Electronic Supporting Information

Modulating the Catalytic Activity of Enzyme-like Nanoparticles Through their Surface Functionalization

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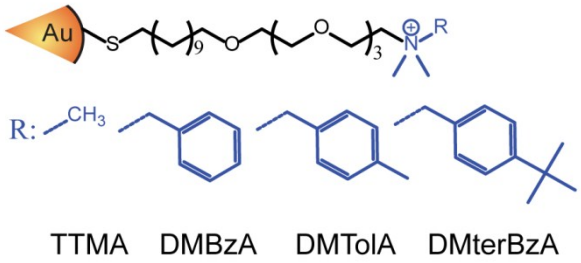
Table of Contents

Experimental Section

Materials All starting chemicals were commercially available. All reagents and solvents, unless mentioned otherwise, were purchased from Fisher scientific. The gold salt was purchased from Strem Chemicals Inc. Allyl carbamate protected rhodamine (**Pro-Rho)** was synthesized as in early report.[1]

ESI-1 Ligands

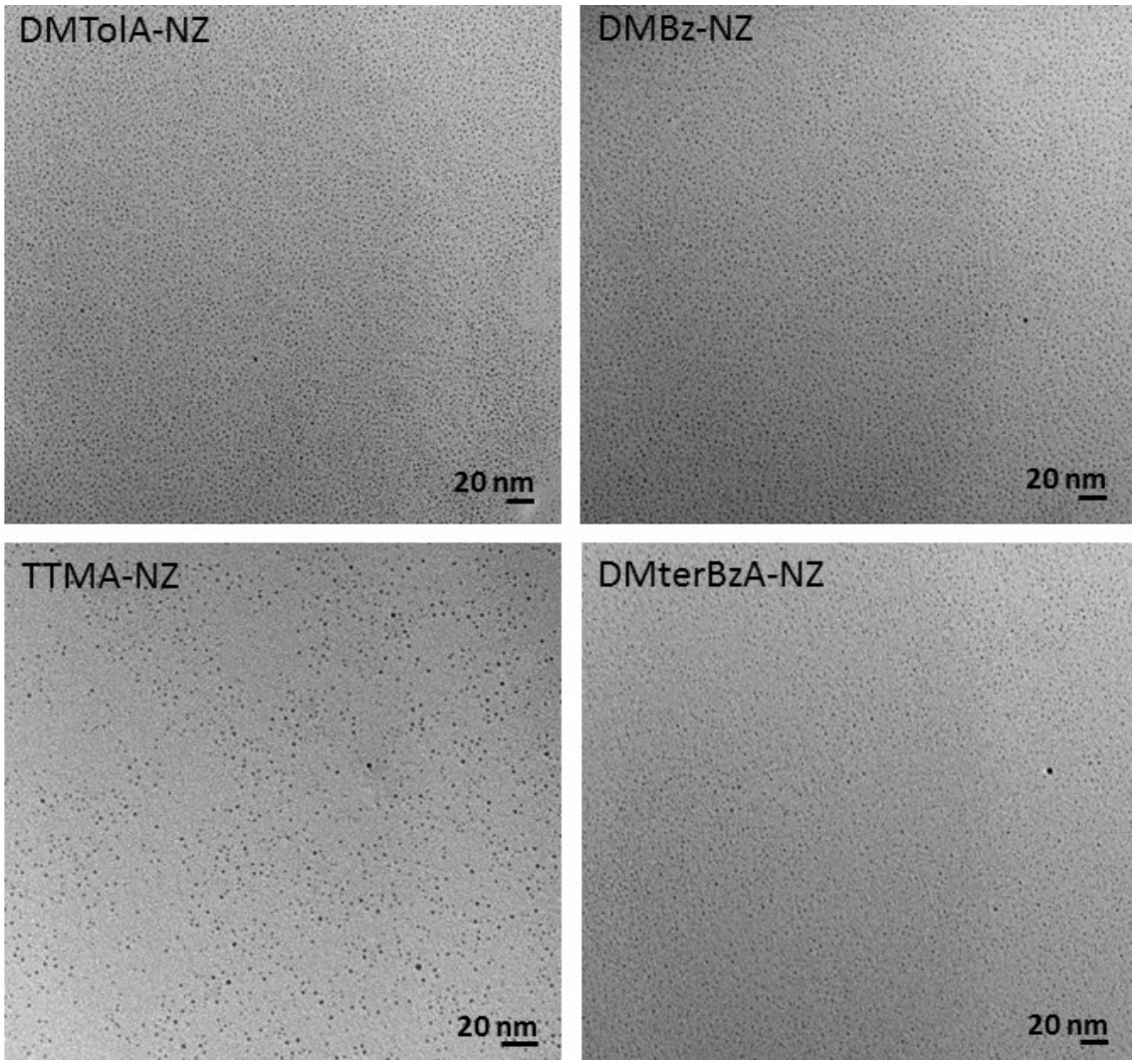
The ligands used for synthesizing the nanoparticle (NP) scaffolds were the following:



TTMA DMBzA DMTolA DMterBzA

TTMA ligand was synthesized as in reference 2. The rest our ligands (the most hydrophobic ones) were synthesized as in reference 3.

ESI-2 Synthesis of nanoparticle. The NPs used in this study were synthesized by the Brust-Schiffrin two-phase method[4] and were post-functionalized using the Murray place exchange reaction.[5] In a typical reaction, 10 mg of NPs capped with pentanetiol is dissolved in 10 mL distilled DCM and purged with argon for 10 min. Subsequently, 50 mg of the corresponding ligand in 2.5 mL of methanol is added to the nanoparticle solution. The reaction mixture was stirred for 2 days followed by removal of solvent mixture. The resulting black colored residue was then washed with a mixture of hexane (90%) and DCM (10%) for five times to remove 1-pentanethiol and excess ligands. The resulting residue was dissolved in distilled water and purified by dialysis with skin membrane (10,000 MWCO) in distilled water for 3 days. Finally, molecular cut off filtration (10,000 MWCO for five times) were performed.



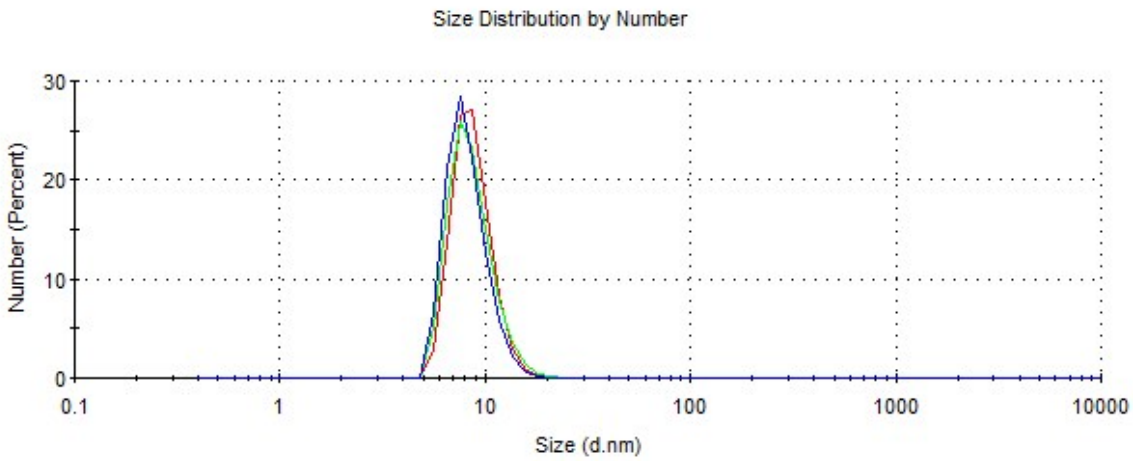
ESI-3 Determination of amount of Ru per NZs

The cuantification of Ru catalysts per particles were carried out by first dissolving 10 μL of NZsolution (10-30 μM) with 0.5 mL of fresh aqua regia. The resulting solution was was then diluted to 10 mL with de-ionized water. Samples composition was then analyzed on a Perkin-Elmer NexION 300X ICP mass spectrometer. ^{197}Au and ^{101}Ru were measured under the standard mode: nebulizer flow rate: 0.95 L/min; rf power: 1600 W; plasma Ar flow rate: 18 L/min; dwell time: 50 ms. Standard Au and Ru solutions (concentration: 0, 0.2, 0.5, 1, 2, 5, 10, and 20 ppb) were prepared for constructing the calibration curve.

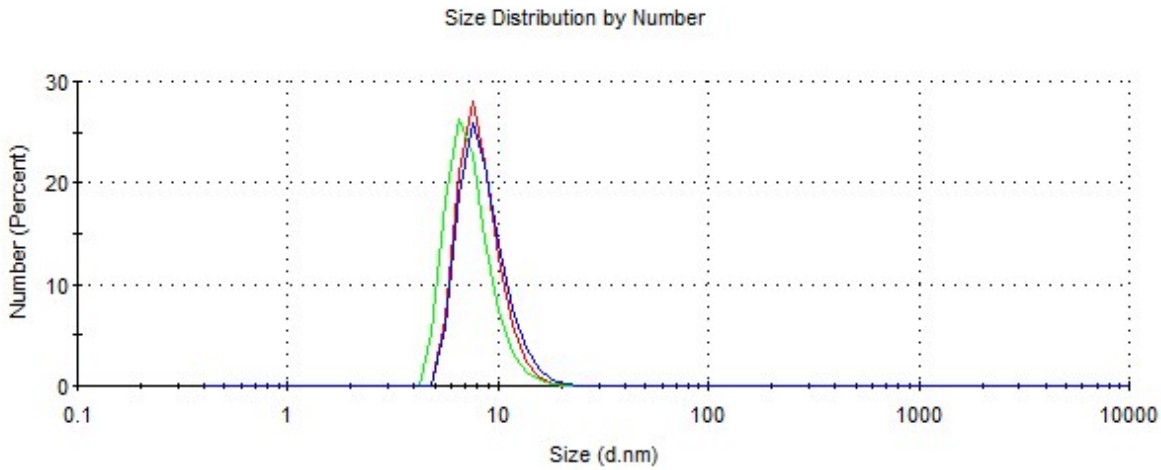
ESI-4 Determination of NZs size by Dynamic Light Scattering

Hydrodynamic diameter of the NZs were measured by dynamic light scattering (DLS) in saline phosphate buffer (PBS, pH=7.4), using a Malvern Zetasizer Nano ZS instrument. The measurement angle was 173° (backscatter). Data were analyzed by the “multiple narrow modes” (high resolution) based on non-negative-least-squares (NNLS).

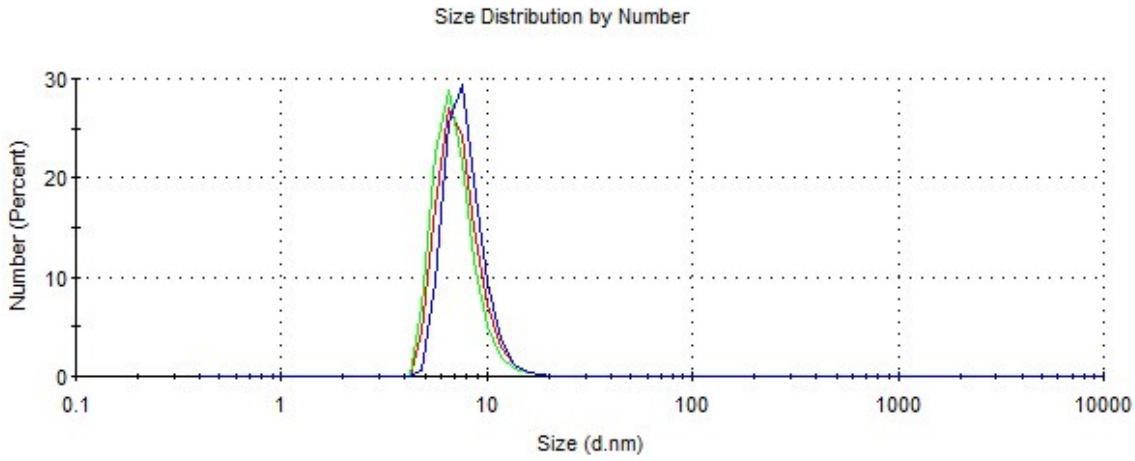
TTMA-NZ (in PBS)



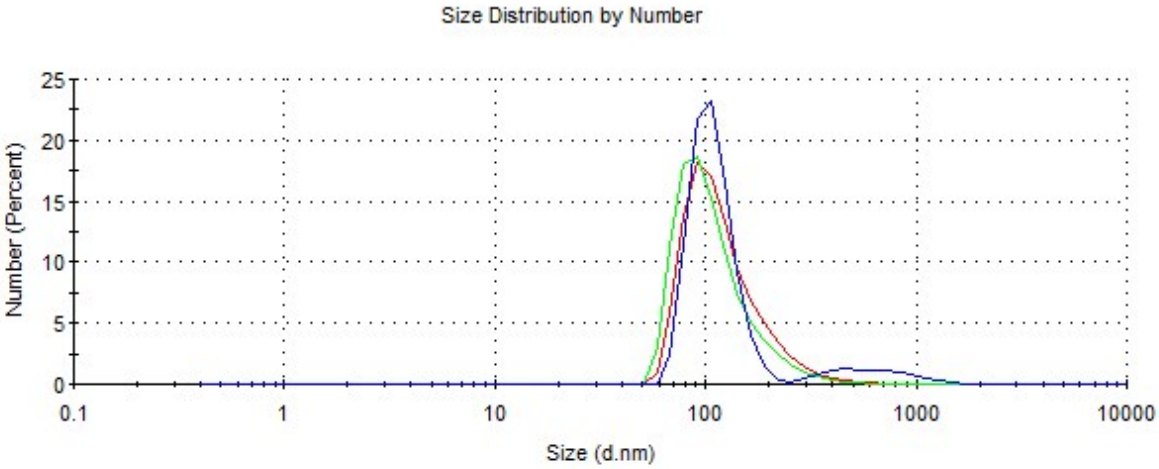
DMBzA-NZ (in PBS)



DMTolA-NZ (in PBS)



DMterBzA-NZ (in PBS)



DMterBzA-NZ

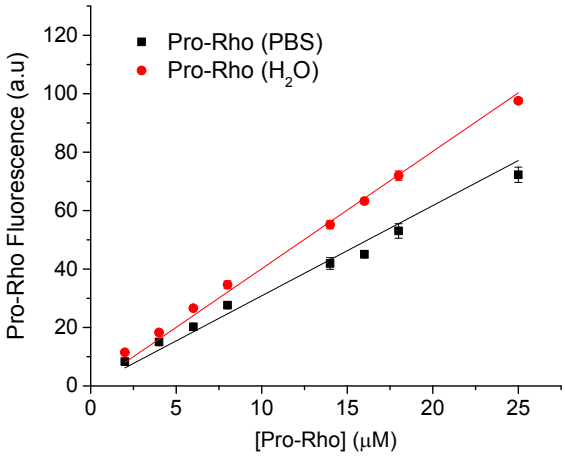
Due to the large aggregation in PBS this nanozyme resulted almost inactive in PBS.

ESI-5 Adsorption experiments

The experiments for determining the adsorption capacity of each nanoparticle scaffold for Pro-Rho were carried out in sodium phosphate buffer (pH 7.4) and deionized water. All nanoparticle and substrate stock solutions were mixed in a 96-well plate to provide 100 μL final solution containing 400 nM of each nanoparticle and **Pro-Rho** at 2, 4, 6, 8, 14, 16, 18 and 25 μM concentrations. Fluorescence variations of adsorbed **Pro-Rho** was registered (Ex: 488 nm, Em: 521 nm, Cut-off: 515 nm) 5 mins after mixing.

The number of adsorbed **Pro-Rho** molecules on each **NP** was determined by analyzing the differences in fluorescence of **Pro-Rho** dissolved in H_2O and PBS media to the mixture containing 400 nM **NPs**. The value of the difference was then divided by the corresponding fluorescence cross section of **Pro-Rho**. The obtained number corresponds to the concentration of Pro-Rho adsorbed on **NPs**. The number of **Pro-Rho** molecules per nanoparticle can be obtained by dividing the concentration of adsorbed **Pro-Rho** by the concentration of **NPs** (400 nM).

The calibration curve for **Pro-Rho** in both media is the following:

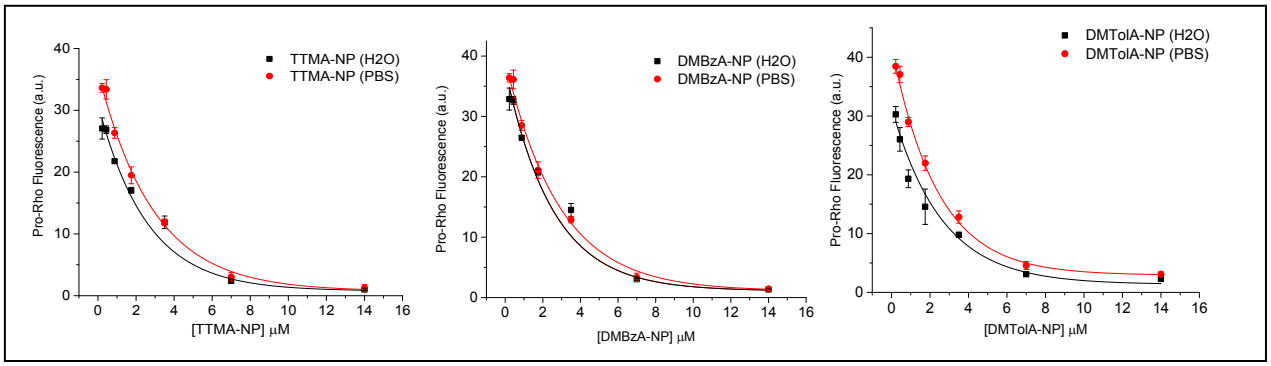


Fl(Pro-Rho, PBS)= 3.09 [**Pro-Rho**] a.u./μM

Fl(Pro-Rho, H_2O)= 4.01[**Pro-Rho**] a.u./μM

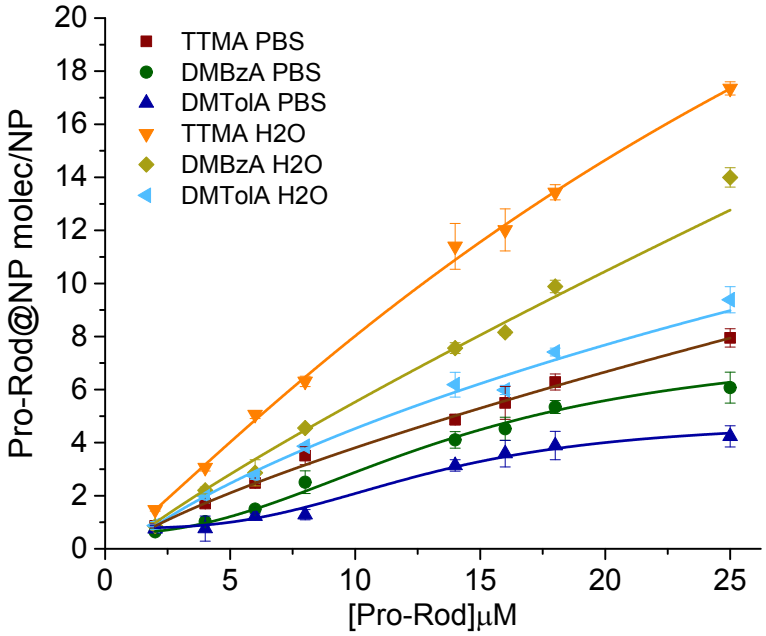
Quenching effect of NPs on Pro-Rho residual Fluorescence

Titration of **Pro-Rho** solution (20 mM) with **NPs** in DI water and PBS.



Adsorption of Pro-Rho on our NPs in DI water

Due to the absence of the screening effect of electrolytes the repulsion among the alkylammonium groups can maximize the expansion of the NPs scaffolds´ monolayer.[20,21] Such conditions constitute a good model to evaluate how the compaction of the monolayer affects the adsorption capacity of our **NPs** scaffolds.

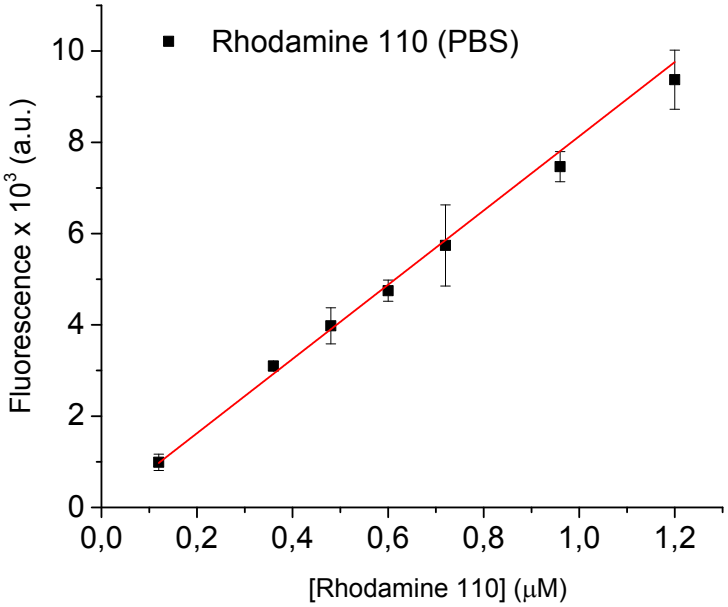


All the nanoparticles displayed a reduced capacity for adsorbing **Pro-Rho** in PBS compared to deionized water. These differences illustrate that increasing the compaction of the monolayer reduces the substrate-**NP** affinity. The observed trend (figure above) suggests that the increase in the hydrophobicity of the alkyl ammonium head group increases the compaction of the monolayer of the nanoparticle (or nanozyme).

ESI-6 Rhodamine 110 standard curve

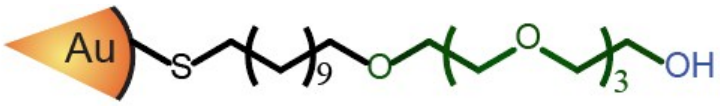
The concentration of the product after catalytic reaction, can be estimated by using the fluorescence intensity coming from rhodamine 110 product. For this purpose, a fluorescence standard curve for rhodamine 110 was obtained. The stock solution of rhodamine 110 (0.3 mM, 1 mL) was prepared in water. Successive dilutions were done, and the fluorescence intensity of

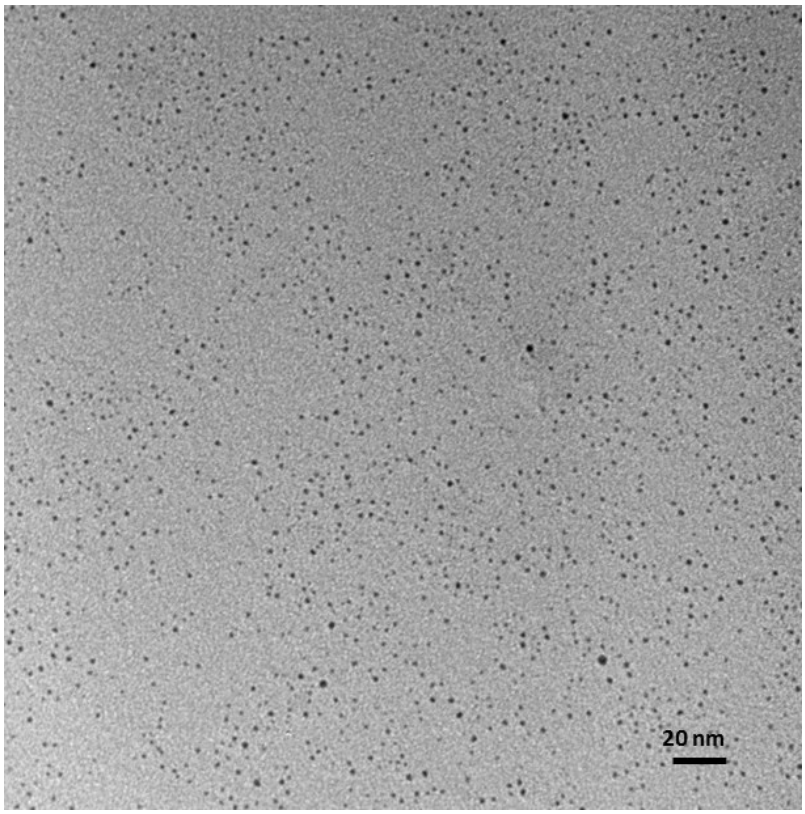
prepared solutions (0.12, 0.36, 0.48, 0.60, 0.72, 0.96, 1.2 μM) were directly measured in a microplate reader (SpectraMax M2). The obtained curve slope is 8.1×10^3 a.u./μM.

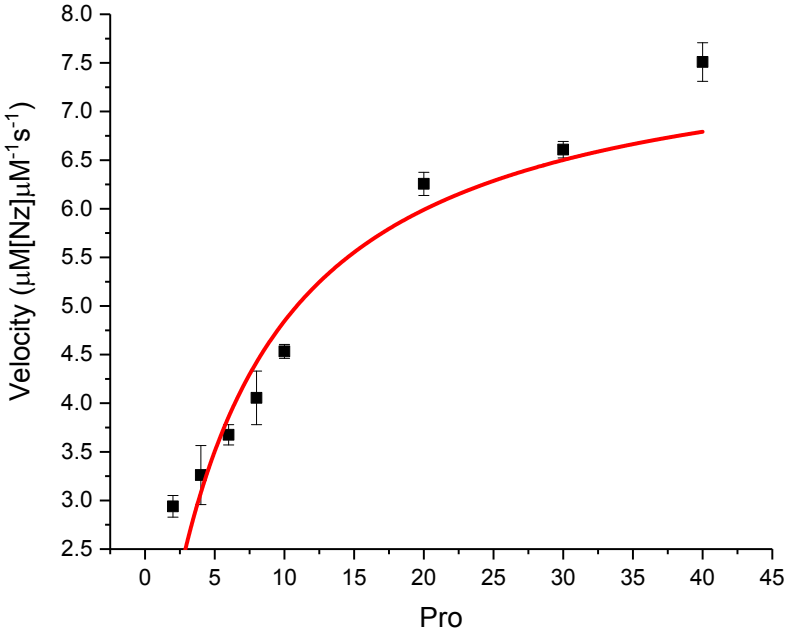


ESI-7 TEGOH-NZ Catalytic activity

Structure of nanoparticle scaffold for TEGOH-NZ



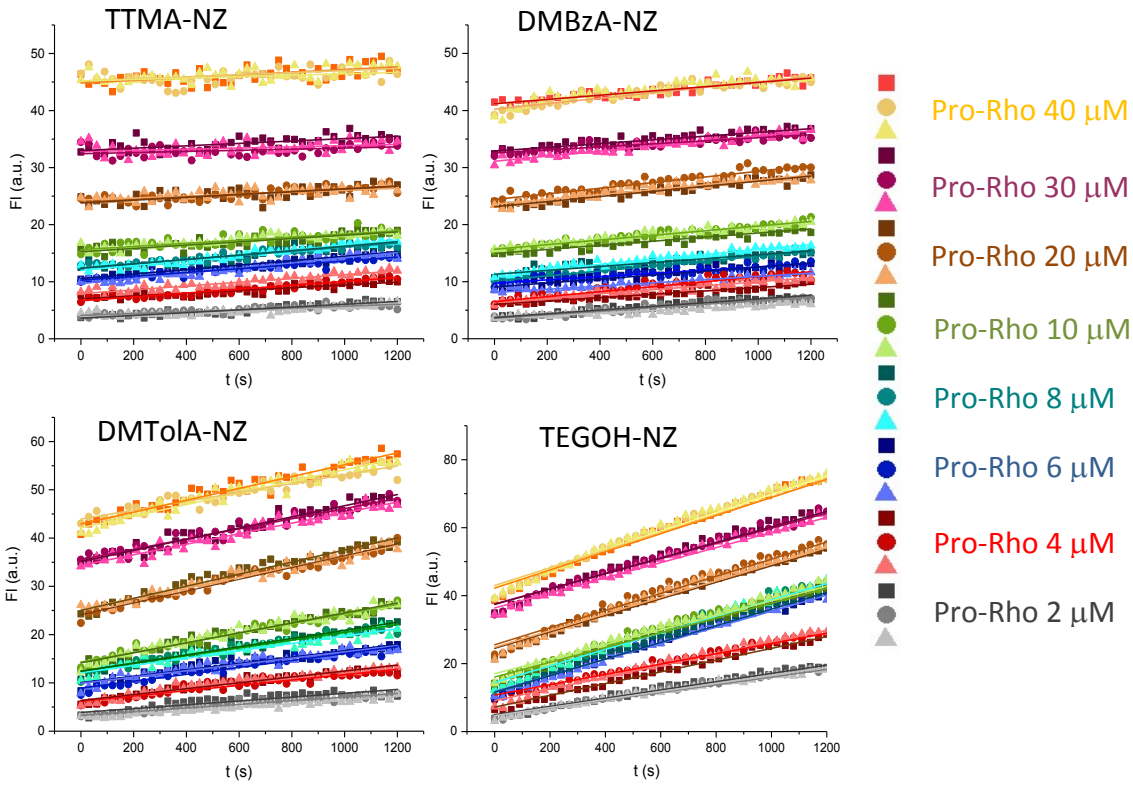




$$\frac{V}{[NZ]} = \frac{k_{Cat}[S]}{K_M + [S]}$$

k_{Cat} = 7.75 ± 0.2 x 10^{-6} s^{-1}
K_M = 6.18 ± 1.5 μM

ESI-8 Catalytic activity of Nanozymes



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[4] M. Brust, M. Walker, D. Bethell, D. J. Schiffrin, R. Whyman, *Chem. Commun.*, 1994, 801–802.

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