Towards ISFET based DNA logic for rapid nucleic acid detection

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Abstract— **This paper presents a novel configuration for Ion sensitive Field Effect Transistors (ISFETs) to be used as a threshold detector during a Single Nucleotide Polymorphism (SNP) base pair match. ISFET-based inverters are used as reaction threshold detectors to convey the chemical reaction level to a logic output once a threshold has been reached. Using this, DNA logic functions are derived for nucleotides allowing identification of multiple SNPs. This logic is extended to facilitate a combination of SNPs, deriving gates which give a simple Yes/No answer to their presence. A DNA logic 'NOR' gate is derived for an application where two SNPs need to absent in order to determine drug delivery.**

I. INTRODUCTION

As we enter a new wave of technology inspired by lifestyle and healthcare, precise and efficient point of care testing is becoming increasingly important. The possibility to accurately detect a gene sequence in real-time using a standalone, fully portable, low power unit would provide end-users with technology as yet unavailable outside a laboratory. This presents opportunities in areas where a real-time result can save time, money and lives [1].

In the past, the feasibility of digital computation in cells by creating in-vivo digital logic circuits using DNA binding Proteins, and gene expression has gained much interest [2]. The key aim being to achieve computation out of biological cells and their genetics. In this paper we propose a novel method of DNA Logic using silicon based transistors with chemical interactions to create so called DNA switches that may perform in-vitro computation. This particular work will be confined to switching based upon the marker for nucleotide insertions, Single Nuclide Polymorphisms, with the application being for diagnosis of disease. SNPs (Single Nucleotide Polymorphisms) are receiving considerable interest due to the potential they offer for disease diagnosis, screening and personalized drug therapy and medicine. A SNP is defined as a mutation of a single base affecting at least 1% of a defined population. Although they do not necessarily cause disease, their association with disease and with effects on the pharmacokinetics of many drugs provides information for diagnosis and pharmacological treatment options for many different diseases. There are currently over 1.8 million identified SNPs [3,4]. In this paper, we propose an extension to the method of applying ISFET technology to the detection of these SNPs [5]. Our method should permit SNP detection outside mainstream laboratory facilities and allow identification by individuals such as clinicians and consumers in a point of care setting.

II. ISFET OPERATION: PH TO VOLTAGE

The Ion Sensitive Field Effect Transistor is a device, which has gained considerable popularity for use as a pH sensor. Being robust, cheap and small in size, it has rapidly become competitive with conventional ion selective glass electrodes. The device, which is well described in the literature [6-8], consists of a transistor similar to a traditional MOSFET but with the insulator surface exposed to an electrolyte solution to form an ion-sensitive area, and a remote gate provided by a reference electrode. The rest of the transistor is encapsulated to isolate the device from the electrolyte. The sensitivity is explained partly by the site binding theory and also by a fast dynamic electrochemical exchange component occurring at the surface of the ISFET [6]. The equilibrium between the binding and unbinding of charged ions at the gate surface becomes a function of analyte concentration, which is measured as a change in the voltage drop across the electrolyte/insulator layers. This change in voltage is often described as a change in ISFET threshold voltage [6,7] or alternatively as an intermediate, pH-dependent chemical potential between insulator surface and reference electrode [8] as illustrated schematically in Fig 1.

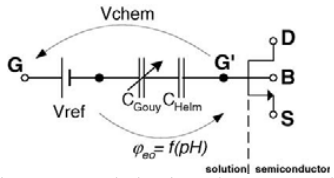

Fig 1: ISFET behavioural macro-model

The dependence of V_{chem} on pH is modeled using a combination of the site-binding theory and the Gouy–Chapman–Stern double-layer model [9], which can be reconciled in a behavioral macro-model representation such as that of Martinoia et al. as shown in Fig 1. ISFET behavioral macromodel. A simplification of this model is to group given by

$$V_{chem} = \gamma + 2.3\alpha U_T pH \qquad (1)$$

where γ is a *pH*-independent grouping of chemical potentials. With α taken as constant to a first-order approximation, the threshold voltage of the ISFET is linearly proportional to *pH*, but is strongly temperature dependent due to scaling by the thermal voltage. It is this change in *pH* representing in a change in transistor threshold voltage which gives rise to a change in the transistor's drain current. The device as with the normal MOSFET, can operate in either strong or weak inversion [8-10].

III. PRIMER EXTENSION: DNA TO PH

DNA is a twisted double-helix structure consisting of four bases called nucleotides, coded for by the letters or 'nucleotides' A, T, C and G. Our unique DNA sequence holds all of our genetic and hereditary information. The double-stranded structure of DNA lends itself to one of its primary functions: DNA replication, the process of copying itself to form new cells. The double-stranded structure breaks into two, whereby each strand then acts as a 'template' to which nucleotides are added in the correct sequence according to

rules of complementary pairs – A can only pair with T and C can only pair with G – in a process known as 'chain extension'. Each single strand of DNA then grows into a double-stranded DNA sequence, resulting in DNA replication – one of nature's most fundamental processes.

The biochemistry of chain extension can be used as a method of DNA detection and has demonstrated for detection of mutations in the DNA known as single nucleotide polymorphisms ("SNPs") [11]. The incorporation of complementary nucleotides into a growing strand of DNA yields pyrophosphate which in-vivo is rapidly hydrolyzed to orthophosphate:

$$HP_2O_7^- + H_2O \Leftrightarrow 2H_2PO_4^- + H^+$$

pyrophosphate orthophosphate (pH 7.4-8.0)

The hydrolysis reaction produces or consumes hydrogen ions depending on the pH in which it occurs [12,13]. Following optimization of the reaction environment in-vitro to maximize the rate of pH change, hydrogen ions are produced, i.e. pH decreases, during nucleotide incorporation. When the ISFET is operated in constant charge configuration with fixed drain current [6], changes in hydrogen ion concentration are detected as changes in the gate-source voltage of the ISFET.

For the purpose of detection of SNPs, where there is a variation of a single nucleotide of the DNA code at a given location, a single stranded DNA "probe" can be designed to uniquely identify that location within the "target" DNA being analysed. If the probe length is *n* nucleotides, then the nucleotide at position *n+1* within the target can be determined by adding different nucleotides in parallel reaction chambers, A, T, C and G, each with its own ISFET sensor and seeing which one incorporates. Only a complimentary nucleotide will be incorporated (extend the probe-target hybrid), thereby releasing protons and reducing pH and leading to a change in ISFET output voltage, and a non-complimentary nucleotide will not. Through a knowledge of which nucleotide has been incorporated by observing which ISFET reaction chamber gives a discrete pH fluctuation representing nucleotide incorporation, one can determine which nucleotide is present at the location of interest and whether this is a mutation (SNP) or "wild type" (no SNP) [14]. We can observe different pH changes as a consequence of different DNA inputs (targets) as shown in Fig 2.

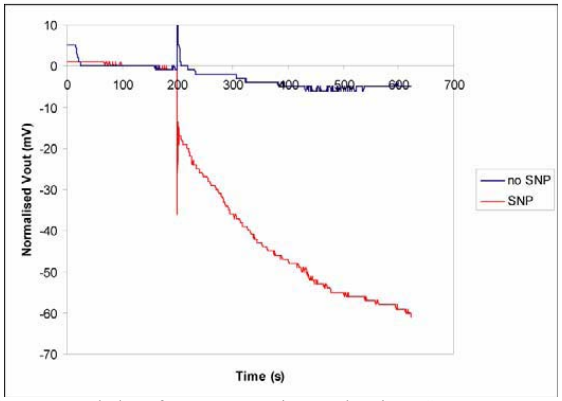

Fig 2: Measured data from an experiment showing ISFET output voltage during (a) complimentary nucleotide incorporation (blue) and (b) no nucleotide incorporation (black) to a DNA template during chain extension

IV. DNA LOGIC

The operation of the ISFET as a means of implementing a "DNA logic" unit capable of calculating truth-table type outputs is an attractive idea for array-based algorithmic processing. The sooner the conversion to digital 0 or 1 signals, the less the effect of degradation from interconnects and sources of interference between the sensor chip and any off-chip interfacing.

To demonstrate how aptly ISFETs can be used for this type of operation, we present an adaptation of the standard CMOS inverter, Fig 3a, which performs a pH-thresholding operation when one of the MOSFETs is replaced by an ISFET, Fig 3b. The circuit can be implemented in strong or weak inversion, but for low power operation, the analysis herein is restricted to weak inversion.

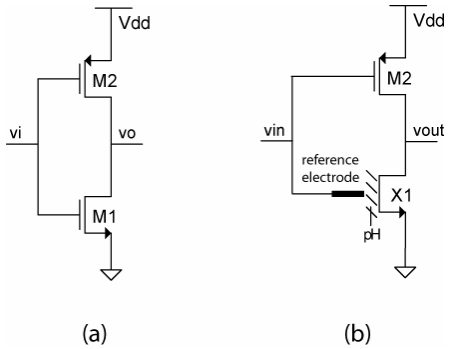

Fig 3: (a) Standard CMOS inverter and (b) ISFET-based inverter or "pH thresholder"

The standard CMOS inverter in weak inversion has the transfer characteristic shown in Fig 4. At the switching threshold V_{inth}, M1 and M2 have equal drain currents giving:

$$V_{\text{int}h} = \frac{V_{DD} - |V_{TOp}| + \ln\left(\frac{I_{sn}}{I_{sp}}\right) V_{TOn}}{1 + \ln\left(\frac{I_{sn}}{I_{sp}}\right)} \quad (2)$$

where the sub-threshold slope for NMOS and PMOS are assumed equal. We have already seen from a behavioral point of view that the ISFET can be considered to be a MOSFET with a chemically sensitive threshold voltage, where V_{chem} varies approximately 55mV/pH. Therefore, if the NMOS in Fig 3a is replaced with by an n-channel ISFET as shown in Fig 3b, the switching threshold will be given as [15]:

$$V_{\text{int}h} = \frac{V_{DD} - |V_{TOp}| + \ln\left(\frac{I_{sn}}{I_{sp}}\right) [V_{TOn} + V_{chem}]}{1 + \ln\left(\frac{I_{sn}}{I_{sp}}\right)} \quad (3)$$

The simulated transfer characteristic of the ISFET-based inverter circuit (Fig 3b) in buffer solutions of pH 6, 7 and 8, is shown in Fig 4. The circuit was simulated in the UMC 0.25µm CMOS technology, Vdd = 2.5V, using a simplified version of the Martinoia macromodel [9] with perfectly linear pH sensitivity (constant α for all pH and temperature). Device dimensions were 50/50 for n-type ISFET X1 and 50/10 for p-type MOSFET M2.

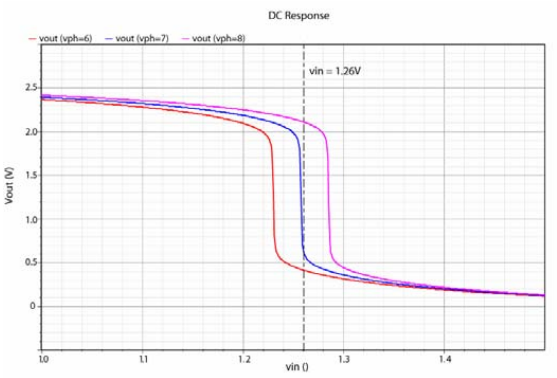

Fig 4: Simulated *Vout* versus *Vin* at pH 6, 7, 8

Table I: Effect of pH on switching threshold

pH	3	4	5	6	7	8	9	10
$V_{inth}(V)$	1.148	1.176	1.204	1.231	1.258	1.286	1.31	1.341

The effect of pH on the switching threshold is shown in Table I and illustrated in Fig 4. A consequence of this characteristic is that if Vin is fixed, then switching will occur exclusively due to a change in pH. Moreover, the pH threshold at which this switching occurs can be set by our choice of vi as shown in Fig 5. For illustration, it is shown that the output is high for pH $\geqslant$ 4 when vin is fixed at 1.18V and for pH 8 when vin =1.26V.

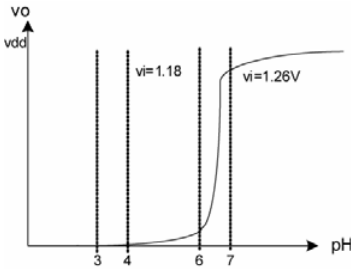

Fig 5: Simulated pH switching thresholds for different input bias voltages

The significance of this simple circuit is that it is a type of pH comparator, not switched by a 0 or 1 on the gate input, but by a pH less than or greater than a chosen threshold. Simulation results have shown the uncertainty region around the threshold pH to be approximately ±0.3 pH units. This is nevertheless a potentially useful technique for array-based thresholding operations where trends and not accuracy are the focus. Discrete pH changes caused by events such as primer extension for SNP detection, extend "pH thresholding" to "DNA logic", which has a practical utility in the direct transduction of a DNA input to a binary output as shown in Table II. Fig. 6 shows circuit simulations showing how the measured data from nucleotide incorporation would be converted to a digital output by the ISFET-based DNA logic "NOT" gate in Fig 3b.

Table II: Truth Table of DNA Logic "NOT" Gate

pH < pHth	DNA Input	Output
0	No SNP detected	1
1	SNP detected	0

V. DNA LOGIC NOR GATE

Thus far, we have shown how the ISFET given a DNA input such as the presence or absence of a SNP will cause a change in pH which can be converted to a binary output using a simple adaptation of the

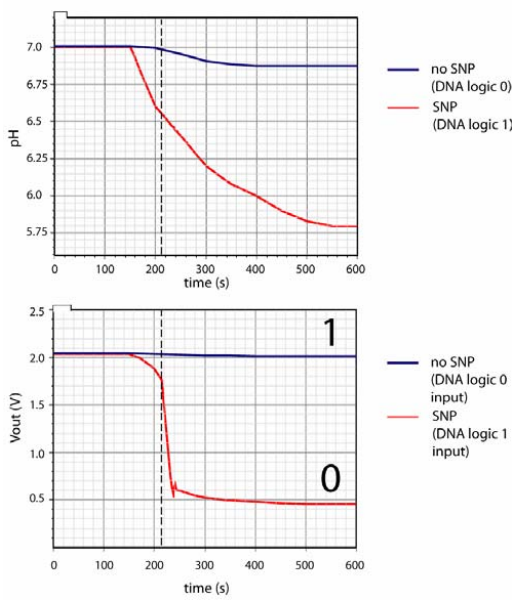

Fig 6: (a) Measured data from pH response during nucleotide incorporation during primer extension when no SNP is present (logic 0) and when SNP is present (logic 1) and (b) simulated output from DNA logic inverter circuit

"NOT" logic gate. That principle can be extended to multiple DNA inputs to create other DNA logic circuits to perform simple logical operations which provide a yes/no answer. Often the presence or absence of a combination multiple SNPs can give information about the risk of genetic predisposition to certain diseases such as heart disease or Type II diabetes [16]. Furthermore, some SNPs are linked to drug metabolism and therefore can be used to predict whether a patient should take a high or low dose of certain prescription drugs. In the case of warfarin, a blood-thinning agent, a high dose in people with certain SNPs could cause excessive bleeding. Two SNPs in particular have been found to correspond with lower dose requirements, due to the fact that they indicate slower metabolism of the drug. These SNPS are on the "CYP2C9*2" and "CYP2C9*3" alleles, and a patient with either or both of these SNPs will require a lower dose of warfarin [17]. For illustration, a simplified "truth table" for warfarin dose as predicted by CYP2C9 genotype, is shown in Table III.

Table III: Truth table of warfarin dose predicted from CYP2C9 genotype

CYP2C9*2	**CYP2C9*3**	**Dose**
Absent (0)	Absent (0)	High (1)
Absent (0)	Present (1)	Low (0)
Present (1)	Absent (0)	Low (0)
Present (1)	Present (1)	Low (0)

Table III corresponds to a "NOR" relationship, which can be implemented using ISFET-based DNA Logic. The circuit of the DNA-NOR gate is shown in Fig. 7 where the classical MOSFET is replaced with ISFETs at the inputs, with appropriate DNA probes and biochemical reagents to detect nucleotide incorporation during primer extension [5]. The simulation results are shown in Fig 8, where the four vertical dash lines divide the simulation into four results corresponding to the truth table inputs shown in Table IV, where either SNP A or SNP B is present in a DNA sample. This simulation clearly demonstrates that when a single base is detected on either ISFET, then the output will be zero. Only if neither ISFET detects a SNP will the result be a digital one.

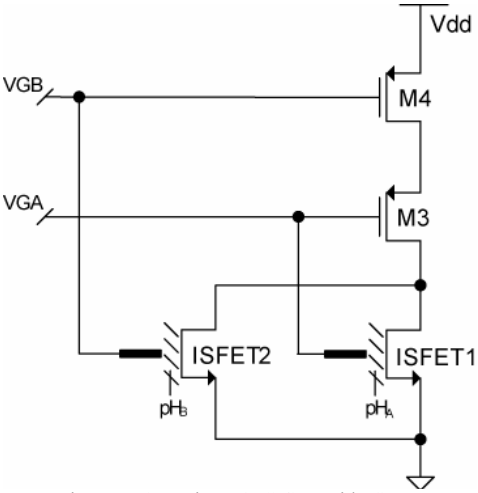

Fig 7: DNA Logic "NOR" Gate with ISFET

Table IV: Truth table of DNA Logic NOR Gate

pH<pHthA (SNP A present)	**pH<pHthB** (SNP B present)	**Vout**
0	0	1
0	1	0
1	0	0
1	1	0

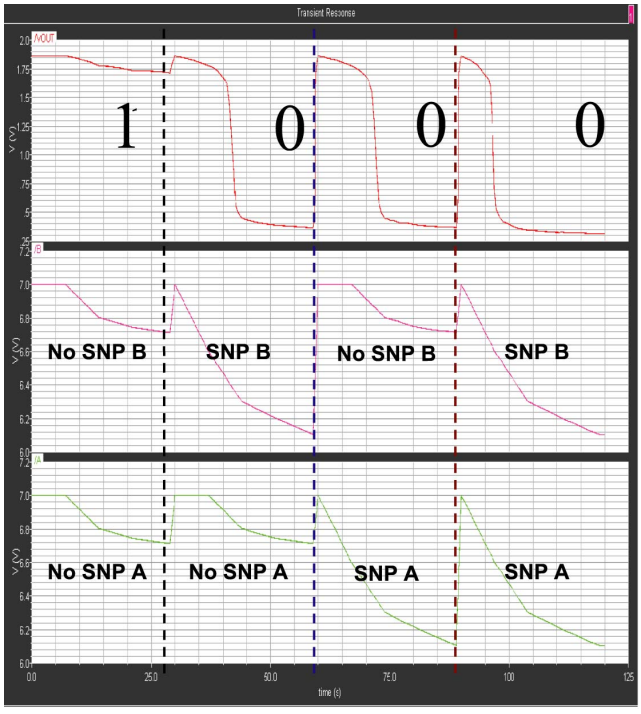

Fig 8: Simulated output response from DNA-NOR circuit for reactions in truth Table III

VI. Conclusion

In this paper, we have demonstrated the feasibility of using DNA to switch on and off transistors to create a synthetic form of DNA logic. This will be useful in two applications, either to create DNA computation as a extension to the work already being pioneered within cellular computing or as described in this letter for point-of care SNP logic on silicon chip based technologies with the advantages therefore of disposability, portability as well as leveraging on the economy of scales of semi-conductor technologies.

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