



Biologie Structurale 'Une histoire de France'

Dino Moras

RENAFOBIS
Oléron, 1^{er} juin 2014

Wilhelm Röntgen



Wilhelm Röntgen

Naissance	27 mars 1845 Remscheid (Royaume de Prusse)
Décès	10 février 1923 (à 77 ans) Munich (Allemagne)
Nationalité	Allemand
Champs	Physique
Institutions	Université de Strasbourg Université Louis-et-Maximilien de Munich
Diplômé de	École polytechnique fédérale de Zurich
Renommé pour	Rayon X
Distinctions	Prix Nobel de physique (1901)



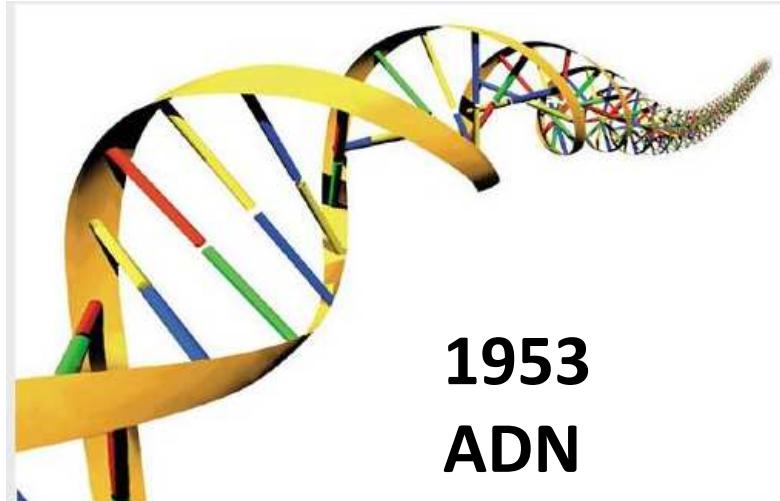
Max von Laue



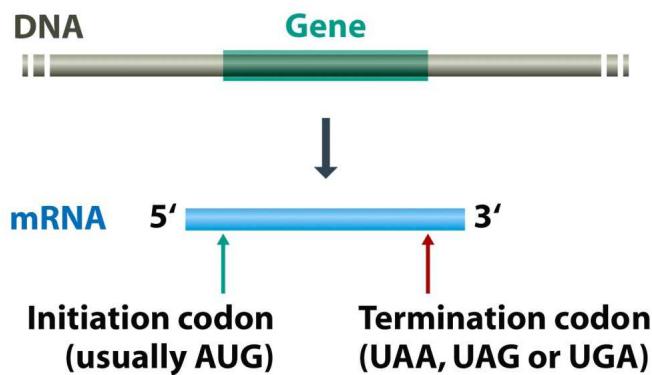
Naissance	9 octobre 1879 Coblence (Empire allemand)
Décès	24 avril 1960 (à 80 ans) Berlin (Allemagne de l'Ouest)
Nationalité	Allemande
Institutions	Université de Zürich Université Goethe Université de Berlin Max-Planck-Institut
Diplômé de	Université de Strasbourg Université de Göttingen Université de Munich Université de Berlin
Renommé pour	sa découverte de la diffraction des rayons X
Distinctions	Prix Nobel de physique



La biologie moléculaire est le fruit du mariage de la génétique avec la cristallographie



**De la structure moléculaire de l'ADN à la production de protéines :
la traduction du code génétique**



L'hypothèse de l'adaptateur
Crick, F.H.C. (1958)

La synthèse fidèle des protéines à partir des ARN messagers dépend de la présence d'ARNt correctement acétylés (porteurs de l'acide aminé correspondant à leur anticodon).

L'hypothèse postulait que chaque aminoacyl-ARNt serait chargé par un seul enzyme

20 aa

20 synthétases

> 60 ARNt

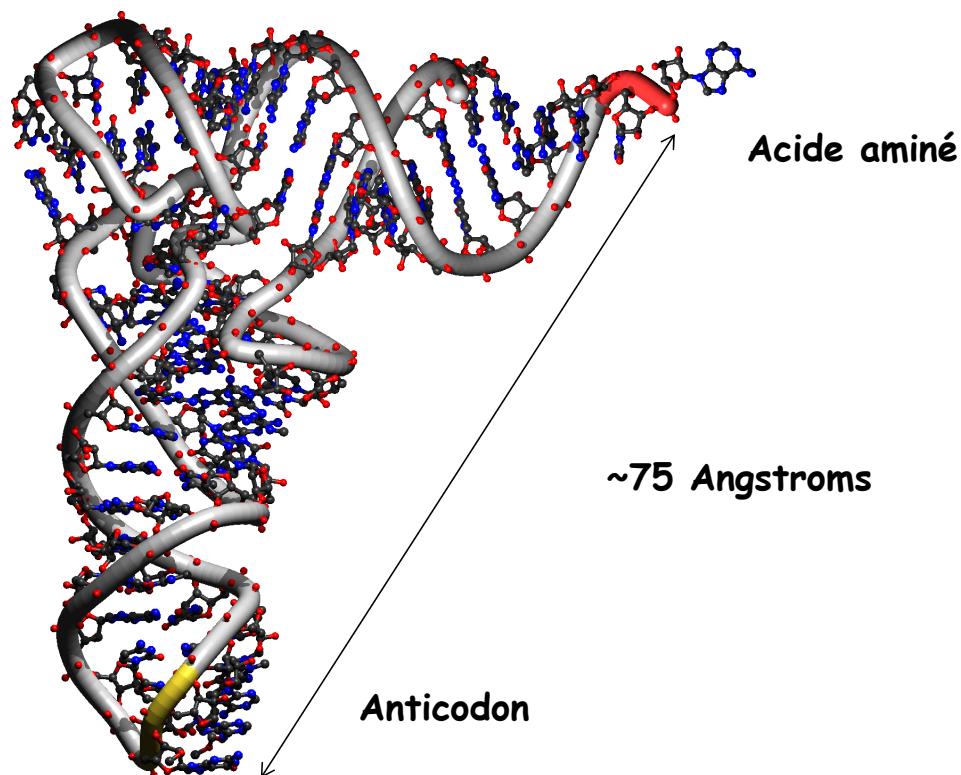
Le code génétique (1966)

2nd base in codon

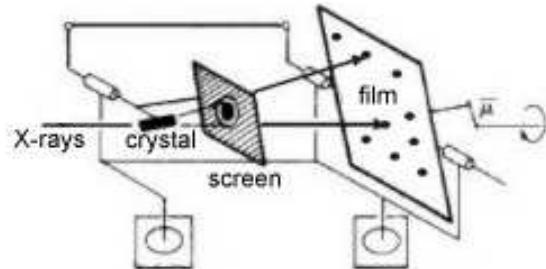
	U	C	A	G	
U	Phe F Phe F Leu L Leu L	Ser S Ser S Ser S Ser S	Tyr Y Tyr Y STOP STOP	Cys C Cys C STOP Trp W	U C A G
C	Leu L Leu L Leu L Leu L	Pro P Pro P Pro P Pro P	His H His H Gln Q Gln Q	Arg R Arg R Arg R Arg R	U C A G
A	Ile I Ile I Ile I Met M	Thr T Thr T Thr T Thr T	Asn N Asn N Lys K Lys K	Ser S Ser S Arg R Arg R	U C A G
G	Val V Val V Val V Val V	Ala A Ala A Ala A Ala A	Asp D Asp D Glu E Glu E	Gly G Gly G Gly G Gly G	U C A G

1974 :

1^{ère} Structure 3D d'un ARN de transfert ARNt de la phenylalanine



Les années 70 en France De la chimie à la Biologie Structurale



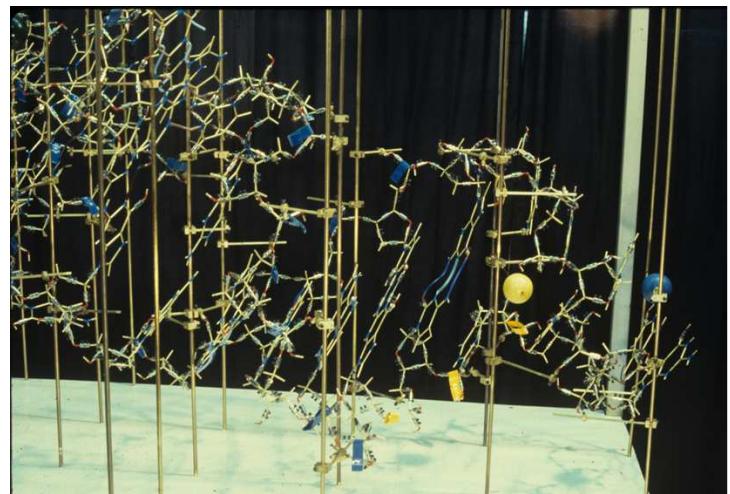
Precession
camera

CAD4 Nonius / Institut Le Bel 1977

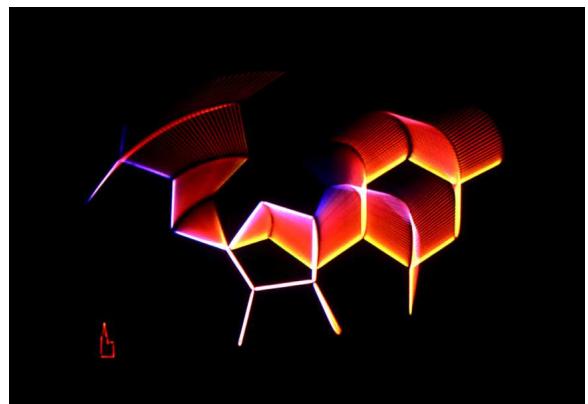
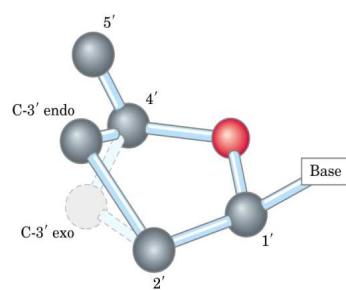
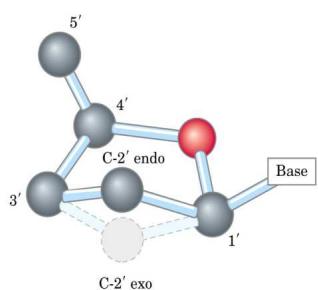


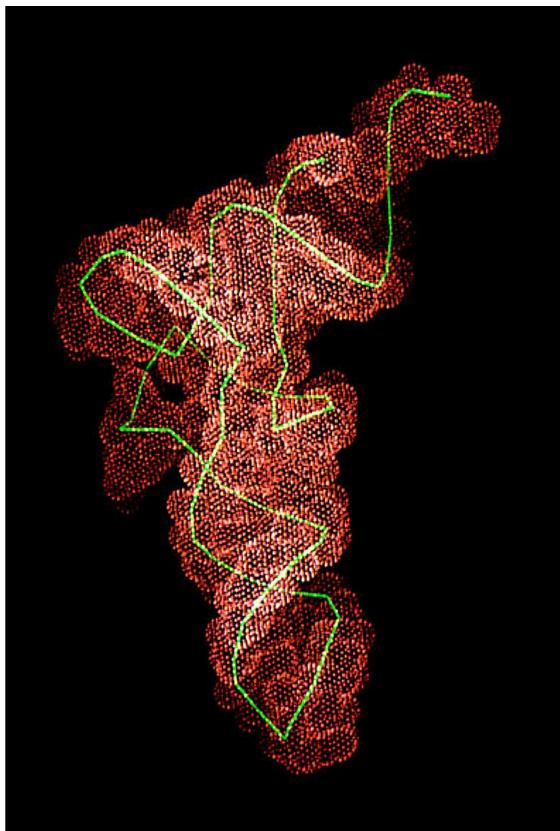
Richard Box / Institut Le Bel 1979

tRNAasp : anticodon stem



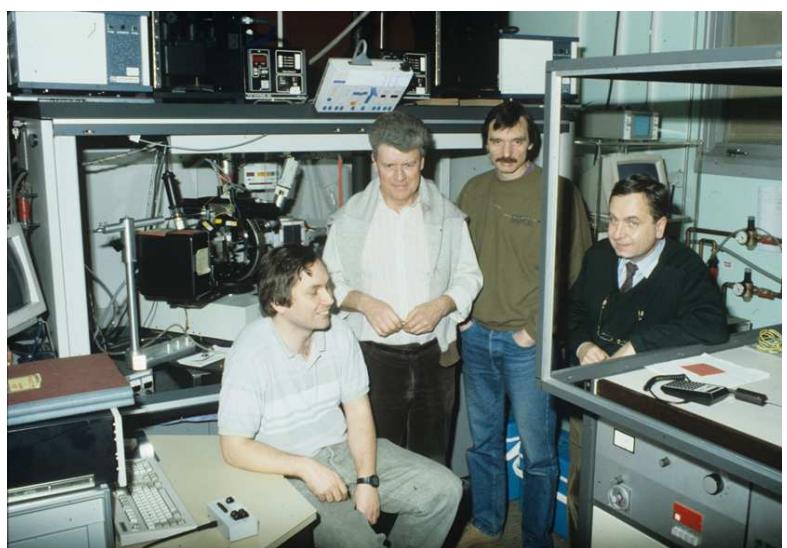
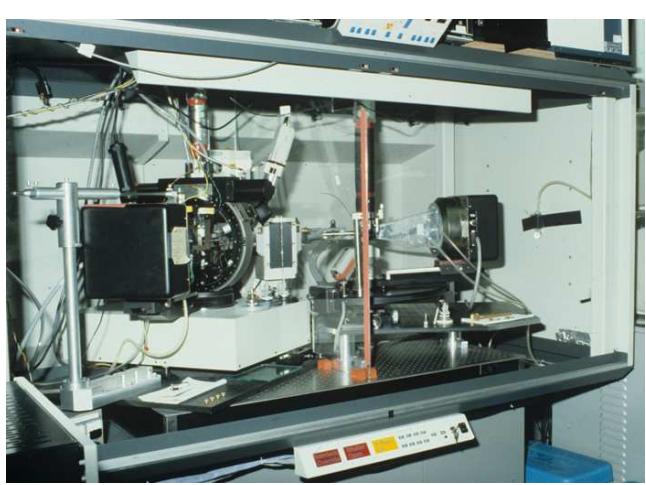
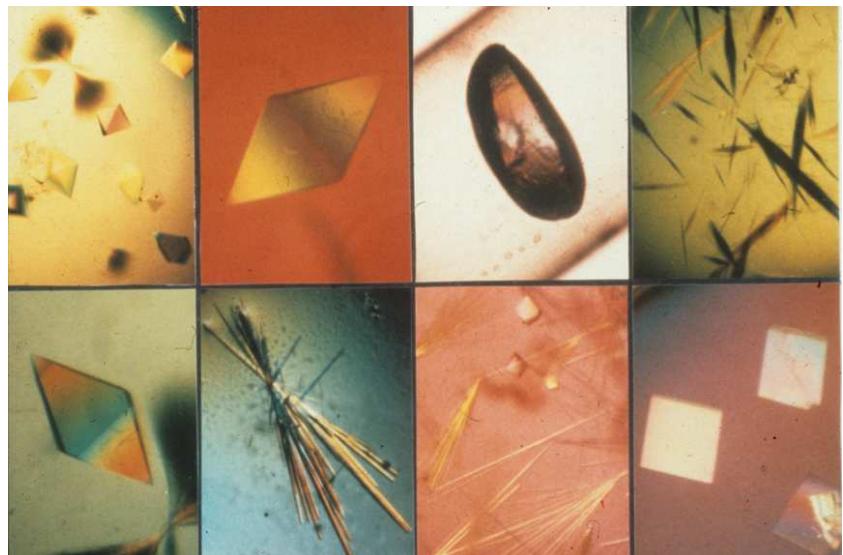
- E&S color workstation (first in europe)



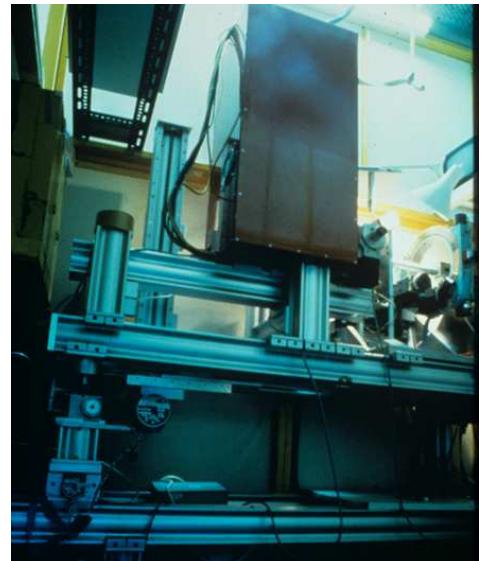
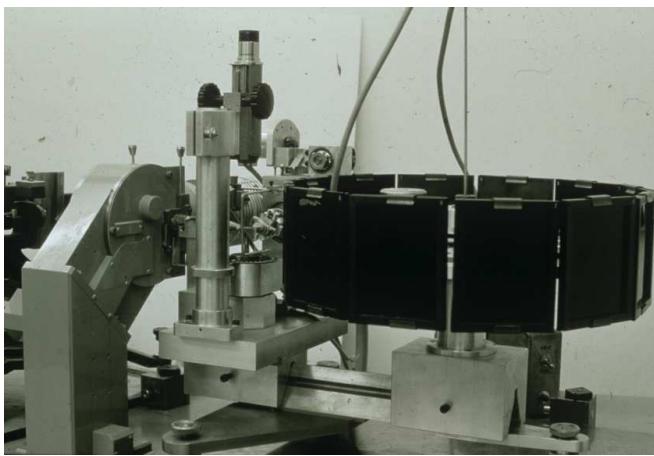


Les années 80

1980 : structure du tRNAasp
1981 : cristaux du complexe tRNAasp- aspRS

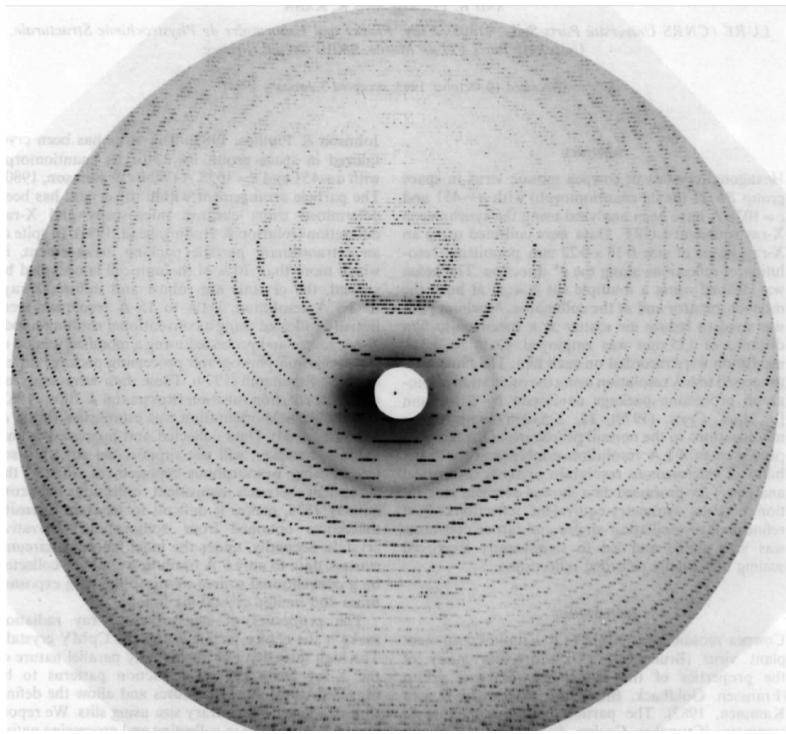


**Les années 80
La camera d'oscillation
Le synchrotron
LURE
ESRF**



Roger Fourme

**DEA National de Biologie Structurale
Paris-Orsay/Lure ULP/IBMC Strasbourg
Grenoble, Marseille**



J. Appl. Cryst. (1984). 17, 147-153

Macromolecular Crystallography with Synchrotron Radiation: Collection and Processing of Data from Crystals with a Very Large Unit Cell

BY R. USHA AND J. E. JOHNSON* Department of Biological Sciences, Purdue University, W. Lafayette, Indiana 47907, USA

D. MORAS AND J. C. THIERRY Institut de Biologie Moléculaire et Cellulaire, 15 rue Descartes, 67084 Strasbourg CEDEX, France

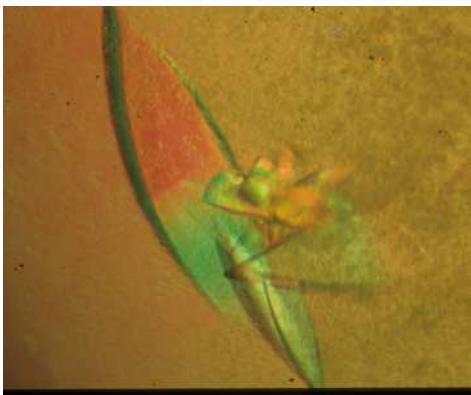
AND R. FOURME AND R. KAHN LURE (CNRS Université Paris-Sud), 91405 Orsay, France and Laboratoire de Physicochimie Structurale, Université Paris Val de Marne, 94010 Creteil, France

(Received 10 October 1983; accepted 3 January 1984)

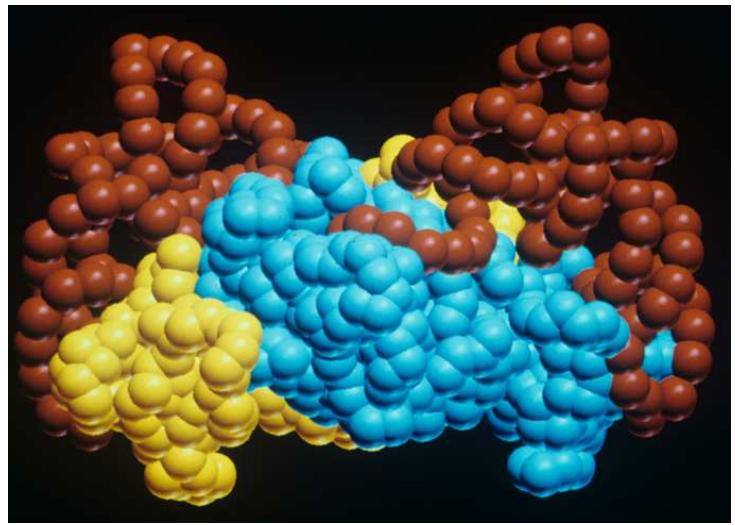
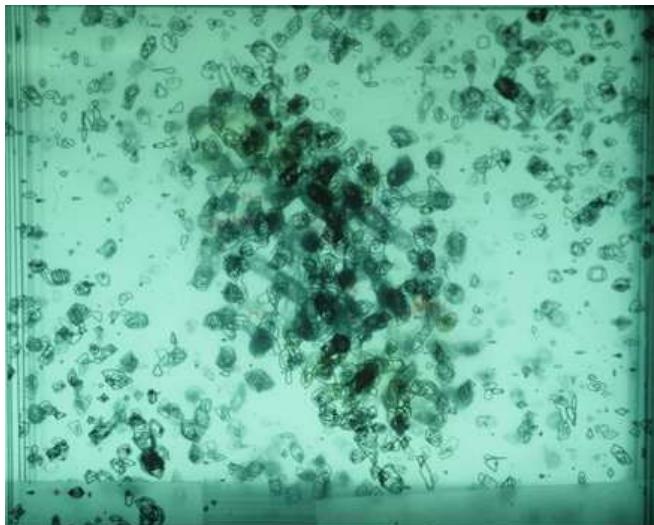
L'international : les Regios Meeting

Alsace
Kleebach 1988



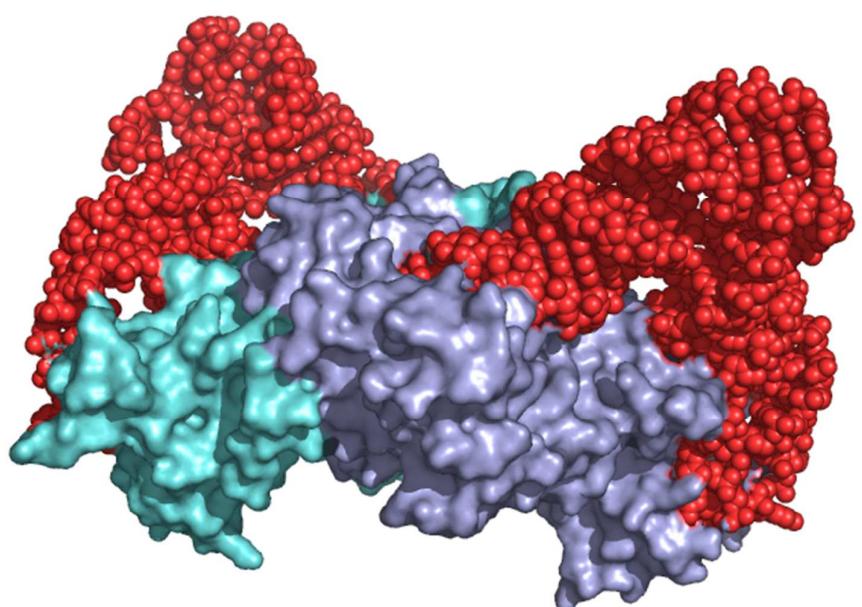
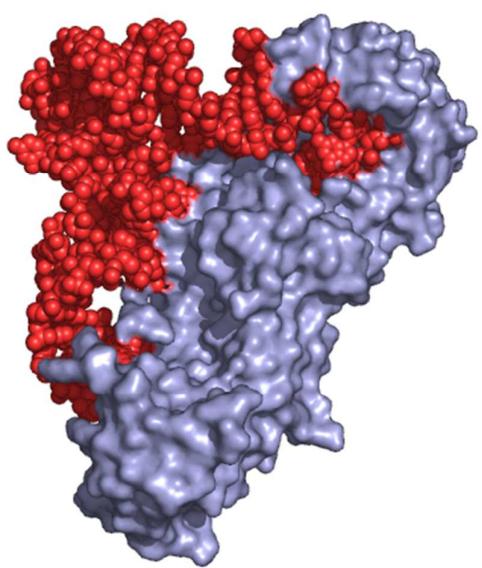


Marc Ruff



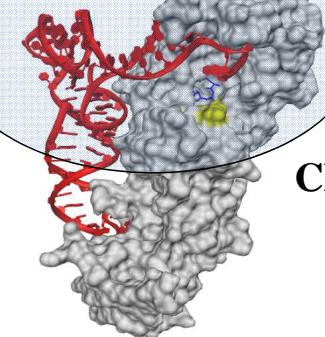
GlnRS-tRNA^{Gln}

AspRS-tRNA^{Asp}

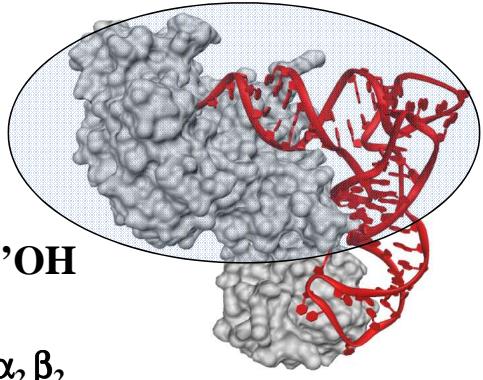


Les 2 classes d'aa-tRNA synthétases

Class I / 2'OH



Class II / 3'OH



Arg α
Cys α 2'-3' OH
Ile α
Leu α
Met α
Val α

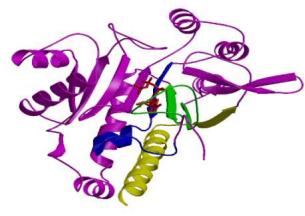
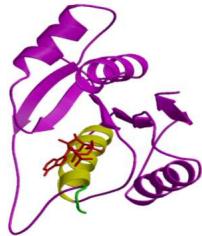
Gly α_2 $\alpha_2\beta_2$
His α_2
Pro α_2
Thr α_2
Ser α_2
Ala α_2

GluRS α
GlnRS α
Lys α

Asn α_2
Asp α_2
Lys α_2

Trp α 2
Tyr α 2 2'-3' OH

Phe $\alpha_2\beta_2$ 2' OH

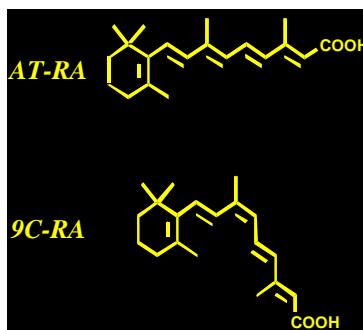
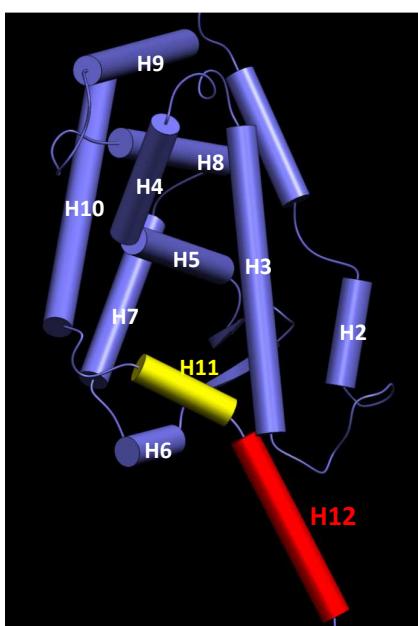


1994

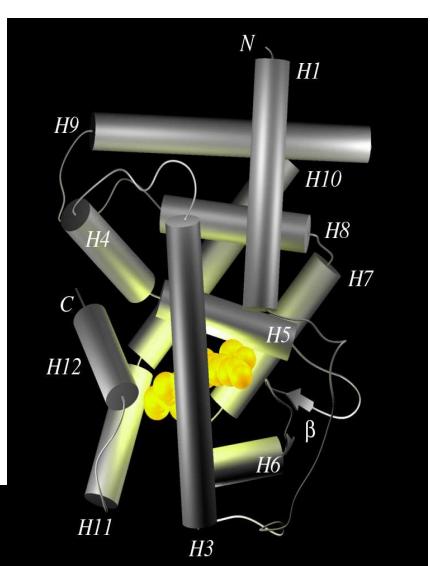
IGBMC



Du gène au médicament



RXR Bourguet et al. (1995) Nature
RAR Renaud et al. (1995) Nature



hRAR γ LBD / AT-RA

DEA

XENON

RXR

2000...

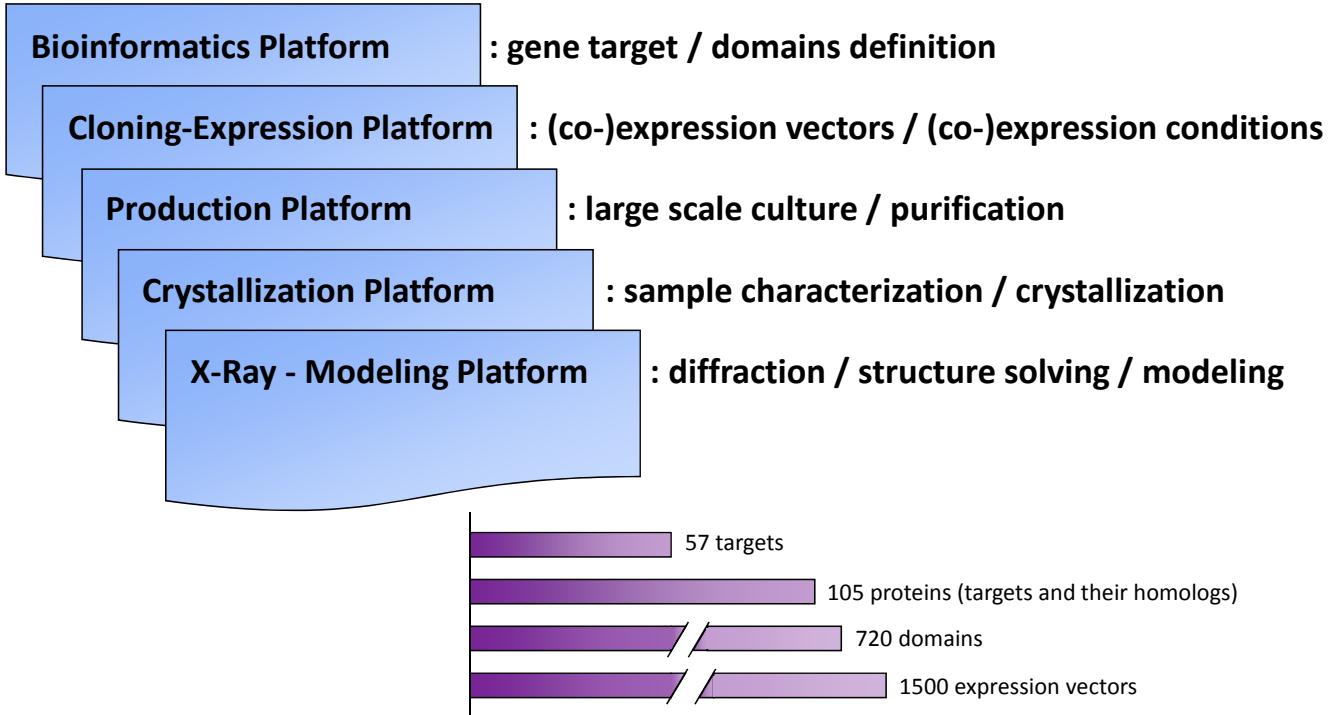
Structural Genomics,

SPINE (2002-...)

Integrative Structural Biology

INSTRUCT

Structural Genomics Platforms

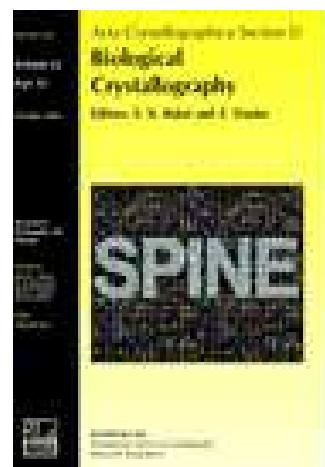


SPINE (FP5) Structural proteomics in Europe October 2002-March 2006

Main results :

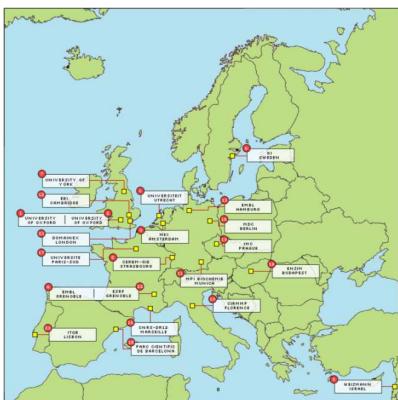
- a pipeline procedure for structure determination
- structures of important human proteins, protein involved in diseases and disorders, pathogens : 308 novel protein and a further 61 derivative structures
- European standards in protein crystal handling for X-ray cristallography

Acta Crystallographica Section D
V.62, p.1103-1285 (October 2006)
Structural Proteomics IN Europe



SPINE 2-Complexes (FP6)
From Receptor to Gene: Structures of Complexes from signalling pathways
linking Immunology, neurobiology and cancer.

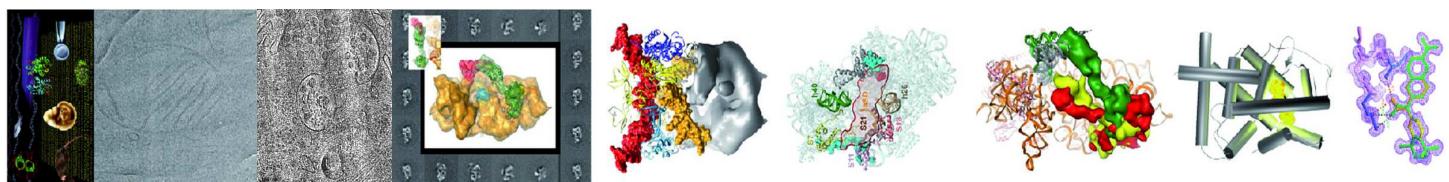
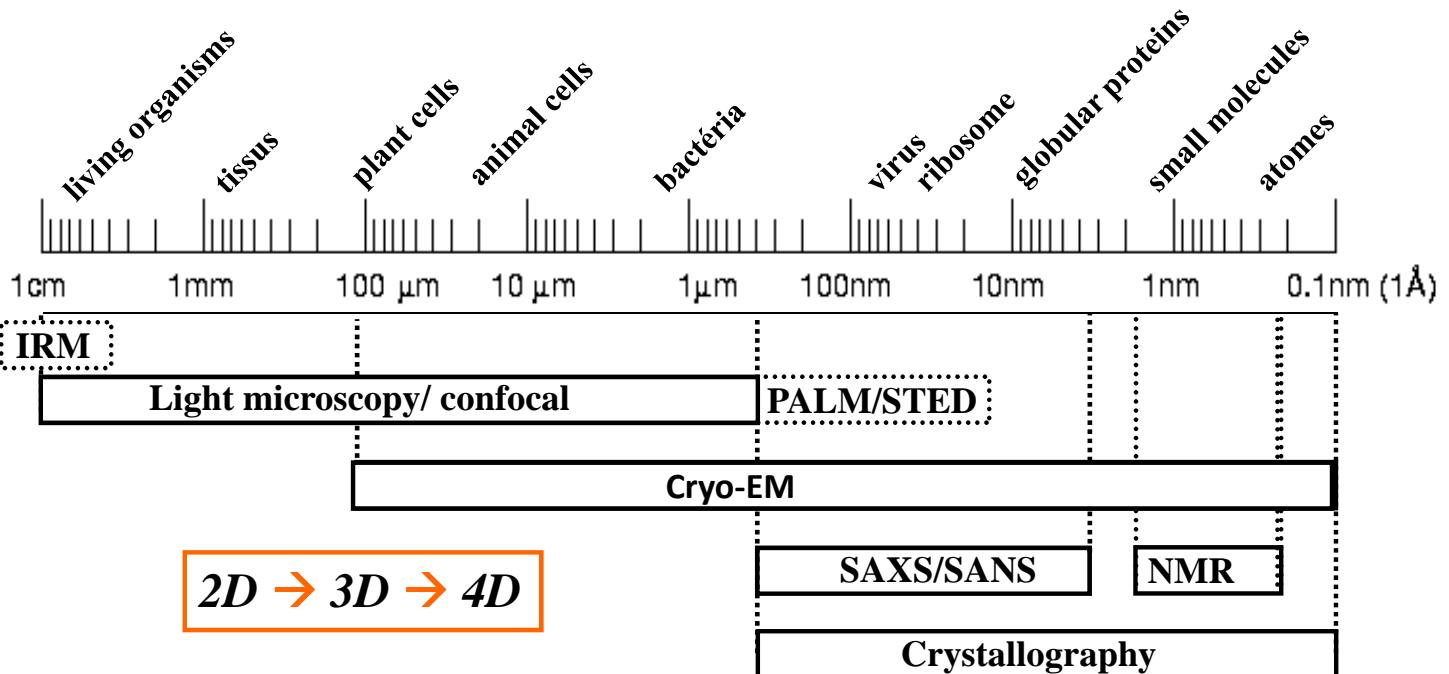
- Combining knowledge of genomes with HTP methods for structural proteomics
- 3D structures of human proteins and their complexes:
protein-protein, protein-ligand and protein-nucleic acids complexes
- Development of HTP procedures for :
cloning, expression and purification
biophysical and biochemical characterization
structure determination by X-Rays, NMR or EM



Montecatini 2006 : Integrated Structural Biology



« De la molécule à la cellule »



instruct
Integrating
Biology

*Integrated Structural Biology – the emerging role
of an infrastructure to support European
competitiveness in structural biology*

www.structuralbiology.eu

A distributed infrastructure for integrated structural biology

ESFRI Assessment 130213

Solid State NMR

In Cell NMR

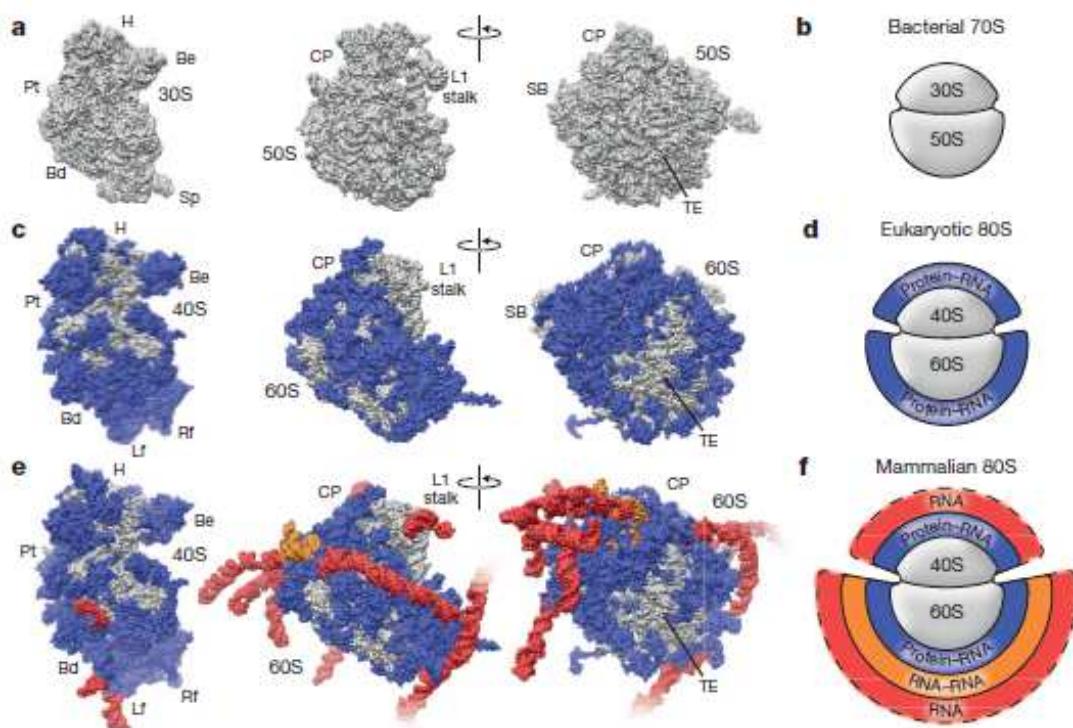
DNP

Ultra-high resolution photonics

Tomography

Mass Spectrometry

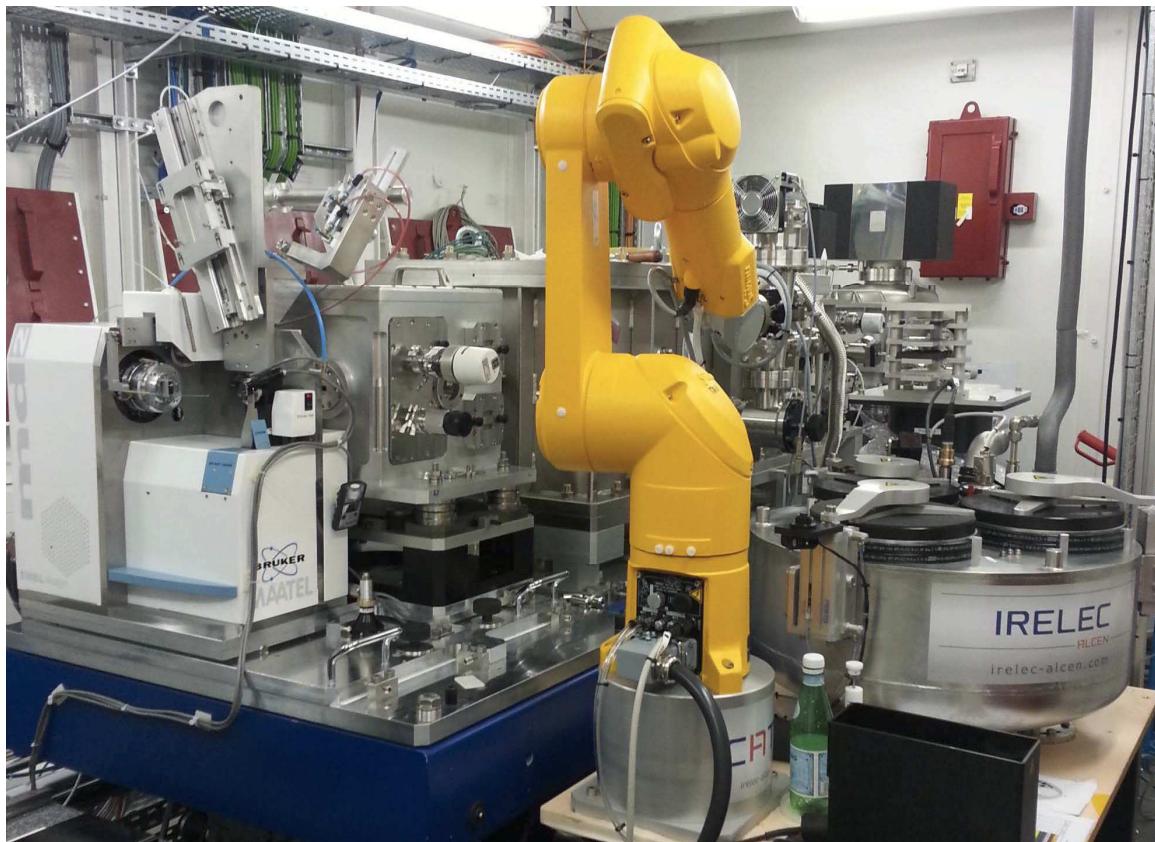
Structures moléculaires 3D de ribosomes :
bactéries (1990), levures (2011) et mammifères (2013)



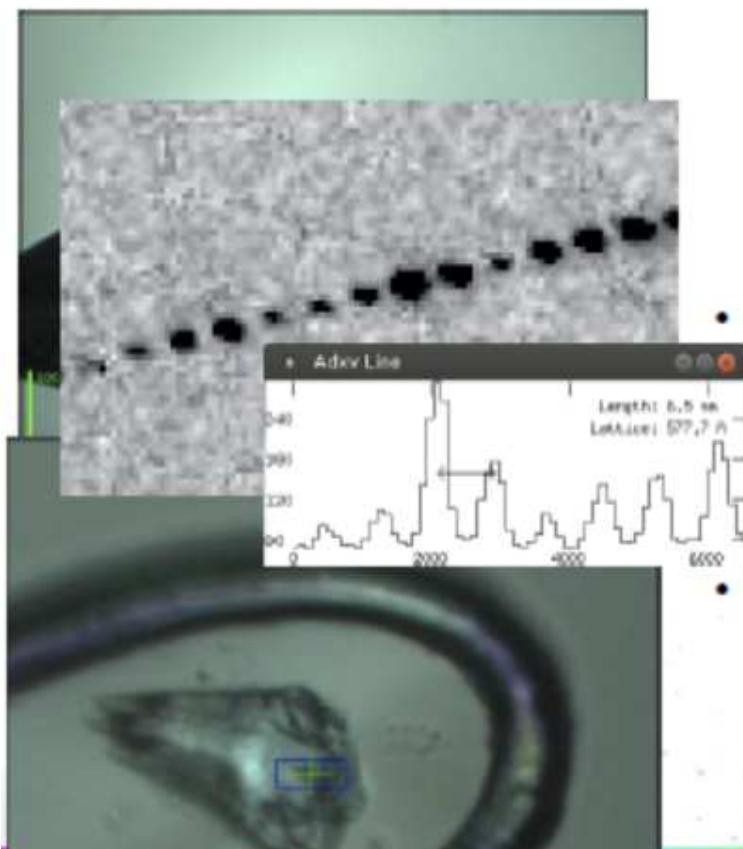
Les structures moléculaires de mammifères ont été obtenues
par cryosmicroscopie électronique

μ -MX : SOLEIL Proxima 2

William SHEPARD



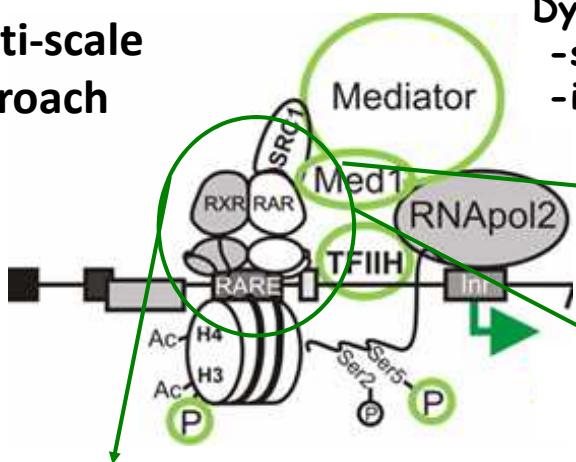
Future Directions



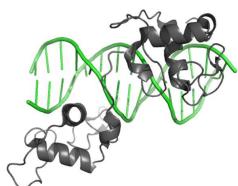
- Radiation Damage
 - Example
 - 180° collection & 0.3 % flux
 - Lower X-ray doses
 - Weaker S/N
 - Poorer resolution
- Large unit cells
 - Unit cell axes > 500Å
 - more common...
 - Requires slitting down
 - Consequently less flux
- Automation
 - Screening strategies
 - Fast grid scans
 - Auto-screen entire pucks
 - Pipelining & Faster collects

Integrative structural biology analysis of nuclear receptors mediated transcription regulation

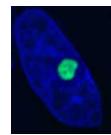
Multi-scale approach



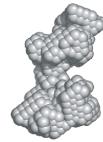
Crystal structures



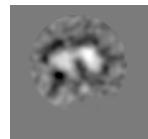
Dynamics of the complexes
-single molecule (FRET)
-in the cell (FRET/FLIM)



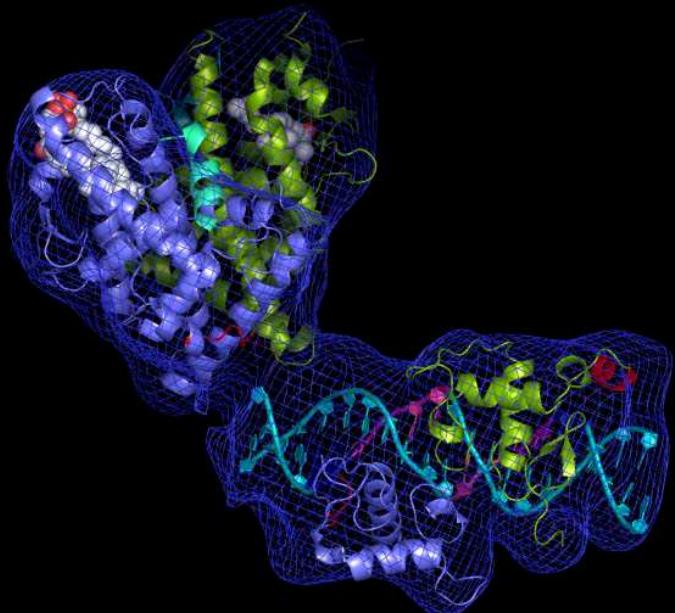
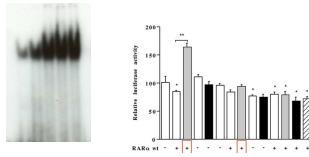
Solution structures
(SAXS, SANS)



CryoEM

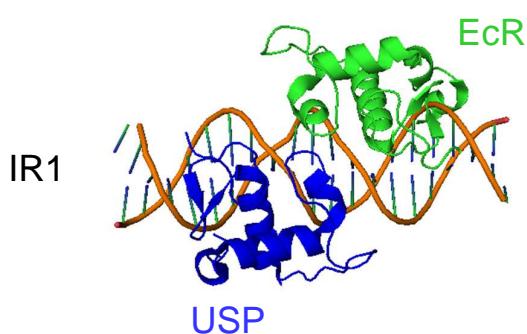


Functional data



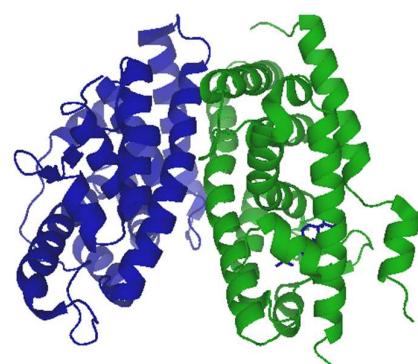
Crystal structures of USP and EcR isolated domains:

DBDs:



Devarakonda *et al.*, *EMBO J.* 2003.
Jakób *et al.*, *NAR* 2007

LBDs:



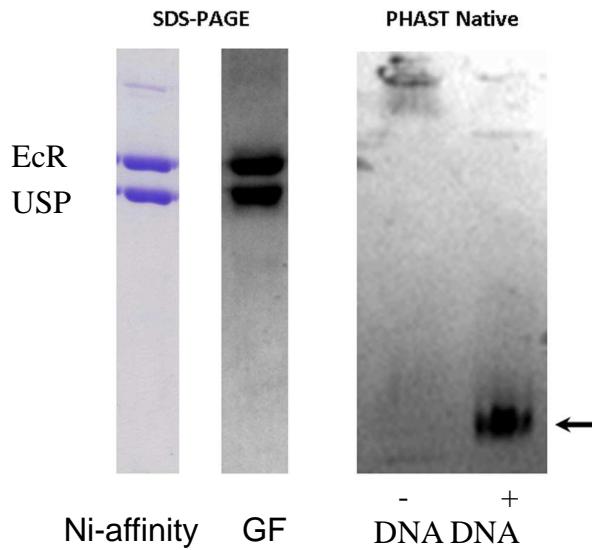
Billas *et al.*, *Nature* 2003.
Carmichael *et al.*, *JBC* 2005.

The palindromic DNA-bound USP/EcR nuclear receptor adopts an asymmetric organization with allosteric domain positioning

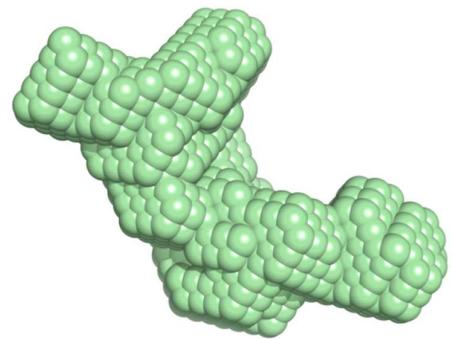
Massimiliano Maletta, Igor Orlov, Pierre Roblin, Yannick Beck, Dino Moras, Isabelle M.L. Billas & Bruno P. Klaholz
Nature Comm, in press

SAXS study of USP/EcR complex on a natural DNA inverted repeat (IR1)

Complex preparation:

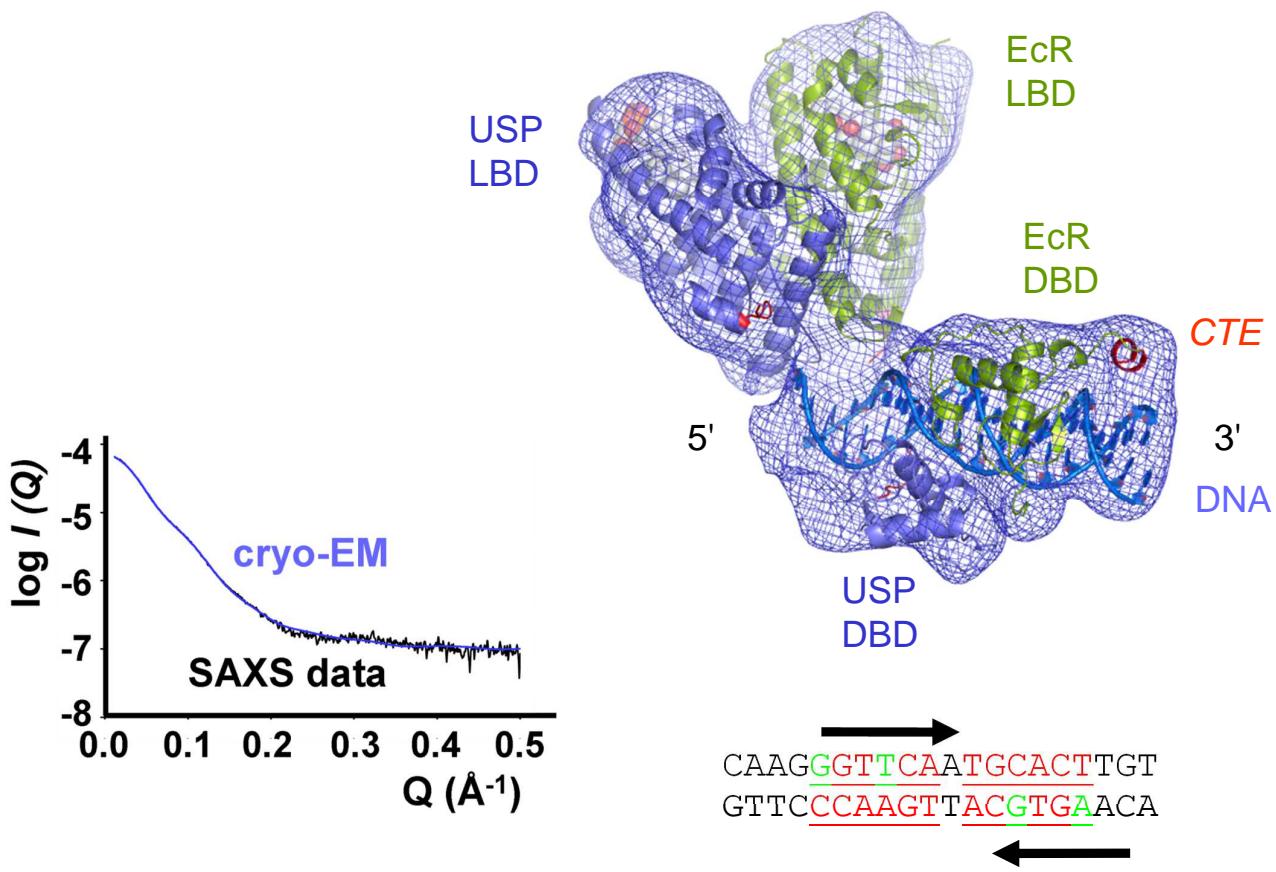


Ab initio SAXS envelope:

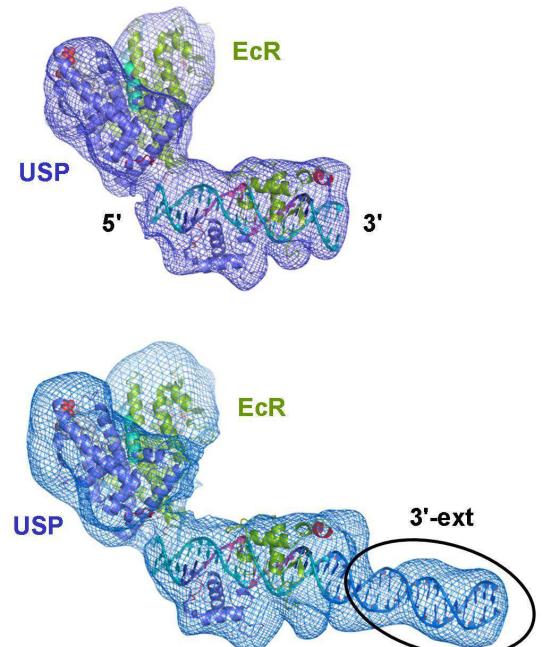
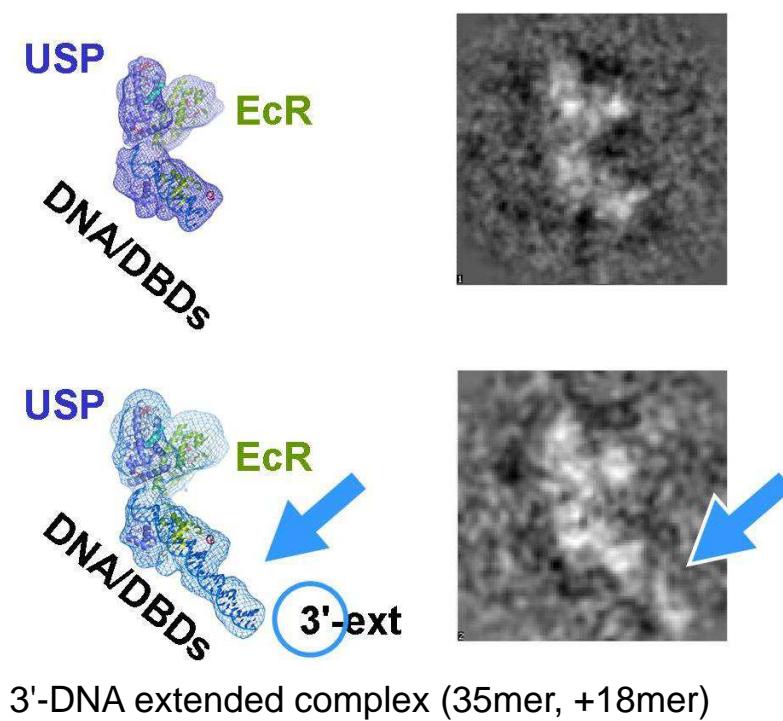


SOLEIL synchrotron, Paris
HPLC-coupled SAXS

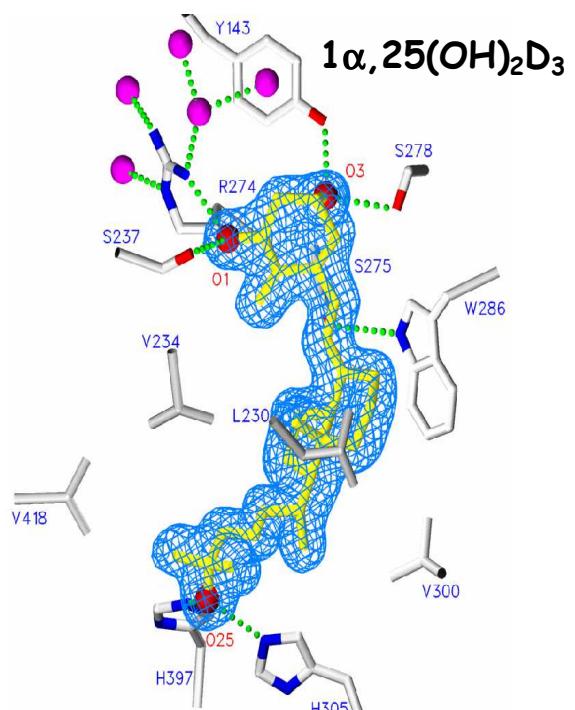
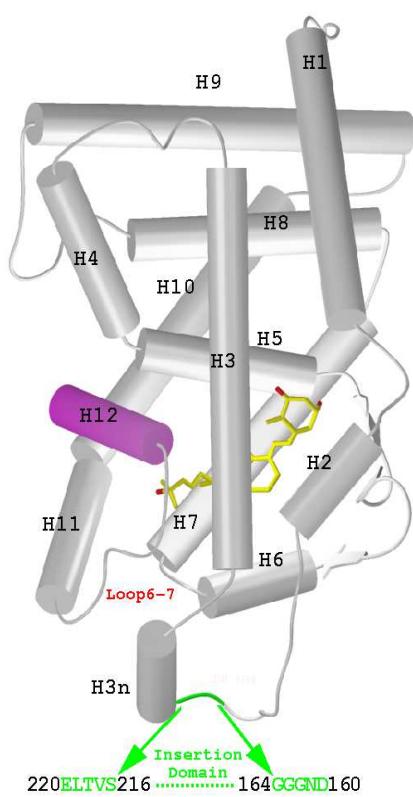
Cryo-EM Structure of the USP/EcR complex



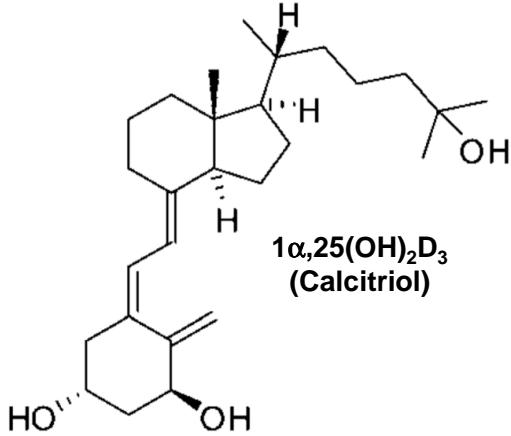
Assignment of the polarity on the DNA:



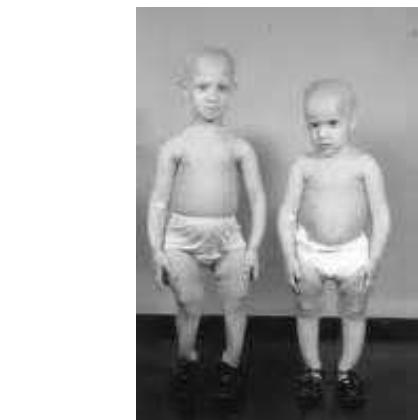
Le récepteur de la vitamine D



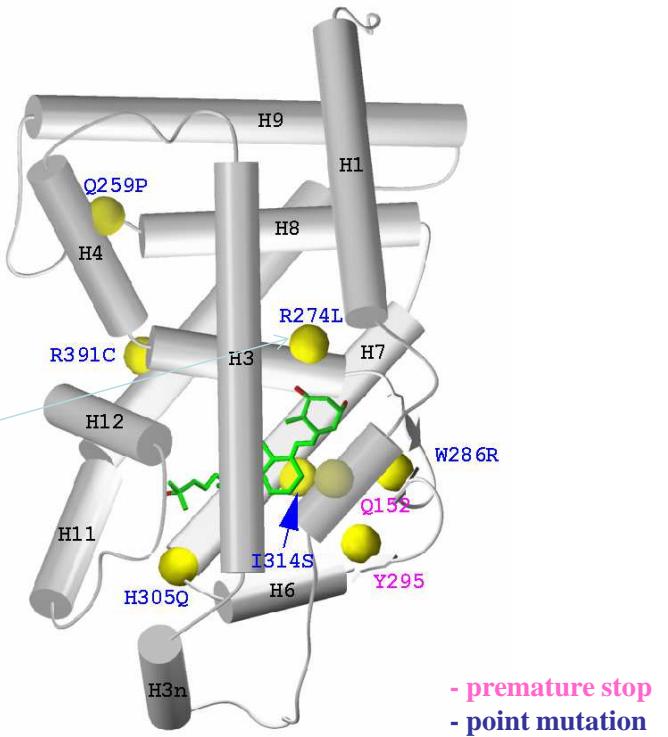
Le ligand (calcitriol, dérivé de la vitamine D) dans l'enveloppe expérimentale de la structure cristallographique



Mutations naturelles observées dans le domaine de fixation du ligand liées au syndrome de rachitisme héréditaire avec résistance à la 1,25-dihydroxyvitamine D



Rosen, JF et al., J Pediatr. 1979

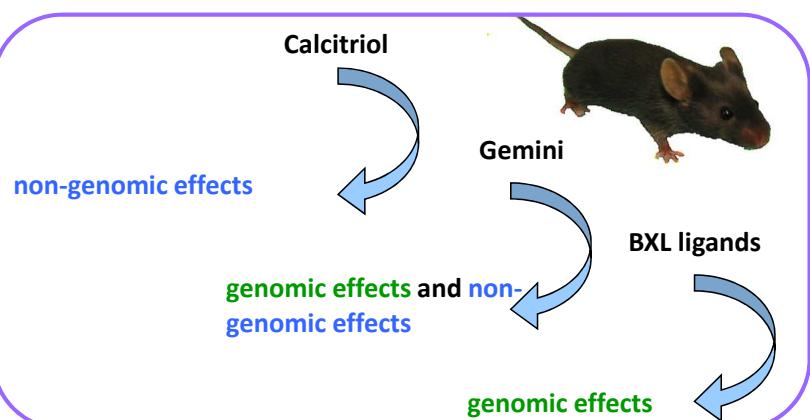


From atomic resolution structure to in vivo model:

Rational design of the *vdr*-knock-in mouse model L304H

(Coll. with Daniel Metzger, Dept of Functional genomics, IGBMC)

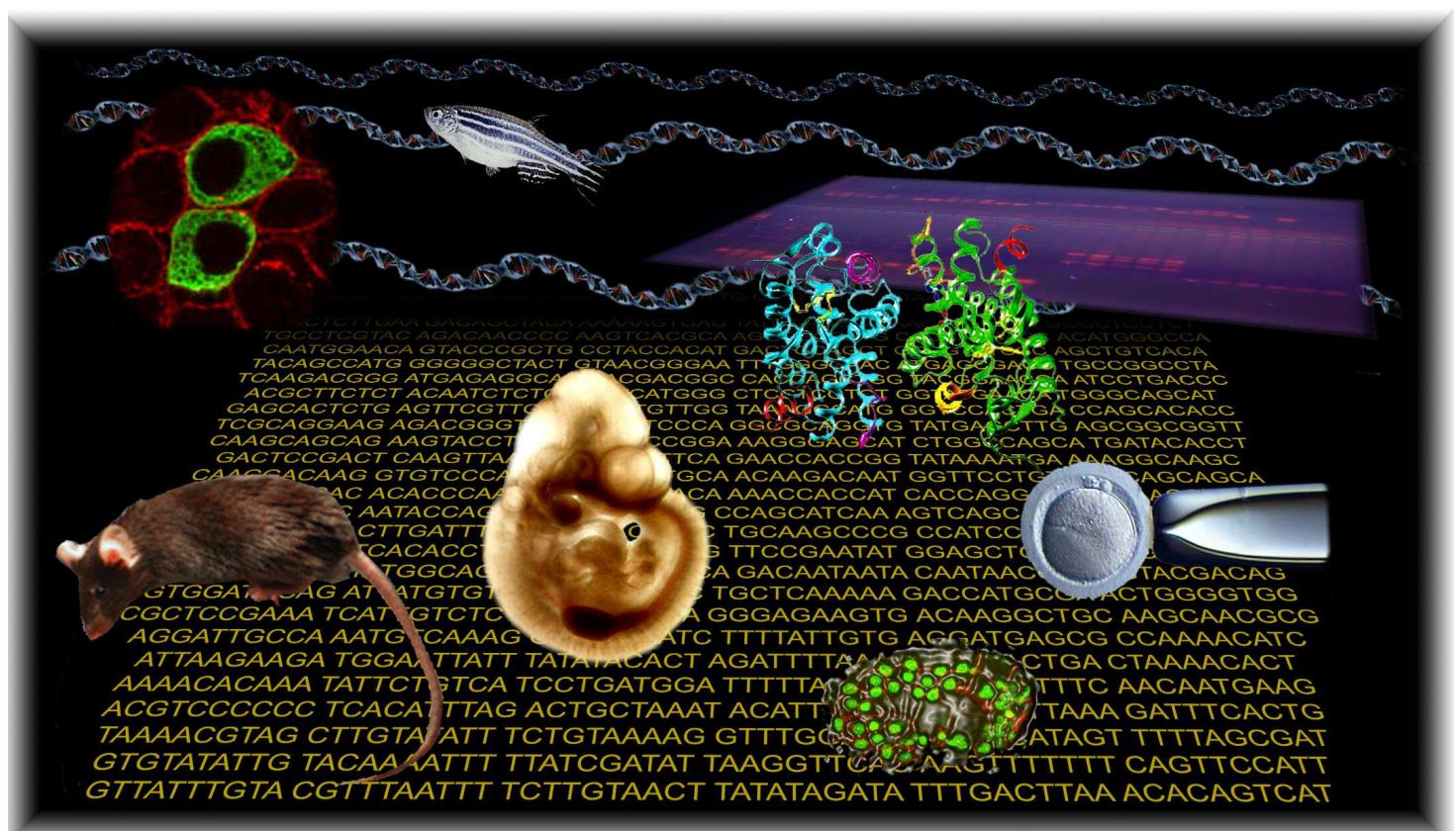
Knock-in mouse expressing the VDR_{L304H}
(Mouse Clinical Institute)



Discriminate the genomic from the non-genomic pathways

Le futur : de la structure moléculaire des complexes fonctionnels à leur positionnement dans la cellule, le tissu et l'animal :

le Code Cellulaire

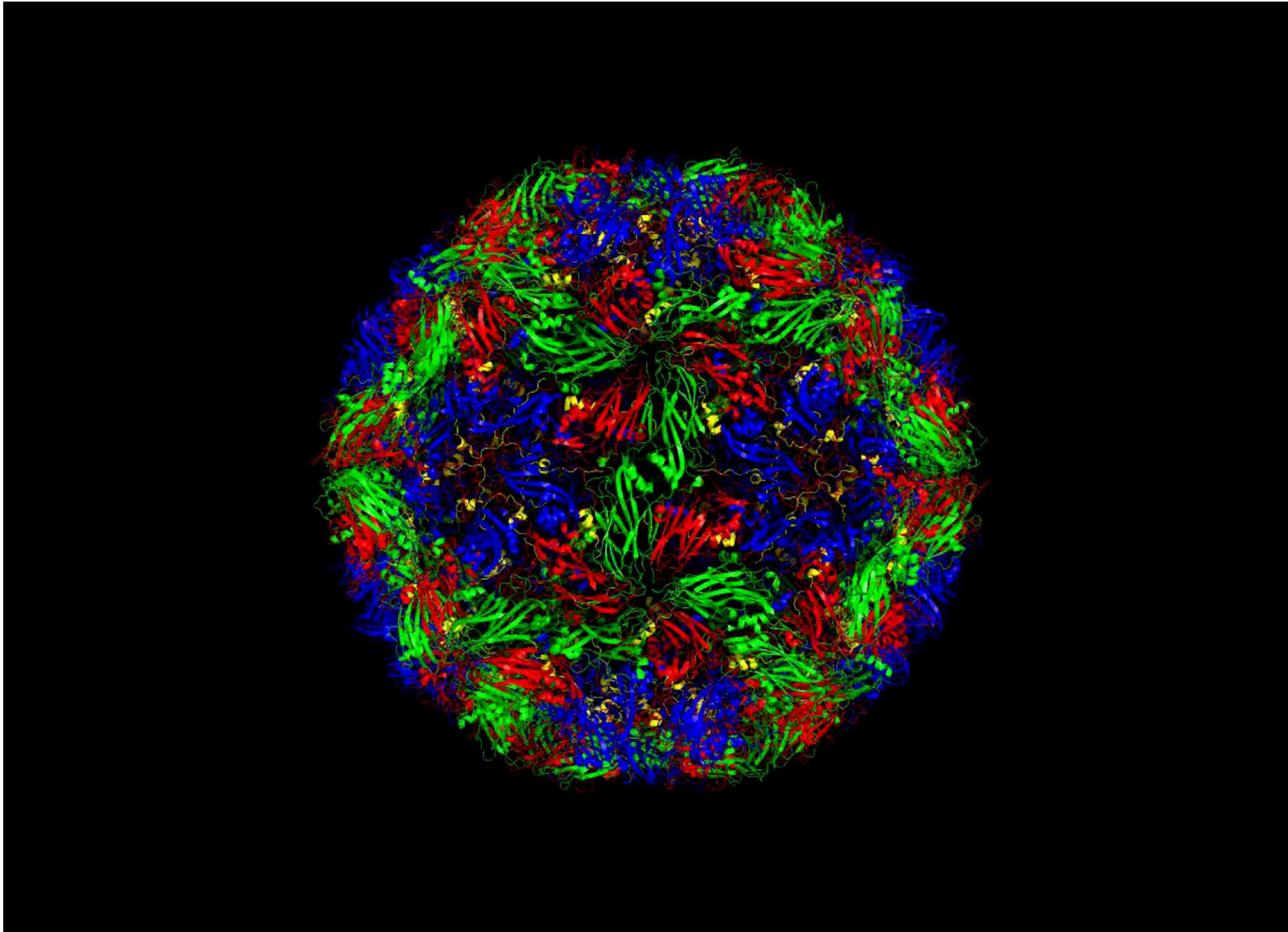


Can we avoid future outbreaks of foot-and-mouth disease through vaccination?

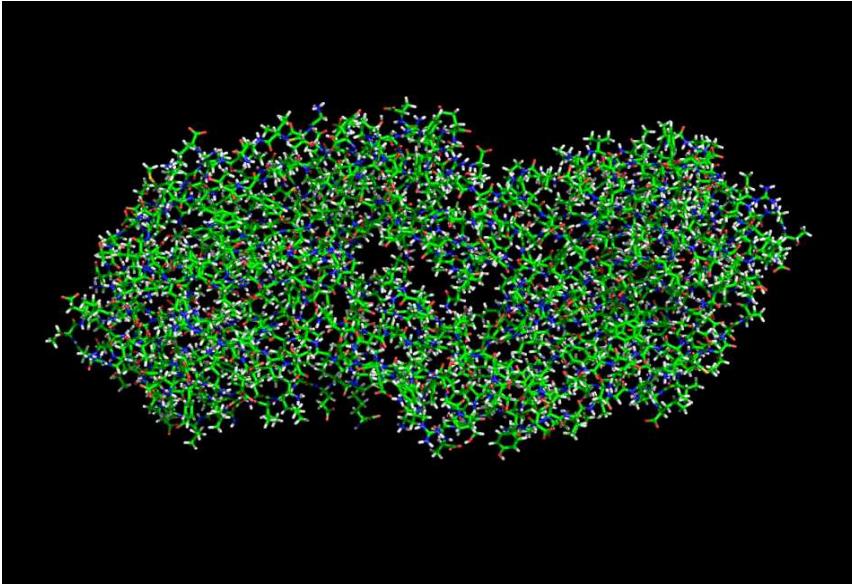
David Stuart, Oxford University and Diamond Light Source



FMD vaccines are fragile - capsids are unstable

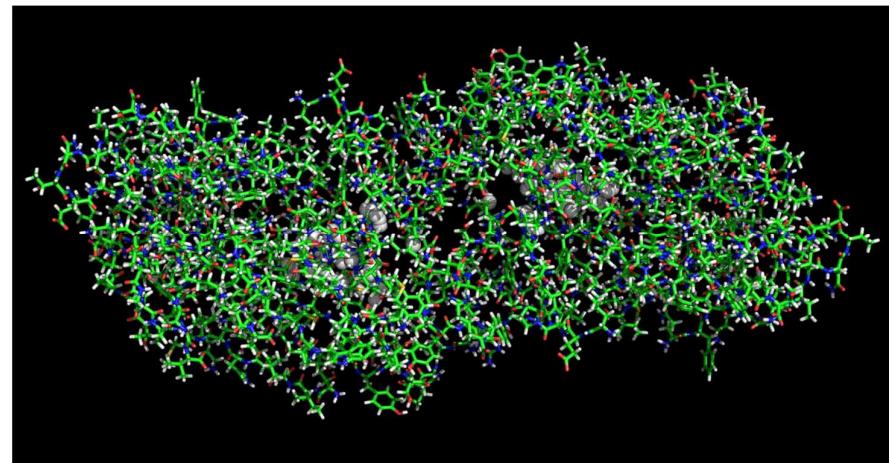


- If we look in detail and come up with ideas of how we might re-engineer the shell to make it more stable, can we develop an ‘in silico’ method to estimate how much the changes stabilise or destabilise the particles?
- ... amazingly the answer seems to be yes!



“Textbook” simulation
– noise dominates

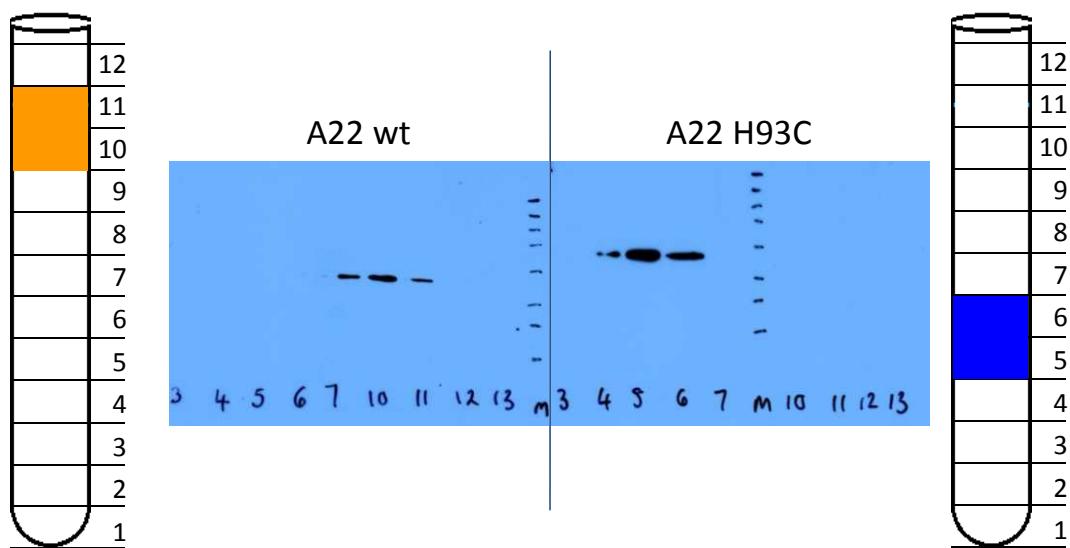
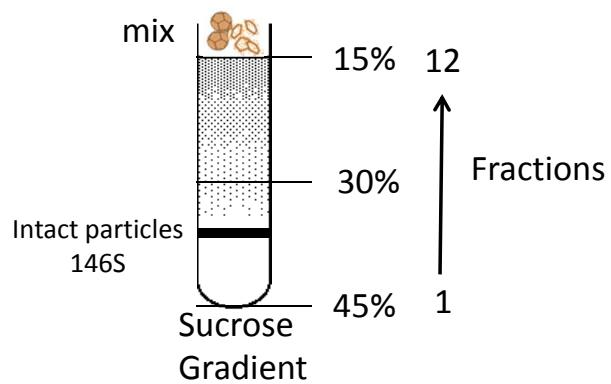
Modified simulation
- target the weakspot



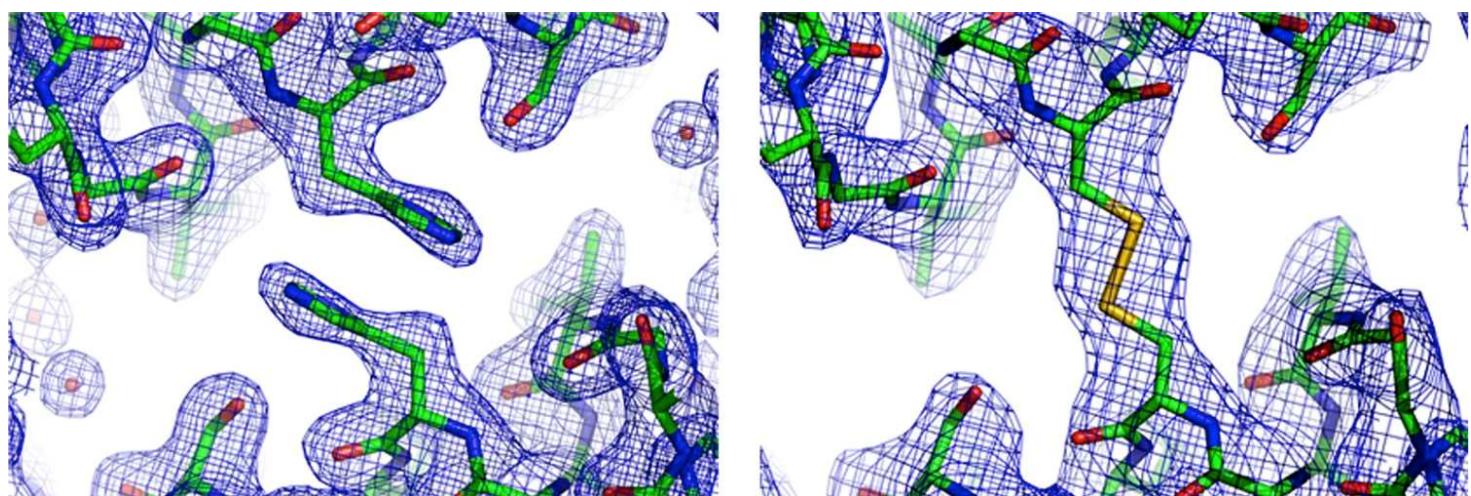
- And it works:

A serotype validation: temperature stability

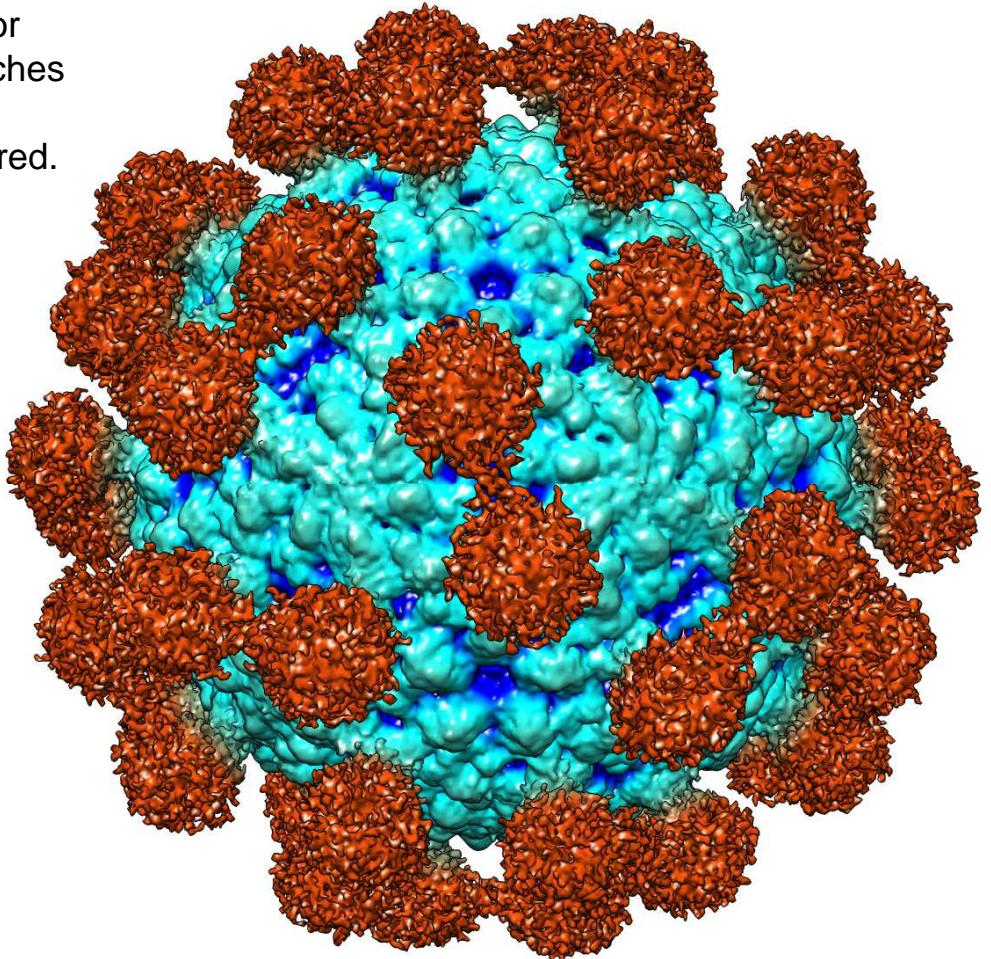
Heating at 56°C for 2 hrs



We use Diamond to validate the molecular engineering



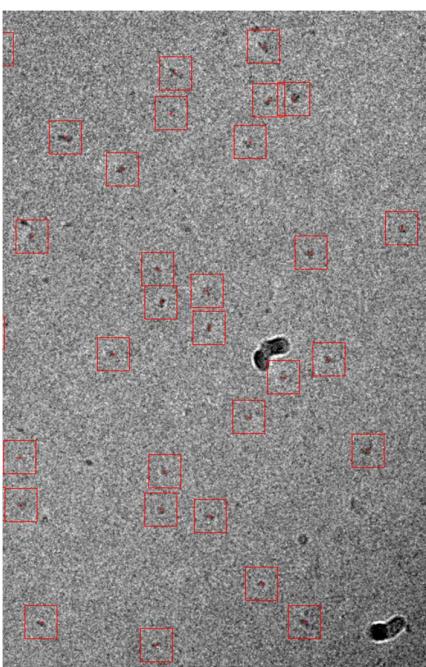
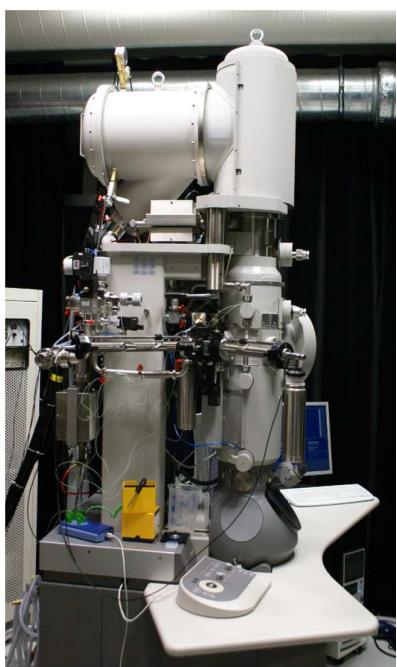
And if we step back we can learn many more things – for instance how the virus attaches to cells (via the cell-surface integrin molecule shown in red). Exciting times in structural biology!



Mont Ste Odile 2006



3 cryo-EM structures:



Polar electron microscope data,
CCD, 100kV, 59k, 50 000 particles

→ independent
structure validation

