DNA Remodeling by Tyrosine Recombinases

Tom Ellenberger

Department of Biochemistry and Molecular Biophysics

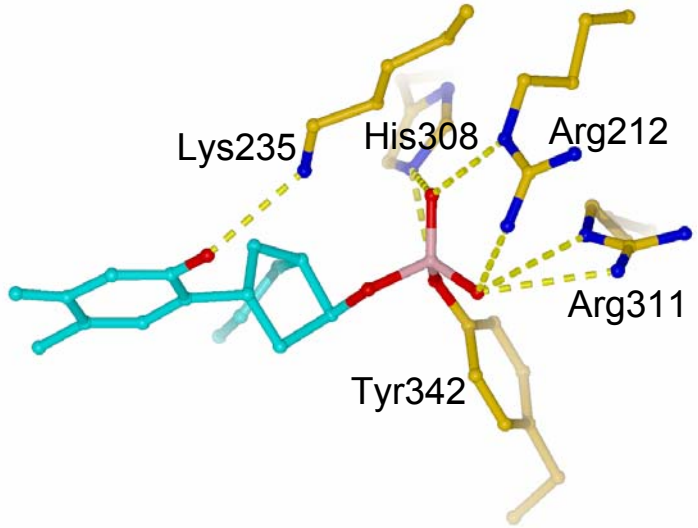
Washington University in St.Louis
SCHOOL OF MEDICINE

Diverse Biological Activities of Tyrosine Recombinases

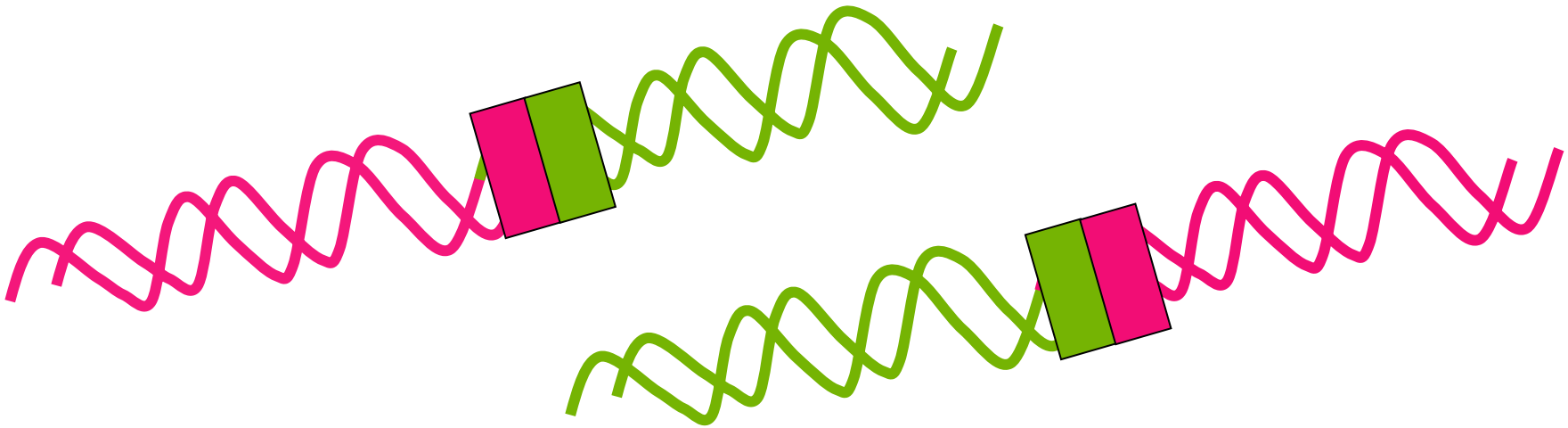
Viral integration and excision

Control of gene expression

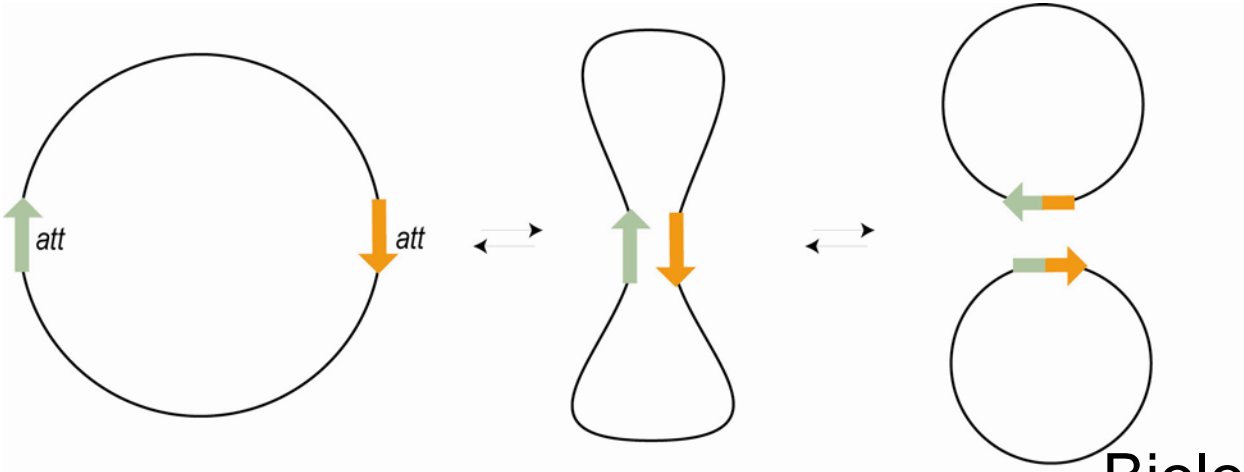
Gene rearrangements

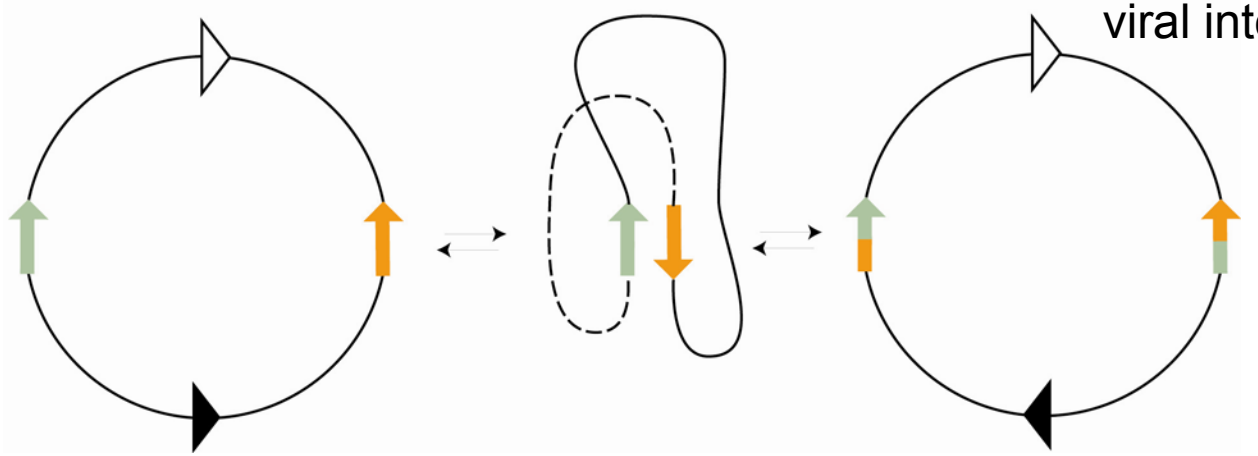


Segregation of newly replicated chromosomes



Phage Integrases Catalyze Site-Specific Recombination





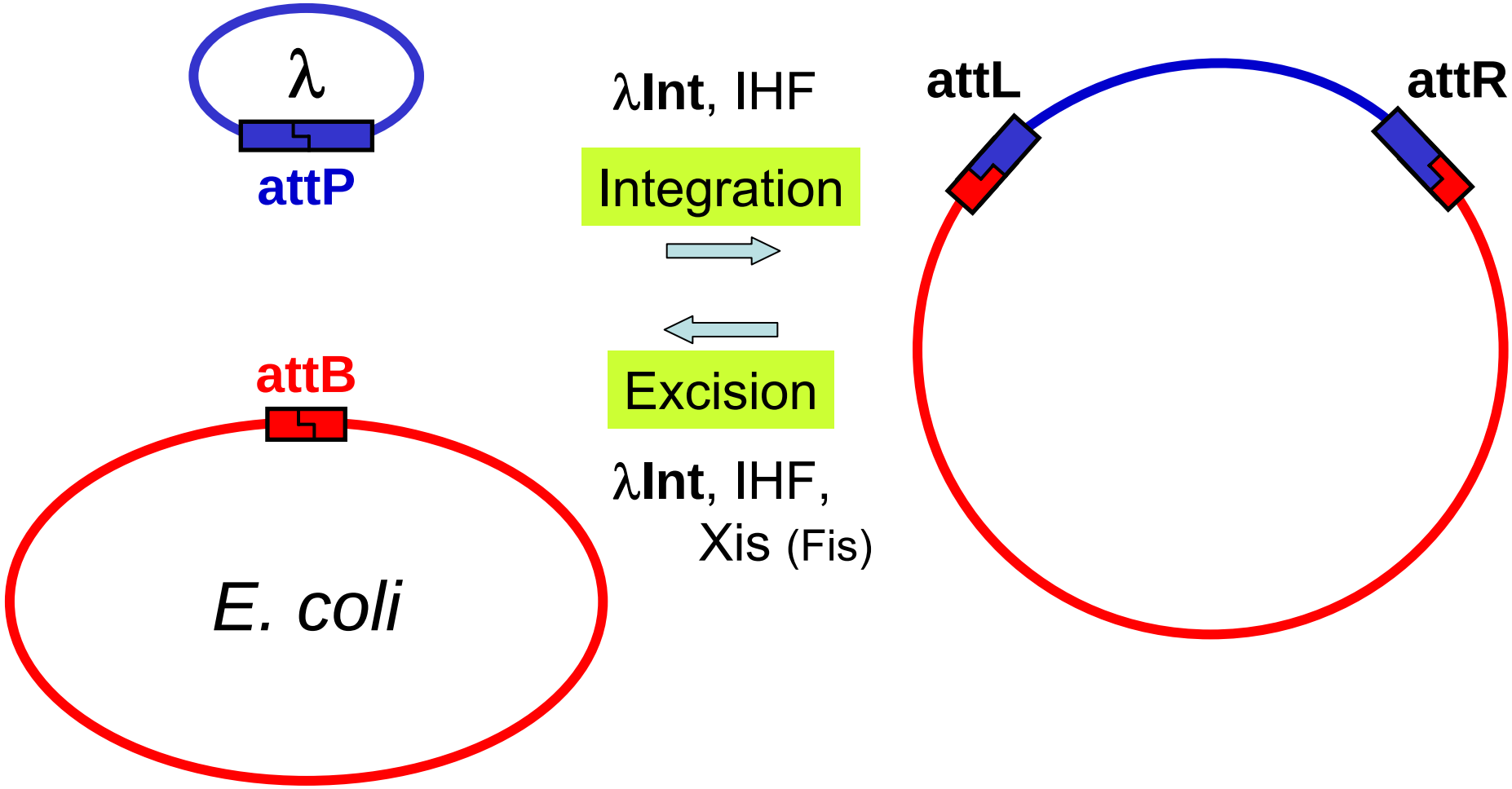
Biological Roles:

resolution of concatamers

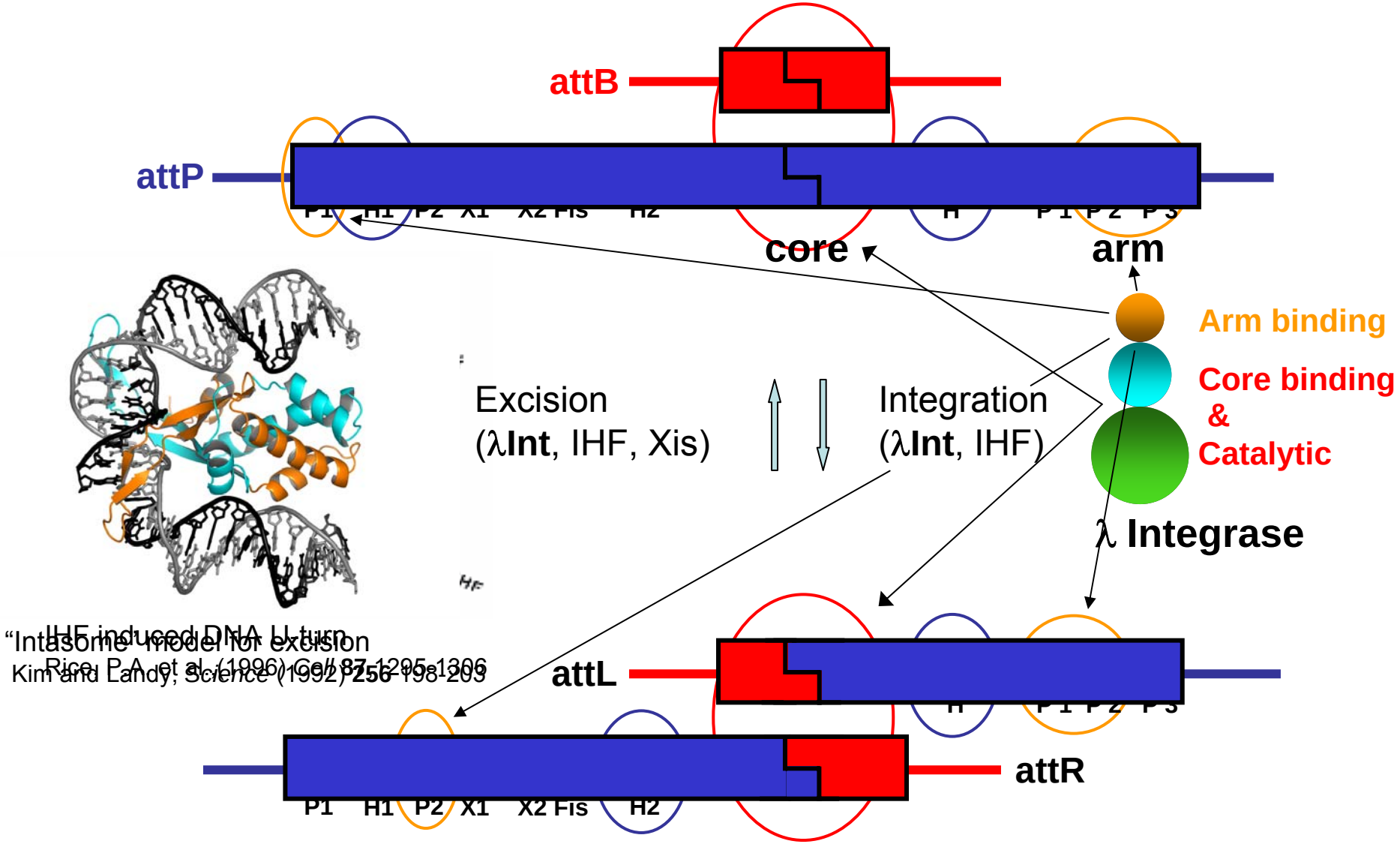
inversion/control of gene expression

viral integration/excision

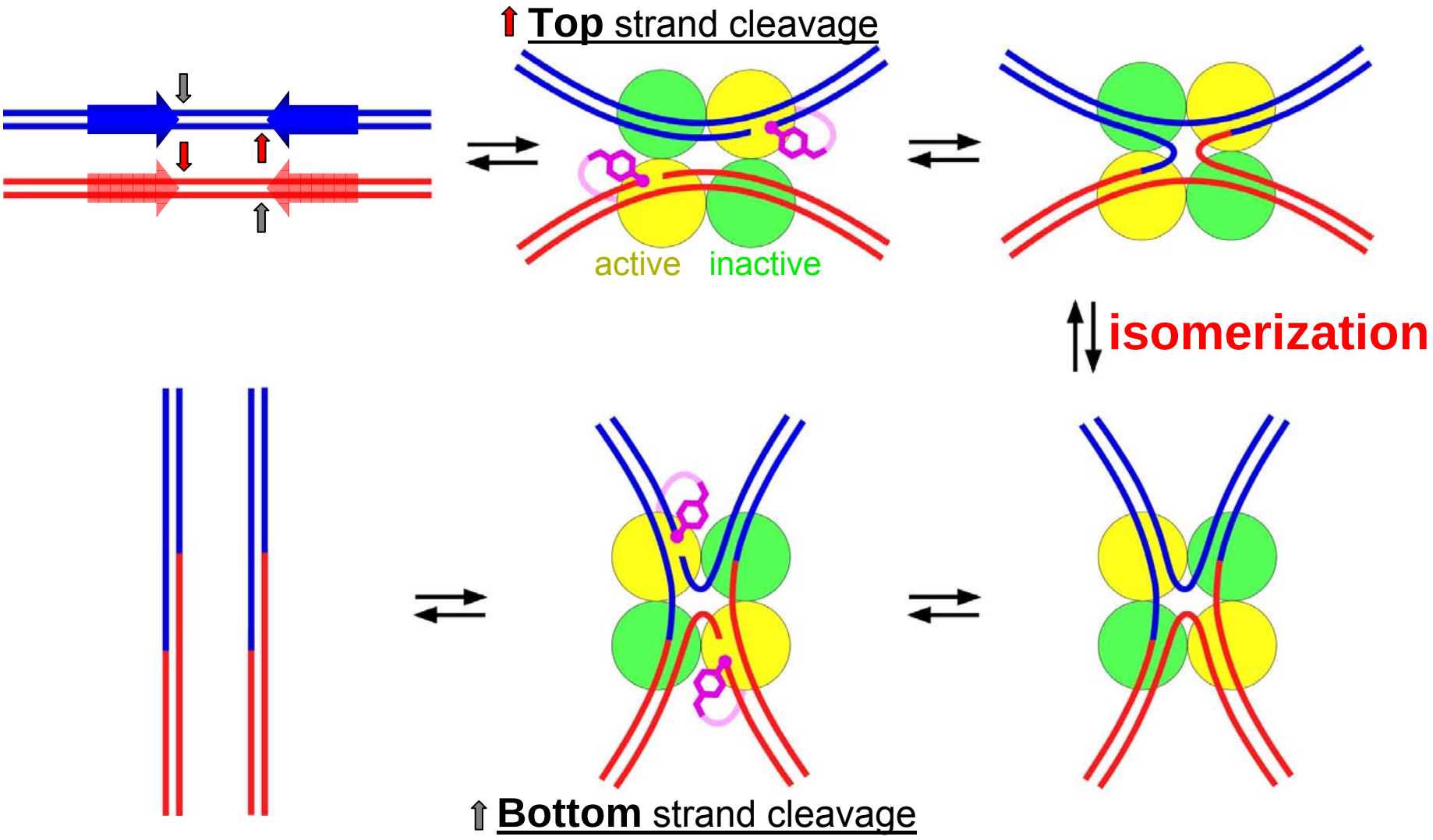
λ Recombination



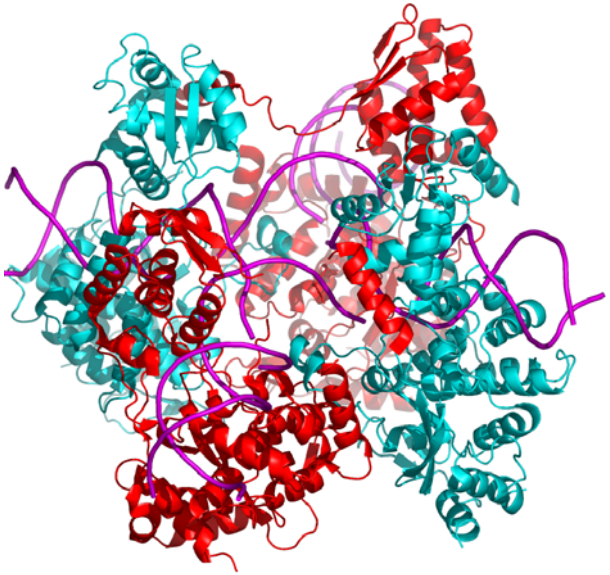
λ-int binds simultaneously to "Core" and "Arm" DNA sites



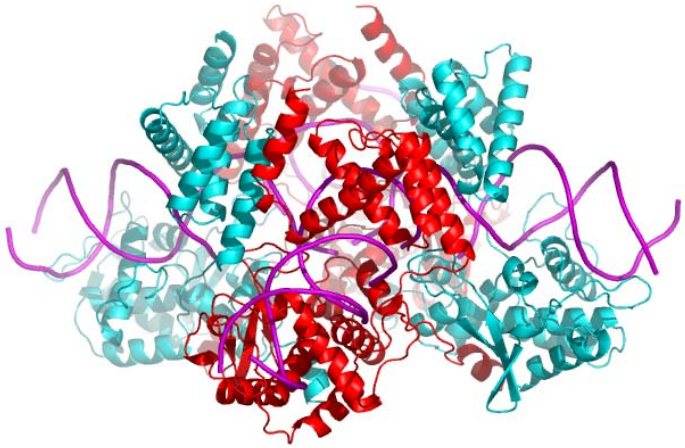
Ordered, Pairwise Exchange of DNA Strands



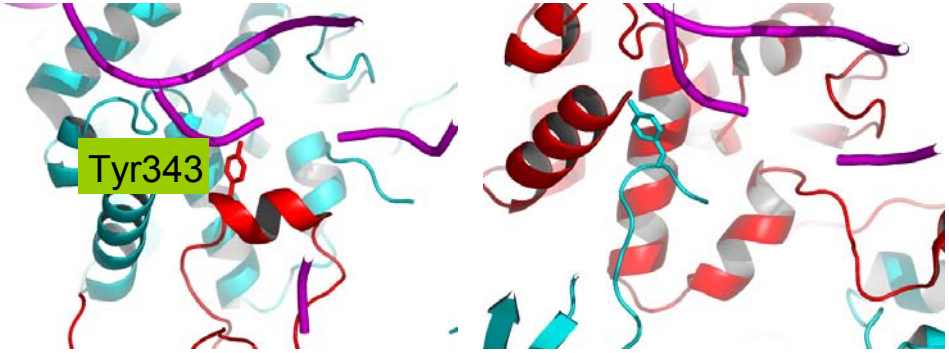
Structures of Cre and Flp Recombinases

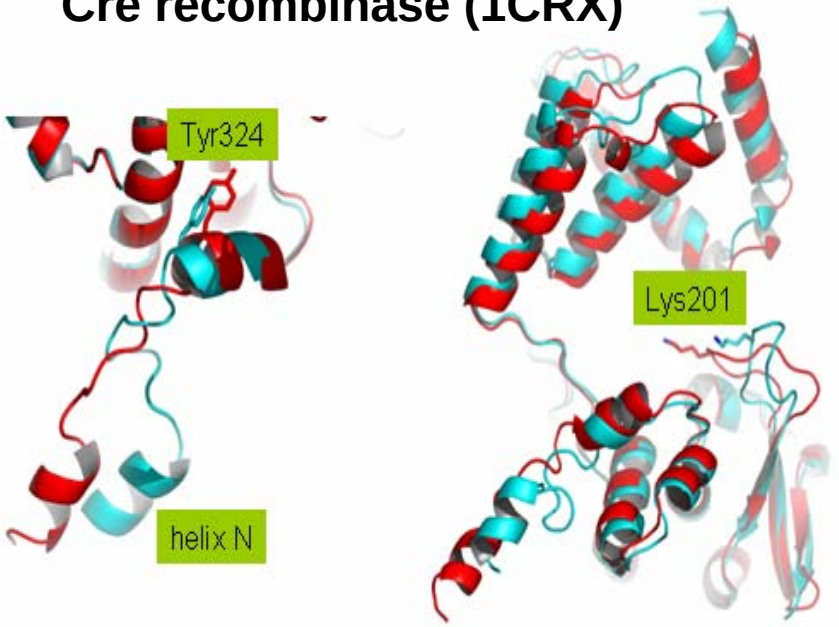


Flp recombinase (1FLO)



Cre recombinase (1CRX)

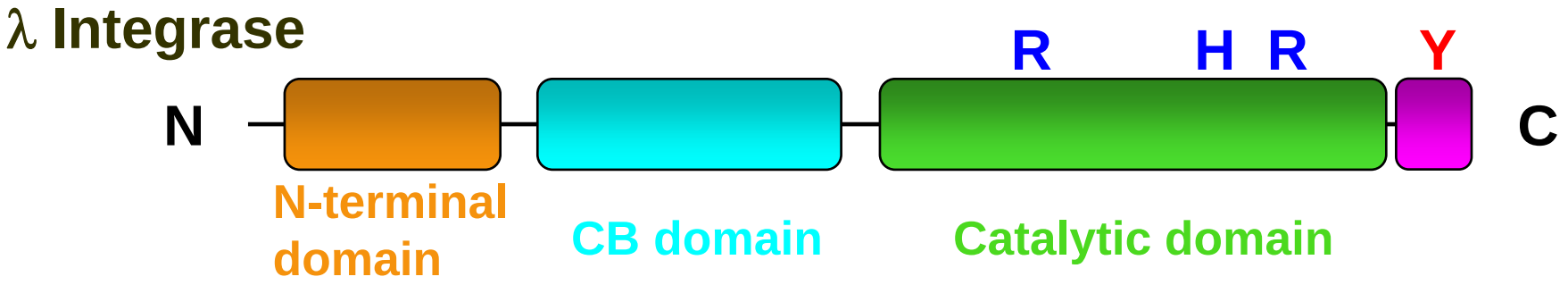


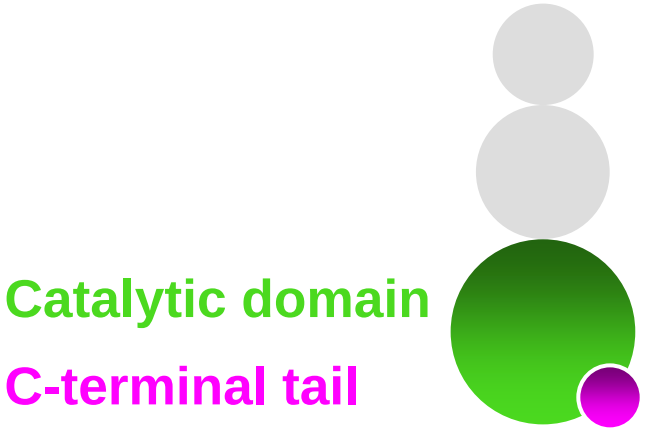


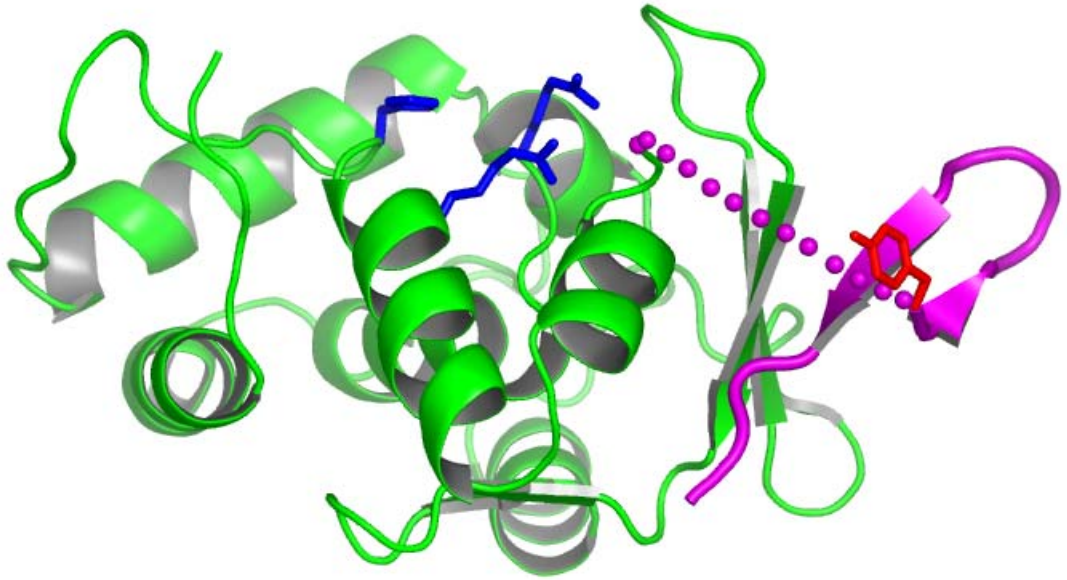
Chen, Y., Narendra, U., Iype, LE., Cox, MM., Rice, PA. (2000) *Mol Cell* **6**, 885-897.

Guo, F., Gopaul, DN., van Duyne, GD. (1997) *Nature* **389**, 40-46.

Structure of λ-int's catalytic domain showed an inactive conformation

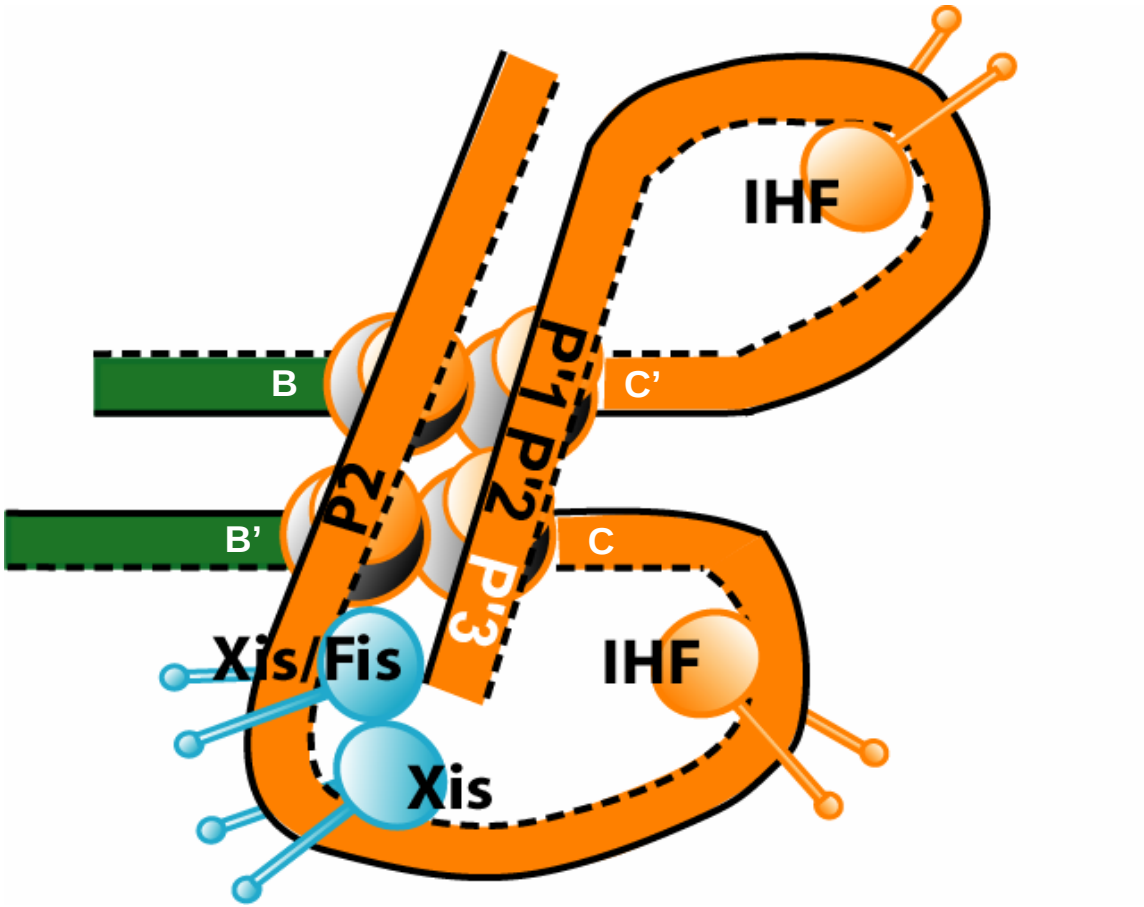






Crystal structure of the unliganded catalytic domain
Kwon, HJ. *et al*., (1997) *Science* **276**,126-131

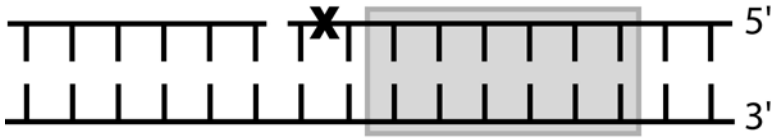
*att*P Arms Deliver λ-int to the Sites of DNA Strand Exchange

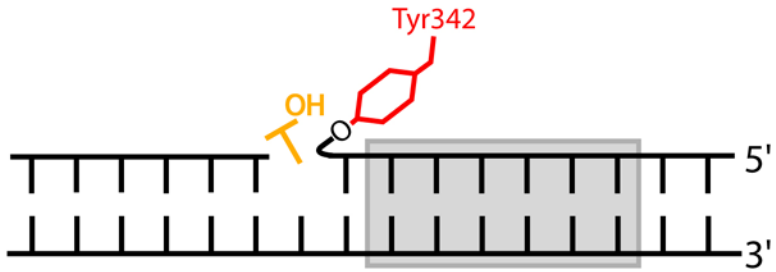


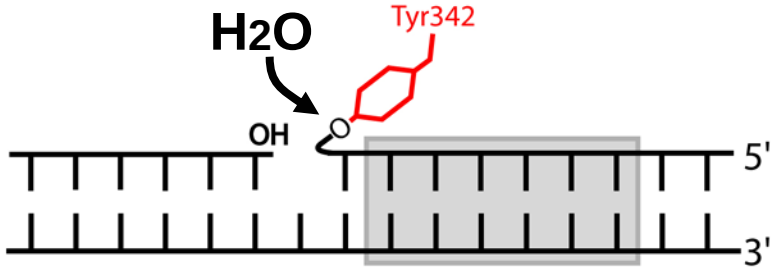
Kim and Landy 1992. Science 256, 198-203

A new type of suicide DNA substrate traps covalent λ-int:DNA complexes

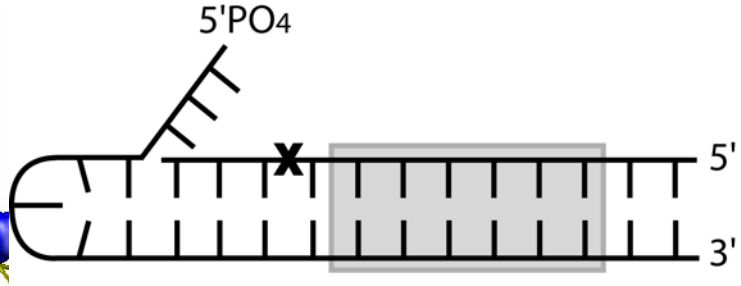
Conventional nicked-suicide substrate

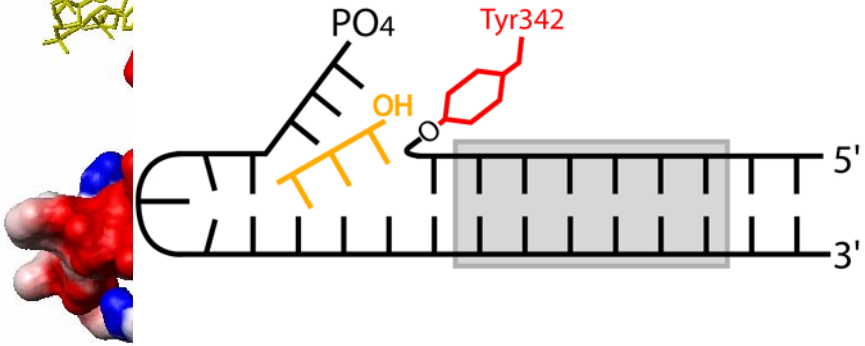


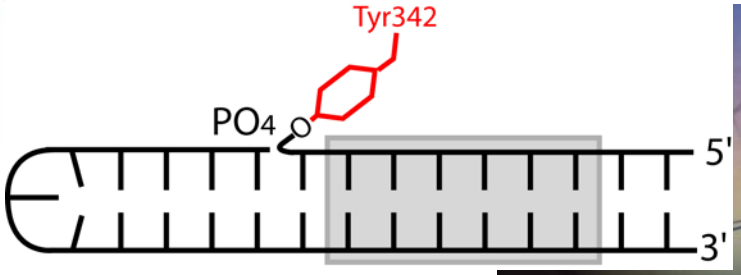




Flap-suicide substrate





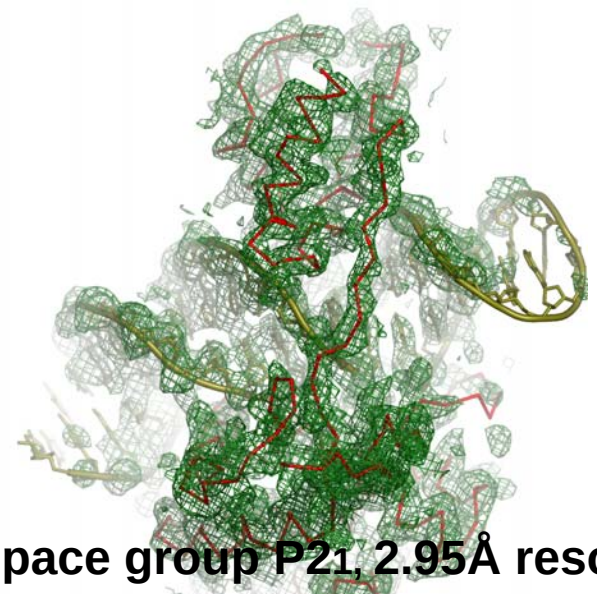


The DNA-bound structure of λ-int shows a conformation active for DNA cleavage

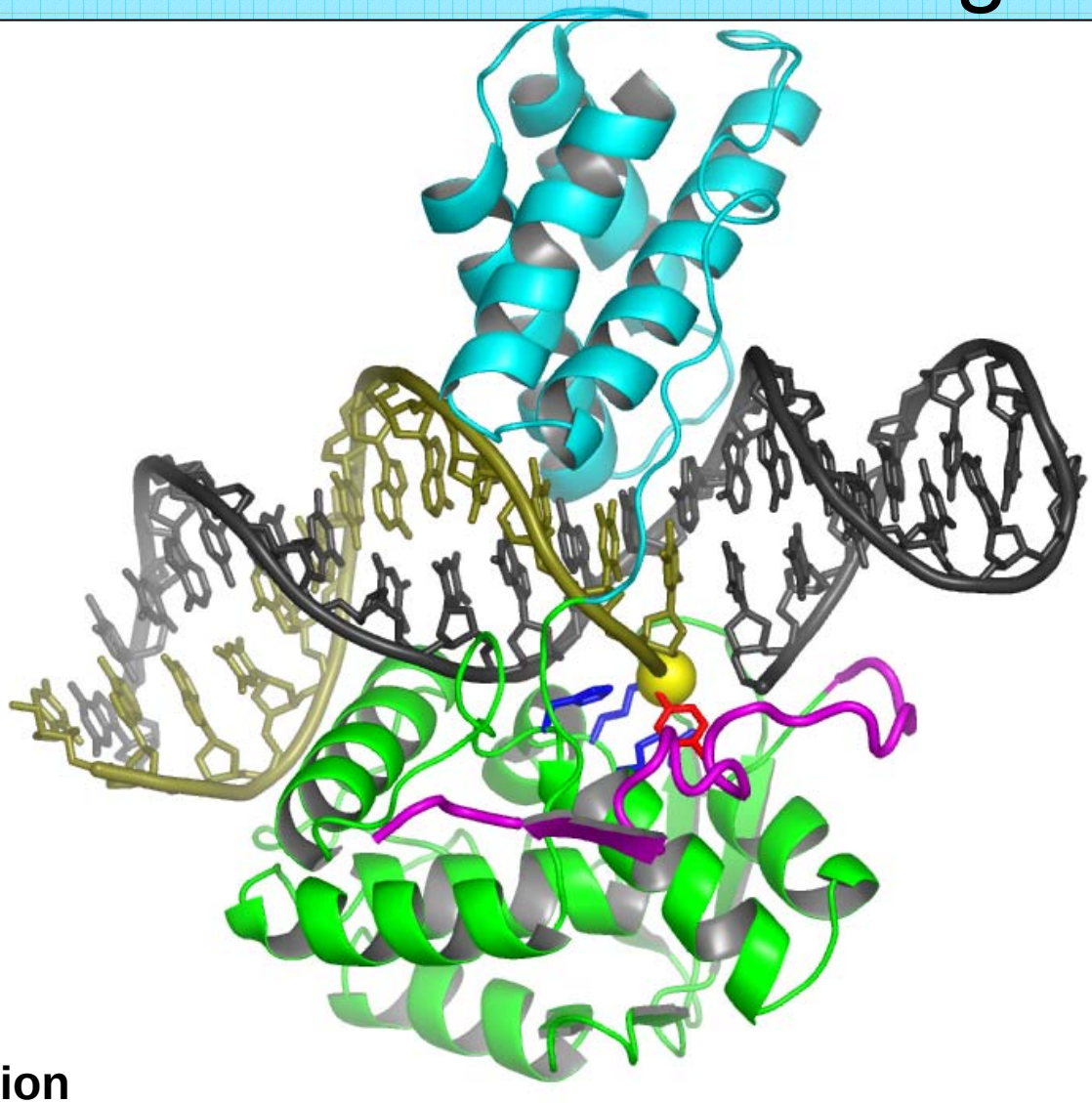
CB domain

Catalytic domain

C-terminal tail

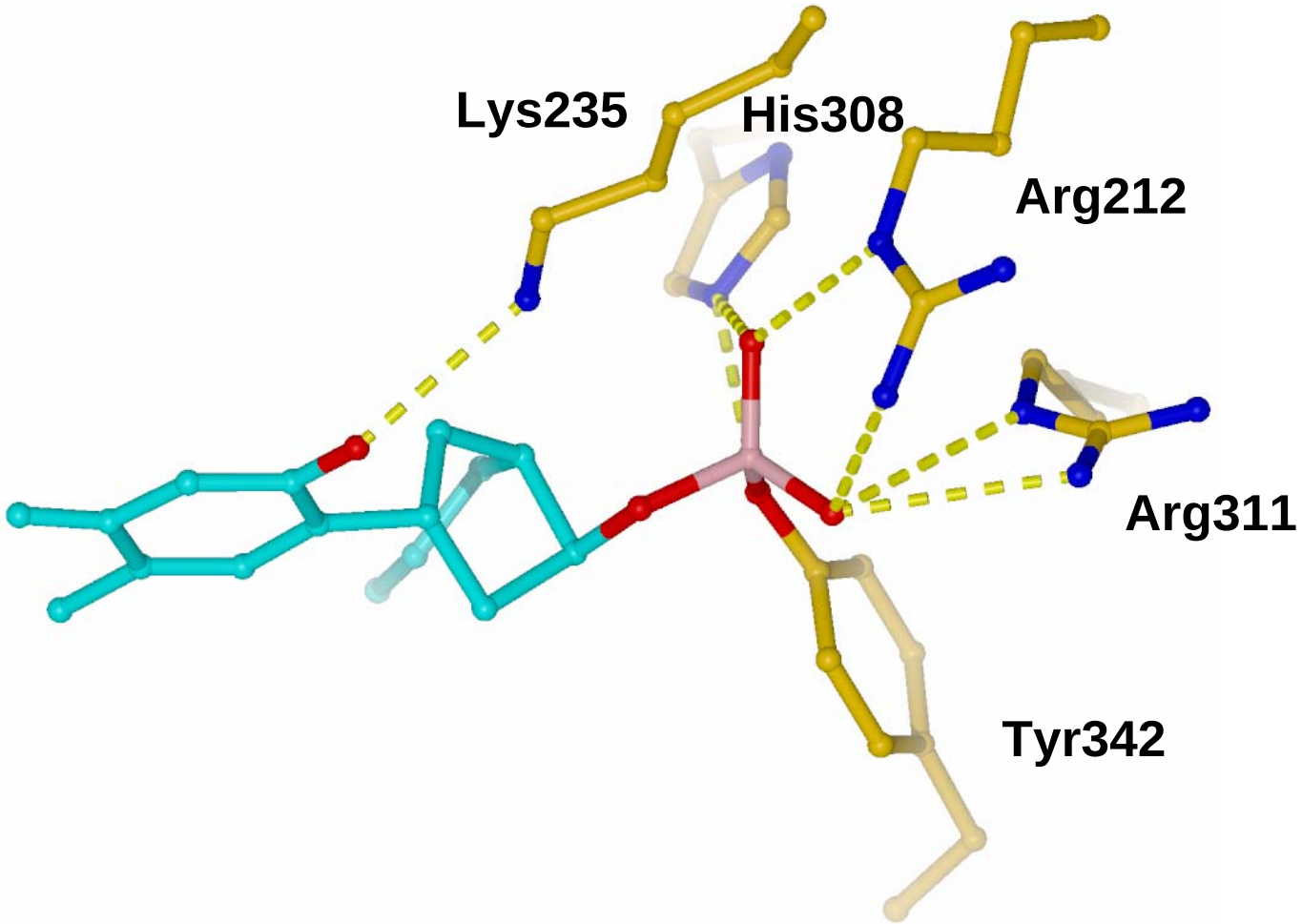


Space group $P2_1$, 2.95Å resolution
Rcryst = 23.1 % , Rfree = 25.9 %

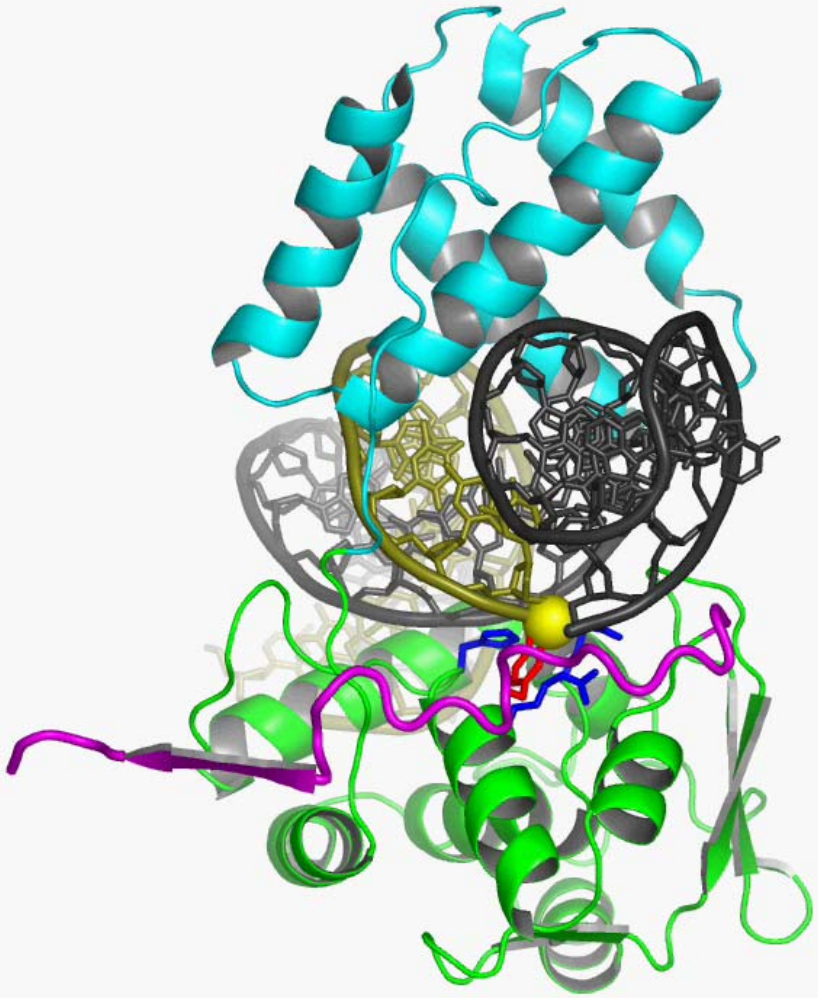


Aihara et al. 2003. Mol. Cell 12, 187-98

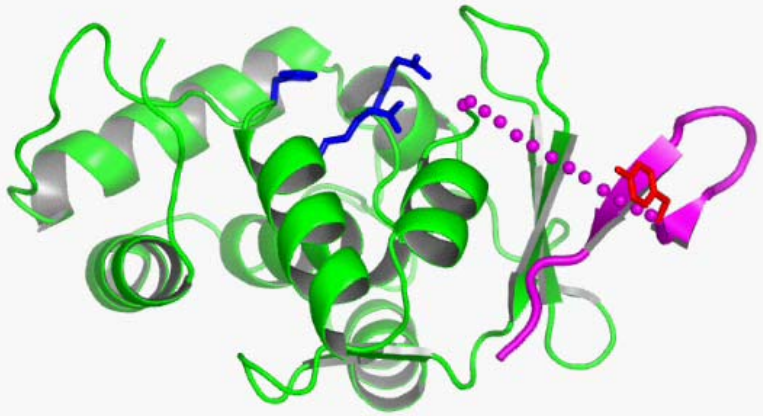
Basic residues stabilize the phosphotyrosine protein-DNA linkage



Tyr342 nucleophile moves into the active site for DNA cleavage in *cis*

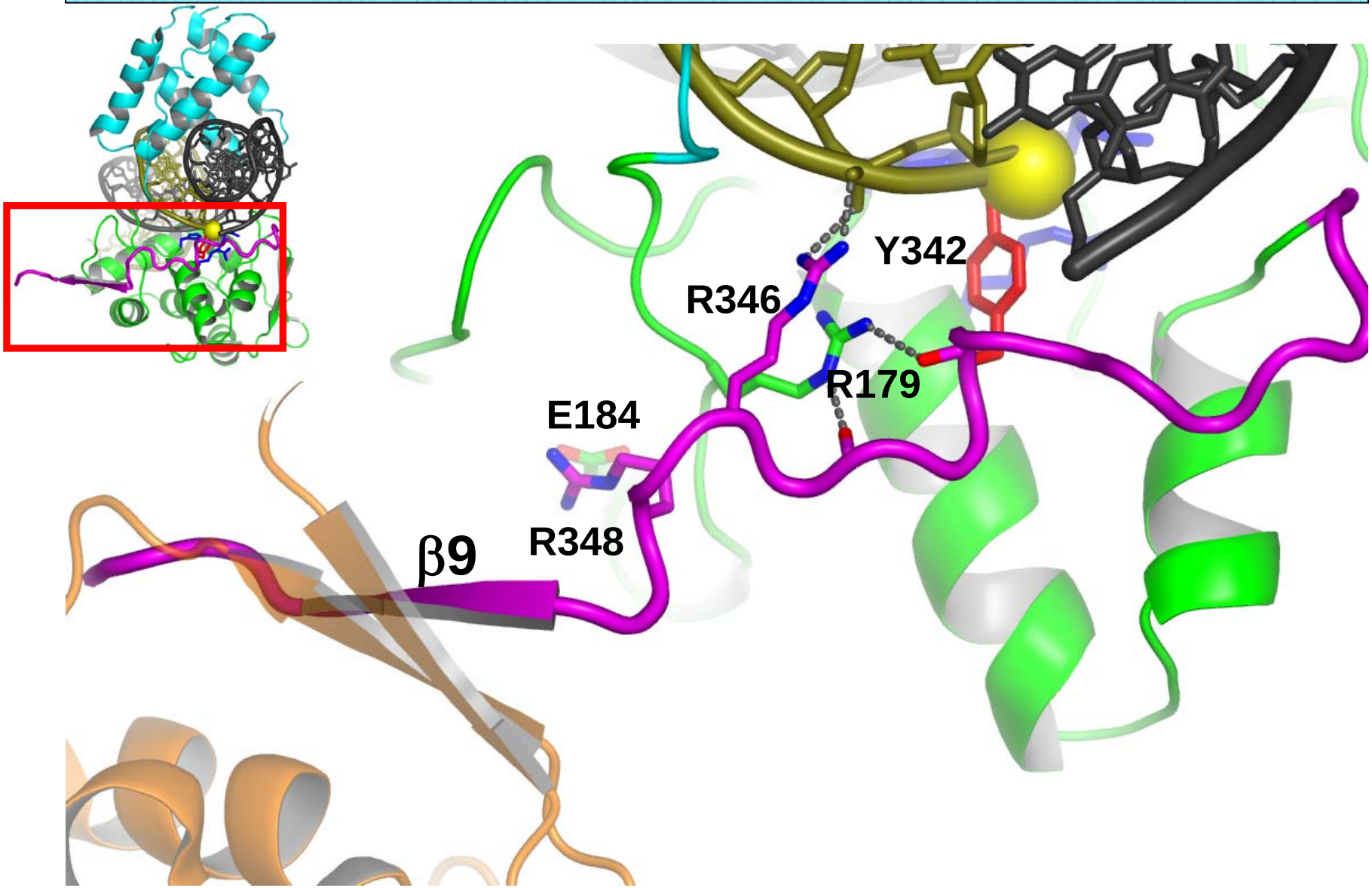


DNA bound (active)

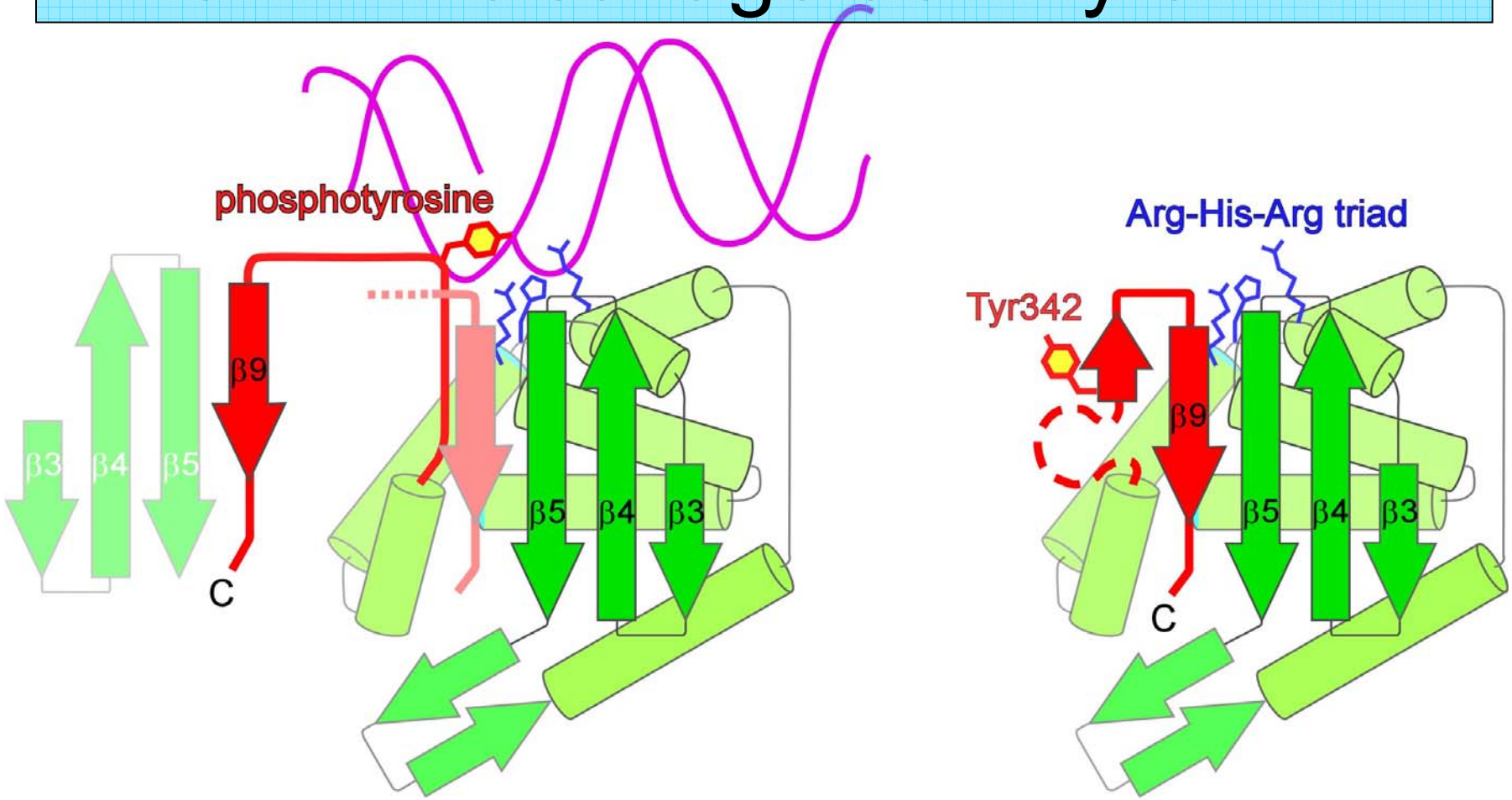


Free (inactive)

Tyr342 Moves Into the Active Site and Ejects β9 For *Trans* Interactions



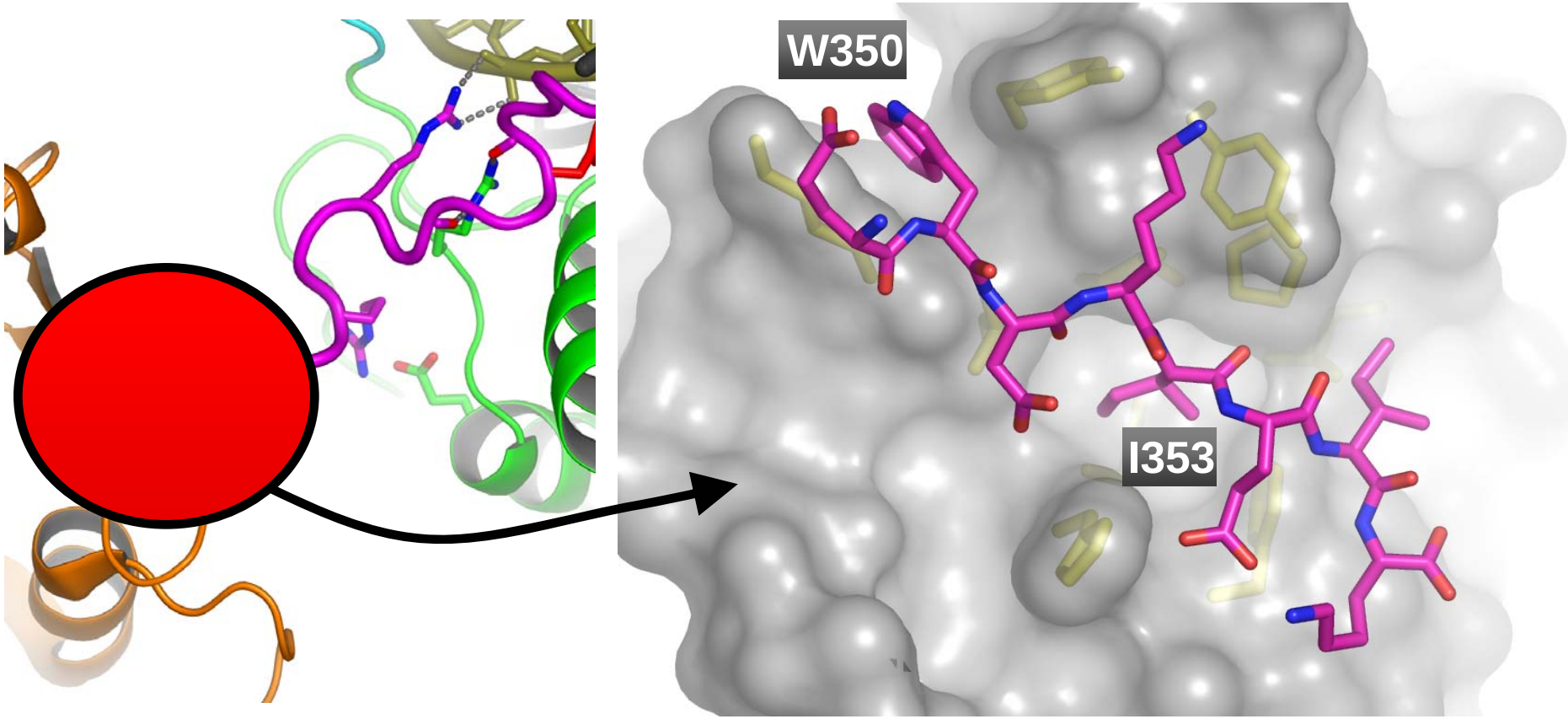
A conformational switch controls the DNA cleavage activity of λ-int



Active (*trans*)

Inactive (*cis*)

Trans interactions of β9 coordinate the recombination reaction?

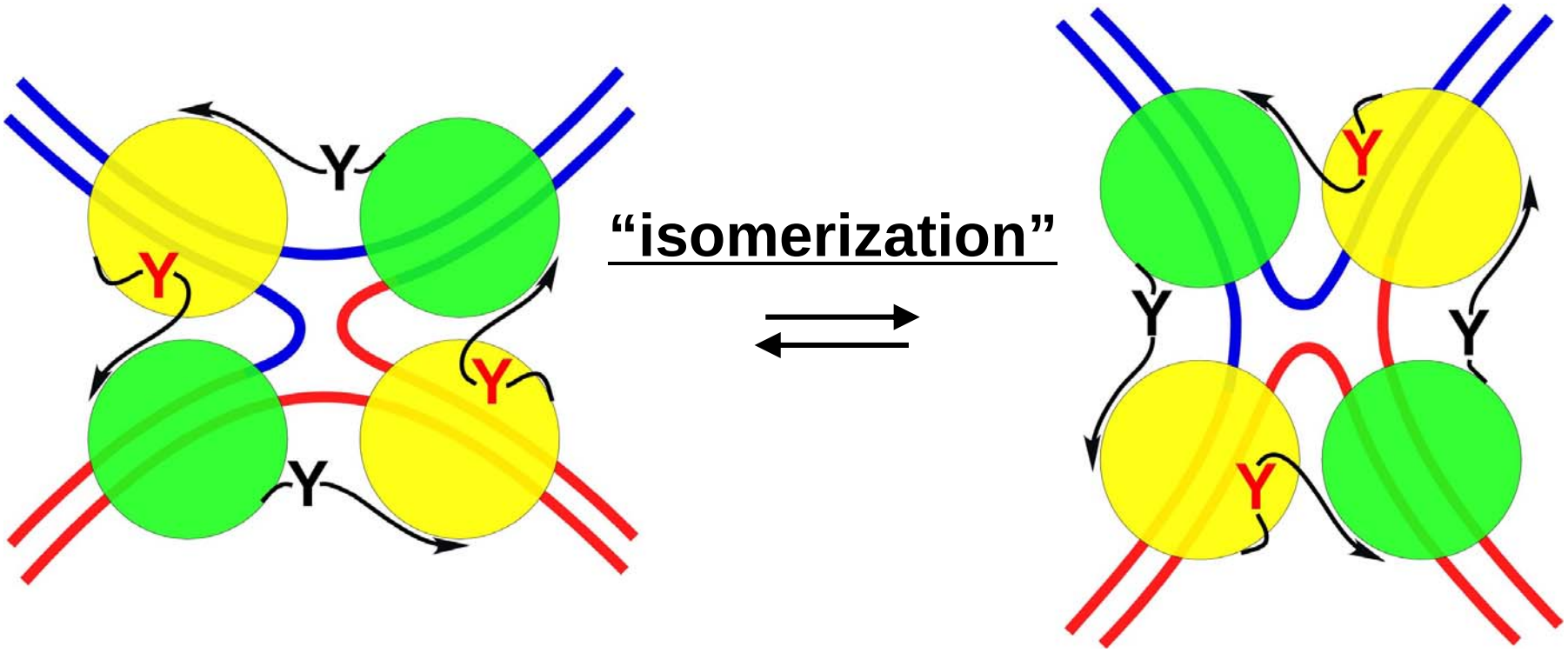


W350A, I353A, W350ter mutations cause elevated DNA cleavage activity of λ-int monomers while abolishing recombination.

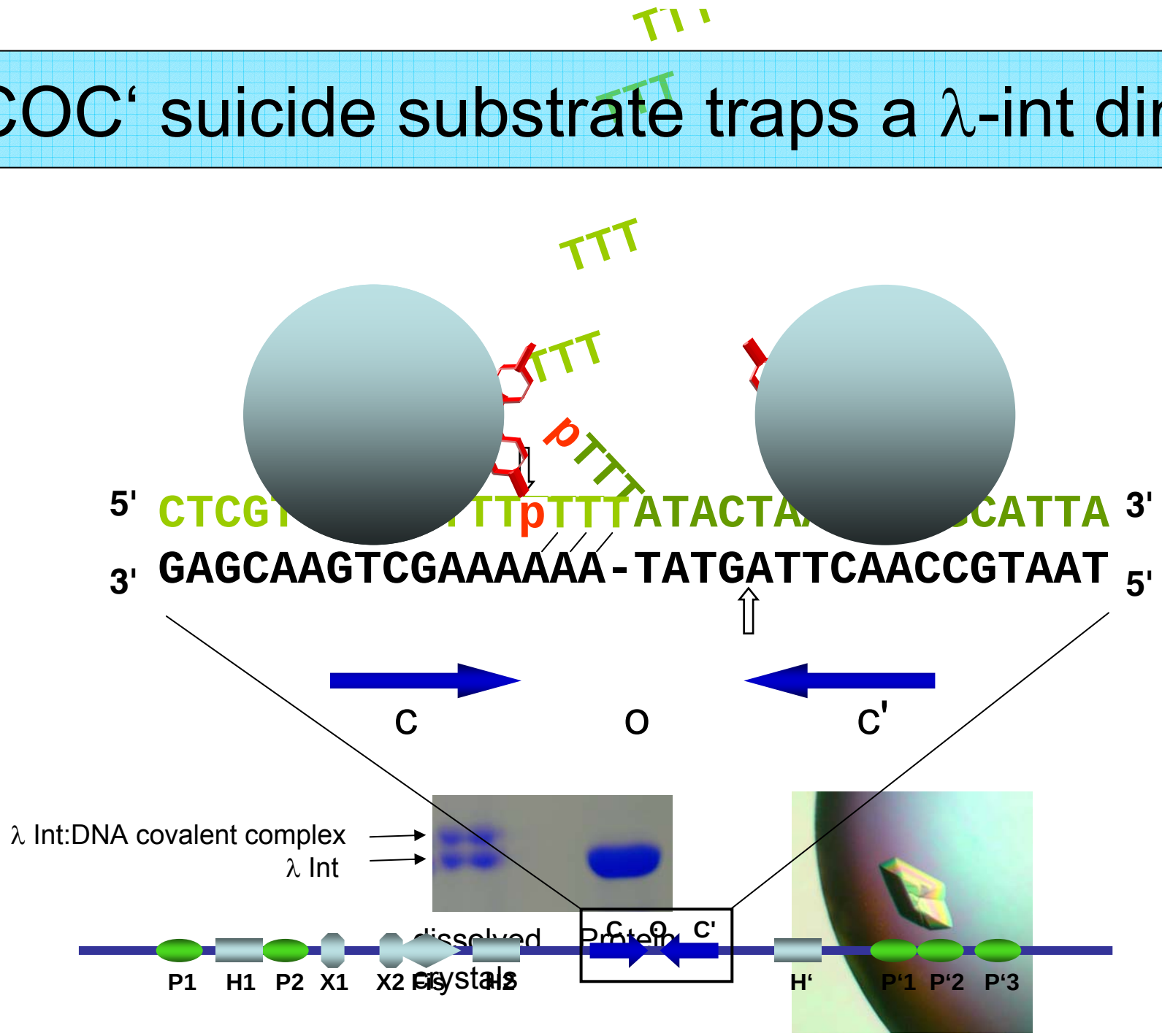
Tekle et al., (2002) *J Mol Biol*. **324**, 649-665

Kazmierczak et al., (2002) *Nucleic Acids Res*. **30**, 5193-5204

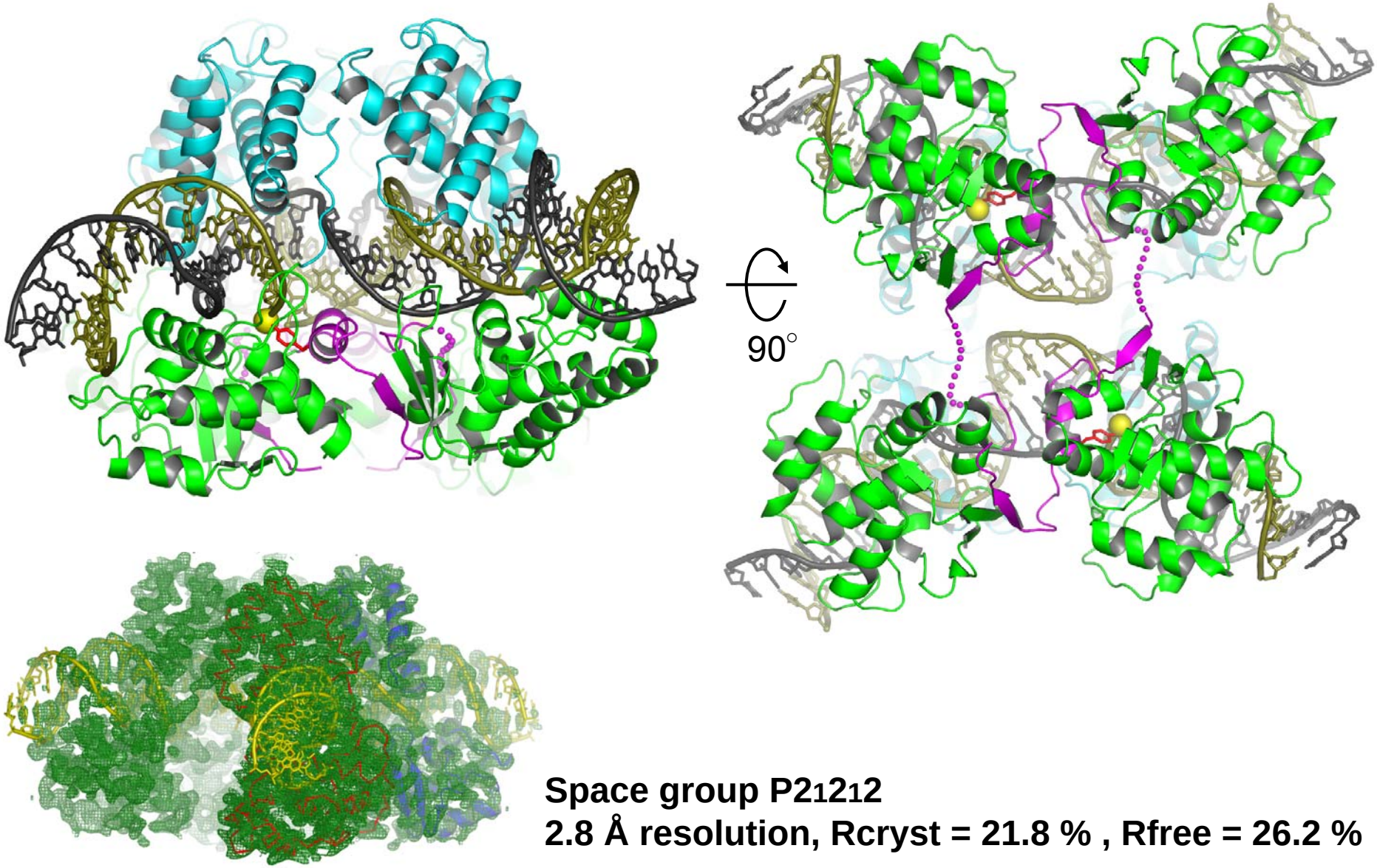
The β9 tail of λ-int controls half-the-sites reactivity?



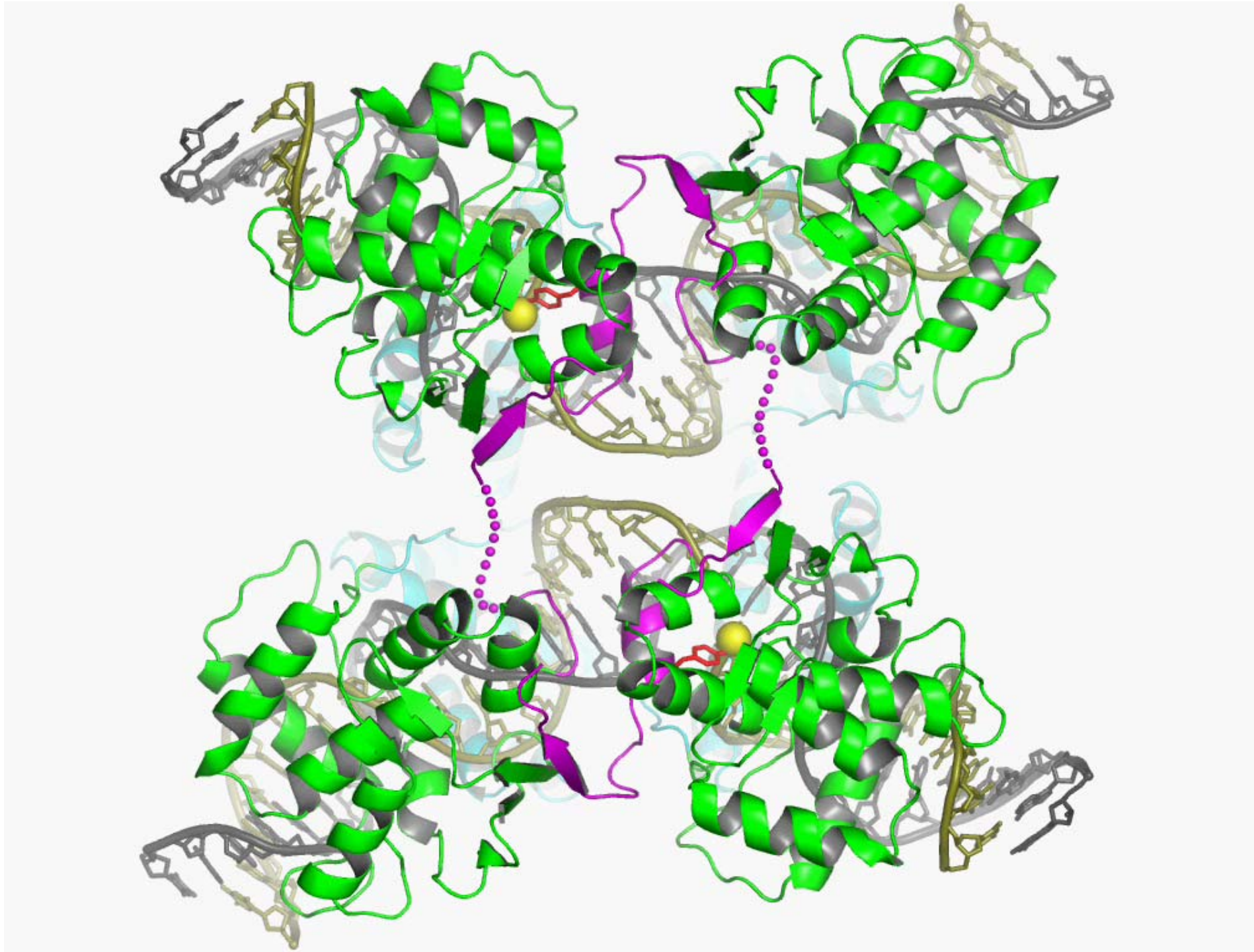
COC‘ suicide substrate traps a λ-int dimer

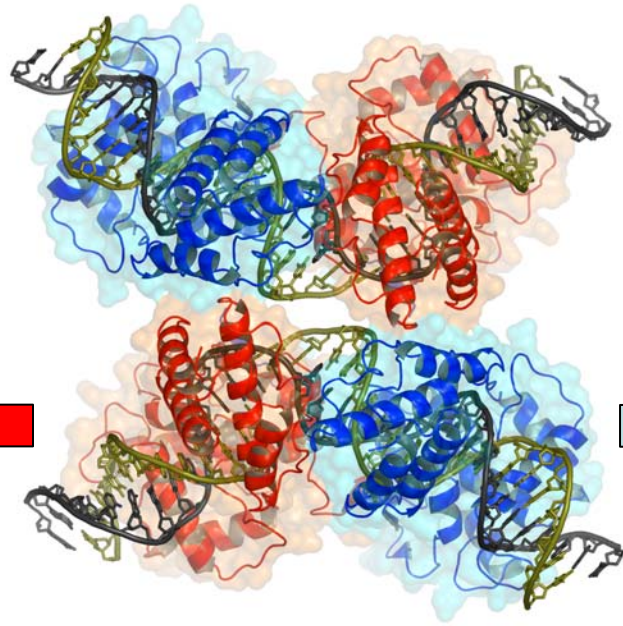


Cyclic permutation of the β9 "tails" in λ-int tetramers



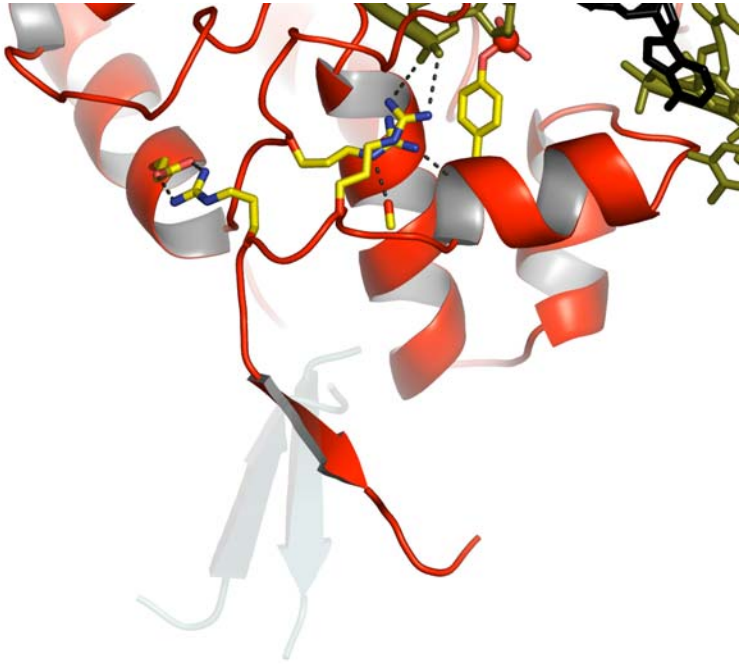
Space group $P2_12_12$
2.8 Å resolution, Rcryst = 21.8 % , Rfree = 26.2 %

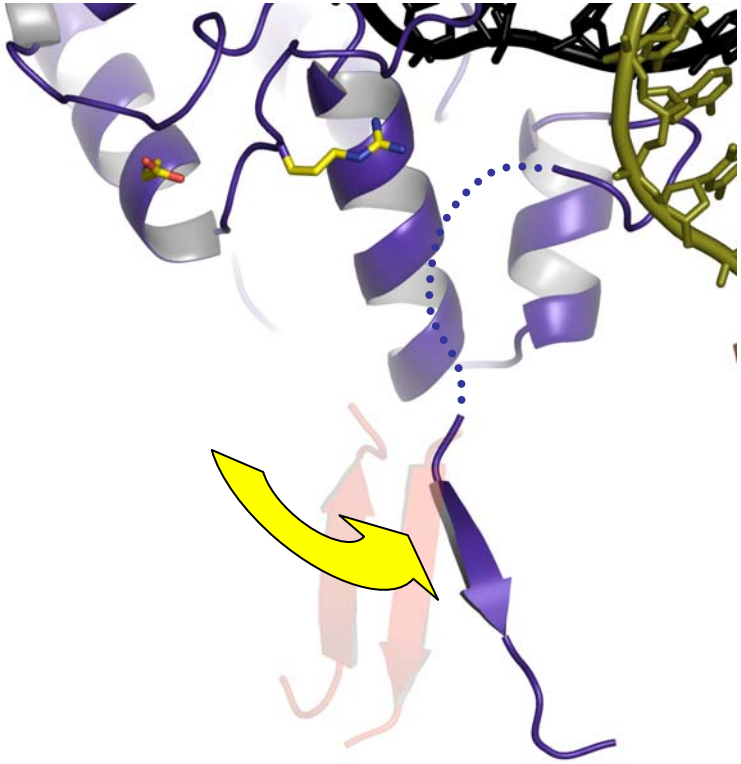




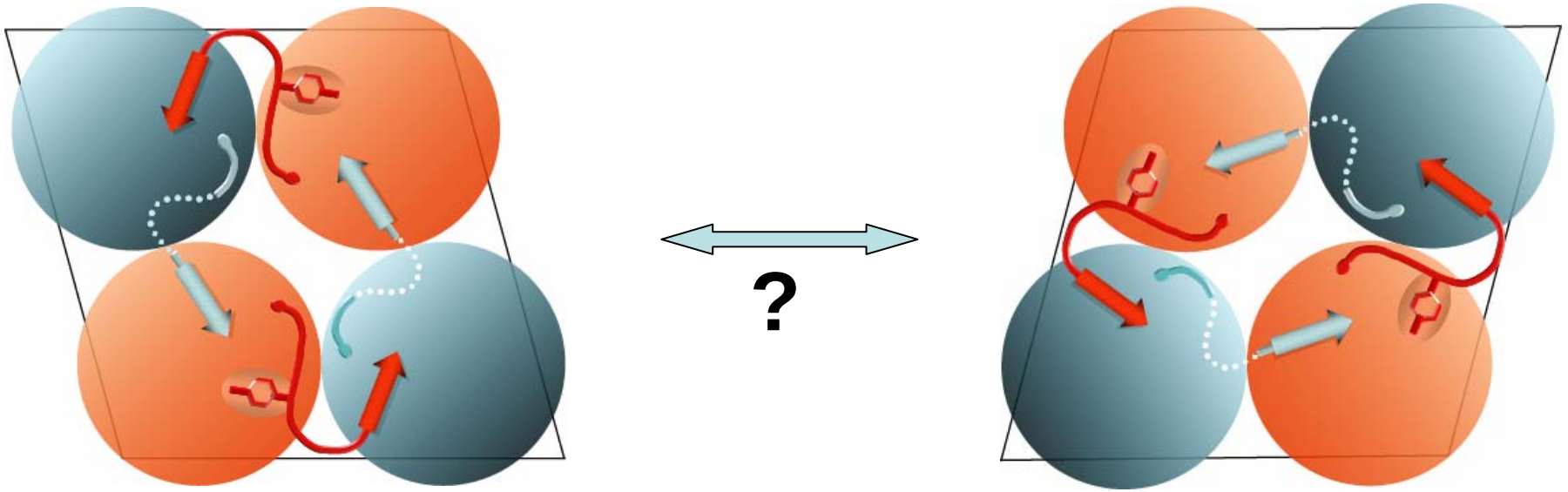
active

inactive

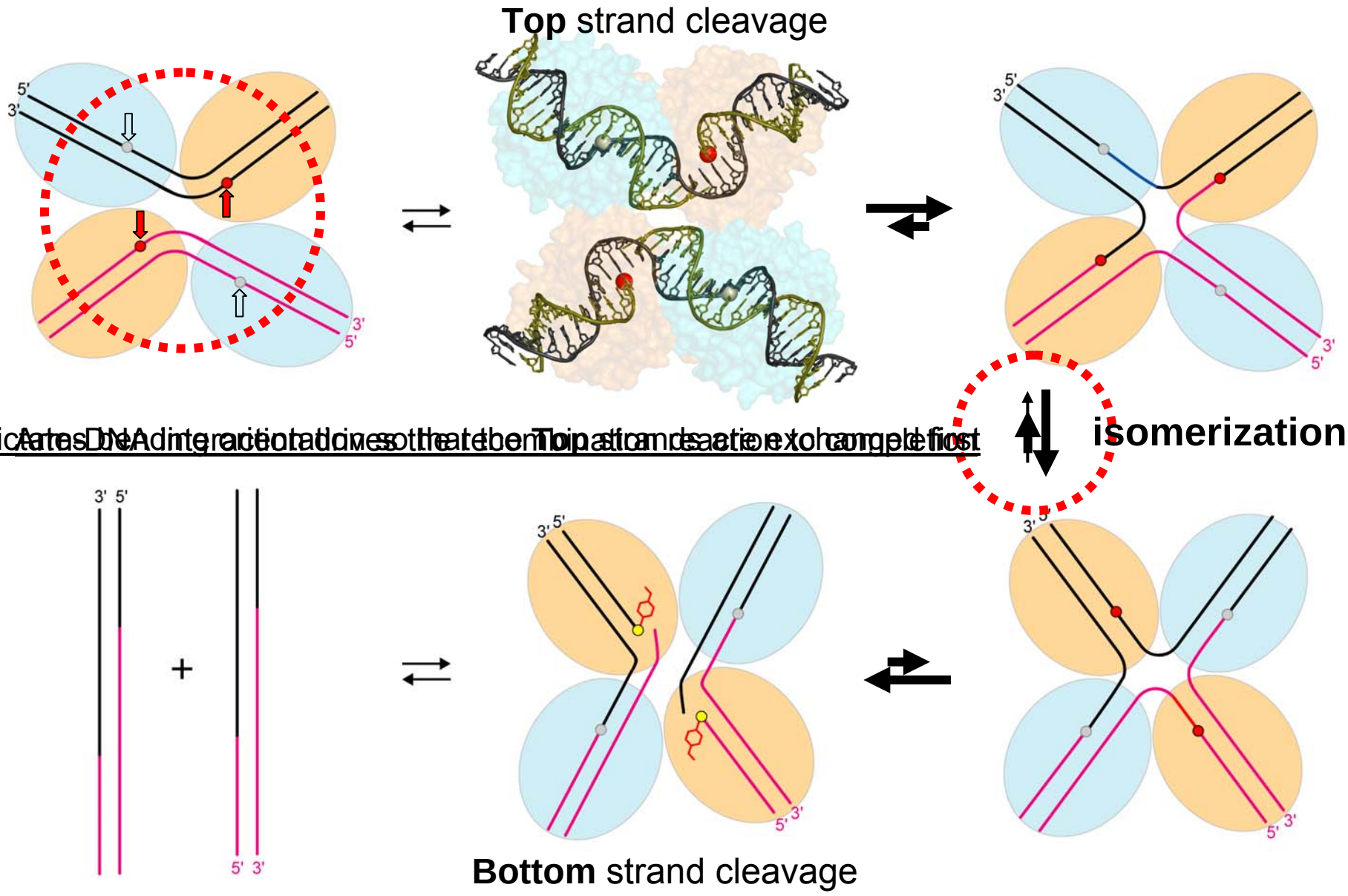




How Do Arm-binding Interactions Promote Isomerization?



Recombination with the arm-DNA



The 2-arm Holliday Junction Complex

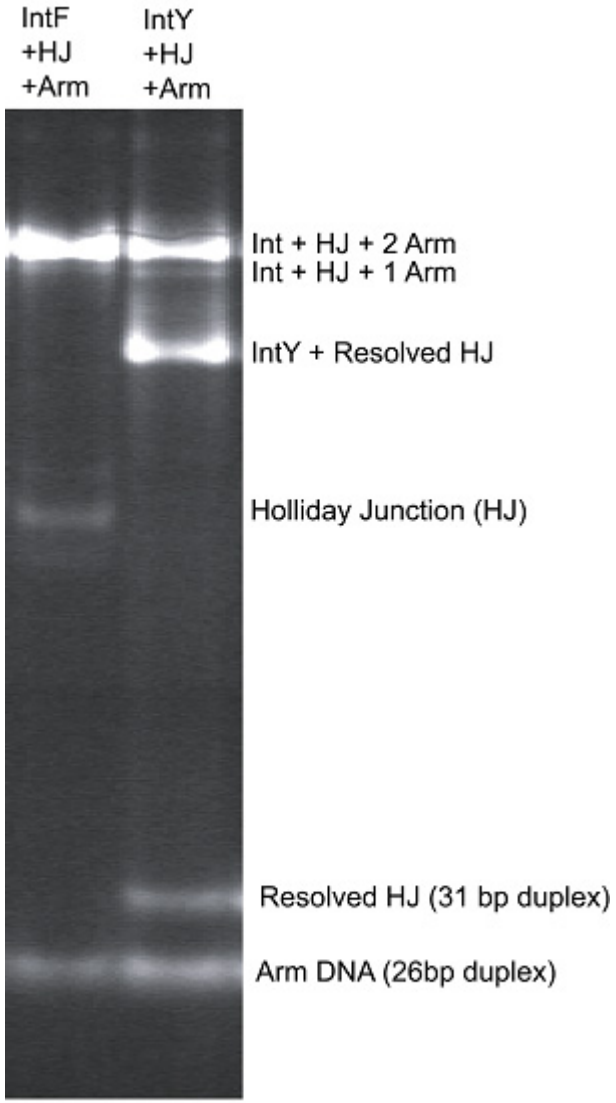
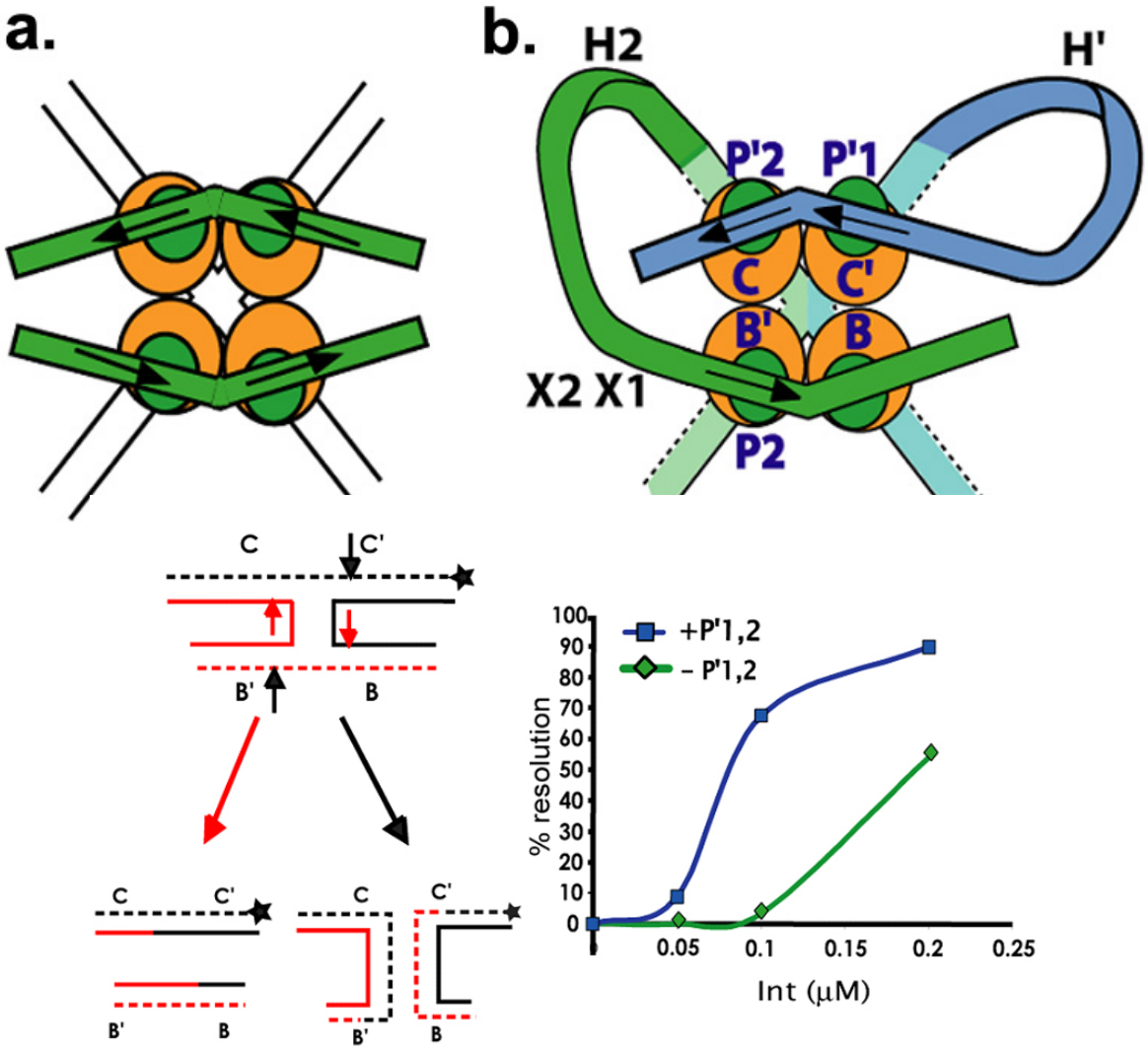
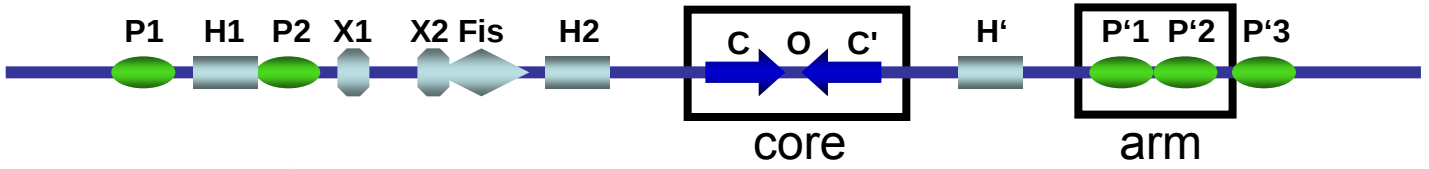


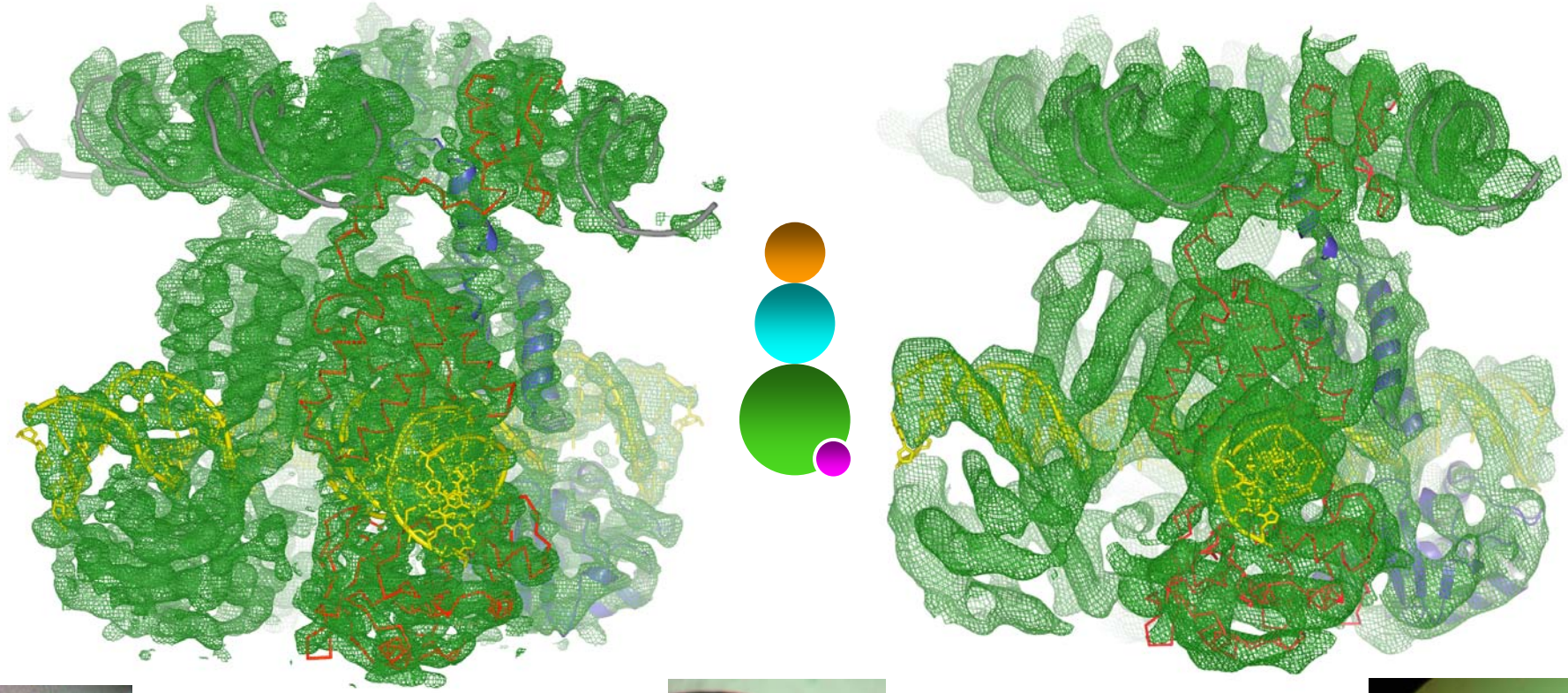
Fig2: Native PAGE of complexes formed with Integrase (IntY and IntF), HJ and Arm sites

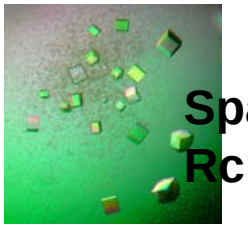


Radman-Livaja et al. 2003. Mol. Cell 11, 783.

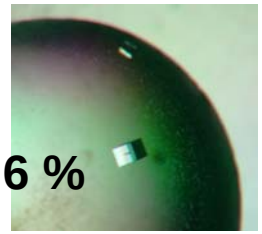
Structures of full-length λ-int complexed with core and arm DNAs



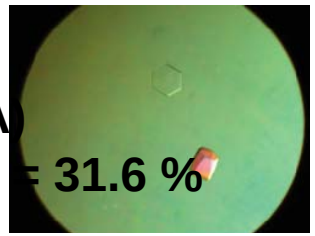




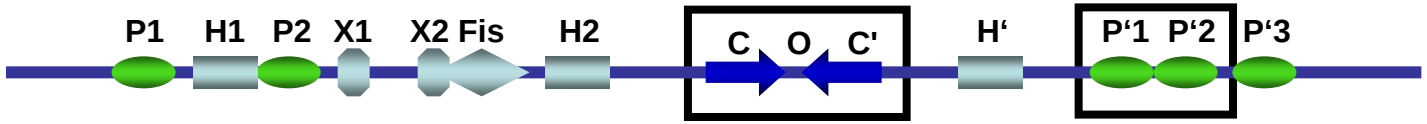
Space group $C222_1$ (3.8Å)
Rcryst = 24.8 % , Rfree = 29.6 %



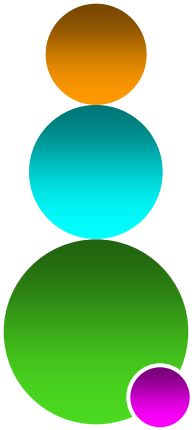
Space group $P3_1$ (4.4 Å)
Rcryst = 28.1 % , Rfree = 31.6 %



λ Recombination Complexes



IHF induced DNA U-turn
Rice, P.A. et al.,(1996) *Cell* **87**,1295-1306

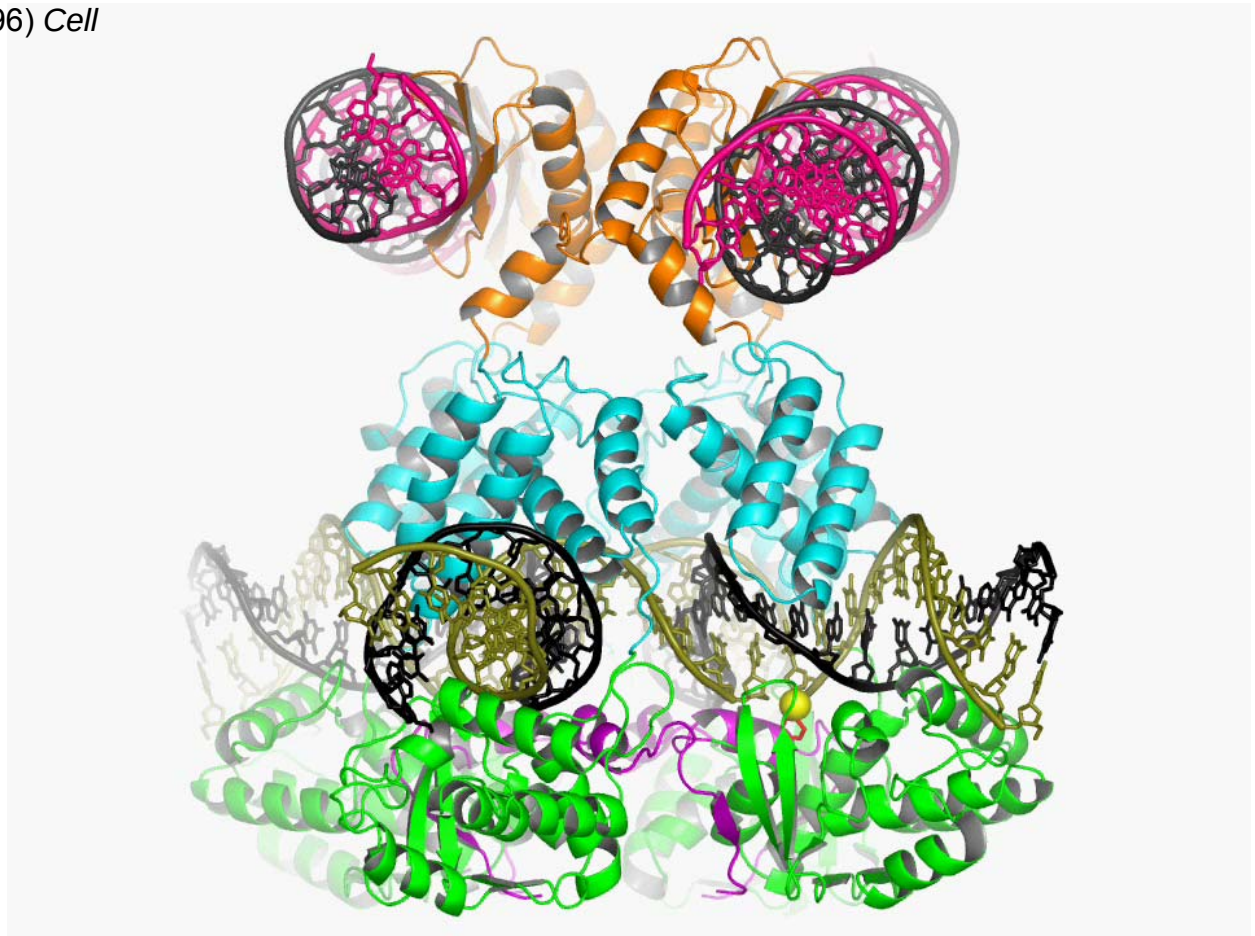


N-terminal

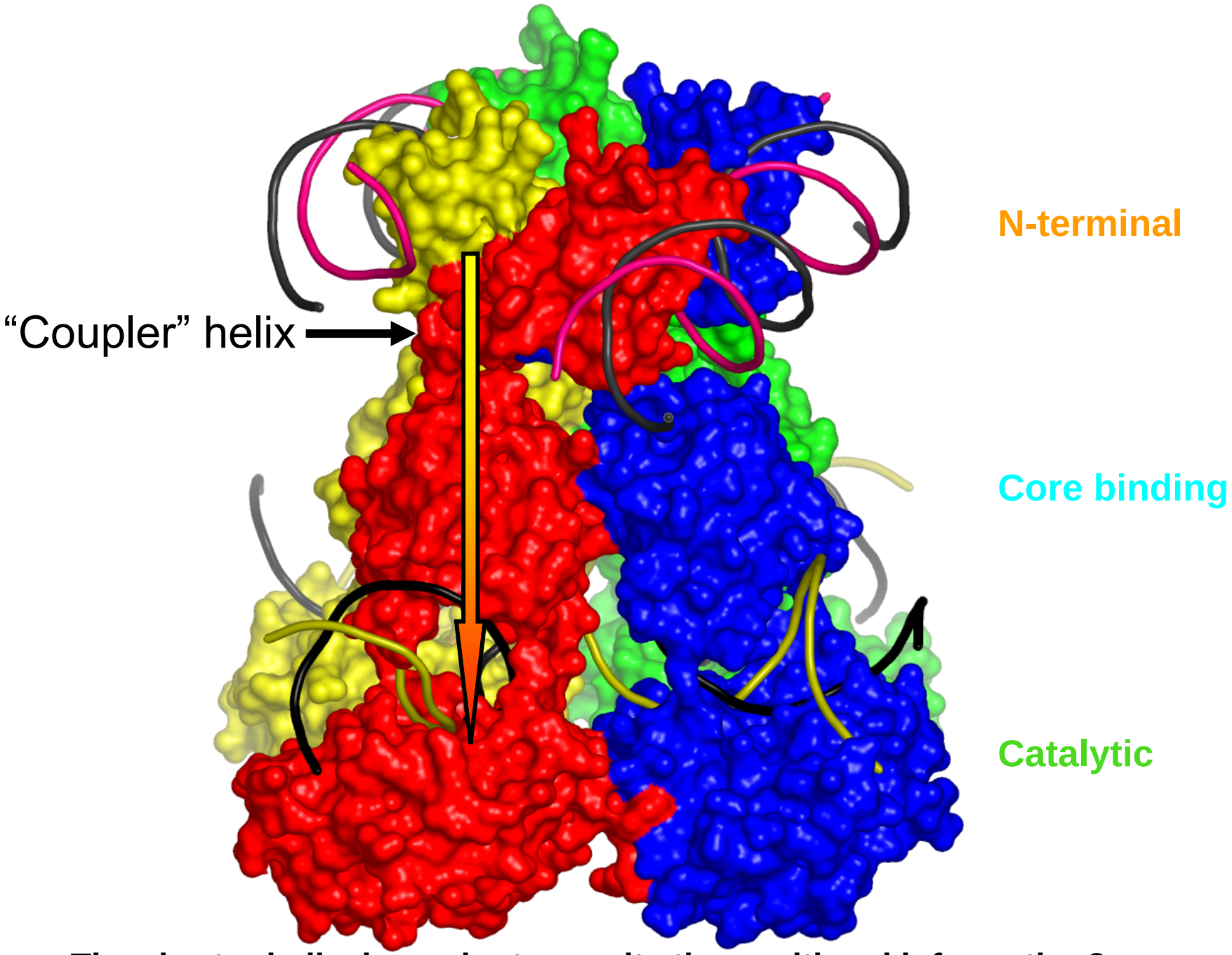
CB

Catalytic

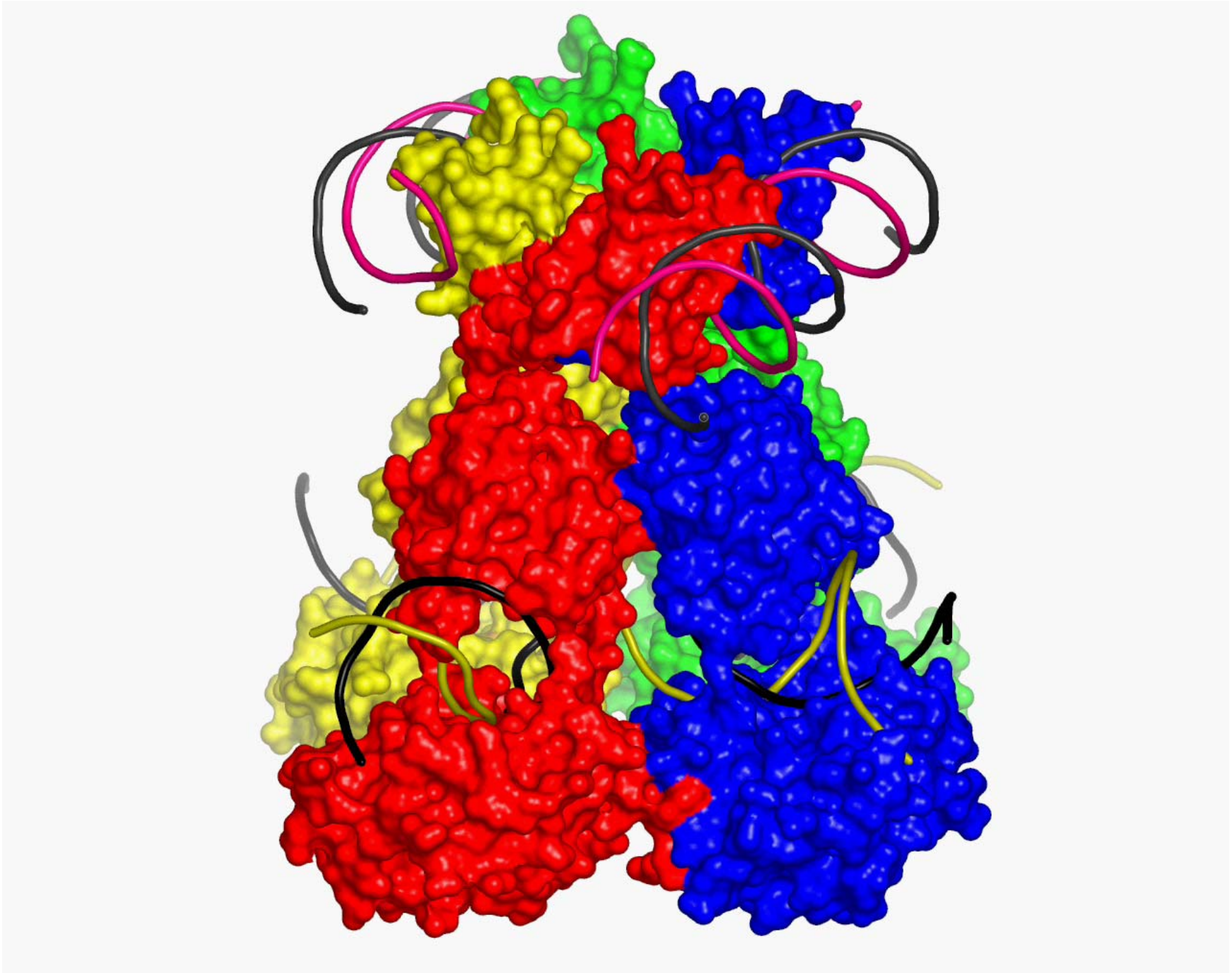
C-terminal tail

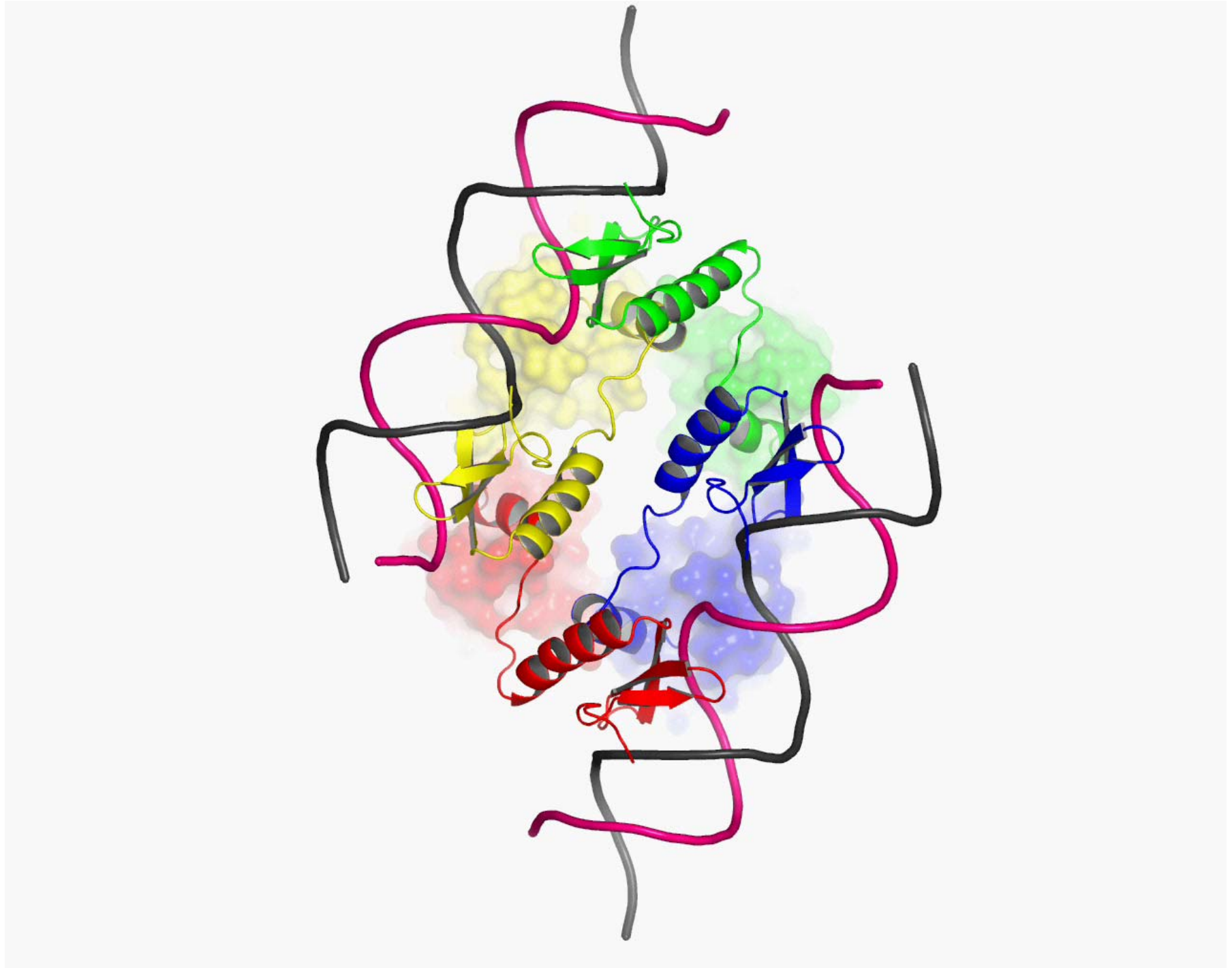


A structural basis for allosteric control of DNA recombination by lambda integrase.
Biswas et al. Nature (2005) **435**, 1059-1066.

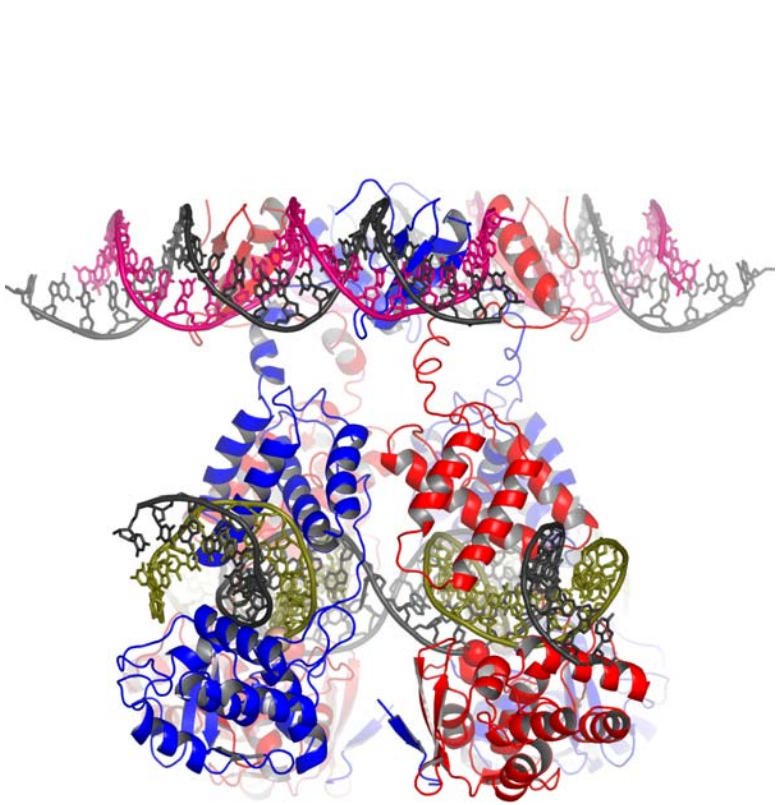


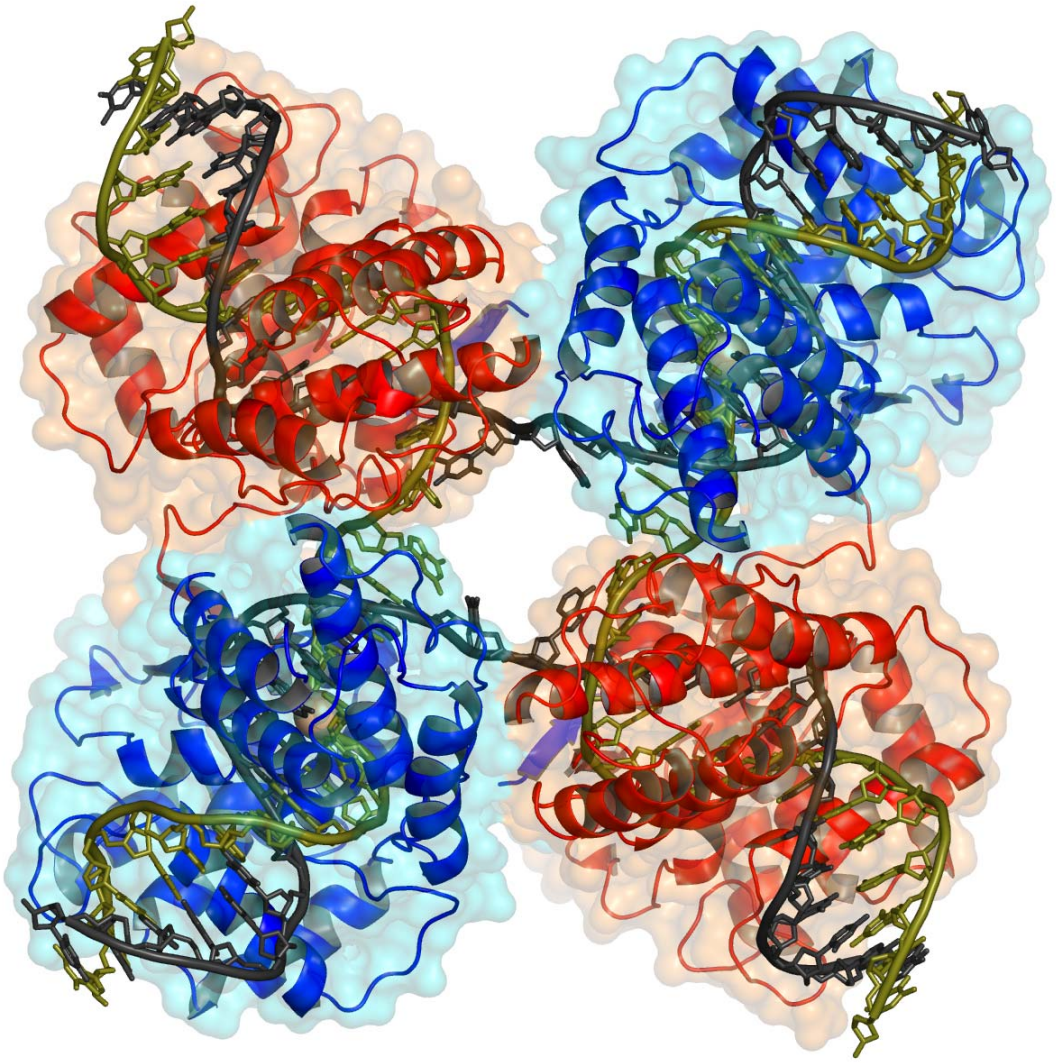
The short α-helical coupler transmits the positional information?



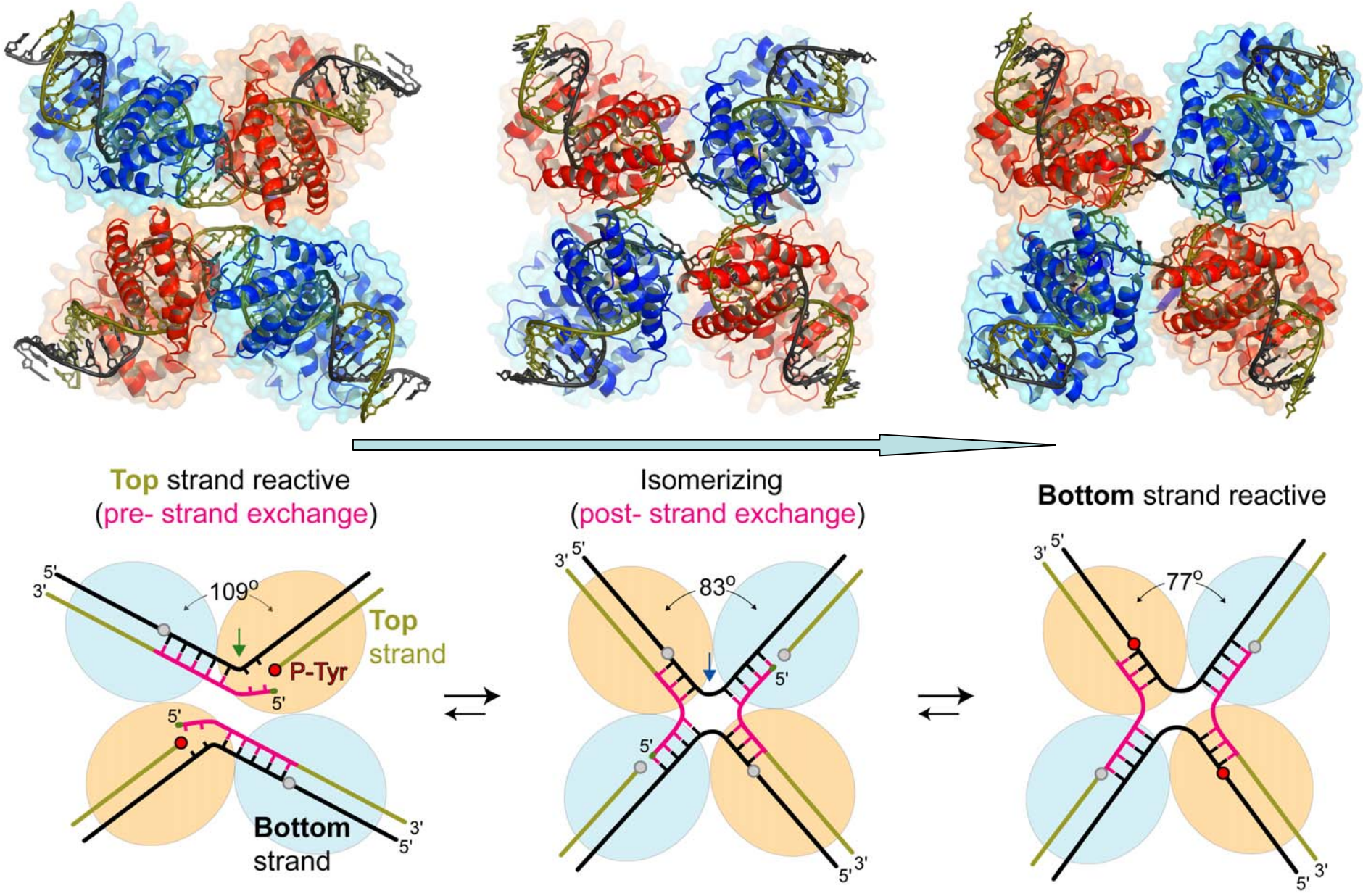


Arm-DNA interactions induce strand exchange and HJ isomerization

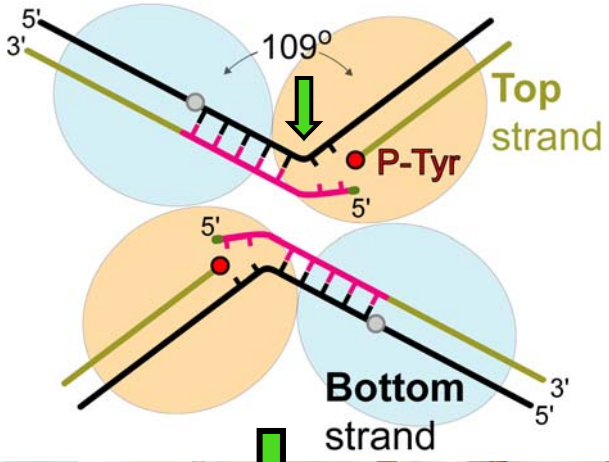




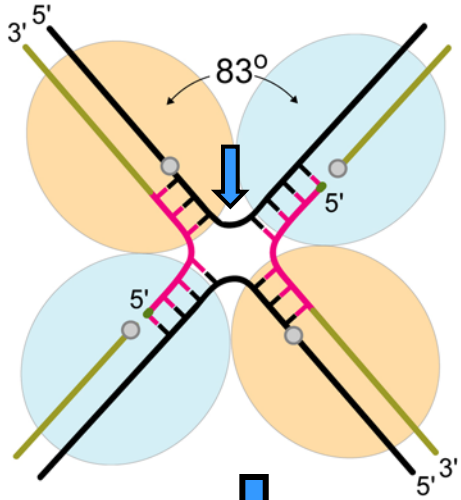
Snapshots During Isomerization

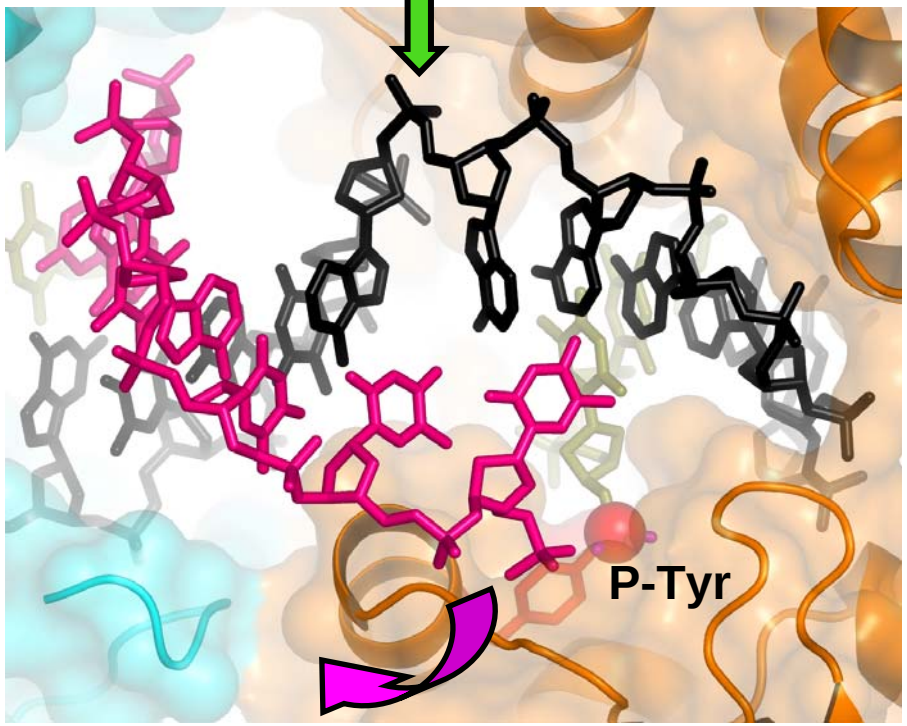


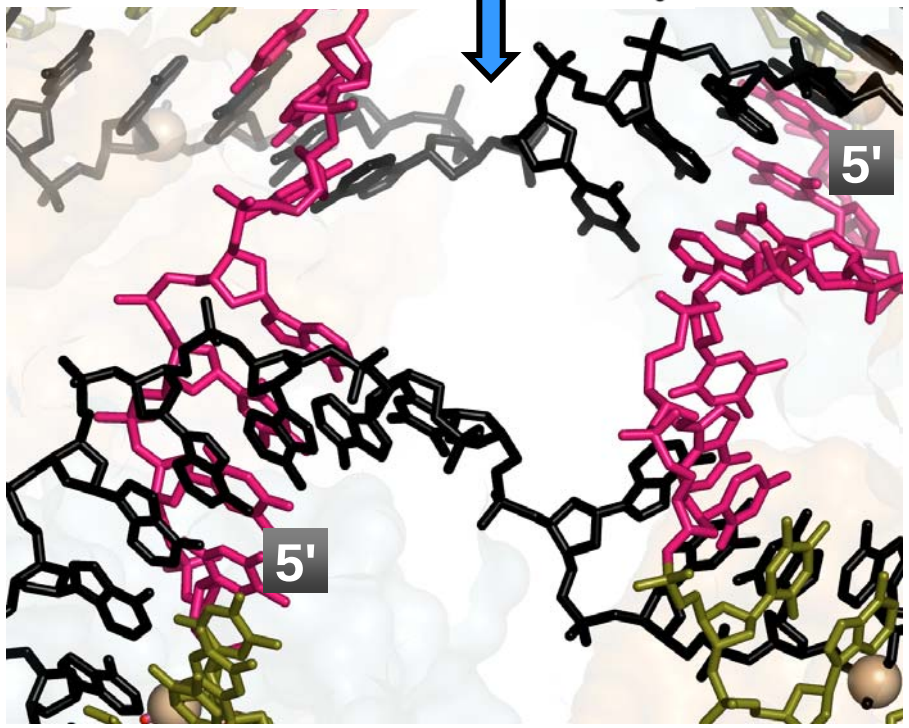
Migration of the DNA bend (branch migration)



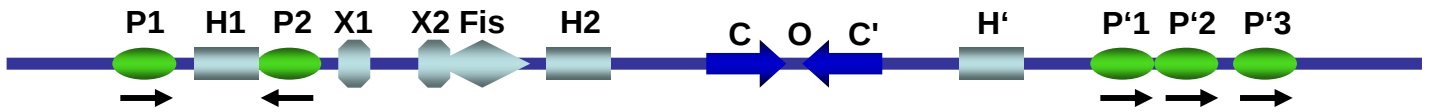
Top strand exchange

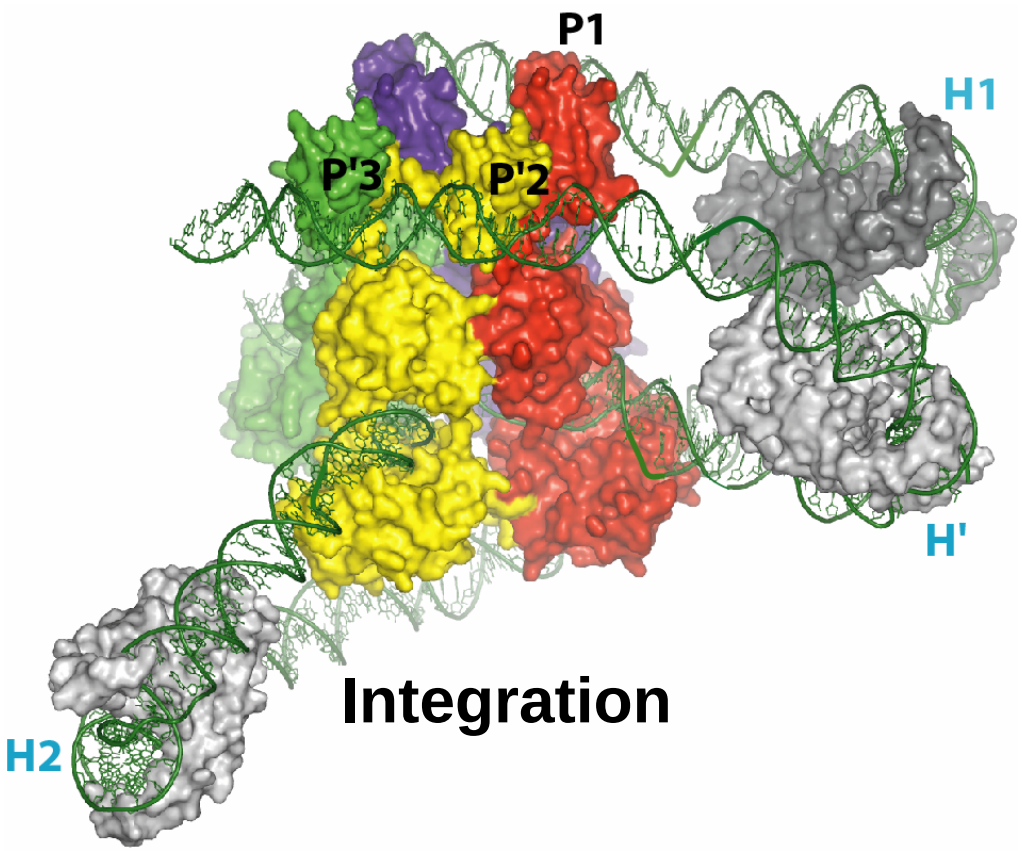




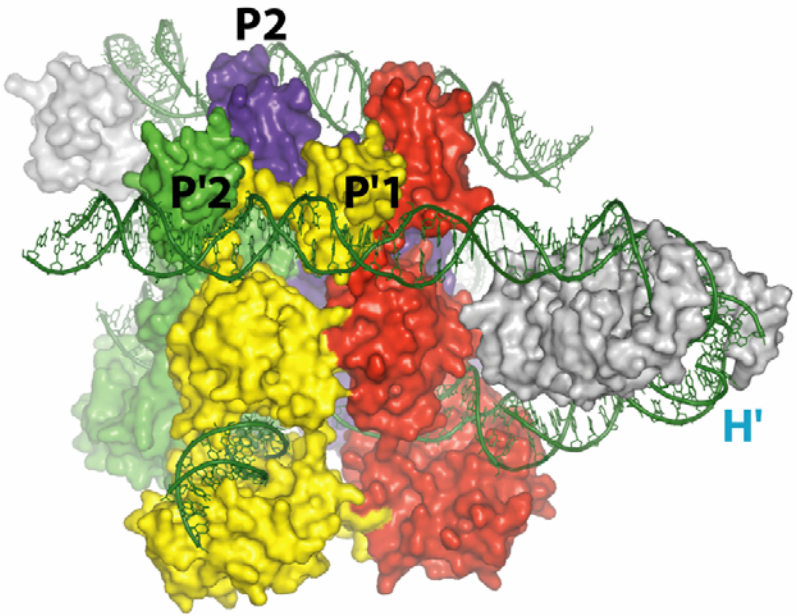


“Intasome” models for integrative and excisive recombination



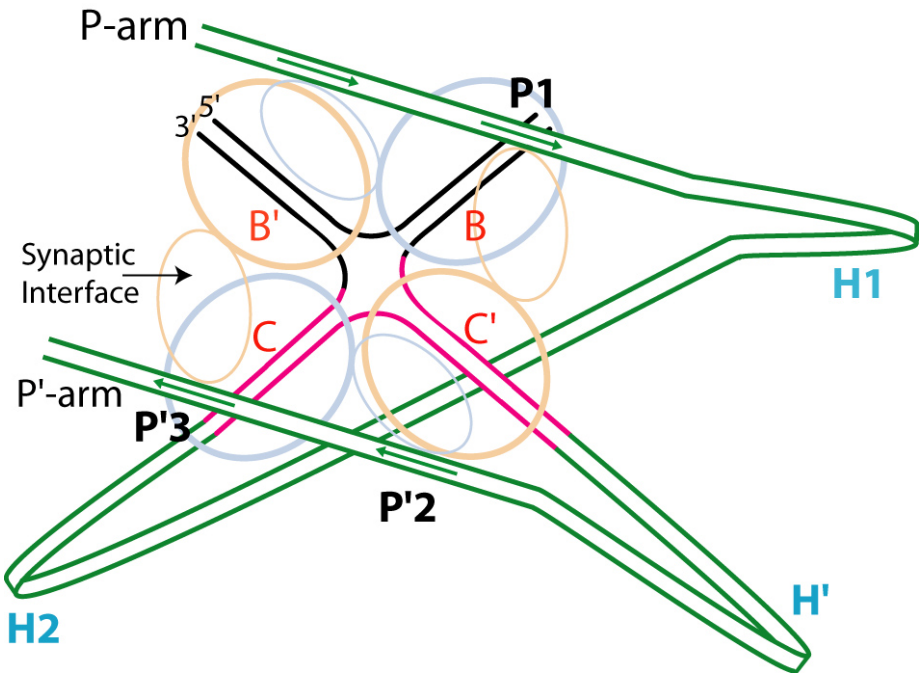


Integration

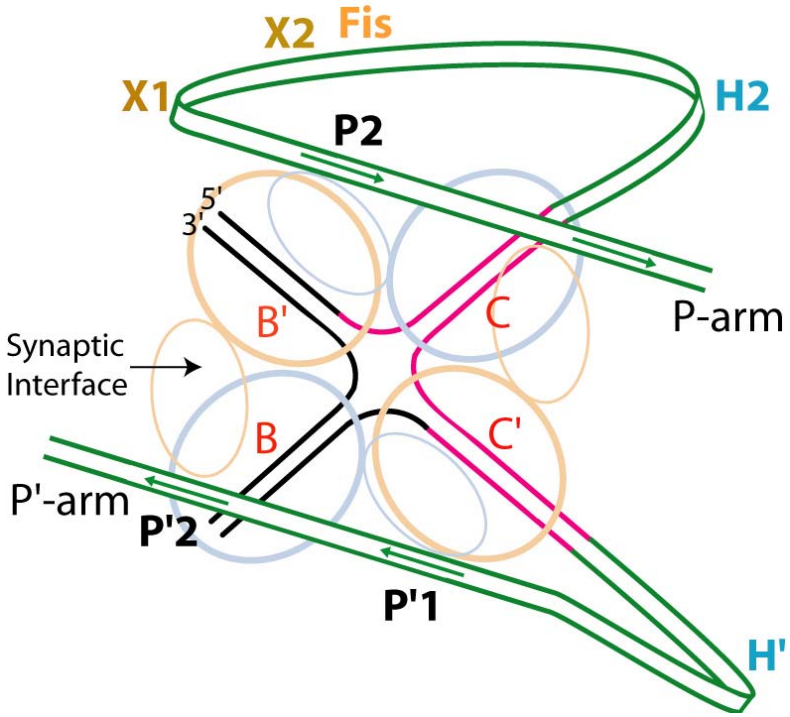


excision

Modeling the “Intasome”



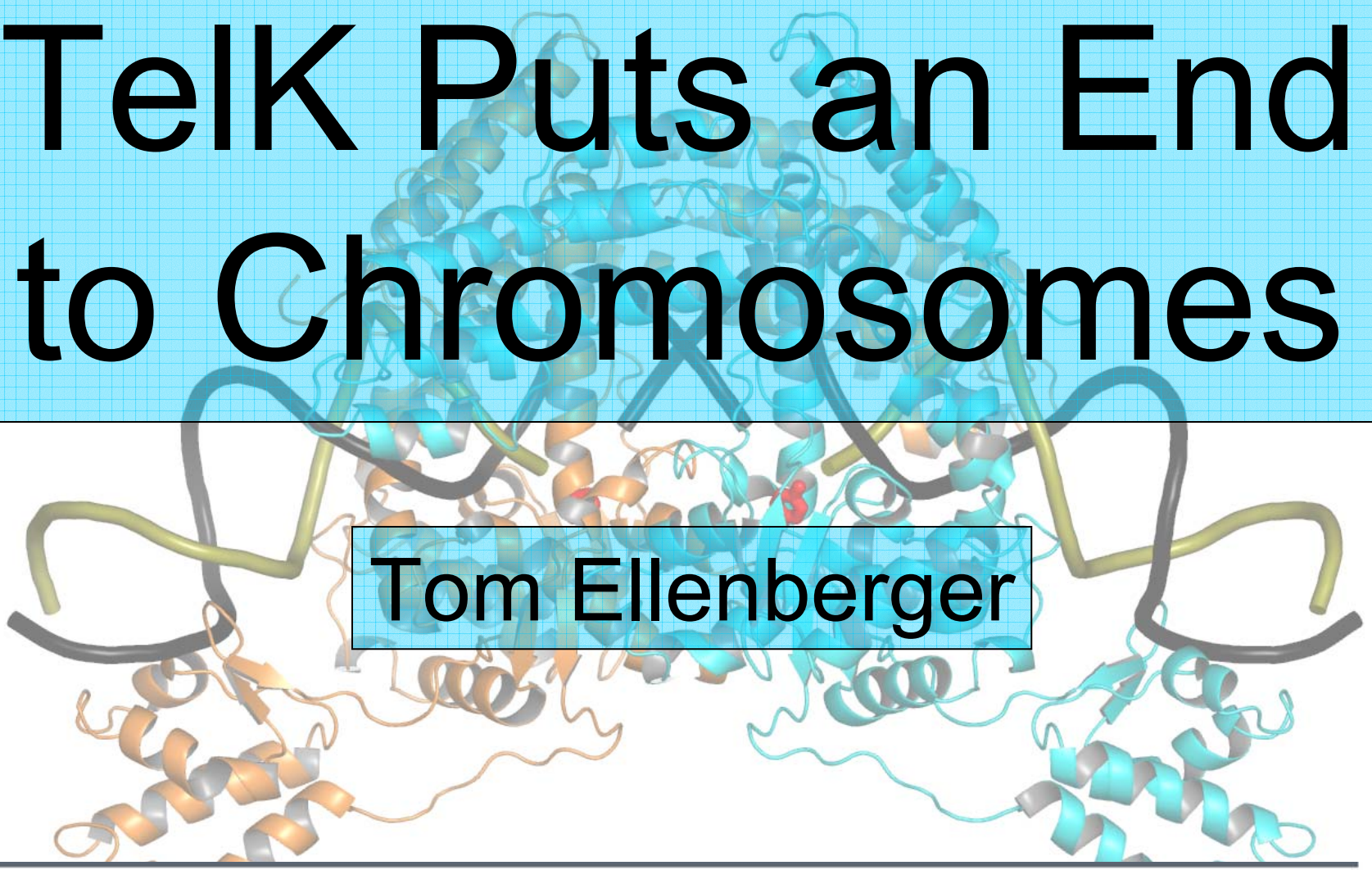
Integration



Excision

A structural basis for allosteric control of DNA recombination by lambda integrase. Biswas et al. Nature (2005) **435**, 1059-1066.

TelK Puts an End to Chromosomes



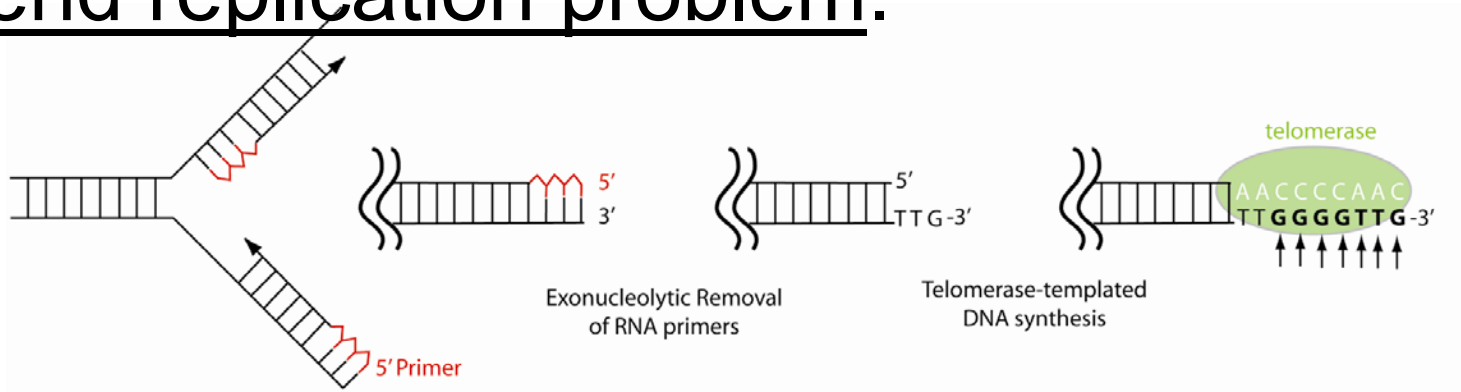
Tom Ellenberger

Department of Biochemistry and Molecular Biophysics

Washington University in St. Louis School of Medicine

Chromosome Ends

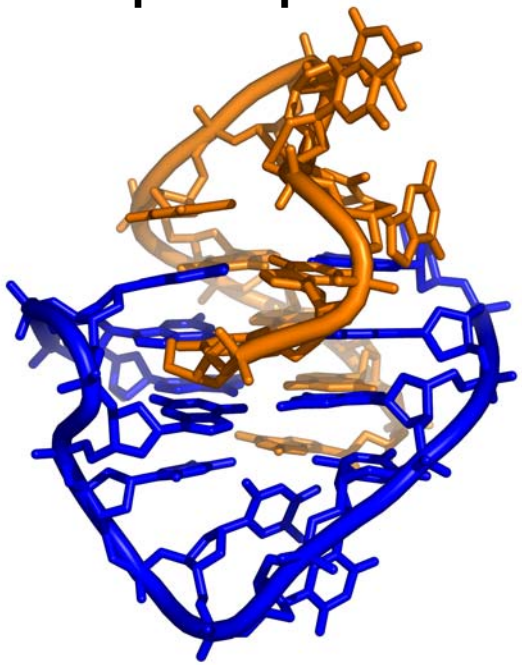
- A unique structure distinguishes telomeres from dsDNA breaks.
- Chromosome ends are nonrecombinogenic, transcriptionally silent, and bind to multiple proteins.
- Specialized enzymes process chromosome ends after replication, in order to solve the "end replication problem."



Chromosome Ends

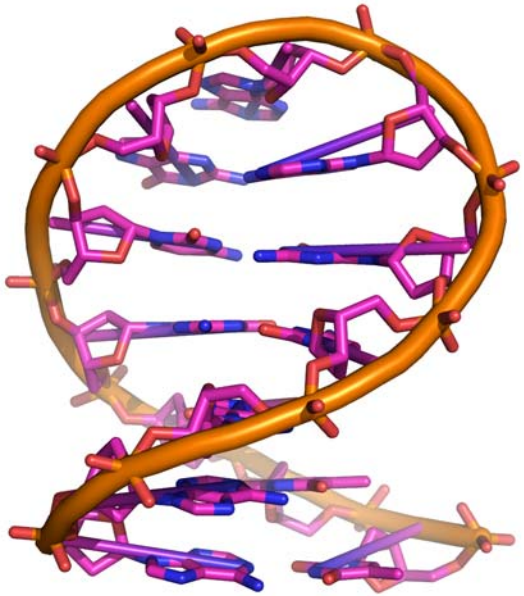
Telomeres

- G-rich repeats
- Heterogeneous in length
- Synthesized by a specialized RNA polymerase, telomerase
- Predicted quadruplex structure

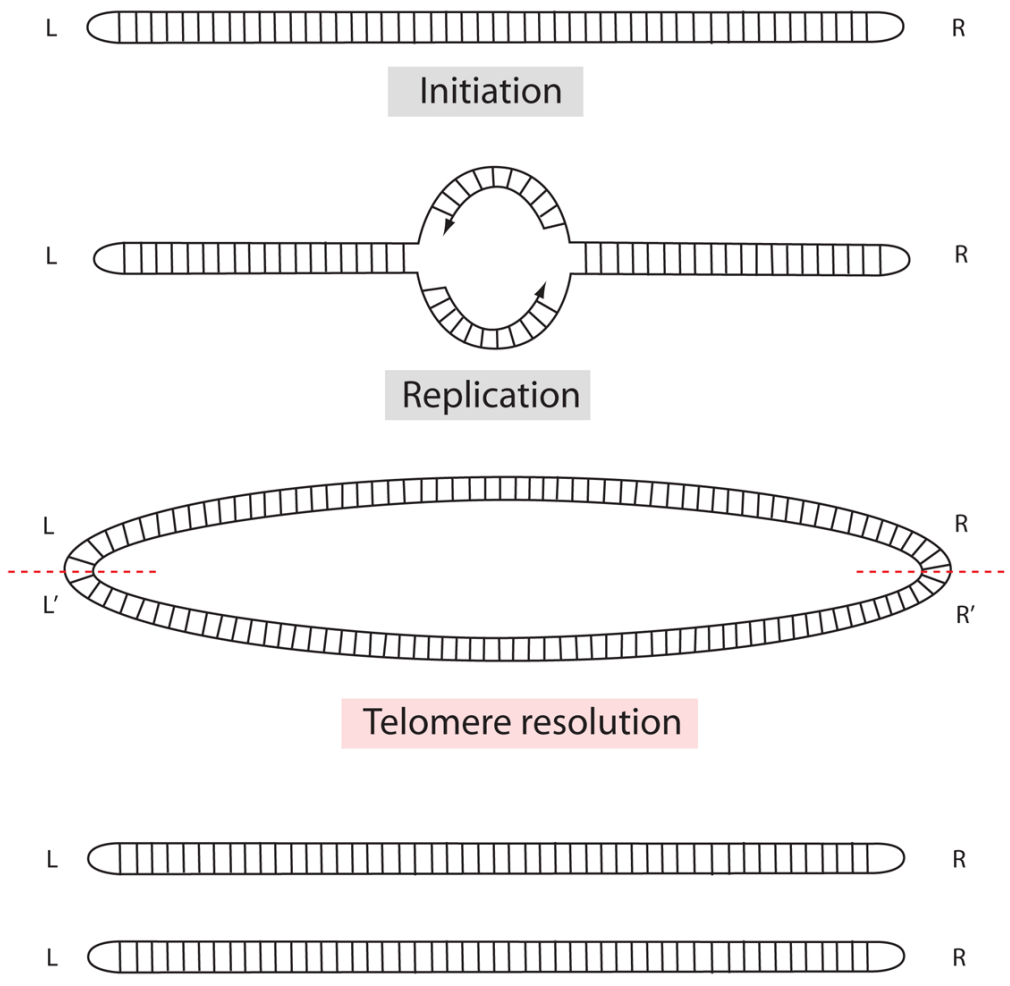


Linear Plasmid Ends

- Short palindromic sequence
- Fixed length
- Generated by endonucleolytic cleavage and ligation
- Predicted hairpin structure

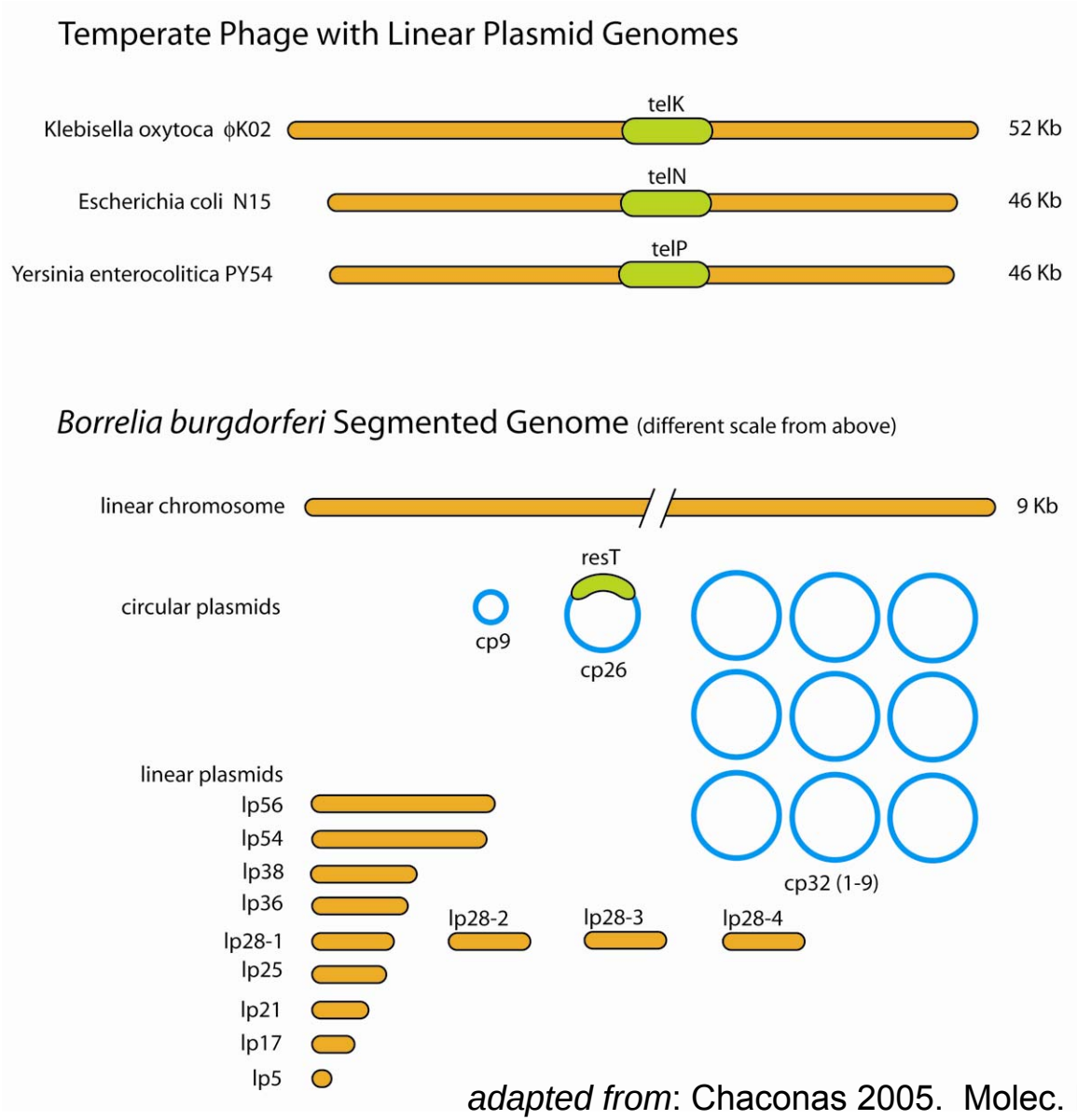


Replication of Linear Plasmids



adapted from Kobryn and Chaconas 2002. Molec. Cell 9, 195

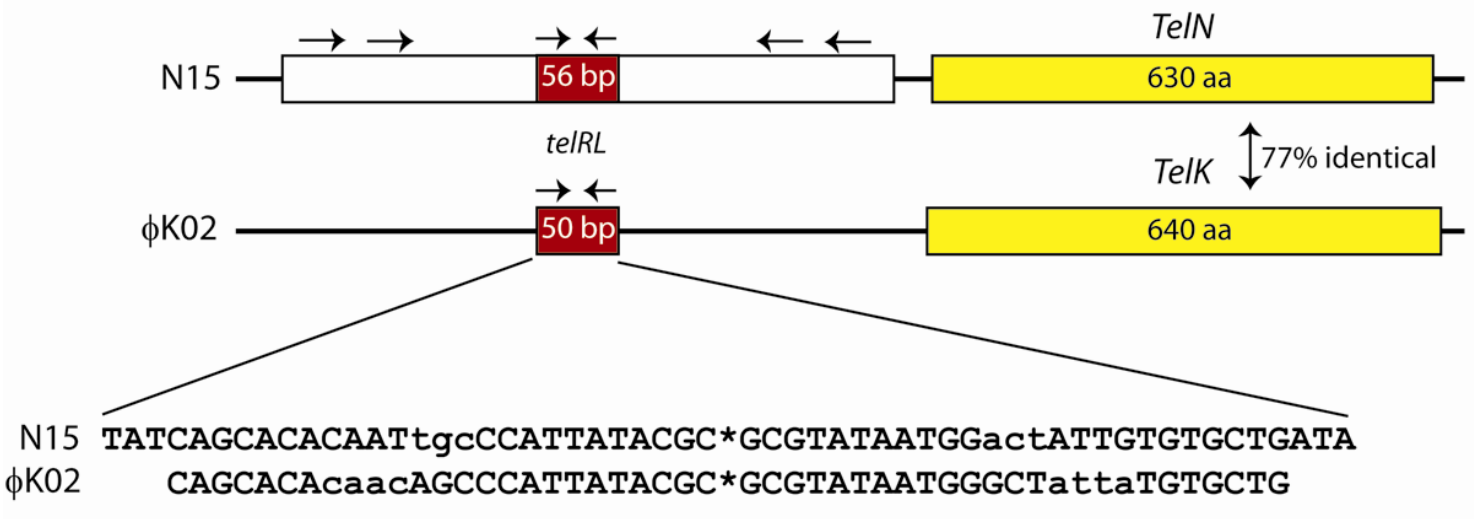
Linear Replicons With Hairpin Ends



adapted from: Chaconas 2005. Molec. Microbiology 28, 625.

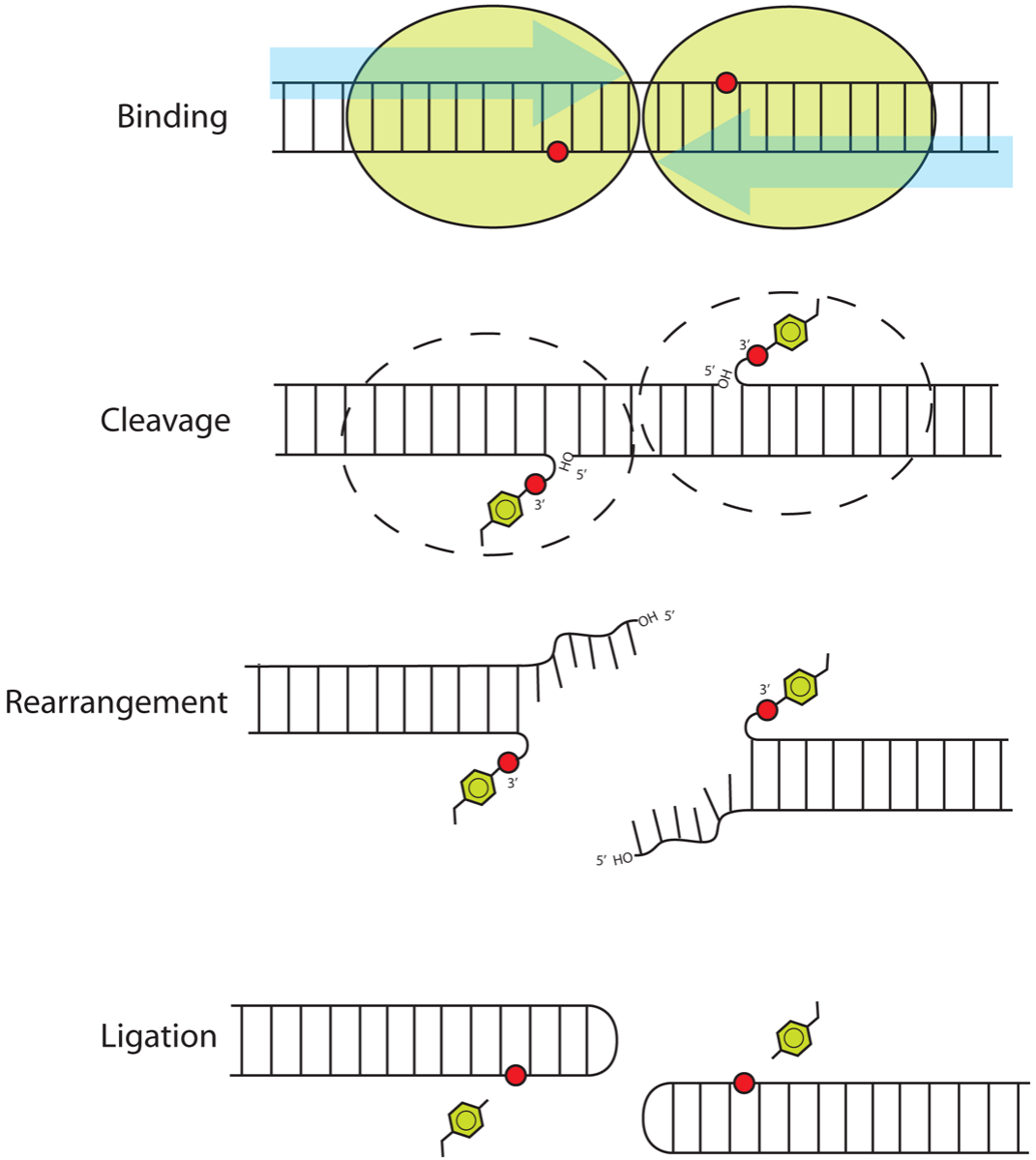
Telomere Resolvases

- TelK (*Klebsiella oxytoca* phage ϕK02)
- TelN (*E. coli* phage N15)
- TelP (*Yersinia enterocolitica* phage PY54)
- ResT (*Borrelia burgdorferi*)

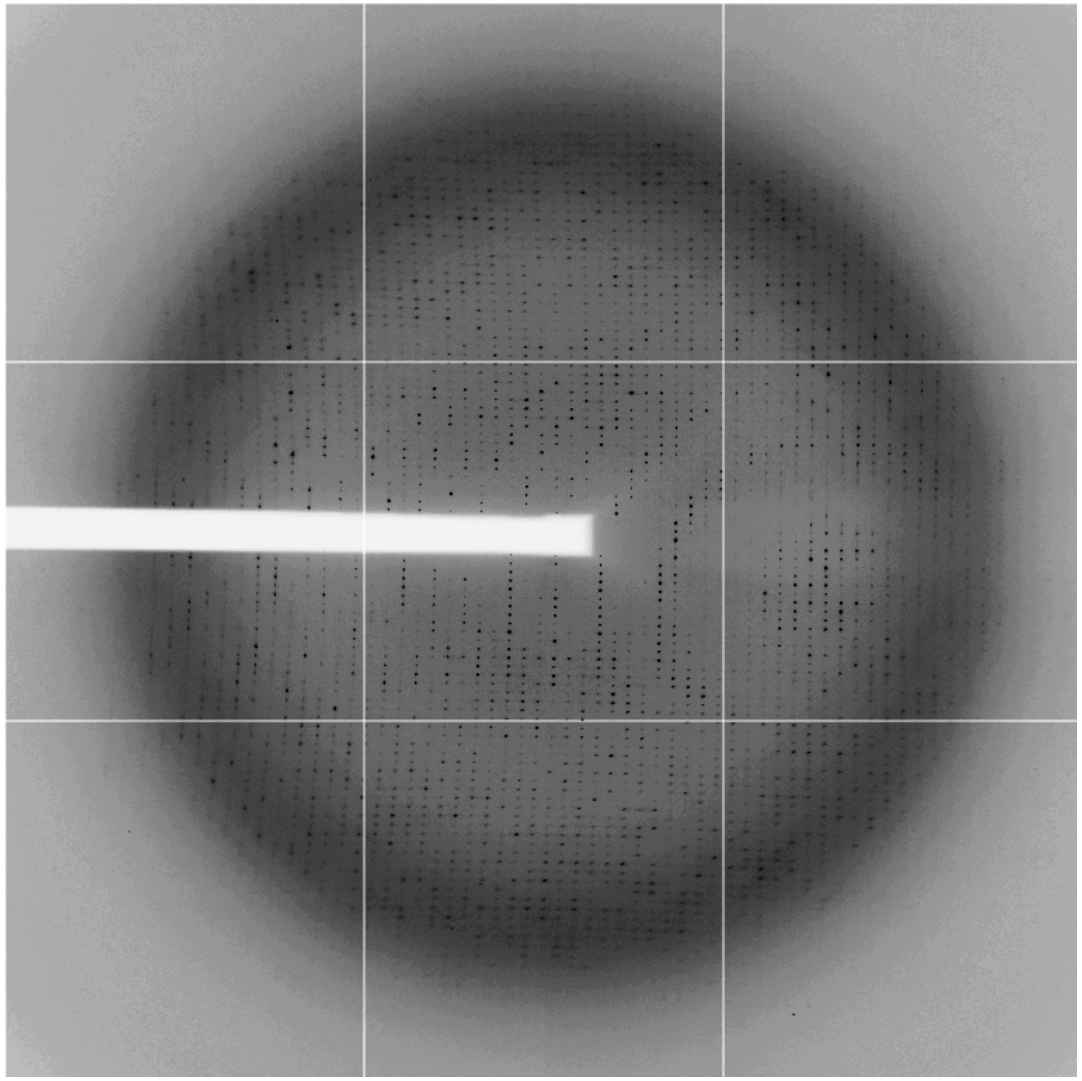


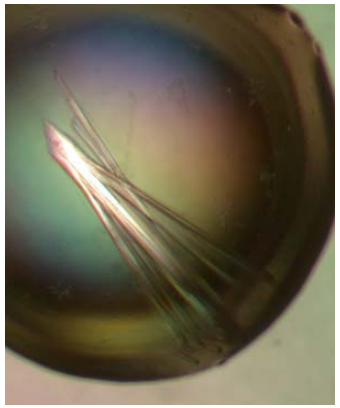
adapted from Huang et al. 2004. JMB 337, 77.

Reaction Scheme for Telomere Resolution



TelK(1-538) -DNA Complex





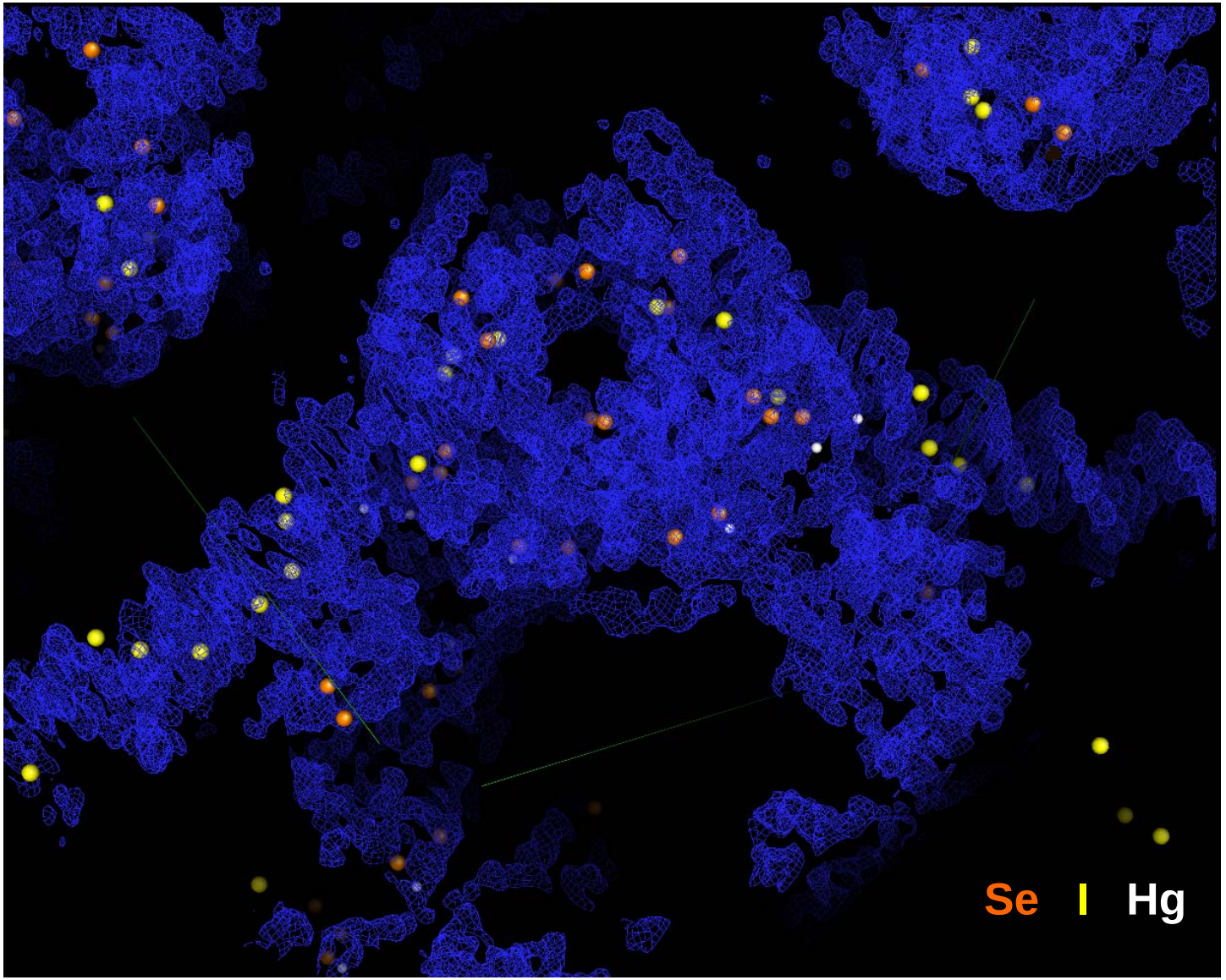
Space group $P4_1$

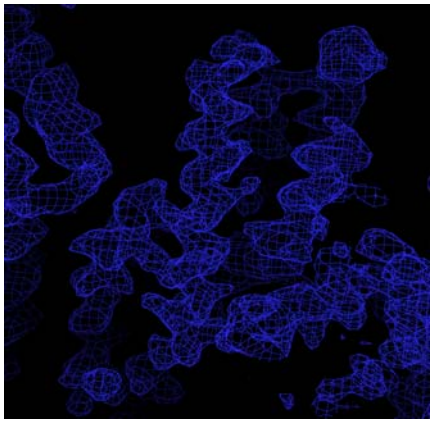
Unit cell parameters
***a* = *b* = 158Å, *c* = 91Å**

a.s.u. contains two TelK538 Molecules bound to a 44bp "replicated telomere."

Diffraction image from native crystal of TelK with nicked "suicide DNA"

Crystallographic Studies of TelK-DNA Complexes





Protein helices

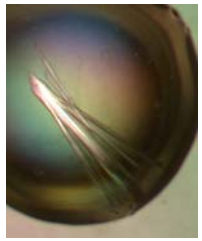
Phases obtained using selenium, iodine, and mercury derivatives.

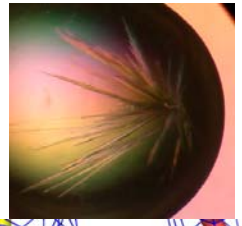
Structure Determination and Model Refinement

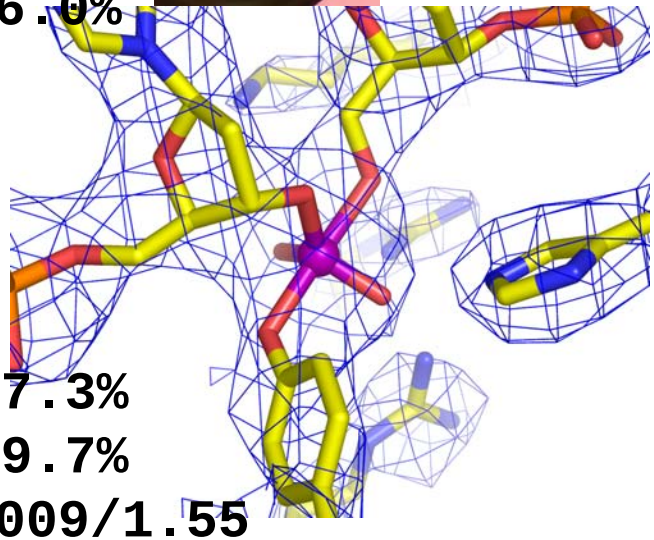
Space group $P4_1$

Unit cell a = b = 158Å, c = 91Å

	Nicked-native	Vanadate-complex
Resolution	50-3.0Å	50-3.0Å
Rmerge	12.1%	14.2%
I/s	11.0	7.9
Redundancy	5.1	2.9
Completeness	91.0%	96.0%





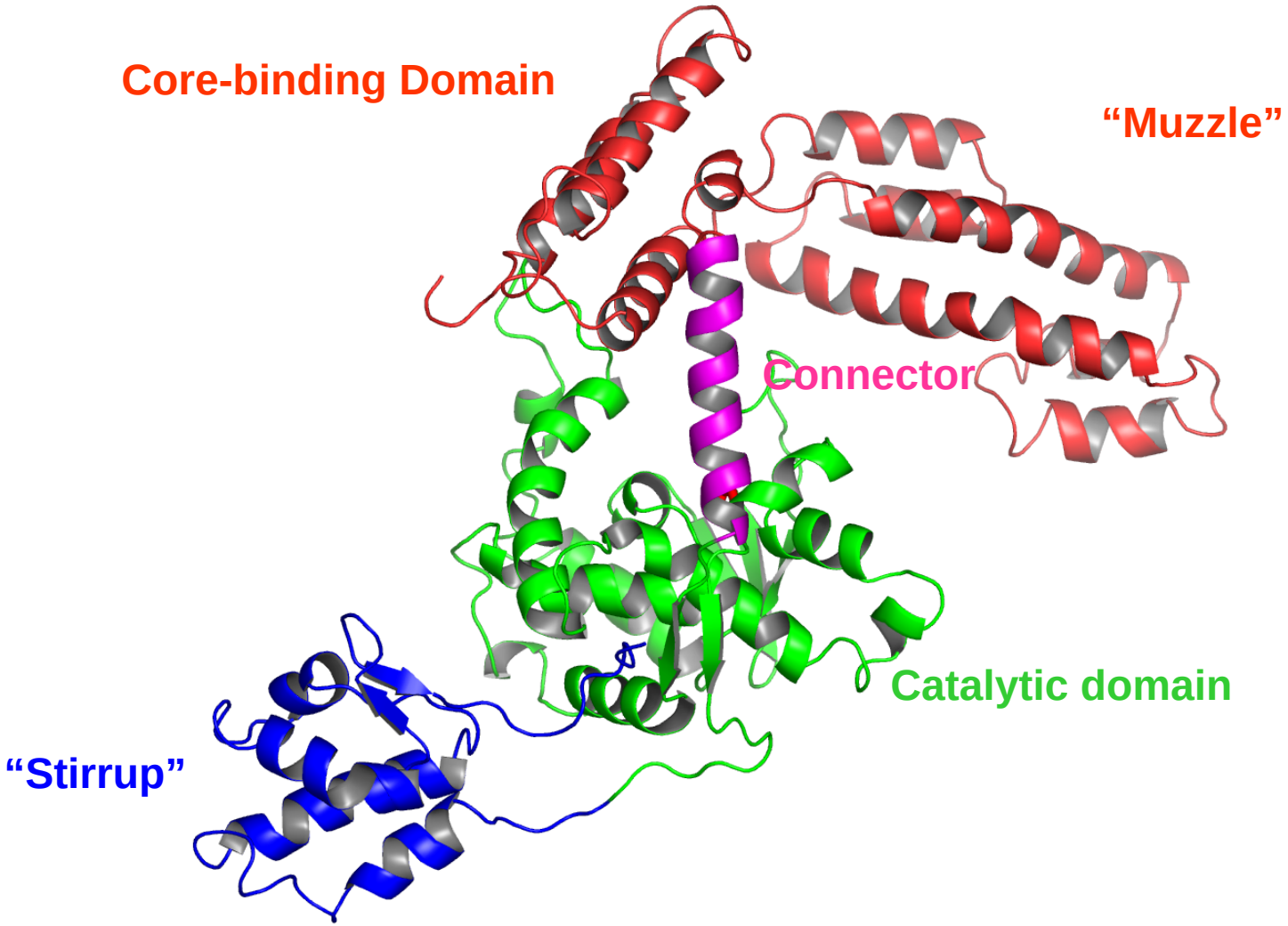


FOM – SHARP 0.35

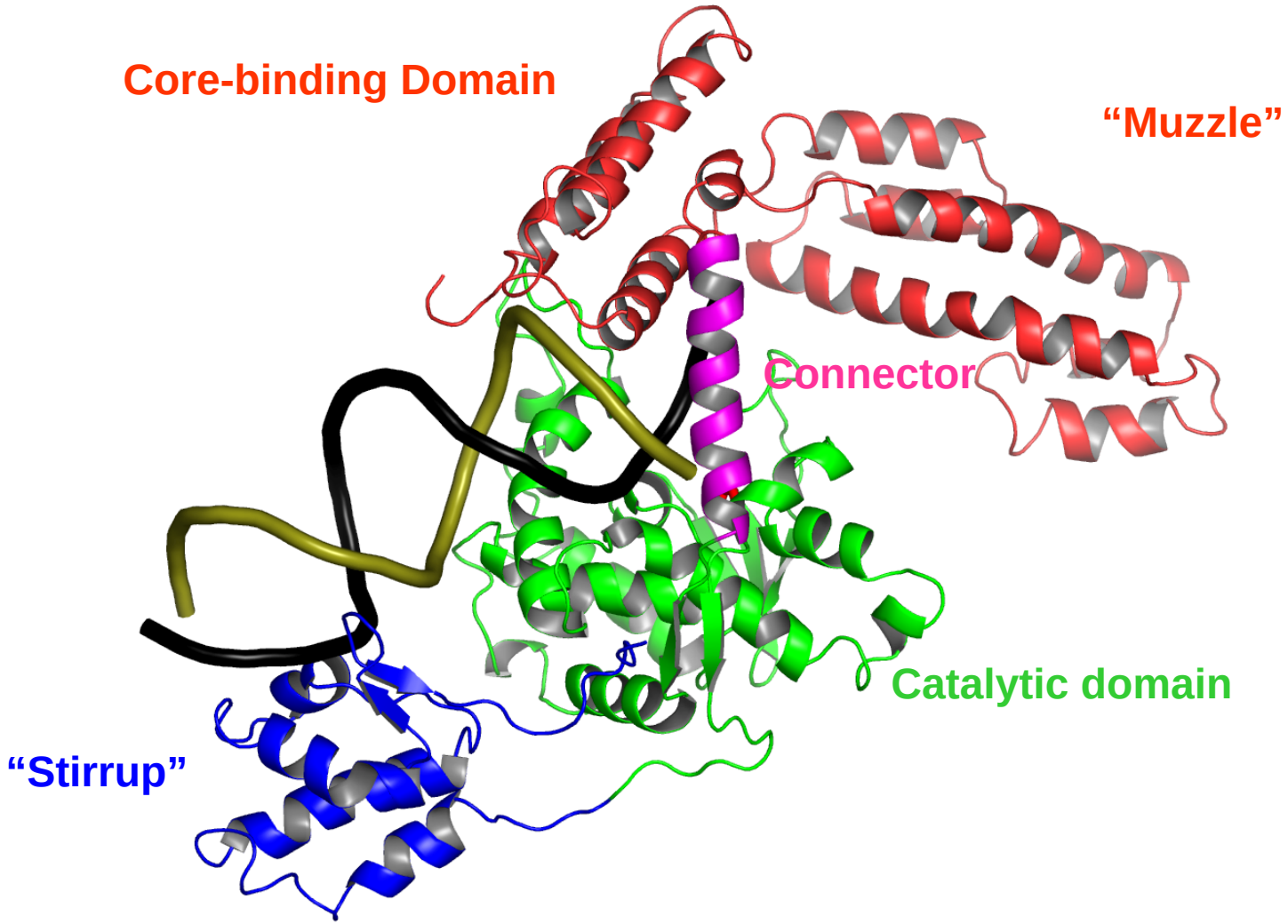
Selenium(24),Iodine(13),Hg(6)

Refinement		
Rwork	32.7%	27.3%
Rfree	33.6%	29.7%
Rmsd (bond/angle)	0.01/1.60	0.009/1.55

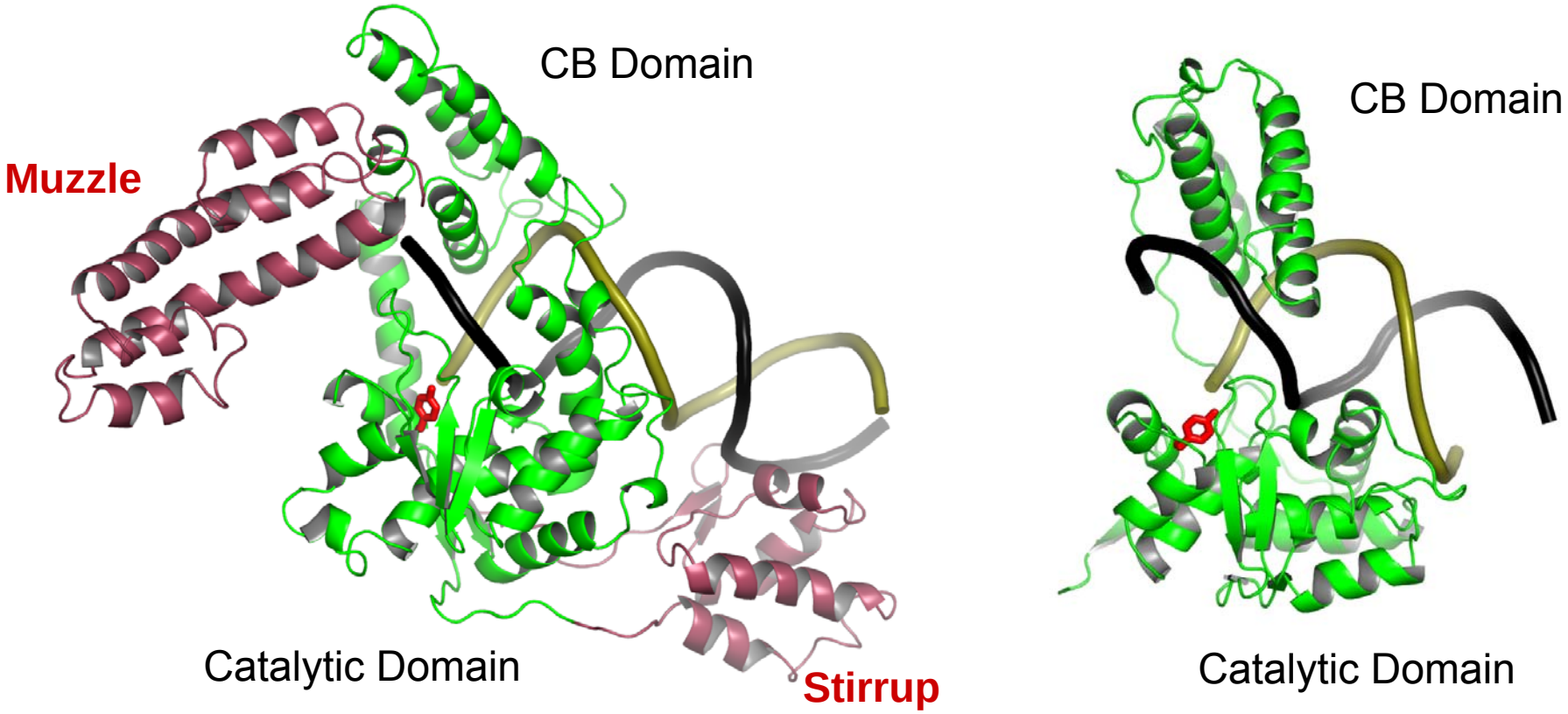
TelK Monomer



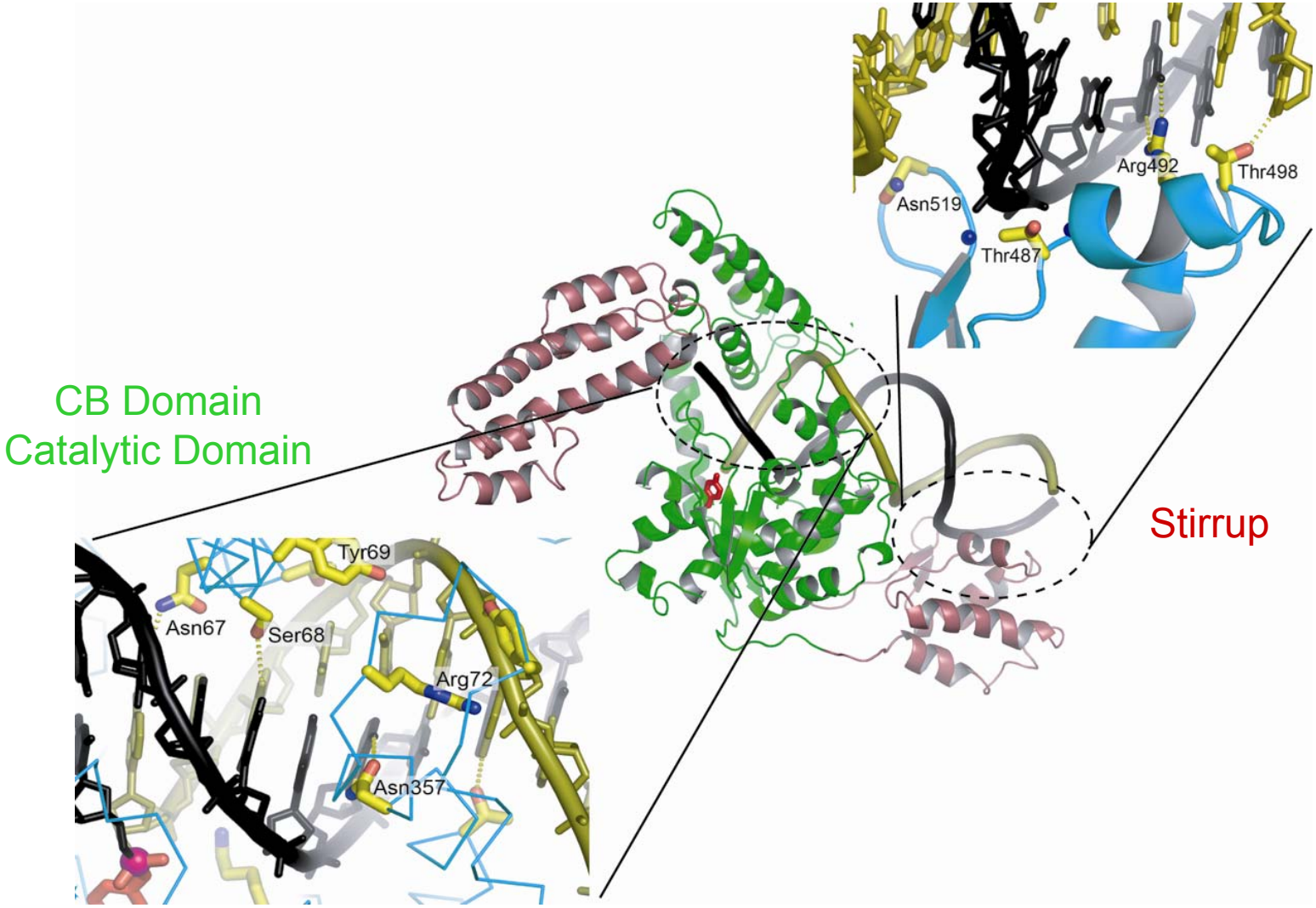
TelK Monomer



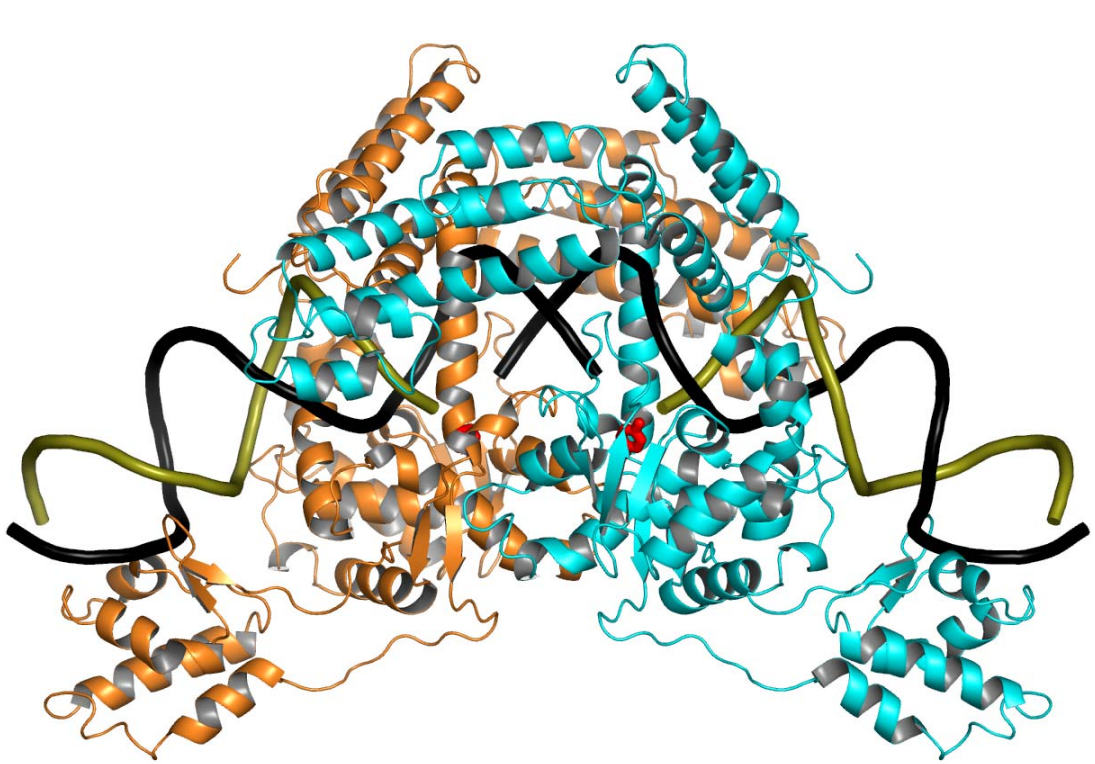
Adaptive Structural Elements

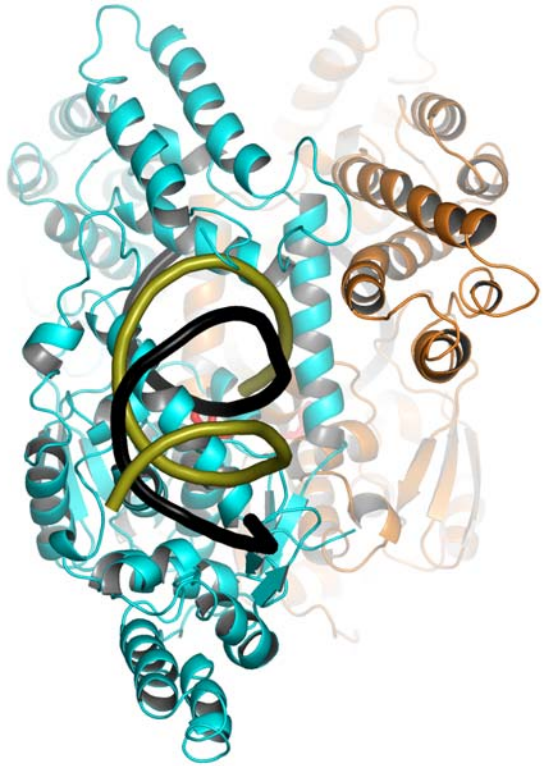


Sequence-specific DNA Contacts

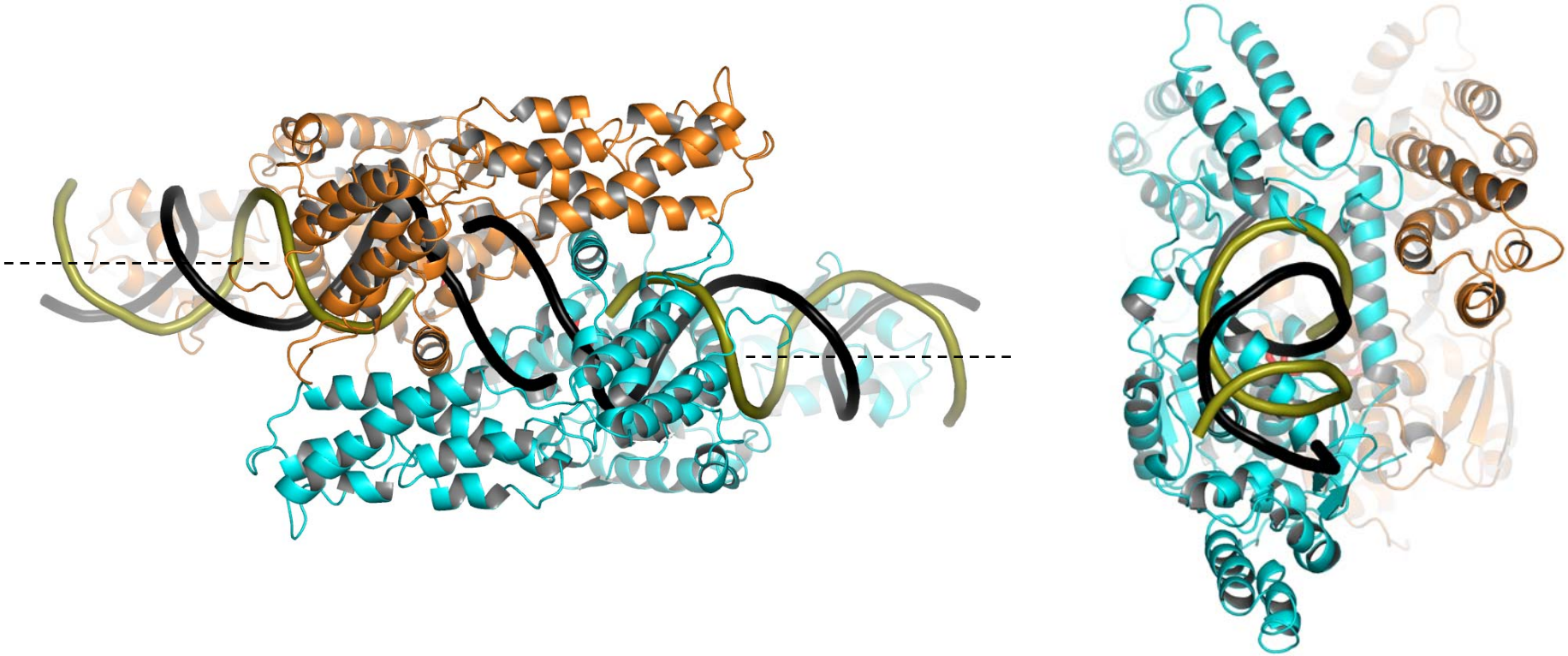


TelK Dimer Functions as a *"DNA Crimping Tool"*

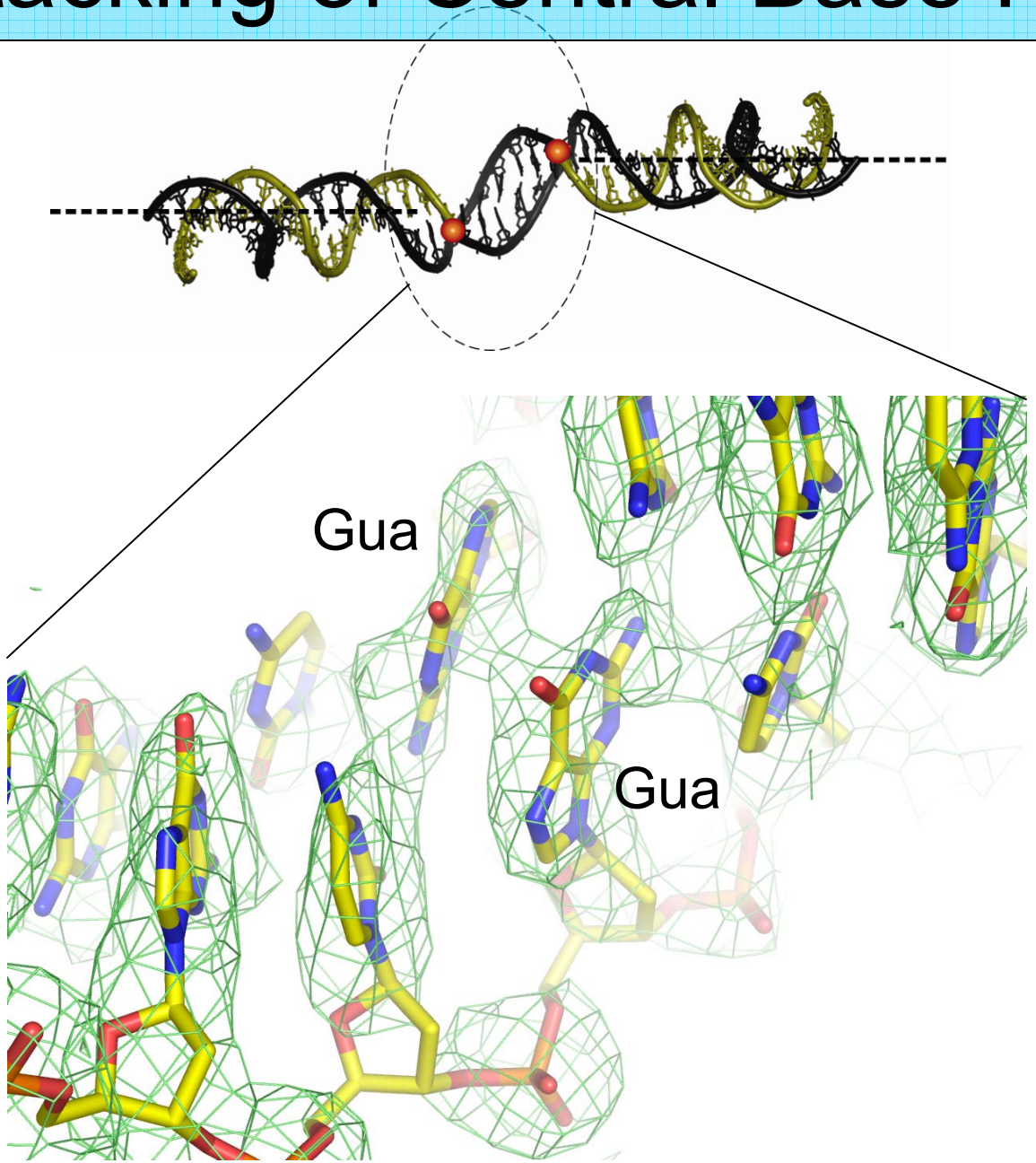




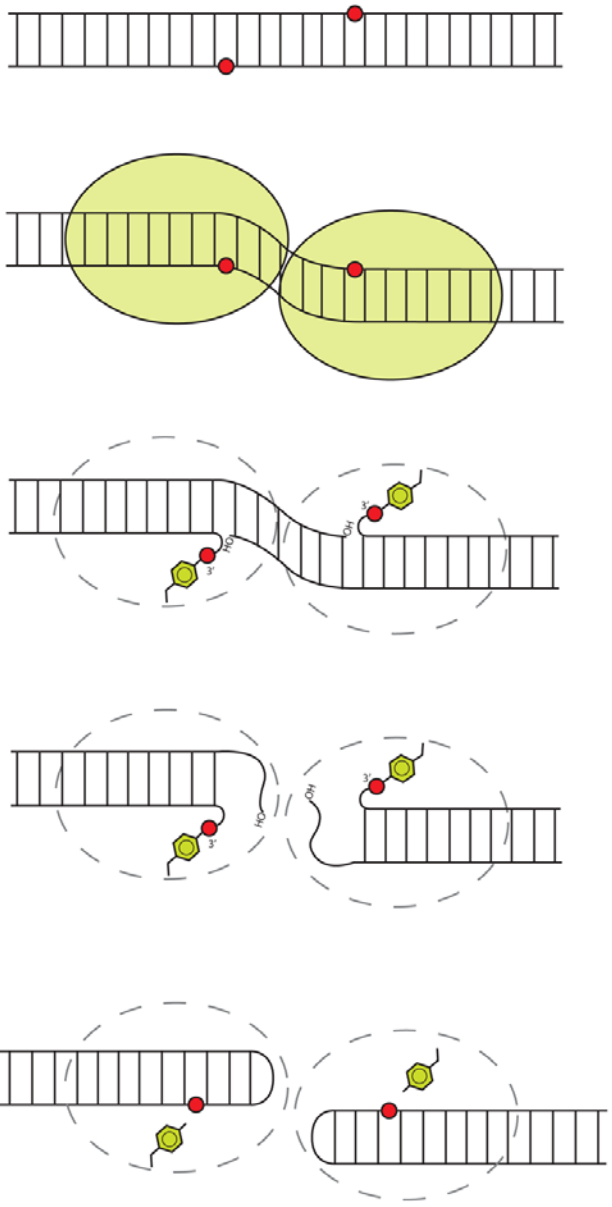
TelK Dimer Functions as a *"DNA Crimping Tool"*

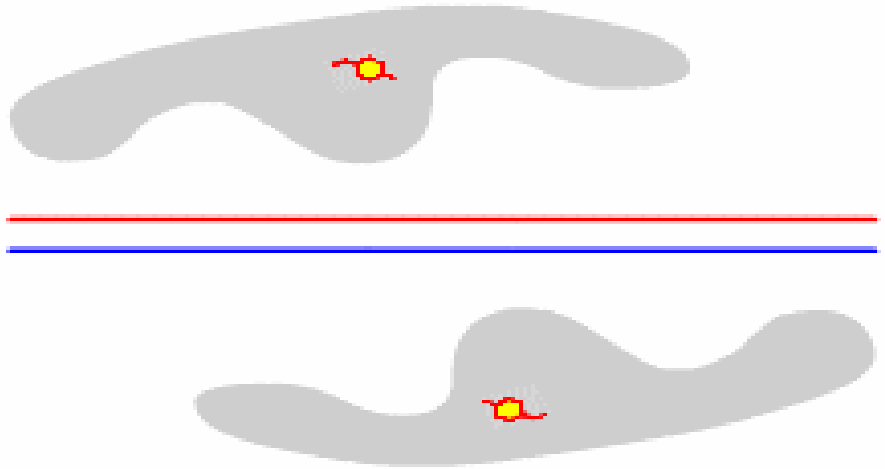


Unstacking of Central Base Pairs



Working Model for Telomere Resolution





What Have We Learned?

- TelK forms a compact dimer stabilizing a highly distorted DNA conformation.
- DNA binding surface is extended by the C-terminal “stirrup” of TelK.
- Structure is inconsistent with proposal of “pre-hairpinning” prior to DNA cleavage.

Predictions

- DNA binding energy is coupled with distortion of the DNA substrate.
- “Crimping” of the DNA serves to drive the reaction forward.
- Following DNA cleavage, hairpin formation is spontaneous, i.e., the enzyme does not chaperone the 5’-OH end of the cleaved DNA.

The Credits

Hideki Aihara (λ int, TelK)

Tapan Biswas (λ int)

Collaborators:

Art Landy (Brown U.)- λ recombination

Wai Mun Huang (U. of Utah)- telomere resolution

Funding:

NIGMS, Human Frontier Science Program