

The Impact of Structural Genomics ?

Pedro Alzari

Structural Microbiology



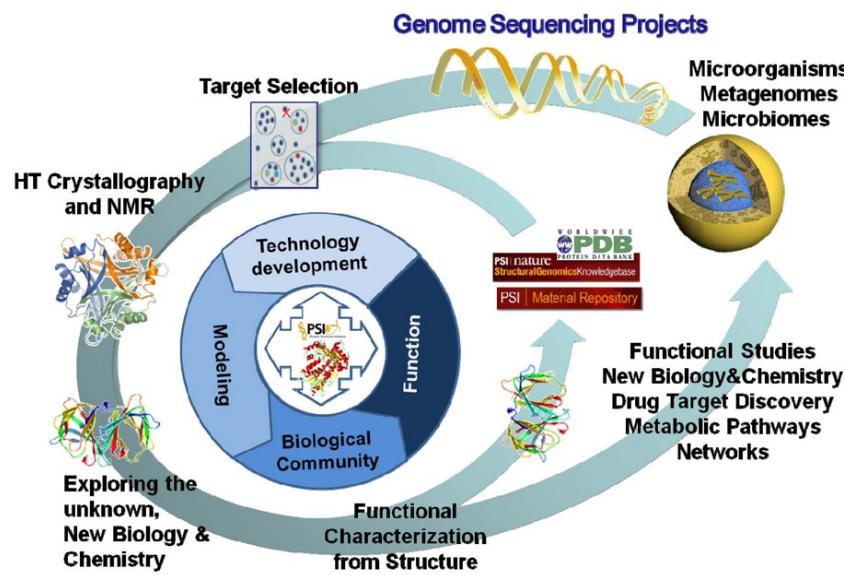
Bioinformatics and Genome Analyses Course
Institut Pasteur Tunis, Tunisia. September 18 – December 15, 2017

Institut Pasteur

What is Structural Genomics ?

Is the process of **high-throughput** determination of 3-D structures of biological macromolecules

What is the Goal of Structural Genomics ?



To develop improved methods that will result in high-throughput biology and protein structure determination

Provision of enough structural templates to facilitate homology modeling of most proteins.

Structural elucidation of complete biological pathways

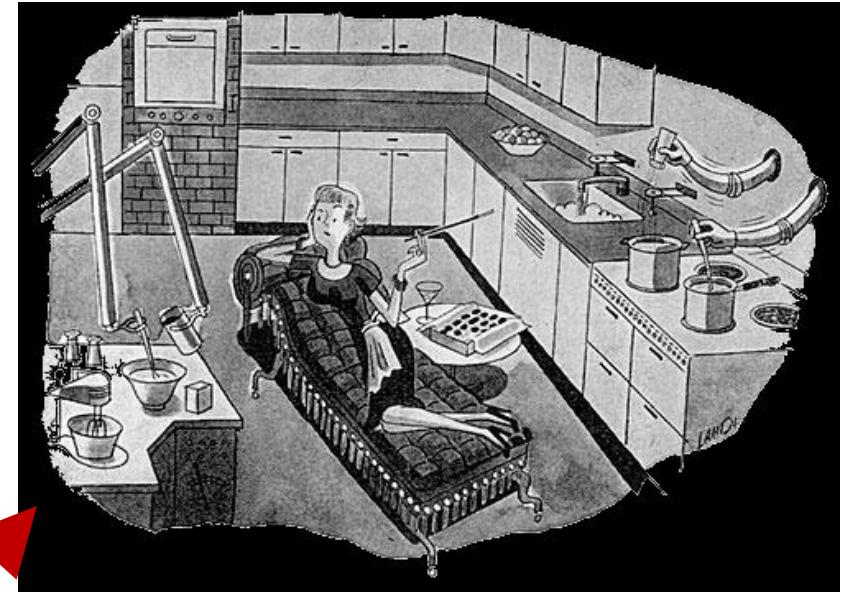
To determine new protein folds

To determine the functions of unknown proteins

Structural Genomics Aims



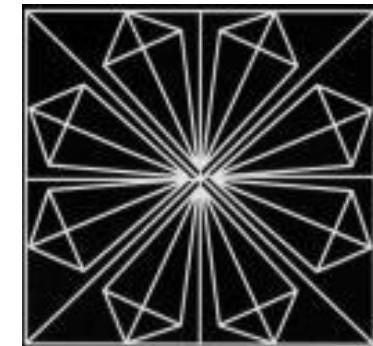
Pathogens and disease



Automation / High Throughput

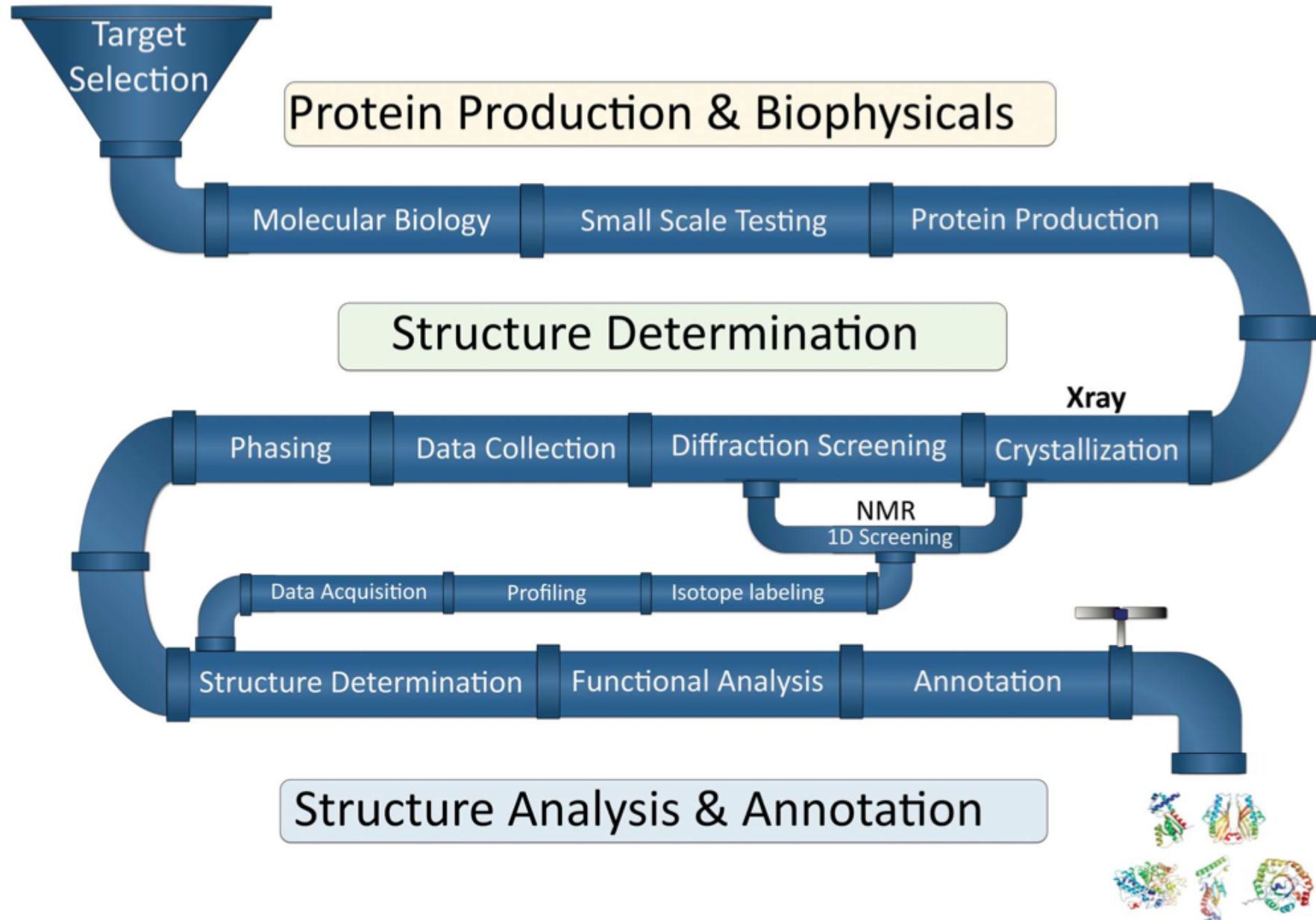


Human Proteins

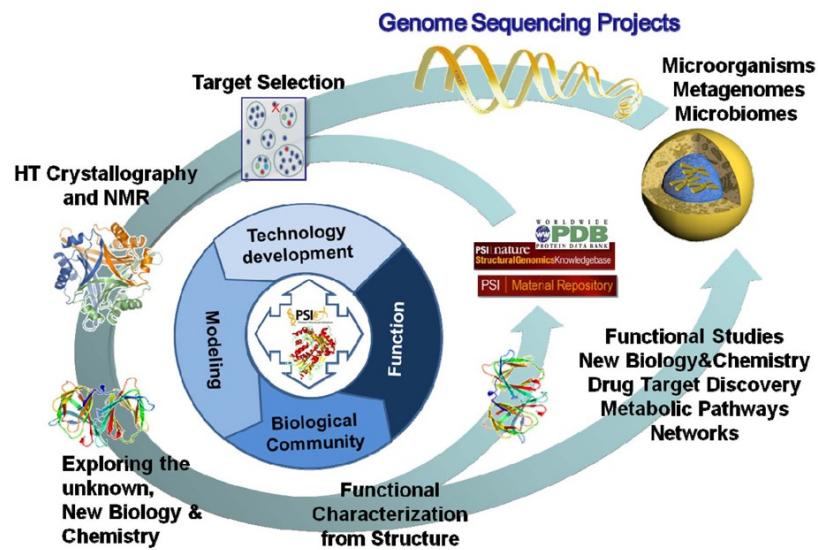


Coverage of Fold Space

The Structural Genomics Pathway



Target Selection



Complete organisms;
thermophilic, mesophilic or halophilic
eukaryotic or prokaryotic

HUMAN DISEASE

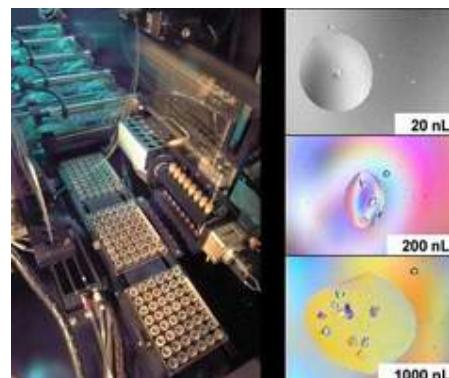
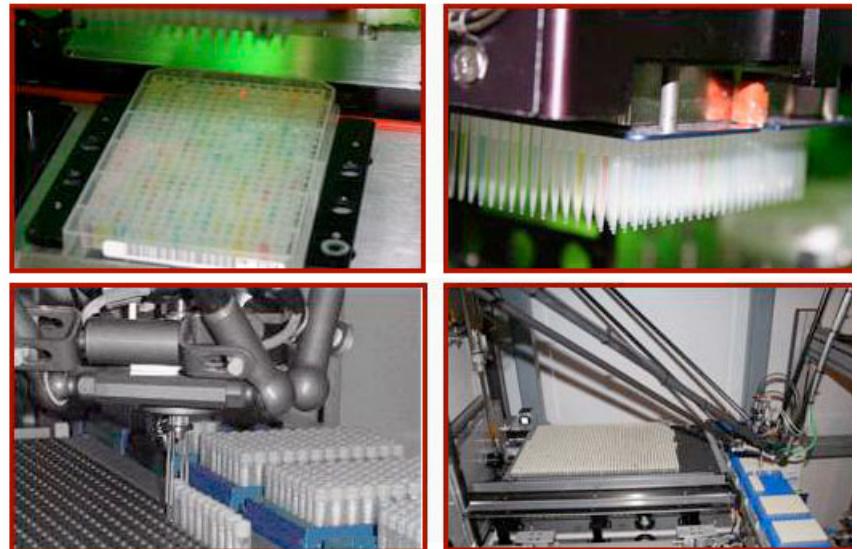
Targets in this area of structural genomics are G-protein coupled receptors, ion channels and kinases etc.

The development of techniques to allow over-expression, purification and crystallization of these targets is required

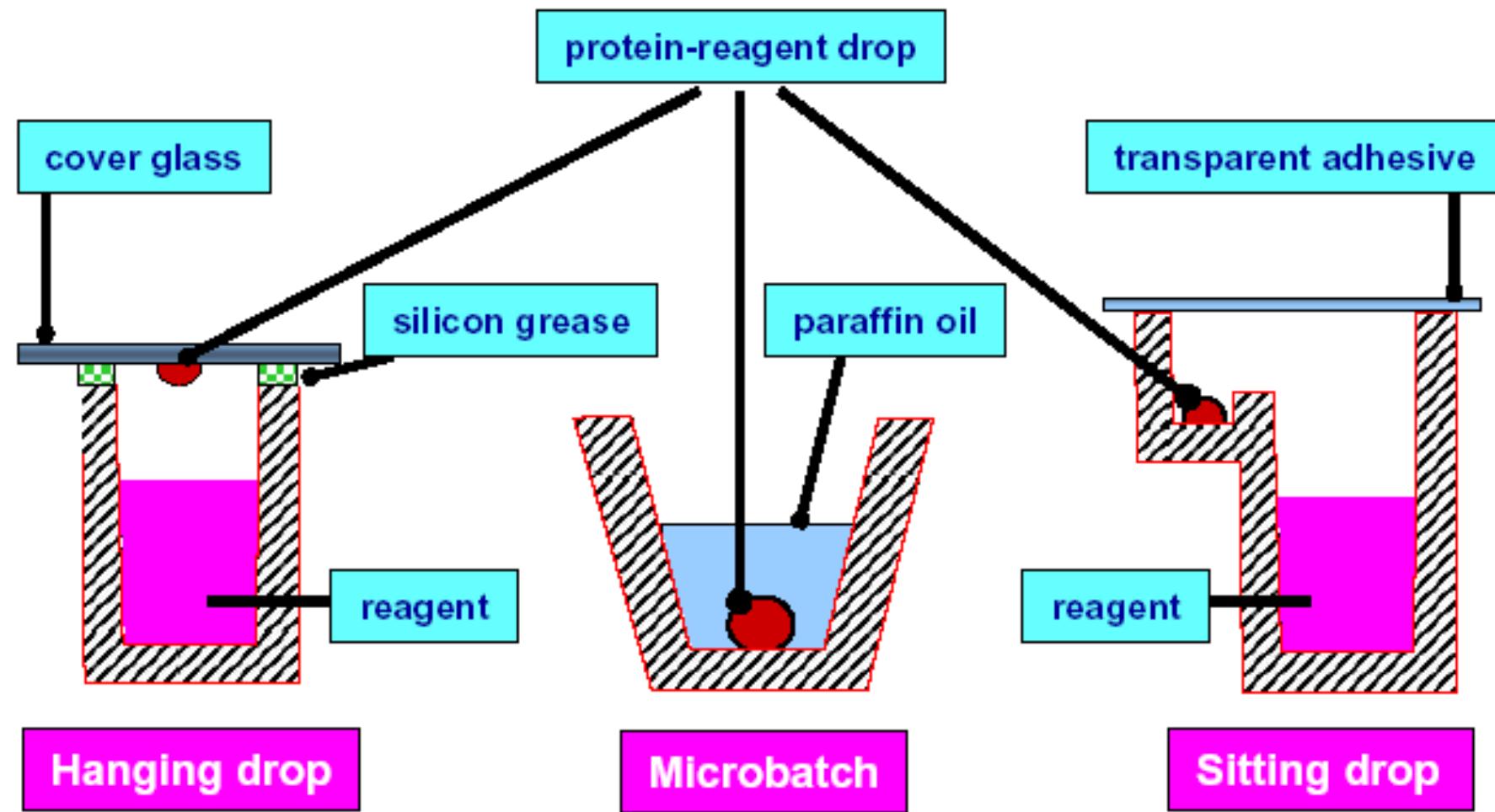
Cloning, Expression and Purification

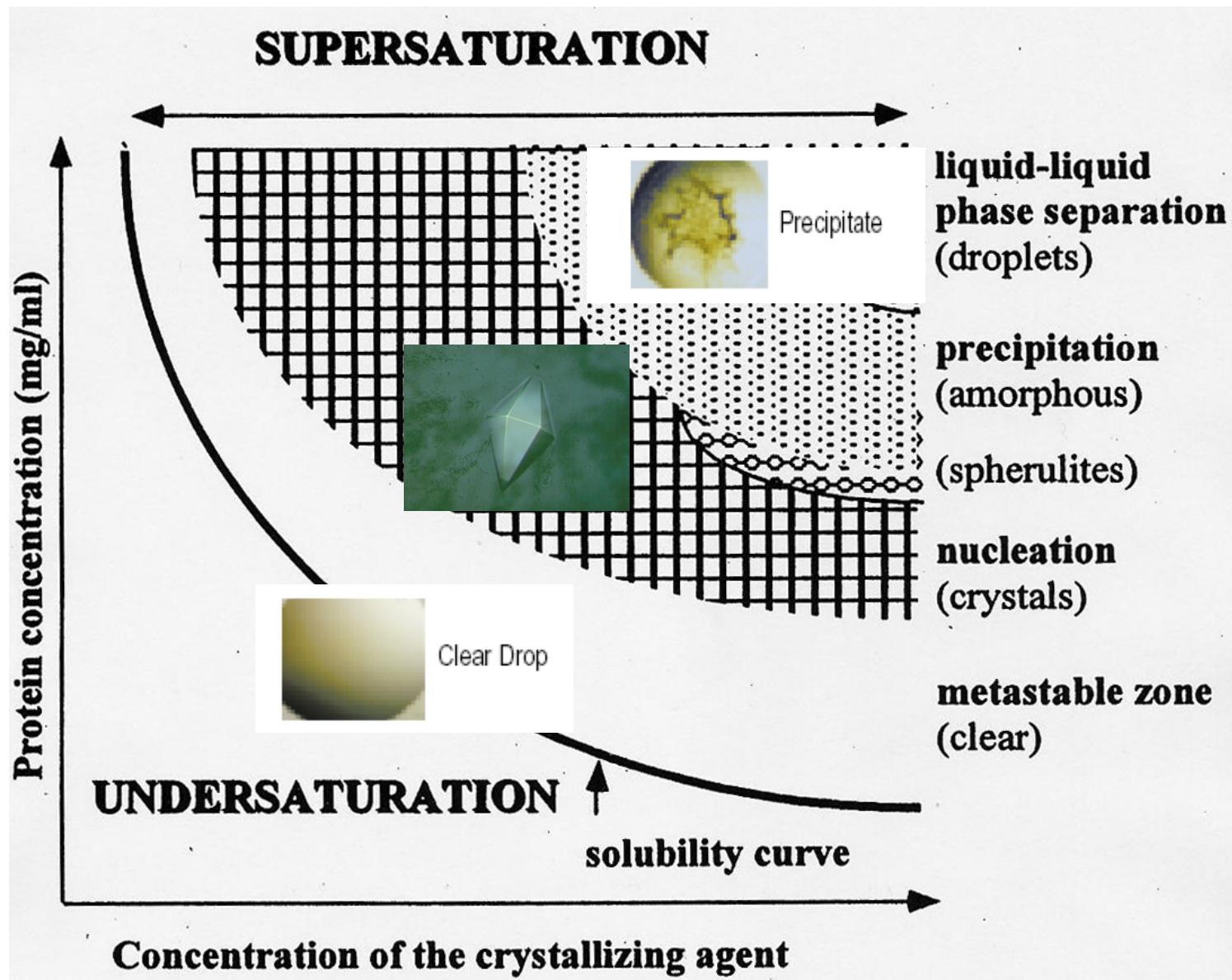
Develop technologies necessary for large-scale initiatives

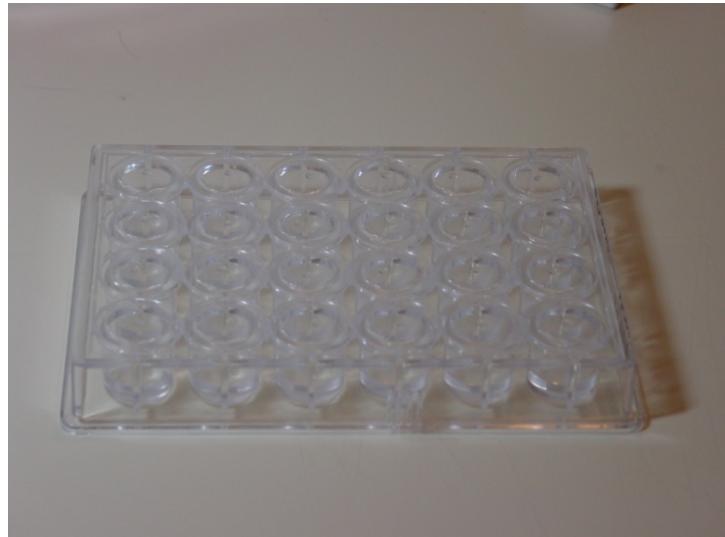
- develop high-throughput (HTP) cloning
- develop high-throughput expression
- develop high-throughput purification



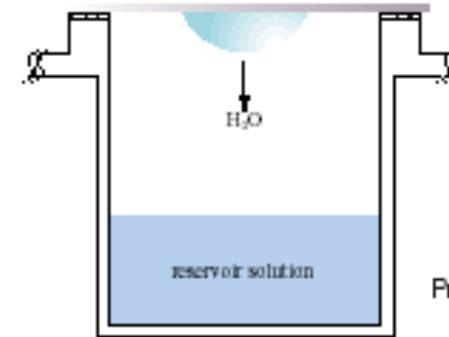
Protein Crystallization Methods



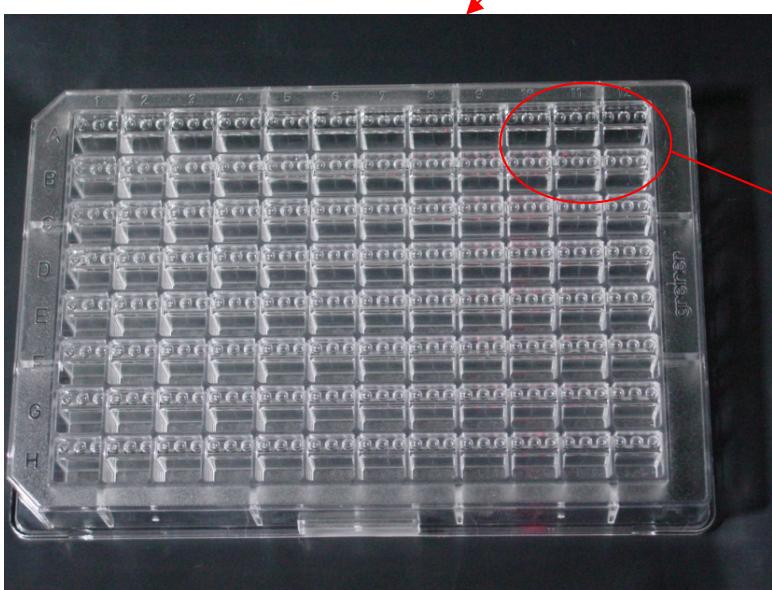




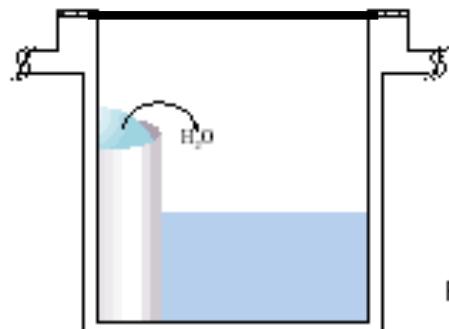
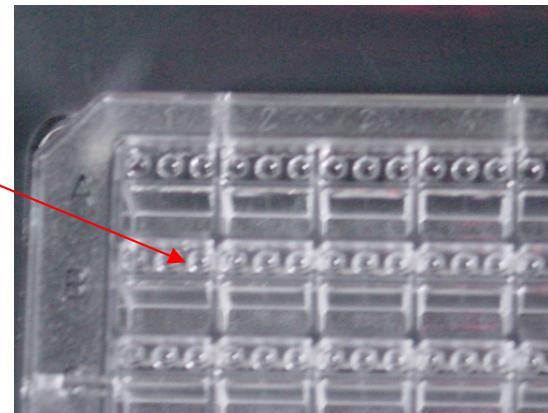
Plaque VDX, linbro
24 trous



Process of vapor diffusion.



Plaque Greiner
96 trous



Process of vapor diffusion.

Robot de Cristallogenèse

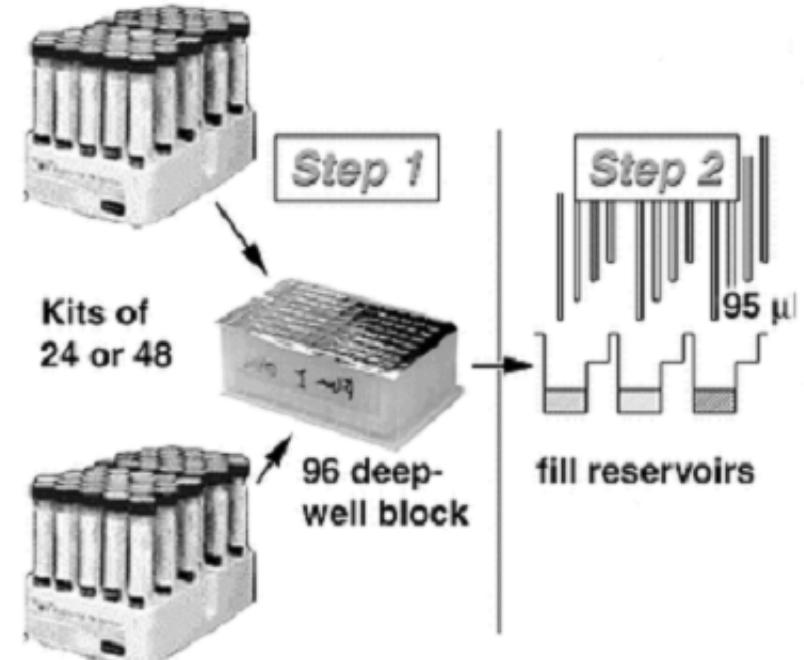
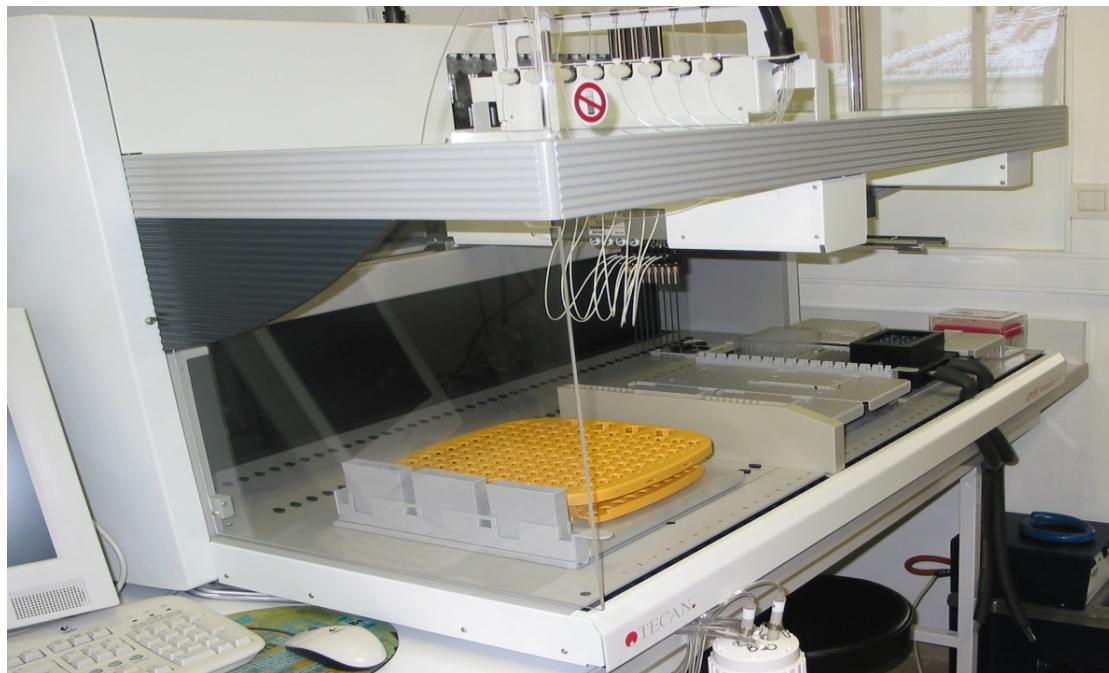
- Robot Tecan : Genesis workstation150:**

- Préparation des solutions de cristallisation.
 - Remplissage des plaques deep well de stockage.

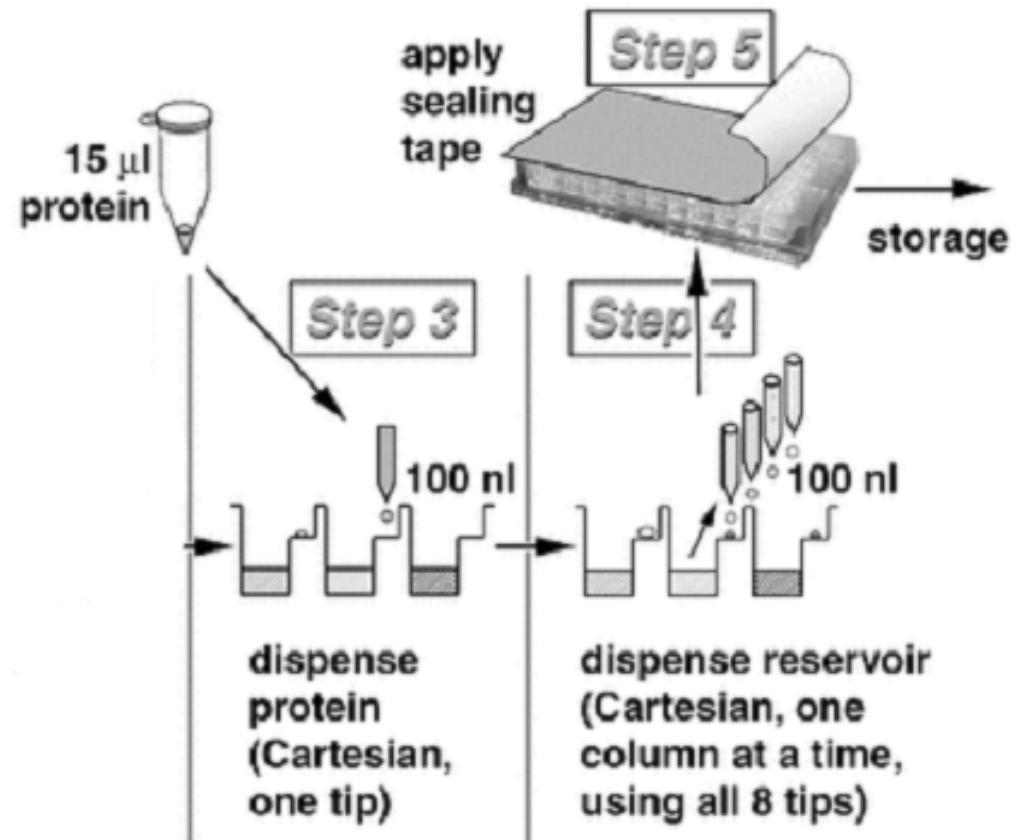
- Remplissage des plaques de cristallisation.

- Faire les mélanges protéine et solution de cristallisation :

- microgoutte : sitting drop

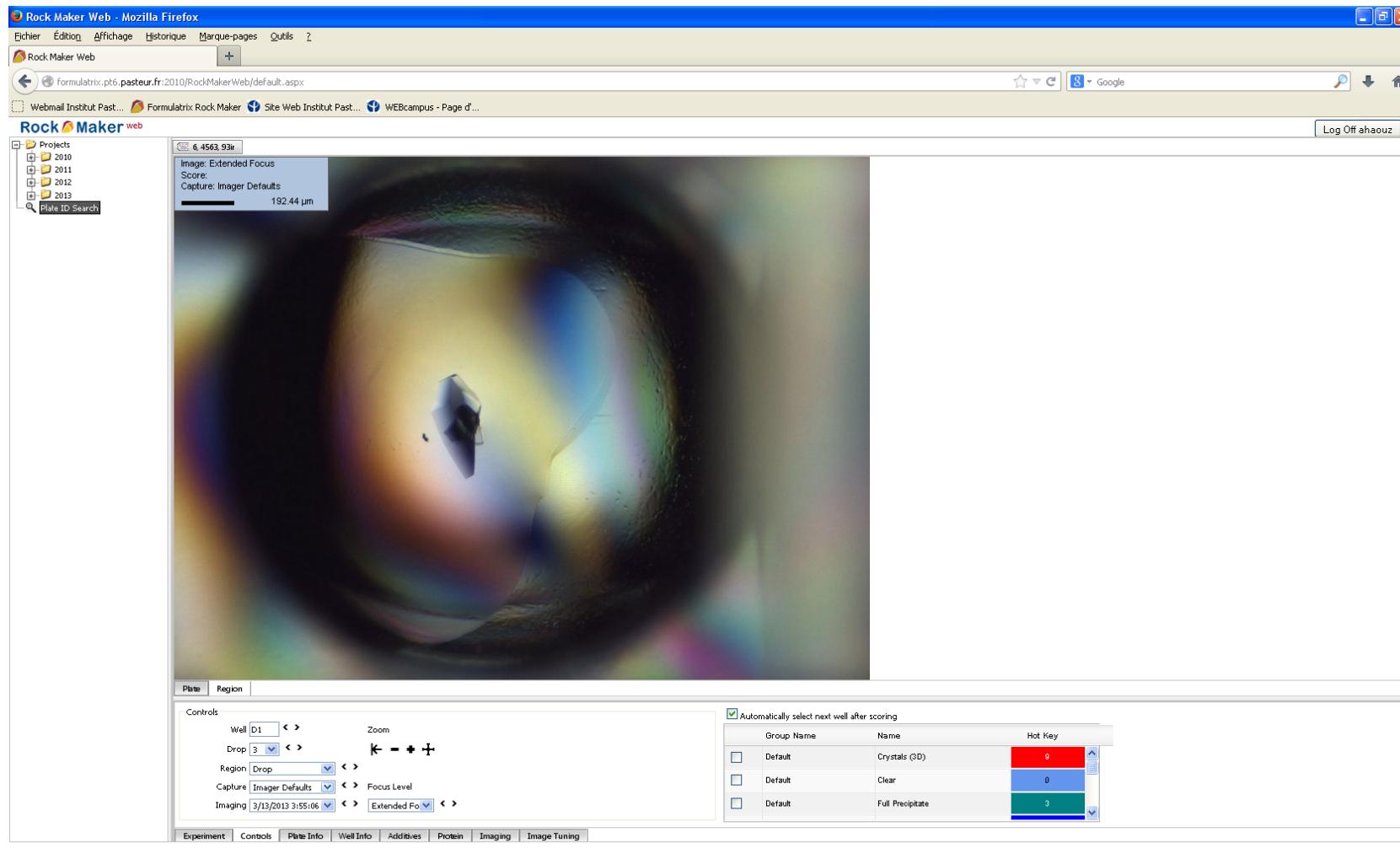


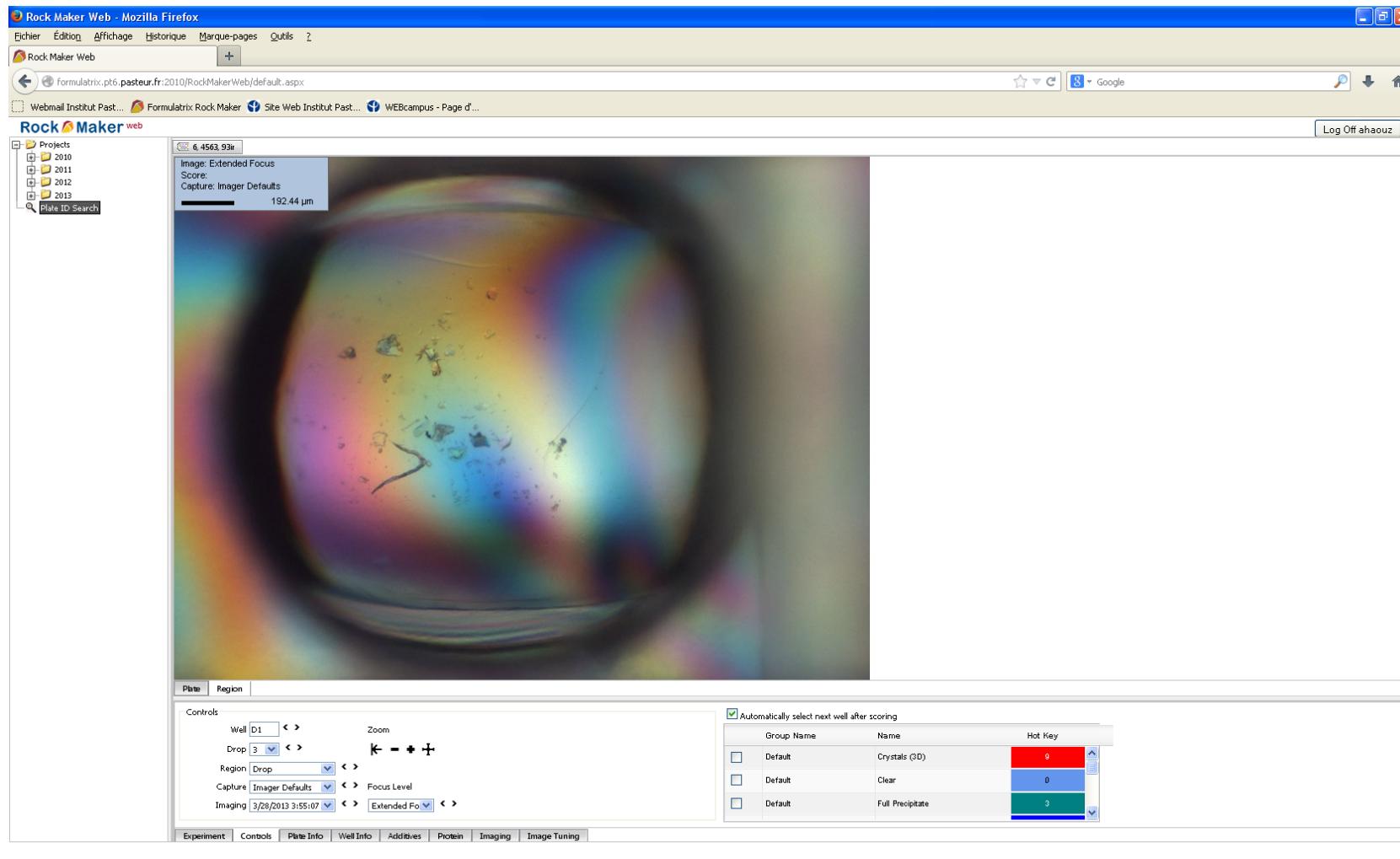
Robot cartesian pour les nanogouttes

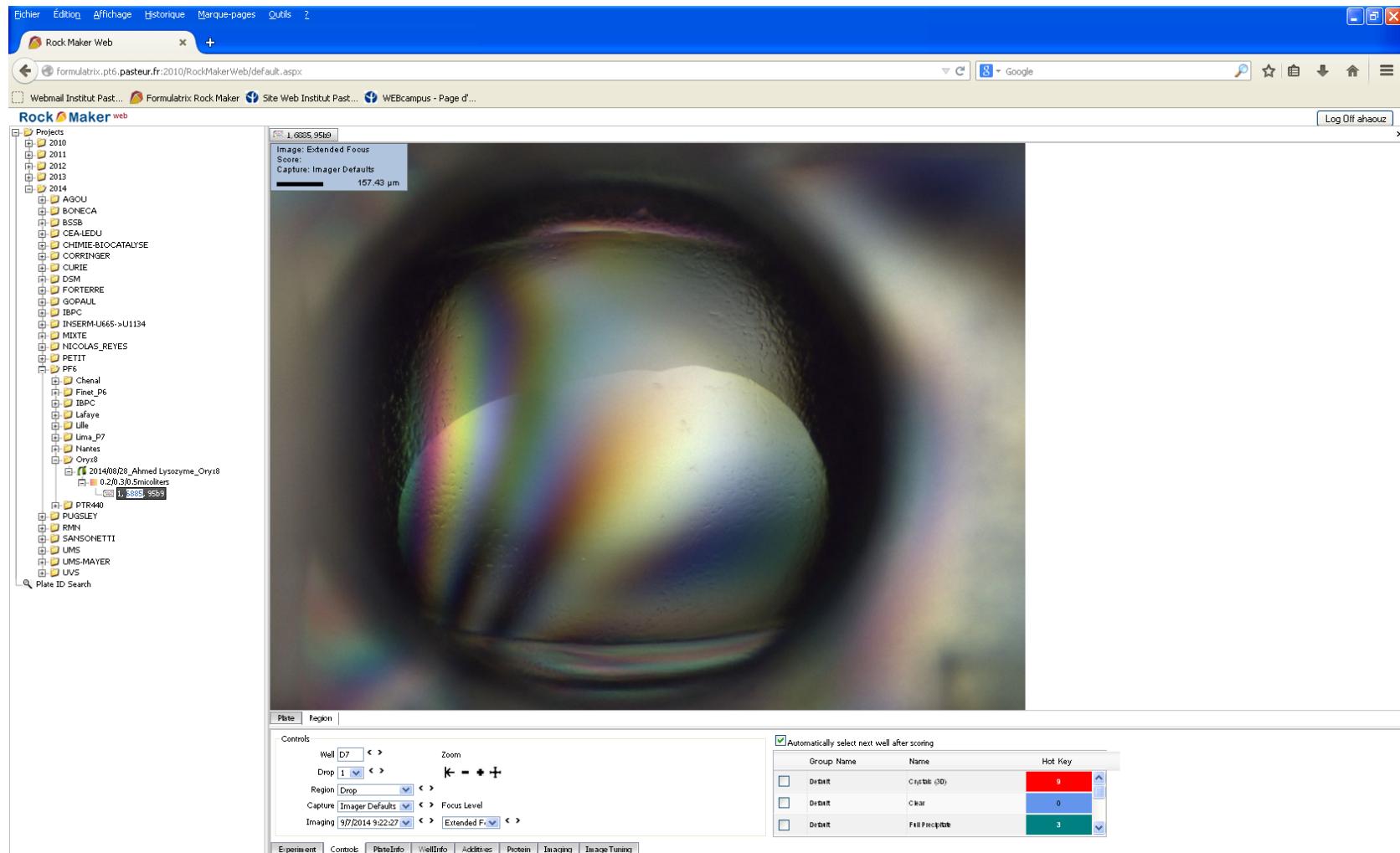


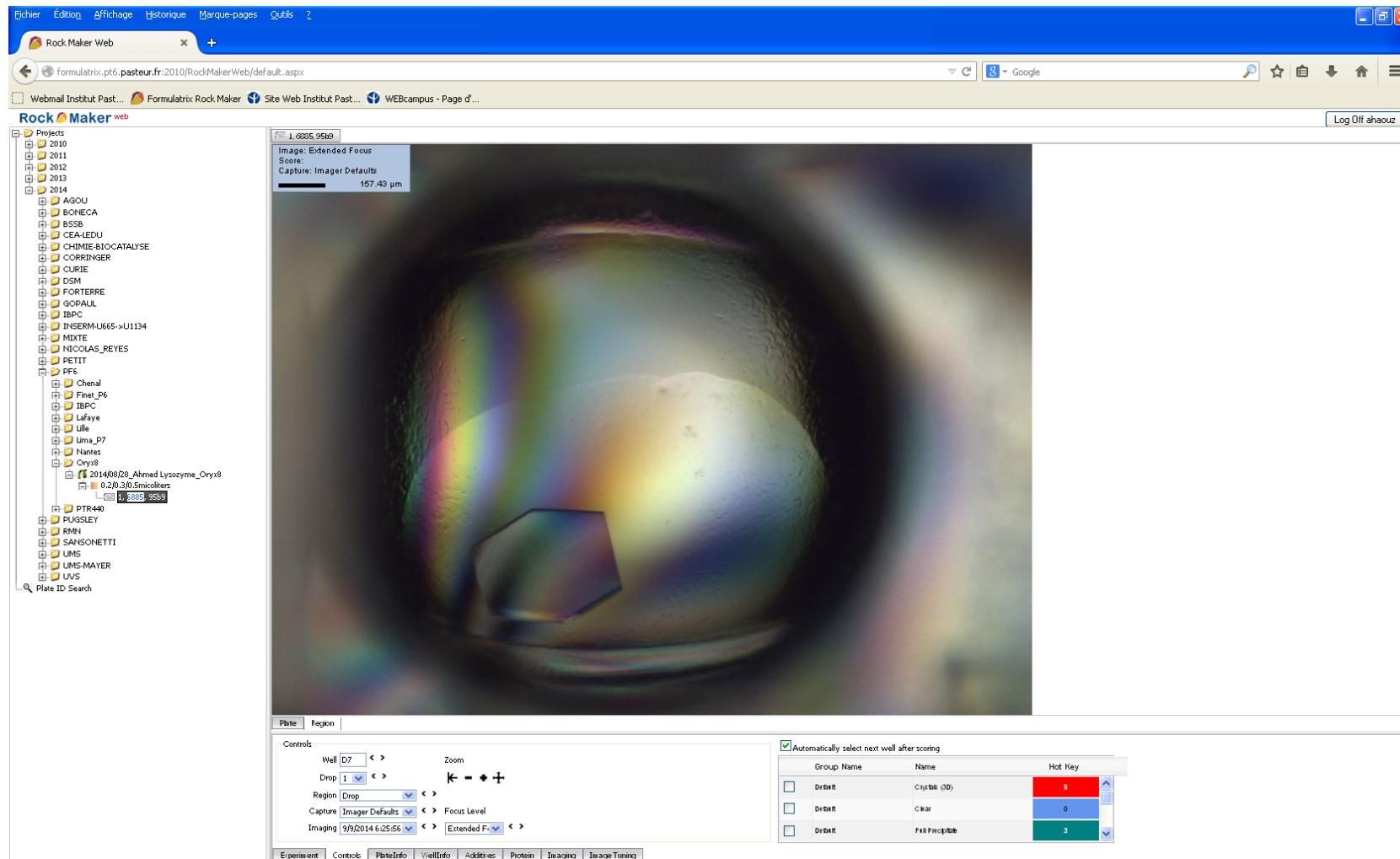
Suivi de l' apparition des cristaux

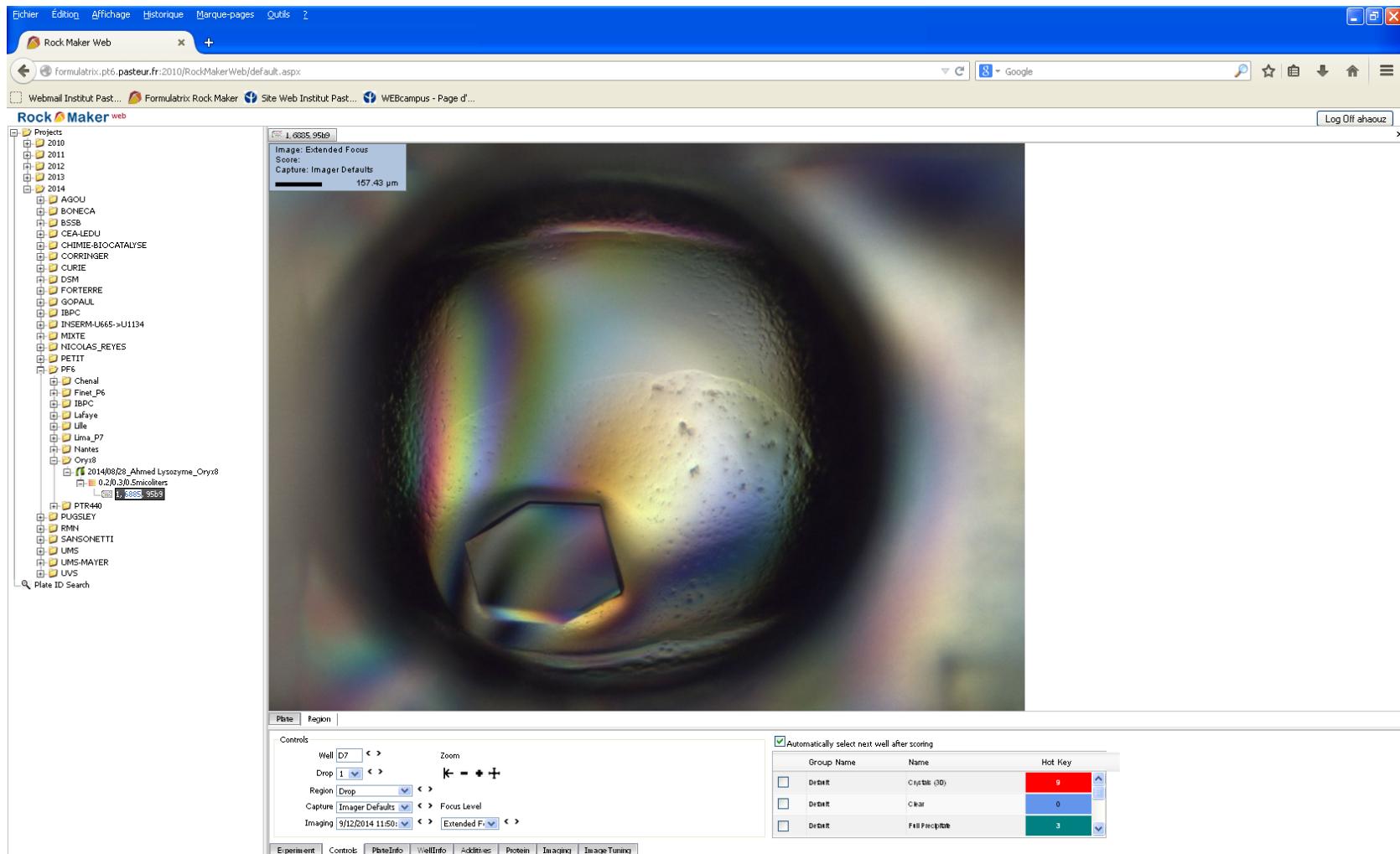


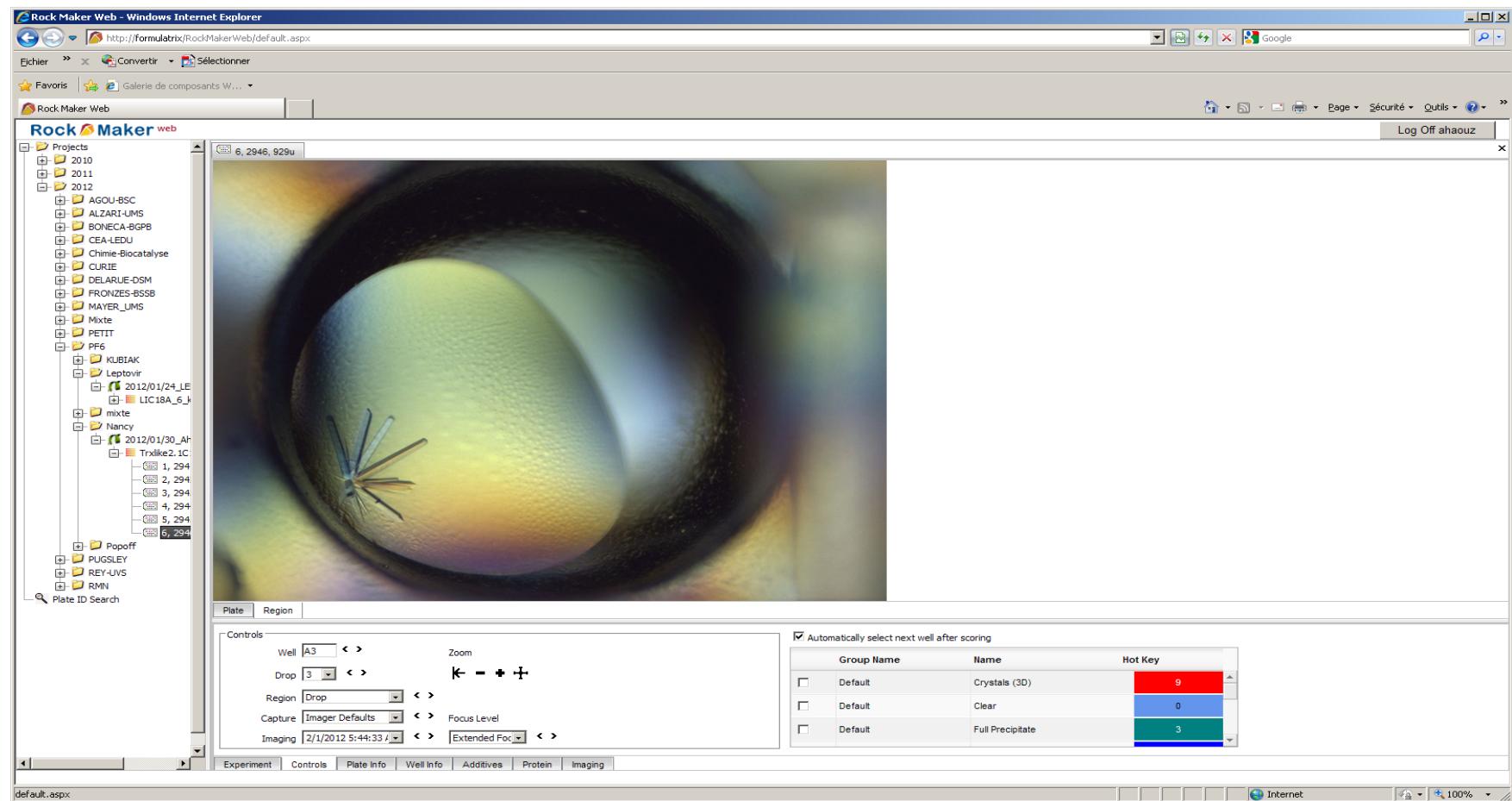


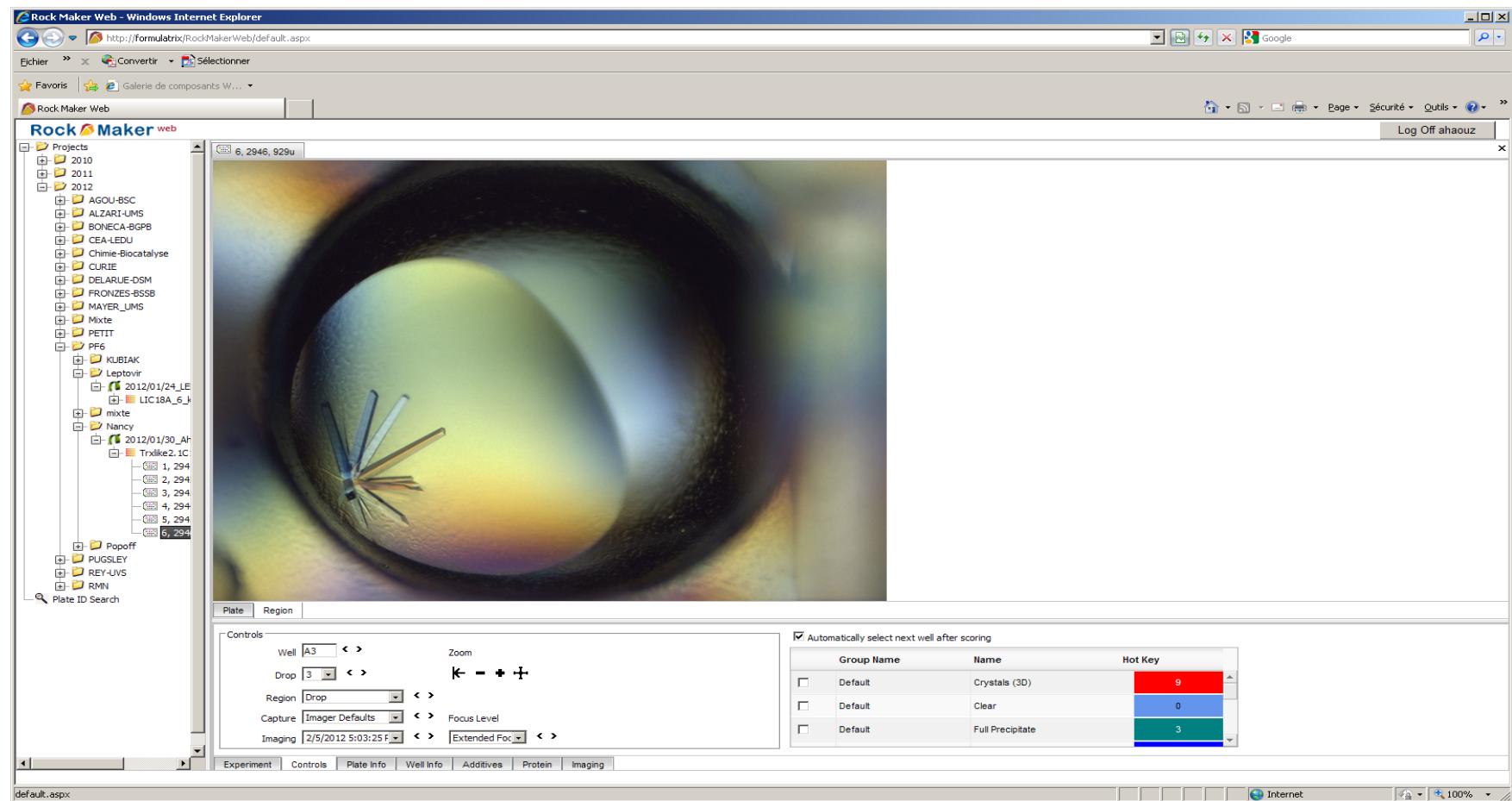








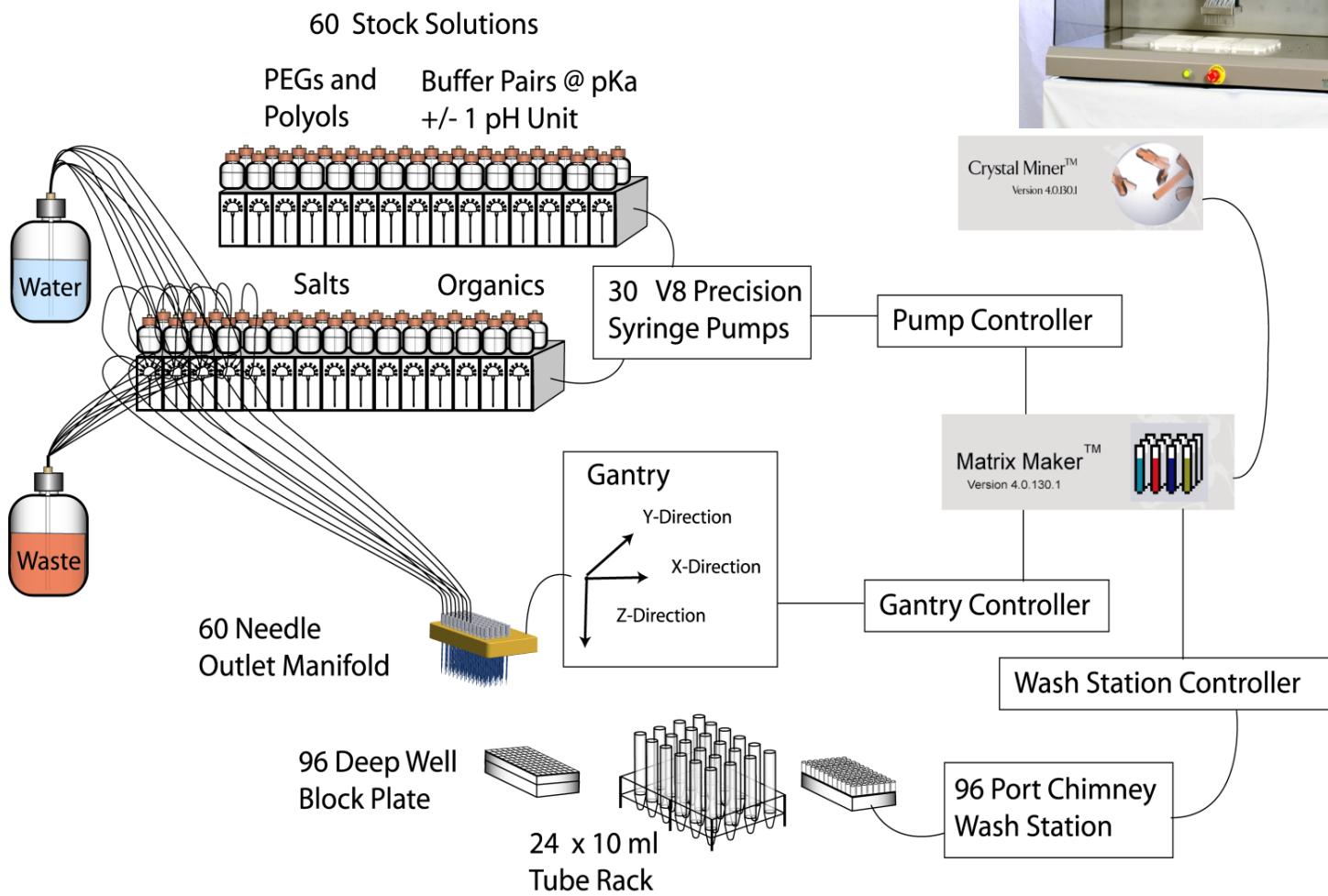




Matrix Maker™

Emerald BioSystems

integrated solutions for biotechnology



Proteins: known sequences and 3D structures

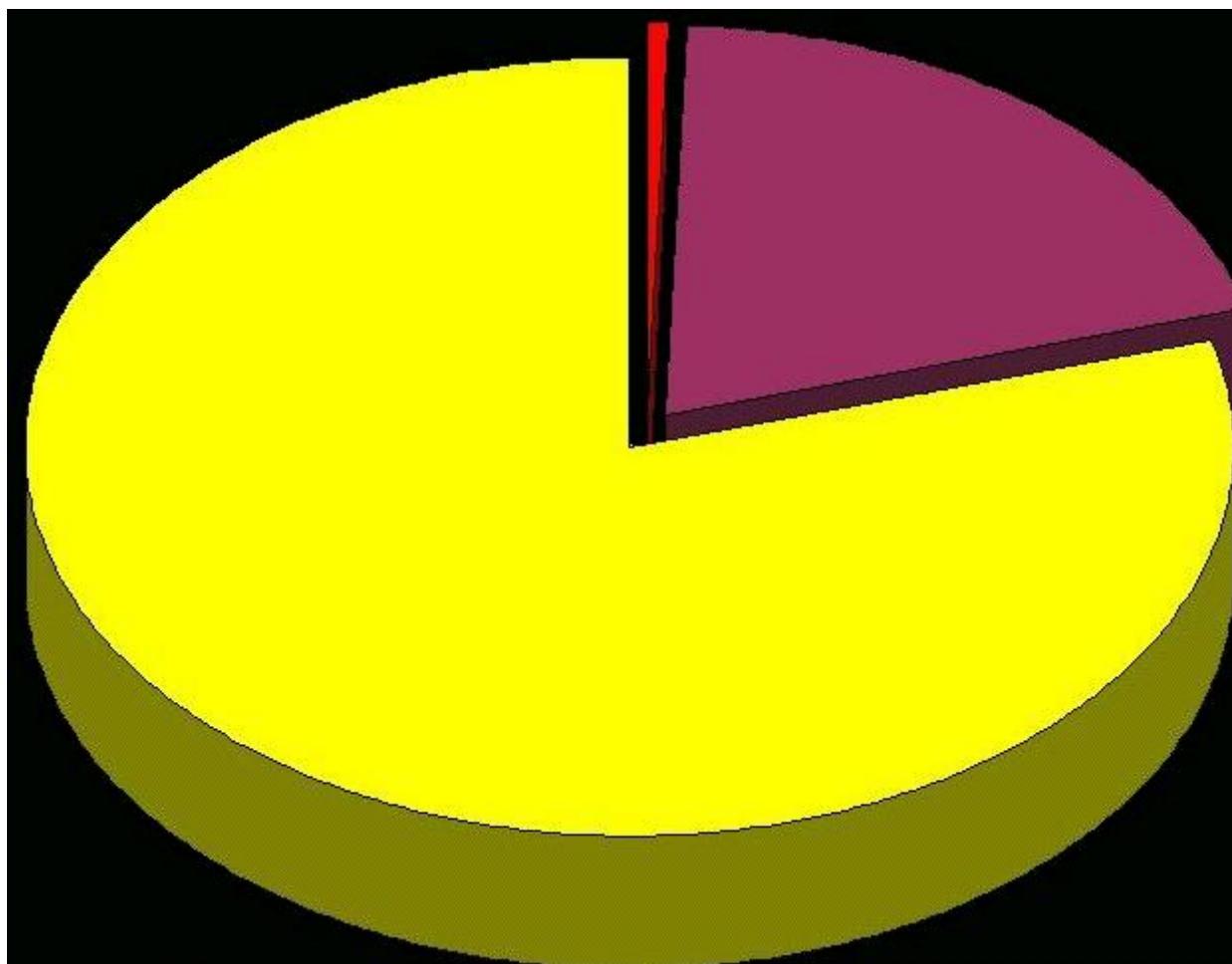
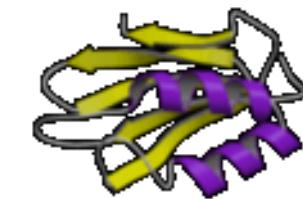
**~1.3m non-redundant
protein sequences**

MRTKSPGDSKFHEITKTPPKNQVSNS...
MIVISGENVDIAELTDFLCAA...
PPRIPYSMVGPCCVFLMH...
MDVVDSLFLVNGSNITSACELGFENE...
VYAWETAHFLDAAPKLIEWEV...
MAQQRRGGFKRRKKVDFIAANKIE...
CELGFENETLFCLDPRPRPSKE...
MAQQRRGGFKRRKKVDFIAANKIE...
MGMKKNRPRRGSLAFSPRKRAKKLVP...
MQILKENASNQRFVTRESEV...
MEKFEGYSEKQKSQQYFVYPFLF...
MEEFVNPKIKVIGVGGGGSNAVRMY...
MAVTQEIIAGIAEIEEVVTGIEP...
...

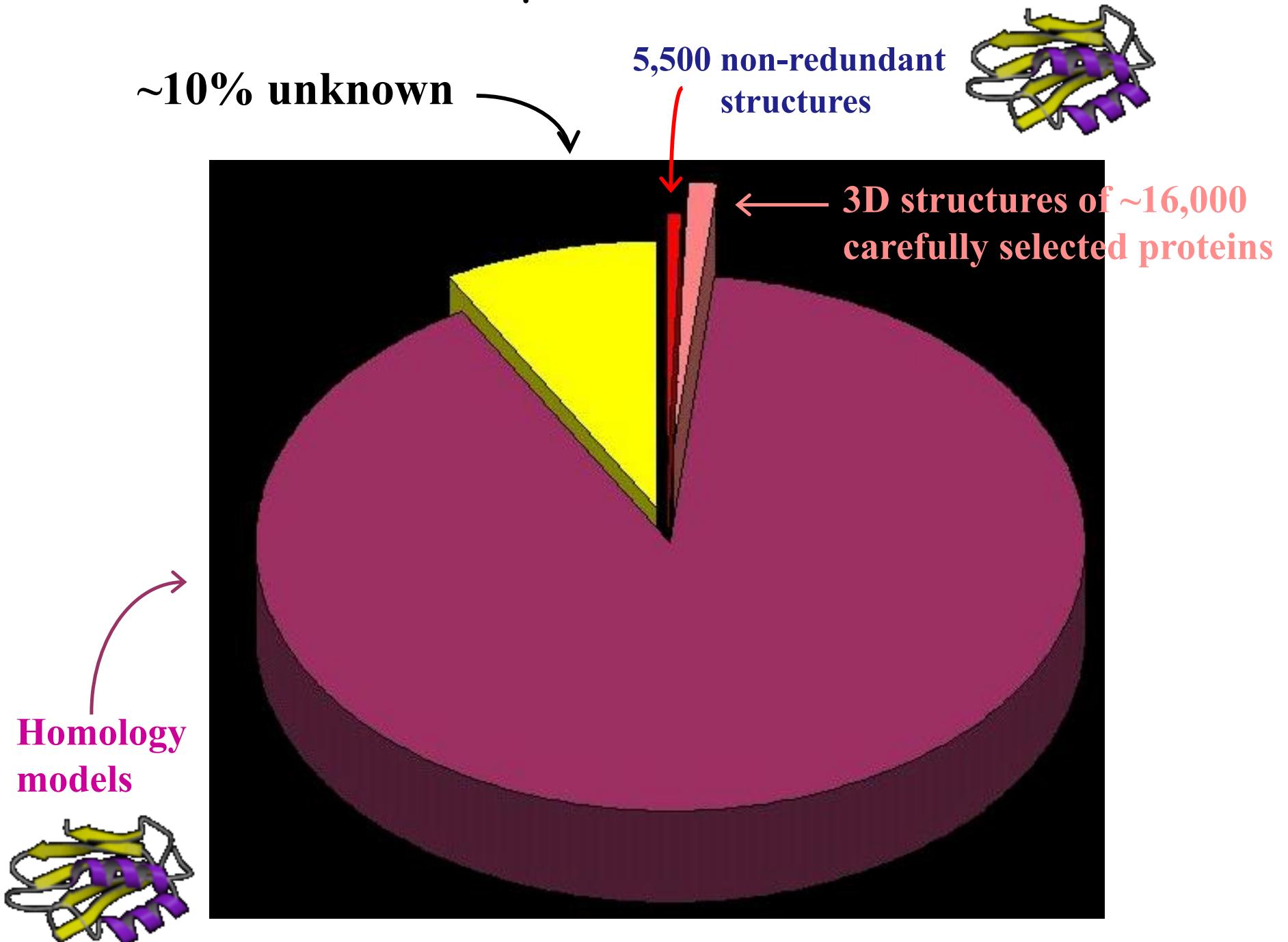
**5,500 non-redundant
structures**



**~260,000
homology
models**



Proteins: known sequences and 3D structures



Yearly Growth of Total Structures

number of structures can be viewed by hovering mouse over the bar

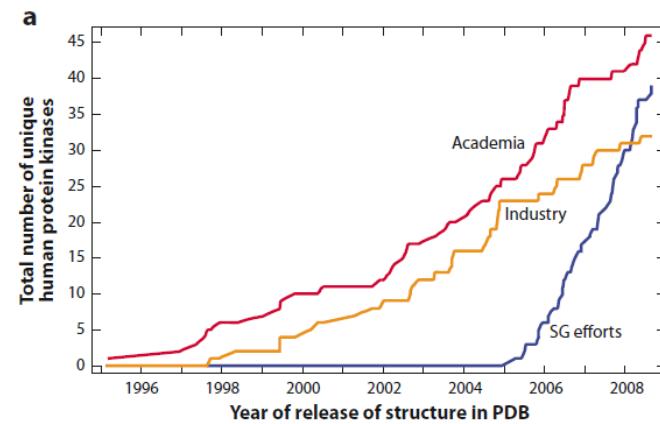
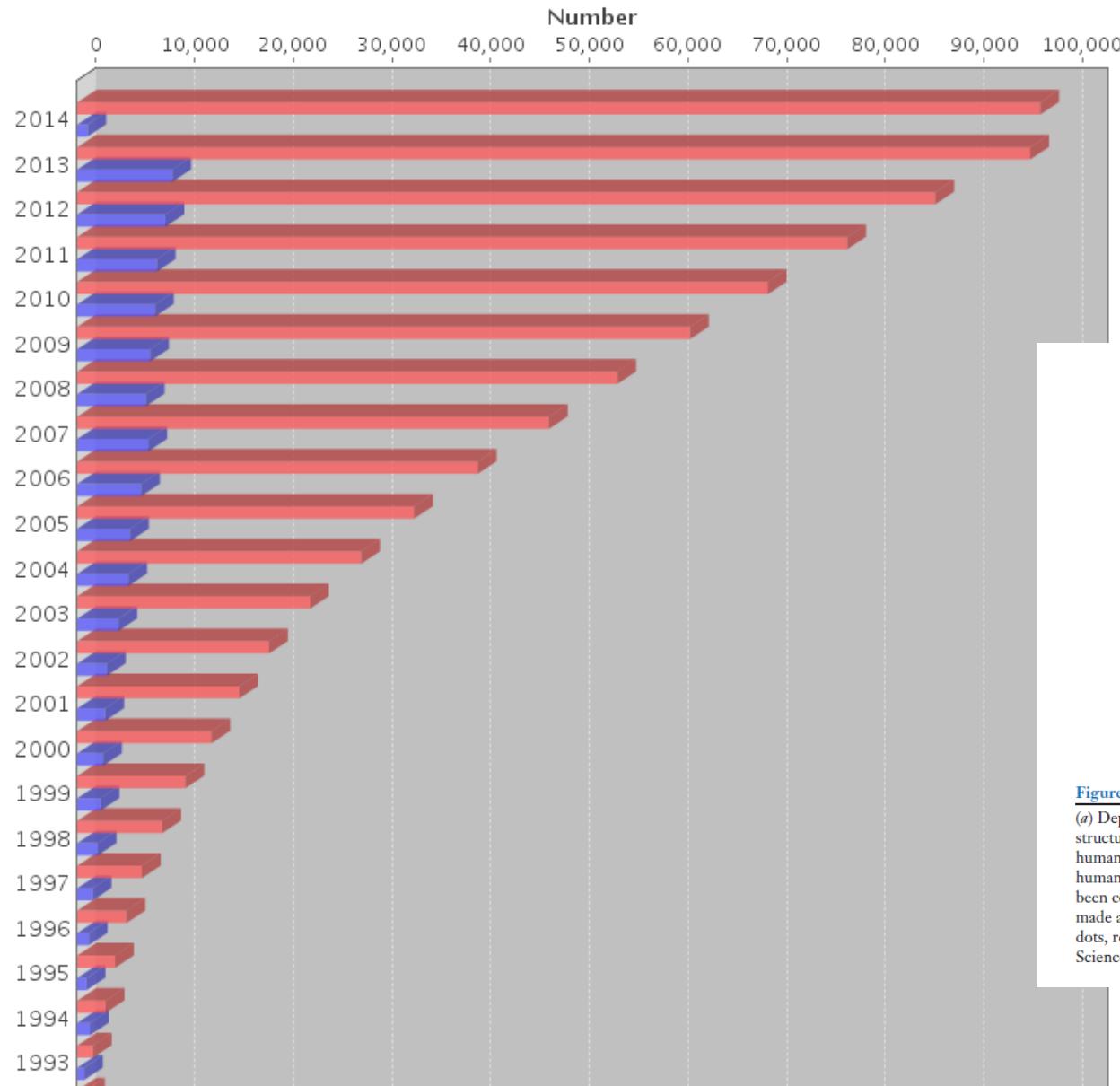


Figure 2

(a) Deposition of protein kinase catalytic domains into the public databases by academia, industry, and structural genomics projects. (b) Structural coverage and phylogenetic tree of the catalytic domains of the human protein kinase family. Targets with available 3D structure that share at least 95% identity to the human reference sequence and a resolution of at least 3 Å are indicated by dots. Atypical kinases have not been considered. Structures deposited by academic laboratories are indicated by red dots, and structures made available by industrial laboratories and structural genomics efforts are highlighted by yellow and blue dots, respectively. The phylogenetic tree has been reproduced with permission from Cell Signaling and Science (76).

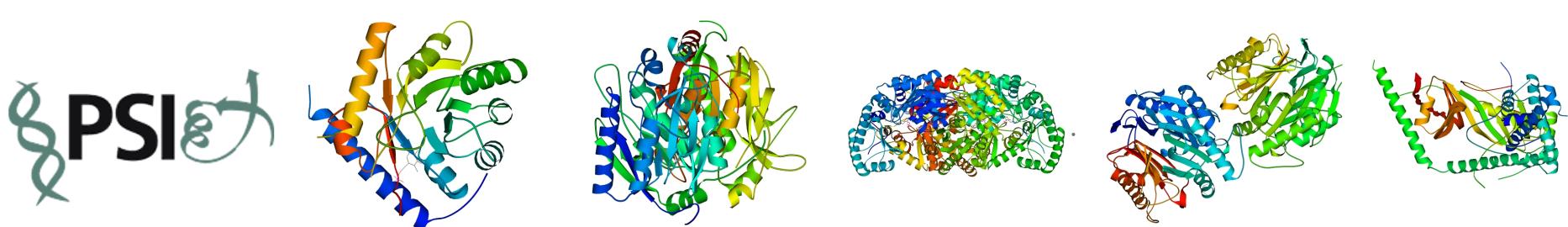
Structural Genomics

“The next step beyond the human genome project”

From the NIH Request for Proposals for Structure Genomics Centers:

“These studies should lead to an **understanding of structure/function** relationships and the ability to obtain **structural models** of all proteins identified by genomics.

This project will require the determination of a **large number** of protein structures in a **high-throughput mode**.”





- TargetDB progress "summary statistics" report for Structural Genomics Centers in Asia
Summary reports and sequence lists are maintained by both TargetDB and the contributing site.

The contributing Centers in the table below are grouped by project/geographical region:

[PSI Centers](#), [Centers in Europe](#), [Centers in North America](#), [Centers in Asia](#)

 Protein Structure Initiative Centers	
Accelerated Technologies Center for Gene to 3D Structure (ATCG3D) – PSI-2	
The Berkeley Structural Genomics Center (BSGC) – PSI-1	
Reports: Targets Status Organisms PDB Redundancy	
The Center for Eukaryotic Structural Genomics (CESG) – PSI-2	
Reports: Targets Status Organisms PDB Redundancy	
Center for High-Throughput Structural Biology (CHTSB) – PSI-2	
Center for Structure of Membrane Proteins (CSMP) – PSI-2	
The Joint Center for Structural Genomics (JCSG) – PSI-2	
Reports: Targets Status Organisms PDB Redundancy	
The Integrated Center for Structure and Function Innovation (ISFI) – PSI-2	
Reports: Targets Status Organisms PDB Redundancy	
The Midwest Center for Structural Genomics (MCSG) – PSI-2	
Reports: Targets Status Organisms PDB Redundancy	
The NorthEast Structural Genomics Consortium (NESG) – PSI-2	
Reports: Targets Status Organisms PDB Redundancy	
New York Consortium on Membrane Protein Structure (NYCOMPS) – PSI-2	
The New York Structural Genomics Research Consortium (NYSGRC) – PSI-2	
Reports: Targets Status Organisms PDB Redundancy	
The Southeast Collaboratory for Structural Genomics (SECSG) – PSI-1	
Reports: Targets Status Organisms PDB Redundancy	
Structural Genomics of Pathogenic Protozoa Consortium (SGPP) – PSI-1	
Reports: Targets Status Organisms PDB Redundancy	
Mycobacterium tuberculosis Structural Genomics Consortium (TBSGC) – PSI-1	
Reports: Targets Status Organisms PDB Redundancy	

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PSI - Structures

(Sep-2005 1246 images)

[Fichier](#) [Edition](#) [Affichage](#) [Favoris](#) [Outils](#) ?[Précédente](#) [Suivante](#) [Rechercher](#) [Favoris](#) [Média](#)Adresse http://sg.pdb.org/target_centers.htmlLiens [Hotmail](#) [Personnaliser les liens](#) [Windows](#) [Windows Media](#)

Structural Genomics projects in Europe

Bacterial Targets at IGS-CNRS, France (BIGS)

Reports: [Targets](#) | [Status](#) | [Organisms](#) | [PDB](#) | [Redundancy](#)

The Israel Structural Proteomics Center (ISPC)

Reports: [Targets](#) | [Status](#) | [Organisms](#) | [PDB](#) | [Redundancy](#)

Marseilles Structural Genomics Program, France (MSGP)

Reports: [Targets](#) | [Status](#) | [Organisms](#) | [PDB](#) | [Redundancy](#)

Oxford Protein Production Facility, England (OPPF)

Reports: [Targets](#) | [Status](#) | [Organisms](#) | [PDB](#) | [Redundancy](#)

Structural Proteomics in Europe, England (SPINE)

Reports: [Targets](#) | [Status](#) | [Organisms](#) | [PDB](#) | [Redundancy](#)

Mycobacterium Tuberculosis Structural Proteomics Project, Germany (XMTB)

Reports: [Targets](#) | [Status](#) | [Organisms](#) | [PDB](#) | [Redundancy](#)

Paris-Sud Yeast Structural Genomics, France (YSG)

Reports: [Targets](#) | [Status](#) | [Organisms](#) | [PDB](#) | [Redundancy](#)

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Structural Genomics projects in North America

Montreal-Kingston Bacterial Structural Genomics Initiative, Canada (BSGI)

Reports: [Targets](#) | [Status](#) | [Organisms](#) | [PDB](#) | [Redundancy](#)

Structure 2 Function Project, USA (S2F)

Reports: [Targets](#) | [Status](#) | [Organisms](#) | [PDB](#) | [Redundancy](#)

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Structural Genomics projects in Asia

RIKEN Structural Genomics Initiative, Japan (RSGI)

Reports: [Targets](#) | [Status](#) | [Organisms](#) | [PDB](#) | [Redundancy](#)

© RCSB PDB



Internet

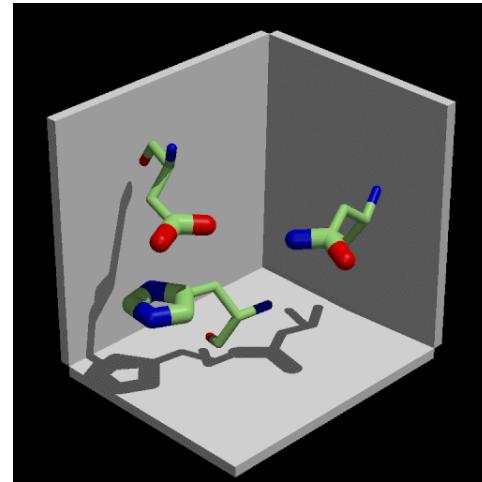
SG consortia 2000-2015

- Investment of 2 billion U\$S (PSI 1 billion)
- 13500 protein structures solved
- 4400 publications (>100000 citations)
- Structural data freely available to the scientific community
- Important software and database developments
- Creation, improvement and validation of many protocols for protein production and crystallization, data collection, and functional analysis

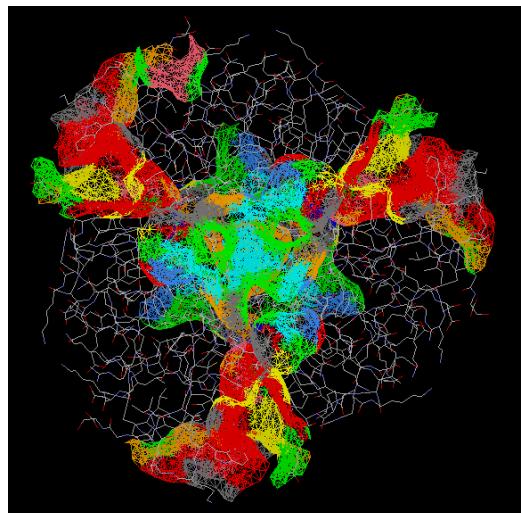
Function through homology

Sequence similarity

Motif searches



Active Site Templates



Surface comparison



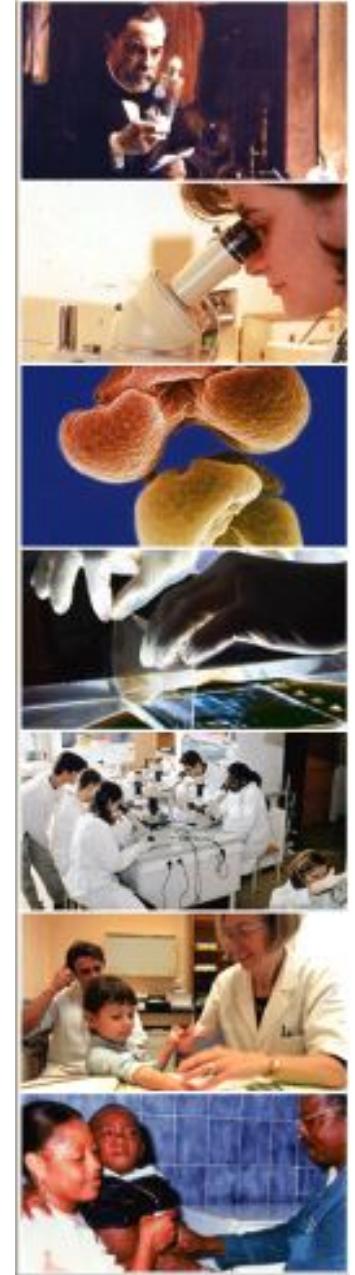
HTH motifs



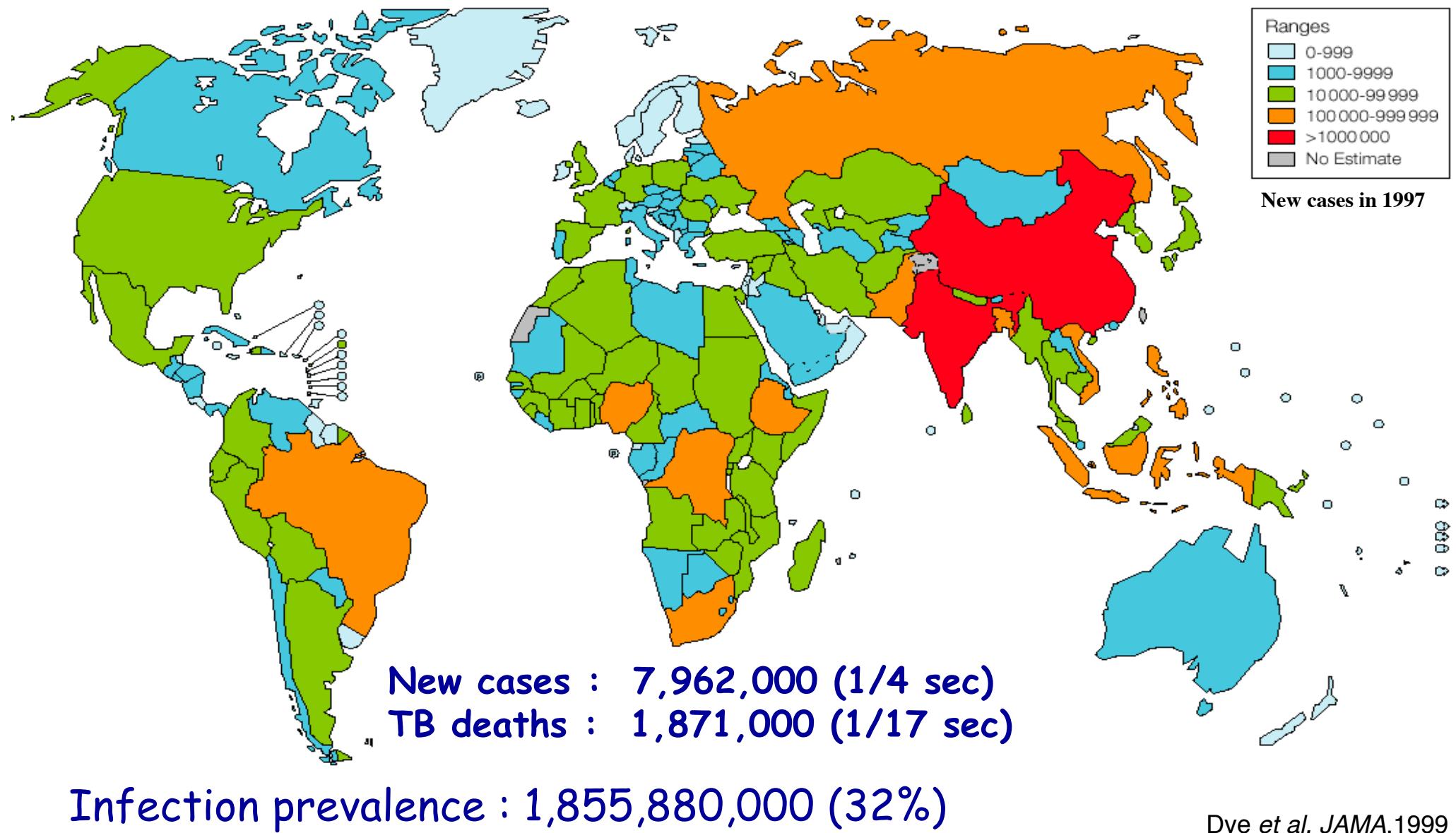
Structural Similarity

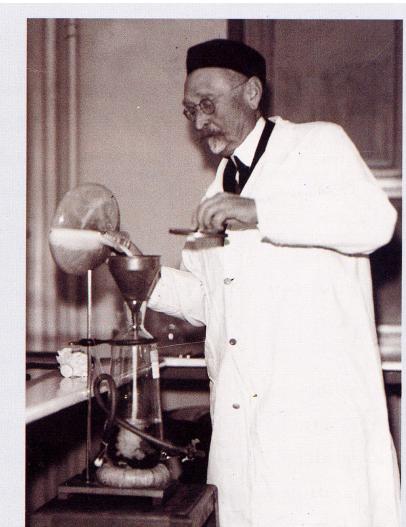
Structural Genomics of Mycobacteria

Pedro M. Alzari



Estimates of TB burden (1997)





BCG: A Calmette, C Guerin

article

Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence

S. T. Cole¹, R. Brosch¹, J. Parkhill¹, T. A. James², N. R. Thomson³, P. R. Wheeler⁴, H. Hauzeur⁵, T. Garnier⁶, C. Churchar⁷, D. Harris⁸, K. Mungall⁹, D. Basham⁹, D. Brown¹⁰, T. Chillingworth¹¹, R. Connor¹², R. M. Davies¹³, K. Devlin¹⁴, S. Duthoy¹⁵, T. Feltwell¹⁶, A. Fraser¹⁷, N. Hamlin¹⁸, S. Holroyd¹⁹, T. Hornsby²⁰, K. Jagels²¹, C. Lacroix²², J. Maclean²³, S. Moulé²⁴, L. Murphy²⁵, K. Oliver²⁶, M. A. Quail²⁷, M.-A. Rajandream²⁸, K. M. Rutherford²⁹, S. Rutledge³⁰, K. Seeger³¹, S. Simon³², M. Simmonds³³, J. Skelton³⁴, R. Squares³⁵, S. Squares³⁶, J. Strelkowa³⁷, K. Tekaia³⁸, J. Woodward³⁹ & B. G. Barrell⁴⁰

¹Unit de Génomique Moléculaire Bactérienne, Institut Pasteur, Paris, France
²Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
³Tuberculosis Research Unit, Laboratory of International Health, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA
⁴§ Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark
⁵Centre de Recherche en Microbiologie, Université de Montréal, Montréal, Québec, Canada
⁶Centre de Recherche en Microbiologie, Université de Montréal, Montréal, Québec, Canada
⁷Unité de Génomique Moléculaire Bactérienne, Institut Pasteur, Paris, France
⁸Unité de Génomique Moléculaire Bactérienne, Institut Pasteur, Paris, France
⁹Tuberculosis Research Unit, Laboratory of International Health, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA
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¹¹Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
¹²Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
¹³Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
¹⁴Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
¹⁵Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
¹⁶Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
¹⁷Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
¹⁸Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
¹⁹Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
²⁰Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
²¹Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
²²Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
²³Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
²⁴Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
²⁵Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
²⁶Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
²⁷Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
²⁸Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
²⁹Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
³⁰Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
³¹Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
³²Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
³³Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
³⁴Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
³⁵Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
³⁶Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
³⁷Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
³⁸Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
³⁹Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
⁴⁰Wellcome Trust Genome Campus, Hinxton, Cambridge, UK

nature
International weekly journal of science

Mycobacterial genome revealed

Glasses Exploring the energy landscape

Comet chemistry Processing the comet

Altruism The importance of image building

Countless millions of people have died from tuberculosis over the last 2,000 years. The complete genome sequence of the bacterium that causes the disease has now been determined and analysed in order to improve the conception of new prophylactic and therapeutic measures. The genome contains around 4,000 genes, and has a high content of the proteins. *M. tuberculosis* has a low capacity devoted to the production of glycine-rich proteins with a repetitive sequence.

Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK

*Unit de Génomique Moléculaire Bactérienne, Institut Pasteur, Paris, France
†Tuberculosis Research Unit, Laboratory of International Health, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA
§Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark

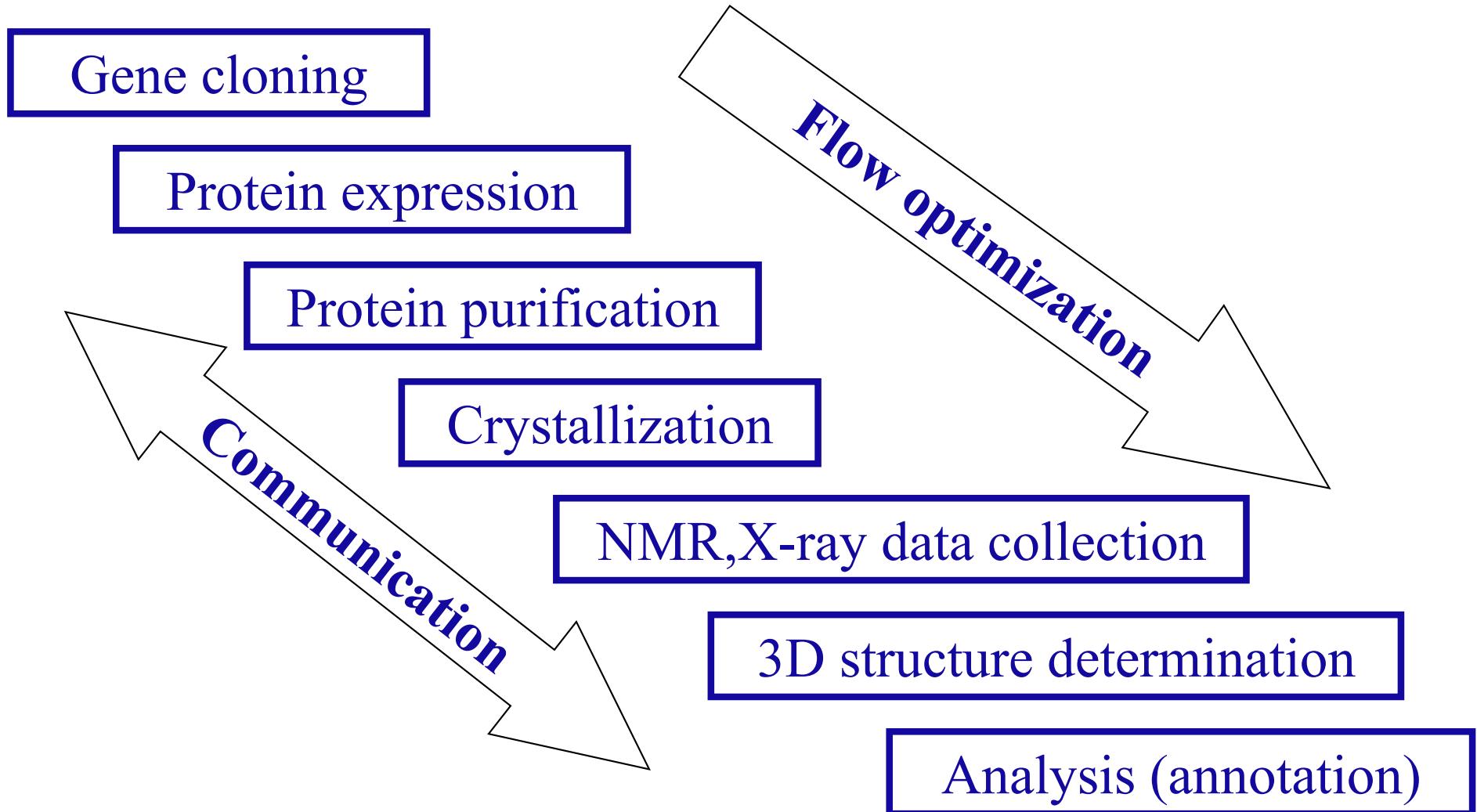
articles

Massive gene decay in the leprosy bacillus



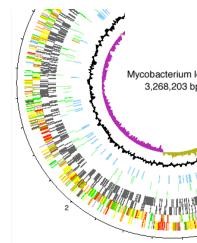
Mycobacterial genomics:
ST Cole & Sanger Center

Structural Genomics of Mycobacteria

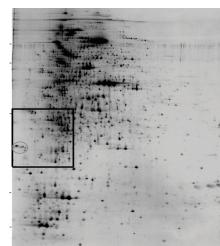




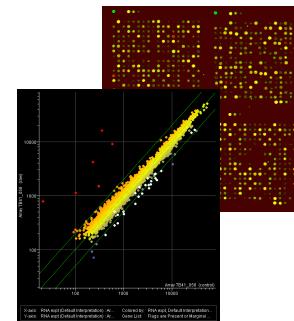
INSTITUT PASTEUR



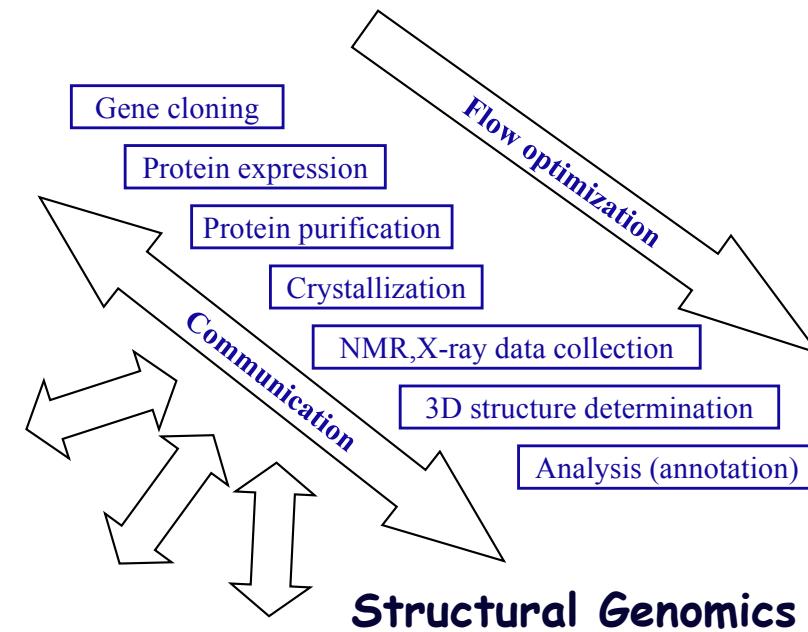
Genomics



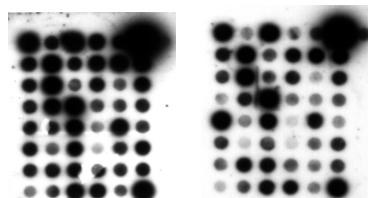
Proteomics



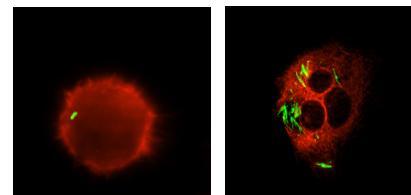
Microarrays



Structural Genomics



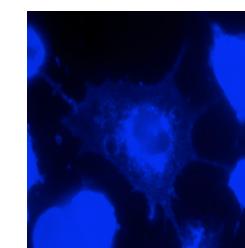
ST Mutagenesis



Functional genetics



TB microbiol.



TB immunol.

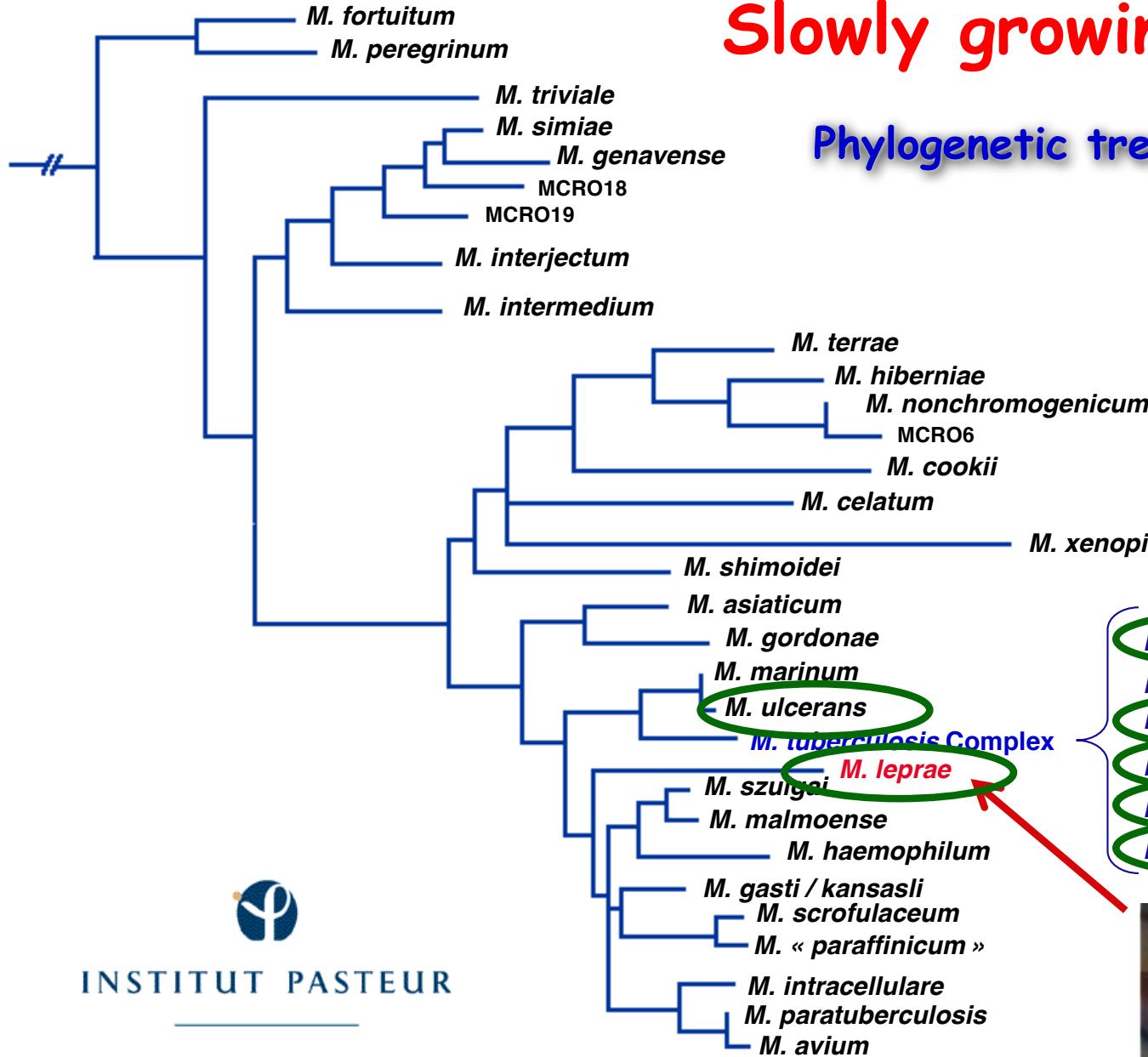


Animal infection

Drug Target Discovery and Validation

Slowly growing mycobacteria

Phylogenetic tree (16S rRNA gene seqs)



Tuberculosis



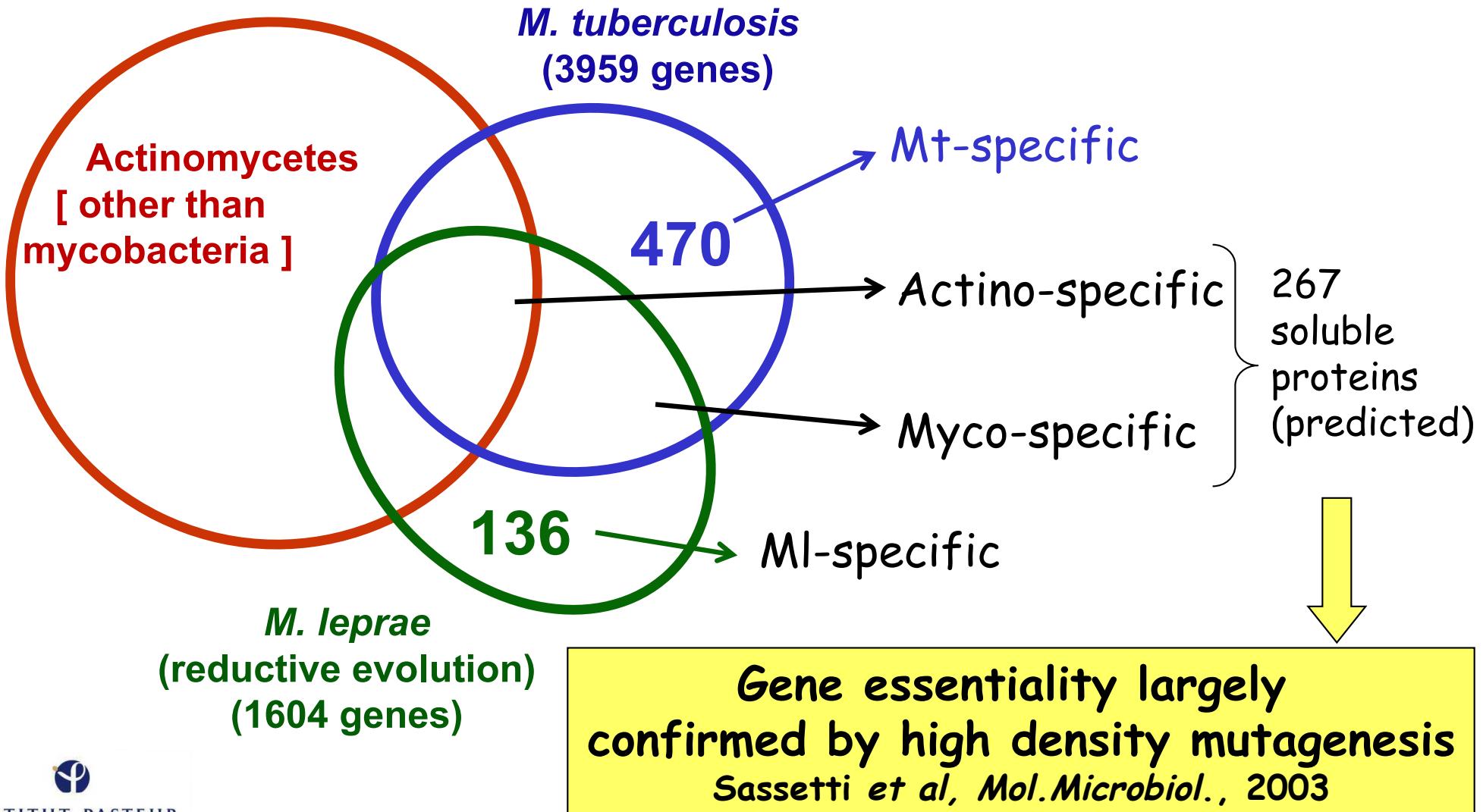
Leprosy

M. smegmatis



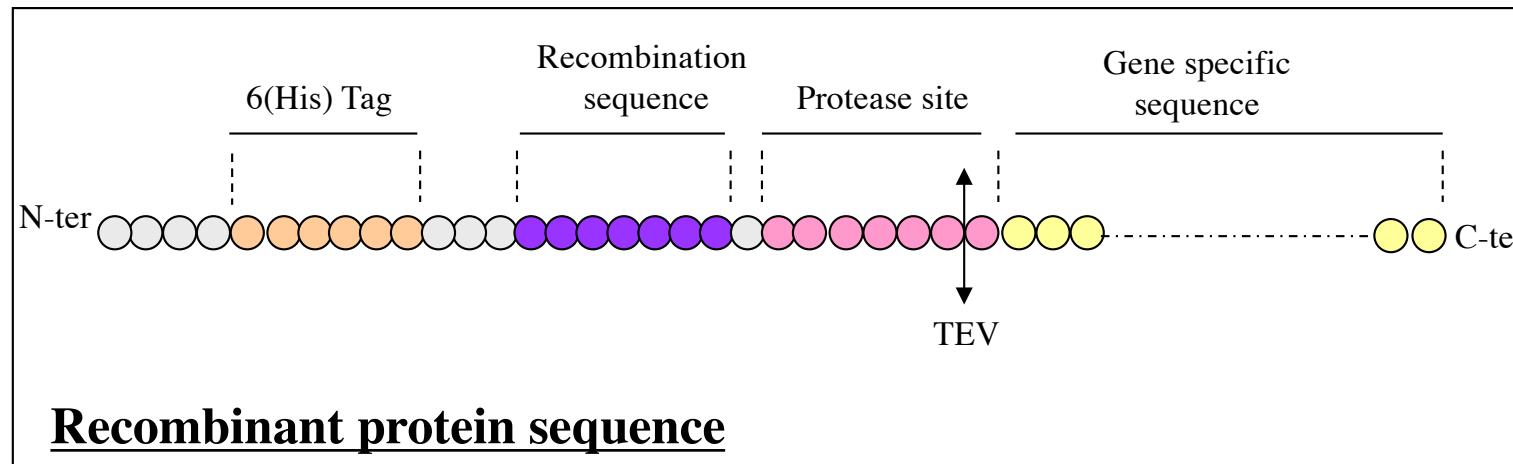
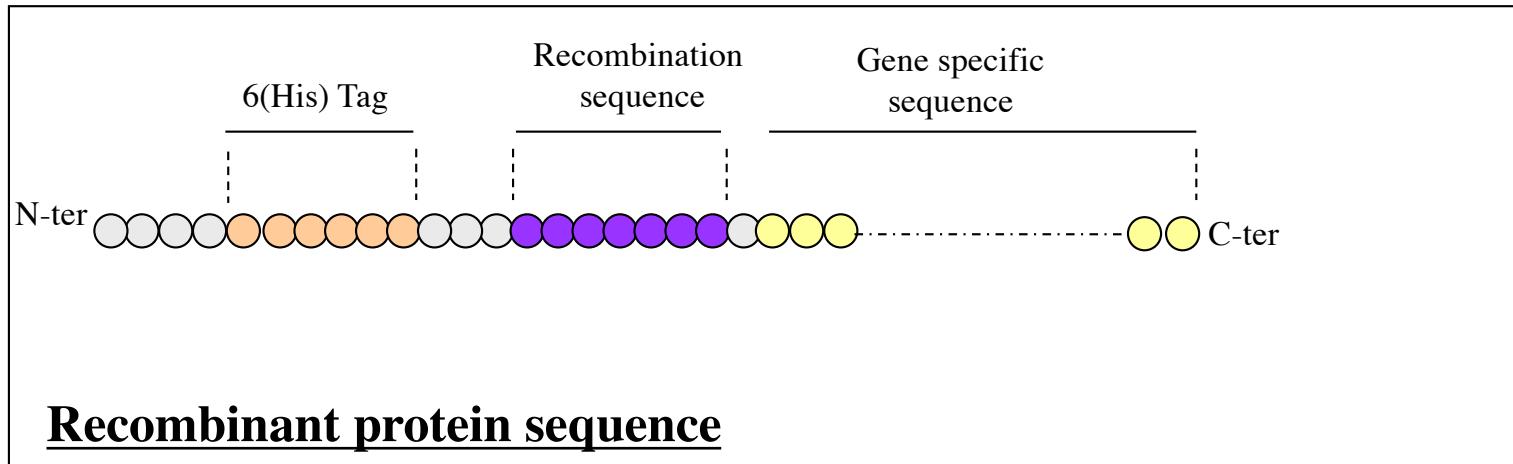
INSTITUT PASTEUR

Restricted genes of unknown function

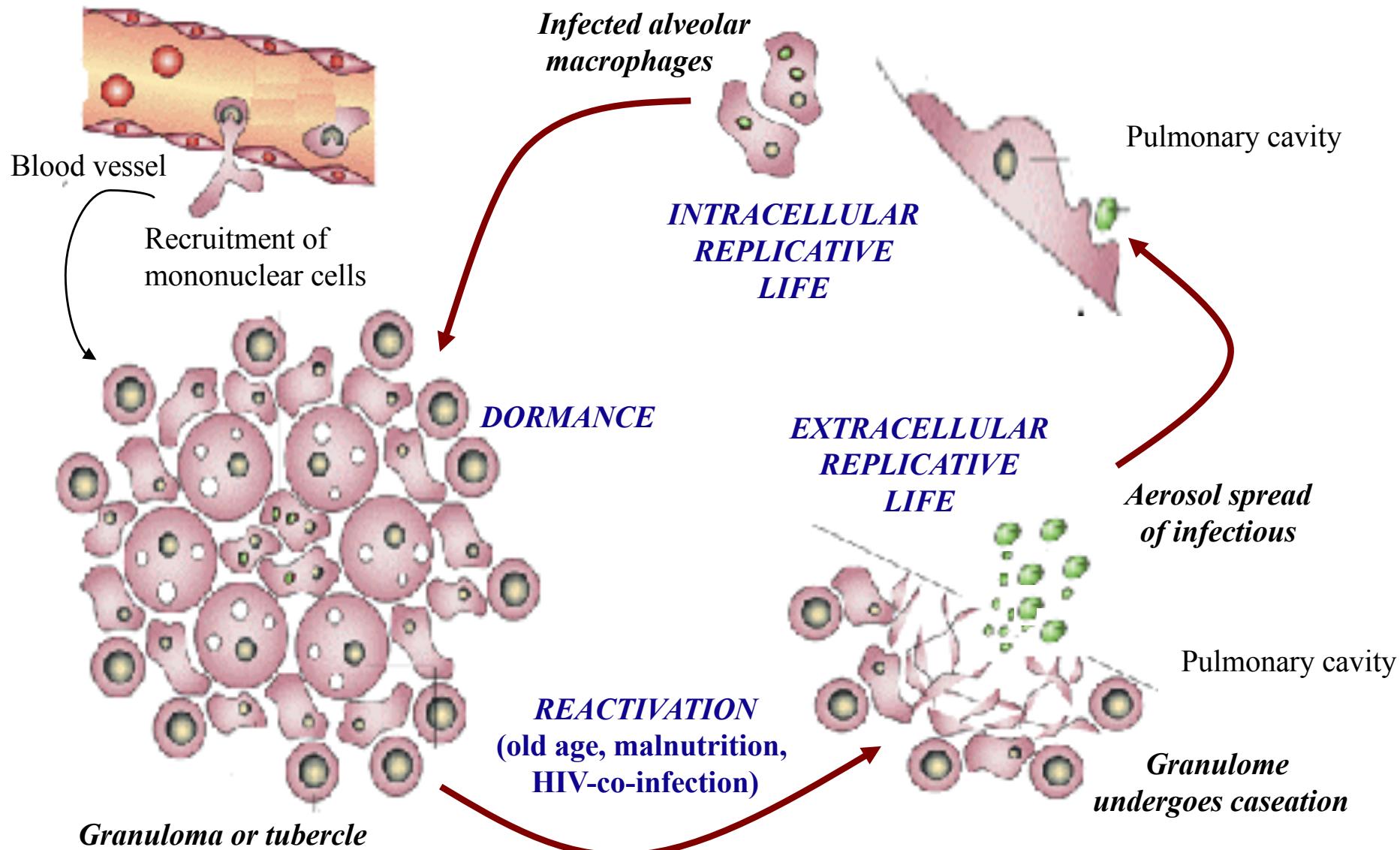


Cloning strategy

Genetic recombination (Gateway) from invitrogen

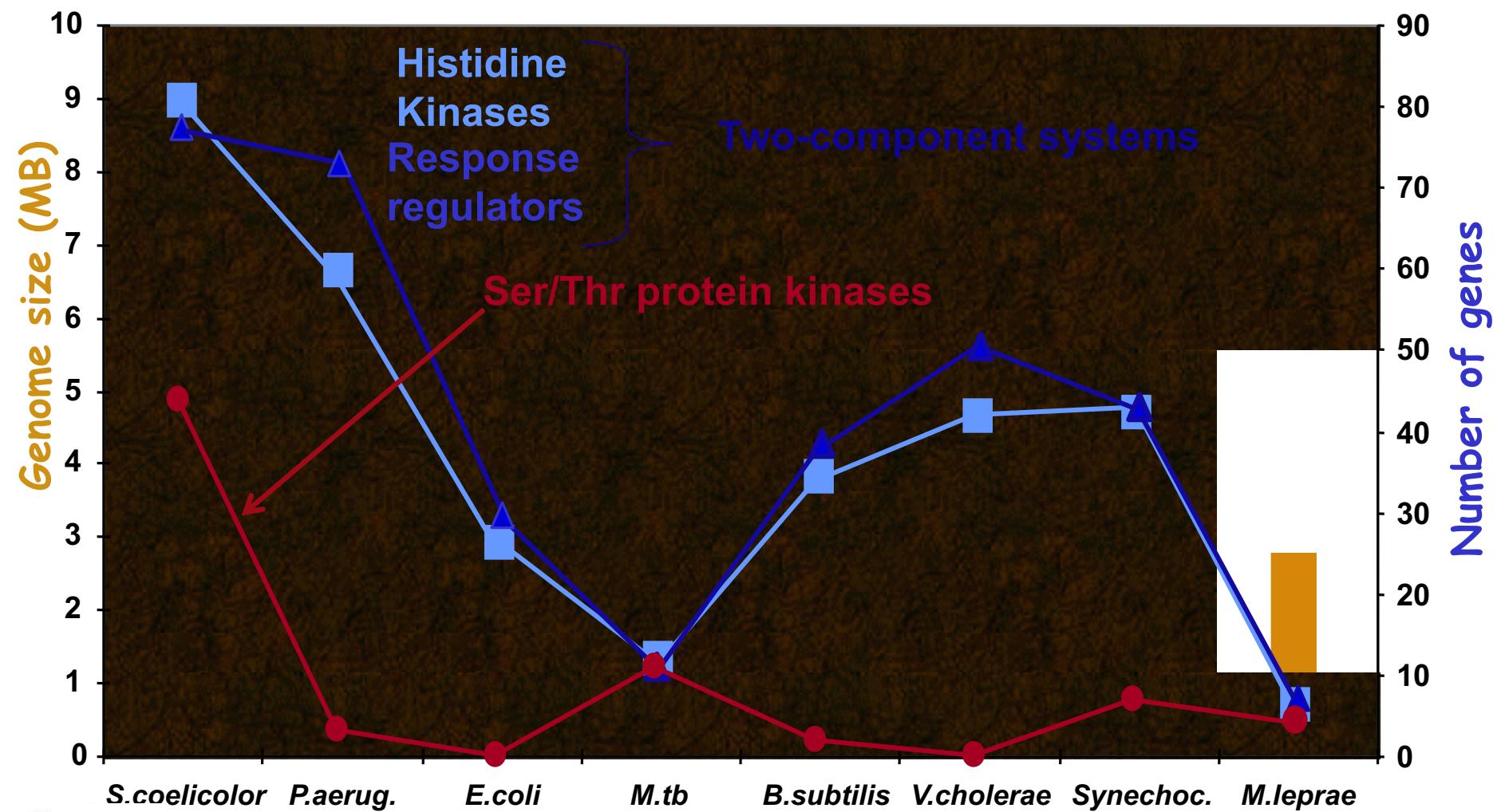


Life cycle of *M. tuberculosis*

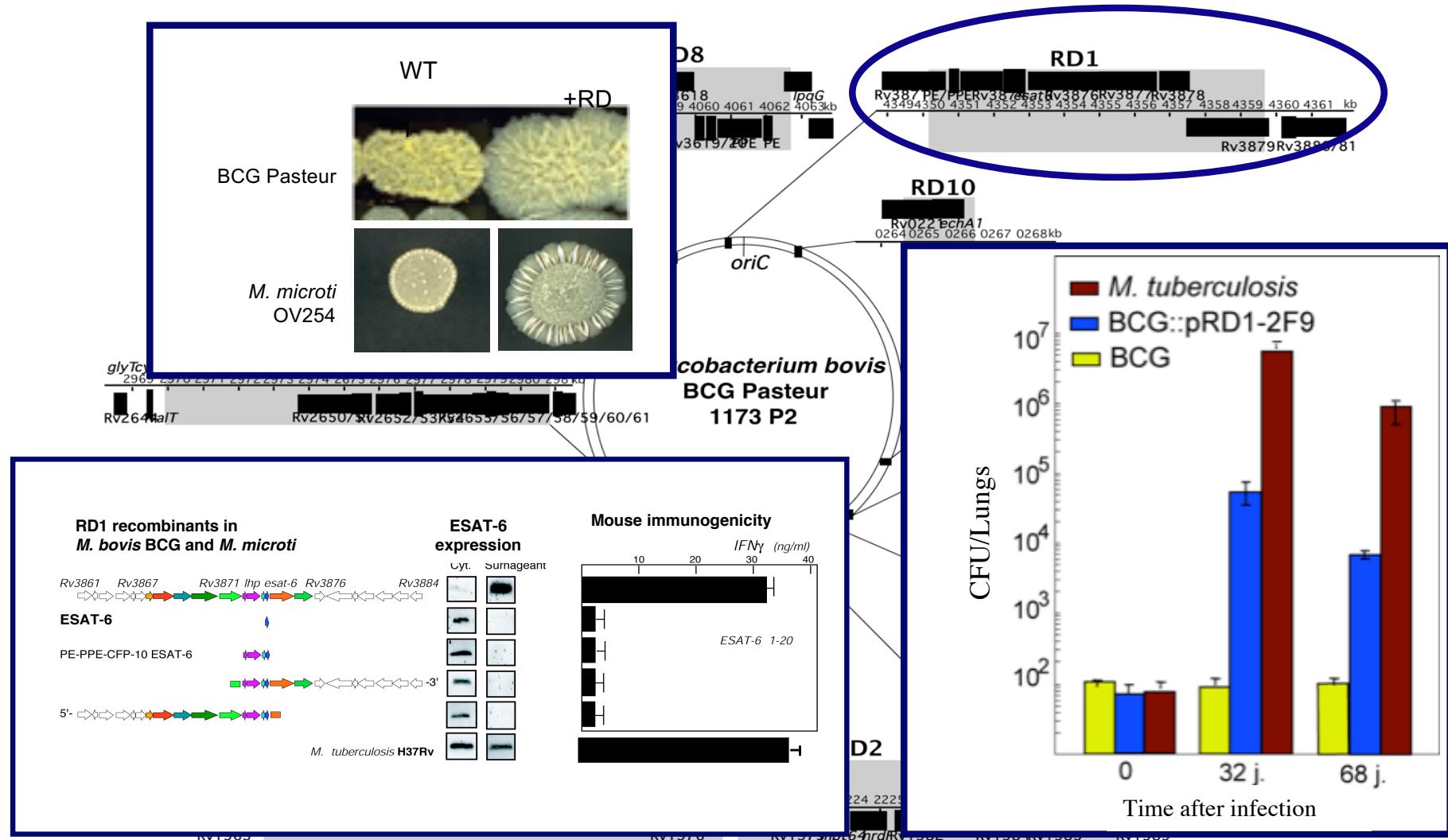


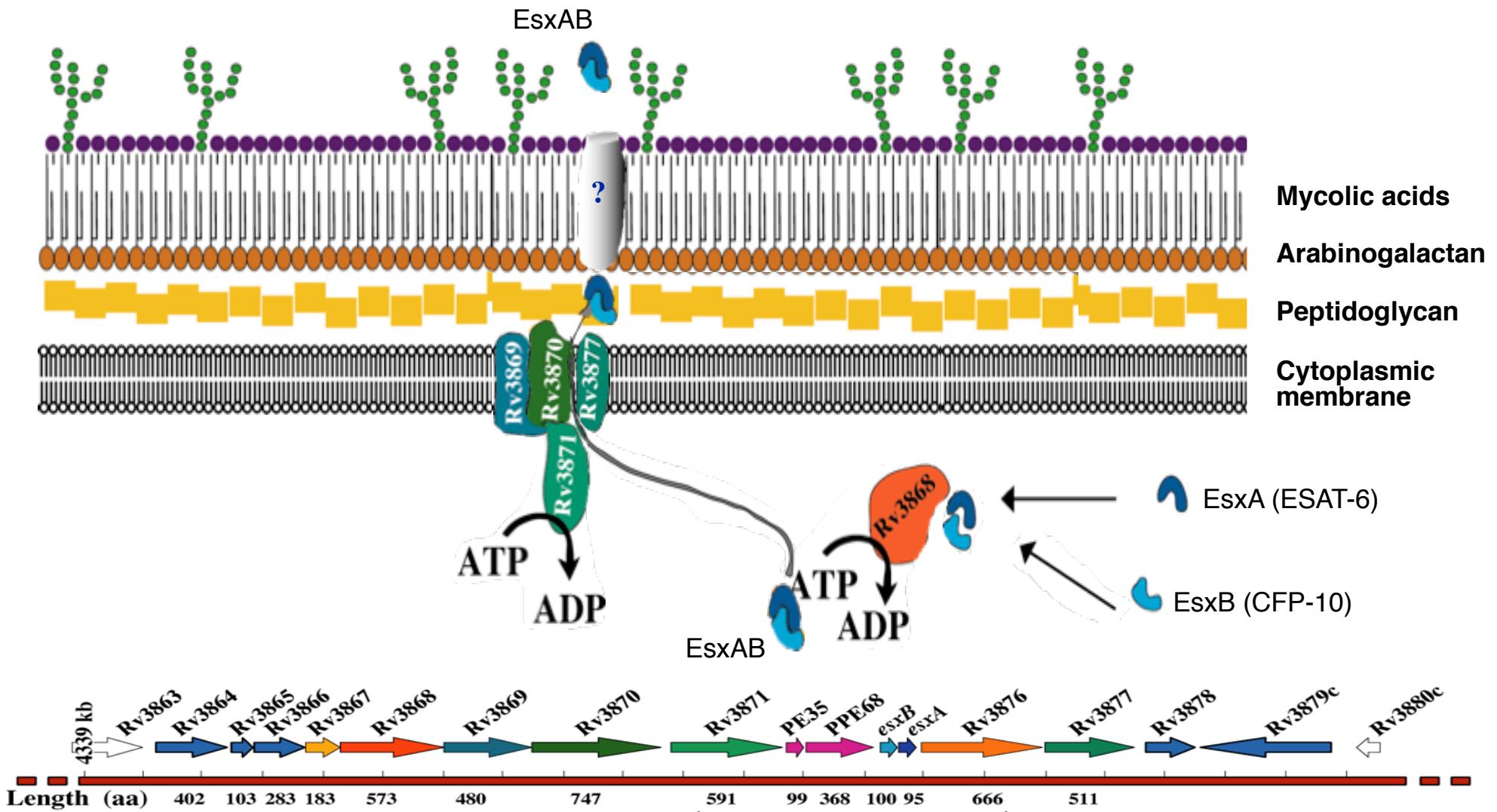
Adapted from, *Nature Rev. Mol. Cell Biol.*, 2, 569-586 (2001)

Eukaryotic-like signaling elements



Virulence factors

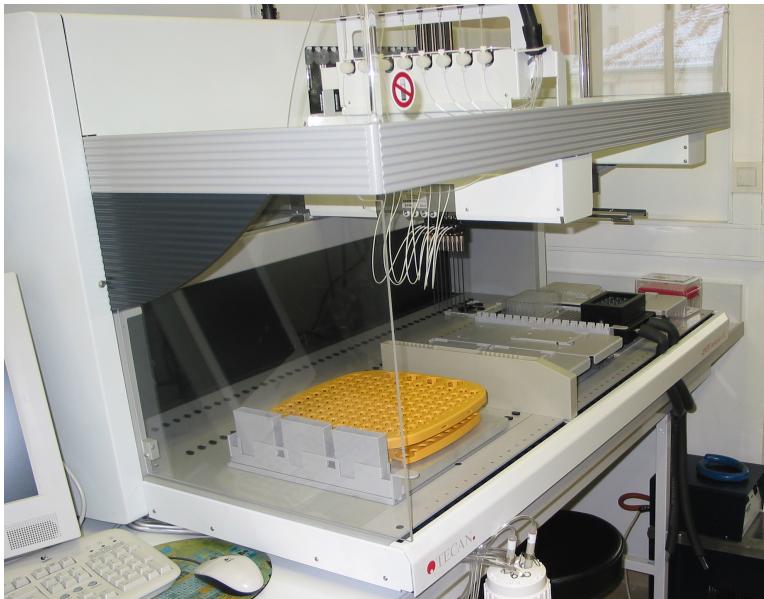




Systematic gene knock-out (secretion machinery)
Virulence of EsxA mutants (host-parasite interactions)

R.Brosch, IP

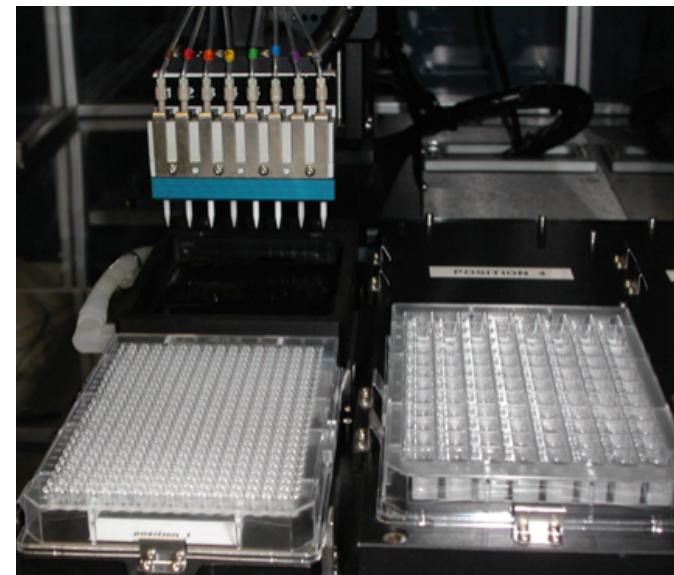
Crystallization



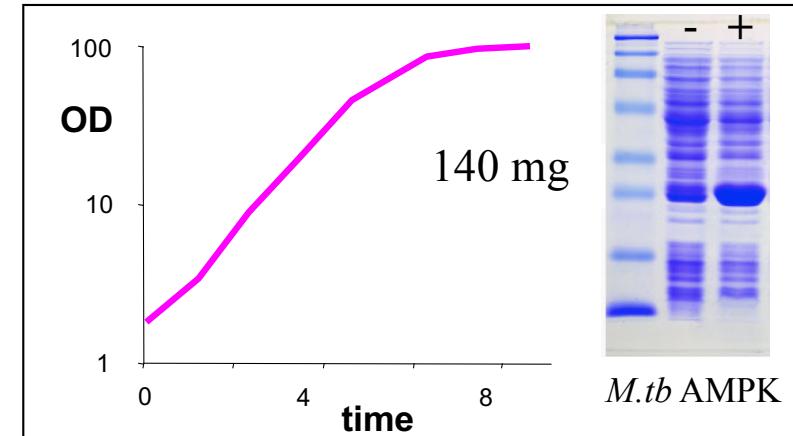
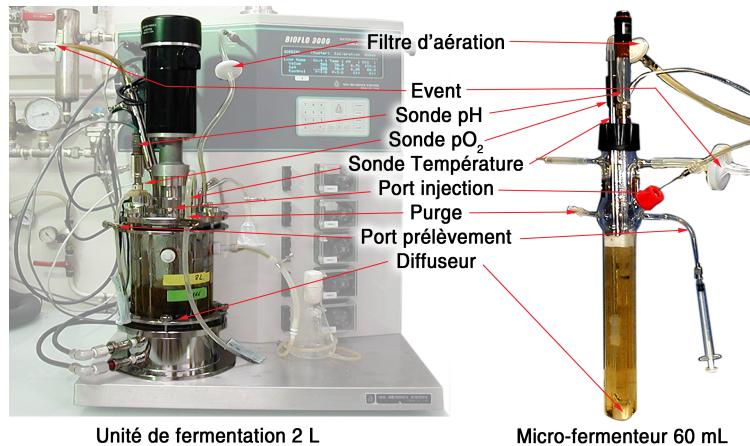
TECAN Genesis
(Crystallization, inhibitor screening)



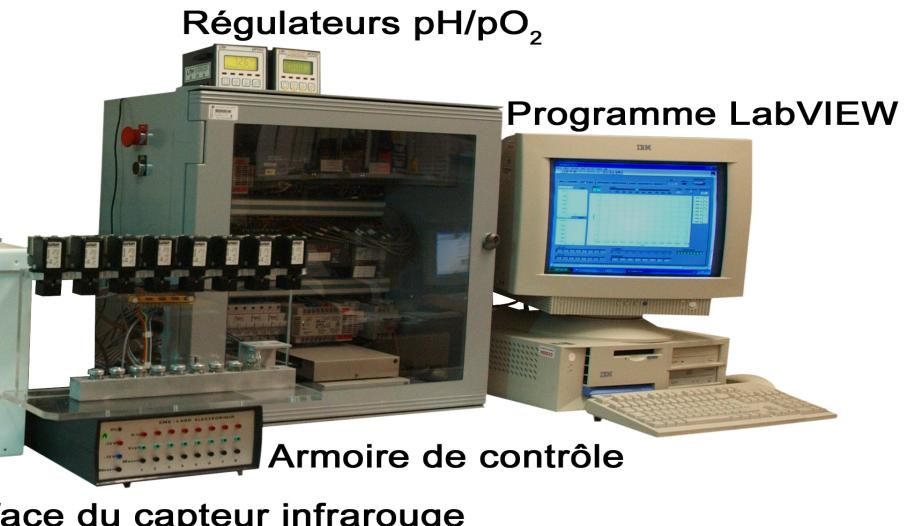
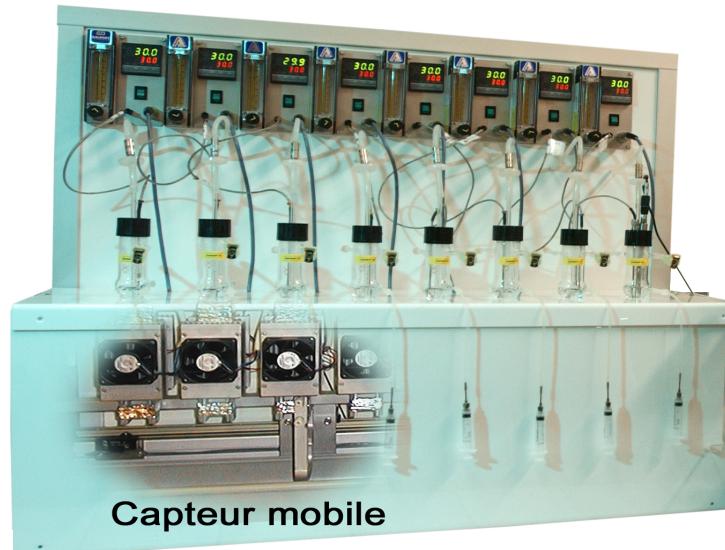
Cartesian Technologies nano-dispenser



Parallel multi-microfermentors (MMF)



Batterie de 8 micro-fermenteurs



Web Databases

Screenshot of the SGPLIMS web interface showing a search for targets. The search results table lists 535 items, with the first five rows shown:

Accession number	Gene name	Organism	Keywords	Access. restriction	Responsible person	Criterion	Global status
Rv0007		Mycobacterium tuberculosis	Conserved hypothetical	Public	Stewart T. Cole	Unknown function restricted to actinomycetes	selected
Rv0010c		Mycobacterium tuberculosis	Conserved hypothetical	Public	Stewart T. Cole	Unknown function restricted to mycobacteria	selected
Rv0014c	pknB	Mycobacterium tuberculosis	Ser/Thr protein kinase trans-membrane	Public	Pedro Alzari	Signal transduction	Structure determination
Rv0015c	pknA	Mycobacterium tuberculosis	Ser/Thr protein kinase trans-membrane	Public	Pedro Alzari	Signal transduction	Insoluble
Rv0016c	pbpA	Mycobacterium tuberculosis	peptidoglycan synthesis (rod shape)	Public	Dario Alzari	Signal transduct	Soluble

Below the search results, three small images are shown: a crystal image, a gel electrophoresis image, and a radial distribution function (RDF) plot.

On the left, there is a server rack and a computer monitor displaying a blue screen.

At the bottom, logos for EXPASy, Tuberculist, LEROMA, and PDB are displayed.

Screenshot of the Xtalo web interface showing a collection overview. The collection code is Deskc-mutantV188_2, and the description is DesK + AMPPCP low resolution dataset.

Beamline	Beamlne name: ID14-3 Detector: CCD Source: Synchrotron Site: Grenoble	Status	
Protein(s)	Protein name: Deskc-mutantV188 Expression clone name: Leader SwissProt code: Deskc-mutantV188		
Project	Project name: Deskc-mutantV188 UBS-PF6 Group name: Leader Project description: full description		
Collected by	anne-marie wehenkel	Responsible	name: ahmed haouz pedro alzari
Date	19/09/04	Hour	19.30

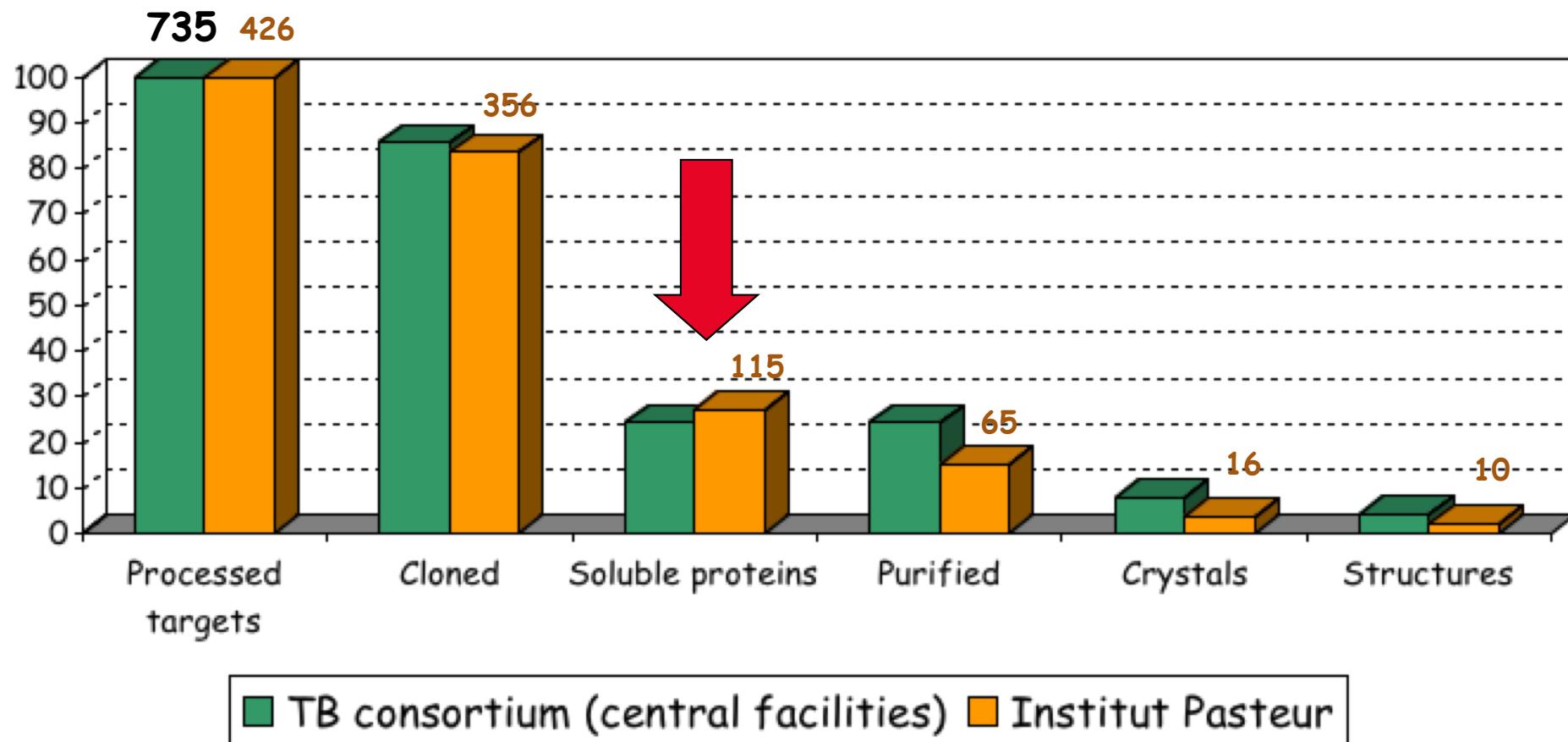
Below the table, several navigation links are available: Collection details, Data Reduction, Structure Solution, Refinement, Analysis, Deposition, Summary, All about Collection, and Main Page.

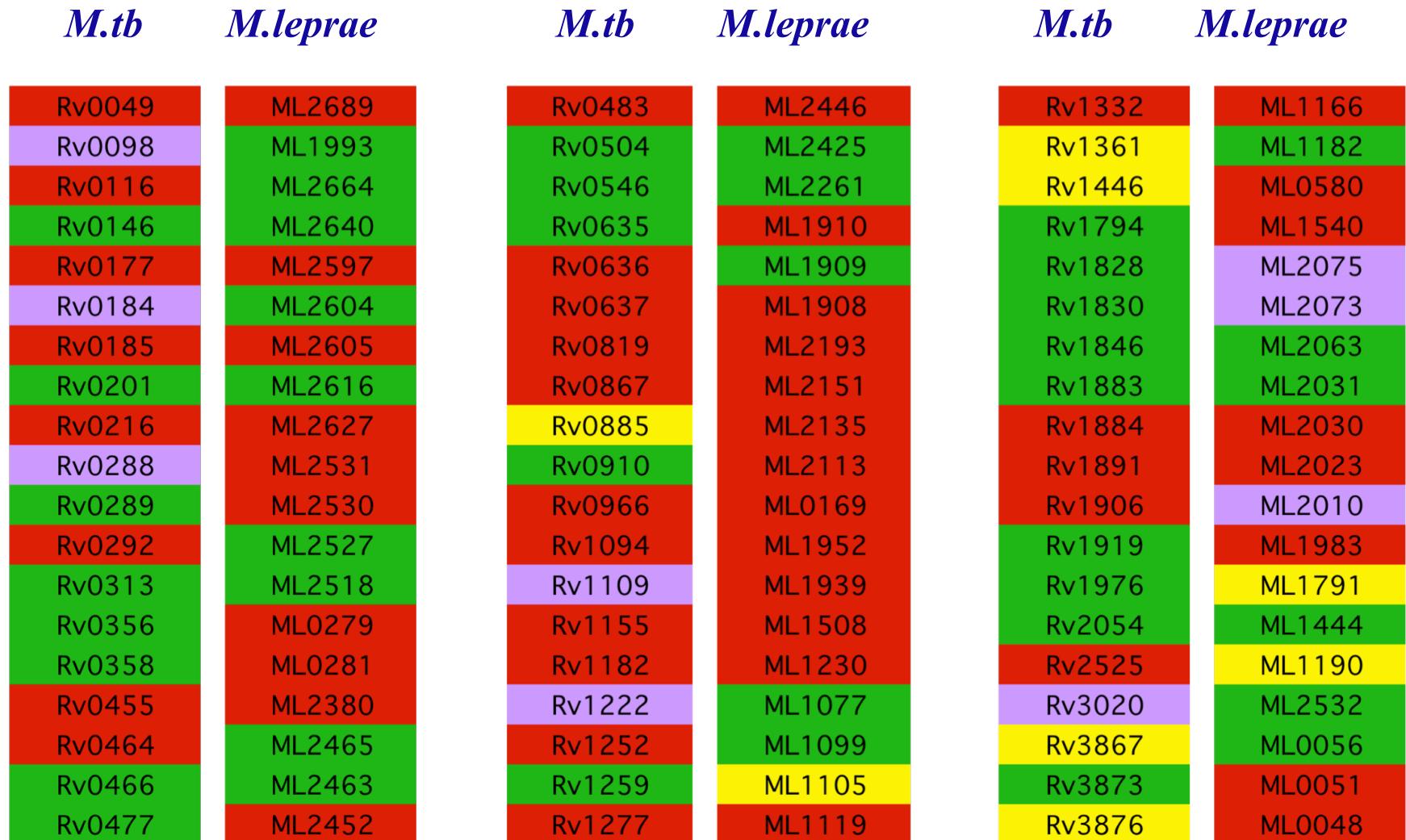
<http://www.pasteur.fr/SGM>

F. Guillemot, IP

The solubility bottleneck

(sept 2004)

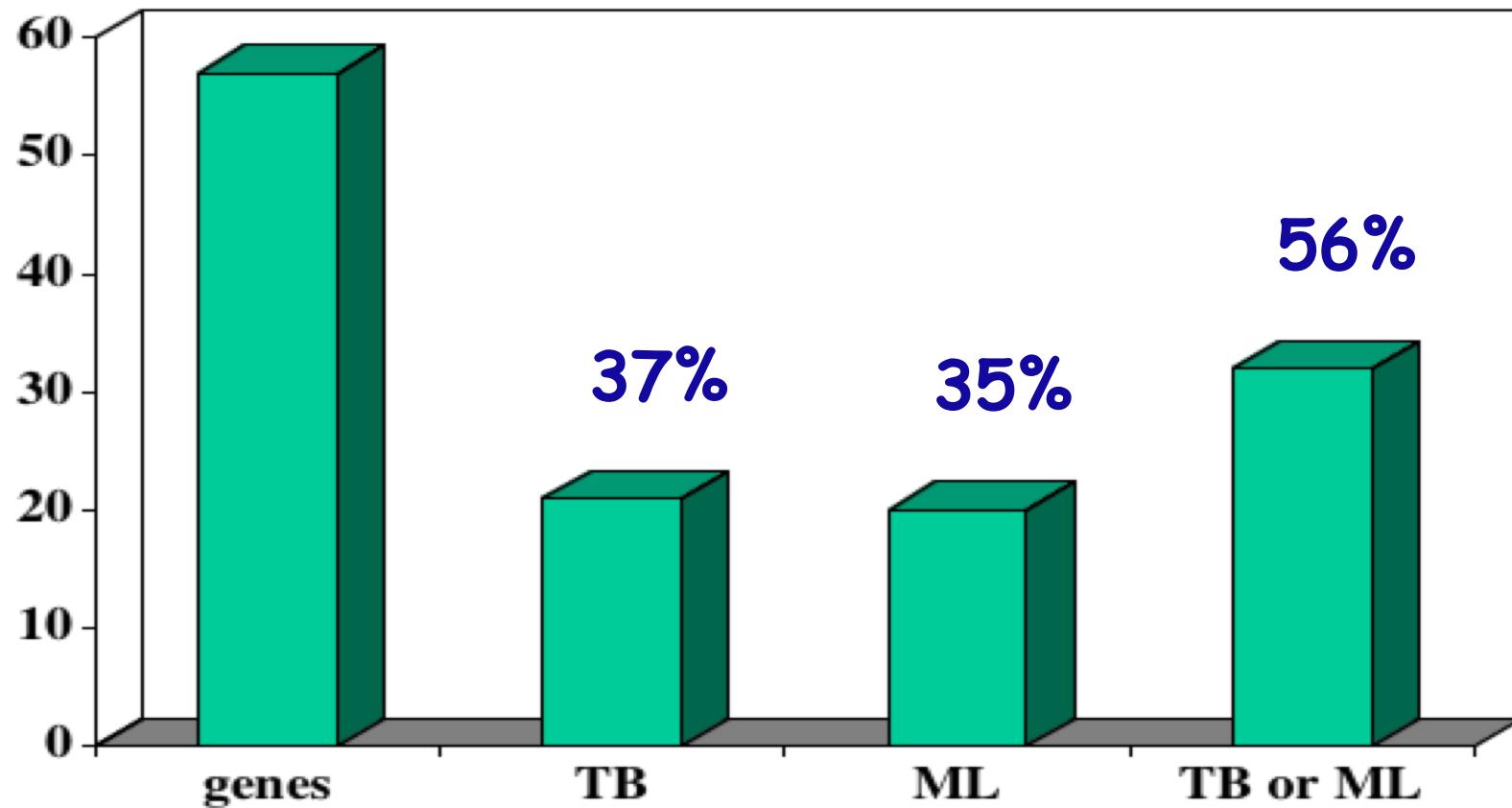




Orthologous genes

cloned no expr. insoluble soluble

Soluble proteins

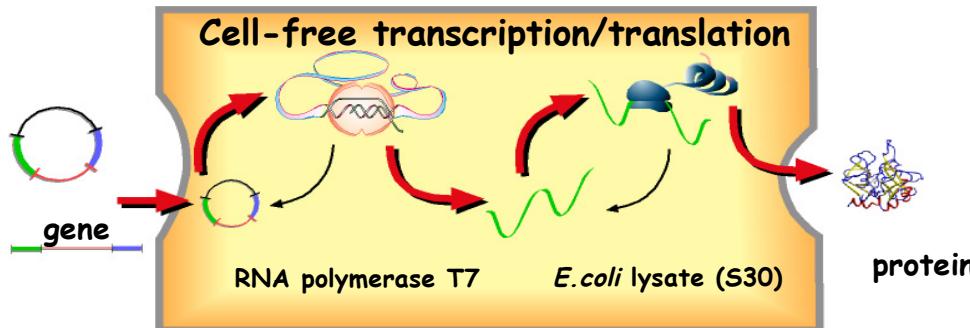


Next: multi-species cloning (*M. smeg*)

Optimizing protein expression parameters

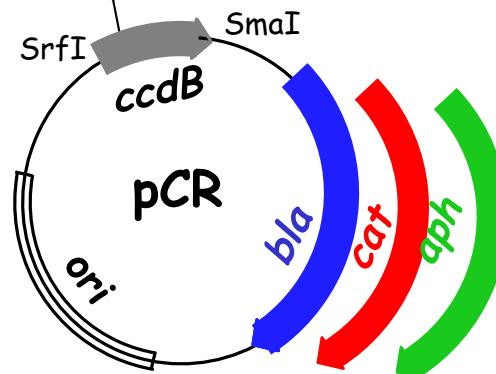


PCR:



Parameter optimization

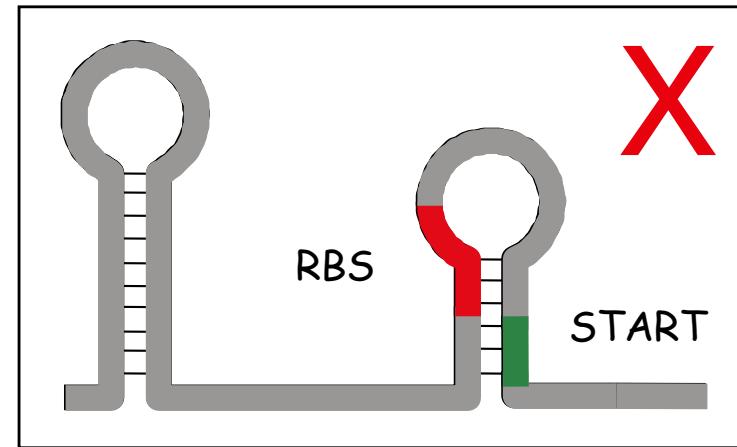
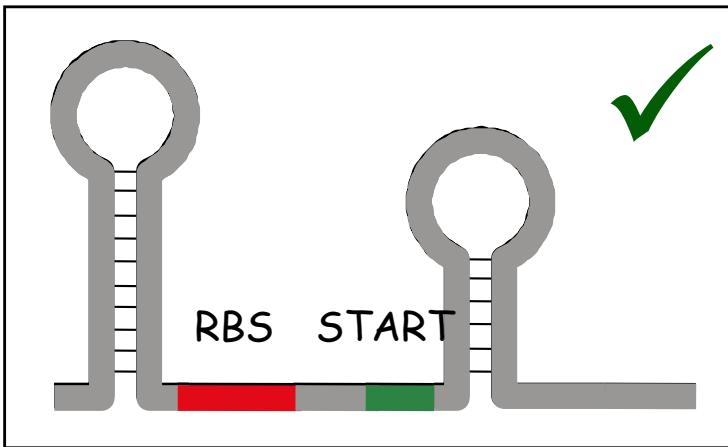
Bi-directional cloning of optimally expressed PCR fragments



E. coli
(BL21)

J.M.Betton, IP

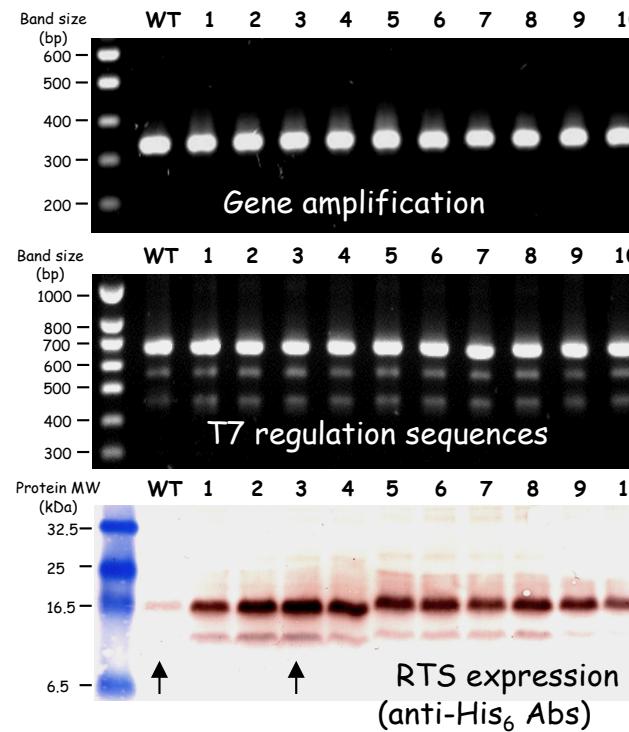
Optimizing for mRNA secondary structure



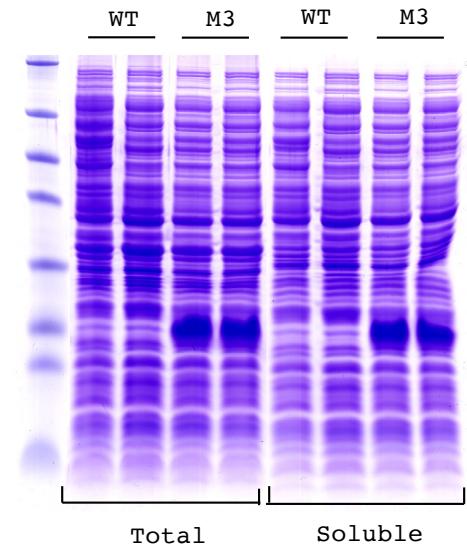
ML0180

WT	Met Pro Thr Tyr Ser Tyr Glu ATG CCG ACC TAC AGC TAC GAG
M1	ATG CCA ACT TAT TCA TAT GAA
M2	ATG CCA ACT TAT TCA TAT GAG
M3	ATG CCA ACA TAT TCA TAT GAG
M4	ATG CCA ACC TAT TCA TAT GAA
M5	ATG CCA ACT TAC TCA TAT GAA
M6	ATG CCA ACT TAC TCT TAT GAA
M7	ATG CCA ACC TAT TCA TAT GAG
M8	ATG CCA ACT TAT TCA TAC GAG
M9	ATG CCA ACT TAT TCA TAC GAA
M10	ATG CCA ACA TAT TCA TAC GAG

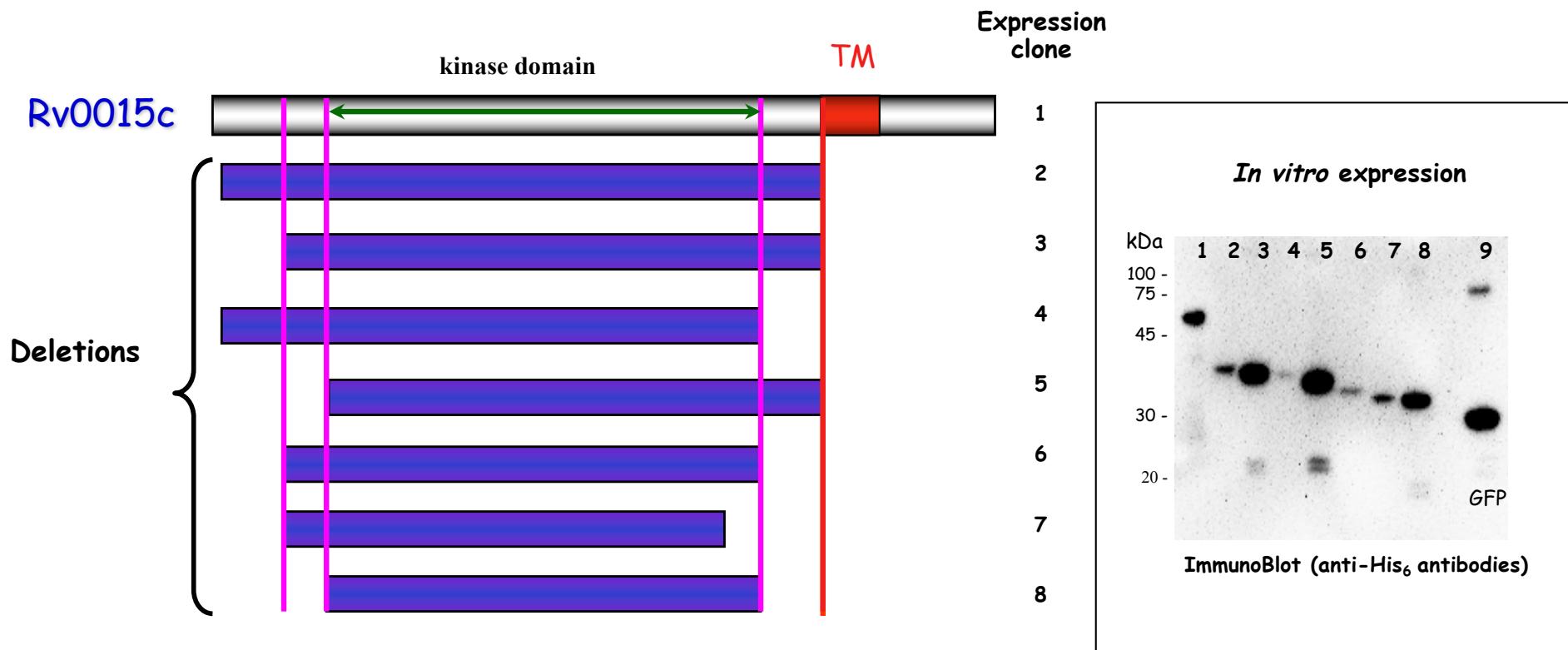
Silent mutations (ProteoExpert)



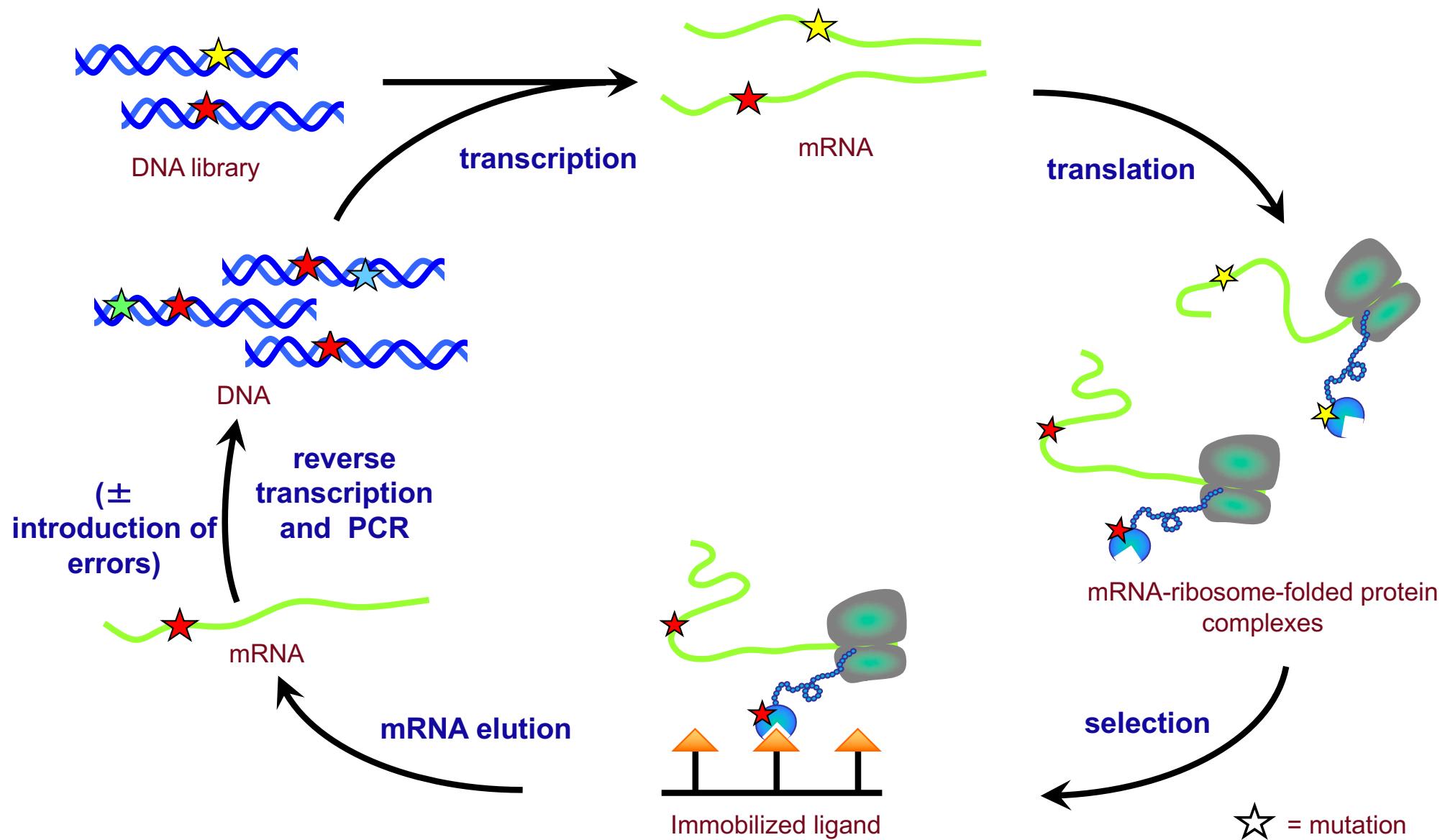
BL21 expression



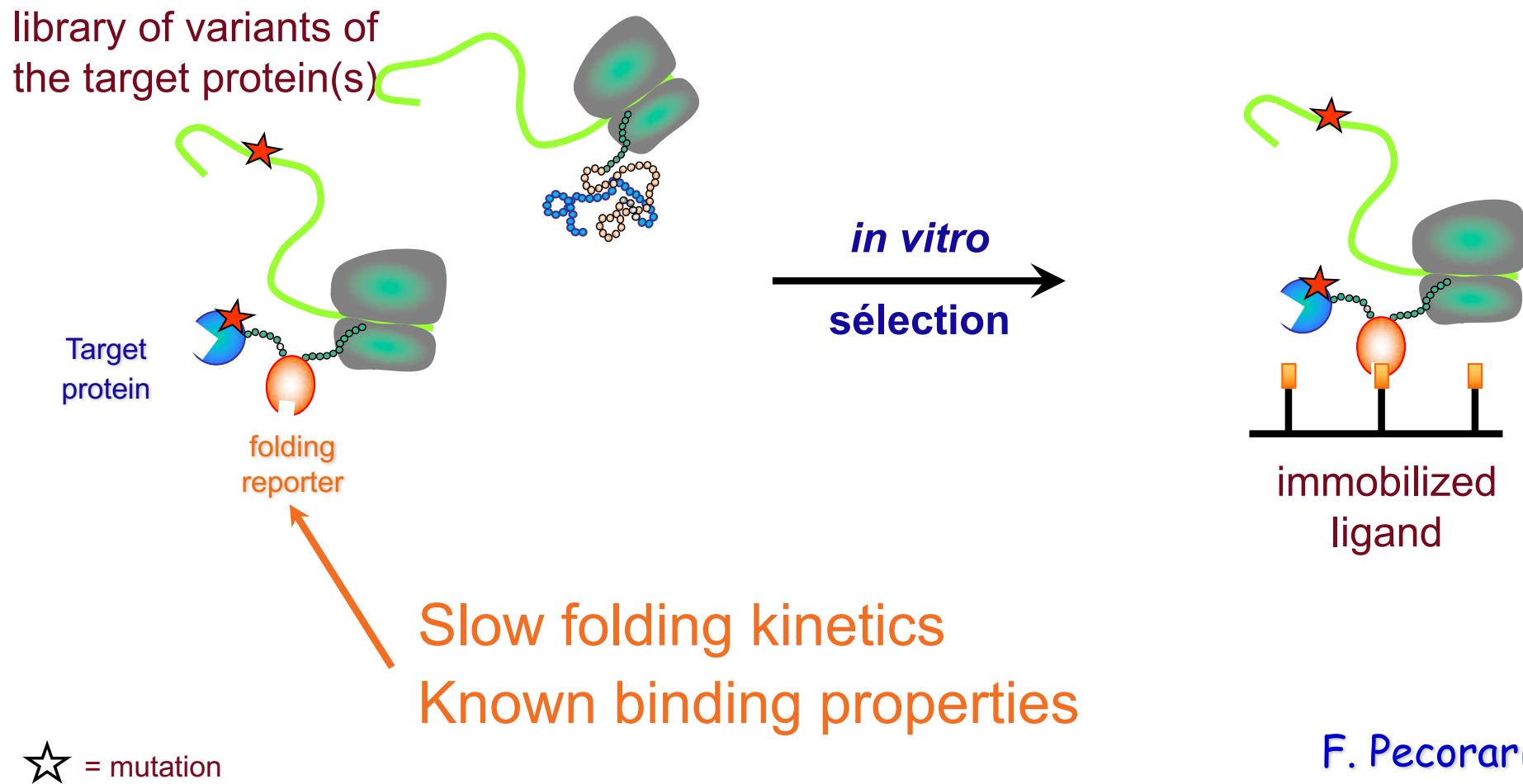
Optimizing the expression of soluble domains



In vitro evolution (ribosome display)



Selection for solubility



F. Pecorari, IP

The project :
Production of a large collection
of recombinant proteins



The goal :
**Reduction of volume and size vessel keeping
the same protein yields**

The solutions :
**Miniaturization of the reactors
Cultivation in high density media**

Protein production

Multi-fermentor technology

Cell cultures in 60 ml reactors

Online turbidity measurements (0.5-100 OD range)

Computer control of temperature, pH and pO₂

Battery of 8 reactors

10-100 mg of pure protein from 60 ml cell culture

Protein purification

Akta-Explorer 3D for parallel purification

(IMAC, ionic exchange and/or gel filtration)

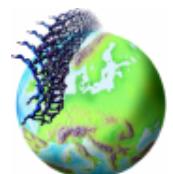
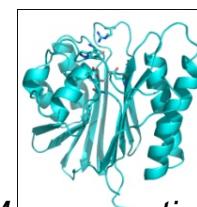
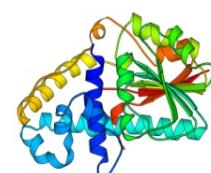
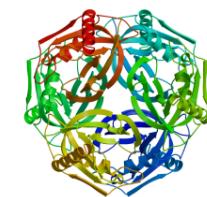
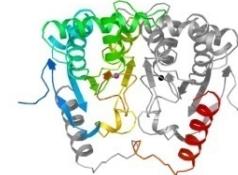
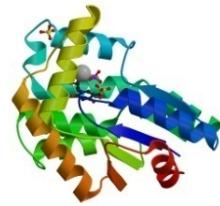
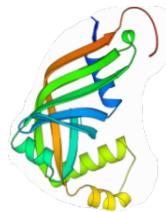
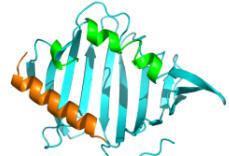
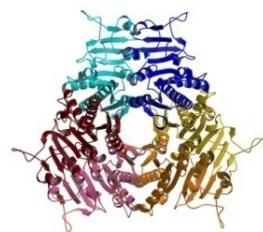
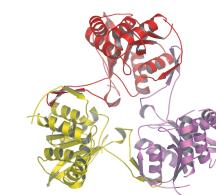
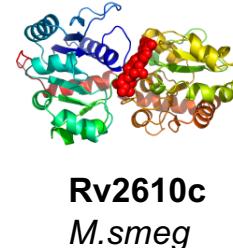
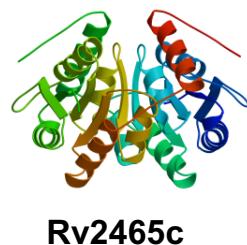
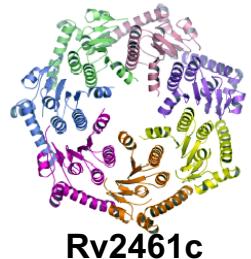
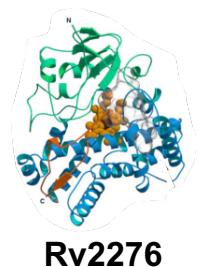
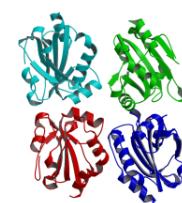
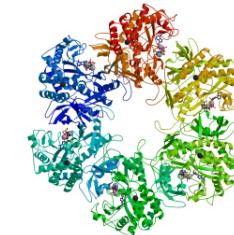
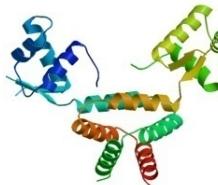
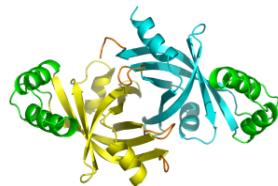
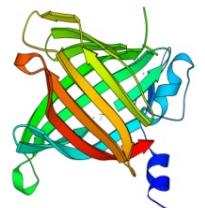
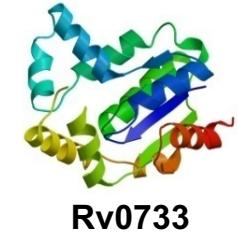
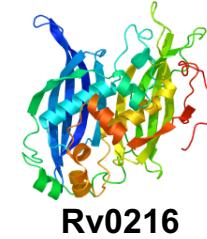
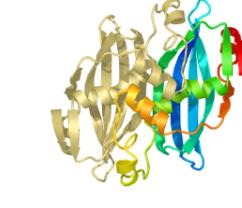
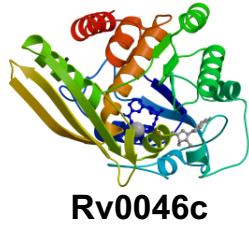
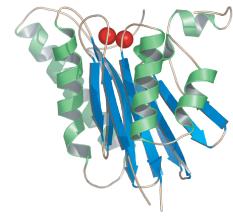
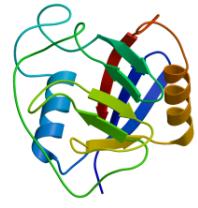


Quality controls:

DNA sequence, SDS page, mass spectrometry, CD spectra, DLS, NMR

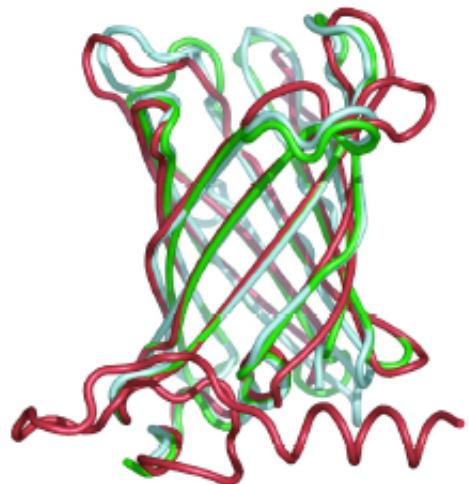


Processed targets	564
PCR fragments	610
Cloned genes	535
No expression	47
Expression, insoluble	280
Expression, soluble	175
Overexpressed proteins	120
Purified (in crystallization trials)	95
Crystals	30
Diffraction data	29
Structure	27
HSQC	1
NMR assigned	1

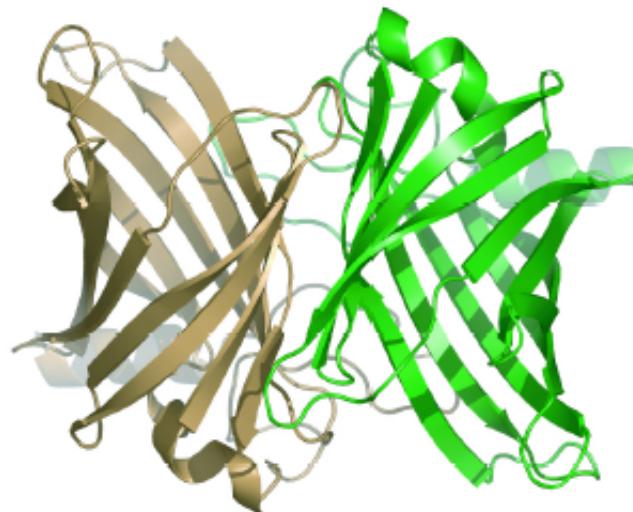


The crystal structure of Rv0813c from *Mycobacterium tuberculosis*
reveals a new family of FABP-like proteins in bacteria.

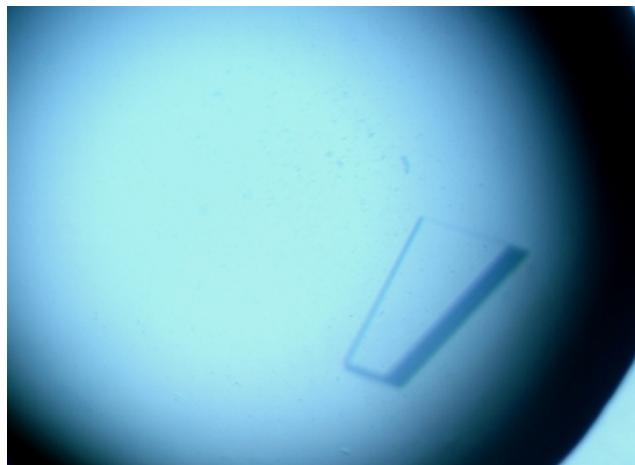
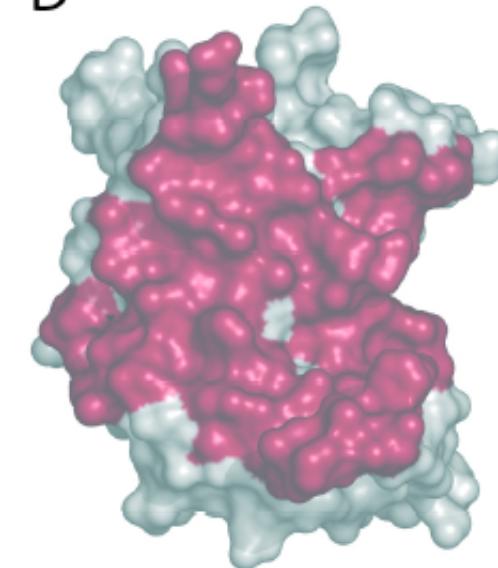
B



C



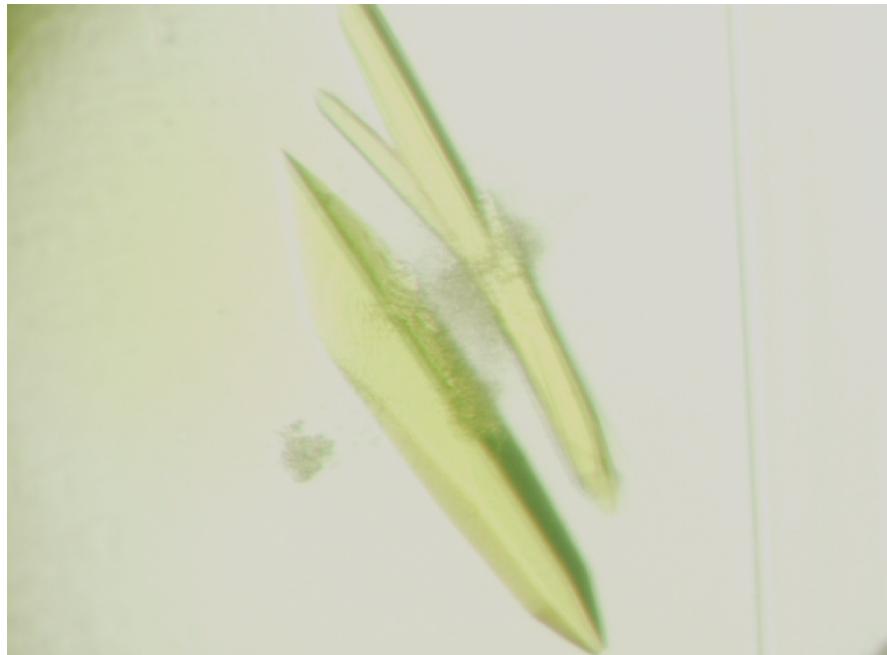
D



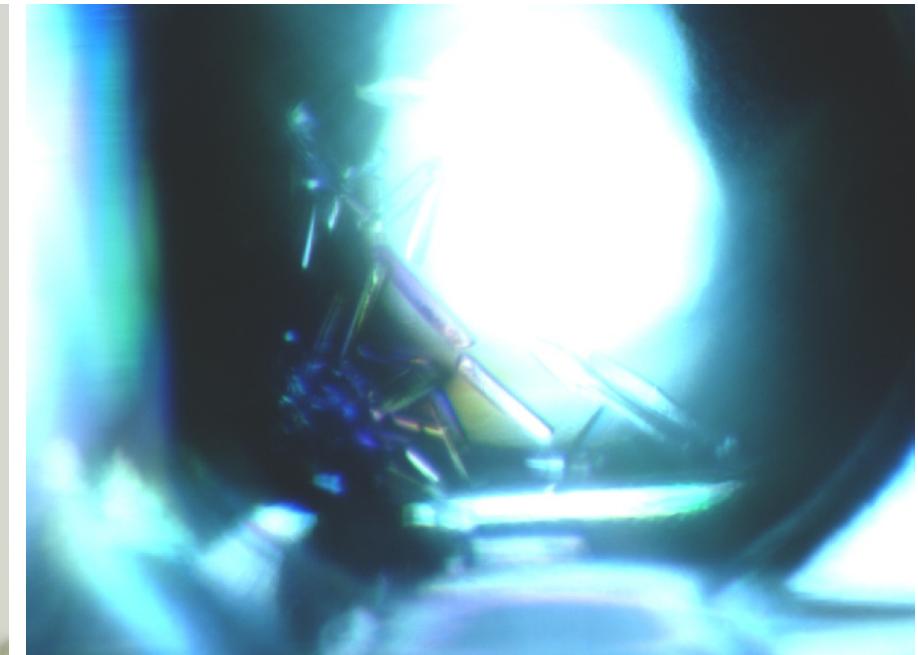
tr|O53827|O53827_MYCTU Hypothetical protein - *Mycobacterium tuberculosis*.
MSSGAGSDATGAGGVHAAGSGDRAAAVERAKATAARN**I**PAFDLDPVPADTANLREGAD
LNNALLALLPLVGWWRGEGEGRGPDGDYRGQQ**I**VSHDGGDYLNWESRSWRLTATGDYQ
EPGLREAGFWRFVADPYDPSESQAIELLAHSAGYVELFYGRPRTQSSWELVDALARS
SGVLVGGAKRLYGIVEGGDLAYVEERVDADGLVPHLSARLSRFVG

Methionine

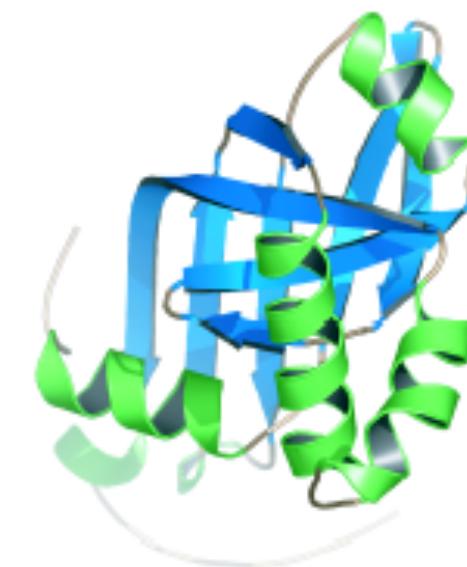
Rv2991: a possible FMN binding protein



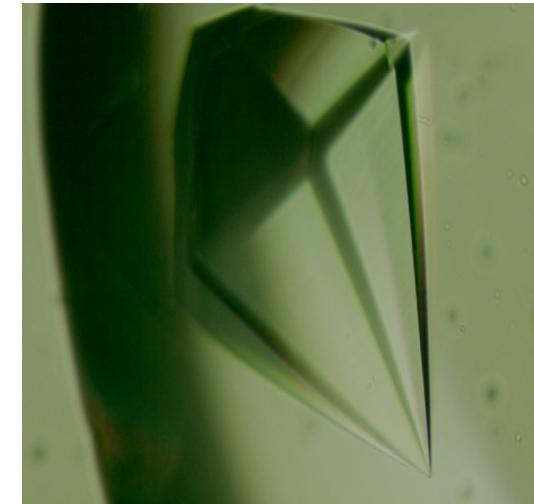
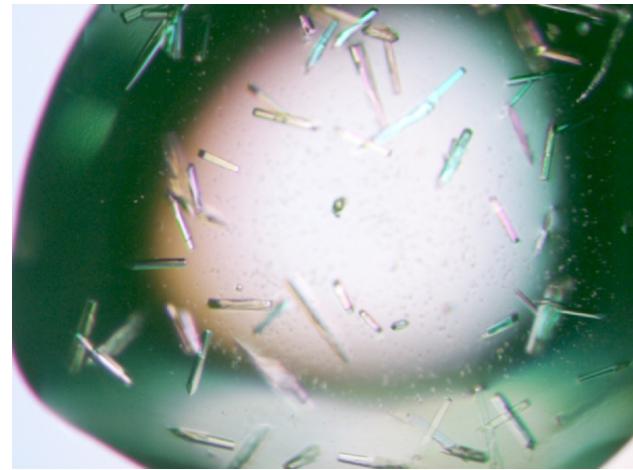
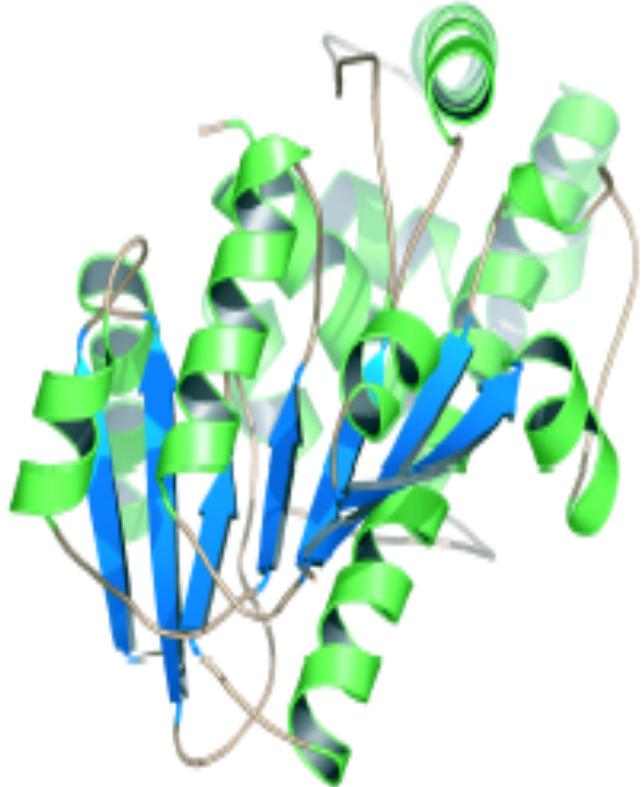
Rv2991+FMN



Rv2991 : apo-protein



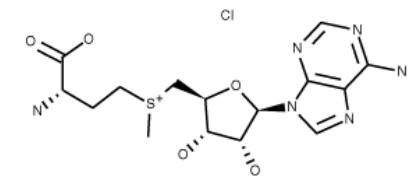
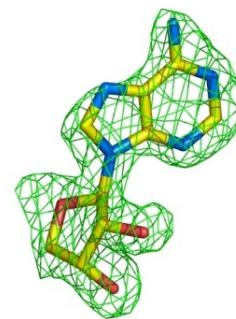
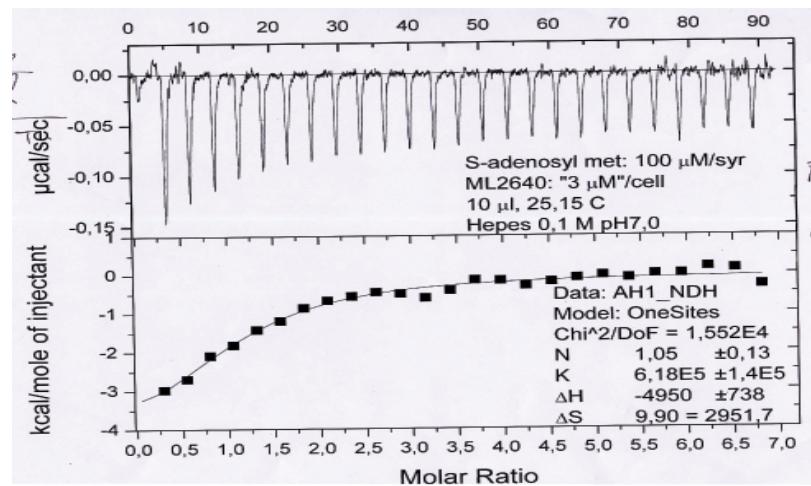
ML2640



Resolution 2.8 Å

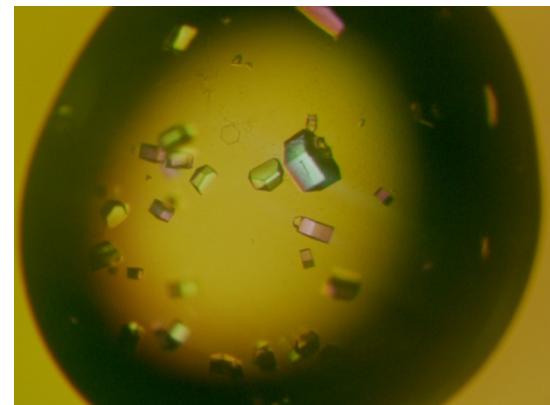
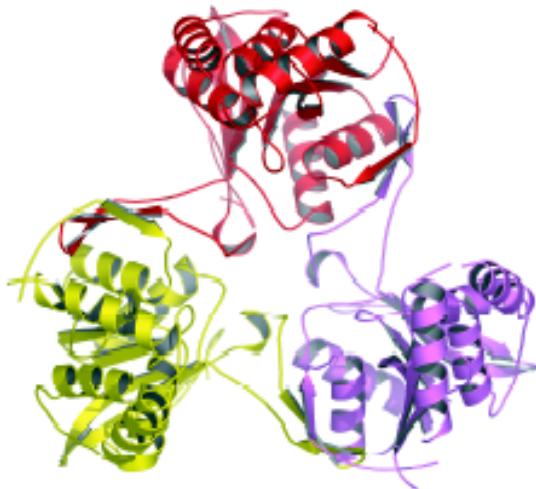
Resolution 1.7 Å

Function : methyl-transferase



S-(5'-Adenosyl)-L-methionine chloride

Rv2714: a possible purine phosphorylase



Résolution 2.2Å

