End-specific covalent photo-dependent immobilisation of synthetic DNA to paramagnetic beads

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ABSTRACT

A novel approach for light-dependent covalent immobilisation of synthetic DNA oligomers to amino-coated paramagnetic beads is described. A hetero-bifunctional photo-reactive cross-linking chemical, 4-nitrophenyl 3-diazopyruvate, is applied to attach 5′ amino-modified DNA to both silica and polystyrene paramagnetic beads. The coupling yields are comparable with similar methods in which no photo-reactive chemicals are used. The immobilised DNA on the polystyrene and silica beads was used efficiently in hybridisation experiments. An extension of this approach to light-directed immobilisation of specific DNA to beads, located at different positions in micro-flow reactors, opens up a range of integrated applications to complex diagnostics, evolutionary biotechnology and novel areas such as DNA computing.

INTRODUCTION

In recent years, DNA chip-based assays have become a familiar approach suitable for a broad range of applications such as expression analysis (1,2), polymorphism analysis and genotyping (3,4), and the detection of pathogens (5). The expansion of DNA chip technology has encouraged the rapid development of light-directed oligonucleotide synthesis (6,7). Light-directed oligonucleotide synthesis has proven to be an efficient method for fabricating probe arrays with densities as high as 10^6 unique sequences/cm^2 (8). Nevertheless, there are some common errors in optical oligonucleotide synthesis, such as premature truncations of the growing strand and base deletions, which seem difficult to avoid. Potentially, the error rate in DNA chips due to these factors could be reduced by replacing the light-directed oligonucleotide synthesis with light-directed oligonucleotide immobilisation. Despite the existence of a great number of different chemical methods for specific 5′- and 3′-end covalent DNA attachment, there are only a few approaches for the photo-immobilisation of DNA (9). In this article, we propose a novel method for light-dependent covalent immobilisation of 5′-end amino-modified single-stranded DNA, based on the hetero-bifunctional, photo-reactive cross-linking agent 4-nitrophenyl 3-diazopyruvate (DAPpNP).

As a solid support for photo-immobilisation of DNA, we employ silica and polystyrene amino-coated super-paramagnetic beads. Our goal is to apply this photo-dependent DNA immobilisation to allow a programmed immobilisation of DNA to beads at specific sites in micro-flow reactors. This patterned immobilisation can then direct the sequence specific selection from complex DNA populations (J.S.McCaskill, submitted) and thereby enable an optical specification of different problem instances in the field of DNA computing (10). A super-paramagnetic bead-based module for integrated complex selection in micro-flow reactors has been described (J.S.McCaskill, submitted) together with a sample application (to the maximal clique problem) designed to make use of the current light-directed immobilisation procedure.

In contrast to chemical cross-linking reagents, which have been successfully applied for DNA immobilisation (11,12), photo-reactive cross-linking reagents have primarily been employed as tools for defining interactions among proteins, nucleic acids, ligands and their receptors (13,14). The major disadvantage in using photo-reactive cross-linking reagents for DNA immobilisation is that such agents form extremely reactive groups after photo-activation, usually causing many non-specific side reactions (9).

In this work, for the attachment of DNA we avoid directly utilising the amino-ketene group, formed after photolysis of a diazo group of diazopyruvic acid, because of its high reactivity and its instability in aqueous solutions. Instead, we facilitate the transformation of the ketene group into a carboxyl group, which is then utilised for reaction with a 5′ amino-modified deoxynucleotide oligomer in the presence of carbodiimide.

MATERIALS AND METHODS

Paramagnetic beads and oligodeoxynucleotides

Silica and polystyrene super-paramagnetic beads were purchased from Micromod GmbH (Rostock, Germany). All beads are essentially monodisperse. The properties of the beads are presented in Table 1. Oligomers were obtained from IBA-NAPS (Göttingen, Germany). The DNA was 5′ amino-labelled using a C6 linker. All DNA was purified to HPLC grade. The oligomer sequences used are shown in Table 2.

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Table 1. Properties of the paramagnetic beads used (as specified by the manufacturer, see Materials and Methods)

Bead type	Size (μm)	Content of magnetite (%)	Functional groups on the surface	Particle charge density (nmol/mg)
Carboxyl polystyrene-co-maleic	15 ± 2	15–20	Acrylic acid	6.0
Amino polystyrene-co-maleic	15 ± 2	15–20	$(CH_2)_4NH_2$	3.1
Amino silica	15 ± 5	20	$(CH_2)_3NH_2$	3.26
Carboxyl silica	15 ± 5	20	Citric acid	2.1

Table 2. DNA oligonucleotides employed

DNA	Type of modification	Sequence 5′–3′	Length (nt)
1	5′ Amino C6	TCCCGAAAATACTAAAAAAGCA	22
2	5′ Amino C6	TTCCCGGACGGTCACAGCTTGTCTG	25
3	5′ Amino C6 or 5′ amino C6 and 3′ fluorescein	TTTTTTTTCAGACAAGCTGTGTCCGTCTCCCGGGA	35
4	5′ Rhodamine 6G or 5′ amino C6	TCCCGGGAGACGGACACAGCTTGTCTG	27

Modification of amino-coated beads with the photo-reactive bifunctional cross-linker DAPpNP

DAPpNP was obtained from Molecular Probes (Eugene, OR). The cross-linker was dissolved to produce a 200 mM solution in dry DMSO. 100 μl of this solution was added to 5 mg amino-coated beads resuspended in 400 μl dry DMSO. The reaction was performed for 2 h at room temperature with continuous shaking in the dark (see Fig. 1, reaction 1). The beads were washed several times in the dark to remove any traces of nitrophenol with deionized H_2O until there was no longer any measurable absorbance at 412 nm in the rinsing solution.

Photolysis of the diazo group to amino ketene and its transformation to the carboxyl group

The diazo group attached to beads (as above) was photolysed by exposure to UV radiation for 30 s applying a mercury lamp with a power of 15 W from 280 to 315 nm and 75 W from 315 to 400 nm (UV-F400, Panacol-Elosol GmbH, Oberursel, Germany). In the case of immobilisation of amino-modified DNA applying 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDAC) (Sigma) (see Fig. 1, left-hand route) 5 mg beads were placed into a quartz cuvette in 500 μl deionized H_2O. The reaction mix was incubated for 0.5 h at room temperature while continuously shaking in the dark (see Fig. 1, reactions 2 and 4).

In the case of direct immobilisation of amino-modified oligomers (see Fig. 1, right-hand route), 25 μl (100 pmol/μl) of the 5′ amino-modified 35 nt oligomer was added. The reaction was incubated for 2 h at room temperature with continuous shaking in the dark. Negative controls were performed, as for the reactions described above, but in the dark without exposure to radiation.

Covalent attachment of 5′ amino-modified DNA oligomers to a photo-induced carboxyl group

An aliquot (2.5 nmol) of the 35 nt 5′ amino-modified oligomer was used for the covalent linkage with a photo-induced carboxyl group on 5 mg beads dissolved in 500 μl deionized H_2O in the presence of 10 mM EDAC. The reaction was performed for 3 h at room temperature with continuous shaking in the dark (see Fig. 1, reaction 5).

In order to remove non-covalently bound DNA, the beads were treated with SPSC buffer (50 mM sodium phosphate, 1 M NaCl, pH 6.5) for 1.5 h at 42°C, and then with 100 mM Tris–acetate pH 8.0 for 1 h at 42°C in the dark.

Quantification of the DNA attached to the beads

The peptide bond formed between the immobilised DNA and the bead spacer was destroyed with 28% NH_4OH (Sigma). The reaction was performed at 48°C for 3 h with continuous shaking. NH_4OH was removed by evaporation with a SpeedVac Concentrator (Savant Instrument, Inc., Holbrook, NY). The cleaved DNA was purified on a NAP-10 column (Pharmacia Biotech, Uppsala, Sweden) as described by the manufacturer. The concentration of DNA cleaved from the beads was estimated by UV spectroscopy with a Cary 3E photometer (Varian Inc., Walnut Creek, USA) at 260 nm as described by Sambrook *et al.* (15).

HPLC analysis

HPLC analysis was performed on an HPLC liquid chromatograph (Shimadzu LC-10AT with system controller SCL-10A and UV-vis detector SPD-10 Avi at 260 nm) using the software package Class VP v.4.0 (Shimadzu, Japan). An anion-exchange column (mono-Q HR5/5, Pharmacia) was loaded with a linear salt gradient (buffer A: 10 mM NaOH in H_2O; buffer B: 1 M NaCl in 10 mM NaOH) at a flow rate of 1.5 ml/min, increasing buffer B from 0 to 100% in 20 min.

DNA hybridisation on beads and its detection

Beads (2.5 mg) with immobilised DNA (see Table 2, row 3) were resuspended in 500 μl 100 mM Tris–acetate buffer pH 8.0 in the presence of 2 μM 5′ rhodamine 6G labelled DNA (see Table 2, row 4). The hybridisation reactions took place at 42°C for 1 h while continuously shaking in the dark, after

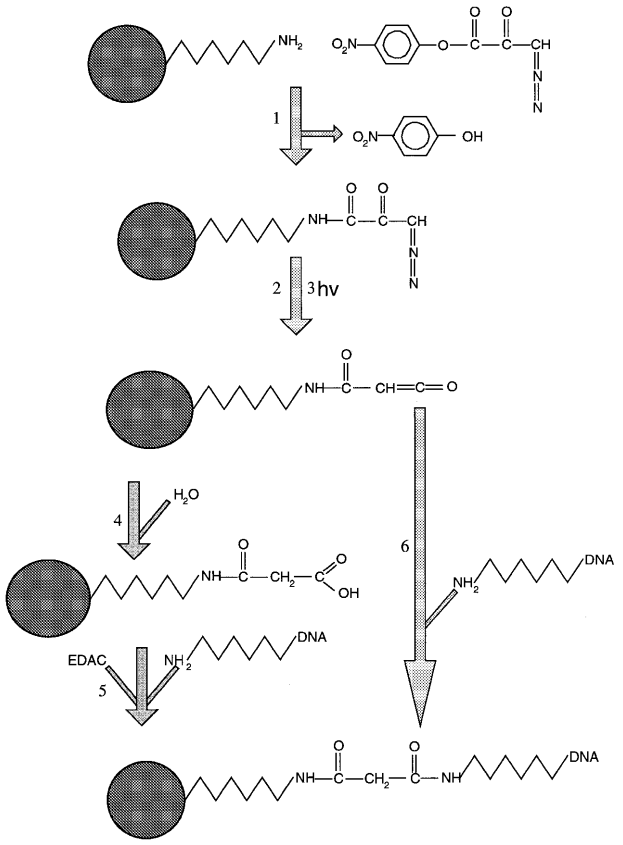

Figure 1. Overall scheme of photo-immobilisation. Left-hand route: the main photo-immobilisation scheme using the zero-crosslinker EDAC to attach 5′ amino-modified DNA oligomers to beads. (**1**) The 4-nitrophenyl ester group of DAPpNP reacts with amines on the beads producing diazopyruvic. (**2**) Photolysis of the diazo group attached to the beads by UV illumination and (**3**) formation of ketene amides with (**4**) conversion of the ketene group to carboxyl in water. (**5**) Covalent immobilisation of 5′ amino-modified oligomer to the photo-induced carboxyl group (attached to the beads) in the presence of EDAC via peptide-bond formation. Right-hand route: (**6**) Direct utilisation of the amino-ketene group for immobilisation of 5′ amino-modified DNA oligomers.

which the hybridisation solution was removed and the beads were washed three times with 500 µl 100 mM Tris–acetate buffer pH 8.0 at 42°C for 15 min. Finally, the beads were resuspended in 100 µl water and incubated at 95°C for 2 min. The fluorescent signal of the denatured DNA in the supernatant was measured by a spectrofluorimeter with excitation at 524 nm and emission measured at 557 nm (FluorMax-2, Instruments S.A., Inc., Edison, NJ).

Light-directed DNA immobilisation to beads in a micro-flow reactor

In order to demonstrate a spatially defined light-directed DNA immobilisation the beads were incorporated in a micro-flow reactor. The design and the fabrication of the micro-reactor are described in McCaskill *et al.* (16). The micro-reactor was anodically bonded to two 500-µ-thick pyrex (borosilicate) wafers, where 1 mm of the wafer has a 50% transmission at 306 nm. The reactor has two inlet and two outlet channels (see Fig. 3C). Both inlet channels are filled with polystyrene amino-coated beads (see Table 1, row 2) in the dark. The beads are restrained in inlet channels by a ledged bead barrier. They were previously treated with a solution of DAPpNP and washed as described above.

Water was pumped with a flow rate of 2 µl/min via both inlet channels of the micro-flow reactor in the dark using a precision syringe pump model 260 manufactured by World Precision Instruments Inc. (Sarasota, FL). The reactor was placed under a UV lamp with part of one of the inlet channels covered by an opaque foil (see Fig. 3B). The micro-flow reactor was irradiated by the lamp twice for 30 s. The aqueous solution was replaced after 2 h by a solution of 10 mM EDAC and 2.5 nmol of the 35 nt 5′ amino-modified and 3′ fluorescein-modified oligomer (see Table 2, row 3). The reaction was performed for 2 h at a pump rate of 1 µl/min. In order to remove as much of the non-covalently attached DNA as possible, the micro-flow reactor was washed three times with 1 ml 50% DMSO and three times with 1 ml 100 mM Tris–acetate pH 8.0 buffer at a 2 µl/min flow rate.

Spatially resolved detection of DNA immobilisation to beads

A CCD camera model LN/1024 TKB/1 (Princeton Instrument Inc., Monmouth Junction, NJ) was employed to capture images from the micro-flow reactors.

The illumination was performed either by white light or by laser beam at 514.5 nm from an argon-ion laser model 2080-15S manufactured from Spectra-Physics Lasers, Inc. (Mountain View, CA). A homogenous pool of light of diameter ~10 cm was created by acoustic vibration of a multi-mode optical fibre at 80 Hz. An interference filter was placed between the camera and the sample, custom designed from AHF-Analysentechnik (Tübingen, Germany) with an OD of 6 at 514 nm and transmission of 85–90% at 523–600 nm.

For data acquisition and image processing, a customised version of the software PMIS v.2.0.1 (GKR Computer Consulting, Boulder, CO) was employed.

RESULTS AND DISCUSSION

The overall scheme of the photo-dependent immobilisation is shown in the left-hand route of Figure 1. The 4-nitrophenyl ester group of DAPpNP reacts with amines producing diazo-pyruvic acid amides that undergo UV photolysis to generate ketene amides through carbene formation and Wolf rearrangement (17,18). The ketene group is unstable in water, reacting readily to form a carboxyl group (17). This photo-induced carboxyl group can be used for a covalent immobilisation of 5′- or 3′-end amino-labelled DNA in the presence of EDAC, via the formation of a peptide bond (11). An alternative, slightly more involved, mechanism shown in the right-hand route of Figure 1 has also been investigated. It starts with the same photo-activated ketene group, but coupling occurs directly with 5′-end amino-modified DNA.

Chemical stability of the diazo group

The key point in our photo-immobilisation strategy is the chemical stability of the diazo group. Diazomethane and other diazoalkyl derivatives have been used in the HPLC analysis of low molecular weight compounds to label carboxyl groups of fatty acids (19). Diazoalkanes and diazoacetyl compounds (amides and esters) react with carboxylate groups even without catalysts. This should not be a problem here because both

carboxyl and diazo groups are attached to the bead surface spacers (see Table 1) so that their reaction should be limited by geometric constraints.

The diazo compound was found to be stable in water at room temperature (20). Because this point was critical for the current method, we tested the stability of the diazo compound in the presence of EDAC and 5′ amino-modified DNA oligomer in water, using an HPLC analysis as described in Materials and Methods.

First, we examined the retention time of a 5′ amino-modified oligomer (see Table 1, oligomer 1) in deionized H_2O using the HPLC system. A retention time of 11.9 min for this DNA oligomer was found (peak UV absorption). Secondly, 40 mM of DAPpNP with a 2 µM aqueous solution of the same DNA in the presence of 100 mM EDAC were incubated at room temperature for 2 h and for 8 h in the dark. An identical HPLC analysis showed that the retention time of the DNA was unchanged for both incubation times, 11.9 min (data not shown).

To ascertain the sensitivity of this measurement to potential reactions of the diazo group, we repeated the experiment in a buffer solution in which it proved reactive. We incubated 40 mM of DAPpNP with a 2 µM solution of the DNA oligomer in 100 mM borate buffer pH 8.6 for 2 and 8 h. For both incubation times, two peaks of DNA were observed, at 11.9 and 12.5 min (data not shown). The second peak indicates a reaction between the 5′ amino group of DNA and DAPpNP, resulting in the production of diazopyruvic acid amides at the 5′-end of the DNA oligomer. Since such reactive products can be discriminated by HPLC analysis, we can conclude from the experiment above that the diazo-compound is stable in water in the presence of EDAC and amino-modified DNA at room temperature for at least 8 h in the dark.

Photo-dependent immobilisation of DNA oligomers to amino-coated paramagnetic beads

The immobilisation reaction was performed as described in Materials and Methods together with negative controls. Amino-coated silica and polystyrene beads, 15 µm in diameter, were used as a solid support in the first set of experiments. The DNA products were cleaved from the beads and were purified as described in Materials and Methods. The DNA products were quantified by UV absorbance as reported in Table 3.

The negative control reaction 2 (see Fig. 2 and Table 3, column 2) was performed in the same way as the positive reaction 1 (see Fig. 2 and Table 3, column 1), but without exposure to UV radiation. Negative control 3 (see Fig. 2 and Table 3, column 3) was made without treatment of beads with DAPpNP negative control 4 (see Fig. 2 and Table 3, column 4) was made with neither DAPpNP nor EDAC and negative control 5 (see Table 3, column 5) was made without EDAC. As one can see from Figure 2 and Table 3, the non-specific immobilisation in all negative controls is the same, so that the non-specific immobilisation is due to surface interaction but not to chemical reactions.

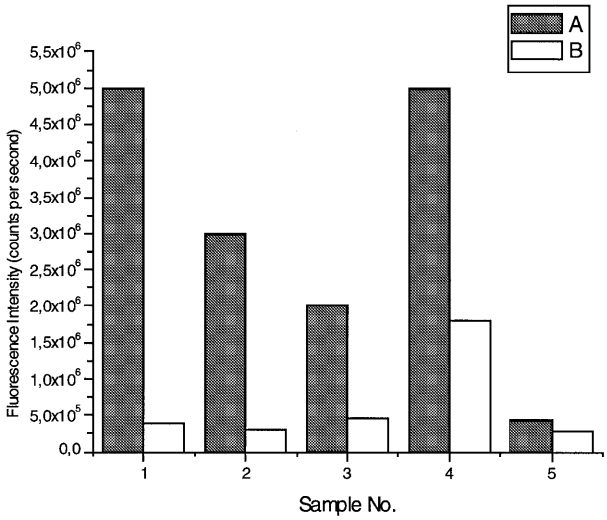

Figure 2. Hybridisation analysis of DNA to bead-bound oligomers. Filled bars, total hybridisation yield; unfilled bars, background hybridisation. Sample 1, carboxyl-coated polystyrene beads: immobilisation using the zero-crosslinker EDAC. Sample 2, carboxyl-coated silica beads: immobilisation using the zero-crosslinker EDAC. Sample 3, amino-coated silica beads: photo-immobilisation using the zero-crosslinker EDAC. Sample 4, amino-coated polystyrene beads: photo-immobilisation scheme using the zero-crosslinker EDAC. Sample 5, amino-coated silica beads: direct utilisation of the amino-ketene group for immobilisation of 5′ amino-modified DNA oligomer (see Materials and Methods).

We demonstrated a spatially defined light-directed DNA immobilisation to magnetic beads incorporated in a micro-flow reactor. As one can see from the fluorescent image in Figure 3B, the beads in the channel not exposed to radiation show a very low fluorescent signal in comparison with the fluorescent signal from the beads exposed to radiation. Both inlet channels of the micro-flow reactor are indeed filled with beads as shown in the white light image of Figure 3A. The quantification of the fluorescent signal is shown in Figure 4.

Comparison of various beads and surface coatings for immobilisation

Carboxyl-coated beads may be used directly for immobilisation of amino-modified DNA using EDAC (without the above photo-activation technique). A comparison with such direct immobilisation provides a measure of the efficiency of the photo-immobilisation procedure introduced above.

To implement the above efficiency comparison, the yield of our photo-immobilisation strategy on polystyrene amino-coated beads as described in the previous section was compared with the DNA immobilisation yield on polystyrene carboxyl-coated beads of the same size (15 µm) but applying only a one-step

Table 3. Yields for photo-immobilisation of DNA to silica paramagnetic beads

Conditions	1. UV irradiation	2. Dark	3. No DAPpNP	4. No EDAC nor DAPpNP	5. No EDAC
DNA concentration in pmol/mg beads	460 ± 75	76 ± 12	81 ± 13	77 ± 12	81 ± 13

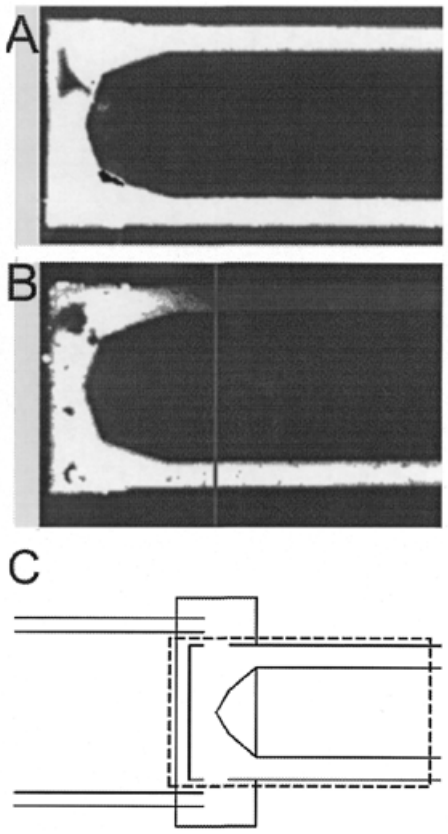

Figure 3. Light-dependent DNA immobilisation to beads in a micro-flow reactor. (**A**) White light image. Both inlet channels of a micro-reactor are filled with beads. (**B**) Fluorescence image. A fluorescent signal from the beads incorporated in a micro-flow reactor. The greyed column indicates the area profiled in Figure 4 and shows the line of segregation between the irradiated part (on the left-hand side) and non-irradiated part (on the right-hand side) of the inlet channel on the top of the picture. (**C**) Micro-flow reactor scheme. The two inlet channels (on the right-hand side of the scheme), two outlet channels (on the left-hand side of the scheme) and the bead barrier are shown. The dashed square shows the part of the micro-flow reactor presented in (A) and (B).

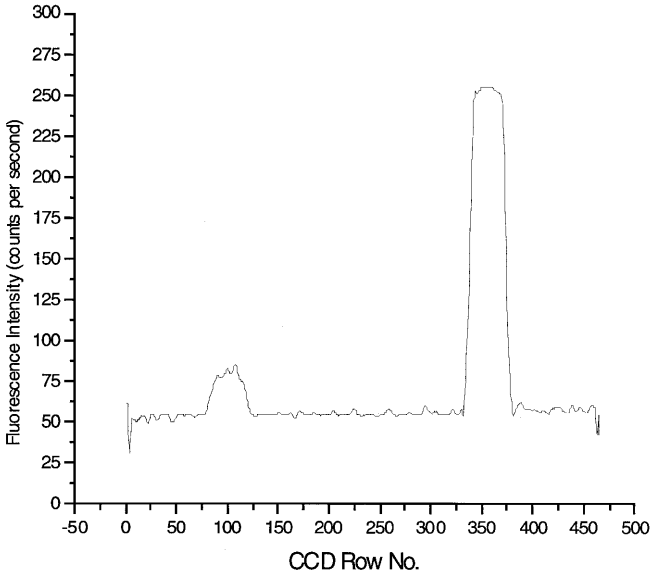

Figure 4. Quantification of the fluorescent signal of spatially defined light-directed DNA immobilisation to beads in a micro-flow reactor. An intensity section of Figure 3B is shown. Between rows 75 and 125: the signal from the beads in the channel not exposed to radiation with an intensity of 80 counts per second (c.p.s.). Between row 325 and 375: the signal from beads in the channel exposed to radiation with intensity >250 c.p.s. The background signal is ~50 c.p.s.

reaction with 5′ amino-coated 35 nt DNA oligomer in the presence of EDAC.

As can be seen from Table 4, the immobilisation yield of the photo-immobilisation procedure (210 pmol/mg beads) is even higher than the immobilisation yield from the well-known EDAC synthesis (150 pmol/mg beads). This is due to less non-specific immobilisation on carboxyl-coated beads than on amino-coated ones.

Hybridisation analysis

Hybridisation analyses were made in order to estimate the efficiency of the applied immobilisation methods on carboxyl- and amino-coated polystyrene and silica beads. A 35 nt DNA oligomer (see Table 2, oligomer 3) was immobilised on carboxyl-coated polystyrene beads as described in Materials and Methods. Immobilised DNA on 2.5 mg beads was hybridised with the complementary 5′ rhodamine-labelled 27 nt oligomer (see Table 2, oligomer 4) and measured by fluorescence as described in Materials and Methods. As one can see in Figure 2, the hybridisation yield on amino-coated silica beads after applying the photo-dependent immobilisation is ~25% less in comparison with the hybridisation yield on carboxyl-coated silica beads after direct immobilisation of 5′ amino-modified DNA by EDAC. At the same time, the background signal is almost the same (with the exception of amino-coated polystyrene beads after applying the photo-dependent immobilisation) despite the fact that silica beads showed more non-specific DNA attachment. This could imply that the non-specifically attached DNA is not accessible to hybridisation.

CONCLUSIONS

We have shown that the hetero-bifunctional photo-reactive cross-linking agent DAPpNP can be employed for light-directed immobilisation of DNA via a photo-induced formation of a carboxyl group and final immobilisation of 5′- or 3′-end

Table 4. Comparison of suitability of different super-paramagnetic beads for specific immobilisation of DNA

Bead type	DNA immobilisation (pmol DNA/mg beads)	Non-specific DNA attachment (pmol DNA/mg beads)	Non-specific DNA immobilisation (%)
Carboxyl polystyrene-co-maleic	170 ± 20	15 ± 3	9
Amino polystyrene-co-maleic	210 ± 40	50 ± 9	24

amino-modified synthetic DNA in the presence of EDAC. This photo-dependent DNA immobilisation strategy for light-directed attachment of DNA oligomers to paramagnetic beads may be applied to parallel investigations of DNA hybridisation in micro-flow reactors (J.S.McCaskill, submitted). Beads placed in different positions in micro-flow reactors may be selectively addressed (i.e. loaded with oligomers by photo-dependent DNA immobilisation) using a photo-lithographic mask and analysed individually in parallel. We expect the technique to further extend our ability to perform parallel *in vitro* analyses with DNA.

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REFERENCES

1. Gray,N.S., Wodicka,L., Thunnissen,A.W., Norman,T.C., Kwon,S., Espinoza,F.H., Morgan,D.O., Barnes,G., LeClerc,S., Meijer,L., Kim S., Lockhart,D. and Schultz,P. (1998) *Science*, **281**, 533–537.
2. Lockhart,D.J., Dong,H., Byrne,M.M., Follettie,M.T., Gallo,M.V., Chee,M.S., Mittmann,M., Wang,C., Kobayashi,M., Horton,H. and Brown,E.L. (1996) *Nat. Biotechnol.*, **14**, 1675–1680.
3. Cargill,M., Altshuler,D., Ireland,J., Sklar,P., Ardle,K., Patil,N., Lane,C.R., Lim,E.P., Kalyanaraman,N., Nemesh,J. *et al*. (1999) *Nature Genet.*, **22**, 231–238.
4. Wang,D.G., Fan,J.B., Siao,C., Berno,A., Young,P., Sapolsky,R., Ghanour,G., Perkins,N., Winchester,E., Spencer,J., Kruglyak,L. *et al*. (1998) *Science*, **280**, 1077–1082.
5. Hacia,J.G., Sun,B., Hunt,N., Edgemon,K., Mosbrook,D., Robbins,C., Fodor,S.P.A., Tagle,D. and Collins,F.S. (1998) *Genome Res.*, **8**, 1245–1258.
6. Pirrrung,M.C., Fallon,L. and McGall,G. (1998) *J. Org. Chem.*, **63**, 241–246.
7. Beier,M. and Hoheisel,J.D. (1999) *Nucl. Nucl.*, **18**, 1301–1304.
8. McGall,G.H., Barone,A.D., Diggelmann,M., Fodor,S.P.A., Gentalen,E. and Ngo,N. (1997) *J. Am. Chem. Soc.*, **119**, 5081–5090.
9. Kalachikov,S.M., Adarichev,V.A. and Dymshits,G.M. (1992) *Bioorg. Khim.*, **18**, 52–62.
10. Adleman,L.M. (1994) *Science*, **266**, 1021–1024.
11. Lund,V., Schmid,R., Richwood,D. and Hornes,E. (1988) *Nucleic Acids Res.*, **16**, 10861–10880.
12. Chrisey,L.A., Lee,G.U. and O´Ferrall,C.E. (1996) *Nucleic Acids Res.*, **24**, 3031–3039.
13. Tate,J.J., Persinger,J. and Bartholomew,B. (1998) *Nucleic Acids Res.*, **26**, 1421–1426.
14. Harrison,J.K., Lawton,R.G. and Gnegy,M.E. (1989) *Biochemistry*, **28**, 6023–6027.
15. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
16. McCaskill,J., Penchovsky,R., Gohlke,M., Ackermann,J. and Ruecker,T. (2000) *DNA6 Sixth International Meeting on DNA Based Computers—Preliminary Proceeding*. Leiden Center for Natural Computing, The Netherlands, pp. 239–246.
17. Zeller,K., Meier,H., Kohlshorn,H. and Mueller,E. (1972) *Chem. Ber.*, **105**, 1875–1886.
18. Goodfellow,V.S., Settineri,M. and Lawton,R.G. (1989) *Biochemistry*, **28**, 6346–6360.
19. Dermar,J.C., Disher,R.M. and Wensel,T.G. (1992) *FASEB J.*, **6**, A81–A81.
20. Friedman,A.L., Ismagilowa,G.S., Salesov,V.S. and Nivikov,S.S., (1972) *Uspechi Chimii*, **41**, 722–757.