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JZUS-B

Selenocystine-based fluorescence assay combined with structural prediction for functional analysis of cystine transporter variants in cystinuria

Key words: Cystinuria; *SLC3A1*; *SLC7A9*; Selenocystine; Diagnosis; Mutation

PAPER SUMMARY

A nonradioactive route to cystinuria variant function

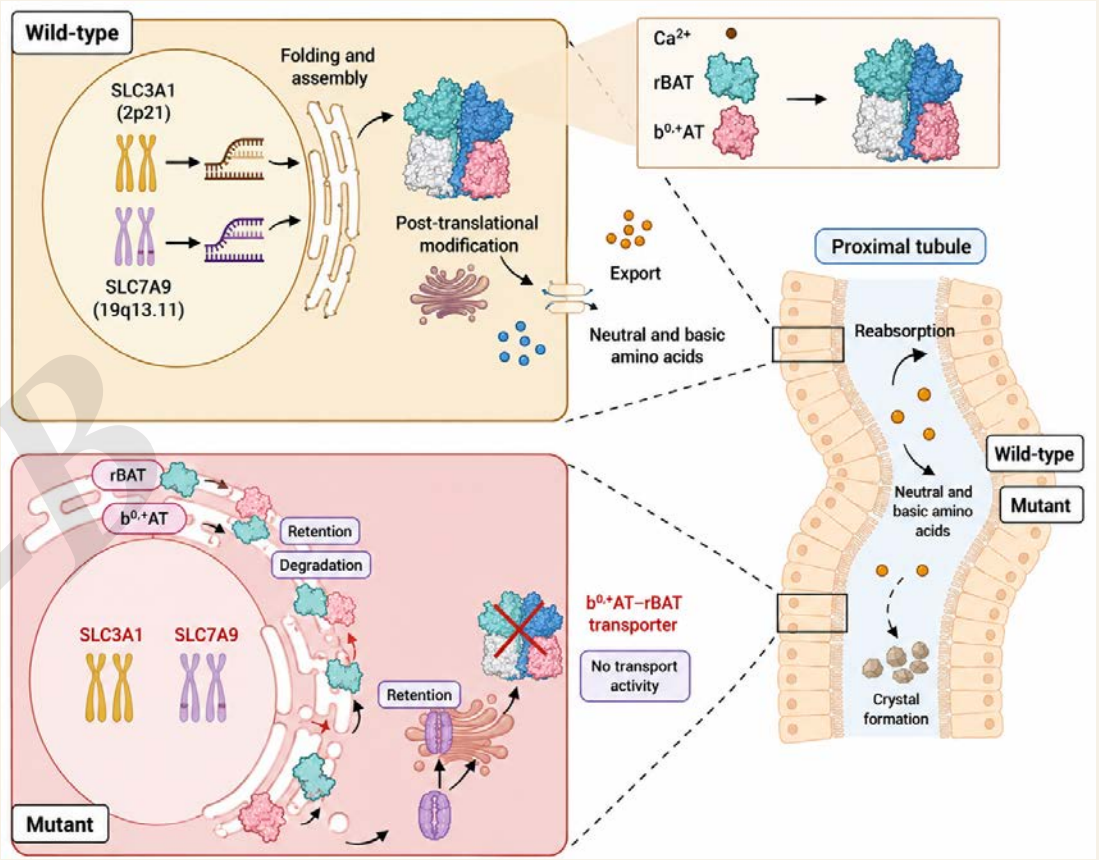
Selenocystine fluorescence assay + AlphaFold3 structural interpretation for SLC3A1/SLC7A9 variants

Clinical gap

Radioisotope assays and mass spectrometry are difficult to scale for routine variant interpretation.

Platform answer

A microplate-readable fluorescent surrogate measures transporter activity and connects the signal to structural mechanisms.



Mechanism of $b^{0,+}$AT–rBAT Transporter Dysfunction in Cystinuria

1%-2%
adult kidney stones
cystine stones

up to 10%
pediatric stones
reported burden

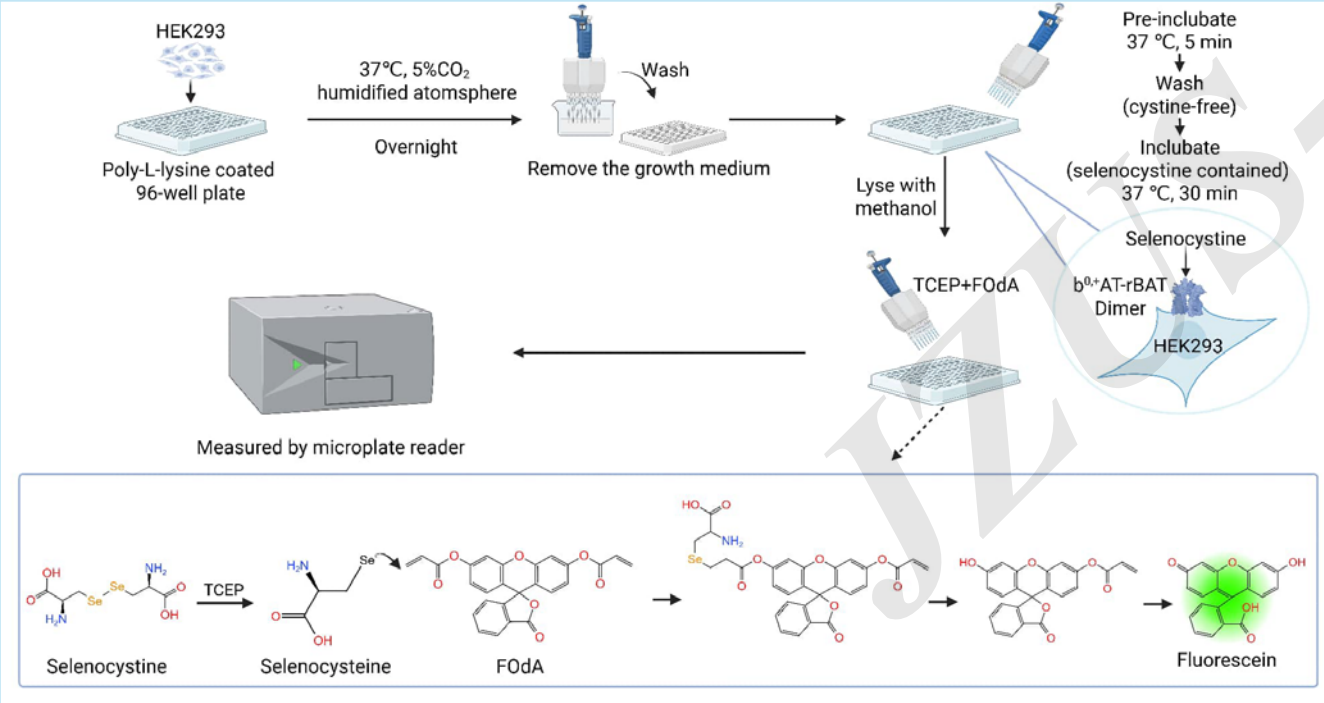
460+
reported pathogenic mutations
HGMD: SLC3A1 + SLC7A9

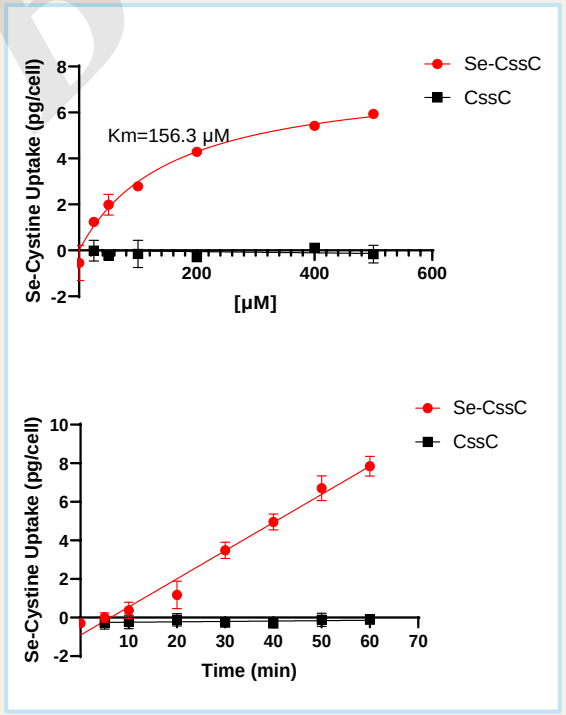
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■ ASSAY DESIGN

Selenocystine converts transporter uptake into a microplate signal

The workflow replaces radioactive cystine uptake with a cell-based fluorescent readout compatible with standard laboratory infrastructure.





- nonradioactive
- 30 min uptake
- 535 nm readout
- variant-ready

Innovation Selenocystine acts as a cystine analog, but its selenium chemistry creates a sensitive fluorescence handle after cellular uptake.

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He et al., ZUSB-D-25-00767, 2026

VALIDATION

Selenocystine behaves like cystine, but is much easier to see

Selenocystine produced a high-sensitivity signal while retaining transporter competition and selectivity patterns expected for SLC3A1/SLC7A9.

>100-fold
stronger in-vitro fluorescence
Se-CssC vs cystine

Km 156.3 +/- 24.2
umol/L apparent affinity
near literature cystine Km ~200

7.65 +/- 0.45x
Vmax over base value
saturable uptake

25 umol/L
detectable uptake
cystine not detectable at 600

Remaining signal after competition tracks substrate identity



Interpretation: the fluorescent surrogate is not just visible; it follows the expected amino-acid transport logic.

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VARIANT STRATIFICATION

Clinical variants separate into functional severity bands

Operational thresholds translated residual transport into mild, moderate, or severe functional categories consistent with prior radioisotope-based reports.

MILD
>60% residual activity

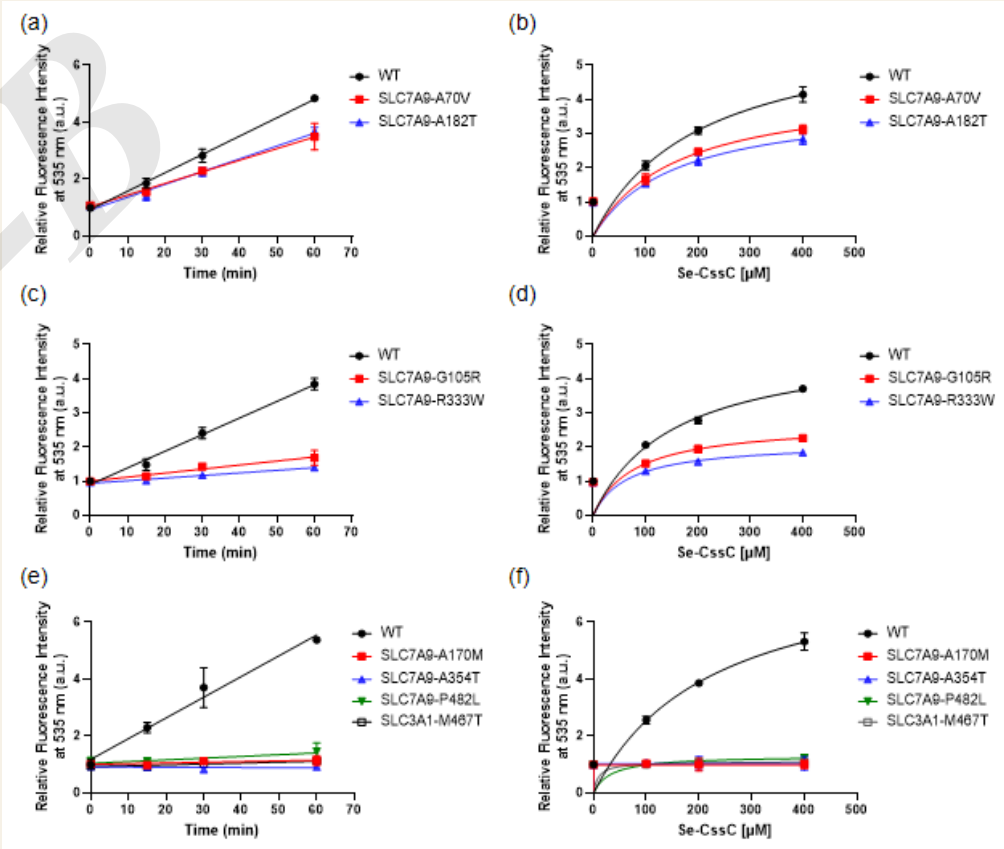
- SLC7A9 A70V
- SLC7A9 A182T

MODERATE
20%-60% residual activity

- SLC7A9 G105R
- SLC7A9 R333W

SEVERE
<20% residual activity

- SLC7A9 P482L
- SLC7A9 V170M
- SLC7A9 A354T
- SLC3A1 M467T



Why it matters A scalable activity readout can help reclassify cystinuria variants and prioritize follow-up for genotype-guided care.

He et al., ZUSB-D-25-00767, 2026

INTEGRATED INTERPRETATION

Function plus structure turns VUS triage into a testable pathway

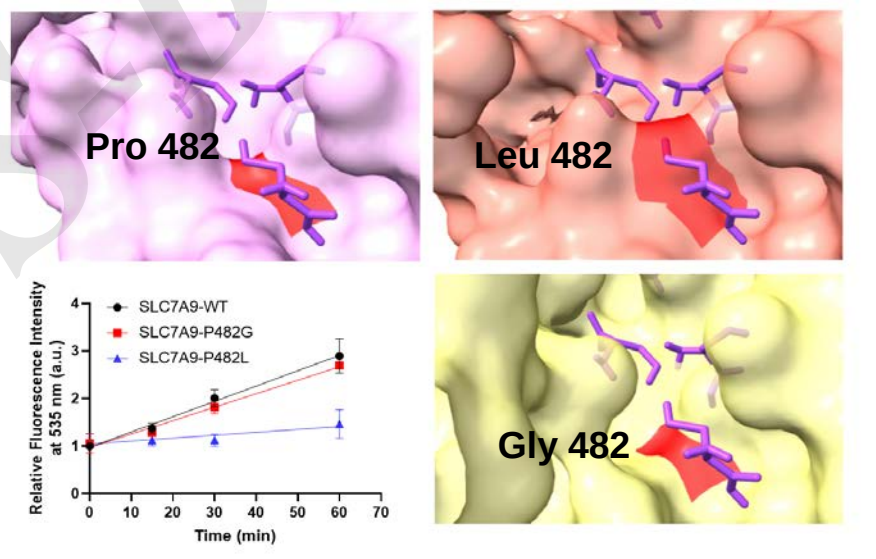
The assay supplies measured activity; AlphaFold3/docking adds mechanistic hypotheses when predictions are discrete and locally confident.

P482L: channel occlusion

Bulky leucine protrudes into the predicted substrate path.

~5% wild-type activity retained

While **P482G** remains open and retains ~100%, acting as an internal contrast.



Workflow

genotype → cell assay → severity band → structure model → clinical validation

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He et al., ZUSB-D-25-00767, 2026