Electronic Supplementary Material (ESI) for RSC Advances.

SUPPORTING INFORMATION

Lanthanide-doped Nanoparticles for Specific Recognition of Toll-like Receptor (TLR) in Human Neutrophils

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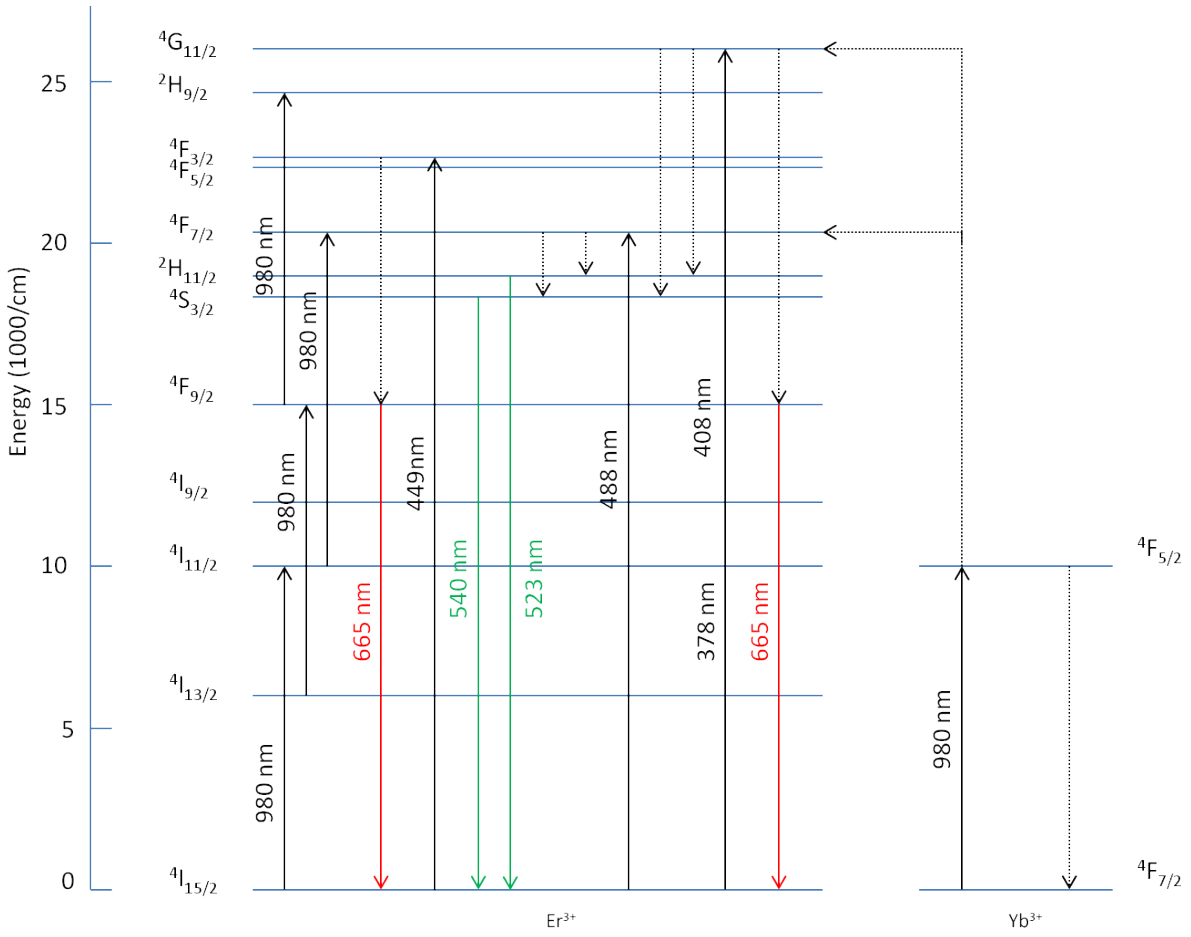


Scheme SI 1. Energy transfer processes of Er^{3+} and Yb^{3+} at different excitation wavelengths.

Table SI 1. Zeta-potential data from the UCNP after each functionalization step. Numbers in the name of the sample correspond to mg/mL of biomolecules added.

Sample	Charge (meV)	s.d
UCNP-APTMS	+14.34	0.59
UCNP-APTMS-Glutaraldehyde	+32.3	0.97
UCNP-APTMS-BSA	+7.38	0.37
UCNP-APTMS-Dex	+7.09	0.25
UCNP-APTMS-BSA/Dex	-22.93	0.83
UCNP-APTMS-BSA/Dex-Strv0.5	-24.18	0.62
UCNP-APTMS-BSA/Dex-Strv5.0	-28.6	1.07

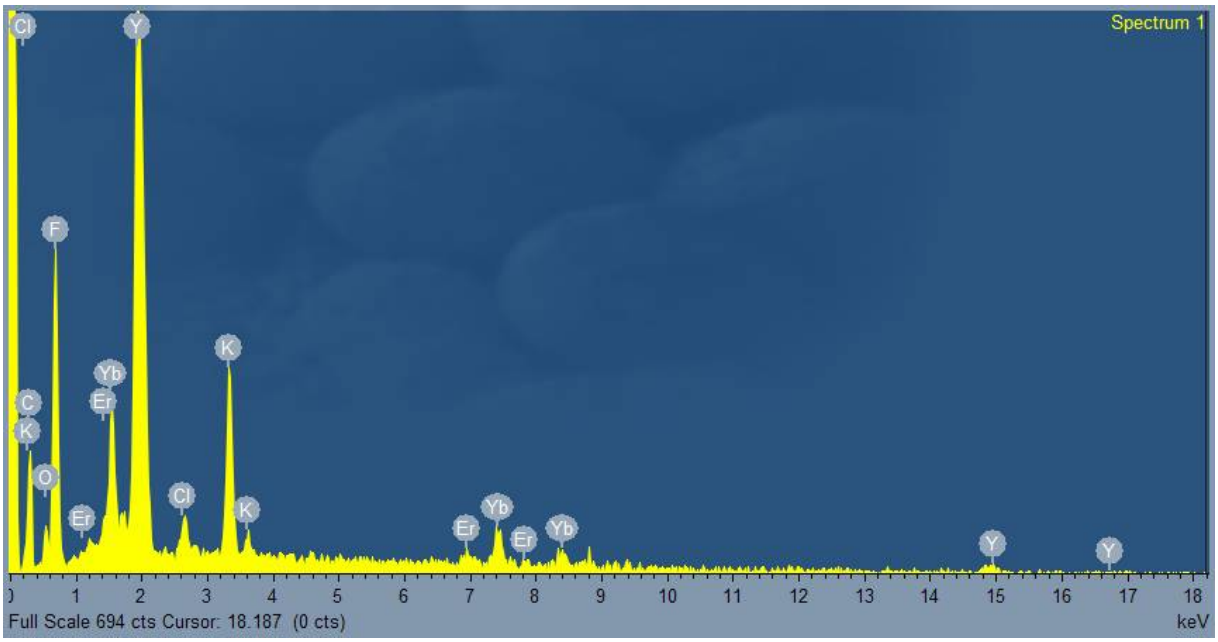


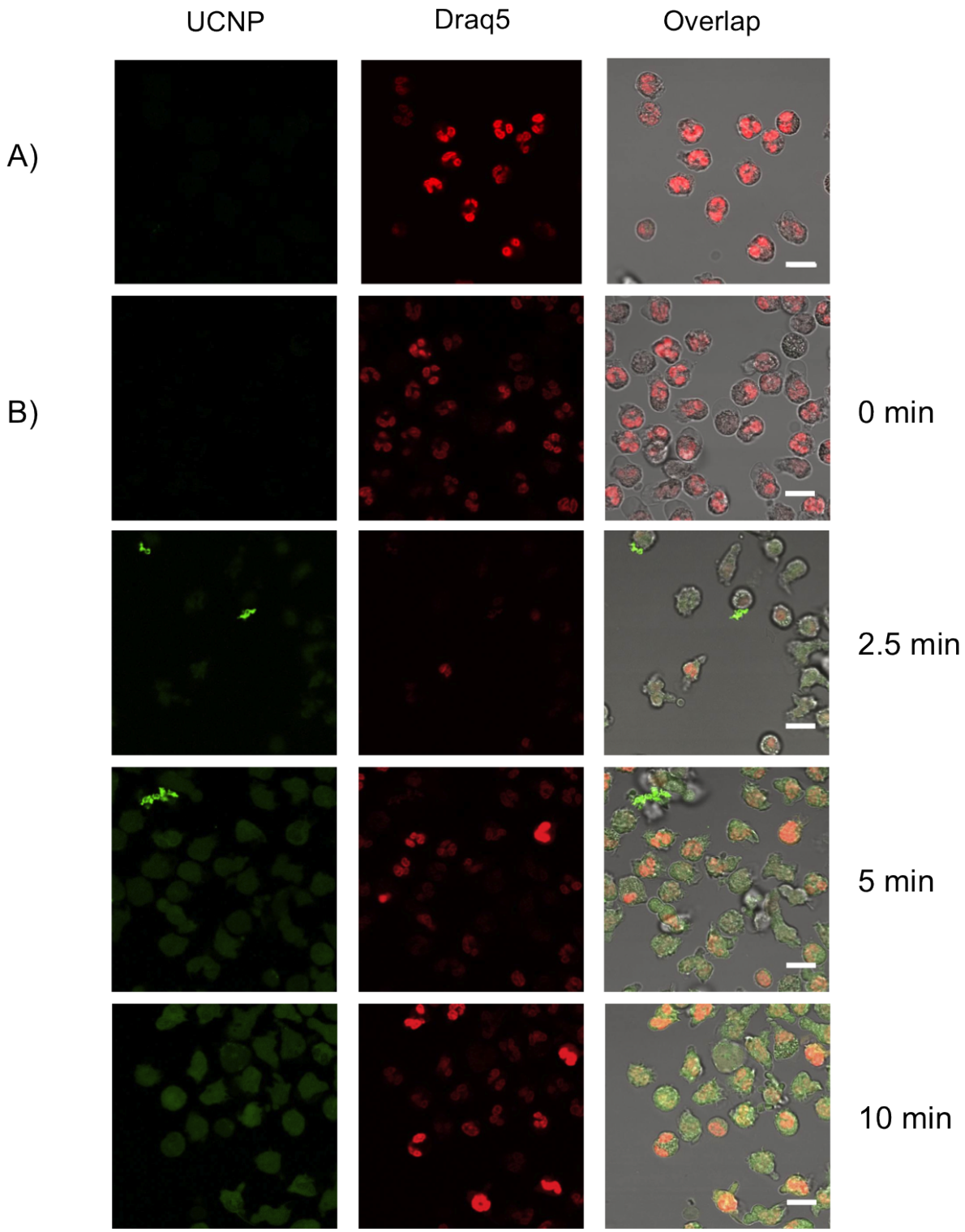
Fig. SI 1. Elemental mapping with EDX of KYF_4:Yb,Er.

Fig. SI 2. Electrophoresis of UCNP before and after functionalization. Lanes correspond to samples (1) UCNP-APTMS, (2) UCNP-APTMS-BSA, (3) UCNP-APTMS-Dex, (4) UCNP-APTMS-BSA/Dex, and (5) UCNP-APTMS-BSA/Dex-Strv5.0. UCNP were revealed under 405 nm excitation wavelength.

Mechanisms of nanoparticles cellular uptake. Nanoparticles (NP) uptake by cells may occur through several different mechanisms, which can be classified into phagocytosis and pinocytosis. Pinocytosis includes macropinocytosis with uptake of particles with sizes > 1 μm, clathrin or caveolae-mediated endocytosis, or clathrin/caveolae independent endocytosis. Caveolae consist of plasma membrane invaginations of sizes between 50-80 nm containing cholesterol and sphingolipids, receptors and caveolins[1,2]. Endocytosis of various membrane receptors may also occur via lipid rafts [3]. Lipid rafts provide a platform for the assembly of receptors, adaptors, regulators, and other downstream proteins as a signaling complex, and may be joined with caveolae. Clathrin-coated pits of 100–200 nm have been

shown to be associated with the key protein clathrin and other scaffold proteins such as AP-2 and eps15 [4]. Macropinocytosis is a form of endocytosis related to cell surface ruffling and provides a route for non-selective endocytosis of solute macromolecules. Macropinosomes are more than 0.2 μm in diameter, and may be as large as 5 μm.[5] Specialized phagocytosis via protease activation receptor 2 occurs in HEK that up take melanosomes from adjacent melanocytes. It is possible that NP may be uptaken by cells *via* their size selectivity that may match those of endocytic pits. Neutrophils granulocytes mainly trap nanoparticles via extracellular networks.[6,7]

NP endocytosis by cells not only depends on the size of the NP, but also on the surface coating and charge [7]. Carboxydextran-coated superparamagnetic iron oxide nanoparticles (SPION) were internalized by human mesenchymal stem cells, and the efficiency of uptake was correlated with the amount of carboxyl groups on the NP surface.[8] Cationic D,L-polylactide (PLA)-NP entered HeLa cells in greater amounts than anionic PLA-NP.[9,10] NP uptake may also depend on the length of the surface coating,[11] or the type of cells.[12]


UCNP
Draq5
Overlap
A)
B)
0 min
2.5 min
5 min
10 min

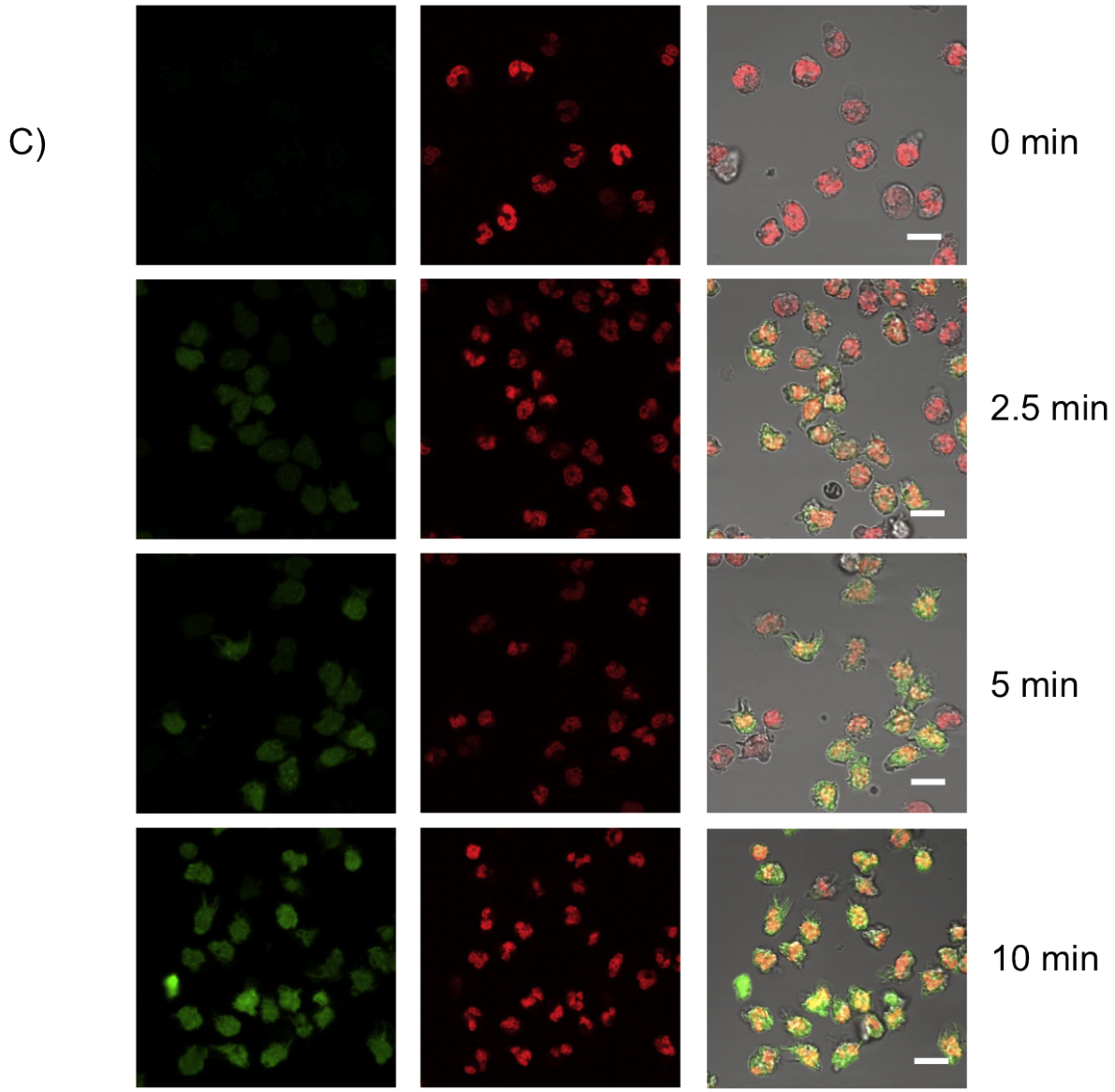


Fig. SI 3. Confocal images of A) neutrophils without UCNP, B) neutrophils with UCNP-APTMS and C) neutrophils with UCNP-APTMS-BSA/Dex-Strv5.0 under 488 nm excitation incubated at 37ºC. Neutrophils were first treated with biotin-LPS. From left to right, the images correspond to nanoparticles (green), Draq5 (red) and overlap (green and red), respectively. Incubation times (in minutes) are on the right hand side. Scale bar corresponds to 10 μm.

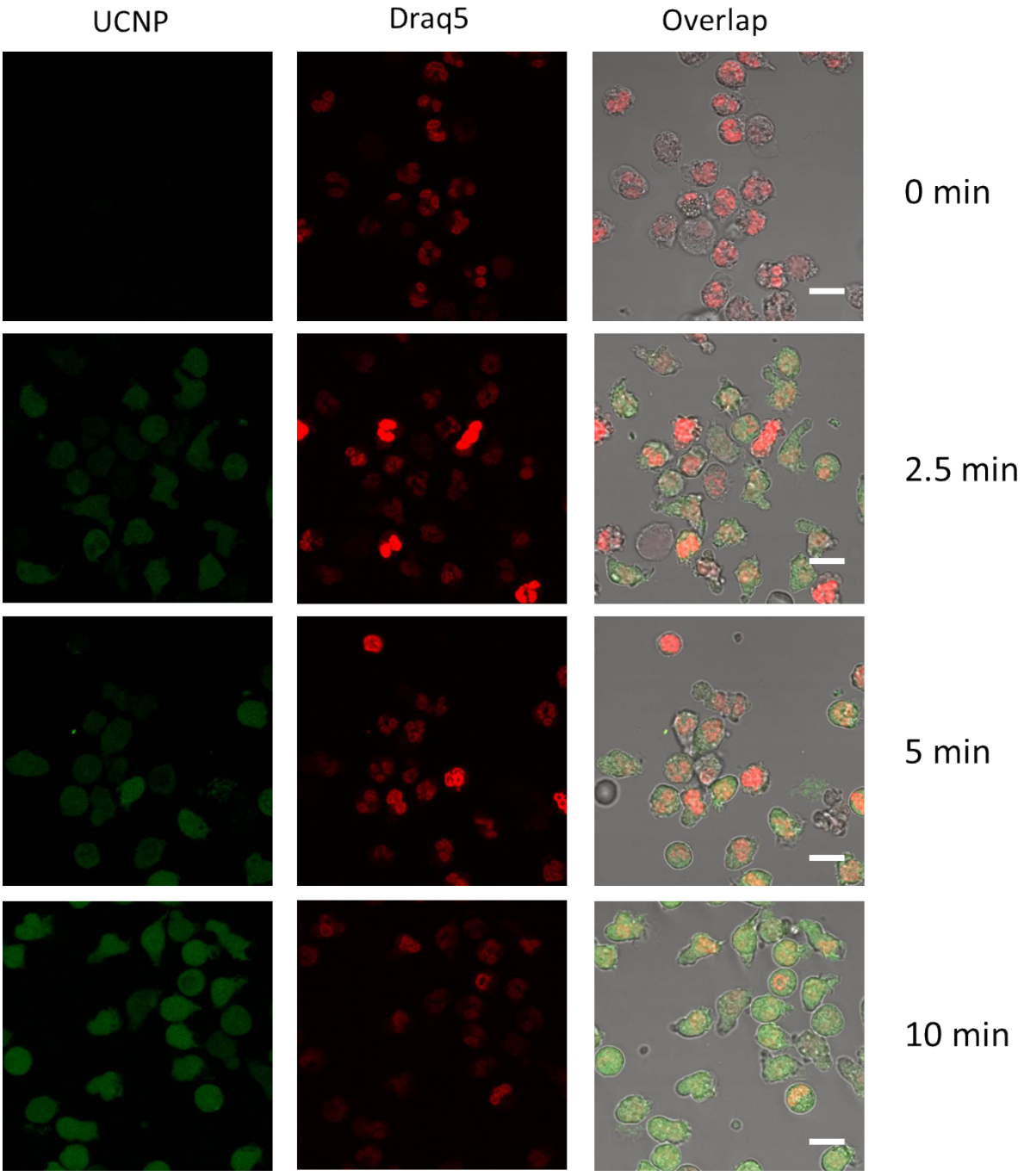


Fig. SI 4. Confocal images of neutrophils incubated at 37ºC with UCNP-APTMS-BSA/Dex-Strv5.0-Biotin-LPS0.1 (0.1mg/mL of Biotin-LPS added) under 488 nm excitation. From left to right, the images correspond to nanoparticles (green), Draq5 (red) and overlap (green and red), respectively. Incubation times (in minutes) are on the right hand side. Scale bars correspond to 10 μm.

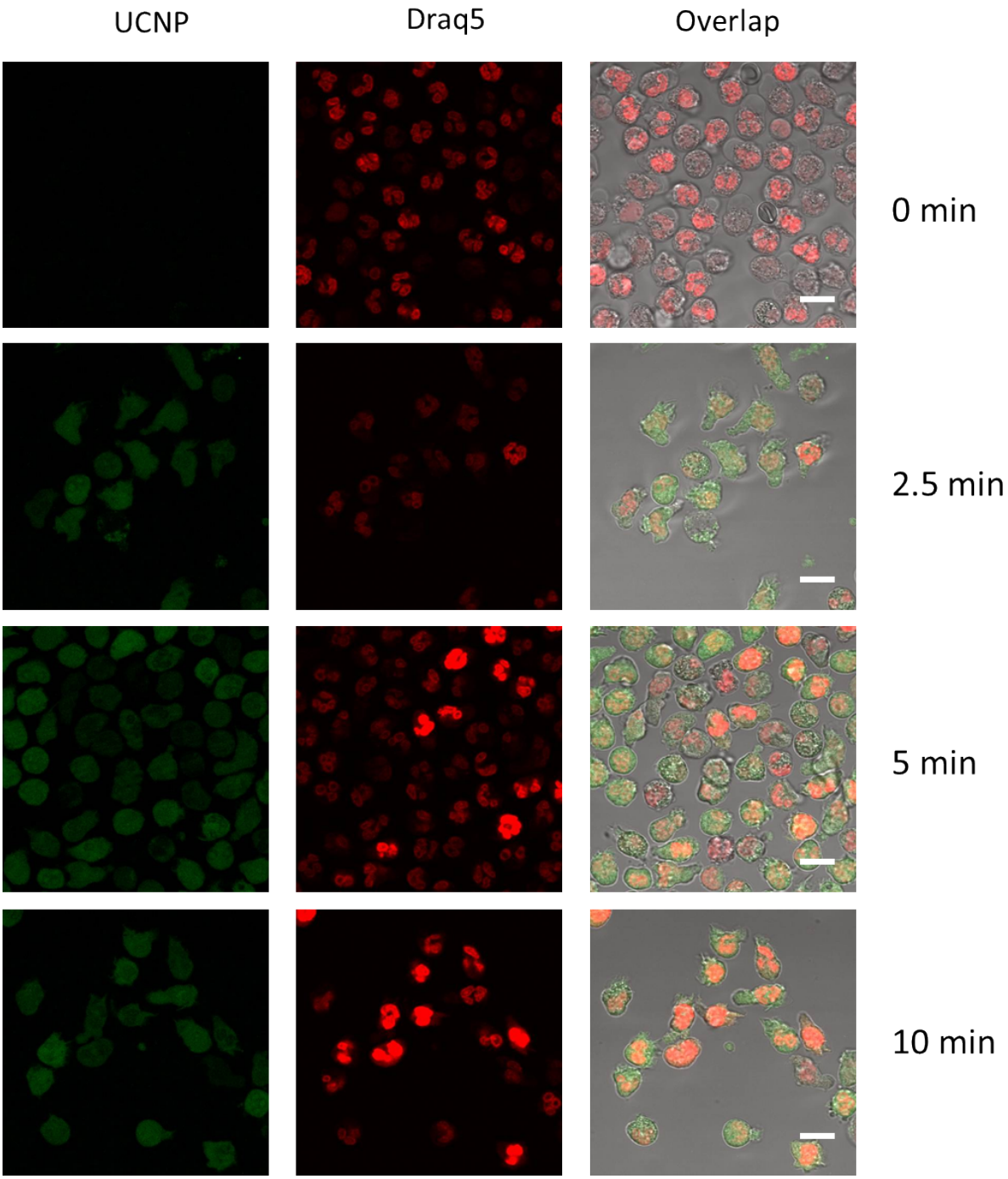
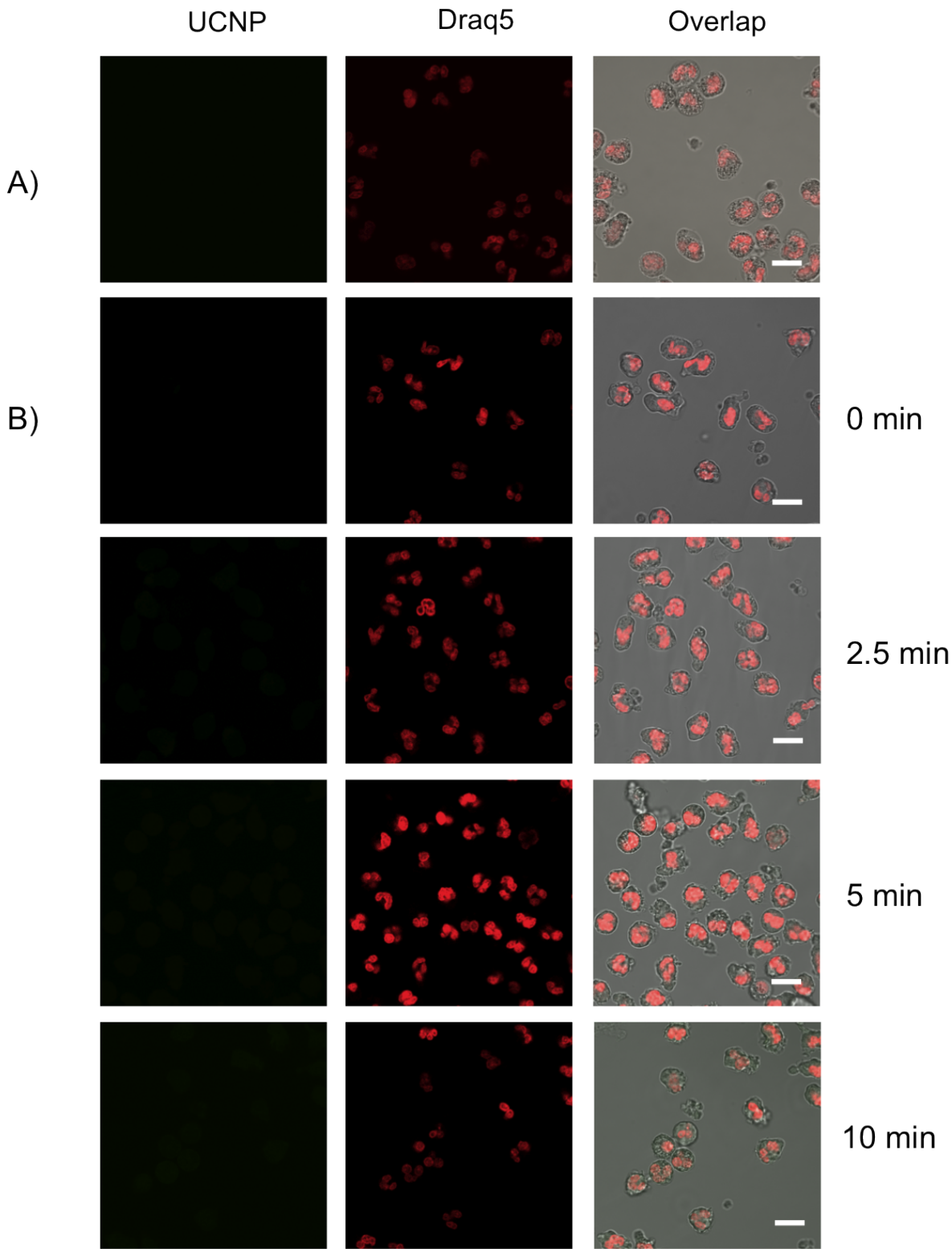


Fig. SI 5. Confocal images of neutrophils incubated at 37ºC with UCNP-APTMS-BSA/Dex-Strv5.0 under 488 nm excitation. From left to right, the images correspond to nanoparticles (green), Draq5 (red) and overlap (green and red), respectively. Incubation times (in minutes) are on the right hand side. Scale bars correspond to 10 mm.


UCNP
Draq5
Overlap
A)
B)
0 min
2.5 min
5 min
10 min

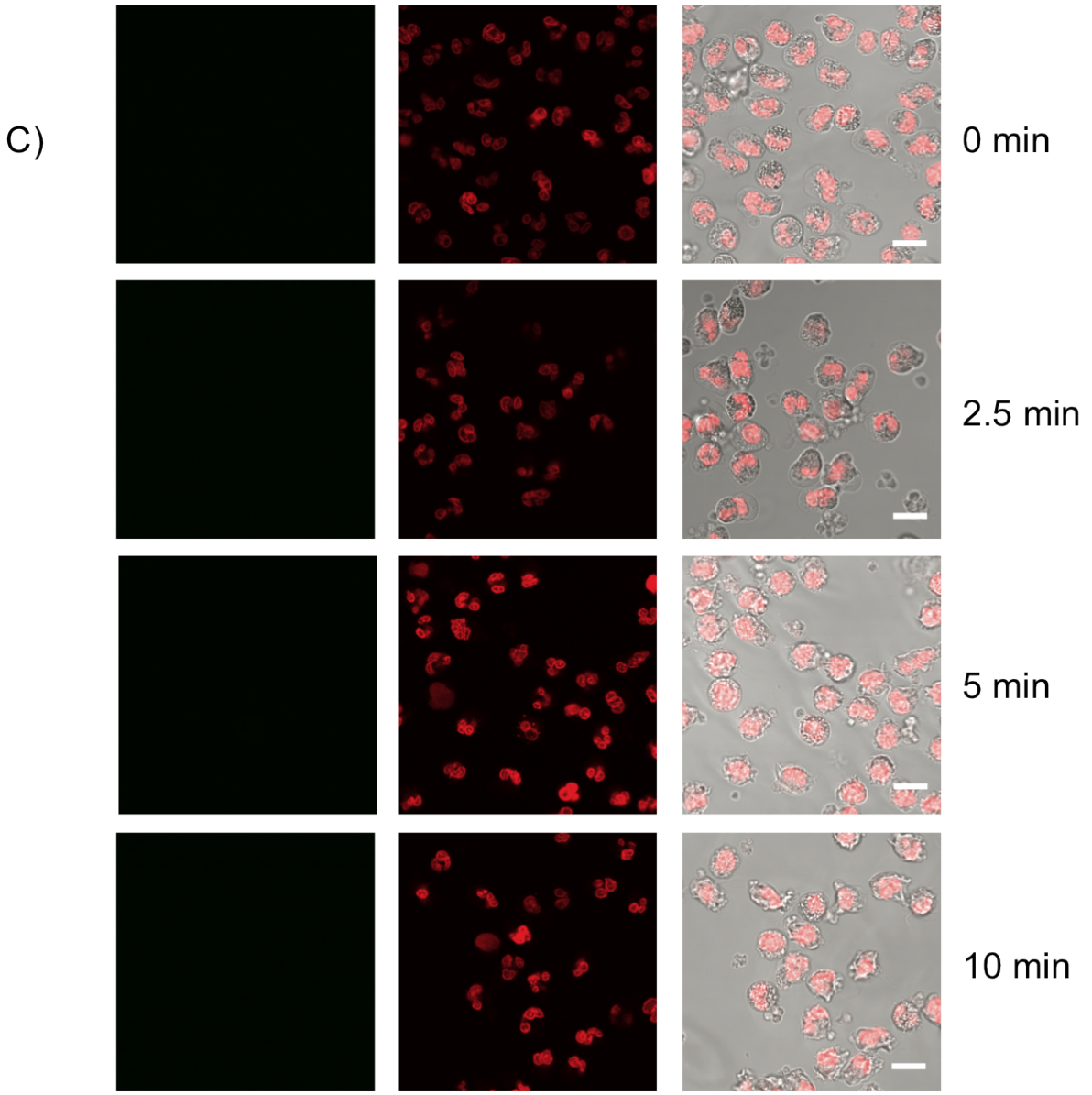


Fig. SI 6. Confocal images of A) neutrophils without UCNP, B) neutrophils with UCNP-APTMS and C) neutrophils with UCNP-APTMS-BSA/Dex-Strv5.0 under 488 nm excitation incubated at 4ºC. Neutrophils were first treated with biotin-LPS. From left to right, the images correspond to nanoparticles (green), Draq5 (red) and overlap (green and red), respectively. Incubation times (in minutes) are on the right hand side. Scale bar corresponds to 10 μm.

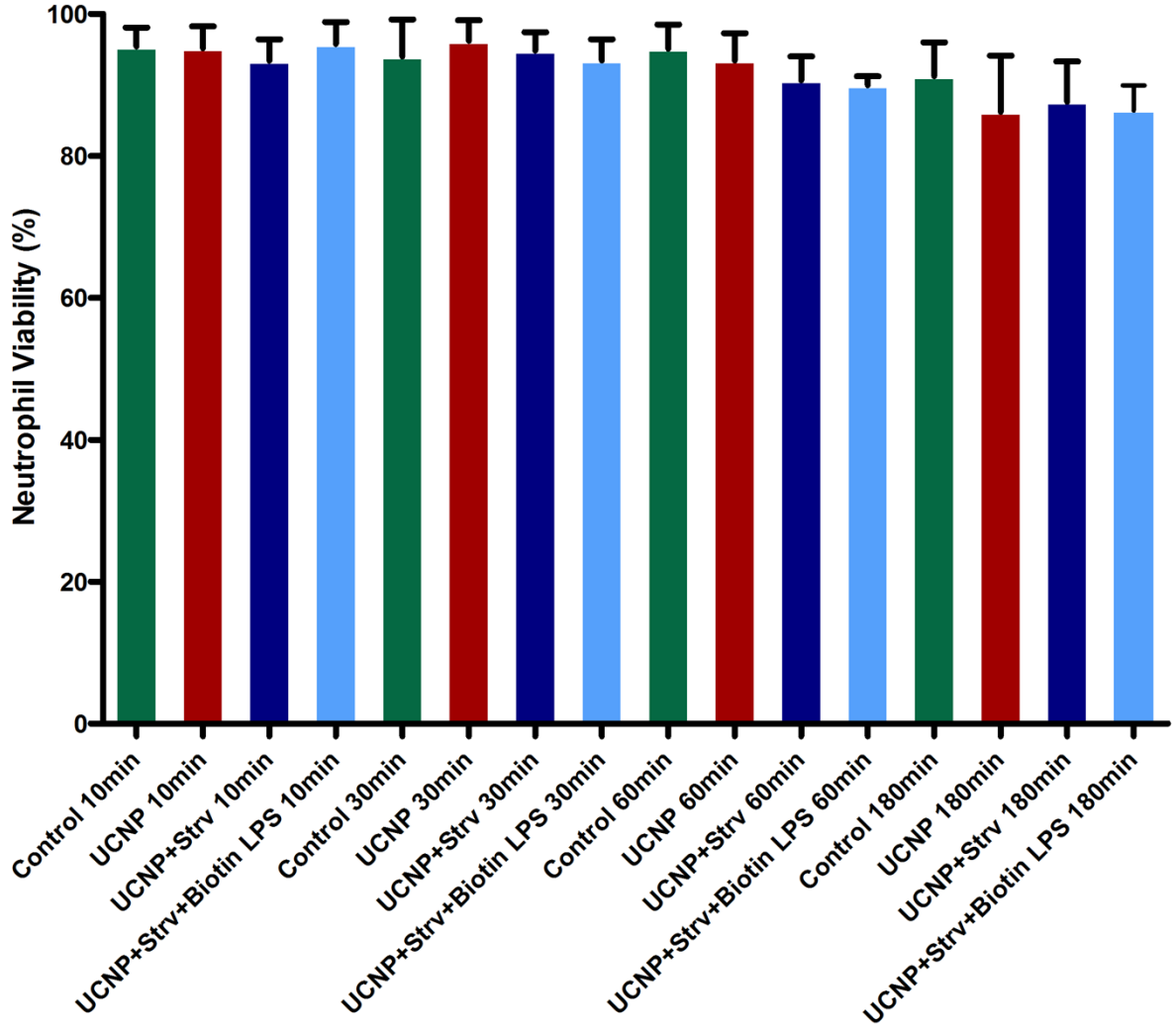


Fig. SI 7. Trypan Blue exclusion test for neutrophil viability testing at 10, 30, 60 and 180 min coincubation in the presence of UCNPs.

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