Time-dependent size of BLInCs

A Zetasizer Nano ZS system (Malvern Instruments Ltd.) was used to measure the diameters of a 1% v/v solution of as-prepared BLInCs, at 15 measurements per run. The measurement was repeated at various time points up to ~6 hours. An intensity-weighted distribution is reported (Fig. S1).

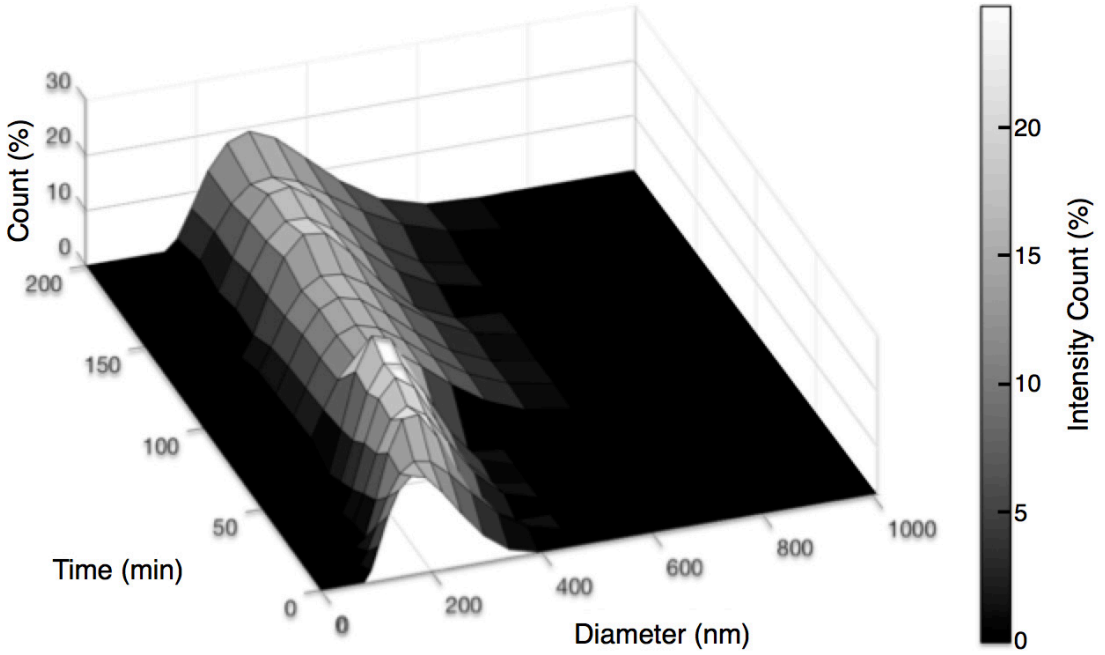

Figure S1
Distribution of diameters of BLInCs as a function of time.

Formation of BLInCs Signal

A vector of linear US signal data as a function of time for a single pixel—vector A—has length 1,000. Each number in vector A is a value of US intensity for that moment in time. Two examples of vector A are shown below.

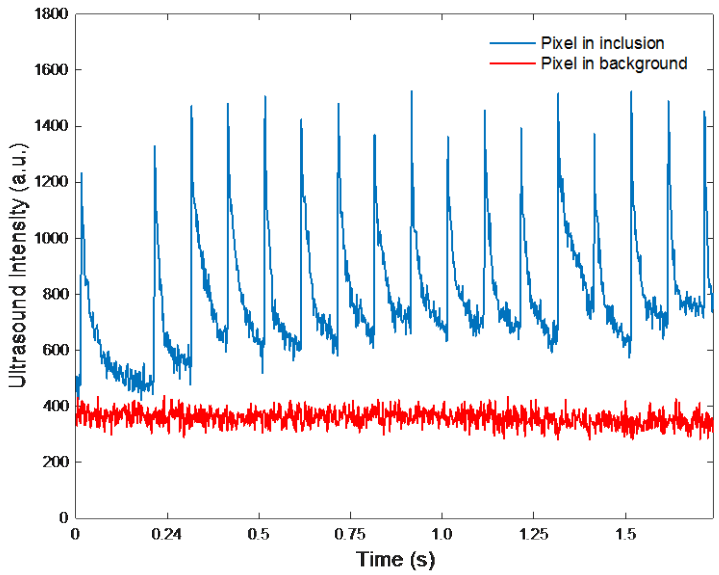

Figure S2
Linear ultrasound signal of a pixel within an inclusion of BLInCs and of a pixel in the background, over 1000 frames, collected at 580 frames per second.

1. For each pixel, calculate a differential, A_diff, which is a vector of 999 frames.

$$A\_diff[1] = A[2] - A[1],\ A\_diff[2] = A[3] - A[2],\ A\_diff[3] = A[4] - A[3],\ \ldots$$

An full image of differential pixels at a given time point is shown below (Fig. S3a).The complete vectors A_diff for a blinking pixel and for a non-blinking pixel are shown below (Fig. S3).

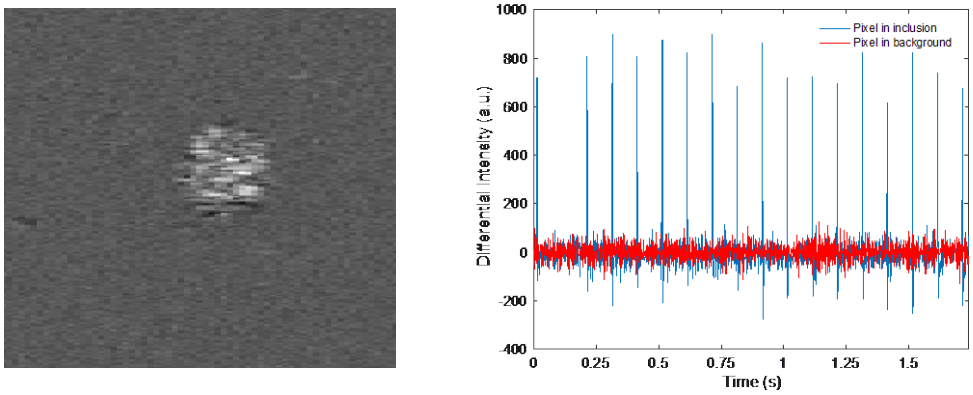

Figure S3
Image formed by subtracting subsequent ultrasound image frames at the time of BLInCs activationDifferential ultrasound signal as a function of frame for a pixel within an inclusion of BLInCs and of a pixel in the background.

2. For the values of A_diff that exhibit the peak values (circled below), calculate the average value of A_diff. This is called ‘mean_peaks’, and is a scalar value for each pixel. The values of mean_peaks for blinking pixels were ~300, and ~30-40 for non-blinking pixels.

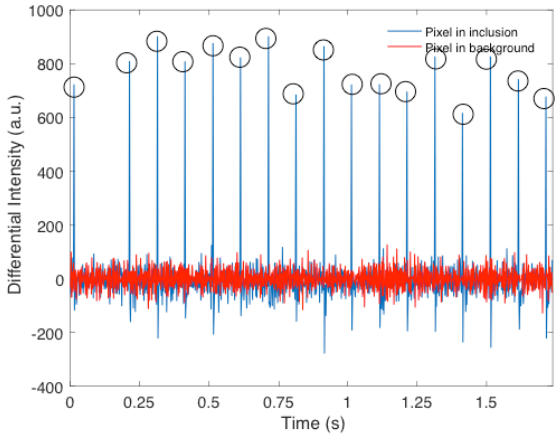

Figure S4
Differential ultrasound signal as a function of frame for a pixel within an inclusion of BLInCs and of a pixel in the background. The values of the signal averaged to obtain ‘mean_peaks’ are circled.

3. Calculate the autocorellation of A_diff, called A_corr. Because A_diff is 999 in length, then the length of A_corr is 1997, or (2*n – 1).

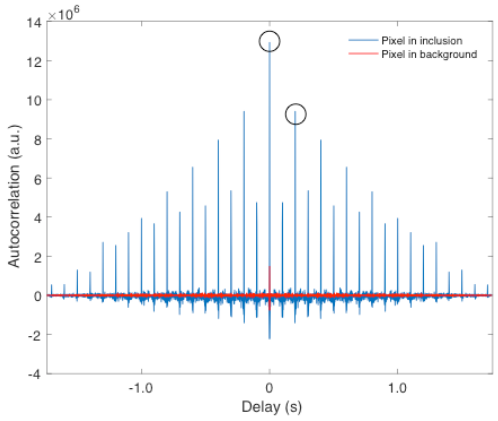

Figure S5
Autocorrelation of the differential ultrasound signal for a pixel within an inclusion of BLInCs and of a pixel in the background. The values used to calculate 'blinking_ratio' are circled.

4. The autocorrelation of a blinking pixel contains peaks (to the side) outside of the highest, central peak. The A_corr of a non-blinking pixel only contains this central peak, and no additional peaks. The next step is to calculate blinking_ratio, which is the value of A_corr[1028] / A_corr[999] (circled in above figure). This blinking_ratio, a scalar value, will be much higher for a blinking pixel than for a non-blinking pixel. For blinking pixels, the ratio values are ~0.5; for a non-blinking pixel, these ratios are ~0.05.

5. For each pixel, multiply the value of blinking_ratio by the value of mean_peaks. This scalar value is much larger for a blinking pixel than a non-blinking pixel. This value, called BLInCs_signal, is shown in Fig. 6e.

The multiplicands to achieve the final BLInCs signal are 'mean_peaks' and 'blinking_ratio' (both not shown). The product of the two, BLInCs_signal, is shown in Fig. 6e, and also overlaid onto a B-mode image in Fig. 6g.

Imaging technique with no BLInCs injection

Obtaining a 'ground truth' experiment is of major concern. Although the phantom experiments should help to validate that the signal originates only from areas where there are BLInCs, this is more difficult to know certainly in *in vivo* applications. In addition to the lymph node imaging following an injection of BLInCs (Fig. S8a), two other organs were imaged without an injection of BLInCs: a mouse lymph node (Fig. S8b) and stomach (Fig S8c). In these experiments, no BLInCs signal was detected above noise levels, except for surface artifacts from oscillating bubbles. This gives us more confidence that the signal is originating from the BLInCs.

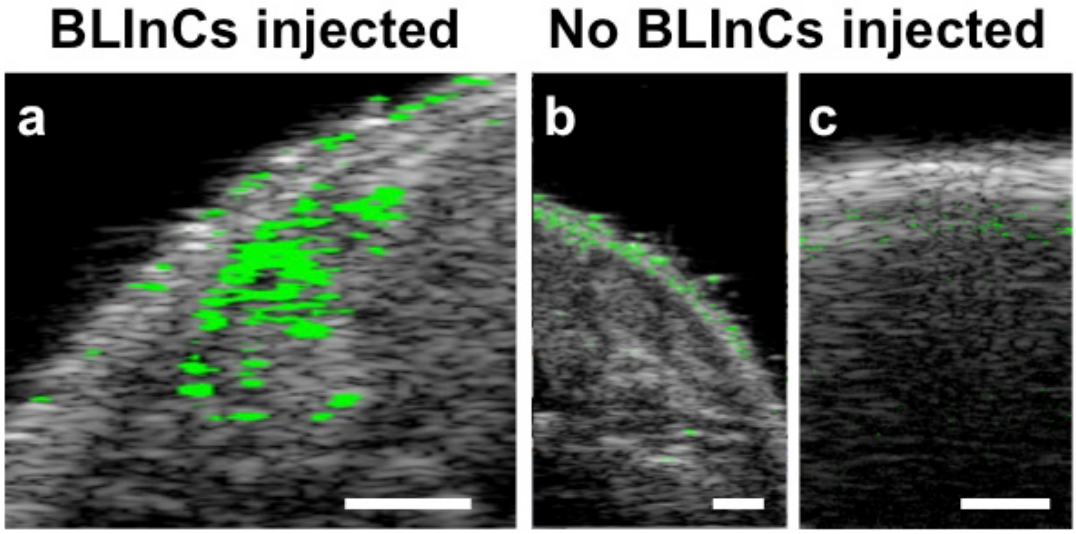

Figure S6
(a) Signal of particles overlaid onto a B-mode image of the lymph node 30 minutes following an injection of BLInCs. (b) Signal of BLInCs overlaid onto a B-mode image of the lymph node when no injection was performed. (c) Signal of BLInCs overlaid onto a B-mode image of a mouse stomach when no injection was performed. Scale bars = 1 mm.