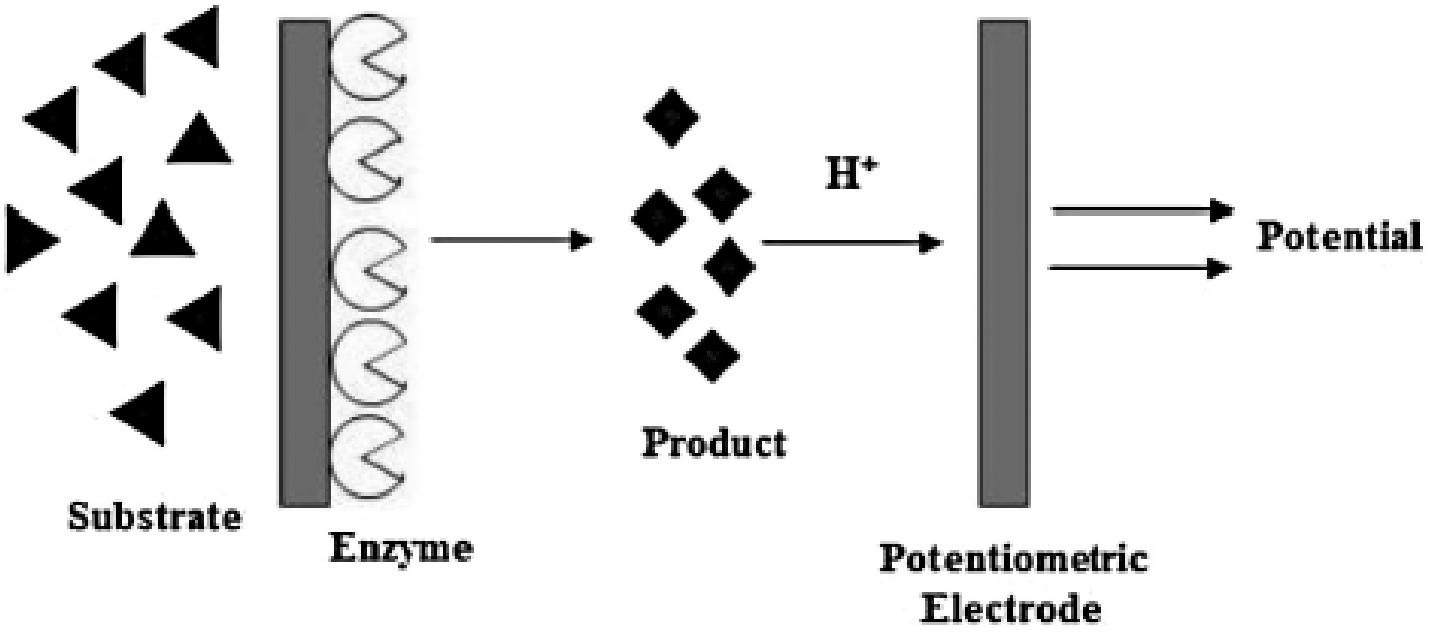
POTENTIOMETRIC BIOSENSORS

Potentiometric measurements involved determination of the potential difference between either an indicator and a reference electrode or two reference electrodes separated by a permselective membrane, in the absence of significant current between them.

The most common potentiometric devices are pH electrodes.

Several other ions (Na^+, K^+, Ca^{2+}, NH^{4+}, F^-, I^-, CN^-) or gas (CO_2, NH_3) selective electrodes are also available.



The potential differences between these indicators and reference electrodes are proportional to the logarithm of the ion activity or gas fugacity (or concentration), as described by **Nernst-Donnan.**

$$E_{cell} = E^{0}_{cell} - \frac{RT}{nF} \ln Q$$

E_{cell} is the observed cell potential at zero current that is called the electromotive force (EMF),
E^{0}_{cell} is a standard potential contribution to the cell,

R and T are univ. gas constant and absolute temp. (in degrees Kelvin), respectively,
n is the number of charges, F is the Faraday constant, and

***Q* is the ratio of ion concentration at the anode to ion concentration at the cathode**

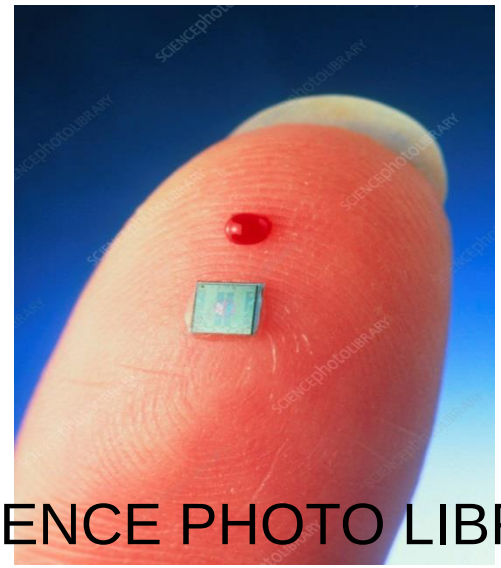
ISFET: BioFET

The ion selective field effect transistor (ISFET)-based potentiometric devices have received intensive interest recently due to their several advantages over the conventional potentiometric biosensors.

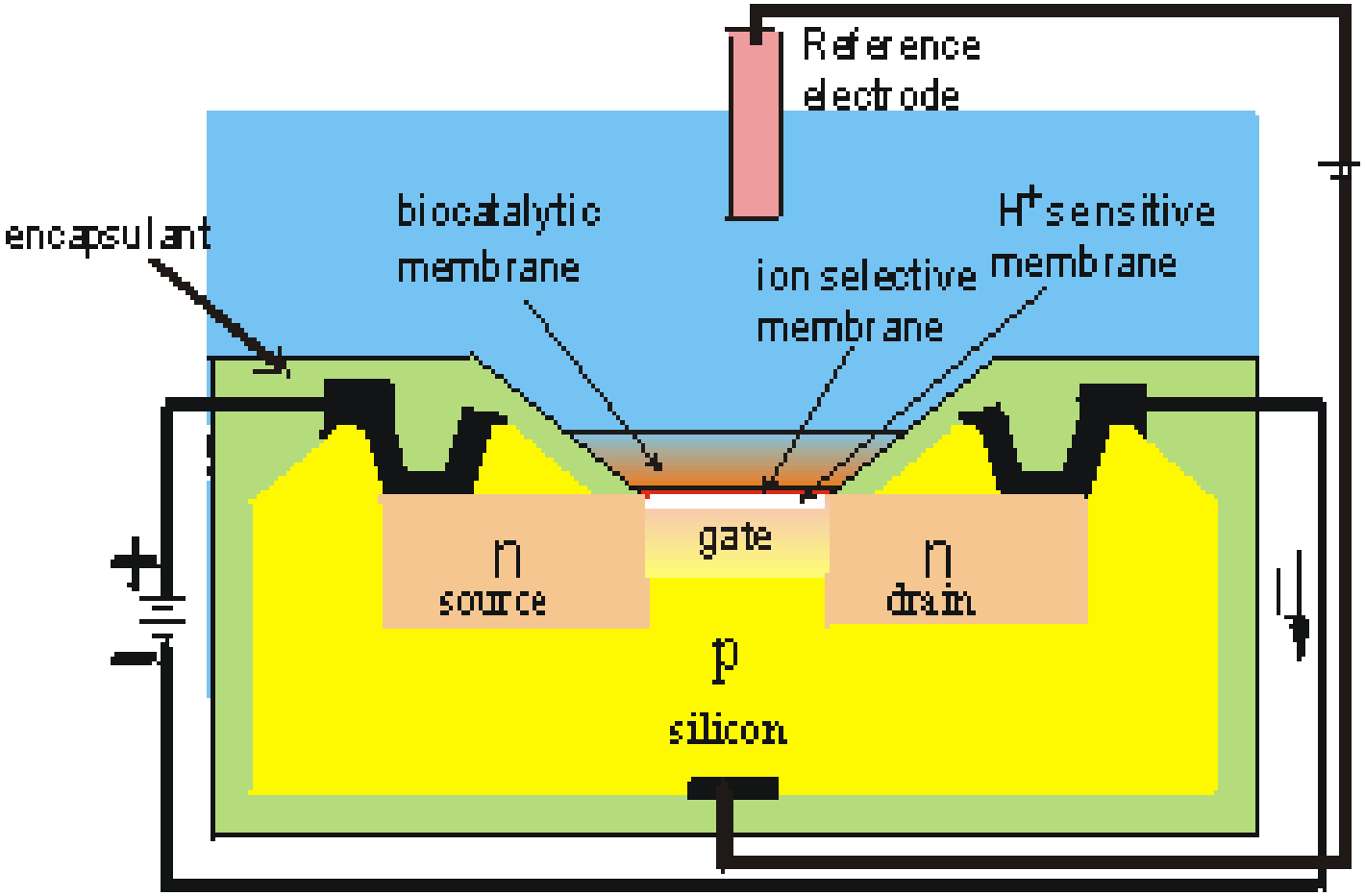
[A transistor is a miniature semiconductor that *regulates or controls* current or voltage flow in addition *amplifying and generating* these electrical signals and acting as a switch/gate for them.]

The key advantages of ISFETs over conventional potentiometric sensors (such as ion-selective electrodes): **small size, short response time, and their compatibility with semiconductor fabrication methods, giving scope for monolithic integration of the sensors and processing circuitry.**

The extremely small size (<< 0.1 mm^2) allows cheap mass-produced fabrication using integrated circuit technology.



Schematic diagram (section across) of an ENFET [when enzyme is used as electrode catalyst ISFET is termed enzyme-linked field effect transistors (ENFET). And termed BioFET when any biorecognition element is used in ISFET].



Terminals:
Gate,

Drain,

Source,

Body
(base/bulk/substrate).

Dimension of the active area : ~500 µm long x 50 µm wide x300 µm thick.

Fabrication of ISFET

The chip is insulated by a thin layer (0.1 μm thick) of silica (SiO_2) which forms the gate of the FET.

The main body is a p-type silicon chip with two n-type silicon areas: the negative source and the positive drain all are created by doping.

Doping refers to the process of intentionally introducing impurities into an extremely pure semiconductor in order to change its electrical properties.

An n-type semiconductor (n for Negative) is obtained by carrying out adding an impurity of valence-V elements to a valence-IV semiconductor in order to increase the number of free, in this case negative, charge carriers.

Doping with group III elements, such as boron, which are missing the fourth valence electron creates "broken bonds", or holes, in the silicon lattice that are free to move.

Thus group V element is said to behave as an electron donor, and a group III element as an acceptor**.**

Above this gate is a thin layer of H^+-sensitive material, a protective ion selective membrane, the biocatalyst and the analyte solution, which is separated from sensitive parts of the FET by an inert encapsulating ***polyimide photopolymer***.

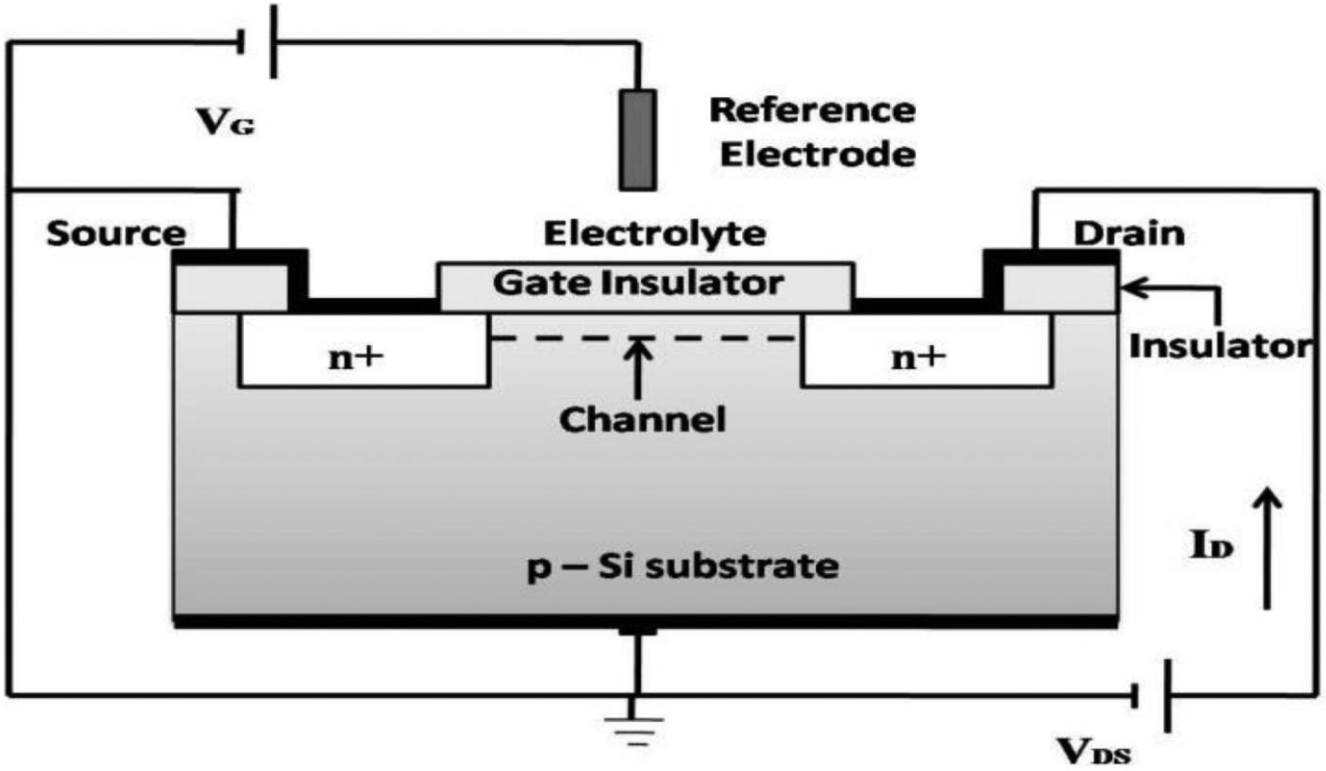
Typical pH sensitive gate materials are Si_3N_4, Al_2O_3 and Ta_2O_5.
In practice, these layers are deposited on the top of the first layer of SiO_2 by means of chemical vapour deposition (CVD).

The gate electrode is separated from the channel by a barrier which is sensitive to hydrogen ions and a gap to allow the substance under test to come in contact with the sensitive barrier.

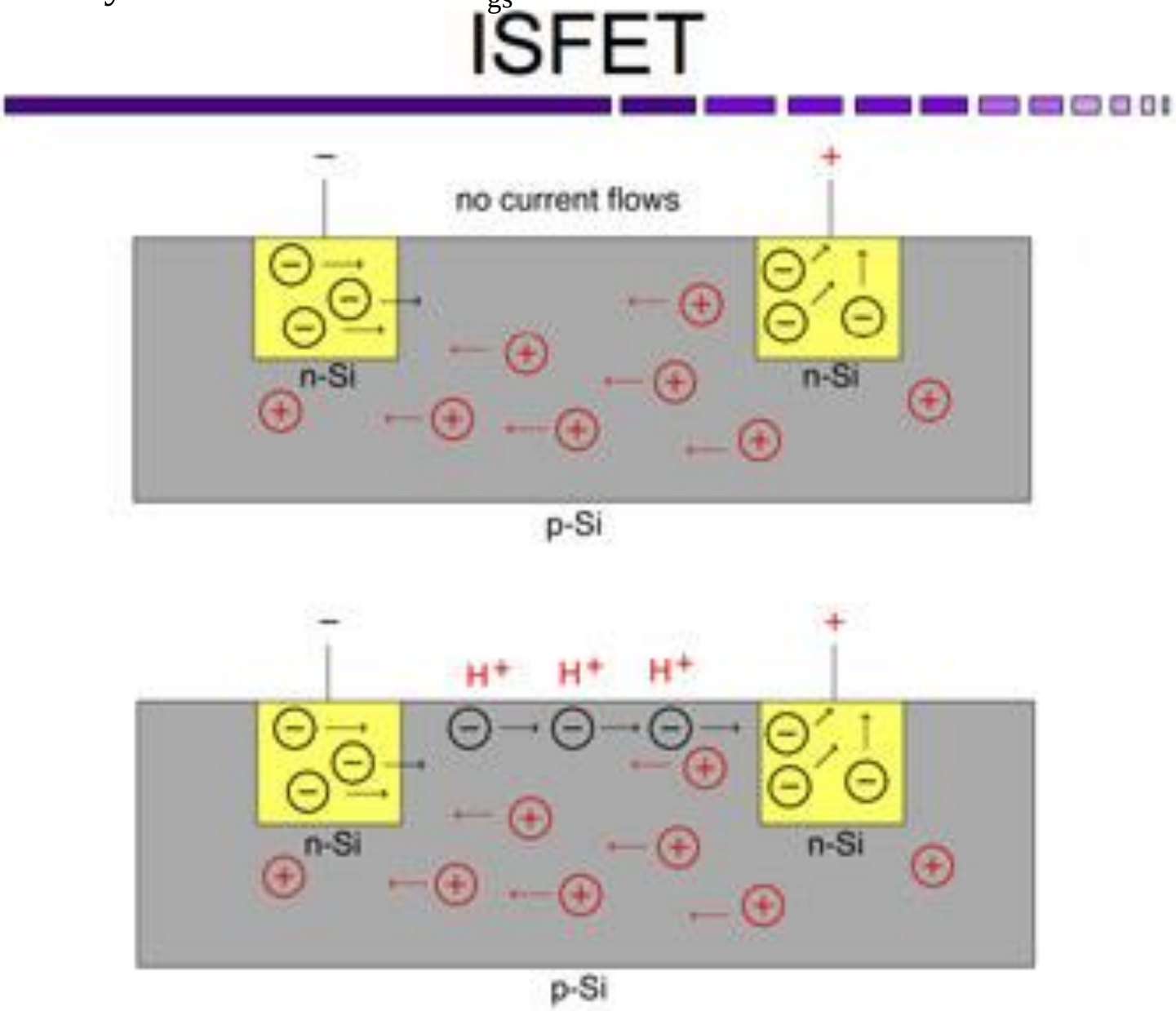
WORKING PRINCIPLE

Electrons flow from the source to drain terminal if influenced by an applied voltage. When a +Ve V (w.r.t to the silicon) is applied to the gate electrode, electrons are attracted to the surface of the semiconductor. Consequently, a conducting channel is created between the source and the drain, near the silicon dioxide interface.

The conductivity of this channel can be modulated by adjusting the strength of electrical field between the gate electrode and the silicon, perpendicular to the substrate surface. At the same time a voltage can be applied between the drain and the source (V_{ds}), which results in a drain current (I_d) between the n-regions.



The source-drain current is influenced by the interface potential at the oxide/aqueous solution. At a fixed source-drain potential (V_{ds}), changes in the gate potential can be compensated by modulation of the V_{gs}.



The surface of the gate oxide contains OH-functionalities, which are in **electrochemical equilibrium with ions in the sample solutions (H+ and OH-).**

The hydroxyl groups at the gate oxide surface can be protonated and deprotonated and thus, when the gate oxide contacts an aqueous solution, a change of pH will change the SiO_2 surface potential.
Typical pH sensitivities measured with SiO_2 ISFETs are 37-40 mV/ pH unit

The gate electrode is separated from the channel by a barrier which is sensitive to hydrogen ions and a gap to allow the substance under test to come in contact with the sensitive barrier.

The site-binding model

It describes the equilibrium between the S–OH surface sites and the H^+ ions in the solution.

The hydroxyl groups coating an oxide surface such as that of SiO_2 can donate or accept a proton and thus behave in an **amphoteric way** following acid-base reactions occurring at the oxide-electrolyte interface:

$$—Si–OH + H_2O \leftrightarrow —Si–O^- + H_3O^+$$
$$—Si–OH + H_3O^+ \leftrightarrow —Si–OH_2^+ + H_2O$$

An ISFET's threshold voltage depends on the pH of the substance in contact with its ion-sensitive barrier.

The biosensor responding to the electrical potential change via the current output. Thus, these are potentiometric devices although they directly produce changes in the electric current.

The drain to source current of an ISFET (I_D or I_{DS}) can be deduced:

$$I_D = \mu C_i \left(\frac{W}{L}\right) V_{DS} \left[\left(V_{GS} - V_{TH(ISFET)}\right) - 0.5\ V_{DS}\right]$$

where C_i is the oxide capacitance per unit area, W and L are the channel width and length respectively, and μ is electron mobility in the channel.

Here the term $\mu C_i \left(\frac{W}{L}\right)$ is constant.

V_{GS} and V_{DS} are kept constant through biasing; therefore, $V_{TH(ISFET)}$ is the only input parameter.

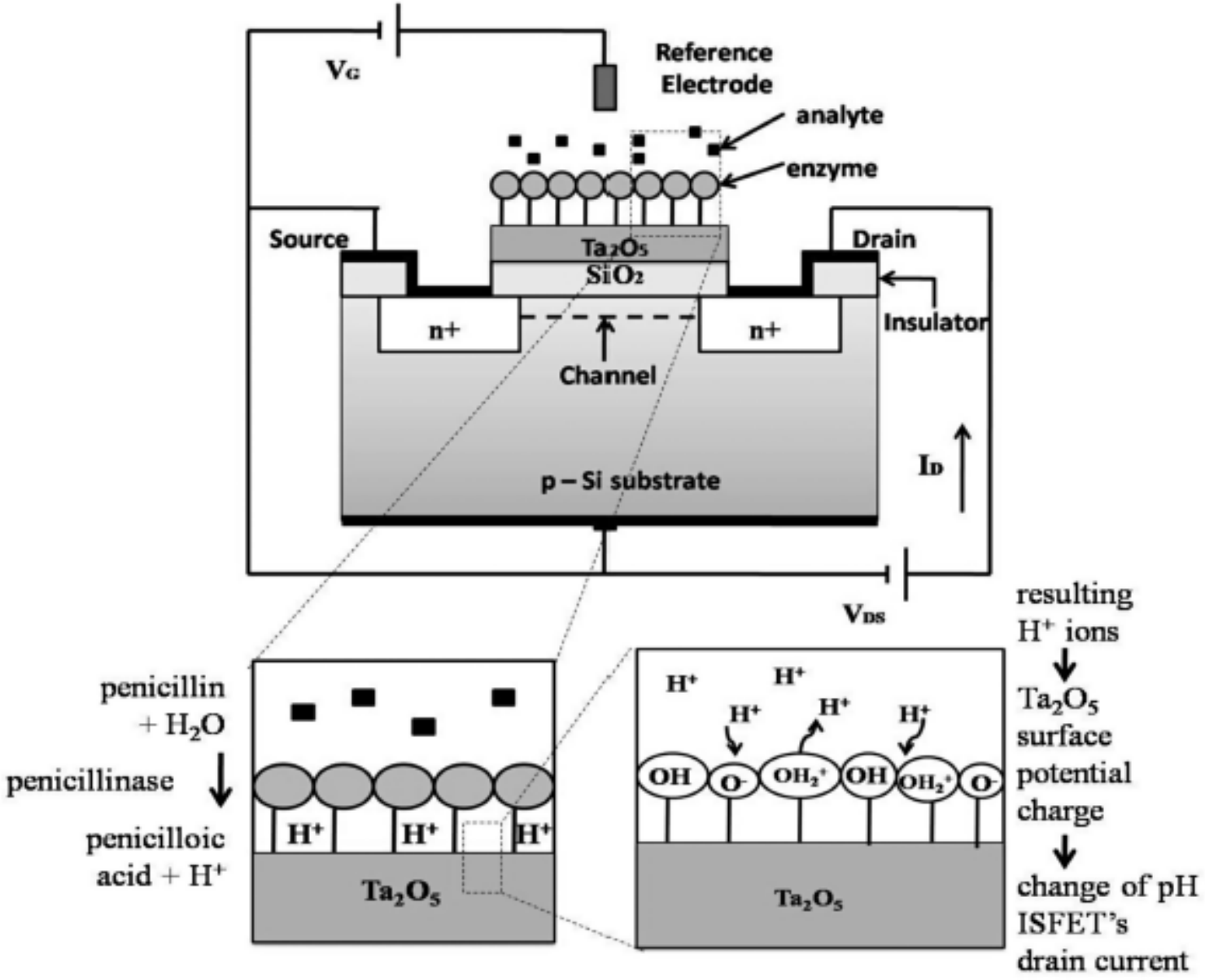
The threshold voltage of ISFET is given by:

$$V_{TH(ISFET)} = (E_{ref} - \psi_0 + \chi_{sol} - (\Phi_{Si} / q) - (Q_i + Q_{SS} + Q_B) / C_i) + 2\Phi_f)$$

E_{ref} is the potential of the reference electrode;
Φ_{Si} is the silicon electron work function;
q is the elementary charge;
Ci is the capacitance of the gate insulator;
Qi, QSS, and QB are the charges located in the insulator, in the surface and interfac
states, and in the depletion region, respectively;
Xsol is the surface dipole potential of the solution, which is constant;
Φf is the potential difference between the Fermi level of doped and intrinsic silicon
2Φf is the surface inversion voltage;and
0 is the potential at the electrolyte-insulator interface that depends on the activity c
ions in the analyte.

The **threshold voltage**, is the minimum gate-to-source voltage $V_{GS(th)}$ that is needed to create a conducting path between the source and drain terminals.

Detection of penicillin



ENFET (penicillin-sensitive ENFET) structure and functional principle.

Following a protein kinase activity using label-free FET device

Freeman et al. Chem. Commun., 2007, 3450–3452

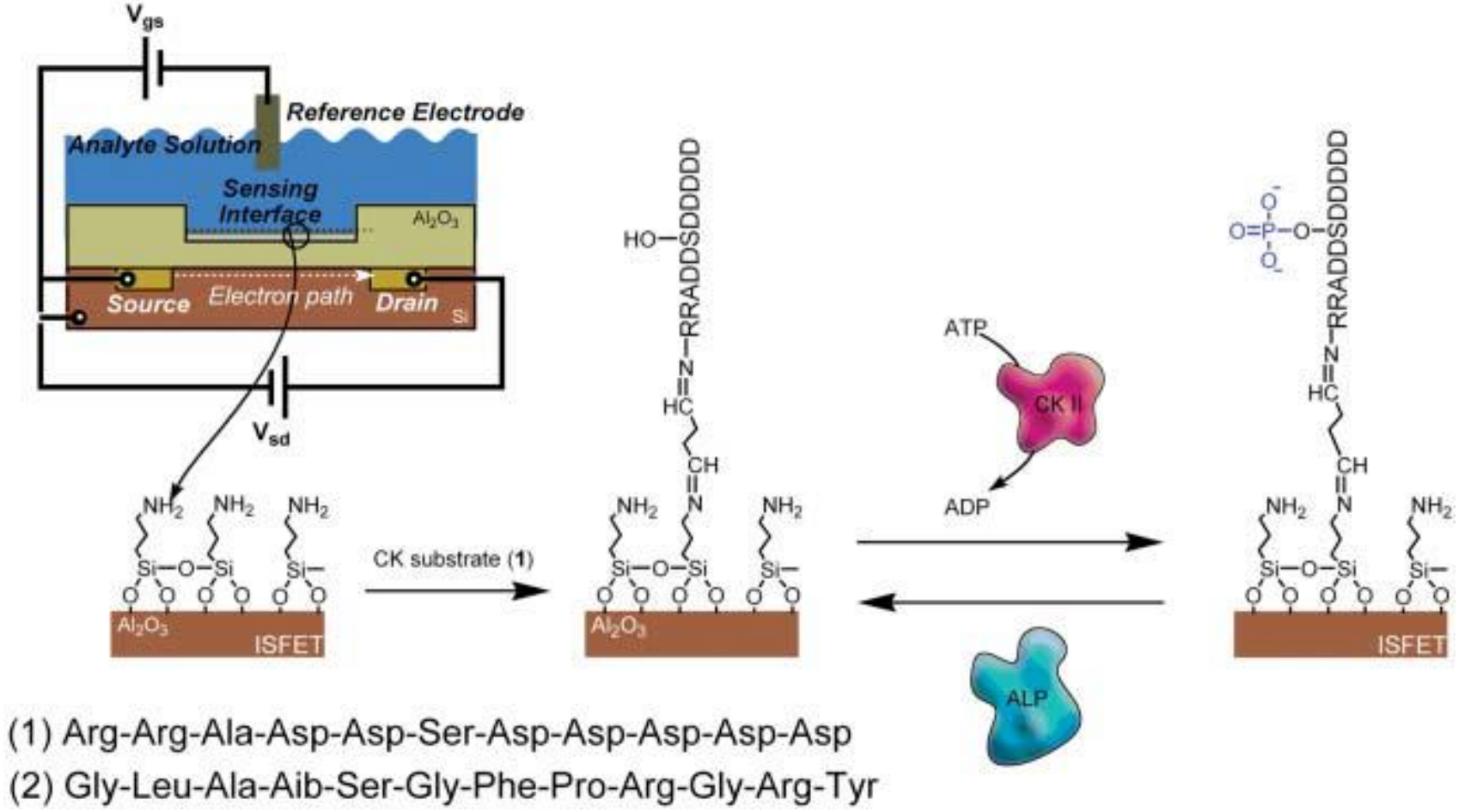
The protein kinases are a large family of enzymes that modulate the activity of proteins by phosphorylation.

One of the most versatile of the protein kinases is casein kinase II (CK2), a serine/threonine-selective protein kinase, which can phosphorylate more than 160 proteins.

CK2 is involved in signal transduction, transcriptional control, apoptosis, cell cycle and more.

Aberrant activity of CK2 has been implicated in a number of Diseases: reduced activity in neuron of Alzheimer's disease, elevated amounts in various types of cancer.

It also plays a major role in the life cycle of HIV-1, and it has been found that CK2 is a selective target of HIV-1 transcriptional inhibitors.



Substrate sequence-specific peptide includes the serine residue adjacent to aspartic acid, is recognized by CK2.

The Al_2O_3 gate of an ISFET device was modified by the treatment of the ISFET with 3-aminopropyltriethoxysilane.

The Peptide was covalently linked to the aminosiloxane-functionalized gate interface by treatment of the gate with glutaric dialdehyde.

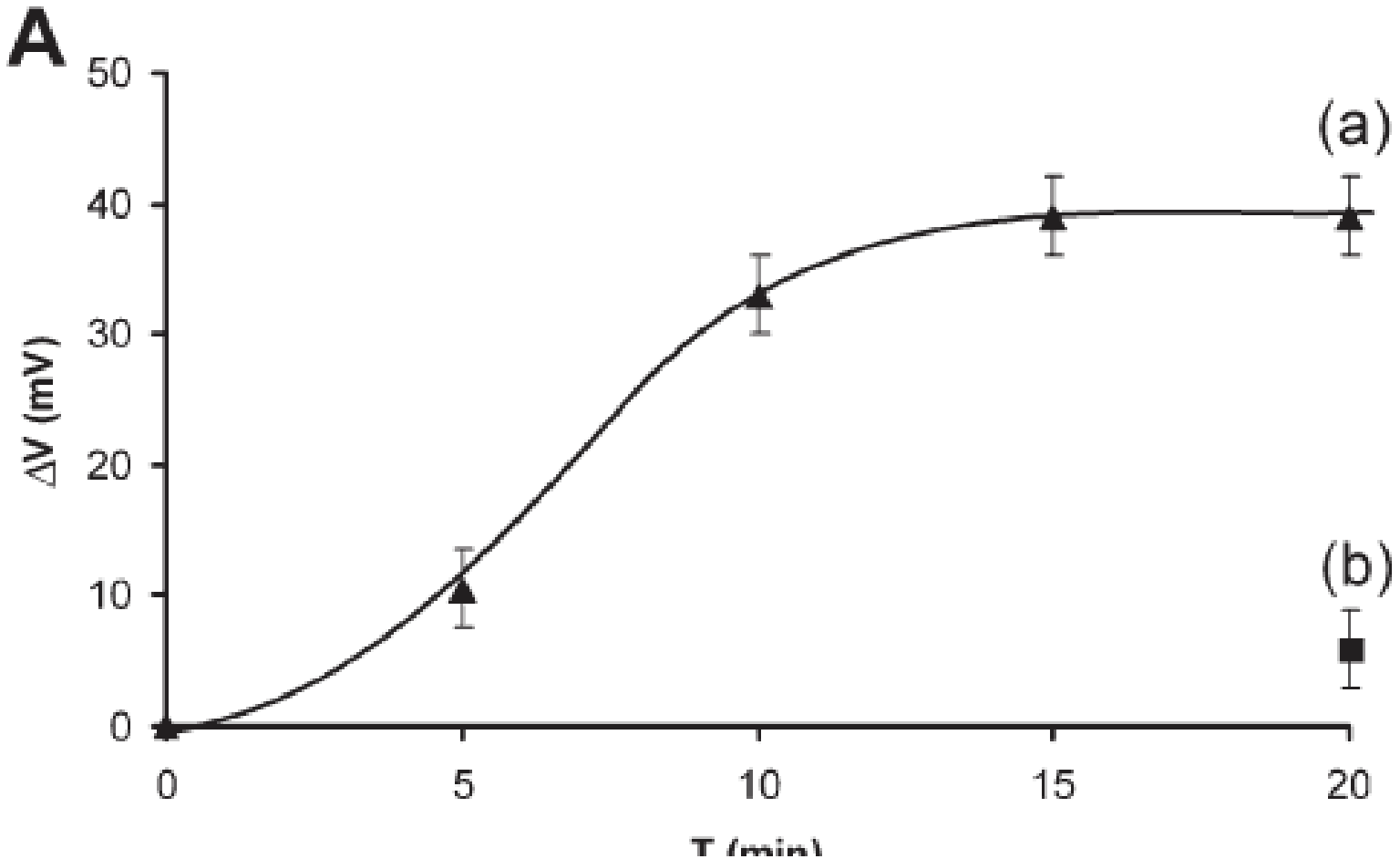
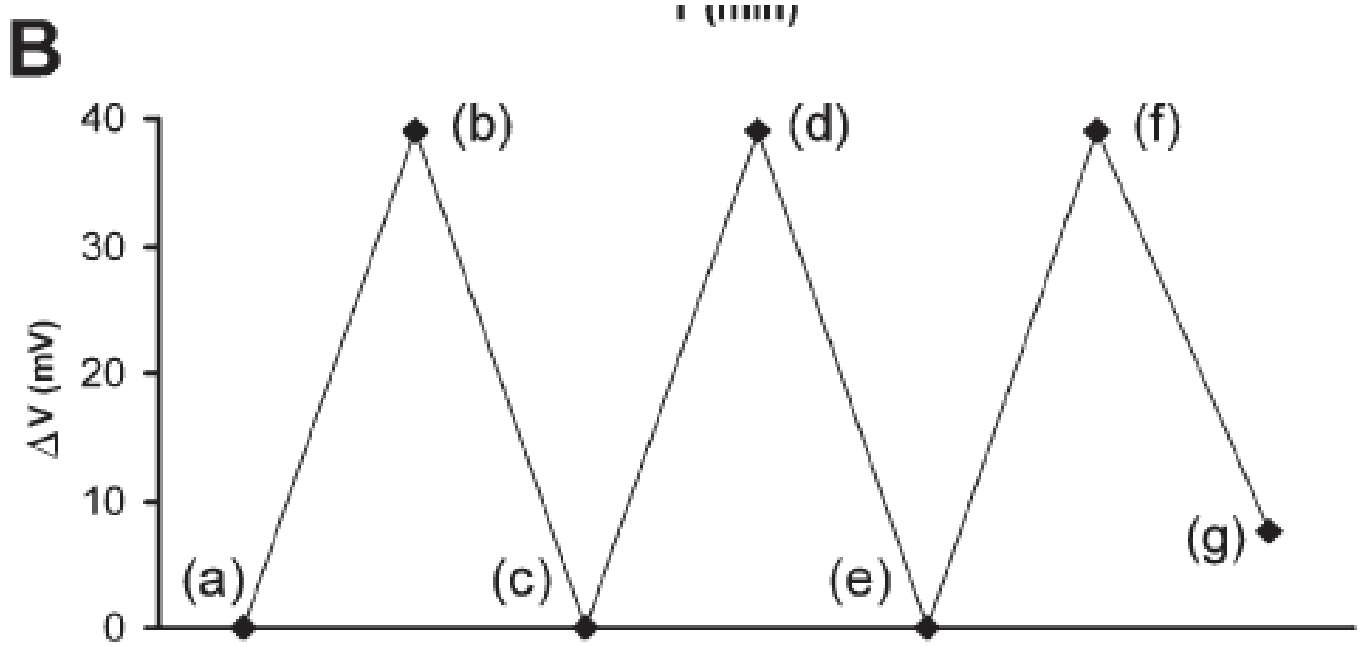


Fig. 1 (A) (a) Time-dependent changes in the source-to-gate potential upon the treatment of the (**1**)-functionalized device with CK2, 0.012 U, and ATP, 100 μM. (b) Potential change of the (**2**)-modified device upon treatment with CK2, 0.012 U, and ATP, 100 μM, for 20 minutes. (B)



One proof that phosphorylation indeed occurred on the (1)-modified surface was obtained by treatment of the phosphorylated gate with alkaline phosphatase that catalyzed the hydrolysis of the phosphate ester group to the neutral peptide (1). Upon application of the hydrolyzing enzyme, the gate potential returned to the original value after 20 minutes, Fig. 1(B), point (c). This is

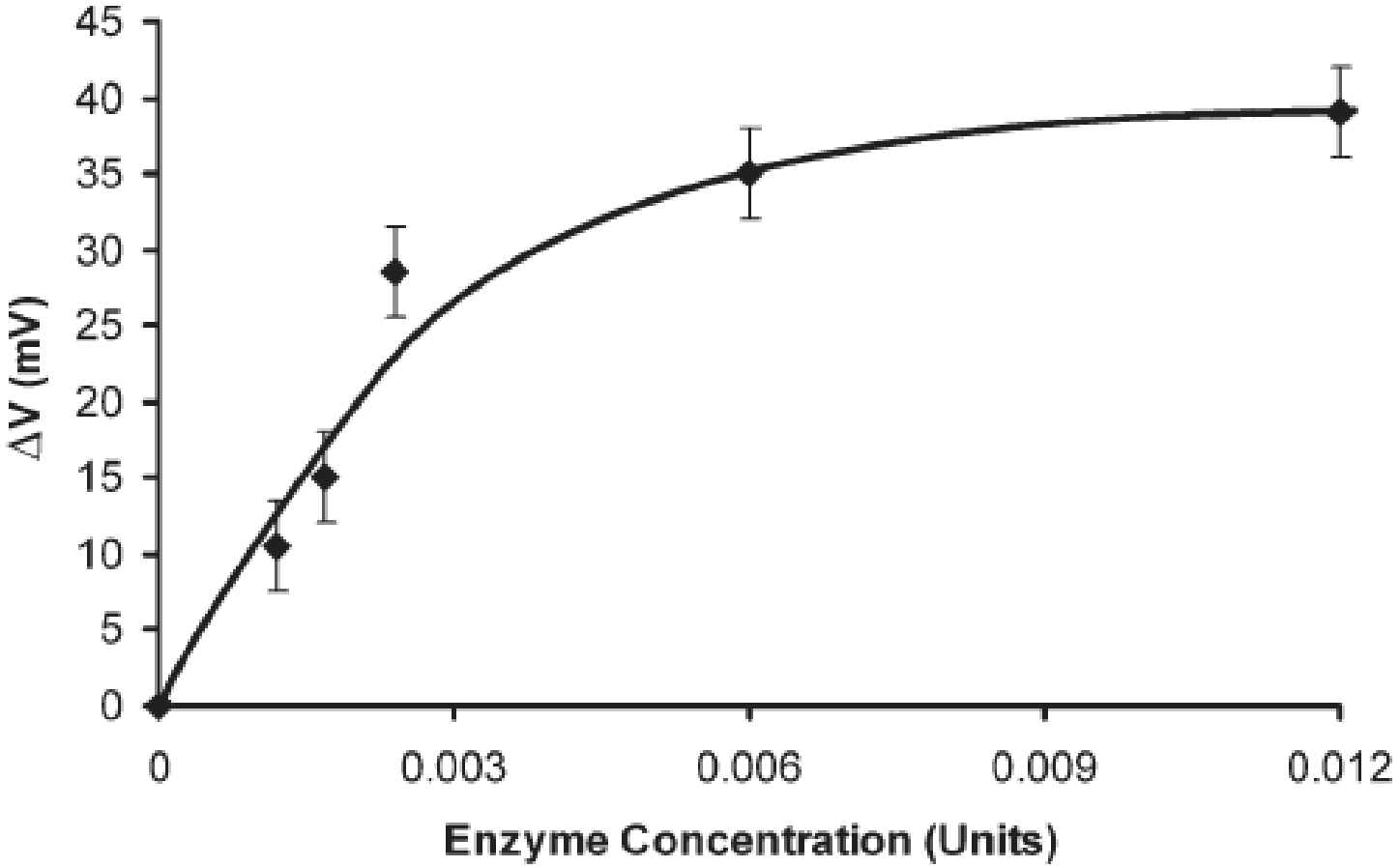


Fig. 3 Source-to-gate potential changes upon phosphorylation of the (1)-functionalized device with different concentrations of CK2 in the presence of ATP, 100 μM, for 20 minutes. Readouts were made in 20 mM

CK is *ca.* 1.5×10^{-3} U mL^{-1}. The shape of the curve depicted in Fig. 3 is consistent with the fact that the FET response is a logarithmic function of the phosphate surface charges

Disadvantages:

The sensitivity of FETs, may be affected by the composition, ionic strength and concentrations of the solutions analyzed.

END