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Biocatalytic Analysis of Biomarkers for Forensic Identification of Gender

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Electronic supplementary information (ESI)

Chemicals and reagents used:

The following enzymes and organic/inorganic chemicals were purchased from Sigma-Aldrich and used as supplied: creatine kinase from rabbit muscle, Type I (CK, E.C. 2.7.3.2); pyruvate kinase from rabbit muscle, Type III (PK, E.C. 2.7.1.40); L-lactate dehydrogenase from bovine muscle, Type X (LDH, E.C. 1.1.1.27); alanine transaminase from porcine heart, (ALT, E.C. 2.6.1.2), human serum (type AB), creatine anhydrous (Crt), adenosine 5′-triphosphate disodium salt hydrate (ATP), phospho(enol)pyruvic acid monopotassium salt (PEP), β-nicotinamide adenine dinucleotide reduced dipotassium salt (NADH), L-alanine (Ala), α-ketoglutaric acid (KTG), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), glycyl-glycine (Gly-Gly), potassium hydroxide (KOH), and magnesium acetate tetrahydrate (MgAc). Ultrapure (18.2 MΩ·cm) water from a NANOpure Diamond (Barnstead) source was used in all experiments.

Instrumentation and measurements:

A Shimadzu UV-2450 UV-Vis spectrophotometer, with a TCC-240A temperature-controlled holder and 1 mL poly(methyl methacrylate) (PMMA) cuvettes, was used for all optical measurements. The signals corresponding to the concentration of NADH and formazan were measured optically at λ = 340 and 580 nm, respectively.

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Composition and operation of the model systems:

To mimic the male and female groups, characteristic levels of CK (234 U/L for male; 122 U/L for female) and ALT (32.1 U/L for male, 22.6 U/L for female) were used to activate the biocatalytic cascades (A), (B) and (C) described below.

(A) System for the single biomarker CK-assay (see Scheme 1(A) in the paper): The system responding to the variable concentrations of CK was designed and optimized in the present study and realized in 50 mM Gly-Gly buffer, pH 7.95, containing 2 U/mL PK, 1 U/mL LDH, 15 mM Crt, 1 mM ATP, 0.6 mM PEP, and 0.25 mM NADH. The biocatalytic cascade activated by the CK input resulted in the oxidation of NADH to yield NAD^+, thus resulting in the decrease of the absorbance detected optically at λ = 340 nm. The reaction and optical measurements were performed at 25˚± 0.1 °C.

(B) System for the two-biomarker CK-ALT-assay (see Scheme 1(B) in the paper): The system responding to the variable concentrations of CK and ALT was designed and optimized in the present study and realized in 50 mM Gly-Gly buffer, pH 7.95, containing 2 U/mL PK, 1 U/mL LDH, 15 mM Crt, 1 mM ATP, 0.6 mM PEP, 0.25 mM NADH, 200 mM Ala, 1 mM KTG. In the next set of experiments the CK and ALT were dissolved in human serum and then the serum solution was mixed with the Gly-Gly buffer (50% v/v) containing all components listed above, so that each component was at half its original concentration. The biocatalytic cascade activated by the CK and ALT inputs resulted in oxidation of NADH to yield NAD^+, thus resulting in the decrease of the absorbance detected optically at λ = 340 nm. The reaction and optical measurements were performed at 25˚± 0.1 °C.

(C) System for the two-biomarker CK-ALT-assay (see Scheme 1(B) in the paper) with PMS and NBT (added part (C) in Scheme 1): The system responding to the variable concentrations of CK and ALT and generating a color difference depending on the gender was designed and optimized in the present study and realized in 50 mM Gly-Gly buffer, pH 7.95, containing 2 U/mL PK, 1 U/mL LDH, 15 mM Crt, 1 mM ATP, 0.6 mM PEP, and 0.25 mM NADH. The biocatalytic cascade activated by the CK and ALT inputs resulted in the oxidation of NADH to yield NAD^+. The remaining NADH was oxidized to NAD^+ through the catalytic action of PMS, converting NBT to formazan and resulting in the increase of its absorbance detected optically at λ = 580 nm. The assay was performed in a buffer solution. The reaction and optical measurements were performed at 25˚± 0.1 °C.

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Serum stains analysis:

To mimic the male and female groups, characteristic levels of CK (234 U/L for male; 122 U/L for female) and ALT (32.1 U/L for male, 22.6 U/L for female) were dissolved in human serum (type AB; Sigma-Aldrich). The serum samples were dried/aged on a glass surface at 35˚C under reduced air pressure (ca. 25 mm Hg) using a vacuum pump Buchi Vac V500 (Buchi Labs, AG, Switzerland) for various time intervals (maximum up to 24 hours). Then, the samples were re-dissolved in 0.5 mL of 50 mM glycyl-glycine buffer, pH 7.95, containing 2 U/mL PK, 1 U/mL LDH, 15 mM Crt, 1 mM ATP, 0.6 mM PEP, 0.25 mM NADH, 200 Mm Ala**,** and 1 mM KTG. The obtained solutions were analyzed according to the CK-ALT-assay procedure. The experiment was repeated 3 times for each aging time period.