Modern Aspects of Electrochemistry 52

Noam Eliaz *Editor*

Applications of Electrochemistry and Nanotechnology in Biology and Medicine I

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Applications of Electrochemistry and Nanotechnology in Biology and Medicine I

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Preface

The emergence of nanoscience and nanotechnology has led to new developments in and applications of electrochemistry. These two volumes of Modern Aspects of Electrochemistry, entitled: "Applications of Electrochemistry and Nanotechnology in Biology and Medicine, I and II" address both fundamental and practical aspects of several emerging key technologies. All Chapters were written by internationally renowned experts who are leaders in their area.

The chapter by A. Heiskanen and J. Emnéus provides a lucid and authoritative overview of electrochemical detection techniques for real-time monitoring of the dynamics of different cellular processes. First, biological phenomena such as the cellular redox environment, release of neurotransmitters and other signaling substances based on exocytosis, and cellular adhesion, are discussed thoroughly. Next, the capabilities of electrochemical amperometric and impedance spectroscopic techniques in monitoring cellular dynamics are highlighted, in comparison to optical and other techniques. The applications of such techniques already include biosensors and microchip-based biological systems for cell biological research, medical research and drug development. Finally, the state-of-the-art and future developments, e.g. miniaturization of planar interdigitated electrodes in order to achieve a gap/width size regime on the nanometer scale and thus considerable signal amplification, are summarized.

Electron transfer by thermally activated hopping through localized centers is an essential element for a broad variety of vital biological and technological processes. The use of electrode/self-assembled monolayer (SAM) assemblies to explore fundamental aspects of long- and short-range electron exchange between electrodes and redox active molecules, such as proteins, is reviewed comprehensively in a chapter by D.H. Waldeck and D.E. Khoshtariya. The authors, who are pioneers in this area, nicely demonstrate that such bioelectrochemical devices with nanoscopically tunable physical properties provide a uniquely powerful system for fundamental electron transfer studies and nanotechnological applications. Studies on protein systems also reveal how the binding motif

of the protein to the electrode can be changed to manipulate its behavior, thus offering many promising opportunities for creating arrays of redox active biomolecules.

A microbial fuel cell (MFC) is a bio-electrochemical transducer that converts microbial biochemical energy directly to electrical energy. In their authoritative chapter, J. Greenman, I.A. Ieropoulos and C. Melhuish overview lucidly the principles of biofilms, biofilm electrodes, conventional fuel cells, and MFCs. Potential applications of both biofilm electrodes and MFCs are suggested, including sensing, wastewater treatment, denitrification, power packs, and robots with full energy autonomy. The symbiotic association between microbial life-forms and mechatronic systems is discussed in detail by the authors, who are internationally renowned experts in this field.

The last three chapters in Volume I deal with surface modification of implants, namely surface biofunctionalization or coating. First, R. Guslitzer-Okner and D. Mandler provide concise survey of different electrochemical processes (electrodeposition, electrophoretic deposition, microarc deposition, electropolymerization, and electrografting) to form different coatings (conducting polymers, non-conducting polymers, sol-gel inorganic-organic polymer materials, oxides, ceramics, bioglass, hydroxyapatite and other calcium phosphates) on different substrates (titanium and its alloys, stainless steels, cobalt-chrome alloys, nitinol, and magnesium alloys). The authors who are highly experienced in this field demonstrate the applicability of these coatings for medical devices such as drug eluting stents and orthopedic implants.

Different electrochemical processes to render metal implants more biofunctional and various electrochemical techniques to characterize the corrosion resistance of implants or the adsorption of biomolecules on the surface are reviewed by T. Hanawa in his authoritative chapter. Electrodeposition of calcium phosphates or polyethylene glycol (PEG), as well as anodizing and micro-arc oxidation processes to obtain TiO_2 nanotube-type oxide film on Ti substrate, or electrochemical treatment to obtain nickel-free oxide layer on nitinol alloys, are described. The effects of different surfaces on phenomena such as cell adhesion, bacterial attachment and calcification are presented.

The last chapter in Volume I, by T. Kokubo and S. Yamaguchi, lucidly summarizes the pioneering work and inventions

of these authors in the field of bone-bonding bioactive metals for orthopedic and dental implants. The metals include titanium, zirconium, niobium, tantalum and their alloys. The main surface modification technique presented in this chapter is chemical, followed by heat treatment, although other techniques such as ion implantation, micro-arc treatment, hydrothermal treatment and sputtering are also described. The bone-bonding ability of metals with modified surfaces is attributable to the formation of apatite on their surface in the body environment, which can be interpreted in terms of the electrostatic interaction of the metal surface with the calcium or phosphate ions in a body fluid. These findings open numerous opportunities for future work.

Volume II begins with a chapter by P.S. Singh, E.D. Goluch, H.A. Heering and S.G. Lemay which provides a lucid overview of the fundamentals and applications of nanoelectrochemistry in biology and medicine. First, some key concepts related to the double layer, mass transport and electrode kinetics and their dependence on the dimension and geometry of the electrode are discussed. Next, various fabrication schemes utilized in making nano-sized electrodes are reviewed, along with the inherent challenges in characterizing them accurately. Then, the "mesoscopic" regime is discussed, with emphasis on what happens when the Debye length becomes comparable to the size of the electrode and the diffusion region. Quantum-dot electrodes and charging and finite-size effects seen in such systems are also described. Then, recent advances in the electrochemistry of freely-diffusing single molecules as well as electrochemical scanning probe techniques used in the investigations of immobilized biomolecules are presented by the authors, who have pioneered several of the developments in this area. Finally, a brief survey of the applications of nanoelectrodes in biosensors and biological systems is provided.

During the last decade, nanowire-based electronic devices emerged as a powerful and universal platform for ultra-sensitive, rapid, direct electrical detection and quantification of biological and chemical species in solution. In their authoritative chapter, M. Kwiat and F. Patolsky describe examples where these novel electrical devices can be used for sensing of proteins, DNA, viruses and cells, down to the ultimate level of a single molecule. Additionally, nanowire-based field-effect sensor devices are discussed as promising building blocks for nanoscale bioelectronic interfaces

with living cells and tissues, since they have the potential to form strongly coupled interfaces with cell membranes. The examples described in this chapter demonstrate nicely the potential of these novel devices to significantly impact disease diagnosis, drug discovery and neurosciences, as well as to serve as powerful new tools for research in many areas of biology and medicine.

The Human Genome Project has altered the mindset and approach in biomedical research and medicine. Currently, a wide selection of DNA microarrays offers researchers a high throughput method for simultaneously evaluating large numbers of genes. It is anticipated that electrochemical detection-based DNA arrays will provide many advantages over radioisotope- or fluorophore-based detection systems. Due to the high spatial resolution of the scanning electrochemical microscope (SECM), this technology has been suggested as a readout method for locally immobilized, micrometer-sized biological recognition elements, including a variety of DNA arrays with different formats and detection modes. In his concise review, K. Nakano explains the underlying electrochemistry facets of SECM and examines how it can facilitate DNA array analysis. Some recent achievements of Nakano and his colleagues in SECM imaging of DNA microdots that respond toward the target DNA through hybridization are presented.

Biological membranes are the most important electrified interfaces in living systems. They consist of a lipid bilayer incorporating integral proteins. In view of the complexity and diversity of the functions performed by the different integral proteins, it has been found convenient to incorporate single integral proteins or smaller lipophilic biomolecules into experimental models of biological membranes (i.e. biomimetic membranes), so as to isolate and investigate their functions. Biomimetic membranes are common in pharmaceuticals, as well as for the investigation of phase stability, protein-membrane interactions, and membrane-membrane processes. They are also relevant to the design of membrane-based biosensors and devices, and to analytical platforms for assaying membrane-based processes. The last two chapters in Volume II are dedicated to these systems. In their thorough chapter, R. Guidelli and L. Becucci review the principles and types of biomimetic membranes, the advantages and disadvantages of these systems, their applications, their fabrication

methodologies, and their investigation by electrochemical techniques – mainly electrochemical impedance spectroscopy (EIS). This definitie chapter was written by two authors who are among the leaders leaders in the field of bioelectrochemistry worldwide.

Ion channels represent a class of membrane spanning protein pores that mediate the flux of ions in a variety of cell types. They reside virtually in all the cell membranes in mammals, insects and fungi, and are essential for life, serving as key components in inter- and intracellular communication. The last chapter in Volume II, by E.K. Schmitt and C. Steinem, provides a lucid overview of the potential of pore-suspending membranes for electrical monitoring of ion channel and transporter activities. The authors, who are internationally acclaimed experts in this area, have developed two different methods to prepare pore-suspending membranes, which both exhibit a high long-term stability, while they are accessible from both aqueous sides. The first system, nowadays known as nano black lipid membrane (nano-BLM), allows for ion channel recordings on the single channel level. The second system – pore-suspending membranes obtained from fusing unilamellar vesicles on a functionalized porous alumina substrate – makes it possible to generate membranes with high protein content. The electrochemical analysis of these systems is described thoroughly in this chapter, and is largely based on EIS.

I believe that the two volumes will be of interest to electrochemists, chemists, materials, biomedical and electrochemical engineers, surface scientists, biologists and medical doctors. I hope that they become reference source for scientists, engineers, graduate students, college and university professors, and research professionals working both in academia and industry.

I wish to thank Professor Eliezer Gileadi who was the driving force making me edit these two volumes. I dedicate this project to my wife Billie and our two daughters, Ofri and Shahaf, for their infinite love and support.

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Modern Aspects of Electrochemistry

Topics in Number 50 include:

- Investigation of alloy cathode Electrocatalysts
- A model Hamiltonian that incorporates the solvent effect to gas-phase density functional theory (DFT) calculations
- DFT-based theoretical analysis of ORR mechanisms
- Structure of the polymer electrolyte membranes (PEM)
- ORR investigated through a DFT-Green function analysis of small clusters
- Electrocatalytic oxidation and hydrogenation of chemisorbed aromatic compounds on palladium
  Electrodes
- New models that connect the continuum descriptions with atomistic Monte Carlo simulations
- ORR reaction in acid revisited through DFT studies that address the complexity of Pt-based alloys in electrocatalytic processes
- Use of surface science methods and electrochemical techniques to elucidate reaction mechanisms in electrocatalytic processes
- In-situ synchrotron spectroscopy to analyze electrocatalysts dispersed on nanomaterials

Topics in Number 51 include:

- Temperature effects on platinum single-crystal and aqueous solution interphases
- Surface thermodynamics of the metal and solution interface
- XAS investigations of PEM fuel cells
- Palladium-Based electrocatalysts required for alcohol oxidation in direct alcohol fuel cells
- Structure and Reactivity of transition metal chalcogenides used for molecular oxygen reduction reactions
- Proton conductivity and electrocatalysis in high temperature PEM fuel cells

1

Monitoring of Cellular Dynamics with Electrochemical Detection Techniques

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I. Introduction

The elucidation of the human and *Saccharomyces cerevisiae* genomes has opened new possibilities in biology, medicine and drug discovery. This knowledge has increased the number of targets and ways to treat diseases; clinical studies of disease manifestation can effectively be translated to the level of certain genes and their products, proteins. The traditional way of finding active compounds to treat different diseases has relied on biochemical assays to modulate the function of a certain target, such as an enzyme, a cell surface receptor or an ion channel. The targets have been isolated from the natural environment to be used in high-throughput screening (HTS) assays to test huge libraries of possible active compounds. At the same time has come the realization that cells and organisms are more than just the sum of their functional units. More needs to be known in order to fully understand the living organisms in terms of their functional and constructional units, the cells and proteins. This has further led to the development of ana-

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lytical systems, screening platforms, which rely on cell-based assays instead of dissecting cells to the component level. Techniques that have been applied for assaying biological parameters in intact cells range from intracellular monitoring of temperature[1], pH[2] and oxygen consumption[3] as well as extracellular monitoring of oxygen consumption[4] and acidification[4,5] to cellular adhesion[6] and wound healing,[7] G-protein coupled receptor (GPCR) activation,[8, 9] monitoring of enzyme activity,[10-13] cofactor availability,[14-17] cellularly released secondary metabolites,[18-21] gene expression detected as appearance of mRNA[22] and ion channel activity.[23]

A new development in treating diseases has also emerged; instead of solely relying on medication, cells that die due to the pathological manifestation of a disease could potentially be replaced by new cells that can restore the functions impaired by the disease. Such development is on the way to treat Type 1 diabetes mellitus (T1DM)[24] and Parkinson's disease (PD).[25] In both cases, a possible treatment could rely on embryonic stem cells (ESCs), which can differentiate into the desired type of cells to replace the destroyed pancreatic insulin secreting β-cells of T1DM patients or dopaminergic neurons in striatum of PD patients. Despite the great possibilities, these treatments are still far from being realized in clinical trials. The major obstacles are insufficient knowledge on how to control the differentiation process of ESCs and their integration into the host tissue to restore the impaired functions. Miniaturization of analytical platforms has emerged as a possibility to address these challenges encountered in biology and medicine. Developments in microfluidic technology can be adopted to alleviate problems in liquid handling at the same time increasing capabilities of parallelization.[26,27] However, no matter how much improvement microfluidics can provide to handling and cultivation of cells, the development has to be conducted hand-in-hand with development of analytical techniques to more effectively discover the dynamics of cellular processes. This emphasizes the significance of research on finding and understanding ways of detecting biologically relevant cellular parameters and then integrating the protocols into analytical systems to achieve a new generation of tools for biology, medicine and drug discovery.

At present, the primary detection technique in systems that are used to study the behavior of living cells is optical or fluorescence detection that can be automated in X-Y direction to monitor cellu-

lar responses and behavior at multiple positions.[28] Due to, for instance, photodamage on the studied cells, these techniques do not always provide well-developed capabilities to conduct real-time measurements that could provide more information than just an endpoint. Capability for real-time measurements that facilitate monitoring of cellular dynamics under conditions, where an observed cell or cell population can function as its own control, is crucial for effective realization of systems applied for cellular studies in biology, medicine and drug discovery. Electrochemical detection can provide the necessary capability for real-time monitoring and has been applied in different formats to study the dynamic processes of living cells based on, for instance, amperometric,[12,16,17,20,29-31] potentiometric[14] and impedance spectroscopic[6,9] measurements. However, despite ongoing research, the full potentials of electrochemical techniques have not yet been utilized. Micro- and nanofabrication of electrode systems[32] with the inherent capability for scaling of electrode dimensions can provide possibilities for parallel detection of different cellular parameters to suit equally well single-cell and cell population monitoring. Additionally, systems built for the application of electrochemical techniques can also be integrated with capabilities for simultaneous microscopic observations, facilitating more effective multiparameter monitoring of cellular dynamics.

This chapter illustrates the potentials of amperometric and impedance spectroscopic monitoring of cellular dynamics. In order to provide a complete understanding of what biological and medical aspects can be studied using these electrochemical techniques, the following sections contain a thorough description of biological phenomena, such as cellular redox environment (CRE), release of neurotransmitters and other signaling substances based on Ca^{2+} ion triggered mechanism (exocytosis), as well as cellular adhesion with emphasis on cell-cell and cell-growth substrate interactions. The discussion of the biological phenomena is then lifted to the context of electrochemical measurements to highlight the capabilities of amperometry in monitoring of the dynamic changes in CRE and exocytotic behavior of cells as well as impedance spectroscopy in monitoring of factors influencing cellular adhesion. Additionally, the capabilities of other, especially optical, techniques are discussed to provide a perspective to what benefits and possible limitations electrochemical techniques possess.

II. MONITORING OF CELLULAR REDOX ENVIRONMENT

1. Biological Significance of Cellular Redox Environment

(*i*) *Cellular Redox Processes*

Cellular functions, such as locomotion, contraction and biosynthesis of cellular building blocks require energy, which organisms acquire by digesting the main constituents of food, carbohydrates, proteins and fats, to form the biopolymeric structures from monomers. Digestion of carbohydrates, proteins and fats results in formation of hexoses (glucose, fructose, galactose, etc.), amino acids, glycerol and fatty acids that are taken up by the cell, in which they undergo further degradation in enzymatic processes referred to as catabolism. Ultimately, the catabolic processes provide precursors for the synthesis of new biomolecules (anabolic processes) needed for the maintenance of cellular structures and growth as well as energy. Based on purely thermodynamic considerations, the energy contents stored in biomolecules is too large to be released in a single process without being detrimental to the cells. Instead, all types of cells, from prokaryotes to eukaryotes, store the released energy in the form of catabolic intermediates that facilitate energy release in a stepwise manner. Examples of such catabolic intermediates are the reduced cofactors nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide ($FADH_2$) as well as acetyl coenzyme A (Acetyl-CoA). NADH, NADPH and $FADH_2$ as well as their corresponding oxidized forms, NAD^+, $NADP^+$ and FAD, respectively, are known as cellular redox couples. An examples of other redox couples, aside from those that are involved in metabolic processes, is glutathione (GSSG) and its oxidized form (GSSG), involved in cellular detoxification processes to alleviate, for instance, oxidative stress [33]. The different cellular redox couples participate in enzymatic processes that catalyze oxidation or reduction of biomolecules. In these processes, the reduced form of a redox couple functions as an electron donor and, in an analogous manner, the oxidized form functions as an electron acceptor.

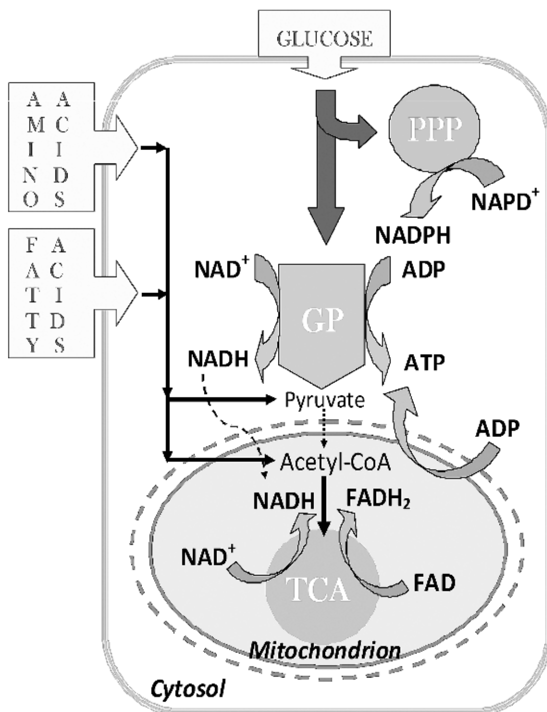
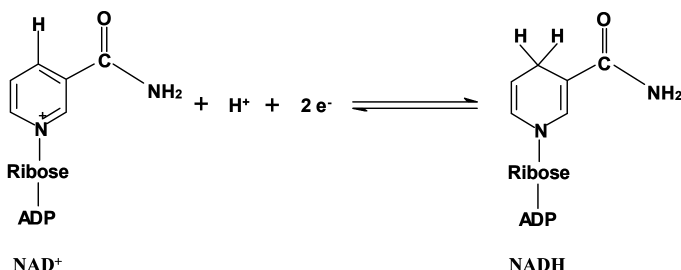


Figure 1. Glucose catabolism proceeds through the cytosolic pentose phosphate pathway (PPP) and glycolytic pathway (GP) generating NADPH and NADH, respectively. GP also produces ATP in product level phosphorylation. The GP endproduct, pyruvate, enters the mitochondria and upon conversion into acetyl coenzymeA (Acetyl-CoA) contributes to formation of NADH and $FADH_2$ in the tricarboxylic (TCA) cycle and further ATP through oxidative phosphorylation. Electrons from cytosolic NADH are shuttled into the mitochondria. Degradation of amino acids and fatty acids primarily leads to formation of pyruvate or Acetyl-CoA, and further processing in the mitochondria.

Figure 1 shows a schematic overview of the main catabolic pathways. In respiratory eukaryotic cells, the metabolism of different biomolecules leads to a common intermediate, the mitochondrially formed Acetyl-CoA, which is catabolized further in the tricarboxylic acid (TCA) cycle, resulting in the formation of, for



Scheme 1. The reduction of NAD^+ to NADH as part of enzyme-catalyzed reactions proceeds as a transfer of two electrons and one proton, i.e., a hydride.

instance, three molecules of NADH and one molecule of $FADH_2$ per each cycle. Amino acids form, however, a heterogeneous group, some of which are catabolized to other TCA cycle intermediates than Acetyl-CoA.[34] The enzymatic reactions resulting in the formation of these compounds are oxidation-reduction (redox) reactions, in which NAD^+ gains two electrons and one proton (the combination is also referred to as a hydride) (Scheme 1) and FAD gains two electrons and two protons. These compounds can then deliver their electrons in subsequent redox processes as part of the mitochondrial electron transport chain (ETC) (also referred to as the respiratory chain). NADH is oxidized by NADH-quinone oxidoreductase (Complex I) and $FADH_2$ by Succinate-coenzyme Q reductase (Complex II), resulting in reduction of ubiquinone (UQ) to ubiquinole (UQH_2) in the UQ-pool of the mitochondrial membrane. Further activity of the ETC, i.e.,

(*a*) oxidation of UQH_2 by Coenzyme Q: cytochrome *c*-oxidoreductase (Complex III)
(*b*) upon reduction of cytochrome c (Cyt c), and
(*c*) the subsequent oxidation of Cyt c by Cytochrome c oxidase (Complex IV), finally results in reduction of molecular oxygen (O_2) to water.

The gradually lost potential energy of the electrons of NADH and $FADH_2$ is stored in the form of a proton gradient (ΔH^+) that is built up and maintained by Complexes I, III and IV, which are able to expel protons from the mitochondrial matrix into the mitochondri-

al intermembrane space (IMS).[35] ATP synthase utilizes the formed ΔH^+ to synthesize ATP that serves as the universal cellular energy, predominantly formed in the mitochondria but transported into the cytosol,[35] where it is utilized for biosynthetic processes and other energy-requiring functions.

The cytosolic catabolism of glucose may follow two different pathways depending on cellular needs. The glycolytic pathway (GP) provides two molecules of NADH, ATP and pyruvate for each catabolized glucose molecule. The formed NADH can, to some extent, be utilized either in cytosolic redox processes, such as lactic (in muscle cells) and alcoholic fermentation (in *Saccharomyces cerevisiae*), or the electrons of NADH may be shuttled into the mitochondria to be processed in the ETC.[35] The formed pyruvate is also transported into the mitochondria, where pyruvate dehydrogenase converts it into Acetyl-CoA followed by processing in the TCA cycle. The enzymatic reactions of the oxidative part of the pentose phosphate pathway (PPP) form two molecules of NADPH per glucose molecule. These serve as redox equivalents in biosynthetic processes, such as synthesis of fatty acids. The non-oxidative part of the PPP continues the catabolism of glucose, resulting in the formation of ribulose-5-phosphate, which is further converted into ribose-5-phosphate, the precursor of nucleotide synthesis.[34]

(*ii*) *Definition of Cellular Redox Environment*

The oxidized and reduced cofactors, such as NAD^+-NADH and $NADP^+$-NADPH (collectively denoted as $NAD(P)^+$-NAD(P)H), form redox couples, the reduction potential (E) of which is defined by the Nernst equation (Eq. 1) for the half reaction shown in Scheme 1, where E° is the standard reduction potential under standard conditions, where T = 298 K, activity $a_{NA(P)D}{}^+ = a_{NAD(P)H} = a_H{}^+ = 1$ ($[NAD(P)^+] = [NAD(P)H] = 1$ molal and pH = 0), R is the gas constant (8.314 J K^{-1} mol^{-1}), F is the Faraday's constant (96485 C mol^{-1}) and n is the number of transferred electrons, n = 2,

$$E_{NAD(P)^+/NAD(P)H} = E^\circ_{NAD(P)^+/NAD(P)H} + \frac{RT}{nF}\ln\frac{\alpha_{NAD(P)^+}\alpha_{H^+}}{\alpha_{NAD(P)H}} \quad (1)$$

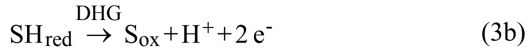
The standard conditions are not, however, applicable to biological systems. If concentrations are used instead of activities, these can be replaced by $\gamma_i[i]$, where γ_i is the activity coefficient and [i] the molar concentration of the species i. In literature, tabulated values of reduction potential for biological systems are usually valid at pH = 7 and defined at an equimolar composition with respect to the redox couple. Under such conditions the standard reduction potential, E°, is replaced by the formal potential, $E^{\circ'}$, which implicitly comprises the contribution of pH and the activity coefficients. Equation (2) shows this relation for the $NAD(P)^+$/ NAD(P)H redox couple,

$$E_{NAD(P)^+/NAD(P)H} = E^{\circ'}_{NAD(P)^+/NAD(P)H} + \frac{RT}{nF}\ln\frac{[NAD(P)^+]}{[NAD(P)H]} \quad (2)$$

where only the concentrations of the components of the redox couple affect the formal potential.

In redox reactions catalyzed by enzymes, generally referred to as redox enzymes, electrons are shuttled from a donor compound that is oxidized to an acceptor compound that is reduced. According to some estimations, about one fourth of all known proteins belong to the category of redox enzymes.[36] These can be classified based on the employed natural electron donating and accepting compounds into the following classes: dehydrogenases, oxidases, peroxidases and oxygenases. Dehydrogenases catalyze oxidation of a substrate (donor) through hydride transfer to a cofactor (acceptor), which can be a prosthetic group permanently bound to the enzyme, such as flavin mononucleotide (FMN) and FAD, or a soluble co-substrate transiently associated with the enzyme, such as $NAD(P)^+$.[37] In a dehydrogenase catalyzed reduction, however, the cofactor functions as the donor, while the substrate to be reduced is the acceptor. In a redox reaction, the substrate forms the other half reaction needed for the complete process. Equations (3a) and (3b) show the two half reactions for a dehydrogenase (DHG) catalyzed oxidation of an arbitrary substrate using $NADP^+$ as the cofactor,

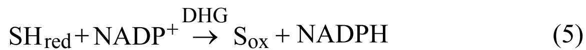
$$NADP^+ + H^+ + 2e^- \xrightarrow{DHG} NADPH \quad (3a)$$



$$SH_{red} \xrightarrow{DHG} S_{ox} + H^{+} + 2\,e^{-} \tag{3b}$$

where S_{red} and S_{ox} are the reduced and oxidized forms of the substrate, respectively. In an analogous way as was shown above for the $NAD(P)^{+}$-NAD(P)H redox couple, the substrate forms another redox couple, the reduction potential of which is also determined by the Nernst equation. Equation (4) shows the corresponding form of the Nernst equation for the substrate with the earlier described convention, using the formal potential, $E^{\circ\prime}$. Although Eq. (3b) indicates the involvement of a proton, its contribution to the reduction potential is included in the formal potential. Equation (5) shows the overall oxidation of the substrate,

$$E_{S_{ox}SH_{red}} = E^{\circ\prime}_{S_{ox}SH_{red}} + \frac{RT}{nF}\ln\frac{[S_{ox}]}{[SH_{red}]} \tag{4}$$



$$SH_{red} + NADP^{+} \xrightarrow{DHG} S_{ox} + NADPH \tag{5}$$

The overall electromotive force (ΔE) of the enzymatic reduction is obtained as the difference between the reduction potentials of the half reactions as shown by Eq. (6):

$$\Delta E = \left(E^{\circ\prime}_{NAD(P)^{+}/NAD(P)H} + \frac{RT}{nF}\ln\frac{[NAD(P)^{+}]}{[NAD(P)H]} \right) - \left(E^{\circ\prime}_{S_{ox}SH_{red}} + \frac{RT}{nF}\ln\frac{[S_{ox}]}{[SH_{red}]} \right) \tag{6}$$

The validity of Eq. (2) implies that inside living cells the actual reduction potential is primarily dependent on the value of the ratio $[NAD(P)^{+}]/[NAD(P)H]$. At pH 7 and equimolar concentration of both components, the reduction potential of the NAD^{+}/NADH and $NADP^{+}$/NADPH redox couple is –320 mV vs. the normal hydrogen electrode (NHE).[38] However, if the pH of interest for a redox reaction differs from pH 7, which is the reference value for tabulated biological reduction potentials, the actual

reduction potential ($E_{pH=x}$) may be calculated based on Eq. (7) below,[33]

$$E_{NAD(P)^+/NAD(P)H\,(pH=X)} = E^{\circ\prime}_{NAD(P)^+/NAD(P)H} + \left[(pH - 7.0) \times \left(\frac{\Delta E}{\Delta pH} \right) \right] mV \tag{7}$$

which indicates that for $NAD(P)^+/NAD(P)H$, involving a transfer of two electrons (n = 2), the actual reduction potential is decreased by 30.8 mV per unit increase in pH at 37ºC. Although the reduction potential of $NAD^+/NADH$ and $NADP^+/NADPH$ is the same, their participation in different metabolic processes is rigorously controlled by the existence of enzymes with strict dependence on either one of the cofactors as well as compartmentalisation of the metabolic processes.[39,40]

Living cells exert a rigid control of the catabolic and anabolic processes, which maintain the $NADP^+/NADPH$ and $NAD^+/NADH$ ratios as well as pH in different sub-cellular compartments at a constant level.[41] Additionally, the involvement of the GP and the PPP in the catabolism of glucose varies between different type of cells, depending on whether they have a greater need for NADH and Acetyl-CoA to be used for production of ATP in the mitochondria or NADPH and intermediates of the PPP for biosynthesis.[42] The $NADP^+/NADPH$ and $NAD^+/NADH$ ratios in different type of mammalian cells have been shown to be of the order of magnitude of 0.01[43] and 10–1000,[41] respectively. These values implicate that the reduction potential of the $NADP^+$-NADPH redox couple is kept on average about 60 mV more negative than the formal potential, whereas that of the NAD^+-NADH redox couple is kept 30–90 mV more positive than the formal potential (the estimated values are valid at 37°C). The general consequence is that the $NADP^+$-NADPH redox couple maintains the intracellular environment reductive as is necessary in reductive biosynthesis, whereas the NAD^+-NADH redox couple effectively serves as a sink of electrons as needed in oxidative catabolism.

Based on the description above, the formal potential of the cellular redox couples, such as $NADP^+$-NADPH and NAD^+-NADH, is determined by the concentration ratio of the individual