




Elche. Spain



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***“Computer-Aided Protein Design
of Enzymes to
Recognize Specific Peptide
and
DNA Sequences”***

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JUSTIFICATION OF THE TOPIC

Enzymes are proteins that catalyze essential reactions in a specific way:

- Proteases recognize and cleave another proteins
- Nucleases recognize and cleave nucleic acids

We already use some of them as molecular tools in the Biochemistry laboratory.

Computer Protein Design (CPD):

- Use **structural data, software and computers** to look for new molecules.
- **Avoid big scale** production and screenings
(as in Pure Combinatorial Methods used in Pharmaceutical companies).
- **Save** time and money.
- Important **source of molecular tools**.

RESEARCH QUESTION

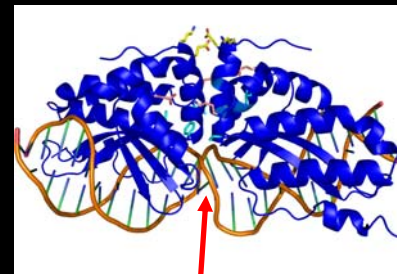
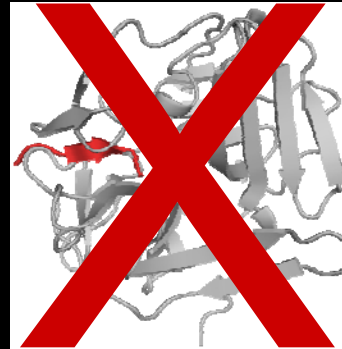
Can Computer Protein Design be use
to modify enzymes “a la carte”?

OBJETIVES OF THE RESEARCH

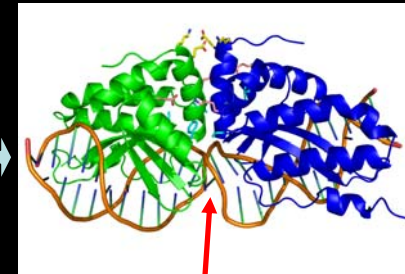
The main objective is to **redesign new enzymes “a la carte”** in order to **change affinity and specificity** in protein complexes and enzyme-peptide **interactions**.

The particular objectives are:

- To redesign a protease to change the canonical recognition site.
- To redesign the interaction interface of one meganuclease homodimer to make an obligate heterodimer.



Palindromic

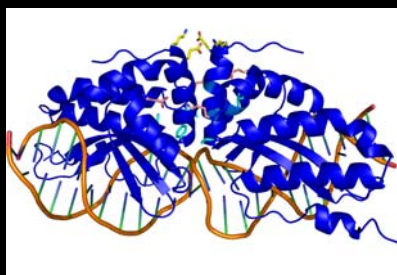


Non-Palindromic

The long term objective is to **use these modified enzymes as molecular tools** to open the way for **new therapies**.

RESEARCH METHODS

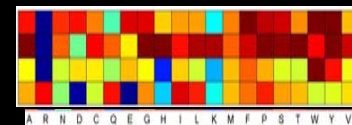
We used structural information available in the Protein Data Bank and **FoldX**, a computational protein design algorithm developed by our group. (<http://foldx.embl.de/>)



Homodimer



In silico STRUCTURAL POSITION-SCAN MUTATIONS,
SCREENING
AND
ENERGY CALCULATION

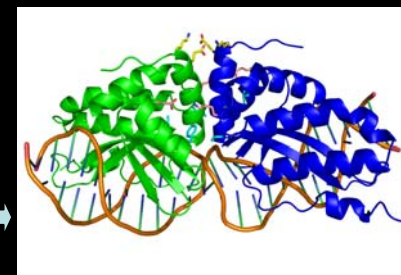
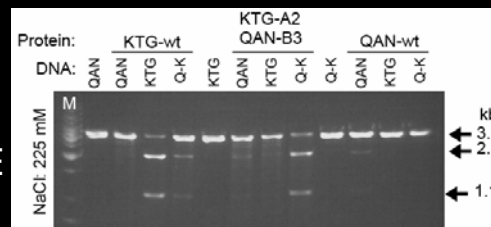


DATA ANALYSIS



PRODUCTION
of the
BEST MUTANTS

In vitro
CLEAVAGE
ASSAYS



Heterodimer

The meganuclease has been redesigned to an obligate heterodimer, and so recognizing a novel DNA sequence.

RESEARCH RESULTS AND CONCLUSIONS

- FoldX** has been **successfully used** as a protein design software, for *in silico* screening of enzyme-substrate and protein-protein interactions.
- A **new meganuclease solely recognizing a non-palindromic target site** has been obtained.
- A **new protease recognizing a non canonical target site** has been obtained.
- These enzymes can be used as **new molecular tools** and they have a **potencial use in genetic therapies (Gene correction, etc)**.
- The general **methodology used can serve** as a starting platform to tackle **any other redesign of interest**.

THANKS

FOR YOUR
ATTENTION