

# Directed evolution of Bxb1 for the development of Modular Integrases (MINTs)

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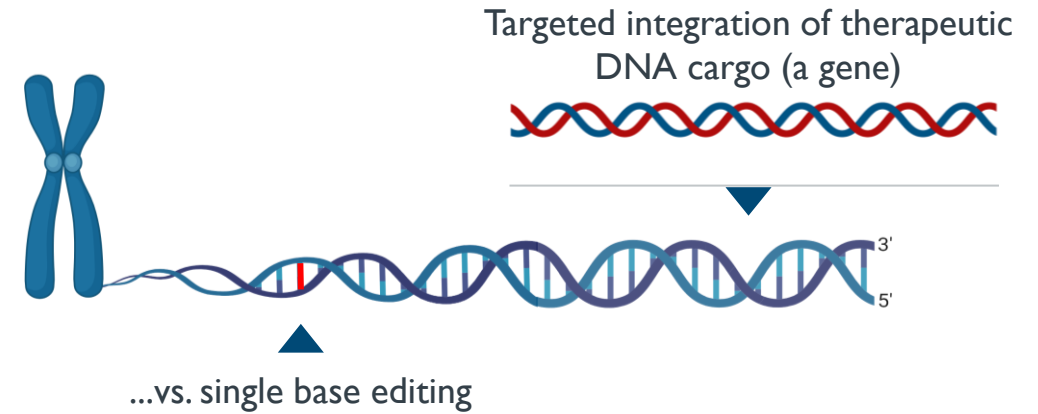
ASGCT, May 7-10, 2024

I am a full-time employee of Sangamo Therapeutics

# Integrases will usher in a new era of genomic medicine

## Integrases meet requirements for ideal therapeutic agents

- ✓ Capable of delivering large payloads - 10 kb+
- ✓ No copying required - low error rate
- ✓ Self sufficient - no dependence on cell DNA repair machinery
- ✓ No DNA breaks - reduced translocation risk



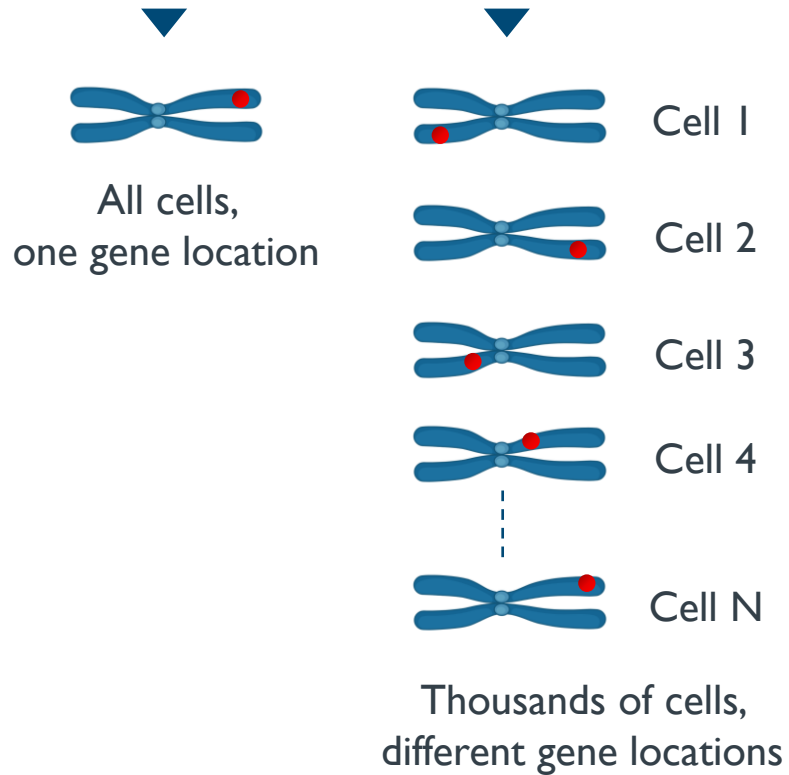
*“if it was possible to integrate very large DNA sequences where you could integrate entire genes or sets of genes into a targeted position in the genome would be very powerful” –Jennifer Doudna*

*“a hypothetical fully programmable recombinase would be in some respects an ultimate genome editing agent” –David Liu*

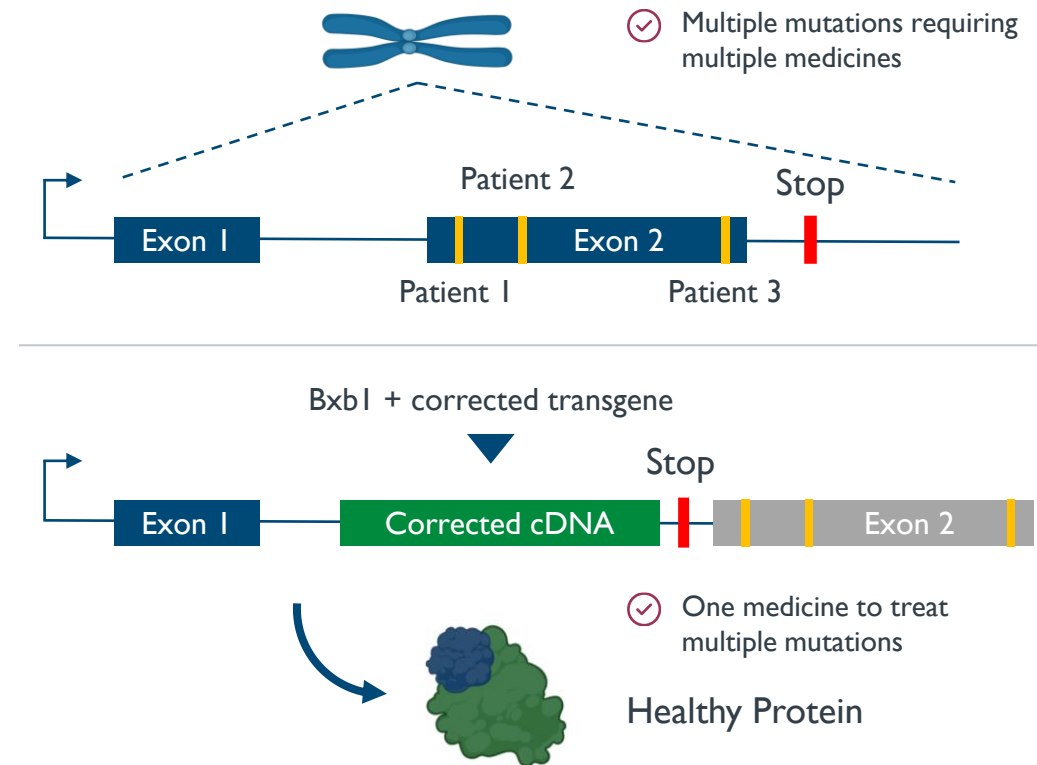
CRISPR roundtable

# Targeted integration improves existing therapies, and enables new therapies

## Targeted vs. Random Integration

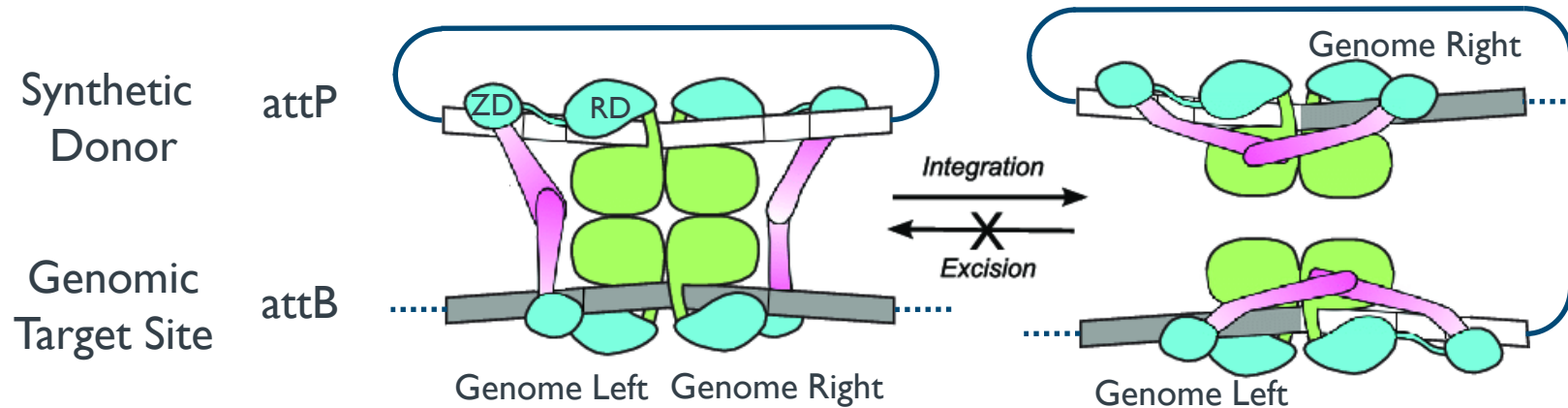


## One medicine vs. multiple variants for each mutation



Images by Biorender

# Bxb1 is a serine integrase that displays an ideal mechanism for therapeutic targeted integration



Adapted from Gupta et al., NAR (2017)

- **Break-free:** Enzyme protects genome from double stranded breaks and ligates after recombination
- **Irreversible integration:** Integrase coiled-coil domains ensure integration is unidirectional
- **Large cargo delivery:** Native enzyme integrates 50kb cargo into host genome

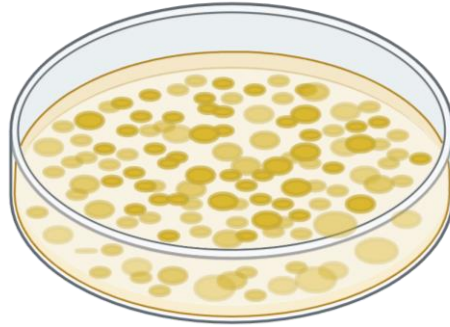
# Overview of the integrase reprogramming process

## Model



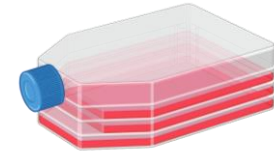
- 3D Structural model
- Mapping DNA-protein interactions

## Directed evolution

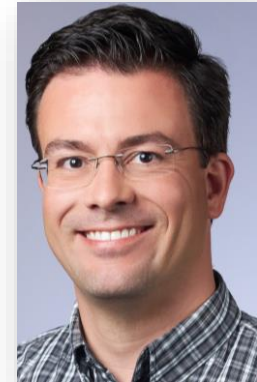


- Bacterial selection
- Up to 1 billion mutants

## Test and validate

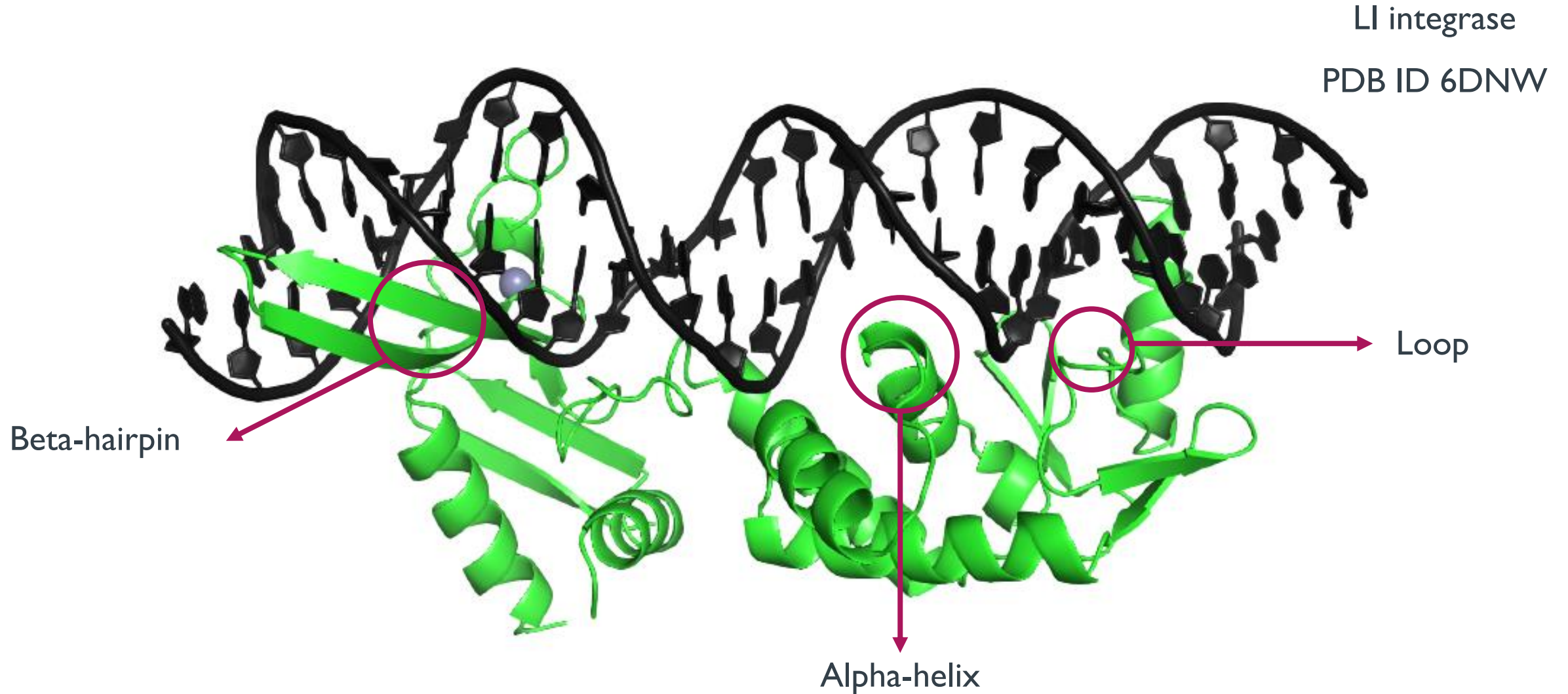


- Activity in human cells

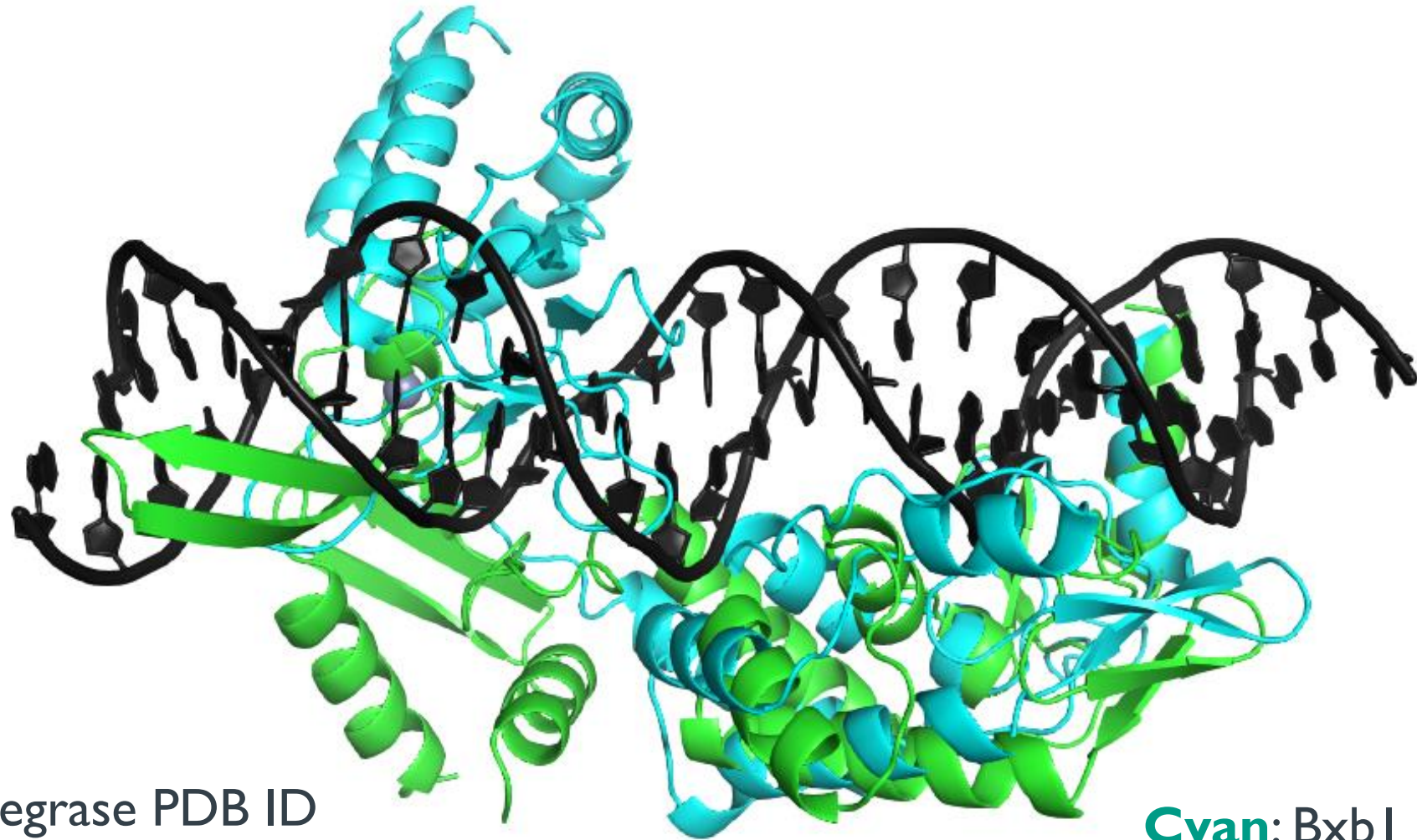


**Friedrich Fauser**  
Poster I 680 05/10/24

The only known structure of a serine integrase bound to DNA is from the *Listeria innocua* prophage



— Alignment of a Rosettafold model of Bxb1 to LI integrase is inadequate

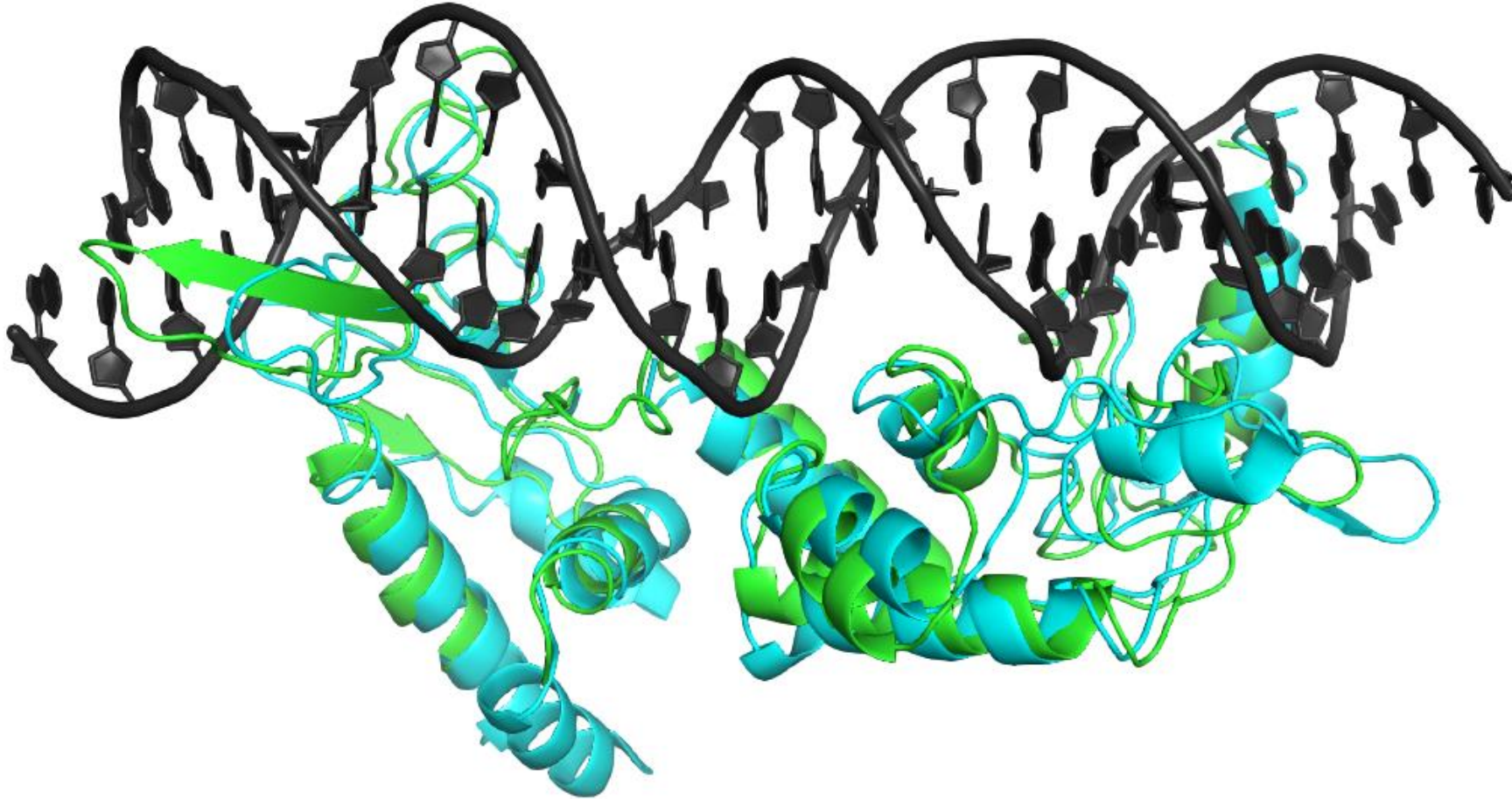


**Green:** LI integrase PDB ID  
6DNW

**Cyan:** Bxb I model



— Separating Bxb1 domains yields a useful model for experimental testing



**Green:** LI integrase PDB ID  
6DNW

**Cyan:** Bxb I model

# Experimentally mapped Bxb1 protein-DNA target interactions

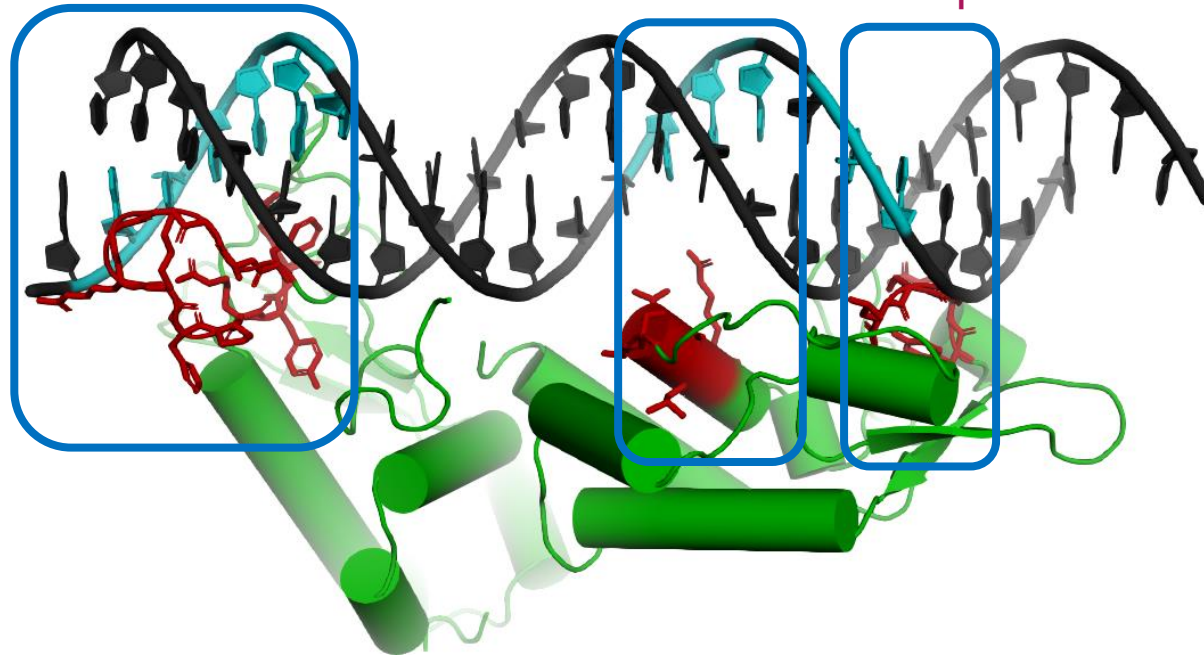
Bxb1 target DNA  
(attP left half-site)

5' - G **GCTTGT** TGTTCT **GAC** **GAC** GGCG - 3'

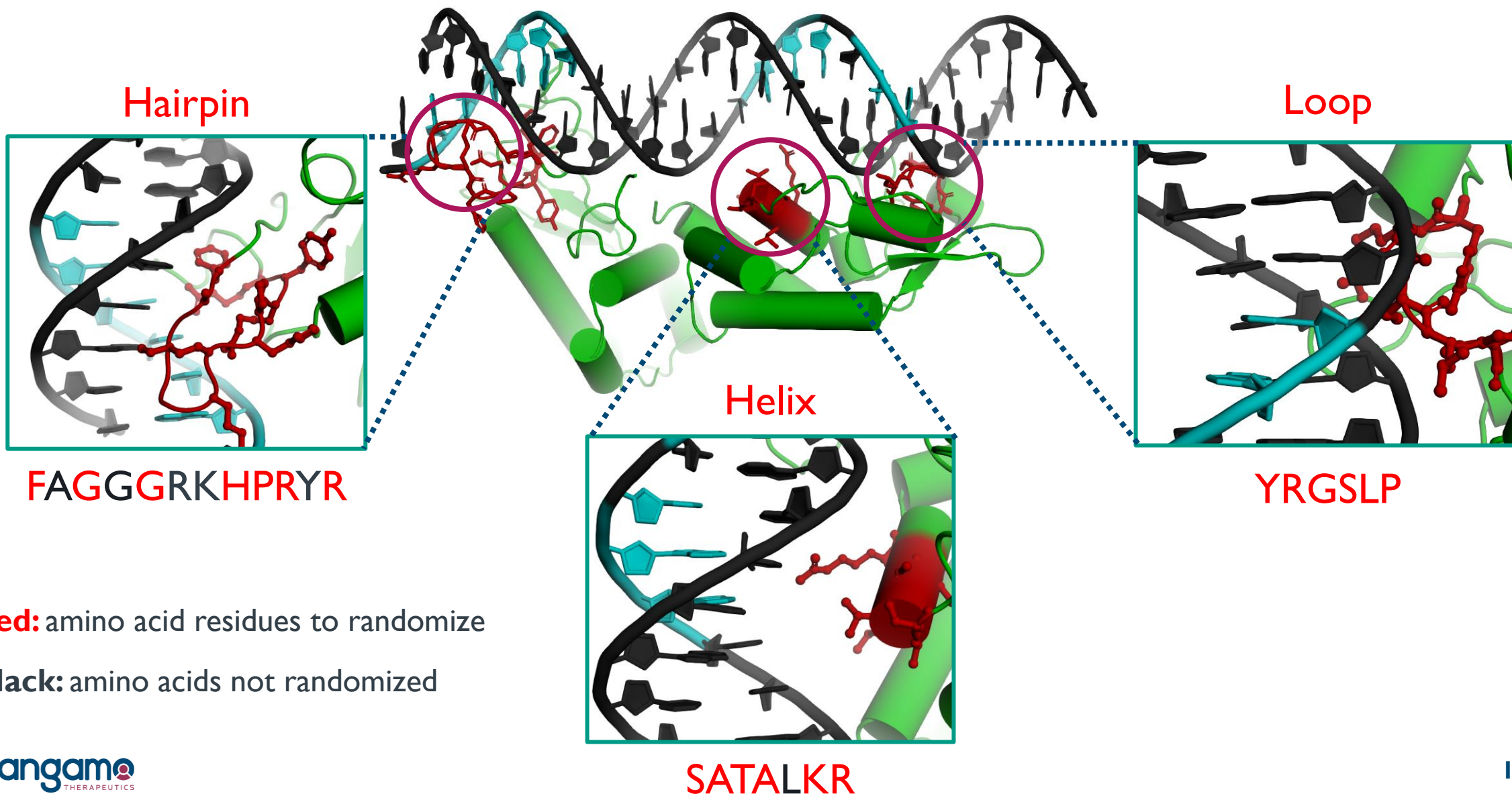
Hairpin

Helix

Loop



Experimental model points to distinct residues that can be randomized in a selection experiment



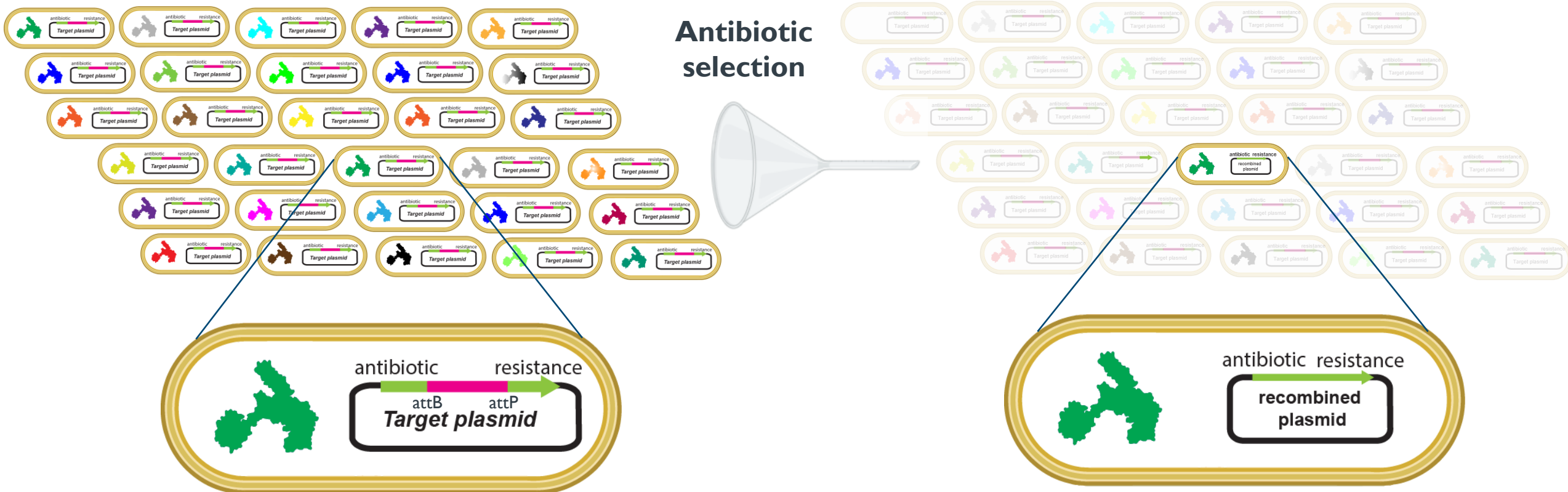
**Red:** amino acid residues to randomize

**Black:** amino acids not randomized

# Bacteria are the ideal platform for integrase directed evolution

- Single BxbI variants and a desired target sequence in each bacterial cell
- Library of one billion individual integrase variants was tested
- Only a small subset of variants survive antibiotic challenge

Inspired by Gersbach et al. NAR (2010)



# Bacterial selections can reprogram the Bxb1 helix

~1 billion variants

P T D V L P K  
 A W R I L S K  
 Y G H K L A I  
 Y H L R L P S  
 W T A W L G S  
 R R T M L S P  
 V M L L L V W  
 V V V Q L A R  
 I P L S L L T  
 I W P P L M G  
 L L N V L I P  
 Y M N C L S W  
 P H R V L A A  
 E T C S L L H  
 P Y V A L K W  
 T Q R P L S L  
 P A G P L K P  
 R R T G L K A  
 T K P G L A R  
 L R A H L C L  
 V W E A L P G  
 A T A S L T H  
 S H V K L G R  
 R K Q L L G P  
 A M I C L A P  
 L L V R L E E  
 I S W G L N K  
 A G T V L R T  
 N A L K L G L  
 R F S L L F R  
 M L W C L M A  
 D T R D L S Q  
 E E R Q L G L  
 D D L R L R R  
 T P G T L L P

Chaos

Bxb1 natural half-site

GGCTTGTGACGGCG  
 ATGCTCCTGTCGGCG

Human DNA target half-site, identified computationally using Bxb1 in vitro specificity data from Bessen et al. Nat. Comm. (2019)

Antibiotic selection

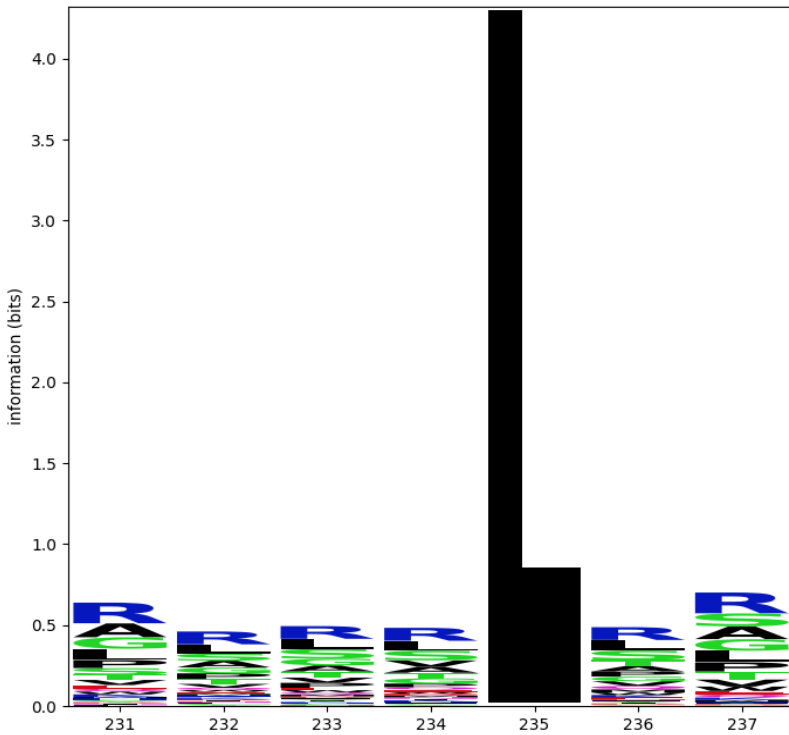


~100 candidate integrases

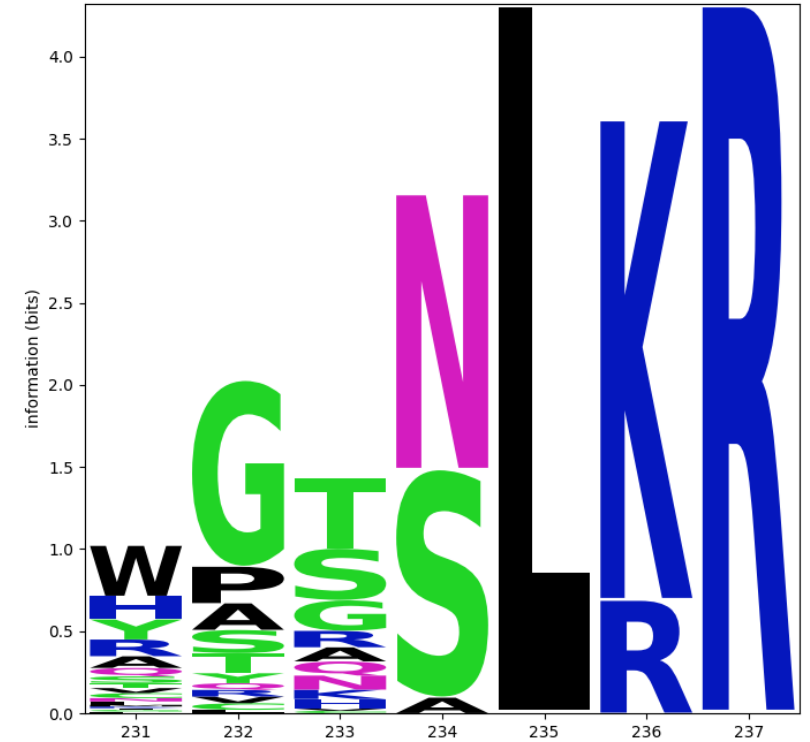
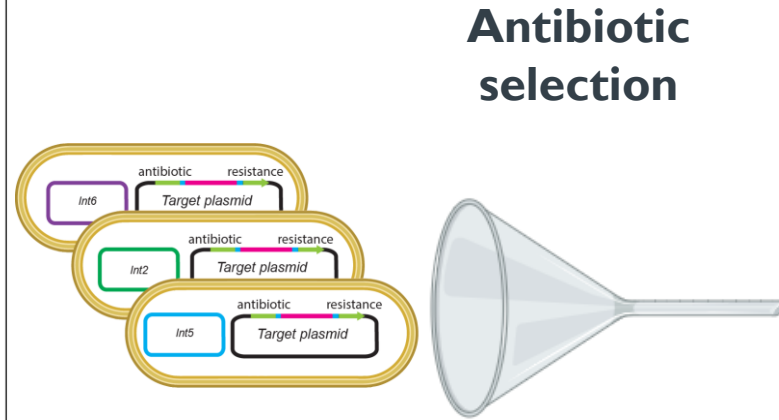
R P G N L K R  
 R G T S L K R  
 H A G S L K R  
 A P Q N L K R  
 Y P T S L R R  
 H T T S L K R  
 R S G N L R R  
 Y A T S L K R  
 R A G N L R R  
 T G G N L R R  
 R G N N L R R  
 W A S S L R R  
 A G N N L R R  
 R S N N L K R  
 H A T S L K R  
 H A Q N L R R  
 W A T S L R R  
 R G G N L K R  
 W S Q N L K R  
 R G H N L K R  
 Y G S N L K R  
 R G G N L R R  
 R G N S L K R  
 Y G N N L K R  
 W S G S L R R  
 W P G S L K R  
 W A S T L K R  
 H Q G S L K R  
 R S S S L K R  
 Y A S T L K R  
 H A R N L K R  
 W P S T L K R  
 F S T S L K R  
 T S G N L K R

Order

# Bacterial selections can reprogram the Bxb1 helix

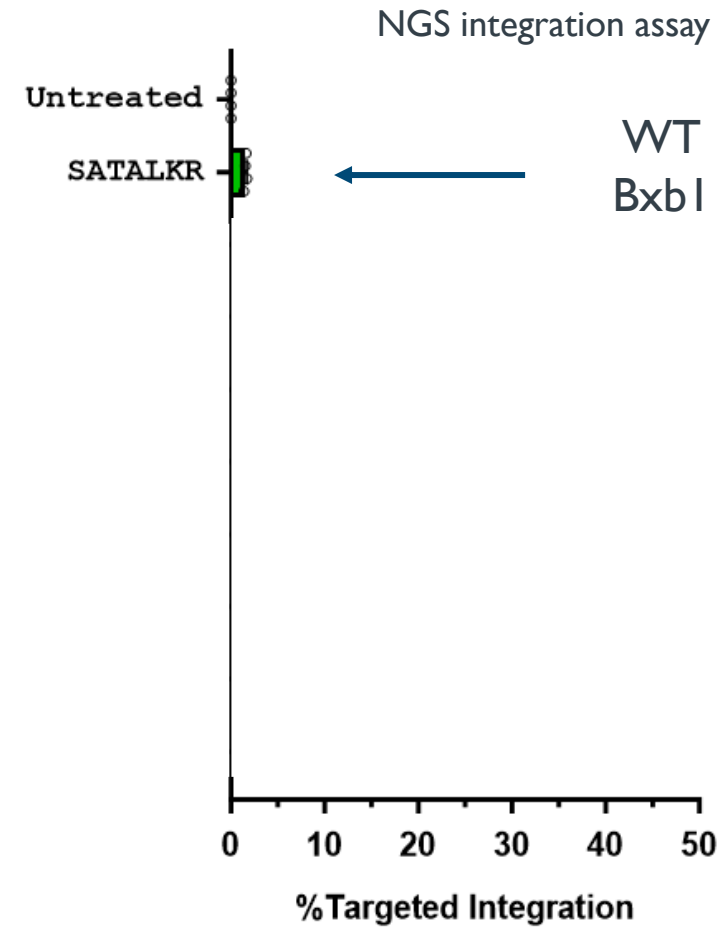
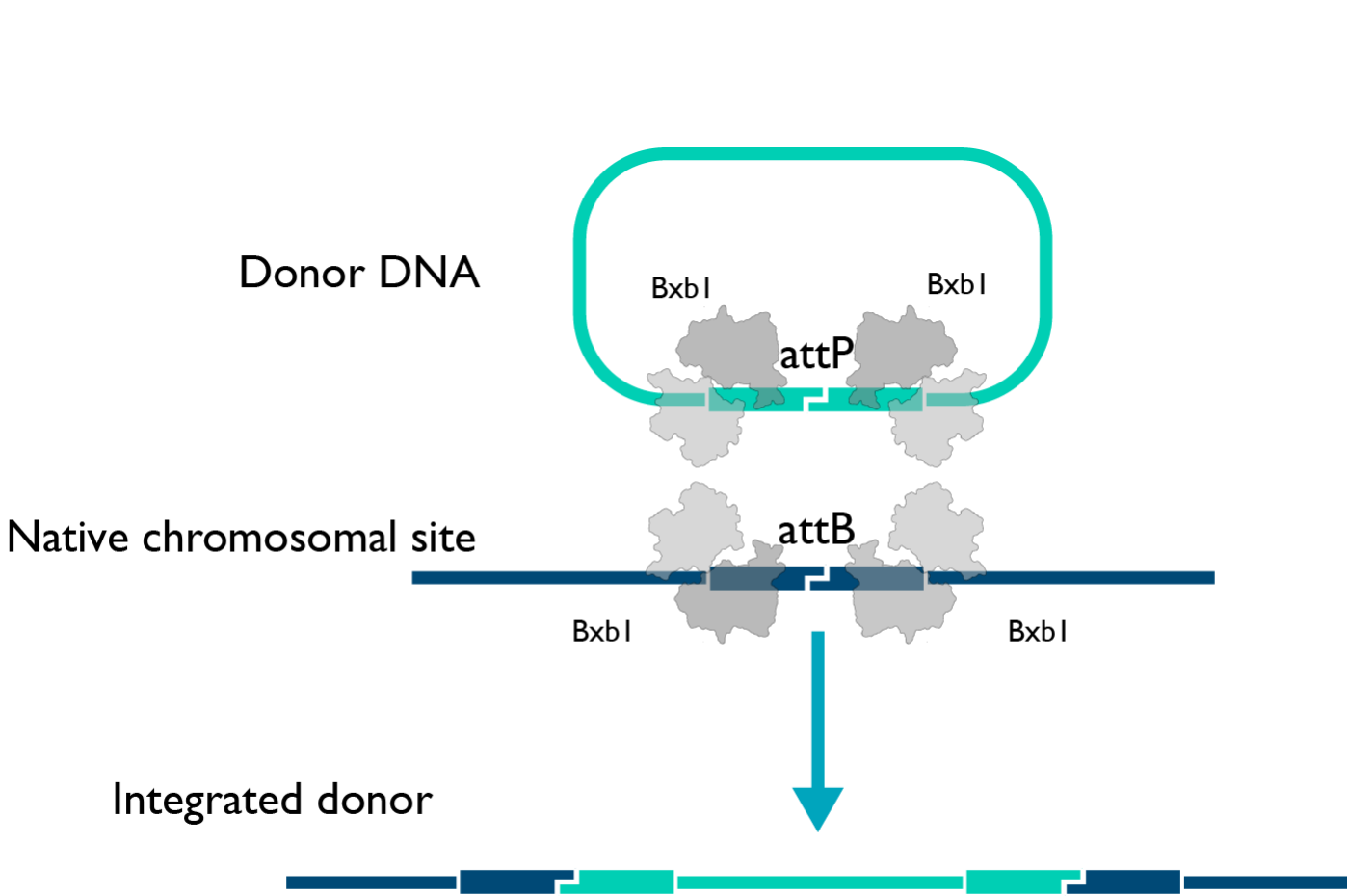


Chaos



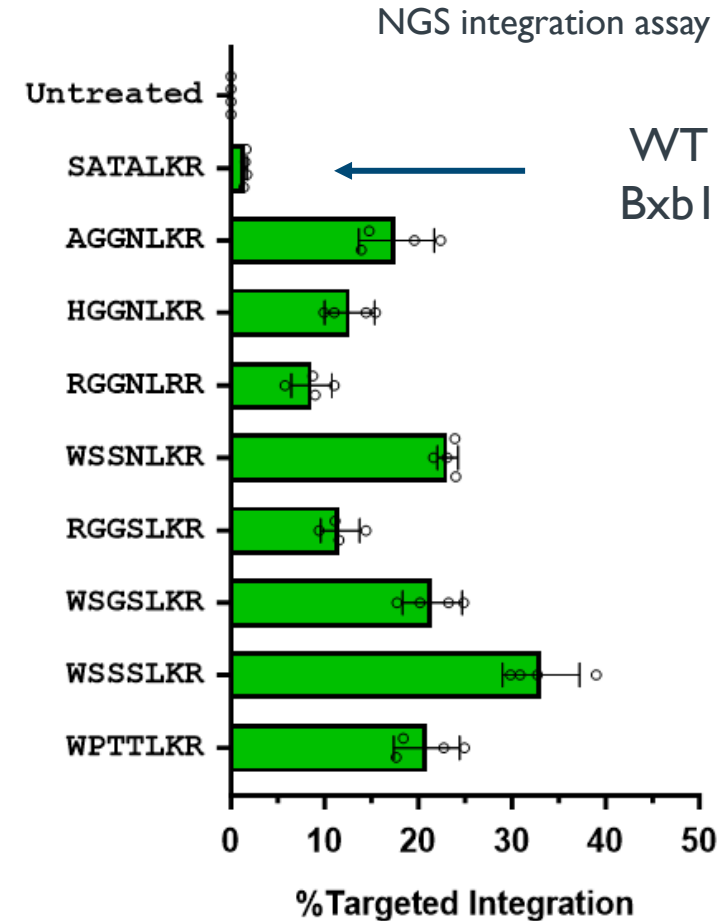
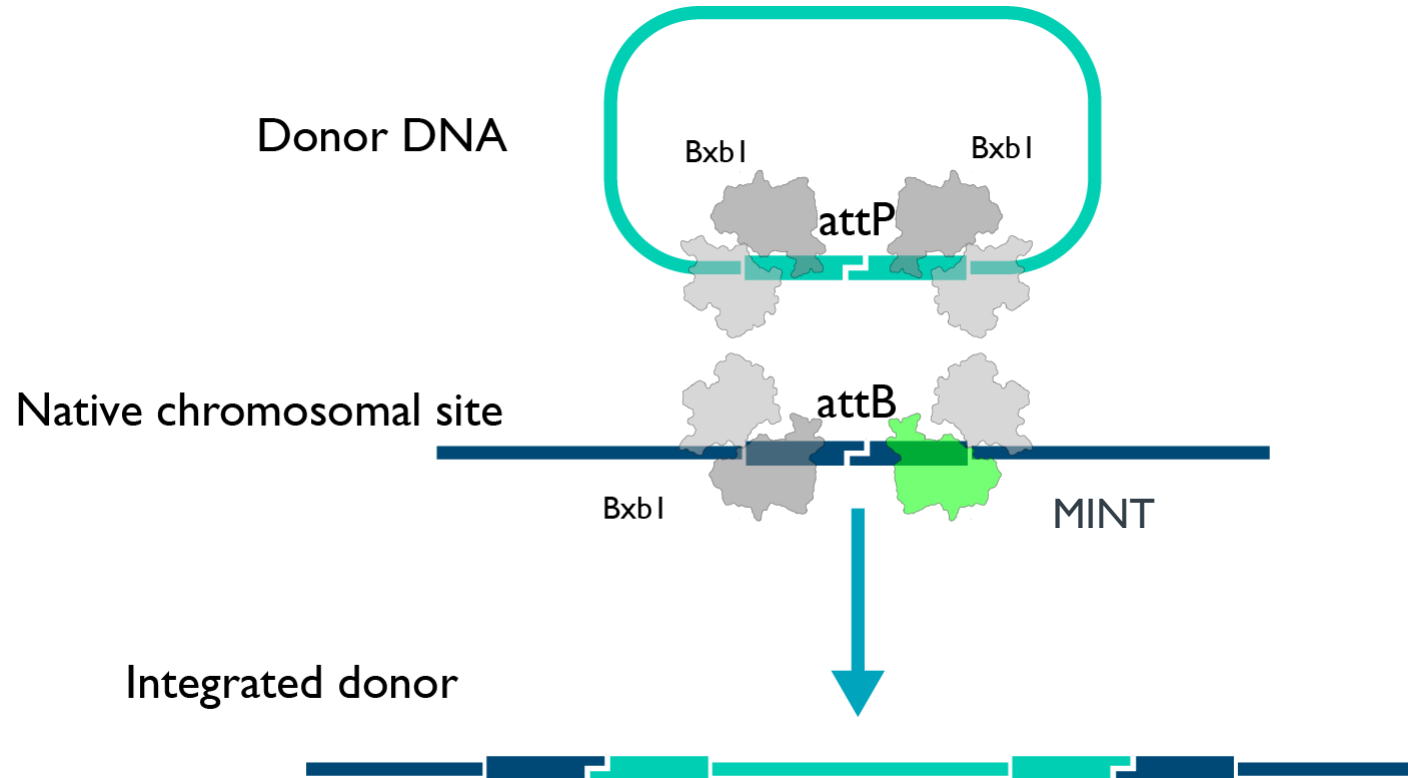
Order

# Engineered Modular Integrases (MINTs) with selected helices enable efficient targeted integration into the genome of human cells



Preview of human cell data from Fauser et al.  
Poster 1680 on 05/10/24

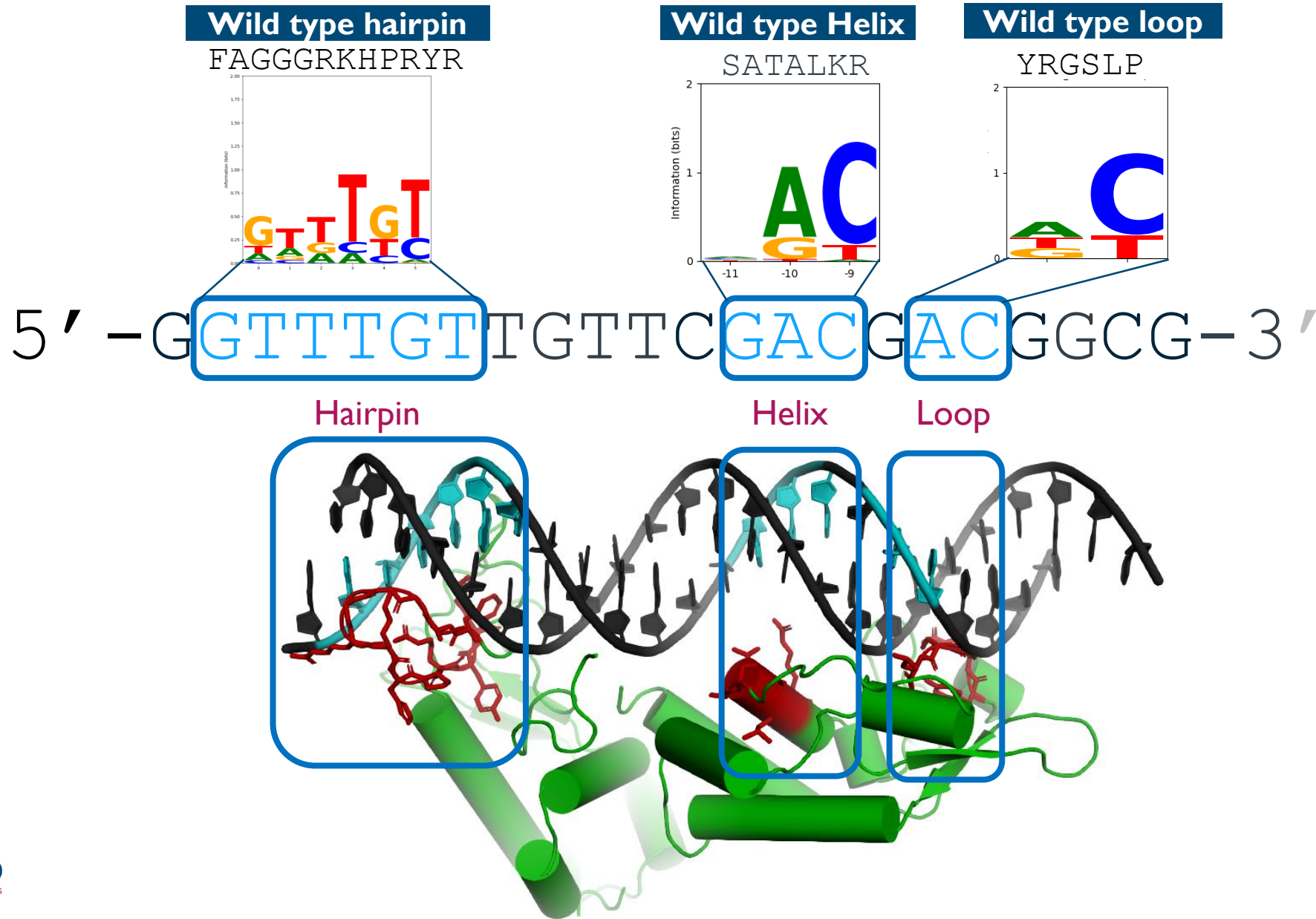
# Engineered Modular Integrases (MINTs) with selected helices enable efficient targeted integration into the genome of human cells



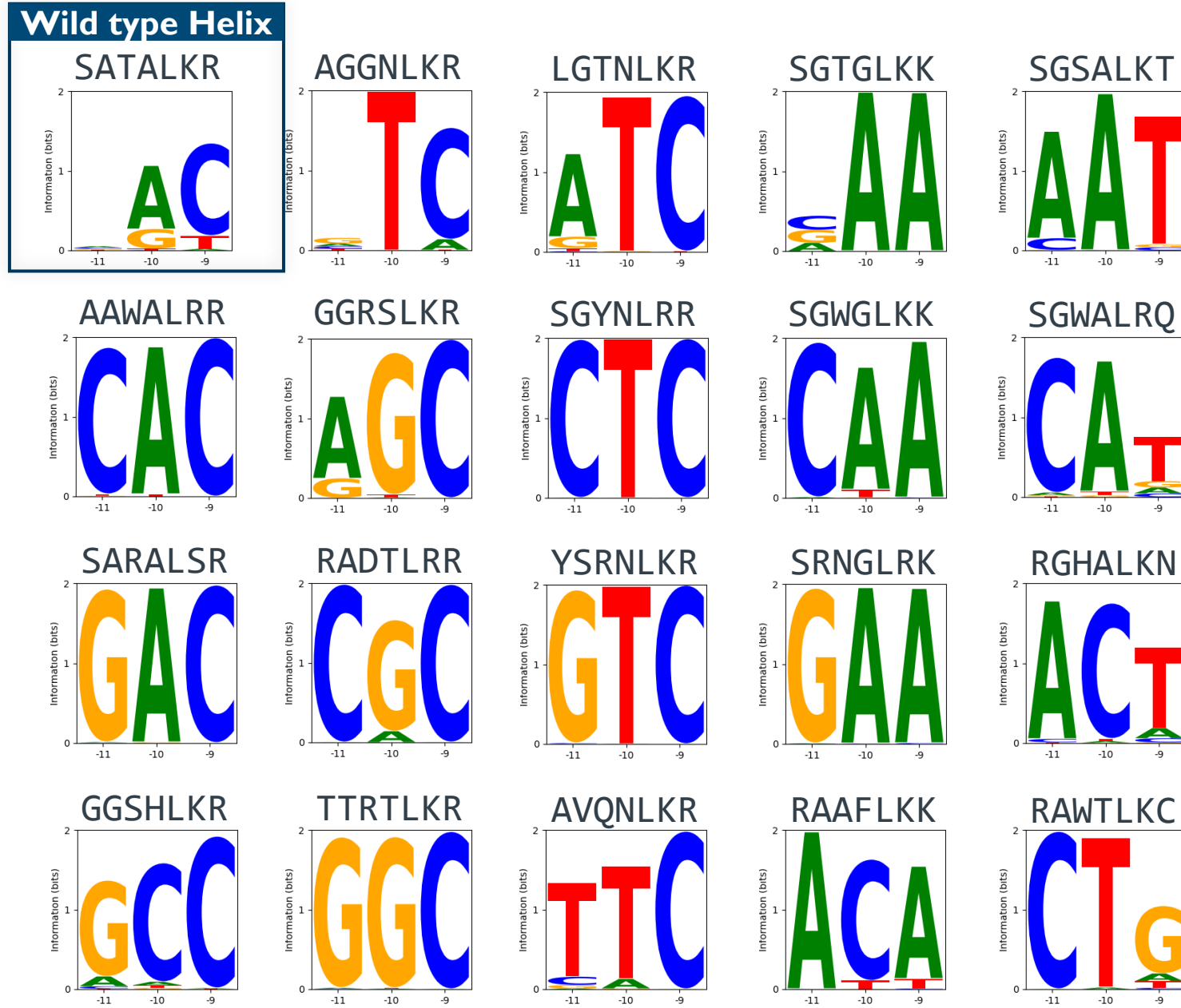
Preview of human cell data from Fauser et al.  
Poster 1680 on 05/10/24



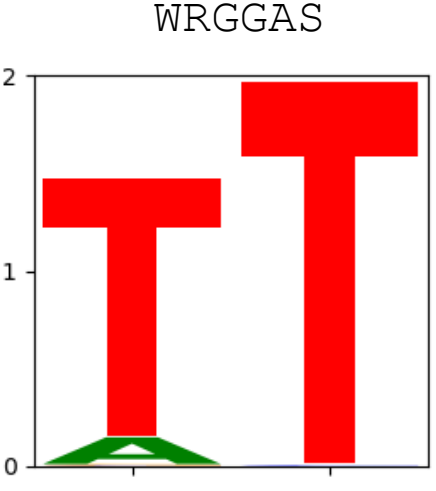
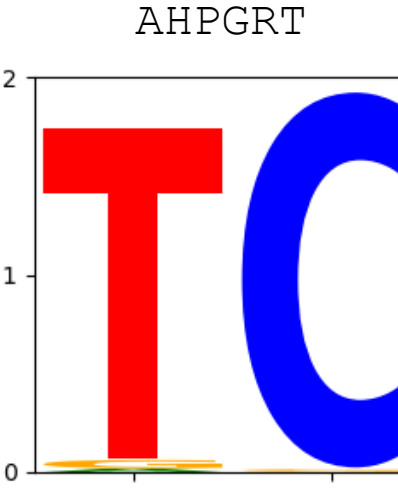
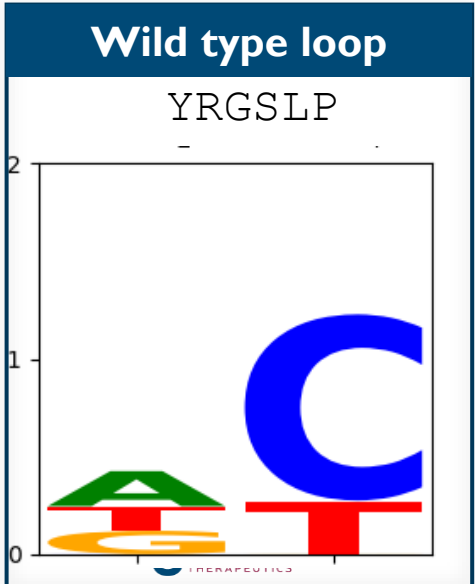
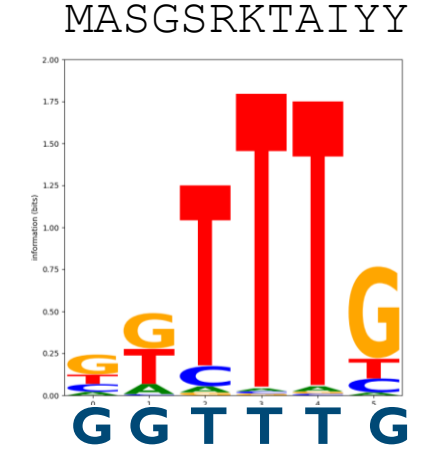
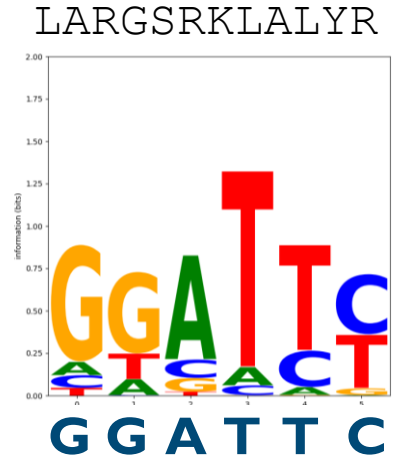
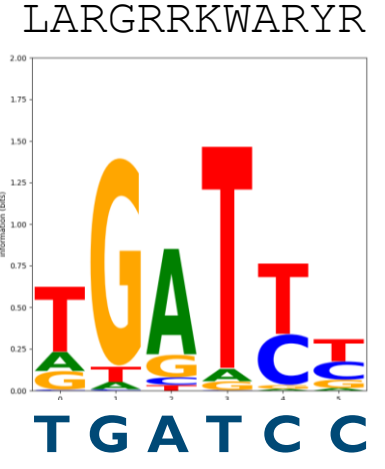
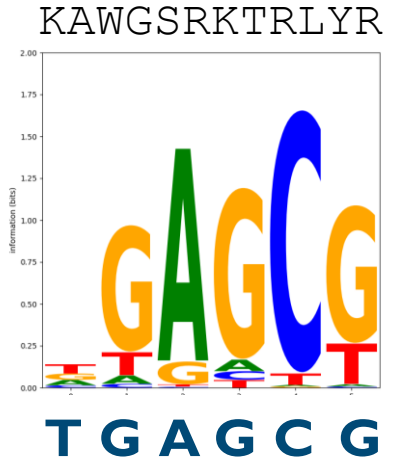
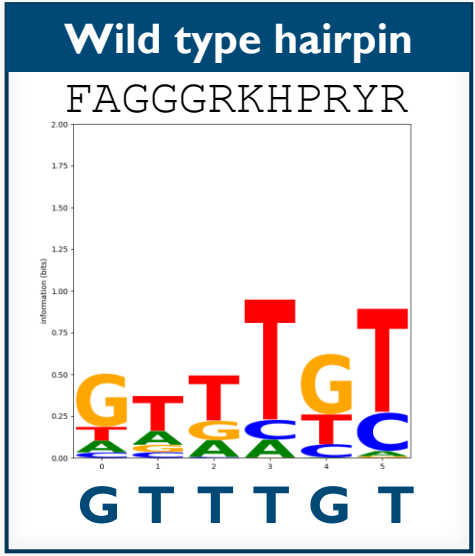
# Specificity determining motifs can be reprogrammed systematically



# We have systematically reprogrammed the Bxb1 recognition helix



# Hairpin and loop submotifs can also be systematically reprogrammed



# Reprogrammed Bxb1 submotifs give rise to Modular Integrases (MINTs)

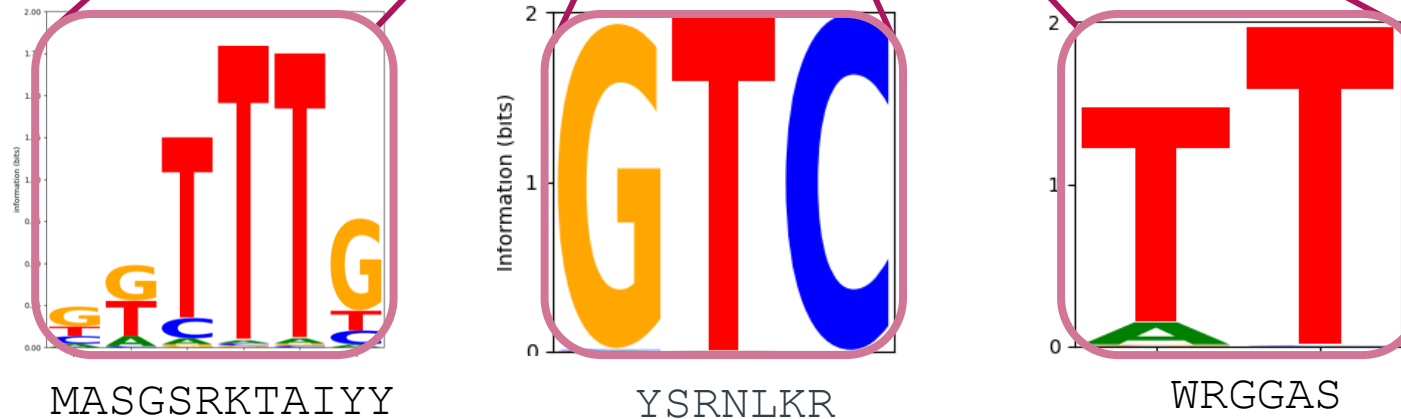
*Natural target half-site*

**GGCTTGTCGACGACGGCG**

*Human safe-harbor locus half-site*

**G**GGTTT**G**AGTC****CCTT**GGCA**

*Engineered submotifs with novel DNA specificity*



— We believe our strategy can be used to reprogram a wide variety of natural integrases

BxbI

Pa0I

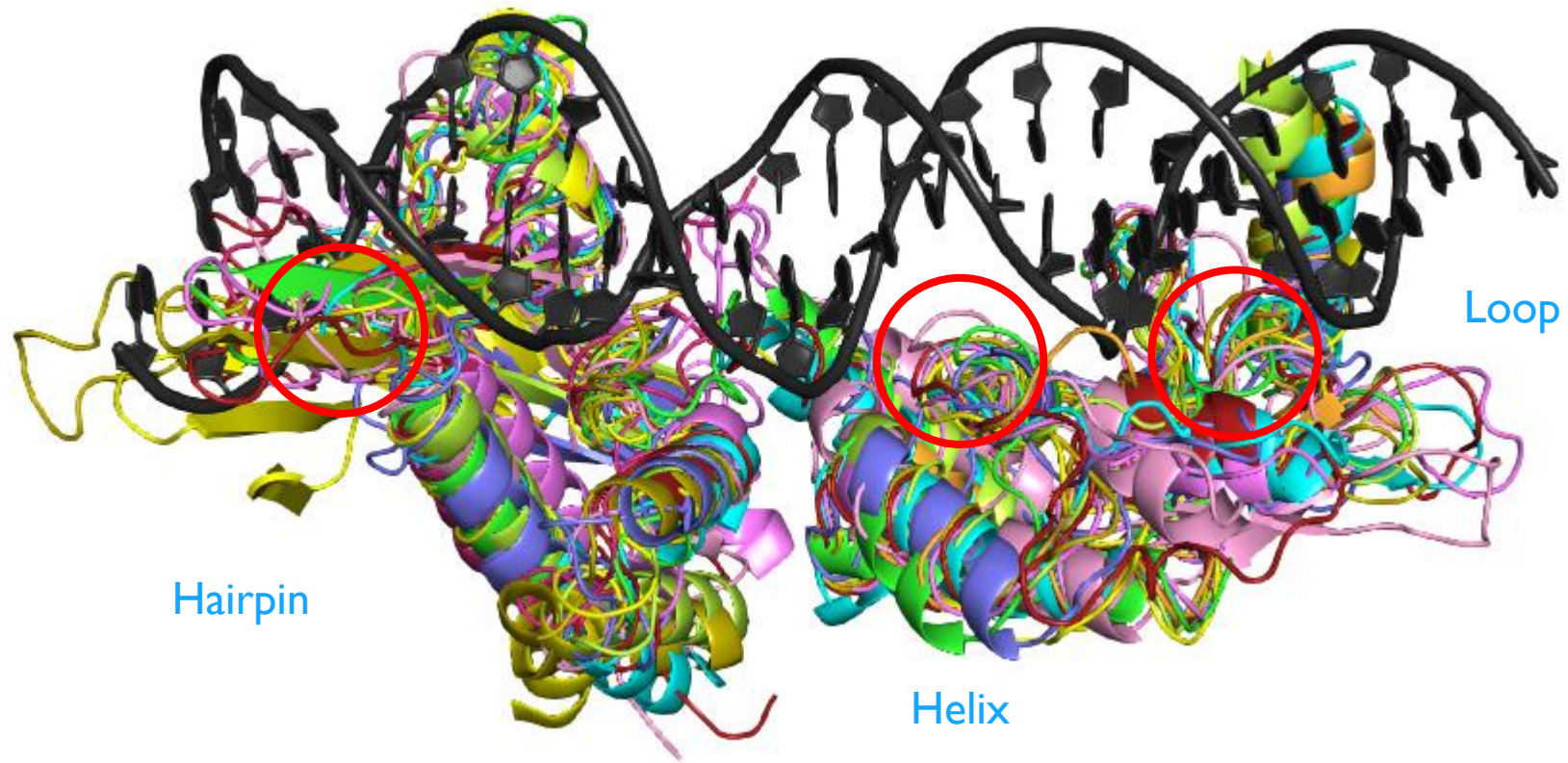
Kp03

Nm60

Si74

BcyInt

SscInt



Durrant et al. Nat Biotech (2022)

Yarnall et al. Nat Biotech (2023)

## Summary

- ✔ Demonstrated serine integrase reprogramming for the first time
- ✔ MINTs enable insertion of large DNA cargo into the human genome
- ✔ MINTs unlock new ways to treat genetic diseases
- ✔ Reprogramming strategy will likely apply to other integrases



## Thanks - Q&A



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