

Delivering antisense oligonucleotides to the murine brain using image-guided focused ultrasound

Dr Kara L. Vine-Perrow

kara@uow.edu.au

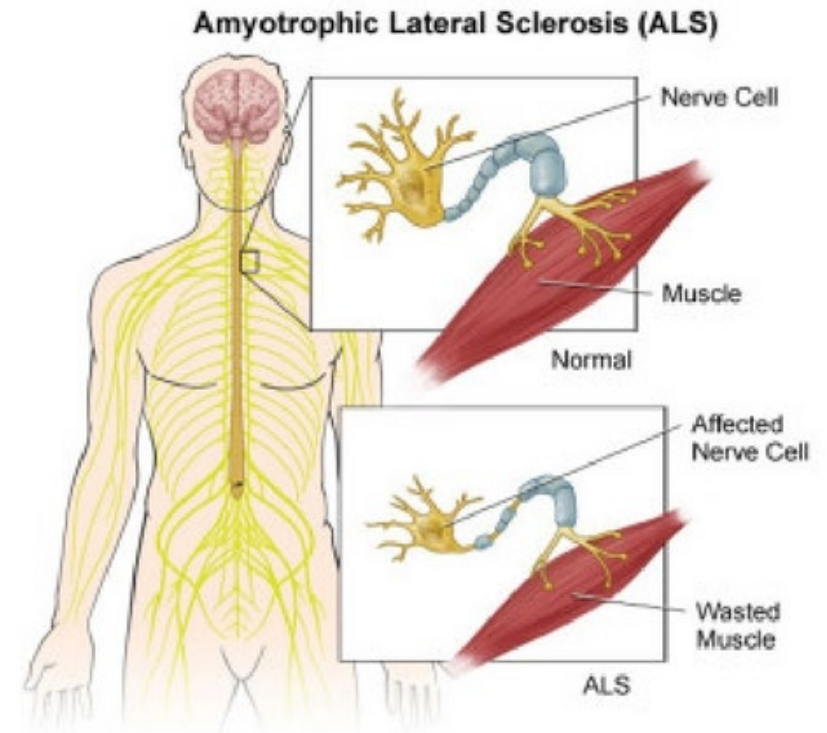
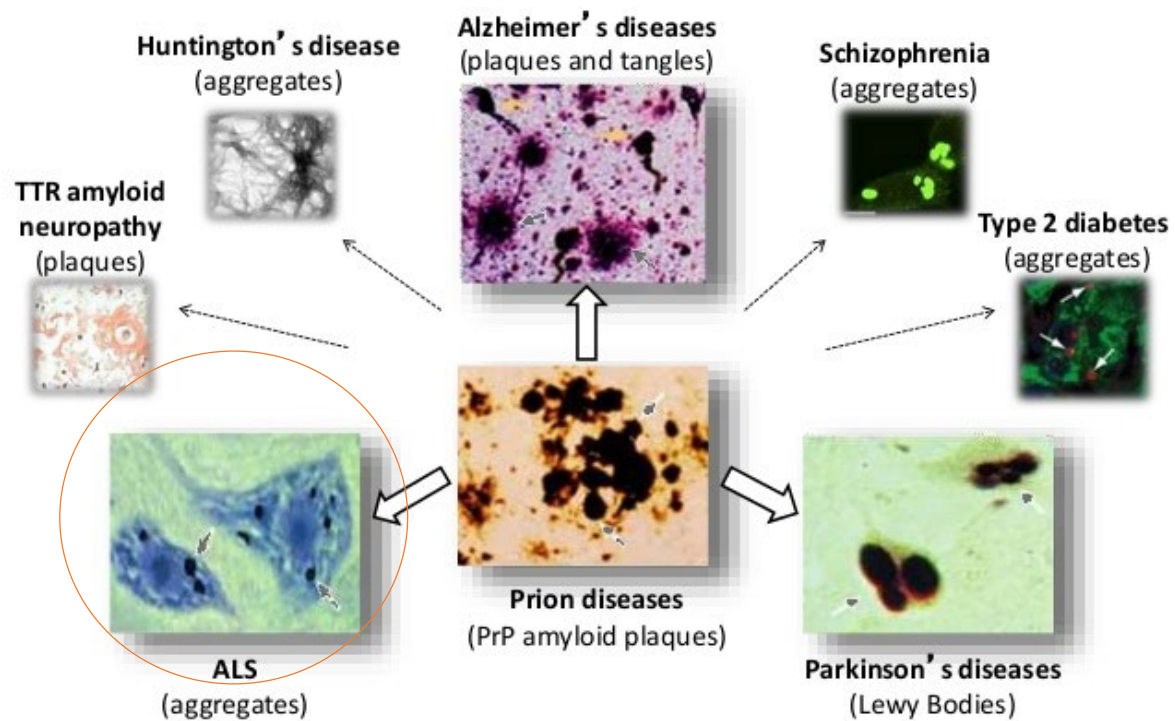
CRS 2022 Annual Meeting & Expo

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

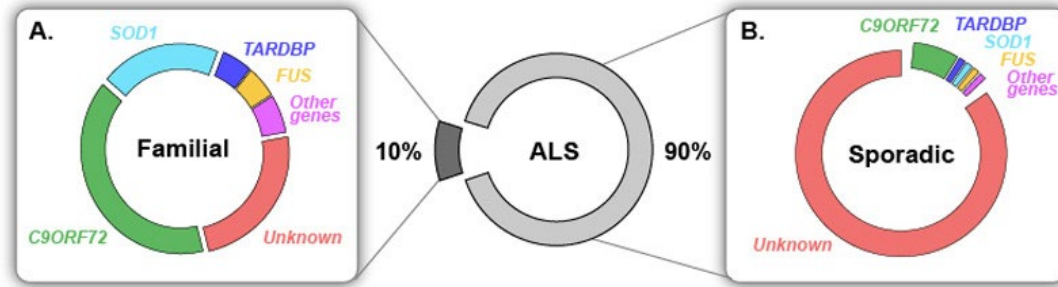
Advanced Delivery Science



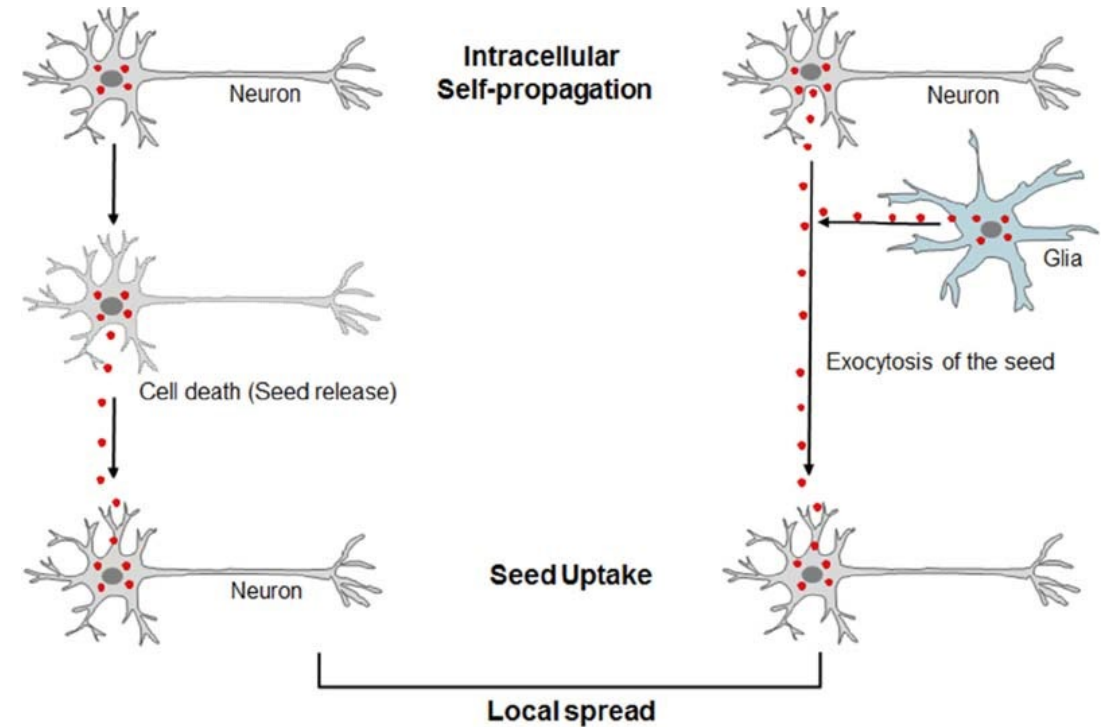
Amyotrophic Lateral Sclerosis (ALS)



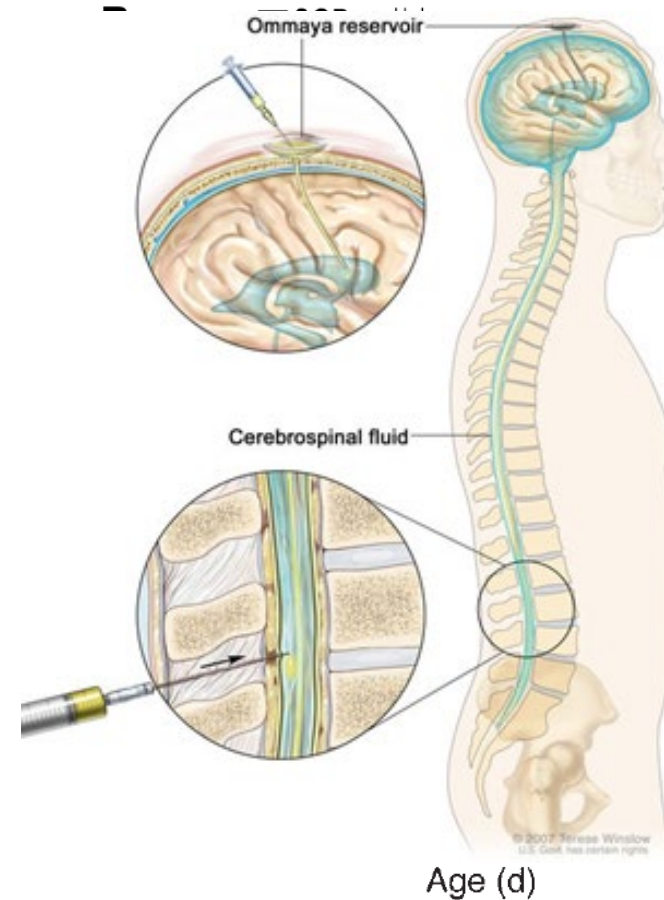
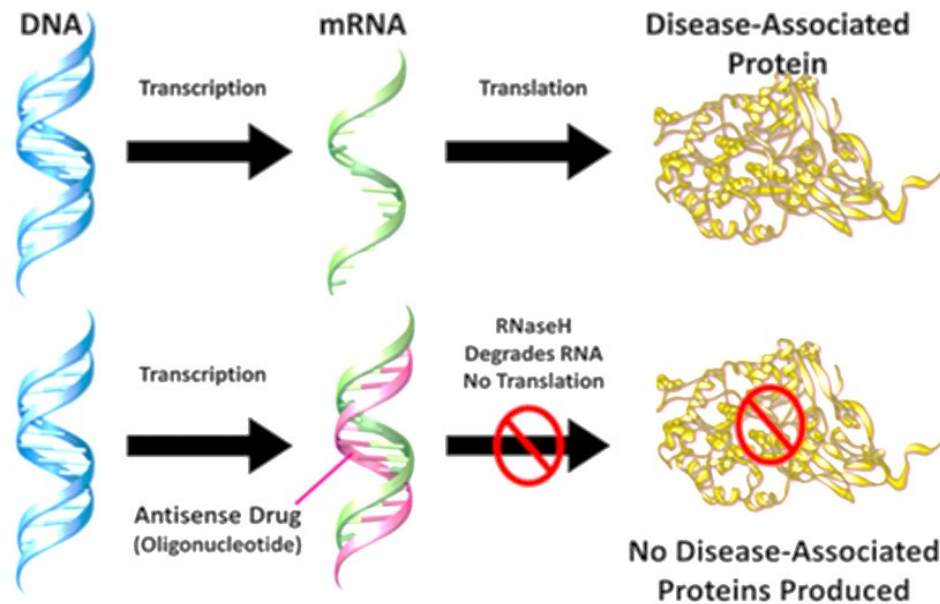
SOD1 best studied causative gene



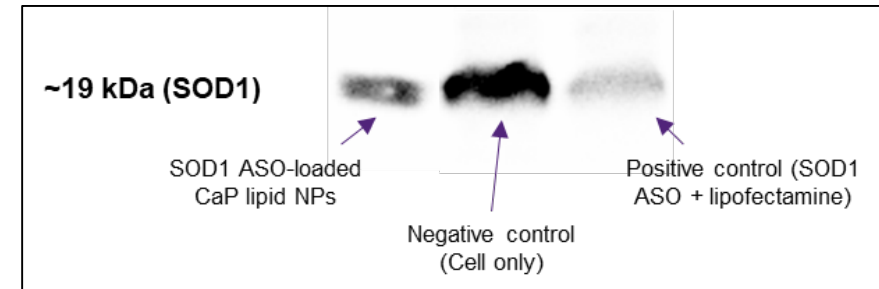
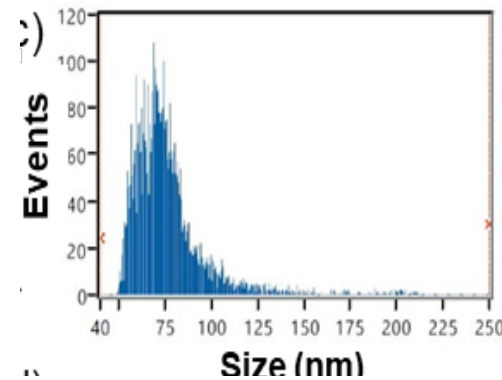
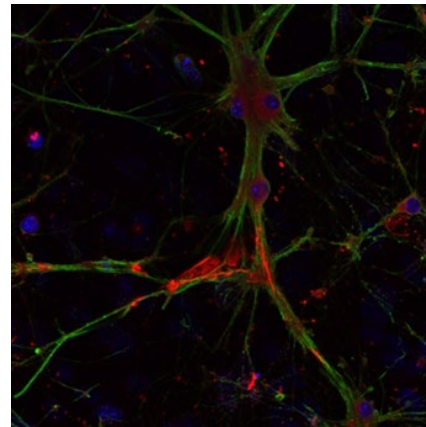
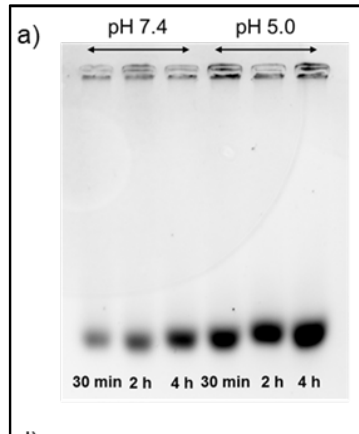
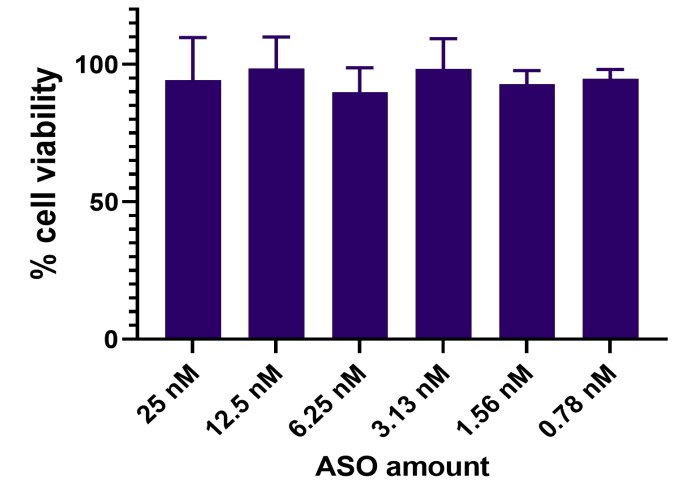
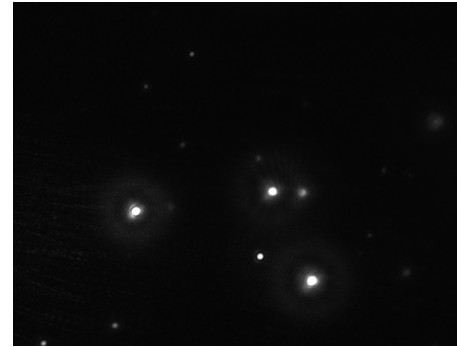
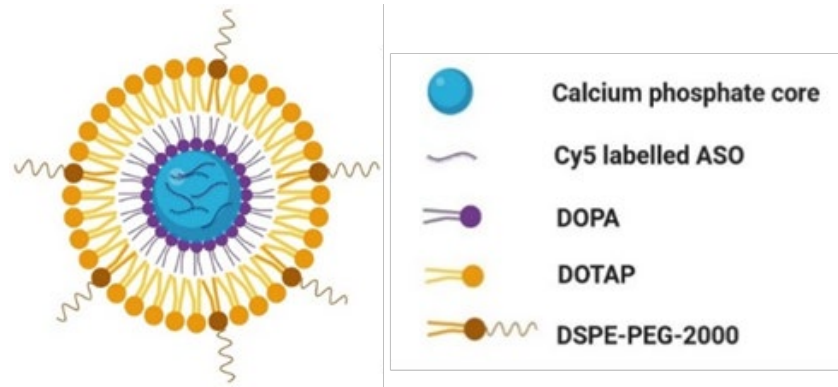
- best-studied mutation is in the gene encoding copper/zinc superoxide dismutase (Cu/Zn SOD, SOD1)
- SOD1 is a metalloenzyme that catalyses the destruction of free superoxide radicals in the body maintaining homeostasis
- Mutations in SOD1 cause ALS through a toxic gain of function



Antisense oligonucleotides targeting SOD1



CaP-lipid nanoparticles (NPs) to deliver ASOs



Chen et al., Frontiers in Neuroscience 2017
Ediriweera *et al.*, submitted JCR

Gayathri Ediriweera



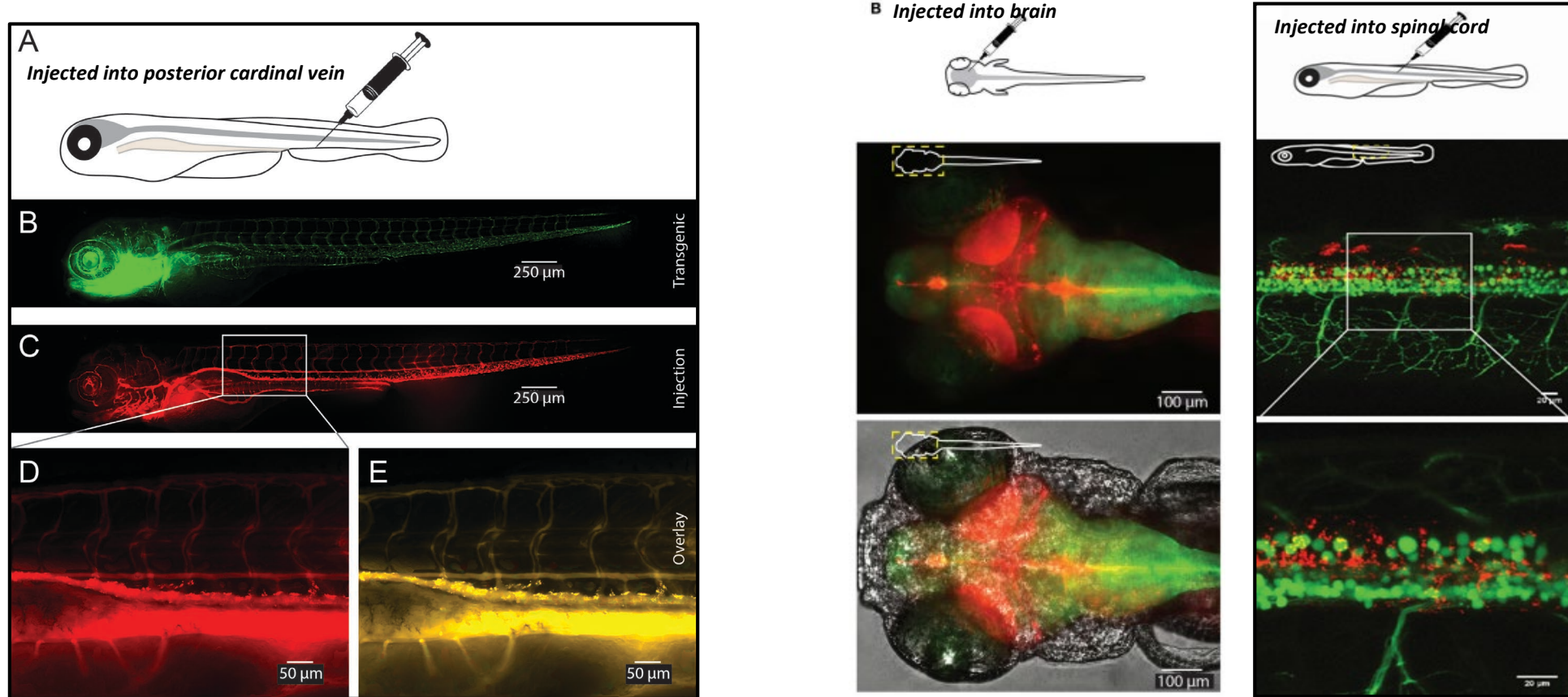
CRS 2022 Annual Meeting & Expo

Advanced Delivery Science

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada



Accumulation NPs in zebrafish brain and spinal cord



Chen et al., Frontiers in Neuroscience 2017

Marco Morsch

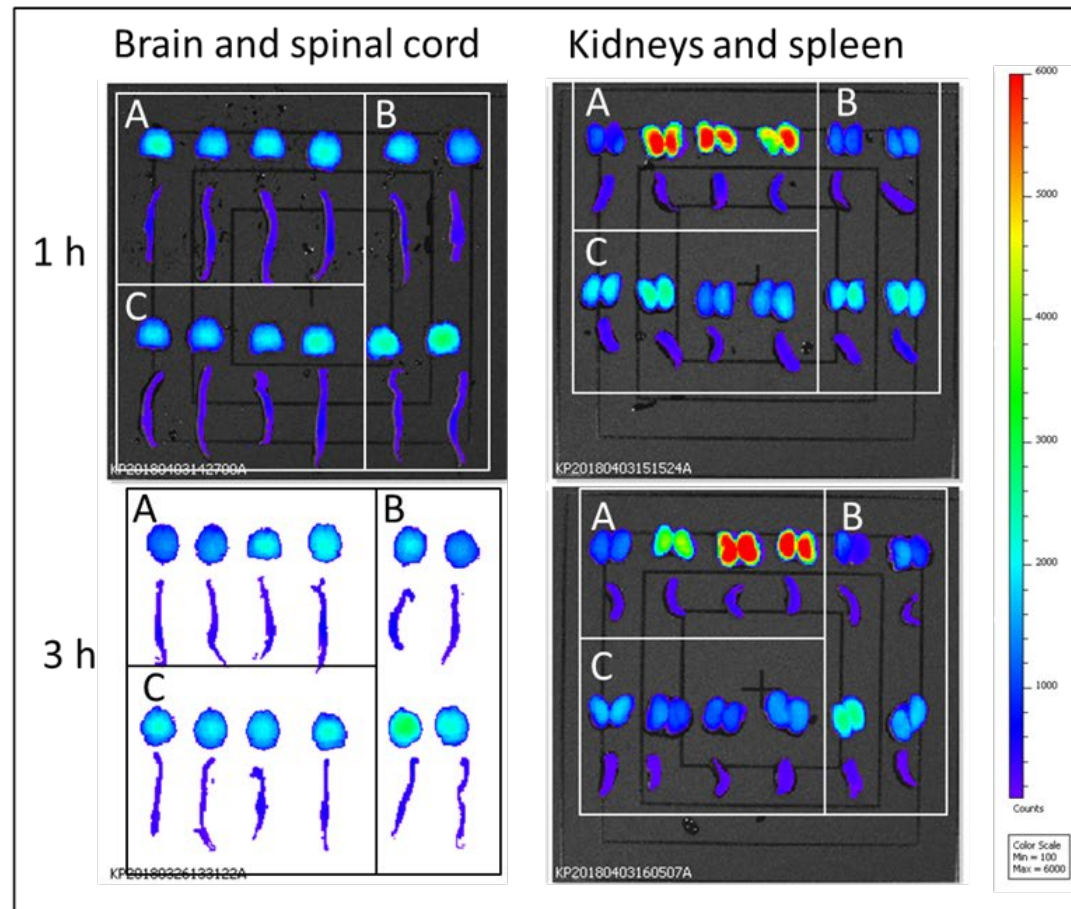


CRS 2022 Annual Meeting & Expo

Advanced Delivery Science

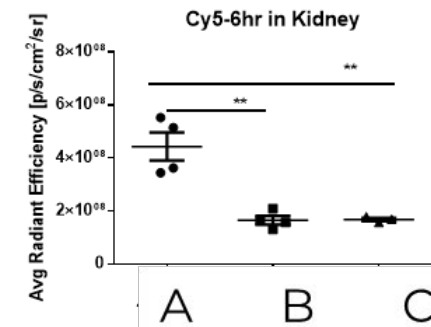
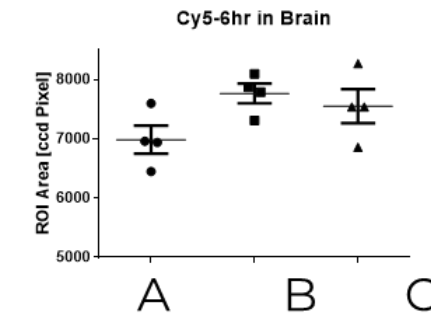
July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

NPs reduce renal clearance of SOD1 ASO



Cy5-labelled SOD1 (Ex 640 nm/Em 700 nm)

(A) Naked SOD1 ASO
(B) Non-functionalised CaP-lipid NPs
(C) Functionalised CaP-lipid NPs



Liyu Chen
Clare Watson

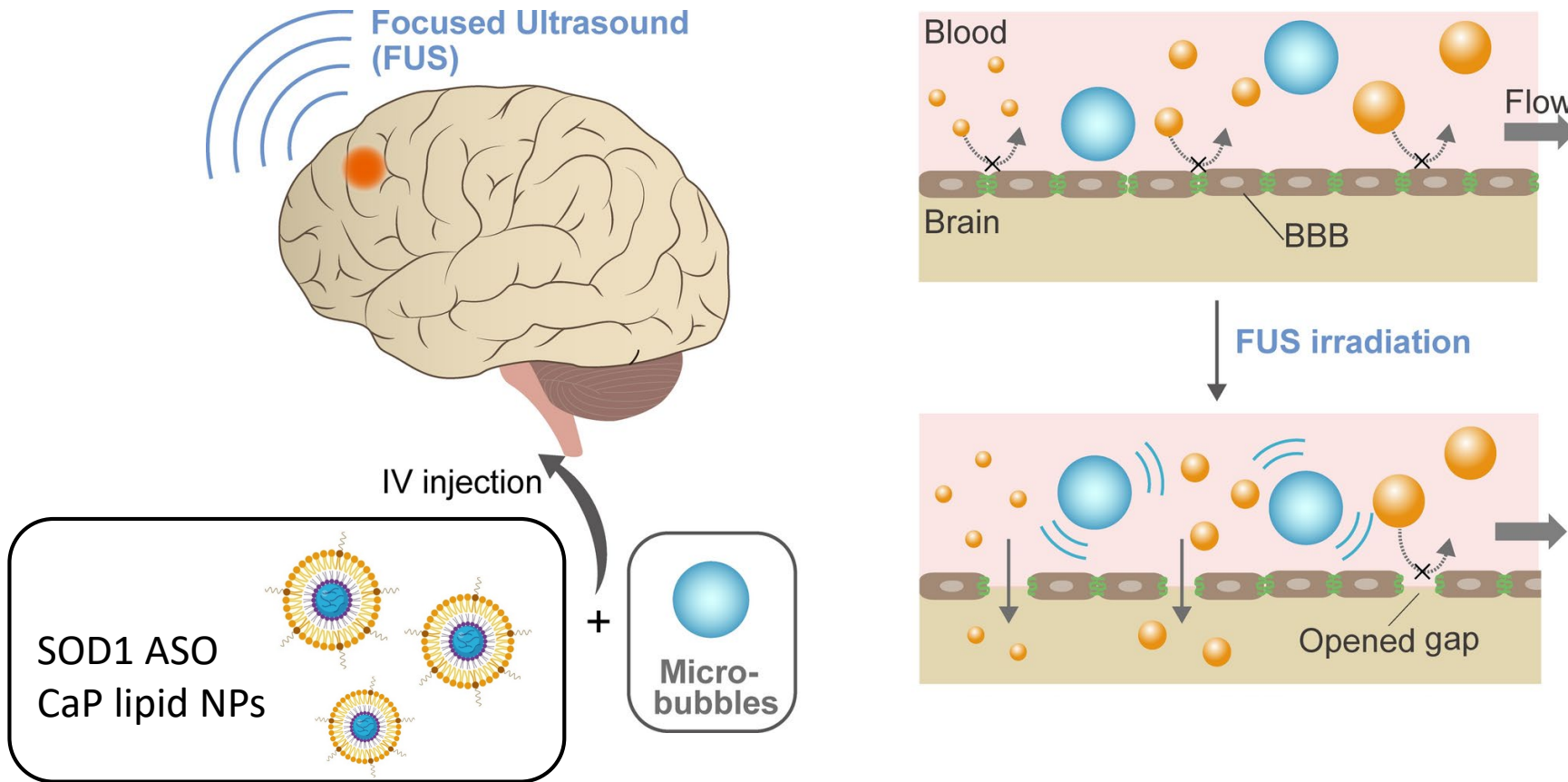


CRS 2022 Annual Meeting & Expo

Advanced Delivery Science

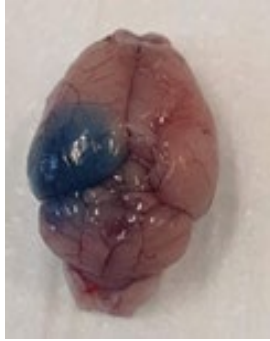
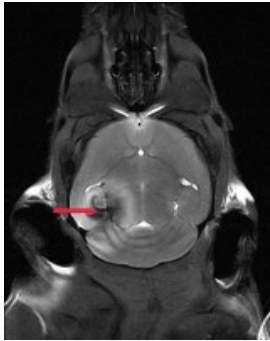
July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

FUS non-invasive approach to open BBB



Optimisation of ultrasound conditions

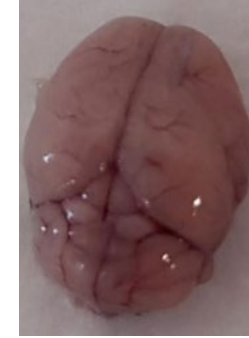
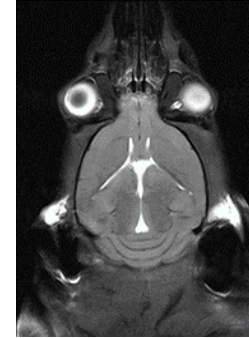
T2 weighted MRI Evans blue



Frequency	Sonication length	MB dose
0.33 MI	120 sec	0.4 mL/kg

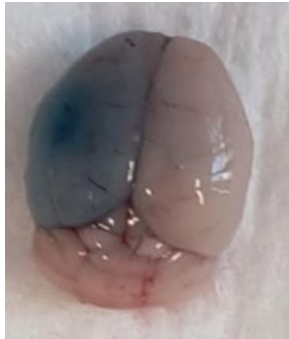
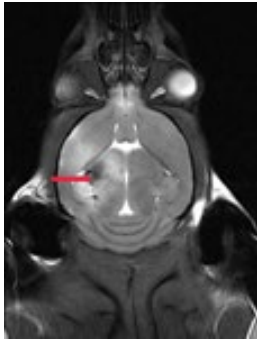
Excessive BBB opening

T2 weighted MRI Evans blue



Frequency	Sonication length	MB dose
0.14 MI	120 sec	0.4 mL/kg
0.22 MI	120 sec	0.4 mL/kg
0.319 MI	120 sec	0.15 mL/kg
0.319 MI	120 sec	0.4 mL/kg
0.325 MI	120 sec	0.15 mL/kg
0.325 MI	120 sec	0.4 mL/kg

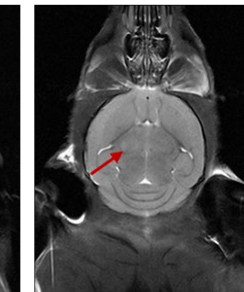
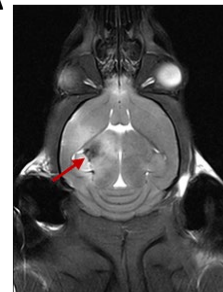
Absence of BBB opening



Frequency	Sonication length	MB dose
0.33 MI	120 sec	0.15 mL/kg

Transient opening of BBB

A



Day 0

Day 1

Day 5

Gayathri Ediriweera

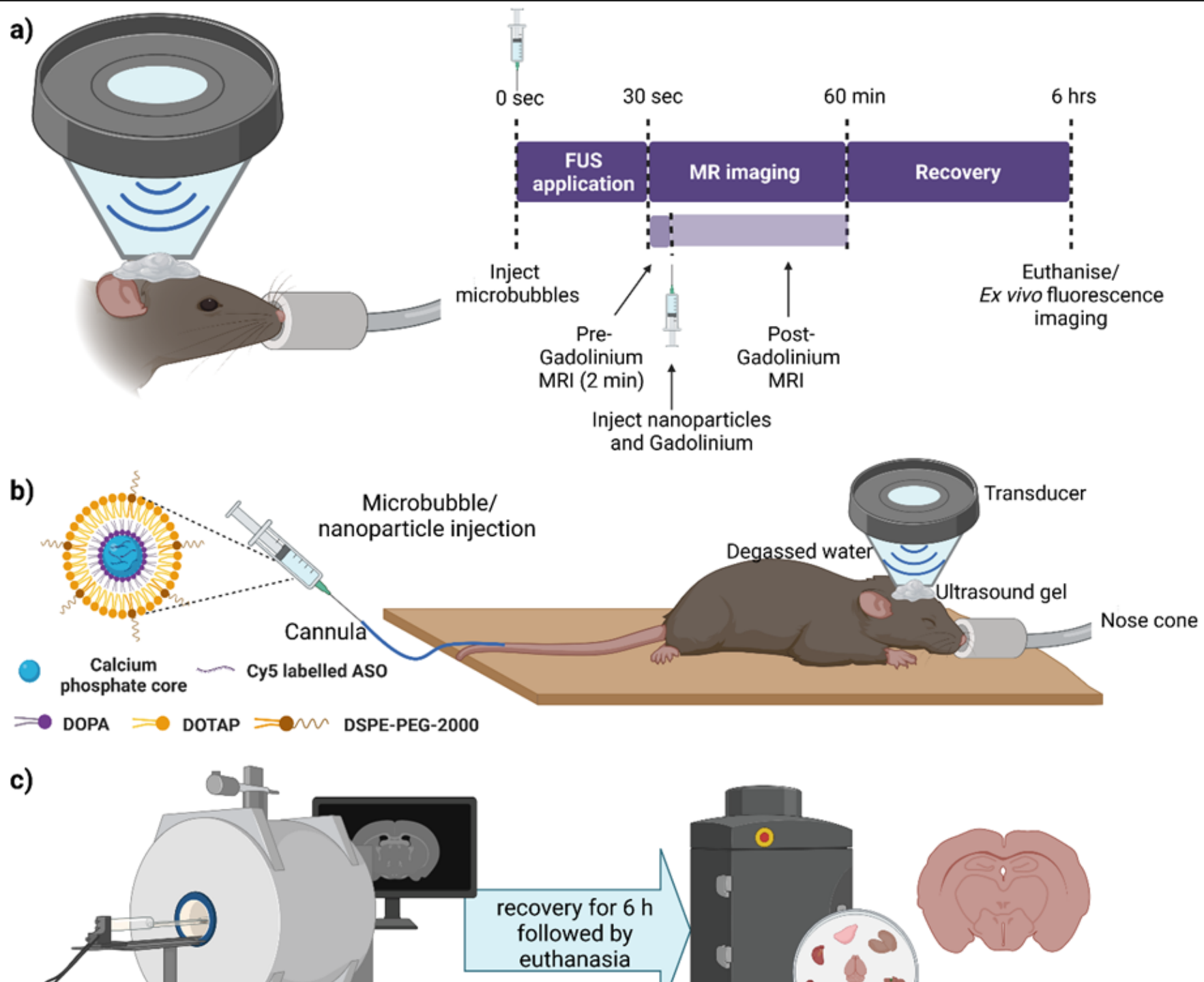
Ediriweera *et al.*, submitted JCR



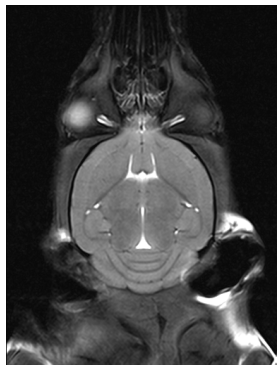
CRS 2022 Annual Meeting & Expo

Advanced Delivery Science

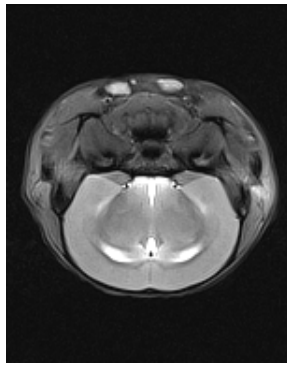
July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada



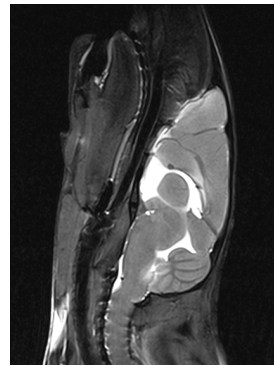
FUS increases ASO-loaded NP delivery to the brain



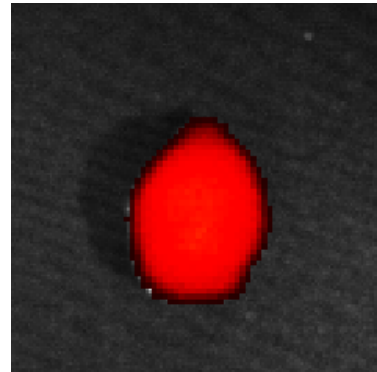
Coronal



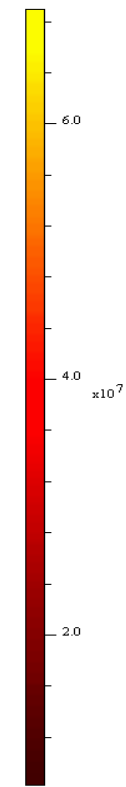
Transverse



Sagittal



Epi-fluorescence



Radiant Efficiency
($\frac{p/sec/cm^2/sr}{\mu W/cm^2}$)

Color Scale
Min = 8.21e6
Max = 6.90e7

Ediriweera *et al.*, submitted JCR

Gayathri Ediriweera

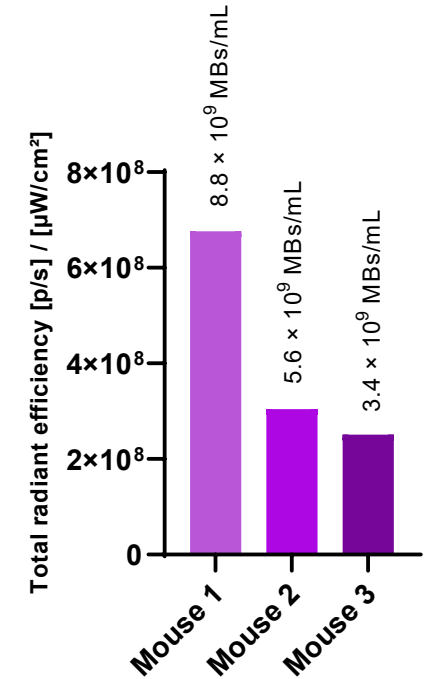
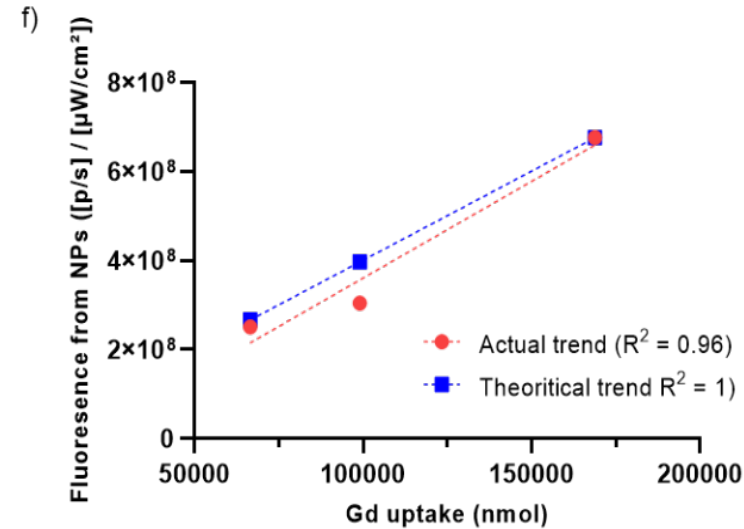
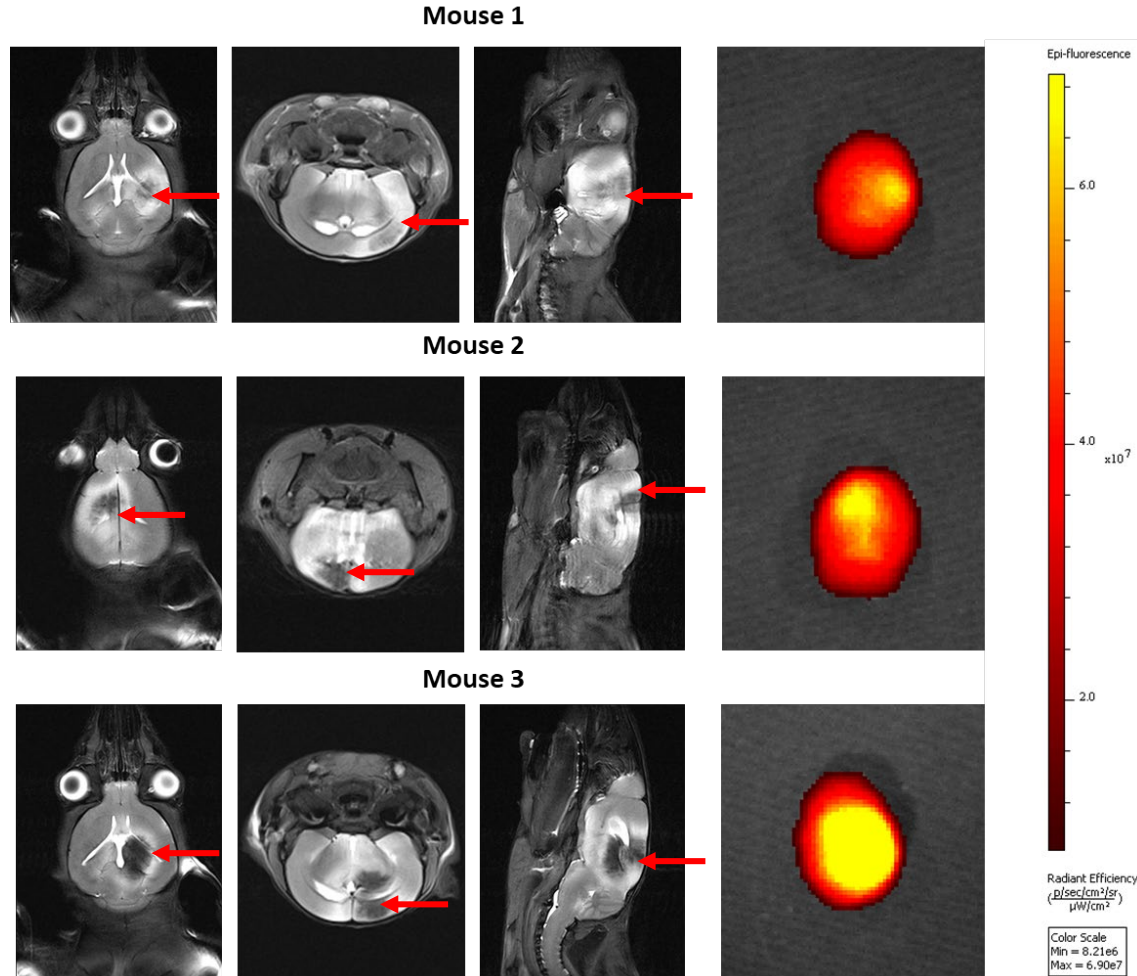


CRS 2022 Annual Meeting & Expo

Advanced Delivery Science

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

Extent of BBB opening correlates to NP uptake in brain



Gayathri Ediriweera

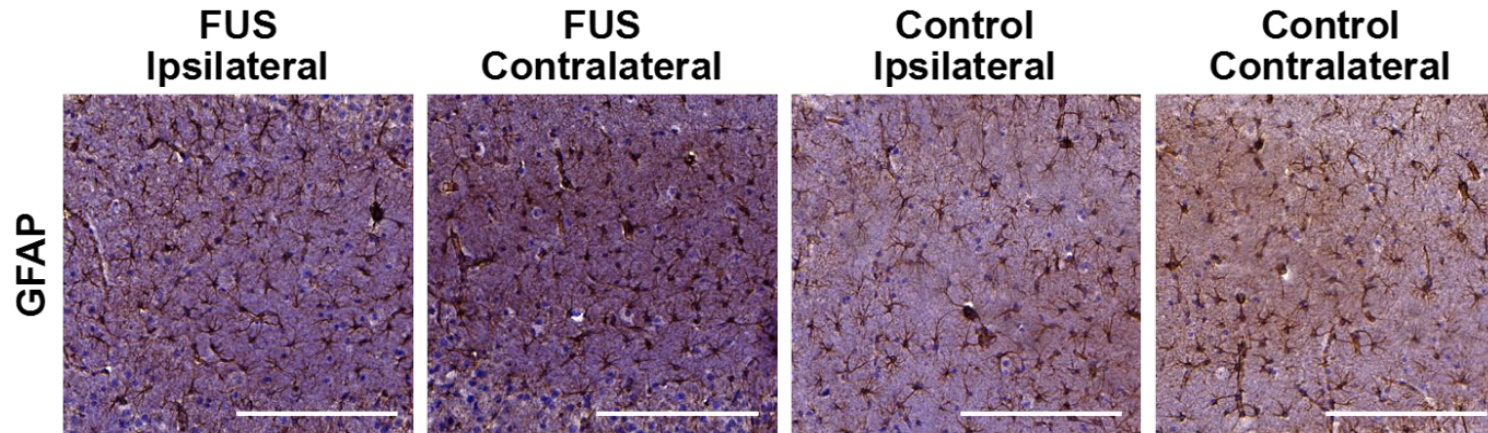


CRS 2022 Annual Meeting & Expo

Advanced Delivery Science

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

FUS-mediated BBB opening did not induce astrocyte or microglial activation



Scale bar = 200 μ m.



CRS 2022 Annual Meeting & Expo

Advanced Delivery Science

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

Summary and study conclusions

- CaP-lipid NPs encapsulating SOD1-ASO significantly down-regulate SOD1 protein expression (HEK293), rapid accumulation in primary motor neuron cell cultures and zebrafish model of ALS.
- FUS + MBs significantly improves the delivery of ASO-loaded CaP-lipid NPs to the brain compared to control mice receiving FUS without microbubbles.
- FUS-mediated opening of the BBB is transient and without evidence of any perturbation in the neural microenvironment, parenchymal injury or necrosis indicating the treatment is well tolerated.
- Efficacy of SOD1-ASO-loaded CaP-lipid NPs in the transgenic G93A SOD1 ALS mouse model.



CRS 2022 Annual Meeting & Expo

Advanced Delivery Science

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada



Acknowledgements



Dr Gayathri Ediriweera

University of Queensland

Kristofer Thurecht
Nicholas Fletcher
Gary Cowin
Joshua Simpson
Joanna Wasielewska

University of Wollongong

Justin Yerbury
Jeremy Lum
Luke McAlary
Mikayla Brown

University of Adelaide

John Finnie & Jim Manavis



UNIVERSITY
OF WOLLONGONG
AUSTRALIA



THE UNIVERSITY
OF QUEENSLAND
AUSTRALIA

CREATE CHANGE



THE UNIVERSITY
OF QUEENSLAND
AUSTRALIA

Centre for
Advanced Imaging



THE UNIVERSITY
OF QUEENSLAND
AUSTRALIA

AIBN Australian Institute for
Bioengineering and Nanotechnology



Health
Illawarra Shoalhaven
Local Health District



UNIVERSITY
OF WOLLONGONG
AUSTRALIA

IHMRI is a joint initiative of the Illawarra Shoalhaven
Local Health District and the University of Wollongong



MOLECULAR
HORIZONS



UNIVERSITY
OF WOLLONGONG
AUSTRALIA



Dr Liyu Chen



Clare Watson



CRS 2022 Annual Meeting & Expo

Advanced Delivery Science

July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

