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Tailored biocatalyst achieved by the rational anchoring of imidazole groups on a natural polymer: furnishing a potential artificial nuclease by the sustainable material engineering

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1. Experimental details: synthesis

DEDNPP was prepared by standard methods from $POCl_3$, as described previously.[1] Inorganic salts were of analytical grade and used without further purification.

GAIMZ was prepared as described in the literature and modified as follows: (i) 0.5 g of commercial GA(from *Acacia senegal* and *Acacia seyal*) was dissolved in 50 mL of deionized water and the mixture was left stirring overnight at room temperature; (ii) after, 1.52 g (13.2 mmol) of N-hydroxysuccinimide (NHS) and 2.53 g (13.2 mmol) of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were added to the GA solution into a round-flask, under ice-bath. The mixture was left stirring for 2 h, followed by the addition of 1.57 mL (13.2 mmol) of N-(3-Aminopropyl)-imidazole (API), which was left stirring overnight at room temperature. The resulting GAIMZ was dialyzed against deionized water for 3 days. The final stock solution of GAIMZ was kept in the freezer.

We estimate that the stock solution of GAIMZ is ~8.0 mg/mL, from previous data of GA (Absorbance vs concentration). This is reasonable since the starting solution of GA is 10 mg/mL, which is functionalized and finally dialyzed, whereby, more dilute. Due to the small degree of functionalization, the incorporated mass of IMZ moieties should not

significantly affect this concentration. Actually, we consider most important evaluating the concentration of GAIMZ as a function of the reactive IMZ groups anchored, *i.e.*, local concentration, and the stock solution is estimated as 2 mM, represented herein as [IMZ^{local}]. This was confirmed by the colorimetric assay of glucuronic acid content and potentiometric titration analysis. Given the concentration of the stock solution, all reactions were followed with concentrations $\leq 2 \times 10^{-3}$ M.

2. Colorimetric assay

The uronic acid content of GA and GAIMZ was determined by the *m*-hydroxydiphenyl method described by Filisetti-Cozzi and Carpita.[2] To 400 µL of each sample solution were added 40 µL of 4 M sulfamic acid/potassium sulfamate solution (pH 1.6) and 2.4 mL of 75 mM sodium tetraborate in sulfuric acid solution and vortex vigorously. The tubes were heated to 100 ºC in a boiling water bath for 10 min and then plunged into an ice bath for 10 min. After, 80 µL of *m*-hydroxidiphenyl solution was added to each tube, vortex vigorously and the absorbances were read at 525 nm on a Shimadzu UV-Vis spectrophotometer. The uronic acid content was calculated based on a glucuronic acid standard curve (Fig S1).

Analysis with GA and GAIMZ was carried out in triplicate and the last showed a 15.5±0.9 % in mass of glucuronic acid.

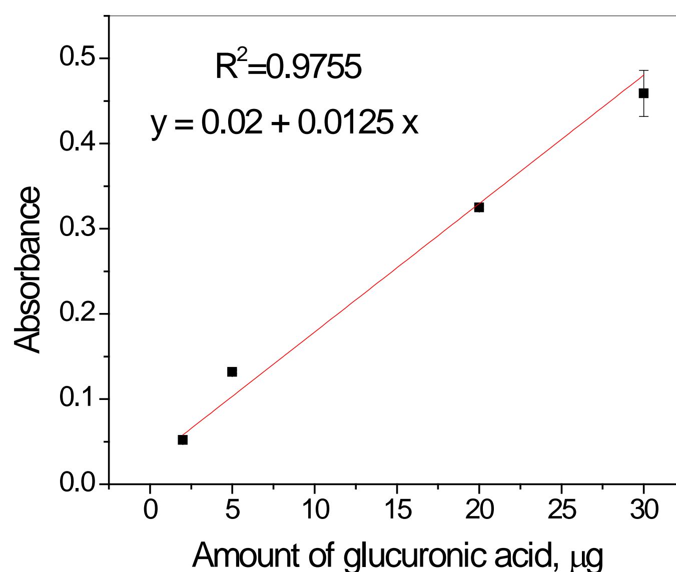

Figure S1 –– Standard curve of glucuronic acid measured at 525 nm

3. FTIR Analysis

GAIMZ was lyophilized and the solid was characterized by FTIR, recorded on a FTIR Bio-Rad spectrophotometer over the range of 4000-400 cm^{-1}, using the KBr pellet method. Comparison spectrum for GA was obtained directly from the stock solid.

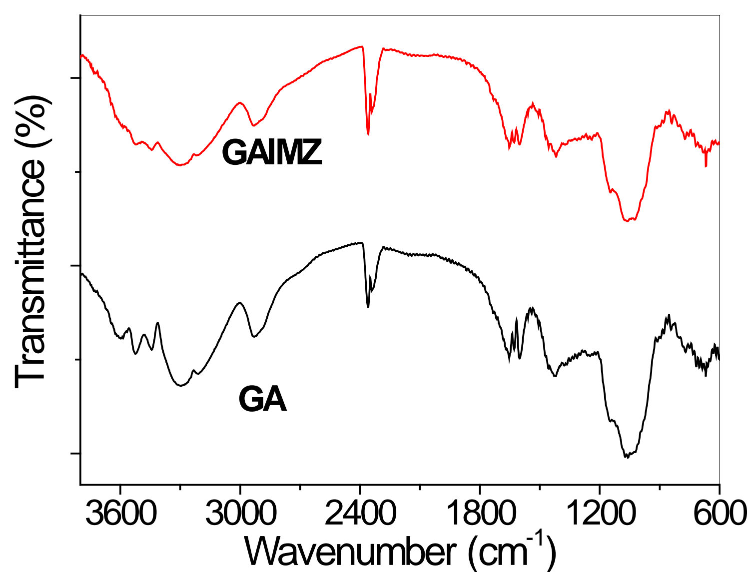

Figure S2. FTIR spectra for GA and GAIMZ.

4. NMR Analysis

An amount of the stock solution of GAIMZ was heated under vacuum yielding 0.3 mL of a solution with estimate concentration of 30 mg/mL, which was mixed to 0.3 mL of D_2O. It was submitted to ^{13}C NMR analysis at 50°C using a 400 MHz Bruker DRX Avance spectrometer equipped with a 5 mm inverse probe. Chemical shifts were expressed in δ ppm relative to a standard of acetone (δ 30.2).

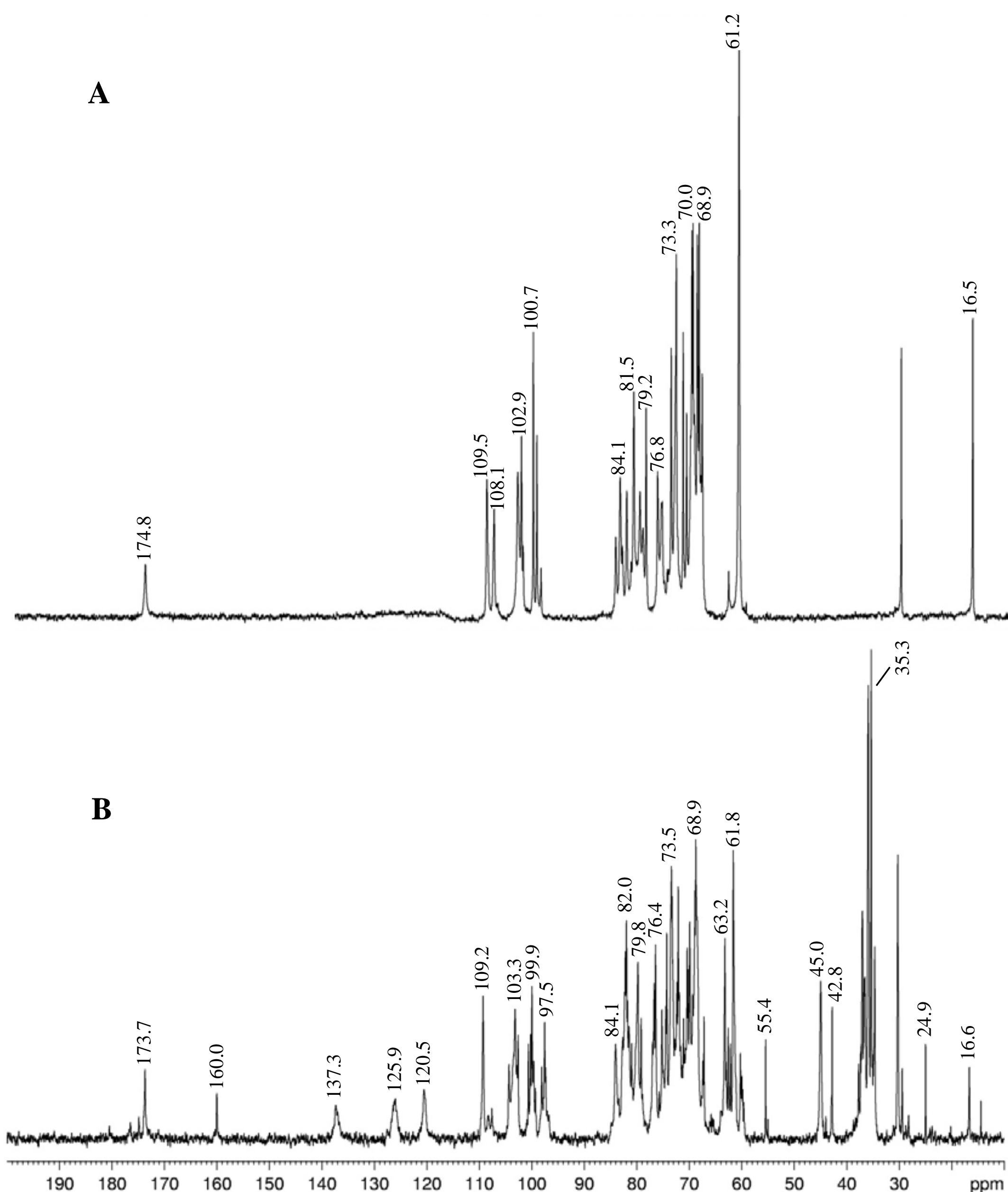

Figure S3.^{13}C NMR spectra for (**A**) GA and (**B**) GAIMZ, in H_2O +D_2O at 50 ºC. Numerical values in δ (ppm)

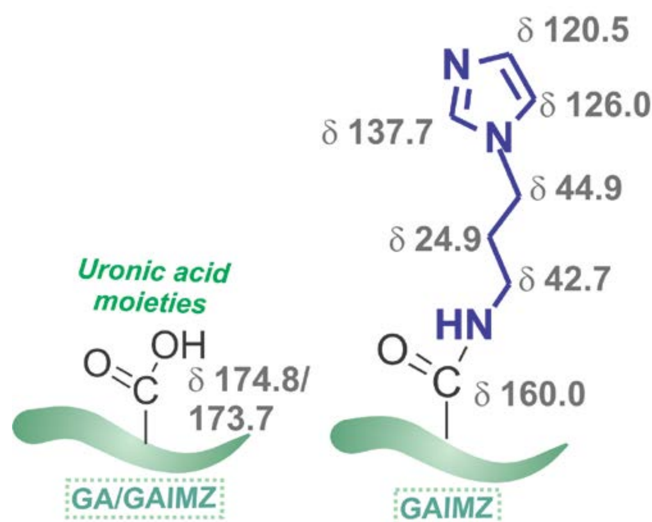

Figure S4. ^{13}C NMR assignments.

5. Kinetics

Reactions of DEDNPP were followed spectrophotometrically (UV-Vis Shimadzu UV2401PC) by monitoring the appearance of 2,4-dinitrophenolate (DNP) at 400 nm. Reactions were started by adding 10 μL of 7.5 mM stock solutions (in acetonitrile) of the substrate to 3 mL of aqueous solutions of GAIMZ, at different pH values (pH 8-10) and kept under controlled temperature (25°C). Solutions were buffered with 0.01 M of $KHCO_3$ (pH 7.0) and K_2HPO_4 (7.5-10.5). Absorbance versus time data were stored directly on a microcomputer, and observed pseudo-first-order rate constants, k_{obs}, were calculated, using an iterative least-squares program; correlation coefficients were > 0.999 for all kinetic runs. For the recycling of GAIMZ, reaction with DEDNPP at pH 8.5 was carried out as previously described. After completion, the reaction medium was left standing for 1 day and another aliquot of DEDNPP was added (10μL of stock solution). This "second" reaction as followed and the procedure was repeated for 4 cycles.

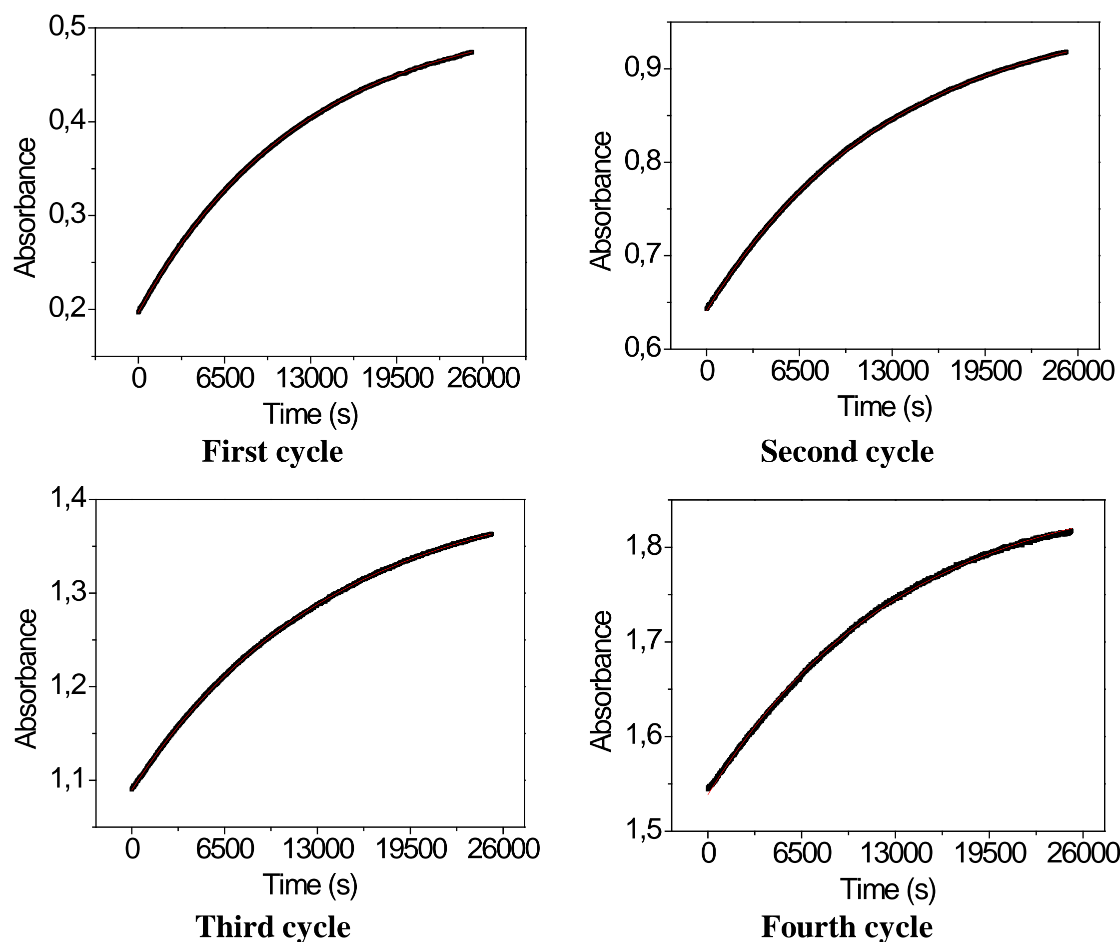

Figure S5. Kinetic profiles for the recycling (four consecutive cycles) for the reaction of GAIMZ with DEDNPP at pH 8.53. Solid line is the pseudo-first order fit.

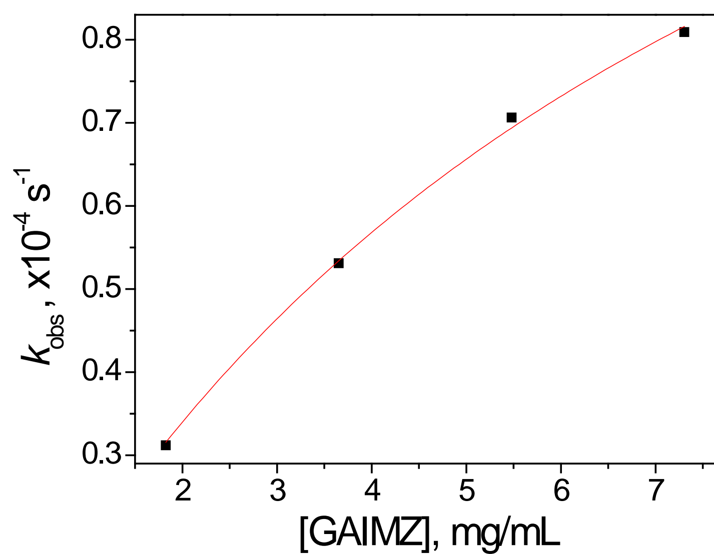

Figure S6. Dependence of k_{obs} with the concentration of GAIMZ in the reaction with DEDNPP, at 25°C and pH 8.53.

6. Potentiometric titration

Potentiometric titrations were carried out in a 100.0 mL thermostated cell, at 25°C. A solution containing 20 mL of the sample was acidified with HCl (0.1 M) and titrated with small increments of KOH, which was CO_2 free. The pH was monitored by a pHmeter. The concentration of KOH used was 7.965 x10^{-3} M and 9.953x10^{-3} M for GA and GAIMZ, respectively. The concentration of GA titrated was 10.00 mg/mL, while for GAIMZ was 1.83 mg/mL. The program BEST7[3] was used to determine the equilibrium constants and also to quantify the percentage of the ionic groups, using the value -13.78 for pK_w. Three pK_a´s values were determined for GAIMZ, which refer to (i) uronic acid groups (~4.5); (ii) cationic species of amino acid residues (~6.5) and (iii) imidazole groups (~7.7). These pK_a´s are expected, since the values for uronic acid and amino acid residues have been reported for other complex structures.[4-6] Values for imidazole groups are high, but also expected for complex samples where neighboring groups can lead to less acidic imidazole groups, such as in proteins (histidine residues in enzymes).[7,8] This approach has been successfully used by other authors in complex systems[9,10] such as the presented here, thus validating the present considerations.

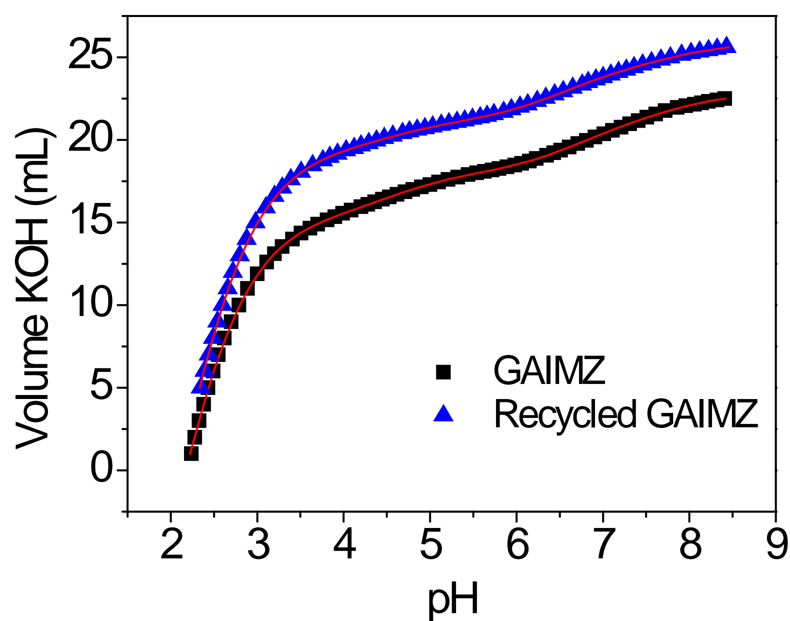

Figure S7. Potentiometric titration curve for GAIMZ before and after recycling in the reaction with DEDNPP. The base used was KOH 0.09 M, at 25°C. Solid line is the fit calculated using the program BEST7.[3]

Table S1 – pK_a values determined for GAIMZ before and after recycling**.**

Constants	**GAIMZ**	**Recycled GAIMZ**
pK_{a1} (uronic acids)	4.47±0.01	4.24±0.01
pK_{a2} (amino acids)	6.49±0.01	6.47±0.01
pK_{a3} (imidazole)	7.48±0.01	7.51±0.01
% of imidazole groups	17.0%	18.3%*

* The slight increase is probably due to the residual phenolic product after recycling.

7. DNA Cleavage

For the DNA cleavage assay, 120 ng of plasmid pUC19 (New England Biolabs) were incubated with the indicated amounts of GA and GAIMZ for 16 hours at 37°C in the presence of Tris-HCl buffer pH8.0. The DNA was submitted to 1% agarose electrophoresis in TBE buffer (Tris 44.5 mM, boric acid 44.5 mM, EDTA 1 mM pH8.3) and stained with ethidiumbromide. Gels were run at 80V for 90 minutes on 8 cm long horizontal gels. Stained gels were visualized under UV-light (312 nm) with an EC3 transilluminator from UVP from BioImaging Systems (Upland-CA, USA) equipped with CCD camera. Plasmid integrity was evaluated by the presence of linear form of the plasmid on the basis of its distinct electrophoretic mobility.

Figure S8. Control reaction with plasmid DNA with buffered Tris-HCl pH 8 solution, followed for 16 h at 37°C.

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