Programmed Nanopores

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Directed Assembly of Sub-Nanometer Thin Organic Materials with Programmed-Size Nanopores**

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Nanothin materials with selective, molecular-size pores[1] are critical for technological breakthroughs in DNA sequencing,[2] the fabrication of microreactors,[3] molecular electronics,[4] and advanced drug-delivery devices.[1,5] Recent studies of pores in self-assembled lipid bilayers reinforced the potential of stable nanoporous organic materials.[6] Although there has been remarkable progress in the fabrication of nanopores with diameters greater than 2 nm,[1,4b] precise pore-size control of those with diameters of 0.5–2 nm remains a challenge. Herein, we show an efficient method for creating nanothin materials with uniform imprinted nanopores. We synthesized polymer materials with sub-nanometer thickness and programmed size pores (0.8 and 1.3 nm) in high yield from inexpensive components.

Using temporary self-assembled scaffolds, we directed the assembly of building blocks into the desired shape. In this approach, the scaffold can be recycled and used again to fabricate a new batch of nanomaterials. The directed assembly method potentially allows for great flexibility in shaping nanomaterials by varying scaffolds and/or building blocks.

Amphiphilic molecules, such as phospholipids, spontaneously form self-assembled structures in water whose shape and size can be well-controlled.[7] Hydrophobic monomers can be dissolved and polymerized within the bilayer interior.[8] Our work introduces a method for the creation and size control of nanopores. Hydrophobic monomers, crosslinkers, and pore-forming templates are loaded into the hydrophobic interior of self-assembled bilayers (Figure 1 A). The geometry of the bilayers permits lateral propagation of polymerization but prohibits material growth orthogonal to the plane of the bilayers, thus ensuring uniform thickness. Polymerization yields a crosslinked polymer film with embedded pore-forming templates (Figure 1 B), which can be removed together with the scaffold (Figure 1 C). In this work, we used a liposomal bilayer to demonstrate successful pore-size control. Many other amphiphilic bilayers, such as supported bilayers, black lipid membranes in microapertures, and surface-mounted hybrid bilayers have been reported,[9] and we anticipate that the technique for pore size control will be readily adaptable to create a variety of nanothin membranes with programmed permeability.

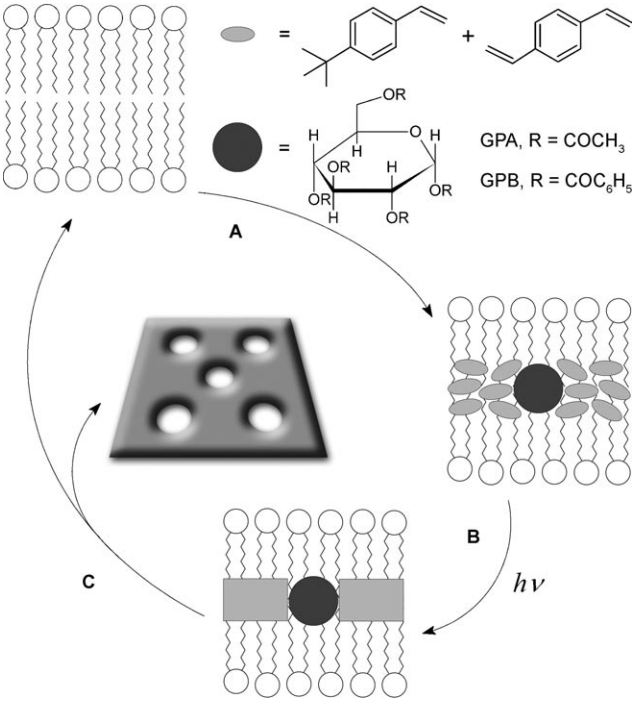

Figure 1. Directed assembly of nanothin polymer films with uniform nanopores: A) The self-assembled phospholipid bilayer is loaded with hydrophobic monomers (*tert*-butylstyrene and divinylbenzene) and pore-forming templates (glucose pentaacetate, GPA or glucose pentabenzoate, GPB); B) Polymerization produces a nanothin film with embedded template molecules in the bilayer interior; C) Removal of phospholipids with the help of a detergent or solvent exchange yields nanothin film with uniformly sized pores. Pore-forming templates are removed either by solvent extraction or chemical degradation.

We selected glucose pentaacetate (GPA, approximate size 0.8 nm), and glucose pentabenzoate (GPB, approximate size 1.3 nm), as pore-forming templates (Figure 2). These molecules are lipophilic, fairly symmetric, and commercially available. They are also chemically degradable, which is useful for the removal of tightly held molecules from the crosslinked polymer film. Upon hydrolysis, they form small hydrophilic molecules of glucose and either acetic or benzoic acid that can easily diffuse from the pore into the aqueous solution.

We loaded a 1:1 mixture of 4-*tert*-butylstyrene and *p*-divinylbenzene in the bilayer interior and determined the molar ratio of monomers to lipids to be approximately 0.9, by

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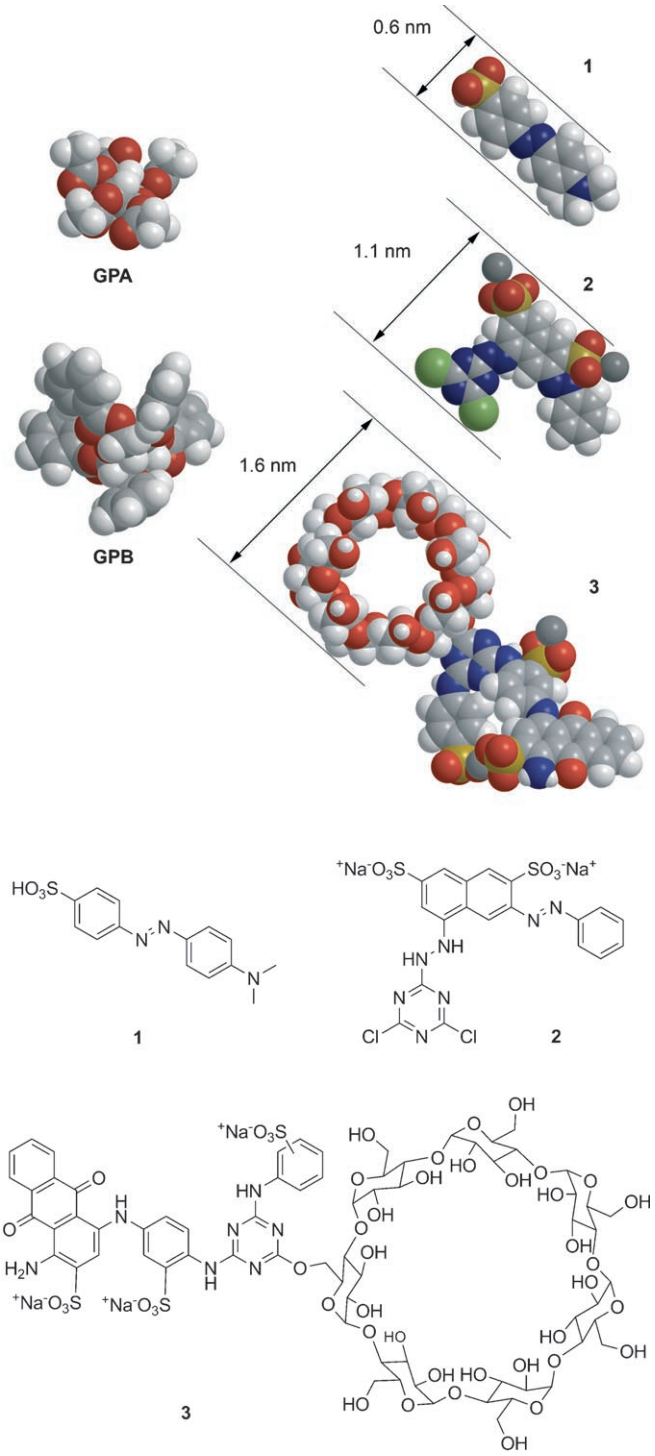

Figure 2. Pore-forming templates: GPA, glucose pentaacetate, and GPB, glucose pentabenzoate. Size probes: **1**, methyl orange (0.6 nm); **2**, Procion Red (1.1 nm); **3**, Reactive Blue 2/β-cyclodextrin conjugate (1.6 nm). The smallest cross-section corresponds to the smallest pore the probe can cross in its most tightly packed conformation.

extraction with hexane followed by gas chromatography (see Supporting Information). This ratio correlates well with previously reported maximum molar ratios of approximately 0.9 for alkanes in multilamellar liposomes,[10] 0.9 for benzene[11] in multilamellar liposomes, and 0.85 for styrene in surfactant admicelles.[12] The UV-initiated polymerization was complete, as evidenced by the absence of unreacted monomers determined by gas chromatography. Formation of polymer nanocapsules was unambiguously demonstrated by transmission electron microscopy (TEM) (see Supporting Information).

TEM images show no size difference among samples of liposomes and nanocapsules with or without pore-forming templates. In our capsules, about 1.8 monomer and cross-linker molecules were sandwiched between each pair of lipids in the bilayer, which translates into an average thickness of 0.7 nm for the polymerized material.

We designed a size-probe retention assay to evaluate the permeability of newly prepared nanoporous materials. A capsule is formed with a mixture of entrapped size probes, after which the lipid scaffold and pore-forming templates are removed, releasing any probes smaller than the pore size. The capsules are separated from the released probes and the quantities of retained probes are determined by UV-vis spectroscopy. For simplicity of visual observation, we selected three probes of primary colors: yellow (methyl orange, **1**, MW 328 $g\,mol^{-1}$, approximate smallest dimension 0.6 nm), red (Procion Red, **2**, MW 615 $g\,mol^{-1}$, approximate smallest dimension 1.1 nm), and blue (Reactive Blue 2 conjugated to β-cyclodextrin, **3**, MW 1938 $g\,mol^{-1}$, approximate smallest dimension 1.6 nm).[13]

To prepare nanocapsules with 0.8 nm pores, we codissolved GPA with monomers in the bilayer interior (Figure 1). Similarly, we used GPB to form 1.3 nm pores. The control sample had no pore-forming templates in the bilayer. After polymerization and detergent-assisted lipid removal, hydrolysis of templates with sodium hydroxide opened the pores. Capsules were separated from released probes by size-exclusion chromatography (Figure 3). The mobile phase contained a detergent to prevent liposomes from reassembling and to solubilize hydrophobic polymer capsules. The nanocapsule fraction was collected immediately following the column void volume. The control sample was colored brown (a ternary color produced by mixing yellow, red, and blue) indicating the retention of all three size probes (Figure 3). The capsules prepared with GPA as the pore-forming template released 0.6 nm yellow probes and retained 1.1 nm red and 1.6 nm blue probes (pore-size range 0.6–1.1 nm), as evidenced by its purple color (Figure 3). The sample with GPB as the pore-forming template released 0.6 nm yellow and 1.1 nm red probes and retained 1.6 nm blue probes (pore size range 1.1–1.6 nm), resulting in blue colored capsules (Figure 3). These data confirm successful creation of programmed size pores with narrow size distribution.

Retention of each probe was determined quantitatively by UV/Vis spectroscopy. In each experiment, an aliquot of liposome solution was taken as a reference immediately before the polymerization. The unentrapped probe was removed by size-exclusion chromatography and the amount of encapsulated probe was determined from its absorbance. Light scattering was subtracted in all measurements.

When using GPA, we detected complete release of 0.6 nm probes and substantial (> 80 %) retention of 1.1 and 1.6 nm probes (Table 1). Using GPB led to complete release of both

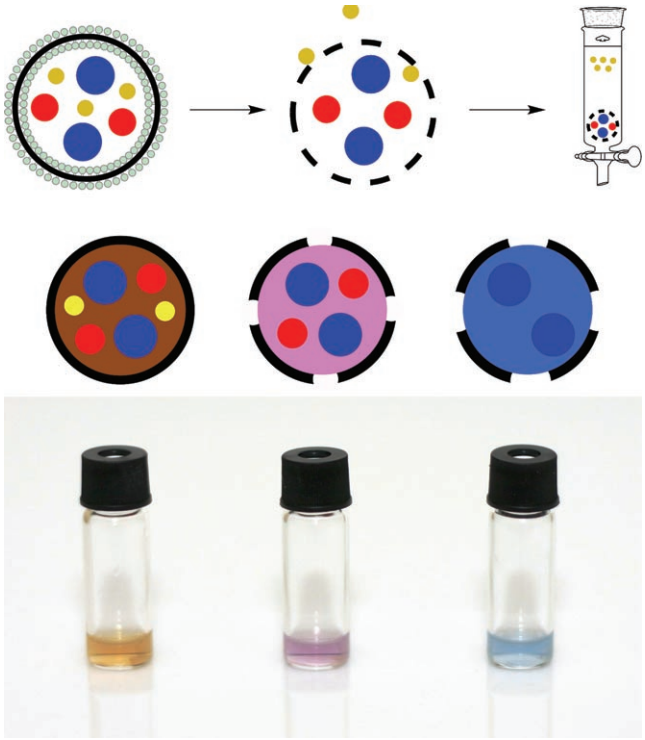

Figure 3. Selective permeability demonstrated by the size probe retention assay. Liposomes were loaded with the mixture of colored size probes: 0.6 nm yellow (**1**), 1.1 nm red (**2**), and 1.6 nm blue (**3**). Monomers and crosslinkers with or without pore-forming templates were loaded into the liposomal membrane and polymerization was initiated, after which the phospholipids and pore-forming templates were removed, and capsules were separated from released size probes on a size-exclusion column. The nanocapsules fraction is shown. In the absence of pore-forming templates, no probes escaped, and the capsules remained brown (left sample). When GPA was used as pore forming template, 0.6 nm yellow probe was released, and the capsules were colored purple (middle sample). With GPB as a pore forming template, 0.6 nm yellow and 1.1 nm red probes were released, and capsules were colored blue (right sample).

Table 1: Retention (%) of size probes in nanocapsules fabricated with different pore-forming templates.

Probe	No template	GPA[a]	GPB[a]
1 (0.6 nm)	84 ± 3	ND[b]	ND[b]
2 (1.1 nm)	85 ± 6	86 ± 7	ND[b]
		84 ± 8[c]	
		83 ± 9[d]	
		81 ± 11[e]	
3 (1.6 nm)	87 ± 6	84 ± 5	86 ± 6

[a] 100 template molecules per capsule were used except where indicated; [b] not detectable; [c] 5 GPA molecules per capsule were used; [d] after second separation, performed 1 h after the first one; [e] after second separation, performed 24 h after the first one.

0.6 and 1.1 nm probes and substantial retention of 1.6 nm probes. Capsules produced with no templates exhibited substantial retention of all three probes. These results indicate a high (> 80 %) yield of nanocapsules with well defined pores and no pinholes or intrinsic pores exceeding the size of the templated pores.

The colored size probe retention assay was corroborated by studying the release of self-quenching fluorescence dyes: calcein, similar in size to probe **2** and fluorescein isothiacyanate-dextran (FITC-D, average MW 4000), slightly larger than probe **3**. Calcein was released through GPB-templated pores but not through GPA-templated pores. No release of FITC-D through GPA- or GPB-templated pores was detected (see Supporting Information).

We varied the number of pore-forming templates between 5 and 100 per capsule and detected no difference in the retention (Table 1). Considering that with an average of 5 templates per capsule random distribution should give a significant number of capsules containing one or two templates, we find it likely that a substantial number of templates, if not each one, results in a well-defined pore. We further conclude that using 100 templates per capsule did not lead to template aggregation or phase separation from monomers; otherwise no retention of probes would be possible.

In several experiments, we eluted porous nanocapsules containing colored probes through a size exclusion column, 1 and 24 h after the first separation. No further release of entrapped size probes was detected (Table 1), suggesting that efflux from porous capsules was fast in the chromatography time scale (5-15 min.) and that pores did not noticeably expand with time, even through temporary fluctuations.

We anticipate that a wide range of pore sizes can be fabricated by using the same directed-assembly strategy. Templates can be designed to explore alternative pore opening mechanisms (enzymatic, photochemical, etc.). Pore formation described here has similarities to molecular imprinting that has been successfully used for separation, sensors, and catalysis.[14] We envision that pores capable of molecular recognition can be synthesized by imprinting an analyte surrounded by noncovalently bound molecules with polymerizable moieties.[13] It is conceivable to achieve uniform orientation of templates and, correspondingly, uniform recognition sites, as a result of a high degree of organization in the bilayers. Alternatively, a polymerizable and degradable template can be designed to create a functionalized pore suitable for further modifications (for example, creating stimuli-responsive porous materials).

In summary, we have demonstrated an efficient method for the directed assembly of sub-nanometer thin organic materials with programmed-size nanopores, using controlled polymerization in the organized interior of temporary self-assembled scaffolds. These materials open exciting opportunities for technological advances in diverse disciplines.

Experimental Section

Reactive Blue 2/β-Cyclodextrin conjugate: A freshly prepared solution of β-cyclodextrin (500 mg, 0.4 mmol) in water (40 mL) was mixed with a freshly prepared solution of Reactive Blue (550 mg, 0.4 mmol, 60 % of dye in reagent) in water (20 mL). After stirring the mixture for 5 min at ambient temperature, NaCl (2 g) was added. 30 min later, Na_2CO_3 (100 mg) was added, and the reaction mixture was stirred overnight. The mixture was purified using size exclusion chromatography (Sephadex G-25, deionised water). ^{1}H NMR (D_2O): δ = 8.51–8.24 (m, 4H), 8.11–7.62 (m, 5H), 7.51–7.06 (m,

3H), 5.05 (d, $J = 3.4$ Hz, 7H), 3.96–3.52 ppm (m, 42H). Electrospray MS: $[M + 4H^+ - Na^+]^{3+}$ 639.55 (calcd 639.46).

Porous nanocapsules: Unilamellar liposomes were formed by extrusion of multilamellar vesicles prepared from 20 mg of DLPC using a standard literature protocol. GPA or GPB were mixed with lipids prior to hydration with an aqueous solution (1 mL) of size probes (**1**, 100 mM; **2**, 10 mM; **3**, 3.7 mM). We used tris(hydroxymethyl)aminomethane (TRIS) buffer (50 mM, pH 7.4 at 25 °C), phosphate buffer (100 mM, pH 7.6 at 25 °C) and water for vesicle preparation with identical results. 4-*tert*-butylstyrene (6 μL) and *para*-divinylbenzene (5 μL) were added to 1 mL of liposome solution, and the solution was stirred for 24 h at 4 °C. Liposomes containing monomers in the lipid bilayer were irradiated by UV light in a home-built apparatus (two 4 W UV lamps, $\lambda = 254$ nm) for four hours at ambient temperature. Triton X-100 (1 mL, 2% in water) and NaOH (0.5 mL, 0.1M) were added to the solution to remove the outer shell of lipids and hydrolyze the pore-forming template. The mixture was separated on a size-exclusion chromatography column (Sepharose 4B) to remove released size probes. The nanocapsules fraction was collected and analyzed with UV/Vis spectroscopy (Agilent 8453 UV/Vis spectrophotometer) to quantify the amounts of entrapped colored size probes (see Supporting Information for full experimental details).

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[1] *Nanoporous Materials: Science and Engineering* (Eds.: G. Q. Lu, X. S. Zhao), Imperial College Press, London, **2004**.

[2] a) H. Bayley, C. R. Martin, *Chem. Rev.* **2000**, *100*, 2575–2594; b) J. J. Kasianowicz, E. Brandin, D. Branton, D. W. Deamer, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 13770–13773; c) P. Chen, J. Gu, E. Brandin, Y. R. Kim, Q. Wang, D. Branton, *Nano Lett.* **2004**, *4*, 2293–2298.

[3] a) D. M. Vriezema, M. C. Aragones, J. A. A. W. Elemans, J. J. L. M. Cornelissen, A. E. Rowan, R. J. M. Nolte, *Chem. Rev.* **2005**, *105*, 1445–1489; b) C. Nardin, S. Thoeni, J. Widmer, M. Winterhalter, W. Meier, *Chem. Commun.* **2000**, 1433–1434.

[4] a) Z. Lin, D. H. Kim, X. Wu, L. Boosahda, D. Stone, L. LaRose, T. P. Russell, *Adv. Mater.* **2002**, *14*, 1373–1376; b) U. Jeong, D. Y. Ryu, J. K. Kim, D. H. Kim, X. Wu, T. P. Russell, *Macromolecules* **2003**, *36*, 10126–10129.

[5] a) Y. Wei, K.-Y. Qiu, *Nanoporous Mater.* **2004**, 873–892 (Eds.: G. Q. Lu, X. S. Zhao); b) T. A. Desai, D. J. Hansford, L. Kulinsky, A. H. Nashat, G. Rasi, J. Tu, Y. Wang, M. Zhang, M. Ferrari, *Biomed. Microdevices* **1999**, *2*, 11–40.

[6] a) V. Gorteau, G. Bollot, J. Mareda, D. Pasini, D.-H. Tran, A. Lazar, A. W. Coleman, N. Sakai, S. Matile, *Bioorg. Med. Chem.* **2005**, *13*, 5171–5180; b) D. T. Bong, T. D. Clark, J. R. Granja, M. R. Ghadiri, *Angew. Chem.* **2001**, *113*, 1016–1041; *Angew. Chem. Int. Ed.* **2001**, *40*, 988–1011; c) R. J. Brea, L. Castedo, J. R. Granja, *Chem. Commun.* **2007**, 3267–3269.

[7] a) J. C. Stendahl, M. S. Rao, M. O. Guler, S. I. Stupp, *Adv. Funct. Mater.* **2006**, *16*, 499–508; b) M. J. Boerakker, N. E. Botterhuis, P. H. H. Bomans, P. M. Frederik, E. M. Meijer, R. J. M. Nolte, N. A. J. M. Sonunerdijk, *Chem. Eur. J.* **2006**, *12*, 6071–6080; c) *Liposomes: a Practical Approach* (Eds.: V. Torchilin, V. Weissig), Oxford University Press, Oxford, **2003**.

[8] a) N. Poulain, E. Nakache, A. Pina, G. J. Levesque, *J. Polym. Sci.* **1996**, *34*, 729–737; b) J. Hotz, W. Meier, *Langmuir* **1998**, *14*, 1031–1036; c) C. Nardin, T. Hirt, J. Leukel, W. Meier, *Langmuir* **2000**, *16*, 1035–1041; d) J. Kurja, R. J. M. Noelte, I. A. Maxwell, A. I. German, *Polymer* **1993**, *34*, 2045–2049; e) C. A. McKelvey, E. W. Kaler, J. A. Zasadzinski, B. Coldren, H. T. Jung, *Langmuir* **2000**, *16*, 8285–8290; f) M. Jung, D. H. W. Huber, P. H. H. Bomans, P. M. Frederic, J. Meuldijk, A. M. van Herk, H. Fischer, A. I. German, *Langmuir* **1997**, *13*, 6877–6880; g) J. F. P. d. S. Gomes, A. F.-P. Sonnen, A. Kronenberger, J. Fritz, M. A. N. Coelho, D. Fournier, C. Fournier-Noel, M. Mauzac, M. Winterhalter, *Lamgmuir* **2006**, *22*, 7755–7759.

[9] a) E. T. Castellana, P. S. Cremer, *Surf. Sci. Rep.* **2006**, *61*, 429–444; b) D. Berti, *Curr. Opin. Colloid Interface Sci.* **2006**, *11*, 74–78; c) E. Sackmann, *Science* **1996**, *271*, 43–48.

[10] T. J. McIntosh, S. A. Simon, R. C. MacDonald, *Biochim. Biophys. Acta Biomembr.* **1980**, *597*, 445–463.

[11] R. V. McDaniel, S. A. Simon, T. J. McIntosh, V. Borovyagin, *Biochemistry* **1982**, *21*, 4116–4126.

[12] B. Kitiyanan, J. H. O'Haver, J. H. Harwell, S. Osuwan, *Langmuir* **1996**, *12*, 2162–2168.

[13] β-cyclodextrin-Reactive Blue conjugate was prepared in a similar fashion to the previously described dextran staining method: W. F. Dudman, C. T. Bishop, *Can. J. Chem.* **1968**, *46*, 3079–3084.

[14] a) W. Li, S. Li, *Adv. Polym. Sci.* **2007**, *206*, 191–210; b) J. D. Matry, M. Mauzac, *Adv. Polym. Sci.* **2005**, *172*, 1–35; K. Haupt, K. Mosbach, *Chem. Rev.* **2000**, *100*, 2495–2504.

