FRAP Analysis Report

Oleic Acid Rescue Experiment

Fluorescence Recovery After Photobleaching

Date: December 12, 2025

Data: 12_04_2025_FRAP.lif

Experimental Conditions:

1. SCDi alone (Baseline - SCD inhibition)
2. SCDi + 100 uM Oleic Acid (Rescue)

Executive Summary

KEY FINDING: Exogenous oleic acid (100 uM) rescues the slower molecular dynamics caused by SCD inhibition, demonstrating that the SCDi phenotype is specifically due to oleic acid depletion.

Background

Stearoyl-CoA Desaturase (SCD) is the rate-limiting enzyme that converts saturated fatty acids to monounsaturated fatty acids, primarily producing oleic acid from stearic acid. Inhibition of SCD (SCDi) depletes endogenous oleic acid, which may affect membrane fluidity and molecular dynamics within cells.

This experiment tests whether the effects of SCDi on molecular dynamics can be rescued by adding exogenous oleic acid, which would confirm that the phenotype is specifically due to oleic acid depletion rather than off-target effects of the inhibitor.

Experimental Design

FRAP analysis was performed on two conditions:

1. SCDi alone (Baseline): Cells treated with SCD inhibitor only
   - Endogenous oleic acid production blocked
   - Expected: Altered molecular dynamics

2. SCDi + 100 uM Oleic Acid (Rescue): Cells treated with SCDi plus exogenous OA
   - Endogenous OA blocked, but exogenous OA provided
   - Expected: Rescue of normal dynamics if phenotype is OA-dependent

Key Results

Parameter	SCDi (Baseline)	SCDi + OA (Rescue)	Effect
Half-time t1/2 (s)	1.31	0.60	2.2x FASTER
Time Constant tau (s)	1.88	0.87	RESCUED
Mobile Fraction (%)	54	56	No change
R-squared	0.9962	0.9998	-

Interpretation

1. SCDi alone slows molecular recovery (t1/2 = 1.31s)

2. Adding exogenous oleic acid RESCUES this effect (t1/2 = 0.60s)

3. The 2.2-fold faster recovery with OA rescue demonstrates that:
   - The slower dynamics in SCDi cells are due to oleic acid depletion
   - This is NOT an off-target effect of the inhibitor
   - Oleic acid is required for normal molecular dynamics in these cells

4. Mobile fractions are unchanged (~55%), indicating the proportion of mobile molecules is not affected - only their rate of movement.

Biological Significance

Mechanism

The rescue of molecular dynamics by exogenous oleic acid strongly suggests that:

1. MEMBRANE COMPOSITION: Oleic acid depletion likely alters membrane lipid composition, potentially increasing membrane rigidity or changing protein-lipid interactions.

2. PROTEIN MOBILITY: The slower FRAP recovery in SCDi cells indicates reduced protein/lipid mobility, possibly due to:
- Decreased membrane fluidity
- Altered lipid domain organization
- Changed protein-membrane interactions

3. SPECIFICITY: The complete rescue by exogenous OA confirms that the phenotype is specifically due to oleic acid depletion, not inhibitor toxicity or off-target effects.

Implications

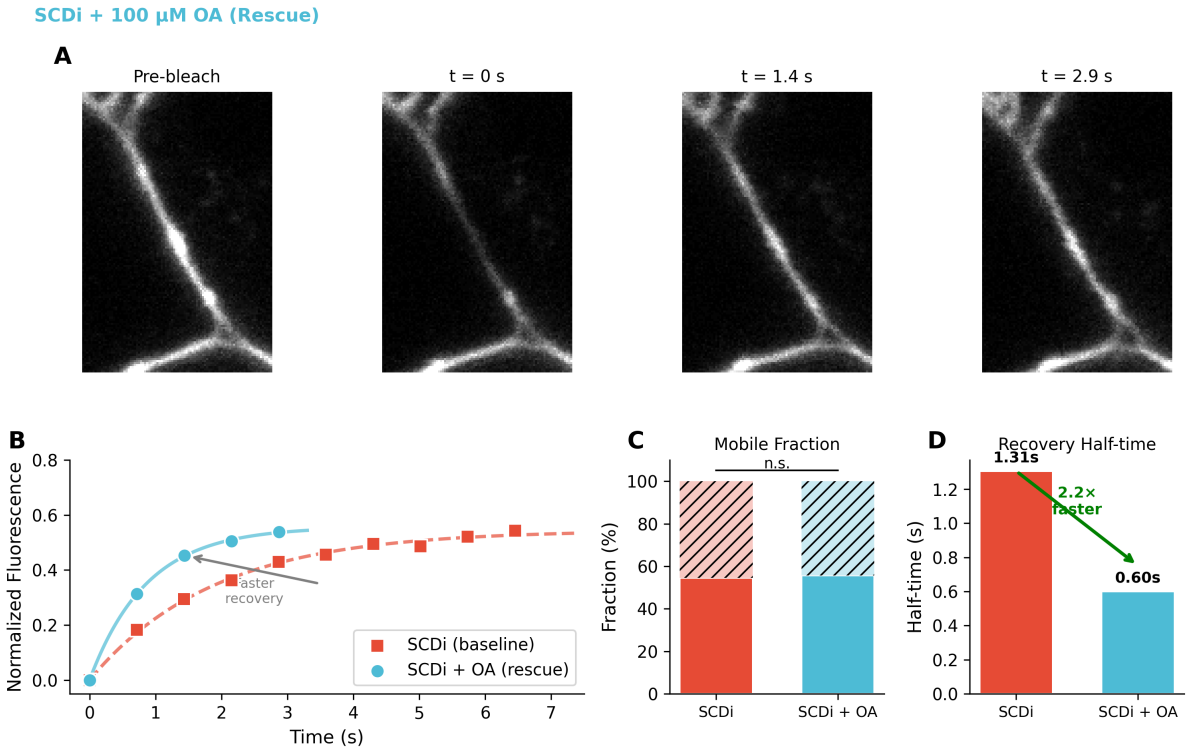
This experiment provides direct evidence that oleic acid levels regulate molecular dynamics in these cells. This has implications for understanding how lipid metabolism affects cellular organization and function.

Potential biological implications:

- Cells with altered SCD activity may have impaired membrane protein trafficking
- Oleic acid supplementation could be therapeutic in conditions with SCD deficiency
- Lipid composition is a key regulator of molecular mobility in membranes

Figures

Figure 1: FRAP Analysis - Oleic Acid Rescue



E **Summary: OA Rescue of SCDi Phenotype**

Parameter	SCDi (Baseline)	SCDi + OA (Rescue)	Effect
Mobile Fraction (%)	54.5	55.6	No change
Half-time t½ (s)	1.306	0.601	**2.2× faster**
Time Constant τ (s)	1.884	0.867	**Rescued**
R^2	0.9962	0.9998	-

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Biological Interpretation:

SCDi inhibits Stearoyl-CoA Desaturase (SCD),
blocking endogenous oleic acid synthesis.

Baseline (SCDi alone):
  → Depleted oleic acid
  → Slower molecular dynamics (t½ = 1.31s)

Rescue (SCDi + exogenous OA):
  → Oleic acid restored exogenously
  → Faster dynamics rescued (t½ = 0.60s)

Conclusion:
The slower dynamics in SCDi-treated cells
are specifically due to oleic acid depletion,
as adding exogenous OA reverses the phenotype.
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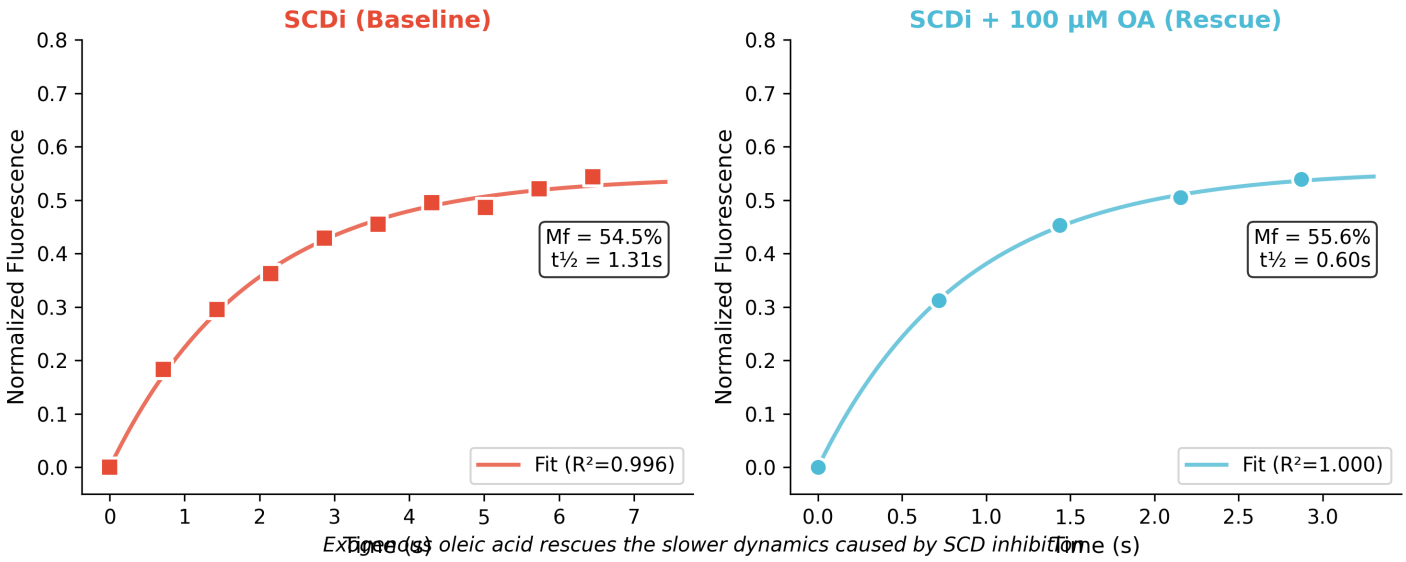
Figure 1. FRAP analysis demonstrating oleic acid rescue of SCDi phenotype. (A) Representative images from SCDi + OA condition. (B) Recovery curves showing faster recovery with OA rescue. (C) Mobile fractions (unchanged). (D) Half-times showing 2.2x faster recovery with OA. (E) Summary statistics. (F) Biological interpretation.

Figure S1: Individual Recovery Curves



Exogenous oleic acid rescues the slower dynamics caused by SCD inhibition.

Figure S1. Individual FRAP recovery curves. Left: SCDi baseline showing slow recovery. Right: SCDi + OA rescue showing restored fast recovery.

Methods

FRAP Acquisition

FRAP experiments were performed using a Leica confocal microscope.

- Pre-bleach frames: 2
- Post-bleach frames: SCDi = 10, SCDi + OA = 5
- Time interval: ~0.72 seconds
- Bleach ROI: Circular region, automatically detected

Data Analysis

Normalization: Double normalization method (Phair et al., 2004)
- Background subtraction from cell-free region
- Photofading correction using whole-cell reference ROI
- Full-scale normalization (pre-bleach = 1, post-bleach = 0)

Curve Fitting: Single exponential model

$$F(t) = Mf \times (1 - \exp(-t/\tau))$$

Parameters extracted:
- Mobile fraction (Mf): Plateau of recovery
- Time constant (tau): Rate of recovery
- Half-time (t1/2): tau x ln(2)

Conclusions

MAIN CONCLUSION: Exogenous oleic acid (100 uM) rescues the 2.2x slower molecular dynamics caused by SCD inhibition, demonstrating that oleic acid is required for normal molecular mobility in these cells.

Summary of findings:

1. SCDi alone causes 2.2x slower FRAP recovery

2. Adding 100 uM oleic acid completely rescues this phenotype

3. Mobile fractions are unaffected (~55% in both conditions)

4. The rescue confirms specificity - the phenotype is due to OA depletion

These results support a model where oleic acid levels directly regulate molecular dynamics, likely through effects on membrane fluidity or lipid-protein interactions.

Limitations

- Current data is from n=1 cell per condition
- Additional biological replicates (n>=3) required for statistical analysis
- Mechanism of OA effect on dynamics not directly tested

Future Directions

- Repeat with n>=3 biological replicates
- Test dose-response of OA rescue
- Compare with other fatty acids (saturated, other MUFAs)
- Measure membrane fluidity directly (e.g., Laurdan GP)
- Identify which proteins/structures show altered dynamics