

# (SD2022-279) Mutant ZRANB2 zinc finger proteins with GGG RNA sequence targeting specificity

Tech ID: 33860 / UC Case 2021-Z08-1

## BACKGROUND

Existing RNA-targeting tools for sequence-specific manipulation include anti-sense oligos (ASOs), designer PUF proteins and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas systems. However, there are significant limitations to each of the current tools. ASOs are usually not available for most RNA manipulations other than gene silencing. Designer proteins, such as PUF (Pumilio and FBF homology protein), possess low RNA recognition efficiency and it remains challenging to target RNA sequences >8-nucleotides (nt) in length. The bulky Cas protein (Cas13d: average 930 amino acids) leads to complication for transgene delivery and concerns of its immunogenicity due to its bacterial origin.

Mutants of zinc finger(ZnF) proteins in ZRANB2 recognize a single-strand RNA containing a novel GGG motif with micromolar affinity, compared to the original motif GGU. These mutants serve as a foundation for RNA-binding ZnF designer protein engineering for in vivo RNA sequence-specific targeting.

ZnFs are generally compact domains (~3kDa each) that have been successfully engineered for DNA recognition as modular arrays. A ZnF-based system has unique advantages, especially in a therapeutic context: (1) Broad application with the possibility to fuse with other effector domains; (2) High efficiency of RNA recognition (3 RNA bases recognized per 30-amino-acid ZnF) with a small size of protein. Only 4 ZnFs (~100 aa) is required for specific targeting in the transcriptome. (3) Humanized components without immunogenic concern.

By engineering new sequence specificity of the ZRANB2 ZnF1, researchers from UC San Diego identified 13 mutants that altered their preferred RNA binding motif from GGU to GGG. They are N24R, N24H, N14D/N24R, N14D/N24H, N14R/N24R, N14R/N24H, N14H/N24R, N14H/N24H, N14Q/N24R, N14Q/N24H, N14E/N24R, N14S/N24R, N14E/N24H.

## TECHNOLOGY DESCRIPTION

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## OTHER INFORMATION

### CATEGORIZED AS

- Medical
  - Therapeutics
- Research Tools
  - Nucleic Acids/DNA/RNA

### RELATED CASES

2021-Z08-1



FIG. 1A

(57) **Abstract:** Provided herein are zinc finger peptides comprising the amino acid sequence of SEQ ID NO: 1 (SDGDWICPDKKCGN-VNFARRTSCNRCGREKTT) and at least one substitution compared to SEQ ID NO: 1, wherein the zinc finger peptide is an engineered zinc finger peptide.

APPLICATIONS

The mutant ZnF proteins could be used as a component of a RNA-targeting designer array.

The ZnF arrays could be fused with RNA effector proteins for various RNA manipulations (RNA degradation, RNA splicing control, RNA translation etc.) with potential applications in both biological research and RNA-targeting therapeutics.

Only 6 ZnFs are required to target RNA specifically (18-nt) in the human transcriptome.

The designer ZnF-array size is only ~100 amino acids, which is much more convenient to use and especially beneficial in therapeutic applications considering the limited packaging capacity of vectors like adenovirus-associated virus (AAV).

ADVANTAGES

STATE OF DEVELOPMENT



# Engineering Sequence Specificity in an RNA-Binding Zinc Finger Protein

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## Introduction:

### ZRANB2 Zinc Finger Is an excellent scaffold for developing an RNA-targeting toolbox

Targeting and manipulating endogenous RNAs in a sequence-specific manner is important for both understanding RNA biology and developing RNA-targeting therapeutics. RNA-recognizing zinc fingers (ZnFs) are excellent candidates as designer proteins due to their compact size (~3kDa each) and modular sequence recognition.

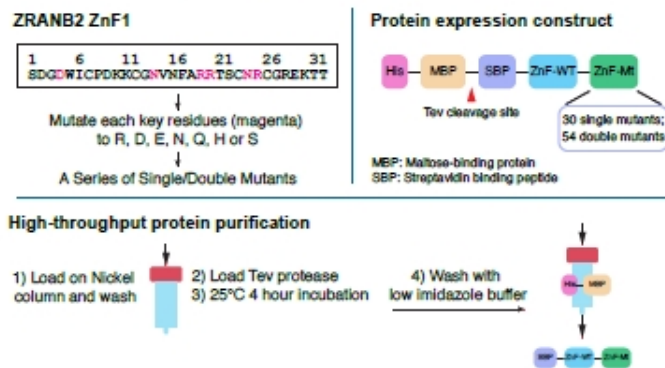


Previous research showed that a single ZnF in ZRANB2 recognizes a single-strand RNA containing a GGU motif with micromolar affinity<sup>1</sup>. Although structures of ZRANB2 ZnF in complex with the RNA have been solved, rules that govern its sequence-specific recognition are still unclear for engineering purpose.



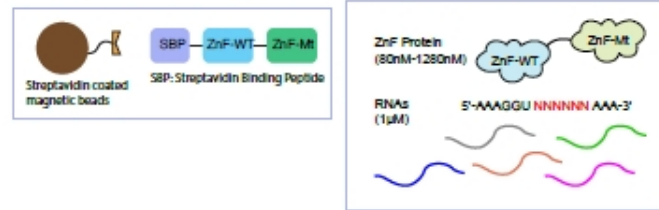
ZRANB2-ZnF1 binding the RNA "GGU" core motif

### Systematic mutagenesis and high-throughput purification of ZRANB2 ZnF



## Characterization of RNA-binding profile of each ZnF: RNA-blind-n-seq Assay<sup>2</sup>

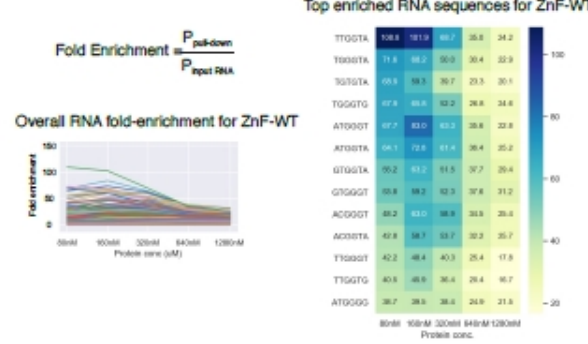
(1) Immobilization of ZnF proteins (2) Incubation with a pool of random RNAs



(3) Pull-down bound RNAs and preparation for deep-sequencing



(4) Calculation of fold-enrichment score

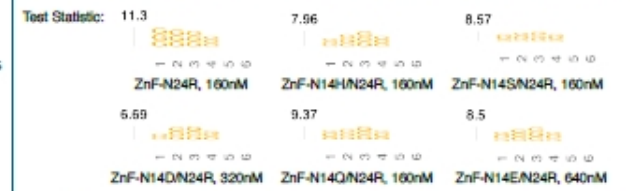


## Identification of core motif: kpLogo analysis<sup>3</sup>

Use kpLogo (k-mer probability logo) server to reports 3-mer enrichment at each position using student's t-test.



## ZnF mutants with altered RNA binding preferences



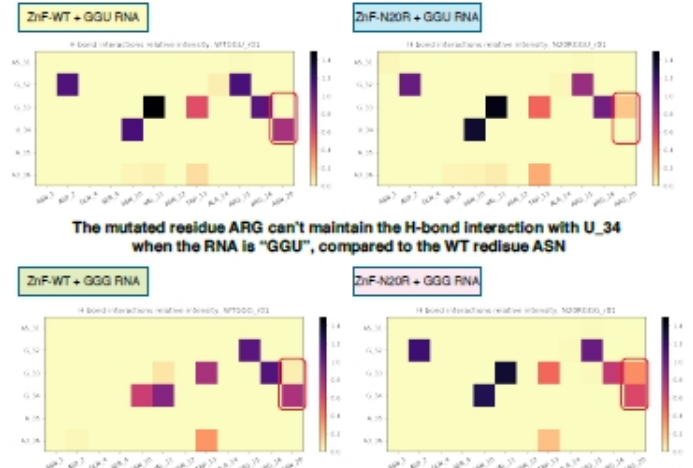
## All-atom MD simulation of ZnF-RNA Interaction

(1) Three replicates of 50ns NPT simulation for each condition. (based on PDB structure 3G9Y) Conditions:

ZnF-WT, GGU RNA	ZnF-N20R, GGU RNA
ZnF-WT, GGG RNA	ZnF-N20R, GGG RNA

\* N20 in 3G9Y correspond to N24 in ZRANB2 ZnF1

(2) Calculate H-bond intensity score for a unique pair of protein residue and RNA base (from CPPTRAJ<sup>4</sup> H-bond statistics) and plot on heatmaps



The mutated residue ARG can't maintain the H-bond interaction with U\_34 when the RNA is "GGU", compared to the WT residue ASN

The mutated residue ARG is able to make H-bond interaction with the G\_34 when the RNA is "GGG", which leads to its preference of "GGG" motif

## Conclusions

- We identified several ZRANB2 ZnF mutants with an altered sequence specificity, preferring GGG motif instead of GGU motif.
- We rationalized this change is due to alternations in hydrogen bonding patterns between the protein and the bound RNA using MD simulation.
- Our analysis of ZRANB2-RNA interactions both *in vitro* and *in silico* expands the understanding of general ZnF-RNA recognition rules, and thus serves as a foundation for RNA-binding ZnF designer protein engineering.

## INTELLECTUAL PROPERTY INFO

UC San Diego is securing patent rights

(<https://patents.google.com/patent/WO2023164549A2>) on the technology and welcomes

interest from companies in a position to commercialize this invention.

(51) International Patent Classification:	GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).	
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(54) Title: ZINC FINGER PEPTIDES, PEPTIDE ARRAYS, AND METHODS OF USE THEREOF		

RELATED MATERIALS

- Qishan Liang, Joy S. Xiang, Kevin D. Corbett, Gene Yeo, Engineering sequence specificity in an RNA-binding zinc finger protein, Biophysical Journal, Volume 121, Issue 3, Supplement 1, 2022, Pages 479a-480a - 02/11/2022

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