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Electronic Supplementary Information for

Construction of DNA ligase-mimicking nanozymes via molecular imprinting

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MATERIALS AND METHODS

1. Reagents and Materials

3-Aminopropyltriethoxysilane (APTES), 2',4',6'-trihydroxyacetophenone monohydrate (THAP), tetraethoxysilane (TEOS), and 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) were obtained from Sigma (St. Louis, MO, USA). 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) and 2-morpholinoethanesulfonic acid (MES) were from purchased from Aladdin (Shanghai, China). Modified octadeoxyribonucleotide ($_{HO}$GTTGTTGT), modified decadeoxyribonucleotide ($_{HO}$GTGTTGTTGT), modified hexadeoxyribonucleotides (including $_{HO}$TGTTGT, $_{HO}$TGGTGT, $_{HO}$TGTTGG, $_{HO}$TGGGGT, $_{HO}$TGTTTG, $_{HO}$TTGGGT, $_{HO}$TGTGTG, $_{HO}$ACAACA, and $_{HO}$CACCAC) and modified trideoxyribonucleotides (including $_{HO}$TGT$_{p}$, and $_{HO}$TGT) that have a hydroxyl group at the 5' end rather than a phosphate group as normal deoxyribonucleotides do were purchased from Sangon (Shanghai, China). The secondary structures of these ssDNAs were simulated by the Oligoevaluator software (http://www.oligoevaluator.com/OligoCalcServlet) and no secondary structures were confirmed. The structures of the trideoxyribonucleotides and hexadeoxyribonucleotides are shown in Figure S1 and S2. Cetyltrimethylammonium bromide (CTAB), dimethyl sulfoxide (DMSO), glycine, and all other chemical reagents were of analytical grade and obtained from Sinopharm (Shanghai, China). Water used in all the experiments was purified by a Milli-Q Advantage A10 ultrapure water purification system (Millipore, Milford, MA, USA). Fused-silica capillaries with 75 μm i.d × 365 μm o.d were from Yongnian Optic Fiber Plant (Hebei, China).

2. Instrumentation

Transmission electron microscopy (TEM) characterization was performed on a JEM-2100 system (JEOL, Tokyo, Japan). The X-ray diffraction (XRD) patterns of samples were acquired on an ARL XTRA diffractometer (Thermo Fisher Scientific, Waltham, MA, USA) with Cu Kα radiation in the 2θ range of 0.5-6°. UV absorbance measurement

was performed on a NanoDrop 2000/2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), the wavelength was set at 260 nm for all the analytes. Nitrogen adsorption-desorption measurements were conducted at 77 K on an ASAP2020 instrument (Micromeritics, Norcross, GA, USA). Surface areas were calculated by the Brunauer-Emmett-Teller (BET) method and the pore size distributions were calculated by Barrett-Joyner-Halenda (BJH) method. Fourier transform infrared (FTIR) spectrometry was carried out on a Nicolet 6700 FT-IR spectrometer (Thermo Fisher, MA, USA). The energy dispersive X-ray (EDX) spectroscopic analysis was carried out on a JSM 7800F system (JEOL, Tokyo, Japan). The AGEC-TCA reaction was carried out on a Biometrica Tadvanced PCR thermo-circler (Analytikjena, Germany) with special temperature programs. Capillary electrophoresis (CE) separation and analysis was carried out on a P/ACE MDQ system (Beckman Coulter, Fullerton, CA, USA). A fused-silica capillary (60 cm in total, 50 cm to the detector) was used as the separation column. The running buffer contained 25 mM sodium borate, 25 mM glycine and 5 mM monopotassium phosphate, pH 9.0. The separation voltage was 25 kV unless otherwise specified. The UV detection wavelength was 214 nm. Sample was injected by a pressure of 0.5 psi for 5 s. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometric (MALDI TOF MS) analyses were carried out on a 4800 plus MALDI TOF/TOF Analyzer (Applied Biosystems, Framingham, MA, USA) with a pulsed nitrogen laser operated at 337 nm. The laser energy was adjusted to slightly above the threshold to obtain good resolution and signal-to-noise ratio (S/N). All mass spectra reported were obtained in the negative ion mode. The accelerating voltage was 20 kV. The matrix for MALDI-TOF MS analysis was 300 mM THAP dissolved in 50% (v/v) ACN.

3. Optimization of imprinting conditions

In order to optimize the synthetic procedure of the imprinted material, the molar ratio of APTES to TEOS was changed and evaluated in terms of imprinted factor. For details, totally 6 different molar ratios of APTES:TEOS were investigated, including 1:9, 0.8:9.2, 0.6:9.4, 0.4:9.6, 0.2:9.8, and 0.1:9.9. In the optimization procedure, the molar ratio of

the template to APTES was fixed at 1:1. Among the six ratios, the ratio 0.2: 9.8 was found to provide the highest IF value.

4. Comparison of the imprinting of oligo-deoxyribonucleotides of different length

To investigate the effect of the oligo-deoxyribonucleotide length on the imprinting, modified deoxyribonucleotides of different length, including $_{HO}$TGTTGT, $_{HO}$GTTGTTGT and $_{HO}$GTGTTGTTGT, were used as templates and their imprinted MSNs were prepared according to the imprinting procedure specified in the main text. Meanwhile, their corresponding non-imprinted MSNs were prepared using otherwise identical procedure except that no imprinting templates were present. To investigate the binding capability of the prepared imprinted and non-imprinted MSNs for different template length, a 1.0-mg amount of imprinted or non-imprinted MSNs was added to a 1.5-mL centrifugal vial, and 500 μL of deoxyribonucleotide template solution (about 25 μM containing 50 mM HEPES, 100 mM NaCl, 5 mM $MgCl_2$, pH 7.6) was added. The vial was vigorously shaken on a vibrator under room temperature for 1 h. After the extraction, the material was separated by centrifugation. The obtained precipitate was washed with 200 μL washing solution (50 mM HEPES, 100 mM NaCl, 5 mM $MgCl_2$, pH 7.6) and the washing step was repeated for three times. After that, the mixture was centrifuged again. The precipitate was redispersed in 50 μL of 100 mM acetic solution. The obtained mixture was shaken on a vibrator under room temperature for 1 h. Subsequently, the mixture was centrifuged. The obtained supernatant was taken out and its UV absorbance was measured at 260 nm. The procedure for each type of material was repeated for three times.

5. Measurement of binding capacity and imprinting factor

The binding capacity of imprinted MSNs was evaluated using the template hexadeoxyribonucleotide as the analyte. For details, 500 μL of the $_{HO}$TGTTGT used as the template (8 OD/mL) dissolved in 50 mM HEPES buffer containing 50 mM $MgCl_2$ was mixed with 1 mg of imprinted MSNs within a microcentrifuge tube. The tube was

shaken on a rotator for 1 h at room temperature. The imprinted MSNs were then collected by centrifugation and washed with 50 mM HEPES buffer containing 50 mM $MgCl_2$ for three times. After washing, the imprinted MSNs were resuspended and eluted in 50 μL of 100 mM acetic acid solution for 1 h on a rotator. Finally, the imprinted MSNs were centrifuged again and the eluate was collected by pipetting carefully. The UV absorbance of the eluate at 260 nm was measured. The binding capacity was calculated through the corresponding calibration curve. The measurement was repeated for 5 parallel experiments and the averaged value was used as the final binding capacity. The same procedure was carried out for non-imprinted MSNs. The imprinting factor (IF) was calculated according to the ratio of the binding capacity of $_{HO}$TGTTGT captured by imprinted MSNs over that by non-imprinted MSNs.

The measurement procedure of the IF value for DNA ligase nanomimic was the same as that in the measurement of $_{HO}$TGTTGT-imprinted MSNs except that naturally occurring DNA sequence TGTTGT was used as template.

6. Selectivity test

To examine the selectivity of the $_{HO}$TGTTGT-imprinted MSNs, a series of template analogues with different base sequence were used as interfering sequences. Standard solutions of the template or its analogues (8 OD/mL) dissolved in 50 mM HEPES buffer containing 50 mM $MgCl_2$ were prepared. Within a microcentrifuge tube, 1 mg of imprinted MSNs was added to 500 μL of above-mentioned solutions, respectively. The tubes were shaken on a rotator for 1 h at room temperature. The imprinted MSNs were collected by centrifugation and then rinsed with 200 μL of 50 mM HEPES buffer containing 50 mM $MgCl_2$ for three times. After washing, the imprinted MSNs were resuspended and eluted in 50 μL 100 mM acetic acid solution for 1 h on a rotator. Finally, the imprinted MSNs were centrifuged again and the eluates were collected by pipetting carefully. UV absorbance of the eluates was measured at 260 nm.

7. Binding constant

The binding strength of $_{HO}$TGTTGT-imprinted MSNs was evaluated using the reaction

products and substrates at test compounds. A 1-mg aliquot of imprinted MSNs was added to solutions (500 μL) of a test compound at different concentrations in 1.5-mL plastic microcentrifuge tubes. The tubes were shaken on a rotator for 1 h at room temperature. The washing and elution procedures were the same as described above. The amounts of test compounds extracted by the imprinted MSNs were determined by measuring the UV absorbance at 260 nm of the eluates. Three parallel measurements were carried out for each concentration. The amounts of test compounds bound to the imprinted MSNs were plotted according to the Scatchard equation as described below to estimate the binding properties of the imprinted MSNs.

$$Q_e/[S] = (Q_{max}-Q_e)/K_d$$

where Q_e, $[S]$, Q_{max} and K_d are the amount of test compound bound to the imprinted MSNs at equilibrium, the free concentration of test compound at adsorption equilibrium, the saturated adsorption capacity and the dissociation constant, respectively. By plotting $Q_e/[S]$ versus Q_e, K_d and Q_{max} can be calculated from the slope and the intercept, respectively.

8. Spontaneous ligation test

To check whether or not the ligation can occur spontaneously, 10 μL of mixture solution of $_{HO}TGT_p$ (0.02 μmol), $_{HO}TGT$ (0.02 μmol), and EDC (0.4 μmol) dissolved in 50 mM HEPES, 50 mM $MgCl_2$ buffer (pH 5.40) was added into a 200-μL centrifugal vial. The vial was placed in the PCR thermo-circler and the temperature was set at the TCA mode (25 °C for 24 min and 95 °C for 5 min, repeated for 24 cycles) or constant temperature (25 °C). After reaction, the reaction mixtures were separately combined with 10 μL of 100 mM acetic solution, which was denoted as TCA-Control-1 and RT-Control.

To check whether or not there was template leakage from the imprinted MSNs during the TCA processing, 1.0 mg of imprinted MSNs and 10 μL of 50 mM HEPES buffer (pH 5.40) were added into a 200-μL centrifugal vial. The vial was placed in the PCR thermo-circler and the temperature cycle was set as 25 °C for 24 min and 95 °C for 5 min, repeated for 24 cycles. After reaction, the reaction mixture was centrifuged. The

obtained supernatant was transferred into another centrifugal vial while the precipitate was washed with 10 μL of 100 mM acetic solution and the eluate was combined with previous supernatant, which was finally supplemented with 100 mM acetic solution to a total volume of 20 μL. The obtained mixture was denoted as TCA-MIP in buffer. The finally obtained mixtures were separated and analyzed by CE under 30 kV.

9. Template leakage test

To check whether there was template leakage from the imprinted MSNs, elements content was evaluated using EDX spectroscopy. For details, 500 μL of $_{HO}$TGTTGT (8 OD/mL) was first dissolved in 50 mM HEPES buffer containing 50 mM $MgCl_2$ and then mixed with 1 mg of imprinted MSNs in a microcentrifuge tube. The tube was shaken on a rotator for 1 h at room temperature. The imprinted MSNs were then collected by centrifugation and rinsed with 200 μL of 50 mM HEPES buffer containing 50 mM $MgCl_2$ for three times to remove the unbound template. The washed template-bound MIP was collected by centrifugation and freeze-dried before EDX characterization. For comparison, freeze-dried $_{HO}$TGTTGT-imprinted MSNs, non-imprinted MSNs and $_{HO}$TGTTGT were also analyzed.

10. Effectiveness of the TCA program

An amount of 1.0 mg of imprinted MSNs was added to a 200-μL centrifugal vial, and 10 μL of reacting solution containing 0.1 μmol substrate ssDNA each dissolved in 50 mM HEPES, 100 mM NaCl and 5 mM $MgCl_2$ (pH 7.6) was added. The vial was placed in the PCR thermo-circler and the temperature cycle was set as 25 °C for 1 h and 95 °C for 5 min, repeated for 12 cycles. After reaction, the reaction mixture was centrifuged. The obtained supernatant was transferred into another centrifugal vial while the precipitate was washed with 20 μL of 100 mM acetic solution and the eluate was combined with previous supernatant, which was finally supplemented with 100 mM acetic solution to a total volume of 100 μL. The obtained mixture was separated and analyzed by CE. The result was denoted as TCA-MIP. The above experiment was repeated but the imprinted MSNs were replaced with non-imprinted MSNs and the

other conditions were identical. The obtained result was denoted as TCA-NIP. The above experiment was repeated but no imprinted or imprinted MSNs were present and the other conditions were identical. The obtained result was denoted as TCA-Control-2. The above experiment was repeated but the temperature was kept constant at 25 °C, and the obtained result was denoted as RT-MIP.

11. Optimization of AGEC-TCA reaction

For optimization of the release temperature, 1 mg of imprinted MSNs was added into a 200-μL centrifugal vial, and 10 μL of mixture solution of $_{HO}TGT_p$ (0.02 μmol), $_{HO}TGT$ (0.02 μmol), and EDC (0.4 μmol) dissolved in 50 mM HEPES, 50 mM $MgCl_2$ buffer (pH 5.40). The vial was placed in the PCR thermo-circler and the temperature cycle was set as 25 °C for 24 min and specified release temperature (75, 80, 85, 90 or 95 °C) for 5 min, repeated for 24 cycles. After reaction, the reaction mixture was centrifuged. The obtained supernatant was transferred into another centrifugal vial while the precipitate was washed with 10 μL of 100 mM acetic solution and the eluate was combined with previous supernatant, which was finally supplemented with 100 mM acetic solution to a total volume of 20 μL. The obtained mixture was separated and the hexadeoxyribonucleotide product was analyzed by CE. The experiments were repeated for three times.

To optimize the reaction time, 1 mg of imprinted MSNs was added into a 10-μL solution of $_{HO}TGT_p$ (0.1 μmol), $_{HO}TGT$ (0.1 μmol), EDC (2 μmol) in 50 mM HEPES buffer containing 50 mM $MgCl_2$ (pH 5.40). The mixture was allowed to react for 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, or 6 min at 25 °C, shaken on a rotator in a water bath. The molecularly imprinted MSNs were then collected by centrifugation and then rinsed with 10 μL of 50 mM HEPES buffer containing 50 mM $MgCl_2$ (pH 5.40) for three times. After washing, the molecularly imprinted MSNs were resuspended and eluted in 50 μL of 100 mM acetic acid solution for 1 h on a rotator. Finally, the molecularly imprinted MSNs were centrifuged again and the eluates were collected by pipetting carefully. UV absorbance of the eluates was measured at the wavelength 260 nm.

For optimization of the release time, 1 mg of imprinted MSNs was added into a 10-μL

solution of $_{HO}TGT_p$ (0.1 μmol), $_{HO}TGT$ (0.1 μmol), EDC (2 μmol) in 50 mM HEPES buffer containing 50 mM $MgCl_2$ (pH 5.40). The tube was shaken on a rotator for 24 min in water bath of 25 °C. The molecularly imprinted MSNs were then collected by centrifugation and then rinsed with 10 μL of 50 mM HEPES buffer containing 50 mM $MgCl_2$ (pH 5.40) for three times. After washing, the molecularly imprinted MSNs were added with 50 μL of 50 mM HEPES buffer containing 50 mM $MgCl_2$ (pH 5.40). The obtained solution was then heated in the PCR thermo-circler to 85°C for different release time (0.5, 1, 1.5, 2, 2.5, 3, 3.5, or 4 min). Finally, the molecularly imprinted MSNs were centrifuged again and the eluates were collected by pipetting carefully. UV absorbance of the eluates at 260 nm was measured.

12. AGEC-TCA reaction of modified trideoxyribonucleotides

Molecularly imprinted MSNs (1 mg) were added to a 2.5 μL solution of $_{HO}TGT_p$ (0.1 μmol) in 50 mM HEPES buffer containing 50 mM $MgCl_2$ (2.5 μL) at 25 °C. A 2.5-μL solution of $_{HO}TGT$ (0.1 μmol) in 50 mM HEPES buffer containing 50 mM $MgCl_2$ was added. The mixture was stirred at 25 °C for 15 min, then added with 0.4 M EDC (5 μL). The mixture was then submitted to temperature-circled AGEC reaction. Temperature circle was 25 °C for 5 min and 85 °C for 3 min per round. Since temperature cooling down from 85 °C to 25 °C took 1 min, the total reaction time took per round was 9 min. After reacting for 18, 36, 54, 72, 81, 90, 99, 108, and 117 minutes, the mixture was centrifuged and the supernatant was collected. The MSNs were washed with 100 mM acetic acid solution (10 μL) and centrifuged again. The supernatant was pooled with that obtained from the previous centrifugation. The amount of product obtained from the HEPES solution and that recovered by acetic acid wash was quantified by CE analysis.

For control reaction, a volume of 2.5 μL of $_{HO}TGT_p$ (0.1 μmol) in 50 mM HEPES buffer containing 50 mM $MgCl_2$ was shook with 2.5 μL of $_{HO}TGT$ (0.1 μmol) in 50 mM HEPES buffer containing 50 mM $MgCl_2$. The mixture was added with 2 μmol EDC (5 μL) and then reacted at 25 °C by water bath. After reacting for 6, 12, 24, 36, 48, 60, 66, 72, or 78 h, the mixture was analyzed by CE.

13. Reaction specificity

To examine the reaction specificity of the $_{HO}$TGTTGT-imprinted MSNs, the same AGEC-TCA reaction and control reaction were carried out with a series of analogues of the modified trideoxynucleotides, including $_{HO}$TGG$_p$, $_{HO}$TGG, $_{HO}$TTG$_p$, $_{HO}$TTG, $_{HO}$GTG$_p$ and $_{HO}$GTG. Each pair of analogues was processed and then reacted for 108 min in the same conditions as in the AGEC-TCA reaction of $_{HO}$TGT$_p$ and $_{HO}$TGT. After the same post-processing procedures, the amount of product was quantified by CE analysis.

To examine the reaction specificity of TGTTGT-imprinted MSNs, the same AGEC-TCA reaction and control reaction were carried out with three substrate analogues, including TGG, TTG, and GTG. After the same procedures, the reaction product was quantified by CE analysis.

14. Effect of substrate concentration on the reaction rate and its enhancement

To investigate the effect of substrate concentration on the reaction rate of $_{HO}$TGTTGT-imprinted MSNs, three concentrations of $_{HO}$TGT$_p$ and $_{HO}$TGT (10, 2 and 1 mM for each substrate) were investigated. The solutions were prepared through diluting a 10-μL solution of $_{HO}$TGT$_p$ (0.1 μmol), $_{HO}$TGT (0.1 μmol), EDC (2 μmol) with 50 mM HEPES buffer containing 50 mM $MgCl_2$ by certain folds. The mixture was then submitted to temperature-circled AGEC reaction. Temperature circle was 25°C for 5 min 85 °C for 3 min and temperature cooling back for 1 min per round. Since the equilibrium time of AGEC-TCA and control reaction at the concentration of 10 mM were 1.8 and 66 h, respectively, the reaction time was set at the same duration for comparison. After the same procedure of reaction, the product was quantified by CE.

To investigate the effect of substrate concentration on the reaction rate of TGTTGT-imprinted MSNs, three concentrations (20, 4 and 2 mM) of TGT were investigated. The solutions were prepared through diluting a 5-μL solution of TGT (0.2 μmol) with 0.1 M MES buffer containing 50 mM $MgCl_2$ (pH 6.15) by certain folds. The mixtures were then submitted to temperature-circled reaction. Temperature circle for the reaction was 4

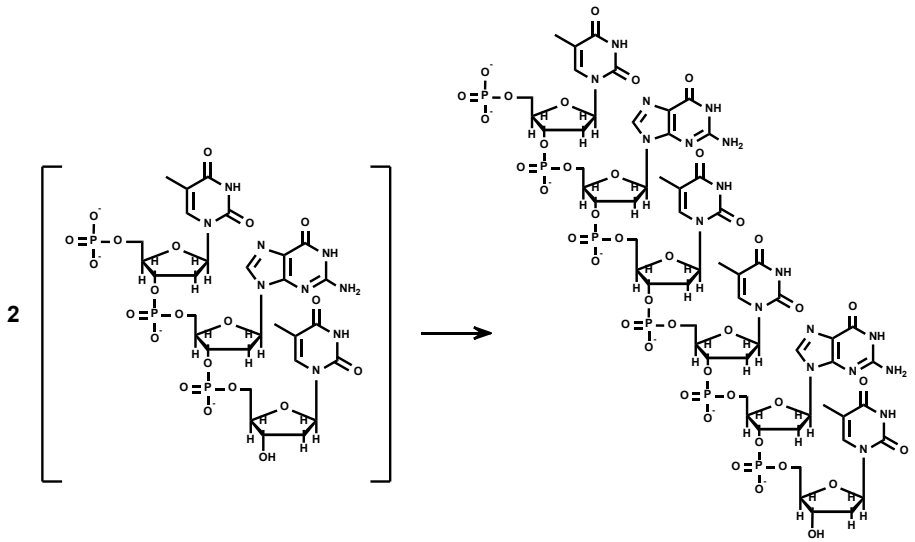
°C for 5 min, 85 °C for 3 min and temperature cooling back for 1 min per round. The reaction time for AGEC-TCA reaction and control reaction at all concentrations was set at 6.75 and 288 h, respectively. After the same procedures, the product was quantified by CE.

15. Thermal stability and reusability of $_{HO}$TGTTGT-imprinted MSNs

The thermal stability and reusability of $_{HO}$TGTTGT -imprinted MSNs were investigated by measuring the amount of $_{HO}$TGTTGT product generated at each round for 28 consecutive rounds. An amount of 1 mg of imprinted MSNs was added into a 10-μL solution containing $_{HO}TGT_p$ (0.1 μmol), $_{HO}$TGT (0.1 μmol), EDC (2 μmol) dissolved in 50 mM HEPES buffer containing 50 mM $MgCl_2$ (pH 5.40). The mixture was then submitted to temperature-circled AGEC reaction. The mixture was allowed to react for 5 min at 25 °C and to release the product for 3 min at 85 °C per round. After reacting for 1 round, the mixture was centrifuged and the supernatant collected. The MSNs were washed with 100 mM acetic acid solution (10 μL) and centrifuged again. The supernatant was combined with that obtained from the previous centrifugation. The amount of product obtained from the HEPES solution and that recovered by acetic acid wash was quantified by CE analysis. Then the MSNs were further washed with 50 μL 100 mM acetic acid solution and dried under high vacuum. After that, the MSNs were used for a new round of reaction.

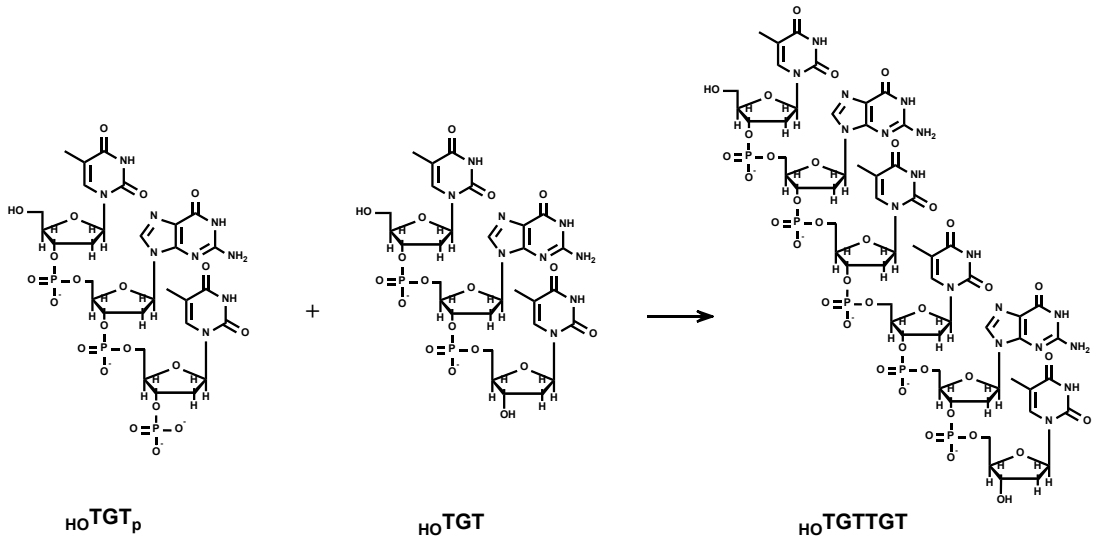
SUPPLEMENTARY DATA

A



TGT + TGT → TGTTGT

B



$_{HO}$TGT$_p$ + $_{HO}$TGT → $_{HO}$TGTTGT

Fig. S1 The diagram for (A) the ligation of two natural triadeoxynucleotides (TGT) and (B) the ligation between artificial triadeoxynucleotides ($_{HO}$TGT$_p$ and $_{HO}$TGT).

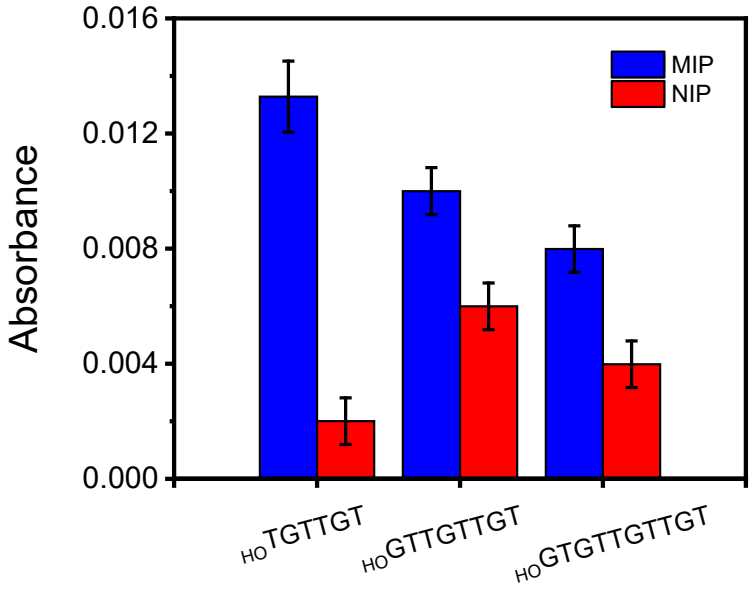


Fig. S2 Comparison of the binding capability of imprinted MSNs (MIP) with modified ssDNA with different lengths and corresponding non-imprinted MSNs (NIP) towards the templet. Error bars represent standard deviations for 3 parallel experiments.

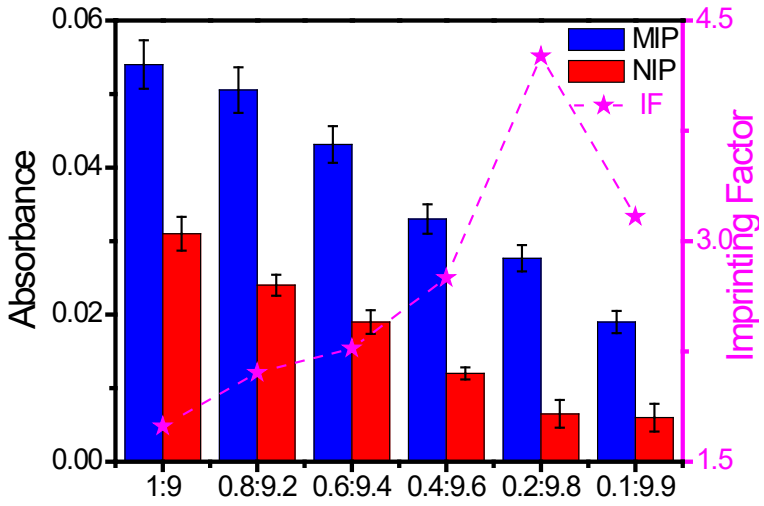


Fig. S3 Effects of the molar ratio of APTES: TEOS for the imprinting on the target amount captured by $_{HO}$TGTTGT-imprinted MSNs and nonimprinted MSNs and the imprinting factor. Error bars represent standard deviations for 3 parallel experiments.

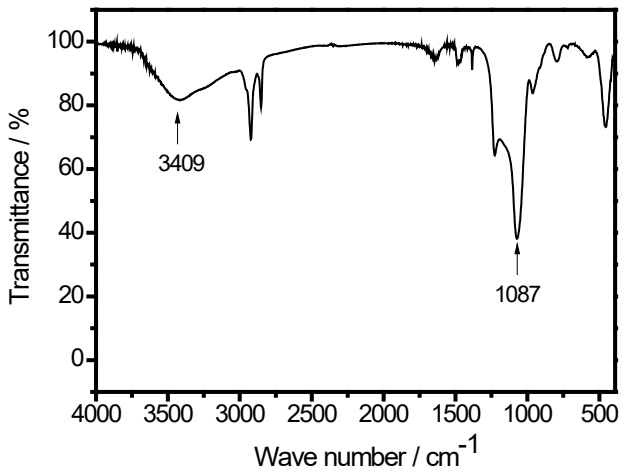


Fig. S4 FTIR spectrum for $_{HO}$TGTTGT-imprinted MSNs. Peak at 1087 cm^{-1} can be attributed to the Si-O bond of the backbone of silica-based materials. Peak at 3409 cm^{-1} can be assigned to the amino group of APTES.

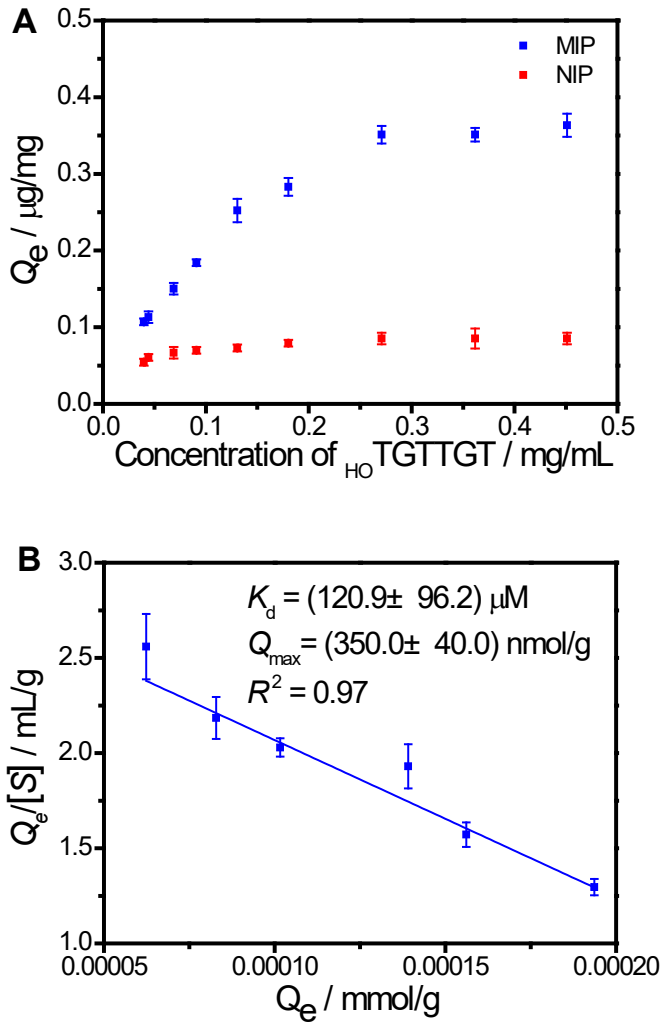


Fig. S5 (A) Binding isotherms for $_{HO}$TGTTGT-imprinted and nonimprinted MSNs towards $_{HO}$TGTTGT and (B) Scatchard plot for $_{HO}$TGTTGT-imprinted MSNs towards $_{HO}$TGTTGT. Error bars represent standard deviations for 3 parallel experiments.

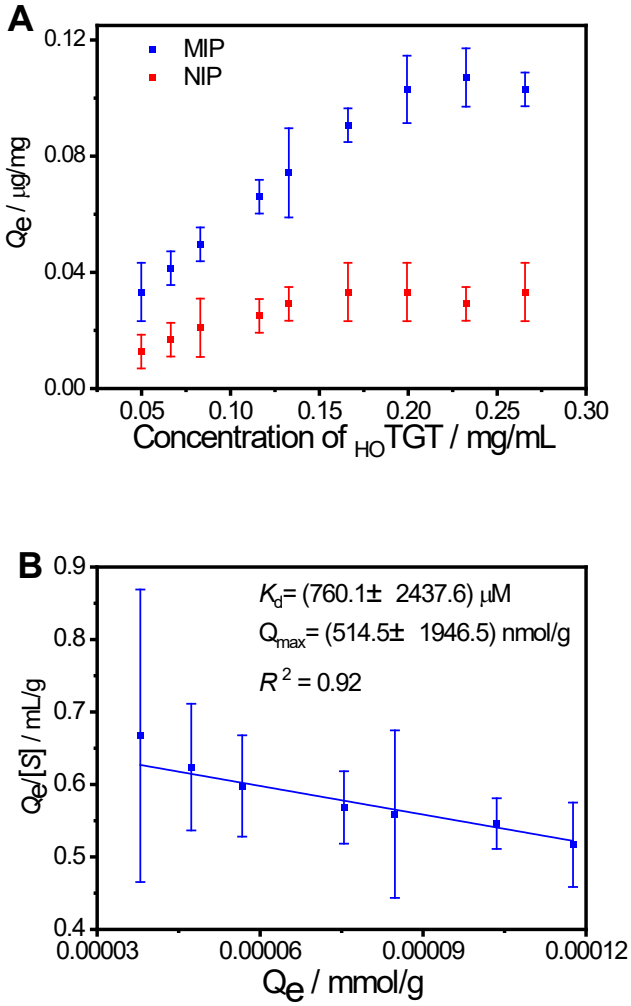


Fig. S6 (A) Binding isotherms for HOTGTTGT-imprinted and nonimprinted MSNs towards HOTGT and (B) Scatchard plot for HOTGTTGT-imprinted MSNs towards HOTGT. Error bars represent standard deviations for 3 parallel experiments.

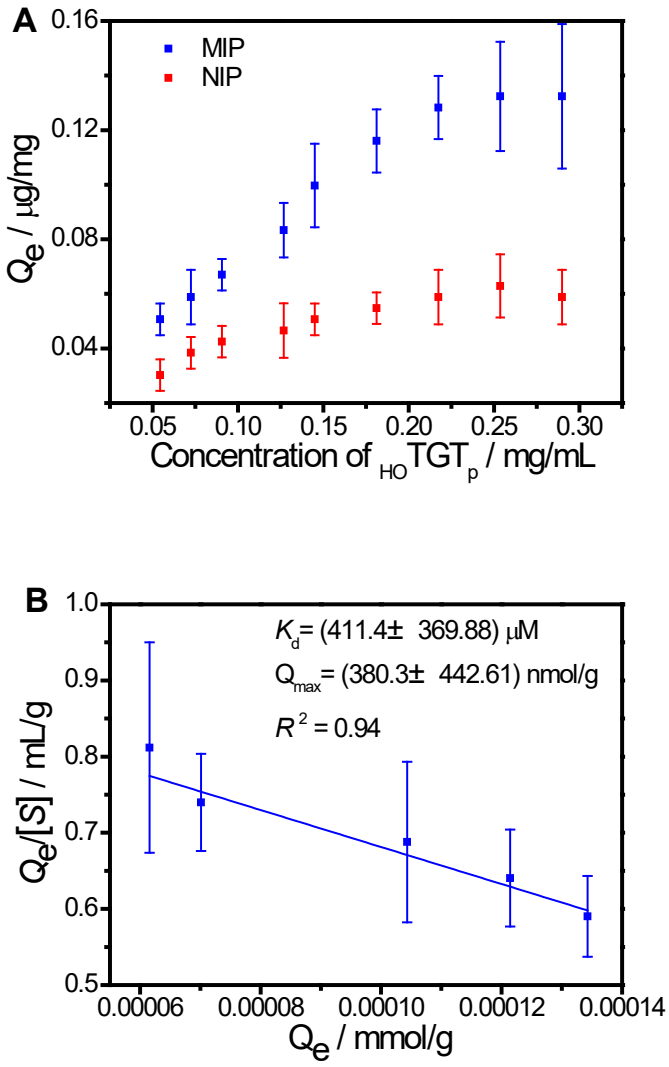


Fig. S7 (A) Binding isotherms for $_{HO}$TGTTGT-imprinted and nonimprinted MSNs towards $_{HO}TGT_p$ and (B) Scatchard plot for $_{HO}$TGTTGT-imprinted MSNs towards $_{HO}TGT_p$. Error bars represent standard deviations for 3 parallel experiments.

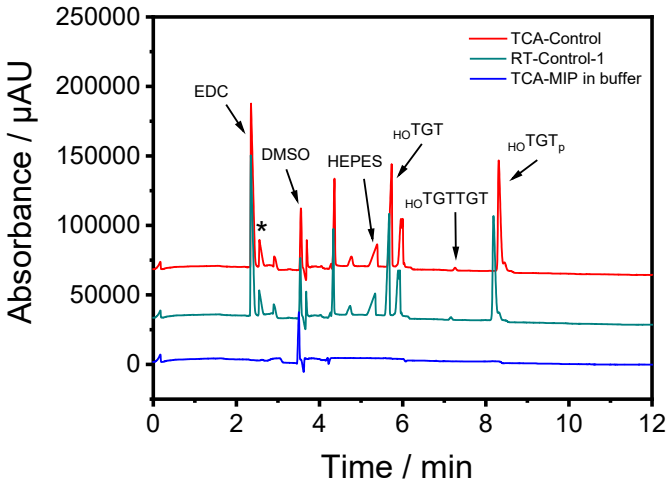


Fig. S8 Electropherograms of the products generated by different reactions. TCA-Control: reaction between $_{HO}$TGT and $_{HO}$TGT$_p$ in the absence of $_{HO}$TGTTGT-imprinted MSNs under TCA program (25 °C for 24 min and 95 °C for 5 min, repeated for 24 cycles); RT-Control-1: reaction between $_{HO}$TGT and $_{HO}$TGT$_p$ in the absence of $_{HO}$TGTTGT-imprinted MSNs under 25 °C for the same duration as TCA; TCA-MIP in buffer: reaction of $_{HO}$TGTTGT-imprinted MSNs with HEPES buffer under the same TCA program. The peak with asterisk is system peak.

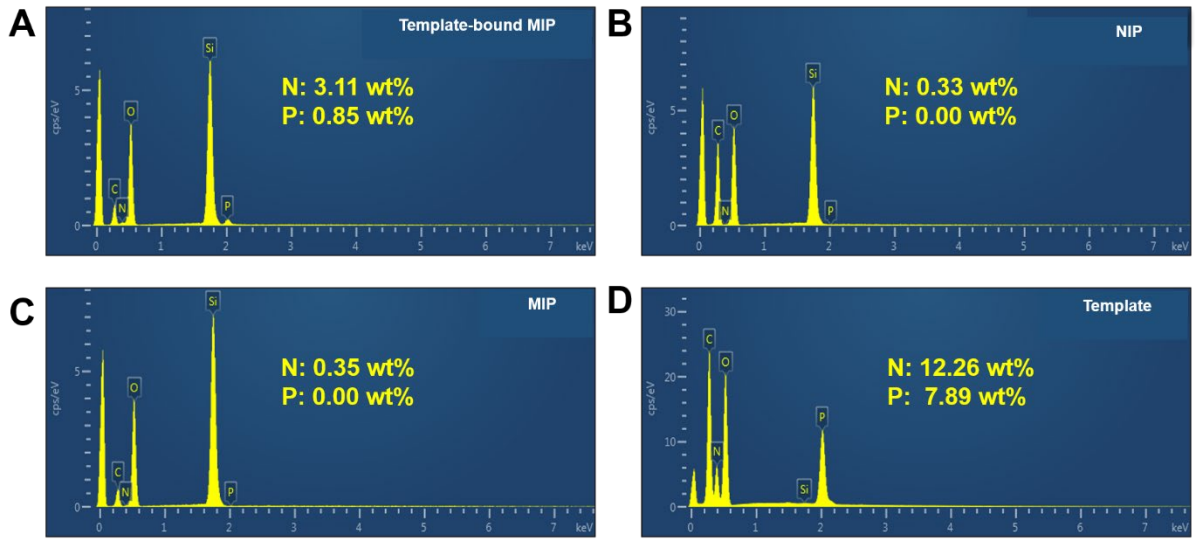


Fig. S9 Energy dispersive X-ray spectroscopic analysis of different materials. (A) $_{HO}$TGTTGT-bound MIP ($_{HO}$TGTTGT-imprinted MSNs); (B) NIP: non-imprinted MSNs; (C) MIP: $_{HO}$TGTTGT-imprinted MSNs; (D) Template ($_{HO}$TGTTGT).

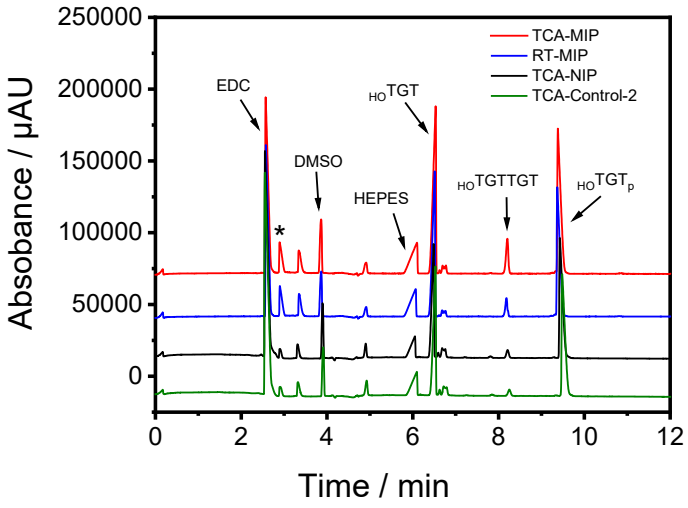


Fig. S10 Electropherograms of the products generated by different reactions. TCA-MIP: reaction with $_{HO}$TGTTGT-imprinted MSNs under TCA program (25 °C for 1 h and 95 °C for 5 min, repeated for 12 cycles); RT-MIP: reaction with $_{HO}$TGTTGT-imprinted MSNs under 25 °C for the same duration as TCA; TCA-NIP: reaction with non-imprinted MSNs under the same TCA program; TCA-Control-2: reaction without imprinted or non-imprinted MSMs under the above TCA program. The peak with asterisk is system peak.

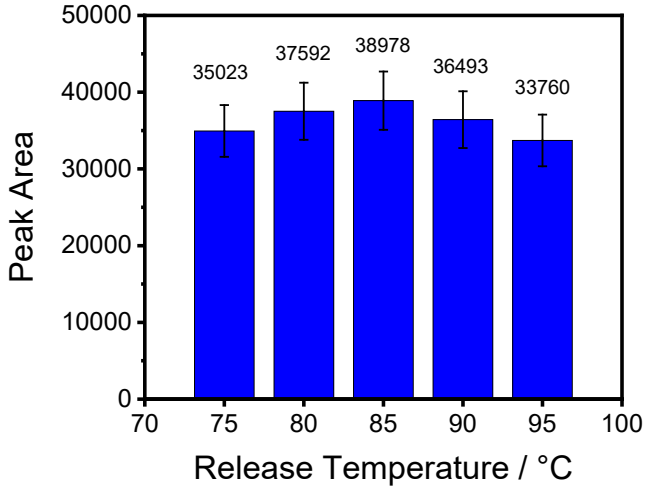


Fig. S11 The effect of product release temperature on the amount of product generated by the $_{HO}$TGTTGT-imprinted MSNs. The temperature cycle was set as: 25 °C for 24 min and then at specified product release temperature for 5 min, repeated 24 cycles. Error bars represent standard deviations for 3 parallel experiments.

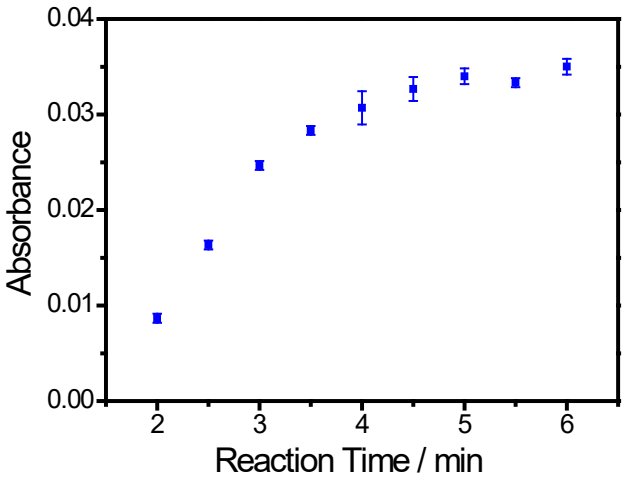


Fig. S12 Dependence of the amount of $_{HO}$TGTTGT produced by $_{HO}$TGTTGT-imprinted MSNs on the reaction time in a single heating cycle. Error bars represent standard deviations for 3 parallel experiments. It is noteworthy that the solutions under test for this figure experienced dilution so that the absorbance values were much lower as compared with those in Figure S12.

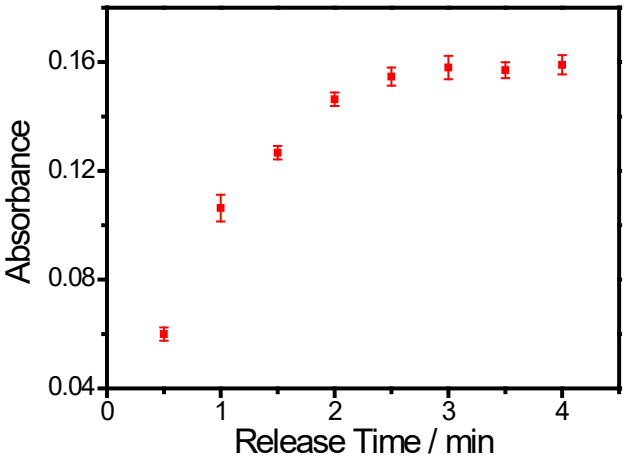


Fig. S13 Dependence of the amount of HOTGTTGT generated by HOTGTTGT-imprinted MSNs on the release time in a single heating cycle. Error bars represent standard deviations for 3 parallel experiments.

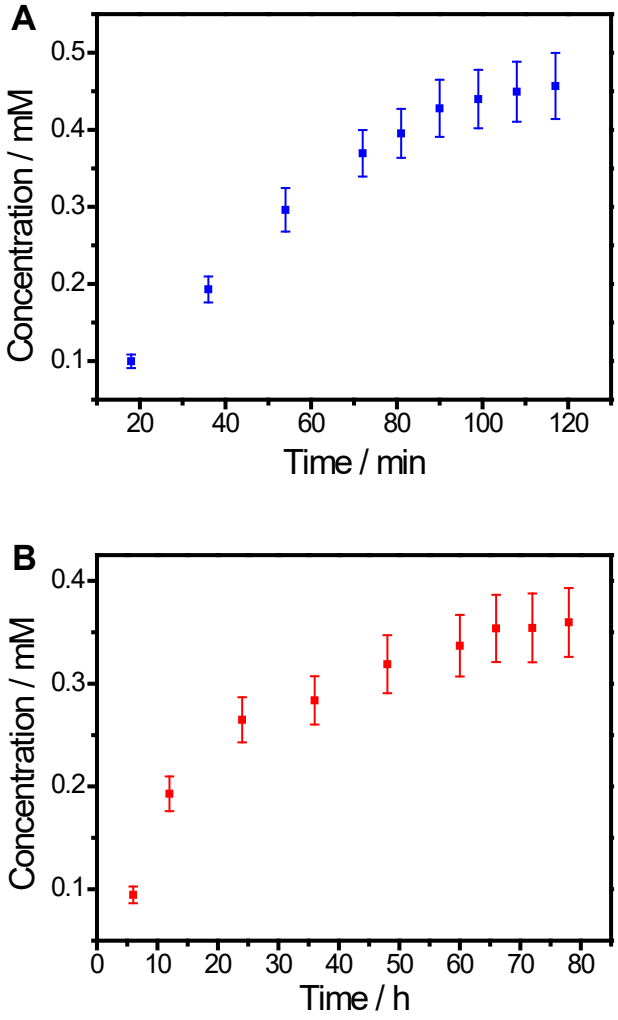


Fig. S14 Reaction equilibrium for (A) $_{HO}$TGTTGT-imprinted MSNs-based AGEC-TCA reaction and (B) control reaction in the absence of $_{HO}$TGTTGT-imprinted MSNs. Error bars represent standard deviations for 3 parallel experiments.

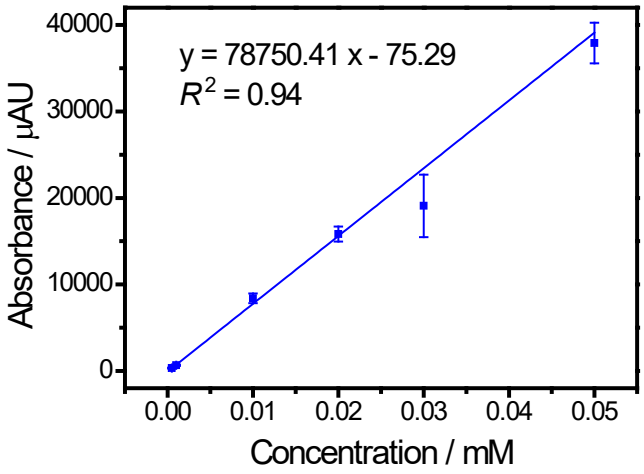


Fig. S15 Dependence of the UV absorbance on the concentration of $_{HO}$TGTTGT. Error bars represent standard deviations for 3 parallel experiments.

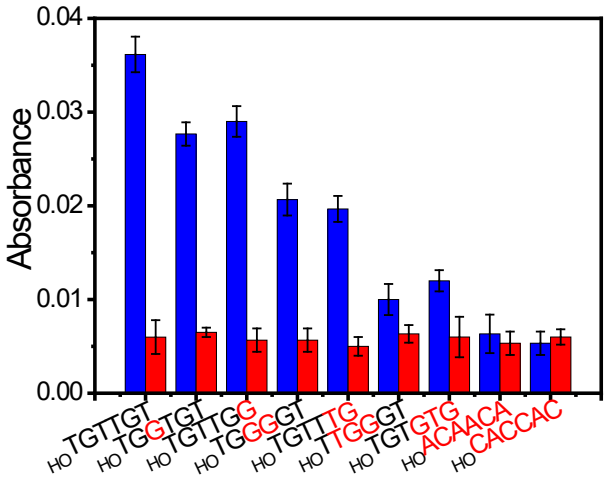
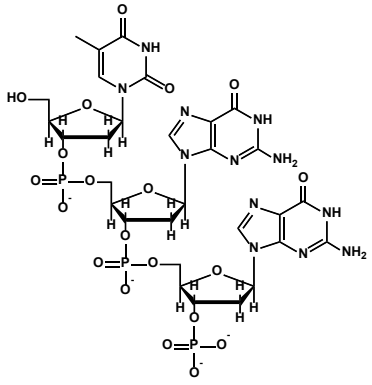
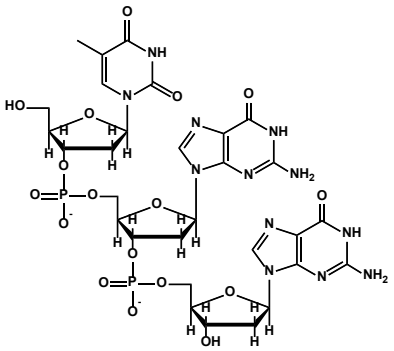


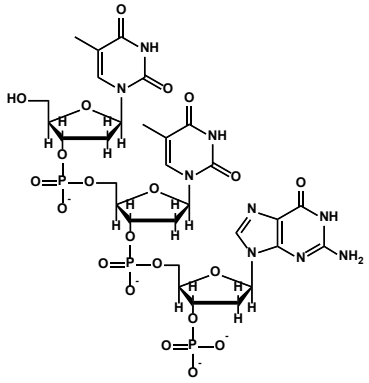
Fig. S16 Selectivity of $_{HO}$TGTTGT-imprinted MSNs (blue bars) and non-imprinted MSNs (red bars) towards the template $_{HO}$TGTTGT and its analogues. Error bars represent standard deviations for 3 parallel experiments. Red letters indicate the mismatched nucleotides.



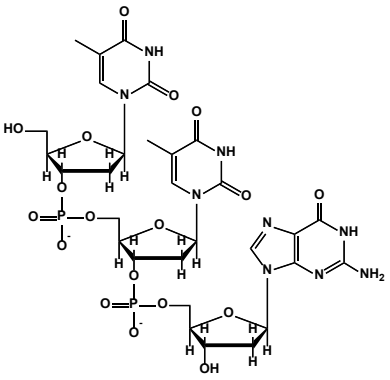
$_{HO}TGG_{p}$



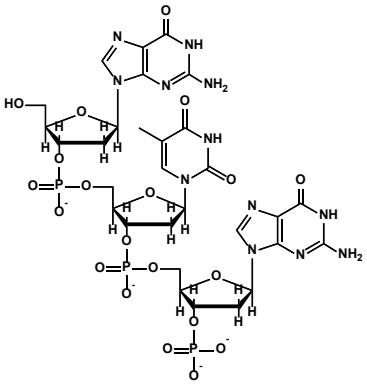
$_{HO}TGG$



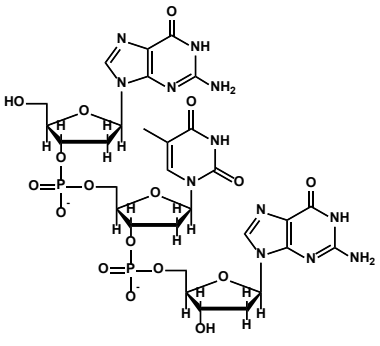
$_{HO}TTG_{p}$



$_{HO}TTG$



$_{HO}GTG_{p}$



$_{HO}GTG$

Fig. S17 The structure of the analogues of $_{HO}$TGT and $_{p}$TGT$_{OH}$.

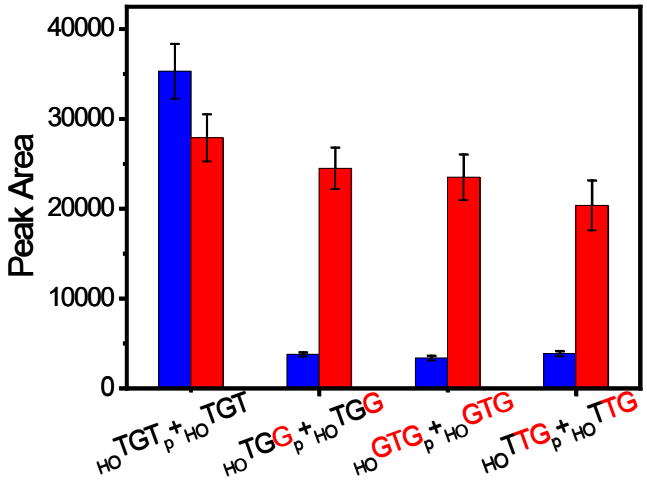


Fig. S18 Selectivity of HOTGTTGT-imprinted MSNs-based AGEC-TCA reaction towards different substrates. Blue bars: MIP-based AGEC-TCA reaction for 108 min; Red bars: control reaction for 66 h. Error bars represent standard deviations for 3 parallel experiments. Red letters indicate the mismatched nucleotides.

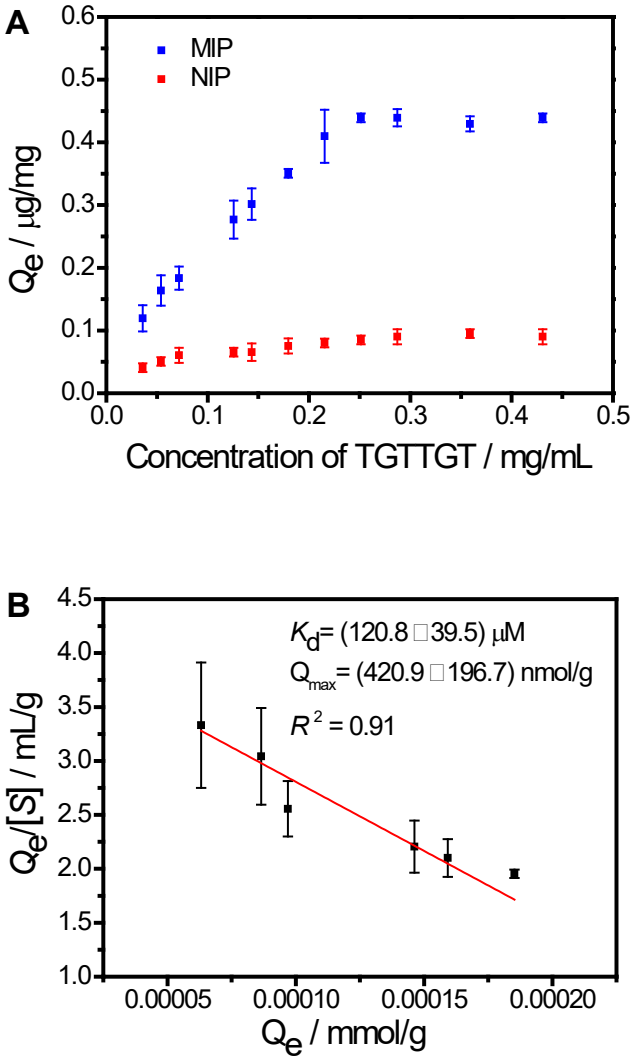


Fig. S19 (A) Binding isotherms for the TGTTGT-imprinted MSNs and non-imprinted MSNs towards TGTTGT and (B) corresponding Scatchard plot. Error bars represent standard deviations for 3 parallel experiments.

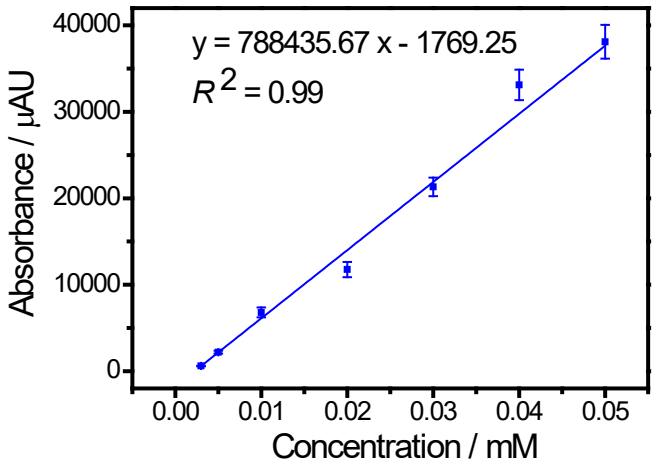


Fig. S20 Dependence of the UV absorbance on the concentration of TGTTGT. Error bars represent standard deviations for 3 parallel experiments.

Table S1. Comparison of the enhancement factor on reaction rate of $_{HO}$TGTTGT-imprinted MSNs at different substrate concentrations.*

Substrate conc. (mM) for each	Reaction type	Product conc. (μM)	Average reaction rate (μM / h)	Enhancement factor
10	AGEC-TCA	45.14	25.08	46.4
	Control	35.64	0.54	
2	AGEC-TCA	7.35	4.08	58.3
	Control	4.59	0.07	
1	AGEC-TCA	1.50	0.83	59.3
	Control	0.91	0.014	

* Substrate: $_{HO}$TGT and $_{HO}$TGT$_{p}$; TCA program: react at 25 °C for 5 min, product release at 85 °C for 3 min; temperature cooling back in 1 min; Control reaction: react at 25 °C in the absence of $_{HO}$TGTTGT-imprinted MSNs. The average reaction rates for the AGEC-TCA reaction were averaged for a reaction period of 1.8 h while those for the control reaction were averaged for 66 h.