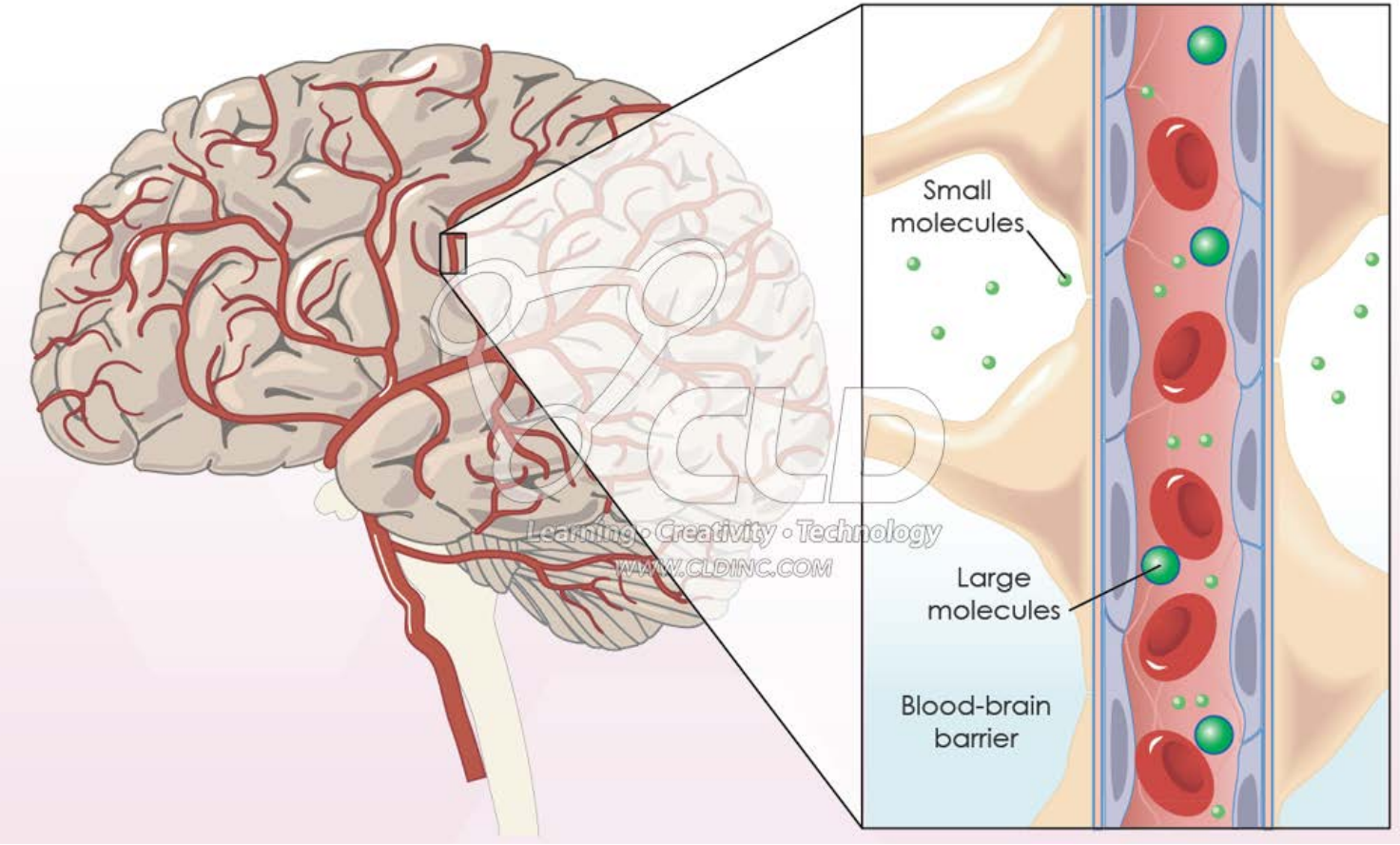
Improving Methods for Traversing the Blood-Brain Barrier: A Workshop

Keck Center of the National Academies
of Sciences, Engineering, and Medicine

September 8th, 2017

Getting Biologics Into the Brain



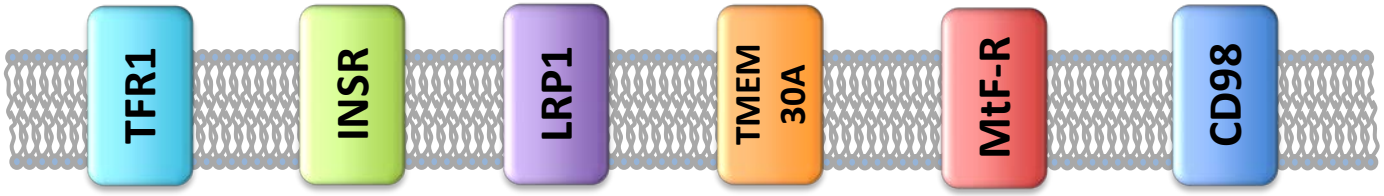
The blood brain barrier (BBB) protects the brain and prevents most therapeutics, including antibodies and most other large and small molecules, from passing through blood vessels into the brain.

Trojan Horse Approach to the BBB

Receptor-mediate transfer without disrupting the barrier

Desirable properties of a BBB shuttle:

- Rapid – immediate uptake to the parenchymal
- Efficient – high capacity transfer
- Potent – effective at low therapeutic doses
- Versatile – works with multiple types of cargo
- Safe – unaltered transport of endogenous ligand
- Translatable – rodent/human cross-reactivity



TfR1 VNARs with vastly greater BBB transfer were isolated using combination of *in vitro/in vivo* phage selection

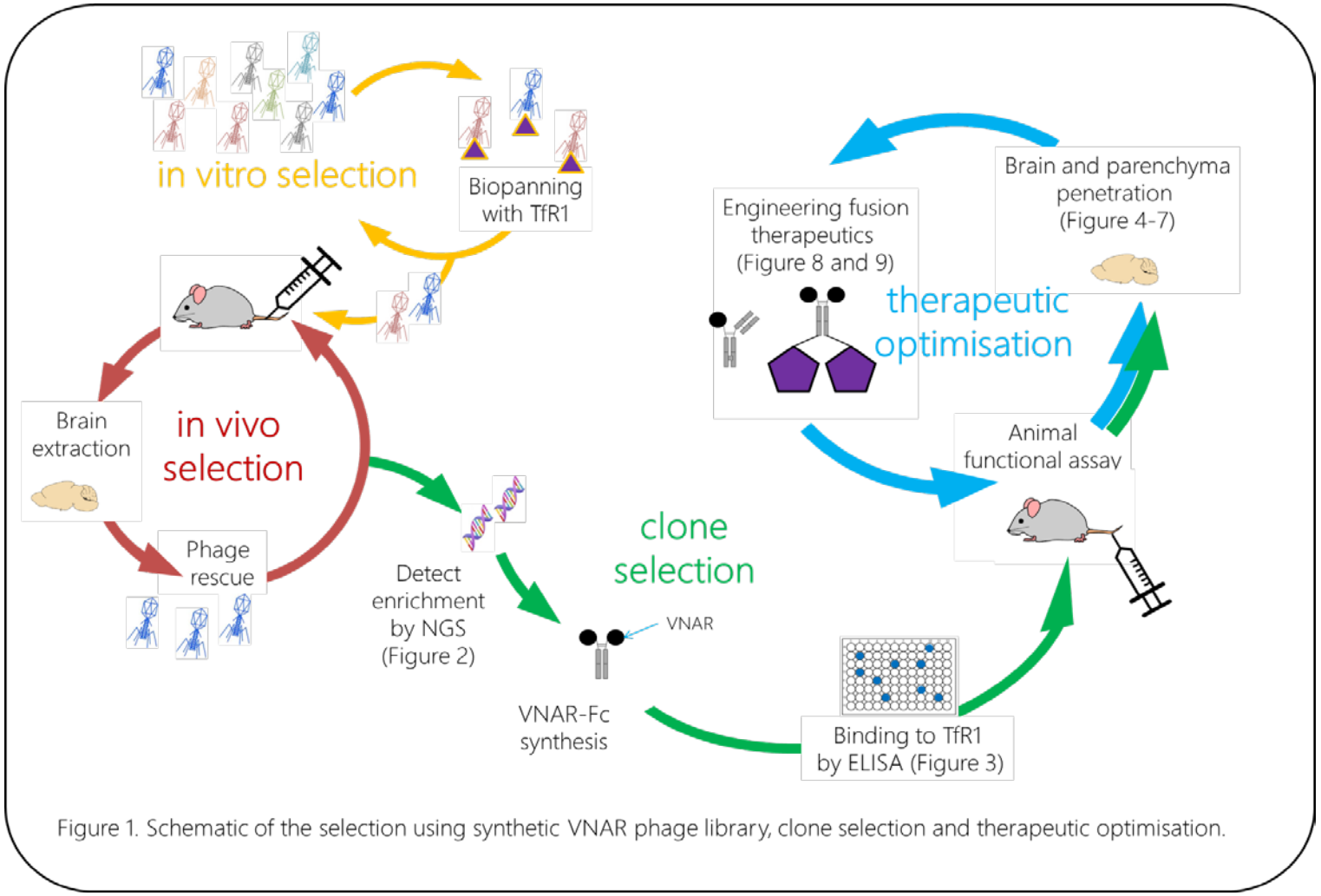
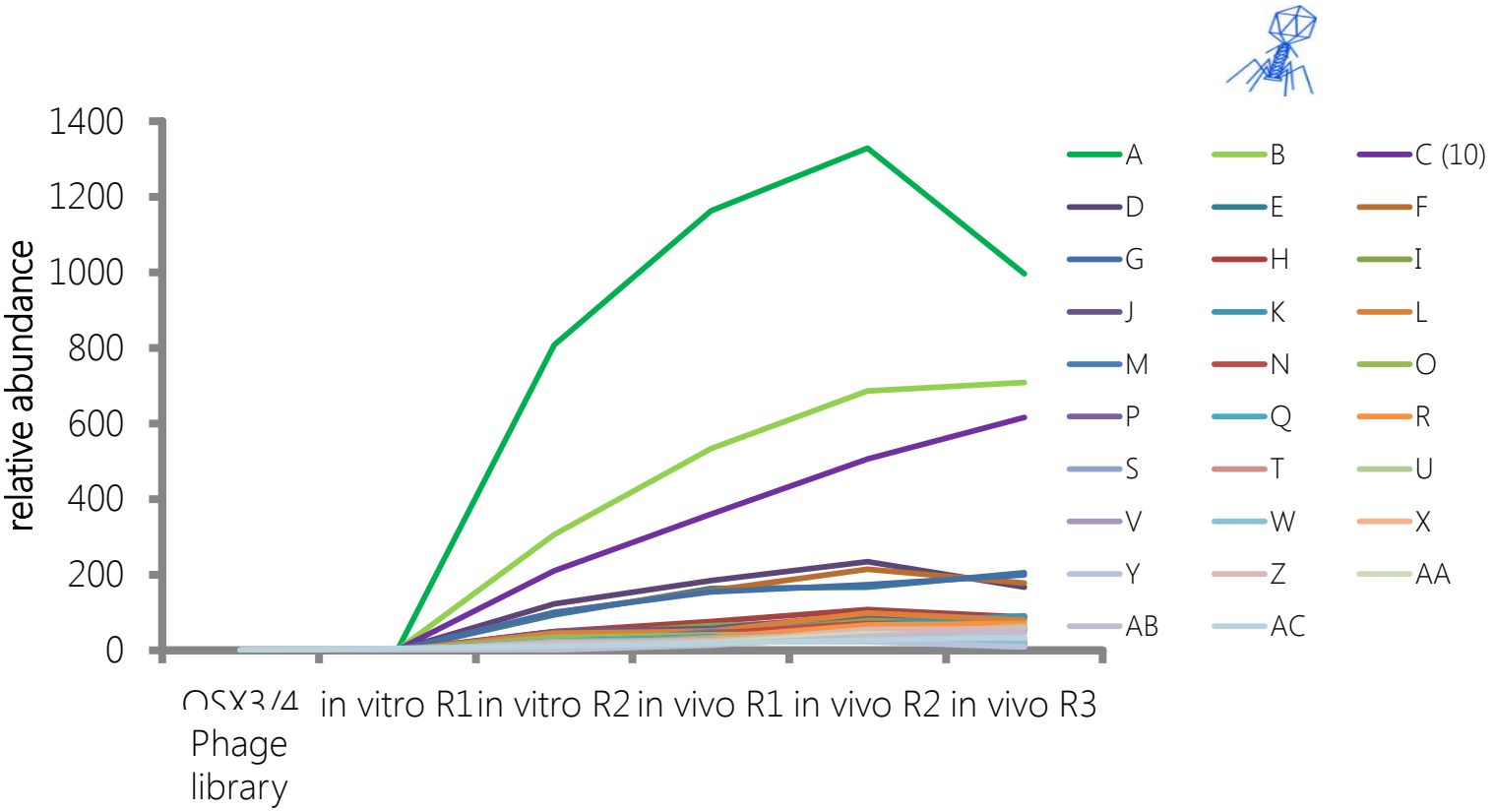
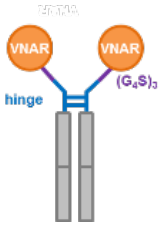


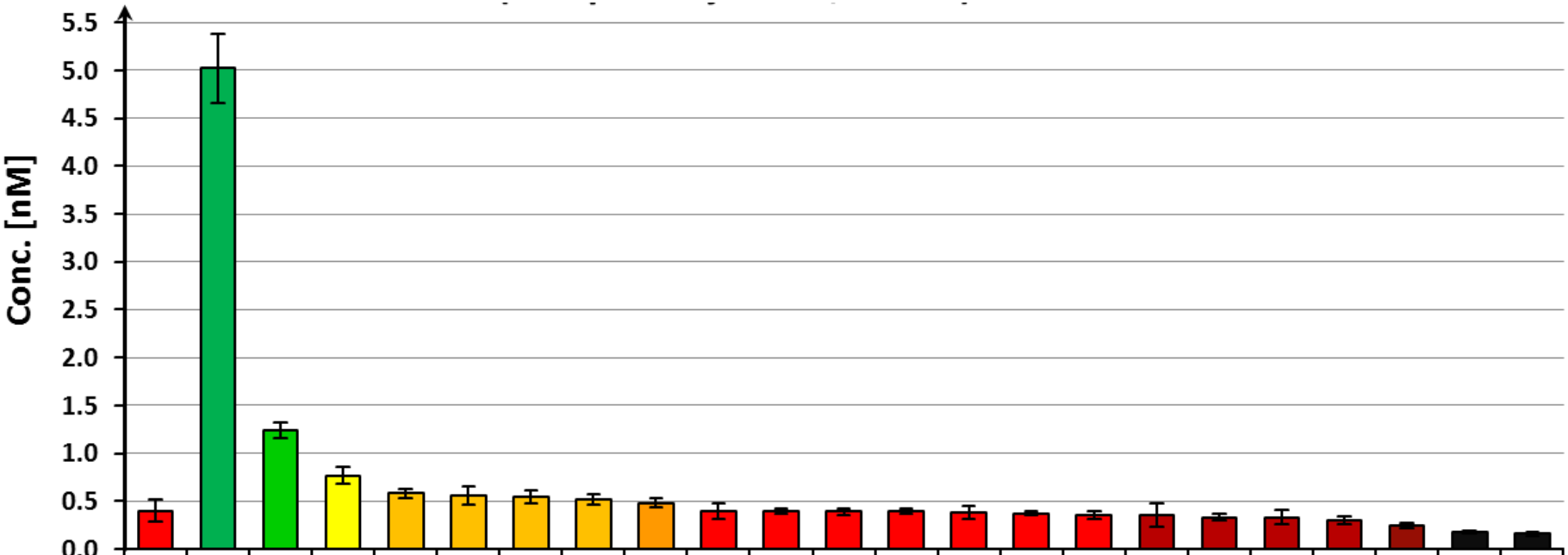
Figure 1. Schematic of the selection using synthetic VNAR phage library, clone selection and therapeutic optimisation.

VNAR phage clones enriched in the brain were identified by next generation sequencing (NGS)





VNARs formatted as bivalent Fc fusions were screened for brain uptake after a single 2 mg/kg, IV dose

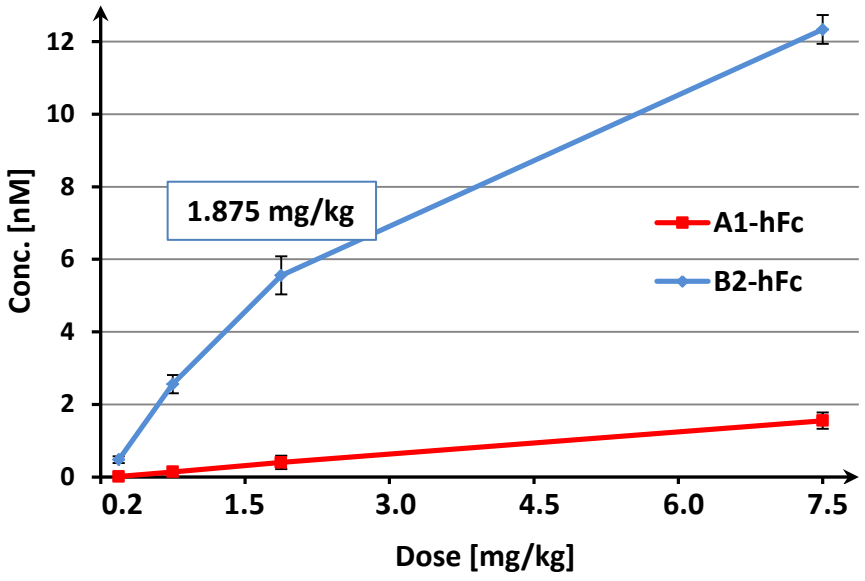


	A1	B2	31	15	10	23	14	19	11	27	30	12	24	20	35	26	33	37	34	25	32	17	16
Conc. [nM]	0.40	5.02	1.24	0.77	0.59	0.56	0.55	0.52	0.48	0.40	0.40	0.39	0.39	0.39	0.37	0.35	0.35	0.33	0.33	0.30	0.25	0.18	0.16

- **B2-hFc was far superior to any other VNAR to TfR1 identified to date**
- **Clone 31 & 15 were the next best and 5 other have appreciable uptake, which would be more apparent at higher doses**

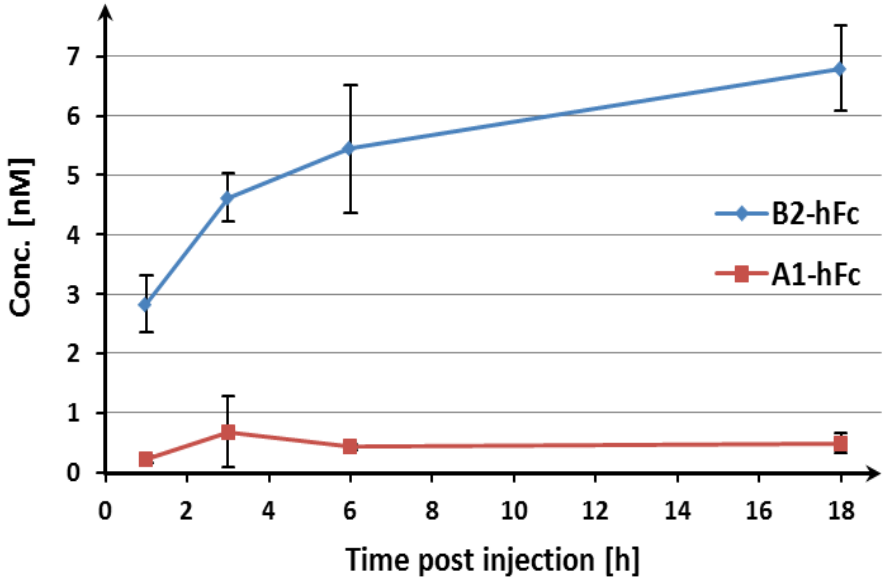
Brain uptake is rapid, robust & prolonged at therapeutic doses

Single ascending dose: 18 hr post inj



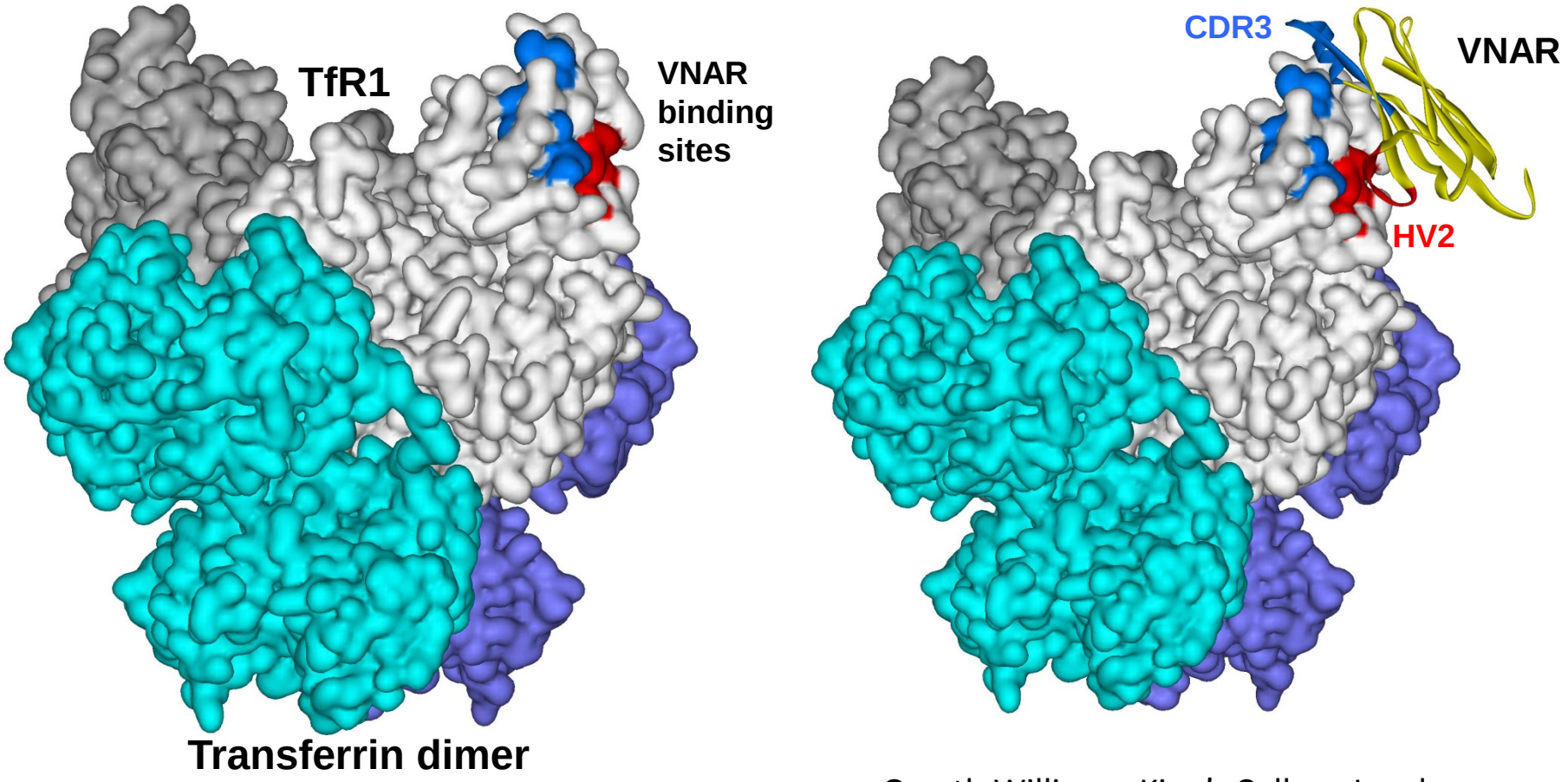
- Brain uptake is readily detected 18 hr after < 2 mg/kg

Time course of single dose: 2 mg/kg



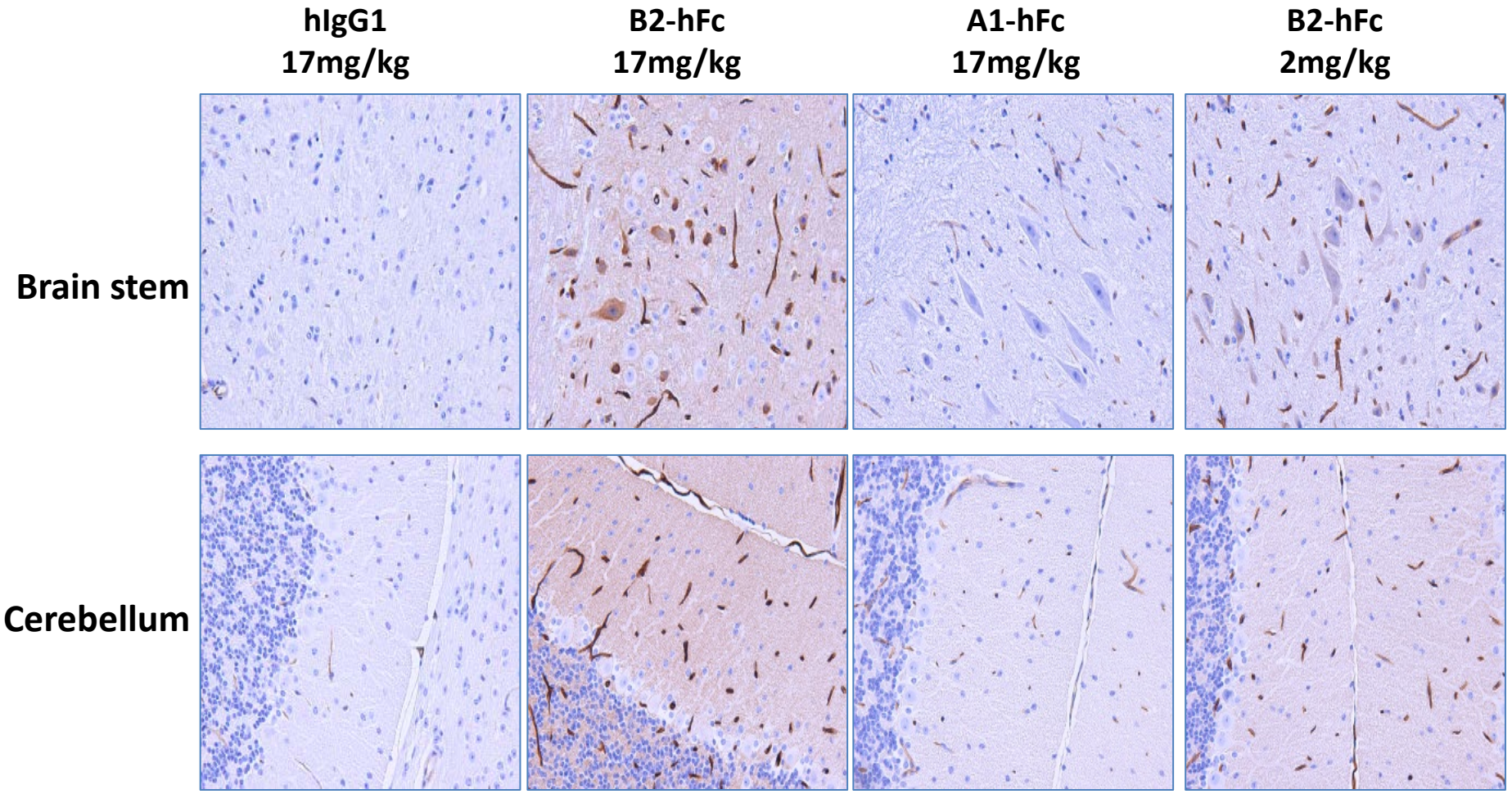
- Brain uptake is detectable 1 hr after IV injection & the level increases for at least 18 hr

VNAR binds the apical region of TfR1, distant from the transferrin binding domain



Gareth Williams, King's College London

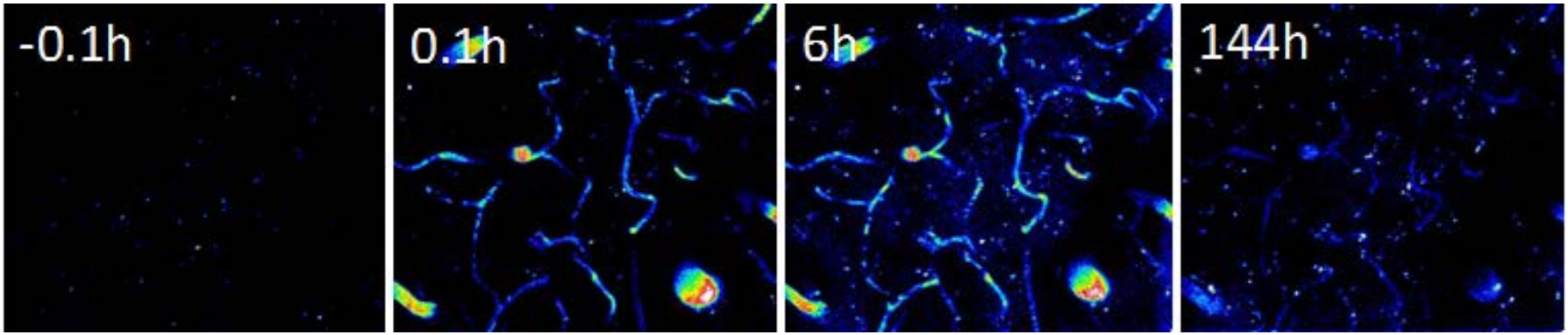
Immunohistochemistry shows transfer of the lead TfR1 VNAR across blood vessels and into the parenchyma & neurons



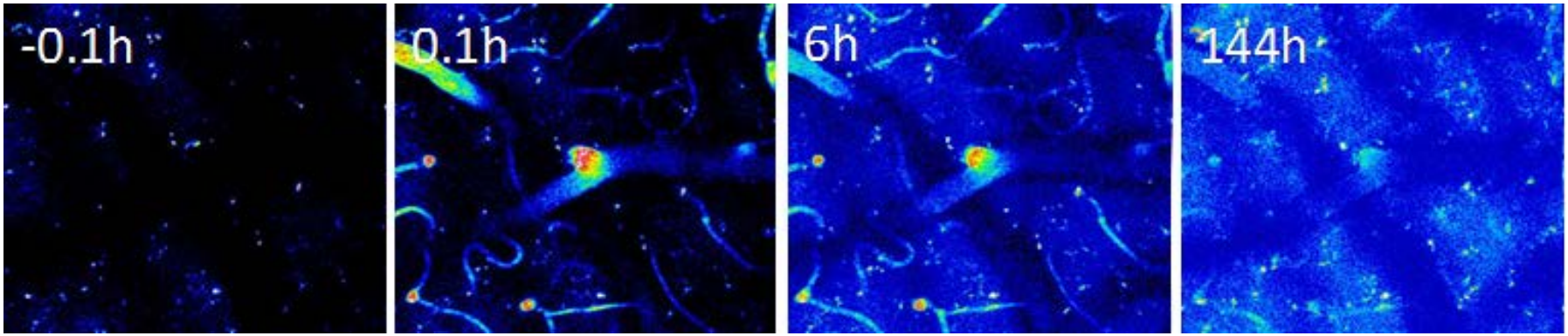
18 hrs after single IV injection; IHC for human Fc after cardiac perfusion

Two-photon imaging shows transfer of a TfR1-VNAR/CD20 bispecific antibody to the brain parenchyma

RITUXIMAB

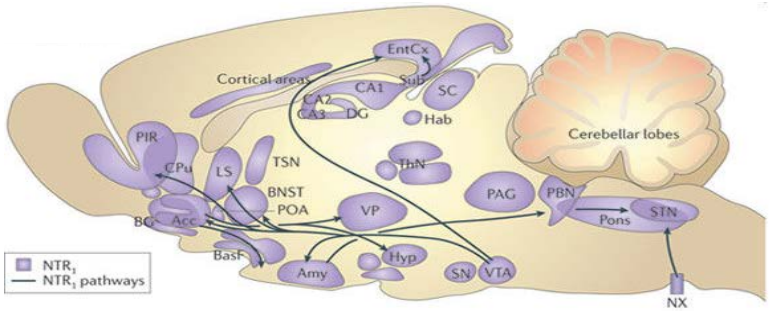


RITUXIMAB-VNAR bispecific



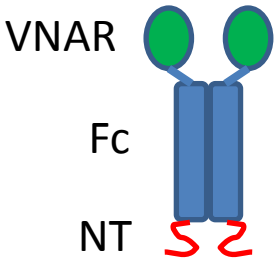
Time course after 4mg/kg, IV of Alexa488 labelled antibodies

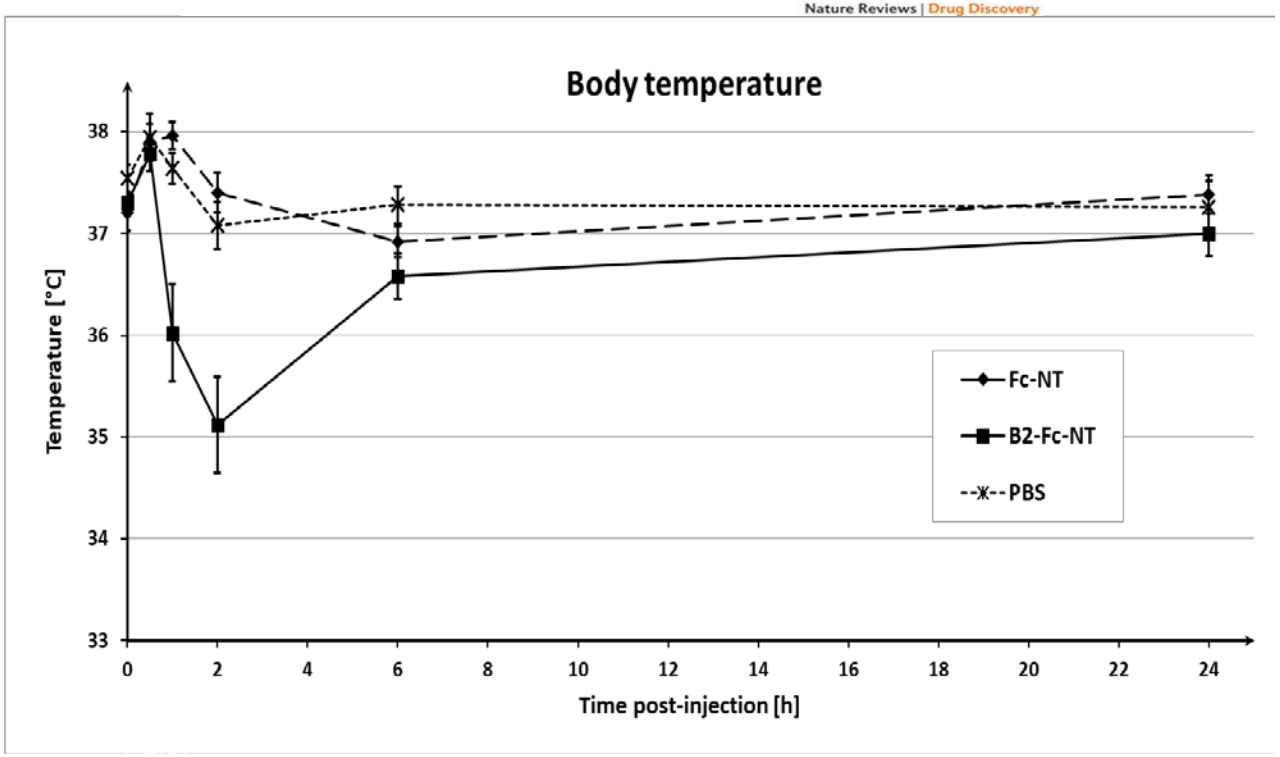
Reduction in body temperature mediated by neurotensin (NT) as a physiological measure of parenchymal transport



Nature Reviews | Drug Discovery

B2-hFc-NT





A truly effective BBB carrier system opens up a wide range of therapeutic mechanisms and options for CNS disease