

Supporting Information

A target-driven DNA-based molecular machine for rapid and homogeneous detection of arginine-vasopressin

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Table S1. Peptides and ODN sequences used in this work

Peptide (N terminus to C terminus)	
AVP	CYFQNCPRG-NH ₂ (disulfide bond 1-6)
AVP-N ₃	N ₃ -KCYFQNCPRG-NH ₂ (disulfide bond 1-6)
OXT	CYIQNCCPLG-NH ₂ (disulfide bond 1-6)
Oligodeoxynucleotides (5' to 3') ^a	
Acetylene-modified ODN	TCCACAGACACAG ACCGATCCT <u>AACGATGGACGAAGTGT</u> <u>CCATCGTT-CHCH</u>
MB-1	FAM- <u>CGGCGGTCCACAGACACACCGCCG</u> -BHQ1
MB-2	ROX- <u>CGGCGGTCCACAGACACACCGCCG</u> -BHQ2

^a The complementary parts are underlined and the recognition sequence of Nt.AlwI endonuclease are shown in bold. FAM is fluorescein. ROX is 6-carboxy-X-rhodamine. BHQ1 and BHQ2 are Black Hole Quencher 1 and Black Hole Quencher 1, respectively.

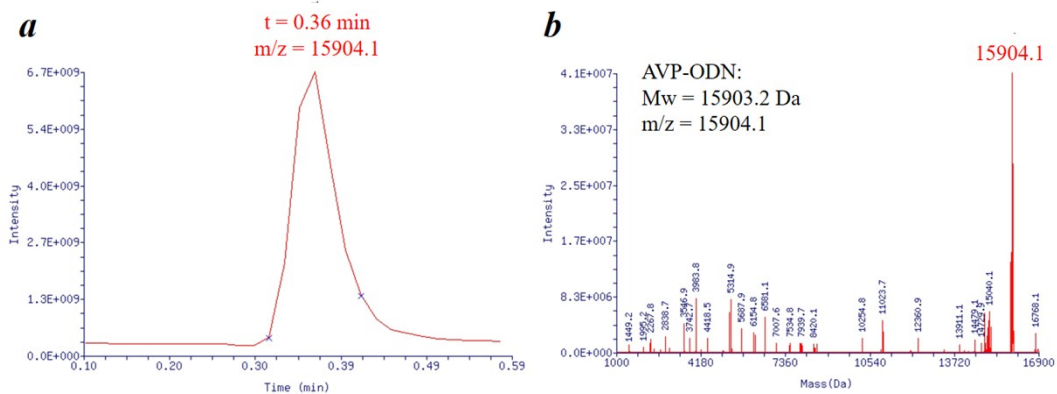


Figure S1. Characterization of the synthesized AVP-ODN conjugate. (a) LC-MS chromatogram. (b) ESI mass spectrum.

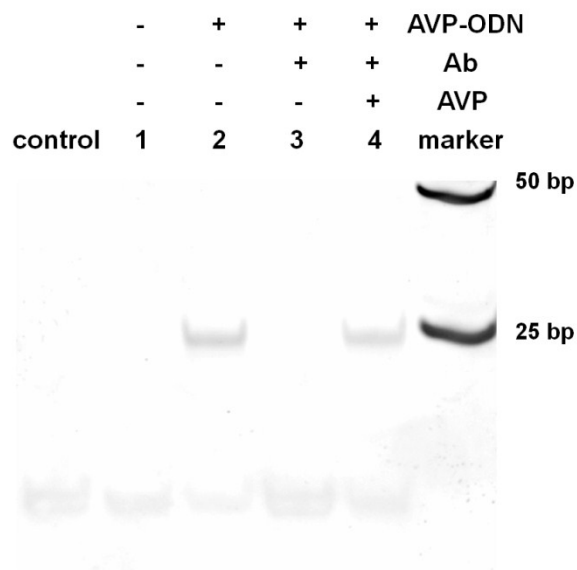


Figure S2. Gel electrophoresis analysis of the DNA duplexes formed between the ssDNA (generated by the nicking enzyme) and the molecular beacon (MB-1). Control lane, MB-1 only; lane 1, without AVP-ODN; lane 2, AVP-ODN only; lane 3, AVP-ODN + anti-AVP antibody; lane 4, AVP-ODN + antiAVP-antibody + AVP (700 pM)

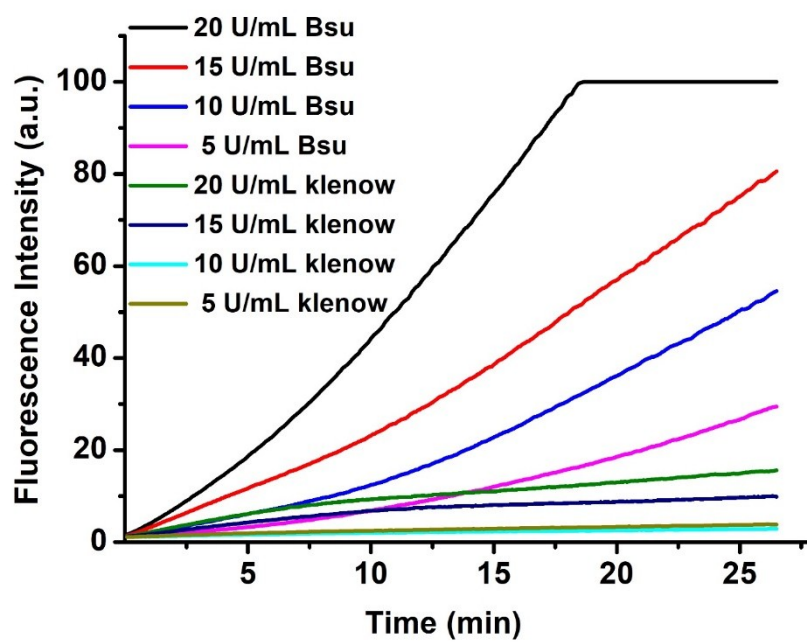


Figure S3. Time curves of the fluorescence signals of 10 nM AVP-ODN at different concentrations of *Bsu* or Klenow polymerase.

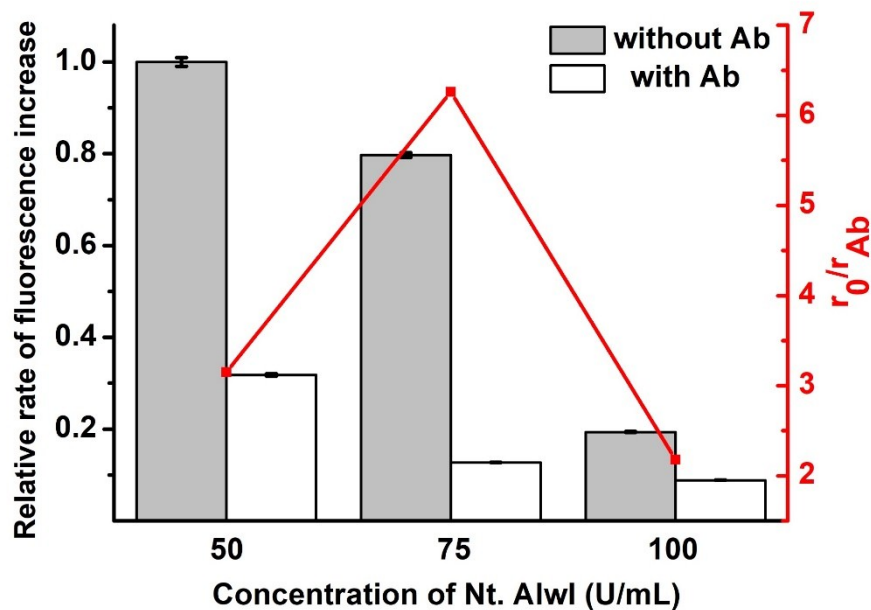


Figure S4. Relative rate of fluorescence increase of 10 nM AVP-ODN with and without addition of anti-AVP antibody (300 nM) at different concentrations of Nt. AlwI (column). The rate of fluorescence increase of AVP-ODN without addition of antibody at 50 U/mL of Nt. AlwI was set as 1.0. Ratio of the rate obtained without and with anti-AVP antibody represents the detection window (line in red).

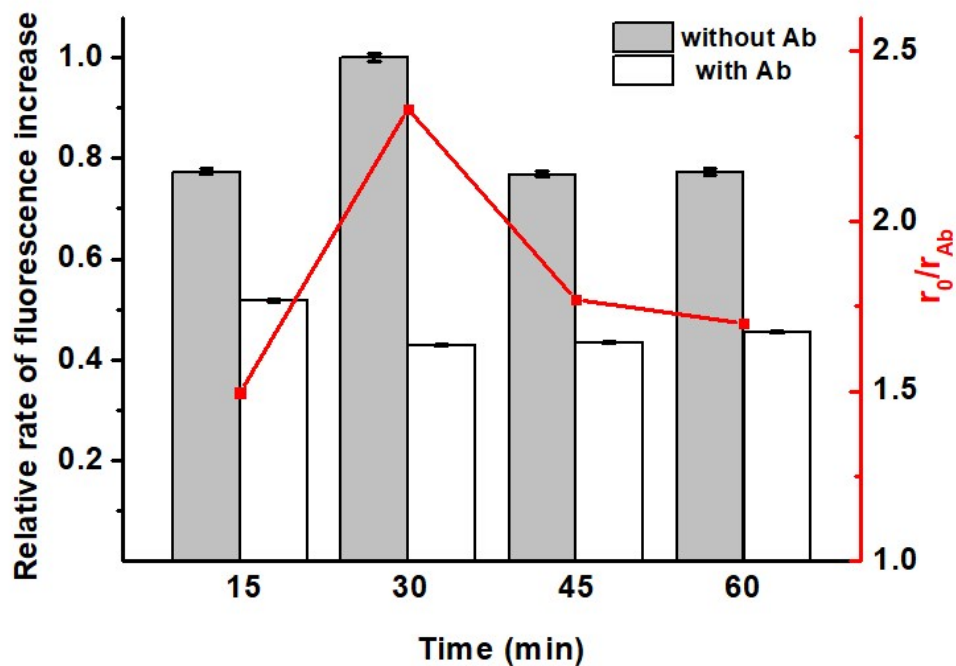


Figure S5. Relative rate of fluorescence increase of 10 nM AVP-ODN with (white column) and without (grey column) addition of anti-AVP antibody (300 nM) at different incubation time. The rate of fluorescence increase of AVP-ODN incubated for 30 min without anti-AVP antibody was set as 1.0. Ratio of the rate obtained without and with antibody represents the detection window (line in red).

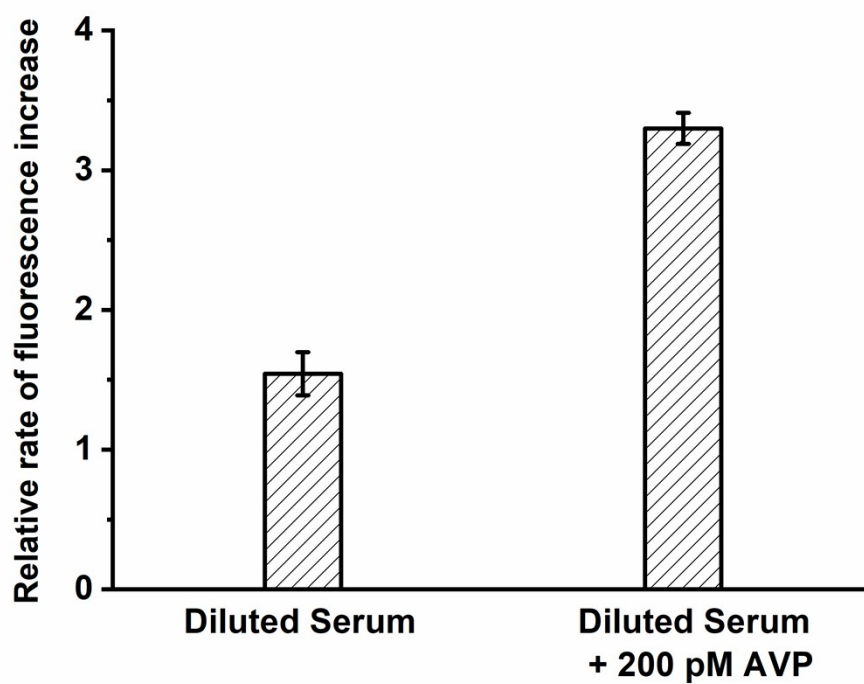


Figure S6. Recovery test of 200 pM AVP in 10 times diluted serum by using MB-1.

The recovery of AVP was observed to be $92\pm 2\%$ ($n=3$).

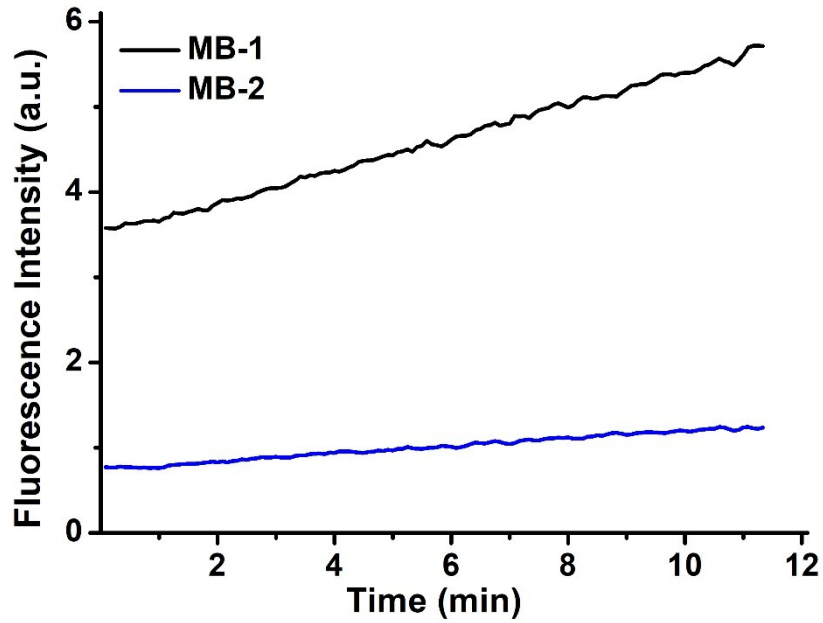


Figure S7. Influences of the serum matrices on the stability of MB-1 and MB-2 under the detection conditions. 200 nM MB and 400 μ M dNTPs were mixed in Cutsmart Buffer. After the heating-annealing procedure, 300 nM anti-AVP antibody and 2.5 μ L serum without any pretreatment were added. After incubation at 37 $^{\circ}$ C for 30 min, 40 U/mL DNA polymerase and 75 U/mL Nt.AlwI were added and the time dependent fluorescent curves were recorded. No AVP-ODN was added to the reaction solution.