Supplementry Information

Sonophoric Nanoprobe aided pH Measurement *in vivo* using Photoacoustic Spectroscopy

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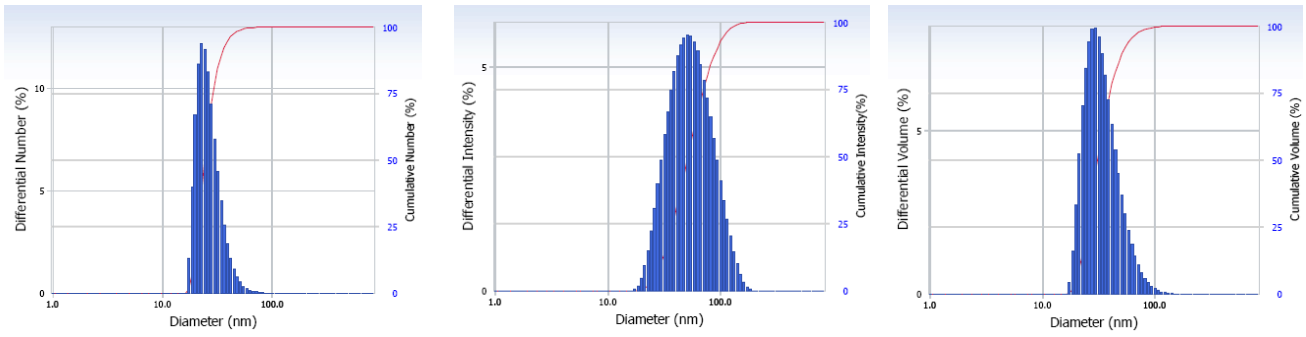
Characterization of the nanoprobes:

1. Size and Zeta Potential measurement

The nanoprobes were suspended in water at a concentration of 1mg/ml and the particle size distribution in aqueous solution and the Zeta potential were measured by dynamic light scattering (DLS), using a Beckman-Coulter DelsaNano C Zeta potential/submicron size analyzer.

The DLS measurement for size distribution is expressed by intensity, volumer and number. For the nanoprobes the size was recorded as 28±8nm, 62±28nm and 37±14nm when expressed as number, intensity, volume distribution respectively. Thus the average hydrodynamic size was recorded as 53nm with a polydispersity index of 0.19, which shows good monodispersity of the nanoprobes in solution.



(a) (b) (c)

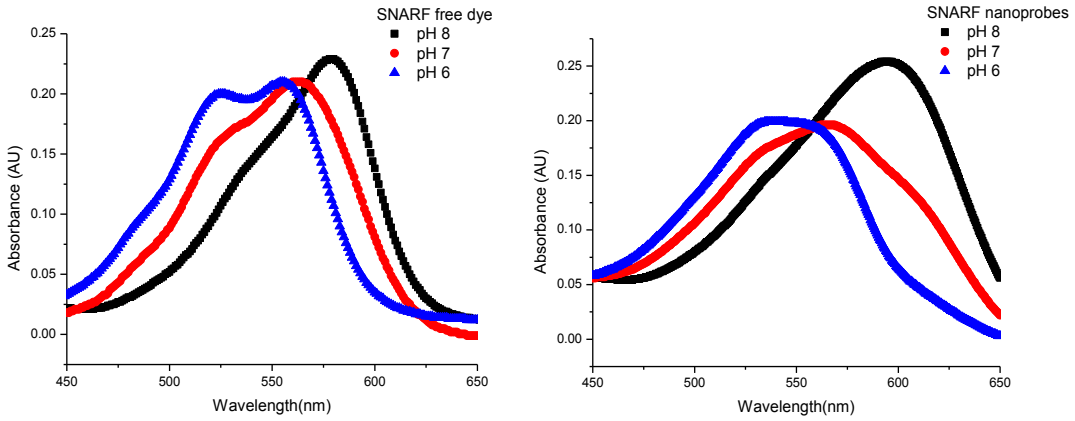
Figure S1. The size distribution of the nanoparticle when suspended in water is shown according the (a) Number distribution, (b) Intensity distribution and (c) Volume distribution.

2. Dye leaching

The nanoprobes presented here consists of the SNARF dye physically entrapped within the relatively hydrophbic matrix, without any chemical conjugation. Thus there may be a tendency of the dye to leach out of the nanoprobes. In order to check for any dye leaching the nanoprobe were suspended in water at a concentration of 2mg/ml and stirred in an Amicon ultra filtration cell for 48 hours, at constant volume. Occasionally the filtrate was collected and the concentration of the dye was determined by comparing the absorbance of the filtrate with the absorbance of a known concentration of dye. The absorption studies were done using a Shimadzu UV-VIS spectrometer. We do not observe any signifiant dye leaching from the nanoppobes.

3. Comparison between free dye and nanoprobes

The acid-base properties of the dye are always likely to undergo a minor change as a result of encapsulation inside the polymer matrix. Thus calibration is not based on the free dye, only on the dye encapsulating nanoprobe; each batch of nanoprobes is first calibrated, using buffers of known pH, before using it for the biological application. The changes in the optical properties of the SNARF free dye and nanoprobes were determined by comparing their absorption spectra. The free dye and nanoprobes were each suspended in a series of pH buffers and their absorption spectra were measured using a spectrophotometer. The following figure shows the spectrum of the free dye and the nanoprobes at pH 6, 7 and 8. We observe a slight red shift in the absorption spectrum for the nanoprobes and also a slight reduction in the pH sensitivity as compared to free dye. The extinction coefficient for SNARF dye is 53×10^3 $cm^{-1}M^{-1}$. The extinction coefficient of the dye in the free form and encapsulated form are the same.



(a) (b)

Figure S2. The pH dependent absorption spectra of the (a) free dye and (b) dye encapsulated nanoprobes. The measurements were performed by using equal amounts of free dye and nanoprobe encapsulated dye, suspended in various pH buffers. We observe a slightly higher extinction coefficient for the dye encapsulated nanoprobes at lower wavelengths, due to scattering from the nanoprobes.