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De Novo Designed TfR1 Binding Peptide To Shuttle Antibody Therapeutics Across Blood Brain Barrier

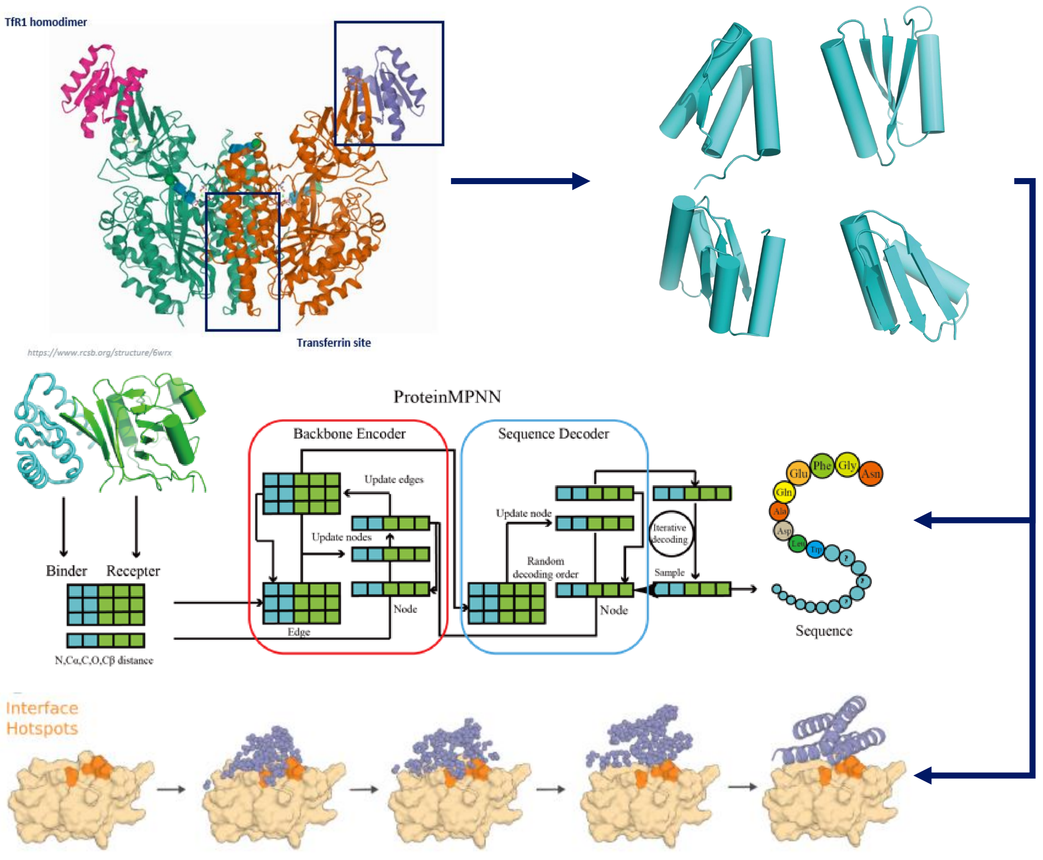
Hao Wu*, Yibo Qiu, Fei Sheng, Yue Wu
ChainGen Bio, Shanghai, China *Email: hao.wu@chaingenbio.com

Introduction

- Some TfR1 binding moieties are capable of crossing blood-brain-barrier (BBB) through receptor mediated transcytosis
- TfR1 binding peptides are discovered by *de novo* design and demonstrate capability of shuttling antibody across BBB

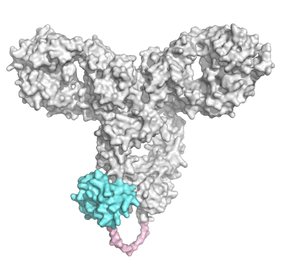
Peptide discovery

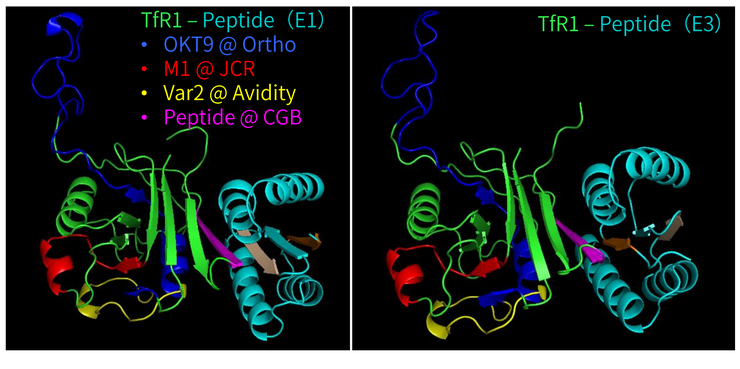
- *De novo* design by RosettaFold, ProteinMPNN & RFdiffusion etc[1-2]
- Previous work[3] by Baker Lab focused on 2 structures and screened 600+ sequences; our work evaluated 419 structures and screened 200,000 sequences
- High-throughput DNA synthesis and display expediated the discovery



Peptide characterizations

- Forty-six peptides were identified, with average length of 70aa, single nM affinity & fast on-and-off kinetics
- Peptides bound TfR1 apical domain with 2 conformations
- Fusion on C-terminal of human IgG did not impact TfR1 binding affinity
- No competition with transferrin & no binding to TfR2

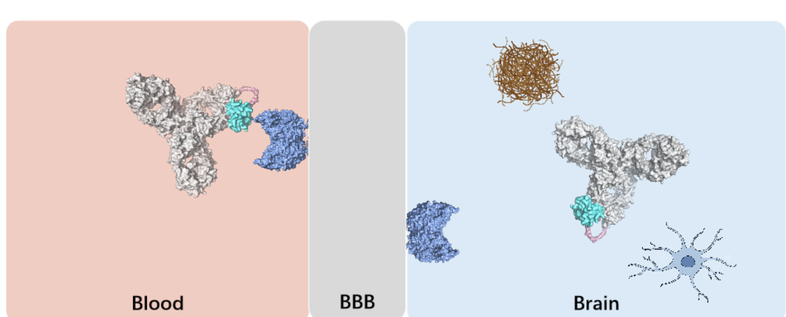


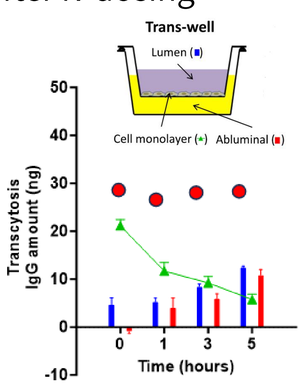


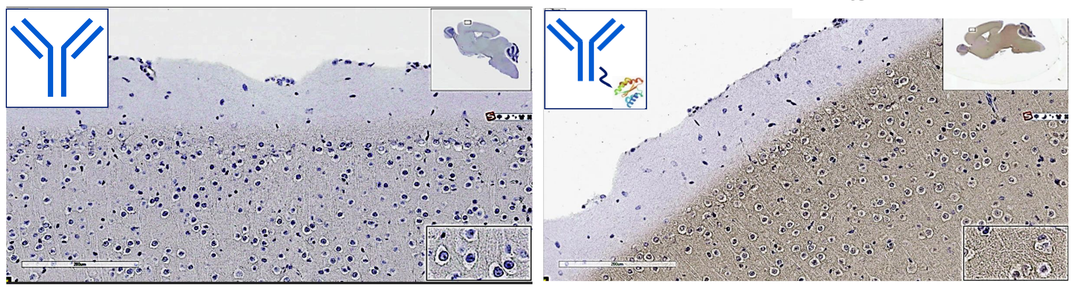
	ka (1/Ms)	kd (1/s)	KD (M)	
TfR1 antibody	1.26E+05	1.00E-06	7.94E-12	
Peptide	1.53E+06	5.62E-03	3.67E-09	↓ No change
IgG-peptide	6.82E+05	3.16E-03	4.64E-09	
VHH	4.72E+05	4.04E-05	8.57E-11	↓ 2-log lowering
IgG-VHH	1.50E+05	7.61E-04	5.10E-09	

Delivery of antibody across BBB

- Fusion protein crossed BBB through TfR1 mediated transcytosis[4]
- Brain delivery was confirmed in parenchymal of hTfR1 transgenic mice and in cerebrospinal fluid of non-human primates after iv dosing

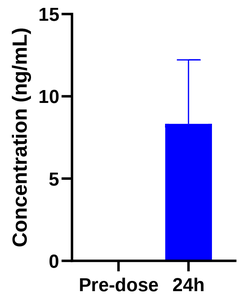






CSF Concentration

Mean ± SEM, n = 2, single dose = 10mpk, determined by a hi-sensitive aβ-based ELISA assay

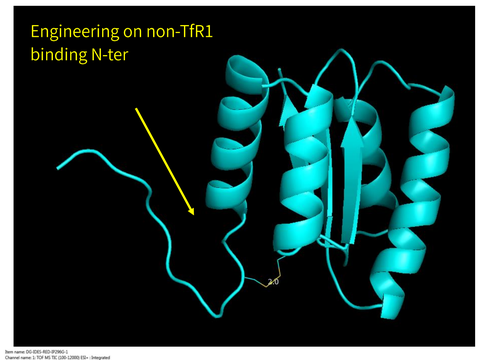


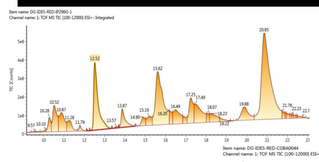
Efficacy evaluations

- Peptide fusion on C-terminal does not affect antibody binding to Aβ
- Surrogate fusion protein with mouse IgG2a is capable of stimulating phagocytosis of Aβ by primary mouse microglia
- Intravenous injected fusion protein is capable of binding to ICV injected Aβ in brain section of transgenic mice

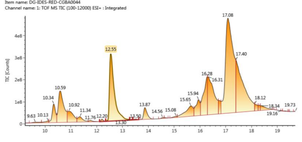
Optimizations & CMC readiness

- Peptide engineering is to improve bioactivity and CMC developability
- For example, introducing disulfide bond(s) on non-TfR1 binding domains improved titer and stability





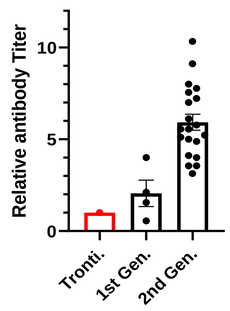
Peptide mapping 1st Gen.



Peptide mapping 2nd Gen.

Antibody Titer in CHO-K1

Mean ± SEM, trontinemab is normalized to 1



	1st Gen.	2nd Gen.
Linker breaks	18.7%	1.6%
LC lost	16.5%	N.D
Enzymatic breaks	3.9%	2.8%

N.D: not detected

Summary

- *De novo* design is a novel approach of peptide discovery
- Unlike Fab or VHH, peptide is smaller in size, reveals unique epitope/paratope and kinetics, and has plenty room of optimizations
- TfR1 Peptide offers an alternative approach of shuttling antibody across BBB

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References: 1. Dauparas et al. Science 378 (2022); 2. Watson et al. Nature 620 (2023) 3. Sahtoe et al. PNAS 118 (2021); 4. Transwell assay is described in US20200071413A1.