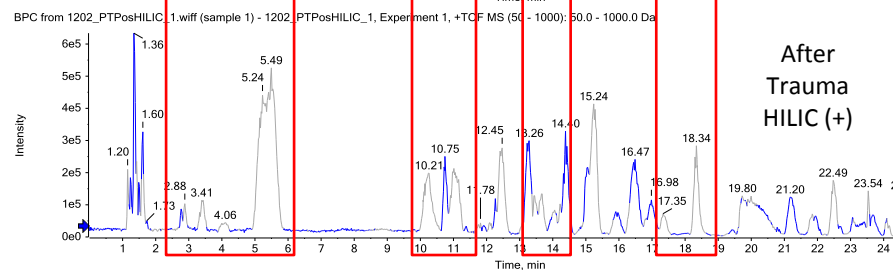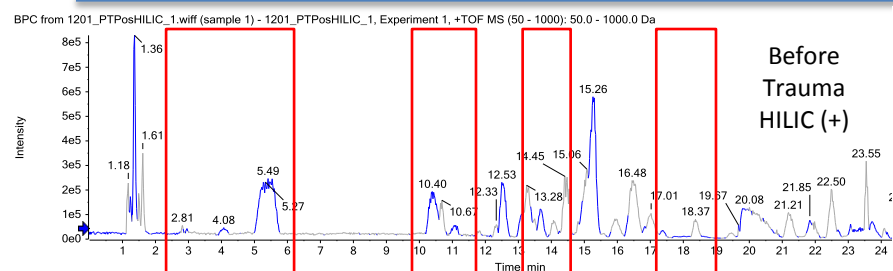
LC-MS Analysis - OPLS-DA Score Plots

LC-MS Analysis - Significant Features
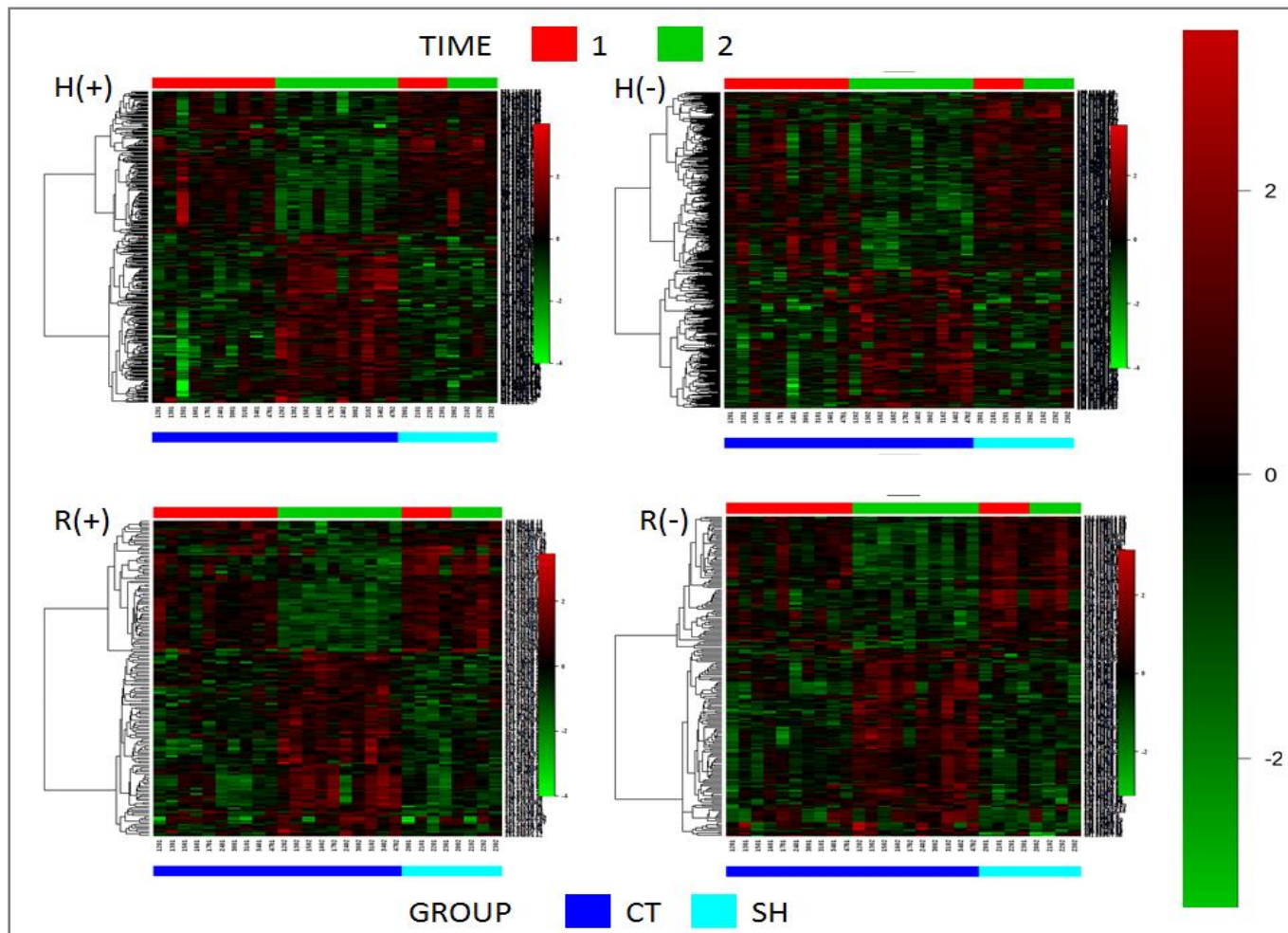
Group:
CT- Combat Trauma
SH- Sham

Time:
1- Before Sham/Trauma
2- After Sham/Trauma

Heatmap shows that significant features can be clearly divided into two groups: those that increase after trauma and those that decrease

LC-MS Analysis
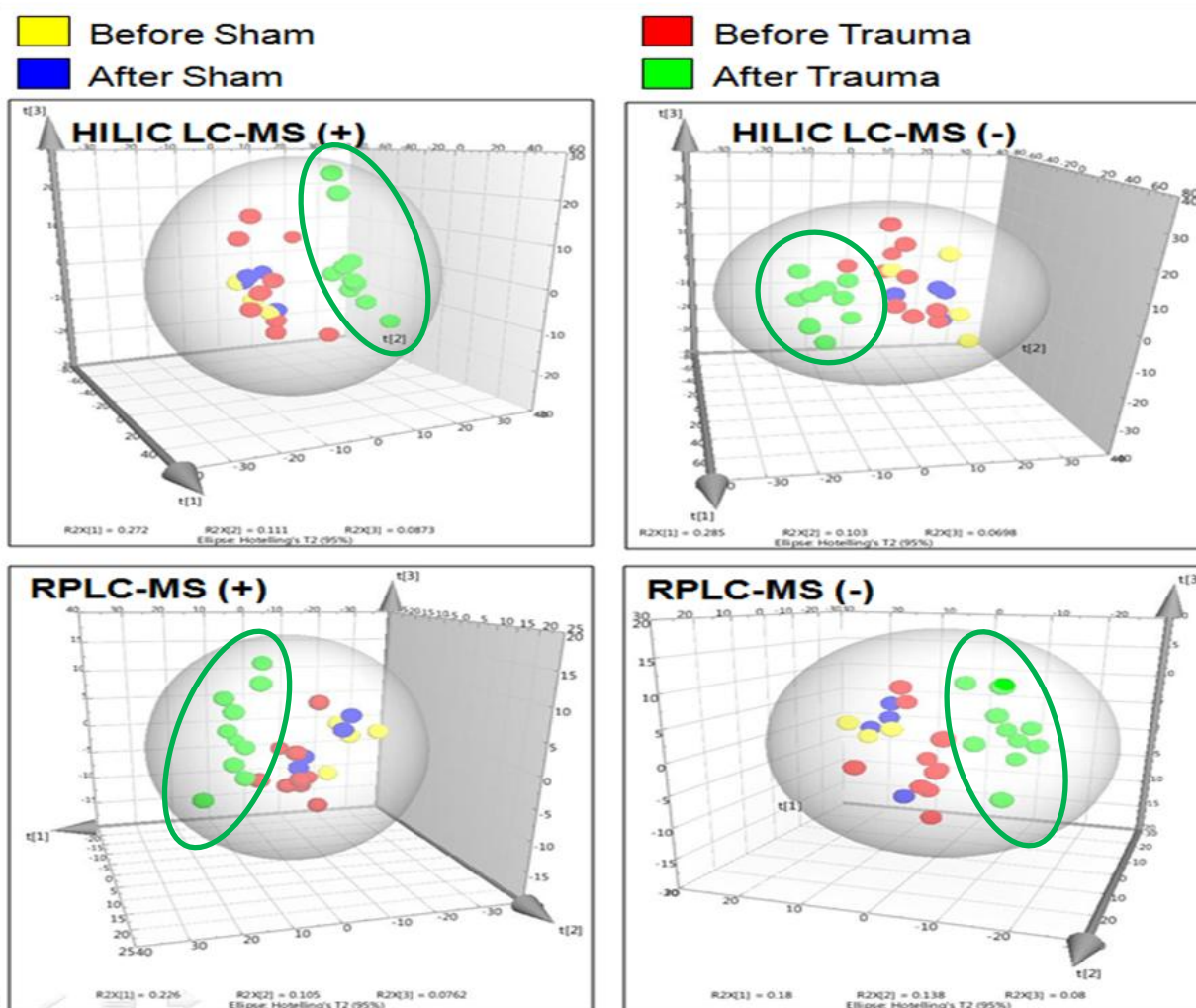
PAs, PCs, PEs, PIs, PGs, SM, Cer

Methylated nucleosides & amino acids

Sat'd & Unsat'd Fatty Acids, Oxo & Hydroxy Fatty Acids

Organic Acids
Uric Acid
Ascorbic Acid
Hippuric Acid

Pos HILIC

Neg HILIC

Pos RPLC

Neg RPLC

54

21

9

1

2

10

27

0

0

0

Amino Acids
L-Cystine
Choline
L-methionine
S-Adenosylmethionine

0

2

5

6

1

Purines & Pyrimidines
Inosine
Guanosine
Hypoxanthine
Adenosine
5'-CMP
Thymidine
Uridine
Cytosine
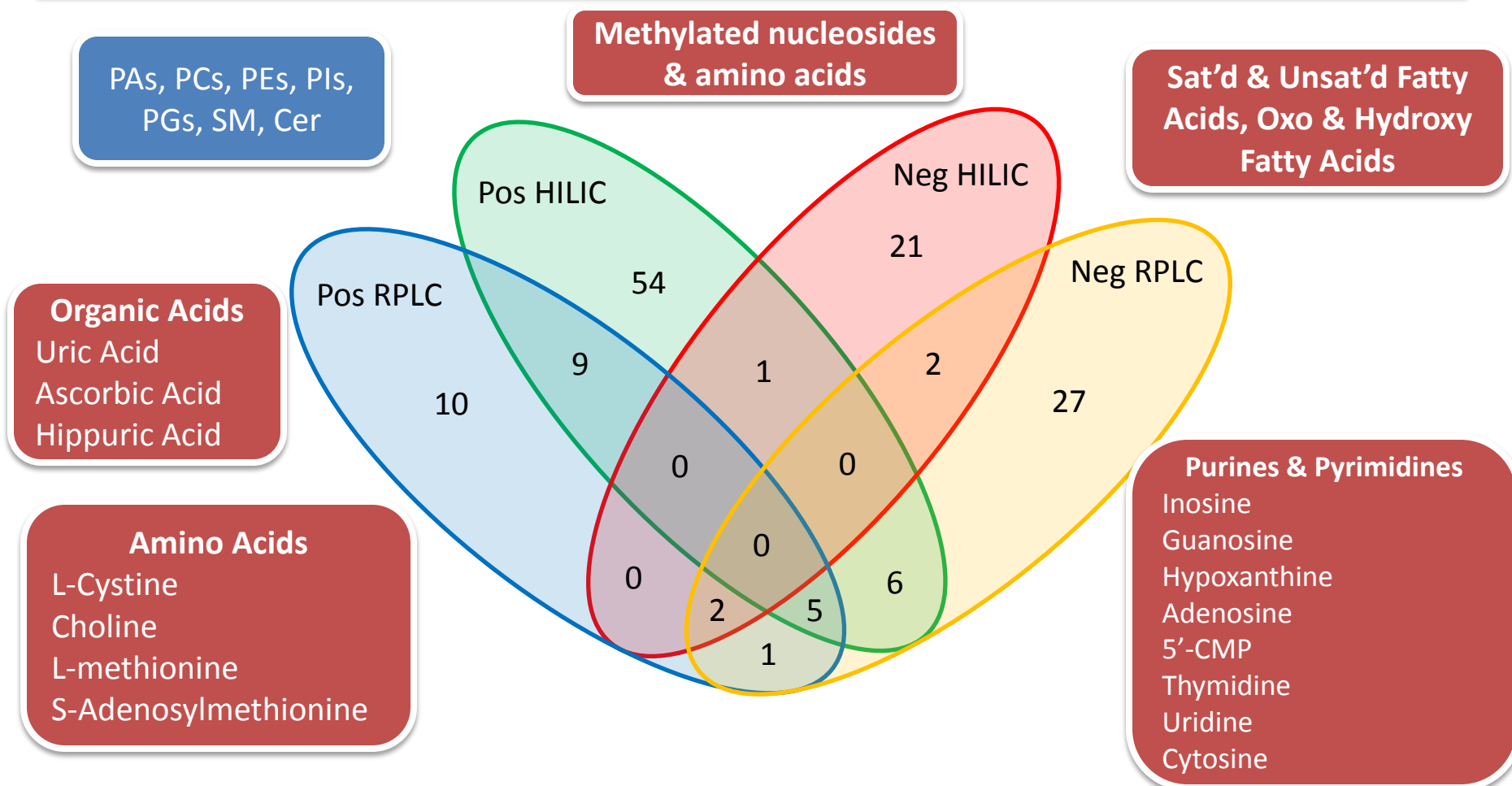
- 112 unique metabolites for different LCMS modes
- 26 metabolites detected in more than one mode

Pathway Analysis

Main **pathways** affected after injury

|	Total Cmpd	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact
Glycerophospholipid metabolism	39	9	0.0010	6.899	0.023	0.003	0.363
Purine metabolism	92	7	0.0050	5.295	0.068	0.008	0.046
Pyrimidine metabolism	60	6	0.0006	7.461	0.014	0.002	0.210
Sphingolipid metabolism	25	3	0.0000	10.254	0.001	0.001	0.304
Arginine and proline metabolism	77	3	0.0024	6.039	0.048	0.006	0.146
Cysteine and methionine metabolism	56	3	0.0328	3.418	0.262	0.042	0.159
Glycerolipid metabolism	32	2	0.0001	8.831	0.004	0.001	0.204
Phenylalanine metabolism	45	2	0.0004	7.728	0.011	0.002	0.032

Network Analysis
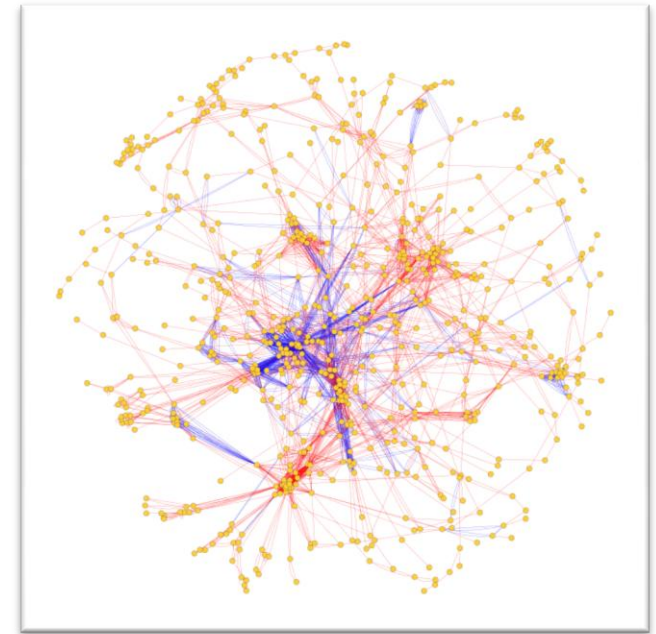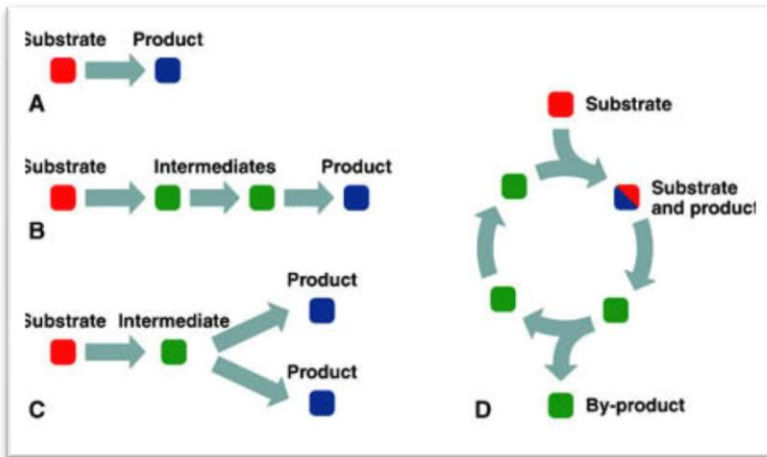
Pathways

Networks

- Recognition of inter-dependence of pathways - Networks
- "Omics" platforms used to determine correlations and networks

Correlation Analysis

- Explanation of relationships or interactions
- Novel insights into biological mechanisms
- Easier visualization of associations

Correlation Analysis

Metabolites showing correlations with inflammatory and organ damage markers

| Metabolites	IL-10	TNF-a	CKMB	CRP	Kim1	PAI-1	NGAL	hFABP	H2S	Ang-2
LDL	-0.695	-0.406	-0.082	-0.137	-0.550	-0.587	-0.802	-0.571	-0.544	-0.172
2-amino-6-oxo-2,4-hexadienoic acid	-0.736	-0.433	-0.383	-0.508	-0.531	-0.510	-0.621	-0.247	-0.704	0.054
4,7,10,13,16-docosapentaenoic acid	0.521	0.521	0.273	0.214	0.186	0.242	0.366	0.657	0.471	-0.112
Adrenic Acid	-0.811	-0.685	-0.262	-0.315	-0.337	-0.377	-0.560	-0.370	-0.686	0.060
Eicosadienoic Acid	-0.769	-0.628	-0.259	-0.402	-0.367	-0.451	-0.645	-0.164	-0.585	-0.093
Glutarylcarnitine	0.400	0.192	0.427	0.596	0.563	0.488	0.461	0.343	0.637	0.236
LPC(20:3/0:0)	-0.712	-0.540	-0.262	-0.388	-0.179	-0.269	-0.449	-0.464	-0.674	0.151
LPC(22:5)	-0.783	-0.622	-0.314	-0.478	-0.306	-0.429	-0.540	-0.407	-0.688	0.126
PA(18:1(9Z)/0:0)	0.635	0.443	0.327	0.372	0.516	0.547	0.726	0.366	0.540	0.379
PC(18:1/0:0)	-0.782	-0.741	-0.186	-0.558	-0.438	-0.585	-0.633	-0.374	-0.669	0.033
PC(18:2/0:0)	-0.592	-0.558	-0.432	-0.472	-0.521	-0.577	-0.725	-0.515	-0.549	-0.082
PC(18:3(9Z,12Z,15Z)/0:0)	-0.530	-0.467	-0.472	-0.548	-0.496	-0.500	-0.680	-0.447	-0.664	-0.028
PC(20:4/0:0)	-0.823	-0.615	-0.266	-0.429	-0.412	-0.498	-0.744	-0.224	-0.577	-0.120
PC(20:5(5Z,8Z,11Z,14Z,17Z)/0:0)	-0.525	-0.357	-0.347	-0.739	-0.705	-0.576	-0.673	-0.196	-0.636	-0.142
PC(22:4(7Z,10Z,13Z,16Z)/0:0)	-0.752	-0.620	-0.304	-0.369	-0.337	-0.363	-0.534	-0.467	-0.683	0.161
PC(22:6/0:0)	-0.757	-0.536	-0.375	-0.427	-0.326	-0.448	-0.773	-0.303	-0.594	-0.134
SN-Glycero-3-phosphocholine	-0.704	-0.438	-0.328	-0.525	-0.236	-0.350	-0.401	-0.427	-0.746	0.096
Deoxyguanosine	0.557	0.369	0.408	0.488	0.488	0.632	0.743	0.422	0.613	0.380
Guanosine	0.499	0.307	0.397	0.513	0.609	0.670	0.720	0.436	0.552	0.288
Inosine	0.505	0.404	0.319	0.514	0.554	0.687	0.722	0.465	0.483	0.299
Succinoadenosine	0.625	0.461	0.340	0.498	0.697	0.554	0.768	0.379	0.649	0.383
Uric Acid	0.564	0.418	0.407	0.478	0.235	0.501	0.563	0.573	0.527	0.082
1-Methylguanosine	0.539	0.381	0.627	0.447	0.426	0.514	0.684	0.524	0.628	0.169
1-methylnicotinamide	0.568	0.436	-0.037	0.414	0.578	0.510	0.613	0.051	0.420	0.345
1-methyluric Acid	0.161	0.134	0.479	0.511	0.385	0.398	0.406	0.580	0.512	0.297
3'-O-Methyladenosine	0.574	0.314	0.440	0.427	0.536	0.579	0.747	0.350	0.501	0.483
3'-O-Methylguanosine	0.606	0.484	0.377	0.357	0.403	0.552	0.722	0.472	0.484	0.412
Thymine	0.554	0.576	0.010	0.209	0.239	0.576	0.578	0.320	0.438	0.384
Uridine	0.385	0.596	0.263	0.235	0.455	0.498	0.745	0.455	0.425	0.513
Arginine	-0.736	-0.428	-0.088	-0.391	-0.410	-0.360	-0.449	-0.375	-0.610	0.011
Citrulline	0.801	0.521	0.322	0.466	0.457	0.559	0.747	0.061	0.560	0.147
Cysteine-Homocysteine disulfide	0.438	0.275	0.468	0.486	0.647	0.378	0.641	0.267	0.621	0.500
L-Cysteinylglycine disulfide	0.562	0.352	0.219	0.594	0.525	0.434	0.485	0.080	0.618	0.268
L-Cystine	0.687	0.524	0.189	0.408	0.633	0.510	0.808	0.242	0.600	0.367
L-methionine	0.723	0.586	-0.023	0.476	0.495	0.410	0.598	0.205	0.577	0.232
N1-acetylspermidine	0.723	0.511	0.425	0.408	0.351	0.457	0.700	0.495	0.536	0.225
S-Adenosylmethionine	0.639	0.437	0.352	0.697	0.546	0.558	0.585	0.417	0.641	0.088

Color of the cells indicates the significance of the correlation. Red: $p < 0.05$, Green: $p < 0.01$, Blue: $p < 0.001$.

Correlation Network

- ➢ Network connections show correlations among metabolites
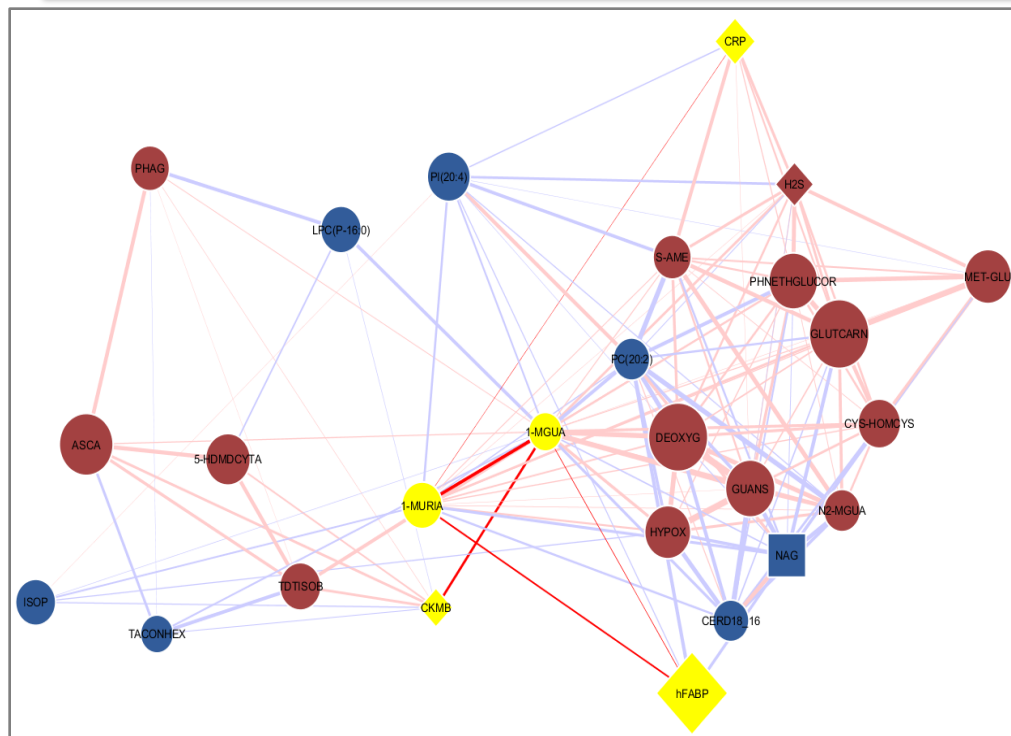- ➢ Thicker lines indicate stronger correlations

Correlation Network

Red: up-regulated,
Blue: down-regulated
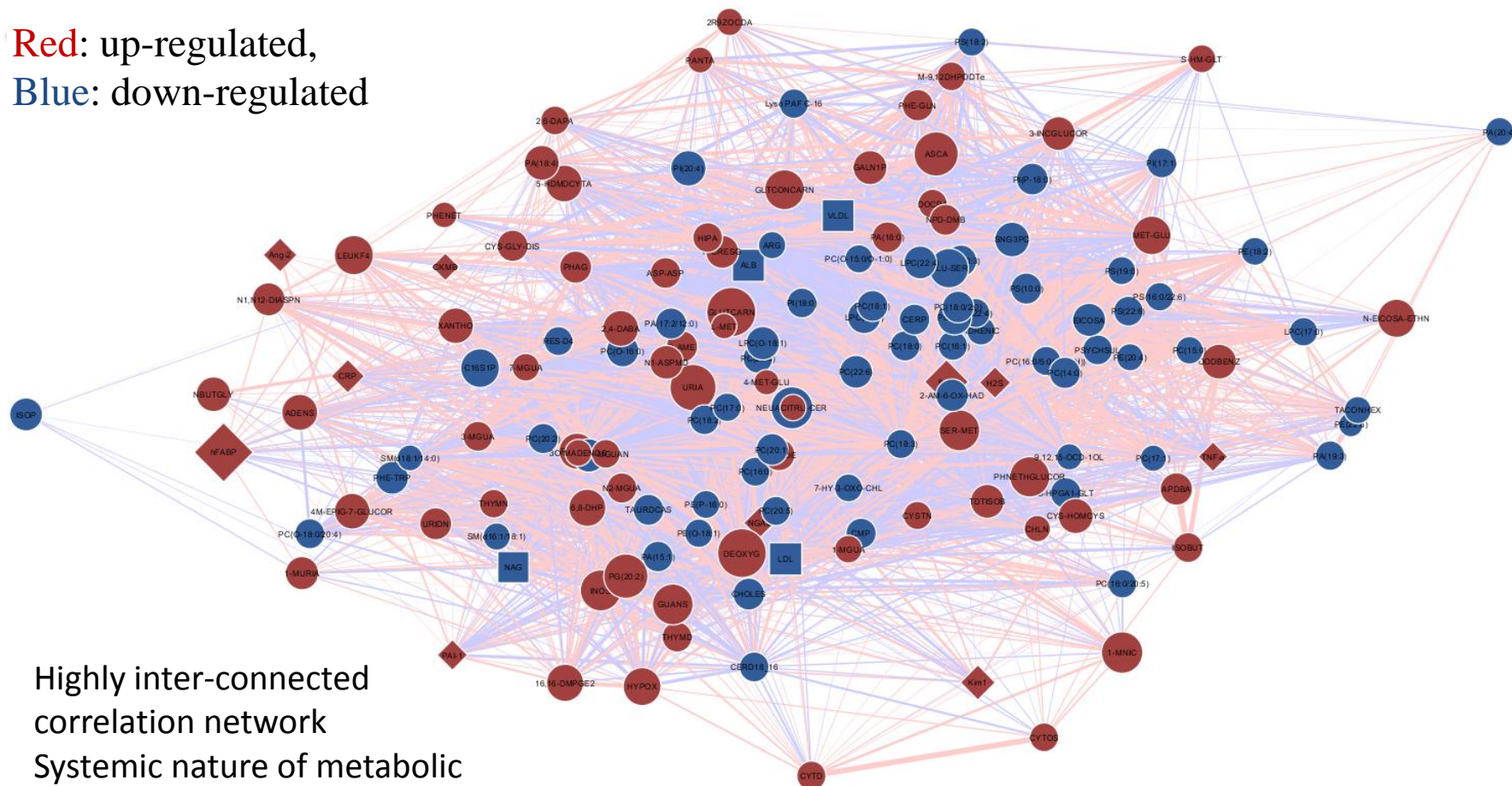
- Highly inter-connected correlation network
- Systemic nature of metabolic response
- Metabolic response intricately interwoven with inflammatory response
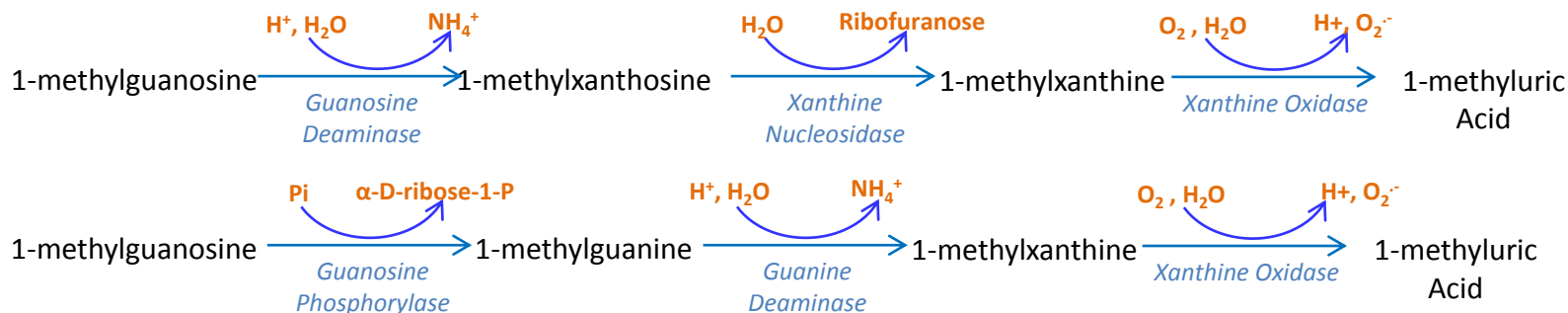
29

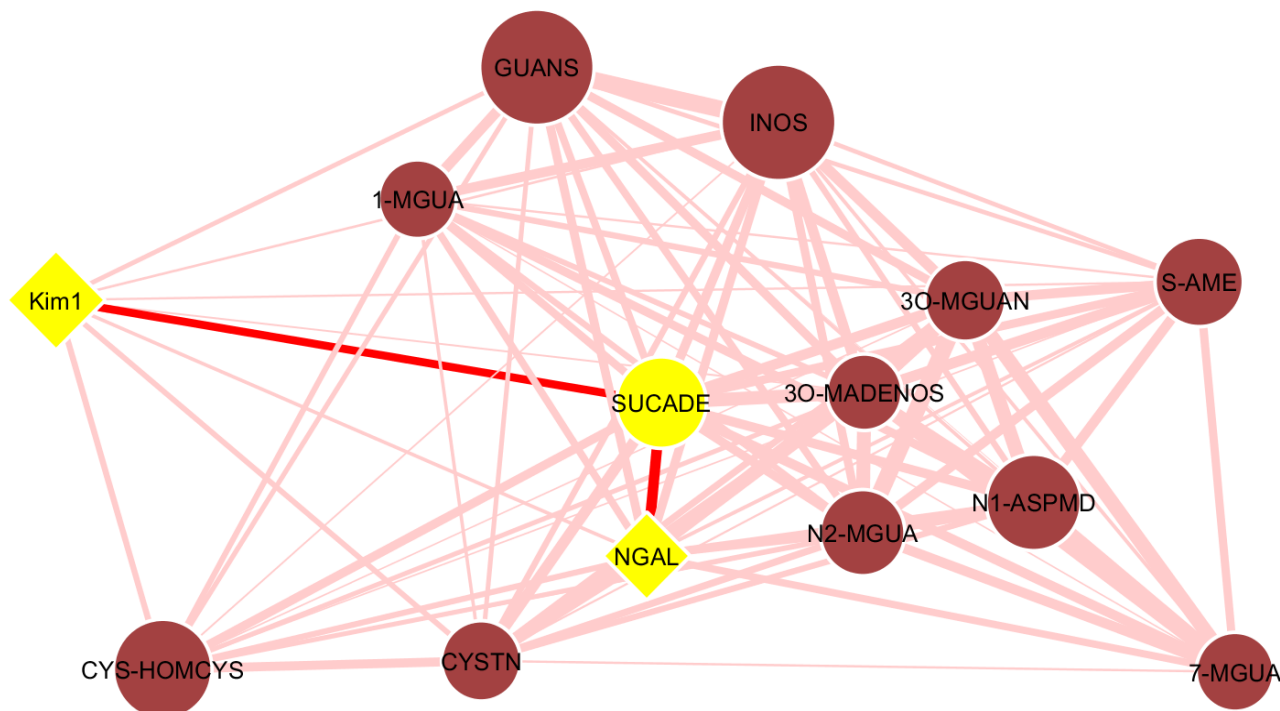# 1-methyluric Acid Sub-network

1-methyluric acid (1-MURIA) is the product of the metabolism of 1-methylxanthine.

1-methyluric acid is linked to:
1. Oxidative stress
2. Apoptosis due to GTP depletion
3. Highly activated inflammatory response (increased methylation)

- 1-methyluric acid may be a good marker of ischemic myocardial injury.
- Generation of 1-methyluric acid may also be a protective mechanism as it was found to be an effective free radical scavenger

1-methylguanosine →(H⁺, H₂O / NH₄⁺) *Guanosine Deaminase* → 1-methylxanthosine →(H₂O / Ribofuranose) *Xanthine Nucleosidase* → 1-methylxanthine →(O₂, H₂O / H+, O₂⁻) *Xanthine Oxidase* → 1-methyluric Acid

1-methylguanosine →(Pi / α-D-ribose-1-P) *Guanosine Phosphorylase* → 1-methylguanine →(H⁺, H₂O / NH₄⁺) *Guanine Deaminase* → 1-methylxanthine →(O₂, H₂O / H+, O₂⁻) *Xanthine Oxidase* → 1-methyluric Acid

30

Succinoadenosine Sub-network

- Based on correlation matrix, succinoadenosine (SUCADE) was found to be highly correlated to both KIM-1 and NGAL.
- A sub-network was then generated based on highest correlations of succinoadenosine in order to better see its association with NGAL and KIM-1.
- Sub-network shows both purine metabolites and cysteine-methionine pathway metabolites.
- Most of them are strongly associated with NGAL (inflammatory) and have less association with KIM-1 (renal).
- This could be an indication that they are more associated with inflammatory response than with renal injury.
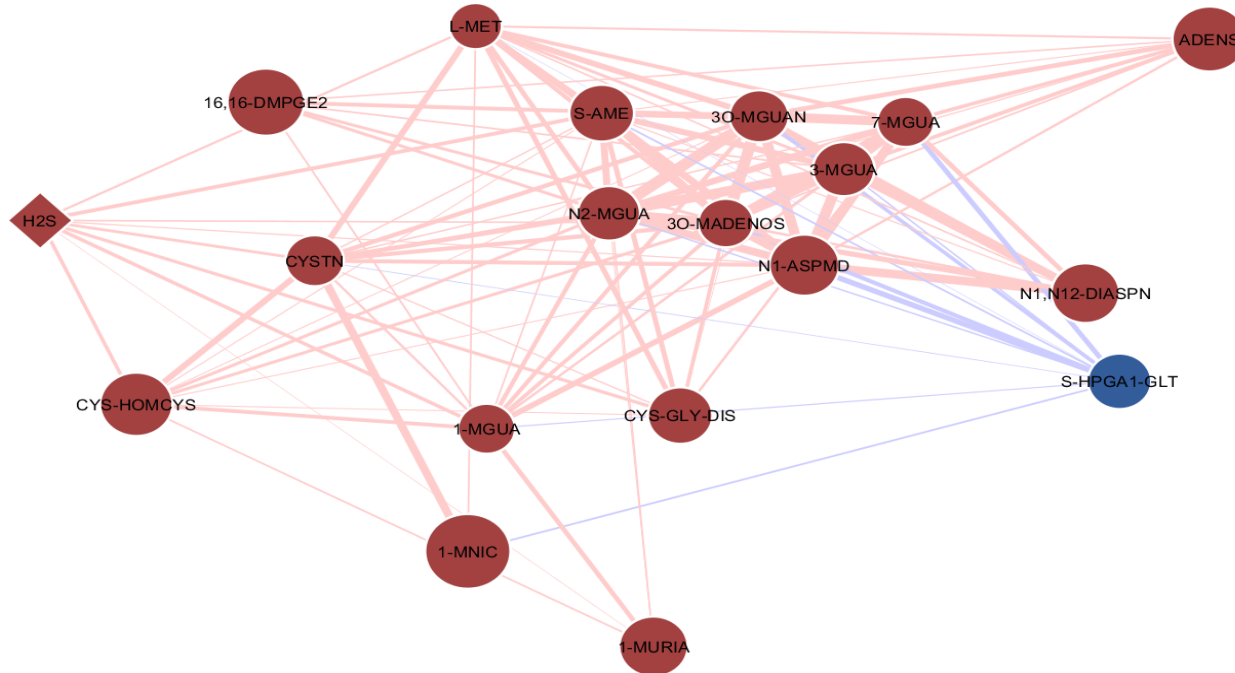
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Purine Biosynthesis

Observations

- Elevation of succinodenosine after trauma
- Inhibition of ADSL activity
- Down-regulation of ATP production

- Inhibition of ADSL enzyme leads to formation of Succinoadenosine
- ATP production is inhibited
- Since ATP and GTP co-regulate each other, GTP production is also affected
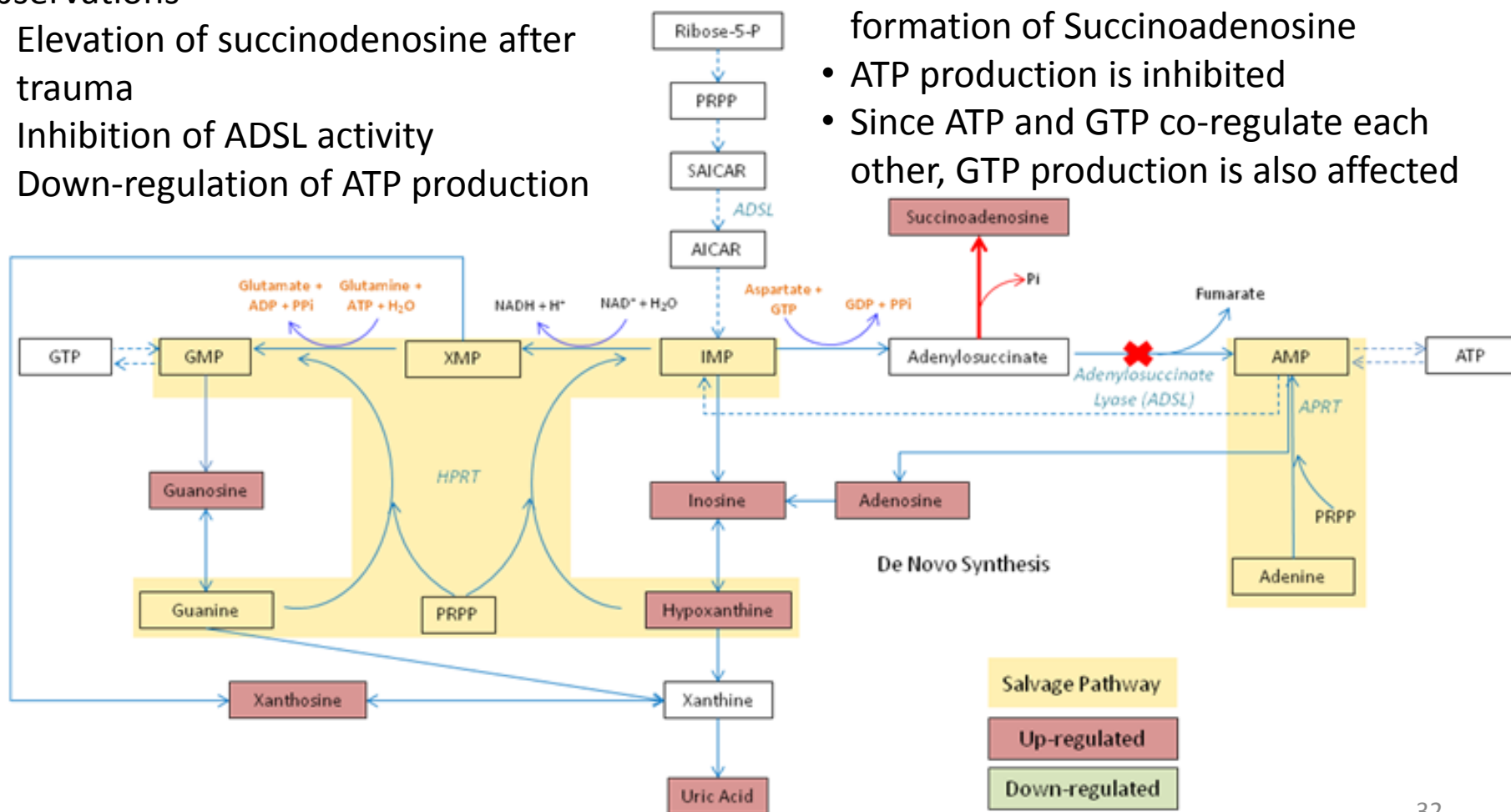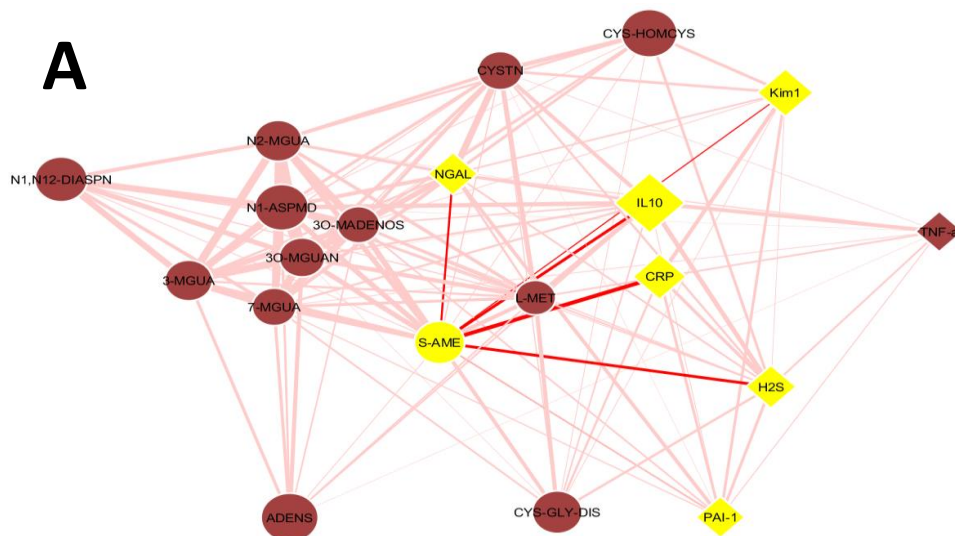
S-Adenosylmethionine (S-AME) Sub-network

- Up-regulation of methylated purines as well as up-regulation of other metabolites indicates that S-adenosylmethionine-homocysteine methylation cycle is highly activated after truama.
- There is also an observed up-regulation of formation of H_2S whereas formation of glutathione conjugate of Prostaglandin A1 (S-HPGA1-GLT) was observed to be down-regulated.
- This could be due to decreased availability of glutathione as its formation is being sacrificed in favour of increased rate of H_2S formation, oxidation of L-cysteine to disulfide form (L-cystine) and re-methylation of homocysteine for methylation cycle.
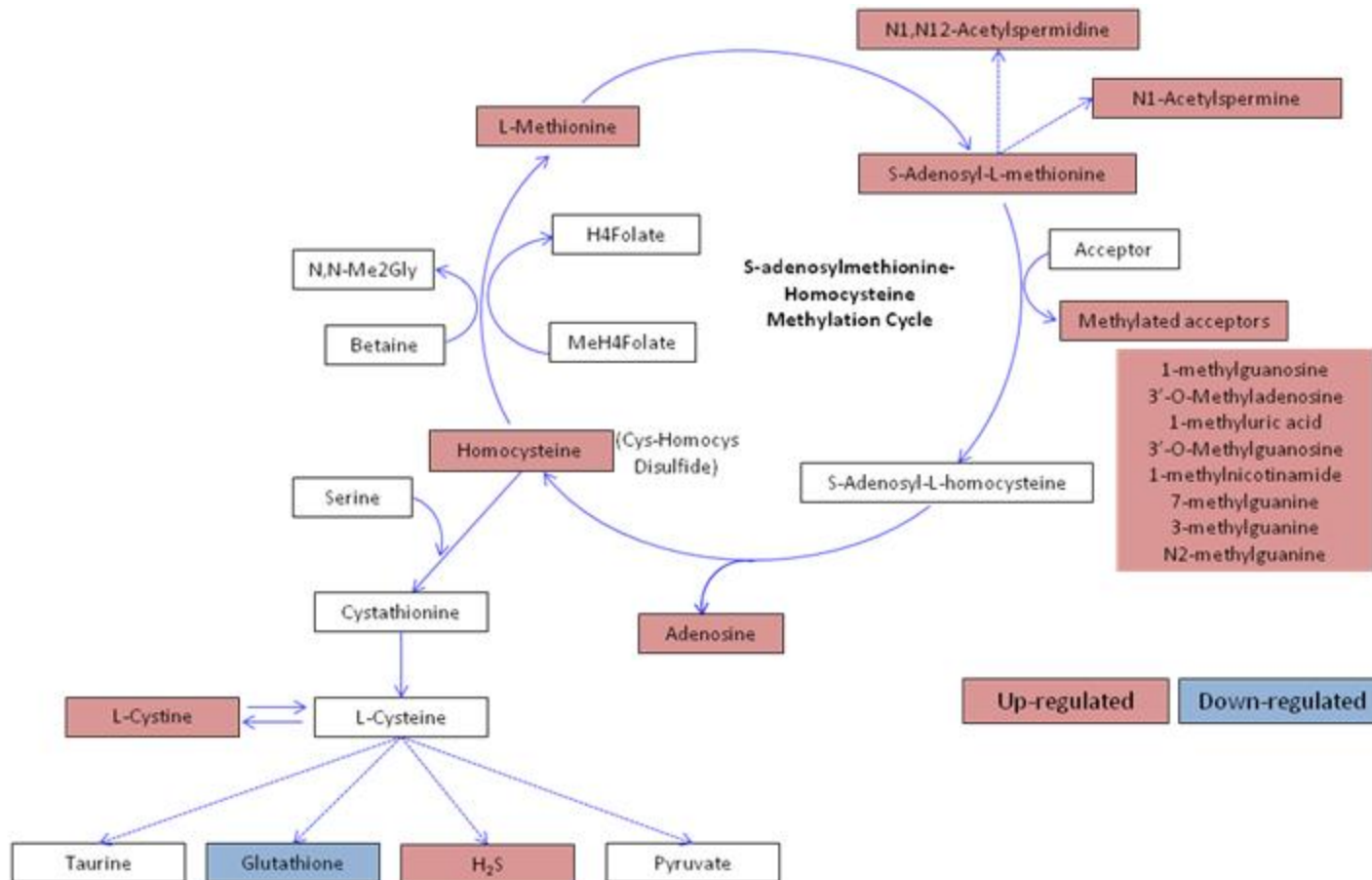
- Metabolites in S-AME sub-network are also reflected in this pathway
- Correlation network generated reflects actual biological processes

S-Adenosylmethionine Sub-network

A

- S-adenosylmethionine (S-AME) and L-cystine (CYSTN) are both highly correlated with inflammatory markers.
- Given the association of these pathways with inflammatory response, involvement of these metabolites in organ injury sub-networks reflects effects of systemic inflammary response syndrome (SIRS) and its role in development of organ failure.
- Therefore, regulation of these pathways may potentially be relevant in treatment of traumatic hemorrhagic shock.

B

Associations of S-AME (A) and L-Cystine (B) with the inflammatory markers.
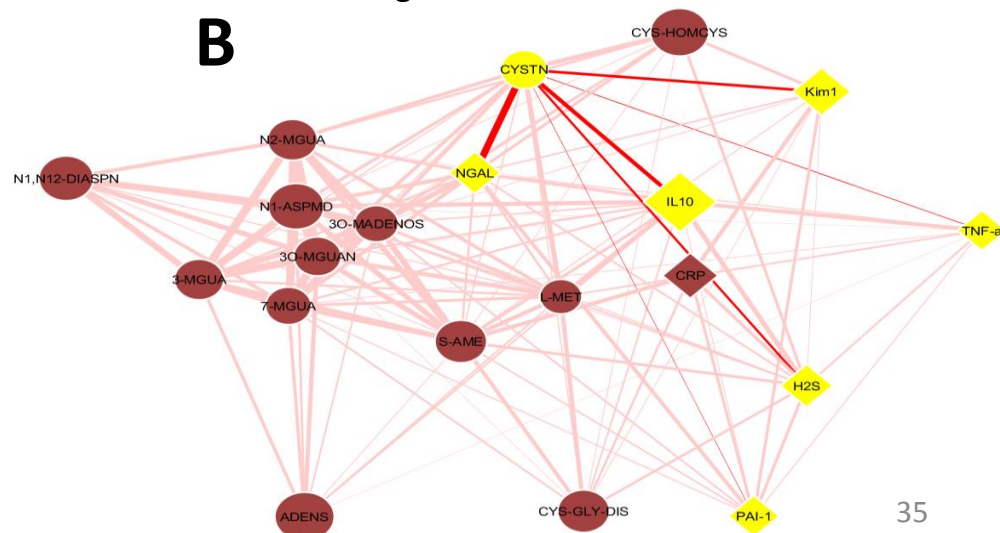
Summary

- Correlation network analysis gave new insights into the metabolic and inflammatory response to traumatic hemorrhagic shock

- Potential renal and myocardial injury markers were identified

- Cysteine-methionine pathway is highly associated with inflammatory response and development of organ failure

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