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

Funding sources

Imaging biology with genetic reporters

Green fluorescent protein (GFP)

Firefly luciferase

Pioneered by Tsien, Chalfie, Shimomura
Pioneered by DeLucca, Green, McElhony

Why genetic reporters for imaging?

(1) Targeted Delivery
(2) Genetic specificity
(3) Genetic Tunability
Imaging biological dark matter: beyond GFP

In vivo biology
- Depth → photons fail
- Magnetic resonance imaging
- H₂O diffusion
- Paramagnetism

Anaerobic biology
- Low O₂ → GFP fails
- O₂ independent fluor. imaging
- Photoreception

Depth → photons fail
- Magnetic resonance imaging
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Depth → photons fail
- Magnetic resonance imaging
- H₂O diffusion
- Paramagnetism

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

Reporter genes for MRI
- Metal-based
  - ferritin
  - P450-based
- Diamagnetic CEST
- Gas-vesicles
  - Xe HyperCEST, susceptibility MRI

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

Mukherjee et al., 2017, Prog. in NMR Spec.

Diffusion-weighted MRI – fundamental concepts

Diffusion MRI can be used to image & quantify self diffusion of H₂O

Signal intensity is proportional to how fast molecules diffuse

Fast diffusion → darkening, restricted diffusion → brightening

Factors affecting water diffusion in tissues
- Geometry
- D_intra & D_extra
- Membrane permeability

Diffusion-weighted MRI
- Dark → CSF
- Bright → swelling

Diffusion-weighted MRI
- Dark → CSF
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Aquaporins mediate transmembrane H$_2$O flux

Concept: increase H$_2$O diffusion $\rightarrow$ hypointensity in diffusion MRI

Aquaporin exchange across cell membrane

- (-) Aquaporin cell
- (+) Aquaporin cell

Monte Carlo simulations of water diffusion

Diffusion increases with permeability & the effect is most pronounced at long diffusion times ($\Delta_{\text{eff}}$)

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.

AQP1 is nontoxic in various cell lines

Cellular viability

Cellular morphology

AQP1 expression does not impact cell shape or viability

Scale bar: 10 μm
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%

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

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
Imaging gene expression in a xenograft using AQP1

Experimental approach

Immunofluor.  H&E histology

Diffusion MRI  AQP1 contrast in vivo

Scale bar: 3 mm  Scale bar: 10 μm

AQP1 does not affect tumor growth

AQP1+ & GFP+ tumor growth  AQP1+ & GFP+ tumor mass

Summary

Aquaporins: new, metal-free, nontoxic, orthogonal to T₁ & T₂, sensitive, autologous, readily implementable

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
AQP1 as a reporter gene immunotherapy

AQP1 expression increases water diffusivity by 160% in Jurkat cells

Imaging mixed Jurkat + K562 using AQP1

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

Imaging Jurkat cells in xenografts

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

Imaging CAR-T cells with AQP1
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

Acknowledgements

Mukherjee Lab

Illinois Team (Fluor.)

Caltech Team (MRI)

Funding (to Arnab)
S1. Diffusion-weighted MRI 101

- H₂O molecule

Stationary H₂O molecules

Thermal motion

S2. Enhancing water diffusion using aquaporins (AQP)

Decreased diffusion in AQP4 knockdown rats

<table>
<thead>
<tr>
<th>Sample</th>
<th>ADC (μm²/s)</th>
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<tbody>
<tr>
<td>CHO AQP1-</td>
<td>745 ± 31 (n=8)</td>
</tr>
<tr>
<td>CHO GFP+</td>
<td>695 ± 25 (n=7)</td>
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% increase is modest (7.19%) relative to control cells expressing GFP

S3. Molecular Imaging of ATP with MRI

Optical reporters for ATP imaging

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

Glutamine synthetase (GS)

Hypothesis: ATP sensing based on ATP-dependent H₂O access to Mn²⁺
S4. ATP imaging with $\text{GS}_{\text{ATP}}$  

$T_2$ weighted MRI of increasing ATP levels  

$\text{GS}_{\text{ATP}}$ can “sense” physiological levels of ATP  

$\text{GS}_{\text{ATP}}$ can image ATP levels across a large, physiologically relevant range.

S5. Engineering GS – sensor specificity  

Glut cross-talk in wild type  

Inactivating mutations  

Specificity in engineered $\text{GS}_{\text{ATP}}$  

$\text{GS}_{\text{ATP}}$ has ~60-fold reduced enzymatic activity, while retaining ability to “sense” ATP  

S6. Dynamic ATP imaging with $\text{GS}_{\text{ATP}}$  

Sensor specificity: crosstalk with ATP-like molecules  

$\text{GS}_{\text{ATP}}$ displays minimal cross-talk with ADF, AMP, and adenosine.
S7. Beyond metals: Engineering H₂O diffusion

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 H₂O molecules

Ensemble of H₂O 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
Summing it up: “Lighting” up biology beyond GFP