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Mini Review

New Approach in Forensic Analysis – Biomolecular Computing Based Analysis of Significant Forensic Biomarkers

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Abstract

Novel biochemical assays based on biomolecular information processing realized in enzyme-biocatalytic systems were developed for forensic analysis of various biomarkers. On-site analyses of biomarkers for recognition of ethnic origin and gender of biological fluids were developed. Future perspectives for forensic analysis and scientific challenges are discussed in the paper.

INTRODUCTION

Biochemical assay of biological substances found on a crime scene is an important part of forensic investigation aiming at identification of crime victims and suspects. A biochemistry/molecular biology-based subarea of forensic analysis, forensic serology, deals with the complex task of gathering information on type, age, origin or gender from biological fluids (blood, saliva, etc.) found on a crime scene [1-5]. Modern forensic serology [6-8] relies on two major methods: immunoassay [8] and DNA [9]/RNA [10] analysis. While simple primary steps in this investigation (e.g., identification of human blood vs. other colored spots) can be easily done in field conditions, more specific analytical steps require sophisticated laboratory equipment and highly qualified staff [6]. This introduces time-delay in the investigation procedure and increases the complexity of the investigation itself. Despite the modern trend of designing portable equipment for on-site forensic biochemical analysis [6,11], including microfluidic [12] and lab-on-a-chip [13] systems, there is an obvious lack of on-site detection/characterization technologies, analogous to point-of-care diagnostic approaches, which have become common in medical areas (e.g. diabetes management, pregnancy tests, etc.).

A novel approach to the analysis of biological substances for simplifying recognition of biomarkers in forensic applications can be borrowed from recent advances in unconventional chemical computing [14], specifically, from recently pioneered biomolecular computing systems [15]. Such biocomputing systems based on proteins/enzymes [16,17], DNA [18], RNA [19], DNAzymes [20] and whole cells [21] can perform logic operations processing multiple biochemical input signals. Based on biomolecular systems various Boolean logic operations such as AND, OR, XOR, NOR, NAND, INHIB, XNOR, etc., were realized [15]. Sophisticated networks composed of several concatenated biomolecular logic gates performing complex logic operations were designed for unconventional computing applications [15]. Particularly rapid progress was recently achieved in the area of enzyme-based biocomputing systems allowing their assembling in the form of concatenated logic networks composed of many gates performing various Boolean logic operations and including non-Boolean elements, such as enzyme-based multiplexer and demultiplexer, amplifier, filter, etc., mimicking their electronic counterparts [16]. Although chemical information processing systems were originally considered exclusively for computational applications, [22,23] it has recently been realized that they have features suitable for analytical/bioanalytical use [24,25]. These systems can analyze several biochemical signals according to a "preprogrammed" algorithm and generate a binary "YES/NO" answer without using a computer. Such chemical testing, analogous to a pregnancy test, might be convenient for certain end-user and point-of-care applications, being particularly useful for on-site forensic investigations. Networks with computational steps that involve only biochemical processes [15,16] are being investigated for new technological capabilities that include multi-input biosensors with new functionalities [24,25]. After developing biomedical applications (e.g., analysis of injury-signaling biomarkers) [26-28] and military/homeland security applications (e.g., analysis of explosives and chemical weapons) [29], the biocomputing approach was successfully implemented to forensic applications [30,31].

The important feature of the novel analytical approach is the rapid qualitative analysis in the binary form "YES/NO" in response to the presence/absence of various combinations of

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biomarkers. Notably, instead of a quantitative analysis of a single highly specific biomarker, the present approach is based on the logic processing (e.g., in the format of Boolean AND logic gate) of several less specific biomarkers with all information processing steps performed by biochemical reactions, Figure 1A.

One of the new applications based on biocatalytic cascades analyzing simultaneous presence of creatine kinase (CK) and lactate dehydrogenase (LDH) was developed aiming at the recognition of biofluids of different ethnic origin for forensic analysis, Figure 1B [30]. Knowing the difference in the concentrations of CK and LDH in the blood of healthy adults of two ethnical groups, Caucasian (CA) and African American (AA), and taking into account the human population distribution patterns, samples of different ethnic origin with various CK/LDH concentrations were mimicked and analyzed. The analysis was performed using a multi-enzyme / multi-step biocatalytic cascade where the differences in both included enzymes resulted in an amplified difference in the final analytical response. The statistically established analytical results confirmed excellent probability to distinguish samples of different ethnic origin (CA vs. AA). The results obtained on the model solutions were confirmed by the analysis of real serum samples collected from human subjects of different ethnic origin.

Another biocatalytic assay analyzing simultaneous presence of creatine kinase (CK) and alanine transaminase (ALT) was developed aiming at the recognition of biofluids of different gender for forensic applications, Figure 1C [31]. Knowing the difference in the concentrations of CK and ALT enzymes in the blood of healthy adults of male and female groups we mimicked the samples of different gender with various CK/ALT concentrations. The analysis was performed using a multi-enzyme / multi-step biocatalytic cascade where the differences in both included enzymes resulted in an amplified difference in the final analytical response. The analysis performed in human serum solutions allowed discrimination of samples corresponding to male/female groups. The robustness of the developed analysis allowed determination of the gender for serum solutions after their drying and ageing at least for 1 hour. Importantly for forensic application, reaction with a chromogenic reactant nitroblue tetrazolium allowed qualitative discrimination of the "male" and "female" samples with a naked eye.

Applying novel concepts from the area of unconventional computing (specifically from biomolecular computing) [15,16] to biosensing and bioanalytical assays [24,25] has resulted in the design of biomolecular systems logically processing several chemical signals and converting them to a single binary output in the format of YES/NO. Information processing in biomolecular systems does not require electronic computers and proceeds at the level of chemical reactions. The "program" for processing chemical inputs can be implemented in the composition of the biomolecular system and can include various logic operations applied in different combinations. The systems exemplified above demonstrated the simplest logic processing based on logic AND/OR gates, applied to biochemical input signals. However, many other logic operations integrated in various logic circuitries are possible with the use of different enzymes [16] and other biomolecules [15], to allow high-fidelity detection of various biomarkers. The resulting digital biosensors would thus benefit different important fields, being particularly useful in rapid on-field forensic analysis. An important challenge in developing this kind of the digital multi-signal biosensor system is obtaining a

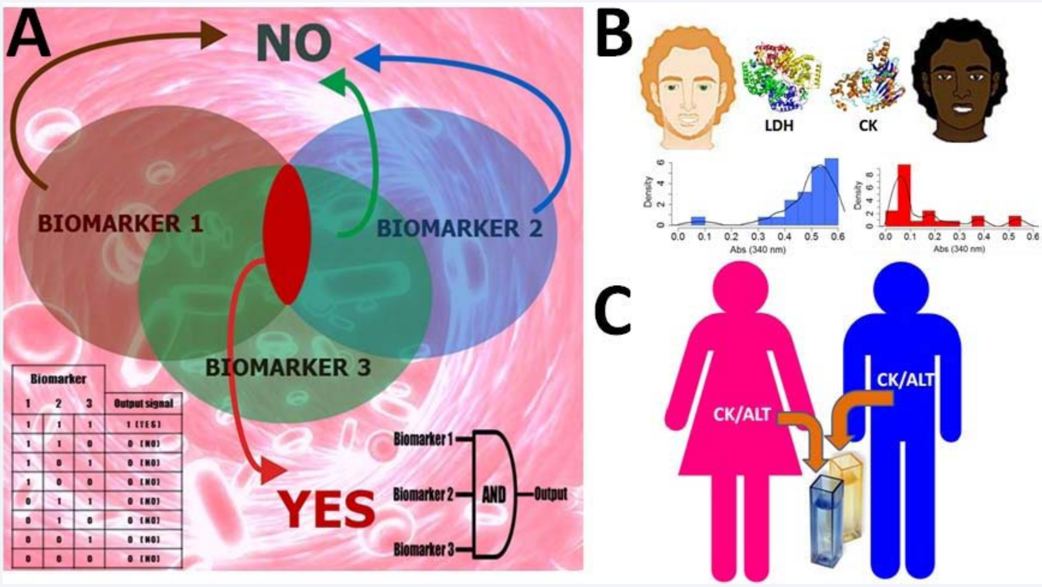

Figure 1 (A) Simultaneous presence of biomarkers with limited specificity indicates a definitive YES conclusion, schematically represented by the overlapping region in the diagram. In the biochemical computing approach, this conclusion is realized as the output YES (or 1) of a multi-input AND logic gate, obtained only when all the three inputs are in the ranges corresponding to 1. The output NO (or 0) is obtained for all the other combinations of inputs. The "truth table" for such a three-input gate and its logic diagram are also shown. (B) A biocatalytic assay analyzing simultaneous presence of creatine kinase (CK) and lactate dehydrogenase (LDH) recognizes biofluids of different ethnic origin for forensic applications. (C) A biocatalytic assay analyzing simultaneous presence of creatine kinase (CK) and alanine transaminase (ALT) recognizes biofluids of different gender for forensic applications. (Parts A, B and C are adopted from refs. 25, 30 and 31, respectively, with permission).

significant difference between the logic 1 and 0 output values (in other words, a well-defined YES/NO answer). One should remember that in case of forensic analysis the input signals (biomarkers) appear with small difference. In order to obtain significant difference in the output signals, thus being able to distinguish biological samples of different origin, the response-function in the information processing steps should be sigmoid rather than linear [32-34]. In other words, the system should demonstrate a non-linearity with a sharp transition between the 0 (NO) and 1 (YES) states. The first steps in this direction have been already done experimentally and analyzed theoretically [35]. However, extensive research effort aimed at designing chemical "filter"-systems similar to the electronic counterparts is needed. The thresholds separating the logic 0 and 1 values could be tailored for a specific application by following expected concentrations of biomarkers in the biological fluids. This will be an important step towards future on-site forensic analysis.

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