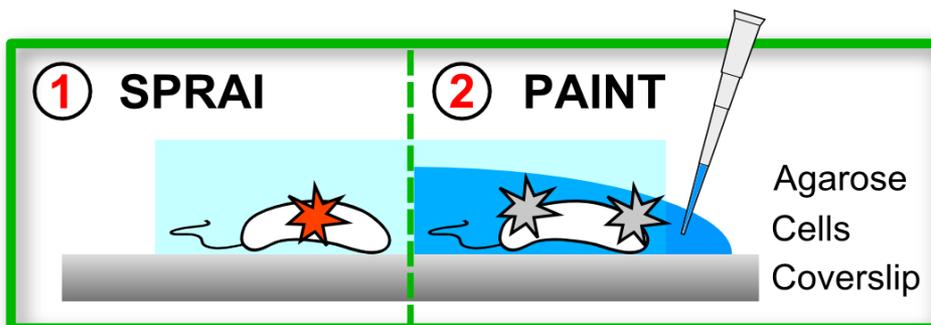
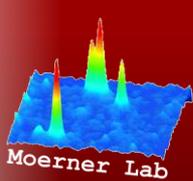


# Three-dimensional superresolution colocalization of intracellular protein superstructures and the cell surface in live *C. crescentus*



## Sequential two-color imaging with a single pumping laser

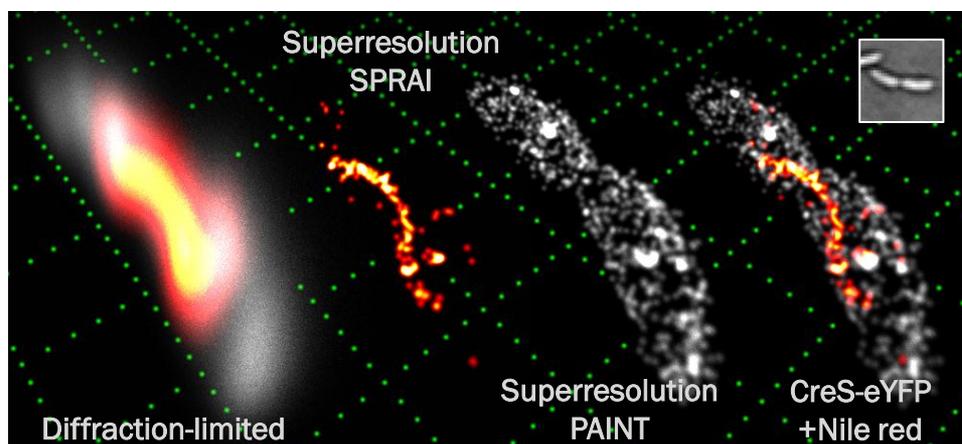
### Step 1: SPRAI

Image **single Crescentin-enhanced YFP (CreS-eYFP) fusions** via **photo-induced blinking** until they irreversibly photobleach

### Step 2: PAINT (Hochstrasser et al., 2006)

Introduce solution containing a PAINT dye (e.g., Nile Red) that **binds transiently** to the cell surface, lighting up when localized near the membrane

Measure 3D location of single molecules using double-helix (DH-PSF) microscope to provide z position



Grid/scale bars = 1  $\mu\text{m}$ , **red/orange: CreS-eYFP SPRAI**, gray: Nile red PAINT,  $\sigma_x = \sigma_y = 19 \text{ nm}$ ,  $\sigma_z = 34 \text{ nm}$