Supplemental Material



Figure S-1: Diagram of the apparatus used to oxidize elemental sulfur in the synthesis of 33 S-SO₄²⁻ from 33 S⁰.



Figure S-2: Isotope and element ratio properties of the five clusters for the killed control sample showing minimal uptake of the ¹³C and ¹⁵N label in the killed control and a subset of ROIs with higher S/CN. All ROIs plot on a reduced scale for ¹³C/¹²C, ¹⁵N/¹⁴N and ³²S/³³S as compared to

the live, labeled experiments. The killed control did not result in the affiliation of isotope phenotypes from clusters 'a' – 'e' with Gammaproteobacteria ('g') or Deltaproteobacteria ('d') as observed in the labeled substrate incubations.



Figure S-3: Isotope and element ratio properties of the five clusters for the unlabeled control sample. All ROIs plot within one standard deviation of the calculated natural abundance value resulting in a reduced scale for ${}^{13}C/{}^{12}C$, ${}^{15}N/{}^{14}N$ and ${}^{32}S/{}^{33}S$ ratios as compared to the live, labeled experiments. The unlabeled control did not result in the affiliation of isotope phenotypes from clusters 'a' – 'e' with Gammaproteobacteria ('g') or Deltaproteobacteria ('d') as observed in the labeled substrate incubations.



Figure S-4: Comparison of FISH and NanoSIMS cluster analysis for multiple images from 2, 7 and 10 days of incubation. FISH experiments (1st and 3rd panels) were performed with probes for

Deltaproteobacteria (purple) and Gammaproteobacteria (green). Clusters 'a', 'b' and 'c' are primarily affiliated with filamentous Gammaproteobacteria. Cluster 'd' is primarily affiliated with clusters of coccoid Gammaproteobacteria. Cluster 'e' is primarily affiliated with Deltaproteobacteria.