SUPPLEMENTARY INFORMATION

Aptasensors Based on Supramolecular Structures of Nucleic Acid-Stabilized Ag Nanoclusters

Etery Sharon‡, Natalie Enkin‡, H. Bauke Albada and Itamar Willner*

Institute of Chemistry, The Center for Nanoscience and Nanotechnology,

The Hebrew University of Jerusalem, Jerusalem 91904, Israel

E-mail: willnea@vms.huji.ac.il.

Phone: 972-2-6585272. Fax: 972-2-6527715

Author Contributions

E. S and N. E. ‡ contributed equally to this work.

Experimental Section

Materials

Ultrapure water from NANOpure Diamond (Barnstead International, Dubuque, IA, USA) was used for all experiments. All other reagents were purchased from Sigma-Aldrich Inc. The DNA strands were purchased from Integrated DNA Technologies Inc. (IDT). All oligonucleotides were HPLC-purified and freeze-dried by the supplier. The oligonucleotides were used as provided and dissolved in an ultrapure water to give stock solutions of 100 μM.

The list of nucleic acids sequences used in the study include:

(**1a**) 5'-CTC TGC TCG ACG GAT TCC TCC TGG GGG AGT ATT GCG GAG GGA GGA AGG TTA AGT GT-3'

(**2a**) 5'-ACC CGA ACC TGG GCT ACC ACC CTT AAT CCC CAA TCC GTC GAG CAG AG-3'

(**3a**) 5'-ACA CTT AAC CTT TTT- IAbRQSp -3'

(**1b**) 5'-GCT GCA GAA TGG GAT CTT CAT GAC AAG GAA AAT CCT TCA ATG AAG TGG GTC AAT TAT-3'

(**2b**) 5'-CCC TTC CTT CCT TCC AAC CAA CCC ATC CCA TTC TGC AGC-3'

(**3b**) 5'-ATA ATT GAC CCA TTT-BHQ2 -3'

Synthesis of Fluorescent Ag NCs

The 615 nm luminescent Ag NCs were synthesized by mixing 10 μL of the nucleic acid (**2a**), 100 μM, with 20 μL of a phosphate buffer solution, 20 mM, pH = 7.0. To this solution, 4 μL of a freshly prepared aqueous solution of $AgNO_3$, 1.5 mM, were

added, followed by the vigorous shaking of the solution for 30 seconds. After 15 minutes, 4 μL of freshly prepared aqueous solution of $NaBH_4$, 1.5 mM, was added to the solution, followed by vigorous shaking of the mixture for 30 seconds. The solution was kept in dark at room temperature and was allowed to react for 3 hours. The 560 nm luminescent Ag NCs were synthesized by mixing of the nucleic acid (**2b**), as described above.

Preparation of the sensing modules

The analyses were performed in a reaction volume of 120 μL that included phosphate buffer solution, 10 mM, pH = 7.0, HEPES buffer solution, 10 mM, pH = 7.0, and 200 mM $NaNO_3$. The sensing modules: (**1a**)/(**2a**)/(**3a**), and (**1b**)/(**2b**)/(**3b**), 5 μM and 7 μM, respectively, were reacted for 30 minutes and then subjected to the respective targets. The (**1a**)/(**2a**)/(**3a**) sensing module was reacted with AMP, ATP, ADP, GMP, UMP, and the (**1b**)/(**2b**)/(**3b**) sensing module was reacted with cocaine, for different time intervals, at fixed concentrations of the targets, or for a fixed time intervals for variable concentrations of the targets.

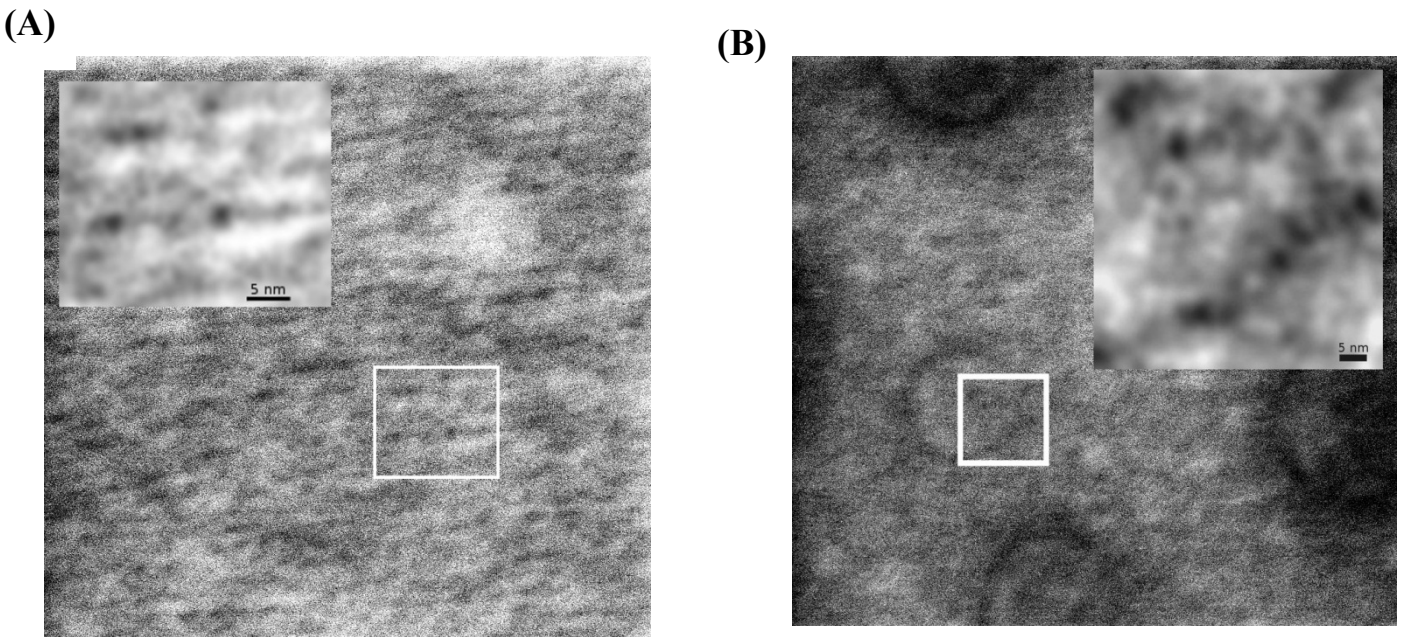


Figure S1: TEM images of: (A) The (2a)-stabilized AgNCs (B) The (2b)-stabilized AgNCs.

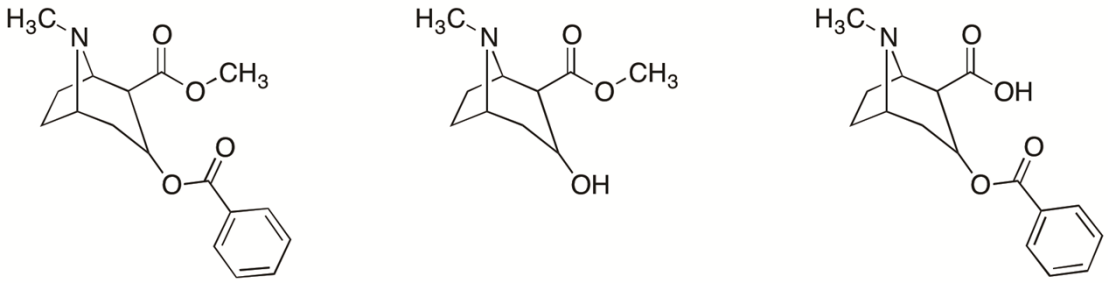
Table S1: Characterization of the nucleic acid modified AgNCs by ESI-MS spectometry

ESI-MS analysis revealed the presence of 6–7 atoms in the (2a)-stabilized Ag NCs. The formula weight of the DNA is 14282.2 g/mol, and its $[M+H]^+$ parent ion peak is detected at m/z =14284.6 (calc: 14283.2). Using this value, the following m/z values could be identified.

615 DNA	foundm/z	calcm/z	assignment
6 Ag NCs	2487.0	2487.2	for $[DNA–6H+6Ag]^{6–}$
7 Ag NCs	2505.0	2505.2	for $[DNA–6H+7Ag]^{6–}$

ESI-MS analysis revealed the presence of 4–5 atoms in the (2b)-stabilized Ag NCs. The formula weight of the DNA is 11612.9 g/mol, and its $[M+H]^+$ parent ion peak is detected at m/z = 11614.9 (calc: 11614.6). Using this value, the following m/z values could be identified.

560 DNA	foundm/z	calcm/z	assignment
4 Ag NCs	2408.9	2408.1	for $[DNA–5H+4Ag]^{5–}$
5 Ag NCs	2429.4	2429.6	for $[DNA–5H+5Ag]^{5–}$



cocaine (**1S**)

ecgonine methyl ester (**2S**)
"crack"

benzoylecgonine (**3S**)

Cocaine (**1S**) and its derivatives ecgonine methylester (**2S**) and the benzoylecgonine (**3S**), the illicit drug, "crack", are metabolites of cocaine. We examine the selectivity of the sensing module shown in Figure 1 toward the discrimination of the two metabolites from cocaine.

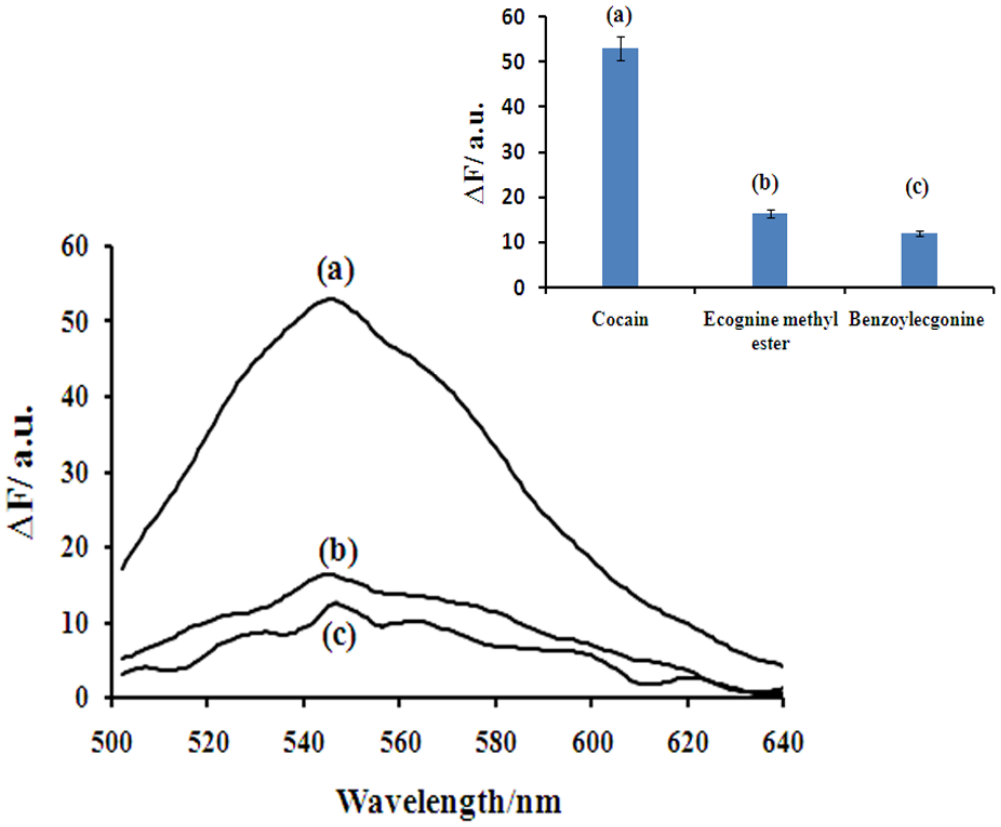


Figure S2: Evaluation of the sensitivity of the cocaine-sensing module shown in Figure 1

Figure S2 depicts the luminescence spectra generated by the sensing module (**1b**)/(**2b**)/(**3b**) upon analyzing cocaine, (**1S**), 12 μM, curve (a) and upon analyzing ecgonine methylester, (**2S**), and of benzoylecgonine (**3S**), 20 μM each, curves (b) and (c), respectively. The luminescence spectra were recorded after a fixed time-interval corresponding to 85 minutes. Note that minute luminescence changes are observed for (**2S**) and (**3S**), although the concentrations of these derivatives are 1.8-fold higher than the concentration of cocaine (**1S**). These results indicate that the sensing module is selective towards the analysis of cocaine.