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DNA-Templated Silver Nanorings**

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Nanostructures of noble metals with well-defined shapes and sizes are increasingly attracting the attention of scientists in the fields of catalysis, electronics, photonics, information storage, optoelectronics, biological labeling, etc. The further development and practical applications of nanostructures are expected to increase rapidly because of their interesting optical, electronic, and magnetic properties.[1] In this context, important knowledge of the direct preparation of metallic nanostructures of controlled size and shape has been developed over the past few years, and various morphologies, such as spherical nanoparticles,[2] nanocubes,[3] nanoprisms,[4] nanoplates,[5] or nanobelts,[5] can now be prepared in a controlled way. However, since these techniques are based on the directed growth of particles in the reaction medium, they can only lead to shapes of a simple topology, such as spheroids, ellipsoids, or polyhedrons. In contrast, nanoparticles with a toroidal shape (nanoring) can not be produced by a direct growth technique. Hence, the only way to produce such a morphology is to use a toroidal template of nanometer-scale dimensions. Elaborate and successful methods to prepare silver or gold rings based on the use of a nanoparticle array or a mesoporous membrane as a primary template, were recently described by Xia and co-workers[6] and Yan and Goedel,[7,8] respectively. However, these techniques provide rings with a minimal size of 0.5 μm that can not be directly dispersed in an aqueous medium. On the other hand, due to the specific interaction between DNA and silver ions, DNA is an ideal template to build silver nanostructures. This principle has been used successfully to produce nanoparticle arrays on a DNA scaffold,[9,10] or DNA-templated silver nanowires.[11,12] However, the ability of DNA chains to form toroidal condensates[13] as a result of the DNA-folding transition (DNA condensation[14]) has not been hitherto noticed by materials scientists. The ability of DNA to condense into well-defined toroids provides a unique opportunity to use them as templates to create silver toroidal nanostructures (nanorings) of controlled shape and dimensions. In this communication, we describe a one-pot, three-step, simple preparation of well-defined silver nanorings (100 nm in diameter) dispersed in water, based on the use of dilute solutions of DNA condensates as nanostructured templates.

DNA is a semiflexible, highly charged polyelectrolyte that assumes an elongated-coil conformation in water due to the electrostatic repulsion between the negatively charged monomers. DNA molecules usually fold into tightly packed toroidal condensates with an outer diameter of typically 70–90 nm[13,14] in the presence of hydrophilic neutral polymers,[15] or upon the addition of a small amount of condensing agent, such as cationic polyamines,[13] multivalent metal cations,[16] and cationic surfactants,[17] to a dilute DNA solution. The role of the condensing agents is to induce an attraction between the DNA monomers (chain neutralization or crowding effect), and the toroidal morphology is adopted because of the native rigidity of the DNA double-stranded chain.[18,19]

Figure 1 is a schematic representation of the preparation of DNA-templated silver nanorings. First, unfolded DNA molecules (T4 DNA, 166 000 base pairs, contour length 57 μm) were condensed by the addition of tetravalent cation spermine (SPM^{4+}, *N*,*N*′-bis(3-aminopropyl)-1,4-diaminobutane) to obtain uniform toroids (made of a single DNA chain) with an average inner and outer diameter of about 30 and 80 nm, respectively (Figs. 1A,B). A transmission electron microscopy (TEM) image of a typical T4 DNA toroid is shown in Figure 2A. The DNA toroids obtained by this method were

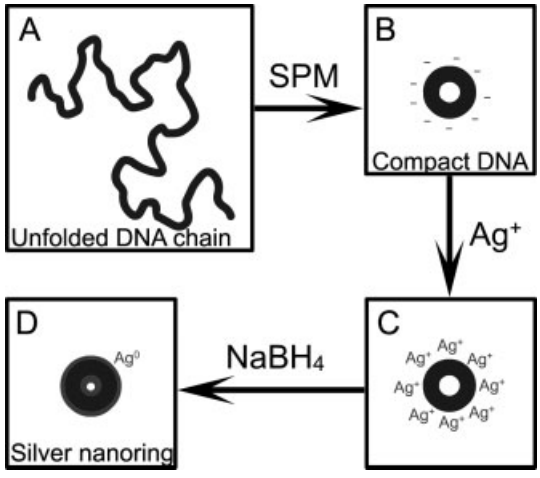

Figure 1. Schematic representation of the silver-nanoring synthesis. A) Unfolded DNA chain. B) DNA toroidal condensate formed after the addition of a condensing agent (in this study, spermine, noted SPM^{4+}, a tetravalent polycation). C) After the addition of $AgNO_3$, silver ions bound to the surface of the DNA toroid. D) DNA-templated silver nanorings were formed after Ag^+ was reduced by $NaBH_4$.

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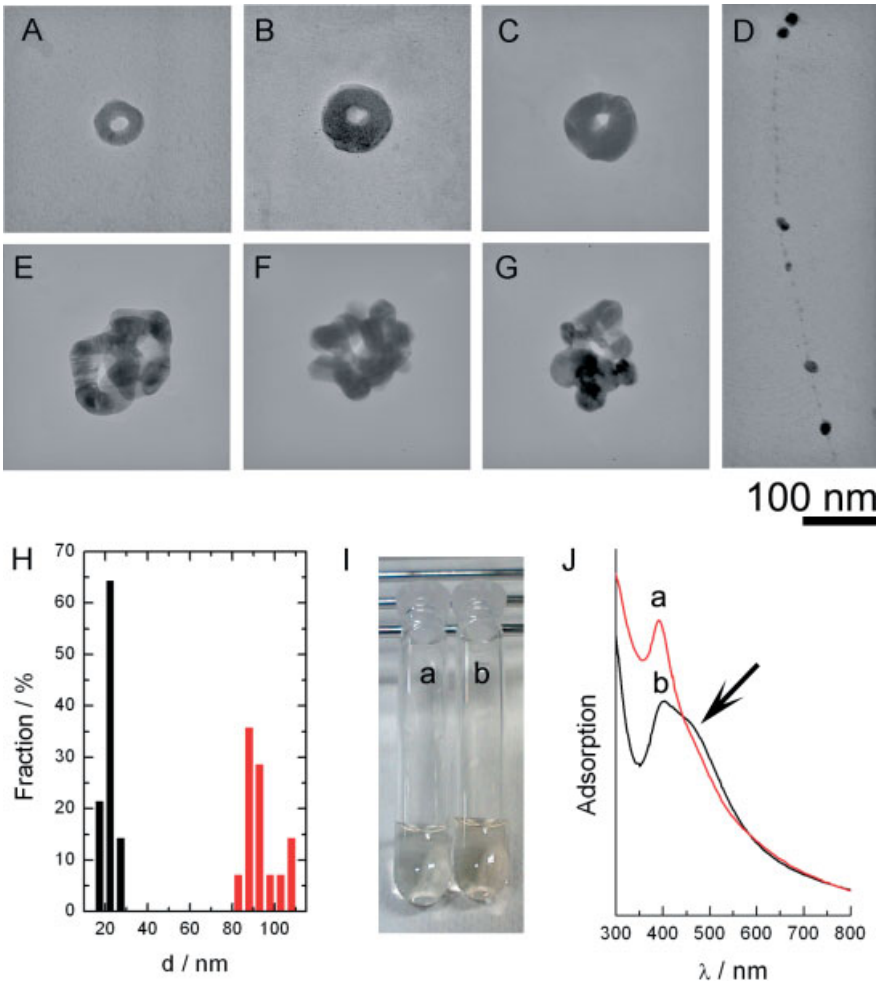

Figure 2. TEM images of various T4 DNA/silver nanostructures. A) Single-chain DNA toroidal condensate obtained by compaction of T4 DNA (1×10^{-6} M nucleotides) with spermine (20×10^{-6} M) in pure water. B,C) DNA-templated silver nanorings obtained after the successive addition of $AgNO_3$ (100×10^{-6} M) and $NaBH_4$ (100×10^{-6} M) to the DNA/spermine solution (image A). D) Fragment of unfolded DNA chain complexed with silver nanoparticles observed under the conditions used in (B,C) except with a spermine concentration of 100×10^{-6} M. E) Typical multiring nanostructure obtained under the conditions used in (B,C) at high DNA concentration (10×10^{-6} M). F,G) Irregular DNA-templated nanostructures observed under the conditions used in (B,C) but using 100×10^{-6} M of $AgNO_3$ and 20×10^{-6} M of $NaBH_4$. H) Statistical distributions of inner (black bars) and outer (red bars) diameters (d) of silver nanorings obtained at optimal conditions ([T4 DNA] = 1×10^{-6} M, [SPM^{4+}] = 20×10^{-6} M, [$AgNO_3$] = 100×10^{-6} M, [$NaBH_4$] = 100×10^{-6} M). Parameters of 100 nanorings were analyzed to build the distribution. I) Control solution obtained by mixing spermine (20×10^{-6} M), $AgNO_3$ (100×10^{-6} M), and $NaBH_4$ (100×10^{-6} M) in pure water (a), and a solution of silver nanorings obtained by compaction of T4 DNA (1×10^{-6} M nucleotides) with spermine (20×10^{-6} M) in pure water and successive addition of $AgNO_3$ (100×10^{-6} M) and $NaBH_4$ (100×10^{-6} M) (b). J) UV-Vis spectra of the control solution without DNA (solution a, red curve) and the silver-nanorings solution (solution b, black curve) prepared as described in (I). The arrow indicates the characteristic long-wavelength (λ) absorption shoulder (470 nm) inherent to the silver-ring morphology.

used as templates for the preparation of silver nanorings (Fig. 1B,D). Silver nitrate solution was added to the solution containing the DNA toroids (Figs. 1B,C), and the Ag^+ ions were then quickly reduced by adding the common reducing agent sodium borohydride ($NaBH_4$) (Figs. 1C,D).

It is known that an unfolded DNA chain acts as a source of nucleation centers for Ag^0-nanostructure formation due to binding of Ag^+ with DNA.[9–11,20] Once the DNA chain has been folded, the toroidal condensate that results is usually described as having a neutral, very dense, crystal-like core, in which Ag^+ ions most probably cannot penetrate. However, the surface of the DNA toroid has a residual negative charge[21] and DNA nucleotides that are accessible to Ag^+ ions. Therefore, we assume that Ag^+ ions bind to the toroid surface, which acts as a source of nucleation centers, as depicted in Figure 1C. After reduction of Ag^+ to Ag^0 by $NaBH_4$ (Figs. 1C,D), the DNA toroids are covered by a thin silver shell to finally yield silver nanorings (Fig. 1D) dispersed in water. Typical TEM images of silver nanorings templated by the T4 DNA toroids are shown in Figures 2B,C.[22]

As a general rule, the synthesis of uniform nanostructures requires well-determined conditions (concentration of reagents,

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order and time of mixing, etc.) In our four-component system, precisely adjusted conditions are also important to achieve a satisfactory shape of the silver nanorings. These conditions can be summarized as follows. First, the concentration of DNA should not be higher than 1×10^{-6} M unless the aim is to synthesize multiring nanostructures, because DNA toroids tend to aggregate at the stage of compaction at higher DNA concentrations. Figure 2E shows such a multiring structure containing two toroids. Second, a deficit or excess of condensing agent (here, spermine) should be avoided because DNA condensates exist only over a precise range of condensing-agent concentrations.[23,24] Under our experimental conditions, the spermine concentration [SPM^{4+}] should be between 5 and 50×10^{-6} M. For [SPM^{4+}] $<5\times10^{-6}$ M, the DNA is not completely neutralized (< 90 %) and all the DNA chains are in the elongated coil state.[25,26] For [SPM^{4+}] $>50\times10^{-6}$ M, the DNA chains that had been condensed at a low spermine concentration unfold into an elongated coil state due to the so-called re-entrant transition.[23,24] Under these conditions (deficit or excess of spermine), the addition of silver nitrate and further reduction by $NaBH_4$ leads to the formation of silver nanoparticles on the unfolded DNA chain, as shown in Figure 2D. Third, the DNA/$AgNO_3$/$NaBH_4$ molar ratio primarily controls the shape and the characteristic size of the nanorings. In our experiments, a 100-fold molar excess of $AgNO_3$ over DNA nucleotides (100×10^{-6} M), and from equivalent amounts to a fivefold excess of $NaBH_4$ over $AgNO_3$ were the optimal conditions to insure a satisfactory toroidal shape of the silver nanorings. For higher $AgNO_3$ concentrations, the resultant nanorings are very thick or even contain no hole. On the other hand, if the $NaBH_4/AgNO_3$ ratio is significantly below unity, the growth of individual silver nanoparticles on the DNA toroid is induced, rather than homogeneous growth of the silver shell. This leads to the formation of irregular rings (e.g., a flower-like shape, Figs. 2F,G). When the experimental conditions are optimized as described above ([T4 DNA] 1×10^{-6} M, [SPM^{4+}] 20×10^{-6} M, [$AgNO_3$] and [$NaBH_4$] both 100×10^{-6} M), the silver nanorings have a well-defined toroidal shape (Figs. 2B,C), with an average outer diameter of 93 ± 7 nm and an inner diameter of 22 ± 4 nm, and narrow distributions of inner and outer diameters, as shown in Figure 2H. The difference between the average parameters of the initial DNA toroids and the final nanorings suggests the formation of a silver shell with a thickness of about 5 nm.

The optical properties of the silver nanorings in solution differ from those of the corresponding solutions of silver nanostructures prepared without DNA toroidal seeds (Figs. 2I,J). The UV-vis absorption spectra of the nanorings are characterized by a broad absorption with a maximum at 400–500 nm. Two closely located overlapping bands can be distinguished and are assigned to the typical absorption for silver nanoparticles (400 nm) and an absorption that results from the toroidal shape of the silver rings (about 470 nm) (Fig. 2J). The second band, which is specific to the silver rings, was not observed in the absence of DNA toroidal seeds. Similarly shaped spectra were reported for nanorods made of noble metals with very low aspect ratios.[27]

The formation of the silver nanoring shell can be used to encapsulate DNA and protect it from changes in the environment (pH, chemical composition, biochemical environment, presence of enzymes, etc.) or to block the DNA activity (chemical or biological). The ability to protect DNA from a chemical modification of the environment was confirmed by fluorescence microscopy (FM) observations of single-chain DNA (1×10^{-6} M) labeled by the fluorescent dye YOYO-1 ((1,1′-(4,4,7,7-tetramethyl-4,7-diazaundecamethylene)-bis-4-[3-methyl-2,3-dihydro-(benzo-1,3-oxazole)-2-methylidene]-quinolinium tetraiodide)) (0.2×10^{-6} M). In the absence of condensing agent, all the DNA chains were unfolded and were observed as elongated coils with large intrachain fluctuations and a very slow diffusive motion (Fig. 3A). When spermine was added (20×10^{-6} M), FM reveals that all the DNA chains folded abruptly into very fast diffusing globules that

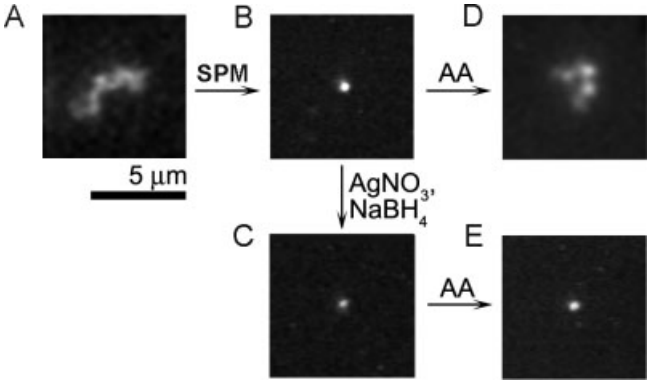

Figure 3. FM images of single molecules of T4 DNA (1×10^{-6} M) labeled with the fluorescent dye YOYO-1 (0.2×10^{-6} M) in various aqueous environments. A) Elongated coil conformation in pure water. B) Compact DNA (globule) in the presence of 20×10^{-6} M of spermine. C) Compact DNA covered by silver (silver nanoring) observed after addition of $AgNO_3$ (100×10^{-6} M) and $NaBH_4$ (100×10^{-6} M) to the solution used for (B). D) After addition of AA (100×10^{-6} M) into the solution used for (B), all DNA molecules unfolded into a coil state. E) After addition of AA (100×10^{-6} M) into the solution used for (C), all DNA molecules were still in a compact state.

correspond to the DNA toroidal condensates (Fig. 3B). After further addition of $AgNO_3$ and reduction by $NaBH_4$, DNA condensates (i.e., DNA silver nanorings) could still be observed, most probably due to absorption of the fluorescent dye (YOYO) on the silver nanorings (Fig. 3C).

It is important to note that the silver nanorings diffuse freely in the solution, and almost as fast as the original DNA toroidal condensates, which indicates that i) they have an average size of 100 nm and ii) they are well dispersed in the water solution. To model the chemical modification of the DNA environment, we added acetyl anhydride (AA, 1×10^{-3} M) to the DNA medium before and after the silver treatment. The addition of AA induces a partial acetylation of free spermine in the solution and, thereby, displaces the equilibrium towards the unfolded form of DNA. When AA was added before silver treat-

ment, FM observations show that all the DNA condensates unfolded into the initial elongated coil state (Fig. 3D). However, after silver modification, the addition of AA had essentially no effect on the behavior of the DNA condensates, which still diffuse rapidly and freely in the solution (Fig. 3E). These observations demonstrate that the encapsulation of DNA provides protection from chemical modifications of the environment. Release of the DNA from the silver shell can be achieved using a previously described methodology.[28]

The potential scope of nanostructures that can be obtained by the DNA-condensate-templated method is rather broad. If DNA molecules are longer than 400 base pairs,[29] they can be condensed by a majority of multications to adopt a toroidal conformation, which can then be used as a template for silver (or other suitable noble metal) nanorings. Essentially, the diameter of the nanorings can be varied from 30 to ~200 nm.[30,31] Furthermore, other self-assembled long DNA chain morphologies, such as rods, rackets, and toroids-on-a string structures,[32,33] as well as programmed DNA constructions,[34] can potentially serve as seeds to obtain a large variety of corresponding metal nanostructures.

In conclusion, the unique ability of DNA molecules to be organized into toroidal condensates of a well-defined shape and size upon their interaction with multications enables the creation of monodisperse silver rings of nanometer size. Adaptation of the concept described in this communication together with already known principles of the synthesis of nanostructures (directed growth, specific adsorption, lithography) is awaited to gain new, yet hitherto impossible to prepare, sophisticated nanostructures of noble metals. This method might also be used to protect DNA from chemical or biological modifications to the environment or to block DNA activity by encapsulating DNA condensates into silver nanorings.

Experimental

Materials: Bacteriophage T4 DNA (166 000 base pairs, Nippon Gene Co., Ltd., Japan); $AgNO_3$ (99.9999 % purity, Aldrich, Japan), spermine (Naclai Tesque, Japan), fluorescent dye YOYO-1 (Molecular Probes, USA), and sodium borohydride (Naclai Tesque, Kyoto, Japan) were used as received.

Methods: TEM observations were performed using a JEM-1200EX microscope (JEOL, Tokyo, Japan) at an acceleration voltage of 100 kV. We used carbon-coated grids with a 300 mesh size at room temperature. Each grid was placed for 3 min on top of a 15 μL droplet of DNA solution on a Parafilm sheet, and the solution was blotted with filter paper before microscopic observation. The sample was stained by placing the grid on a 15 μL droplet of uranyl acetate (1 % in water) for 15 s before observation.

FM observations were performed using an Axiovert 135 TV (Carl Zeiss, Germany) microscope equipped with a 100× oil-immersion lens. Fluorescent images were recorded using an EB-CCD camera and an Argus 10 image processor (Hamamatsu Photonics, Hamamatsu, Japan).

UV-Vis spectra were recorded on a Jasco U-550 UV/VIS spectrophotometer in 1.0 cm × 0.2 cm × 0.5 cm quartz cells.

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[1] *Nanoparticles: From Theory to Applications* (Ed.: G. Schmid), Wiley-VCH, Weinheim, Germany **2004**.
[2] D. L. Van Hyning, C. F. Zukoski, *Langmuir* **1998**, *14*, 7034.
[3] Y. Sun, Y. Xia, *Science* **2002**, *298*, 2176.
[4] C. Xue, Z. Li, C. A. Mirkin, *Small* **2005**, *1*, 513.
[5] B. Wiley, Y. Sun, B. Mayers, Y. Xia, *Chem. Eur. J.* **2005**, *11*, 454.
[6] J. M. McLellan, M. Geissler, Y. Xia, *J. Am. Chem. Soc.* **2004**, *126*, 10 830.
[7] F. Yan, W. A. Goedel, *Nano Lett.* **2004**, *4*, 1193.
[8] F. Yan, W. A. Goedel, *Angew. Chem. Int. Ed.* **2005**, *44*, 2084.
[9] H. Nakao, H. Shiigi, Y. Yamamoto, S. Tokonami, T. Nagaoka, S. Sugiyama, T. Ohtani, *Nano Lett.* **2003**, *3*, 1391.
[10] G. Wei, H. Zhou, Z. Liu, Y. Song, L. Wang, L. Sun, Z. Li, *J. Phys. Chem. B* **2005**, *109*, 8738.
[11] E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, *Nature* **1998**, *391*, 775.
[12] A. Ongaro, F. Griffin, L. Nagle, D. Iacopino, R. Eritja, D. Fitzmaurice, *Adv. Mater.* **2004**, *16*, 1799.
[13] L. C. Gosule, J. A. Schellman, *Nature* **1976**, *259*, 333.
[14] V. A. Bloomfield, *Curr. Opin. Struct. Biol.* **1996**, *6*, 334.
[15] U. K. Laemmli, *Proc. Natl. Acad. Sci. USA* **1975**, *72*, 4288.
[16] J. Widom, R. L. Baldwin, *Biopolymers* **1983**, *2*, 1595.
[17] S. M. Mel'nikov, V. G. Sergeyev, K. Yoshikawa, *J. Am. Chem. Soc.* **1995**, *117*, 2401.
[18] A. Y. Grosberg, A. V. Zhestkov, *J. Biomol. Struct. Dyn.* **1986**, *3*, 859.
[19] J. Ubbink, T. Odijk, *Europhys. Lett.* **1996**, *33*, 353.
[20] *Nucleic Acid–Metal Ion Interactions* (Ed.: T. G. Spiro), Wiley Interscience, New York **1980**.
[21] Y. Yamasaki, Y. Teramoto, K. Yoshikawa, *Biophys. J.* **2001**, *80*, 2823.
[22] The use of highly diluted DNA solutions, which is critical for monomolecular DNA compaction into toroids and high colloidal stability of the final silver nanorings in solution, results in a weak adsorption of the silver nanorings on TEM carbon-coated grids. Therefore, the adsorbed silver nanorings are distributed on the TEM grid with an average separation distance of several micrometers.
[23] J. Pelta, F. Livolant, J.-L. Sikorav, *J. Biol. Chem.* **1996**, *271*, 5656.
[24] Y. Murayama, Y. Sakamaki, M. Sano, *Phys. Rev. Lett.* **2003**, *90*, 018 102.
[25] R. W. Wilson, V. A. Bloomfield, *Biochemistry* **1979**, *18*, 2192.
[26] D. Baigl, K. Yoshikawa, *Biophys. J.* **2005**, *88*, 3486.
[27] N. R. Jana, L. Gearheart, C. J. Murphy, *Adv. Mater.* **2001**, *13*, 1389.
[28] C. A. Mirkin, S.-J. Park, R. Jin, *WO Patent Application 02/46483 A2*, **2002**.
[29] J. Widom, R. L. Baldwin, *J. Mol. Biol.* **1980**, *144*, 431.
[30] C. C. Conwell, I. D. Vilfan, N. V. Hud, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9296.
[31] Y. Yoshikawa, K. Yoshikawa, T. Kanbe, *Langmuir* **1999**, *15*, 4085.
[32] H. Noguchi, S. Saito, S. Kidoaki, K. Yoshikawa, *Chem. Phys. Lett.* **1996**, *261*, 527.
[33] A. A. Zinchenko, V. G. Sergeyev, S. Murata, K. Yoshikawa, *J. Am. Chem. Soc.* **2003**, *125*, 4414.
[34] N. C. Seeman, P. S. Lukeman, *Rep. Prog. Phys.* **2005**, *68*, 237.

