## **Supplementary information**

## POZylation; a new approach to enhance nanoparticle diffusion through mucosal barriers

Edward D.H. Mansfield<sup>a</sup>, Katy Sillence<sup>b</sup>, Patrick Hole<sup>b</sup>, Adrian C. Williams<sup>a</sup>,

and Vitaliy V. Khutoryanskiy a\*

- a) School of Pharmacy, University of Reading, PO Box 224, Whiteknights, Reading, Berkshire, RG6 6AD
- b) Malvern Instruments Limited, London Road, Amesbury, Wiltshire, SP4 7RT



Fig 1s. Fluorescence spectra for a 1% w/v mucus suspension (orange), compared to 1% (blue), 5% (red), and 25% (purple) BODIPY C5 TMR maleimide labelled silica nanoparticles. Based on this, 5% coverage was chosen as it is detectable above the background fluorescence of the mucus.



Fig 2s. Viscosity of a 1% w/v mucin dispersion as a function of temperature. The equation for the trend line was used to determine the viscosity of mucin at 25 and 37  $^{0}$ C respectively.



Fig 3s. Exemplar description of ImageJ protocol. A and b) show subtraction of the background (process>subtract background). C and d) show how the scale was set. A line was drawn to the same length as the scale bar determined from the Leica software, followed by analysis>set scale. The units were changed to match that pf the scale bar (default inches was changed to mm). E, f, and g) show the analysis. A line was drawn (1.5 mm in length) crossing the mucus barrier, followed by analyse>plot profile (ctrl+k). The determined plot (shown in g) was then saved into excel format. This was repeated for a further 4 lines per image, at random locations along the mucus barrier. In excel, each plot profile was assessed individually, and the size of the initial peak measured was measured by determine the start point, and end point along the x-axis, and subtracting the two values. This was repeated for each profile, for each image.



Fig 4s. Size distributions for unfunctionalised thiolated (blue), PEGylated (red), and POZylated (green) silica nanoparticles as determined by Nanoparticle Tracking Analysis.



Fig 5s. TGA thermograms of thiolated, PEGylated and POZylated nanoparticles



Fig 6s. Distribution of diffusion coefficients for thiolated (blue), PEGylated (red), and POZylated nanoparticles though a 1% w/v gastric mucin dispersion at 25  $^{0}$ C. Data represent the mean of three repeats.



Fig 7s. Exemplar images of stomach sections showing the diffusion of thiolated, PEGylated, and POZylated silica through a mucosal membrane at 0, 15, 30, and 45 minutes. The control sample was deionised water without any particles.