## **Electronic Supplementary Material**

## Intracellular *in situ* labeling of TiO<sub>2</sub> nanoparticles for fluorescence microscopy detection

Koshonna Brown<sup>1</sup>, Ted Thurn<sup>1,†</sup>, Lun Xin<sup>1</sup>, William Liu<sup>1,‡</sup>, Remon Bazak<sup>1,∥</sup>, Si Chen<sup>2</sup>, Barry Lai<sup>2</sup>, Stefan Vogt<sup>2</sup>, Chris Jacobsen<sup>3</sup>, Tatjana Paunesku<sup>1</sup>, and Gayle E. Woloschak<sup>1</sup> (云)

<sup>1</sup> Department of Radiation Oncology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, USA

<sup>2</sup> X-ray Science Division, Advanced Photon Source, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois 60439, USA

<sup>3</sup> Department of Physics & Astronomy, Weinberg College of Arts and Sciences, 2145 Sheridan Road, Evanston, Illinois 60208, USA

<sup>†</sup> Present Address: U.S. Department of State, 2201 C Street, NW Washington, DC 20520, USA

<sup>*+*</sup> Present Address: Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, Maryland 20993, USA

Present Address: Department of Otorhinolaryngology and Head & Neck Surgery, University of Alexandria Medical School, Azarita Medical Campus, Champlollion Street, Khartoum Square, Alexandria 21547, Egypt

Supporting information to DOI 10.1007/s12274-017-1654-8

**Table S1** Zeta potentials and dynamic light scattering of NPs. Bare  $TiO_2$  nanoparticles and azide-coated nanoparticles were diluted 1:200 in water and their hydrodynamic diameters and zeta potentials measured. Due to aggregation and the non-spherical shape of aggregates, sizing data obtained were deemed not reliable and cryo TEM images were used for nanoparticle sizing

Nanoparticle:dispersant	Zeta potential (mV)	Hydrodynamic diameter (nm)
Bare NPs: $dH_2O$ (1:200)	$-34.07 \pm 0.91$	$364.7 \pm 18$
NP-azide:dH <sub>2</sub> O (1:200)	$-31.30 \pm 0.78$	$387.9\pm79$



**Figure S1** Cryo-TEM images of bare and dopamine-azide coated  $TiO_2$  nanoparticles. Bare  $TiO_2$  nanoparticles and azide-coated nanoparticles were diluted 1:50 in distilled H<sub>2</sub>O, plunge frozen in liquid ethane and imaged. Compared to uncoated nanoparticles (a), azide-coated (b) nanoparticles and aggregate sizes appear slightly increased.

Address correspondence to g-woloschak@northwestern.edu

TSINGHUA DINIVERSITY PRESS 2 Springer



**Figure S2** Cells treated with nanoparticles simultaneously coated with ARS and dopamine-azide and stained *in situ* by Click reaction with Alexa Fluor 488 alkyne. High nanoparticle concentration in this experiment (20 fold higher than e.g., in Fig. 4) leads to formation of substantial aggregates. This thickness of nanoparticle deposits was not compatible with proportional Click labeling, therefore, only those areas of nanoparticle aggregates that are relatively thin show an obvious red (ARS) and green (Alexa Fluor 488) fluorescence overlap. Importantly, this early experiment has also shown that a Click reaction with Alexa Fluor 488 alkyne may result in weak staining of nucleoli. In our subsequent experiments we have decreased nanoparticle concentration and refined Click reaction (1,500 × dilution of Alexa Fluor 488 alkyne) to develop the protocol detailed in the Experimental section.