

Inserm

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Comprehensive map of DNA repair signalling network: computational analysis of synthetic lethality in DNA repair and application for cancer treatment design

Inna Kuperstein

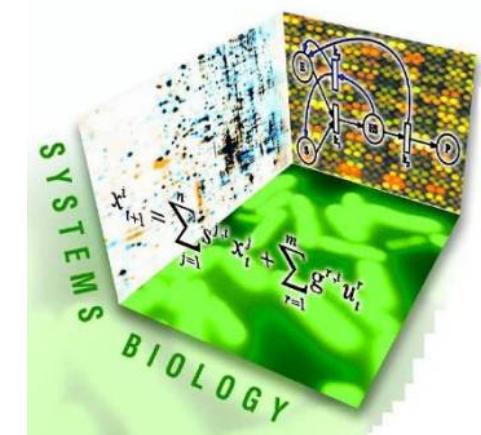
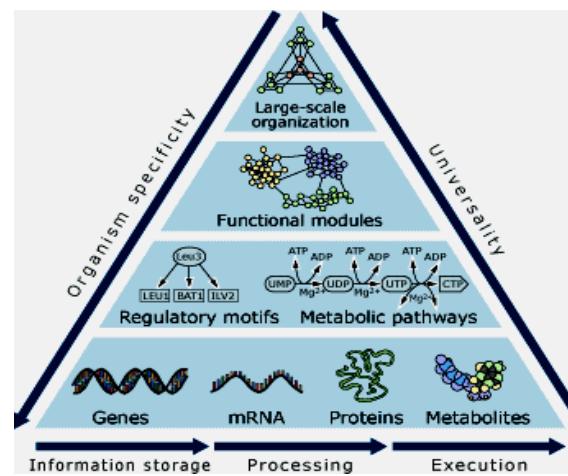
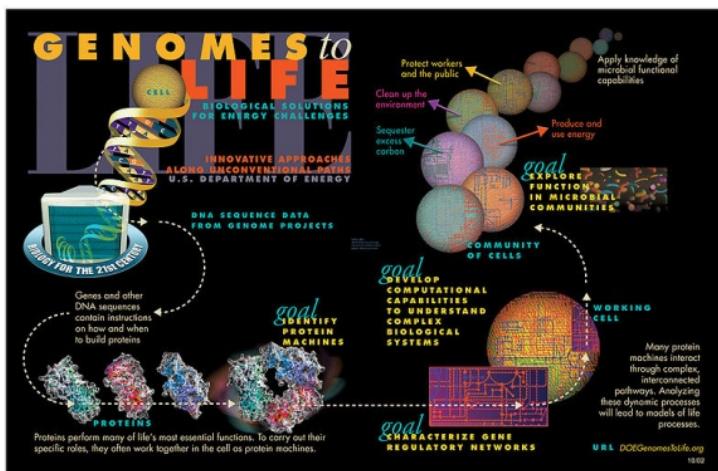
“Computational Systems Biology of Cancer”
U900 Institut Curie/INSERM/Ecole des Mines ParisTech, Paris, France

Systems biology

Inter-disciplinary field that studies complex interactions
in biological systems

Representation and analysis of biological processes
as signalling networks

Systems biology and computational modeling. (2011) Cell, Volume 144, Issue 6

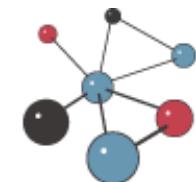


Existing resources of cell signaling pathways

- Commercial pathway databases



- Publicly available pathway databases



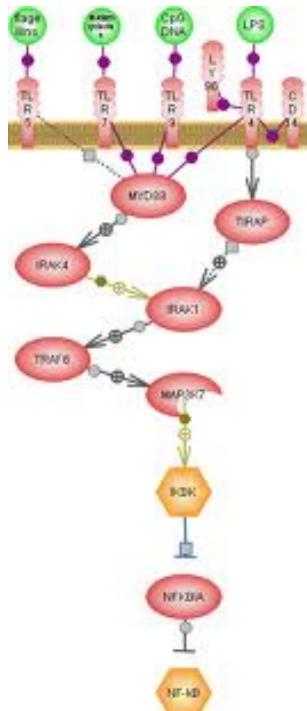
- Cancer-specific pathway databases



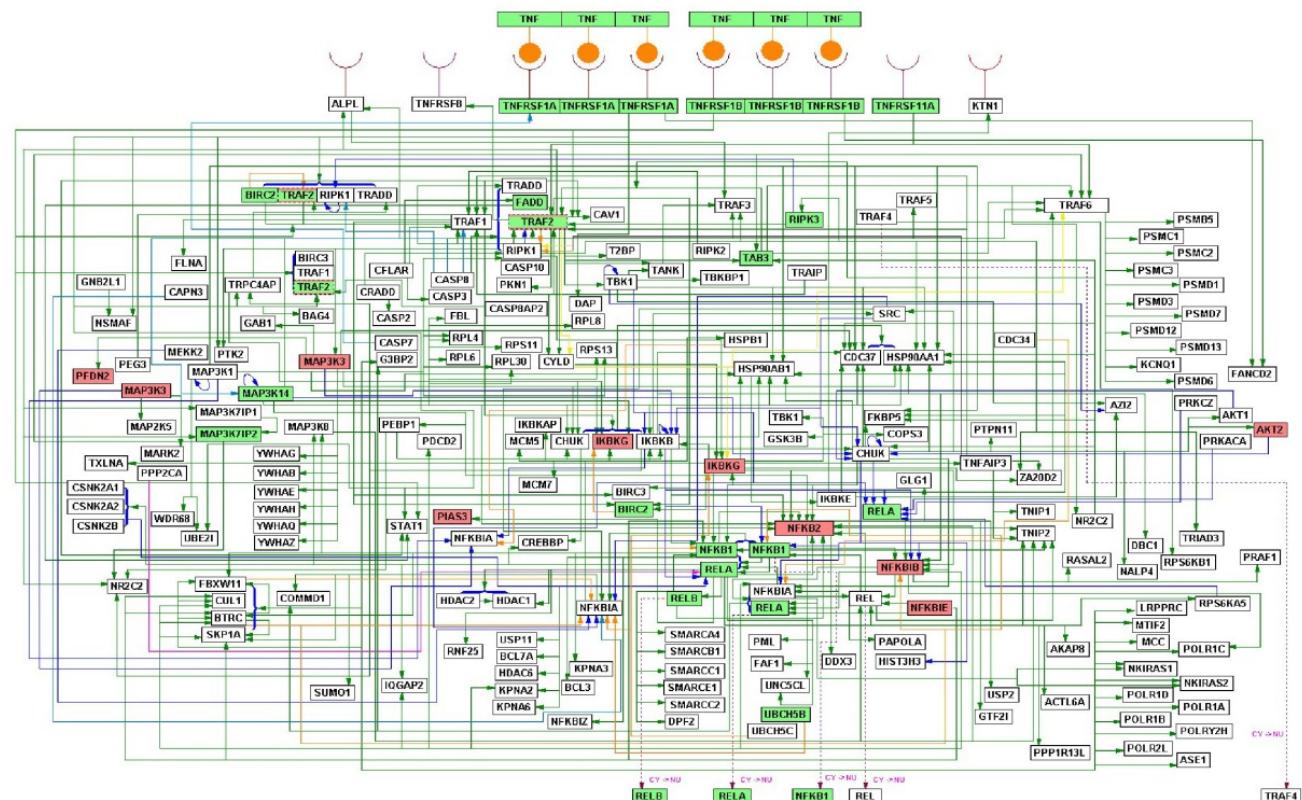
Disadvantages:

- ✗ Standard
- ✗ Visualization
- ✗ Navigation
- ✗ Maintenance

Signalling pathway

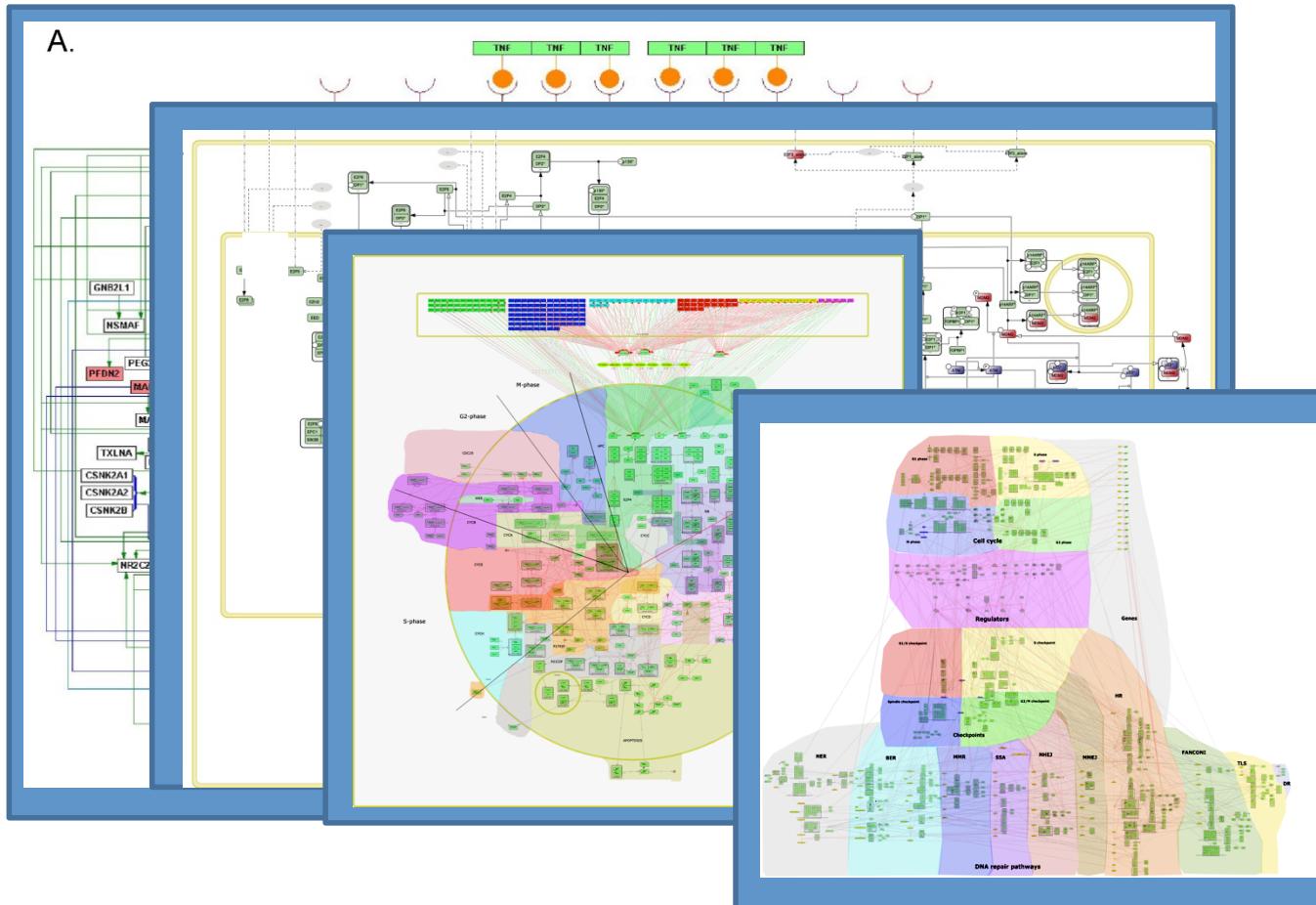


Signalling network



Construction map of signaling networks

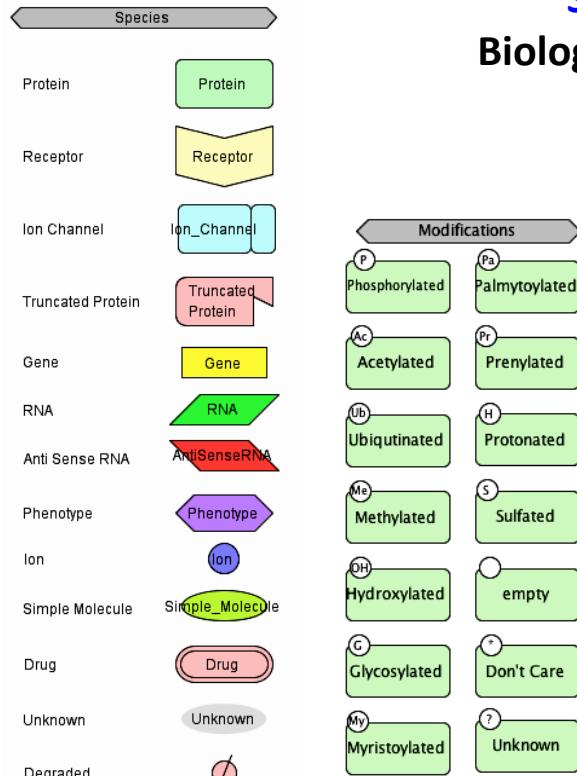
Systems biology approach for representation of signalling pathways as comprehensive networks amenable for analysis



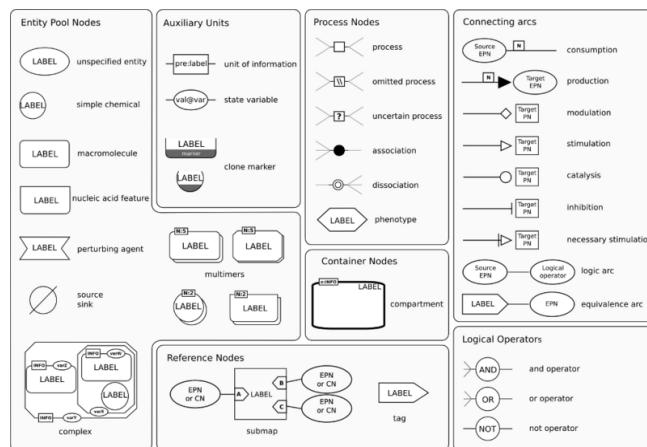
Networks types:

- Gene-gene interaction
- Protein-protein interaction
- Transcription regulation
- Metabolic
- Drug-target interaction
- Signal transduction

Standards and tools for signaling networks construction



Visual syntax
Systems Biology Graphical Notation (SBGN)
Biological molecules and interactions representation

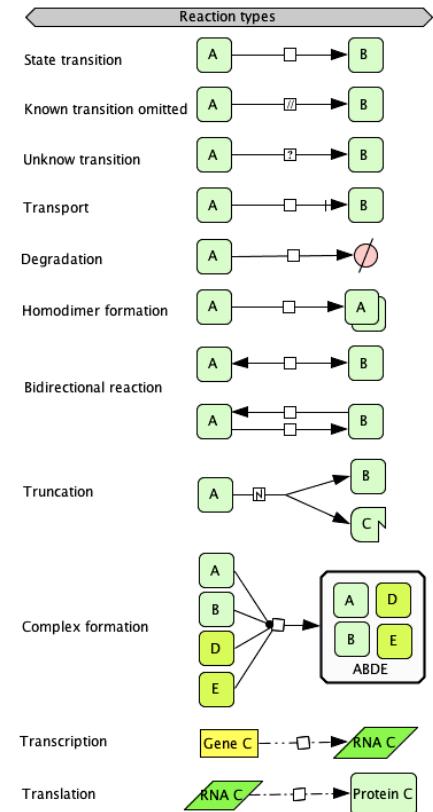


Tool: CellDesigner
Diagram editor for signalling networks representation



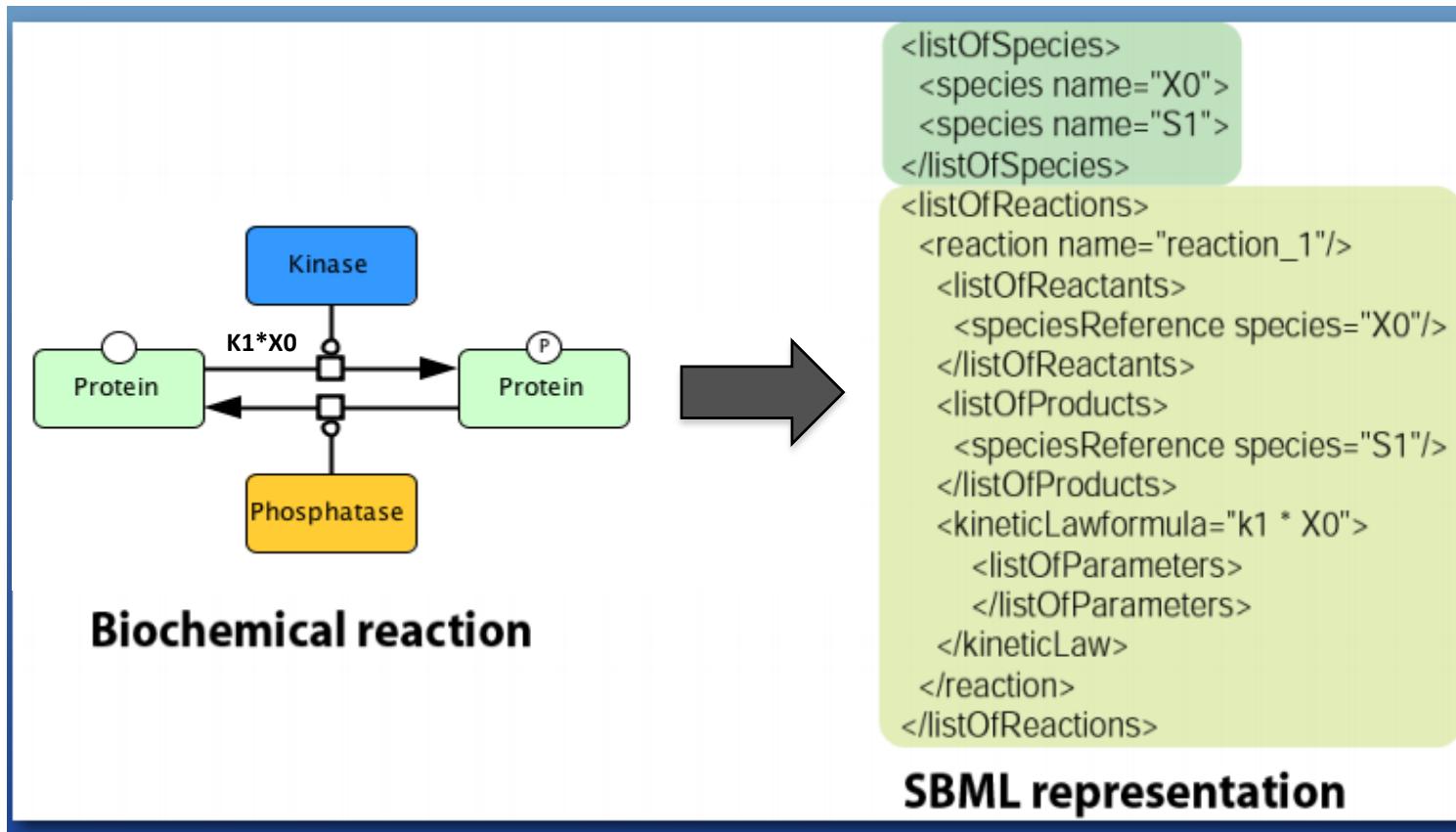
The Systems Biology Graphical Notation.
Le Novère N, et. al
Nat Biotechnol. 2009 Aug;27(8):735-41.

Biochemical modeling with Systems Biology Graphical Notation.
Jansson A, Jirstrand M.
Drug Discov Today. 2010 May;15(9-10):365-70.

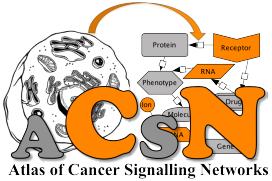


Standards and tools for signaling networks construction

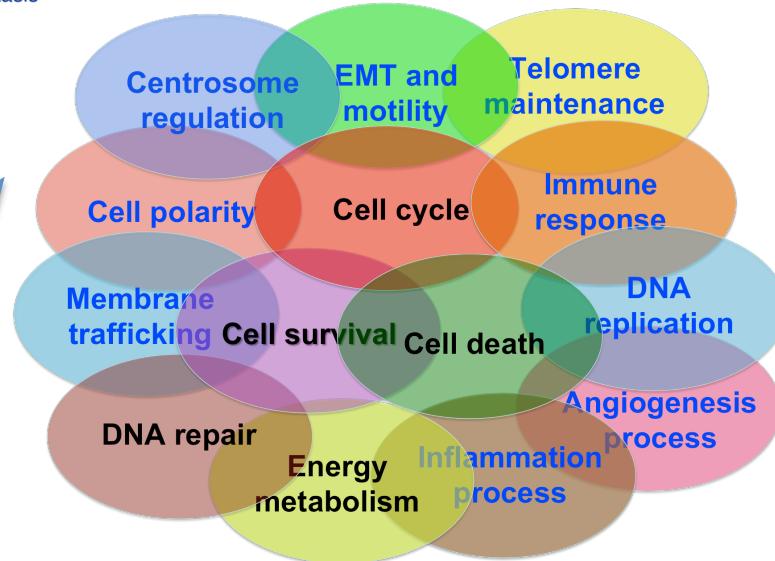
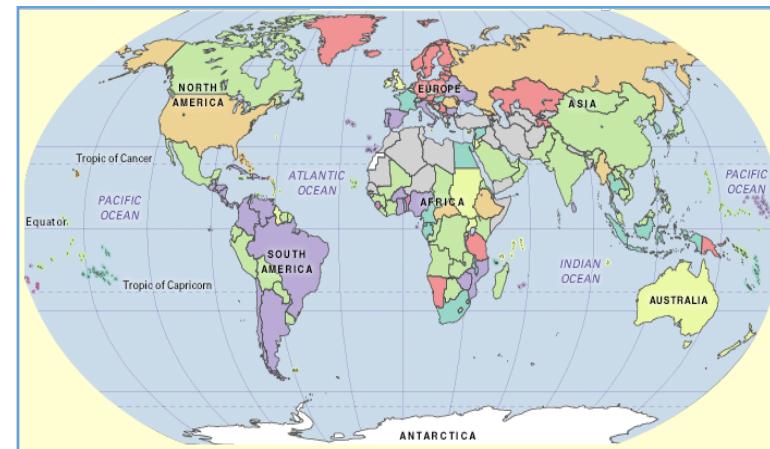
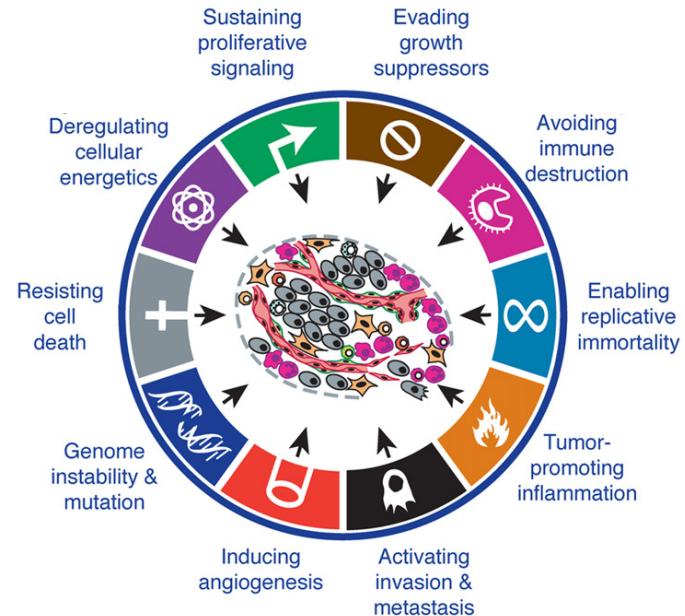
Systems Biology Markup Language (SBML)
Computational representation of biochemical processes



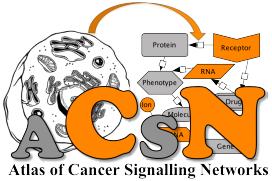
Evolving a lingua franca and associated software infrastructure for computational systems biology: the Systems Biology Markup Language (SBML) project.
Hucka M, Finney A, Bornstein BJ, Keating SM, Shapiro BE, Matthews J, Kovitz BL, Schilstra MJ, Funahashi A, Doyle JC, Kitano H.
Syst Biol (Stevenage). 2004 Jun;1(1):41-53.



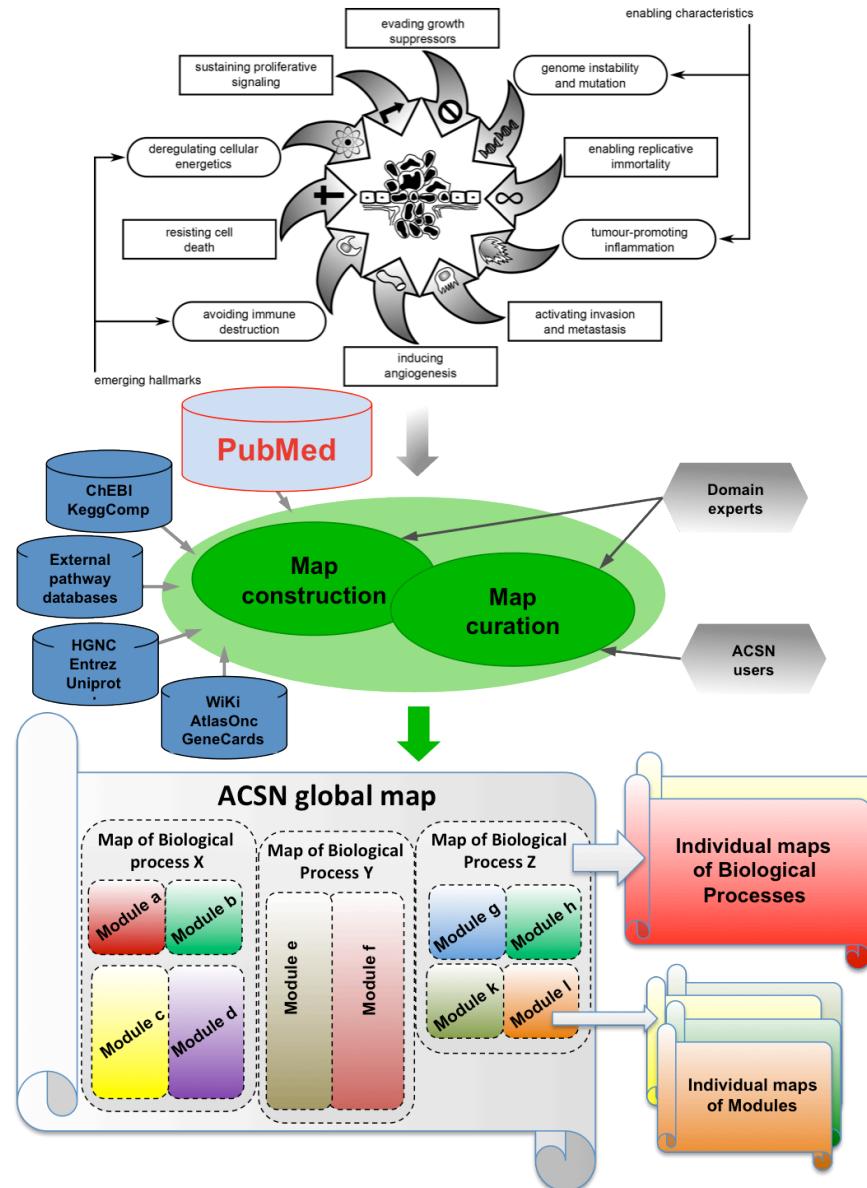
Atlas of Cancer Signalling Networks (rationale)

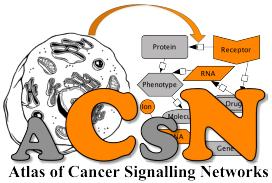


<http://acsn.curie.fr>
acsn@curie.fr



Atlas of Cancer Signalling Networks (structure)



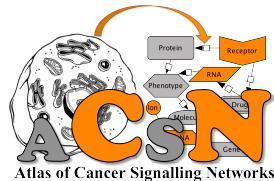


Atlas of Cancer Signalling Networks (features)

The screenshot shows the homepage of the **institutCurie** website, featuring the **Atlas of Cancer Signalling Networks**. The main content area displays a complex, interconnected network diagram titled "ATLAS OF CANCER SIGNALING NETWORKS". The diagram is organized into several large, overlapping colored regions representing different biological processes: "DNA REPAIR" (blue), "IMMUNE RESPONSE" (light blue), "EMT & POLARITY" (orange), "SURVIVAL" (pink), "APoptosis" (red), and "MITOCHONDRIAL METABOLISM" (yellow). Within these regions, numerous specific molecular pathways and proteins are labeled, such as "Regulators", "Checkpoints", "DNA damaging agents", "NER", "BER", "MMR", "SSA", "NHEJ", "HR", "FANCOMI", "TLS", "G1/S checkpoint", "S-phase checkpoint", "G2/M checkpoint", "Spindle checkpoint", "G1 phase", "S phase", "G2 phase", "M phase", "G2-phase", "M-phase G1-early", "G1-late", "R-point", "Tight junctions", "Adherens junctions", "Desmosomes", "Gap junctions", "Cell-matrix adhesions", "Extracellular matrix", "WNT canonical", "MapK", "PI3K-AKT mTOR", "Hedgehog", "TNF response", "HIF1", "AKT mTOR", "Mitochondrial metabolism", "Caspase", "MOMP regulation", "Apoptosis genes", and "WNT non-canonical". A sidebar on the right lists "Entities" (Proteins, Genes, RNAs, antisense RNAs, Simple molecules, Ions, Drugs, Phenotypes, Unknown, Complexes, Reactions) and "Maps" (Apoptosis, Survival, EMT, Cell cycle, DNA repair). The bottom of the page includes a "Supported browsers" note, a "Search" bar, and a "Terms of Use" link.

Features:
Cancer-related
Manually curated
Comprehensive
Interconnected
Browsable and zoomable
Applicable for data integration

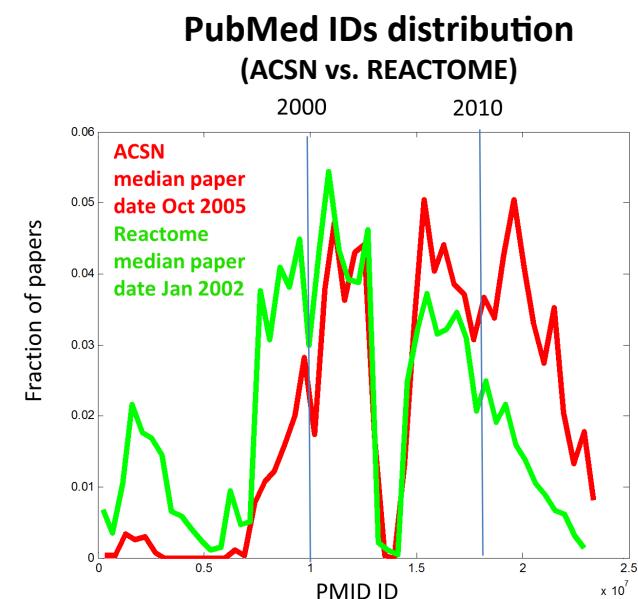
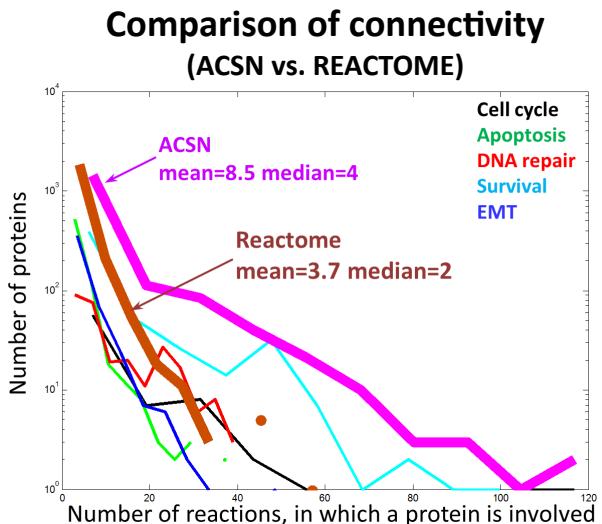
<http://acsn.curie.fr>
acsn@curie.fr



Atlas of Cancer Signalling Networks (content)

Map/Module	Chemical species	Proteins	Reactions	References	Creation date	Last update
Apoptosis map	1640	687	1166	595	2010	2013
AKT_MTOR	142	67	98	61		
CASPASES	197	110	118	109		
HIF1	59	28	30	20		
MITOCH_METABOLISM	704	411	397	212		
MOMP_REGULATION	255	120	184	143		
TNF_RESPONSE	212	113	142	62		
APOPTOSIS_GENES	213	113	142	208		
Cell cycle map	165	78	165	235	2008	2011
APOPTOSIS_ENTRY	49	16	24	46		
APC	40	16	24	11		
CDC25	21	9	14	29		
CYCLINA	18	15	8	23		
CYCLINB	31	16	31	37		
CYCLINC	5	7	2	2		
CYCLIND	32	12	20	18		
CYCLINE	31	24	14	19		
CYCLINH	15	12	7	12		
E2F1	34	15	20	27		
E2F4	32	16	17	21		
E2F5	28	18	14	5		
INK4A	10	5	5	11		
P21CIP	31	24	15	23		
P27KIP	30	23	15	21		
RB	23	18	10	19		
WEE	10	6	4	11		
Cell survival map	1926	554	1304	846	2011	2013
WNT_NON_CANONICAL	442	179	285	179		
WNT_CANONICAL	201	336	492	221		
HEGEGHOG	351	97	245	251		
PI3K_AKT_MTOR	393	128	262	203		
MAPK	248	100	176	64		
DNA repair map	709	377	505	593	2010	2013
CHECKPOINTS	N.a.	N.a.	N.a.	N.a.		
G1_S_CHECKPOINT	66	38	34	141		
S_PHASE_CHECKPOINT	83	43	45	122		
G2_M_CHECKPOINT	98	47	61	176		
S_G2_M_CHECKPOINT	39	38	38	77		
CEL_CC_PHASE	N.a.	N.a.	N.a.	N.a.		
G1_CC_PHASE	77	40	46	122		
S_CC_PHASE	114	80	47	140		
G2_CC_PHASE	16	15	26	46		
M_CC_PHASE	65	40	35	75		
DNA_REPAIR_PATHWAYS	N.a.	N.a.	N.a.	N.a.		
BLM	111	57	57	90		
NER	90	48	36	125		
MMR	57	35	30	130		
HR	121	69	49	230		
NHEJ	58	37	25	158		
MMEJ	36	23	13	115		
FANCONI	115	82	54	182		
TLS	35	24	17	88		
SSA	29	19	13	75		
DR	14	3	8	44		
EMT_and_cell_motility_map	1233	571	1078	522	2012	2013
EMT_REGULATORS	296	82	199	172		
ECM	250	98	130	146		
CELL_MATRIX_ADHESIONS	218	88	169	59		
CYTOSKELETON_POLARITY	279	206	269	198		
CELL_CELL_ADHESIONS	341	162	324	230		
TIGHT_JUNCTIONS	N.a.	N.a.	N.a.	N.a.		
ADHERENS_JUNCTIONS	N.a.	N.a.	N.a.	N.a.		
DESmosomes	N.a.	N.a.	N.a.	N.a.		
CAD_JUNCTIONS	N.a.	N.a.	N.a.	N.a.		
ACSN global map	5905	1821	4600	2774	2013	

	Chemical species	Proteins	Reactions	References	Creation date
ACSN global map	5905	1821	4600	2774	2013



Atlas of Cancer Signalling Networks (ACSN): a highly curated pathway database and a discussion forum for cancer systems biology
Kuperstein I, CohenDPA, Nguyen HA, Bonnet E, Viara E, Grieco L, Fourquet S, Calzone L, Barillot E and Zinovyev A (submitted)



A web tool for navigation, curation and maintenance of signaling networks

NaviCell = Google map + Semantic zoom + Blog

Google map

phase

G1-early

G2-phase

APC

E2F6

R-point

INK4

CDC25

WEE

CYCB

CYCA

CYCE

CYCH

P27KIP

P21CIP

apo

S-phase

semantic zoom

Protein p54
ATR

Identifiers
Ataxia telangiectasia and Rad3 related
HUGO-ATR, HGNC-882, ENTREZ-545,
UNIPROT-Q13535

Modules
MODULE-APOPTOSIS_ENTRY
MODULE-E2F1

References

Modification: s378
ATR
in nucleus

Entities

- Proteins
- Genes
- RNAs
- antisense RNAs
- Simple molecules
- Ions
- Drugs
- Phenotypes
- Unknown
- Complexes
- Reactions
- Modules

Complex composition:

- CDK2
- cyclin A2*
- p27Kip1*

Identifiers

Modules

References

Modifications:

Participates in complexes:

In compartment: nucleus

Participates in reactions:

As Reactant or Product:

As Catalyst:

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A2*:p27Kip1*

Complex CDK2:cyclin A2*:p27Kip1* p24_p27_p73

Complex composition:

- CDK2
- cyclin A2*
- p27Kip1*

Identifiers

Modules

References

Modifications:

Participates in complexes:

In compartment: nucleus

Participates in reactions:

As Reactant or Product:

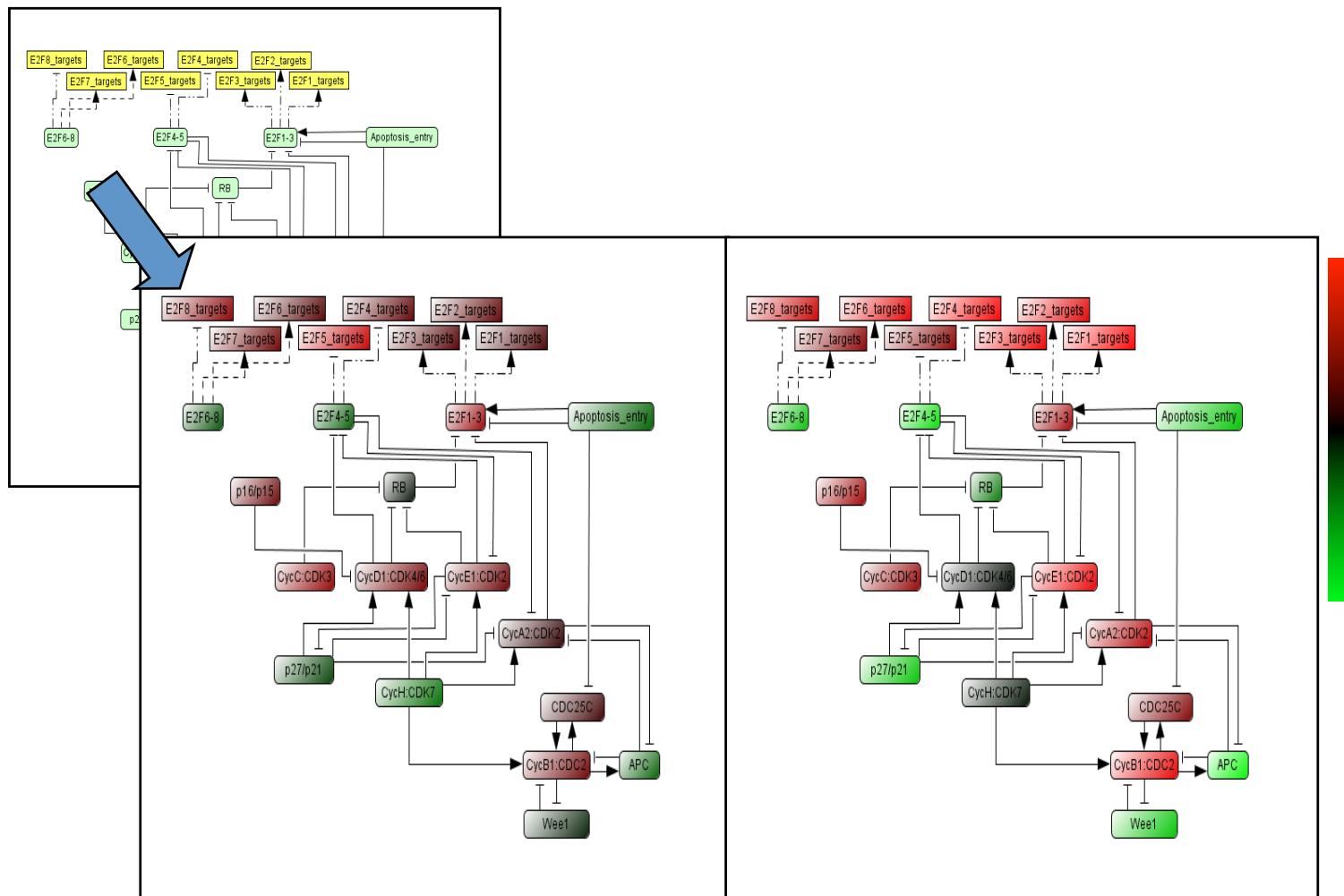
As Catalyst:

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Data visualisation and analysis in the context of signalling networks

‘Protein staining’ with transcription data of breast cancer

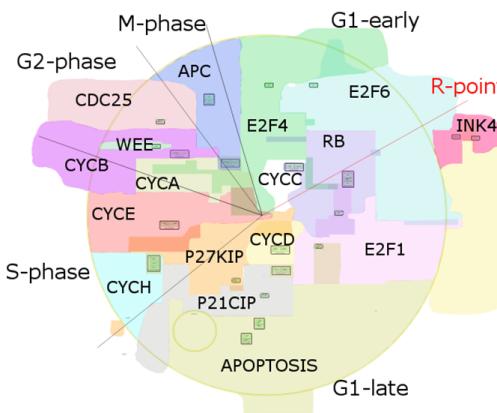


A comprehensive modular map of molecular interactions in RB/E2F pathway.
Calzone L, Gelay A, Zinovyev A, Radvanyi F, Barillot E (2008) Mol Syst Biol 4: 173.

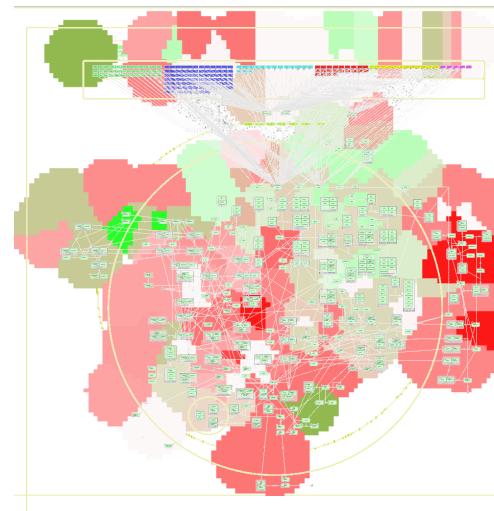
Data visualisation analysis in the context of signalling networks

'Pathway staining' with transcription data of breast cancer

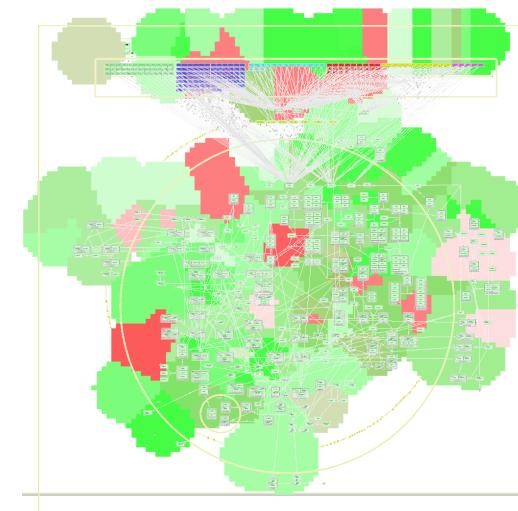
Cell cycle signalling network



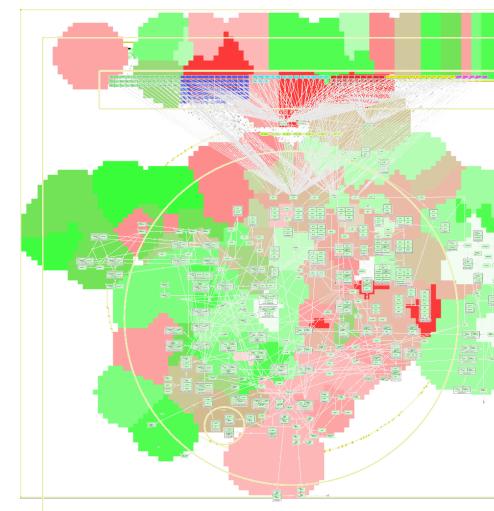
Data overlay:
Copy number
Gene expression



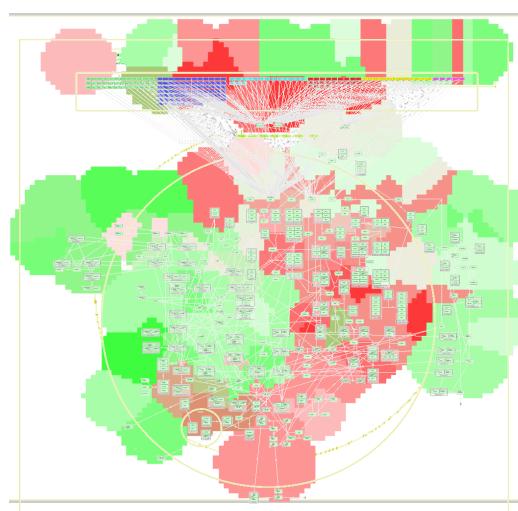
Cancer status: G3, T4, invasive



Cancer status: G2, T2, invasive



Normal



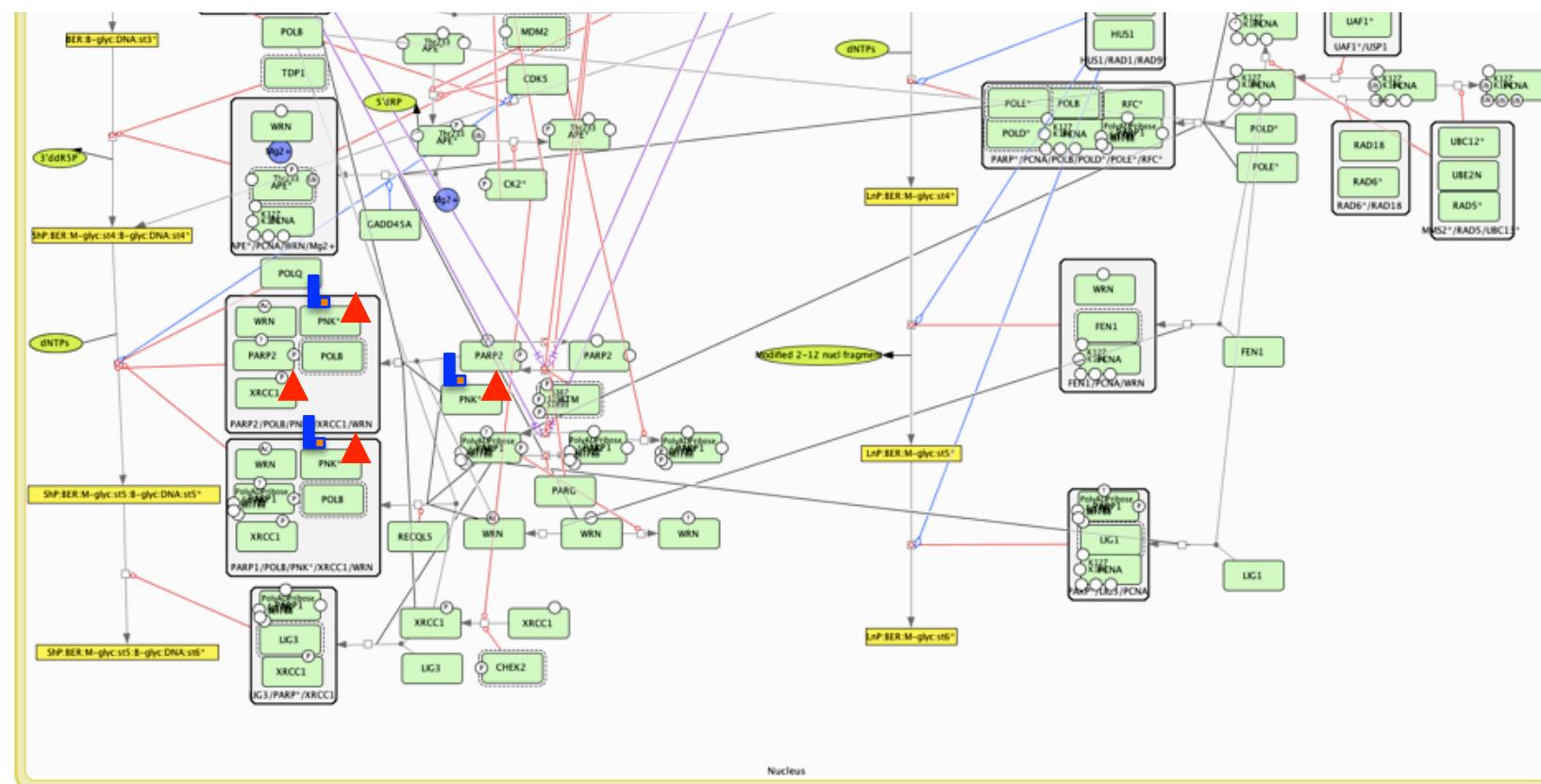
Normal

Data analysis and visualization in the context of signalling networks

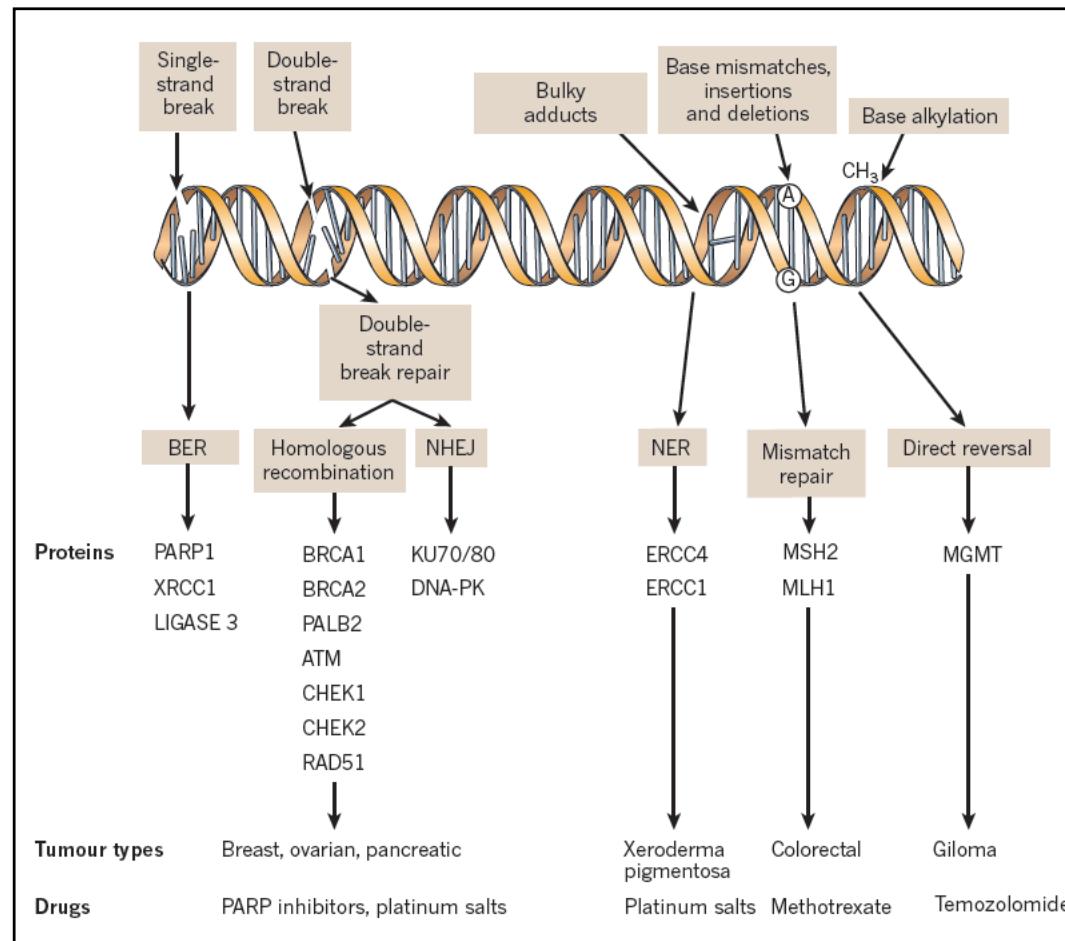
Data overlay:

Mutation status (glyph)

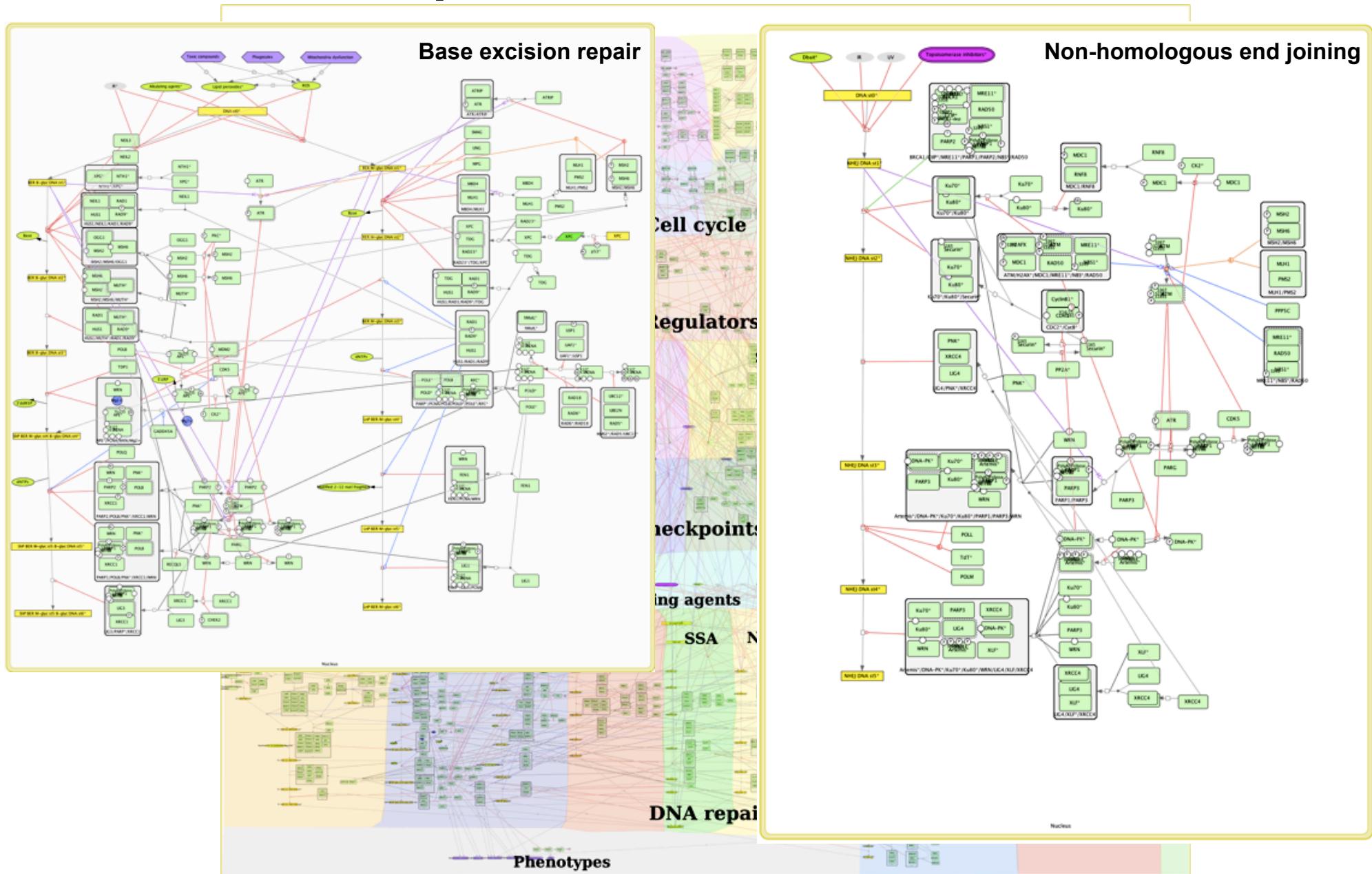
Expression data (bar plot)



DNA repair pathways

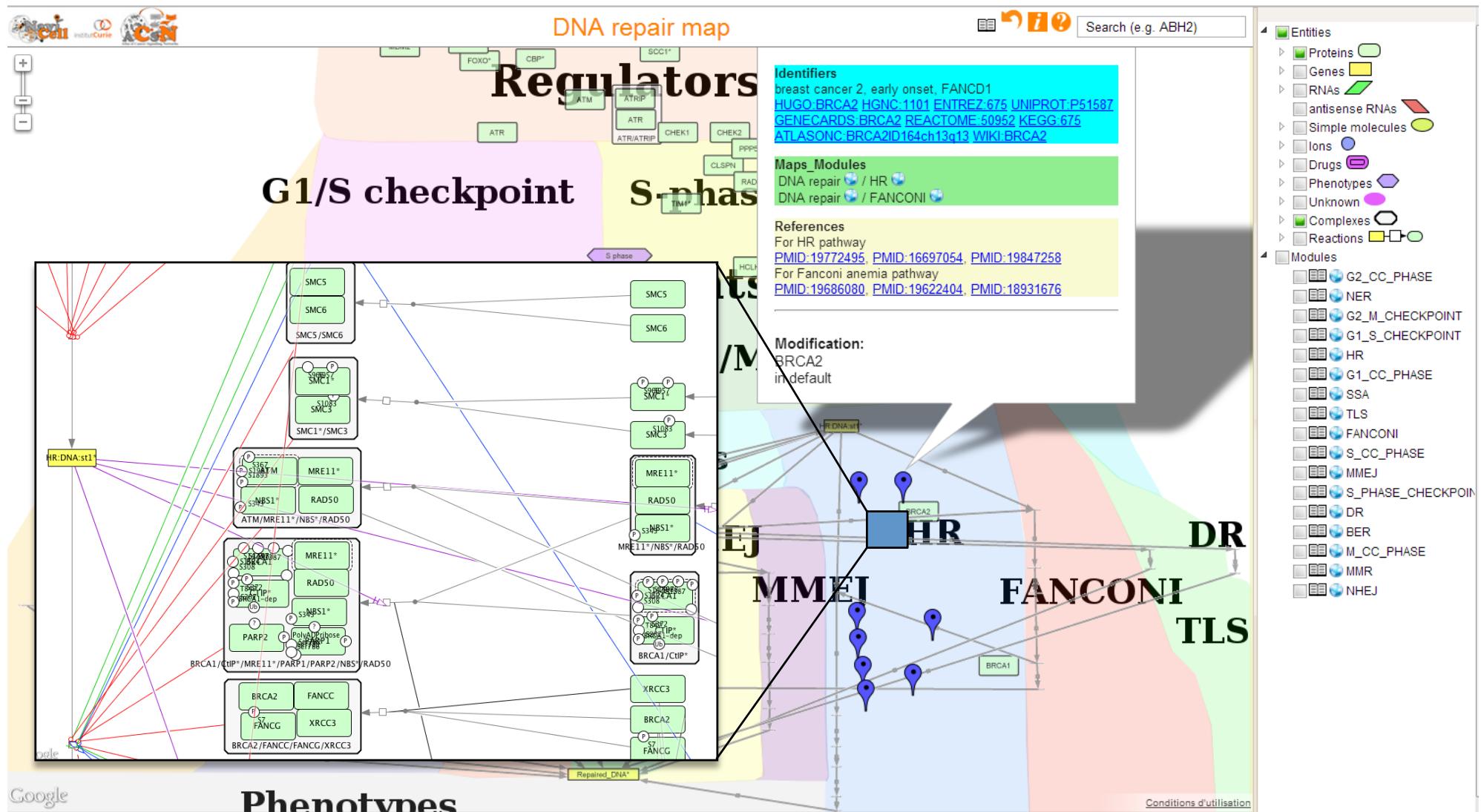


Network of DNA repair pathways: comprehensive reconstruction



Network of DNA repair pathways: comprehensive reconstruction

Part of ATLAS OF CANCER CELL SIGNALLING (<http://acsn.curie.fr>)



Synthetic genetic interactions

Unexpectedly larger or smaller effect of mutations combination on a compared to their individual effects.

Types of synthetic interactions

Negative or aggravating when the combined effect of two or more gene defects is more severe than it is expected from a simple multiplicative model (cause decrease of fitness).

Positive or alleviating interaction when the effect is less severe than expected (increase of fitness). This is observed in the situation when mutation of one gene compensates or buffers the effect of the mutation in the other gene.

Synthetic lethality

An extreme case of negative genetic interactions

<u>Gene A</u>	<u>Gene B</u>	<u>Cell fate</u>
+	+	
-	+	
+	-	
-	-	

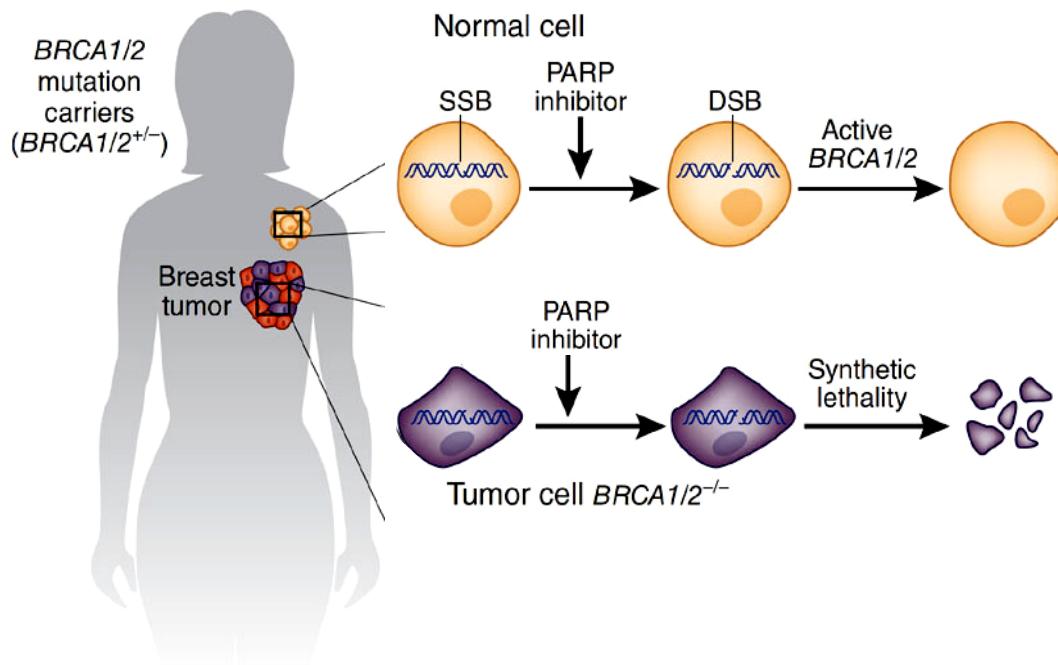
Synthetic lethality

An extreme case of aggravating genetic interaction leading to the cell death when two non-essential genes are perturbed.

Dobzhansky Genetics (1946)

Synthetic lethality paradigm in cancer treatment

BRCA/PARP synthetic lethal pair



Approach

Exploit genetic ablation in cancer cell and chemical inhibition to achieve synthetic lethality in tumors.

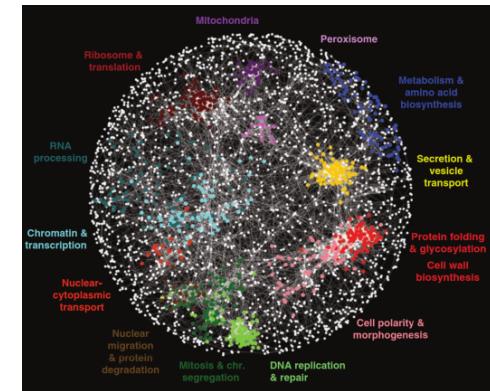
Application example

Tumor cells deficient for the BRCA are synthetic-lethal to inhibition of PARP activity.

Hartwell et al. Science (1997)

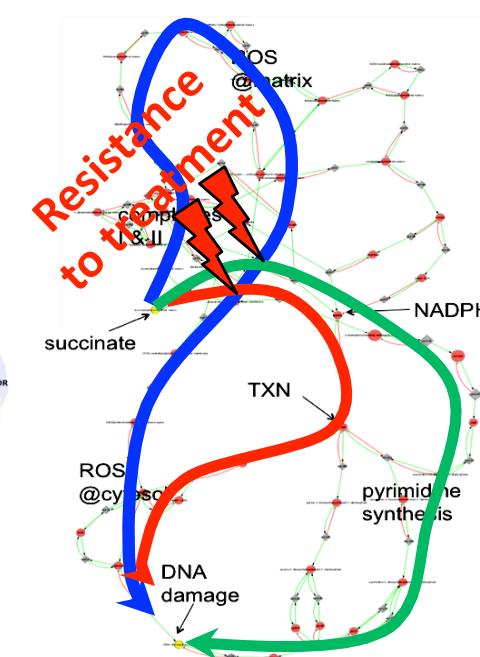
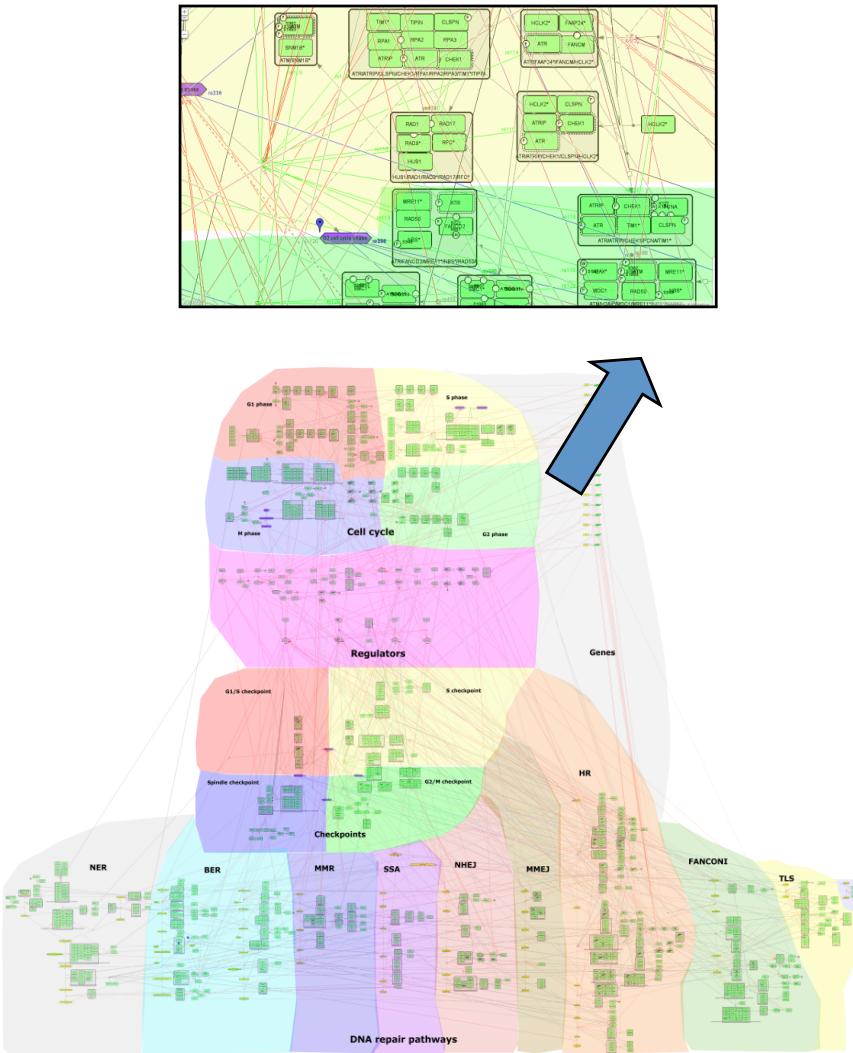
Discovering synthetic lethal pairs *screenings and modeling*

- Examples of large-scale screenings
 - in yeast (Constanzo et al, Science ,2010)
 - in C. elegans (Lehner et al, Nat Gen, 2006)
 - in human
 - MYC: Toyoshima et al, PNAS, 2012
 - TP53: Krastev et al, Nat Cell Biol, 2011; Xie et al, PLoS Gen, 2012
 - KDACs: Lin et al, Nature, 2012
- **There might be synthetic lethal cocktails (triples)!**

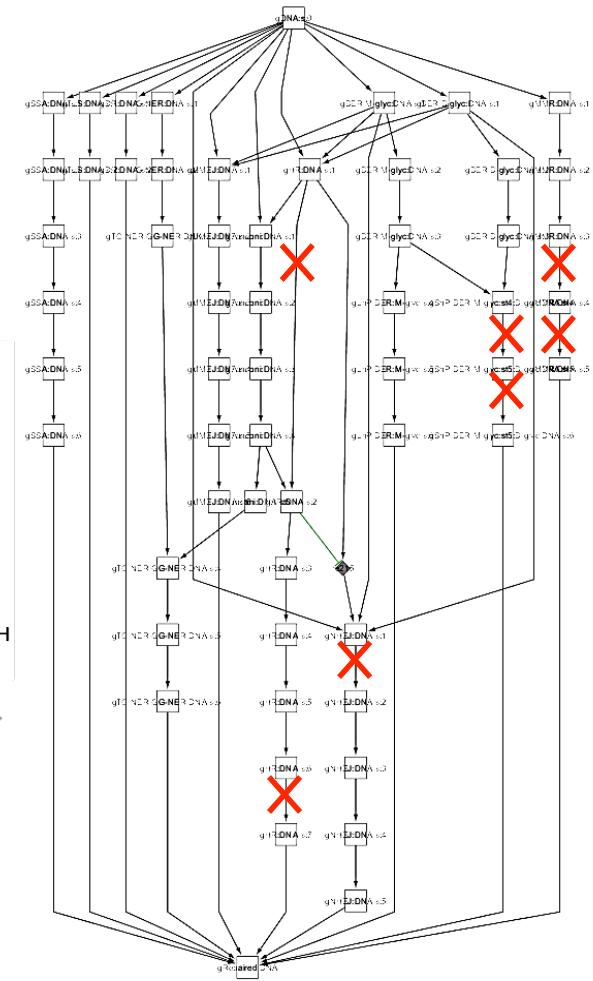


Signalling networks for intervention strategy design

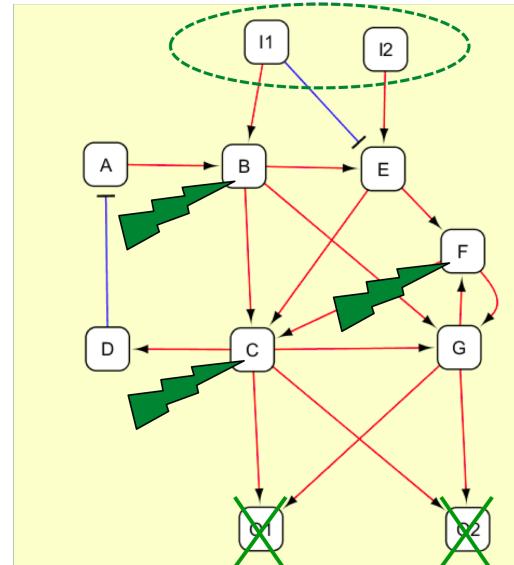
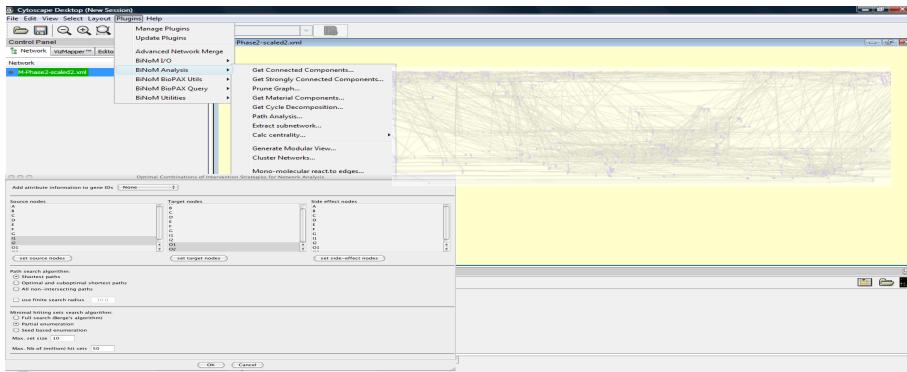
Structural analysis



PARP inhibitor action



OCSANA: an Integrative pathway analysis to reveal synthetic lethal cocktails



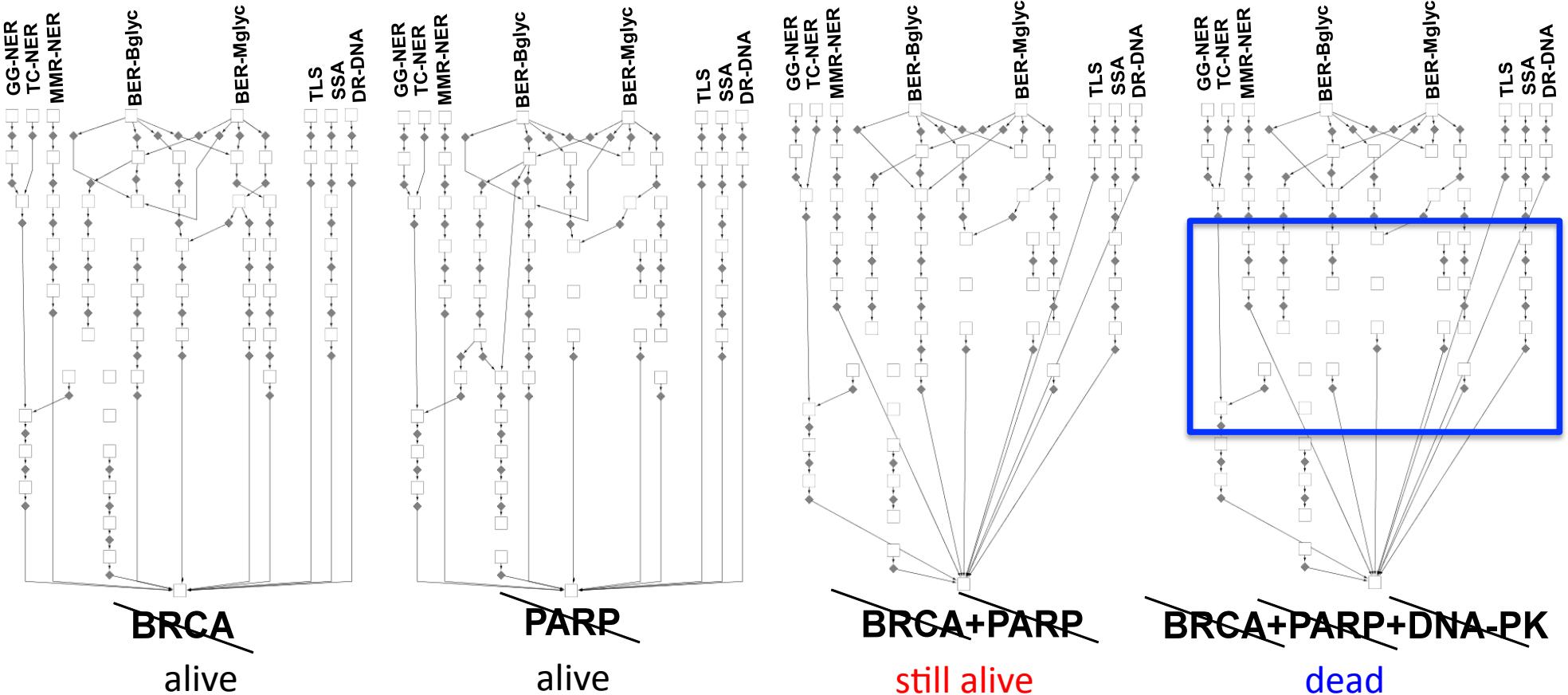
Prioritizing the list of master regulators
 Identifying points of fragility in the network
 Identifying synthetic lethal combinations

Elementary Pathways	Elementary Nodes	Computation Time	Total Number of MinHitSets	MinHitSets Size 1	MinHitSets Size 2	MinHitSets Size 3	MinHitSets Size 4	MinHitSets Size 5	Comments
2300	198	5.51	252	0	0	108	42	102	
2214	131	7.43	74	9	5	12	7	42	
15	71	7.40	38336	6	0	160	4848	33322	
198	126	2.04	74	8	5	12	7	42	
529	171	2.40	112	0	6	30	32	44	Antia apoptotic
1476	121	1.21	74	1	5	12	7	42	Downregulated by BRCA1 and p53. Upregulated by USF-1 (which is upregulated by BRCA2)
246	119	0.24	86	21	0	12	7	42	Study of Combinations

Prediction of synthetic lethal cocktails from structural analysis of DNA repair network

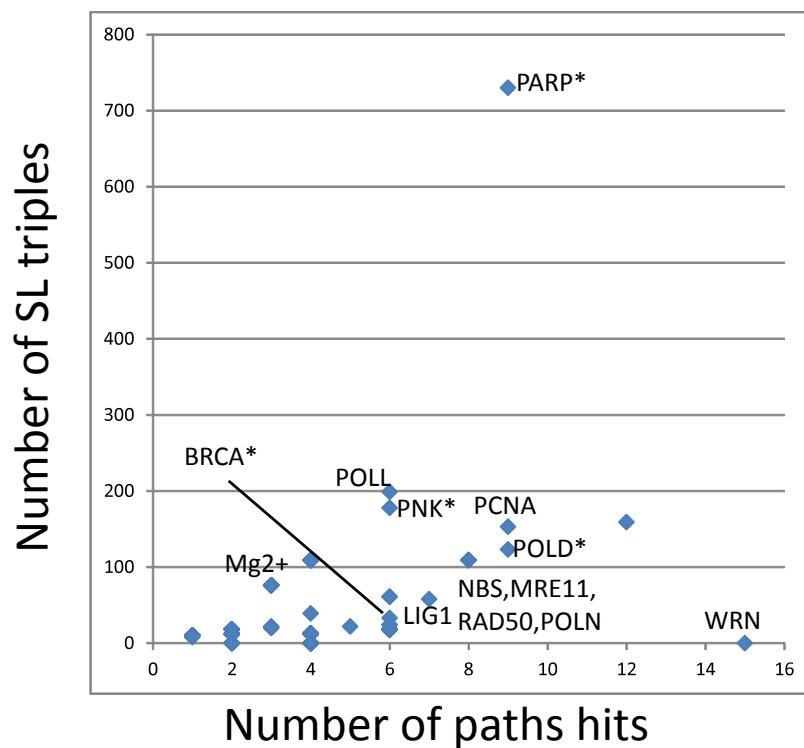
DNA state transition graph

20 paths from
different types of DNA
damage to the state
of repaired DNA



Systematic prediction of synthetic lethality (SL): minimal hit sets

Synthetic lethality by hitting triplets: **1452 sets of size 3**



Known synthetic lethality
(e.g., BRCA1+PARP,
MRE11A+PRKDC)
have high statistical score
compared to a random
pairs of genes from DNA repair

Studying mechanisms of synthetic lethality: reversibility of signalling pathways

Linear pathway



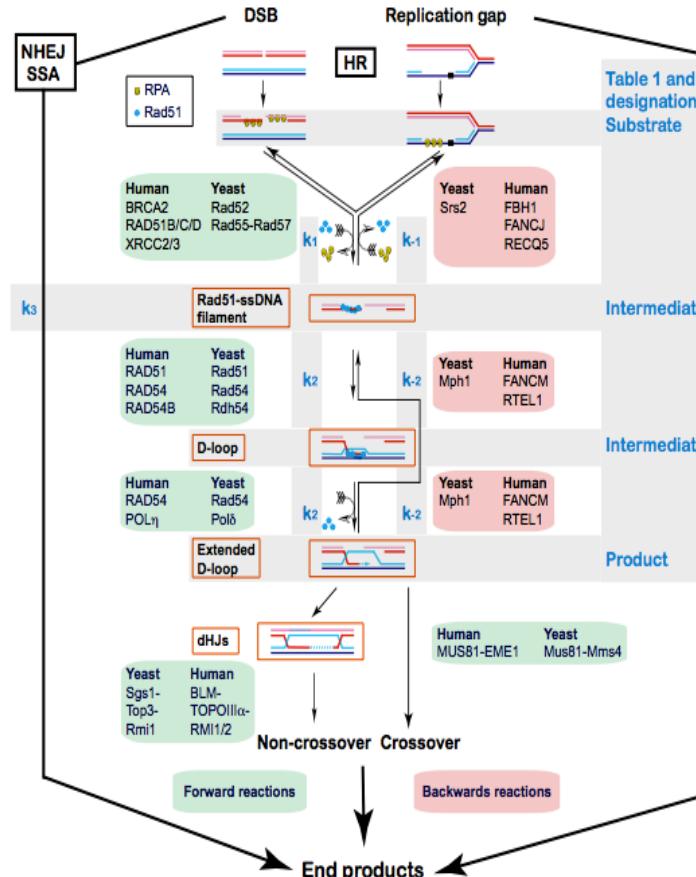
Pathway with reversible steps



Reversible steps in homologous recombination pathway

Role in:

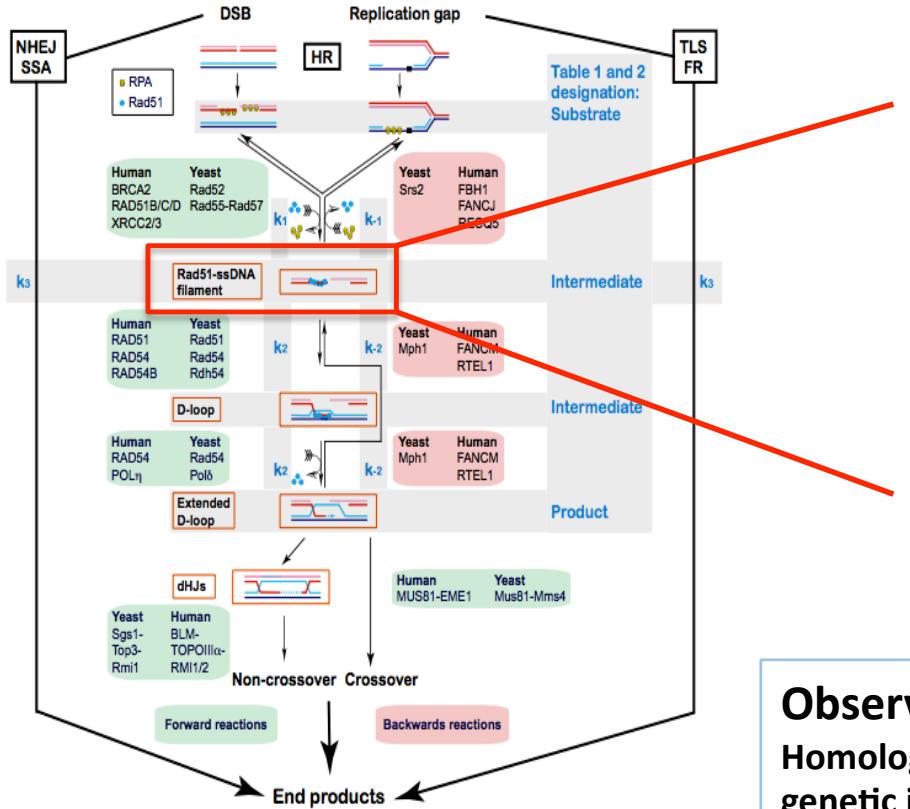
Maintaining genome integrity
Double strand breaks repair
Interstrand crosslinks repair
Single-stranded DNA gaps filling



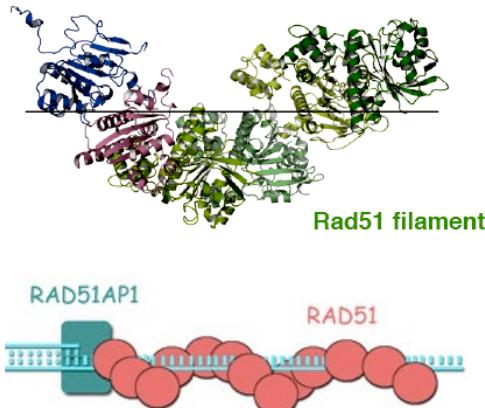
Fabre et al. PNAS (2002)

Schwartz and Heyer Chromosoma (2011)

Homologous recombination as a model pathway for synthetic lethality modelling



Toxic intermediate



Observation 1

Homologous recombination pathway is enriched with negative genetic interactions within single pathways

Observation 2

Homologous recombination pathway contains reversible steps

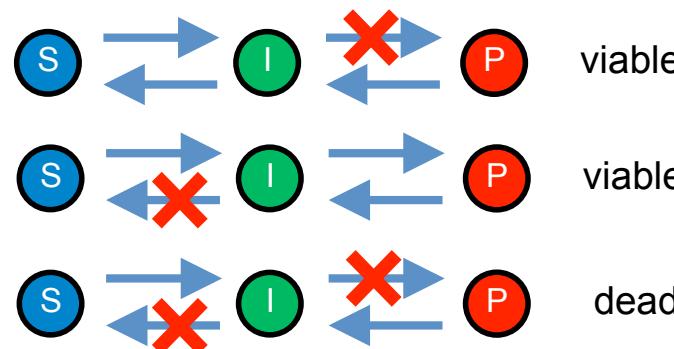
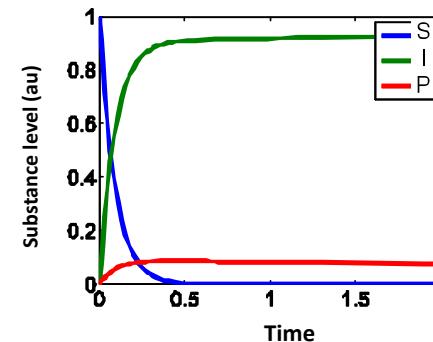
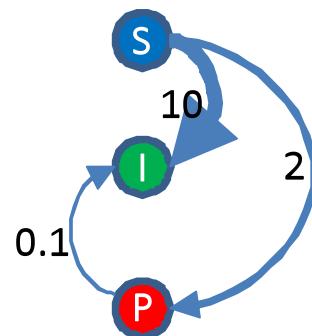
Observation 3

RAD51 – ssDNA intermediate filament is toxic to cells

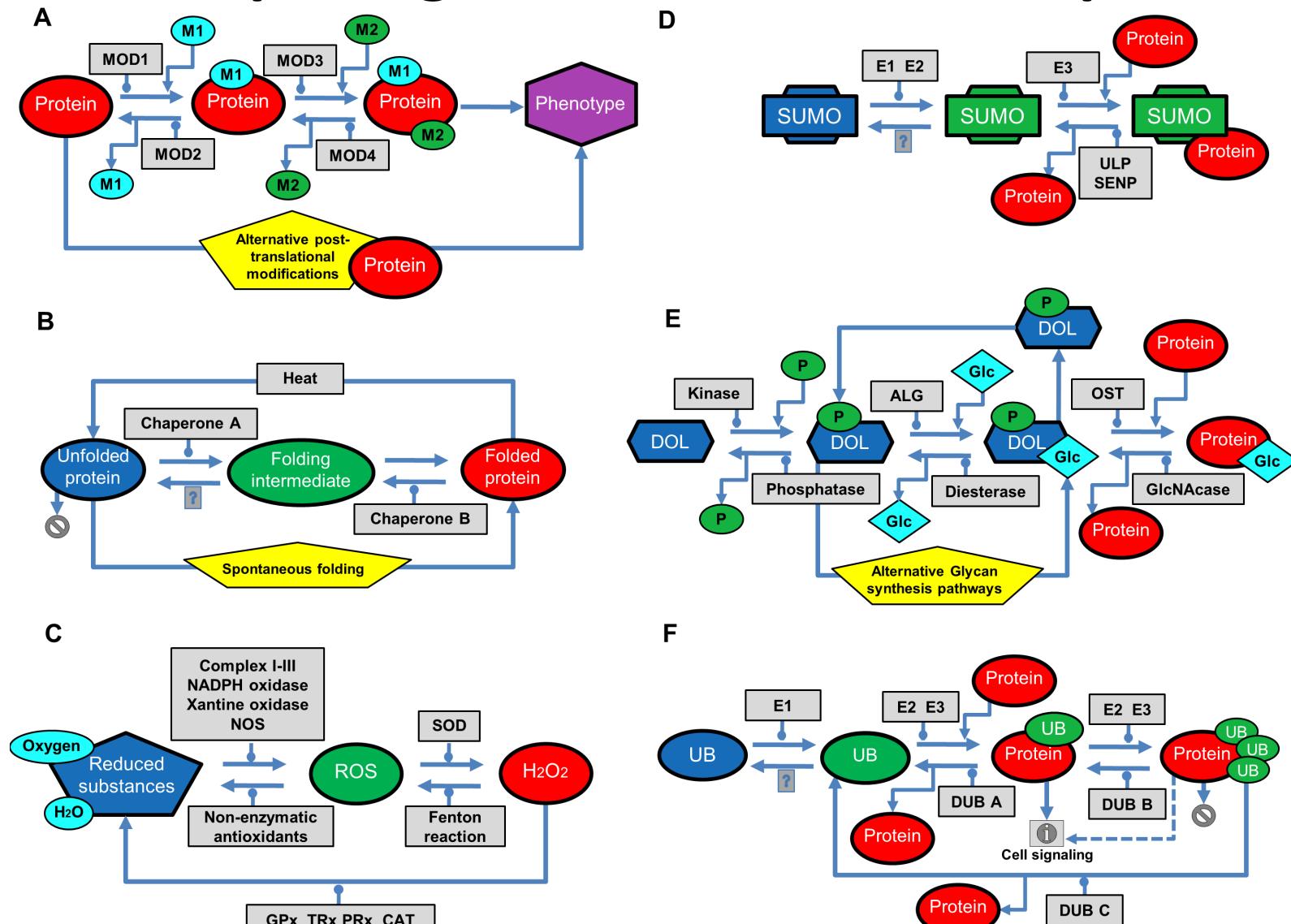
Synthetic lethality within single reversible pathway

Kinetic trap: cell death due to toxic intermediates accumulation

Srs2-Rad54 double mutant in yeast leading to accumulation of un-resolved RAD51 – ssDNA toxic intermediates



New mechanism of synthetic lethality: kinetic trap is a generic feature of cell pathways



Synthetic Lethality between Gene Defects Affecting a Single Non-essential Molecular Pathway with Reversible Steps.

Zinovyev A, Kuperstein I, Barillot E and Heyer WD (2013) PLoS Comput Biol.

PARP inhibitors mechanism of action

Between pathways SL or within reversible pathway SL?

Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors.

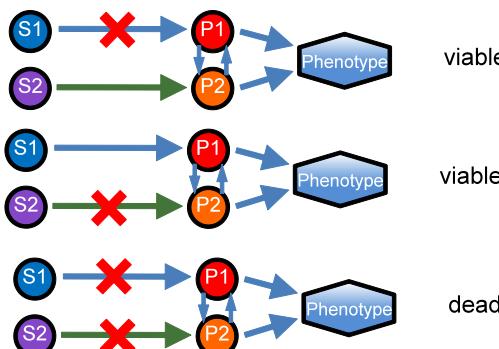
Murai J, Huang SY, Das BB, Renaud A, Zhang Y, Doroshov JH, Ji J, Takeda S, Pommier Y.

PARP inhibitors trap the PARP1 and PARP2 enzymes at damaged DNA. Trapped PARP-DNA complexes were more cytotoxic than unrepaired SSBs caused by PARP inactivation, arguing that PARP inhibitors act in part as poisons that trap PARP enzyme on DNA.

postreplication repair, the Fanconi anaemia pathway, polymerase η , and FEN1 are critical for repairing trapped PARP-DNA complexes. In summary, our study provides a new mechanistic foundation for the rational application of PARP inhibitors in cancer therapy.

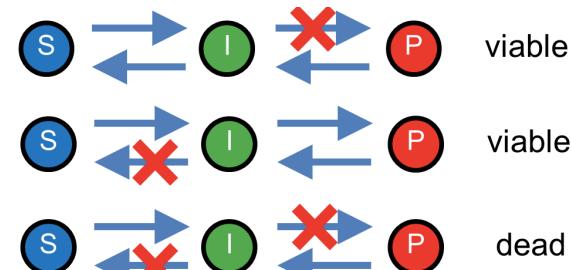
Classical mechanism

Between pathways synthetic lethality
Lack of DNA repair and accumulation of damaged DNA



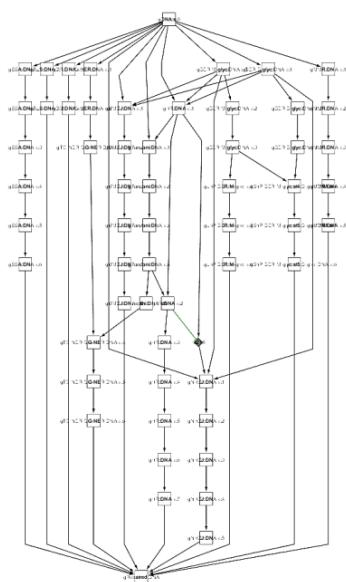
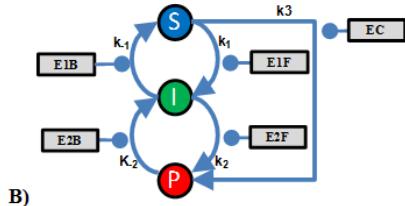
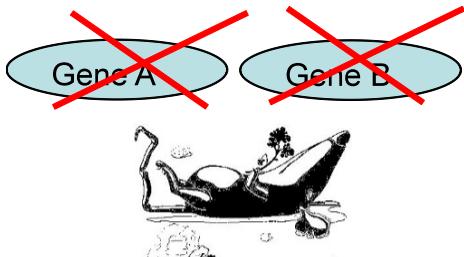
New mechanism

Within pathway synthetic lethality
Kinetic trap into toxic intermediate accumulation



Conclusions

Synthetic lethality: rationalized paradigm in finding anti-cancer drugs and predicting response



- Detailed representation of DNA repair mechanisms allows integrated data analysis (*data overlay and visualization, network structure analysis*)
- Structural analysis of signalling networks can help in predicting combinations of genetic interactions (*Synthetic Lethal triplets*)
- Mathematical modeling can help in discovery of new mechanisms of synthetic lethality (*kinetic trap*)

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