Collaborative Virtual Organisation & Infrastructure for Anti-Malarial Drug Design

Barry Hardy PhD
Director of Communities & Research Activities
Douglas Connect, Switzerland

Bio-IT World Europe Conference, Hannover, Germany
11-13 October 2011

Community of Practice starting point

innovationwell

echeminfo

We started with community development interactions, both virtual and face-to-face…

Knowledge Cafe Discussions

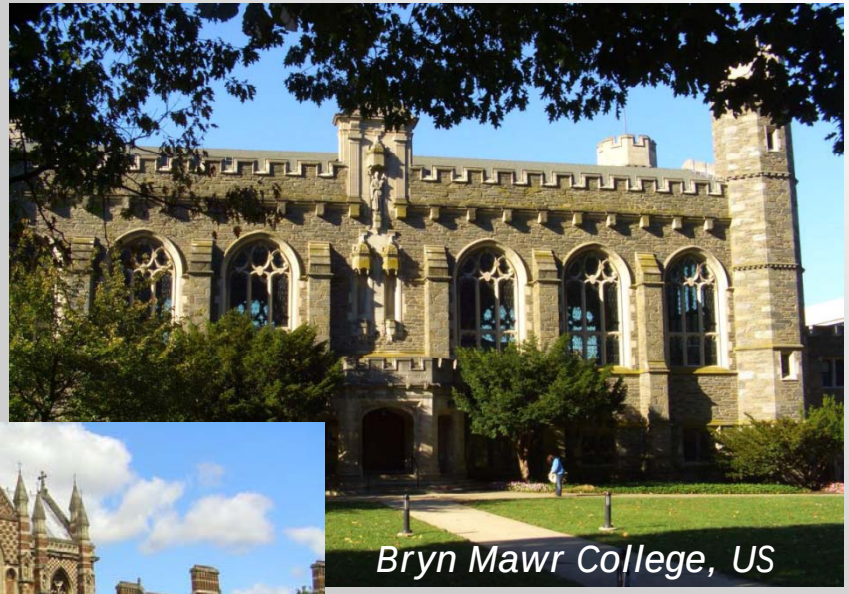
SAM

SYNERGY

Community of Practice starting point

innovationwell echeminfo

…and continued by holding workshops and InterAction Meetings in US and Europe


Bryn Mawr College, US


Oxford University, UK

SAM SYNERGY

Community of Practice starting point

echeminfo

Community of Practice for
Drug Discovery & Development

Latest Advances in Drug Discovery & Planning Methods

Advanced Training Workshop

Oxford University, June 25-29, 2007

Interactive pragmatic workshops with leading experts and industry practitioners...

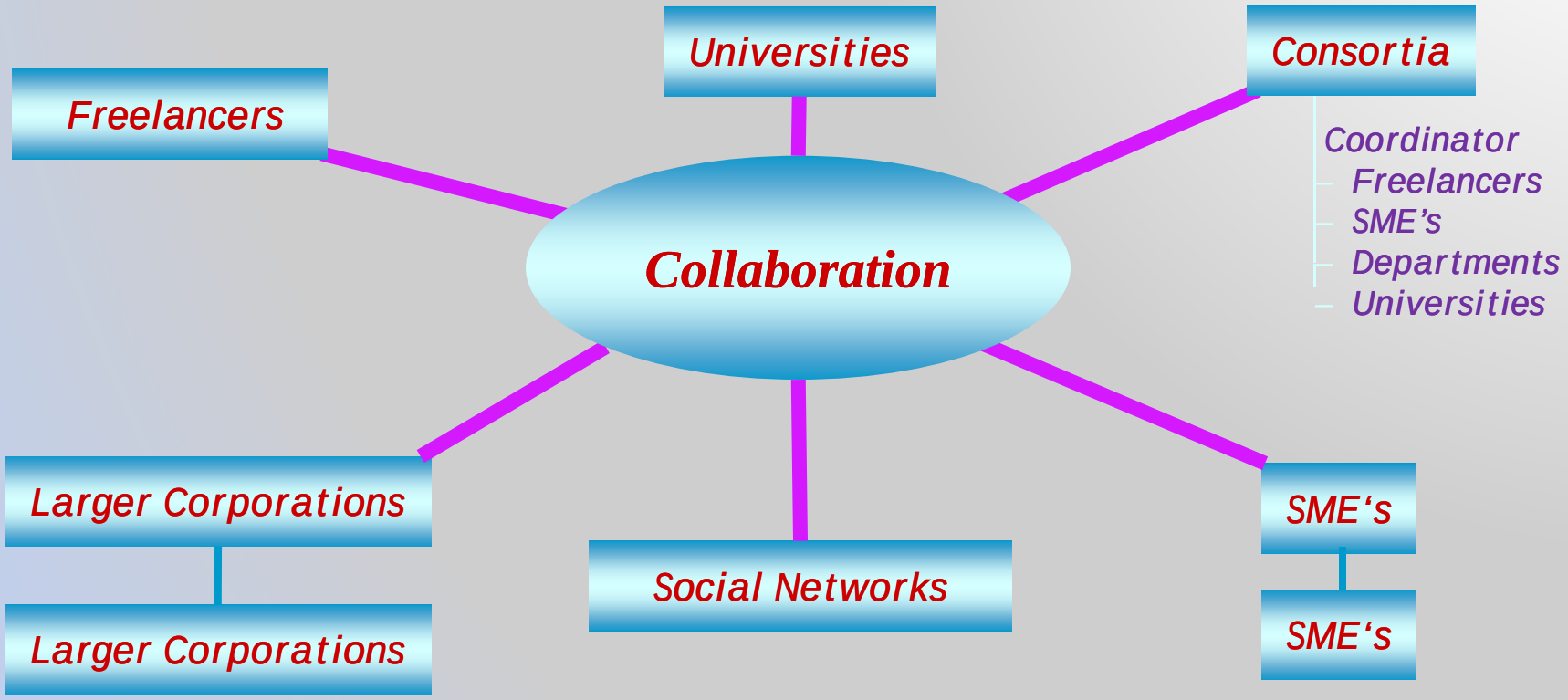
- Protein Target & Ligand Modelling
- Virtual Screening & Docking
- Structure-based Drug Design
- Pharmacophore Models
- Focused Library Design
- ADME, QSAR & Predictive Toxicology

Study problems in detail using leading-edge

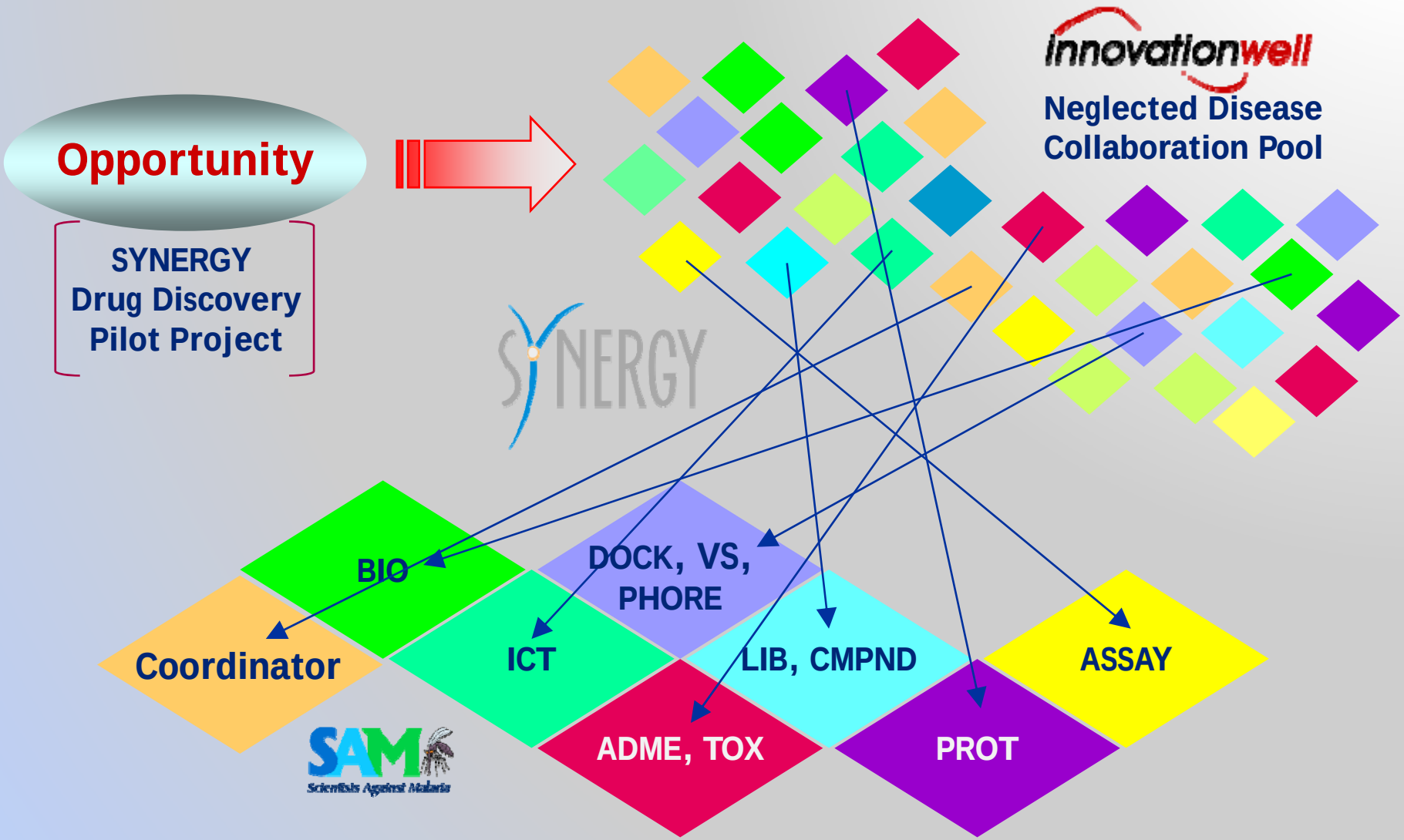
Community of Practice starting point

Community and workshop interactions created a valuable environment and network from which to build collaborations

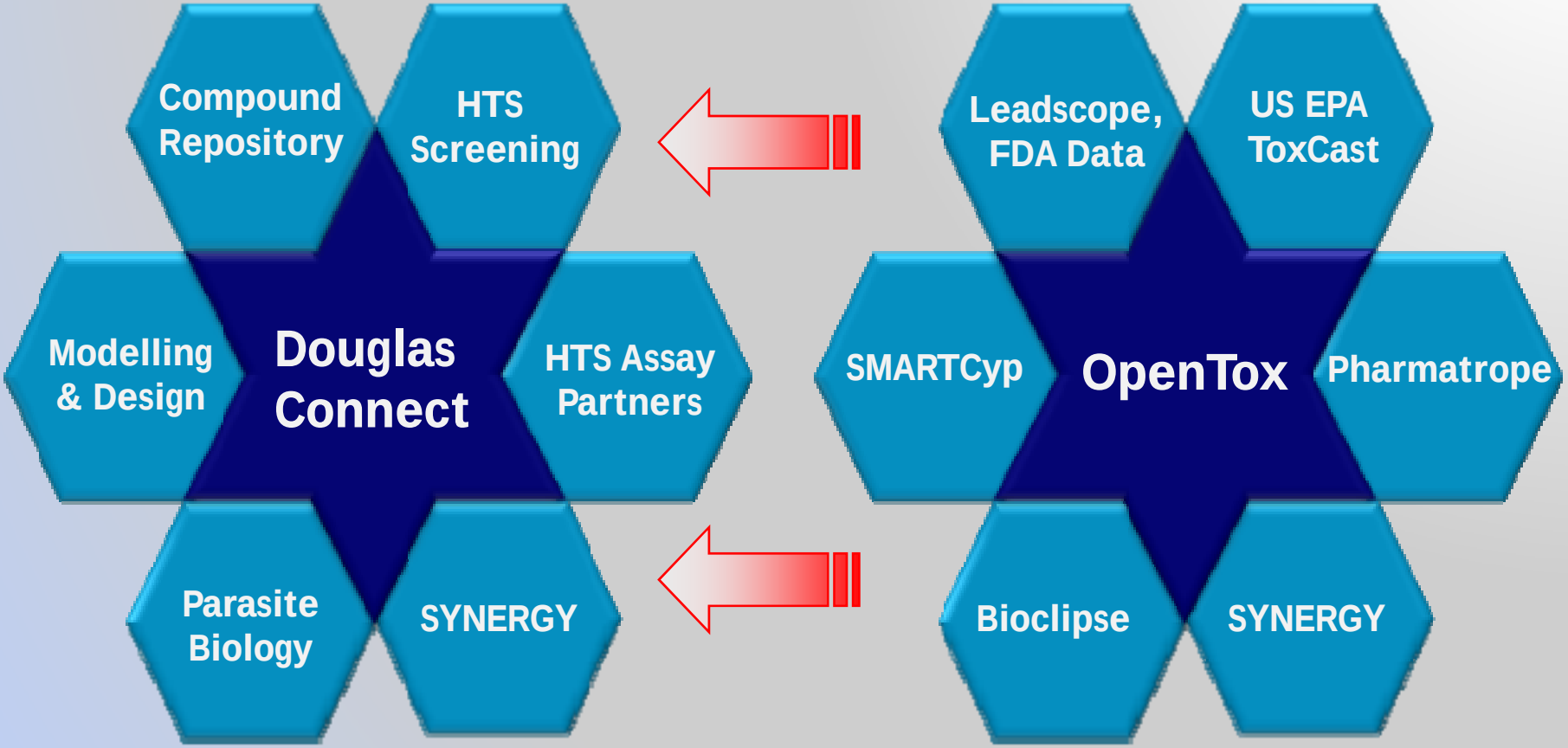
Emerging Multilateral Collaboration Landscape



Formation of VO from Collaboration Pool



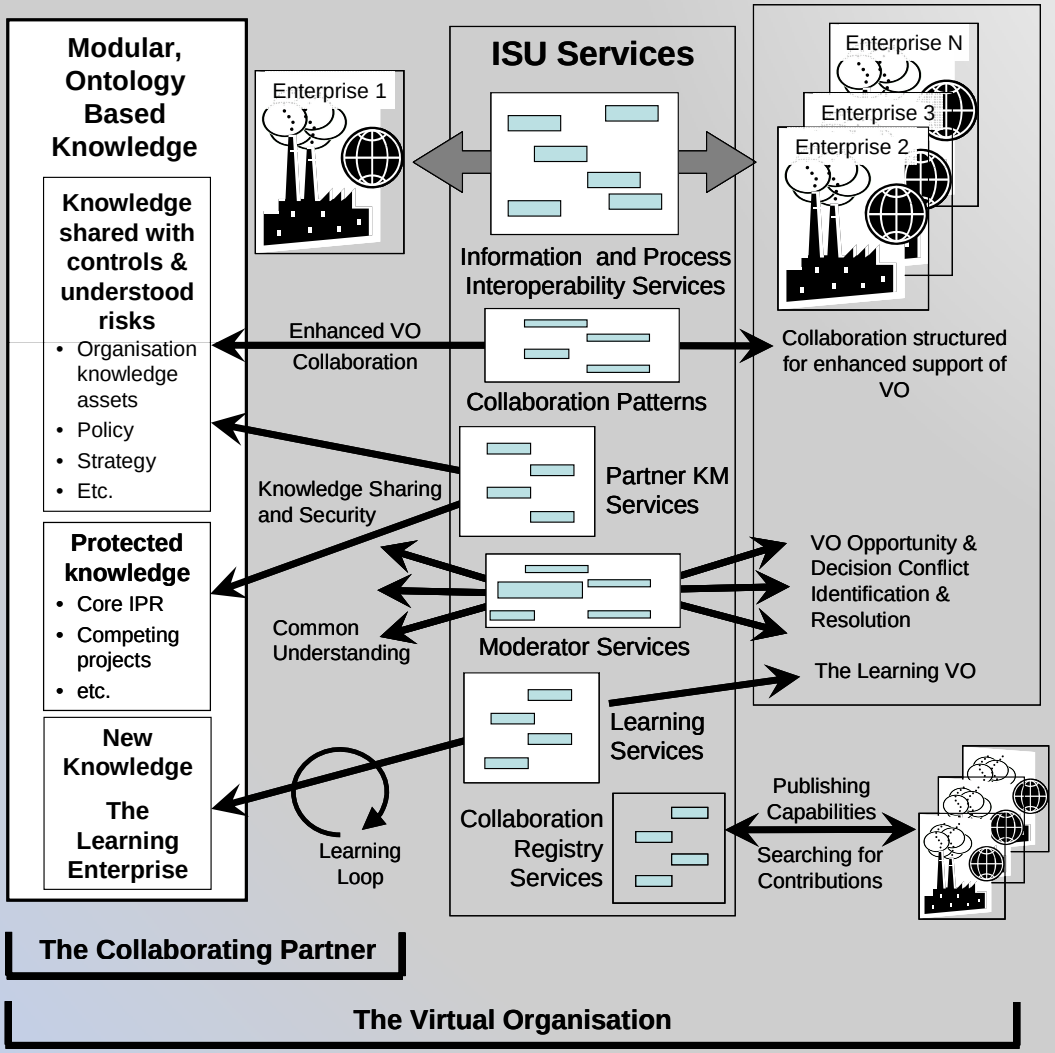
Virtual Organization Operational Pilots



Scientists Against Malaria VO

Predictive Toxicology VO

SYNERGY Collaboration Services



Operational Pilot 1 – Collaborative Drug Discovery

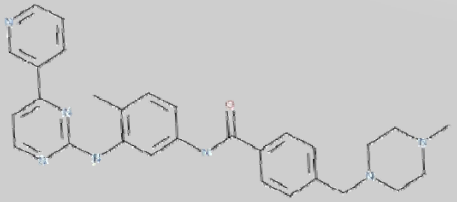
Target Protein Structure

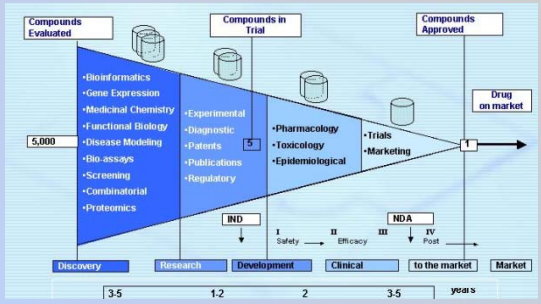
VO

- Drug Design Techniques
- Molecular Modelling
- Virtual Screening
- Biological Assays
- Synthesis or Acquisition
- Testing

Output result

Drug Leads

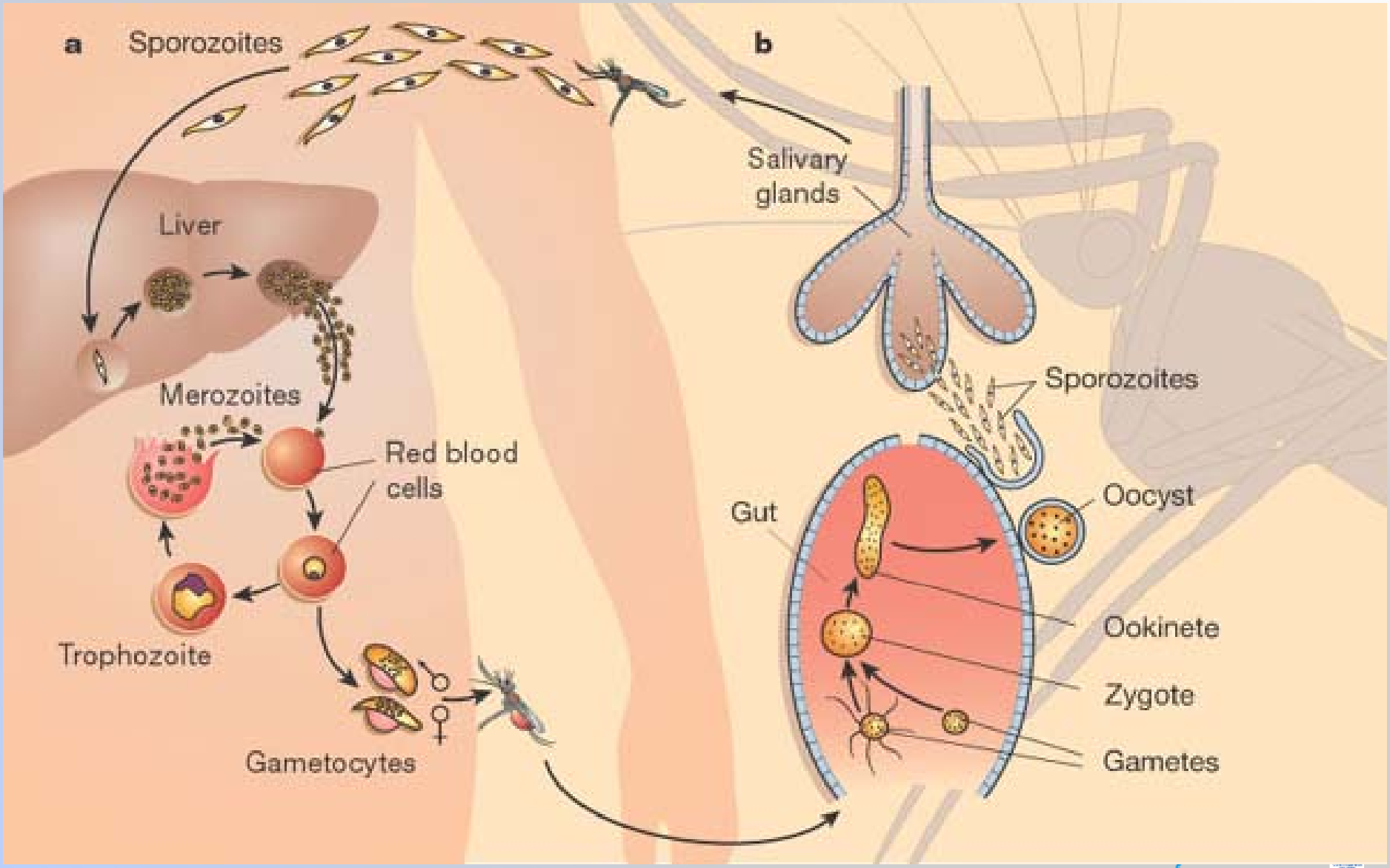




Drivers

- *Pharmaceutical Industry pressures on R&D environment*
- *Progressing innovation and discoveries for Neglected Diseases*

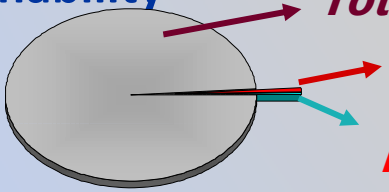
The (complicated) Plasmodium Life Cycle



Malaria Treatment: Lack of Investment

- 2.5 billion people at risk
- 500 M cases yearly
- ca. 1 M deaths yearly
- Many child fatalities
- Brain Damage, Impaired Development
- Few drugs, no vaccine yet
- Impact on

Education, Community, Income, Conservation, Sustainability



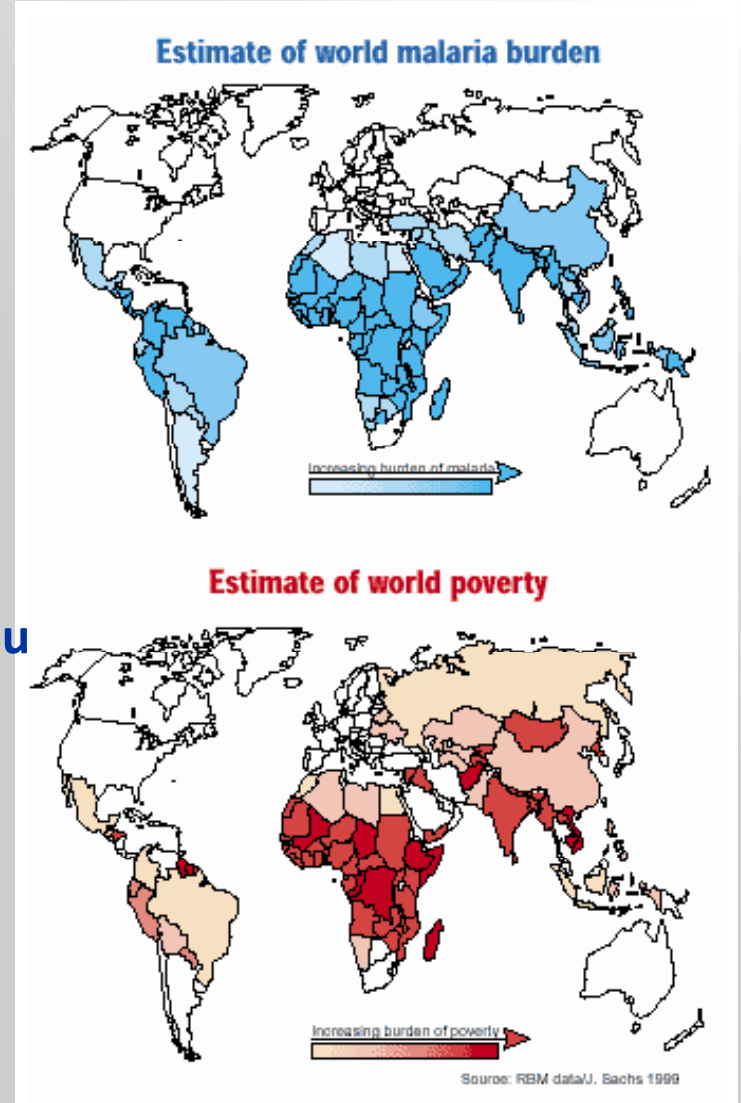
Total: 1223

Tropical diseases: 13

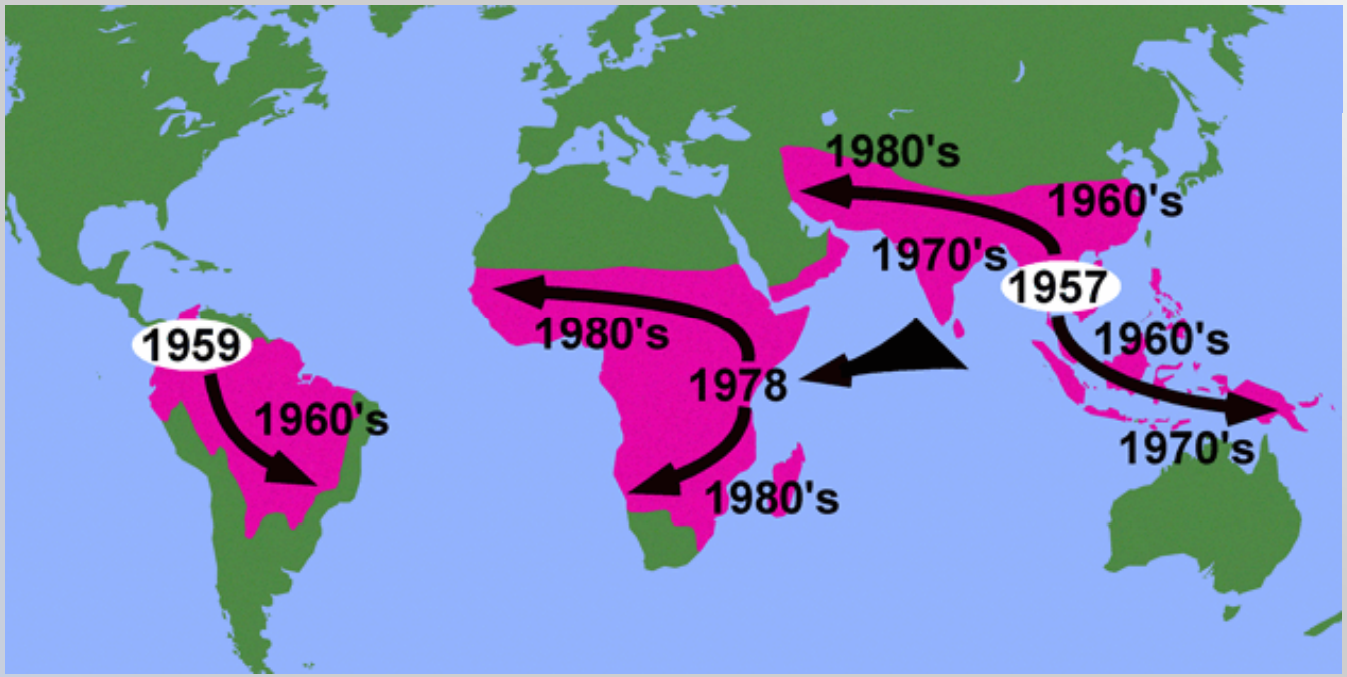
Malaria: 3

Drug development outcome, last quarter of the XXth century

Greenwood & Mutabingwa, Nature 415:670-672



Drug Resistance is a challenge



Emergence and spread of chloroquine resistance

Scientists Against Malaria Founding Partners

- **Barry Hardy** & **Roman Affentranger** (Douglas Connect)
- **Alessandro Contini** (University of Milan)
- **Hugo Gutierrez de Teran** (Public Galician Foundation of Genomic Medicine)
- **Jeffrey Wiseman & Matt Clark** (Pharmatrope)
- **Jeff Spitzner** (Rescentris)
- **Ruben Papoian**, **William Seibel** & **Sandra Nelson** (Univ. of Cincinnati Drug Discovery Center)
- **Sharon Bryant** (Inte:Ligand)
- **Andrew Wilks** & **Isabelle Lucet** (Monash University)
- **Christian Doerig** Coordinator of the FP7 MALSIG project on signalling in Malarial parasites
- **Matteo Dal Peraro** (EPFL, Lausanne)

www.scientistsagainstmalaria.net

SAM

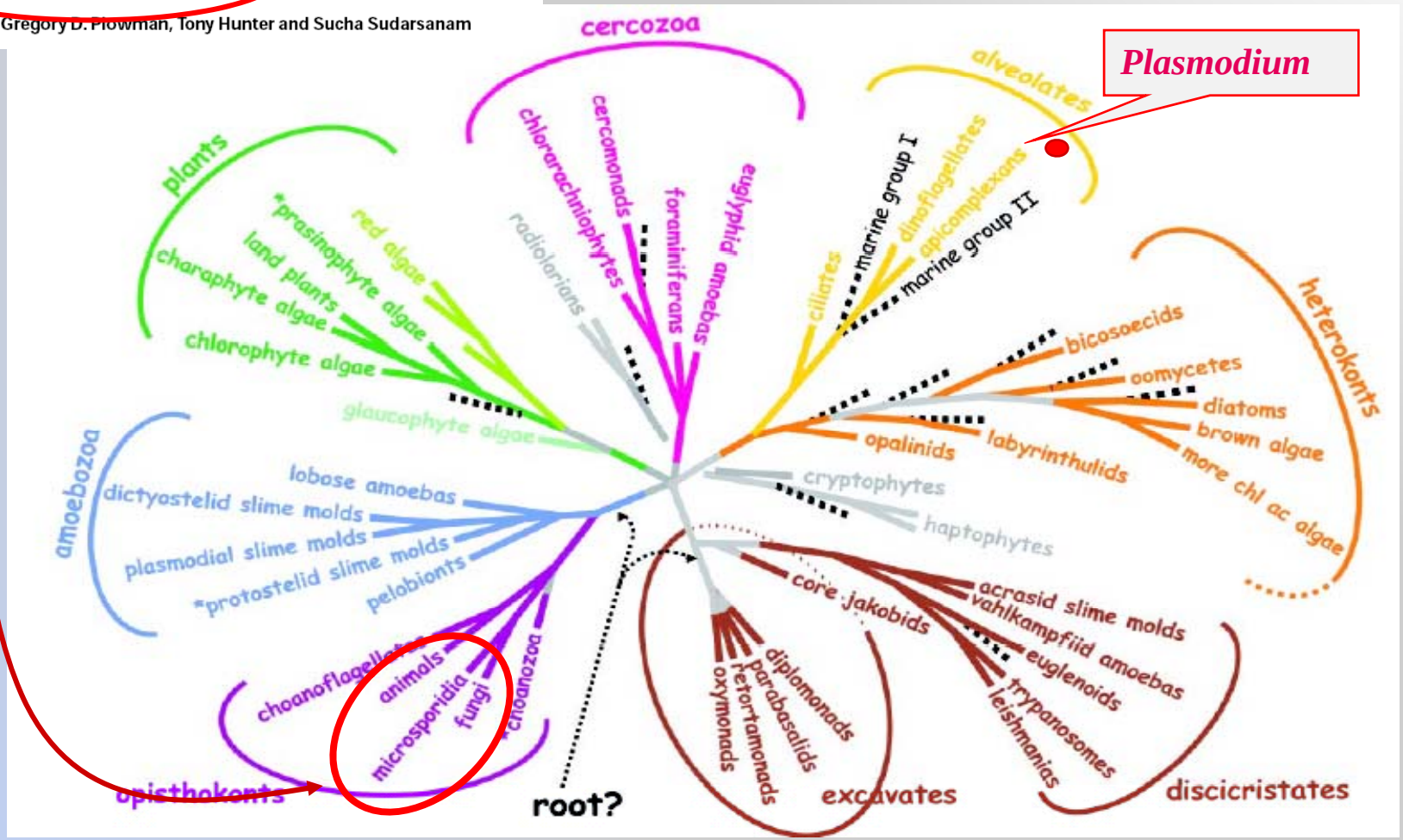
SYNERGY

Plasmodium Life Cycle & Kinome

514 Review TRENDS in Biochemical Sciences Vol.27 No.10 October 2002

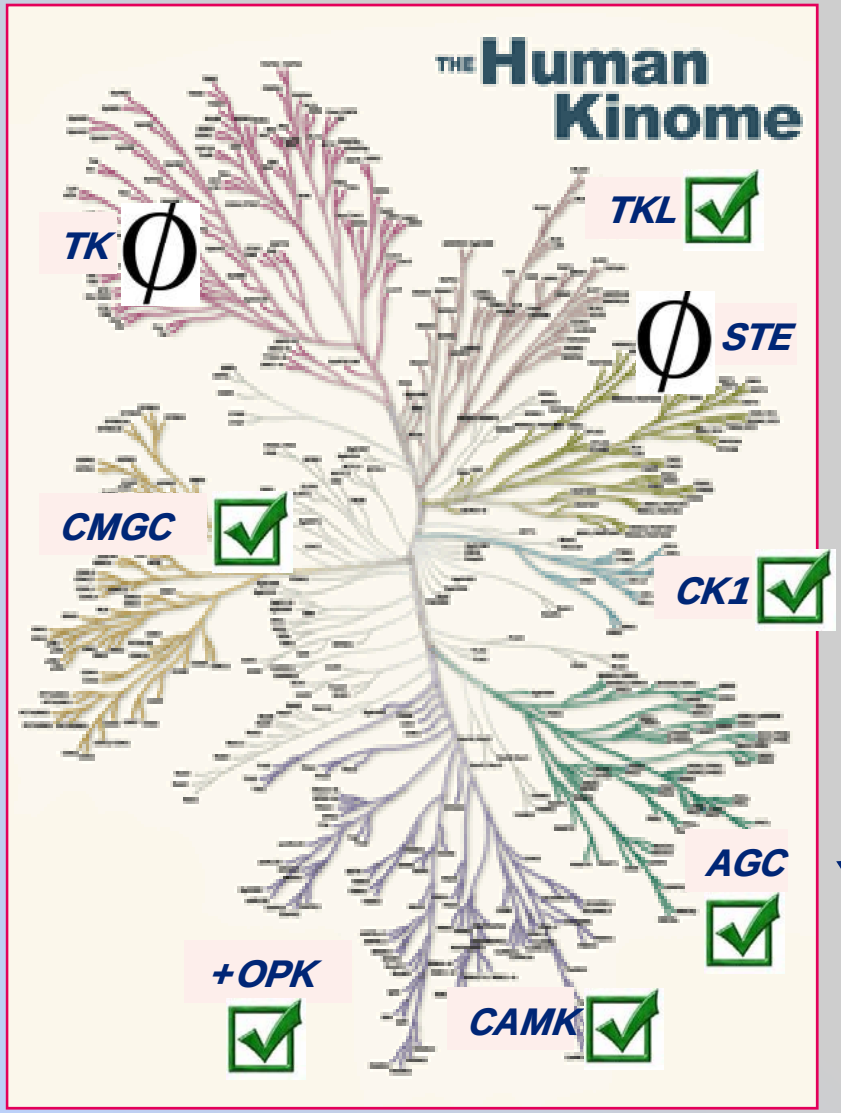
Evolution of protein kinase signaling from yeast to man

Gerard Manning, Gregory D. Plowman, Tony Hunter and Sucha Sudarsanam



Baldauf, Science, 2003

Plasmodium Life Cycle & Kinome



PlasmoDB mining

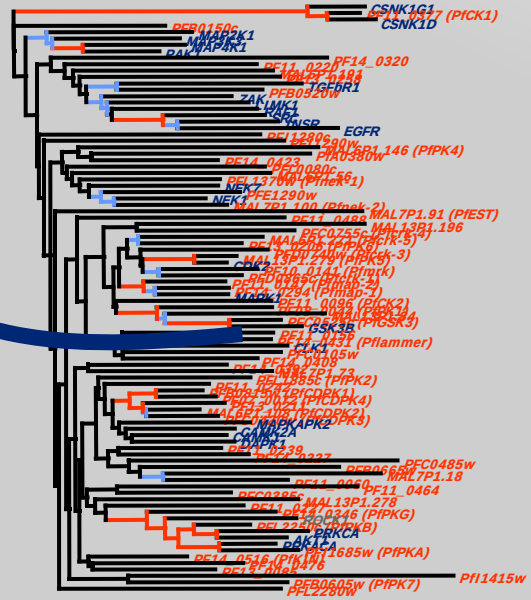
(HMM, BLAST, Gene Ontology prediction)

↓

Alignment with selected human PKs, manual edition

↓

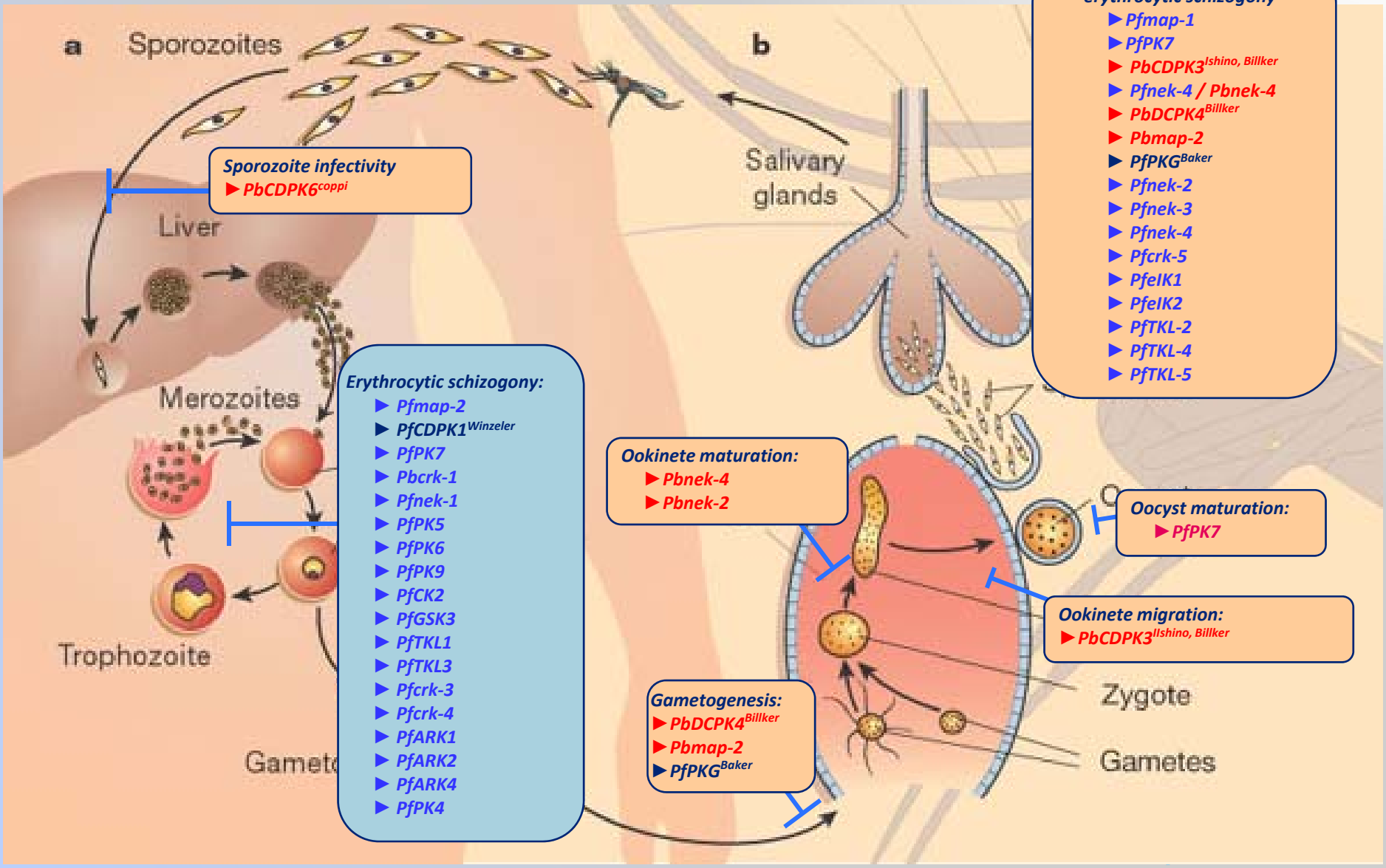
Phylogenetic tree



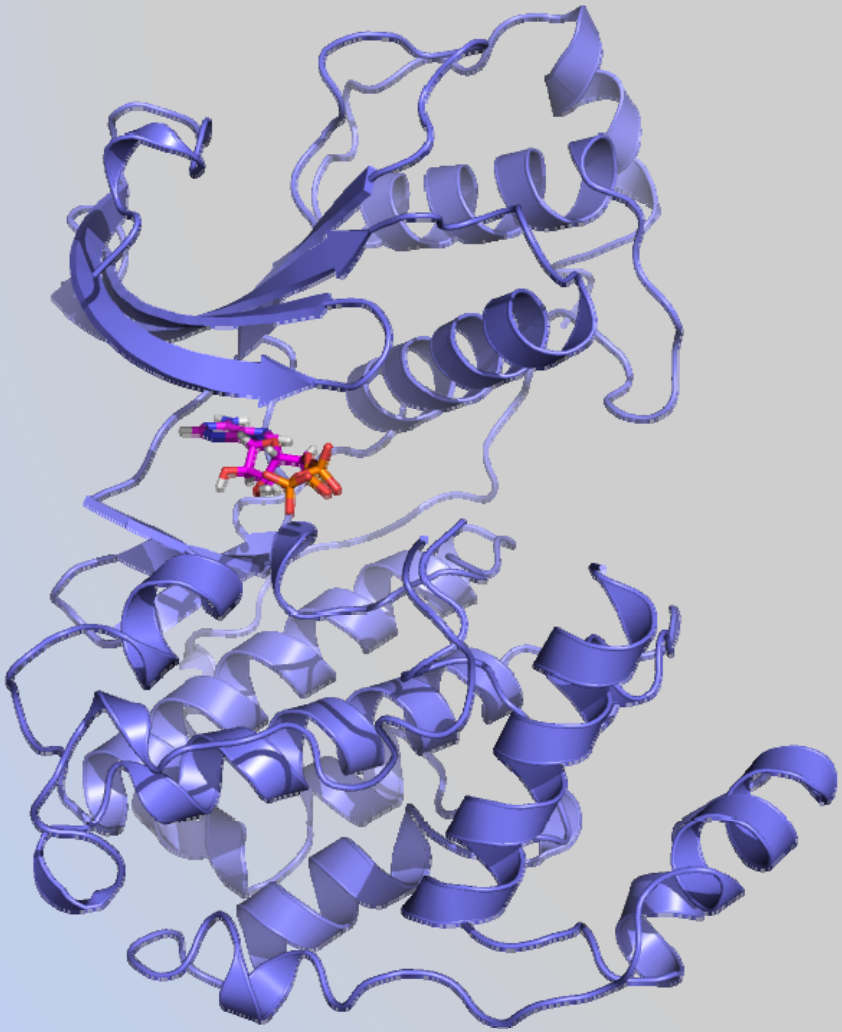
- **518 protein kinases (2% of genes)**
- **Involved in essentially all cellular processes (30% of the proteome is phosphorylated)**

SYNERGY

Plasmodium Life Cycle & Kinome



Target Model



- Starting point – no protein structure, no known ligand/inhibitor
- Initial model of PfMAP2 Kinase Protein built based on existing knowledge
- Library of Potential Inhibitors created (ca. 1.2 M structures)
- Virtual screening runs carried out at computing centres in Italy, Spain and USA
- Protein expressed in Monash, Australia and shipped to screening centre at Univ. Cincinnati where assays were developed and run

Homology Modelling

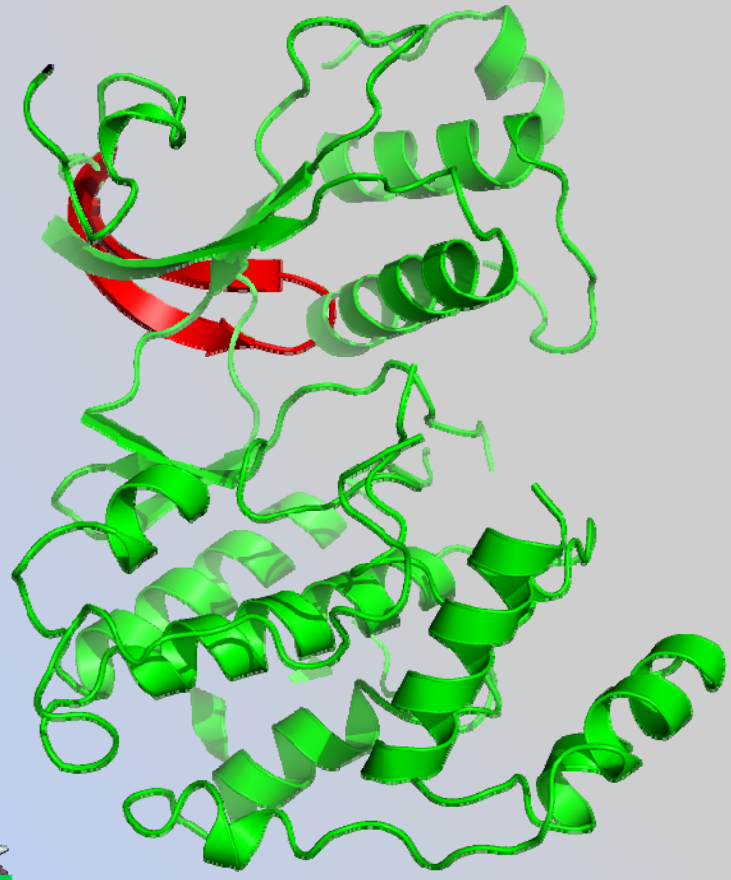
Alignment of Pfmap-2 with 1GOL and 1CM8

```
 _aln.p   70        80        90       100       110       120       130
1golA     ---------------AAAAAAGPEMVRGQVFDVGPRYTNLSYIGEGAYGMVCSAYDNLNKVRVAIRK
1cm8A     --------------RSGFYRQEVTKTA---WEVRAVYRDLQPV------AVCSAVDGRTGAKVAIKK
pfmap2    DNISKNCNIVEKKNNKSKEEKINIKEAIIKNVKVPDNYEIKHLIGRGSYGYVYLAYDKNANKNVAIKK
 _consrvd                                  *   *             *  * *      *** *

1golA     I-SPFEHQTYCQRTLREIKILLRFRHENIIGINDIIR-APTIEQMKDVYIVQDLMETDLYKLLKTQ-H
1cm8A     LYRPFQSELFAKRAYRELRLLKHMRHENVIGLLDVFTPDETLDDFTDFYLVMPFMGTDLGKLMKHE-K
pfmap2    VNRMFEDLIDCKRILREITILNRLKSDYIIRLHDLIIPEDLL-KFDELYIVLEIADSDLKKLFKTPIF
 _consrvd     *       *  **   *        *   *              * *      ** ** *

1golA     LSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKICDFGLARVADP------------
1cm8A     LGEDRIQFLVYQMLKGLRYIHAAGIIHRDLKPGNLAVNEDCELKILDFGLARQADS------------
pfmap2    LTEQHVKTILYNLLLGEKFIHESGIIHRDLKPANCLLNQDCSVKICDFGLARTINSDKDIHIVNDLEE
 _consrvd *         *  * *   **     ****** *   *  *  ** ******

1golA     --DHDHTG--------FLTEYVATRWYRAPEIMLNSKGYTKSIDIWSVGCILAEMLS-----------
1cm8A     --EM.-------------G.VVTRWYRAPEVILNWMRYTQTVDIWSVGCIMAEMIT-----------
pfmap2    KEENEEPGPHNKNLKKQLTSHVVTRWYRAPELILLQENYTNSIDIWSTGCIFAELLNMMKSHINNPTN
 _consrvd                        * ********  *    **   **** *** **

1golA     NRPIFPGK----------------HYLDQLNHILGILGSPSQEDLNCIINLKARNYLLSLPHKNKVP
1cm8A     GKTLFKGS----------------DHLDQLKEIMKVTGTPPAEFVQRLQSDEAKNYMKGLPELEKKD
pfmap2    RFPLFPGSSCFPLSPDHNSKKVHEKSNRDQLNIIFNVIGTPPEEDLKCITKQEVIKYIKLFPTRDGID
 _consrvd     * *                     ***  *    * *  *           *    *

1golA     WNRLFPNADSKALDLLDKMLTFNPHKRIEVEQALAHPYLEQYYDPSDEPIAEAPFKFDMEL-DDLPKE
1cm8A     FASILTNASPLAVNLLEKMLVLDAEQRVTAGEALAHPYFESLH-------QVQKYDDS------RTLD
pfmap2    LSKKYSSISKEGIDLLESMLRFNAQKRITIDKALSHPYLKDVRKENLENFSTEKIILPFDDWMVLSET
 _consrvd               **  **      *     ** ***
```

PfMAP2 modelling

Three template pdbs selected:

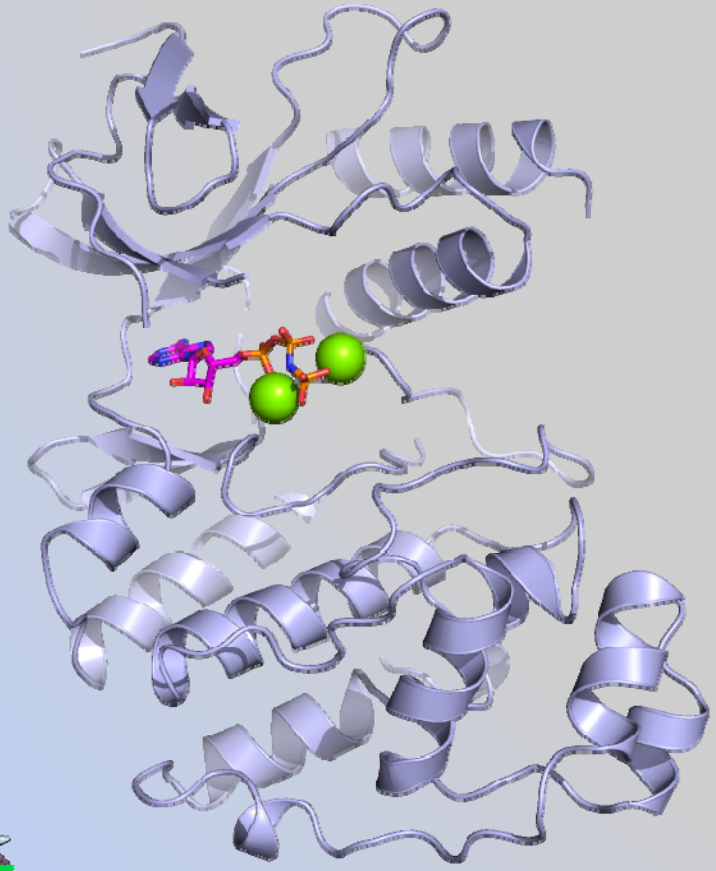
3N9X_PRE-RELEASE.pdb



PfMAP2 modelling

Three template pdbs selected:

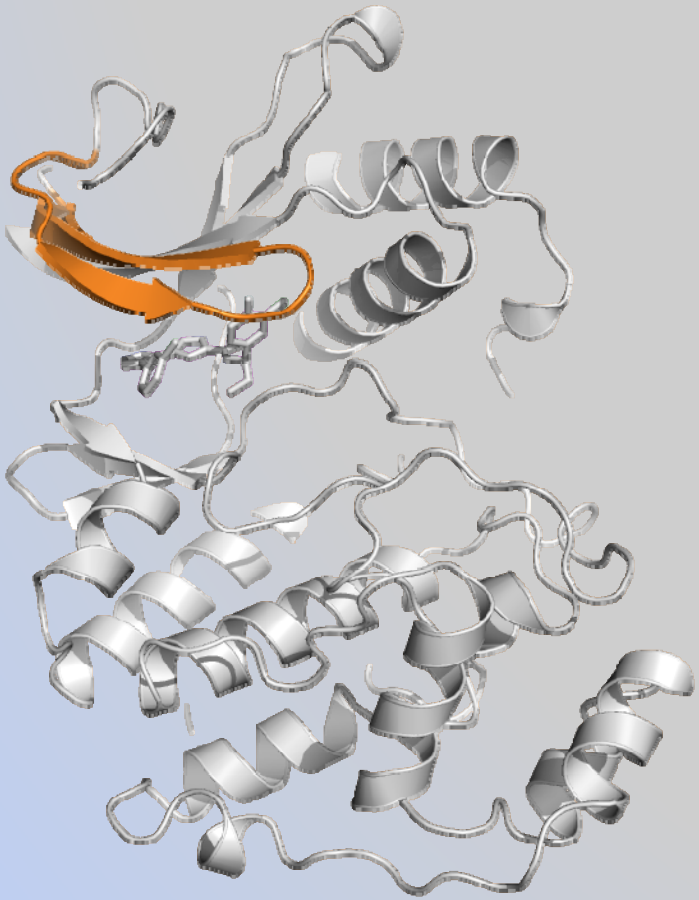
1CM8.pdb

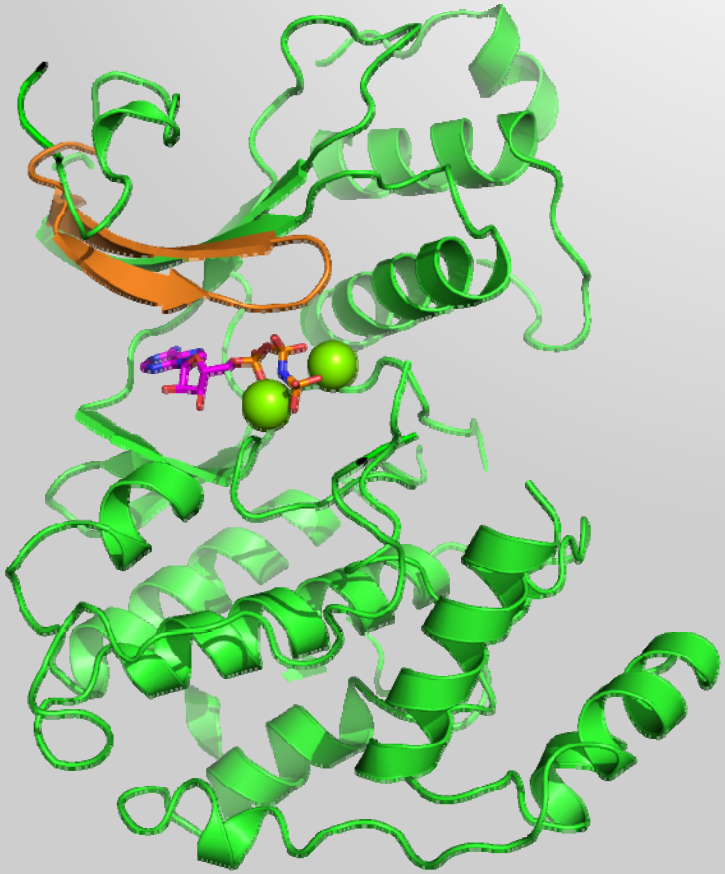


PfMAP2 modelling

Three template pdbs selected:

2OJJ.pdb





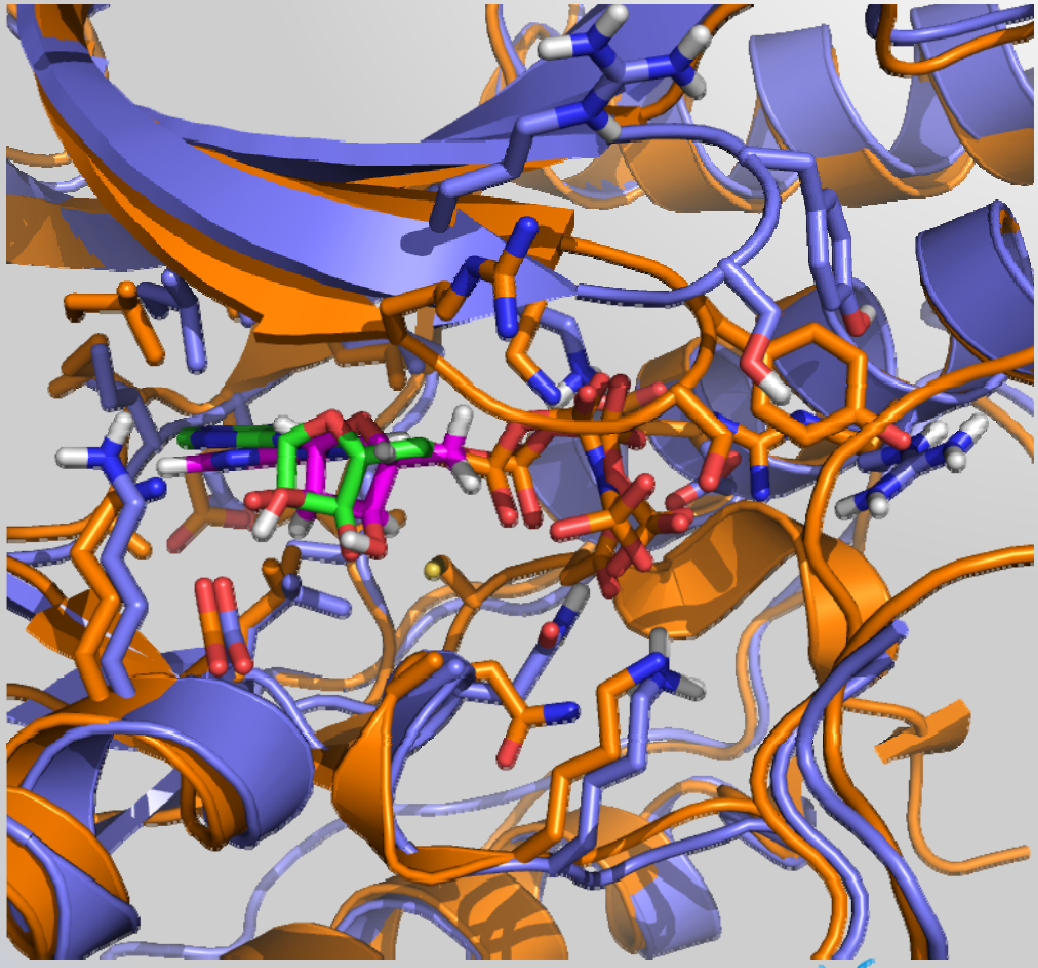
PfMAP2 Crystal Structure Model Comparison

3NIE.pdb protein structure by SGC was released August 11, 2010

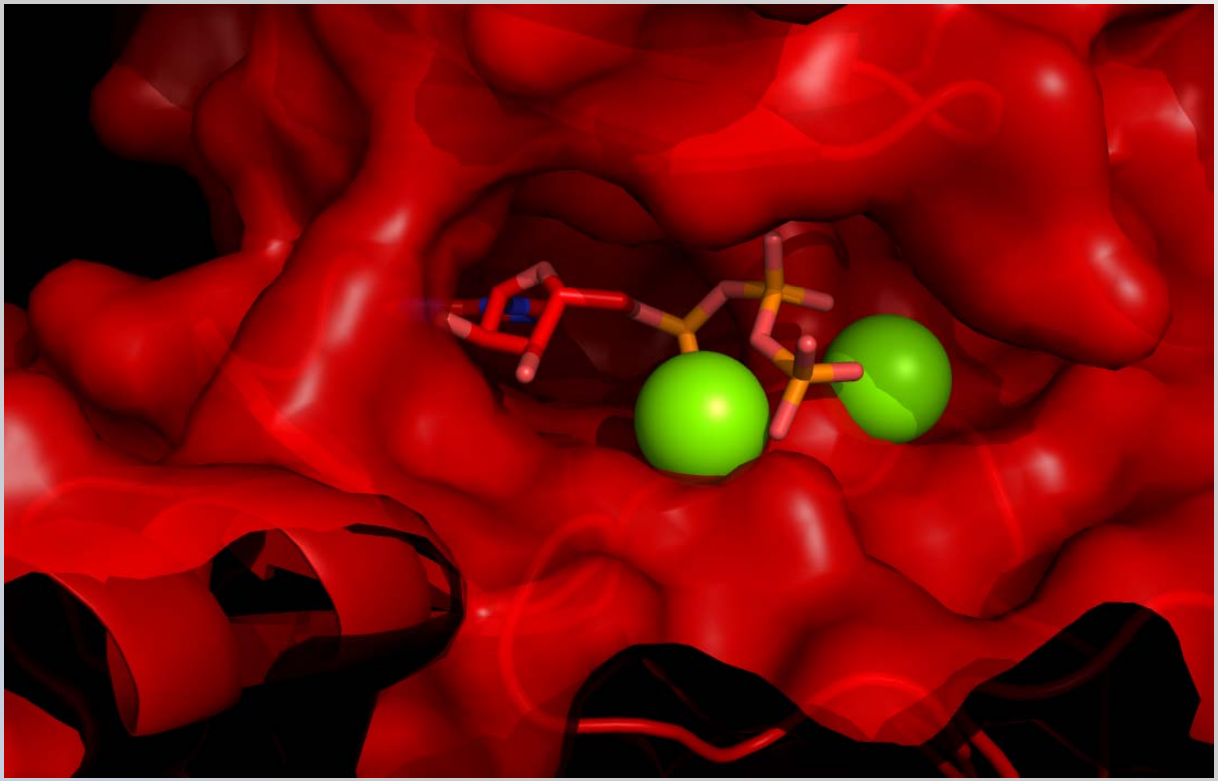
Overall, our model was quite accurate

Conformation of ATP was modelled well

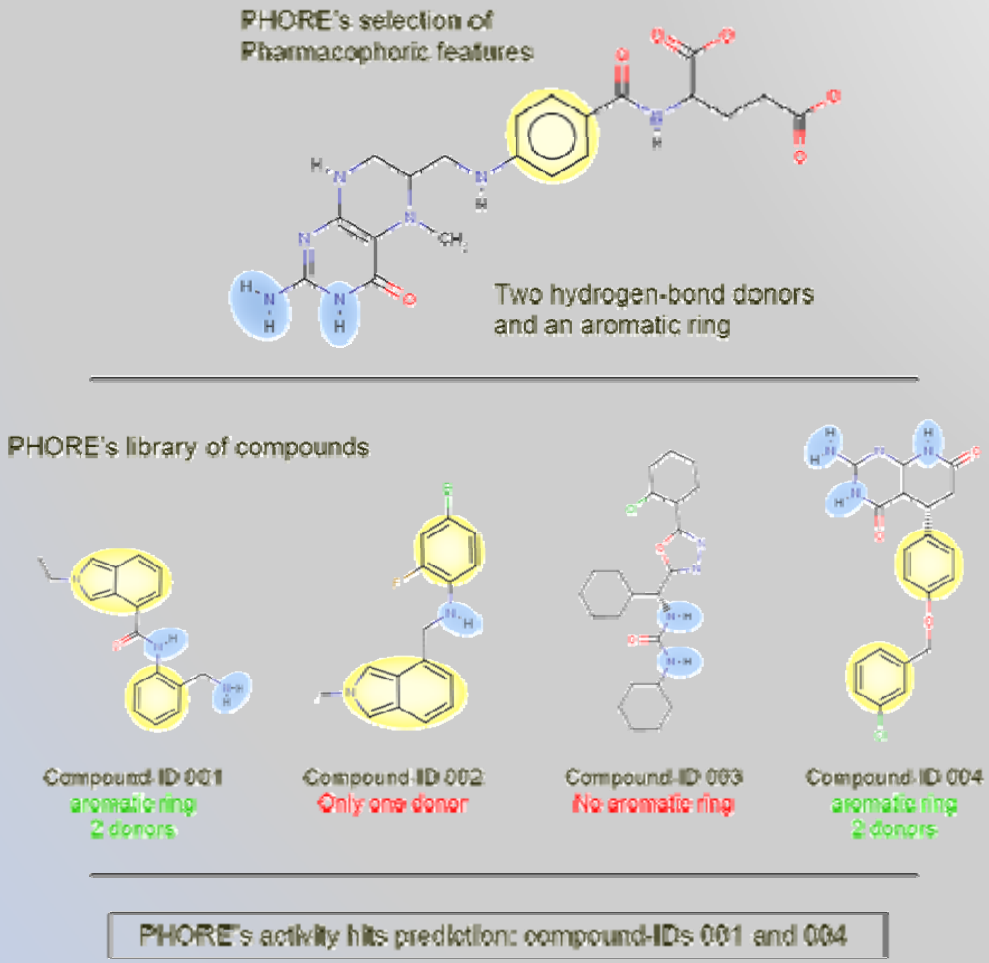
Binding pocket residues were mostly ok



Binding Pocket

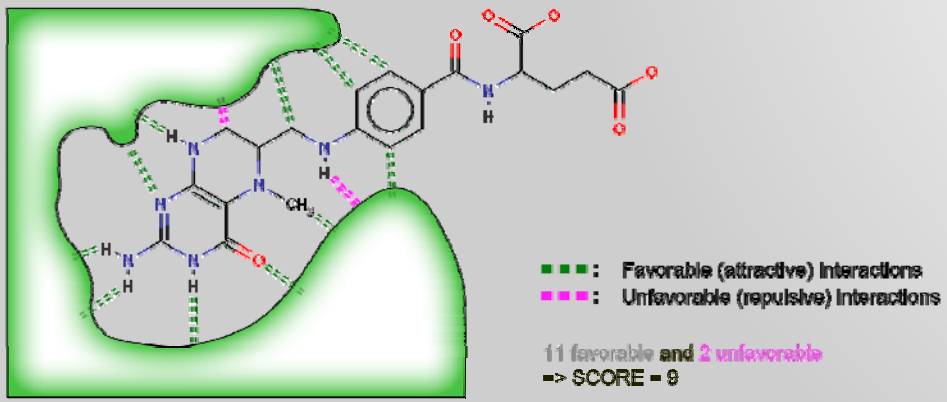


Pharmacophore-based Screening

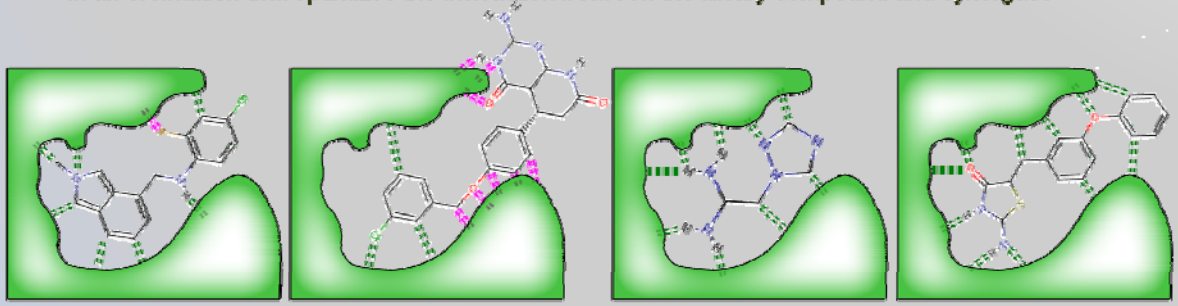


Dock Screening Prediction

γ-synergine bound to synergase (schematic depiction)



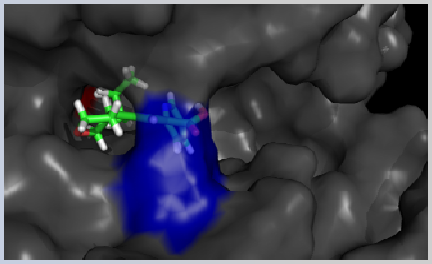
Each member of DOCK's library of compounds is placed in the binding pocket of synergase in an oreintation that optimizes the interaction between the library compound and synergase

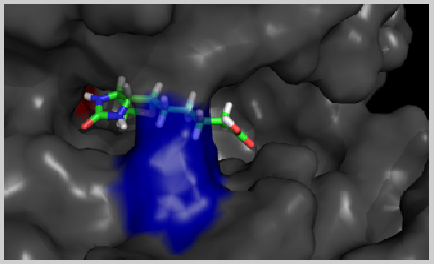


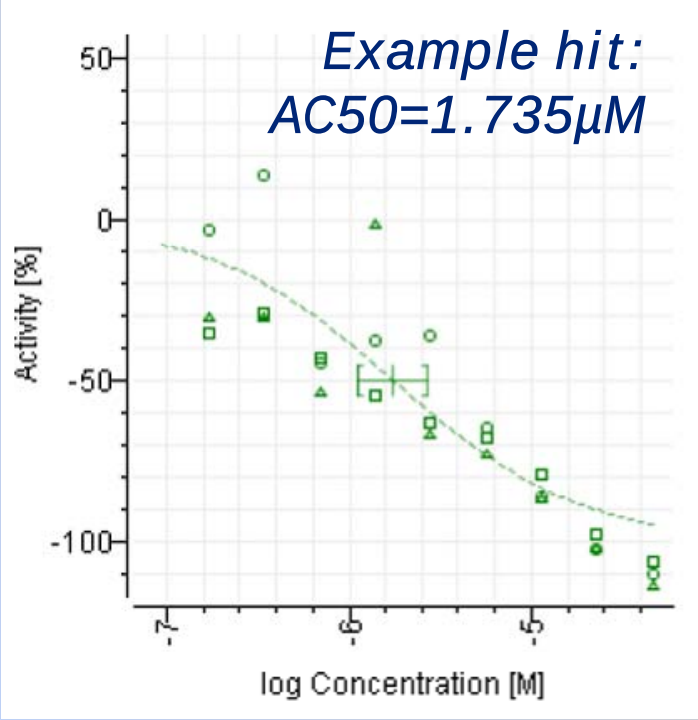
Compound-ID 002	Compound-ID 004	Compound-ID 005	Compound-ID 006
7 favorable	3 favorable	9 favorable	11 favorable
1 unfavorable	8 unfavorable	0 unfavorable	0 unfavorable
SCORE = 6	SCORE = -5	SCORE = 9	SCORE = 11

DOCK's activity hits prediction: compound-IDs 005 and 006

Preliminary Results







Pharmacophore Search

- Found 696 fits in library of >300,000 compounds
- Evaluated energies by free energy simulations

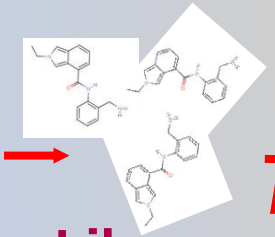
Docking predictions

- 996 compounds predicted as consensus between three docking screens (AutoDock, Vina, Glide)

Binding Assay

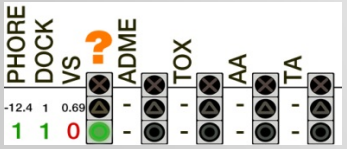
- Assay required Development & Optimisation Time
- Several micromolar hits with dose-response curves obtained from initial screenings.

SAM Workflow



Library

Data

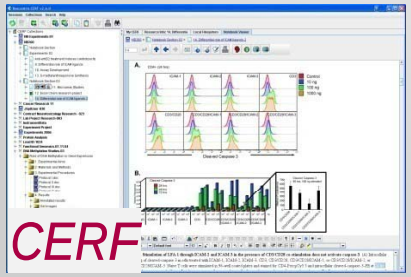


Refine Predictions

SYNERGY

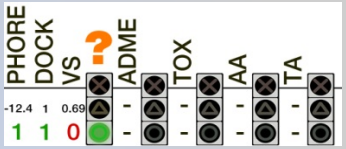
Binding Assay

Data



OpenTox

Toxicity Predictions

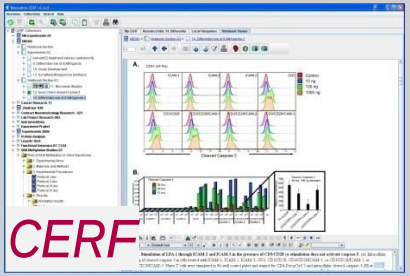


Refine Predictions

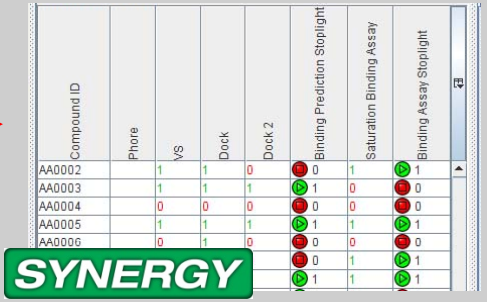
SYNERGY

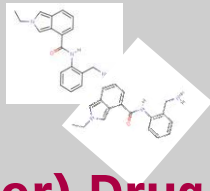
Toxicity Assay

Data



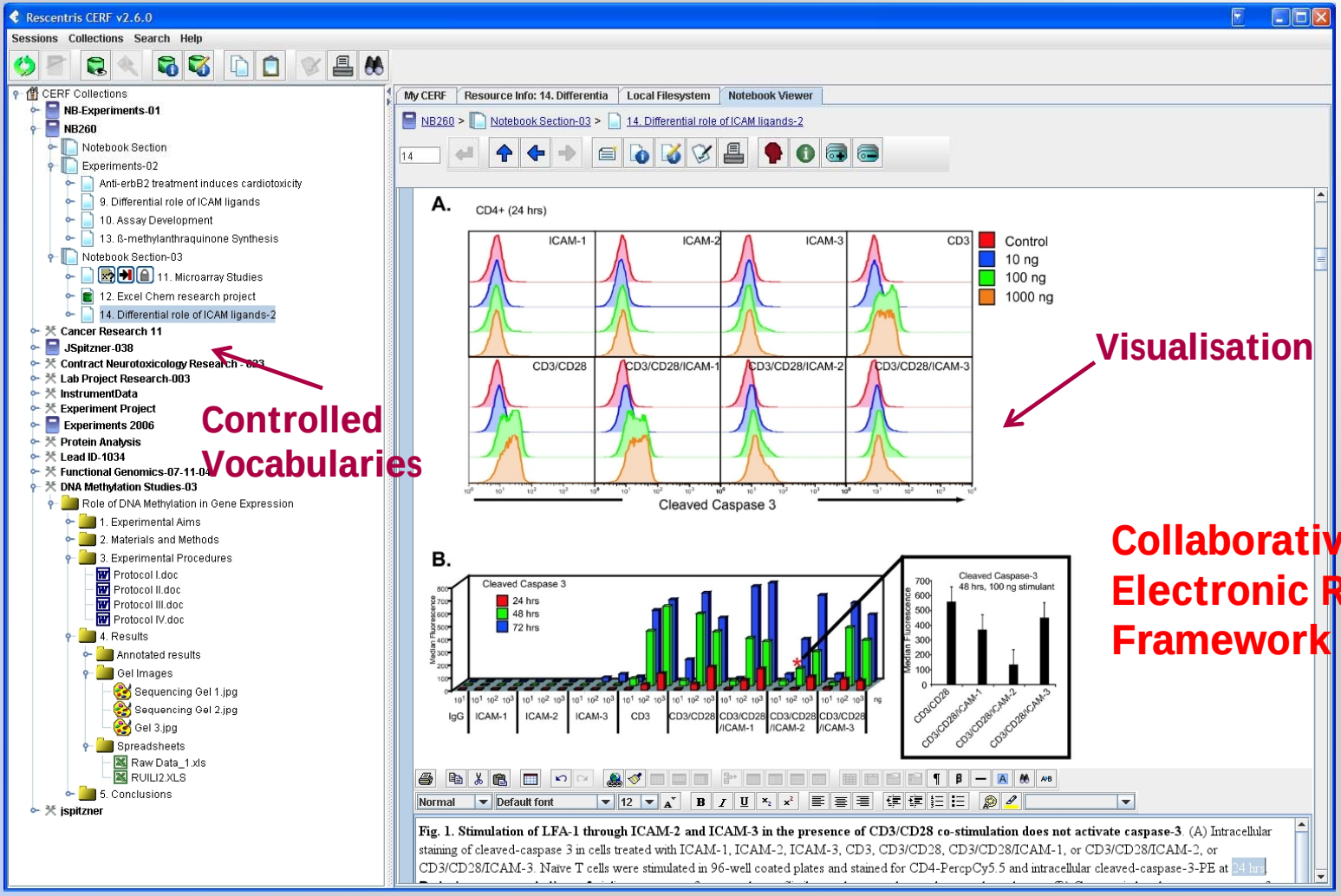
Decision Dashboard





(Safer) Drug Leads

Data Entry & Analysis



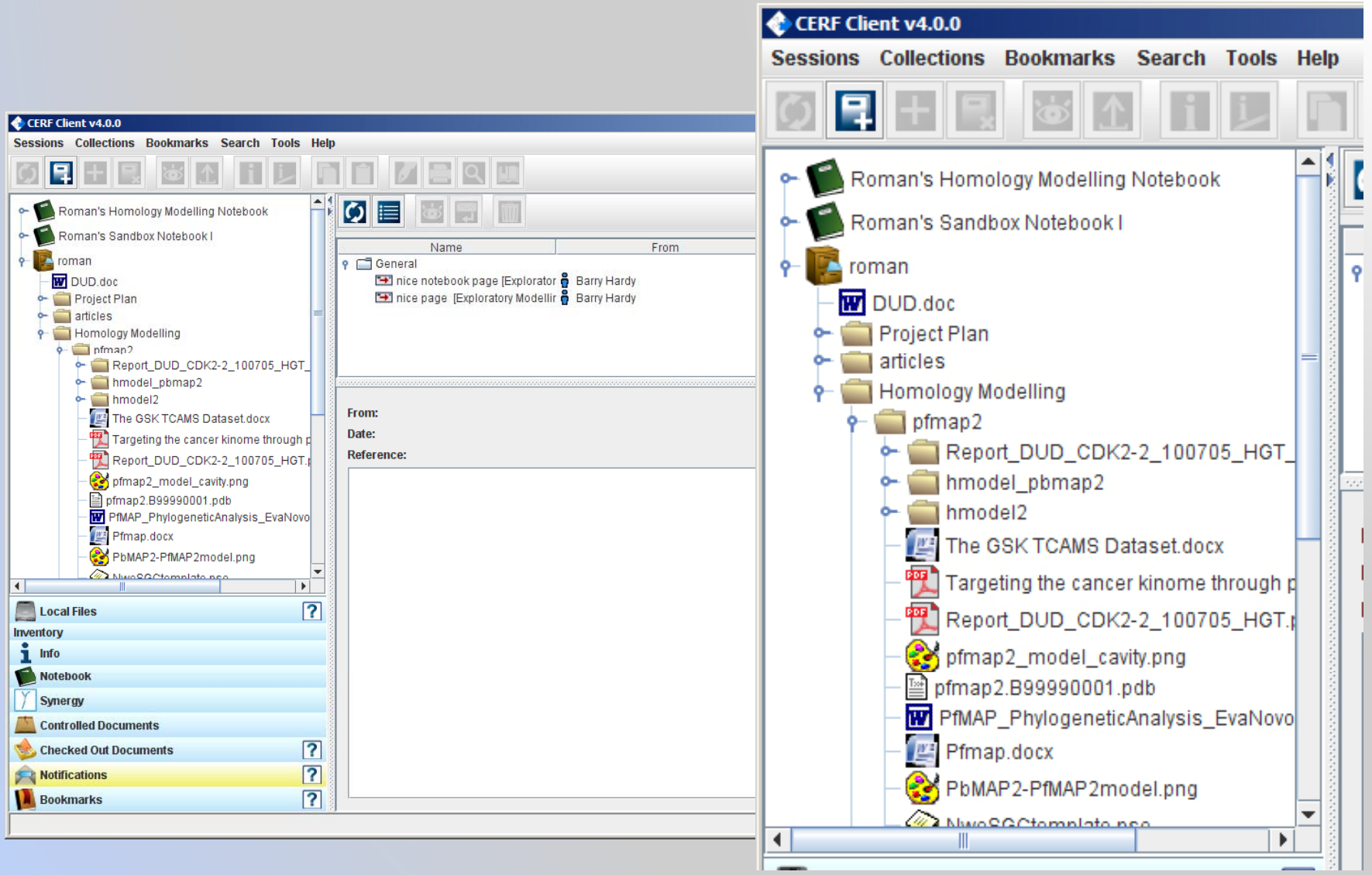
Controlled Vocabularies

Visualisation

Collaborative Electronic Research Framework (CERF)

www.rescentris.com

File cabinets to store data etc



Template Spreadsheets to Document Computations

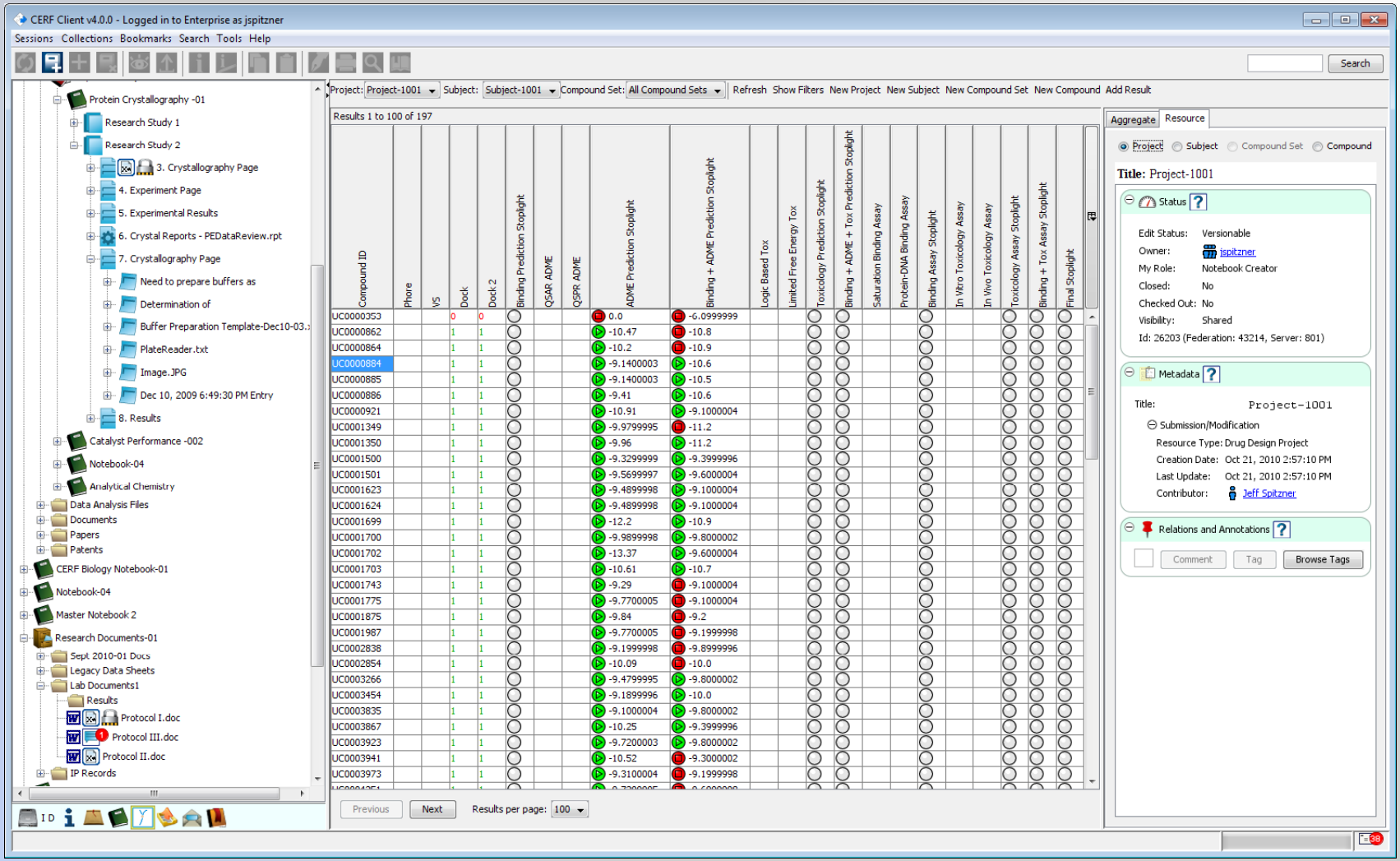
Computational Analysis Form (Roman Affentranger, Tuesday, September 7, 2010 10:55:19 AM)

fx:

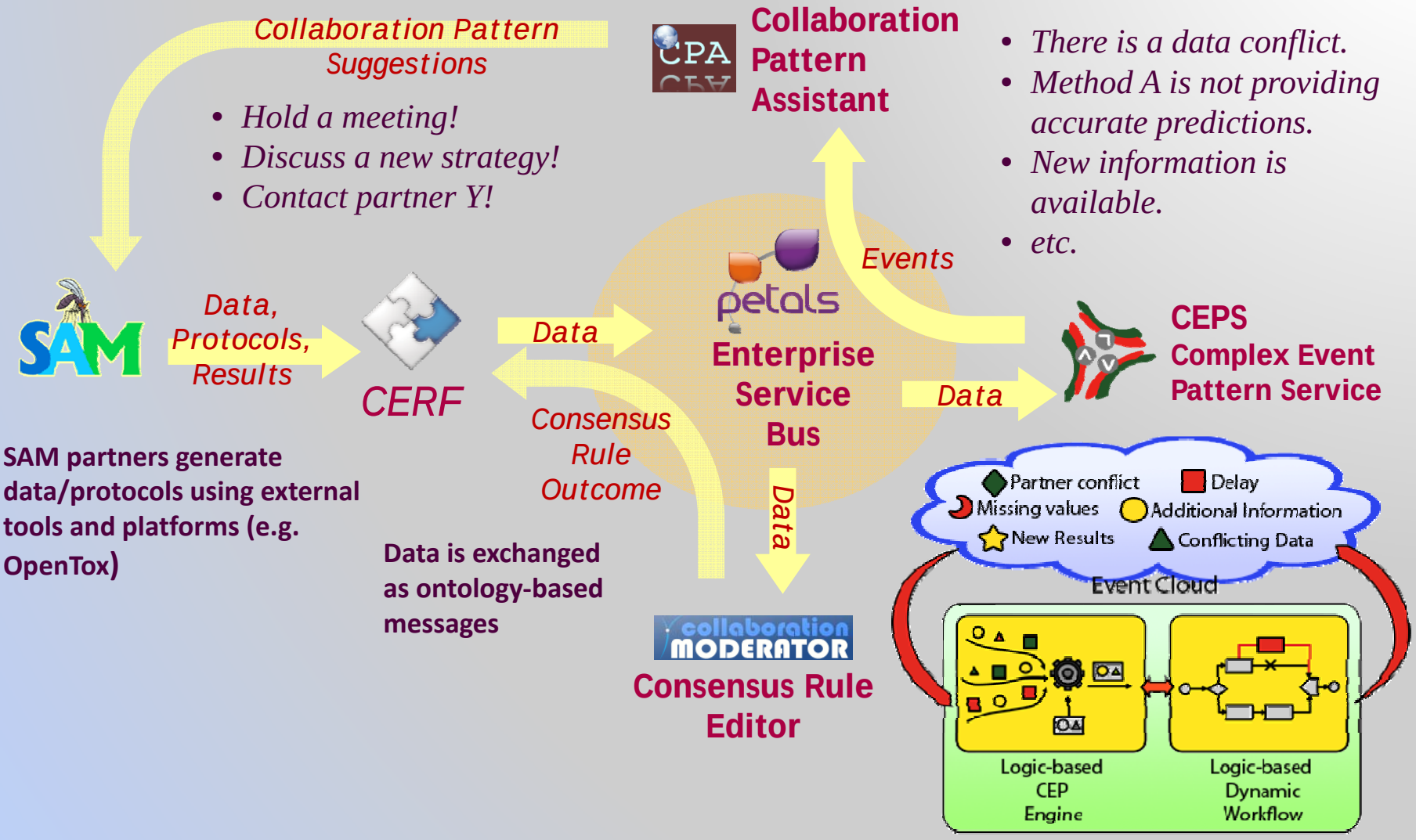
	A	B	C	D	E	F
1						
2	**Operator Name (if different from Notebook user):**	Roman	Operator Organization:	Douglas Connect		
3	**Computer Operating System & Version**	Fedora 10	Computer architecture	x86_64		
4	**Name of computational software**	MODELLER	Version of software	9v8		
5	**Analysis Performed**	Template Selection for PfMAP2 step 1: get list of template candidates				
6	**Method (Steps - use as many as you need):**					
7	1. Preparation of PfMAP2 sequence in MODELLER format		pfmap2.ali			
8	2. Building a sequence profile		build_profile.py			
9	3. Remove template candidates with low ID or small overlap		manual			
10	4. Download selected pdb files		get_pdb.sh			
11	5. Structural alignment of template candidates		salign.py			
12						
13	**Parameters used**	Default parameters were used for all MODELLER scripts				
14	**Sample input file**	pfmap2.ali				
15		Script	Input	Output		
16	**Reference Files (inputs and outputs) - attach as links**	build_profile.py	pfmap2.ali	build_profile.prf		
17				build_profile.log		
18				build_profile.ali		
19		get_pdb.sh	pdb-list.dat	get_pdbs		
20		salign.py	get_pdbs	salign.log.gz		
21				pfmap2-salign-templates.tree		
22				pfmap2-salign-templates.pap		
23				pfmap2-salign-templates.ali		
24						

Sheet 1

Event-driven Collaboration Dashboard



SAM ICT Architecture

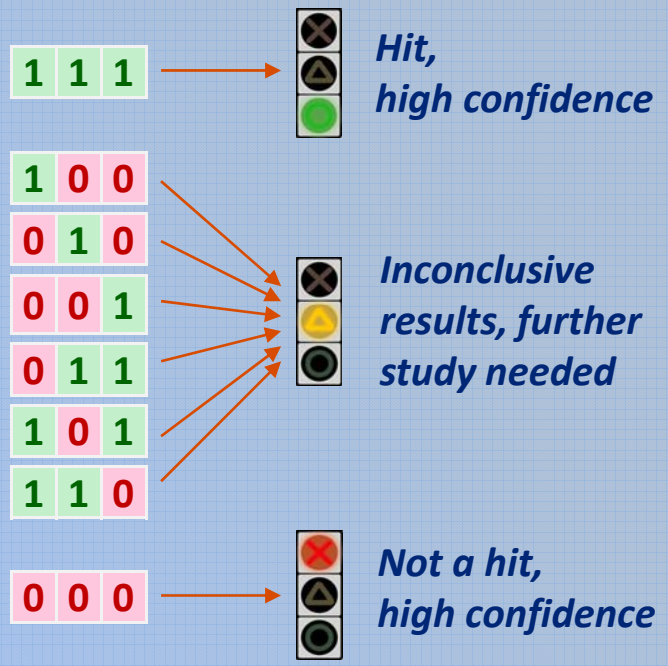


SAM partners generate data/protocols using external tools and platforms (e.g. OpenTox)

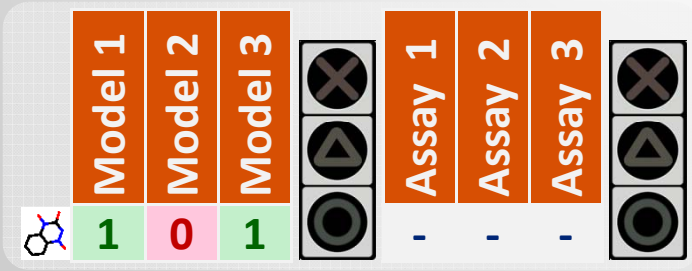
Data is exchanged as ontology-based messages

Resolving Inconclusives

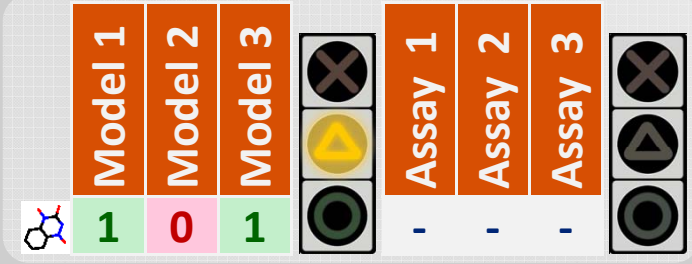
Recommendation Rules:



ELN



Synergy



OpenTox

Resolving Inconclusives

?

ELN

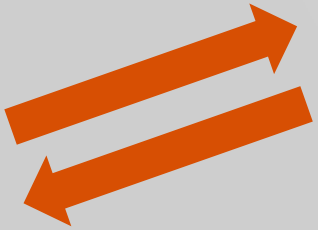
Model 1	Model 2	Model 3	Assay 1	Assay 2	Assay 3
1	0	1	-	-	-

Synergy

Model 1	Model 2	Model 3	Assay 1	Assay 2	Assay 3
1	0	1	-	-	-

OpenTox

Resolving Inconclusives



ELN

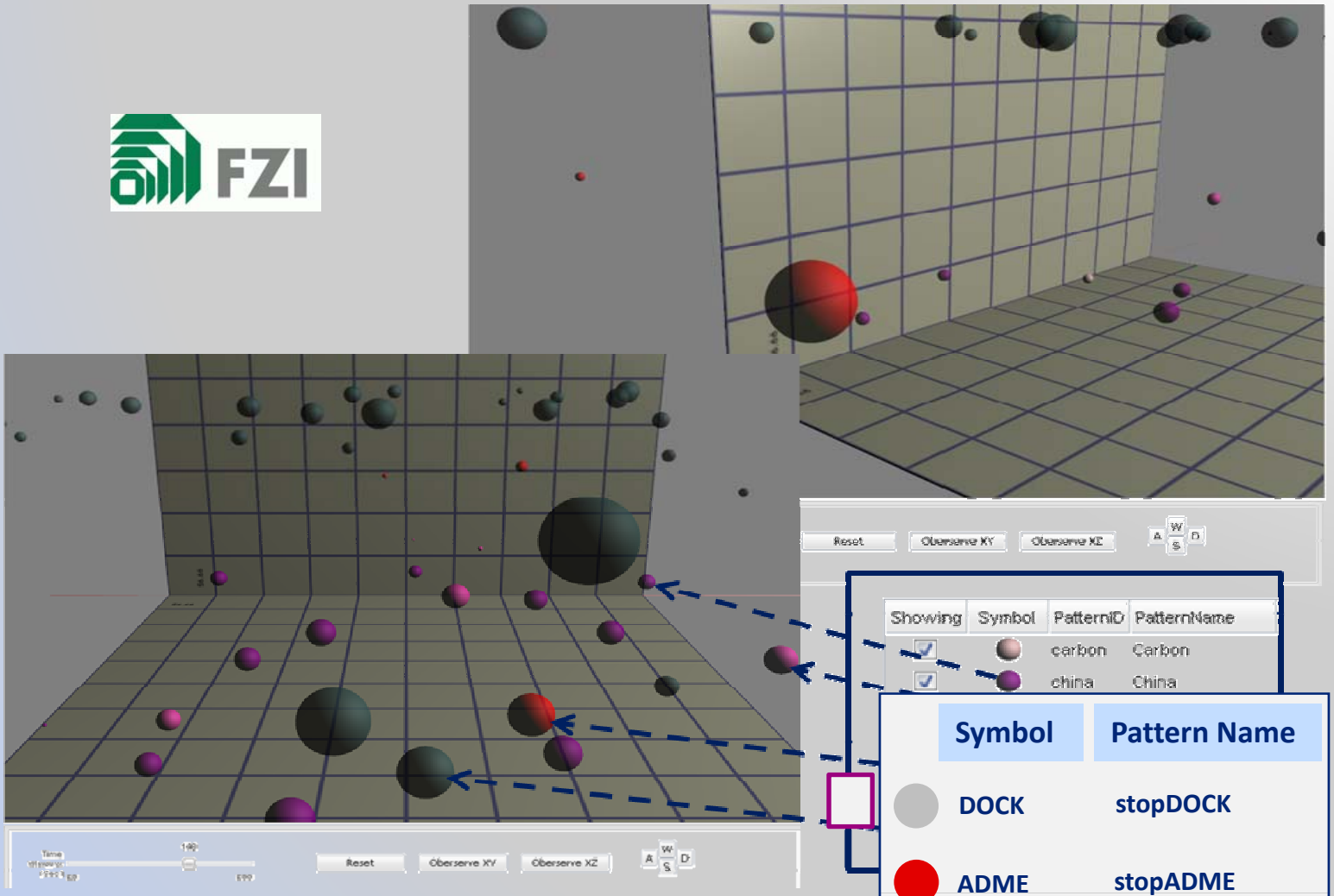
Model 1	Model 2	Model 3	Assay 1	Assay 2	Assay 3
1	0	1	-	1	1

Synergy

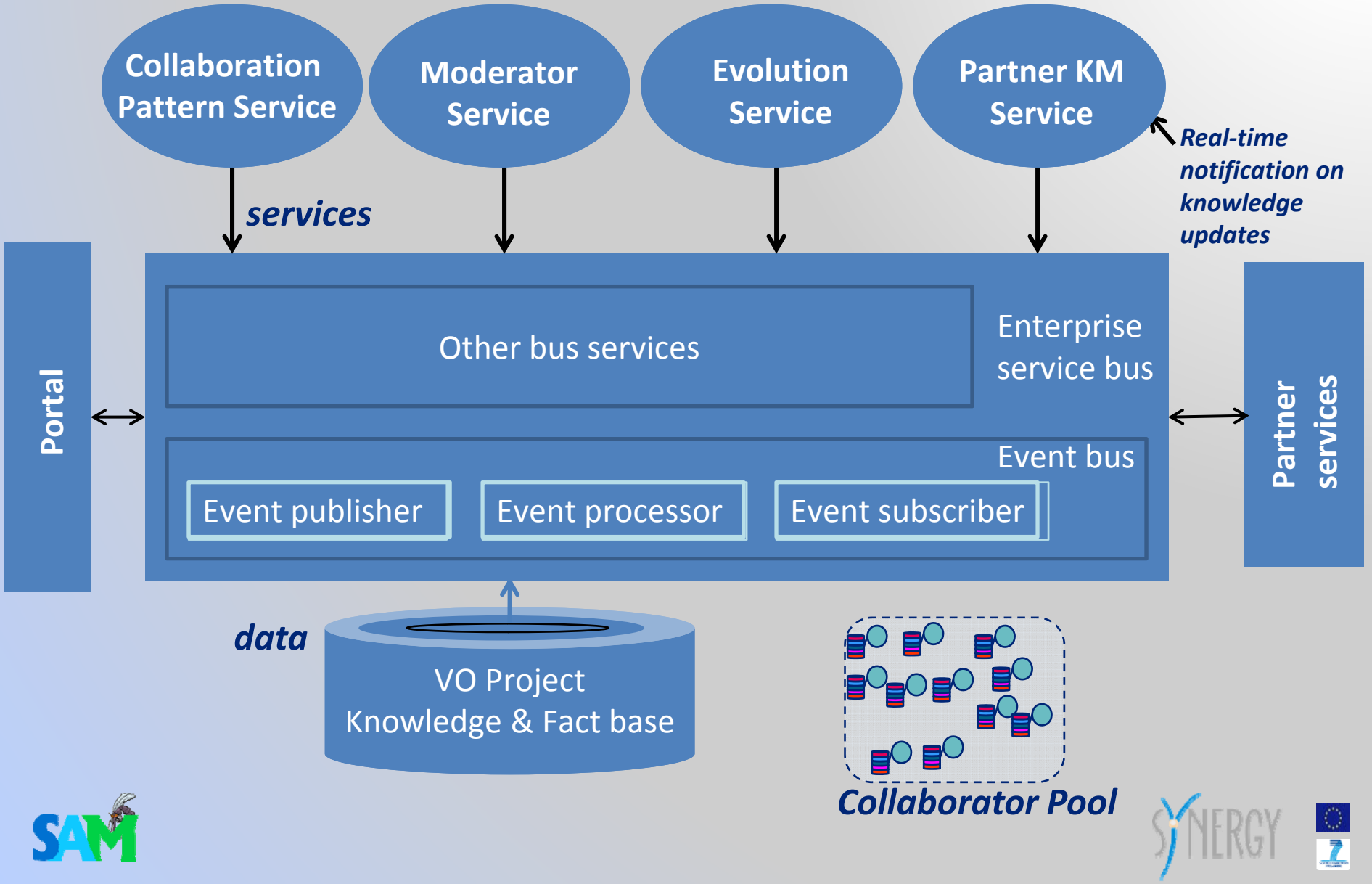
Model 1	Model 2	Model 3	Assay 1	Assay 2	Assay 3
1	0	1	-	-	-

OpenTox

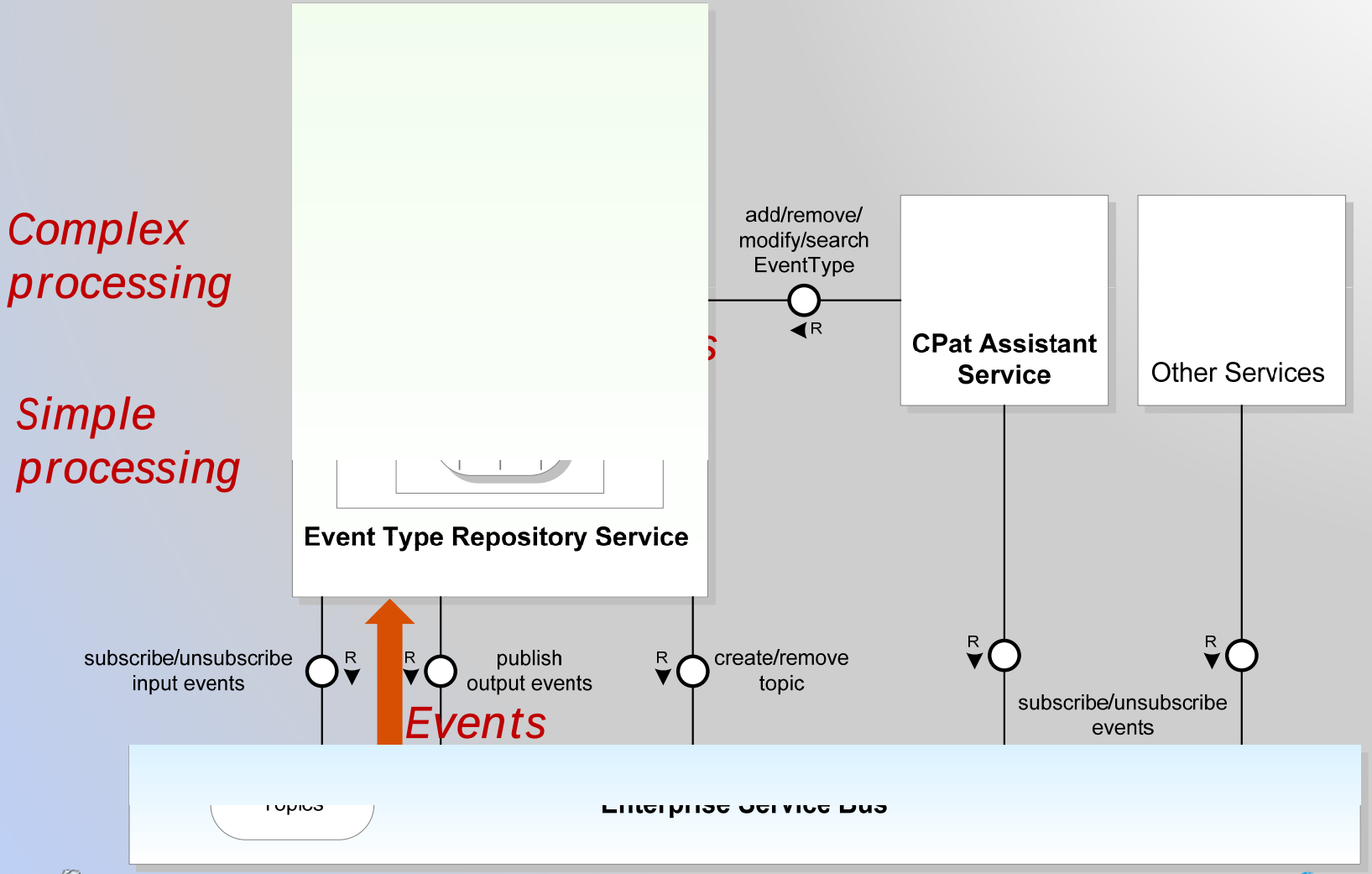
Complex Events Stream



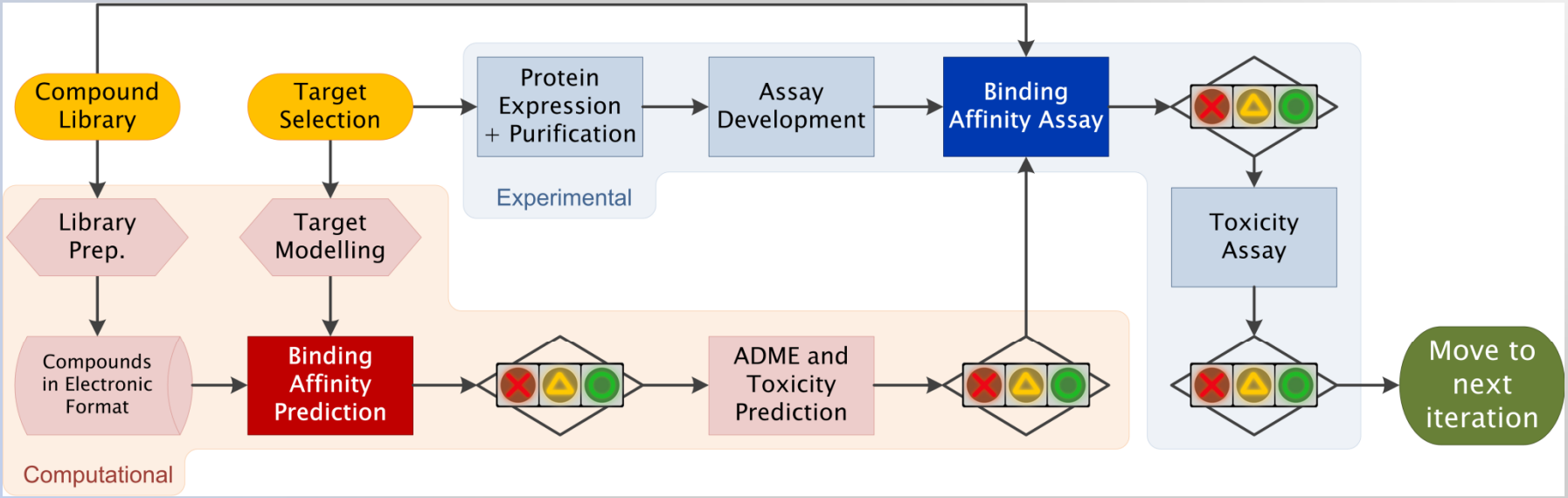
SYNERGY Service Support



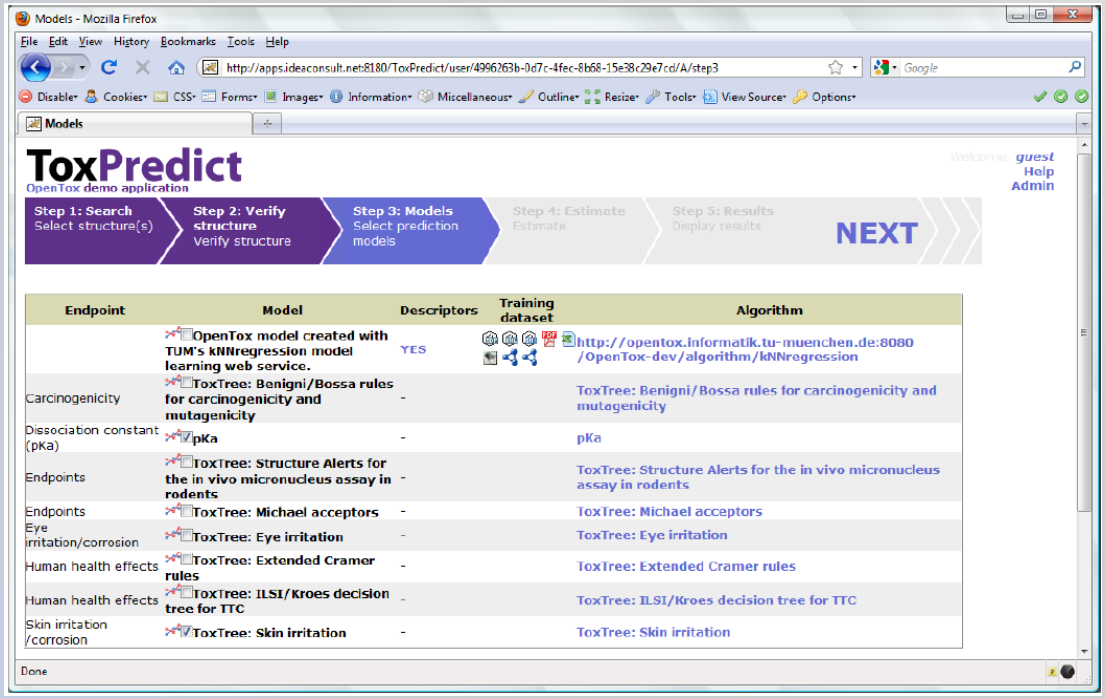
SYNERGY Complex Event Processing



Incorporation of Holistic Predictive ADME & Toxicity



Predictive toxicology model building

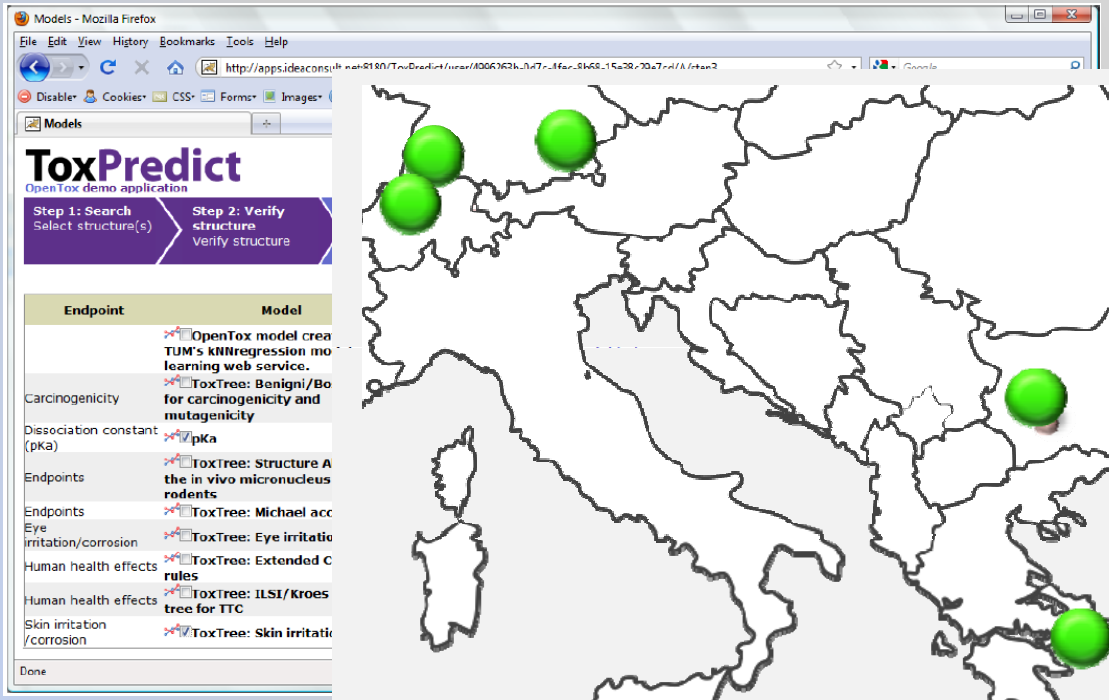


Simple building of predictive toxicology applications based on well-established methods and databases

Applications and Models based on OpenTox Web Services are applied holistically to drug design libraries to help guide decisions on chemistry directions and classes…

www.opentox.org

Predictive toxicology model building

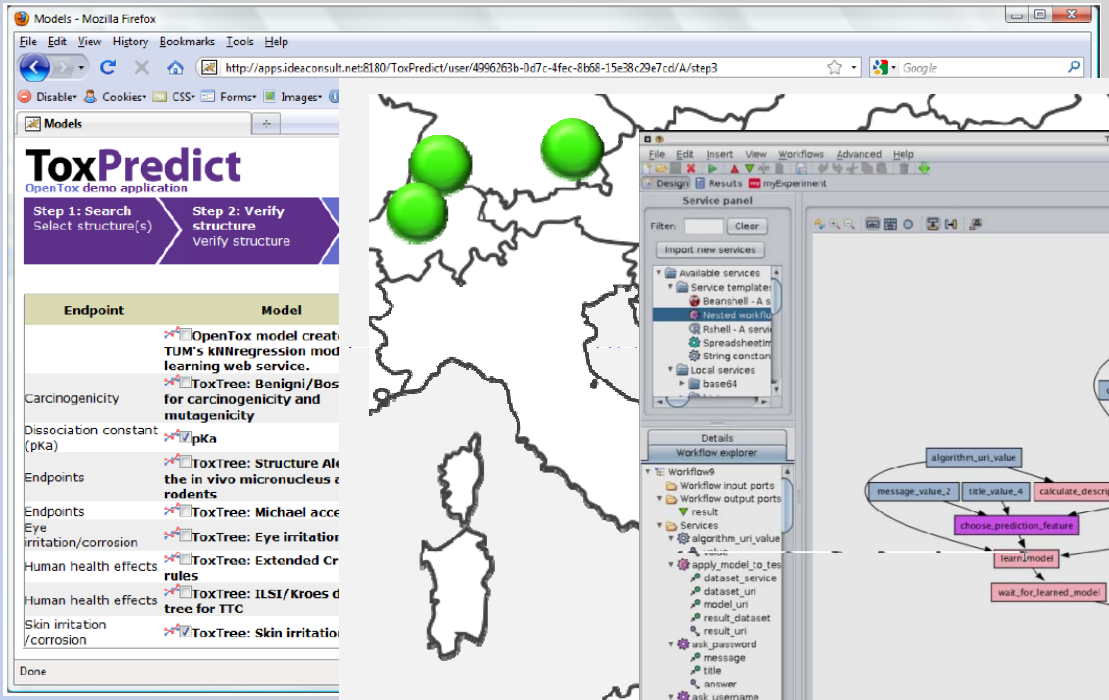


Simple bui
applicatio
methods a

Distributed applications, integrating wide range of data, models, prediction methods

Predictive toxicology model building

OpenTox



Simple buildi
applications
methods and

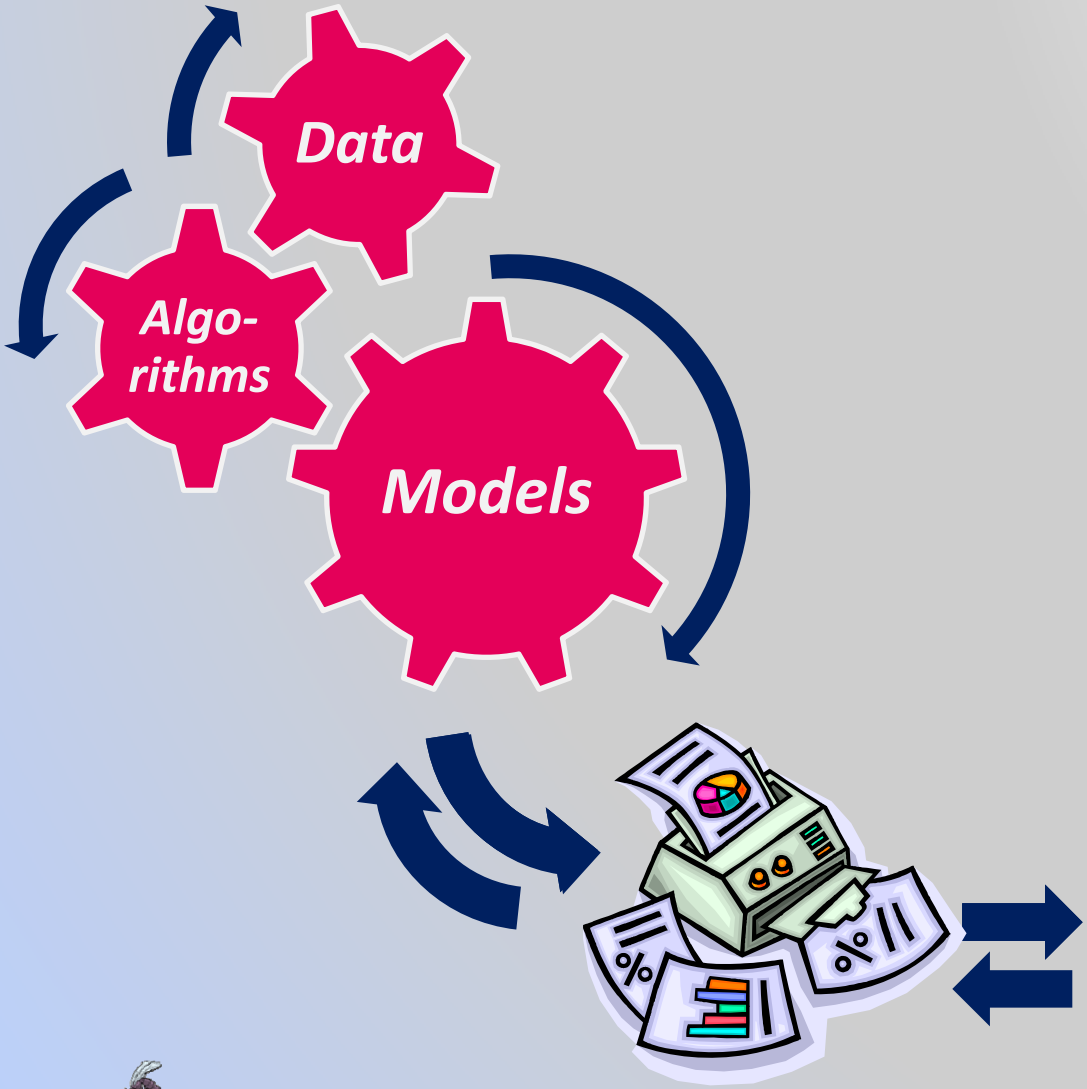
Distributed a
wide range o
methods

Integration into workflow systems
for computational biology (Taverna)

SAM

SYNERGY

Predictive toxicology model building



ELN

	Model 1	Model 2	Model 3	Assay 1	Assay 2	Assay 3
	-	-	-	-	-	-

Synergy

	Model 1	Model 2	Model 3	Assay 1	Assay 2	Assay 3
	-	-	-	-	-	-

OpenTox

	Model 1	Model 2	Model 3	Assay 1	Assay 2	Assay 3
	1	0	1	-	-	-

Predictive toxicology model building

ELN

Model 1	Model 2	Model 3	Assay 1	Assay 2	Assay 3
1	0	1	-	-	-

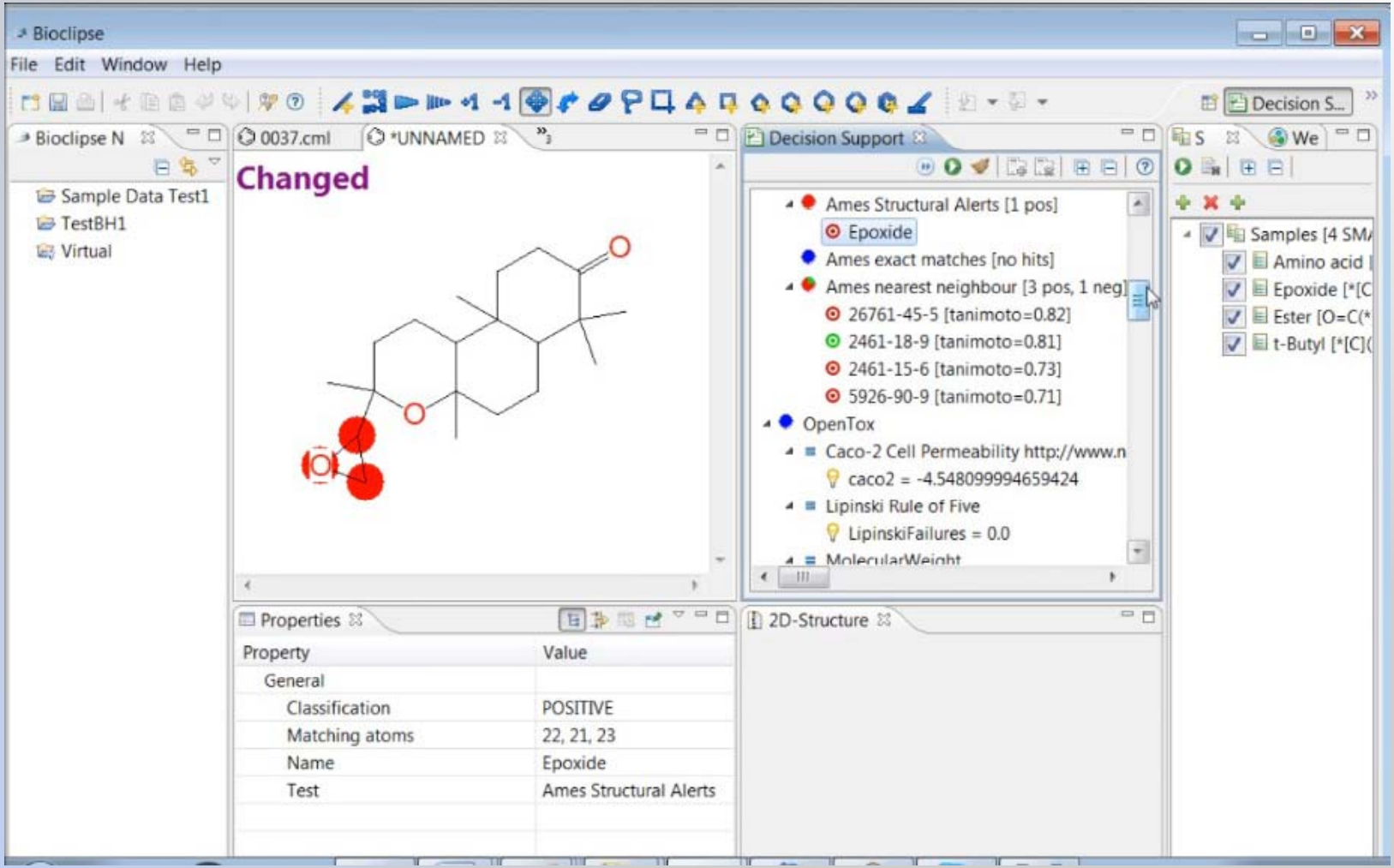
Synergy

Model 1	Model 2	Model 3	Assay 1	Assay 2	Assay 3
-	-	-	-	-	-

OpenTox

Model 1	Model 2	Model 3	Assay 1	Assay 2	Assay 3
1	0	1	-	-	-

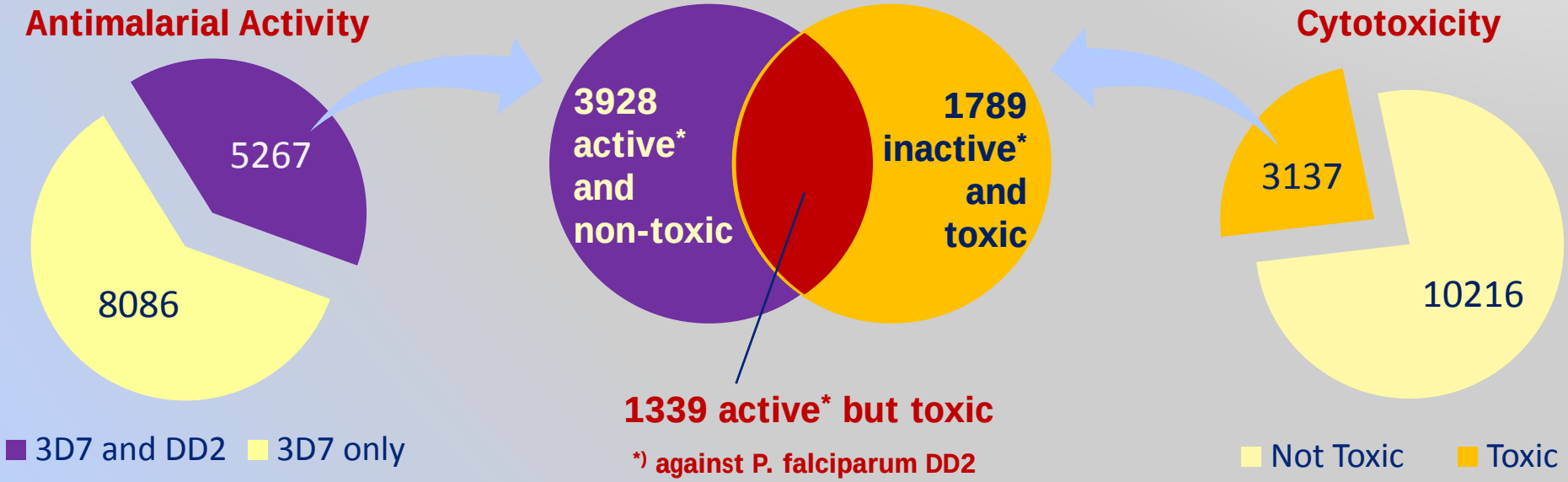
Bioclipse – OpenTox Interoperation



"Malaria Box"*: A collection of chemical compounds active against *(i.e. inhibiting growth of)* the malaria parasite *Plasmodium falciparum*

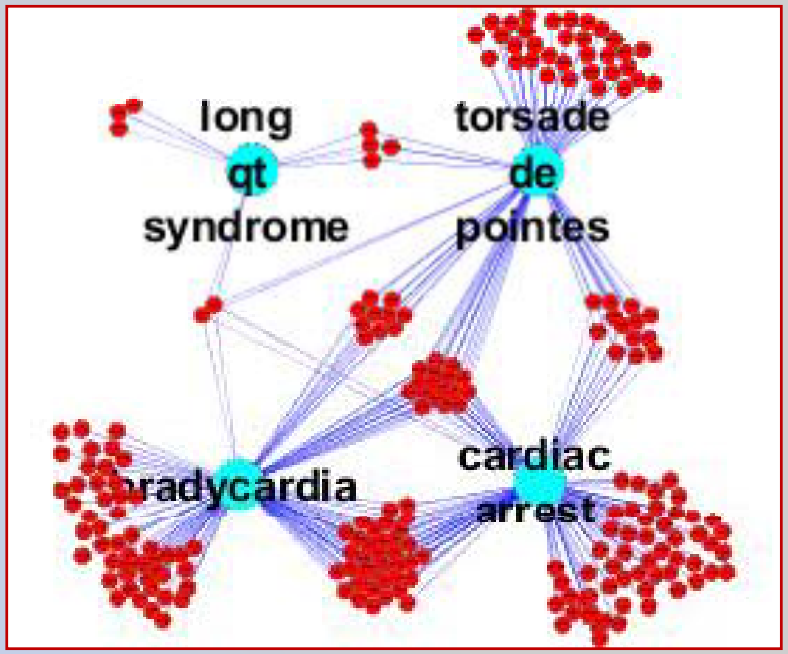
Data provided that is relevant for this project:

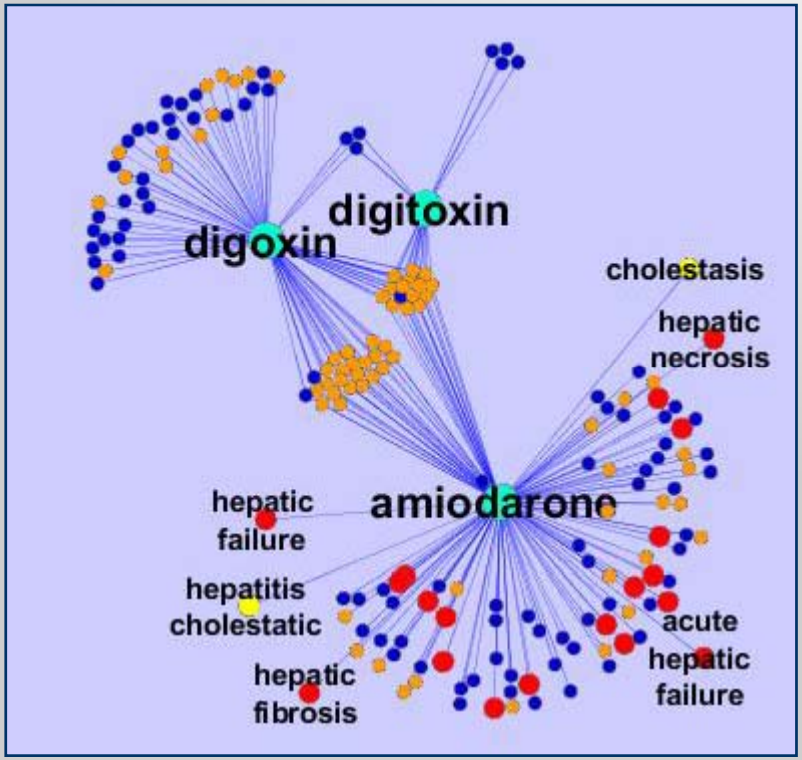
- **Activity against (growth inhibition of) *P. falciparum* strain 3D7 (common strain)**
- **Activity against (growth inhibition of) *P. falciparum* strain DD2 (multi-drug resistant strain)**
- **Cytotoxicity against (growth inhibition of) human hepatocytes, HepG2 (hepatoma cells)**



*Gamo et al., *Nature* **465**(7296), 305-10 (2010)

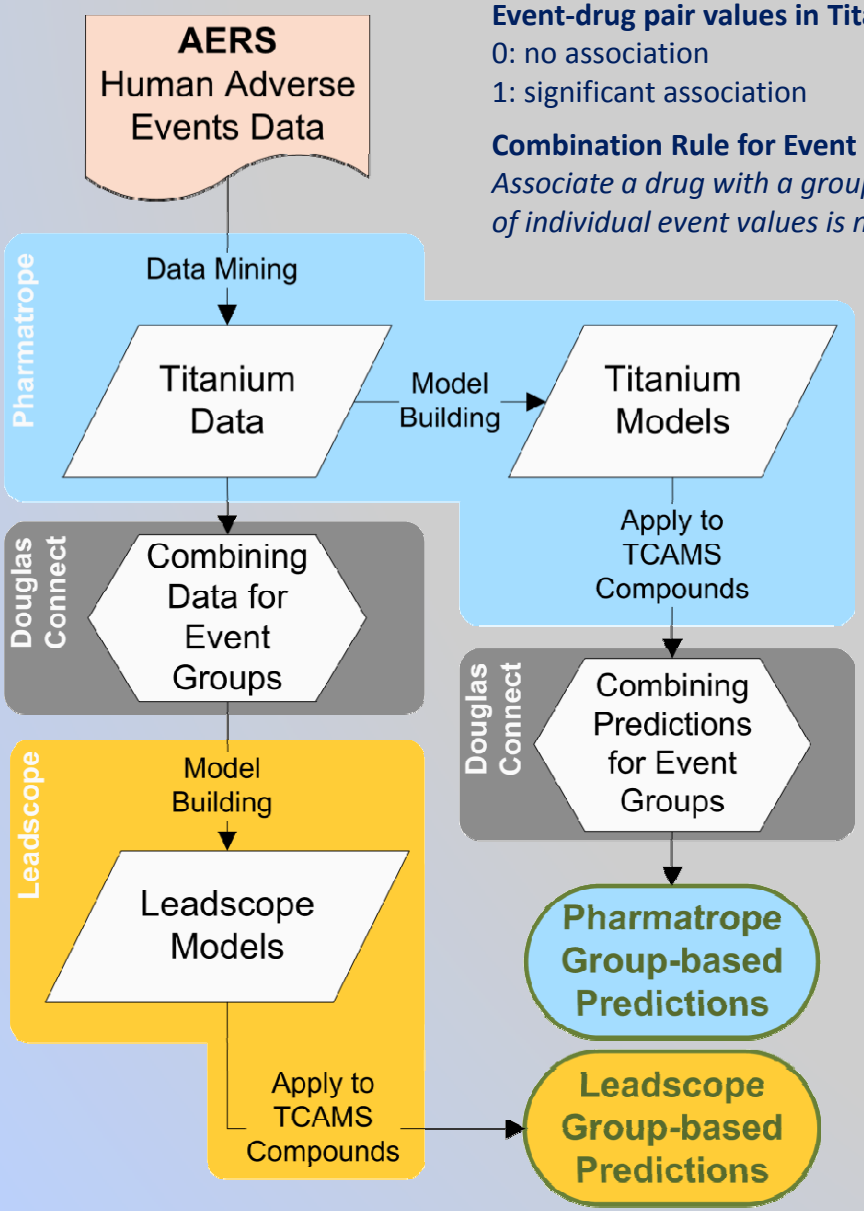
Mining Human Adverse Events





PHARMATROPE

Adverse Events Model Development



Event-drug pair values in Titanium Data:
0: no association
1: significant association

Combination Rule for Event Groups:
Associate a drug with a group if the sum of individual event values is non-zero

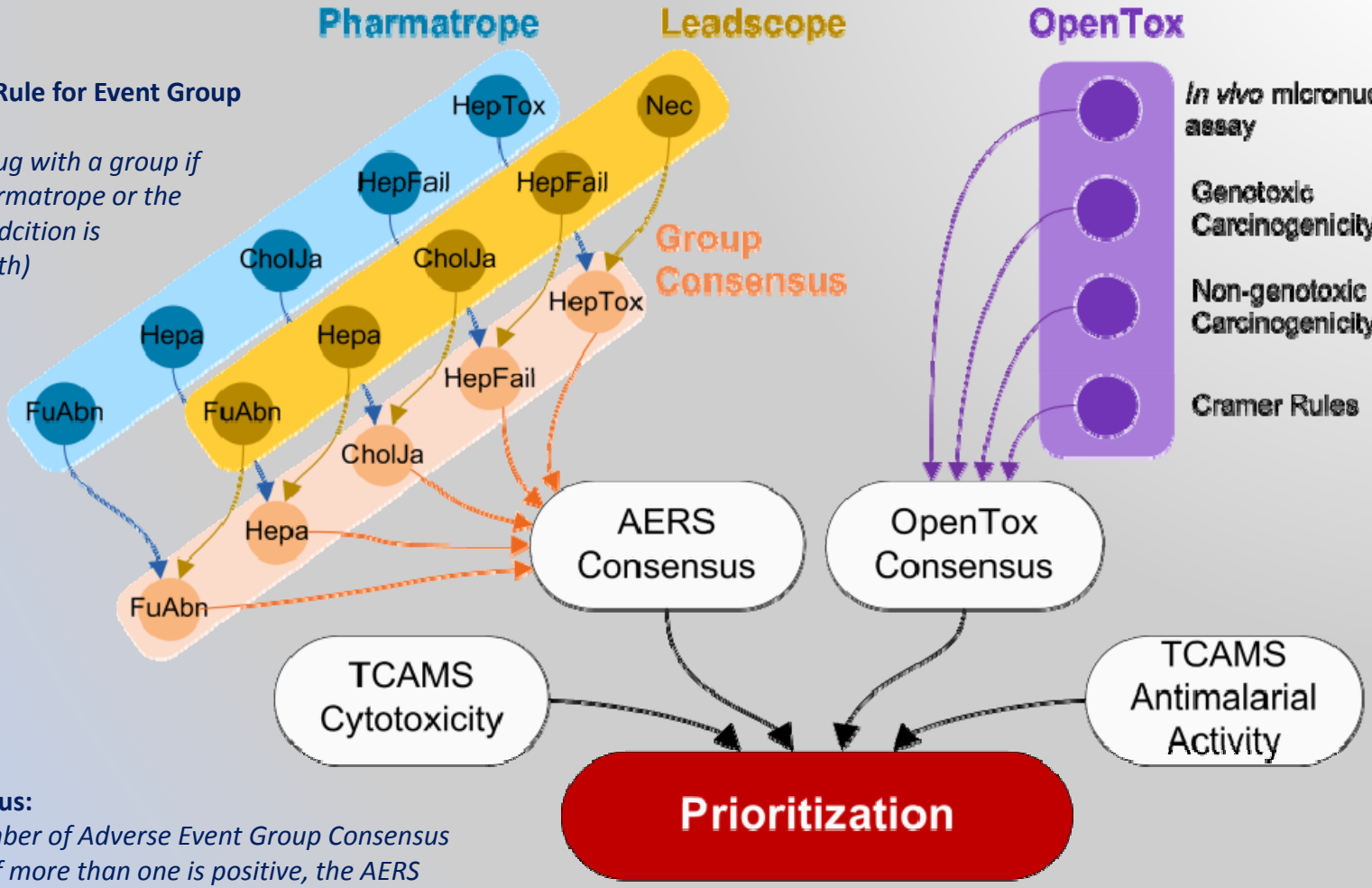
Event-drug pair values in Titanium Predictions:
0 : no association (0)
0.35-0.4 : non-significant association (0)
> 0.4 : significant association (1)

Combination Rule for Event Groups:
Associate a drug with a group if the sum of individual event values is larger of equal to 0.4.

Adverse Event Groups	Group Name
Hepatic function abnormal Liver disorder	**FuAbn**
Hepatic necrosis	**Nec**
Cytolytic hepatitis Hepatitis Hepatitis acute Hepatitis toxic	**Hepa**
Cholestasis Jaundice Hepatitis cholestatic jaundice cholestatic Yellow skin	**CholJa**
Hepatic failure Hepatitis fulminant Acute hepatic failure Hepatorenal failure	**HepFail**
Hepatotoxicity Hepatomegaly Hyperbilirubinaemia Hepatosplenomegaly	**HepTox**

Combining Predictions & Experimental Data

Combination Rule for Event Group Predictions:
Associate a drug with a group if either the Pharmatrope or the Leadscope predcition is positive (or both)



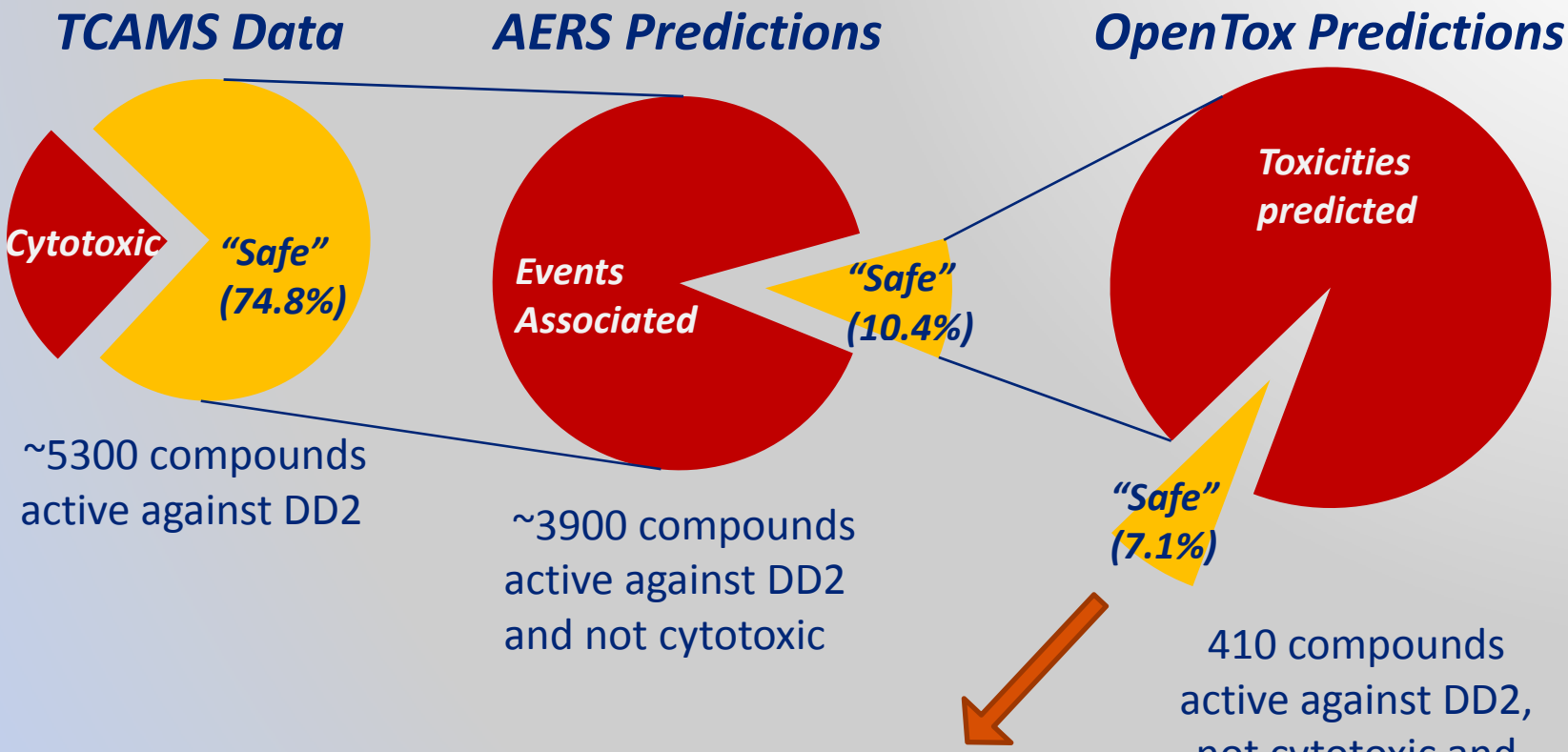
AERS Consensus:
Count the number of Adverse Event Group Consensus associations. If more than one is positive, the AERS Consensus is positive.

OpenTox Consensus:
Negative if both carcinogenicity and the micronucleus assay predictions are negative, OR if the Cramer Rule classification is Class I. Positive otherwise.

TCAMS Cytotoxicity:
Positive if > 30% growth inhibition at 10 µM.

TCAMS Antimalarial Activity:
Positive if > 80% growth inhibition of P. Falciparum DD2 at 2 µM.

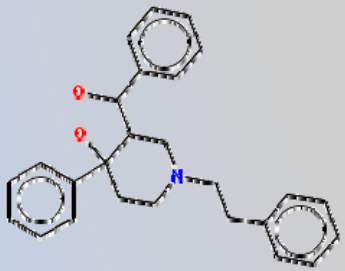
Compound Prioritisation Filtering



29 compounds without any indication of adverse events or toxicities

Example Classified Compounds

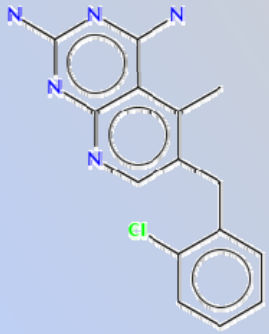
"Safe"



TCMDC-131287:

- No predicted association with adverse events (consistent)
- Negative for carcinogenicity and mutagenicity
- No inhibition of HepG2 growth
- Strong inhibition of *P. falciparum* DD2 growth

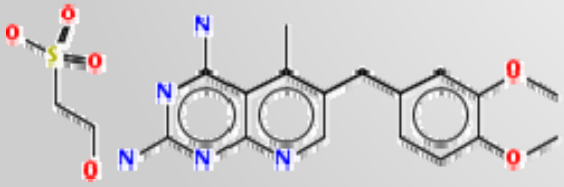
"Toxic"



TCMDC-137245:

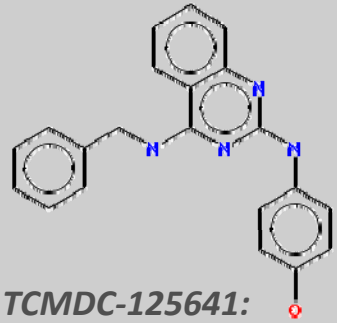
- Associated with 4 and 5 (out of 5) adverse events group by Pharmatrope and Leadscope, respectively
- Positive for carcinogenicity and mutagenicity, Cramer Class III
- 67% HepG2 inhibition (10 µM)
- 91% *P. falciparum* DD2 growth inhibition (at 2 µM)

Ambiguous, Further Data Required



TCMDC-138057:

- Predicted association with many adverse events groups
- Positive for carcinogenicity and mutagenicity
- Considered safe (Class I) with Cramer rules
- Inhibition of HepG2 growth could not be measured
- Strong inhibition of *P. falciparum* DD2 growth



TCMDC-125641:

- No adverse event association predicted with Pharmatrope models
- Strong association with all five adverse events groups predicted with the Leadscope models
- Negative for carcinogenicity and mutagenicity
- Intermediate inhibition of HepG2 growth (33% at 10 µM)
- Strong inhibition of *P. falciparum* DD2 growth (100% at 2 µM)

Future Directions

Collaborations

- **Develop Malaria R&D project (e.g., chemistry, targets, libraries, Kinome Knowledge Base)**
- **Support New Collaborative Discovery Projects**
- **Multi-Project Resource Management**
- **Progress Collaborative Innovation in “Blue Ocean” areas**
- **Develop Collaboration Pool, Member Profiles & Legal/IP Templates**

Collaboration Infrastructure

- **Add Services in the Cloud Capability for data management, model building**
- **Integrated Omics Analysis, Biomarkers**
- **Weight of Evidence Methods and Workflows**
- **Support of hESC and iPSC protocols, assays, data**
- **Add Partners who can plugin value-add services, resources**

Caprivi Delta, Namibia

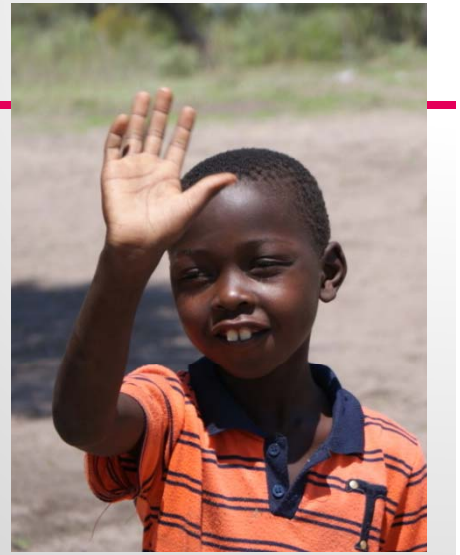
Many High Risk areas for Malaria lack local resources (e.g., doctors, medicines, roads, nutrition, water etc.,).

Simple solutions (e.g., nets, needles) can have significant impact.

New innovation and bottom-of the pyramid business models are needed.

Caprivi Delta, Namibia



Current Realities (we can impact on):

1) Many of the parents of these children may die of AIDS

2) Many of these children will be infected with malaria

3) Many will not receive medical care or drugs for either of these diseases

If you would like to get involved in our Scientists Against Malaria work, please contact us!

barry.hardy -(at)- douglasconnect.com

www.scientistsagainstmalaria.net