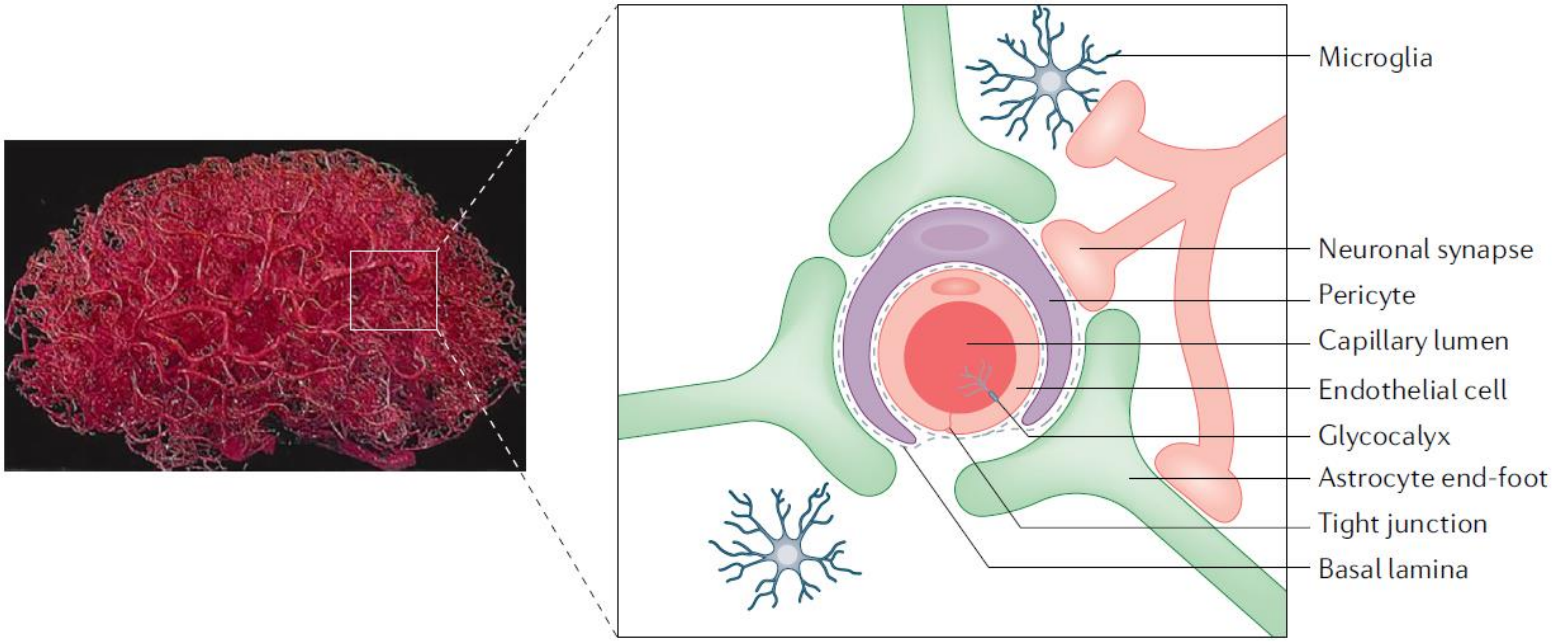
Progress in the development of delivering technologies for brain therapeutics

Simone Hornemann, TJC, 09.03.2021

The blood brain barrier (BBB) and its effect on treatment strategies for central nervous disorders



- The BBB controls the transport of hydrophilic substances (e.g. antibodies) from the periphery into the CNS
- Sufficient exposure in the central nervous system is the major hurdle.
- Approaches to bypassing the blood-brain barrier are under development.

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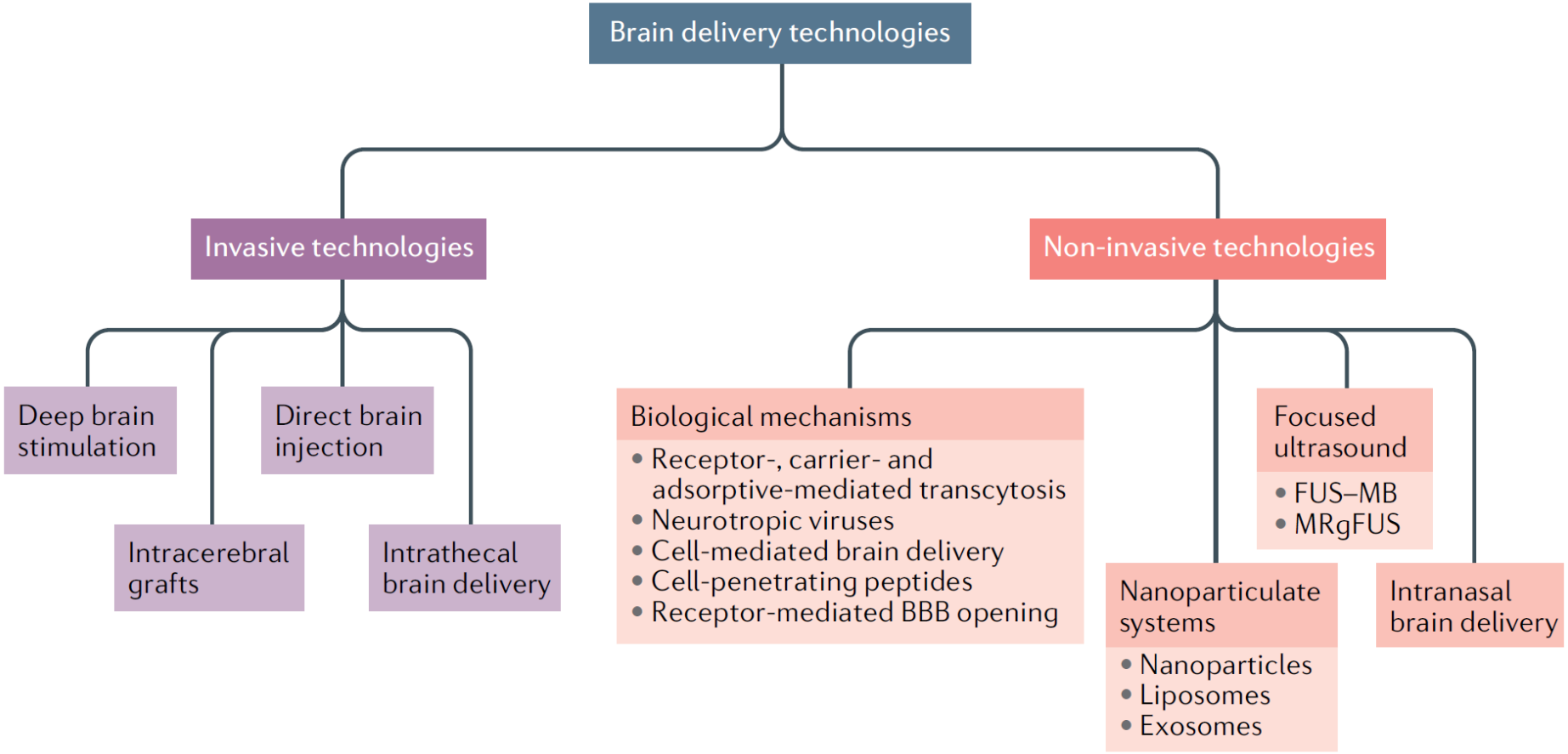
Spectrum of brain delivery technologies



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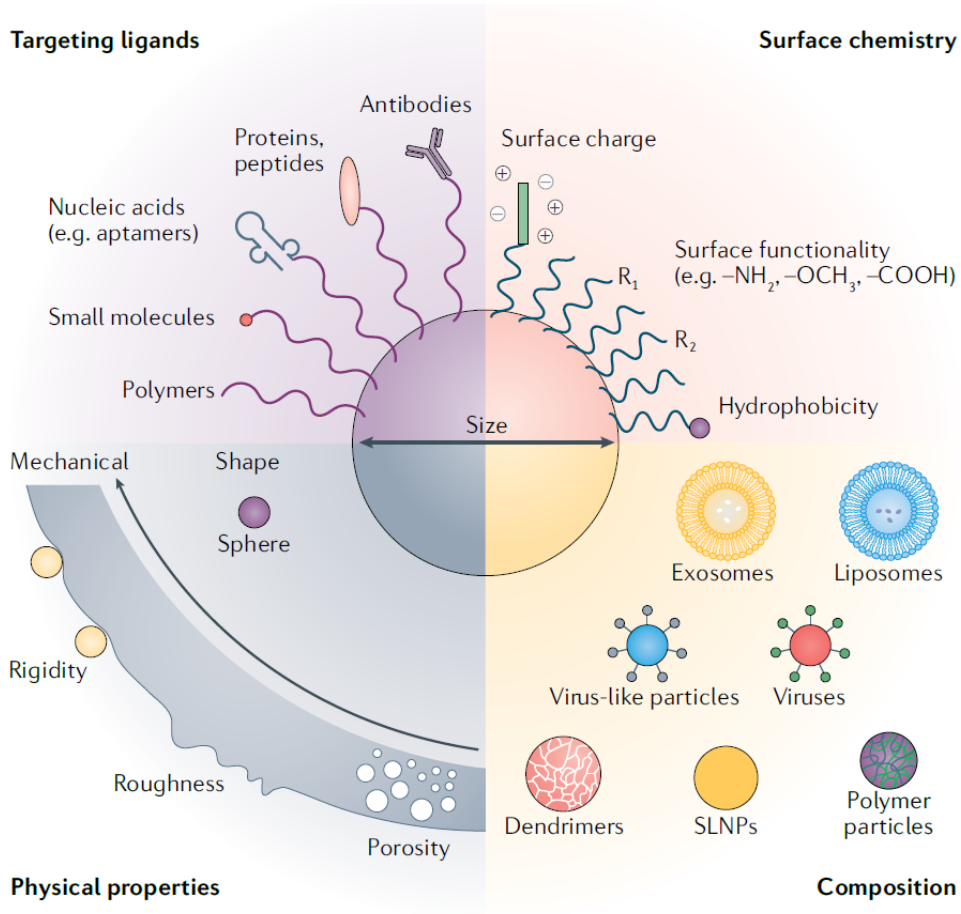
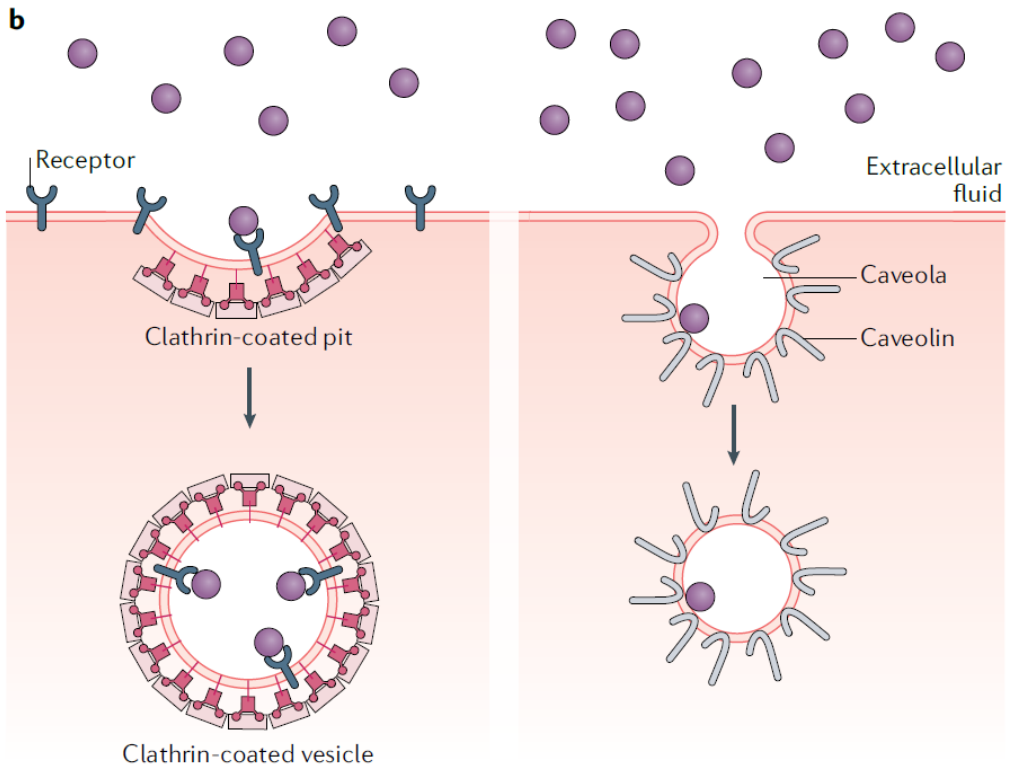
Synthetic nanocarriers for non-invasive brain delivery





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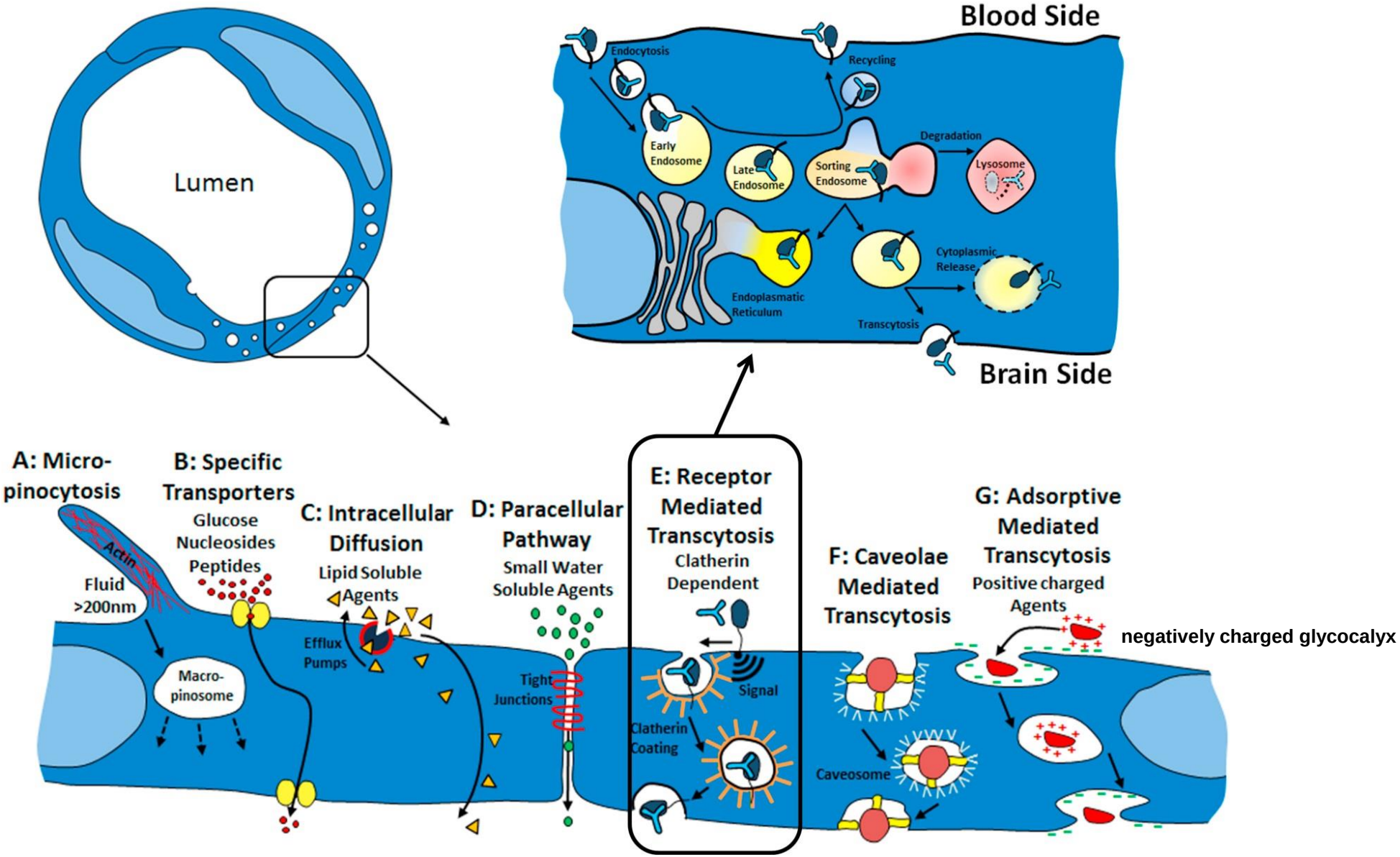
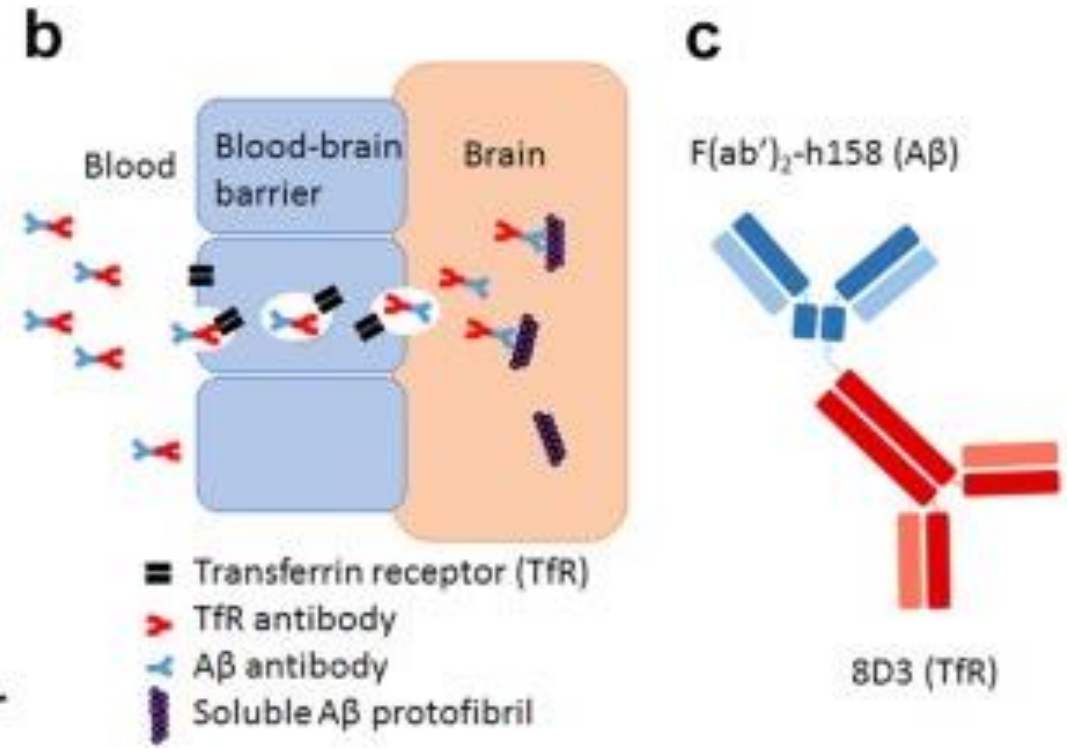
Different mechanisms and routes to cross the BBB.



Per-OlaFreskgård and EduardUrich Neuropharmacology 2017

Transcytosis by the transferin receptor to overcome the BBB



- The most well-studied BBB target for brain delivery is the transferrin receptor (TfR),
- Early studies with antibodies that bound to rat and mouse TfR showed increases in brain exposure relative to control antibodies, demonstrating the potential of targeting the TfR for BBB transcytosis
- Although these antibodies accumulated in brain, they were later found to be mostly trapped within the brain endothelium.
- More recently, low-affinity antibodies that bind monovalently to human TfR (hTfR) were shown to improve brain uptake in mice and nonhuman primates.
- An anti-Aβ antibody fused at its C terminus to an anti-TfR single-chain Fv (scFv) showed improved brain Aβ reduction in a mouse model of Alzheimer's disease (AD) as compared to the parental antibody.

BLOOD-BRAIN BARRIER

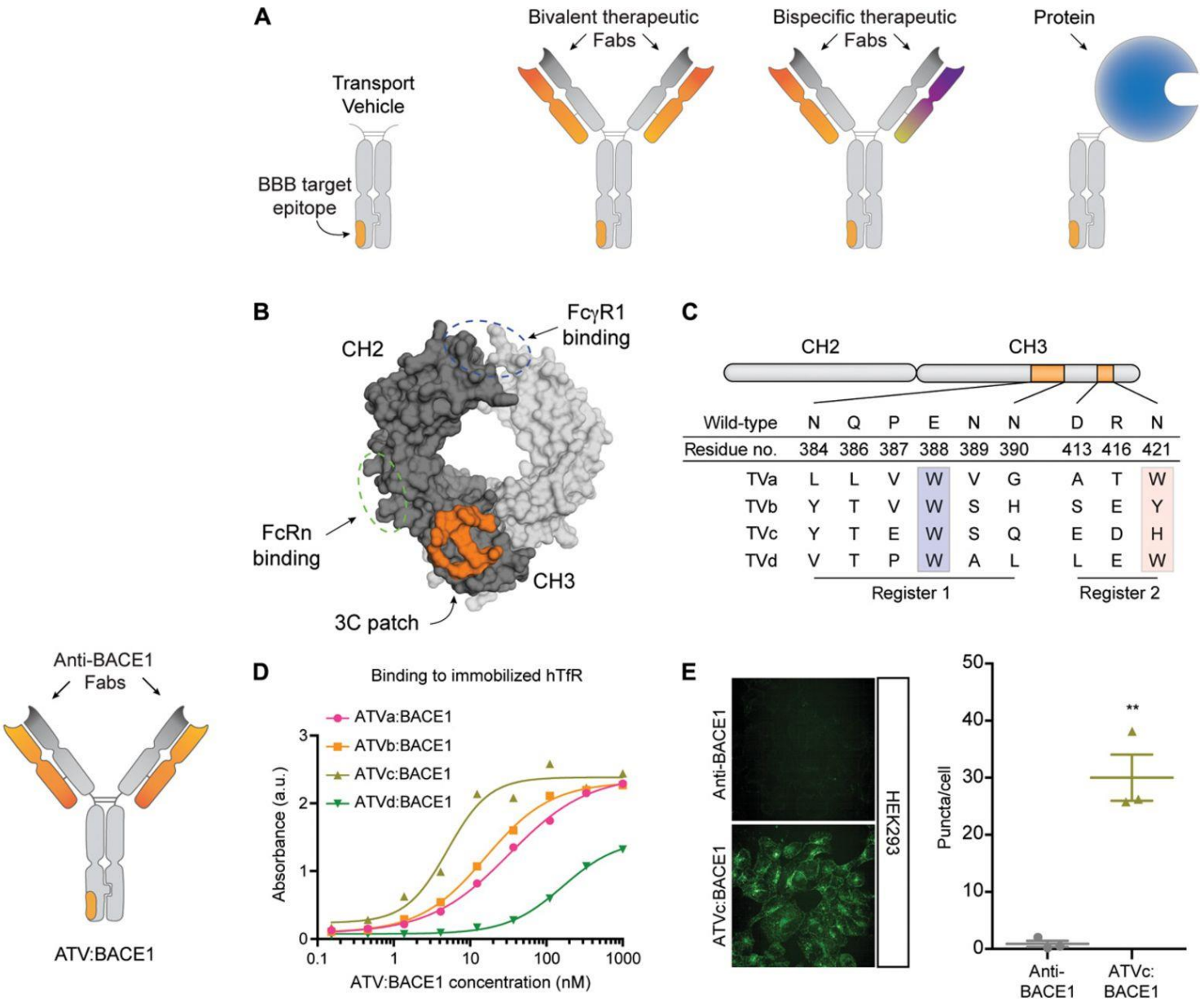
Brain delivery of therapeutic proteins using an Fc fragment blood-brain barrier transport vehicle in mice and monkeys

Mihalis S. Kariolis*†, Robert C. Wells†, Jennifer A. Getz, Wanda Kwan, Cathal S. Mahon, Raymond Tong, Do Jin Kim, Ankita Srivastava, Catherine Bedard, Kirk R. Henne, Tina Giese, Victoria A. Assimon, Xiaocheng Chen, Yin Zhang, Hilda Solanoy, Katherine Jenkins, Pascal E. Sanchez, Lesley Kane, Takashi Miyamoto, Kylie S. Chew, Michelle E. Pizzo, Nicholas Liang, Meredith E. K. Calvert, Sarah L. DeVos, Sulochanadevi Baskaran, Sejal Hall‡, Zachary K. Sweeney, Robert G. Thorne, Ryan J. Watts, Mark S. Dennis, Adam P. Silverman†, Y. Joy Yu Zuchero*†

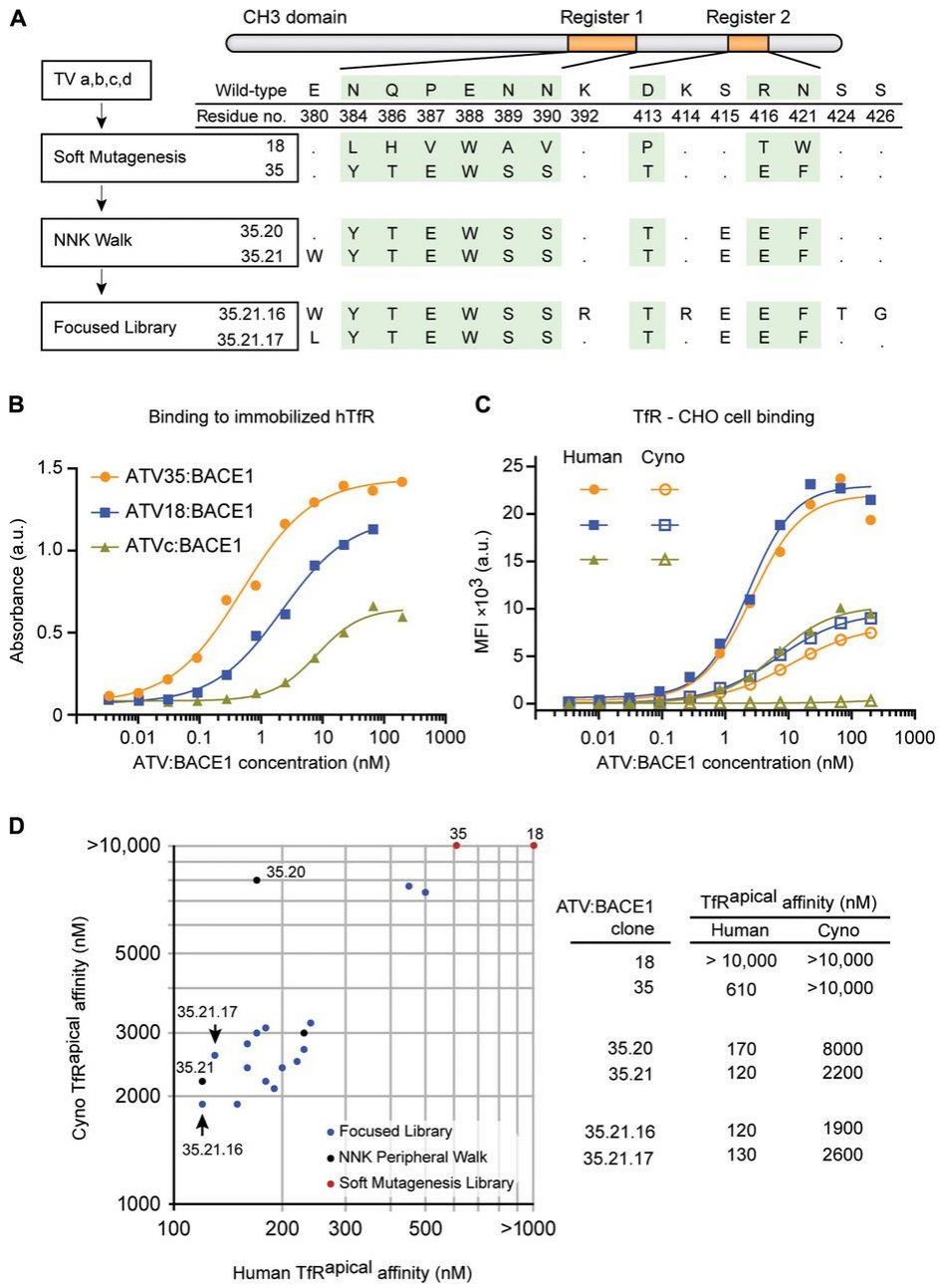
Science Translational Medicine
AAAS

Sci Transl MedVolume 12(545):eaay1359, 2020

Modularity and engineering of the TV platform

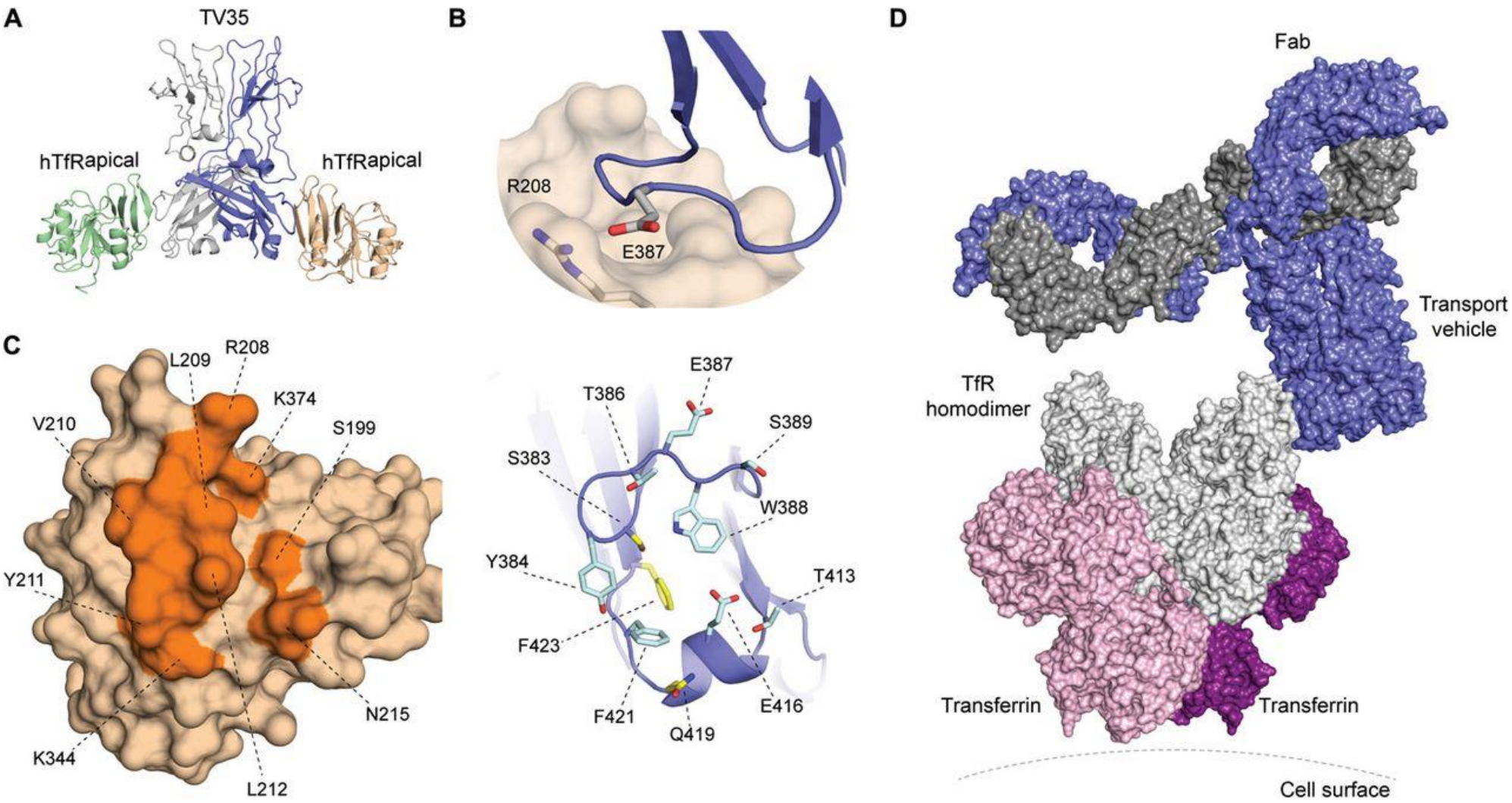


Affinity maturation of TV



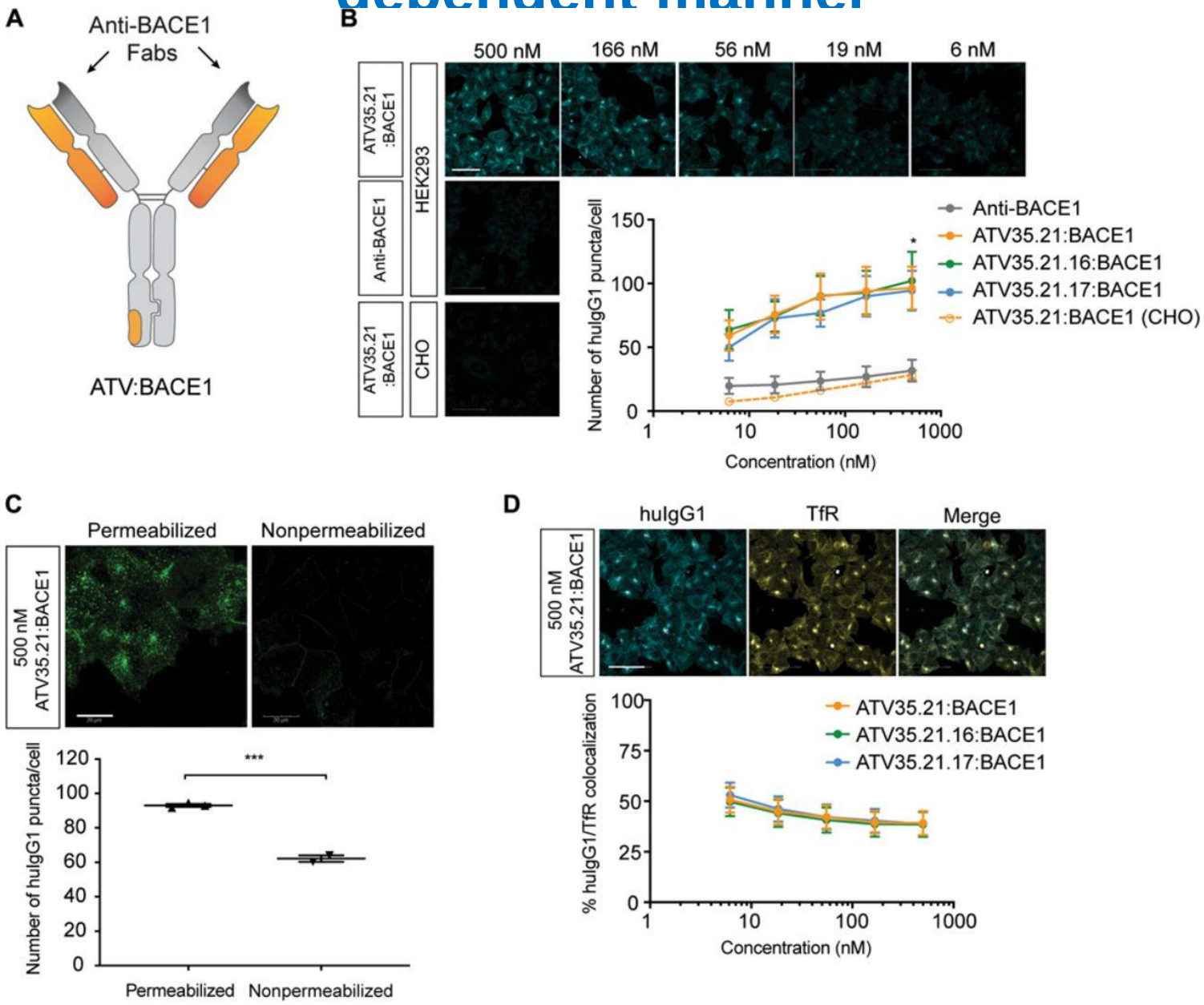
ATV:BACE1 clone	TfR^apical affinity (nM) Human	TfR^apical affinity (nM) Cyno
18	> 10,000	>10,000
35	610	>10,000
35.20	170	8000
35.21	120	2200
35.21.16	120	1900
35.21.17	130	2600

Structural characterization of TV35 in complex with hTfR^{apical}

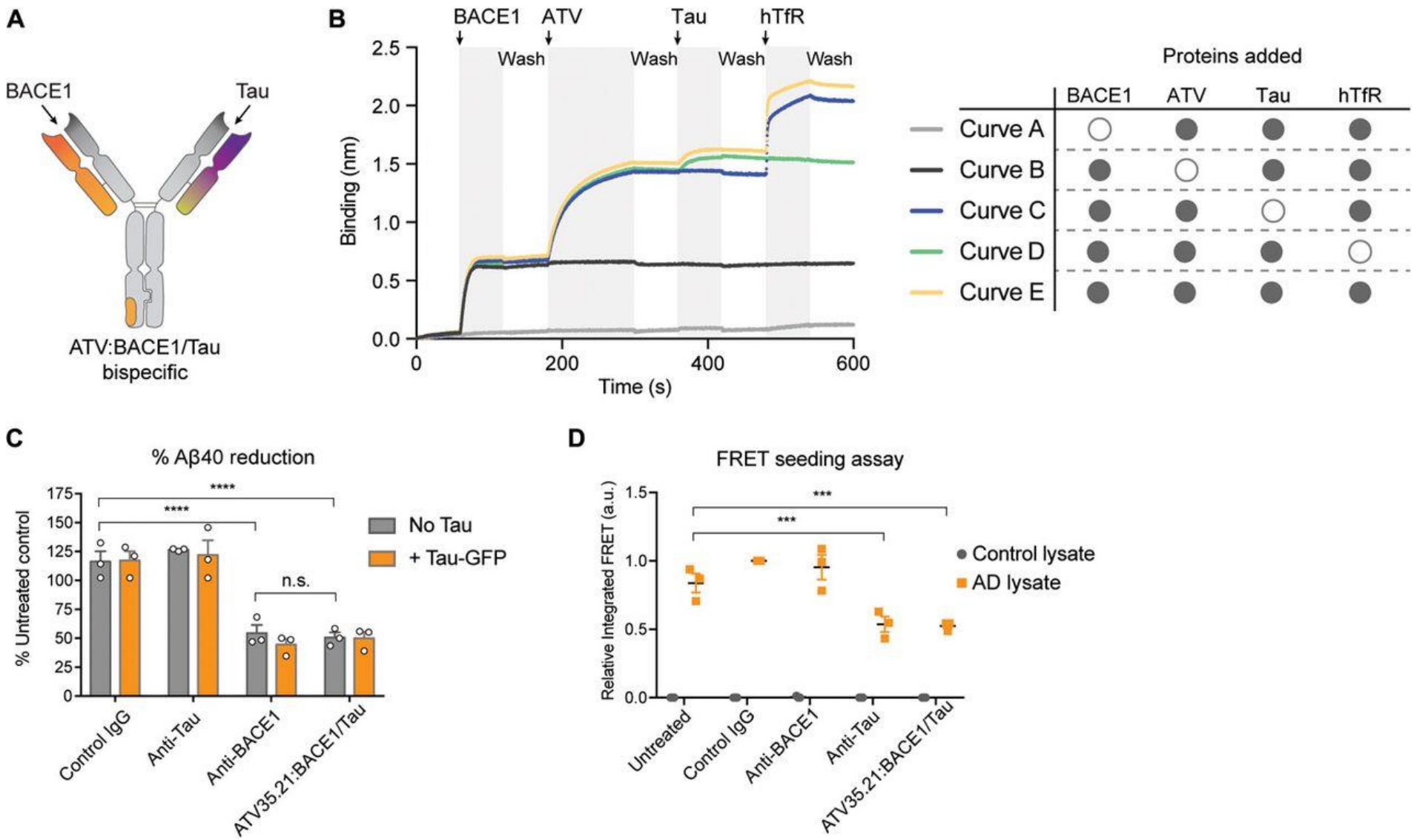


Open book view of the TV35 CH3:hTfR^{apical} binding interface

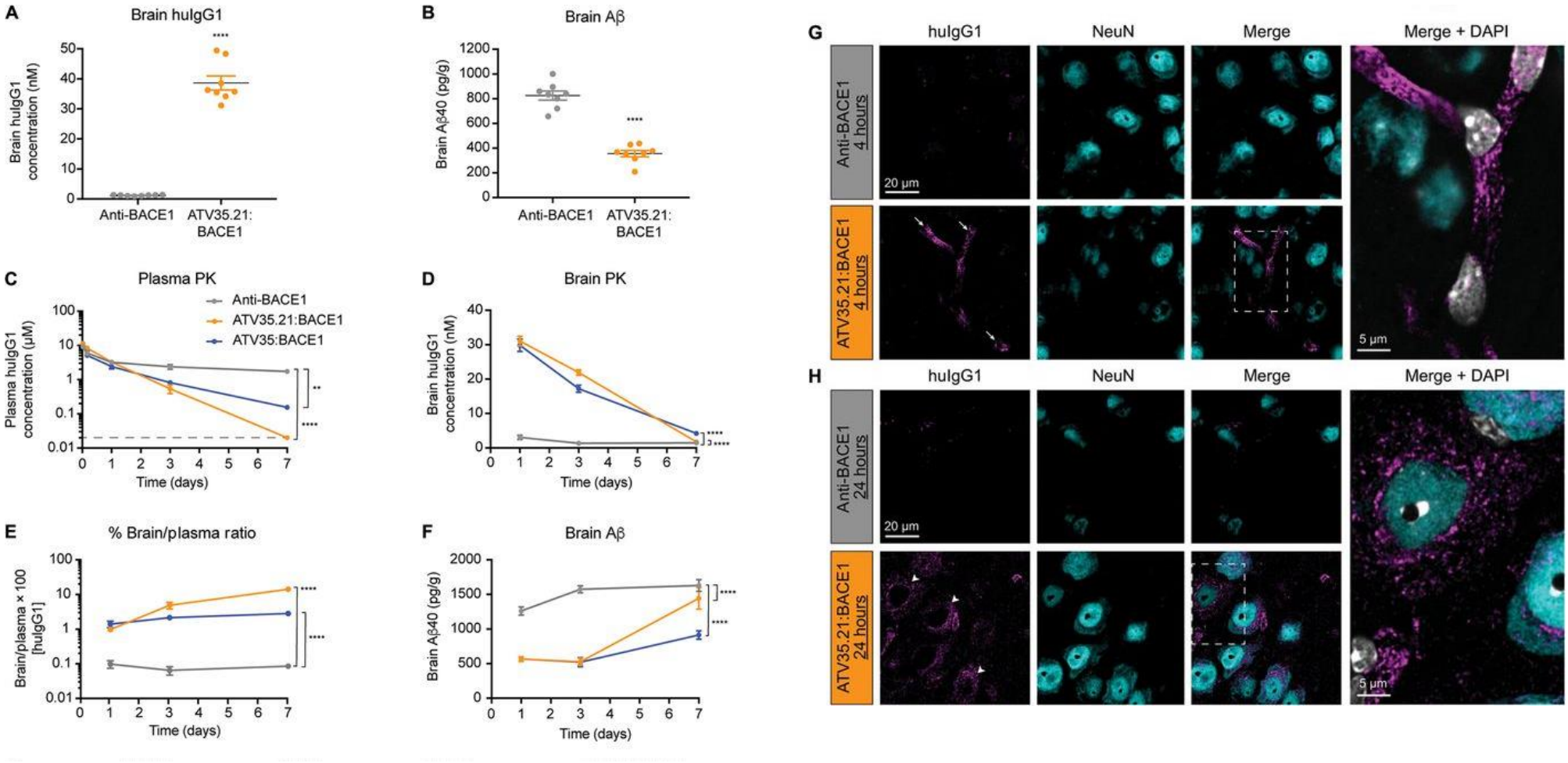
ATV:BACE1 is internalized into human cells in a dose-dependent manner



Example of TV platform applied to a bispecific antibody

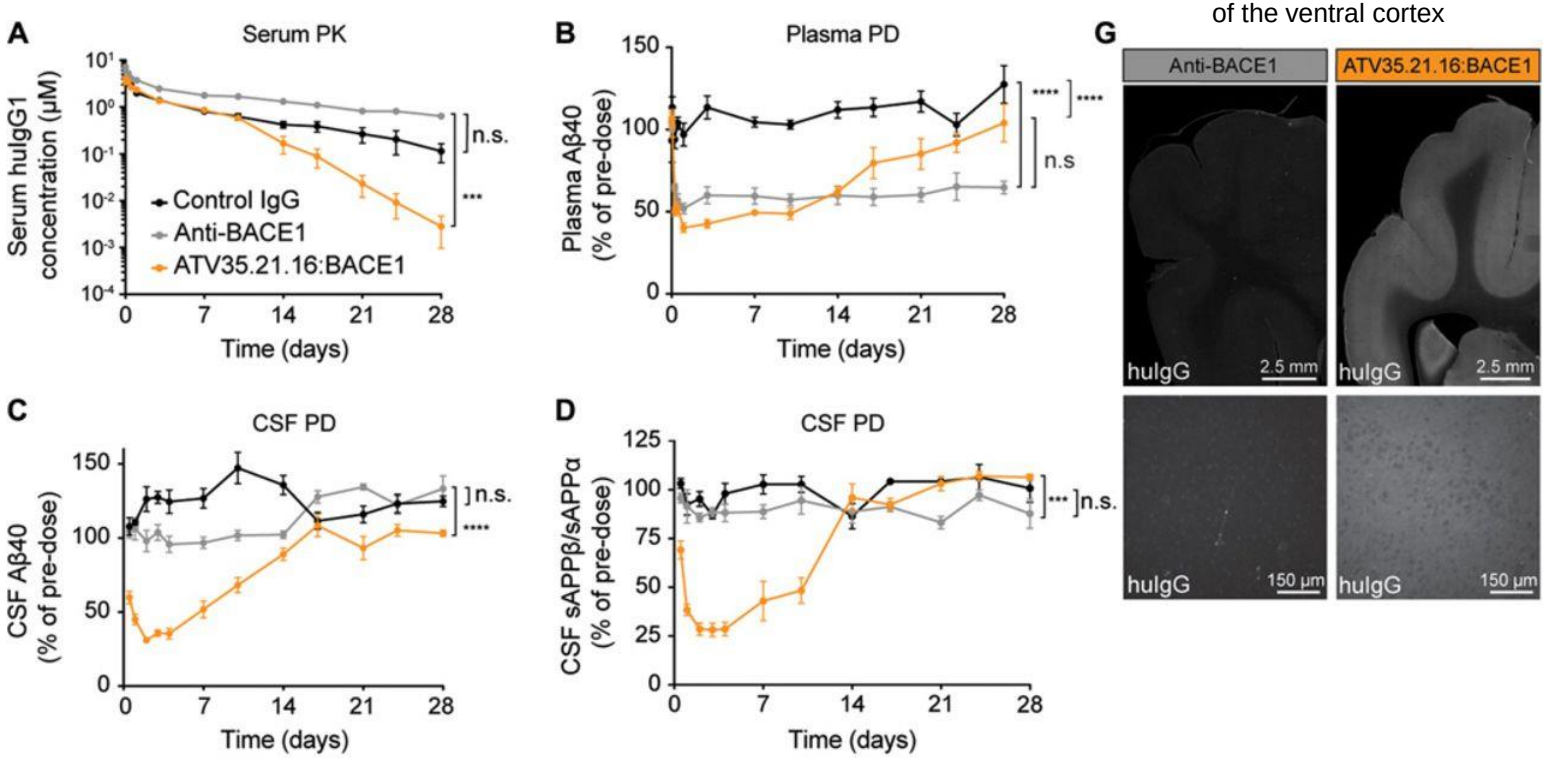


PKPD and brain distribution of ATV:BACE1 in TfRmu/hu KI mice.

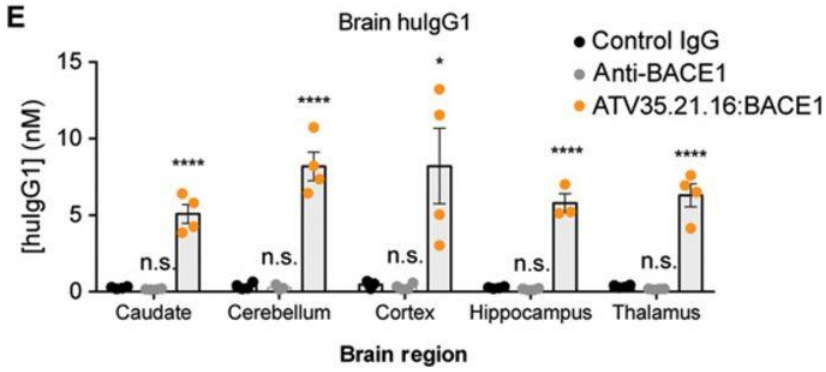


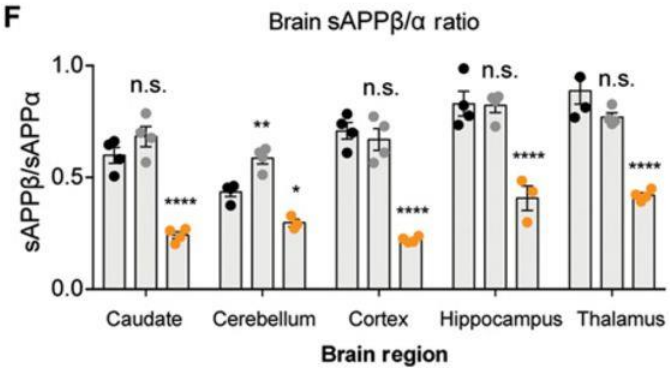
PKPD of ATV:BACE1 in nonhuman primates.

Immunohistochemistry of the ventral cortex



After 48h

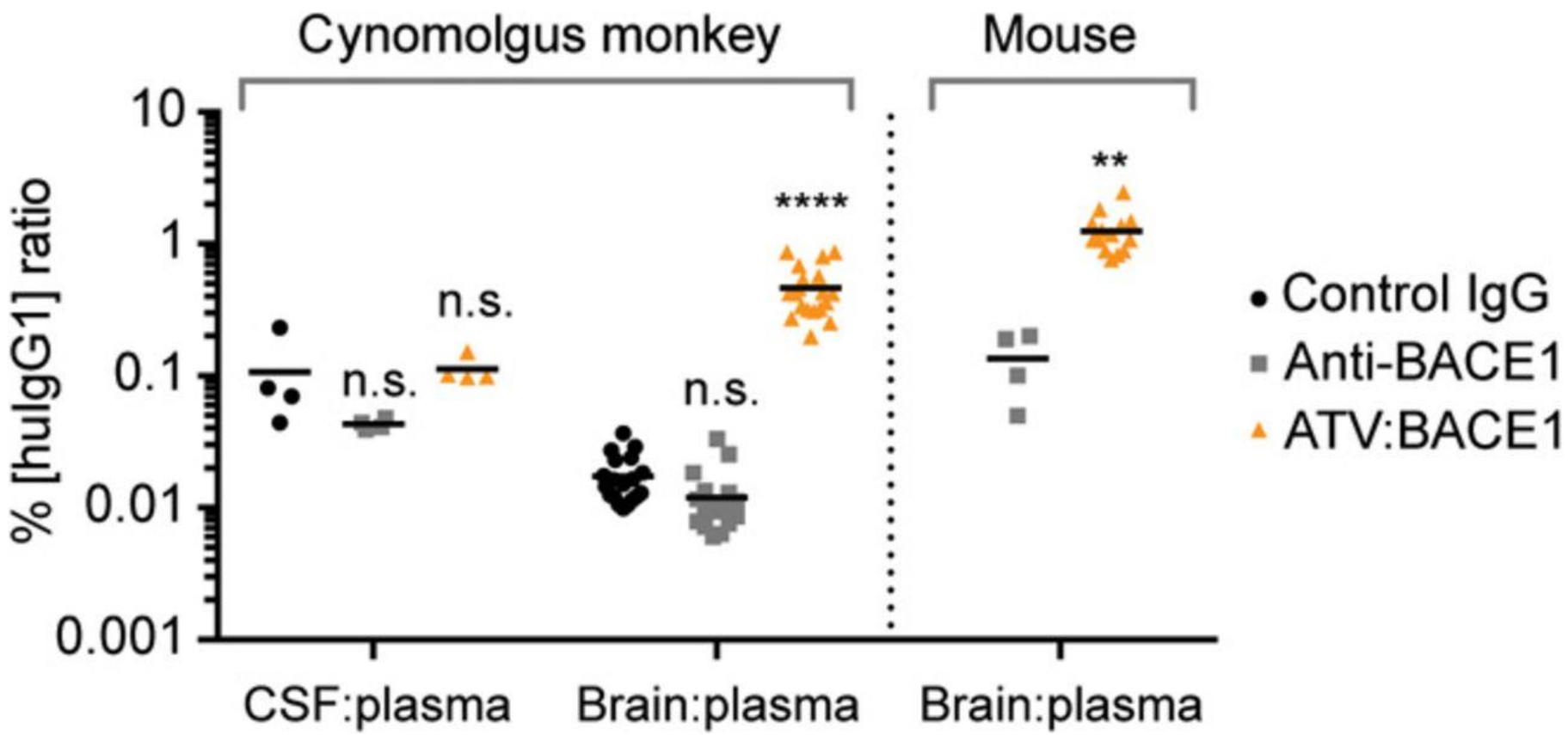




ATV:BACE1 were 26- to 35-fold higher than that of anti-BACE1

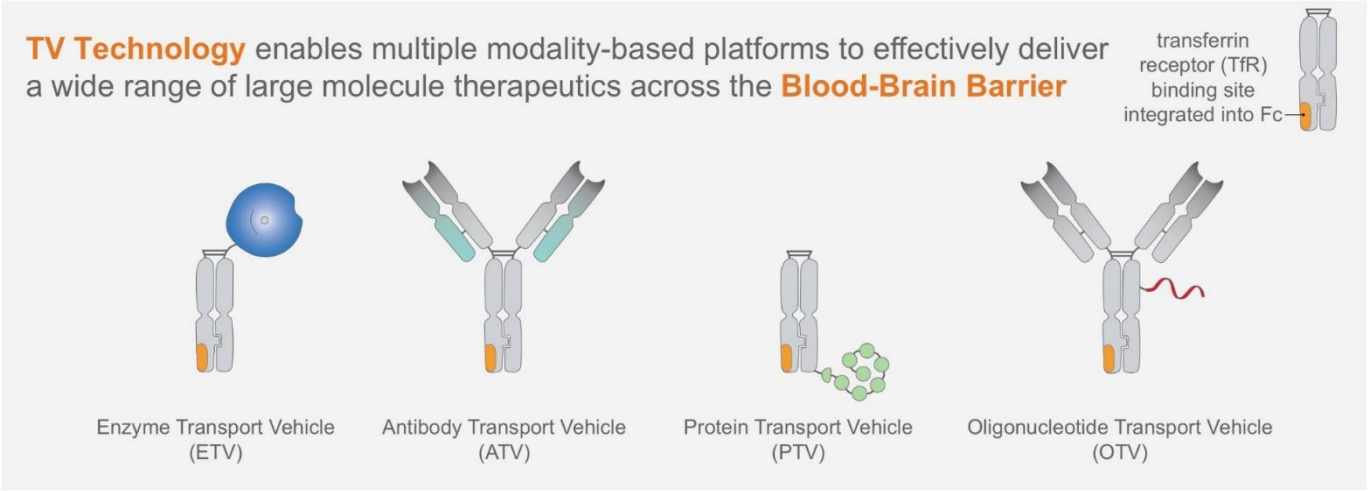
32 to 69% reduction in sAPPβ/α as compared to control IgG

Comparison of plasma and brain exposures across species.



Summary

- A highly modular TV platform that binds a brain endothelial cell–enriched target (TfR) to enable brain delivery of biotherapeutics has been developed
- TVs were engineered using directed evolution to bind the apical domain of the human transferrin receptor (hTfR) without the use of amino acid insertions, deletions, or unnatural appendages.
- A crystal structure of the TV-TfR complex revealed the TV binding site to be away from transferrin and FcRn binding sites, which was further confirmed experimentally in vitro and in vivo.
- Recombinant expression of TVs fused to BACE1 Fabs yielded antibody transport vehicle (ATV) molecules with native immunoglobulin G (IgG) structure and stability.
- Peripheral administration of anti-BACE1 ATVs to hTfR-engineered mice and cynomolgus monkeys resulted in substantially improved CNS uptake and sustained pharmacodynamics responses.



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Article
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Identification and Characterization of DNA Aptamers Specific for Phosphorylation Epitopes of Tau Protein

I-Ting Teng,[⊥,†] Xiaowei Li,[⊥,†] Hamad Ahmad Yadikar,[§] Zhihui Yang,[§] Long Li,[†] Yifan Lyu,[‡] Xiaoshu Pan,[†] Kevin K. Wang,[*,§,∥] and Weihong Tan[*,†,‡]

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Article

Enhanced in Vivo Blood−Brain Barrier Penetration by Circular Tau−Transferrin Receptor Bifunctional Aptamer for Tauopathy Therapy

Xiaowei Li, Yu Yang, Hengzhi Zhao, Tian Zhu, Zhihui Yang, Haiyan Xu, Yueqiang Fu, Fan Lin, Xiaoshu Pan, Long Li, Cheng Cui, Min Hong, Lu Yang, Kevin K. Wang,* and Weihong Tan*

Cite This: J. Am. Chem. Soc. 2020, 142, 3862−3872
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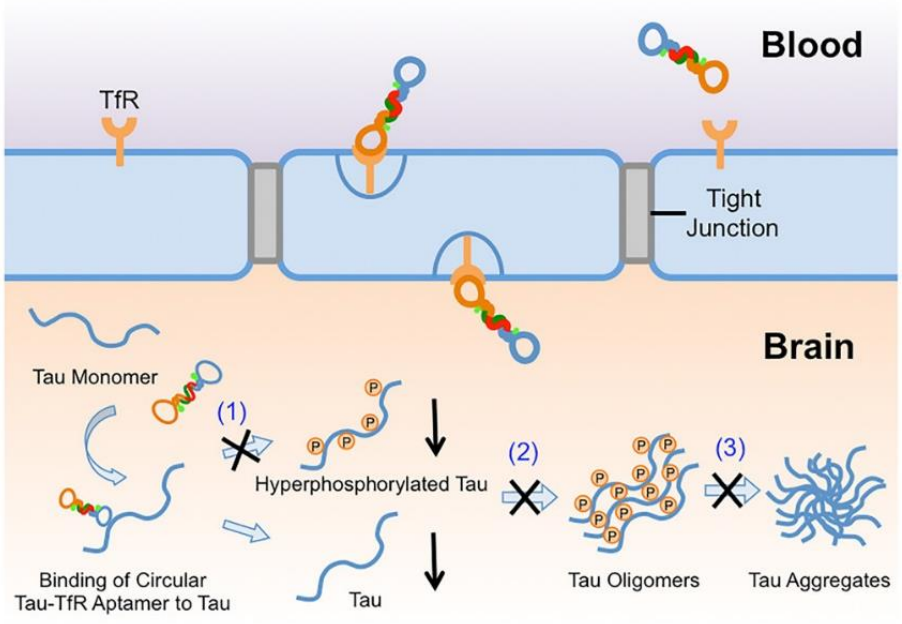
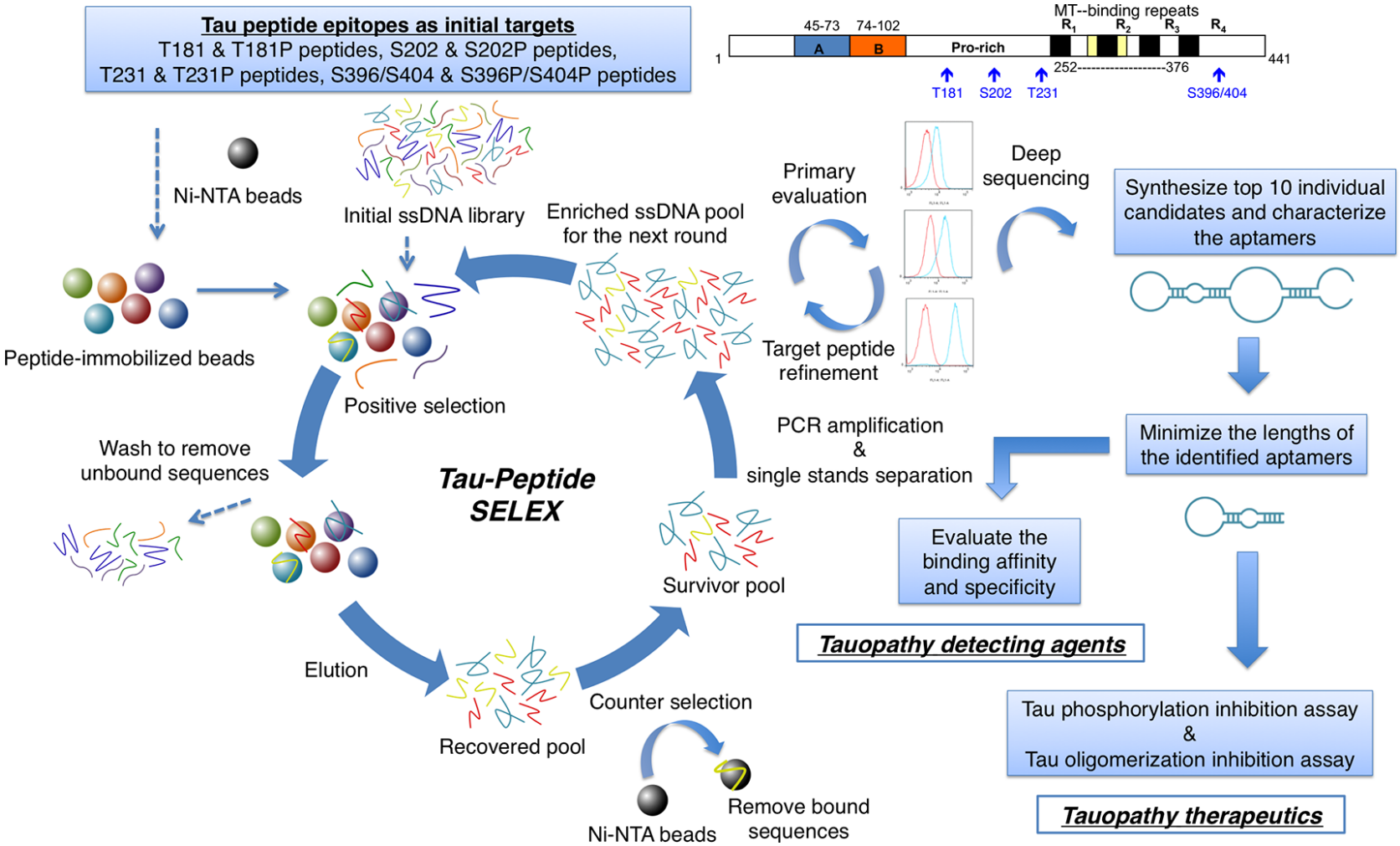
Introduction

- To tackle the problems of current approaches to the treatment of tauopathies, such as low specificity toward Tau protein, difficult quality control, and inefficient BBB penetration, aptamers capable of selective binding to defined targets are being considered as an alternative therapeutic strategy.
- Aptamers capable of selective binding to defined targets are being considered as an alternative therapeutic strategy.
- Chemically synthesized aptamers can be reproducibly scaled up, and they are more readily modified with functional groups to meet specific therapeutic and diagnostic needs.
- Emerging aptamer probes relevant to the central nervous system (CNS) appear to display protective benefits in the context of neuronal functions or neurodegeneration, as well.

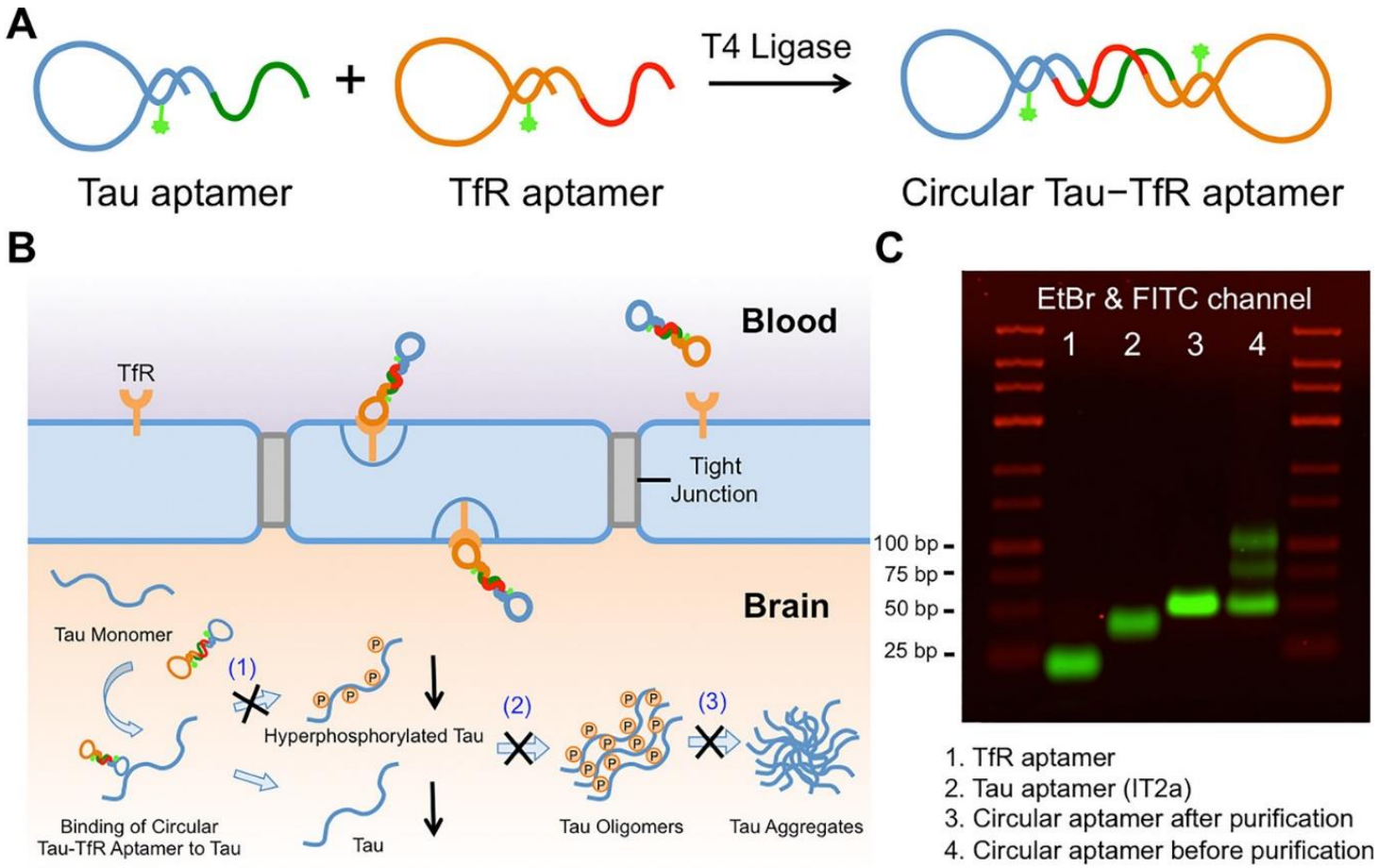


Tau aptamer discovery by "Systematic Evolution of Ligands by EXponential enrichment (SELEX)" and characterization workflow

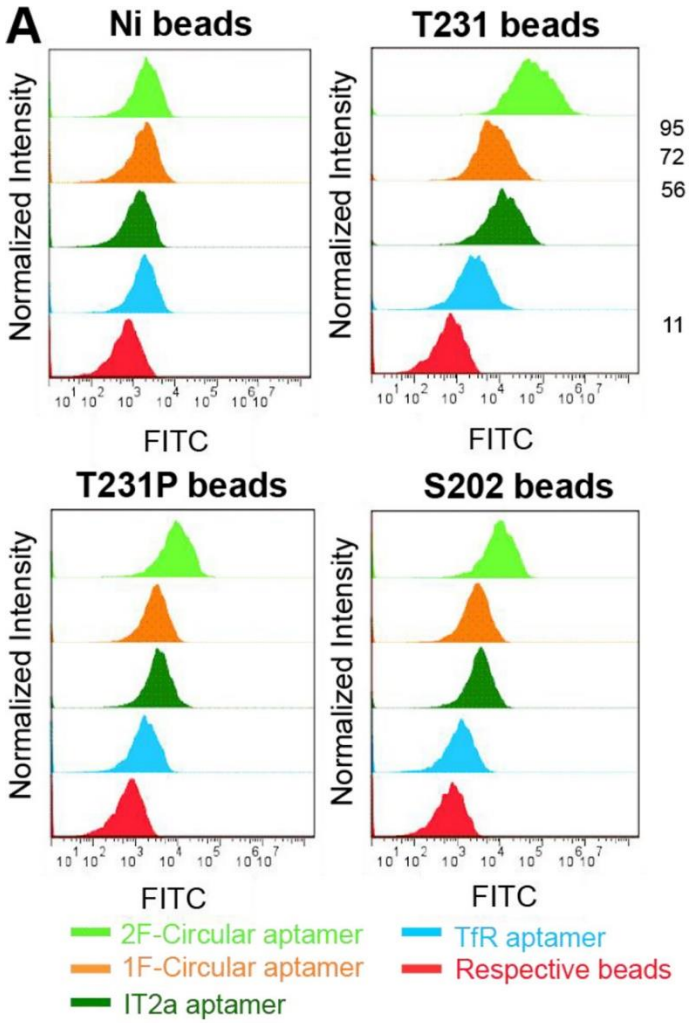


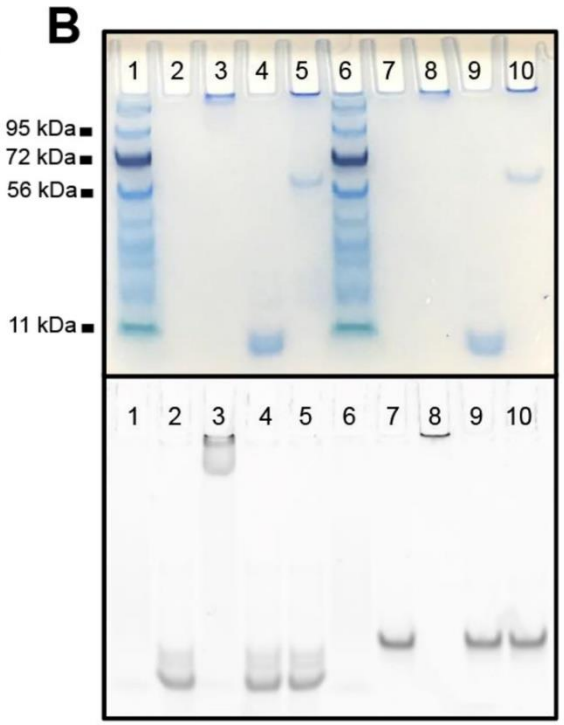
Teng et al., JACS 2018

Schematic of formation of circular Tau and transferrin receptor (TfR) bispecific aptamer



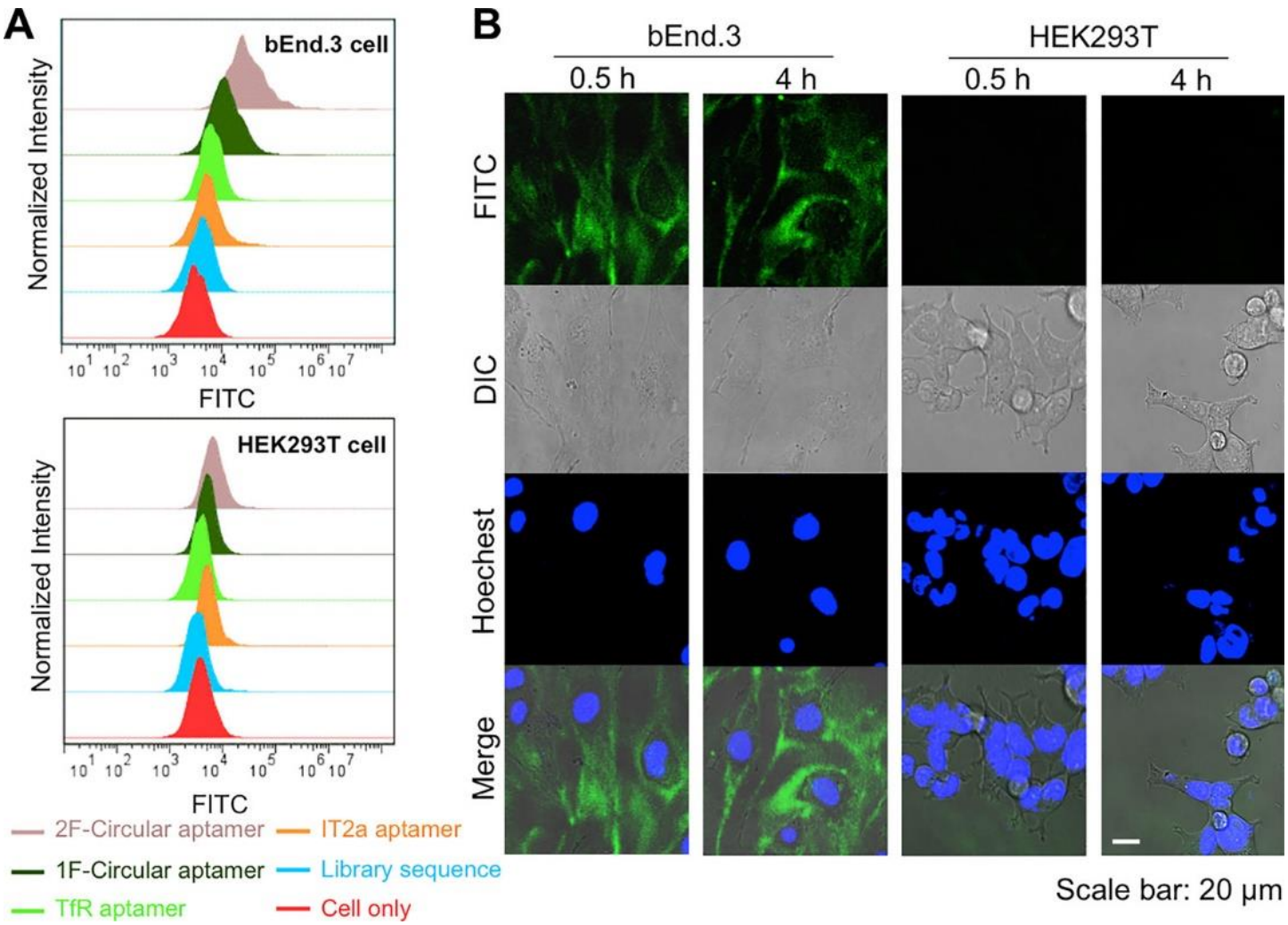
Specific binding tests of Tau, TfR, and Tau–TfR bispecific aptamers against Tau 441 protein and Tau peptide targets



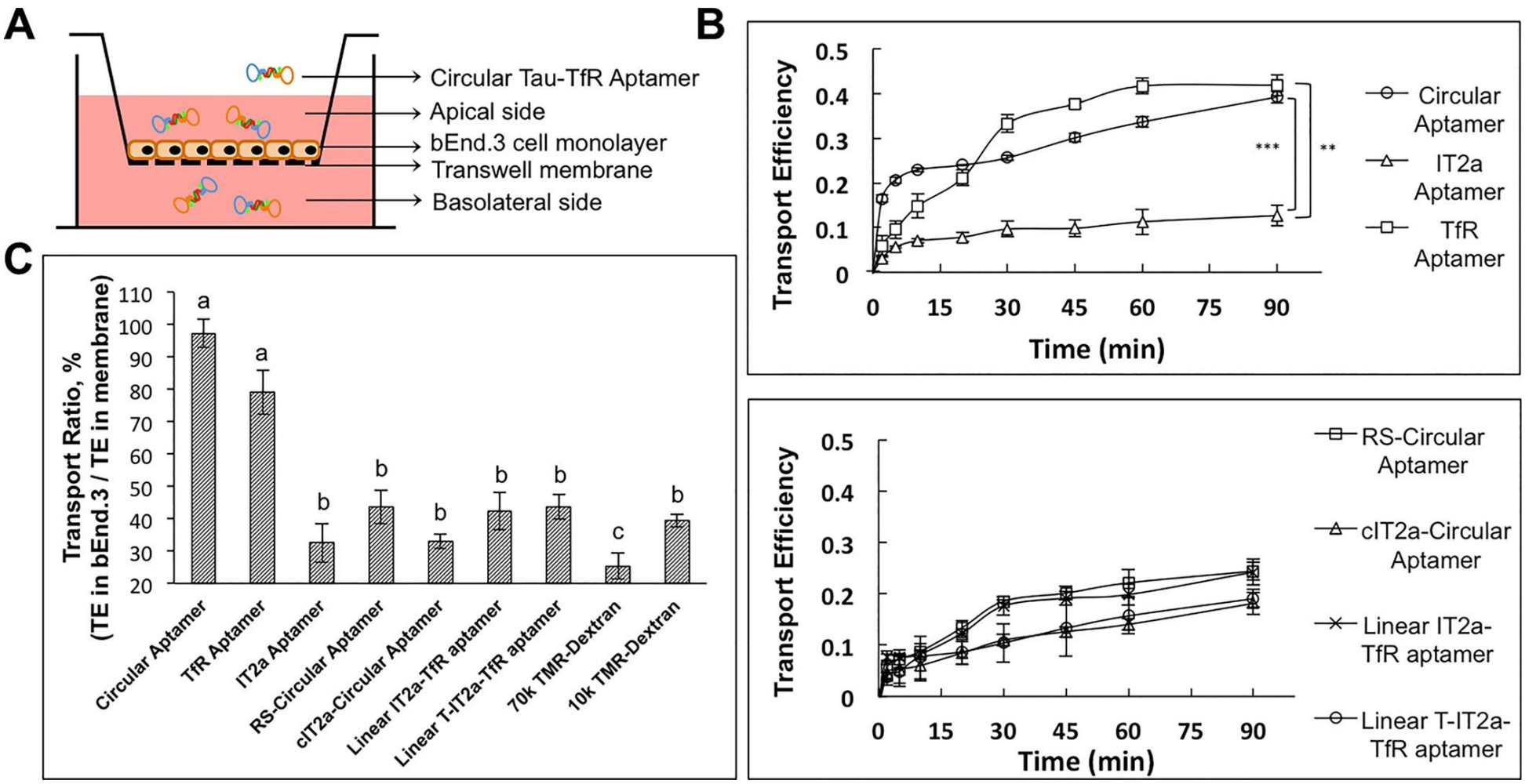


1, 6. Ladder
2. IT2a aptamer
3. IT2a aptamer + Tau441 (67 kDa)
4. IT2a aptamer + S100B (9 kDa)
5. IT2a aptamer + BSA (66 kDa)
7. Circular aptamer
8. Circular aptamer + Tau411 (67 kDa)
9. Circular aptamer + S100B (9 kDa)
10. Circular aptamer + BSA (66 kDa)

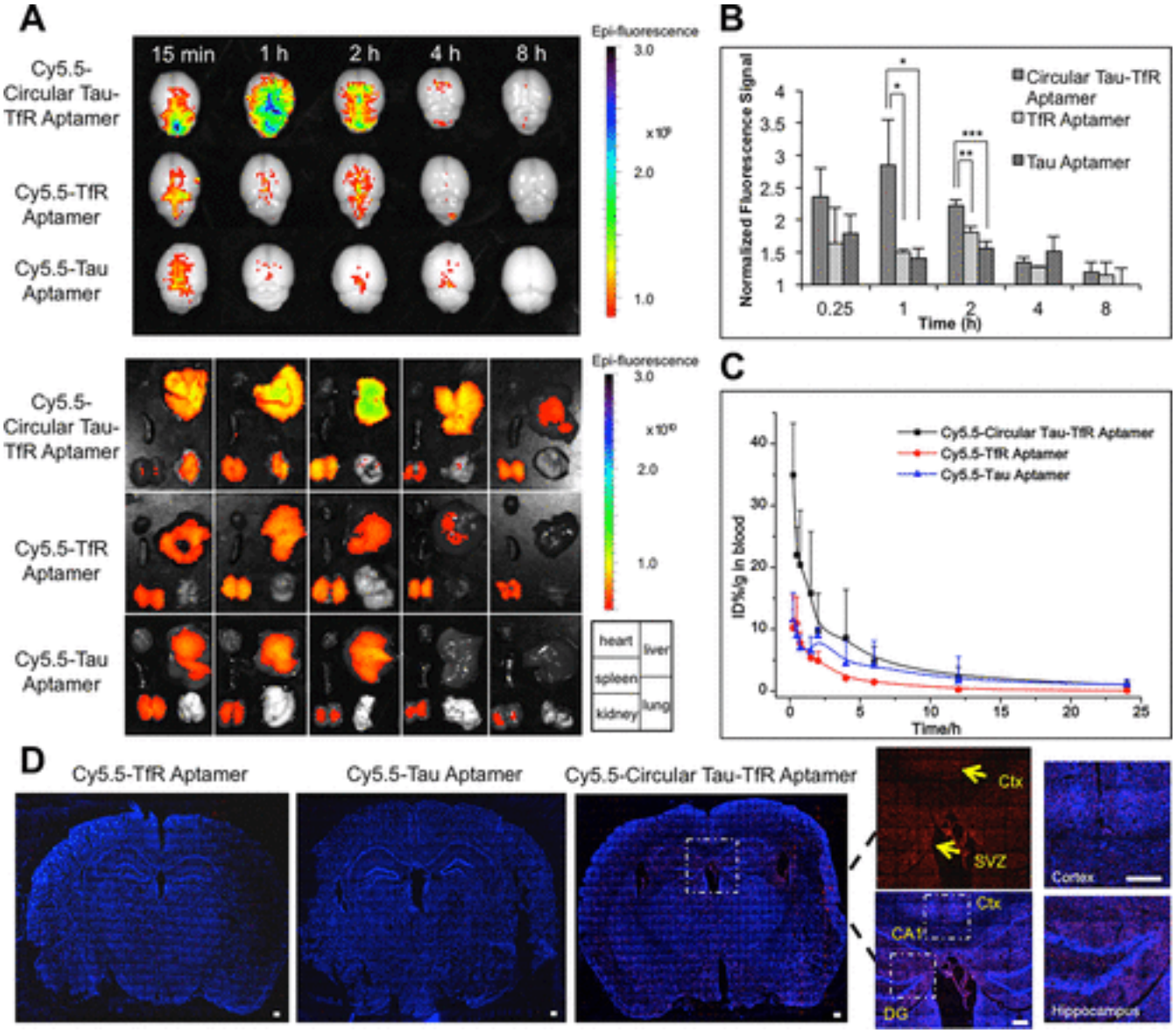
Flow cytometry and confocal microscopy analysis



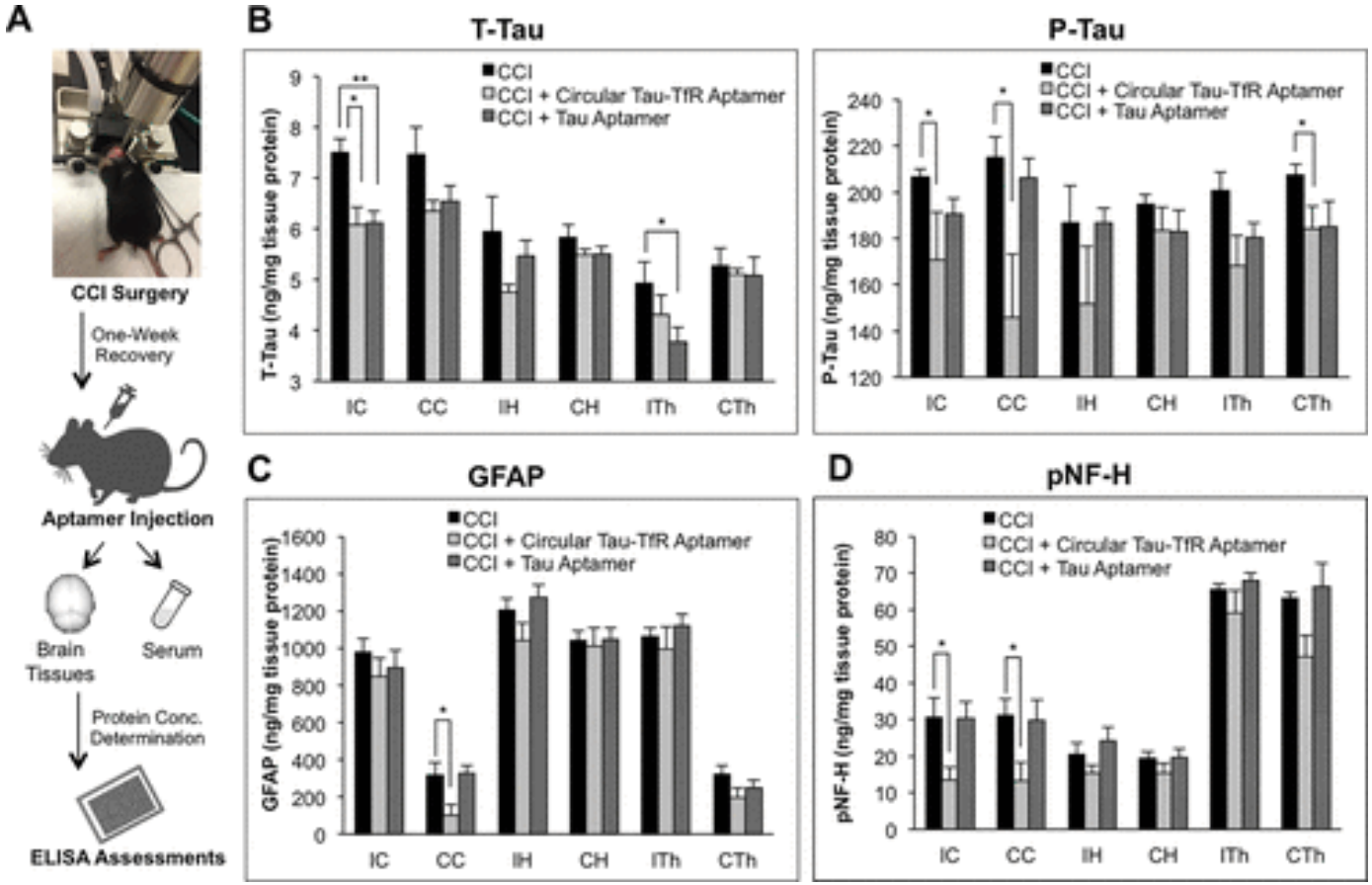
Examination of circular bispecific aptamer crossing the in vitro BBB model



Brain and blood exposure to circular Tau–TfR bispecific aptamers.

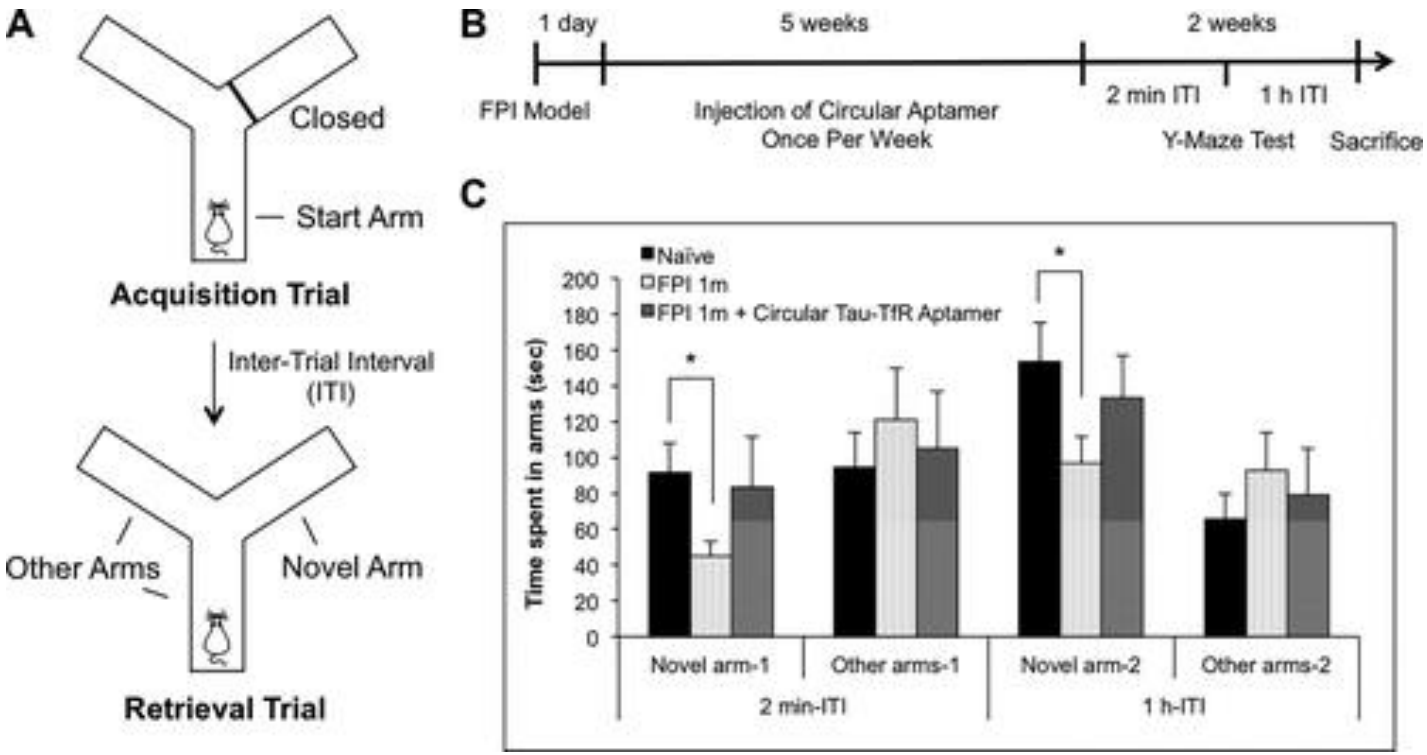


ELISA assessment of TBI-related biomarker levels in different brain tissues of untreated or aptamer-treated hTau mice after CCI surgeries



IC/CC, injured/control cortex; IH/CH, injured/control hippocampus; ITh/CTh, injured/control thalamus.

Y-maze behavior test to evaluate the effects of circular aptamer on improving spatial memory of hTau mice



Summary

- The synthesis of a circular bifunctional aptamer to enhance the in vivo BBB penetration for better tauopathy therapy has been reported.
- The circular aptamer consists of one reported transferrin receptor (TfR) aptamer to facilitate TfR-aptamer recognition-induced transcytosis across BBB endothelial cells, and one Tau protein aptamer
- This novel circular Tau−TfR bifunctional aptamer displays significantly improved plasma stability and brain exposure
- It also exhibit the ability to disrupt tauopathy and improve traumatic brain injury (TBI)-induced cognitive/memory deficits in vivo, providing important proof-of-principle evidence that circular Tau−TfR aptamer can be further developed into diagnostic and therapeutic candidates for tauopathies.

Distinct engineering of brain cargos might lead to improved properties

Adptamers

- Small
- Production can easily be scaled up
- High specificity and affinity
- High chemical stability
- Low immunogenicity
- Able to target specifc protein-protein interactions
- Can be easily be modified with functional groups

Transport vehicle (TV)

- **Natural IgG Fc structure** without linkers or appended sequences
- **Monovalent, optimized affinity binding** to avoid vascular trapping and TfR degradation
- Ability to introduce or silence **effector function**
- Ability to deliver antibodies (**ATV**), enzymes (**ETV**), proteins (**PTV**), and ASOs (**OTV**)

Higher brain exposure

Greater modularity

Longer half-life

Better safety

Both approaches need further validation in clinical studies, but these technologies might translate into suitable therapeutics for several diseases.

Thank you for your attention!