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Controlling the lifetime of cucurbit[8]uril based self-abolishing nanozymes

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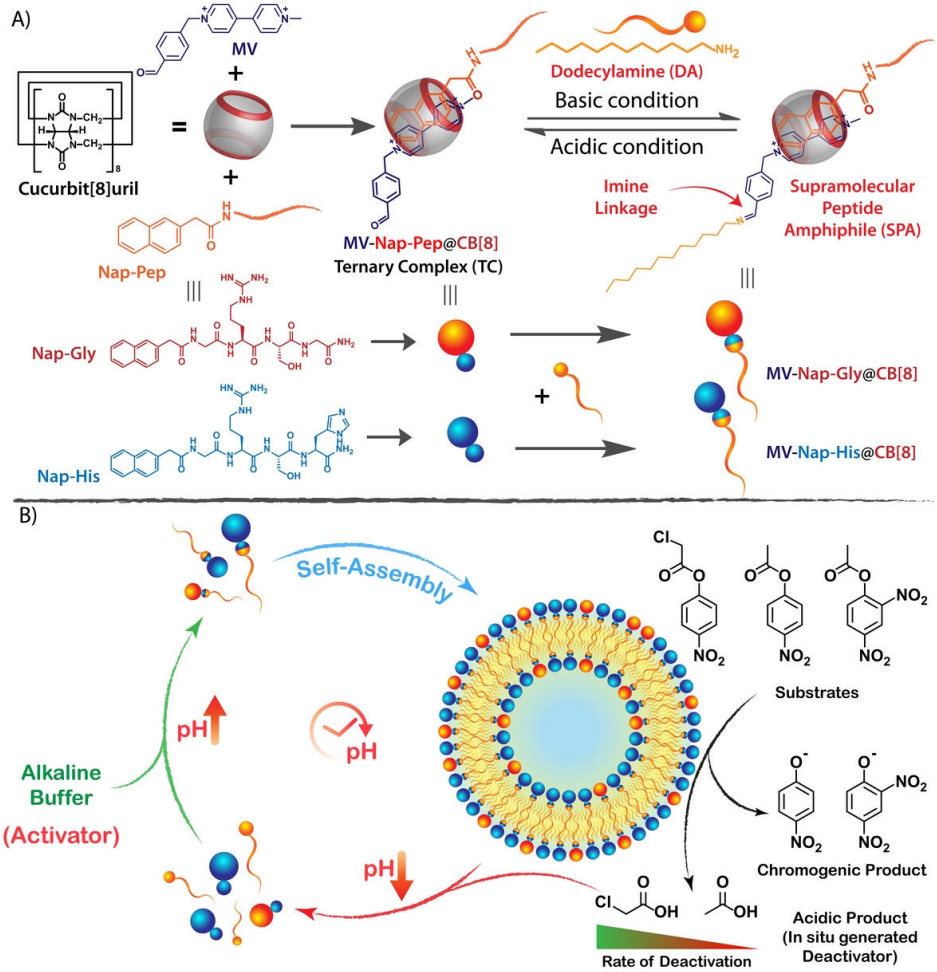
Nature has evolved a unique mechanism of self-regulatory feedback loops that help in maintaining an internal cellular environment conducive to growth, healing and metabolism. In biology, enzymes display feedback controlled switchable behavior to upregulate/downregulate the generation of metabolites as per the need of the cells. To mimic the self-inhibitory nature of certain biological enzymes under laboratory settings, herein, we present a cucurbit[8]uril based pH responsive supramolecular peptide amphiphile (SPA)[1-3] that assembles into hydrolase mimetic vesicular nanozymes upon addition of alkaline TRIS buffer (activator) but disintegrates gradually owing to the catalytic generation of acidic byproducts (deactivator). The lifetime of these nanozymes could be manipulated in multiple ways, either by varying the number of catalytic groups on the surface of the vesicles, by changing the acid generating substrate, or by changing the ratio between the activator and the substrate. The self-inhibitory nanozymes displayed highly tunable lifetimes ranging from minutes to hours, controlled and in situ generation of deactivating agents and efficient reproducibility across multiple pH cycles.



Scheme 1: Chemical structures of different components and graphical presentation of **(A)** the formation of CB[8] assisted SPA under alkaline condition and **(B)** the self-inhibitory feedback driven temporal formation of nanozymes showing hydrolase like catalytic activity.

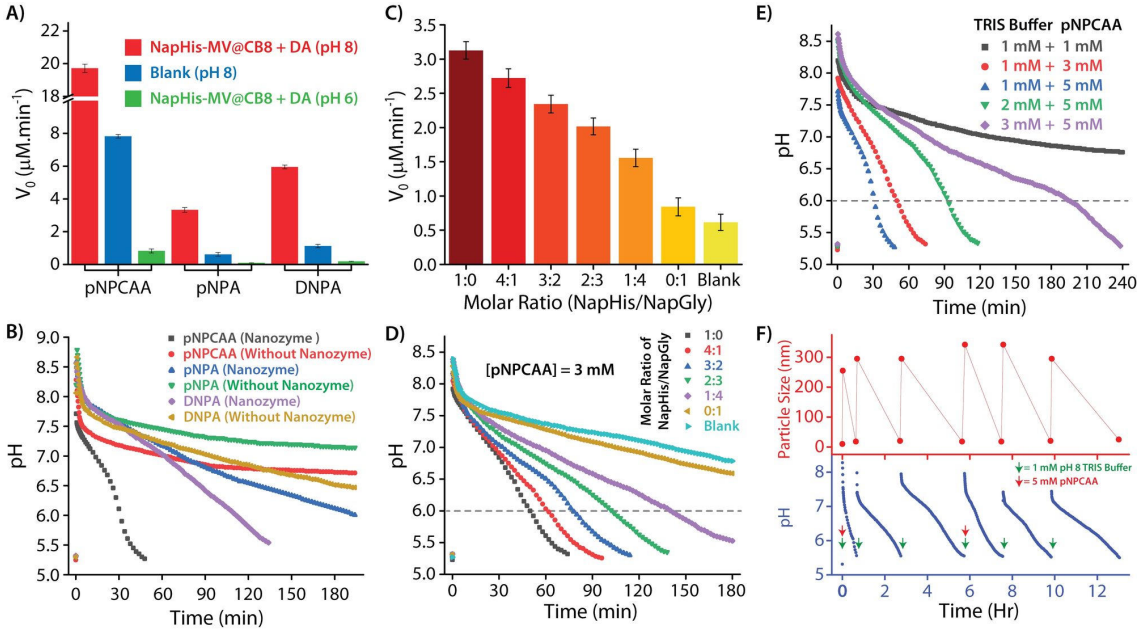


Figure 1: (A) Rate of hydrolysis of pNPA/pNPCAA/DNPA (0.5 mM) by the alkaline buffer (blank, pH 8) and the assembled/disassembled nanozyme (100 μM) at pH 8/6, respectively. **(B)** pH clock with/without the nanozyme (100 μM) using pNPA/pNPCAA/DNPA (5 mM) as substrates and 1 mM pH 9 TRIS buffer as the activator. **(C)** Rate of hydrolysis of pNPA (0.5 mM) using different molar ratios of NapHis/NapGly ([nanozyme] = 100 μM) in pH 8 buffer. **(D)** pH clock using different molar ratios of NapHis/NapGly nanozyme (100 μM), 1 mM pH 9 TRIS buffer and 3 mM pNPCAA. **(E)** pH clock using various molar ratios of pH 9 TRIS buffer and pNPCAA in the presence of 100 μM nanozyme **(F)** Time dependent variation in pH (bottom) and particle size (top) across six consecutive pH cycles.

As outlined above, the first approach to regulating the lifetime of the transient nanozymes involves varying the acid generating ester substrates. Depending upon the rate of hydrolysis of the esters and the acidic strength of the generated acids, the lifetime of the nanozymes could be easily tuned **(Fig. 1A/B)**. Second, the propensity of the nanozyme to hydrolyze the esters and generate acidic deactivators depends on the number of hydrolytically active head groups (His) on the surface of the vesicular nanozymes and thus, by varying the ratio of active (His) and inactive (Gly) head groups on the surface, the rate of dissipation of the nanozymes can be modulated **(Fig. 1C/D)**. Another approach to tune the lifetime of the transient state is to vary the molar ratio of the activator (pH 9 TRIS buffer) and the substrate (pNPCAA). For a fixed concentration of TRIS buffer (1 mM), a higher concentration of pNPCAA resulted in shorter lifetimes of the nanozymes owing to the faster rate of hydrolysis **(Fig. 1E)**. Higher concentrations of TRIS buffer against a fixed concentration of pNPCAA (5 mM), however, resulted in longer lifetimes on account of the decrease in the ability of the generated acid to neutralize higher concentrations of the alkaline buffer. Furthermore, akin to natural counterparts, the vesicular nanozymes could be reactivated by fresh addition of the activator after consecutive pH cycles and the activation/deactivation process could be efficiently cycled across six consecutive cycles without significant dampening **(Fig. 1F)**. In nutshell, the self-inhibitory feedback mechanism employed herein presents a new approach to generation of transient functional assemblies and may serve as a novel addition to the progressively developing field of out-of-equilibrium systems chemistry.

References

1. Das, S.; Das, P.; Dowari, P.; Das, B. K.; Das, D., Bi-directional feedback controlled transience in Cucurbituril based tandem nanozyme. *Journal of Colloid and Interface Science* **2022**, *614*, 172-180.

2. Das, S.; Das, T.; Das, P.; Das, D., Controlling the lifetime of cucurbit[8]uril based self-abolishing nanozymes. *Chemical Science* **2022**. DOI: 10.1039/D1SC07203J

3. Das, S.; Dowari, P.; Pramanik, B.; Das, D., pH clock instructed transient supramolecular peptide amphiphile and its vesicular assembly. *Chemical Communications* **2019**, *55 (94)*, 14119-14122.