Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

"Fix and Assay": Separating in-cellulo sphingolipid reactions from analytical assay in time and space using an aldehyde-based fixative

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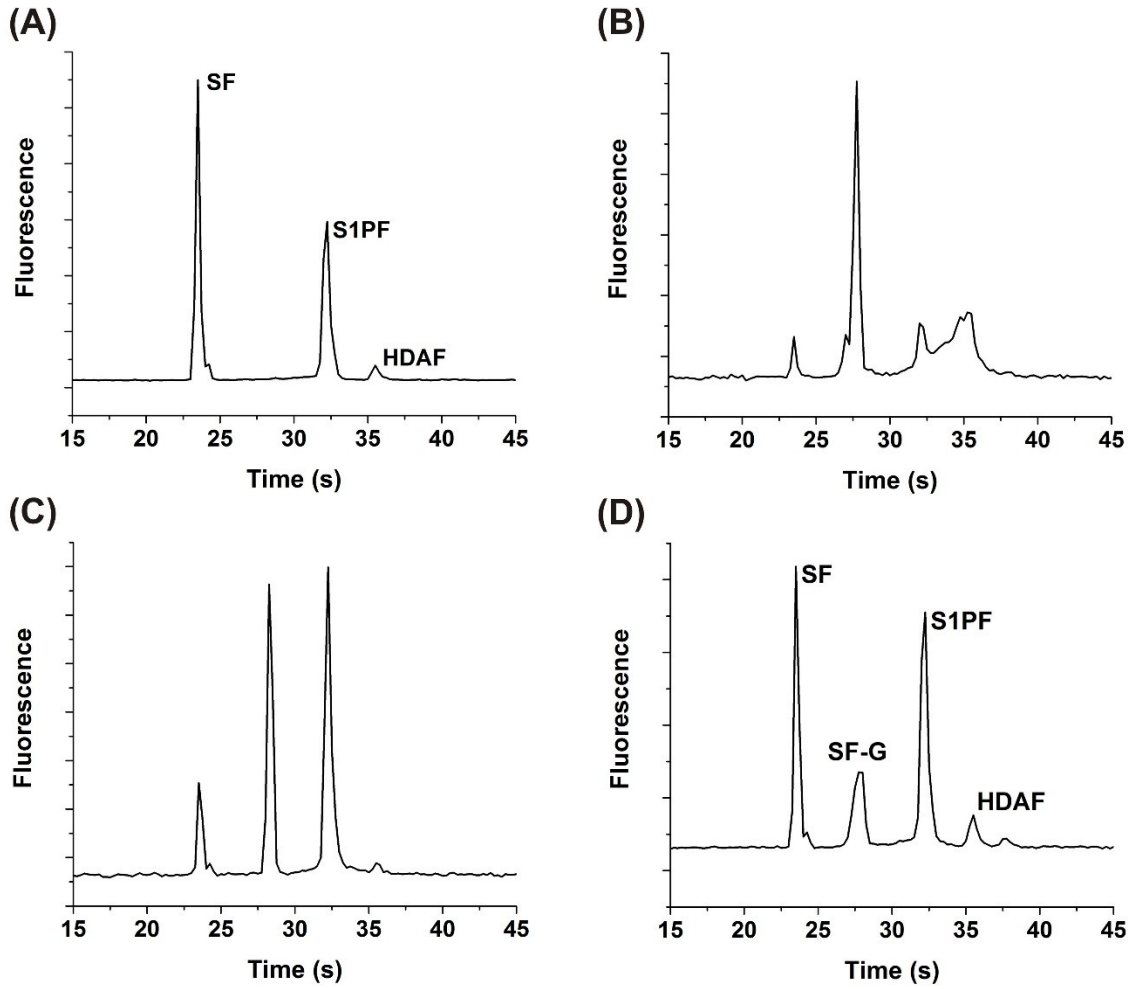
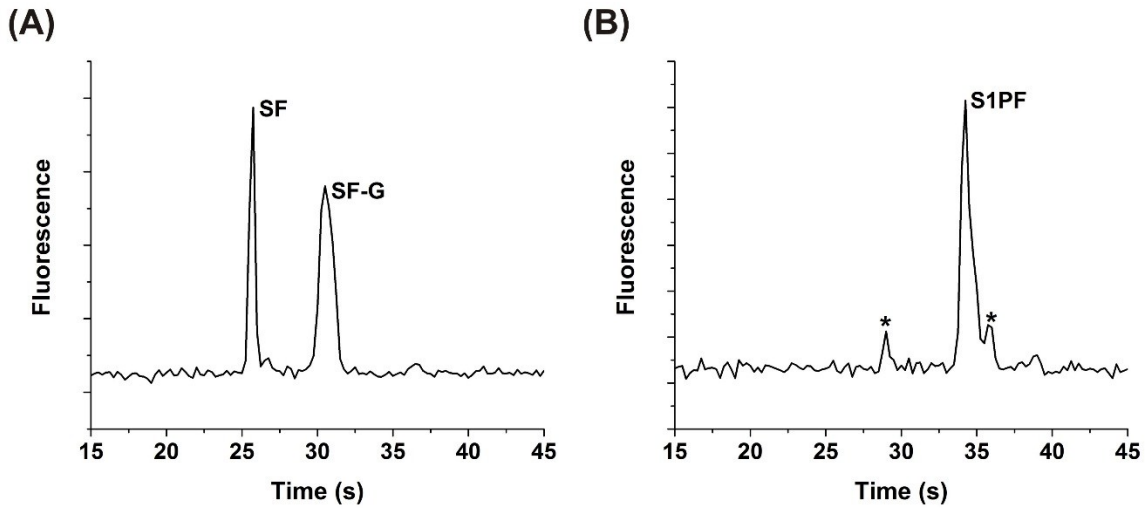
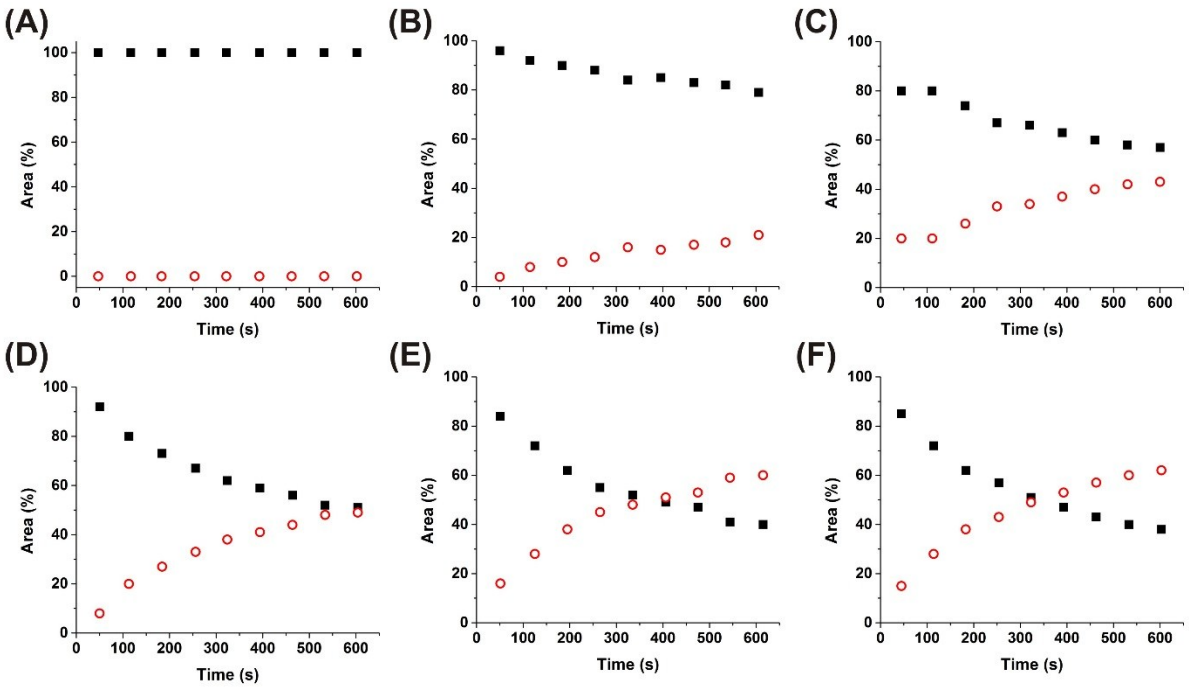


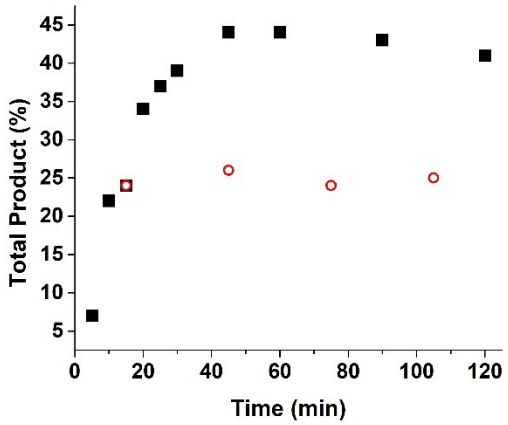
Supplemental Figure S1: Electropherograms of K-562 lysates of cells that were (A) unfixed or fixed with (B) glutaraldehyde (5%), (C) formaldehyde (5%), or (D) glyoxal-based fixative (no dilution). SF = sphingosine fluorescein; S1PF = sphingosine-1-phosphate fluorescein; HDAF = hexadecanoic acid fluorescein; and SF-G = sphingosine fluorescein-glyoxal adduct.



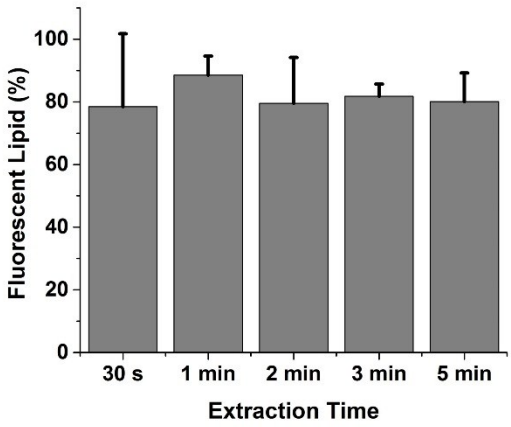
Supplemental Figure S2: Electropherograms of (A) SF standard or (B) S1PF standard incubated with glyoxal. * indicates a synthetic impurity in the S1PF standard (present in the absence of the glyoxal). SF = sphingosine fluorescein; S1PF = sphingosine-1-phosphate fluorescein; and SF-G = sphingosine fluorescein-glyoxal adduct.



Supplemental Figure S3: Formation of the unidentified fourth peak over time when SF was incubated with varying dilutions of the glyoxal-containing fixative. Shown on the Y-axis is the percentage of total lipid present as SF (closed black squares) or the fourth species (open red circles). SF was incubated with dilutions of 100% (A), 90% (B), 75% (C), 50% (D), 25% (E), or 0% (F) of the glyoxal-based fixative.



Supplemental Figure S4: Product formation over time in unfixed (closed black squares) and fixed (open red circles) K-562 cells. To verify that fixation with glyoxal terminated cellular reactions, K-562 cells were loaded with SF and incubated; a subset of the sample was fixed with glyoxal 15 min after reaction start time and product amounts in cell lysates were measured over time in both sample populations. In unfixed cells, the total product amount increased until 45 min reaction time. In fixed cells, the total product amount remained constant over time, indicating that cellular reactions were terminated with the fixative. Shown on the Y-axis is the amount of total product (S1PF+HDAF+Peak 5+Peak 6) formed while the X-axis shows the reaction time.



Supplemental Figure S5: Fluorescent lipid extracted from K-562 cells fixed with a glyoxal-based fixative when incubated with extraction solution for the indicated times. Shown on the Y-axis is the percentage lipid relative to unfixed control cells while the X-axis indicates incubation time with the extraction solution. Bar height represents the average of triplicate measurements and the error bars indicate one standard deviation.