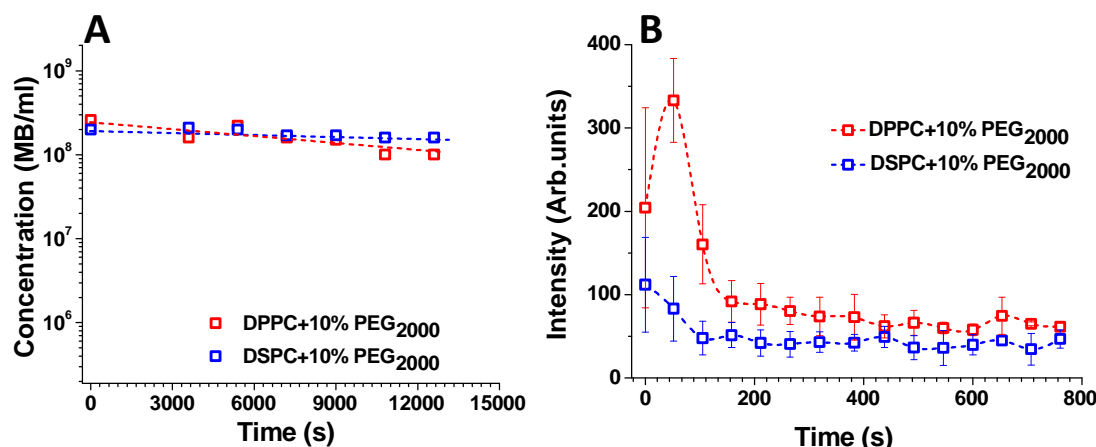


Improving *in vivo* microbubbles lifetime via intercalation of C₆F₁₄ into the lipid shell

Supplementary 1:

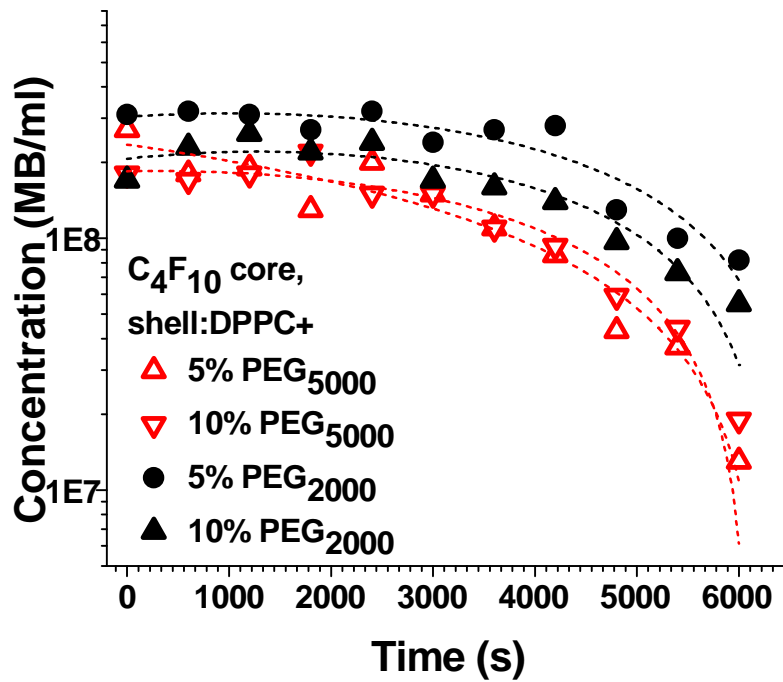


Supp 1: Graphical representation comparing lifetime of MBs. **(A):** *in vitro* MB lifetime for C₃F₈ gas encapsulated MB with different lipids DPPC and DSPC, in the shell at room temperature. **(B):** *in vivo* Time Intensity Curves (TICs) for the same composition MBs, in mice aorta

In vitro, (supp 1A) samples were collected and imaged every 30 min to measure the MB concentration. The presented results show long lived MBs in both cases (>3 hrs) and no significant difference for either of the lipid coated MB samples. *In vivo*, supp 1B. The same samples were injected into mice via the tail and time intensity curves (TICs) obtained in the mouse aorta. Supp1B shows the TIC curves with very short *in vivo* stability (~2min) but detectable difference in signal intensity between the two samples.

These results suggested that the *in vitro* experiment do not simulate the *in vivo* conditions adequately. The *in vivo* TIC curves suggested DPPC based MBs provide stronger contrast than the DSPC based MBs.

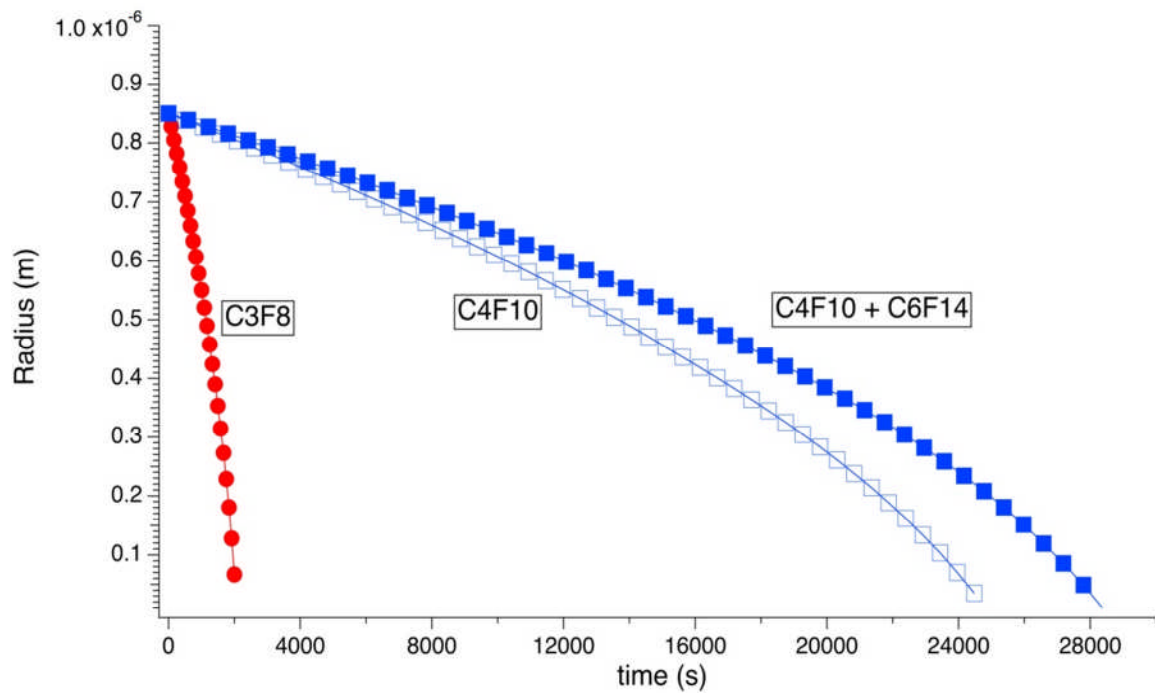
Supplementary 2:



Supp 2: Concentration of MBs with DPPC as the main lipid component and different PEG chain lengths and concentrations. In this case the gas core is fixed to C₄F₁₀.

Supp.2 Effect of changing the PEG concentration and PEG chain length on MB stability. MBs in this case are all encapsulating C₄F₁₀ in the core and have DPPC as the major shell component, with either 5 or 10% of either DSPE-PEG₂₀₀₀ or DSPE-PEG₅₀₀₀. The results here show that the longest lived MBs are those with 5% PEG₂₀₀₀ in the shell.

Supplementary 3:



Supp 3: Modelling MB lifetime after Epstein-Plesset (ref K. Sarkar)

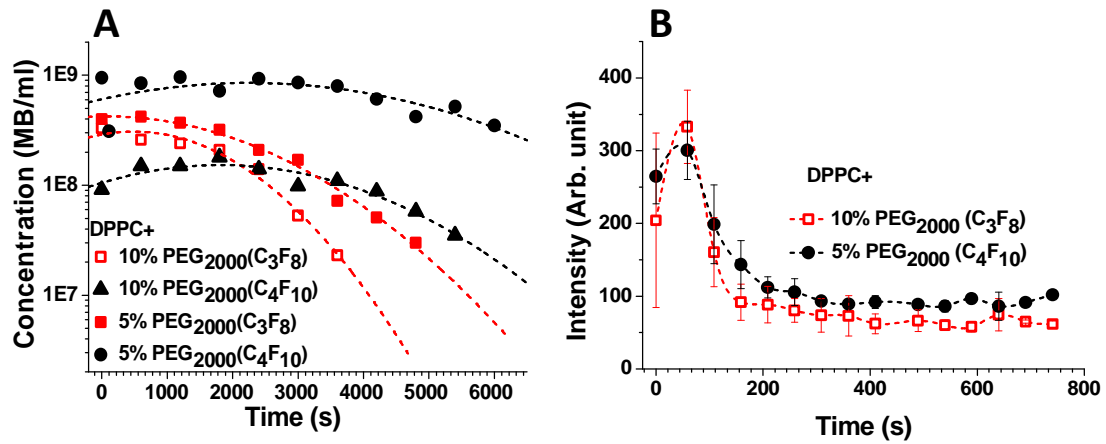
Supp.3 shows modelling of MB size as a factor of changing the gas core, this modelling is calculated Following Sarkar and using these constants for the calculations:

L_g = Ostwald Coeff.: $C_3F_8 = 5.2e-4$; $C_4F_{10} = 2.02e-4$;

K_g = diffusivity of gas in water (m^2s^{-1}): $C_3F_8 = 7.45e-10$; $C_4F_{10} = 6.9e-10$

$h_g = 1/R_{shell}$ = permeability of shell (ms^{-1}): $C_3F_8 = 1.2e-6$; $C_4F_{10} = 2.57e-7$

Supplementary 4:



Supp 4: MBs lifetime with changing gas component and PEG₂₀₀₀ concentration in the shell . (A): *in vitro* MB lifetime in cell media at 37 °C. (B): *in vivo* Time Intensity Curves (TICs) comparing the difference between the shortest and improved lifetime MBs in mice aorta.