The discovery of innovative therapeutic approaches:

Under the street light is not necessarily the right place to search.

Dr. François Iris, Founder & C.S.O. BIO-MODELING SYSTEMS

Forum TERATEC 2013

Atelier A5

26th June 2013, Palaiseau, France

Contact: www.bmsystems.net email: manuel.gea@bmsystems.net

The discovery of therapeutics.

It is first and foremost a matter of integrating huge masses of information.

But there are first THREE problems & ONE paradox to resolve.

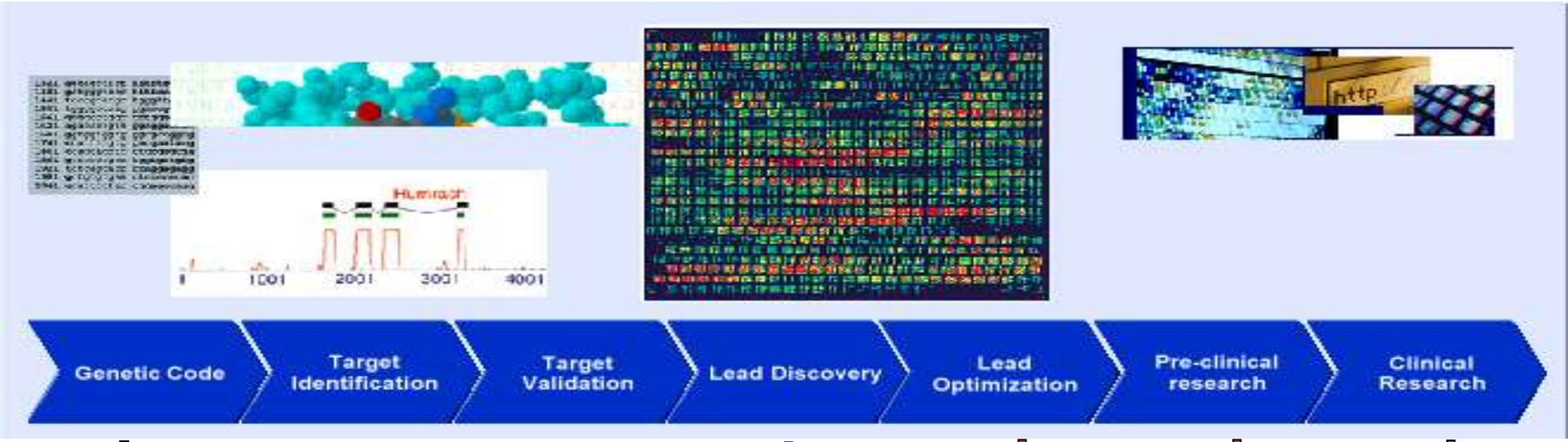
Problem 1. The nature of information

All that goes by the name « ***Information*** » is not necessarily **Useful** and/or **Utilisable!**

In the bio-medical realm, the information available is **ALWAYS**:

- **incomplete,** to an unknown extent;
- **biased,** to an unknown extent; and
- **erroneous,** to an unknown extent.

This takes particular significance in drugs development



Genetic Code → Target Identification → Target Validation → Lead Discovery → Lead Optimization → Pre-clinical research → Clinical Research

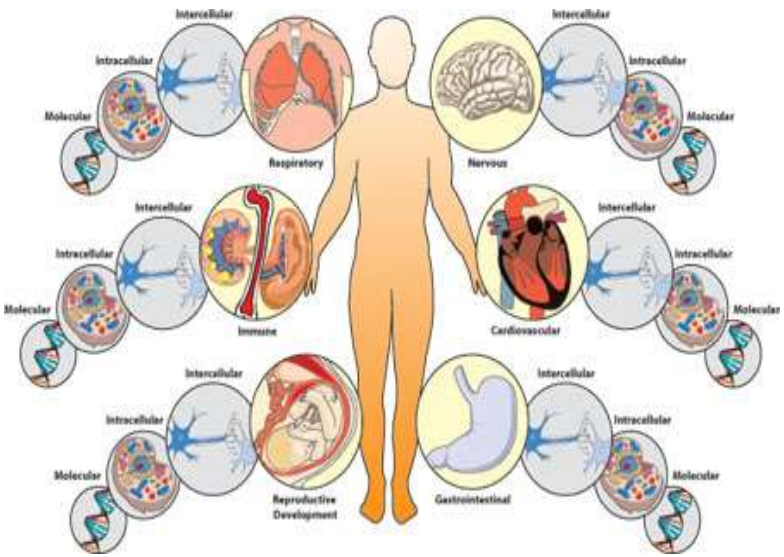
Genetic Code	Target Identification	Target Validation	Lead Discovery	Lead Optimization	Pre-clinical research	Clinical Research
❖ Sequence analysis ❖Annotations ❖Comparative genomics ❖ Pharmaco-genomics	❖Functional genomics ❖Expression analysis ❖Proteomics ❖Structural genomics ❖Pathway discovery	❖Expression analysis (arrays) ❖Proteomics ❖Structural genomics ❖Pathway discovery ❖Pharmaco-genomics	❖Expression (arrays) ❖Proteomics ❖Pathway discovery ❖HTS	❖Combi. chemistry ❖Expression (arrays) ❖Proteomics ❖Structural genomics	❖Animal models ❖In siliso simulations ❖Expression (arrays) ❖Proteomics ❖PK/PD	❖Expression (arrays) ❖Pharmaco-Genomics ❖PK/PD

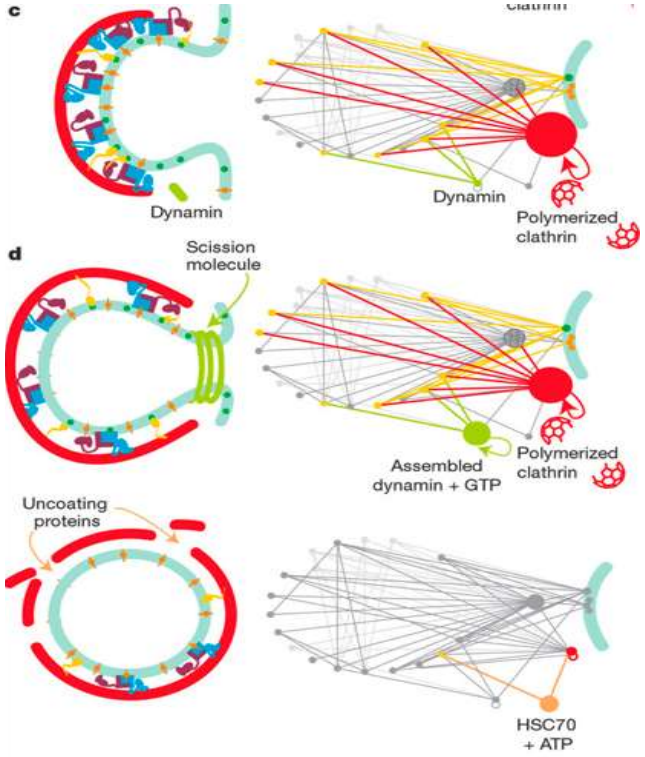
A process generating a flood of heterogeneous information

Problem 2. Therapeutic success.

The success of a therapeutic approach largely arises from the coherent manipulation of a physiological system as a whole

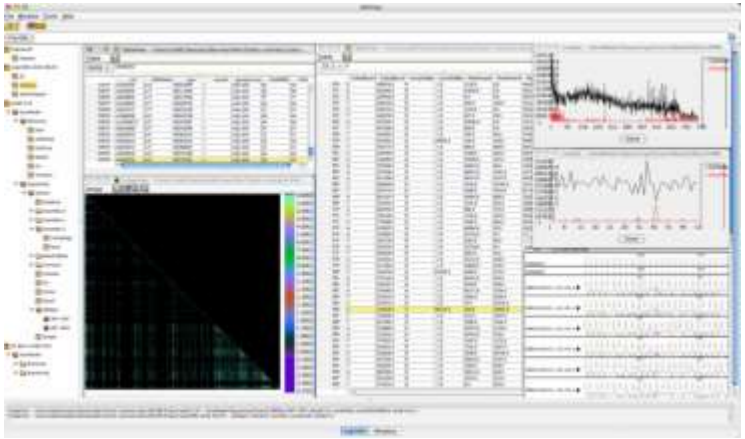
and not from that of a target in a molecular context.



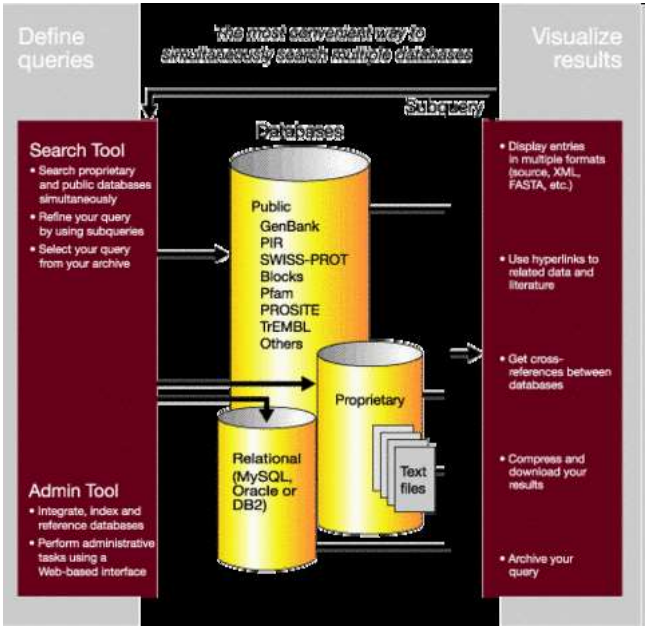


Therefore, any given medical problem must be approached from a systems standpoint.

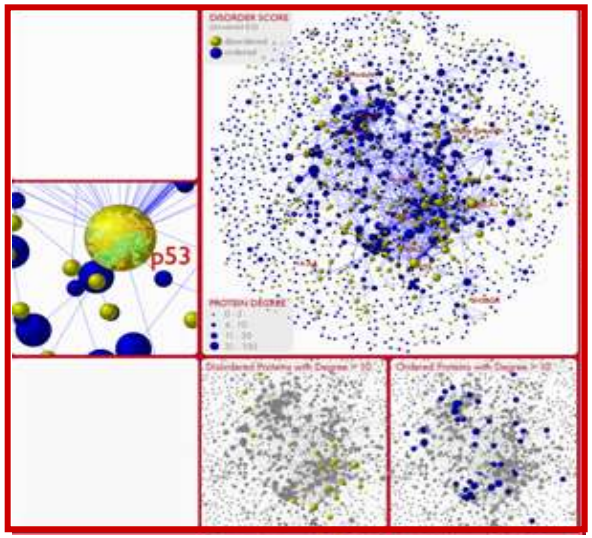
Problem 3. The data analysis & integration tools we must utilise in systems-level approaches.



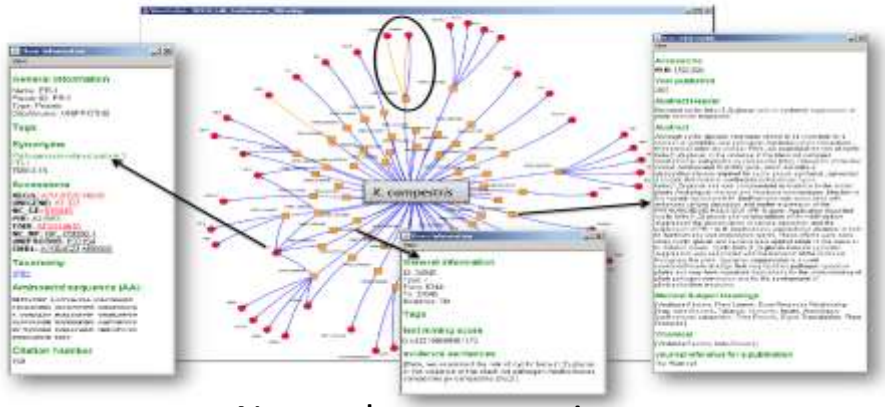
Data flat-file



Analytical WEB resources

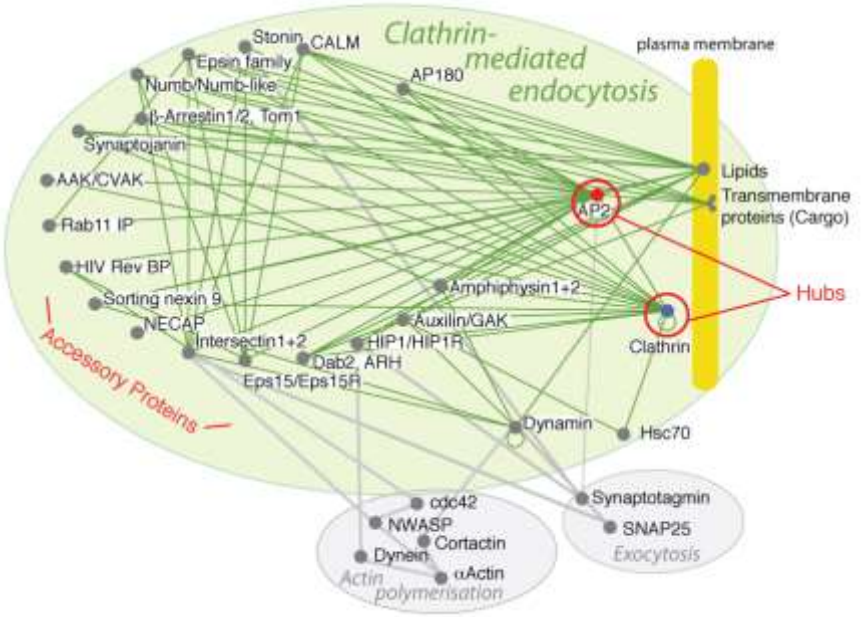


Visualisation tools



Networks construction

These approaches results in a highly misleading vision of protein interactions & networks.



How can a single hub protein bind so many different partners?

The problem is largely non-existent and resides in the construction and the representation of protein interaction networks within data-bases.

Proteins derived from a single gene, even if different, are clustered in maps into a single node.

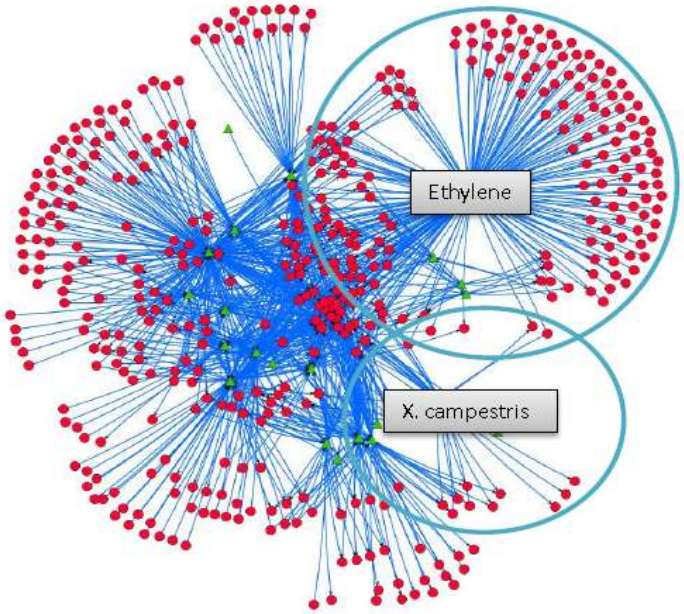
This leads to the impression that a single protein binds to a very large number of partners.

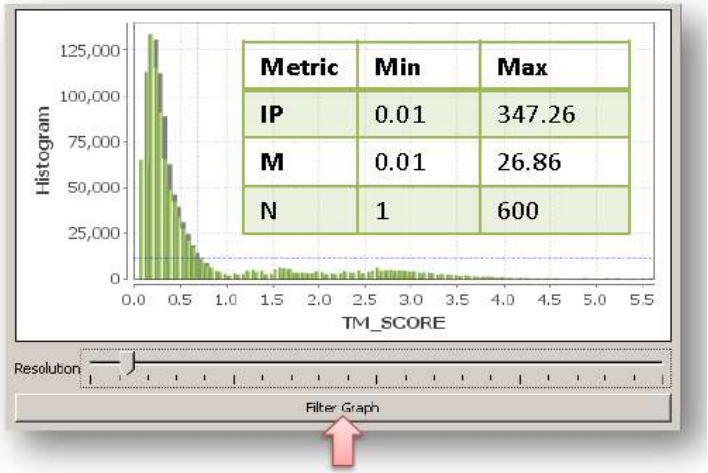
In reality, it does not.

Protein networks reflect confusions involving combinations of functional plasticity addressing a same protein together with distinct physiological roles of different proteins encoded by one gene.

And the more complex the organism being analysed, **the worse it gets.**

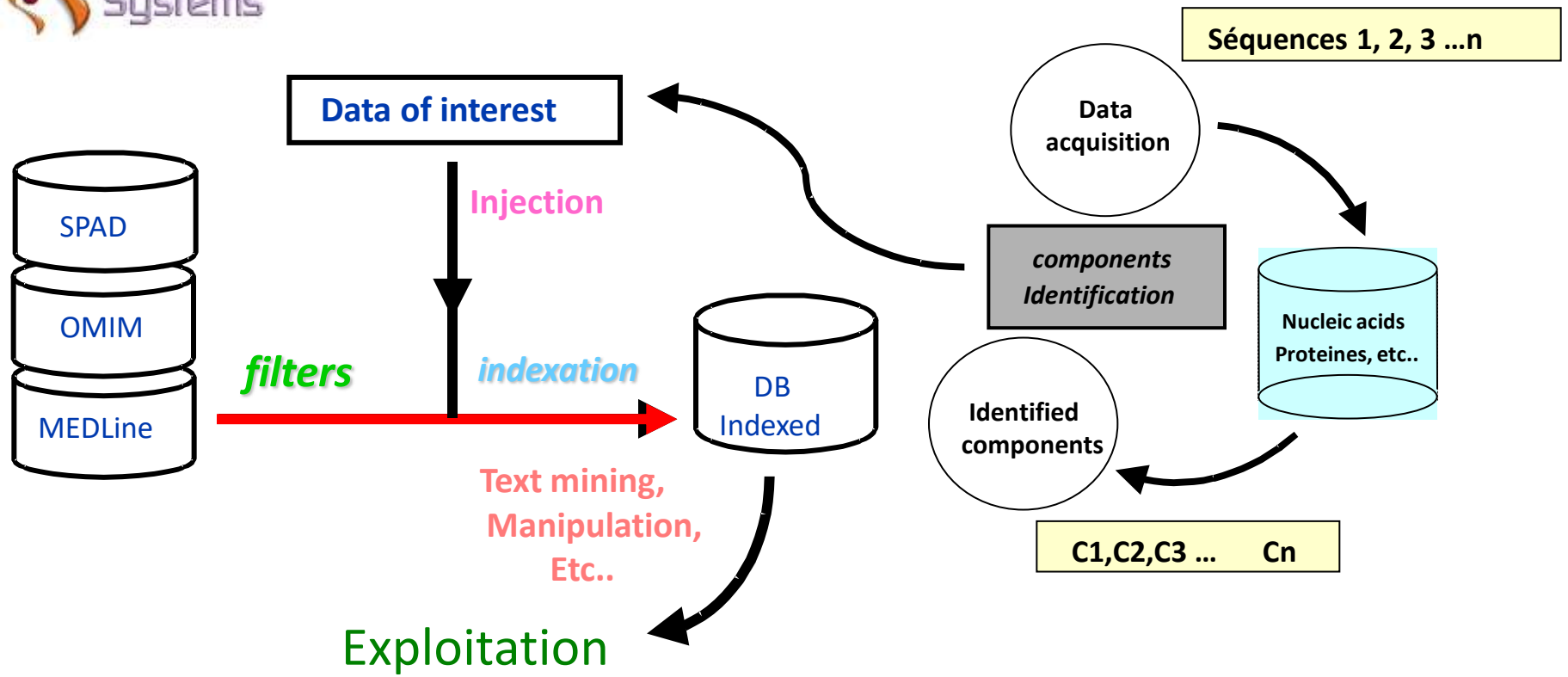
Protein-Stress Association Network





- 3145 proteins linked to 32 stresses by 10777 relations
- On average
  - each protein associated with 3.4 stresses
  - each stress associated with 337 proteins
- Filtering associations based on three scoring metrics IP, M and N
- Which metric and cut-off are most suited for filtering noise?

This significantly affects the Classical analytical Process



What constitutes a GOOD filter?

What constitutes a GOOD indexation strategy?

ALL information entered into the DB is ALWAYS *biased*, *incomplet*, *erroneous*, etc…

} Accumulation of inconsistencies

The challenge is clearly not a question of technologies only!

The paradox that must be resolved.

If you dream to create the first operational bird model…

… a "basic" living Complex system that not only flies…

Be sure to use the appropriate modeling concepts & tools. If not…

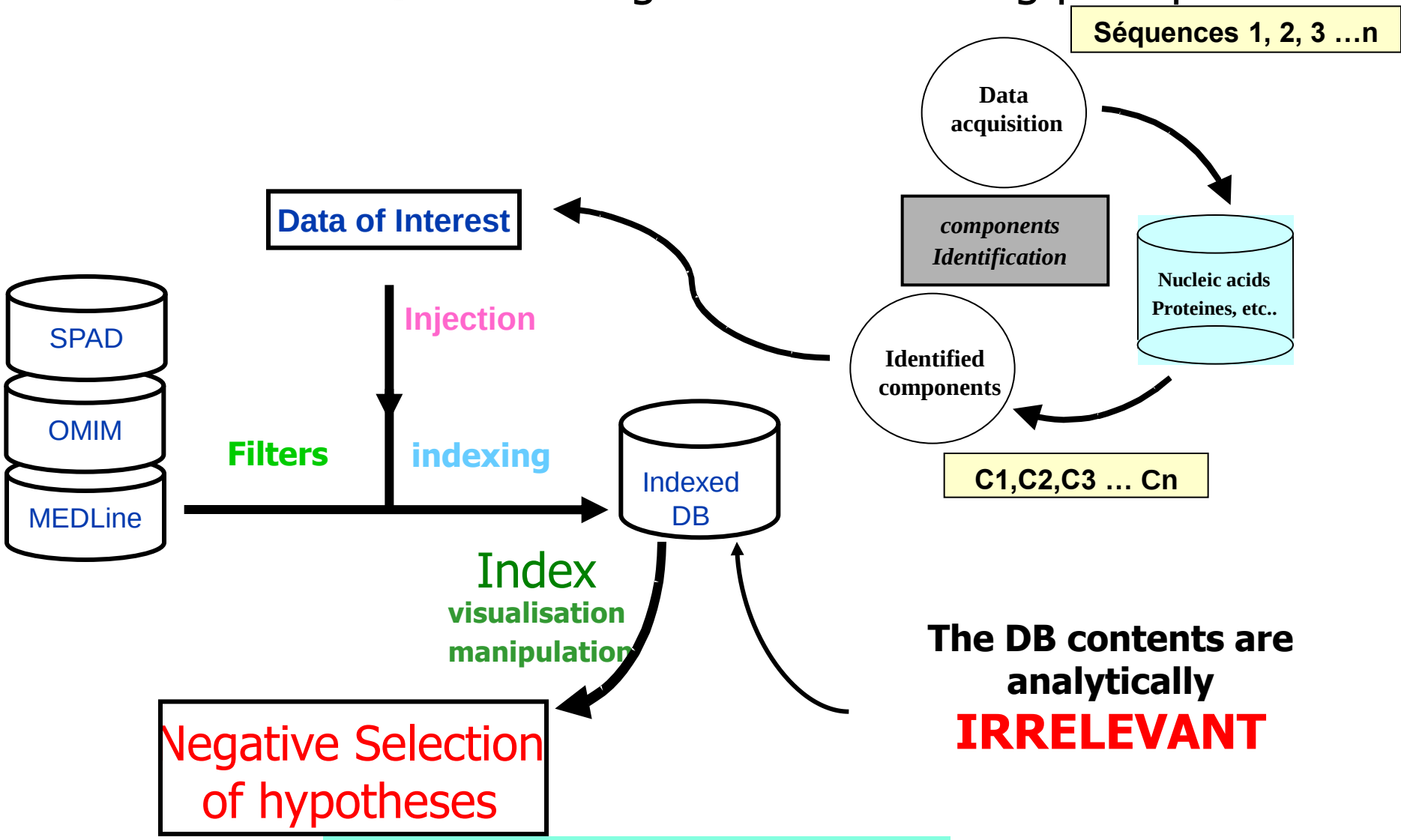
…you get a Complicated "Cartesian" system. It does fly, but…

How to escape the paradox whereby we have NO OTHER CHOICE but utilise tools & approaches that CANNOT enable us to reach our goal?

By changing our intellectual approach!

Another way of thinking:

The *CADI™* Integration & Modelling principles.



The differences between « heuristic » and « mathematical » approaches.

Heuristics:

A problems solving approach evaluating each step in a process, searching for satisfactory solutions rather than for optimal solutions, using all available *qualitative* information instead of *quantitative* information.

Thus,

Heuristic modelling starts from accumulated information to produce a model capable of describing the mechanisms that generated the observed outcome / data and predict their modifications associated with a different outcome**;**

It plays the role of an architect

While

mathematical (Bayesian) modelling starts from quantitative data to produce models capable of reiterating this data and predict the outcome of a different experimental paradigm.

It plays the role of an engineer

and

Far from being incompatible, these two approaches can be complementary.

Bayesian and Heuristic approaches can be complementary, provided they are harnessed in the proper order.

Bayesian approaches are of limited usefulness when applied to poorly defined multicellular physiological systems because they cannot efficiently reveal & define the functional states within such a system (cross-talks alterations, etc…).

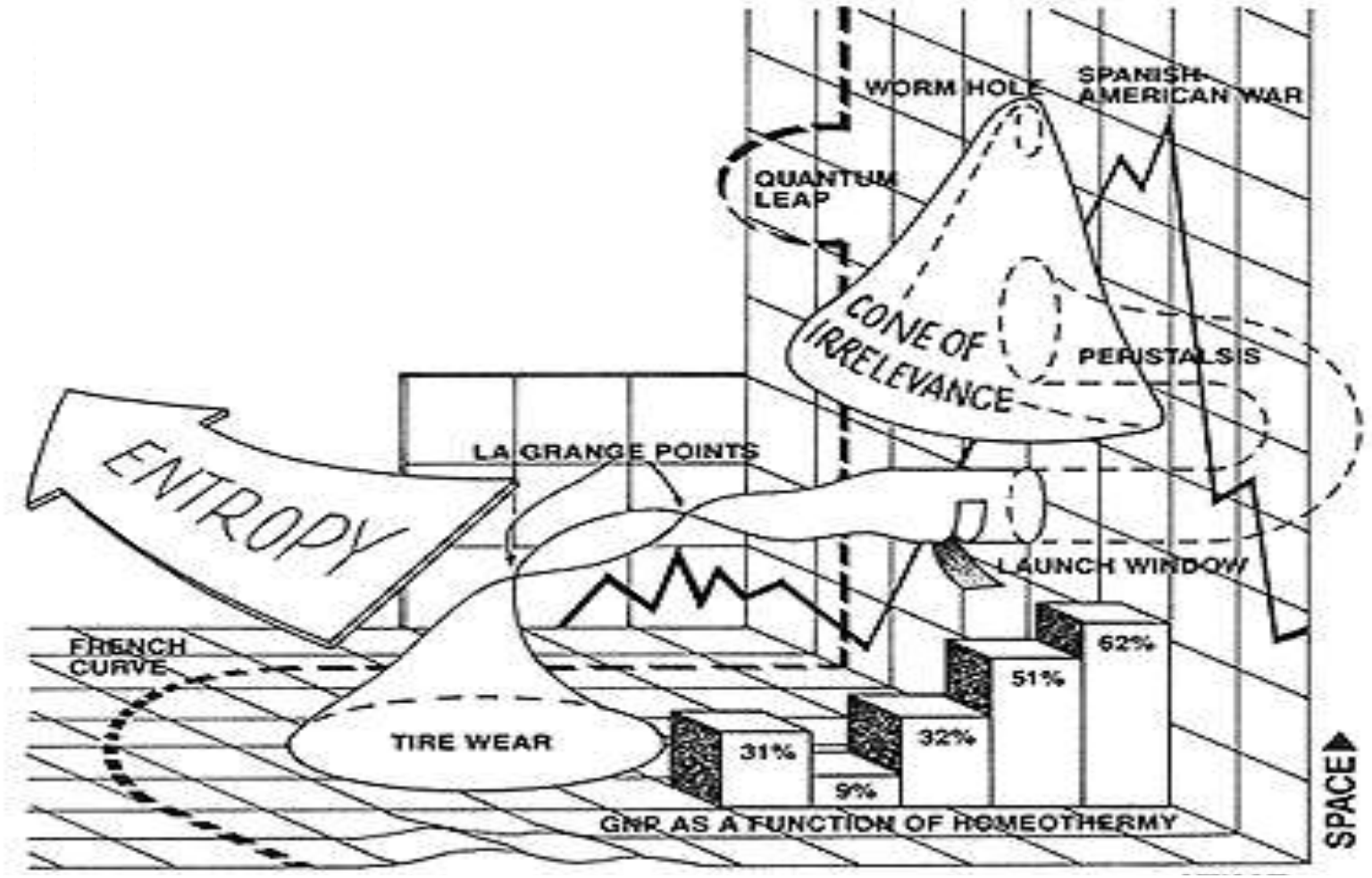
→ But heuristic approaches are very efficient at doing precisely this.

Heuristic models are of limited usefulness when addressing the dynamics of defined complex physiological pathways structures and cross-talks because they are not open to mathematical manipulations.

→ But Bayesian models are very efficient at doing precisely this.

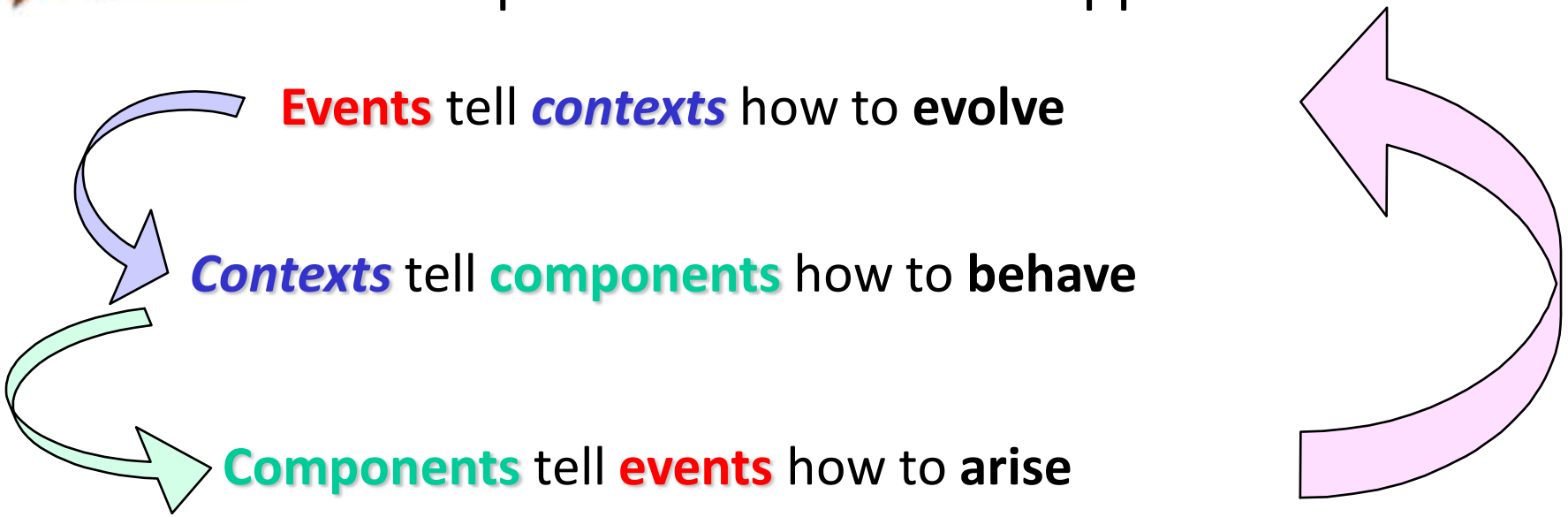
To efficiently address the translation of systems biology to clinical & medical interventions (dominated by patient's data heterogeneity and largely unstructured documents), ways to achieve synergy between Heuristic and Bayesian approaches can be effectively designed.

The heuristic analytical process must follow a « relativistic » approach.



Within this framework, *Non-linearity*, *Irrelevance*, *Wear*, *Relative weights* & *Contexts* are key concepts.

Why? Because of a very simple set of rules which imposes « relativistic » approaches.



Analyses in terms of biological components and functions are now IRRELEVANT.

EVENT-DRIVEN (relativistic) analytical approaches become necessary.

This, in turn, imposes analytical procedures based upon the negative selection of working hypotheses.

Why “negative selection” of working hypotheses?

“While it is not always possible to demonstrate that a statement is true, it is always possible to demonstrate it to be false” Karl Popper, 1963.

Mathematical approaches are based on “positive selection”: it is assumed that every data-set/statement is actually valid.

Yet, “an estimated 85% of current published research findings are false or exaggerated”

J.P.A Joannidis, 2014 [PLoS Med. 11(10): e1001747]; F. Prinz et al., 2011 [Nat Rev Drug Discov. 10 (9):712]

Positive selection becomes a killer!

How to identify what is NOT false and/or exaggerated?

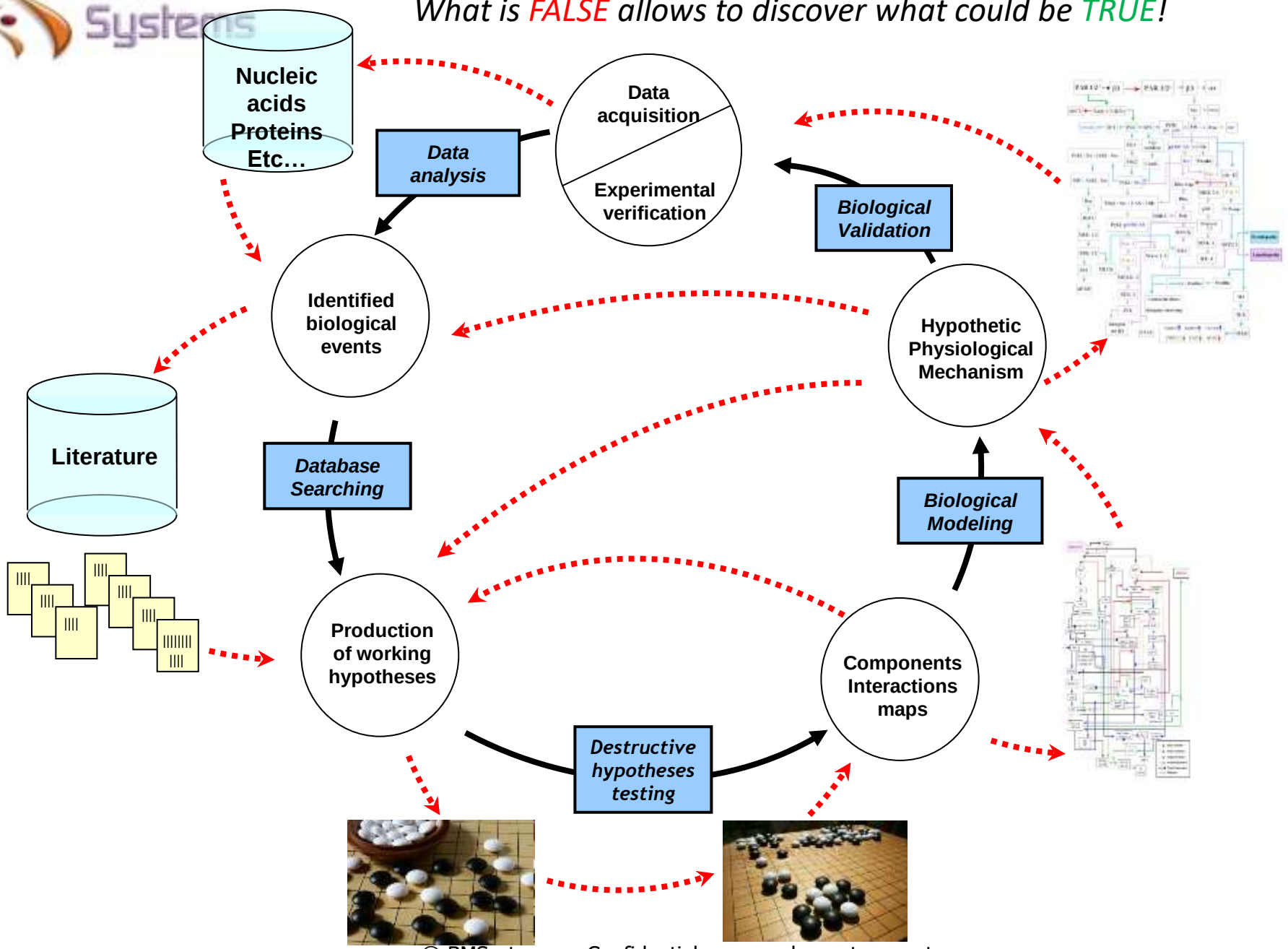
By doing every thing possible to destroy working hypotheses!

Only hypotheses that resist destruction are worth retaining.

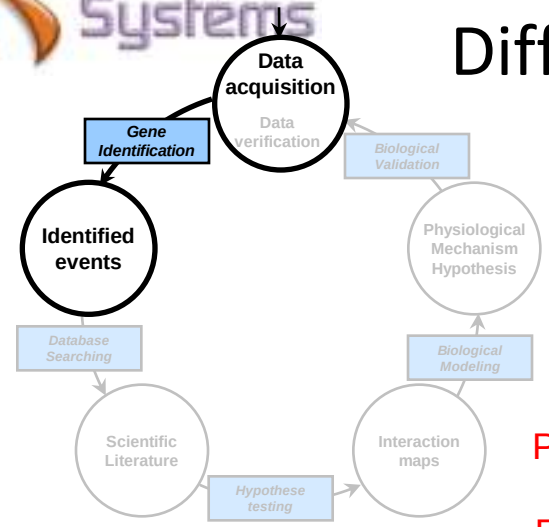
Hence, what is demonstrated “False” can now be used to discover what could be “True”.

The *CADI* ™ Integration workflow

What is FALSE allows to discover what could be TRUE!



MCF-7/MCF-7 ras Differentially Expressed Genes

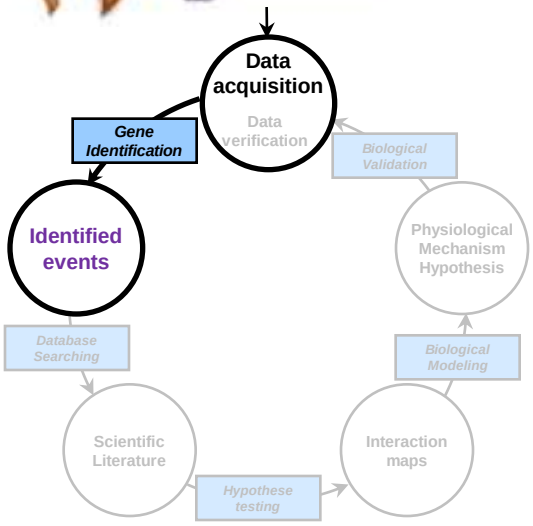


Over-expressed
Under-expressed
over 600 genes

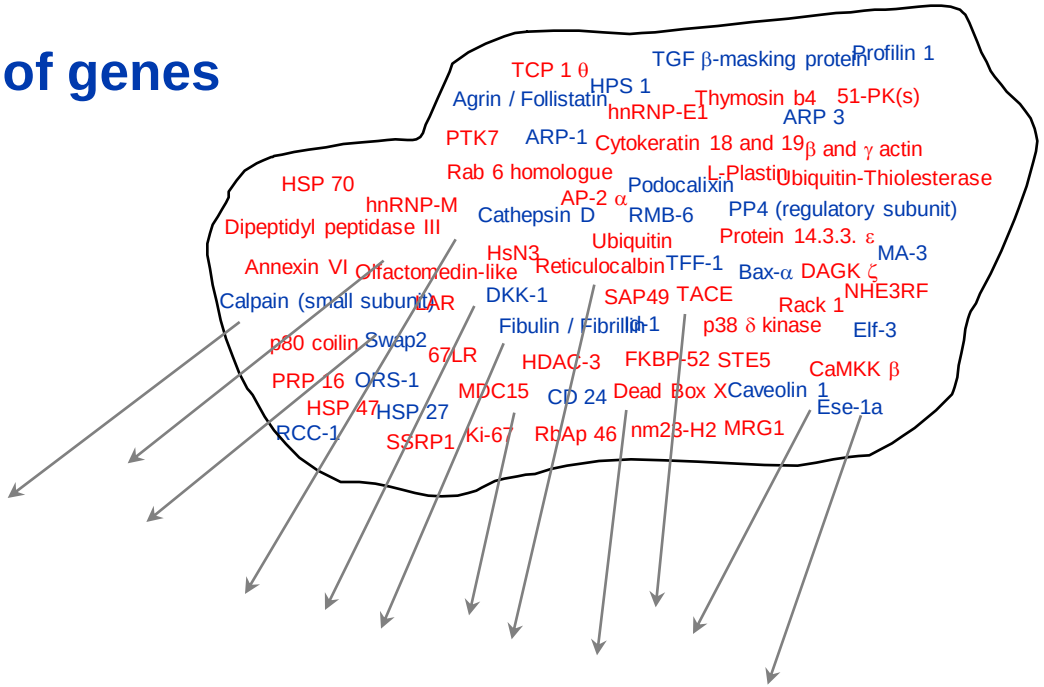
TGF β-masking protein
Profilin 1
TCP 1 θ
HPS 1
Agrin / Follistatin
Thymosin b4
51-PK(s)
hnRNP-E1
ARP 3
PTK7
ARP-1
Cytokeratin 18 and 19
β and γ actin
Rab 6 homologue
L-Plastin
Ubiquitin-Thiolesterase
HSP 70
Podocalixin
AP-2 α
hnRNP-M
PP4 (regulatory subunit)
Cathepsin D
RMB-6
Dipeptidyl peptidase III
Protein 14.3.3. ε
Ubiquitin
HsN3
MA-3
Annexin VI
Reticulocalbin
TFF-1
DAGK ζ
Olfactomedin-like
Bax-α
NHE3RF
SAP49
TACE
LAR
DKK-1
Rack 1
Calpain (small subunit)
p38 δ kinase
Fibulin / Fibrillin
Id-1
Elf-3
p80 coilin
Swap2
67LR
HDAC-3
FKBP-52
STE5
CaMKK β
PRP 16
ORS-1
MDC15
Dead Box X
Caveolin 1
HSP 47
CD 24
Ese-1a
HSP 27
Ki-67
RbAp 46
nm23-H2
MRG1
RCC-1
SSRP1

Gadal F. *etal*. (2003) *Nucleic Acids Research* 19 (31): 5789-5804

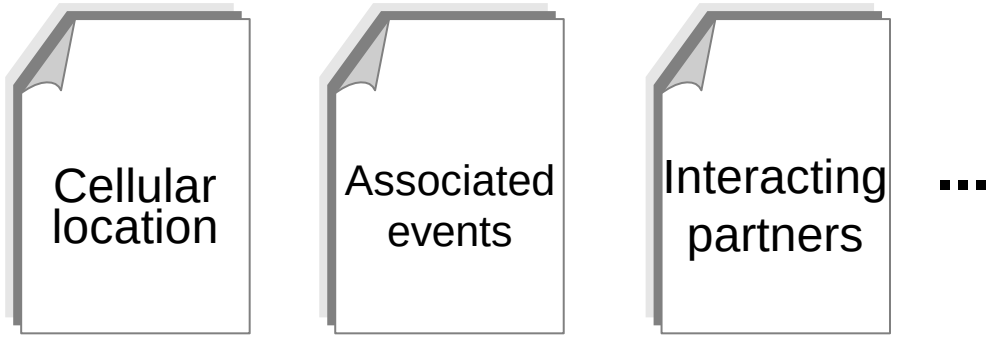
Information Retrieval & Extraction



Set of genes



Set of papers



Gadal F. *etal*. (2003) *Nucleic Acids Research* 19 (31): 5789-5804

Information MEDLINE

text only

UI - 98057316
TI - The human 37-kDa laminin receptor precursor interacts with the prion protein in eukaryotic cells.
AU - Rieger R
AU - Edenhofer F
AU - Lasmezas CI
AU - Weiss S
LA - eng
MH - Actins/metabolism
MH - Animal
MH - Binding Sites
MH - COS Cells
MH - Cell Line
MH - Eukaryotic Cells
MH - Hamsters
MH - Human
MH - Mice
MH - Mice, Inbred C57BL
MH - PrPSc Proteins/*metabolism
MH – ProteiPrecursors/chemistry/*metabolism
MH - Rabbits
MH - Laminin/chemistry/genetics/*metabolism
MH - Saccharomyces cerevisiae/metabolism
MH - Spodoptera/cytology
SO - Nat Med 1997 Dec;3(12):1383-8.

AB - Prions are thought to consist of infectious proteins that cause transmissible spongiform encephalopathies. According to overwhelming evidence, the pathogenic prion protein PrPSc converts its host encoded isoform PrPC into insoluble aggregates of PrPSc, concomitant with pathological modifications (for review, see refs. 1-3). Although the physiological role of PrPC is poorly understood, studies with PrP knockout mice demonstrated that PrPC is required for the development of prion diseases. Using the yeast two-hybrid technology in Saccharomyces cerevisiae, we identified the 37-kDa laminin receptor precursor (LRP) as interacting with the cellular prion protein PrPC. Mapping analysis of the LRP-PrP interaction site in S. cerevisiae revealed that PrP and laminin share the same binding domain (amino acids 161 to 180) on LRP. The LRP-PrP interaction was confirmed in vivo in insect (Sf9) and mammalian cells (COS-7). The LRP level was increased in scrapie-infected murine N2a cells and in brain and spleen of scrapie-infected mice. In contrast, the LRP concentration was not significantly altered in these organs from mice infected with the bovine spongiform encephalopathic agent (BSE), which have a lower PrPSc accumulation. LRP levels, however, were dramatically increased in brain and pancreas, slightly increased in the spleen and not altered in the liver of crapie-infected hamsters. …

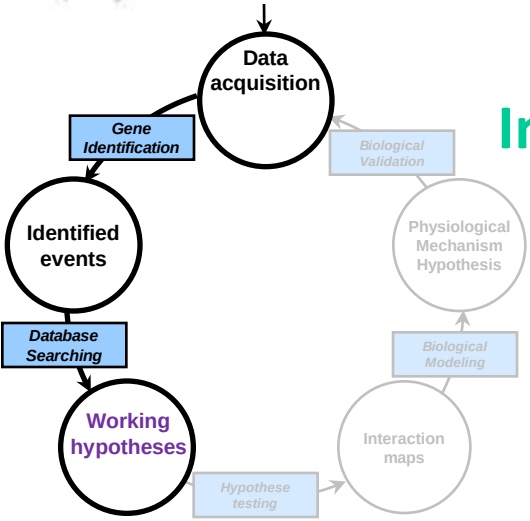
Information MEDLINE

text only

UI - 98057316
TI - The human 37-kDa laminin receptor precursor interacts with the prion protein in eukaryotic cells.
AU - Rieger R
AU - Edenhofer F
AU - Lasmezas CI
AU - Weiss S
LA - eng
MH - Actins/metabolism
MH - Animal
MH - Binding Sites
MH - COS Cells
MH - Cell Line
MH - Eukaryotic Cells
MH - Hamsters
MH - Human
MH - Mice
MH - Mice, Inbred C57BL
MH - PrPSc Proteins/*metabolism
MH – ProteiPrecursors/chemistry/*metabolism
MH - Rabbits
MH - Laminin/chemistry/genetics/*metabolism
MH - Saccharomyces cerevisiae/metabolism
MH - Spodoptera/cytology
SO - Nat Med 1997 Dec;3(12):1383-8.

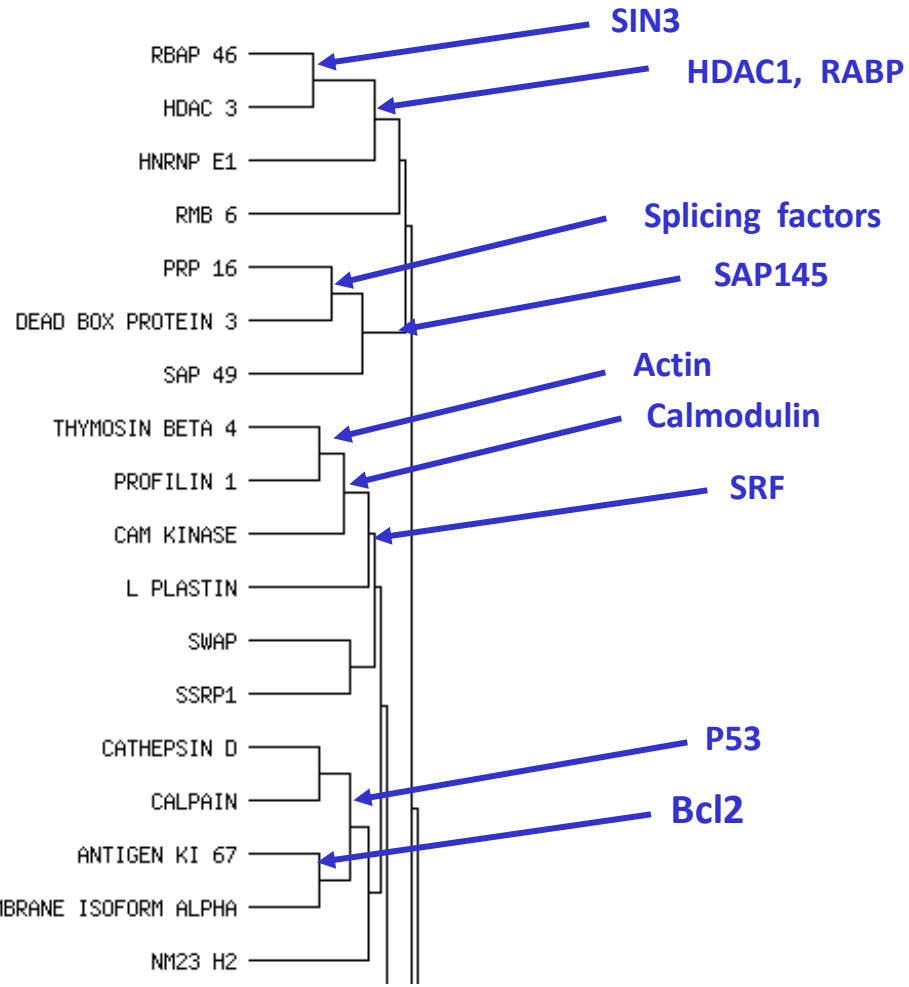
AB - Prions are thought to consist of infectious proteins that cause transmissible spongiform encephalopathies. According to overwhelming evidence, the pathogenic prion protein PrPSc converts its host encoded isoform PrPC into insoluble aggregates of PrPSc, concomitant with pathological modifications (for review, see refs. 1-3). Although the physiological role of PrPC is poorly understood, studies with PrP knockout mice demonstrated that PrPC is required for the development of prion diseases. Using the yeast two-hybrid technology in Saccharomyces cerevisiae, we identified the 37-kDa laminin receptor precursor (LRP) as interacting with the cellular prion protein PrPC. Mapping analysis of the LRP PrP interaction site in S. cerevisiae revealed that PrP and laminin share the same binding domain (amino acids 161 to 180) on LRP. The LRP-PrP interaction was confirmed in vivo in insect (Sf9) and mammalian cells (COS-7). The LRP level was increased in scrapie-infected murine N2a cells and in brain and spleen of scrapie-infected mice. In contrast, the LRP concentration was not significantly altered in these organs from mice infected with the bovine spongiform encephalopathic agent (BSE), which have a lower PrPSc accumulation. LRP levels, however, were dramatically increased in brain and pancreas, slightly increased in the spleen and not altered in the liver of crapie-infected hamsters. …

Generating hypotheses & systematic destructive testing.

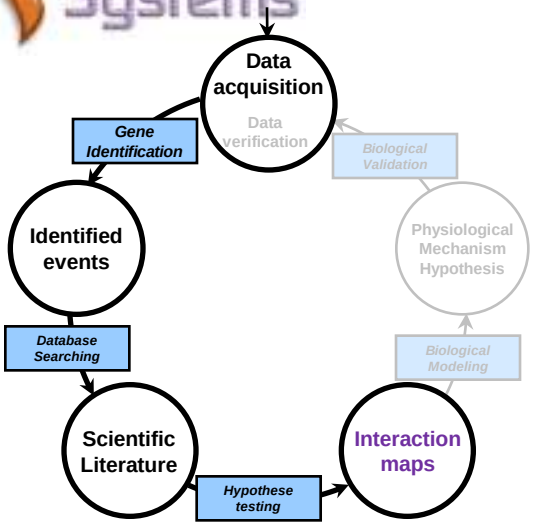


Index visualisation & manipulation

RNA Splicing & expression



Apoptosis & cell survival



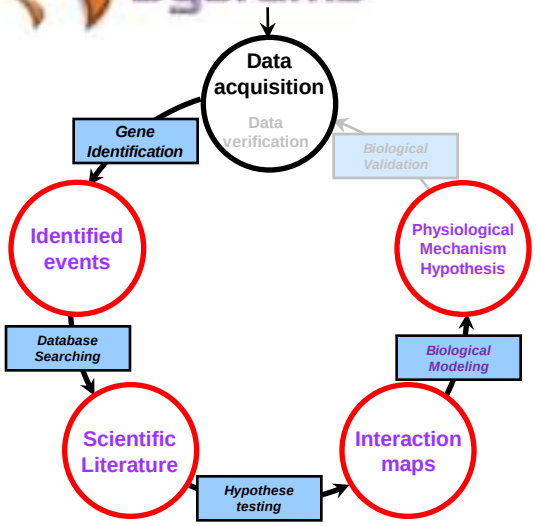
Interaction maps

describe the pathways that have become functional and those that have become forbidden in response to local conditions imposed by the activation of defined biological mechanisms.

Specific biological events do not occur because they are fated to.

They occur because other events could not!

The CADI™ Integration & Modelling Process.

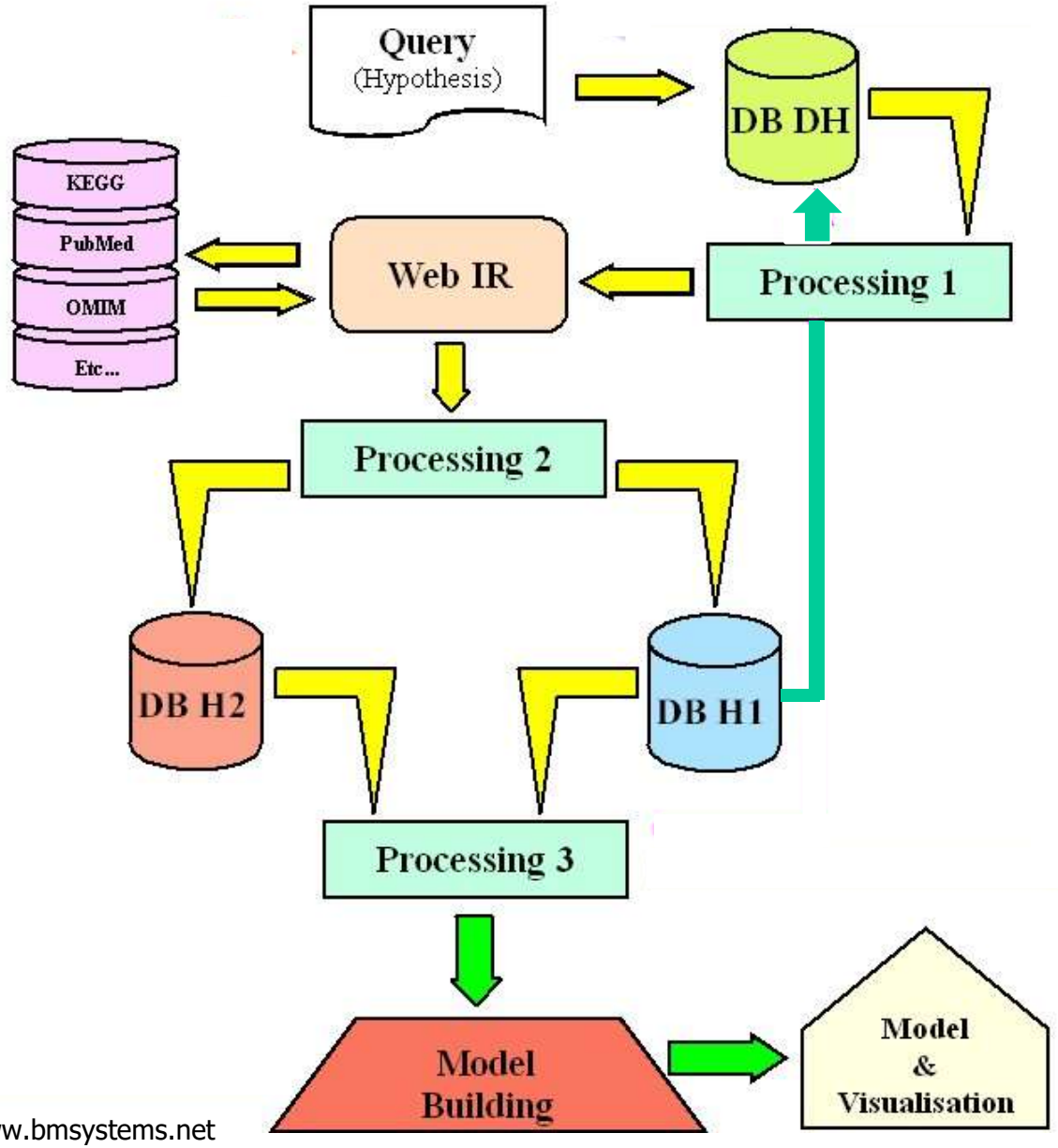


This iterative process does three things:

- It largely resolves the coherence issues attached to the classical approach;

- It reveals hitherto unknown mechanisms/processes, and

- It allows the translation of systems biology to clinical & medical interventions.



As a result,

Searching under the street lamp because that is where there is light may be much less helpful than expected.

Indeed, since it is the presence/absence of specifiable events which govern phenotypic characteristics, it follows that

The mechanisms that characterise a given phenotype (be it pathological or not) within a given biological system can be utilised as analytical tools to unravel those associated with any other phenotypic transition affecting the same biological system.

Here, symptomatology ceases to function as defining criteria to become contextual and relative functional end-products.

Therefore, the solution to a given biological problem can be efficiently obtained through entirely indirect investigations.

This is particularly true for problems resistant to direct approaches because characterised by

- High symptomatologic heterogeneity, and/or
- High functional/phenotypic uncertainty.

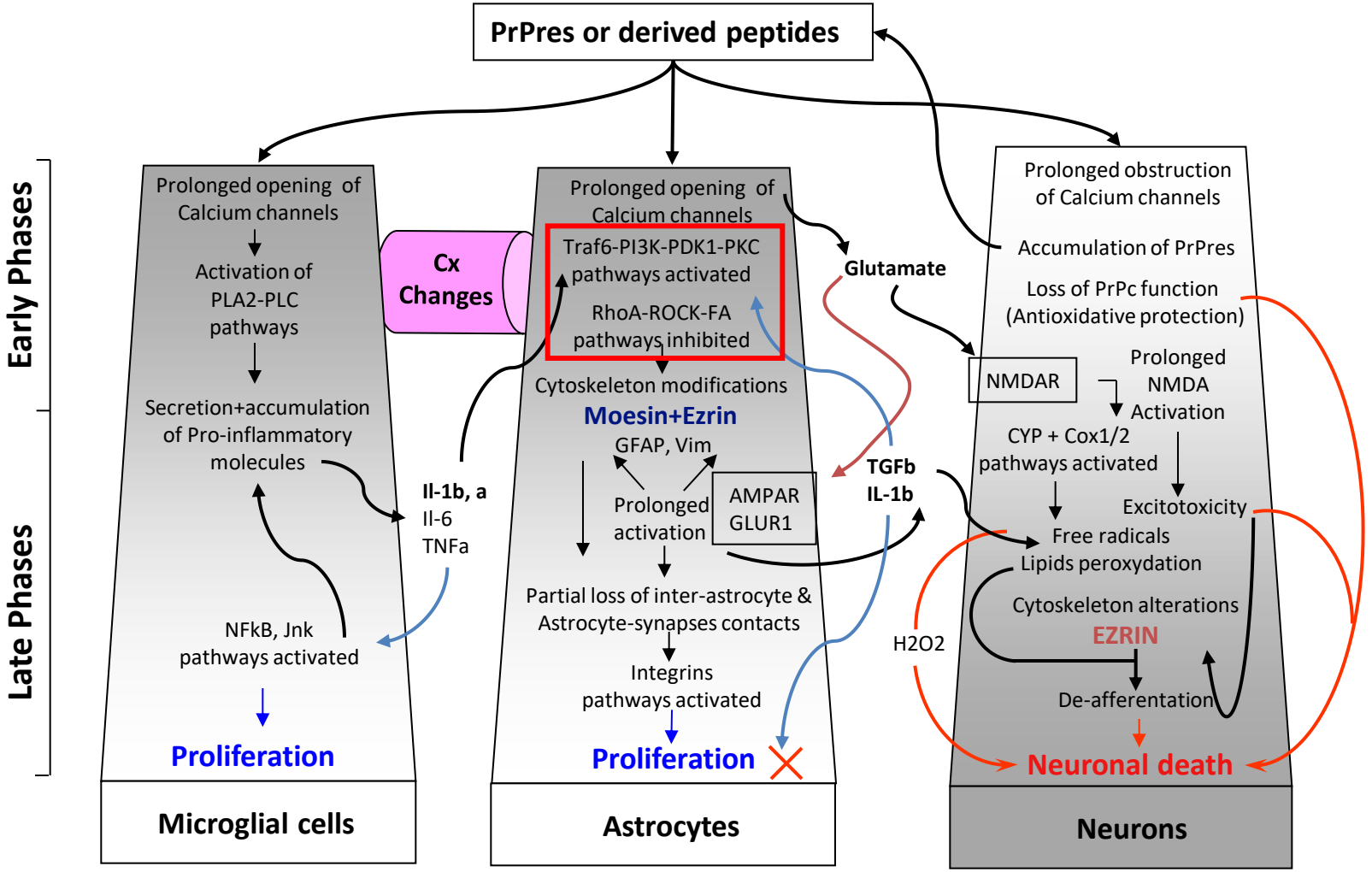
Example 1:

Understanding the *in vivo* mechanisms of Creutzfeldt-Jakob Disease pathogenesis & progression leads to the discovery of improved treatment for non-degenerative psychiatric disorders.

CJD: A few facts.

- Progressive neurodegenerative disease, invariably fatal.
- Long asymptomatic incubation phase (30 years + in man)
- Short clinical phase (death within 6 months - 2 years)
- Pathological agent: abnormally folded form of the PrP protein
- Pathological mechanisms : Unknown
- Clinical progression mechanisms : Unknown
- Mode of propagation within the CNS: Unknown
- Therapeutic or preventive means: Unknown
- Pathogenesis Biomarkers: Clinical symptoms + CNS spongiosis (post-mortem).

The pathogenesis & clinical progression model

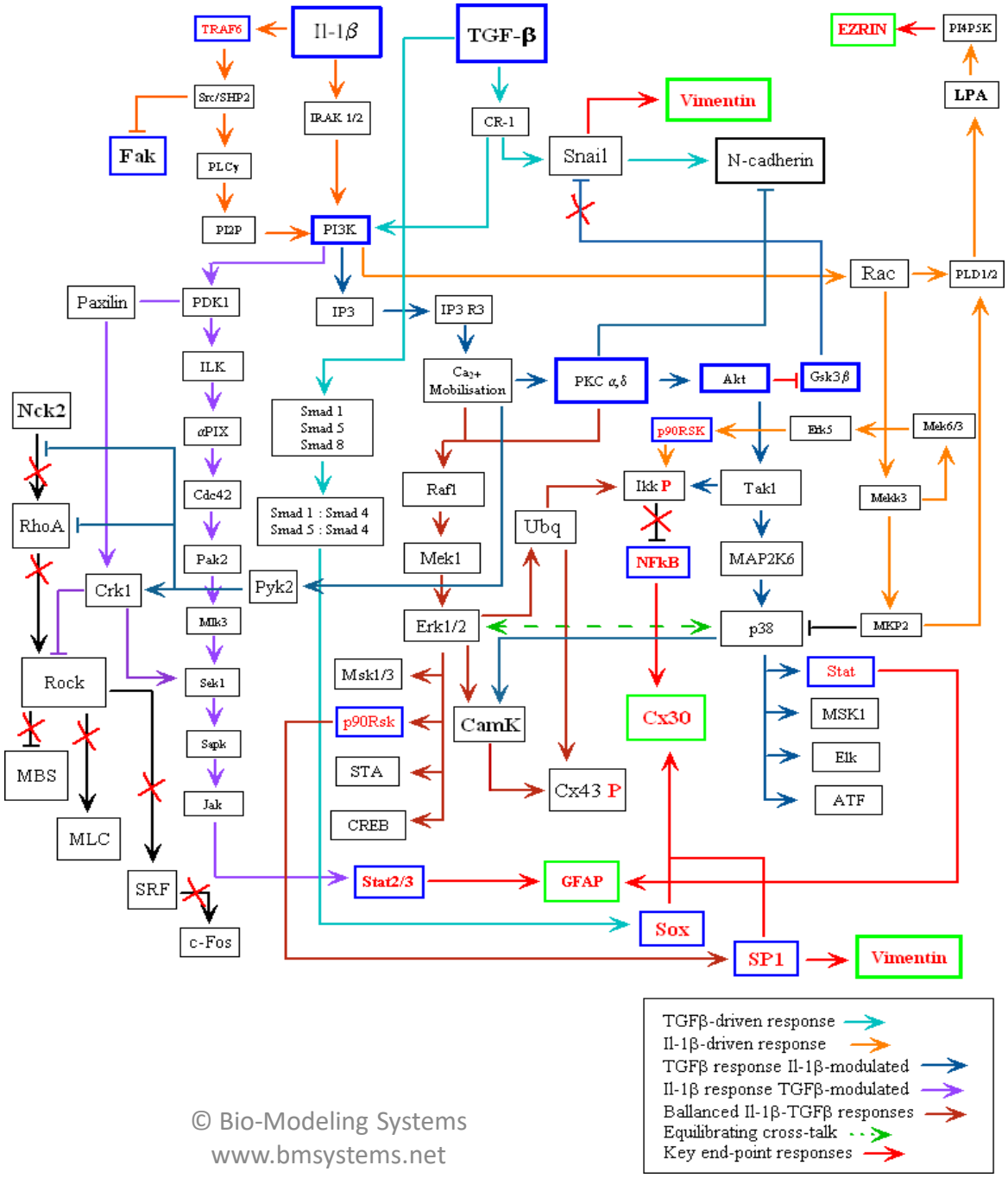


Iris F (2012); Pharmacopsych. **45** (Suppl. 1): S12-21.

The main driving mechanism.

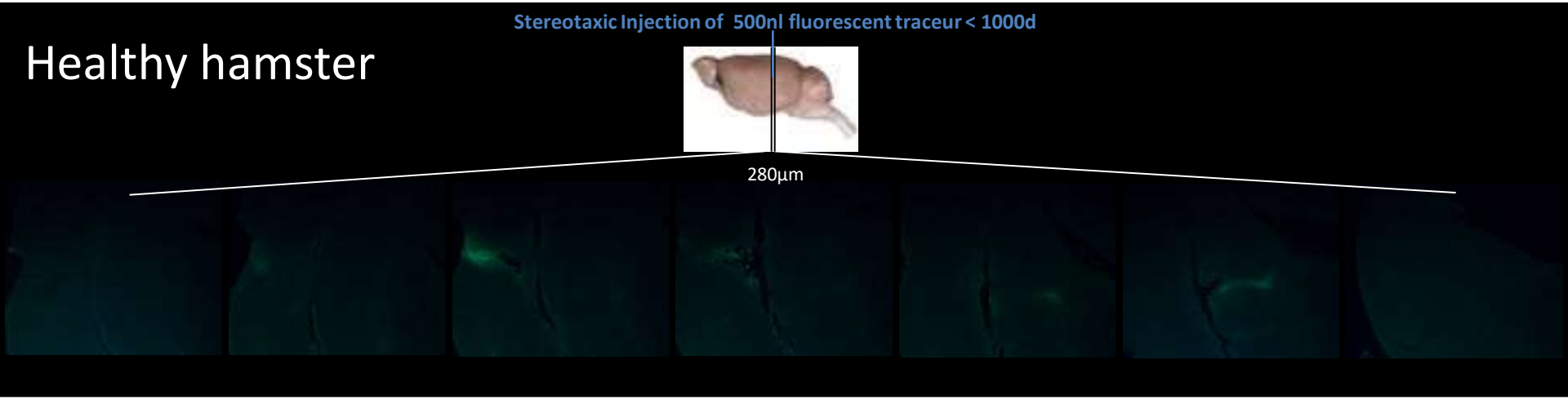
Il-1b & TGFb-mediated signalling in hippocampus astrocytes

The pathways through which chronic neuronal stress signalling and concurrent glial pro-inflammatory responses lead to reactive astrocyte activation (GFAP + Vim) associated with cytoskeleton reorganisation (ezrin). This leads to a major switch in Cx targeting & distribution, resulting in the formation of a syncythium with massively altered diffusive properties and neurotrophic functions.

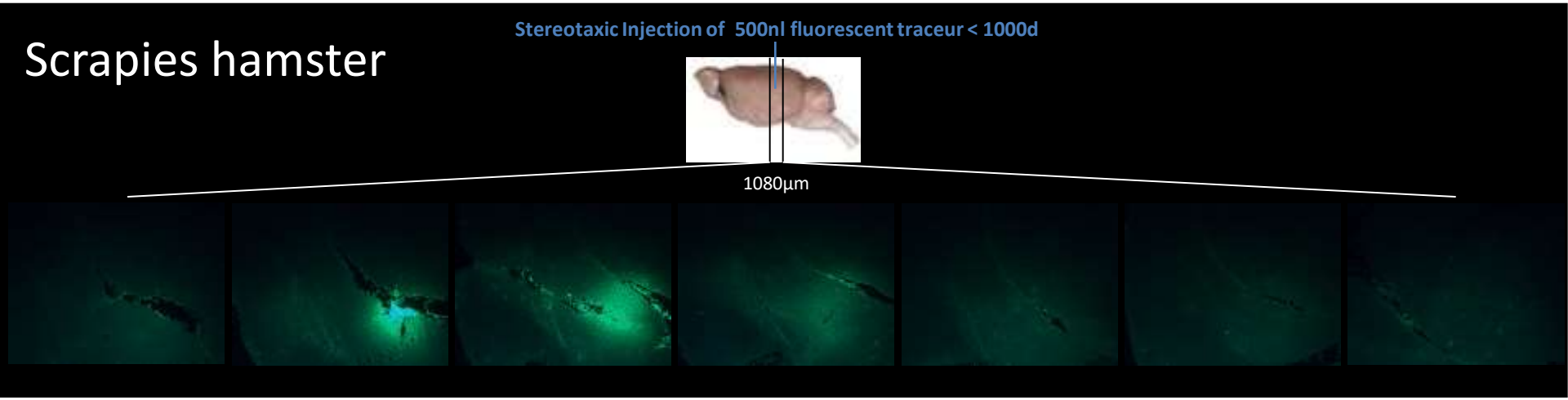


Effects of the modifications of glial connexions upon intercellular diffusion processes.

Healthy hamster

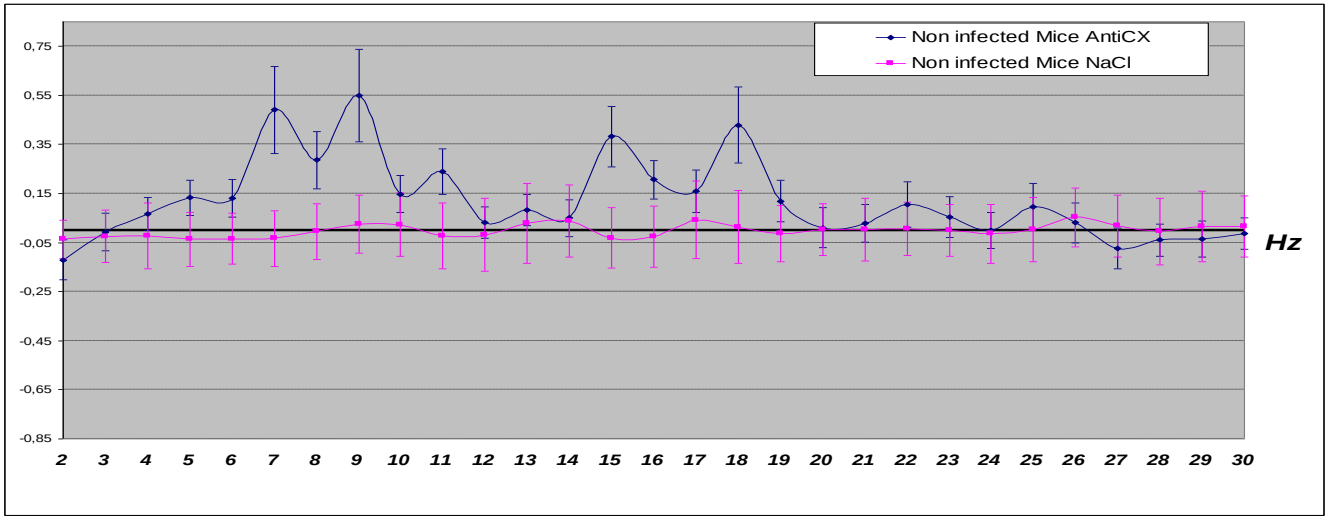


Scrapies hamster

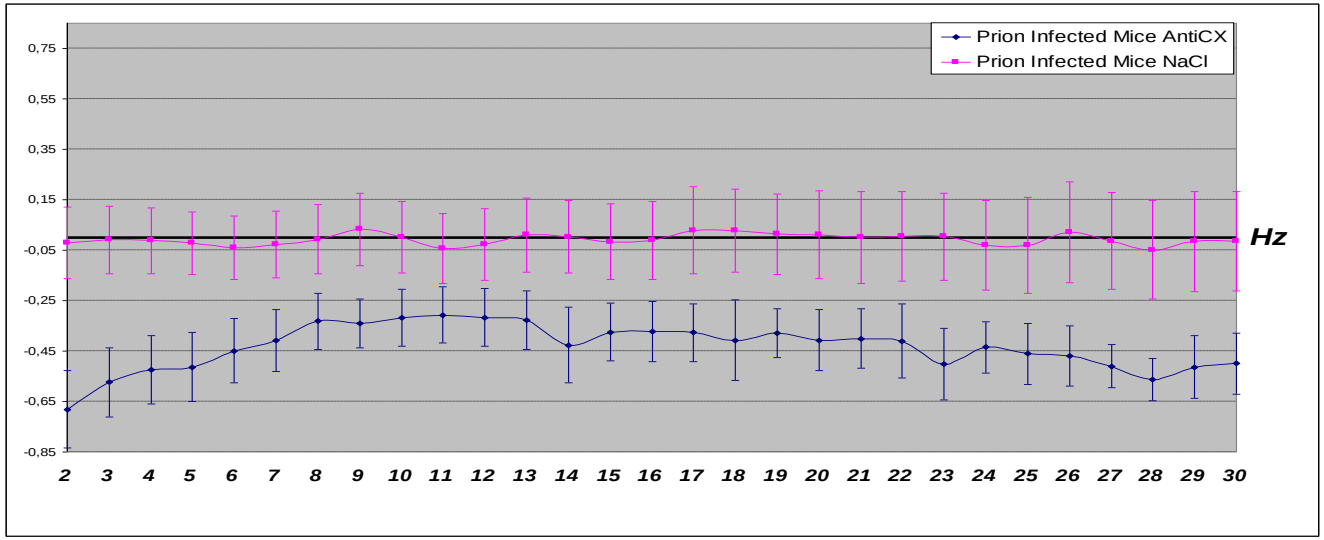


The effects are much more wide-spread than anticipated!

Functional alterations linked with modifications of glial connections.



Healthy mice



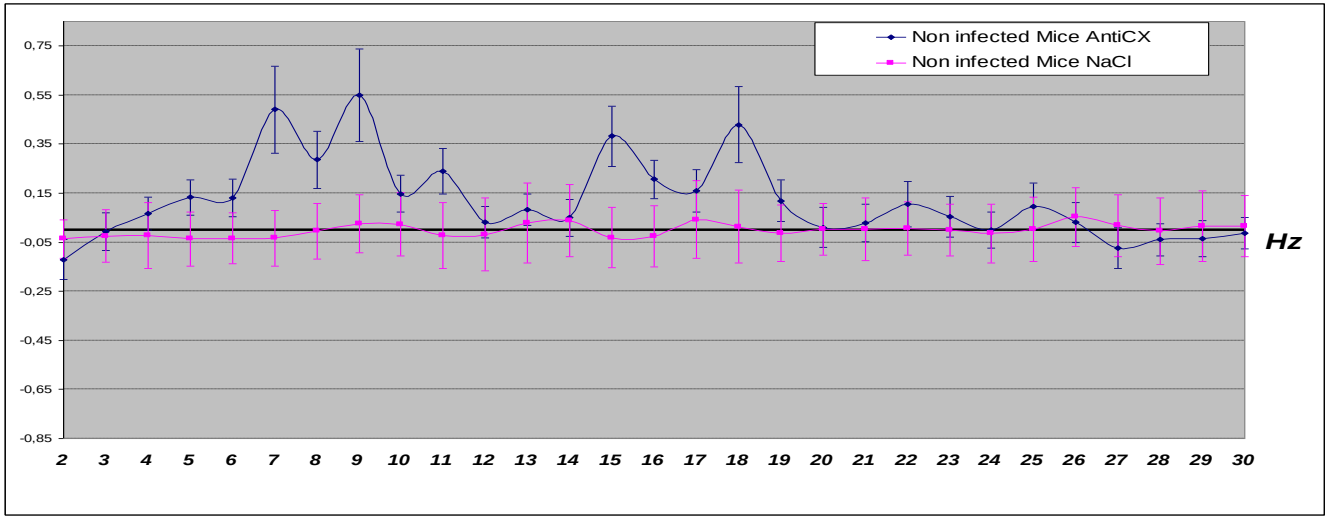
Scrapies mice
(asymptomatic phase).

NaCl

Anticonnexin

Practical consequences.

One of the roles of connexins is to dampen neuronal synchronisation.



Healthy mice

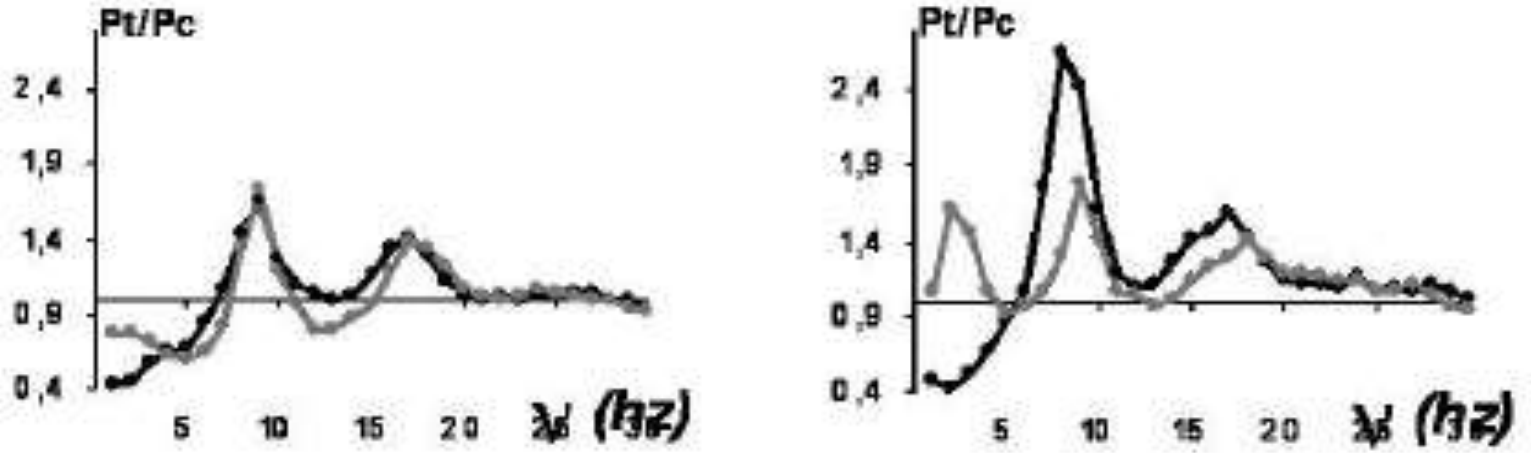
- NaCl
- Anticonnexin

In healthy animals, pharmacological blockade of Cx activity results in quantitative EEG patterns closely resembling an epileptic crisis
(frequency range-specific hyper-synchronisations).

Hence, in cases where the aim of treatment is to affect synchronisation, the adjunction of Cx blockers could potentiate current drugs therapeutic effects (allow to decrease doses while preserving activity) thereby reducing unwanted side effects.

A new model was constructed and the predicted approach tested in vivo.

Effects Cx blocker adjunction to Paroxetin treatment.



1st hour **Illustrative** 2nd hour

——— Paroxetin 1 mg/kg (2 to 5-fold below usual doses)

——— Paroxetin 0.5 mg/kg + Cx blocker 0.4 mg/kg

Similar boosting effects of Cx blockers are seen with
Clozapin; Modafinil, Diazepam; Venlafaxin; Escitaloprame; Bupropion; Sertralin; etc.

The dose-reducing effects are in the order of 5 to 20-fold (depending on the therapeutic molecule) without any loss of therapeutic effects.

Therapeutics Class Patent (WO/2010/029131) filed in 2010 covers two options:
"Safety" improvement: Lower dose for same output and better "safety"
"Efficacy" improvement: Same dose for better output and same "safety"

NB: one of the molecules utilised as Cx blocker is already on the market for a completely different indication and is used here at 1/X th of its approved dosage (the predicted dose-dependent effects were observed).

The net results.

CJD is not a neurological disease *stricto sensus*.
It is a disease that primarily affects astrocytes structures and functions which, over time, lethally affects healthy glial & neuronal cells through « bystander effects », leading to widespread CNS disorganisation (spongiosis) and functional failure.

But this model also provides an understanding of key mechanisms associated with psychiatric & neurology disorders.

An entirely new approach for their effective treatment was designed, tested in vivo and validated.

Patent covering novel therapeutics for psychiatry & neurodegenerative disorder (CEA/BMSystems).

This CEA/BMSystems collaborative research in CNS (psychiatric and neurological disorders) led to the co-owned patent WO201029131 with a worldwide exclusive license to Theranexus CEA's spin-off currently in Phase II.

Neither of which have much to do with CJD *per se*…

This work received a Bio-IT World « Best Practices » award from the Cambridge HealthTech Institute (USA).

AND

Was selected as 1 of the 3 pan-European « state of the art examples of systems biology approaches of benefit to medicine » by the European Commission's DG Research, Directorate of Health (June 2010).

Example 2:

Understanding the co-evolutionary interplays between bacteria and bacteriophages leads to the discovery of the means whereby ***undefined*** multi-resistant bacterial pathogens can be efficiently controlled.

The questions (French Defence)

- How to rapidly (less than 30 min) and efficiently ***detect*** the presence of any given LIVE bacterial pathogen?

- How to rapidly and efficiently ***destroy*** any ***unknown*** bacterial pathogen or emerging strain ***without using***

A) Antibiotics: too many resistant strains, and very rapid resistance acquisition.

B) Vaccines: much too slow to act, and small strain variations often lead to inefficacy.

In other words, what is required is a "detector-killer".

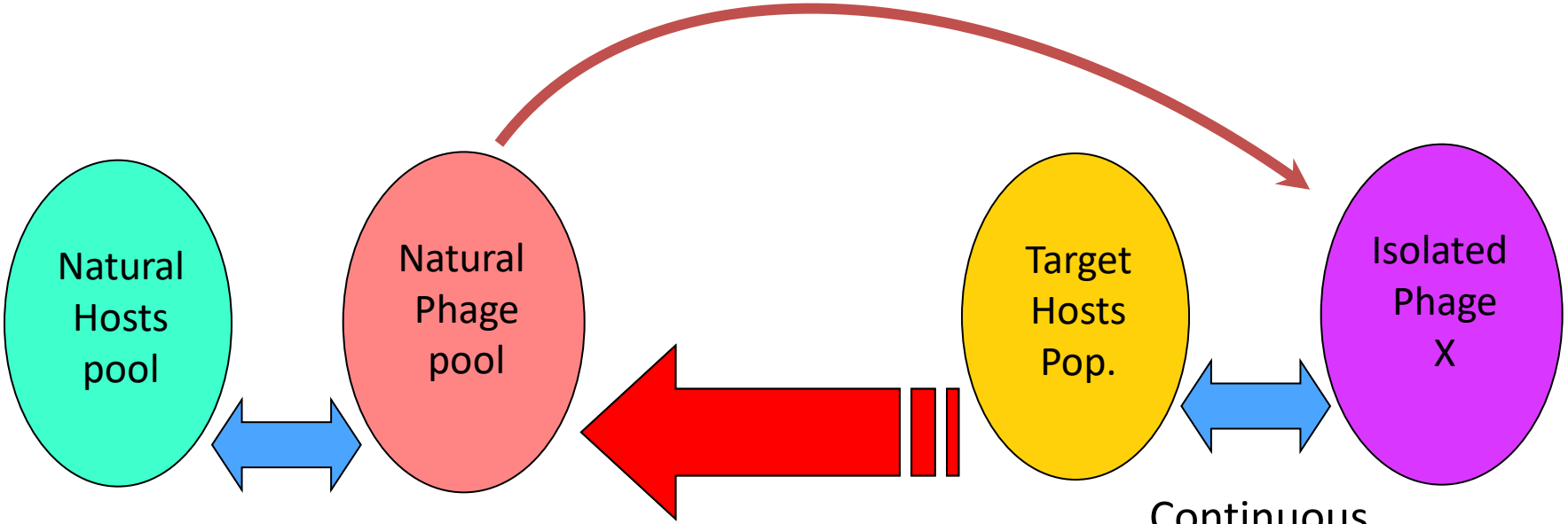
The first apparent answer

Bacteriophages, the natural predators of bacteria, could present the best potential to act as detectors-killers.

- Many are very host-specific,
- They only replicate in LIVE bacterial cells,
- Many kill the cells in which they replicate,
- As the phage progeny population increases that of the target diminishes (in a « closed » environment, few targets, if any, should escape), and
- They are extremely numerous and varied (they probably represent the most numerous « life forms » on the planet).

BUT the matter is NOT as simple as it first appears!

Co-evolution versus unidirectional predatory pressure.



Bacteria have existed for nearly 4 BILLION Years. They have so far resisted to EVERYTHING.
And it is certainly **NOT** for lack of phages!

The model-derived solution.

- ***What, in essence, is the problem?***

The bacterial targets will try anything to escape predation and we have no idea what will be the successful strategy. Furthermore, this strategy is likely to vary between locations (populations) for a same target.

- ***What do we need to achieve?***

We must be capable of always preceding the targets escape strategies, no matter what they could be.

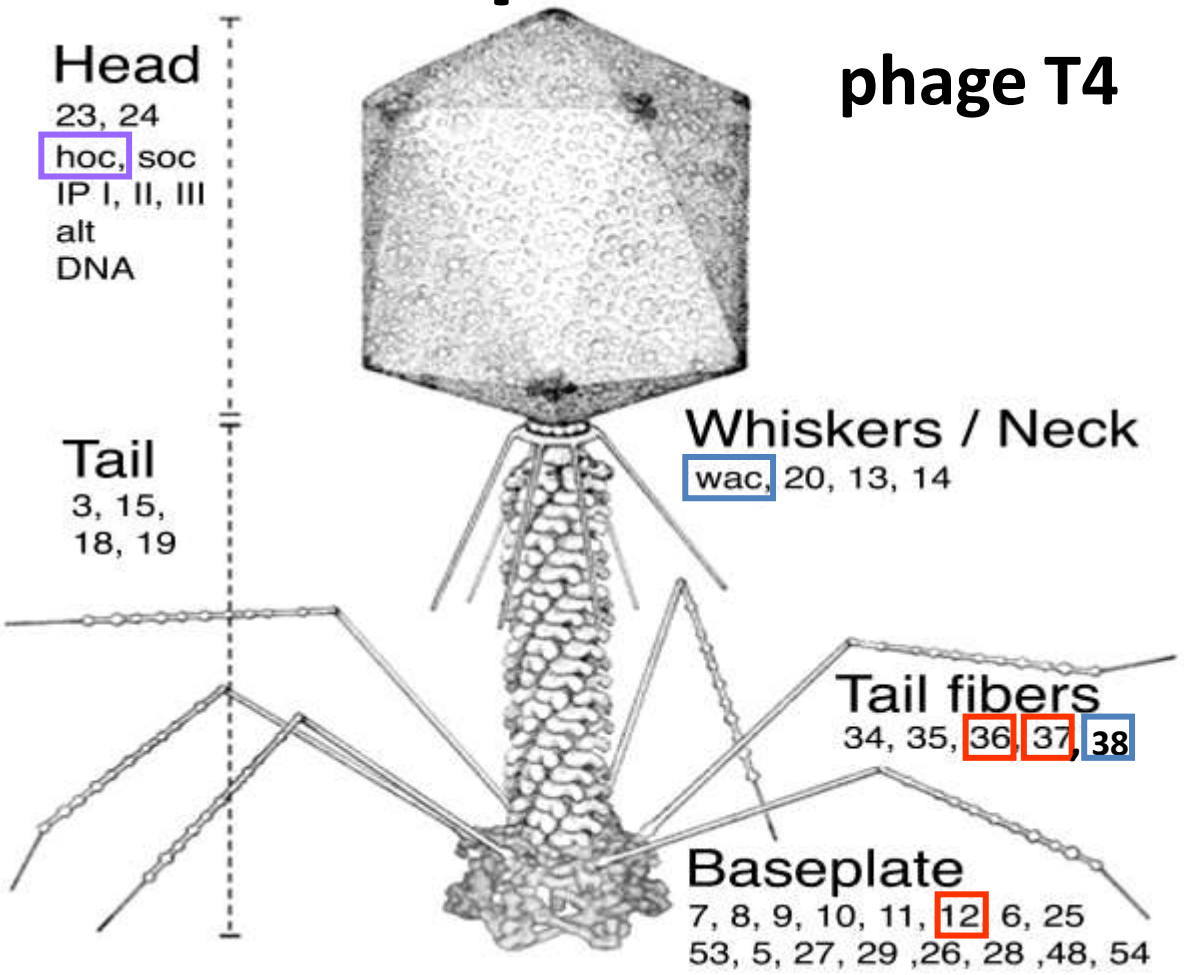
- ***The best-fit solution*** (model-derived):

we MUST adopt a stochastic approach.

It becomes necessary to

- abandon all idea of « natural phage pools » and,
- stochastically engineer phage banks in order to produce particles capable of targeting anything and everything while maintaining their capacity to replicate in the face of targets evasion attempts.

The problems:



phage T4

How to modify any of these proteins in N different regions, at X different sites, in Z different manners, all this simultaneously and then recombine **the multitude of variants** generated in a **population** of **obligate lytic** phages?

The technological answers.

Three proprietary technologies (invented at BMSystems) allowing the production of stochastically engineered phage banks.

TAPE™ Ⓟ (WO 2008/093009):

A technology allowing to rapidly & simultaneously introduce defined densities of random mutations in any number of selected regions within a gene while conserving intact any number of defined coding domains in this same gene.

Applicable to any known coding sequence.

RipH™ Ⓟ (WO 2009/090081):

A technology allowing to reversibly inactivate the genome of an obligate lytic phage within its host and carry out high efficiency homologous recombinations targeting multiple genes simultaneously without adversely affecting the host bacteria and the replicative capacity of the phage.

Ab-ACCUS™ Ⓟ (WO 2008/093010):

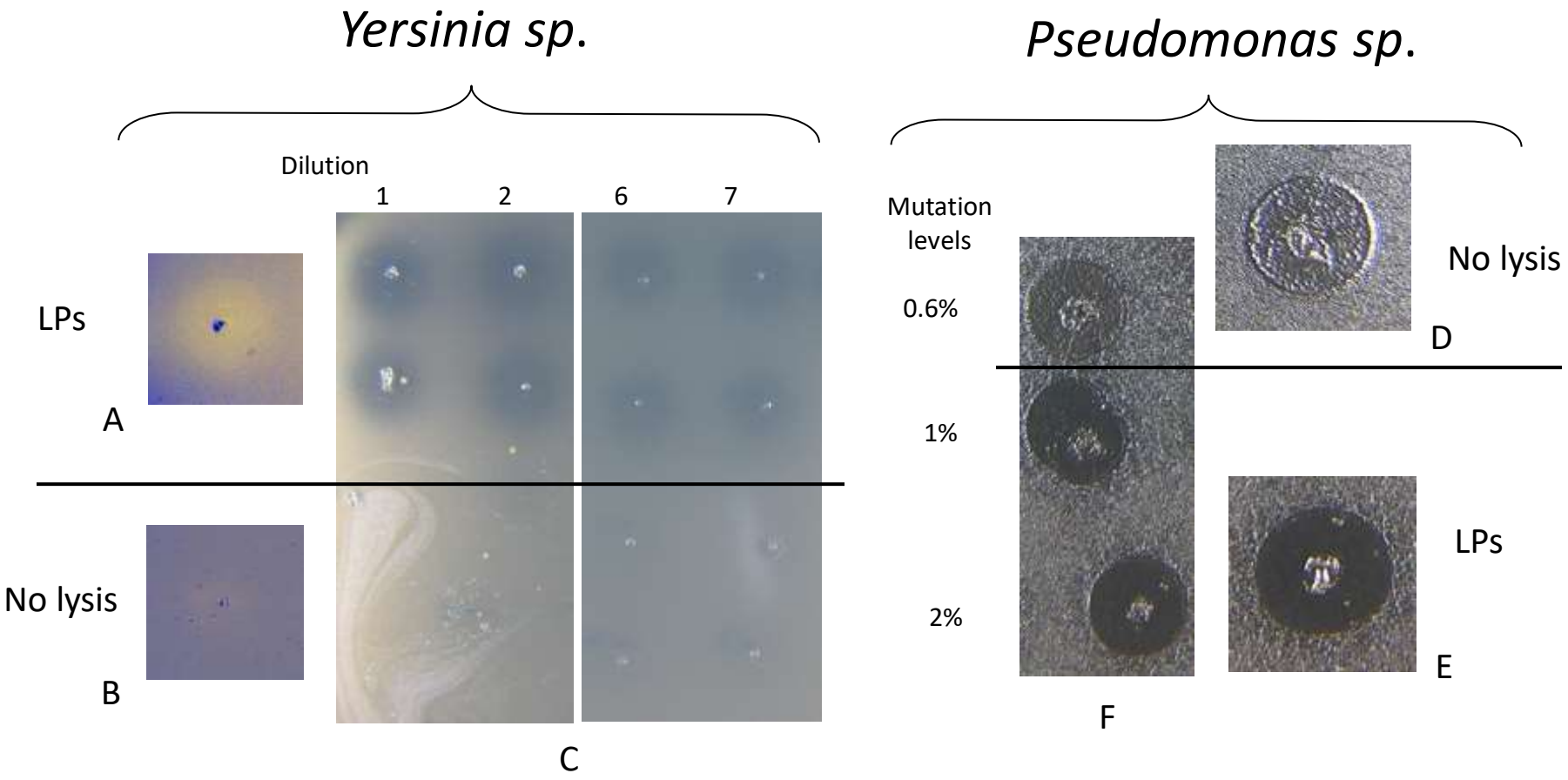
A recombination technology allowing the rapid & efficient production of lytic phage banks in which every individual differs from all others for any number of selected genes or other sequences.

Applicable to any phage and to any known sequence.

The results.

While T4 is specific to a narrow range of E. coli strains,

An engineered T4 bank contains variants capable of detecting and killing gram$^-$ bacteria far removed from E. coli.



Pouillot F, Blois H & Iris F (2010); Biosecur Bioterror. **8** (2): 155-169.

Practical consequences

(besides high-profile publications)

- Three technological patents with broad applications,
- Creation and financing of a Bio-Pharma company (Pherecydes Pharma) specialised in biodefense & biosecurity (40% owned by BM-Systems),

- Research program with French civil and defence health-services,
- Discussions with US food industry firms,
- Contract with anti-bodies producer & discussions with enzymes producers.

All this in less than 3 years.

From our point of view, under the street light is definitely not the right place to search!

Nevertheless, it MUST be remembered that

Models are AIDS to thought

NOT a replacement for it.

Collaborations (2004-2012):

(that we are allowed to mention)

L'Oreal: Industrial biotech Program "Synthons"; *Success, 1 patent (pending).*

On going, 3 publications.

Max Planck Institute (Munich): Projet "Chronic Anxiety". *Both successfully completed.*

US, EU & French Awards; 1 therapeutics class patent; 1 publication.

CEA: Projects "CJD" ; "

All 3 successfully completed, 3 publications.

Inserm: Projects "Tumoral Progression"; "Therapeutic Resistance"; "ADAM 15 RGD". *Success, 1 publication.*

CNRS: Projet "Müllerian Regression".

Useful Downloads

For more information about information quality & reliability

- A new evidence published in Sciences confirms the poor reproducibility (less than 1/3) of studies published in peer-reviewed.
- An estimated 85% of current published research findings are false or exaggerated: How to Make More Published Research True. Published in PLOS Medicine by John P. A. Ioannidis Meta-Research Innovation Center at Stanford (METRICS), Stanford University.
- Diagnosing the decline in pharmaceutical R&D efficiency. Published Nature Review Drug Discovery. The diagnostic is clear for our industry.
- Believe it or not: how much can we rely on published data on potential drugs targets? Their title is crystal clear. Published Nature Review Drug Discovery

Heuristic modeling principles and case studies

- The discovery of Innovative Therapeutic Approaches: Under the street light is not the right place to search BIT's 10th Annual Congress International Drug Discovery Science and Technology 2012 November 8-10, 2012, Nanjing, China
- The Differences & Complementarities Between « Heuristic » and « Mathematical» approaches. The scientific presentation given by Dr. François IRIS (CSO BMSystems) during the EPA (European Psychiatric Association) conference in 2011.

For more information Email: manuel.gea@bmsystems.net

Questions

Contact: www.bmsystems.net
Email: manuel.gea@bmsystems.net