Supporting Information

Small-molecule Labeling of Live Cell Surfaces for Three-dimensional Super-resolution Microscopy

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S1
**Figure S1.** Jablonski diagram
Simplified Jablonski diagram showing photoswitching of rhodamine spirolactam in response to purple (405 nm) excitation. Upon absorbing purple light, the non-fluorescent closed isomer can either non-radiatively return to the ground state, or transform into the open isomer via the bond cleavage between the lactam nitrogen and the xanthene ring. The open isomer is less stable and will thermally return to the closed isomer (on the time scale of ms in polar solvents). The open isomer can also enter its first excited state by absorbing green (561 nm) laser light. From this excited state, it can either emit photons (orange arrow), return to the open isomer ground state (gray dashed arrow between S1 and S0), or photobleach (gray dashed arrow to photobleached state).

**Figure S2.** Photoactivation of molecules of 8 doped into polymer film.
(A) Molecules of 8 doped into PVAVA film (1% in water) before (left), during (right), and immediately after 0.5 s (5 frames at 100 ms/frame) photoactivation with a 405 nm laser (97 W/cm²). The activated molecules are excited with a 561 nm laser (32 W/cm²). (B) The mean photons per pixel over three photoactivation sequences is plotted with time. The plot is color-coded by which laser is exciting the sample (purple for 405 nm, green for 561 nm). The fluorescence of the SM is ~25x above background. No fluorescence is detectable above background from the closed isomer before photoactivation, indicating a very large contrast ratio between the two isomers. (C) PVAVA film control without doped 8 showing before (left), during (right), and immediately after the same photoactivation sequence shown in (A). No evidence of photoactivation is seen in the polymer. (D) Corresponding mean photons per pixel over the three photoactivation sequences plotted for the PVAVA control. All scale bars are 2 μm.
The photoactivation quantum efficiency ($\Phi_P$) is the probability of generating the open isomer per photon absorbed by the closed form when pumped at 405 nm. This probability was quantified for molecules 6-8 by irradiating dye-doped PVAVA (1% in water) with different doses of 405 nm activation light and measuring the increase in fluorescence arising from the photo-generated open isomer. For more details about experimental procedure and results, see Table S1 and Materials and Methods.

Figure S4. Spectra of closed and open isomer in polymer and in solution
(A) Fluorescence excitation (red curve) and emission (green curve) spectra of the photogenerated open isomer of 8 stabilized in polymer (10% PMMA/toluene). (B) Absorbance (red curve) and fluorescence emission (dark yellow curve) of the open isomer of 8 in solution (1:1 acetonitrile:water) stabilized by the addition of strong acid (1M HCl). The absorbance spectrum of the closed isomer of 8 in solution (blue curve) is overlaid on both plots. The open isomer spectra in polymer matches well with the spectra obtained by stabilizing the open isomer with acid. This similarity justified using the acid-stabilized open isomer spectra to determine the photophysical characteristics of the open isomers (see Table S1).
**Figure S5.** Super-resolution statistics

(A) Example single-molecule time trajectories of immobilized 9 attached to cells. Most (98%) molecules only emit for one frame (50 ms per frame); however, a subset of molecules are fluorescent over many frames. (B) Scatter plots showing the extracted localization estimate for each frame that the single molecule was detected. The standard deviation of these positions is the localization precision of the experiment. (C) Histograms of the extracted standard deviation of fitted positions for 350 single molecules emitting for six frames or more. The mean precision is 14.9 nm in x, 13.1 nm in y, and 16.8 nm in z. (D) Histogram of photons detected per fit with mean of ~3800 photons per 50 ms frame. (E) The background is estimated by a median filter in Matlab and is ~32 photons/pixel.
Figure S6. Cross-sectional thickness of a single stalk
(A) 2D projection of localizations of 9 on a single cell. Localizations on the cell body are colored in black. The stalk is divided into five sections (blue, green, red, teal, and purple). (B) Panel showing each section of localizations in the stalk, viewed down the stalk long axis. (C) Histogram of the transverse cross-section of points in each stalk section. The histogram fits well to a single Gaussian (solid line) and the sigma of the fit is an estimate of the cross-sectional thickness of each stalk section.
Figure S7. Simulation of stalk thickness
(A) Schematic of simulation procedure for a given axial cross-section (gray circle) of the cell stalk. The underlying stalk structure is simulated as a hollow cylinder with a radius of 50 nm (from cryoEM measurements\(^1\)) and a transverse thickness of 8 nm (from estimates of the thickness of the RsaA surface layer). Localizations are simulated randomly on this stalk surface (green star). (B) An \(xy\) projection of one representative stalk simulation on the hollow cylinder shown in (A) with one slice highlighted in green. (C) The localizations in the green section of (B) rotated to look down the cell long axis. The hollow nature of the underlying structure is apparent. (D) Schematic of the same simulation procedure but with the addition of the uncertainty incurred from the experimental localization precision (light green circle around the green star). (E) An \(xy\) projection of one representative stalk simulation with the localization error included. (F) The localizations of the highlighted section (green points) in (E) rotated to look down the long cell axis. Adding uncertainty in position from the experimental localization precision obscures the hollow nature of the underlying stalk structure. (G) Histogram of the radial distances to the fitted center of the stalk for the mean of 25 simulations including localization precision uncertainty (black line) and the 95% confidence bounds (gray dashed lines) compared to the raw data (green line). The distribution for the raw data is similar to the simulation. (H) An \(xy\) projection of the raw stalk localizations and (I) the rotated highlighted section.
Figure S8. Cross-sectional thickness of example cell surface
(A) 2D projection of representative predivisional cell with eight cross-sections (150-nm thick) highlighted in color. (B) Rotating each of the cross-sections to look down the long cell axis shows the localizations roughly sample a circle. The radius of the circle decreases at the division septum. (C) The distribution of radial distances to the center of a circle fitted to the points in (B), color-coded by the corresponding cell section. These distributions can be fit well to single Gaussians (solid curve overlaid on the histograms). The sigma of the Gaussian fits is an estimate of the thickness of the cell surface. This thickness is a convolution of the true underlying surface thickness and the uncertainty incurred from the experimental localization precision. The thicknesses range from ~23 nm to ~31 nm, except for the section at the division septum (light green).

Figure S9. Comparison of Nile Red PAINT and rhodamine spirolactam label
(A) An axial cross-section (75 nm thick) of rhodamine spirolactam localizations on cell surface. Scale bar is 1 μm. (B) The distribution of radial distances to the fitted circle center can be fit to a Gaussian (red line) yielding a measured thickness of 28 nm. (C) An axial cross-section (400-nm thick) of Nile Red localizations obtained with PAINT. A ~6x thicker axial section was necessary for this data because of lower overall sampling of the cell surface. The box in (C) has dimensions of 1x1 μm. (D) The distribution of the Nile Red radial distances from (C) can also be fit to a Gaussian with a measured thickness of 58 nm, roughly 2x larger than what is obtained using the rhodamine spirolactam labeling method. (C) and (D) are reproduced with permission from reference 2.
Figure S10. Gel of labeled lysed cells
(A) Fluorescence emission (pump at 532 nm) from protein gel of *C. crescentus* cells labeled with 9, then washed and lysed. The lanes are (1) ladder, (2) unlabeled cells, (3) cells incubated with 8, (4) cells labeled with 9, (5) blank, (6) ladder, (7) unlabeled cells, (8) cells incubated with 8, (9) cells labeled with 9, and (10) cells labeled with 9. Fluorescence is evident from lanes (4), (9) and (10) at the approximate mass of RsaA (~100 kDa), but not from the other control lanes. The yellow rectangle guides the eye. (B) Same protein gel, stained with Coomassie blue for protein.

Figure S11. Sampling uniformity of labeling with time
(A) Percentage of total fits as a function of position along the cell axis for an example cell imaged immediately after labeling with 9 (red) and a cell imaged after waiting 60 min after labeling. (B) 2D SR reconstructions of the two cells shown in (A).
Figure S12. Variance of pulse-chase cells and cells imaged immediately (A) 2D histogram of the number of localizations (color-scaled from black to white) along the normalized cell axis for cells imaged 60 minutes after labeling. Each row represents a different cell (24 cells total). Each row could be plotted analogously to the curves in Fig S11. (B) 2D histogram for cells imaged immediately after imaging (24 cells). The cells in both (A) and (B) are ordered by cell length (top row of 2D histogram has shortest cell, bottom row has longest cell). The longest cells in (B) are predivisional, and a dip in the number of localizations along the cell axis is expected at the septum between the cells (because of the decreased surface area). This dip is indicated with a black arrow in (B). (C) 2D super-resolution images of three rows of cells in (A), colored arrows indicate which cells correspond to which row. (D) Comparison of the variance in the number of fits along the cell axis between (A) and (B) shows the much larger variance in the cells imaged 60 minutes after labeling.
Table S1. Photophysical characterization of rhodamine spirolactam derivatives

|          | Closed isomer<sup>a</sup> | Open isomer<sup>e</sup> |                      |                  |                  |                  |
|----------|---------------------------|-------------------------|---------------------|---------------------|---------------------|
|          | \( \lambda_{\text{max}}^{\text{abs}} \) [nm] | \( \varepsilon \) [M\(^{-1}\) cm\(^{-1}\)]<sup>b</sup> | \( \varepsilon \) [M\(^{-1}\) cm\(^{-1}\)]<sup>c</sup> | \( \Phi_p \) [x10\(^{-4}\)]<sup>d</sup> | \( \lambda_{\text{max}}^{\text{abs}} \) [nm] | \( \lambda_{\text{max}}^{\text{em}} \) [nm] |
| 1        | 317                       | 9580                    | -                   | -                   | 560                | 583                |
| 2        | 315                       | 12790                   | -                   | -                   | 560                | 585                |
| 3        | 292                       | 59890                   | -                   | -                   | 562                | 585                |
| 4        | 278                       | 39380                   | -                   | -                   | 561                | 587                |
| 5        | 316                       | 13100                   | -                   | -                   | 562                | 587                |
| 6        | 360                       | 56770                   | 5380                | 1.3±0.08            | 558                | 584                |
| 7        | 325                       | 35040                   | 2701                | 3.7±0.3             | 560                | 585                |
| 8        | 365                       | 32860                   | 17040               | 89±8                | 560                | 585                |
| 9        | 350                       | -                       | -                   | -                   | 560                | 582                |
| 10       | 369                       | 31160                   | -                   | -                   | 559                | 583                |

<sup>a</sup>Spectra taken in 1:1 acetonitrile:water  
<sup>b</sup>Measured at \( \lambda_{\text{max}} \)  
<sup>c</sup>Measured at \( \lambda = 405\text{nm} \)  
<sup>d</sup>Photoactivation quantum efficiency at 405 nm, determined in 1% PVAVA films  
<sup>e</sup>Stabilized by acid

Movie S1. Raw 3D data
(A) Individual frame of raw 3D data for the region of cells shown in (B). The movie plays at half real time (10 fps). Single molecules of 9 appearing as two spots with the angle between the spots encoding axial position are visible when the sample is excited with 561 nm. About a third of a way into the movie, the 561 nm pump is blocked and the sample is briefly irradiated with 405 nm laser light to activate a sparse subset of 9.
**Movie S2.** Surface section through cell

3D Super-resolution reconstruction of surface localizations with 75 nm slice highlighted in yellow. The slice moves by 25 nm along the cell axis as the movie progresses. The localizations are plotted as 3D spheres with sigma of 14 nm.
Materials and Methods

Synthesis and characterization
The NMR characterization of the synthesized materials was carried out at $^1$H (400 MHz), $^{13}$C (100 MHz), (Bruker Avance 400 MHz spectrometer using Topspin version 2.1 software) in CDCl$_3$, CD$_3$OH, D$_2$O or DMSO-$d_6$ with tetramethylsilane as internal standard. EI-MS was obtained at 70 eV using a Finnigan Polaris ion trap MS coupled with a Trace GC instrument. Differential scanning calorimetry (DSC) measurements were performed using a TA Instruments Differential Scanning Calorimeter 2920 at heating and cooling rates of 5-10°C per minute (unless otherwise stated). Temperatures are calibrated with an indium standard. Thin Layer Chromatography (TLC) was carried out using Silicycle brand plastic backed plates (250 μm thick layer of 60 Å silica gel with UV 254 and 354 nm fluorescence indicator). Column chromatography (flash) was carried out using Silicycle brand 60 Å, 40-63 μm particle size silica. THF, Et$_2$O, benzene and toluene were dried by distilling over sodium benzophenone. Anhydrous DMF and DMSO were purchased from Sigma Aldrich. All chemicals were used as received. UV Vis (Ultra Violet and Visible) spectra were recorded on Agilent/HP8453 diode array spectrometer. Samples were prepared by dissolving in an appropriate solvent and measurements were done in standard quartz cuvettes of 1.0 cm path-length. Melting points were obtained using a Nikon eclipse E600 POL with temperature controller (Metter FP90). On some occasions a microwave reactor (CEM Discover) was used due to better yield and shorter reaction time. The purity and molecular mass of samples were checked in house by using GC-MS (Finnegan Trace GC Ultra equipped with mass detector (Finnegan Polaris Q)) and the HRMS were run at the Ohio State University Mass Spectrometry and Proteomics Facility.

Rhodamine salts
Rhodamine salts were synthesized by literature procedures. A direct fusion of phthalic anhydride and 3-dialkylaminophenol at a temperature above 160°C$^1$ (Scheme 1) with successive addition of acids of interest affords rhodamine salts. For example; perchlorate salt of rhodamine dyes can be obtained when the fused mixture of phthalic anhydride and 3-dialkylaminophenol was subsequently treated with perchloric acid. The dye was collected as a green solid in 25% to 35% yield after cooling overnight in a refrigerator.$^4$

\[
\begin{align*}
\text{PhO}_{2} + \text{C}_{6}H_{4}O & \xrightarrow{H_3PO_4} \text{C}_{18}H_{17}N_{3}O_3Cl_4^{-} \\
R & = \text{CH}_3 (25\%); \text{CH}_2\text{CH}_3 (35\%)
\end{align*}
\]

Scheme 1 Synthesis of $N$-(9-(2-carboxyphenyl)-6-(dialkylamino)-3H-xanthen-3-ylidene)-$N$-methyl/ethylmethanaminium perchlorate/hydrochloride
Synthesis of rhodamine spirolactams:
The rhodamine spirolactams were synthesized by activating the carboxylic group of rhodamine salts with p-toluenesulfonyl chloride and DMAP in dry DCM at room temperature as described by Funasaka et. al.5
The addition of aryl amine to the activated rhodamine salts affords an amide, which undergoes cyclization to make a spirolactam. Intermediate 1, and compounds 1, 2, 4, 5, and 7 were synthesized as shown in Scheme 2.

A representative procedure for the rhodamine lactam (Intermediate 1) synthesis

Scheme 2 synthesis of rhodamine spirolactam, Intermediate 1

To a stirred solution of rhodamine B base (3.0 g, 6.78 mmol) in dichloromethane (36 mL) was added toluenesulfony chloride (2.21 g, 11.64 mmol). To this stirred mixture after 15 minutes was added DMAP (1.82 g, 15.83 mmol) at room temperature. After stirring the mixture for 15 min, a solution of 4-iodoaniline (1.35 g, 6.17 mmol) in dichloromethane (36 mL) was added. The reaction mixture was stirred for three hours and reaction progress was monitored by TLC using ethyl acetate:hexane (1:1) as eluent (a strong halochromic spot was appeared at the top of the TLC plate). The mixture was quenched with saturated aqueous sodium bicarbonate and the organic phase was extracted with ethyl acetate. The organic solution was washed with saturated aqueous sodium bicarbonate, dried over anhydrous magnesium sulfate and evaporated. The crude reaction mixture was purified by column chromatography using a mixture of hexane:ethyl acetate (1:1) as eluent. The desired compound was obtained from the first fraction as a creamy white solid (2.76 g, 85%); mp 173-176ºC; FTIR (neat, cm⁻¹) 3016, 2975, 2935, 1700, 1615, 1120, 786; ¹H NMR (400 MHz, CDCl₃) δ 7.99-7.97 (m, 1H), 7.49-7.46 (m, 2H), 7.44-7.42 (m, 2H), 7.14-7.11 (m, 1H), 6.62 (d, J = 8.72 Hz, 2H), 6.58 (d, J = 8.84 Hz, 2H), 6.30-6.29 (m, 1H), 6.27-6.26 (m, 3H), 3.29 (q, J = 7.2 Hz, 8H), 1.15 (t, J = 7.2 Hz, 12H); ¹³C NMR (100 MHz, CDCl₃) δ 167.7, 153.4, 153.0, 148.8, 137.6, 136.7, 133.0, 130.4, 128.6, 128.6, 128.5, 123.9, 123.3, 108.1, 106.0, 97.8, 91.7, 67.3, 44.3, 12.6; UV-Vis (DCM) λmax (lg ε) 240 (1.63), 280 (1.22), 320 (0.45) and cut off at 420 nm; C₃₄H₃₄N₅O₂, theoretical exact mass [M+H] 644.1774, and found [M+H] 644.1795

Compound 1
Yield 326 mg, 57%: mp 230ºC; FTIR (neat, cm\(^{-1}\)) 3012, 2975, 1691, 1613; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.02-7.98 (m, 1H), 7.50-7.47 (m, 2H), 7.16-7.08 (m, 4H), 6.80-6.78 (m, 2H), 6.63 (d, \(J = 8.80\) Hz, 2H), 6.30 (dd, \(J = 8.86\) Hz, 2.6 Hz, 2H), 6.24 (d, \(J = 2.60\) Hz, 2H), 3.31 (q, \(J = 7.11\) Hz, 8H overlapping), 1.14 (t, \(J = 7.10\) Hz, 12H); \(^{13}\)C NMR (400 MHz, CDCl\(_3\)) \(\delta\) 167.7, 153.2, 153.1, 148.7, 136.6, 132.8, 131.0, 128.8, 128.5, 128.1, 127.3, 126.6, 124.0, 123.3, 108.1, 106.4, 97.7, 44.3, 12.5; UV-Vis (DCM) \(\lambda_{\text{max}}\) (lg \(\varepsilon\)) 241 (1.78), 278 (1.13), 320 (0.43) and cut off at 350 nm; C\(_{34}\)H\(_{35}\)N\(_3\)O\(_2\), theoretical exact mass [M+H] 518.2809, and found [M+H] 518.2862.

**Compound 2**

Yield (127 mg, 68%): mp 197.5ºC; FTIR (neat, cm\(^{-1}\)) 3020, 2970, 1689, 1616. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.04-8.02 (m, 1H), 7.69-7.67 (m, 1H), 7.59 (d, \(J = 8.80\) Hz, 1H), 7.52-7.50 (m, 3H), 7.39-7.34 (m, 2H), 7.24 (d, \(J = 1.7\) Hz, 1H), 7.25-7.19 (m, 1H), 7.01 (dd, \(J = 8.78\) Hz, 2.02 Hz, 2H), 6.73 (d, \(J = 8.80\) Hz, 2H), 6.35 (dd, \(J = 8.85\) Hz, 2.7 Hz, 2H), 6.25 (d, \(J = 2.60\) Hz, 2H), 3.33 (q, \(J = 7.10\) Hz, 8H overlapping), 1.13 (t, \(J = 7.0\) Hz, 12H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 167.8, 153.4, 153.1, 148.7, 134.4, 133.4, 132.9, 131.9, 131.0, 128.8, 128.1, 127.3, 125.6, 125.5, 124.0, 123.3, 108.2, 106.4, 97.8, 67.6, 44.3, 12.5; UV-Vis (DCM) \(\lambda_{\text{max}}\) (lg \(\varepsilon\)) 241 (1.78), 278 (1.13), 320 (0.43) and cut off at 350 nm; C\(_{38}\)H\(_{37}\)N\(_3\)O\(_2\), theoretical exact mass [M+H] 568.2964, and found [M+H] 568.2992.

**Compound 3**

To a solution of compound 5 (0.143 g, 0.276 mmol) in 10 mL of anhydrous acetonitrile was added methyl iodide (0.0432 g, 0.304 mmol) in 1.36 mL of anhydrous acetonitrile. The mixture was allowed to reflux under nitrogen for 1 hour before being treated with an additional 0.0432 g of methyl iodide in 1.36 mL of acetonitrile. After another hour of reflux, thin layer chromatography revealed the complete consumption of the starting material. The reaction mixture was rotovapped to dryness affording methylated rhodamine lactam as pure amber colored solid. (0.172 g, Yield 95%). FTIR (neat, cm\(^{-1}\))): 2969, 2928, 1721, 1632, 1610, 1544, 1511, 1465, 1426, 1402, 1374, 1352, 1320, 1303, 1266, 1214, 1197, 1114, 1075, 1014, 930; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.66 (d, \(J = 7.28\) Hz, 2H), 8.06 (d, \(J = 7.28\) Hz, 2H), 8.02 (d, \(J = 7.56\) Hz, 1H), 7.68 (t, \(J = 7.32\) Hz, 1H), 7.60 (t, \(J = 7.38\) Hz, 1H), 7.06 (d, \(J = 6.42\) Hz, 1H), 6.58 (d, \(J = 8.88\) Hz, 2H), 6.48 (d, \(J = 2.00\) Hz, 2H), 6.316 (dd, \(J = 8.88\) Hz, 2.08 Hz, 2H), 4.04 (s, 3H), 3.31 (q, \(J = 7.04\) Hz, 8H), 1.07 (t, \(J = 6.86\) Hz, 12H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 169.5, 154.4, 152.0, 149.8, 149.3, 146.0, 136.4, 129.6, 127.7, 126.0, 124.5, 124.1, 115.1, 109.0, 104.5, 97.9, 67.2, 46.8, 44.0, 12.8; UV-Vis (DCM)
\( \lambda_{\text{max}} \) (lg \( \varepsilon \)) 241 (1.78), 278 (1.13), 320 (0.43) and cut off at 350 nm; \( \text{C}_{34}\text{H}_{37}\text{N}_{4}\text{O}_{4}^+ \), theoretical exact mass for positive ion 533.2911, and found 533.2935.

**Compound 4**

![Compound 4 structure](image)

Yield (198 mg, 49%): mp 207°C; FTIR (neat, cm\(^{-1}\)) 3010, 2972, 2930, 2250, 1697, 1614, 1546, 1511, 1429, 1401, 1329; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 8.0-7.97 (m, 1H), 7.52-7.44 (m, 2H), 7.43-7.39 (m, 2H), 7.26-7.23 (m, 2H), 7.12-7.09 (m, 1H), 6.57 (s, 1H), 6.55 (s, 1H), 6.32 (s, 1H), 6.31 (s, 1H), 6.27 (d, \( J = 2.8 \) Hz, 1H), 6.25 (d, \( J = 2.8 \) Hz, 1H), 3.31 (q, \( J = 7.2 \) Hz, 8H), 1.15 (t, \( J = 6.8 \) Hz, 12H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 168.3, 153.7, 148.9, 141.6, 133.7, 132.5, 129.3, 128.24, 125.2, 123.8, 123.5, 118.8, 108.3, 105.8, 97.8; UV-Vis (DCM) \( \lambda_{\text{max}} \) (lg \( \varepsilon \)) 240 (1.63), 280 (1.22), 320 (0.45) and cut off at 420 nm; theoretical exact mass \([\text{M+H}]^+\) 543.2760, and found \([\text{M+H}]^+\) 543.2782.

**Compound 5**

![Compound 5 structure](image)

Yield (0.81 mg, 72%); mp 222°C; FTIR (neat, cm\(^{-1}\)) 3025, 2970, 2885, 2745, 1702, 1633, 1511, 1317 1115, 788; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 8.31 (d, \( J = 6.32 \) Hz, 2H), 7.97 (d, \( J = 6.96 \) Hz, 2H), 7.47-7.43 (m, 2H), 7.36-7.35 (m, 2H), 7.06 (d, \( J = 7.32 \) Hz, 1H), 6.54 (d, \( J = 8.84 \) Hz, 2H), 6.37 (d, \( J = 2.32 \) Hz, 2H), 6.22 (dd, \( J = 8.84 \) Hz, 2.04 Hz, 2H), 3.30 (q, \( J = 7.04 \) Hz, 8H), 1.14 (t, \( J = 7.00 \) Hz, 12H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 168.8, 154.2, 152.3, 150.1, 148.9, 144.9, 133.8, 128.4, 128.2, 127.9, 123.7, 123.5, 116.8, 108.3, 105.9, 97.9, 66.8, 44.3, 12.5; UV-Vis (DCM) \( \lambda_{\text{max}} \) (lg \( \varepsilon \)) 245 (1.9), 275 (1.3), 320 (0.45) and cut off at 350 nm \( \text{C}_{33}\text{H}_{34}\text{N}_{4}\text{O}_{2} \), theoretical exact mass \([\text{M+H}]^+\) 519.2749, and found \([\text{M+H}]^+\) 519.2760.

Not all the compounds were synthesized following Scheme 1. Reaction paths were modified for compounds 6, 8, 9 and 10. Compound 6 was synthesized using a Jeffer modification of the Heck reaction between intermediate 1 and 4-[(E)-2-(4-vinylphenyl)vinyl]pyridine\(^6\) and intermediate 2 was synthesized by the same method in a reaction between intermediate 1 and 4-vinylpyridine.
**Compound 6**

In a 100 mL round bottom flask, equipped with stirbar, condenser, oil bath, and nitrogen inlet was placed the intermediate 1 (0.25 g, 0.4 mmol), 4-[(E)-2-(4-vinylphenyl)vinyl]pyridine\(^7\) (0.081 g, 0.4 mmol), DMF (2 mL), TDA-1 (0.012 mL) and potassium carbonate (0.24 g, 0.42 mmol). The reaction mixture was heated to 80\(^\circ\)C and palladium acetate (8.8 mg) was added all at once. The reaction was then heated to 110\(^\circ\)C and monitored by TLC. The reaction was completed in 1 hour and to make sure the reaction was further heated half an hour more. When water was added to the mixture, a dense creamy white precipitate was appeared. It was filtered off, washed with water, air dried and then dissolved in ethyl acetate and dried with magnesium sulfate. The solvent was removed by rotary evaporation. The product was collected using hexane and filtered to obtain a creamy white mass (0.235 g, 82%). mp 145-165\(^\circ\)C; FTIR (neat cm\(^{-1}\)) 3018, 2968, 1694, 1613, 1512; \(^1\)H NMR (400 MHz, CDCl\(_3\), δ 8.54 (d, \(J = 5.08\) Hz, 2H), 7.90-7.88 (m, 1H), 7.65-7.53 (m, 2H), 7.56-7.54 (m, 7H), 7.39 (d, \(J = 8.76\) Hz, 2H), 7.26 (d, \(J = 16.36\) Hz, 1H), 7.15 (s, 2H), 7.07-7.06 (m, 1 H), 6.89, (d, \(J = 8.64\) Hz, 2H), 6.57 (d, \(J = 8.84\) Hz, 2H), 6.38 (dd, \(J = 8.89\) Hz, 2.54 Hz, 2H), 6.30 (d, \(J = 2.50\) Hz, 2H), 3.30 (q, \(J = 7.04\) Hz, 8H), 1.06 (t, \(J = 6.96\) Hz, 12H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) δ 167.2, 153.9, 152.6, 150.4, 148.8, 144.7, 137.8, 136.8, 135.9, 135.2, 133.8, 133.0, 129.9, 128.8, 128.5, 128.4, 127.9, 127.3, 127.0, 126.2, 124.2, 123.3, 121.2, 108.6, 106.0, 97.6, 66.9, 44.0, 12.3; UV-Vis (DCM) \(\lambda_{\text{max}}\) (lg ε) 240 (2.2, 325 (1.13), 367 (1.8) and cut off at 430; C\(_{49}\)H\(_{46}\)N\(_4\)O\(_2\), theoretical exact mass [M+H] 723.3699, and found [M+H] 723.3707

**Compound 7**

Yield (408 mg, 55%; mp 238\(^\circ\)C; FTIR (neat cm\(^{-1}\)) 3021, 2974, 2212, 1692, 1634, 1510 ; \(^1\)H NMR (400 MHz, DMSO) δ 8.26-8.22 (m, 2H), 7.92-7.89 (m, 1H), 7.75-7.72 (m, 2H), 7.6-7.52 (m, 2H), 7.43-7.39 (m, 2H), 7.08-7.04 (m, 3H), 6.56 (s, 1H), 6.54 (s, 1H), 6.37 (d, \(J = 2.0\) Hz, 1H), 6.35 (d, \(J = 2.8\) Hz, 1H), 6.33 (s, 1H), 6.32 (s, 1H), 3.29 (q, \(J = 7.2\) Hz, 8H); 1.06 (t, \(J = 6.8\) Hz, 12H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) δ 167.5, 153.8, 152.7, 149.2, 147.4, 138.8, 134.0, 132.8, 132.2, 129.7, 129.6, 128.9, 128.5, 125.6, 124.2, 124.1, 123.4, 119.0, 109.0, 106.4, 98.2, 94.4, 88.4, 67.2, 44.1, 12.8; UV-Vis (DCM) \(\lambda_{\text{max}}\) (lg ε) 240 (1.83), 279 (1.36), 350 (0.86) and cut off at 430 nm; C\(_{42}\)H\(_{38}\)N\(_4\)O\(_4\), theoretical exact mass [M+H] 663.2971, and found [M+H] 663.3014
Intermediate 2

In a 100 mL round bottom flask, equipped with stir bar stirrer, condenser, oil bath, nitrogen was placed intermediate 1 (0.2527 g, 0.39 mmol), 4-vinyl pyridine (0.042 mL, 0.39 mmol), DMF (16 mL), TDA-1 (0.01 mL) and potassium carbonate (0.05 g, 0.39 mmol). The reaction mixture was heated to 80ºC and palladium acetate (0.88 mg) was added all at once. The reaction was then heated to 110ºC and monitored by TLC. The reaction was completed in 1 hour and to make sure the reaction was complete it was heated half an hour more. The reaction mixture was cooled to room temperature. Water was added to this cooled mixture and a dense creamy white precipitate appeared. It was filtered and dissolved in ethyl acetate and dried over magnesium sulfate. The solvent was removed by rotary evaporation. The product was stuck on the wall of flask. It was scraped off and collected by using hexane and filtered to obtain a creamy white mass (0.21 g, 86%). mp 153-158 ºC; FTIR (neat cm\(^{-1}\)) 3030, 2967, 2868, 1705, 1676, 1632, 1613, 1589, 1547, 1512, 1344, 1324, 1219, 1118; \(^{1}H\) NMR (400 MHz, CDCl\(_3\)) \(\delta 8.52 (d, J = 6.2 Hz, 2H), 8.01-7.99 (m, 1H), 7.51-7.46 (m, 2H), 7.30-7.28 (m, 4H), 7.16-7.12 (m, 2H), 6.94 (d, \(J = 8.52 Hz, 2H\)), 6.85 (d, \(J = 16.28 Hz, 2H\))

Intermediate 2 (0.10 g, 0.16 mmol) was dissolved in acetonitrile (2 mL). To this solution was added methyl iodide (0.02 mL, 0.32 mmol) dissolved in acetonitrile (2 mL) and refluxed under nitrogen for two hours. The reaction was monitored by TLC. When the TLC of the sample was taken after three hours, it showed the consumption of all of the starting material. The solvent and excess methyl iodide was removed under reduced pressure. A yellowish compound resulted which was dissolved in a minimal amount dichloromethane then hexane was used to triturate the product (115 mg, 94%); mp >260ºC; FTIR (neat cm\(^{-1}\)) 3036, 2968, 2928, 1689, 1614, 1513, 1464, 1329, 1216, 1181, 1115; \(^{1}H\) NMR (400 MHz, DMSO \(d_6\)) \(\delta 8.82 (d, J = 6.76 Hz, 2H), 8.11 (d, J = 6.80 Hz, 2H), 7.91 (d, J = 6.68 Hz, 1H), 7.83 (d, \(J = 16.32 Hz, 1H\)), 7.58-7.55 (m, 4H), 7.39 (d, \(J = 16.28 Hz, 1H\)), 7.09-7.06 (m, 3H), 6.56 (d, \(J = 8.88 Hz, 2H\)), 6.38 (dd, \(J = 8.94 Hz, 2.5 Hz, 2H\)), 6.32-6.32 (m, 2H), 4.23 (s, 3H), 3.31 (q, \(J = 6.8, 8H\)), 1.07 (t, \(J = 6.8, 12H\)); \(^{13}C\) NMR (100 MHz, DMSO \(d_6\)) \(\delta 167.4, 154.0, 152.7, 152.5, 148.8, 145.5, 140.1, 139.4; \(\text{C}_{41}\text{H}_{40}\text{N}_{4}\text{O}_{2}\), theoretical exact mass [M+H] 621.3230, and found [M+H] 621.3280
134.1, 132.9, 129.5, 129.0, 128.7, 125.6, 124.2, 123.8, 123.4, 123.0, 122.7, 125.6, 124.2, 123.8, 123.4, 123.0, 122.7, 108.7, 105.9, 97.6, 66.9, 47.3, 44.0, 12.8. UV-Vis (DCM) $\lambda_{\text{max}}$ (lg $\varepsilon$) 245 (1.7), 280 (0.89), 325 (0.4), 390 (0.7) and cut off at 495 nm; $C_{42}H_{43}N_4O_2^+$, theoretical exact mass for positive ion 635.3381, and found 635.3396

**Compound 9**

![Chemical structure](image)

Intermediate 2 (0.20 g, 0.32 mmol) was dissolved in 6 mL of warm acetonitrile and 2,5-dioxopyrrolidin-1-yl 3-iodopropanoate (0.1 g, 0.34 mmol) was added in the solution of acetonitrile (6 mL) and refluxed overnight. The TLC of the reaction indicated the presence of starting material rhodamine lactam. The reaction was refluxed for another 24 hours until complete consumption of starting material. The excess acetonitrile was removed under reduced pressure. A brown solid was obtained which was crushed into powder and stirred in ethyl acetate overnight and filtered to afford a brown solid (0.44 g, 71%); mp >280°C; FTIR (neat cm$^{-1}$): 3035, 2969, 2928, 2869, 1696, 1613, 1513, 1465, 1327, 1216, 1115; $^1$H NMR (400 MHz, DMSO $d_6$) $\delta$ 8.97 (d, $J = 6.7$ Hz, 2H), 8.17 (d, $J = 6.7$ Hz, 2H), 7.92-7.88 (m, 2H), 7.6 - 7.5 (m, 4H), 7.40 (d, $J = 6.9$ Hz, 1H), 7.11-7.06 (m, 3H), 6.59-6.56 (m, 2H), 4.86 (t, $J = 6.8$ Hz, 2H), 3.63 (t, $J = 6.8$ Hz, 2H), 3.3 (q, $J = 6.9$ Hz, 8H), 2.81 (s, 4H), 1.07 (t, $J = 6.9$ Hz, 12H); $^{13}$C NMR (100 MHz, DMSO $d_6$) $\delta$ 170.4, 167.5, 166.9, 154.0, 153.7, 152.5, 148.8, 145.3, 140.8, 139.6, 134.2, 132.8, 129.4, 129.0, 128.8, 128.7, 125.5, 124.2, 123.9, 123.6, 123.4, 108.7, 105.9, 97.6, 66.98, 54.8, 44.1, 31.7, 25.9, 12.8; UV-Vis (DCM) $\lambda_{\text{max}}$ (lg $\varepsilon$) 242 (1.63), 279 (0.86), 325 (0.45), 395 (0.7), and cut off at 490 nm; $C_{48}H_{48}N_5O_6^+$, theoretical exact mass for positive ion 790.3599, and found 790.6358

**Compound 10**

![Chemical structure](image)

Intermediate 2 (0.2 g, 0.32 mmol) was dissolved in 6 mL of warm acetonitrile. 2-(2-(2-ethoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (0.5 mL, 0.58 g, 1.76 mmol) was added in the solution of acetonitrile and refluxed overnight. The TLC of the reaction indicated the presence of starting material-rhodamine lactam so additional 0.1 mL of 2-(2-(2-ethoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate was added. The reaction was run for 6 h with the complete consumption of starting material. The reaction was stopped and the solvent was removed under reduced pressure and the excess of the 2-(2-(2-ethoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate was also removed under high vacuum (25µ) at 150°C. A brown sticky solid compound was obtained. The HNMR indicated that the compound was still not pure. After cooling in a refrigerator overnight the brown mass was broken up with a spatula and further crushed into powder by stirring overnight in hexane. The product was finally obtained as a yellow powder (0.41 g, 88%); mp >280°C, FTIR (neat cm$^{-1}$) 3350, 2917, 2850, 1687, 1614, 1513, 1464, 1327, 1216, 1115; $^1$H NMR (400 MHz, DMSO $d_6$) $\delta$ 9.0 (s, 1H), 8.1 (d, $J = 6.7$ Hz, 2H), 7.9 (d, $J = 6.7$ Hz, 2H), 7.6-7.5 (m, 4H), 7.40 (d, $J = 6.9$ Hz, 1H), 7.11-7.06 (m, 3H), 6.59-6.56 (m, 2H), 4.86 (t, $J = 6.8$ Hz, 2H), 3.63 (t, $J = 6.8$ Hz, 2H), 3.31 (q, $J = 6.9$ Hz, 8H), 2.81 (s, 4H), 1.07 (t, $J = 6.9$ Hz, 12H); $^{13}$C NMR (100 MHz, DMSO $d_6$) $\delta$ 170.4, 167.5, 166.9, 154.0, 153.7, 152.5, 148.8, 145.3, 140.8, 139.6, 134.2, 132.8, 129.4, 129.0, 128.8, 128.7, 125.5, 124.2, 123.9, 123.6, 123.4, 108.7, 105.9, 97.6, 66.98, 54.8, 44.1, 31.7, 25.9, 12.8; UV-Vis (DCM) $\lambda_{\text{max}}$ (lg $\varepsilon$) 242 (1.63), 279 (0.86), 325 (0.45), 395 (0.7), and cut off at 490 nm; $C_{42}H_{43}N_4O_2^+$, theoretical exact mass for positive ion 635.3381, and found 635.3396
1514, 1460, 1351, 1075; $^1$H NMR (400 MHz, CDCl$_3$) δ 9.11-9.09 (m, 2H), 8.0-7.98 (m, 1H), 7.82-7.79 (m, 4H), 7.51-7.44 (m, 3H), 7.38-7.36 (m, 2H), 7.15-7.12 (m, 5H), 6.93 (d, $J = 16.20$ Hz, 1H), 6.61 (d, $J = 8.7$ Hz, 2H), 6.31-6.27 (m, 4H), 4.91 (t, $J = 4$ Hz, 2H), 4.00 (t, $J = 4$ Hz, 2H), 3.63-3.54 (m, 8H), 3.50 (q, $J = 7.2$ Hz, 2H), 3.31 (q, $J = 7.2$ Hz, 8H), 2.31 (s, 3H), 1.17 (t, $J = 7.2$ Hz, 3H), 1.15 (t, $J = 4$ Hz, 12H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 172.2, 158.7, 158.1, 157.3, 153.6, 151.0, 149.9, 145.2, 144.2, 142.7, 138.9, 137.6, 135.3, 134.2, 133.8, 133.5, 133.4, 133.2, 132.8, 130.7, 130.3, 128.9, 128.5, 128.4, 128.2, 113.4, 110.7, 102.3, 74.9, 74.8, 74.7, 74.3, 73.8, 70.7, 48.8, 25.9, 20.2, 17.5; UV-Vis (DCM) λ$_{\text{max}}$ (lg ε) 240 (1.4), 277 (0.8), 325 (0.4), 390 (0.67), and cut off at 480 nm; C$_{49}$H$_{57}$N$_4$O$_5^+$, theoretical exact mass for positive ion 781.4323, and found 781.4383
**Bulk spectroscopic characterization and analysis**

Absorbance and fluorescence spectra were measured using standard 1-cm path length quartz cuvettes in a Cary 6000i UV-vis spectrometer or a Jobin-Yvon Horiba Fluorolog3 fluorometer, respectively. Unless otherwise noted, the compounds were dissolved at ~μM concentration in 1:1 acetonitrile (Fisher spectroscopic grade): NanoPure water. Molar extinction coefficients of the closed isomer were determined by measuring the absorbance of a series of dilutions with known concentrations. Since the open isomer has ~ms lifetime in polar solvents, acid (HCl, 1M, Fisher) was added to stabilize the open form. Neutralizing the solution (KOH, 1M, Fisher) recovered the closed isomer. The spectral properties were shown to be the same as photo-generated open isomer in polymer (Figure S4). To photo-generate the open isomer in polymer, a μM concentration of dye was doped into 10% (by mass) of poly(methyl methacrylate) (PMMA, 200 μm beads, MW ~ 75,000 g/mol, Polysciences Inc.) in distilled toluene. Once dissolved, 400μL of dye/PMMA mixture was drop-cast onto glass slides (Fisher Finest, No.1, 22x22 mm) and irradiated by UV light for five minutes. The fluorescence emission and excitation spectra were collected in front-face geometry.

To determine the closed isomer’s integrated absorbance for wavelengths > 400 nm, absorbance spectra were converted into units of epsilon (M⁻¹cm⁻¹) by dividing by the solution concentration. The absorbance between 400 and 500 nm was numerically integrated in Matlab using the trapz function.

**Imaging rhodamine spirolactams doped in polymer**

Rhodamine spirolactams were immobilized in polymer films to measure sensitivity to the 405 nm activating laser intensity. Polymer films were prepared using 1% (by mass) solutions of poly(vinyl alcohol-vinyl acetate) (PVAVA, 88% hydrolyzed, MW ~31,000 g/mol, Polysciences Inc.) in Nanopure water. The polymer sample was doped with rhodamine spirolactam (concentrations ranged from ~100 to 1 nM) and then spin–cast onto Argon-plasma-etched coverslips (Fisher Finest, No.1, 22x22 mm). These samples were imaged on the microscope set-up described below, but without the DH-PSF phase masks. To convert the ADC counts recorded by the EMCCD camera into detected photons, the counts (with the dark offset removed) were multiplied by (conversion gain)/(electron multiplication gain), as previously described. The conversion gain determined for our camera is 26.93 (photoelectrons/ADC count).

The photoactivation quantum efficiency (Φ_P) was determined for molecules 6, 7, and 8 from PVAVA polymer samples doped with ~100 nM dye by following a procedure similar to that outlined in reference 13. The closed isomer of molecules 6, 7, and 8 have absorbance values at 405 nm which are converted to extinction coefficient in Table S1. The extinction coefficient of 9 (the reactive form of 8) was not measured due to limited quantity of material, but the absorption spectra of 8 and 9 had the same shape, so we take the behavior of 8 as a reasonable proxy for 9. The Φ_P values were determined by irradiating dye-doped polymer samples with 405 nm laser light for different irradiation times and measuring the relative increase in fluorescence, (I₂-I₁)/I₁, where I₂ is the intensity after 405 nm irradiation, and I₁ is the starting intensity from pre-activated molecules; background fluorescence is subtracted from both. This relative increase in fluorescence intensity was measured in triplicate for a variety of 405 nm irradiation times. The characteristic growth rate (k_p) for photoactivation was extracted by determining the initial slope of the relative increase curve. The fitted values of k_p were then used in the photoactivation equation13:
where \( R_p \) is the rate of photoactivation, \( R_{abs} \) is the rate of absorbing photons, \( \sigma_i \) is the absorption cross-section of the closed isomer (related to the molar absorption coefficient by \( \sigma_i = \left(1000 \times 2.303 \epsilon \right) / N_A \)), \( \lambda \) is the activation wavelength, \( h \) is Planck’s constant, \( c \) is the speed of light, and \( N_A \) is Avogadro’s number. Of course, the overall yield of product is given by the quantum efficiency times the photons absorbed.

**Live cell sample preparation and labeling**

Colonies of the wild type (CB15N) strain of *Caulobacter crescentus* were grown overnight in test tubes containing 5-mL of PYE growth medium with shaking in a 28°C water bath. Cultures for imaging were grown by diluting 1:1000 into the defined minimal media M2G without antibiotic present. When the cells had grown to midlog phase, 1-mL aliquots were washed at least 3x by centrifuging for 3 minutes at 8,000 RPM (Eppendorf MiniSpin F-45-12-11) and resuspending the pellet in 1-mL clean M2G. To label the cells with \( ^9\text{F} \), 50 μL of \( ^9\text{F} \) (dissolved in DMSO (Fisher) at ~100 nM - 1 μM) was added slowly to the cell suspension and left to incubate (covered with aluminum foil) for 30 minutes. Unreacted \( ^9\text{F} \) was removed by five washes with M2G (centrifuge 3 min at 8,000 RPM at room temperature). All steps involving \( ^9\text{F} \) were done under red lights to minimize exposure to UV radiation before imaging. The cleaned cells were resuspended in a small amount of M2G to produce a concentrated cell suspension. Fiducial markers were added to this cell suspension (≈1 nM, Molecular Probes, 540/560 carboxylate-modified FluoSpheres, 100 nm diameter) and 1-μL of this mixture was deposited onto an agarose pad (1.5% (by mass) low melting point agarose (Invitrogen) in M2G buffer) and mounted onto a Ar plasma-etched glass slide (Fisher Finest, No.1, 35x50 mm) and imaged immediately. This procedure is very similar to that described in reference 16. For pulse-chase labeling experiments, the cells were resuspended in 5-mL of clean M2G media and allowed to grow in the cell shaker for between 30 and 120 minutes before imaging.

**Protein gel of labeled cells**

A protein gel of lysed, labeled cells was used to support our hypothesis that we primarily label the RsaA protein in the outer S-layer. Cells were grown as described above, then incubated with 50 μL of either \( ^9\text{F} \) (~1 μM), \( ^8\text{F} \) (~1 μM), or DMSO (Fisher) for thirty minutes (covered with aluminum foil) and washed as previously described. The cells were lysed by heating to 90°C for five minutes, then separated on an acrylamide gel. The fluorescence from the gel was imaged using a Typhoon 9400 using 532 nm excitation. The laser light was filtered with a 555 nm BP. After fluorescence imaging, the gel was stained with Coomassie dye.

**Three-dimensional super-resolution imaging and data processing**

Three-dimensional super-resolution imaging was performed on the dual channel DH-PSF microscope previously described. Briefly, samples were imaged on an inverted fluorescence microscope (Nikon
Diaphot 200) with the internal tube lens removed. Fluorescence emission was collected through high NA oil-immersion objective (Olympus UPlanSApo 100X/140NA) and filtered through a dual pass dichroic mirror (Chroma, zt440/514/561rpc), a 561 nm dichroic beam splitter (Semrock, FF560-FDi01), a 514 nm long pass filter (Semrock, LP02-514RE) and a 561 nm notch filter (Semrock, NF03-561E). Modulation by transmissive phase masks generated the DH-PSF, which was imaged onto an electron-multiplying Si charge coupled camera (Andor Ixon DU-897E). Fluorescence excitation was pumped by a 561 nm laser (Coherent Sapphire 100 mW, 1.9 kW/cm², beam radius of 27 μm (1/e²)). Photoactivation was provided by a 405 nm laser (Coherent, < 100 W/cm²). Single-molecule positions were determined using a fiducial bead to generate a z-calibration curve. The microscope objective was stepped relative to the fiducial bead in 50 nm increments using a piezoelectric objective scanner (Mad City Labs, C-focus). This bead was used to calibrate the DH-PSF angle, midpoint, and statistical localization as a function of the z defocus. Using this calibration removes apparent motion of single-molecule localizations in x and y arising from tilt and estimator inaccuracy. The calibration bead is also used to generate template DH-PSF images, which are used to identify potential single-molecule signals. Single molecules were fit using code built in Matlab (Mathworks) and available online.

The localization precision was defined as the standard deviation of the fitted positions of the same molecule of 9 emitting over several frames. In general, the laser power was optimized such that the majority of molecules only emit for one frame (97.6 ± 0.1 %) before photoswitching back to the dark state or photobleaching. However, a subset of long-lived molecules emit over many frames. Candidate molecules were identified by filtering the localizations by time and space. Single molecule emission was assumed to arise from the same molecule if the 3D positions were within a 60 nm radius and turned on within two frames (50 ms frames). The mean localization precision from the standard deviation of the position of 351 molecules (>5 frames on) was \( \sigma_x = 14.9 \text{ nm}, \sigma_y = 13.1 \text{ nm}, \) and \( \sigma_z = 16.8 \text{ nm}. \) In the super-resolution reconstructions, each localization was plotted as an identical 3D Gaussian spot with a width of sigma = 14 nm.

**Image analysis**

Individual cells were selected manually. To determine the central axis of a cell, the localizations were rotated to be aligned with the x-axis. If the cell had a stalk, the stalk localizations were ignored for the rotation and cell body axis determination. The user defined an approximate central axis by selecting between 5 and 10 points along the cell axis for both the xy and xz projections. This approximate axis was smoothed using cubic interpolation. Next, the initial axis was iteratively improved by fitting circles to sections of localizations perpendicular to the cell axis (50 nm thick sections). The center of the fitted circle was used as the improved axis, and the axis was smoothed by cubic interpolation. Varying the thickness of the sections had only a minimal effect on the final cell axis. The final cell axes were reproducible with different initial input by the user. The surface thickness of the cell body was determined by dividing the cell into windows along the central axis (75 nm thick), rotating these localizations into the transverse plane, and fitting the points to a circle. The distribution of radial distances to the fitted circle center was fit to a single Gaussian and the surface thickness reported is the \( \sigma \) of this fit. The number of localizations found within the cell (as opposed to on the cell surface) was determined by finding the percentage of localizations (not including the endcaps of the cell or the septum for predivisional cells) within 150 nm of the cell axis.
The stalk central axis was determined analogously to the procedure for the cell body, but only using localizations in the stalk. The reported stalk length was determined using a MATLAB script to determine the length of a curve. The cross-sectional thickness of the stalk was determined by dividing the stalk into five windows (~200 nm thick), rotating these localizations into the transverse plane, then potting the histogram of the number of localizations in one dimension perpendicular to the stalk. The cross-sectional thickness reported is the σ of the single Gaussian fit to the histogram. Stalk localizations were simulated by approximating the stalk as a hollow cylinder with a radius of 50 nm and a thickness of 8 nm. Localizations were randomly scattered on the surface of the cylinder and allowed to penetrate up to 8 nm towards the central axis. Localization precision was added by shifting each localization by a randomly chosen precision error (pulled from the experimental distribution of localization precisions).
NMR spectra

Compound 2 $^1$H NMR (400 MHz) CDCl$_3$

Compound 2 $^{13}$C NMR (100 MHz) CDCl$_3$
Compound 3 $^1$H NMR (400 MHz) DMSO $d_6$

![NMR Spectrum](image)

Compound 3 $^{13}$C NMR (100 MHz) DMSO $d_6$

![NMR Spectrum](image)
Compound 4 $^1$H NMR (400 MHz) CDCl$_3$

![NMR spectrum of Compound 4](image)

Compound 4 $^{13}$C NMR (100 MHz) CDCl$_3$

![NMR spectrum of Compound 4](image)
Compound 5 $^1$H NMR (400 MHz) CDCl$_3$

\[ \text{Diagram of Compound 5} \]

\[ \text{1.126} \quad 1.143 \quad 1.161 \quad 3.280 \quad 3.297 \quad 3.315 \quad 3.332 \quad 6.211 \quad 6.217 \quad 6.233 \quad 6.239 \quad 6.372 \quad 6.377 \quad 6.535 \quad 6.557 \quad 7.056 \quad 7.074 \quad 7.260 \quad 7.350 \quad 7.353 \quad 7.366 \quad 7.436 \quad 7.451 \quad 7.455 \quad 7.472 \quad 7.963 \quad 7.981 \quad 8.311 \quad 8.326 \quad 11.821 \quad 8.143 \quad 2.036 \quad 2.117 \quad 1.976 \quad 1.042 \quad 1.975 \quad 2.024 \quad 1.061 \quad 2.000 \]

Compound 5 $^{13}$C NMR (100 MHz) CDCl$_3$

\[ \text{Diagram of Compound 5} \]

\[ \text{168.816} \quad 154.250 \quad 148.925 \quad 144.924 \quad 133.836 \quad 128.478 \quad 128.227 \quad 128.227 \quad 127.927 \quad 123.713 \quad 123.532 \quad 116.825 \quad 116.825 \quad 115.987 \quad 115.987 \quad 114.825 \quad 114.825 \quad 113.532 \quad 113.532 \quad 112.301 \quad 112.301 \quad 108.301 \quad 108.301 \quad 105.987 \quad 105.987 \quad 97.508 \quad 97.508 \quad 44.524 \quad 44.524 \quad 43.836 \quad 43.836 \quad 168.816 \]
Compound 6 $^1$H NMR (400 MHz) DMSO $d_6$

[Chemical structure image]

Compound 6 $^{13}$C NMR (100 MHz) DMSO $d_6$

[Chemical structure image]
Compound 7 \(^1\)H NMR (400 MHz) DMSO \(d_6\)

Compound 7 \(^{13}\)C NMR (100MHz) DMSO \(d_6\)
Compound 8 \(^1\)H NMR (400 MHz) DMSO \(d_6\)

\[
\begin{array}{c}
\text{Compound 8}^{15}\text{C NMR (100 MHz) DMSO } d_6
\end{array}
\]
Intermediate 1 $^1$H NMR (400 MHz) CDCl$_3$

![NMR谱图1](attachment:image1)

Intermediate 1 $^{13}$C NMR (100 MHz) CDCl$_3$

![NMR谱图2](attachment:image2)
Intermediate 2 $^1$H NMR (400 MHz) CDCl$_3$

Intermediate 2 $^{13}$C NMR (100 MHz) CDCl$_3$
Compound 9 $^1$H NMR (400 MHz) DMSO $d_3$

Compound 9 $^{13}$C NMR (100 MHz) DMSO $d_3$
Compound 10 $^1$H NMR (400 MHz) CDCl$_3$

Compound 10 $^{13}$C NMR (100 MHz) CDCl$_3$
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