



Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting

Cermak, Doyle, Christian, Wang, Zhang, Schmidt,
Baller, Somia, Bogdanove, Voytas
Nucleic Acids Research, March 24, 2011

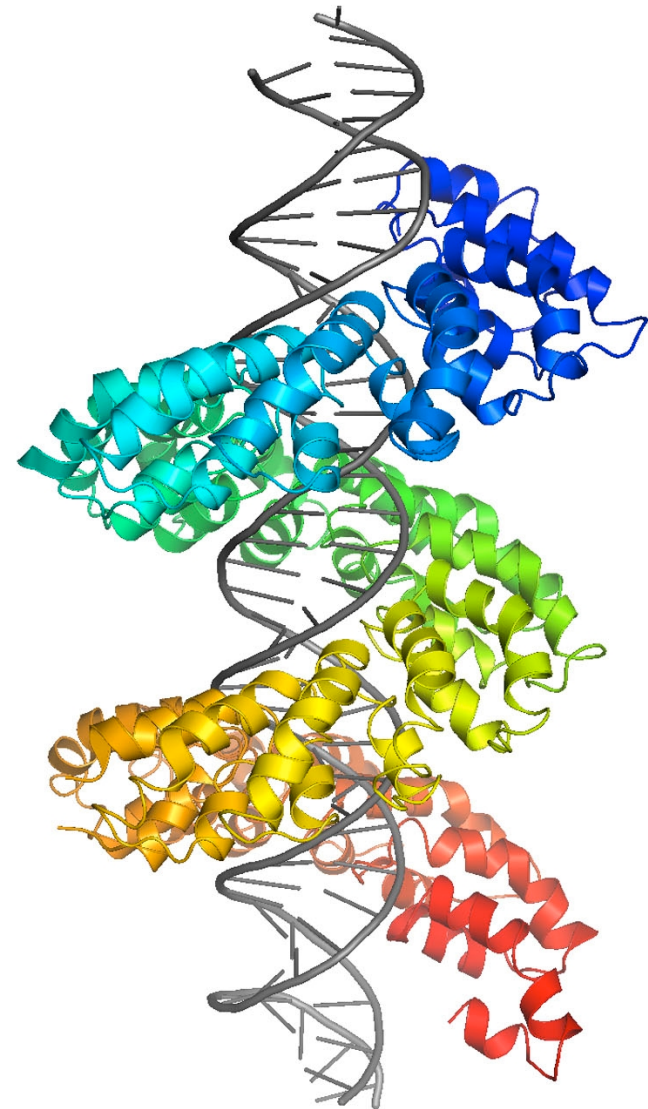
Shelley Ackerman
20.385 Journal Club II

TALENs and TAL Effectors

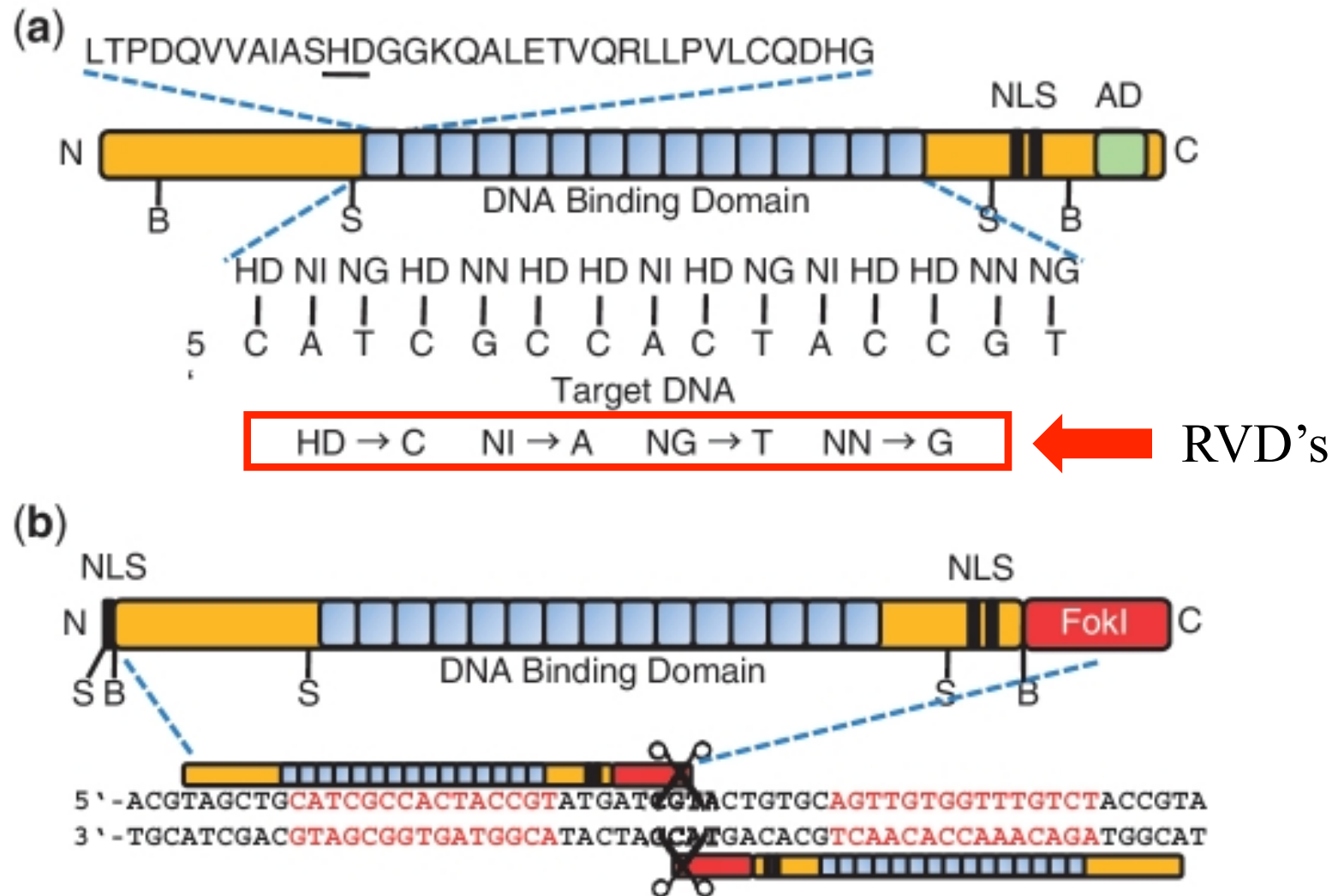
- TAL: Transcription Activator-Like
- Class of specific DNA binding protein
- Produced by plant bacteria in *Xantomonas*
 - Modulates host gene expression
- RVD: Repeat-variable di-residue
 - Residues at position 12 & 13 that give target specificity
- Double Strand Break Repair:
 - Non-homologous End Joining (NHEJ)
 - Homologous Recombination (HR)

Goals & Motivation

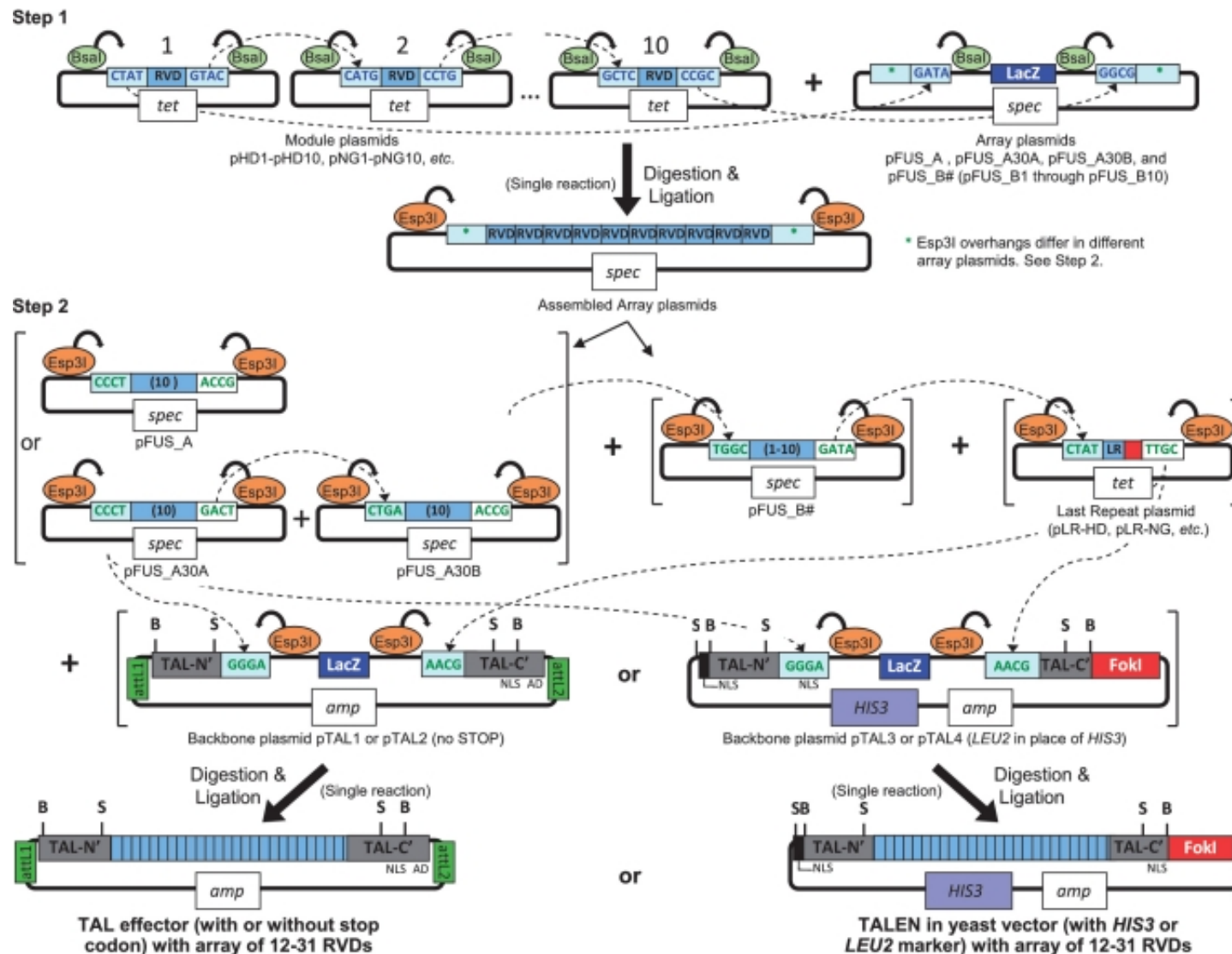
- Develop tools for Genome Engineering
- DNA Targeting
- Goal: Develop system for assembly of custom TALEN & TAL Effectors



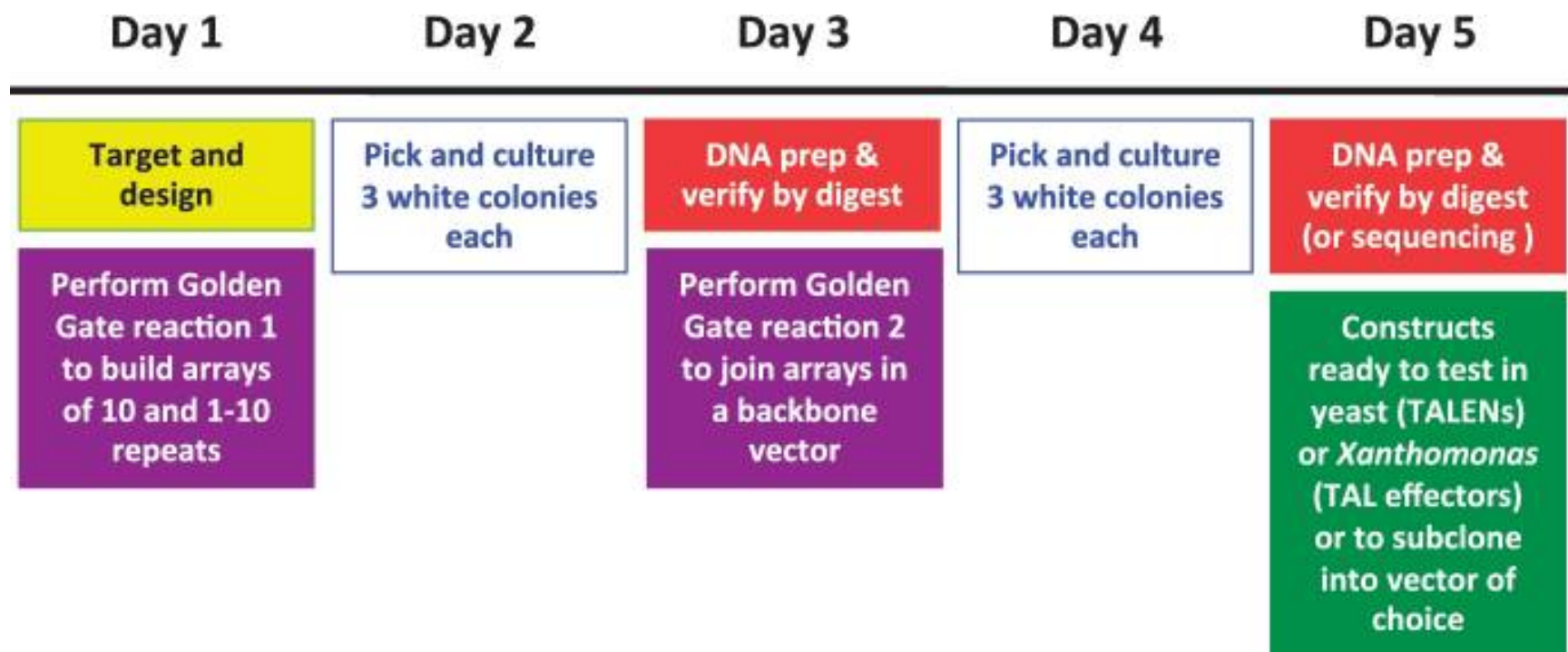
Structure of TAL Effector & TALEN



Creating TAL Effectors and TALENs via Golden Gate assembly

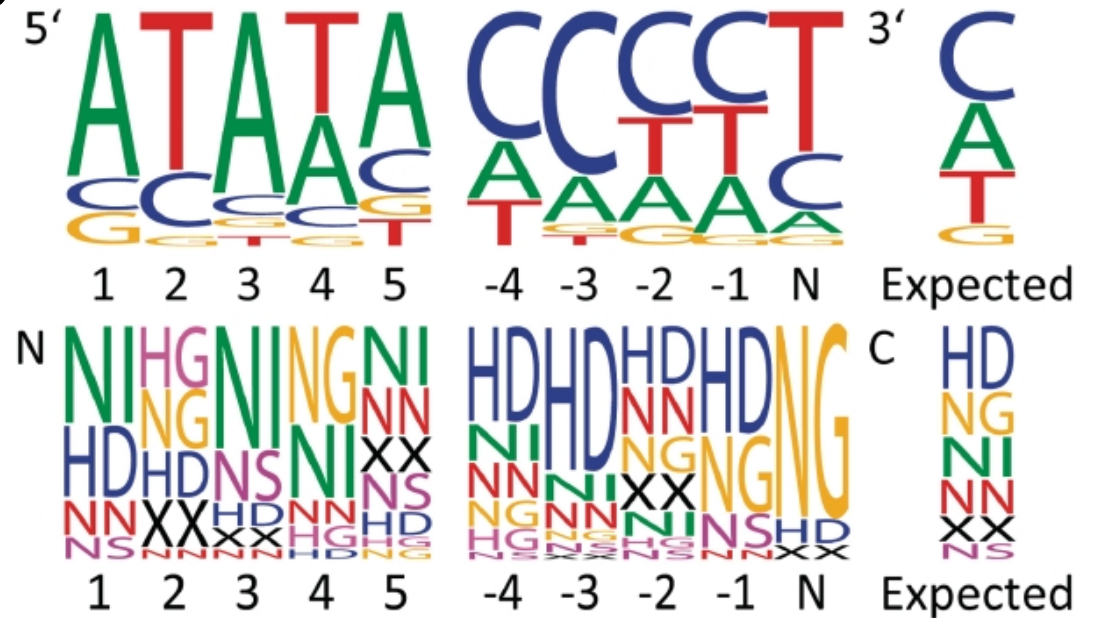


Construct Assembly Timeline



TALEN Design Guidelines

1. TALEN monomer binding sites must be preceded by 5'-T
2. No T at position 1
3. No A at position 2
4. Must end with a T
5. Base composition within two S.D. of observed average



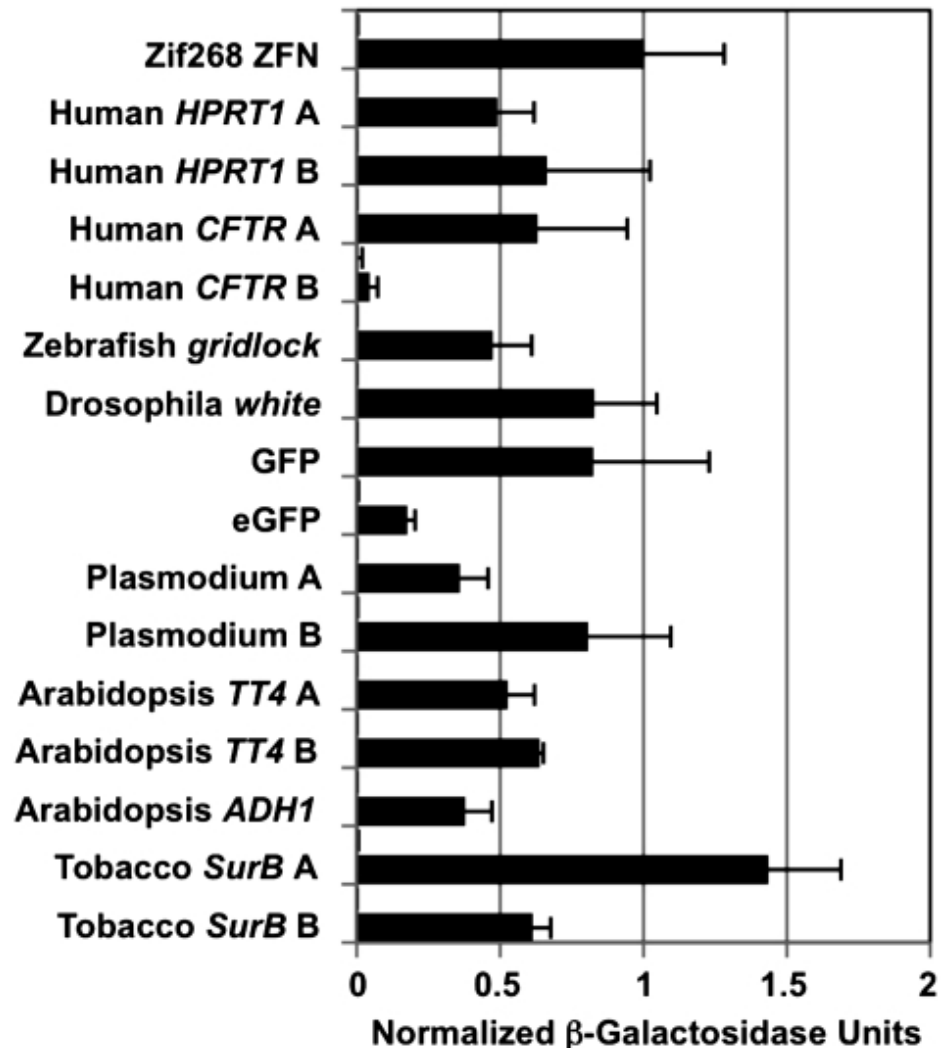
Software for TALEN design

The screenshot shows the TALE-NT web interface. At the top, there's a red header with the text "TALE-NT" and "TAL Effector-Nucleotide Targeter". Below the header are navigation links: "Home", "Help", and "About". The main section is titled "Enter FASTA sequences" and includes instructions: "Enter up to 5000 total bases. (e.g. You may have 1 sequence of 5k bases, 5 sequences with 1k bases, or any such combinations)". A text area labeled "FASTA sequence" contains a long DNA sequence. Below the text area are input fields for "Spacer", "Min", and "Max". A note states: "(Default spacers are 15, 18-30 bases. Check this option to enter a min and max to search a range of spacer sizes)". There are several checkboxes: "Require A, C, or G at position 1 (not a T)", "Require C, G, or T at position 2 (not an A)", "Require a T at position N", "Allow sites to end in G", and "Percent composition". At the bottom are "Submit" and "Reset" buttons.

- Used for design of TALEN and TAL effectors for genome editing
- Guidelines reflect naturally occurring TAL effectors
 - Binding sites
 - Spacer lengths

Activity measured for 15 custom TALEN pairs

- Reporter-based yeast assay
- Significant activity above target-only negative controls
- 14/15 resulted in $\geq 25\%$ Zif268 ZFN's activity



Genome Editing using Custom TALENs

- Successful targeted mutagenesis in human embryonic cells and plant protoplasts
 - *HPRT1* gene
 - *Arabidopsis ADH1* gene
- Mutations at sites via NHEJ
- Could not quantify frequency of mutagenesis

Significance

- Designed method for custom assembly of TALENs and TAL effectors
- Made all parts available through AddGene, non-profit clone repository
- Software developed is free online tool

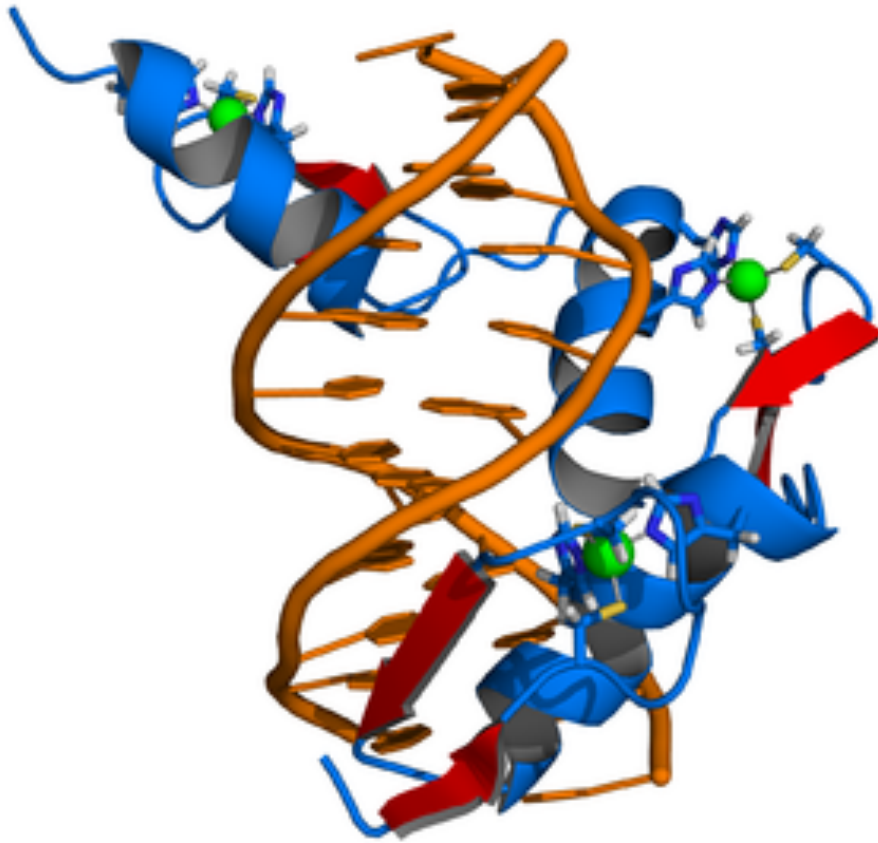
Concerns with the Paper

- Counterarguments against other papers and strategies
 - Spent more time discussing why better than explaining actual data
- No explanation for variability in activity levels in TALENs tested

Questions?



Zinc Fingers



- Coordinate Zn with Cysteine or Histidine
- Bind to DNA/RNA/ Proteins
- Hard to target for specific binding sites
- Can target every ~500 BP
- Difficulty targeting AT rich sequences

Proper function of TALEN exhibited on leaves

