

Supplementary Information

The Cocktail Method: Influence of Microbubble Shell Homogeneity on Acoustic Behavior and Stability

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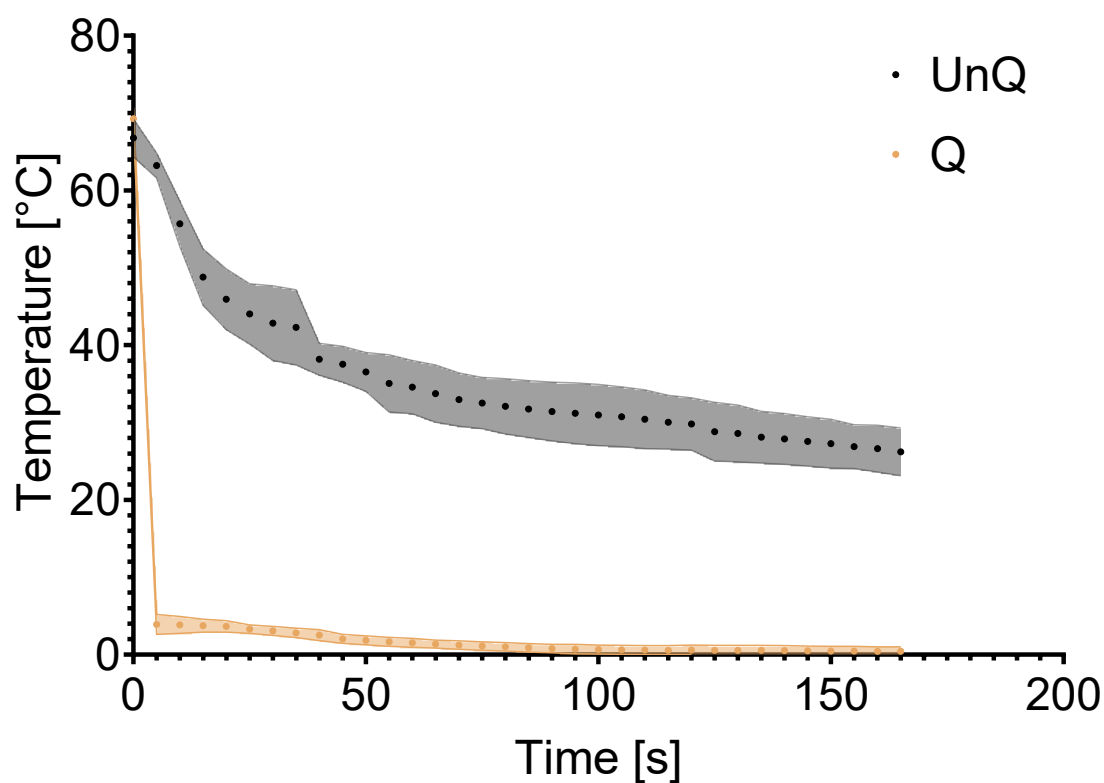


Figure S 1 Temperature gradient of standard cooling procedure (UnQ) versus cooling gradient of the cocktail method (Q).

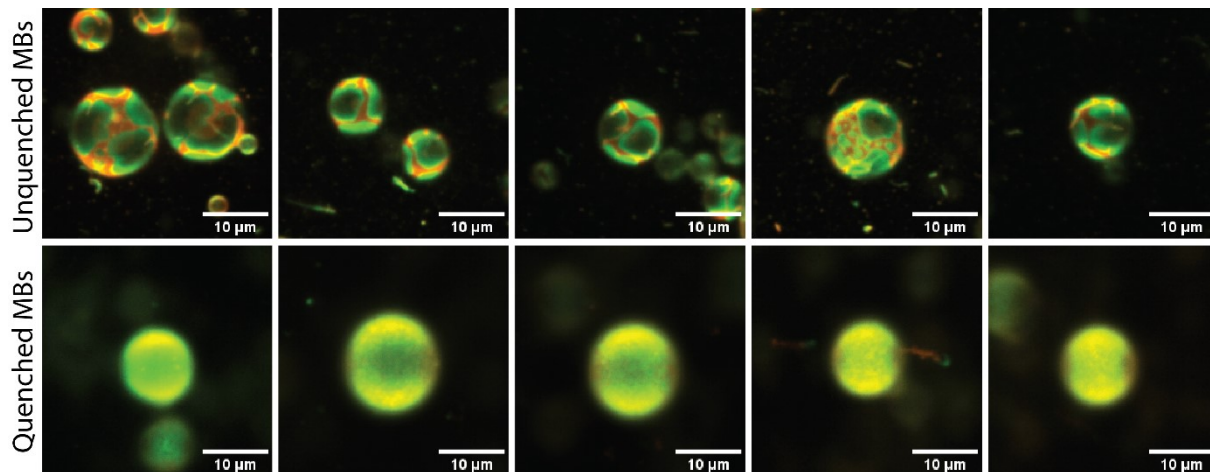


Figure S 2 Representative images of Unquenched and Quenched MBs for co-localization analysis.

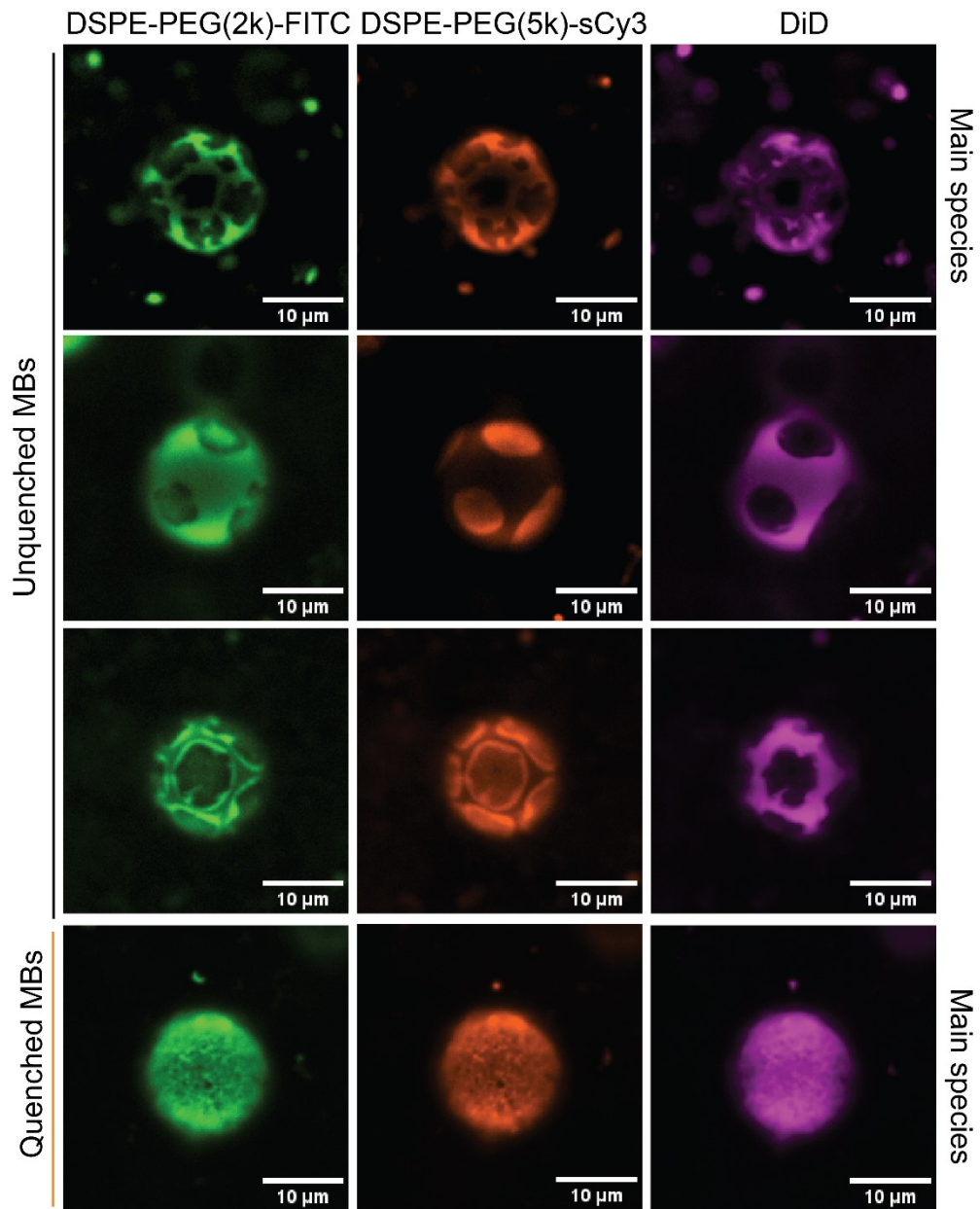


Figure S 3 Multi color co-localization of different emulsifier lipids DSPE-PEG2000-FITC and DSPE-PEG5000-sCy3 and membrane probe DiD. Three different types of lipid unmixing patterns could be detected for Unquenched MBs, while having one preferred species versus a single, seemingly homogenous species for the quenched MBs.

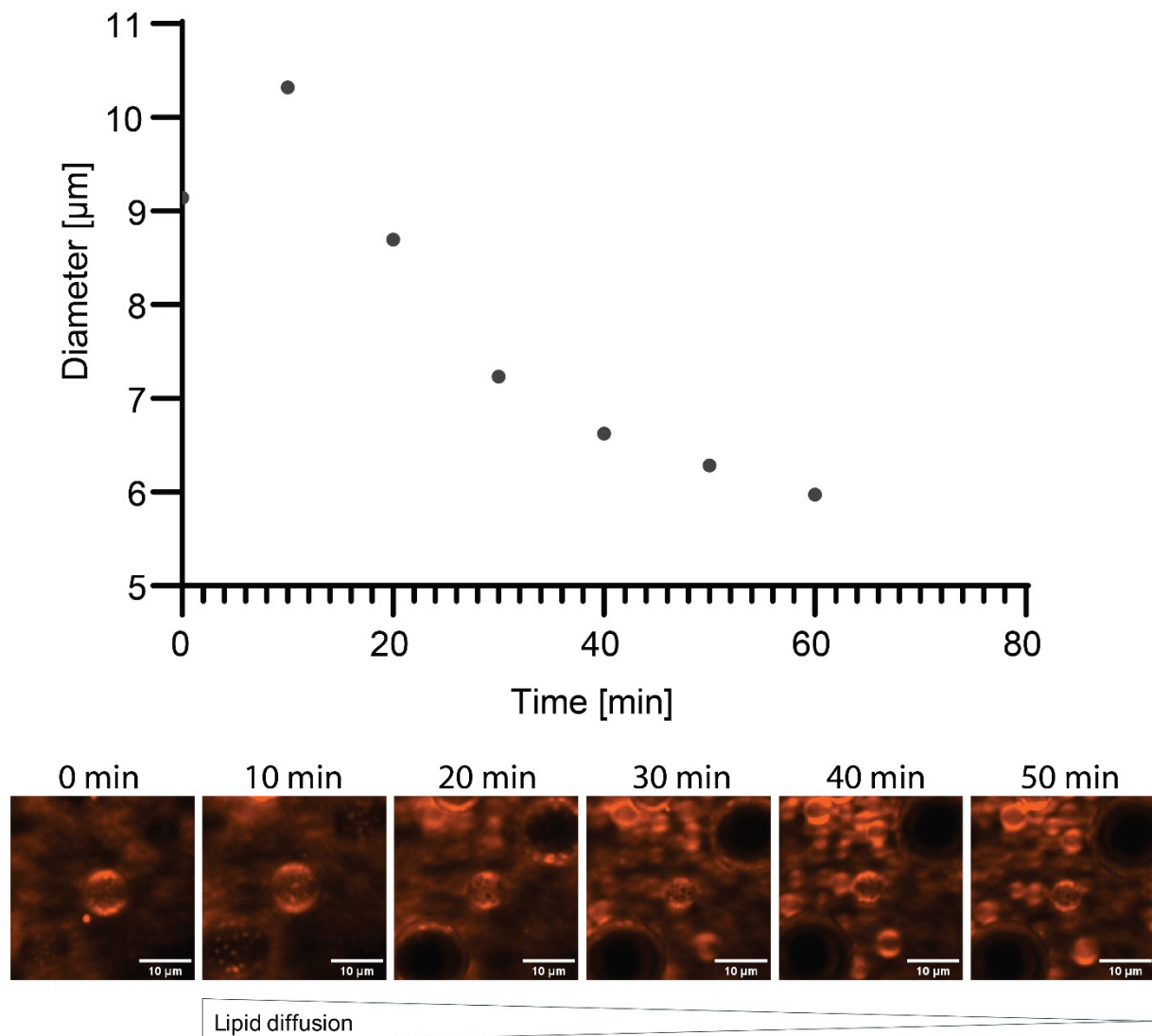


Figure S 4 Tracking of MB radius during stabilization. A single MB was recorded over the period of 60 minutes to follow the correlation between radial decrease and visual decrease of lipid diffusion.

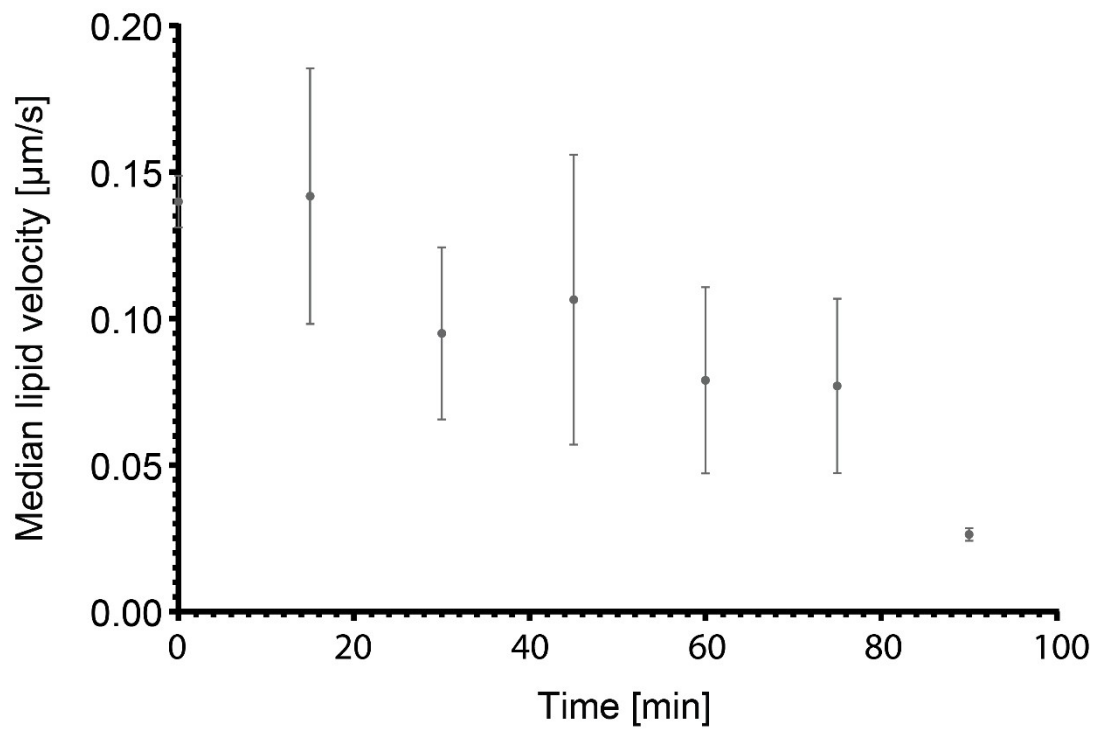


Figure S 5 Tracking of lipid diffusion velocity during MB stabilization. After production of MBs using the cocktail method, every 15 minutes a sample of the batch was taken and the surface structures of multiple MBs were recorded using confocal microscopy. Using a MB detection algorithm and Farnebeck's optical flow algorithm, the diffusion speed of the lipids was determined.

Dil

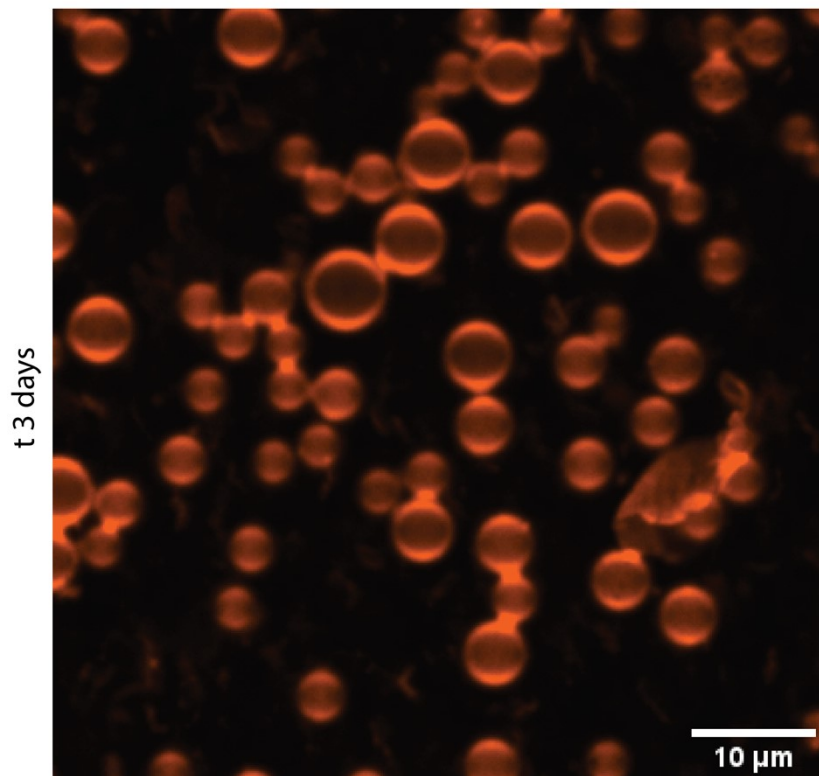


Figure S 6 Confocal image of a quenched microbubble population after 3 days of storage, visualized using the membrane probe Dil.

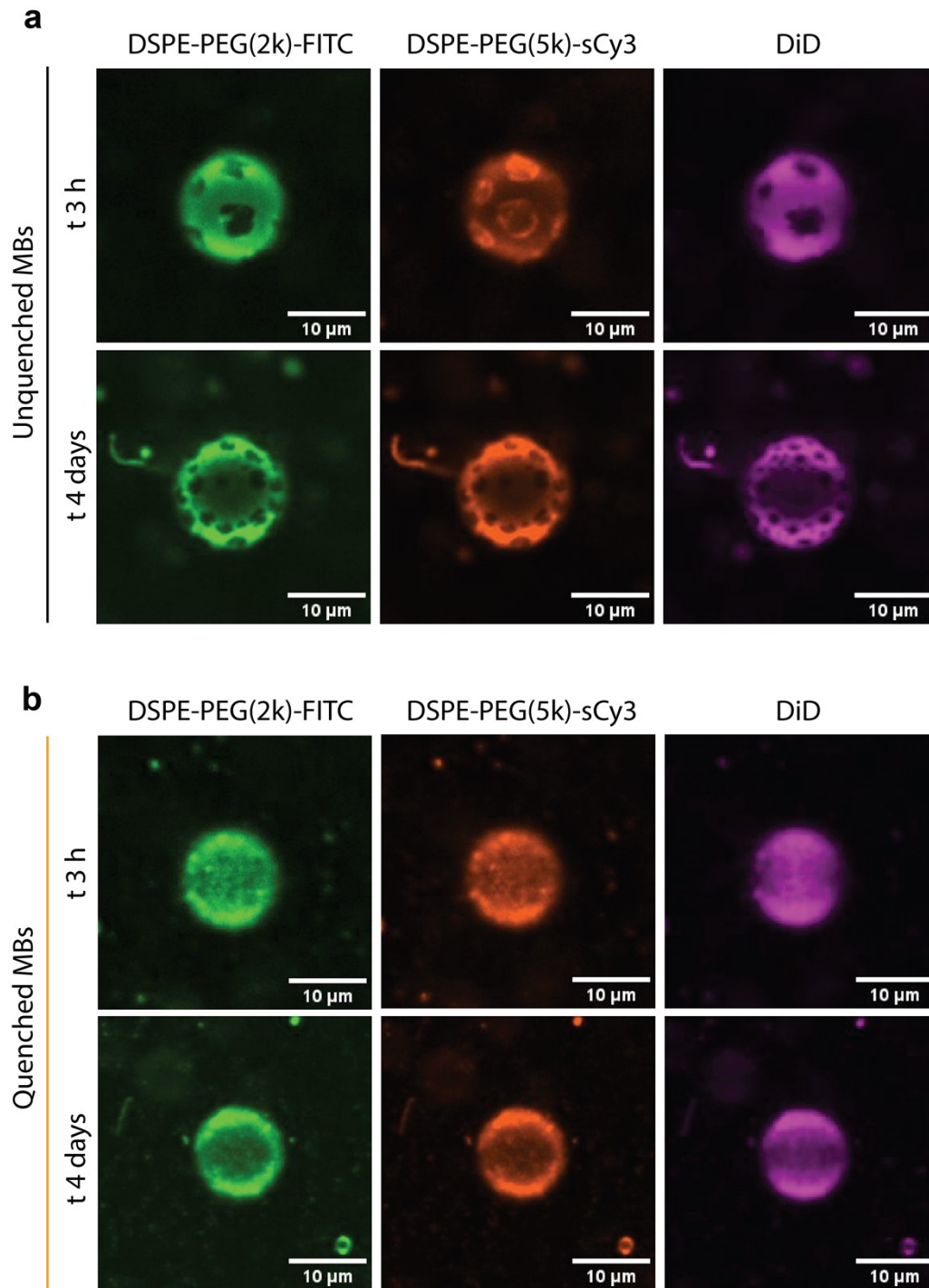


Figure S 7 Representative images comparing quenched and unquenched MB batches at 3 hours versus 4 days post-production. Images were performed using labeled emulsifiers DSPE-PEG(2k)-FITC, DSPE-PEG(5k)-sCy3 and membrane probe DiD (note: these are population-representative, not the identical MBs)

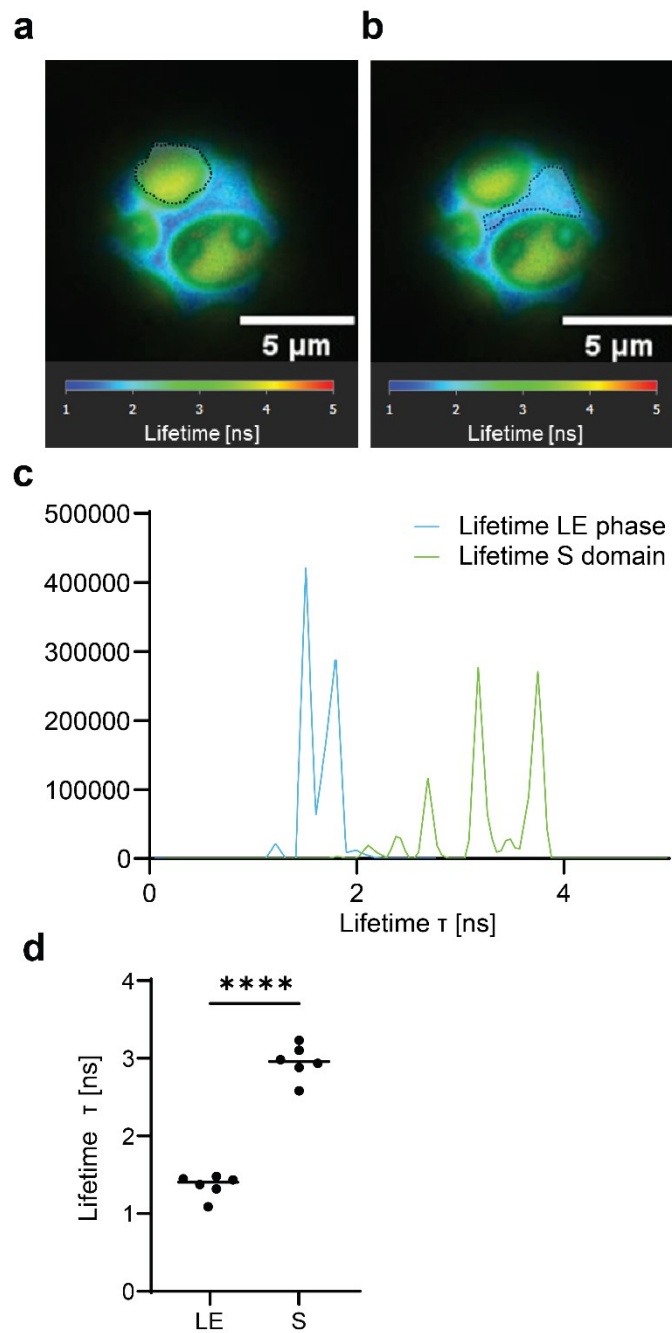


Figure S 8 Lifetime comparison between surface domains. Representative images of defined regions of interest (ROI) for (a) S phase and (b) L_E phase in unquenched MBs. (c) Lifetime Histogram corresponding to defined ROIs in (a) and (b). (d) Mean lifetime of L_E versus S domain.

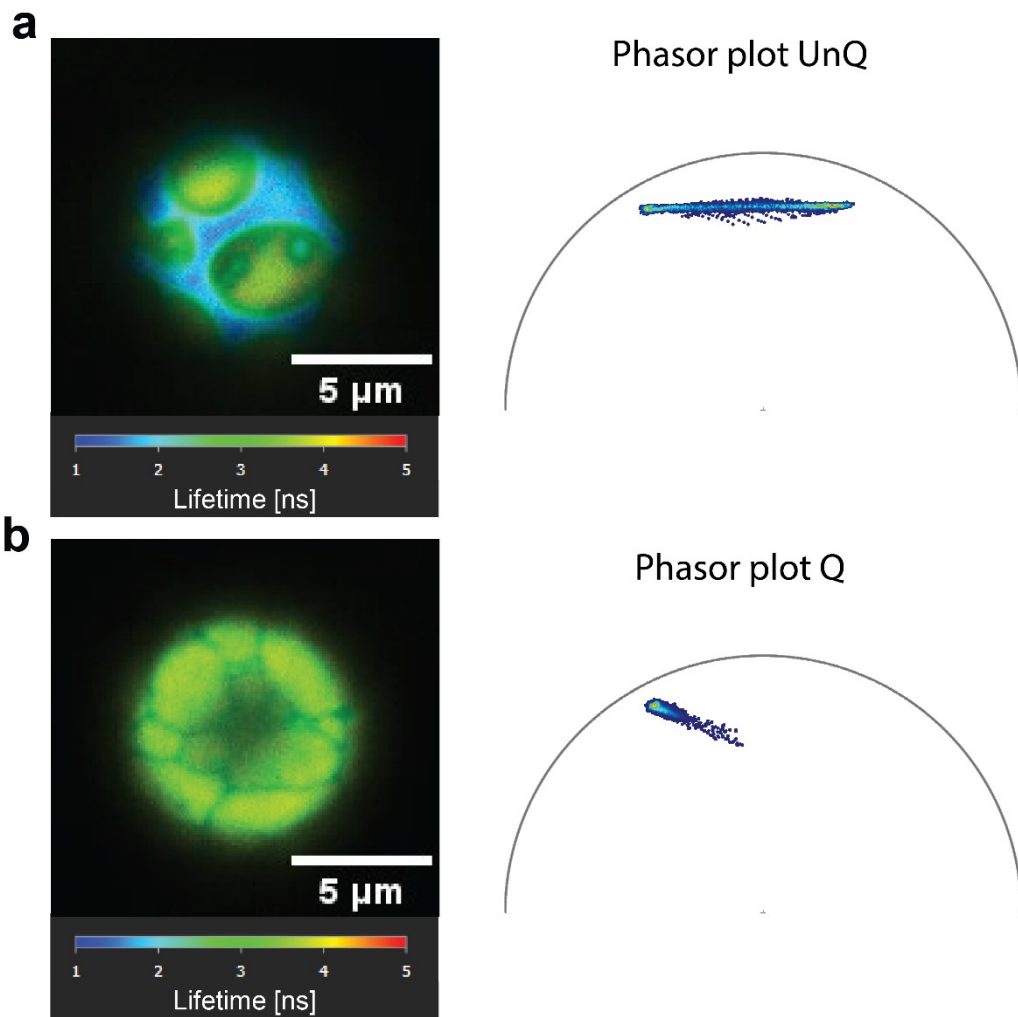


Figure S 9 FLIM phasor plot of an unquenched and quenched MB. Spatial pixels are transformed into a two-dimensional phasor plot based on their respective fluorescence decay profiles. The coordinates within this plot represent the average lifetime and decay multiplicity; longer lifetimes are positioned toward the left, while shorter lifetimes shift to the right. Proximity to the universal semicircle indicates a mono-exponential (uniform) decay transition.

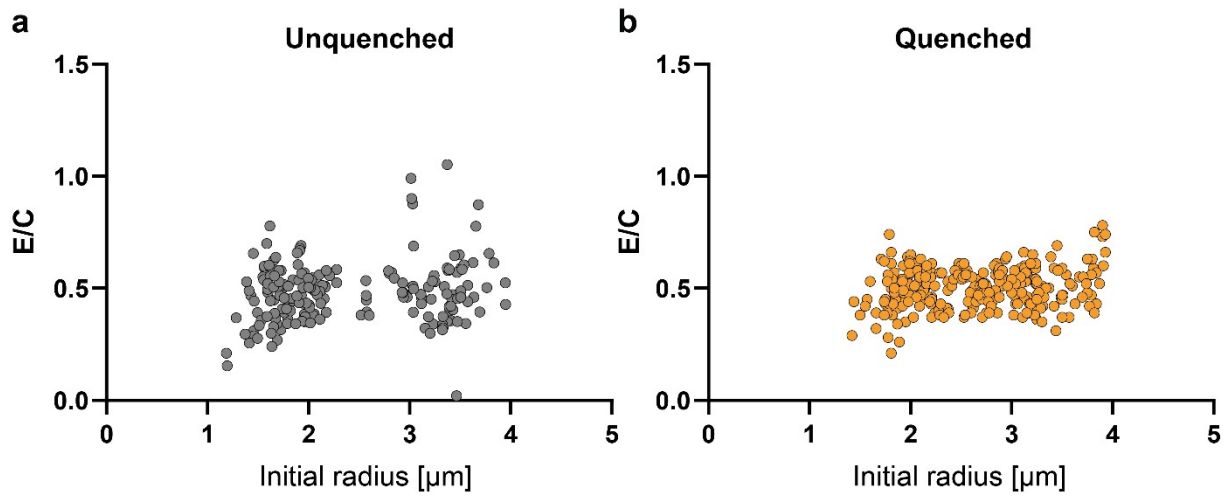


Figure S 10 E/C ratios of Unquenched versus Quenched MBs as a function of their initial radius

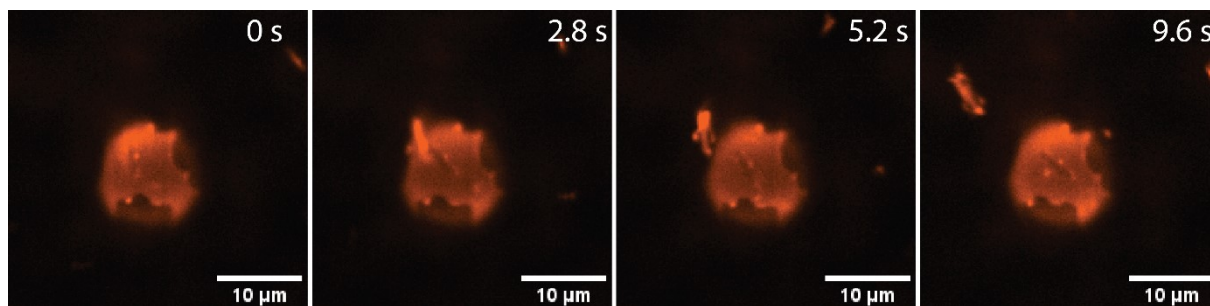


Figure S 11 Time series of lipid loss in LE phase during stabilization process.

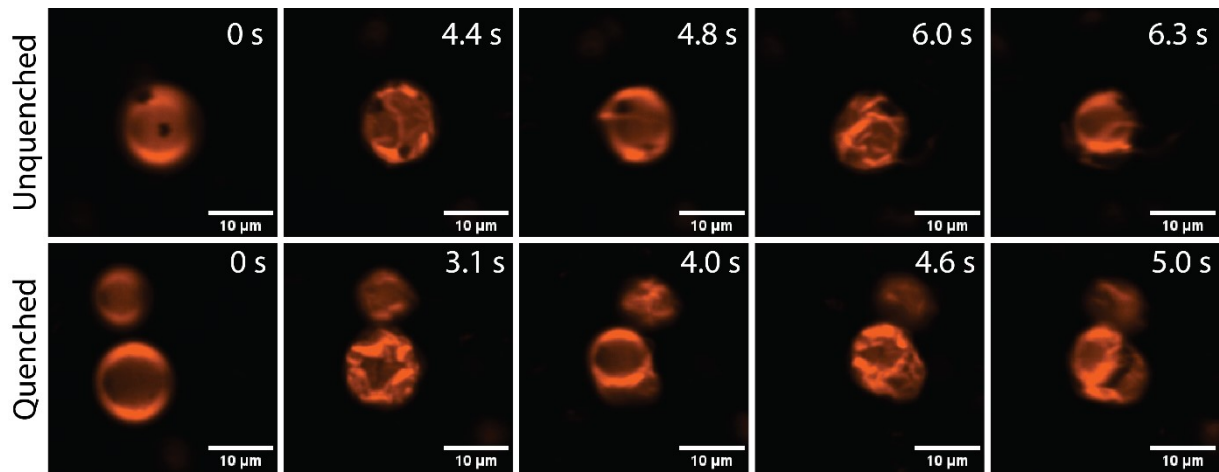


Figure S 12 Compression time series of Unquenched and Quenched MBs. Both MBs show similar wrinkling to smooth transitions, while wrinkling happening mainly in the LE phase.