# Supplementary Information

Preparation and Noninvasive Imaging of Biogenic Gas Vesicle Nanostructures

Authors: Anupama Lakshmanan#, George J. Lu#, Arash Farhadi#, Suchita P. Nety#, Martin Kunth, Audrey Lee-Gosselin, David Maresca, Raymond W. Bourdeau, Melissa Yin, Judy Yan, Christopher Witte, Dina Malounda, F. Stuart Foster, Leif Schröder and Mikhail G. Shapiro*
#Equal contribution, *Corresponding author (mikhail@caltech.edu)
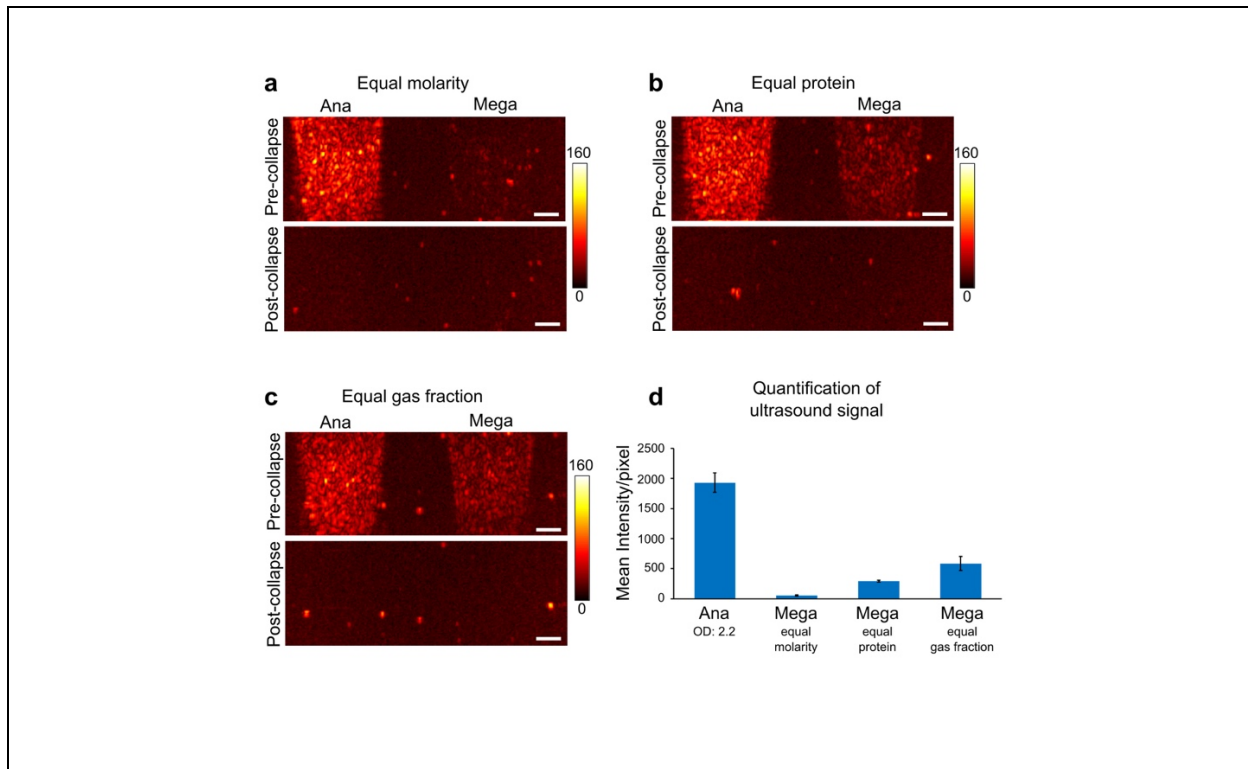
Supplementary Figure 1

Ultrasound imaging of Mega GVs

B-mode ultrasound images of purified, wild-type Ana GVs (OD500,ps : 2.2) versus a purified and unclustered batch of Mega GVs at (a) equal molarity, (b) equal protein concentration and (c) equal gas fraction. Scale bars are 1 mm. Images were acquired using the Verasonics L22-14v transducer and the ray-lines script with the following parameters: transmit frequency: 18MHz, number of cycles of the transmitted pulse: 6, F number: 2, imaging voltage: 3V, with the transducer focus (8 mm depth) aligned close to the center of the sample well. Images were processed and analyzed using MATLAB. Images are shown before (top panel) and after collapse (bottom panel) using a high-power burst from the transducer at 25V for 10 s. (d) Quantification of ultrasound signal was performed by selecting a region of interest (ROI) of defined size within the sample well and calculating the mean intensity per pixel for the selected ROI, after post-collapse background subtraction (n=12 for Ana GVs, n=4 for Mega GVs at each condition shown in a, b and c; error bars are SEM).