Biomolecular Diffusion-based Reporters for Magnetic Resonance Imaging

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Talk outline

(1) Overview of research vision: imaging biological *dark* matter

(2) Genetically engineering water diffusion for MRI

(3) Tracking T cells using biomolecular water diffusion

Acknowledgements


Di Wu


Mohamad Abedi


Prof. Mikhail Shapiro


Prof. Vladimir Ponomarev


Prof. Russ Jacobs

Funding sources

Imaging biology with genetic reporters

Green fluorescent protein (GFP)

Pioneered by Tsien, Chalfie, Shimomura

Firefly luciferase

Pioneered by DeLucca, Green, McElroy

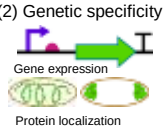
Why **genetic reporters** for imaging?

(1) Targeted Delivery


C. elegans

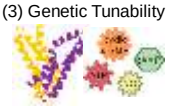

Tobacco

(2) Genetic specificity



Protein localization

(3) Genetic Tunability


Biochemical sensors

Imaging biological *dark matter :* beyond GFP

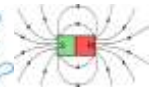
In vivo biology

Depth → photons fail

Magnetic resonance Imaging

H_2O diffusion

Paramagnetism



Anaerobic biology

Low O_2 → GFP fails

O_2 independent fluor. imaging

Photoreception

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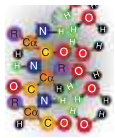
Reporter genes for MRI

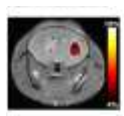
Metal-based

ferritin

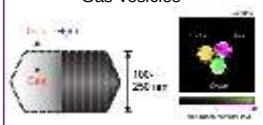
P450-based

Diamagnetic CEST





Gas-vesicles



Xe HyperCEST, susceptibility MRI

Mukherjee *et al.*, 2017, *Prog. In NMR Spec.*

*Can we develop a sensitive, **metal-free**, autologous reporter gene for MRI?*

Pioneered by Gilad, Bar-Shir, Neeman, Bulte, Brindle, Ahrens, Meade, van Zijl, Jasanoff, Yang, McMahon, Weissleder, Sherry, Shapiro, & other labs

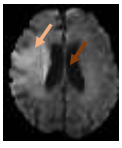
Diffusion-weighted MRI – fundamental concepts

Diffusion MRI can be used to **image** & **quantify** self diffusion of H_2O

Signal intensity is proportional to how fast molecules diffuse

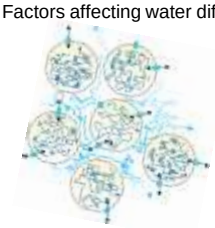
Fast diffusion → **darkening**, restricted diffusion → brightening

Diffusion weighted MRI



Dark → CSF
Bright → swelling

Factors affecting water diffusion in tissues



- Geometry
- D_{intra} & D_{extra}
- Membrane permeability

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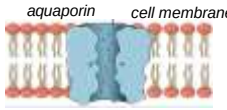
Aquaporins mediate transmembrane H_2O flux

Aquaporins mediate H_2O exchange

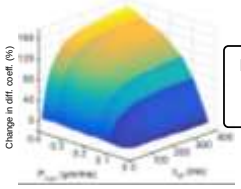


Concept: increase H_2O diffusion → hypointensity in diffusion MRI

(-) Aquaporin cell

(+) Aquaporin cell

Monte Carlo simulations of water diffusion



Diffusion increases with **permeability** & the effect is most pronounced at **long diffusion times (Δ_{eff})**

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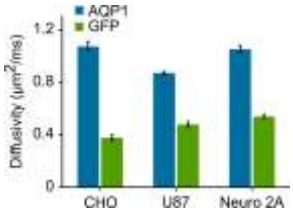
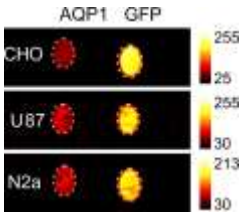
AQP1 as a reporter gene for MRI

Diffusivities in AQP1+ cells compared to control cells expressing GFP



Diffusion weighted images of AQP1 & control cells



AQP1 expression increases water diffusion by 82-187 % across various mammalian cell lines

Mukherjee *et al*., 2016, *Nat. Commun*.

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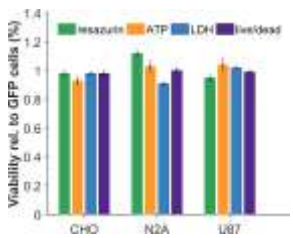
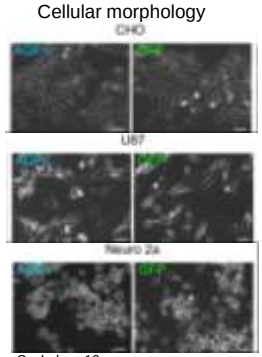
AQP1 is nontoxic in various cell lines

Cellular viability



AQP1 expression does not impact cell shape or viability

Cellular morphology



Scale bar: 10 μm

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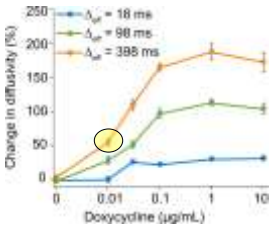
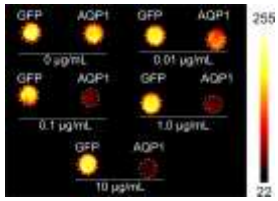
Sensitivity & dynamic range

Dose-dependent change in diffusivity using titratable AQP1



Diffusion weighted images of dose dependent contrast



AQP1 expression at sub-μM levels (457 nM) is sufficient to enhance diffusion by ~50%

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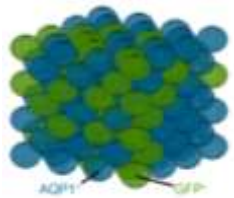
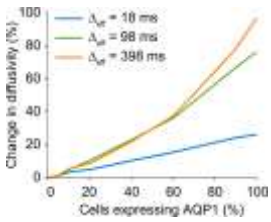
Imaging mixed populations with AQP1: simulations

108 AQP1+ & control GFP+ cells randomly distributed



Predicted change in diffusivity as a function of % of AQP1 cells



Simulations predict **≈ 10% AQP1** cells is sufficient for observing diffusion weighted MRI contrast

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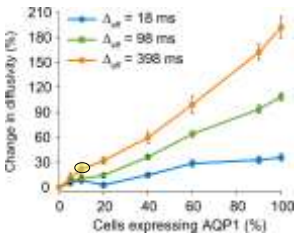
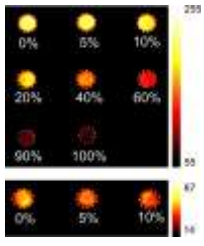
Imaging mixed populations with AQP1: experiments

Increase in diffusivity as a function of AQP1+ fraction



Diffusion weighted imaging of mixed cells using AQP1



~10% AQP1 cells is sufficient to result in a statistically significant increase in water diffusion

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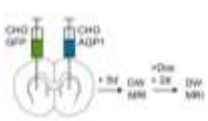
Imaging gene expression in a xenograft using AQP1

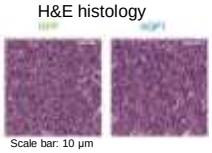
Experimental approach



Immunofluor.

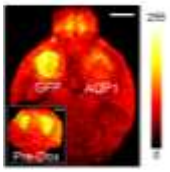
Scale bar: 3 mm

H&E histology



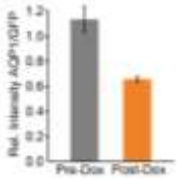
Scale bar: 10 μm

Diffusion MRI



Scale bar: 3 mm

AQP1 contrast *in vivo*



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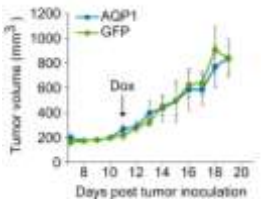
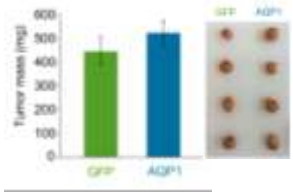
AQP1 does not affect tumor growth

AQP1+ & GFP+ tumor growth



AQP1⁺ & GFP⁺ tumor mass



Summary

Aquaporins: new, metal-free, nontoxic, orthogonal to T_1 & T_2, sensitive, autologous, readily implementable

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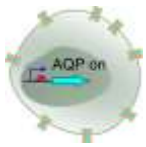
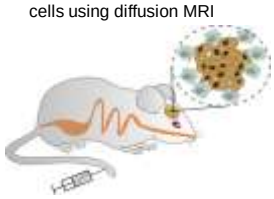
Tracking cells with MRI reporter genes

Immune cells engineered to express AQP1



Tracking injected immune cells using diffusion MRI



Why aquaporins?

No signal loss through dilution

In-built viability tracking

No false positives from metal phagocytosis

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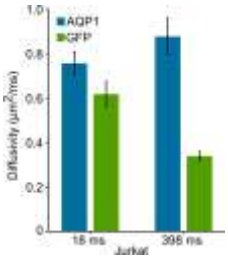
AQP1 as a reporter gene immunotherapy

Diffusivities in AQP1+ Jurkat cells compared to control cells



Diffusion weighted images of AQP1 Jurkat & control cells

AQP1 expression increases water diffusivity by 160% in Jurkat cells

Abedi*, Mukherjee* *et al*., in preparation.

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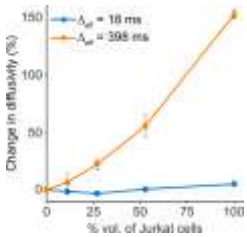
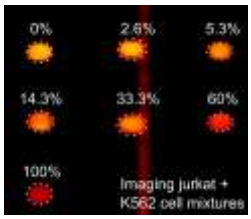
Imaging mixed Jurkat + K562 using AQP1

Increase in diffusivity as a function of AQP1+ fraction



Diffusion weighted imaging of mixed cells using AQP1



≈30% AQP1-labeled Jurkat cells is sufficient to result in a significant increase in water diffusion

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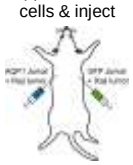
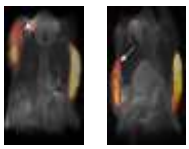
Imaging Jurkat cells in xenografts

Approach: mix cells & inject



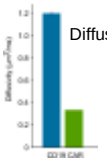
Diffusion imaging of Jurkat cells in tumor *in vivo*



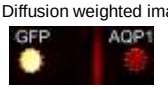
A 30:70 Jurkat/tumor cell mixture can be imaged *in vivo*

Imaging CAR-T cells with AQP1

Diffusivity



Diffusion weighted images



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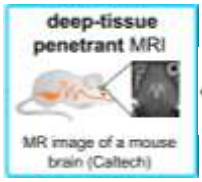
Summing it up: "*Lighting*" up biology beyond GFP

Developing aquaporins for MRI



Upcoming goals

Engineer aquaporins: **sensitivity**, **dynamic range**, **background-free** imaging

Genetic reporters of function and activity based on aquaporins

Multiplexed imaging capabilities

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Acknowledgements

Mukherjee Lab


Kang-Ching Fan


Nolan Anderson


Dr. Harun Ozbakir

Illinois Team (Fluor.)


Kevin Weyant


Josh Walker

Caltech Team (MRI)


Hunter Davis


Di Wu


George Lu

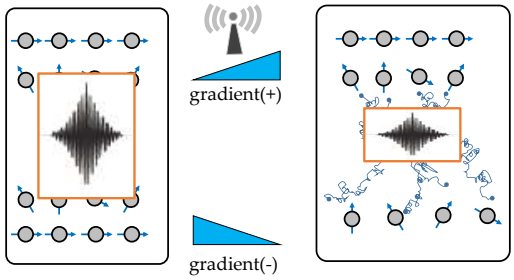
Funding (to **Arnab**)

S1. Diffusion-weighted MRI 101

H_2O molecule

Stationary H_2O molecules

Thermal motion



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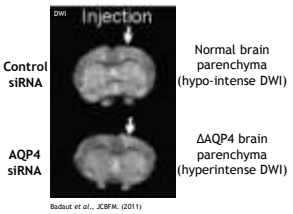
S2. Enhancing water diffusion using aquaporins (AQP)

Decreased diffusion in AQP4 knockdown rats



Badaut *et al.*, JCBFM. (2011)

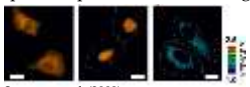
AQP1 expression enhances diffusion in CHO cells (PGSE, Δ_{eff} = 15 ms)

Sample	ADC (μm²/s)
CHO AQP1⁺	745 ± 31 (n=5)
CHO GFP⁺	695 ± 25 (n=7)

% increase is modest (7.19 %) relative to control cells expressing GFP

S3. Molecular Imaging of ATP with MRI

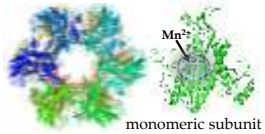
Optical reporters for ATP imaging



Imamura *et al.* (2009)

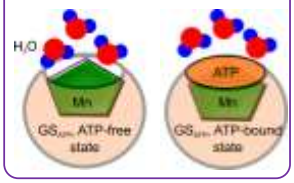
Can we develop a reporter gene for imaging ATP using MRI?

Glutamine synthetase (GS)



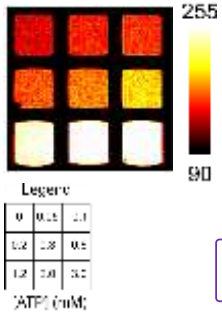
Glutamate + NH_3+ **ATP** → Glutamine + Pi+ ADP

Hypothesis: ATP sensing based on ATP-dependent H_2O access to Mn^{2+}

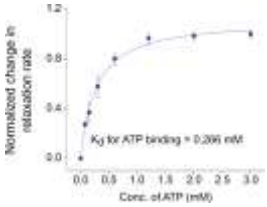


S4. ATP imaging with GS_{ATP}

T_2 weighted **MRI** of increasing ATP levels



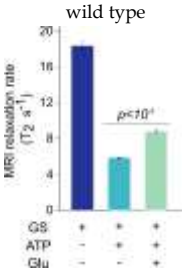
GS_{ATP} can "sense" **physiological** levels of ATP



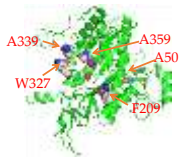
GS_{ATP} can image ATP levels across a large, physiologically relevant range

S5. Engineering GS – sensor specificity

Glut **cross-talk** in wild type

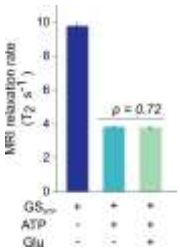


Inactivating mutations



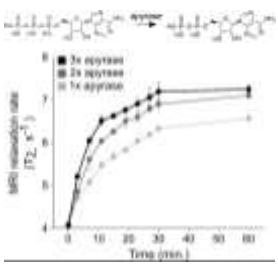
Engineered ATP sensor → **GS_{ATP}**

Specificity in engineered GS_{ATP}

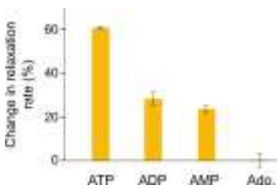


GS_{ATP} has ~ 60-fold reduced enzymatic activity, while retaining ability to "sense" ATP

S6. Dynamic ATP imaging with GS_{ATP}



Sensor **specificity**: crosstalk with ATP-like molecules



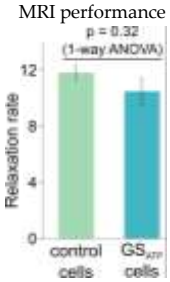
GS_{ATP} displays minimal cross-talk with ADP, AMP, and adenosine

S7. Beyond metals: Engineering H_2O diffusion

Intracellular **expression**

western blot

Intracellular MRI performance

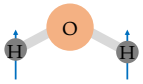


Can we engineer MRI reporters that entirely **obviate the need for metal supplementation**?

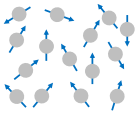
Engineer water diffusion for MRI contrast

S8. Image formation in MRI

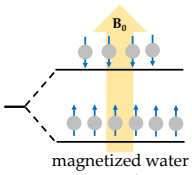
Magnetic moment in individual H_2O molecules



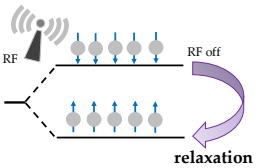
Ensemble of H_2O molecules



zero net magnetization

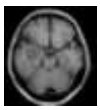


magnetized water sample

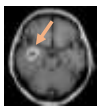


relaxation

Rate of relaxation – MRI contrast

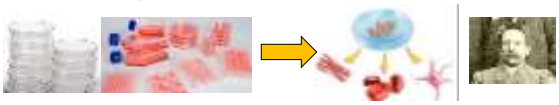


Contrast agents – regulate relaxation



The Petri dish & the Green Fluorescent Protein

Petri dish – to grow cells



Green fluorescent protein (GFP) – to visualize

Jellyfish

GFP X-ray structure

Cells labeled with multi-color GFPs

1

Summing it up: "*Lighting*" up biology beyond GFP

Engineering of LOV proteins