

# Optimization of poly(*N*-isopropylacrylamide) as an artificial amidase

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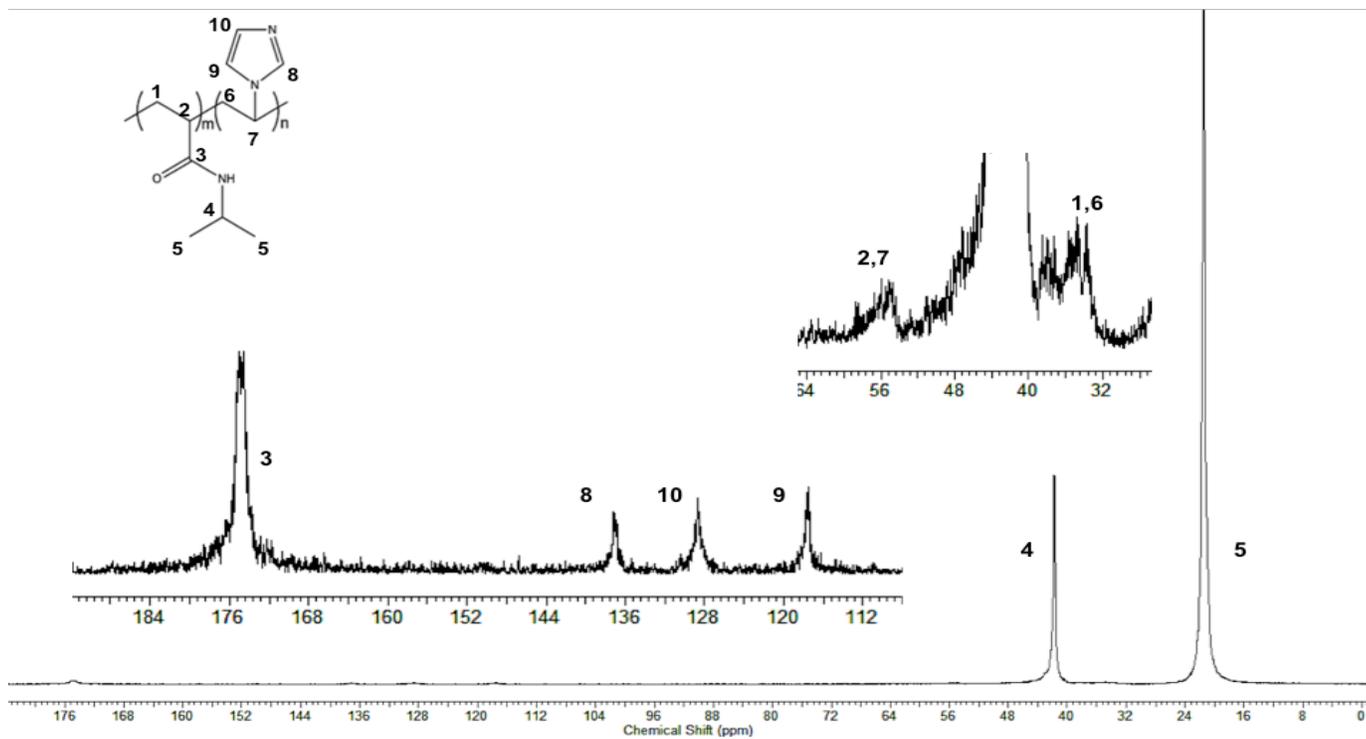
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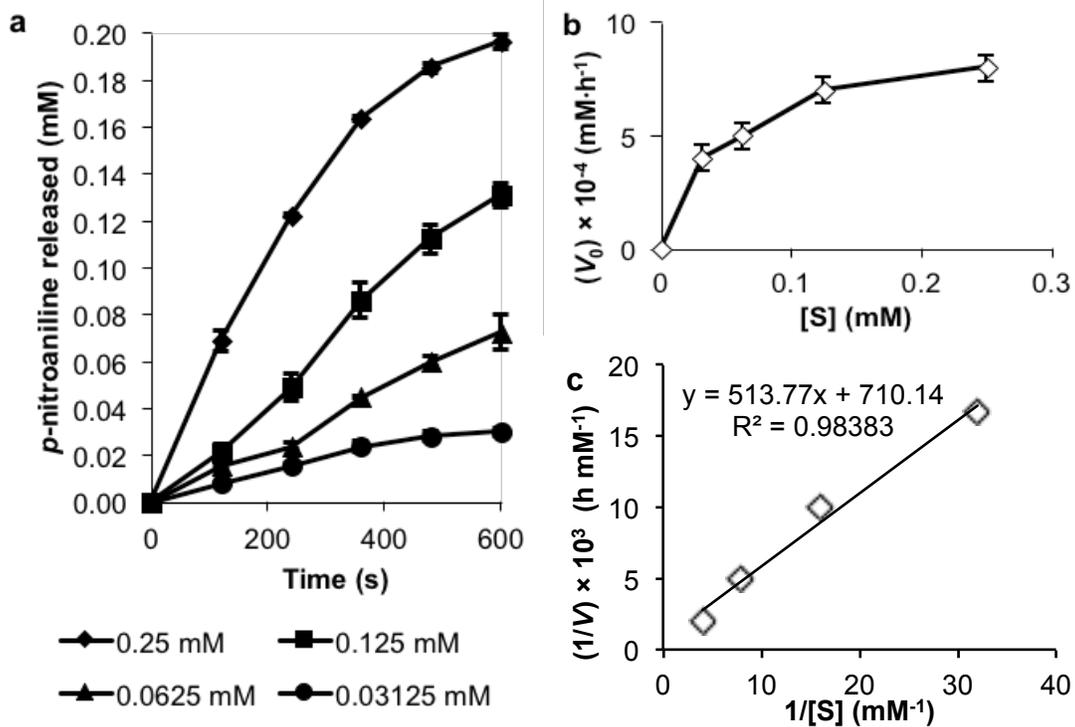
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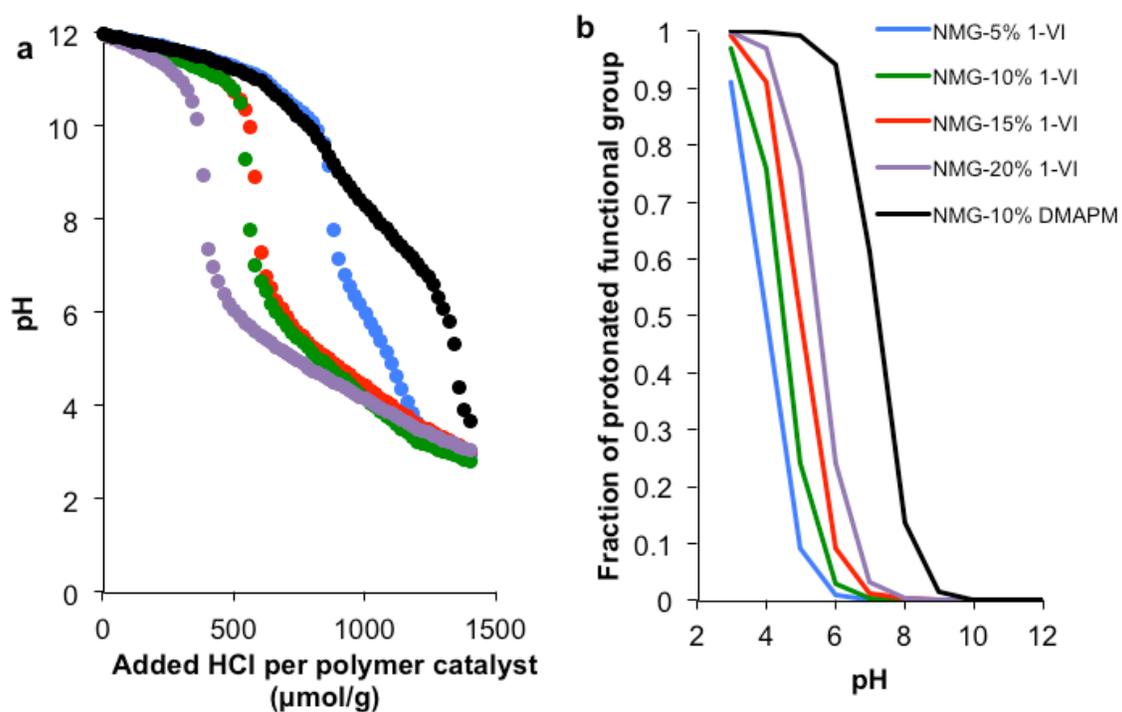
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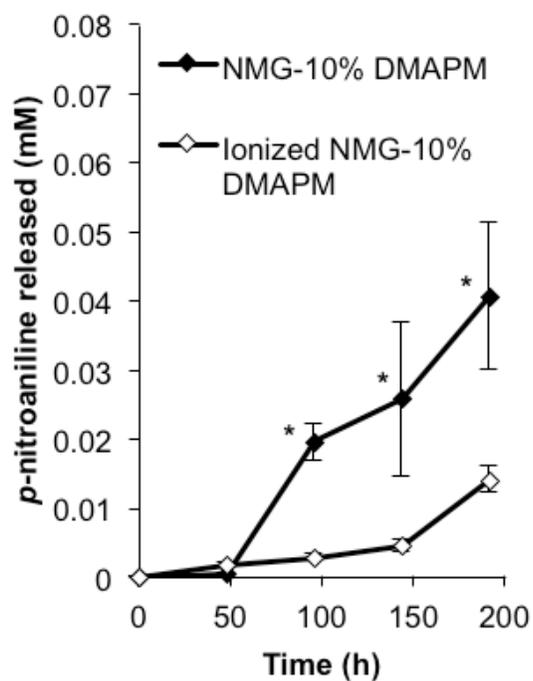
**Figure S1.**  $^{13}\text{C}$  NMR of NMG-20% 1-VI in  $\text{D}_2\text{O}$ .



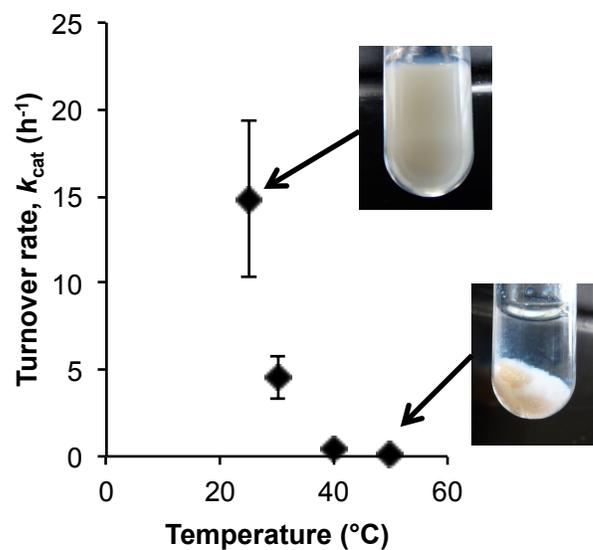
**Figure S2.** Esterase activity on substrate, *p*-nitrophenyl acetate using 40 g/L of NMG-1-VI 20% at 25°C, pH 8. (a) Amount of *p*-nitrophenol released as a function of time. Initial velocity obtained was plotted as (b) Michaelis-Menten plot and followed by (c) Lineweaver-Burk plot to determine the catalytic constants. All data shown are the means of triplicate tests.



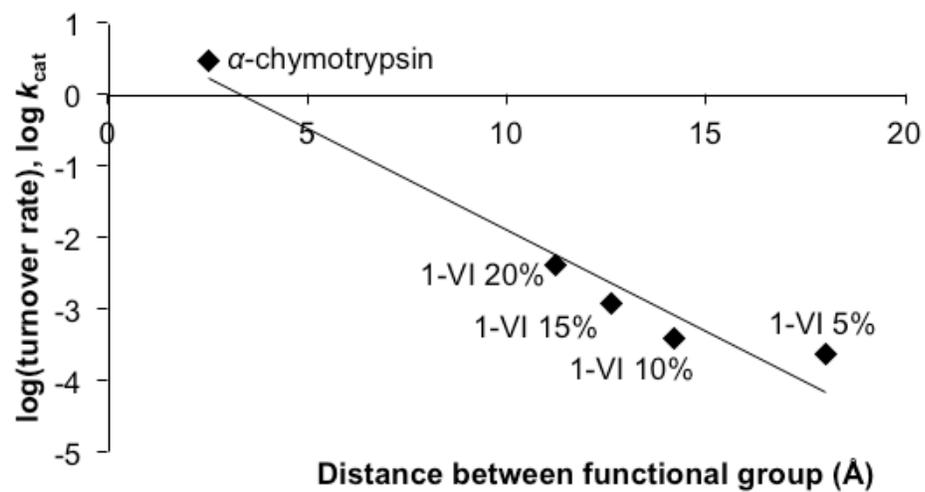
**Figure S3.** (a) Acid–base titration of polymer catalyst solution of NMG–5% 1-VI (●), NMG–10% 1-VI (●), NMG–15% 1-VI (●), NMG–20% 1-VI (●), and NMG–10% DMAPM (●) at 25°C against HCl. (b) The fraction of protonated functional group for different NMGs.



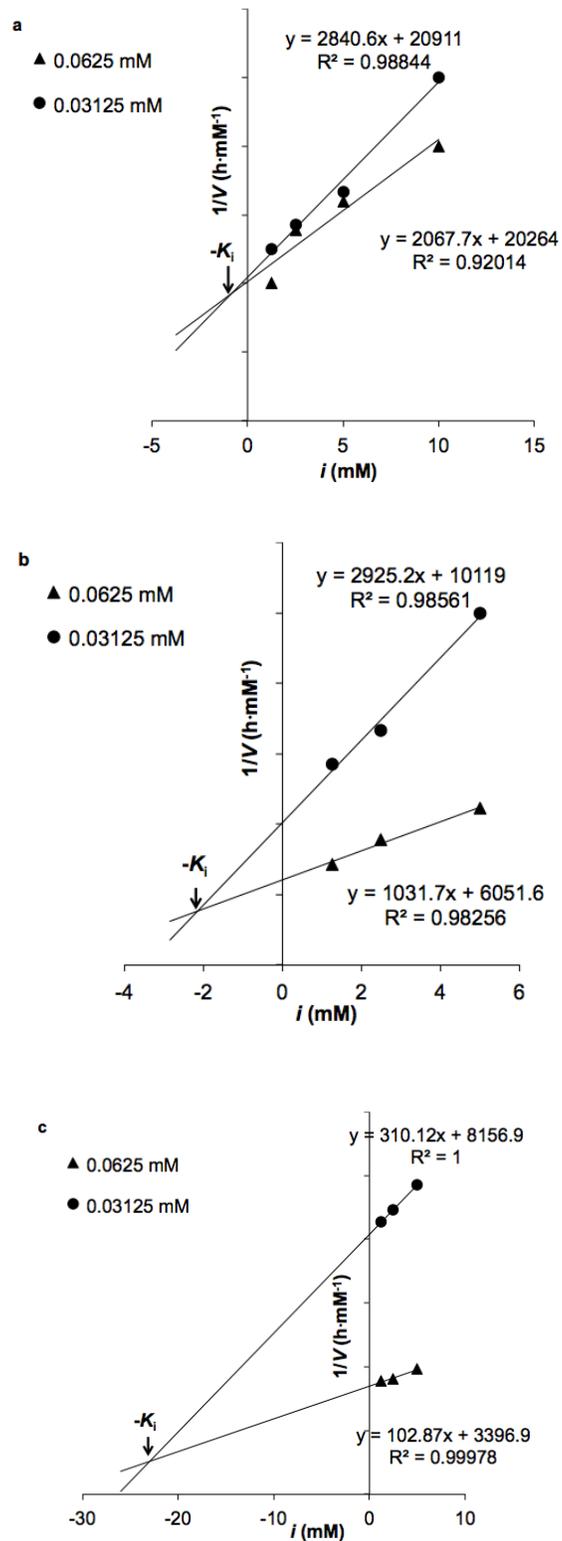
**Figure S4.** Amidase activity on substrate, L-alanine *p*-nitroanilide (0.25 mM) using ionized NMG–10% DMAPM and NMG–10% DMAPM (as control) at 25°C, pH 8. All data shown are the means of triplicate tests, and mean data accompanied by asterisks are significantly different (Tukey's HSD test,  $p < 0.05$ ).



**Figure S5.** The influence of temperature on the amidase activity on substrate, L-alanine *p*-nitroanilide using 40 g/L of NMG-1-VI 20% at pH 6. Insets show the transition of NMG from swollen state (25°C) to shrunken state as the temperature increased to 50°C. All data shown are the means of triplicate tests.



**Figure S6.** Linear plot of the relationship between catalytic rate and calculated distance. Turnover rate for  $\alpha$ -chymotrypsin was taken from the reported result as mono-peptide-*p*-nitroanilide substrates were used.



**Figure S7.** Dixon plots of NMG–20% 1-VI with different types of inhibitors (a) UV, (b) PMSF and (c) E-64 using L-alanine *p*-nitroanilide as substrate at 25°C, pH 6.

Figure S5 shows the Dixon plot which was used to calculate the inhibitor constant,  $K_i$  of the NMG. According to the Dixon method,  $K_i$  is determined when the straight lines generated from different substrate concentrations intercept each other at a point on the left of the vertical axis. Therefore, the value of  $-K_i$  can be determined directly.<sup>1</sup>

**Table S1.** Distance between functional group of the NMG based on the proposed theoretical average distance.

<b>NMG-<i>x</i> 1-VI</b>	<b>Distance,</b>
<b>(%)</b>	<b>(Å)</b>
5	18.0
10	14.2
15	12.6
20	11.2
<i>α</i> -chymotrypsin	2.6 – 2.9 <sup>a</sup>

<sup>a</sup>Distance among the catalytic triads at the active site of *α*-chymotrypsin are Ser 195 – His 57, 2.74 – 2.82 Å and His 57 – Asp 102, 2.61 – 2.64 Å as reported.<sup>2</sup>

**Table S2.** Debye-Huckel length of NMGs.

<b>Sample</b>	<b>Debye-Huckel length, <math>1/\kappa^a</math> (<math>\times 10^2</math> nm)</b>
NMG–5% 1-VI	3.76
NMG–10% 1-VI	3.84
NMG–15% 1-VI	4.71
NMG–20% 1-VI	7.44
NMGs in 0.1 M Acetate buffer	0.01
$\alpha$ -chymotrypsin	0.40
$\alpha$ -chymotrypsin in 0.1 M Sodium phosphate buffer <sup>b</sup>	0.01

<sup>a</sup>Debye-Huckel length is calculated based on the equation,

$$\frac{1}{\kappa} = \frac{304 \text{ pm}}{\sqrt{c} \text{ mol/L}}$$

where,  $1/\kappa$  denotes Debye-Huckel length and  $c$  is concentration of electrolyte.

<sup>b</sup>Condition of  $\alpha$ -chymotrypsin is based on the catalysis using mono-peptide-*p*-nitroanilide substrates.<sup>3</sup>

## REFERENCES

- (1) Dixon, M. *Biochem. J.* **1953**, *55*, 170–171.
- (2) Blow, D. M. *Acc. Chem. Res.* **1976**, *9*, 672–678.
- (3) Ascenzi, P.; Menegatti, E.; Guarneri, M.; Bortolotti, F.; Antonini, E. *Biochemistry* **1982**, *21*, 2483–2490.