PLS Biologia/Biotecnologie
Corso di formazione per insegnanti

L'utilità del disordine: approcci biotecnologici allo studio delle proteine intrinsecamente disordinate

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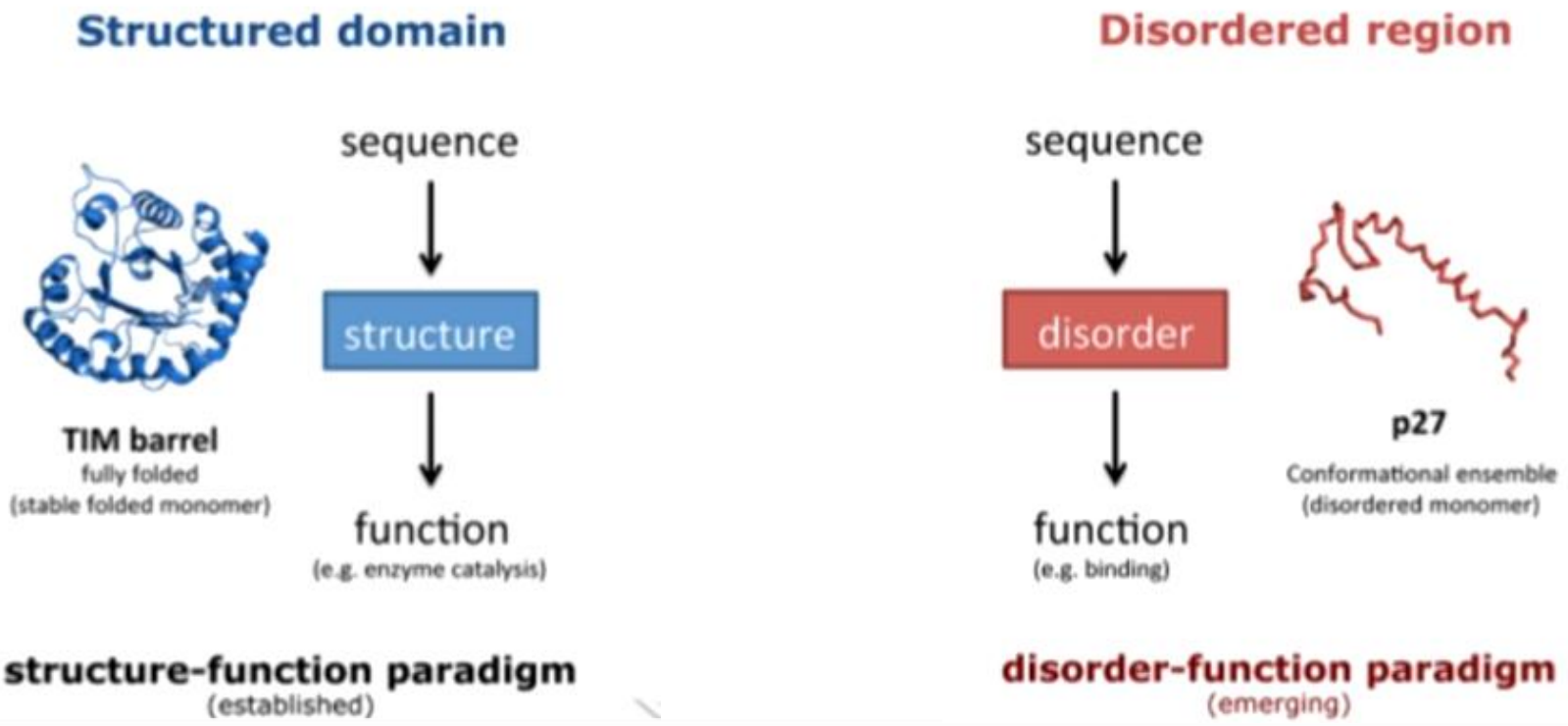
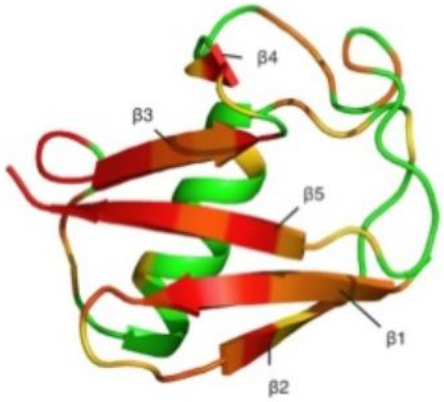
Struttura delle proteine: vecchi e nuovi concetti



NMR as a tool to observe disorder

To perform and analyze NMR experiments that provide information on protein flexibility in terms of *conformational ensembles*.

Single domain



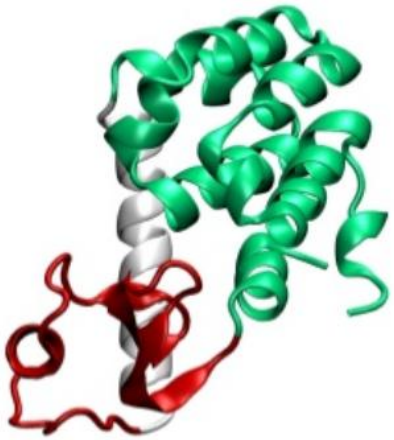
JACS **2011**

Angew Chem **2011**

JCTC **2013**

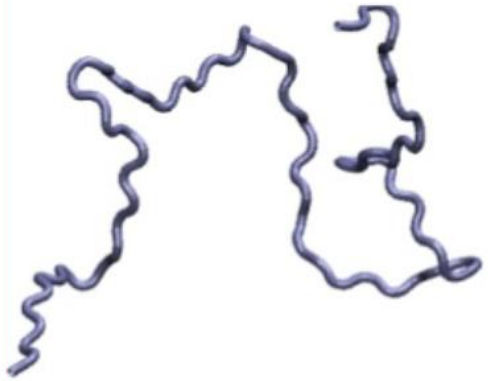
Nat Commun **2014**

Multidomain



PLoS CB **2014**

Disordered



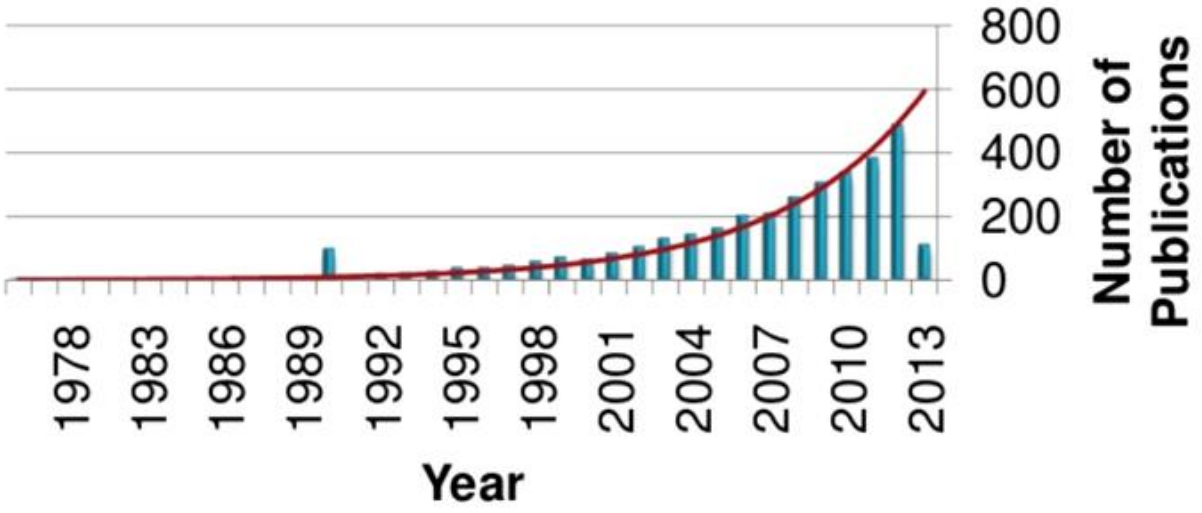
JACS **2010**

PNAS **2013**

BJ **2013a, 2013b**

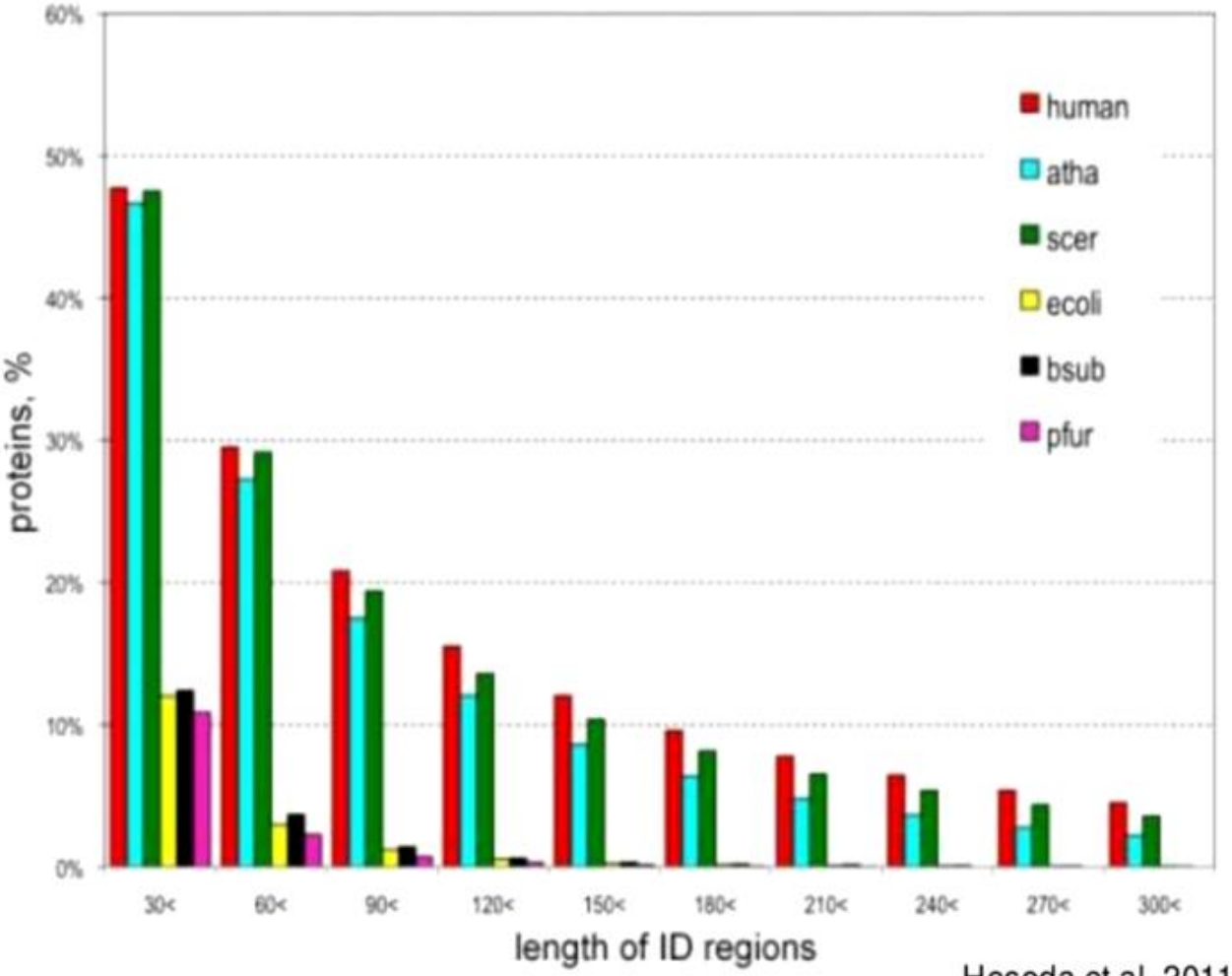
Disorder Becomes Apparent

- Early discovery
  - Bovine serum albumin binding sites (Karush,1950)
- Later…
  - Rapid rise of genomic data (~1990)
    - ***P***redictors ***o***f ***n***atural ***d***isordered **r**egions (PONDRs)
  - Early proton NMR experiments (Daniels et al, 1978)



Disorder, Disorder, (most)Everywhere!

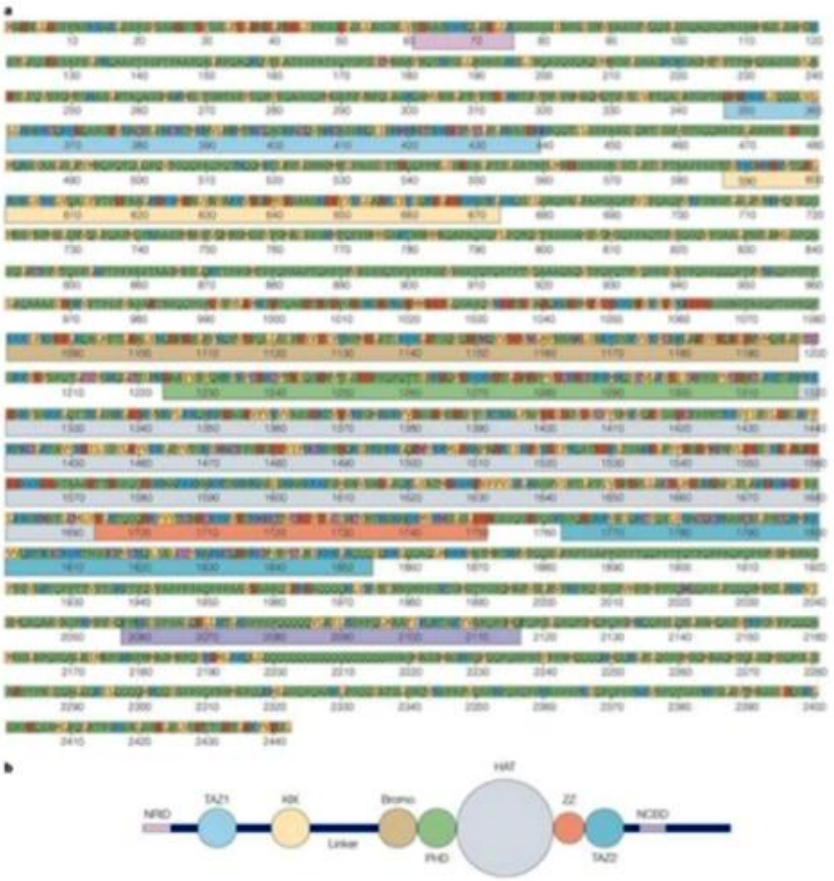
- Generally:
  - ↑complexity of organism → ↑disorder
  - Some exceptions
- **35–51%** of eukaryotic proteome (Dunker et al, 2000)



Hosoda et al, 2011

Why Did We Miss It?

- Unobserved
  - Bias of experiment
  - Access to genomic data limited before ~1990
  - Crystal structure relatively uninformative
- Ignored
  - Crystal structure artifacts dismissed
  - Disorder thought to be an artifact



Nature Reviews | Molecular Cell Biology

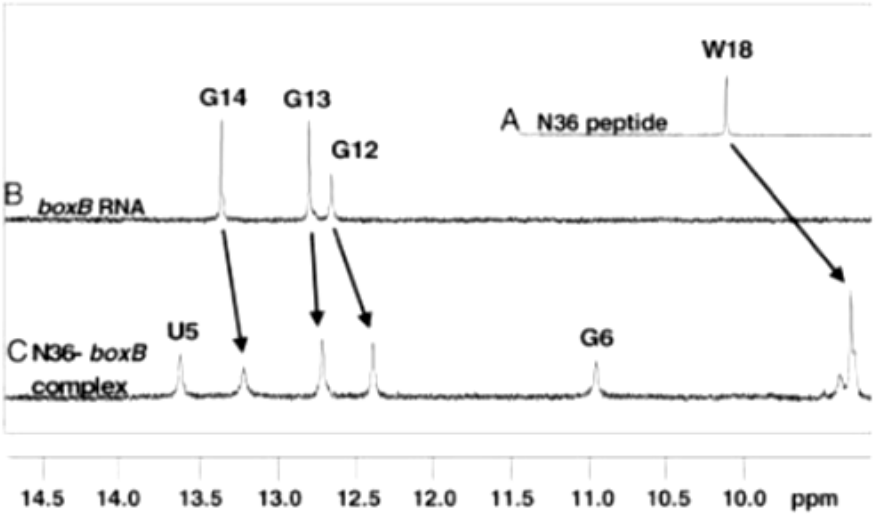
Dyson & Wright, 2005

What is Disorder in Proteins?

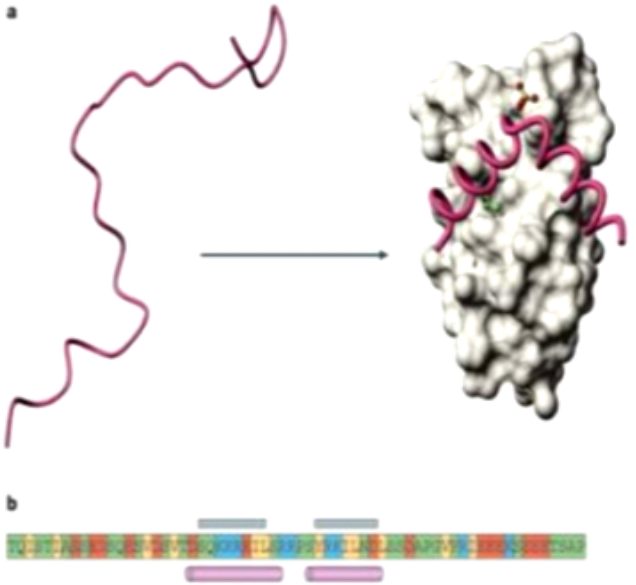
- Definition:
  - A protein that does not adopt a well-defined native structure when isolated in solution under near-physiological conditions (Eliezer, 2009)
- 2 types
  - Denatured state ensembles (DSEs)
  - Intrinsically disordered proteins (IDPs)
- Vast and malleable configurational ensembles (CEs)
- Charged
- What can impact disorder?

Proposed Mechanisms

- Regulation
  - Folding upon binding
  - Highly specific / low affinity binding
- Multiple interaction sites
- Aggregation



Schärpf et al, 2001



Nature Reviews | Molecular Cell Biology

Dyson & Wright, 2005

Structured domain

TIM barrel
fully folded
(stable folded monomer)

sequence → structure → function
(e.g. enzyme catalysis)

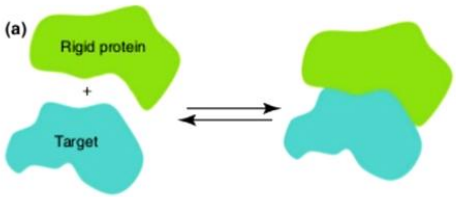
structure-function paradigm
(established)

Disordered region

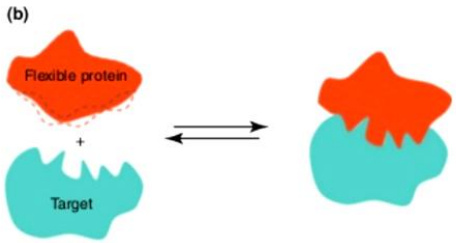
sequence → disorder → function
(e.g. binding)

p27
Conformational ensemble
(disordered monomer)

disorder-function paradigm
(emerging)



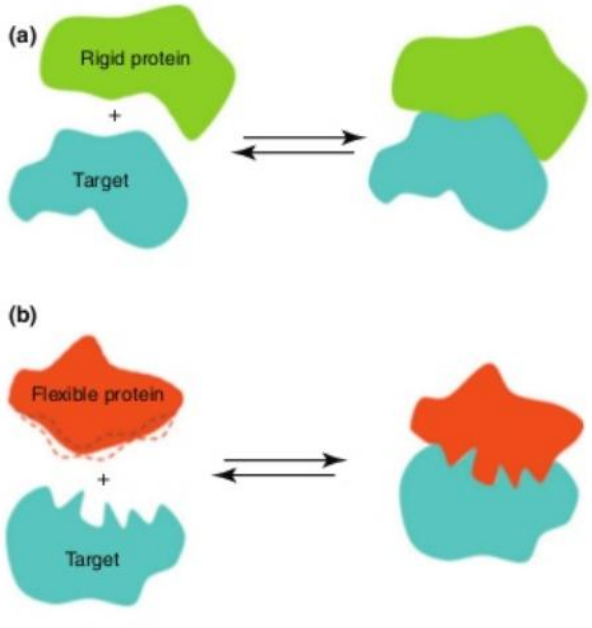
High affinity + high specificity



Low affinity + high specificity

Zhou H-X *TIBS* **2011**

Why has evolution favoured intrinsic disorder



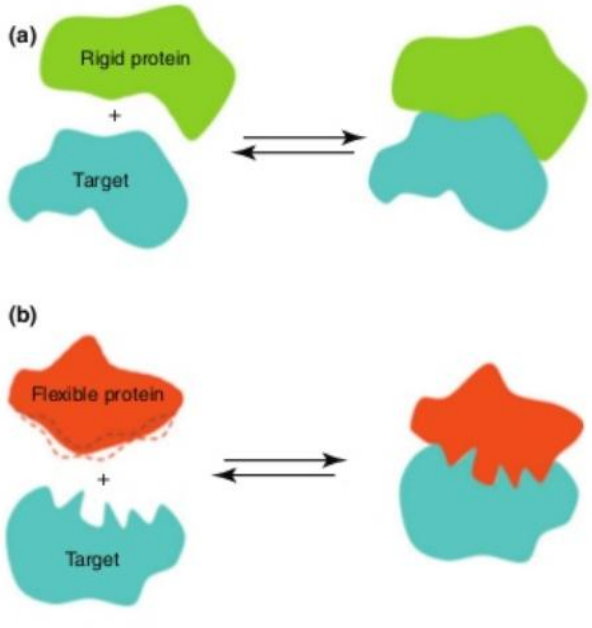
High affinity + high specificity

Low affinity + high specificity

Zhou H-X *TIBS* **2011**

One intriguing proposal is that intrinsic disorder makes low affinity compatible with high specificity.

Why has evolution favoured intrinsic disorder



High affinity + high specificity

Low affinity + high specificity

Zhou H-X *TIBS* **2011**

This is because in many scenarios it is desirable that protein protein interactions are of low affinity e.g. in signaling.

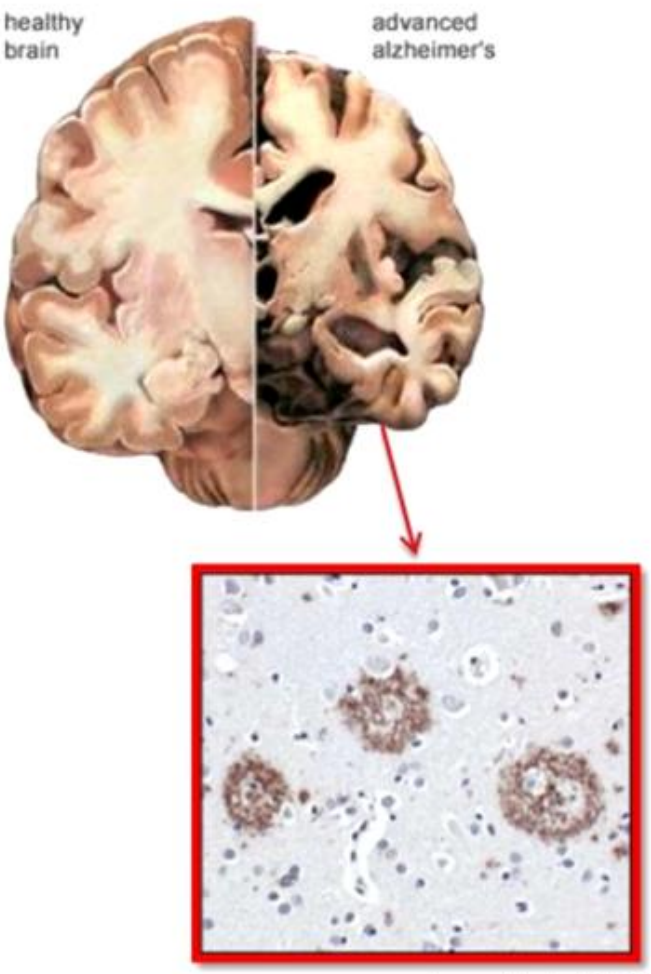
Why are IDPs Interesting?

Diverse Roles!

- Regulatory
  - Homeostasis of signaling pathways
  - Translation/Transcription
- Structural
  - Flexible Linkers

AND….. They can kill you.

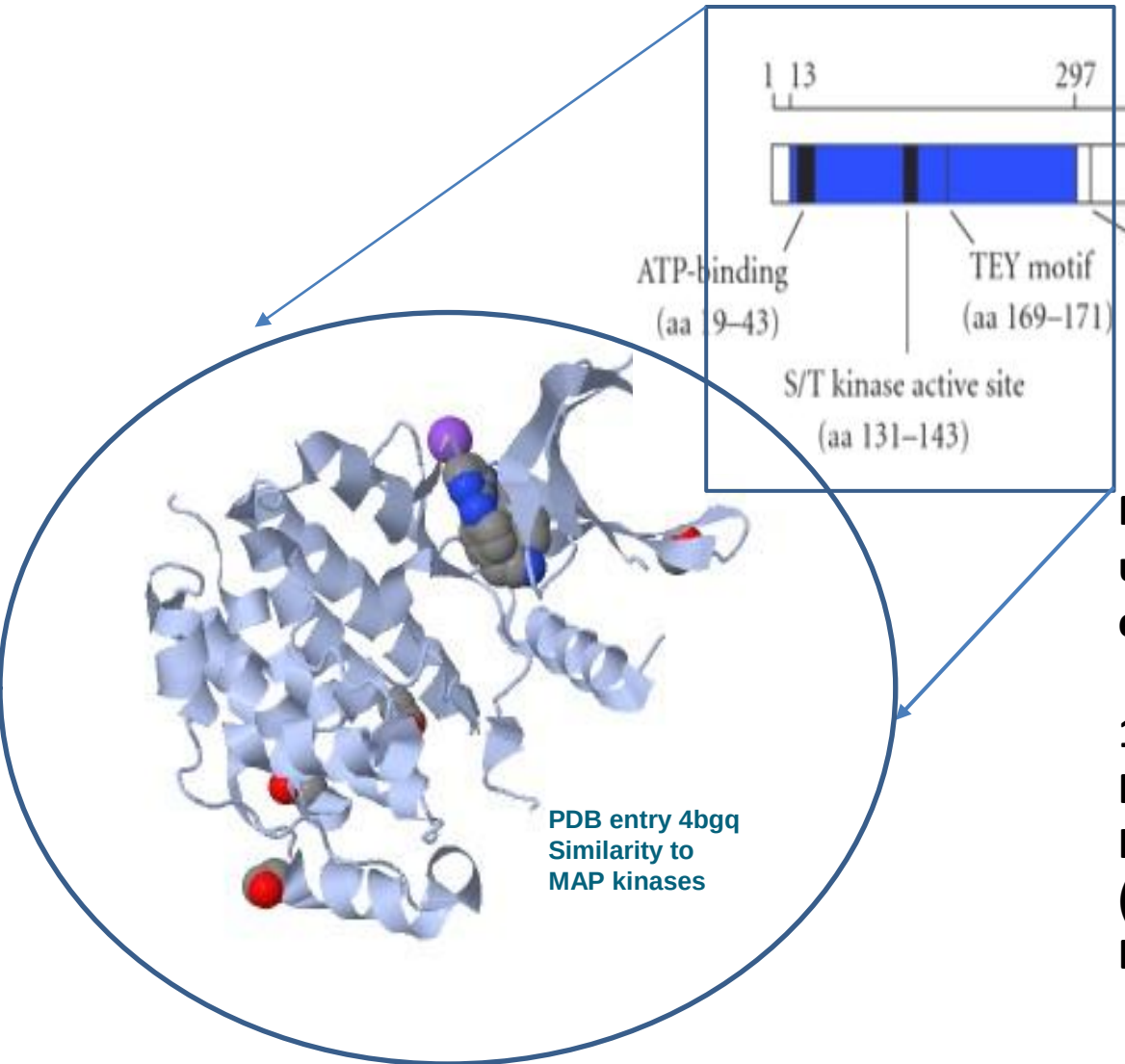
- Disease states
  - Cancer (lack of cell cycle regulation)
  - Brain (amyloid plaque formation)



Lee et al, 2003

facciamo un esempio…

Una proteina umana IDPR coinvolta in una malattia genetica ultrarara: CDKL5



Mutazioni in CDKL5 determinano una encefalopatia epilettica ad esordio precoce (CDD)

1: circa 50.000 bambine (*X-linked)*
Ritardo mentale grave
Incapacità di usare le mani (stereotipie)
Epilessia farmaco resistente!

CDKL5 Protein Replacement Therapy (PRT) rescues neurological phenotypes of a mouse model of CDKL5 disorder*

- TAT -> N-terminal tag with the ability to deliver macromolecules and proteins into cells and into the brain
- When administred as intracerebroventricular infusion (ICV) or systemically (EV), TAT-CDKL5_1 restored hippocampal development, hippocampus-dependent memory and breathing pattern, rescuing various neuroanatomical and behavioral defects in Cdkl5-null mice.

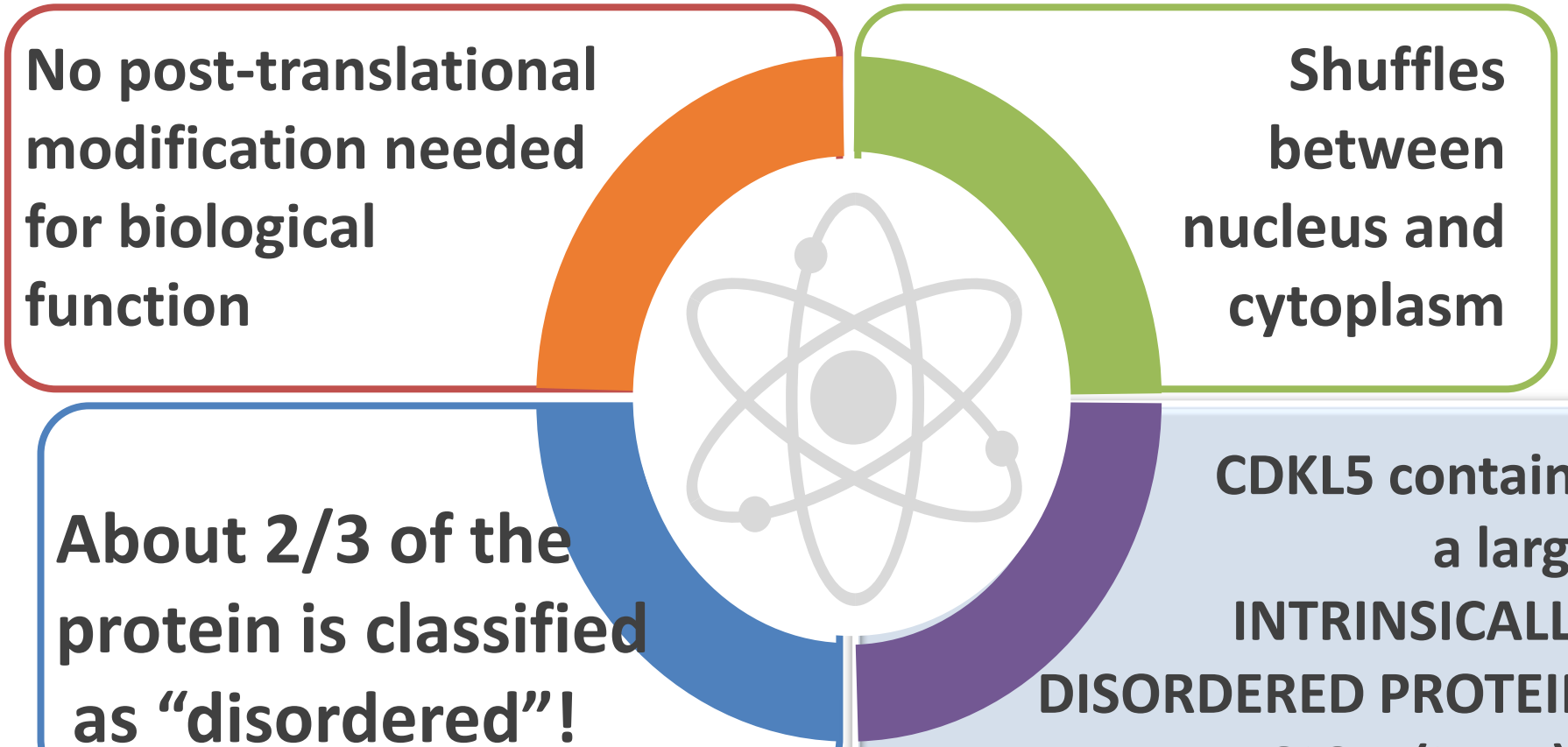
TATk28-CDKL5 protein therapy as promising clinical tool

for the treatment of CDKL5 deficiency disorder!

WE NEED TO PRODUCE LARGE QUANTITIES OF RECOMBINANT CDKL5

* Trazzi S. *et al., Human Molecular Genetics, 2018, 27, 1572-1592.* *USA patent application 20150247134*

CDKL5 is a difficult protein to be produced

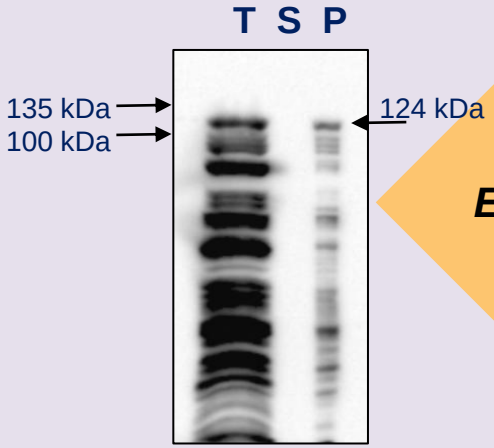


* Kilstrup-Nielsen C, *et al.*, Neural Plast. 2012:728267

Recombinant CDKL5 production in bacterial cell factories

Production of TAT-CDKL5_5 at suboptimal growth temp in rich media (growth at 20°C, induction at 0,7 OD_{600nm} 100 µM IPTG, downshift at 15°C, 18 hrs induction)

The protein is heavily proteolysed and accumulates as insoluble inclusion bodies even at lower production temperature.

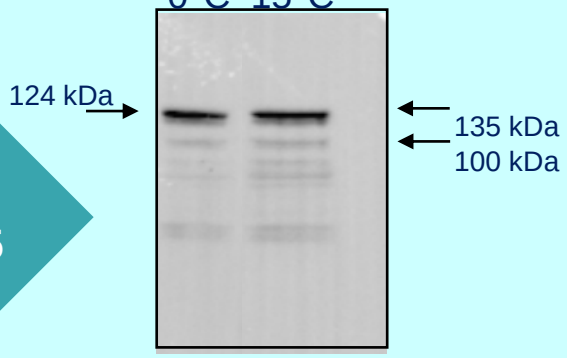


Anti-His Tag Ab

***E. coli* BL21(DE3)**

***Pseudoalteromonas haloplanktis* TAC125**

*Ph*TAC125 soluble extracts



Anti-CDKL5 N-term Ab

Production of TAT-CDKL5_5 in synthetic medium at two growth temp (0 and 15°C) (induction at 0,7 OD_{600nm} 10mM galactose, different induction times)

The protein is fully soluble and largely preserved from proteolysis at both production temperatures!

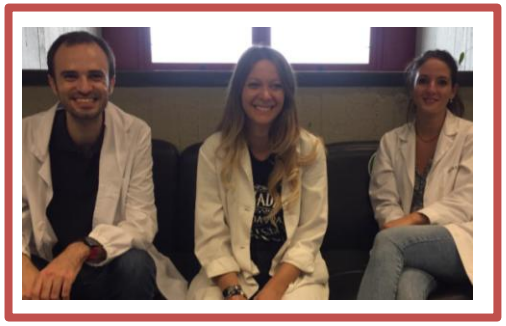
THE PSYCHROPHILIC CELL FACTORY...

Pseudoalteromonas haloplanktis TAC125

- ✓Gram negative, gamma proteobacterium, from Antarctic sea water
- ✓Full genome knowledge[1]
- ✓Growth in a wide range of temperatures (-5/25°C)
- ✓Growth at high cell density in flasks or automatic fermentors
- ✓Short replication time in complex media
- ✓Medium copy number cryptic plasmid pMtBL[2]

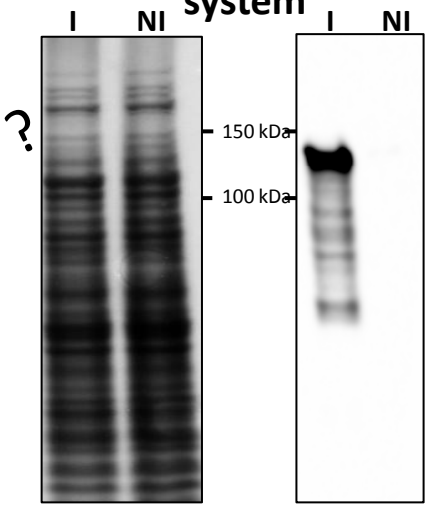
✓Established gene-expression technology[3]

(1) Médigue et al. (2005), *Genome Research*, 15: 1325-35; (2) Tutino et al. 2001 *Extremophiles* (2001) 5, 257-264
(3) Parrilli et al., (2008) in *Psychrophiles: from Biodiversity to Biotechnology* Margesin, R.et al. (Eds.) Springer-Verlag pp.365-379



High quality rec*h*CDKL5_1 is produced by *Ph*TAC125

Original expression system



Coomassie

Anti-CDKL5 Wb

~ 15 µg of protein in 1 L culture

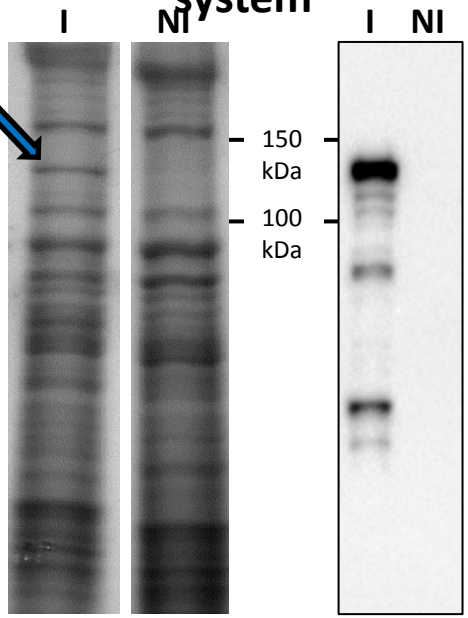
01 Tuning transcriptional efficiency

02 Tuning translational efficiency

03 Host improvement by genome engineering

04 Enhancing vector copy number

Current expression system

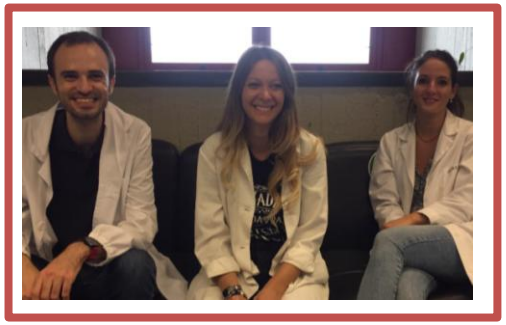


Coomassie

Anti-His Wb

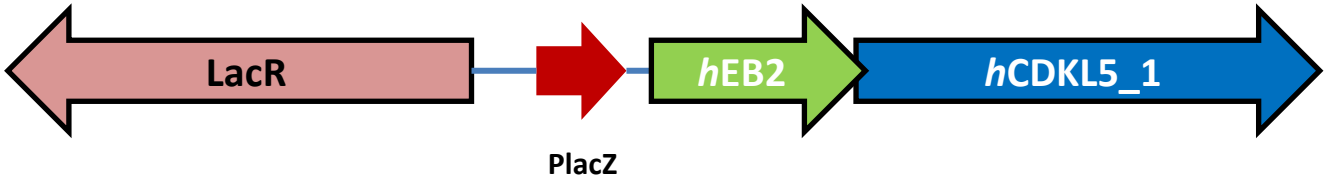
5-10 mg of protein in 1 L culture!

Is the recombinant enzyme active??

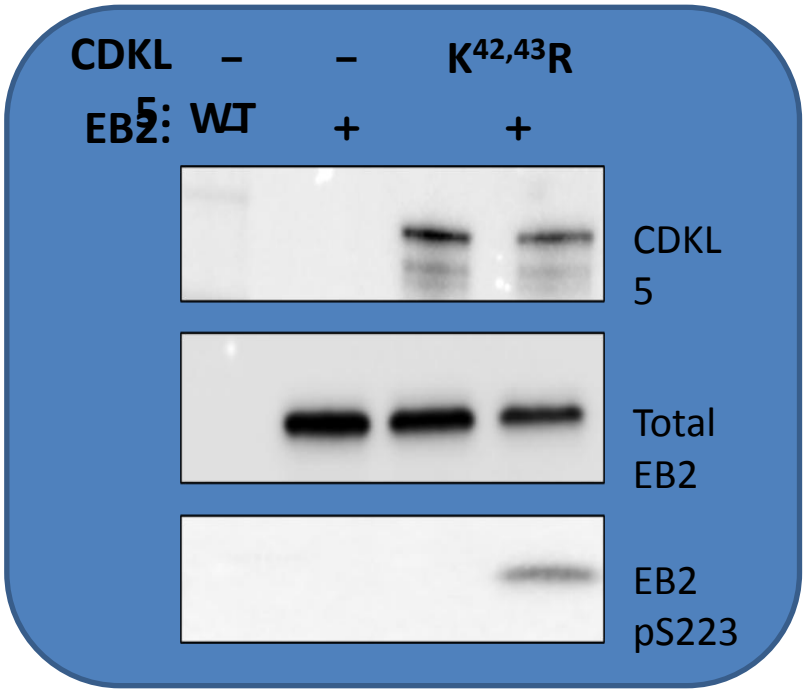


Rec *h*CDKL5_1 produced in *Ph*TAC125 is active!!

***In vivo* co-expression activity assay**



*h*EB2 and *h*CDKL5_1 are co-expressed in *Ph*TAC125 under the control of the same promoter at 15°C



EB2 is phosphorylated in *Ph*TAC125 only when co-expressed with CDKL5 WT

This approach will be used to preliminary characterize if the missense mutations may alter CDKL5 activity on EB2!

Conclusioni:

- Il disordine è molto diffuso nel proteoma umano
- Nuove metodiche per studiare le IDP
- Nuovi approcci biotecnologici per la produzione di IDP per motivi di studio e di terapia
  - *P. haloplanktis* TAC125 come ospite innovativo per la produzione ricombinante di IDP (tecnologia *made in Naples*)