Molecular computing by PNA:PNA duplex formation

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Molecular computing is potentially one of the most powerful tools for the development of massive parallel computing protocols. In the present paper, a first example of the use of PNA:PNA interactions in molecular computing is described. A series of short PNA sequences have been designed with a four base stretch coding for variables and solutions. Hybridization of the components in different combinations was tested both in solution and in a microarray format. A series of PNA representing the solutions were spotted on a microarray surface in order to simulate the hardware. A series of PNA representing the variables, labeled with TAMRA, were used to interrogate the device enabling to solve non-deterministic logic operations. The system was shown to be able to solve a two-variable equation with a high signal to noise ratio. This paper intends to provide a proof of principle that PNA, on account of their stability and specificity of binding, are most suitable for constructing organic-type computers.

Introduction

The increasing role that electronic devices play in our daily lives, as well as our constant need to pursue superior technologies, have raised a wide interest for the development of molecular systems mimicking the operation of electronic logic gates and circuits.^{1–4} Besides their integration in the heart of digital computers, electronic logic circuits control the operation of a variety of devices, from calculators and store automation to video games and music equipment.

An interesting possibility for improving computing devices is to use molecular interactions, such as those occurring in the complementary base recognition scheme, for solving mathematical problems. The pioneer of this idea was Adleman, who first solved a non-deterministic problem using DNA molecules, in a study that can be considered as the first relevant achievement of molecular-recognition based computing strategies.⁵

Furthermore, many studies have reviewed the possibility to use supramolecular concepts for building electronic gates at the molecular level.^{6–9} For example, recently, an interesting study¹⁰ has reported the development of an important electronic device, mimicking computing at the molecular level, named keypad lock, which differs from a simple logic gate by the fact that its output signals are dependent not only on the proper combination of inputs but also on the correct order by which these inputs are introduced. In other words, one needs to know the exact password that opens this lock. This device could be used for numerous applications in which access to an object or data is to be restricted to a limited number of persons; it represents a new approach for protecting information at the molecular level.

In the past years, considerable efforts have been focused on developing new generation of molecular logic gates and molecular computers, based on DNA;^{5,11–16} a biomolecule possessing well-regulated structures and the ability to store genetic information. DNA computer has been intensively used for solving a class of intractable computational problems,^{13,17,18} such as 3-satisfiability (3-SAT) problems, in which the computing time can grow exponentially with the problem size.

The basis for the use of DNA:DNA duplex formation as computing tool is illustrated in **Figure 1**. A set of DNA sequences can be used as variables and another set as possible solutions, so that each solution forms a duplex only if it is a solution of a particular clause containing the corresponding variable. Combining a set of molecular events of this type is equivalent to performing parallel computing in normal calculators.

The most attractive advantage of DNA computation is its massive parallelism computation power and huge memories. Up to now, many progresses have been made to improve its performance and increase its reliability,^{14,19} though improvement in specificity of interaction and chemical stability are likely to be necessary to make these devices robust enough.

Compared to DNA probes, PNAs²⁰ have been shown to be efficient tools in numerous applications, since they can form duplexes more stable than double-stranded DNA and are superior to oligonucleotide probes in the recognition of single base mutations.^{21–23} Another interesting property of PNAs, which is useful in biological applications, is their stability to both nucleases and peptidases, since their unnatural skeleton prevents recognition by natural enzymes, making them more persistent in biological fluids.²⁴ PNA can be used as smart materials in several

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Figure 1. Basic principles of DNA computing.

applications and have been proposed as very interesting scaffolds for nanotechnological fabrication.^{25,26} PNA:DNA interactions have been used in previous studies to induce a stricter sequence selectivity in DNA-based computing schemes.²⁷

Since synthetic DNA encoding for variables and solutions have to be appropriately designed and synthesized, the substitution of DNA with completely artificial peptide nucleic acid (PNA) molecules can allow to improve the same operation, if only hybridization of complementary components is used for computation and not enzymatic processing.

In the present study, we investigated the possibility to use PNA:PNA interactions on a PNA-based microarray system as a possible way for performing parallel computational experiments at the molecular level. The advantages of using PNA:PNA interactions are: (1) the possibility to use the same scheme as DNA computers with the same type of code; (2) the higher stability of the duplex formed, which allows to use short sequences with still high response thus reducing the possible interfering sequences;²⁸ (3) the higher specificity of PNA:PNA interactions, with single mismatch discrimination, which increases the precision of calculation (4) the higher chemical and enzymatic stability of the PNA components as compared to DNA-based devices. A simple model has been constructed as a proof of concept and evaluation of performances.

Results and Discussion

Sequence design. Using an approach similar to that described by Pirrung and coworkers for DNA computing,²⁹ we considered as a model the two-variable SAT problem:

$$F = (X \vee Y) \wedge (X \vee \bar{Y}) \quad (1)$$

where the variables are Boolean and can assume values of 1 (true) and 0 (false); $\vee$ is the logical “OR” operation, $\wedge$ is the logical “AND” operation; $\bar{Y}$ is the negation of Y .

The library for a two-variable SAT problem (possible assignments $X = 1; 0$ and $Y = 1; 0$) was encoded by four different PNAs of 8 bp each. The minimal length of the PNA which allows sufficiently high stability of the duplexes was chosen. All probes, representing solutions of the problem, and the fluorescent targets, representing all variables, have two distinct regions: a common fixed region designed to maximize the affinity of the probe to its complementary target and a variable (coding) region which codes the data contained in each strand. Two units of the spacer [2-(2-aminoethoxy)ethoxy acetic acid] were used for the PNA encoding the solutions, in order to avoid possible interactions of the probes with the surface. 5(6)-Carboxytetramethylrhodamine (TAMRA; $\lambda_{\text{ex}} = 545 \text{ nm}$ and $\lambda_{\text{em}} = 570 \text{ nm}$) was used as a fluorescent reporter group for the PNA encoding the variables. The general structures of the PNA oligomers are reported in Figure 2; PNA sequences are shown in Table 1.

UV melting temperatures. In order to verify if the hybridization was sufficiently strong and if aspecific binding of mismatched sequences could interfere with the computing process, the thermal stability of various duplexes and the sequence-selectivity were initially evaluated by determining the melting temperatures (T_m) at 260 nm of all full-matched and of several mismatched duplexes. The T_m data are summarized in Table 2. Very high melting temperatures in the same range for all perfectly matched duplexes (52.0–55.0°C) were observed. The high stability of the duplexes is one of the factors in favour of the use of PNA:PNA instead of DNA:DNA interactions, since the analogous 8-mer oligonucleotide duplexes would have low T_m , according to the widely accepted nearest neighbor model,³⁰ as reported in Table 2; PNA:DNA duplexes of the same length and similar sequences would have melting temperatures in the range 30.0–40.0°C.³¹ Furthermore, the PNA:PNA as described in detail by Tomac et al. are almost insensitive to ionic strength and can be used in the absence of salt, whereas both DNA:DNA and to a lesser extent PNA:DNA have melting temperatures depending on ionic strength, with opposite effects. When two or three Watson-Crick base-pair mismatches were introduced at any position in the coding region (middle of the sequence), a large decrease in T_m (>30°C) was observed, thereby providing a compelling evidence that the PNA:PNA interaction is highly specific. Three mismatches (represented by the case of PNA2:PNA8) gave rise to no hybridization at all. The specificity of interaction is a crucial point in DNA computing, since for each calculation a “yes or no” response should be obtained, and clear cut results require all the full matched pairing to be much more stable than the mismatched.

Though we did not perform calculation using single-mismatched PNA sequences, in a recent study the effect of different mismatches were studied for PNA:PNA duplexes, showing a drop from 70°C to 52–54°C for a decameric sequence.²⁸ Thus it is possible to envisage that appropriate conditions can be chosen also when using PNA differing only of one base.

Surface effects are very important when considering the stability of the duplexes on microarrays, as outlined by hybridization studies at different temperatures performed on PNA microarrays fabricated by microcontact printing. PNA:DNA hybridization showed a decrease of about 15°C in melting temperature in

Figure 2. General structures of PNA strands with the coding region (XYZW). O, aminoethoxyethoxyacetyl-spacer; TAMRA, 5(6)-carboxytetramethyl-rhodamine.

comparison with that obtained in solution.³³ Thus, the subsequent hybridization study was performed at $T = 40^{\circ}\text{C}$ in order to allow full match PNA:PNA to be formed and to minimize non-specific interactions.

PNA computing using microarray technology. The computational protocol was as follows: Step 1: a microarray slide containing covalently bound unlabeled PNA library strands encoding all possible solutions ($X/Y = 1/1; 1/0; 0/1; 0/0$, for two-variable problem) was prepared. Four mixtures containing, each, two PNA strands represented possible values of X and Y , were deposited in quadruplicate. Step 2: for each clause, the array was hybridized with a mixture containing two fluorescently-labeled PNA strands which represented all variables in the clause. Only library strands encoding “truth” assignments satisfying the clause form duplexes, and give a fluorescence signal. Step 3: the outcome was read by scanning the microarray slide and assigning truth values to the variables X and Y above a given threshold.

Thus, PNA strands (1–4) representing possible assignments were deposited onto the surface (Fig. 3) so that all possible solutions were represented. In each spot a 1:1 mixture of two PNA strands was deposited, each strand representing the value of a different variable: PNA1/PNA2 for 1/1, (i.e., $X = 1$ and $Y = 1$); PNA1/PNA4 for 1/0 ($X = 1$, $Y = 0$); PNA3/PNA2 for 0/1 ($X = 0$, $Y = 1$) and PNA3/PNA4 for 0/0 ($X = 0$, $Y = 0$). The array/PNA probes should be the same for all problems of n or fewer variables, equivalent to computing hardware.

Moreover, the complementary fluorescent targets used depend of the logic expression to be verified, for example PNA5/PNA6 for “ $X \text{ OR } Y$ ” or PNA5/PNA8 for “ $X \text{ OR } \bar{Y}$ ”, and were equivalent to the software. The fluorescence signals indicate the zones containing probes complementary to the targets used, and potentially encoding the solution of the problem.

Table 1. PNA Library encoding possible solutions (1–4) and variables (5–8)

Probes	Solutions
PNA 1	H-OO-TCATCTAC-NH ₂ ($X = 1$)
PNA 2	H-OO-TCTCATAC-NH ₂ ($Y = 1$)
PNA 3	H-OO-TCAGTTAC-NH ₂ ($X = 0$)
PNA 4	H-OO-TCAAATAC-NH ₂ ($Y = 0$)
Targets	Variables
PNA 5	TAMRA-Lys-GTAGATGA-NH ₂ (X)
PNA 6	TAMRA-Lys-GTATGAGA-NH ₂ (Y)
PNA 7	TAMRA-Lys-GTAACTGA-NH ₂ ($\bar{X}$)
PNA 8	TAMRA-Lys-GTATTTGA-NH ₂ ($\bar{Y}$)

Logic operation “ $X \text{ OR } Y$.” Using the protocol described above, we constructed a 2-input logic gate “OR” that performs Boolean calculations and a single-input gate “Report” that releases a fluorescent output signal in response to specific single-stranded PNA input sequences. The experimental study of this two-variable problem used the above described array, on which all possible solutions ($X/Y = 1/1; 1/0; 0/1; 0/0$) were immobilized, followed by hybridization with fluorescent PNA (PNA5 + PNA6) representing variables X and Y , respectively. The output of the computation is reported in Figure 4.

According to Watson-Crick base pairing interactions, upon hybridization with PNA5 + PNA6, one set of spots (1/1) should contain two perfectly matched duplexes; two sets of spots (1/0; 0/1) should contain one perfectly matched duplex each and the last set (0/0) should contain no duplex. Then, after hybridization, only spots 1/1; 1/0; 0/1 should be fluorescent. The results obtained are fully consistent with the mathematical predictions and provide solutions to the problem ($X \text{ OR } Y = 1$) of $(X,Y) = (1,1); (1,0); (0,1)$.

Table 2. Melting temperatures T_m (°C) for full-matched and mismatched PNA:PNA duplexes

Duplexes	Number of mismatches	T_m (°C) measured	T_m PNA:DNA (°C) calculated ^b	T_m DNA:DNA (°C) calculated ^c
PNA1:PNA5	0	55.0	38.2	15.7
PNA2:PNA6	0	54.0	32.9	15.7
PNA3:PNA7	0	54.0	38.6	17.4
PNA4:PNA8	0	52.0	38.2	12.7
PNA1:PNA8	2	25.0		
PNA1:PNA7	2	<20 ^a		
PNA2:PNA8	3	<20 ^a		

^aNo melting transition was observed in the range 20–95°C. ^bCalculated at 55 μ M of each strand according to the model described in reference 31.

^cCalculated at 5 μ M of each strand and 0.1 M NaCl using the nearest neighbor model with parameters from Allawi et al.³⁰

Figure 3. Computation protocol. (A) deposition: 30 μ M PNA probes (1–4) representing all possible solutions were spotted in quadruplicate on the microarray. Each spot contains two different PNA strands. (B) Hybridization with fluorescent targets (i.e., PNA5 + PNA6; 1 μ M strand concentration). The solutions of the problem were read out using a scanner.

Under the experimental conditions used, the signal intensity is levelled at higher signal intensity due to saturation, and this explains the equivalence of the three solutions (1/1, 0/1 and 1/0).

Logic operation “X OR NotY.” Analogously, when hybridization was performed using PNA5 + PNA8 representing the variables X and (NotY), respectively, two sets of spots (1/1 and 0/0) should contain one fully matched duplex each; one set (1/0) should contain two matched duplexes and the last one (0/1) should contain no duplex. Therefore, one must expect only spots 1/1; 1/0; 0/0 containing PNA probes complementary to

the targets X and (Not Y) to be fluorescent. The experimental data reported in **Figures 5** are fully consistent with the theoretical predictions, providing (X,Y) = (1,1); (1,0); (0,0) as solutions of the problem.

Combined operation “(X OR Y) AND (X OR NotY).” Using the results obtained with the two independent clauses, the combined “OR-AND-OR” gate was designed so that outputs from single “OR” gates can serve as input for the further “AND” gate. This corresponds to the selection of solutions which are compatible both with the first AND the second clause at the same time, as illustrated by the scheme in **Figure 6A**. The combined operation can thus be performed by simply multiplying the intensities obtained for each possible solution in the two experiments. In an ideal case, solutions having a zero value (i.e., false) either in the first or in the second clause will give a zero (false) result, whereas only solutions positive intensities in both measurements will have a significant intensity (i.e., 1 value or true). In the real case, the residual intensities observed in the case of false values give rise to a very low overall value, which can be assigned to zero (i.e., false) result, and is clearly distinguishable from positive (i.e., true) values. The fluorescence intensity reported in **Figure 6B** is the product of the intensities of the signal exhibited by single gates in correspondence with the same set of spots (i.e., $F(1/1) = F_1(1/1) \times F_2(1/1)$). According to the data reported in **Figure 6**, the equation is true only if (X,Y) = (1,1); (1,0).

Although in this example, we performed a very simple calculation, the number of calculations needed (two hybridization steps and product of intensities) is less than that of the systematic test of all possible solutions, which is 2^n , in this case 4. This advantage is crucial when performing the same type of operations with a very high number of variables.

It should be emphasized that the set reported with four bases used as code is capable of representing $4^4 = 256$ different

combinations, which allows in principle to code for 128 variables and their negation, still with a very simple system easily made with only 8 nucleobases. Since PNA:PNA interactions are very sensitive to even single base mismatches, it is possible to envisage that appropriate conditions for this type of experiments could be found. The same model can also be extended to longer PNA sequences allowing to further extend the coding sequence and to avoid the use of singly mismatched sequences or to address even more complicated problems avoiding. This is to our knowledge the shortest oligonucleotide model described so far for DNA-like computing. Nevertheless, the efficiency of the computing process in the combined logical equation is rather remarkable, with at least a 10:1 intensity ration between the correct and the incorrect solutions.

Conclusions. The examples described above demonstrate that our approach to Boolean control based on PNA:PNA interactions is general, and that PNAs could be potential candidates for integration into molecular circuits and computers. Although the prototype of the PNA computer constructed here can process a limited number of operations so far, it allows to envisage a new generation of processing devices based on PNA, able to solve in short time, more complicated non deterministic problems. DNA-based devices (computers and logic gates) have been intensively studied; however, compared to DNA probes, PNA form more stable duplexes and with higher specificity, and are more resistant to enzymes and chemical agents. The current study, although still not suitable for practical applications, demonstrate that it should be possible to employ PNA molecules in a broad spectrum of nanobiotechnological applications, such as computers and logic gates. For DNA computing, extension of simple technologies such as those described in the present work has already led to the solution of a 20-variable SAT problem, which is a computing challenge hardly addressable with normal computers. Therefore extension of the present protocol can be easily foreseen.

PNAs have also been successfully used as candidates for therapeutic drugs³⁴⁻³⁶ and it is generally believed that the power of Boolean computing would find application in molecular agents able to diagnose biological disorders and produce the right signaling or even therapeutic outputs.²⁹ Then, molecular devices based on biomolecules such as PNA capable of highly discriminating between fullmatched and mismatched sequences, might have considerable advantages over simple molecular logic gates.

Figure 4. (A) Diagrammatic representation of the logic gate "X OR Y" wired to a fluorescent reporter. (B) Observed fluorescence output from calculation. (C) Normalized Fluorescence Intensity: Each value is the average of the intensities corresponding to four spots in each column reported in (B).

Materials and Methods

Synthesis of PNA oligomers. The synthesis was performed on an ABI 433A peptide synthesizer with a software modified to run the PNA synthetic steps (scale: 5 μ mol), using Boc chemistry and standard protocols, with HBTU/DIEA coupling. A MBHA resin was used and downloaded manually from the first monomer. In the case of the PNA encoding the variables, upon completion of the PNA oligomer synthesis, 5(6)-carboxytetramethylrhodamine (the Fluorophore unit) was linked manually using DIC/DHBTCH as coupling reagent. The crude PNA were purified by RP-HPLC with UV detection at 260 nm. Semi-prep column C18 (5 microns, 250 x 10 mm, Jupiter Phenomenex, 300 Å) was utilized, eluting with water + 0.01% TFA (eluent A) and a mixture 60: 40 of water/acetonitrile + 0.01% TFA (eluent B); elution gradient: from 100% A to 100% B in 50 min, flow: 4 ml/min. The resulting pure PNA oligomer was characterized by ESI-MS which gave positive ions consistent with the final products.

UV melting analysis. Solutions of 1:1 PNA/PNA were prepared in a pH = 7.0 buffer consisting of 100 mM NaCl, 10 mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.1 mM EDTA. Strand concentrations were 5 μ M for each component. Thermal denaturation profiles (Abs vs. T) of the hybrids were measured at 260 nm with an UV/Vis Lambda Bio 20 Spectrometer equipped with a Peltier Temperature Programmer PTP6, interfaced to a personal computer. For the temperature range 95°C to 20°C, the UV absorbance was recorded at 260 nm every 0.5°C. A melting curve was recorded

Figure 5. (A) Diagrammatic representation of the logic gate “X or NotY” wired to a fluorescent reporter. (B) Observed fluorescence output from calculation. (C) Normalized Fluorescence Intensity: Each value is the average of the intensities corresponding to four spots in each column reported in (B).

Figure 6. (A) Diagrammatic representation of a combined gate “(X OR Y) AND (X OR NotY)” wired to a fluorescent reporter. (B) Normalized Fluorescence Intensity: Each value is given by: $F1(i) \times F2(i)$; where F1 and F2 are average intensities corresponding to each i^{th} column reported in Figures 4B and 5B, respectively.

for each duplex. The melting temperature (T_m) was determined from the maximum of the first derivative of the melting curves.

Array preparation. CodeLink Activated Slides (Amersham Biosciences) were used as solid supports modified with isothiocyanate groups to which the amino-terminal group of the PNA probes encoding all possible solutions was covalently. The deposition of the probes was carried out using a SpotArrayTM24 (PerkinElmer) with a pin-and-ring deposition system. The manufacturer’s instructions for the deposition protocol were slightly changed in order to comply with the special requirement of the chemical structures of PNAs: in particular a 100 mM carbonate buffer (pH 9.0) containing 10% acetonitrile and 0.001% sodium dodecyl sulphate (SDS) was used as deposition buffer. Moreover, after every deposition, the pin-and-ring system was purged with water for 10 s and further washed with acetonitrile/water (1:1), in order to avoid dragging of the

probes in subsequent depositions. The probes were coupled to the surface and the remaining reactive sites were blocked by leaving the slides in a humid chamber (relative humidity 75%) at room temperature for 12 h, followed by immersion in a glass rack containing a 50 mM solution of ethanolamine, 0.1 M TRIS, pH 9, pre-warmed at 50°C, for 30 min. The slides were washed twice with bidistilled water at room temperature and then slowly shaken for 30 min in plastic tubes containing a 4x saline/sodium citrate (SSC) solution and a 0.1% SDS buffer pre-warmed at 50°C. Each slide was then washed with bidistilled water at room temperature and centrifuged in a plastic tube at 800 rpm for 3 min. Slides were then ready to undergo the hybridization protocol.

Hybridization, scanning and signal quantification. Labeled PNA samples (1 μ M strand concentration) encoding two variables were prepared by diluting stock solutions to a final volume of 65 μ l and a final concentration of 4x SSC and 0.1% SDS buffer. Hybridization was performed by loading the samples to “in situ frame” chambers and leaving the slides under slow shaking for 1 h at 40°C. After the hybridization step, all the slides were treated individually to prevent cross contamination. The slides were washed under slow shaking for 5 min at 40°C with a 2x

SSC, 0.1% SDS buffer pre-warmed at 40°C, followed by treatment for 1 min with 0.2x SSC and for 1 min with 0.1x SSC at room temperature. The slides were then spin-dried at 1,000 rpm for 5 min.

The fluorescent signal deriving from the hybridization was acquired using a ScanArray Express (PerkinElmer) with a green laser (λ_{ex} = 545 nm and λ_{em} = 570 nm). In order to correctly compare the hybridization data, all the images reported were acquired with laser power = 100 and photomultiplier gain = 52.

Quantification of the spots was obtained with the QuantArray software using the Adaptive Circles method. Briefly, the fixed circle option was used to define the grid for spot detection, and the mean signal intensity of each spot was quantified; the local background was subtracted. The signal intensity for each code was expressed as the mean of four spots. Signal intensities reported refer to the mean fluorescence and were normalized against the higher signal measured.

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References

- Magri DC, Brown GJ, McClean GD, de Silva AP. Communicating chemical congregation: a molecular AND logic gate with three chemical inputs as a “lab-on-a-molecule” prototype. *J Am Chem Soc* 2006; 128:4950-1.
- Callan JF, de Silva AP, Magri DC. Luminescent sensors and switches in the early 21st century. *Tetrahedron* 2005; 61:8551-88.
- Balzani V, Credi A, Venturi M. Molecular Logic Circuits. *ChemPhysChem* 2003; 4:49-59.
- Shirashi Y, Tokitoh Y, Hirai T. A fluorescent molecular logic gate with multiply-configurable dual outputs. *Chem Comm* 2005; 5316-8.
- Adleman L. Molecular Computation of Solutions to Combinatorial Problems. *Science* 1994; 266:1021-4.
- Stojanovic MN, Semova S, Kolpashchikov D, Macdonald J, Morgan C, Stefanovic D. Deoxyribozyme-based ligase logic gates and their initial circuits. *J Am Chem Soc* 2005; 127:6914-5.
- Raymo FM, Giordani S. All-optical processing with molecular switches. *Proc Natl Acad Sci USA* 2002; 99:4941-4.
- De Sousa M, de Castro B, Abad S, Miranda MA, Pischel U. A molecular tool kit for the variable design of logic operations (NOR INH, EnNOR). *Chem Comm* 2006; 2051-3.
- Kompa KL, Levine RD. A molecular logic gate. *Proc Natl Acad Sci USA* 2001; 98:410-4.
- Margulies D, Felder CE, Melman G, Shanzler A. A Molecular Keypad Lock: A photochemical device capable of authorizing password entries. *J Am Chem Soc* 2007; 129:347-54.
- Okamoto A, Tanaka K, Saito I. DNA Logic Gates. *J Am Chem Soc* 2004; 126:9458-63.
- Miyoshi D, Inoue M, Sugimoto N. DNA logic gates based on structural polymorphism of telomere dna molecules responding to chemical input signals. *Angew Chem Int Ed* 2006; 45:7716-9.
- Braich RS, Chelyapov N, Johnson C, Rothmund PWK, Adleman L. Solution of a 20-Variable 3-SAT problem on a DNA computer. *Science* 2002; 296:499-502.
- Lin CH, Cheng HP, Yang CB, Yang CN. Solving satisfiability problems using a novel microarray-based DNA computer. *Biosystems* 2007; 90:242-52.
- Ogasawara S, Ami T, Fujimoto K. Autonomous dna computing machine based on photochemical gate transition. *J Am Chem Soc* 2008; 130:10050-1.
- Xiong F, Spetzler D, Frasch WD. Solving the fully-connected 15-city TSP using probabilistic DNA computing. *Integrative Biol* 2009; 1:275-80.
- Lipton RJ. DNA solution of hard computational problems. *Science* 1995; 268:542-5.
- Liu Q, Wang L, Frutos AG, Condon AE, Corn RM. DNA computing on surfaces. *Nature* 2000; 403:175-9.
- Wu H. An improved surface-based method for DNA computation. *Biosystems* 2001; 59:1-5.
- Nielsen PE, Egholm M, Berg RH, Buchardt O. Sequence-selective recognition of dna by strand displacement with a thymine-substituted polyamide. *Science* 1991; 254:1497-500.
- Wittung P, Nielsen PE, Buchardt O, Egholm M, Nordén B. DNA-like double helix formed by peptide nucleic acid. *Nature* 1994; 368:561-3.
- Egholm M, Buchardt O, Christensen L, Behrens C, Freier SM, Driver DA, et al. PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen-bonding rules. *Nature* 1993; 365:565-8.
- Chakrabarti MC, Schwarz FP. Thermal stability of PNA/DNA and DNA/DNA duplexes by differential scanning calorimetry. *Nucleic Acids Res* 1999; 27:4801-6.
- Demidov VV, Potaman VN, Frank-Kamenetskii MD, Egholm M, Buchardt O, Sonnichsen SH, Nielsen PE. Stability of peptide nucleic acids in human serum and cellular extracts. *Biochem Pharmacol* 1994; 48:1310-3.
- Nielsen PE. PNA directed sequence addressed self-assembly of DNA nanostructures. *AIP Conference Proceedings (DNA-Based Nanodevices)* 2008; 1062:13-8.
- Lukeman PS, Mittal AC, Seeman NC. Two dimensional PNA/DNA arrays: estimating the helicity of unusual nucleic acid polymers. *Chem Comm* 2004; 1694-5.
- Rose JA, Hagiya M, Deaton RJ, Suyama A. A DNA-based in vitro Genetic Program. *J Biol Phys* 2002; 28:493-8.
- Sen A, Nielsen PE. On the stability of peptide nucleic acid duplexes in the presence of organic solvents. *Nucleic Acids Res* 2007; 35:3367-74.
- Pirrung MC, Connors RV, Odenbaugh AL, Montague-Smith MP, Walcott NG, Tollett JJ. The arrayed primer extension method for dna microchip analysis. Molecular computation of satisfaction problems. *J Am Chem Soc* 2000; 122:1873-82.
- Allawi HT, SantaLucia J Jr. Thermodynamics and NMR of internal G.T mismatches in DNA. *Biochemistry* 1997; 36:10581-94.
- Giesen U, Kleider W, Berding C, Geiger A, Orum H, Nielsen PE. A formula for thermal stability (Tm) prediction of PNA/DNA duplexes. *Nucleic Acids Res* 1998; 26:5004-6.
- Tomac S, Sarkar M, Ratilainen T, Wittung P, Nielsen PE, Nordén B, Gräslund A. Ionic effects on the stability and conformation of peptide nucleic acid complexes. *J Am Chem Soc* 1996; 118:5544-52.
- Calabretta A, Wasserberg D, Posthuma-Trumpie GA, Subramaniam V, van Amerongen A, Corradini R, et al. Patterning of peptide nucleic acids using reactive microcontact printing. *Langmuir* 2011; 27:1536-42.
- Nielsen PE. Gene targeting using peptide nucleic acid. *Methods Mol Biol* 2005; 288:343-58.
- Kaihatsu K, Huffman KE, Corey DE. Intracellular uptake and inhibition of gene expression by PNAs and PNA-Peptide conjugates. *Biochemistry* 2004; 43:14340-7.
- Tonelli R, Fronza R, Purgato S, Camerin C, Bologna F, Alboresi S, et al. Anti-gene Peptide Nucleic Acid (PNA) specifically inhibits n-myc expression in human neuroblastoma cells leading to persistent cell-growth inhibition and apoptosis. *Mol Cancer Ther* 2005; 4:779-86.
- Margolin AA, Stojanovic MN. Boolean calculations made easy (for ribozymes). *Nature Biotechnol* 2005; 23:1374-6.