Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Rapid and Room Temperature Detection of Single Nucleotide Variation with Enhanced Discrimination by Crowding Assisted Allele Specific Extension

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1. **Sample preparation and operation for the measurement of kinetics**

Template, Primer and Blocker DNA were dissolved and diluted using 1×Blue Buffer (NaCl (50 mM), Tris-HCl (10 mM), $MgCl_2$ (10 mM), DTT (1 mM), pH 7.9 at 25 ^oC, Enzymatics) and formed gapped DNA (2 μM). DNA polymerase I (Klenow fragment (3'-5' exo-), 50 U/ μL, Enzymatics) and dNTPs (10 mM, Takara) were diluted to 5U/ μL and 375 μM respectively by using 1×Blue Buffer. The final concentrations for DNA, enzyme and dNTPs mixture were 500 nM, 1.8 μM and 125 μM, respectively. Different crowding agents including Dextran 6000 (Sigma), Dextran 12000 (Sigma), Ficoll 70 (Sigma), Bovin serum albumin (BSA, Sigma) and PEG 20000 (Sigma) were diluted to the concentrations of 30% (w/v), 24% (w/v) and 12% (w/v).

For the rapid kinetics with the matching target, the extension reactions were nearly completed within several seconds. Therefore, we need a rapid mixing method with minimal reagent consumption. The droplet-based microfluidic platform provides rapid mixing on the scale of millisecond and consumes reagents on the scale of nanoliter, which meets the two requirements with the matching target. Precision syringe pumps (PHD 2000, Harvard Apparatus) were utilized to drive the flows of the gapped DNA and enzyme mixture, diluting buffer or crowding agents, dNTPs mixture into the microchannel. Silicone oil (50 cP, Brookfield) was used as the carrier fluid. Aqueous solutions containing DNA substrate with enzyme, diluting buffer or crowding agents and dNTPs mixture formed droplets at the T-junction. The flow rates of the oil phase and each water phase were 1.0 μL/ min and 0.4 μL/ min, respectively. Images were taken at room temperature by confocal microscopy (Nikon C1si) equipped with a photomultiplier tube detection system. The excitation and emission wavelengths were set as 543 nm and 650 nm, respectively. Because the reaction time was linearly proportional to moving distance in the microchannel, we could obtain reaction results at different reaction time by taking one picture. The time interval between each capture was 20 s.

For the slow kinetics with the mismatching targets, mixing was performed with the small centrifuge within several seconds. The detection was accomplished within the sealed glass capillary.

2. **Sample preparation and operation for the measurement of the binding affinity between the template-primer duplex and Klenow fragment (exo-)**

The binding affinity between the template-primer duplex and the Klenow fragment (exo-) was measured by isothermal titration calorymetry (Microcal ITC_{200}). Template, Primer and Blocker DNA were dissolved and diluted using 10%, 8% and 4% Ficoll 70 solutions and formed gapped DNA (0.5 μM). Klenow fragment (exo-) was diluted to 10 μM by 10%, 8% and 4% Ficoll 70. For each measurement, titration solution (300 μl) and sample solution (60 μl) diluted by the same concentration of the Ficoll 70 were inserted into the titration syringe and the sample cell, respectively. The sample cell was filled with the gapped DNA solution by using the Hamilton syringe. The control cell was filled with ddH_2O. The titration syringe was loaded

with Klenow fragment (exo-) solution prior to the measurement. The cell temperature was maintained at 25 ºC during the measurement.

3. The method for obtaining the k_{obs}

The extension of the primer would lead to the increase of the fluorescence signal. Therefore, by using the confocal microscope to measure the fluorescence signal change at different time points, the data points were fit to the single exponential model: $\boldsymbol{Y = Y_0 - A \times \exp\ (-k_{obs}\ t)}$ to obtain k_{obs}, the initial rate constant of dNTPs incorporation (Figure S1).

4. Calculation of the fraction volume occupancy

(1) Volume of each crowding molecule:

Each crowding molecule is considered as a sphere with a radius r, the value of which was from either literature or the measurement of dynamic light scattering. Volume of a sphere $V_1=4/3\ \pi r^3$

(2) The number of each types of crowding molecules with the concentration (c) and volume (V):

The molecular weight of each macromolecule was M_W.

The number of the crowding molecules $N=(c\times V) \times (6.023\times10^{23}) / M_W$

(3) The fraction volume occupancy (Φ) in the volume (V):

$\Phi= V_1\times N/V\times100\%$

5. Measurement of the viscosity

The viscosity of the buffer solutions containing the crowding agents was measured in an Ubbelohde viscometer with an inner diameter of 1 mm. The experiments were carried out at 20 ºC. DI water (viscosity = 1.0050 mPa·s) was used as a reference fluid.[1]

6. The quantitative relationship between target DNA concentration and the kinetic parameters

k_{obs} refers to the observed rate constant in each extension process. As suggested by the previous works[2,3], k_{obs} was also the rate constant of the initial burst phase in fluorescence and the first-order rate for dNTPs incorporation, and the process could be fit using a single exponential model:

$F = F_0 - A \times exp(-k_{obs}\ t)$ (F_0 and A are constants) (1)

where F is the fluorescence intensity and is proportional to the concentration of the target DNA ([target DNA]):

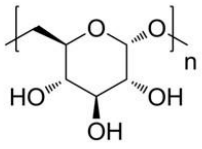
$F = a \times$[target DNA] (a is a constant) (2)

Therefore, we could obtain the quantitative relationship between the target DNA concentration and the kinetic parameters:

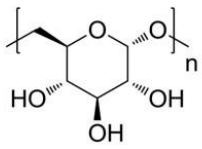
[target DNA] $= F_0/a - A/a \times exp(-k_{obs}\ t)$ (3)

Table S1. DNA substrates used in the study for detecting single nucleotide substitution. "I" denoted the single nucleotide substitution occurred at the primer terminus.

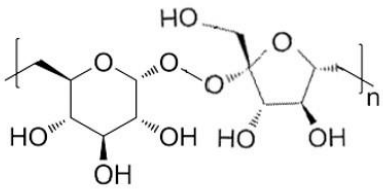
DNA sequence
Matching template and primer sequence: Match: 3′-GCAATCGGTGAGGCCTGACGCCATATTGCGCGT-5′-Cy3 5′-CGTTAGCCACTCCG CGGTATAACGCGCA-3′-BHQ-2 **Mismatching at the template-primer terminus:** Mismatch-T-I: 3′-GCAATCGGTGAGGCCTGACGCCATATTGCGCGT-5′-Cy3 5′-CGTTAGCCACTCCT CGGTATAACGCGCA-3′-BHQ-2 Mismatch-A-I: 3′-GCAATCGGTGAGGCCTGACGCCATATTGCGCGT-5′-Cy3 5′-CGTTAGCCACTCCA CGGTATAACGCGCA-3′-BHQ-2 Mismatch-C-I: 3′-GCAATCGGTGAGGCCTGACGCCATATTGCGCGT-5′-Cy3 5′-CGTTAGCCACTCCC CGGTATAACGCGCA-3′-BHQ-2



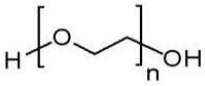
Dextran 6000 $(C_6H_{10}O_5)n$ n=37 Mw=6000



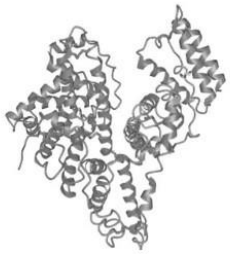
Dextran 12000 $(C_6H_{10}O_5)n$ n=74 Mw=12000



Ficoll 70, $(C_{12}H_{22}O_{11})n$ n=204 Mw=70000



PEG 20000 $HO(C_2H_4O)_nH$ n=453 Mw=20000



BSA Albumin from bovine serum which contains of 583 amino acids Mw=66430

Figure S1. Structures of different macromolecules.

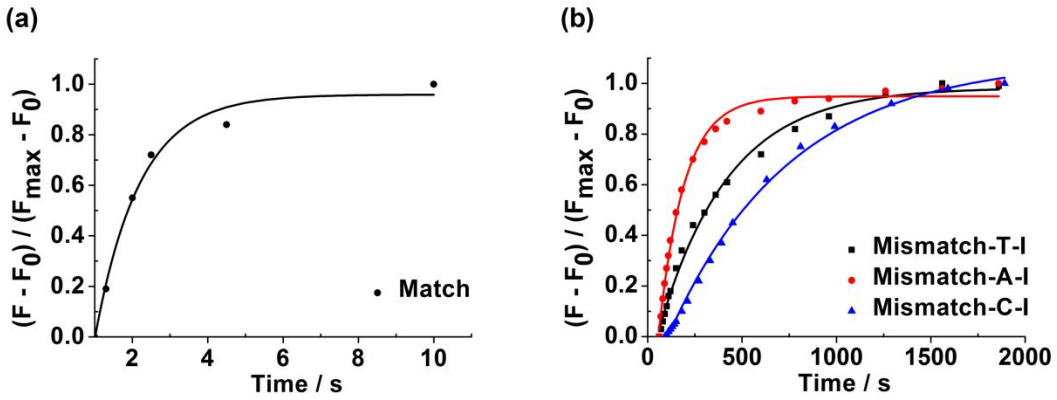


Figure S2. Kinetic profiles with Klenow fragment (exo-) in buffer solutions. Data points were fit to a single-exponential model: $\boldsymbol{Y = Y_0 - A \times exp\ (-k_{obs}\ t)}$. In each process, F_{max} and F_0 were the maximum and initial fluorescence intensity, respectively. (a) Kinetic profiles with a matching target. (b) Kinetic profiles of Mismatch-T-I, Mismatch-A-I and Mismatch-C-I with a single-base mismatch at the target terminus.

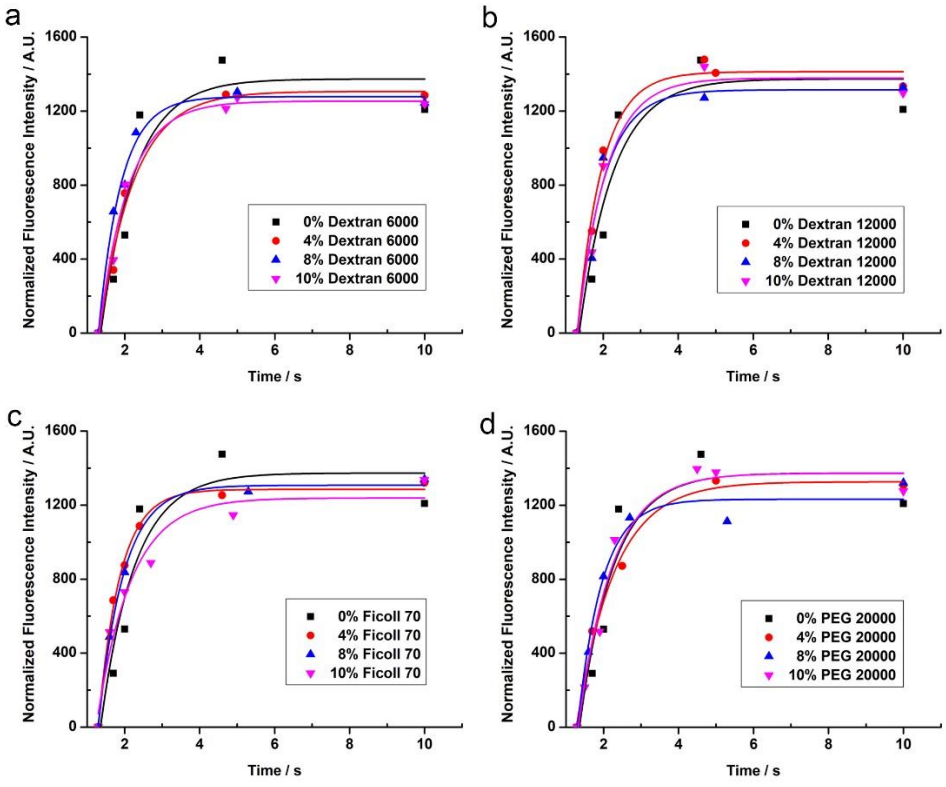


Figure S3. Fluorescence data of the Match sequence in different concentrations of Dextran 6000 (a), Dextran 12000 (b), Ficoll 70 (c), PEG 20000 (d).

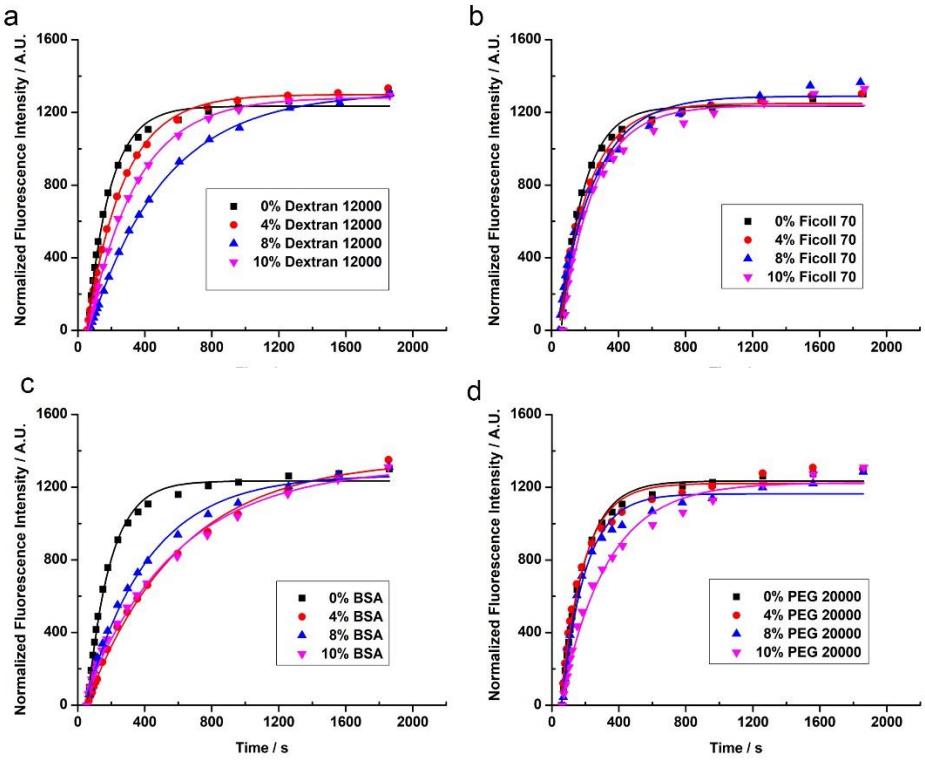


Figure S4. Fluorescence data of the Mismatch-A-I sequence in different concentrations of Dextran 12000 (a), Ficoll 70 (b), BSA (c), PEG 20000 (d).

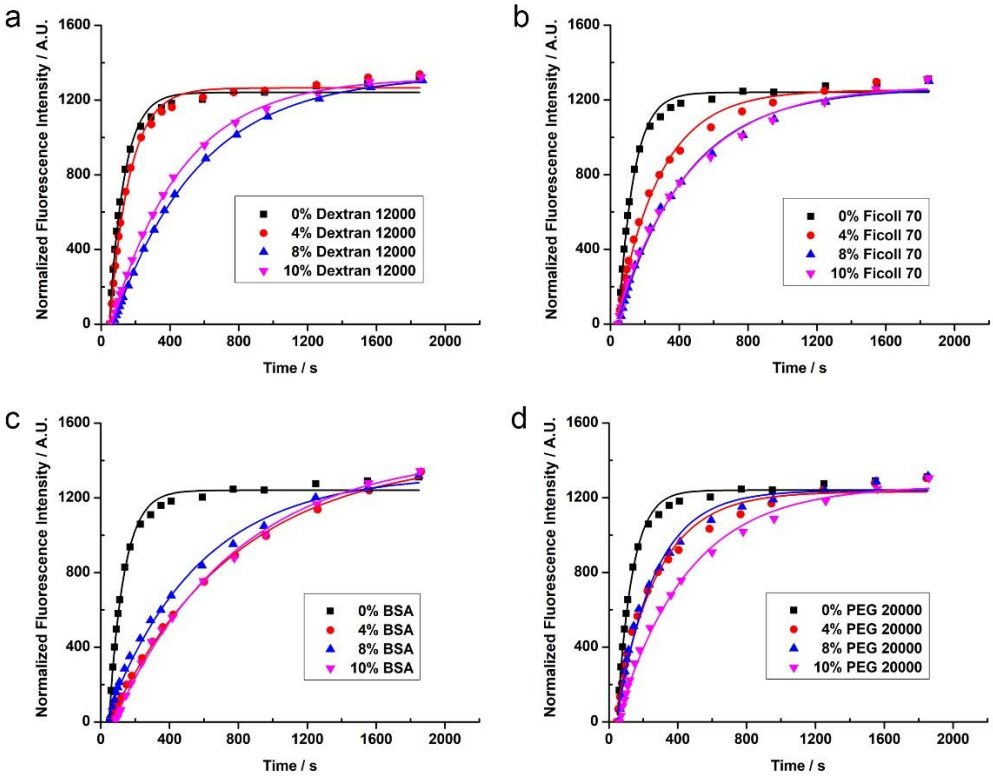


Figure S5. Fluorescence data of the Mismatch-T-I sequence in different concentrations of Dextran 12000 (a), Ficoll 70 (b), BSA (c), PEG 20000 (d).

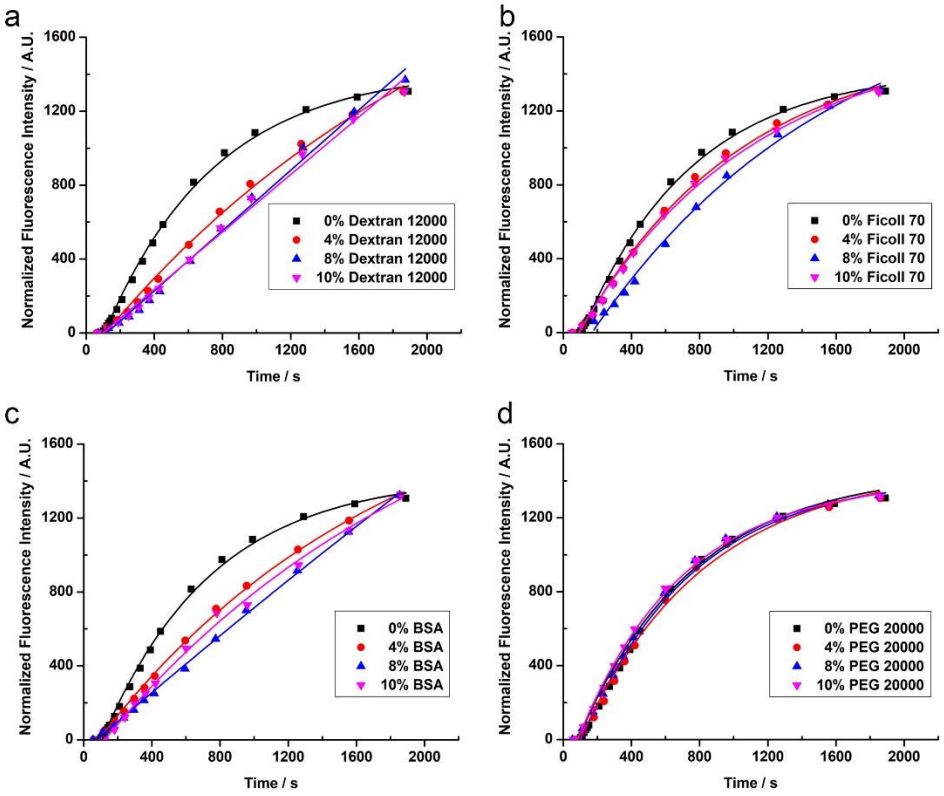


Figure S6. Fluorescence data of the Mismatch-C-I sequence in different concentrations of Dextran 12000 (a), Ficoll 70 (b), BSA (c), PEG 20000 (d).

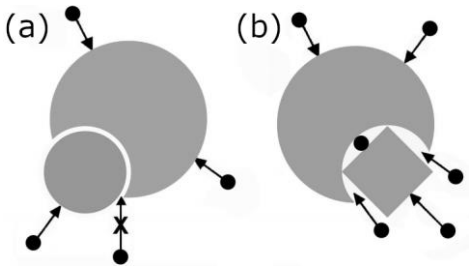


Figure S7. The depletion effects by macromolecular crowding are sensitive to shape match. (a) For the matching target, the template-primer duplex fits well with the shape of the Klenow fragment (exo-) and experiences a relatively strong entropic attraction because the crowding agents (small black dots) cannot enter the interface. (b) The mismatching target causes shape mismatch between the template-primer duplex and the Klenow fragment (exo-), so the crowding agents can enter the interface and weaken the depletion effects.[4]

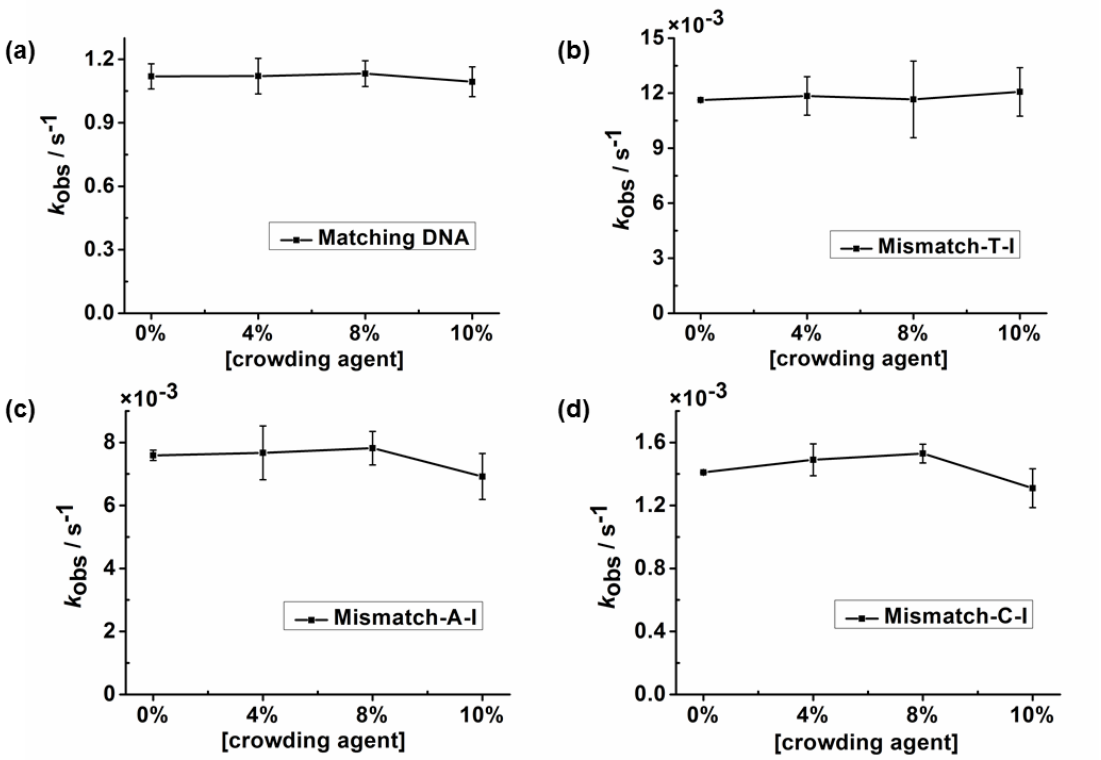


Figure S8. Rate constants derived from the kinetic profiles in different concentrations of glucose with Match sequence (a), Mismatch-T-I (b), Mismatch-A-I (c) and Mismatch-C-I (d).

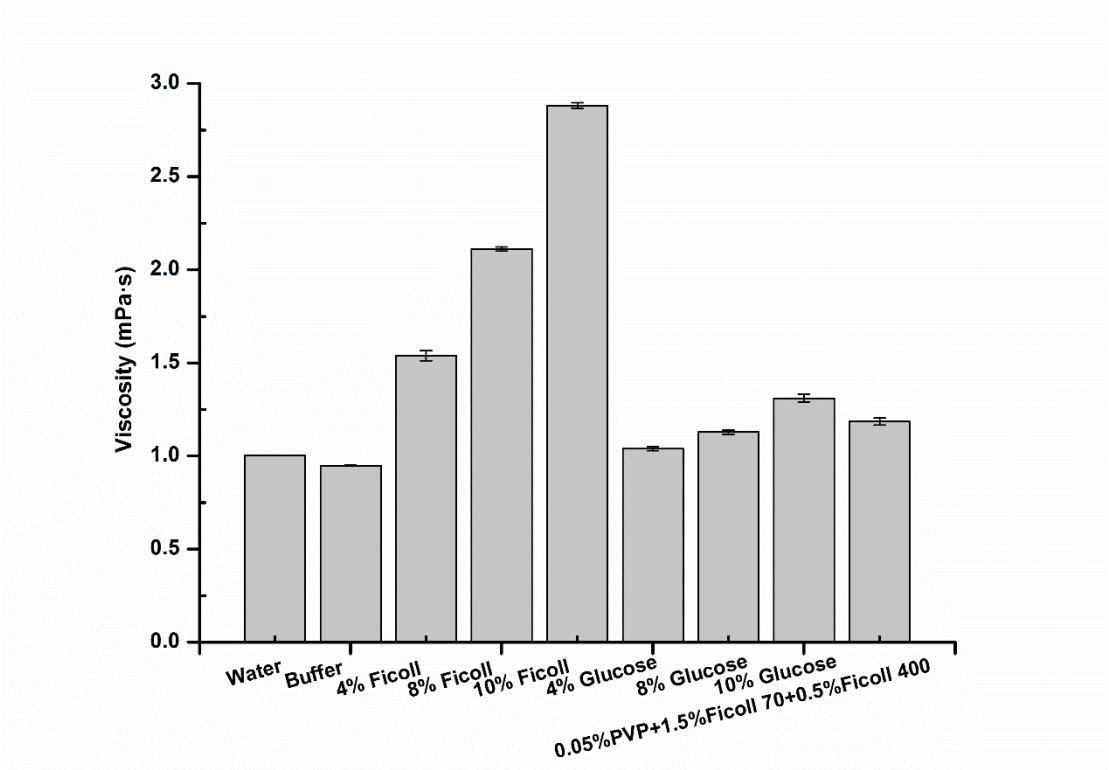


Figure S9. Viscosity measured in water, buffer solutions and buffer solutions containing different kinds of crowding agents.

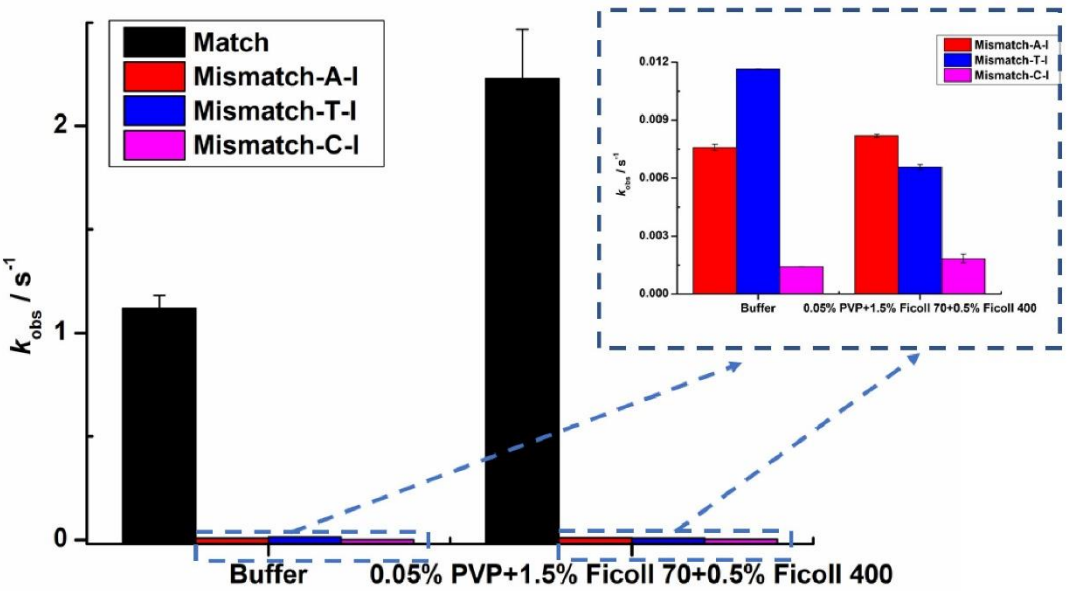


Figure S10. Rate constants derived from the kinetic profiles in buffer solution and 0.05% PVP+1.5% Ficoll 70+0.5% Ficoll 400 solution with Match sequence, Mismatch-T-I, Mismatch-A-I and Mismatch-C-I. Inset was the enlarged picture of the rate constants with the three mismatching sequences.

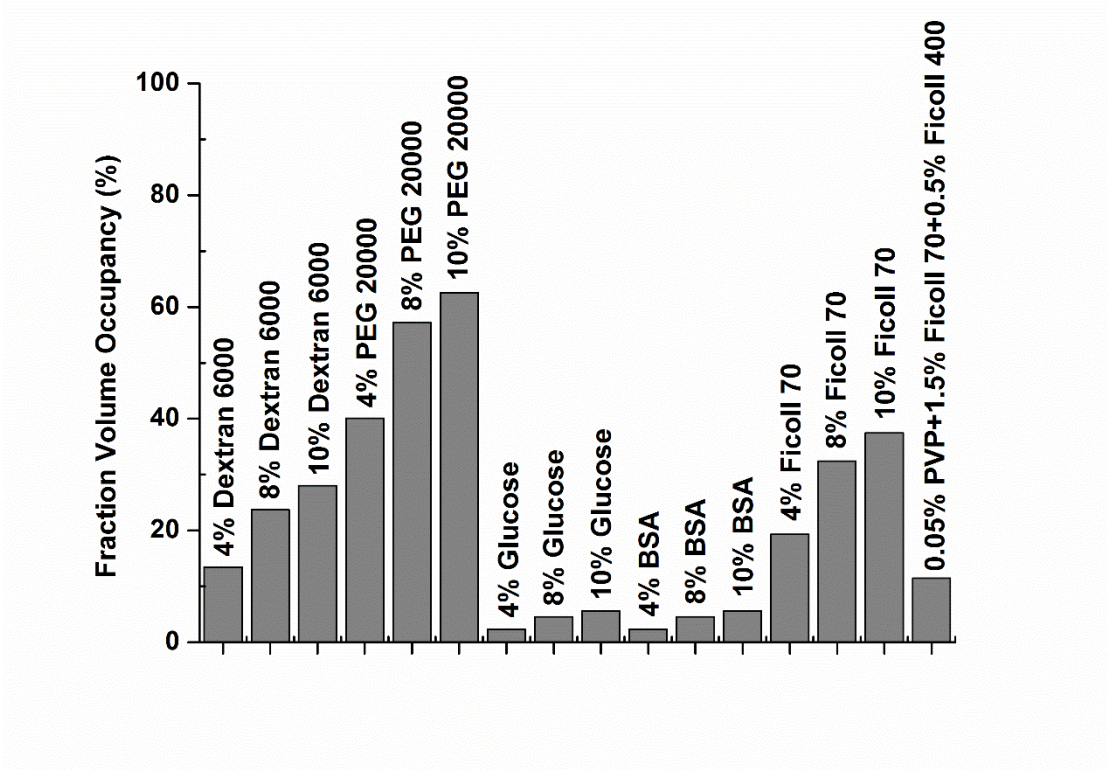


Figure S11. Fraction volume occupancy from different kinds and concentrations of crowding agents.

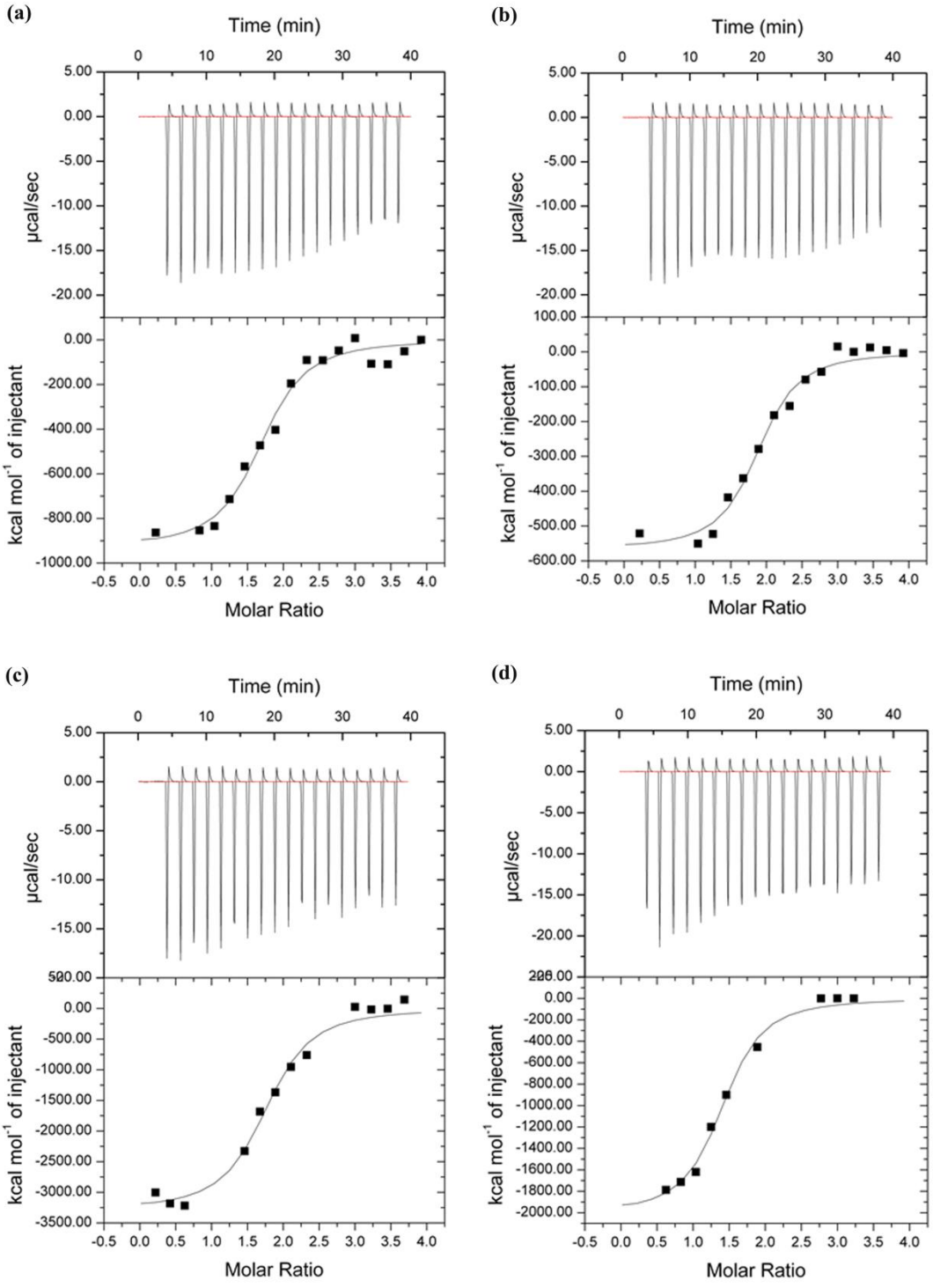


Figure S12. Isothermal titration calorimetry profiles of the Match sequence in Table S1 with the concentrations of 0% Ficoll 70 (a), 4% Ficoll 70 (b), 8% Ficoll 70 (c) and 10% Ficoll 70 (d).

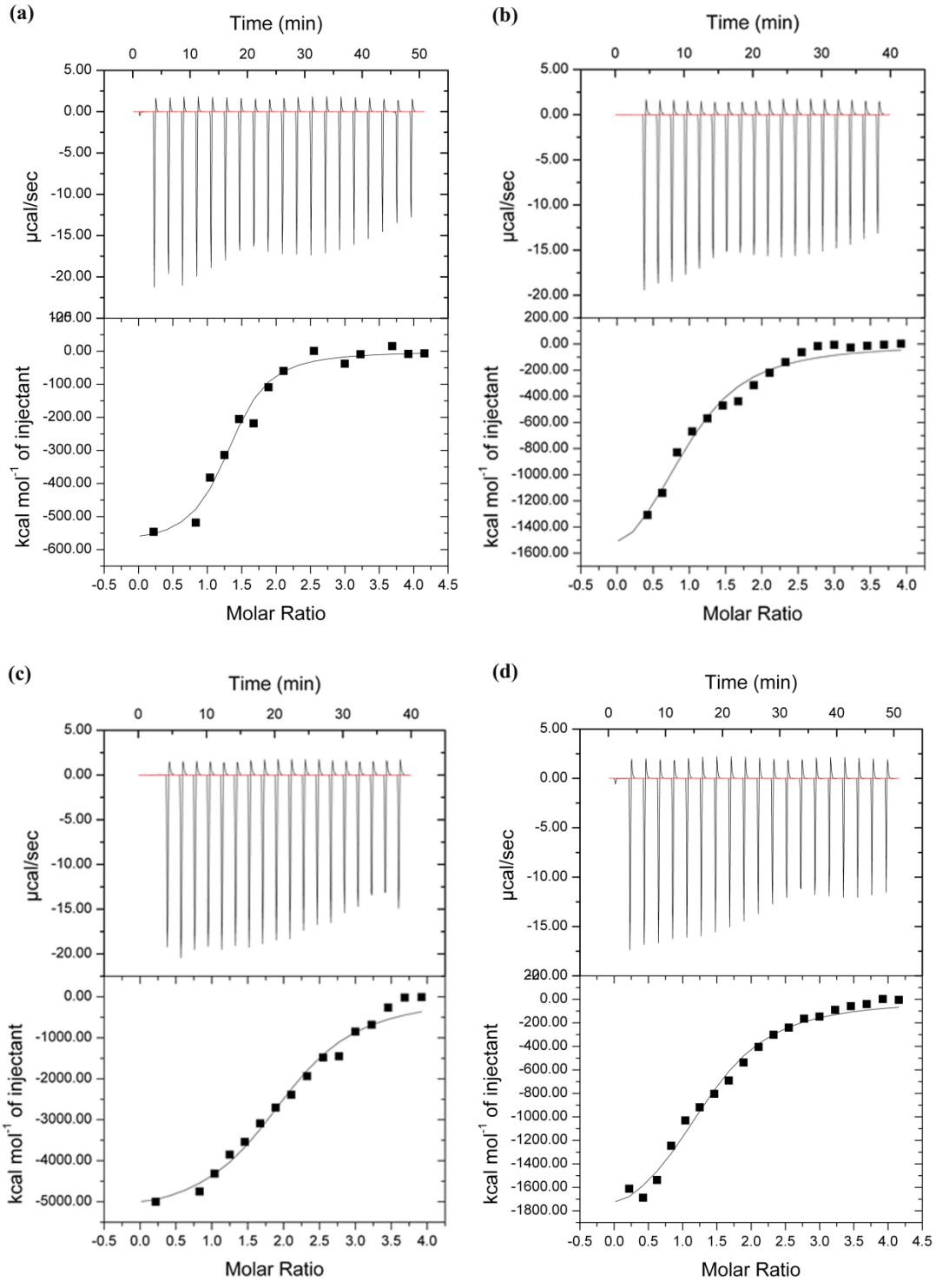


Figure S13. Isothermal titration calorimetry profiles of the Mismatch-T-I sequence in Table S1 with the concentrations of 0% Ficoll 70 (a), 4% Ficoll 70 (b), 8% Ficoll 70 (c) and 10% Ficoll 70 (d).

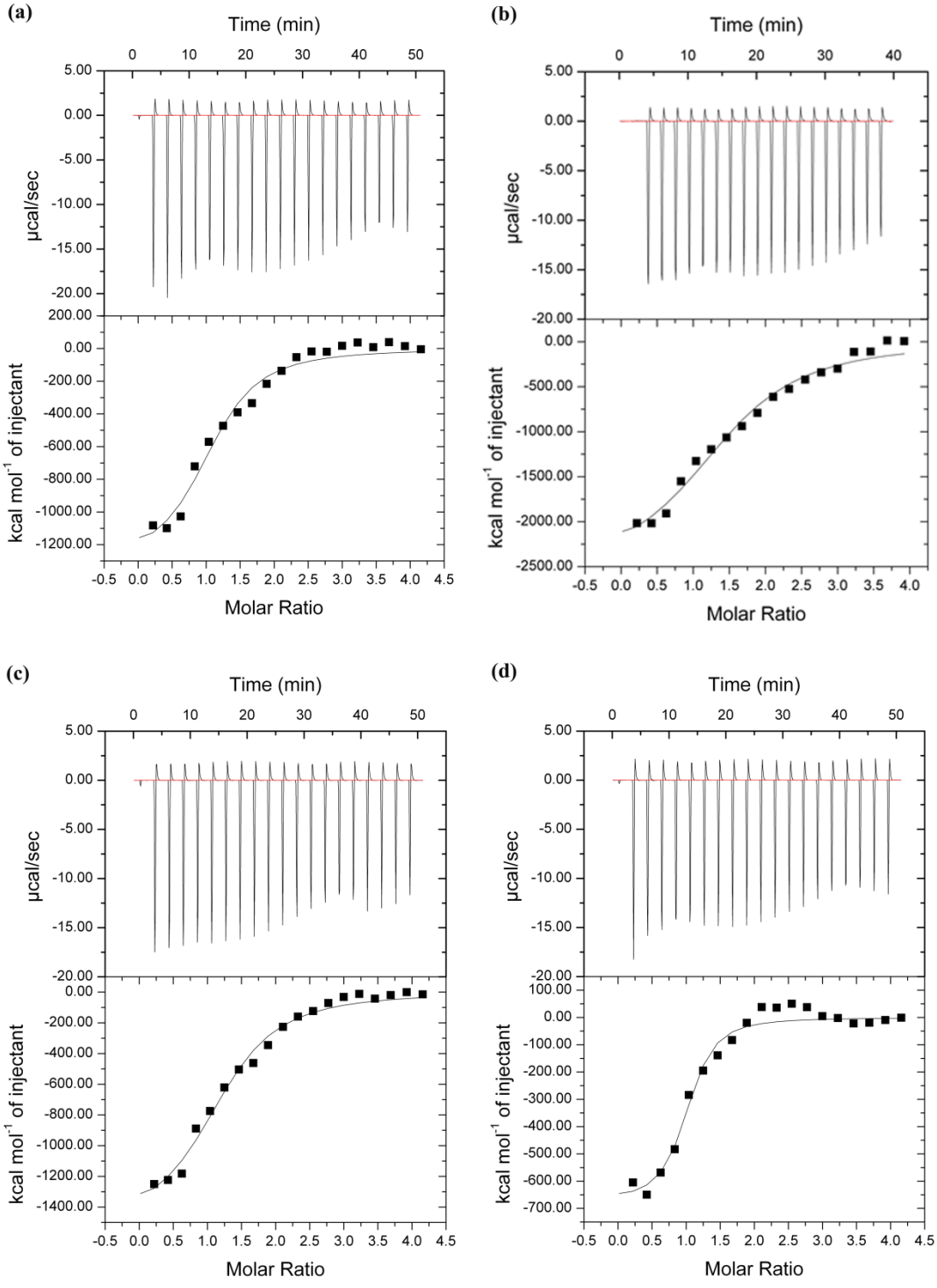


Figure S14. Isothermal titration calorimetry profiles of the Mismatch-A-I sequence in Table S1 with the concentrations of 0% Ficoll 70 (a), 4% Ficoll 70 (b), 8% Ficoll 70 (c) and 10% Ficoll 70 (d).

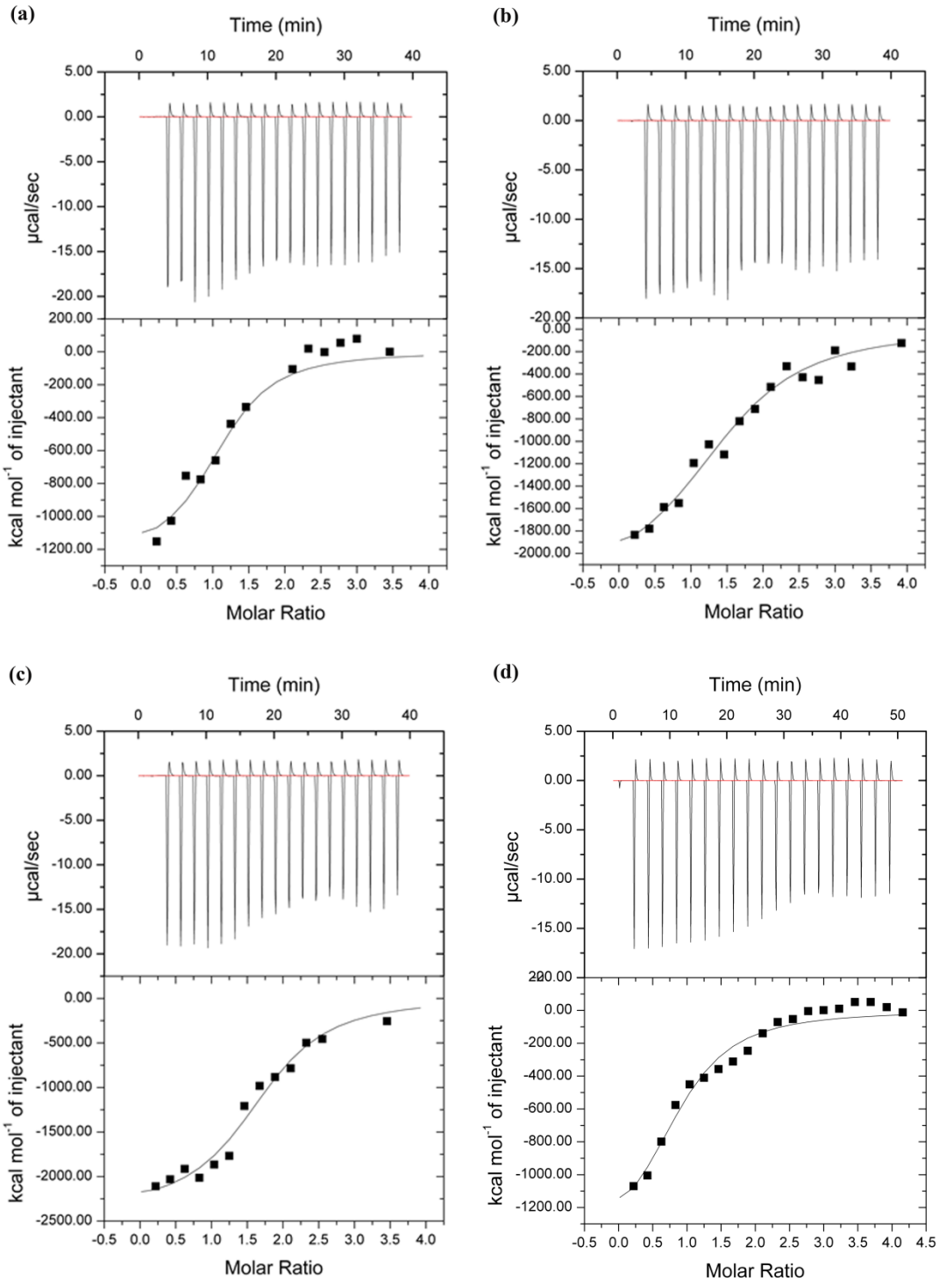


Figure S15. Isothermal titration calorimetry profiles of the Mismatch-C-I sequence in Table S1 with the concentrations of 0% Ficoll 70 (a), 4% Ficoll 70 (b), 8% Ficoll 70 (c) and 10% Ficoll 70 (d).

Reference

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