

MOLECULAR AND CELL BIOLOGY

Deciphering Iron Redox Changes in Alzheimer's Disease using DNAzyme Sensors that can Simultaneously Monitor Fe²⁺ and Fe³⁺

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Abstract

Background: Imbalanced Fe levels can lead to oxidative stress and initiate ferroptosis, an Fe-dependent cell death that involves lipid peroxidation and can lead to neuron cell loss in neurodegenerative diseases including Alzheimer's disease (AD). While the Fe³⁺/Fe²⁺ ratio has been identified as the primary determining factor for lipid peroxidation, the role of Fe redox equilibrium and dynamic in AD is not well understood, due to limited tools for visualizing Fe²⁺ and Fe³⁺ simultaneously. To overcome this limitation, we recently reported DNAzyme-based sensors for simultaneous imaging of Fe²⁺ and Fe³⁺. In this research update, we have integrated the sensors with brain-wide immunohistochemistry staining to identify cellular correlations between Fe redox changes and AD progression.

Method: We obtained DNAzymes that are highly selective for either Fe²⁺ or Fe³⁺ from a DNA library of up to 10¹⁵ sequences and used counter-selection to remove sequences binding competing metal ions. We converted the DNAzymes into fluorescent turn-on sensors using a method called "catalytic beacon" approach. With these sensors, we imaged Fe²⁺ and Fe³⁺ simultaneously in AD mouse brains. We also performed immunohistochemistry to evaluate neurodegeneration (NeuN), gliosis (Iba1&GFAP), amyloid beta pathology (HJ 3.4), and their correlation with Fe redox changes.

Result: We observed correlated signal changes with the regulation of iron levels. We further applied these sensors in ferroptosis and observed a decrease in Fe³⁺/Fe²⁺ redox ratio over time, indicating Fe redox changes as a potential source of oxidative stress in ferroptosis. These sensors also detected an elevated Fe³⁺/Fe²⁺ ratio in the AD mouse brain, particularly in amyloid plaque regions, suggesting a correlation between amyloid plaques and the accumulation of Fe³⁺ and/or conversion of Fe²⁺ to Fe³⁺.

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Furthermore, by co-staining the Fe sensors with immunohistochemistry biomarkers, we found correlations between Fe, Fe redox changes, and neurodegeneration among mice groups differing in genotype, sex, and age.

Conclusion: We have developed highly selective sensors for simultaneously imaging Fe²⁺ and Fe³⁺. By integrating these sensors with immunohistochemistry, we have identified correlations between Fe redox, amyloid plaques, and neurodegeneration in AD mice. Our sensors can offer deep insights into the detailed mechanism of ferroptosis and its role in AD.

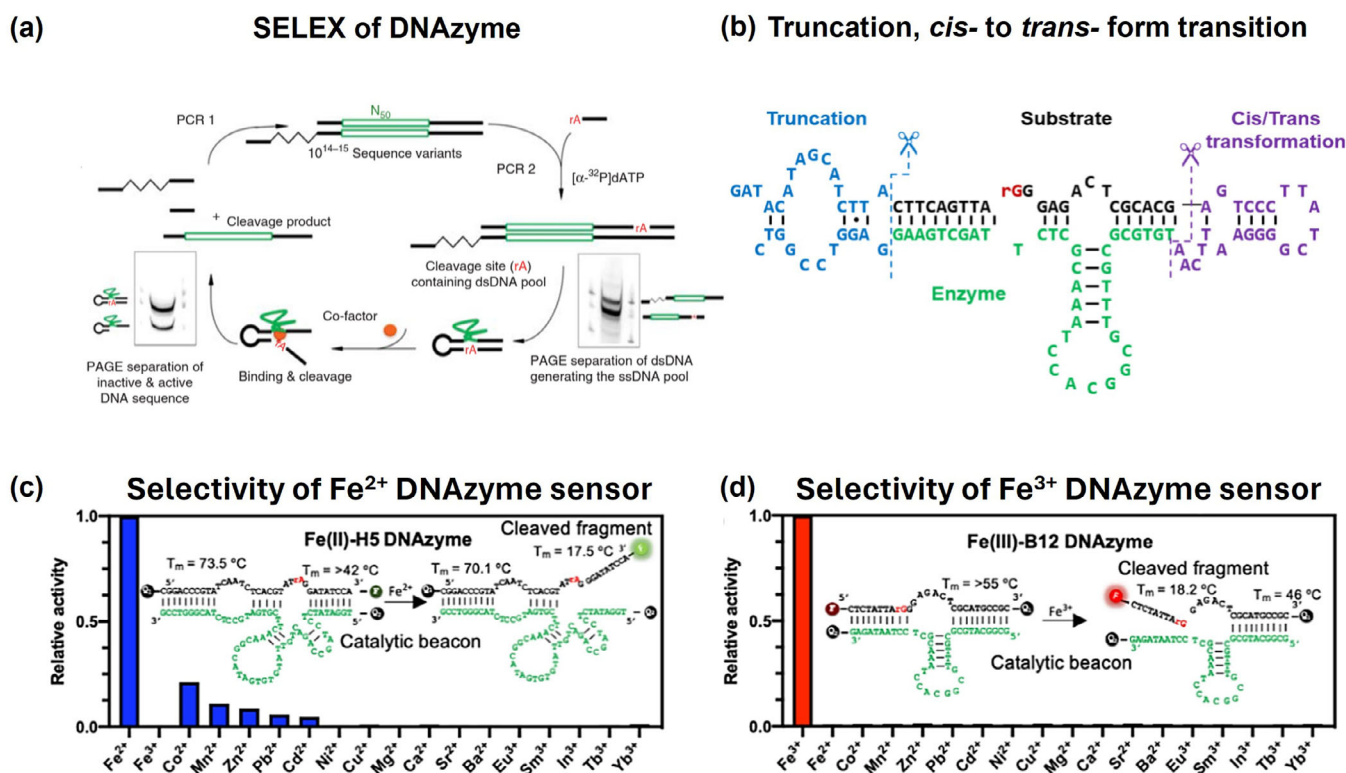


Figure 1. The stepwise process involved in the development of DNAzyme-based sensors. (a) In vitro selection entails the iterative screening of potential DNAzyme candidates. **(b)** A schematic representation of sequence truncation and the conversion from cis- to trans- form is demonstrated, using Fe³⁺ DNAzyme as an example. **(c)** High selectivity of Fe²⁺ DNAzyme sensor. Insert shows the secondary structure of the Fe²⁺ DNAzyme sensor. It consists of an enzyme strand shown in green with an Iowa Black FQ quencher (Q1) at the 5' end, and a substrate strand shown in black with the same quencher (Q2) at the 5' end and an Alexa Fluor 488 fluorophore (F1) at the 3' end. The fluorescence is quenched in the absence of target metal ions. In the presence of Fe²⁺, the RNA base in the substrate strand (red) was cleaved, and the DNA strand became shorter, which reduced the melting temperature between the strands. This change allows the dissociation between the fluorophore and the quencher, and results in fluorescence turn-on for sensing Fe²⁺. **(d)** High selectivity of Fe³⁺ DNAzyme sensor. Insert shows the secondary structure of the Fe³⁺-specific DNAzyme sensor. The reaction is similar to Fe²⁺ DNAzyme but only recognizes Fe³⁺ specifically.

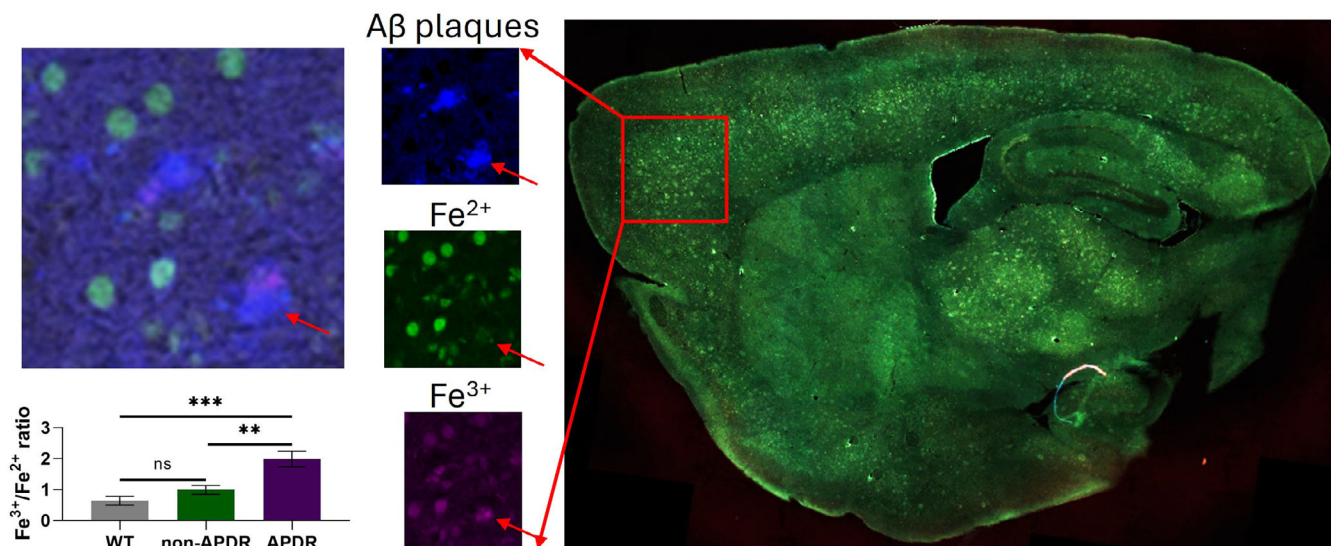


Figure 2. Imaging Fe²⁺ and Fe³⁺ in 5xFAD mice brains with our DNAzyme sensors. The brain slices were also stained with HJ3.4 antibody, which labels immunoreactive Aβ plaques. Fe³⁺/Fe²⁺ ratio was increased in the Aβ plaques deposited region (APDR) but not in other regions (non-APDR) in the 5xFAD mouse cortex.

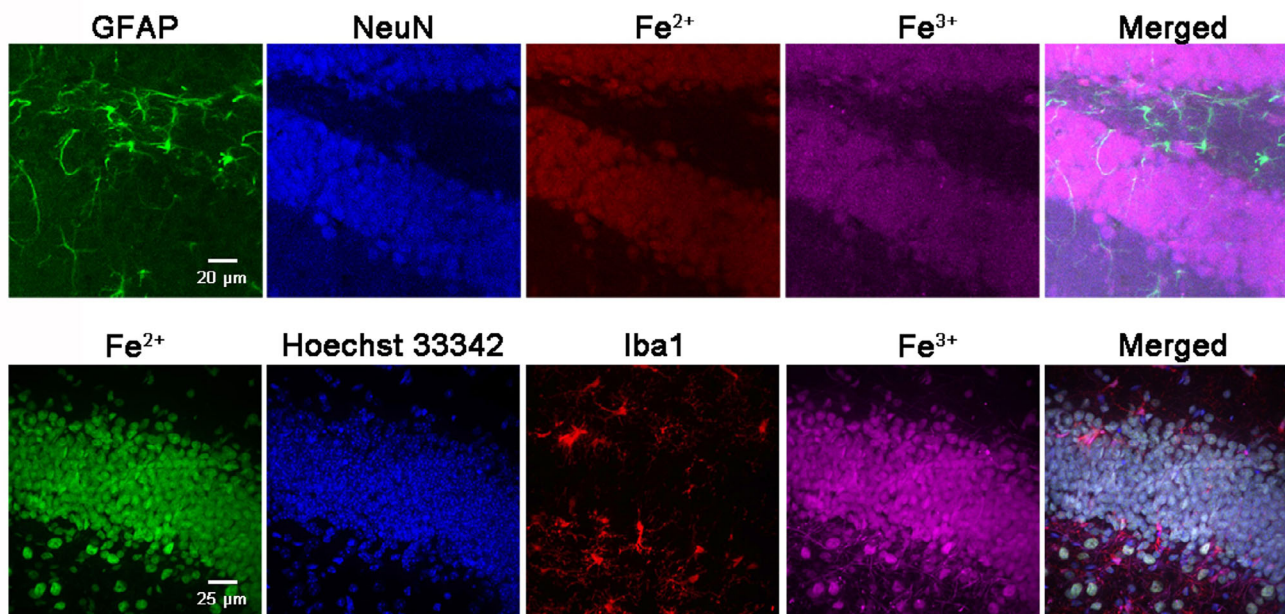


Figure 3. Co-staining of Fe²⁺, Fe³⁺, and immunohistochemistry biomarkers. Both Fe²⁺ and Fe³⁺ showed colocalization in NeuN-labeled neuron cells, but not GFAP-labeled glial cells.