Tracking Individual Endogenous Dopamine Transporters Using Antagonist-Conjugated Quantum Dots

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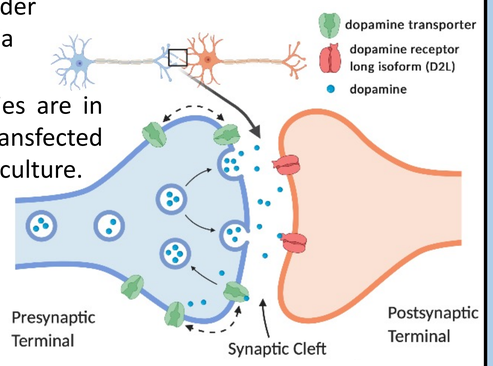
Motivation

Protein Dysfunction is Linked to Disease

- Dopamine transporter (DAT) is a **transmembrane protein** that drives **dopamine reuptake** at presynaptic terminal.
- Abnormalities in DAT function have been linked to **neurological disorders.**
  - Bipolar disorder
  - Schizophrenia
  - ADHD
- Previous studies are in transiently transfected monolayer cell culture.

Goal: study DAT diffusion in endogenous cell systems



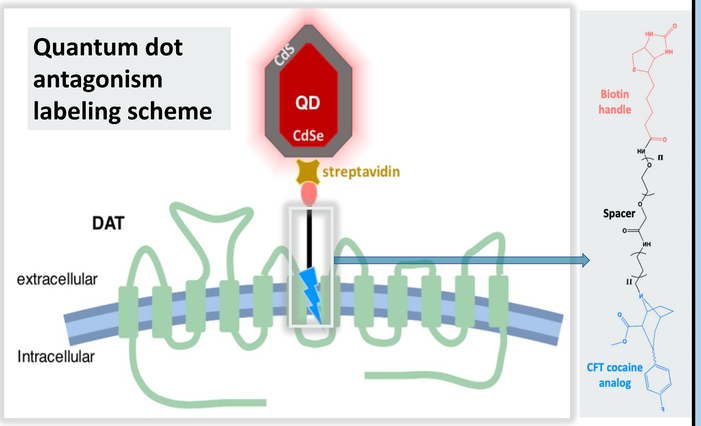
Using Quantum Dots to Label and Track DAT

Why Quantum Dots?

Semiconductor nanocrystals

1. extremely bright
2. broad absorption, high tunability, narrow Gaussian emission
3. high photostability

Quantum dot antagonism labeling scheme



Identification and Tracking

Confirming the presence of DAT in PC-12

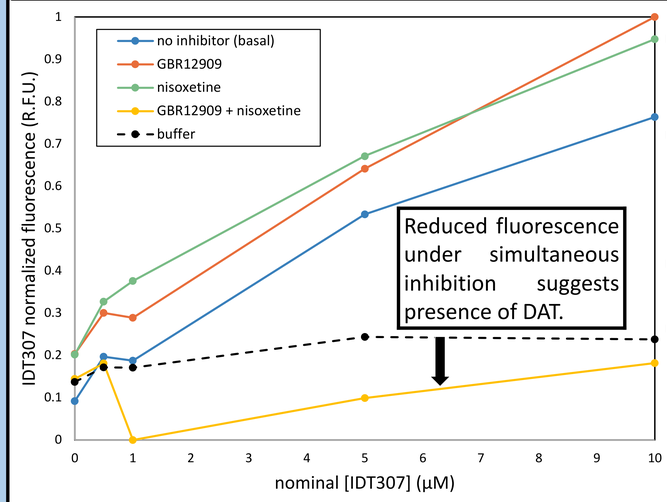
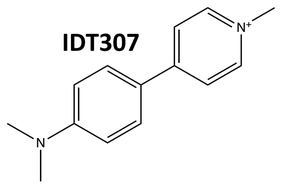


Figure 1: IDT307 transport assay in DAT expressing PC-12 cells under DAT inhibition (GBR12909, orange), NET inhibition (nisoxetine, green), and both DAT and NET inhibition (yellow).

- IDT307 is a molecule that fluoresces once transported into cell.
- Neuronal PC-12 cells express both DAT and norepinephrine. transporter (NET).
- Both NET and DAT uptake IDT307.
- GBR12909 inhibits DAT, nisoxetine inhibits NET.



Imaging IDT307 Intake:

- Previous studies report DAT localized in **neurite projections.**
- DAT inhibition shows decreased fluorescence in neurites.

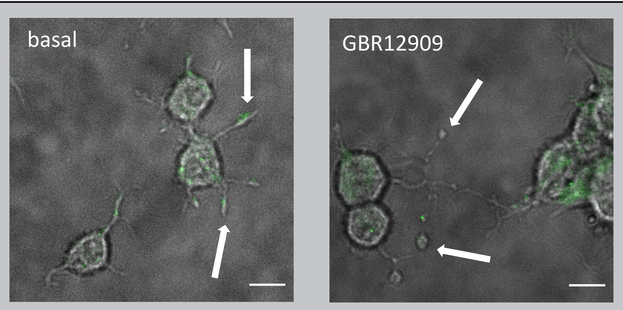
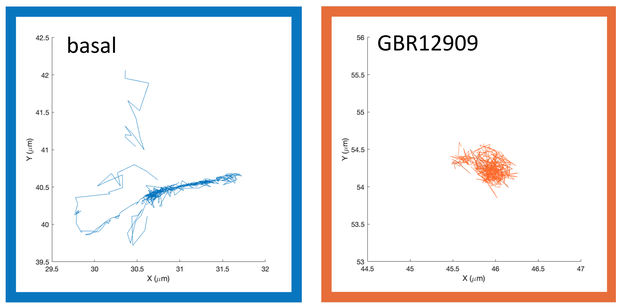


Figure 2: IDT307 uptake: basal (left) GBR12909 (right) scale:10 μm

Single Particle Tracking of DAT in PC-12



High mobility in basal trajectory supports tracking active DAT membrane diffusion.

Figure 3: Example of mobile quantum dot trajectory reconstructions for basal (blue) and GBR12909 (orange) .

Identification and Tracking Cont.

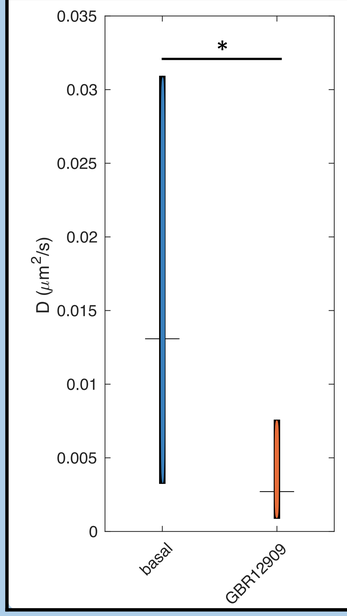


Figure 4: Quantum dot mobile phases in DAT expressing neuronal PC-12 cells. Diffusion coefficients box plots of trajectories analyzed under basal (blue; mean ± SEM: 0.018 ± 0.002 μm²/s) and GBR12909 (orange; mean ± SEM: 0.0057 ± 0.0012 μm²/s), (Kolmogorov-Smirnov 2-sample test, *p<0.00001; Ranksum test, *p<0.0001).

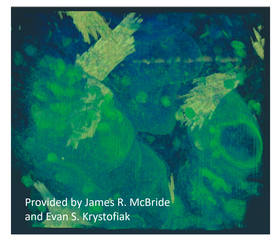
Two mobile populations were identified. The basal population is significantly more mobile than GBR12909 population, further **supporting DAT labeling and tracking**.

Future Goals

Immediate: Carry out additional QD tracking replicates to further increase difference between the number of diffusion coefficients under basal (N=69) and GBR12909 (N=46) conditions.

Immediate: Investigate effects of membrane composition on DAT diffusion.

Future: Incorporate cryo-electron microscopy tomography and image reconstruction to visualize the QD probe bound to DAT in PC-12.



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Images created with BioRender.com and InkScape

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