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

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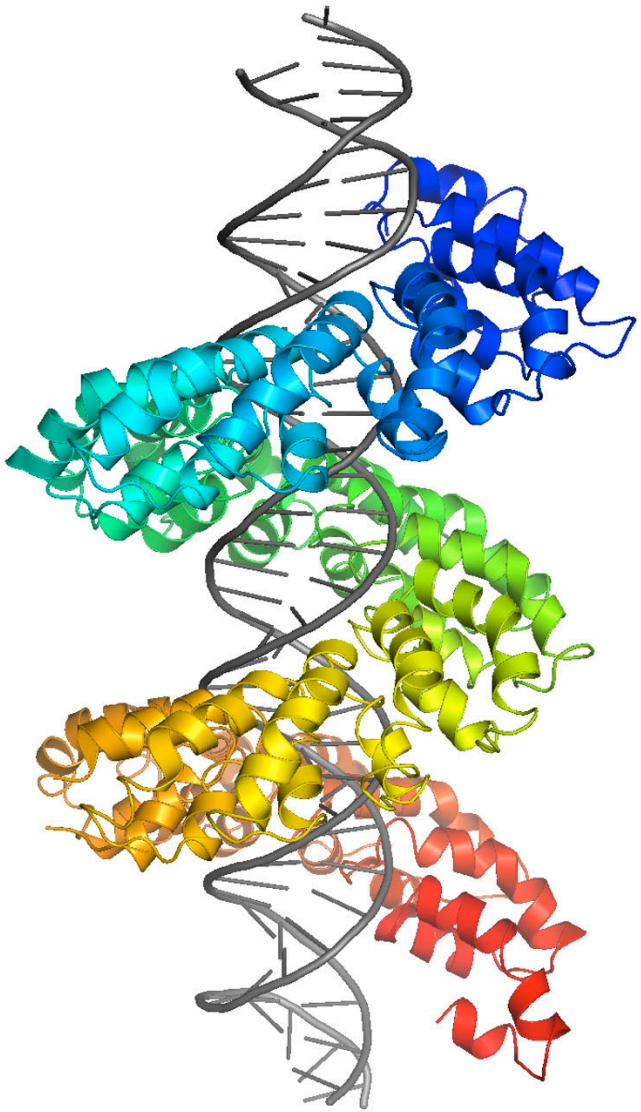
20.385 Journal Club II

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)

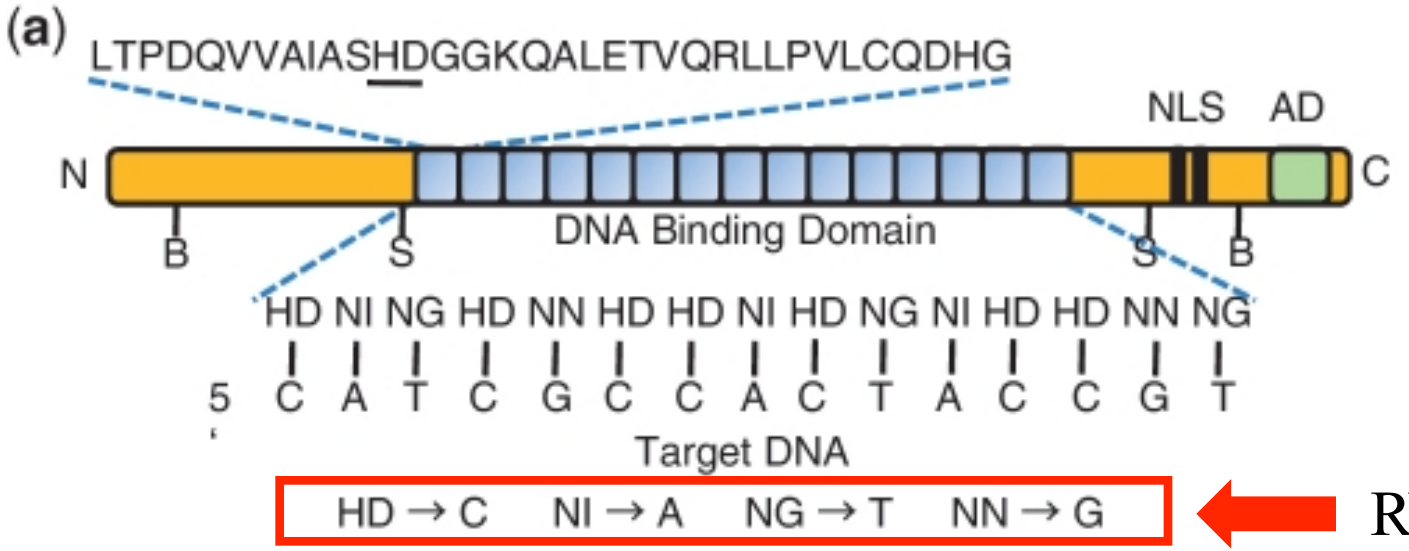
Goals & Motivation

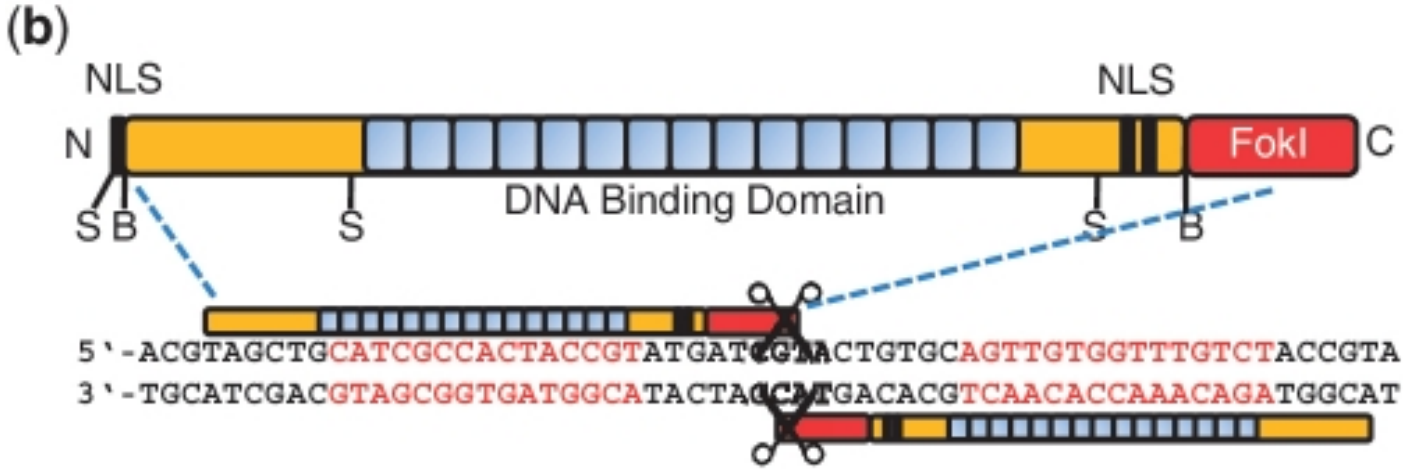
- Develop tools for Genome Engineering
- DNA Targeting
- Goal: Develop system for assembly of custom TALEN & TAL Effectors



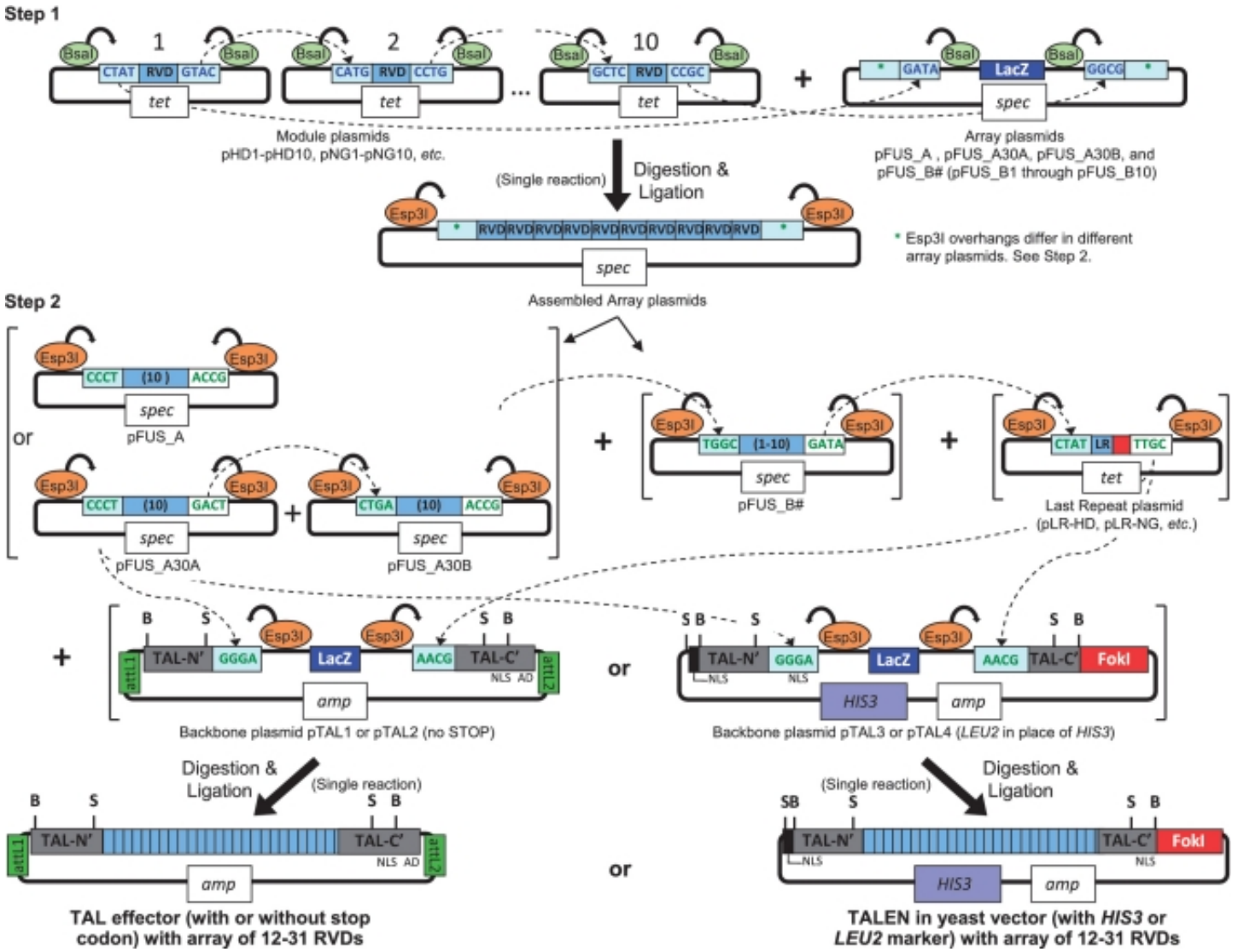
http://www.newswise.com/images/uploads/2012/01/4/TAL_effector_DNA_color_1.JPG

Structure of TAL Effector & TALEN

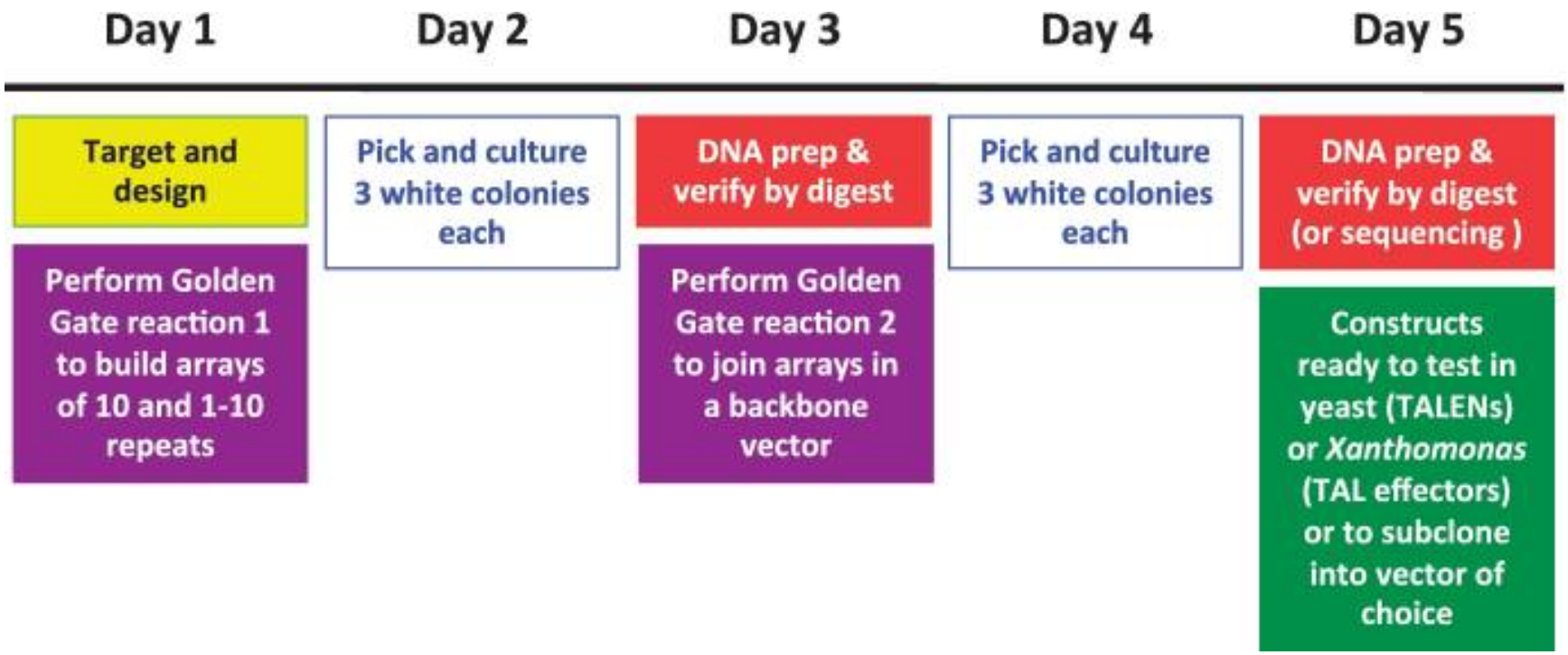




Creating TAL Effectors and TALENs via Golden Gate assembly

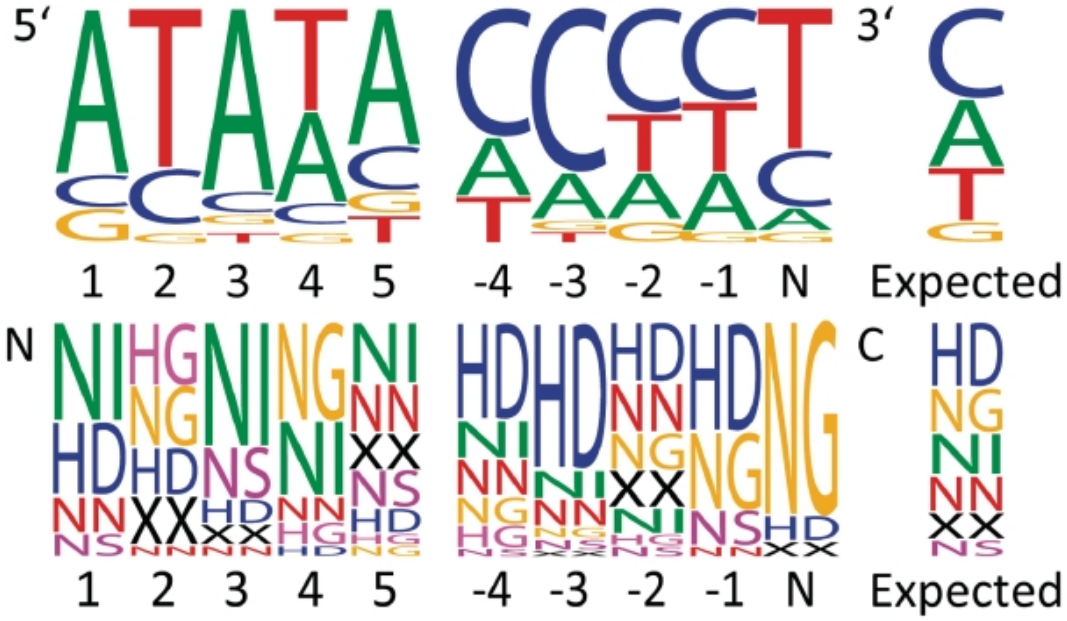


Construct Assembly Timeline



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

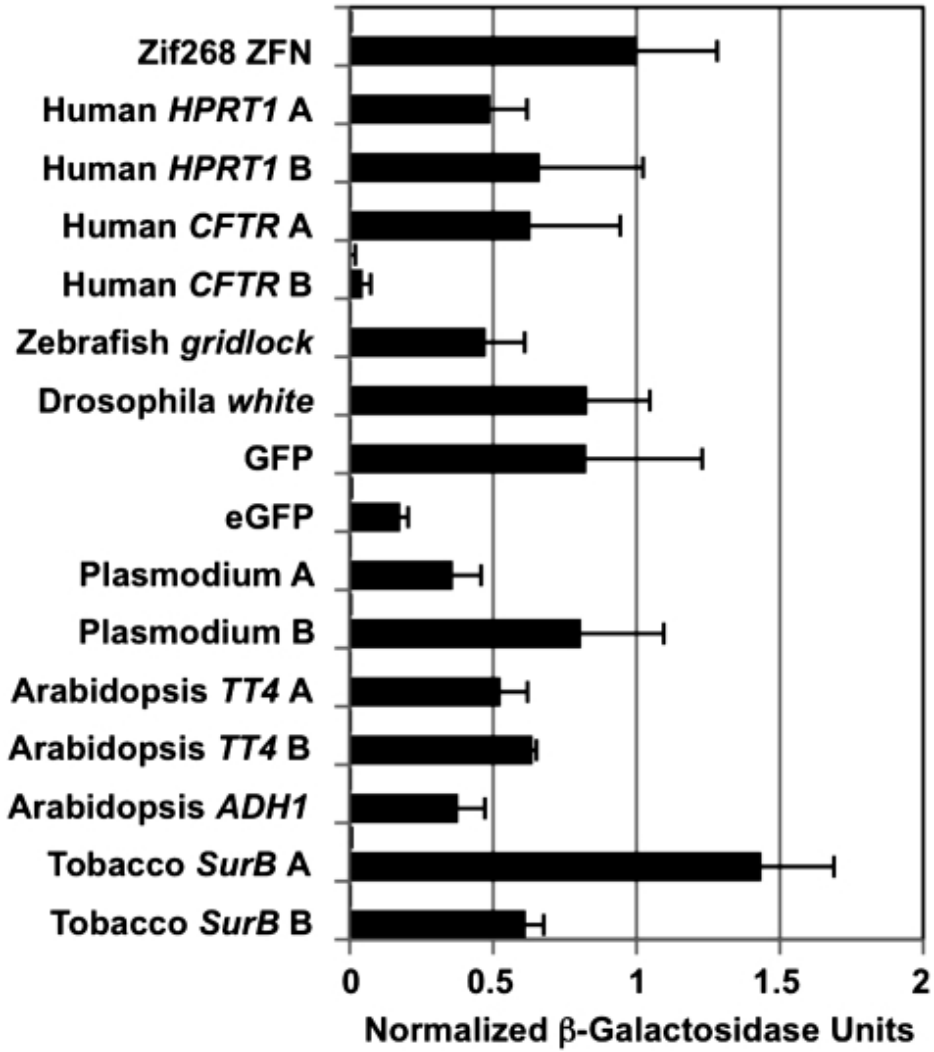


Software for TALEN design

- 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 ≥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

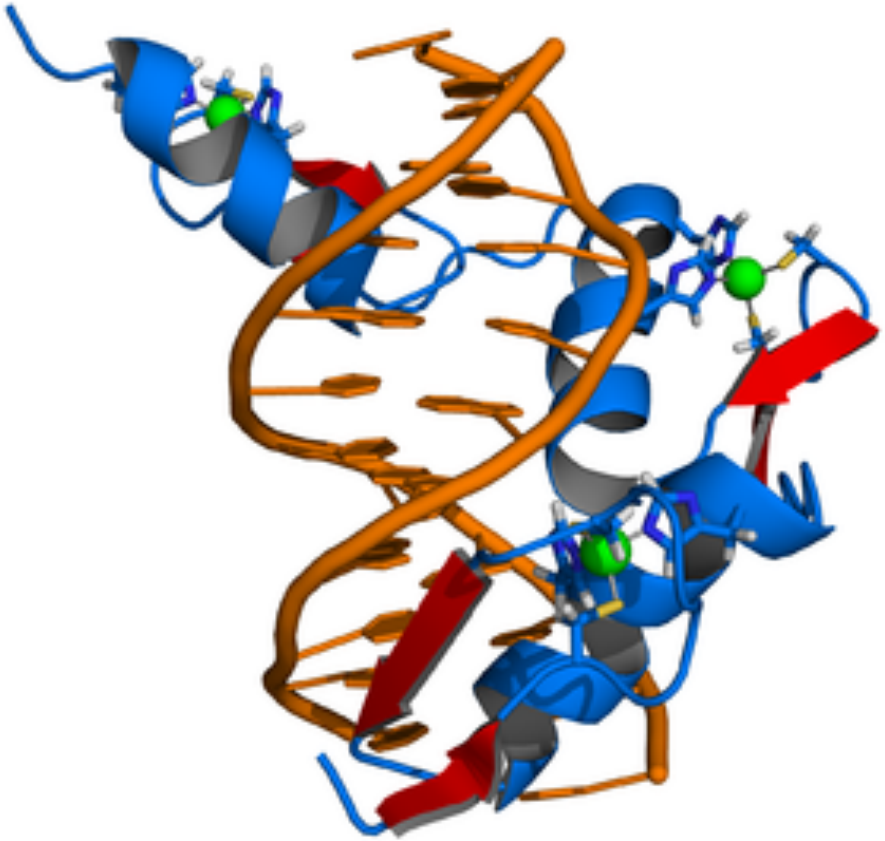
- Designed method for custom assembly of TALENs and TAL effectors
- Made all parts available through AddGene, non-profit clone repository
- Software developed is free online tool

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

http://en.wikipedia.org/wiki/Zinc_finger

Proper function of TALEN exhibited on leaves