

# The Combinatorics of Allosteric Activation

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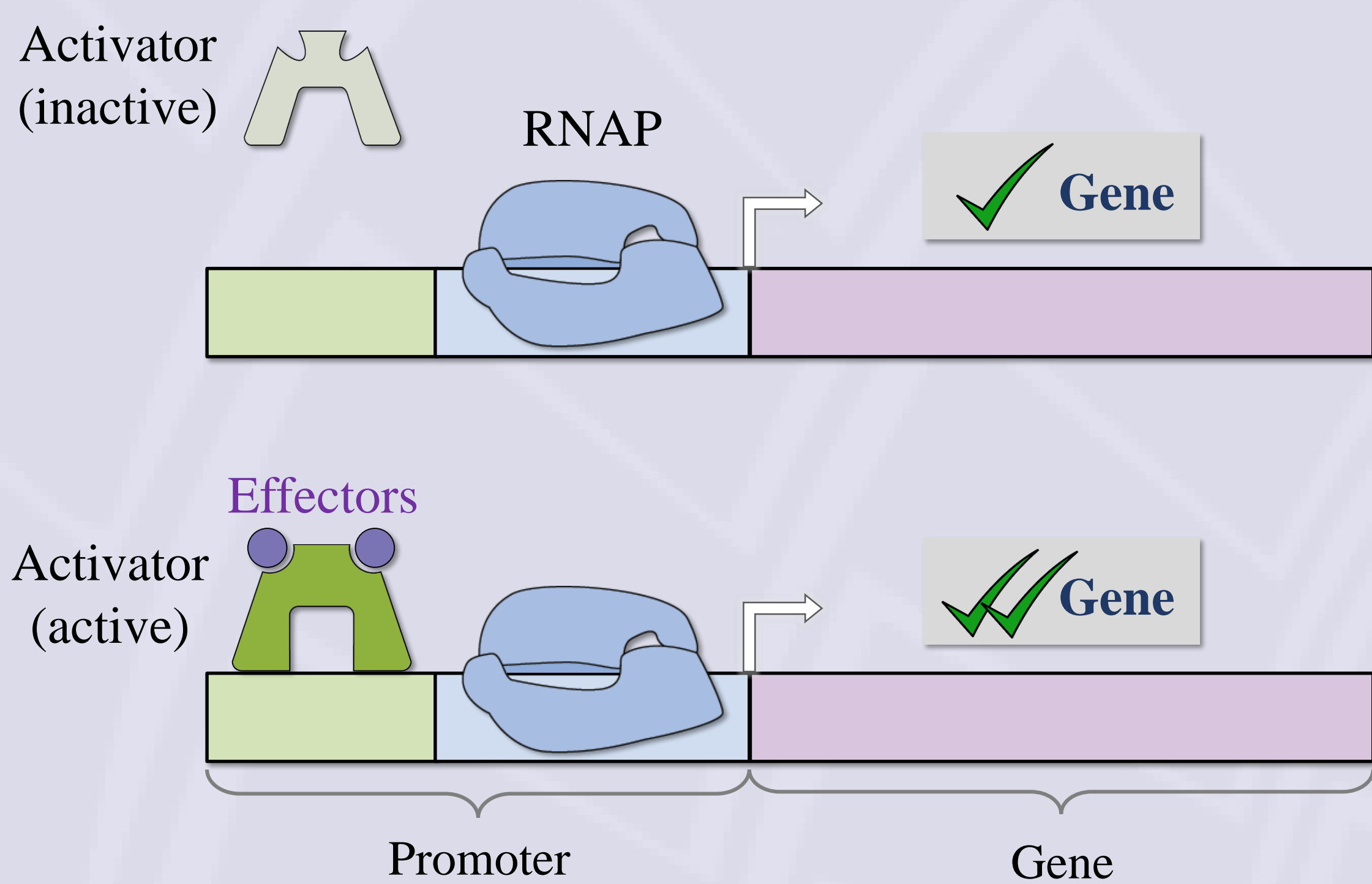


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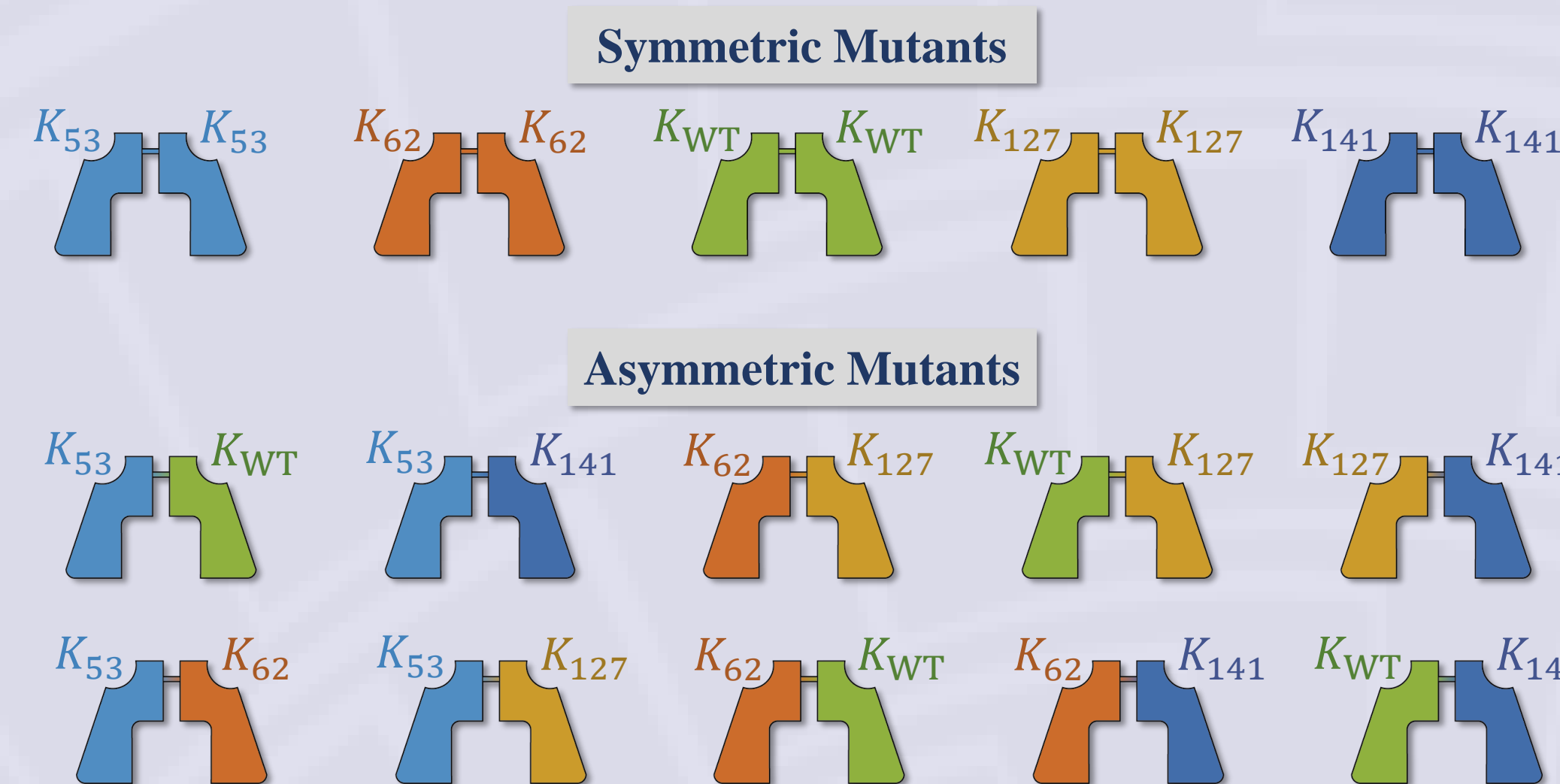
## I Mechanism of Activation

CRP (cAMP Receptor Protein)

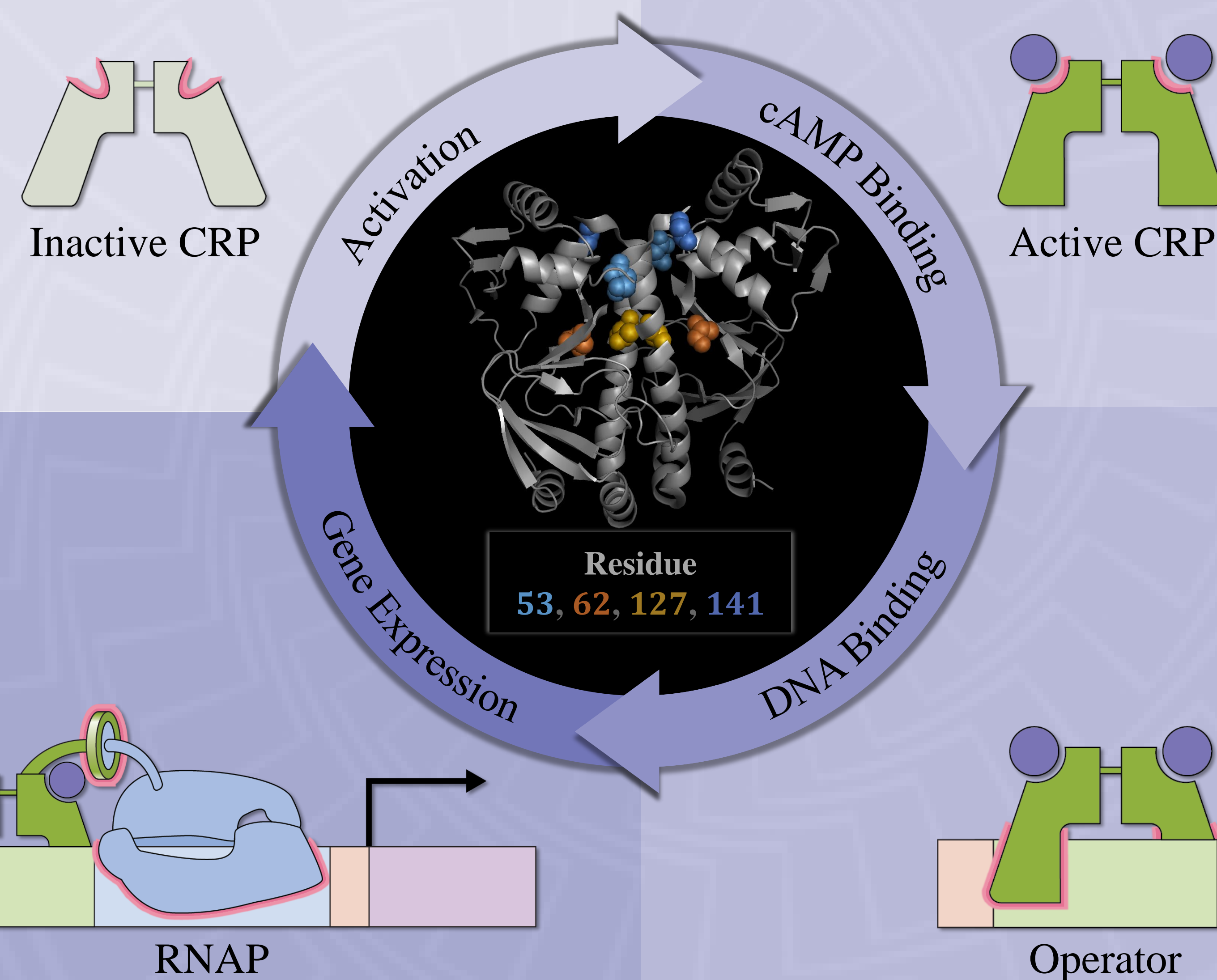
- Homodimeric transcriptional activator
- Global transcriptional regulator (100+ genes)
- Lanfranco *et al.* created linked dimer to probe the combinatorics of mutations [1]



## A Linked Homo-Oligomer



- $N$  subunits can generate  $\frac{N(N+1)}{2}$  CRP mutants
- Combinatorial explosion in predictive power (# of parameters)  $\propto$  (# of subunits)



## II MWC Model

Active CRP		Inactive CRP	
State	Relative Probability	State	Relative Probability
	1		$e^{-2\beta\epsilon}$
	$\frac{c}{K_{62}^{Act}}$		$e^{-2\beta\epsilon} \frac{c}{K_{62}^{Inact}}$
	$\frac{c}{K_{WT}^{Act}}$		$e^{-2\beta\epsilon} \frac{c}{K_{WT}^{Inact}}$
	$\frac{c}{K_{62}^{Act}} \frac{c}{K_{WT}^{Act}}$		$e^{-2\beta\epsilon} \frac{c}{K_{62}^{Inact}} \frac{c}{K_{WT}^{Inact}}$

## Parameter Degeneracy

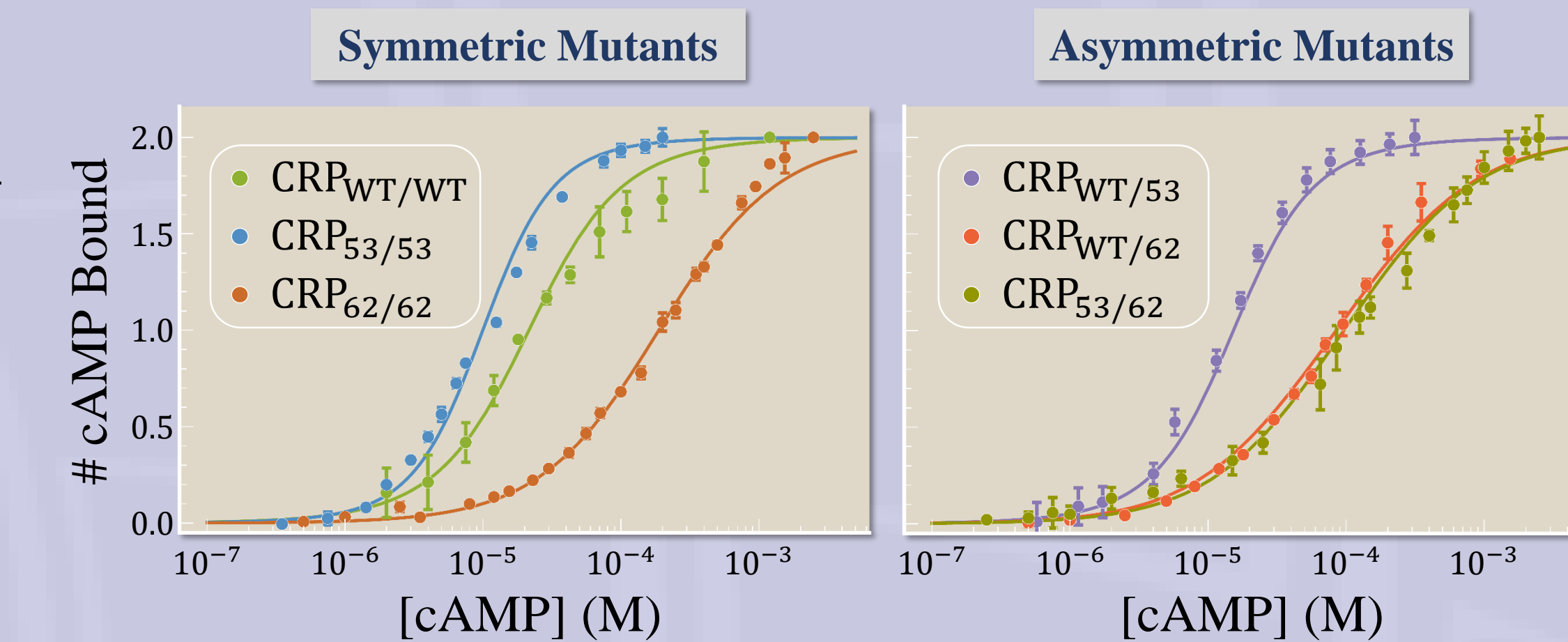
Average # cAMP Bound

$$\left( \frac{c}{K_{62}^{Act}} + \frac{c}{K_{WT}^{Act}} \right) + 2 \frac{c}{K_{62}^{Act}} \frac{c}{K_{WT}^{Act}} + e^{-2\beta\epsilon} \left( \frac{c}{K_{62}^{Inact}} + \frac{c}{K_{WT}^{Inact}} \right) + 2 e^{-2\beta\epsilon} \frac{c}{K_{62}^{Inact}} \frac{c}{K_{WT}^{Inact}}$$

$$\approx \frac{2 \frac{c}{K_{62}^{Act}} \frac{c}{K_{WT}^{Act}} + \left( \frac{c}{K_{62}^{Inact}} + \frac{c}{K_{WT}^{Inact}} \right) + 2 \frac{c}{K_{62}^{Inact}} \frac{c}{K_{WT}^{Inact}}}{\frac{c}{K_{62}^{Act}} \frac{c}{K_{WT}^{Act}} + \left( 1 + \frac{c}{K_{62}^{Act}} \right) \left( 1 + \frac{c}{K_{WT}^{Act}} \right)}$$

$\tilde{K}_{62/WT}^{Act} = e^{-\beta\epsilon} \tilde{K}_{62/WT}^{Inact}$

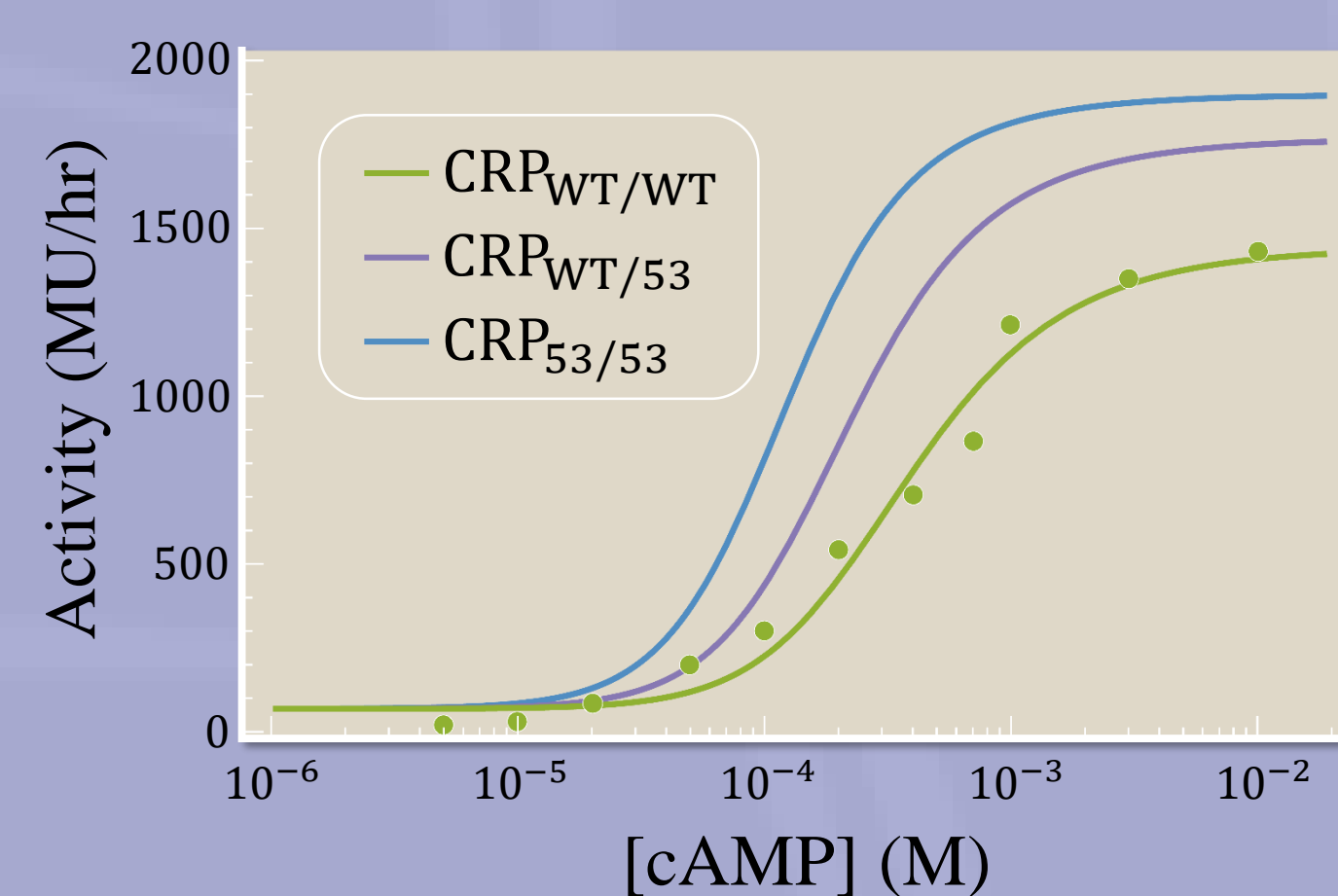
$e^{\beta\epsilon} \ll 1$



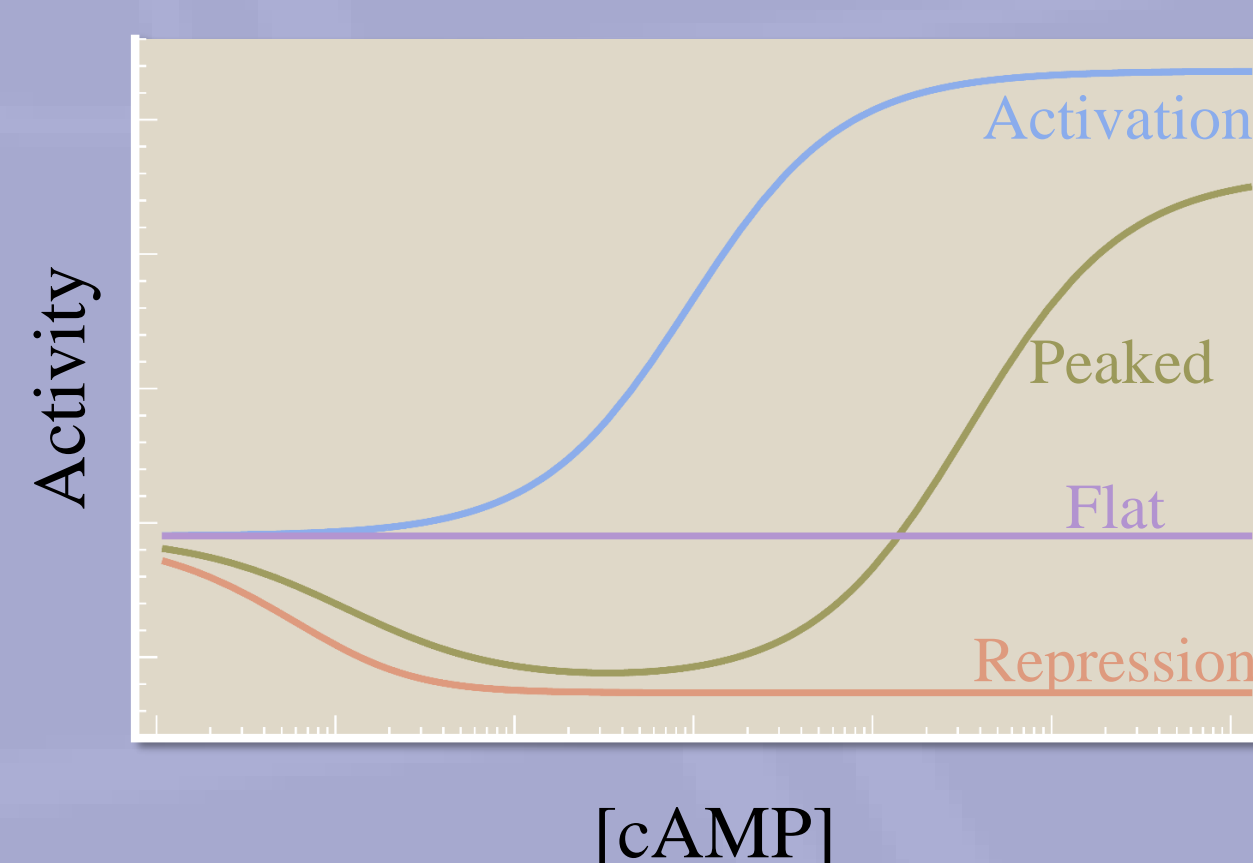
Parameters	Parameter Set 1 ( $10^{-6}$ M)	Parameter Set 2 ( $10^{-6}$ M)
$K_{WT}^{Act}, K_{WT}^{Inact}$	1, 40	0.2, 40
$K_{53}^{Act}, K_{53}^{Inact}$	0.5, 50	0.1, 50
$K_{62}^{Act}, K_{62}^{Inact}$	70, 200	10, 200
$\epsilon$	$-3 k_B T$	$-5 k_B T$

## IV In Vivo Predictions

- Calibrated model to WT CRP gene expression data [3]
- Model can then predict activation within *in vivo* systems



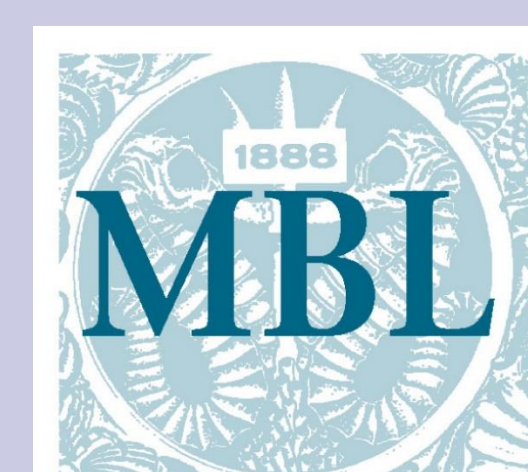
- Theoretically explored the spectrum of activation curves for any dimeric transcriptional activator



$M_L^A < M_L^I, M_R^A < M_R^I$   
 $M_L^A > M_L^I, M_R^A > M_R^I$   
 $M_L^A < M_L^I, M_R^A > M_R^I$   
 $M_L^A = M_L^I = M_R^A = M_R^I$

## Conclusions

- A holistic, quantitative understanding of transcription factor activation
- Combinations of mutations can be characterized from single mutations
- Model being tested for non-global activators (*in vitro* and *in vivo*)



Woods Hole Biological Discovery



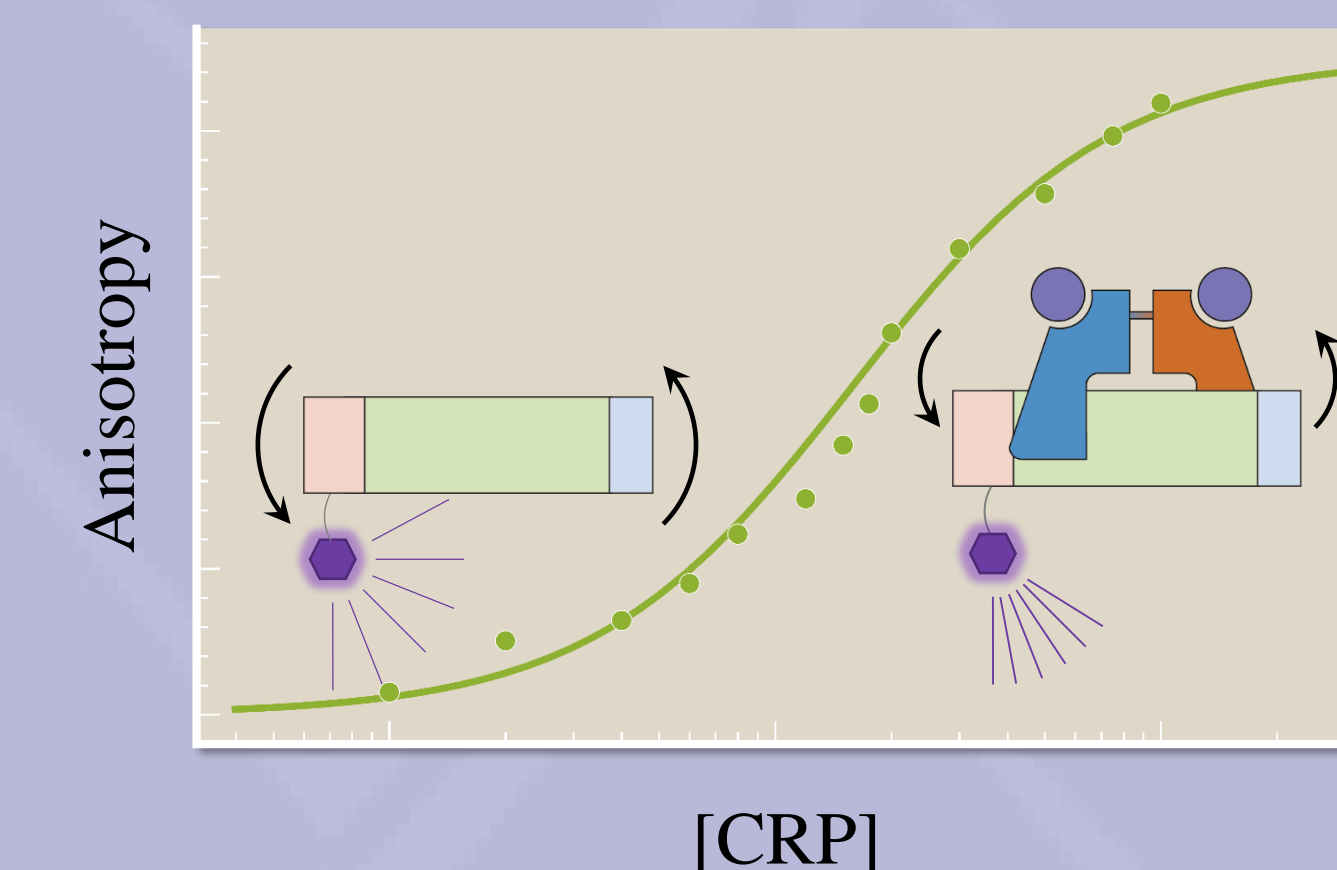
National Institutes of Health



[1] Lanfranco 2017 *J Biol Chem*. [2] Sharma 2009 *PNAS*. [3] Kuhlman 2007 *PNAS*. [4] Lin 2002 *Biochem (PDB)*

## III Anisotropy

- Anisotropy measured for tagged promoter binding to CRP<sub>53/62</sub>
- Inferred CRP-DNA affinity differs based on the # of bound cAMP



- Singly cAMP-bound CRP binds tightest to the promoter
- Results are in line with structural knowledge of CRP [2]

