

## Supporting Information

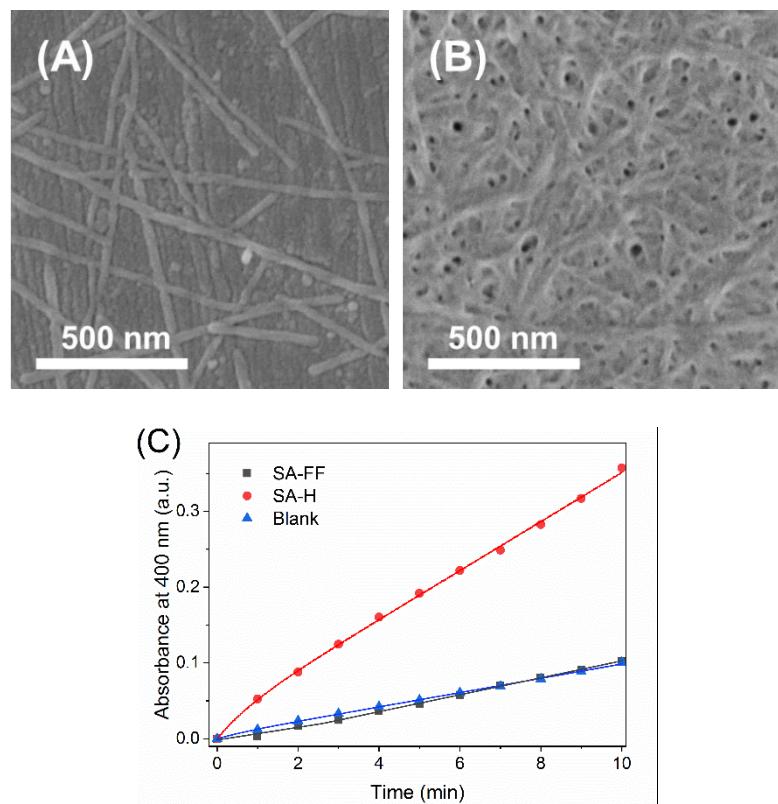
### Constructing peptide-based artificial hydrolase with customized selectivity

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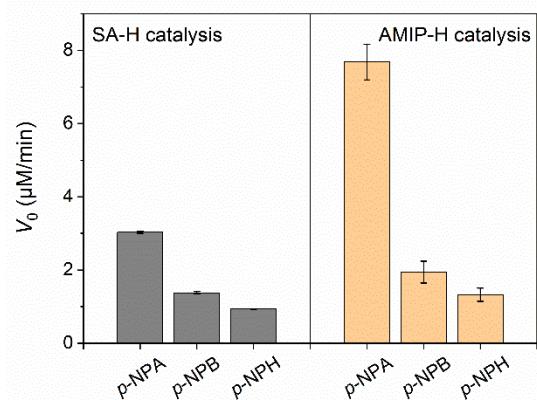
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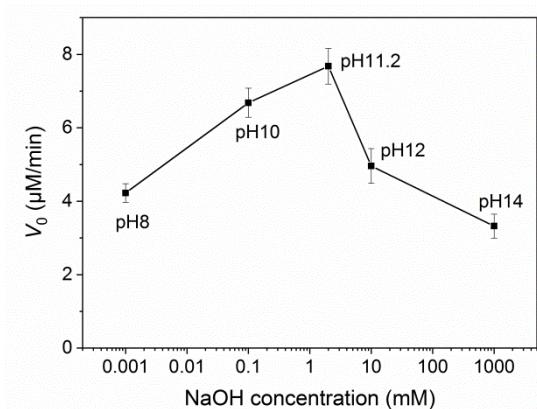
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**Figure S1.** SEM images of (A) SA-FF and (B) SA-H. (C) Plots of absorbance at 400 nm vs time for the hydrolysis of *p*-NPA.

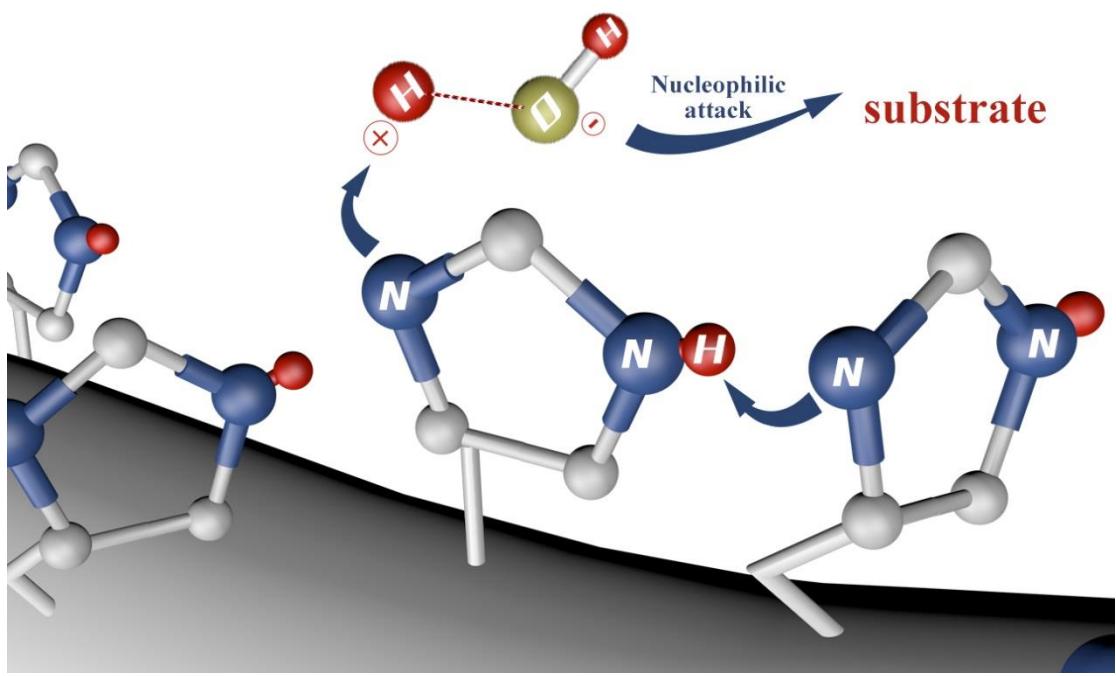


**Figure S2.** The hydrolytic rates of SA-H (left) and AMIP-H (right) toward *p*-NPA, *p*-NPB and *p*-NPH.



**Figure S3.** The catalytic activity ( $V_0$ ) of AMIP-H that washed by different concentration/pH of NaOH solution.

The highest catalytic activity of AMIP-H was obtained when using 2 mM of NaOH solution (pH11.3) as elution solution. When the concentration of eluent is lower than 2 mM (pH<11.3), imprinting template cannot be completely removed and the catalytic activity of AMIP-H is unfavorable. When increasing the concentration of eluent (pH>11.3), the polymer is destroyed under the high alkaline condition, which also decreases the catalytic activity. As a result, 2 mM of NaOH solution was used to elute imprinting templates.



**Figure S4.** The catalytic mechanism of peptide nanofibers.

**Table S1.** Catalytic parameters of the SA-H and other imprinted hydrogels for hydrolysis of *p*-NPA, *p*-NPB and *p*-NPH<sup>a</sup>

Substrate	Enzyme	$V_{\max}$ ( $\mu\text{M}/\text{min}$ )	$k_{\text{cat}}$ ( $10^{-3}\text{min}^{-1}$ )	$K_m$ (mM)	$k_{\text{cat}}/K_m$ ( $10^{-3}\text{min}^{-1}\text{mM}^{-1}$ )
<i>p</i> -NPA	SA-H	$20.2 \pm 1.71$	$4.04 \pm 0.34$	$7.79 \pm 0.70$	$5.27 \pm 0.82$
	NIP-H	$17.6 \pm 1.53$	$3.56 \pm 0.56$	$4.28 \pm 0.70$	$8.38 \pm 0.43$
	<u>AMIP-H</u>	<u><math>20.6 \pm 0.82</math></u>	<u><math>4.12 \pm 0.16</math></u>	<u><math>2.66 \pm 0.28</math></u>	<u><math>15.5 \pm 0.40</math></u>
	BMIP-H	$16.9 \pm 1.38$	$3.68 \pm 0.28$	$5.96 \pm 0.60$	$6.27 \pm 0.47$
	HMIPI-H	$14.7 \pm 1.67$	$3.58 \pm 1.02$	$4.86 \pm 0.59$	$7.36 \pm 0.44$
<i>p</i> -NPB	SA-H	$5.61 \pm 0.35$	$1.12 \pm 0.41$	$3.74 \pm 0.85$	$3.34 \pm 0.33$
	NIP-H	$3.17 \pm 0.48$	$0.64 \pm 0.09$	$1.04 \pm 0.27$	$6.54 \pm 0.30$
	AMIP-H	$5.44 \pm 1.67$	$0.89 \pm 0.33$	$1.14 \pm 0.22$	$7.84 \pm 0.43$
	<u>BMIP-H</u>	<u><math>3.93 \pm 0.70</math></u>	<u><math>0.79 \pm 0.14</math></u>	<u><math>0.83 \pm 0.29</math></u>	<u><math>11.2 \pm 0.58</math></u>
	HMIPI-H	$3.63 \pm 0.26$	$0.35 \pm 0.03$	$0.96 \pm 0.09$	$7.85 \pm 0.23$
<i>p</i> -NPH	SA-H	$3.31 \pm 0.69$	$0.66 \pm 0.14$	$3.55 \pm 0.17$	$1.93 \pm 0.29$
	NIP-H	$4.54 \pm 0.63$	$0.91 \pm 0.19$	$3.81 \pm 0.27$	$2.63 \pm 0.27$
	AMIP-H	$4.79 \pm 0.45$	$0.96 \pm 0.09$	$2.55 \pm 0.28$	$3.84 \pm 0.37$
	BMIP-H	$4.24 \pm 0.22$	$0.85 \pm 0.13$	$2.61 \pm 0.37$	$3.87 \pm 0.44$
	<u>HMIPI-H</u>	<u><math>1.92 \pm 0.08</math></u>	<u><math>0.35 \pm 0.05</math></u>	<u><math>0.62 \pm 0.06</math></u>	<u><math>5.65 \pm 0.27</math></u>

<sup>a</sup> $V_{\max}$  is the maximal reaction velocity,  $k_{\text{cat}}$  is the catalytic constant,  $k_{\text{cat}} = V_{\max}/[E]$ , [E] is the molar concentration of Fmoc-FFH, and  $K_m$  is the Michaelis constant,

**Table S2.** Comparison of hydrolytic activity of some peptide-based artificial enzymes.

Catalyst	Substrate	Conditions <sup>a</sup>	$V_0$ ( $\mu\text{M}/\text{min}$ ) <sup>a</sup>	Reference
Au@E3H15	<i>p</i> -NPA	Tris-HCl buffer pH 7.3, 25 °C $C_{\text{pep}} = 0.05 \text{ mM}$ $C_{\text{sub}} = 0.5 \text{ mM}$	0.54	Mikolajczak <i>et al.</i> <sup>1</sup>
Q11R/H	<i>p</i> -NPA	PBS buffer pH 7.4, 25 °C $C_{\text{pep}} = 0.2 \text{ mM}$ $C_{\text{sub}} = 0.5 \text{ mM}$	0.85	Zhang <i>et al.</i> <sup>2</sup>
PepNTs-His-Arg	<i>p</i> -NPA	HEPES buffer pH 8.0, 25 °C $C_{\text{pep}} = 0.1 \text{ mM}$ $C_{\text{sub}} = 0.5 \text{ mM}$	2.44	Huang <i>et al.</i> <sup>3</sup>
CNT– (SHE/W) <sub>2:1</sub> – LKLKLKL	<i>p</i> -NPA	Tris-HCl buffer pH 8.0, 37 °C $C_{\text{pep}} = 3.5 \text{ }\mu\text{g/mL}$ $C_{\text{sub}} = 2.6 \text{ mM}$	1.32	Zhang <i>et al.</i> <sup>4</sup>
D/H/S	<i>p</i> -NPA	PBS buffer pH 7.4 $C_{\text{pep}} = 0.1 \text{ mM}$ $C_{\text{sub}} = 1 \text{ mM}$	1.71	Gulseren <i>et al.</i> <sup>5</sup>
SA-H	<i>p</i> -NPA	HEPES buffer pH 7.5, 35 °C $C_{\text{pep}} = 0.5 \text{ mM}$ $C_{\text{sub}} = 5 \text{ mM}$	11.52	Wang <i>et al.</i> <sup>6</sup>
AMIP-H	<i>p</i> -NPA	PBS buffer pH 8.0, 25 °C $C_{\text{pep}} = 0.5 \text{ mM}$ $C_{\text{sub}} = 1 \text{ mM}$	7.68	<b>This study</b>

<sup>a</sup>The reaction conditions and  $V_0$  values are cited directly from the original reference.

## References

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