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(54) Title: MICROELECTRONIC MOLECULAR DESCRIPTOR ARRAY DEVICES, METHODS, PROCEDURES, AND FORMATS FOR COMBINATORIAL SELECTION OF INTERMOLECULAR LIGAND BINDING STRUCTURES AND FOR DRUG SCREENING

(57) Abstract: These inventions relate to microelectronic molecular descriptor array devices, methods, procedures, and formats for combinatorial selection of intermolecular ligand binding structures and for drug screening. More particularly, those devices and methods rapidly carry out higher order selectivity of combinatorially produced intermolecular ligand binding components, supramolecular structures and supramolecular complexes by application of unique stringency parameters. Preferably, the invention includes the formation of exponential libraries by aggregation of sublibraries through the influence of electronic stringency to influence formation or detection of supramolecular structures or complexes. In addition, this invention relates to microelectronic array devices, procedures, methods and formats for molecular recognition processes, new drug discovery, generation of new affinity reagents, generation of synthetic antibodies, and for immunoassays.

# DESCRIPTION

## Microelectronic Molecular Descriptor Array Devices, Methods, Procedures, And Formats For Combinatorial Selection Of Intermolecular Ligand Binding Structures And For Drug Screening

### Field of the Invention

The field of this invention relates to devices and methods for carrying out multi-step and multiplex affinity binding reactions in microscopic formats. In particular, those devices and methods rapidly carry out higher order selectivity of combinatorially produced intermolecular ligand binding components, supramolecular structures and supramolecular complexes by application of unique stringency parameters. In addition, this invention relates to microelectronic array devices, procedures, methods and formats for molecular recognition processes, new drug discovery, generation of new affinity reagents, generation of synthetic antibodies and for immunoassays.

### Related Applications

This application is related to Application Serial No. 08/968,065, entitled "Methods and Procedures for Molecular Biological Analysis and Diagnostics", filed December 5, 1997, Application Serial No. 08/855,058, entitled "Methods for Electronic Perturbation Analysis of Biological Materials", filed May 14, 1997, United States Patent No. 5,849,486, entitled "Apparatus and Methods for Active Programmable Matrix Devices", filed September 27, 1995, U.S. Patent No. 5,632,957, entitled "Molecular Biological Diagnostic Systems Including Electrodes", filed September 9, 1994, Application Serial No. 08/271,882, entitled "Methods for Electronic Stringency Control for Molecular Biological Analysis and Diagnostics", filed July 7, 1994, and U.S. Patent No. 5,665,662, entitled "Active Programmable Electronic Devices for Molecular Biological Analysis and Diagnostics", filed November 1, 1993. This application is also related to Application PCT/US99/03080, entitled "Advanced Active Devices and Methods for Molecular Biological Analysis and Diagnostics", filed February 11, 1999. This application is also related to WO 97/43232, entitled "Novel Substance Library and Supramolecular Complexes Produced Therewith", published November 20, 1997; WO 99/15539, entitled "Pentopyranosyl Nucleoside, and Production and Use of the Same", filed September 22, 1997; WO 99/15541, entitled "Pentopyranosyl Nucleoside for Producing an Electronic Component, and Conjugates of Said Pentopyranosyl Nucleoside", filed September 22, 1997; WO 99/15509, entitled "Cyclohexyl and Heterocyclyl Nucleoside Derivatives, Method for Producing These Derivatives, and the Use of the Derivatives and Their Oligomers or Conjugates in Pairing and/or Testing Systems, filed September 22, 1997;

WO 99/15542, entitled "Linker Nucleoside, and Production and Use of the Same", filed September 22, 1997; WO 99/15893, entitled "Addressable Modular Recognition System, Production Mode and Use", filed September 22, 1997; WO 98/25943, entitled "Non-Helical Supramolecular Nanosystems", filed December 11, 1996. The foregoing are hereby incorporated by reference as if fully set forth herein.

Background of the Invention

It is now possible using automated combinatorial synthetic processes to create large libraries of peptides, oligonucleotides, and other interesting combinations of organic and inorganic moieties. In the case of peptide libraries, these can be combined with unique pairing systems, which then form ligand binding component structures. These ligand binding component structures can be designed such that they can assemble into larger structures, "supramolecular structures", which have molecular recognition properties and the potential for forming selective and stable complexes with important biologically active molecules and structures. Methods for rapidly carrying out combinatorial selection of large numbers of these ligand binding structures could lead to considerable information concerning the nature of the ligand binding process, particularly for known drugs and biologically active compounds. If very large numbers of these complexes could be rapidly evaluated with known compounds, then processes for screening and evaluating new drugs and other important compounds might be developed (see Miculka, C., Hoppe, H.U., and Windhab, N., in European BioPharmaceutical Review, June 1998, pp. 52-57).

Supramolecular binding structures have been described by a number of workers in the field. Classes of supramolecular polymers with repeating units have been described by Lehn J.M., in J. Chem. Soc. Chem. Commun. 479, 1990. Polymeric tubular type supramolecular structures formed, form cyclic peptides has been described by Ghadiri, M. R., et al., in Nature 366, pp. 324-327, 1993. Two and three dimensional DNA structures have been designed and synthesized enzymatically by Zhang, Y., and Seeman, N.C., in J. Am. Chem. Soc., 116, pp. 1661-1669, 1994. Individual type supramolecular complexes composed of nucleic acid and peptide components have been described by Virtanen, J., and Virtanen, S., in PCT WO 96/13522, May 9, 1996. While methods for the synthesis of nucleic acid peptide structures are proposed, there is no mention of how complexes would be selected, particularly from large combinatorial libraries of components and structures. Other groups have been interested in self-assembling versions of model combinatorial systems or "Virtual Combinatorial Libraries" (see I. Huc and J.M. Lehn, in Proc. Nat Acad.Sci. USA, 94 pp. 2106-2110, 1997; and A. Brady and J.K.M. Sanders, J. Chem. Soc., Perkin Trans., 1, pp. 3237-3253, 1997).

While a number of synthetic techniques exist for preparing complicated supramolecular and other three dimensional structures, rapid and selective processes are needed to actually determine optimal structures for the many intended applications. Microarray technology could be of value for rapid evaluation and screening of large libraries of ligand binding components and for the selection of supramolecular structures and supramolecular complexes. DNA array technology might potentially be useful for selection of supramolecular structures, particularly those involving second order self-assembly via hybridization of the component structures. While many DNA array technologies are available and could be applied to this type of combinatorial selection processes, their limited stringency parameters may present problems with obtaining high level selectivity (see A. Marshall and J. Hodgson, Nature Biotechnology, Vol. 16, pp. 27-31, 1998, and G. Ramsay, Nature Biotechnology, Vol. 16, pp.40-44, 1998).

Many hybridization techniques involve carrying out numerous operations that are often complex and time consuming. Many hybridization techniques are also limited by a lack of sensitivity, specificity, or reproducibility. For example, problems with sensitivity and specificity have so far limited the applications of nucleic acid hybridization. Nucleic acid hybridization analysis generally involves the detection of very small numbers of specific target nucleic acids (DNA or RNA) with probes among a large amount of non-target nucleic acids. In order to keep high specificity, hybridization is normally carried out under the most stringent condition, achieved through a combination of temperature, salts, detergents, solvents, chaotropic agents, and denaturants. Multiple sample nucleic acid hybridization analysis has been conducted on a variety of filter and solid support formats (see G. A. Beltz et al., in Methods in Enzymology, Vol. 100, Part B, R. Wu, L. Grossmam, K. Moldave, Eds., Academic Press, New York, Chapter 19, pp. 266-308, 1985). One format, the so-called "dot blot" hybridization, involves the attachment of target DNAs to a filter, which are subsequently hybridized with a labeled probe(s). "Dot blot" hybridization gained wide-spread use, and many versions were developed (see M. L. M. Anderson and B. D. Young, in Nucleic Acid Hybridization - A Practical Approach, B. D. Hames and S. J. Higgins, Eds., IRL Press, Washington DC, Chapter 4, pp. 73-111, 1985). The "dot blot" hybridization has been developed for multiple analysis of genomic mutations (Dattagupta Nanibhushan and D. Rabin, in EPA 0228075, July 8, 1987) and for the detection of overlapping clones and the construction of genomic maps (G. A. Evans, in US Patent No. 5,219,726, June 15, 1993).

Another format, the so-called "sandwich" hybridization, involves attaching oligonucleotide probes covalently to a solid support and using them to capture and detect multiple nucleic acid targets. (M. Ranki et al., Gene, 21, pp. 77-85, 1983; A. M. Palva, T. M. Ranki, and H. E. Soderlund, in UK Patent Application GB 2156074A, October 2,

1985; T. M. Ranki and H. E. Soderlund in US Patent No. 4,563,419, January 7, 1986; A. D. B. Malcolm and J. A. Langdale, in PCT WO 86/03782, July 3, 1986; Y. Stabinsky, in US Patent No. 4,751,177, January 14, 1988; T. H. Adams et al., in PCT WO 90/01564, February 22, 1990; R. B. Wallace et al. 6 Nucleic Acid Res. 11, p. 3543, 1979; and B. J. Connor et al., 80 Proc. Natl. Acad. Sci. USA pp. 278-282, 1983).

Using the current nucleic acid hybridization formats and stringency control methods, it remains difficult to detect low copy number (i.e., 1-100,000) nucleic acid targets even with the most sensitive reporter groups (enzyme, fluorophores, radioisotopes, etc.) and associated detection systems (fluorometers, luminometers, photon counters, scintillation counters, etc.). This difficulty is caused by several underlying problems associated with direct probe hybridization. One problem relates to the stringency control of hybridization reactions. Hybridization reactions are usually carried out under stringent conditions in order to achieve hybridization specificity. Methods of stringency control involve primarily the optimization of temperature, ionic strength, and denaturants in hybridization and subsequent washing procedures. Unfortunately, the application of these stringency conditions causes a significant decrease in the number of hybridized probe/target complexes for detection.

Another problem relates to the high complexity of DNA in most samples, particularly in human genomic DNA samples. When a sample is composed of an enormous number of sequences that are closely related to the specific target sequence, even the most unique probe sequence has a large number of partial hybridizations with non-target sequences. A third problem relates to the unfavorable hybridization dynamics between a probe and its specific target. Even under the best conditions, most hybridization reactions are conducted with relatively low concentrations of probes and target molecules. In addition, a probe often has to compete with the complementary (target) strand for the actual target sequence. A fourth problem for most present hybridization formats is the high level of non-specific background signal. This is caused by the affinity of DNA probes to almost any material. These problems, either individually or in combination, lead to a loss of sensitivity and/or specificity for nucleic acid hybridization in the described formats.

New techniques are being developed for carrying out multiple nucleic acid hybridization analysis on micro-formatted multiplex or matrix devices (e.g., DNA chips and arrays) (see M. Barinaga, 253 Science, pp. 1489, 1991; W. Bains, 10 Bio/Technology, pp. 757-758, 1992). These methods usually attach specific DNA sequences to very small specific areas of a solid support, such as micro-wells of a DNA chip. These hybridization formats are micro-scale versions of the conventional "dot blot" and "sandwich" hybridization systems.

Some micro-formatted hybridization can be used to carry out "sequencing by hybridization" (SBH) (see M. Barinaga, 253 Science, pp. 1489, 1991; W. Bains, 10 Bio/Technology, pp. 757-758, 1992). SBH makes use of all possible n-nucleotide oligomers (n-mers) to identify n-mers in an unknown DNA sample, which are subsequently aligned by algorithm analysis to produce the DNA sequence (R. Drmanac and R. Crkvenjakov, Yugoslav Patent Application No. 570/87, 1987; R. Drmanac et al., 4 Genomics, 114, 1989; Strezoska et al., 88 Proc. Natl. Acad. Sci. USA 10089, 1991; and R. Drmanac and R. B. Crkvenjakov, US Patent No. 5,202,231, April 13, 1993. There are two formats for carrying out SBH. One format involves creating an array of all possible n-mers on a support, which is then hybridized with the target sequence. This is called "reverse dot blot." Another format involves attaching the target sequence to a support, which is sequentially probed with all possible n-mers. Both formats have the fundamental problems of direct probe hybridizations and additional difficulties related to multiplex hybridizations. Southern, United Kingdom Patent Application GB 8810400, 1988; E. M. Southern et al., 13 Genomics 1008, 1992, proposed using the "reverse dot blot" format to analyze or sequence DNA. Southern identified a known single point mutation using PCR amplified genomic DNA. Southern also described a method for synthesizing an array of oligonucleotides on a solid support for SBH. However, Southern did not address how to achieve optimal stringency condition for each oligonucleotide on an array. See, e.g., PCT WO89/10977, entitled "Analysing Polynucleotide Sequences", priority claim May 3, 1988. Other workers using the reverse dot blot microarray format, required very long hybridization and stringency washing time in order to achieve minimal single base mismatch discrimination (Z. Guo et al., Nucleic Acids Res., Vol. 22, #24, pp. 5456-5465, 1994).

Fodor et al., 251 Science 767-773, 1991, used photolithographic techniques to synthesize oligonucleotides on a glass matrix. Pirrung et al., in US Patent No. 5,143,854, September 1, 1992, teach large scale photographic solid phase synthesis of polypeptides in an array fashion on silicon substrates. Fodor et al., 364 Nature, pp. 555-556, 1993, used an array of 1,024 8-mer oligonucleotides on a solid support to sequence DNA. In this case, the target DNA was a fluorescently labeled single-stranded 12-mer oligonucleotide containing only nucleotides A and C. 1 pmol (~6 x 1011 molecules) of the 12-mer target sequence was necessary for the hybridization with the 8-mer oligomers on the array. The results showed many mismatches. Like Southern, Fodor et al. did not address the underlying problems of direct probe hybridization, such as stringency control for multiplex hybridizations. In order to achieve optimal stringency and selectivity for target DNA sequences a large number of immobilized capture probes must be incorporated into the array (usually 20 or more for determining single base mismatches, see M. J. Kozal et

al., Nature Medicine, Vol. 2, No. 7, pp. 753- 759, 1996. See, e.g., U.S. Patent No. 5,744,305, Fodor et al., entitled "Arrays of Materials Attached to a Substrate".

Drmanac et al., 260 Science 1649-1652, 1993, used the above discussed second format to sequence several short (116 bp) DNA sequences. Target DNAs were attached to membrane supports ("dot blot" format). Each filter was sequentially hybridized with 272 labeled 10-mer and 11-mer oligonucleotides. A wide range of stringency conditions were used to achieve specific hybridization for each n-mer probe; washing times varied from 5 minutes to overnight, and temperatures from 0° C to 16° C. Most probes required 3 hours of washing at 16°C. The filters had to be exposed for 2 to 18 hours in order to detect hybridization signals. The overall false positive hybridization rate was 5% in spite of the simple target sequences, the reduced set of oligomer probes, and the use of the most stringent conditions available. See, e.g., U.S. Patent No. 5,695,940, Drmanac et al, entitled "Method of Sequencing by Hybridization of Oligonucleotide Probes".

In another approach of matrix hybridization, Beattie et al., in The 1992 San Diego Conference: Genetic Recognition, November, 1992, used a microrobotic system to deposit micro-droplets containing specific DNA sequences into individual microfabricated sample wells on a glass substrate. The hybridization in each sample well is detected by interrogating miniature electrode test fixtures, which surround each individual microwell with an alternating current (AC) electric field. See, e.g., U.S. Patent No. 5,653,939, Hollis et al., entitled "Optical and Electrical Methods and Apparatus for Molecule Detection".

Regardless of the format, current micro-scale DNA hybridization system and passive DNA chip/array approaches do not overcome some of the underlying problems associated with nucleic acid hybridization reactions. They require very high levels of relatively short single-stranded target sequences or PCR amplified DNA, and can still produce a high level of false positive hybridization signals even under the most stringent conditions. In the case of multiplex formats using passive arrays of short oligonucleotide sequences, it is not possible to optimize the bulk stringency condition for each individual sequence with any conventional approach because the arrays or devices used for these formats can not change or adjust the temperature, ionic strength or denaturants at individual test sites or locations relative to other locations. Therefore, a common stringency condition must be used for all the sequences on the device. This results in a large number of non-specific and partial hybridizations and limits the application of the passive hybridization arrays and devices. The problem becomes more compounded as the complexity and number of different target sequences on the array increases.

Thus, the realistic evaluation and screening of large combinatorial libraries of ligand binding components; the selection of supramolecular structures and supramolecular complexes which involve pairing (hybridization) and multiple ligand binding components;

the evaluation of sets of known drugs and related compounds; and the development of the actual molecular descriptor array for drug screening would best be carried out using some type of microarray system. Many passive DNA array technologies are available, which could potentially be used for selecting ligand binding components which involve hybridization pairing components. Unfortunately, the passive nature of these DNA array technologies presents a problem. These passive arrays are unable to independently provide the huge diversity of stringency conditions that are needed to select the most specific ligand binding structures from vast number of potential combinations. For example, using a 10,000 member library of ligand binding components can produce huge numbers of "potential" supramolecular structure combinations ($>10^{12}$). Because of the diverse nature of the ligand binding components and the requirement for the base pairing/hybridization properties, using passive array technology with limited bulk stringency parameters (temperature, pH, ionic strength, etc.) would require inordinate amounts of time to carry out the combinatorial selection processes. Secondly, when large numbers of bulk stringency conditions are applied, each bulk stringency change can reduce the effective number of complexes on the array; therefore, only a limited number of stringencies can be applied. In essence, there are limitations for passive or conventional DNA arrays formats, in that they may not provide the high speed and high selectivity necessary to evaluate and screen vast combinatorial libraries of ligand binding components and the large numbers of subsequent supramolecular structures and complexes which form on the array.

Summary of the Invention

In one aspect, this invention relates to apparatus and methods for the use of active microelectronic array devices to carry out combinatorial processes where component structures form intermolecular or supramolecular ligand binding structures and subsequent supramolecular complexes (or aggregates) with improved speed and higher order specificity. The intermolecular or supramolecular ligand binding structures are composed of programmable self-assembling/pairing components that contain one or more ligand binding components. The supramolecular complexes or aggregates are formed when supramolecular ligand binding structures with the appropriate or selective molecular recognition and binding properties, bind or aggregate with a specific ligand molecule or structure which include, drug molecules, or other biologically active molecules or structures.

Once an optimal supramolecular complex pattern formation on the array is determined for a known specific ligand molecule (drug or other biologically active compound), the array can then serve as a "molecular descriptor" device. This "molecular descriptor" device can be used for a variety of applications, some of which include: new

drug discovery and screening processes, generation of novel affinity reagents, or generation of high affinity/high specificity synthetic antibodies. Additionally, other versions of these arrays can be used for multiplex proteomic analyses and for multiple immunoassay applications.

In a non-array, single site version, the invention may be considered as a device for the formation and detection of supramolecular aggregates comprising an attachment surface, the attachment surface being located in a variable electronic environment, a molecular recognition system including at least a first and a second molecular recognition component, at least the first component being attached to the surface, a separate molecular species bound to the molecular recognition system, and a third structure for formation of a supramolecular aggregate with the separate molecular species. Preferably, the molecular recognition system comprises a pairing system. Most preferably, the pairing system is a complementary and coded pairing system, such as p-RNA. Again preferably, the separate molecular species comprises a peptide sequence.

In the combinatorial selection processes of this invention, microelectronic arrays are used to affect the affinity binding of small molecules (drugs, metabolites, metal ions, etc.), large molecules (proteins, enzymes, antibodies, etc.), and larger structures (organelles, cells, etc.) to the supramolecular ligand binding structures. These processes enable the identification of those intermolecular ligand binding structures that form the most selective and/or stable supramolecular complexes in the presence of a drug or other biologically active molecule or structure. The use of microelectronic arrays allows the combinatorial selection process to be carried out in what might be considered a near real-time evolutionary or learning mode. This process not only identifies the more stable supramolecular complexes, but also provides information on the structure's important molecular recognition properties. These molecular recognition properties include: conformational, chemical, physical and mechanistic reasons as to why certain supramolecular structures and their component intermolecular ligand binding structures have more optimal binding characteristics. Thus, the molecular recognition properties of the array can provide more useful information about the structure/function relationships of the bound drug or molecule, the ligand binding components and process, and even the actual biological receptor site. Feedback of information on the supramolecular complex formation allows iterative processes to be designed either for more focused selection of specific classes of supramolecular ligand binding structures, or very wide screening for newer classes of supramolecular ligand binding structures. Microelectronic arrays are able to achieve this higher order specificity because they provide the added parameter of selective electric field stringency control at each test site on the array. Thus, in addition to the conventional or classical stringency parameters which include, temperature, pH, ionic

strength, and chemical agents (detergents, denaturants, chaotropic agents), the application of electric field stringency to supramolecular complex formation provides a powerful parameter for selecting the improved and/or more specific supramolecular ligand binding structures. In addition to providing site independent general electronic stringency, the electric field can also be used to, in effect, perturb the intermolecular suprastructures themselves producing lower energy configurations which can lead to the formation of better ligand binding environments and complexes.

For new drug discovery and screening applications, supramolecular complex binding patterns are first determined for known drugs, agonists, antagonists, inhibitors, toxins and other biological agents that are known to interact at a targeted biological receptor site. The binding patterns of these known drugs or biological agents on the combinatorial microelectronic array can be correlated with both good or desirable characteristics (for example, drug effectiveness) and bad or undesirable characteristics (for example, drug toxicity). Thus, the molecular recognition properties of the microelectronic array allow it to become a useful molecular descriptor for the drug/receptor site structure/function relationships. The subsequent testing of new drugs, therapeutic agents, or unknown compounds and the determination of their particular supramolecular complex formation patterns on the "molecular descriptor" array, can now provide potential information as to the new drug's or compound's effectiveness, side effects, and toxicity.

One important form of an intermolecular ligand binding structure relevant to this invention utilizes pyranosyl-RNA or p-RNA as the programmable self-assembling pairing component and peptides as the ligand binding component. In particular, the use of p-RNA as the programmable pairing component provides significant advantages for producing self-assembling intermolecular ligand binding structures. p-RNA is a nucleic acid-like molecule in which the sugar group is a pentopyranose (see Figure 1). (See Pitsch, S., et al., Helv. Chim. Acta, 76, pp. 2161-2183, 1993) The replacement of the normal deoxyribose (or ribose) with the pentopyranose sugar leads to a planar form for the hybridized double-stranded p-RNA (see Figure 2). The planar form for double-stranded p-RNA structure has unique attributes for forming stable intermolecular structures and supramolecular complexes when a drug or other molecule is bound. Additionally, p-RNA does not hybridize with DNA or RNA, and thus represents a unique programmable pairing system. p-RNA molecules can also be derivatized with peptide sequences at almost any position in their sequence. The peptide sequences, containing various combinatorial arrangements of amino acids, form the actual ligand binding structures. Other classes of self-assembling intermolecular ligand binding supramolecular structures can be produced using nucleic acids (DNA or RNA) and peptides. In these suprastructures, the nucleic acid moiety provides the programmable self-assembling pairing component, and the peptide moieties

serve to form the ligand binding component. The p-RNA and the DNA (or RNA) based classes represent just some of the possible intermolecular ligand binding suprastructures that are relevant to this invention. (It should be pointed out that "supramolecular structure" and "suprastructure" are used interchangably in describing this invention).

One particularly useful form of intermolecular ligand binding components leads to supramolecular structures that produce a "triad" type ligand binding configuration. These "triad" binding structures are produced by designing two short p-RNA sequences (A) and (B), which are complementary to a third longer capture p-RNA sequence (C). The two p-RNA sequences (A) and (B) are preferably designed so as to be contiguous, when paired (hybridized) with the complementary capture p-RNA (C) sequence (see Figure 3). The combinatorial binding formats and assays that utilize the "triad"-type ligand binding suprastructure involve producing a microelectronic array in which the capture p-RNA-peptide (C) ligand binding component structures are selectively addressed and immobilized to specific test sites or microlocations on the array. In these immobilized p-RNA-peptide (C) structures, the p-RNA component is one common or generic sequence that is complementary for the two p-RNA (A) and (B) sequences. While the p-RNA sequence is preferably the same for all the immobilized capture p-RNA's (C) on the array, each test site (microlocation) on the array is addressed with a p-RNA-peptide set containing a "different peptide sequence" from the known combinatorial peptide library. The peptides sequences on the p-RNA's (A) and (B) components, preferably contain the same sets of known peptide sequences as does the immobilized capture p-RNA-peptides on the array. Thus, the p-RNA-peptides (A), (B) and (C) form peptide sublibraries.

When the p-RNA–peptide (A), (B) and (C) components are combined under appropriate pairing (hybridization) conditions, a large number of intermolecular ligand binding suprastructures are formed (the cube of the number of peptides in the library). This process is called the formation of an "exponential library by aggregation of sublibraries" or ELIAS (see Figure 4). (See generally, WO 97/43232, entitled "Novel Substance Library and Supramolecular Complexes Produced Therewith", published November 20, 1997, WO 98/25943, entitled "Non-Helical Supramolecular Nanosystems", filed December 11, 1996, and WO 99/15893, entitled "Addressable Modular Recognition System, Production Mode and Use", filed September 22, 1997, hereby incorporated by reference as if fully set forth herein.). When the two complementary sequences of the p-RNA-peptide (A) and (B) components hybridize to the complementary sequence of the capture p-RNA-peptide (C) sequence, they produce the intermolecular ligand binding suprastructure in which the three peptide strands form the triad binding structure or pocket. This triad binding structure or pocket has the potential to be an affinity binding site for a specific drug, or other biologically active molecule or structure (see Figure 5).

One basic ELIAS combinatorial selection process, relevant to this invention, involves using a microelectronic array selectively addressed with capture p-RNA-peptide (C) ligand binding components, which is subsequently contacted with a solution containing the drug molecule and the p-RNA-peptide (A) and (B) ligand binding components. The combinatorial selection process is carried out under appropriate conventional and/or electronic stringency conditions for forming supramolecular ligand binding structures and supramolecular complexes. The supramolecular complex patterns on the array are detected, and the specific ligand binding components (peptides) are subsequently identified. This process is called "ELIAS on a chip" or the ELOC process (see Figure 6).

This combinatorial selection or ELOC process can be carried out using either passive or active microelectronic arrays. Using active microelectronic arrays for the ELOC process has significant advantages in certain circumstances. Active microelectronic chip/array technologies have been demonstrated which provide capability for selectively addressing arrays with DNA sequences, carrying out rapid multiplex hybridization and then providing electronic stringency for improving DNA hybridization selectivity. These same basic microelectronic arrays can be used for the combinatorial selection processes that are the subject of this invention. The basic designs and procedures for fabricating microelectronic DNA chips and arrays, particularly higher density devices (10,000 active sites) are applicable to the combinatorial and ELOC processes of this invention. The various electronic methods, procedures and formats for carrying out electronic addressing of the arrays, active electronic hybridization and electronic stringency all serve as a basis for developing the combinatorial and ELOC processes of this invention.

One important aspect of this invention involves microelectronic array based ELOC formats that provide speed and higher order selectivity for carrying out combinatorial selection processes. One of several ELOC formats relevant to this invention involves a transient dynamic equilibrium process (Format 1). In this format, the general stringency condition on the microelectronic array is set at or near the Tm (thermal melting mid-point) for the hybridization of the two p-RNA (A) and (B) sequences to the complementary capture p-RNA (C) sequence. In this ELOC format, "triad" formation on the array is in a dynamic equilibrium state, and any initial binding of a specific drug or target molecule to any combination of peptide sequences from the p-RNA-peptide (A), (B) and (C) sets will now stabilize that particular "triad" supramolecular complex. The ligand binding event effectively raises the thermal melting temperature (Tm) for the hybridized p-RNA-peptide (A) and/or (B) components to the selected capture p-RNA-peptide (C) sites on the array. Thus, only those drug/p-RNA-peptide (A) and/or (B) complexes that have some degree of stability with a peptide (C) can hybridize to the capture p-RNA-peptides (C) sites on the array. Other ELOC formats include a homogeneous combinatorial selection process that is

designed to allow a specific drug molecule to first complex with specific p-RNA-peptide (A) and (B) structures in the solution phase, and then bind to the array (ELOC Format 2); and a heterogeneous combinatorial selection process designed to initially form very large numbers of triad ligand binding superstructures on the microarray, and then select and bind the drug molecule (ELOC Format 3).

Electronic stringency may be used in different fashions and at different levels to achieve higher order selectivity. A first level can involve low electric field stringency to remove non-specific or partially bound structures. The second level can involve application of a more precise intermediate level of electric field strength to select the more specific and/or stable supramolecular complexes involving p-RNA-peptide (A) and (B) sets. The third level of electronic stringency can involve application of the most precise and the highest level of electric field strength to select the most stable supramolecular complexes involving both p-RNA-peptide sets (A) and (B), and the capture p-RNA-peptide sets (C); i.e., the true "triad" drug/peptide complexes. Additionally, the electric field can be used to effectively perturb the intermolecular superstructures producing lower energy configurations that can lead to better drug or target molecule binding environments and more stable supramolecular complexes.

After the combinatorial selection process has been carried out, the test site positions on the array at which the supramolecular complexes form identifies all the basic sets of intermolecular p-RNA-peptide structures which are responsible for the drug binding event. This electronic ELOC process allows the combinatorial selection for a large number intermolecular ligand binding structures to be made very rapidly. By way of example, a 100 test site array with 100 different peptide sequences would select through $10^6$ possible triad ligand binding structures for the most unique combinations. A 1,000 test site array with 1000 different peptide sequences would select through $10^9$ possible triad ligand binding structures for the most unique combinations. A 10,000 test site array with 10,000 different peptide sequences would select through $10^{12}$ possible triad ligand binding structures for the unique combinations. Once supramolecular ligand binding complex patterns are determined, and the unique p-RNA-peptide intermolecular ligand binding structures are identified for known drugs or biologically active compounds, the microelectronic array can be used as a molecular descriptor for screening new drugs or unknown compounds.

Brief Description of the Drawings

Figure 1 shows the basic p-RNA unit structure and the phosphodiester backbone structure of the p-RNA molecule.

Figure 2 shows the basic double-stranded helical structure of DNA compared to the p-RNA planar or ladder-like structure.

Figure 3 shows the basic structure of the three p-RNA sequences (A, B, and C) used to form the hybridizing or pairing system for the combinatorial ELIAS process.

Figure 4 shows the basic form of the three p-RNA peptide derivatized (A, B, and C) structures, which produce the intermolecular ligand binding supramolecular structures for the ELIAS process.

Figure 5 shows formation of a basic supramolecular complex between a ligand molecule and the p-RNA-peptide "triad" structures. The intermolecular structure can form both with and without the ligand binding molecule. However, the intermolecular structure with the bound ligand molecule ordinarily is a more stable form.

Figure 6 shows the basic features of the ELOC process in which supramolecular complexes form on specific test sites on the microelectronic array.

Figure 7 shows the complete structure for the fully hybridized 7-mer p-RNA (A), the 7-mer p-RNA (B), and the complementary 14-mer p-RNA (C).

Figure 8 shows the dimensional geometry and stereostructure of the hexamer peptide "triad" structure. The figure also gives the relative distance of the amino acid R-groups from the peptide "triad" convergence point.

Figure 9 shows a molecular model of a full p-RNA-peptide triad suprastructure binding a biotin ligand molecule. The biotin portion is constructed using a CPK space-filing model.

Figure 10a shows the basic structure of the acetylcholine molecule. Figure 10b shows the acetylcholine molecule in a favorable peptide triad binding structure within a negative binding pocket. Figure 10c shows an unfavorable peptide triad binding structure with positive binding pocket.

Figure 11a shows an intermolecular ligand binding supramolecular structure with a duplex peptide ligand binding structure, Figure 11b shows shows an intermolecular ligand binding supramolecular structure with a quadraplex peptide ligand binding structure, and Figure 11c shows an intermolecular ligand binding supramolecular structure with multiplex triad peptide ligand binding structures.

Figure 12 shows an example of a molecular descriptor array surface in which certain test site areas indicate supramolecular complex formation relating to drug effectiveness and other areas relate to toxicity or ineffectiveness.

Figures 13a and 13b show an intermolecular ligand binding supramolecular structure where electronic perturbation is used to flex an unfavorable p-RNA-peptide structure (13a) into a more favorable conformation (13b) that can now bind a specific drug molecule.

Figure 14 shows the addressing of a portion of a 10,000 test site microelectronic array having 30 micron test sites with p-RNA complementary and non-complementary sequences and subsequent hybridization with the complementary fluorescent labeled p-RNA sequence.

Figure 15 shows the ELOC Format 1, also known as the Transient Dynamic Equilibrium Triad Formation Process.

Figures 16a, 16b, and 16c show the ELOC Format 2 or Homogeneous Triad Formation Process.

Figure 17 shows the ELOC Format 3 or Heterogeneous Triad Formation Process.

Figure 18 shows multiplexing using p-RNA-peptide triads as synthetic antibodies.

Figure 19 shows modular immunoassays using p-RNA-antibody conjugates and a microelectronic array.

Figure 20a shows the structure of a synthesized 7-mer p-RNA labeled in the 2'-terminal position with Texas Red (TR-90 4'-I-G-A-A-G-G-G-TR-2', I = tryptamine, and TR = Texas Red). Figure 20b also shows the mass spectrum for the molecule.

Figure 21 shows the structure of the Texas Red labeled TR90 sequence (4'-I-G-A-A-G-G-G-TR-2') hybridized with a complementary biotinylated B92 sequence (4'-Biotin-C-C-C-T-T-C-T-I-C-C-C-C-C-G-2'). Figure 21 also shows the UV hypochromicity curves for the thermal denaturation of the hybridized pairs.

Figure 22 shows the structure of the Texas Red labeled TR90 sequence (4'-I-G-A-A-G-G-G-TR-2') and a Cyanine-3 fluorescent dye labeled Cy3-91 sequence (4'-Cy3-C-G-G-G-G-G-I-2') both hybridized with the complementary biotinylated B92 sequence (4'-Biotin-C-C-C-T-T-C-T-I-C-C-C-C-C-G-2'). Figure 22 also shows the UV hypochromicity date for the thermal denaturation of the hybridized pairs.

Figure 23 shows an example of a p-RNA sequence with multiple-tryptamine linkers (4'-C-C-I-I-I-G-G-2') that has been synthesized and characterized. Figure 23 also shows the UV hypochromicity and melting characteristics for the hybridized pair.

Figure 24a shows a double tryptamine p-RNA structure with two peptide chains attached via the tryptamine linker. Figure 24b shows the mass spectrum for the molecule.

Figure 25 shows four peptide residues incorporated into one supramolecular structure. Figure 24b also shows the UV hypochromicity curves which demonstrate the melting of the double-stranded p-RNA-peptide structure occurs around 50 $^{0}$C.

Figure 26 shows a simulation of a typical measurement starting from a normalized intensity at three positions of a set of 30 points (due to 30 conditions, time, temperature, stringency etc. here normalized to a total of 1) superimposed with typical noise.

Figure 27 shows a graph of the underlying idealized signal development.

Figure 28 shows a plot of factory analysis clustering of signal development on a 10,000 site array with a simulation of noise of absolute +/- 25% (50%), showing factor 1 (horizontal axis) versus factor 2 (vertical axis).

Detailed Description

In one aspect, this invention relates to the design and fabrication of addressable active microelectronic array devices, and the processes, procedures, techniques, formats, methods and uses of these devices to carry out multi-step and multiplex affinity binding reactions in microscopic formats. In a first aspect, this invention relates to the use of these microelectronic array devices to rapidly carry out combinatorial selection and molecular recognition processes involving large numbers of potential intermolecular binding components and supramolecular complexes. In a second aspect, this invention relates to using microelectronic arrays and the electronic stringency parameter to affect higher order selectivity of intermolecular and supramolecular ligand binding structures and the formation of supramolecular complexes. In a third aspect, this invention relates to using microelectronic arrays and the electronic stringency parameter to effectively perturb supramolecular structures to their lowest energy configurations, to dynamically flex the supramolecular structures, and to generally enhance the formation of selective and stable ligand binding complexes.

The combinatorial array devices of this invention can be used for a variety of applications, some of which include:

(1) new drug discovery and screening processes,
(2) correlation of known drug effectiveness and toxicity with structure,
(3) elucidation of structure/function relationships for hormones,
(4) elucidation of structure/function relationships for neurotransmitters,
(5) elucidation of hapten/antigen antibody epitope binding site characteristics,
(6) elucidation of structure/function relationships for ligand binding moieties which bind to cell surface receptors,
(7) elucidation of structure/function relationships for enzyme substrates,
(8) generation of high affinity/high specificity synthetic antibodies,
(9) generation of novel affinity reagents,
(10) generation of novel synthetic enzymes and catalyst,
(11) generation of novel chelate reagents,
(12) subsequent generation of novel synthetic antibody arrays,
(13) subsequent generation of novel affinity reagent arrays,
(14) subsequent generation of novel synthetic enzyme and catalyst arrays,
(15) subsequent generation of novel chelate arrays,

The devices, processes, procedures, techniques, formats and methods of this invention can be used for combinatorial and molecular recognition processes involving a variety of other biological and synthetic affinity and ligand receptors. Additionally, other versions of these arrays can be used for multiplex proteomic analyses and immunoassay applications.

Specific Ligand Molecules & Structures

In one aspect, this invention relates to the use of active microelectronic array devices to carry out combinatorial processes for the selection of supramolecular complexes with improved speed and higher order specificity. The supramolecular complexes are formed by the affinity binding of various specific ligand molecules and structures to intermolecular/supramolecular ligand binding structures. For purposes of this invention, a specific ligand molecule or structure is defined as a targeted molecule or structure that has specific affinity for another molecule, synthetic ligand binding structure, or a biological receptor. Generally, a given specific ligand molecule or structure is a biologically active molecule (drug, metabolite, hormone, peptide) or structure (protein, antibody) that has a specific shape and fits into a specific biological or physiological receptor molecule, site or structure, where one or more non-covalent binding interactions stabilize the complex. Receptor sites can include, but are not limited to: chelates, peptides, proteins, antibodies, enzymes, nucleic acid/protein complexes, membranes, and cells. The specific or selective fit of a given specific ligand molecule or structure into its specific receptor site is sometimes referred to as a "lock and key" fit. The non-covalent interactions which bind the specific ligand molecule to the receptor site can include: hydrogen bonding, hydrophobic bonding, aromatic ring stacking, electrostatic interactions, chelation (with metal ion ligands) and van der Waals interactions. In addition to the physical/chemical parameters which determine ligand binding, the ligands and receptor sites can also have stereoselective properties. For enantiomeric ligands or molecules where chirality is based on optical asymmetry at a single atom, there are no differences in physical/chemical properties in the absence of a dissymmetric receptor site or binding surface. However, diastereomer ligands with more than one asymmetric center can have different physical/chemical properties. It is well known that small molecule enantiomeric ligands can have large differences (several orders of magnitude) in binding to a receptor site. These types of ligands should be considered "stereospecific as well as stereoselective." (see P. Taylor and P. A. Insel in "Principles of Drug Action – The Basis of Pharmacology" Edited by W. B. Pratt and P. Taylor, Third Edition, Churchill Livingston, pp. 1- 74, 1990).

In addition to drug molecules with specific target receptor sites, some other important classical biological ligand/receptor interactions include: substrate molecules with enzymes, haptens/antigens with antibodies, toxins and carcinogens with biological, receptors, biotin with avidin or streptavidin, hormones with their receptor sites and neurotransmitters with nerve cell receptors. In some cases, the same molecule or structure can serve both as a ligand and a receptor; for example, an antibody molecule may serve as a receptor for a specific hapten molecule and also as a ligand for another antibody. Some of the specific ligand target molecules and structures relevant to this invention include, but are not limited to:

(1) **Small Ligand Molecules** - drugs, therapeutic agents, agonists, antagonists, inhibitors, metabolites, amino acids, peptides, hormones, ACTH, angiotensins, bradykinins, cytokines, endomorphins, endorphins, enkephalins, exorphins, lymphokines, neurotransmitters, vitamins, nucleotides, oligonucleotides, synthetic anti-sense oilgonucleotide agents, lectins, haptens, sugars, lipids, fatty acids, biological co-factors (NADPH, FAD, thiamine pyrophosphate, etc.), metal ions, metal chelates, dyes, porphyrins, toxins, carcinogens, mutagens and other natural or synthetic small molecules with biological activity or binding properties.

(2) **Large Ligand Molecules** - proteins, hormones, Interleukins, Tumor Necrosis Factors, enzymes, antibodies, associated antibody epitope sites, DNAs, RNAs, synthetic polynucleotides, synthetic polypeptides, toxins, natural and synthetic polysaccharides, aptamers, and other natural or synthetic large molecules or polymers which have biological activity or binding properties.

(3) **Larger Ligand/Receptor Structures** - cells, bacteria, virus, cell surface membranes, cell surface proteins, cell surface receptor and effector sites, organelles, nuclei, mitochondria, ribosomes, synthetic micelles, and other natural or synthetic surfaces which have biological activity or binding properties.

Recapitulating to some extent, the combinatorial selection processes of this invention involve the use of a microelectronic array to carry out selection of supramolecular complexes which are formed by the binding of a specific ligand target molecule to one or more "intermolecular ligand binding structures". For purposes of this invention, intermolecular ligand binding structures are defined as molecular structures which have one component with programmable pairing properties, and a second component with ligand binding properties. These structures are designed such that they can self-assemble under appropriate conditions into larger intermolecular structures "supramolecular structures" via their programmable pairing properties. The structures are further designed such that in the presence of an appropriate specific ligand target molecule or structure, the binding of the ligand molecule by two or more of the ligand binding

components can also lead to the formation and stabilization of the larger supramolecular structures. The binding of the specific ligand molecule by the ligand binding components effectively produces intermolecular stabilization of the self-assembled supramolecular structure through the pairing components. The formation of the complete specific ligand molecule/intermolecular ligand binding structure is called the "supramolecular complex".

Programmable Pairing Structures

One type of a programmable pairing structure or component, of particular importance to this invention, which can be used to form self-assembling intermolecular ligand binding structures is pyranosyl–RNA or p-RNA. p-RNA is a nucleic acid like molecule in which the sugar group is a pentopyranose. Figure 1 shows the basic p-RNA structure. Some of the important characteristics of p-RNA which are similar to DNA, include: Classical Watson-Crick type base pairing, enantioselective base pairing, and antiparallel strand orientation for double-stranded structure.

Some important p-RNA characteristics which are different than DNA include: higher duplex stability and selectivity than DNA or RNA, p-RNA does not base pair with DNA or RNA, and p-RNA duplexes form quasi-ladder structures, not the classical helix.

While p-RNA has some characteristics similar to DNA/RNA, the fact that it does not hybridize to DNA means that it is uniquely different from other nucleic acid variants or derivatives i.e., p-RNA represents a unique pairing system. Additionally, the replacement of the normal deoxyribose (or ribose) with the pentopyranose sugar leads to planar or ladder-like form for the hybridized double-stranded p-RNA structure. The planar form of double-stranded p-RNA structure provides unique attributes for forming stable self-assembling intermolecular structures, suprastructures, and supramolecular complexes when ligand molecules are bound. The basic procedure for the synthesis and purification of p-RNA is given in the Experimental Section (see Example 1). See also, WO 99/15539, entitled "Pentopyranosyl Nucleoside, and Production and Use of the Same", filed September 22, 1997; WO 99/15540, entitled "Method for Producing a Pentopyranosyl Nucleoside", filed September 22, 1997; WO 99/15541, entitled "Pentopyranosyl Nucleoside for Producing an Electronic Component, and Conjugates of Said Pentopyranosyl Nucleoside", filed September 22, 1997, incorporated herein by reference as if fully set forth herein. The p-RNA molecules can be derivatized with many of the same components and by many of the same procedures that have been developed for DNA and RNA modification. Thus, p-RNA can be derivatized (functionalized or modified) with biotin moieties, aromatic and aliphatic amine groups, aromatic and aliphatic thiol groups, aromatic and aliphatic aldehyde groups. p-RNA can also be functionalized by incorporation of a tryptamine ribopyranosyl (Tr) phosphoramidite at the terminal position

or anywhere within the sequence. See, e.g., WO 99/15542, entitled "Linker Nucleoside, and Production and Use of the Same", filed September 22, 1997, incorporated herein by reference as if fully set forth herein. The procedure for incorporation of a tryptamine ribopyranosyl **(I)** phosphoramidite into p-RNA is given in the Experimental Section (see Example 1). Subsequently, functionalized p-RNAs can be further derivatized or coupled with fluorophores (see Example 2), chromophores, biotin, chelates, amino acids, and peptides, proteins, streptavidin, nucleic acids (DNA/RNA), nanoparticles, and a variety of other molecules and structures. p-RNAs functionalized with amines, thiols, aldehydes, and/or tryptamine **(I)** nucleosides (see Example 3) can also be subsequently attached to solid supports and surfaces, these include, but are not limited to: glass, silicon, plastics, nylon, nitrocellulose, ceramics, metals, metal oxides, agarose, polyacrylamide and other polysaccharides. p-RNAs can be functionalized at their 2' or 4' terminal positions or at any position within the sequence (see Example 2). Derivatization of p-RNA can be carried out via modification of the base moieties, sugar, or the phosphate groups.

While p-RNA is the preferred programmable pairing structure, other potentially useful programmable pairing components which can be used to form self-assembling intermolecular ligand binding structures include homologues with monomers containing 6 membered rings, which are called cyclohexyl nucleic acids or CNA's (CNA-peptide pairing systems are disclosed in WO 99/15509, entitled "Cyclohexyl and Heterocyclyl Nucleoside Derivatives, Method for Producing These Derivatives, and the Use of the Derivatives and Their Oligomers or Conjugates in Pairing and/or Testing Systems, filed September 22, 1997). CNA's have an uncharged backbone structure, which means that they could have advantages for forming pairing structures under low ionic strength conditions.

Yet, another group of programmable pairing components which can be used to form self-assembling intermolecular ligand binding structures includes, but is not limited to: deoxyribonucleic acids (DNA), ribonucleic acids (RNA), synthetic polynucleotides, synthetic oligonucleotides, methylphosphonate nucleic acid analogues, phosphorothioate nucleic acid analogues, phosphorodithioate nucleic acid analogues, peptide nucleic acids (PNA) and other modified nucleic acids. In these structures, the nucleic acid moieties serve to form the intermolecular pairing system via classical hybridization. (It is important to point out again that the pairing system for p-RNA is distinct from DNA/RNA and all other nucleic acid derivatives).

Finally, other intermolecular pairing systems may be based on peptides, proteins, modified polysaccharides, lectins, electrostatic (cation/anion) type polymers, and metal/chelate systems, and a variety of synthetic structures which can be designed with

programmable self-assembling pairing properties. The above, are all hereby incorporated into this invention.

Ligand Binding Structures

The second important component of the intermolecular ligand binding structure is the actual ligand binding structure itself. The ligand binding structures of this invention are defined as those structures which, in the presence of an appropriate specific ligand target molecule or structure, can provide one or more of the following;

(1) A two-dimensional coordinate structure or three-dimensional structure (pocket) which can accommodate and/or properly fit the specific ligand molecule,

(2) Provide the ligand molecule with appropriate stereoselective and/or stereospecific fit,

(3) Provide the ligand molecule with one or more hydrogen bonds,

(4) Provide the ligand molecule with one or more electrostatic interactions,

(5) Provide the ligand molecule (metal) with one or more chelation interactions or coordination sites,

(6) Provide the ligand molecule with one or more hydrophobic interactions,

(7) Provide the ligand molecule with one or more base stacking interactions, and/or

(8) Provide the ligand molecule with one or more van der Waals interactions.

A first class of ligand binding structures of this invention includes, but is not limited to: amino acids, peptides, cyclic peptides, antibodies, proteins, avidin, streptavidin, lectins, carbohydrates, polysaccharides, chelates, metal chelates, membranes, micelles, fluorophores, chromophores, crown ethers, cyclodextrins, cells and composites of two or more of any of the above. A second class of ligand binding structures includes, but is not limited to: p-RNA, CNA, DNA, RNA, aptamers, oligonucleotides, PNA, and composites of two or more of the above.

In the second class of structures, the p-RNA and/or nucleic acid components are used for both pairing and ligand binding. Ligand binding structures can be composed of components from both group one and group two.

Programmable pairing components and ligand binding components can be combined in an almost unlimited number of configurations. Generally, the programmable pairing component and the ligand-binding component are covalently coupled. For example, a peptide ligand-binding component could be covalently coupled to a p-RNA pairing component to form an intermolecular ligand binding structure.

Intermolecular ligand binding structures of this invention include, but are not limited to, the following:

(1) p-RNA BASED INTERMOLECULAR BINDING STRUCTURES

p-RNA Molecular Derivatives/Structures/Compositions

Examples of p-RNA molecular derivatives, structures and/or compositions include:

- p-RNA-amino acid(s), L, D, natural, un-natural forms,
- p-RNA-peptide,
- p-RNA-DNA-peptide (double recognition/pairing with ligand binding),
- p-RNA-antibody (protein, enzyme),
- p-RNA-hapten derivative,
- p-RNA-drug derivative,
- p-RNA-neurotransmitter derivative,
- p-RNA-hormone derivative,
- p-RNA-toxin derivative,
- p-RNA-radioisotope derivative,
- p-RNA-lectin (carbohydrate, disaccharide, polysaccharide derivatives),
- p-RNA-DNA (RNA, PNA, methylphosphonate),
- p-RNA-DNA (RNA, PNA, methylphosphonate)-peptide,
- p-RNA-fluorophore(s),
- p-RNA-donor/acceptor fluorophores (arrangements for photonic energy transfer),
- p-RNA-fluorophore-peptide,
- p-RNA-chromophore(s),
- p-RNA-chromophore-peptide,
- p-RNA-chromophore/fluorophore (arrangements for photonic energy transfer),
- p-RNA- electron donor/acceptor moieties,
- p-RNA-metal chelator,
- p-RNA-metal chelator -peptide,
- p-RNA-multiple metal chelator arrangements, and
- p-RNA-metal chelator–chromophore/fluorophore (arrangements for photonic to electronic transfer systems).

p-RNA Nanoscale, Meso-Scale and Solid Support Structures

Examples of p-RNA nanoscale, meso-scale and/or solid support structures include:

- p-RNA-nanoparticles/nanostructures/nanodevices (metal nanoparticles, gold

nanoparticles, metal oxide nanoparticles, quantum dots, carbon nanotubes, polysaccharide nanobeads, and organic polymer nanobeads),

- p-RNA-meso-scale structures/devices (metal particles/structures/devices, metal oxide particles/structures/devices, silicon particles/structures/devices, semiconductor particles/structures/devices, gallium arsenide particles/structures/-devices, lift-off microelectronic devices, lift-off photonic devices, lift-off mechanical devices, and micro sensor devices),
- p-RNA-macroscopic surface/support materials (glass, quartz, mica, metal, metal oxides, silicon, silicon dioxide, GaAs, plastics, organic polymers, natural polymers, cell surfaces, agarose gels, polyacrylamide gels, hydrogels, silica gels, nylon, nitrocellulose, and ceramics),

p-RNA-Peptide Nanoscale, Mesoscale and Solid Support Structures

Examples of p-RNA and peptides nanoscales, mesoscales and solid support structures include:

- p-RNA-peptide-nanoparticles/nanostructures/nanodevices (metal nanoparticles, gold nanoparticles, metal oxide nanoparticles, quantum dots, carbon nanotubes, polysaccharide nanobeads, and organic polymer nanobeads),
- p-RNA-peptide-meso-scale structures/devices (metal particles/structures/devices, metal oxide particles/structures/devices; silicon particles/structures/devices, semiconductor particles/structures/devices, gallium arsenide particles/structures/devices, lift-off microelectronic devices, lift-off photonic devices, lift-off mechanical devices, and micro sensor devices), and
- p-RNA-peptide-macroscopic surface/support materials (glass, quartz, mica, metal, metal oxides, silicon, silicon dioxide, GaAs, plastics, organic polymers, natural polymers, cell surfaces, agarose gels, polyacrylamide gels, hydrogels, silica gels, nylon, nitrocellulose, and ceramics).

p-RNA-Peptide-Fluorophore Nanoscale, Mesoscale, and Solid Support Structures

Examples of p-RNA and peptide-flurophore nanoscale, mesoscale, and solid support structures include:

- p-RNA-peptide-fluorophore-nanoparticles/nanostructures/nanodevices (metal nanoparticles, gold nanoparticles, metal oxide nanoparticles, quantum dots, carbon nanotubes, polysaccharide nanobeads, and organic polymer nanobeads),
- p-RNA-peptide-fluorophore-meso-scale structures/devices (metal particles/structures/devices, metal oxide particles/structures/devices; silicon particles/structures/devices, semiconductor particles/structures/devices, gallium

arsenide particles/structures/devices, lift-off microelectronic devices, lift-off photonic devices, lift-off mechanical devices, and microsensor devices), and

- p-RNA-peptide-fluorophore-macroscopic surface/support materials (glass, quartz, mica, metal, metal oxides,/silicon, silicon dioxide, GaAs, plastics, organic polymers, natural polymers, cell surfaces, agarose gels, polyacrylamide gels, hydrogels, silica gels, nylon, nitrocellulose, and ceramics).

(2) CNA BASED INTERMOLECULAR LIGAND BINDING STRUCTURES

Examples of CNA based intermolecular ligand binding structures include:

- CNA-Amino Acid,
- CNA-Peptide,
- CNA-DNA (RNA, PNA, methylphosphanate),
- CNA-DNA-peptide (double recognition/pairing with ligand binding),
- CNA-fluorophore,
- CNA-fluorophore-peptide,
- CNA-antibody (protein, enzyme),
- CNA-lectin (carbohydrate, disaccharide, polysaccharide),
- CNA-chromophore, metal ligand, nanoparticle, nanobead, and meso-scale derivatives,
- CNA-macroscopic surface (glass, silicon, silicon dioxide, GaAs, plastics, ceramics, metals), and
- CNA –including, but not limited to all derivatives listed above for p-RNA.

p-RNA-PEPTIDE INTERMOLECULAR LIGAND BINDING STRUCTURES

p-RNA-peptide structures represent the class of intermolecular ligand binding structures that are of major importance to this invention. In these structures, the p-RNA moiety provides a programmable self-assembling intermolecular pairing system (via base sequence and hybridization), and the peptide moieties serve to form the actual ligand binding structures. One particular advantage of using p-RNA, is that upon pairing (hybridization) the double-stranded p-RNA is a planar or ladder-like structure. This structure is the result of the replacement of the normal deoxyribose (or ribose) with the pentopyranose sugar moiety. Figure 2 shows the basic double-stranded p-RNA planar/ladder structure. The planar or ladder-form of paired double-stranded p-RNA provides more predicable supramolecular ligand binding structures and complexes. p-RNA has further advantages of providing more stable and selective duplex structures than does DNA or RNA. The fact that p-RNAs are much more stable than DNA, means that significantly shorter sequences can be used to form the double-stranded planar structures.

p-RNA also does not pair or hybridize with DNA. This means that any extraneous DNA in a sample does not interfere with p-RNA pairing/hybridization.

Another advantage is that DNA can be used to create a separate pairing system or composite system with two programmable pairing components (e.g., p-RNA-DNA or p-RNA-RNA). Thus, unlike most other nucleic acid analogues (PNA, methy-phosophonates), p-RNA represents a unique programmable pairing system, involving the same basic purine and pyrimidine base pairing (adenine with thymidine, and guanine with cytosine) as DNA or RNA, but with no affinity to DNA or RNA.

The p-RNA structure can be derivatized, functionalized and modified in the same fashion and by most of the same procedures that are used for DNA or RNA. p-RNA molecules can be derivatized with peptide sequences at basically any position in the sequence. The peptide sequences can contain any arrangement of amino acids, to form large combinatorial groups of potential ligand binding structures. One particular useful form of p-RNA-peptide intermolecular ligand binding structures are able to self-assemble into a "triad" type ligand binding superstructure. Designing two short p-RNA sequences (p-RNA sequence (A) and p-RNA sequence (B)) which are complementary to a third longer p-RNA sequence (p-RNA sequence (C)) produces these triad ligand binding suprastructures. The longer p-RNA sequence (C) functions as a template for positioning the two shorter sequences (A and B). The two shorter p-RNA sequences (A and B) are designed so as to be contiguous, or nearly contiguous, when hybridized to the complementary "template" p-RNA sequence (C). Figure 3 shows the generic design for the three complementary p-RNA sequences (A, B, & C), and the pairing or hybridized double-stranded planar/ladder structure. The third "template" p-RNA sequence (C) can be further functionalized so it can be immobilized on beads, particles, or support material (glass, silicon, silicon dioxide, silicon nitride, metals, metal oxides, plastic, nylon, agarose, polyacrylamide, hydrogels, silica gels, sol-gels, etc.).

For the combinatorial selection processes of this invention, the appropriate functionalization allows the template p-RNA sequence (C) to be either covalently or noncovalently attached to a specific test site on the microelectronic array. By way of example, the p-RNA, can be functionalized with a biotin moiety which allows it to be attached to the microelectronic array test sites via streptavidin incorporated in the permeation layer of test site. It is also possible to covalently attach or immobilize p-RNA sequences that have been functionalized with amines, thiols, aldehydes, carboxyl groups, hydrazines, azido groups, and with phenylboronic acid. Generally, the template p-RNA sequence (C) is functionalized at either its 4' or 2' terminal position when attachment to a solid support is the objective. However, it is possible to functionalize the p-RNA at any position in its sequence, and it still can be used for attachment to solid supports.

The peptide sequences that form the ligand binding component of the intermolecular ligand binding structures are generally covalently coupled to the p-RNA sequence. However, non-covalent attachments are possible (biotin/avidin, etc.), and can sometimes have applicability. Covalent coupling of the peptide can be achieved by either using a functional "R" group provided by one or more of the amino acids in the peptide itself, or by incorporating additional functionalization into the peptide sequence. Functional groups, which can provide by one or more of the amino acids in the peptide sequence itself, include the following:

Amino Acid Functional "R" Groups for Coupling include:

- Cysteine (thiol),
- Lysine (amino),
- Serine (hydroxyl),
- Tyrosine (hydroxyl),
- Glutamate (carboxyl),
- Aspartate (carboxyl),
- Any N-terminus (amino),
- Any C-terminus (carboxyl),

Generally, the use of an amino acid functional (R) group for coupling to the p-RNA means that, the "R" group from amino acid used for coupling will play less of a role in the actual ligand binding process. Also, consideration must be given to the full synthetic route, including peptide synthesis, de-blocking procedures, and the final p-RNA/peptide coupling procedure, so that amino acid "R" groups which are expected to be available for ligand binding and combinatorial selection remain viable after coupling to the p-RNA.

Coupling of the peptide sequence to the p-RNA molecule can generally be carried out at any position within either the p-RNA sequence or the peptide sequence. This includes:

p-RNA and Peptide Coupling Positions

- Functionalized 3' and or 5' terminal position on the p-RNA,
- Functionalized internal p-RNA sequence positions (base, sugar, phosphate ester),
- Functionalized intervening nucleotides (tryptamine, DNA, RNA, etc.),
- Functionalized spacer moieties (phosophoramide nucleoside spacers),
- N-terminus of peptide (amine),
- C-terminus of peptide (carboxyl),

- R-group terminus of peptide,
- Internal R group of peptide, and
- Added spacer/functional group to peptide.

Functional groups for coupling reactions that can be incorporated into p-RNA include: tryptamine nucleotides, amines, thiols, aldehydes, hydroxyl (ribose), carboxyl, phosphate, maleimides, and a number of others. One particular method relevant to this invention for coupling a peptide via the cysteine thiol group involves using a peptide sequence with a cysteine in the terminal position, which is then reacted with one or more tryptamine nucleotides within the p-RNA sequence. The procedure for linking cysteine peptides to tryptamine (I) containing p-RNA sequences is given in the Experimental Section (see Example 3). Additionally, in some cases it may be desirable to have peptides in which the basic combinatorial peptide structures are extended a further distance from the p-RNA structure. This would be important when it is necessary to create supramolecular triad structures for binding larger ligand molecules and structures (antibodies, cell surfaces, etc.) Thus, the use of so-called spacer groups is also incorporated into this invention. Examples of these spacer groups can include, but are not limited to: short run of amino acids (-gly-gly-gly-), aliphatic chains (-$CH_2$ -$CH_2$ -$CH_2$ -$CH_2$ -), polyglycols, and polysaccharide structures. Spacer groups can be designed to provide either ridge or flexible intervening structures between the p-RNA and the peptide.

Another general class of intermolecular ligand binding structures relevant to this invention can utilize nucleic acids as the pairing system component and peptides as ligand binder component. These structures can include DNA-peptides, RNA peptides, or various nucleic acid analogues with peptides attached. When DNA is used to form the pairing system, the double-stranded structures that are formed are helical. When peptide components are on one or more members of pairing DNA strands, the helical nature of the DNA produces a relatively complex structure; this can make it somewhat more difficult to determine the best positions for the peptide ligand binding structures.

## PEPTIDE LIBRARIES, ELIAS, ELOC, AND TRIPLEX SUPRASTRUCTURES

### Peptide Libraries

The various combinatorial binding assays, formats, and procedures which are relevant to this invention, first involve producing a microelectronic array in which a first group of capture p-RNA-peptide intermolecular ligand binding structures (each with a different peptide sequence) are selectively addressed and immobilized to specific test sites on the array. Addressing of p-RNA-peptides to the microelectronic array can be carried out by electronic addressing (as is described in the Experimental Section of this

invention), or by other mechanical deposition (ink jetting, micropipeting, microcapillary application, etc.). In the addressed group of p-RNA-peptide structures, the capture p-RNA (C) component has a common or generic sequence that is complementary for the other two p-RNA sequences (A & B). p-RNA sequences A and B are not complementary to each other, but only pair/hybridize with their own complementary section of capture p-RNA (C) sequence. While the capture p-RNA (C) sequence is the same for all test sites, each test site on the array contains a different peptide sequence from a known peptide library. The use of the term "each" is not meant to exclude the situation where repetitive or redundant test sites are included within the array.

Peptide libraries can be created with any given number of peptides containing permutations of any number of amino acids within the sequence. One particularly useful peptide library that is relevant to the combinatorial aspects of this invention includes a group of 10,000 hexamer peptide (containing six amino acids). In this group of hexamer peptides, the C-terminus amino acid is usually a glycine, which is generally used as the starting amino acid resin in the synthesis procedure, and the N-terminus amino acid is usually a cysteine which provides a thiol group that is used for coupling to a tryptamine within the p-RNA molecule.

While other amino acids may be included, the four internal amino acids of the hexamer peptide are preferably chosen form a group of ten amino acids that include:

Amino Acids in Peptide Library

- Arginine (guanidinium R-group: positive charge, electrostatic interactions)
- Asparagine (amide R-group: hydrophilic & hydrogen bonding interactions)
- Glutamic Acid (aliphatic carboxyl R-group: negative charge electrostatic interactions)
- Histidine (imidazole R-group: pH sensitive positive charge & metal coordination)
- Leucine (aliphatic chain R-group: hydrophobic interactions)
- Phenylalanine (aromatic ring R-group: hydrophobic & ring stacking interactions)
- Proline (closed ring amino acid: stereochemistry, produces bend in peptide chain)
- Serine (aliphatic hydroxyl R-group: hydrophilic & hydrogen bonding)
- Tryptophan (aromatic ring R-group: hydrophobic & ring stacking interactions)
- Tyrosine (phenol ring R-group: hydrophobic ring stacking & hydrogen bonds)

The 10,000 peptide hexamer library contains all possible permutations of the ten amino acids listed above. The ten chosen amino acids provide a good representation of

the important "R" groups which are available to form ligand binding structures and stabilize the ligand complexes via hydrogen bonds, hydrophobic interactions, ring stacking interactions, electrostatic interactions, and van der Waals interactions. The generic structure of the hexamer peptide is shown in the diagram below:

**p-RNA-I-$S_{R-1}$-Cys(1)-AA(2)$_{R-2}$-AA(3)$_{R-3}$-AA(4)$_{R-4}$-AA(5)$_{R-5}$-$Gly_{R-6}$-COOH**

The hexamer peptide library, or other peptide libraries can be constructed using reagents and automated solid phase peptide synthesis procedures that are well known to those who practice the art of peptide synthesis.

The particular 10,000 peptide hexamer library described above represent just one of numerous libraries that can be used for combinatorial selection processes and other applications. Other peptide libraries anticipated by this invention include, but are not limited to:

(1) libraries which have longer peptides (e.g., 7-mers, 8-mers, 9-mers, and up to 100-mers, and various sublibrary combinations),

(2) libraries with shorter peptides (5-mers, 4-mers, 3-mers, 2-mers, and single amino acids, and various sublibrary combinations),

(3) libraries with other groupings of amino acids, and sublibrary combinations, and

(4) libraries which include all twenty one amino acids.

Also, envisioned by this invention are libraries with combinations of peptides with other moieties; as well as combinations of non-peptide ligand binding moieties, examples of which include, but are not limited to:

(1) peptides and metal chelates,

(2) selective metal chelates,

(3) peptides and fluorophores and/or chromophores,

(4) fluorophores and chromophores,

(5) chromophores and electron donor/acceptor moieties,

(6) peptides and enzymes,

(7) peptides and coenzymes,

(8) peptides and antibodies,

(9) peptides and hormones,

(10) peptides and neurotransmitters,

(11) peptides and drugs,

(12) peptides and lectins,

(13) peptides and polysaccharides,

(14) peptides and lipids,

(15) peptides and DNA/RNA/oligonucleotides, etc.,

(16) peptides and p-RNA/CNA (where these moieties are available for ligand binding),

The ELIAS and ELOC Concepts and Processes

In a completed addressed microelectronic array there is a known peptide sequence from a peptide library at a known test site on the array. While the peptide sequence at each site is different, the capture p-RNA (C) sequence is generic or common and complementary to both p-RNA sequences (A & B). Generally, the p-RNA sequences A and B have been coupled with the same sets of peptide sequences (from the known peptide library) as were the immobilized capture p-RNA-peptides. The p-RNA peptide of groups A, B, and C now represent sublibraries of the original peptide library. When the three groups of p-RNA–peptides are mixed together under hybridization conditions, a large number of intermolecular ligand binding superstructures are formed (the cube of number of peptides in the library or on the array).

This process represents the formation of an "exponential library by aggregation of sublibraries" and is called "ELIAS". The basic ELIAS concept is shown in Figure 4. When the two complementary sequences of the p-RNA-peptide (A) and (B) components hybridize to complementary sequence of the capture p-RNA-peptide (C) sequence, they produce the intermolecular ligand binding structure in which the peptide strands form a "triad" binding structure or pocket. This "triad" binding structure or pocket has the potential affinity for a specific ligand molecule or structure (see Figure 5).

By way of example, a 100 test site array addressed with 100 different sets of p-RNA-peptide (C), and contacted with 100 different sets of p-RNA-peptides (A) and 100 different sets p-RNA-peptides (B) under hybridization conditions, would select through $10^6$ possible supramolecular or triad ligand binding structures for the unique combinations. A 1000 test site array with 1000 different sets of p-RNA-peptide (C) sequences contacted with 1000 different sets of p-RNA-peptides (A) and 1000 different sets of p-RNA-peptides (B) under hybridization conditions, would select through $10^9$ possible supramolecular or triad ligand binding structures for the unique combinations. A 10,000 test site array with 10,000 different sets of p-RNA-peptide (C) sequences, and contacted with 10,000 different sets of p-RNA-peptides (A) and 10,000 different sets of p-RNA-peptides (B) under hybridization conditions, would select through $10^{12}$ possible supramolecular or triad ligand binding structures for the unique combinations.

When a microelectronic array or another array platform is used to carry out "ELIAS", the process is called "ELIAS on a chip", or "ELOC". The basic ELOC concept is shown in Figure 6. The microelectronic array based ELOC process and the various electronic formats represent an important aspect of this invention in certain embodiments.

Depending on the objective of a particular combinatorial assay and the nature of the specific target molecules or structures, different formats can be more effective in providing information about the structure-function relationships between the ligand and the binding site and lead to more useful "molecular descriptor" devices. The basic ELOC combinatorial selection processes can be carried out in a number of different formats that involve the use of different conventional and electronic stringency parameters to affect the p-RNA pairing/hybridization process and to affect and/or perturb the peptide triad structures and the ligand binding process. Additionally, the formats can involve different order as to when and how ligands and p-RNA-peptide components A and B are added to the system. The ELOC processes and formats, and the p-RNA-peptide structures themselves are designed to achieve the following:

(1) Produce supramolecular structures with optimal peptide arrangement "triads" for accepting and selectively binding specific molecules and structures.

(2) Produce the optimal stringency conditions that allow non-specific binding interactions and/or less specific peptide/ligand supracomplexes to be differentiated from the more specific and/or stronger binding supramolecular peptide/ligand supramolecular complexes.

(3) To rapidly detect "supramolecular complex" formation, and identify the optimal intermolecular ligand binding structures (p-RNA-peptides).

(4) To produce useful "molecular descriptor" arrays for new drug screening and selection.

Triad Suprastructures

A particular group of p-RNA peptide intermolecular structures relevant to this invention, are those structures that can self-assemble, via the p-RNA pairing/hybridization, into arrangements that produce a "triad" peptide ligand binding structure. Ability to form the "triad" peptide ligand binding structure is achieved by appropriate design of the p-RNA-peptide structures. In one method, the capture p-RNA (C) sequence is functionalized in the 4'-terminal position with a biotin moiety for subsequent immobilization to microelectronic array test sites, via streptavidin within the agarose permeation layer of the test site. Thus, the immobilized capture p-RNA has a "test site surface-biotin-4'-p-RNA-2' terminal" orientation. The lengths for capture p-RNA (C) sequences can most broadly range from about six nucleotides (bases) to over one hundred nucleotides (bases); more ideal lengths are from about seven nucleotides to sixty nucleotides; and the most ideal lengths are from about eight nucleotides to forty nucleotides. Generally, the peptide sequence for the capture p-RNA (C) sequence is coupled at or near (within three bases), of

the middle of the p-RNA (C) sequence. In most cases, the peptide is coupled through the modified tryptamine nucleoside (I) within the p-RNA sequence (see Example 3). The p-RNA-peptide sequence (A) is designed to hybridize with the 2' end of the capture p-RNA (C) sequence. The lengths for the p-RNA (A) sequences can most broadly range from about three nucleotides (bases) to over one hundred nucleotides (bases); more ideal lengths are from about four nucleotides to thirty nucleotides; and the most ideal lengths are from about five nucleotides to twenty nucleotides. Generally, the peptide sequence for the p-RNA (A) sequence is coupled at or near (within three bases) of the 2'-terminus of p-RNA (A). The coupling of the peptide to the p-RNA (A) is via the cysteine thiol (peptide) to the modified tryptamine nucleoside **(I)** at or near the 2' end of p-RNA (A), using the procedure described above for the p-RNA (C). The p-RNA sequence (B) is designed to hybridize with the 4' end of the immobilized capture p-RNA (C) sequence. The lengths for the p-RNA (B) sequences can most broadly range from about three nucleotides (bases) to over one hundred nucleotides (bases); more ideal lengths are from about four nucleotides to thirty nucleotides; and the most ideal lengths are from about five nucleotides to twenty nucleotides. Generally, the peptide sequence for the p-RNA (B) sequence is coupled at or near (within three bases) of the 4'-terminus of p-RNA (B). The coupling of the peptide to the p-RNA (B) sequence is via the cysteine thiol (peptide) to the modified tryptamine **(I)**, at or near the 4' end of p-RNA (B), using the procedure described above for the p-RNA (C)

In order to detect and identify the formation of supramolecular structures or supramolecular complexes which have formed at a specific test site(s) on the array surface; either the (A) or (B) p-RNA (or both A & B) sequences are derivatized with a reporter group. The usual reporter group(s) used are fluorophores; however, also included in the invention are chromophores, biotin/avidin detection systems, chemiluminescent agents, metal chelates, radioisotopes, proteins, enzyme detection systems, antibodies and nanoparticles. By way of example, p-RNA-peptide (A) might be labeled with a Cyanine-3 fluorophore (Ex 530 nm, Em 570 nm) and p-RNA-peptide (B) might be labeled with a Texas Red fluorophore (Ex 590 nm, EM 620 nm). See Example 2. In this case, two color fluorescent analysis can be used to detect the formation of intermolecular ligand binding complexes on the array surface. Fluorescent analysis is not limited to these two fluorophores, as a variety of fluorescent labels may be used (fluorescein, Rhodamine, Bodipy Texas Red, Bodipy Far Red, Cyanine–5, etc.) Most detection formats and systems used for DNA hybridization and immunodiagnostic analysis, which are well know to those practicing the art, can also be employed for many combinatorial applications. These general detection methodologies are hereby incorporated into this invention.

An example of a useful fully modified 7-mer p-RNA (A) sequence, 7-mer p-RNA (B) sequence, and complementary 14-mer p-RNA (C) sequence are shown below:

p-RNA (A) 4'-(Fluorophore)-C-G-G-G-G-G-I-(Peptide)-2'

p-RNA (B) 4'-(Peptide)-I-G-A-A-G-G-G-X-2'

p-RNA (C) 4'-(Biotin)-C-C-C-T-T-C-I-(Peptide)-T-C-C-C-C-C-G-2'

The paired or hybridized form of fully modified p-RNA-peptide (A), (B), and (C) sequences is shown in Figures 7.

When all three p-RNA sequences are hybridized, the combination of the peptide positioning within p-RNA (A), (B), and (C) sequences, and the intrinsic p-RNA property of forming planar/ladder like structures, produces the "triad" intermolecular superstructure in which the peptide strands form a pocket like ligand binding structure. This pocket like structure has an ideal generic configuration for accepting and binding ligand molecules or structures. The basic peptide triad structure generically represents a three dimensional small molecule ligand binding receptor. For a peptide triad structure where the three hexamer peptide chains diverge at about a $60^0$ angle from the focal point of attachment to the p-RNA structure, each chain extends a distance of approximately 2.0 nanometers (nm) form the focal point (see Figure 8). For the hexamer peptide library discussed above, the first amino acid (cysteine) and its thiol R-1 group (-SH) are involved in the covalent attachment to the p-RNA. The second amino acid with the R-2 group is potentially available for ligand interaction and is approximately 0.6 nanometer (nm) form the attachment focal point. The third amino acid with the R-3 group is approximately 0.95 nm from the focal point. The fourth amino acid with the R-4 group is approximately 1.3 nm from the focal point. The fifth amino acid with the R-5 group is approximately 1.7 nm from the focal point. The sixth and last amino acid is approximately 2.0 nm from the focal point. In the case of the hexamer peptide library that uses glycine in the C-terminal position, the R-6 group is a hydrogen atom (-H) which would not be expected to have significant interaction with ligand molecules. However, the glycine's free C-terminal carboxyl ($-COO^-$) could potentially be involved in ligand binding interactions. By way of example, Figure 9 shows a molecular model of a full p-RNA-peptide triad suprastructure binding a biotin ligand molecule. The biotin portion is constructed using a CPK space filling model system.

Acetylcholine Ligand Binding Triad Models

The binding of the acetylcholine molecule and related substrates, agonists, antagonists, inhibitors, and drugs to the anionic binding site of acetylcholinesterase and the Nicotinic Cholinergic receptors and Muscarinic Cholinergic receptors ($M_1$, $M_2$ , $M_3$), provides a good model for developing peptide ligand binding systems. Acetylcholine is a

relatively small molecule (MW 146) which is the acetyl ester of choline (2-hydroxy-N,N,N-trimethylethanaminium). The acetylcholine molecule carries a formal positive charge which plays a role in binding and orienting the molecule in the enzyme active site and in receptor sites (see Figure 10a). Thus, it would not be unexpected that a first group of peptide "triad" structures that had reasonable affinity for the acetylcholine molecule, would have an anionic binding site for electrostatic interaction with the positively charged choline group, and a slightly hydrophobic binding pocket which could accommodate the acetyl group. By way of intuitive example, a potentially favorable ligand binding "triad" structure might be composed of the following hexamer peptide (A, B, and C) sequences:

Potential Favorable Acetylcholine Binding "Triad" Peptides

p-RNA –(A)-Cys-Leu-Ser-Leu-Glu-Gly

p-RNA –(B)-Cys-Ser-Leu-Glu-Ser-Gly

p-RNA –(C)-Cys-Leu-Leu-Ser-Glu-Gly

The potentially favorable binding of acetylcholine to the above triad peptide structure is shown in Figure 10b. It would also be reasonable to expect that a triad with peptides that contained cationic amino acids (Arginine, Histidine) would be unfavorable binding sites for the acetylcholine molecule. By way of intuitive example, a potentially unfavorable ligand binding "triad" structure might be composed of the following hexamer peptide (A, B, and C) sequences:

Potential Un-Favorable Acetylcholine Binding "Triad" Peptides

p-RNA –(A)-Cys-Ser-Arg-Ser-Arg-Gly

p-RNA –(B)-Cys-Ser-Arg-His-Arg-Gly

p-RNA –(C)-Cys-His-Arg-Tyr-Arg-Gly

The potentially unfavorable binding of acetylcholine to the above triad peptide structure is shown in Figure 10c.

It would also be reasonable to expect that many of the Acetylcholine agonist (Nicotine Muscarine, Phenyltrimethylammonium, etc.), and antagonist (d-Tubocurarine, Trimethaphan, Hexamethomium, Decamethonium, Atropine, etc.); would be favorably bound by the first peptide triad structure, and unfavorably by the second peptide triad structure. While one can predict to some degree what might be a generically favorable or unfavorable binding ligand structures for certain ligands, it would be very difficult to empirically determine which are the "optimal" structures that would provide the basis for the molecular descriptor device. This is particularly true when parameters of stereoselectivity and stereospecificity are involved for both the ligand molecules and the "very large" numbers of supramolecular triad structures that are possible. This is, of

course, the primary reason why a rapid combinatorial selection process with higher order selectivity is needed.

Other Supramolecular Arrangements

Also included in this invention are combinations of intermolecular ligand binding structures that lead to the formation of other types of supramolecular arrangements, some which include but are not limited to:

(1) Structures with two p-RNA-peptide component structures (of which one could be immobilized), that form a dimer supramolecular ligand binding structure (see Figure 11a). These structures represent somewhat simpler configuration, but would still be able to from relatively stable complexes; in particular metal coordination and chelates structures which frequently can have more planar or two dimensional structures.

(2) Structures with four p-RNA-peptide structures (of which one could be immobilized), that form a quadraplex supramolecular ligand binding structure (see Figure 11b). These structures can be used to form "binding pockets" which involve four peptide chains. Such structures can be used to create more protected ligand binding "pockets" and involve more amino acid R-groups in providing stereoselectivity and binding interactions. The procedure for the synthesis of a quadraplex p-RNA-peptide structure is given in the Experimental Section (see Example 3)

(3) Structures with an immobilized p-RNA-peptide component that can form more than one triad supramolecular binding structure along its length (see Figure 11c). These unique supramolecular structures with more than one triad ligand binding component can be used for binding more than one ligand to the same base structure. More importantly, such structures can be designed with different triad ligand binding component and used to bind larger and/or polymeric ligands that have multiple binding sites.

MICROELECTRONIC ARRAY COMBINATORIAL FORMATS AND PROCESSES

An important aspect of this invention involves electronic combinatorial selection processes and formats where microelectronic arrays are used to affect the affinity binding of small molecules (drugs, agonist, antagonists, substrates, metabolites, metal ions, etc.), large molecules (proteins, enzymes, antibodies, etc.) and larger structures (organelles, cells, etc.) to various supramolecular structures. One example of a supramolecular structure which is important to this invention is a p-RNA-peptide intermolecular triad ligand binding structure. The use of microelectronic arrays allows the combinatorial

selection process to be carried out in what might be considered a near real time evolutionary or learning mode. Thus, very large combinatorial libraries of peptides (e.g, 10,000) can be tested in formats that select through near astronomical numbers of potential supramolecular ligand binding structures ($10^{12}$). These electronic based combinatorial processes allow rapid selection of the more stable supramolecular complexes, while also providing information as to the conformational, chemical and mechanistic reasons why certain intermolecular ligand binding structures have more or less optimal characteristics. That is, the arrays provide structure/function relationships concerning the binding process. Feedback of information on supramolecular complex formation allows iterative processes to be designed either for more focused selection of specific classes of intermolecular ligand binding structures, or very wide screening for newer classes of intermolecular ligand binding structures. In relation to drug discovery applications, known classes of drugs (agonist, antagonists, inhibitors, toxins, etc.) could be rapidly analyzed and the structure/function relationships of their binding process determined. From the correlation and analyses of these known drugs (agonist, antagonists, etc.), the microelectronic array can then be used as a molecular descriptor device for screening of new drugs, to determine their effectiveness, side effects, and toxicity. Example 9 in the Experimental Section gives a description of the type of algorithms that can be used to deconvolute and analyze the large amount of data that would come off an ELOC combinatorial array after a drug/ligand binding experiment. This type of analysis will be important for determining the molecular descriptor properties of the ELOC arrays. **Figure 12** demonstrates conceptually how a microelectronic molecular descriptor array would show a grouping of test sites where supramolecular complexes have formed, which are indicative of drug effectiveness and, another grouping of test sites that are indicative of drug toxicity.

Combinatorial Complexity and Stringency

The availability of large combinatorial peptide libraries and the supramolecular formation process provides the potential to form a significant number of highly selective ligand binding complexes. Unfortunately, the combination of these very large numbers of mixed peptide sequences (with different interacting and binding R-groups), p-RNA sequences and ligand molecules also creates a large amount of non-specifically bound materials. This nonspecifically bound material leads to a high background signal production that can make it very difficult to detect the "true" supramolecular complex formation. Additionally, some of the specific p-RNA peptide sequences involved in non-productive or non-specific binding will not be available to form the truly selective supramolecular complexes. By way of example, those peptides from the combinatorial peptide library that contain several arginine moieties and possess a net positive charge

would be expected to have significant interaction with the negatively charged backbone of the p-RNA sequence, in particular with their intermolecular connected p-RNA component. Such non-specific binding interactions could cause useless aggregation of these peptides and depletion of these p-RNA-peptide components. **Figure 13a and 13b** show how electronic perturbation and stringency can be used to flex such nonproductive structures, allowing them to now create a viable triad binding structure for some potential negatively charged ligand molecule. Thus, the application of appropriate stringency processes which allows one to reduce nonspecific interaction while still maintaining the ability to produce viable and detectable supramolecular triad complexes is one of the key objectives of this invention.

Microelectronic arrays have the potential to achieve higher order specificity for combinatorial selection processes, because they provide the added parameter of selective electric field stringency control at each binding (test) site on the array. Thus, in addition to the classical stringency parameters which include: temperature, pH, ionic strength, and chemical agents (detergents, denaturants, chaotropic agents), the application of an electric field stringency to supramolecular complex formation provides a totally new and powerful parameter for selecting the improved and more specific intermolecular ligand binding structures. Active microelectronic chip/array technologies have been demonstrated which provide capability for rapid multiplex hybridization and electronic stringency for improving hybrid selectivity. This technology also includes procedures and methods for selective addressing DNA sequences, oligonucleotides, amplicons, and other moieties to the array device.

Basic designs and procedures for fabricating microelectronic DNA chip and array devices, and basic methods, procedures and formats for carrying out electronic addressing, active electronic hybridization and stringency are found in: US Patent No. 5,605,662 - "Active Programmable Electronic Devices for Molecular Biological Analysis and Diagnostics"; US Patent No. 5,632,957 – "Molecular Diagnostic Systems Including Electrodes" ; US Patent No. 5,849,486 – "Apparatus and Methods for Active Programmable Matrix Devices"; US Patent No. 4,787,963, "Methods and Means for Annealing Nucleic Acid Molecules at an Accelerated Rate"; SN 08/271,882 – "Methods for Electronic Stringency Control for Molecular Biological Analysis and Diagnostics" (207/263).

In particular, those electronic stringency parameters (DC, AC/DC, and electronic pulsing protocols) which concern electronic perturbation, have been described in patents and applications which deal with the so-called area of fluorescent perturbation. See, e.g., U.S. Patent No. 5,849,486, entitled "Apparatus and Methods for Active Programmable

Matrix Devices", and Application Serial No. 08/855,058, entitled "Methods for Electronic Perturbation Analysis of Biological Materials", filed May 14, 1997.

Additionally, the design and fabrication procedures for higher density microelectronic arrays (e.g., 400, 1200, 10,000, and higher numbers of test sites) that have active on-board electronic control have been described in the following patent applications. See, e.g., PCT/US99/03080, entitled "Advanced Active Devices and Methods for Molecular Biological Analysis and Diagnostics", filed February 11, 1999.

The designs, procedures and formats described in the above patents and patent applications provide the underlying basis for developing microelectronic descriptor arrays for carrying out selective electronic addressing of the p-RNA-peptides to the arrays. This material also provides the basis for the electronic stringency parameters that are to be used in the combinatorial selection and screening processes described in the present invention. The above patents and applications are hereby incorporated into this invention. Further information on the basics of electronic hybridization and stringency are also discussed in the following reference articles (Heller, M. J. , IEEE Engineering in Medicine and Biology, pp.100-104, March/April 1996; Sosnowski, R., et al., Proc. Nat. Acad. Sci. 94, pp. 1119-1123, 1997; Edman, C. F., et al., Nucleic Acid Research 25, pp. 4907-4914, 1997; Cheng, J., et al., Nature/Biotechnology 16, pp. 541-546, June 1998; and Gilles, P. N., et al., Nature/Biotechnology 17, No. 4, pp. 365-370, 1999). Experiments demonstrating electronic stringency for p-RNA-peptide intermolecular structures are shown in the Experimental Section (see Example 4). Example 5 shows further experiments which demonstrate supramolecular complex formation and "significant" increase in intermolecular complex stability (increase electronic Tm) for p-RNA histidine rich peptides in the presence of nickel ions. This is a particularly important experiment as it provides verification that certain intermolecular complexes can be significantly stabilized upon proper ligand molecule binding.

Addressing of Arrays with p-RNA-Peptides

Microarrays can be selectively addressed with p-RNA peptides sets by either electronic or mechanical means. Electronic addressing of p-RNA-peptides to the microelectronic array involves exposing the array to a solution of the specific p-RNA peptide (C), and biasing the selective site on the array positive relative to a set of negatively biased sites or counter electrodes on the array. Generally, the addressing solution contains between 1 to 100 nM of the biotinylated p-RNA-peptide (C) sequence in a 50-100 mM hisitidine (zwiterionic) solution. Electronic addressing is carried out by application of between 200nA to 600nA (to the positively biased site) for a period of 60 to 120 seconds. Electronic addressing is normally carried out in a serial fashion, however

parallel electronic addressing devices and methods are described in the patents listed in the above sections. The above listing of patents and publications also provides detailed information on the methods and procedures for electronic addressing, and is hereby incorporated into this invention. Mechanical means of selectively addressing microarrays includes ink jetting, micropipeting, microcapillary deposition and other techniques, many of which are known to those who practice DNA or other microarray art. Example 6 (Experimental Section) describes the addressing of a 10,000 test site microelectronic array (30 micron test sites) with complementary and non-complementary p-RNA capture sequences and the subsequent hybridization with the complementary fluorescent labeled p-RNA target sequence. **Figure 14** shows the results for addressing and hybridization on the 10,000 test site microelectronic array.

Key Objectives of Electronic ELOC Formats

Three basic electronic ELOC formats are described which provide advantages for carrying out combinatorial selection processes that involve different libraries (smaller and larger), different ligands, and/or require different combinatorial selection criteria such as speed, selectivity and sensitivity. The formats are designed to take advantage of both the self-assembling properties of the p-RNA sequences (hybridization of p-RNA A and B sequences to an immobilized p-RNA C sequence) and the overall stabilization of intermolecular structures that occurs upon ligand binding. The formats are also designed to utilize conventional stringency (temperature, pH, ionic strength, etc.), electronic assisted hybridization, electronic stringency and electronic perturbation to overcome the combinatorial complexity issues which lead to non-specific binding and non-specific background problems. Reduction of nonspecific binding and background is of key importance to the combinatorial process, as it directly leads to the corresponding improvement in sensitivity for detecting supramolecular complexes. In general, the formats are designed to produce the maximum number of selective and stable supramolecular complexes and allow them to be detected as rapidly as possible.

Electronic ELOC Format 1 – Dynamic Equilibrium Triad Formation Process

This format is designed to take advantage of transient supramolecular structure formation produced by rapid forming and breaking p-RNA-peptides (A) and p-RNA peptides (B) hybrids with the immobilized p-RNA-peptides (C) site on the array. Additionally, the format takes advantage of the stabilization effect that occurs when a favorable ligand binding event stabilizes a specific triad structure that is produced in the transient process. This electronic ELOC format involves a first step in which the general stringency condition on the microelectronic array is, at or near (plus/minus $5^0$ C), the

thermal melting mid-point (Tm) for the hybridization of the two p-RNA (A) and (B) sequences to the immobilized complementary capture p-RNA (C) sequence. The p-RNA-peptide (A) and (B) sublibraries can be added at concentrations from about 1nM to 100 μM of each sublibrary. For a 10,000 member combinatorial peptide library this is a concentration of about 100 fM to 10 nM for each peptide sequence. These p-RNA peptide libraries can be in either low conductance solution or higher conductance solutions. Under low conductance conditions (50 mM to 100 mM histidine) electronic assisted hybridization can be used to accelerate both the on and off rates for hybrid formation. When higher conductance solutions are used, hybridization can proceed at its normal rate and electronic stringency or perturbation can be used to accelerate the hybrid off rates. Higher conductance solutions can be composed of 1 mM to 1M of sodium chloride/sodium phosphate, sodium citrate, Tris, or any common buffer system used for normal hybridization or affinity binding reactions that are well known this art. These procedures are designed to achieve the condition where the supramolecular triad structure formation for most of the potential p-RNA-peptides (A, B, and C) exist in a transient or dynamic equilibrium state. At or near the Tm for the p-RNA hybridization, supramolecular complexes are rapidly forming and breaking at all test sites on the array. Thus, under these conditions very large numbers of combinatorial supramolecular triad structures are being tested at all of the p-RNA-peptide (C) sites on the array. This process is driven primarily by the hybridization of the p-RNA sequences. In addition to temperature and/or electronic assisted hybridization, stringency on the array can also be controlled by other factors that include pH, ionic strength, detergents, denaturants and chaotropic agents. Combinations, of some, or all of these general stringency parameters (including temperature) can be used to achieve the transient or dynamic equilibrium state.

The ligand molecules can be added into the system at any time during this process, however depending on the nature of the ligand, addition after the dynamic equilibrium state is reached may sometime be preferred. Ligand molecule concentrations can range from about 1 pM to 100 nM. Under these conditions, the binding of a ligand molecule to any favorable triad combination of peptide sequences will effectively stabilize that particular supramolecular structure/complex formation. This is an intermolecularly produced stabilization that effectively raises the thermal melting temperature (Tm) for the hybridization of those selected p-RNA-peptide (A) and/or (B) components to the selected capture p-RNA-peptide (C) site(s) on the array. Thus, only those ligand/p-RNA-peptide (A) and/or (B) complexes that have this higher degree of stability remain hybridized to capture p-RNA-peptides (C) sites on the array. Under these dynamic equilibrium conditions, this increased stability is due almost totally to the ligand/peptide complex formation process. Electronic assisted hybridization, stringency and perturbation can be

used in several modes both during and after the initial process. During the process, the electric field can be used to effectively perturb the intermolecular superstructures producing lower energy configurations that can lead to better ligand binding environments and to more stable complexes. Both positive and negative electric field stringency in contiguous or pulsed DC and/or offset AC scenarios, can be used to perturb peptide triad structures and ligand/peptide triad complexes. The multitude of combinatorial triad structures that can potentially be produced would have numerous peptide combinations containing both positively and negatively charged amino acid R-groups. These charged peptide R-groups would be strongly influenced by the application of the electric field. In particular, the electric field could be used to flex the peptide triad structures, helping them to achieve lowest energy conformations that are more suitable to ligand binding. Additionally, the electric field can be used to perturb the ligand/supramolecular complexes allowing them to find more stable configurations. Any charged ligand molecules can themselves be influenced or perturbed by application of negative and/or positive electric field. Figure 13a and 13b show an example of the electronic perturbation effect on charged p-RNA-peptide structures. By way of further example, an acetylcholine ligand molecule that has a formal positive charge would be moved toward and concentrated at a microelectronic array test site that is biased positive, and moved away from a test site which is biased negative. Additionally, a positively charged ligand molecule could be removed from its ligand binding site by application of positive electronic stringency to that site. For a methotrexate ligand molecule which has a formal negative charge, application of a negative bias would move the molecule away from the test site, while application of a positive bias would move the molecule toward and concentrate it at that test site. Additionally, a negatively charged ligand molecule could be removed from its ligand binding site by application of negative electronic stringency.

After the dynamic equilibrium and complex formation stage of the process, electric field stringency can be used at three other levels to achieve higher selectivity and sensitivity for detecting supramolecular complexes. The first level involves low electric field stringency to remove nonspecific or partially bound structures. The second level involves application of a more precise intermediate level of electric field strength to select only the most selective and/or stable supramolecular complexes involving p-RNA peptide (A) and (B) sets. The third level of electronic stringency involves application of the most precise and highest level of electric field strength to select the most stable supramolecular complexes involving both p-RNA-peptide sets (A) and (B), and the capture p-RNA peptide sets (C); i.e., the optimal "triad" peptide/ligand complexes. The positions on the array at which the supramolecular complexes form, identifies all the basic sets of intermolecular p-RNA-peptide structures which are responsible for the ligand binding

event. This electronic ELOC format allows the combinatorial selection for a large number intermolecular ligand binding structures to be made very rapidly. Figure 15a and 15b shows the ELOC Format 1 or the Transient Dynamic Equilibrium Triad Formation process.

By way of example, a combinatorial selection process to produce a molecular descriptor array for screening new drugs and therapeutics which affect the Nicotinic and Muscarinic Cholinergic receptors might be carried out in the following manner. The combinatorial selection process involving the 10,000 member p-RNA-peptide library would be carried in electronic ELOC format 1 for a group of ligands which include the cholinergic substrate (acetylcholine) and related drugs, agonist, and antagonists (nicotine, muscarine, phenyltrimethylammonium, d-tubocurarine, hexamethonium, atropine,etc.). A 10,000 test site microelectronic array would be selectively addressed with all 10,000 p-RNA-peptide (C) sequences. The ELOC format 1 would be carried out for each of the known cholinergic ligands (acetylcholine, nicotine, muscarine, etc.) with the p-RNA-peptide (A) and (B) sublibraries. The positions of supramolecular complex formation on the microelectronic array would be detected and correlated for all the known chloinergic substrates, agonists, and antagonists. The microelectronic array would now be a useful molecular descriptor for screening new drugs which affect the cholinergic receptors.

Electronic ELOC Format 2 – Homogeneous Triad Formation Process

Electronic array ELOC format 2 is a combinatorial selection process that is designed to allow a specific ligand to complex with specific p-RNA-peptide (A) and (B) structures in the solution phase. Under one set of low stringency conditions (below the Tm for p-RNA hybridization) the homogeneous phase would be carried out separate from the microelectronic array. In this case, stringency conditions would be optimized for formation of ligand/p-RNA-peptide (A)/p-RNA-peptide (B) complexes. Once the solution phase (homogeneous) complex formation has taken place, the solution can be placed on the microelectronic array that has been selectively addressed with the p-RNA-peptide (C) library. The dimer complexes (p-RNA-peptide A/ligand/p-RNA-peptide B) are then allowed to form the triad supramolecular complexes by binding to specific positions (p-RNA-peptide C) sites on the array. The same basic general and electronic procedures that were described for ELOC Format 1 can now be used for carrying out combinatorial selection of the optimal supramolecular ligand complexes.

A second variation of Format 2 involves carrying out the homogeneous phase formation of the ligand/p-RNA-peptide (A)/p-RNA-peptide (B) complexes on the microlelectronic array. In this case, the initial procedure has to be carried out at high stringency or above the Tm for p-RNA-peptide hybridization. The second phase, would

involve lowering the general stringency conditions to be at or near the Tm for p-RNA hybridization, so that triad complex formation could start occurring on the array. Electronic assisted hybridization, stringency and perturbation can be used in several modes both during and after the second phase. During the process, the electric field can be used to effectively perturb the intermolecular superstructures producing lower energy configurations that can lead to better ligand binding environments and to more stable complexes. Both positive and negative electric field stringency in contiguous or pulsed DC and/or offset AC scenario, can be used to perturb peptide triad structures and ligand/peptide triad complexes. The multitude of combinatorial triad structures that can potentially be produced would have numerous peptide combinations containing both positively and negatively charged amino acid R-groups. These charged peptide R-groups would be strongly influenced by the application of the electric field. In particular, the electric field could be used to flex the peptide triad structures, helping them to achieve lowest energy conformations that are more suitable to ligand binding. Additionally, the electric field can be used to perturb the ligand/supramolecular complexes allowing them to find more stable configurations. Any charged ligand molecules can themselves be influenced or perturbed by application of negative and/or positive electric field. After the complex formation stage of the process, electric field stringency can be used at three other levels to achieve higher selectivity and sensitivity for detecting supramolecular complexes. The first level involves low electric field stringency to remove nonspecific or partially bound structures. The second level involves application of a more precise intermediate level of electric field strength to select only the most selective and/or stable supramolecular complexes involving p-RNA-peptide (A) and (B) sets. The third level of electronic stringency involves application of the most precise and highest level of electric field strength to select the most stable supramolecular complexes involving both p-RNA peptide sets (A) and (B), and the capture p-RNA-peptide sets (C); i.e., the optimal "triad" peptide/ligand complexes. The positions on the array at which the supramolecular complexes form, identifies all the basic sets of intermolecular p-RNA peptide structures which are responsible for the ligand binding event. Figure 16a, 16b, and 16c show the ELOC Format 2 or Homogeneous Triad Formation process.

Electronic ELOC Format 3 – Heterogeneous Triad Formation Process

Electronic array ELOC format 3 is a combinatorial selection process designed to initially form a very large number of triad type ligand binding superstructures. This process involves a first step of carrying out a low stringency hybridization of the complete peptide sublibrary sets of the two p-RNA peptide (A & B) sequences to the capture p-RNA sequence (C) immobilized to the test sites on the array. The low stringency condition

for hybridization on the array would be the equivalent of $5^0$ C or more below the Tm (thermal melting mid-point) for the p-RNA hybrids. This low stringency condition allows a very large number p-RNA peptide triad superstructures to be rapidly formed on the array. In the next step, the ligand molecule (drug or biologically active compound) is added to the system under the low stringency condition. Electronic stringency can be used in several modes both during and after the initial process. During the process, the electric field can be used to effectively perturb the intermolecular superstructures producing lower energy configurations that can lead to better ligand binding environments and to more stable complexes. Both positive and negative electric field stringency in contiguous or pulsed DC and/or offset AC scenarios, can be used to perturb peptide triad structures and ligand/peptide triad complexes. The multitude of combinatorial triad structures that potentially can be produced would have numerous combinations of both positively and negatively charged amino acid R-groups. These would be strongly influenced by the application of the electric field. In particular, the electric field could be used to flex the peptide triad structures, helping them to achieve lowest energy conformations that are more suitable to ligand binding. Additionally, the electric field can be used to perturb the ligand/supramolecular complexes allowing them to find more stable configurations. Electric field stringency can be used further for three levels of selectivity. The first level involves low electric field stringency to remove non-specific or partially bound structures. The second level involves application of a more precise intermediate level of electric field strength to select only the most selective and/or stable supramolecular complexes involving p-RNA peptide (A) and (B) sets. The third level of electronic stringency involves application of most precise and highest level of electric field strength to select the most stable supramolecular complexes involving both p-RNA peptide sets (A) and (B), and the capture p-RNA peptide sets (C); i.e., the true "triplex" peptide/ligand complexes. The positions on the array at which the supramolecular complexes form, identifies all the basic sets intermolecular p-RNA peptide structures which are responsible for the ligand binding event. This electronic ELOC format allows the combinatorial selection for a large number intermolecular ligand binding structures to be made very rapidly. Figure 17a, 17b, and 17c shows the ELOC Format 3 or Heterogeneous Triad Formation process.

OTHER APPLICATIONS DERIVED FROM COMBINATORIAL SELECTION PROCESSES

In addition to using the combinatorial processes of this invention for producing molecular descriptor arrays for drug screening applications, the identification of the ligand specific supramolecuar structures can also lead to their direct use and applications for a

number of other areas. Some of these unique supramolecular structures include but are not limited to:

(1) Synthetic Antibodies,
(2) New Affinity Regents,
(3) Synthetic Catalysis/Enzymes, and
(4) New Metal Chelate Structures.

Synthetic Antibodies

Once a combinatorial process has been used to identify supramolecular structures which have high selectivity and binding for a specific ligand, those specific triad structures represent the equivalent of a "synthetic antibody". While a normal mammalian response to some haptin/antigen may produce as many as $10^6$ antibodies, the peptide libraries and combinatorial processes described in this invention can produce $10^{12}$ different triad binding structures. Thus, the possibility for producing very unique and selective supramolecular structures which have antibody-like binding properties is enormous. Since, all three peptide sequences in any given supramolecular triad structure can be identified (because of pseudo 3-fold symmetry on the array), the "specific" triad structures can now be produced very easily in higher amounts and used as immunodiagnostic reagents, replacing traditional polyclonal or monoclonal antibodies. These specific p-RNA-peptide supramolecular "synthetic antibody" structures can now be used in variety of homogeneous and heterogeneous (immobilized) immunoassay formats. These formats include the generation of synthetic antibody arrays for multiple ligand (hapten/antigen) analysis. Specific p-RNA peptide supramolecular structures with specific peptide sequences can be produced with common p-RNA sequences as was described for the combinatorial selection processes, or with specific p-RNA sequences. Specific p-RNA-peptide supramolecular triads with common p-RNA sequences can be selectively addressed to the array using the same electronic procedures described for the electronic combinatorial processes. Other formats can involve using specific p-RNA sequences A, B, and C, which would produce the "specific" p-RNA-peptide triad structures when all the component structures were simply mixed together. Additionally, other formats would involve using specific p-RNA C type structures, with an added specific p-RNA extension (4 to 30 bases) which would allow the whole triad structure to be subsequently hybridized to a complementary specific p-RNA sequence which has been immobilized to the microelectronic array (see Figure 18). In this format, the specific complementary p-RNA sequences are first pre-addressed to the array. The specific p-RNA-peptide supramolecular triads "synthetic antibodies" are reacted in solution (homogeneously) with samples containing the target haptens/antigens. After complex formation between the

specific p-RNA-peptide triad and the haptens/antigens, the complexes can be selectively immobilized by hybridization to the complementary p-RNA sequences on the array. The microelectronic arrays can be used for selective addressing of structures, for electronic perturbation of structures and complexes, and for applying electronic stringency to achieve better assay sensitivity and selectivity. The above description represents just some of the many homogenous and heterogeneous synthetic antibody "immuno" type assay formats which are envisioned by this invention. Further aspects of this invention include the development of sandwich type synthetic antibody related assays where one specific p-RNA-peptide triad structure is used to selectively bind and immobilize the targeted hapten/antigen, and a second specific p-RNA-peptide triad labeled with a reporter group is used to detect the complex. Some of the reporter groups which can be used to label these p-RNA peptide triads include, but are not limited to, fluorophores, chromophores, enzymes, chemiluminescent moities, biotin/avidin detection forming complexes, gold particles, nanobeads, magnetic beads, and radioisotopes.

By way of example, one important "immuno" type assay utilizing the synthetic antibodies of this invention would be designed to detect and quantify the levels of Cytokinins (TNF, IL-1, IL-6, IL-8, etc.) and other cellular agents (Bradykinins, tissue factors, adhesion molecules, etc.) which are the initial indicators of bacterial/endotoxin septic infections, systemic inflammatory response syndrome (SIRS), sepsis, and impending septic shock and multiple organ dysfunction syndrome (MODS).

IMMUNODIAGNOSTIC AND PROTEOMIC APPLICATIONS

Other broad classes of applications which are the subject of this invention involve the use of p-RNA-antibody, p-RNA-protein, and p-RNA-enzyme derivatives which can be addressed to microelectronic arrays and then used in subsequent applications such as immunodiagnostics, protein binding assays, and enzyme assays.

Immunodiagnostic p-RNA-Antibody Arrays

Multiple or modular type immunodiagnostic assays can be developed by preparing specific p-RNA-antibody conjugates which are then selectively addressed to a microelectronic or other array type device or substrate **(see Figure 19).** In one such format, specific complementary p-RNA sequences (capture sequences) are first pre-addressed to the array. In the next step, specific p-RNA-antibody conjugates are reacted in solution with samples containing the target haptens or antigens. These p-RNA-antibody conjugates have both a specific antibody and a specific p-RNA sequence which allows it to be subsequently captured (via hybridization) to its complementary p-RNA on the array. The p-RNA-antibody/hapten/antigen complexes are then selectively immobilized by

hybridization to the complementary p-RNA sequences on the array. A number of procedures can be used to detect the antibody/hapten/antigen complex, one of which is a so-called sandwich format. In the sandwich assay format, the first specific p-RNA-antibody conjugate is used to selectively bind and immobilize the targeted hapten/antigen, and a second specific antibody labeled with a reporter group is used to detect the complex. Some of the reporter groups which can be used to label the reporter antibody include but are not limited to fluorophores, chromophores, enzymes, chemiluminescent moities, biotin/avidin reporter complexes, gold particles, nanobeads, magnetic beads, and radioisotopes. Additionally, an important aspect of this invention involves the use of the electronic properties of the microelectronic array for:

(1) The selective addressing of initial p-RNA capture sequences and/or the specific p-RNA-antibody conjugates.

(2) For electronic perturbation of p-RNA antibodies and the hapten or antigen complexes which are formed.

(3) For applying electronic stringency to the p-RNA-antibody/hapten/antigen complexes to achieve better assay sensitivity and selectivity.

The above descriptions represent just some of the many homogenous and heterogeneous immunodiagnostic assay formats that are envisioned by this invention.

Antibodies can easily be attached to p-RNA molecules by a number of covalent and non-covalent methods. Example 7 of this invention describes a procedure where complementary p-RNA constructs were used as immobilization tethers for a protein conjugate consisting of Streptavidin and a goat anti-human $F(ab')_2$ antibody. p-RNA No. 81 was used to provide a capture sequence for p-RNA No. 80 by binding the biotin of p-RNA No. 81 to Streptavidin which had been immobilized in the permeation layer covering a microelectronic array (APEX chip). The biotin of p-RNA No. 80 was then used to bind to a mobile streptavidin which had been chemically conjugated to a goat anti-human $F(ab')_2$ antibody. The goat anti-human $F(ab')_2$ antibody was used to capture its specific antigen target which is human IgG $F(ab')_2$ antibody. This represents just one of a number of methods by which antibodies can be modified with specific p-RNA molecules and addressed to a microelectronic or other array device. In further experiments, complementary p-RNAs #54 and #79 were used to form another immobilization tether or p-RNA-antibody derivative. In this case p-RNA #54 was immobilized to a permeation layer overlaying the microelectronic array by binding its 4' biotin to immobilized Streptavidin in the permeation layer. p-RNA #79 was hybridized to its complementary strand #54 and the 4' biotin of #79 was used to immobilize the Streptavidin half of a solubilized conjugate of Streptavidin and goat anti-human $F(ab')_2$ antibody. The goat anti-human F(ab')2 antibody was then used as an immunosorbent to capture its target antigen

which is human IgG F(ab')$_2$ antibody. Selectivity of the respective capture p-RNA capture strands (either #81 or #54) was evidenced that when the complementary strands of p-RNA (either #80 or #79, respectively) were bound to the Streptavidin-goat anti-human antibody protein complex in solution and then hybridized to an APEX chip containing the captures, the p-RNAs attached to the protein conjugate bound only to their respective complementary immobilized capture strands. This demonstrates the basis for enabling geographical sorting of immunological reagents which are simultaneously present in a homogeneous solution. Example 8 demonstrates novel methods for achieving simultaneous multiple homogeneous assays combined with discrete analyte detection.

Using the complementary pairs of p-RNA sequences from Example 3, the immunological reagents from Example 3, and including a second protein conjugate consisting of Streptavidin chemically coupled to a murine monoclonal anti-subunit of human Chorionic Gonadotropin a simultaneous immunological detection of two different antigens was accomplished. The p-RNA capture strands successfully differentiated between their respective complementary strands such that the two antigen targets were differentially detected by the appropriate p-RNA capture strands.

This demonstrates the capability for multiple simultaneous immunological reactions to be performed in solution coupled with individual detection of the specific antigen targets of each of the individual immunological reactions. The separation of antigen target detection is accomplished by employing the selectivity of p-RNA strands for their respective complements to achieve selective antigen target detection.

Selectivity of the respective capture p-RNA capture strands (either #81 or #54) was evidenced that when the complementary strands of p-RNA (either #80 or #79, respectively) were bound to the Streptavidin-goat anti-human antibody protein complex in solution and then hybridized to an APEX chip containing the captures, the p-RNAs attached to the protein conjugate bound only to their respective complementary immobilized capture strands. This demonstrates the basis for enabling geographical sorting of immunological reagents that are simultaneously present in a homogeneous solution.

## EXPERIMENTAL SECTION

### Example 1 – Synthesis and Purification of p-RNA

The automated synthesis of the p-RNA oligonucleotides in a typical 15 μM scale was carried out using an ECOSYN$^{TM}$D 300$^{+}$– Eppendorf, BIOTRONIC DNA Synthesizer. Solid support derivatives derived from DMT-protected pyranosyl-nucleoside (A,T,G,C) precursers free at the 2'-position and benzoylated at the 3'-position were used for the p-

RNA synthesis. CPG solid support materials were also used in carrying out the standard phosphoramidite DNA synthesis and for incorporating the tryptamine ribopyranosyl **(I)** phosphoramidite monomer. All of these derivatives standard DNA phosphoramidites, DNA phorphoramidite pyranosyl nucleotide monomers, tryptamine ribopyranosyl **(I)** phosphoramidite monomers, as well as commercial phorphoramidite dyes (cyanine 3, cyanin 5, etc.), amino linker moieties, and biotin moities, can all be linked via the standard succinate linker to the CPG-support. The phosphoramidite synthesis methodology used followed preferably the allyl-oxy-phosphoramidite strategy described for DNA by Noyori et.al. (Y. Hayakawa, S. Wakabayashi, H. Kato, R. Noyori, J. Am. Chem. 1990, *112*, 1691).

The synthesis protocol included the following steps: (1) DMT deblocking was carried out using 6% dichloroacetic acid (v/v) in dichloromethane (100 ml); (2) washing with dichloromethane (20ml), washing with acetonitrile (20ml), and flushing with argon; (3) coupling procedure involved first washing the CPG solid support material with the activator (0,5 M pyridinium hydrochloride in dichloromethane (0,2ml), then 30 minutes treatment with 1/1- activator (0,76ml of the phosphoramidites (8 eq; 0,1 M dissolved in acetonitrile); (4) washing with acetonitrile (20ml); (5) the capping procedure involved a 2 minute treatment with 50% Cap A (10,5ml) and 50% Cap B (10,5ml) regents from PerSeptive (Cap A: THF, lutidine, acetic-anhydride, Cap B: 1-methylimidazole, THF, pyridine); (6) washing with acetonitrile (20ml); (7) the oxidation procedure involved a 1 minute treatment with 120 ml of the oxidation solution (a freshly prepared solution of 400 mg of iodine in 100 ml of acetonitrile, adding 9,2 ml of 2,4,6-collidine and 46 ml of water.).

Before cleavage from the solid support, the p-RNA-oligonucleotide is first allyl-deprotected at the phosphotriester linkages and at the guanine bases under the conditions described by *Noyori* and coworkers. (Y. Hayakawa, S. Wakabayashi, H. Kato, R. Noyori, J. Am. Chem. 1990, *112*, 1691). This is carried out by suspending the support in a mixture of 272 mg of $Pd(PPh_3)_4$, 272 mg of $PPh_3$ and 272 mg of $Et_2NH_2HCO_3$ in 15 ml of dichloromethane at room temperature. The suspension is vigorously shaken for 4 to 5 hours. The support is then carefully washed with dichloromethane (30 ml), acetone (30 ml) and water (30 ml) suspended for 30 minutes in a 0,1M solution of sodium diethyldithiocarbamate in water, and washed again with water (15 ml), acetone (15ml) and dichloromethane (15 ml). The cleavage from the solid support and the deacylation of the bases and sugars was effected by hydrazinolysis at 4°C within 25-40 hours (25% hydrazine hydrate in water, 6ml). Hydrazine is removed from the crude oligonucleotide by desalting over a Sep-Pak-cartridge (elution with acetonitrile/triethyl-ammonium-hydrogencarbonate 0,1M). The oligonucleotide containing fractions were combined and

evaporated to dryness. After a first purification of the oligonucleotide by HPLC (LiChrospher 100 RP-18 (10µM) Merck; buffer A: trietyl-ammonium-acetate 0,1M; buffer B: trietyl-ammonium-acetate 0,1M/ acetonitrile 1/4), the dimethoxytrityl group at the 4′-end was cleaved off by dissolving the oligonucleotide (dry) in formic acid/water = 3 / 2 at room temperature. After evaporation of the acid, the now fully deprotected oligonucleotide was dissolved in water and purified on a preparative HPLC-column (RP18). The combined product fractions were evaporated to dryness and then dissolved in a 0,1 M triethyl-ammonium-hydrogencarbonate and desalted over a Sep-Pak-C18 (Waters) cartridge. The eluted product was evaporated in vacuum, once dissolved with 2 ml of water and re-evaporated to dryness and then dissolved in 1 ml of water for the determination of the optical density. For checking the purity, the oligonucleotide was injected on an analytical RP18 column. (>95%). The product was characterized and identified by ESI-MS.

Example 2 – Procedure for Fluorescent Dye Labeling of p-RNA at 2'-Terminus

The following gives the basic procedure for incorporating a fluorescent dye into the 2'-terminal position of a p-RNA sequence. This provides just one of the many options for incorporation of reporter labels and other moieties into the p-RNA sequence.

About 100 mg of 3′-Amino-Modifier C3 CPG (purchased by Glenn Research) was poured in 2 ml of DMF and gently agitated for 5 minutes. The Fmoc protection group on the 3'-amino moiety was removed by a 10-minute treatment with 3.5 ml of DMF/Piperidine (6/4). The procedure was repeated a second time. The deprotected 3'-amino-C3-CPG-support material was then washed with 10 ml of DMF and 10 ml Acetonitrile. The 3'-amino-C3-CPG-support material was then dried under high vacuum for 10 minutes. Approximately 71 mg of deprotected 3′-amino-C3 CPG support material (43µmol/g, ~3,06µmol) was then placed into 0.4 ml of DMF with 0.05 ml of a saturated aqueous $NaHCO_3$ solution. About 5 mg (3,06µmol) of Texas Red-X succinimidyl ester (purchased from Molecular Probes) was dissolved in 0.1 ml of DMF (0,1 ml, and then added to deprotected 3′-amino-C3 CPG support material. The reaction mixture was then shaken for 4 hours at room temperature. The Texas Red dye reacted 3′-amino-C3 CPG support material was then washed with 10 ml of DMF/Pyridine. The reacted material had a blue color and red fluorescence. The remaining free amino groups on the modified CPG were capped with 0.4 ml acetic anhydride (with 0.2 g DMAP) in 2 ml Pyridine for 30 minutes. The dye modified CPG support was then washed with Pyridine, DMF, Methanol and Methyl-t-Butyl-Ether, and then dried under high vacuum. The Texas Red labeled 3′-Amino-C3 CPG support was then ready for the synthesis of a p-RNA oligonucleotide sequence using standard phosphoramide synthesis procedure after deblocking the DMT

protection group. This 2'-labelling procedure can be used for those fluorescent dyes which are stable through the remaining p-RNA synthesis procedure.

The structure of a synthesized 7-mer p-RNA labeled in the 2'-terminal position with Texas Red (TR-90 4'-**I**-G-A-A-G-G-G-**TR**-2', **I** = tryptamine, and **TR** = Texas Red) is shown in **Figure 20.** The mass spectrum for the TR-90 molecule is also shown in Figure 20. The structure of the TR90 sequence (4'-I-G-A-A-G-G-G-TR-2') hybridized with the complementary biotinylated B-92 sequence (4'-Biotin-C-C-C-T-T-C-T-I-C-C-C-C-G-2') is shown in **Figure 21**. The UV hypochromicity curves for the thermal denaturation of the hybridized pairs are also shown in Figure 21. This hybridization was carried out using 5 μM concentrations of each of the p-RNA sequence in 0.01 M Tris/HCL pH 7/0.15 M $NaCl_2$. The UV hypochromicity results shows the Tm for the hybridized pair was about 59 °C. The hybridized structure of the TR90 sequence (4'-I-G-A-A-G-G-G-TR-2') and the Cyanine-3 fluorescent dye labeled sequence Cy3-91 (4'-Cy3-C-G-G-G-G-G-I-2') now both hybridized with the complementary biotinylated B-92 sequence (4'-Biotin-C-C-C-T-T-C-T-**I**-C-C-C-C-C-G-2') is shown in **Figure 22.** The UV hypochromicity curves for the thermal denaturation of the hybridized pairs is also shown in Figure 22. This hybridization was carried out using 5 μM concentrations of each of the p-RNA sequence in 0.01 M Tris/HCL pH 7/0.15 M $NaCl_2$. The hypochromicity results show the Tm for the hybridized complex was about 61 °C.

Example 3 – Procedure for Attachment of Peptide Moiety to p-RNA via Tryptamine Linker

The following example is the procedure that would be used to attach a peptide moiety to a p-RNA oligomer containing a tryptamine group in place of a normal base moiety. This procedure first involves the incorporation of a tryptamine nucleoside into the p-RNA sequence, which then provides an aliphatic amine group for the subsequent coupling of the peptide to the p-RNA.

An iodacetylation of p-RNA with N-(iodacetyloxy)-succinimide was carried out using 1 equivalent of the tryptamine amino-linked p-RNA-oligomer in a 0.1 molar solution of sodium-bicarbonate (pH 8.4; 1 ml per 500 nmol oligo) mixed with a solution of 70 equivalent of N-(iodacetyloxy)-succinimide (M = 283.018) in DMSO (10 % = 0.1 ml per 500 nmol oligo). The tube was kept in the dark at ambient temperature (25°C) for approximately 30-90 minutes. Completion of reaction was monitored by HPLC [Buffer A : 0.1 M triethylammonium acetate in water buffer B : 0.1 M triethylammonium acetate in water : $CH_3CN$ = 1 : 4 method 1 : starting with 10 % buffer B; proceeding to 50 % buffer B in 40 minutes; observation wavelength 260 nm method 2 : starting with 10 % buffer B; proceeding to 45 % buffer B in 100 minutes; observation wavelength 260 nm on a Merck

10 μM LiChrosphere ™ 100 RP-18 column; 250 x 4 mm. The product was eluted anywhere from 4 to 9 minutes after the unmodified iodacetylated oligonucleotide. The product was desalted and further purified by standard work-up procedures on a Sep Pak™ cartridge. The solution was poured over an activated Sep Pak™ cartridge, washed with 20 ml 0.1 M TEAB buffer solution and eluted with pure acetonitrile. The product yield was determined by UV absorption at 260 nm, and then the product was lyophilyzed to dryness using a vacuum centrifuge.

The iodacetylated p-RNA was dissolved in a buffer system containing Borate-HCL buffer and $Na_2EDTA$ (pH 7.6; 100 μl per 10 nmol oligo) and mixed with 30-60 equivalents of the peptide in 100 μl DMF. (Buffer system: Borax/HCl-buffer; Riedel-de Haën, pH 8.0, mixed with an equal amount of a 10 mM solution of $Na_2EDTA$ in water, the pH was adjusted to 7.6 with HCl, the resulting solution contained ~5 mM $Na_2EDTA$). The reaction was carried out in the dark, at ambient temperature (25°C), for approximately 6-12 hours. The completion of reaction was monitored by HPLC. The HPLC buffer system was buffer A 0.1M triethylammonium acetate in water, and buffer B 0.1M triethylammonium acetate/$CH_3CN$ in water at a 1 to 4 ratio. Elution was started with 90% buffer A/10% buffer B, and proceeded to 50% buffer A/50% buffer B by 40 minutes; the elution was monitored at 260nm and 220nm, the HPLC column material used was Merck 10μM LiChrosphere ™100 RP-18 in a 250mm by 4mm column. After the iodacetylated oligomer had disappeared, the final product was isolated by the same HPLC procedure and desalted using the above Sep Pak™ cartridge procedure.

Procedure for Multiple p-RNA-Tryptamine Attachment Sites

Other p-RNA-peptide intermolecular architectures can be created by modification of p-RNA with multiple tryptamine linker sites within the same p-RNA oligonucleotide structure. An example of one such p-RNA sequence with multiple-tryptamine linkers (4'-C-C-I-I-I-G-G-2') that has been synthesized and characterized is shown in Figure 23. The UV hypochromicity and melting characteristics for the hybridized pair is also shown in Figure 23. The melting temperatures (Tm) and thermodynamic properties for other hybridized pairs of p-RNA's with one, two and three tryptamine linkers (includes multiple tryptamine linkers in series and with intervening nucleotides) are given in Table 1 below.

TABLE 1

| | elting emp. | Thermodynamic Pairing Data for Hybridized p-RNA -Tryptamine Oligomers (Kcal/Mol) | | | |
|---|---|---|---|---|---|
| **Sequence** | $T_m$(°C) 10μM | $\Delta H_{VH}$ | $T\Delta S_{VH}$ 298°K | $\Delta G_{VH}$ 298°K | K (M) 298°K |
| PRNA - CCCGGG * | 68 | -48,5 | -35,5 | -13,0 | - |
| pRNA - **CCCIGGG** | 63 | -54,1 | -41,2 | -12,9 | $2,8 .10^9$ |
| pRNA - **CCCIIGGG** | 64 | -51,4 | -38,6 | -12,8 | $2,3 .10^9$ |
| pRNA – CCIIIGG | 24 | -43,5 | -36,9 | -6,6 | $7,5 .10^4$ |
| pRNA - CCICGIGG | 49 | -88,5 | -75,3 | -13,2 | $4,7 .10^9$ |
| pRNA - CICIGIG | no hybrid pairing detected | | | | |

The solution hybridization properties for the sequences in Table 1 showed the desired cooperative behaviors, including Tm, melting curve, and thermodynamic properties. Additionally, the sequences were characterized by mass spectroscopy (see Table 2). Those examples demonstrate that two or three juxtaposed tryptamine linkers in a short p-RNA oligomer sequence still exhibit appropriate hybridization properties.

TABLE 2

| **Sequence** | $MH^+$] found | $MH^+$] calc. |
|---|---|---|
| pRNA - CCCIGGG | 2243,4 | 2243,4 |
| pRNA - CCCIIGGG | 2597,5 | 2597,5 |
| pRNA - CCIIIGG | 2303.6 | 2302.7 |
| pRNA - CCICGIGG | 2597,6 | 2597,5 |
| pRNA - CICIGIG | 2301,4 | 2302,7 |

An example of a multiple tryptamine p-RNA-peptide conjugate (4'-CCC-I*-I*-GGG-2' with *two-Cys-Phe-Pro-Tyr-Trp-Gly peptides) is shown in **Figure 24**. The $NH_2$-Cys-Phe-Pro-Tyr-Trp-Gly-$CO_2H$ peptides were linked through the thiol group of the cysteine amino acid to the primary amino group of tryptamine via a heterobifunctional linker (succinimidyl ester of iodoacetic acid). The mass spectrometric analysis of the p-RNA –peptide structure is also shown in Figure 24, and in Table 3 below.

TABLE 3

| **Sequence** | $M-3H]^{3-}$ found | $M-3H]^{3-}$- calc. |
|---|---|---|
| pRNA – CCCI*I*GGG | 1407,9 | 1406,2 |

The double tryptamine p-RNA architecture allows four peptides residues to be incorporated into one supramolecular structure when two p-RNA peptide sequences hybridize to the complementary capture p-RNA peptide sequence (this is shown in **Figure 25)**. The UV hypochromicity curves show the melting ($T_m$) of the double-stranded p-RNA-peptide structure is around 50 $^0$C. This is consistent with the predicated $T_m$ for these p-RNA sequences. The fact that hybridization occurs in the presence of the large and bulky peptide structures indicates that the tryptamine linker arrangement produces favorable stereochemistry for attachment of peptides. Additionally, measurement of the tryptophan fluorescence could be used to follow the formation of the p-RNA hybrids in solution.

Example 4 - Demonstration of Electronic Stringency for a p-RNA-Peptide Triad Intermolecular Structure

This experiment involves the use of electronic stringency to determine the electronic $T_m$ for combinations of the p-RNA sequences #70:#72, #71:#72 and #71:#72. The sequences of the p-RNA's are shown below:

#70 4'-I-G-A-A-G-G-2'

#71 4'-G-G-G-G-G-I-2'

#72 4'-C-C-T-T-C-I-C-C-C-C-C-2'

The #70 sequence is complementary to the 4' end of the #72 sequence, and the #71 sequence is complementary to the 2' end of the #72 sequence. When all three are hybridized together #70/#71: #72, there is only one unpaired base remaining in the #72 sequence, which is the tryptamine (I) at base position 6. The 4'-terminal (I) of the #70 sequence and the 2'-terminal (I) of the #71 sequence also remain unpaired. (The Tryptamine (I) moieties are the points of attachment for adding peptide sequences or other ligand binding structures).

The thermal $T_m$ for the hybridized combination of the p-RNA sequences #70:#72 was 49$^0$ C; the #71:#72 was 49$^0$ C; and fully paired structure #70/#71:#72 was 49$^0$ C. The electronic $T_m$s for hybridized combination of the p-RNA sequences #70:#72 was 230 nanoamperes (nA); the #71:#72 was 215 nA; and #71:#72 was 250 nA. These results show that there is reasonable consistency between the thermal and the electronic $T_m$s. In fact, the electronic stringency seems to better differentiate full structure #70/#71:#72, from the high $T_m$, G:C rich #70:#72 hybrid pair. There is only weak evidence (from the electronic $T_m$) that there is very much additional stabilization of the fully paired structure #70/#71:#72 due to any base stacking effects. This is an advantage because it now means that the stabilized full structures with peptide sequences (triad) and bound ligand molecules (supramolecular complexes) can be more readily detected and differentiated.

Example 5 - Demonstration of Supramolecular Structures via Alteration of Electronic $T_m$'s for Metal (Nickel) Ligand Binding p-RNA-Peptide Triad Suprastructures

These experiments are designed to show the basic concept that specific ligand binding to a specific p-RNA-peptide supramolecular structure "triad" will produce a more stabilized structure "supramolecular" structure which can be differentiated by an increase in it's thermal and/or electronic $T_m$. In this example, nickel ($Ni^{2+}$) is used as a specific metal ligand for p-RNA-peptide structures in which the peptide sequences are rich in histidine (the imidazole R-groups of histidine are expected to be strong binders of metal ions, nickel in particular). The capture p-RNA structures used in this experiment were derived from p-RNA sequence #72 (see Example), and included a #72 p-RNA capture sequence with no peptide attached, the Cap-72-NP component, a # 72 p-RNA capture sequence with the hexapeptide N-Cys-His-His-His-His-Gly-C (-CHHHHG) attached, the Cap-72-CHHHHG component, and a # 72 p-RNA capture sequence with the hexapeptide N-Cys-Phe-Pro-Ser-Phe-Gly-C (-CFPSFG) attached, the Cap-72-CFPSFG component. The #71 p-RNA sequence was derivatized with the hexapeptide N-Cys-His-His-His-His-Gly-C (-CHHHHG) and also with the fluorescent dye (Cy3) to form the Cy3-71-CHHHHG component. The #70 p-RNA sequence was derivatized with the hexapeptide N-Cys-His-His-His-His-Gly-C (-CHHHHG) to form the 70-CHHHHG component. Formation of all five hybridized "triad" suprastructures was demonstrated, which included: (1) 70-CHHHHG/ Cy3-71-CHHHHG:Cap-72-NP; (2) 70-CHHHHG/ Cy3-71-CHHHHG:Cap-72- CHHHHG; (3) 70-CHHHHG/ Cy3-71-CHHHHG:Cap-72- CFPSFG. In further experiments, hybridization was demonstrated for the three capture p-RNA #72 sequences (Cap-72-NP, Cap-72-CHHHHG and Cap-72-CFPSFG), with a 70-BTR (Bodipy Texas Red fluorophore) sequence and the 70-CHHHHG component; and with a Cy3-71 and a Cy3-71-CHHHHG component.

ELOC type experiments were carried out using the addition of $NiCl_2$ (10 μM) as the specific metal ligand for full p-RNA-peptide "triad" containing the CHHHHG ligand binding CHHHHG peptides. At 10 μM NiCl the 70-CHHHHG/ Cy3-71-CHHHHG:Cap-72-NP hybridized "triad" electronic $T_m$ was increased from 260 nA –280 nA range with 50% signal loss (without Ni present) to 700 nA with only a 20% loss of signal (with Ni present). This is indicative of a highly stabilized "Supramolecular Complex" when the Ni ligand is present. As a control the a Cy3-71-CHHHHG:Cap-72-NP hybridized with and with out Ni present in the solution and no change was observed in the electronic $T_m$.

Example 6 - Demonstration of Moderate Density Addressing of p-RNA 's to 400 Test Sites on a 10,000 Site Microelectronic Array

An APEX chip with 10,000 sites using individual site current control was coated with an agarose:streptavidin permeation layer. 300 sites were simultaneously electronically biased with a positive potential and a current of 100 nanoamperes (nA). A solution containing 1 M of p-RNA #92 was overlayed over the biased sites and the p-RNA immobilized by binding between the permeation layer immobilized Streptavidin and the 4'-biotin. The solution containing p-RNA #92 was removed and replaced with a solution containing 1 M of p-RNA #72 and an additional 100 sites were electronically biased with a positive potential at a current of 100 nanoamperes and the p-RNA immobilized by the binding of the 4'-biotin to the immobilized Streptavidin. The solution containing the p-RNA #72 was removed and replaced by a solution containing 1 M of p-RNA #91 which had been labeled with the fluorescent dye Cyanine3.

#92 4' Biotin-C-C-C-T-T-C-T-I-C-C-C-C-C-G-2'

#91 4' C-G-G-G-G-G-I-Cy3-2'

#72 4' Biotin-C-C-T-T-C-I-C-C-C-C-C-2'

The 400 sites represented by the previous two p-RNA binding events were electronically biased with a positive potential at a current of 100 nanoamperes and the mobile p-RNA oligmer, Cy3 labeled #91, was allowed to hybridize for 30 seconds with the immobilized capture strands, #92 and #72. The fluid containing p-RNA #91 was removed and the chip washed to remove residual fluid and then imaged on a fluorimeter. The resulting image demonstrated that the p-RNA oligmer #91 hybridized only to its matching complementary strand #92 and not to the non-complementary strand #72. This demonstrates moderate scale electronically mediated multi-site hybridization with hybridization mediated strand discrimination (see Figure 14).

Example 7 - Demonstration of Novel Immunoreagent Immobilization Techniques via p-RNA Tethers

For this experiment the following pairs of complementary p-RNA constructs were used as immobilization tethers for a protein conjugate consisting of Streptavidin and a goat anti-human $F(ab')_2$ antibody:

#80 4' Biotin-I-G-G-G-A-A-G-G-G-2'

#81 4' Biotin-C-C-C-T-T-C-C-C-2'

#54 4' Biotin-T-A-G-G-C-A-I-T-2'

#79 4' Biotin-A-I-T-G-C-C-T-A-2'

p-RNA #81 was used to provide a capture sequence for p-RNA #80 by binding the biotin of p-RNA #81 to Streptavidin which had been immobilized in the permeation layer

covering an APEX chip. The biotin of p-RNA #80 was then used to bind to a mobile streptavidin which had been chemically conjugated to a goat anti-human $F(ab')_2$ antibody. The goat anti-human $F(ab')_2$ antibody was used to capture its specific antigen target which is human IgG $F(ab')_2$ antibody.

Similarly the complementary pair of p-RNAs #54 and #79 were used to form another immobilization tether. p-RNA #54 was immobilized to a permeation layer overlaying an APEX chip by binding its 4' biotin to Streptavidin which was immobilized in the permeation layer. p-RNA #79 was hybridized to its complementary strand #54 and the 4' biotin of #79 was used to immobilize the Streptavidin half of a solubilized conjugate of Streptavidin and goat anti-human $F(ab')_2$ antibody. The goat anti-human F(ab')2 antibody was then used as an immunosorbent to capture it's target antigen which is human IgG $F(ab')_2$ antibody.

Selectivity of the respective capture p-RNA capture strands (either #81 or #54) was evidenced when the complementary strands of p-RNA (either #80 or #79, respectively) were bound to the Streptavidin-goat anti-human antibody protein complex in solution and then hybridized to an APEX chip containing the captures, the p-RNAs attached to the protein conjugate bound only to their respective complementary immobilized capture strands. This demonstrates the basis for enabling geographical sorting of immunological reagents which are simultaneously present in a homogeneous solution.

Example 8 - Demonstration of Novel Methods for Achieving A Simultaneous Multiple Homogeneous Assays Combined with Discrete Analyte Detection

Using the complementary pairs of p-RNA sequences from Example 7, the immunological reagents from Example 3, and including a second protein conjugate consisting of Streptavidin chemically coupled to a murine monoclonal anti-subunit of human Chorionic Gonadotropin, a simultaneous immunological detection of two different antigens was accomplished. The p-RNA capture strands successfully differentiated between their respective complementary strands such that the two antigen targets were differentially detected by the appropriate p-RNA capture strands. Optionally, a sandwich format detection may be utilized, such as where a fluorescently labeled antigen interacts with the antibody.

This demonstrates the capability for multiple simultaneous immunological reactions to be performed in solution coupled with individual detection of the specific antigen targets of each of the individual immunological reactions. The separation of antigen target detection is accomplished by employing the selectivity of p-RNA strands for their respective complements to achieve selective antigen target detection. Detection

may be by direct detection, e.g., electrical, optical or other direct detection, or by a sandwich format, such as through use of another fluorescently labeled antibody.

Example 9 - Demonstration of an Analysis Algorithm for Deconvolution of Data from Targeting with a Sublibrary

The following is a description of the type of algorithms that will be used to deconvolute and analyze the large amount of data that would come off a ELOC combinatorial arrays after a drug/ligand binding experiment. This type of analysis will be important for determining the molecular descriptor properties of the ELOC arrays. Malinowski and Howery (E. R. Malinowski and D.G. Howery, Factor Analysis in Chemistry, John Wiley & Sons, New York, 1980) have described a mathematical technique for a model independent approach to find from such a set of measurement data sets of abstract data in the same dimensions as the measurements called Factors that can be combined to reproduce the original data of the measurements. The mathematical derivation of these factors is such, that each factor explains the maximum amount of variance in the data set not accounted for by other factors.

Any set of data that can be represented as a matrix can be treated by factor analysis (P. M. Fredericks, J. B. Lee, P. R. Osborn and D. A. J. Swinkels Materials Characterization using FT-IR Spectra. Part 2: Mathematical and Statistical Considerations, Applied Spectroscopy, 39, 2 (1989), p. 311). Such data can be treated as a matrix where each column contains the value measured of one set (intensities against time, voltage concentration etc.). A data matrix containing variables of the conditions (temperature, pH, ionic strength, stringency, etc.) coming from 30 moments or snapshots of the detected intensities at 10,000 test sites (on the combinatorial chip/array) throughout the experiment, would be a dynamic representation of signals evolving through 30 moments in time, temperature or any other changing parameter. As the intensity measurements may be regarded in first order as a representation of molecules found in the optical focus, the use of a linear correlation model proves very useful for cluster analysis of similar chip events per position as well as for a noise reduction and a reconstruction (recombination) of idealized signal evolution and signal correlation in reasonable time for the large data sets created.

A simulation of data handling using the Factor Analysis format, would show the intensities measured at 10,000 test sites (on the combinatorial test chip/array) at moment 1 in column 1, at moment (condition) 2 in column 2, and so on. Thus, a reconstruction of the Data set by **(f)** chosen orthogonal Factor for a maximum of 30 Factor sets is given by the equation:

**Data Matrix** [10 000 x 30] = **Variable loading matrix** (Factors) [10 000 x f] **x Score-matrix** [f x 30].

The factor scores are the weighting factor attributed to each factors (1 to 30). Factors with contributions below the signal to noise ratio may be regarded as non-correlating noise without substantial physical or experimental meaning. Chip positions however, that show similar events (in shape or intensity) will show similar contribution in their major factors. A plot of the first two factor columns (variable loading matrix) often yields sufficient information to identify the position similarities with respect to the event. **Figure 26** shows a simulation of a typical measurement starting from a normalized intensity at three positions of a set of 30 points (due to 30 conditions, time, temperature, stringency etc. here normalized to a total of 1) superimposed with typical noise. **Figure 27** shows the underlying idealized signal development. In fact, we calculated realizable signals of pairing events for 7 position with the growth and sigmoid decay, 3 positions with the simple sigmoid decay and the remaining 9990 with the linear intensity decay throughout the experiment. **Figure 28** shows the excellent and striking clustering in a plot of only the first main attributing factors as described above 7 correlating positions showing similar events as a cluster at the lower left part and three positions at the upper right part of the cloud of clustered events with all the same linear signal development. A recalculation of the events using the linear equation above and f = 2 yields idealized noise reduced events for these positions. This procedure can be used with pretreated data to look for similarities in binding, clustering of consensus peptide sequences and the possibility to identify peptide sequences that correspond to the same set of subunits but distributed differently with respect to the immobilized species and the soluble subunits.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it may be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Claims:

1. An array based molecular descriptor device for interacting with a specific ligand molecule or structure comprising:

an array of electronic microlocations,

capture intermolecular ligand binding components coupled to the microlocations, the intermolecular ligand binding components including a capture programmable pairing component and a ligand binding component, the capture programmable pairing component being the same at the various microlocations and the ligand binding component being different at the various microlocations, and

supramolecular complexes formed at least certain of the microlocations, the supramolecular complex comprising said capture intermolecular ligand binding component, a first programmable pairing component complementary to, at least, a portion of the capture programmable pairing component, including a ligand binding component, and a second programmable pairing component complementary to the capture programmable pairing component and having a ligand binding component, and a specific ligand molecule or structure, such that upon binding of the specific ligand molecule or structure a specific supermolecular complex forms, via the pairing of the specific first programmable pairing component, the second programmable pairing component and the capture programmable pairing component.

2. The array based device of claim 1 wherein the programmable pairing components include p-RNA.

3. The array based device of claim 1 wherein the programmable pairing component includes CNAs.

4. The array based device of claim 1 wherein the programmable pairing component includes a composite system.

5. The array based device system of claim 4 wherein the composite system includes both p-RNA and DNA.

6. The array based device of claim 1 wherein the programmable pairing component includes nucleic acid.

7. The array based device of claim 6 wherein the nucleic acid is DNA.

8. The array based device of claim 6 wherein the nucleic acid is RNA.

9. The array based device of claim 1 wherein the electronic microarray has independently controllable electronic microlocations.

10. The array based device of claim 1 wherein the ligand binding components include peptides.

11. The array based device of claim 10 wherein the ligand binding components comprise peptide sequences.

12. The array based device of claim 11 wherein the peptide sequences are hexamer peptide sequences.

13. The array based device of claim 1 wherein the ligand binding component includes an amino acid.

14. The array based device of claim 1 wherein the ligand binding component includes an antibody.

15. The array based device of claim 1 wherein the ligand binding component includes a protein.

16. The array based device of claim 1 wherein the ligand binding component includes an enzyme.

17. The array based device of claim 1 wherein the ligand binding component includes a metal chelator.

18. The array based device of claim 1 wherein the ligand binding components are selected from a combinatorial library.

19. The array based device of claim 1 wherein the ligand binding components comprise a combination of various ligand binding components.

20. The array based device of claim 19 wherein one ligand binding component comprises a peptide ligand binding component and another ligand binding component comprises a metal chelator.

21. The array based device of claim 1 wherein the specific ligand molecule comprises a small molecule.

22. The array based device of claim 21 wherein the small molecule comprises a drug.

23. The array based device of claim 21 wherein the small molecule comprises a metabolite.

24. The array based device of claim 21 wherein the small molecule comprises a metal ion.

25. The array based device of claim 21 wherein the small molecule comprises a peptide.

26. The array based device of claim 1 wherein the specific molecule comprises a large molecule.

27. The array based device of claim 26 wherein the large molecule comprises a protein.

28. The array based device of claim 26 wherein the large molecule comprises an enzyme.

29. The array based device of claim 26 wherein the large molecule comprises an antibody.

30. The array based device of claim 26 wherein the specific structure comprises an organelle.

31. The array based device of claim 26 wherein the specific structure comprises a cell.

32. The array based device of claim 1 further including an electronic stringency system.

33. The array based device of claim 32 wherein the electronic stringency system includes a perturbation system.

34. The array based device of claim 1 further including an illumination source.

35. The array based device of claim 34 further including a detector.

36. The array based device of claim 1 further including an analysis system.

37. The array based device of claim 1 further including a display.

38. The array based device of claim 1 further including an electronic control system.

39. The array based device of claim 1 further including a data processing system.

40. An array based molecular descriptor device for interacting with a specific ligand molecule or structure comprising:

an array of electronic microlocations,

intermolecular ligand binding components coupled at the microlocations, the intermolecular ligand components including a p-RNA capture programmable pairing component and a ligand binding component, the capture p-RNA programmable pairing component being the same at the various microlocations, and the peptide ligand binding component being different at the various microlocations, and

supermolecular complexes formed at least certain of the microlocations, the supermolecular complex comprising said capture intermolecular ligand binding structure, a first p-RNA programmable pairing component complementary to at least a portion of the capture programmable pairing component, including a peptide ligand binding component, and a second p-RNA programmable pairing component complementary to the capture programmable pairing component and having a peptide ligand binding component, and a specific ligand molecule or structure, such that in the presence of the specific ligand molecule or structure a specific supermolecular structure forms via the selective binding

by the specific first programmable pairing component, the second programmable pairing component and the capture programmable pairing component.

41. A method for the combinatorial selection of supermolecular complexes on an electronic array based device comprising the steps of:

providing capture intermolecular ligand binding structures at a plurality of sites on the array, each capture intermolecular ligand binding structure having a common capture programmable pairing component and a different ligand binding component,

providing complementary intermolecular ligand binding structures to the device, having a programmable pairing component which is complementary to the capture programmable pairing component and includes a ligand binding component, and a specific ligand molecule or structure,

setting the stringency conditions on the device to effect supermolecular complex formation, and

detecting the microlocations at which supermolecular complexes have been formed.

42. The method of claim 41 wherein the stringency conditions include electronic stringency.

43. The method of claim 42 wherein the electronic stringency conditions include electronic perturbation.

44. The method of claim 41 wherein the stringency conditions include conventional stringency conditions.

45. The method of claim 44 wherein the stringency conditions include temperature.

46. The method of claim 44 wherein the stringency conditions include pH.

47. The method of claim 44 wherein the stringency conditions include ionic strength.

48. The method of claim 44 wherein the stringency conditions include chemical agents.

49. The method of claim 44 further including the step of determining the ligand binding components involved in a given supermolecular complex.

50. The method of claim 44 wherein the identity of the ligand binding components is used in the selection of ligand binding components for a second array.

51. The method of claim 44 wherein the identity of the ligand binding components is utilized to form synthetic antibodies.

52. The method of claim 44 wherein the identity of the ligand binding components is utilized to form new affinity reagents.

53. The method of claim 44 wherein the identity of the ligand binding components is utilized to form synthetic catalysis/enzymes.

54. The method of claim 44 wherein the identity of the ligand binding components is utilized to form new metal chelate structures.

55. A method for determining an expected biological response of a test substrate (molecule) comprising the steps of:

providing a molecular descriptor array on which supramolecular complexes may form at definable locations,

applying at least one known substrate, monitoring the response of the known substrate on the molecular descriptor array and developing a response profile therefrom,

applying a test substrate (molecule) to the molecular descriptor array and monitoring the response thereto, and

analyzing the response of the test substrate (molecule) on the molecular descriptor array in relationship to the response from the known substrate so as to determine the expected biological response of the test substrate.

56. A method for drug discovery comprising the steps of:

providing a molecular descriptor array having a plurality of definable locations,

providing a drug to the molecular descriptor array, and monitoring the response of the molecular descriptor array to the drug, thereby developing a response profile for the drug,

subsequently providing in series related compounds to the molecular descriptor array, monitoring the response of the molecular descriptor array to the related compounds, thereby developing further response profiles for the related compounds, and

providing a test compound to the molecular descriptor array, monitoring the response of the molecular descriptor arrays to the test compound and analyzing the response of the test compound relative to the response profiles for the drug and related compounds so as to predict expected response of the test compound.

57. The method of claim 56 wherein the related compound is an agonist.

58. The method of claim 56 wherein the related compound is an antagonist.

59. The method of claim 56 wherein the related compounds are inhibitors.

60. The method of claim 56 wherein the related compounds are toxins.

61. A method for evolving an improved molecular descriptor array comprising the steps of:

providing a first molecular descriptor array,

applying a specific ligand molecule or structure to the first molecular descriptor array, monitoring the response to the specific ligand molecule or structure on the molecular descriptor array, and identifying supermolecular structures and their specific ligand binding components, and

providing a second library of ligand binding components, the components of the library being selected at least in part based upon the identified ligand binding components from the first molecular descriptor array interaction, and providing a second molecular descriptor array utilizing the second combinatorial library.

62. The method of claim 61 wherein the libraries include peptide sequences.

63. The method of claim 61 wherein the second library includes peptide sequences which are longer than the peptide sequences in the first library.

64. The method of claim 61 wherein the second library includes other amino acids not included in the first library.

65. The method of claim 61 wherein the second library comprises a subset of the first library.

66. A method for formation of supermolecular complexes on an array, comprising the steps of:

providing an array of microlcations,

providing capture intermolecular ligand binding structures at the microlocations, the capture intermolecular binding structures having capture programmable pairing components which are common to the various microlocations and ligand binding components which vary from microlocation to microlocation,

contacting the array with a solution containing intermolecular ligand binding components a and intermolecular ligand binding components b, under conditions where the programmable pairing components of the intermolecular binding structures a and b are in dynamic equilibrium with the pairing components of the capture intermolecular ligand binding structure,

introducing a specific ligand molecule or structure, and

forming supermolecular complexes at certain of the microlocations.

67. The method of claim 66 wherein the dynamic equilibrium conditions are at, or near, the melting temperature of the supermolecular complex comprising the capture intermolecular ligand binding structure and the intermolecular ligand binding structures a and b.

68. The method of claim 66 wherein the dynamic equilibrium conditions are at 5°C or less than the melting temperature.

69. The method of claim 66 wherein the array is an active microelectronic array.

70. The method of claim 66 wherein the dynamic equilibrium conditions include electronic stringency conditions.

71. The method of claim 66 wherein the electronic stringency conditions include electronic perturbation.

72. A method for the formation of supermolecular complexes comprising the steps of:

providing in a solution phase a specific ligand molecule or structure, and intermolecular ligand binding structure a and an intermolecular ligand binding structure b under conditions to form a dimer complex of the specific ligand molecule or structure, intermolecular ligand binding structure a and intermolecular ligand binding structure b, and

contacting the dimer complex with an array of capture intermolecular ligand binding structures, each capture intermolecular ligand binding structure having a common capture programmable pairing component and a different ligand binding component, under conditions so as to form a supermolecular structure consisting of a dimer complex and a capture intermolecular ligand binding component.

73. The method of claim 72 wherein the formation of the dimer complex is done separate from the array.

74. The method of claim 72 wherein the dimer complexes are formed in solution on the array.

75. The method of claim 72 wherein the array is an electronic array.

76. The method of claim 72 wherein the electronic array is placed in a repulsive condition to the components during the formation of the dimer complexes.

77. A method for the formation of supermolecular structures on an electronic microarray comprising the steps of:

providing an array of microlocations, each microlocation including a capture intermolecular ligand binding structures, the intermolecular ligand binding structure having a common programmable pairing component and varying ligand binding components,

contacting the array with intermolecular ligand binding structures a and intermolecular binding structures b having programmable pairing components adapted to pair with the capture programmable pairing component, and ligand binding components,

introducing the specific ligand molecule or structure, and

varying the stringency conditions to determine the specific supermolecular complex locations on the array.

78. The method of claim 77 wherein the stringency conditions include electronic stringency conditions.

79. The method of claim 77 wherein the electronic stringency conditions include electronic perturbation.

80. A method for performing multiple immunological reactions on a microelectronic array device comprising the steps of:

providing a plurality of microelectronic sites, each site having a programmable pairing component couple thereto, the programmable pairing components varying from site to site,

providing a plurality of different types of antibodies, each type of antibody being labeled with a different programmable pairing component, the different programmable pairing components being complementary to the programmable pairing components coupled to the microelectronic sites,

providing an antigen,

reacting the antigen and the plurality of labeled, different types of antibodies with the programmable pairing components at the microelectronic sites,

interacting the programmable pairing component and the complement programmable pairing, and

determining the sites at which an antibody coupled to the antigen coupled to the complementary programmable pairing component is present.

81. The method of claim 80 wherein the antigen and plurality of labeled, different types of antibodies are provided together in a homogeneous format.

82. The method of claim 80 wherein the programmable pairing component and the complement to the programmable pairing component are p-RNA.

83. A device for the formation and detection of supramolecular complexes comprising:

an attachment surface, the attachment surface being located in a variable electronic environment,

a molecular recognition system, including at least a first and a second molecular recognition component, at least the first component being attached to the surface,

a separate molecular species bound to the molecular recognition system, and

a third structure for formation of a supramolecular complex with the separate molecular species.

84. The device for the formation and detection of supramolecular complexes of claim 83 wherein the molecular recognition system comprises a pairing system.

85. The device for the formation and detection of supramolecular complexes of claim 84 wherein the pairing system is a complementary pairing system.

86. The device for the formation and detection of supramolecular complexes of claim 85 wherein the pairing system is a complementary and coded pairing system.

87. The device for the formation and detection of supramolecular complexes of claim 86 wherein the complementary pairing system is a p-RNA pairing system.

88. The device for the formation and detection of supramolecular complexes of claim 83 wherein the separate molecular species includes a peptide.

89. The device for the formation and detection of supramolecular complexes of claim 88 wherein the separate molecular species comprises a peptide sequence.

90. The device for the formation and detection of supramolecular complexes of claim 88 wherein the separate molecule species comprises an antibody.

91. The device for the formation and detection of supramolecular complexes of claim 88 wherein the separate molecule species comprises an antibody fragment.

92. The device for the formation and detection of supramolecular complexes of claim 88 wherein the separate molecule species comprises a specific binding protein.

93. The device for the formation and detection of supramolecular complexes of claim 88 wherein the separate molecule species comprises a specific biological binding site.

94. The device for the formation and detection of supramolecular complexes of claim 93 wherein the physiological binding site is an isolated biological binding site.

95. The device for the formation and detection of supramolecular complexes of claim 93 wherein the physiological binding site is a cloned biological binding site.

96. The device for the formation and detection of supramolecular complexes of claim 93 wherein the physiological binding site is a mimicry biological binding site.

97. The device for the formation and detection of supramolecular complexes of claim 93 wherein the physiological binding site is a fused biological binding site.

98. The device for the formation and detection of supramolecular complexes of claim 83 wherein the separate molecular species are from a combinatorial library.

99. The device for the formation and detection of supramolecular complexes of claim 98 wherein the library includes more than one species.

100. The device for the formation and detection of supramolecular complexes of claim 98 wherein the library includes at least 10 species.

101. The device for the formation and detection of supramolecular complexes of claim 98 wherein the library includes at least 100 species.

102. The device for the formation and detection of supramolecular complexes of claim 98 wherein the library includes at least 1000 species.

103. The device for the formation and detection of supramolecular complexes of claim 98 wherein the library includes at least 10,000 species.

104. The device for the formation and detection of supramolecular complexes of claim 98 wherein the library is a sublibrary aggregated to form a higher order library.

105. The device for the formation and detection of supramolecular complexes of claim 83 wherein a single separate molecular species is bound to the molecular recognition system.

106. The device for the formation and detection of supramolecular complexes of claim 105 wherein a cooperative ensemble of molecular species are bound to the molecular recognition system.

107. The device for the formation and detection of supramolecular complexes of claim 106 wherein there are two molecular species (a diad).

108. The device for the formation and detection of supramolecular complexes of claim 106 wherein there are three molecular species (a triad).

109. The device for the formation and detection of supramolecular complexes of claim 98 wherein the components of the library identify specific molecular properties.

110. The device for the formation and detection of supramolecular complexes of claim 98 wherein the components of the library identify specific chemical properties.

111. The device for the formation and detection of supramolecular complexes of claim 109 wherein the property is binding.

112. The device for the formation and detection of supramolecular complexes of claim 109 wherein the property is molecular recognition.

113. The device for the formation and detection of supramolecular complexes of claim 109 wherein the property is chemical.

114. The device for the formation and detection of supramolecular complexes of claim 109 wherein the property is physical activity.

115. The device for the formation and detection of supramolecular complexes of claim 83 wherein the separate molecular species is covalently bound to the molecular recognition system.

116. The device for the formation and detection of supramolecular complexes of claim 83 wherein the separate molecular species is supermolecularly bound to the molecular recognition system.

117. The device for the formation and detection of supramolecular complexes of claim 116 wherein the supramolecular binding include a strepdavidin/biotin interaction.

118. The device for the formation and detection of supramolecular complexes of claim 83 wherein the separate molecular species is bound to both the first and the second molecular recognition component.

119. The device for the formation and detection of supramolecular complexes of claim 83 wherein the supramolecular complex includes covalent binding.

120. The device for the formation and detection of supramolecular complexes of claim 83 wherein the supramolecular complex includes supramolecular binding.

121. The device for the formation and detection of supramolecular complexes of claim 98 wherein there are n species of the types and the number of variations is $b^n$.

122. The device for the formation and detection of supramolecular complexes of claim 83 further including a detector.

123. The device for the formation and detection of supramolecular complexes of claim 122 where the detector detects the presence of a supramolecular complex.

124. The device for the formation and detection of supramolecular complexes of claim 122 where the detector detects a property respecting the assembly of the supramolecular complex.

125. The device for the formation and detection of supramolecular complexes of claim 122 where the detector detects a property respecting the disassembly of the supramolecular complex.

126. The device for the formation and detection of supramolecular complexes of claim 125 wherein the detector detects a property respecting the disassembly of the supramolecular complex includes the off-rate.

127. The device for the formation and detection of supramolecular complexes of claim 122 wherein the detection includes a competitive binding format.

128. The device for the formation and detection of supramolecular complexes of claim 122 wherein the detection includes a sandwich format.

129. The device for the formation and detection of supermolecular complexes of claim 83 wherein a plurality of physically distinct sites each comprising an attachment surface being located in a variable electronic environment.

130. The device for the formation and detection of supramolecular complexes of claim 129 wherein each site has a common first molecular recognition component.

131. The device for the formation and detection of supramolecular complexes of claim 129 wherein each site has different components.

132. The device for the formation and detection of supramolecular complexes of claim 83 wherein at least one of the molecular recognition components, the separate molecular species or the supramolecular complex is labeled.

133. The device for the formation and detection of supramolecular complexes of claim 132 wherein the label comprises a fluorescent label.

134. The device for the formation and detection of supramolecular complexes of claim 83 comprising a diagnostic device.

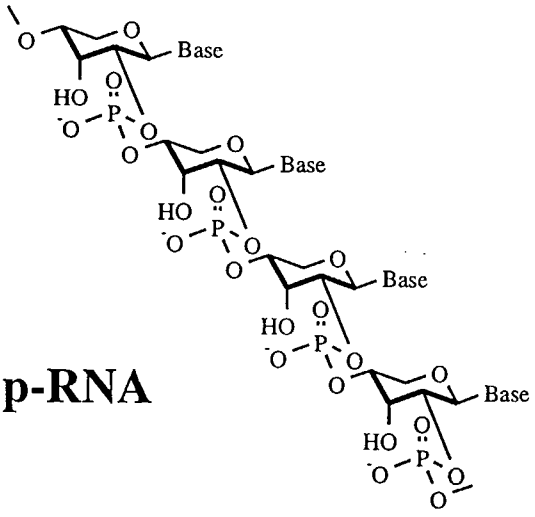
135. The device for the formation and detection of supramolecular complexes of claim 134 wherein the diagnostic device tests materials from the group consisting of: blood, tissue, feces, cells, cell compartments, and cell factions.

136. A method for forming supramolecular structures in an exponential library by aggregation of sublibraries (ELIAS), the improvement comprising subjecting at least certain of the components for formation of a supramolecular complex to electronic stringency.

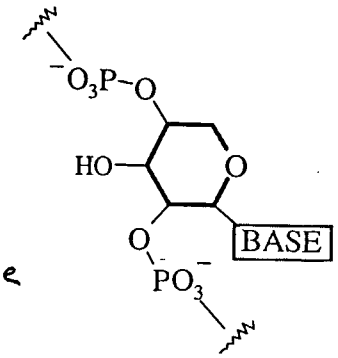
137. The method of claim 136 wherein the electronic stringency conditions include electronic perturbation.

# Figure 1 - p-RNA Structure

## Pyranosyl-RNA




**p-RNA**

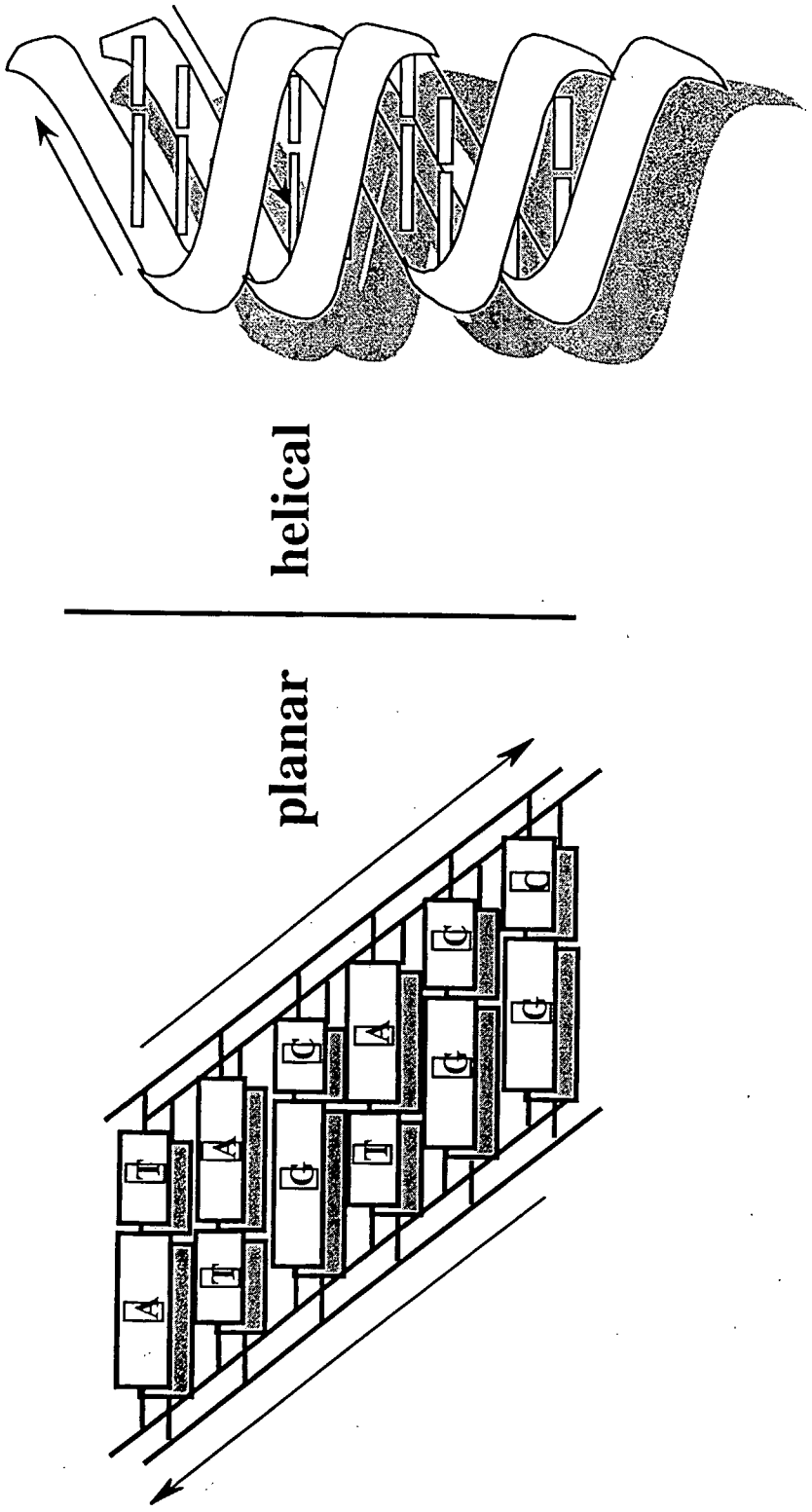



**p-RNA**
unit
structure

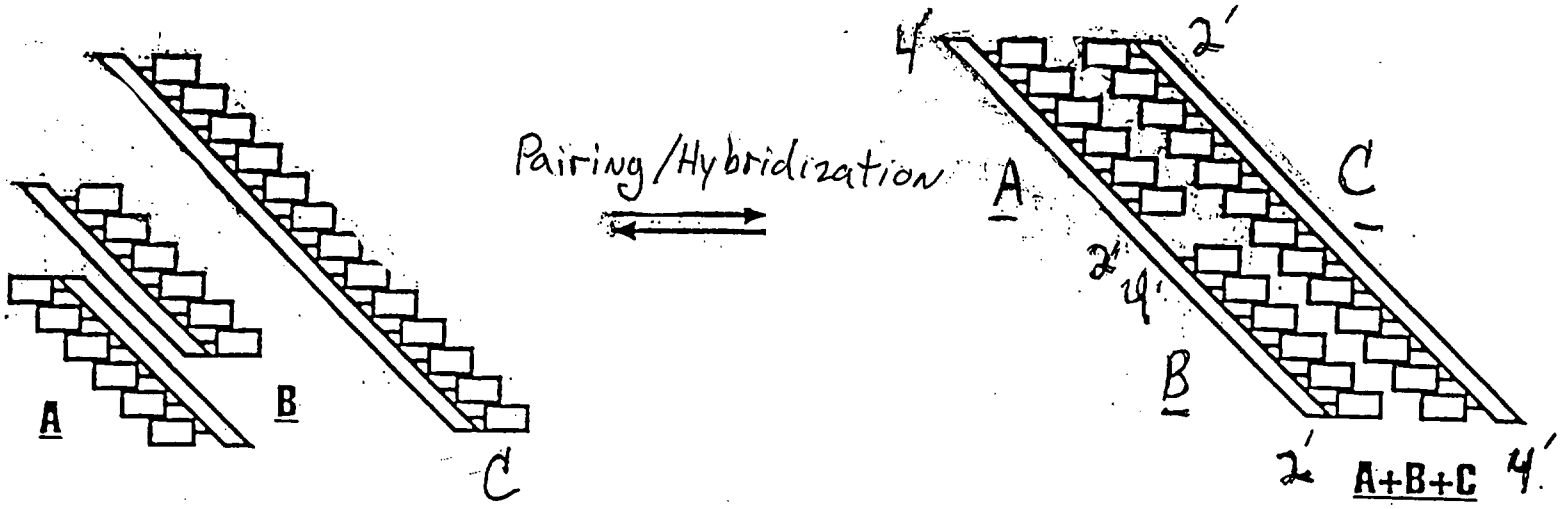
## Advantageous properties:

*Watson-Crick* pairing mode exclusively
*Antiparallel* strand orientation exclusively
- Duplexes *more stable* than in DNA or RNA
- Duplexes have a *quasi-linear* ladder structure
- Pairing is *enantioselective*
- Potential to replicate *without enzymes*
(thus prebiotic ancestor to RNA?)
- No pairing with DNA or RNA

Figure 2 - p-RNA Planar and DNA Helical Structures



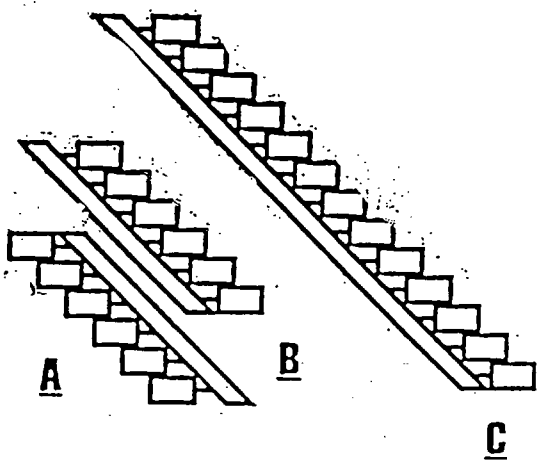
# Figure 3- p-RNA Components A, B, and C




p-RNA Components
A, B, and C
un-paired

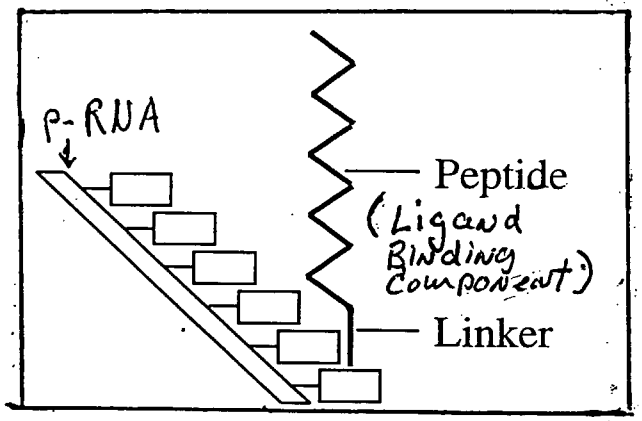
p-RNA Components
A, B, and C
paired or hybridized

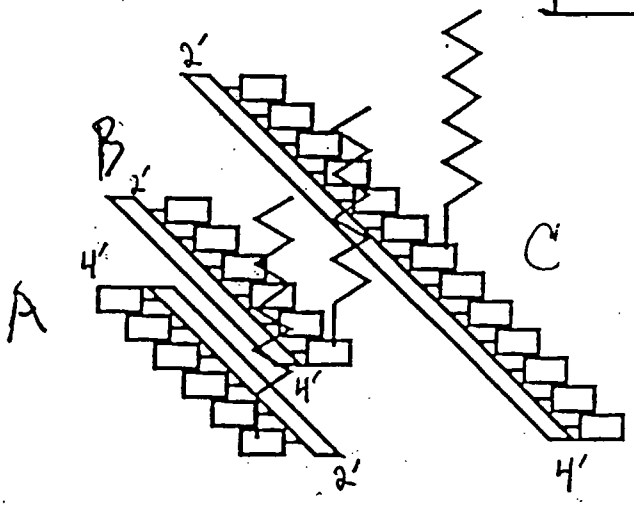
# Figure 4- p-RNA Peptide Derivatized A, B, + C Components




p-RNA Components
← A, B, and C
(Programable Pairing Components)

Linking of peptide library with p-RNA Components A, B, + C




← p-RNA Peptide derivatized A, B, and C Components

(Intermolecular Ligand Binding Components)

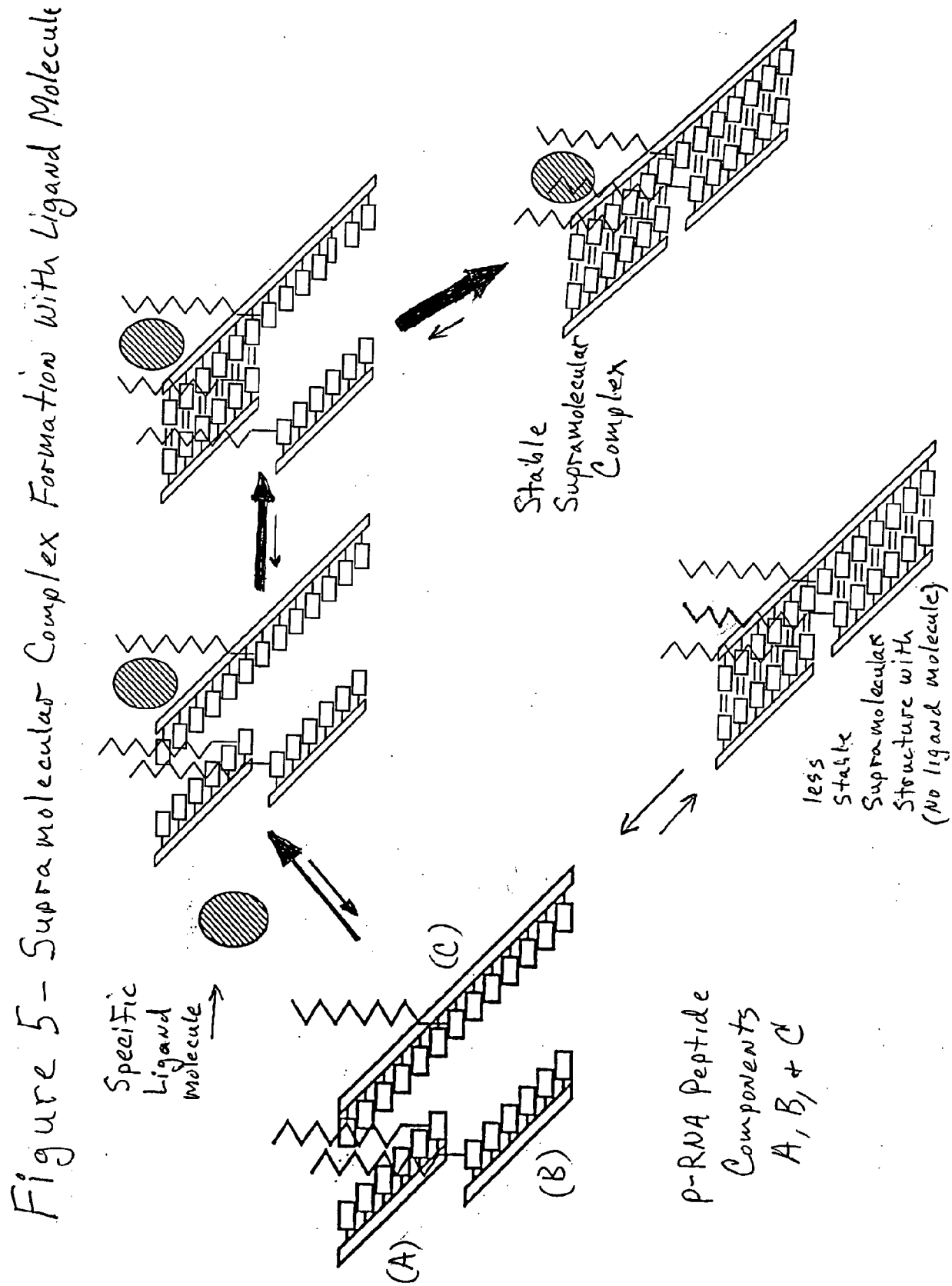


Figure 5 - Supramolecular Complex Formation With Ligand Molecule
Specific Ligand Molecule
(A)
(B)
(C)
P-RNA Peptide Components A, B, + C
Stable Supramolecular Complex
less stable Supramolecular Structure with (No ligand molecule)

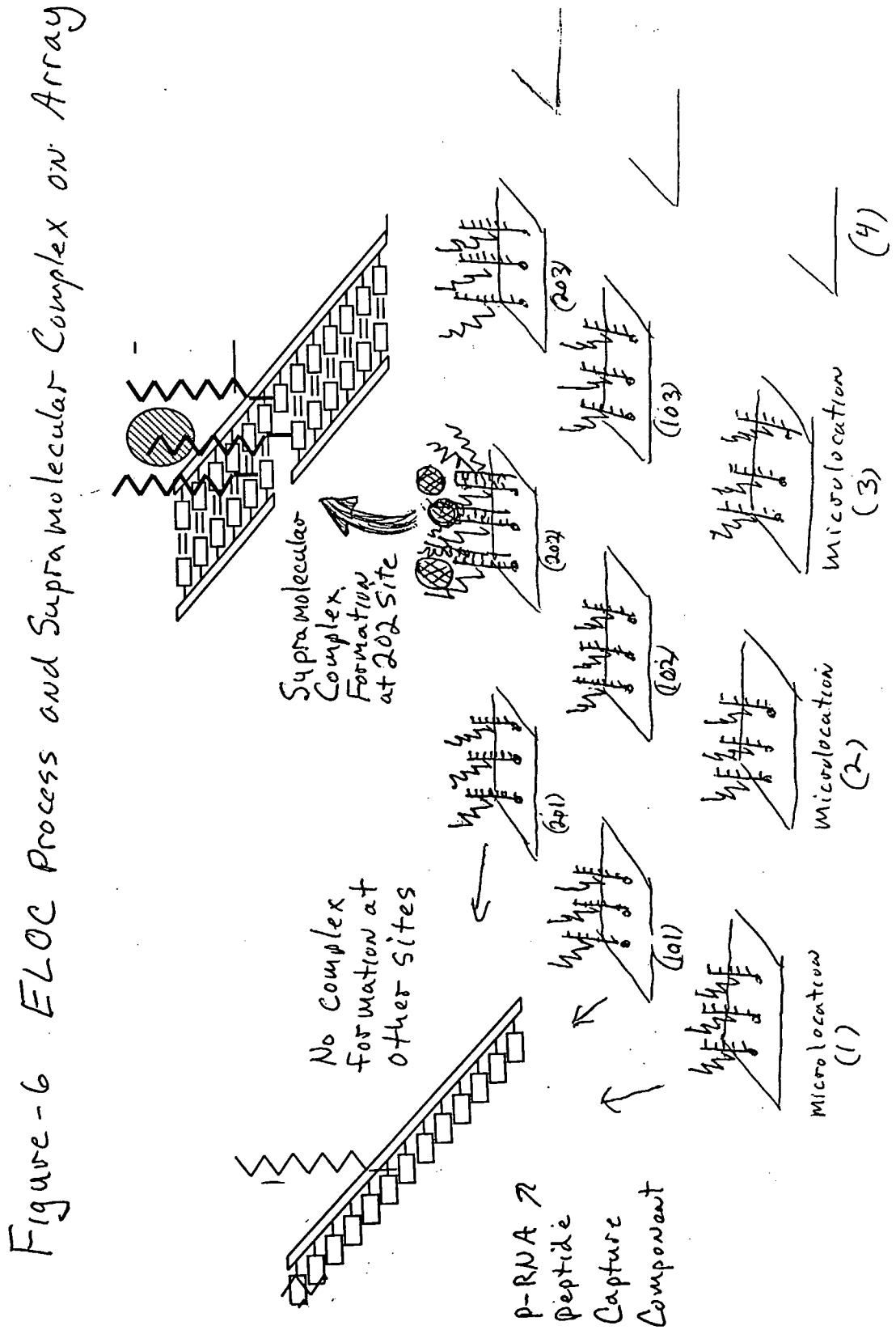
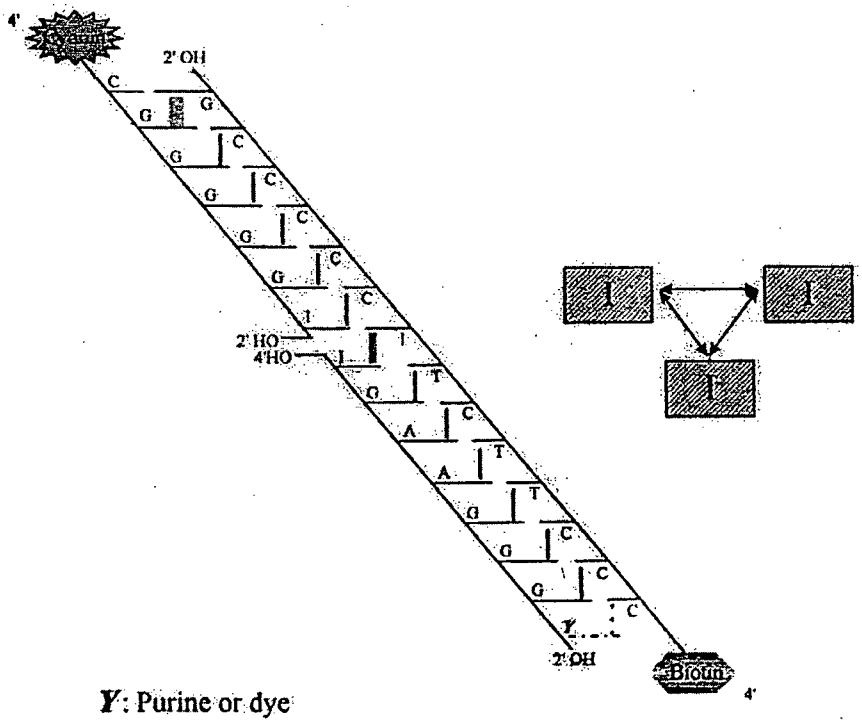



Figure-6 ELOC Process and Supramolecular Complex on Array

Portion of Microelectronic ELOC Chip

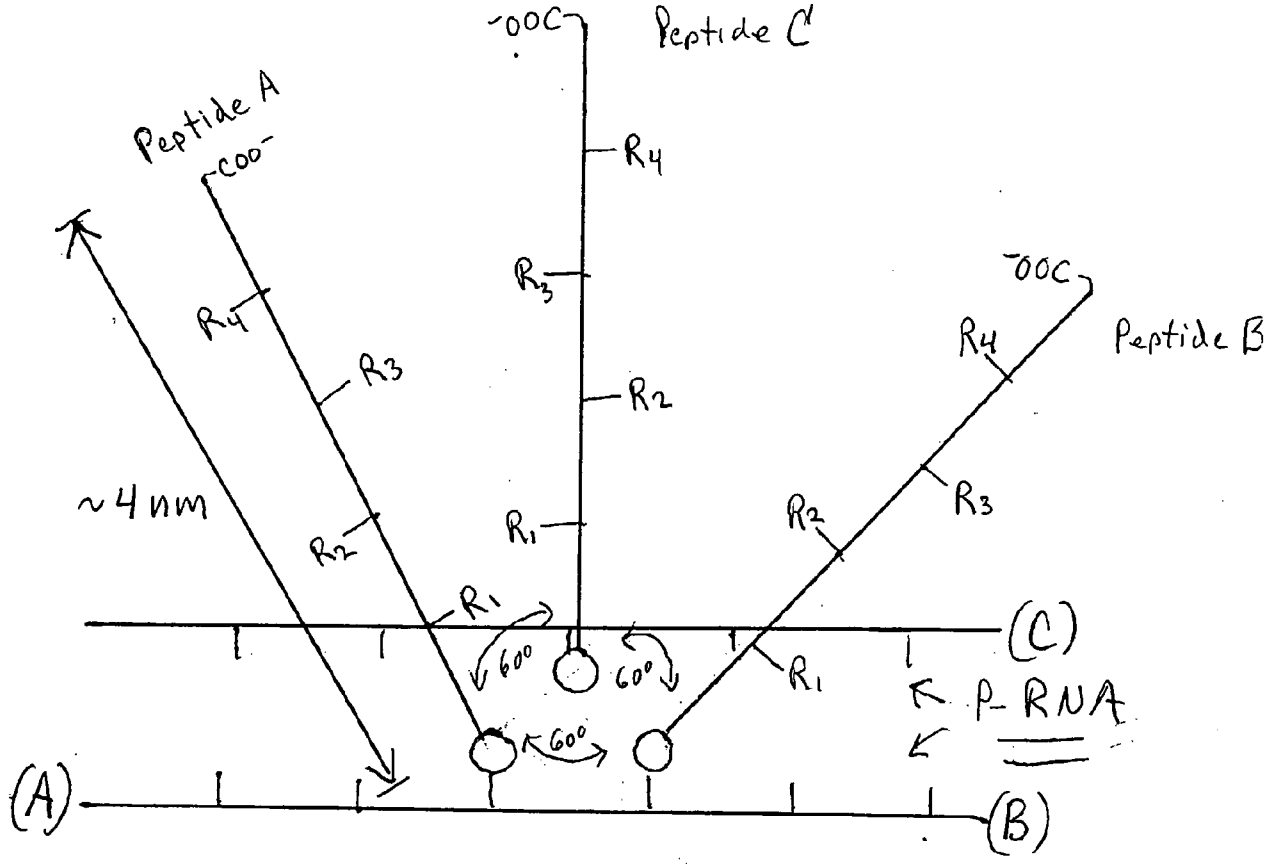
Figure 7- Fully hybridized 7-mer p-RNA A, 7-mer p-RNA B, and complementary capture p-RNA C.

Oligo 90: 4' IGAAGGGY 2'
Oligo Cy91: 4' Cyanine-CGGGGGI 2'
Oligo Biot92: 4' Biotine-CCCTTCTICCCCCG 2'




Y: Purine or dye

Figure 8- Dimensional Geometry of Hexamer Peptide Triad




Amino Acid R Groups

$R_1 \sim 0.6$ nm

$R_2 \sim 0.96$ nm

$R_3 \sim 1.3$ nm

$R_4 \sim 1.7$ nm

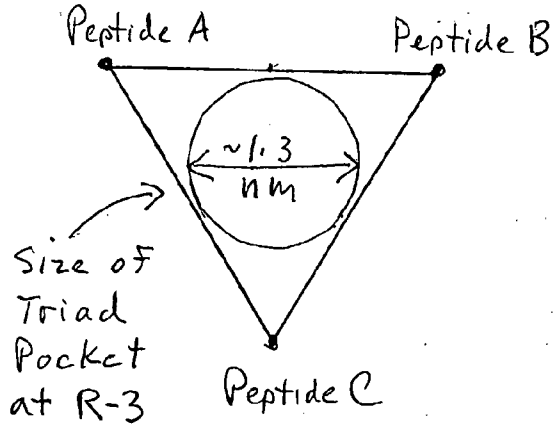


Figure 9 - p-RNA-Peptide "Triad" Supra structure Complex Binding a Biotin Molecule

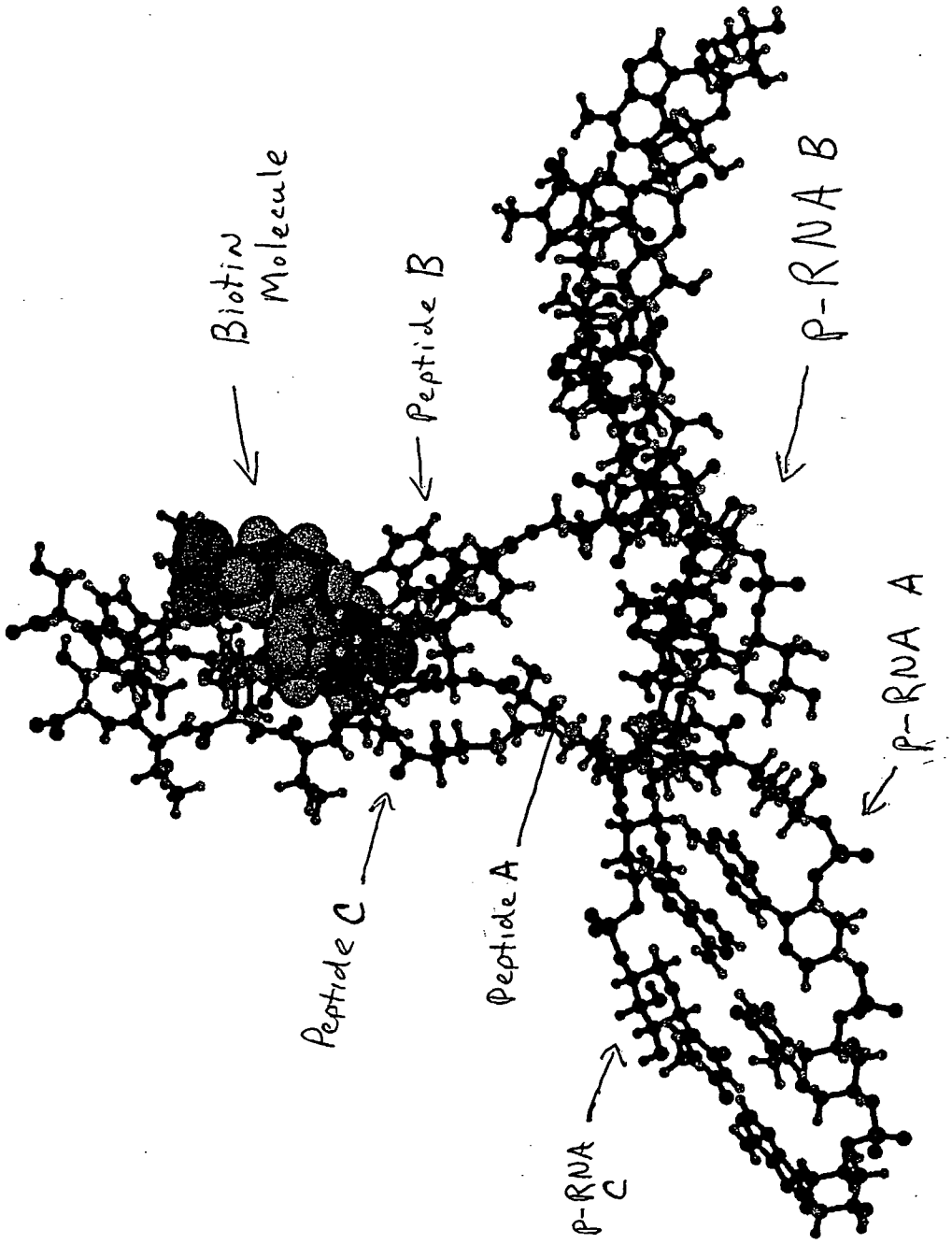
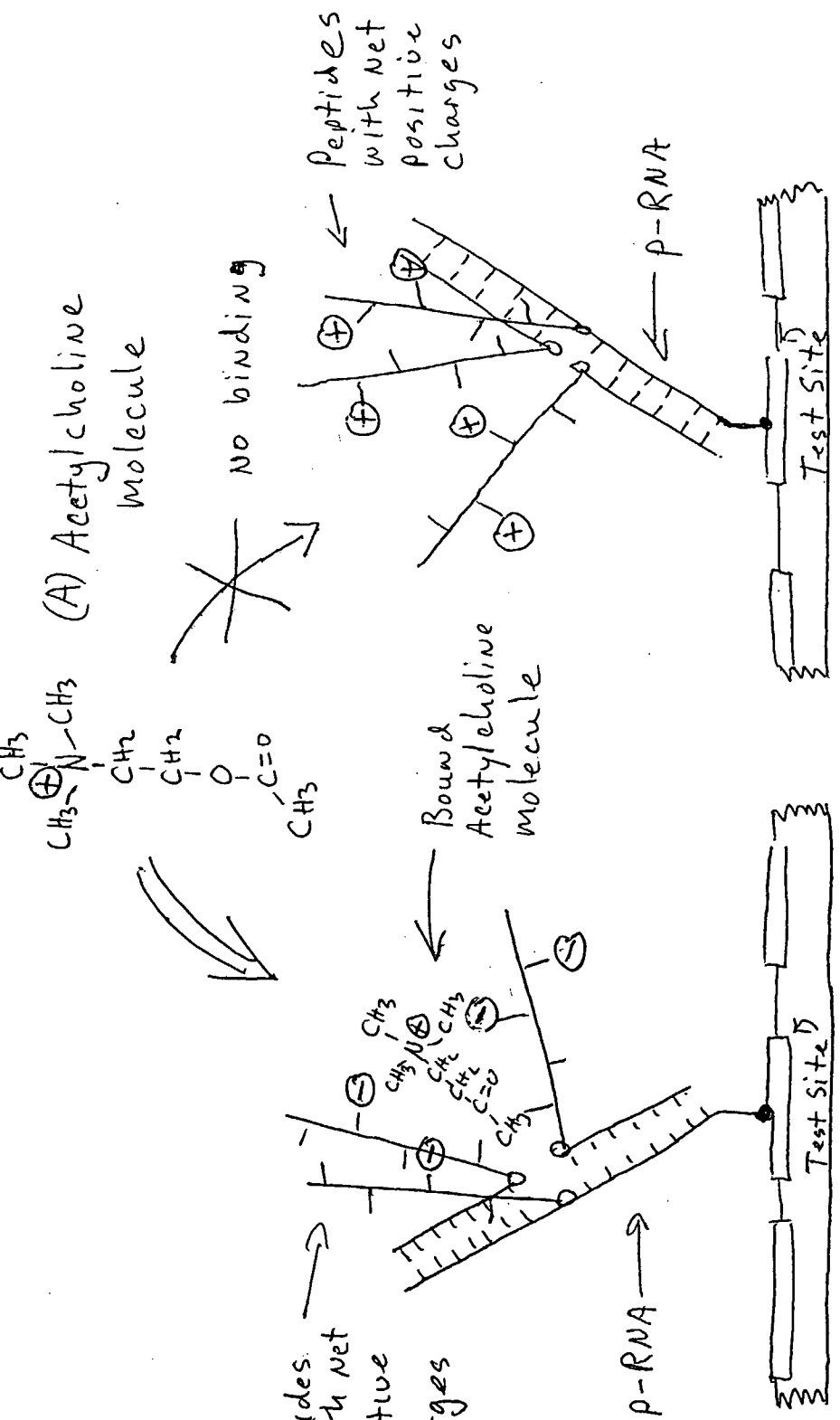


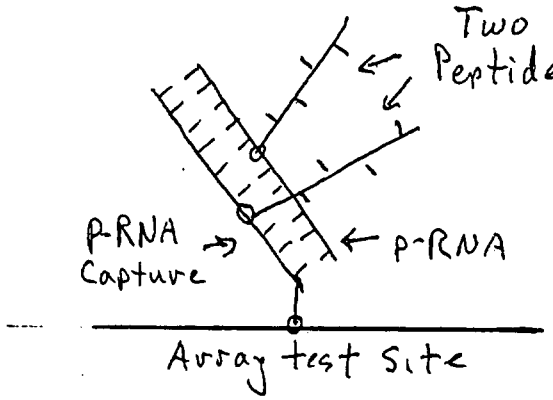
Figure 10 - Favorable and Unfavorable Peptide Triads For Binding Acetylcholine



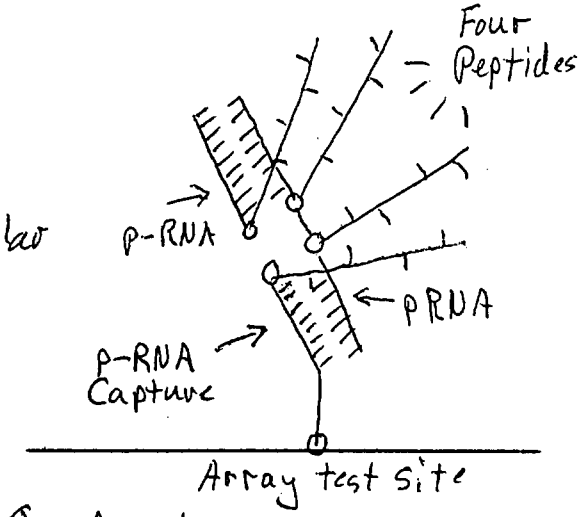

(B) Favorable Peptide Triad Binding Site For Acetylcholine

(C) Unfavorable Peptide Triad Binding Site for Acetylcholine

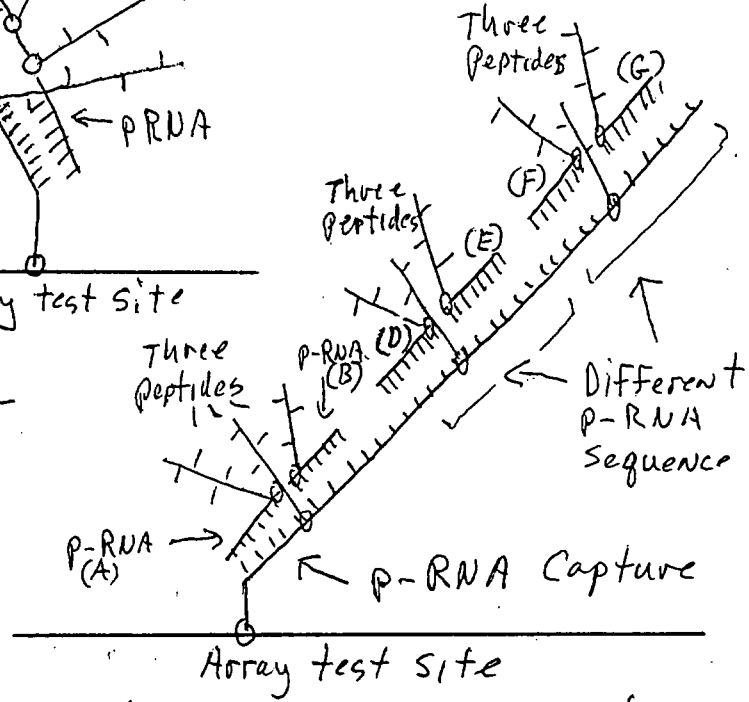
# Figure 11 - Other Supramolecular Structures




(A) Duplex Supramolecular Structure

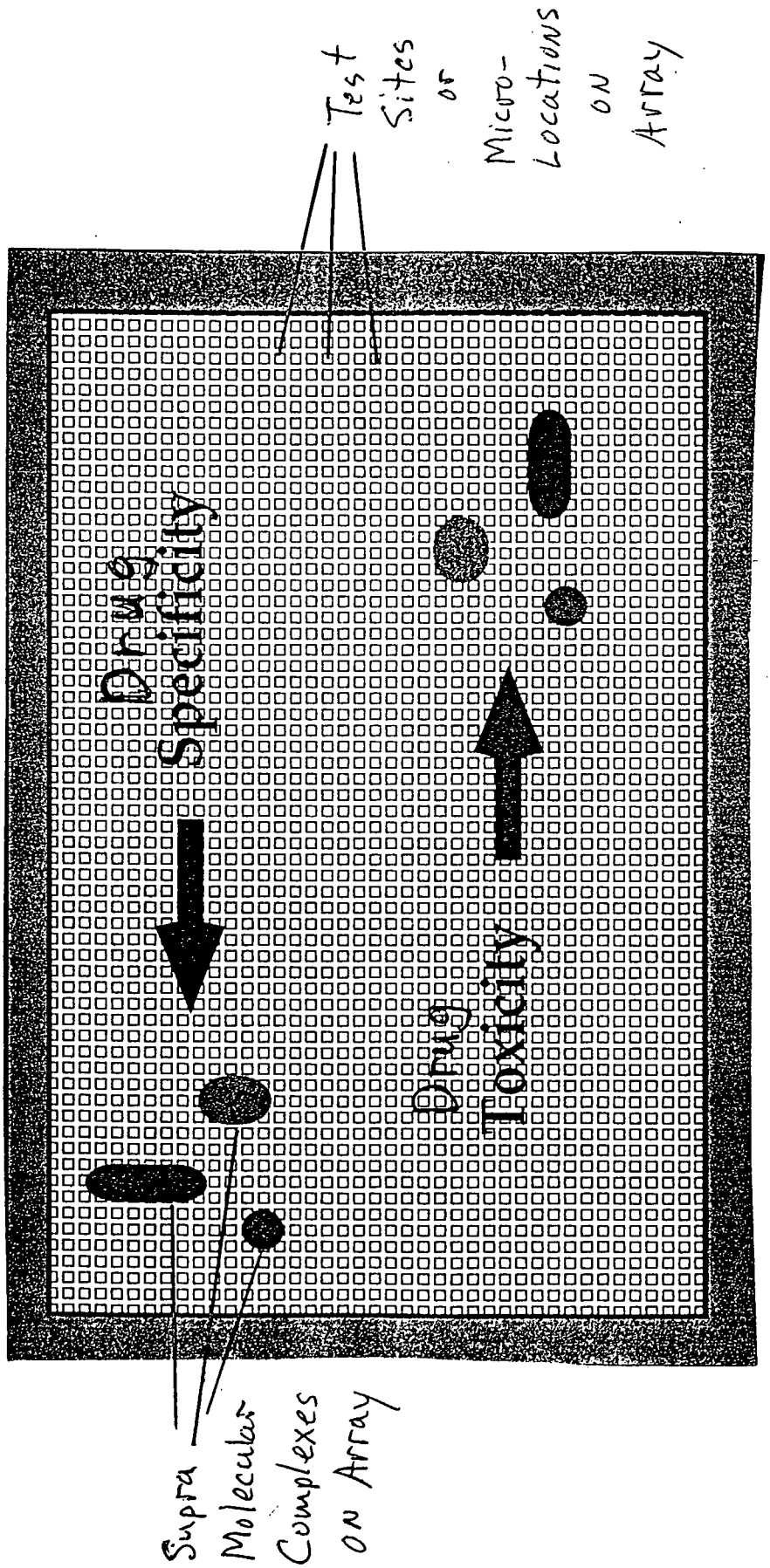



(B) Quadraplex Supramolecular Structure

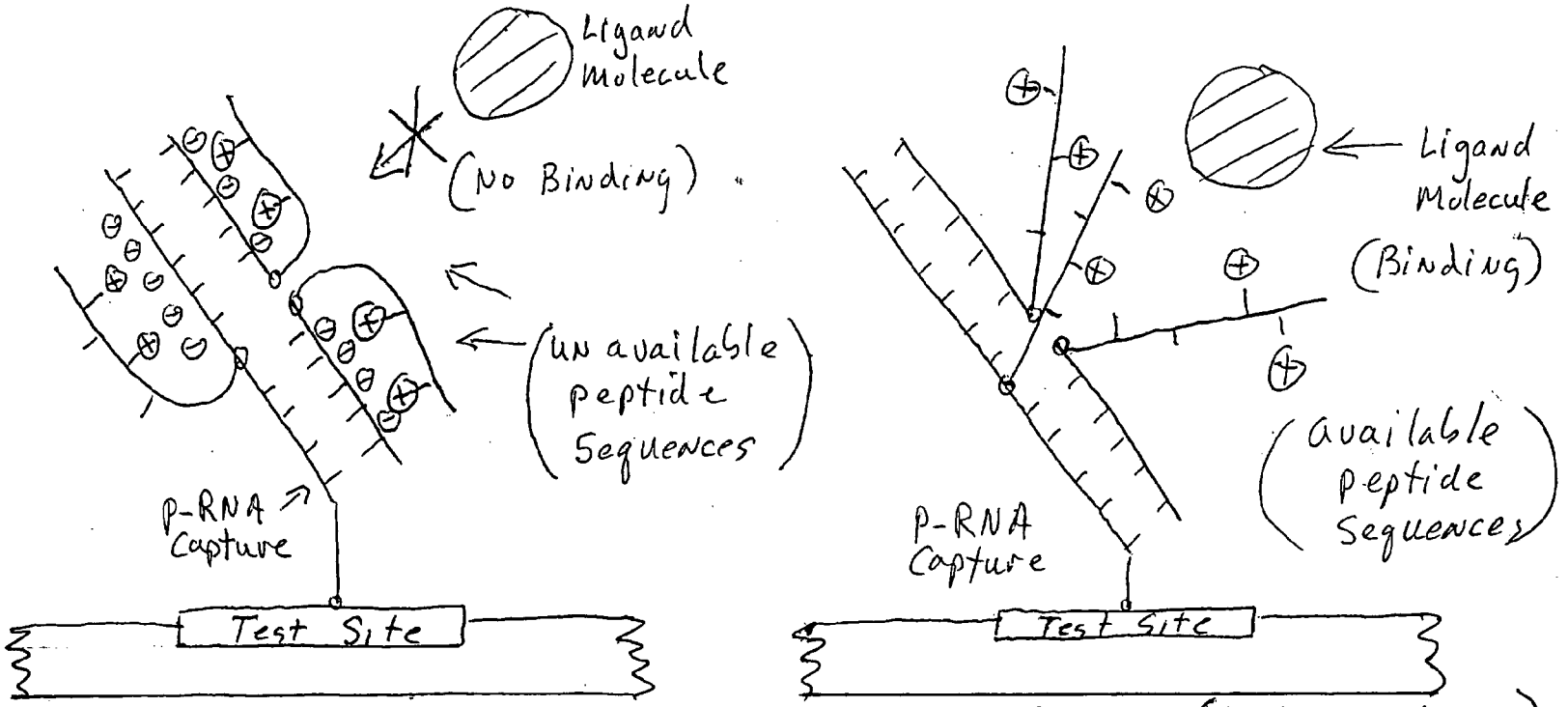



(C) Multiplex Triad Supramolecular structures

Figure 12 - Molecular Descriptor Array



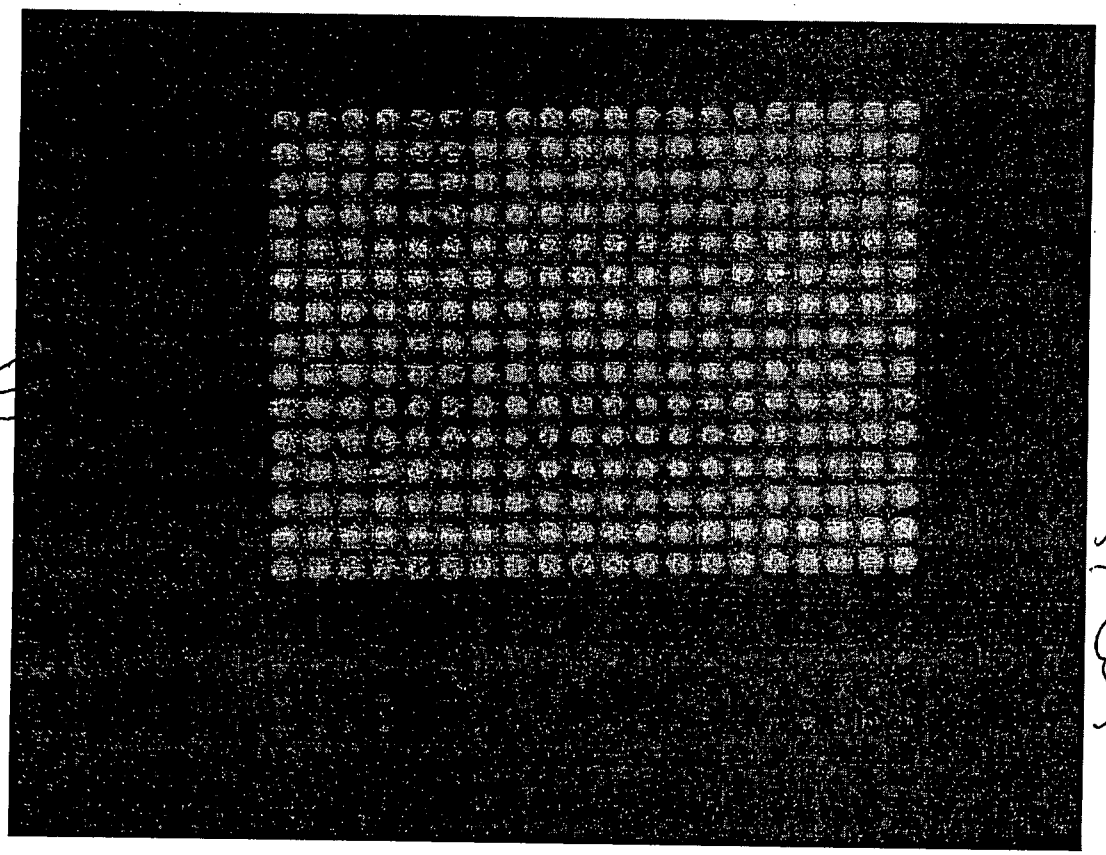
# Figure 13 - Electronic Perturbation to Improve Ligand Binding




(A) In Active Structure

(B) Active Binding Structure During Electronic Perturbation

Figure 14- Specific p-RNA Hybridization on 10,000 Site Microelectronic Array




Portion of 10,000 site Microelectronic Array

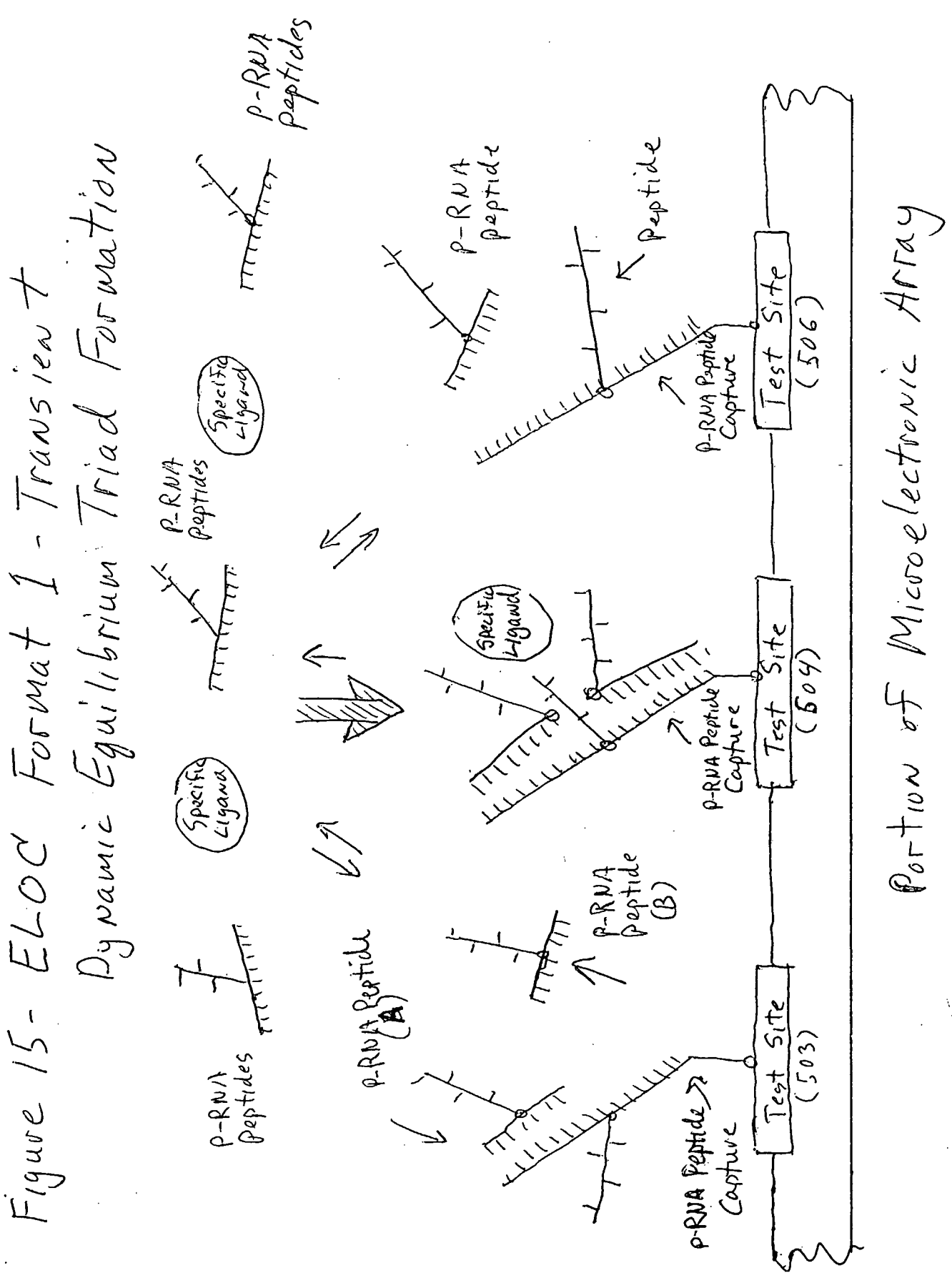
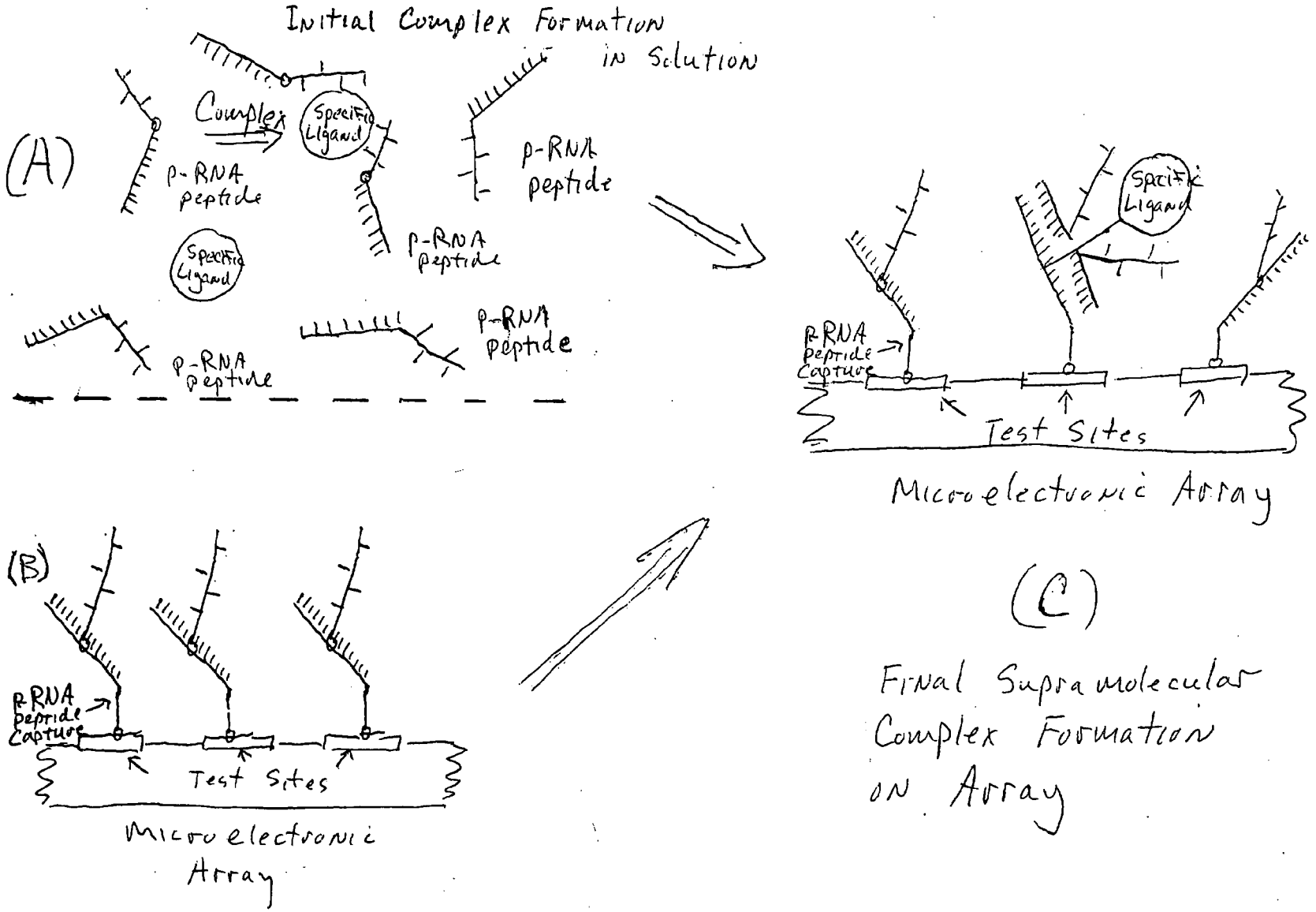
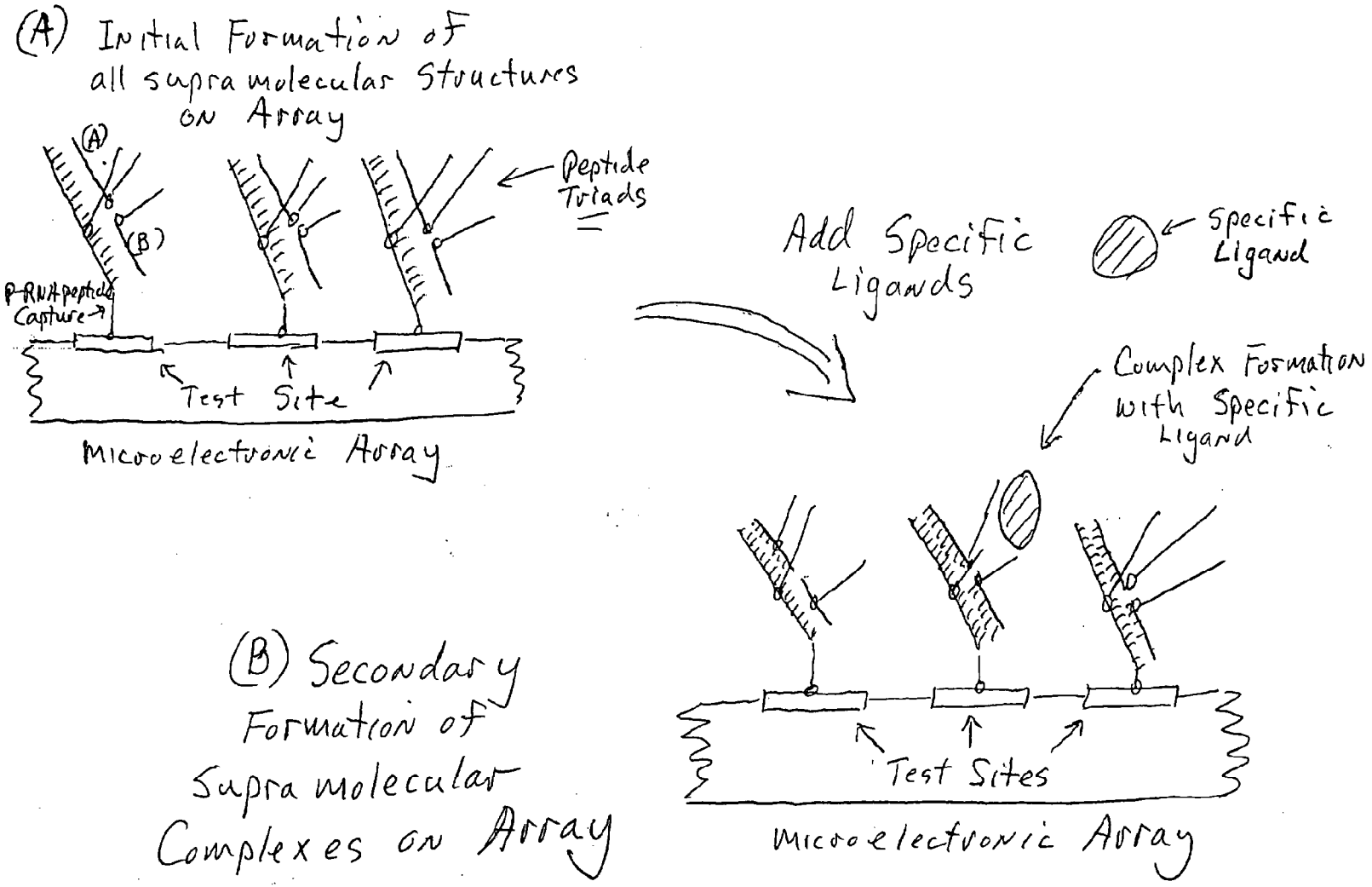


Figure 15- ELOC Format 1 - Transient
Dynamic Equilibrium Triad Formation
P-RNA Peptides
Specific Ligand
P-RNA Peptides
P-RNA Peptide (A)
P-RNA Peptide (B)
P-RNA Peptide
Peptide
P-RNA Peptide Capture
P-RNA Peptide Capture
P-RNA Peptide Capture
Test Site (503)
Test Site (504)
Test Site (506)
Portion of Microelectronic Array

# Figure 16 - ELOC Format 2 - Homogeneous Triad Formation Process



# Figure 17- ELOC Format 3 - Heterogeneous Triad Formation Process



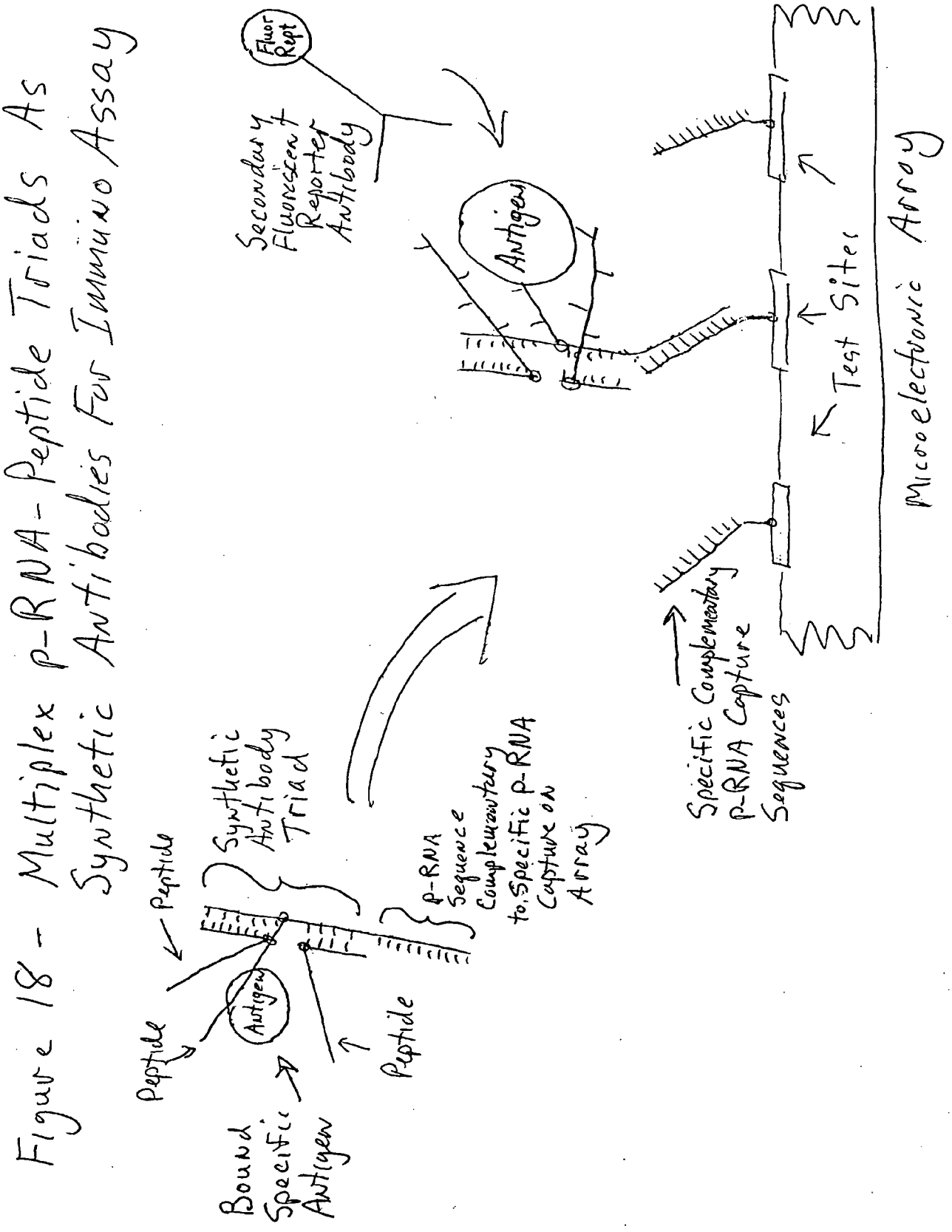
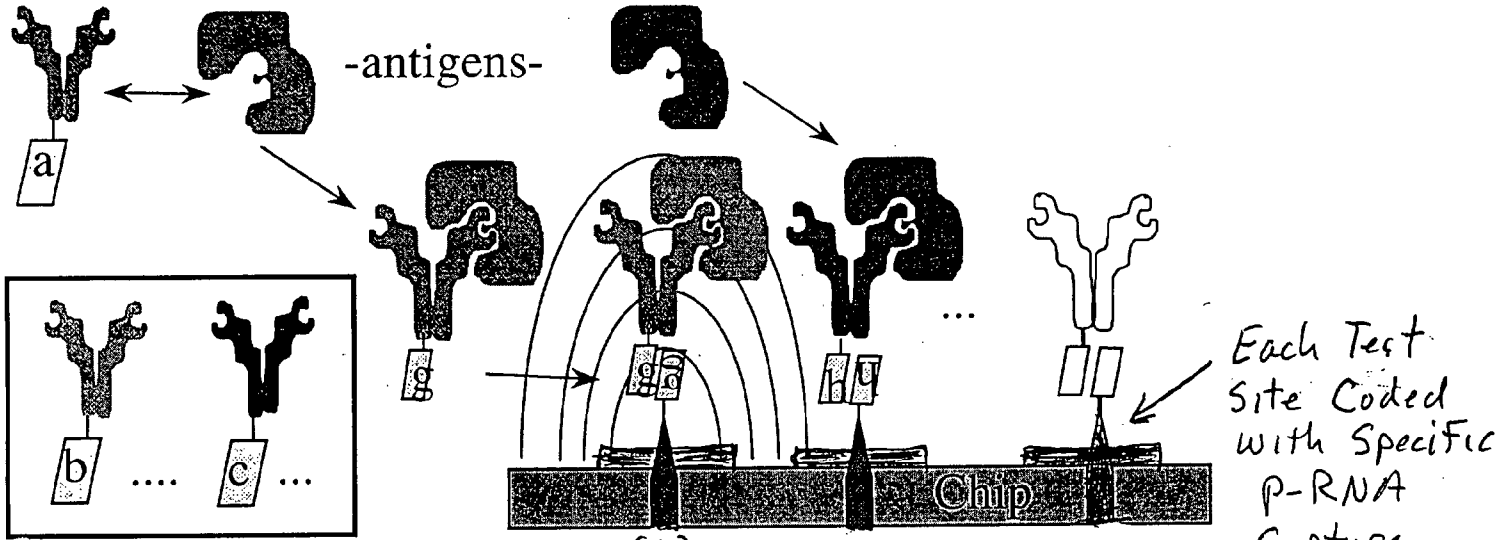
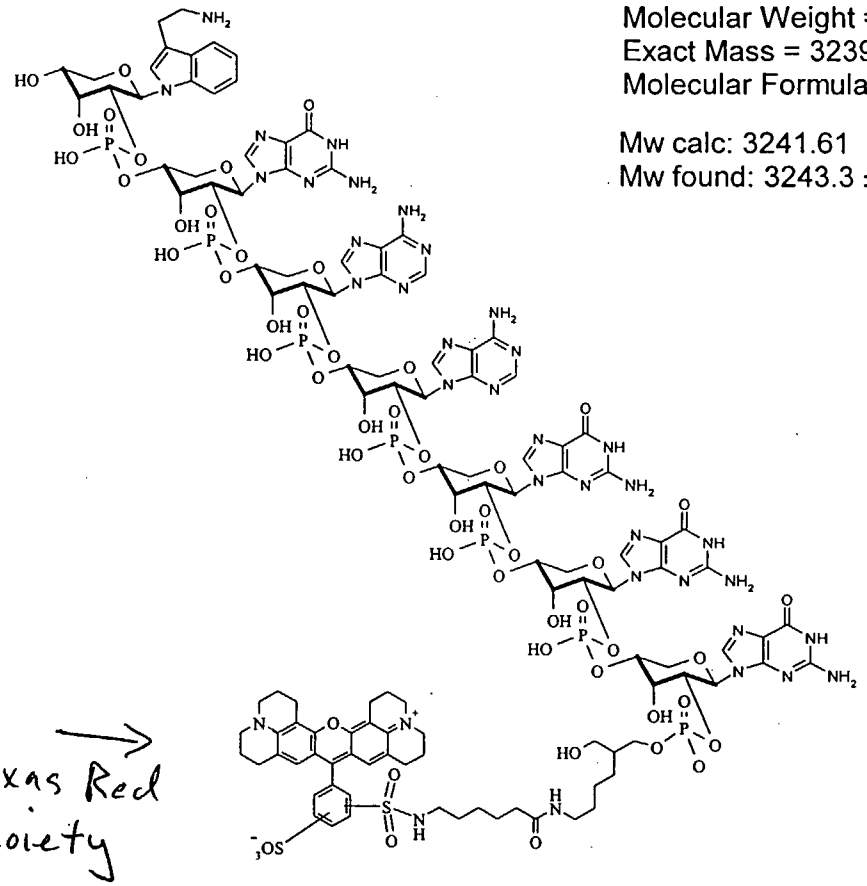


Figure 18 - Multiplex p-RNA-Peptide Triads As Synthetic Antibodies For Immuno Assay
Peptide
Synthetic Antibody Triad
Antigen
Peptide
Bound Specific Antigen
Peptide
p-RNA Sequence Complementary to Specific p-RNA Capture on Array
Secondary Fluorescent Reporter Antibody
Fluor Rept
Antigen
Specific Complementary p-RNA Capture Sequences
Test Sites
Microelectronic Array

Figure-19 - Modular Immuno Assay Using p-RNA Antibody Conjugates on Microelectronic Array



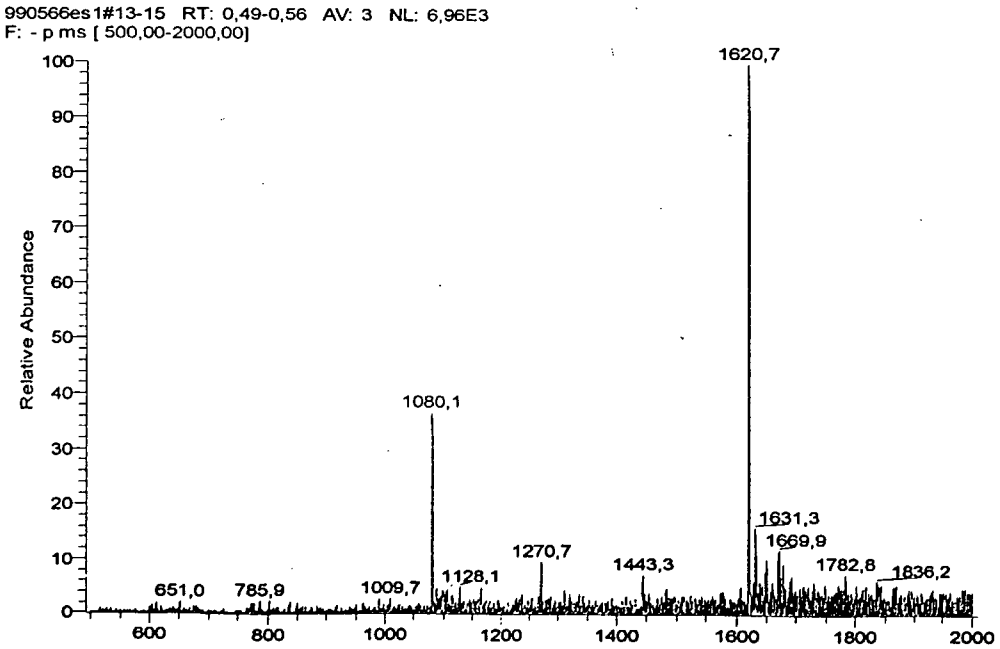
# Figure 20 - Structure and Mass Spec for TR-90-p-RNA Conjugate



Molecular Weight = 3241.61
Exact Mass = 3239.73
Molecular Formula = $C_{119}H_{146}N_{36}O_{55}P_7S_2$

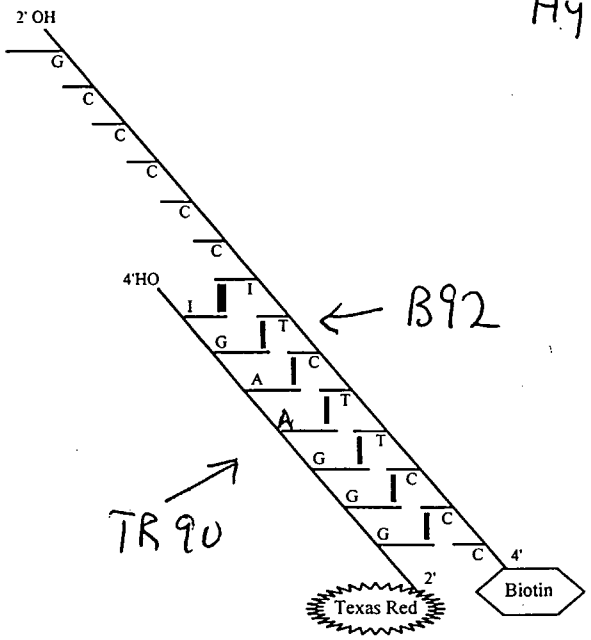
Mw calc: 3241.61
Mw found: 3243.3 ± 0.5 (error~ 0.4‰)

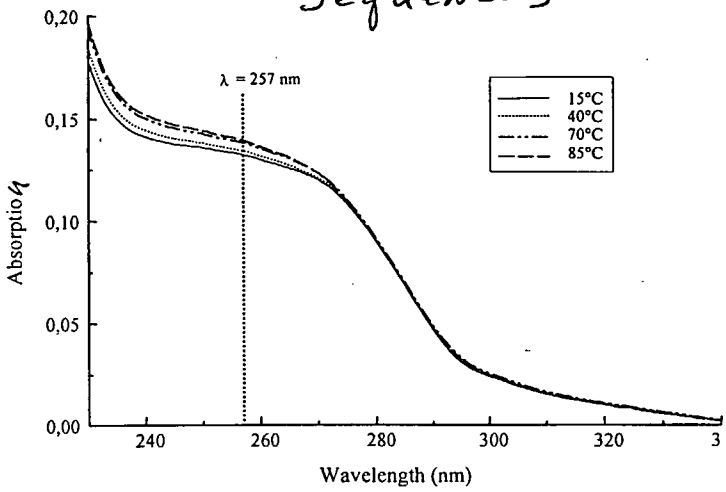
Texas Red Moiety



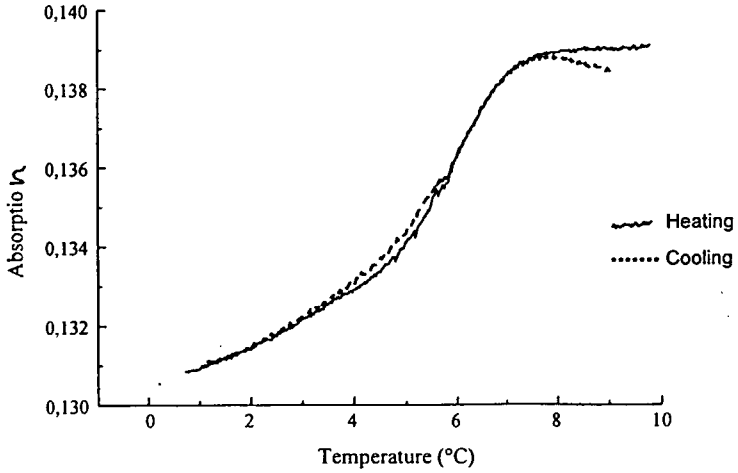
Oligo TR90: $^{4'}$ IGAAGGG-TexasRed $^{2'}$
Oligo B92: $^{4'}$ Biotin-CCCTTCTICCCCCG $^{2'}$

Figure 21- Structure for Hybridized TR-90 and B92 Sequences



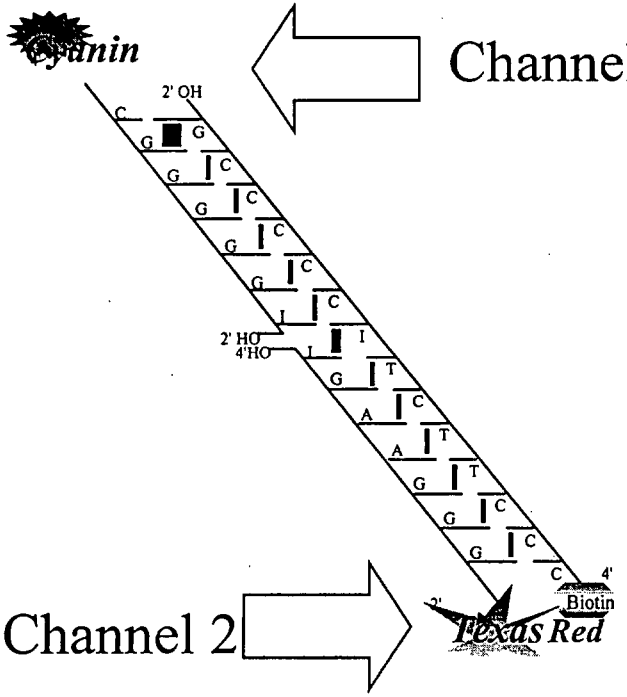




$T_m$ = 59 °C
Tris/HCl 0.01M ; pH 7
0.15 M NaCl
c = 5 μM + 5 μM

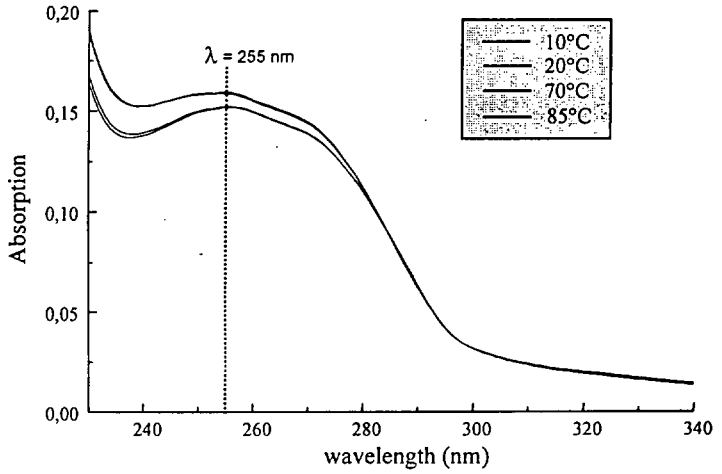


Oligo TR-90: 4' IGAAGGG-TexasRed 2'
Oligo Cy3-91: 4' Cyanine3-CGGGGGI 2'
Oligo Biot-92: 4' Biotin-CCCTTCTICCCCCG 2'

Figure 22
Structure for Hybridized TR90, Cy3-91, and B92 P-RNA Conjugates




$T_m$ = 61 °C
Tris/HCl 0.01M ; pH 7
0.15 M NaCl
c = 5 µM + 5 µM + 5µM



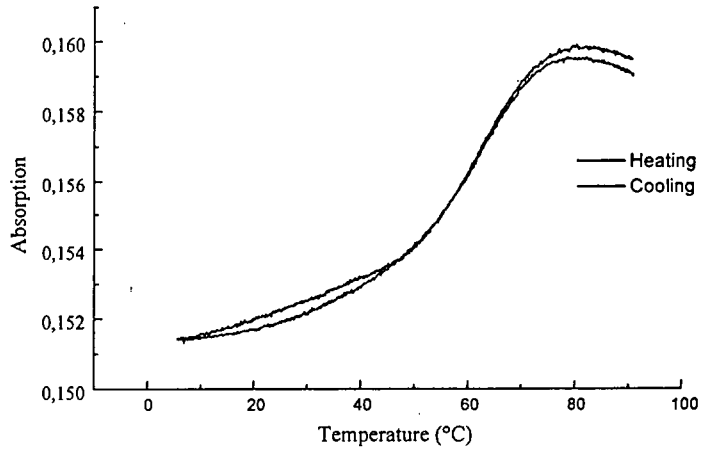
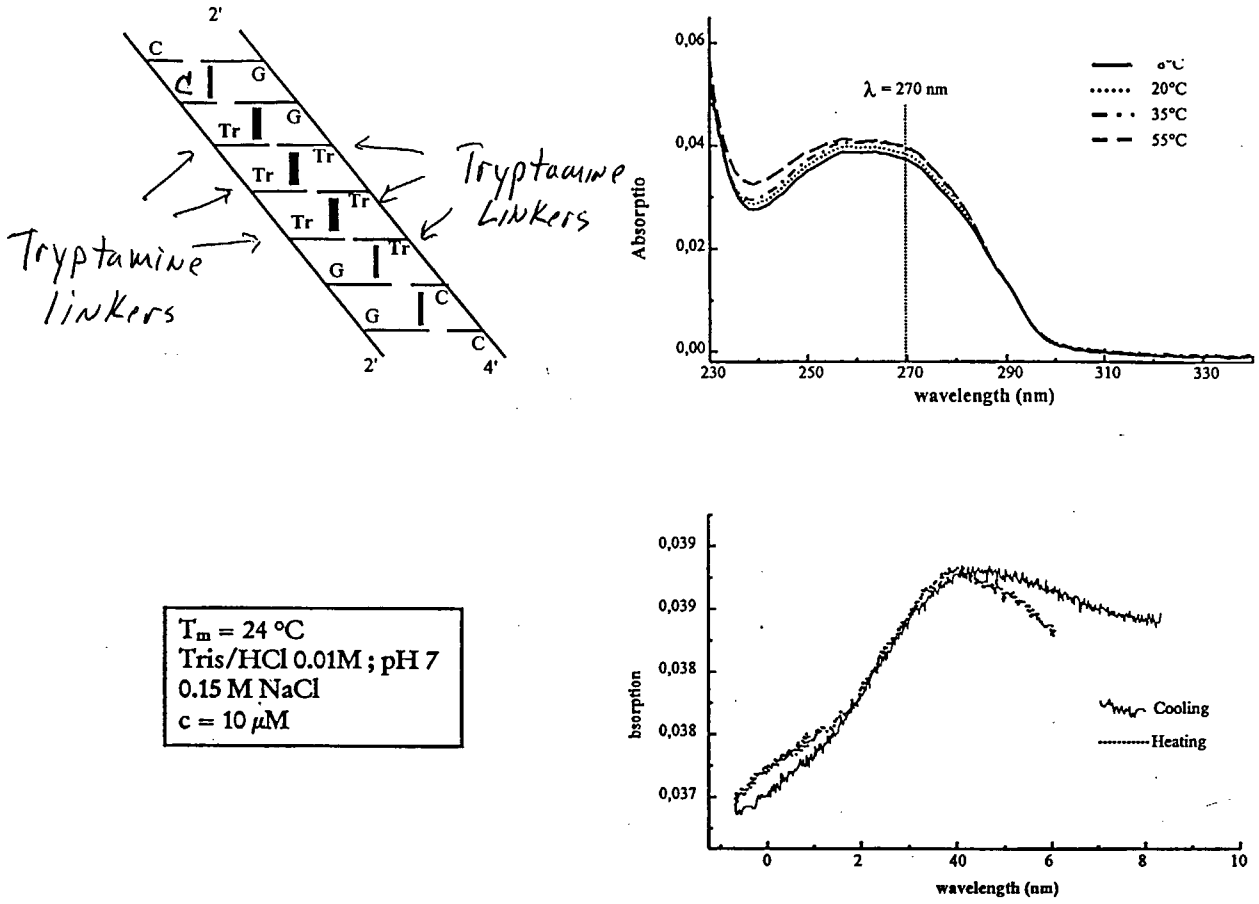



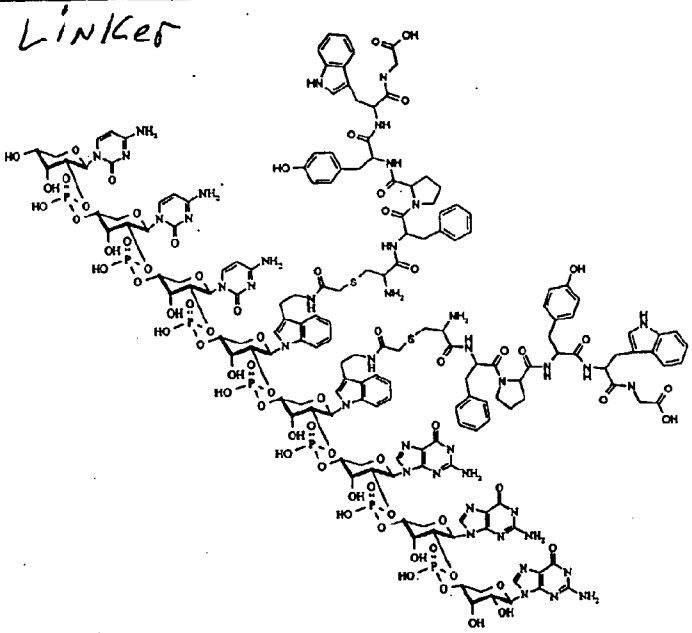
Figure 23- p-RNA Sequence with Multiple Tryptamine Linkers




$T_m$ = 24 °C
Tris/HCl 0.01M ; pH 7
0.15 M NaCl
c = 10 μM

Thermodynamic data of the self pairing p-RNA oligomer $^{4'}$ CC**TrTrTr**GG $^{2'}$

Figure 24 - p-PNA with Two Peptides via Tryptamine Linker



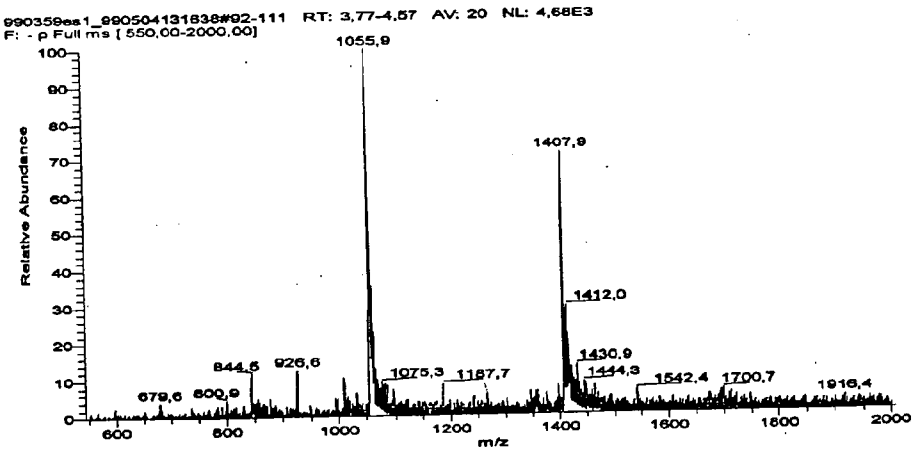
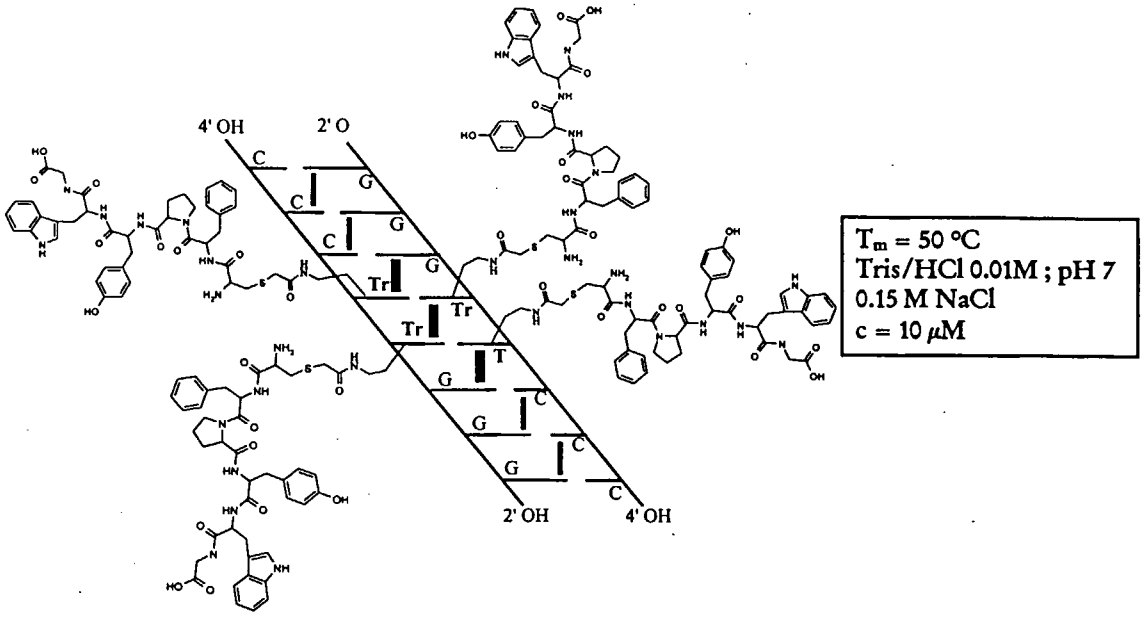
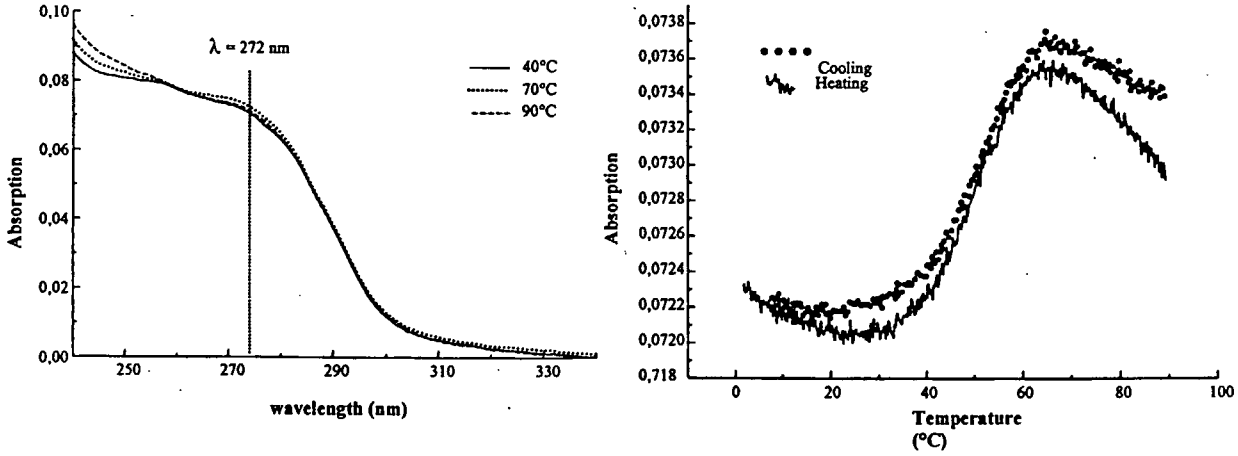


Figure 25- p-RNA Hybrid Structure with Four Peptides




Thermodynamic data of the p-RNA peptide conjugate

Figure 26 - Simulation of Measurements with Noise

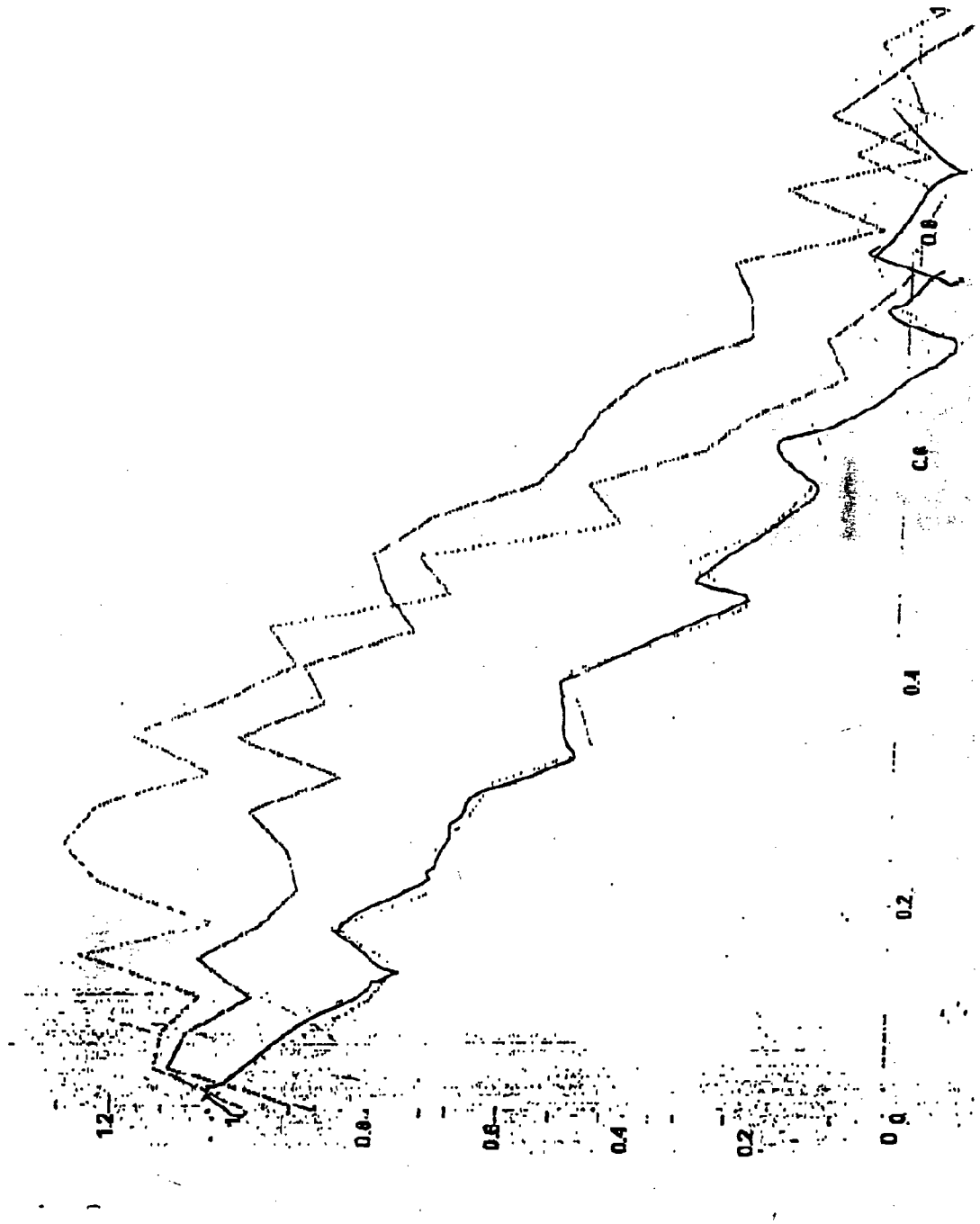


Figure 27 - Idealized Signal Development

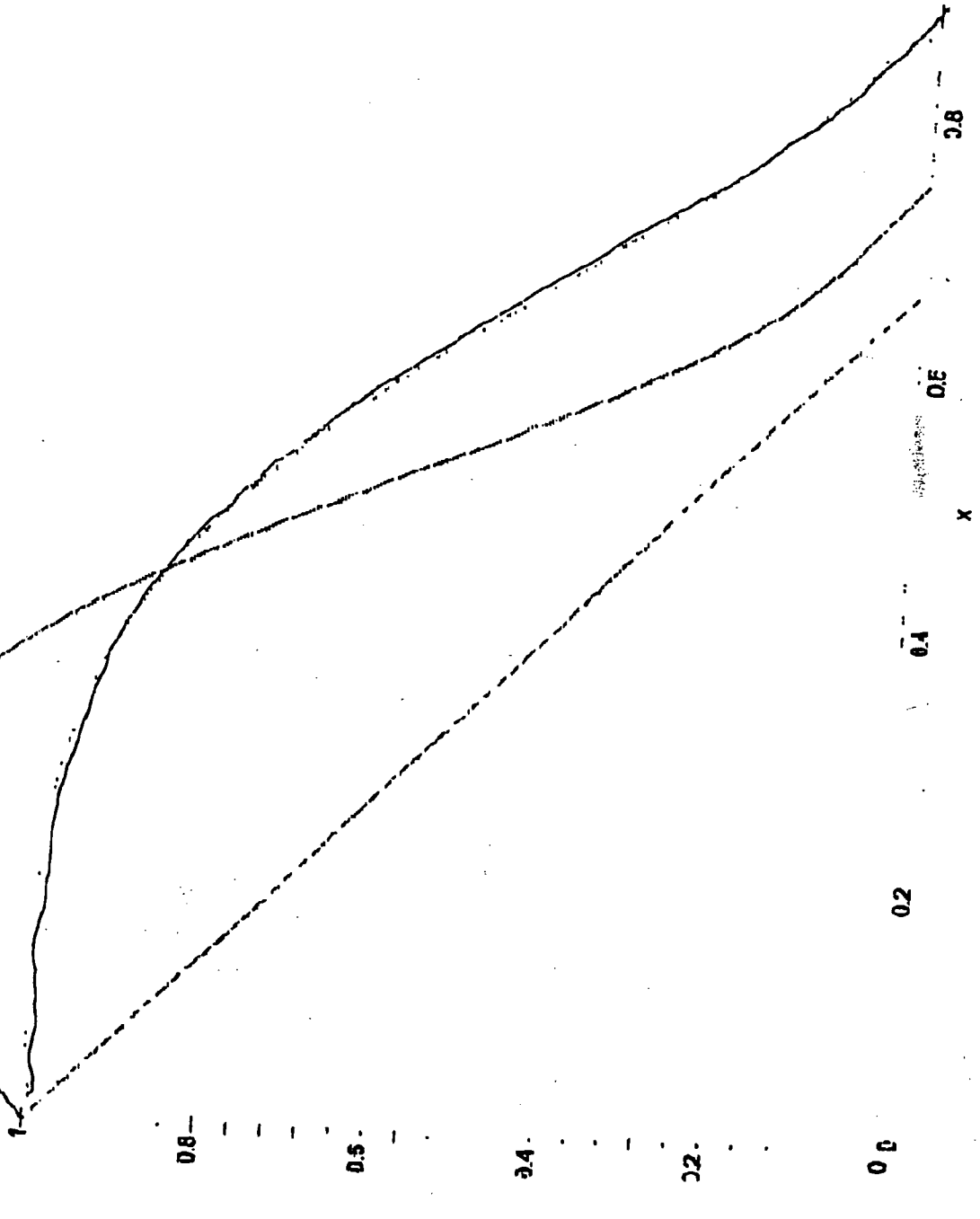
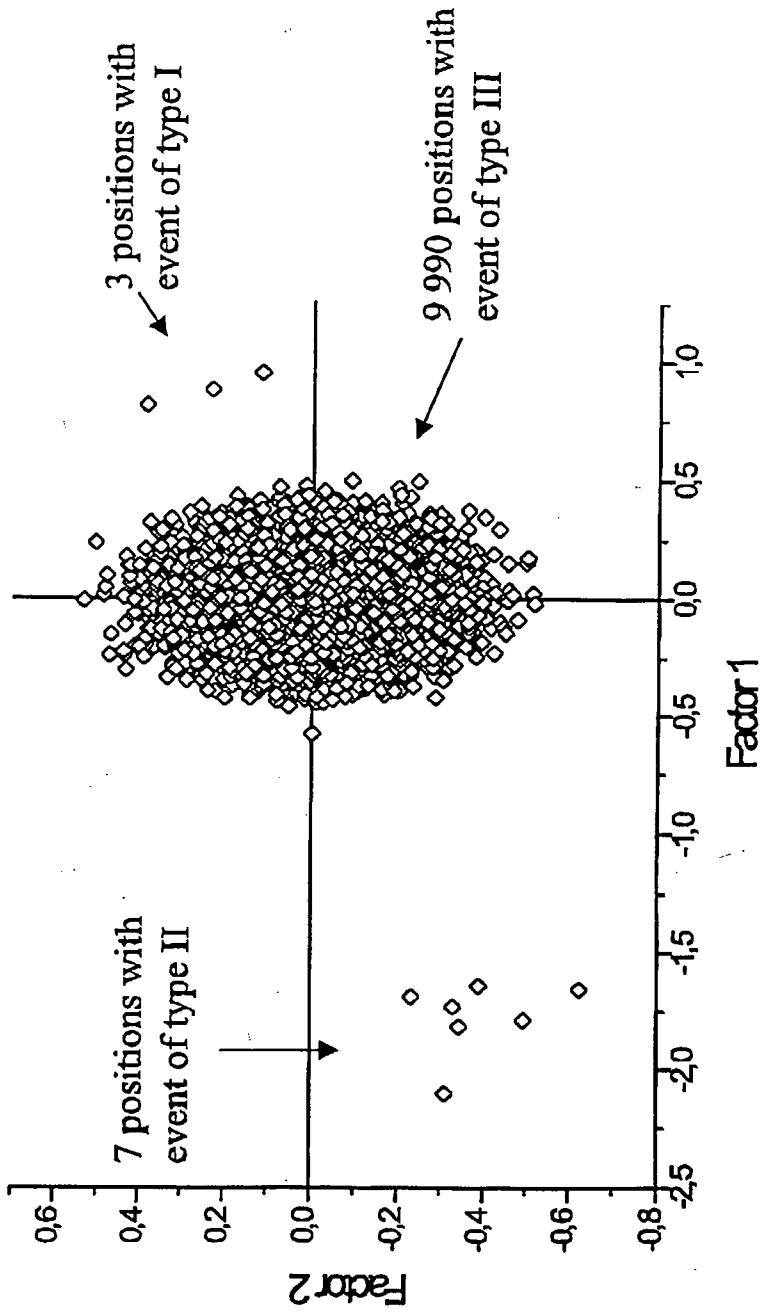


Figure 28

Factoranalysis clustering of the signal development on 10 000 different chip position events with a simulation of noise of absolute +/- 25 % (50%) .



# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/20847

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/50, 68.1, 76, 82.01, 82.02, 82.05; 435/6, 7.1, 7.2, 285.2; 436/149, 150, 806, 809

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 98/51819 A1 (NANOGEN, INC.) 19 November 1998, see entire document, especially the abstract and the associated figure. | 1,4, 6-39, 83-86, 88-135 |
| Y | US 5,811,239 A (FRAYNE) 22 September 1998, see entire document, note the multicomponent nucleic acid complexes. | 1, 4, 6-39, 83-86 and 88-135 |
| Y | US 5,653,939 A (HOLLIS et al) 05 August 1997, see the entire document. | 1, 4, 6-39, 83-86 and 88-135 |
| Y | US 5,632,957 A (HELLER et al) 27 May 1997, see entire document. | 1, 4, 6-39, 83-86 and 88-135 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

Date of the actual completion of the international search
23 OCTOBER 2000

Date of mailing of the international search report
29 DEC 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)★

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/20847

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US 5,605,662 A (HELLER et al) 25 February 1997, see the entire document. | 1, 4, 6-39, 83-86 and 88-135. |
| Y | US 5,871,902 A (WEININGER et al) 16 February 1999, see entire document, note the multicomponent complexes used. | 1, 3-4, 6-39, 83-86 and 88-135 |
| Y,P | WO 99/42558 A1 (NANOGEN, INC.) 26 August 1999, see the entire document. | 1, 4, 6-39, 83-86 and 88-135 |
| Y | WO 99/29711 A1 (NANOGEN, INC.) 17 June 1999, see the entire document. | 1, 4, 6-39, 83-86, 88-135. |
| X,P | US 6,077,668 A (KOOL) 20 June 2000, see the entire document, especially claim 15 and note the teaching of pRNAs. | 2, 5, 40 and 87 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/20847

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-40 and 83-135

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

mational application No.
PCT/US00/20847

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

G01N 35/00, 15/06, 25/08, 25/18, 27/02, 27/00, 27/02, 21/29, 33/53; C12Q 1/68

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

422/50, 68.1, 76, 82.01, 82.02, 82.05; 435/6, 7.1, 7.2, 285.2; 436/149, 150, 806, 809

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

Dialog, USPAT and Derwent using WEST:
search terms:microelectronic, electrode, arrays, DNA oligonucleotides, RNA, pRNA, CNA pyranosyl, cyclohexyl, nucleic acids

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-40 and 83-135, drawn to an array device.
Group II, claim(s)41-54, drawn to method of combinatorial selection.
Group III, claim(s)55-60, drawn to methods of drug discovery and determining an expected biological response.
Group IV, claim(s)61-65, drawn to methods of evolving an a descriptor array.
Group V, claim(s) 66-82 and 136-137 drawn to methods of forming complexes on an array
The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The use of microelectronic arrays is known in the art, hence the feature joining the groups does not constitute a special technical feature.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

The species correspond to the different combinations of components used to form the supramolecular complexes. Note that many of the explicitly recited subspecies are directed subgeneric categories (e.g., a combinatorial library). Election of such a subgeneric will not provide a species for examination on the merits.

| | | | |
|---|---|---|---|
| 专利名称(译) | 用于组合选择分子间配体结合结构和用于药物筛选的微电子分子描述符阵列装置，方法，程序和格式 | | |
| 公开(公告)号 | EP1210607A4 | 公开(公告)日 | 2004-12-22 |
| 申请号 | EP2000955293 | 申请日 | 2000-07-28 |
| [标]申请(专利权)人(译) | 内诺金有限公司<br>阿温提斯研究技术两合公司 | | |
| 申请(专利权)人(译) | NANOGEN INC.<br>AVENTIS研究与技术公司 | | |
| 当前申请(专利权)人(译) | NANOGEN INC.<br>AVENTIS研究与技术公司 | | |
| [标]发明人 | HELLER MICHAEL J<br>WINDHAB NORBERT<br>ANDERSON RICHARD R<br>ACKLEY DONALD E<br>NOVA TINA S<br>HOPPE HANS ULLRICH<br>HAMON CHRISTIAN J | | |
| 发明人 | HELLER, MICHAEL, J.<br>WINDHAB, NORBERT<br>ANDERSON, RICHARD, R.<br>ACKLEY, DONALD, E.<br>NOVA, TINA, S.<br>HOPPE, HANS-ULLRICH<br>HAMON, CHRISTIAN, J. | | |
| IPC分类号 | G01N33/53 B01J19/00 C12M1/00 C12Q1/68 C40B30/04 C40B40/06 C40B40/10 G01N21/17 G01N21/59 G01N33/15 G01N33/50 G01N33/68 G01N37/00 G01N35/00 G01N33/543 | | |
| CPC分类号 | C40B30/04 B01J19/0046 B01J2219/00653 B01J2219/00659 B01J2219/00707 B01J2219/00722 B01J2219/00725 B01J2219/00729 C12Q1/6825 C40B40/06 C40B40/10 G01N21/171 G01N21/59 G01N33/6845 G01N2800/52 | | |
| 优先权 | 09/374338 1999-08-13 US | | |
| 其他公开文献 | EP1210607A1 | | |
| 外部链接 | Espacenet | | |


摘要(译)

这些发明涉及微电子分子描述符阵列装置，用于组合选择分子间配体结合结构和用于药物筛选的方法，程序和形式。更具体地，这些装置和方法通过应用独特的严格性参数，快速地对组合产生的分子间配体结合组分，超分子结构和超分子复合物进行更高阶选择性。优选地，本发明包括通过子库的聚集通过电子严格性的影响来形成指数文库，以影响超分子结构或复合物的形成或检测。此外，本发明涉及用于分子识别过程，新药物发现，新亲和试剂的产生，合成抗体的产生和免疫测定的微电子阵列装置，程序，方法和形式。