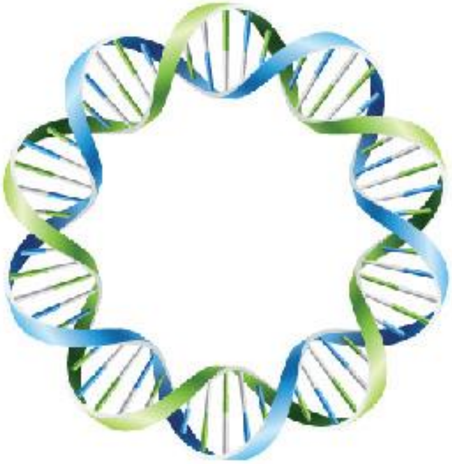
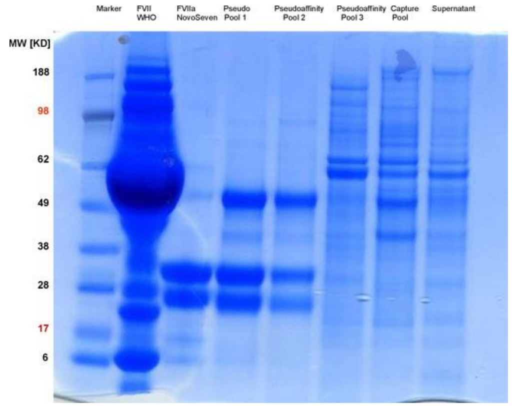
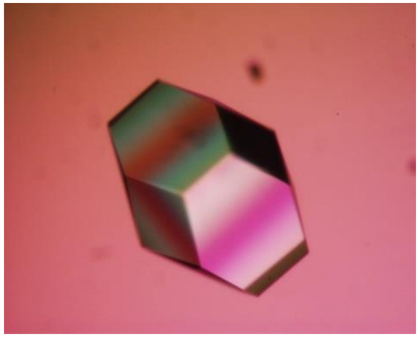
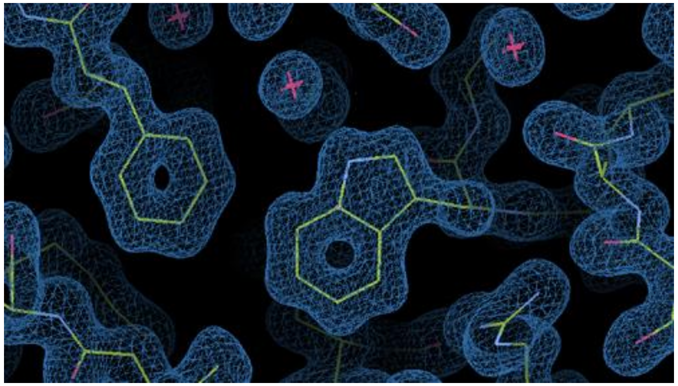






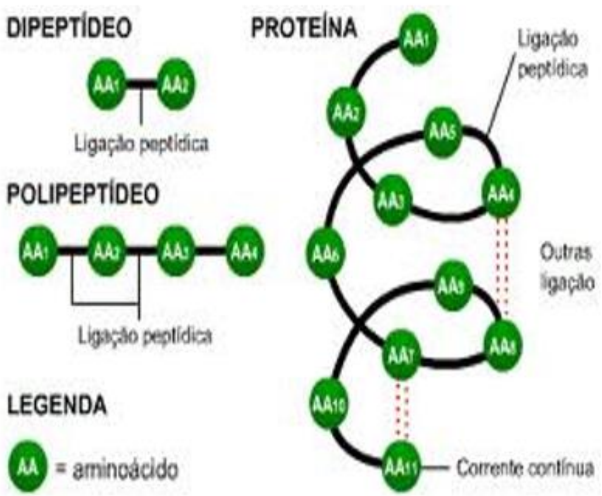
Biologia Estrutural e aplicações

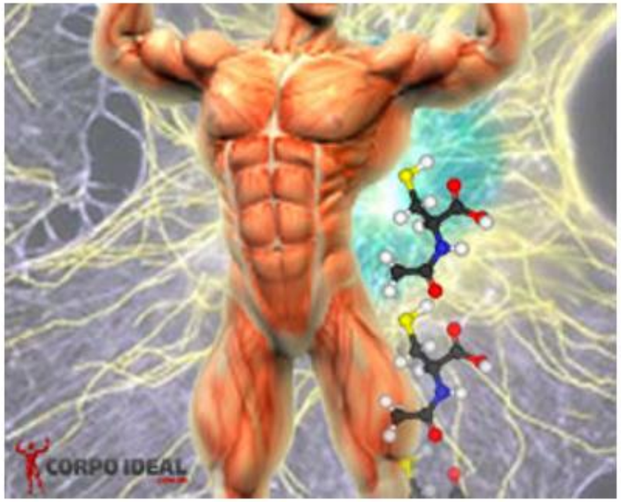
Prof Marcio V B Dias
E-mail:mvbdias@usp.br

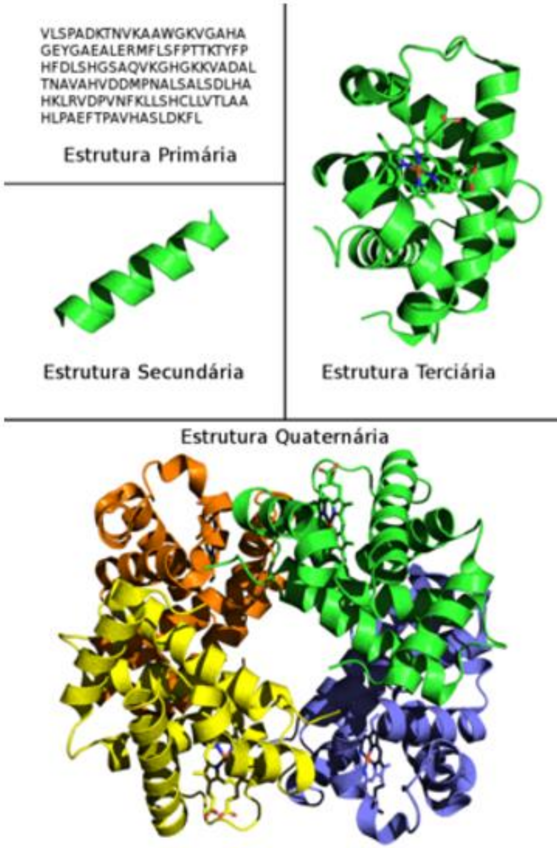


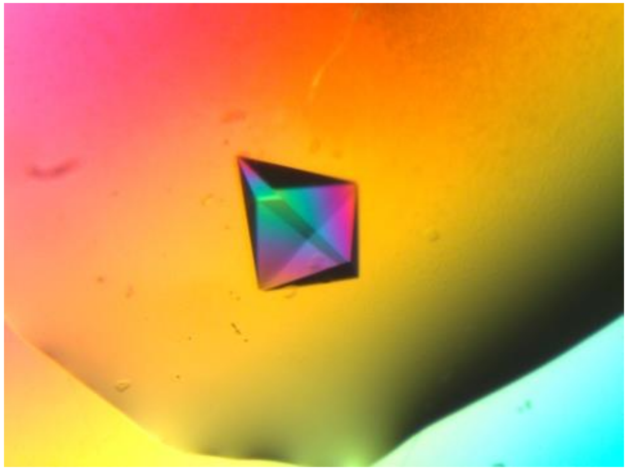
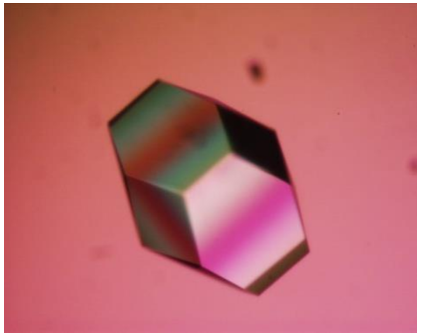
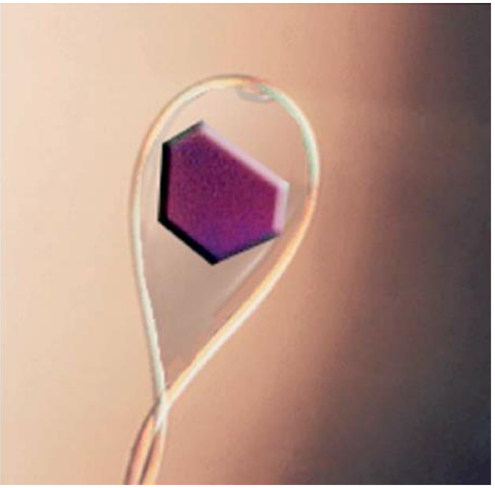
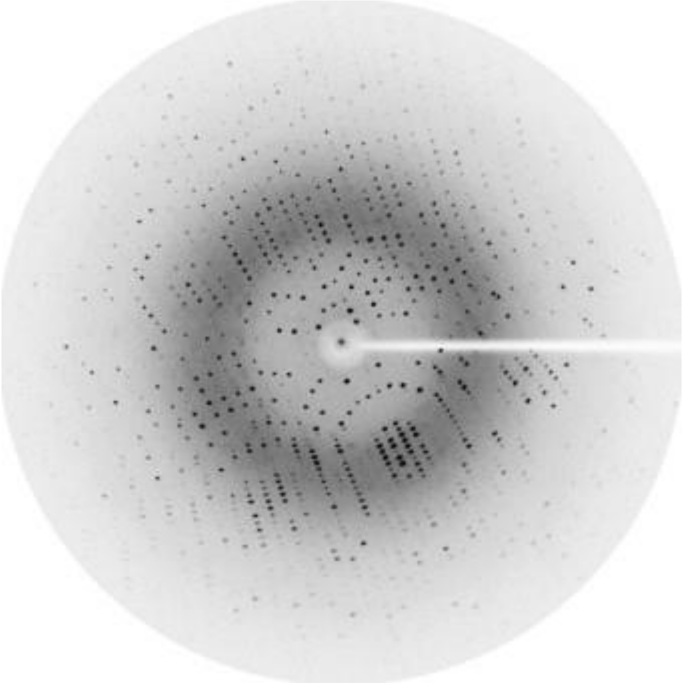
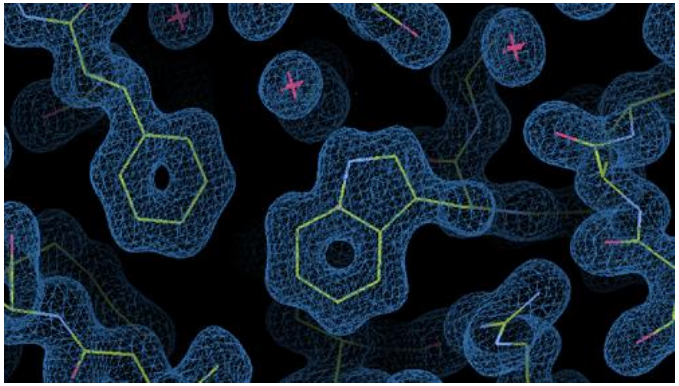
Por que estudar proteínas????

- ✓ essenciais à estrutura, à função, e ao regulamento celular
- ✓ responsáveis pela homeostasia do organismo
- ✓ inúmeras finalidades: diagnóstica, terapêutica e de prevenção (vacinas)







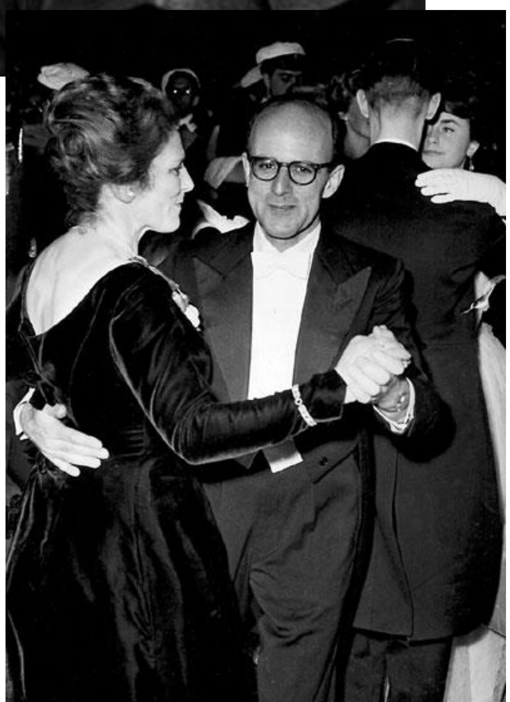






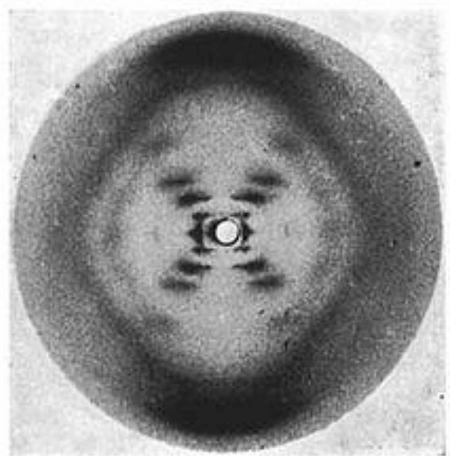
Dorothy C. Hodgkin

Venkatraman Ramakrishnan

Thomas A. Steitz

Ada E. Yonath

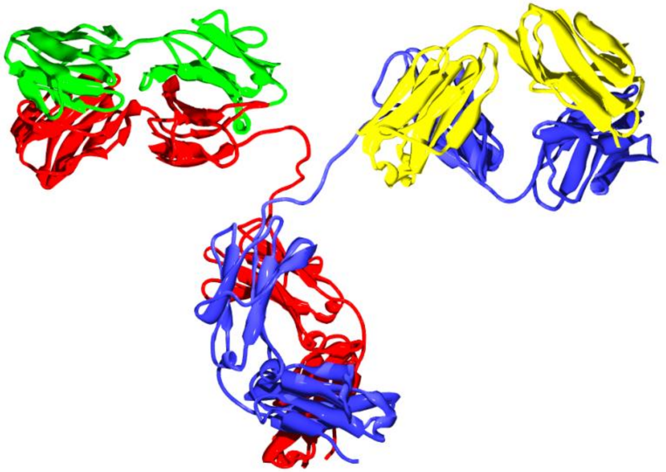




Max Perutz

Rosalind Franklin

Para que cristalizar uma proteína?

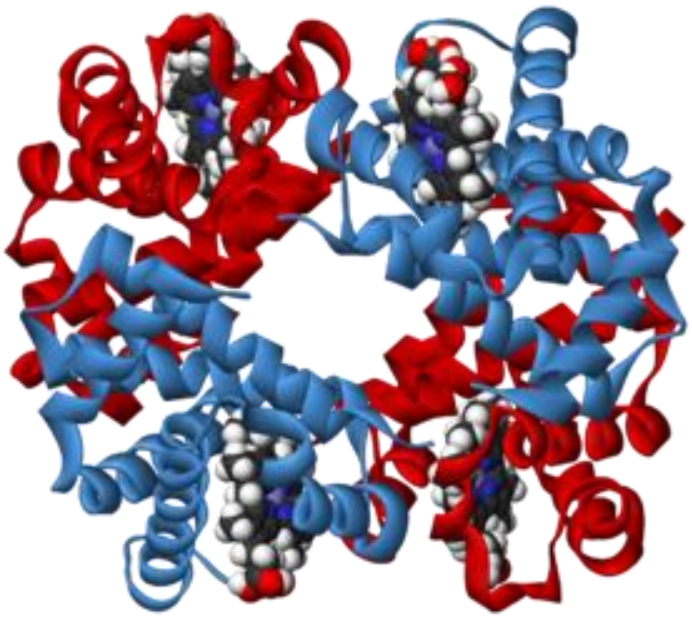


Primeira resposta:

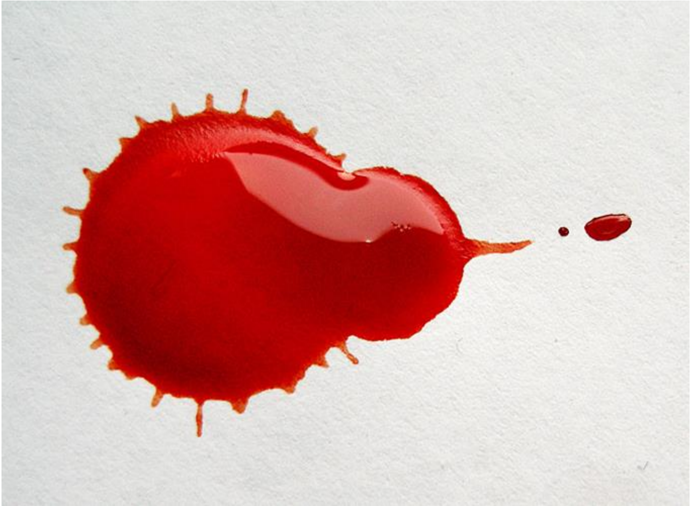
-entender sua estrutura

Mas para que entender a estrutura?

-entender o processos biológicos
-como transporte de oxigênio
-anticorpos
-interação de proteínas com ligantes
-mecanismo de catálise
-desenho de medicamentos
-regulação alosteríca
-mecanismo de doenças

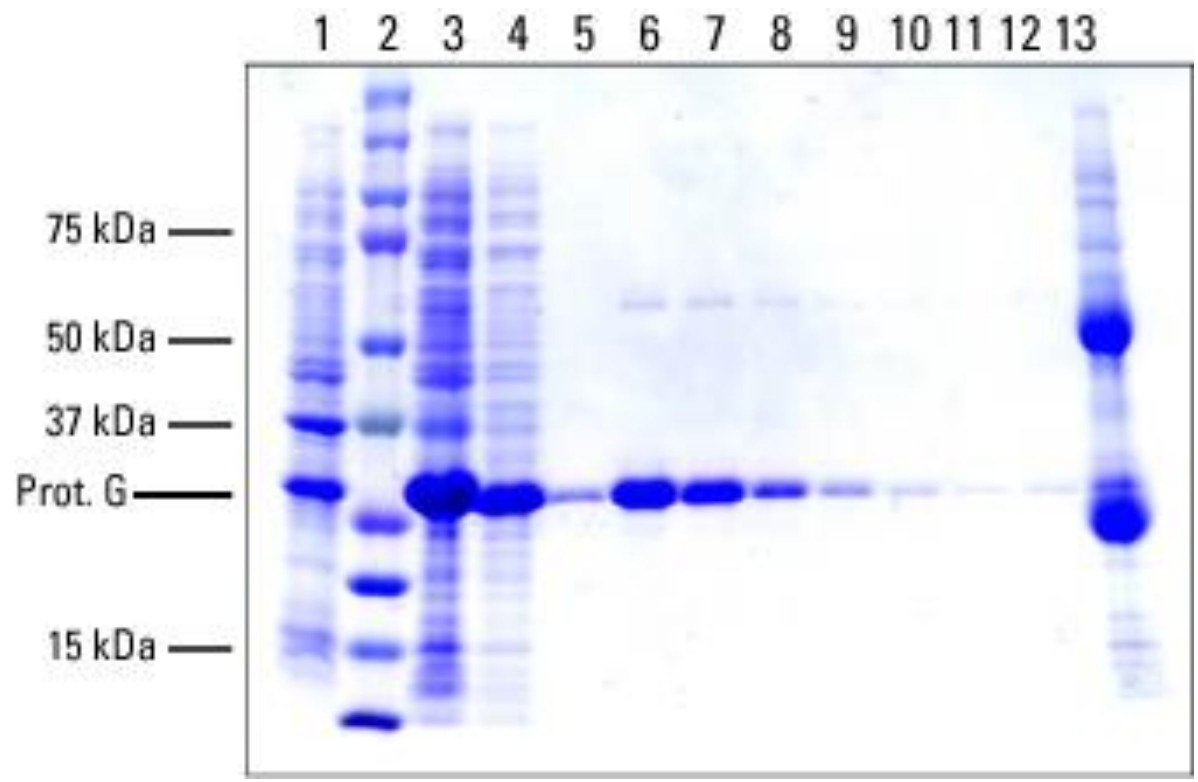


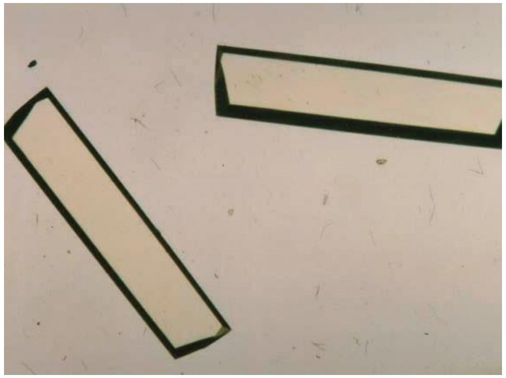
Primeiro Passo



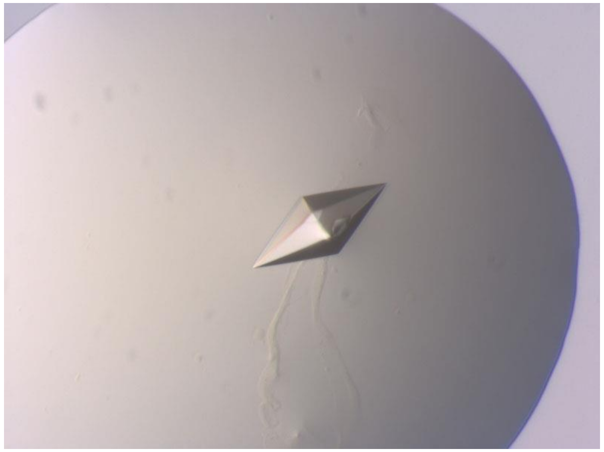
Purificação de proteínas

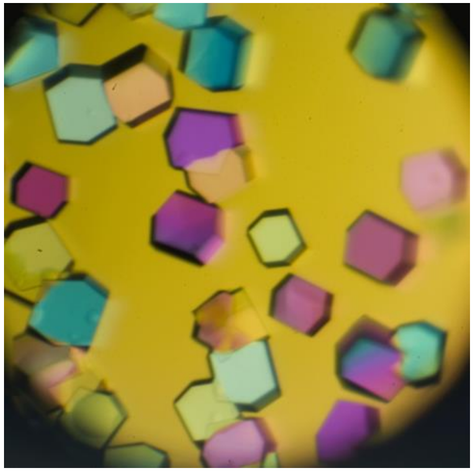
Purificação de proteínas baseado em afinidade

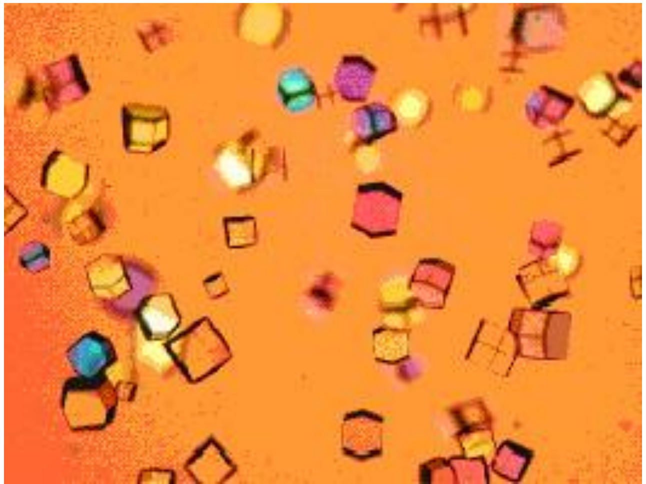


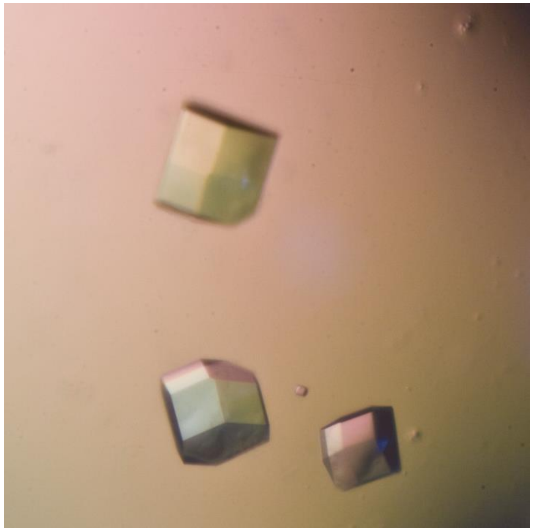


Cristais de proteína

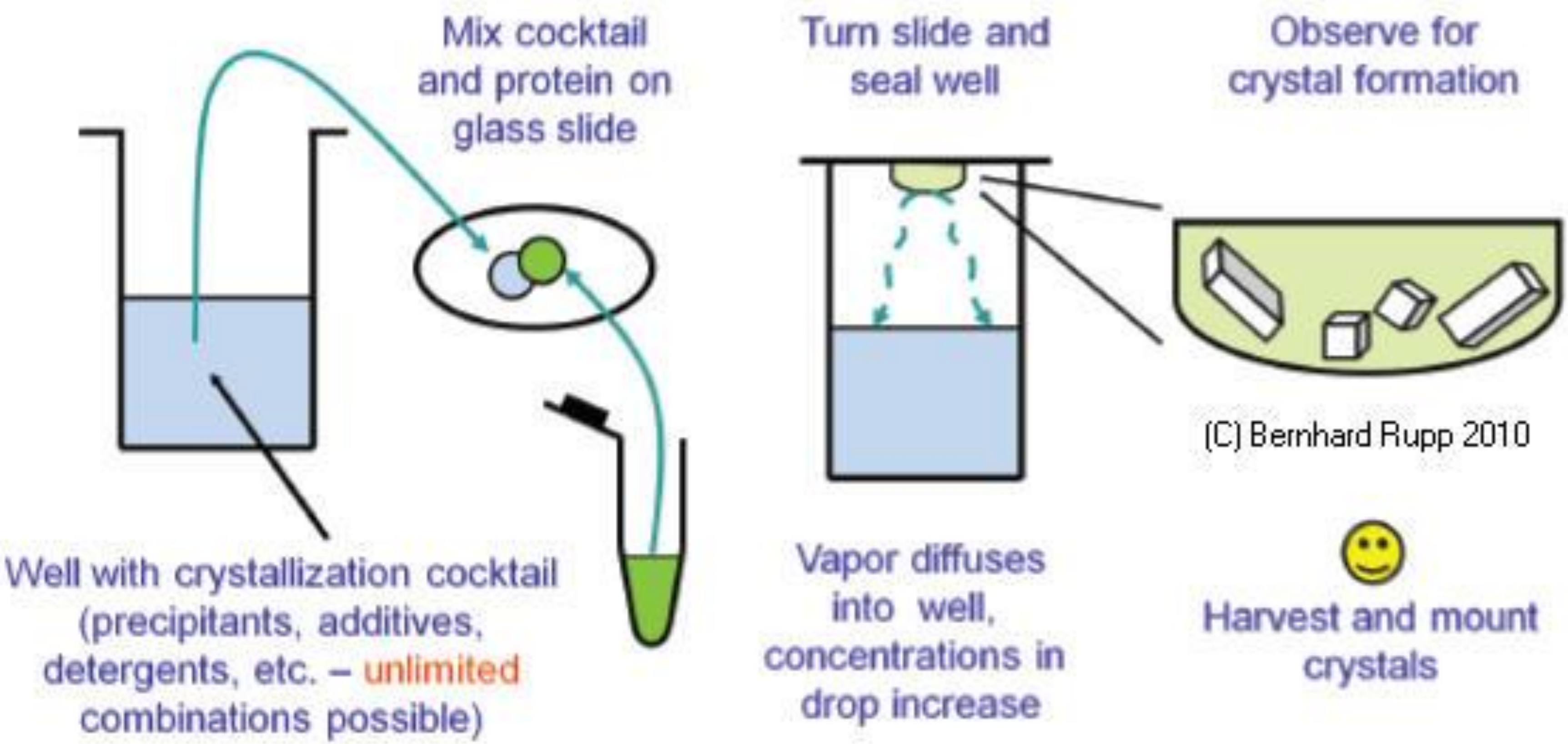






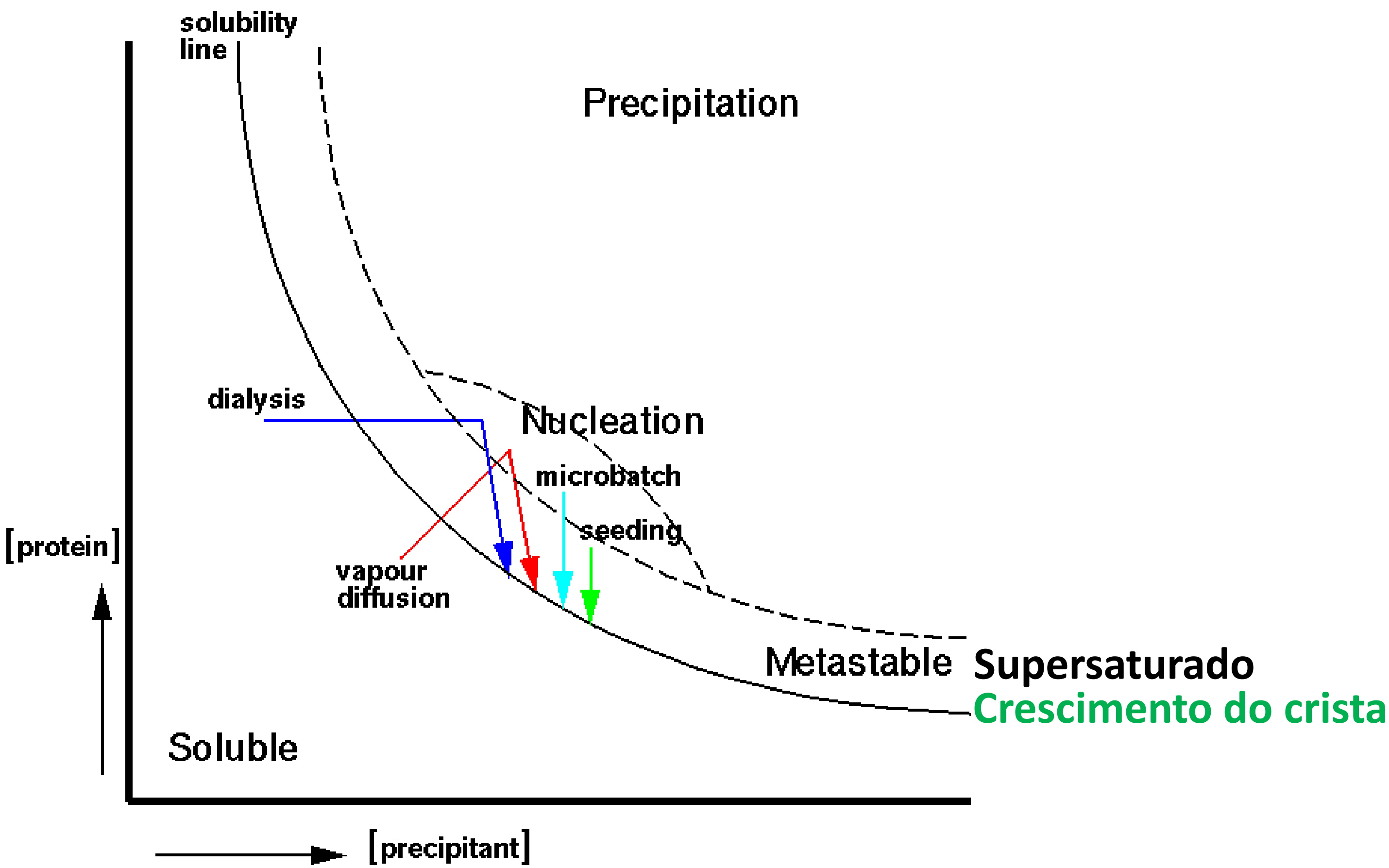


O Processo de Cristalização



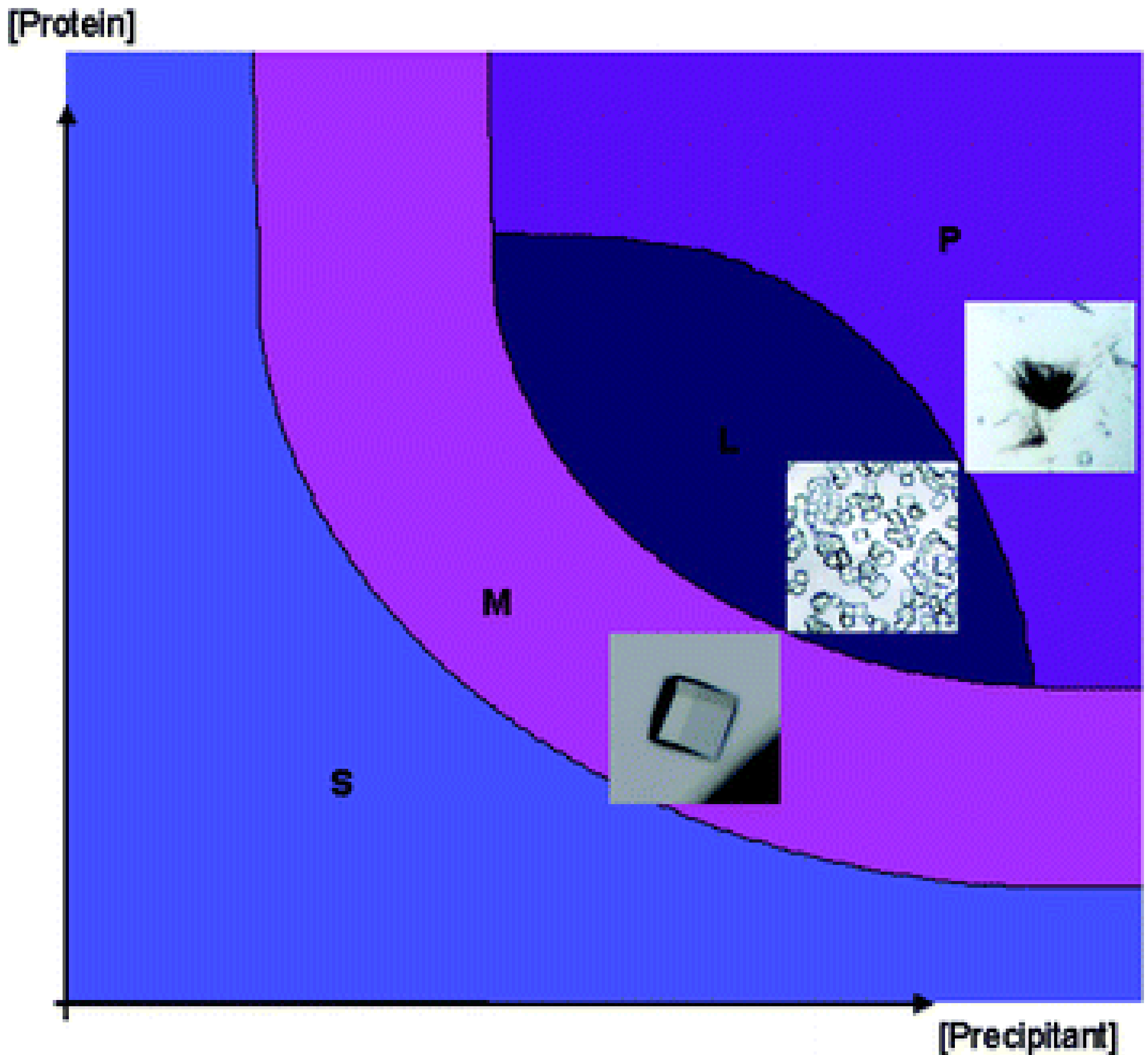
Como obter um cristal de Proteína?

Diagrama de fase

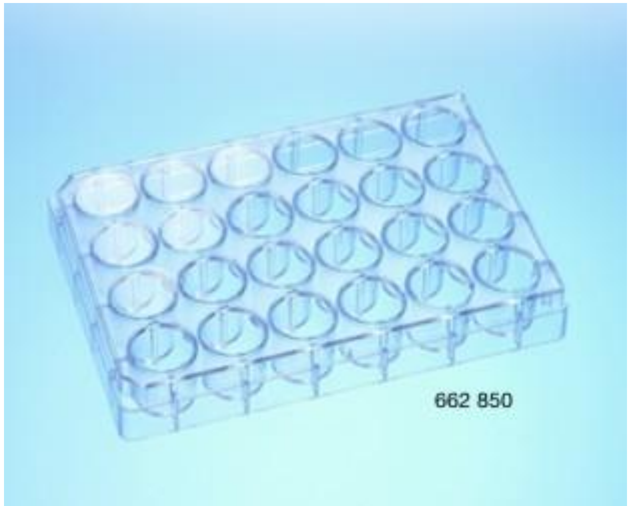


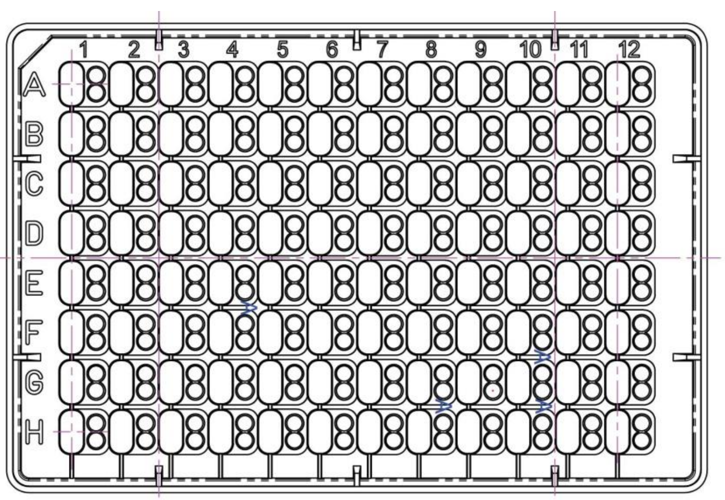
Protein Solubility Phase Diagram and Crystallisation

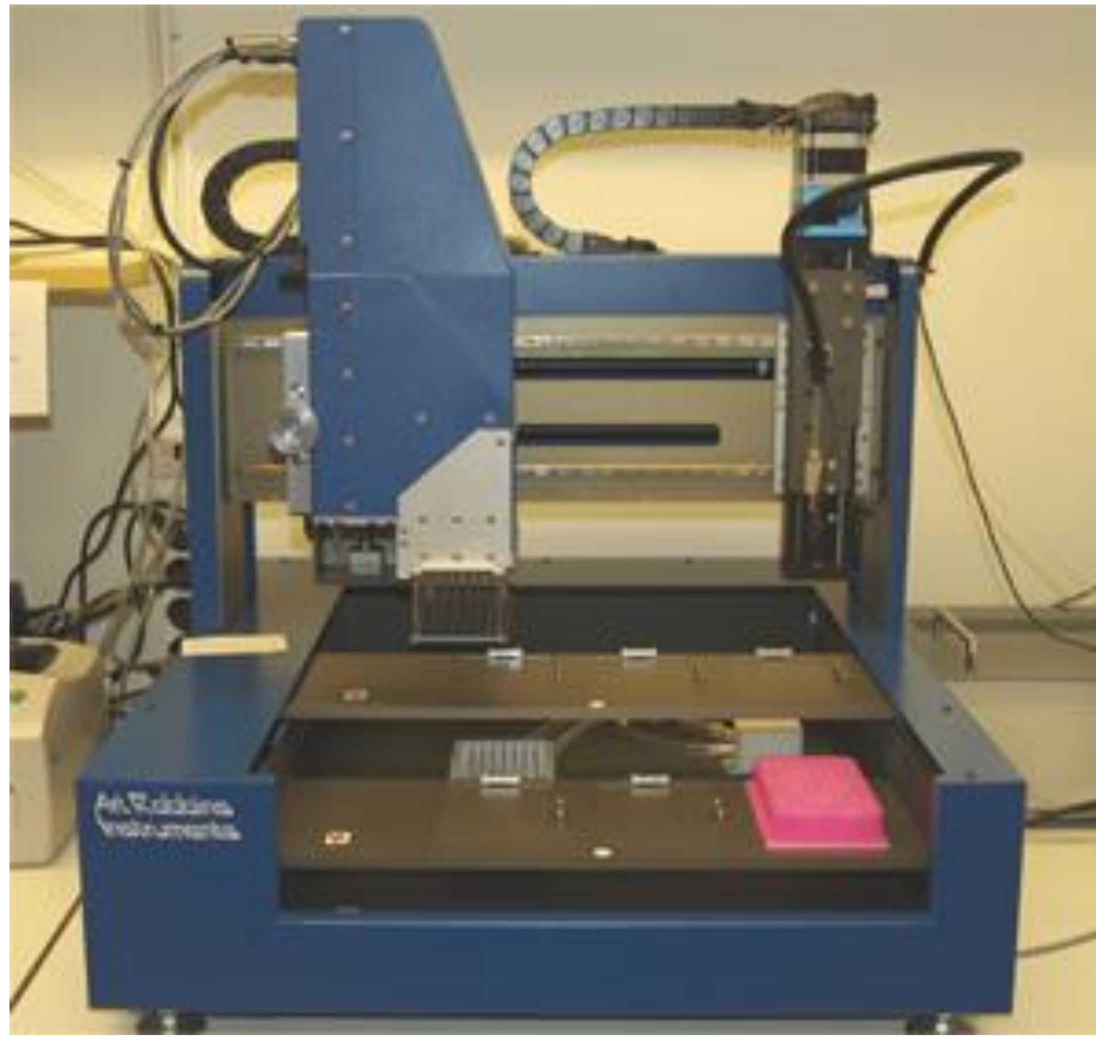
Diagrama de fase



Cristalização rototizada







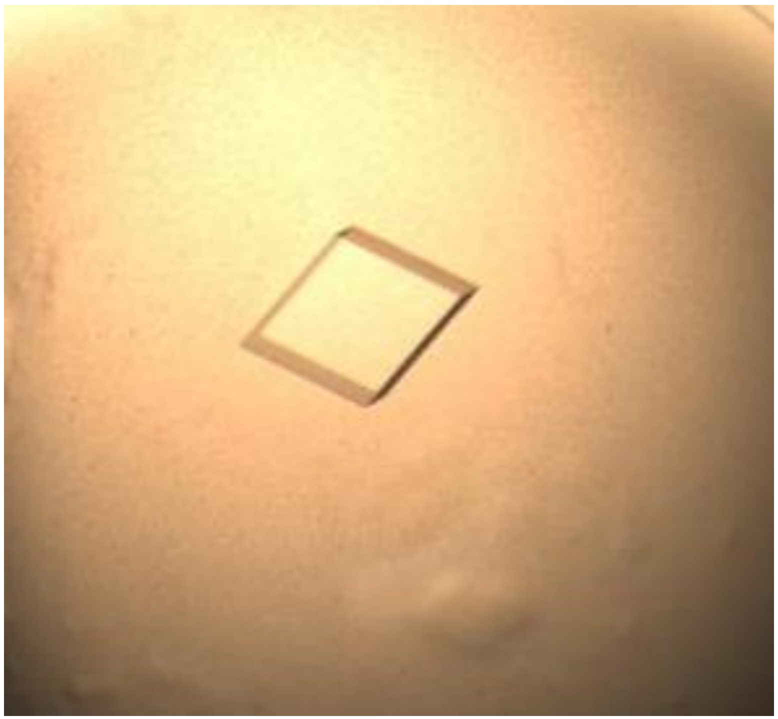
Diferentes Kits de Cristalização

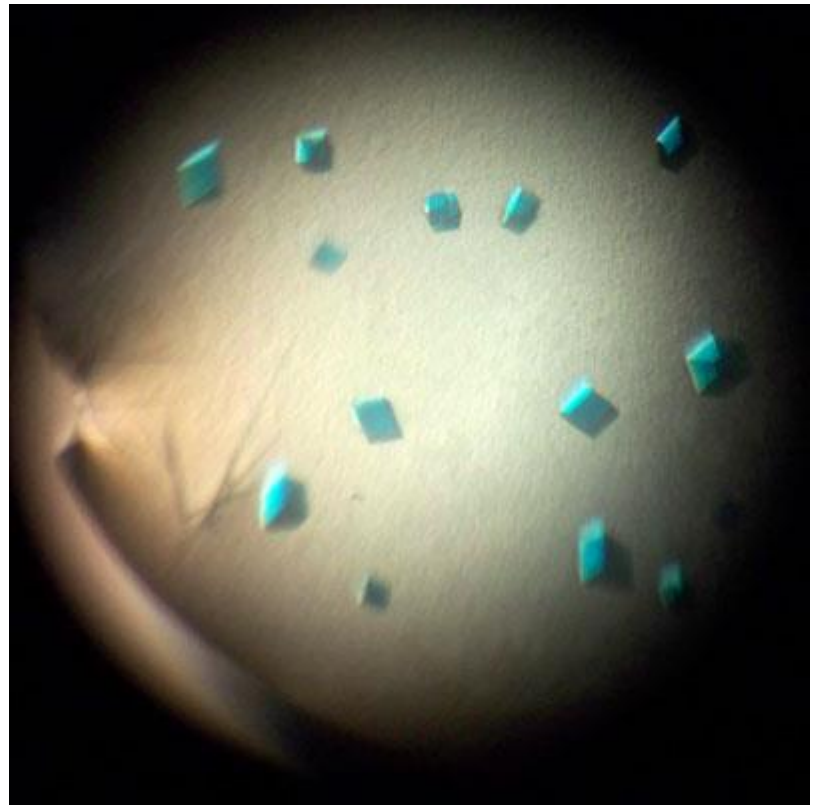
- Tampão

- Sal

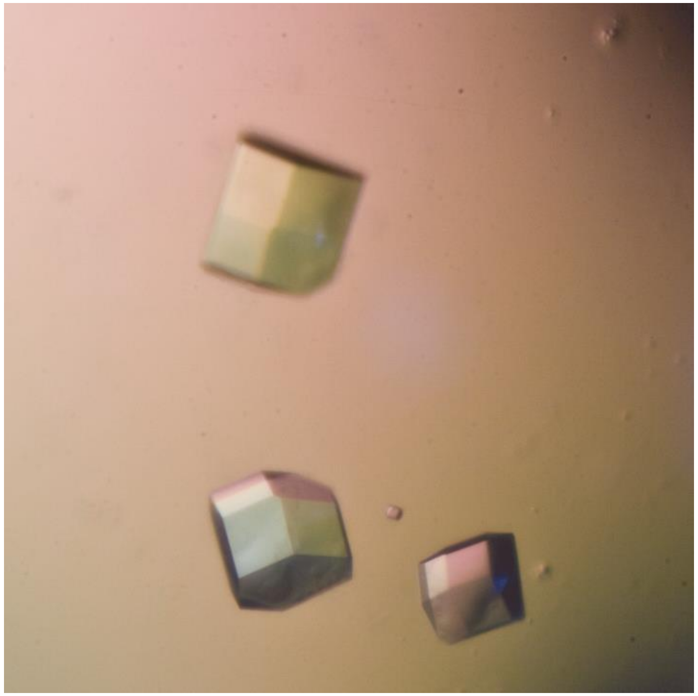
- Agente precipitante

Diferenciar Cristal de Proteína com de Sal





Montagem dos cristais

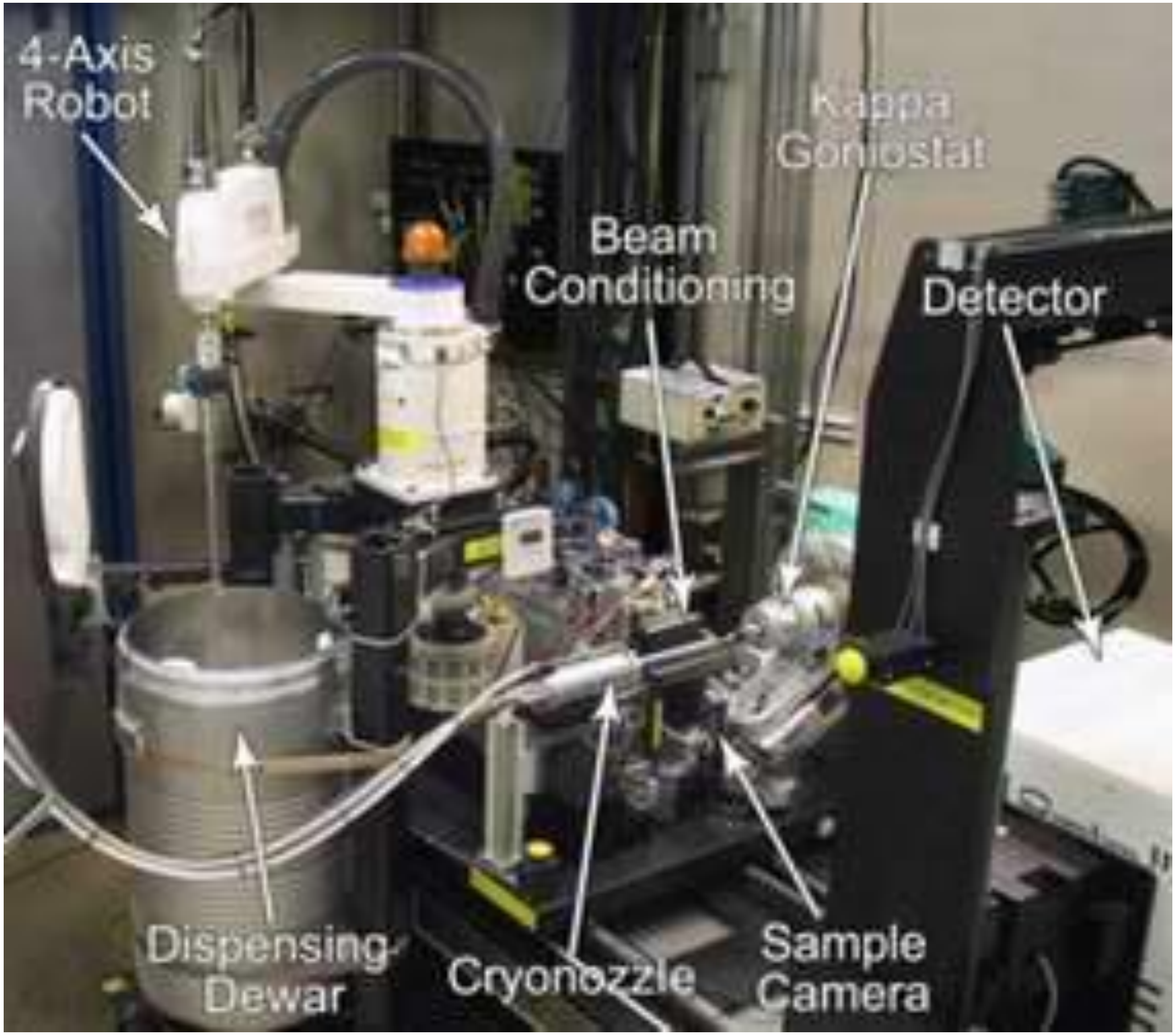


Sírius

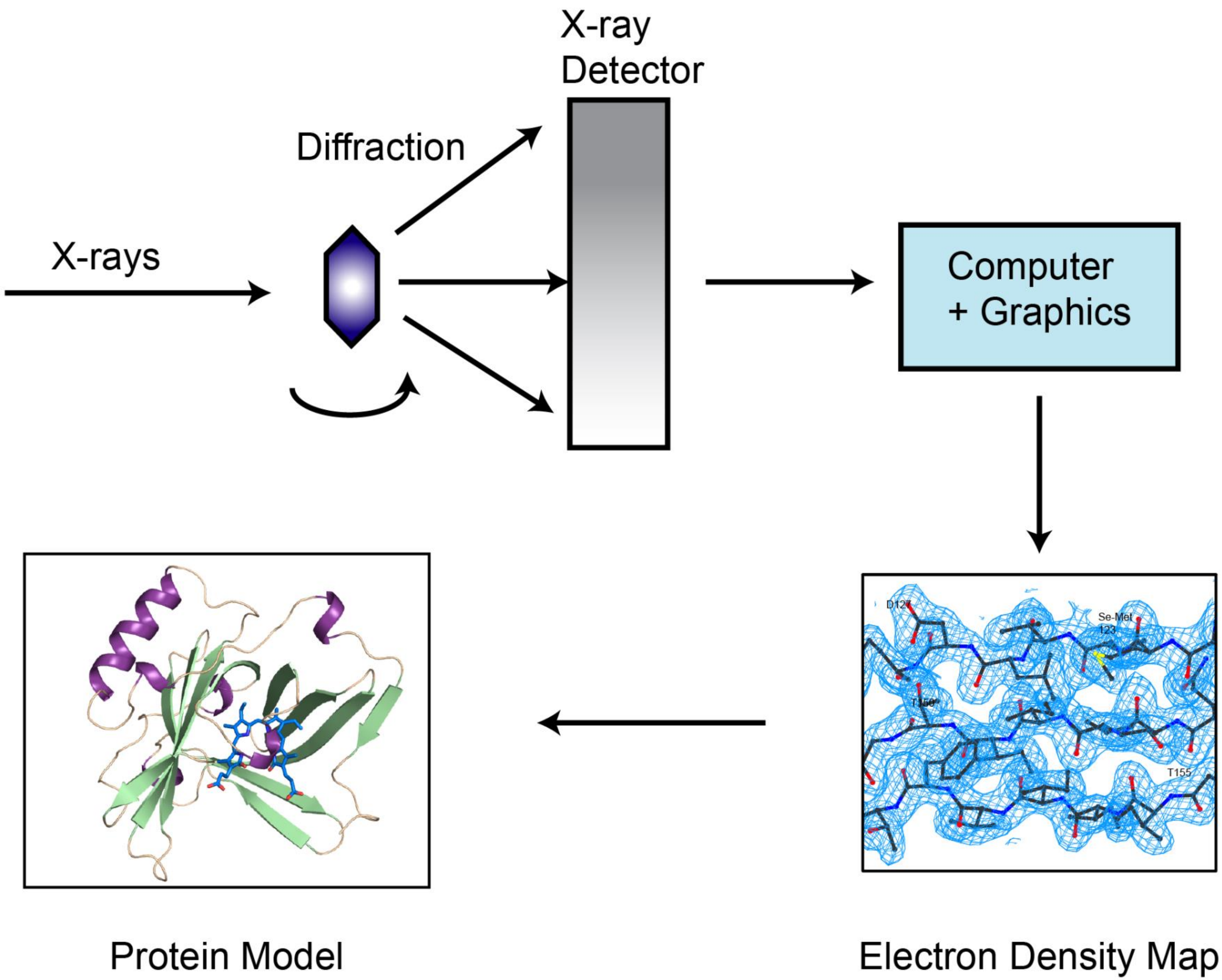
https://www.youtube.com/watch?v=lbxOSSUkgv0&list=RDCMUCYhTgGdeaBbbZ-h_LVJ_6bw&start_radio=1&rv=lbxOSSUkgv0&t=549

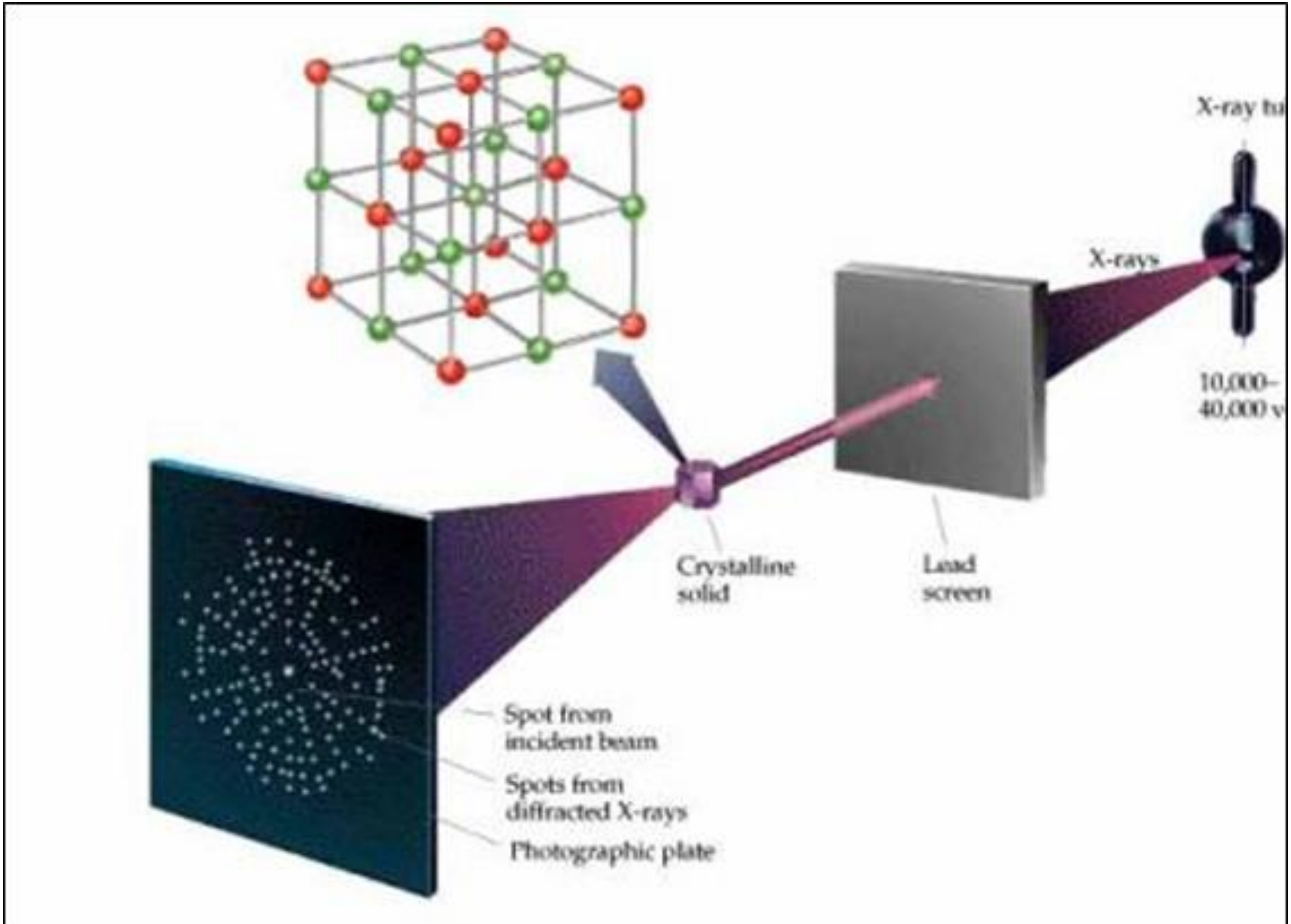
MX2

Instrumentação de uma estação de coleta

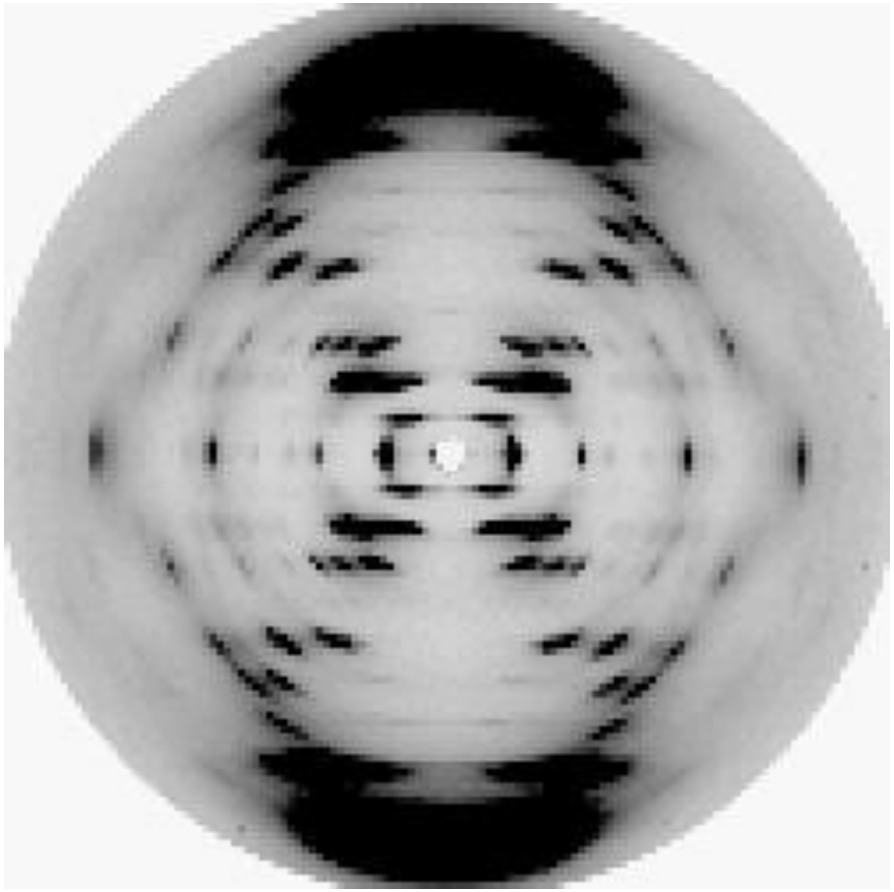


Overview of the X-ray Crystallographic Method

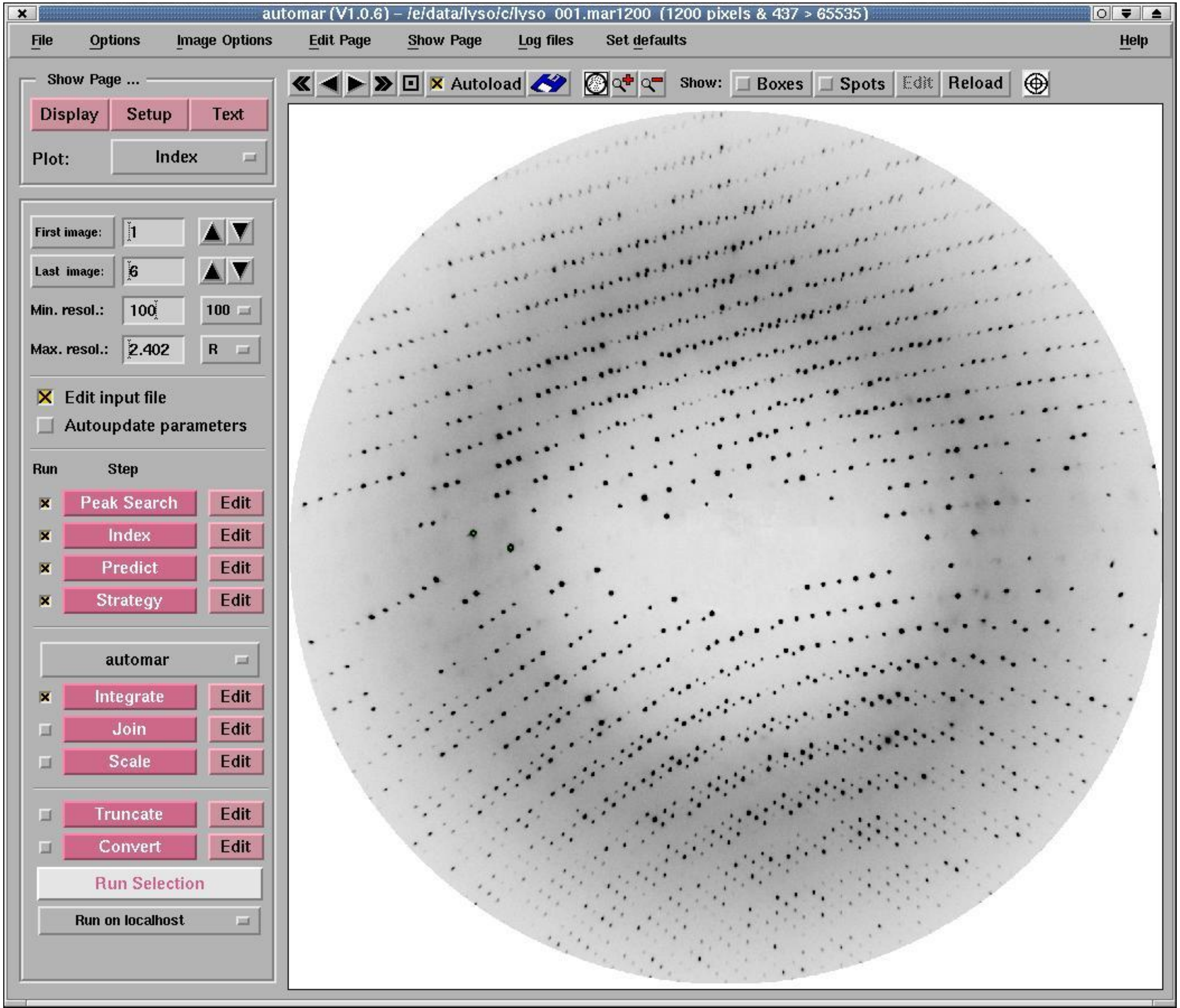



X-ray tu
X-rays
10,000–
40,000 v
Crystalline
solid
Lead
screen
Spot from
incident beam
Spots from
diffracted X-rays
Photographic plate

Padrão de difração para o DNA



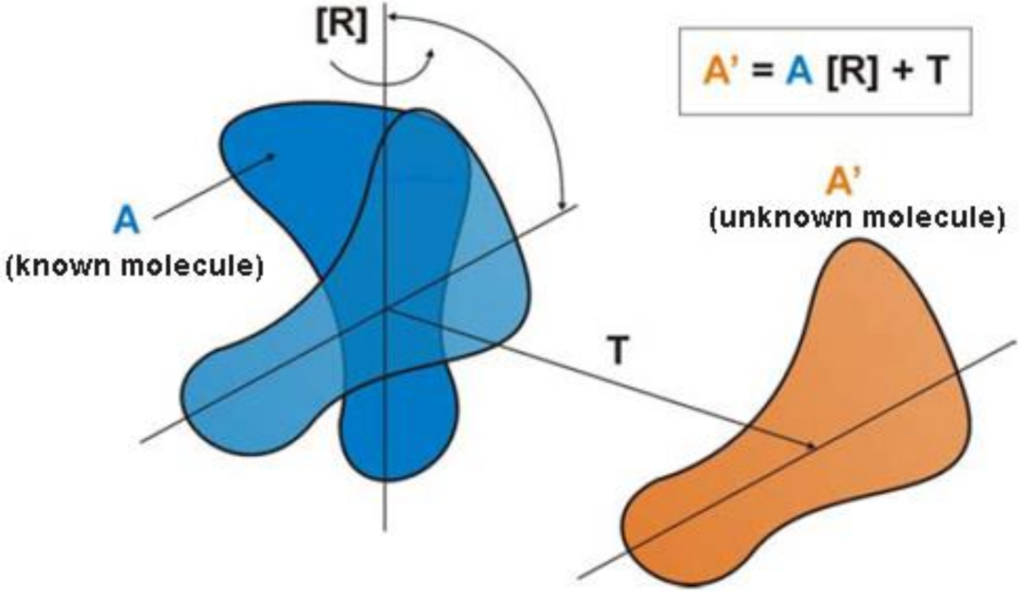
Padrão de difração de proteína



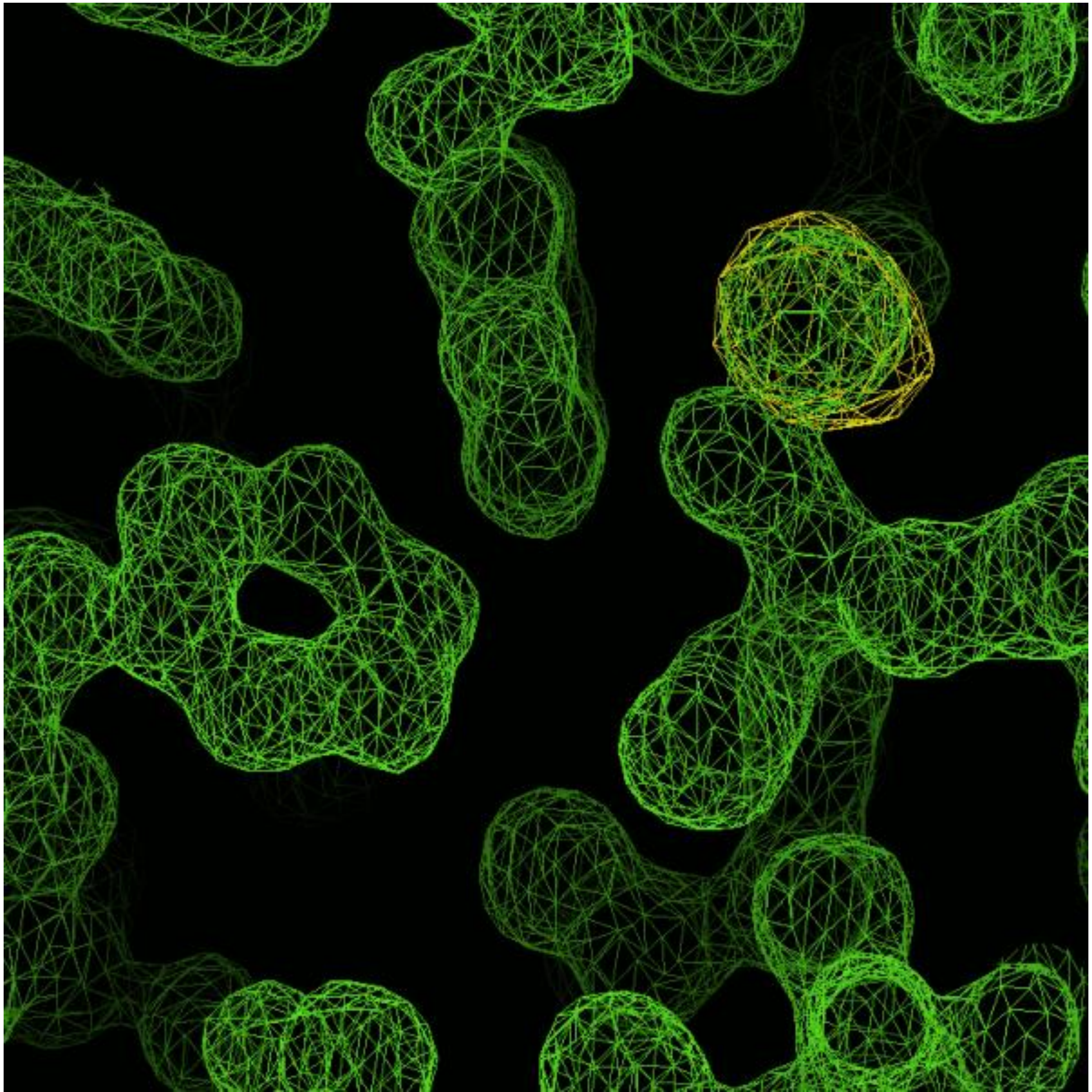
Métodos de determinação da fase

-**Substituição molecular**

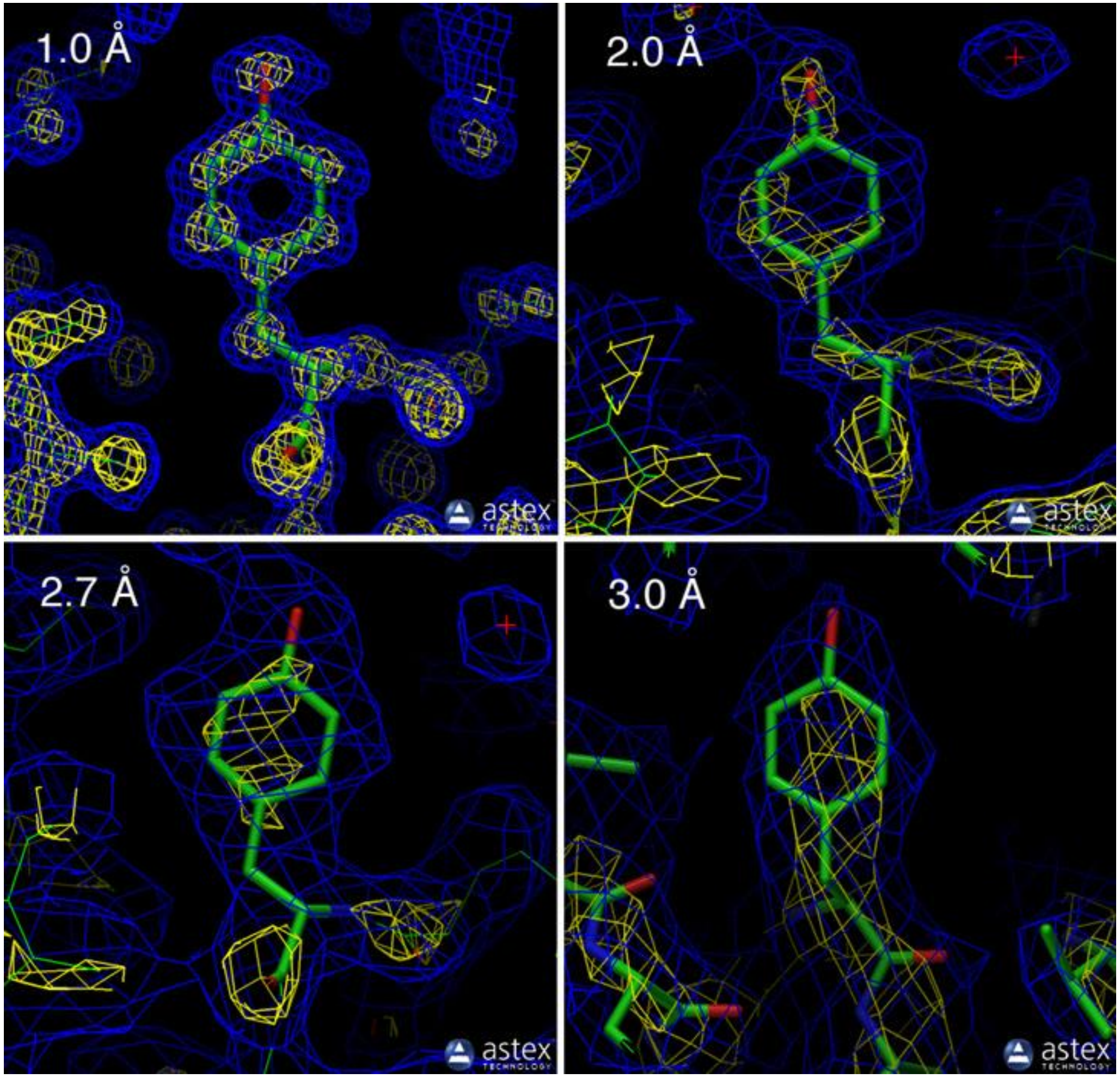
-**Métodos experimentais**



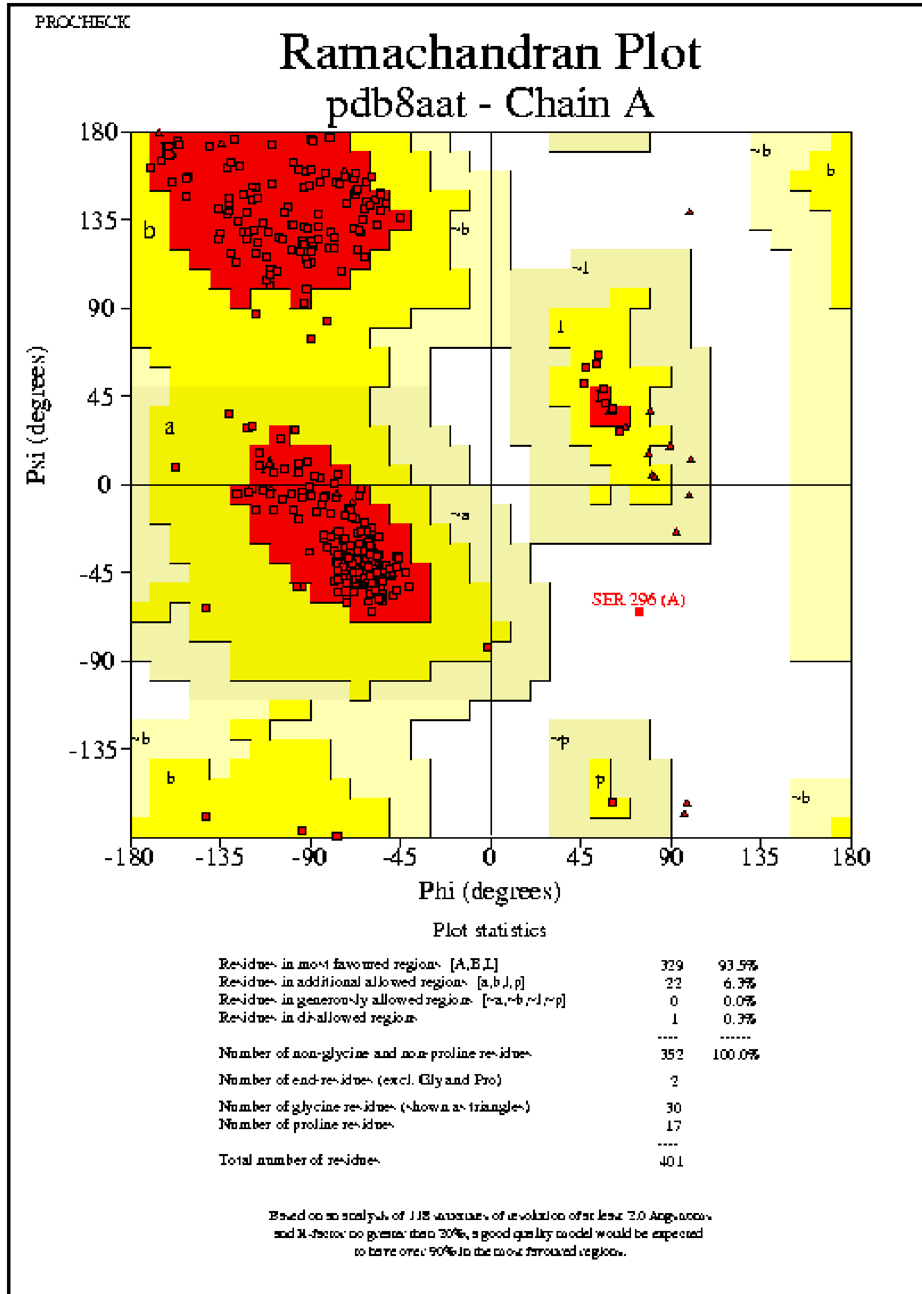
Mapa de densidade eletrônica



Resolução estrutural



Análise da qualidade da estrutura
Baseado em restrições conformacionais



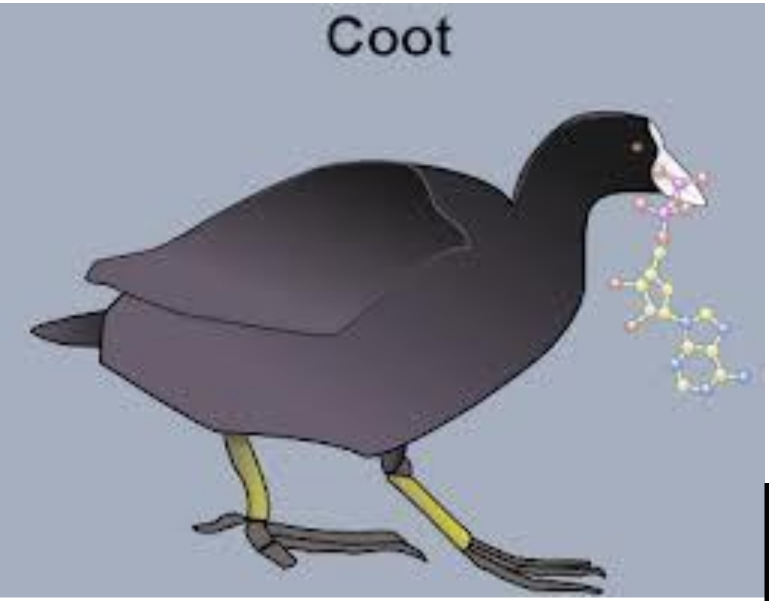
Fatores importantes para averiguar a qualidade de uma estrutura cristalográfica:

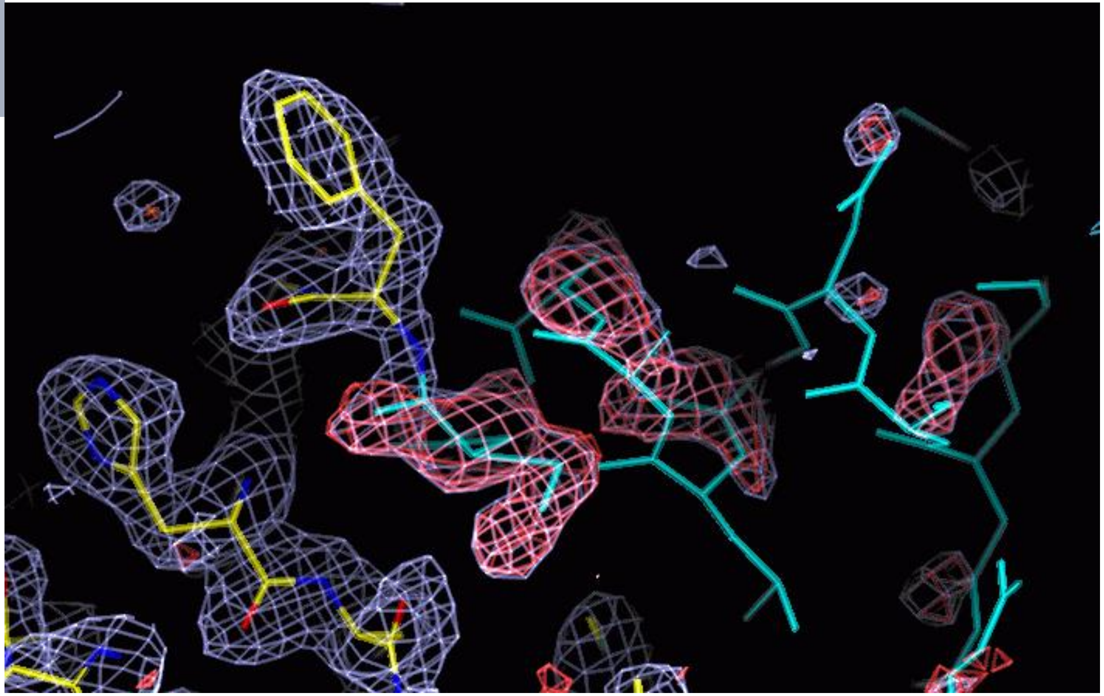
- Resolução

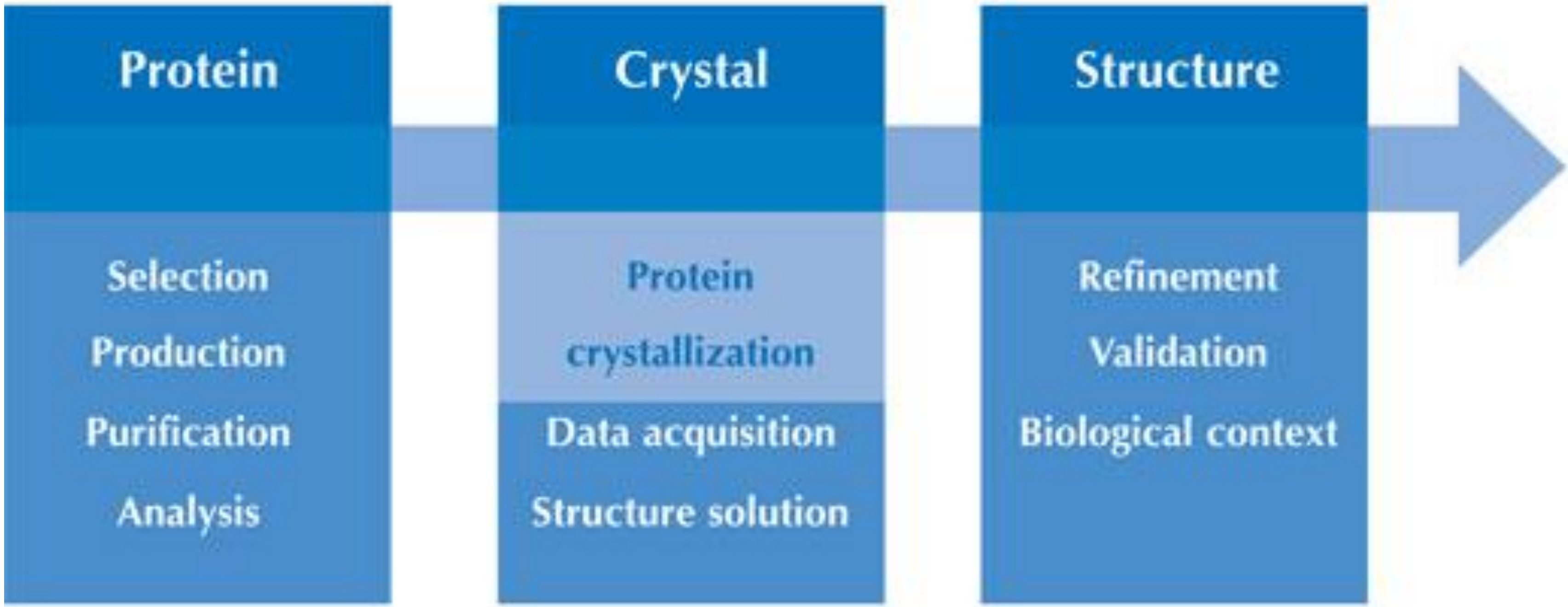
- Fator-R e R-free

- Qualidade estereoquímica

Refinamento cristalográfico






Protein
Crystal
Structure
Selection
Production
Purification
Analysis
Protein
crystallization
Data acquisition
Structure solution
Refinement
Validation
Biological context

Exemplos de aplicação da biologia estrutural:

- **Planejamento de fármacos baseado em estrutura**

- **Melhoramento em biocatálise**

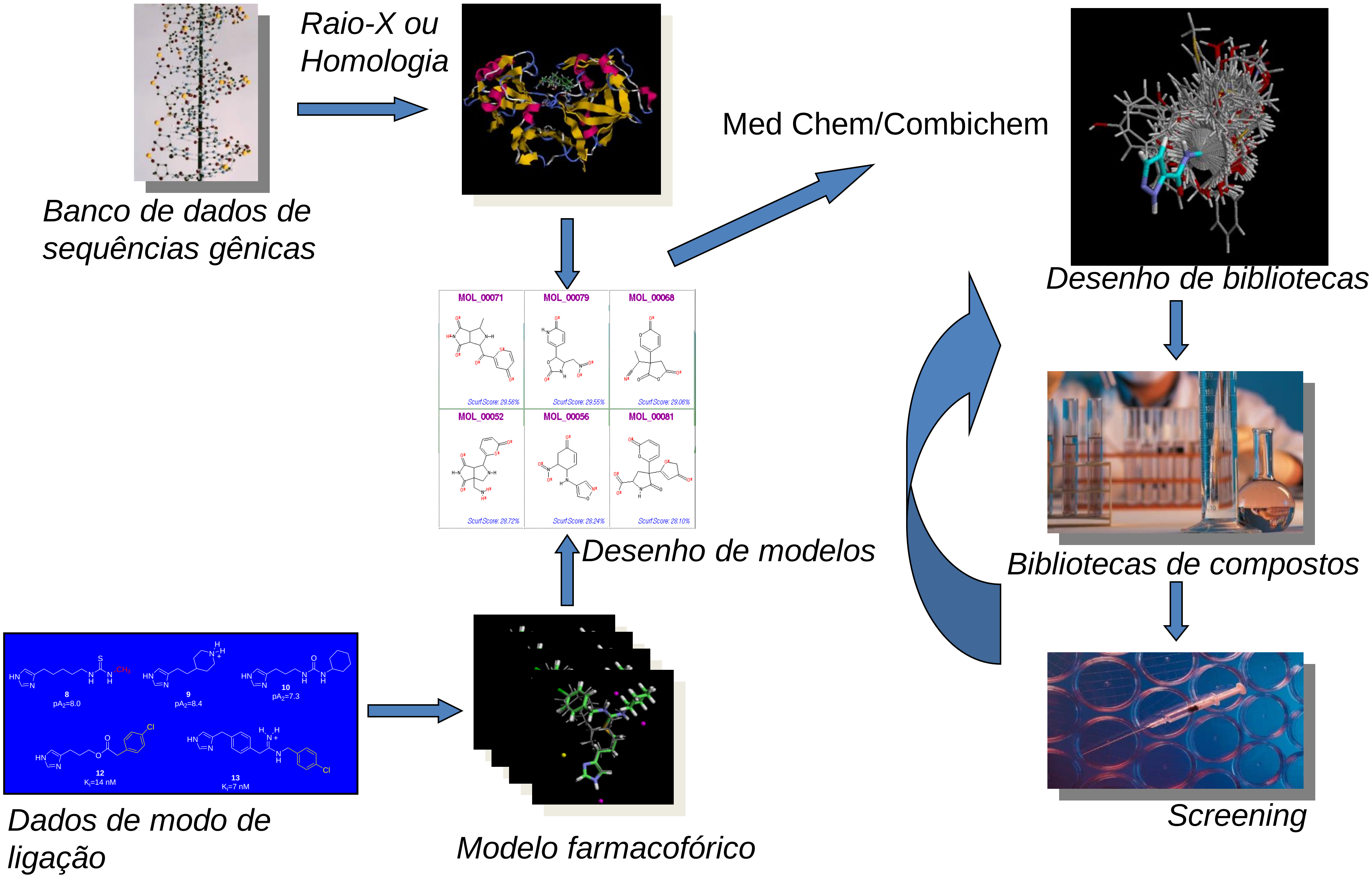
Planejamento de fármacos e Desenvolvimento

Como os fármacos são descobertos e desenvolvidas?

Trabalhando em conjunto

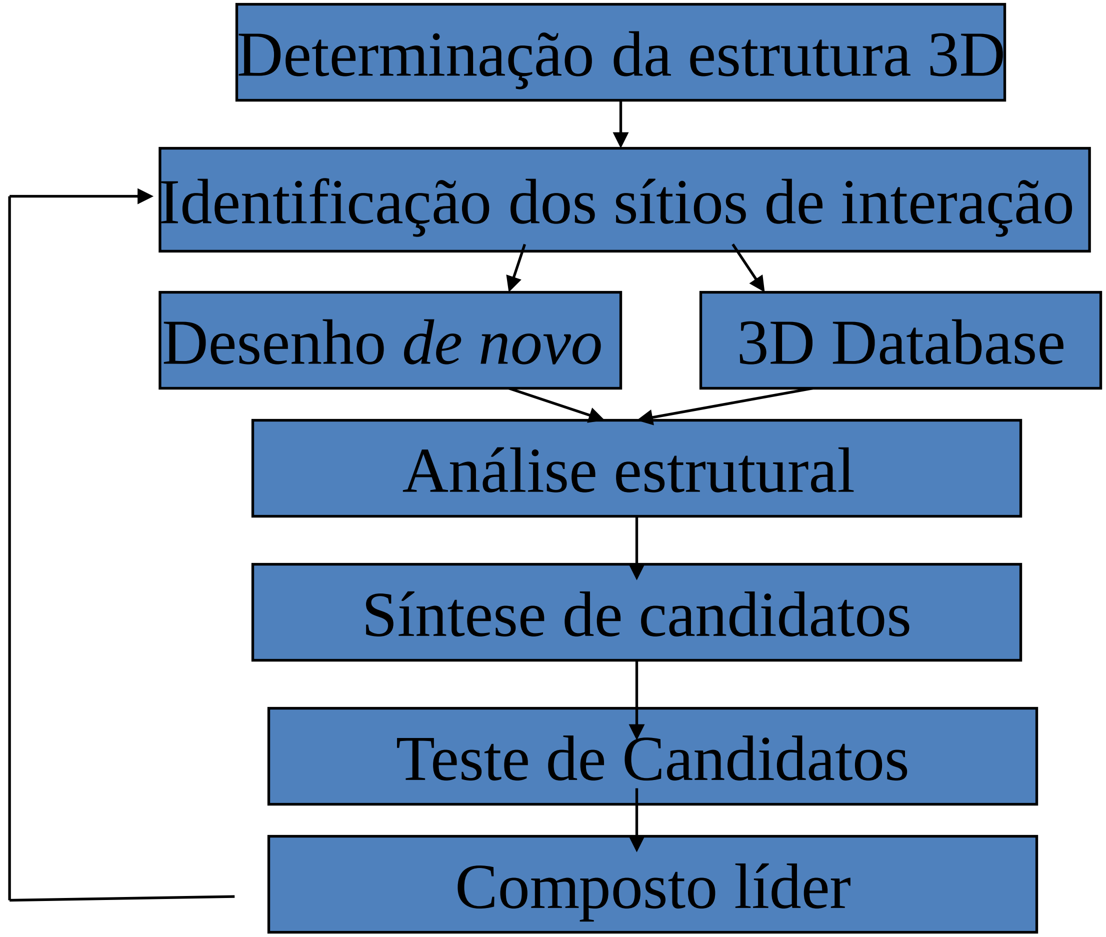
- **Biologia estrutural**
- **Bioquímica**
- **Química medicinal**
- **Toxicologia**
- **Farmacologia**
- **Biofísica química**
- **Tecnologia de informação**

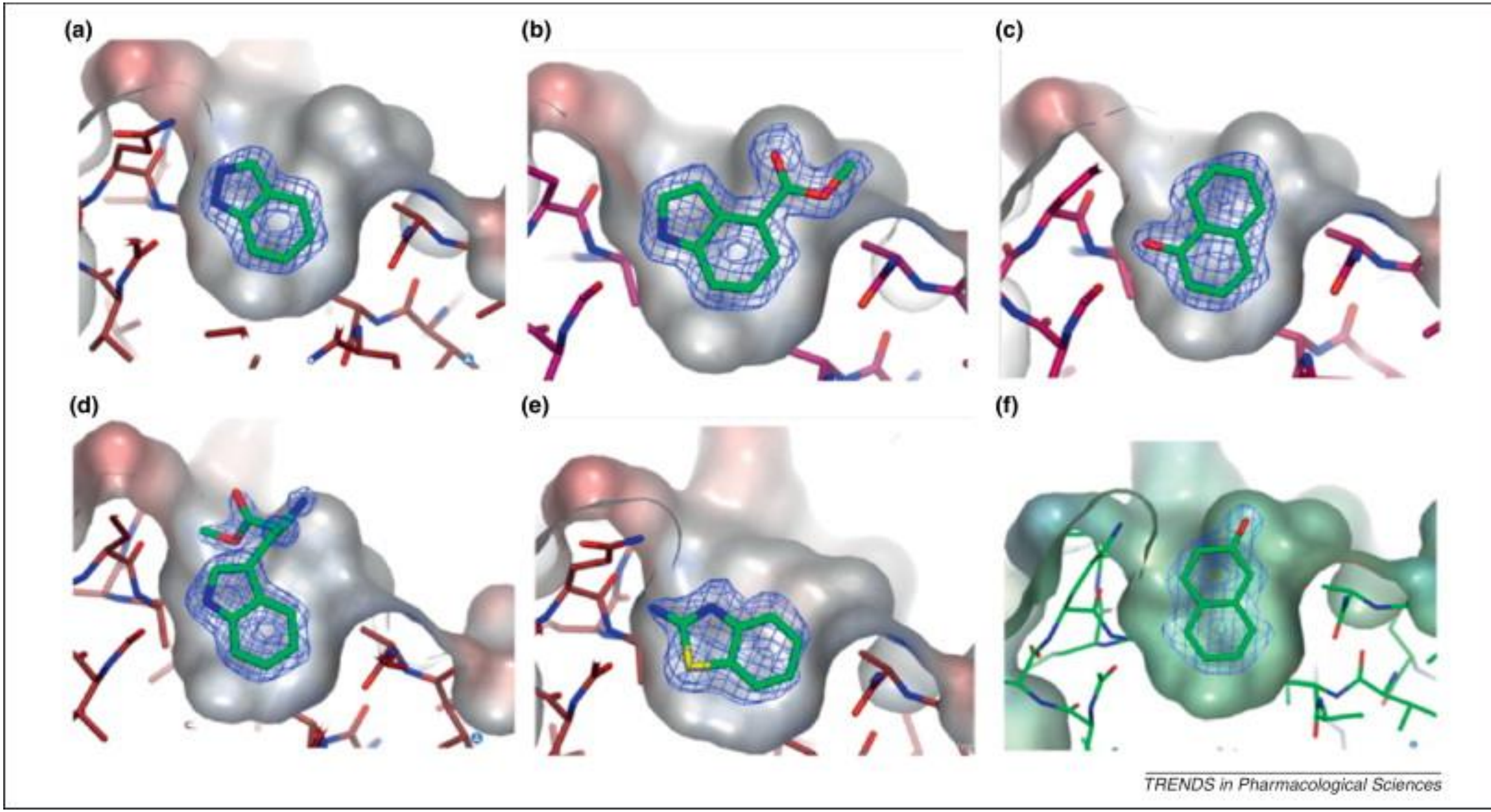
Processo de descoberta e planejamentode fármacos



Planejamento de fármacos baseado em estrutura

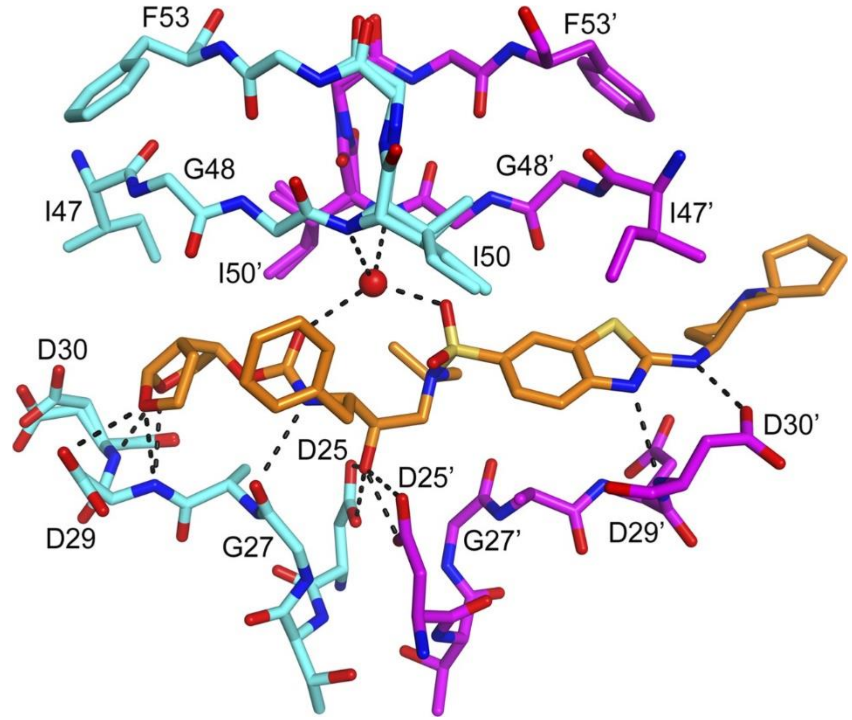
Descoberta ou desenho de moléculas que interagem com alvos bioquímicos de estrutura tridimensional conhecidas

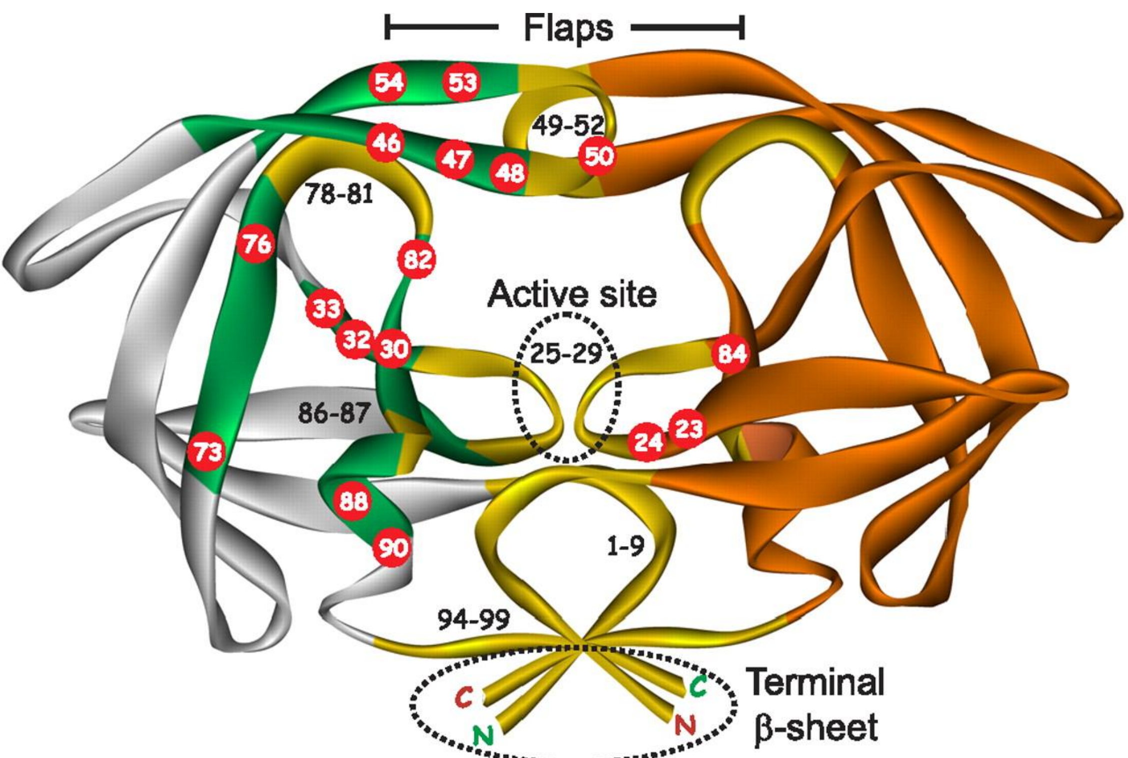




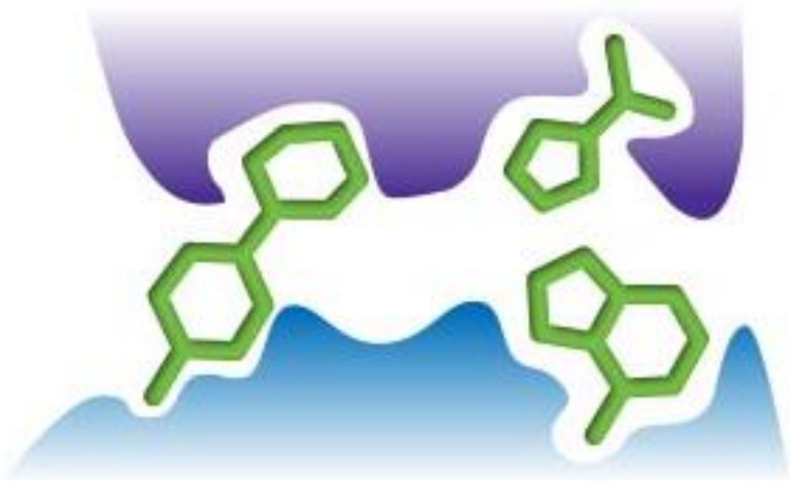
(a)
(b)
(c)
(d)
(e)
(f)
TRENDS in Pharmacological Sciences

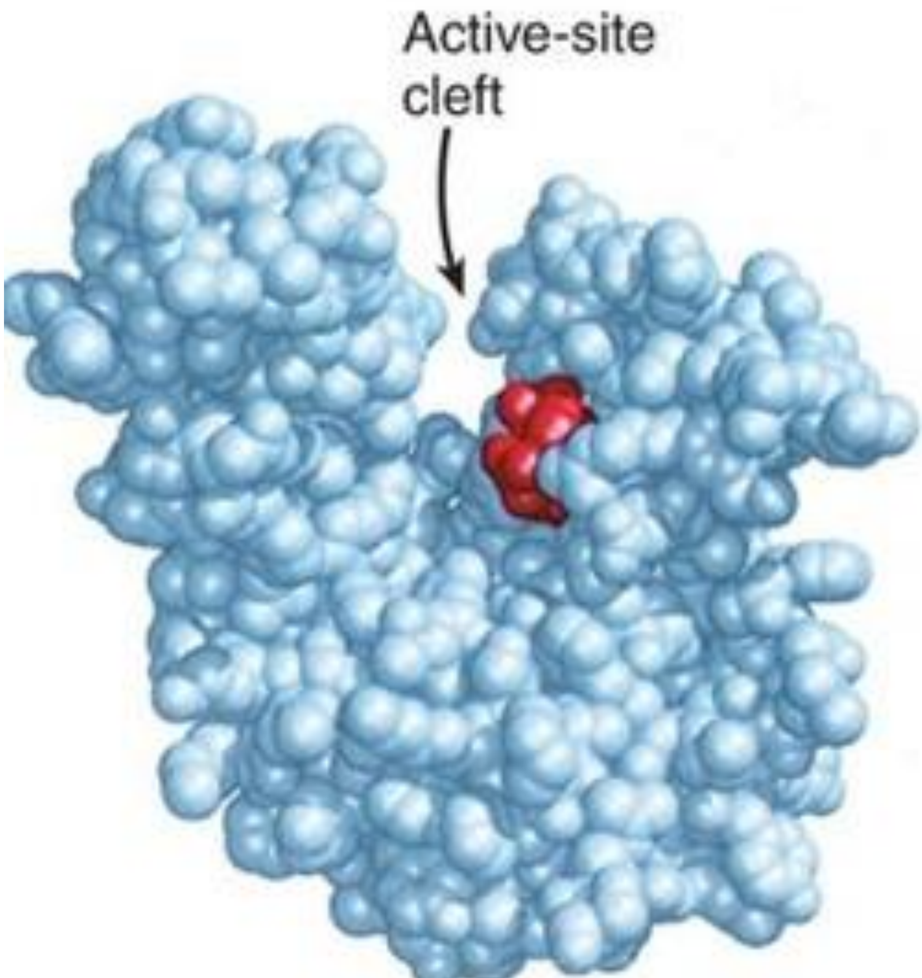
HIV protease – um exemplo clássico do processo de desenho de fármacos baseado em estrutura

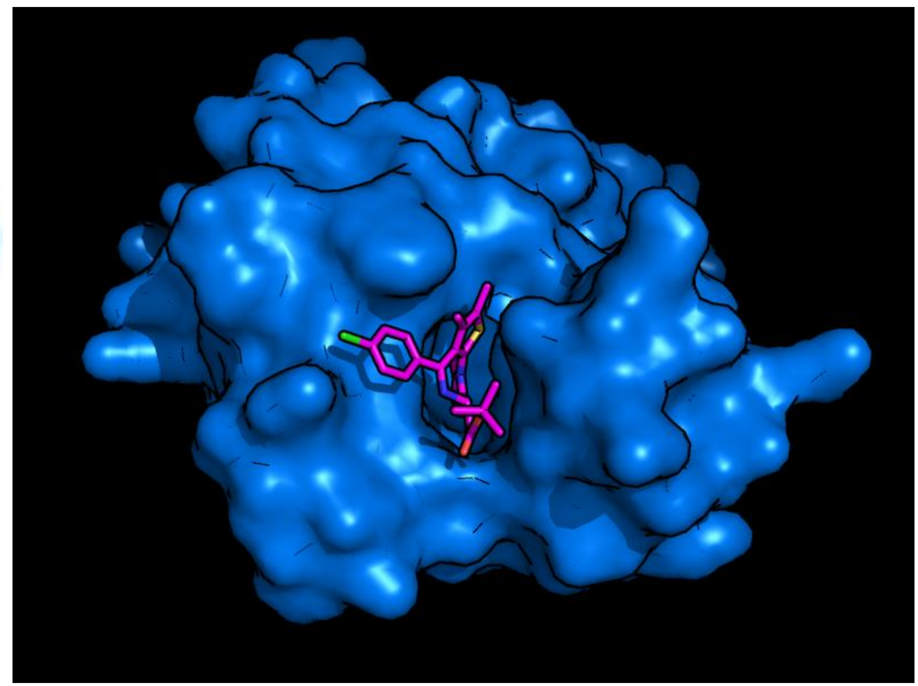


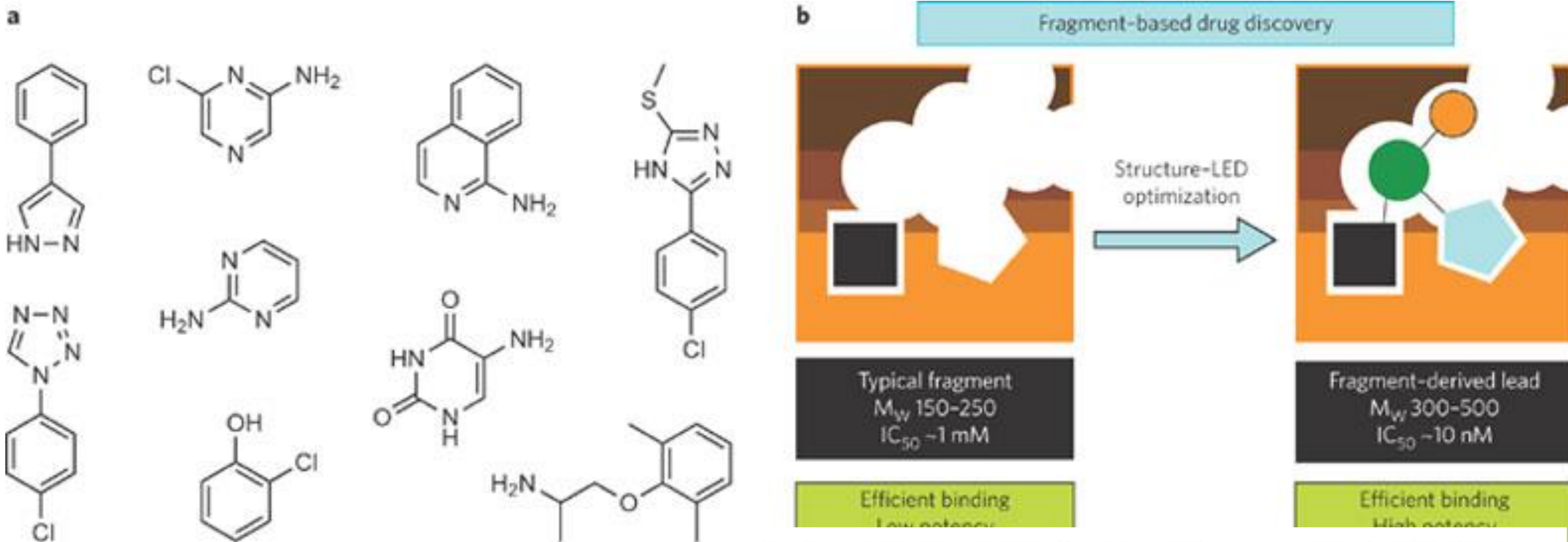


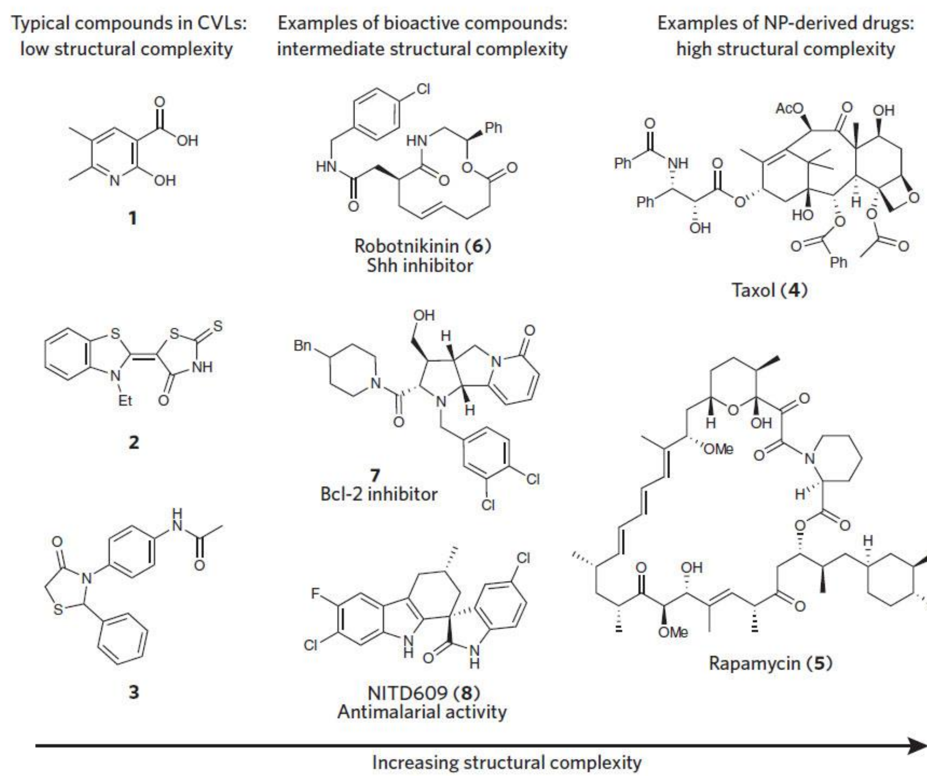
Um exemplo moderno da aplicação da estrutura de proteínas – planejamento de fármacos baseado em fragmentos



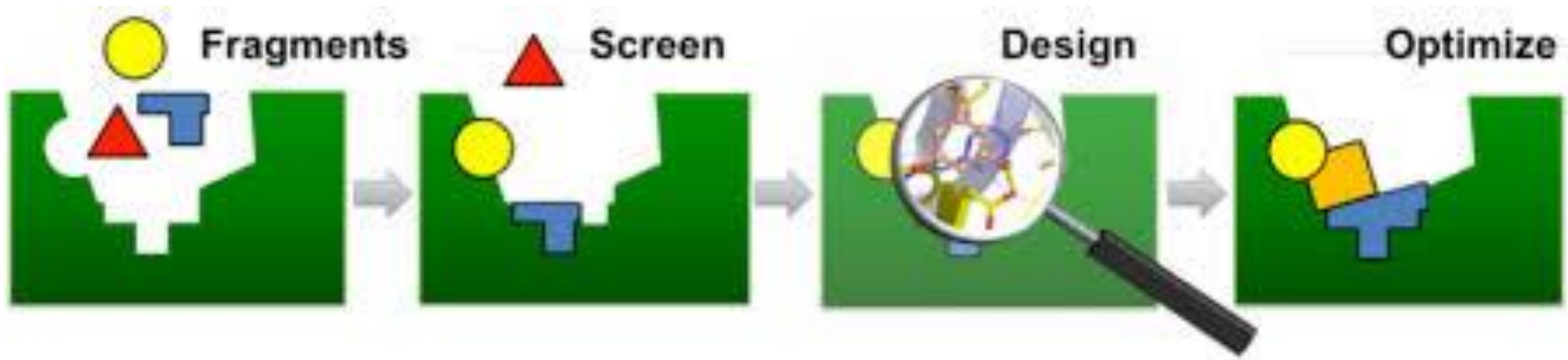

Active-site
cleft




a
b
Fragment-based drug discovery
Structure-LED optimization
Typical fragment
M_W 150–250
IC_{50} ~1 mM
Fragment-derived lead
M_W 300–500
IC_{50} ~10 nM
Efficient binding
Efficient binding


Typical compounds in CVLs: low structural complexity
Examples of bioactive compounds: intermediate structural complexity
Examples of NP-derived drugs: high structural complexity
1
2
3
Robotnikinin (6)
Shh inhibitor
7
Bcl-2 inhibitor
NITD609 (8)
Antimalarial activity
Taxol (4)
Rapamycin (5)
Increasing structural complexity

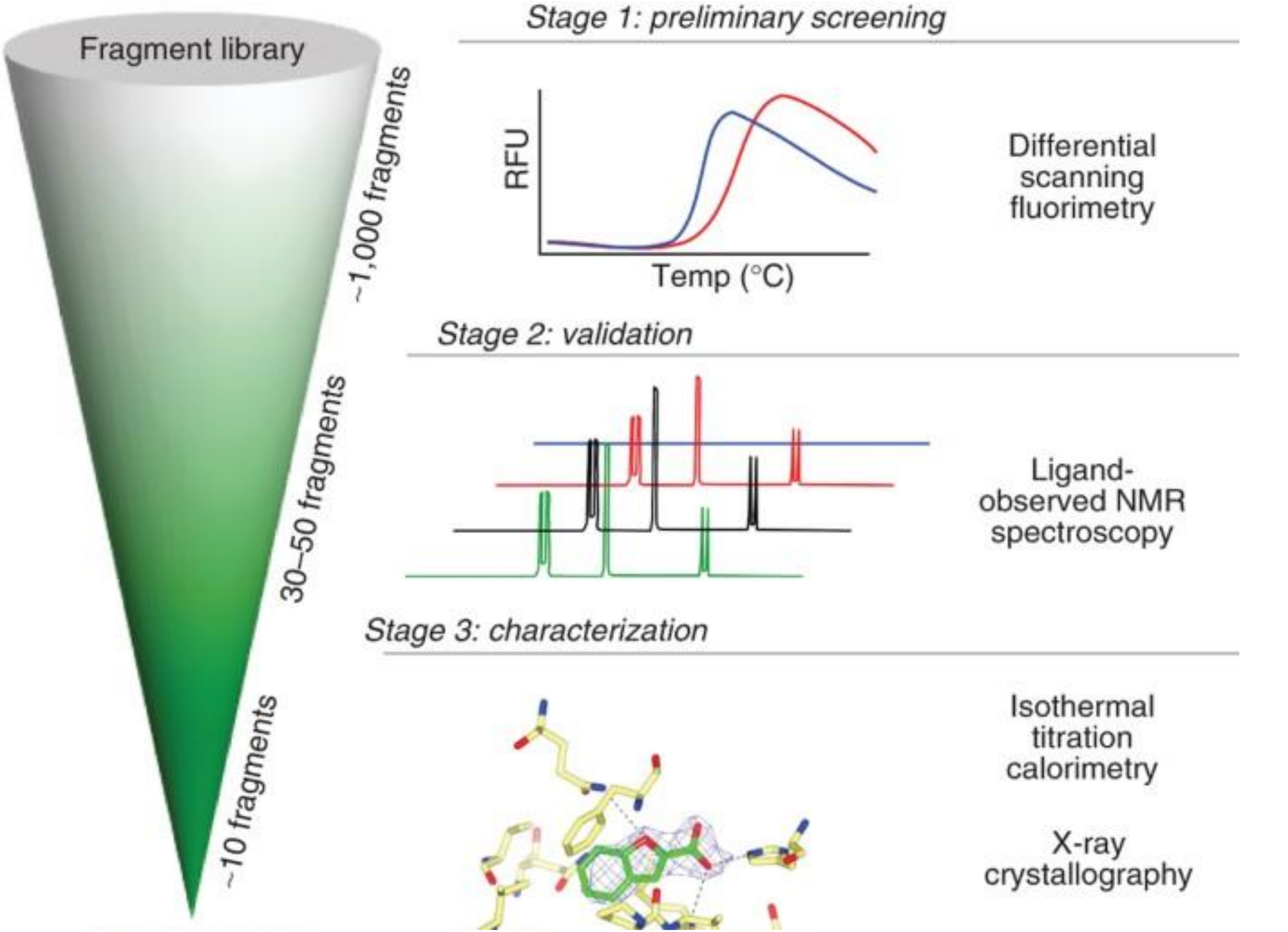
How we know which fragments are the best?



-1000 compounds (about 300 da)

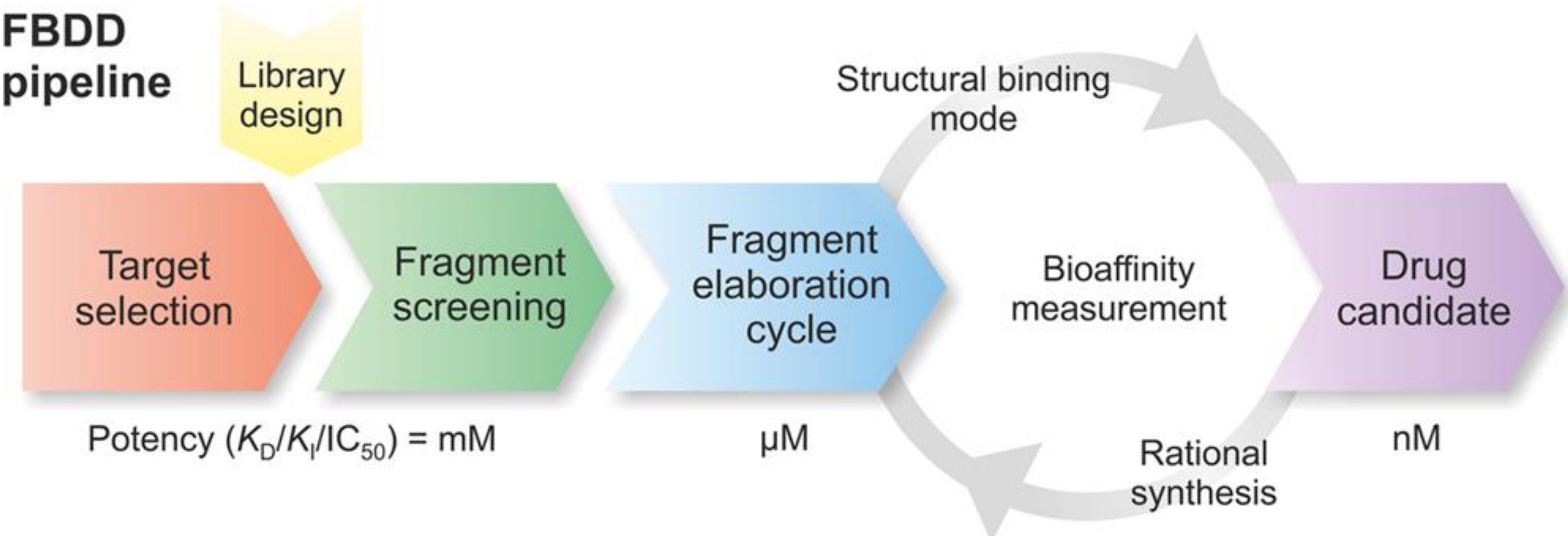
How to screen?

-biophysical techniques, as NMR, MS, ITC, crystallography, TS, BLI, SPR



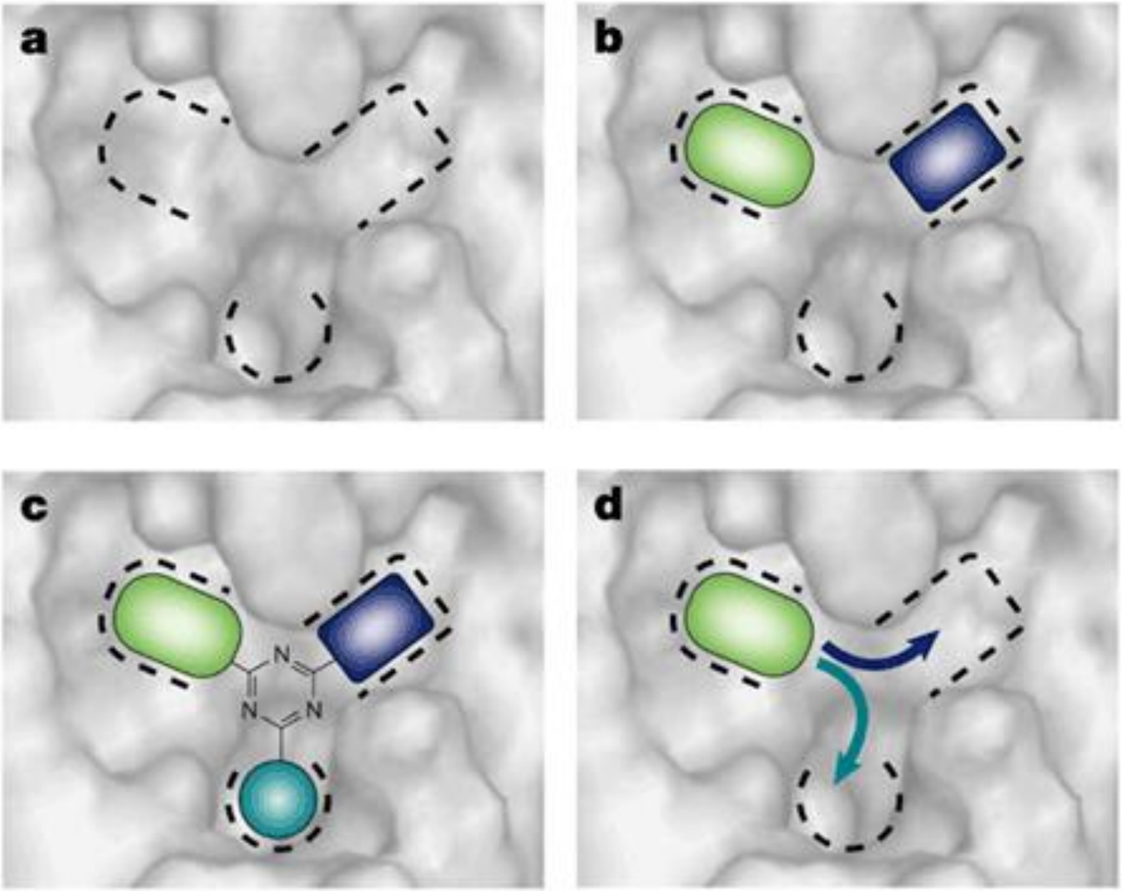
Fragment library
~1,000 fragments
30–50 fragments
~10 fragments
Stage 1: preliminary screening
RFU
Temp (°C)
Differential scanning fluorimetry
Stage 2: validation
Ligand-observed NMR spectroscopy
Stage 3: characterization
Isothermal titration calorimetry
X-ray crystallography

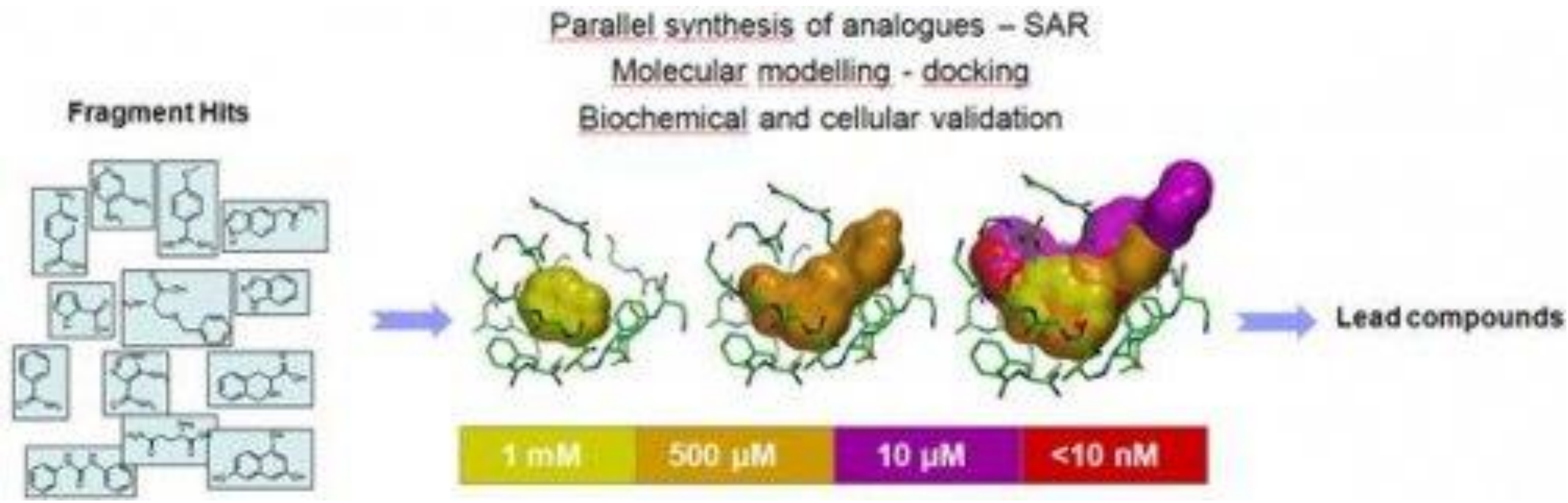
FBDD pipeline

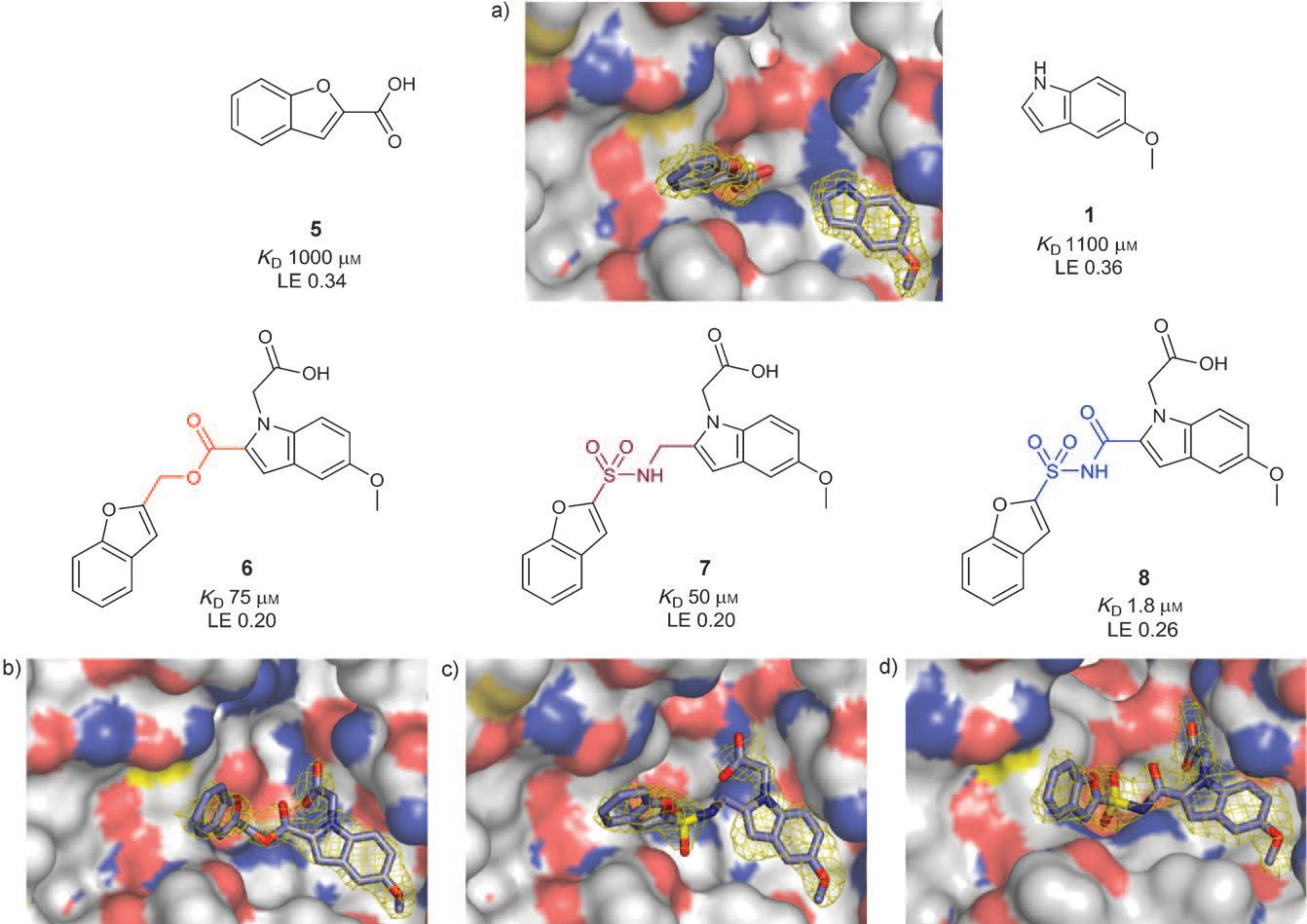


FBDD biophysical toolkit

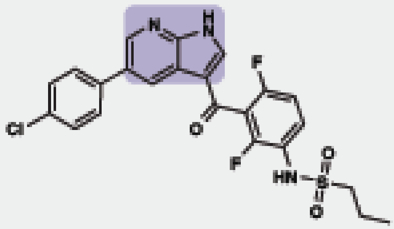
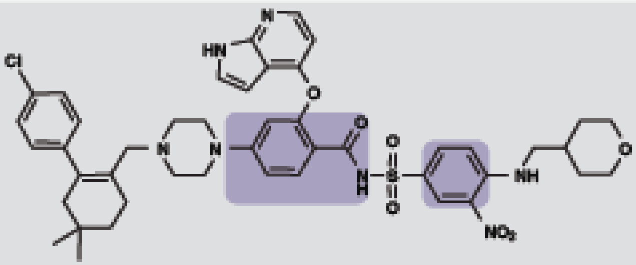
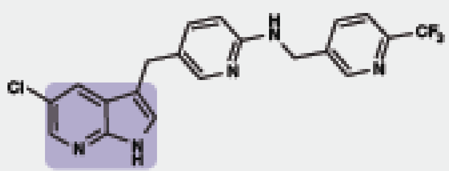
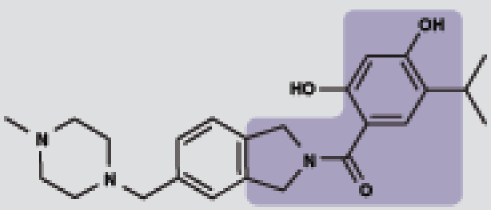
Strategies of FBDD




Parallel synthesis of analogues – SAR
Molecular modelling - docking
Biochemical and cellular validation
Fragment Hits
Lead compounds
1 mM
500 µM
10 µM
<10 nM



Huang et al. (2009). Angew. Chem. Int. Ed. 2009, 48, 8452 –8456

Structure*	Drug	Drug Target	Status
	Vemurafenib	BRAF-V600E	Approved
	Venetoclax	BCL-2	Approved
	Pexidartinib	FMS, KIT and FLT3–ITD	Phase III
	Onalespib	HSP90	Phase II

**shading indicates initial fragment*

https://www.technologynetworks.com/drug-discovery/articles/advances-in-fragment-based-drug-discovery-303059

nature reviews
drug discovery

Review Article | Published: 15 July 2016

Twenty years on: the impact of fragments on drug discovery

Daniel A. Erlanson, Stephen W. Fesik, Roderick E. Hubbard, Wolfgang Jahnke & Harren Jhoti

Nature Reviews Drug Discovery **15**, 605–619(2016) | Cite this article

nature chemistry

Perspective | Published: June 2009

The rise of fragment-based drug discovery

Christopher W. Murray & David C. Rees

Nature Chemistry **1**, 187–192(2009) | Cite this article

Biologia estrutural e biocatálise

Biocatálise é a aplicação de enzimas e microrganismos em química sintética, e usar esses biocatalisadores naturais para novas propostas: aplicação em que enzimas NÃO foram evoluídas para tal.

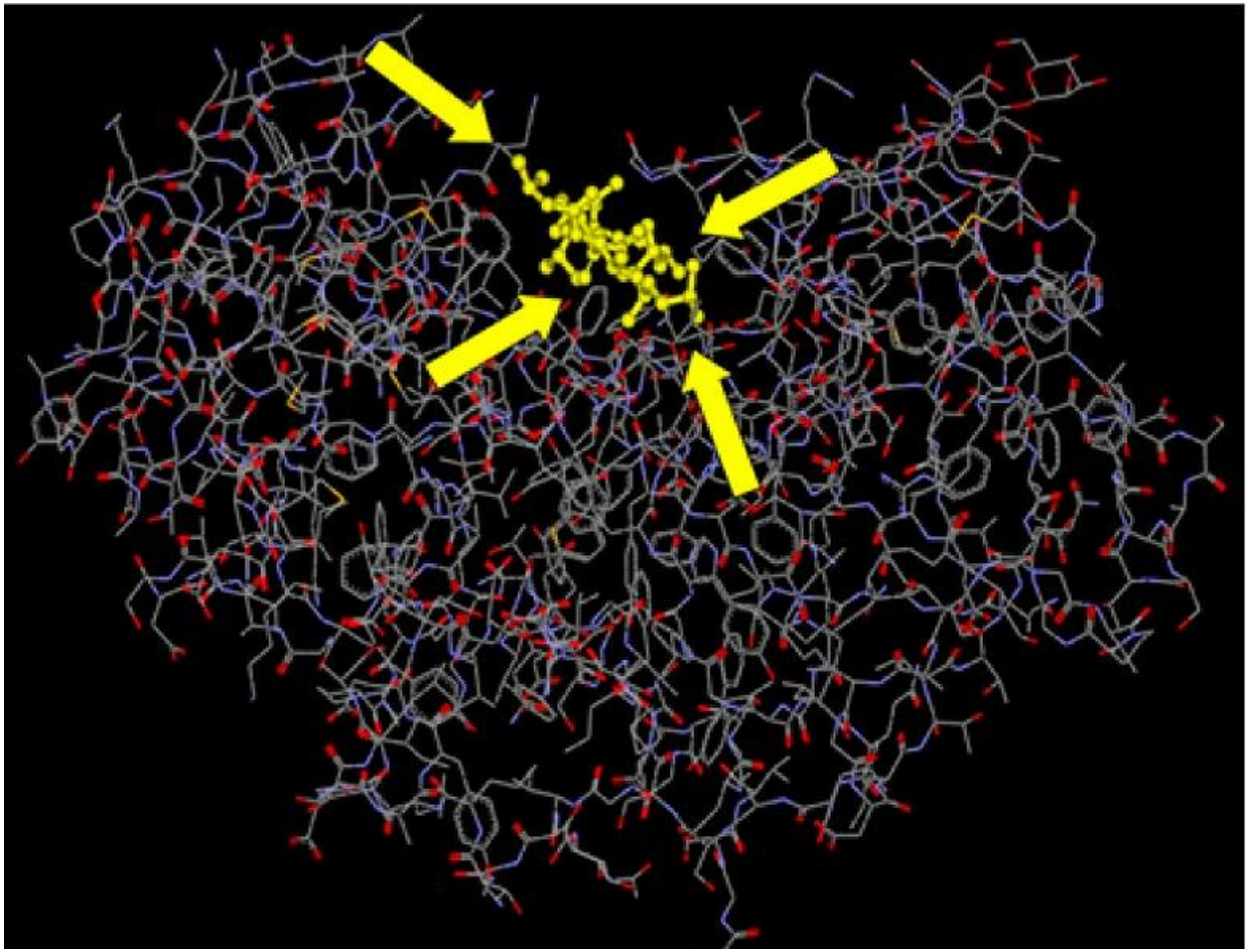
Francis Arnould – Pioneira em biocatálise
Prêmio Nobel em 2018

A biocatálise tem 2 objetivos principais:

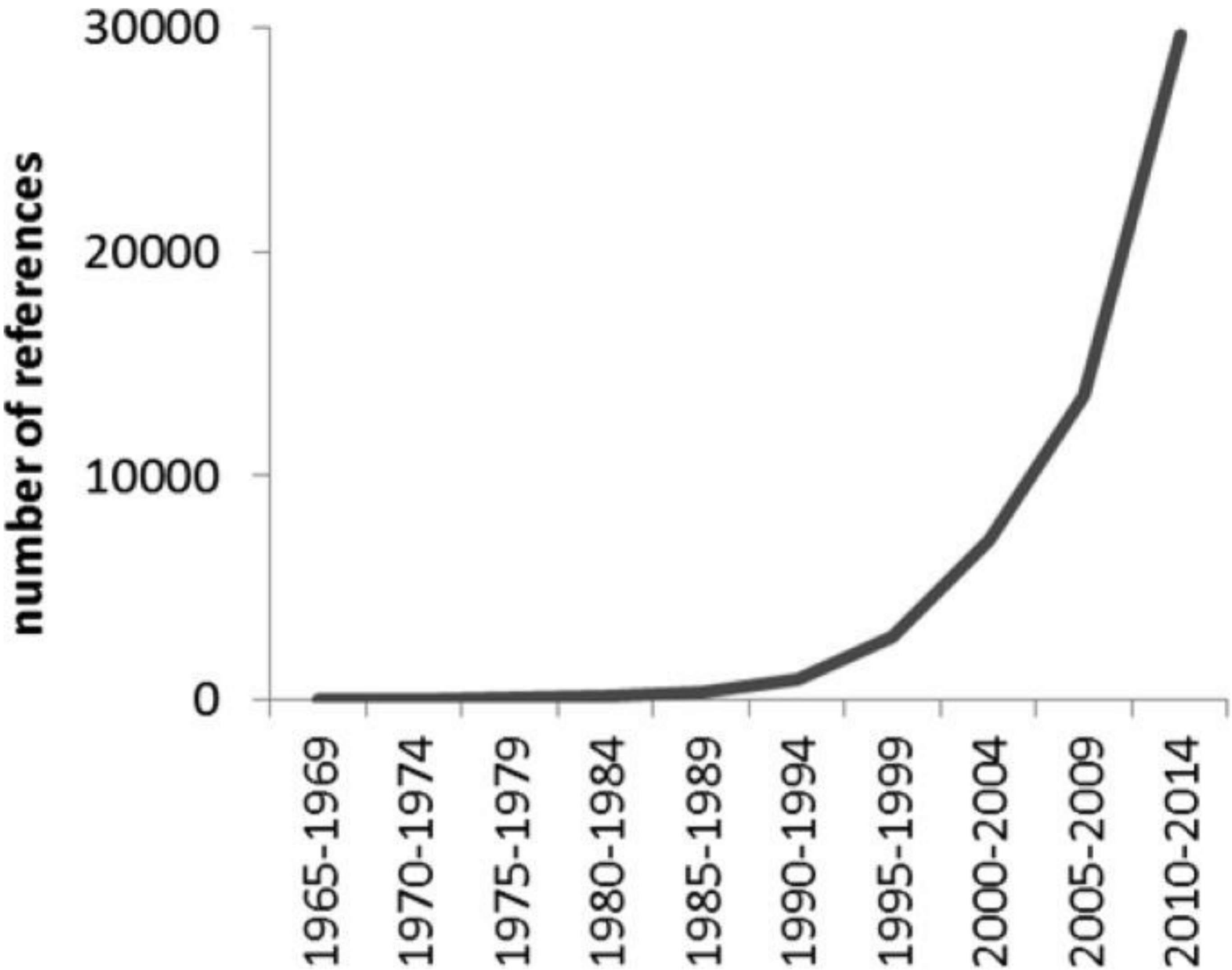
- Acelerar reações químicas ou transformações;

- Utilizar da "química verde" diminuindo a quantidade de compostos tóxicos despejados no ambiente

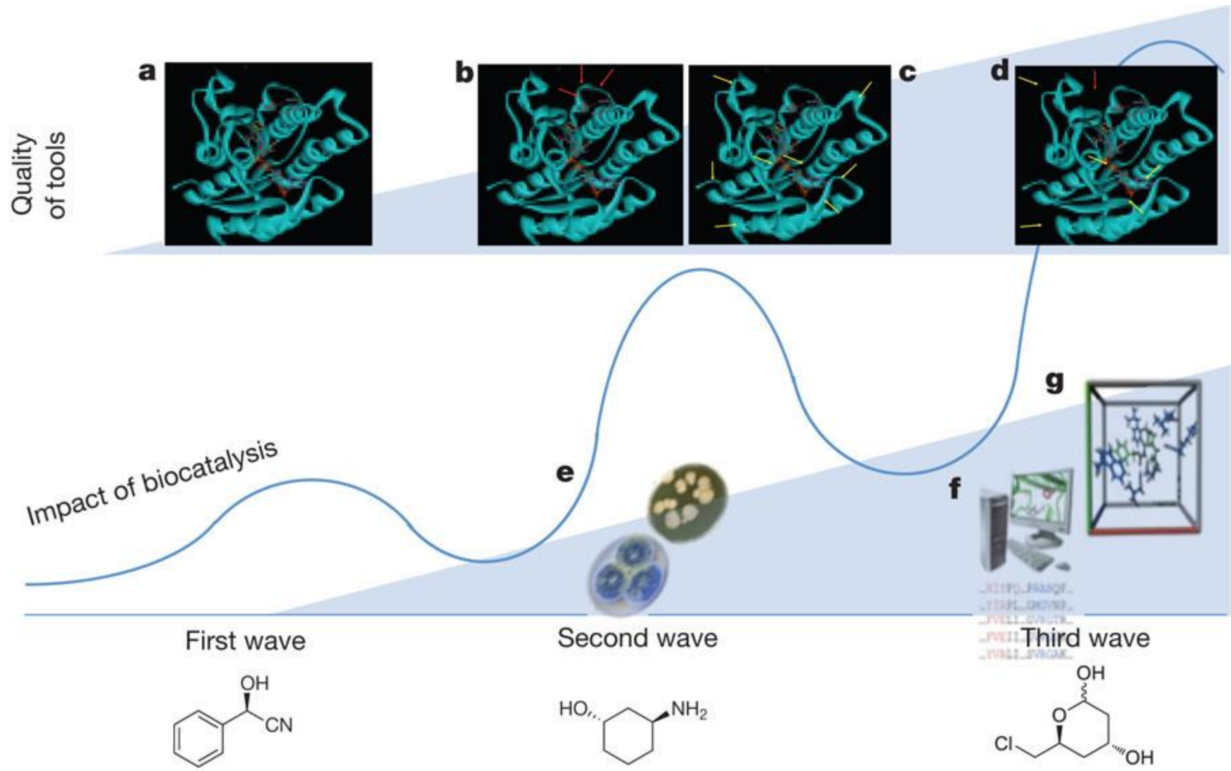
A biologia estrutural traz informação sobre os pontos de contato de substratos com a proteína



A biocatálise é um tópico de amplo interesse nos últimos anos pela indústria farmacêutica



The evolution of enzyme discovery and protein engineering strategies used to identify desired catalysts.



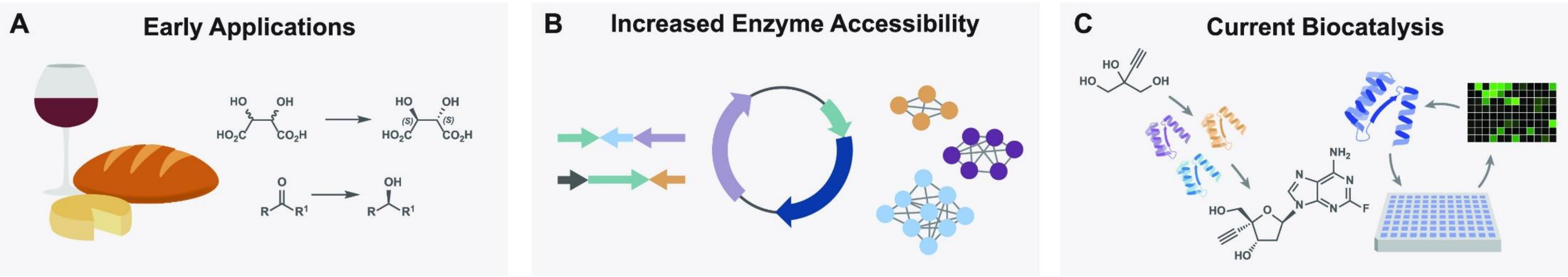
(*R*)-mandelonitrila

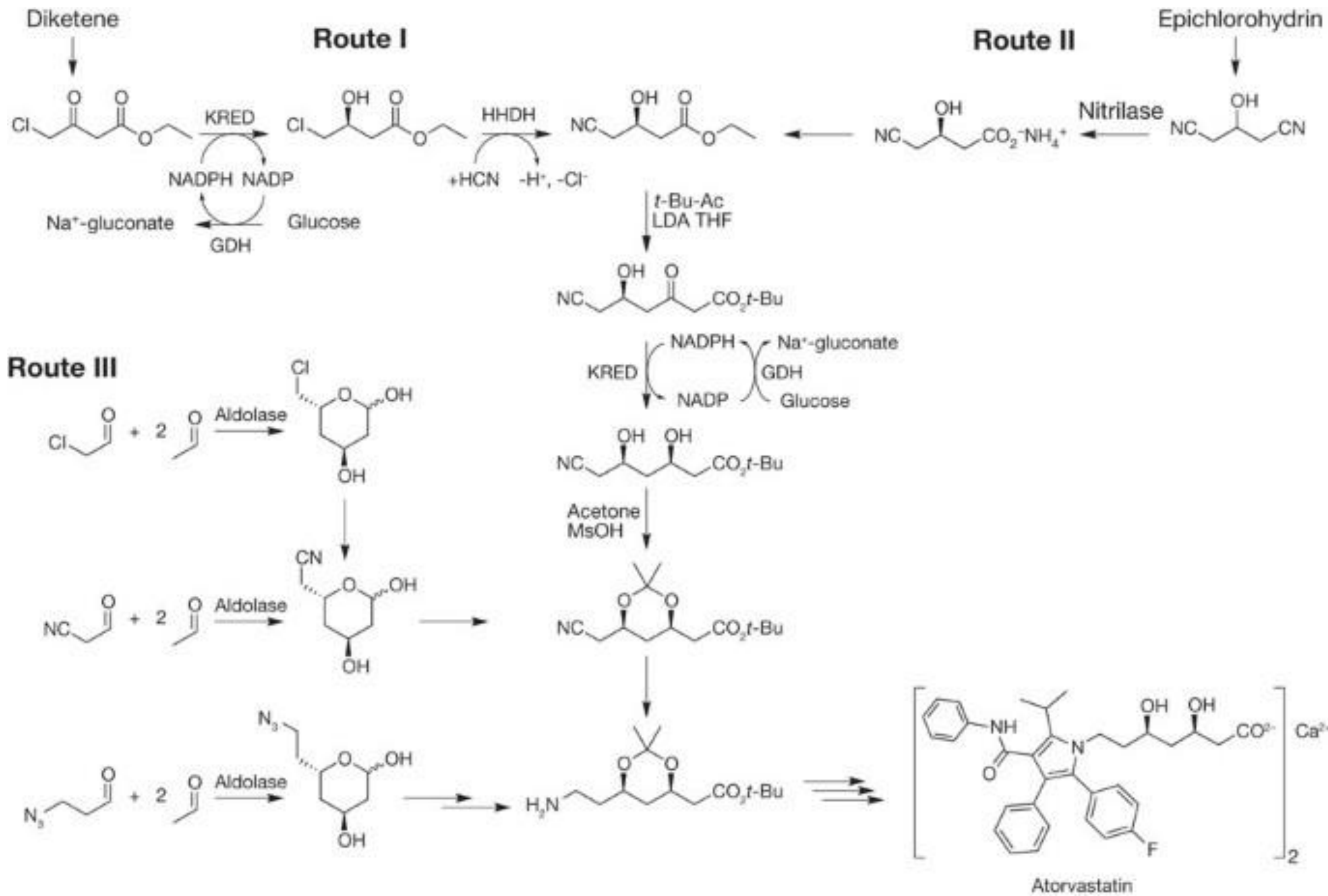
(1*S*,3*S*)-3-aminociclohexanol

6-cloro-2,4,6-trideoxi-D-eritrohexapiranosídeo

UT Bornscheuer *et al.* *Nature* **485**, 185-194 (2012) doi:10.1038/nature11117

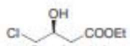
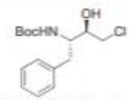
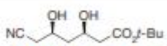
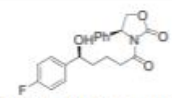
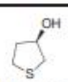
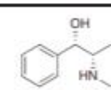
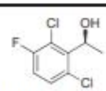
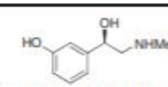
Evolução da aplicação da biocatálise




Diketene
Route I
Route II
Epichlorohydrin
KRED
NADPH NADP
Na+-gluconate
GDH
Glucose
HHDH
+HCN -H+, -Cl-
Nitrilase
t-Bu-Ac
LDA THF
KRED
NADPH
NADP
Na+-gluconate
GDH
Glucose
Route III
Aldolase
Acetone
MsOH
Aldolase
Aldolase
Atorvastatin

Alguns exemplos da aplicação de ceto-oxidoredutases na indústria

Table 1 | Recently developed biocatalytic processes in the pharmaceutical industry

Product	Technology (company, reference)	Product	Technology (company, reference)
	KREDs		
 Ethyl (*S*)-4-chloro-3-hydroxybutanoate (**1**)	Various protein engineering approaches, including directed evolution, to increase activity, stability and coenzyme specificity. Initial intermediate for atorvastatin (Lipitor) manufacture on the industrial scale. (Kaneka[81], Codexis[52])	 *Tert*-butyl (2*S*,3*R*)-4-chloro-3-hydroxy-1-phenylbutan-2-yl-carbamate (**10**)	Recombinant expression and also protein engineering for activity. Intermediate for atazanavir (Reyetaz). (Bristol Myers Squibb[82]; Codexis, WO/2011/005527)
 *Tert*-butyl 6-cyano-3,5-dihydroxyhexanoate (**2**)	Protein engineering for activity and stability to 30% organic substrate (the hydroxyketone substrate and a *tert*-butyl acetoacetate impurity, both liquids). Intermediate for atorvastatin. (Codexis, US/2011/7879585; Pfizer, US/2008/0118962)	 (4*S*)-3[(5*S*)-5-(4-fluorophenyl)-5-hydroxy-pentanoyl]-4-phenyl-1,3-oxazolidin-2-one (**11**)	Protein engineering for activity and solvent stability. Intermediate for ezetimibe (Zetia, Vytorin). (Codexis, WO/2010/025085)
 (*R*)-3-hydroxythiolane (R3HT) (**3**)	Directed evolution primarily for enantioselectivity. Intermediate for sulopenem-type antibiotics (since discontinued). (Codexis[83], WO/2009/029554)	 D-pseudoephedrine (**12**)	Protein engineering to overcome product inhibition. The ketone substrate is racemic at C_2 and racemizes during the reaction to give high-e.e. product. The product of the reaction is a generic API. (Daiichi[84])
 (*S*)-1-(2,6-dichloro-3-fluorophenyl)-ethanol (DCFPE) (**4**)	Directed evolution of a variant identified from the R3HT (**3**) project for increased activity. Note that the ketone is hindered owing to the ortho chloro-substituents. Raw material for crizotinib (Xalkori). (Codexis, WO/2009/036404)	 (*R*)-phenylephrine (**13**)	Protein engineering for activity and tolerance to aminoketone substrate. The product of the reaction is a generic API. (Codexis, WO/2011/022548)

REVIEW

doi:10.1038/nature11117

Engineering the third wave of biocatalysis

U. T. Bornscheuer[1], G. W. Huisman[2], R. J. Kazlauskas[3,4], S. Lutz[5], J. C. Moore[6] & K. Robins[7]

Over the past ten years, scientific and technological advances have established biocatalysis as a practical and environmentally friendly alternative to traditional metallo- and organocatalysis in chemical synthesis, both in the laboratory and on an industrial scale. Key advances in DNA sequencing and gene synthesis are at the base of tremendous progress in tailoring biocatalysts by protein engineering and design, and the ability to reorganize enzymes into new biosynthetic pathways. To highlight these achievements, here we discuss applications of protein-engineered biocatalysts ranging from commodity chemicals to advanced pharmaceutical intermediates that use enzyme catalysis as a key step.

1980s and 1990s, initial
ıre based, extended the
is of unusual synthetic
; to the manufacture of
. Examples include the
r synthesis of diltiazem

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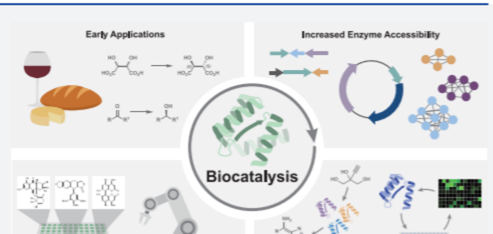
Outlook

State-of-the-Art Biocatalysis

Joshua B. Pyser, Suman Chakrabarty, Evan O. Romero, and Alison R. H. Narayan*

Cite This: *ACS Cent. Sci.* 2021, 7, 1105–1116

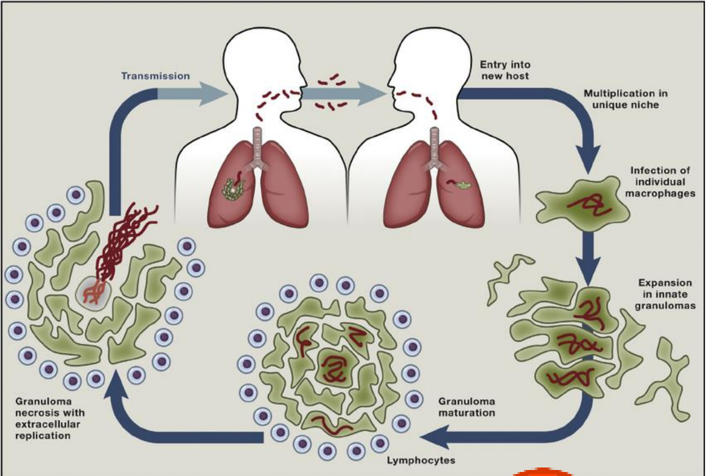
ABSTRACT: The use of enzyme-mediated reactions has transcended ancient food production to the laboratory synthesis of complex molecules. This evolution has been accelerated by developments in sequencing and DNA synthesis technology, bioinformatic and protein engineering tools, and the increasingly interdisciplinary nature of scientific research. Biocatalysis has become an indispensable tool applied in academic and industrial spheres, enabling synthetic strategies that leverage the exquisite selectivity of enzymes to access target molecules. In this Outlook, we outline the technological advances that have led to the field's



Consideração final sobre o uso da biologia estrutural:

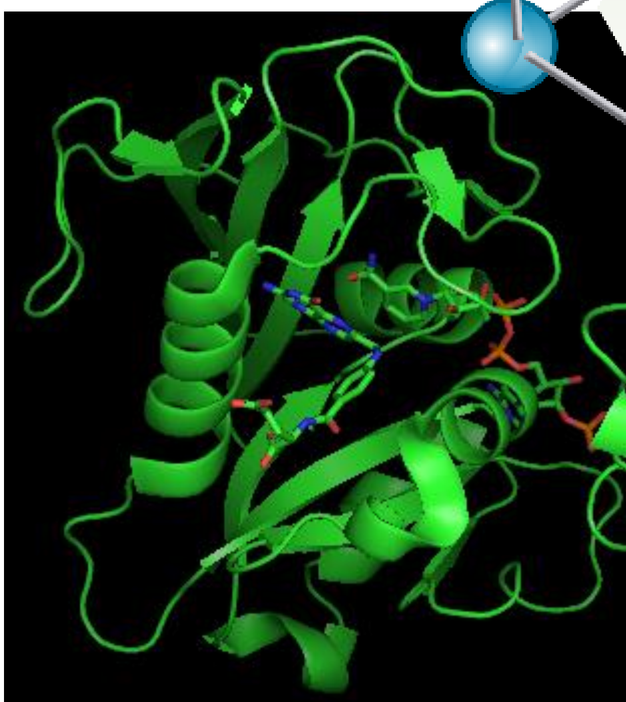
- **Desenho de fármacos – aumentar pontos de interação para ganho de afinidade e inibição**

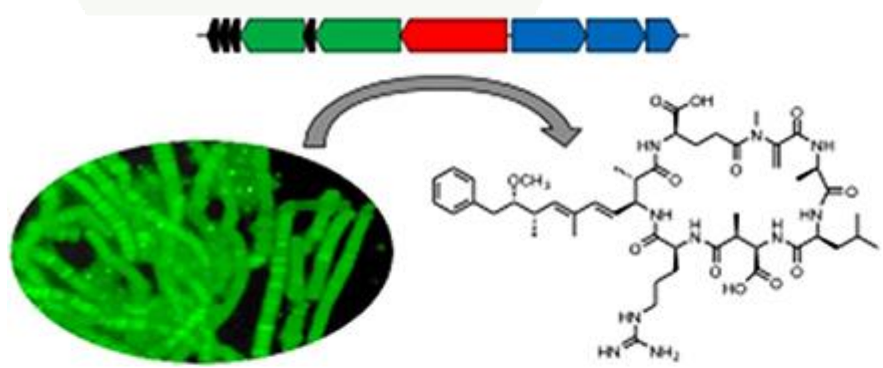
- **Biocatálise – aumentar pontos de interação ou promiscuidade para aumentar eficiência de catálise ou atividade contra substratos alternativos.**



Laboratório de Biologia Estrutural Aplicada

Divisão Fármacos





Exercício:

1) COVID-19 mudou drasticamente nosso modo de vida nos últimos 18 meses, levando à morte de um grande número de pessoas. Embora hoje tenhamos vacina, ainda há poucas moléculas que estão em estudos clínicos para o tratamento desta doença. Assim, a biologia estrutural tem sido extremamente importante neste momento e em um ano e meio de pandemia, o número de estruturas para proteínas depositados no “Protein Data Bank” é bastante expressivo, incluindo na presença de potencias inibidores. Vocês, como futuros biomédicos, podem trabalhar na interpretação de resultados estruturais. Assim, entrem no PDB, código de acesso **7D1M** e analise as interações do composto CG376 com a *main protease de SARS-CoV-2*, indicando quais são essas interações (hidrofóbicas e interações de hidrogênio) que garantem a afinidade desse composto. Tente encontrar pelo menos uma modificação no composto que poderia aumentar sua afinidade pela proteína.

Para facilitar: Entre em “https://www.rcsb.org/” digite **7D1M** (em procura)/abaixo da estrutura vai em **3D View**/ligand interactions/components/polimer/add representation/line|label/ Observe os amino ácidos que interagem com o ligante.

1) Biocatálise tem se tornado uma importante abordagem para a síntese de novos compostos utilizando técnicas menos agressivas ao meio ambiente (química verde) e mais baratas. Portanto para que uma enzima possa ser utilizada nessas técnicas, modificações são necessárias. Baseado no que se sabe sobre as proteínas de organismos termofílicos, que modificações poderiam ser propostas para aumentar a estabilidade de proteínas a altas temperaturas? Como a biologia estrutural poderia auxiliar nesse processo para uma enzima que não apresenta similaridade com outras já resolvidas?