



Bioinformaticians + Experimentalists = Successful Protein Analysis

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Research subjects



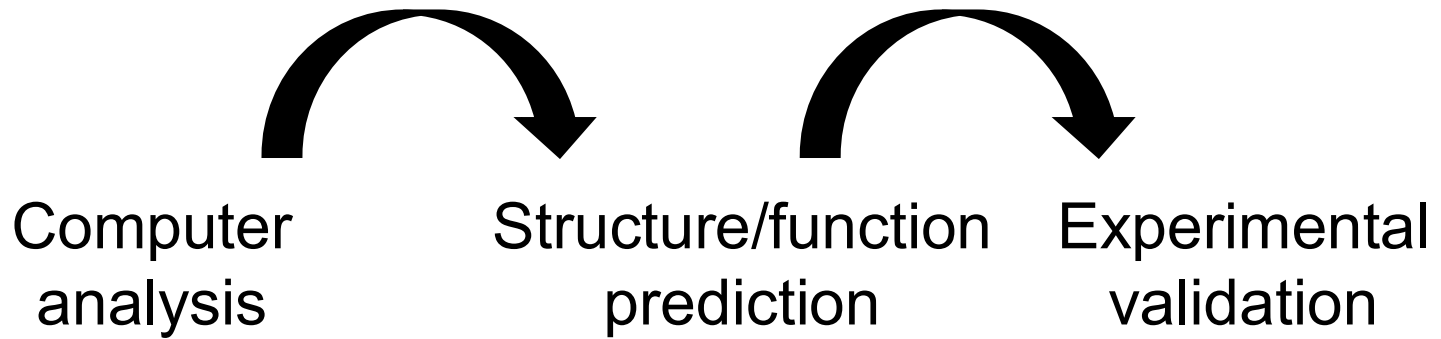
Enzymes acting on:

DNA

RNA

- Discovering novel enzymes
- Protein engineering
- Characterization of protein complexes

Structure of the laboratory



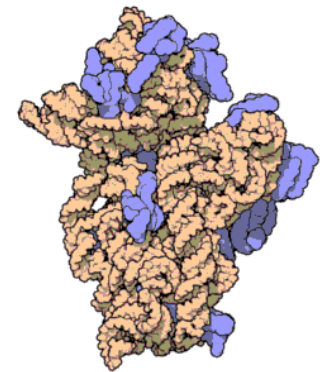
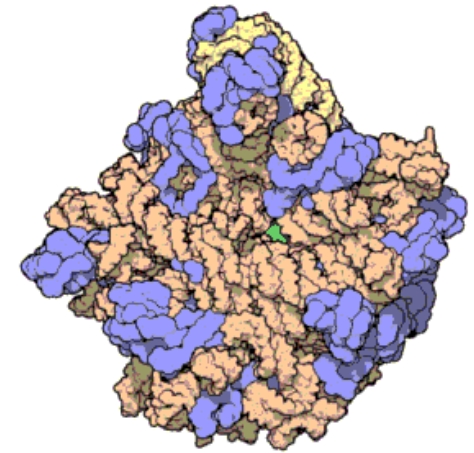
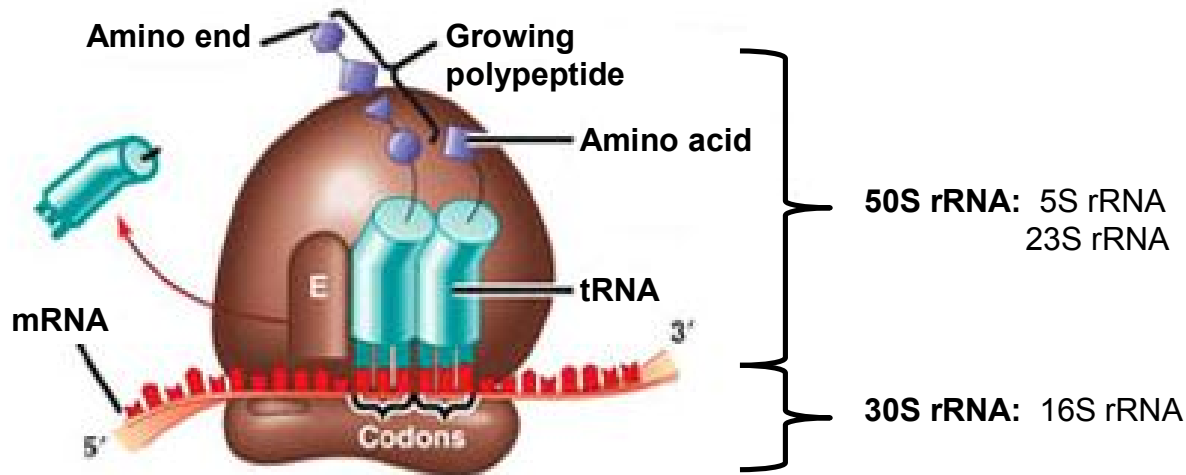
What information do we get from protein structure?

- Protein function
- Biological processes
- Mechanism of reaction and interaction

Discovering new RNA methyltransferases

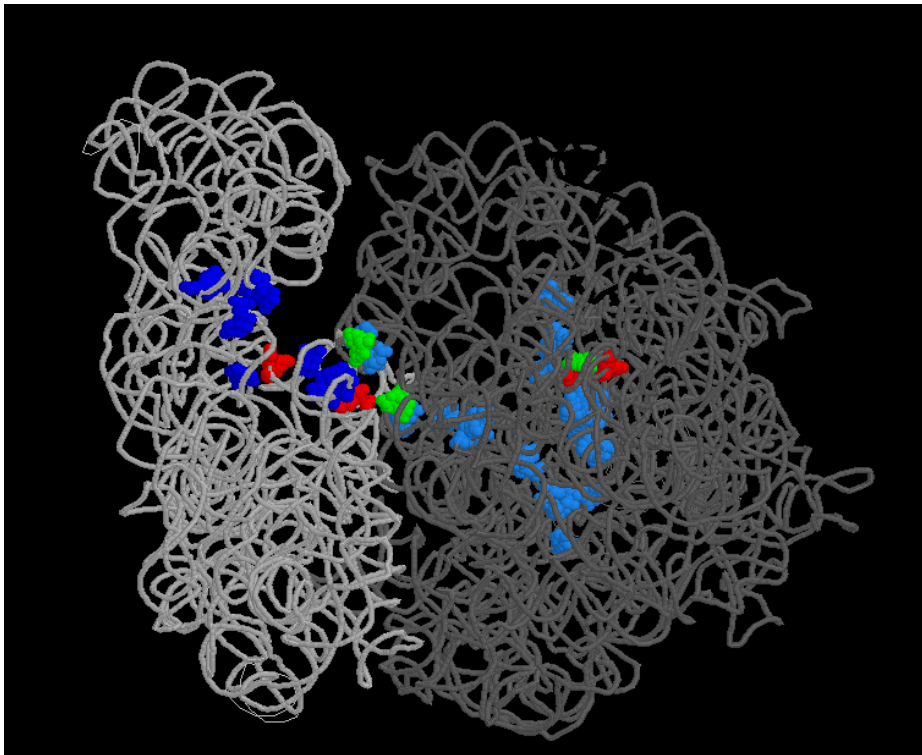
Elżbieta Purta

Protein synthesis in bacteria



Modified nucleosides in rRNA

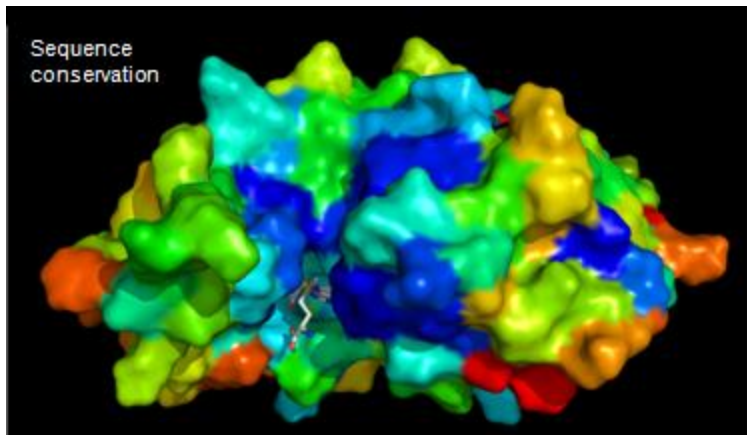
- 11 modified nucleosides in 16S; 26 in 23S
- Main function: reinforce the tertiary structure essential for catalysis
- Other functions: resistance to antibiotics that bind to rRNA



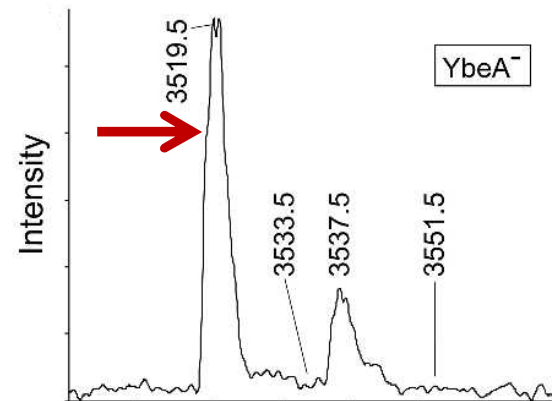
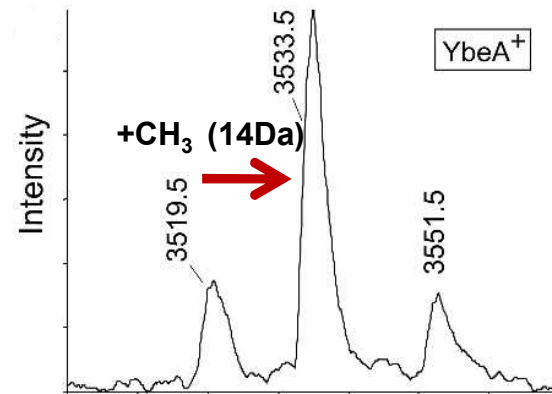
Modifying enzyme unknown

Modifying enzyme
identified in this lab

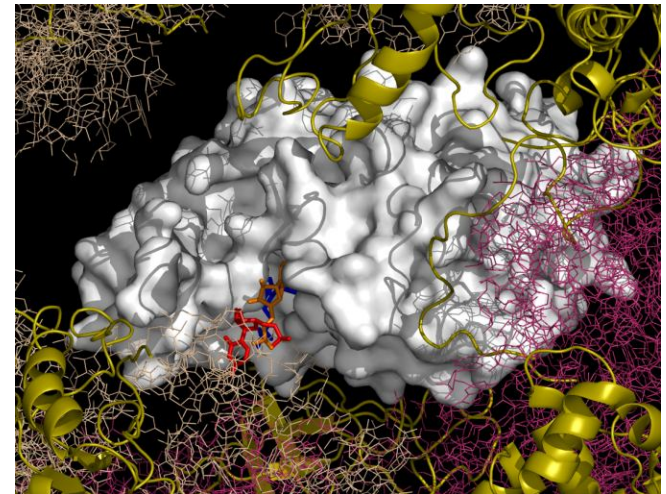
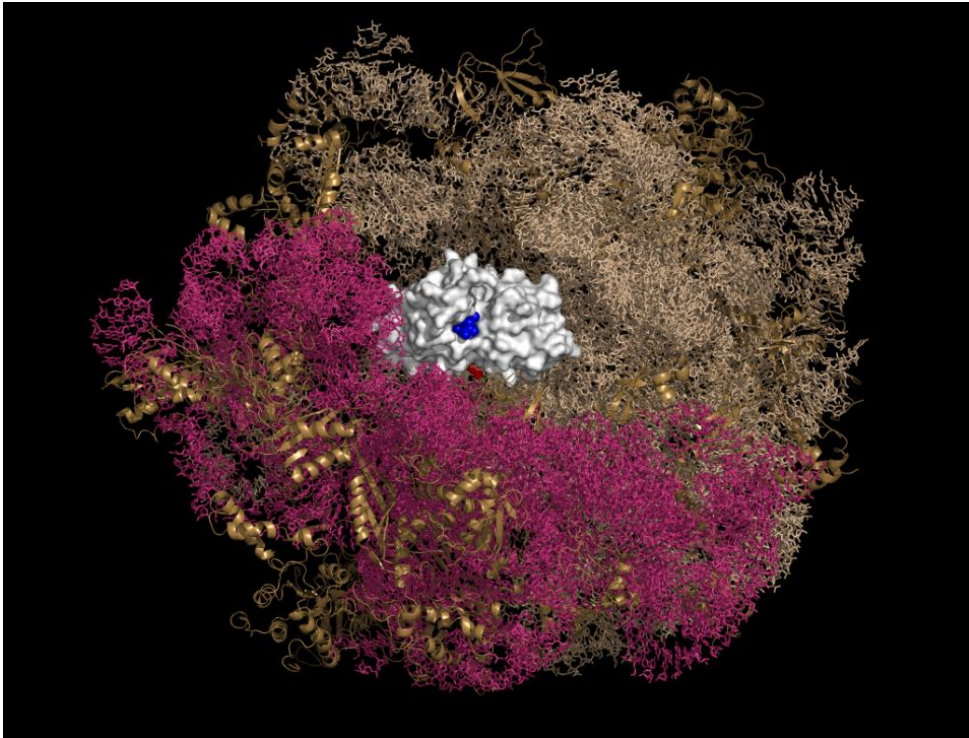
YbeA: novel MTase specific for 23S rRNA



Mass spectrometry analysis



Interaction of YbeA with the ribosome

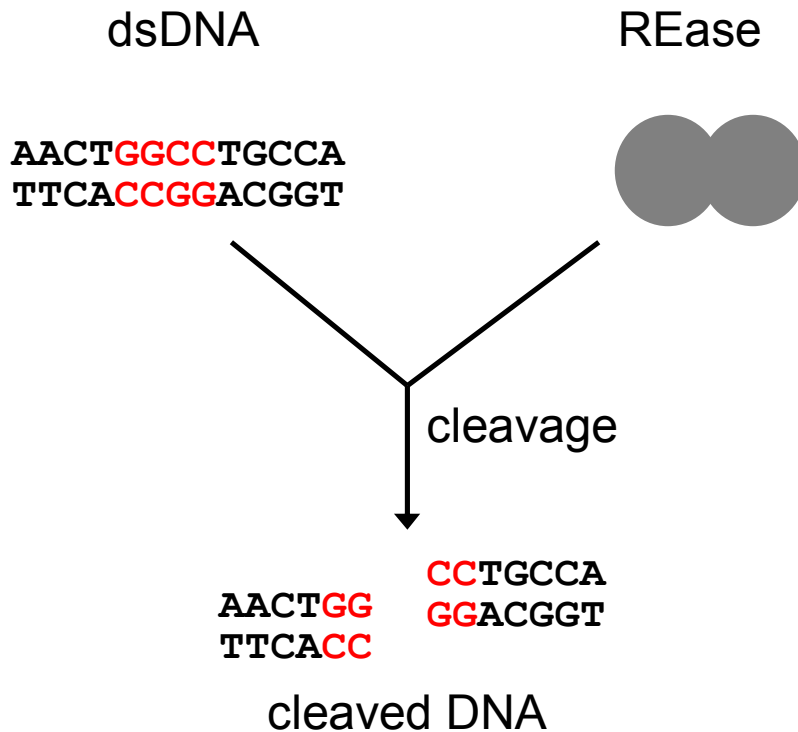


Protein engineering of restriction endonucleases

Sebastian Pawlak

Restriction endonucleases (REases)

- occur frequently in bacteria and archaea
- cleave double-stranded DNA in a sequence-specific manner



Why engineer REases?

Important tools in biology:

recombinant DNA technology

diagnostic tool

DNA physical mapping

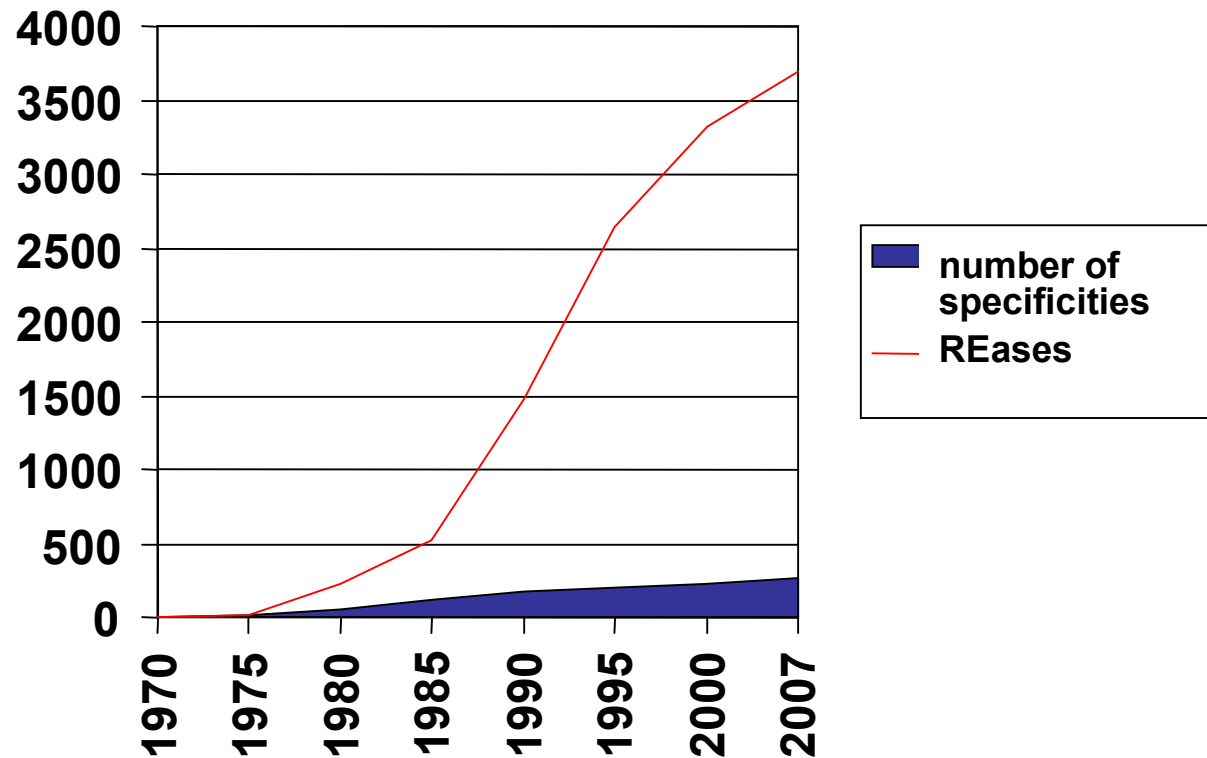
various cleaved sequences

~ 3700 REases



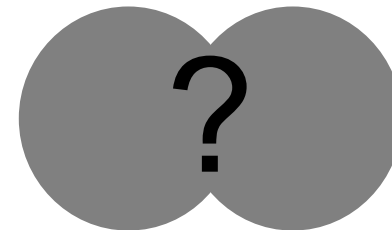
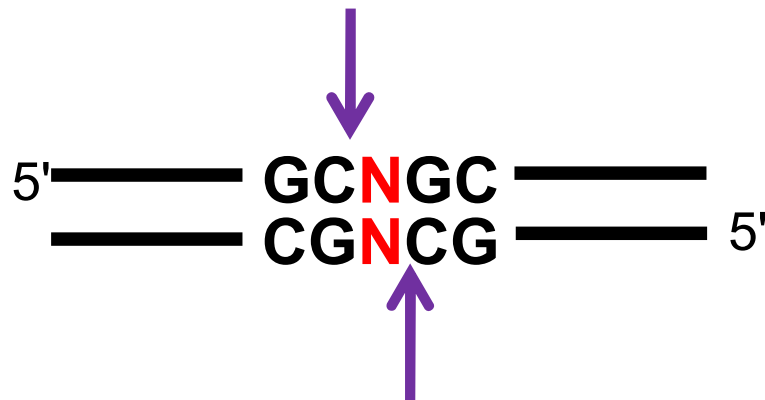
266 distinct
specificities

Increase in amount of discovered REases versus specificities



Engineering of restriction endonuclease Bsp6I

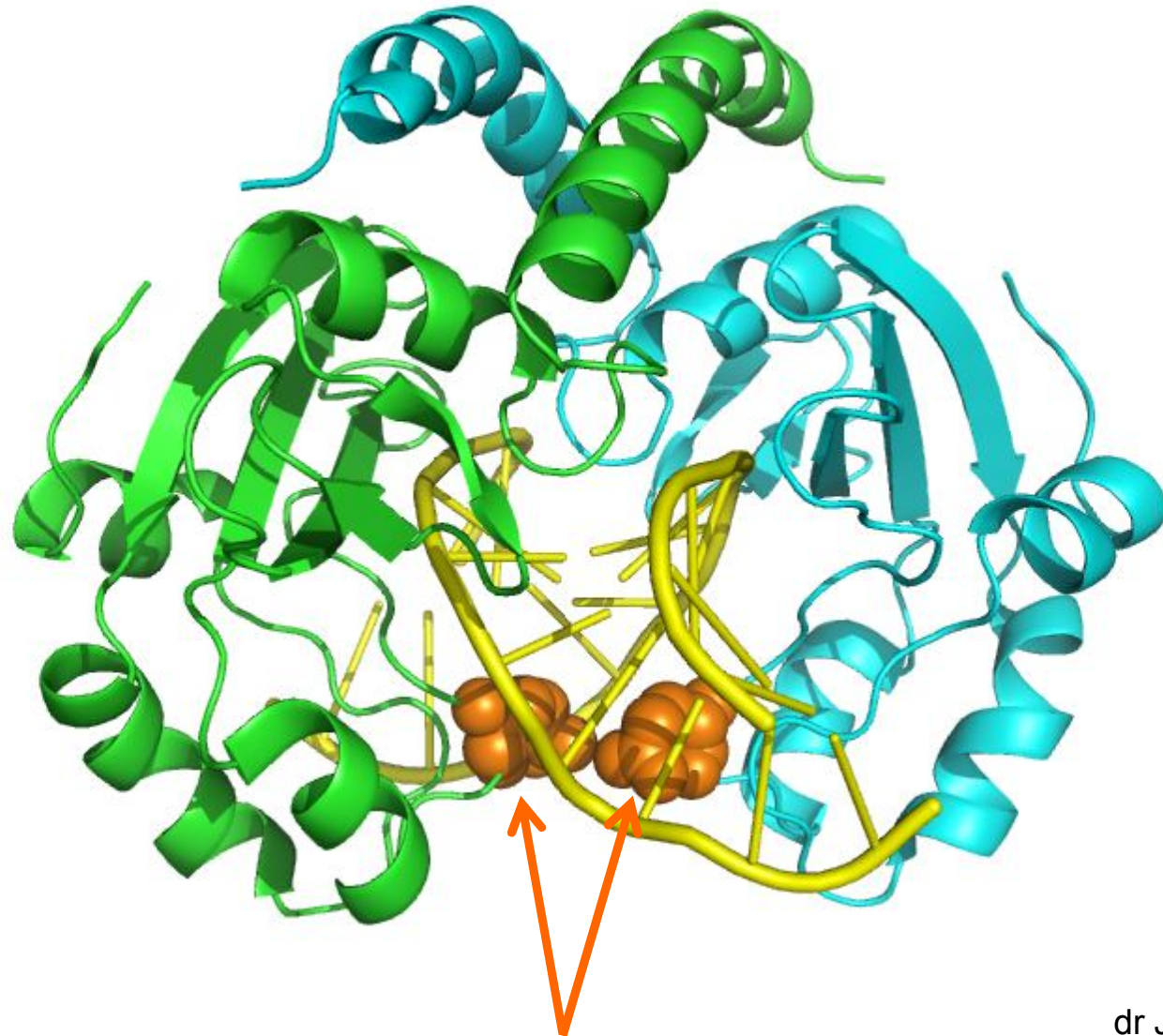
Bsp6I



structure
unknown

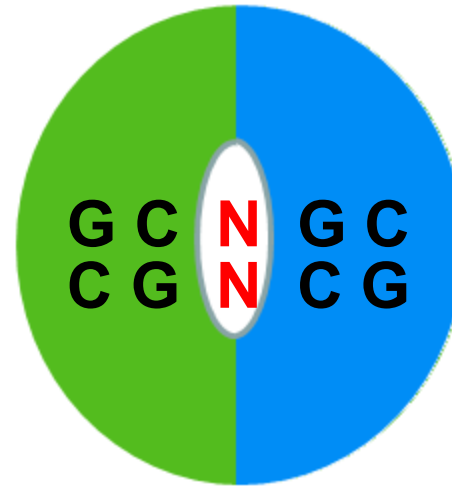
N = C, G, A, T

Bsp6I model

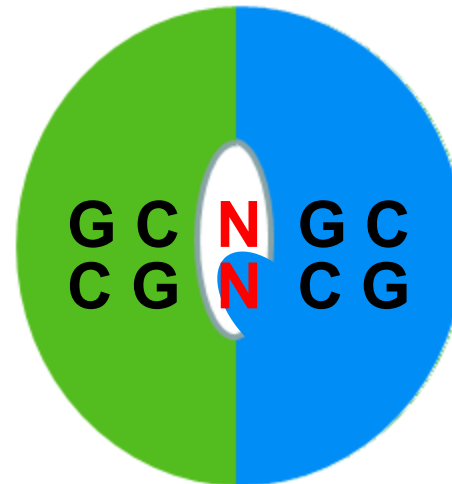


Predicted effect of Bsp6I mutagenesis

No contact with
middle bases

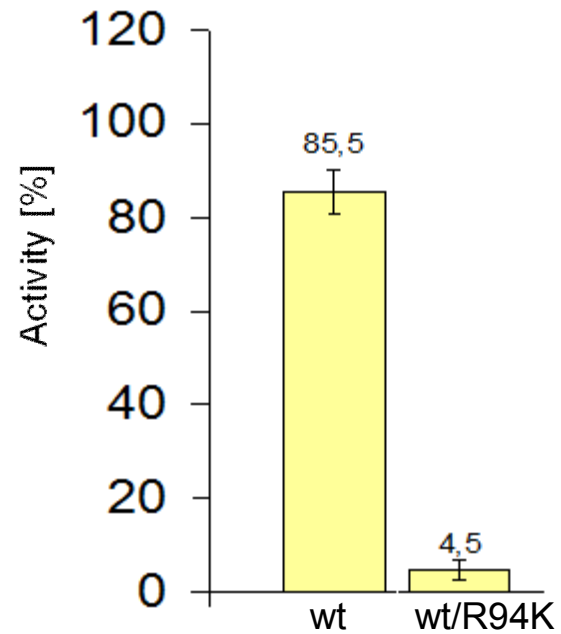
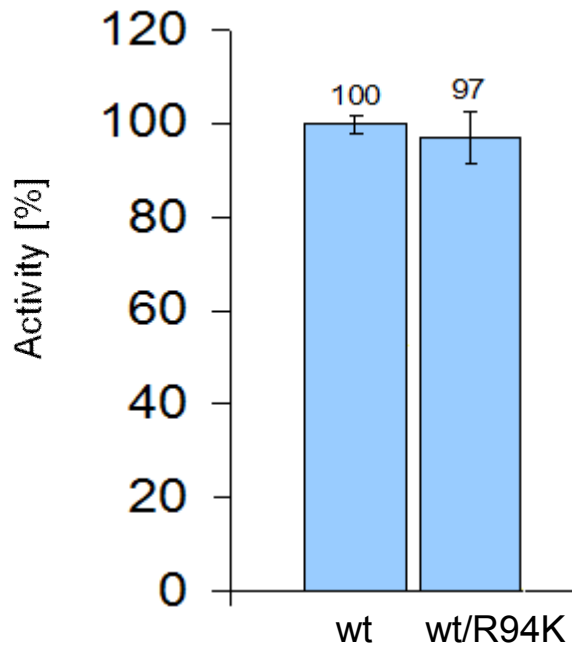
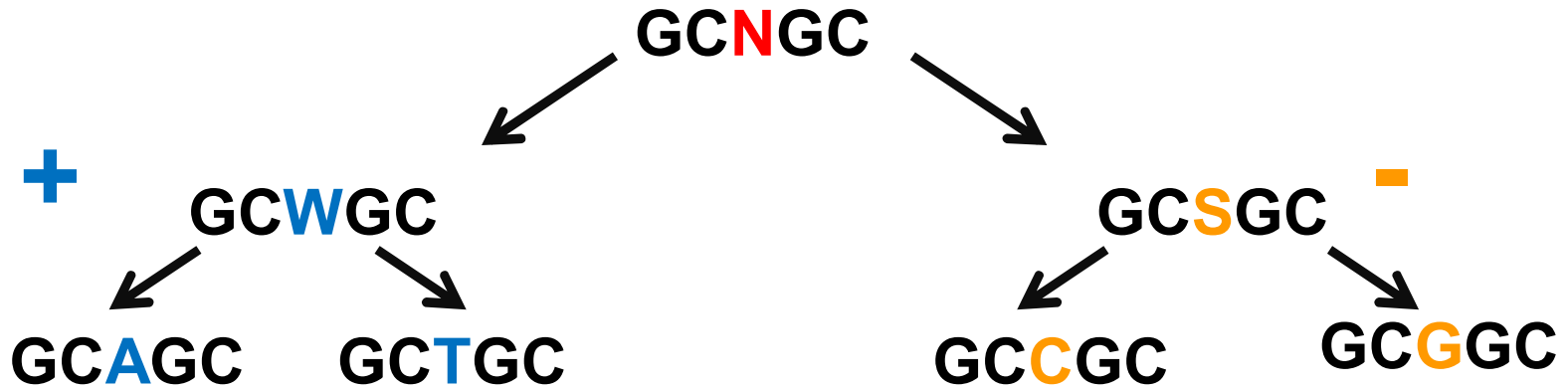


Creating contact
with middle bases



E94 → K

Bsp6I with novel specificity



Characterization of protein complexes involved in DNA repair

Katarzyna Poleszak

Why study protein complexes involved in DNA repair?

DNA repair

→ Processes correcting DNA damages

→ Crucial to maintain genome stability

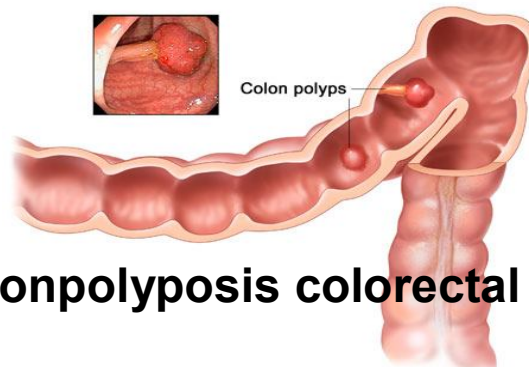
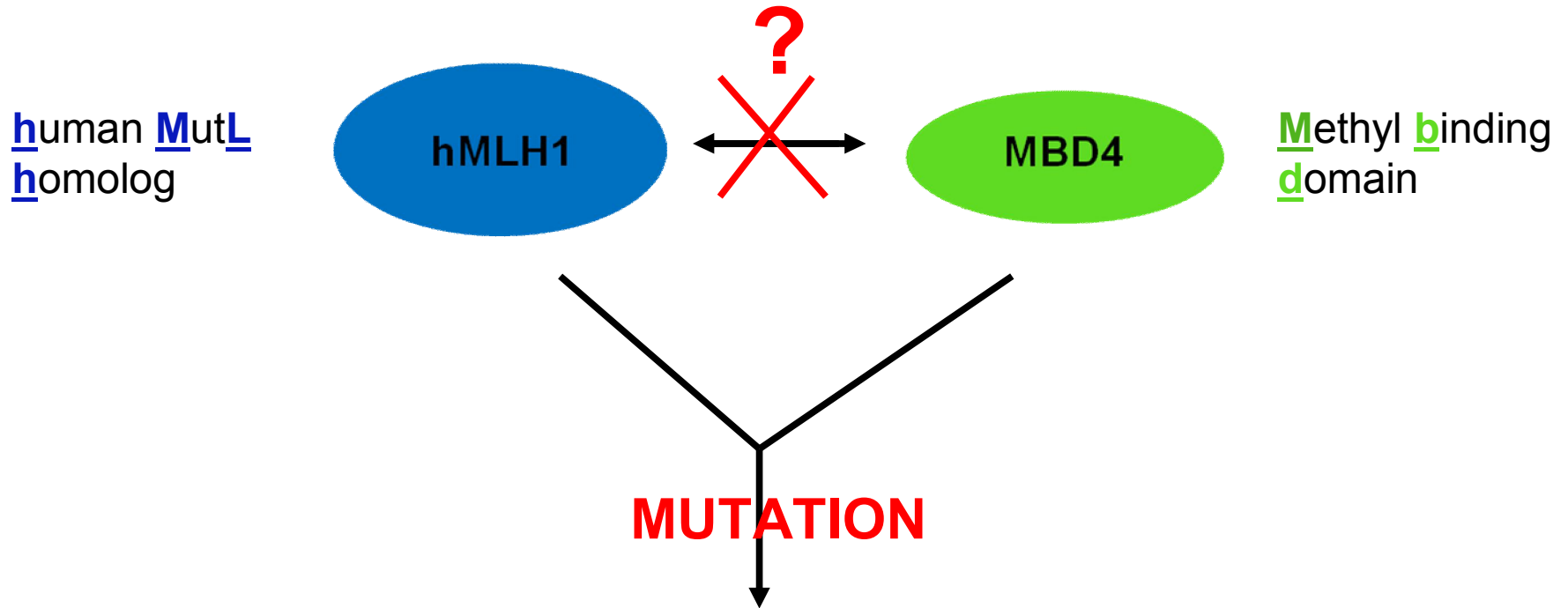
Protein-protein interactions

→ Critical to most biological processes

Protein interaction sites

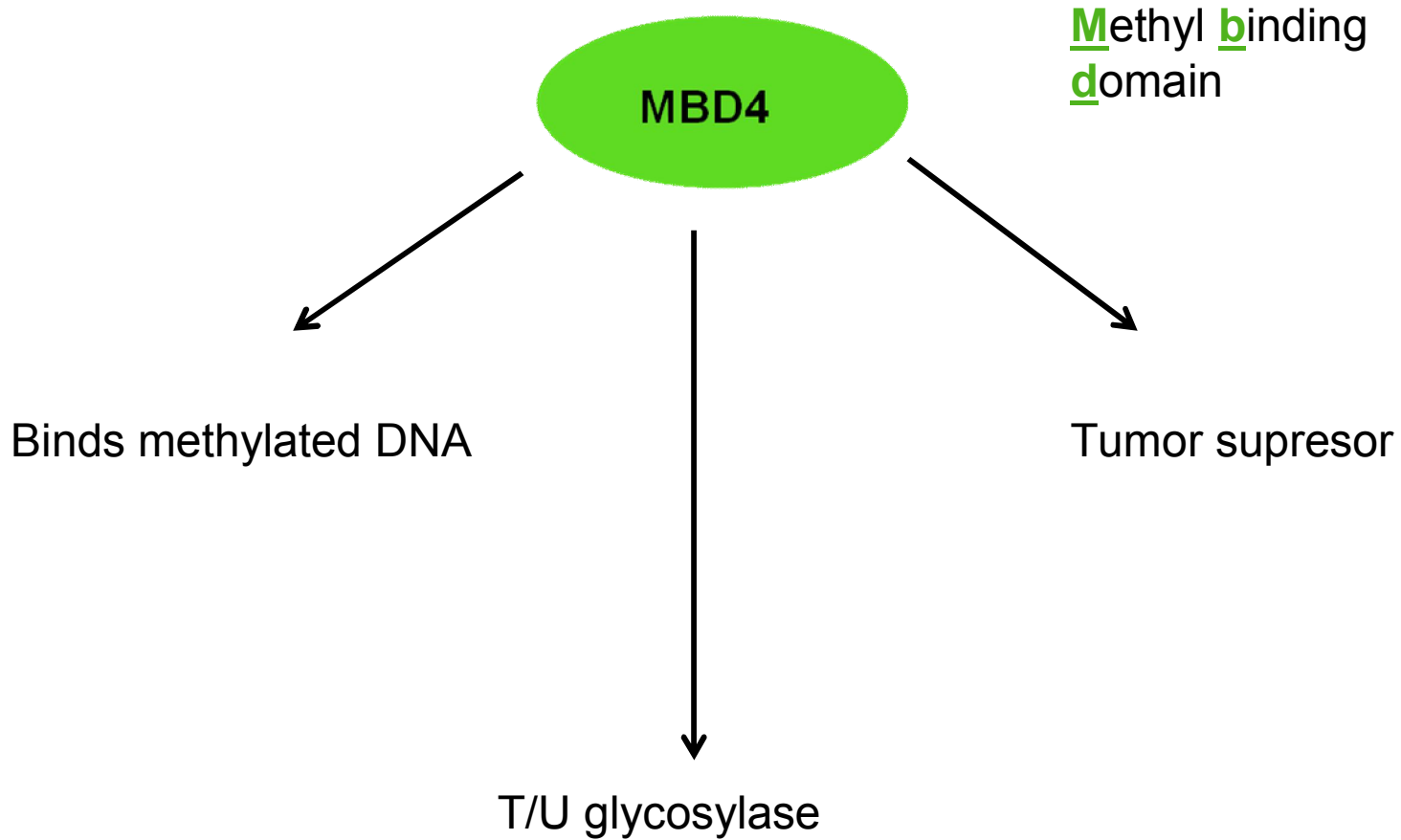
→ Potential drug targets

Analysing hMLH1-MBD4 interactions



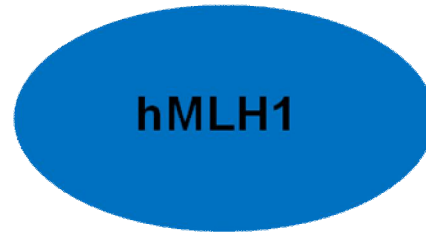
Hereditary nonpolyposis colorectal cancer (HNPCC)

Functions of MBD4

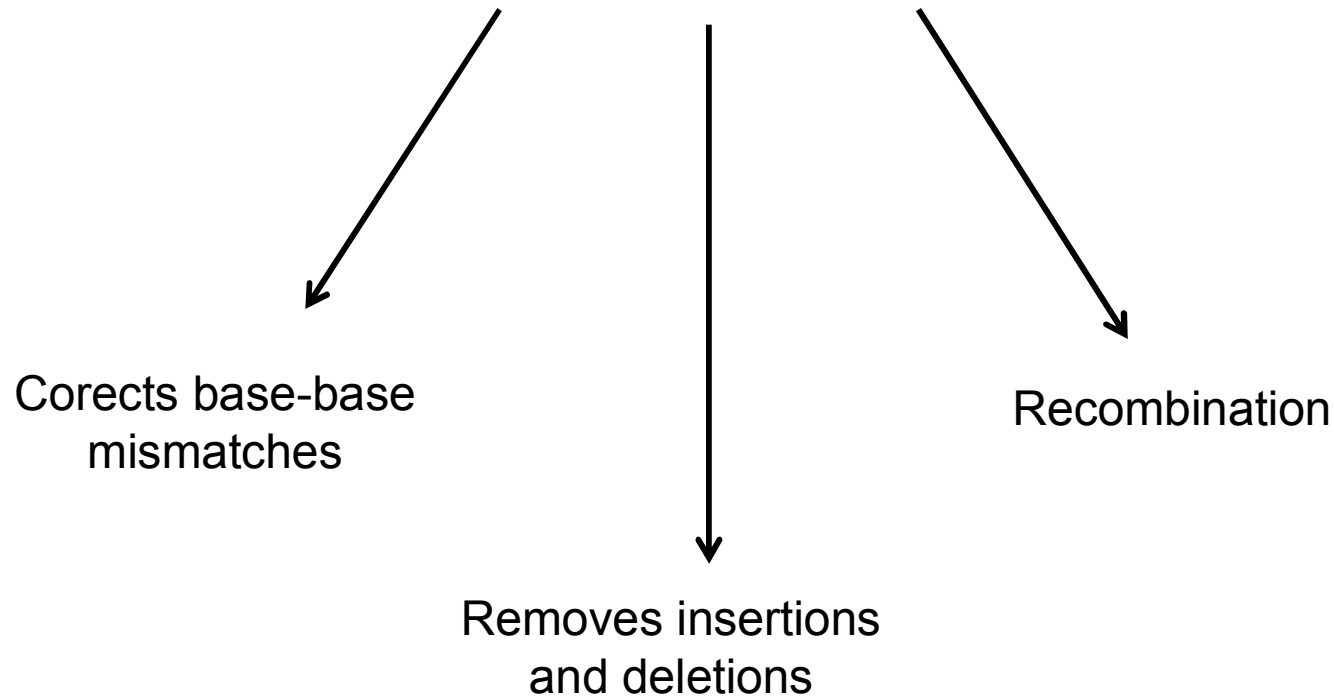


Functions of hMLH1

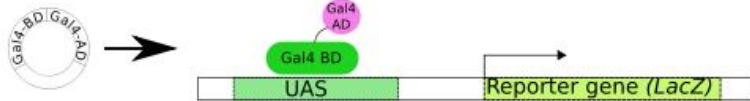
human MutL
homolog



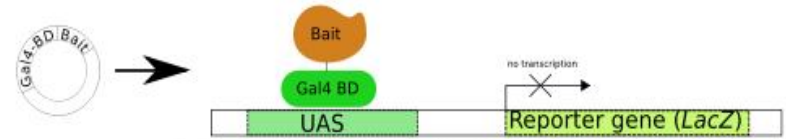
Depending on the interaction partner



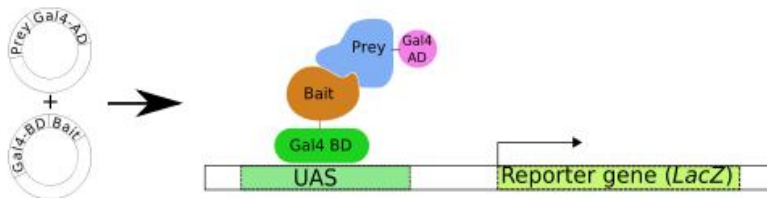
Yeast two-hybrid system (Y2H)



A. Regular transcription of the reporter gene



B. One fusion protein only (Gal4-BD + Bait) - no transcription

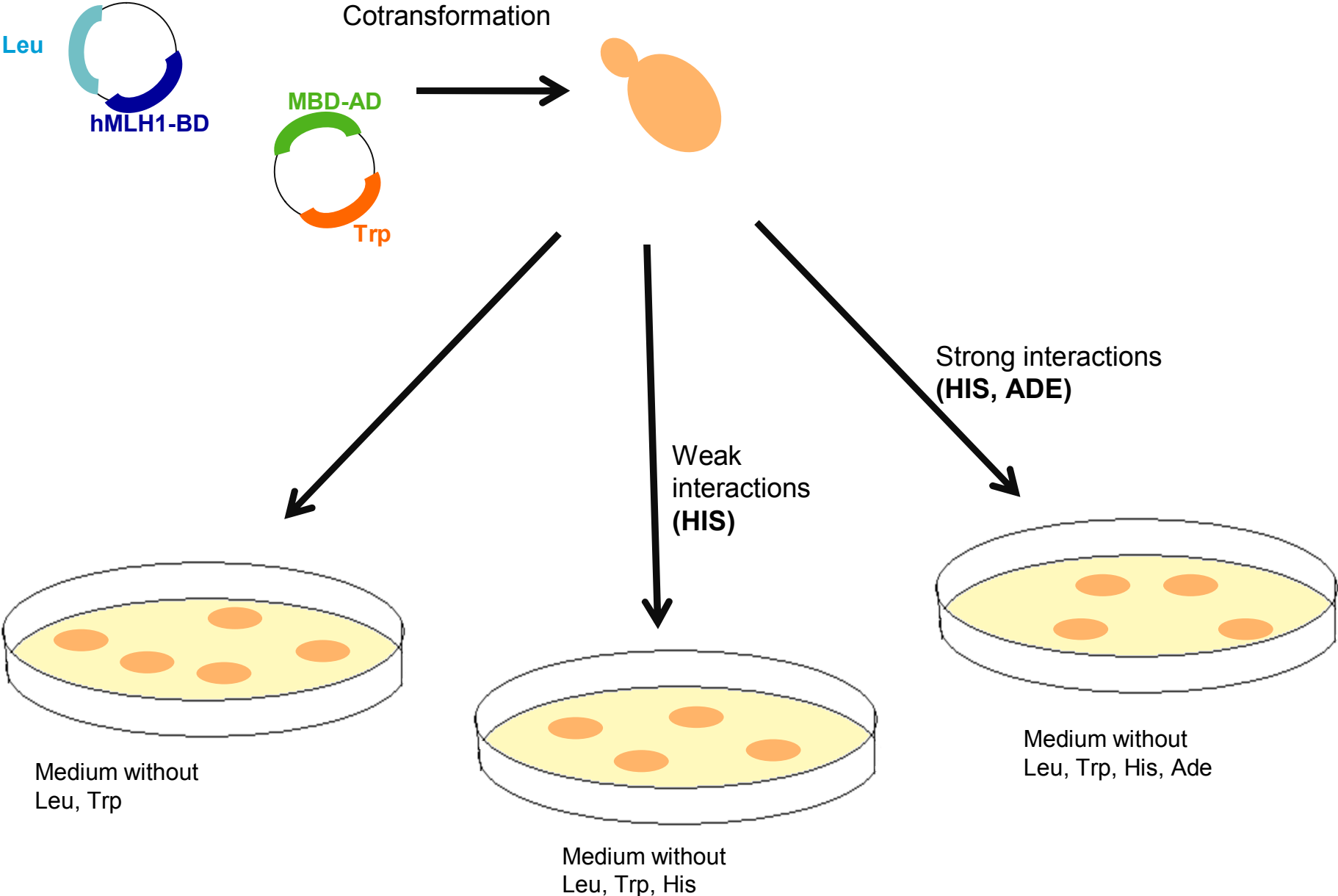


D. Two fusion proteins with interacting Bait and Prey

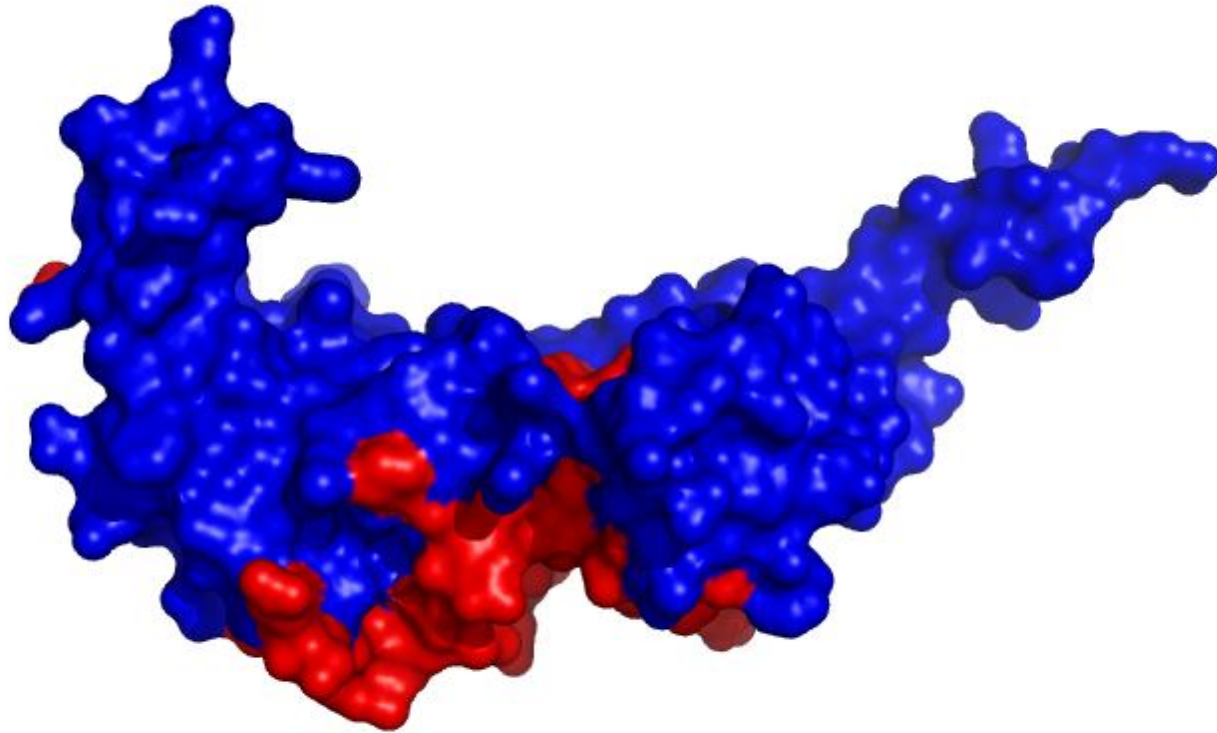


C. One fusion protein only (Gal4-AD + Prey) - no transcription

Screening protein-protein interactions



Prediction of hMLH1 protein interaction sites

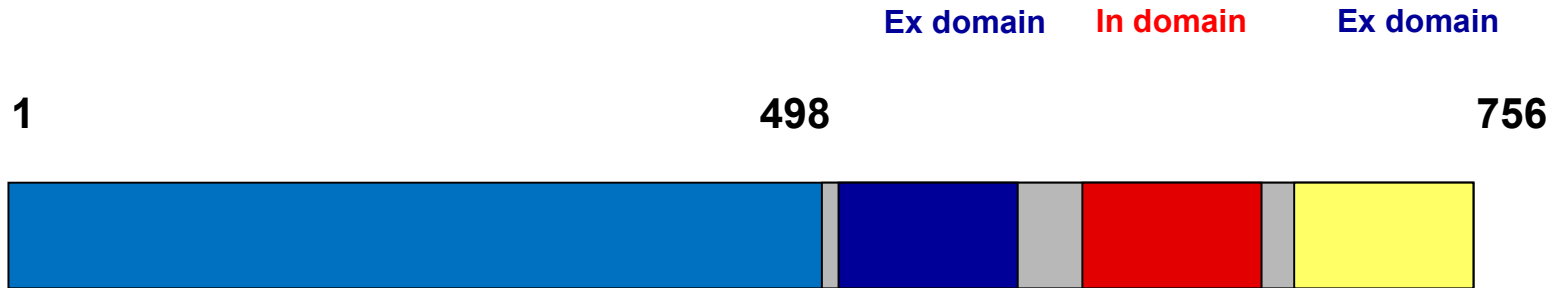


hMLH1

human MutL homolog

NTD
N – terminal domain

CTD
C – terminal domain

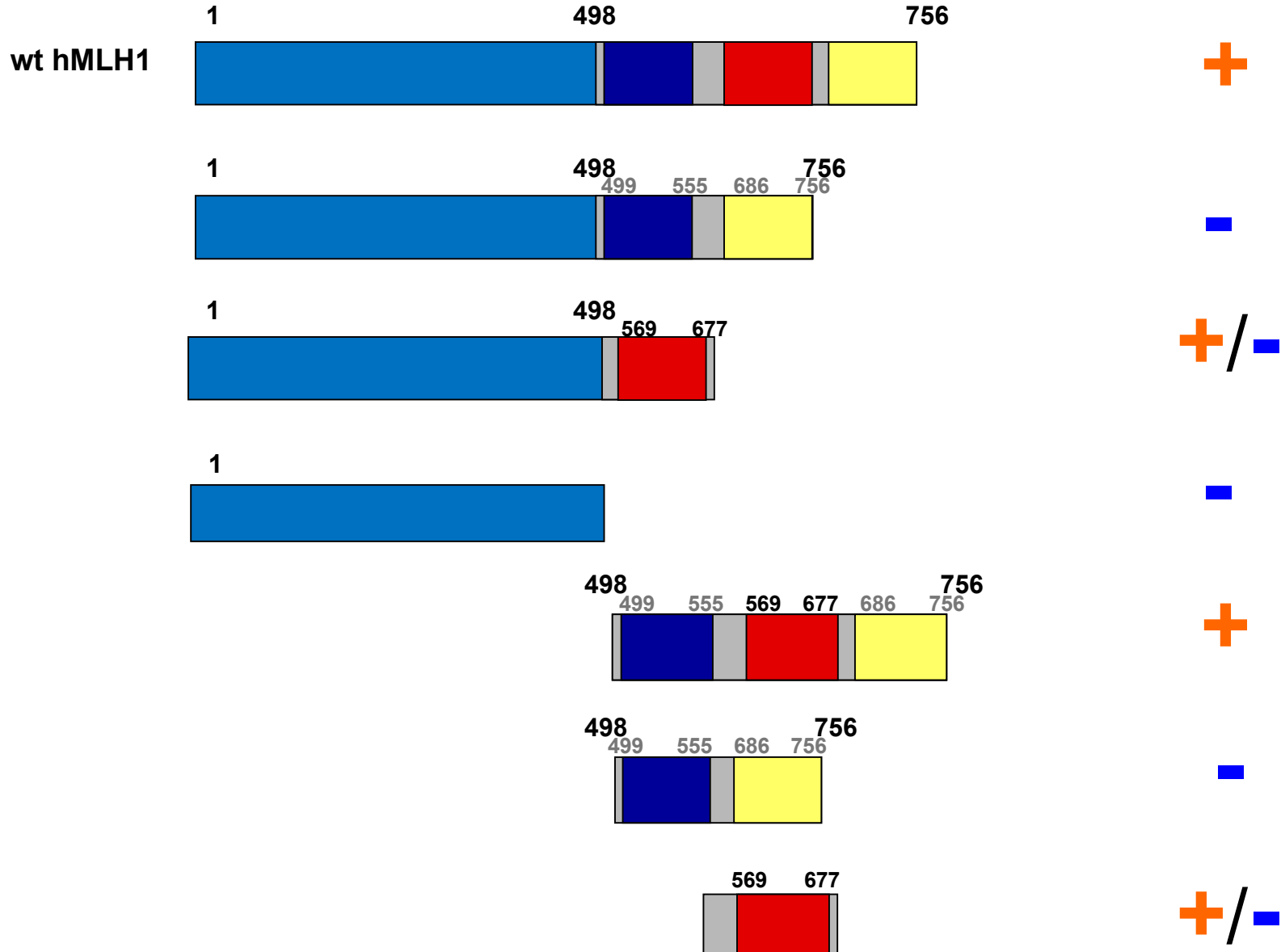


- highly conserved

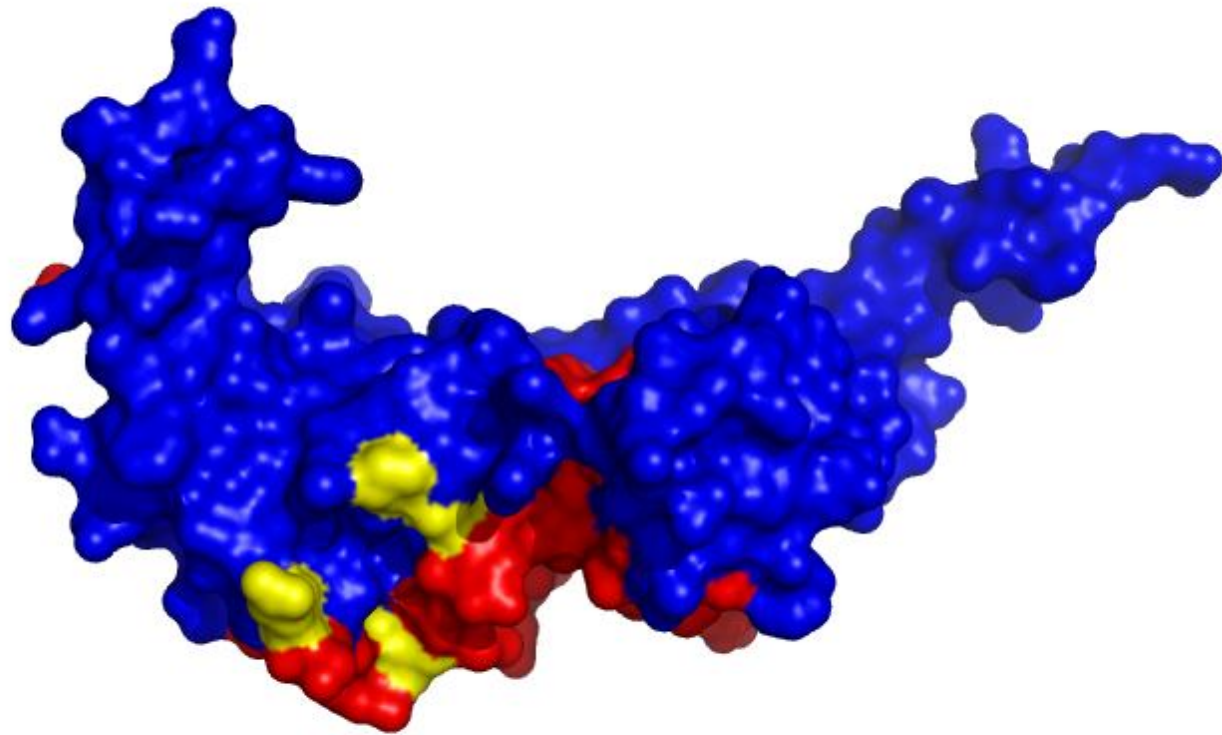
- less conserved
- involved in homo-, and heterodimerization

hMLH1 – MBD4

Y2H



hMLH1 mutations



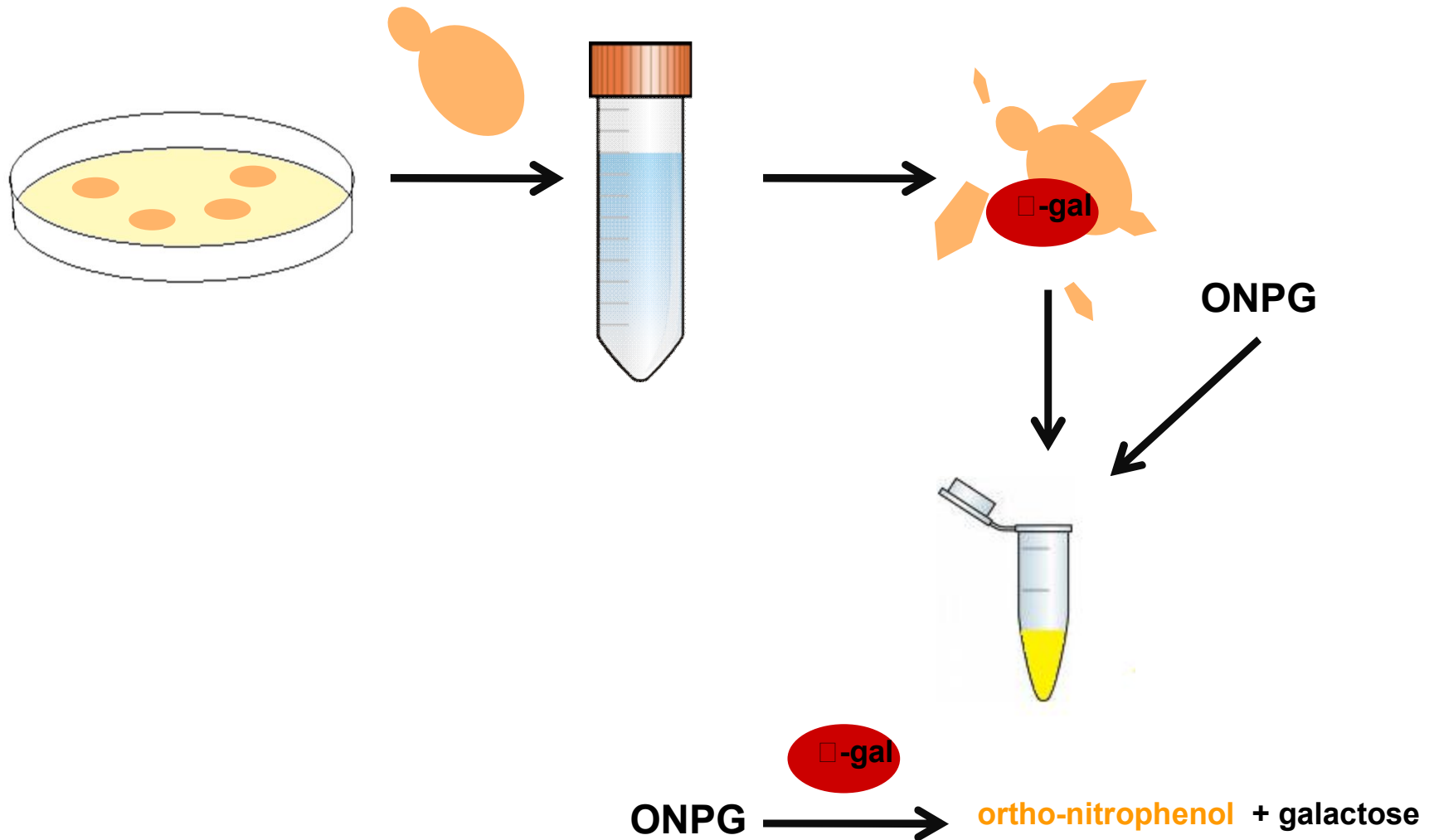
L574P

P640S

P648L

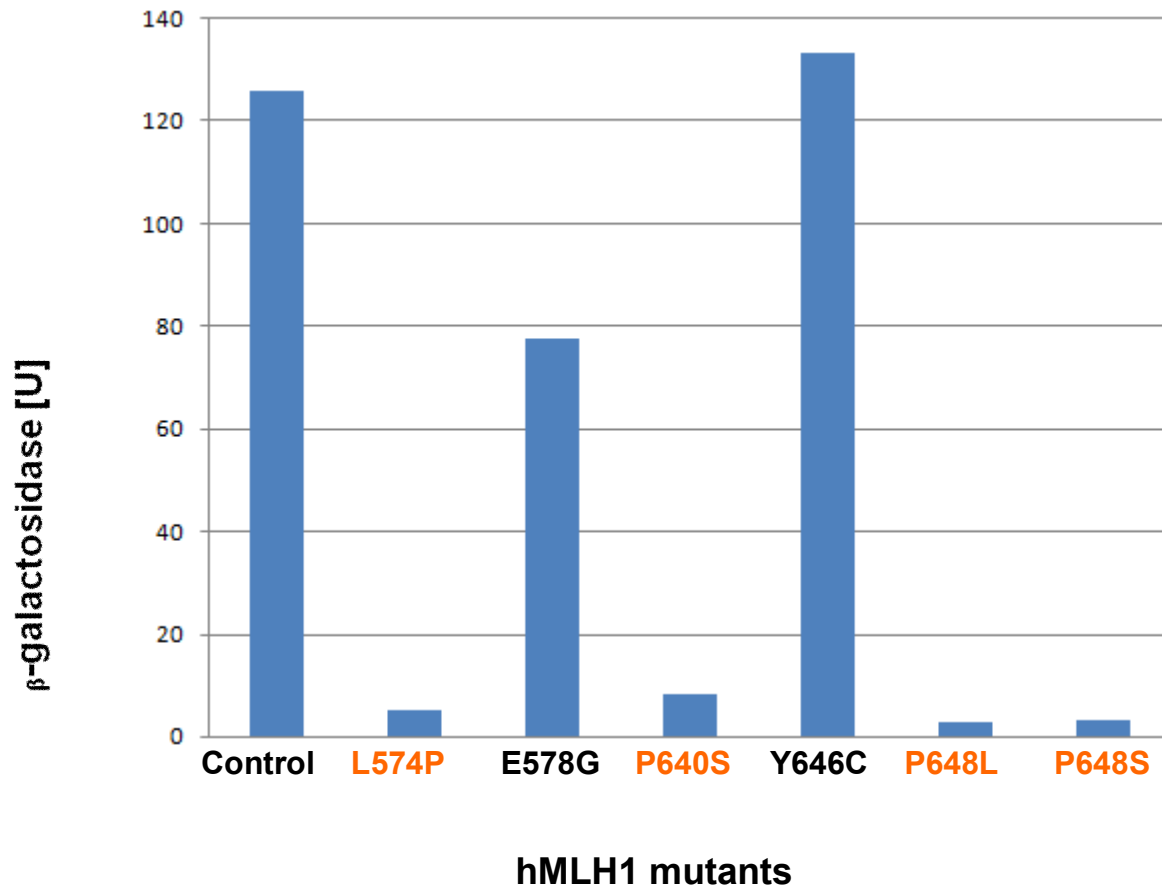
P648S

Measuring the strength of interaction

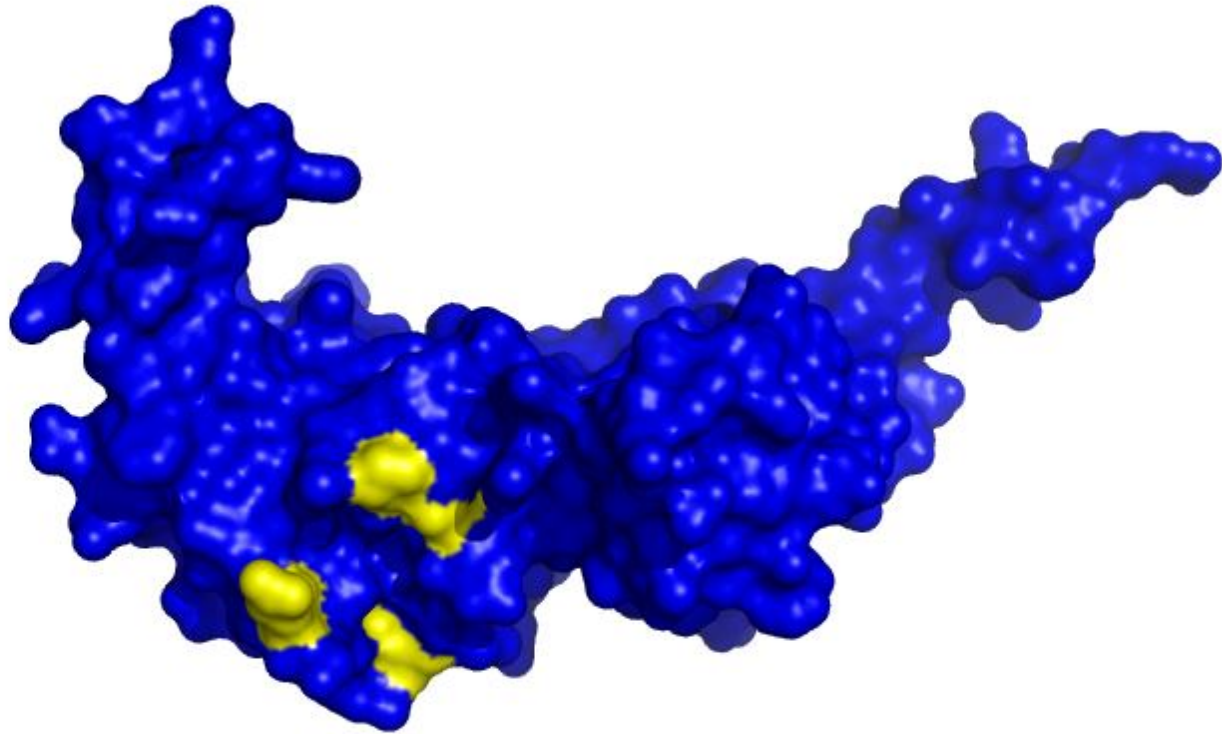


hMLH1 mutants-MBD4

Relative strength of interaction



Mutations abolishing hMLH1-MBD4 interaction



Summary

Bioinformaticians + Experimentalists
= Successful Protein Analysis



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[Colorado3D](#) (protein model analysis)

[MODOMICS](#) (a database of RNA modification pathways)

[PLASTOMICS](#) consortium server

[DNA ENZYMES](#) consortium server

Acknowledgements



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**Thank you for
your attention**