## Supplementary Information for "Photoactivatable fluorescent probes reveal heterogeneous nanoparticle permeation through biological gels at multiple scales"



Benjamin S. Schuster, Daniel B. Allan, Joshua C. Kays, Justin Hanes, and Robert L. Leheny

**Fig. S1.** Effect of particle diameter and surface chemistry on nanoparticle diffusion in cystic fibrosis sputum, as measured by multiple particle tracking (MPT). 100 and 500 nm PEG-coated polystyrene particles (PS-PEG) are compared with 100 nm carboxylated PS (PS-COOH). The MSDs of individual particle trajectories from MPT in one patient sample are plotted. The enhanced mobility of the 100 nm PS-PEG particles vs. 100 nm PS-COOH and 500 nm PS-PEG aligns with previous results [1–3] and justifies our present focus on small, PEG-coated particles.



**Fig. S2.** Gallery of PANDA experiments to assess nanoparticle transport in sputum from four cystic fibrosis patients. For each patient, three PANDA trials (from three distinct locations of the sputum sample) are presented. The images shown are all from 10 minutes after photoactivation, illustrating substantial spread of particles from the initial activation region for patients 1, 2, and 3. In patient 4, little particle diffusion is seen in any of the locations. The white scale bar, shown for Patient 1 - Location 1, applies for all 12 trials and represents 25  $\mu$ m.



**Fig. S3.** This plot revisits the video shown in Fig. 5 (which was Patient 3, Location 1) to show the percentage of particles that have diffused at least 25  $\mu$ m from the initial activation region as a function of time. For comparison, the data for Patient 2, Location 2 from Fig. 4G is replotted here. Percent traversed was calculated as explained in the main text. The data is only shown until approximately 10 minutes because a 10-minute video was collected for Patient 3, Location 1.

Particle batch	Diameter (Z-Average, nm)	PDI	ζ-potential (mV)
1	156	0.09	-6
2	156	0.07	-3
3	168	0.11	-3
4	170	0.09	-3
5	162	0.11	-5
6	164	0.08	-3
average	162	0.09	-3
standard deviation	5	0.01	1

Table S1. Size and ζ-potential of photoactivatable PLGA-PEG nanoparticles.\*

\*Size (hydrodynamic diameter) and PDI were measured by dynamic light scattering (DLS), and  $\zeta$ -potential by laser Doppler electrophoresis, using a Malvern Zetasizer Nano ZS90. Data for each batch of nanoparticles represents the mean of *n* = 3 measurements. Measurements were taken on particles suspended in 10 mM NaCl at pH 7.4. The average diameter measured by DLS was approximately 50% larger than that measured by transmission electron microscopy (TEM) (Fig. 1A); this discrepancy has been reported previously and is due to several factors, including dehydration of the particles during sample preparation for TEM [4,5].

## References

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