

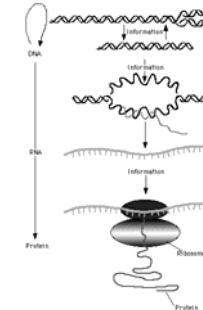
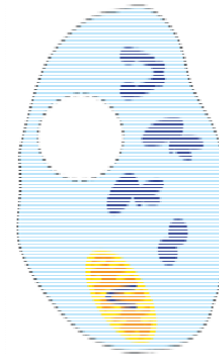
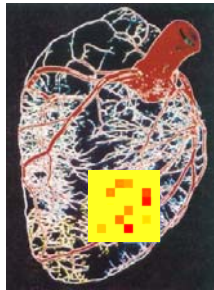
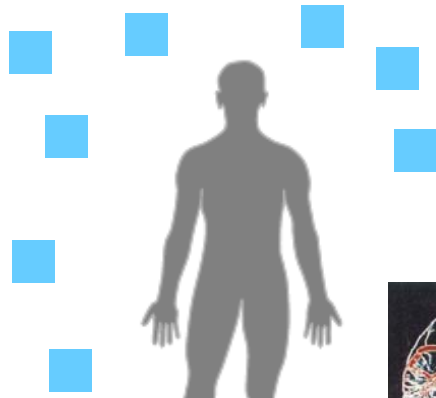
Triple Negative Breast Cancer: towards a more integrated approach



Renaud Seigneuric, Dr., PhD., HDR.
Cancer and ADaptive Immune Response (CADIR) Team
UMR 1231 Lipides, Nutrition, Cancer
Faculté des Sciences de Santé, Dijon
renaud.seigneuric@u-bourgogne.fr



A reductionist approach



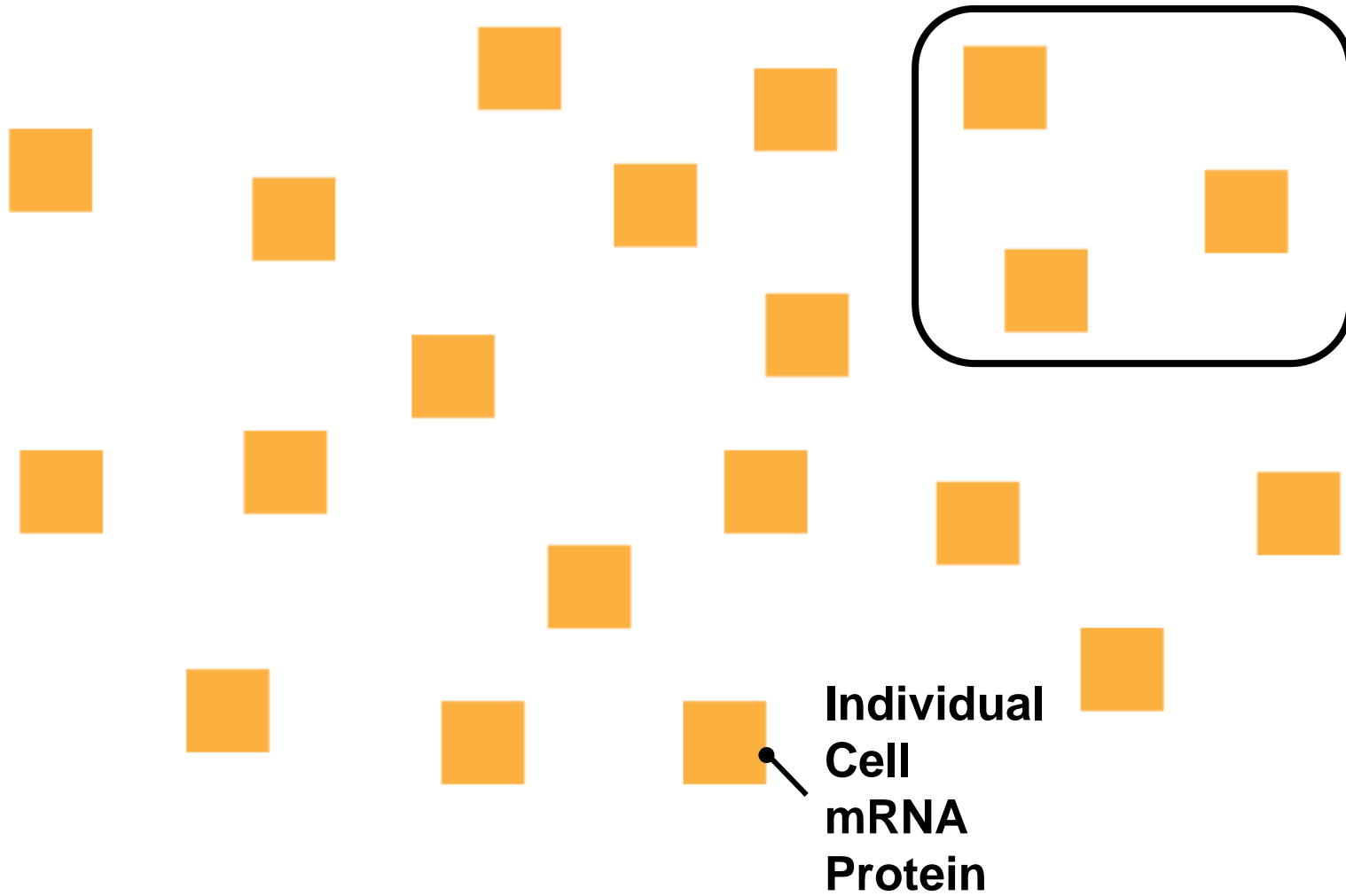
Microfluidics
Nanosciences
Protein crystallization



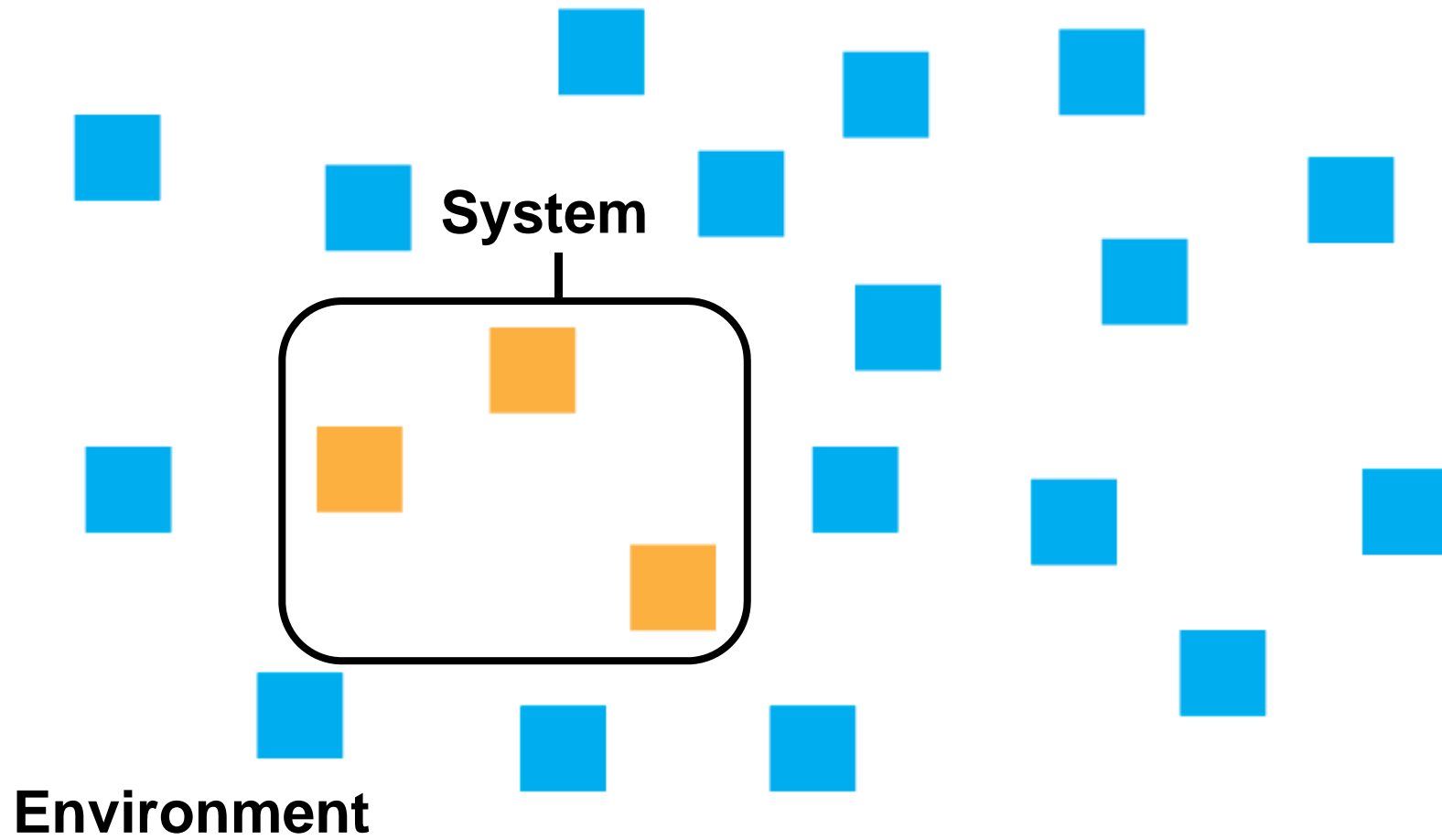
Concepts

System

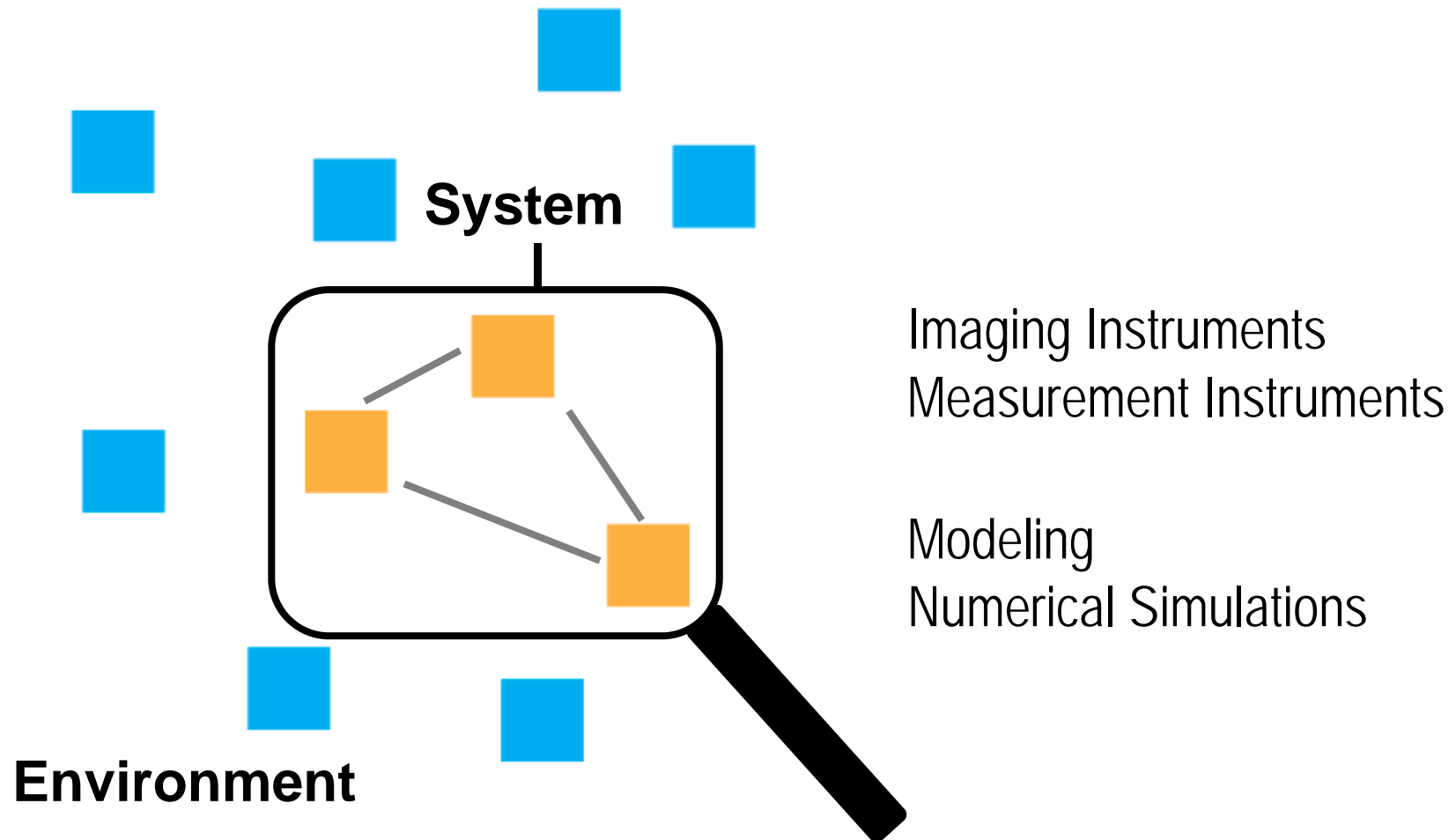
System



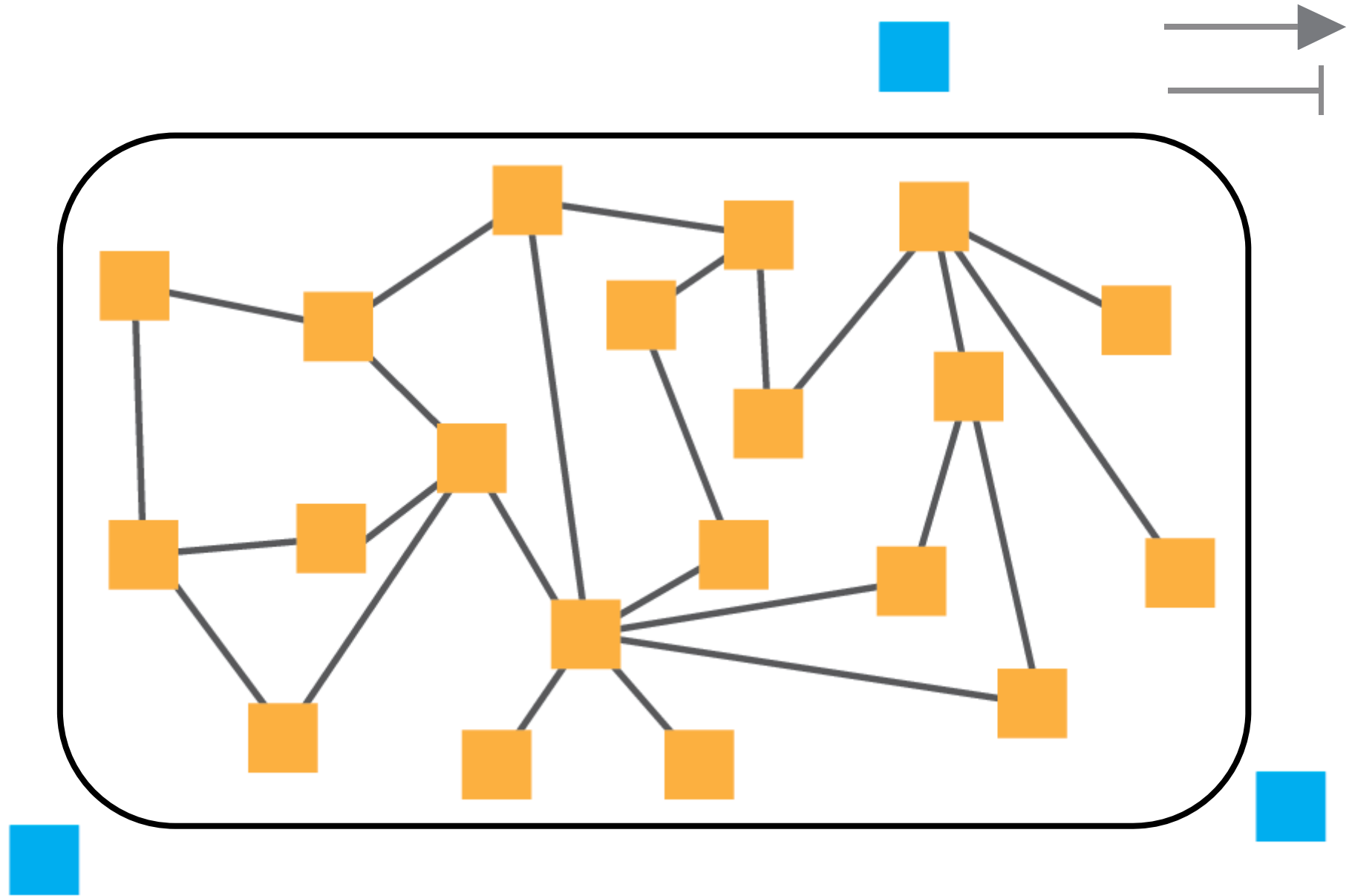
System



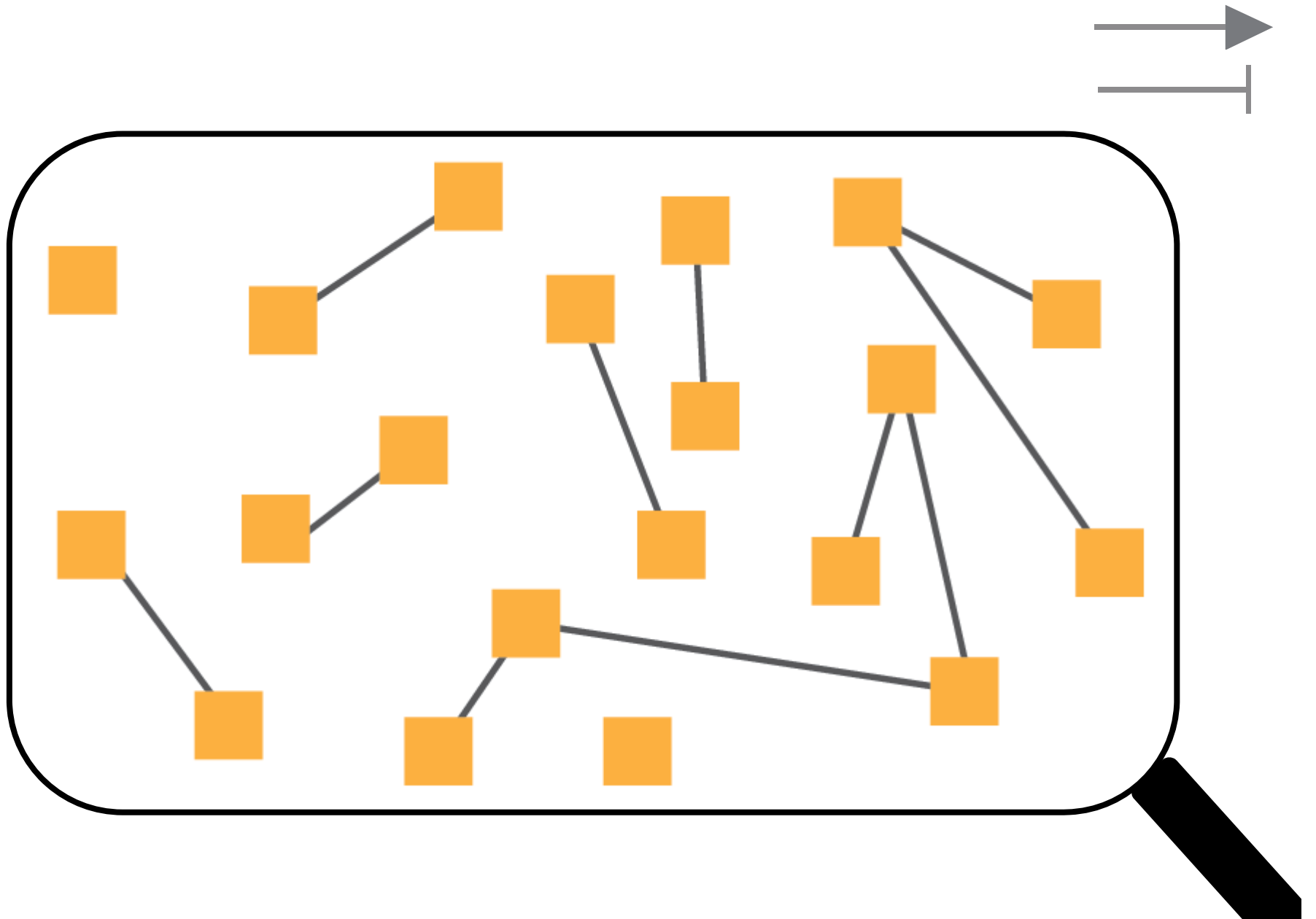
System



System



System



Complex System

System



Boeing 747; $3 \cdot 10^6$ pieces, HSP meeting, D.C.



Canadian geese, Prostate Cancer Institute, Vancouver

Complicated System

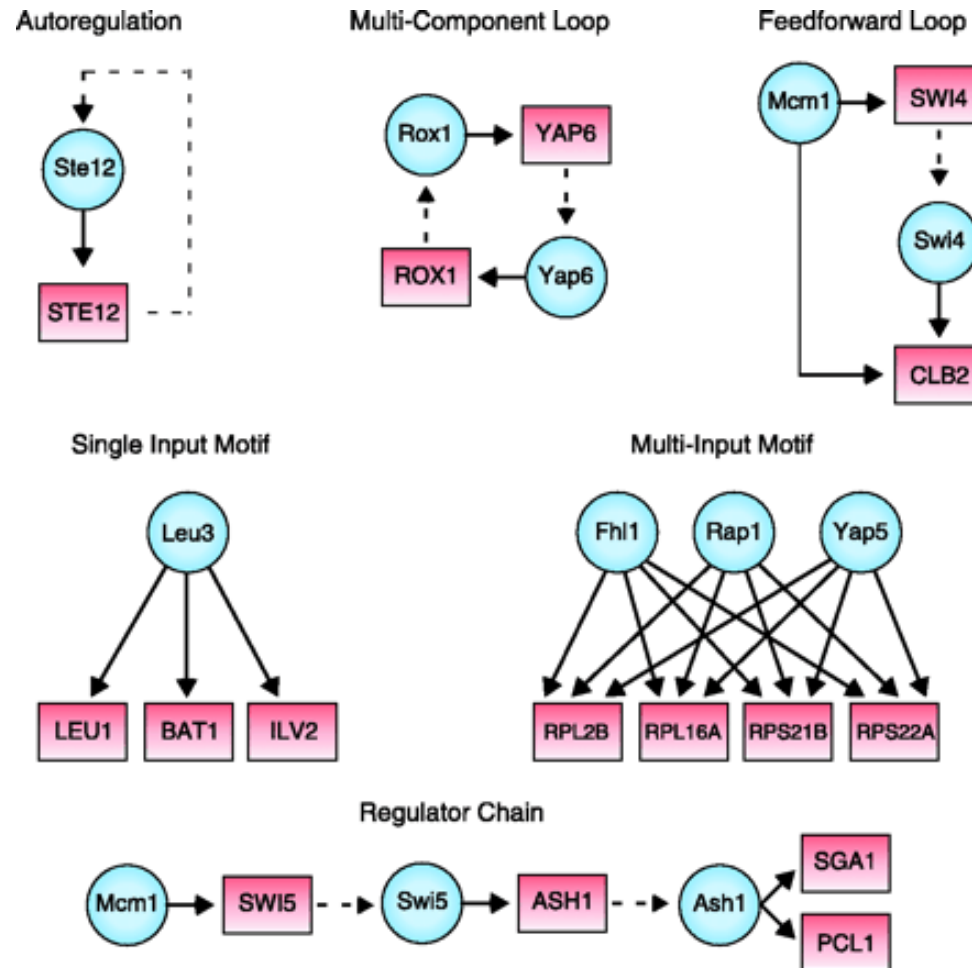
- composed of a large number of interacting components
- its properties can be accurately predicted
- it **is** the sum of its parts
- Central organization

« **Complicated is not Complex** »
[Nat. Biotech 1999]

Complex System

- displays properties are *NOT* predictable from a complete description of its components
- The whole is more than the sum of its parts
- Its elements can adapt and play different roles
- No element is essential (distributed system)
- auto-organization

Interaction / Regulation

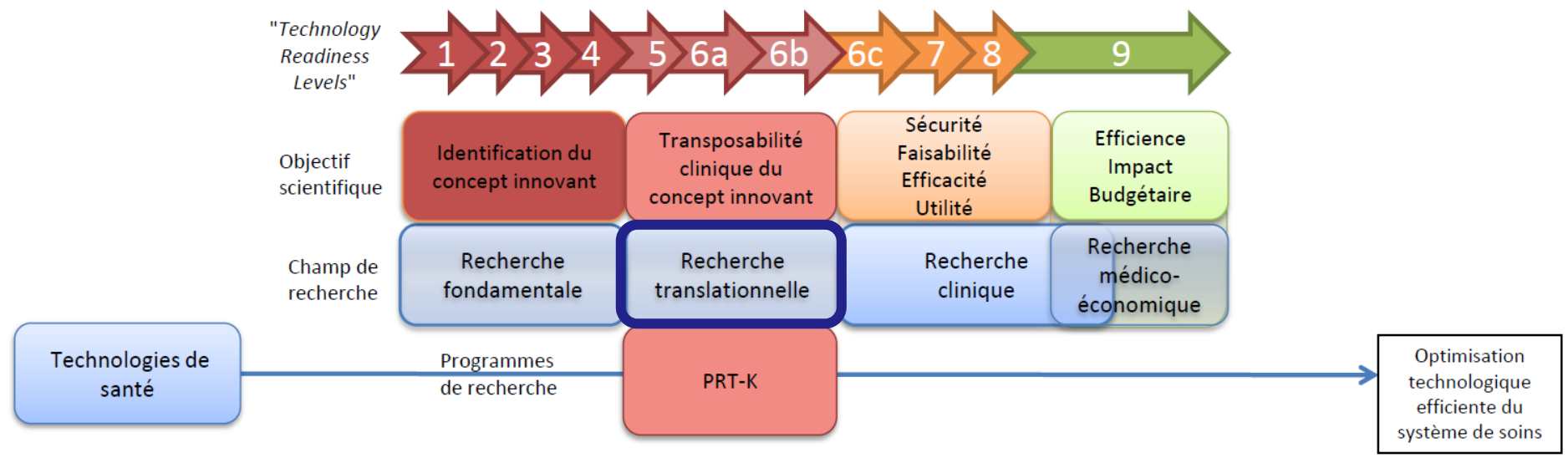
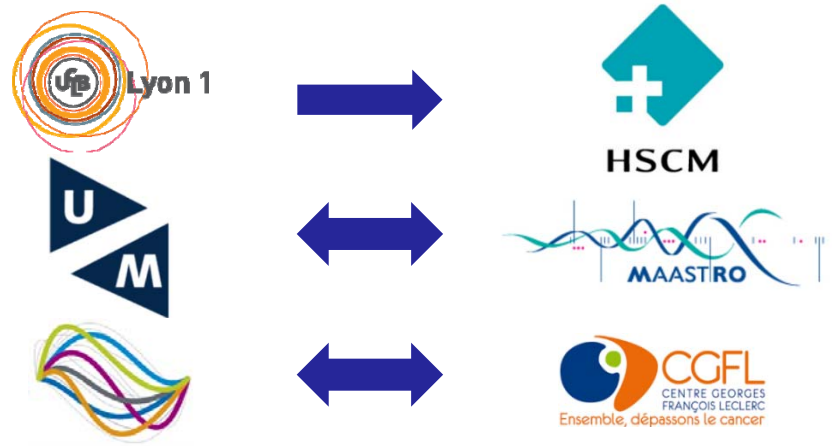


The 6 basic regulatory motifs

○ : transcription factor; □ target.

Translational Research

Translational Research



Biomarkers

Oncology / Bioinformatics / Personalized Medicine



Omics

Signatures

Sampling

Biomarkers

Variables



~ 10^{14} cells

~ 200 cell types

~ 20.000 genes

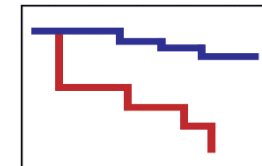
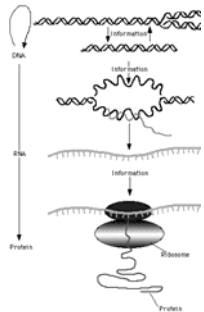
~200.000 mARNs

~ 3.000 metabolites

~ 1.000.000 proteins
(alt.splicing + PTM)



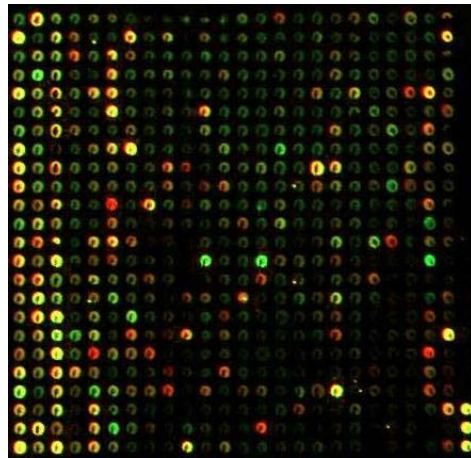
- Survival
- Diagnosis
- Treatment efficacy



DNA Microarrays

Microarrays

- ① Solid support (glass, silicon wafer, nylon membrane) = Slide
- ② Molecular probes (cDNA, Oligos [25-65mers]) = detectors
- ③ Printing probes on the slide
(presynthesized then deposited, synthesized *in situ*)



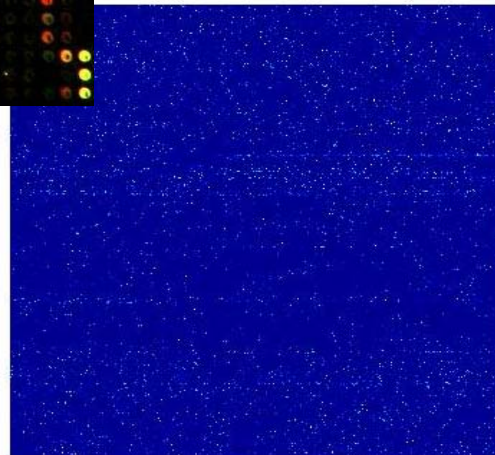
- ④ Target isolation, reverse transcription and amplification

- ⑤ Target Labeling

- ⑥ Hybridizing

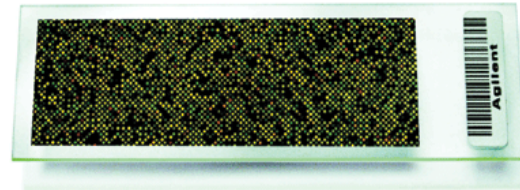
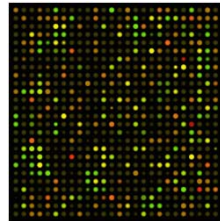
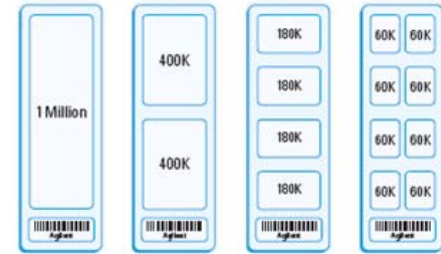
- ⑦ Scanning

- ⑧ Analyses



Microarrays

- Underdetermined problem:
- 1 DNA microarray has 20,000+ probe sets



- This Affymetrix HG U133 Plus 2.0 microarray has 54,675 probe sets



Breast Cancer



Breast Cancer in France

- In France, in 2017:
- ~59,000 / 400,000 new cancer cases
- ~12,000 / 150,000 cancer deaths

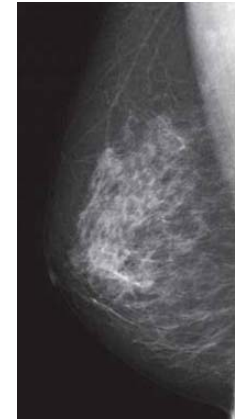
Classe d'âge	Incidence	Mortalité
[00 ; 14]	0	0
[15 ; 49]	11 135	856
[50 ; 64]	<u>19 034</u>	2 374
[65 ; 74]	15 180	2 620
[75 ; 84]	8 574	2 582
[85; ++]	5 045	<u>3 451</u>
Total	58 968	11 883



[Inca 2017]

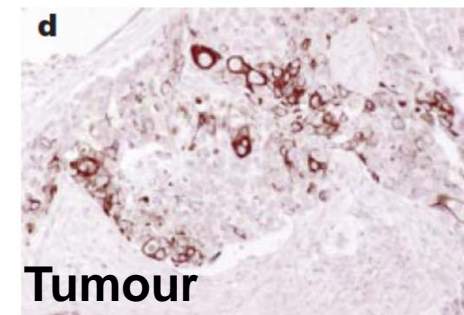
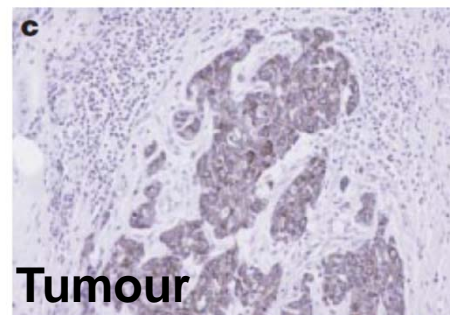
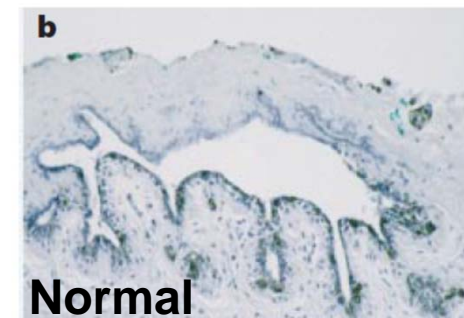
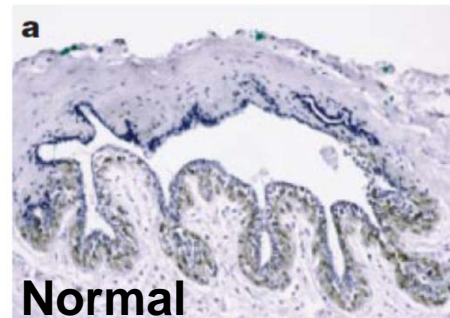


Breast Cancer



- Breast cancer is a **heterogeneous** disease with respect to:
 - cellular composition,
 - molecular alterations, and
 - clinical outcome

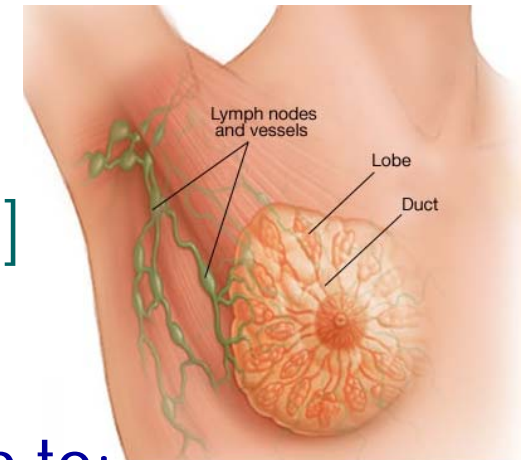
- Challenges:
 - Classification
 - Prognosis





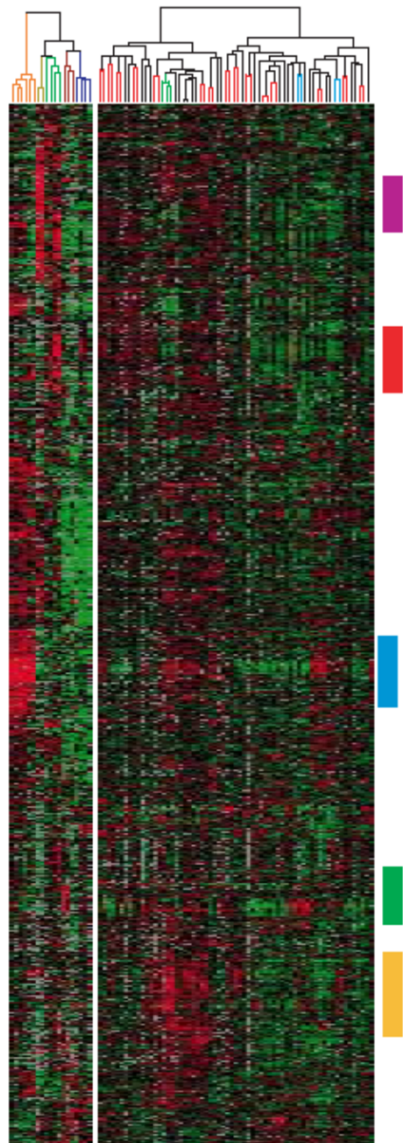
Breast Cancer

- Main clinical parameters:
 - age, lymph node status, tumor size, histological grade
 - estrogen receptor [ER],
 - progesterone receptor [PR]
 - epidermal growth factor receptor 2 [HER2]



- All of which are routinely used in the clinic to:
- Stratify (partitioning of) patients for prognostic predictions and
 - to select treatments

« intrinsic » molecular subtypes [Perou et al Nature 2000]



65 surgical specimens of human breast tumours from 42 different individuals, using complementary DNA microarrays representing 8,102 human genes

letters to nature

.....

Molecular portraits of human breast tumours

**Charles M. Perou^{*†}, Therese Sørlie^{†‡}, Michael B. Eisen^{*},
Matt van de Rijn[§], Stefanie S. Jeffrey^{||}, Christian A. Rees^{*},
Jonathan R. Pollack[¶], Douglas T. Ross[¶], Hilde Johnsen[‡],
Lars A. Akslen[#], Øystein Fluge[☆], Alexander Pergamenschikov^{*},
Cheryl Williams^{*}, Shirley X. Zhu[§], Per E. Lønning^{**},
Anne-Lise Børresen-Dale[‡], Patrick O. Brown^{¶††} & David Botstein^{*}**

TNBC



Triple-Negative Breast Cancer (TNBC)

- Main clinical parameters:
 - age, lymph node status, tumor size, histological grade
 - estrogen receptor [ER-],
 - progesterone receptor [PR-]
 - epidermal growth factor receptor 2 [HER2-]

Currently:

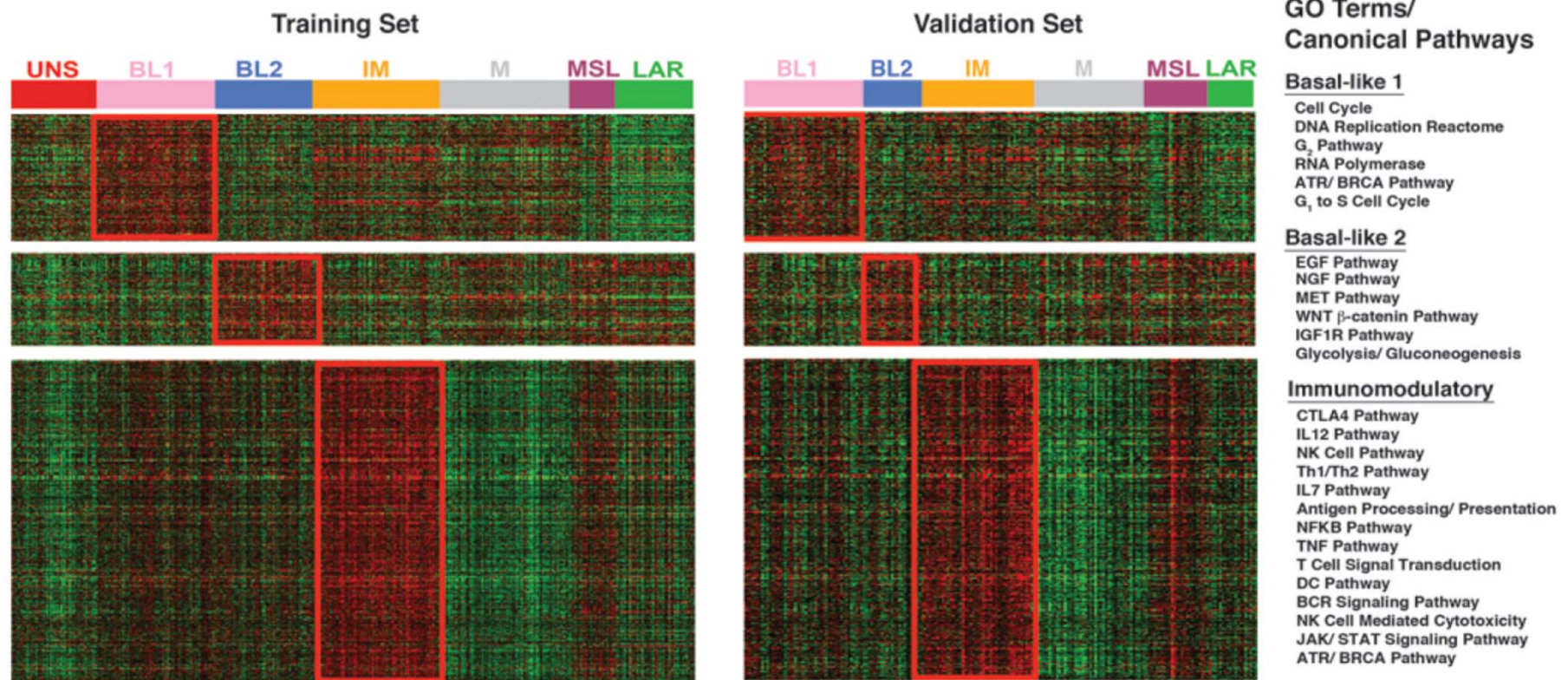
- No validated biomarkers
- No approved targeted therapies

Questions:

- Can we better understand TNBC?
- Identify potential resistance biomarkers?

TNBC Subtypes

Subtyping Triple-Negative Breast Cancer

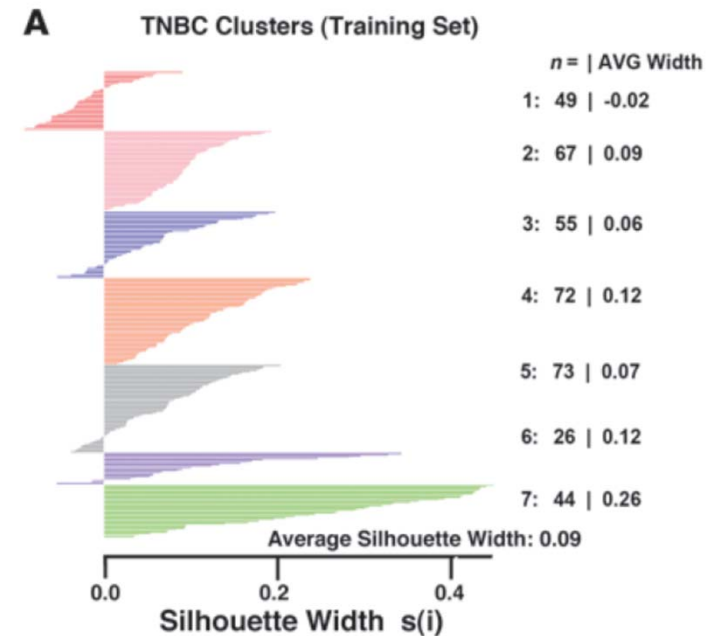


Lehman et al. JCI 2011

Subtyping Triple-Negative Breast Cancer

Seven (7) subtypes:

- basal-like 1,
- basal-like 2,
- immunomodulatory,
- luminal androgen receptor
- mesenchymal,
- mesenchymal stem-like,
- unstable



Outline

- Concepts

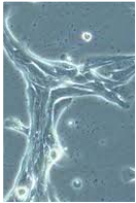


- An integrative approach to TNBC
- Next steps?

Towards an integrative approach to TNBC

Approach

Wet-bench experiments



- TNBC cell lines culture: HCC70, HCC1937 et MDA-MB-231



- MTT viability tests after treatment (4 chemotherapies)



- Proliferation kinetics/dynamics by impedance-metry, dose-response

Bioinformatics



n Search



Array22
Array16
Array28
Array15
Array12
Array30



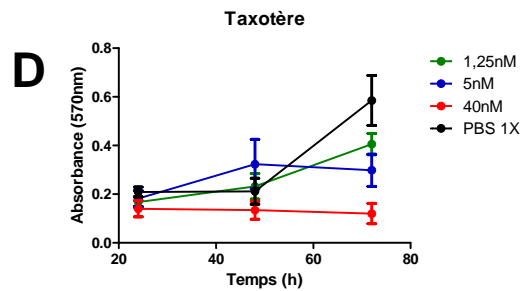
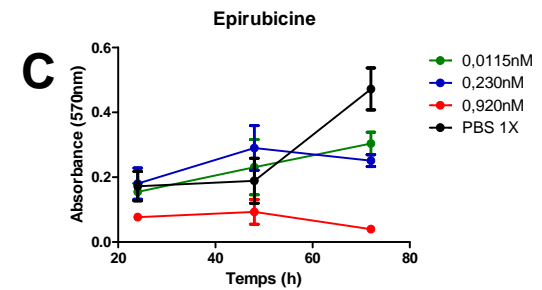
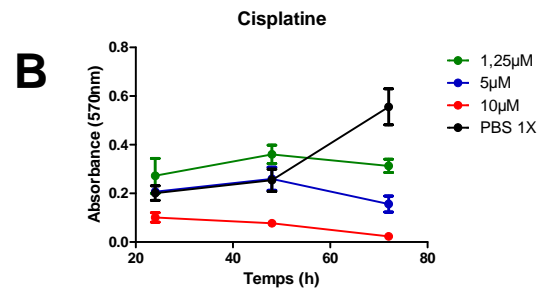
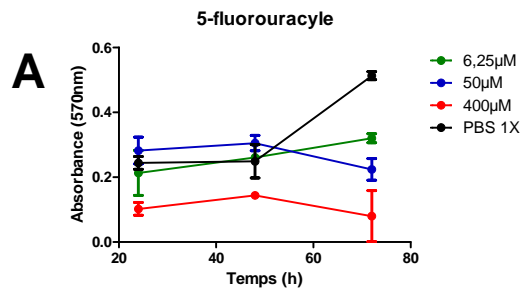
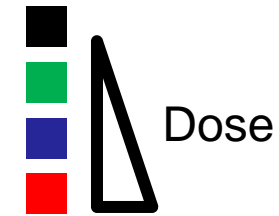
- Download microarray data (e.g.: from GEO, TCGA)

- QA/QC, Normalization, Batch-correction

- Find differentially expressed genes, GO, Pathway analyses

Treatments

- MTT tests on TNBC cell lines:
TNBC cells are killed by un-targeted agents

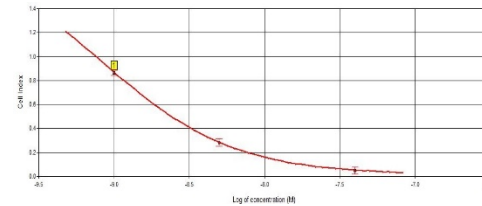
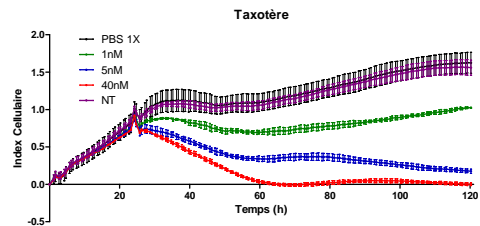
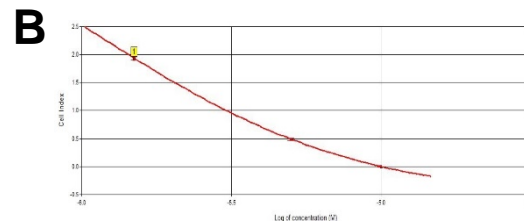
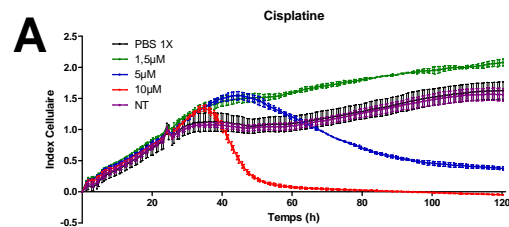


E

Lignée	HCC70			HCC1937			MDA-MB-231		
Traitement	IC25	IC50	IC75	IC25	IC50	IC75	IC25	IC50	IC75
5-fluorouracyle (µM)	6,25	35	400	12,5	50	100	12,5	50	200
Cisplatine (µM)	1,5	5	10	1,25	7,5	20	1,25	5	20
Epirubicine (nM)	0,1	0,25	1	0,1	1	2	0,1	0,5	1,5
Taxotère (nM)	1	5	40	2,5	10	20	5	10	20

Treatments

- Dose-response kinetics of TNBC cell lines



**HCC70 and
MDA-MB-231
cell lines
exhibit various
responses**

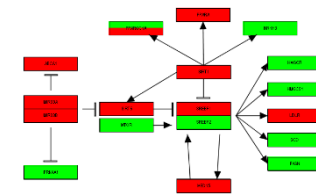
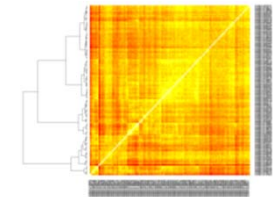
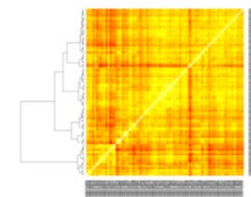
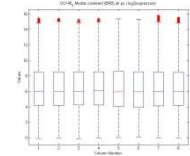
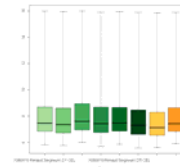
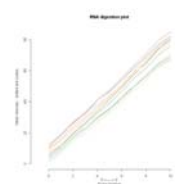
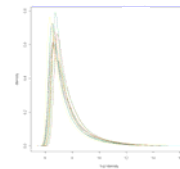
C

Traitement	5-fluorouracyle		Cisplatine		Epirubicine		Taxotère	
Lignée	IC50 (μM)	R ²	IC50 (μM)	R ²	IC50 (nM)	R ²	IC50 (nM)	R ²
HCC70	100	0,984	3,16	1	0,447	1	2	1
MDA-MB-231	28	0,95	3,98	0,696	0,355	0,781	11	0,902

Bioinformatics Pipeline

- Download Microarrays
- QA/QC
- Normalize data
- Correct for Batch effect
- Differential Expression (gene level)
- Differences in Pathways
- Map existing drugs
- Interpretation for treatment success/failure

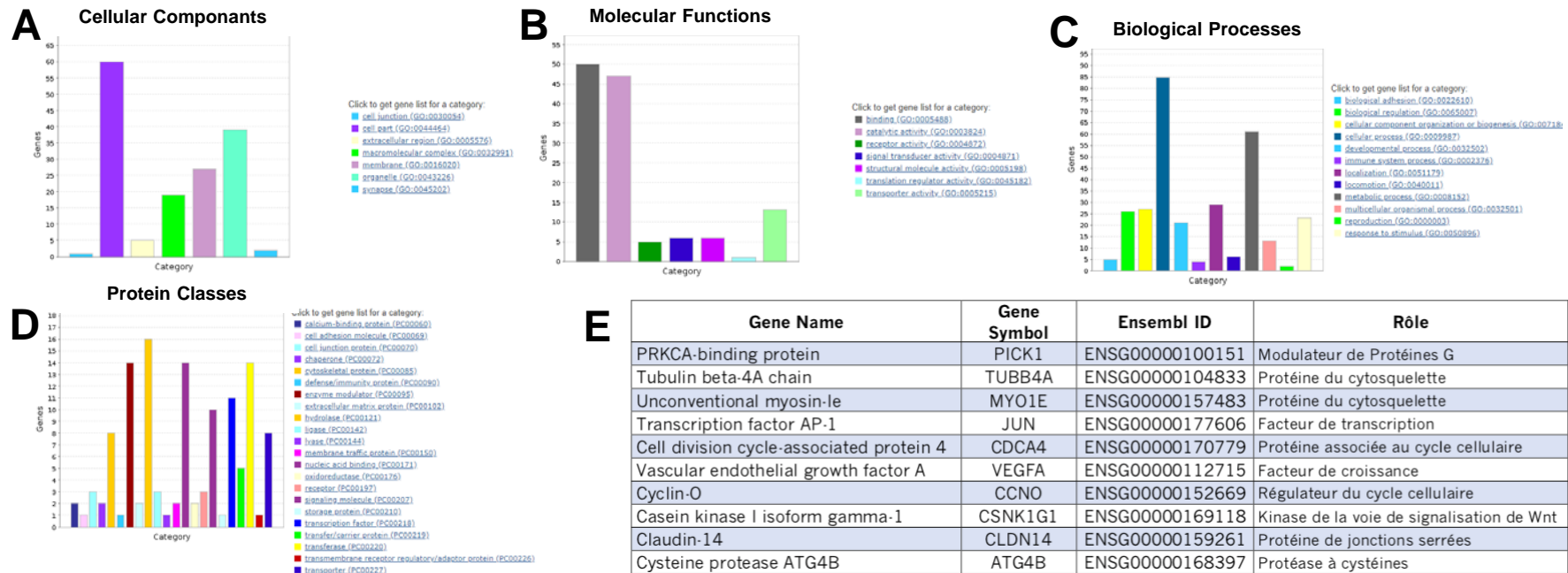
GEO



Finding differentially
expressed
(genes and) pathways

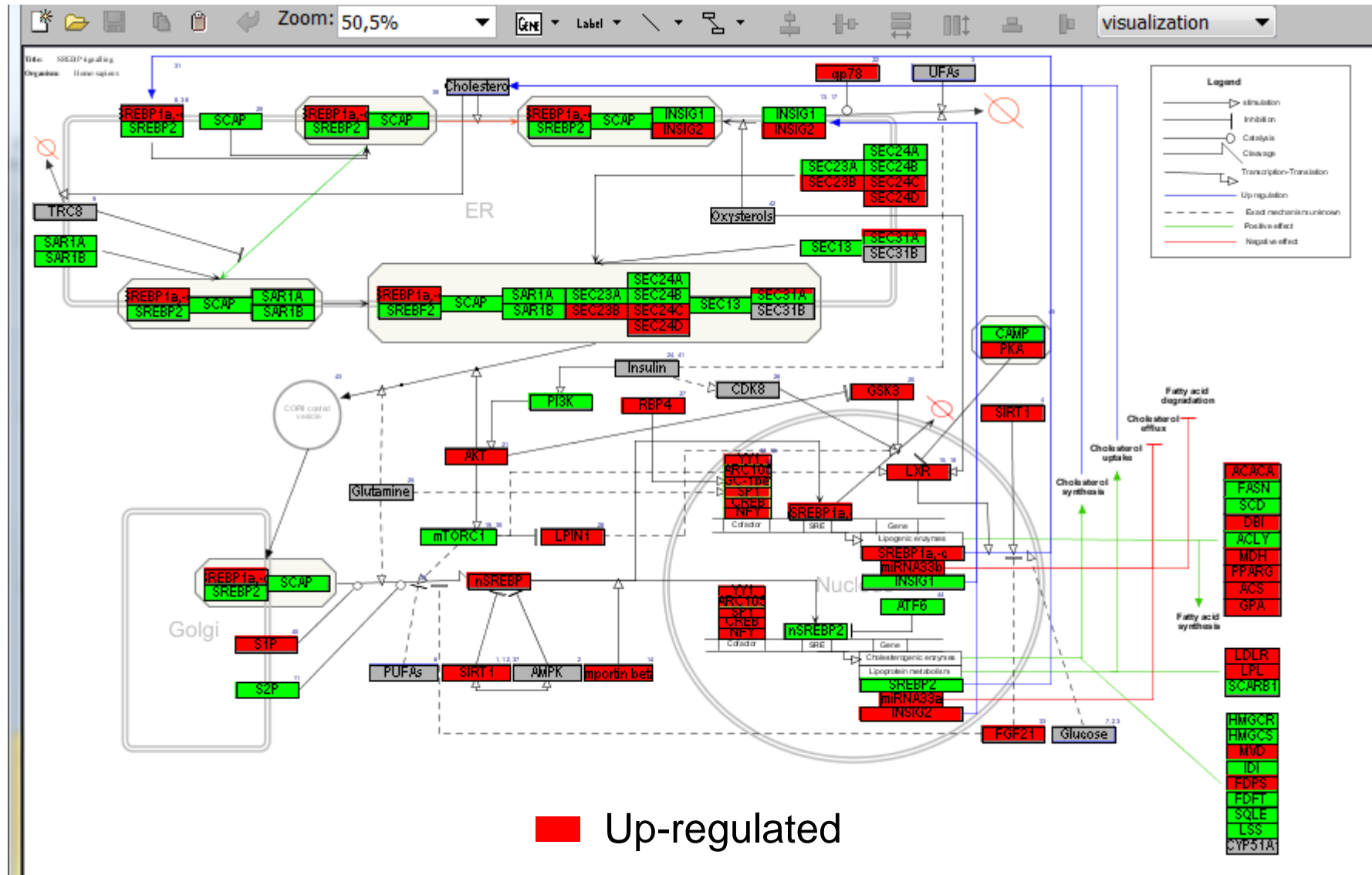
Treatments

- Gene signatures (231 transcripts, FDR=10%),
- Gene Ontology analysis



Possible cell proliferation differences between survivor vs non-survivor TNBC patients

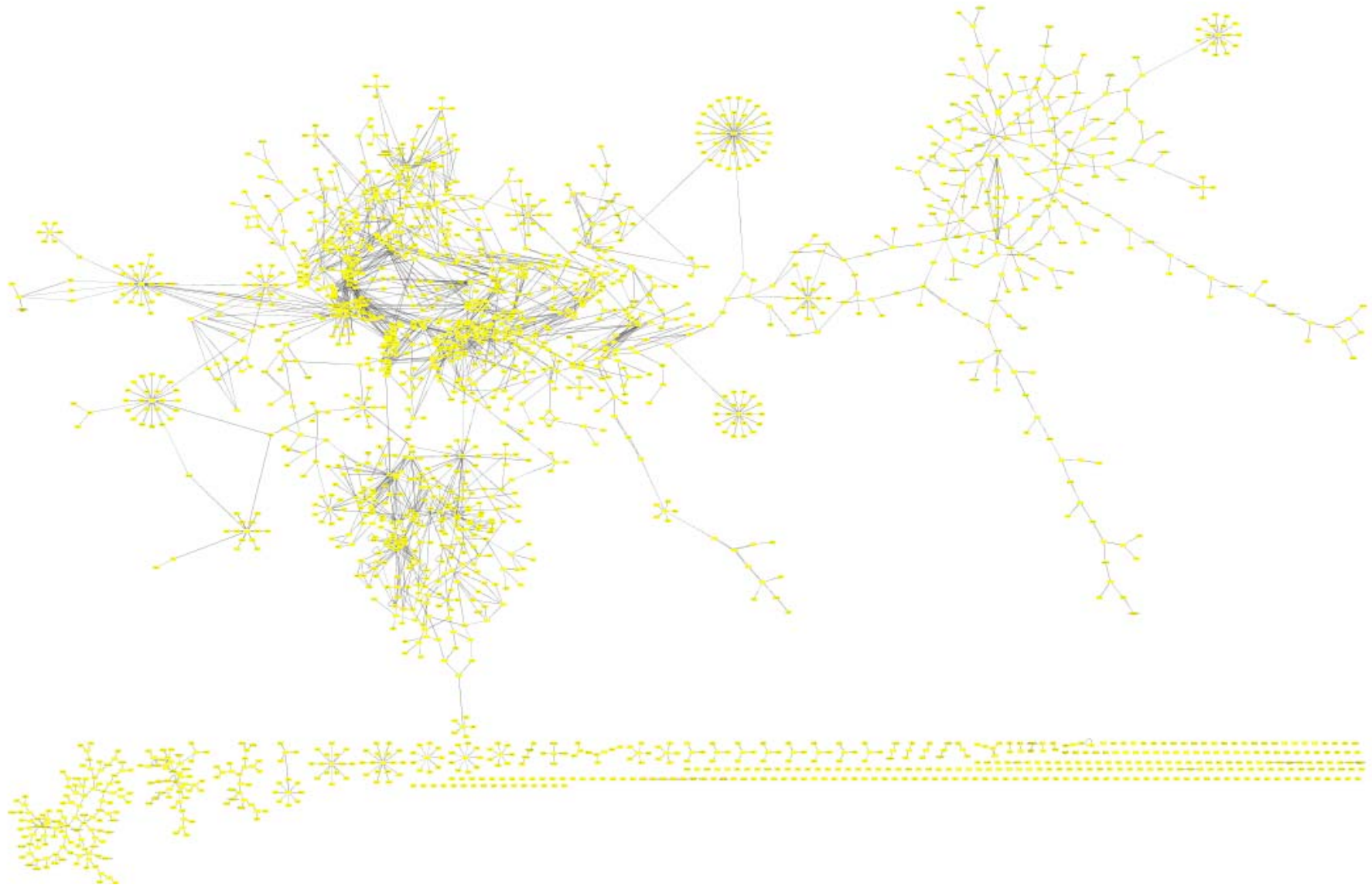
Pathways



■ Up-regulated
■ Down-regulated

Pathway Integration

Merged network (2172 nodes | 2541 edges) \int

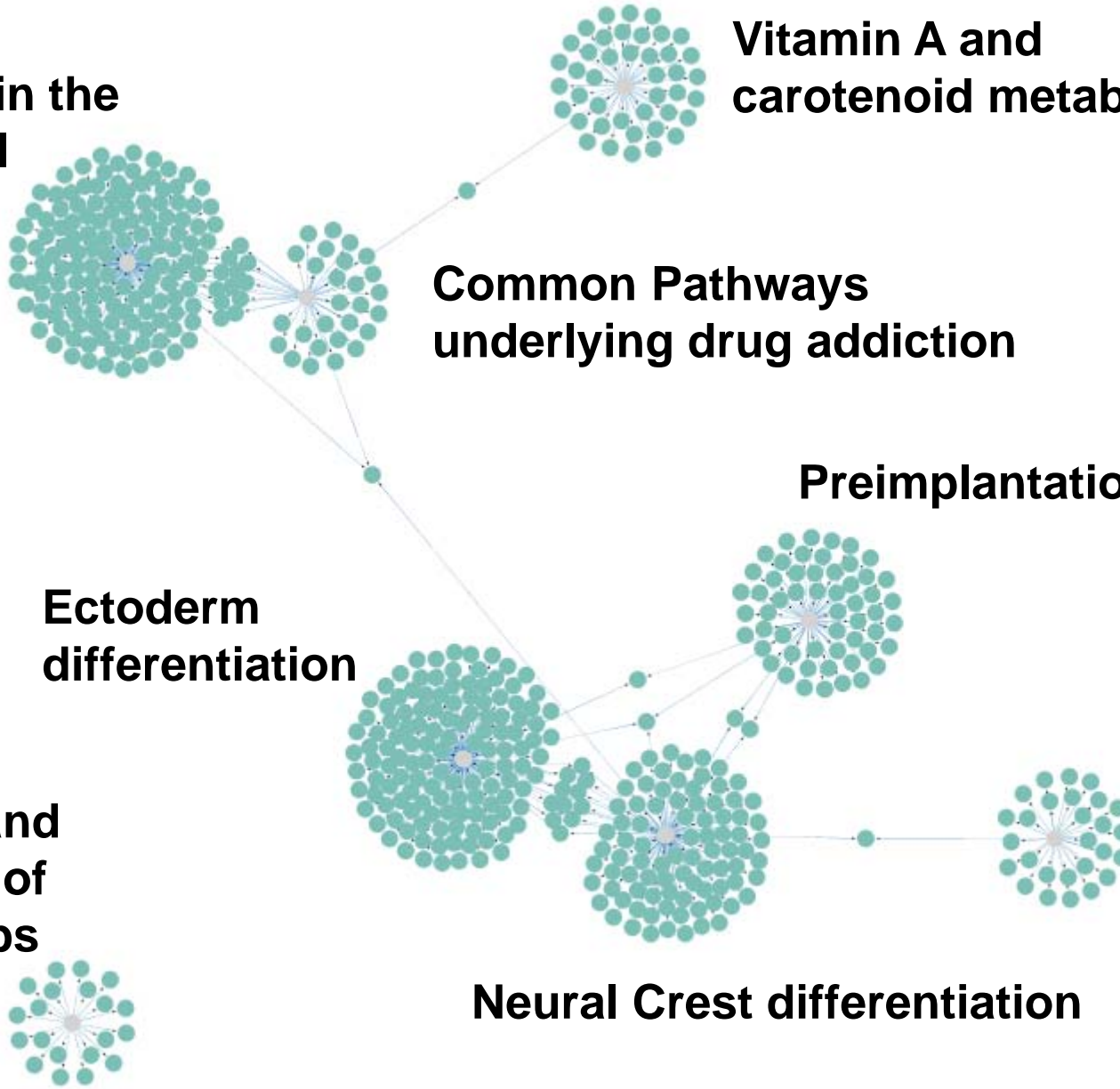


8 pathways (557 nodes | 581 edges)

∫

**Calcium
Regulation in the
Cardiac Cell**

**Vitamin A and
carotenoid metabolism**



**Common Pathways
underlying drug addiction**

Preimplantation embryo

**Ectoderm
differentiation**

**Urea cycle and
metabolism of
amino groups**

**Gastric
cancer
network 1**

Neural Crest differentiation

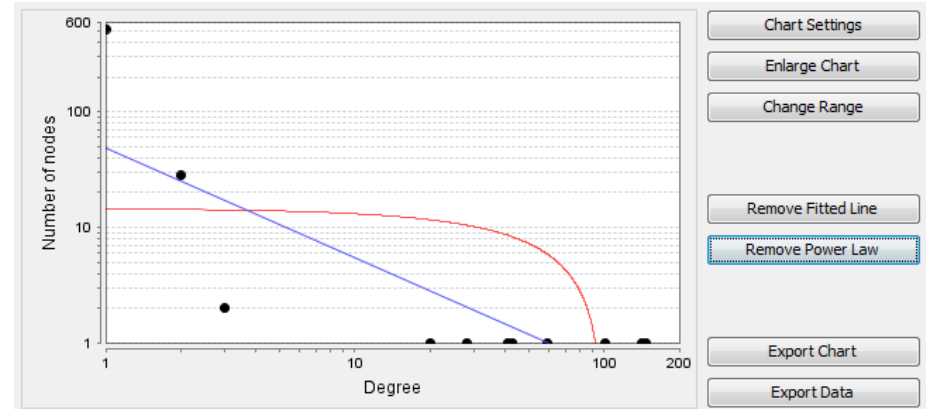
8 pathways (557 nodes | 581 edges)



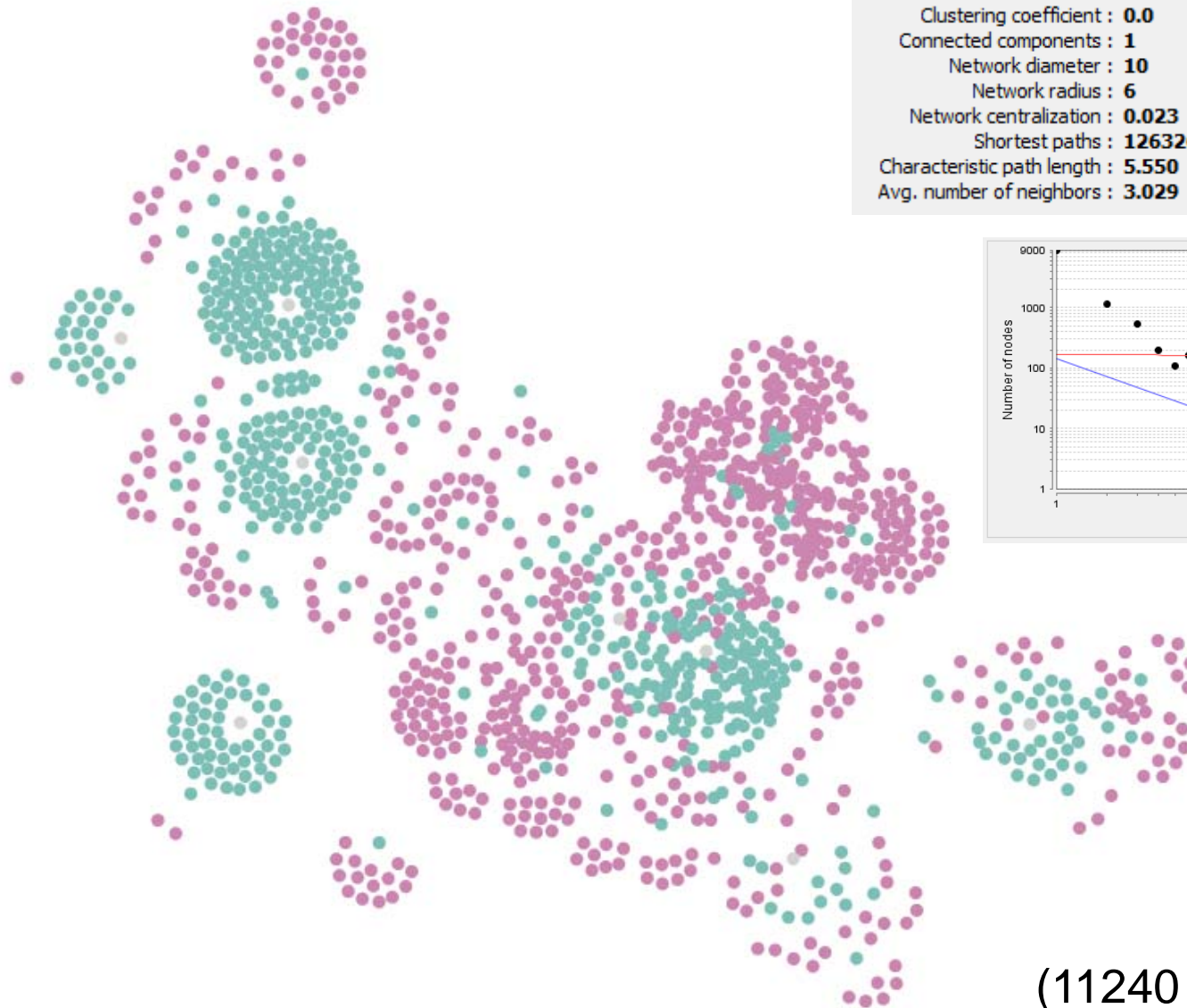
Betweenness Centrality	Closeness Centrality	Stress Centrality Distribution	
Shortest Path Length Distribution	Shared Neighbors Distribution	Neighborhood Connectivity Distribution	
Simple Parameters	Node Degree Distribution	Avg. Clustering Coefficient Distribution	Topological Coefficient

Clustering coefficient : **0.0**
Connected components : **2**
Network diameter : **8**
Network radius : **1**
Network centralization : **0.262**
Shortest paths : **287180 (92%)**
Characteristic path length : **4.552**
Avg. number of neighbors : **2.086**

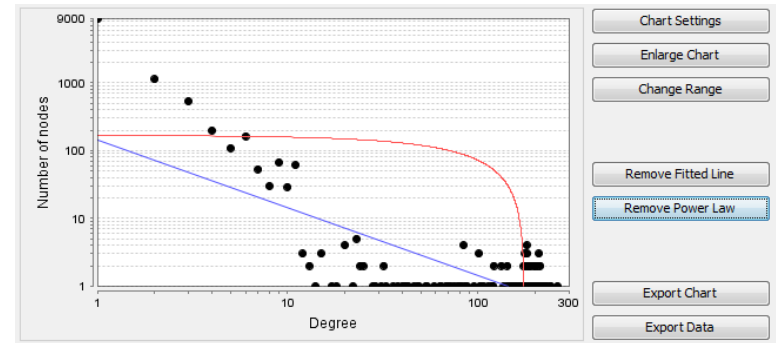
Number of nodes : **557**
Network density : **0.004**
Network heterogeneity : **4.908**
Isolated nodes : **0**
Number of self-loops : **0**
Multi-edge node pairs : **0**
Analysis time (sec) : **0.555**



Proteins + Drugs + miRNAs + TF



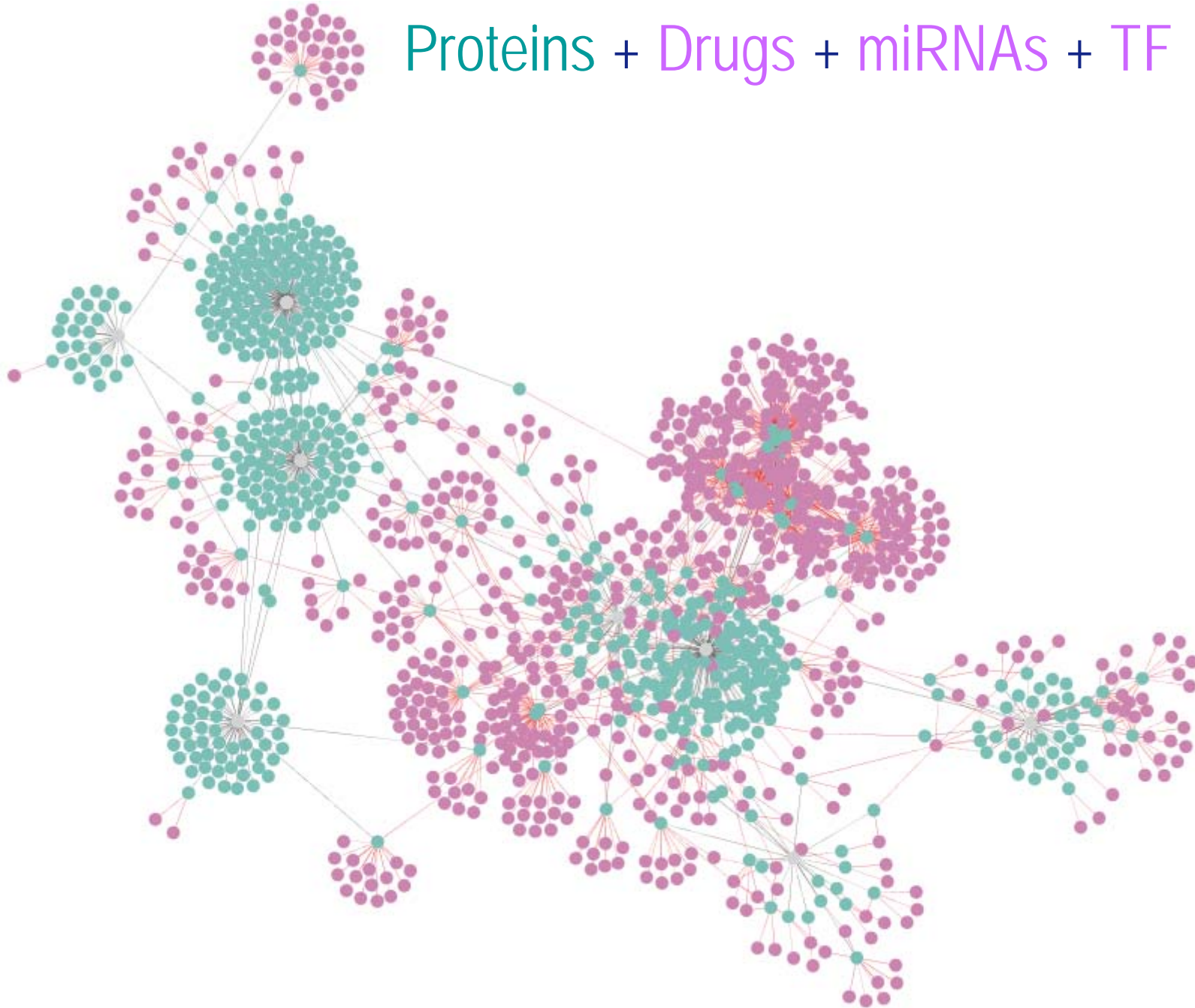
Clustering coefficient : 0.0	Number of nodes : 11240
Connected components : 1	Network density : 0.000
Network diameter : 10	Network heterogeneity : 5.150
Network radius : 6	Isolated nodes : 0
Network centralization : 0.023	Number of self-loops : 0
Shortest paths : 126326360 (100%)	Multi-edge node pairs : 0
Characteristic path length : 5.550	Analysis time (sec) : 7.258
Avg. number of neighbors : 3.029	



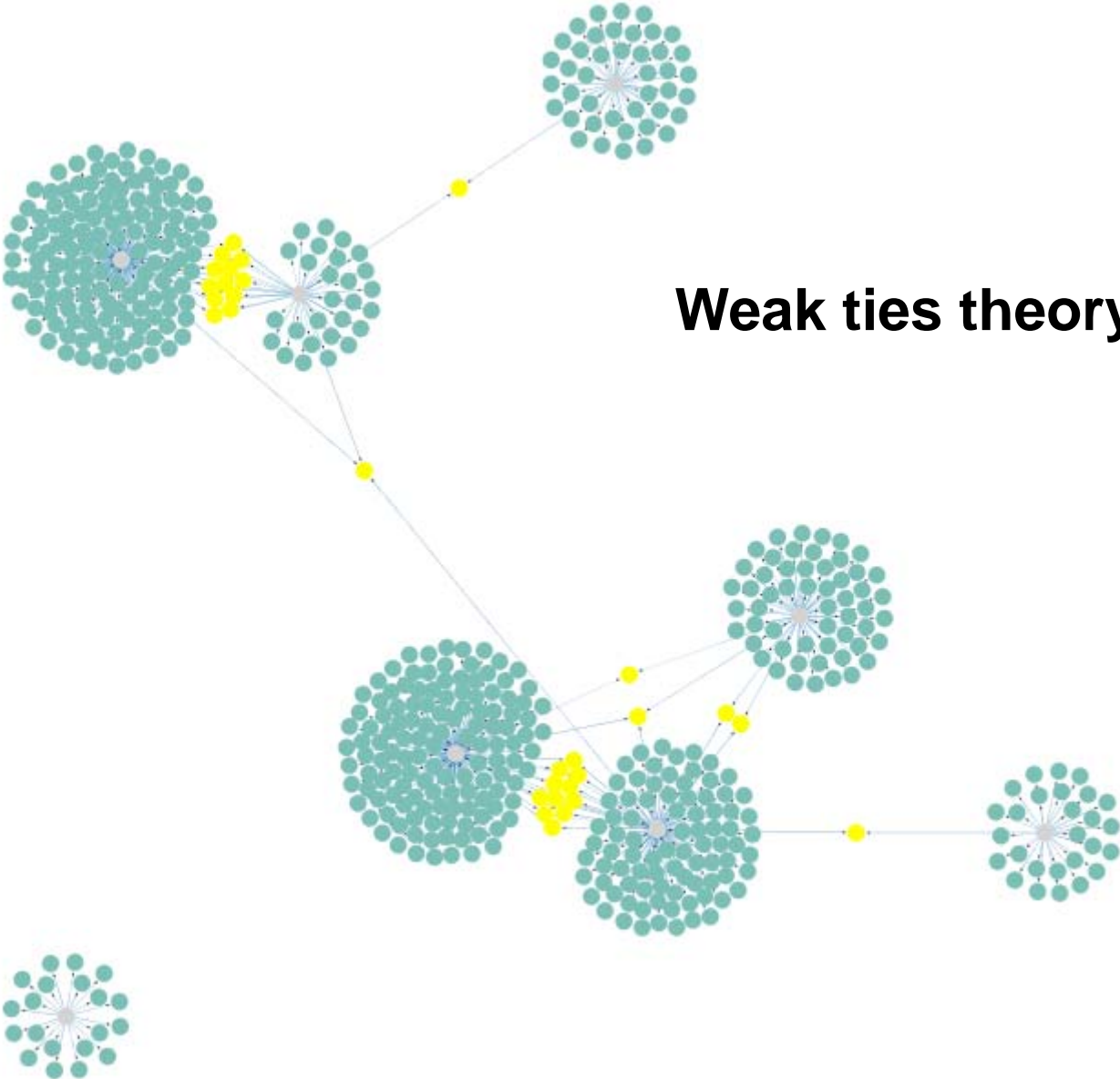
(11240 nodes | 17022 edges)

Proteins + Drugs + miRNAs + TF

∫



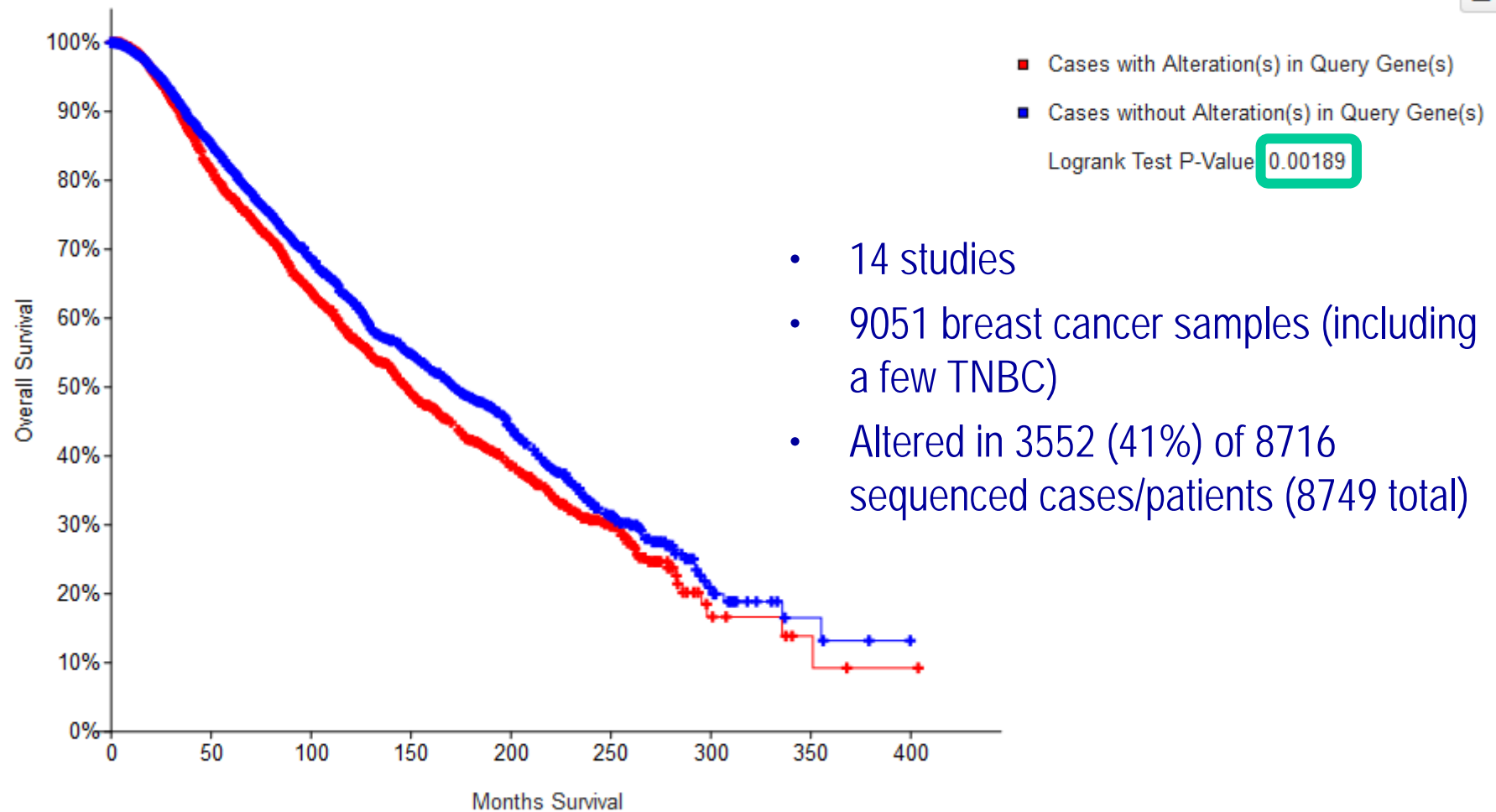
30 Linkers



Weak ties theory

Testing Set (TCGA)

Overall Survival Kaplan-Meier Estimate

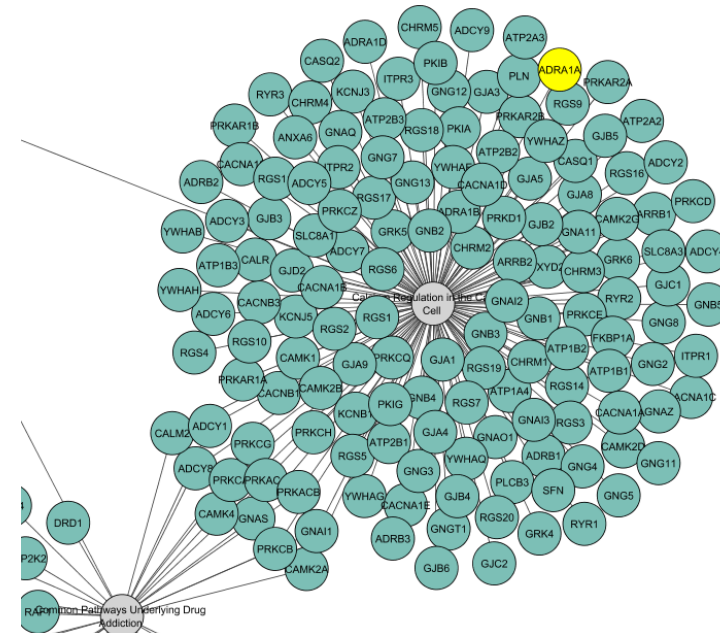


- 14 studies
- 9051 breast cancer samples (including a few TNBC)
- Altered in 3552 (41%) of 8716 sequenced cases/patients (8749 total)

Descending Degree of Nodes

CTL.Type	Degree	CTL.GeneID	name	CTL.proteinName
gene	261	ENSG00000120907	ADRA1A	Alpha-1A adrenergic receptor
gene	242	ENSG00000133019	CHRM3	Muscarinic acetylcholine receptor M3
gene	239	ENSG00000168539	CHRM1	Muscarinic acetylcholine receptor M1
gene	230	ENSG00000149295	DRD2	D(2) dopamine receptor
gene	226	ENSG00000072062	PRKACA	cAMP-dependent protein kinase catalytic subunit alpha
gene	224	ENSG00000169252	ADRB2	Beta-2 adrenergic receptor
gene	219	ENSG00000181072	CHRM2	Muscarinic acetylcholine receptor M2
gene	217	ENSG00000043591	ADRB1	Beta-1 adrenergic receptor
gene	215	ENSG00000100030	MAPK1	Mitogen-activated protein kinase 1
gene	214	ENSG00000170214	ADRA1B	Alpha-1B adrenergic receptor
gene	213	ENSG00000087586	AURKA	Aurora kinase A
gene	213	ENSG00000137193	PIM1	Serine/threonine-protein kinase pim-1
gene	211	ENSG00000184845	DRD1	D(1A) dopamine receptor
gene	209	ENSG00000077150	NFKB2	
gene	209	ENSG00000109320	NFKB1	
gene	209	ENSG00000082701	GSK3B	
gene	206	ENSG00000171873	ADRA1D	
gene	205	ENSG00000165092	ALDH1A1	
gene	205	ENSG00000088832	FKBP1A	
gene	202	ENSG00000205268	PDE7A	

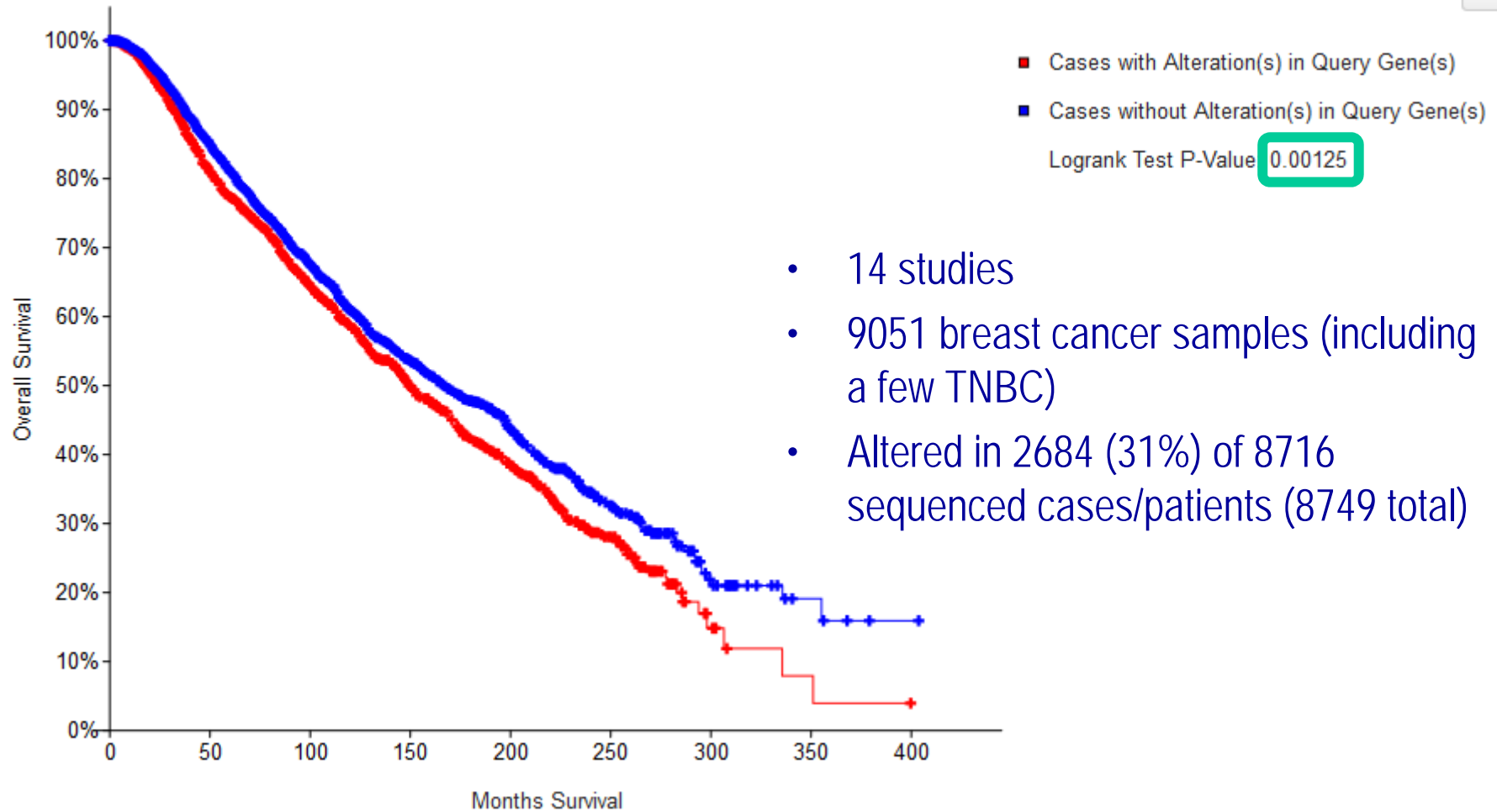
Calcium Regulation in the Cardiac Cell



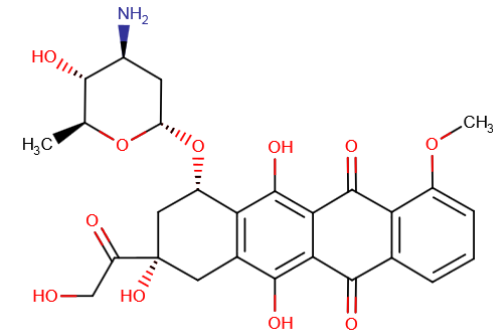
Centrality / Hub theory

Testing Set (TCGA)

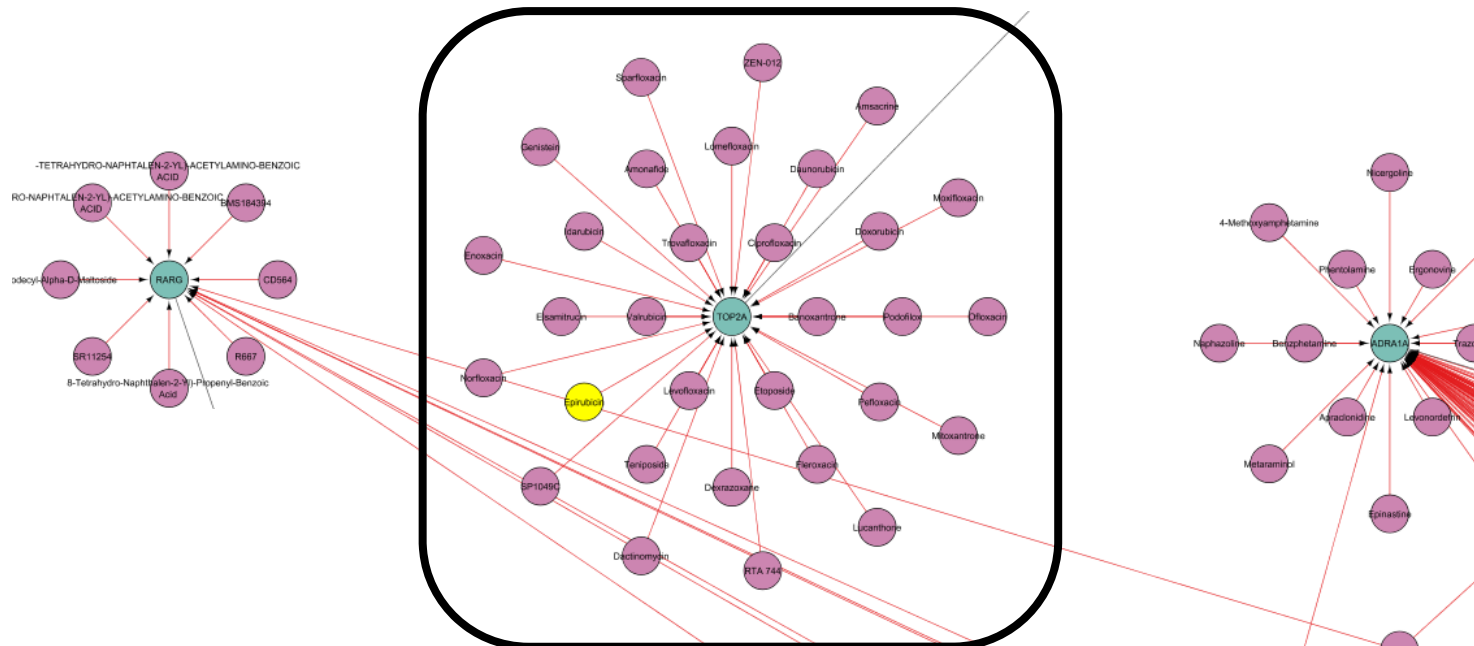
Overall Survival Kaplan-Meier Estimate



DB00445 = Epirubicin



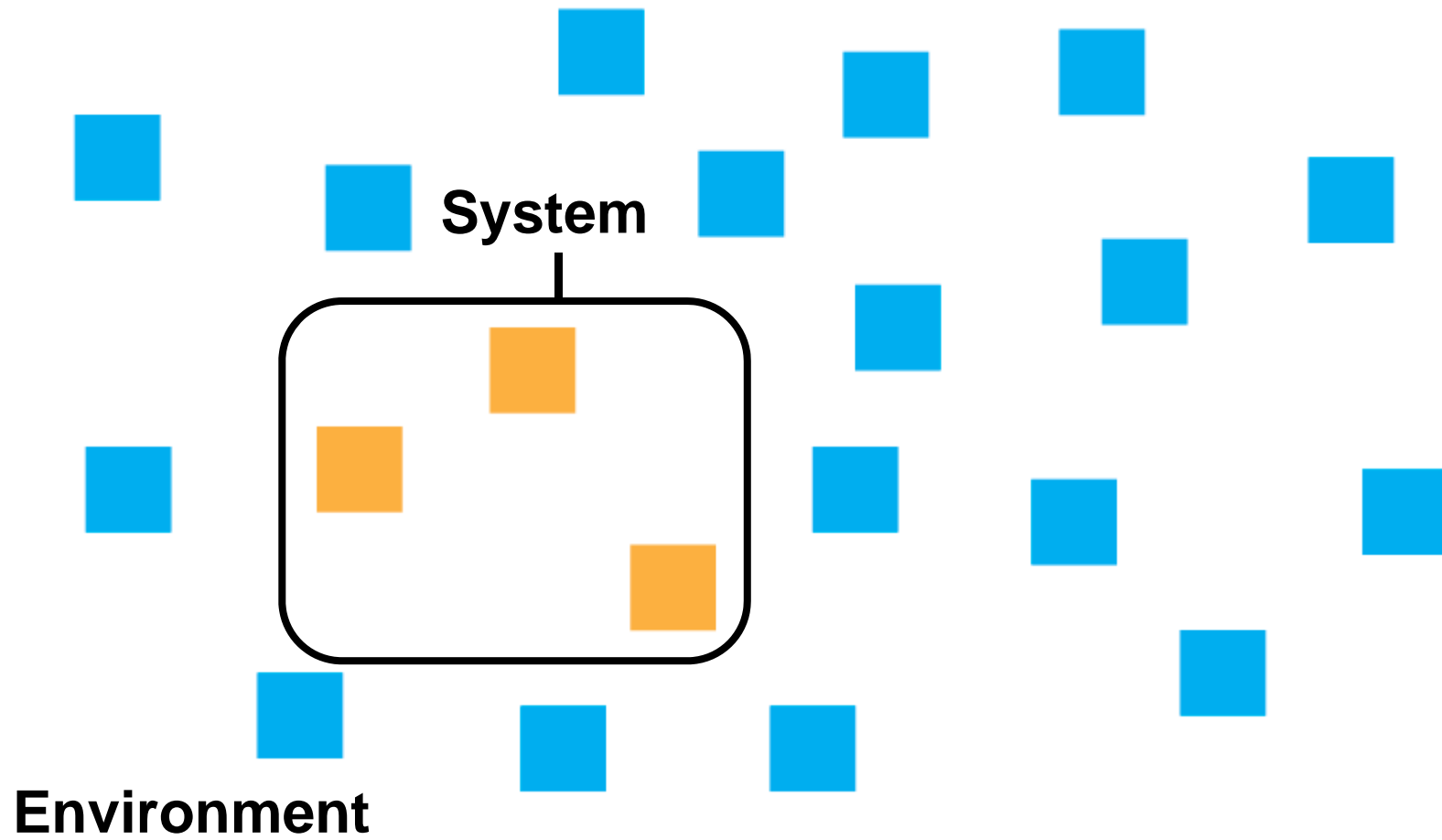
- Total Number of Approved Drugs: ~3,200
- Total Number of Approved Small Molecule Drugs: ~2,300



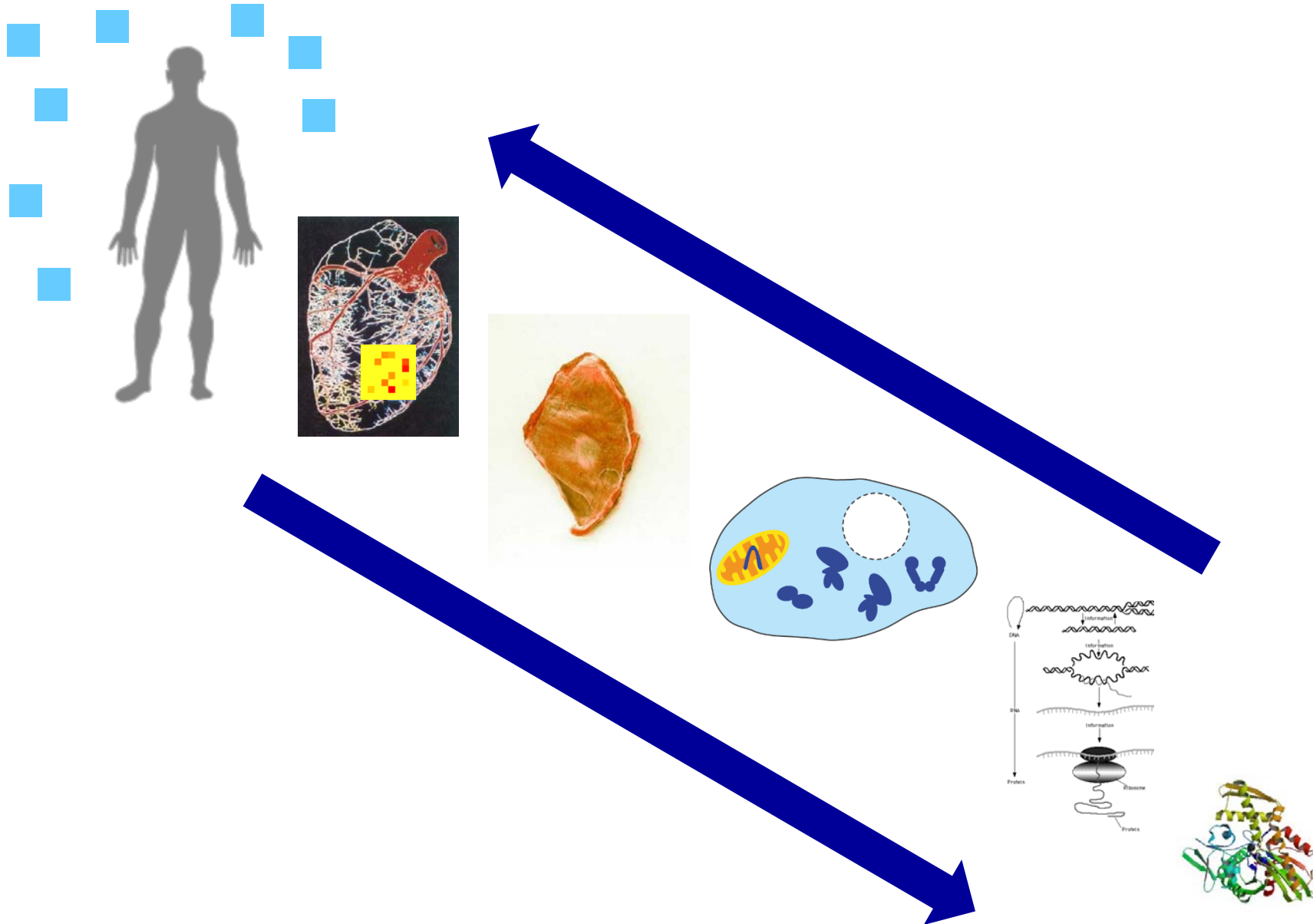
Outline

- Concepts
- An integrative approach to TNBC
- • Next steps?

System



Towards an integrative approach

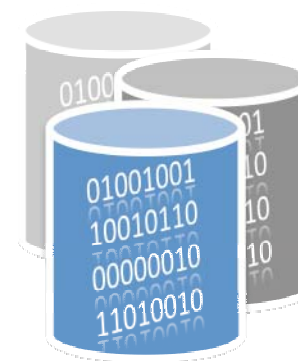


THE WORLD NEEDS BETTER **HEALTH DATA**

More integration

∫

- Confidentiality
- Managed by an organization without any potential conflicts of interest
- Qualitative & quantitative
- Baseline value
- ↗ Data quality
- ↗ Sampling « frequency »
- Integrative framework



Acknowledgements



- S. De Almeida
- A. Plagos

- F. Ghiringhelli's Team
- S. Ladoire
- R. Boidot
- L. Arnould

- C. Evelo
- M. Kurtmon

