Extended Applications of Picomolar Technology to Measure Immunoactive Biomarkers

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ABSTRACT

The picotechnology extended use in marine and biology applications are reported after our report on picotechnology to measure picomolar quantities of single stranded DNA in evaluation of Texotere chemosensitivity (TCRT,2008, 28(6):175). Lead and mercury poisoning is a life threatening to fish and animals with irreversible neurological, cardiovascular and reproductive damage. The biophysical interactions of minute quantities of lead and mercury with blood are not detectable. Fish albumin protein was major transporter in blood to interact with mercury and lead ions in solution. Single strand DNA, interleukin IL-8, nitric oxide and fucosidase estimations posed difficulty due to their picomolar quantities. Our hypothesis was that picomolar concentrations of mercury and lead strongly affect albumin properties in solution. We present the applications of major biomolecules in picomolar quantities of single strand DNA, mercury levels, gold, anti-HIV proteins, corynbacteriumfucosidase, interleulin IL-8, nitric oxide, sub-picomolarfluorescein measurements useful in detection of cancer, HIV and monitoring disease. The basic principles of dilutions, spectroscopic picomolarquantitation by genomics, picomolar fluorescent spectrometer and detection limits are displayed for readers interested in picotechnology instruments in clinics and labs. In conclusion, picomolar spectroscopy is very effective in detecting mercury, lead poisoning, immunogens, HIV and enzymes at a very early stage. It may set a new bio-inspired inexpensive platform of bio-sensing in clinical, marine applications.

Keywords: picotechnology, picofluorescein spectrometer, HIV, nitric oxide, mercury, lead

1 INTRODUCTION

Picotechnology is the method to measure picomolar quantities in the sample. It may be water sample of river, acquarium, tank or body fluid. The picomolar concentration is the downside of 10-12 pMolarmeasurement in the sample. The environmental agencies measure and caliberate the quality of metals and minerals in water and evaluate the toxicity level in drinking water or flora &phona like marine fresh water fish. Mercury, arsenic, cadmium, selenium or radioactive substances are real health hazards specially in the geographical regions where people are dependent on local fisheries industry and water supply. Several metal solutions are known to act with high activity as their concentration in solution is decreased towards picomoles. Best examples are calcariaphos(calcium phosphate), natrummur (sodium sulphate), ferrum ox (iron oxide), arsenica(arsenic oxide) known in Homeopathy based on the physical principle of administration of similar metal ions 'amplify' the bioreaction of same metals in physiological conditions (more than 10^8 times) in the inverse order upon decreased metal administered concentration. If picomoles of metal is administered in the body will cause high magnitude of physiological reaction activation than metal is administered in nanomoles. In keeping this view in mind, reactions requiring calcium, iron, magnesium, zinc etc cofactors in picomole concentration is becoming area of interest to understand the potentials of 'extremely low metal administration' to trigger the battery of intracellular and physiological milieu due to less known or unknown proteins and protein regulatory factors. The protein and metal cofactor interaction in biomolecules is extremely important and its picomolar range needs new technical development. We focus on role of sodium in neurofilament proteins, calcium in

actin-myosin proteins and microtubule proteins. However, ultra small concentration of ions is less likely to trigger at molecular conformation level but more likely triggers thermodynamics and energy activation by increasing enthalpy and entropy.

1.1 Picodevices

In biochemical analysis and physical analysis, electrothermal atomic absorption spectrophotometry (EAAS) was studied using a Perkin-Elmer-Zeeman 3030 spectrophotometer is routine technique [1]. Other example is thyroid hormones measurement by ELISA or chemiluminescence to measure the TSH, T3 (9-26 picomoles/L) and T4 (0.60-1.5 picomoles/L). The art of measuring picomolar concentrations of nucleotide phosphates or energy molecules is age old [2]. In the electronics field, picodevices such as picomole concentration of Cl^- by picoelectrometeric titration, sodium ion monitoring device of quadrupole electron impact mass spectrometry, picobio-inspired environmental biosensors have made advancement.

A biosensor named "Picoscope" was developed for real-time simultaneous detection of several biological agents by measuring picometer-range changes of the thickness of different biorecognition spots on the biochip surface. The Picoscope technology seems significantly increase the power of research instruments for bio-, nano- and pico-technologies.

1.2 Pico-biochip

The Picoscope used immobilization of one antibody on the biochip surface via the biotin–streptavidin bridge. *Bacteria* by the Picoscope can be sensed by biotinilated glass slip: streptavidin, first biotinilated antibody against *bacteria*, and antigen in the form of cell suspension at the concentration of 10^7 cells/ml. The change of the antigen layer thickness averaged over the sensor's surface can be detectable. The picochip technology has tremendous advantage in picoparticles based on antibody-biotin-streptavidin-metal compounds to generate bio-response [2].

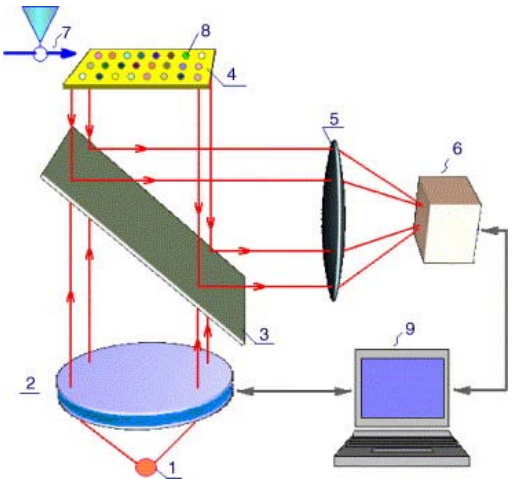

Fig. 1a. Scheme of the Picoscope: 1, superluminescent laser diode; 2, scanned Fabry–Perot interferometer; 3, semi-transparent mirror; 4, glass slip; 5, optics; 6, CCD camera; 7, fluidic system; 8, recognition spots or wells; 9, computer.

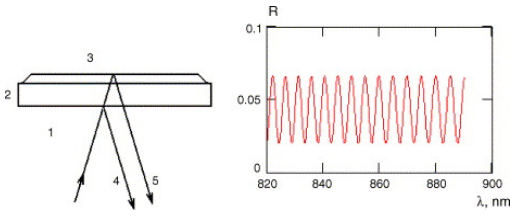

Fig. 1b. Scheme of the interference pattern formation: 1, air; 2, glass slip with a biomolecular layer; 3, biological solution; 4 and 5, reflected beams. (b) Reflection spectrum of a glass slip of 50 μm thick.

Surface plasmon resonance (SPR) "BIAcore 2000" and picoscope can be useful for biosensors based on fluorescent labels. Other attractive applications of the Picoscope based on CCD camera and multi-spot biochip are high throughput screening and multi-agent analysis of liquids, e.g. for food pathogen detection.Thepicochip production is in progress based on immobilization of antibodies and the biotin–streptavidin bridge such as measuring binding kinetics using the two-channel Picoscope.

2 BIOSYSTEMS AND FISH

NSTI-Nanotech 2009, www.nsti.org, ISBN 978-1-4398-1783-4 Vol. 2, 2009

Question arises why live flora fauna like fishes or water borne animals get affected with water toxicity? Let us take example of fish in toxic water. The water toxic minerals interfere with metabolic system in the body and protein synthesis makes defective protein mass such as contaminated toxic fish. The fish muscle structure gets changed. Here we highlight the muscle molecular make up and possible toxic effects. The muscle is made actin and myosin filaments.

2.1Neurofilaments

The network of neurofilaments is further visible at the subnano range of angstrom A units(100 picometers). It enhances the understanding and visualization of proteins in nerve cells containing neurofilaments. The intermediate filaments include the three neurofilament (NF) proteins (designated NF-L, NFM, and NF-H for light, medium, and heavy, respectively). Neurofilaments appear to be anchored to actin filaments and MTs by neuronal members of the plakin family. α-internexin, IF, nestin, are also play role.

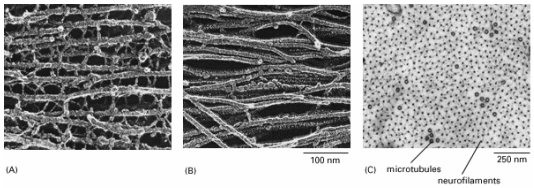

Figure 2. Electron micrographs of two types of intermediate filaments in cells of the nervous system.(A)Freeze-etch image of neurofilaments in a nerve cell axon, showing the extensive cross-linking through protein cross-bridges - an arrangement believed to provide great tensile strength in this long cell process. The cross-links are formed by the long, nonhelical extensions at the carboxyl terminus of the largest neurofilament protein. (B)Freeze-etch image of glial filaments in glial cells illustrating that these filaments are smooth and have few crossbridges.(C) Conventional electron micrograph of a cross-section of an axon showing the regular side-to-side spacing of the neurofilaments, which greatly outnumber the MTs [3].

Overexpression of NF-L or NF-H in transgenic mice indicated the role of neurofilaments in the pathogenesis of motor neuron disease.

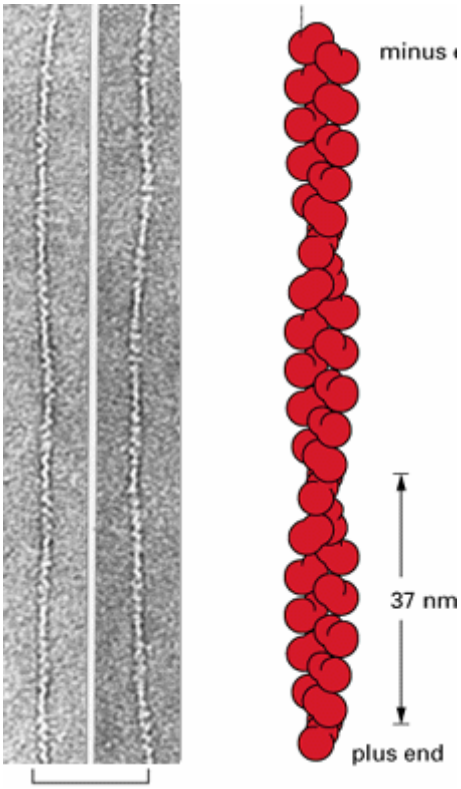

Figure 3. Actin filaments. (A) Electron micrographs of negatively stained actin filaments. (B) The helical arrangement of actin molecules in an actin filament.

1.2 Actin

It is cytoskeletal protein 7 nm in diameter polar structure and form three dimensional networks of actin cytoskeleton.
The actin monomers bind with ATP and hydrolysis play a key role with tubulin to provide dynamic instability, which is very important for their functions.Tropomyosin, binds with actin filaments to act cooperatively in generating the movements of the cell surface, including cytokinesis, phagocytosis, and cell locomotion.

1.3 Microtubules (MTs)

MTs are the polymers play role in cell movements, intracellular transport of organelles, and the separation of chromosomes during mitosis. MTs have a

cylindrical form with a diameter 25 nm. made of tubulins. Dynamic instability due toMT plus end binding proteins, also called "plusend-tracking proteins", or +TIPs are able to "surf" the dynamic ends of MTs.EB1,EB3, APC,CLASP2,LIS1,CLIP-170, and CLIP 115 and dynactin complex. Thus when +TIPs are expressed as GFP-fusions, fluorescence is brightest at the growing tip of the MT and trails off in intensity toward the minus-end of the MT, thus forming a "comet tail."

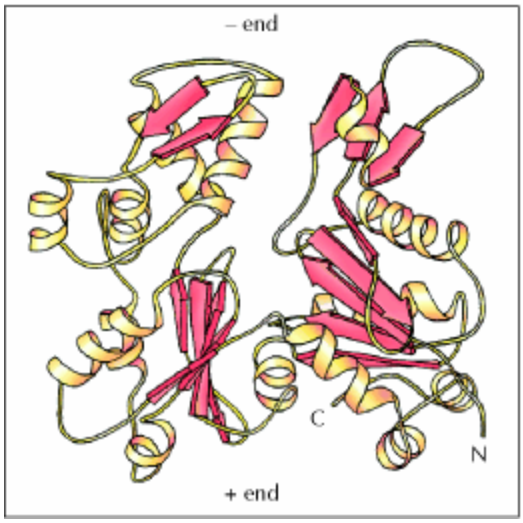

Figure 4. Assembly and structure of actin filaments (A) Actin monomers (G actin) polymerize to form actin filaments (F actin). The first step is the formation of dimers and trimers, which then grow by the addition of monomers to both ends. (B) Structure of an actin monomer.

This specific association of +TIPs with MT distal ends was initially explained by treadmilling. Other factor is "delivery (or deposition) of Kinesin" driven. MTsdepolymerise and GFP-TIP speculate dynamic behaviour of MTs. However, MT behaviour using specific GFP-tagged MT plus end binding proteins as markers has become very popular. Visualization of GFP-TIPs in cells by time-lapse fluorescence microscopy revealed that all fusion proteins move in a comet-like pattern and demonstrated that, at least in CHO cells, most MTs grow persistently from the cell centre towards the cell periphery [3].

1.4 Functions of +TIPs

Several categories of +TIPs, EBs, CLIPs, CLASPs regulate MT behaviour as rescue factors at the MT tip. CLASPs interact with the CLIPs. The EBsmodulate the dynamic instability of MTs.The +TIPs can link the MT cytoskeleton such as CLIP-associating proteins (CLASPs), cytoplasmicdynein/dynactin complex. The first +TIP found (CLIP-170) targets dynein and dynactin to the MT ends and links dynein to MTs. MTs specifically at the leading edge of migrating cells show glycogen synthase kinase-3β (GSK-3β) inhibition to cause asymmetric distribution of CLASPs.

3 CONCLUSION

The concept of pico scale of measurement in biology and chemistry is highlighted with examples of metal ions, bioassemblies. The integrated proteins with metals in supramolecular macromolecules is described with potentials of picomolar science. The proteins and their regulatory metal cofactors play a significant role in structural-functional actions of biomolecules in the body. Picodevices have paved the way to determine minute amounts of metabolites, hormones, nucleotides. Picochips and pico-inspired biological applications remain further attraction in future. Possibly the picomolar concentration of water toxicity will enhance the accuracy and detection of health hazards much better.

REFERENCES

[1].Shalmi, M., Kibble, J. D., Day, J. P., Christensen, P. & Atherton, J. C. American Journal of Physiology 267, F695—701,1994..
[2].Nitkin PI, Gorshkov BG, Nitkin EP, Ksnevich TI. Sensors and Actuators B: Chemical111-112, 11;500-504,2005.
[3].Skoog L. Eur J Biochem. 17:202-2-8, 1970.