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Molecular screening on a compact disc

James J. La Clair *[a] and Michael D. Burkart *[b]

[a] *BIONIC BROS GmbH, Postfach 870330, Berlin D-13162, Germany. E-mail: i@bionicbros.de*

[b] *Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0358, USA. E-mail: mburkart@ucsd.edu; Fax: 001-858-822-2182; Tel: 001-858-534-5673*

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A method is described to screen the recognition between small molecule ligands and biomolecules using a conventional compact disc (CD) player. A procedure was developed to attach ligands to the reading face of a CD by activating the terminus of polycarbonate, a common polymer composite within the reading face of a CD. Terminal residues of the polycarbonate surface **1** were phosphorylated by reaction with ethyl-(*N*,*N*)-diisopropylamine-buffered dichloro-(*N*,*N*)-diisopropylaminophosphate to yield surface-bound chlorophosphate **2**. Ligands containing a primary alcohol were condensed with **2** providing a polycarbonate capped with phosphodiester linked ligands **3**–**6**. Displays were generated on the surface of a CD by printing tracks of ligands **3**–**6** on the disc with an inkjet printer. Using this method, discs were created with entire assemblies of ligand molecules distributed into separate blocks. A molecular array was developed by assembling collections of these blocks to correlate with the CDROM-XA formatted data stored within the digital layer of the disc. Regions of the disc containing a given ligand or set of ligands was marked by its spatial position using the tracking and header information. Recognition between surface expressed ligands and biomolecules was screened by an error determination routine.

Introduction

With the advent of intelligent devices for molecular screening, considerable attention has focused on developing media to screen associations within complex molecular assemblies.[1] In particular, attenuated methods (*i.e.*, methods that reduce amplitude of the biological signal required for identification) have become critical for information rich diagnostics such as that required by combinatorial chemical, genomic, epigenomic, and proteomic investigations.[2] While methods exist to perform high-throughput molecular screening, the expense of these systems precludes their implementation in the common laboratory setting.[3]

Identification of molecular recognition events is central to many recent biomedical advances.[4] As scientists continue to improve their ability to screen molecular interactions, impending technologies gradually become accessible to a larger audience. For instance, tests to identify human chorionic gonadotropin (hCG), a biomarker for the onset of pregnancy, are already available for over-the-counter use in the US and Europe, and ELISA-based assays for HIV/AIDS antibodies are routinely performed around the world.[5] To date, however, routine screening of molecular recognition events is limited to well-funded laboratories.[6] We began with the concept of screening molecular recognition with a standard recordable compact disc (CD), a medium that is globally accessible, inexpensive and durable. Conventional compact discs are composed of a polycarbonate substrate, a reflective metalized layer, and a protective lacquer coating (Fig. 1A), as described by the ISO9660 industry standard.[7] A second format, CD-recordable discs (CD-R or CD-RW) have an organic dye data layer between the polycarbonate substrate and the metal reflective layer.

A CD is read by measuring the change in reflection of a polarized infrared laser (λ = 780 nm). As the laser beam travels through a CD, a binary system is generated by modulating the reflection of light according to its reflection from land or lowered pits within an internal layer (Fig. 1A).[7] When the beam hits a pit, the reflected light is destructively interfered by the incoming beam. When the beam hits land positioned ¼ of the wavelength higher than the pit, the reflected light is constructively transmitted as its phase aligns with the incoming beam. The player reads 1 when the beam hits land (reflected light is switched on by constructive interference) or 0 when the beam hits a pit (light is switched off by destructive interference).

A conduit between the informatic process of the CD and molecular processes of applied diagnostics must be developed before a CD can be used to read molecular interactions.[8] In molecular diagnostics, analyte molecules are classified by the manner in which their association with a sensor or ligand alters some electrical, photochemical, radioactive, or thermal field.[1] The CD reading process, therefore, must somehow be modulated by the binding of a biomolecule to a ligand (a molecular recognition event). We envisioned that adhesion of biomolecules to the surface of a CD could interfere with the optical transmission of the polycarbonate layer, and therein create error in reading digital data from an internal layer of the CD. This signal alteration could be collected and identified as a propagation of erroneous bits within a string of bits.[9] The binary nature of this error provides an ideal entry to connect assays of molecular recognition to computational systems. Software could be programmed to depict the propensity for error based on its alteration of an application, image, text, videographic or sound. The fact that this process operates on a digital channel streamlines the process used to display the identification and quantification of binding events. We reasoned that a byte of data (8 bits of 1 or 0) contained enough permutations to screen up to 2^8 or 256 different molecular recognition events on a single CD. Alternatively, larger bytes (16 or 32 bits) could be adopted for high-throughput analyses. As constructed, each molecular recognition event would therefore have a unique byte. Using this scheme, a block of data was constructed for each ligand using repetitions of these bytes. Comparing the surface area of a CD (78 cm²) relative to size of a bit (the average digit is denoted by ~10^{-6} cm²), sufficient physical space is available to allow single assays to be screened with a large set of repeated bytes, therein increasing the accuracy of this method (Fig. 1).

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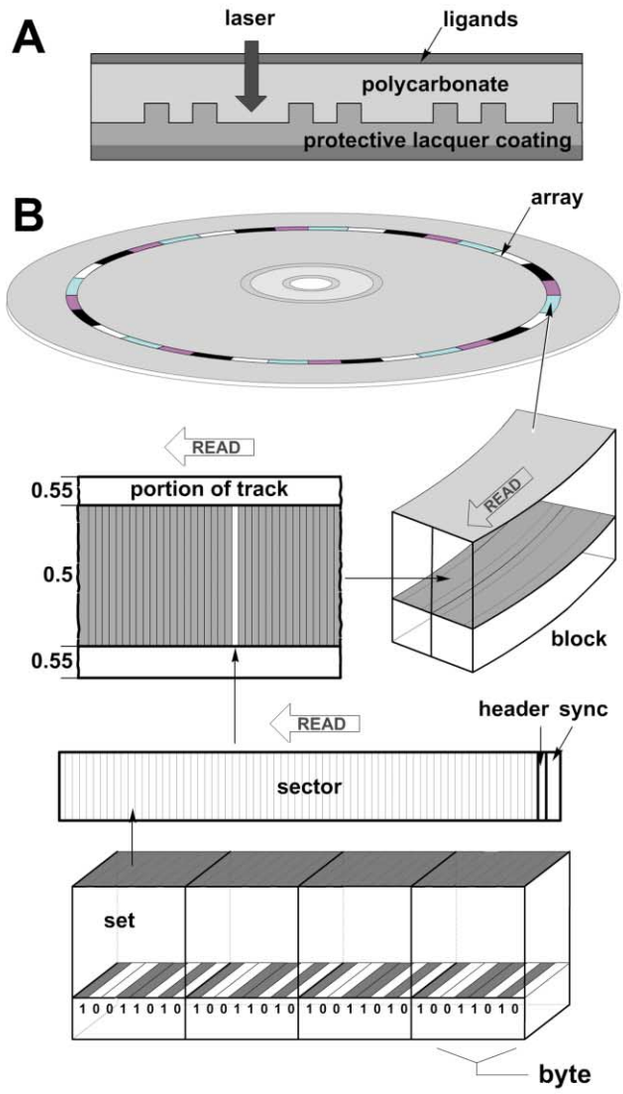

C

	sector	set	byte
array	16,000	9,934,000	37,376,000
block	500	292,000	1,168,000
track	250	146,000	584,000
sector	1	584	2,336

Fig. 1 Composition of screening CD. A. Cross section of CD-R with BIOTE and DIOTE. B. Physical map of screening CD used in this study. Alternating blocks are represented by four unique surface-displayed ligands as indicated by different colors (color assignments provided in Fig. 2). The CD data system is broken down into arrays, blocks, tracks, sectors, sets, and bytes. C. Breakdown of CD data statistics. In the screening CD used in this study, one ligand is screened with the use of a block of data. Since each ligand is represented by one repeated byte of binary data, each block repeats this sequence 1 168 000 times.

Results and discussion

The basic format for molecular recognition on the surface of a CD is as follows. A digital code is imprinted, or "burned", into the imbedded dye of a CD-R disc by standard writing procedures.[10] The code stored in the sectoring of this disc can be any digital string. Here, we used a repeated string of bytes, wherein a given molecular recognition event is coded by a unique byte. This digital information layer is referred to here as a DIOTE. The data structure within this DIOTE is provided in Fig. 1B.

The polycarbonate surface of the disc is then activated and conjugated in a geographically-specific manner with small molecule ligands. The data stored within the DIOTE is used to denote the position on the disc surface where a given ligand or ligand type is placed. Here a direct correlation between the digital data in the DIOTE and the biological data on the disc surface (refered to herein as a BIOTE) provides the recognition element required to decipher recognition events.

Once the BIOTE–DIOTE construct is complete, the disc is read on a standard player using a conventional personal computer (PC). The digital information obtained after this process contains a certain rate of error. This error rate, usually referred to in terms of disc quality and utilized for determining performance, is considered baseline. Subsequently, the CD is exposed to recognition molecules to detect their binding to ligands attached to the BIOTE. The CD is read again by the player, and the error rate is determined. It is expected that binding events will propagate error rates in the digital signal that are proportional to the number of molecules bound and the size of the biomolecular complex. The difference between the baseline error rate and the recognition error rate reveals binding events.

The interaction between ligands **4**–**6** and two proteins, streptavidin[11] and concanavalin A[12], were chosen to explore the utility of this technique. Each of these ligands is attached to the surface of a CD *via* a phosphodiester linkage (Fig. 2). The first ligand contained a coumarin tag **4**, which lacks affinity for streptavidin or concanavalin A and thus serves as a negative control. As a fluorescent molecule, coumarin **4** enables visualization of ligand placement on the CD. The second ligand

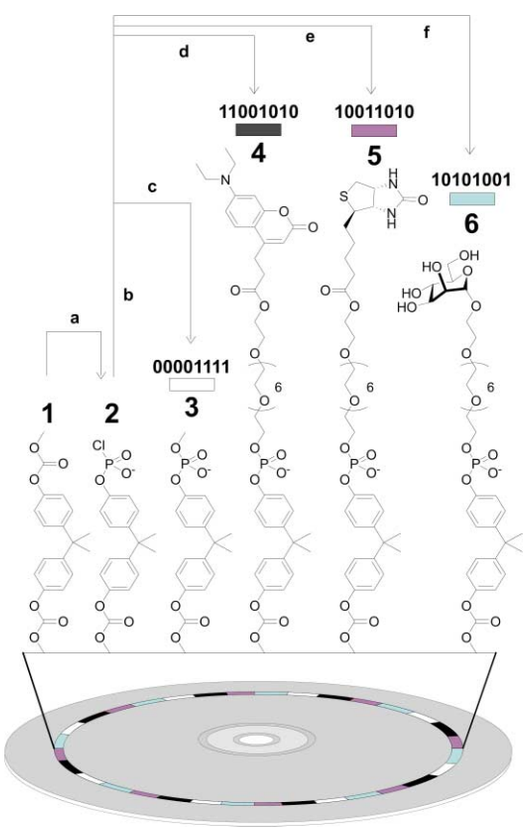

Fig. 2 Synthesis of ligands in BIOTE. The polycarbonate disc terminates in the methoxycarbonate **1**. (**a**) This moiety is deprotected to the alcohol and converted *in situ* to chlorophosphate **2** with excess dichloro-(*N*,*N*)-diisopropylaminophosphate buffered in ethyl-(*N*,*N*)-diisopropylamine. (**b**) Chlorophosphate **2** is converted into ligands **3**, **4**, **5**, or **6** through addition of the appropriate primary alcohol (**d**, **e** and **f**). This addition is performed with the use of an inkjet printer which serves to deliver **4**–**6** to specific locations on the surface of the disc. (**c**) The remaining active sites are capped as blank **3** with methanol. The ligands **4**, **5**, and **6** are shown here with their assigned byte as contained in their DIOTE. Each byte is used to depict a single CMYK color.

contained biotin **5**, which offers potent affinity to streptavidin ($K_d = 4.2 \times 10^{-11}$ M). The third ligand contained an α-mannoside **6**, which offers affinity to Concanavalin A in a more physiological range ($K_d = 10^{-4}$ M). Each of these ligands and blank is assigned an 8-bit digital code that will be matched with the DIOTE of each ligand (see Fig. 2).

Construction of the digital layer – the DIOTE

A single file of digital data was constructed by placing 32 blocks (0.425 ± 0.005 mm wide and 6.280 ± 0.784 mm long) of repetitive 8-digit bytes into two adjacent helical tracks (tracks 32 and 33) within the data layer of the CD (Fig. 1). Each block represented a single recognition event as given by the adhesion of a single ligand. The digital data within each block was written into tracks 32 and 33. Each portion of these tracks contained 250 sectors. These sectors were constructed from binary strings containing a header and sync. The header and sync were required to guide the reading process.[7] The data region of these sectors contained a string of 584 sets. Each set contains a string of four bytes. As shown in Fig. 1 each block contained 2 tracks, 500 sectors, 292 000 sets, 1 168 000 bytes, and 9 344 000 bits.

A disc was constructed to simultaneously screen for recognition of biotin and α-mannose residues. As shown in Fig. 1, a ring was assembled with four different types of blocks. Each type of block was repeated 8 times about the ring. A file containing the digital code representing this ring (*i.e.*, using the data structure described in Fig. 1) was combined with the appropriate CD-ROM XA sectoring and heading and written onto a CD-R disc. Software for this application was programmed in C/C++ and compiled on Linux-based systems (the routines used were adapted from open source routines, see http://k3b.sourceforge.net/ and http://www.xiph.org/paranoia/).

This software was programmed not only to create the digital layer of disc (DIOTE) but also to read the created data sectors and process the data into a recogizable format, using the header and sync to denote geographical positioning. No error correction or error minimization routines were used in either the writing or reading process. The output was displayed by coding 8 digit bytes with a single colored pixel, as given by: regions of the disc that contained blank **1** with white; regions containing the coumarin **4** by black; regions containing biotinylated-ligand **5** by magenta, and regions containing mannosylated-ligand **6** by cyan (Fig. 2). These pixels were arranged according to their header and sync information into a graphic image and were stored as a tiff formatted file. This graphic image provided a scaled model of the digital sectoring in the disc using the CMYK color scheme.

Generation of a layer of biological ligands – the BIOTE

The procedure to convert the terminus of polycarbonate for ligand attachment is shown in Fig. 2. The terminal arylmethoxy carbonate moiety of polycarbonate **1** was deprotected and phosphorylated with excess dichloro-(*N*,*N*)-diisopropylamino-phosphate buffered with ethyl-(*N*, *N*)-diisopropylamine.[13] This process successfully converts the terminus of polycarbonate to a surface-bound chlorophosphate **2**. Now activated, **2** was then condensed with a primary alcohol to provide phosphodiesters **3**–**6**. Two negative controls were generated by reacting the chlorophosphate **2** either with methanol to **3** or a fluorescent dye to **4**. A single primary alcohol was used per block as illustrated in Fig. 2.

Displays were generated using this method by printing tracks of ligands on an activated chlorophosphate disc surface using a commercially available inkjet printer.[14] Using this method, discs were created with assemblies of ligand molecules distributed into separate blocks. The structure of these blocks was developed to print the ligands directly over regions containing comparable data within an underling digital layer or DIOTE. Here, both the spatial position on the surface of the disc and the molecular structure of a given ligand was identified by code stored within CDROM-XA tracking and header information in the DIOTE.

Ligands **4**–**6** were formulated at 0.1 mM in ink (5% bismethylated polyethylene glycol 2500 in ether) and loaded in the cartridge of a CMYK inkjet printer according to their color assignments (Fig. 2). The graphic image generated from writing and reading the DIOTE (as described above) was imported into conventional graphic applications (*i.e.*, Gimp or Photoshop) and printed onto a chlorophosphate-activated CD.[11] The resulting surface was the BIOTE. After printing, the disc contained both digital (in the DIOTE) and molecular (in the BIOTE) code. Each assay was conducted within a single block. Each block had a surface area of 4.2 mm^2 and covered a 2.7 mm^2 wide DIOTE containing a total of 500 sectors, 292 000 sets, 1 168 000 bytes and 9 408 000 bits. An array was then developed (Fig. 1) with 8 blocks for each ligand and blank.

To demonstrate successful attachment of ligands to the CD surface, the CD was fluorescently imaged to identify correct placement of ligand **4**. Imaging was conducted with a CCD camera (SC110, EG&G, Gaithersburg, MD) equipped with a 400 nm cutoff filter (UV-B, LINOS AG, Göttingen Germany) and a fiber optic UV source (LQ1100, LINOS AG, Göttingen Germany). Under the conditions given in Fig. 2, 70 ± 7% of the surface was loaded with coumarin **4**. At this level, each block contained approximately 10^{11} ligand molecules, approximately 10^5 ligand molecules per digital bit.

Standardization of the disc with bound ligands

Once the BIOTE and DIOTE were complete, the disc was read on the same CD-ROM drive and PC used to create it. The digital data in this "native" state is stored as a string $N_n(x)$ where n denotes a given reading and x identifies the block. Fig. 3 depicts the process used to collect $N_n(x)$. The digital code in tracks 31 to 34 mm was read from the disc into a PC. Five reads ($n = 5$) were collected. The routine used for this process not only provided the data stored in CD-ROM XA format sectors but also included the tracking and sectoring information. The sector and tracking data was removed, and the resulting data

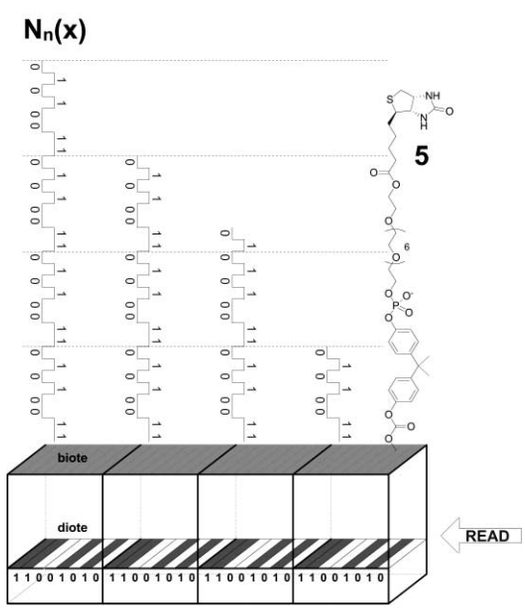

Fig. 3 A model of the collection $N_n(x)$. $N_n(x)$ is collected after writing the DIOTE and attachment of the BIOTE. $N_n(x)$ defines the error rate in the signal created by attached ligands. A single set is shown. As one reads this set from left to right, bits (1 = grey and 0 = white) within the digital layer (DIOTE) are transmitted through the biological layer (BIOTE). This signal is depicted as a departing square wave. Here the BIOTE contains the biotinylated ligand **5**. $N_n(x)$ defines the error rate in the signal created by attached ligands, here with biotinylated ligand **5**.

was processed as $N_n(x)$. This application was programmed for Linux using conventional routines for CD mounting and reading. Comparable routines can be found in CD diagnostic systems (Clover Systems, Laguna Hills, CA).

$N_n(x)$ was then processed with respect to ligands **4**–**6**. As the code used for these ligands contained the same number of digital transitions (*i.e.*, 0 to 1 or 1 to 0), one would expect to find comparable background error. Over the 8 blocks (*i.e.*, the disc had 8 blocks for each ligand), $N_n(x)$ deviated within 78 900 ± 800, 77 800 ± 400,and 76 800 ± 500, bits more than blank **3** for ligands **4**, **5**, and **6**, respectively. This background error rate provided a direct tool to verify production quality. As seen here, a comparable error arose from printing of ligands **3**–**6**.

Screening for molecular interaction

The disc was then exposed to solutions of streptavidin in PBS buffer or concanavalin A in acetate buffer. A PTFE Teflon template machined to match the block structure of the ring (Fig. 1) was mounted on the disc using 6 equally distributed plastic clamps (X-ACTO). Once mounted, this template provided individual 60 µl reaction wells, wherein the bottom of each well contained a BIOTE from a single block. The template was routinely placed on the disc such that it aligned within ±0.01 mm of the center of the disc and had a rotation within ±0.05 mm. This positioning was guided by aligning eight 0.2 mm indented guides on the outer diameter of the disc using a plastic holder containing a male equivalent of the preceding indentations. Each of the resulting wells were filled with 50 µl of 1.0 nM solution of streptavidin in PBS pH 7.2 or 1.0 µM solution of concanavalin A in acetate buffer pH 6.0. After incubation for 1 h, the contents of each well were removed by aspiration. The template was removed and the disc was washed by mild shaking in 400 ml of deionized water. The disc was read and an exposed data string, $E_m(x)$ was collected using the same process used to collect $N_n(x)$. The outcome of this process is depicted in Fig. 4. Again five $E_m(x)$, $m = 5$, were collected for each block (x).

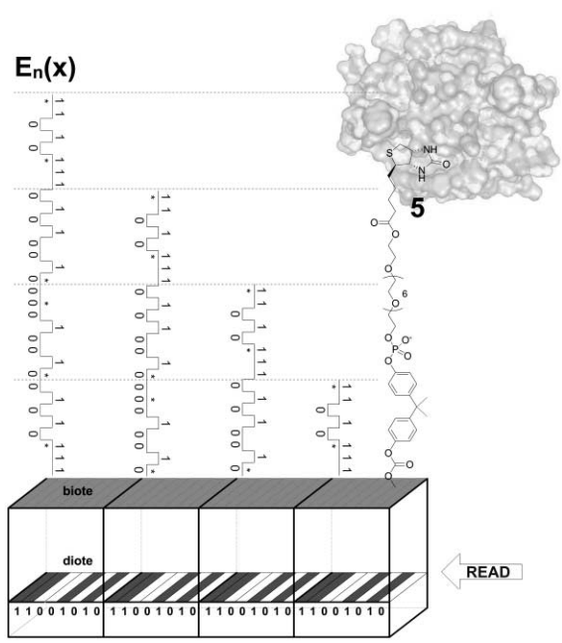

Fig. 4 A model of the collection of $E_m(x)$. $E_m(x)$ defines the error rate in the signal created by the binding of biomolecules to attached ligands. Streptavadin is binding to a BIOTE containing the biotinylated ligand **5**. As one reads this set from left to right, errors (*) in reading appear within the departing square wave.

Using eqn. 1, twenty five $\xi_{nm}(x)$ were generated by subtracting each of the 5 $N_n(x)$ from the 5 $E_m(x)$. Here the subtraction of the two digital strings $N_n(x)$ and $E_m(x)$ yield a third string whose absolute value, $\xi_{nm}(x)$, contains a digit of 1 only when the bit differed between $N_n(x)$ and $E_m(x)$. Each of the bits within $\xi_{nm}(x)$ were then tabulated into a sum using eqn. 2 where i indicates the number of bits in $\xi_{nm}(x)$ and $d\xi$ represents the number of bits that differed between a given $N_n(x)$ and $E_m(x)$. A total of 25 $d\xi$ were generated for each block. Each array contained 8 repetitions of blocks and therefore the disc in Fig. 1 provided a total of 200 $d\xi$ each type of ligand. The average and deviation in this $d\xi$ was reported.

$$\xi_{nm}(x) = |E_m(x) - N_n(x)| \quad (1)$$

$$d\xi = \frac{\Sigma^{i}\xi_{nm}(x)}{i} \quad (2)$$

The average $d\xi$ for interaction between streptavidin and the inert coumarin **4** was 0.1287 ± 0.0084, corresponding to 1 202 201 ± 78 290 bits of 1 (*i.e.*, 1 = error) in total 9 334 000 bits of $\xi_{nm}(x)$. This $d\xi$ was 30% larger than that observed in regions containing blank **3**, $d\xi = 0.0989 \pm 0.0069$. The $d\xi$ for the biotinylated ligand **5**, however, contained 250% more error than blank with an average $d\xi = 0.3542 \pm 0.0131$ over all 8 blocks. This $d\xi$ corresponded to 3 306 671 ± 123 251 bits of 1 in $\xi_{nm}(x)$. This increase in error is significant from the baseline and therefore serves to mark positive molecular binding events. The $d\xi$ for the α-mannosylated-ligand **6** was 0.1432 ± 0.0102. This value compares favorably to the inert ligand **4**, and corresponds to a negative molecular binding event.

Comparable results arose when screening against concanavalin. The $d\xi$ for the interaction between concanavalin A and α-mannosylated-ligand **6** was 0.2157 ± 0.0212, corresponding to an average of 2 097 791 bits of one in $\xi_{nm}(x)$. This value compares favorably with a $d\xi$ of 0.1172 ± 0.0812 for coumarin **4** and $d\xi = 0.1081 \pm 0.0109$ for the biotinylate-ligand **5**, both of which are non-binding events.

To further demonstrate specificity, known non-binding proteins were exposed to the blocks to analyze their interaction to the ligands. Addition of an equivalent of bovine serum albumin ($d\xi = 0.1301 \pm 0.082$) or α-amylase ($d\xi = 0.1247 \pm 0.069$) failed to induce error comparable to that of streptavidin with **5** or **6** for concanavalin A. This observation was further verified by the reduction in $d\xi$ to 0.1347 ± 0.078 upon pre-incubating streptavidin with an excess of biotin prior to application to the disc.

Alternatively, the data content of each block can be determined by examining the relative level of error within each set (4 bytes, 32 bits). Here the number of correct bits are tabluted for each block (292 000 sets in a block). Each set was then tabulated into groups according to the number of correct bits. The number of sets within each group was determined and plotted according its variance, as given by the percentage of sets in each group (*i.e.*, number of sets in a group/the total number of sets or 292 000). Fig. 5 illustrates this method for receptors **4**–**6**. In conditions where the surface does not retain protein (Fig. 5A), comparable variablity within the error is found within both the native ($N_n(x)$) and exposed states ($E_m(x)$). However, in cases where protein adheres to the surface of the disc though its affinity to a ligand, the increased error within the exposed state (00) is clearly observed by a shifting and enlargening of the error variablity. For surfaces coated with biotin, the adhesion of streptavidin corresponds to a shift from the maxium sets from 27 to 18 correct bits/set (Fig. 5B). A comparable shift was observed upon exposing blocks containing α-mannoside **6** with concanavalin A (Fig. 5C).

The CD could also be used to determine the concentration of an analyte. A calibration curve was obtained upon exposure to sub-nanomolar solutions of streptavidin (Fig. 6). This response was limited by the need for >50 µl of 6.5 pM of PBS solution of streptavidin, at which point a variation in 900 000 bits or ±0.089 in $d\xi$ approached the intrinsic error of the reader (±0.0069). At

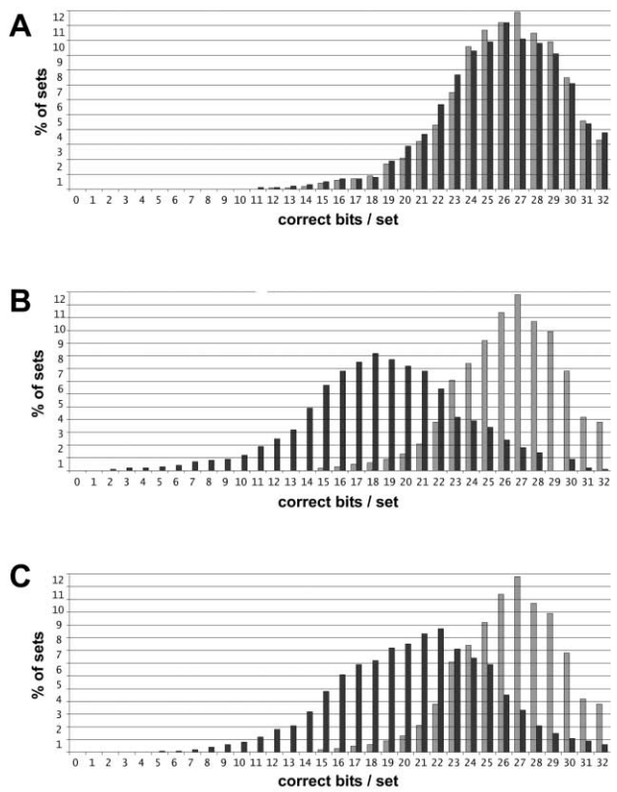

Fig. 5 Variance analysis. The level of error within a block is displayed by the number of erroneous bits in each set (a set contains 4 bytes or 32 bits). The level of error in the "baseline" $N_n(x)$ (light grey bars) is compared to the level of error in "active assay" $E_m(x)$ (dark grey bars). The number of correct bits per set is displayed along the x-axis. The percentage of sets in a block (292,000 sets are in a block) is displayed along the y-axis. (A) The error arising from the interaction between streptavidin and the coumarin tag **4**. (B) The error arising from the interaction between 1.0 nM solution of Streptavidin and the biotin tag **5**. (C) The error arising from the interaction between a 1.0 μM solution of concanavalin and the α-mannosyl ligand **6**.

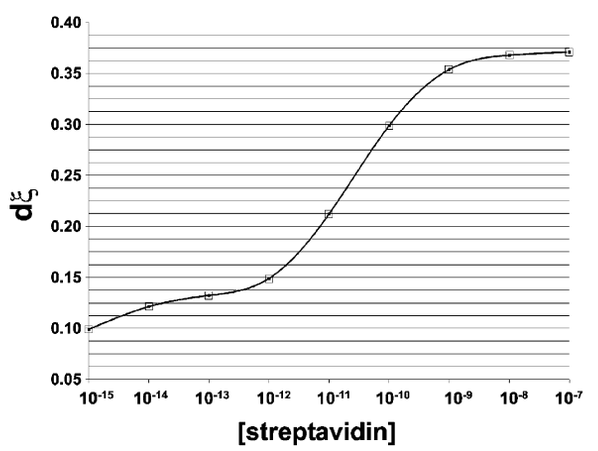

Fig. 6 Concentration profile. The addition of increasing amounts of Streptavidin is directly proportional to the level ($d\xi$) of error generated. This plot depicts the sensitivity of the method and serves as a means to calibrate the affinity between a biotinylated ligand and streptavidin.

this limit, 2×10^8 molecules of streptavidin were delivered per mm^2. This detection was done without the need for the addition of a colorimetric, fluorescent or radioactive label,[15] and therefore eliminates potential losses in affinity and/or selectivity, such as that observed in fluorescent microarraying.[16]

Conclusions

This work identifies a cost-effective method to perform molecular screening with a standard compact disc (CD) player. We demonstrate methods to chemically modify polycarbonate compact discs for functional display of molecules to screen for molecular interactions. We further show a practical example of molecular recognition and its quantification with a personal computer (PC) outfitted with a CD player. We believe that the basic techniques described herein will have utility for applications where molecular recognition is valued, including such fields as biomedical research, medicine, and environmental science.

Experimental

Writing

Creation of DIOTE. A disc was created with a single array containing 8 blocks for each ligand **4**–**6** and blank **3**, as given in Fig. 1. Each ligand was assigned a unique digital name or byte (Fig. 2) This byte also coded for a single CMYK color. A digital file was prepared by placing four of the preceding 8-digit bytes in a set. 584 of these sets were strung sequentially into a sector along with the appropriate CD-ROM XA format 12 byte Sync and 4-byte header. Each block was then completed by aligning two strings containing 250 of these sectors across adjacent tracks (tracks 32 and 33 were used). These tracks provided the complete DIOTE equivalent for a single block. A total of 32 of these blocks, eight for each ligand **3**–**5** and blank **3**, were coded into a file with the proper header and sync to provide a structure that fitted into a ring whose center lay 32 mm from the center of the disc (*i.e.*, tracks 32 and 33). This file was burned onto a CD-R74 compact disc (TDK) using an external CD-R drive (ONE Pro8/8/24) on a personal computer (Intel Celeron 600 MHz PROCESSOR, ASUS i815 motherboard, 10 GB Maxtor drive operating on Redhat Linux 6.1). The resulting disc contained thirty-two blocks 0.425 ± 0.005 mm wide by 6.280 ± 0.784 mm long. The data was then read from the disc using the same CD-R drive and rendered into a 900 dpi TIFF file using the bytes to code for CMYK colors (Fig. 2).

Activation of CD surface. The surface of the CD was activated by placing the disc reading face down on the center of a PTFE mold. The mold was made from PTFE (150 mm × 150 mm × 1 cm) machined with a centered conical well (inner diameter 45 mm, outer diameter 115 mm, depth 3 mm). The well was filled with 25 mM (*N*,*N*)-dichloro-(*N*,*N*)-diisopropylaminophosphate and 100 mM ethyl-(*N*,*N*)-diisopropylamine in dry acetonitrile and the setup was incubated at 35 °C for 6 hours under a dry argon atmosphere. After cooling to room temperature the disc was washed with dry acetonitrile.

Preparation of ligands. Ligands **3**–**5** were prepared from their corresponding polyethylene glycol-adducts.[17]

Creation of BIOTE. The BIOTE layer was printed using a commercial inkjet printer equipped with a CD holder (Precision 2000, SEIKO Tokyo Japan). This printer routinely placed the pattern in Fig. 1 onto a CD with a positional accuracy of ±0.12 mm. To accommodate this error, the area of BIOTE track was expanded to width of 0.6 mm and length of 7 mm. This disc was then activated using the method in Fig. 2 and immediately printed with the ligand ink (1 mM ligand in ether containing 5% bis-methylated polyethylene glycol 2500). Both ligands were printed simultaneously by placing a control ligand in the black cartridge and the active ligands in the color cartridge. This printing process was conducted by printing the tiff file from the PC using the Gimp (http://www.gimp.org). After printing, the disc was incubated for 4 h at 45° C, quenched in a bath of anhydrous methanol, and washed with deionized water and dried.

Reading

After printing of the BIOTE, the disc was read on the same CD-R drive and PC used to write it (a cross-section of 20 other CD-ROM drives offered comparable performance). The disc was read five times ($n = 5$). Raw data was collected from the disc and provided a single string of data containing XA-format information. As the disc was read from the inside outward, the reading process split each block of data in half (*i.e.*, each block contains 2 tracks of 250 sectors). The two halves were identified using the tracking and sectoring information and then recompiled into a single 9 408 000 bit string. The sync and header bytes were then deleted, leaving a string $N_n(x)$ of 292 000 sets or 9 334 000 bits.

The disc was then covered with a 3 mm thick PTFE Teflon mask containing a ring which was cut to have thirty-two spacers centered about radius of 32 mm. When placed on a CD this mask provides 0.21 cm^2 wells that were centered about a radius of 32 mm. An aliquot of 50 μL of a 1.0 nM streptavidin in PBS (0.02 M potassium phosphate, 0.15 M NaCl, pH 7.2) or 50 μL of 1.0 μM concanavalin A in acetate buffer (0.1 M NaOAc, 1 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, 1 mM $MgCl_2$, pH 6.0) was added to the appropriate well and the plate was incubated at 32 °C for 30 min. The reagent and template were removed by aspiration, and the disc was cleaned by soaking in a 500 ml bath of PBS pH 7.2 for 15 min, rinsing with deionized water, and drying with a cotton cloth. The disc was then read five times (m=5) using the same CD-R drive, and the resulting data was cut into $E_m(x)$ using the procedure used above to formulate $N_n(x)$. Again, a single $E_m(x)$ was obtained for each block and reading. The outcome was displayed either by compiling $\xi_{nm}(x)$ and $d\xi$ using eqns 1 and 2 or by variance analysis as given in Fig. 5.

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