HIGH ORDER NUCLEIC ACID BASED STRUCTURES

The present invention relates to nucleic acid based molecular structures that bind to other molecular entities, especially entities other than nucleic acids themselves. In particular, the invention relates to nucleic acid based molecular structures with pharmaceutical activity through binding to specific molecular targets and thereby influencing disease states. The invention also relates to nucleic acid based molecular structures with diagnostic utility.

There is a great desire to provide compositions of matter that are able to specifically alter the activity of particular proteins or modulate the expression of particular gene products. In particular there is a desire for molecules able to form specific binding interactions with other molecules and especially for such molecules to exhibit specific binding within the *in vivo* milieu.

Against these desires, methodologies have been developed to enable the creation of libraries of classes of molecules from which to select such compositions of matter. A pertinent example is the exquisite specificity of binding found in the antibody molecule and several significant technologies now exist for the development of monoclonal antibodies and recombinant derivatives thereof. These have provided a number of therapeutic drugs and many diagnostic and research tools. All such products are protein molecules and as such can only be produced using biological systems. Alternative wholly synthetic binding molecules have been produced. These are molecules selected from a diverse library of similar or variant molecules of the same chemical class, and in general selected using a screening system providing a surrogate of the desired therapeutic target or some aspect of its activity. The screening of vast chemical libraries of small molecules has been a classical route to the development of conventional small molecule pharmaceuticals but libraries of synthetic peptides and synthetic nucleic acid molecules are now also screened for potentially useful therapeutics.

For libraries of nucleic acids, the technical approach has in general been the use of single-stranded RNA or DNA molecules of defined unit length. The basis of

binding to a target molecule is not pre-configured and may be dependent on secondary structure formation within the DNA (or RNA) molecule itself facilitating binding to the other molecular entity (Bock L.C. et al 1992 *Nature* 355: 564-566; Kubrik, M.F. et al 1994 *Nucleic Acids Res.* 22: 2619-2626). The creation of nucleic acid molecules containing pre-configured tracts of secondary (or high order) structure for therapeutic and diagnostic utility has not been previously attempted, and is the object of the present invention.

The present invention relates to novel high order nucleic acid structures and to novel uses of such structures.

Several types of high order nucleic acid structures are known in the prior art. One type of such nucleic acids are termed aptamers and differ from the molecules of the present invention in respect of their size, manufacture and topological complexity. Methods involving either *in vitro* evolution or selection from vast random library pools have been applied to the development of both RNA and DNA aptamers. RNA molecules capable of facilitating enzymatic process such as polynucleotide kinase activity have been evolved by iterative cycles of selection (Lorsch J.R. & Szostak J.W. 1994 *Nature* 371: 31-36), and short single stranded DNA molecules have been selected capable of highly specific inhibition of human phospholipase A_2 (Bennett C.F et al 1994 *Nucleic Acids Res.* 22: 3202-3209). Others have independently developed either RNA or DNA based aptamers capable of binding and inhibiting some of the functions of human thrombin (Bock L.C. et al 1992 *Nature* 355: 564-566; Kubrik, M.F. et al 1994 *Nucleic Acids Res.* 22: 2619-2626).

Other types of high order nucleic acid structures include “branched-DNA” (Horn, T. & Urdea, M. S. 1989, Nucleic-Acids-Res. 17: 6959-6967) whereby one or more regions of a DNA molecule ("probe") which hybridizes to a complememtary nucleic acid molecule can themselves be subjected to hybridization to other DNA molecules in order to amplify the amount of DNA associated with the DNA probe. However, the complexes described by Horn and Urdea (*ibid*) are linearly extended whereby newly hybridized nucleic acid molecules are not designed to hybridise to molecules previously annealed but to other incoming new molecules

to form branched-DNA structures. Indeed, such complexes are simply designed to form as many branches as possible in order to provide more points for annealing of a signaling nucleic acid probe.

Other geometric structures afforded by the base-pairing properties of nucleic acids have been exploited in the fields of material science and nanotechnology (Aliviatos, A.P. et al 1996, Nature 382:609-611; Mao C. et al 2000, *Nature* 407: 493-496; Yurke, B. et al 2000, *Nature* 406: 605-608). Elegant technical methods for the manufacture and analysis of high order nucleic acid based structures including quadrilaterals, cubes octahedra and triangular motifs multiply connected into a lattice have been described (Chen J. et al 1989, *J. Am.Chem. Soc.* 111: 6402-6407; Zhang et al 1994, *J. Am. Chem. Soc.* 116:1661-1669; US. Pat. No 5,278051; US. Pat. No 5,468,851; US. Pat. No 5,386,020 & US. Pat. No 6,072,044). The geometric objects are closed structures fabricated using iterative processes involving restriction enzymes and DNA ligation. Nodal points in the figures may be fixed by cross-over junction between stands to give a rigid form, or more flexible branches achieved by cross-annealing disparate stands. The prior art does not include geometric structures which are not closed (i.e. ends ligated). The prior art does not include geometric structures which include regions of modified nucleic acids and does not include geometric nucleic acid structures conjugated to other molecular entities. The prior art does not include the use of libraries of randomised or semi-randomised nucleic acid structures.

In relation to novel uses of high order nucleic acid structures, it is recognised that exploitation of "low order" nucleic acids as therapeutic and diagnostic molecules *per se* is known in the art. In particular there are many examples of nucleic acid molecules with potential and or actual therapeutic activity. These operate either as antisense molecules, triplex reagents or as RNA molecules with endoribonuclease activity ("ribozymes"). In all of these guises, the modality of the therapeutic nucleic acid is as a modulator of protein expression by a mechanism of action that reduces or blocks protein translation. The specificity of target binding in all of these cases is nucleic acid to nucleic acid. These features (translation modulation, nucleic acid to nucleic acid binding) are in contrast to the modality of the present invention. A distinctive and inventive feature of the

present invention is the use of a high-order nucleic acid structure with binding activity to a target molecule.

Other "low order" nucleic acid structures, especially Aptamers, have been tested as therapeutic and diagnostic molecules. Certain other nucleic acid molecules, especially ribozymes, have been identified as having enzymatic activities with potential pharmaceutical importance. For closed geometric structures, pharmaceutical utility has not been considered although US. Pat No 5,278,051 speculates possible utility as solubilising agents or controlled release vehicles for small molecule therapeutics.

A first aspect of the present invention relates to novel high order nucleic acid based structures, particularly open geometric structures. Furthermore, the invention also relates to the utility of such structures as pharmaceutical and/or diagnostic agents. The invention also relates to high order nucleic acid based structures including nucleotides with modifications. The invention also relates to high order nucleic acid based structures including regions of randomised or semi-randomised nucleotides. The invention also relates to high order nucleic acid based structures conjugated to other molecular entities such as proteins.

Structures of the present invention exploit the Watson-Crick base pairing rules in engineering regions of double stranded structure. Single-stranded nucleic acid molecules have the ability to anneal (hybridise) to other single-stranded molecules by virtue of complementarity between the bases. Whilst such base annealing of two single-stranded molecules usually leads to a linear double-stranded molecule, other structures can be produced for example hairpin loops where one molecule has internal base-pair complementarity and circles where both ends of each single-stranded molecule have mutual complementarity. With strategic design of single-stranded nucleic acid sequences, individual molecules can be designed which can simultaneously anneal to two or more other molecules and if, in turn, these other molecules can also anneal to further molecules including molecules already involved in annealing, then complexes of nucleic acids can be formed.

The overall dimensions and topology of the double stranded DNA molecule are well understood. Double stranded DNA is quite flexible and the helix is able to adopt a number of conformations differing in the angle of rotation between adjacent base pairs along the helix. Naturally occurring single stranded nucleic acid molecules such as RNA adopt preferred conformations in solution. The conformation is dictated by base-pairing interactions within the same molecule leading to the production of a stabilised structure composed of double stranded stems and single stranded loops. The molecules will adopt the conformation of lowest energy and this structure for a known sequence of RNA is capable of prediction by computational approaches (Jaeger J.A. et al 1989 Proc.Natl.Acad. Sci USA 86: 7706-7710). Attempts have been made to produce predictive software for DNA folding and have shown some success (Nielsen D.A. et al 1995 Nucleic Acids Res. 23: 2287-2291). Dimensional comparison between nucleic acid and protein molecules illustrates the very significant difference in structural arrangement between these two classes of molecule, but also support the concept underlying the present invention. A typical globular protein such as myoglobin with molecular weight 17kDa has a size in its longest dimension of 3nm. A larger globular protein such a bovine serum albumin with molecular weight 68kDa is 5nm in its longest dimension (Cohen C., in Wolstenholme G.E.W. & O'Connor M. (eds), Ciba Foundation Symposium, London, J & A Churchill, 1966). The diameter of the double stranded helix is in itself 2nm and DNA strands of a small number of base pairs such as 100, would achieve a contour length approaching 30nm. Thus although the density of DNA or any other nucleic acid molecule is much less than a typical protein, the topology the DNA molecule, even in its most structured native form as a double helix, could readily cover large parts of the exposed surface of almost any protein molecule. In particular if the topology of the usually monofilament DNA were so altered, the DNA could occupy a large area of space in a manner more akin to a much higher density protein molecule. It can be recognised that assembly of nucleic acid structures composed of multiple interconnected strands each of only short (<50) nucleotide tracts can readily result in structures with overall dimensions in the range 10-500 nm. It is a particular objective of the present invention to provide for such a formulation of nucleic acid molecule.

Structures of the present invention are based on the creation of DNA or RNA molecules with secondary structures formed as a result of the interaction of two or more molecules of nucleic acid or, alternatively, as a result of interaction of different defined segments within individual molecules of nucleic acid. In the present invention, the informational content of DNA or RNA is exploited not as a coding entity for expression of a therapeutic protein, nor as a blocking entity for nucleic acid metabolism and gene expression (anti-sense) but to direct assembly of a molecular structure of particular shape in three-dimensions.

The present invention includes nucleic acid molecules, particularly synthetic DNA molecules, which form three-dimensional (non-planar) molecular structures by specific base pairing within the molecules in the set. Specifically, the DNA molecules are designed to have 1 or more regions of sequence ("domains") that can anneal to other molecules in the set ultimately to form a composite three-dimensional nucleic acid structure. Under this scheme, an approximate cuboid structure can be formed by the self-annealing of 6 synthetic DNA molecules each containing 4 domains of complimentarity whereby each molecule interacts with 4 other molecules and whereby each molecule acts effectively like an individual side of a 6-faced cube. The structure is open (not covalently closed), flexible and in particular further embodiments amenable to modification by the addition of other functional or structural groups.

In one high order nucleic acid based structure of the present invention, there are provided nucleic acid molecules each comprising 2 or more domains of self-complementary sequence enabling the nucleic acid molecule to fold upon itself and to interact with each other to form a particular three-dimensional molecular structure via specific base-pairing events. For certain applications, it is recognised that chemical instability of unmodified DNA molecules has been a significant problem for uses such as therapeutic. Several approaches are now available for protecting DNA molecules from degradation by enzymatic attack. These generally include the use of modified phosphodiester backbones (methylphosphonate, phosphorothioate, peptide nucleic acids) or capping 5' and or 3' termini using phosphoramedite, phosphorothioate or phosphorodithioate linkages. It is a particular objective of the present invention to exploit modified or

non-natural nucleic acids in the high order nucleic acid based structures. Moreover a particularly desired feature is the increased flexibility in binding specificity achieved by use of mixed chemistry and alternative non-natural nucleic acid backbones.

The nucleic acid sub-units of a high order nucleic acid structure of the present invention may be homotypic or heterologous in nature, for example DNA containing tracts of RNA. It is known that tracts of RNA within a DNA helix alter the coiling in solution (Wang, A. et al, 1982 Nature, 299: 601-04). The ability to offer conformational diversity within a localised tract of nucleic acid may be significant in altering binding specificity to the target protein, and this phenomenon is known in the art where the binding specificity a thrombin aptamer was dependent on a short tract of highly ordered tertiary structure (Griffin, L. et al 1993 Gene 137:25-31). Additionally, non-natural phosphate backbone analogues may be exploited to enhance stability and also alter the binding specificity to the desired target protein. Latham et al (Latham, J.A. et al 1994 Nucleic-Acids-Res. 22: 2817-22) provide an example whereby the modified nucleotide, 5-(1-pentynyl)-2'-deoxyuridine was used in place of thymidine in a pool of random oligonucleotides. The present invention includes molecules composed of tracts of single stranded nucleic acid, interspersed with tracts of double stranded structure, and other chimeric molecules synthesised to contain different chemical sub-structure but joined exploiting conventional base-pairing rules. Such structures may also combine molecules of DNA and RNA.

Higher order molecular structures of the present invention are assembled from individual or multiple nucleic acid molecules according to any scheme present in the art and may include synthetic nucleic acid species or fragments from much larger molecules such as recombinant plasmids. The structures may be built following self-folding (auto-assembly) or facilitated folding of a single linear molecule of DNA. Facilitated folding may be mediated by proteinacious entities (enzymes such as ligase, topoisomerase, endonuclease, polymerase) or via interaction with non-protein physiochemical conditions (pH, temperature, ionic conditions). Alternatively and or in combination with the above, the molecule may be assembled by interaction with molecules bound to a solid matrix, or whist the

DNA undergoing folding into a higher order structure is tethered or anchored in space during all or part of the assembly process.

A second aspect of the present invention is the provision of libraries of nucleic acid molecules formed to contain a range of semi-random molecules some of which may possess a desired topology capable of interacting in a specific manner with a target molecule. Included in this aspect of the invention are libraries of nucleic acid molecules featuring a guide framework to facilitate assembly of a common structural sub-unit. Within each sub-unit a randomised tract of sequence is incorporated maximising library diversity and potential functional utility with respect to activity in a selective binding assay. In the second aspect of the present invention, an embodiment whereby a library formed from mixtures of *n* separate populations (sets) of synthetic DNA molecules (sub-units) is exploited. In this aspect, the population size of the synthetic nucleic acid sub-units is large and dictated by the degree of randomisation present within a variable segment of the sub-unit. Further sub-unit diversity is inbuilt in other embodiments by variation of the positioning of the variable domain, variation in the number of variable domains (by interspersion with tracts of fixed sequence) and variation in the length of any given variable domain. It is preferred that *n* separate population of sub-unit are mixed in a single cycle of annealing to create a library of multiple nucleic acid structures and individual sequence diversity. It will be obvious that other embodiments may include multiple cycles of annealing and multiple values of the whole integer number *n*.. A particular feature of the library under this scheme is the ability to modulate the degree of complexity of inter-subunit interaction by judicious design and placement of the complementary or guide sequence tract.

A third aspect of the present invention is the novel utility of high order nucleic acid based structures, especially for pharmaceutical and diagnostic use. In this aspect, these structures are capable of binding to a specific target molecule, commonly a protein or proteinaceous target molecule. Where the preferred embodiment encompasses a single protein target, further embodiments are envisaged whereby the target is a protein complex comprised of multiple protein sub-units such as a cell surface receptor, collectively bound by a molecule of the

first aspect of the invention. Other embodiments of the third aspect include the binding to a cellular target or cell species identified by an ability to bind a molecule of the first aspect. Further embodiments include binding to a target or target complex containing non-protein components for example carbohydrate or lipid components of the cell and in particular of the cell surface. Protein, carbohydrate and lipid entities and or complexes thereof may be disease specific entities or present as normal components of a tissue or cell. The target or target complex would include viral particles or viral derived components such as capsid proteins or host derived components of the viral coat. The target or target complexes may include metallic ions or other inorganic chemicals or chemical groups in their composition and may be naturally occurring or introduced by treatments with exogenous agents. Target receptors may include those such as the IL-2 receptor or other cytokine receptors such as receptors for IL-3, M-CSF, GM-CSF and numerous others. Equally, surface molecules such as the IgE receptor whereby blockade of IgE binding together with blockade of a cross-linking activation event at the receptor would be a highly desired outcome. Other surface molecules including members of the cluster differentiation (CD antigens) series are desired targets for disease modulation and in particular in respect of diseases of auto-immune component.

The invention is designed to have particular widespread application in the field of therapeutic molecules. Molecular structures of the invention are desired to agonise or antagonise particular receptors or enzymatic processes for therapeutic benefit whilst contributing none of the disadvantages of conventional protein therapeutics such as immunogenicity. The invention therefore extends to a method for treating or preventing a disease or condition, the method comprising administering to a subject an effective amount of the molecular structure. The invention also extends to the use of such structures in *in vivo* and *in vitro* diagnosis.

A fourth aspect of the present invention comprises high order nucleic acid based structures with modified nucleotides included in the structures. Separate from or in addition to the diversity imposed by the above second aspect, it is particularly desired to impose diversity by the derivitisation of the sub-units of the library and

or by the inclusion of modified bases during their synthesis (thiolated bases, biotinylated bases, epsilon-amino derivatised bases etc.). Thus, a highly diverse library with diversity at the both the level of sequence composition and sequence length is obtained. Such parameters can be fixed within defined limits for different libraries and different target applications. The high order nucleic acid structures will also contain modified nucleotides capable of conferring particular desired properties to the structure additional to features providing stability or binding modulation as above. Such additional desired modifications may be embodied under the first or second aspects of the invention and include the use of hydrophobic tracts, the inclusion of psoralen or acridine groups, linking haptenic group such as biotin or linking to different charged side chains such as amino groups or carboxyl groups to provide facilitated binding to a particular target molecule. In a further preferred embodiment, such groups may act as points for attachment of other molecules such as further nucleic acid molecules or proteins such as an antibody or an enzyme.

A desired feature of molecules of the invention will be high stability *in vitro* and *in vivo*. The chemical composition of the nucleic acid structures is highly influential but also the physical size of the molecule requires control to minimise shear damage in solution and maximise functional utility *in vivo*. For this reason, the preference of the invention is for multi-chain nucleic acid structures constructed from generally small (<80mer) sub-units. Alternatively, the exploitation of a structure composed of larger sub-units (>80mer) may be desired and equally fall into the scope of the present invention.

A fifth aspect of the present invention comprises high order nucleic acid based structures attached to other molecular entities. In particular, this aspect includes nucleic acids attached at one or more specific sites to one or more specific sites on the other molecular entity whereby specific attachment to the nucleic acid is facilitated by modified nucleotides as in the fourth aspect of the invention. In particular, this aspect comprises high order nucleic acid based structures attached to pharmaceutically or diagnostically relevant molecular entities whereby the nucleic acid binds to specific molecular targets relating to disease and the attached molecular entity is then used to combat or detect the disease.

Pharmaceutically relevant entities will include cytokines, Fc portions of antibodies, other antibody-related entities, toxins, enzymes, drugs and pro-drugs, receptor agonists or antagonists, receptor molecules themselves (especially ligand binding domains), radioisotopes, pharmaceutically active nucleic acids, drug transport vesicles such as liposomes, live or attenuated microorganisms, light activatable moieties, and other molecular entities which induce a vaccination effect. Diagnostically relevant entities will particularly include radioisotopes, light activatable moieties such as those producing a chemiluminescent signal, fluorochromes, enzymes, and signal transport vesicles such as beads.

To sum up, the invention comprises the following objects:

- A three-dimensional poly-nucleic acid structure, composed of multiple interconnected strands of nucleic acid molecules or segments thereof by specific base pairing interaction of two or more molecules, characterized in that the structure is not covalently closed.
- A corresponding poly-nucleic acid structure, characterized in that the structure is formed by two or more nucleic acid molecule strands.
- A corresponding poly-nucleic acid structure, characterized in that the structure is formed by three or more nucleic acid molecule strands.
- A corresponding poly-nucleic acid structure, characterized in that said structure is a cube or has essentially the form of a cube.
- A corresponding poly-nucleic acid structure, wherein the cuboid structure is formed by six nucleic acid molecule strands, wherein each molecule strand acts like an individual side of the 6-faced cube.
- A corresponding poly-nucleic acid structure, wherein each nucleic sequence comprises two or more domains that can anneal to other molecules in the set.
- A corresponding poly-nucleic acid structure, wherein each nucleic sequence comprises four domains.
- A corresponding poly-nucleic acid structure, wherein said structure includes nucleic acid molecules which are composed of tracts of single stranded nucleic acid, interspersed with tracts of double stranded structure.
- A corresponding poly-nucleic acid structure according to any of the claims 1 – 8, wherein each nucleic acid strand has less than 80, preferably less than 50, nucleotides.

- A corresponding poly-nucleic acid structure, wherein said structure has the assembly (A1+B1+C1)+(A2+B2+C2) as depicted in Figure 1.
- A poly-nucleic acid structure as defined above, wherein said structure contains sub-units which are composed of a variable randomized sequence tracts in order to get semi-random molecules or segments thereof capable of interacting with a target molecule.
- A three-dimensional poly-nucleic acid structure containing sub-units which are composed of multiple interconnected strands of nucleic acid molecules or segments thereof by specific base pairing interaction of two or more molecules, wherein said structure is covalently closed and contains sub-units which are composed of a variable randomized sequence tracts in order to get semi-random molecules or segments thereof capable of interacting with a target molecule.
- A poly-nucleic acid structure as defined above, wherein the sequence composition and sequence length is variable.
- A corresponding poly-nucleic acid structure, wherein the variable sequence composition is achieved by a one or more modifications of nucleotides within the sequence.
- A poly-nucleic acid structure as defined above, wherein said structure contains sites or groups of nucleic acids which can bind or attach to another molecule or a solid matrix.
- A corresponding poly-nucleic acid structure, wherein said other molecule is a protein, an enzyme, a lipoprotein, a glycosylated protein, an immunglobuline or a fragment thereof.
- A corresponding poly-nucleic acid structure, wherein said other molecule is a nucleic acid.
- A corresponding poly-nucleic acid structure, wherein said molecule is pharmaceutically effective.
- A pharmaceutical composition comprising a poly-nucleic acid structure as defined above and in the claims optionally together with suitable carriers, excipients and diluents and / or other pharmaceutically effective compounds.
- Use of a corresponding poly-nucleic acid structure as diagnostic agent.

- Use of a poly-nucleic acid structure as defined above for the provision of a library for affecting a diversity of specifically randomized topologies in order to obtain different functionalities and / or activities.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1

Depiction of the step-wise assembly of an open cuboid nucleic acid structure from six individual single stranded molecules given as A_1, A_2, B_1, B_2, C_1 and C_2. Assembly proceeds via conventional antiparallel base pairing between nucleic acid molecules. Dimeric intermediates formed between molecules B_1 and C_1, also B_2 and C_2 are shown. Trimeric structures formed between molecules A_1, B_1 and C_1 also A_2, B_2 and C_2 are shown. Assembly of the cuboid structure is achieved by association of the two trimeric moieties and is depicted as molecule $(A_1+B_1+C_1)+(A_2+B_2+C_2)$.

FIGURE 2

Sequence of oligonucleotide sub-units IL2R-1 and IL2R-2 comprising DNA structure with binding activity to IL-2 receptor.

FIGURE 3

Sequence of oligonucleotide sub-units TB-R1 and TB-R2 comprising DNA structure with binding activity to human thrombin.

EXAMPLES

The invention is illustrated by the following examples which should not be considered to be limiting in scope.

EXAMPLE 1

Method for inhibition of an IL2-dependant cell line using a DNA structure selected from a DNA structure library.

Two libraries of synthetic DNA molecules, each of comprising a region of randomised sequence were synthesised.

Library A comprised molecules of structure:

5'AGTCCCAAGCTGGCT$(N)_{13}$CTCCATCGTGAAGTCAGCCAGCTTTGGACT

Library B comprised molecules of structure:

5'GACTTCACGATGGAGGTCAGAATGTGAATA$(N)_{10}$TATTCACATTCTGAC

These sequences were designed to facilitate cross-annealing and represent sub-units of a structure library formed by mixing and cross-annealing of different sub-units according to the scheme of the present invention.

Oligonucleotide (sub-unit) libraries were synthesised with phosphorothioate linkages to maximise stability in the presence of serum factors and purified by HPLC. Purified oligonucleotides were obtained from GenoSys Biotechnologies (Cambridge, UK). A DNA structure library was assembled using a single cycle of cross-annealing. Sub-unit libraries A and B were denatured, mixed and annealed at a temperature of 37°C in a solution of 50mM Tris pH 7.4, 100mM NaCl, 5mM EDTA. Mixing of sub-unit libraries A and B was conducted at equimolar concentration (1□M). In other experiments mixing was conducted using different molar ratios. Assembly of the subunits was verified by gel electrophoresis.

The DNA structure library was screened for structures able to bind the extra cellular domain of the IL-2 receptor (IL2R). This was conducted using soluble recombinant IL2R prepared according to published methods (Meidel, M.C. et al 1988 *Biochem. Biophys. Res. Commun.* 154: 372-379; Meidel, M. C. et al 1989, *J. Biol. Chem.* 264: 21097-21105). Recombinant IL2R was covalently bound to surface activated magnetic beads using protocols recommended by the supplier (Bangs Labs, Fishers, IN, USA). The IL2R-beads were used as an affinity surface to select binding structures from the DNA structure library. IL2R-beads were reacted with the library under a number of experimental conditions including

the presence of chaotrophic salts in control reactions. The library (DNA) concentration was approximately 100nmol in annealing solution as above. Binding molecules were recovered by polymerase chain reaction (PCR) directly from the beads following extensive washing cycles with a solution of 75mM Tris.HCL, 200mM NaCl, 0.5% N-octylglucoside pH8.0. The PCR was conducted using the primer PRA1 (5'-AGTCCCAAGCTGGCT) to recover the library A component using standard reagent systems and conditions. In separate reactions, primers PRB1 (5'GACTTCACGATGGAG) and PRB2 (5'GTCAGAATGTGAATA) were used to recover the library B component. The PCR products were cloned and sequenced using standard reagent systems and procedures.

A number of sequences were recovered and identified as originating from sub-unit library A and sub-unit library B. Of these one pair was synthesised using phosphorothioate chemistry as before. Oligonucleotides IL2-R1 and IL2-R2 (sequences provided in Figure 2) were purified and assembled as previously, and used in a cellular assay for IL-2 antagonism.

TALL-104 (ATCC# CRL-11386) is a human T-cell leukaemia cell line. The cells grow in suspension culture and require IL-2 for optimal growth. The cells may be grown for short period without IL-2 but their growth is significantly reduced. Cells were grown in Iscoves modified Dulbeccos medium (Life Technologies, Paisley, UK) with 50-100u/ml recombinant human IL-2 (Life Technologies, Paisley, UK) and supplemented with 10% (v/v) heat inactivated foetal calf serum. Cells were cultured in an atmosphere of 8-10% CO_2. Dilutions of the annealed IL2-R1/IL2-R2 DNA preparation and a control DNA sample containing random sequence of identical contour length were prepared in culture medium containing IL-2. A parallel dilution series was prepared using medium lacking IL-2. The dilution series ranged from 50□M DNA to 390nM DNA. Assays were performed using sub-confluent TALL-104 cells plated the preceding day in 96 well micro-titre dishes. Cells were collected by centrifugation, washed with pre-warmed (37°C) phosphate buffered saline and the DNA containing medium added for 48hours. Treatments were carried out in quadruplicate. Proliferation was assessed at the end of the 48hour period in a colourimetric assay using a commercially available

tetrazolium compound and following instructions provided by the supplier (Promega, Southampton, UK). Microtitre plates were read at 540nm.

The results showed that the annealed DNA preparation inhibited growth of the TALL-104 cell line under conditions where individual synthetic oligonucleotides IL2-R1 and IL2-R2 were inactive.

EXAMPLE 2

Method for selection of a DNA structure binding to human thrombin.

The library described in example 1 was used to select for a DNA structure able to bind to human thrombin. The library was screened using a human thrombin preparation (Sigma, Poole, UK) linked to surface activated magnetic beads as per example 1. Thrombin-beads were reacted with the DNA structure library as per example 1 except the post binding wash was conducted in a solution of 20mM Tris acetate, pH7.4, 140mM NaCl, 5mM KCl, 1mM $MgCl_2$. Binding molecules were recovered directly from the beads by PCR using reactions and primer sets as for example 1. The PCR products were cloned and sequenced using standard reagent systems and procedures.

A number of sequences were recovered and identified as originating from sub-unit library A and sub-unit library B. Of these, one pair was synthesised using phosphorothioate chemistry as before. Oligonucleotides TB-R1 and TB-R2 (sequences provided in Figure 3) were purified and assembled. The TB-R1/TB-R2 complex was used in a thrombin inhibition assay. Clotting time was measured using a fibrometer at 37°C and adult human plasma freshly prepared from a healthy donor. The extent of thrombin inhibition was determined using a thrombin standard curve plotting clotting time versus thrombin concentration. Clotting time was measured over three logs of DNA structure in the assay.

The results showed inhibition of clotting activity in the presence of the TB-R1/TB-R2 DNA complex.

EXAMPLE 3

Method for the selection of DNA structures binding to recombinant soluble CD4.

The library described in example 1 was used to select for a DNA structure able to bind to a recombinant soluble CD4 (rsCD4) preparation. The DNA structure library was screened using a CD4 preparation (BioDesign, Saco, ME, USA) immobilised to activated magnetic beads as previously. Library screening, washing and selection by PCR was as described for example 2. A single oligonucleotide pair originating from the A and the B sub-unit libraries was synthesised and assembled. The structure was used to inhibit binding of anti-CD4 monoclonal RPAT4 (Serotech, Abingdon, UK) in an enzyme linked immuno absorbant assay (ELISA).

96 well ELISA plates were coated overnight with a 0.2mg/ml solution of rsCD4 in coating buffer (0.05M carbonate-bicarbonate buffer pH9.0) at 4°C. Plates were washed extensively using TBS-T (tris-buffered saline pH8.0 .05% (v/v) Tween 20) and test and control DNA structures were diluted (1:2) across the plate in TBS from a starting concentration of 100□M. The plates were incubated for 40 minutes at 37°C and washed with TBS. A 100ng/ml preparation of antibody RPAT4 in PBS was added to the plate and incubated for 40 minutes at 37°C. Plates were washed and the bound RPAT4 detected using a alkaline phosphtase labelled sheep anti-mouse preparation (Sigma, Poole, UK) and Sigma Fast OPD (Sigma, Poole, UK) as a colour substrate. In some assays, the DNA was co-incubated with the RPAT4 monoclonal. Colour intensity was read using a plate reader and signal compared between test and control wells. The results showed significant inhibition of RPAT4 binding to rsCD4 in the presence of the DNA structure.

Patent Claims

1. A three-dimensional poly-nucleic acid structure, composed of multiple interconnected strands of nucleic acid molecules or segments thereof by specific base pairing interaction of two or more molecules, characterized in that the structure is not covalently closed.

2. A poly-nucleic acid structure according to claim 1, characterized in that the structure is formed by two or more nucleic acid molecule strands.

3. A poly-nucleic acid structure according to claim 2, characterized in that the structure is formed by three or more nucleic acid molecule strands.

4. A poly-nucleic acid structure according to any of the claims 1 – 3, characterized in that said structure is a cube or has essentially the form of a cube.

5. A poly-nucleic acid structure according to claim 3, wherein the cuboid structure is formed by six nucleic acid molecule strands, wherein each molecule strand acts like an individual side of the 6-faced cube.

6. A poly-nucleic acid structure according to any of the claims 1 – 5, wherein each nucleic sequence comprises two or more domains that can anneal to other molecules in the set.

7. A poly-nucleic acid structure according to claim 6, wherein each nucleic sequence comprises four domains.

8. A poly-nucleic acid structure according to any of the claims 1 – 7, wherein said structure includes nucleic acid molecules which are composed of tracts of single stranded nucleic acid, interspersed with tracts of double stranded structure.

9. A poly-nucleic acid structure according to any of the claims 1 – 8, wherein each nucleic acid strand has less than 80 nucleotides.

10. A poly-nucleic acid structure according to claim 9, wherein each nucleic acid strand has less than 50 nucleotides.

11. A poly-nucleic acid structure according to any of the claims 1 – 10, wherein said structure has the assembly (A1+B1+C1)+(A2+B2+C2) as depicted in Figure 1.

12. A poly-nucleic acid structure according to any of the claims 1 – 11, wherein said structure contains sub-units which are composed of a variable randomized sequence tracts in order to get semi-random molecules or segments thereof capable of interacting with a target molecule.

13. A three-dimensional poly-nucleic acid structure composed of multiple interconnected strands of nucleic acid molecules or segments thereof by specific base pairing interaction of two or more molecules, wherein said structure is covalently closed and contains sub-units which are composed of a variable randomized sequence tracts in order to get semi-random molecules or segments thereof capable of interacting with a target molecule.

14. A poly-nucleic acid structure according to any of the claims 1 – 13, wherein the sequence composition and sequence length is variable.

15. A poly-nucleic acid structure according to claim 14, wherein the variable sequence composition is achieved by a one or more modifications of nucleotides within the sequence.

16. A poly-nucleic acid structure according to any of the claims 1 – 15 , wherein said structure contains sites or groups of nucleic acids which can bind or attach to another molecule or a solid matrix.

17. A poly-nucleic acid structure according to claim 16, wherein said other molecule is a protein, an enzyme, a lipoprotein, a glycosylated protein, an immunglobuline or a fragment thereof.

18. A poly-nucleic acid structure according to claim 16, wherein said other molecule is a nucleic acid.

19. A poly-nucleic acid structure according to any of the claims 16 – 18, wherein said molecule is pharmaceutically effective.

20. A pharmaceutical composition comprising a poly-nucleic acid structure of claim 19 optionally together with suitable carriers, excipients and diluents and / or other pharmaceutically effective compounds.

21. Use of a poly-nucleic acid structure of claims 1 – 18 as diagnostic agent.

22. Use of a poly-nucleic acid structure of claim 12 or 13 for the provision of a library for affecting a diversity of specifically randomized topologies in order to obtain different functionalities and / or activities.