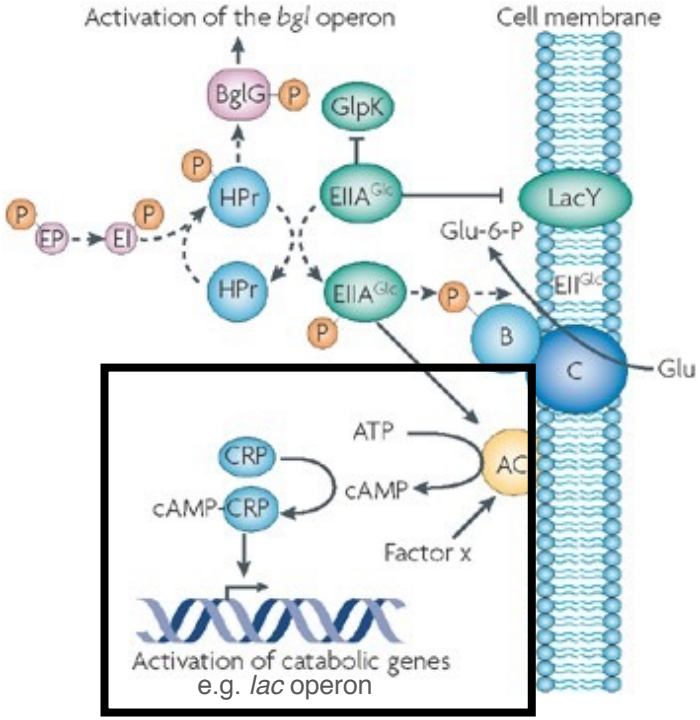
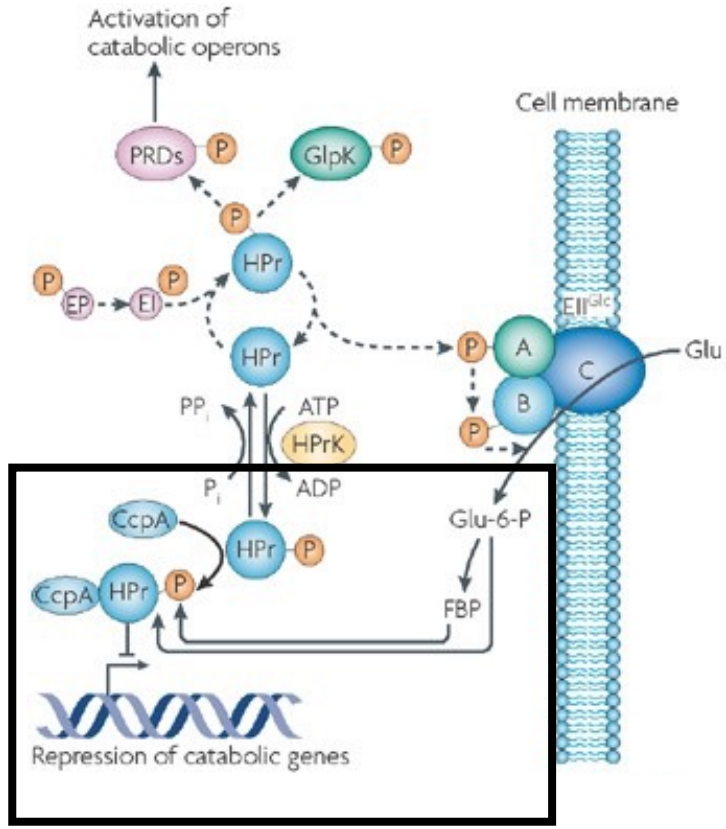
Carbon catabolite repression (CCR) in *E. coli* vs. *B. subtilis*

Same goal – very different mechanism

E. coli



B. subtilis



Figures from "**Carbon catabolite repression in bacteria: many ways to make the most out of nutrients**," Boris Görke & Jörg Stülke

Carbon catabolite repression (CCR) in *E. coli* vs. *B. subtilis*

***E. coli* represses transcription from several catabolic genes by default.**

Induction mechanism:
Transcription is induced only when a small molecule (inducer) binds the TF to release it from the DNA. Otherwise, the operon is repressed.

B. subtilis represses transcription of catabolic genes ***only in the presence of a phosphorylated protein.***

Co-repression mechanism:
Transcription is repressed only when a small protein partner binds the TF, allowing it to bind the DNA. Otherwise, the operon is being transcribed.

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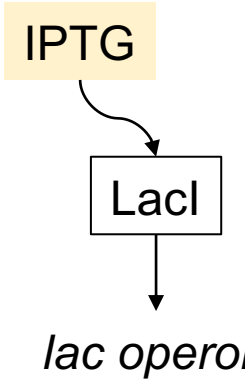

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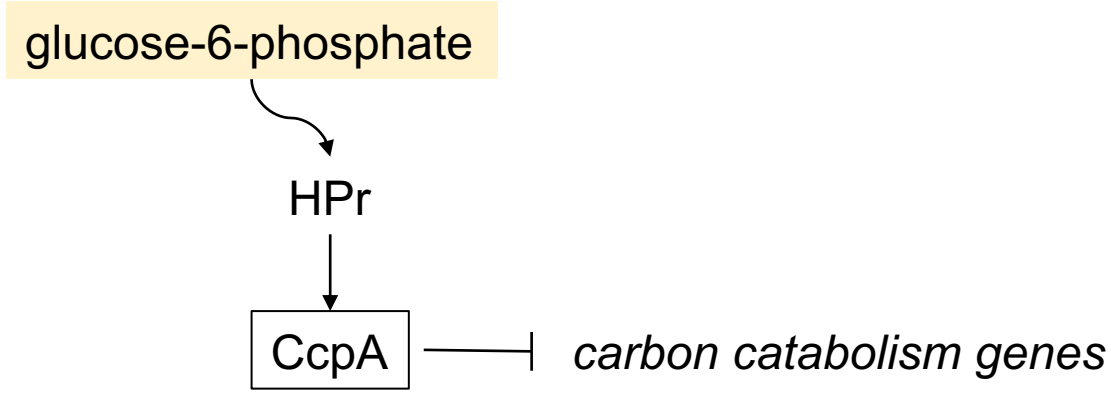
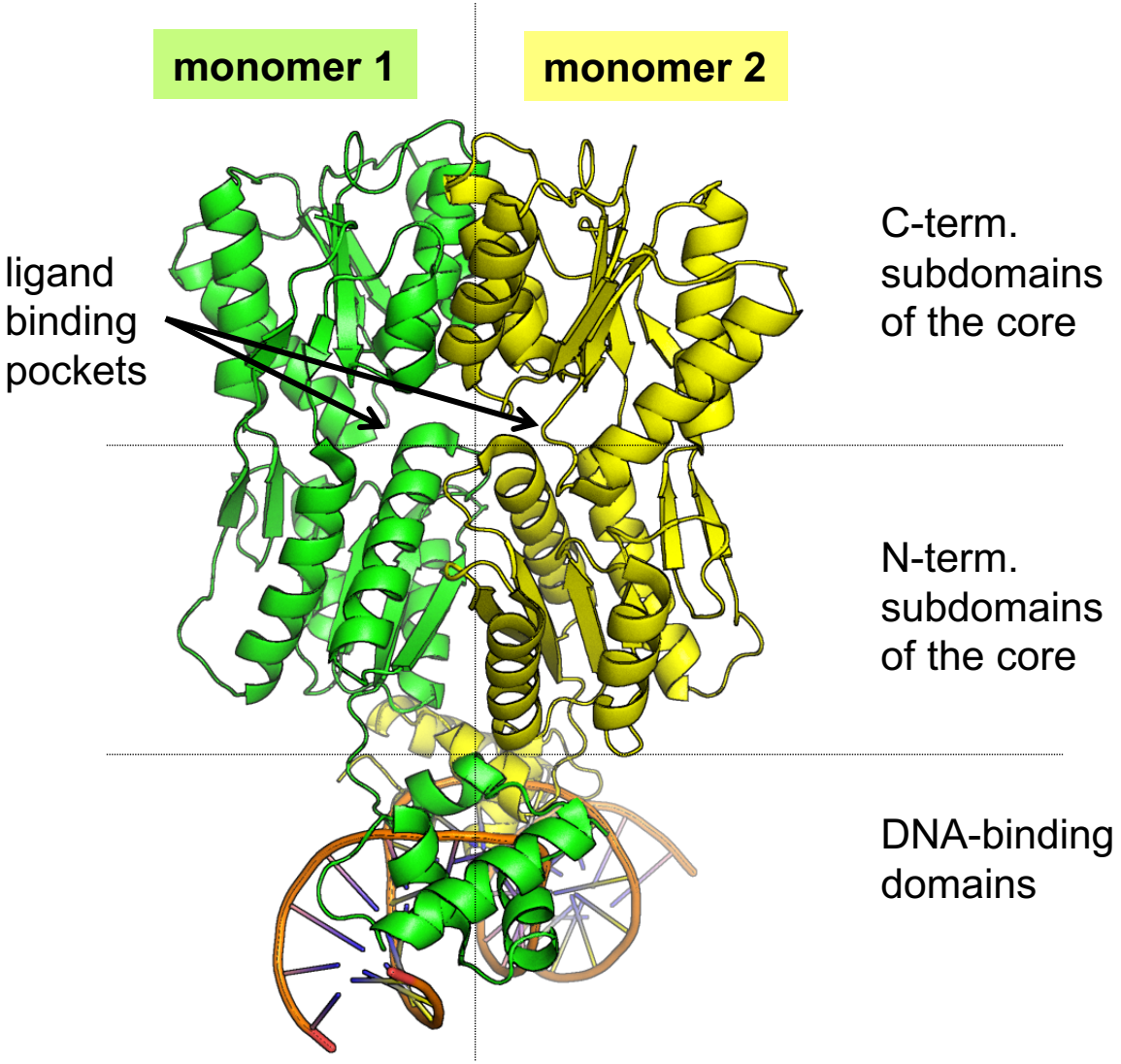


E. coli LacI bound to DNA

Induction mechanism

(releases DNA when bound to ligand)



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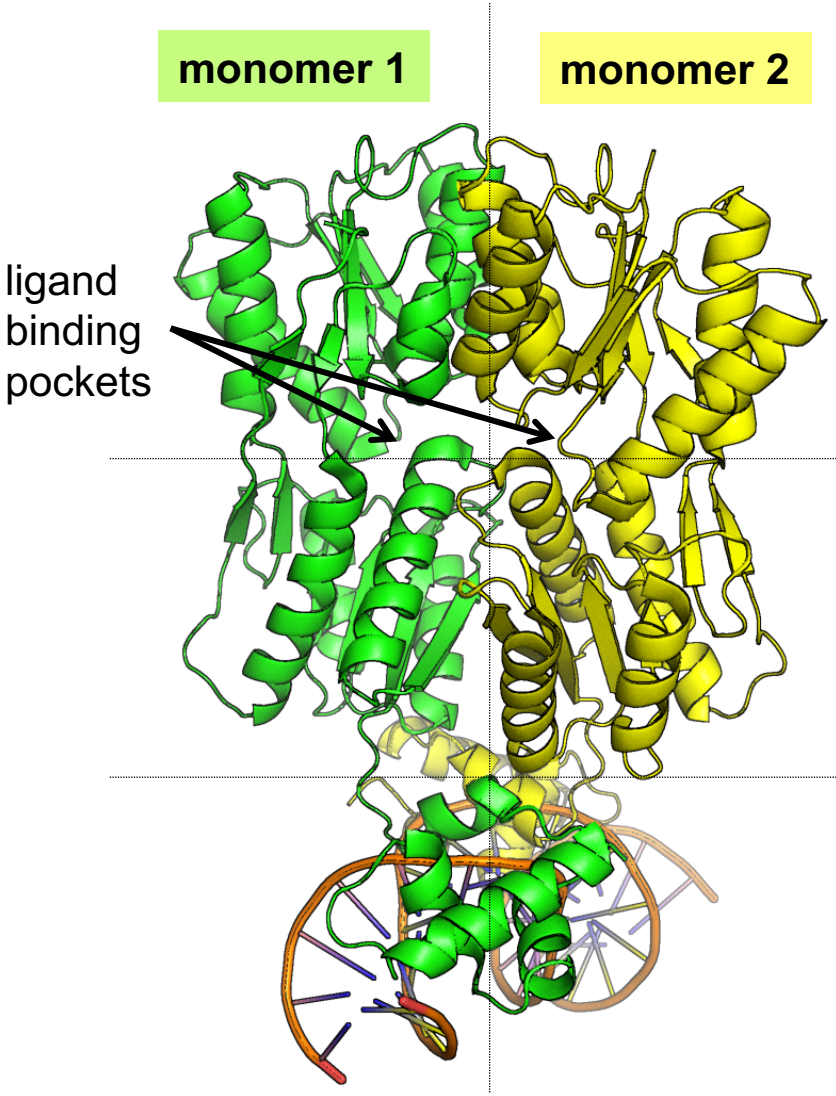
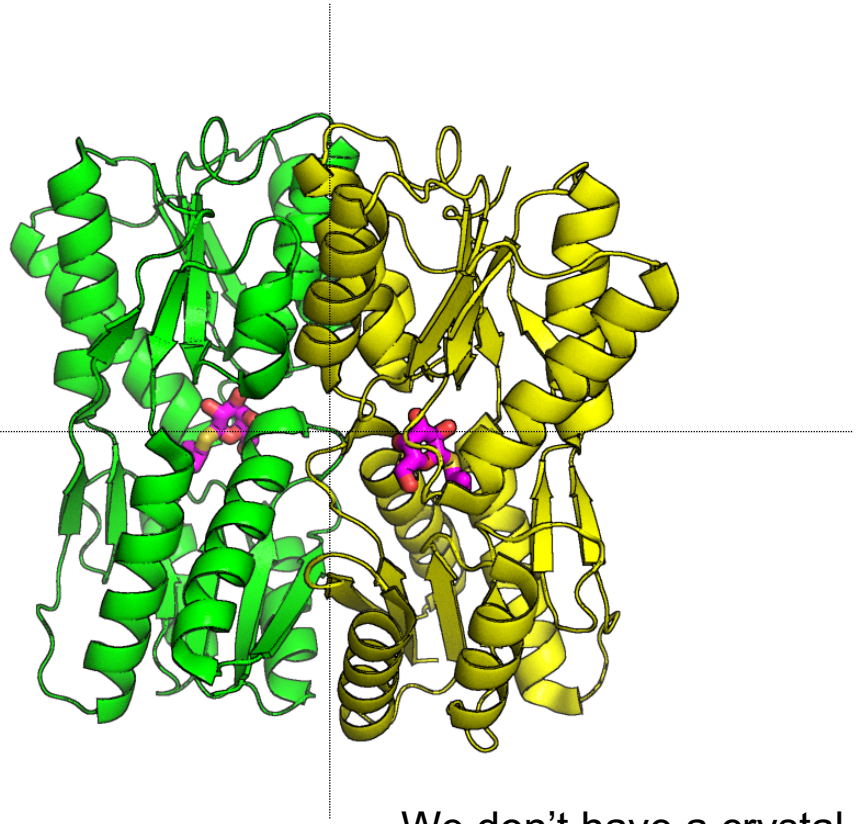


<1.5 Å all-atom RMSD difference!
(PDB IDs 1EFA, 2P9H)

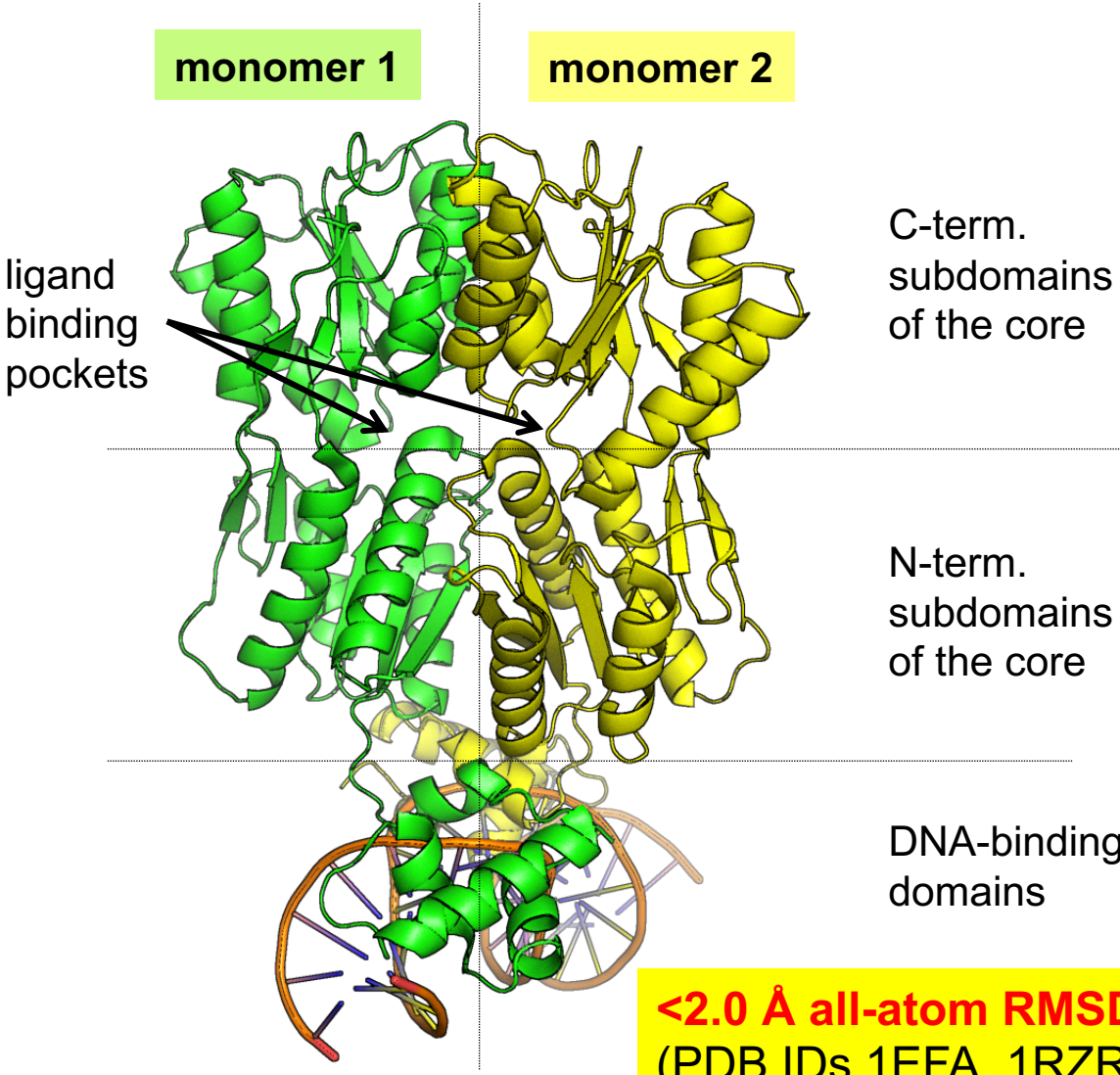
E. coli LacI bound to IPTG, an inducer



We don't have a crystal structure of the DNA-binding domain in the non-DNA-bound state.

E. coli LacI bound to DNA

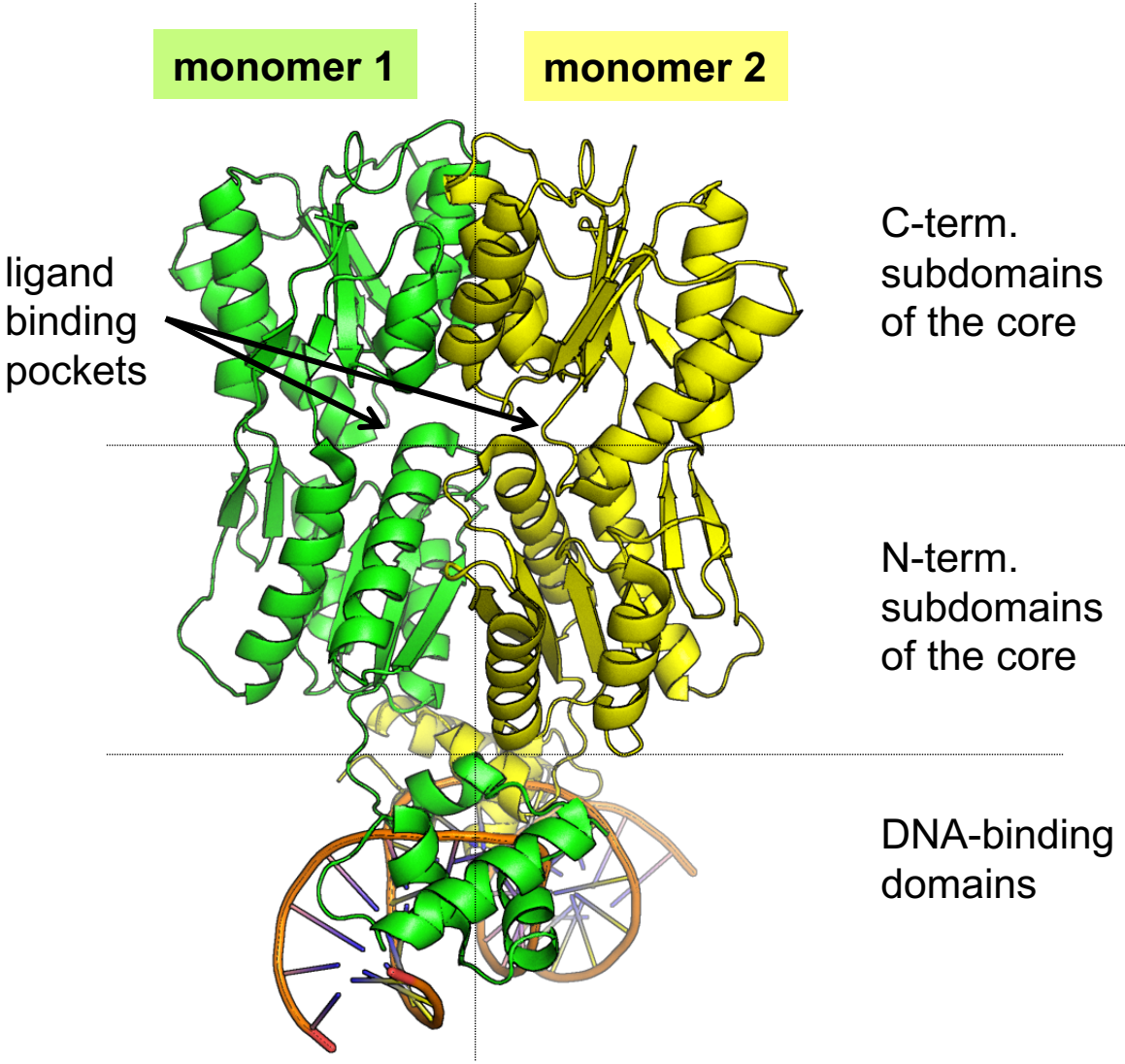
Induction mechanism
(releases DNA when bound to ligand)



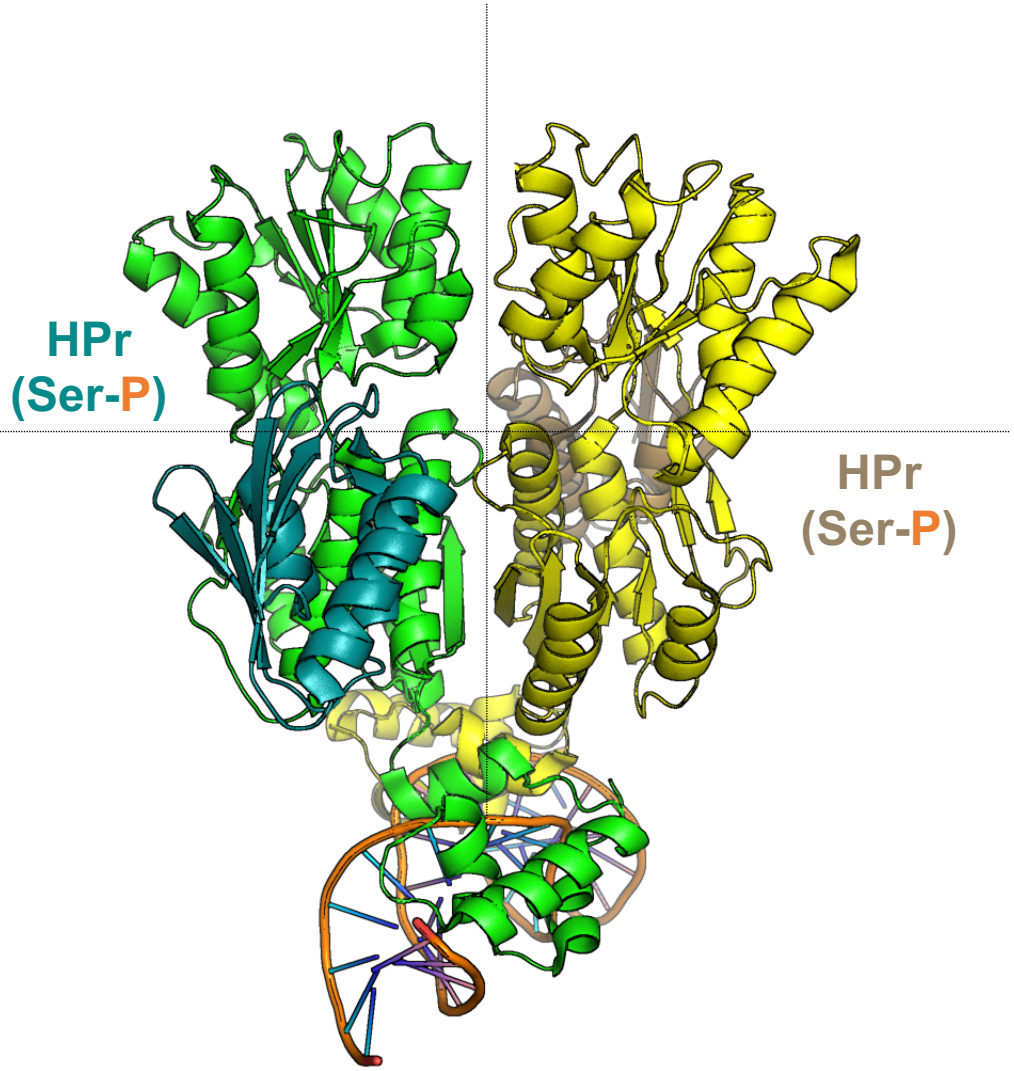
B. megaterium CcpA bound to DNA

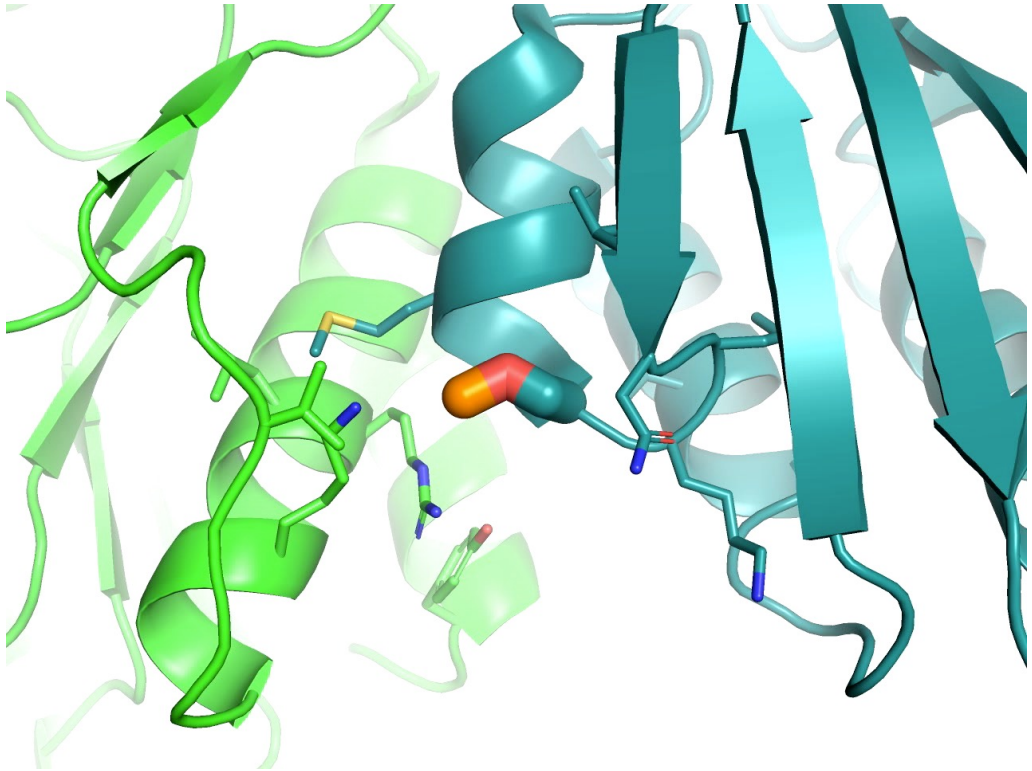
<2.0 Å all-atom RMSD difference!
(PDB IDs 1EFA, 1RZR)

E. coli LacI bound to DNA
Induction mechanism
(releases DNA when bound to ligand)



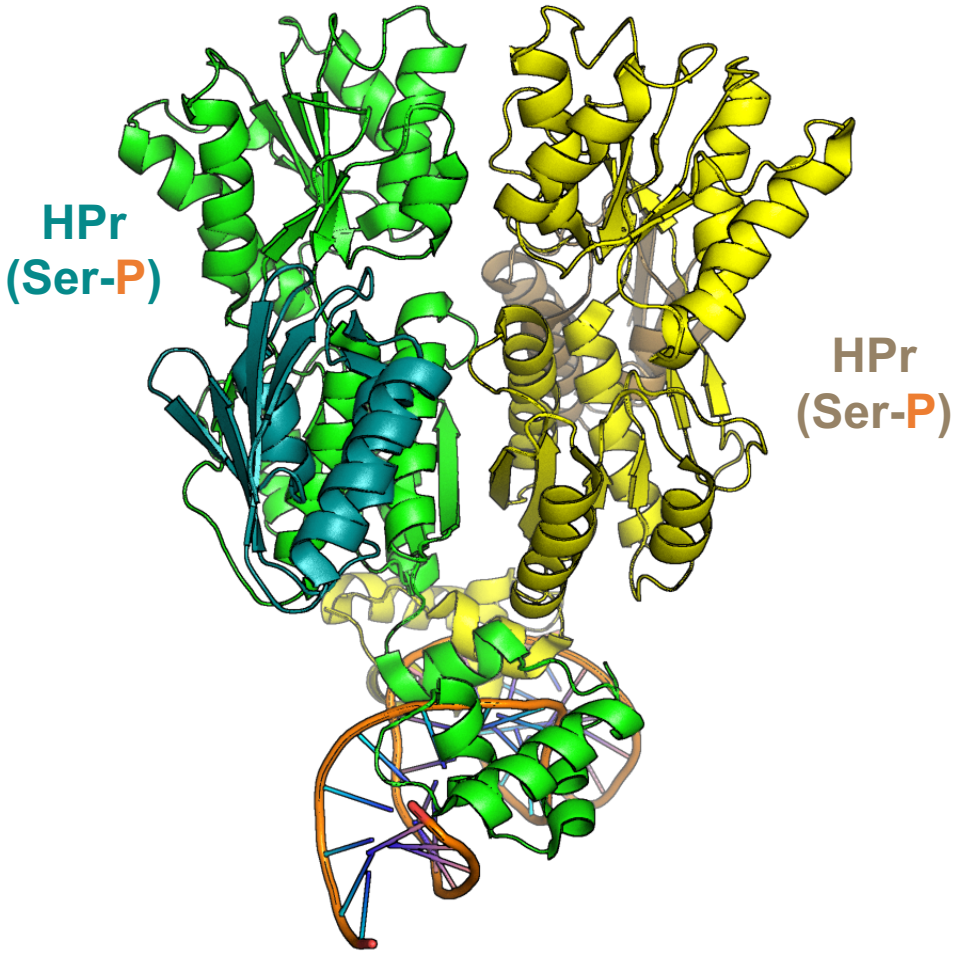
B. megaterium CcpA bound to DNA
Co-repression mechanism
(binds DNA when bound to ligand)





HPr(Ser-P) interactions with CcpA

***B. megaterium* CcpA bound to DNA**
Co-repression mechanism
(binds DNA when bound to ligand)

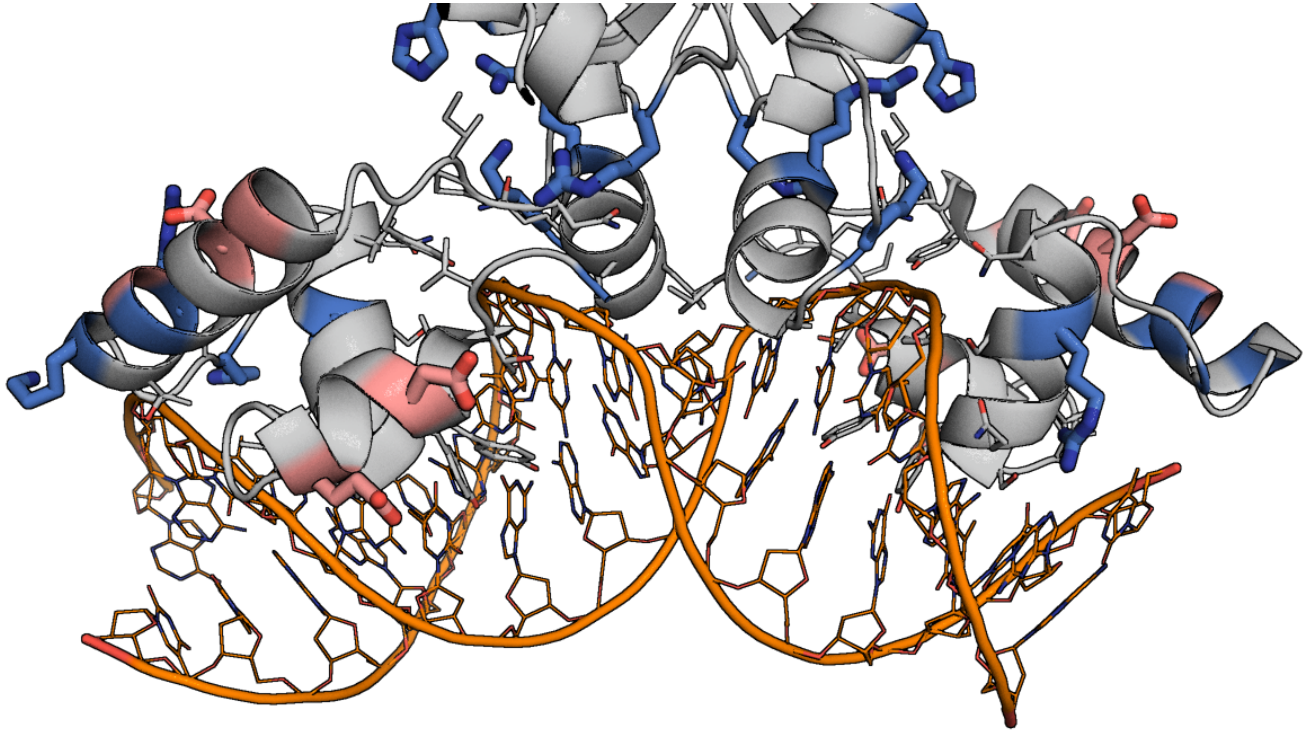


Nonspecific DNA binding

- Affects TF search efficiency
- Mediated by electrostatic interactions with negatively charged phosphate backbone of DNA

Specific DNA binding

- Affects TF dwell time on specific sites
- Mediated by H bonds and VDW interactions between TF DNA-binding domains and specific DNA bases



TF-DNA binding interactions

How much time does a TF spend on its DNA operator?

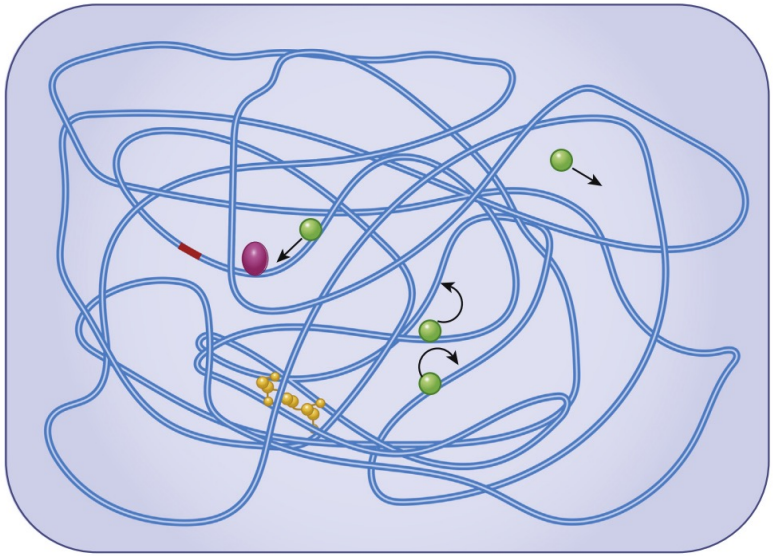
Concentration of TFs bound to DNA

$$\frac{[TF_b]}{[TF][BS]} = \frac{k_{on}}{k_{off}} = K_D$$

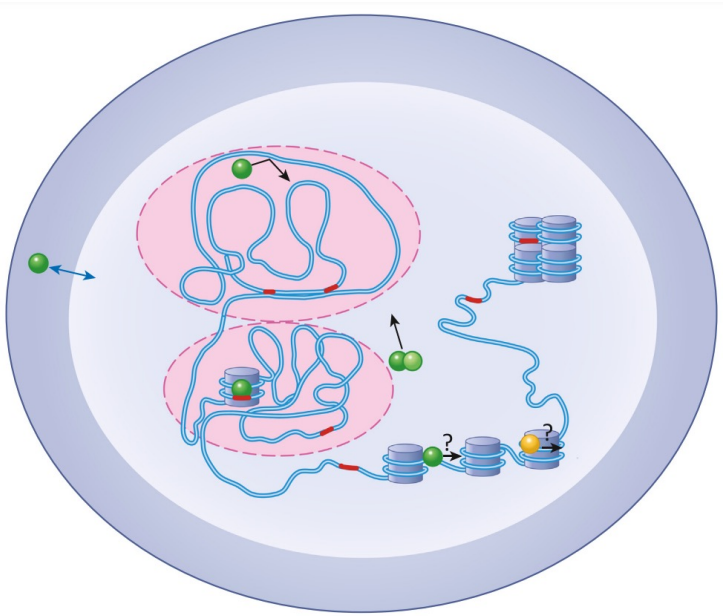
Concentration of binding sites

- TF dwell time on specific DNA sites is inversely proportional to the dissociation constant
- k_{off} ~ binding strength between operator and TF
- k_{on} ~ efficiency of TF finding its site in the genome
- [BS] depends on the operator site accessibility

TF search for the operator site in prokaryotes



TF search for the operator site in eukaryotes



How to understand TF structure

Structural biology

- X-ray crystallography
- Cryo-EM
- NMR/EPR
- HDX/MS

Bioinformatics

- Co-evolution/MSAs
- Homology modeling
- Structure prediction

How to measure TF DNA binding

In vitro

- Electromobility shift assay (EMSA)
- SELEX
- Mechanically Induced Trapping Of Molecular Interactions (MITOMI)
- Single molecule imaging (TIRF)

In vivo

- CHIP-Seq / CUT&RUN
- FRAP
- Fluorescence correlation spectroscopy (FCS)
- Single-particle tracking (SPT)

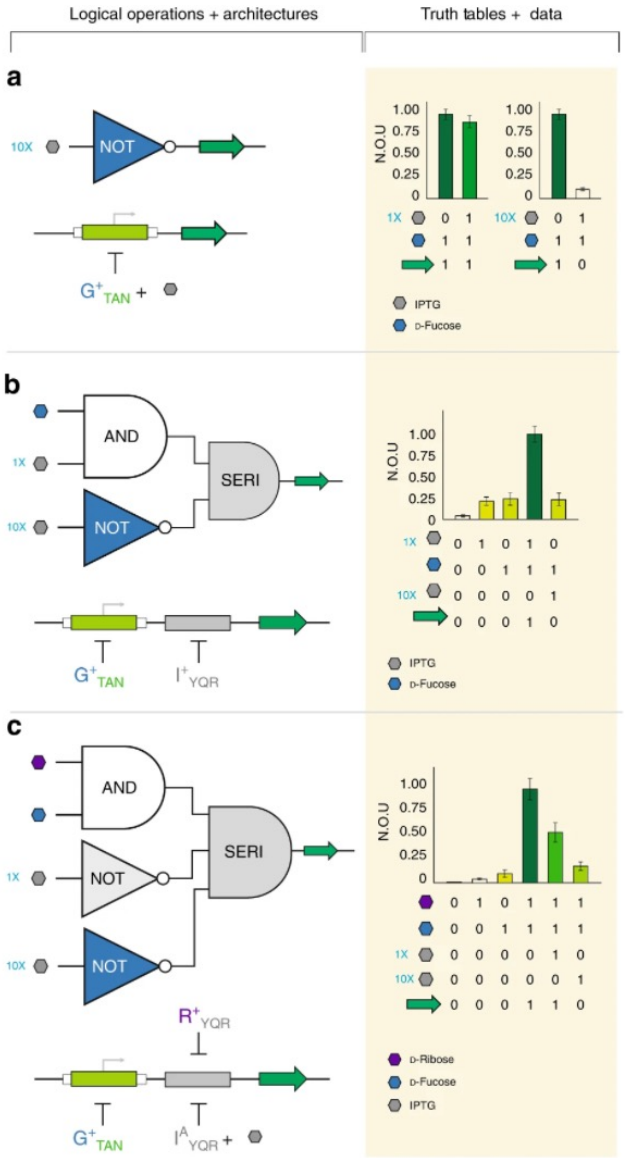
Transcription factors:

- are strongly evolutionarily conserved
  - ★ in their structures
  - ★ … but also in the DNA sequences they recognize
    (one recent study: https://elifesciences.org/articles/04837)

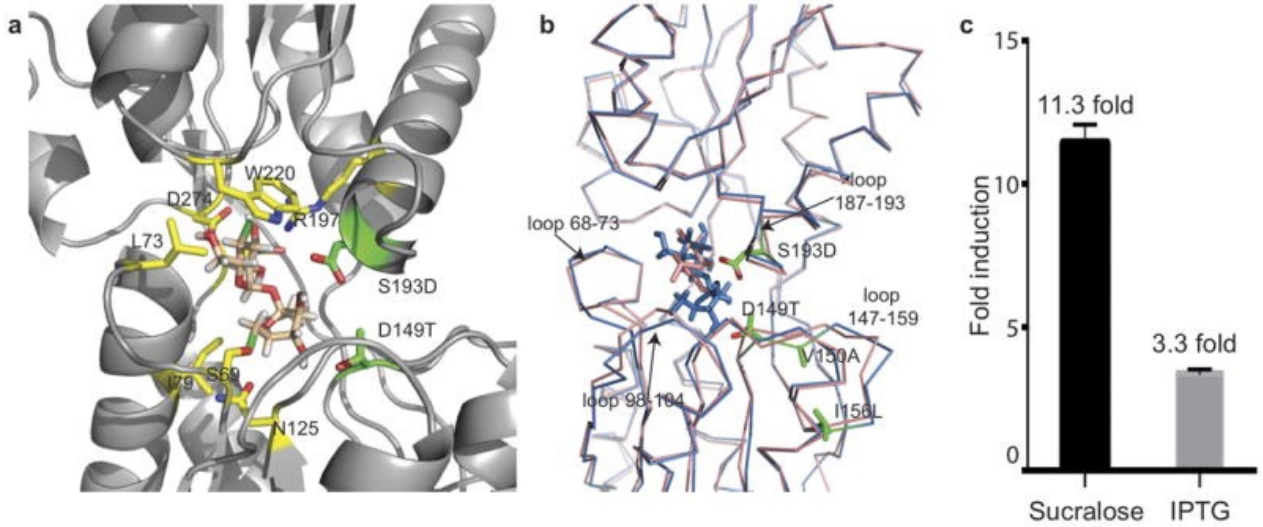
- are usually multimeric (~80%)
  - ★ for ultrasensitive response via cooperativity
  - ★ for intersegmental transfer (?)
    (one recent study: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0108575#pone-0108575-g001)

- are excellent targets for engineering, because we can use them to control cell signaling – if we can understand how they really work!

Recent examples of engineered *lac* repressors

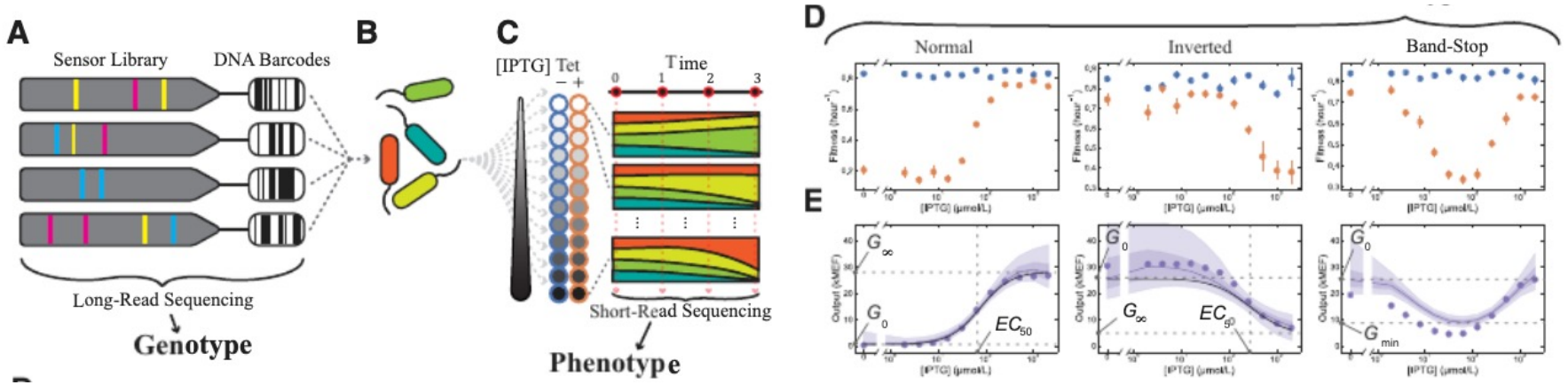


"Transcriptional programming using engineered systems of transcription factors and genetic architectures." Rondon *et al.*, *Nature Comms* 2019

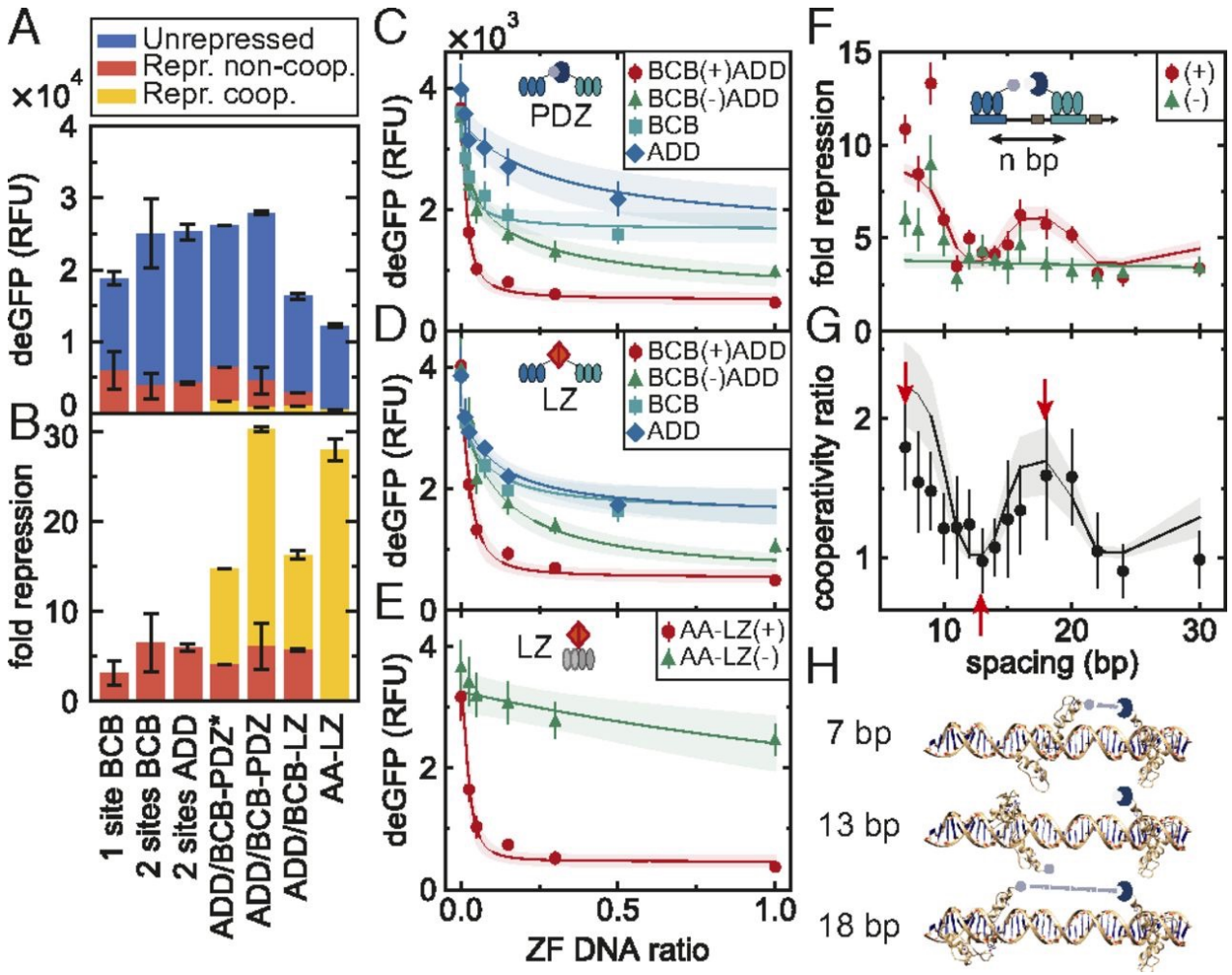


"Engineering an allosteric transcription factor to respond to new ligands." Taylor *et al.*, *Nature Methods* 2016

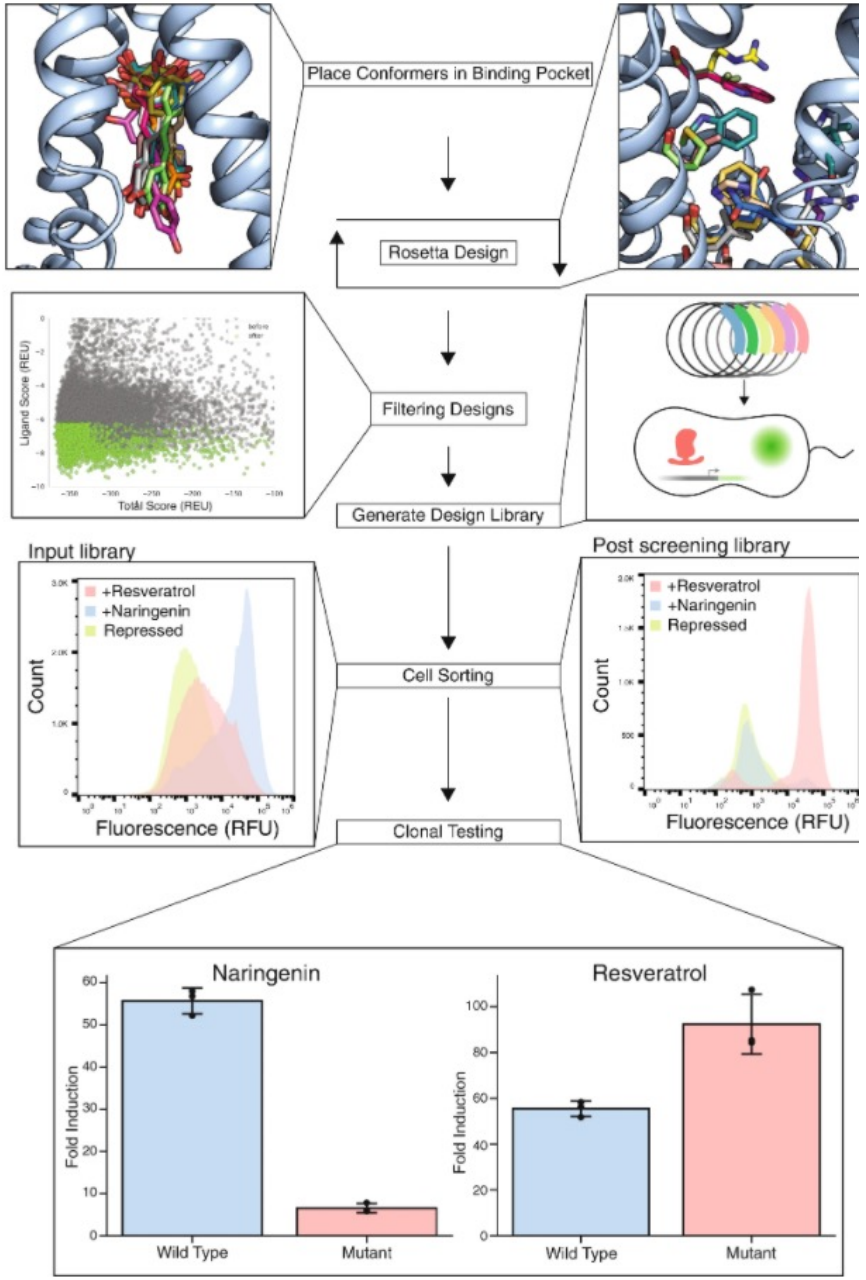
"The genotype-phenotype landscape of an allosteric protein." Tack *et al.*, *Mol Syst Biol* 2021



Recent examples of other engineered transcription factors



"Cell-free gene-regulatory network engineering with synthetic transcription factors." Swank *et al.*, *PNAS* 2019



"Epistasis shapes the fitness landscape of an allosteric specificity switch." Nishikawa *et al.*, *Nature Comms* 2021

Open questions:

- Why did TFs evolve to adopt *these* folds?
- What about all the other ways to bind DNA or ligands?
- What about proteins that have the same fold, but different ‘effector domains’? (like GPCRs)

- Are *conformational dynamics* in TFs conserved?
- How do they evolve?
- Can we predict conformational changes in proteins?

- What biophysical principles underly *functional responses* of TFs, *e.g.*, co-repression vs. induction?