LGN5809 -GenéticaMolecular

MOLÉCULAS CELULARES: UM ENFOQUE NAS PROTEÍNAS

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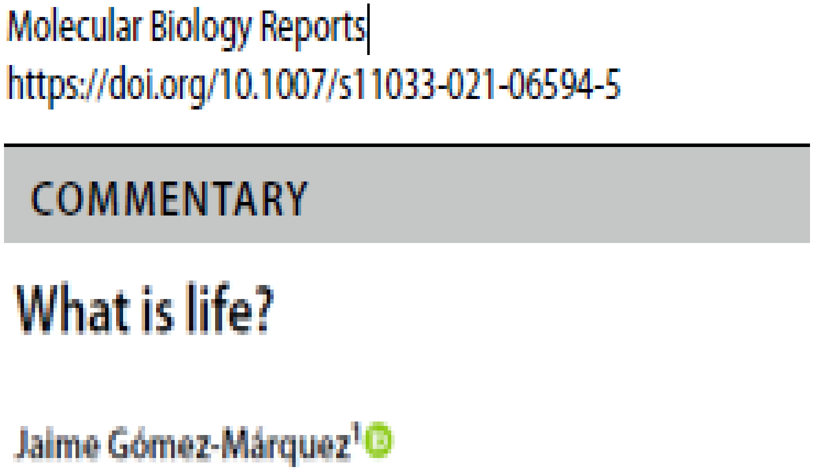
Sumário

- Macromoléculas celulares
- Proteínas–forma e função
- Conceito e princípios da “proteostase”
- Dobramento de proteínas
- Degradação de proteínas
- Proteômica

INTRODUÇÃO

O QUE É A VIDA?



Molecular Biology Reports
https://doi.org/10.1007/s11033-021-06594-5

COMMENTARY

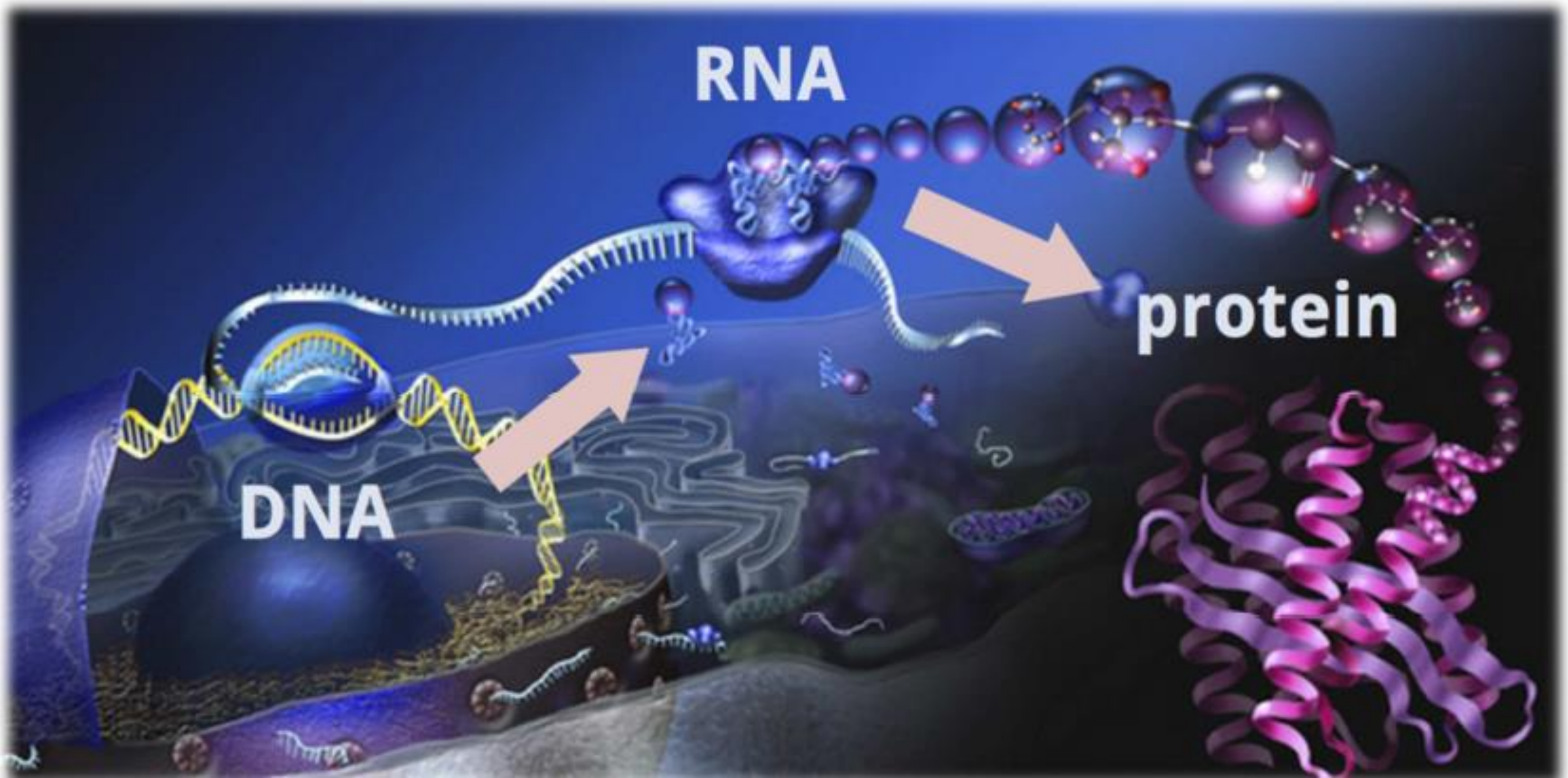
What is life?

Jaime Gómez-Márquez[1]

- **Sistema altamente regulado de síntese e manutenção de moléculas.**

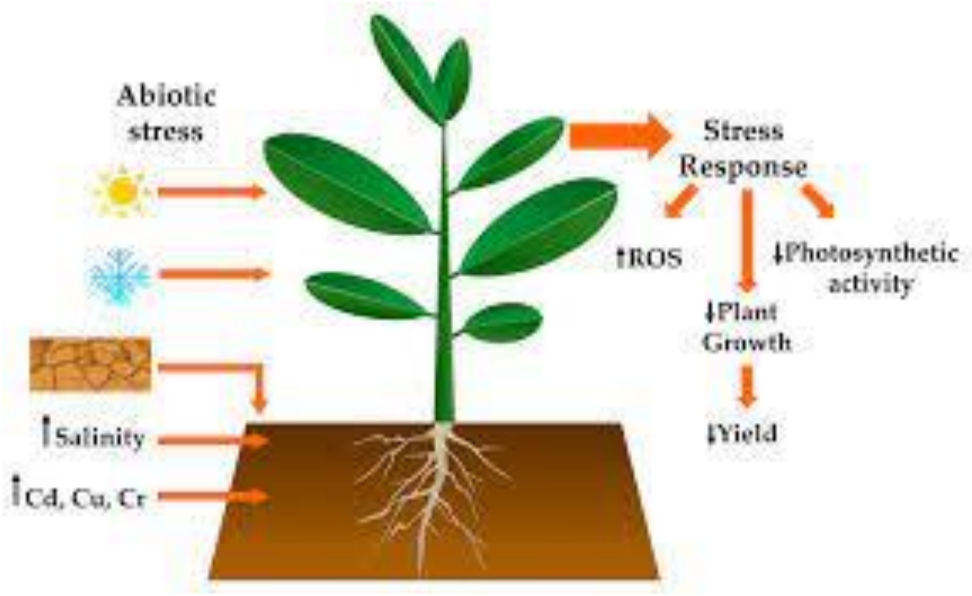
INTRODUÇÃO

- Os organismos vivos utilizam o **mesmo padrão molecular** para assegurar o seu metabolismo, mas cada espécie possui um conjunto específico de **ácidos nucléicos** e consequentemente de **proteínas** responsáveis pela sua **identidade.**

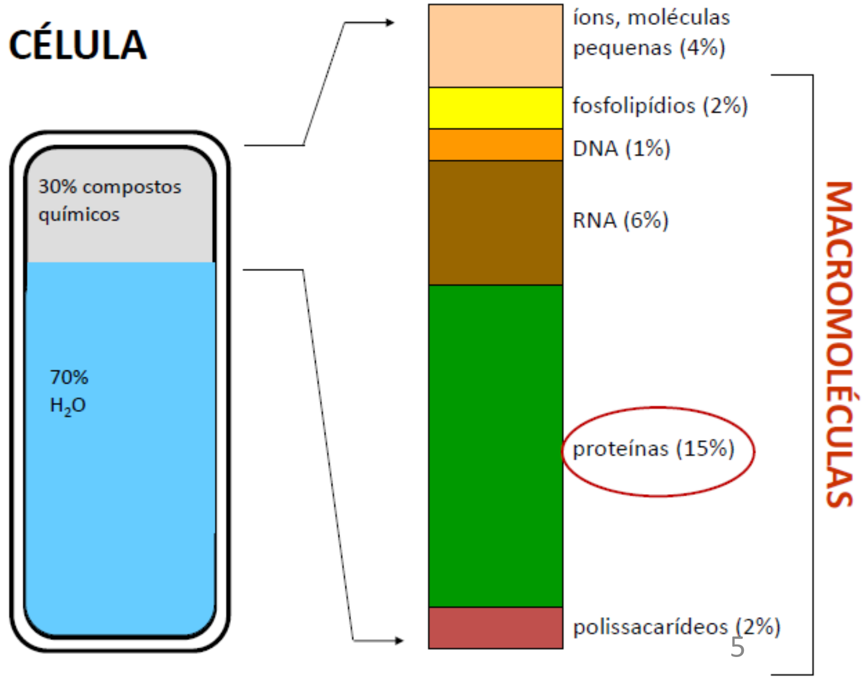


INTRODUÇÃO

- **Vida celular: interações e reações químicas**

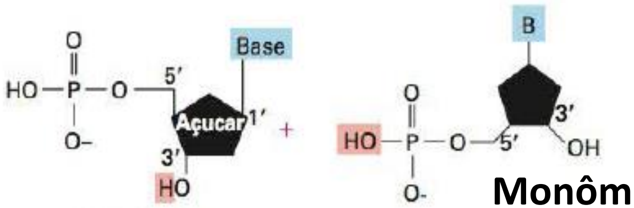


- **Composição química da célula**



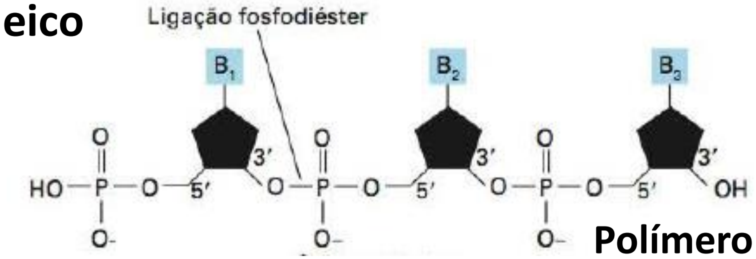
MACROMOLÉCULAS

Nucleotídeo



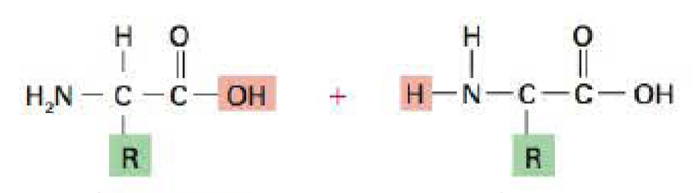
Monômero

Ácido Nucleico



Polímero

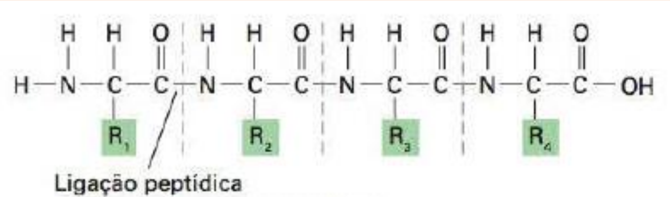
Aminoácido



Monômero

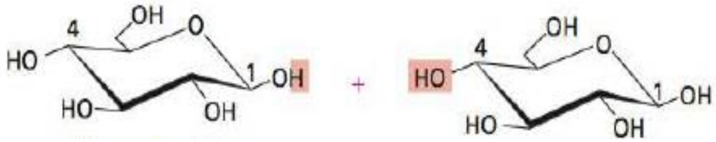
Monômero

Polipeptídeo



Polímero

Monossacarídeo

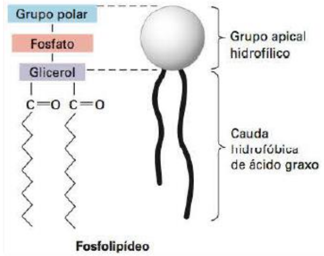


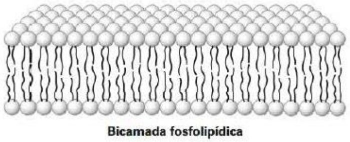
Monômero

Monômero

Polissacarídeo

Polímero



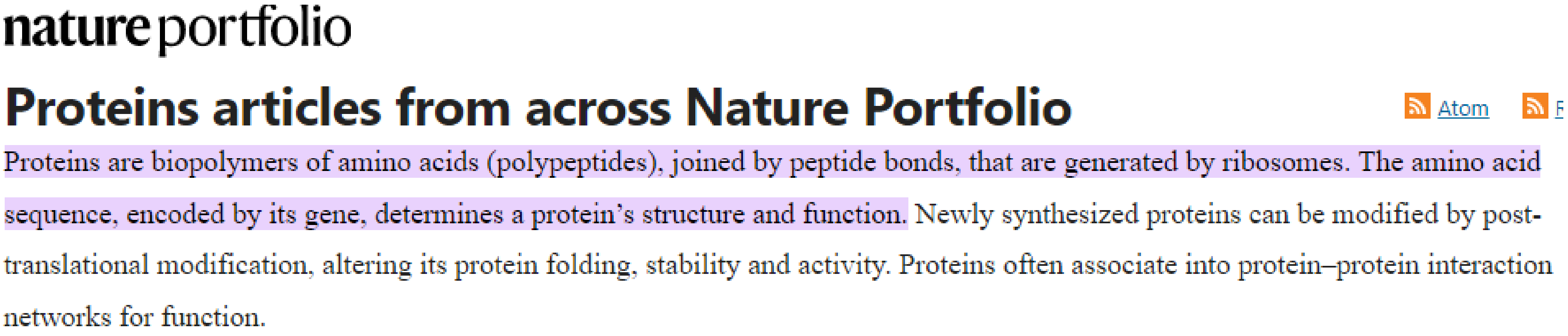


Lipídeos (ácidos graxos + glicerol)

*os lipídios não são polímeros, isto é, não são repetições de uma unidade básica.

PROTEÍNAS: DEFINIÇÃO

MOLÉCULAS QUE FAZEM MOLÉCULAS

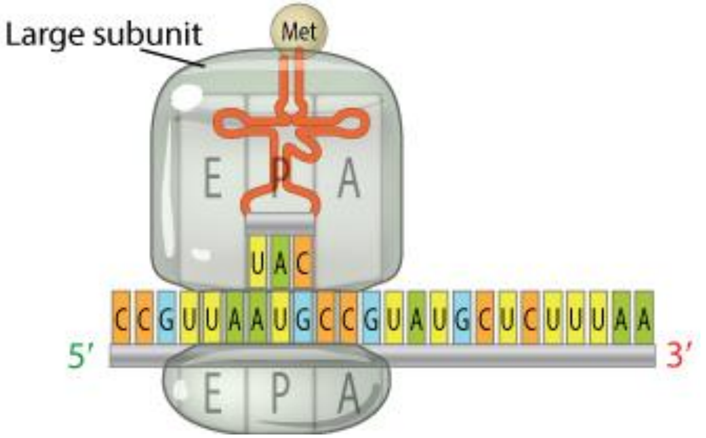


nature portfolio

Proteins articles from across Nature Portfolio

Proteins are biopolymers of amino acids (polypeptides), joined by peptide bonds, that are generated by ribosomes. The amino acid sequence, encoded by its gene, determines a protein's structure and function. Newly synthesized proteins can be modified by post-translational modification, altering its protein folding, stability and activity. Proteins often associate into protein–protein interaction networks for function.

Mais versátil composto celular: estrutura dinâmica, altamente moldável, responsável por inúmeras funções celulares.

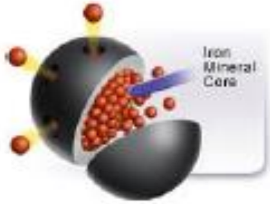


Tradução

PROTEÍNAS

Ex: ferritina

Armazenamento



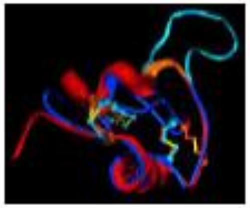
Ex: lipases

Enzimática

Ex: troponina

Sistemas contrácteis

Hormonal

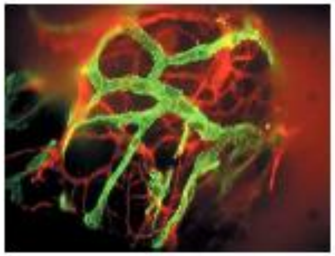


Ex: insulina

Funções das Proteínas

Transporte

Ex: hemoglobina



Nutricional

Ex: caseína

Imunidade

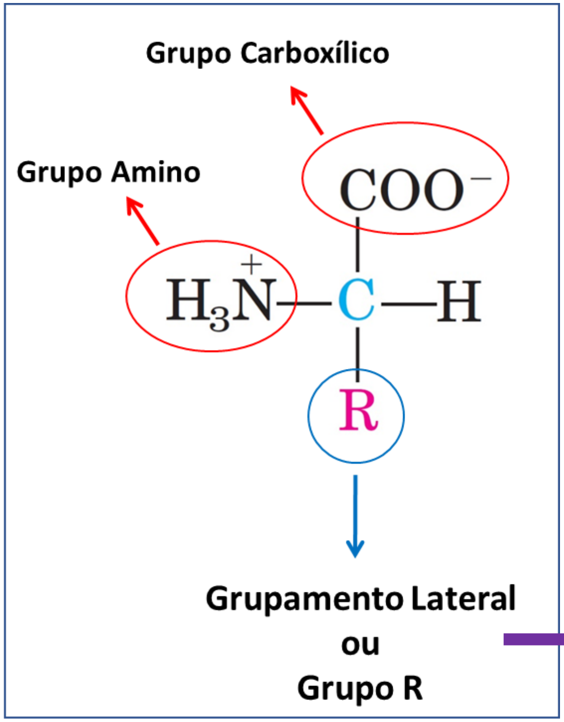
Ex: imunoglobulina

Estrutural

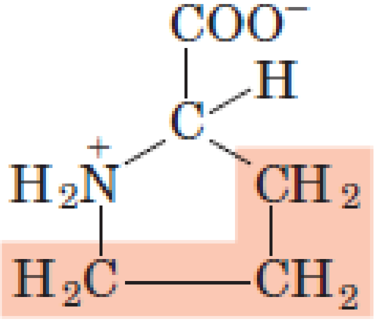
Ex: colágeno

PROTEÍNAS: PROPRIEDADES

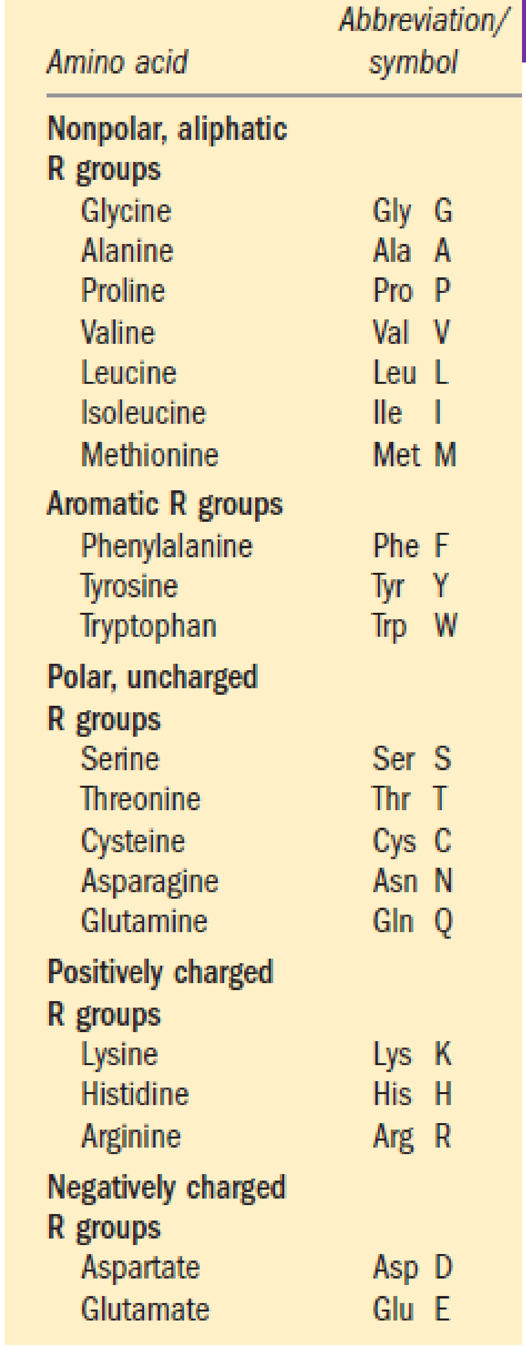
- **Aminoácidos**



- **Forma**
- **Hidrofobicidade**
- **Solubilidade**



Prolina

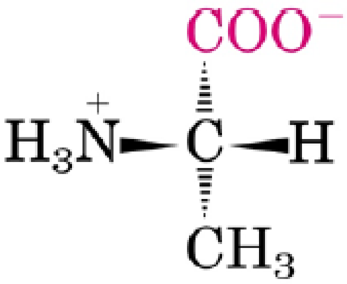


Amino acid	*Abbreviation/ symbol*	
Nonpolar, aliphatic R groups		
Glycine	Gly	G
Alanine	Ala	A
Proline	Pro	P
Valine	Val	V
Leucine	Leu	L
Isoleucine	Ile	I
Methionine	Met	M
Aromatic R groups		
Phenylalanine	Phe	F
Tyrosine	Tyr	Y
Tryptophan	Trp	W
Polar, uncharged R groups		
Serine	Ser	S
Threonine	Thr	T
Cysteine	Cys	C
Asparagine	Asn	N
Glutamine	Gln	Q
Positively charged R groups		
Lysine	Lys	K
Histidine	His	H
Arginine	Arg	R
Negatively charged R groups		
Aspartate	Asp	D
Glutamate	Glu	E

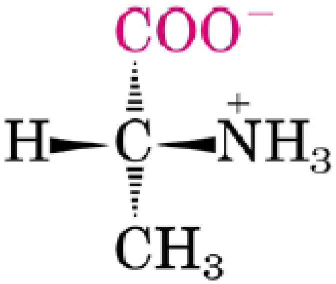
PROTEÍNAS: PROPRIEDADES

➢ ESTEREOQUÍMICA DOS AMINOÁCIDOS

Aminoácidos são denominados "D-" ou "L-“

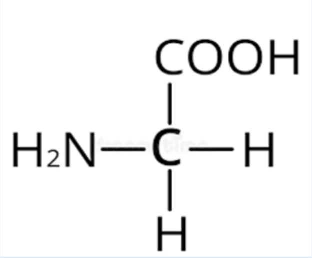


L-alanina



D-alanina

Exceção – aminoácido aquiral



Glicina

- ✓ **Os aminoácidos nas moléculas protéicas são L estereoisômeros;**

PROTEÍNAS: PROPRIEDADES

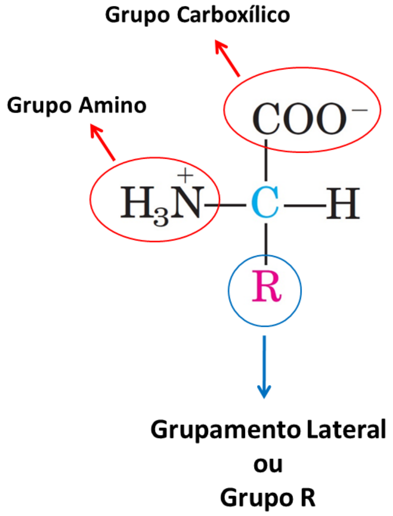
PROPRIEDADES ÁCIDO-BASE DE AMINOÁCIDOS

- Podem ser classificados de acordo com a estrutura da cadeia lateral em neutros, ácidos ou básicos.

Básicos: lisina, arginina e histidina,

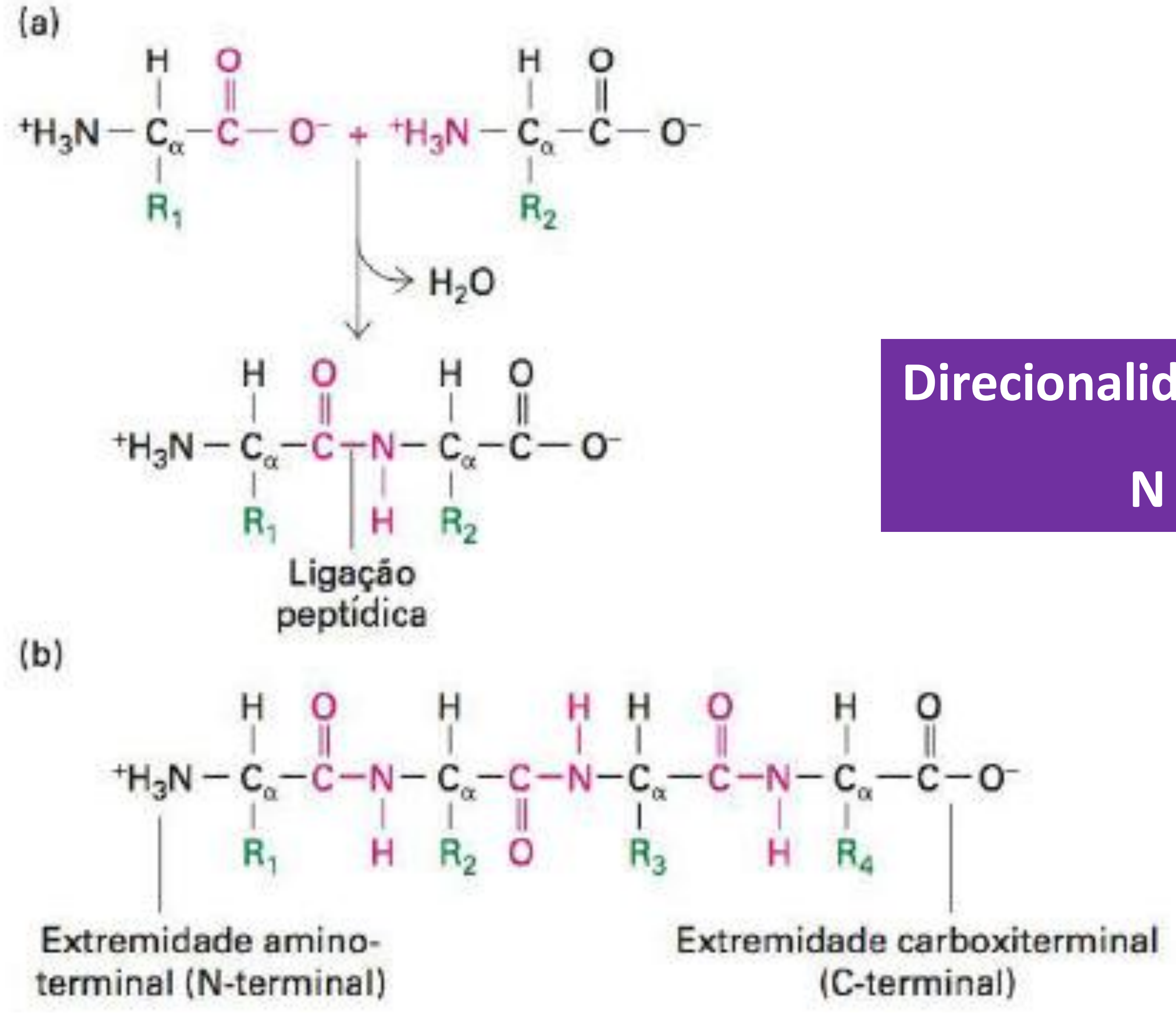
Ácidos: glutamato e o aspartato,

Neutros: os demais



- ✓ **Solúveis em água,**
- ✓ **São Anfóteros – podem doar ou receber prótons (H+),**
- ✓ **A forma predominante do aminoácido depende do pH,**
- ✓ **Ponto de fusão elevado.**

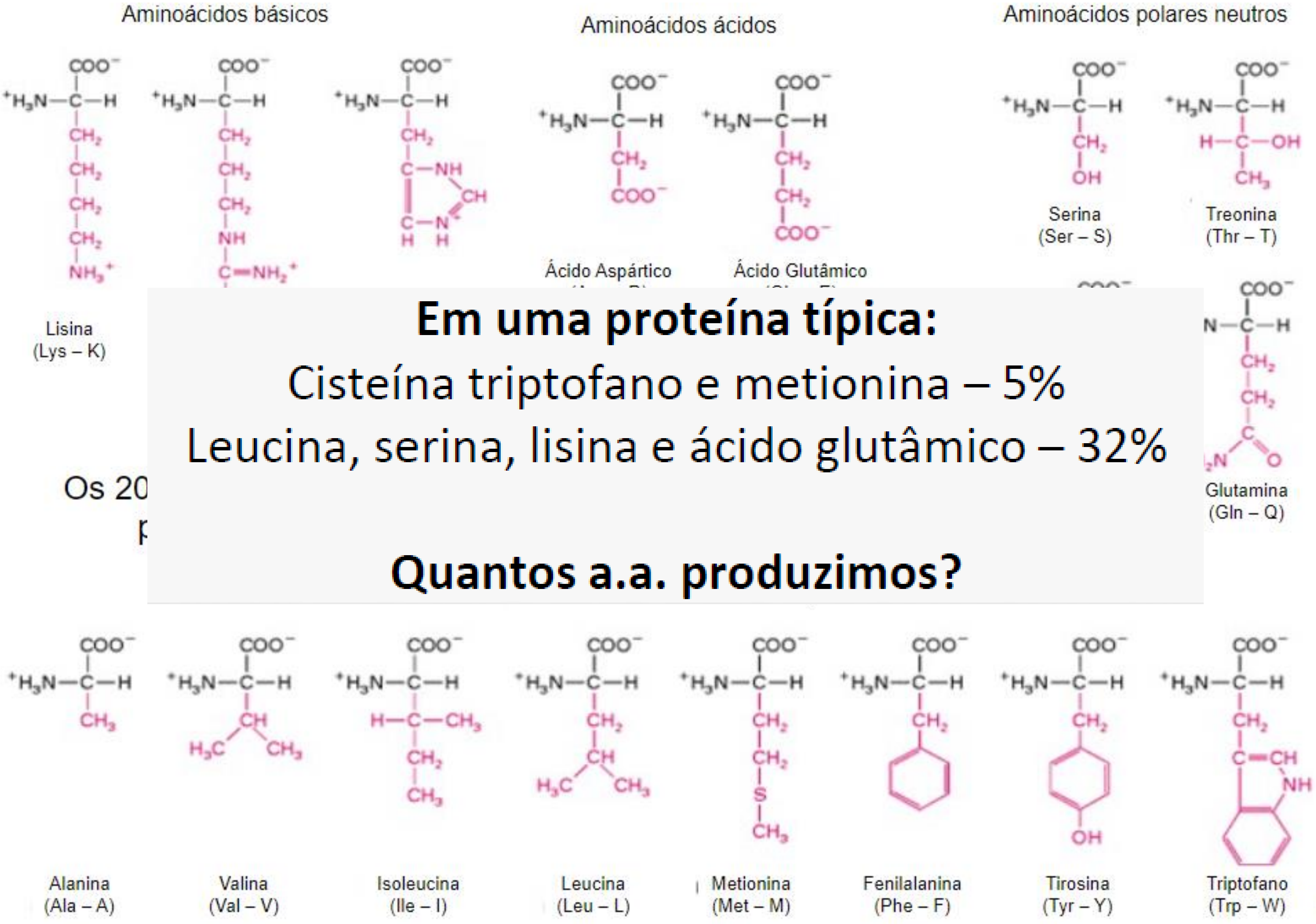
PROTEÍNAS: PROPRIEDADES



Direcionalidade da cadeia

N --- C

PROTEÍNAS: PROPRIEDADES



Em uma proteína típica:

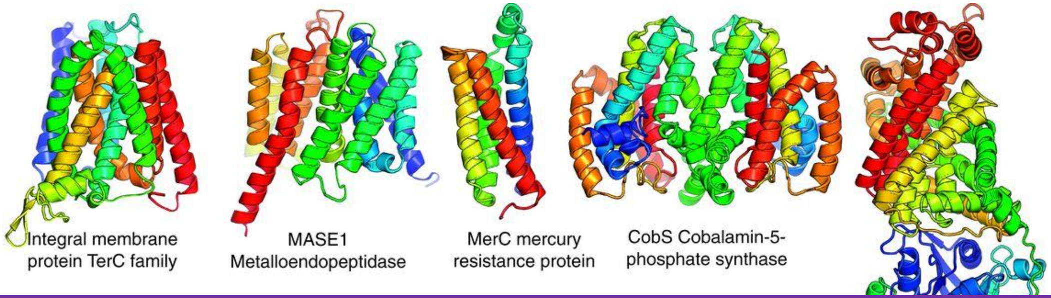
Cisteína triptofano e metionina – 5%

Leucina, serina, lisina e ácido glutâmico – 32%

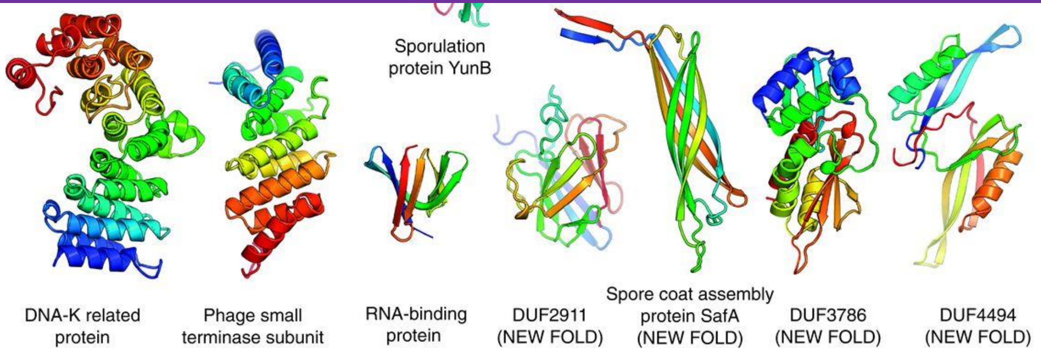
Quantos a.a. produzimos?

PROTEÍNAS: DIVERSIDADE ESTRUTURAL

Mas para que toda essa diversidade estrutural?



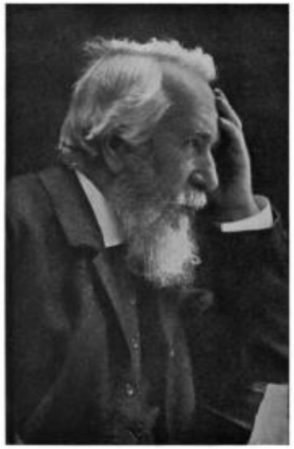
A função de uma proteína é derivada da sua estrutura tridmensional, e a estrutura tridimensional especificada pela sequência de aminoácidos e interações não covalentes.



DOI: 10.1126/science.aah404

PROTEÍNAS: FORMA E FUNÇÃO

Johann Wolfgang von Goethe
1749-1832



Ernst Haeckel 1834-1919

Influência na arquitetura orgânica

“FORMA É FUNÇÃO”

D'Arcy Wentworth Thompson
1860-1948

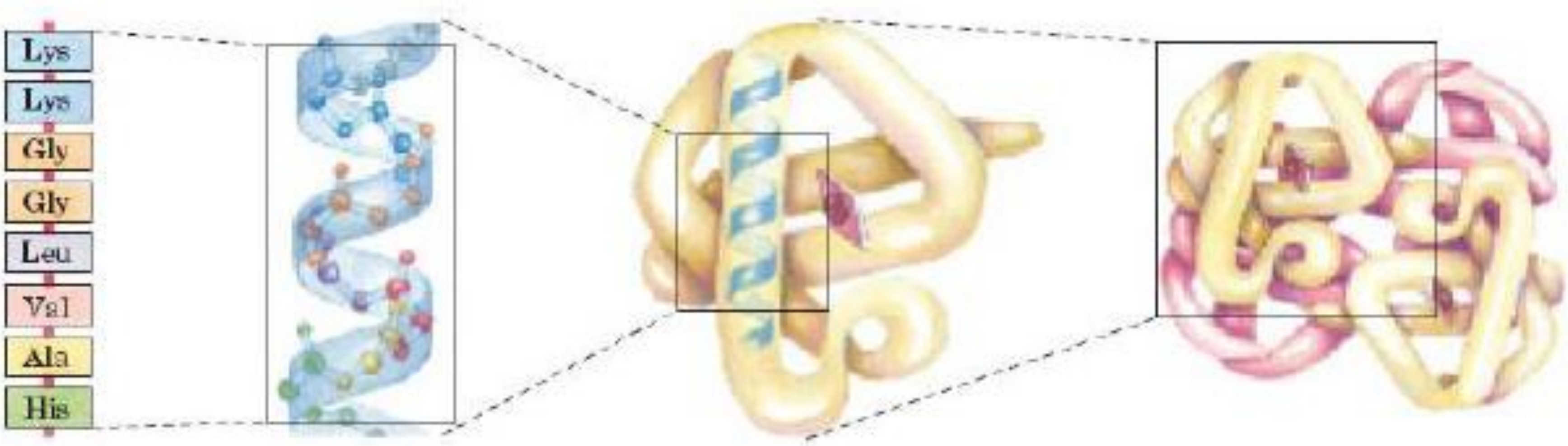
PROTEÍNAS : FORMA E FUNÇÃO

- **CLASSES:**

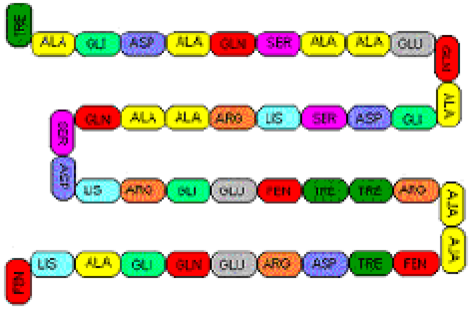
- Proteínas estruturais,
- Proteínas transportadoras de membranas,
- Enzimas,
- Proteínas andaime (*scaffold*),
- Proteínas reguladoras (e.x. proteínas de sinalização),
- Proteínas motoras.

PROTEÍNAS: ESTRUTURA HIERARQUICA

- **Proteínas ativas tem conformação específica**

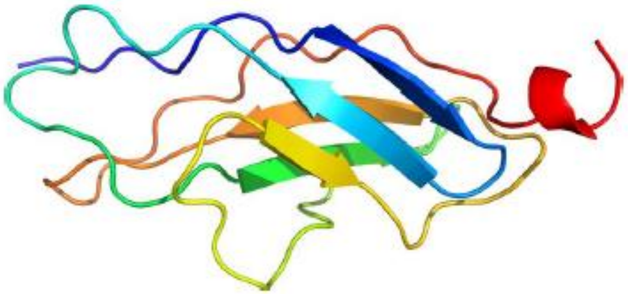


PROTEÍNAS: ESTRUTURA HIERÁRQUICA

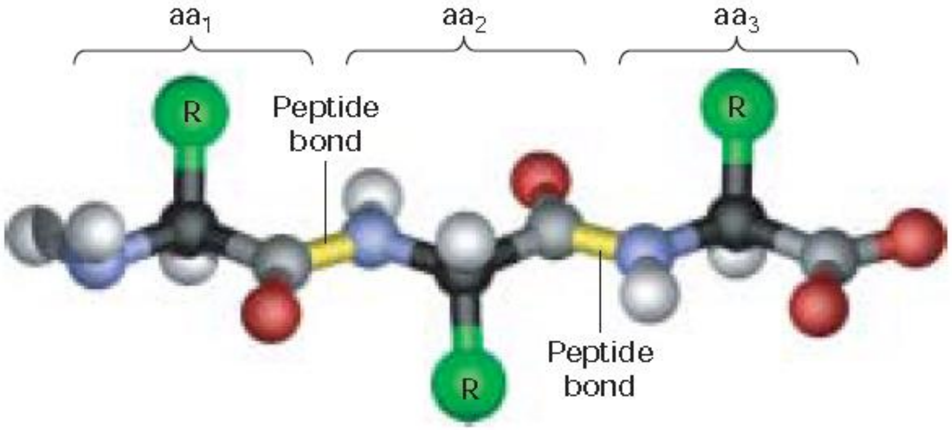


➢ ESTRUTURA PRIMÁRIA

- Arranjo linear de aminoácidos
- Peptideo – até 30 resíduos de aminoácidos
- A massa das proteínas é calculada em Daltons (Da)
- Em média, o peso molecular de aminoácidos em proteínas é de 113 Da



Titina – 35.000 resíduos de aa



PROTEÍNAS: ESTRUTURA HIERÁRQUICA

NEWS | BIOLOGY

Science

‘The game has changed.’ AI triumphs at solving protein structures

In milestone, software predictions finally match structures calculated from experimental data

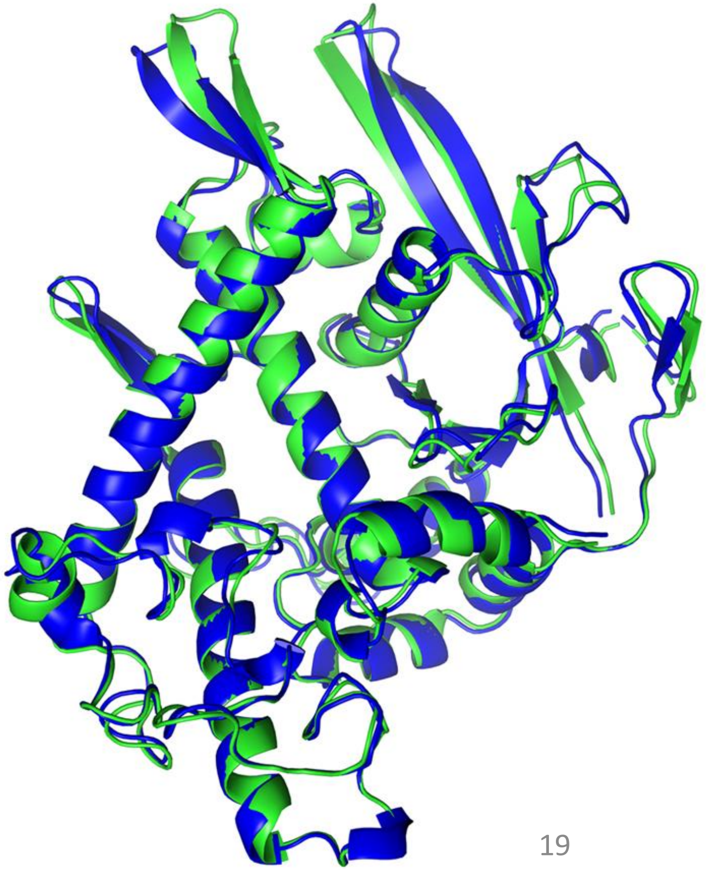
30 NOV 2020 · BY ROBERT F. SERVICE

NEWS | 30 November 2020

nature

‘It will change everything’: DeepMind’s AI makes gigantic leap in solving protein structures

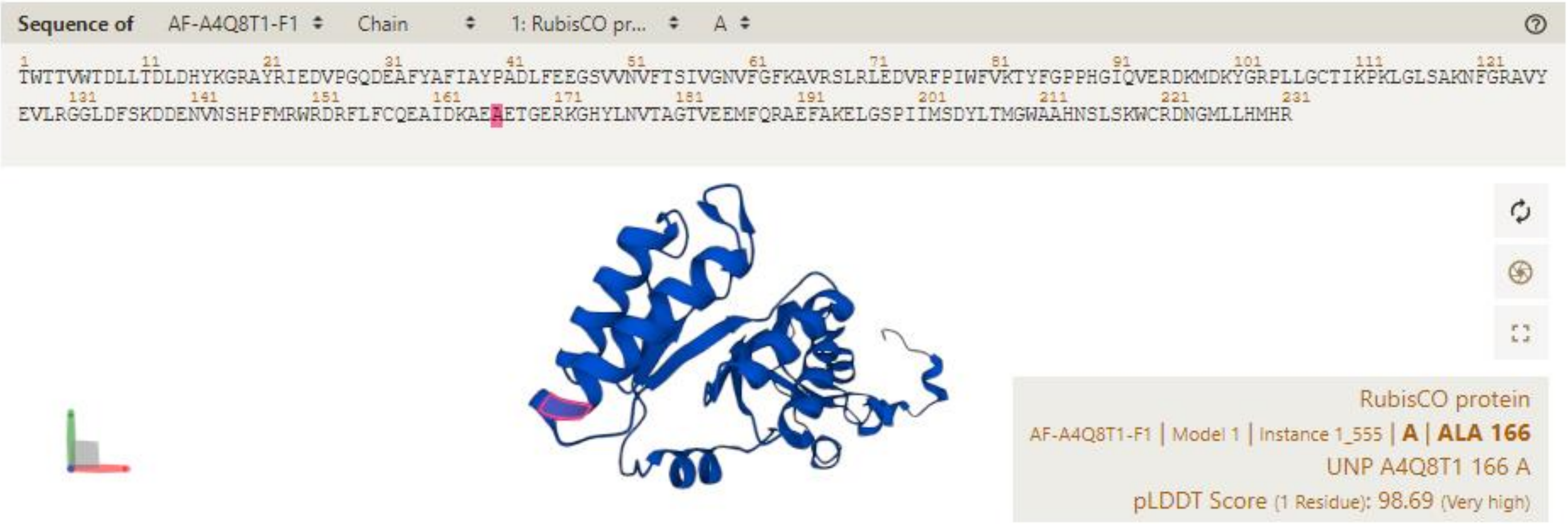
Google’s deep-learning program for determining the 3D shapes of proteins stands to transform biology, say scientists.



PROTEÍNAS: ESTRUTURA HIERÁRQUICA

AlphaFold
Protein Structure Database

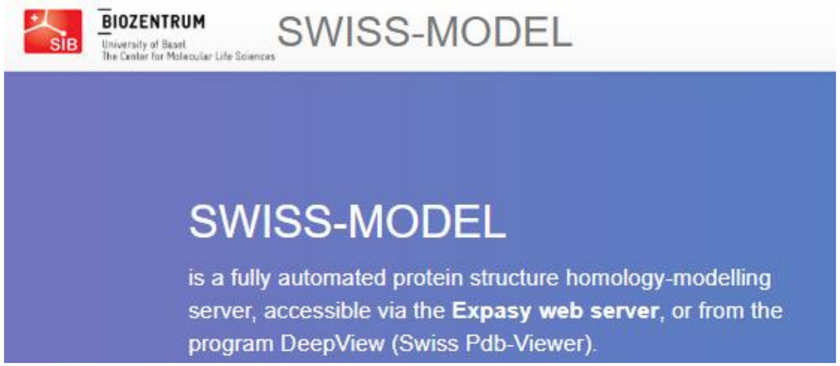
Developed by DeepMind and EMBL-EBI



```
1         11        21        31        41        51        61        71        81        91        101       111       121
TWTTVWTDLLTDLDHYKGRAYRIEDVPGQDEAFYAFIAYPADLFEEGSVVNVFTSIVGNVFGFKAVRSLRLEDVRFPIWFVKTYFGPPHGIQVERDKMDKYGRPLLGCTIKPKLGLSAKNFGRAVY
    131       141       151       161       171       181       191       201       211       221       231
EVLRGGLDFSKDDENVNSHPFMRWRDRFLFCQEAIDKAEAETGERKGHYLNVTAGTVEEMFQRAEFAKELGSPIIMSDYLTMGWAAHNSLSKWCRDNGMLLHMHR
```

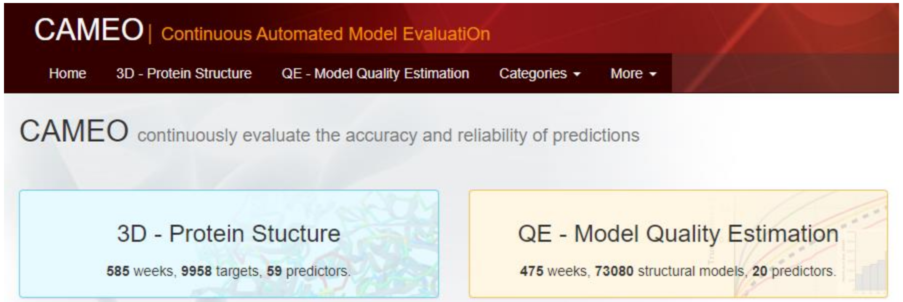
RubisCO protein
AF-A4Q8T1-F1 | Model 1 | Instance 1_555 | **A** | **ALA 166**
UNP A4Q8T1 166 A
pLDDT Score (1 Residue): 98.69 (Very high)

PROTEÍNAS: ESTRUTURA HIERÁRQUICA



SWISS-MODEL

is a fully automated protein structure homology-modelling server, accessible via the **Expasy web server**, or from the program DeepView (Swiss Pdb-Viewer).



CAMEO continuously evaluate the accuracy and reliability of predictions

3D - Protein Stucture

585 weeks, **9958** targets, **59** predictors.

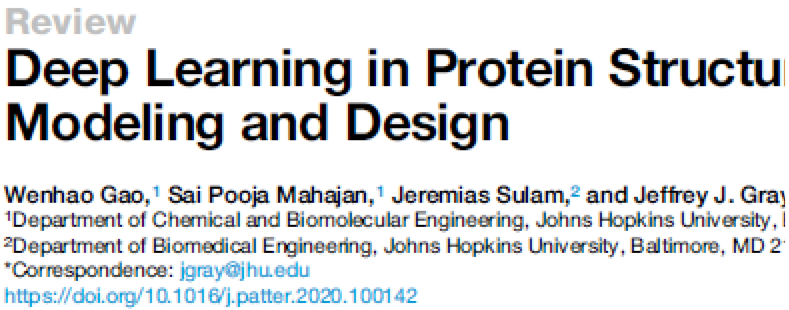
QE - Model Quality Estimation

475 weeks, **73080** structural models, **20** predictors.

ModBase: Database of Comparative Protein Structure Models

Article | Open Access | Published: 15 July 2021

Highly accurate protein structure prediction with AlphaFold



Review

Deep Learning in Protein Structu
Modeling and Design

Wenhao Gao,[1] Sai Pooja Mahajan,[1] Jeremias Sulam,[2] and Jeffrey J. Gra
[1]Department of Chemical and Biomolecular Engineering, Johns Hopkins University,
[2]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 2
*Correspondence: jgray@jhu.edu
https://doi.org/10.1016/j.patter.2020.100142

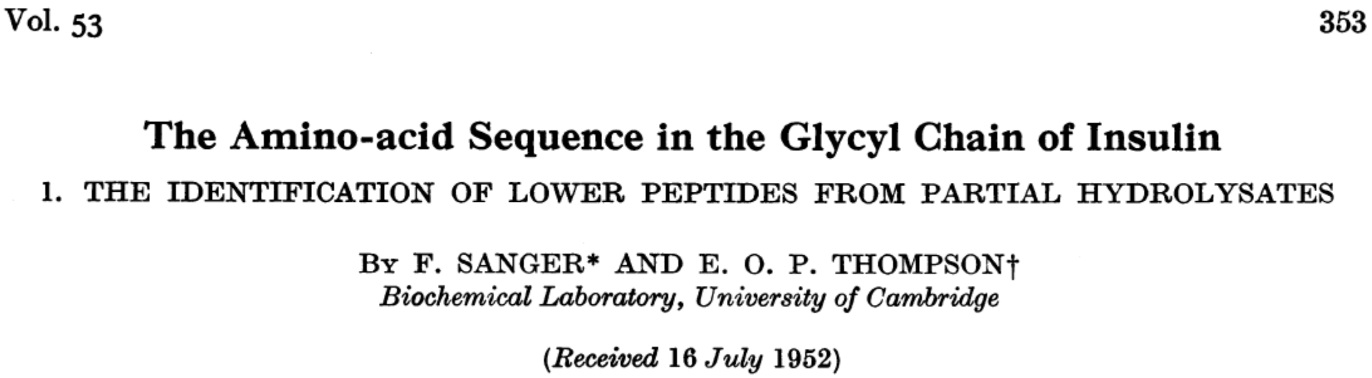
Comment | Published: 11 January 2022

Deep learning and protein structure modeling

Minkyung Baek & David Baker

Nature Methods **19**, 13–14 (2022) | Cite this article

PROTEÍNAS: PRIMEIRA PROTEÍNA SEQUENCIADA



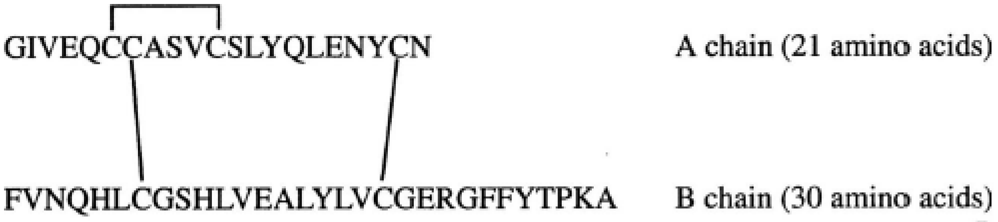
Vol. 53 353

The Amino-acid Sequence in the Glycyl Chain of Insulin

1. THE IDENTIFICATION OF LOWER PEPTIDES FROM PARTIAL HYDROLYSATES

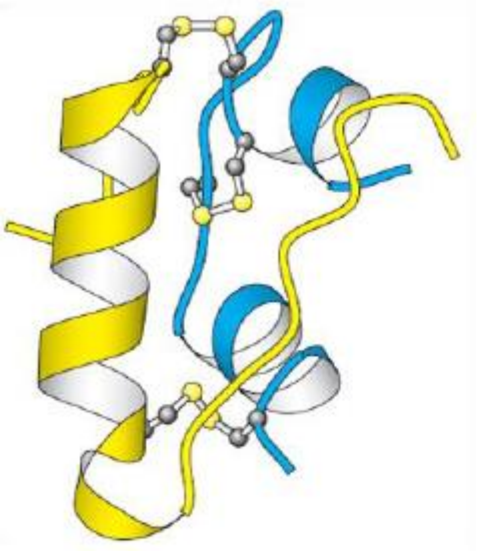
By F. SANGER* AND E. O. P. THOMPSON†
Biochemical Laboratory, University of Cambridge

(*Received* 16 *July* 1952)



GIVEQCCASVCSLYQLENYCN A chain (21 amino acids)

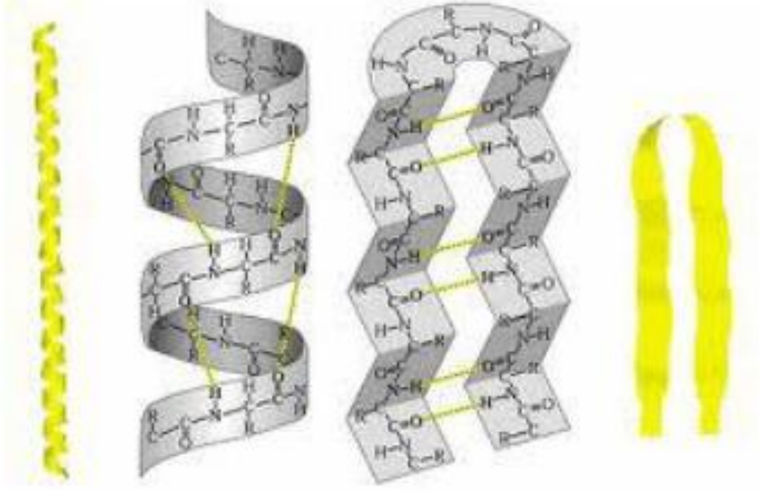
FVNQHLCGSHLVEALYLVCGERGFFYTPKA B chain (30 amino acids)

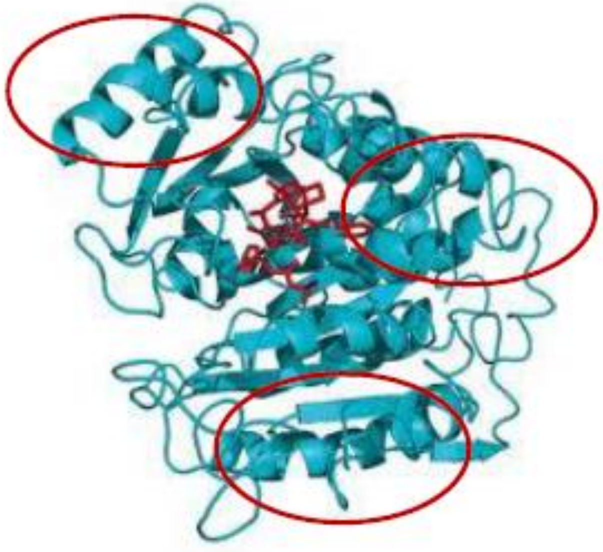


PROTEÍNAS: ESTRUTURA HIERÁRQUICA

➢ ESTRUTURA SECUNDÁRIA

Dobramentos periódicos de porções localizadas de uma cadeia polipeptídica mediados por pontes de hidrogênio

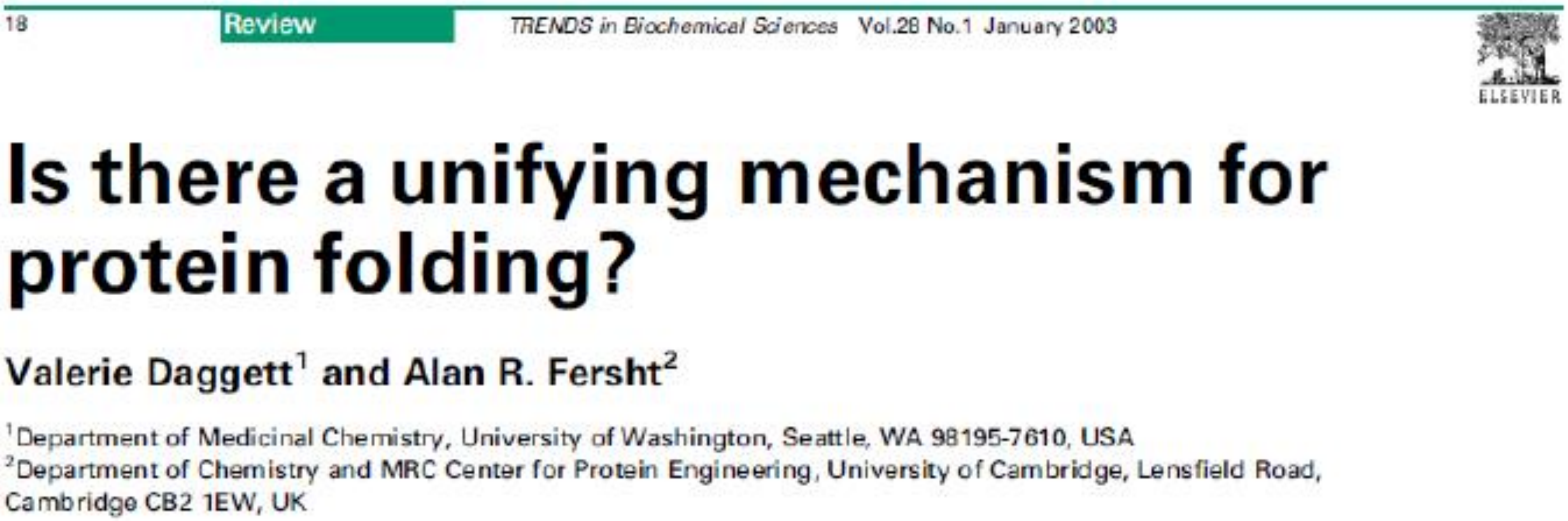




Um polipeptídeo – várias estruturas secundárias

60% compost por **α-hélice e Folha-β**

Restante formas irregulares!!!

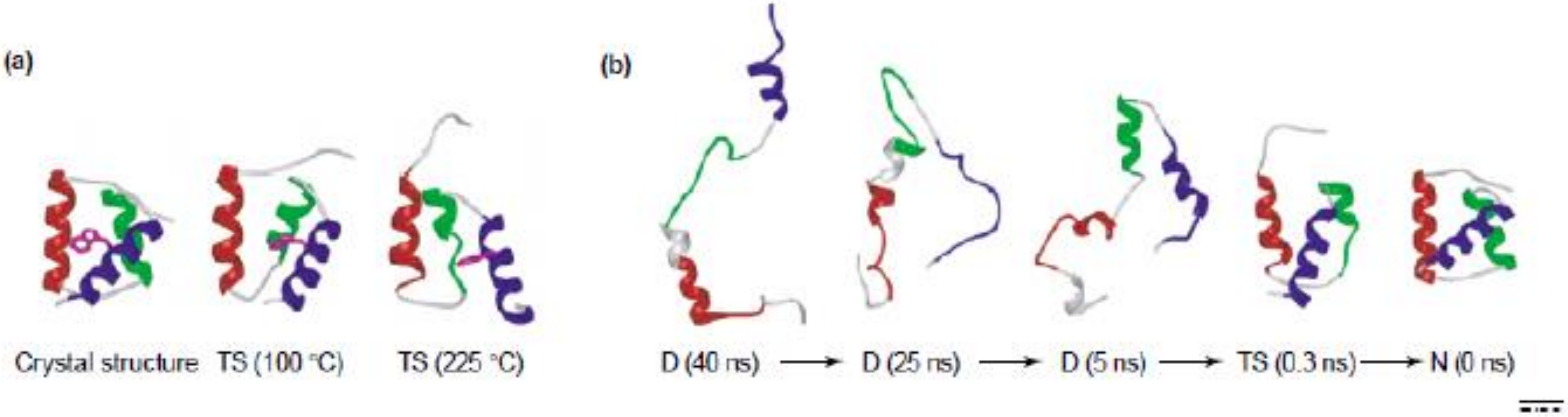


18 Review *TRENDS in Biochemical Sciences* Vol.28 No.1 January 2003

Is there a unifying mechanism for protein folding?

Valerie Daggett[1] **and Alan R. Fersht**[2]

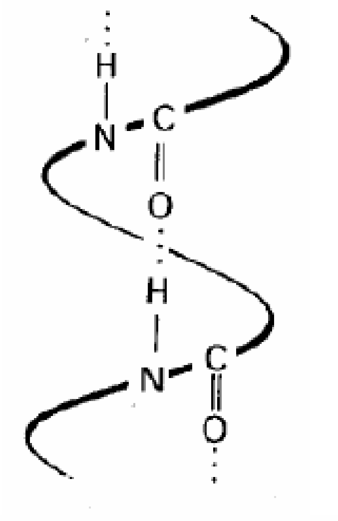
[1]Department of Medicinal Chemistry, University of Washington, Seattle, WA 98195-7610, USA
[2]Department of Chemistry and MRC Center for Protein Engineering, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK

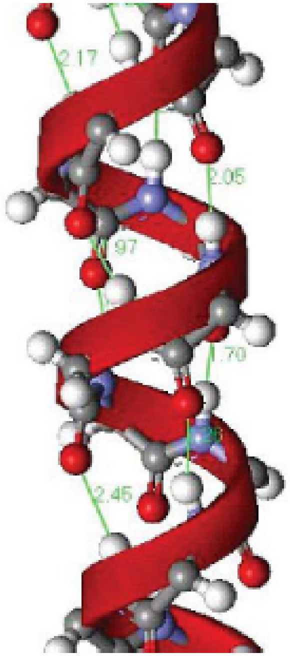


PROTEÍNAS: ESTRUTURA HIERÁRQUICA

α-hélice

O átomo da carbonila faz **ponte de hidrogênio** com o átomo de hidrogênio da amida do quarto resíduo abaixo no **sentido da extremidade C-terminal**





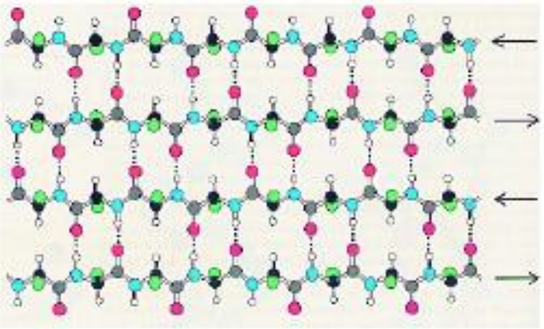
Qual amino-ácido não tem em α-helices???

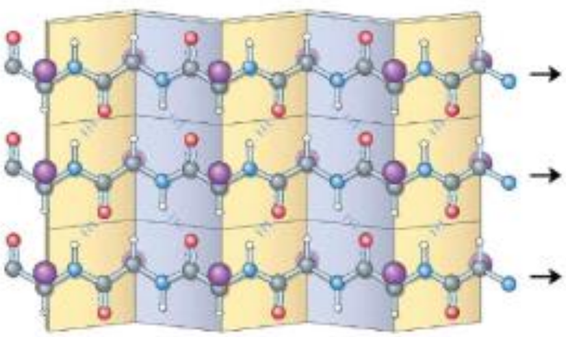
PROTEÍNAS: ESTRUTURA HIERÁRQUICA

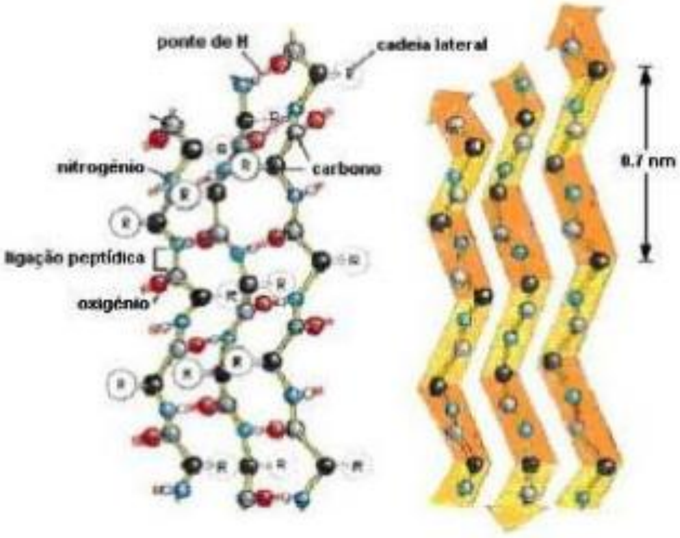
Folha-β

Fitas-β dispostas lateralmente – **cada folha contêm 5-8 residuos**.

Podem ocorrer em sentido **paralelo** ou **anti-paralelo**.





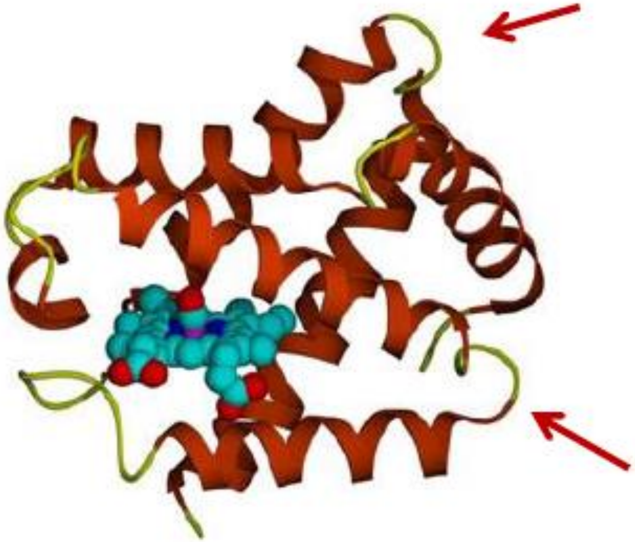


Bolsões de ligação ou núcleos hidrofóbicos

PROTEÍNAS: ESTRUTURA HIERÁRQUICA

Voltas β

Presentes geralmente na **parte externa** da proteína **redirecionando** o esqueleto peptídico. Composta por 3 a 4 resíduos.

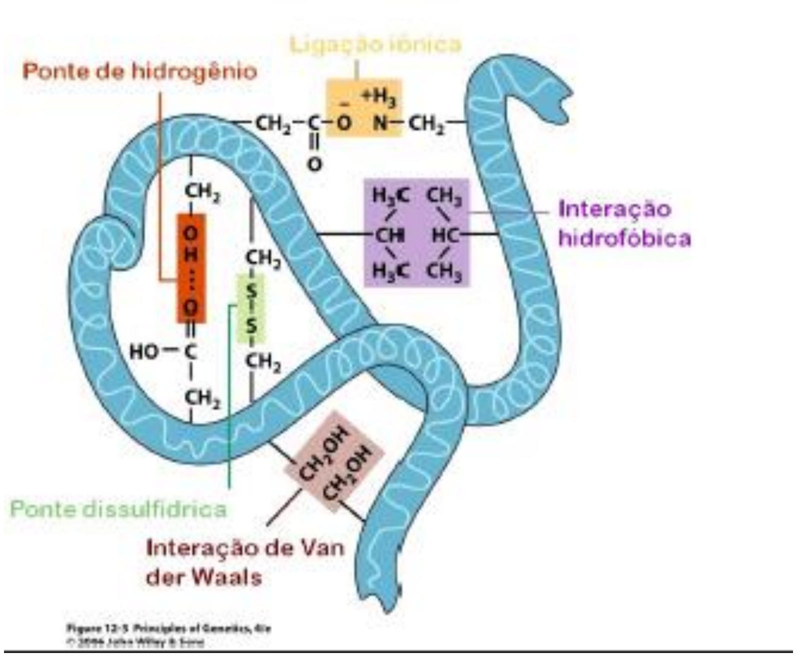


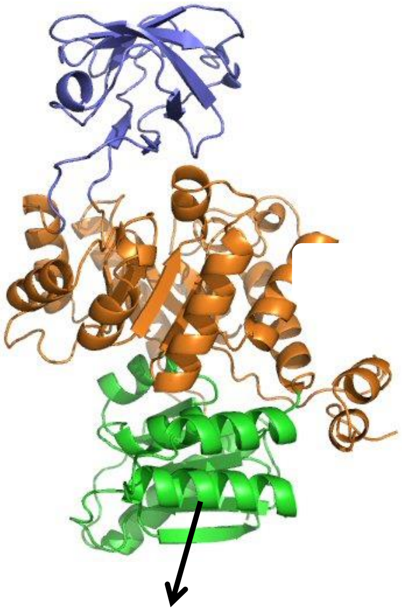
Quais amino-ácidos são frequentemente encontrados em volta-β???

PROTEÍNAS: ESTRUTURA HIERÁRQUICA

➢ Estrutura Terciária

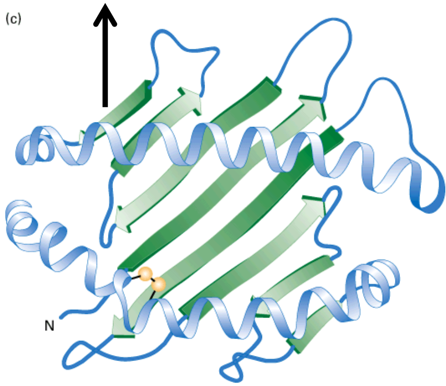
- Arranjo tridimensional de todos os resíduos de aminoácidos
- Estabilizadas por pontes de hidrogênio, interações hidrofóbicas e hidrofílicas
- Não são rígidas





α-hélice

Folha Beta

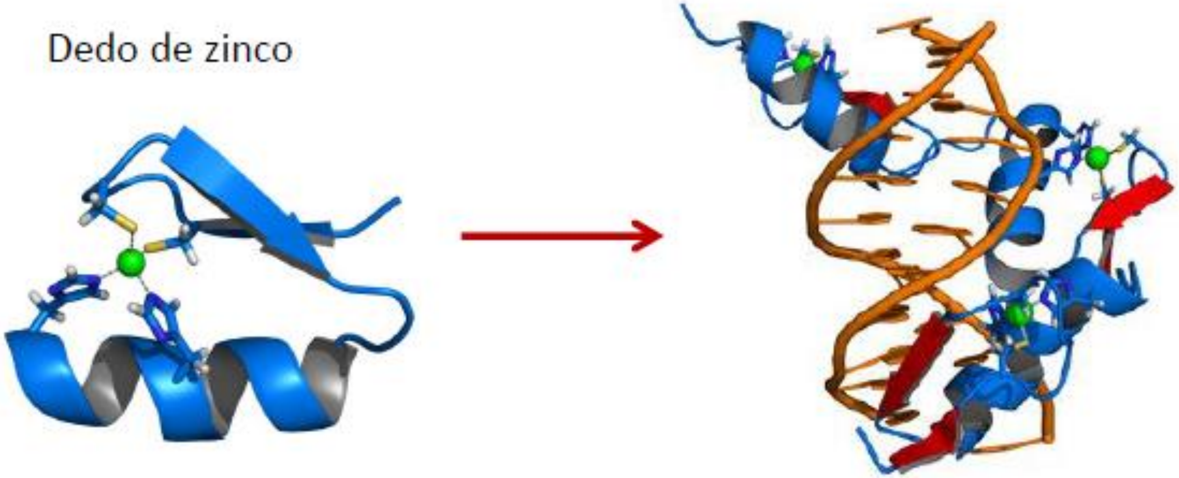


PROTEÍNAS: ESTRUTURA MOLECULAR

➢ MOTIVOS ESTRUTURAIS

Funções como: ligação a um íon específico, ou molécula pequena (e.x. cálcio ou ATP).

Combinações regulares de estruturas secundárias. Assinaturas de alguns grupos de proteínas.



Vários outros – mesmo motivos em proteinas distintas - combinação de estrutura secundária preservadas na evolução!

PROTEÍNAS: ESTRUTURA MOLECULAR

➢ DOMÍNIOS

- Regiões distintas das proteínas onde há dobramentos compactos do polipeptídeo.

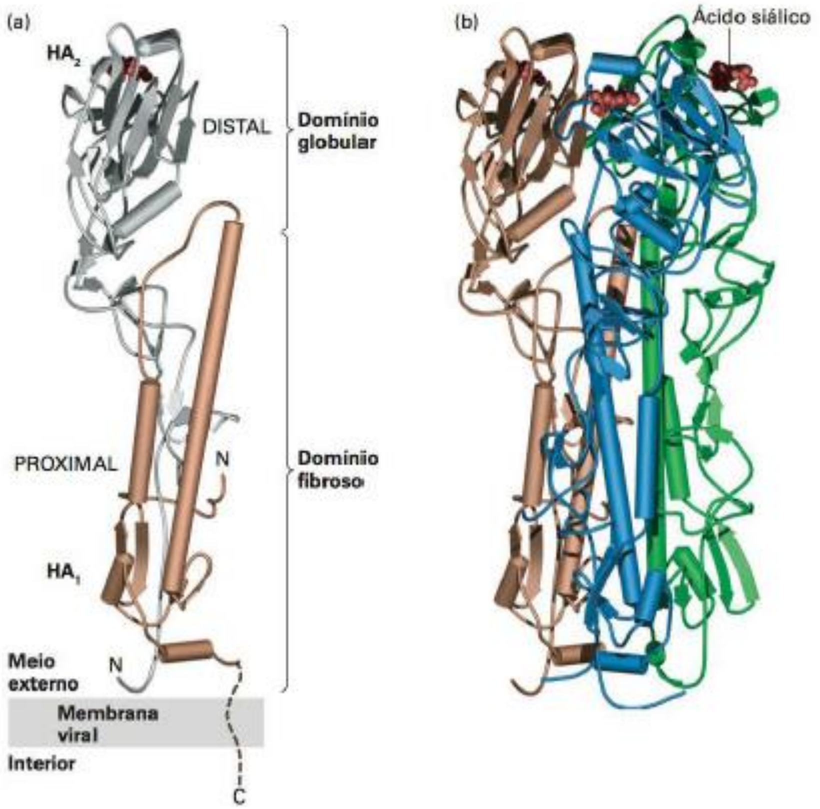
Domínios funcionais

Domínios estruturais

Domínios topológicos

Hemaglutinina – dominioglobular e fibroso

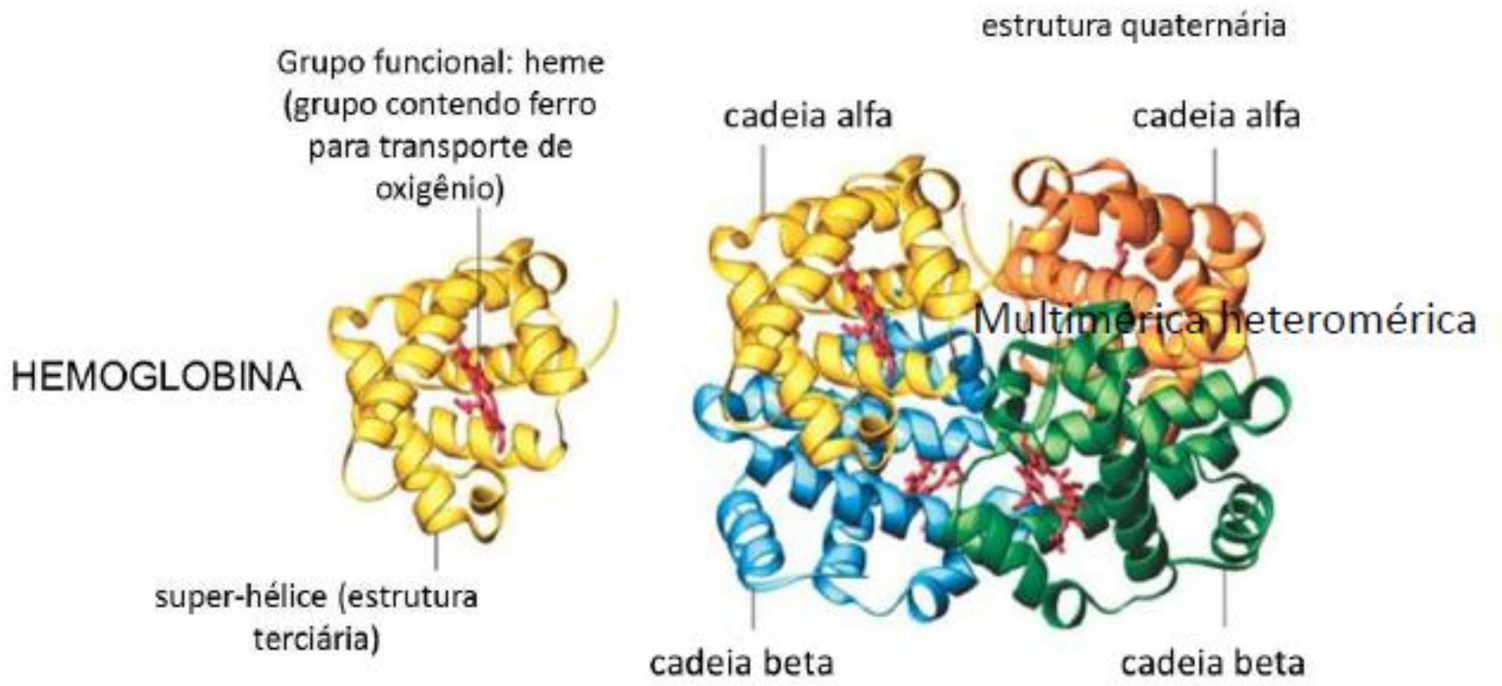
Geralmente domínios estruturais são domínios funcionais!



PROTEÍNAS: ESTRUTURA HIERÁRQUICA

➢ ESTRUTURA QUATERNÁRIA

- Composta por dois ou mais polipeptideos ou subunidades que podem ser iguais ou não.



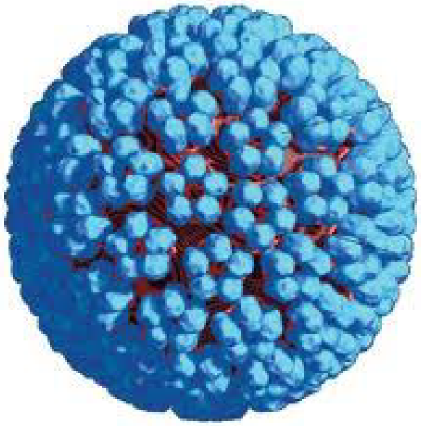
Multimérica heteromérica

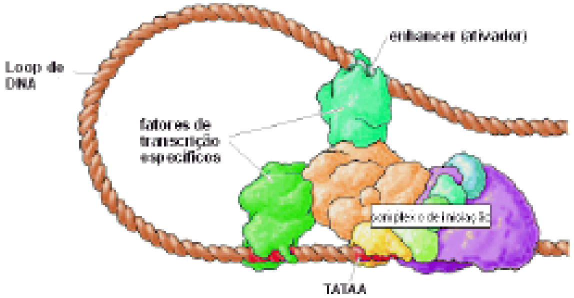
Tetramero α2β2

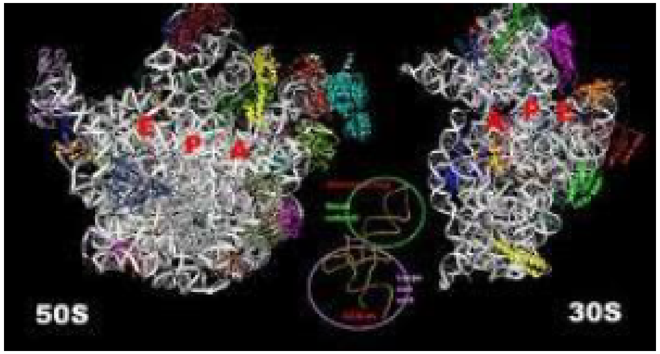
PROTEÍNAS: ESTRUTURA HIERÁRQUICA

Arranjos supramoleculares

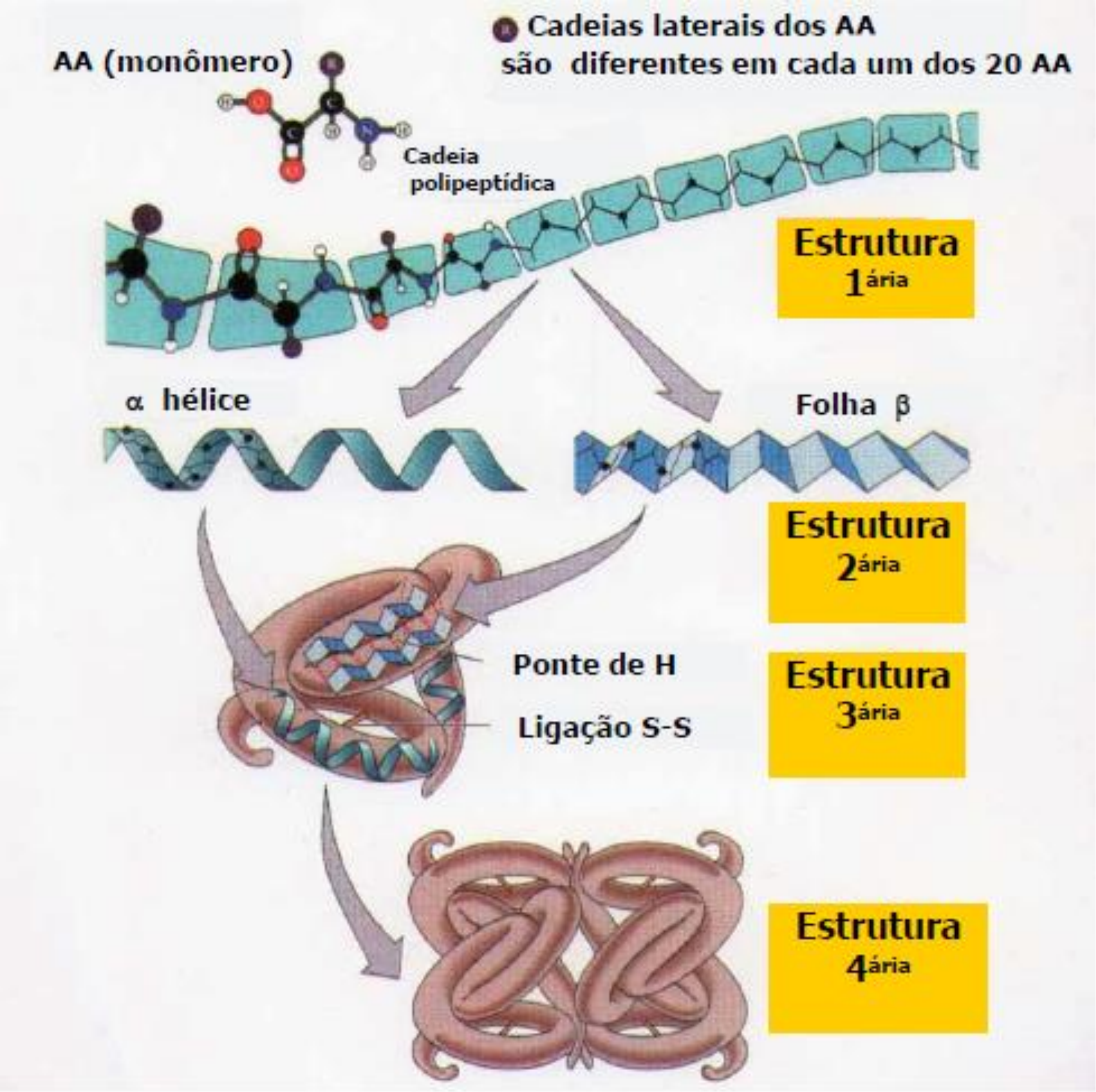
Composto por **dezenas e centenas de subunidades!!!**

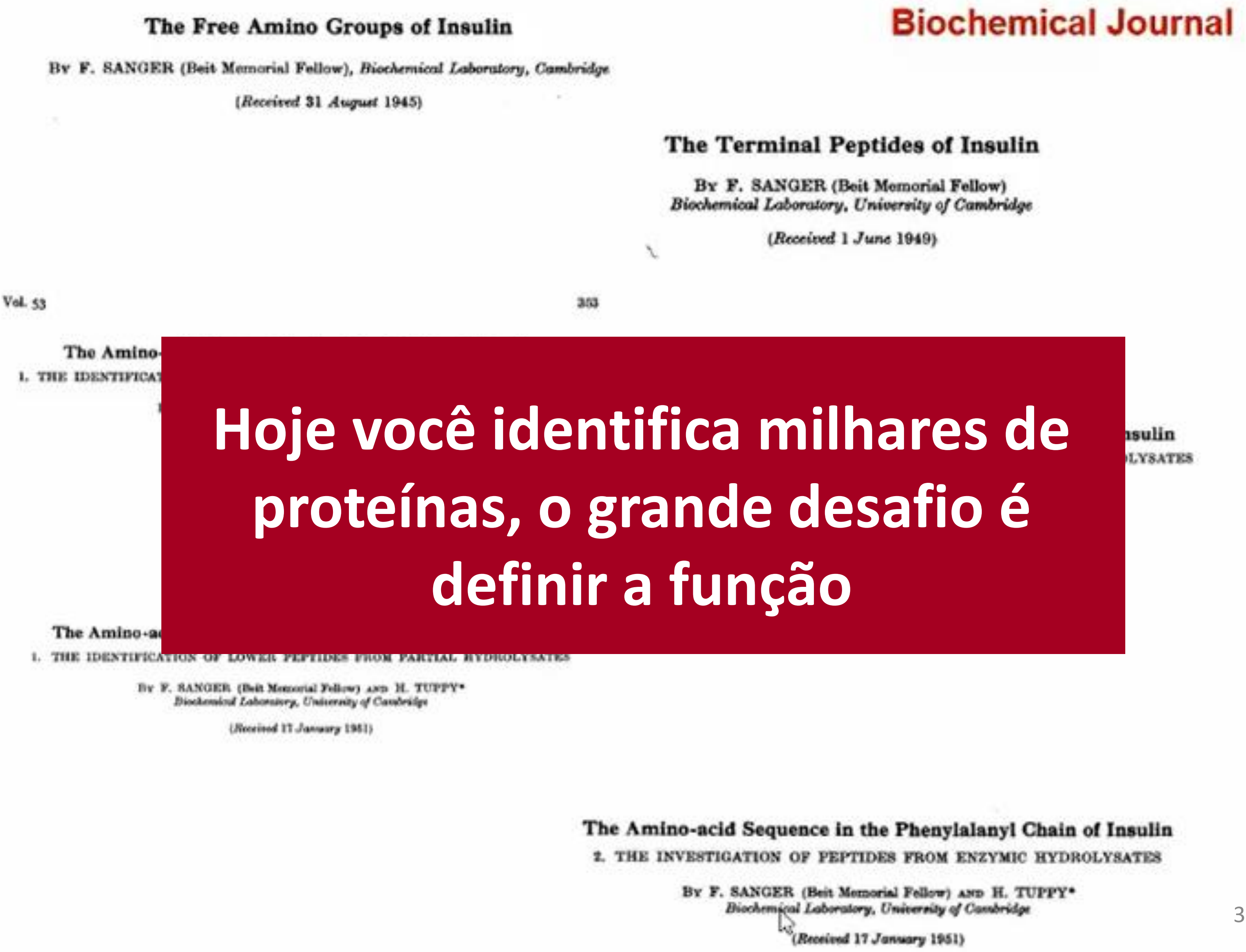






PROTEÍNAS: ESTRUTURA HIERÁRQUICA





Biochemical Journal

The Free Amino Groups of Insulin

By F. SANGER (Beit Memorial Fellow), *Biochemical Laboratory, Cambridge*

(*Received* 31 *August* 1945)

The Terminal Peptides of Insulin

By F. SANGER (Beit Memorial Fellow)
Biochemical Laboratory, University of Cambridge

(*Received* 1 *June* 1949)

Vol. 53

353

The Amino-

1. THE IDENTIFICAT

nsulin

LYSATES

The Amino-a

1. THE IDENTIFICATION OF LOWER PEPTIDES FROM PARTIAL HYDROLYSATES

By F. SANGER (Beit Memorial Fellow) AND H. TUPPY*
Biochemical Laboratory, University of Cambridge

(*Received* 17 *January* 1951)

The Amino-acid Sequence in the Phenylalanyl Chain of Insulin

2. THE INVESTIGATION OF PEPTIDES FROM ENZYMIC HYDROLYSATES

By F. SANGER (Beit Memorial Fellow) AND H. TUPPY*
Biochemical Laboratory, University of Cambridge

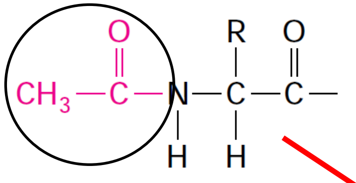
(*Received* 17 *January* 1951)

Hoje você identifica milhares de proteínas, o grande desafio é definir a função

PROTEÍNAS: DIVERSIDADE

- **Modificações pós-traducionais**

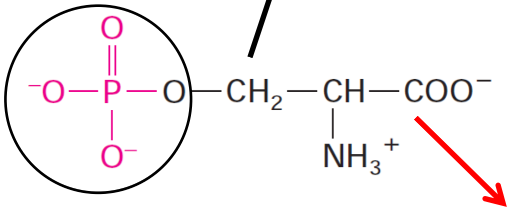
Estabilidade e proteção das proteínas e regulação da interação DNA/Proteína



Acetilação

80% das PTM

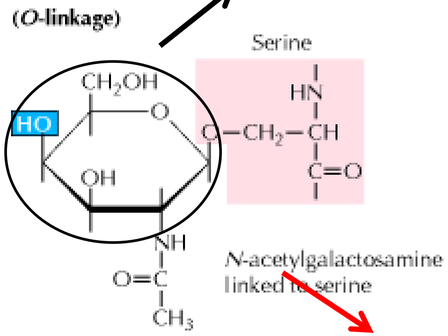
Ativação/Desativação de atividade, sinalização



Fosforilação

Serina, Treonina e Tirosina e Histidina

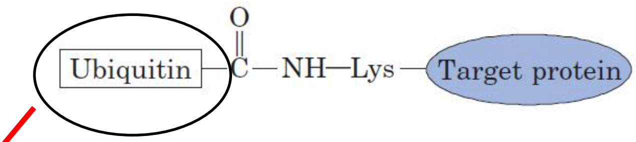
Adição de Carboidratos às proteínas



Glicosilação

Aspargina, Serina e Treonina

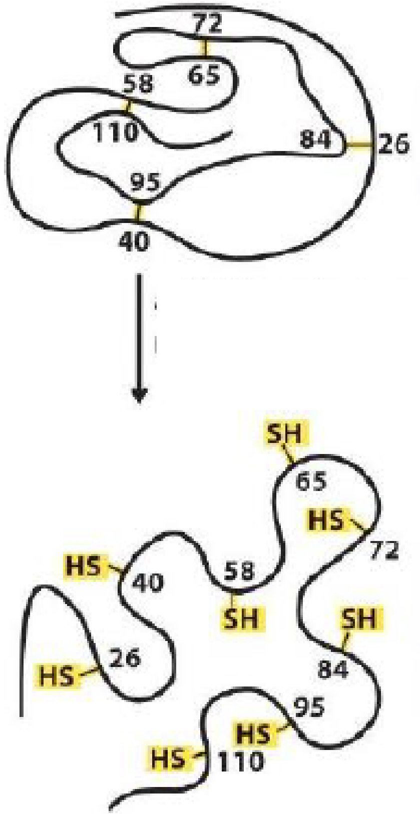
Degradação



Lisina

Ubiquitinação

DOBRAMENTO DE PROTEÍNAS

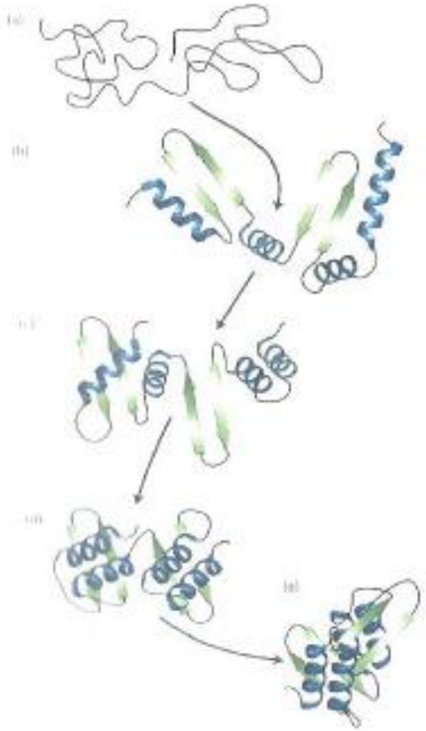


Estrutura primária – define o dobramento, mas leva muito tempo...

Christian B. Anfisen – renaturação para o estado nativo em condições laboratoriais

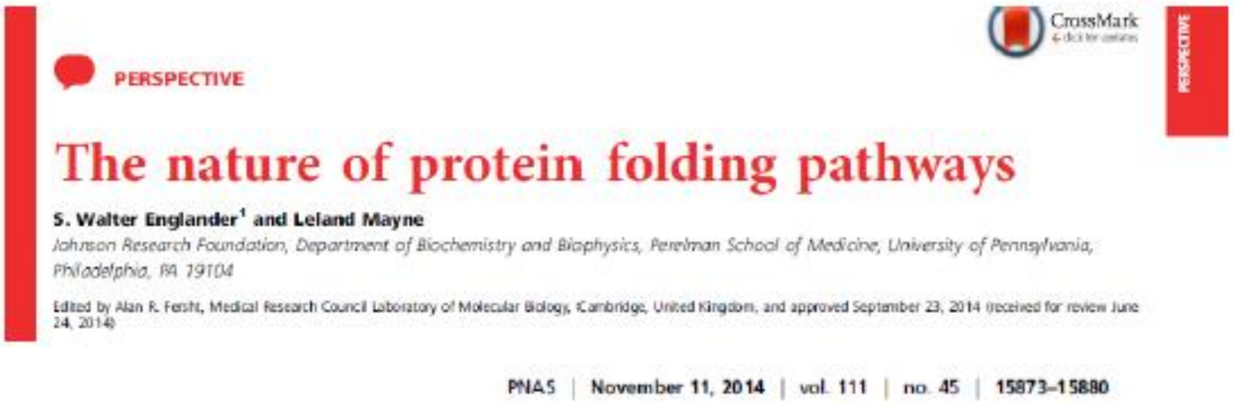
Quantos dobramentos são possíveis? Qual seria o estado nativo?

DOBRAMENTO DE PROTEÍNAS



Há novas descobertas...

Blocos cooperativos de dobramentos - (20 resíduos de aa)



PERSPECTIVE

The nature of protein folding pathways

S. Walter Englander[1] and Leland Mayne
Johnson Research Foundation, Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Edited by Alan R. Fersht, Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom, and approved September 23, 2014 (received for review June 24, 2014)

PNAS | November 11, 2014 | vol. 111 | no. 45 | 15873–15880

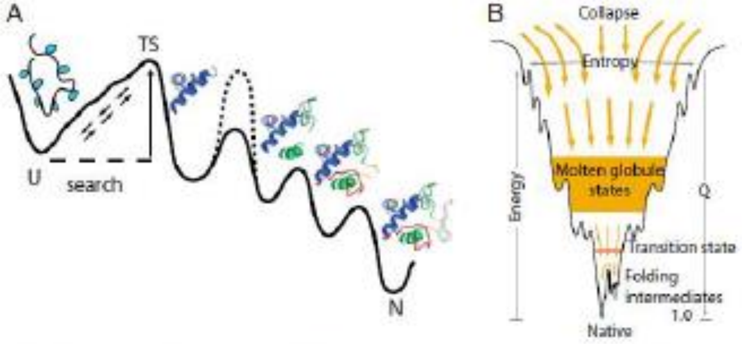


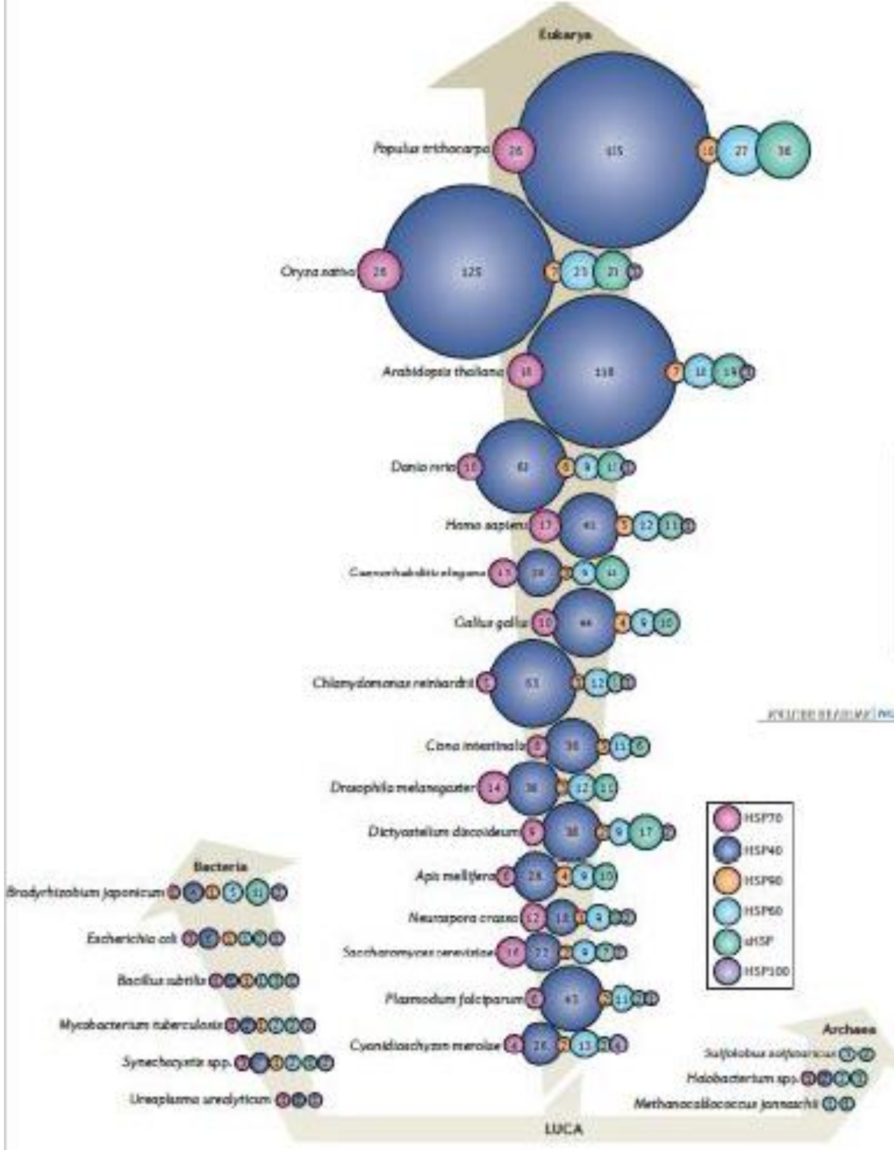
Fig. 1. (*A*) The classical view of a defined folding pathway, and (*B*) the new view of multiple routes through a funneled landscape. Reprinted with permission from ref. 13. Dashed line in *A* illustrates the insertion of an optional error-dependent kinetic barrier, which can affect some population fraction and not others and thus mimic multipathway folding.

PROTEÍNAS: PROTEOSTASE

Dependendo do ambiente, ocorre a adoção de novos padrões de dobramento e o controle da homeostase de proteínas:

- Chaperonas;
- Fatores de dobramento;
- Degradação;
- Sinalização;
- Compartimentos especializados.

PROTEÍNAS: PROTEOSTASE



Diversity in the origins of proteostasis networks — a driver for protein function in evolution

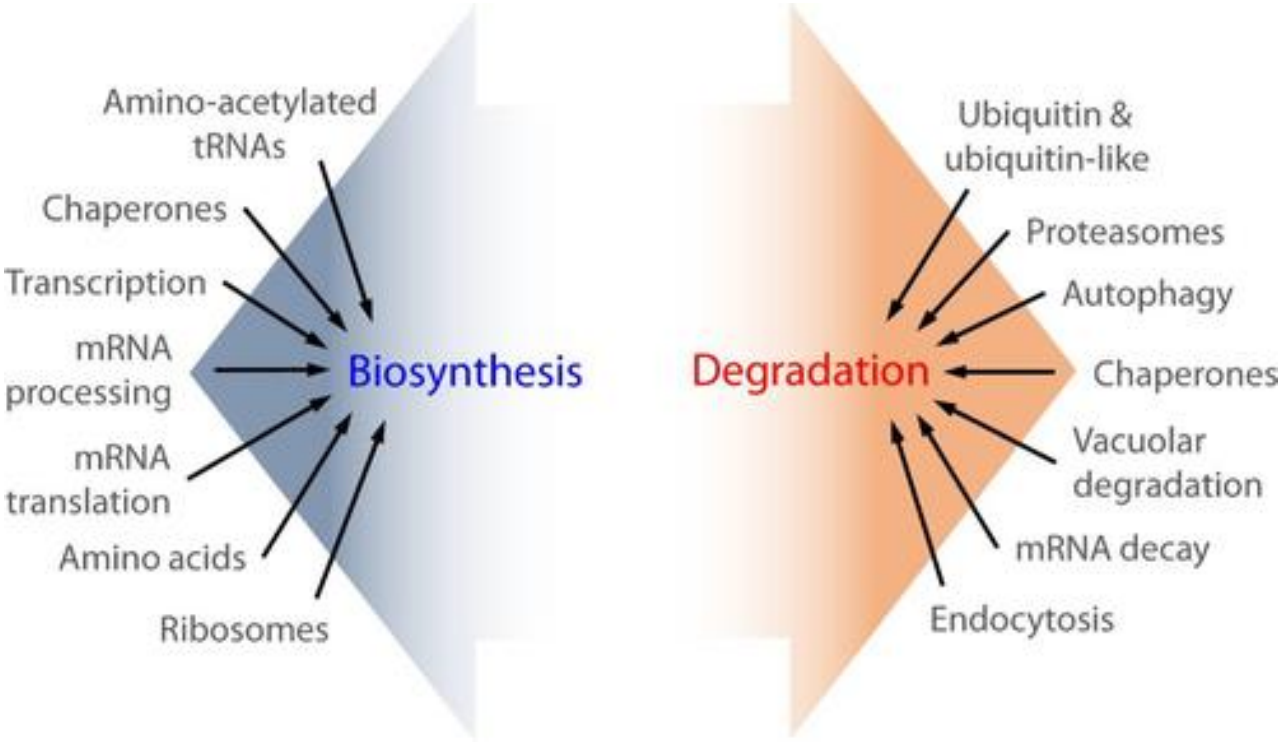
Evan T. Powers[1] and William E. Balch[2]

PROTEÍNAS: PROTEOSTASE

New Phytologist

Plant proteostasis – shaping the proteome: a research community aiming to understand molecular mechanisms that control protein abundance

14 July 2020 | https://doi.org/10.1111/nph.16664 |



Amino-acetylated tRNAs
Chaperones
Transcription
mRNA processing
mRNA translation
Amino acids
Ribosomes
Biosynthesis

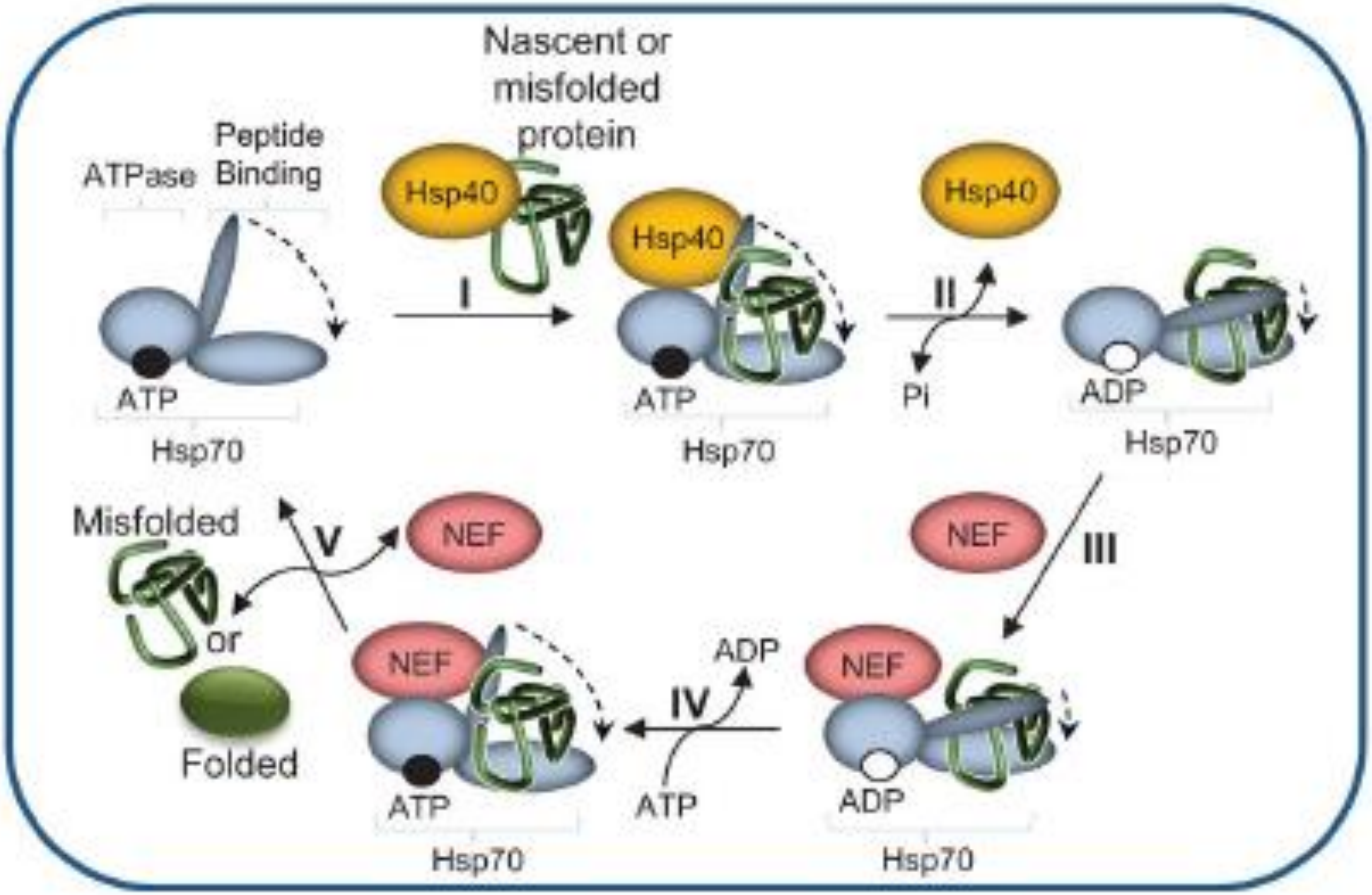
Degradation
Ubiquitin & ubiquitin-like
Proteasomes
Autophagy
Chaperones
Vacuolar degradation
mRNA decay
Endocytosis

PROTEÍNAS: PROTEOSTASE

➢ Chaperonas individuais

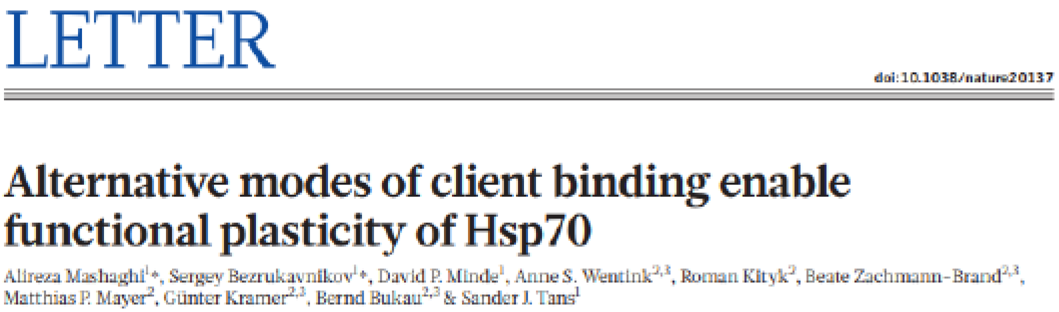
- Eucariotos – **Hsp70**
- Bactérias - **DnaK**

→ Evitam que agredados proteícos se formem



Cochaperonas auxiliam na ligação do substrato e hidrólise do ATP!!

PROTEÍNAS: PROTEOSTASE



LETTER

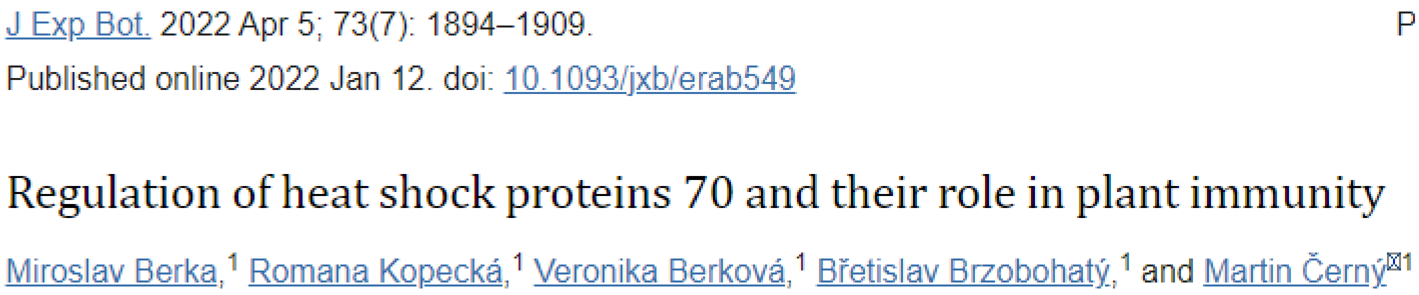
doi:10.1038/nature20137

Alternative modes of client binding enable functional plasticity of Hsp70

Alireza Mashaghi[1]*, Sergey Bezrukavnikov[1]*, David P. Minde[1], Anne S. Wentink[2,3], Roman Kityk[2], Beate Zachmann-Brand[2,3], Matthias P. Mayer[2], Günter Kramer[2,3], Bernd Bukau[2,3] & Sander J. Tans[1]

Cell Stress Chaperones. 2020 Nov;25(6):1071-1081. doi: 10.1007/s12192-020-01144-7. Epub 2020 Jul 27.

Central role of 70-kDa heat shock protein in adaptation of plants to drought stress



J Exp Bot. 2022 Apr 5; 73(7): 1894–1909.

Published online 2022 Jan 12. doi: 10.1093/jxb/erab549

P

Regulation of heat shock proteins 70 and their role in plant immunity

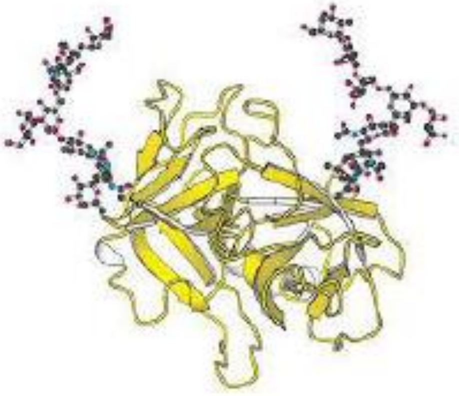
Miroslav Berka,[1] Romana Kopecká,[1] Veronika Berková,[1] Břetislav Brzobohatý,[1] and Martin Černý[1]

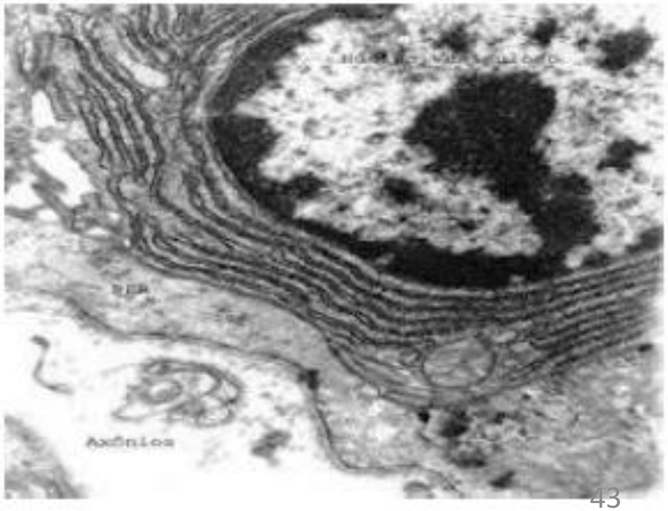
PROTEÍNAS: MODIFICAÇÕES PÓS-TRADUCIONAIS

Modificações químicas também ocorrem ...

Na síntese 20 aminoácidos ... nas células mais de 100 diferentes!!

- Acetilação – aumenta a meia vida
- Fosforilação – serina, treonina, tirosina e histidina
- Glicosilação – asparagina, serina e treonina – cadeias de carboidratos lineares e ramificados



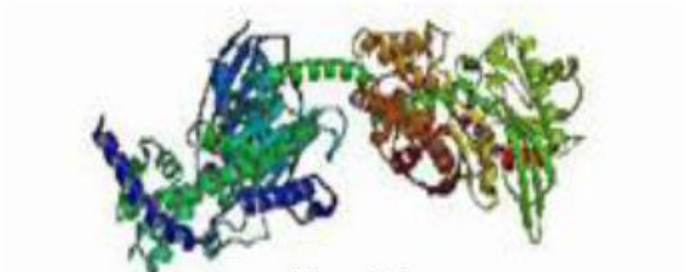


PROTEÍNAS: MODIFICAÇÕES PÓS-TRADUCIONAIS

- **Função é resultado da estrutura!!**

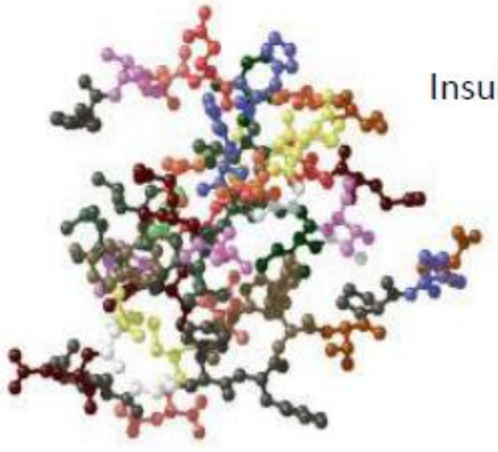
Quase todas as funções dependem da especificidade e afinidade das proteínas!

Comuns em todas as células



Hexokinase

Específicas de tipos celulares



Insulina

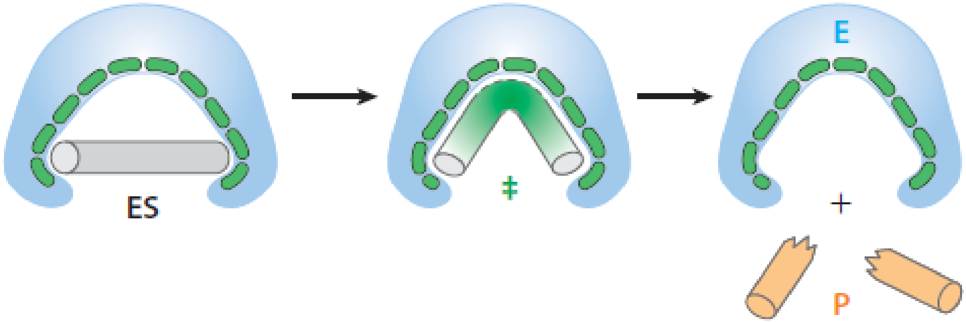
Catalização par aoutras atividades proteícas

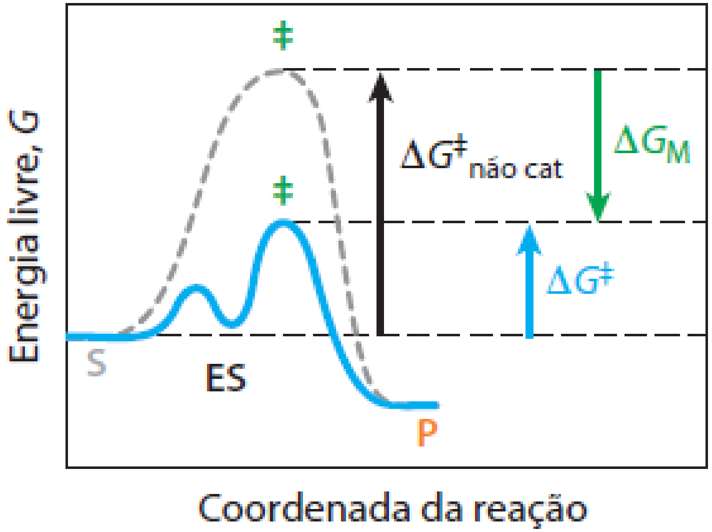
- Fosforilação de proteínas–sinalização
- Hidrólise do ATP

PROTEÍNAS: ENZIMAS

- Alteração química do ligante (substrato)
- Reduz a energia de ativação
- Altamente especializadas (especificidade)

(c) Enzima complementar ao estado de transição



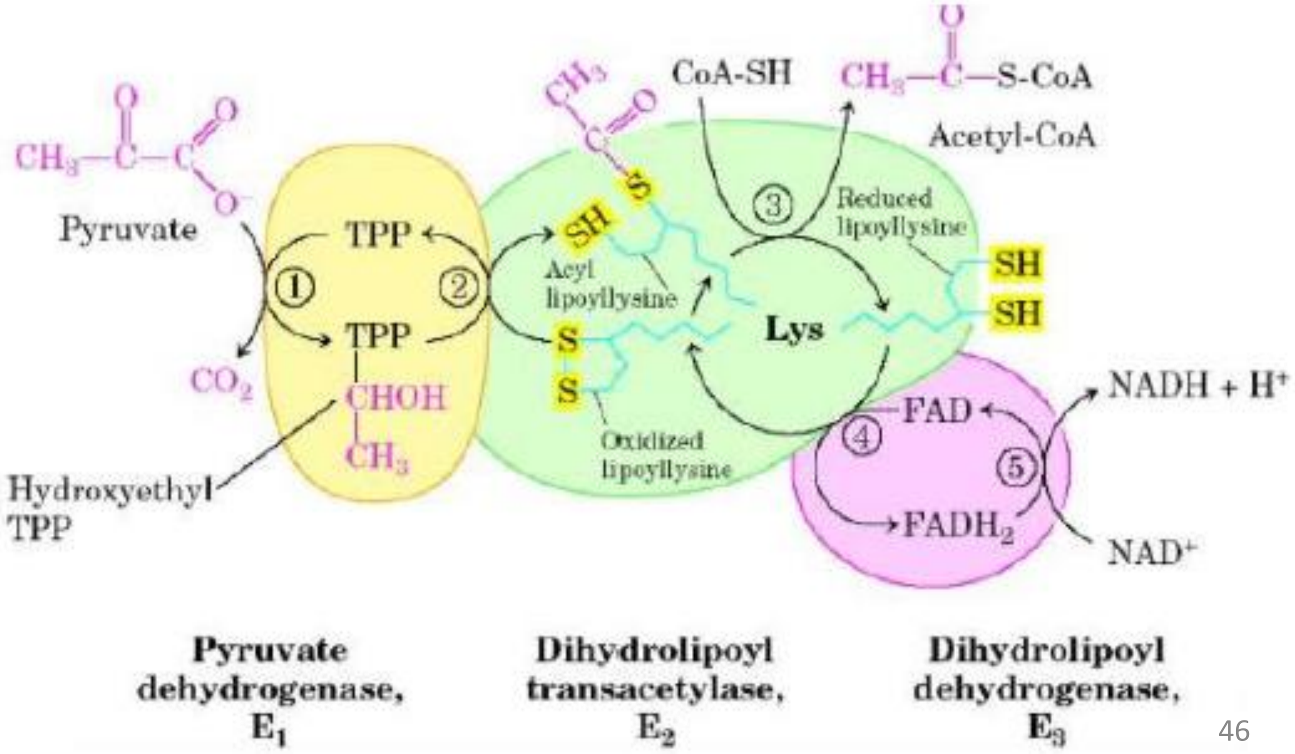


PROTEÍNAS: ENZIMAS

➢ ESTRUTURAS MUTIMÉRICAS

Otimização do processo catalítico:

- Mesma localização
- Estruturas multiméricas
- Coevolução



PROTEÍNAS: MOVIMENTO

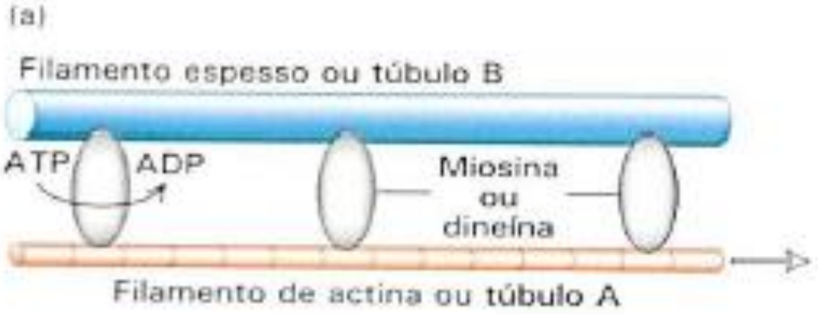
- **Movimento celular e transporte intracelular**

Energia → Movimento

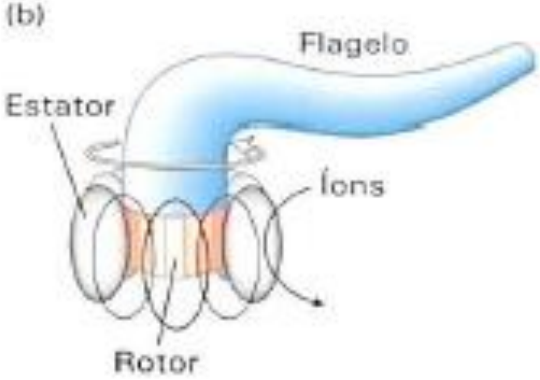
Proteínas motoras

Hidrólise do ATP

Gradiente de íons



Movimento linear



Movimento rotatório

PROTEÍNAS: ALOSTERIA

Nas células tudo é controlado - palavra de ordem é otimização!

Alosteria: qualquer alteração na estrutura terceária ou quartenária induzida pela ligação à um ligante.

Ligante

Sítio Ativo

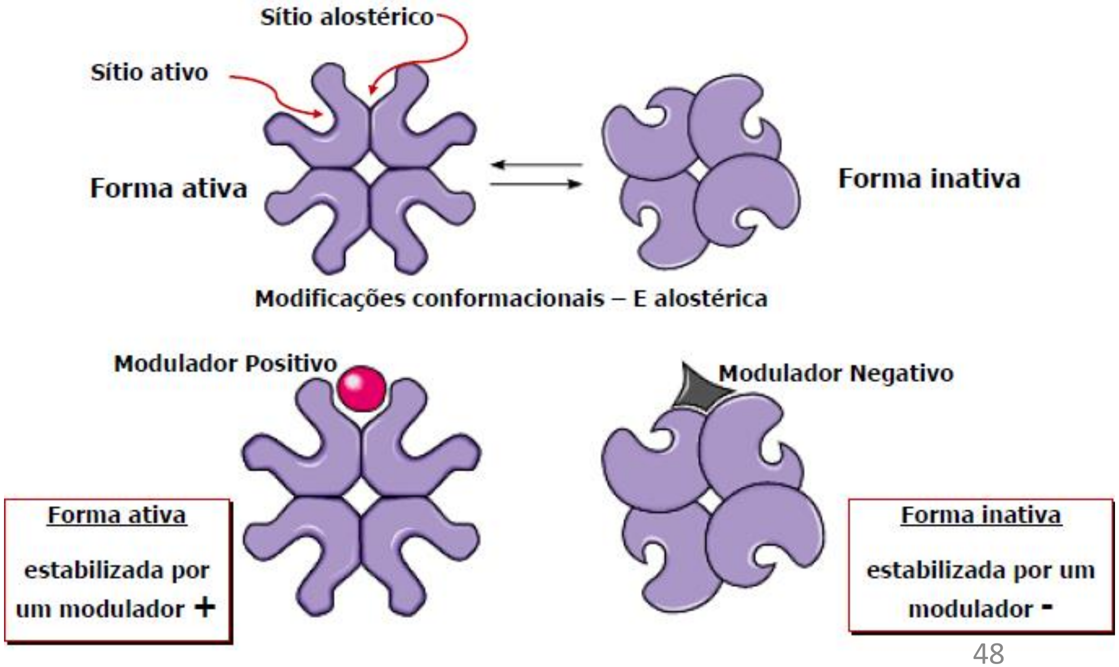
Substrato

Sítio Alostérico

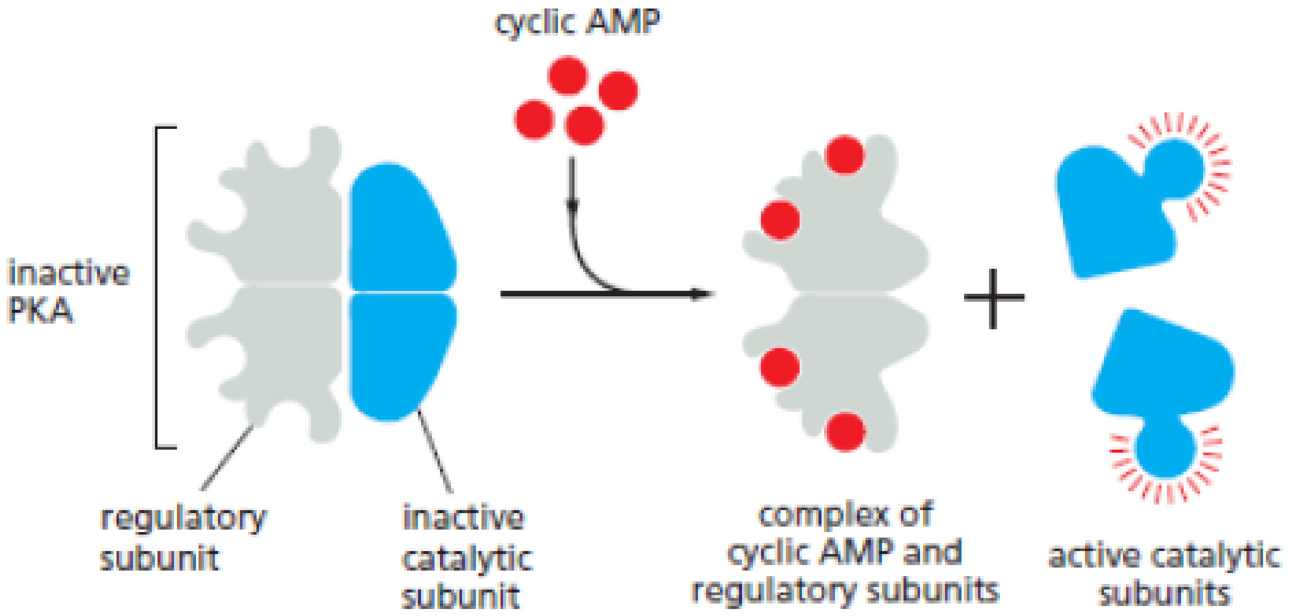
Moduladores ou Efetores

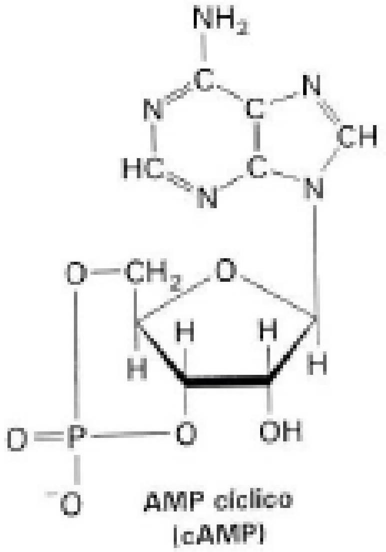
Mudança de conformação

Alteração na atividade



PROTEÍNAS: ALOSTERIA





AMP cíclico
(cAMP)

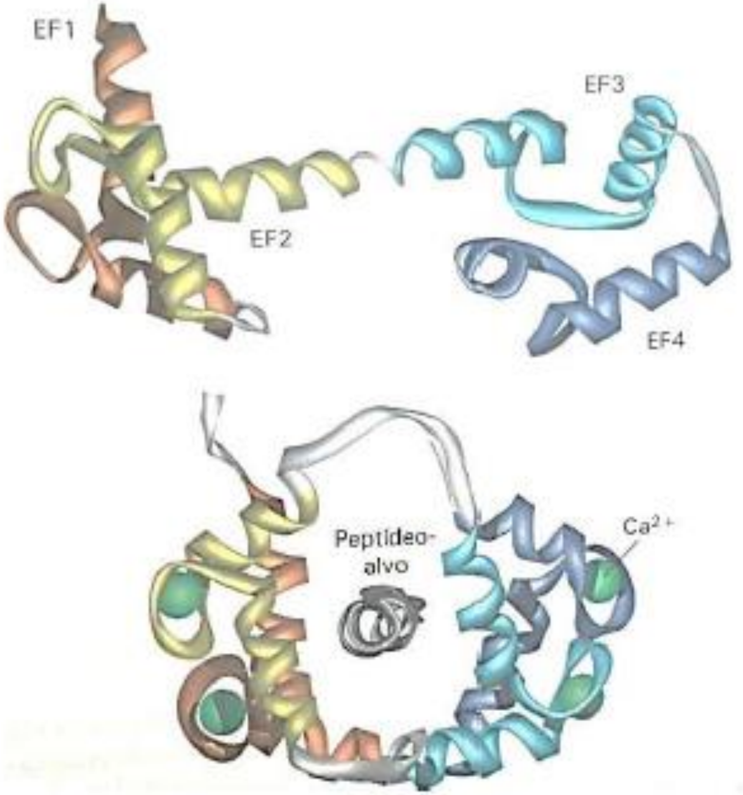
Importantes na regulação de expressão gênica – ativação de fatores de transcrição

PROTEÍNAS: SINALIZAÇÃO

- **Cálcio: modulador da atividade protéica**

Calmodulina – complexo mão EF

Liga e desliga outras proteínas!



PROTEÍNAS: UBIQUITINAÇÃO

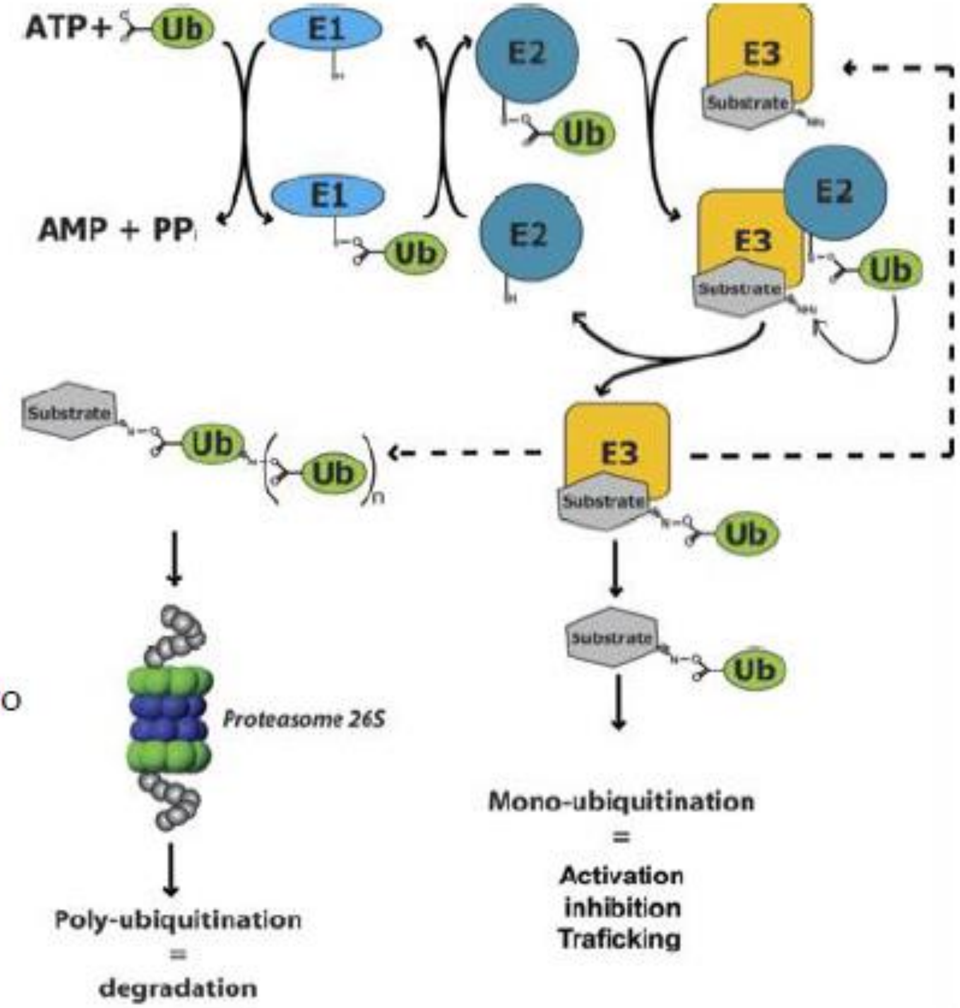
- **Há também o controle da quantidade de proteínas**

Processo de ubuquitinação

E1 – complex de ativação da ubiquitina
E2 – Enzima conjudadora da ubiquitina (resíduo de cisteína)
E3 – Ubiquitina ligase

Transferência para lisina da proteína alvo
Ligação com a glicina C-terminal da ubiquitina

Poliubiquitinação- ligação da nova ubiquitina a lys48...



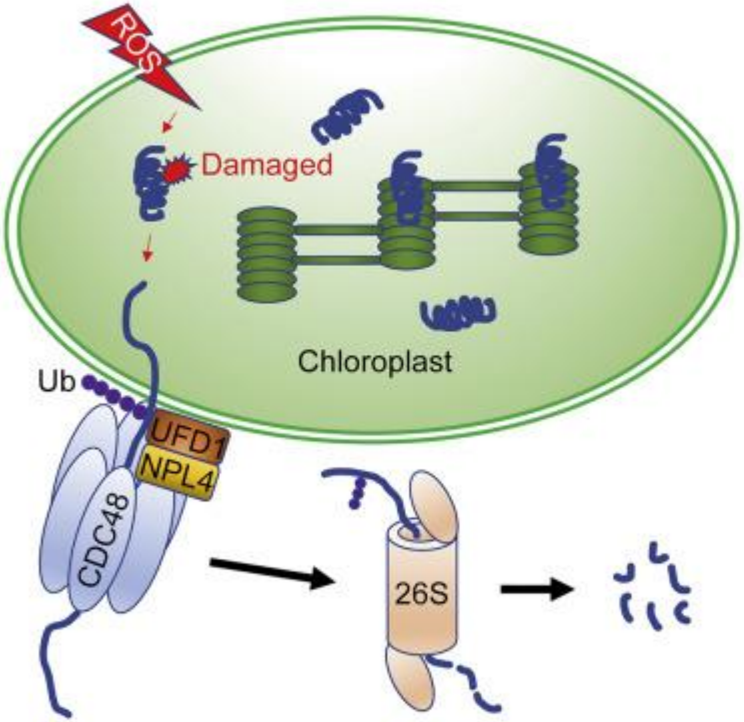
Cell Reports

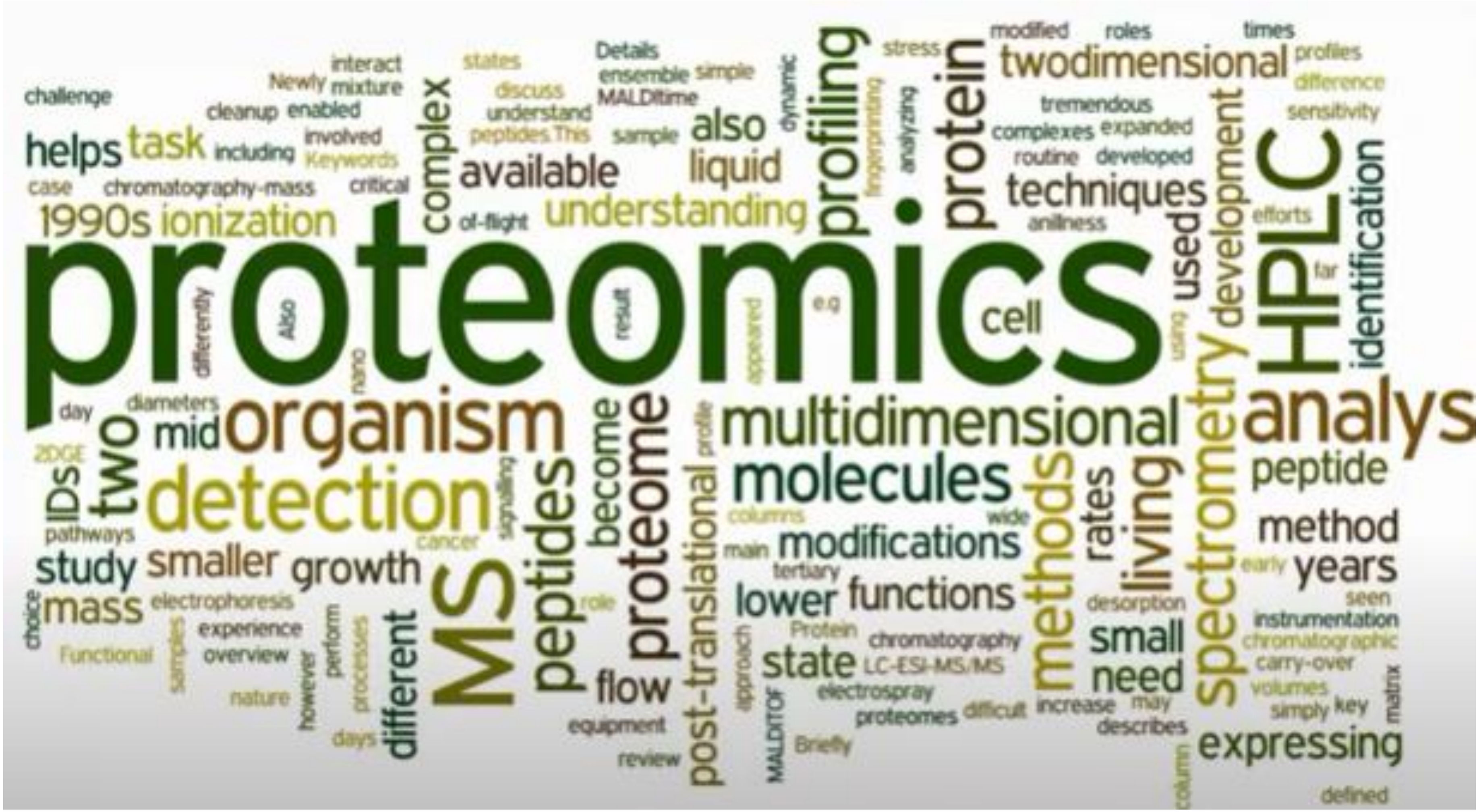
Article

The CDC48 complex mediates ubiquitin-dependent degradation of intra-chloroplast proteins in plants

Authors

Jialong Li, Jiarui Yuan, Yuhong Li, ...,
Wenqiang Yang, Wenhao Zhang,
Rongcheng Lin




proteomics
HPLC
MS
analys
organism
detection
multidimensional
molecules
profiling
protein
twodimensional
techniques
available
understanding
liquid
also
complex
ionization
1990s
helps
task
spectrometry
development
identification
used
cell
proteome
peptides
two
become
post-translational
modifications
functions
lower
state
methods
living
rates
peptide
method
years
small
need
expressing
study
smaller
growth
mass
mid
different
flow
IDs
chromatography
LC-ESI-MS/MS
electrospray
proteomes
electrophoresis
experience
overview
Functional
challenge
interact
Newly
mixture
cleanup
enabled
involved
including
Keywords
case
chromatography-mass
critical
states
discuss
understand
peptides.This
sample
Details
ensemble
simple
MALDItime
of-flight
dynamic
fingerprinting
analyzing
stress
modified
roles
times
profiles
difference
sensitivity
tremendous
complexes
expanded
routine
developed
anilness
efforts
far
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differently
Also
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appeared
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diameters
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2DGE
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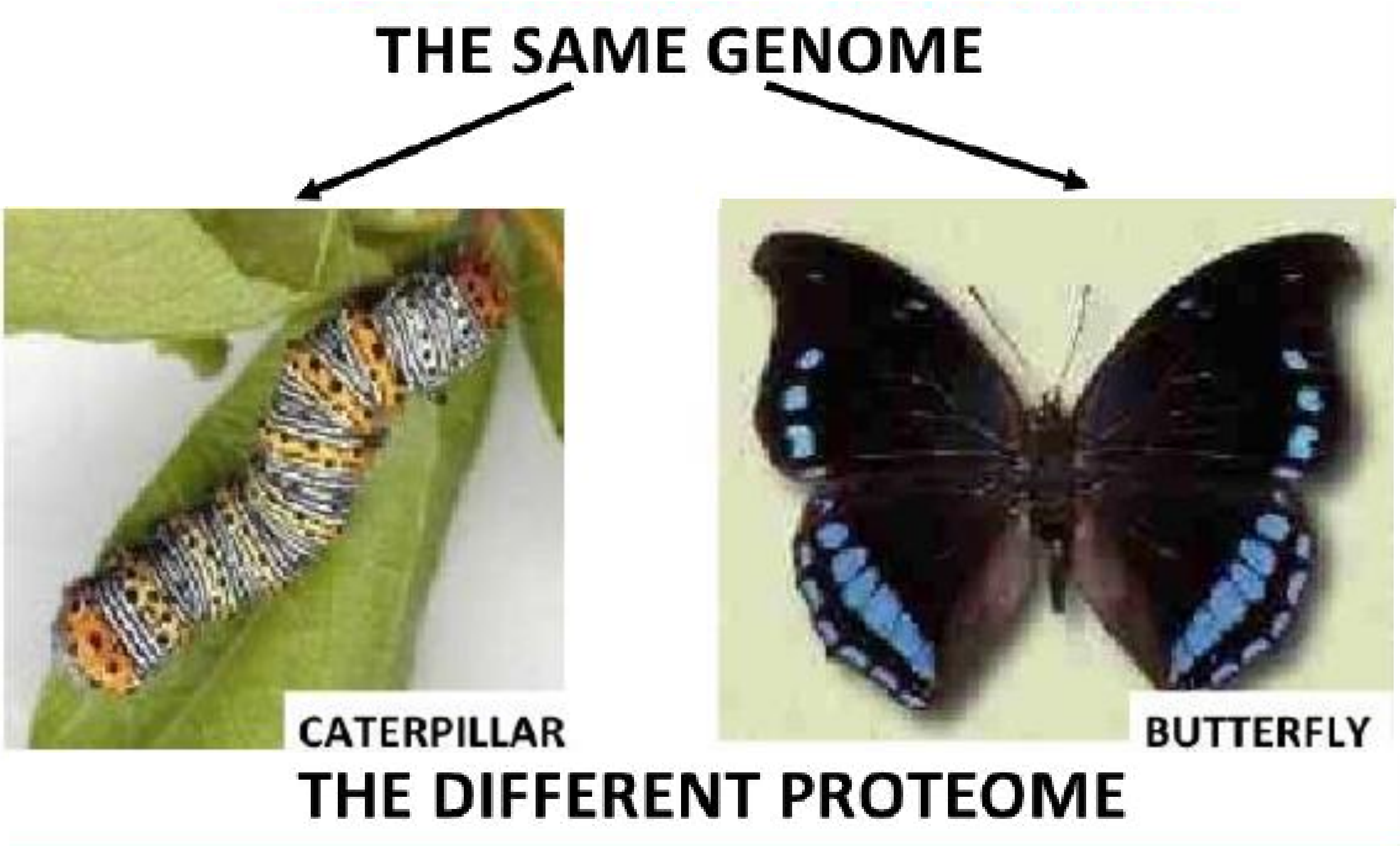
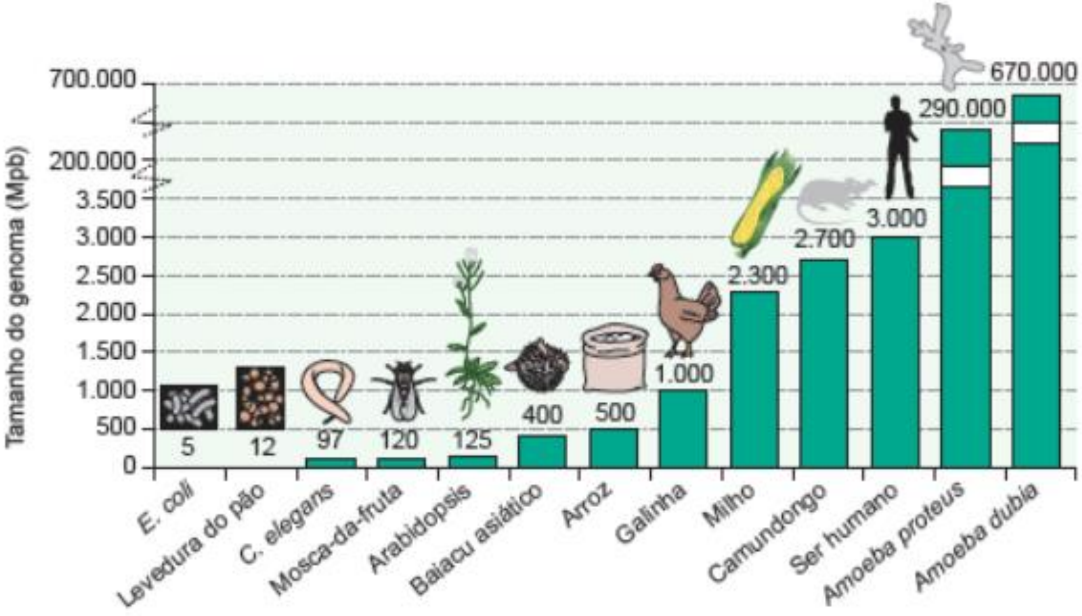
cancer
signalling
columns
wide
main
tertiary
profile
early
desorption
seen
instrumentation
chromatographic
carry-over
volumes
simply
key
matrix
defined
describes
may
increase
difficult
Briefly
MALDITOF
approach
Protein
review
equipment
role
choice
samples
however
perform
processes
nature
days
column

PROTEÔMICA: O INÍCIO

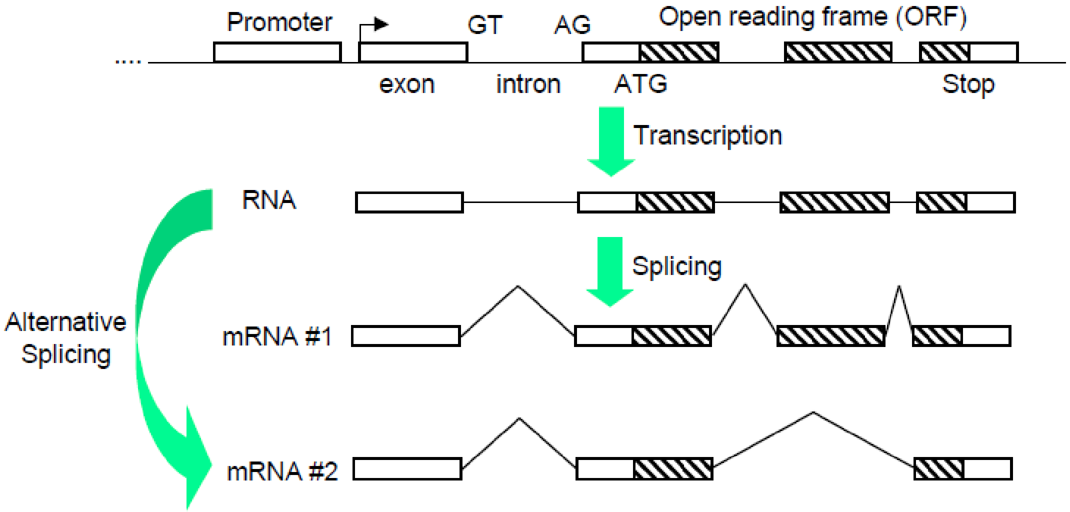
Qual é a diferença?



PROTEÔMICA: O INÍCIO

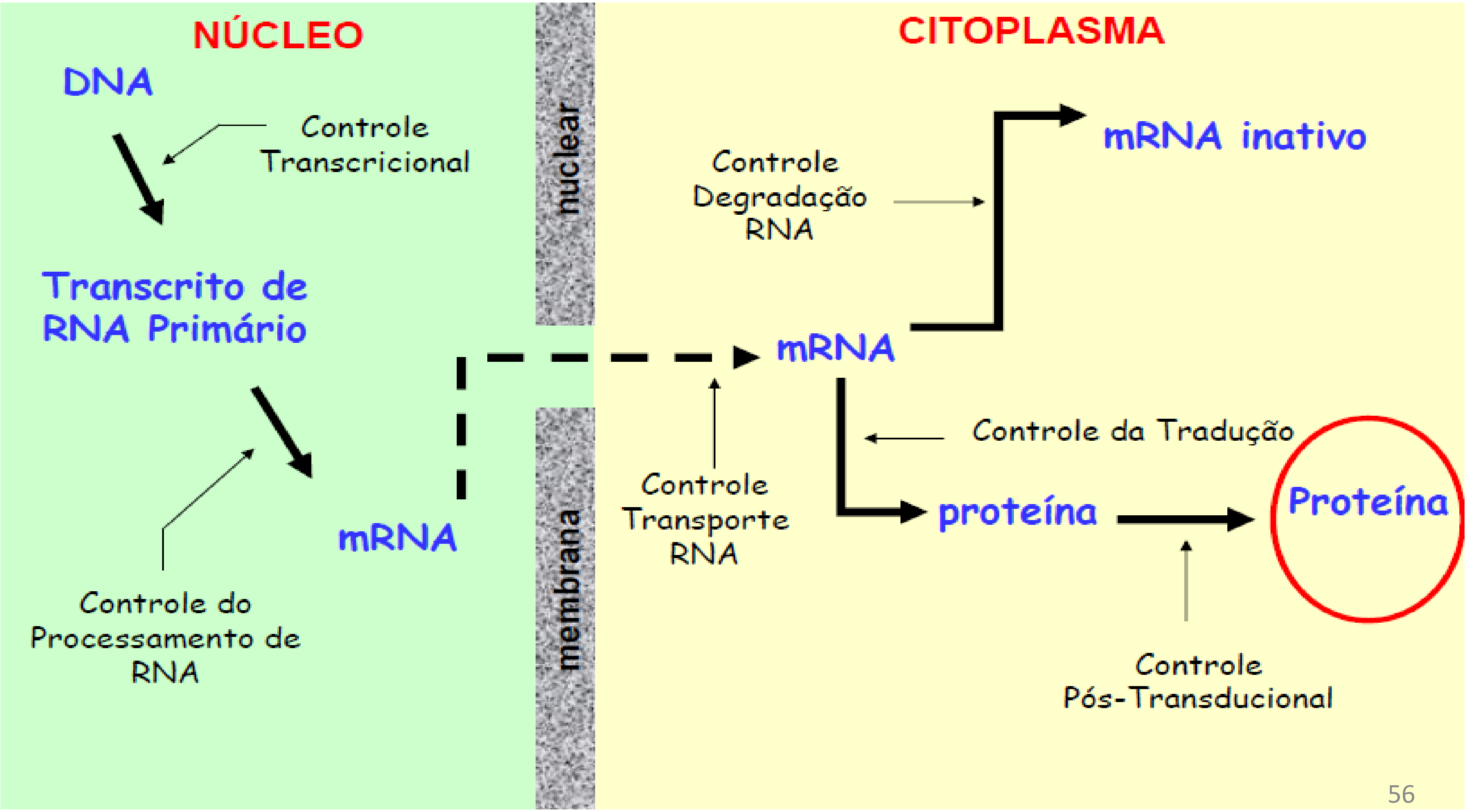


Como esses genes são “usados” ?



PROTEÔMICA: O INÍCIO

- ✓ **As proteínas fornecem informações, em nível molecular, sobre a variabilidade genética que é efetivamente expressa pelo genoma !!!**



PROTEÔMICA: DEFINDO CONCEITOS

❖ PROTEOMA

- Conjunto de todas as proteínas de uma célula, organela, tecido, ou organismo em um dado momento/ condição.

❖ PROTEÔMICA

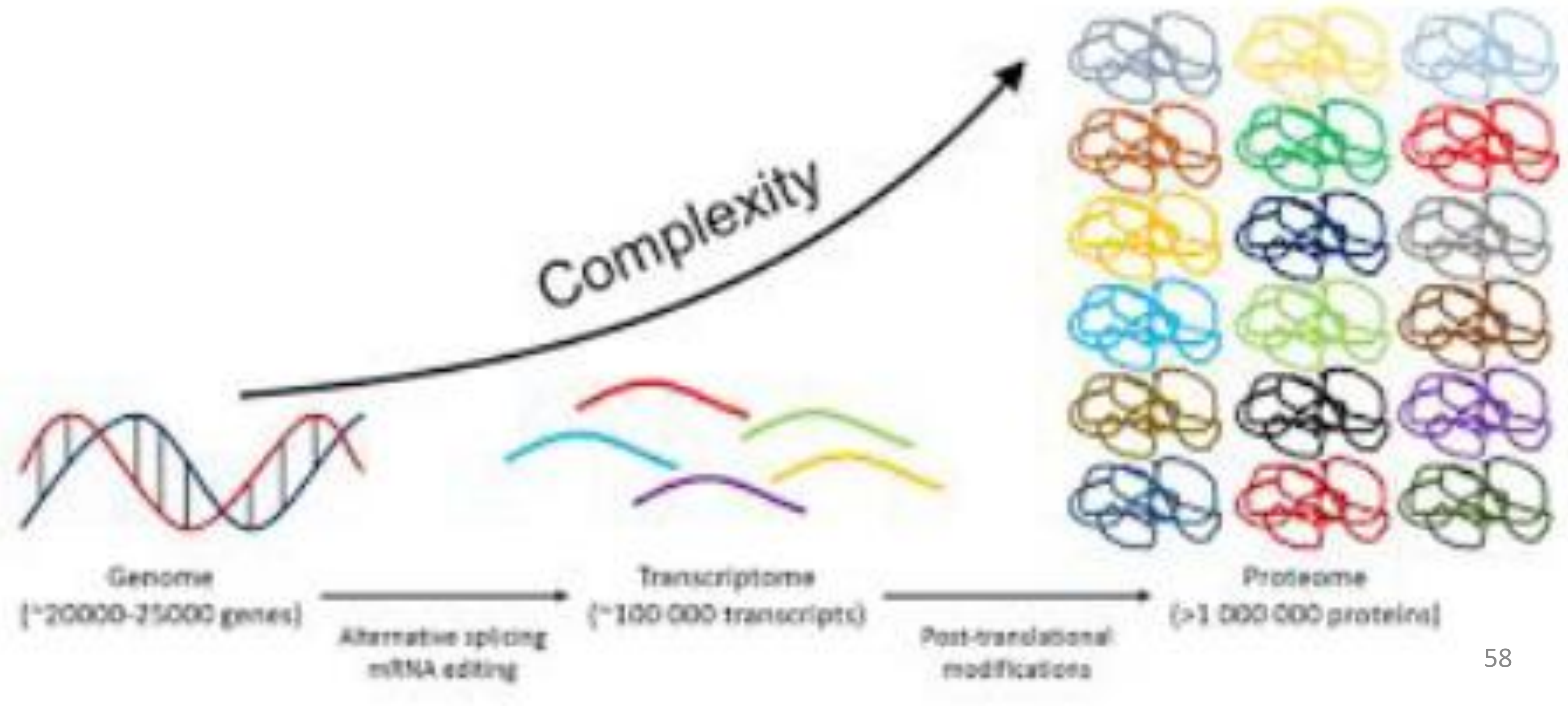
- Metodologia, conjunto de técnicas utilizadas para se estudar o Proteoma.

Análise qualitativa e quantitativa; identificação de modificações pós-traducionais (PTMS), identificação de proteoformas ...

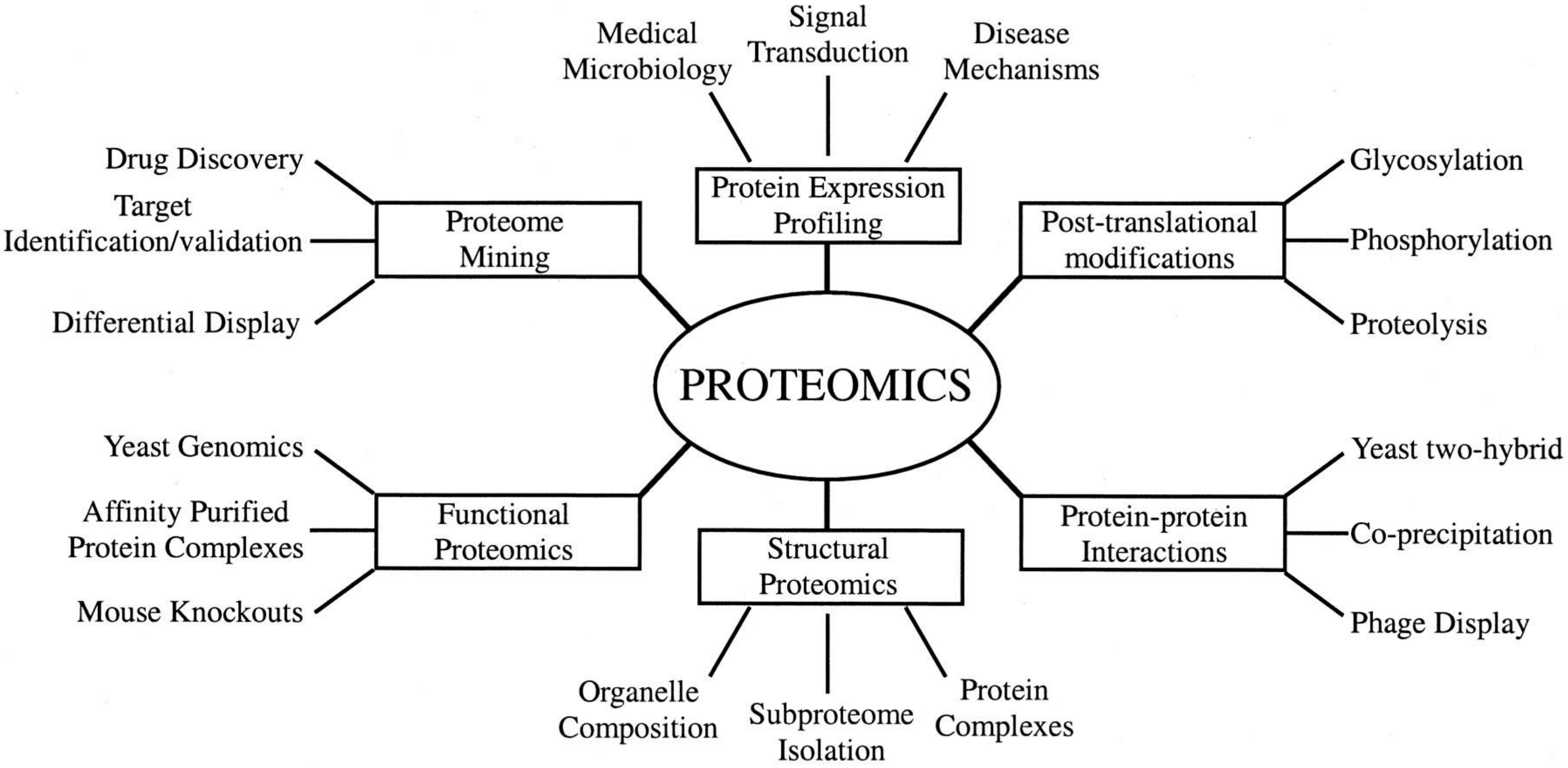
PROTEÔMICA: O INÍCIO

Marc Wilkins em 1994.

“The entire protein complement expressed by a genome, or by a cell or tissue type”



PROTEÔMICA: POSSIBILIDADES

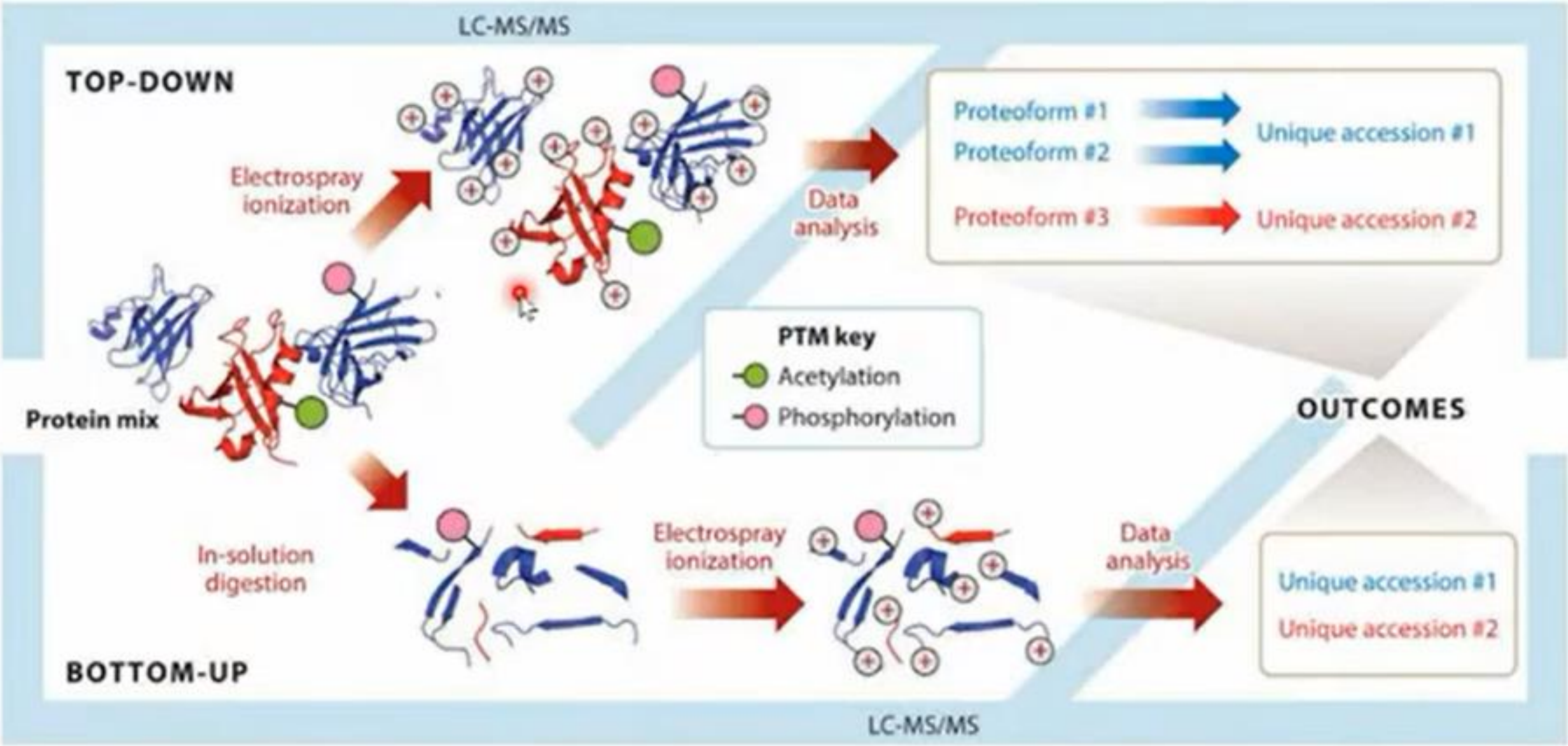


http://mmbr.asm.org/content/66/1/39/F1.large.jpg

PROTEÔMICA: ABORDAGENS EM PROTEÔMICA

➢ BOTTOM-UP E TOP-DOWN

- **Identificação de amostras complexas.**
- **Limitação na identificação de MPT.**



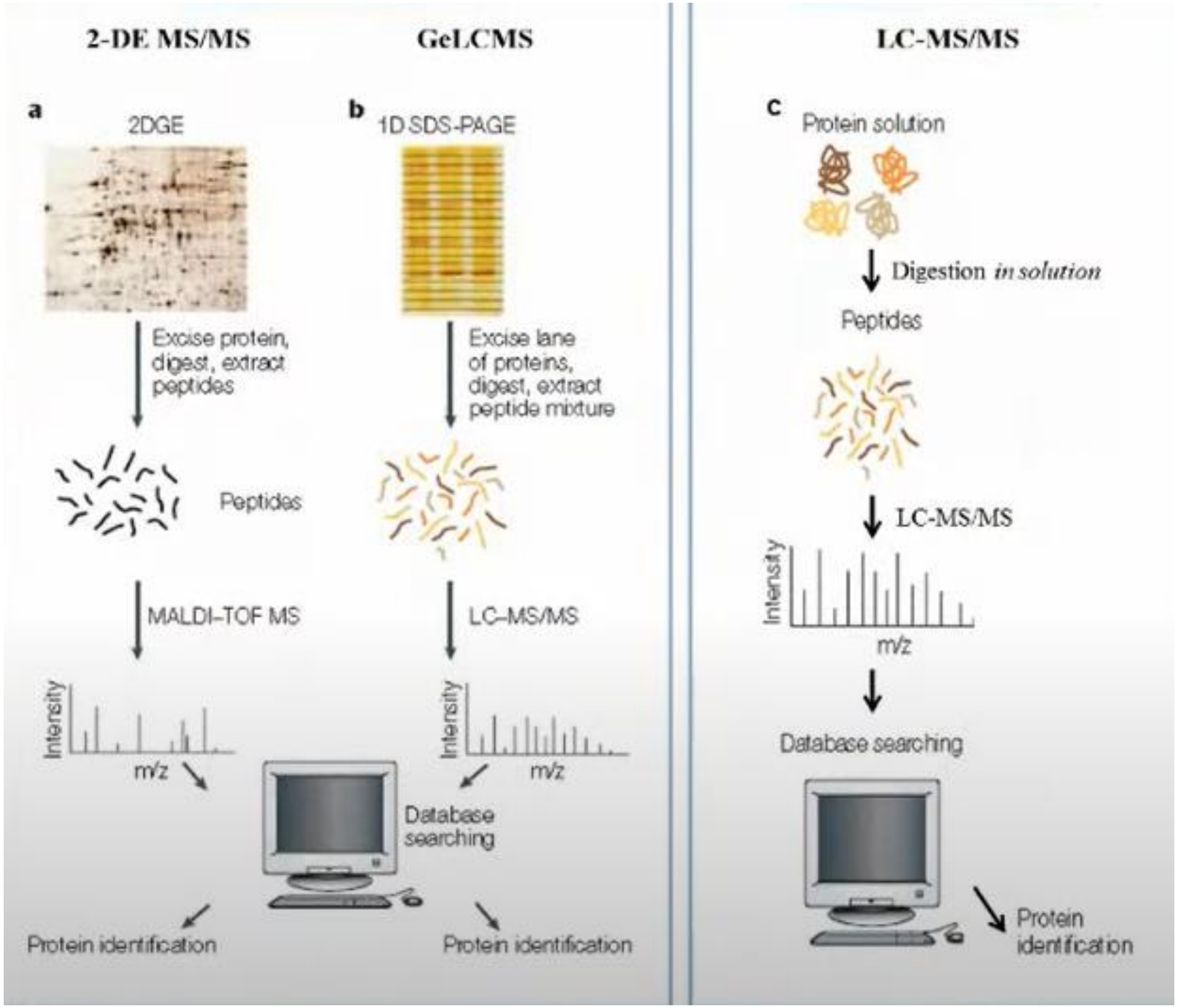
- **Separa proteoformas.**
- **Identificação de proteínas desconhecidas e MPT**

Toby T K, et al.; 2016

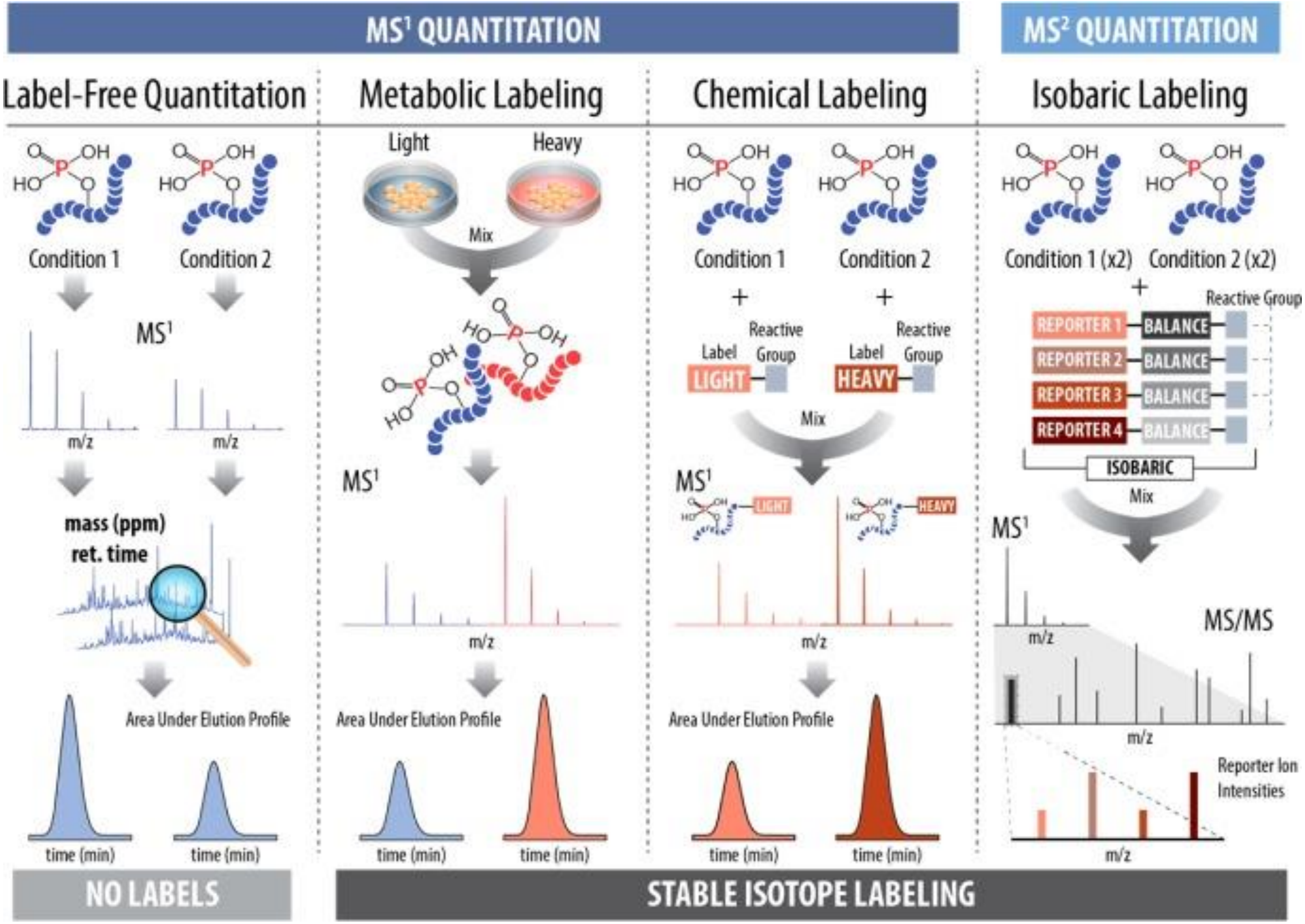
PROTEÔMICA: BOTTOM-UP E TOP-DOWN

- **BOTTOM-UP**

Gel-based **Gel-free**



PROTEÔMICA: ESTRATÉGIAS DE QUANTIFICAÇÃO

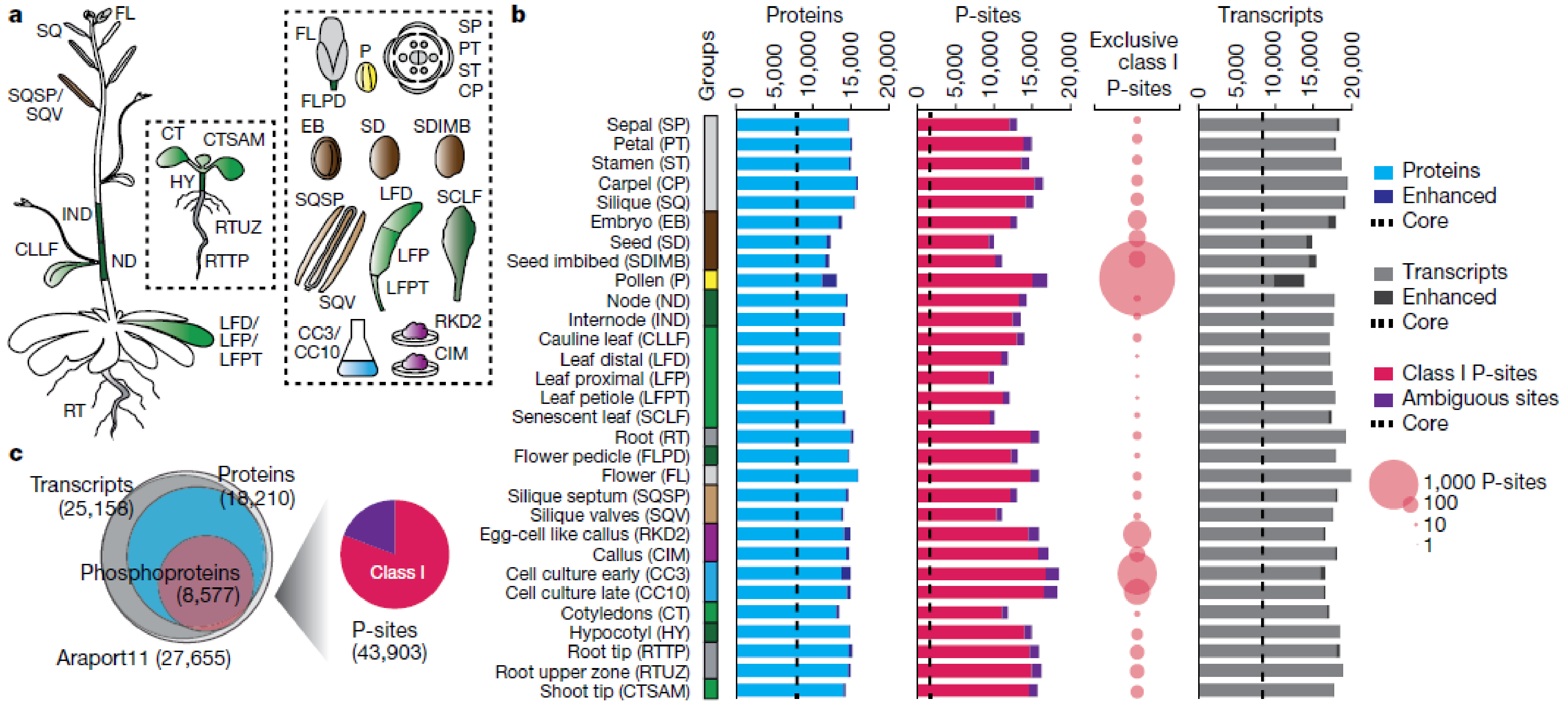


Anal Chem. 2016 Jan 5; 88(1): 74–94.

PROTEÔMICA: EXEMPLO

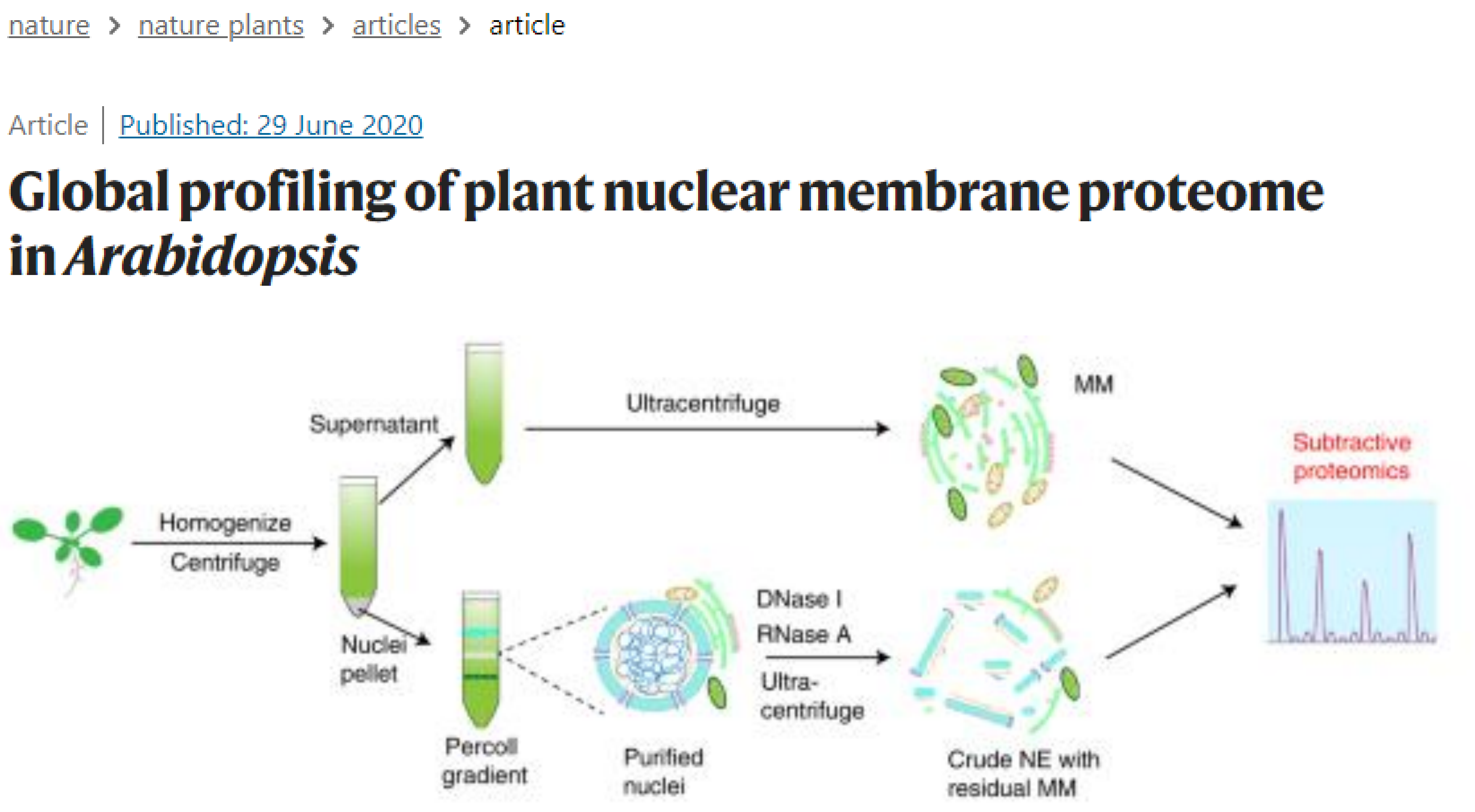
› Nature. 2020 Mar;579(7799):409-414. doi: 10.1038/s41586-020-2094-2. Epub 2020 Mar 11.

Mass-spectrometry-based draft of the Arabidopsis proteome



- Quantitative atlas 30 tissues
- more than 18,000 proteins, representing 66% of the annotated protein-coding genes
- 43,000 sites phosphorylation

PROTEÔMICA: EXEMPLO



nature > nature plants > articles > article

Article | Published: 29 June 2020

Global profiling of plant nuclear membrane proteome in *Arabidopsis*

- Obtained 1,058 candidates for NE-enriched proteins
- Identified ~200 potential candidates for plant nuclear envelope

PROTEÔMICA: EXEMPLO

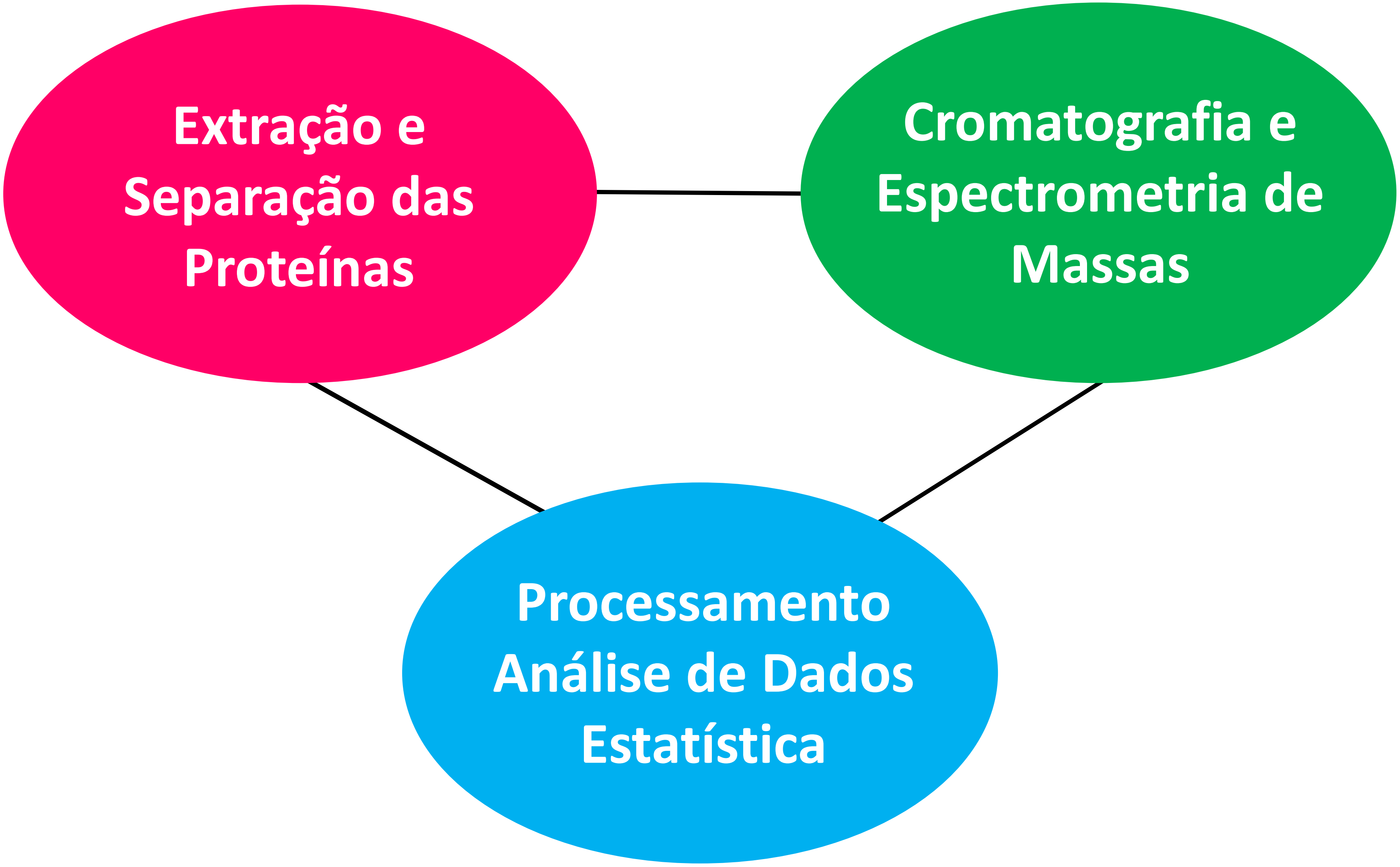
EXPERT REVIEW OF PROTEOMICS
2021, VOL. 18, NO. 2, 93–103
https://doi.org/10.1080/14789450.2021.1910028

PERSPECTIVE

Proteomics and plant biology: contributions to date and a look towards the next decade

J V Jorrin Novo

PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

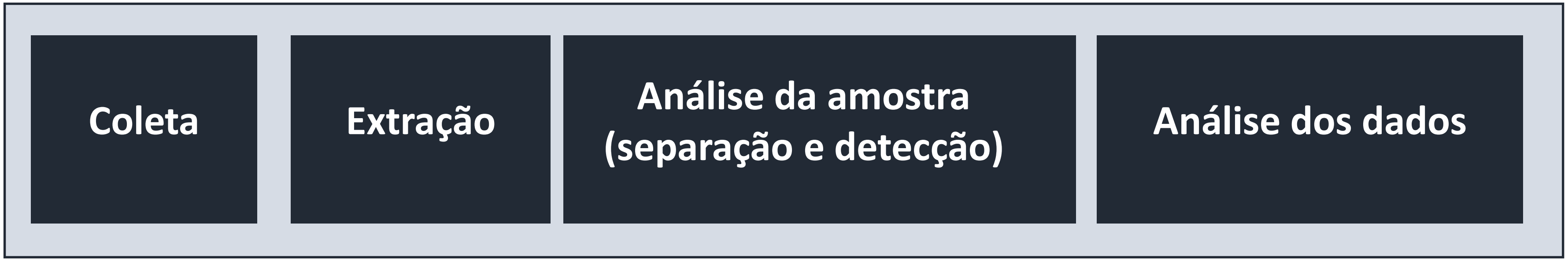


Extração e Separação das Proteínas

Cromatografia e Espectrometria de Massas

Processamento Análise de Dados Estatística

PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

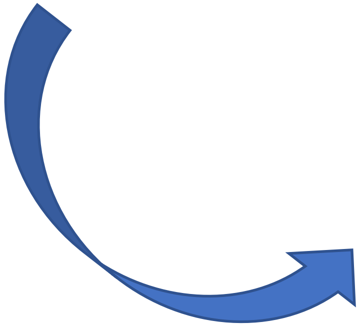


Coleta

Extração

Análise da amostra (separação e detecção)

Análise dos dados



- **Repetição biológica**
- **Interrupção RÁPIDA do metabolismo (*Quenching*)**

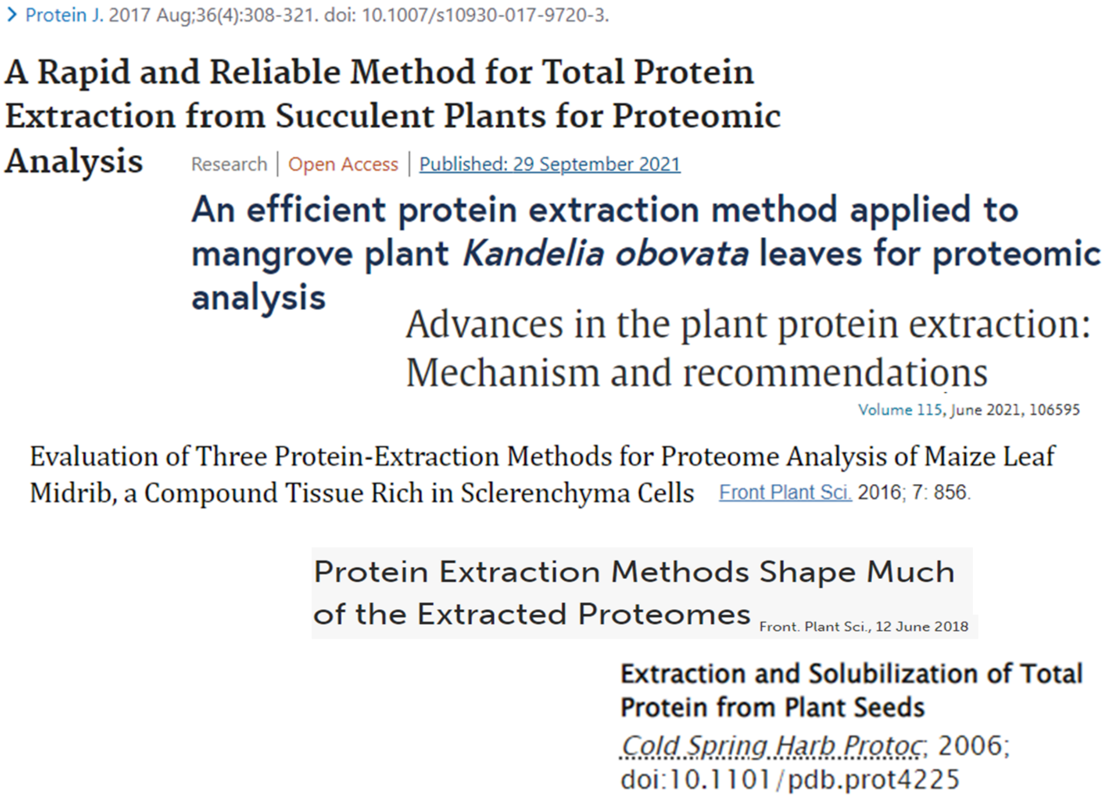
Minimizar a formação ou a degradação de proteínas e metabólitos após a coleta

- **Armazenamento em -80ºC**

PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

➢ EXTRAÇÃO

- ✓ Romper as interações macromoleculares (interações covalentes).
- ✓ Evitar modificações pos-traducionais.



› Protein J. 2017 Aug;36(4):308-321. doi: 10.1007/s10930-017-9720-3.

A Rapid and Reliable Method for Total Protein Extraction from Succulent Plants for Proteomic Analysis

Research | Open Access | Published: 29 September 2021

An efficient protein extraction method applied to mangrove plant *Kandelia obovata* leaves for proteomic analysis

Advances in the plant protein extraction: Mechanism and recommendations

Volume 115, June 2021, 106595

Evaluation of Three Protein-Extraction Methods for Proteome Analysis of Maize Leaf Midrib, a Compound Tissue Rich in Sclerenchyma Cells Front Plant Sci. 2016; 7: 856.

Protein Extraction Methods Shape Much of the Extracted Proteomes Front. Plant Sci., 12 June 2018

Extraction and Solubilization of Total Protein from Plant Seeds

Cold Spring Harb Protoc; 2006;
doi:10.1101/pdb.prot4225

PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

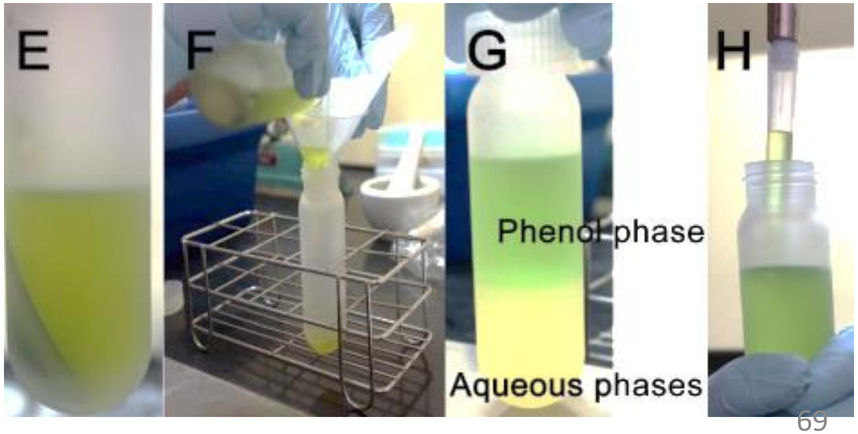
Diferentes protocolos para a extração de proteínas totais:

Extração com ácido tricloroacético (TCA)/Acetona (Damerval et al., 1986): rápida inativação de proteases;

Extração fenólica (Hurkman & Tanaka, 1986): própria para tecidos ricos em compostos fenólicos;

Extração fenólica + TCA/Acetona (Wang et al., 2003): própria para tecidos ricos em fenóis, lipídeos e pigmentos;

Extração de proteínas com remoção da rubico;



Da-Gin et al. 2014. bio-protocol.org/e1277

PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

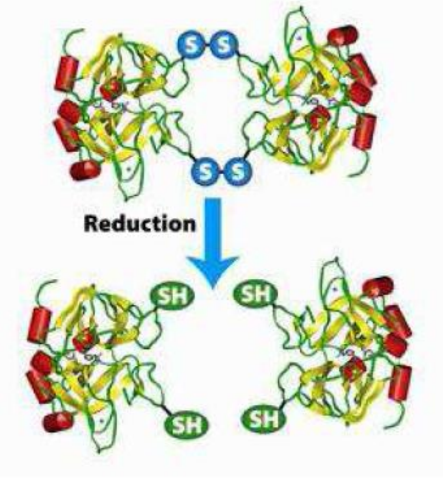
➢ SOLUBILIZAÇÃO DAS PROTEÍNAS:

✓ Agentes caotrópicos (e.x. uréia, tiouréia)

- Rompem as interações não covalentes (ex: interações hidrofóbicas)

✓ Agentes redutores (e.x. DTT, β-mercaptoetanol)

- Rompem as pontes dissulfeto



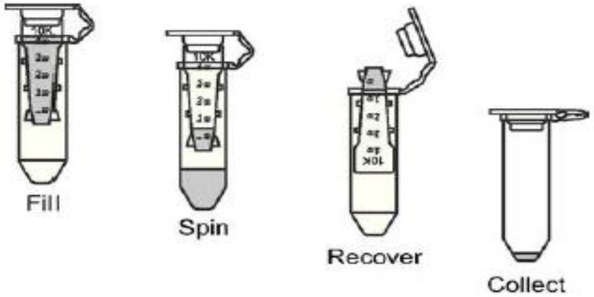
✓ Detergentes (e.x. CHAPS e Triton)

- Rompem as interações hidrofóbicas, promovendo a solubilização de proteínas de membrana e o rompimento das interações entre proteínas.

PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

✓ DESSALINIZAÇÃO

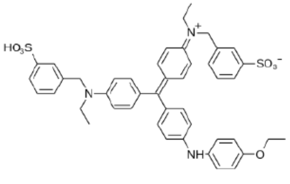
Amicon Ultra-0.5 mL Centrifugal Filters for DNA and Protein Purification and Concentration



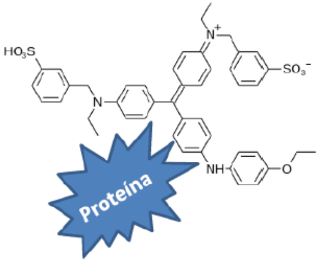
Colunas de dessalinização

✓ QUANTIFICAÇÃO DE PROTEÍNAS

*** Método de Bradford**



Coomassie brilland blue



*** NanoDrop**

*** Bioanalyzer**

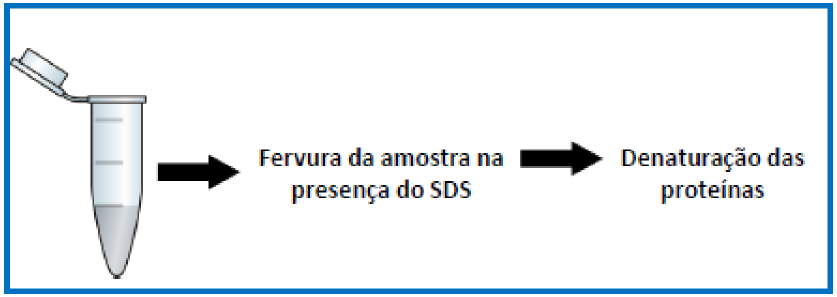
✓ DIGESTÃO DE PROTEÍNAS: Tripsina

PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

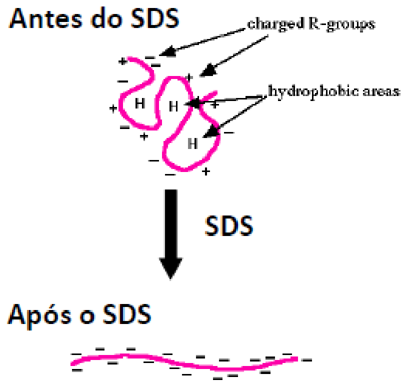
GEL SDS-PAGE

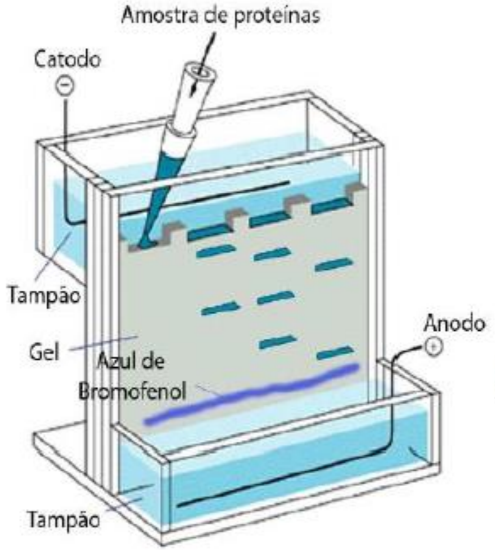
SDS (dodecil-sulfato de sódio) - PAGE (poliacrilamida)

PREPARO DE AMOSTRAS



Fervura da amostra na presença do SDS → Denaturação das proteínas



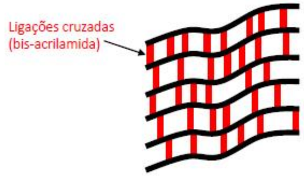


Migração das proteínas no campo elétrico

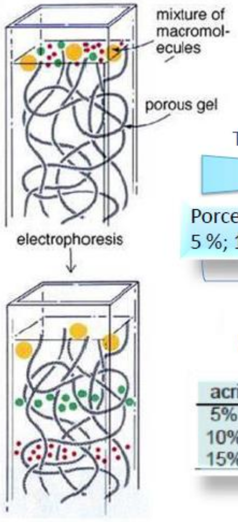
↓

Separação das proteínas por diferença de massa molecular

[monômeros] determina o tamanho dos poros



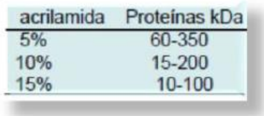
Polímero de acrilamida e bis-acrilamida



Gel serve como uma "peneira"

Porcentagem de acrilamida:
5 %; 10 %, 15 %; 20 %; 30 %

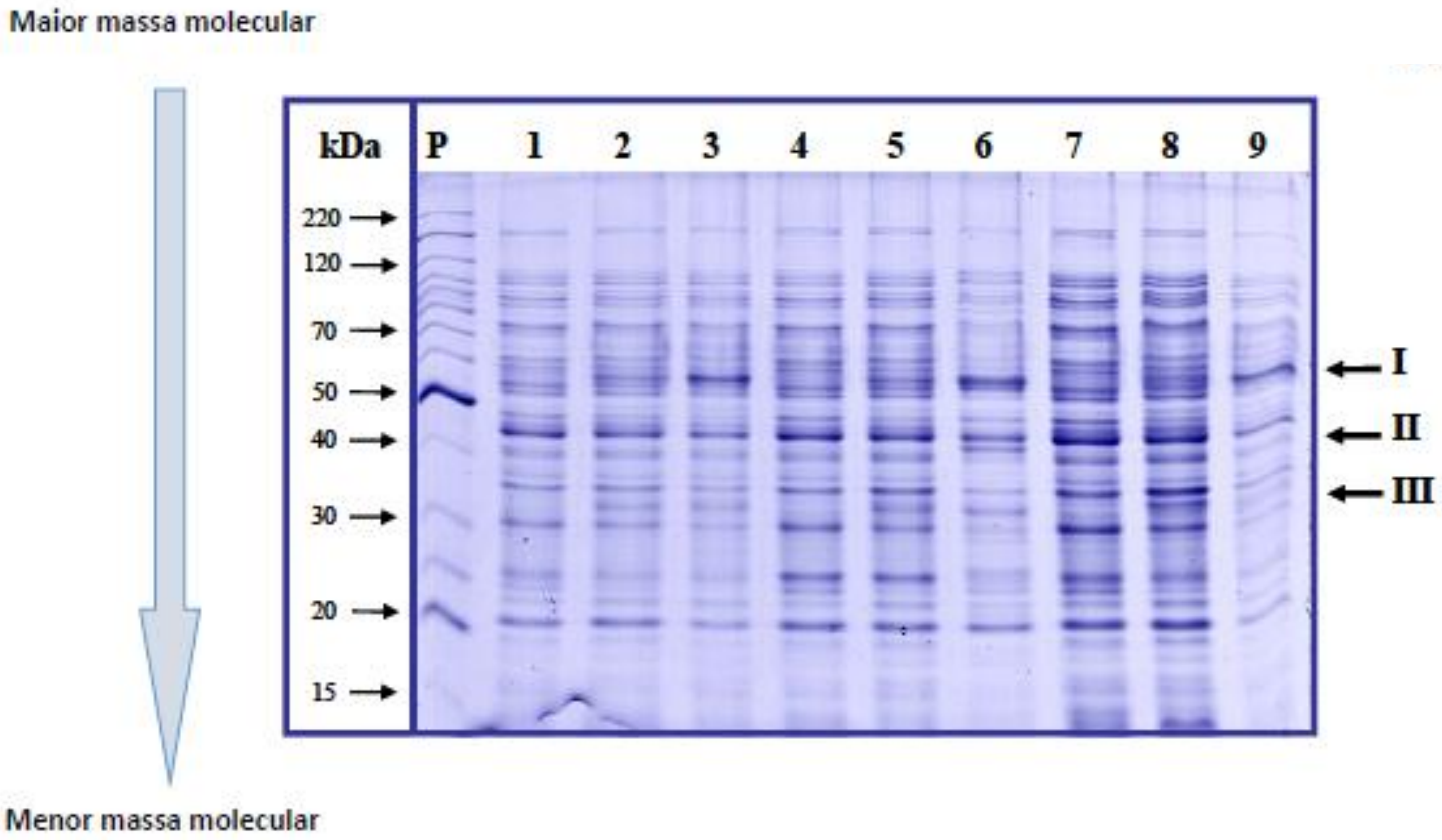
Proteínas (SDS-PAGE)



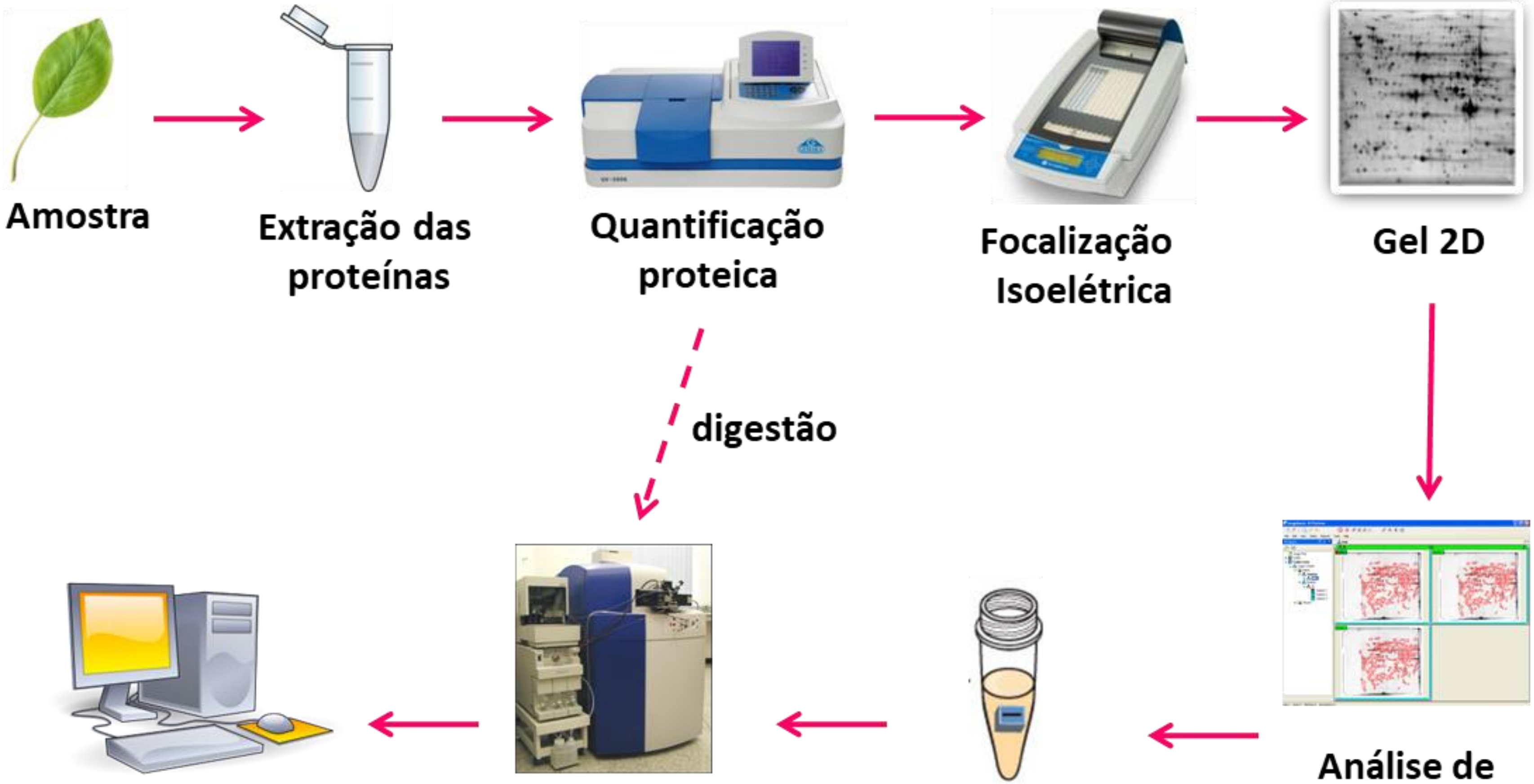
acrilamida	Proteínas kDa
5%	60-350
10%	15-200
15%	10-100

PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

- **Separação das proteínas em gel de sds-page**



PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA



Amostra

Extração das proteínas

Quantificação proteica

Focalização Isoelétrica

Gel 2D

Análise de imagens

Remoção dos spots e digestão

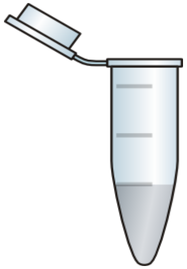
digestão

Cromatografia e Espectrometria de massas

Análise dos dados

PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

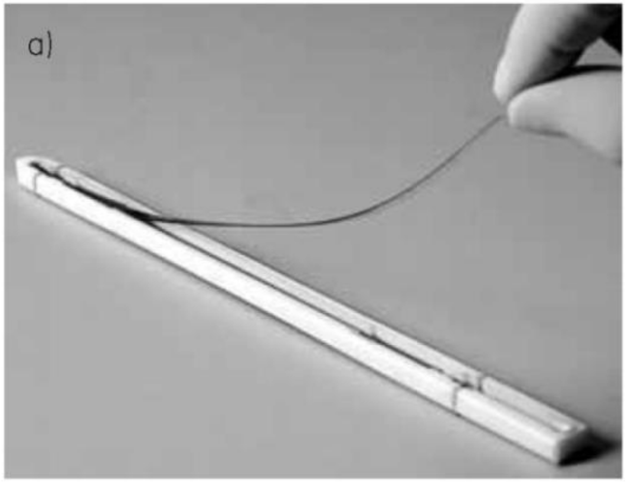
➢ ELETROFORESE BIDIMENSIONAL (2DE)



Pool proteico

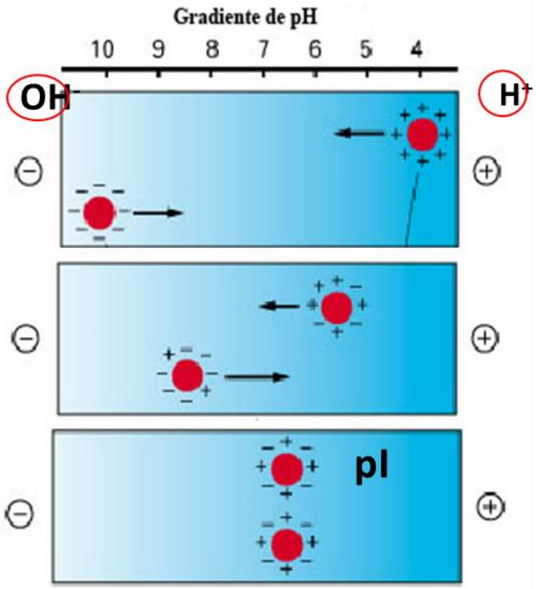
- Focalização Isoelétrica (1D): SEPARAÇÃO POR CARGA

- Eletroforese Bidimensional (2D): SEPARAÇÃO POR MASSA

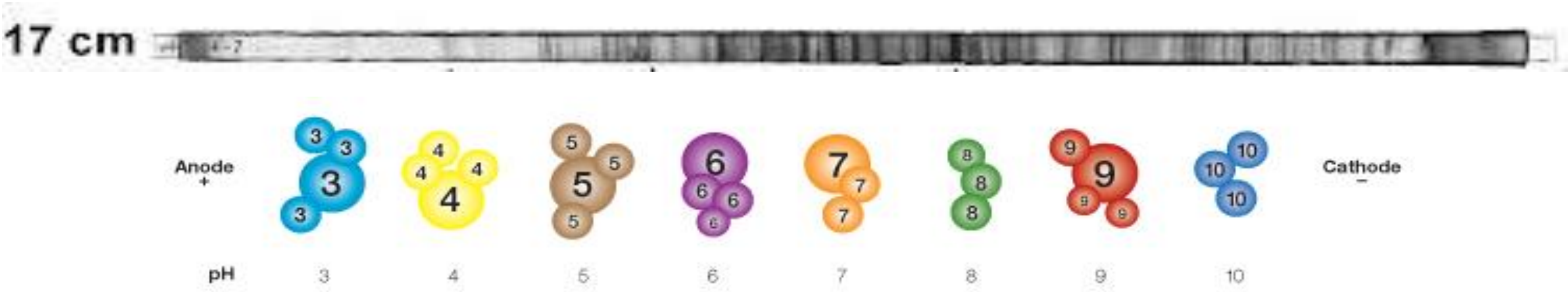


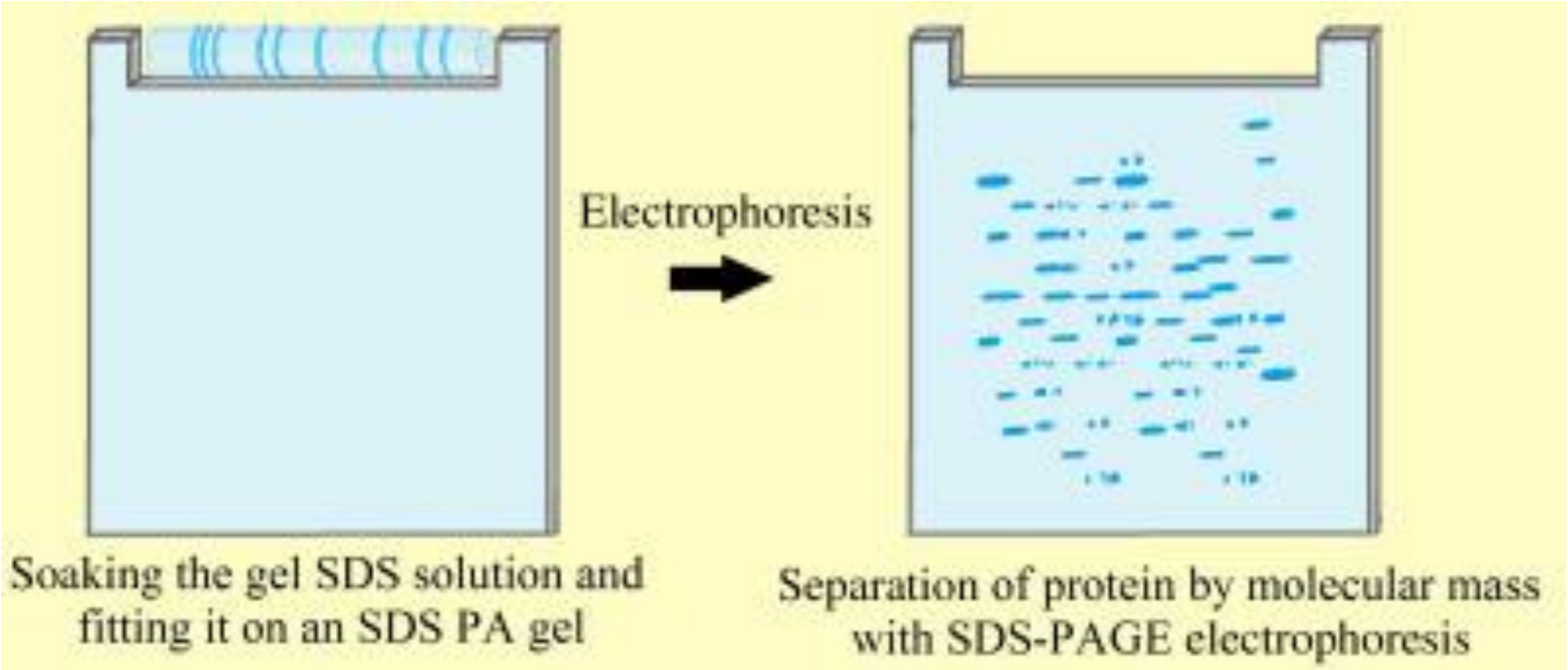
Sarcófago

Focalizador isoelétrico



PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA





Soaking the gel SDS solution and fitting it on an SDS PA gel

Separation of protein by molecular mass with SDS-PAGE electrophoresis

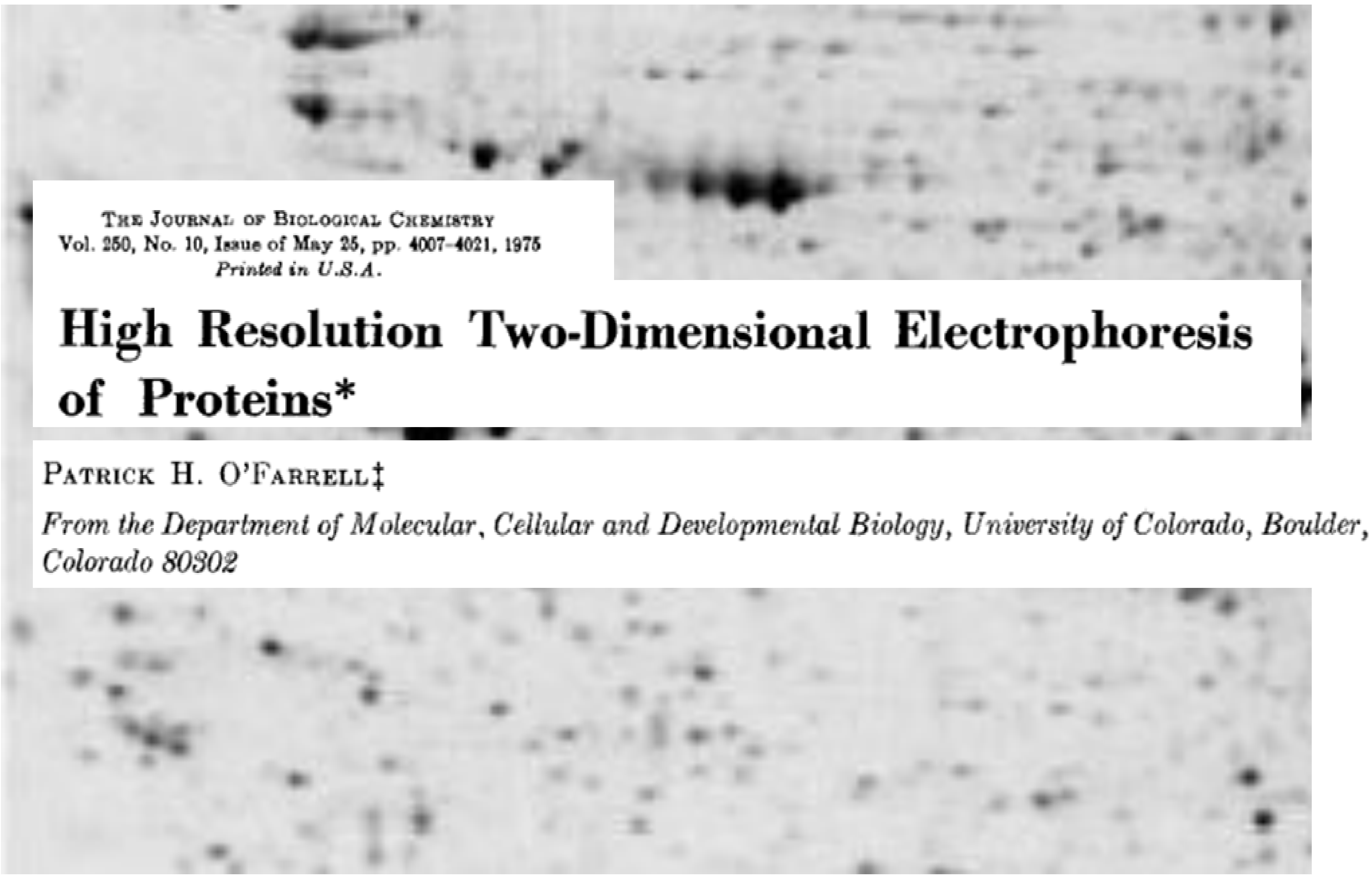
PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

Ácido **Primeira Dimensão** Básico

Alto MW

Segunda Dimensão

Baixo MW



The Journal of Biological Chemistry
Vol. 250, No. 10, Issue of May 25, pp. 4007-4021, 1975
Printed in U.S.A.

High Resolution Two-Dimensional Electrophoresis of Proteins*

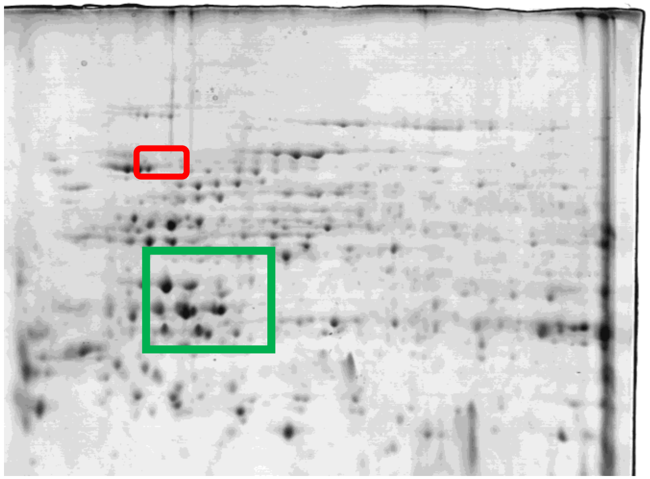
Patrick H. O'Farrell‡

From the Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80302

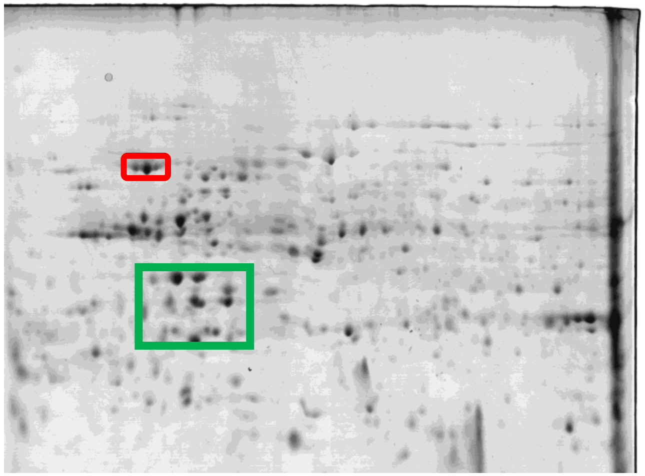
PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

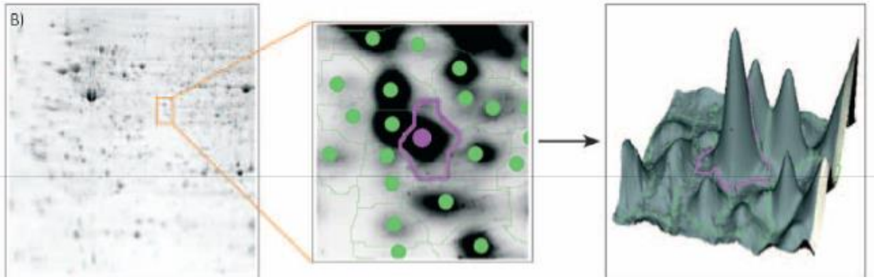
- **ANÁLISE DOS GÉIS**

CONTROLE

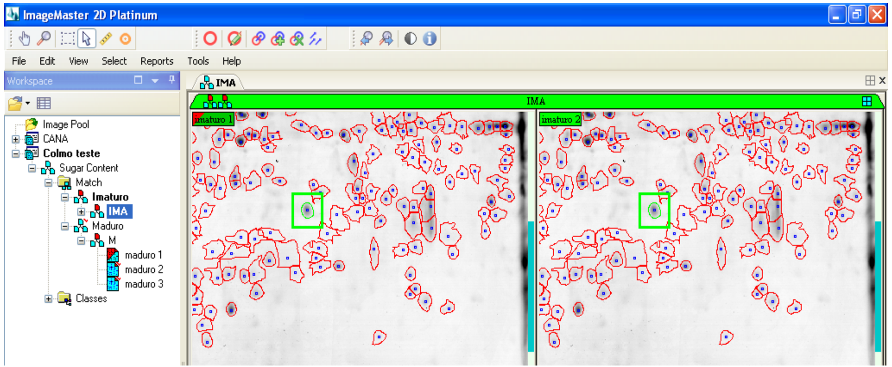


TRATAMENTO



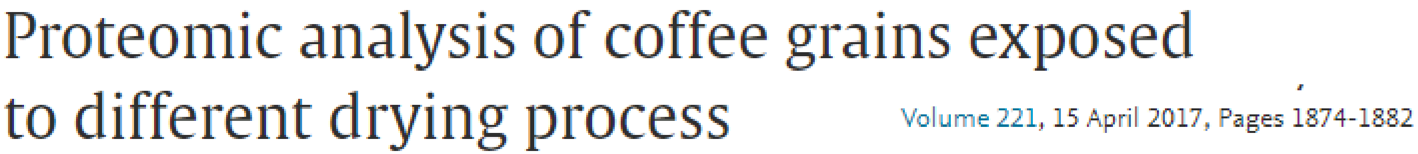


Análise de imagens (ImageMaster)



PROTEÔMICA: PUBLICAÇÕES 2-DE

2017



Proteomic analysis of coffee grains exposed to different drying process

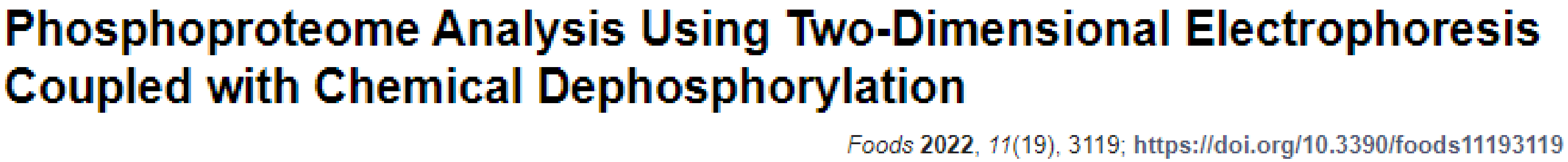
Volume 221, 15 April 2017, Pages 1874-1882

2018

Two-Dimensional Gel Electrophoresis Seed Proteome Map of Pigmented and Non Pigmented Sorghum Genotypes (*Sorghum bicolor L. Moench*)

DOI: 10.21767/2248-9215.100075

2020



Phosphoproteome Analysis Using Two-Dimensional Electrophoresis Coupled with Chemical Dephosphorylation

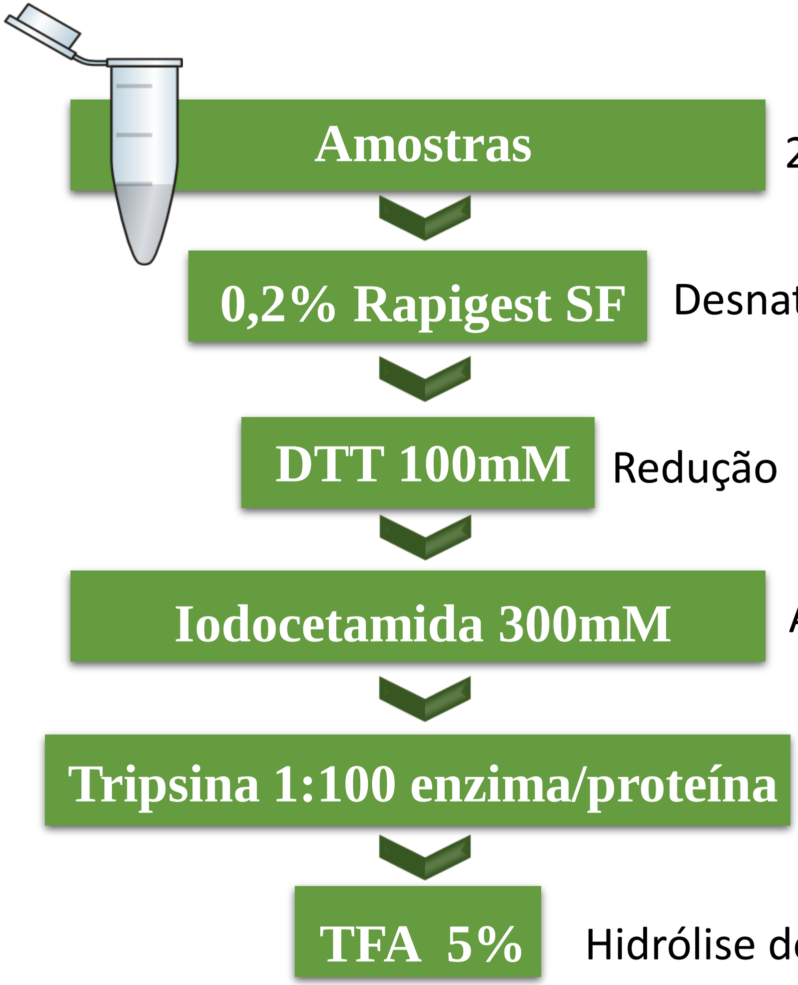
Foods **2022**, *11*(19), 3119; **https://doi.org/10.3390/foods11193119**

2022

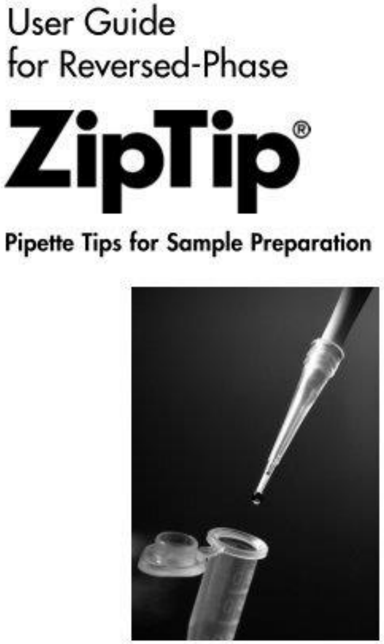
Proteomics Unveils Post-Mortem Changes in Beef Muscle Proteins and Provides Insight into Variations in Meat Quality Traits of Crossbred Young Steers and Heifers Raised in Feedlot

PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

- **Digestão Shotgun (Amostra Complexa)**

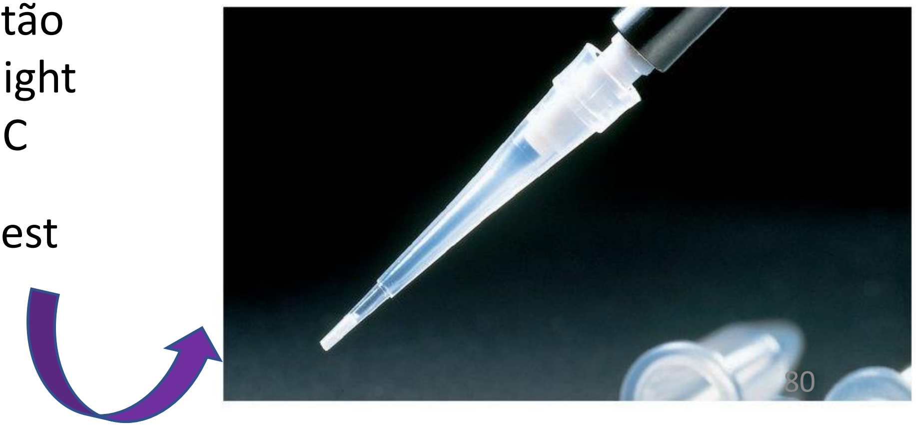


Amostras	200 ng.uL
0,2% Rapigest SF	Desnaturação
DTT 100mM	Redução
Iodocetamida 300mM	Alquilação
Tripsina 1:100 enzima/proteína	Digestão Overnight 37ºC
TFA 5%	Hidrólise do Rapigest



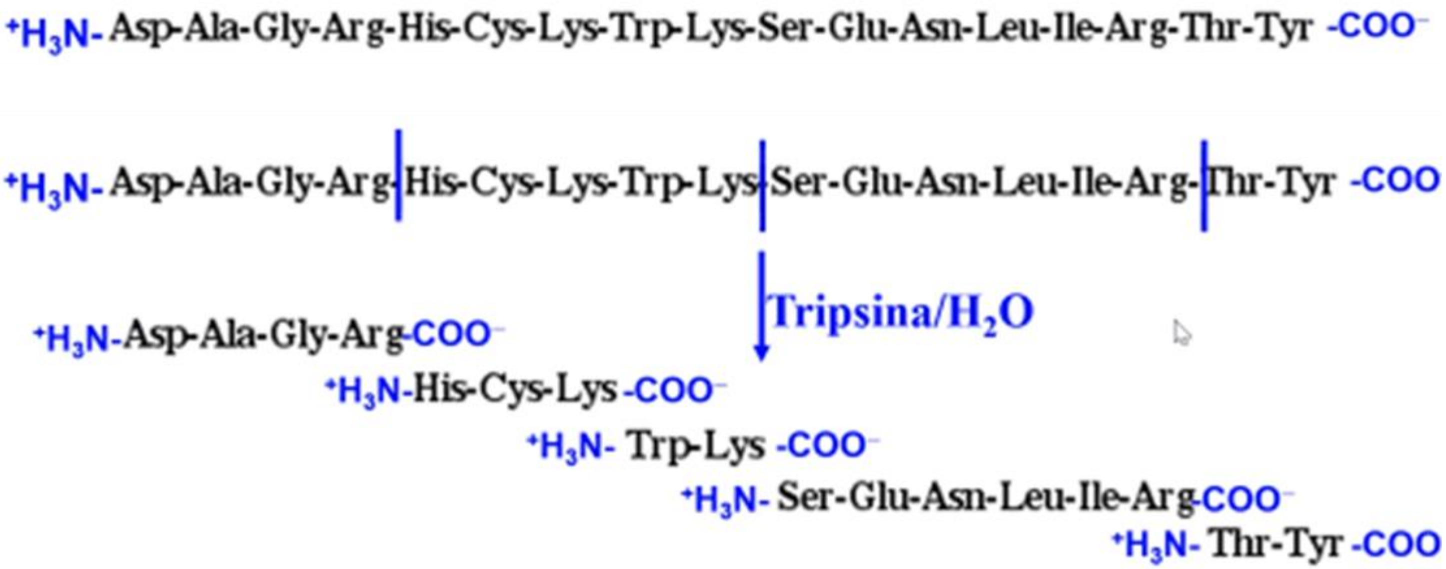
User Guide
for Reversed-Phase
ZipTip®
Pipette Tips for Sample Preparation

MILLIPORE



PROTEÔMICA: FLUXO DE ANÁLISE EM PROTEÔMICA

➢ DIGESTÃO



$^{+}H_3N$- Asp-Ala-Gly-Arg-His-Cys-Lys-Trp-Lys-Ser-Glu-Asn-Leu-Ile-Arg-Thr-Tyr -COO^{-}

$^{+}H_3N$- Asp-Ala-Gly-Arg | His-Cys-Lys-Trp-Lys | Ser-Glu-Asn-Leu-Ile-Arg- | Thr-Tyr -COO

Tripsina/H_2O

$^{+}H_3N$-Asp-Ala-Gly-Arg-COO^{-}

$^{+}H_3N$-His-Cys-Lys -COO^{-}

$^{+}H_3N$- Trp-Lys -COO^{-}

$^{+}H_3N$- Ser-Glu-Asn-Leu-Ile-Arg-COO^{-}

$^{+}H_3N$- Thr-Tyr -COO

PROTEASES ESPECÍFICAS

Enzima	Quebra
Tripsina	**Lys, Arg (C)**
Quimiotripsina	Phe, Trp, Tyr (C)
Pepsina	Phe, Trp, Tyr (N)
Asp-N-protease	Asp, Glu (N)

PROTEÔMICA: CROMATOGRAFIA LIQUIDA E ESPECTROMETRIA DE MASSAS (LC-MS)

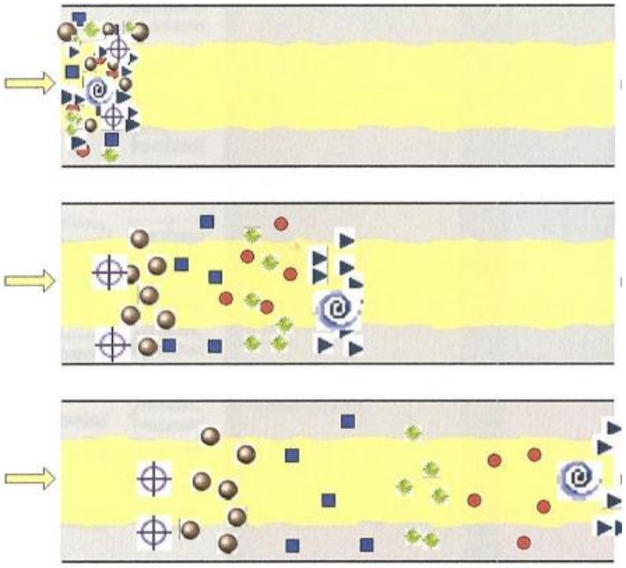
SYNAPT G2 LC-ESI-Q-TOF MS (Waters)

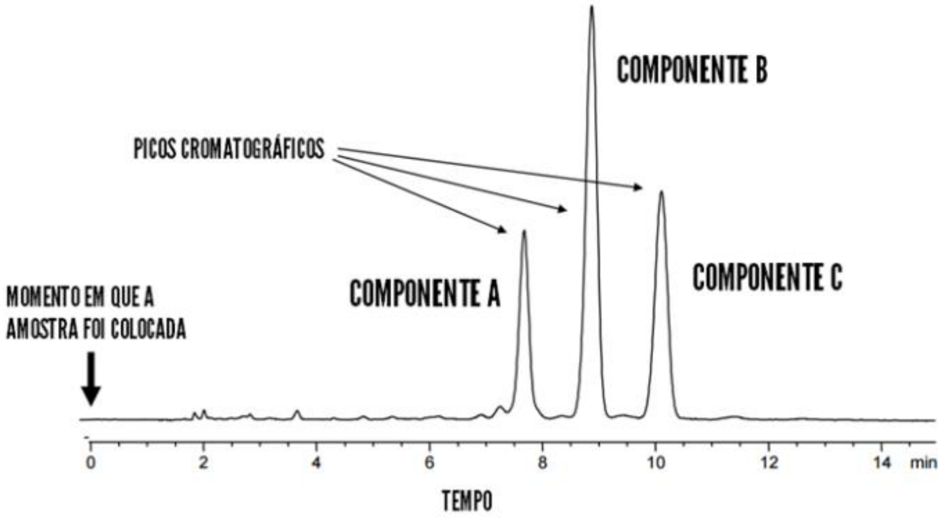
TimsTOF MS
(Bruker)

HRAM Q-TOF MS
(Shimadzu)

PROTEÔMICA: CROMATOGRAFIA LÍQUIDA

- **Métodos físico-químico de separação dos componentes de uma mistura, que ocorre devido a diferentes interações, entre duas fases: a fase móvel (líquidos ou gases) e a fase estacionária (sólidos ou líquido).**
- **Separação de compostos considerando o tempo necessário para que eles eluam da fase estacionária.**

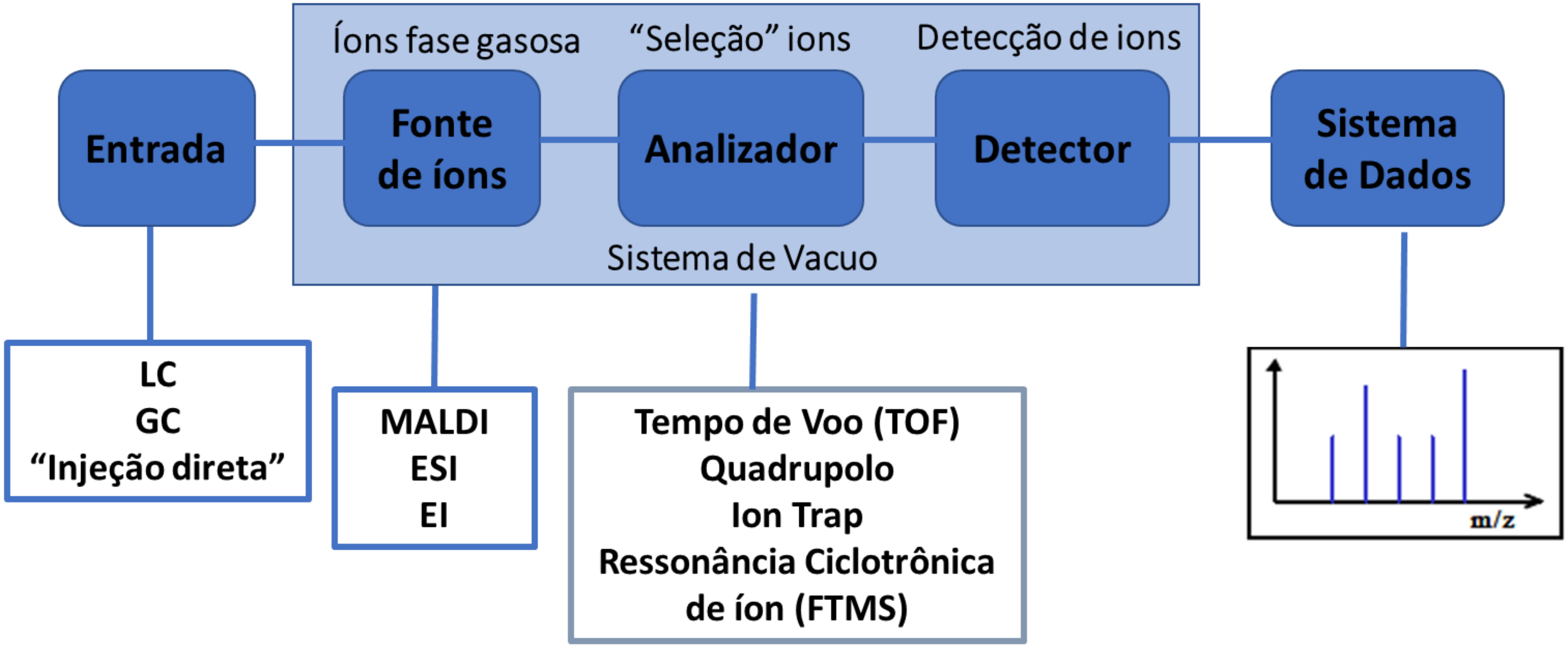




PROTEÔMICA: CROMATOGRAFIA LÍQUIDA

nanoEase M/Z HSS T3 Column

PROTEÔMICA: ESPECTROMETRIA DE MASSAS



Íons fase gasosa

“Seleção” ions

Detecção de ions

Entrada

Fonte de íons

Analizador

Detector

Sistema de Dados

Sistema de Vacuo

LC
GC
“Injeção direta”

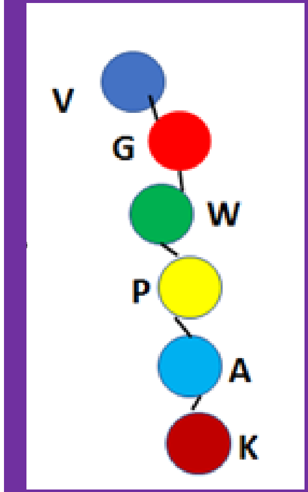
MALDI
ESI
EI

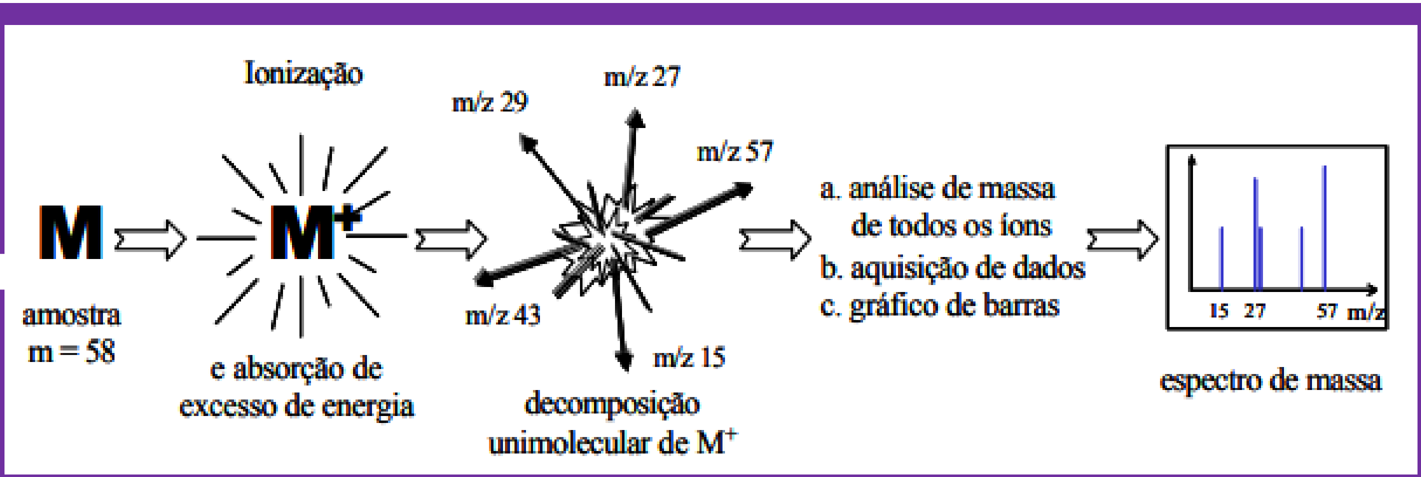
Tempo de Voo (TOF)
Quadrupolo
Ion Trap
Ressonância Ciclotrônica de íon (FTMS)

HPLC – Cromatografia líquida de alta perfomance
UPLC – Cromatografia liquida de ultra performance
MALDI – Ionização assistida por matriz a laser – Matrix assisted laser ionization
ESI – Ionização por eletrospray
EI – Ionização por impacto de elétrons

PROTEÔMICA: ESPECTROMETRIA DE MASSAS

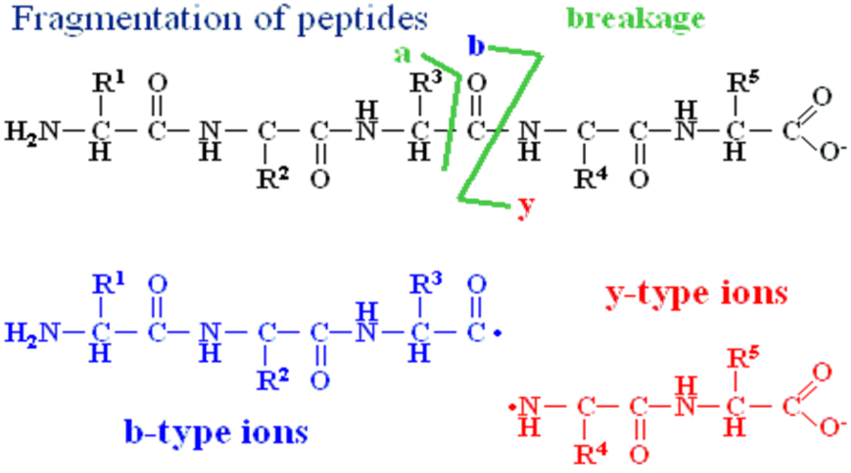
- **Ferramenta analítica para determinar a composição molecular de uma amostra, através da relação massa/carga (m/z) da molécula ionizada.**

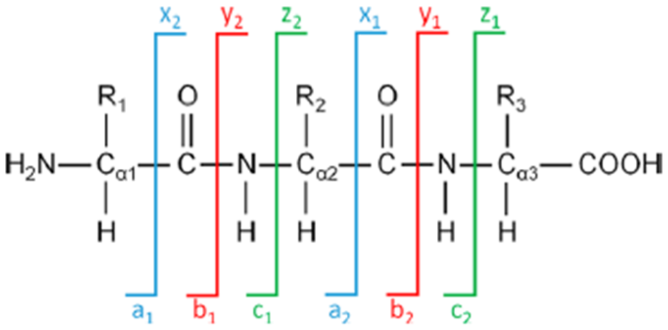




- **O requisito básico para uma análise por espectrometria de massas é a formação de íons livres em fase gasosa.**
- **Em resumo, o MASSAS permite identificar a estrutura molecular e a composição qualitativa e quantitativa de moléculas.**

PROTEÔMICA: FRAGMENTAÇÃO DE PEPTÍDEOS





The Cα–C bonds (in blue)

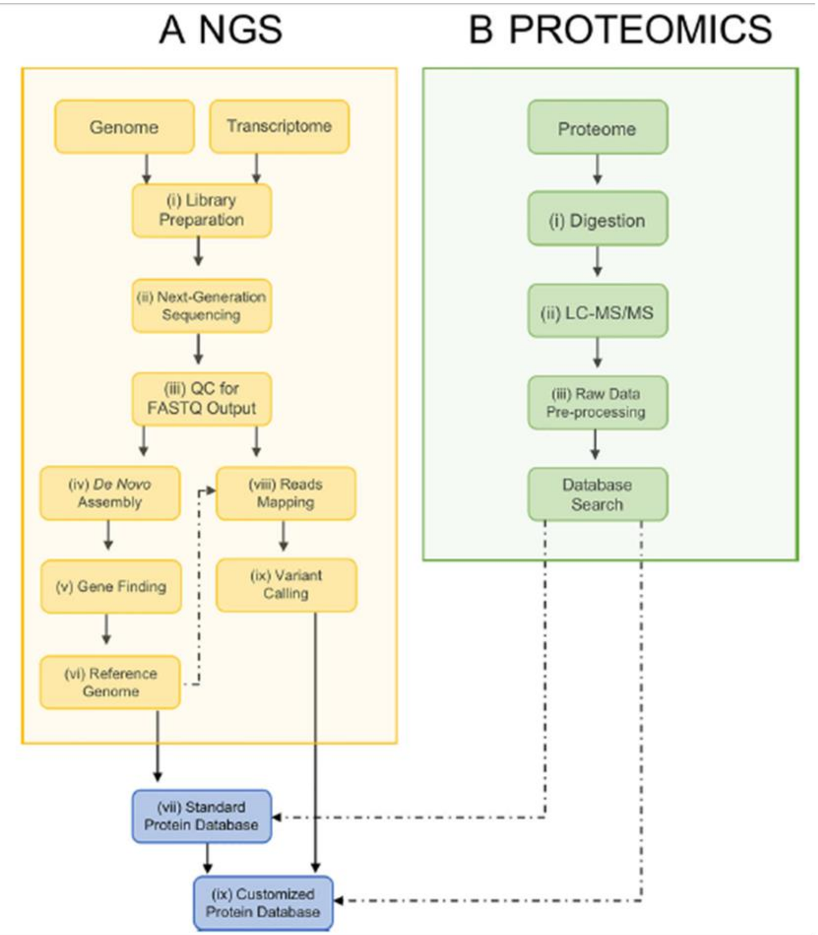
The peptide bonds (in red)

The N–Cα bonds (in green)

Peptide: S-G-F-L-E-E-D-E-L-K

MW	ion			ion	MW
88	b_1	S	GFLEEDELK	y_9	1080
145	b_2	SG	FLEEDELK	y_8	1022
292	b_3	SGF	LEEDELK	y_7	875
405	b_4	SGFL	EEDELK	y_6	762
534	b_5	SGFLE	EDELK	y_5	633
663	b_6	SGFLEE	DELK	y_4	504
778	b_7	SGFLEED	ELK	y_3	389
907	b_8	SGFLEEDE	LK	y_2	260
1020	b_9	SGFLEEDEL	K	y_1	147

PROTEÔMICA: BUSCA EM BANCO DE DADOS




Protein Identification by Database Searching of Mass Spectrometry Data in the Teaching of Proteomics

Vinícius Marquioni,* Francis Morais Franco Nunes, and Maria Teresa Marques Novo-Mansur*

Cite This: *J. Chem. Educ.* 2021, 98, 812–823 Read Online


REVIEW *Proteomics* 2019, *19*, 1800235 **Proteomics**

Proteogenomics www.proteomics-journal.com

Connecting Proteomics to Next-Generation Sequencing: Proteogenomics and Its Current Applications in Biology

Teck Yew Low, M. Aiman Mohtar, Mia Yang Ang, and Rahman Jamal*


Beginner's Guide **Ankit Sinha** Matthias Mann

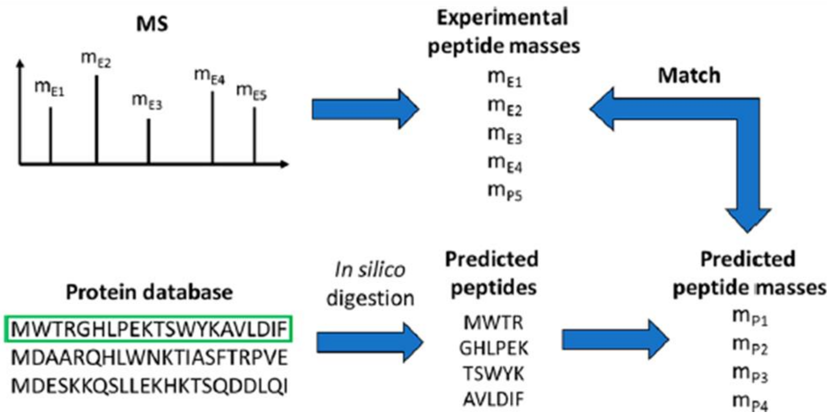
A beginner's guide to mass spectrometry–based proteomics

Biochem (Lond) (2020) 42 (5): 64–69.

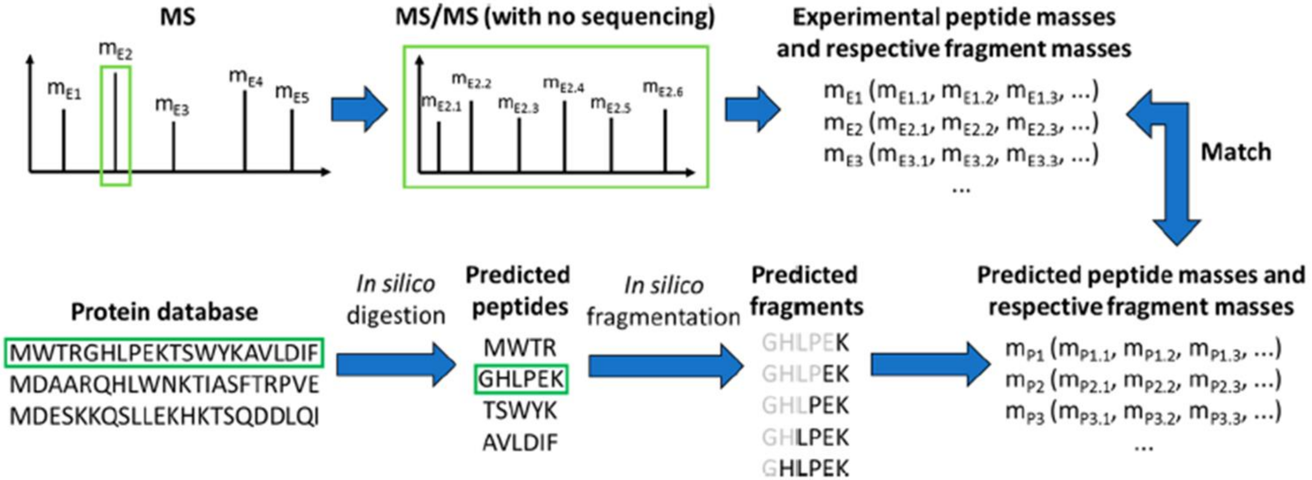
PROTEÔMICA: IDENTIFICAÇÃO BANCO DE DADOS

➢ PEPTIDE MASS FINGERPRINTING

PMF (dados MS)



➢ MS/MS ion search



PROTEÔMICA: IDENTIFICAÇÃO BANCO DE DADOS

MSPQTETKASVG
QYICYVAYPLDLFEEGSV
VFARELGVPIVMHDYLT
GLLLHIHRAMHA

→ **Banco de dados** →

NCBI National Center for Biotechnology Information

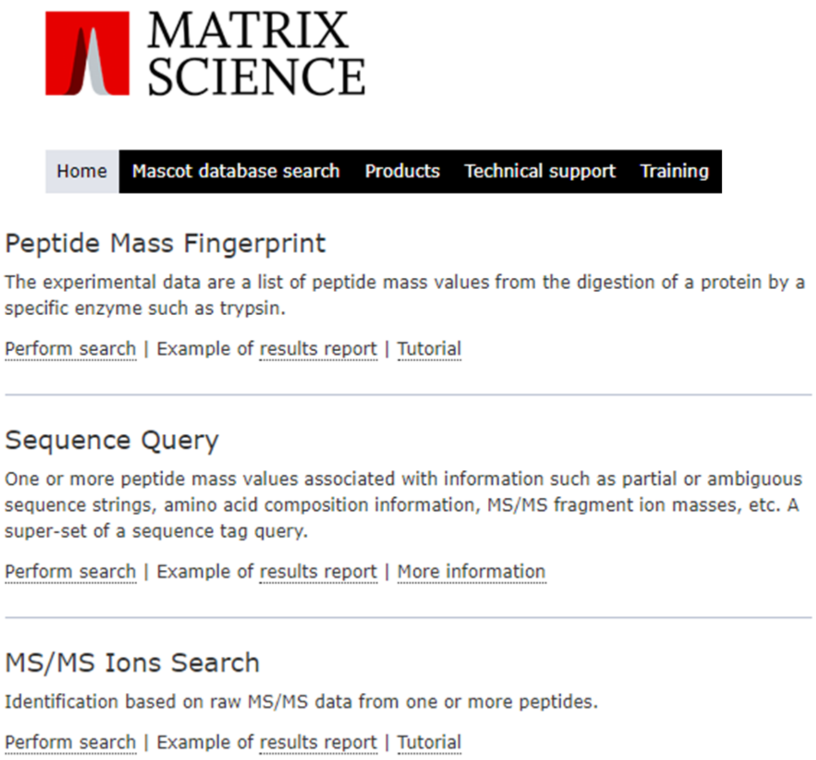
JGI JOINT GENOME INSTITUTE

Phytozome 12 THE PLANT GENOMICS RESOURCE

SIB Swiss Institute of Bioinformatics

>gi|11467200|ref|NP_043033.1| ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [Zea mays]
MSPQTETKASVGFKAGVKDYKLTYYTPEYETKDTDILAAFRVTPQLGVPPEEAGAAVAAESSTGTWTT
VWTDGLTSLDRYKGRCYHIEPVPGDPD**QYICYVAYPLDLFEEGSV**TNMFTSIVGNVFGFKALRALRLE
DLRIPPAYSKTFQGPPHGIQVERDKLNKYGRPLLGCTIKPKLGLSAKNYGRACYECLRGGLDFTKDDEN
VNSQPFMRWRDRFVFCAEAIYKAQAETGEIKGHYLNATAGTCEEMIKRA**VFARELGVPIVMHDYLT**G
GFTANTTLSHYCRDN**GLLLHIHRAMHA**VIDRQKNHGMHFRVLAKALRMSGGDHIHSGTVVGKLEG
EREITLGFVDLLRDDFIEKDRSRGIFFTQDWVSMPGVIPVASGGIHVWHMPALTEIFGDDSVLQFGGG
TLGHPWGNAPGAAANRVALEACVQARNEGRDLAREGNEIIKAACKWSAELAAACEIWKEIKFDGFK
AMDTI

PROTEÔMICA: IDENTIFICAÇÃO BANCO DE DADOS


MATRIX SCIENCE

Peptide Mass Fingerprint

The experimental data are a list of peptide mass values from the digestion of a protein by a specific enzyme such as trypsin.

Perform search | Example of results report | Tutorial

Sequence Query

One or more peptide mass values associated with information such as partial or ambiguous sequence strings, amino acid composition information, MS/MS fragment ion masses, etc. A super-set of a sequence tag query.

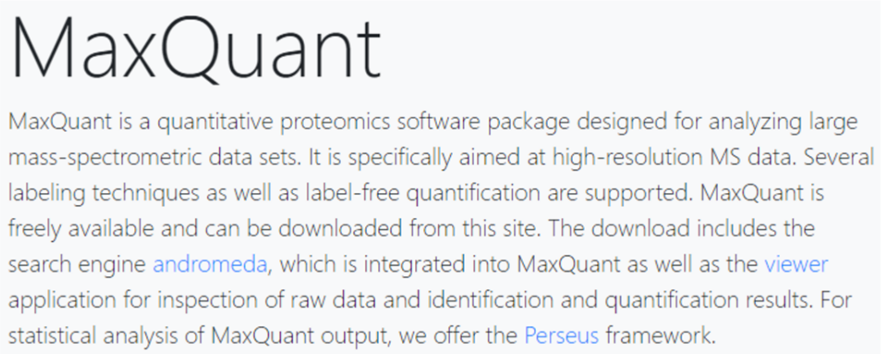
Perform search | Example of results report | More information

MS/MS Ions Search

Identification based on raw MS/MS data from one or more peptides.

Perform search | Example of results report | Tutorial

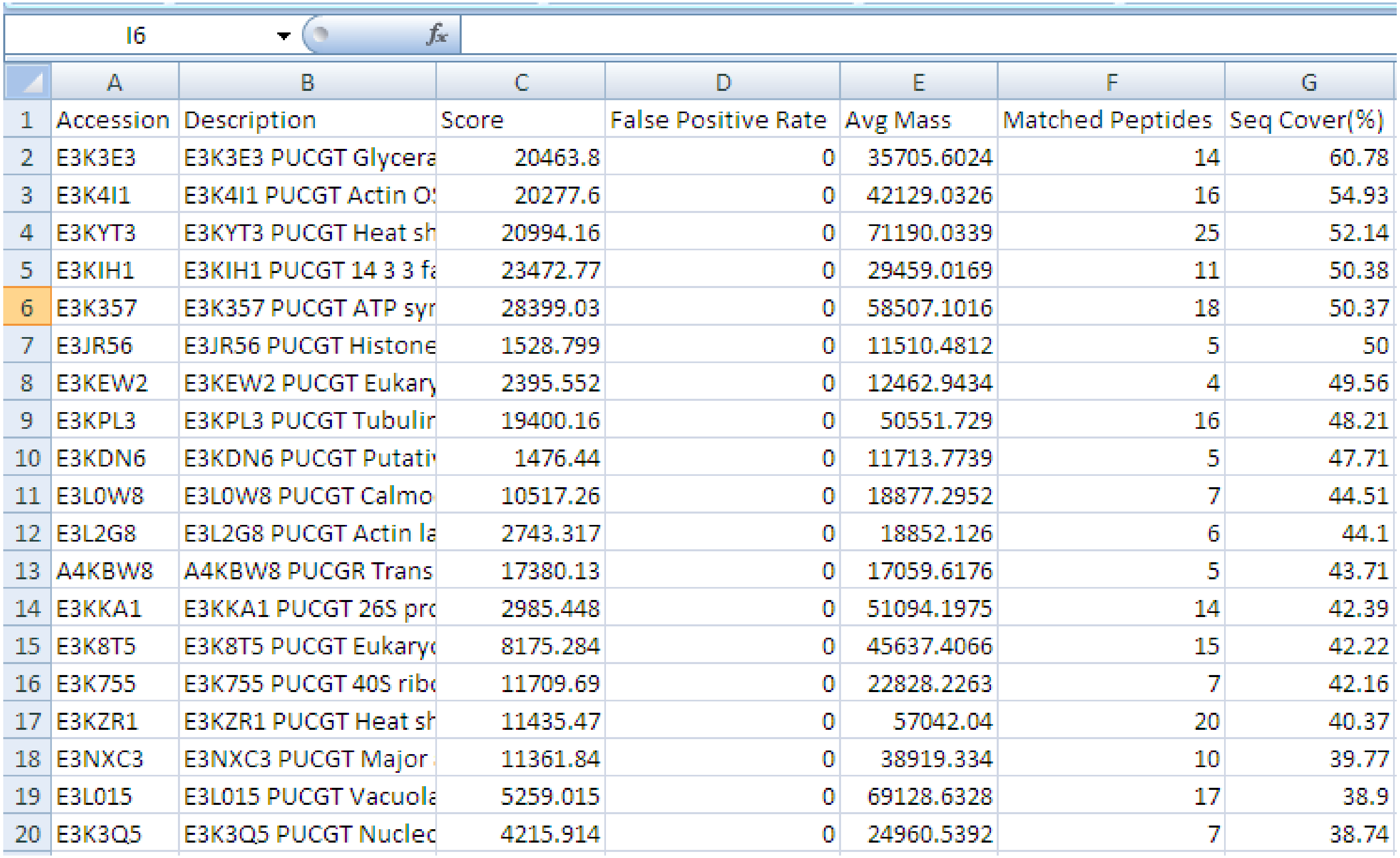
PEAKS



MaxQuant

MaxQuant is a quantitative proteomics software package designed for analyzing large mass-spectrometric data sets. It is specifically aimed at high-resolution MS data. Several labeling techniques as well as label-free quantification are supported. MaxQuant is freely available and can be downloaded from this site. The download includes the search engine andromeda, which is integrated into MaxQuant as well as the viewer application for inspection of raw data and identification and quantification results. For statistical analysis of MaxQuant output, we offer the Perseus framework.

PROTEÔMICA: RESULTADOS



	A	B	C	D	E	F	G
1	Accession	Description	Score	False Positive Rate	Avg Mass	Matched Peptides	Seq Cover(%)
2	E3K3E3	E3K3E3 PUCGT Glycera	20463.8	0	35705.6024	14	60.78
3	E3K4I1	E3K4I1 PUCGT Actin O	20277.6	0	42129.0326	16	54.93
4	E3KYT3	E3KYT3 PUCGT Heat sh	20994.16	0	71190.0339	25	52.14
5	E3KIH1	E3KIH1 PUCGT 14 3 3 fa	23472.77	0	29459.0169	11	50.38
6	E3K357	E3K357 PUCGT ATP syn	28399.03	0	58507.1016	18	50.37
7	E3JR56	E3JR56 PUCGT Histone	1528.799	0	11510.4812	5	50
8	E3KEW2	E3KEW2 PUCGT Eukary	2395.552	0	12462.9434	4	49.56
9	E3KPL3	E3KPL3 PUCGT Tubulin	19400.16	0	50551.729	16	48.21
10	E3KDN6	E3KDN6 PUCGT Putati	1476.44	0	11713.7739	5	47.71
11	E3L0W8	E3L0W8 PUCGT Calmo	10517.26	0	18877.2952	7	44.51
12	E3L2G8	E3L2G8 PUCGT Actin la	2743.317	0	18852.126	6	44.1
13	A4KBW8	A4KBW8 PUCGR Trans	17380.13	0	17059.6176	5	43.71
14	E3KKA1	E3KKA1 PUCGT 26S pro	2985.448	0	51094.1975	14	42.39
15	E3K8T5	E3K8T5 PUCGT Eukary	8175.284	0	45637.4066	15	42.22
16	E3K755	E3K755 PUCGT 40S ribo	11709.69	0	22828.2263	7	42.16
17	E3KZR1	E3KZR1 PUCGT Heat sh	11435.47	0	57042.04	20	40.37
18	E3NXC3	E3NXC3 PUCGT Major	11361.84	0	38919.334	10	39.77
19	E3L015	E3L015 PUCGT Vacuola	5259.015	0	69128.6328	17	38.9
20	E3K3Q5	E3K3Q5 PUCGT Nucleo	4215.914	0	24960.5392	7	38.74

PROTEÔMICA: QUANTIFICAÇÃO

Categorização Funcional:

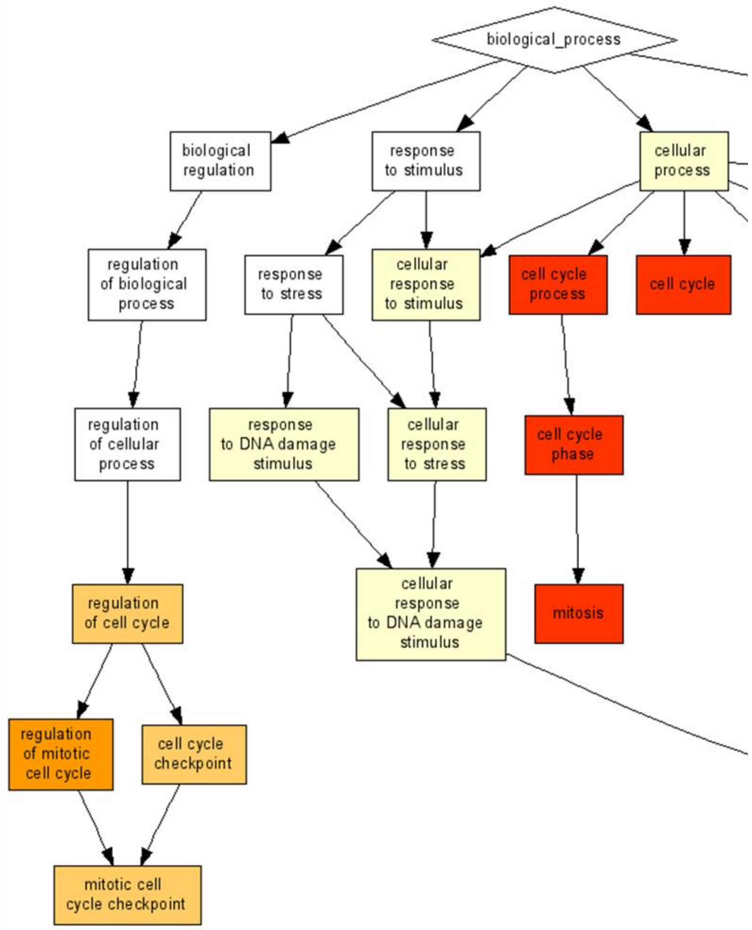
Função Biológica
Função Molecular
Componente celular

blast2go

AGRIGO v2

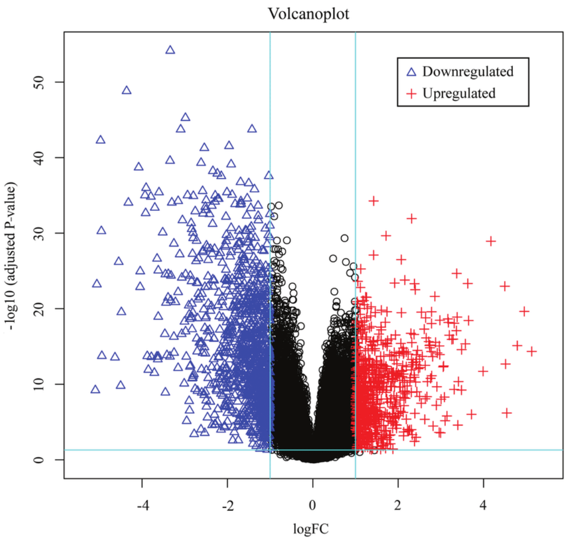
Kyoto Encyclopedia of Genes and Genomes
KEGG

MapMen
Site of Analysis

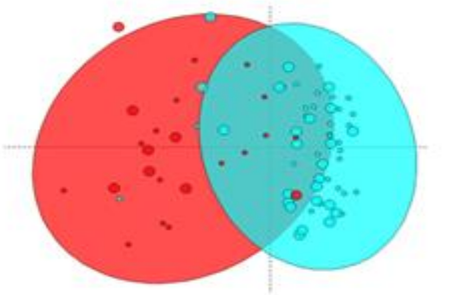


PROTEÔMICA: ESTATÍSTICA

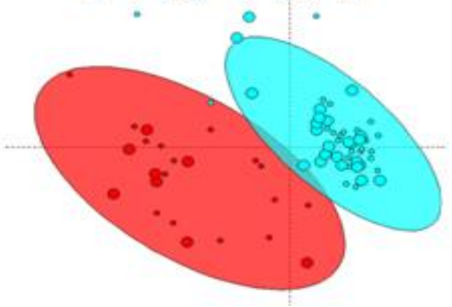
- **Univariada: Teste t, Anova, Volcano Plot**
- **Multivariada: PCA e PLS**

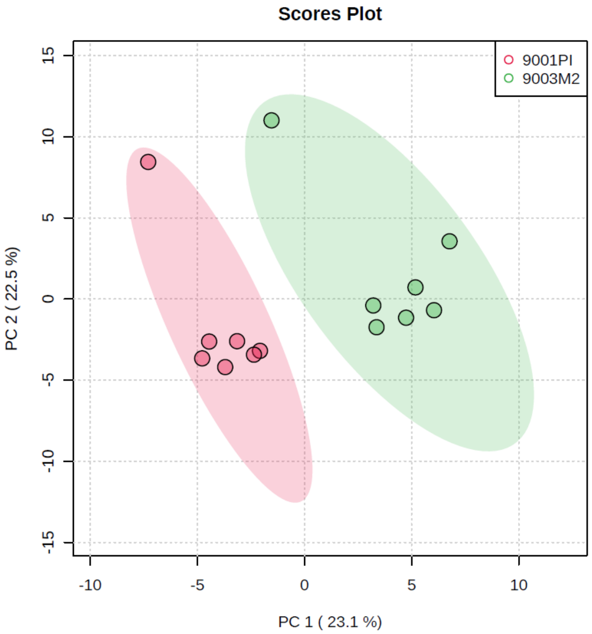


PCA



PLS-DA





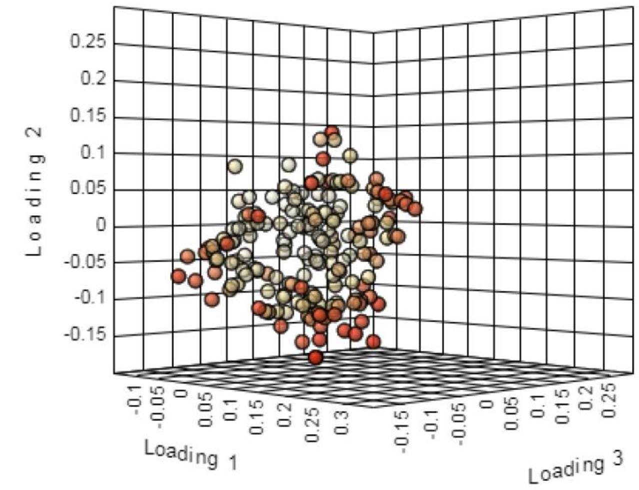
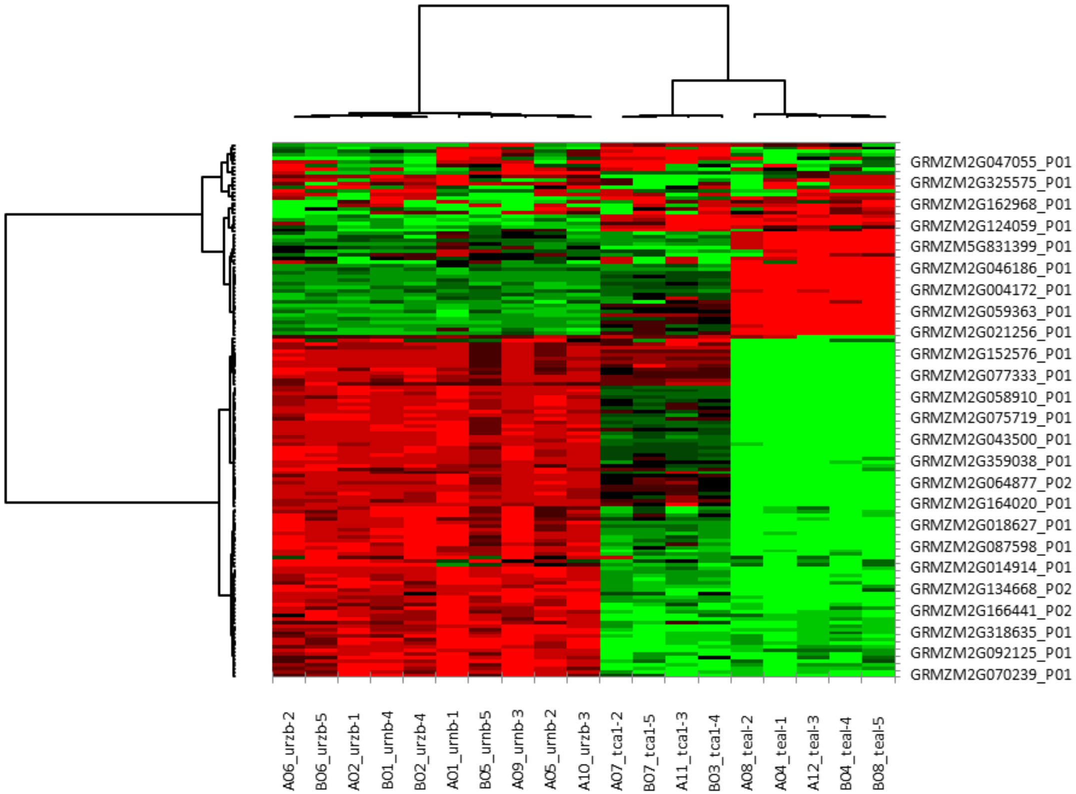
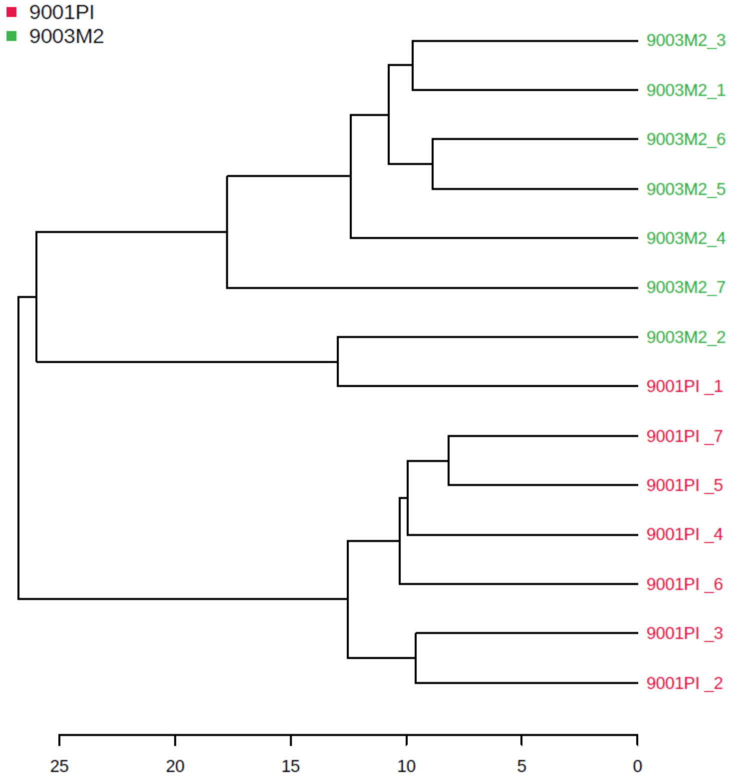


Figure 9: Loadings plot for the selected PCs.

PROTEÔMICA: ESTATÍSTICA

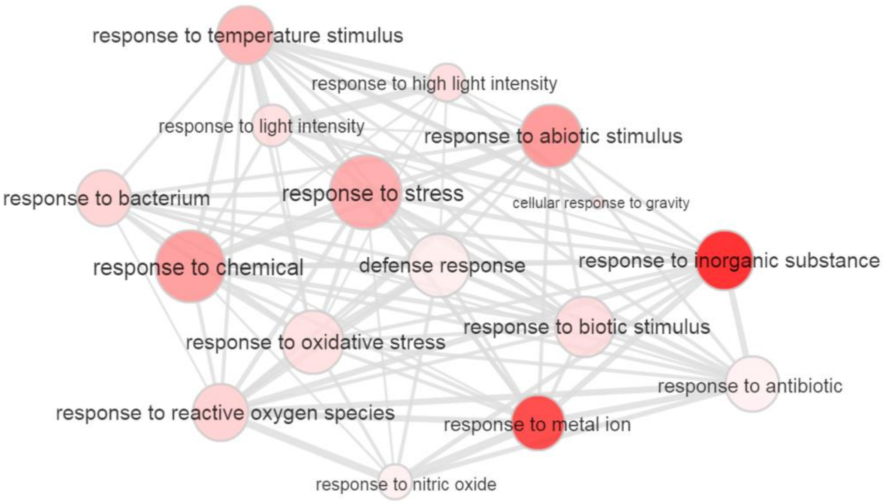




HIERARCHICAL CLUSTERING

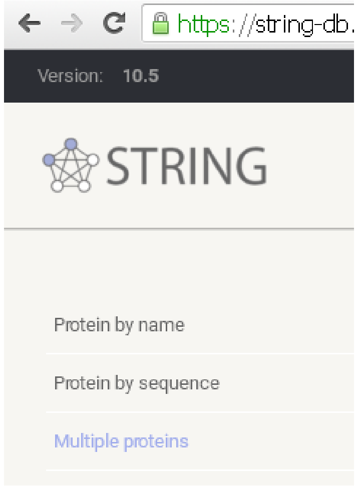
PROTEÔMICA: ESTATÍSTICA

➢ CATEGORIZAÇÃO FUNCIONAL



Cytoscape

INTERAÇÃO PROTEÍNA-PROTEÍNA



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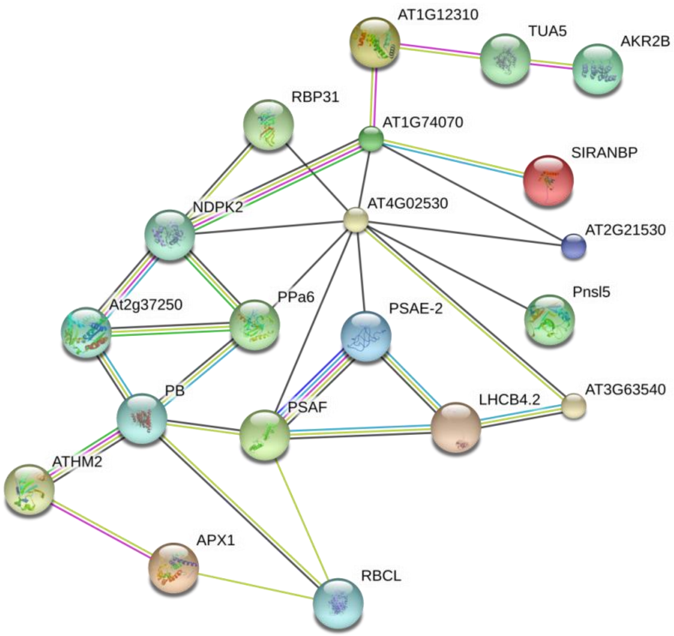

PROTEÔMICA: REPOSITÓRIO

https://www.ebi.ac.uk/pride/