1 **Supporting Information**

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3 **Magnetic resonance detection of gas microbubbles via hyperCEST:**
4 **a path toward a dual modality contrast agent**

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Characterizing the T_1 relaxation of ^{129}Xe in PBS

qHyperCEST fits Z-spectra according to 6 parameters, including the longitudinal relaxation times of Pool A, T_1^A . Upper and lower bounds are required as inputs for each parameter. In order to avoid ending up in a local minimum, T_1^A was measured directly to provide accurate initial guesses. For the measurement, after bubbling xenon gas in the solution for a total of 10 s, a train of 20° RF pulses, spaced 2 s apart, and each followed by signal acquisition, was applied. The normalized signal intensity as function of the RF excitation number k was then fitted by using the following equation:

$$S_k = S_1 \left(\cos \theta e^{-TR/T_1} \right)^{k-1} \text{ with } k = 1, 2, \dots, N$$

where S_1 represent the signal intensity after the first excitation pulse, $\theta = 20^\circ$, and $TR = 2\text{ s}$.

Supporting Information Figure 1: Normalized signal intensity of ^{129}Xe in PBS after each 20° excitation pulse. The fitted T_1 was 160 ± 10 s, in close agreement with the qHyperCEST fitted value of $T_1^A = 140 \pm 60$ s.

Non-steady state of magnetization at the start of Z-spectrum acquisition

In order for ^{129}Xe to become fully incorporated into solution as well as into the MB themselves¹⁻³, the bubbling procedure was started before the acquisition of the first z-spectrum data point. This resulted in an oversaturation of the dissolved-phase magnetization that was easily observed on the first three z-spectra data points (Supporting Information Figure 2), which were purposely acquired using a saturation pulse far away from the dissolved-phase and gas-phase frequency, and discarded during the post processing procedure.

Supporting Information Figure 2: Examples of full Z-spectra including initial points. Data points for each Z-spectrum were collected sequentially from right to left as shown in these examples. A steady state magnetization was easily reached after the acquisition of the first three data points of each z-spectrum, which were discarded from the analysis.

Magnetic field maps of susceptibility-induced frequency shifts

A common feature of all of our NMR spectra was a peak at -192 ppm, which corresponded to gas-phase ^{129}Xe in the PEEK tube used to transfer fresh HP ^{129}Xe gas to the suspension. The Z-spectra indicated that the MB ^{129}Xe peak was at -194.6 ppm, 2.6 ppm upfield from the observable gas-phase peak. Occasionally, a large bubble of ^{129}Xe from the ventilator would get caught in the sensitive region of the spectrometer, and in those instances, an additional peak appeared in the spectrum at -194.6 ppm (Supporting Information Figure 3). In these cases, the spectra were disregarded.

Supporting Information Figure 3: Spectrum of a MB suspension with a large gas bubble trapped in the sensitive region. After the bubbling procedure, a distinct gas-phase ^{129}Xe peak at -194.6 ppm was observed whenever a mm-sized bubble was stuck in the sensitive region of the spectrometer. It is important to note that, even if this signal comes from a much large spherical enclosure, according to magnetostatics, the susceptibility-induced frequency shift felt by the spins in the large bubbles should be the same as the one felt inside the much smaller MB.

In order to confirm that the observed shift was purely due to susceptibility-induced frequency shift, magnetic field maps were simulated using finite element analysis computations (COMSOL Multiphysics, Stockholm, Sweden). Maxwell's equations were solved for a gas-filled vertical cylinder (Figure 4A) and sphere (Figure 4B) submerged in water. The respective susceptibilities of the compartments were considered, and the sphere of Lorentz correction was applied to calculate the local magnetic flux density and the corresponding susceptibility-induced frequency shift⁴. These simulations

confirmed a +2.8 ppm susceptibility induced frequency shift for the gas inside the vertical cylinder compared to that in the sphere. Because these field perturbations are independent of the compartment size, the same susceptibility induced frequency shift is expected for small and large microbubbles, including the mm-sized bubbles trapped in the sensitive region of the spectrometer.

Supporting Information Figure 4: Expected susceptibility-induced frequency shift for a long vertical cylinder and for a sphere. Simulations of the frequency shift expected for an infinite vertical cylinder (A) and a sphere (B) immersed in a more diamagnetic medium. In these simulations, the interior of each geometry is assumed to contain a 75/25 Xe/N₂ mixture ($\chi = -1.904\text{e-}8$), while the exterior is assumed to contain water ($\chi = -9.035\text{e-}6$). According to magnetostatic, the local magnetic flux density felt inside the long cylinder should be about 2.8 ppm greater than the one felt inside the spherical enclosure, regardless of their dimensions. As a result, two separate gas-phase peaks should be expected in the xenon spectrum: one downfield corresponding to ¹²⁹Xe inside the long cylindrical PEEK tube, and one upfield corresponding to ¹²⁹Xe inside the spherical MB.

MB distributions

MB distributions can be either monodisperse or polydisperse, and they are typically specified by their average diameter and number concentration. The MB size distribution for the samples used in our study is shown in the Supporting Information Figure 5.

Supporting Information Figure 5: MB size distributions. Size distributions for small (A), medium (B), and large (C) MB are shown. MB samples were prepared in-house for small ($0.9 \pm 0.4 \mu\text{m}$) and medium ($2 \pm 1 \mu\text{m}$) MB. MB size and concentration were determined via single particle optical sizing (Accusizer 780AD, Particle Sizing Systems, Port Richey, FL, USA). Large ($3.2 \pm 0.7 \mu\text{m}$) MB were purchased from Advanced Microbubbles Inc (Newark, CA, USA).

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