Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Electronic Supplementary Information for:

Multi-Layered Stimuli Responsive DNA Micelles for the Stepwise Controlled Release of Small Molecules

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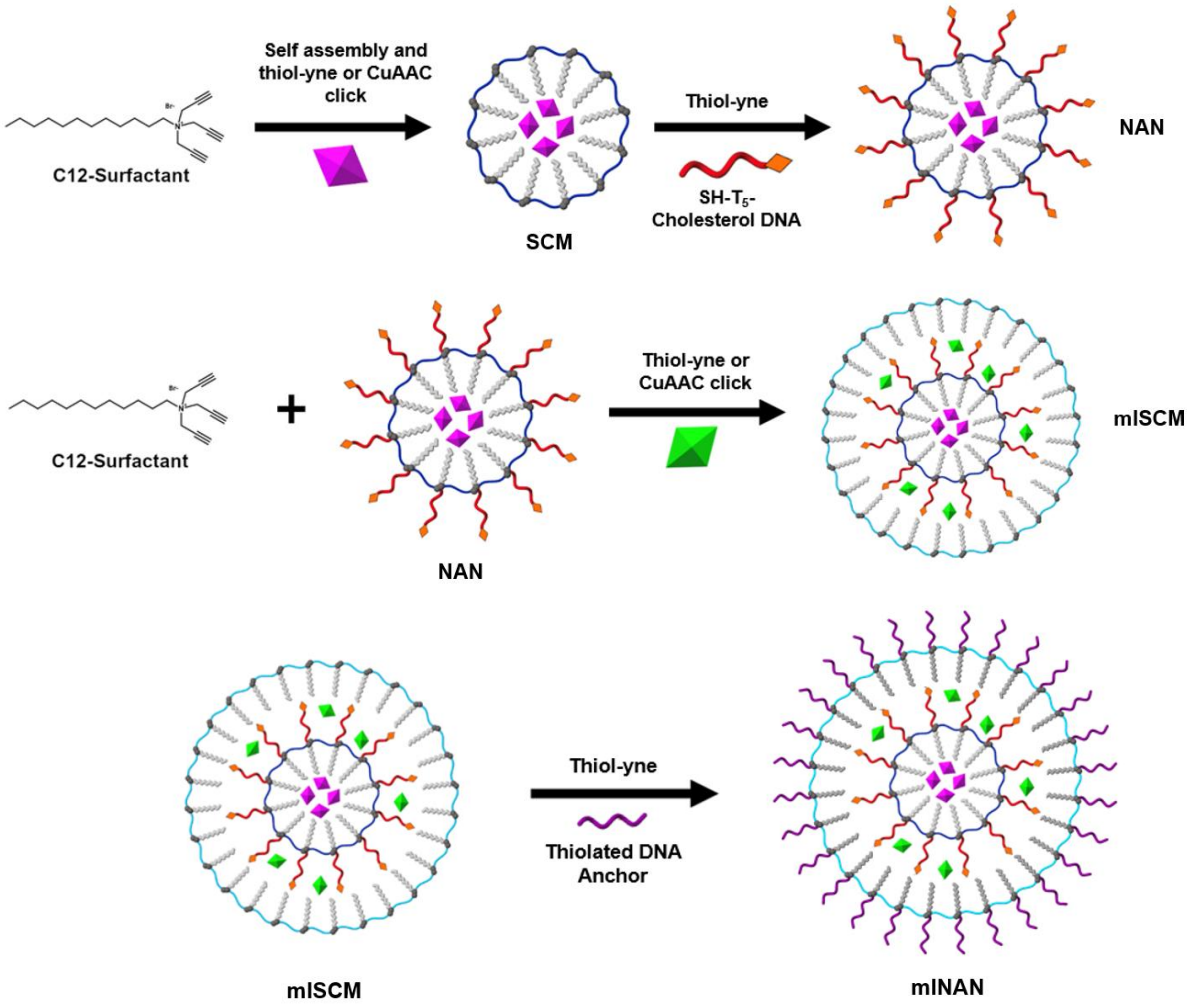
I. Representative Schemes



Scheme S1. Synthetic route for the preparation of mISCMs and mINANs.

II. Crosslinkers

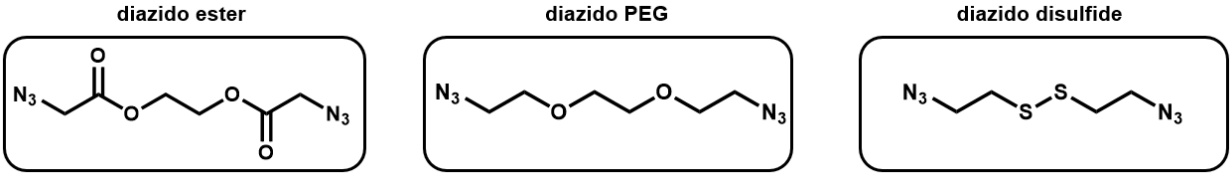


Figure S1. Crosslinkers used for synthesis of mISCMs and mINANs.

III. Dynamic Light Scattering Characterization

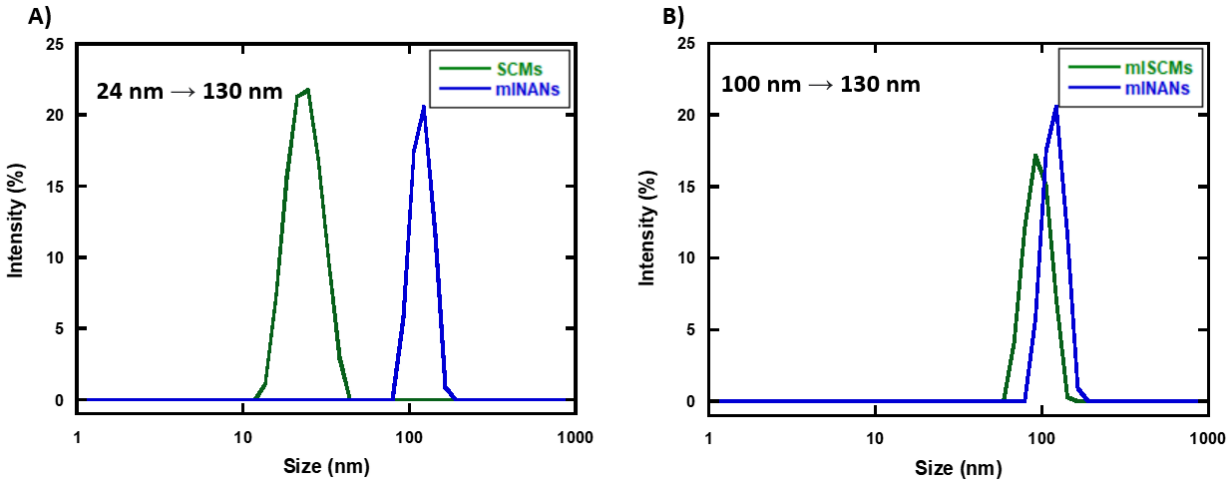


Figure S2. Representative dynamic light scattering analysis indicating the average hydrodynamic size of mlNANs. Particles increased in size with each additional layer assembled upon the SCM core. mlNANs were colloidal as synthesized but partial aggregation and sedimenting occasionally occurred over time. Particles could be resuspended via gentle vortexing before use. **A)** Average particle size increased from SCMs to mlNANs (average of 24 nm to 130 nm). **B)** Size increased from mlSCMs to mlNANs (average of 100 nm to 130 nm). This analysis is reflective of the TEM images of mlSCMs shown below in **Figure S3**.

IV. Additional TEM Micrographs

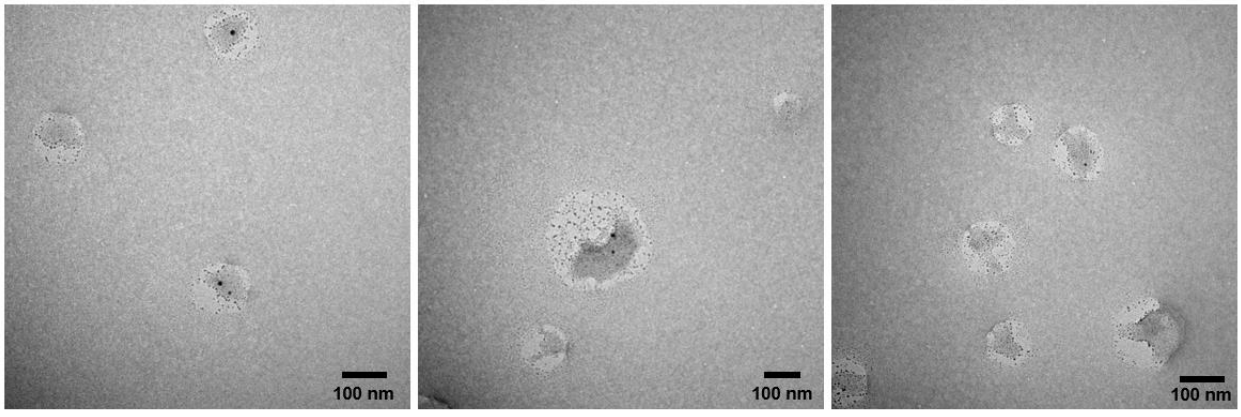


Figure S3. Additional TEM images of mlSCMs with 2 nm and 15 nm AuNPs. Smaller gold nanoparticles can be seen in the outer layer whereas larger gold nanoparticles can be found in the center, representing the core inner micelle.

V. Co-Release of 5-TAMRA and Fluorescein

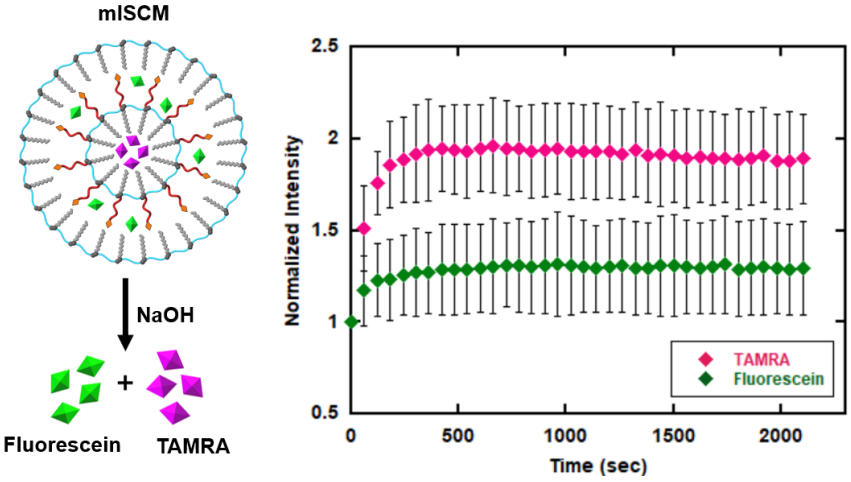


Figure S4. Fluorometric assay for triplicate measurements of 5-TAMRA and fluorescein release from mlSCMs in the presence of sodium hydroxide over 30 minutes.

Fluorometric Cargo Release Assay: A solution of ester crosslinked mlSCMs (inner layer: 5-TAMRA, outer layer: fluorescein) was prepared in water at a total volume of 800 µL. The sample was treated with sodium hydroxide and an initial measurement was taken. Additional measurements were taken every 60 seconds for 35 minutes. In order to track the release of two different dyes from each layer, separate emission profiles were tracked. Fluorescein release (excitation: 488 nm, emission: 512 nm). 5-TAMRA release (excitation: 557 nm, emission: 583 nm).

VI. HPLC Assay Development

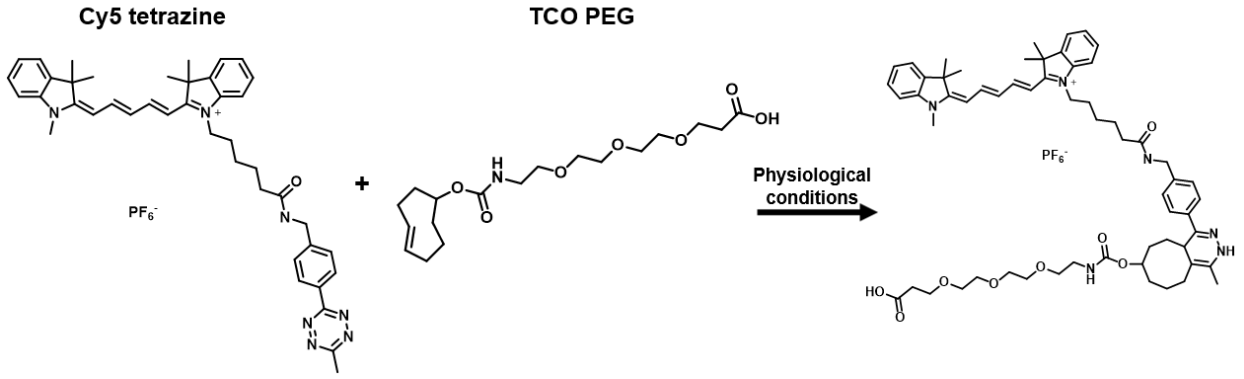


Figure S5. Tetrazine-TCO ligation. Inverse electron demand Diels-Alder reaction completed under physiological conditions.

HPLC Cy5 Tetrazine and Tetrazine-TCO Ligation Data:

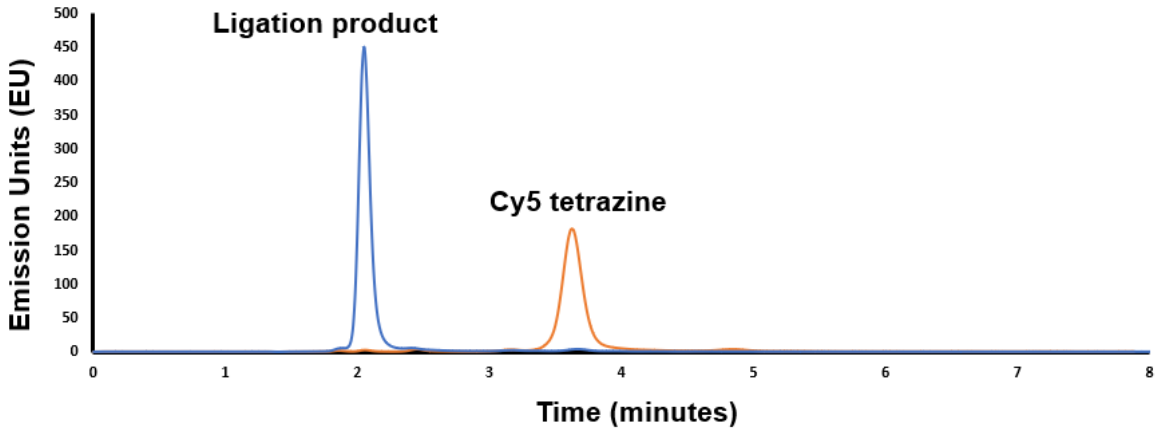


Figure S6. Overlay of HPLC chromatograms of cy5 tetrazine (3.6 minutes) and the cy5 tetrazine-TCO ligation product (2.1 minutes).

HPLC Cyanine 5 Tetrazine Release from mlSCMs (Control Study):

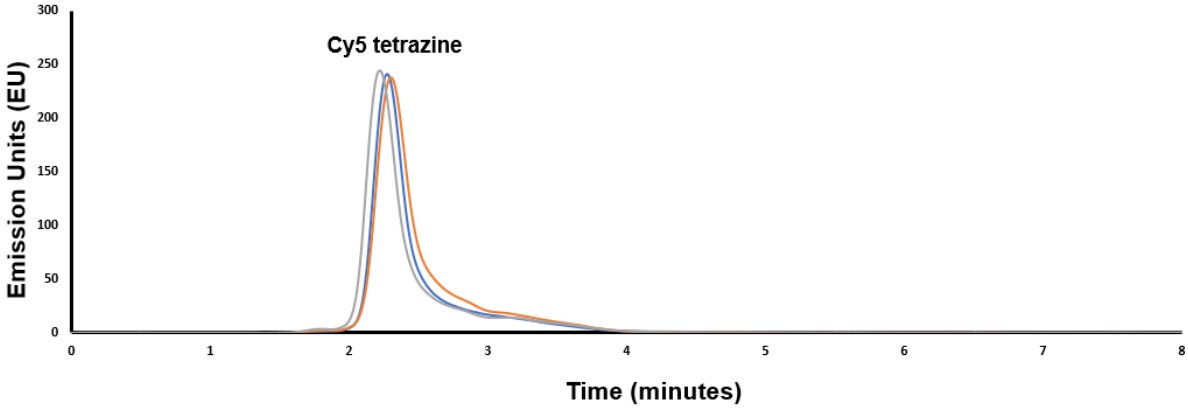


Figure S7. Overlay of HPLC chromatograms of release of cyanine 5 tetrazine from the outer layer of mlSCMs over a period of 1 hour.

VII. Mass Spectrometry Analysis

Mass Spectrometry Analysis: Samples were run using an AB Sciex 4000 QTRAP mass spectrometer.

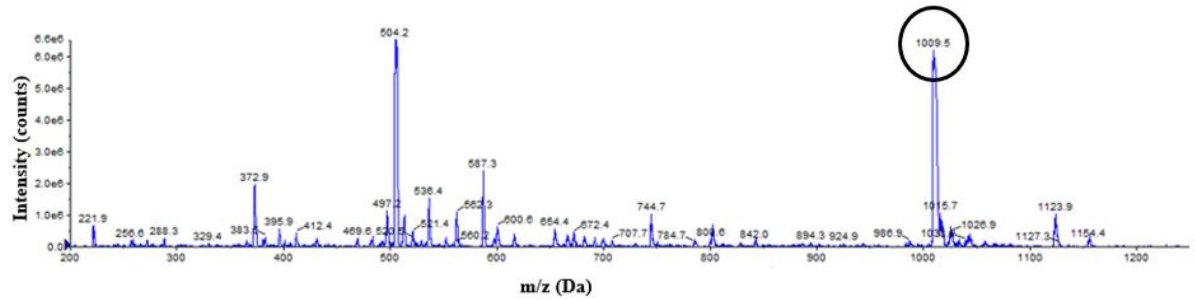


Fig S8. Tetrazine-TCO ligation product: ESI (m/z): $[M - H]^-$ calculated for $C_{60}H_{76}N_6O_8$, 1009.32; found, 1009.5.

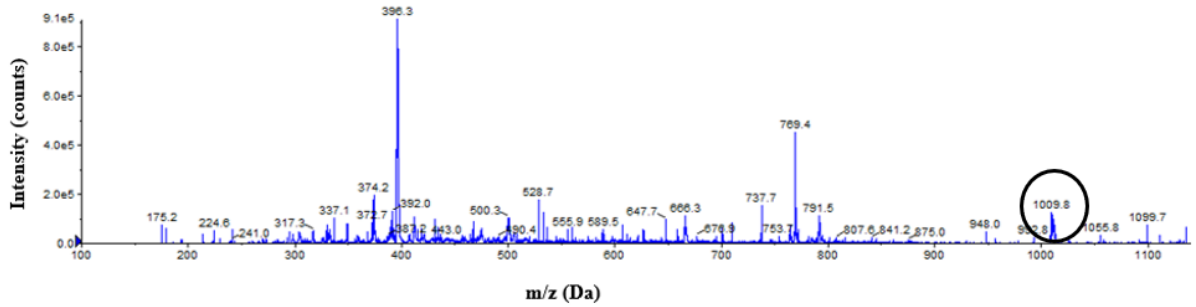


Figure S9. Tetrazine-TCO ligation product after release from mISCMs. ESI (m/z): $[M – H]^-$ calculated for $C_{60}H_{76}N_6O_8$, 1009.32; found, 1009.8.

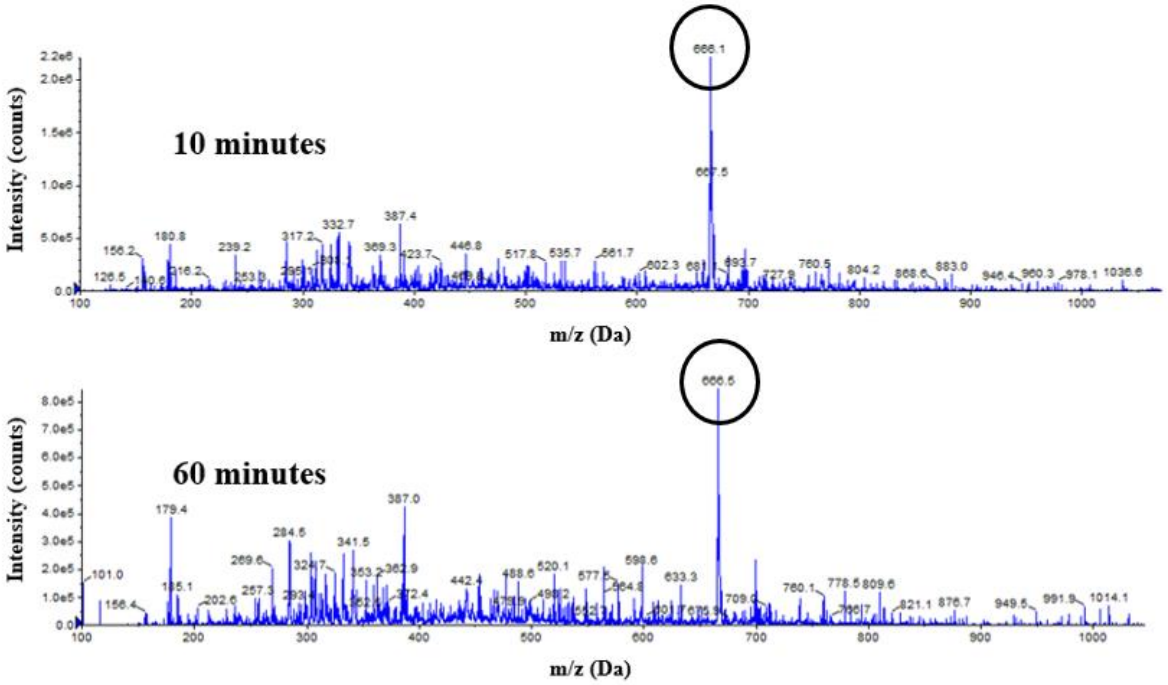


Figure S10. Cyanine 5 tetrazine after release from outer layer of mlSCMs. ESI (m/z): [M – H]$^-$ calculated for $C_{42}H_{47}N_7O$, 665.89; found, 666.1, 666.5.

VIII. mlNAN Stimuli Responsive Cargo Release

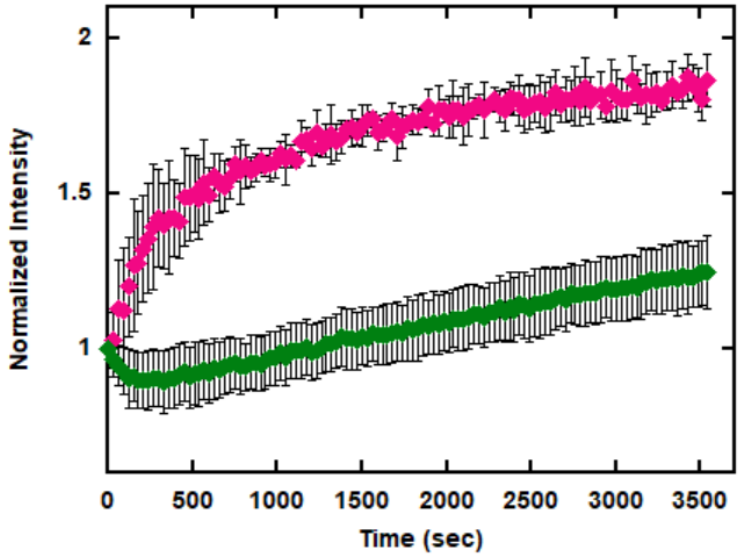


Figure S11. Fluorometric assay data for triplicate measurements of release of ATP Red and MitoTracker Green from ester crosslinked mlNANs (5 μM) in the presence of esterase.

IX. ATP-Red Treatment with ATP and GMP

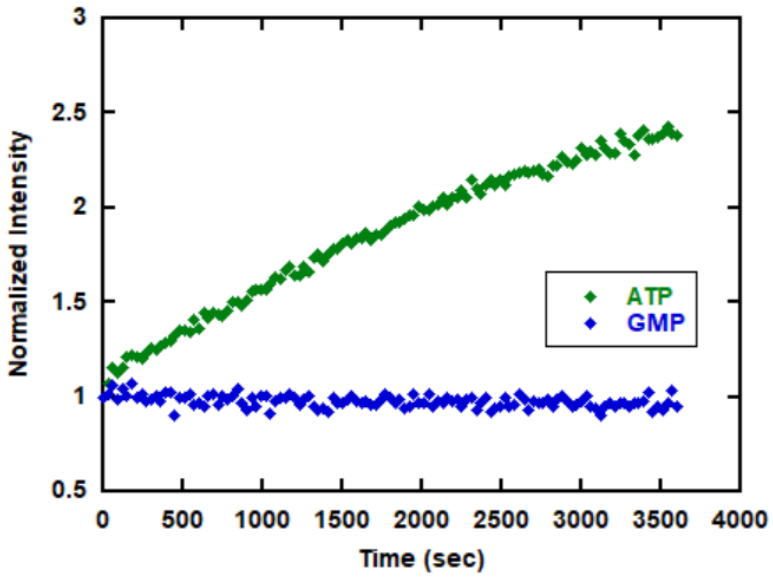


Figure S12. Fluorometric assay data for treatment of ATP-Red with ATP (2.5 mM) and GMP (2.5 mM) after release from NANs treated with esterase.

X. Confocal microscopy of FITC labeled mlNANs

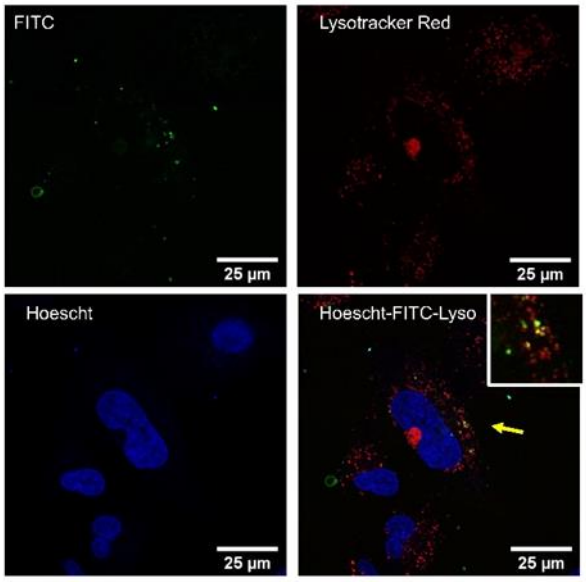


Figure S13. Confocal microscopy images of mlNANs. Confocal images of 30 µM mlNANs labeled with a FITC poly T 20 DNA strand post incubation with A549 cells for 4 hours. Top left: FITC signal from DNA at mlNAN surface. Top right: lysotracker red indicating endosomes/lysosomes. Bottom left: Hoescht staining of cell nuclei. Bottom right: merger

of all three channels. Inset shows a magnified image of where the yellow arrow is. Yellow puncta indicate colocalization of mlNANs and endosomes

XI. References

1. Awino, J. K., Gudipati, S., Hartmann, A. K., Santiana, J. J., Cairns-Gibson, D. F., Gomez, N., and Rouge, J. L. (2017) Nucleic Acid Nanocapsules for Enzyme-Triggered Drug Release. *J. Am. Chem. Soc.* 139, 6278–6281.