Bicycles, bi-cyclic peptides, novel small molecule delivery systems for RNA therapeutics

EuroTIDES - November 2021

IONIS

bicycle therapeutics

Forward-looking statements

This presentation may contain forward-looking statements made pursuant to the safe harbor provisions of the Private Securities Litigation Reform Act of 1995. These statements may be identified by words such as "aims," "anticipates," "believes," "could," "estimates," "expects," "forecasts", "goal," "intends," "may" "plans," "possible," "potential," "seeks," "will," and variations of these words or similar expressions that are intended to identify forward-looking statements. All statements other than statements of historical facts contained in this presentation are forward-looking statements, including statements regarding our future financial or business performance, conditions, plans, prospects, trends or strategies and other financial and business matters; our current and prospective product candidates, planned clinical trials and preclinical activities, current and prospective collaborations and the timing and success of our development of our anticipated product candidates.

Forward-looking statements are neither historical facts nor assurances of future performance. Instead, they are based on our current beliefs, expectations and assumptions regarding the future of our business, future plans and strategies, our development plans, our preclinical and clinical results, our plans to initiate clinical trials and the designs of the planned trials and other future conditions, and are subject to a number of risks and uncertainties that could cause actual results to differ materially and adversely from those set forth in or implied by such forward-looking statements. These risks and uncertainties include, but are not limited to, risks related to the ongoing COVID-19 pandemic, the risk that any one or more of our product candidates will not be successfully developed or commercialized, the risk of cessation or delay of any ongoing or planned clinical trials, the risk that we may not realize the intended benefits of our technology, including that we may not identify and develop additional product candidates for our pipeline, the risk that we may not maintain our current collaborations or enter into new collaborations in the future, or that we may not realize the intended benefits of these collaborations, the risk that our product candidates or procedures in connection with the administration thereof will not have the safety or efficacy profile that we anticipate, the risk that prior results will not be replicated or will not continue in ongoing or future studies or trials, the risk that we will be unable to obtain and maintain regulatory approval for our product candidates, the risk that the size and potential of the market for our product candidates will not materialize as expected, risks associated with our dependence on third-parties and risks relating to our ability to obtain and maintain intellectual property protection for our product candidates. For a discussion of these and other risks and uncertainties, and other important factors, any of which could cause our actual results to differ from those contained in the forward-looking statements, see the section entitled "Risk Factors" in our Quarterly Report on Form 10-Q, filed with the Securities and Exchange Commission (SEC) on November 4, 2021 as well as in other filings Bicycle may make with the SEC in the future, as well as discussions of potential risks, uncertainties and other important factors in our subsequent filings with the Securities and Exchange Commission. New risks and uncertainties may emerge from time to time, and it is not possible to predict all risks and uncertainties. Except as required by applicable law, we do not plan to publicly update or revise any forward-looking statements contained herein, whether as a result of any new information, future events, changed circumstances or otherwise.

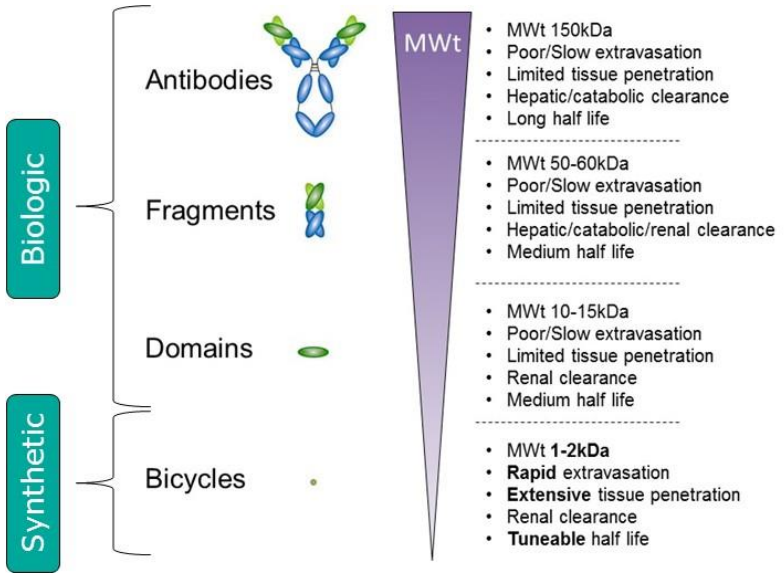
- I am an employee of Bicycle Therapeutics

Bicycle Therapeutics

- Bicycle Therapeutics – drug discovery and development Biopharma

- Unique therapeutic modality conceived by Nobel Prize winner Sir Greg Winter & Christian Heinis
  - platform applicable across many therapeutic areas

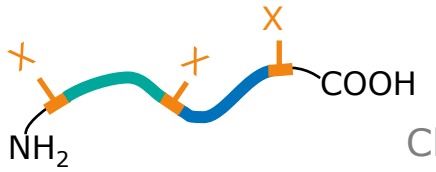
- Five molecules in clinic



bicycle therapeutics

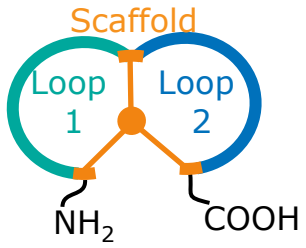
Bicycle® a unique & disruptive therapeutic modality

Linear peptide

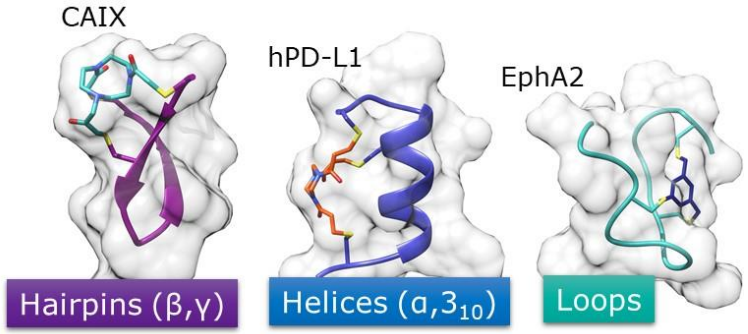


Chemical modification with scaffold

Bicycle



Form biologically relevant 3D structures

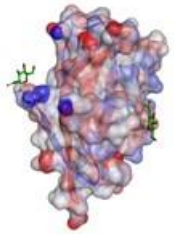


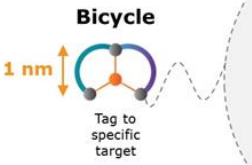
Unique and attractive drug-like attributes

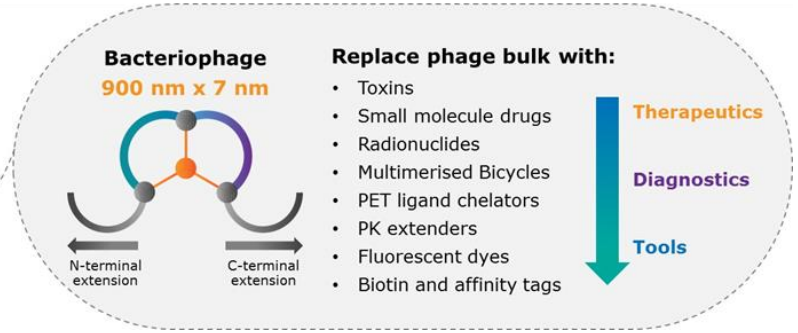
Constraint	pM affinity, absolute selectivity to target
Large binding footprint	Protein-protein "hard to drug" targets"
Fully synthetic	NCEs, scalable mass production
Readily conjugated	**Come off platform conjugation ready – payload enabled**
Routes of admin	Multiple : IV, SQ, inhalation
Rapid platform	Rapid timelines to tools & drug like molecules

Our platform enables conjugation

Target ~1.5–2 kDa ~1–2 MDa



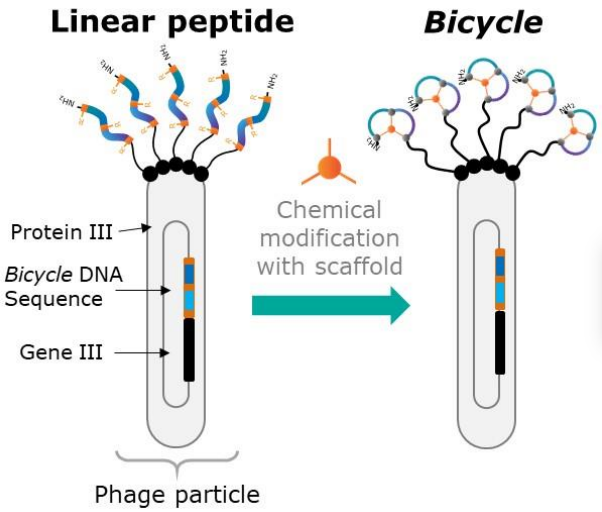




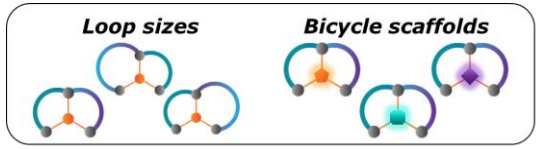
bicycle therapeutics

Bicycle platform – a marriage of phage display and peptide /medicinal chemistry creating novel potential medicines

Bicycle Phage Display - Discovery



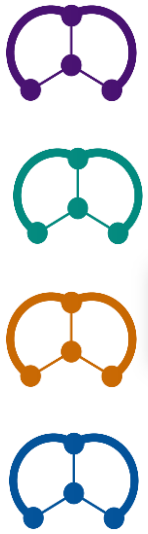
Diverse *Bicycle* phage libraries (>10^{17})



Natural Amino Acids

Optimize Bicycle monomers

Peptide & Medicinal Chemistry



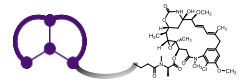
Target binding *Bicycles*

Build and Optimize Therapeutic Bicycle

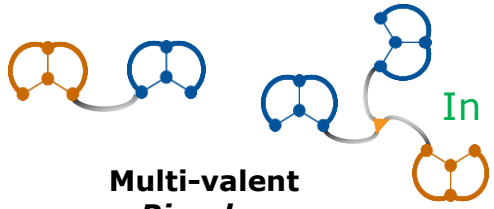
Non-natural Amino Acids

Bicycle Medicines

Monomeric *Bicycles* In the clinic ✓



Targeted Drug Conjugates In the clinic ✓



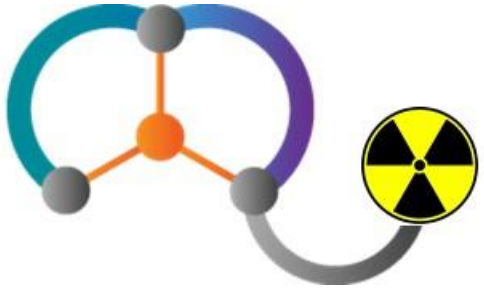
Multi-valent *Bicycles* In the clinic ✓

Targeted delivery

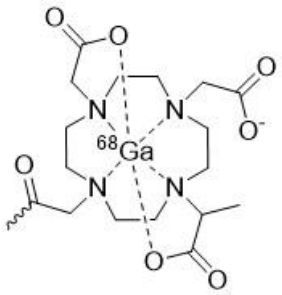
bicycle therapeutics

Bicycles : an ideal tissue targeting delivery system, instructed by our work in oncology

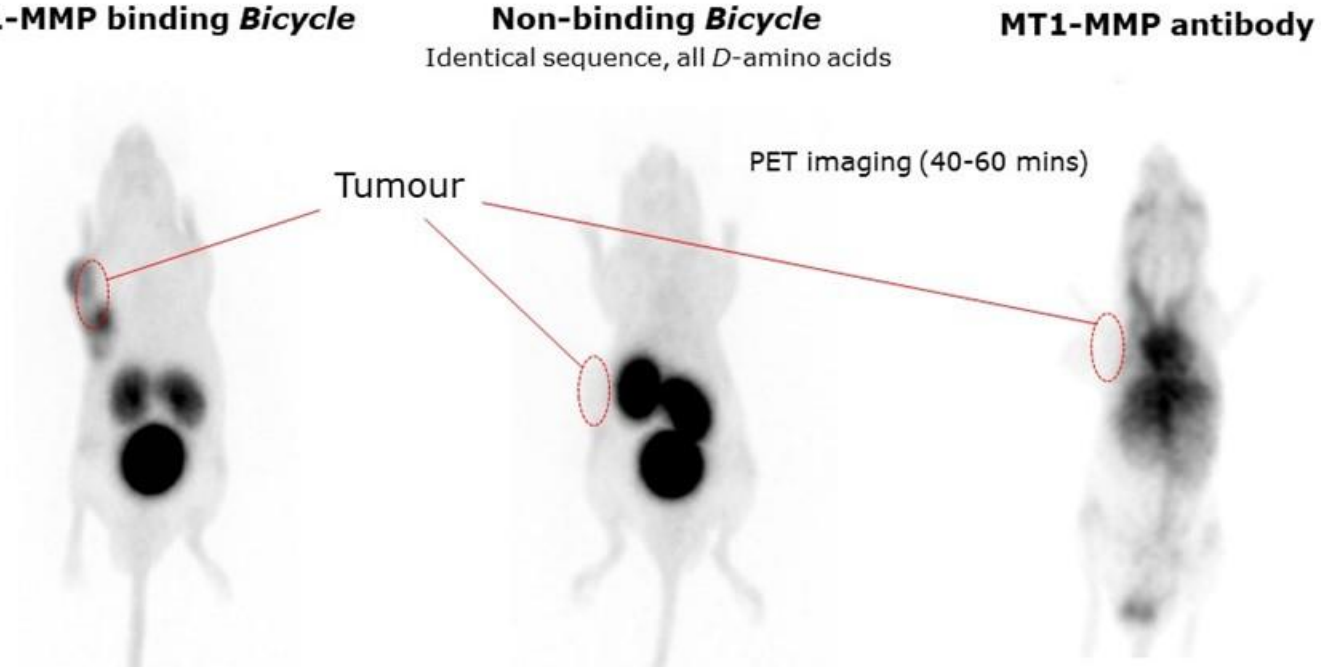
Bicycle® binding to a tumor specific antigen



DOTA caged Ga^{68} imaging agent



Gallium-68 DOTA chelate



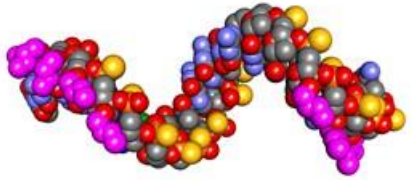
15-20% of ID

<3KDa
(Vd >0.2L/kg)

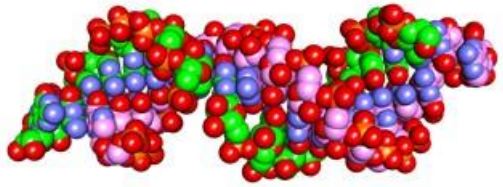
>150KDa
(Vd <0.05L/kg)

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

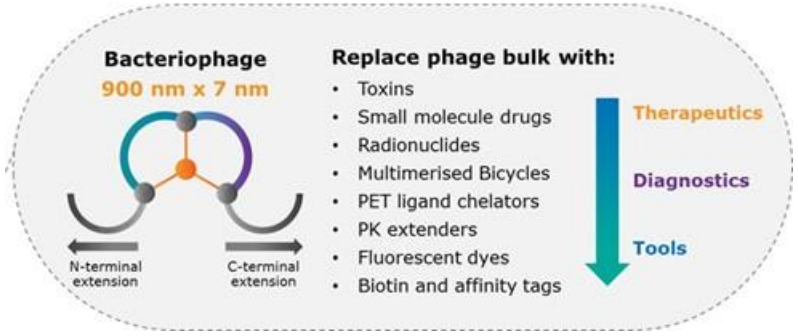
Could we apply the Bicycle technology to deliver antisense therapeutics to specific tissues to treat serious diseases?



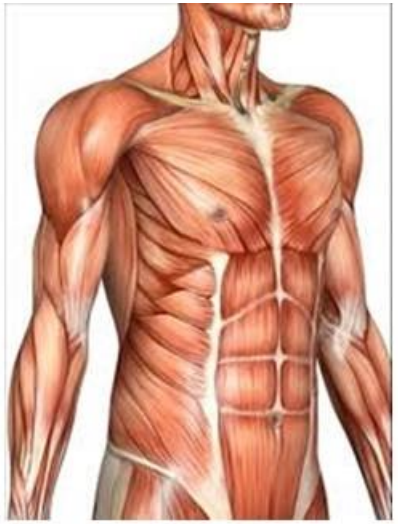
ASO (Mol Wt 5.5-7000)



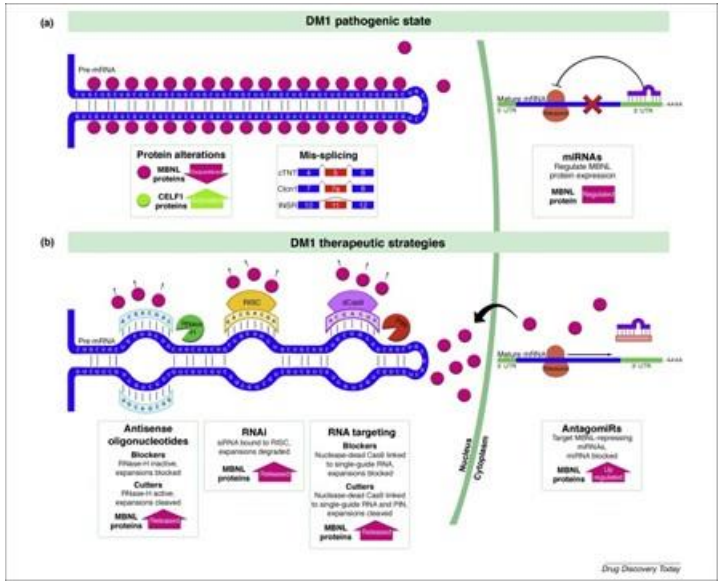
siRNA (Mol Wt 15,000)



M13 phage (Mol Wt 1-2million



Myotonic Dystrophy



IONIS®

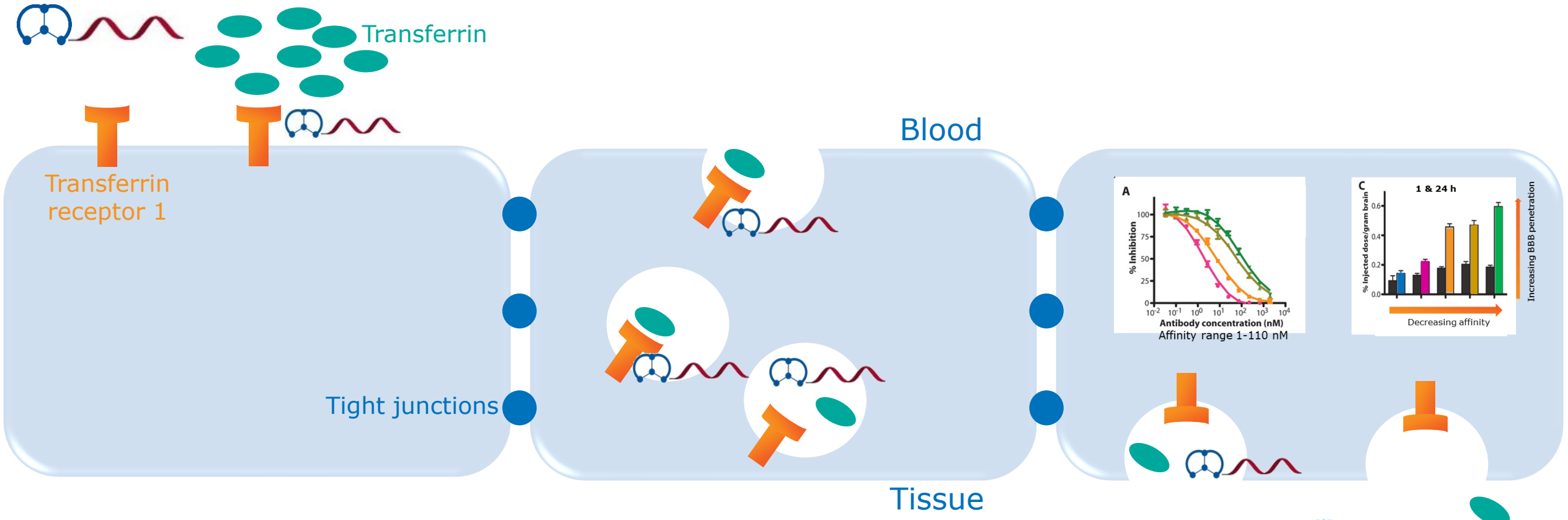
bicycle therapeutics

Targeting TfR1 (Transferrin receptor) as a suitable vehicle to target to deliver ASOs to muscle and brain

Circulating transferrin (~25μM), binder needs to be non-competitive leaving Fe shuttling intact

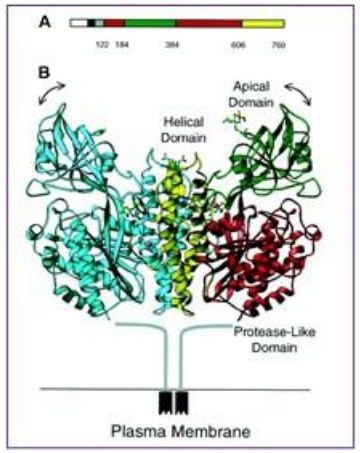
Binder needs to trigger receptor internalization & for CNS endosomal escape and transcytosis

What are the optimal affinities for muscle and CNS delivery

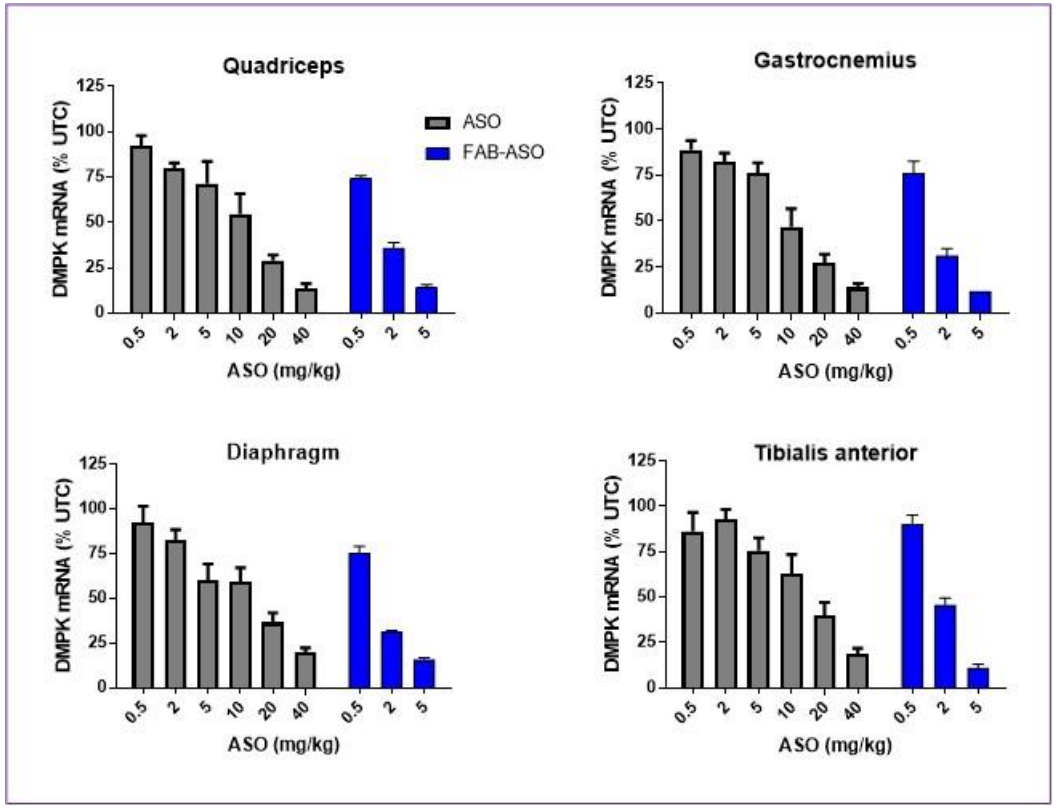


bicycle therapeutics

TfR1 mediated delivery is precedented, ASO potency increased 10-fold in skeletal muscle tissues



Sugo et al, J. Controlled Rel, 2016



ASO	ED_{50} (mg/kg)			
	TA	Quadricep	Diaphragm	Gastroc.
ASO	14	10	11	10
FAB-ASO	1.8	1.2	1.2	1.1

However, dose required to deliver equivalent amounts of ASO are high with biologics

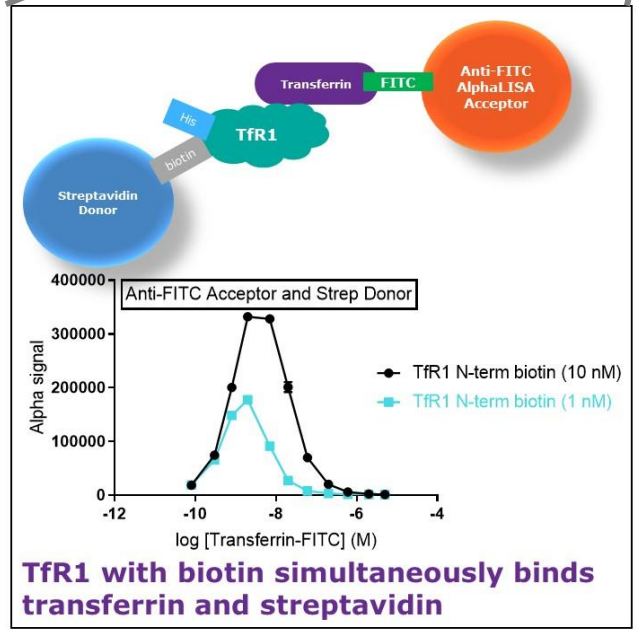
Compound	Mol wt	Conjugate dose (mg/kg)	Theoretical clinical dose (mg)
ASO	~5400	--	210
ASO-FAB	~55000	~33	2310
ASO-MAB	~155000	~93	6510
ASO-centyrin	~15000	~9	600
ASO-Bicycle	~7400	~4	280

(to deliver 3mg/kg ASO)

Screening initially identified TF competitive *Bicycles*

Phase 0 → Pilot → Full Screen

Identify optimum target format

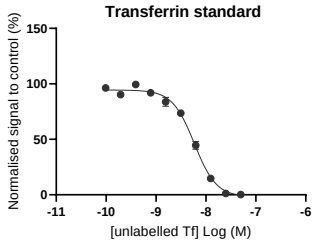


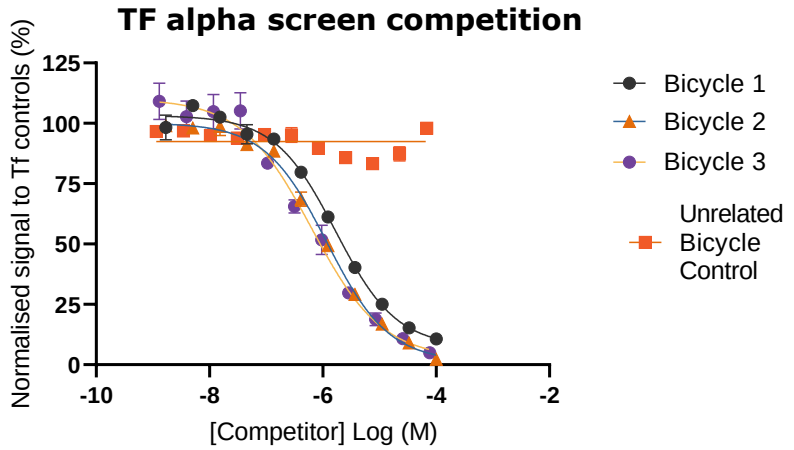
TfR1 with biotin simultaneously binds transferrin and streptavidin

Access maximum *Bicycle* diversity

Define initial pharmacophores

	Blocking tools (transferrin inhibitors)		
	Bicycle 1	**Bicycle2**	**Bicycle 3**
TfR1 FP direct KD (μM)	0.87	1.09	0.52
TfR1 SPR KD (μM)	0.34	0.49	0.47
Alpha direct (Yes/No)	n/d	n/d	n/d
TF Alpha inhibition IC_{50} (μM)	1.19	0.97	0.73

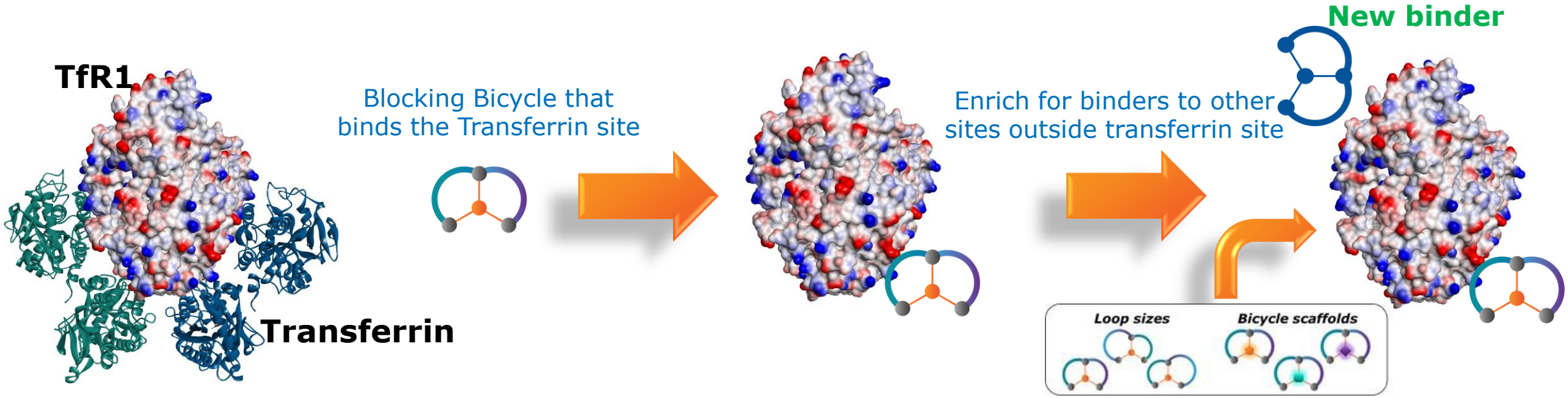




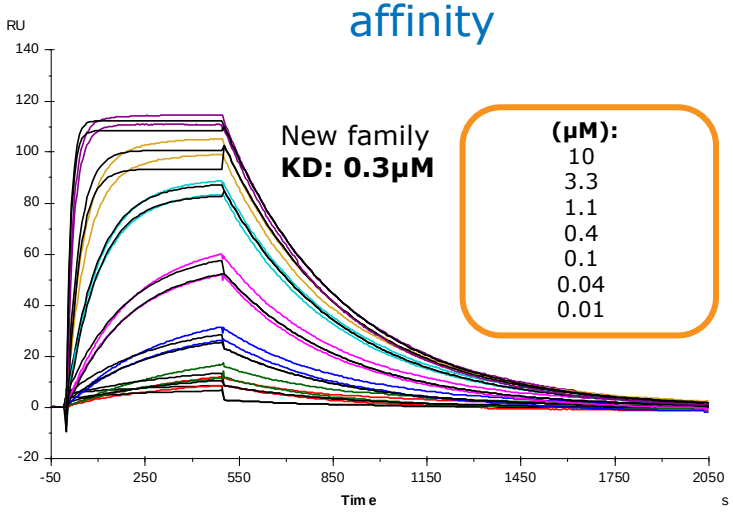
First screen identified TF active site binder "tools"

bicycle therapeutics

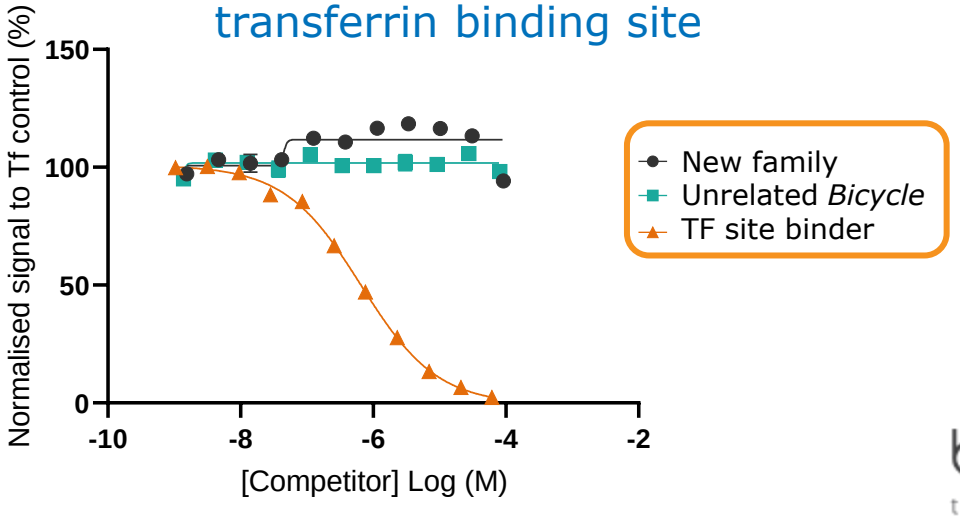
Screening take 2 - generating binders to new epitopes



New family binds TfR1 with sub μM affinity

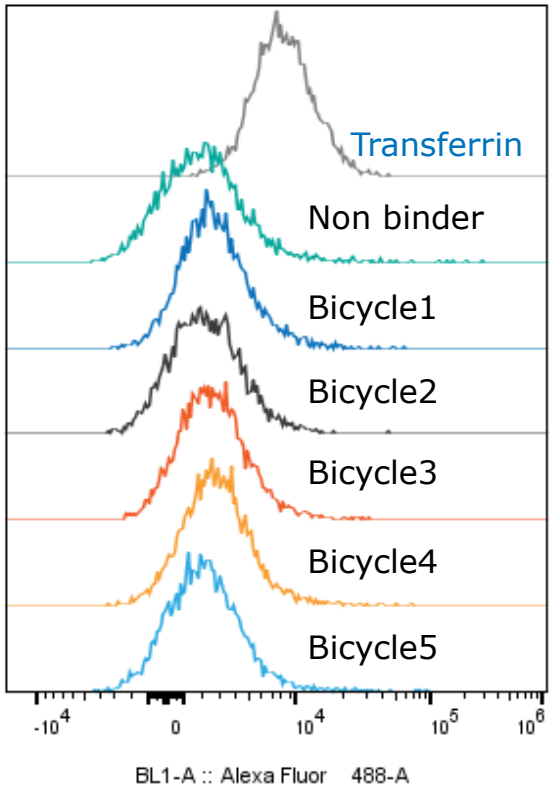


New family does not compete the transferrin binding site



Bicycles are specific and selective for human TfR1

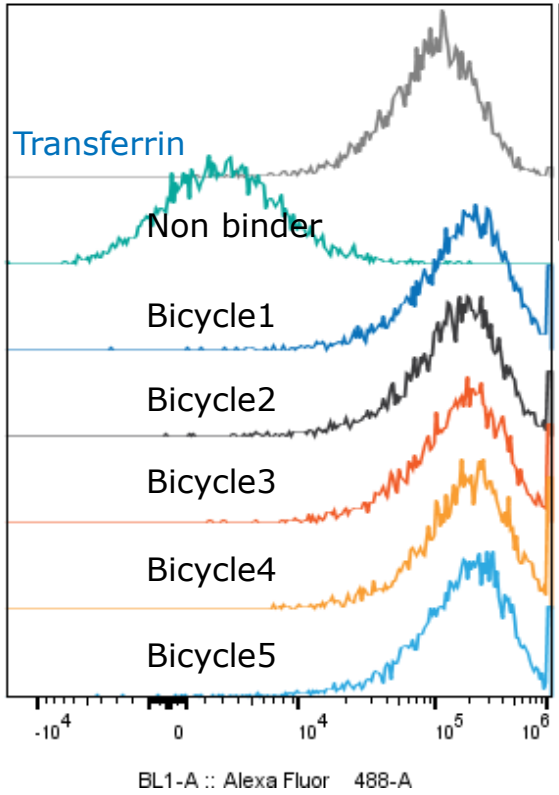
Human TfR2 CHO



	Count	Median : BL1-A
	7625	8168
	7728	1938
	7727	2612
	7691	2160
	7720	2556
	7524	2763
	7776	2068

✓ no binding to human TfR2 cells

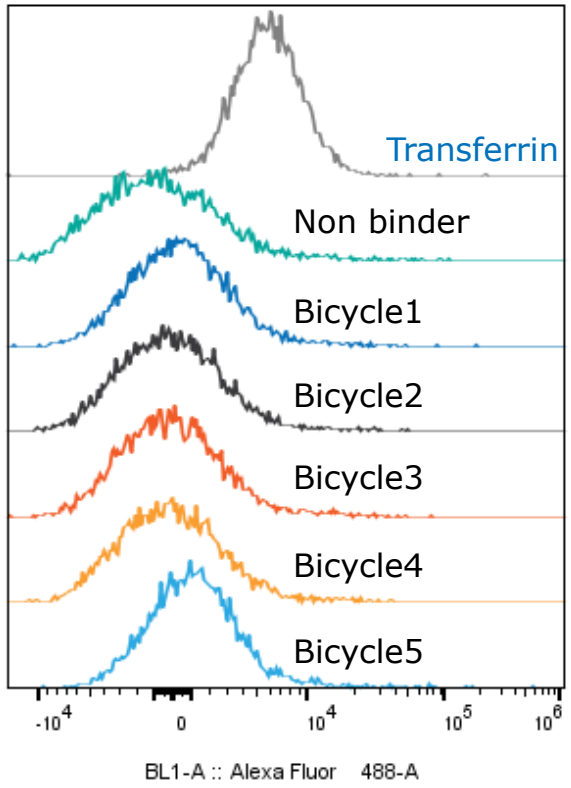
Human TfR1 CHO



	Count	Median : BL1-A
	7446	99941
	7288	3103
	7330	182703
	7195	157168
	7259	171267
	7398	188717
	7289	188208

✓ binding to human TfR1 cells

Parental CHO

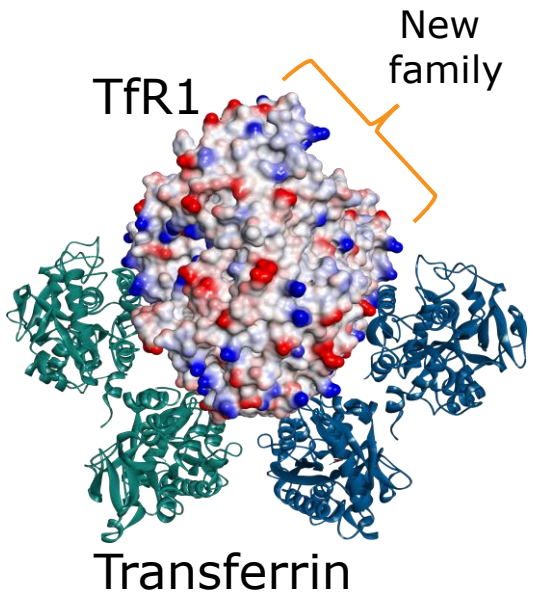


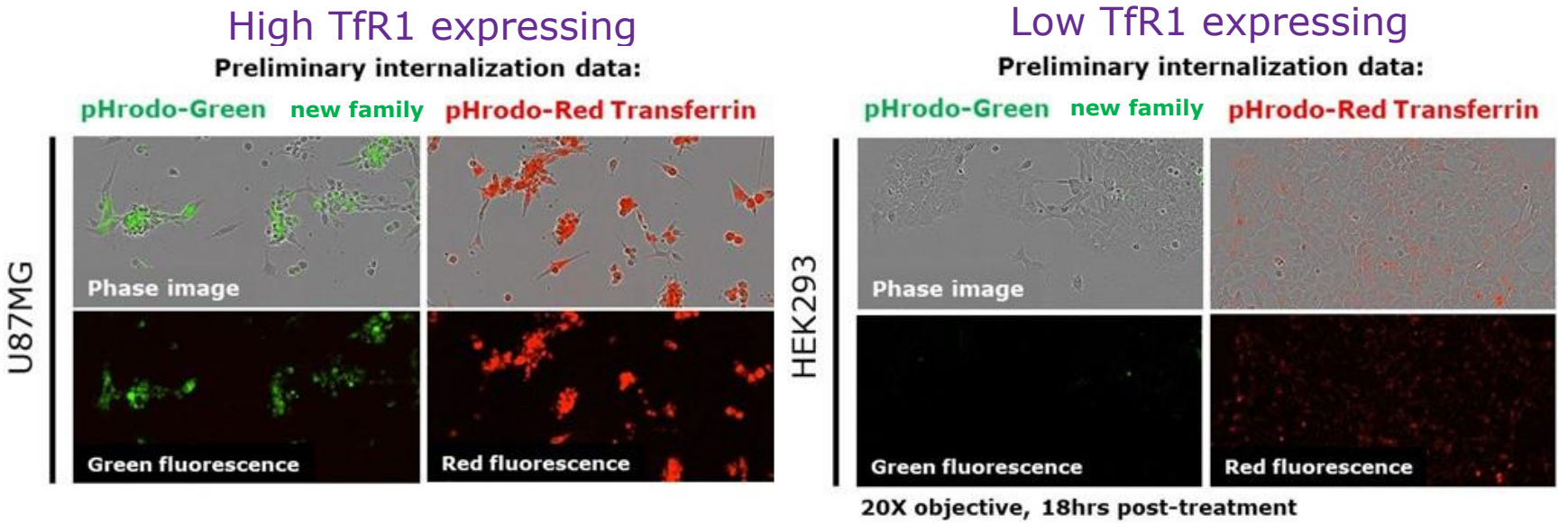
	Count	Median : BL1-A
	7798	5866
	7634	-827
	7800	362
	7703	-145
	7771	-130
	7623	-166
	7573	1053

✓ no binding to parental cells

bicycle therapeutics

Cellular uptake demonstrated for non-active site family



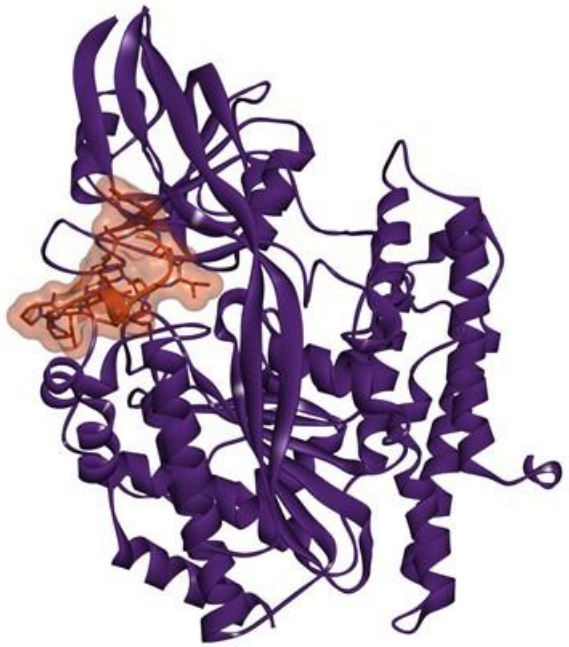


pH sensitive pHrodo tags fluoresce when internalized and trafficked to the acidic endosome/ lysosome

Binders outside ligand binding site show uptake and trafficking to lysosome

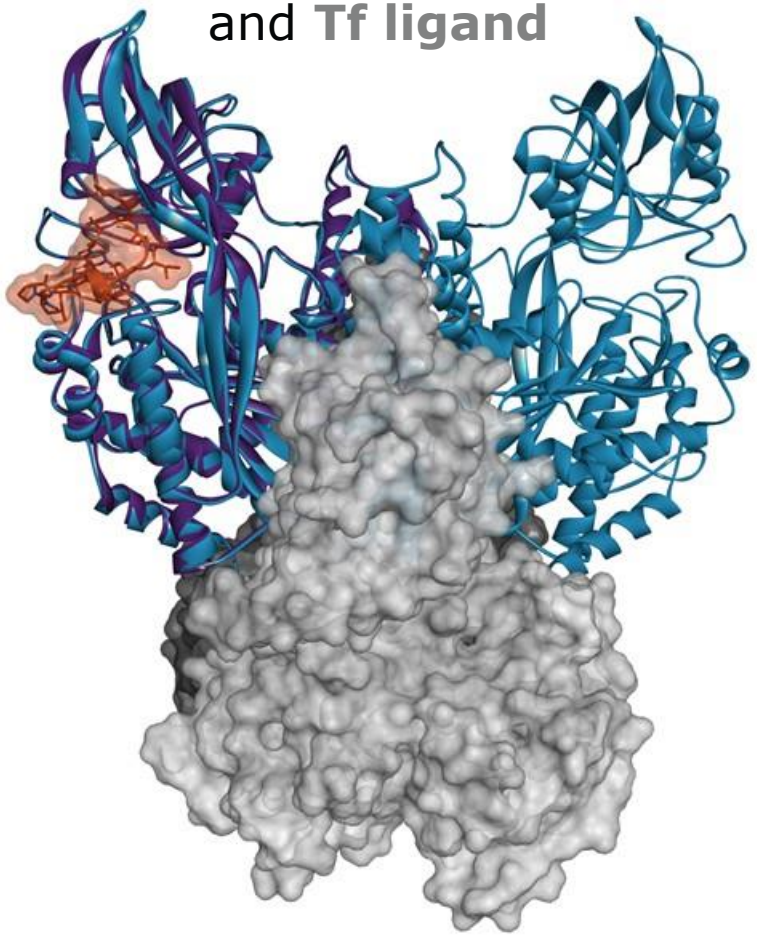
Crystal structure of *Bicycle*® bound into hTfR1

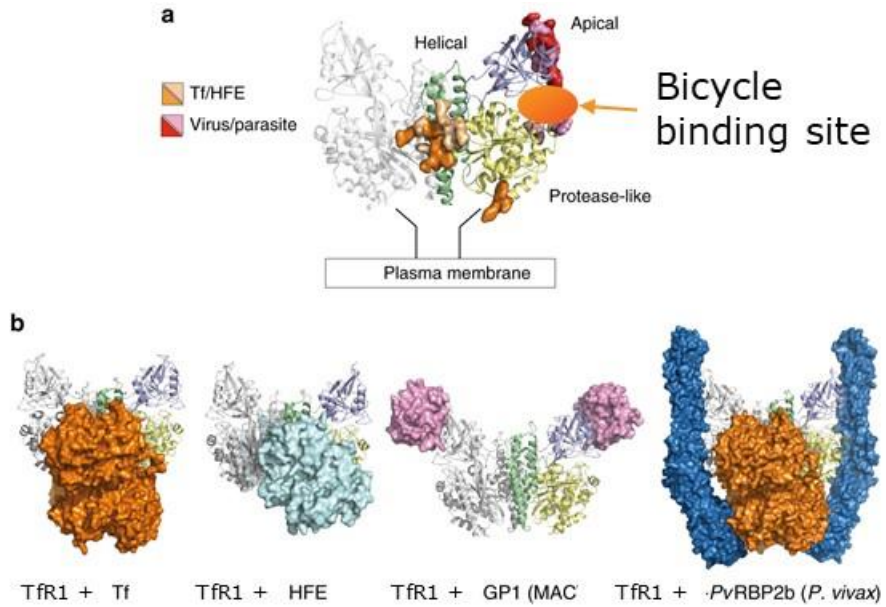
Bicycle
bound to **hTfR1**



(Only 1 TfR1 monomer shown)

Overlay with **TfR1 dimer**
and **Tf ligand**

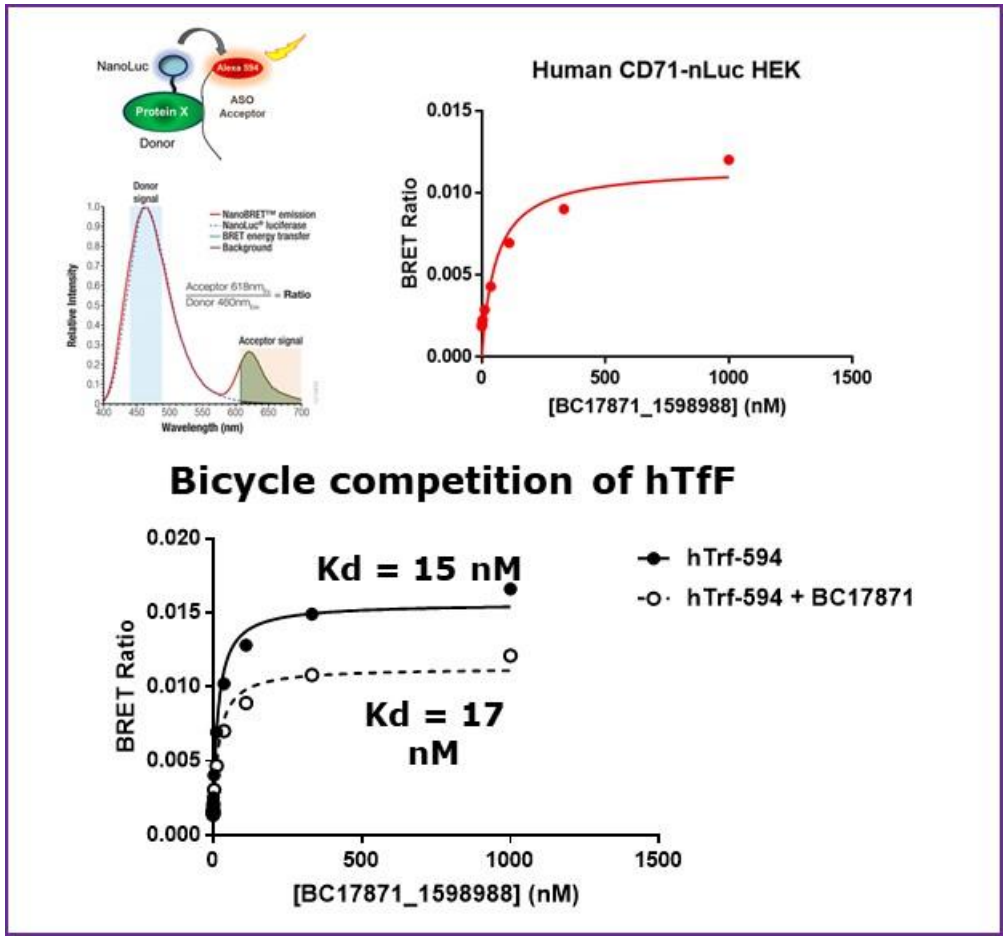


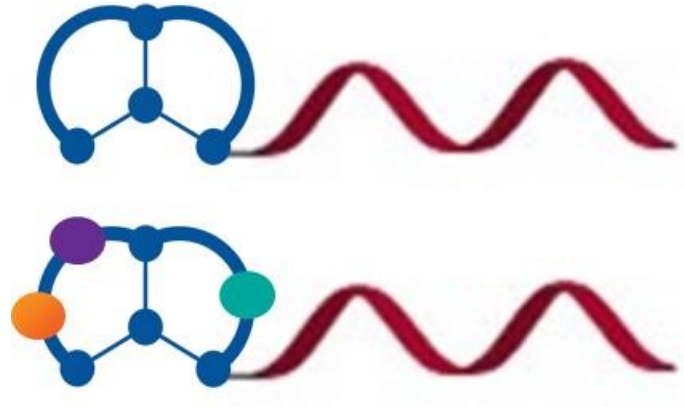


TfR1 with ligands and virus proteins

Bicycle binds to a novel site between apical & protease-like domain, does not compete with transferrin ligand

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

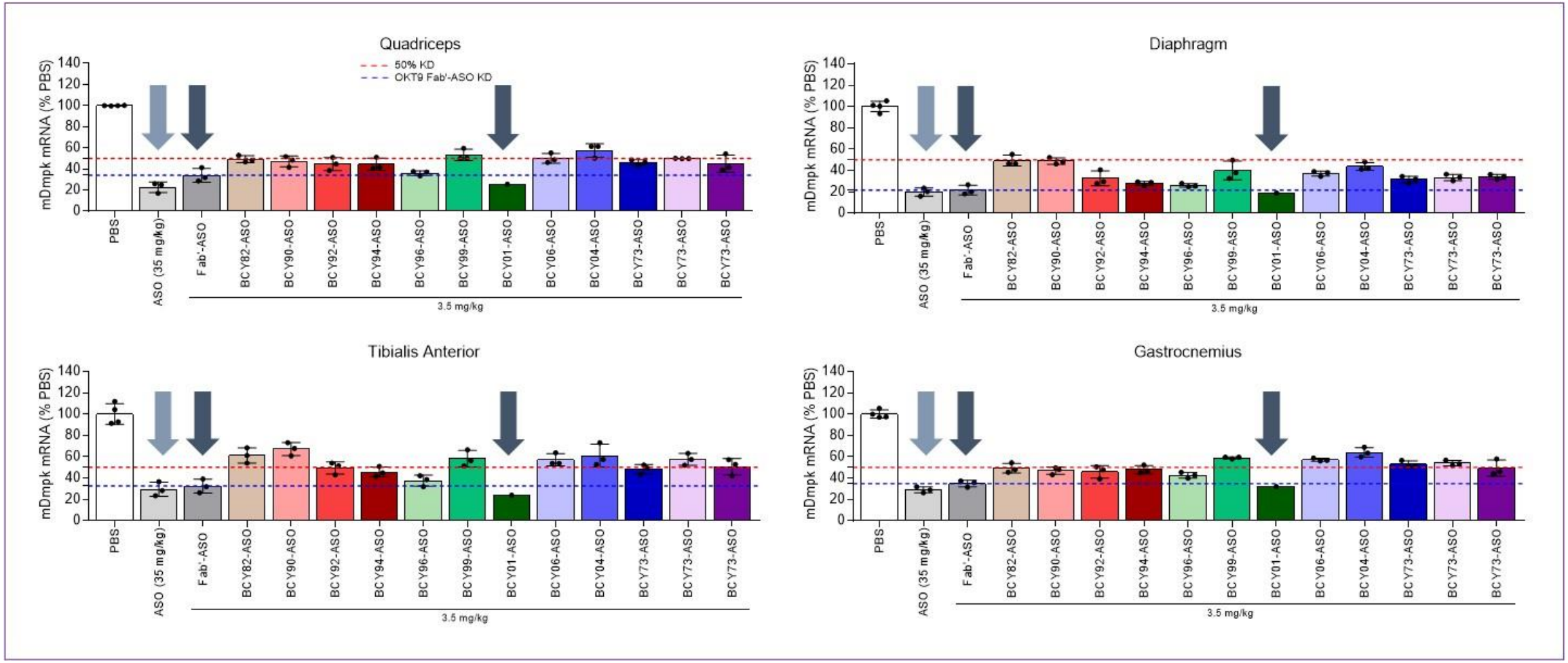




Conjugate	Ki (nM)
BCY82-ASO	55
BCY90-ASO	20
BCY92-ASO	11
BCY94-ASO	10
BCY96-ASO	2
BCY99-ASO	4
BCY01-ASO	1
BCY04-ASO	60
BCY06-ASO	22

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

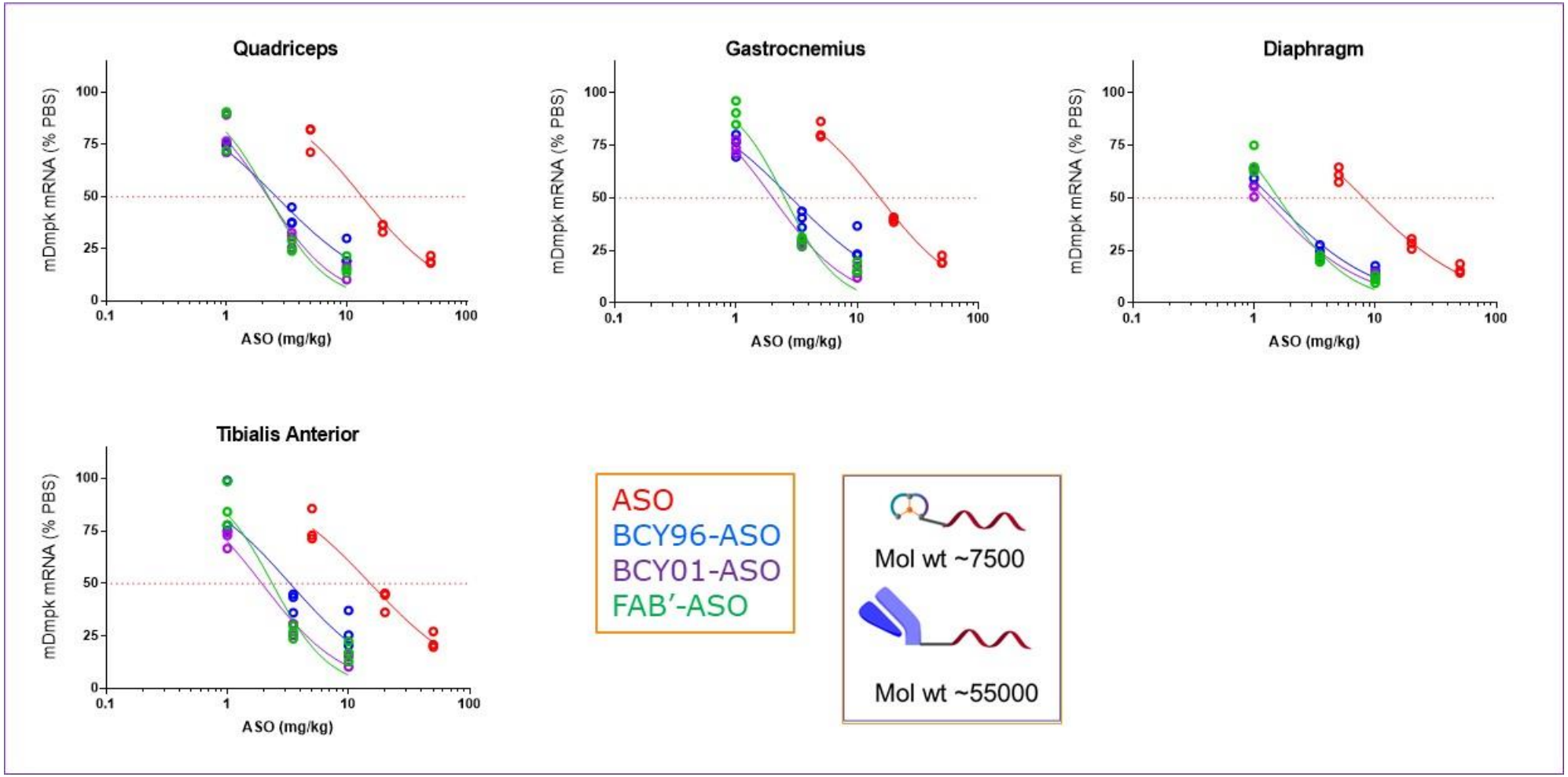
Reduction of DMPK mRNA quantified by qRT-PCR, single dose level



hTfR1$^{KI/+}$ mice were injected with 3.5 mg/kg/wk/3 wks of ASO-conjugates for 3 weeks.

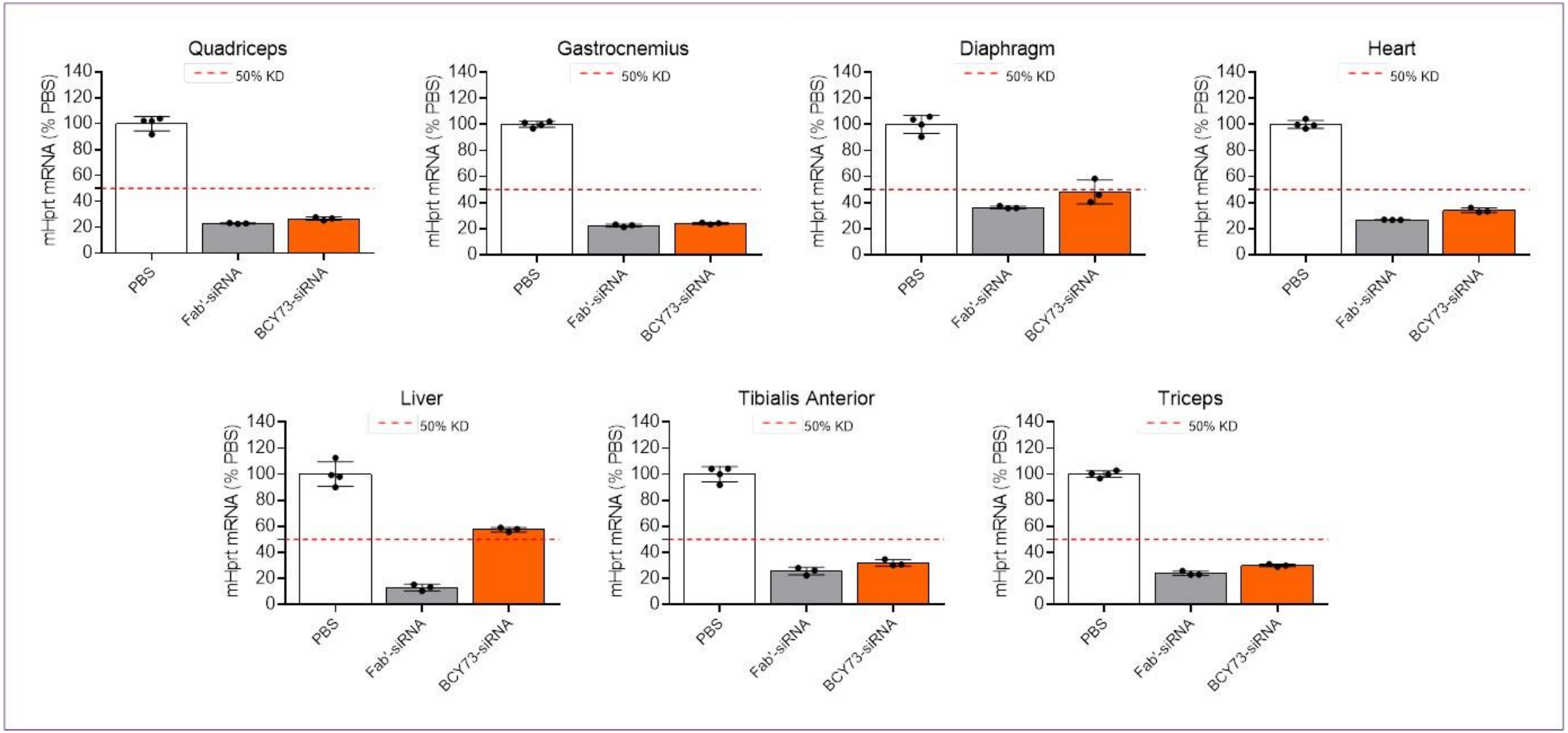
Bicycles targeting hTfR1 enhance ASO potency in striated muscles in hTfR1$^{KI/+}$ mice

Reduction of DMPK mRNA quantified by qRT-PCR, dose response



hTfR1$^{KI/+}$ mice injected with 3.5 mg/kg of ASO conjugates at days 0, 4 and 8 (day 12 data shown)

Bicycle-siRNA conjugate shows similar potency as FAB'-siRNA conjugate in hTfR1$^{KI/+}$ mice



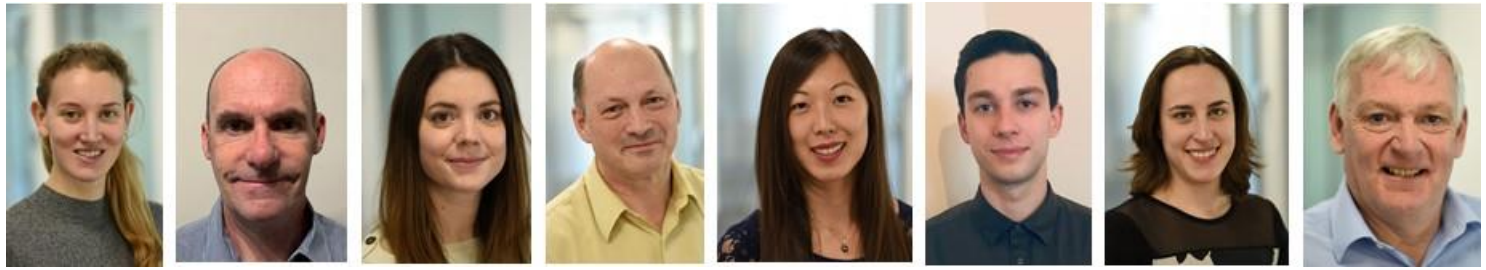
hTfR1$^{KI/+}$ mice were injected with 3.5 mg/kg/wk/3 wks of siRNA-conjugates, 3 week study.

Summary

- Bicyclic peptides (*Bicycle*®) have been identified and tuned to have optimal affinity and specificity for human transferrin receptor 1
- These bind a site distinct from transferrin and other known ligands
- *Bicycles* enhance the potency of gapmer ASOs ~10-fold versus unconjugated ASO in hTfR1$^{KI/+}$ mice with similar potency to Fab'-ASO conjugate
- *Bicycle*-siRNA conjugates show similar potency as Fab'-ASO conjugate in hTfR1$^{KI/+}$ mice
- *Bicycle* ASO & siRNA conjugates were well tolerated in hTfR1$^{KI/+}$ mice

Thank you and acknowledgements

bicycle therapeutics



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