



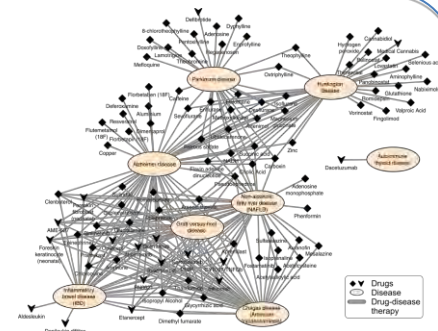
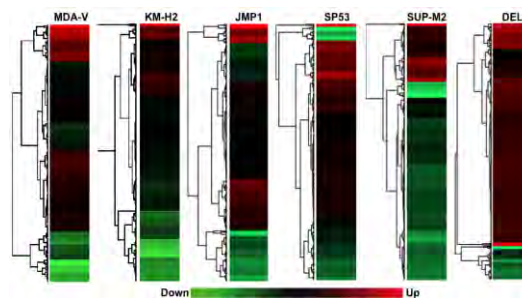
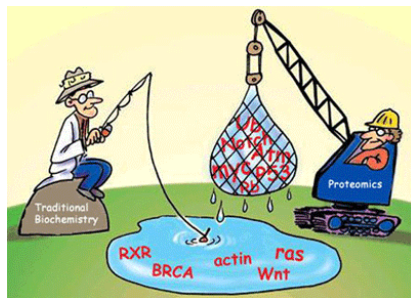
FORTH

INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGY



*COST CLINIMARK TRAINING SCHOOL Approaches for Biomarker Discovery and Validation
Spetses 23-27 September 2019*

Functional and clinical proteomics in the era of precision medicine



Michalis Aivaliotis

Important Definitions

Biomedical research

- The area of science devoted to the study of:
 - the **processes of life**
 - the **prevention and treatment of disease**
 - the **genetic, lifestyle and environmental** factors related to **disease and health**

Important Definitions

Basic research

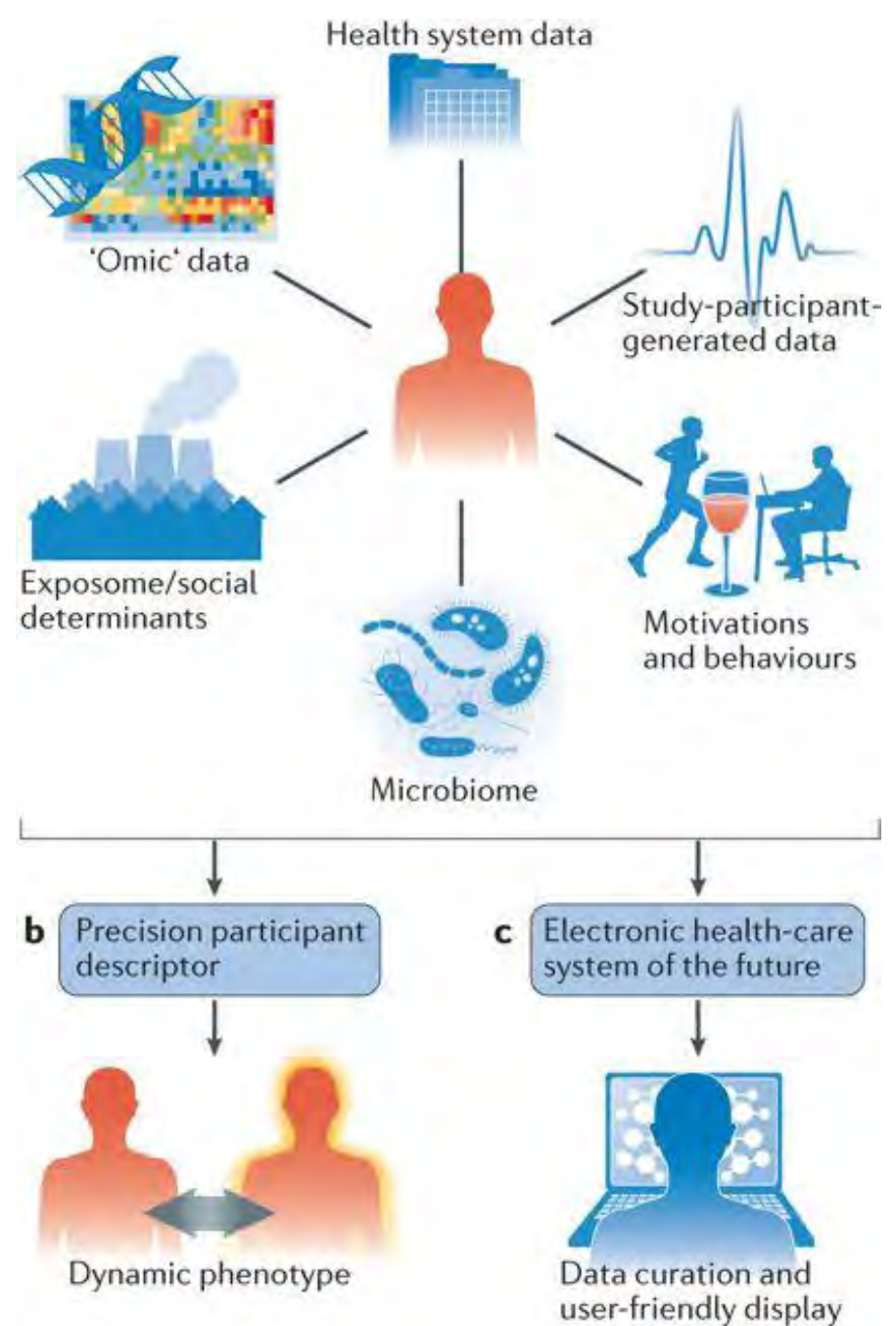
- Research conducted to **increase fundamental knowledge and understanding of the physical, chemical and functional mechanisms of life processes and disease**
 - **Not necessarily directed toward solving any particular problem** in humans or animals
3. Provides the **foundations** of other types of research (e.g. **applied, clinical, translational**)

Important

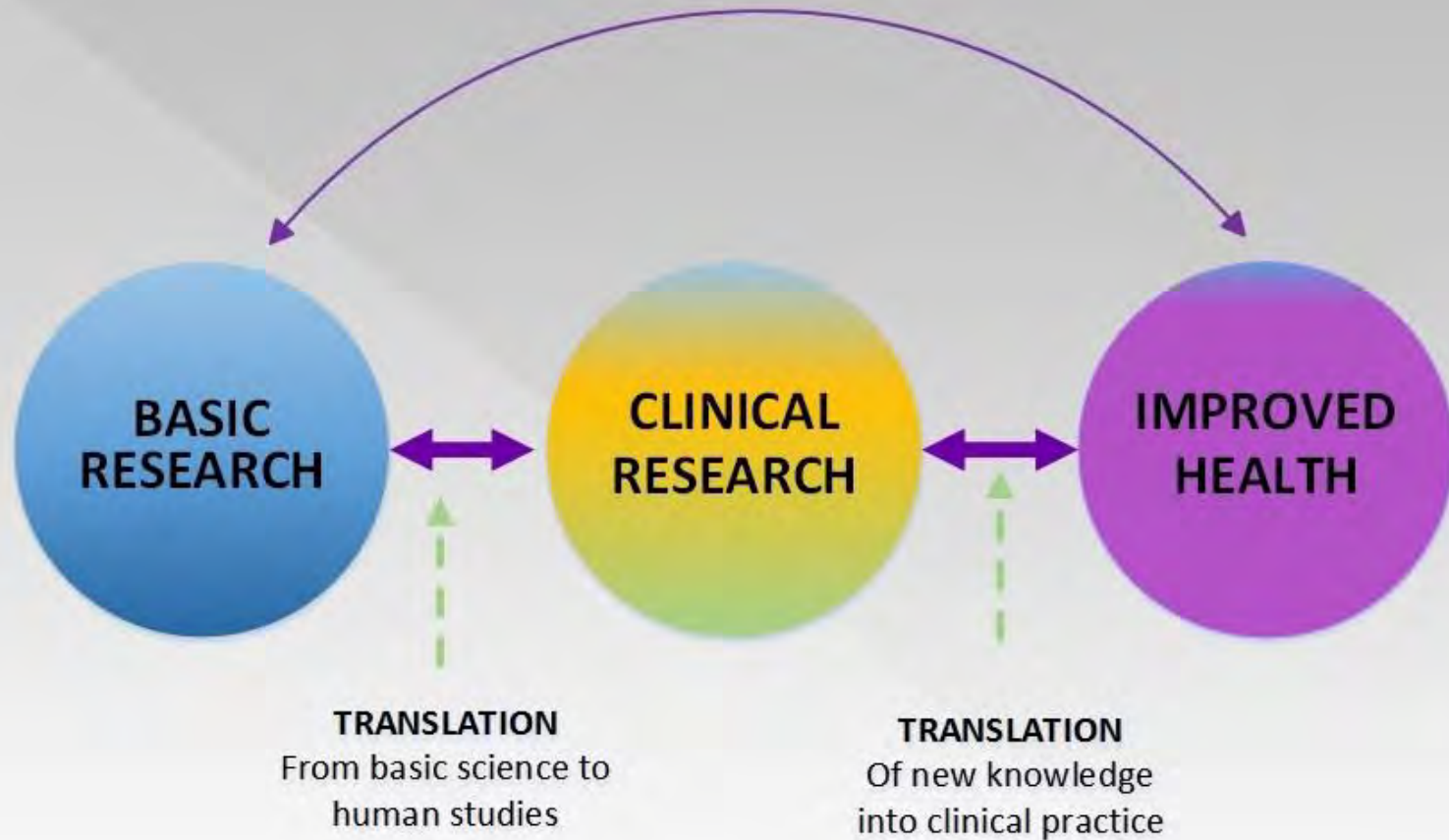
If we don't know how a life process functions normally, we won't know how to recognize and treat it when it functions abnormally.

Precision Medicine

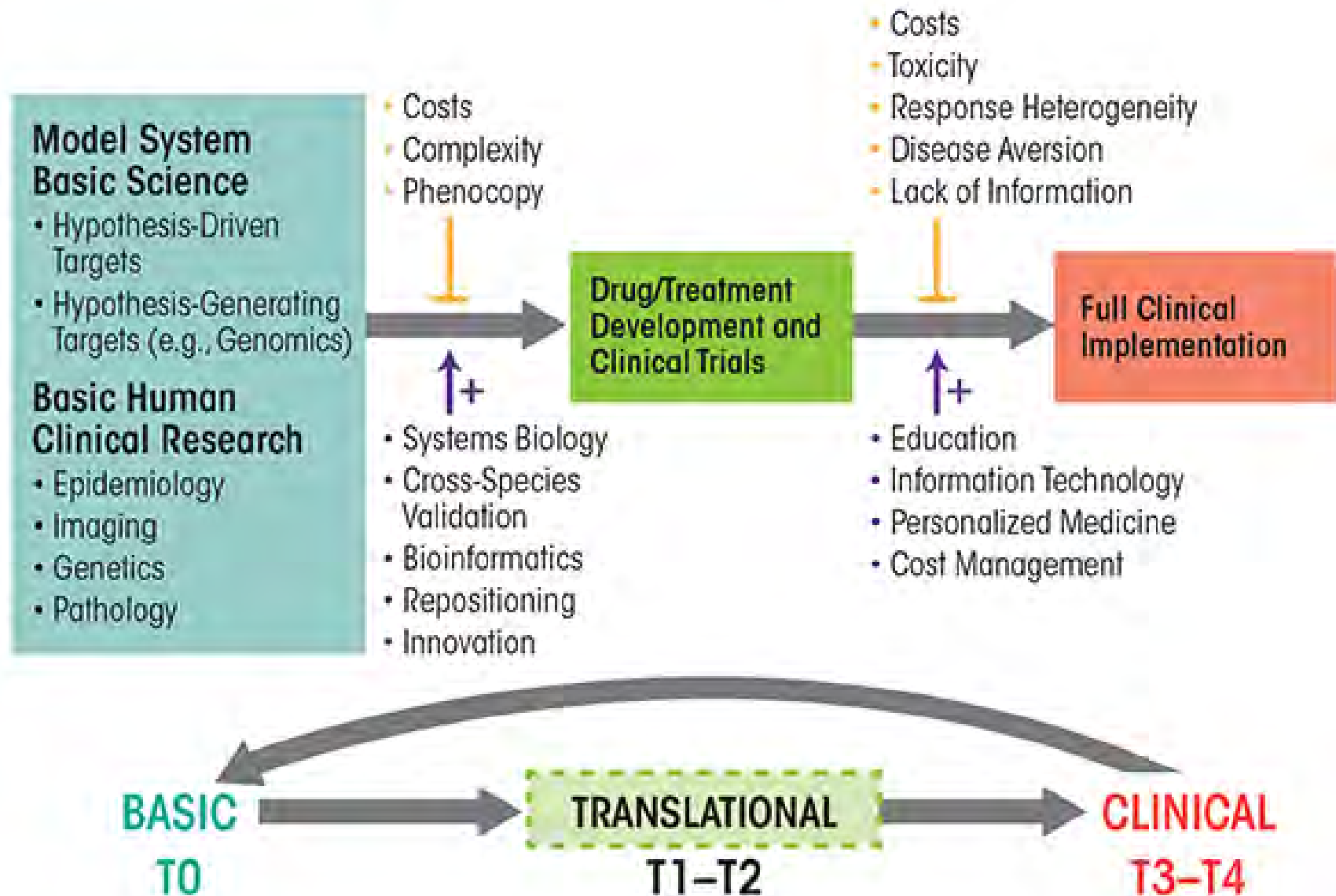
Personalized Medicine



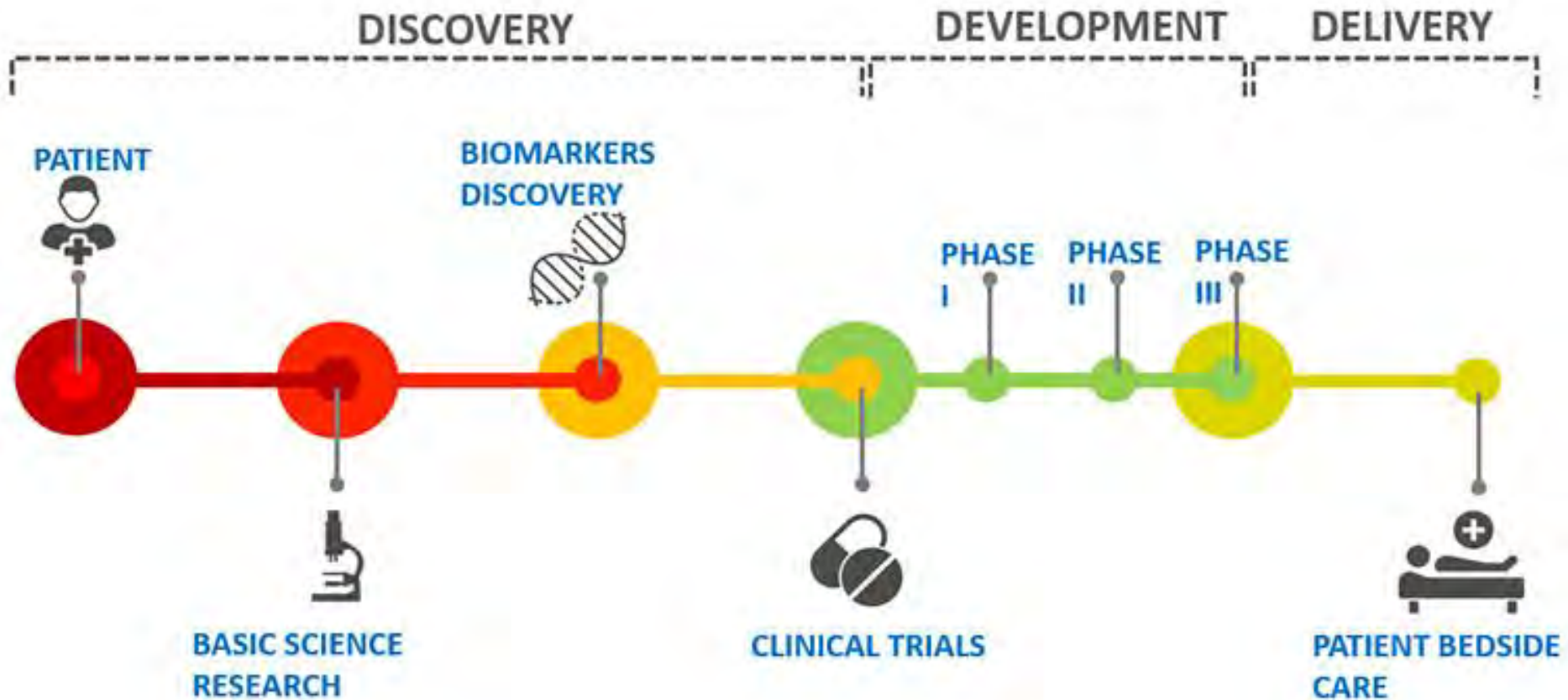
From **Basic Research** to **Precise Personalized Medicine** and **Improved Health**



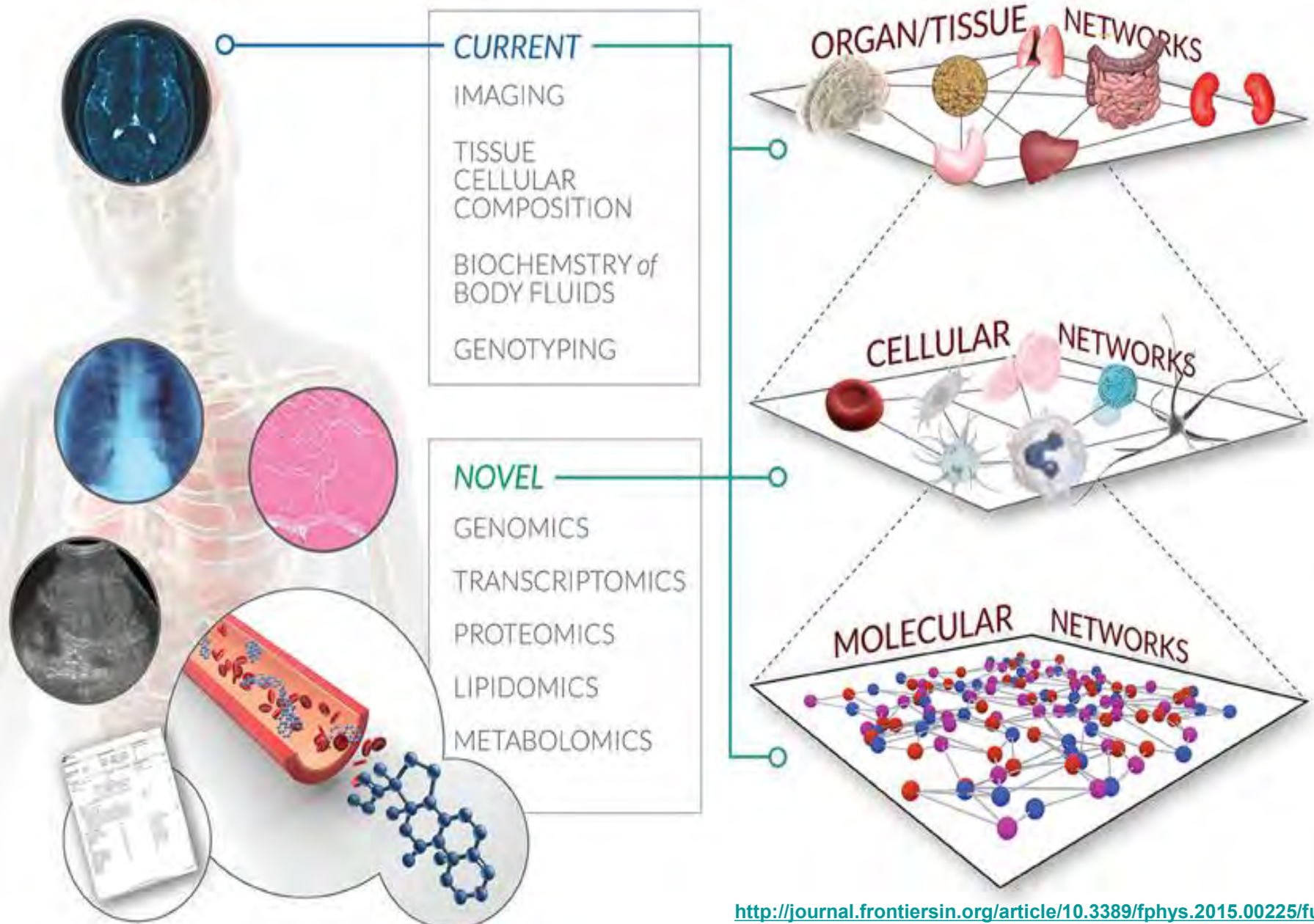
From Basic Research to Precise Personalized Medicine and Improved Health



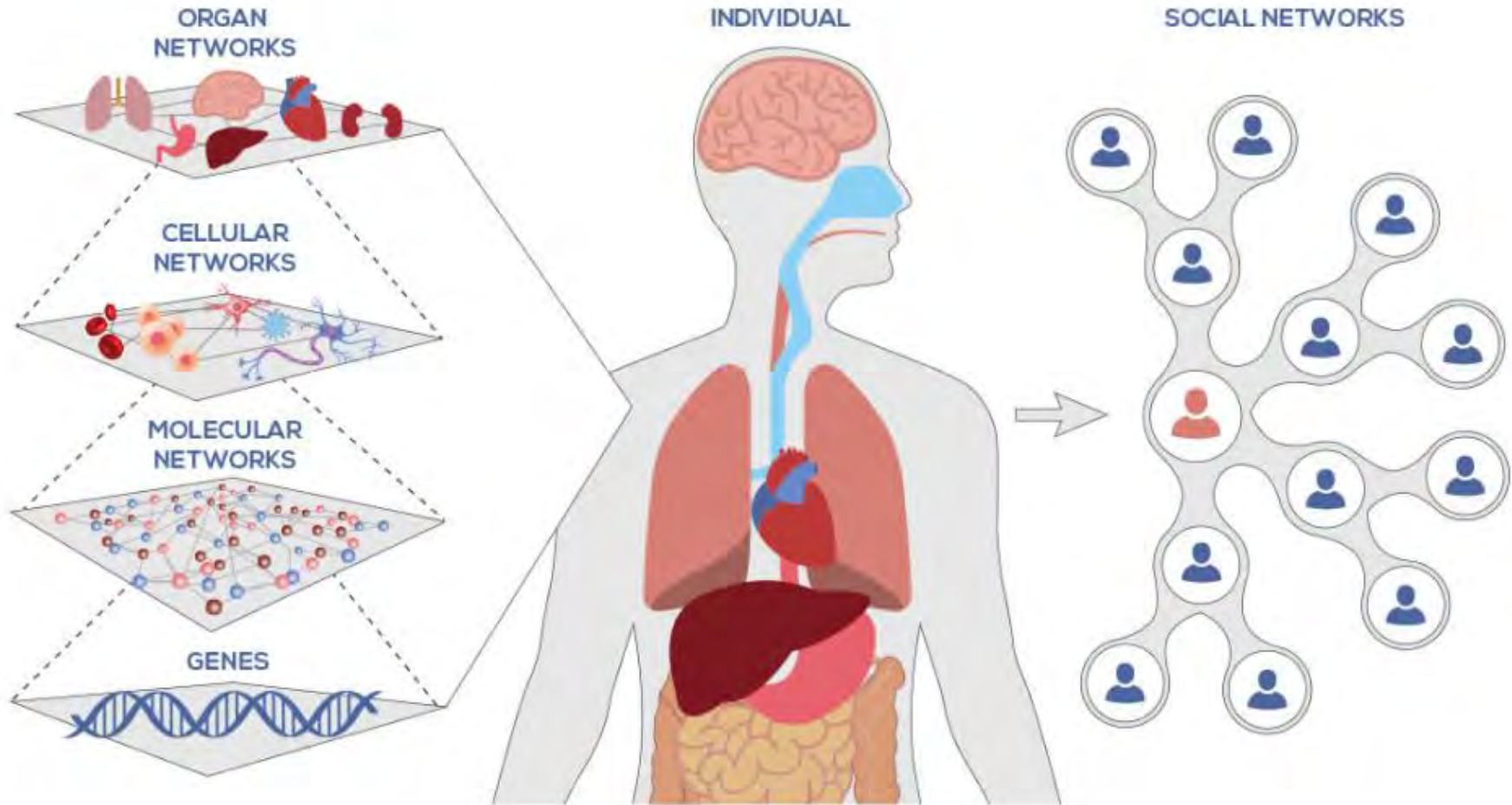
From Basic Research to Precise Personalized Medicine and Improved Health



The era of **-omics** and **Systems Biology** -The New Biology

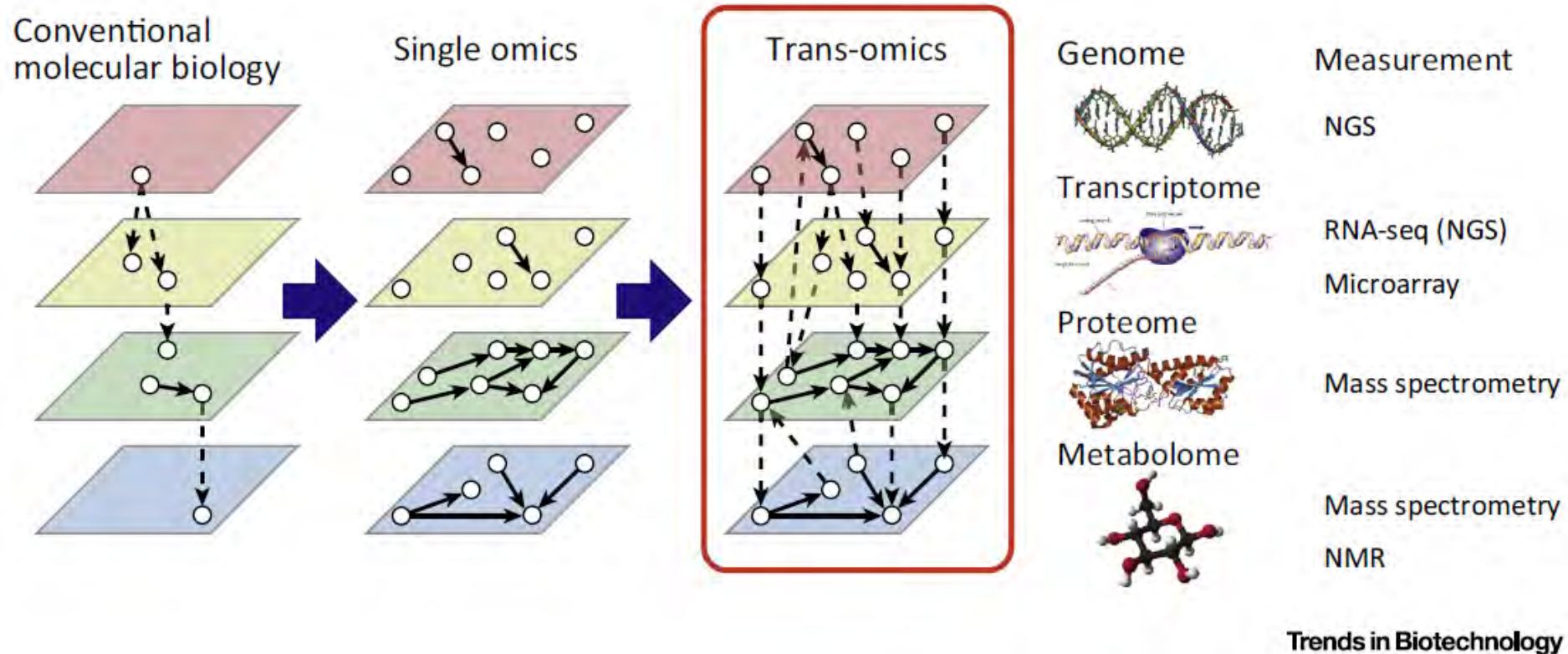


The era of **-omics** and **Systems Biology** -The New Biology



The era of **-omics** and **Systems Biology** -The New Biology

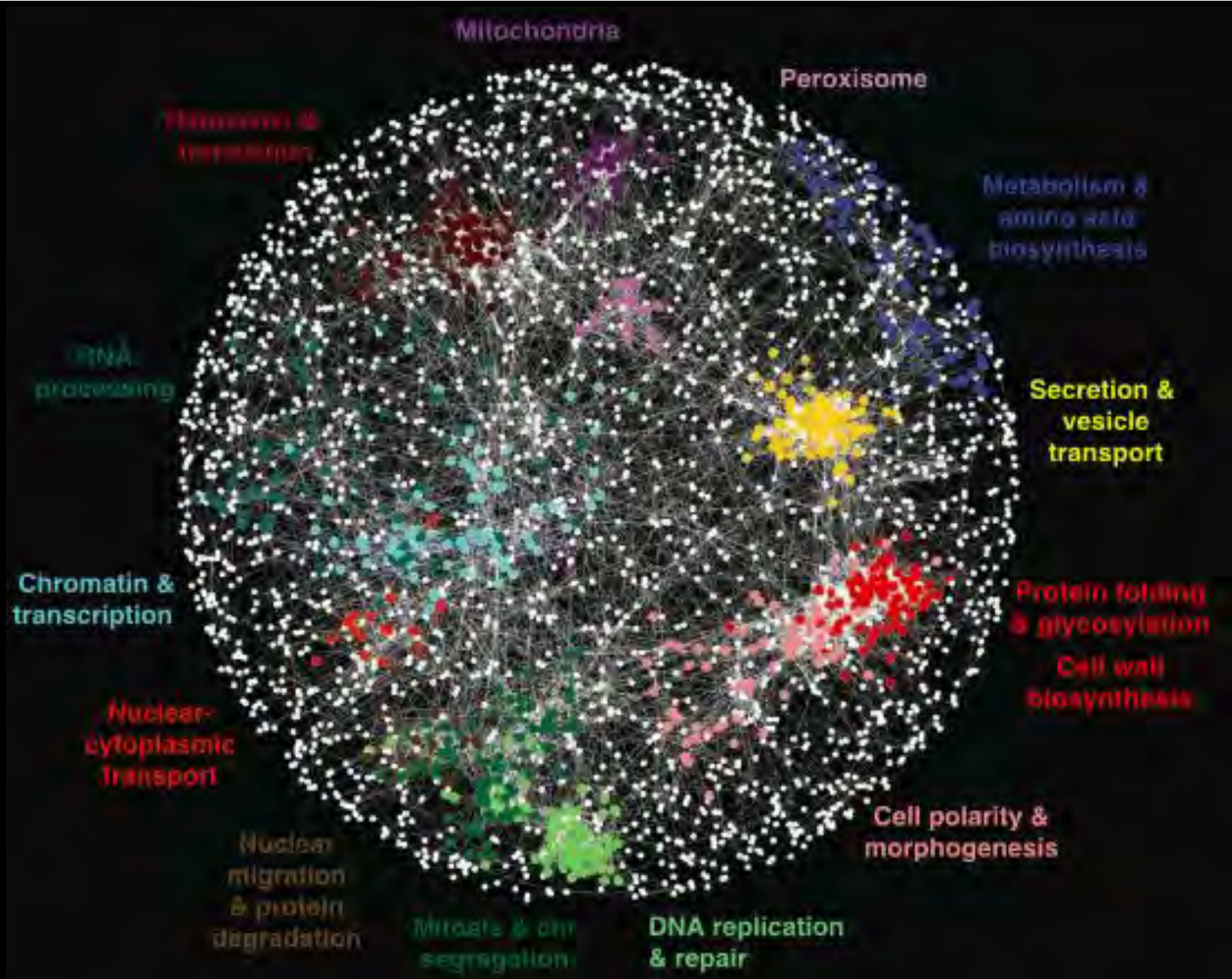
Collection and integration of multi-omics data



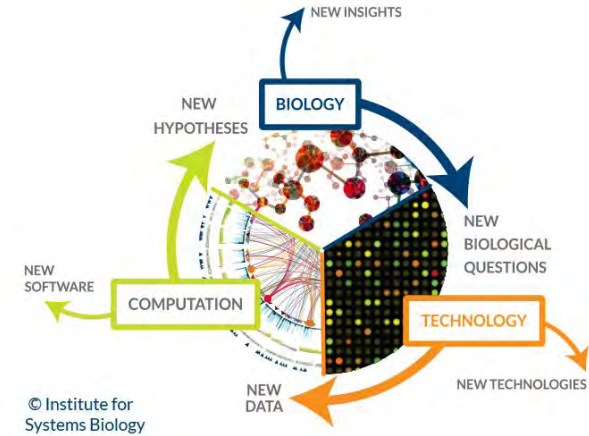
The era of **-omics** and **Systems Biology** -The New Biology

Competency	Relevance
Genomics	Studies of genomes and functional and regulatory elements
Genetic variation	Studies of genome variations
Epigenomics	Studies of hereditary marks in chromatin (histones, DNA)
Transcriptomics	Studies of transcripts, including noncoding RNA and micro RNA
Proteomics	Studies of proteins, including their structure
Metabolomics	Studies of metabolites in cells, tissues, and body fluids
Systems biology	Holistic analysis of the cellular biochemical interaction networks

Biological Interaction Networks



The era of **-omics** and **Systems Biology** -The New Biology



- **Integrative systems biology**

*Extracting biological knowledge from the **-omics** through integration*

- **Predictive systems biology**

Predicting future of biosystem using 'omics knowledge, e.g. in-silico biosystems

Functional Omics

Biological System/Pathway/Process/Condition



Directly relevant sub-ome

genes

DNA methylation

transcripts

proteins

metabolites

Functional Omics

**Health/Disease/Pathobiology/Mechanisms
(Humans, animals, microorganisms, viruses)**

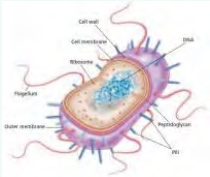
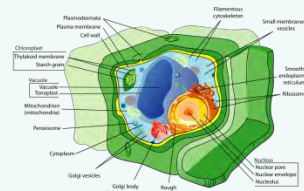
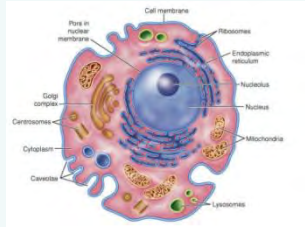


Directly relevant sub-ome

**genes
DNA methylation
transcripts
proteins
metabolites**

Functional Omics

Biological condition(s)



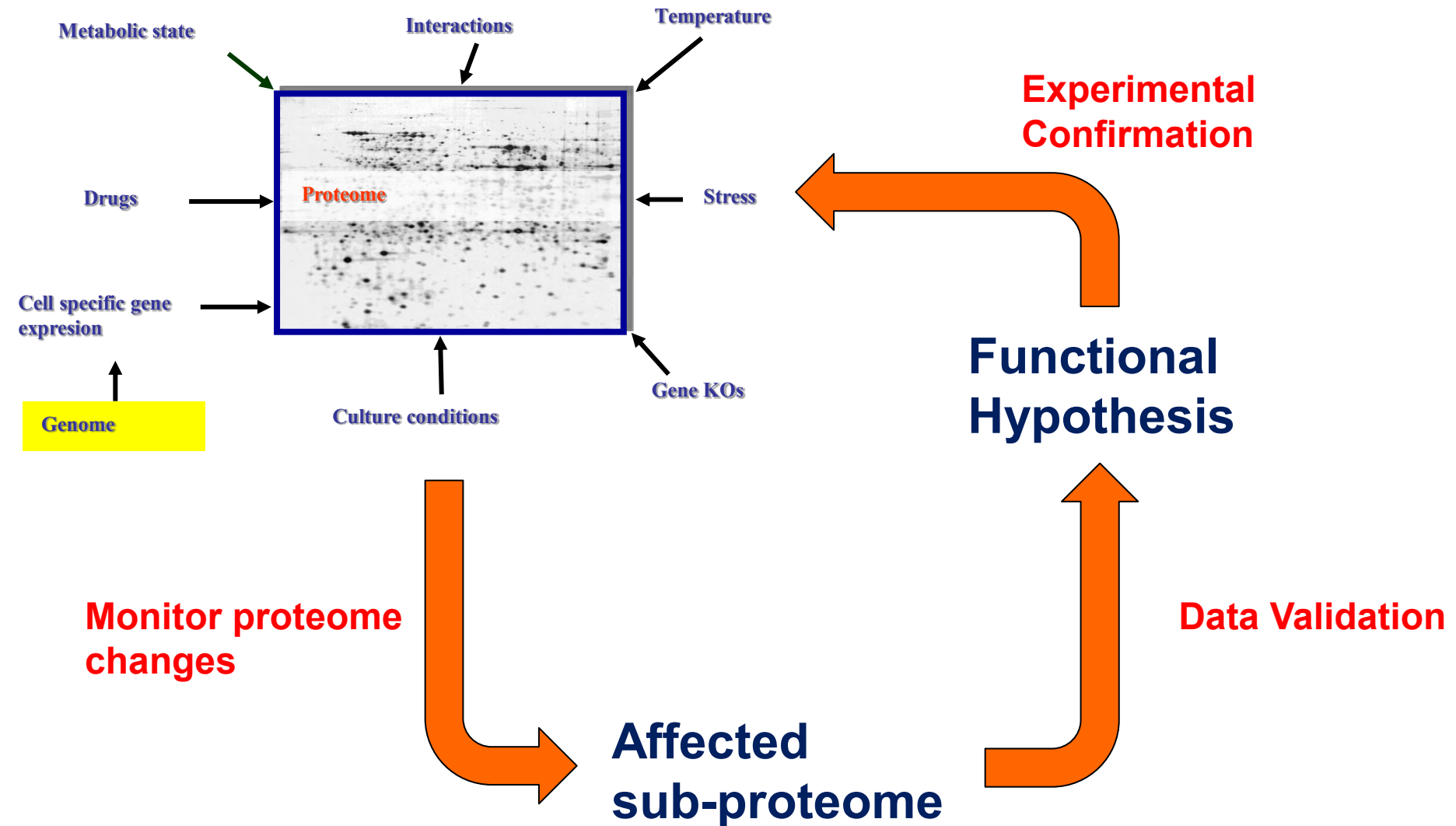
Biological fluids

Biomolecule

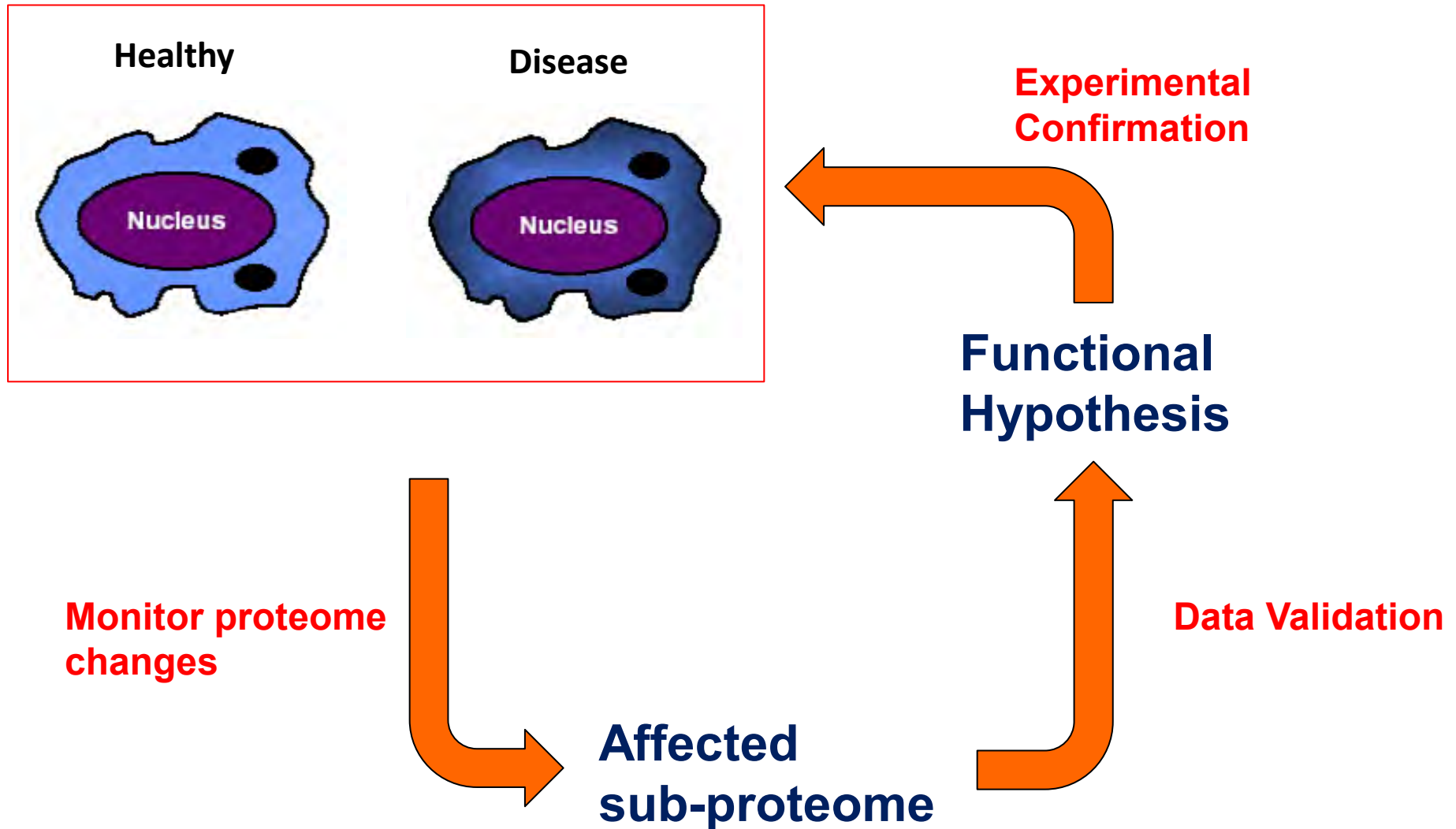
changes in:

- **Abundance**
- **Localization**
- **Modification**
- **Structure**
(primary, quaternary)
- **Interaction**
(binary, complexes)

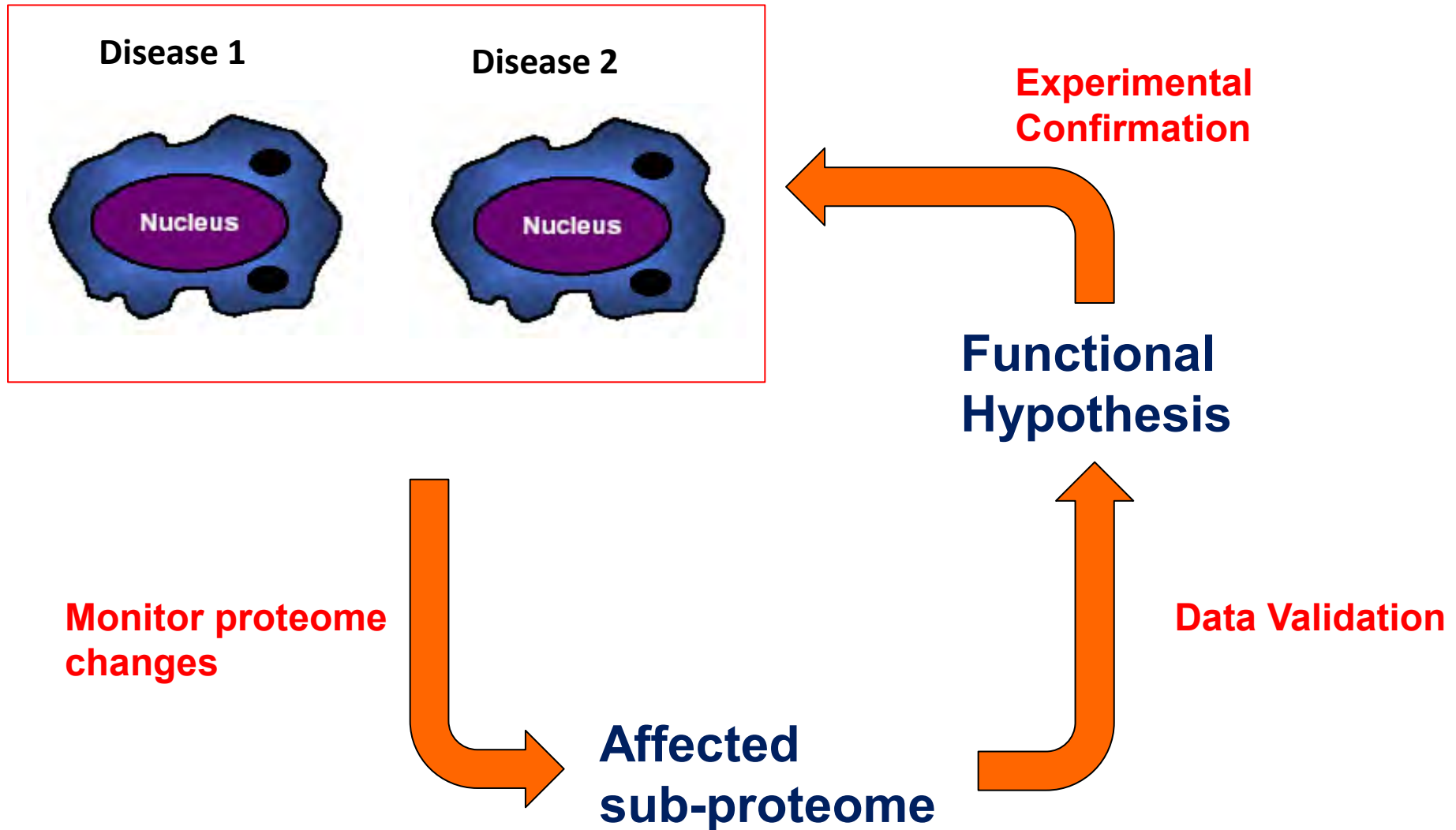
Functional stimulation, perturbation, comparison



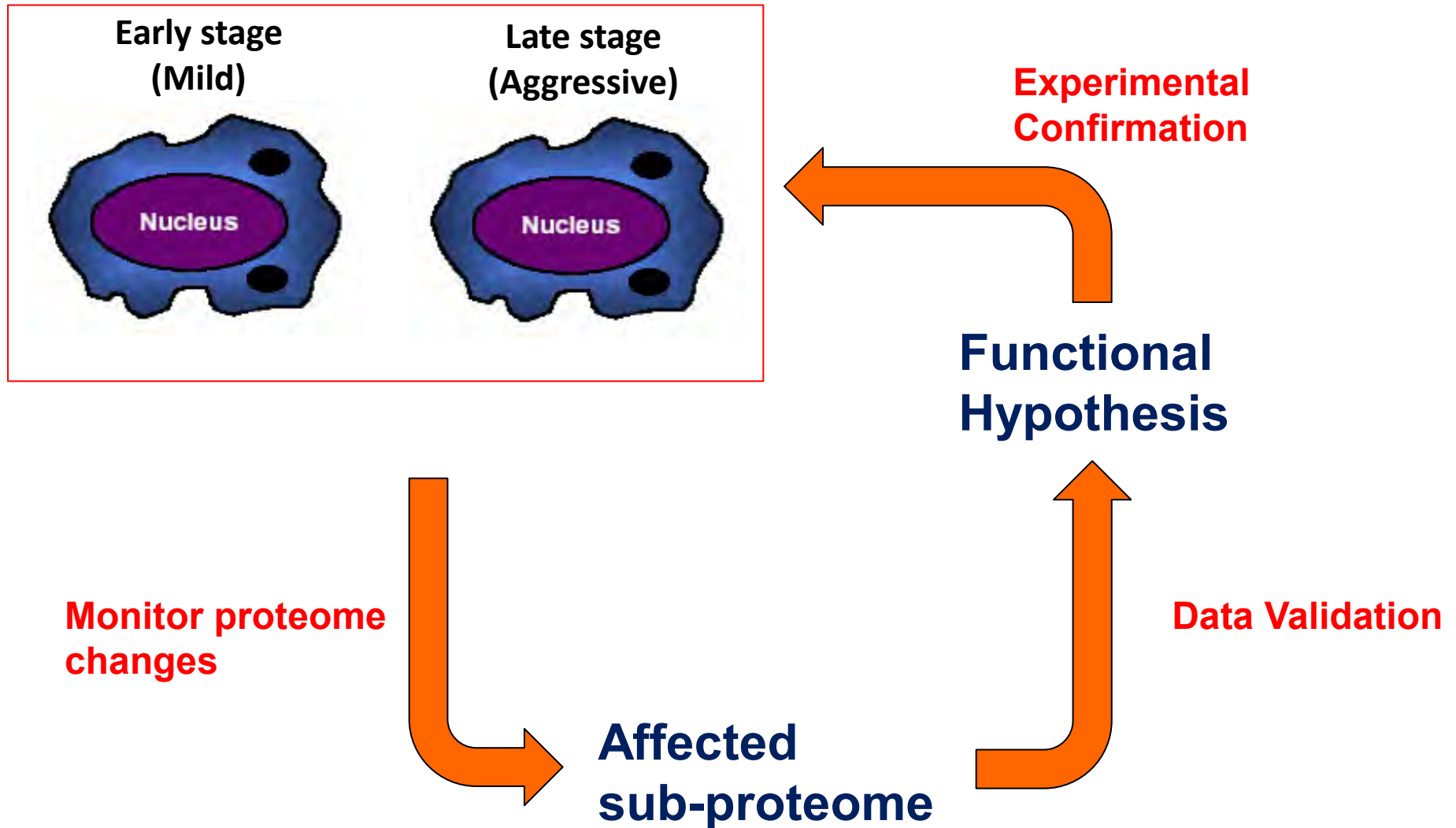
Comparative Proteomics – Healthy vs Disease



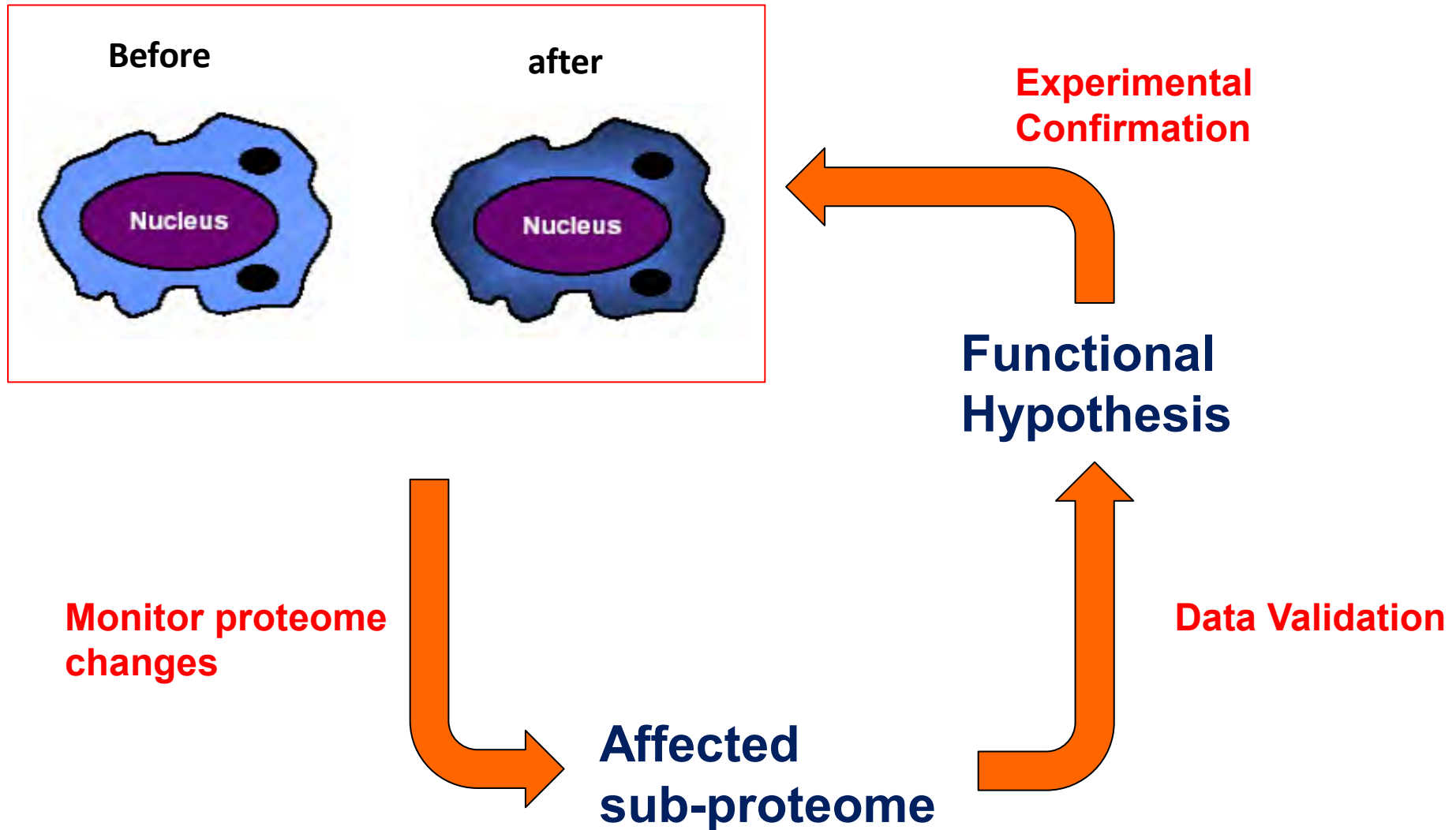
Comparative Proteomics: Disease 1– Disease 2



Comparative Proteomics: Disease (sub) stages



Comparative Proteomics – Diet/Nutrition/drug effect



What is the proteome ?

- **Genome:** 20,000 genes, static (?)
- **Transcriptome:** > 40,000 mRNAs, dynamic (other RNAs)
- **Proteome:** > 47,000 proteins, highly dynamic

Proteome variability: genomic variations,
gene expression
alternative splicing
protein cleavage
modifications

Why perform proteomics ?

Same genome, different proteome



DNA

mRNA

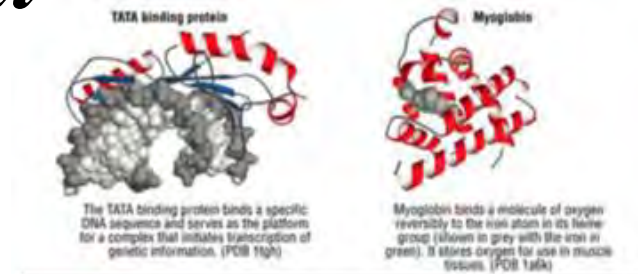
Proteins

tells what **possibly**,
what **probably** and
what **actually** happens.

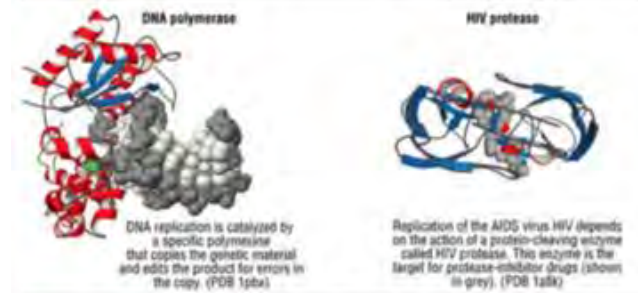
Proteins are flexible multi-tools

The MVPs of cell

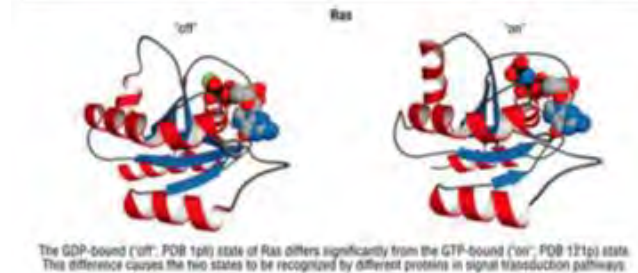
Binding



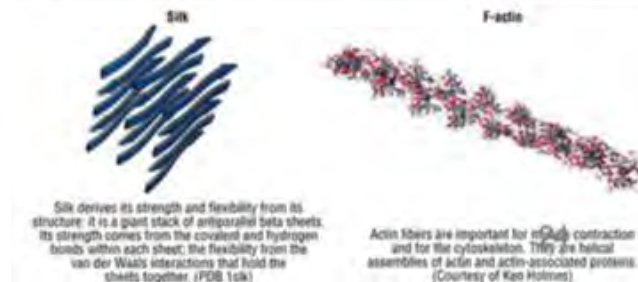
Catalysis



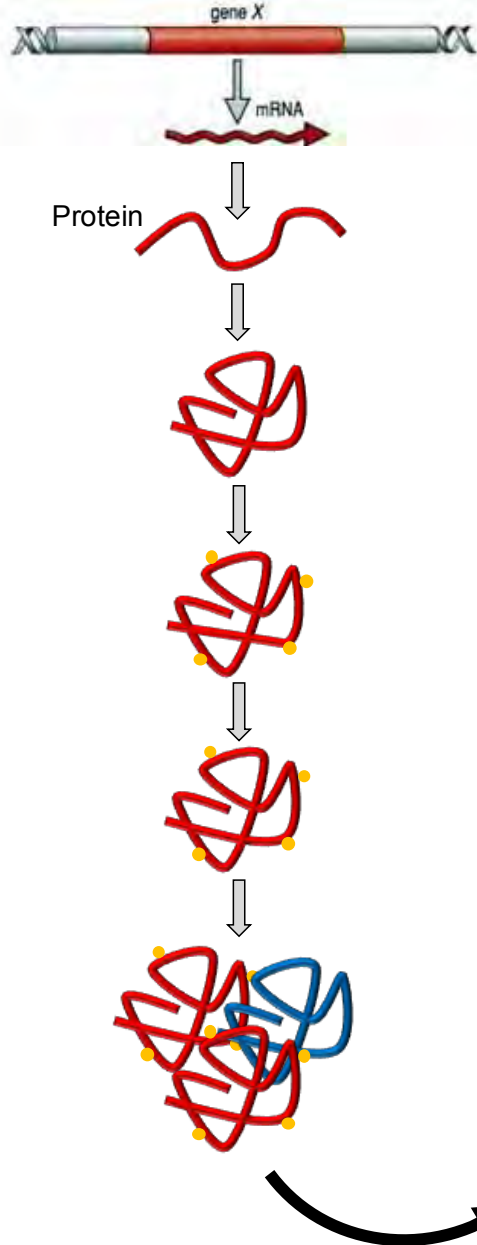
**Switch
(regulation)**



Structure



Protein Maturation/Regulation/Function



Primary and Secondary

Tertiary

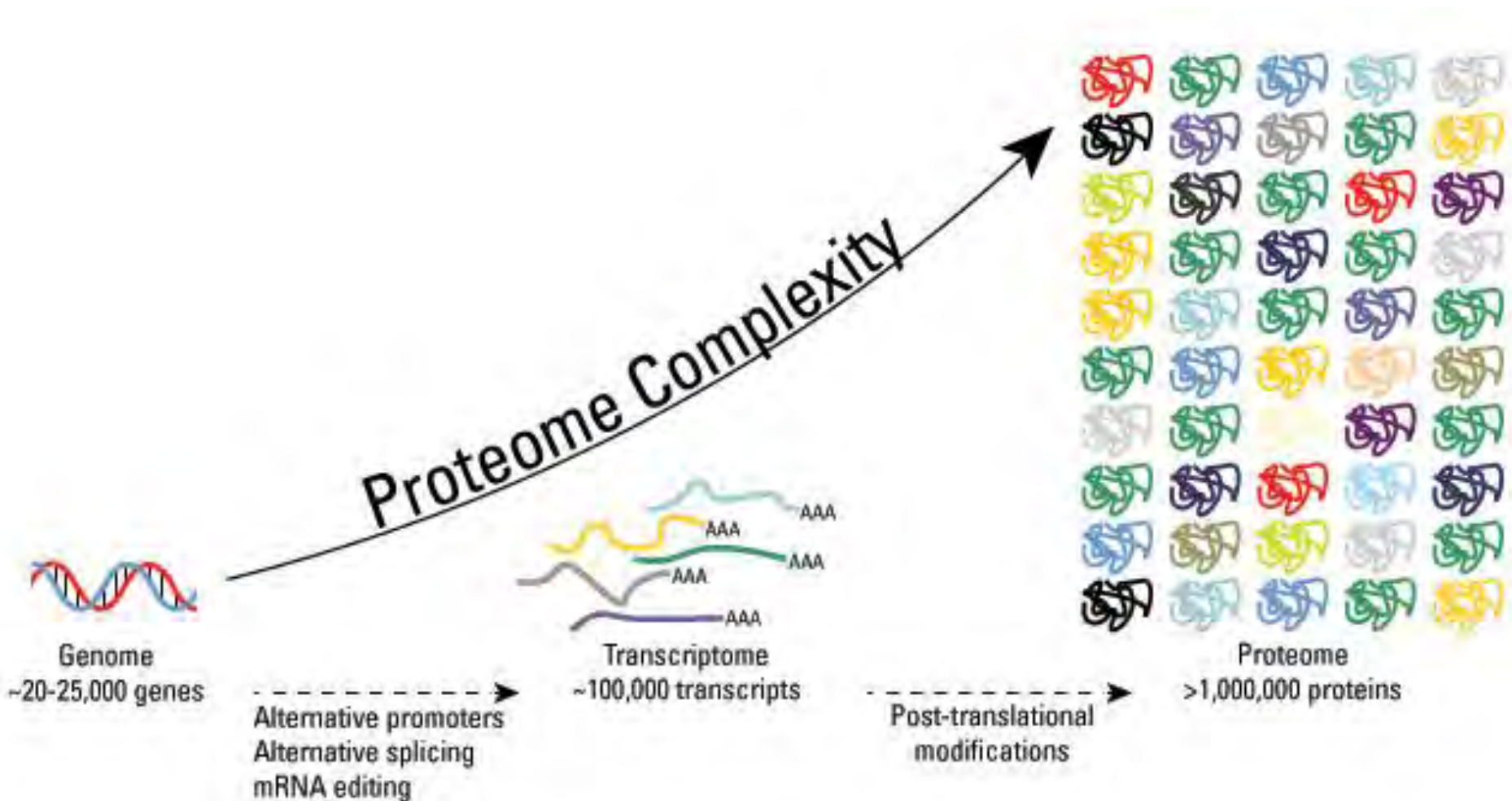
Modifications

Localization

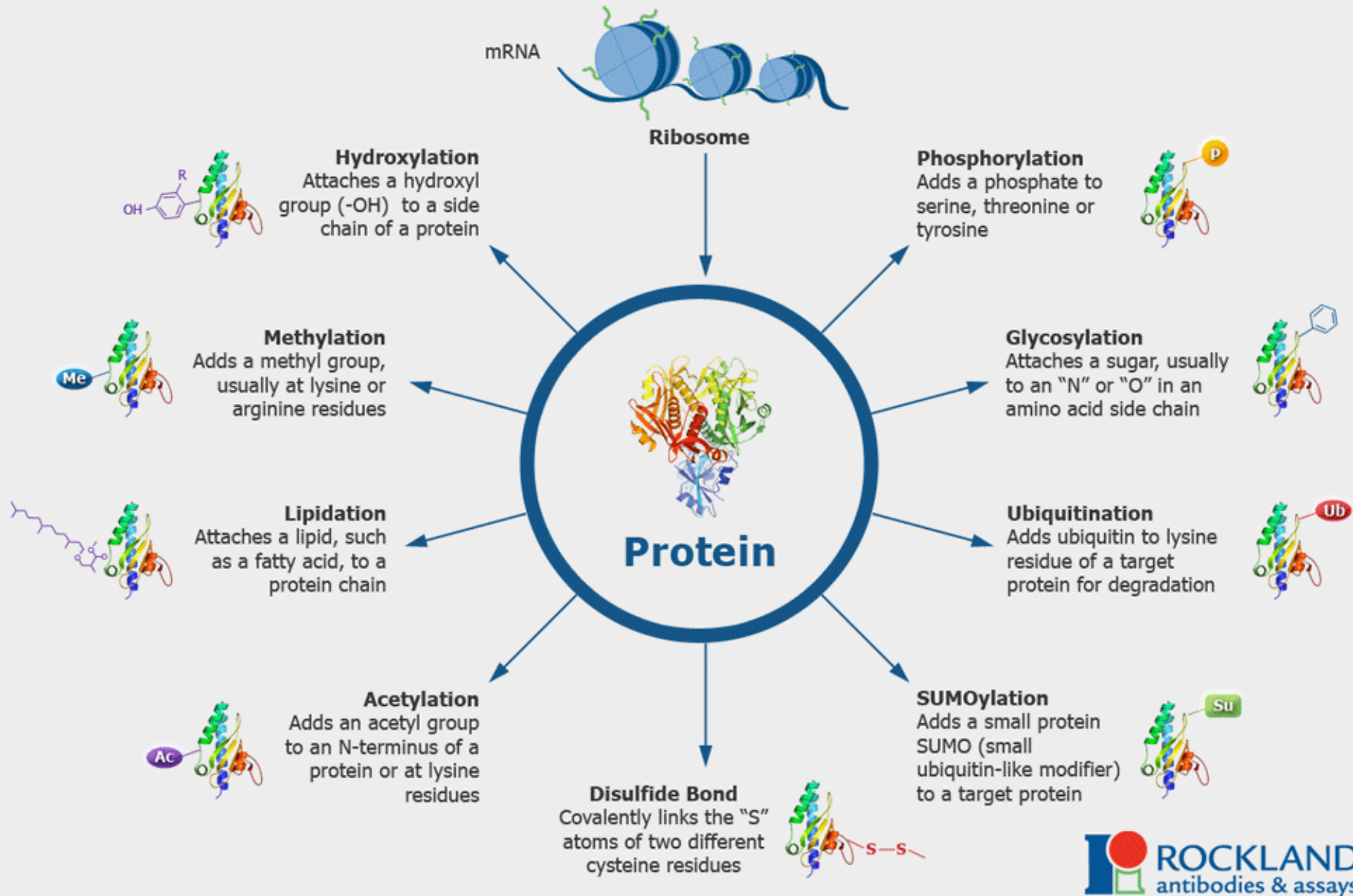
Quaternary (Protein Complex)

Interactions / Regulation/Death

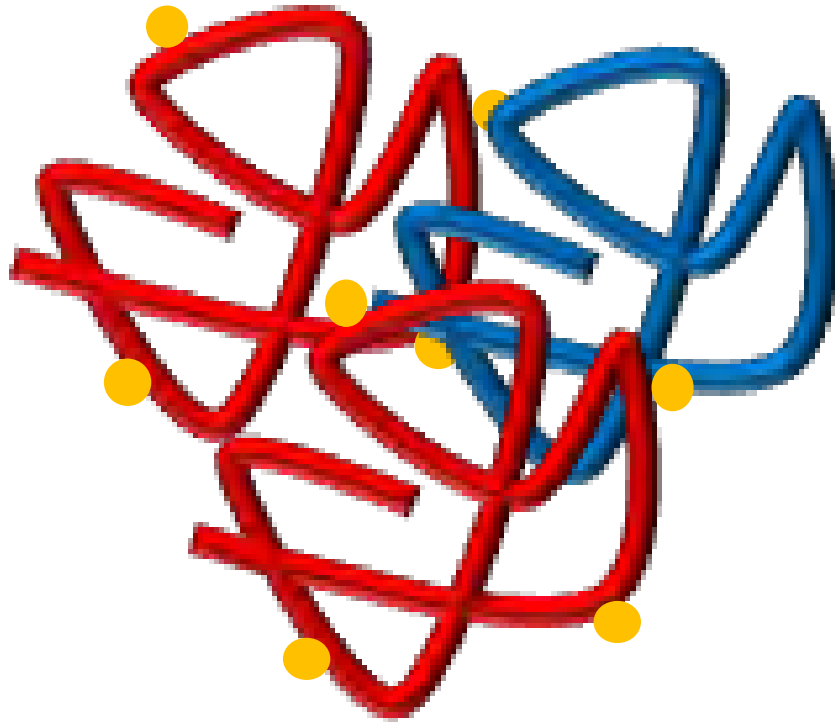
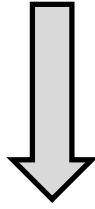
Protein Modification



Protein Modification ~ 200 known PTMs



Protein Complex

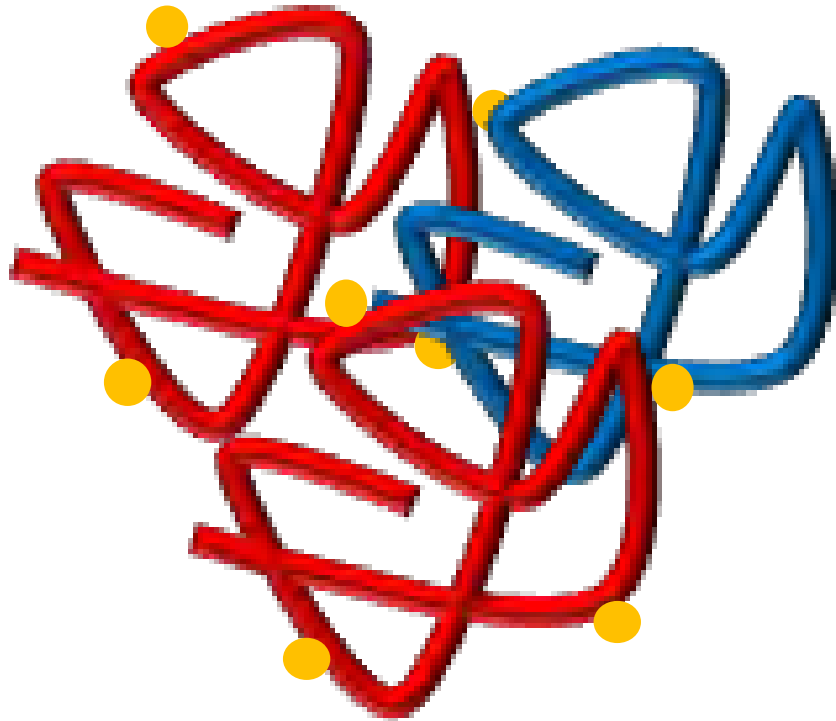
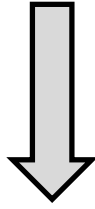


≥ 2 polypeptide chains

Non-covalent interactions

- Hydrogen bonds
- Electrostatic
- Hydrophobic
- Van der Waals

Protein Complex



Highly dynamic

- Size
- Shape
- Interacting partners
- Localization
- Stability
- Abundance
- Properties

Classical biochemistry

[illegible]

Classical biochemistry

[illegible]

Classical biochemistry

[illegible]

Classical biochemistry

[illegible]

Classical biochemistry

[illegible]

Classical biochemistry

[illegible]

Classical biochemistry

[illegible]

Classical biochemistry

[illegible]

Classical biochemistry

[illegible]

Classical biochemistry

[illegible]

Classical biochemistry

???

[illegible]

Omics - strategies

[illegible]

Genomics/Epigenomics

[illegible]

Omics - strategies

Transcriptomics

[illegible]

Omics - strategies

Proteomics

[illegible]

Omics - strategies

Proteomics

bioinformatics

[illegible]

Classical Biochemistry

Genomics/Epigenomics

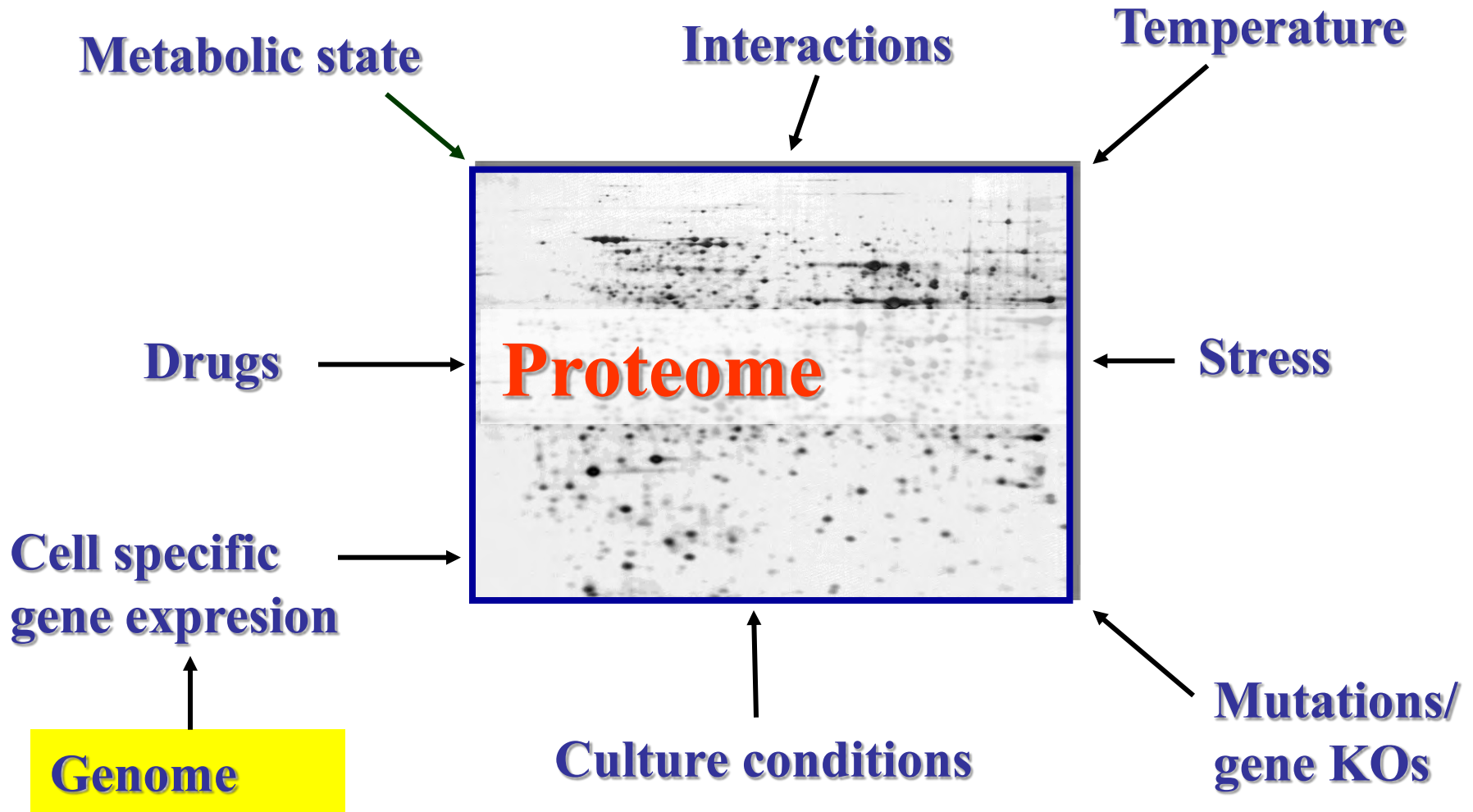
Transcriptomics

Proteomics

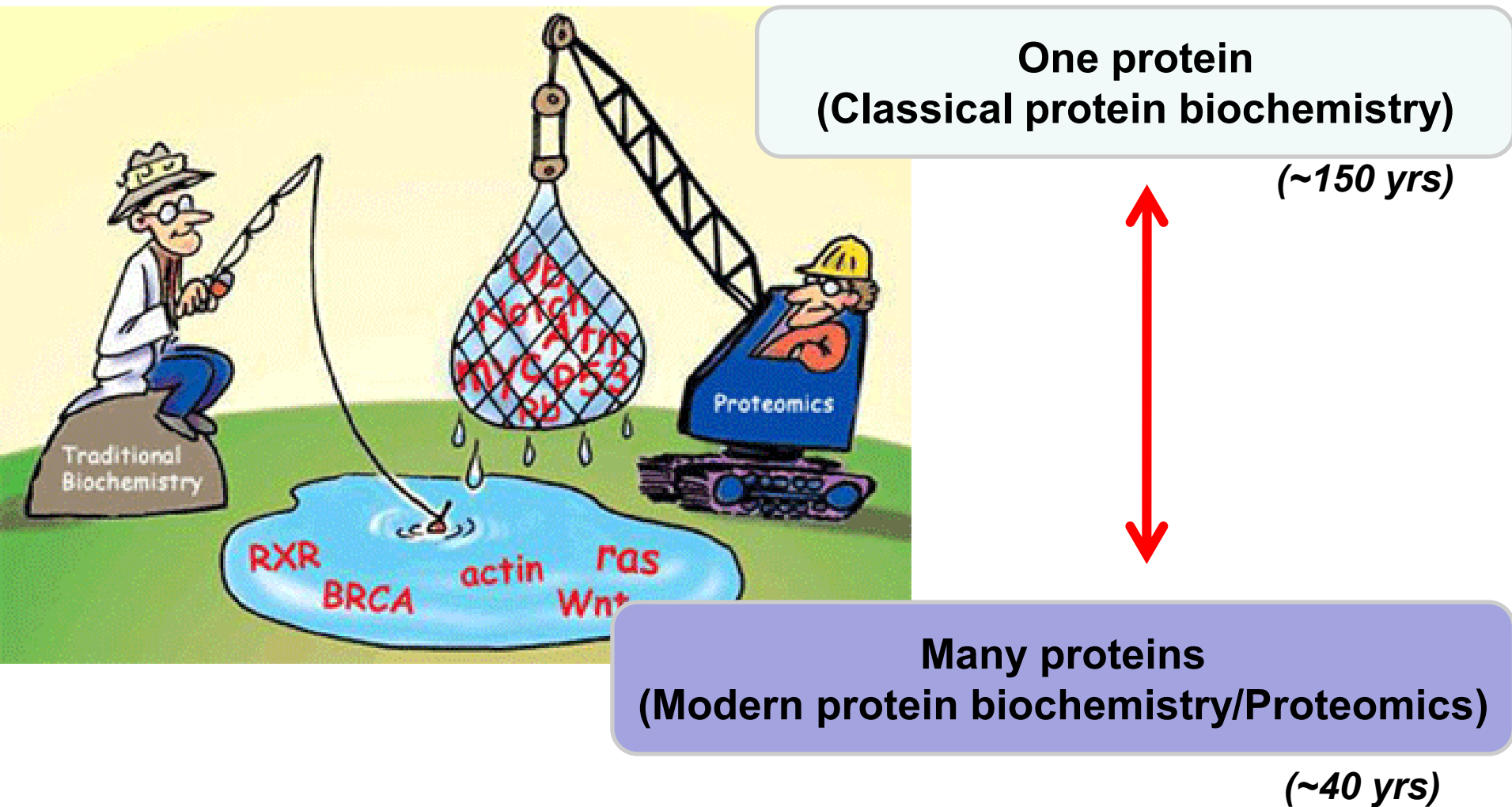
bioinformatics

[illegible]

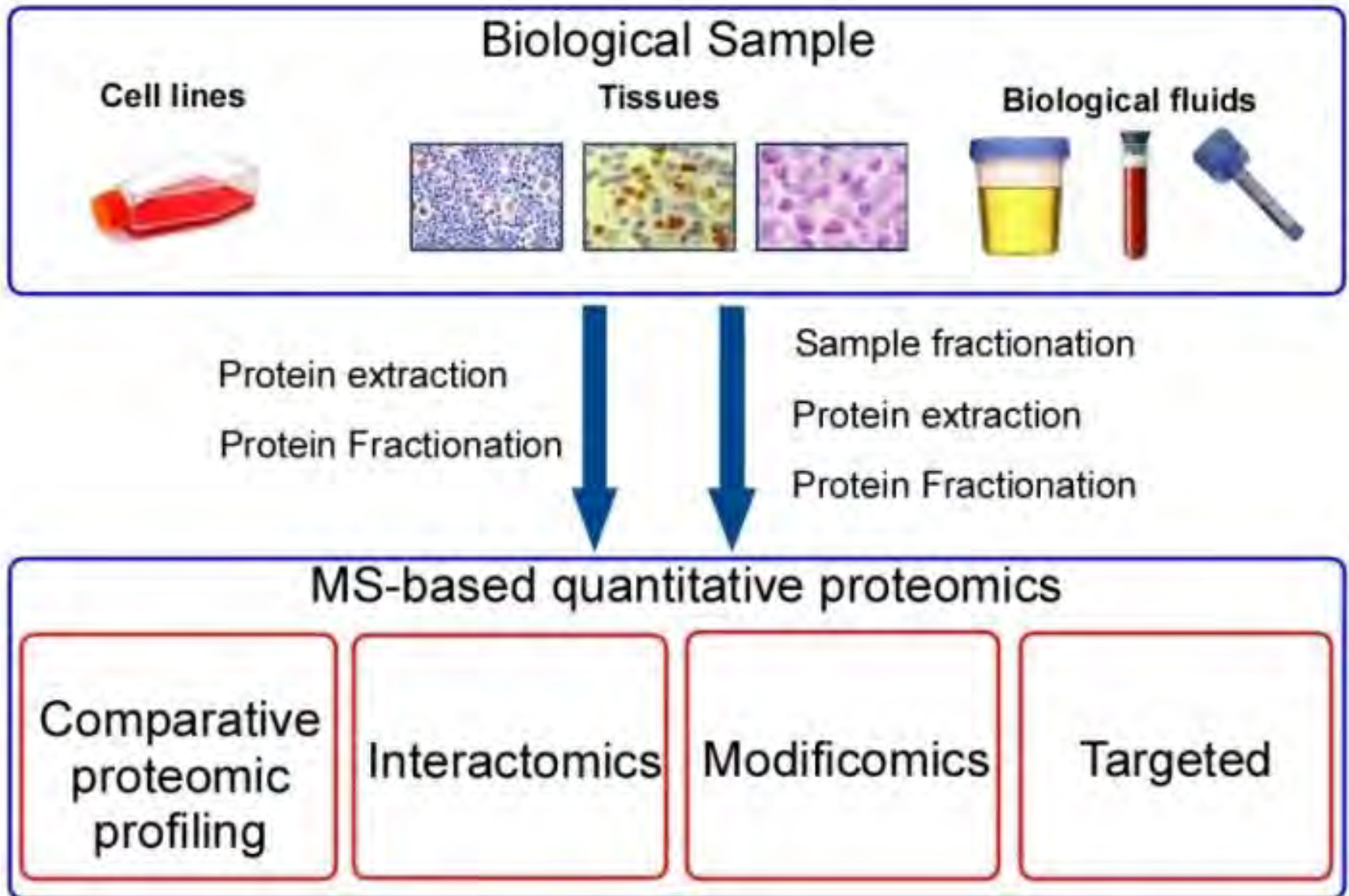
Complexity of Proteome and proteomics



Zoom-in and Zoom-out is required

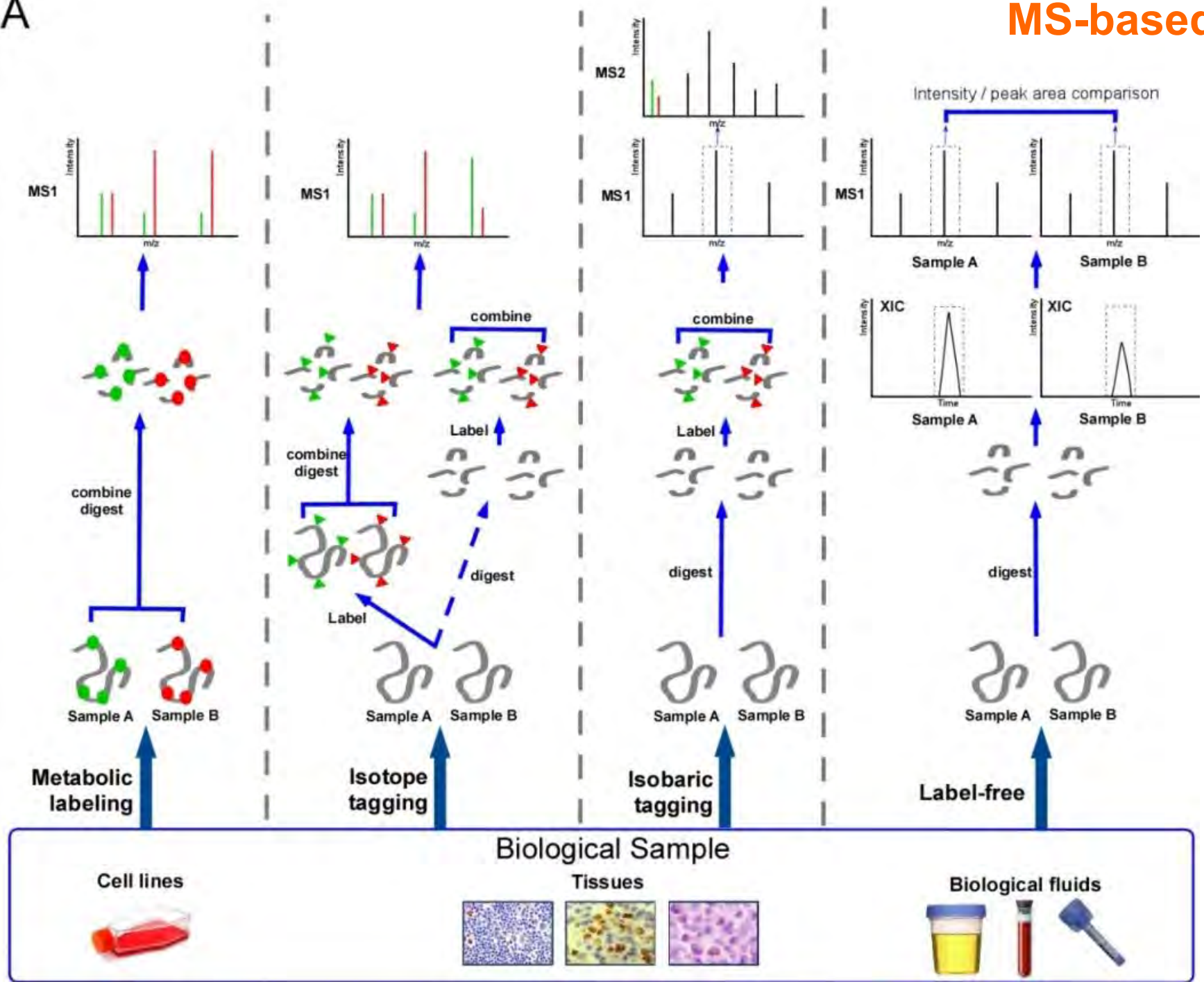


Quantitative Proteomics – Experimental Workflow

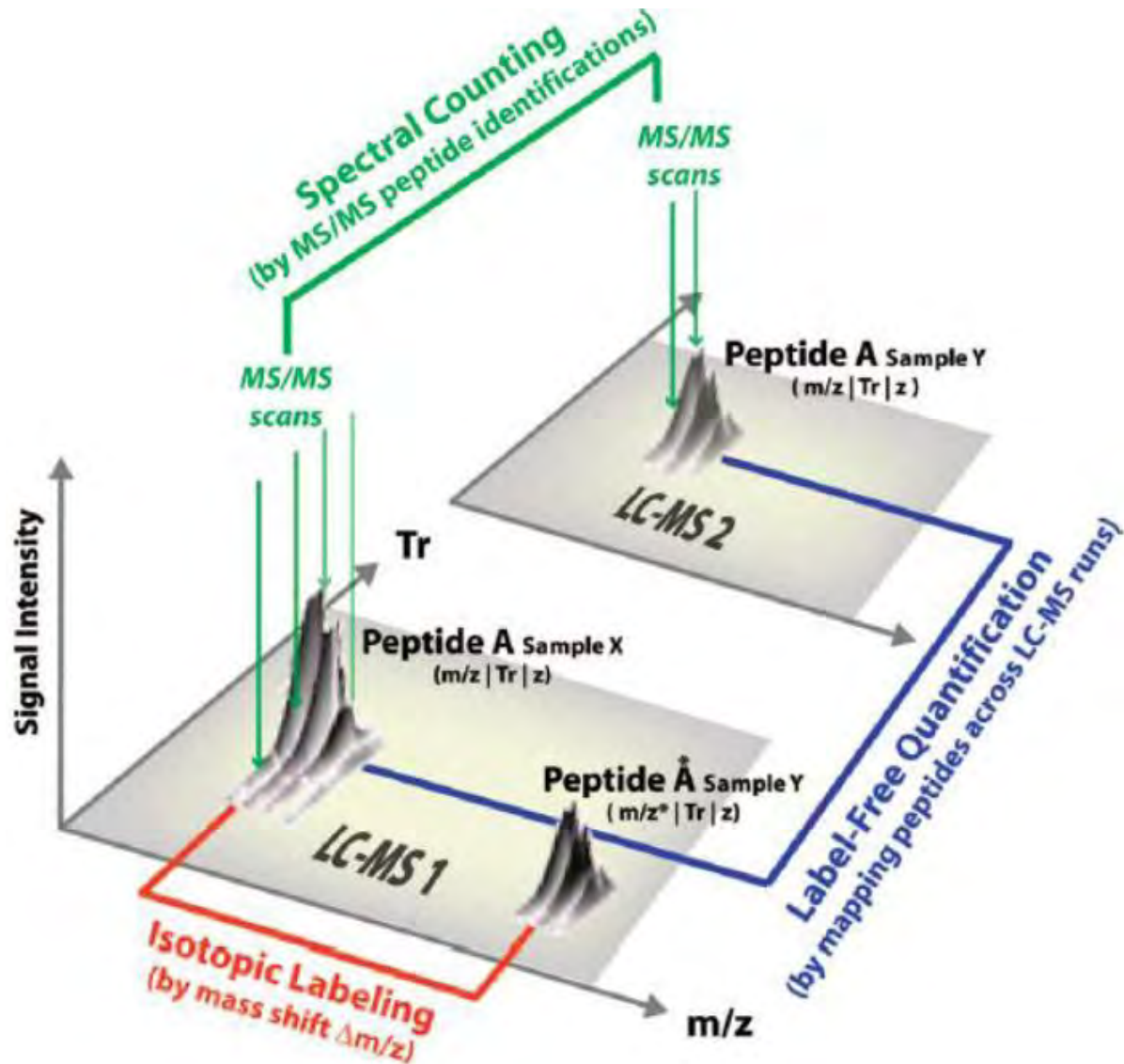


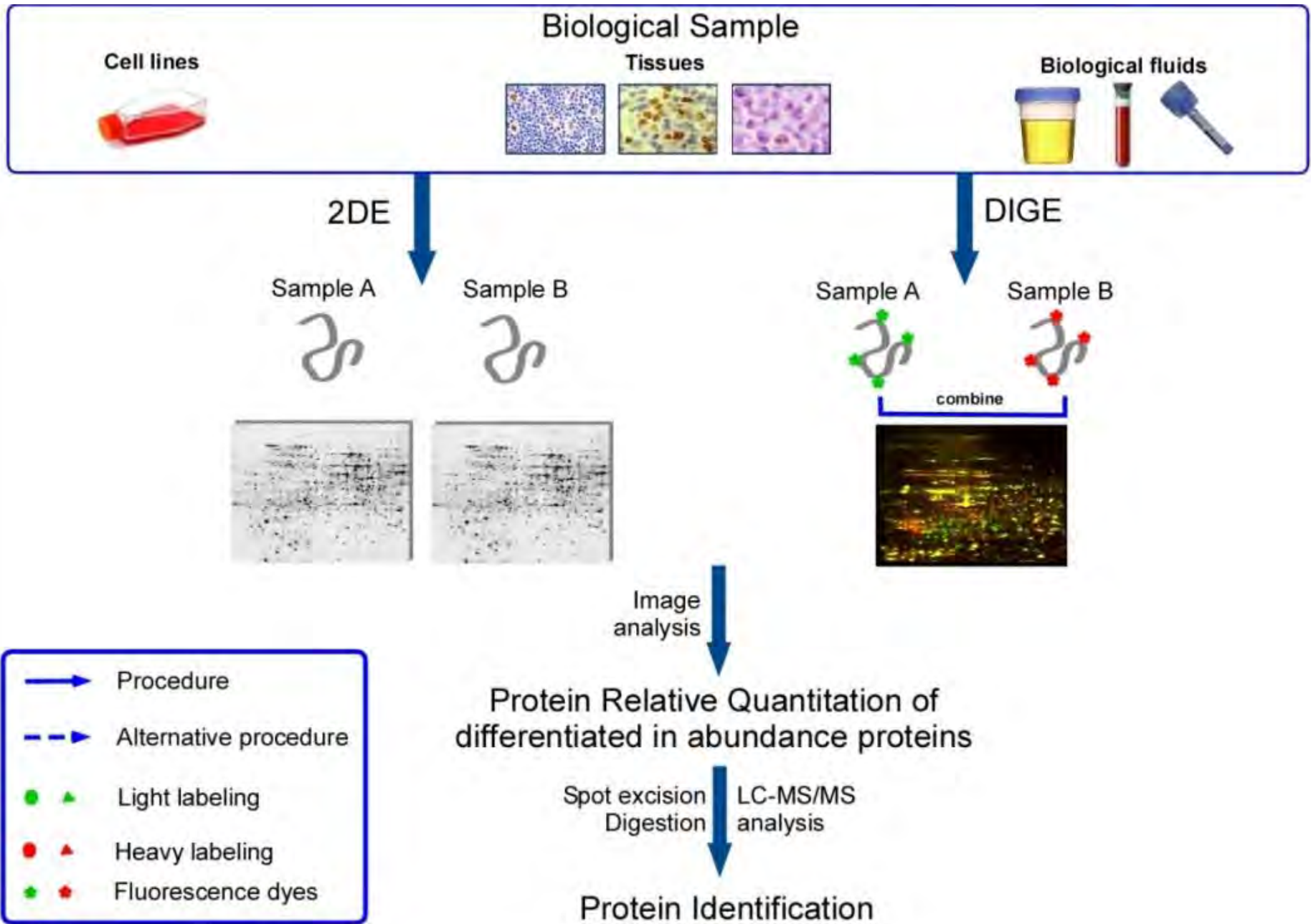
A

MS-based



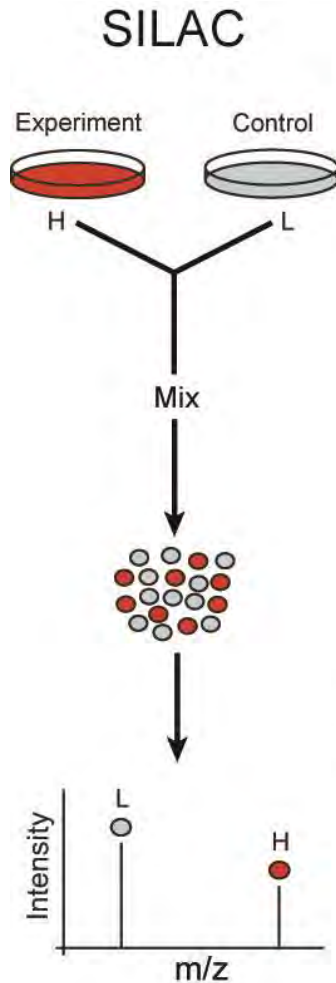
Quantitative MS-Data Analysis





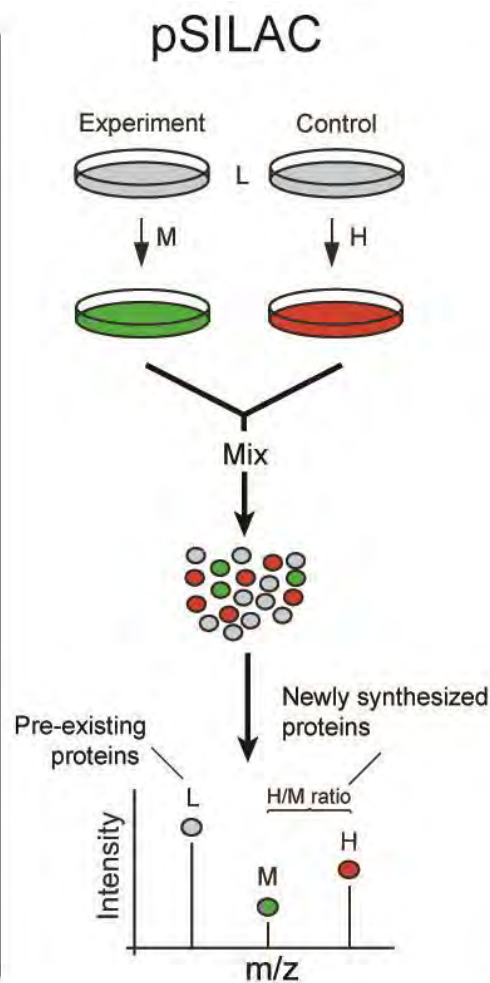
Measuring protein dynamics using SILAC

Relative changes
in protein levels



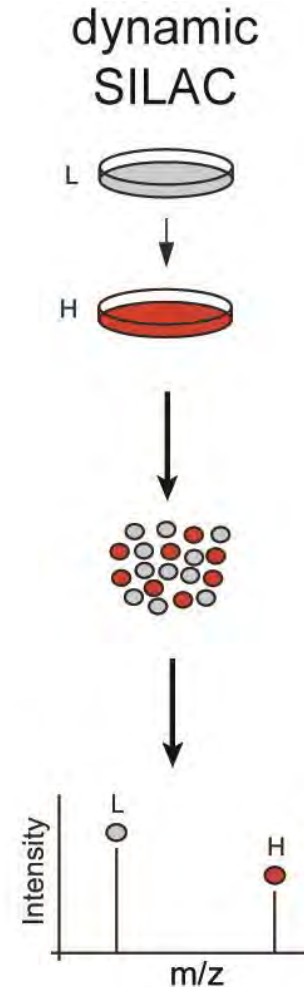
(Ong et al., 2002)

Relative changes
in protein production



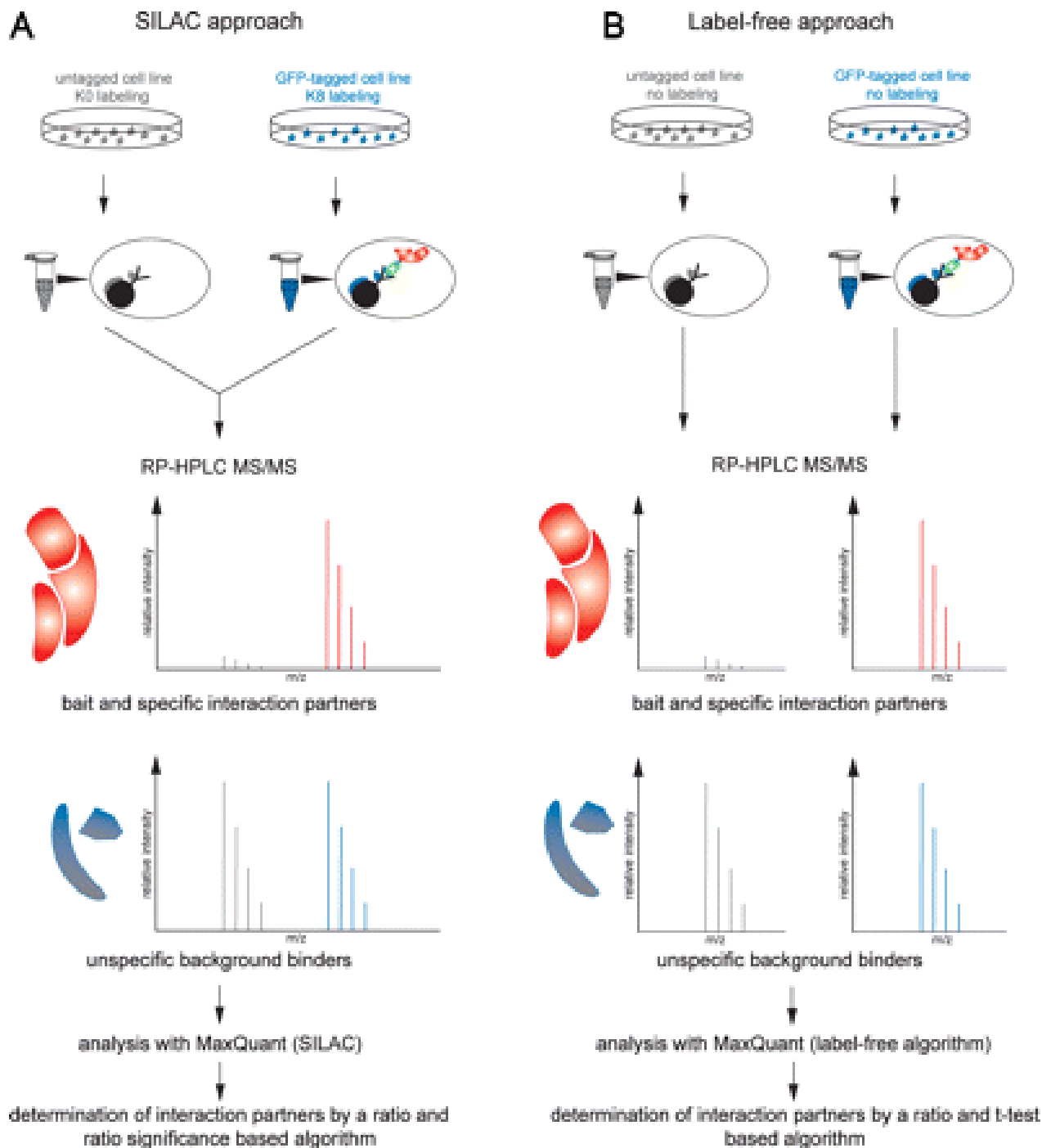
(Schwanhaeusser et al, 2009)

Protein turnover



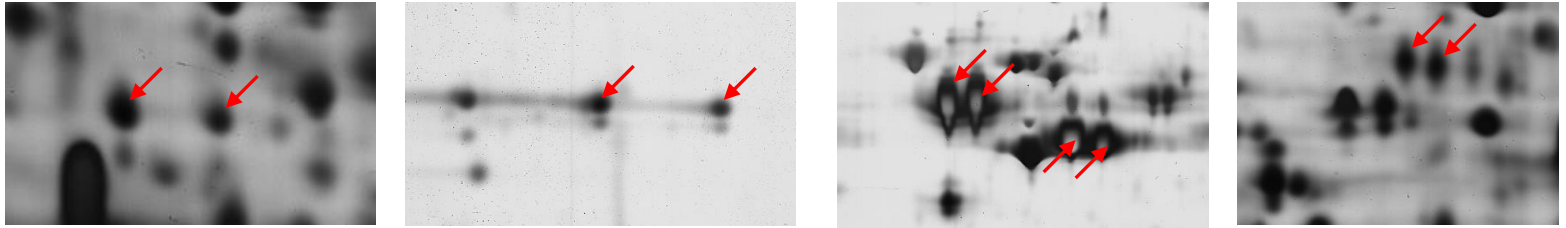
(Doherty et al., 2009)

SILAC vs Label-Free

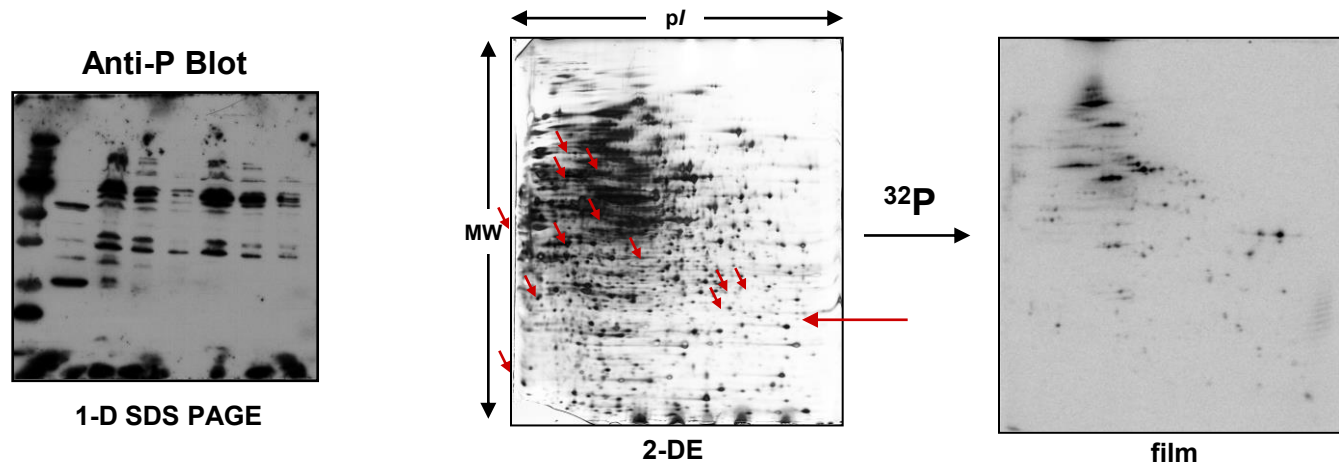


Evidence of PTMs

2-DE

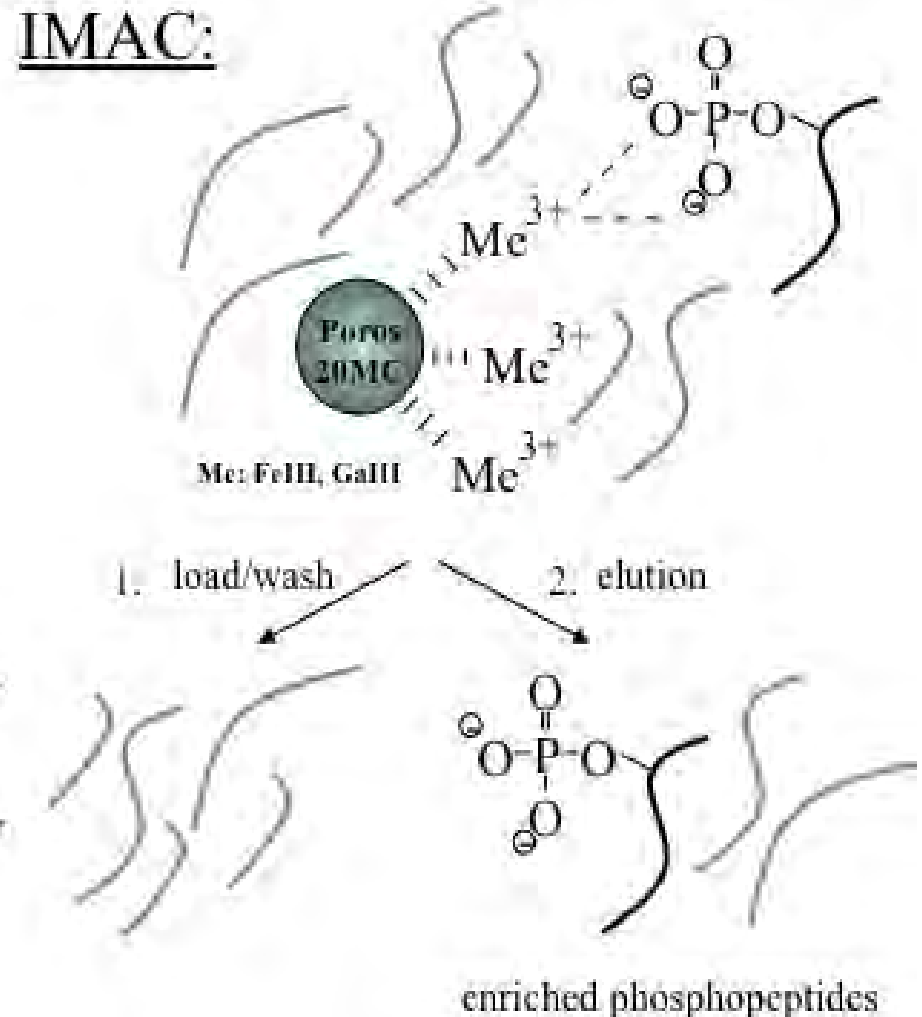
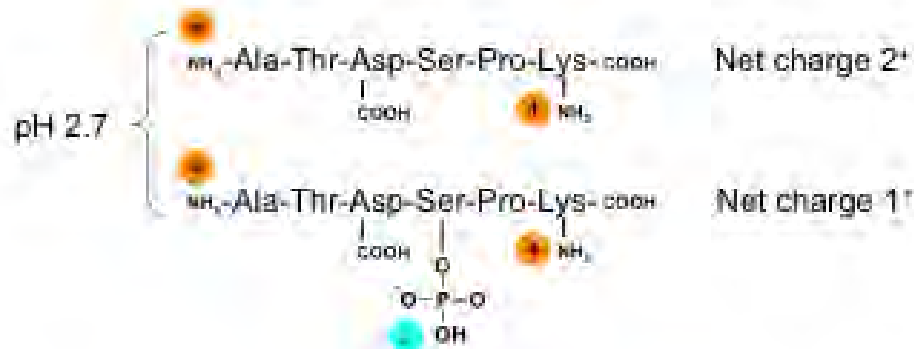
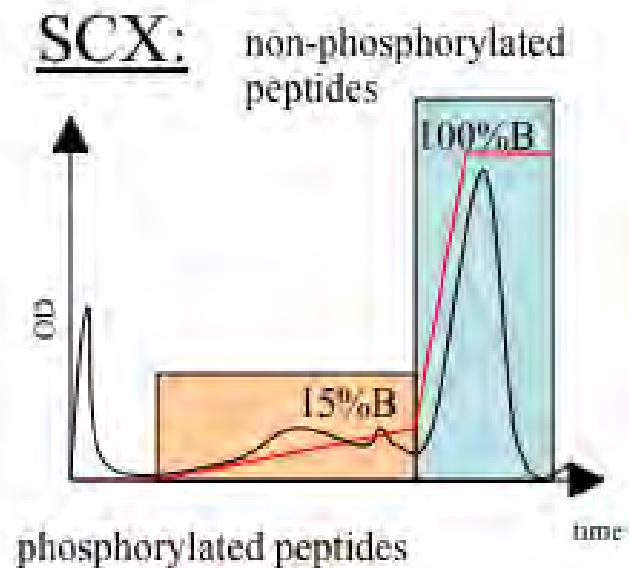


Radioactive labeling/phosphoprotein specific AB



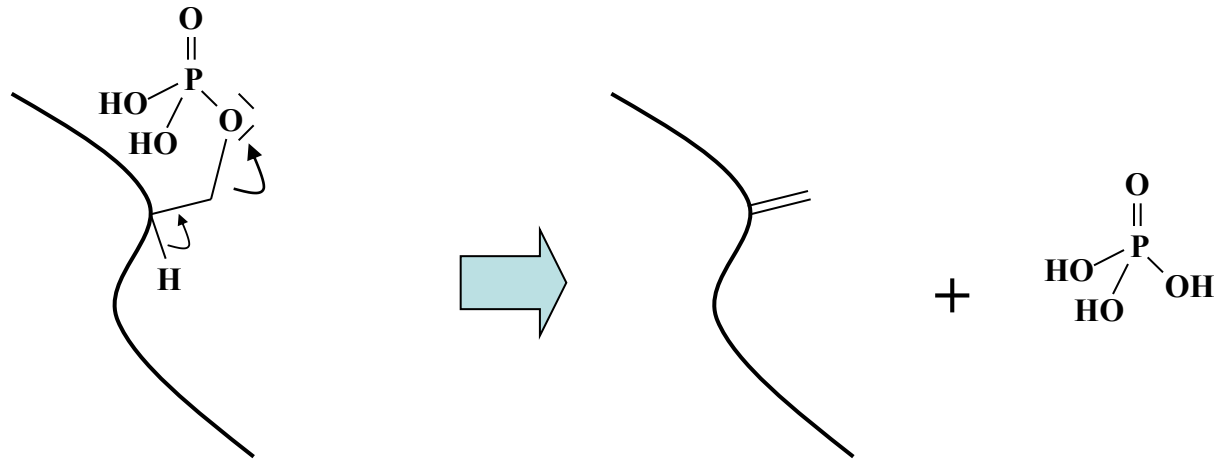
Enrichment of Phosphopeptides

Phosphopeptides are often of low abundance and give bad MS response



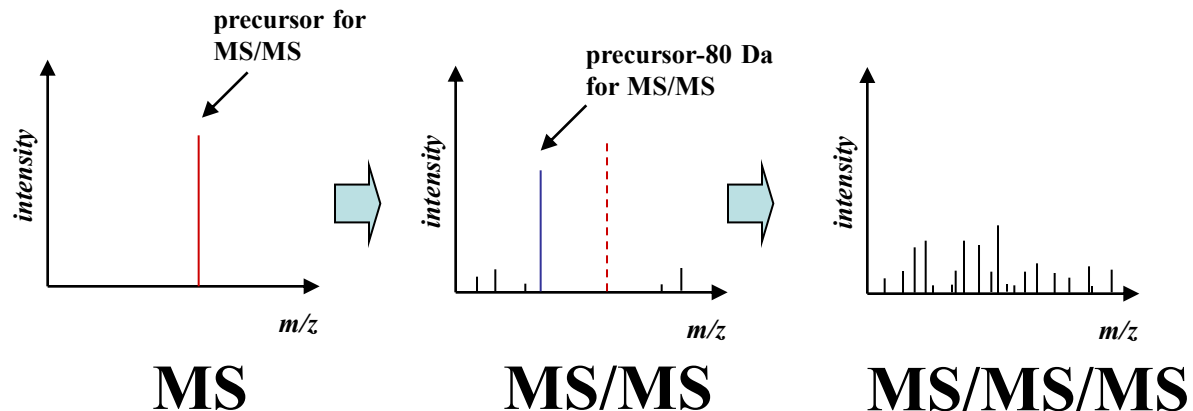
Neutral Loss dependent MS/MS/MS

Neutral Loss of phospho-Ser and phospho-Thr sides

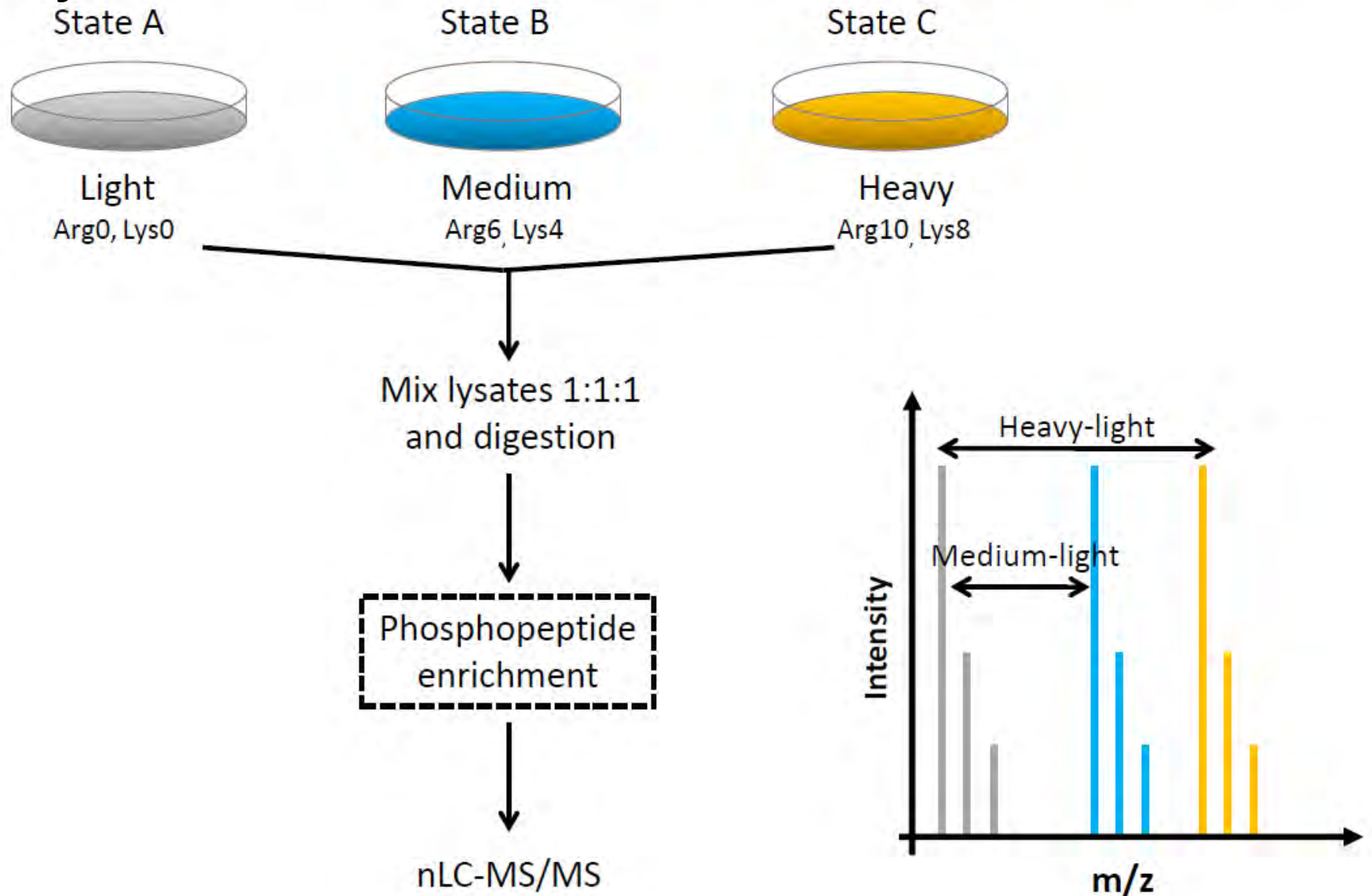


Phosphopeptide

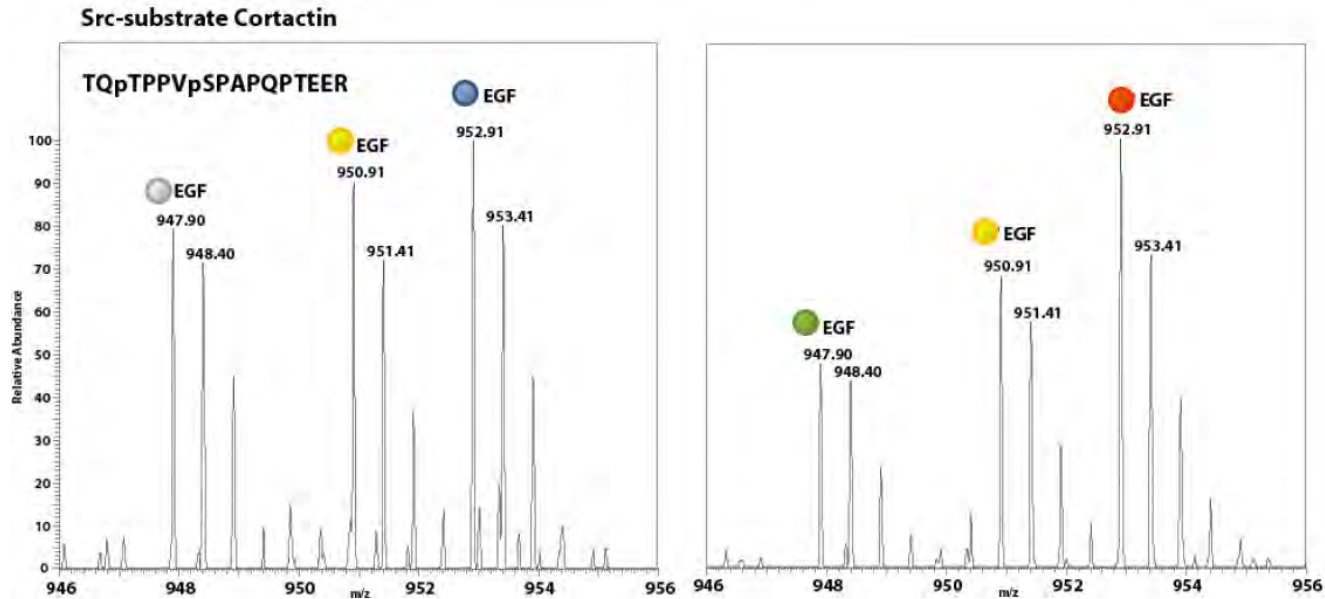
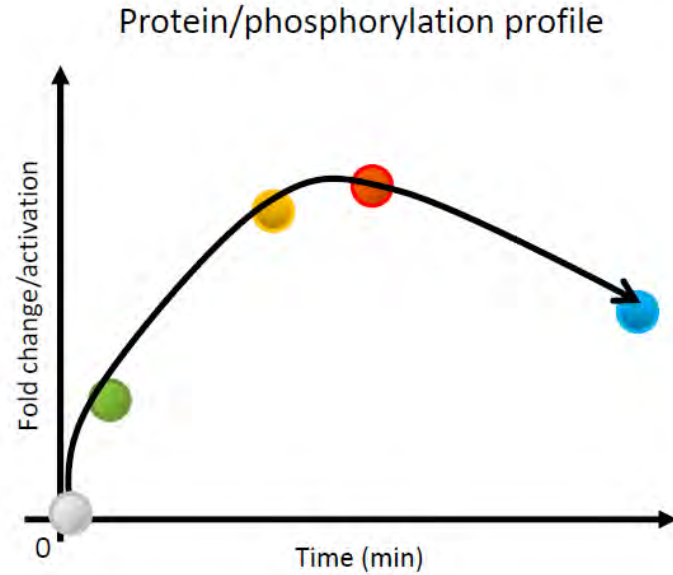
- 80 Da



Phosphoproteome Quantitation: triple SILAC time-resolved analysis



Double triple SILAC – time course



MS-based quantitative proteomics

Comparative
proteomic
profiling

Interactomics

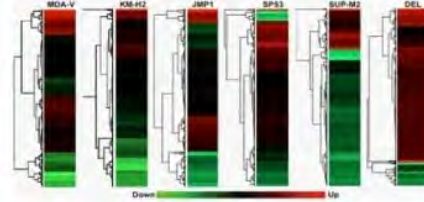
Modificomics

Targeted

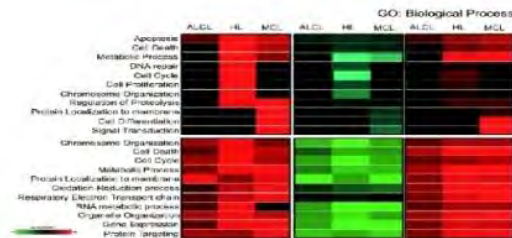


Bioinformatics and Functional analysis

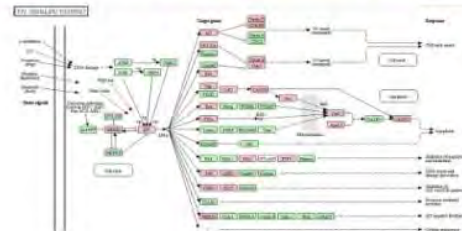
Protein ID/Quant
Comparison



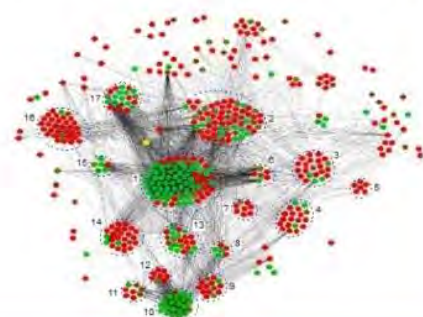
GO terms
Enrichment analysis



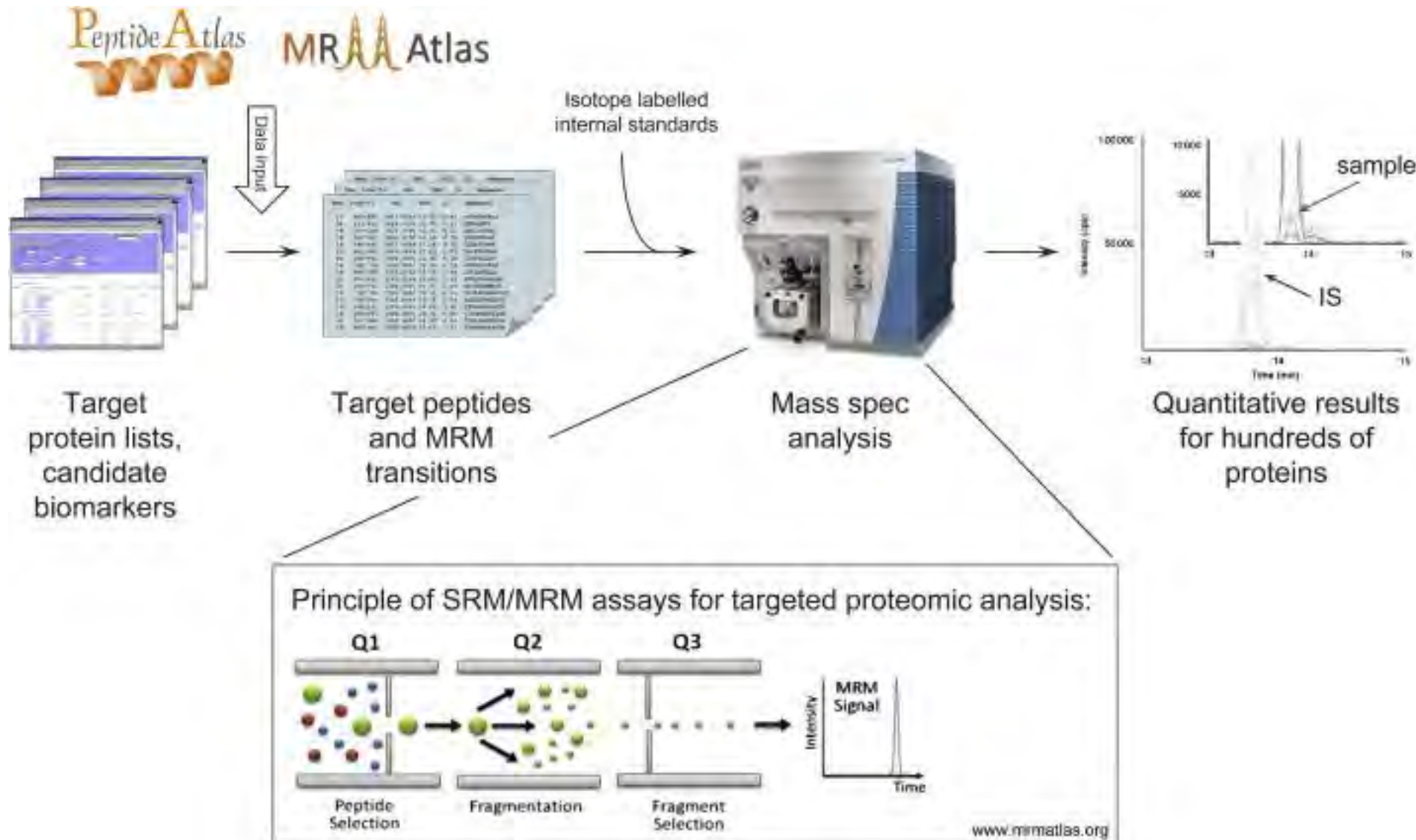
Pathway analysis



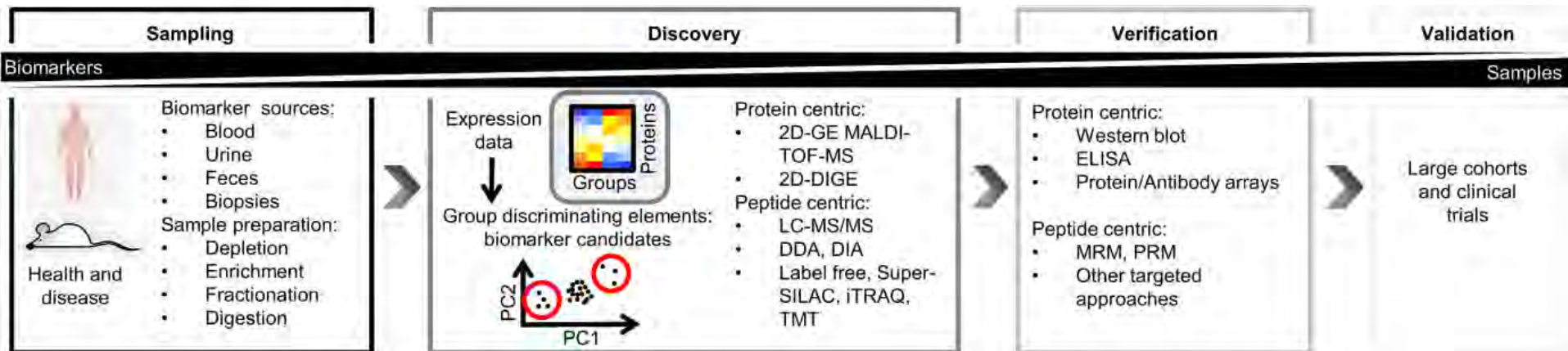
Protein Interactions
network analysis



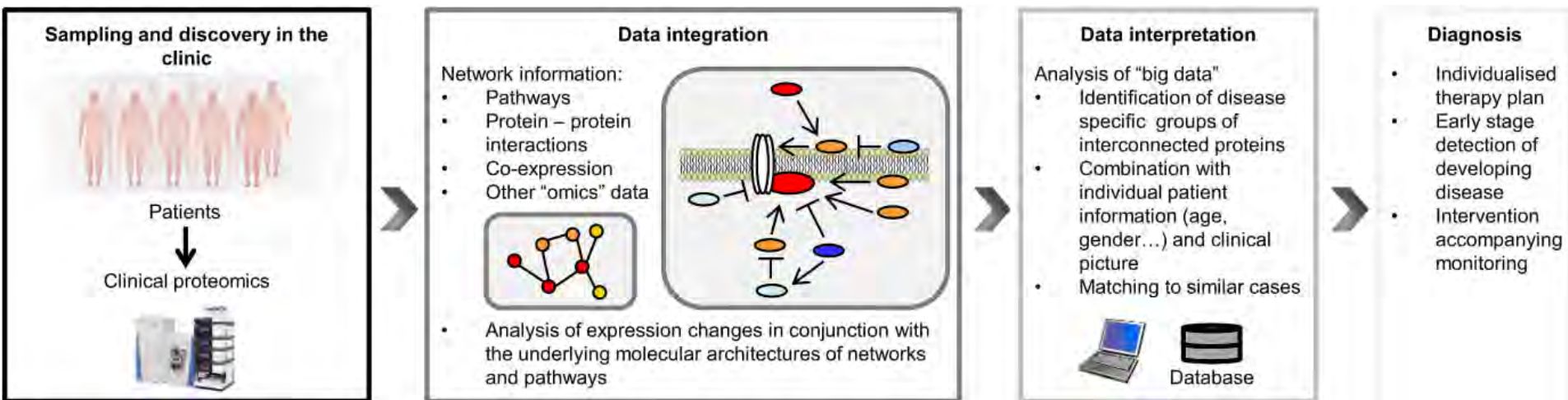
Targeted Proteomics – Detection/Monitoring/Quantitation



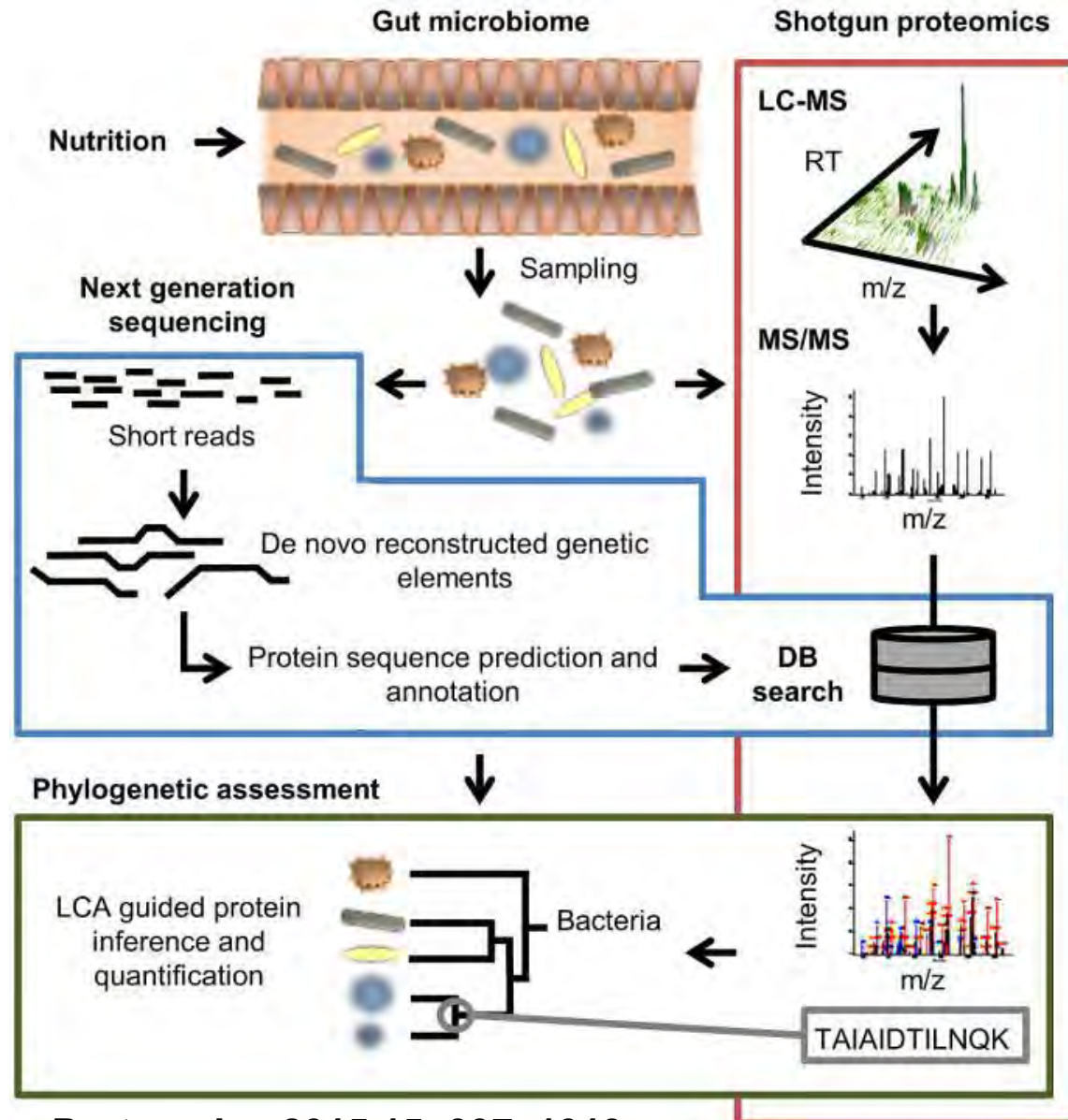
Biomarker development



Emerging proteomic strategies in the clinic

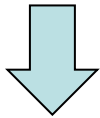


An emerging workflow for shotgun proteomic analysis of complex ecosystems such as the human or animal intestine



Our Approach

**Multi-omics
data**



**Comparative analysis
Network and pathway
analysis
Integration**

Drug-repurposing

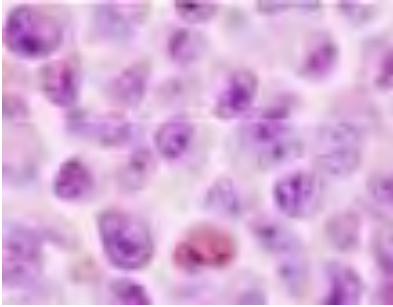


**Health
Disease**

**Understanding
(bio)pathology**

**Biomarkers/drug targets
Drugs/Therapy**

Lymphoma

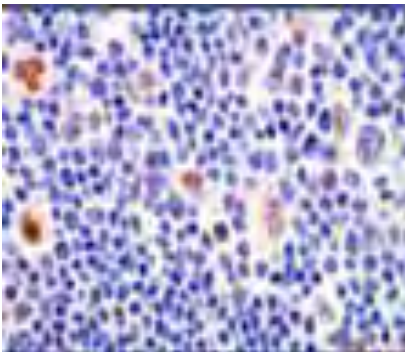
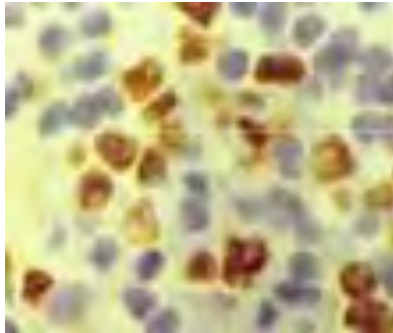


Haematological cancer

Derives from lymphocytes

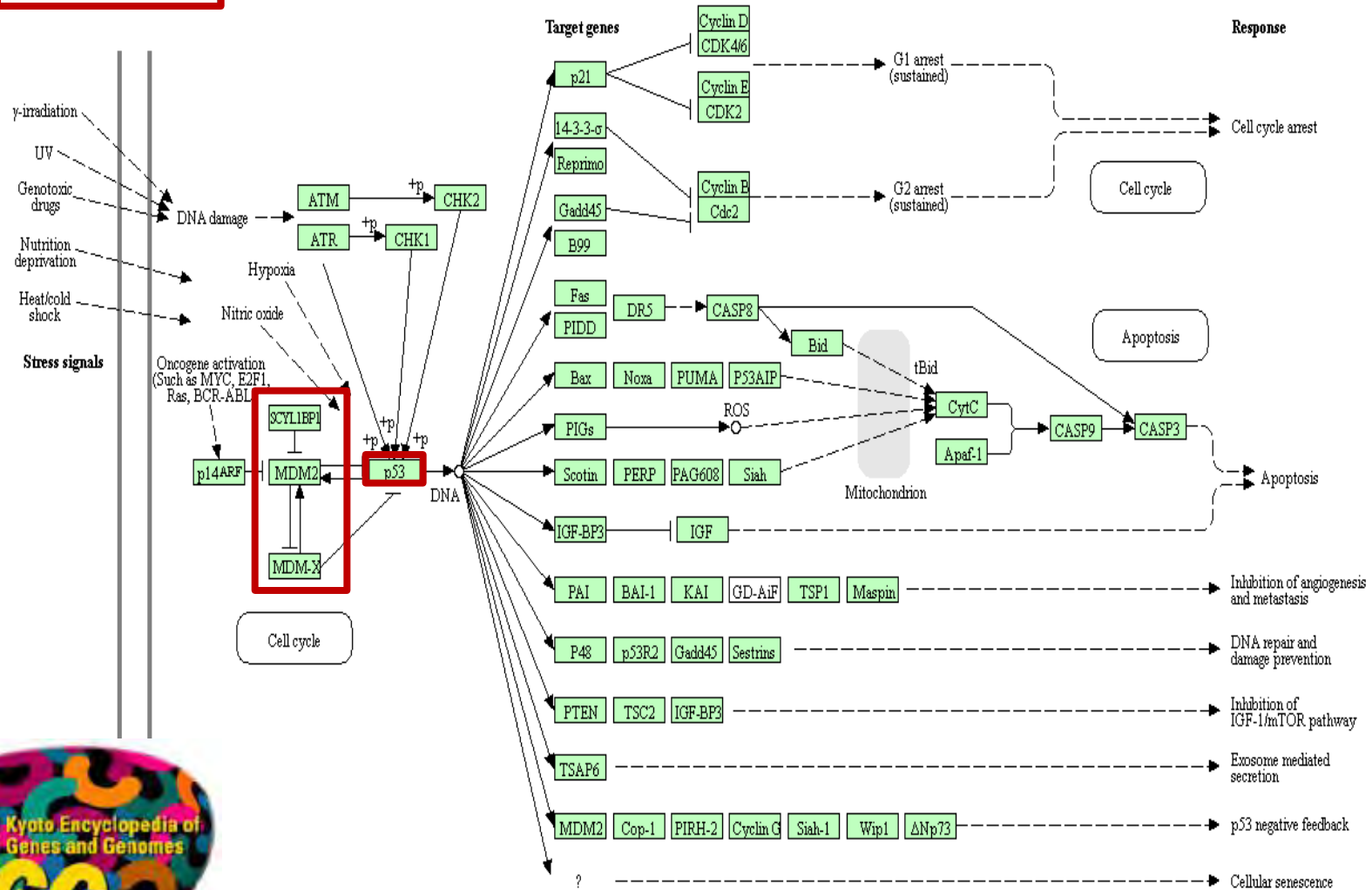
Large heterogeneity

Deactivated wt-p53



Wt p53 deactivated due to MDM2 overexpression

P53 SIGNALING PATHWAY

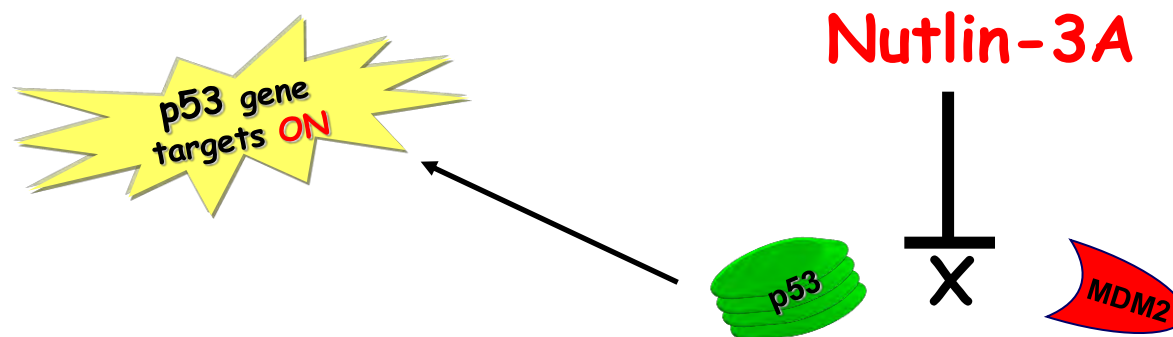


Effect of Nutlin-3a in lymphoma

➤ Non-genotoxic activation of p53



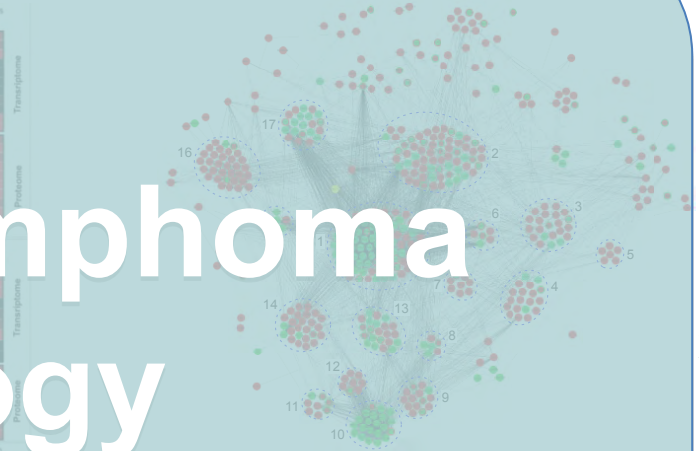
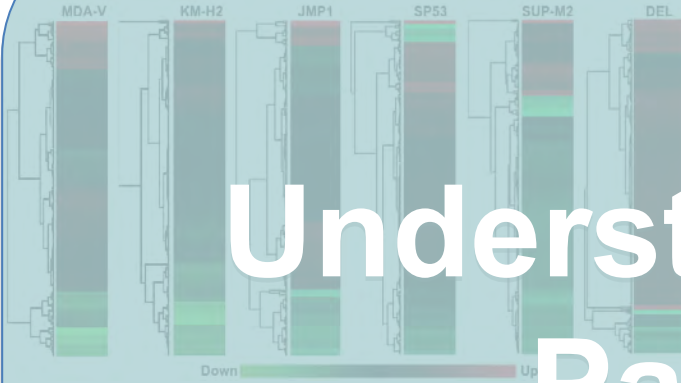
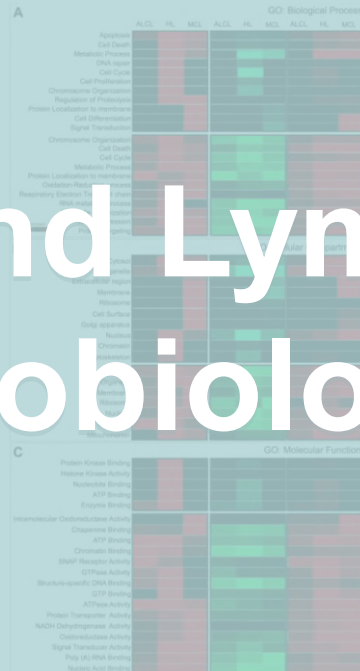
- Cell cycle arrest
- Apoptosis
- Tumor growth inhibition



But...

...it doesn't work in all of the cases.

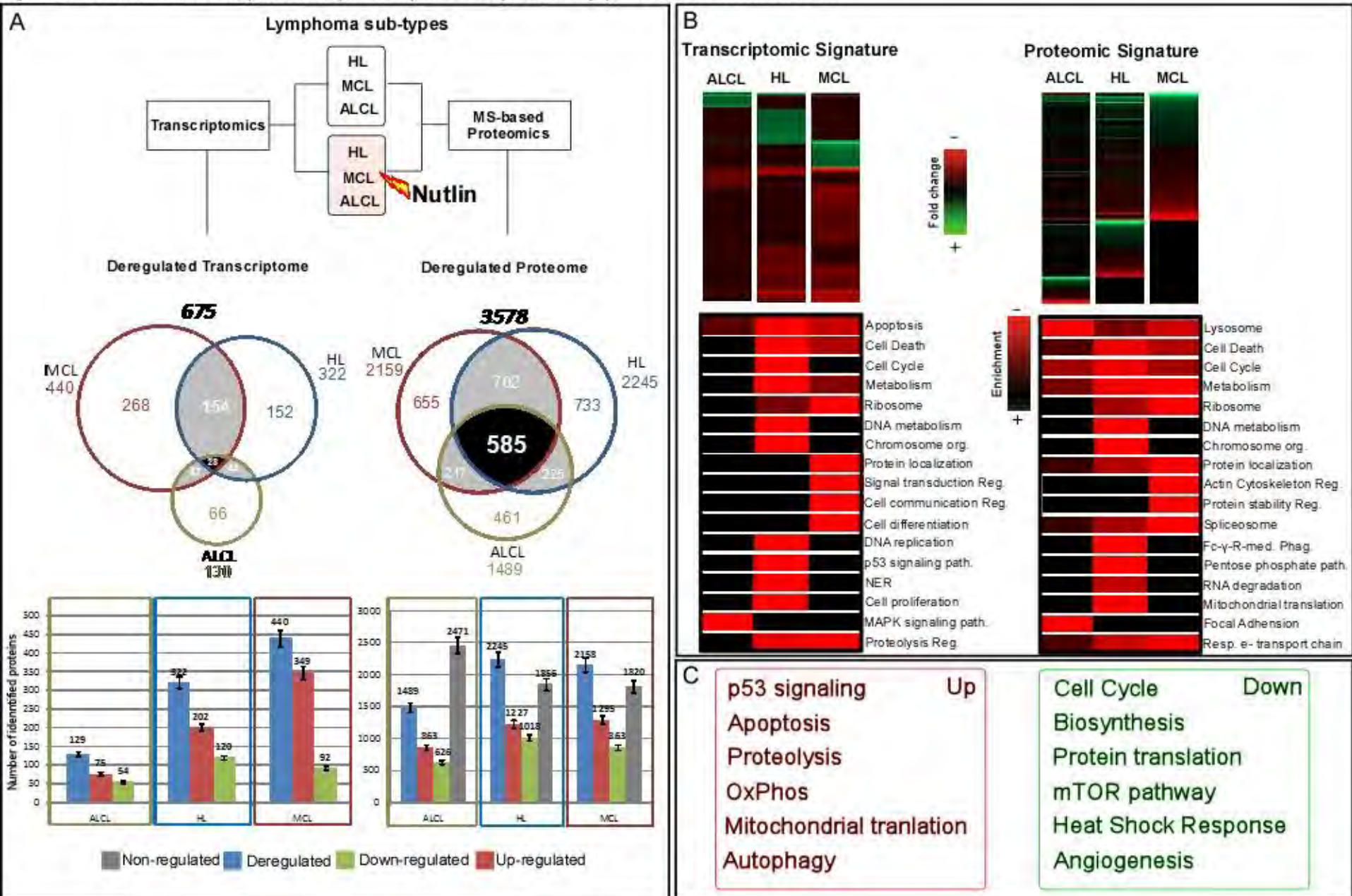
Understand Lymphoma Pathobiology



1. Ce
2. Ap
3. Pr
4. Me
5. SM
6. DM
7. Sig
8. RM
9. Ril
10. Ril
11. Mi
12. SF
tar
13. Tra
14. Pr
15. Bi
16. Ox
17. CH

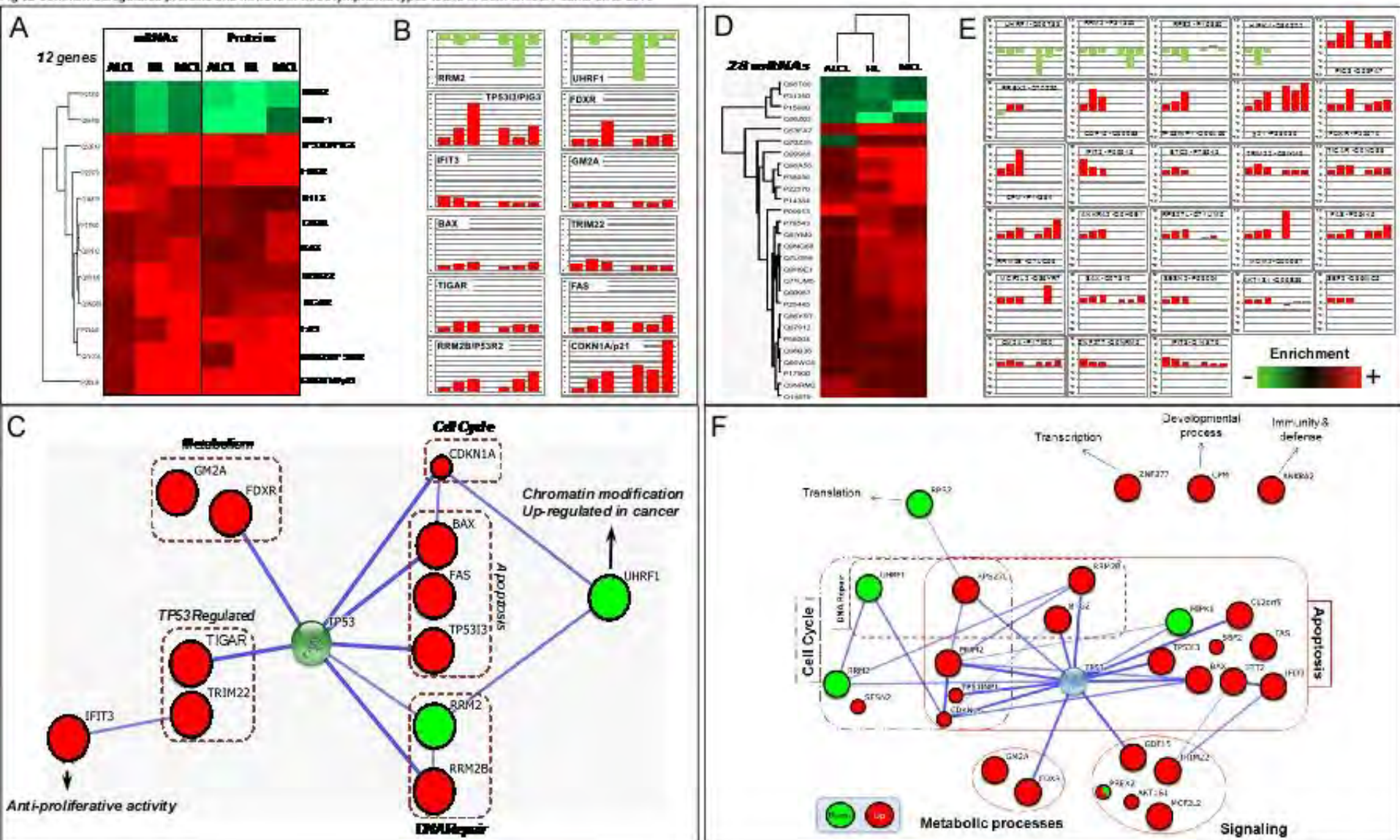
N3a global proteome differential affect

Fig 1: Overview of the results from the comparative transcriptomics and proteomics analysis on model Lymphoma cell lines +/-N3a Psatha et. al 2016



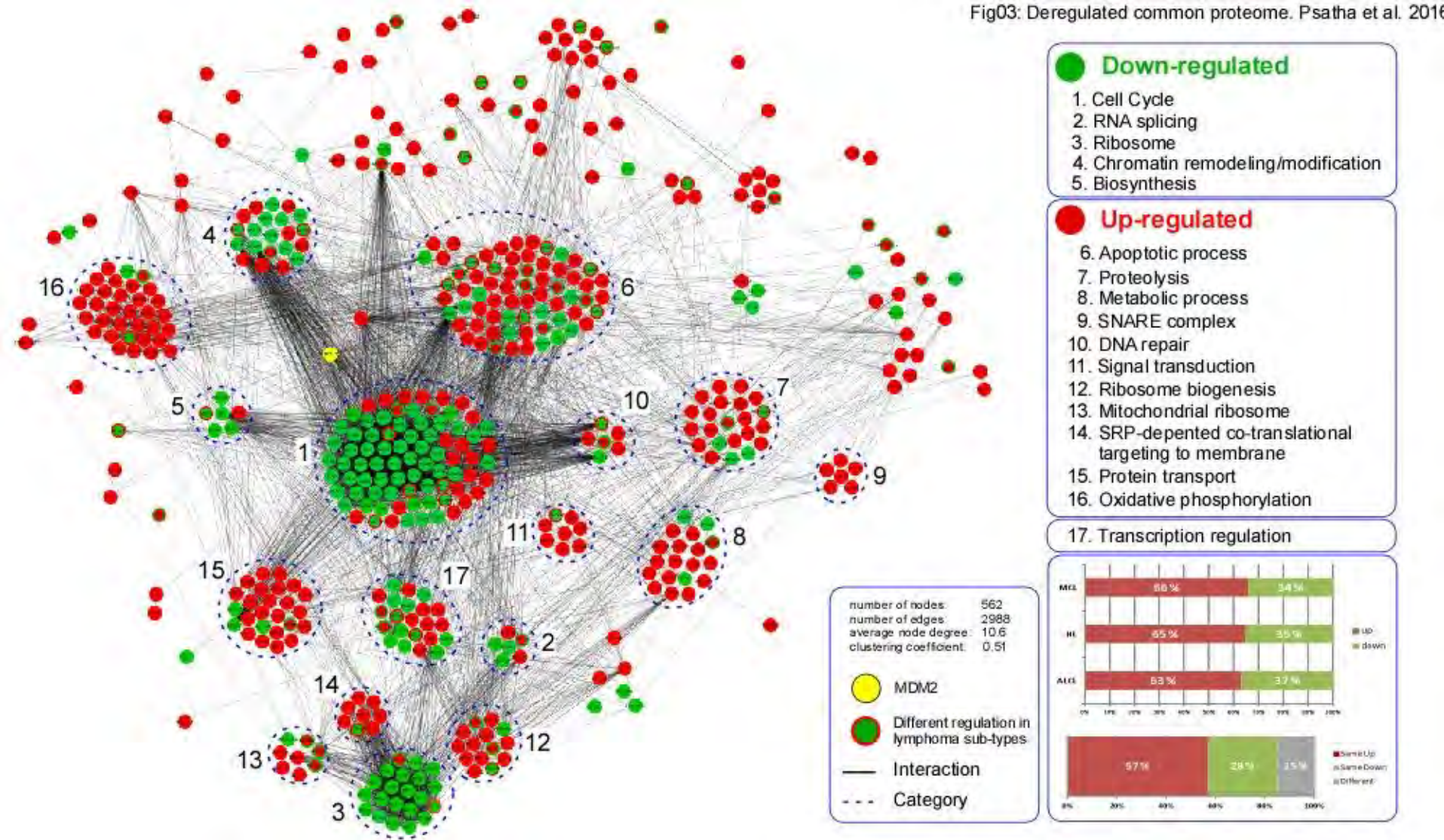
N3a-affected Proteins and pathways

Fig 02 Common deregulated proteins and mRNAs in three lymphoma types found in both omics. Psatha et. al 2016



Common N3a-affected PPIs network analysis

Fig03: Deregulated common proteome. Psatha et al. 2016



P53 signaling pathway

Lymphoma-type dependent activation and rewiring of p53 signaling pathway

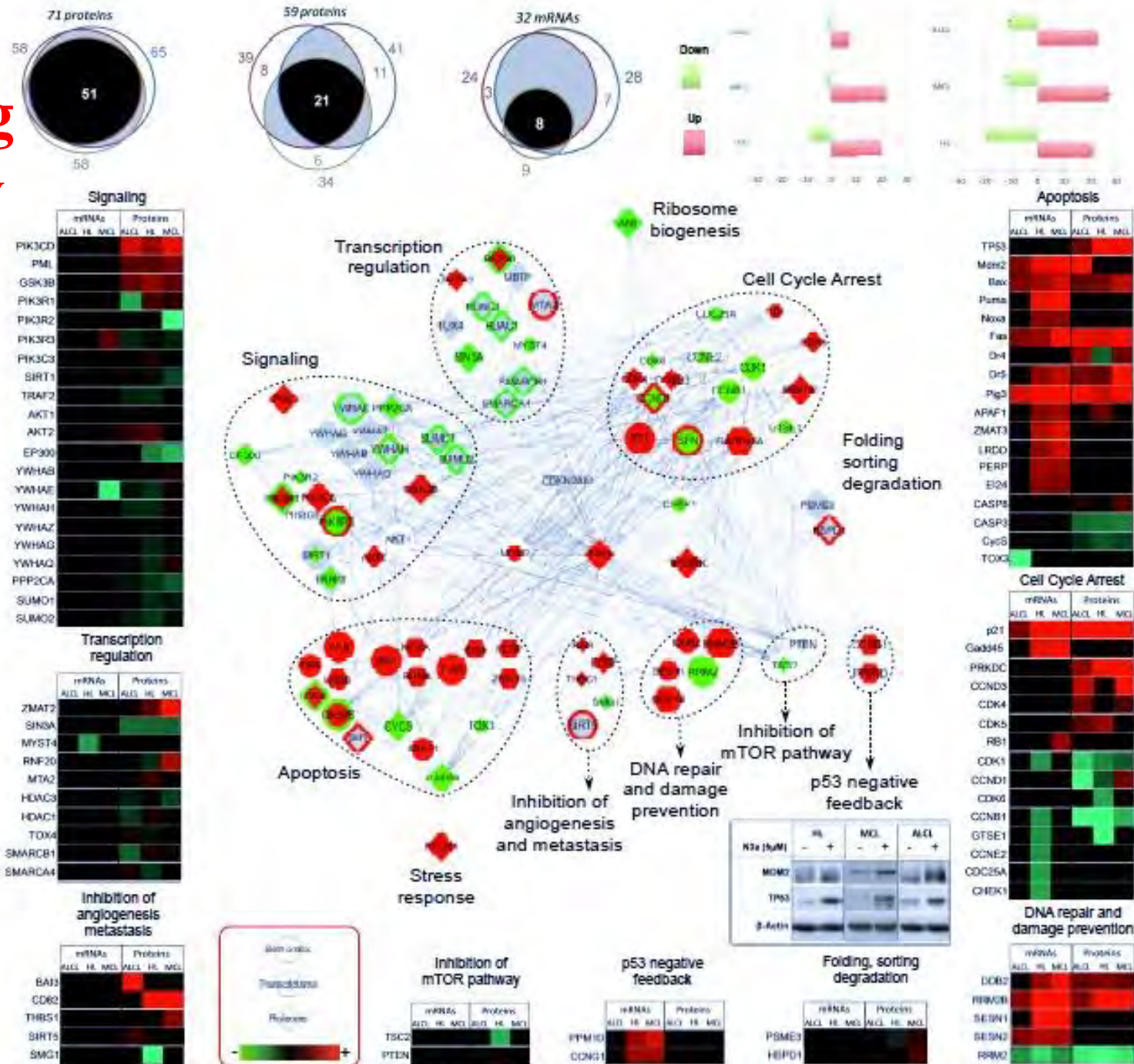
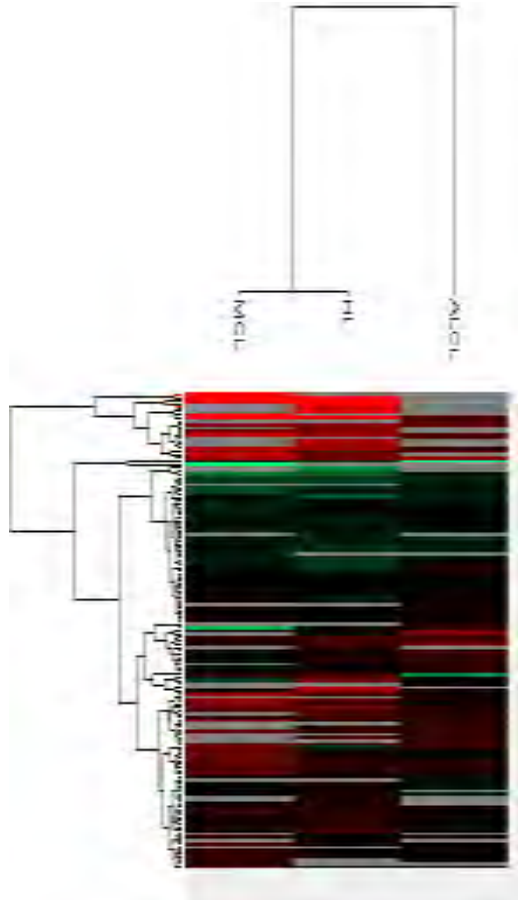


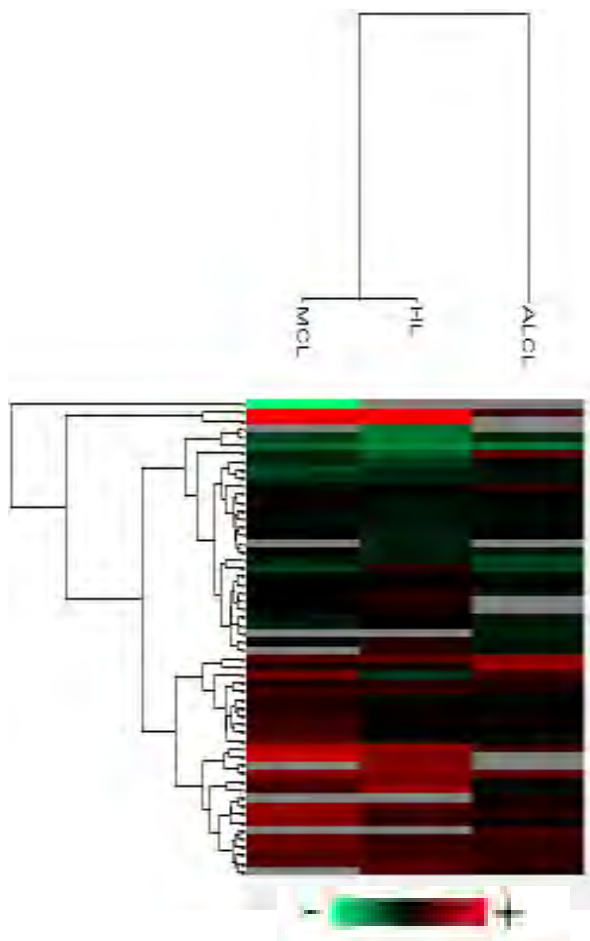
Fig04: Integrative visualization of the effect of N3a in p53 signaling pathway. Psatha et al. 2018

Hierarchical clustering and comparison of affected proteins per lymphoma type and biological process

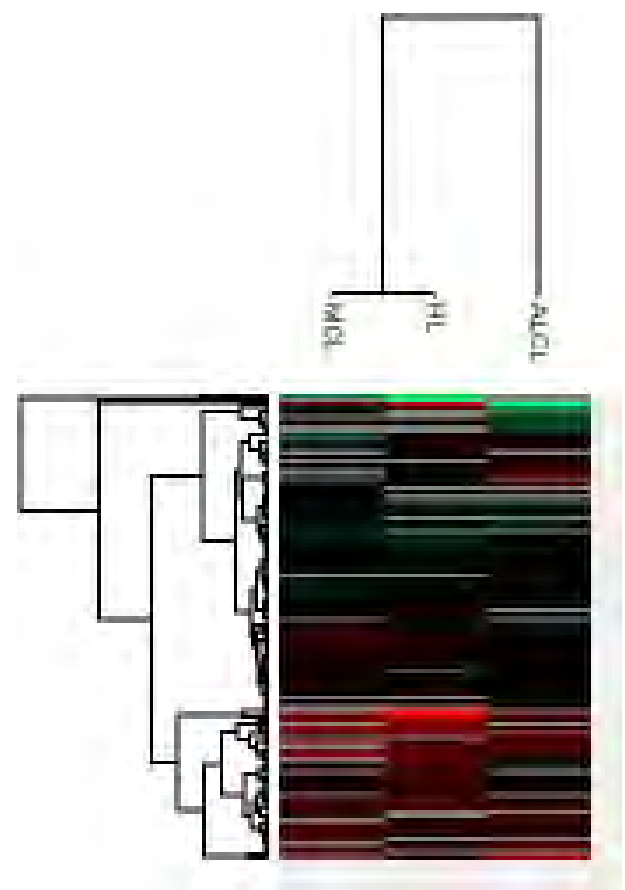
Αποφύγηση



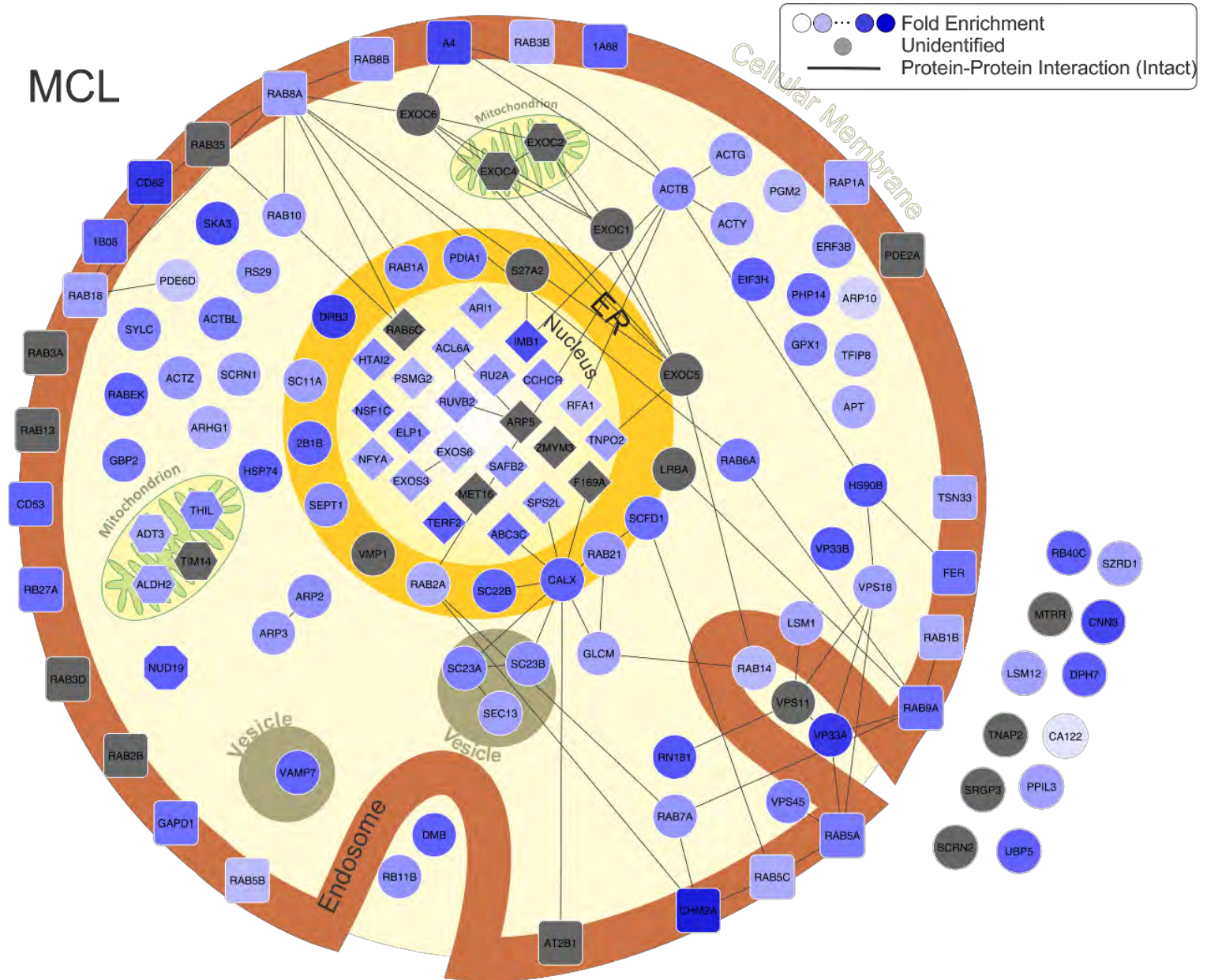
Μιτοφάγηση



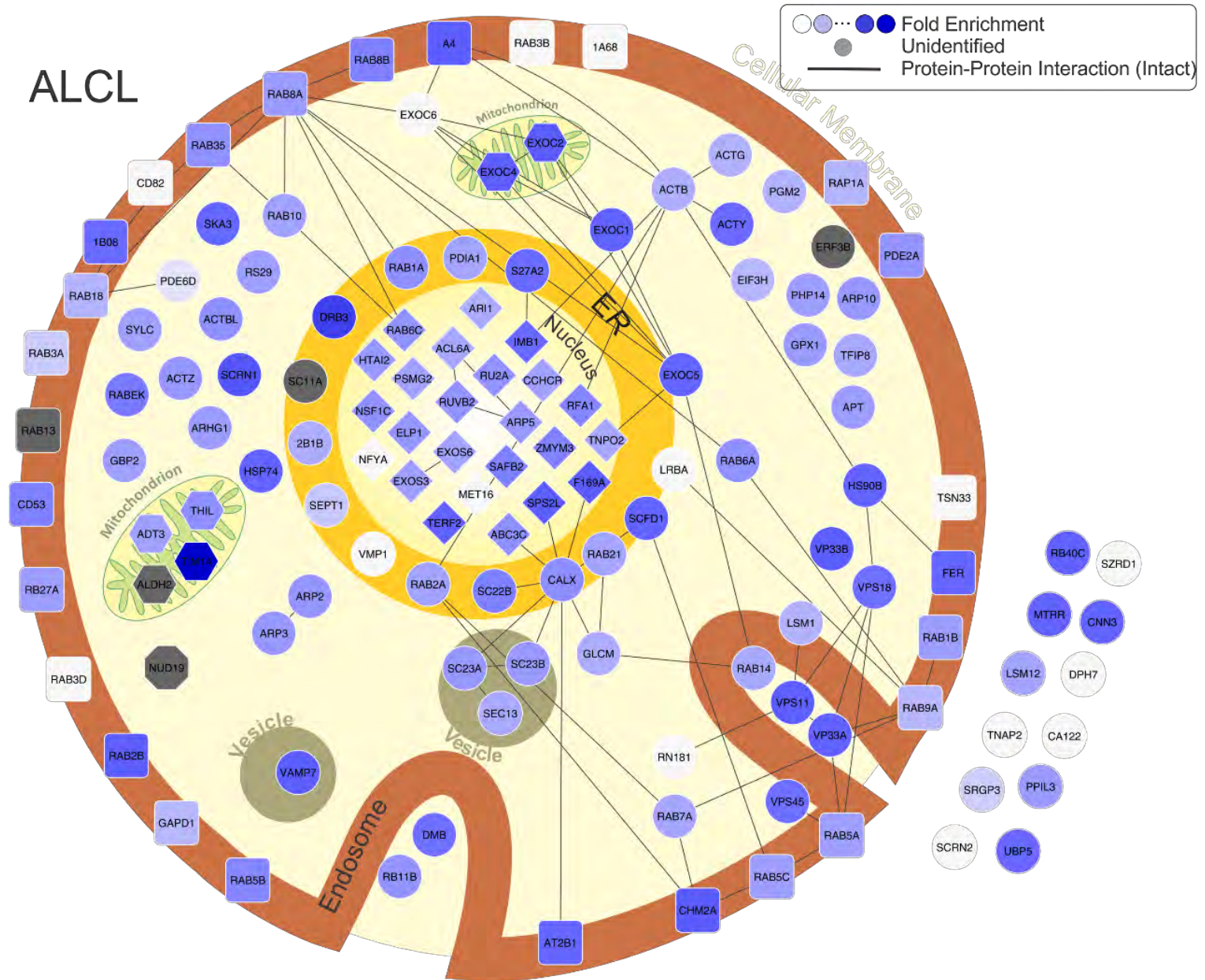
Εξωκυτταρίες



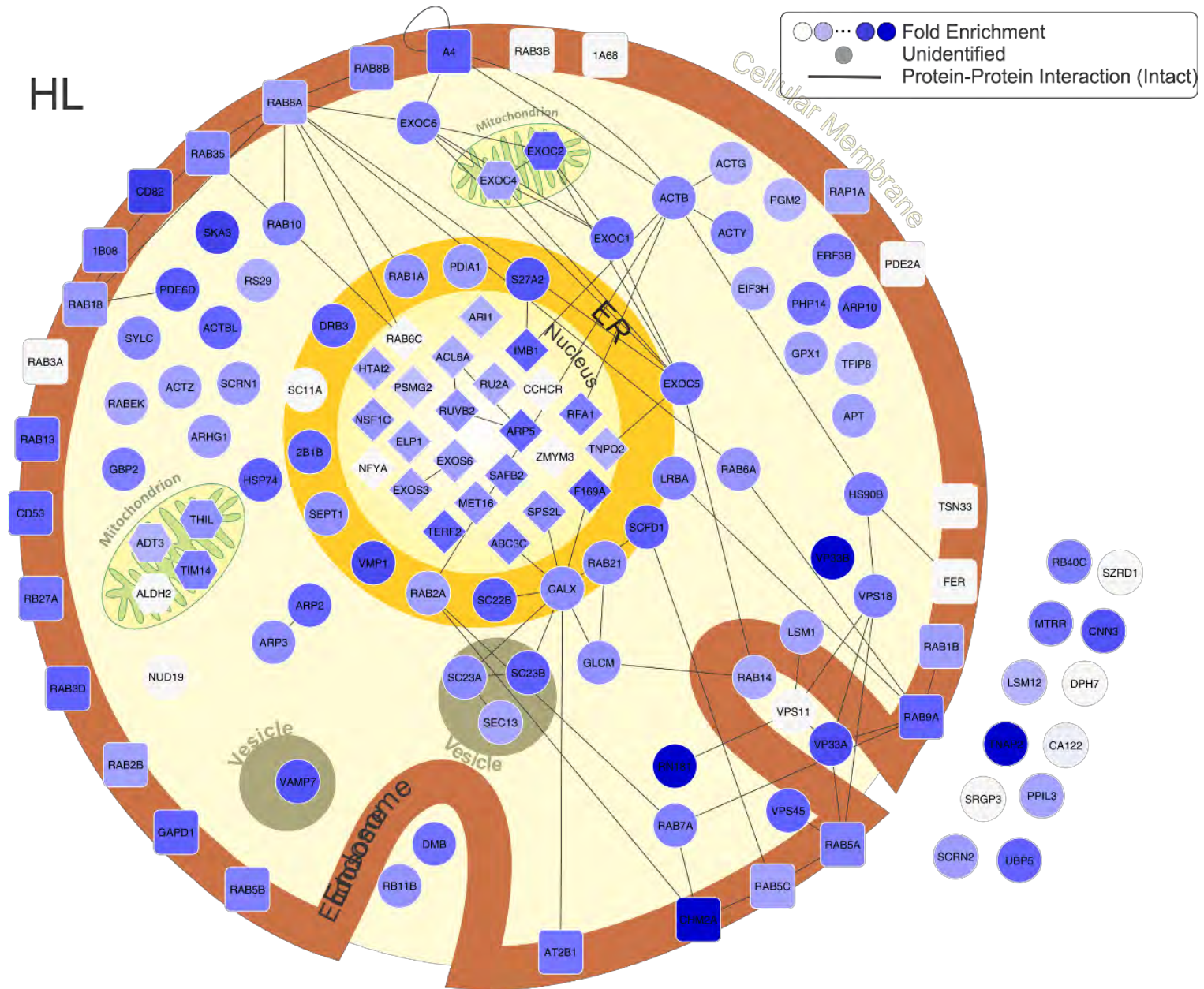
Integrated protein network in MCL



ALCL



HL



N3a-affected signaling pathways

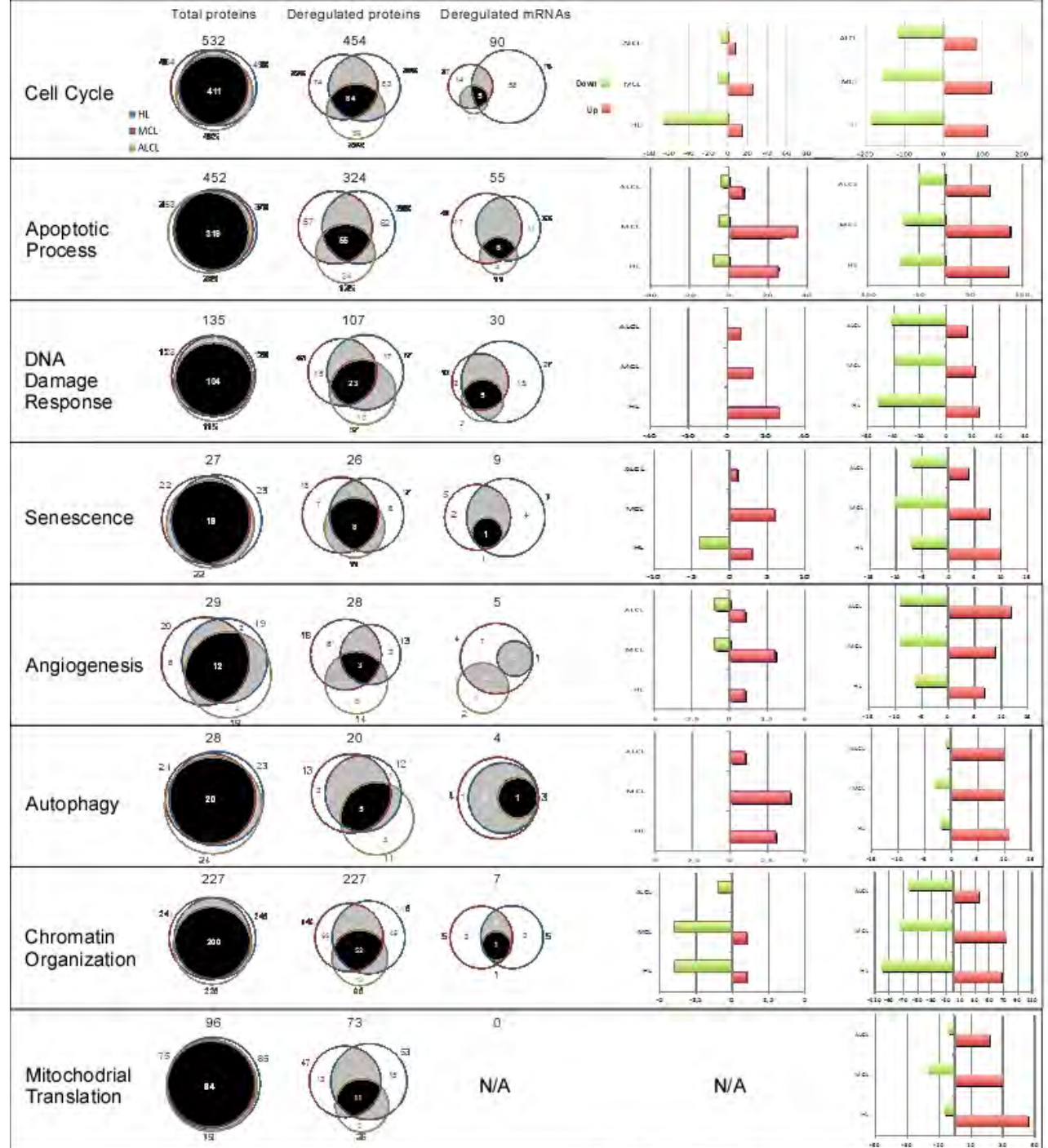
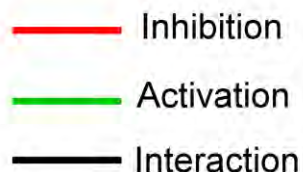
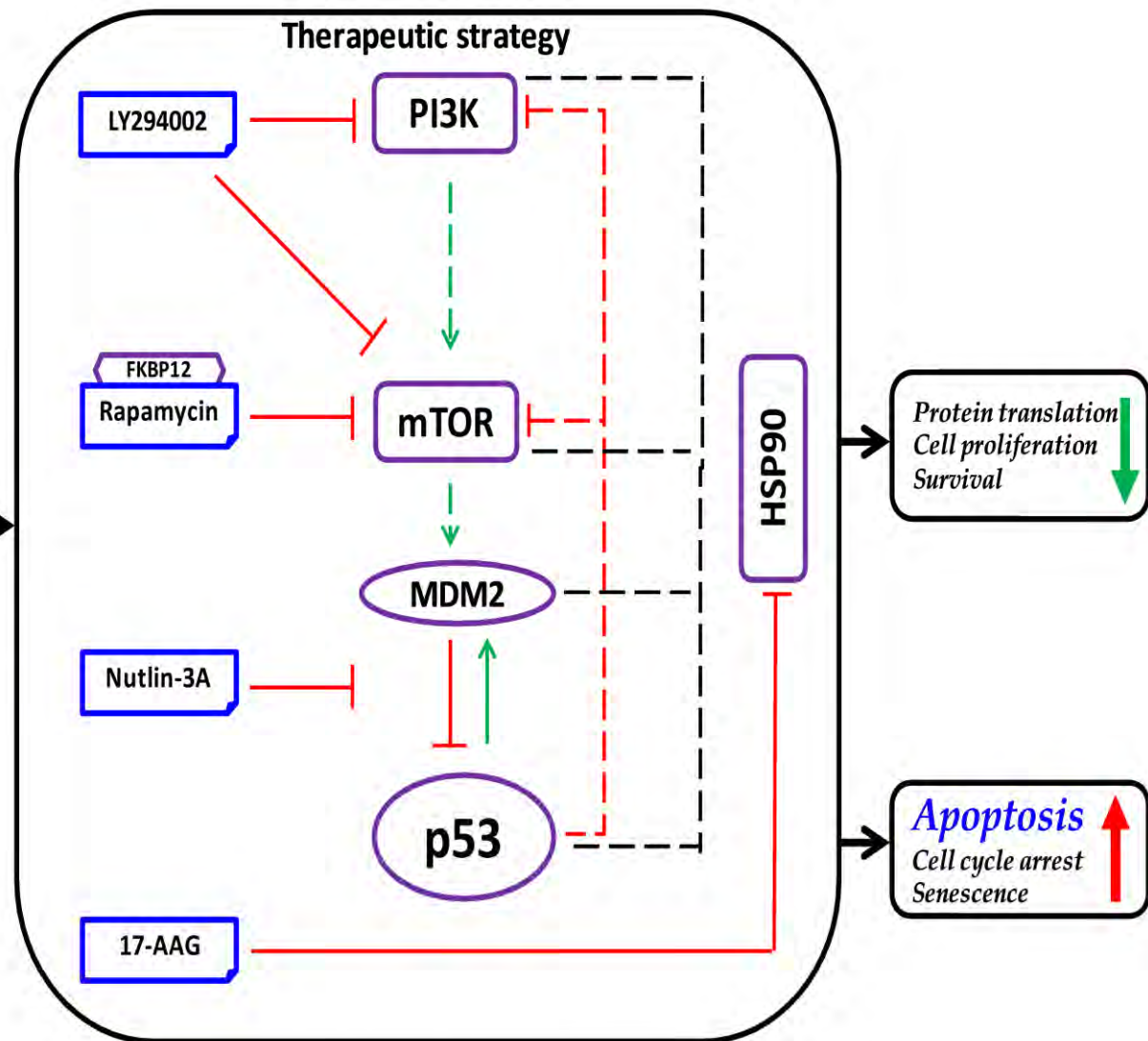
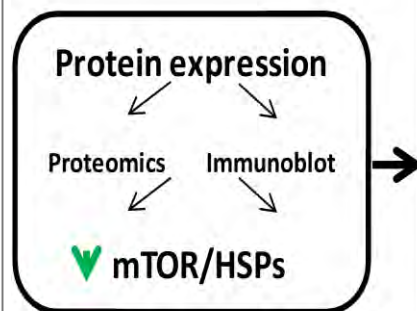


Fig05. PI3K/AKT/mTOR pathway proteins validation. Psatha et al. 2016

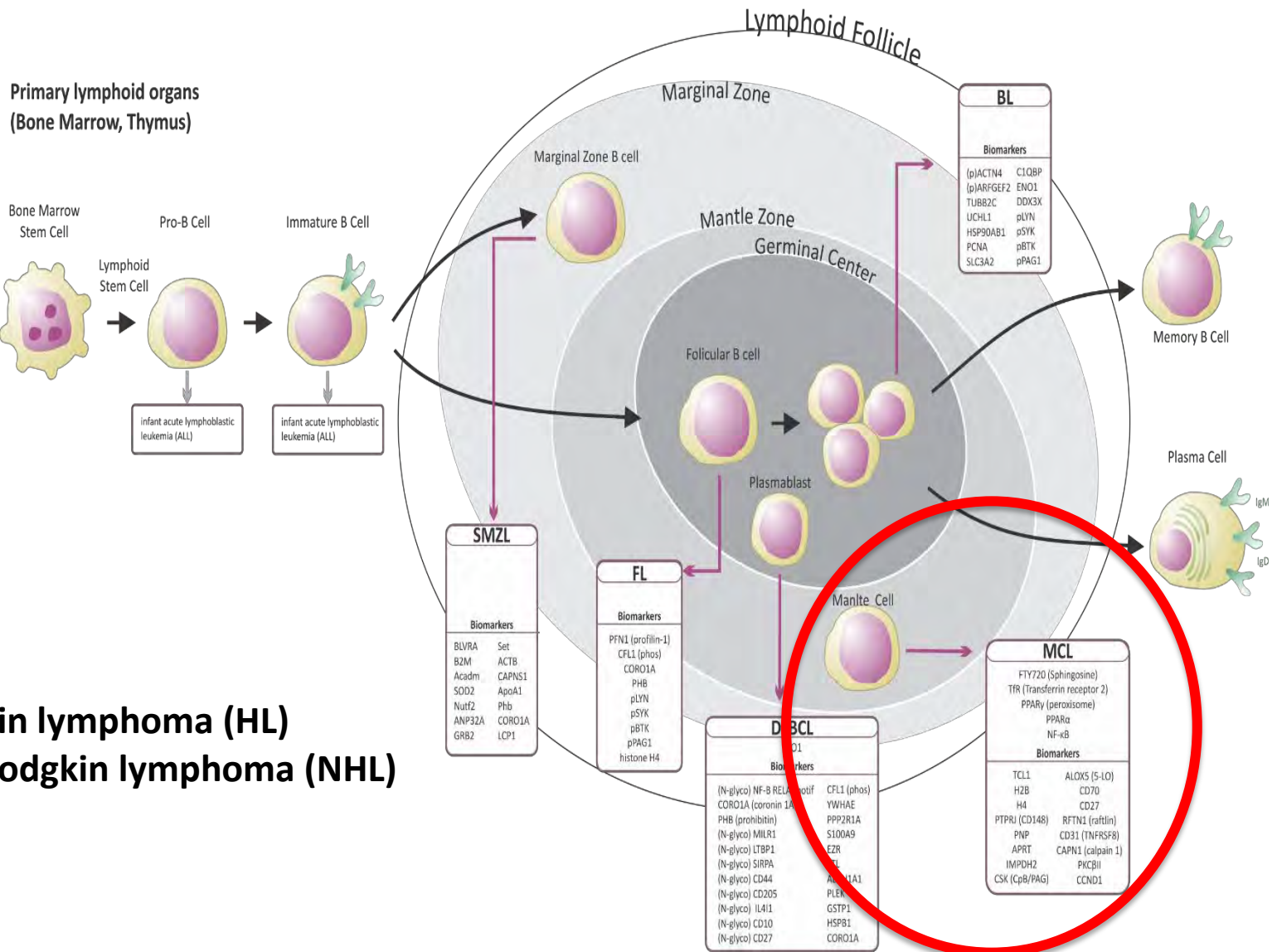
Proposed and tested novel therapeutic strategies



substance	mode of action	targets	concentration
Ly294002	PI3K/mTOR-inhibitor	ATP-competitive	10 nM
Rapamycin	mTOR-inhibitor	FKBP12	30, 50 μ M
Nutlin-3A	p53-activator	MDM2 antagonist	1.5-6 μ M
17-AAG	HSP90-inhibitor	ATP-competitive	0.25-1 μ M

B-cell lymphoma subtypes

Secondary lymphoid organs
(spleene, lymph nodes)



1. Hodgkin lymphoma (HL)
2. Non-Hodgkin lymphoma (NHL)

MCL:

- chromosomal translocation
 $t(11;14)(q13;q32)$ (IgH/CCND1)

Network and pathway analysis

Integration

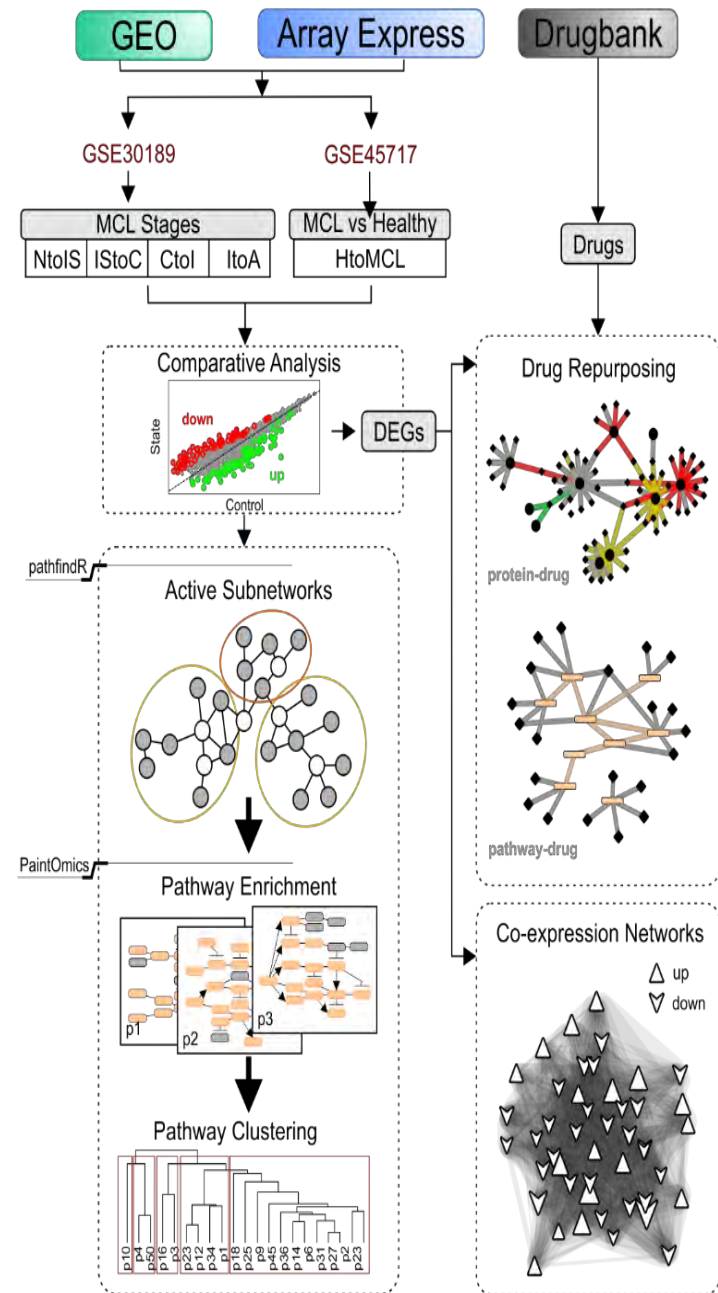
Drug-repurposing

GSE30189 (Stages):

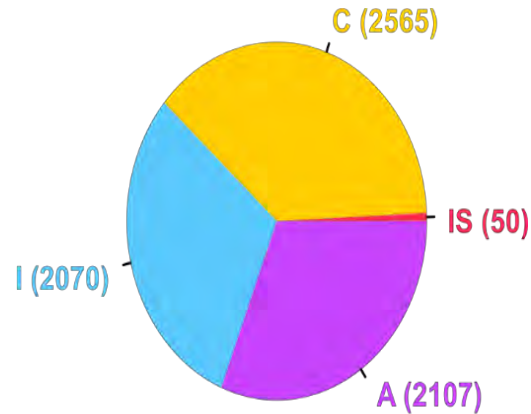
1. Normal (N)
 2. *In Situ* (IS)
 3. Classical (C)
 4. Intermediate (I)
 5. Aggressive (A)
- NtoIS
 IStoC
 CtoI
 ItoA

GSE45717

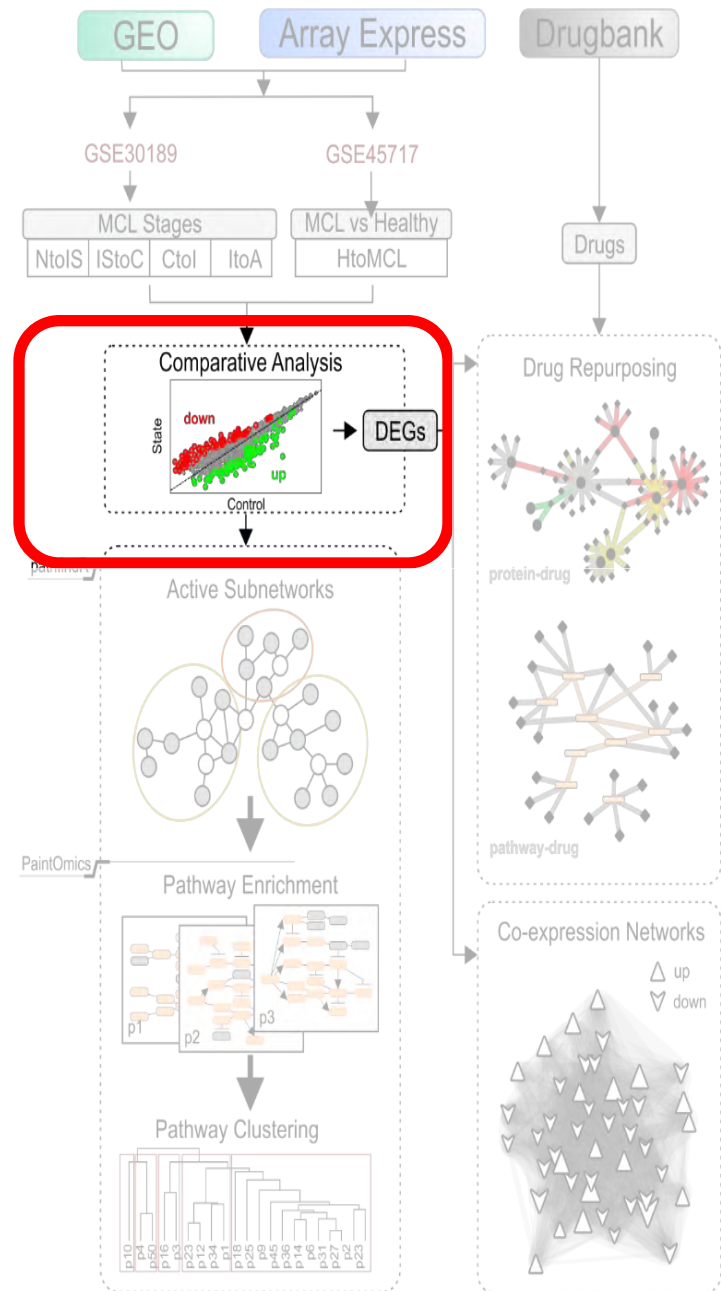
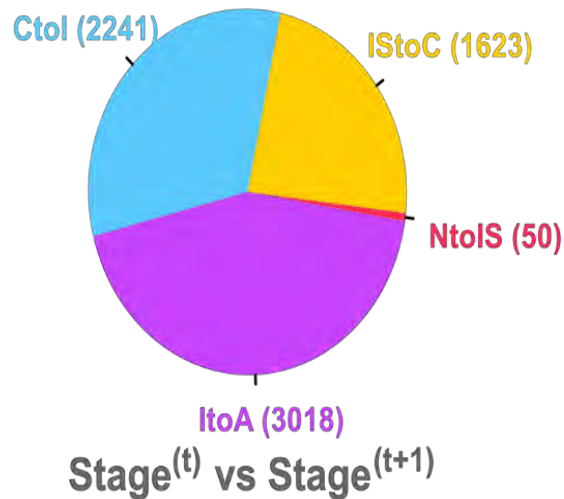
1. Healthy
 2. Typical MCL
- HtoMCL



Differential expressed genes (DEGs)

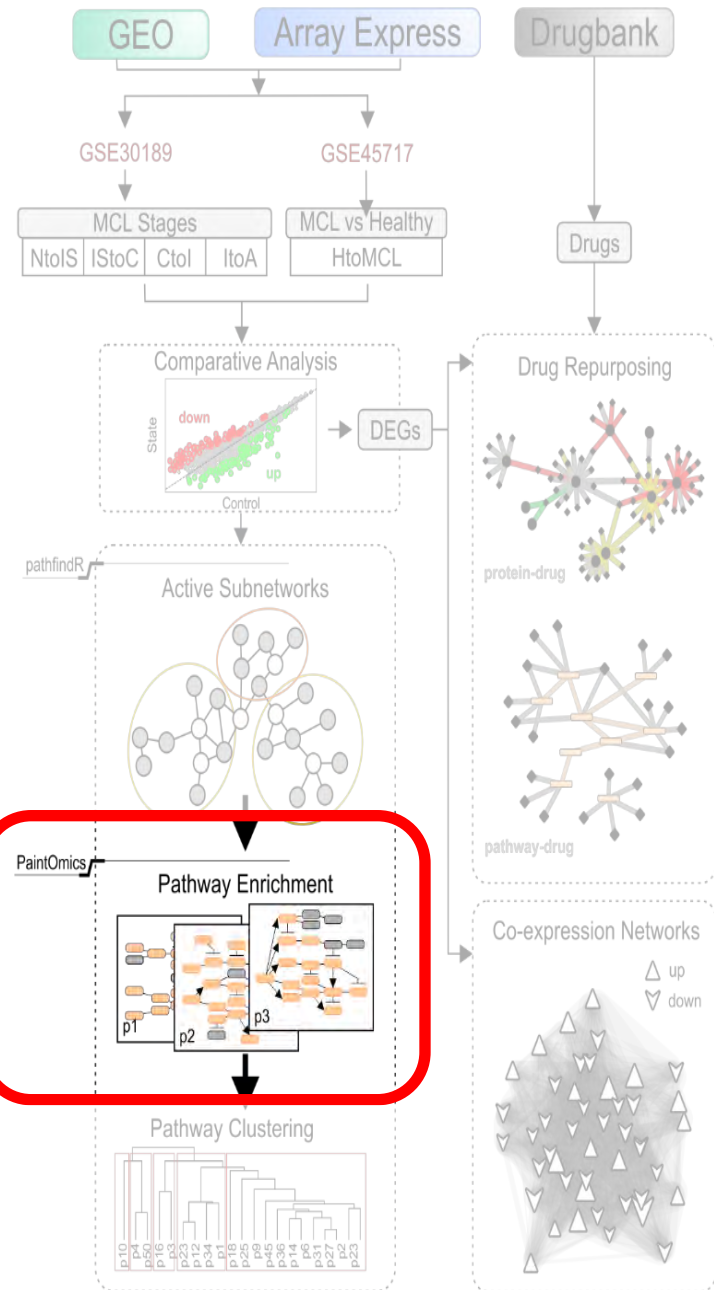


Healthy vs Stage



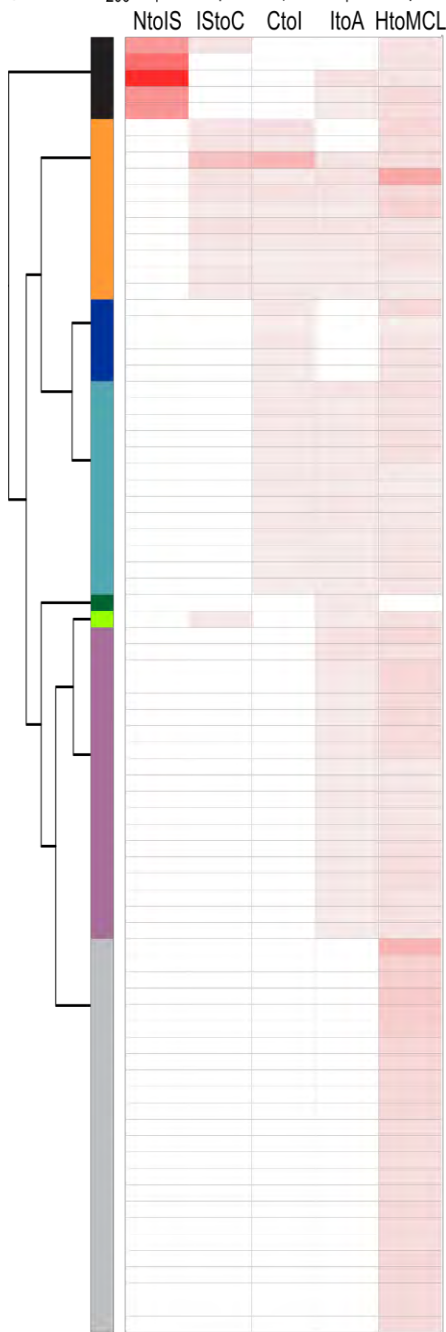
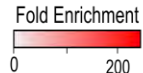
Affected Biological Pathways

	pathfindR	PaintOmics
NtoInS	18	8
IStoC	22	10
Ctol	45	8
ItoA	71	12
HtoMCL	185	58

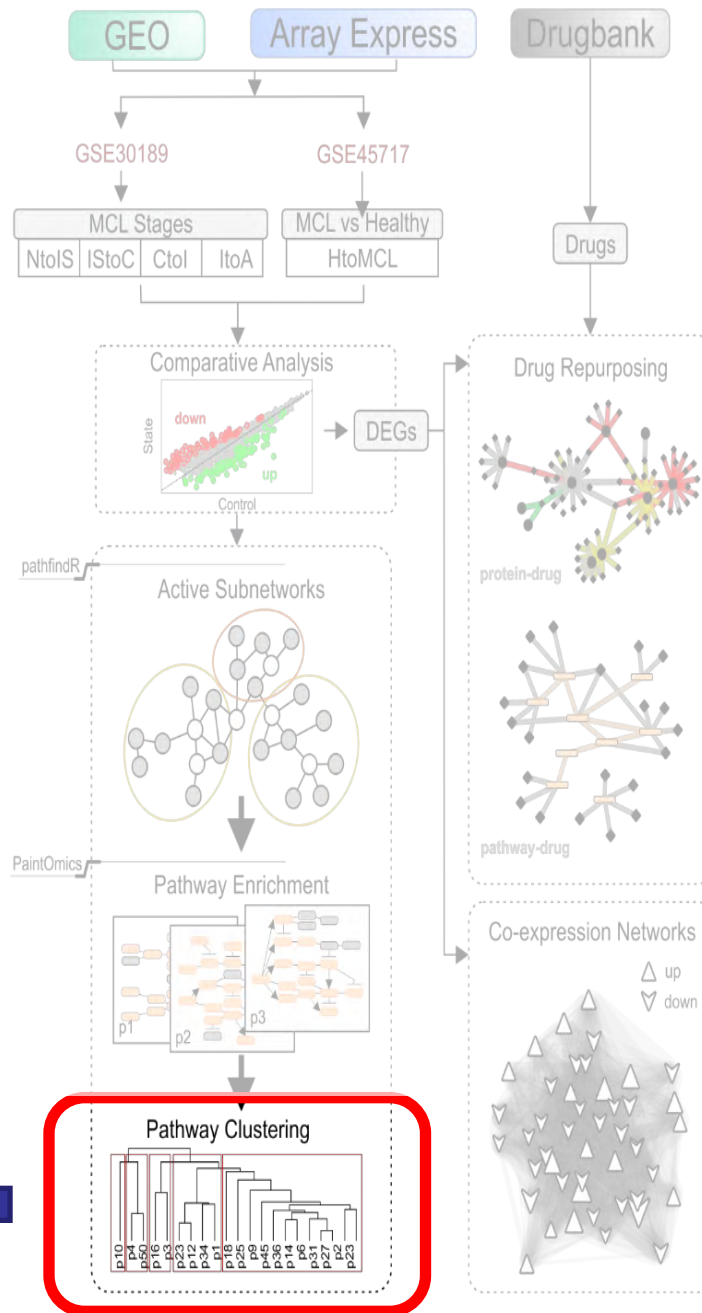


Significantly affected pathways

Pathways	Type
p53 and cell cycle	DLBCL
B-cell receptor (BCR) signaling	DLBCL,MCL,CNS
PI3K/Akt/mTOR signaling pathway	DLBCL
JAK-STAT signaling pathway	DLBCL
NF- κ B pathway	MCL
Jak/STAT pathway	MCL
Wnt/ β -catenin pathway	MCL
WNT pathway	cHL,MCL,CLL
p53 Pathway	MCL, ALCL
NF- κ B prosurvival signals	\CNS
PD-1/PD-L1 pathway	HL,nHL
HGF/c-MET signaling pathway	B cell lymphoma, T and NK cell lymphoma, Hodgkin lymphoma
Hippo pathway	Pancreatic cancer Breast cancer



AGE-RAGE signaling pathway in diabetic complications
Prolactin signaling pathway
 Hedgehog signaling pathway
 Fc epsilon RI signaling pathway
p53 signaling pathway
Ribosome biogenesis in eukaryotes
 Bacterial invasion of epithelial cells
Ribosome
Proteasome
 Pathogenic Escherichia coli infection
Spliceosome
 Adherens junction
 Thyroid hormone signaling pathway
 RNA transport
Ubiquitin mediated proteolysis
 Tight junction
 Fanconi anemia pathway
PI3K-Akt signaling pathway
GnRH signaling pathway
 Proteoglycans in cancer
 Central carbon metabolism in cancer
 Cell cycle
B cell receptor signaling pathway
 Gap junction
 HIF-1 signaling pathway
 mRNA surveillance pathway
 RNA degradation
 Apelin signaling pathway
 Cellular senescence
 ErbB signaling pathway
mTOR signaling pathway
MAPK signaling pathway
 Protein processing in endoplasmic reticulum
 Endocytosis
 Citrate cycle (TCA cycle)
 Leukocyte transendothelial migration
Notch signaling pathway
 Circadian rhythm
Apoptosis - multiple species
 RIG-I-like receptor signaling pathway
 T cell receptor signaling pathway
 IL-17 signaling pathway
NF-kappa B signaling pathway
 Mitophagy - animal
 Transcriptional misregulation in cancer
AMPK signaling pathway
Wnt signaling pathway
Hippo signaling pathway
 Insulin signaling pathway
 Th17 cell differentiation
 Long-term potentiation
Apoptosis
FoxO signaling pathway
 Toll-like receptor signaling pathway
 Neurotrophin signaling pathway
 Mismatch repair
 Hippo signaling pathway - multiple species
 Propanoate metabolism
 DNA replication
 Glycolysis / Gluconeogenesis
 Pyruvate metabolism
 Homologous recombination
 SNARE interactions in vesicular transport
 Vasopressin-regulated water reabsorption
 RNA polymerase
 Pentose phosphate pathway
 TNF signaling pathway
 Other glycan degradation
 Cysteine and methionine metabolism
 Lysine degradation
 Carbohydrate digestion and absorption
 Cytosolic DNA-sensing pathway
 Natural killer cell mediated cytotoxicity
 Base excision repair
 Basal transcription factors
 Oxidative phosphorylation
 Ferroptosis
 Collecting duct acid secretion
 Nucleotide excision repair



Novel findings

- There are “**stage specific**” deregulated biological pathways.
- ***In Situ*** stage show a distinct pathway enrichment phenotype.
- Basic cellular processes related to **protein processing, translation** and **RNA processing** are affected at the **Classical stage**.
- Signaling pathways related to **apoptosis, cellular senescence** and **proliferation** are affected at the **Intermediate and Aggressive stages**.

Angewandte Statistik

-
- Nucleus**
- DNA Damage**
- Oncogene Activation**
- Protein Expression**
- DNA**
- Cellular Membrane**
- Mitochondrion**
- Survival, Proliferation, Differentiation**
- Apoptosis**
- G1 arrest (sustained)**
- Cellular Senescence**
- DNA-repair and damage prevention**
- Inhibition of angiogenesis and metastasis**
- fibinolysis down-regulation**
cell adhesion and spreading
cellular and replicative senescence
- Legend:**
- ■ ■ ■ Fold Change (GSE30189)
 - Differentially Expressed Gene
 - | Inhibition
 - p+ Activation/Phosphorylation
 - e Expression
- Fold Change Scale:**
- 0.28 0.0 0.0 0.0 0.15

Pathways
cross-talk

P53
pathway

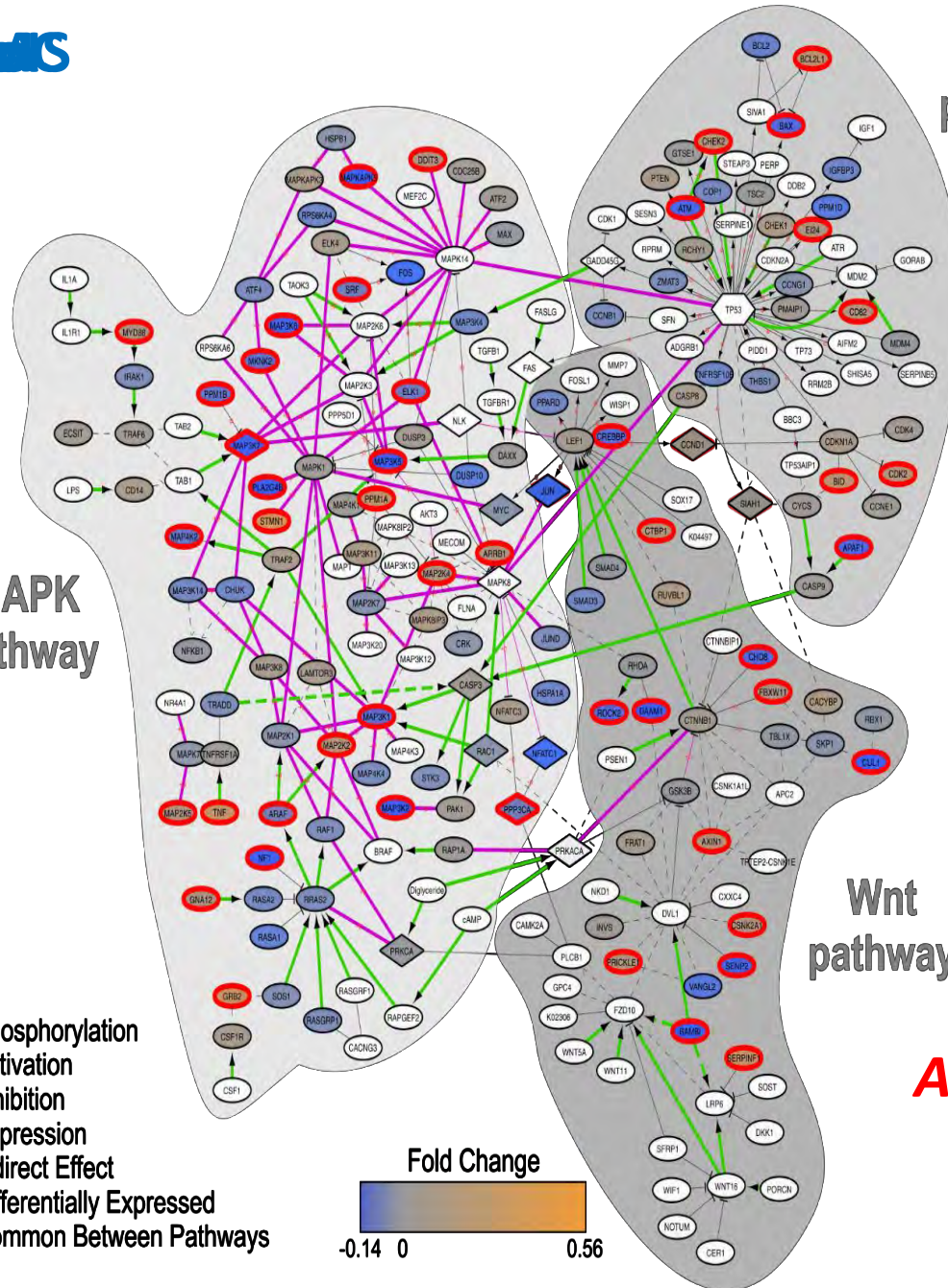
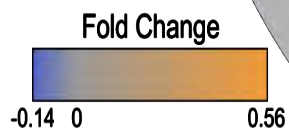
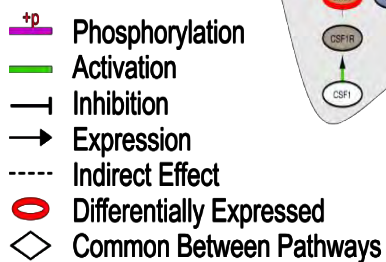
In Situ

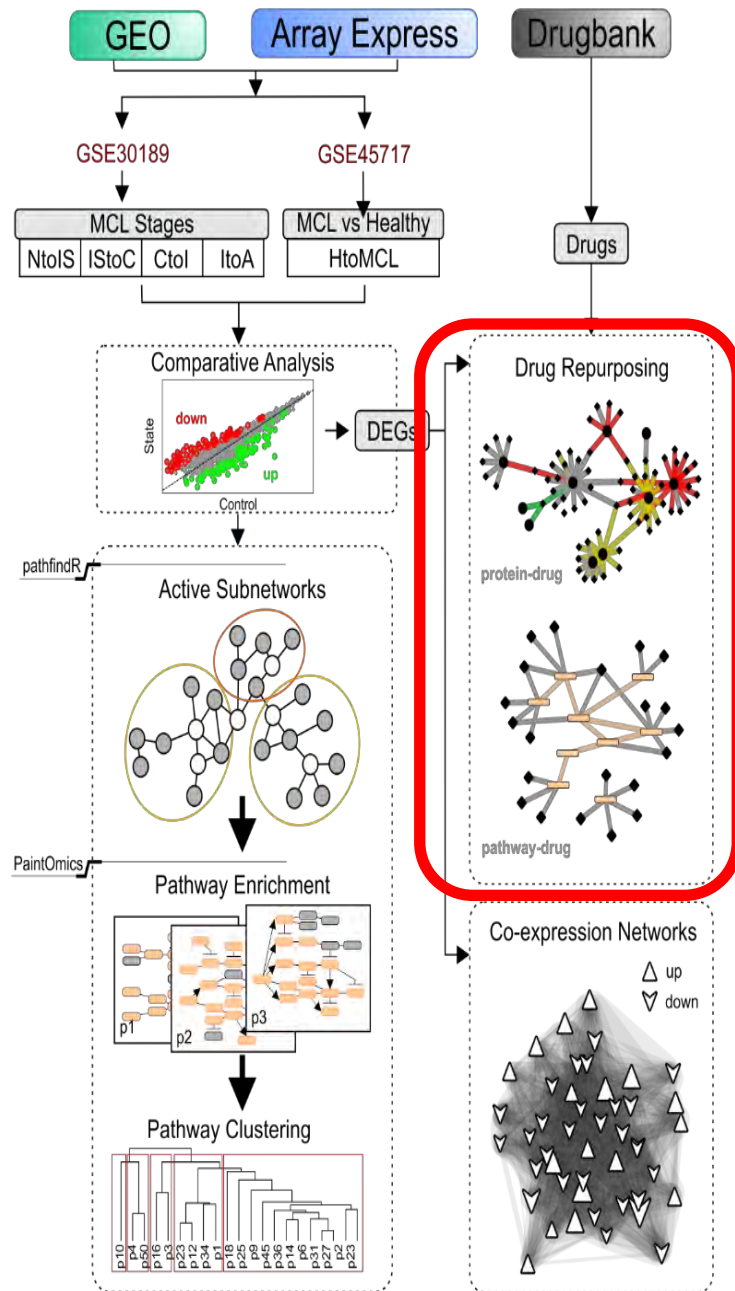
MAPK
pathway

Intermediate

Wnt
pathway

Aggressive



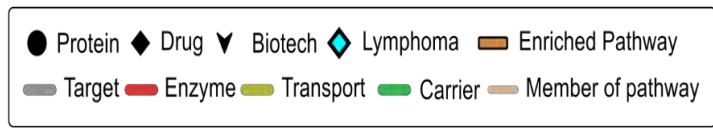
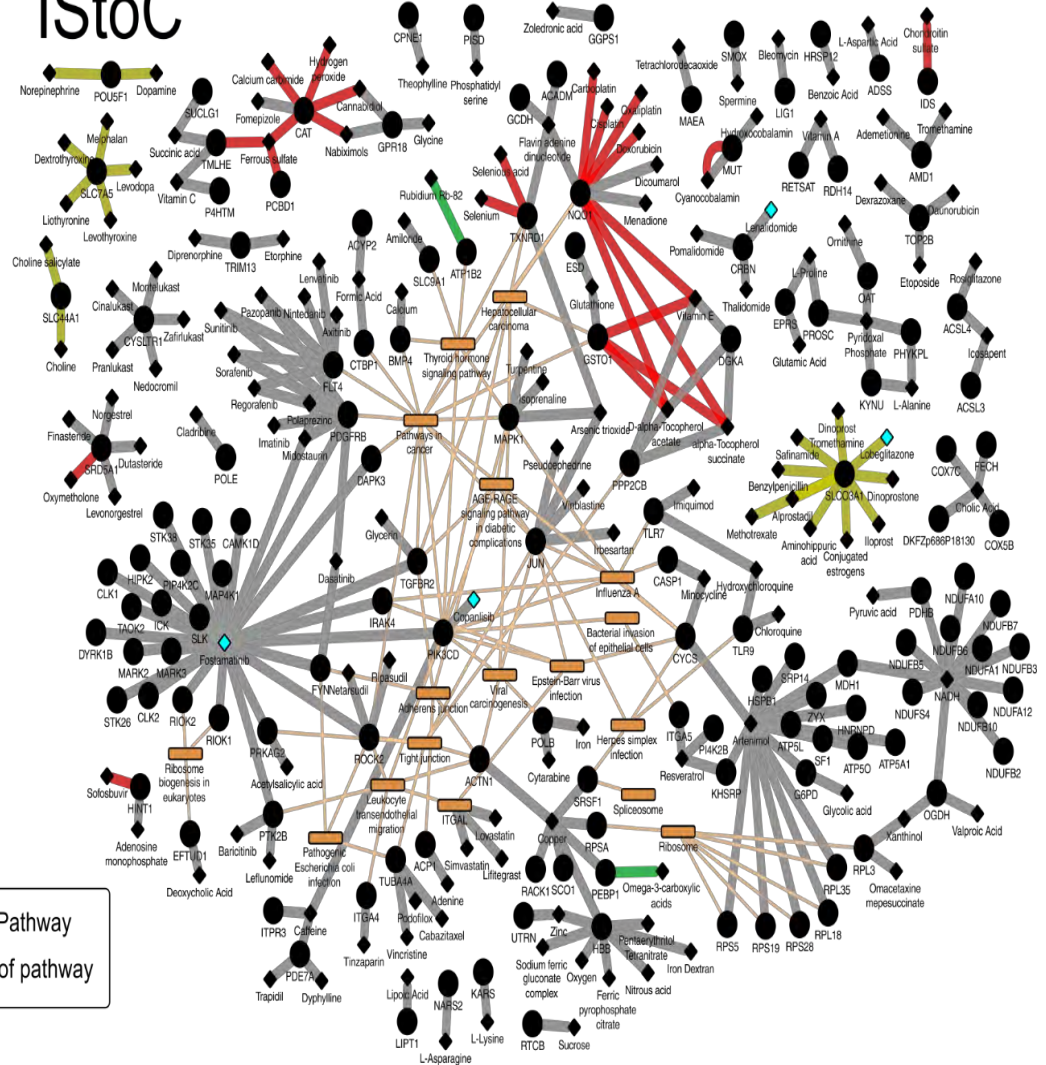


DR

Visualization of DEGs and enriched pathways

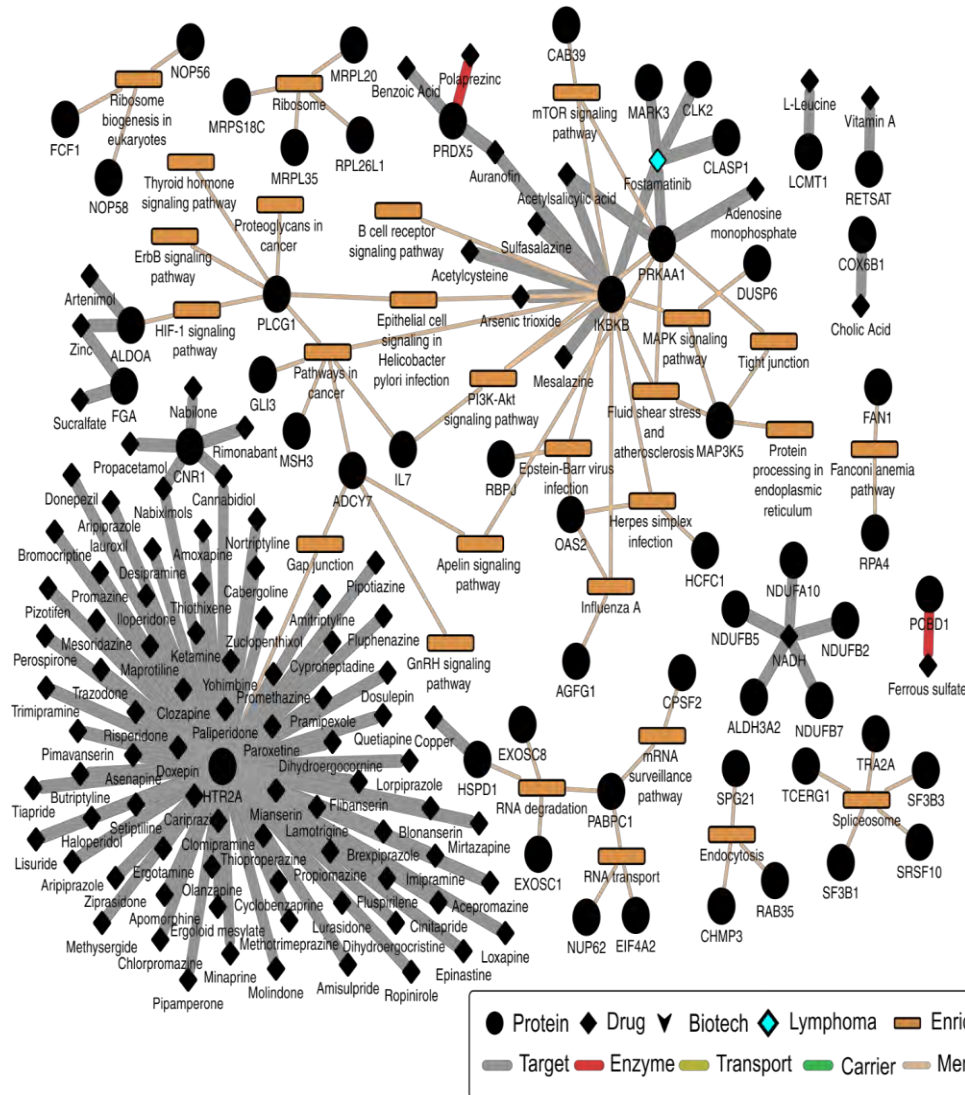
- Drug-target networks
- Drug-pathway-target networks

Ntols

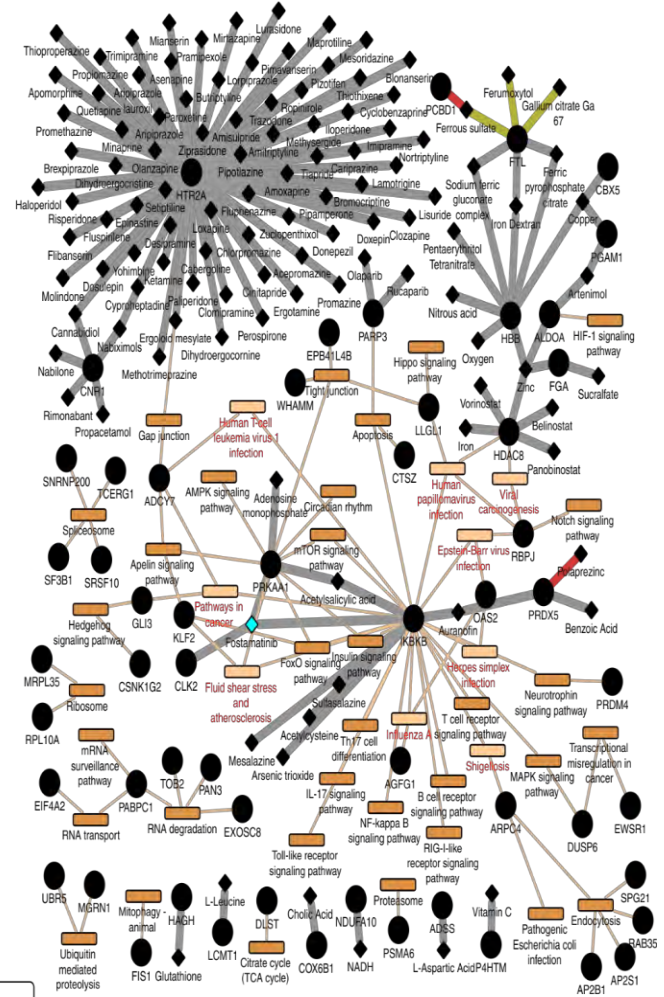


New potential treatments for MCL

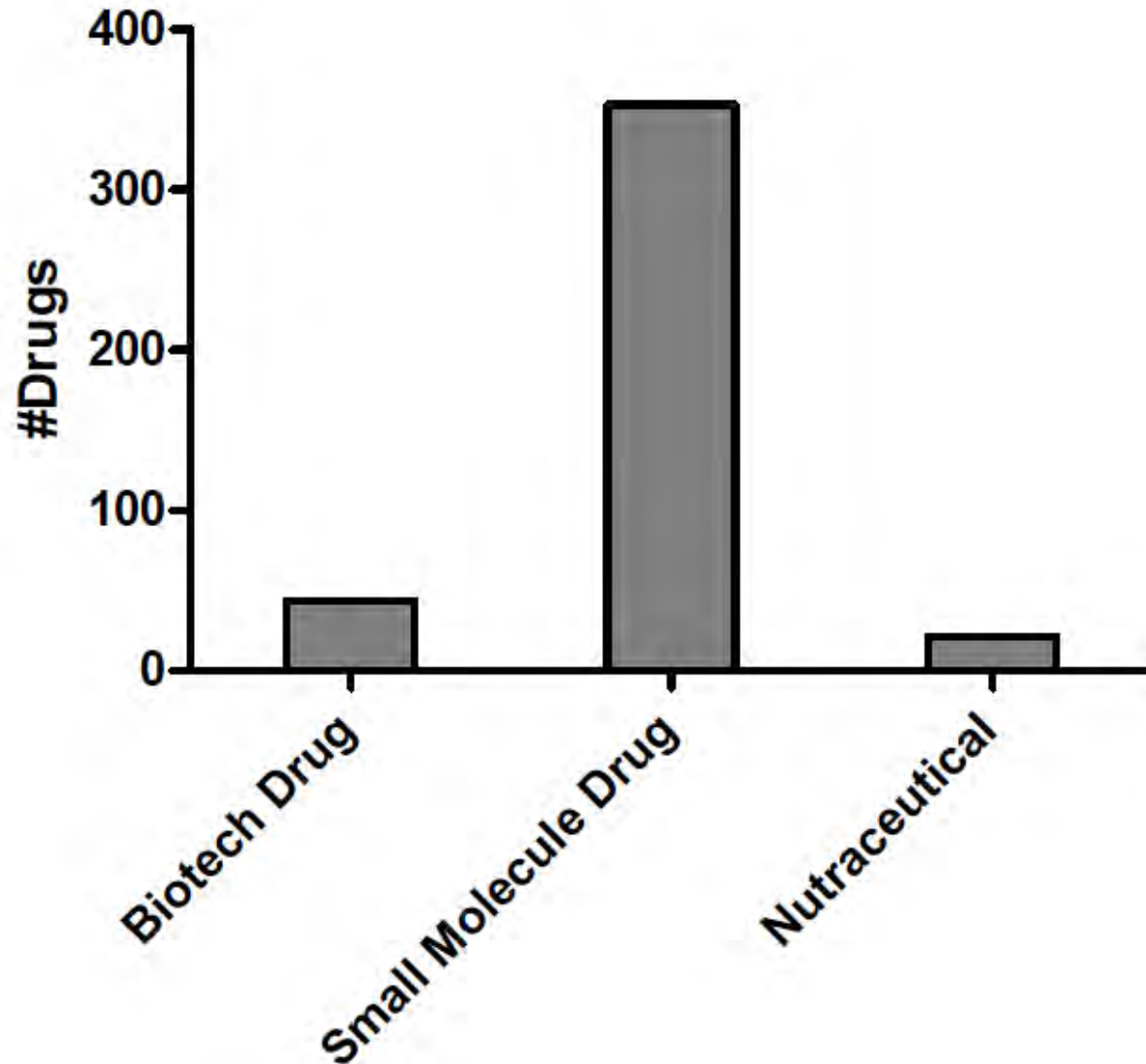
CtoI



ItoA



New potential MCL-drugs categories



MCL – pathways and drugs

BENIGN LYMPHADENITIS

IN SITU

CLASSICAL

INTERMEDIATE

AGGRESSIVE

PATHWAYS

- Prolactin signaling pathway
- Hedgehog signaling pathway
- Fc epsilon RI signaling pathway
- p53 signaling pathway
- AGE-RAGE signaling pathway in diabetic complications

DRUGS

Masoprocol
Mesalazine
Montelukast
Didofenac
Tamoxifen
Zileuton
Balsalazide
Sulfasalazine
Medofenamic acid
Diethylcarbamazine
Aminosalicylic Acid
alpha-Tocopherol succinate
D-alpha-Tocopherol acetate

Minocycline
Diacerein
Binimetinib
Arsenic trioxide
Resveratrol
Cannabidiol
Fostamatinib
Cholecystokinin

PATHWAYS

- Ribosome
- Proteasome
- Spliceosome
- Ubiquitin mediated proteolysis
- Ribosome biogenesis in eukaryotes
- Tight junction
- RNA transport

DRUGS

Ibrutinib
Acalabrutinib
Belinostat
Vorinostat
Panobinostat
Denileukin difitox
Fostamatinib
Muromonab
Vindesine
Tamoxifen
Docetaxel
Paclitaxel
Colchicine
Leflunomide

Dasatinib
Eribulin
Aflibercept
Aldesteukin
Catumaxomab
Baricitinib
Binimetinib
Cholecystokinin
Arsenic trioxide
Alpha-Tocopherol succinate
D-alpha-Tocopherol acetate

PATHWAYS

- Ribosome biogenesis in eukaryotes
- GnRH signaling pathway

- Ribosome
- Proteasome
- Spliceosome
- Cell cycle
- Gap junction
- Tight junction
- Adherens junction
- RNA transport
- RNA degradation
- mRNA surveillance

DRUGS

Ibrutinib
Acalabrutinib
Vorinostat
Belinostat
Panobinostat
Fostamatinib
Doxepin
Loxapine
Lisuride
Ketamine

Zotepine
Clozapine
Promazine
Amoxapine
Dosulepin
Ropinirole
Olanzapine
Imipramine
Ergotamine
Paroxetine
Epinastine

Fibanserin
Iloperidone
Molindone
Mianserin
Cisapride
Trazodone
Minaprine
Donepezil
Tegaserod
Pergolide
Dasatinib

Yohimbine
Molindone
Mianserin
Pizotifen
Sertindole
Nefazodone
Asenapine
Canprazine
Amisulpride
Cannabidiol

Methysergide
Fenfluramine
Mesonidazine
Promethazine
Quetiapine
Lurasidone
Ziprasidone
Cabergoline
Risperidone
Flupentixol

Maprotiline
Desipramine
Piprotiazine
Thiothixene
Fluphenazine
Thioridazine
Trimipramine
Propiomazine
Haloperidol
Aripiprazole

Aflibercept

Clomipramine
Mirtazapine
Remoxipride
Pramipexole
Lamotrigine
Apomorphine
Paliperidone
Fluspirilene
Pimavanserin
Butriptyline

Loripiprazole
Amitriptyline
Nortriptyline
Bromocriptine
Brexipiprazole
Cyproheptadine
Chlorpromazine
Zuclopenthixol
Cyclobenzaprine
Thiopropazine

Ergolid mesylate
Methotrimeprazine
Dihydroergocornine
Dihydroergocristine
Aripiprazole lauroxil

PATHWAYS

- Apoptosis
- Endocytosis
- Mitophagy
- Citrate cycle
- Wnt
- FoxO
- ErbB
- Insulin
- Apelin
- Notch
- Signaling pathways
- AMPK
- IL-17
- Hedgehog
- NF-kappa B
- T cell receptor
- Fc epsilon RI
- Thyroid hormone
- Circadian rhythm

- Cellular senescence
- MAPK signaling pathway
- mTOR signaling pathway
- Ubiquitin mediated proteolysis
- B cell receptor signaling pathway
- Protein processing in ER

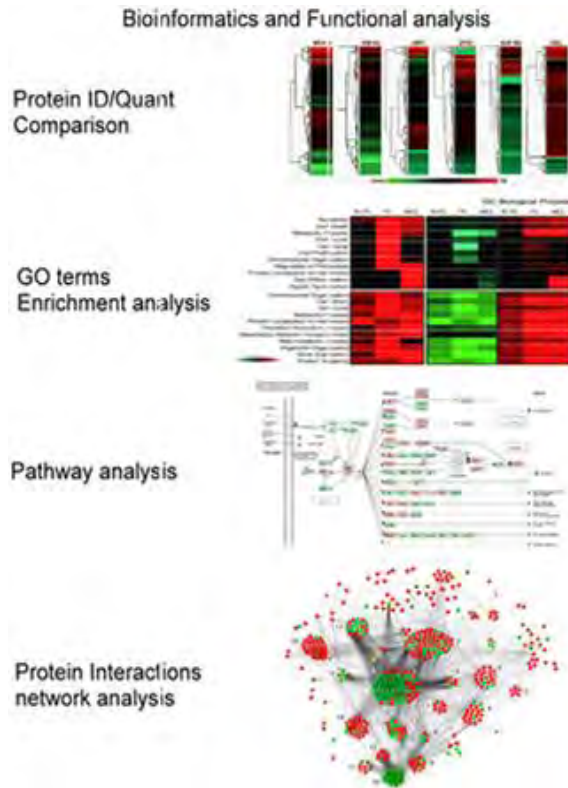
FDA-approved drug for MCL

	Drug ID	Drug Name	Related MCL Stage	Pathway	Original Use
1	DB12010	Fostamatinib	ALL	Ribosome Biogenesis	Rheumatoid Arthritis*
2	DB12483	Aliqopa (Copanlisib Hydrochloride)	Classical	Leukocyte transendothelial Thyroid hormone signaling pathway	FL*
3	DB09198	Lobeglitazone	Classical	-	antidiabetic medication
4	DB00480	Lenalidomide (Revlimid)	Classical	-	multiple myeloma, MCL
5	DB01169	Arsenic trioxide	<i>In situ</i>	p53 signaling pathway prolactin pathway	Acute Promyelocytic Leukemia (experimental on MCL)

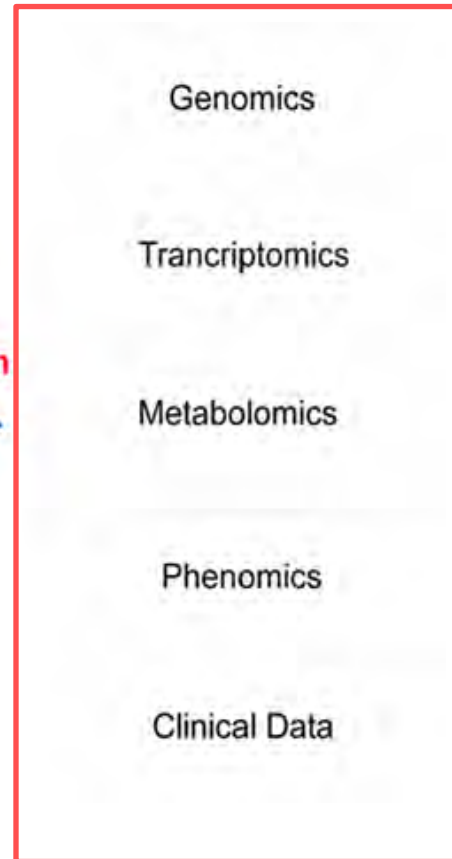
* tested on relapsed Lymphoma (MCL, DLBL, FL,HL)

Future perspectives....

Our Data

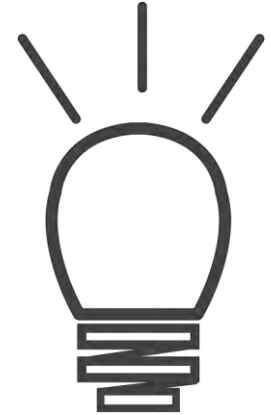


Public Data



Integration

Gained Information



W I S D O M

Personalized Medicine

Disease Mechanisms

Diagnostic/prognostic biomarkers

Novel therapeutic strategies

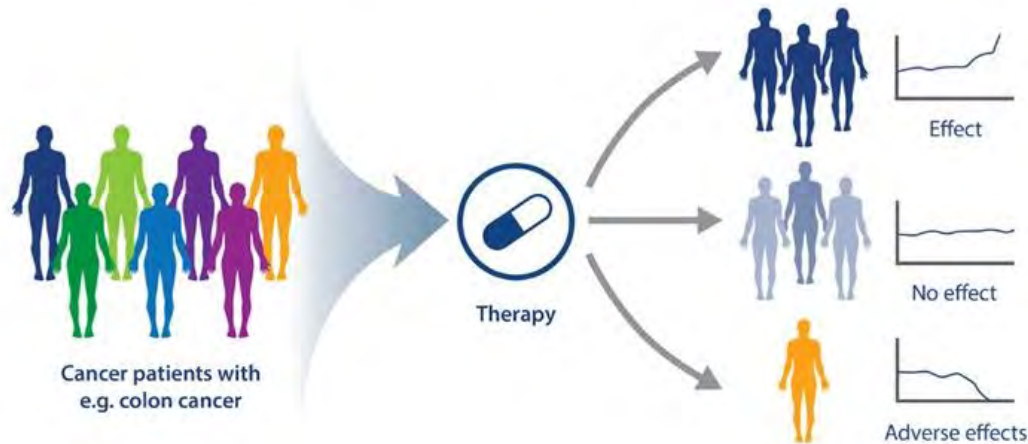
Novel Clinical tools

Integrated Multi-omics Repository

Future perspectives....

Current Medicine

One Treatment Fits All



Future Medicine

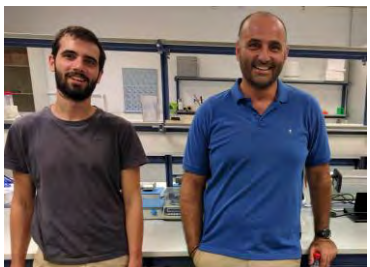
More Personalized Diagnostics







Acknowledgements



Co-financed by Greece and the European Union

Thank you!!!

