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Bicyclic peptidomimetics enable efficient transport of proteins across brain endothelium

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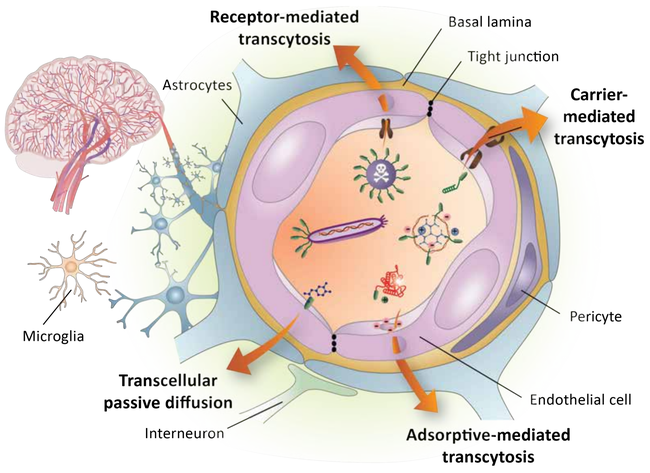
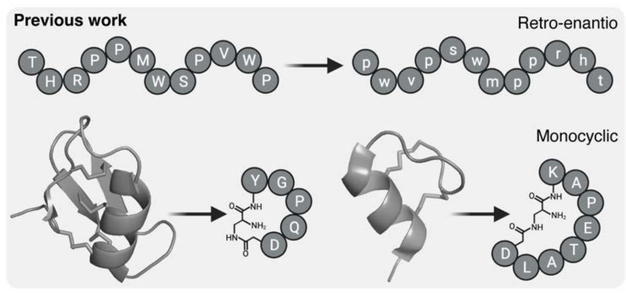
Brain shuttle peptides

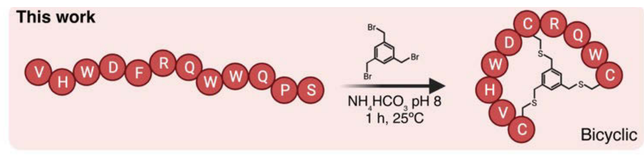


Transport across the **blood-brain barrier** (BBB) is one of the most challenging obstacles in the development of biotherapeutics for brain diseases. **Brain shuttles** are molecules capable of transporting cargoes across the BBB by hijacking endogenous transport mechanisms, including receptor-mediated transcytosis.[1] Most protease-resistant peptide brain shuttles available have low affinity for target receptors & limited protein transport capacity.[2-4]



Objectives

The main goal of this study was to obtain a novel family of bicyclic protease-resistant peptide shuttles, **BrainBikes**, targeting the **transferrin receptor 1 (TfR1)**. We aimed to develop shuttles with high resistance to proteases and suitable affinity for the **transport of therapeutic proteins**.

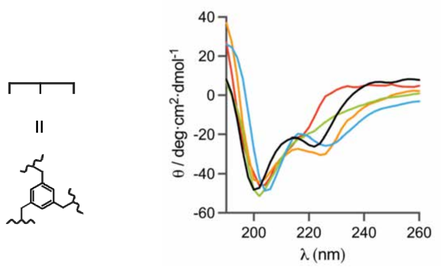


BrainBikes: a new family of brain shuttles

Design of bicyclic peptidomimetics

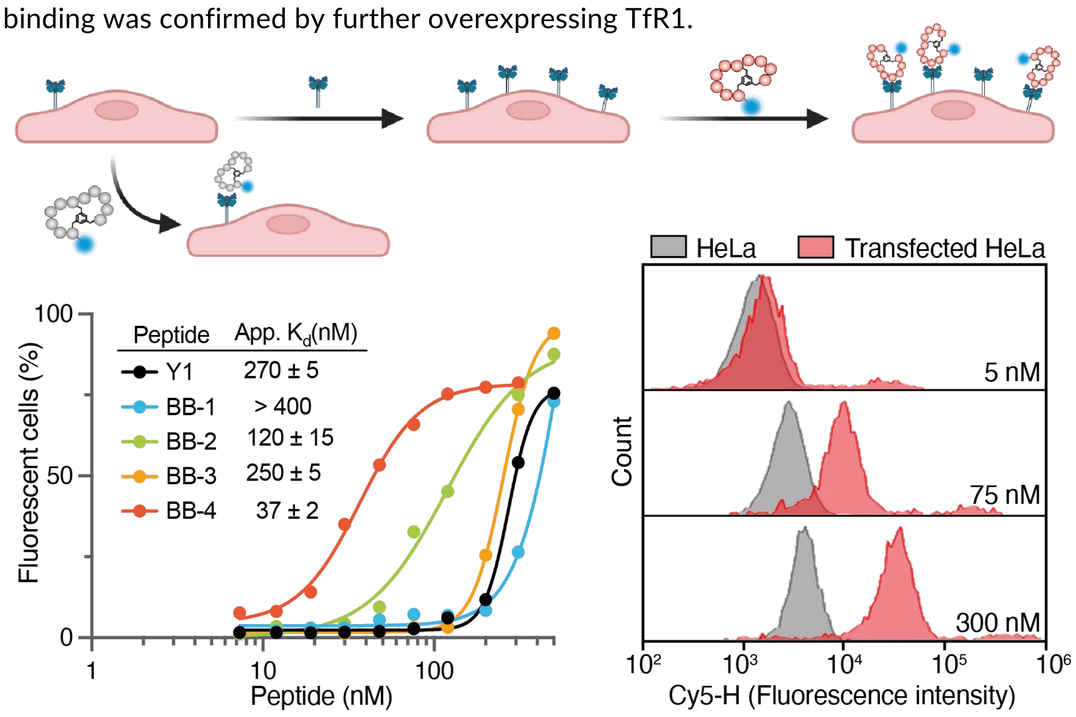
Starting from a sequence identified via phage display against TfR1, Y1, bicyclic variants were obtained by reacting three cysteines with a trifunctional linker. Several analogs were designed enhancing or disrupting the alpha helical conformation of Y1.

Y1	H - V H W D F R Q W W Q P S - NH_2
BrainBike-1	H - **C** V H W D **C** R Q W W Q P S **C** - NH_2
BrainBike-2	H - **C** V H W D F R Q W **C** Q P S **C** - NH_2
BrainBike-3	H - **C** V H W D F R **C** Q W W Q P S **C** - NH_2
BrainBike-4	H - **C** V H W D **C** R Q W **C** - NH_2



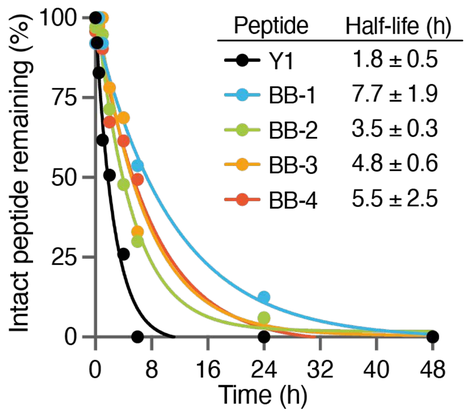
Affinity for cells with high levels of TfR1

All bicyclic peptidomimetics display affinity for cells with high levels of TfR1, especially those with the least alpha helical character. BrainBike-4 (BB4) shows a **7-fold increased cell association** with respect to the parent peptide. Even higher binding was confirmed by further overexpressing TfR1.



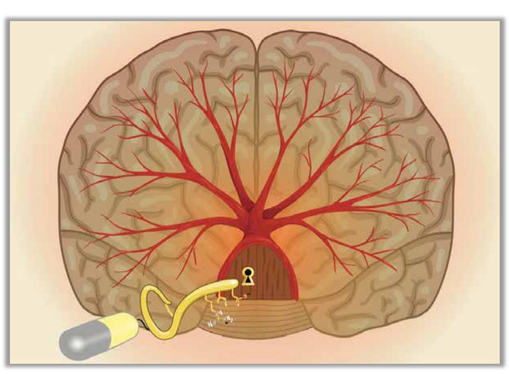
Stability to serum proteases

BrainBikes present **high metabolic resistance**, with half-lives ranging from 3.5 to 7.7 h in human serum. Bicyclization leads to 2- to 4-fold increase in half-life with respect to the parent peptide.



What next?

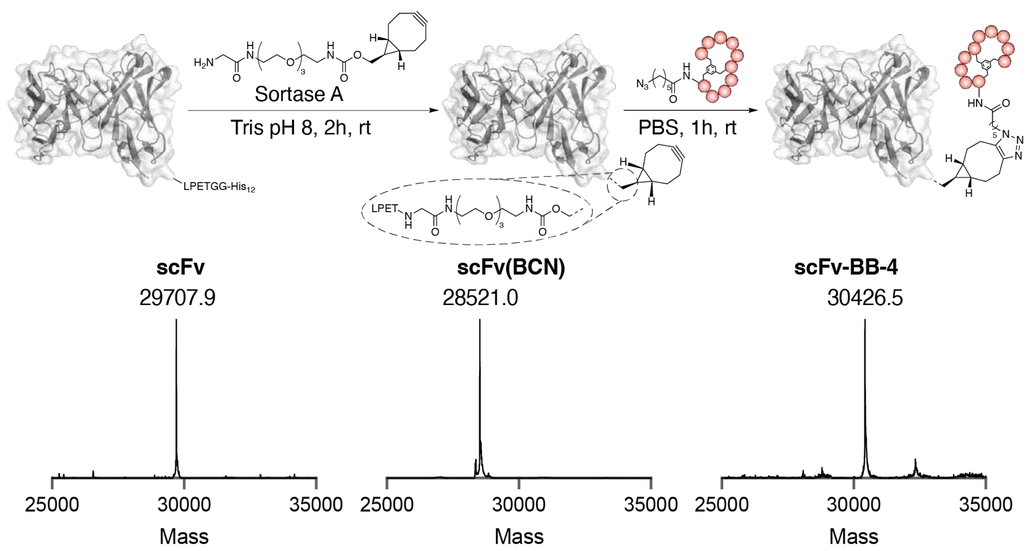
Brain shuttle development is limited by little knowledge on receptor-mediated transcytosis. We are currently developing orthogonal transport systems to shed light on this transport mechanism.



BrainBike-4 increases the transport of proteins

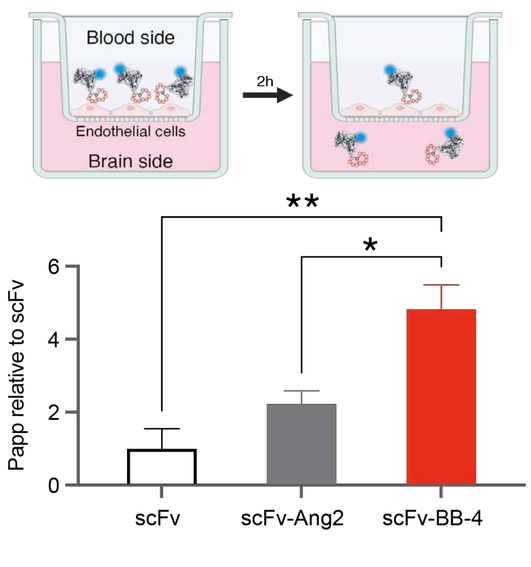
Site-specific conjugation of BrainBike-4 to a single chain antibody

BBB-shuttle peptides were conjugated to a single chain variable fragment (scFv) against a brain tumour target via chemoenzymatic ligation. Peptides were derivatized with an *N*-terminal azide and conjugated to the scFv bearing a complementary reactive handle via an inverse electron-demand Diels-Alder cycloaddition.



Transport of an scFv-BB4 conjugate across brain endothelium

The transport of the conjugate was assessed on a tight monolayer of human brain endothelial cells seeded on Transwell® membranes. **BrainBike-4 enables a 5-fold increase** in the transport of the scFv. This is significantly higher than the transport enhancement provided by Angiopep-2, the current gold-standard peptide brain shuttle. A similar enhancement was observed for other proteins such as GFP.



Conclusions and perspective

Here we report a new class of bicyclic brain shuttle peptidomimetics, BrainBikes. We have shown that linear peptide shuttles binding TfR1 can be turned into BrainBikes utilizing a trifunctional linker. These peptides have enhanced half-lives and BrainBike-4 presents 7-fold higher affinity for cells expressing high levels of TfR1. BrainBike-4 site-specifically conjugated to an scFv enables a 5-fold increase in the transport capacity in a cell-based model of the BBB.[5] We are now assessing the transport of therapeutic protein conjugates *in vivo* and developing new brain shuttles.

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