Modification of anti-DNA antibodies with carbon quantum dots

Simona Dostalova [1,2*], Marketa Vaculovicova [1,2], Sona Krizkova [1,2], Lukas Richtera [1,2], Pavel Kopel [1,2] and Rene Kizek [1,2]

[1] Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic, European Union; simona1dostalova@gmail.com (S. D.), marketa.ryvolova@seznam.cz (M. V.), sonakrizk@centrum.cz (S. K.), oliver@centrum.cz (L. R.), paulko@centrum.cz (P. K.), kizek@sci.muni.cz (R. K.)

[2] Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, Czech Republic, European Union;

* Author to whom correspondence should be addressed; E-Mail: simona1dostalova@gmail.com;
Tel.: +420-5-4513-3350; Fax: +420-5-4521-2044.

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The anti-DNA antibodies are produced in patients with autoimmune disease called systemic lupus erythematodes. They can be reactive against double or single stranded DNA or DNA modified with some other molecules. Using the variety of antibodies it is possible to determine the structure of studied DNA. In this work, we used 4 anti-DNA antibodies produced in egg yolk after immunization of hens with DNA-mBSA antigen – anti-dsDNA, anti-ssDNA, afi-dsDNA and afi-ssDNA. The reactivity of these antibodies was evaluated using the dot blot method with different lengths and concentrations of DNA antigen. The most reactive antibodies (anti-ssDNA) were modified with carbon quantum dots synthesized from multiwall carbon nanotubes and this modification was verified by ELISA-like method with fluorescent detection and fluorescence resonance energy transfer between DNA and quantum dots was observed, increasing the sensitivity of the DNA detection.

Keywords: anti-DNA antibodies; carbon quantum dots; DNA structure; fluorescence imaging

1. Introduction

Antibodies represent a specialized glycoprotein group and form the main base of vertebrate immune system. There are 5 immunoglobulin classes in mammalian serum: IgG, IgM, IgA, IgD and IgE [1]. In avian species the immunoglobulins G are replaced by highly functionally similar immunoglobulins Y and there are also IgA and IgM present [2]. Antibodies are often used in variety of applications, including ELISA for the detection of various analytes [3], virions [4] or tumor markers [5], cell capture for subsequent analysis [6] and magnetic immunoseparation [7,8]. For bulk production, antibodies extracted from chicken egg yolks immunized with the specific antigen are often used [9].

Anti-DNA antibodies were first discovered in 1957 [10] and they can bind to single stranded or double stranded DNA [11,12]. Anti-dsDNA antibodies can be found in the blood of systemic lupus erythematodes (SLE) patients. This autoimmune disease is manifesting by formation of immunocomplexes [13]. The antibodies usually belong to class IgM in normal patients or IgG in SLE patients [14] and they can be transiently found in the blood of patients with some viral infections (HIV, BK or B19 virus) [15]. Anti-dsDNA antibodies usually bind to sugar-phosphate backbone, base pairs or some double strand conformations. Anti-ssDNA antibodies should be able to bind to bases, nucleotides, oligonucleotides and sugar-phospate backbone [16]. Commercially available are also antibodies against DNA adducts such as those with cisplatin [17]. The combination of suitable antibodies can help identify the secondary structures of

DNA [18].

To enhance the antigen detection limit it is possible to label the antibodies with highly fluorescent nanoparticles such as quantum dots (QDs) [3]. QDs have unique fluorescent characteristics, including wide absorbance spectrum with narrow emission spectrum, high quantum yield or excellent photostability [19]. They are usually formed by a semiconductor crystals, such as CdTe, CdS or ZnS, but they can also be prepared from carbon materials with polymer coating [6]. These materials include nanodiamonds [20], graphene [21], graphite [22], single- and multi-wall carbon nanotubes [23], citric acid [24] or sucrose [25]. The prepared carbon quantum dots usually show high fluorescence under UV light [26].

In this work, we studied anti-DNA antibodies produced in chicken egg yolks as a tool for DNA structure detection. We used four different anti-DNA antibodies: anti-dsDNA, afi-dsDNA, anti-ssDNA and afi-ssDNA. Their reactivity towards various sized DNA molecules was evaluated using dot blot method. The successful modification of anti-DNA antibodies with carbon quantum dots was determined by ELISA-like method with fluorescent detection.

2. Results and Discussion

2.1 The reactivity of anti-DNA antibodies and DNA of different lengths

The reactivity of 4 different types of anti-DNA antibodies (anti-dsDNA, afi-dsDNA, anti-ssDNA and afi-ssDNA) to different lengths of double and single stranded DNA was evaluated using the dot blot assay on Zeta Probe membrane with high affinity to DNA. 6 different DNA samples were immobilized on the membrane in the order described in Fig. 1A – double and single stranded oligonucleotide (12 bp), double and single stranded DNA fragment (498 bp) and double and single stranded genomic DNA from bacteriophage λ (48502 bp). To determine which part of DNA serves as an antigen for anti-DNA antibodies, we used two concentrations of different DNA lengths – the same molar concentration (15 nM, Fig. 1B) and same mass concentration (100 μg/mL, Fig. 1C). The antibodies used were produced in chicken egg yolk after hen immunization. Anti-chicken antibody labeled with horseradish peroxidase was used for colorimetric detection of antibody-DNA binding. Table 1 shows the quantification of the observed bands intensity

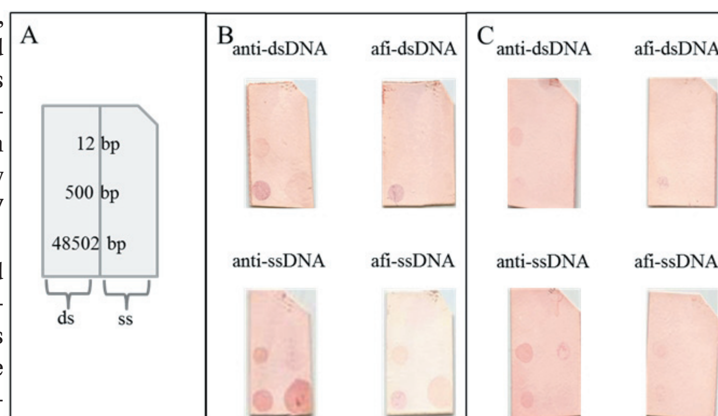

Figure 1: The dot blot assay for evaluation of antibody reactivity with single and double stranded DNA of different length. (A) The sample order on Zeta Probe membrane. (B) Dot blot assay with 15 nM DNA and 22 μg/mL of antibodies. (C) Dot blot assay with 100 μg/mL of DNA and 22 μg/mL of antibodies.

None of the tested antibodies were able to bind to oligonucleotides, either single stranded or double stranded. The anti-dsDNA antibody was able to bind to double stranded DNA fragment and both double and single stranded genomic DNA. With the same molar concentrations of DNA, the anti-dsDNA antibody bound most to the double stranded genomic DNA and the binding to double stranded DNA fragment and single stranded genomic DNA was similar. However, with the same mass concentrations of used DNA, the anti-dsDNA antibody bound equally to all three of these samples. From these results, it can be concluded that this antibody probably binds to the DNA mass (i.e. certain number of bases), not to a special part of DNA

molecule. The afi-dsDNA was prepared by affinity purification of anti-dsDNA antibodies. This antibody was able to bind only to the double stranded genomic DNA, it was non-reactive to any shorter or single stranded DNA.

the single stranded DNA can form double strands with its complementary molecules. The afi-ssDNA was prepared in a similar way to the afi-dsDNA. With the same molar concentrations of DNA, this antibody bound to

Antibody	DNA	15 nM DNA	100 μg/mL DNA
Anti-dsDNA	ds-12 bp	0	0
	ss-12 bp	0	0
	ds-500 bp	40	40
	ss-500 bp	0	0
	ds-48502 bp	56	35
	ss-48502 bp	35	30
Afi-dsDNA	ds-12 bp	0	0
	ss-12 bp	0	0
	ds-500 bp	0	0
	ss-500 bp	0	0
	ds-48502 bp	45	31
	ss-48502 bp	0	0
Anti-ssDNA	ds-12 bp	0	0
	ss-12 bp	0	0
	ds-500 bp	51	46
	ss-500 bp	18	0
	ds-48502 bp	39	50
	ss-48502 bp	56	41
Afi-ssDNA	ds-12 bp	0	0
	ss-12 bp	0	0
	ds-500 bp	18	33
	ss-500 bp	0	0
	ds-48502 bp	24	34
	ss-48502 bp	19	0

Table 1: The colorimetric intensity quantification of dot blot assay.

double stranded DNA fragments and single and double stranded genomic DNA, but in the case of same concentrations of DNA, the afi-ssDNA antibody bound only to double stranded DNA fragment and genomic DNA. These antibodies probably bind to some part of DNA molecule, but the reactivity is also dependent on the DNA mass.

The anti-ssDNA antibody was the most reactive, it was able to bind to both single and double stranded DNA fragment and genomic DNA. With the same molar concentrations of DNA, the antibody bound most equally the double stranded DNA fragment and single stranded genomic DNA. The reactivity to single stranded DNA fragment was poor. With the same mass concentrations of DNA, the antibody equally bound to double stranded DNA fragment and single and double stranded genomic DNA. There was no reactivity to single stranded DNA fragment. Overall, the anti-ssDNA antibody is more reactive to double stranded DNA than single stranded which can be caused by the immunization process during which

2.2 Characterization of carbon quantum dots

The most reactive antibodies (anti-ssDNA) were subsequently modified with carbon quantum dots synthesized from multiwall carbon nanotubes (MWCNT-CQDs). Fig. 2A shows these quantum dots under ambient

and UV (254 and 312 nm). Fig. 2B shows the absorbance spectrum of MWCNT-QDs. The absorption of these quantum dots is the highest for light of smaller wavelengths (UV light). However, their fluorescence intensity increased in dependence to the increasing excitation wavelength with the excitation maximum at 330 nm.

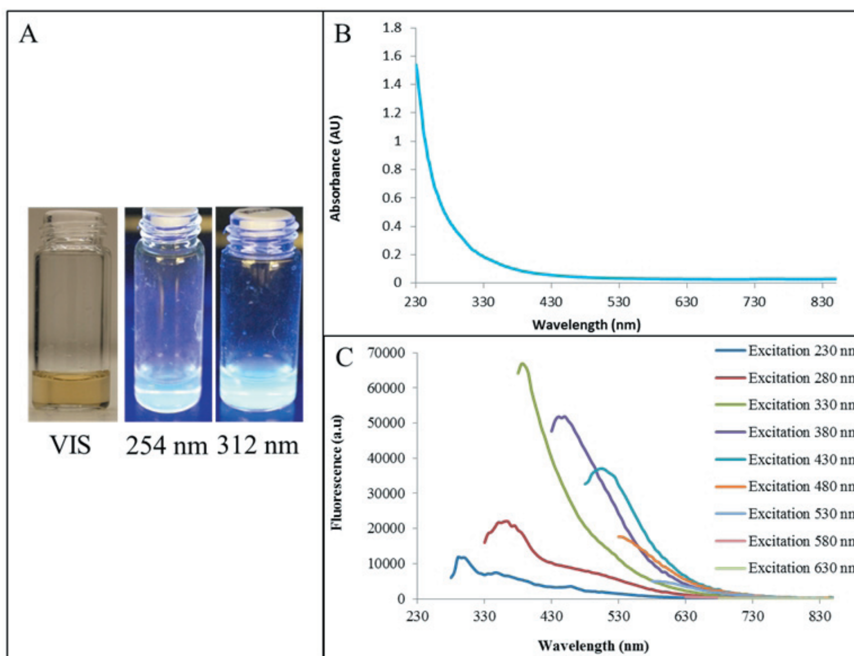

Figure 2: The characterization of MWCNT-CQDs. (A) Visualization under ambient and UV (254 and 312 nm) light. (B) Absorbance spectrum of MWCNT-CQDs. (C) The fluorescence spectra of MWCNT-CQDs after various excitation wavelength.

2.3 Modification of anti-DNA antibodies with carbon quantum dots

These quantum dots were used for the modification of anti-ssDNA antibodies. The successful modification was verified by ELISA-like method with fluorescent detection (Fig. 3). The double stranded DNA fragments were immobilized on the surface of microtiter plate well and the rest of the surface was blocked by milk powder in PBS. The samples were then incubated with antibodies with and without MWCNT-QDs modification and excess molecules of antibodies were removed by washing. The fluorescence of MWCNT-QDs was measured using their excitation maximum at 330 nm. Antibodies with no MWCNT-QDs modification (Fig. 3A) showed no fluorescence using this excitation. In case of antibodies modified with MWCNT--QDS there was very low fluorescence observed and only with the highest DNA concentration (100 µg/mL) (Fig. 3B). However, high fluorescence was observed with the excitation at 230 nm. This fluorescence was observed in samples with antibodies without the MWCNT-QDs modification (Fig. 3C) as well as antibodies with MWCNT-QDs (Fig. 3D). However, the fluorescence was 4 times higher in the case of anti-ssDNA modified with MWCNT-QDs than unmodified antibodies and it increased with the increasing DNA concentration linearly. This difference between the fluorescence of carbon quantum dots and antibodies modified with carbon quantum dots bound to DNA can be probably explained by fluorescence resonance energy transfer between the DNA, antibodies and carbon quantum dots.

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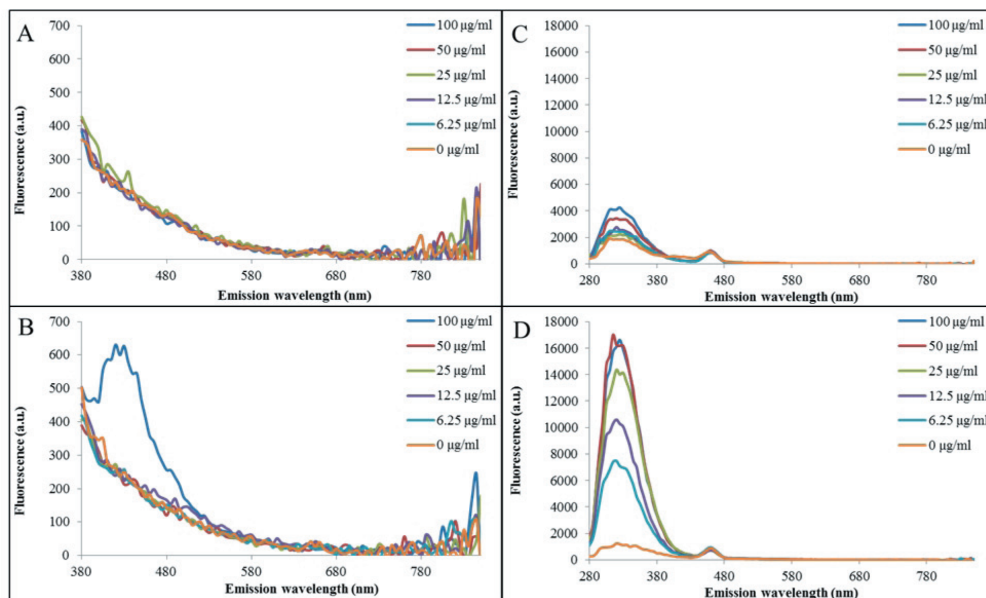

Figure 3: Modification of anti-DNA antibodies with MWCNT-CQDs determined by ELISA-like method with fluorescent detection. (A) Fluorescence spectra (excitation wavelength 330 nm) of microtiter plate wells coated with DNA fragment after incubation with anti-ssDNA antibodies. (B) Fluorescence spectra (excitation wavelength 330 nm) of microtiter plate wells coated with DNA fragment after incubation with anti-ssDNA antibodies modified with MWCNT-CQDs. (C) Fluorescence spectra (excitation wavelength 230 nm) of microtiter plate wells coated with DNA fragment after incubation with anti-ssDNA antibodies. (D) Fluorescence spectra (excitation wavelength 230 nm) of microtiter plate wells coated with DNA fragment after incubation with anti-ssDNA antibodies modified with MWCNT-CQDs.

3. Experimental Section

3.1 Chemicals

All chemicals of ACS purity were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. Deionized water underwent demineralization by reverse osmosis using the instrument Aqua Osmotic 02 (Aqua Osmotic, Tisnov, Czech Republic) followed by further purification using Millipore RG (Millipore, Billerica, MA, USA, 18 MO)–MiliQ water. The pH was measured using WTW inoLab pH meter (Weilheim, Germany).

3. 2 dsDNA and ssDNA preparation

3 different lengths of DNA were used as samples for evaluation of antibody reactivity – genomic DNA (48502 bp), DNA fragment (498 bp) and oligonucleotides (12 bp). Genomic DNA isolated from bacteriophage λ was purchased from New England Biolabs (Ipswitch, MA, USA). From this DNA, xis gene fragment was amplified using Taq PCR kit purchased from New England Biolabs (Ipswitch, MA, USA) and primers synthesized by Sigma-Aldrich (St. Louis, MO, USA) with the sequence 5´-CCTGCTCT-GCCGCTTCACGC-3´ for forward primer and 5´-TCCGGATAAAAACGTCGATGACATTT-GC-3´ for reverse primer. The reaction mixture (100 μL) composed of 1× standard Taq reaction buffer; 0.2 μM deoxynucleotide solution; 0.2 μM of each primer; 6.25 U of Taq DNA polymerase and 750 ng DNA. PCR took place in Mastercycler ep realplex4 S (Eppendorf, New Brunswick, Germany) and the cycling conditions were as follows: denaturation for 120 s at 95 °C; 30 cycles of denaturation for 15 s at 95 °C, annealing for 15 s at 64 °C and elongation for 45 s at 72 °C with a final elongation for 5 min at 72 °C. Obtained DNA fragments were purified using MinElute PCR Purification Kit (Qiagen, Hilden, Germany) and their concentration was determined spectrophotometrically (Analytic Jena, Jena, Germany). The oligonucleotide se-

quences were 5´-ATGGAATGCAGG-3´ (O1) and 5´-CCTGCATTCCAT-3´ (O2), respectively and they were synthesized by Sigma-Aldrich (St. Louis, MO, USA).

To produce double stranded oligonucleotides, O1 and O2 were mixed in 1:1 molar ratio, heated at 99 °C for 4 min and slowly cooled to room temperature to enable the formation of double strand. To dissolve double stranded DNA fragment and genomic DNA and produce single stranded DNA, the samples were heated at 99 °C for 4 min, shock-cooled on ice to prevent the re-formation of double stranded DNA and immediately used for subsequent analysis.

3.3 Preparation of antibodies

Chicken anti-DNA antibodies were prepared by HENA (Prague, Czech Republic). Hens were immunized with DNA-mBSA complex and IgY fraction reactive to DNA-mBSA was obtained from egg yolk. The antibodies were stabilized with 0.1% NaN_3 in PBS and protein concentration was 38.8 mg/mL in immunoglobulin fraction for anti-dsDNA, 30.1 mg/mL for anti-ssDNA, 29.2 mg/mL for afi-dsDNA and 22.2 mg/mL for afi-ssDNA. For the analysis, antibodies were diluted with antibody dilution buffer containing 1 mg/mL BSA in PBS (137 mM NaCl, 2.7 mM KCl, 1.4 mM NaH_2PO_4, and 4.3 mM Na_2HPO_4, pH 7.4) to a concentration of 22 µg/mL.

3.4 Dot bot assay

2 µL of the DNA samples (15 nM or 100 µg/mL) were immobilized on a Zeta Probe membrane (Bio-Rad, Hercules, CA, USA) and dried at 37 °C in an incubator Galaxy 14S (Eppendorf, New Brunswick, Germany). The membrane was blocked for 30 min during rotation at 40 rpm (Multi RS-60, Biosan, Riga, Latvia) in a blocking buffer containing 1% milk powder in PBS. The solution was replaced by primary antibodies (anti-dsDNA, afi-dsDNA, anti-ssDNA or afi-ssDNA antibody). The membrane was incubated with antibodies for 1 h at room temperature during rotation and then washed with PBS with 0.05% Tween 20 (PBS-T). The secondary antibodies labeled with horseradish peroxidase (Dako, Glostrup, Denmark) in dilution of 1:1500 in dilution buffer were added to the membrane and incubated for 1 h during rotation. Visualization in chromogenic substrate followed after washing with PBS-T. The membrane was immersed in the solution composed of substrate buffer (0.5 M acetate buffer, pH 5.4), 0.4 mg/mL 3-amino-9-ethylcarbazole and hydrogen peroxide in the ratio 1000:10:1. The assay was performed according to [27]. Mean intensity of the color was quantified by Carestream Molecular Software (Rochester, NY, USA) in each spot and the color of the background was deducted.

3.5 Multiwall carbon nanotubes-based CQDs (MWCNT-CQDs) preparation and characterization

MWCNT (0.1 g) were heated in 3:1 mixture of H_2SO_4 : HNO_3 (10 ml) under reflux at 140 °C for 8 h. After cooling acetone (10 ml) was added. The mixture was left overnight and Na_2CO_3 (10.6 g) in water (80 ml) was added with stirring. Brown oily viscous liquid was collected on the bottom of the tube after centrifugation (25000 rpm, 20 min, 20 °C). Aqueous layer was used for fluorescence measurement. Absorbance and fluorescence spectra were measured using the TECAN microtitration plate reader Infinite 200 PRO (Männedorf, Switzerland) using 100 µL of the sample in the UV transparent 96-well plate. Each absorbance value is an average of 5 measurements.

3.6 Modification of anti-DNA antibodies with MWCNT-CQDs and ELISA-like verification method

25 µL of MWCNT-CQDs was mixed with 5 µL of anti-ssDNA antibodies (1.4 mg/mL) and 75 µL of water and incubated for 2 h at 20 °C during rotation at 60 rpm. 50 µL of DNA fragment (100 µg/mL) was pipetted on microtiter plate well and incubated at 37 °C for 2 h. The rest of the well surface was blocked for 30 min with a blocking buffer containing 1% milk powder in PBS. The solution was replaced by 100 µL of anti-ssDNA antibodies modified with MWCNT--CQDs and incubated at 37 °C for 1 h and then washed three times with water. Visualization was performed by absorbance and fluorescence

spectra measurement using TECAN microtitration plate reader Infinite 200 PRO (Männedorf, Switzerland) in 100 µL of water.

4. Conclusions

In this work, the reactivity of anti-DNA antibodies was evaluated using the dot blot method. The antibodies were subsequently modified with carbon quantum dots and used in an ELISA-like DNA detection method with fluorescent detection. Using this approach it is possible to increase the sensitivity of DNA immuno-based detection and eliminate the need to use secondary antibodies labeled with HRP in these methods. Moreover, using the combination of different anti-DNA antibodies it is possible to determine different DNA structures.

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements" for biomedical papers.

References

1. Durandy, A.; Kracker, S. Immunoglobulin class-switch recombination deficiencies. Arthritis Res. Ther. 2012, 14.
2. Zhao, Y.; Rabbani, H.; Shimizu, A.; Hammarstrom, L. Mapping of the chicken immunoglobulin heavy-chain constant region gene locus reveals an inverted alpha gene upstream of a condensed upsilon gene. Immunology 2000, 101, 348-353.
3. Janu, L.; Stanisavljevic, M.; Krizkova, S.; Sobrova, P.; Vaculovicova, M.; Kizek, R.; Adam, V. Electrophoretic study of peptide-mediated quantum dot-human immunoglobulin bioconjugation. Electrophoresis 2013, 34, 2725-2732.
4. Lin, J.; Wang, R.; Jiao, P.; Li, Y.; Li, Y.; Liao, M.; Yu, Y.; Wang, M. An impedance immunosensor based on low-cost microelectrodes and specific monoclonal antibodies for rapid detection of avian influenza virus h5n1 in chicken swabs. Biosensors & bioelectronics 2015, 67, 546-552.
5. Li, W.; Jiang, X.; Xue, J.; Zhou, Z.; Zhou, J. Antibody modified gold nano-mushroom arrays for rapid detection of alpha-fetoprotein. Biosensors & bioelectronics 2015, 68, 468-474.
6. Weng, C.-I.; Chang, H.-T.; Lin, C.-H.; Shen, Y.-W.; Unnikrishnan, B.; Li, Y.-J.; Huang, C.-C. One-step synthesis of biofunctional carbon quantum dots for bacterial labeling. Biosensors & bioelectronics 2015, 68, 1-6.
7. Krizkova, S.; Nguyen, H.V.; Stanisavljevic, M.; Kopel, P.; Vaculovicova, M.; Adam, V.; Kizek, R. Microchip capillary electrophoresis: Quantum dots and paramagnetic particles for bacteria immunoseparation : Rapid superparamagnetic-beads-based automated immunoseparation of zn-proteins from staphylococcus aureus with nanogram yield. Methods in molecular biology (Clifton, N.J.) 2015, 1274, 67-79.
8. Zitka, O.; Krizkova, S.; Skalickova, S.; Dospivova, D.; Adam, V.; Kizek, R. Microfluidic tool coupled with electrochemical assay for detection of lactoferrin isolated by antibody-modified paramagnetic beads. Electrophoresis 2013, 34, 2120-2128.
9. Krizkova, S.; Ryvolova, M.; Hynek, D.; Eckschlager, T.; Hodek, P.; Masarik, M.; Adam, V.; Kizek, R. Immunoextraction of zinc proteins from human plasma using chicken yolk antibodies immobilized onto paramagnetic particles and their electrophoretic analysis. Electrophoresis 2012, 33, 1824-1832.
10. Ceppellini, R.; Polli, E.; Celada, F. A DNA-reacting factor in serum of s patient with lupus erythematosus diffusus. Proc. Soc. Exp. Biol. Med. 1957, 96, 572-574.
11. Schur, P.H.; Sandson, J. Immunologic factors and clinical activity in systemic lupus erythematosus N. Engl. J. Med. 1968, 278, 533-&.
12. Tojo, T.; Friou, G.J. Lupus nephritis - varying complement-fixing properties od immunoglobulin g antibodies to antigens of cell nuclei Science 1968, 161, 904-&.
13. Stearns, N.A.; Lee, J.; Leong, K.W.; Sullenger, B.A.; Pisetsky, D.S. The inhibition of anti-DNA binding to DNA by nucleic acid binding polymers. PLoS One 2012, 7.
14. Rothfiel.Nf; Stollar, B.D. Relation of immunoglobulin class pattern of antinuclear antibody and complement-fixing antibodies to DNA in sera from patients with systemic lupus erythematosus J. Clin. Invest. 1967, 46, 1785-&.
15. Oka, Y.; Hirabayashi, Y.; Takahashi, R.; Ishii, T.; Sasaki, T. Can viral infection be a trigger of anti-DNA antibody production through endoplasmic reticulum stress? Arthritis Rheum. 2006, 54, S298-S298.
16. Kalsi, J.K.; Martin, A.C.R.; Hirabayashi, Y.; Ehrenstein, M.; Longhurst, C.M.; Ravirajan, C.; Zvelebil, M.; Stollar, B.D.; Thornton, J.M.; Isenberg, D.A. Functional and modelling studies of the binding of human monoclonal anti-DNA antibodies to DNA. Mol. Immunol. 1996, 33, 471-+.
17. Hirose, J.; Inoue, K.; Morimoto, E.; Iwamoto, H.; Yamaguti, Y.; Kitase, M.; Inagaki, K.; Hiromi, K. Characterization of monoclonal antibodies against (1r,2r)-cyclohexanediamine platinum(ii)-DNA adduct. Biol. Pharm. Bull. 1996, 19, 1220-1222.

18. Xia, Y.M.; Janda, A.; Eryilmaz, E.; Casadevall, A.; Putterman, C. The constant region affects antigen binding of antibodies to DNA by altering secondary structure. Mol. Immunol. 2013, 56, 28-37.
19. Stanisavljevic, M.; Chomoucka, J.; Dostalova, S.; Krizkova, S.; Vaculovicova, M.; Adam, V.; Kizek, R. Interactions between cdte quantum dots and DNA revealed by capillary electrophoresis with laser-induced fluorescence detection. Electrophoresis 2014, 35, 2587-2592.
20. Xiao, J.; Liu, P.; Li, L.H.; Yang, G.W. Fluorescence origin of nanodiamonds. J. Phys. Chem. C 2015, 119, 2239-2248.
21. Du, F.Y.; Li, J.A.; Hua, Y.; Zhang, M.M.; Zhou, Z.; Yuan, J.; Wang, J.; Peng, W.X.; Zhang, L.; Xia, S., et al. Multicolor nitrogen-doped carbon dots for live cell imaging. J. Biomed. Nanotechnol. 2015, 11, 780-788.
22. Pandey, N.; Srivastava, R.K.; Singh, M.K.; Singh, J. Optical properties of carbon nanodots synthesized by laser induced fragmentation of graphite powder suspended in water. Mater. Sci. Semicond. Process 2014, 27, 150-153.
23. Soldano, C. Hybrid metal-based carbon nanotubes: Novel platform for multifunctional applications. Prog. Mater. Sci. 2015, 69, 183-212.
24. Hou, J.; Dong, J.; Zhu, H.; Teng, X.; Ai, S.; Mang, M. A simple and sensitive fluorescent sensor for methyl parathion based on l-tyrosine methyl ester functionalized carbon dots. Biosensors & bioelectronics 2015, 68, 20-26.
25. Chen, X.F.; Zhang, W.X.; Wang, Q.J.; Fan, J.Y. C-8-structured carbon quantum dots: Synthesis, blue and green double luminescence, and origins of surface defects. Carbon 2014, 79, 165-173.
26. Milosavljevic, V.; Nguyen, H.V.; Michalek, P.; Moulick, A.; Kopel, P.; Kizek, R.; Adam, V. Synthesis of carbon quantum dots for DNA labeling and its electrochemical, fluorescent and electrophoretic characterization. Chem. Pap. 2015, 69, 192-201.
27. Krizkova, S.; Adam, V.; Eckschlager, T.; Kizek, R. Using of chicken antibodies for metallothionein detection in human blood serum and cadmium-treated tumour cell lines after dot- and electroblotting. Electrophoresis 2009, 30, 3726-3735.

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