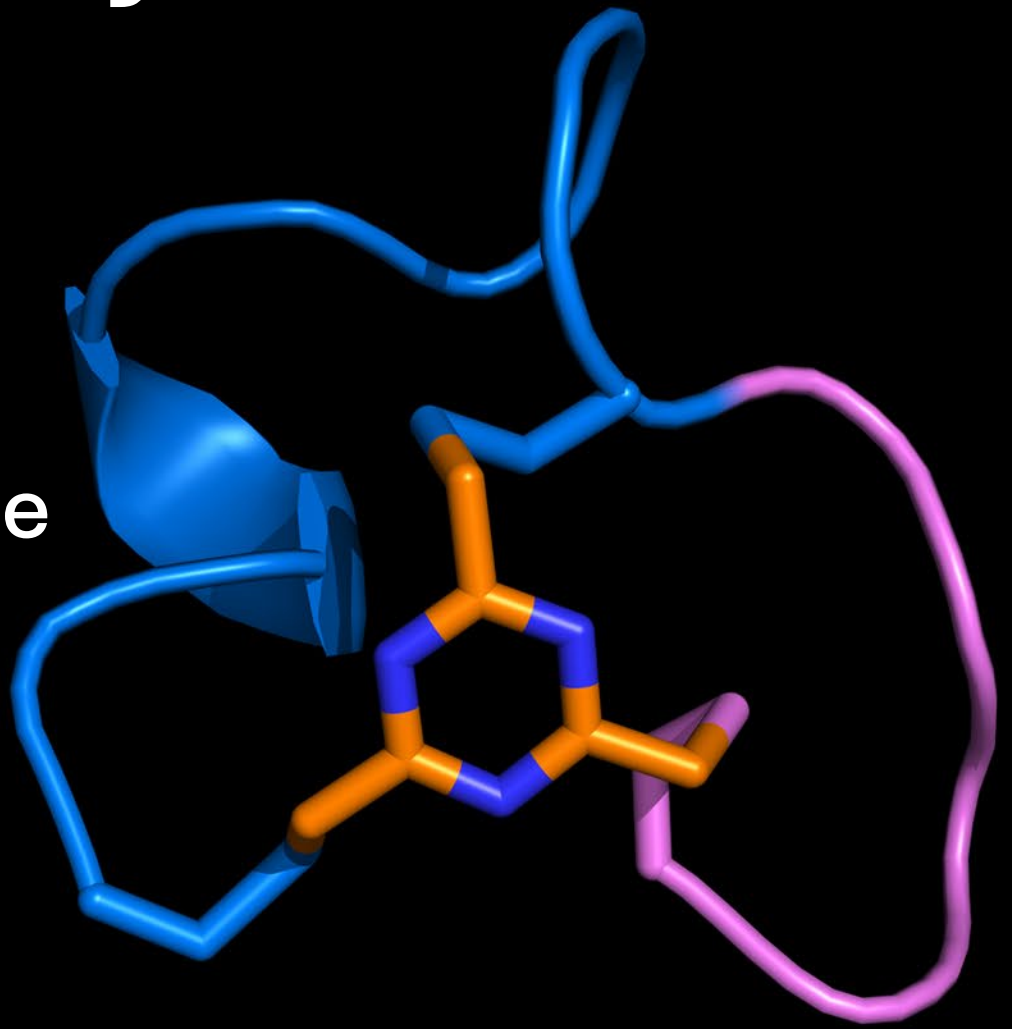
Bicyclic peptides (*Bicycles*) as novel multipurpose delivery systems

Inma Rioja, SVP Biology

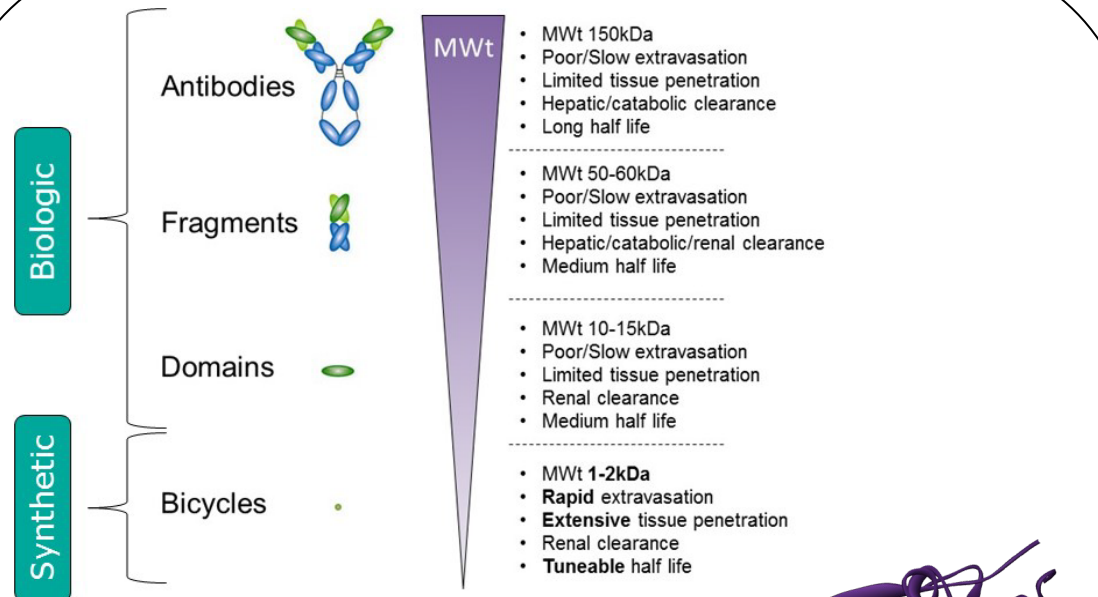
The BNA 2023
International Festival of Neuroscience



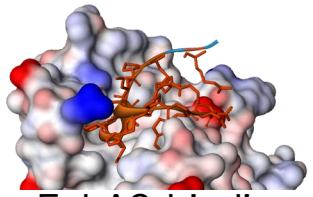
Bicycle®

Bicycle Therapeutics

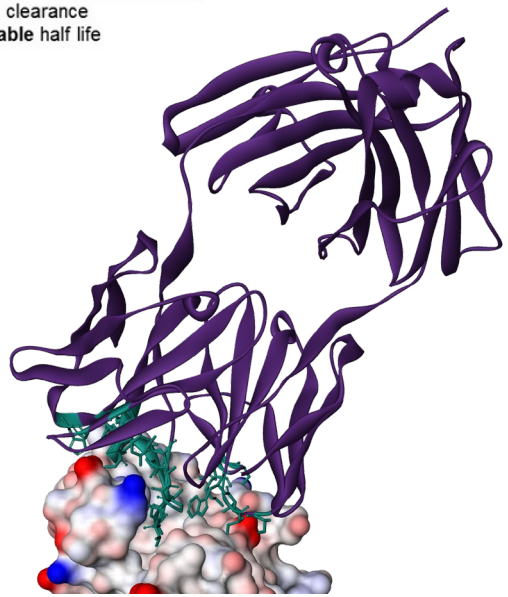
- Clinical-stage biopharma company pioneering *Bicycles*, a new differentiated class of innovative medicines.
- 236 FTEs at Dec 31 2022
- Based in Cambridge (UK) & Boston (USA)
- Unique therapeutic modality:
  - applicable to multiple therapeutic areas
  - enabling to other technologies in delivering other modalities
- Five molecules in the clinic:
  - demonstrated clinical activity
  - demonstrated safety & tolerability



	Bicycles	Fab
Weight	2.3 kDa	48 kDa
Size	19 aa	445 aa
Binding residues	16 aa (85%)	24 aa (5%)

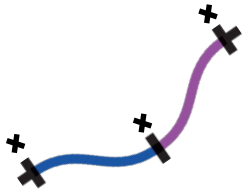


EphA2-binding *Bicycle®*



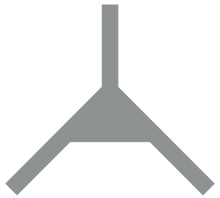
EphA2-binding **Fab**

Bicycle®, a unique & disruptive therapeutic modality



Short linear peptide

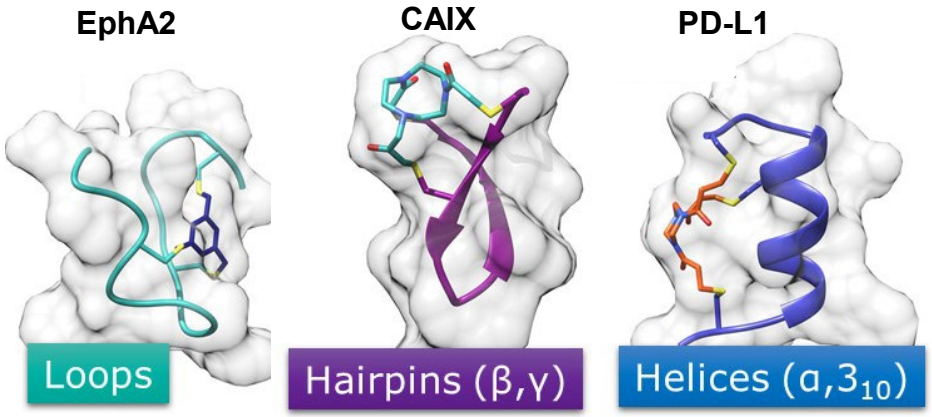
+



Scaffold

- **High affinity and selectivity in a small, fully synthetic format**

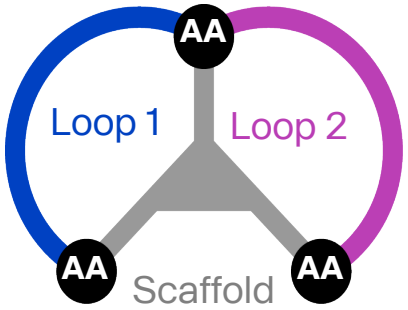
- **Biologically relevant tertiary structures**

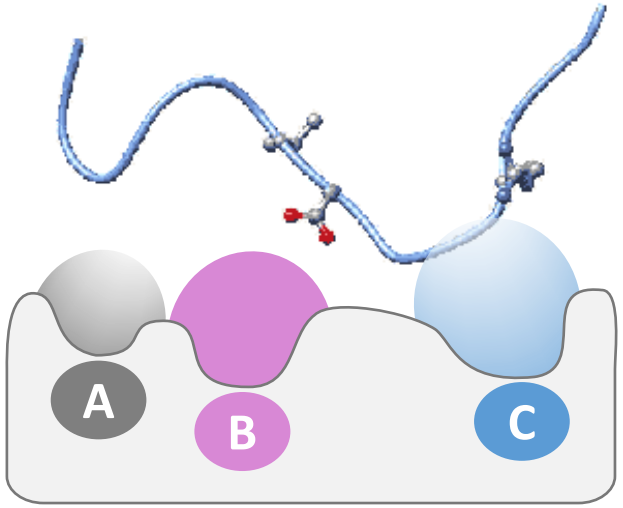
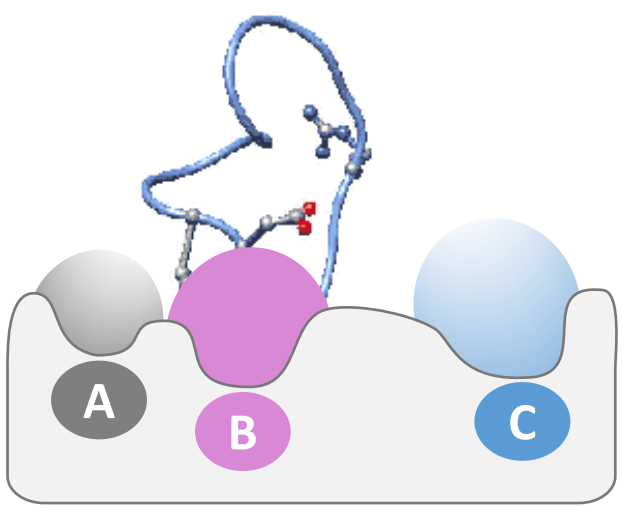
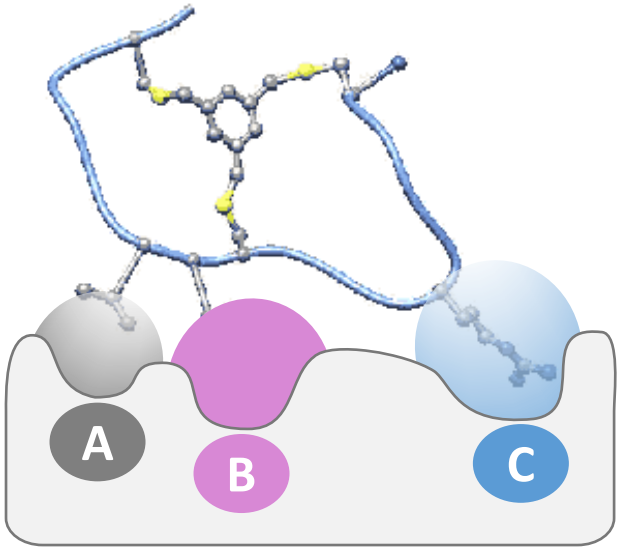
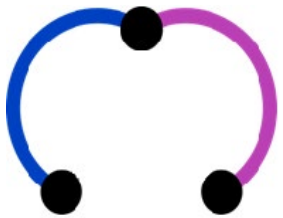
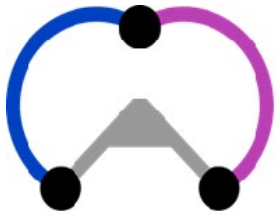
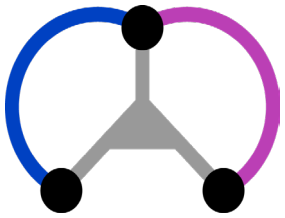


- **Favourable drug-like properties**

Small size (1.5-2 kDa)
High specificity
Chemical synthesis (NCEs)
Rapid tissue penetration
Complex protein targets druggable
Multiple routes of administration
Renal route of elimination
Not immunogenic

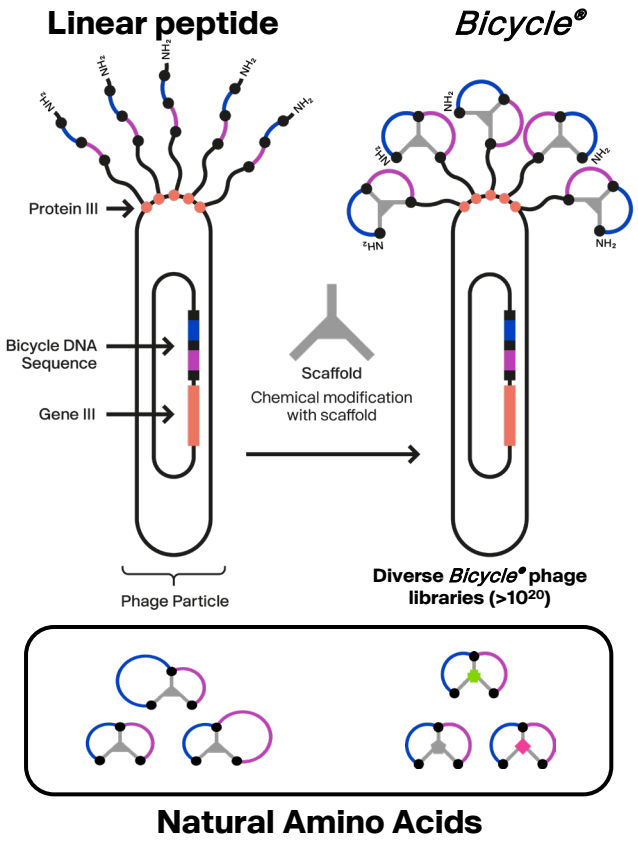
Structural constraints create *Bicycle®* advantage



	Linear peptide	Cyclic peptide	Constrained Bicycle
			
			
MT1-MMP affinity (FP competition)	**K_i (nM)**	**K_i (nM)**	**K_i (nM)**
	>10000	115.1	**1.15 ± 0.07**

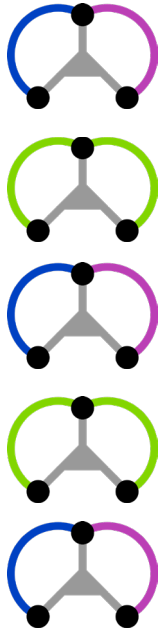
Bicycle® platform delivers a toolkit of building blocks to create novel medicines

Bicycle® Phage Display - Discovery



Optimize *Bicycle*® *monomers*

Peptide & Medicinal Chemistry

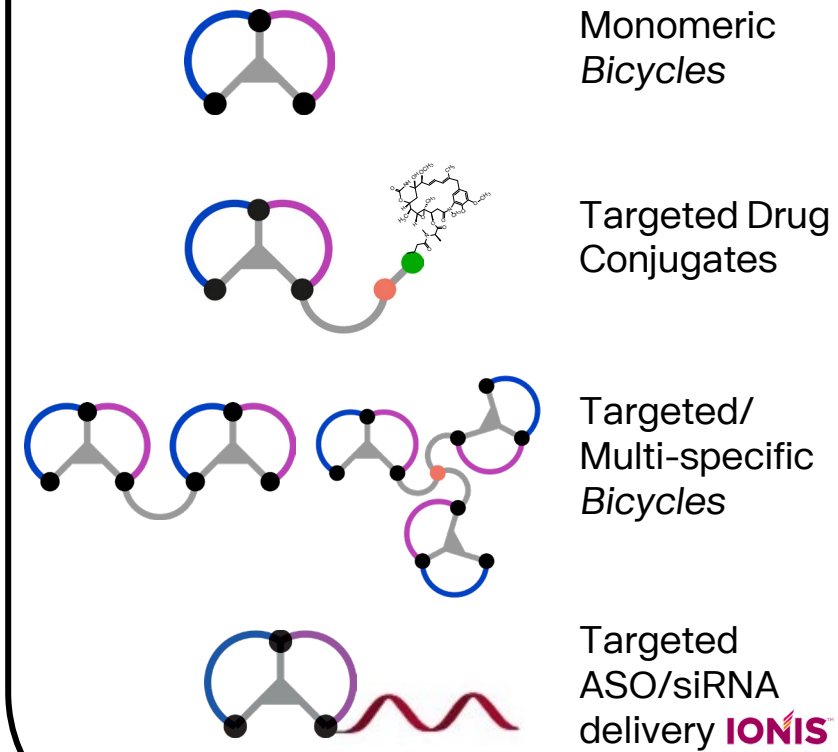


Non-Natural Amino Acids

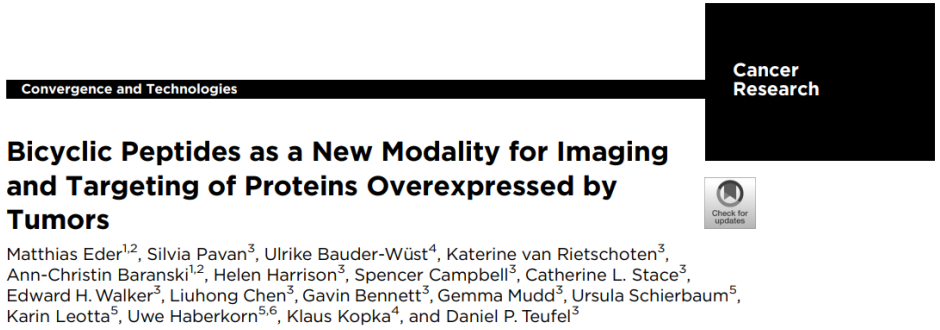
Build and Optimize Therapeutic *Bicycles*

Easy conjugation of Linkers and Payloads

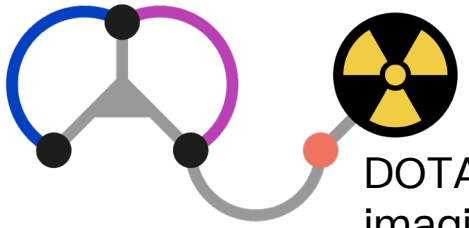
Potential *Bicycle*® Medicines



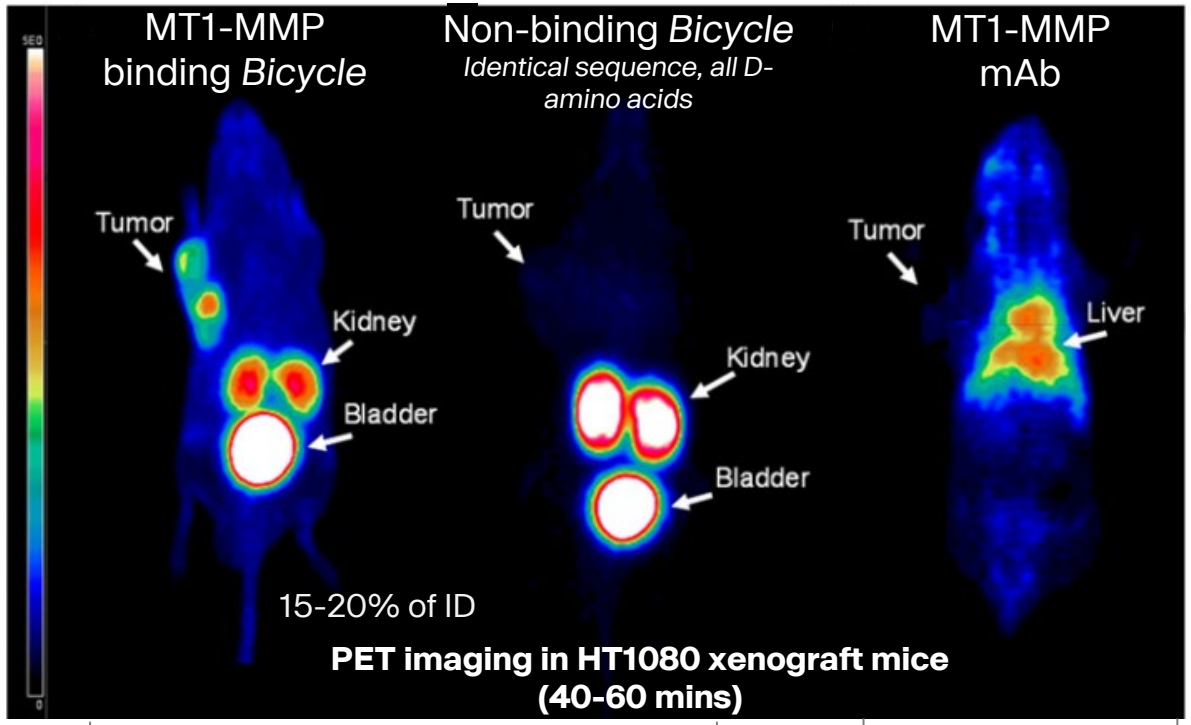
Bicycles enable precise cell targeting: an ideal delivery system, instructed by our work in oncology



Bicycle® binding to a tumor specific antigen



DOTA caged Ga^{68} imaging agent

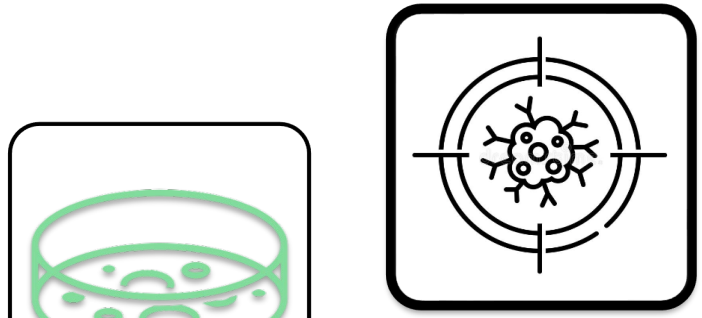


<3KDa
(Vd >0.2L/kg)

>150KDa
(Vd <0.05L/kg)

Bicycles (small molecules) have many advantages over biologics (antibodies)

Oncology focus but therapeutic reach beyond



Oncology

Molecular Cancer Therapeutics | 09.16.2022

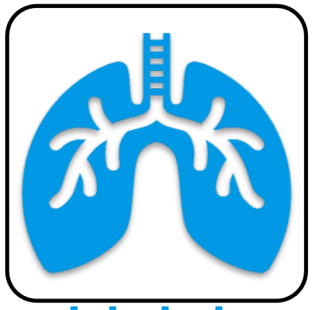
BT8009; a Nectin-4 targeting Bicycle® Toxin Conjugate for treatment of solid tumors.

Journal for ImmunoTherapy of Cancer | 11.02.2021

BT7480, a Novel Fully Synthetic Bicycle Tumor-Targeted Immune Cell Agonist™ (Bicycle TICA™)

Molecular Cancer Therapeutics | 05.12.2020

MMAE delivery using the Bicycle toxin conjugate BT5528



Inhaled

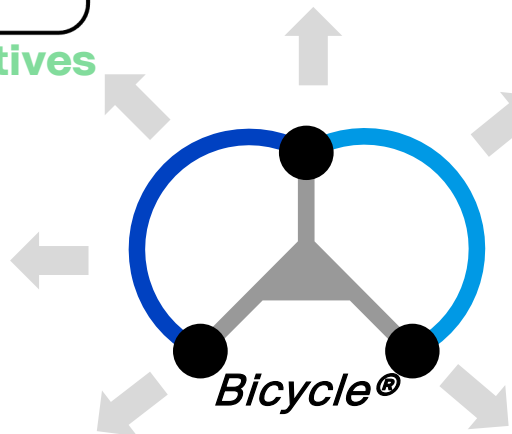
MDPI | 11.18.2022

Antimicrobial and Cell-Penetrating Peptides: Understanding Penetration for the Design of Novel

ACS Infectious Diseases | 08.18.2020

An Assay for Periplasm Entry Advances the Development of Chimeric Peptide Antibiotics

Anti-Infectives

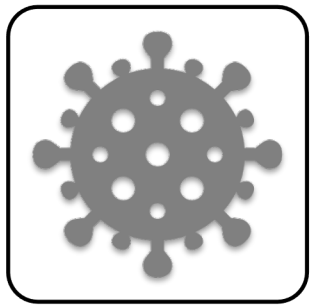


TIDES Asia | 03.15.2023

Bicycles – a new modality in the anti-viral armoury

Microbiology Society Annual Meeting | 04.07.2022

Bicycle®: A novel therapeutic modality for SARS-CoV-2



Virology

Ophthalmology

Journal of Medicinal Chemistry | 03.08.2018

Stable and Long-Lasting, Novel Bicyclic Peptide Plasma Kallikrein Inhibitors for the Treatment of Diabetic macular Edema

ChemMedChem | 07.01.2012

Bicyclic Peptides with Optimized Ring Size Inhibit Human Plasma Kallikrein and its Orthologues While Sparing Paralogous Proteases

PEGS Europe | 11.17.2022

Transferrin Receptor 1-targeting Bicycles: A New Platform for Transcytosis

Neurosciences

Metabolic

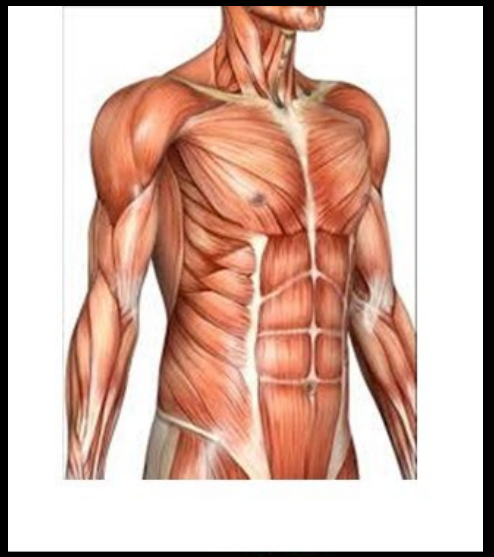
Selected publications shown and available at:
www.bicycletherapeutics.com/media/science-publications/

Harnessing the transferring receptor (TfR1) to enhance delivery of RNA therapeutics to skeletal muscle, cardiac muscle and the CNS

Bicycle Therapeutics Enters Exclusive License and Collaboration Agreement with Ionis to Develop Targeted Oligonucleotide Therapeutics

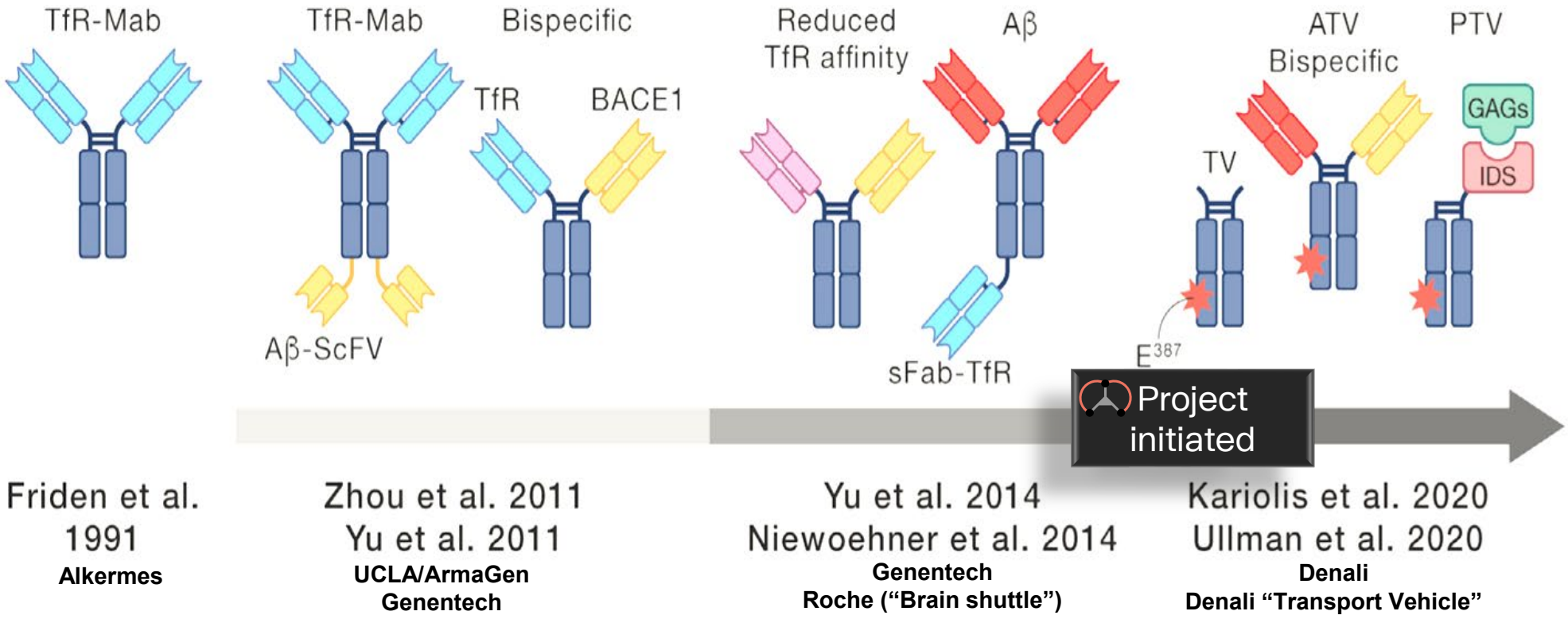
Press release July 13, 2021

- Bicycle to receive a total of $45 million upfront from Ionis and is eligible for development, regulatory and commercial milestone payments and royalties
- The agreement provides Ionis an exclusive license to *Bicycles* that bind with high specificity to the transferrin receptor without modifying natural function
- Bicycle retains rights to use transferrin binding *Bicycles* to deliver all molecular payloads outside of oligonucleotides



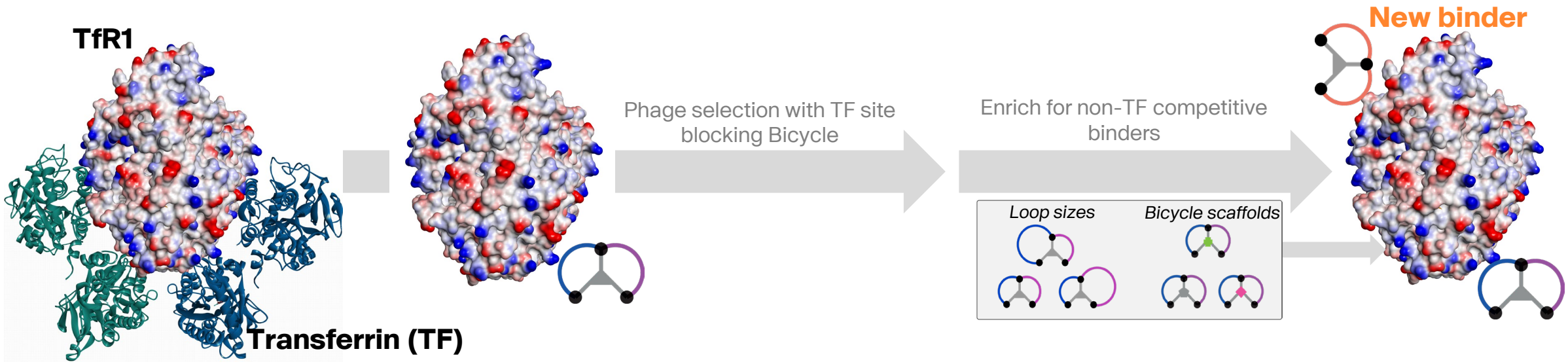
Exploiting TfR1 receptor mediated transcytosis as a drug delivery system

- **Chronology of therapeutic antibody delivery by TfR1 receptor mediated transcytosis**

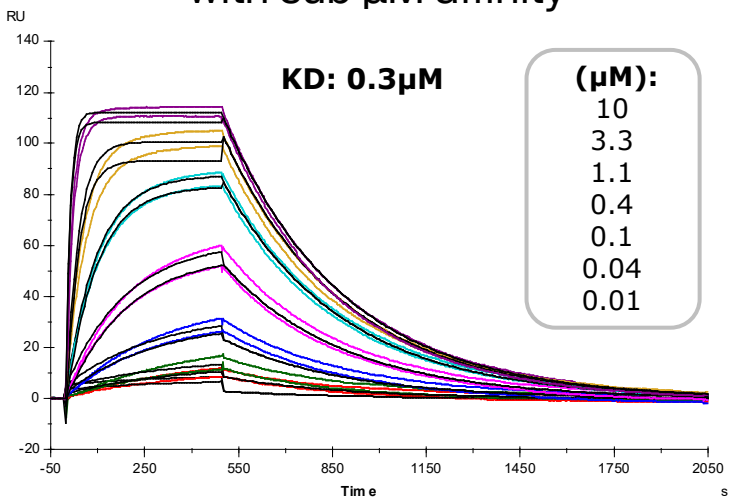


To our knowledge, there are no other small molecule TfR1 shuttles

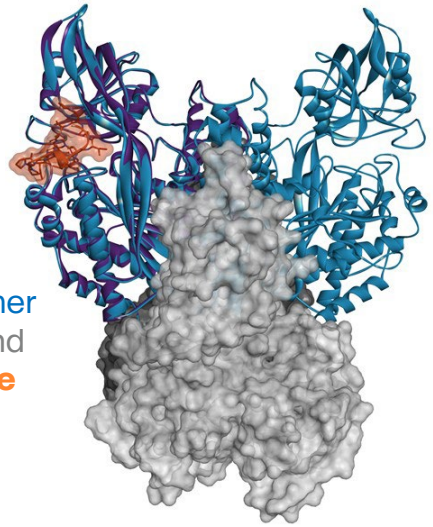
Identification of TfR1 (CD71) *Bicycles*



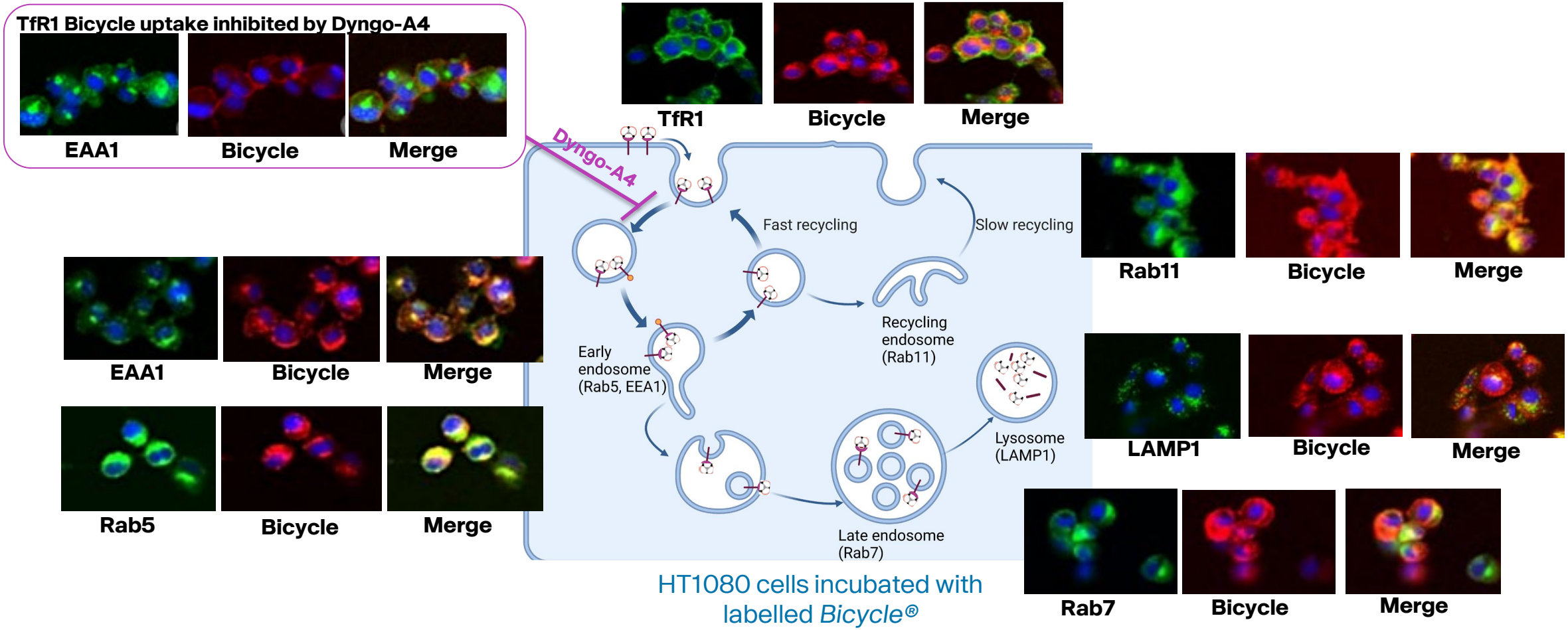
Identified *Bicycles* bind TfR1 with sub µM affinity



Binds to a novel site on TfR1, does not compete TF

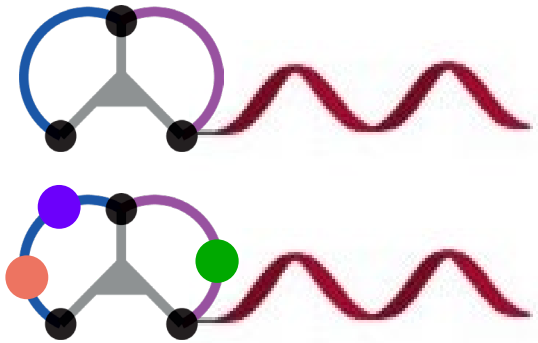


Internalization and colocalization with endosomal markers - fully tunable pharmacology of TfR1 binding *Bicycles*

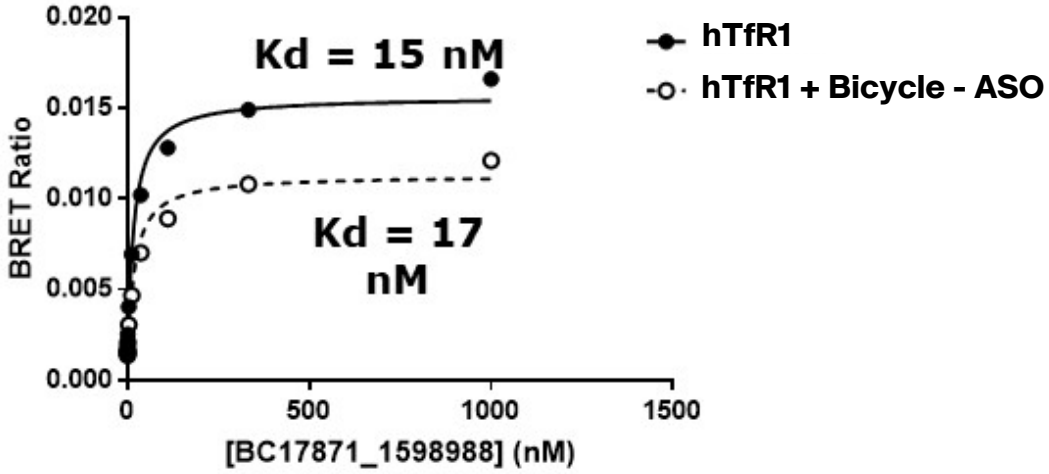


HT1080 cells incubated with labelled *Bicycle®*

Binding to TfR1 is maintained following conjugation of an ASO and affinity can be tuned using medicinal chemistry



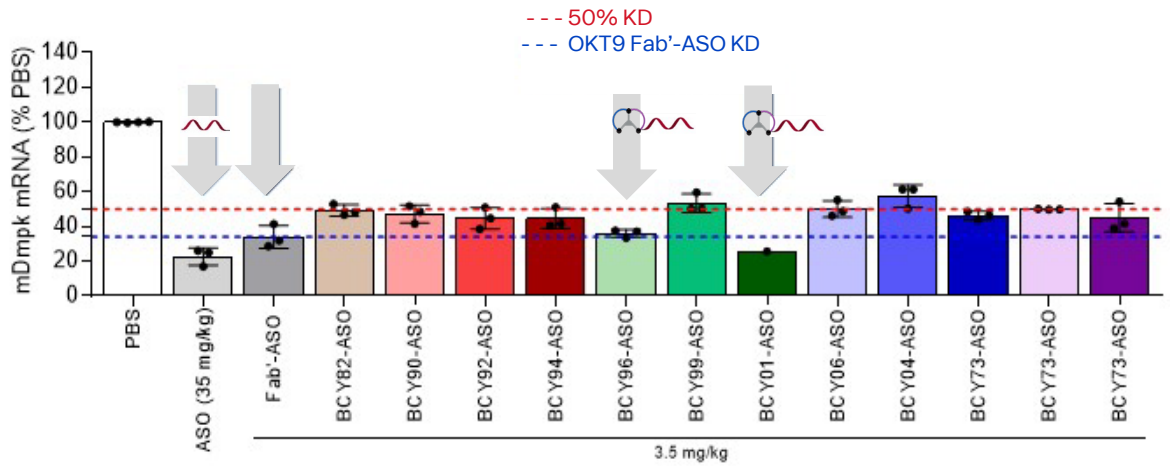
hTfR1 competition assay



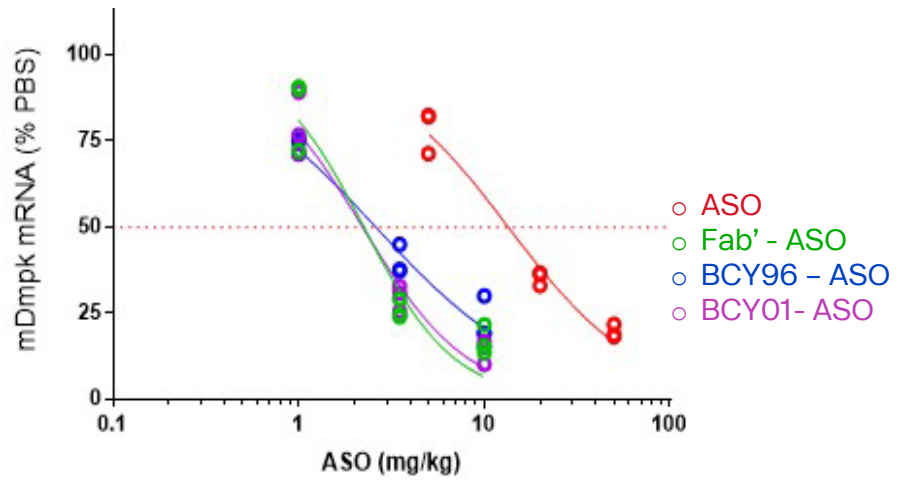
Conjugate	Ki (nM)
BCY04-ASO	60
BCY82-ASO	55
BCY06-ASO	22
BCY90-ASO	20
BCY92-ASO	11
BCY94-ASO	10
BCY99-ASO	4
BCY96-ASO	2
BCY01-ASO	1

Bicycles targeting hTfR1 enhance ASO delivery to skeletal muscles in hTfR1$^{KI/+}$ mice

mDmpk mRNA expression levels (Quadriceps)



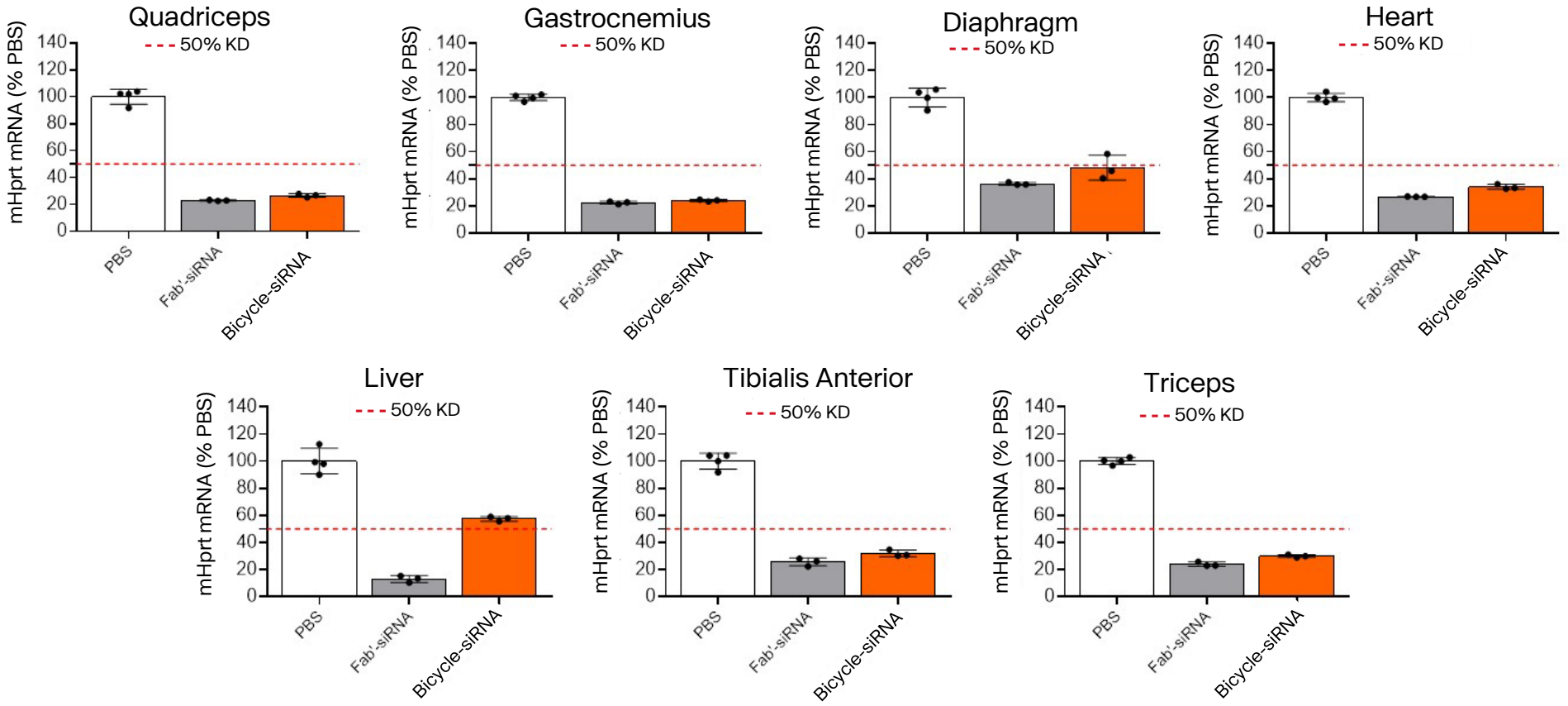
mDmpk mRNA expression levels (Quadriceps)



- hTfR1$^{KI/+}$ mice injected with 3.5 mg/kg/wk of ASO-conjugates for 3 weeks.
- Reduction of *DMPK* mRNA quantified by qRT-PCR.
- Similar results observed in diaphragm, gastrocnemius, tibia anterior.

Bicycles are an optimal delivery system for ASOs

Effective *Bicycle*®-mediated delivery of siRNA in hTfR1$^{KI/+}$ mice



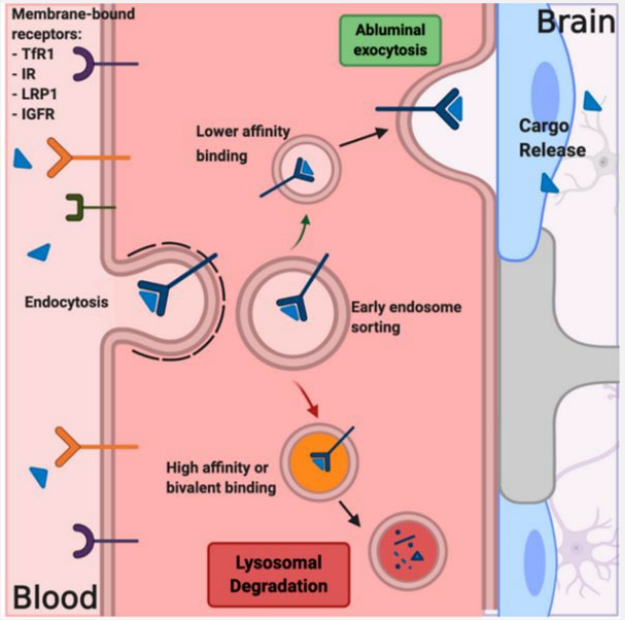
- hTfR1$^{KI/+}$ mice were injected with 3.5 mg/kg/wk/3 wks of siRNA-conjugates, 3-week study.
- Reduction of *mHprt* mRNA quantified by qRT-PCR

Bicycles are an optimal delivery system for siRNAs

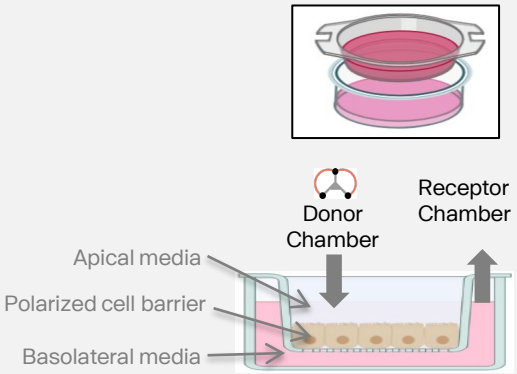
IONIS

TfR1 *Bicycles* show transcytosis across human proximal convoluted tubule cells

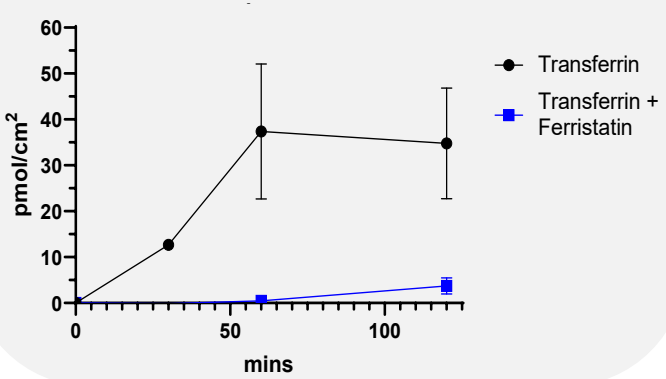
TfR1 can be used as shuttle to permit BBB penetration of therapeutic cargoes via 'receptor-mediated transcytosis'



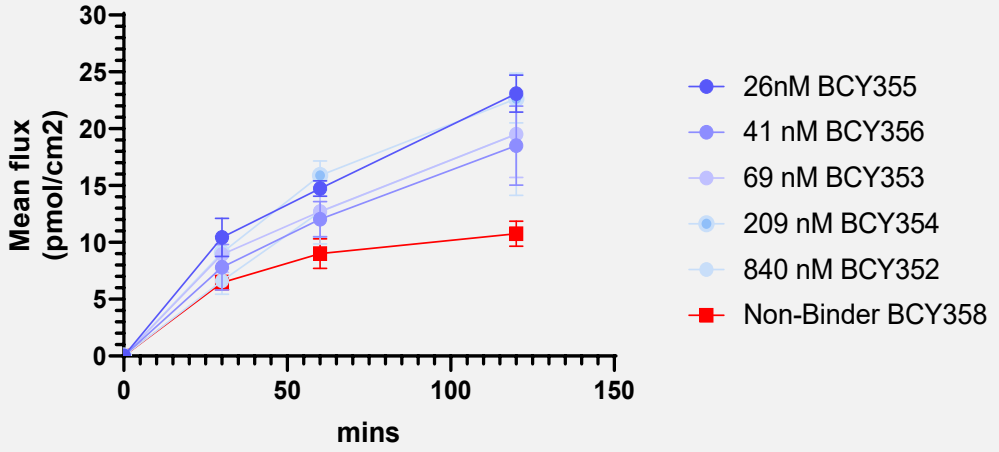
Human PCT (Kidney)



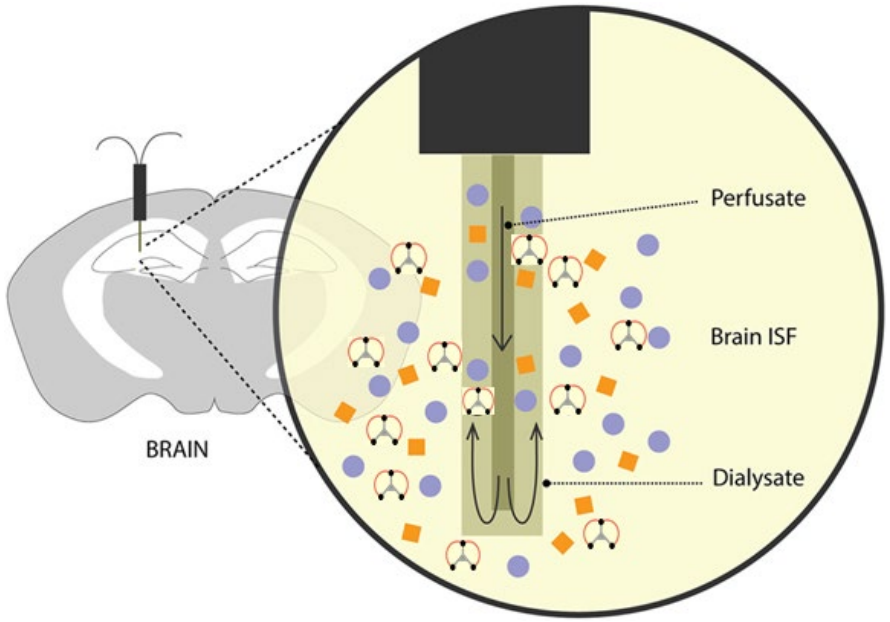
Apical to Basal flux (Transferrin)



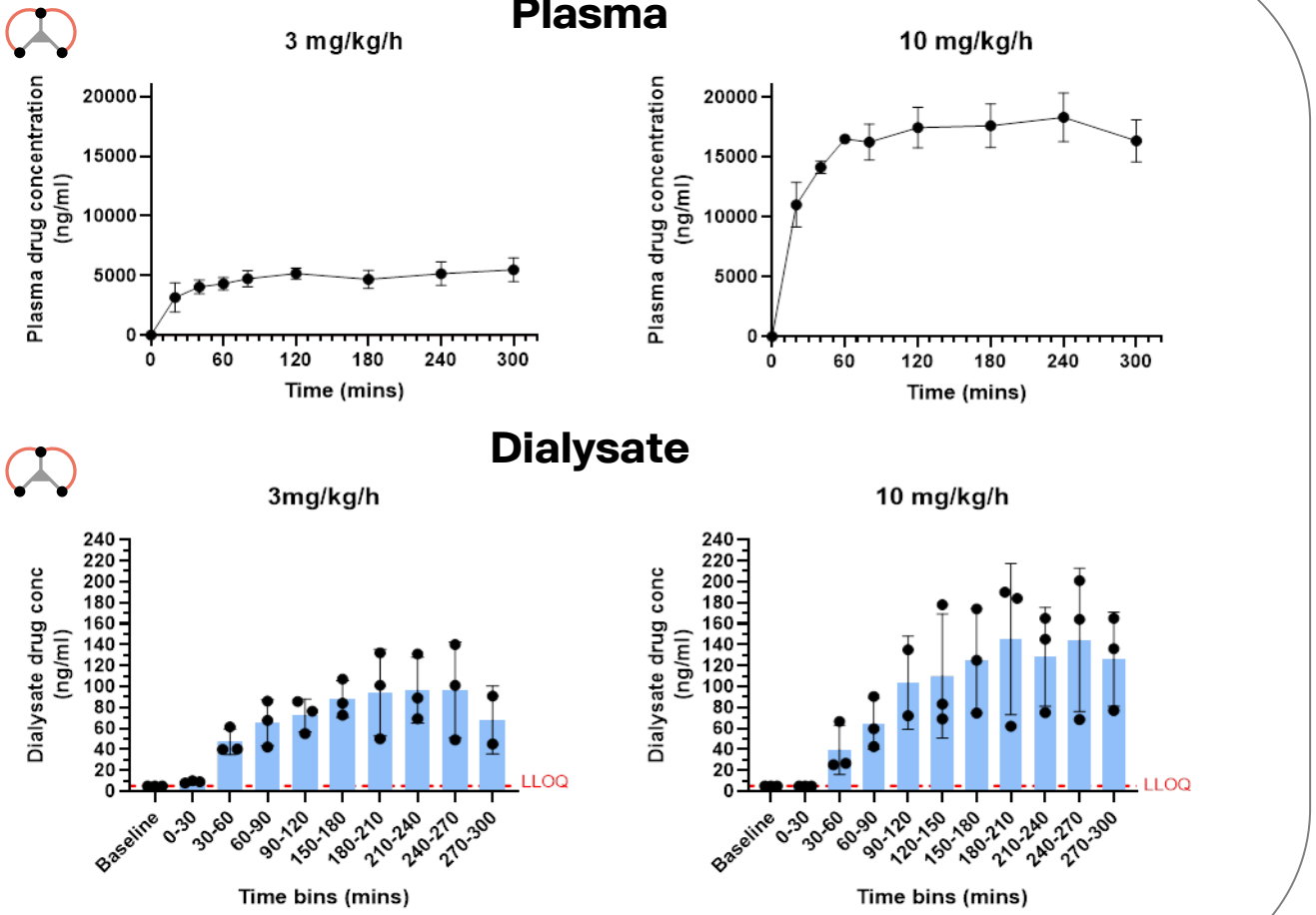
Apical to Basal flux (*Bicycles*)



Transport of a TfR1 Bicycle® across the BBB demonstrated in a non-human primate brain microdialysis study



- *Bicycle®* to TfR1 infused i.v. (3mg/kg/h & 10mg/kg/h) to steady state.



- LLOQ = 5ng/mL.

Leveraging the Bicycle® technology for the discovery of potential novel therapeutics for the treatment of dementia

Bicycle

Alzheimer's Research UK | Make breakthroughs possible

UNIVERSITY OF OXFORD

DRUG DISCOVERY INSTITUTE

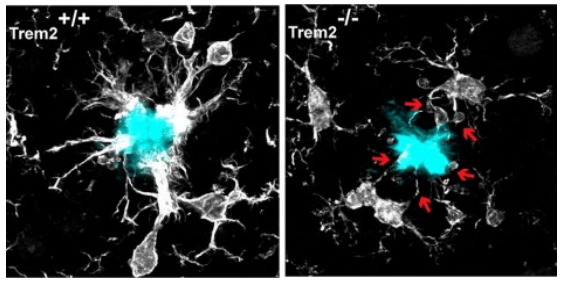
Dementia Discovery Fund

- Identification and characterization of TREM2 Bicycle agonists, a genetically validated dementia target.

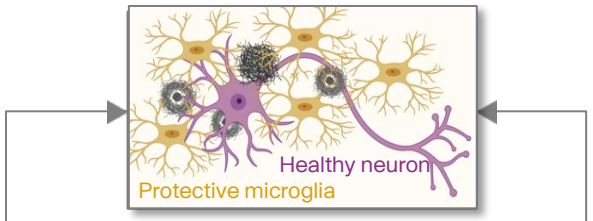
TfR1 *Bicycle*-
TREM2 agonist *Bicycle*

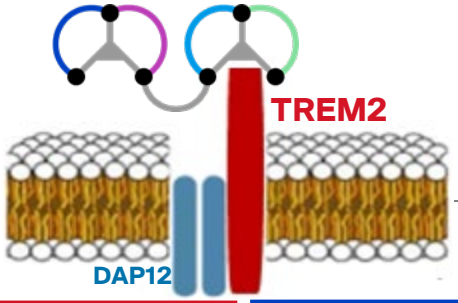
Activating TREM2, a genetically validated dementia target

TREM2 microglia encapsulate Aβ plaques, conferring neuroprotection



Yuan P et al; Neuron. 2016 Oct 5;92(1):252-264.



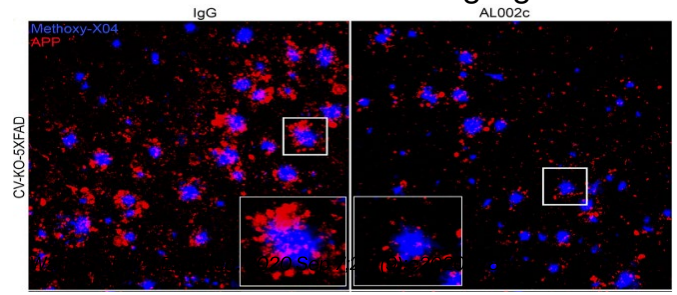


Mutated R47H

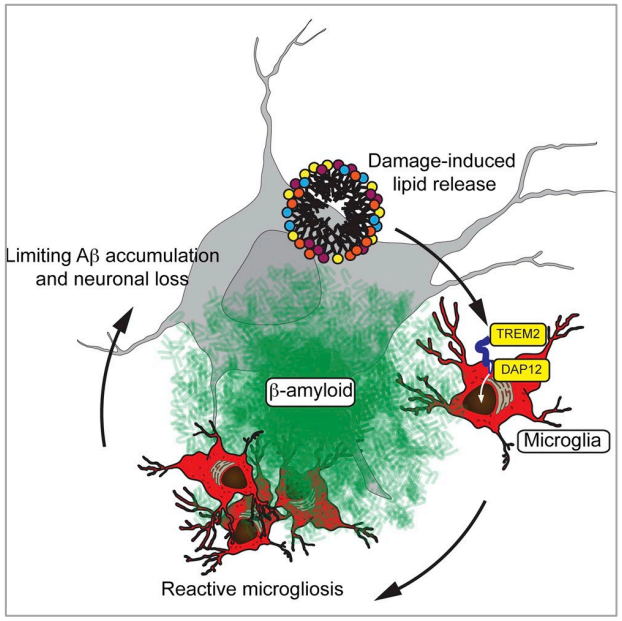
Normal activation

- ↑Phagocytosis
- ↑Neuronal Debris Clearance
- Anti-Inflammatory
- ↓ response to TLR stimulation

Mouse anti-hTREM2 agonistic mAb (AL002c) reduced filamentous plaque & dystrophic neurites in 5XFAD mice at 30 mg/kg



TREM2 timelines for drug discovery



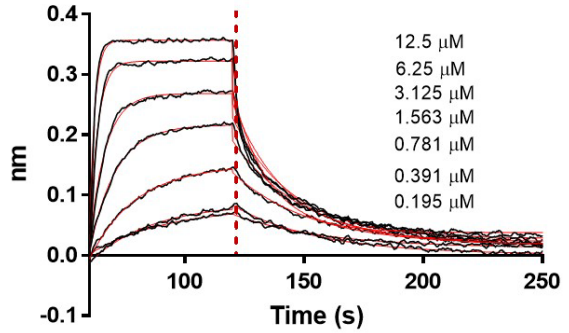
Biologic drug development efforts

- AL002 P1 posted Aug 2018
- AL002 1st patient dosed May 2019
- AL002 P2 posted Oct 2020
- Denali IND enabling Jan 2021
- VGL101 projected start Dec 2021
- Bicycle/ODDI project initiated

- TREM2 variants increase AD odds (Guerriro, 2013 Jonsson, 2013)
- TREM2 KO in 5XFAD increases Aβ pathology (Wang, 2015)
- TREM2 KO in $APPPS_{1-21}$ increases Aβ (Jay 2017)
- Antibody 4D9 reduces shedding & activates signaling (Denali) (Schlepkow, 2020)
- Aug 2021 T3 and Thyromimetics upregulate TREM2 (Ferrara 2021)

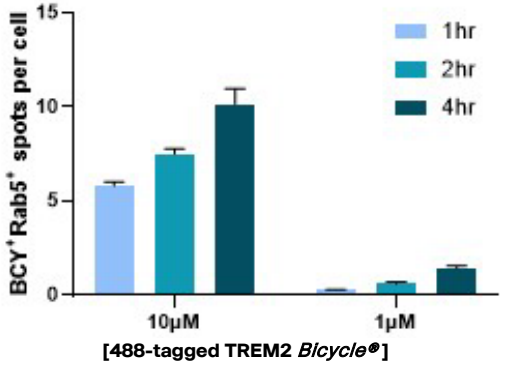
Bicycles display TREM2 pharmacology

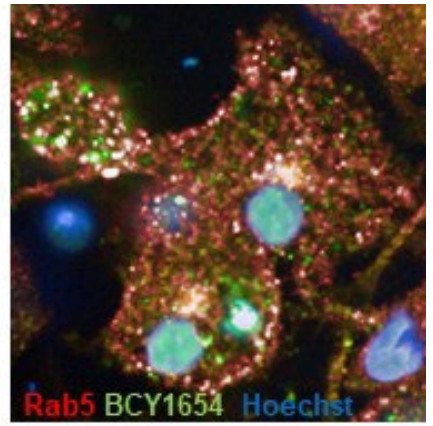
TREM2 *Bicycle®* BLI



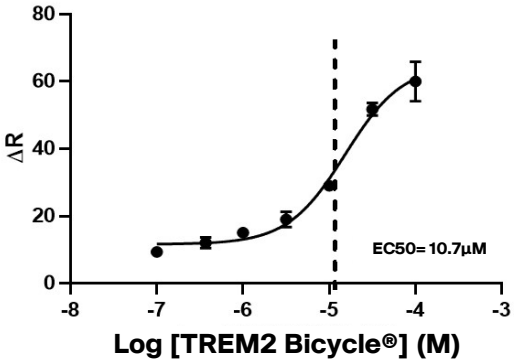
Steady state K_D 0.68 ± 0.07 µM

TREM2 *Bicycle®* Endocytosis (iPSC macrophages)

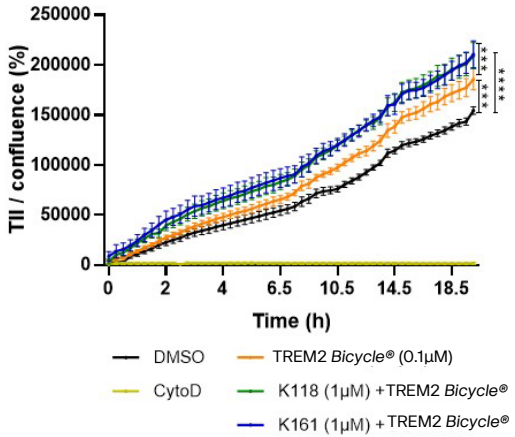




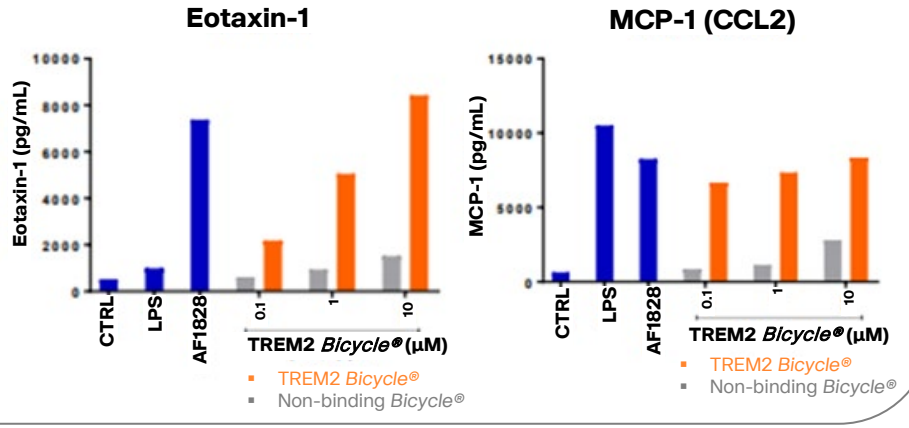
pSYK/tSYK



SHIP1 inhibition enhances TREM2 *Bicycle®*-mediated phagocytosis



TREM2 *Bicycle®* modulates chemokine release in iPSC macrophages



Summary

- *Bicycles* are fully synthetic and readily conjugated precision guided targeting systems
- We have identified the first small molecule TfR1 shuttles
- These have been used to successfully deliver ASO/siRNAs to muscle and initial evidence supports their utility to deliver to the CNS
- We have identified TREM2 agonistic *Bicycles* that exhibit appropriate pharmacology
- We are investigating TfR1 conjugated TREM2 *Bicycles*

Thank you

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