Click Nucleic Acid Mediated Loading of Prodrug Activating Enzymes in PEG-PLGA Nanoparticles for Combination Chemotherapy

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Supplementary Information

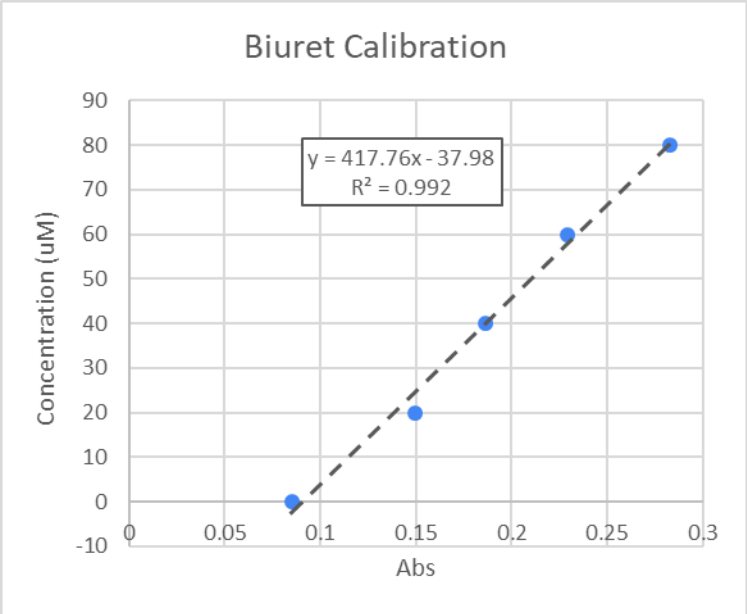

Figure S1. Lowry's assay calibration.

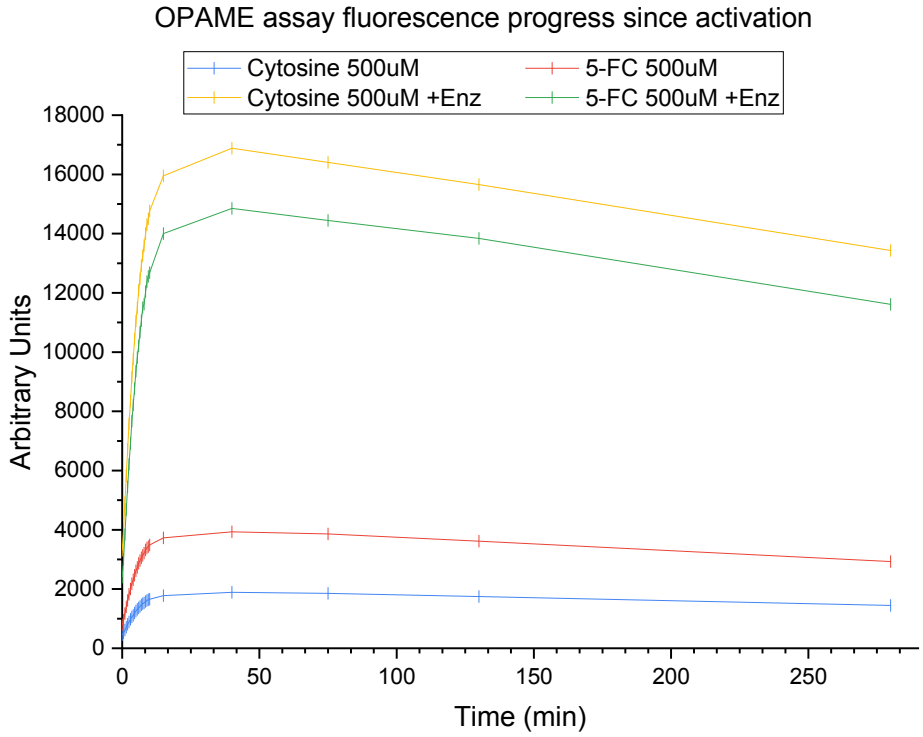

Figure S2. Time stability of the OPAME assay by performing a measurement on 500 µM Cytosine and 5-FC in PBS incubated overnight at 37°C and the same reacted overnight with an arbitrary amount of enzyme.

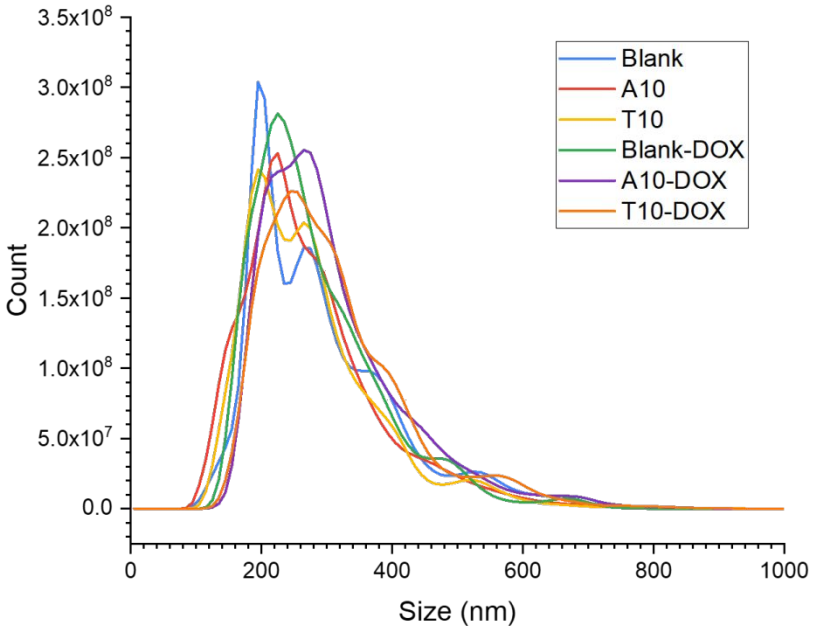

Figure S3. Nano tracking analysis (NTA) results for the size distributions of various NPs samples of interest used in this study.

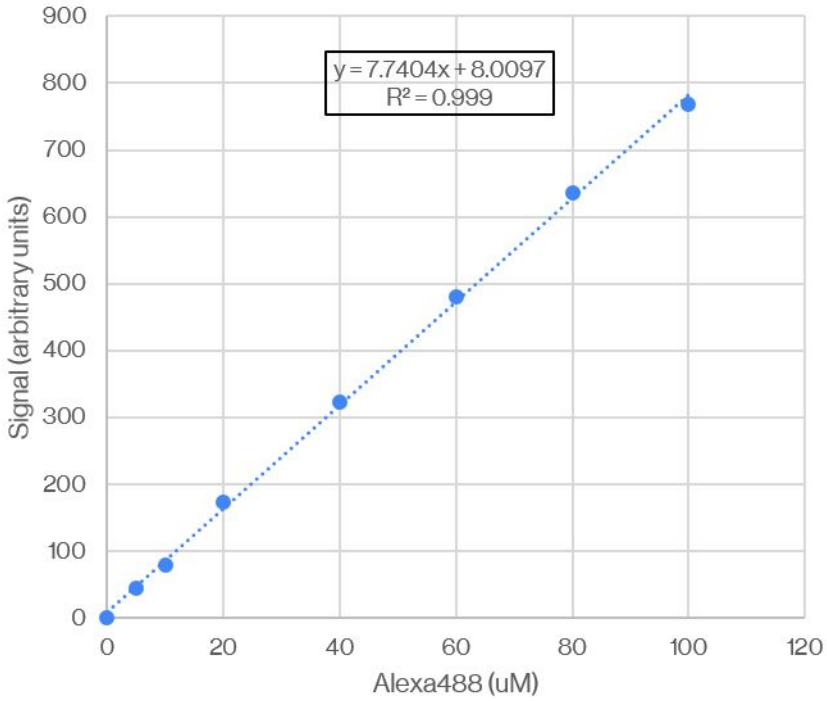

Figure S4. Alexa 488 quantification calibration curve in water.

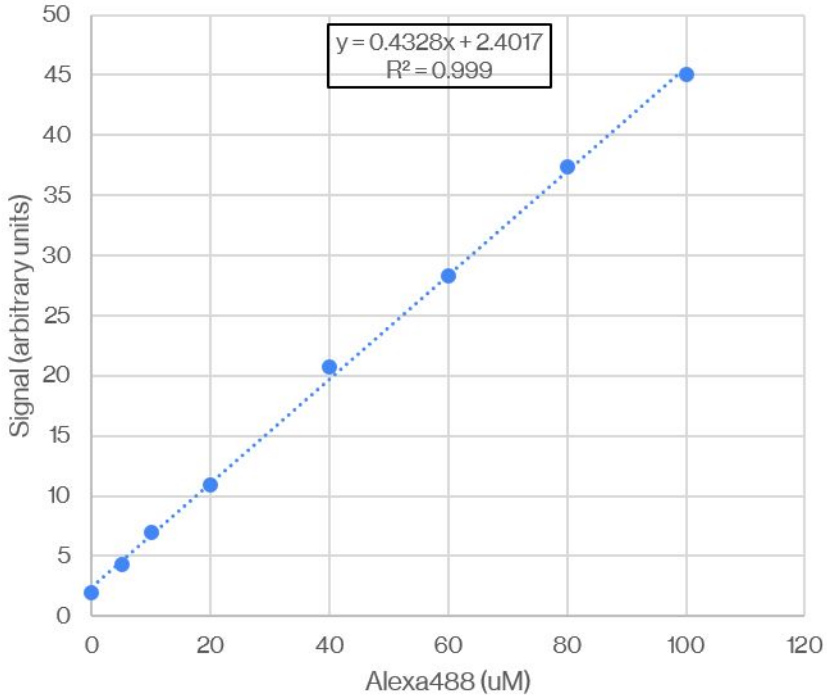

Figure S5. Alexa 488 quantification calibration curve in DMSO.

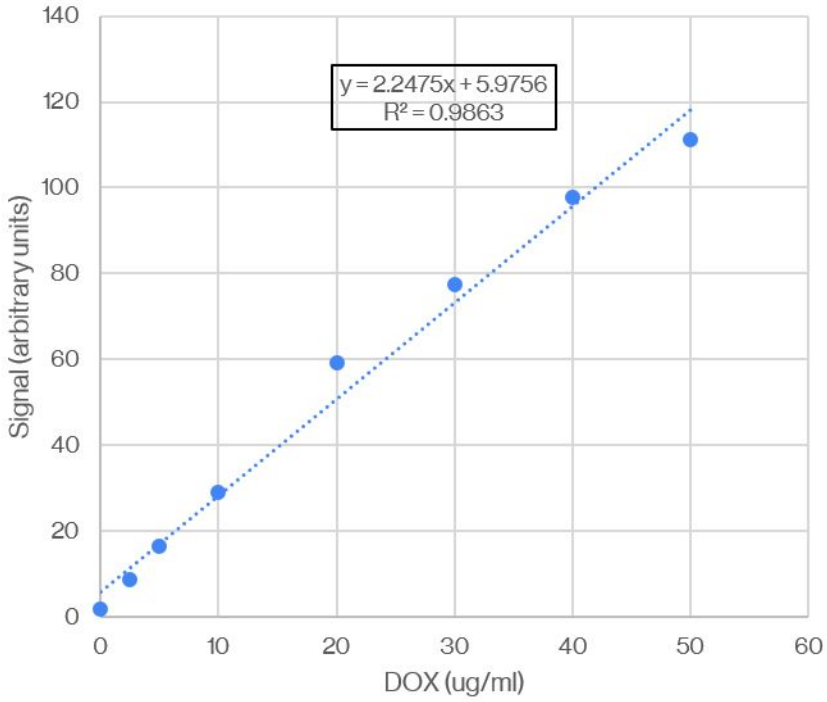

Figure S6. Doxorubicin quantification calibration curve in DMSO.

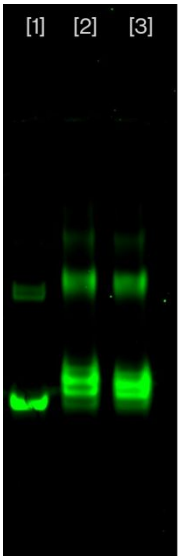

Figure S7. Non-Coomassie stained SDS-PAGE containing [1] CodA after modification with Alexa488 and DBCO and its subsequent modification with [2] poly Adenine and [3] poly Thymine DNA oligos. All samples correspond to the respective dialysis purified product. The scan was done with a 405nm light source and filtering through a BBP1 filter at 50 μm resolution.

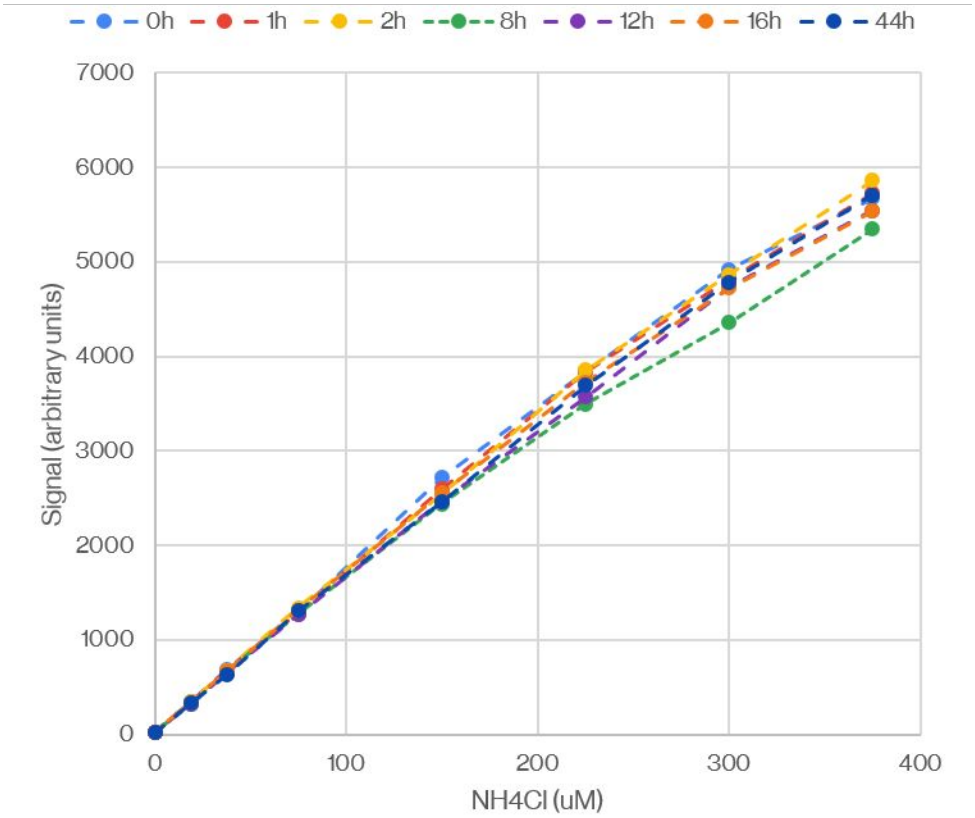

Figure S8. OPAME NH_4Cl calibration curve. Despite the visual effect of being curbed when plotted together none of the calibrations had a calculated r^2 smaller than 0.996.

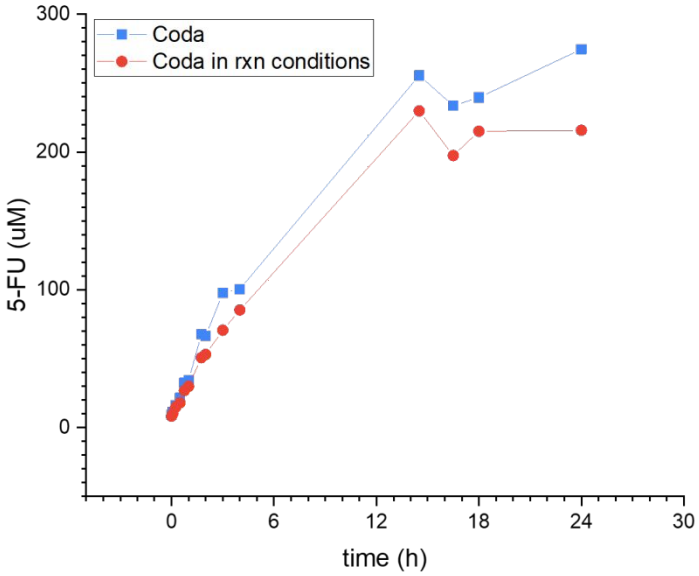

Figure S9. Test performed with the conditions of figure 2b to assess the impact of the raction conditions in CodA activity.

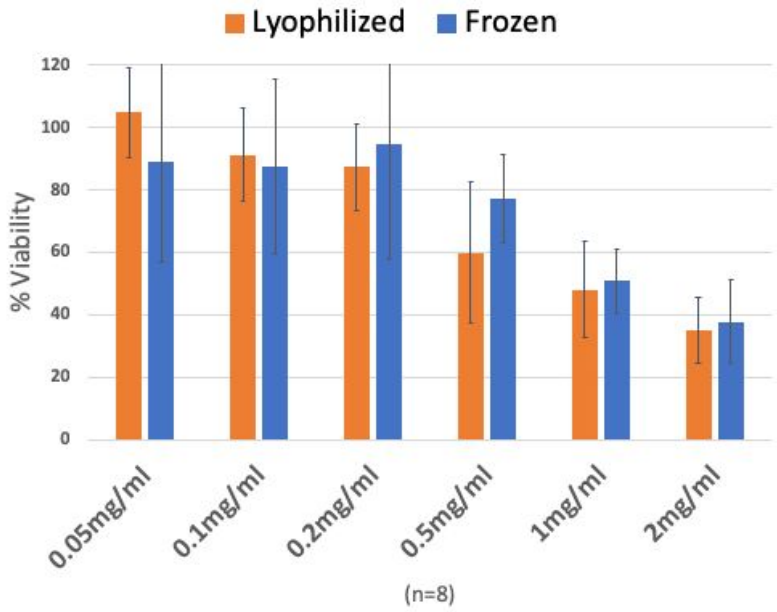

Figure S10. MTT results of incubating varying concentrations of PEG-CNA(T10)-PLGA nanoparticles with MDA-MB-468 cells for 24.

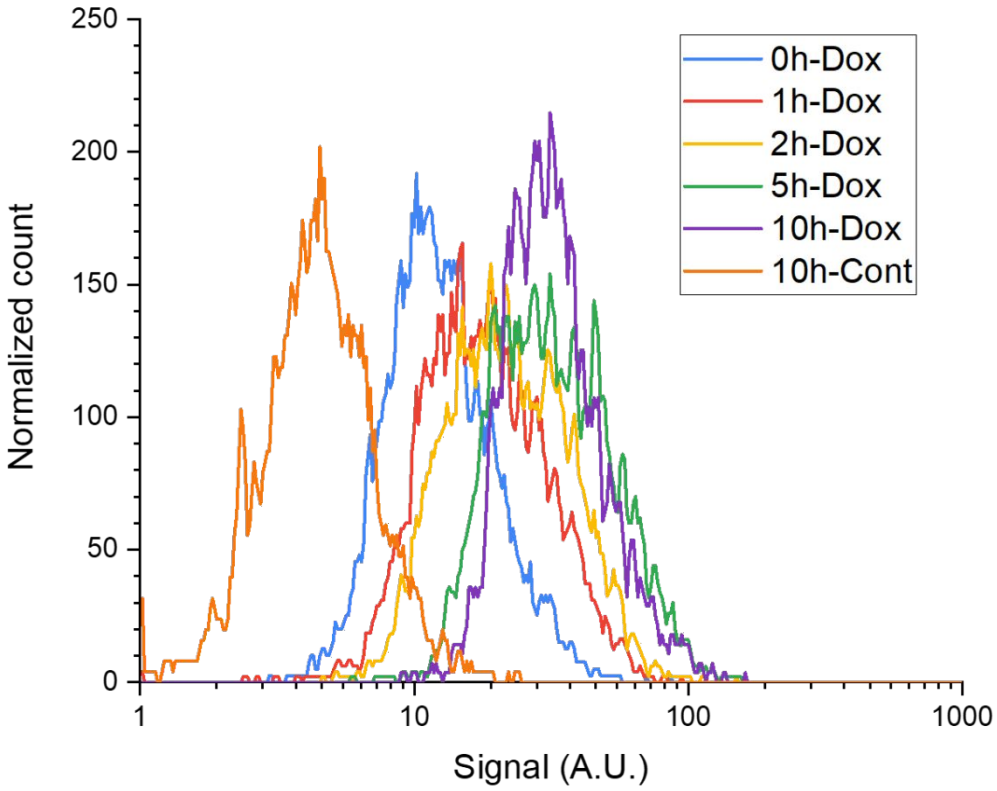

Figure S11. Evaluating nanoparticle entrapment by flow cytometry.MDA-MB-468 cells were seeded in 50000 cells per ml in a 24 well plate and left to adhere overnight. A sample of 0.1 mg/ml PEG-CNA(T10)-PLGA nanoparticles encapsulated with Doxorubicin were added to each appropriate well. As a control, polymer nanoparticles not loaded with DOX were also tested. At each time point the appropriate well was washed twice with PBS followed by adding 200 ul of trypsin and incubated 15 min. The trypsinized samples were next removed, 1 ml of PBS added and centrifuged down 5 min, the pellets of which were resuspended in 1 ml of paraformaldehyde. After 2 h of incubation at RT the samples were centrifuged again and this time pelleted cells were resuspended in PBS and stored at 4 ºC until immediately before their analysis. The analysis was performed by selecting a gate for the cell population and measuring the intrinsic fluorescence of doxorubicin with a 588 nm excitation source and measuring

through a 500-560nm gate. All control samples piled up in the same low range, only the 10 h time point is shown to simplify the representation.