Modulating nanobubble acoustic response by altering shell-rigidity

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Background, Motivation and Objective: The nonlinear response of bubbles in the acoustic field depends on bubble size, shell properties, and the frequency and pressure of the driving ultrasound. Numerical simulations using the Church–Hoff or Marmottant models have shown the dependence of nonlinear bubble oscillation on shell properties such as surface tension, viscosity, thickness, and the shear modulus of the encapsulating shell. (Sojahrood, *Nonlinear Dyn*, 2015) However, experimental evidence demonstrating these effects is limited, especially for nanobubbles (NB). Our objective is to show the dependence of the onset of detectable nonlinear NB response (~200 nm diameter) on bubble shell rigidity and driving ultrasound pressure.

Statement of Contribution/ Methods: Lipid solution with either glycerol (membrane stiffener) or propylene glycol (edge-activator) were used to prepare stiff and flexible NBs, respectively. (Abenojar, *Langmuir*, 2019) NBs with low polydispersity were prepared via mechanical agitation in the presence of C_3F_8, isolation by centrifugation, and filtration through a 400-nm membrane. Size and concentration of filtered NBs were measured by resonant mass measurement (Archimedes®, Malvern Panalytical). The lipid order and packing of the shell were confirmed by measuring the generalized polarization (GP) of C-Laurdan, a fluorescent probe used to sense lipid packing, from ~20 µm bubble images taken using a two-photon confocal microscope (Leica TCS SP2) (Owen, *Nature Protocols*, 2012). The shell- and pressure-dependence of the onset of detectable nonlinear response of NB solutions in PBS were determined using ultrasound in contrast harmonic mode (Toshiba Aplio XG, 12 MHz) at pressures between 74 kPa and 857 kPa (MI = 0.03 to 0.35).

Results/Discussion: Filtered NB solutions had a mean diameter of 200 ± 47 nm and a concentration of 8.2E10 ± 3.9E7 NB/mL. Analysis of the GP confirmed values of -0.003 for flexible and 0.208 for stiff bubbles. Ultrasound images of stiff NB solutions showed a minimal 6% signal increase for pressure increasing from 343 kPa to 465 kPa and a considerable 146% increase from 465 kPa to 710 kPa. Likewise, flexible NB showed a similar trend, but onset of detectable nonlinear response starts at lower pressures (123 kPa to 245 kPa). Selective 'activation' of bubbles using this strategy could enable an additional dimension for multiplexed imaging or pressure sensing applications.

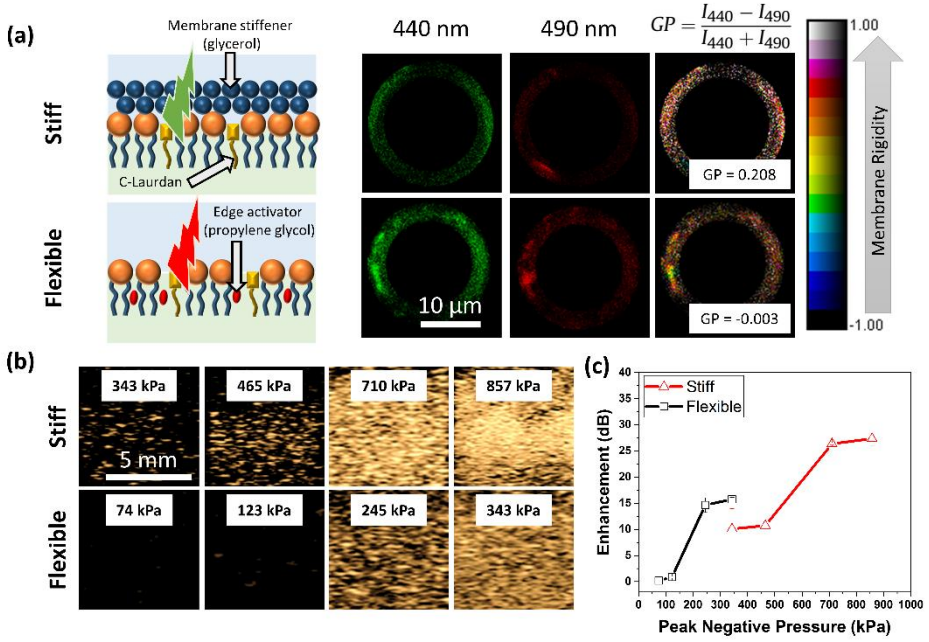

Figure 1. (a) Shell architecture showing predicted position of glycerol, propylene glycol, and the fluorescent dye (C-Laurdan) are shown along with the fluorescent images of bubbles at 440 and 490 nm and the corresponding GP images. (b) Representative ultrasound contrast images and corresponding enhancement (c) of NBs with stiff and flexible shells showing a significant and rapid increase in signal as the driving pressure is increased. Only the pressures adjacent to the largest signal increase are shown.