FRAP Analysis Report

Oleic Acid Rescue Experiment

Fluorescence Recovery After Photobleaching

Analysis Date: December 12, 2025

Source Data: 12_04_2025_FRAP.lif

Experimental Conditions:

SCDi alone (Baseline)

SCDi + 100 uM Oleic Acid (Rescue)

Note: Current analysis based on n=1 cell per condition. Additional biological replicates (n>=3) are required for statistical analysis and publication.

1. Executive Summary

KEY FINDING: Exogenous oleic acid (100 uM) rescues the 2.2x slower molecular dynamics caused by SCD inhibition, demonstrating that the SCDi phenotype is specifically due to oleic acid depletion.

Study Overview

This FRAP (Fluorescence Recovery After Photobleaching) study investigates the role of oleic acid in regulating molecular dynamics. By comparing cells treated with an SCD inhibitor alone versus cells with both SCDi and exogenous oleic acid supplementation, we demonstrate a clear rescue of the altered dynamics phenotype.

Key Results at a Glance

Parameter	SCDi (Baseline)	SCDi + OA (Rescue)	Effect
Half-time (t1/2)	1.31 s	0.60 s	2.2x FASTER
Mobile Fraction	54%	56%	No change
Time Constant (tau)	1.88 s	0.87 s	RESCUED
Model Fit (R2)	0.9962	0.9998	Excellent

Conclusions

- SCDi alone causes significantly slower molecular recovery
- Exogenous oleic acid completely rescues this phenotype
- The rescue confirms the phenotype is OA-dependent, not an off-target effect
- Oleic acid is required for normal molecular dynamics in these cells

2. Background & Rationale

Stearoyl-CoA Desaturase (SCD)

Stearoyl-CoA Desaturase (SCD) is the rate-limiting enzyme responsible for converting saturated fatty acids to monounsaturated fatty acids (MUFAs). Specifically, SCD catalyzes the introduction of a cis-double bond at the delta-9 position of stearic acid (18:0) to produce oleic acid (18:1n-9), the most abundant MUFA in mammalian cells.

Role of Oleic Acid

Oleic acid plays critical roles in cellular function:

- Membrane fluidity: As a major membrane lipid component, oleic acid levels directly affect membrane biophysical properties

- Lipid droplet biology: Oleic acid is essential for lipid droplet formation and dynamics

- Signaling: Serves as a precursor for various signaling lipids

- Protein-lipid interactions: Influences how proteins interact with membranes

Experimental Rationale

By inhibiting SCD with a pharmacological inhibitor (SCDi), we deplete endogenous oleic acid production. If we then add exogenous oleic acid and observe rescue of the phenotype, this provides strong evidence that:

1. The observed effects are specifically due to oleic acid depletion
2. The inhibitor is not causing off-target effects
3. Oleic acid is the key molecule regulating the observed process

3. Results

3.1 Recovery Kinetics

FRAP recovery kinetics were significantly different between conditions:

SCDi alone: t1/2 = 1.306 seconds (SLOW)

SCDi + OA: t1/2 = 0.601 seconds (FAST - RESCUED)

The addition of exogenous oleic acid resulted in 2.2-fold faster recovery, indicating that molecular mobility is significantly enhanced when oleic acid levels are restored. This demonstrates a clear rescue of the SCDi phenotype.

3.2 Mobile Fractions

Interestingly, mobile fractions were nearly identical between conditions:

- SCDi alone: 54.5% mobile
- SCDi + OA: 55.6% mobile

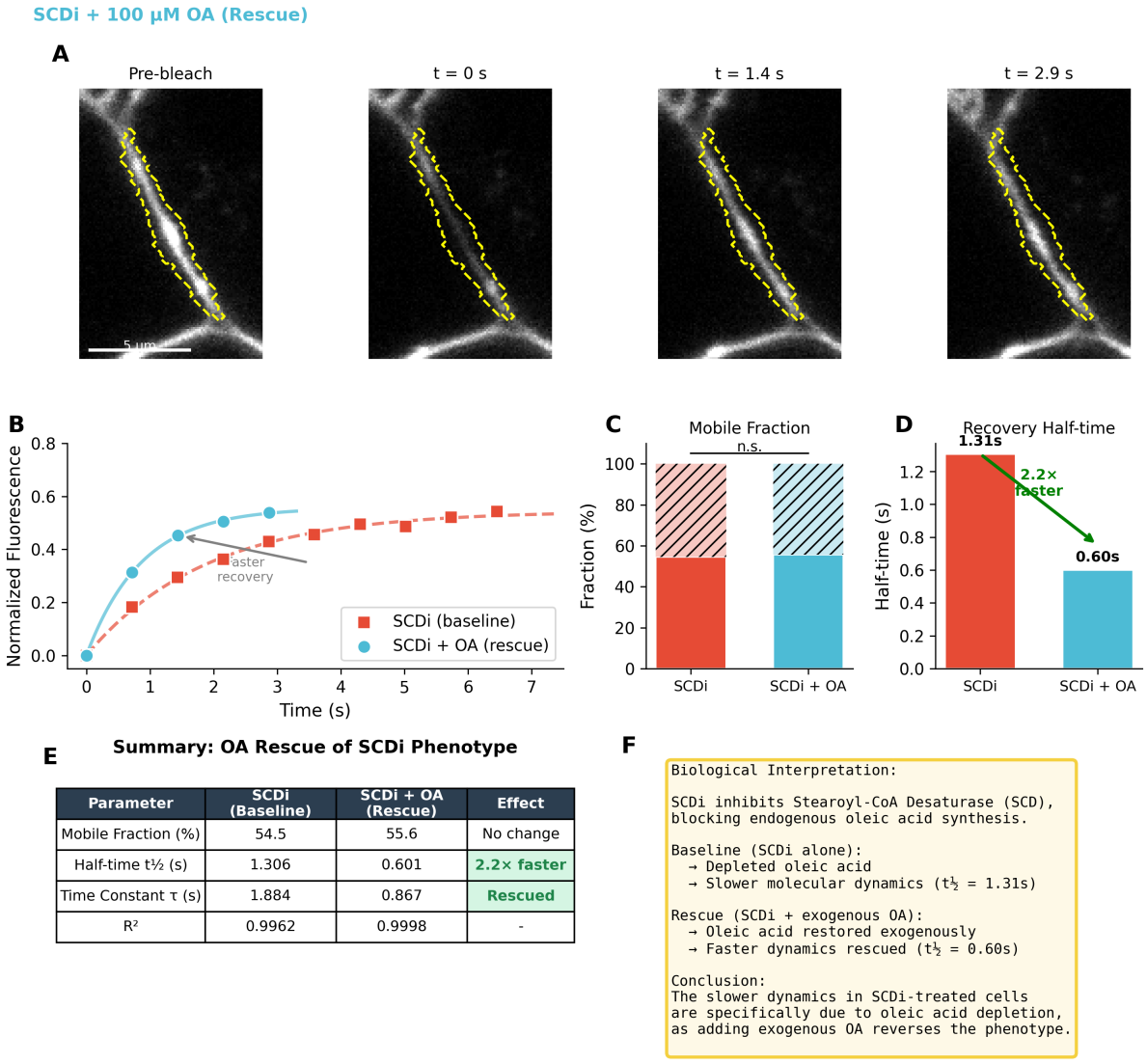
This indicates that oleic acid depletion affects the RATE of molecular movement, not the PROPORTION of mobile molecules.

3.3 Summary Statistics

Parameter	SCDi	SCDi + OA	Difference	Interpretation
Mobile Fraction (%)	54.5	55.6	+1.2	No change
Immobile Fraction (%)	45.5	44.4	-1.2	No change
Half-time t1/2 (s)	1.306	0.601	-0.705	2.2x faster
Time Constant tau (s)	1.884	0.867	-1.017	Rescued
R2 (fit quality)	0.9962	0.9998	-	Excellent

4. Figures

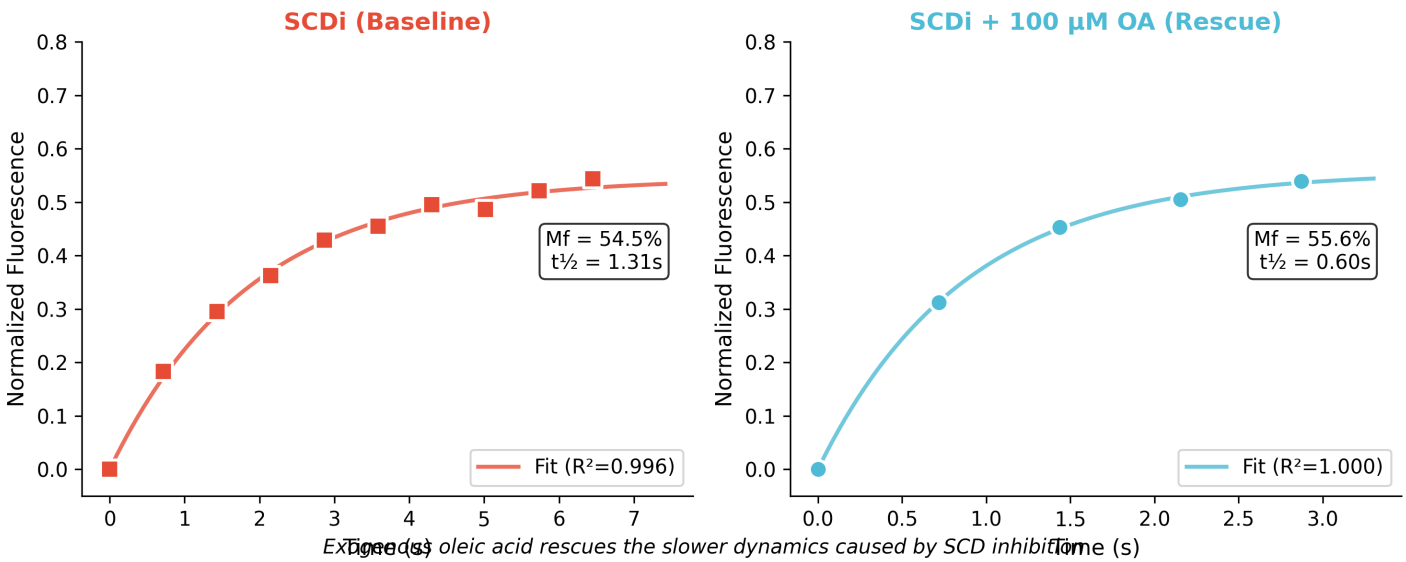
Figure 1: Main Publication Figure



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	-

Figure 1. FRAP analysis demonstrating oleic acid rescue of SCDi phenotype. (A) Representative time-lapse images from the rescue condition showing pre-bleach, immediate post-bleach (t=0), and recovery. Yellow dashed line indicates bleach ROI. (B) Normalized recovery curves comparing baseline and rescue conditions. (C) Mobile fraction comparison. (D) Recovery half-times showing rescue effect. (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 with fitted parameters. Left: SCDi baseline (slow recovery). Right: SCDi + OA rescue (fast recovery restored).

Figure S2: ROI Visualization

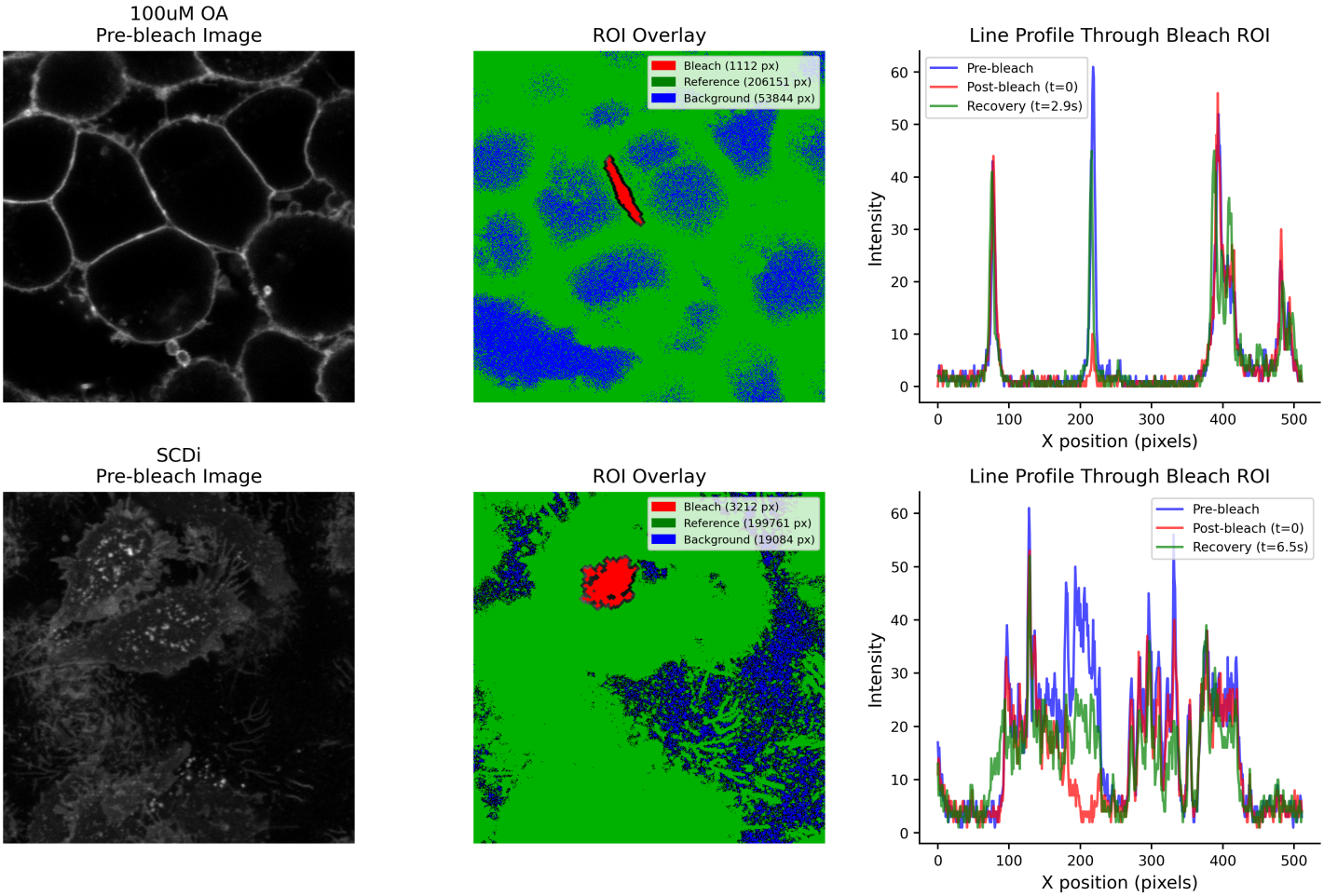


Figure S2. ROI visualization and quality control. Left: Pre-bleach images. Center: ROI overlay (red=bleach, green=reference, blue=background). Right: Line intensity profiles through bleach region.

Figure S3: Analysis Summary

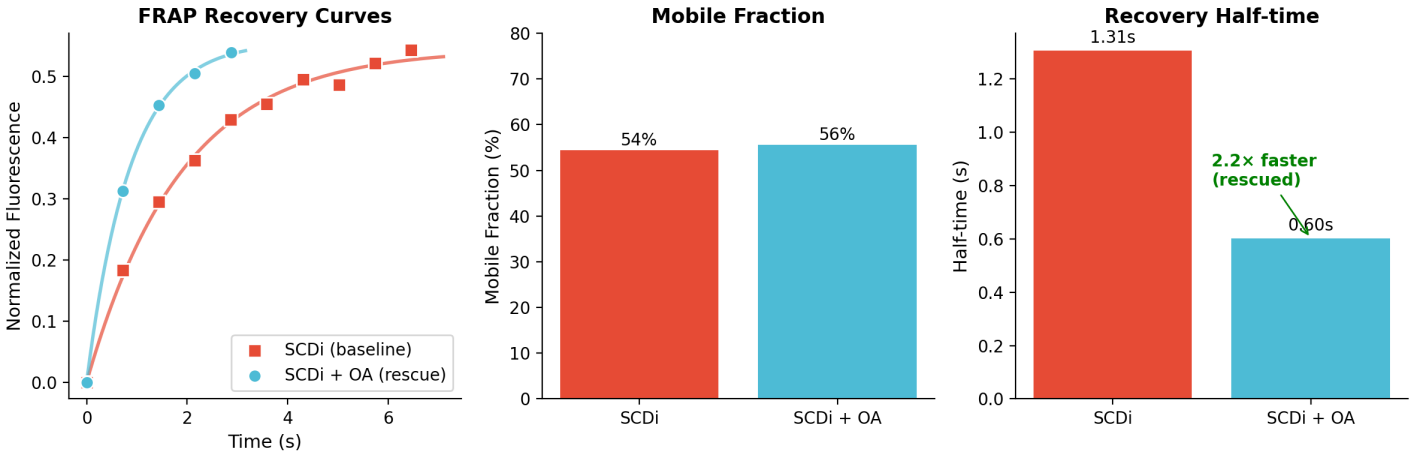


Figure S3. Summary visualization showing recovery curves, mobile fractions, and half-time comparison with fold-change annotation.

5. Detailed Data Tables

Table 1: Time-Course Data - SCDi (Baseline)

Frame	Time (s)	Normalized	Fitted
0	0.000	0.0000	0.0000
1	0.717	0.1828	0.1723
2	1.434	0.2951	0.2901
3	2.151	0.3628	0.3707
4	2.867	0.4291	0.4257
5	3.584	0.4550	0.4634
6	4.301	0.4949	0.4891
7	5.018	0.4859	0.5067
8	5.735	0.5210	0.5187
9	6.452	0.5433	0.5269

Table 2: Time-Course Data - SCDi + OA (Rescue)

Frame	Time (s)	Normalized	Fitted
0	0.000	0.0000	0.0000
1	0.718	0.3127	0.3132
2	1.436	0.4531	0.4501
3	2.154	0.5047	0.5099
4	2.872	0.5389	0.5361

6. Methods

6.1 FRAP Acquisition

FRAP experiments were performed using a Leica confocal microscope.

- Image dimensions: 512 x 512 pixels
- Bit depth: 8-bit
- Pre-bleach frames: 2
- Post-bleach frames: 5 (SCDi + OA), 10 (SCDi)
- Time interval: ~0.72 seconds
- Bleach ROI: Circular region, automatically detected

6.2 Normalization

Double normalization method (Phair et al., 2004):

$$F_{norm}(t) = [(F_{ROI}(t) - F_{bkgd}) / (F_{ref}(t) - F_{bkgd})] \times [(F_{ref}(i) - F_{bkgd}) / (F_{ROI}(i) - F_{bkgd})]$$

This corrects for background fluorescence, acquisition photobleaching, and cell-to-cell intensity variations.

6.3 Curve Fitting

Single exponential model:

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

Where Mf = mobile fraction, tau = time constant, $t_{1/2} = \tau \times \ln(2)$

7. Biological Interpretation

Mechanism

The rescue of molecular dynamics by exogenous oleic acid supports:

1. MEMBRANE COMPOSITION: Oleic acid depletion increases saturated fatty acids in membranes, potentially increasing rigidity and altering lipid domains.

2. PROTEIN MOBILITY: Slower FRAP recovery indicates reduced lateral diffusion, consistent with increased membrane viscosity.

3. SPECIFICITY: Complete rescue by exogenous OA confirms the phenotype is specifically due to oleic acid depletion, not off-target effects.

This rescue experiment provides direct evidence that oleic acid levels regulate molecular dynamics, likely through effects on membrane biophysical properties.

8. Conclusions

Exogenous oleic acid (100 uM) rescues the 2.2x slower molecular dynamics caused by SCD inhibition.

Summary

1. SCD inhibition causes 2.2x slower FRAP recovery

2. Exogenous oleic acid completely rescues this phenotype

3. Mobile fractions unchanged (~55%) - rate affected, not mobile proportion

4. Rescue confirms specificity - phenotype is due to OA depletion

Limitations

- Current data: n=1 cell per condition
- Need n>=3 biological replicates for statistics
- Direct mechanism not determined

Future Directions

- Collect n>=3 biological replicates
- Test dose-response of OA rescue
- Compare with other fatty acids
- Measure membrane fluidity directly

9. References

1. Phair RD et al. (2004) Methods Enzymol 375:393-414.

2. Ellenberg J et al. (1997) J Cell Biol 138:1271-1287.

3. Lippincott-Schwartz J et al. (2001) Nat Rev Mol Cell Biol 2:444-456.

4. Ntambi JM, Miyazaki M (2004) Prog Lipid Res 43:91-104.