(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
02 January 2025 (02.01.2025)

WIPO | PCT

(10) International Publication Number
WO 2025/003065 A1

(51) International Patent Classification:
G01N 27/327 (2006.01) *C12Q 1/00* (2006.01)

(21) International Application Number:
PCT/EP2024/067660

(22) International Filing Date:
24 June 2024 (24.06.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/EP2023/067418
27 June 2023 (27.06.2023) EP

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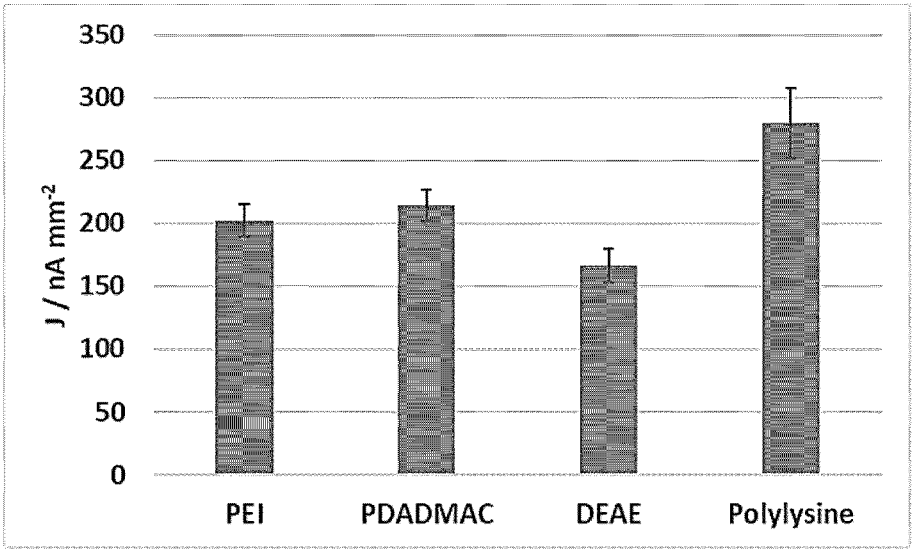
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) **Title:** METHOD OF MANUFACTURING AN ENZYME-ELECTRODE AND ENZYME-ELECTRODE

Fig. 4



(57) **Abstract:** The present invention relates to a method for manufacturing an enzyme electrode, wherein said method comprises the steps of: i) preparing a carbon particle suspension comprising carbon black and a non-conducting cationic polymer; ii) preparing a mixture comprising an enzyme and the carbon particle suspension; iii) applying the mixture to a conductive surface of an electrode; and iv) drying the mixture applied to the conductive surface of the electrode.

WO 2025/003065 A1

[Continued on next page]

Published:

— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

METHOD OF MANUFACTURING AN ENZYME-ELECTRODE AND ENZYME-ELECTRODE

FIELD OF THE INVENTION

The present invention relates to the field of enzyme electrodes and their manufacturing. The invention further relates to the use of enzyme electrodes for the detection and quantification of analytes, in particular to methods and means for the detection and/or quantification of analytes with enzyme-based methods.

BACKGROUND OF THE INVENTION

Enzyme electrodes are used in several different industries. Thereby, these electrodes are incorporated into biosensors for the detection of analytes.

In food analytics and healthcare, carbohydrates such as glucose or lactate can be detected by biosensors using enzyme modified electrodes.

In general, biosensors are analytical devices that leverage the high specificity of biological recognition elements, which are typically enzymes. Thereby, a biosensor consists of a biological recognition element e.g., an enzyme, which is able to specifically interact with a target molecule and a transducer able to convert this interaction into a measurable signal. In biosensors, enzymes are connected to electrodes and electric currents are measured that are proportional to the enzymes' substrate concentration in a sample. Biosensors are easy to operate, highly specific and do not need expensive and heavy equipment like it is the case for HPLC or NMR.

In general, three generations of biosensors are known. A biosensor based on an oxidase such as LOx is dependent on oxygen and is a first-generation biosensor. In second-generation biosensors, redox mediators other than the O_2/H_2O_2 pair are applied to transfer electrons from the enzyme to the electrode. In contrast, third-generation biosensors are based on direct electron transfer from enzyme to electrode. Thereby, the enzymes used in these third-generation biosensors are characterized by direct electron transfer (DET) capability to the electrode. Cellobiose dehydrogenase (CDH), flavocytochrome b2 (FCb2), and flavin adenine dinucleotide glucose dehydrogenase (FADGDH) are examples of such DET-capable enzymes.

One of the major challenges in this field is the material of the electronic circuits and the manufacturing of enzyme modified electrodes. While most applications are based on screen-printed carbon-based electrodes, small embodiments rely on the use of gold or other metals, that can be processed at very small scales. Thereby, enzyme

interaction with e.g., gold-based circuits, yields only in very small signals, that are not useful for commercial applications.

Different approaches for manufacturing enzyme modified electrodes have been developed in the field. Thereby, also different materials have been used.

The manufacturing of electrodes equipped with a carbon black-enzyme ink has been reported. For example, Shimizu, H. and Tsugawa, W. (2012) described glucose monitoring by a Direct Electron Transfer electrode using an electrode equipped with a carbon-enzyme ink comprising a carbon ink. Thereby, the carbon ink comprises a carbon black, an anionic polymer (Nafion), and water.

US2020/024631A1 discloses an electrode made from carbon black, with a polymer matrix containing PEI and enzymes deposited on the electrode.

A method for manufacturing a bioelectrode consisting of carbon black and polyethyleneimine (PEI) was described by Jayapiriya et al. (2023). Carbon black and PEI are applied to the electrode as a solution, and following a drying step, antibodies are immobilized onto the dried layer.

Ibanez-Redin et al. (2020) describe a method to produce a bioelectrode using 3D printing to produce a composite of carbon black, PEI and glucose oxidase.

US2022/133190A1 discloses an electrode comprising cellobiose dehydrogenase coupled to the neutral compound polyvinyl alcohol in the presence of ketjen black which is deposited on a carbon electrode, comprising an additional layer of a polycationic composition is disposed over the analyte modulating layer.

However, prepared carbon inks as described in the literature are often not well dispersed consisting of too large particles leading to sedimentation over time and clogging issues of liquid dispensing devices impeding manufacturability. Limited stability of the enzyme after mixing with the carbon ink also is an issue due to incompatibility of the enzyme with certain ink ingredients.

Thus, there is an urgent need in the art for methods of manufacturing electrodes and methods of manufacturing enzyme modified electrodes which provide improved signals on various electrode materials including also usually hard to modify metallic surfaces.

SUMMARY OF THE INVENTION

It is the objective of the present invention to provide methods of manufacturing enzyme modified electrodes which provide improved signals.

The objective is solved by the subject matter of the present invention.

According to the invention there is provided a method for manufacturing an enzyme electrode, wherein said method comprises the steps of:

i. preparing a carbon particle suspension comprising carbon black and a non-conducting cationic polymer;
ii. preparing a mixture comprising an enzyme and the carbon particle suspension;
iii. applying the mixture to a conductive surface of an electrode; and
iv. drying the mixture applied to the conductive surface of the electrode.

Specifically, the carbon black is graphitized carbon black.

Specifically, the non-conducting cationic polymer is polyethyleneimine (PEI), diethylaminoethyl dextran (DEAE), polylysine, or polydiallyldimethylammonium chloride (PDADMAC).

Specifically, the non-conducting cationic polymer is, diethylaminoethyl dextran (DEAE).

Specifically, in the carbon particle suspension the ratio of carbon black:non-conducting cationic polymer is in the range of 10:1 to 1:10.

Specifically, the carbon particle suspension further comprises water.

Specifically, the carbon particle suspension has a concentration in the range of 1 to 10 % (w/v).

Specifically, the carbon particle suspension is prepared by a sonication treatment.

Specifically, a probe sonicator is used for sonication treatment.

Specifically, the sonication treatment is performed for at least 60 seconds.

Specifically, the sonication treatment is performed at a power density of 0.4 W/cm^3.

Specifically, the sonication treatment is an ultrasonic treatment.

Specifically, agglomerates are removed prior to preparing the mixture comprising the carbon particle suspension and the enzyme.

Specifically, not de-agglomerated, sedimenting agglomerates present in the carbon particle suspension after the sonication treatment are removed prior to preparing the mixture comprising the carbon particle suspension and the enzyme.

Specifically, the conductive surface is gold, platinum, or carbon.

Specifically, the enzyme is a direct electron transfer (DET) enzyme.

Specifically, the DET enzyme is DET enzyme having analyte oxidizing activity.

Specifically, the analyte oxidizing activity is glucose oxidizing activity, lactose oxidizing activity, or lactate oxidizing activity.

Specifically, the DET enzyme is cellobiose dehydrogenase (CDH), flavocytochrome b2 (FCb2), or a functional variant thereof.

Specifically, in the mixture the ratio of enzyme:carbon particle suspension is 1:4.

According to the invention there is further provided an enzyme electrode comprising an analyte sensing layer, wherein said analyte sensing layer comprises carbon black, a non-conducting cationic polymer, and an enzyme.

According to the invention there is further provided an enzyme electrode comprising an analyte sensing layer, wherein said analyte sensing layer is a single layer comprising carbon black, a non-conducting cationic polymer, and an enzyme.

Specifically, carbon black is graphitized carbon black.

Specifically, the non-conducting cationic polymer is polyethyleneimine (PEI), diethylaminoethyl dextran (DEAE), polylysine, or polydiallyldimethylammonium chloride (PDADAMC).

Specifically, the enzyme is a direct electron transfer (DET) enzyme.

Specifically, the DET enzyme is DET enzyme having analyte oxidizing activity.

Specifically, the analyte oxidizing activity is glucose oxidizing activity, lactose oxidizing activity, or lactate oxidizing activity.

Specifically, the DET enzyme is cellobiose dehydrogenase (CDH), flavocytochrome b2 (FCb2), or a functional variant thereof.

Specifically, the electrode is a gold, platinum, or carbon electrode.

According to the invention there is further provided an enzyme electrode produced by the method described herein.

Specifically, the method for manufacturing an enzyme electrode comprises the steps of:

i. preparing a carbon particle suspension comprising carbon black and a non-conducting cationic polymer;

ii. preparing a mixture comprising an enzyme and the carbon particle suspension;

iii. applying the mixture to a conductive surface of an electrode; and

iv. drying the mixture applied to the conductive surface of the electrode.

Specifically, the carbon black is graphitized carbon black.

Specifically, the non-conducting cationic polymer is polyethyleneimine (PEI), diethylaminoethyl dextran (DEAE), polylysine, or polydiallyldimethylammonium chloride (PDADMAC).

Specifically, in the carbon particle suspension the ratio of carbon black:non-conducting cationic polymer is in the range of 10:1 to 1:10.

Specifically, the carbon particle suspension further comprises water.

Specifically, the carbon particle suspension has a concentration in the range of 1 to 10 % (w/v).

Specifically, the carbon particle suspension is prepared by a sonication treatment.

Specifically, a probe sonicator is used for sonication treatment.

Specifically, the sonication treatment is performed for at least 60 seconds.

Specifically, the sonication treatment is performed at a power density of 0.4 W/cm^3.

Specifically, the sonication treatment is an ultrasonic treatment.

Specifically, agglomerates are removed prior to preparing the mixture comprising the carbon particle suspension and the enzyme.

Specifically, not de-agglomerated, sedimenting agglomerates present in the carbon particle suspension after the sonication treatment are removed prior to preparing the mixture comprising the carbon particle suspension and the enzyme.

Specifically, the conductive surface is gold, platinum, or carbon.

Specifically, the enzyme is a direct electron transfer (DET) enzyme.

Specifically, the DET enzyme is DET enzyme having analyte oxidizing activity.

Specifically, the analyte oxidizing activity is glucose oxidizing activity, lactose oxidizing activity, or lactate oxidizing activity.

Specifically, the DET enzyme is cellobiose dehydrogenase (CDH), flavocytochrome b2 (FCb2), or a functional variant thereof.

Specifically, in the mixture the ratio of enzyme:carbon particle suspension is 1:4.

FIGURES

Figure 1: DLS Data describing the size distribution of a carbon/polycation suspension.

Figure 2: A: Catalytic current densities after the addition of 20 mM glucose to various electrode materials modified with carbon/polycation/enzyme ink measured at 0

V vs Ag/AgCl at pH 7.4. B: Current densities for a similar modification but without carbon/polycation using enzyme only.

Figure 3: Catalytic current densities for gold electrodes modified with carbon/polycation and various direct electron transfer enzymes sensitive to either glucose or lactate measured at 0 V (for glucose) and 0.2 V (for lactate) vs. an Ag/AgCl reference electrode.

Figure 4: Catalytic current densities for gold electrodes modified with carbon/ glucose enzyme inks prepared using different polycations measured at 0 V using 20 mM glucose dissolved in 50 mM PBS, pH 7.4.

Figure 5: Catalytic current densities for gold electrodes modified with carbon/ glucose enzyme inks prepared using different carbon black types measured at 0 V using 20 mM glucose dissolved in 50 mM PBS, pH 7.4.

Figure 6: Shelf life of sensors under rapid aging conditions: at 40°C electrodes loose ~20% of the initial currents within the first data point, but are then stable for at least 14 days. At 60°C a PEI based electrodes show residual currents of ~20% while DEAE based sensors retain 50% response.

DETAILED DESCRIPTION

Unless indicated or defined otherwise, all terms used herein have their usual meaning in the art, which will be clear to the skilled person. Reference is for example made to the standard handbooks, such as Sambrook et al, "Molecular Cloning: A Laboratory Manual" (4th Ed.), Vols. 1 -3, Cold Spring Harbor Laboratory Press (2012); Krebs et al., "Lewin´s Genes XI", Jones & Bartlett Learning, (2017); and Berg et al, “Stryer Biochemie” Springer Verlag, 2018.

The subject matter of the claims specifically refers to artificial products or methods employing or producing such artificial products, which may be variants of native (wild-type) products. Though there can be a certain degree of sequence identity to the native structure, it is well understood that the materials, methods, and uses of the invention, *e.g.,* specifically referring to isolated nucleic acid sequences, amino acid sequences, expression constructs, transformed host cells and modified proteins and enzymes, are “man-made” or synthetic, and are therefore not considered as a result of “laws of nature”.

The terms “comprise”, “contain”, “have” and “include” as used herein can be used synonymously and shall be understood as an open definition, allowing further members or parts or elements. “Consisting” is considered as a closest definition without further

elements of the consisting definition feature. Thus "comprising" is broader and contains the "consisting" definition.

The term "about" as used herein refers to the same value or a value differing by +/-5 % of the given value.

As used herein and in the claims, the singular form, for example "a", "an" and "the" includes the plural, unless the context clearly dictates otherwise.

As used herein, amino acids refer to twenty naturally occurring amino acids encoded by sixty-one triplet codons. These 20 amino acids can be split into those that have neutral charges, positive charges, and negative charges:

The "neutral" amino acids are shown below along with their respective three-letter and single-letter code and polarity: Alanine (Ala, A; nonpolar, neutral), Asparagine (Asn, N; polar, neutral), Cysteine (Cys, C; nonpolar, neutral), Glutamine (Gln, Q; polar, neutral), Glycine (Gly, G; nonpolar, neutral), Isoleucine (Ile, I; nonpolar, neutral), Leucine (Leu, L; nonpolar, neutral), Methionine (Met, M; nonpolar, neutral), Phenylalanine (Phe, F; nonpolar, neutral), Proline (Pro, P; nonpolar, neutral), Serine (Ser, S; polar, neutral), Threonine (Thr, T; polar, neutral), Tryptophan (Trp, W; nonpolar, neutral), Tyrosine (Tyr, Y; polar, neutral), Valine (Val, V; nonpolar, neutral), and Histidine (His, H; polar, positive (10%) neutral (90%)).

The "positively" charged amino acids are: Arginine (Arg, R; polar, positive), and Lysine (Lys, K; polar, positive).

The "negatively" charged amino acids are: Aspartic acid (Asp, D; polar, negative), and Glutamic acid (Glu, E; polar, negative).

The term "**electrode**" refers to any suitable material comprising a "**conductive surface**" for accepting electrons e.g., from an enzyme via mediatorless, mediated, or direct electron transfer. The term "**conductive surface**" as used herein refers to the surface of the material capable of accepting electrons.

According to one embodiment of the invention, the conductive surface is gold, platinum, or carbon.

According to one embodiment, the conductive surface may additionally be modified with carbon nanotubes (single or multi-walled), carbon fibers, nanoparticles, e.g. gold nanoparticles, or promoters as e.g., thiols. The electrode or the conductive surface of the electrode may be also of any material to increase the specific surface of the electrode.

According to one embodiment of the invention, the electrode described herein is a working electrode.

According to one embodiment, the electrode described herein enables the detection and/or quantification of an analyte based on direct electron transfer.

The term "**carbon particle suspension**" as used herein refers to a suspension comprising carbon black particles as the solute particles which do not dissolve but get suspended throughout the bulk of the solvent.

The term "**suspension**" as used herein refers to a heterogenous mixture or a fluid that contains solid particles. In other words, a suspension is a heterogenous mixture in which the solute particles do not dissolve, but get suspended throughout the bulk of the solvent.

In general, the two most common types of carbon allotropes are diamond and graphite. Thereby, graphite can be subdivided into carbon black and graphene.

The term "**carbon black**" as used herein refers to a polycrystalline graphite which is produced by combustion.

According to one embodiment, carbon black as described herein is CAS 1333-86-4.

According to one embodiment of the invention, a carbon black particle can be differentiated from other graphite particles by Raman spectroscopy e.g., according to Bokobza L., et al. (2015).

According to one embodiment of the invention, the carbon black described herein is graphitized carbon black.

Non-limiting examples of graphitized carbon black are graphitized mesoporous carbon black with a specific surface area of 50-100 m^2/g (e.g., from Sigma 699624), "Conductex SC Ultra" (from Birla carbon, Alexandria Carbon Black Co SAE), "Conductex K Ultra" (Birla carbon, Alexandria Carbon Black Co SAE), and "Super P Conductive" (from TIMCAL Ltd).

According to one embodiment, the graphitized carbon black described herein has crystalline dimensions with lateral sizes of below 20 nm.

According to a specific embodiment, graphitized carbon black has a pore size of 0.25 cm^3/g pore volume, a surface area of 50-100 m^2/g, a boiling point (bp) of 4827 °C, a melting point (mp) of 3654-3697 °C, and an absolute density of 1.828 g/cm^3.

According to one embodiment of the invention, the carbon black described herein has an NSA surface area m^2/g in the range of 30-250, 40-250, 50-250, 50-210, 60-210,

70-210, 80-210, 90-210, 100-210, 110-210, 120-210, 130-210, 140-210, 150-210, 160-210, 170-210, or 180-210. Specifically, the carbon black described herein has an NSA surface area m^2/g in the range of 180-210.

According to one embodiment of the invention, the carbon black described herein has a STSA surface area m^2/g in the range of 30-130, 40-130, 50-130, 60-130, 70-130, 80-130, 90-130, 100-130, 110-130, or 120-130. Specifically, the carbon black described herein has a STSA surface area m^2/g in the range of 120-130.

The NSA surface area is based on the B.E.T. theory and includes the total surface area, inclusive of micropores, pore diameters less than 2 nm (20 Å).

According to one embodiment, a carbon particle suspension is prepared, wherein said carbon particle comprises carbon black and a cationic polymer. Specifically, said cationic polymer is non-conducting.

The term "**cationic polymer**" as used herein refers to a polymer having a positive charge or incorporating cationic entities in their structure.

According to one embodiment, the cationic polymer described herein has a positive charge at a pH value below pH 7, pH 8, pH 9, pH 10, pH 11, pH 12, pH 13, or pH 14.

The term "**non-conducting**" as used herein e.g., in the context of a cationic polymer refers to a cationic polymer that is not electrically conducting.

According to one embodiment, non-conducting is having an electrical conductance of $<10^{-6}$ S cm^{-1}.

For example, polyethyleneimine (PEI) is a non-conducting cationic polymer because of its positive charge at pH values <8 and its conductivity of $<10^{-6}$ S cm^{-1}.

For example, polyvinyl alcohol is not a non-conducting cationic polymer because of its neutral charge of the hydroxyl groups.

According to one embodiment, the non-conducting cationic polymer is selected from the group consisting of polyethyleneimine (PEI), diethylaminoethyl dextran (DEAE), polylysine, and polydiallyldimethylammonium chloride (PDADAMC).

According to a specific embodiment, the non-conducting cationic polymer is polyethyleneimine (PEI).

According to a specific embodiment, the non-conducting cationic polymer is diethylaminoethyl dextran (DEAE).

According to a specific embodiment, the non-conducting cationic polymer is polylysine.

According to a specific embodiment, the non-conducting cationic polymer is polydiallyldimethylammonium chloride (PDADAMC).

According to one embodiment of the invention, the carbon particle suspension described herein comprises carbon black particles in the size of below 1000 nm after removal of agglomerates or sedimenting agglomerates from the carbon particle suspension. According to a specific embodiment, the carbon particle suspension described herein comprises carbon black particles in the range of 300-700 nm.

According to one embodiment of the invention, in the carbon particle suspension described herein the ratio of carbon black:non-conducting cationic polymer is in the range of 10:1 to 1:10. Specifically, the ratio of carbon black:non-conducting cationic polymer is in the range of 10:1 to 1:1. More specifically, the ratio of carbon black:non-conducting cationic polymer is in the range of 7:1 to 1:1.

According to a specific embodiment, the ratio of carbon black:non-conducting cationic polymer is in the range of 4:1 to 2:1 for PDADMAC. Specifically, the ratio of carbon black:non-conducting cationic polymer is 3.125:1 for PDADMAC.

According to a specific embodiment, the ratio of carbon black:non-conducting cationic polymer is in the range of 4:1 to 2:1 for DEAE. Specifically, the ratio of carbon black:non-conducting cationic polymer is 3.125:1 for DEAE.

According to a specific embodiment, the ratio of carbon black:non-conducting cationic polymer is in the range of 7:1 to 5:1 for PEI. Specifically, the ratio of carbon black:non-conducting cationic polymer is 6.25:1 for PEI.

According to a specific embodiment, the ratio of carbon black:non-conducting cationic polymer is in the range of 1:1 to 5:1 for polylysine. Specifically, the ratio of carbon black:non-conducting cationic polymer is 2:1 for polylysine.

As used herein, the ratio of carbon black:non-conducting cationic polymer is to be understood as weight:weight. For example, a ratio of carbon black:non-conducting cationic polymer of 10:1 refers to 10 mg carbon black and 1 mg non-conducting cationic polymer.

According to one embodiment of the invention, the carbon particle suspension described herein comprises water. In an alternative embodiment, the carbon particle suspension comprises an aqueous solution e.g., a buffer.

According to one embodiment of the invention, the carbon particle suspension has a concentration in the range of 1 to 10 % (w/v). Specifically, the carbon particle suspension has a concentration in the range of 1 to 5 %, 1 to 4 %, or 1 to 3 % (w/v).

According to a specific embodiment, the carbon particle suspension has a concentration in the range of 1 to 2 % (w/v) if PDADMAC is used as non-conducting cationic polymer.

According to a specific embodiment, the carbon particle suspension has a concentration in the range of 1 to 2 % (w/v) if DEAE is used as non-conducting cationic polymer.

According to a specific embodiment, the carbon particle suspension has a concentration in the range of 1 to 2 % (w/v) if DEAE is used as non-conducting cationic polymer.

According to a specific embodiment, the carbon particle suspension has a concentration in the range of 1 to 2 % (w/v) if PEI is used as non-conducting cationic polymer.

According to a specific embodiment, the carbon particle suspension has a concentration in the range of 2 to 3 % (w/v) if polylysine is used as non-conducting cationic polymer.

According to one embodiment of the invention, the carbon particle suspension described herein is prepared by a sonication treatment.

The term "**sonication**" as used herein refers to the process of applying sound energy to agitate particles or discontinuous fibers in a liquid. Ultrasonic frequencies are usually used, so the process is also known as ultrasonication. In general, sonication may be conducted using e.g., an ultrasonic bath or an ultrasonic probe (sonicator).

According to a specific embodiment of the invention, a probe sonicator is used for the sonication treatment.

According to a specific embodiment of the invention, the sonication treatment described herein is an ultrasonic treatment.

The term "**ultrasonic**" as used herein in the context of sonication refers to the use of ultrasonic frequencies. The process of ultrasonic treatment is also known as ultrasonication.

According to a specific embodiment of the invention, the sonication treatment described herein is performed for at least 60 seconds, 120 seconds, 180 seconds, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 60 minutes, or even longer.

According to a specific embodiment of the invention, the sonication treatment described herein may be performed at a power density in the range of 0.01 W/cm^3 to 0.4 W/cm^3 or higher. According to a more specific embodiment, the sonication treatment described herein may be performed at a power density of 0.4 W/cm^3.

According to a specific embodiment, the exposure time of the sonication treatment may be adapted to the specific power density.

In general, the technical effect of preparing the carbon particle suspension described herein by sonication treatment is that the sonication improves the de-agglomeration of carbon particles which are then kept in a deagglomerated state by the polycationic coating.

According to one embodiment of the invention, the carbon particle suspension described herein may comprise agglomerates or sedimenting agglomerates. According to a specific embodiment, the carbon particle suspension described herein may comprise agglomerates or sedimenting agglomerates after the sonication treatment.

According to one embodiment of the invention, agglomerates or sedimenting agglomerates may be removed from the carbon particle suspension described herein. According to a specific embodiment, these agglomerates or sedimenting agglomerates may be removed after the sonication treatment and prior to preparing the mixture comprising the carbon particle suspension and the enzyme.

According to one embodiment of the invention, the carbon particle suspension described herein is prepared prior to mixing the enzyme with the carbon particle suspension.

According to one embodiment, in the method described herein a mixture comprising an enzyme and the carbon particle suspension as described herein is prepared.

The term "**enzyme**" as used herein refers to any substance composed wholly or largely of protein or polypeptides that catalyzes or promotes, more or less specifically, one or more chemical or biochemical reaction(s).

The term "**activity**" as used herein e.g., in the context of an enzyme activity, shall refer to the catalysed reaction of the enzyme. Thereby, an enzyme having an activity is a functionally active molecule such as a functional enzyme. A functional enzyme is specifically characterized by a catalytic centre recognizing the enzyme substrate and catalysing the conversion of the substrate to a conversion product. Enzyme variants are considered functional or functionally active upon determining their enzymatic activity in

a standard test system, e.g., wherein the enzymatic activity is at least 30% of the activity of the parent (not modified or wild-type) enzyme, or at least any of 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%.

Enzyme activity is generally given in units. Thereby, one **unit of enzymatic activity** is defined as the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per min under the respective conditions of the determination method. For example, one unit of enzymatic activity is defined as the amount of enzyme that oxidizes 1 µmol of substrate such as e.g., lactate, per min under the respective conditions of the determination method. The specific activity is given in "U/mg", "U mg^{-1}" or "U per mg". Volumetric activity is given in units per volume such as in "U/mL", "U/ml", "U per mL", "U per ml", "U mL^{-1}", or "U ml^{-1}".

According to one embodiment of the invention, the enzyme is a direct electron transfer (DET) enzyme.

The term "**direct electron transfer enzyme**" or "DET enzyme" as used herein refers to an enzyme which is capable of transferring electrons gained through reaction with its substrate directly to an electron acceptor such as an electrode without the need for mediated electron transfer e.g., by a redox mediator.

According to one embodiment of the invention, the enzyme is a direct electron transfer (DET) enzyme having analyte oxidizing activity.

In general, the term "**oxidizing**" in the context of an oxidizing agent such as an enzyme having oxidizing activity, refers to an agent that oxidizes a substance and gains or "accepts" an electron from said substance. Thereby, the enzyme has "analyte oxidizing activity" or "substance oxidizing activity". Such a substance may also be referred to as substrate. Therefore, e.g., an enzyme having lactate oxidizing activity catalyzes the oxidation of lactate.

In general, an enzyme having substance oxidizing activity gains or accepts one or more electrons from the substance. Thereby, the enzyme itself or a cofactor of the enzyme, gets reduced. In the reduced state, an enzyme cannot catalyze another oxidation reaction of a substance. Therefore, the enzyme or the cofactor of the enzyme needs to be re-oxidized by transferring the gained electrons to an electron acceptor before another oxidation reaction of a substance can be catalyzed.

According to one embodiment, the enzyme described herein comprises two domains i.e., a domain having substrate oxidizing activity and a domain transferring the gained electrons to a terminal electron acceptor such as an electrode.

According to one embodiment, the enzyme described herein is an enzyme having analyte oxidizing activity, wherein the analyte oxidizing activity is glucose oxidizing activity, lactose oxidizing activity, or lactate oxidizing activity.

According to a specific embodiment, the enzyme described herein is a direct electron transfer (DET) enzyme having glucose oxidizing activity, lactose oxidizing activity, or lactate oxidizing activity.

According to one embodiment, the enzyme described herein is cellobiose dehydrogenase (CDH) or flavocytochrome b2 (FCb2).

The term "**cellobiose dehydrogenase**" or “CDH” as used herein refers to an enzyme having a flavin domain and a haem domain connected by a peptide linker, which oxidizes carbohydrates like its natural substrates cellobiose and cello-oligosaccharides and others, like lactose, maltose, and glucose. The reoxidation of the flavin domain cofactor can be achieved by direct oxidation by two-electron acceptors including quinones like 2,6-dichloroindophenol, o- or p-benzoquinone or derivatives thereof, methylene blue, methylene green, and Meldola's blue; or by one-electron acceptors like potassium ferricyanide, ferricenium hexafluorophosphate, and FeCl3; or by intramolecular electron transfer (IET) to the haem domain cofactor and further to a terminal electron acceptor like cytochrome c (cyt c) or an electrode surface.

Cellobiose dehydrogenase is described e.g., in Harreither, W. et al. (2011) and in EP 2223936 A1.

In a specific embodiment, the CDH described herein is a functionally active variant of a CDH peptide sequence and comprises one or more point mutations in the nucleotide sequence encoding the CDH sequence, compared to the respective CDH sequence. Specifically, it comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 point mutations, specifically resulting in one or more amino acid substitutions, additions or deletions, or the like. Specifically, the functional variant of the CDH peptide sequence is a full-length CDH peptide sequence comprising point mutations, or it is a fragment of the full-length CDH peptide sequence with retained enzymatic activity. Specifically, a variant of a CDH sequence is functionally active if it is capable of converting the analyte to be determined with the electrode described herein to the corresponding oxidized form. Specifically, a functionally active variant of a CDH sequence has at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, or even more % of the enzymatic activity of the corresponding CDH sequence with the analyte as substrate. Specifically, a functionally active variant of a CDH sequence has at least 10, 20, 30, 40,

50, 60, 70, or even more % of the enzymatic activity of the corresponding CDH sequence, wherein said enzymatic activity is determined with the CytC assay and the respective analyte, e.g., lactose or glucose as substrate.

According to a specific embodiment, the CDH may comprise the amino acid sequence of a CDH from *Neurospora crassa, Phanerochaete chrysosporium, Corynascus thermophilus,* or *Myriococcum thermophilum.* Specifically, the CDH may be a functional variant of any one of the foregoing and comprise an amino acid sequence having 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99% with the amino acid sequence of an CDH of any one of the foregoing.

According to one embodiment, the enzyme described herein is a CDH selected from the group consisting of SEQ ID NOs:3 to 9 or a functionally active variant thereof.

According to a specific embodiment, the enzyme described herein is a CDH selected from the group consisting of SEQ ID NOs:3 to 9 or an enzyme having 50, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 % sequence identity with any one of SEQ ID NOs:3 to 9.

The following table 1 gives the amino acids sequences of SEQ ID NOs:3 to 9:

Table 1

SEQ ID NO:	Amino acid sequence
3	MRTSSRLIGALAAALLPSALAQNNVPNTFTDPDSGITFNTWGLDEDSPQTQGGFTF GVALPSDALTTDASEFIGYLKCARNDESGWCGISLGGPMTNSLLITAWPHEDTVYTS LRFATGYAMPDVYEGDAEITQVSSSVNSTHFSLIFRCKNCLQWSHGGSSGGASTS GGVLVLGWVQAFDDPGNPTCPEQITLQQHDNGMGIWGAQLNTDAASPSYTDWAA QATKTVTGDCEGPTETSVVGVPVPTGVSFDYIVVGGGAGGIPAADKLSEAGKSVLLI EKGFASTANTGGTLGPEWLEGHDLTRFDVPGLCNQIWVDSKGIACEDTDQMAGCV LGGGTAVNAGLWFKPYSLDWDYLFPDGWKYNDVQPAINRALSRIPGTDAPSTDGK RYYQEGFEVLSKGLAAGGWTSVTANNAPDKKNRTFAHAPFMFAGGERNGPLGTY FQTAKKRNNFDVWLNTSVKRVIREGGHITGVEVEPFRDGGYEGIVPVTKVTGRVILS AGTFGSAKILLRSGIGPEDQLEVVAASEKDGPTMIGNSSWINLPVGYNLDDHLNTDT VISHPDVVFYDFYEAWDDPIESDKNSYLESRTGILAQAAPNIGPMFWEEIVGADGIV RQLQWTARVEGSLGAPNGHTMTMSQYLGRGATSRGRMTITPSLTTIVSDVPYLKD PNDKEAVIQGIINLQNALQNVANLTWLFPNSTITPREYVESMVVSPSNRRSNHWMG TNKLGTDDGRKGGSAVVDLDTRVYGTDNLFVIDASIFPGVPTTNPTSYIVVAAEHAS SRILALPDLEPVPKYGQCGGREWTGSFVCADGSTCEYQNEWYSQCL
4	MGRLGSLAKLLLAVGLNVQQCFGQNGPPTPYTDSETGITFATWSVPDRAEGGNGL APWGGLTFGVALPENALTTDATELIGYLKCGSNGTTTDAWCGLSFGGPMTNSLLLM AWPHEDEILTSFRFASGYTRPDLYTGDAKLTQISSTIDKDHFTLIFRCQNCLAWNQD GASGSASTSAGSLILGWASALRAPTNAGCPAEINFNFHNNGQMIWGATLDESAANP SYSEWAAKATATVTGDCGGATPTTTTTTTTSVPTATGIPVPTGTYDYIVVGAGAGGI PLADKLSEAGKSVLLIEKGPPSSGRWGGTLKPEWLKDTNLTRFDVPGLCNEIWVNS AGVACTDTDQMAGCVLGGGTAVNAGLWWKPYNLDWDYNFPRGWKSRDMAAAT RRVFSRIPGTDNPSMDGKRYLQQGFEILAGGLKAAGWTEVTANDAPNKKNHTYSH SPFMFSGGERGGPMGTYLVSASRRKNFHLWTGTAVKRVVRTGGHITGLEVEPFVN GGYTGVVNVTSITGRVVLSAGAFGSAKILLRSGIGPEDQLEIVKSSTDGPTMISDSS WITLPVGYNLEDHTNTDTVVTHPDVVFYDFYEAGHPNVTDKDLYLNSRAGILAQAAP NIGPMFWEEIKGKDGVVRQLQWTARVEGSAGTPNGYAMTMSQYLGRGAKSRGR MTITKALTTVVSTVPYLQDKNDVEAVIQGIKNLQAALSNVKNLTWTYPPSNTTVEDFV NNMLVSYTNRRSNHWIGTNKLGTDDGRSRGGSAVVDLNTKVYGTDNLFVVDAGIF PGHITTNPTSYIVIAAERASERILDLPPARAQPRFAQCGGRTWTGSFQCAAPYTCQY RNERYSQCR
5	MRPSSRFVGALAAAASFLPSALAQNNAAVTFTDPDTGIVFNSWGLANGAPQTQGG FTFGVALPSDALTTDATEFIGYLECASADNQGWCGVSMGGPMTNSLLITAWPHEDN VYTSLRFATGYAMPDVYSGDATITQISSSINATHFKLIFRCQNCLQWTHDGASGGAS TSAGVLVLGWVQAFPSPGNPTCPDQITLEQHNNGMGIWGAVMDSNVANPSYTEW AAQATKTVEAECDGPSETDIVGVPVPTGTTFDYIVVGGGAGGIPTADKLSEAGKSVL LIEKGIASTAEHGGTLGPEWLEGNDLTRFDVPGLCNQIWVDSKGIACEDTDQMAGC VLGGGTAVNAGLWFKPYSLDWDYLFPSGWKYRDIQAAIGRVFSRIPGTDAPSTDGK RYYQQGFDVLAGGLSAGGWNKVTANSSPDKKNRTFSNAPFMFSGGERGGPLATY LTSAKKRSNFNLWLNTSVKRVIREGGHVTGVEVEPFRTGGYQGIVNVTAVSGRVVL SAGTFGSAKILLRGGIGPADQLEVVKASKIDGPTMISNASWIPLPVGYNLDDHLNTDT VITHPDVAFYDFYEAWNTPIEADKNSYLSSRTGILAQAAPNIGPMMWEEIKGADGIV RQLQWTARVEGSFDTPNGQAMTISQYLGRGATSRGRMTITPSLTTVVSDVPYLKDP NDKEAVIQGIVNLQNALKNVAGLTWTYPNSSITPREYVDNMVVSPSNRRANHWMG TAKIGTDDGRLAGGSAVVDLNTKVYGTDNLFVVDASIFPGTPTTNPSAYIVTAAEHA SQRILGLAAPKPVGKWGQCGGRQWTGSFQCVSGTKCEVVNEWYSQCL

6	MKLLSRVGATALAATLSLKQCAAQMTEGTYTHEATGITFKTWTPSDGSTFTFGLALP GDALTNDATEYIGLLRCQITDPSSPGYCGISHGQSGQMTQALLLVAWASEDVVYTS FRYATGYTLPELYTGDAKLTQIASSVSGDSFEVLFRCENCFSWDQNGATGSVSTSN GALVLGYAASKSGLTGATCPDTAEFGFHNNGFGQWGAVLEGATSDSYEEWAQLA TITPPTTCDGNGPGDKVCVPAPEDTYDYIVVGAGAGGITVADKLSEAGHKVLLIEKG PPSTGLWNGTMKPEWLEGTDLTRFDVPGLCNQIWVDSAGIACTDTDQMAGCVLG GGTAVNAGLWWKPHPADWDDNFPHGWKSSDLADATERVFSRIPGTWHPSQDGK LYRQEGFEVISQGLANAGWREVDANQEPSEKNRTYSHSVFMFSGGERGGPLATYL ASAAQRSNFNLWVNTSVRRAIRTGPRVSGVELECLADGGFNGTVNLKEGGGVIFSA GAFGSAKLLLRSGIGPEDQLEIVASSKDGETFISKNDWIKLPVGHNLIDHLNTDLIITH PDVVFYDFYAAWDNPITEDKEAYLNSRSGILAQAAPNIGPLMWEEVTPSDGITRQFQ WTCRVEGDSSKTNSTHAMTLSQYLGRGVVSRGRMGITSGLTTTVAEHPYLHNDGD LEAVIQGIQNVVDALSQVPDLEWVLPPPNTTVEEYVNSLIVSPANRRANHWMGTAK MGLDDGRSGGSAVVDLNTKVYGTDNLFVVDASIFPGMSTGNPSAMIVIVAEQAAQR ILSLRY
7	MKLLSRVGATALAATLSLKQCAAQMTEGTYTHEATGITFKTWTPSDGSTFTFGLALP GDALTNDATEYIGLLRCQITDPSSPGYCGISHGQSGQMTQALLLVAWASEDVVYTS FRYATGYTLPELYTGDAKLTQIASSVSGDSFEVLFRCENCFSWDQNGATGSVSTSN GALVLGYAASKSGLTGATCPDTAEFGFHNNGFGQWGAVLEGATSDSYEEWAQLA TITPPTTCDGNGPGDKVCVPAPEDTYDYIVVGAGAGGITVADKLSEAGHKVLLIEKG PPSTGLWNGTMKPEWLEGTDLTRFDVPGLYNQIWVDSAGIACTDTDQMAGCVLG GGTAVNAGLWWKPHPADWDDNFPHGWKSSDLADATERVFSRIPGTWHPSQDGK LYRQEGFEVISQGLANAGWREVDANQEPSEKNRTYSHSVFMFSGGERGGPLATYL ASAAQRSNFNLWVNTSVRRAIRTGPRVSGVELECLADGGFNGTVNLKEGGGVIFSA GAFGSAKLLLRSGIGPEDQLEIVASSKDGETFISKNDWIKLPVGHNLIDHLNTDLIITH PDVVFYDFYAAWDNPITEDKEAYLNSRSGILAQAAPNIGPLMWEEVTPSDGITRQFQ WTCRVEGDSSKTNSTHAMTLSQYLGRGVVSRGRMGITSGLTTTVAEHPYLHNDGD LEAVIQGIQNVVDALSQVPDLEWVLPPPNTTVEEYVNSLIVSPANRRANHWMGTAK MGLDDGRSGGSAVVDLNTKVYGTDNLFVVDASIFPGMSTGNPSAMIVIVAEQAAQR ILSLRY
8	MLFKLSNWLLALALFVGNVVAQLVGPTPYTDPDTGIVFQSWVNPAGTLKFGYTYPA NAATVAATEFIGFLECQGAGWCSVSLGGSMLNKPLVVAYPSGDEVLASLKWATGY ANPEPYGGNHKLSQISSSVTSAGFRVVYRCEGCLAWNYQGIEGGSPTNGASMPIG WAYSASSVLNGDCVDNTVLIQHDTFGNYGFVPDESSLRTEYNDWTELPTRVVRGD CGGSTTTSSVPSSTAPPQGTGIPVPTGASYDYIVVGSGAGGIPIADKLTEAGKKVLLI EKGPPSSGRYDGKLKPTWLEGTNLTRFDVPGLCNQIWVDSAGIACRDTDQMAGCV LGGGTAVNAGLWWKPNPIDWDYNFPSGWKSSEMIGATNRVFSRIGGTTVPSQDG KTYYQQGFNVLSSGLKAAGWTSVSLNNAPAQKNRTYGAGPFMFSGGERGGPLAT YLATAKKRGNFDLWTNTQVKRVIRQGGHVTGVEVENYNGDGYKGTVKVTPVSGRV VLSAGTFGSAKLLLRSGIGPKDQLAIVKNSTDGPTMASERDWINLPVGYNLEDHTNT DIVISHPDVVHYDFYEAWTAPIESDKTAYLGKRSGILAQAAPNIGPLFFDEVRGADNI VRSIQYTARVEGNSVVPNGKAMVISQYLGRGAVSRGRMTISQGLNTIVSTAPYLSNV NDLEAVIKSLENIANSLTSKVKNLKIEWPASGTSIRDHVTNMPLDPATRRANHWIGTN KIGTKDGRLTGGDSVVDLNTKVYGTDNLFVVDASIFPGMVTTNPSAYIVIAAEHAASK ILSLPTAKAAAKYEQCGGLEYNGNFQCASGLTCTWLNDYYWQCT

9	MRTTSAFLSGLAAVASLLSPAFAQTAPKTFTHPDTGIVFNTWSASDSQTKGGFTVG MALPSNALTTDATEFIGYLECSSAKNGANSGWCGVSLRGAMTNNLLITAWPSDGEV YTNLMFATGYAMPKNYAGDAKITQIASSVNATHFTLVFRCQNCLSWDQDGVTGGIS TSNKGAQLGWVQAFPSPGNPTCPTQITLSQHDNGMGQWGAAFDSNIANPSYTAW AAKATKTVTGTCSGPVTTSIAATPVPTGVSFDYIVVGGGAGGIPVADKLSESGKSVL LIEKGFASTGEHGGTLKPEWLNNTSLTRFDVPGLCNQIWKDSDGIACSDTDQMAGC VLGGGTAINAGLWYKPYTKDWDYLFPSGWKGSDIAGATSRALSRIPGTTTPSQDGK RYLQQGFEVLANGLKASGWKEVDSLKDSEQKNRTFSHTSYMYINGERGGPLATYL VSAKKRSNFKLWLNTAVKRVIREGGHITGVEVEAFRNGGYSGIIPVTNTTGRVVLSA GTFGSAKILLRSGIGPKDQLEVVKASADGPTMVSNSSWIDLPVGHNLVDHTNTDTVI QHNNVTFYDFYKAWDNPNTTDMNLYLNGRSGIFAQAAPNIGPLFWEEITGADGIVR QLHWTARVEGSFETPDGYAMTMSQYLGRGATSRGRMTLSPTLNTVVSDLPYLKDP NDKAAVVQGIVNLQKALANVKGLTWAYPSANQTAADFVDKQPVTYQSRRSNHWM GTNKMGTDDGRSGGTAVVDTNTRVYGTDNLYVVDASIFPGVPTTNPTAYIVVAAEH AAAKILAQPANEAVPKWGWCGGPTYTGSQTCQAPYKCEKQNDWYWQCV

According to one embodiment, the enzyme described herein is a flavocytochrome b2 (FCb2) or a functional variant thereof.

The term “**FCb2**” refers to a L-lactate-cytochrome c oxidoreductase (EC 1.1.2.3; flavocytochrome b2, FCb2, L-lactate cytochrome c oxidoreductase). In general, FCb2 catalyzes the electron transfer from L-lactate to cytochrome c in yeast mitochondria. In yeast, L-lactate is converted to pyruvate by L-lactate cytochrome c- oxidoreductase (EC 1.1.2.3), which is herein referred to as “Flavocytochrome b2” or "FCb2". Native yeast flavocytochrome b2 (FCb2) has two functional domains that are connected via a “hinge” linker (57 kDa monomer). The FCb2 from *S. cerevisiae* is the best studied representative and has been crystallized (PDB 1FCB).

According to a specific embodiment, the FCb2 described herein may comprise a sequence based on the mature form of FCb2 naturally found in the yeast mitochondrial intermembrane space, which comprises a cytochrome b2 domain, a flavin domain, a hinge region connecting the cytochrome b2 domain and the flavin domain and a tail region at its C-terminus. A mature FCb2 peptide sequence is the sequence of an FCb2 peptide as it is naturally found in the yeast mitochondrion, specifically in the mitochondrial intermembrane space.

According to a specific embodiment, the FCb2 described herein comprises a FCb2 peptide sequence comprising at least a yeast heme domain and a yeast flavin domain.

In a specific embodiment, the FCb2 described herein is a functionally active variant of a FCb2 peptide sequence found in the yeast mitochondrial intermembrane space and comprises one or more point mutations in the nucleotide sequence encoding

the FCb2 sequence, compared to the respective native mature FCb2 sequence. Specifically, it comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 point mutations, specifically resulting in one or more amino acid substitutions, additions or deletions, or the like. Specifically, the functional variant of the FCb2 peptide sequence is a full-length mature FCb2 peptide sequence comprising point mutations, or it is a fragment of the full-length mature FCb2 peptide sequence with retained enzymatic activity. Specifically, a variant of a FCb2 sequence is functionally active if it is capable of converting the analyte to be determined with the electrode described herein to the corresponding oxidized form. Specifically, a functionally active variant of a FCb2 sequence has at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, or even more % of the enzymatic activity of the corresponding wild type FCb2 sequence with the analyte as substrate. Specifically, a functionally active variant of a FCb2 sequence has at least 10, 20, 30, 40, 50, 60, 70, or even more % of the enzymatic activity of the corresponding wild type FCb2 sequence, wherein said enzymatic activity is determined with the CytC assay and the respective analyte, e.g., lactate as substrate.

According to a specific embodiment, the FCb2 may comprise the amino acid sequence of a FCb2 from *S. cerevisiae, W. anomalus, K. marxianus, O. parapolymorpha, Candida glabrata, Kluyveromyces lactis, Lachancea thermotolerans, Saccharomycodes ludwigii, Naumovozyma castelli, Zygosaccharomyces bailii, Zygosaccharomyces parabalii, Lachancea mirantina, Tetrapisispora phaffii, Saccharomyces eubayanus, Saccharomyces kudriavzevii, Saccharomyces paradoxus, Vanderwaltozyma polyspora, Lachancea dasiensis, Wickerhamomyces ciferri, Kluyveromyces dobzhanskii, Kazachstania naganishii, Zygosaccharomyces mellis, Kazachstania saulgeensis, Candida boidinii, Lachancea fermentati, Zygosaccharomyces rouxii, Cyberlindnera fabianii, Cyberlindnera jadinii, Kazachstania africana, Lachancea quebecensis, Kuraishia capsulata, Torulaspora delbrueckii, Komogatella pastoris, Komagatella phaffii, Lachancea nothofagi,* or *Naumovomyces dairenensis.* Specifically, the FCb2 may be a functional variant of any one of the foregoing and comprise an amino acid sequence having 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99% sequence identity with the amino acid sequence of an FCb2 of any one of the foregoing.

Specifically, the recombinant FCb2 described herein comprises a peptide sequence derived from the FCb2 of *Saccharomyces cerevisiae, Kluyveromyces*

marxianus, Wickerhamomyces anomalus, Naumovozyma castelli or *Cyberlindera fabianii*.

Amino acid sequences of polypeptides derived from organisms may be readily derived from publicly available databases such as e.g., from databases provided by the National Center for Biotechnology Information (NCBI).

According to one embodiment, the enzyme described herein is a FCb2 selected from the group consisting of SEQ ID NOs:10 to 50 or a functionally active variant thereof.

According to a specific embodiment, the enzyme described herein is a FCb2 selected from the group consisting of SEQ ID NOs:10 to 50 or an enzyme having 50, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 % sequence identity with any one of SEQ ID NOs:10 to 50.

The following table 2 gives the amino acids sequences of SEQ ID NOs:10 to 50:

Table 2

SEQ ID NO:	Amino acid sequence
10	MMRLQELRNQRYRDLNLRIRITQSEISTSRTVVTTALPVLMFRLISQKTHVSLSRLFLRG QWRAFSKGAGFRGTQRVKRLSSHVALFTALAGLTYILGPGREISLIKNDAAKHSKRRH VSATEVIRHNKEDDCWVVIDGYVYDVTAFIDQHPGGSAVIRGNAGKDVSALFSALHPP DVIQKYIPETQRLGPLEDQMPSDRICSPPAIGETVEDIQRKEELRQKLLDLNSLLNLYDF EYLASQILANQAWAYYSSASDDEFTYRENHAAYHRIFFKPRVLVNVKNVDISTEMLGFK VSVPFYVSATALVKLGNPEEGEKDIARGCGQGEHKCPQMISTFASCSLQEIVEAAPSK EQIQWLQLYVNTNRSATESLLREAETLGLRAIFLTVDTPASGRREKDMKLKFISSNAPQ NARAAKNKSSRGASQALASFIDPTLTWEDVAELKTKTNLPVVIKGVQCVEDVLKAAEIG VDGVVISNHGGRQLDFSRAPLEVLADTMPILKEKHLDDKLEVFIDGGVRRGTDILKALC LGAKGVGLGRPFLYANSCYGKDGVEKAISMLAEELQCSMRLLGAKSIKELGPELLDVT GLKNRSIEIPGDSLCNTVYQKPDFARFVEK
11	MYKRLISKKVYLNTFNSKANFTGKYLSAPKCSFNCIRGYSKFNGNYNVHSAKKYDNNN NNNNSRFSLFAAVAATVITVGTTAIITSNKSDASFLSNTSSVLNKPPIDPKEVVKHNKPD DCWIVINNVVYDLTDFIAKHPGGPDIIQSNAGKDVSAIFNPLHASDVIDKYIKPECQLGPL KEPLPESYICPPLTPGETAEDVARKAELRDKLPPLDSLINLYDFEYLASQILTKQAWGYY SSAADDEVTYRENHAAYHRIFFKPRILINVKECDLSTTMLGTKMDLPFYVSATALCKLG NPSEGEKDIARGCGMGEFNLTQMISTLASCSLKEIVEAKVNDKQTQWFQLYVNADRKI TDNLIKNVEELGLKAIFVTVDAPSLGRREKDMKIKFDPSKTPEITNEDKSHKKQQNTRG ASKALSTFIDPSLTWNDVIDIKKKTKLPVVIKGVQCTADILKAAEIGVDGVVLSNHGGRQ LDFSRAPIEVLAEAMPILKEKGLDKKLEVYIDGGVRRGTDVLKALCLGAKGVGLGRPFL YANSCYGKDGVNRLKEMLADEIEMNMRLLGVTKISDLKPEYLDMISLHARSVNVPKDN LYQEVYIPSTTVEFLDE

12	MLLFIKTSKLKLPKRLLSNFRKTCISPSRNYSRKQTQSFRTVEEYYKAFYKRKYEQTFL WGIPFATVLTLLINQYHTTNIQNDVKLELSSTKSPISTDEVTKHNSENDCWIVINGQVYD LTSFMSIHPGGSDIIKLNAGKDVSAIFNPLHAPNAIERFLPPECYLGPLQGTMPKELICD PYTPGESKEDIARKLKLRNKLPPLESIINLYDFEMIASKILSNQAWAYYSSGSDDEISYR ENHSAYNRIFFKPKVLVDVSQVDLKTEMLGSIVDVPFYATATALCKLGNPEGGEMDIAR GCGQGDTKVVQMISTLASCSVDEVVNAAPSKDQIQWYQVYVNSDRNITKEMIKHVEEL GVKALFVTVDAPYMGTREKDLKIKFSNSTQGAKIMKTRMDAVVNNDKEEEETADVTAG ASRTLSKFIDPSLTWNDIRELKKWTKLPVVIKGVQRSEDVIKAAELGVEGVVLSNHGGR QLDYSRPPIEVLAETVPILKERNLEGKLELFIDGGVRRGTDVIKALCLGAKGVGLGRPFL YSNSCYGKDGVERAIDILKKEIEMSMKLLGVTSIKELTPELLDTSSLKIRSANLPHDILFD NLYQNPTYIKFLDGE
13	MSAMCGRLKPLAQASLRASKRAFNTKSPLRGVQLRPSRCAQRQAHVPFRRTAAVAT AVVAAAAAAAAAGSAAGMSLWGSPIANGTTKDMSKPAISADDVAKHNTDKSIWVVIDG YVYDLTEFVPVHPGGAAVIKANAGKDVTKLFQPIHPPDAIEKFIDPSKRLGPLKDPMPS ELVVGPFTPGETAEDVQRKTELRSRLPPLDSLTNLYDFENLASQILTKQAWAYYSSGS DDEISLRENHSAYHRIFFKPKVLVNVSKVDTSTELLGEKVDVPFYASATALCKLGNPAE GEKDVARGCGMGPKTAPQMISTMASCSLEEICKAANKDQLQWFQLYVNPDHKVMEN MIHDAEDMGCKAIFVTVDAPGFGNREKDAKIKFTSDISGPRTMRKGKEGGGQSKGAA QFMSKFVDASLSWEELAEIKKKTSLPLVVKGVQRVEDVIKAAEMGCSGVVLSNHGGR QLDFSRAPIEVLAEVSPILKEKNYPNFEVFVDGGIRRGTDVIKALCLGAKGVGLGRPILY ANSTYGKEGVQKAIQLLNAEVEMSMRLLGVNSIKELGPELLDTTNIKARCTQVPRDFLY DKTYVSGNLADFLPPSDGGDDAAAGGDDS
14	MSAMCGRLKPLAQASLRASKRAFNTKSPLRGVQLRPSRCAQRQAHVPFRRTAAVAT AVVAAAAAAAAAGSAAGMSLWGSPIANGTTKDMSKPAISADEVAKHNTDKSIWVVIDG YVYDLTEFVPVHPGGAAVIKANAGKDVTKLFQPIHPPDAIEKFIDPSKRLGPLKDPMPS ELVVGPFTPGETAEDVQRKTELRSRLPPLDSLTNLYDFENLASQILTKQAWAYYSSGS DDEISLRENHSAYHRIFFKPKVLVNVSKVDTSTELLGEKVDVPFYASATALCKLGNPAE GEKDVARGCGMGPKTAPQMISTMASCSLEEICKAANKDQLQWFQLYVNPDHKVMEN MIHDAEDMGCKAIFVTVDAPGFGNREKDAKIKFTSDISGPRTMRKGKEGGGQSKGAA QFMSKFVDASLSWEELAEIKKKTSLPLVVKGVQRVEDVIKAAEMGCSGVVLSNHGGR QLDFSRAPIEVLAEVSPILKEKNYPNFEVFVDGGIRRGTDVIKALCLGAKGVGLGRPILY ANSTYGKEGVQKAIQLLNAEVEMSMRLLGVNSIKELGPELLDTTNIKARCTQVPRDFLY DKTYVSGNLADFLPPSDGGDDAAAGGDDS
15	MLFRSQSRLMRLSTRLSLKFERQSVRNFGSSAYARSIGGQRRMRILAGAIALGTTAFSI NYWADASKIKNNELRDSAKAPVSPFEVVKHDKENDCWVVINQYVYDLTEFIPMHPGG QAIIKANAGKDVSAIFGPLHAPDVIEKYIAEEKRIGPLEGHMQPNHVCTPLTPGETADDV ARKEELRGRLPDLDTLLNLYDFEYLASQILTKQAWGYYSSAAEDEFTHRENHAAYHRIF FKPRVLVNVKEIDTTTEMLGHKVSVPFYVSATALVKLGNPEEGEKDIARGCGQGELKC PQMISTFASCSLKEIIEAAPSKDQVQWLQLYVNTDRKATEKLIKEAENLGVKGIFVTVDA PSLGRREKDMKVKFQSPSSPANSKKPRTGSSQGASRALSTFIDPSLNWDDIAELKRKT NLPIIIKGVQRVEDVLKAAEVGVDGVVLSNHGGRQLDFARPPLEILAETMPALKERNLE DKFEIFIDGGVRRGTDVLKALCLGAKGVGLGRPALYANSTYGKDGVQKMIEMLTDEIEY DMRLLGVTSIKDLGPDLIDVTSLSNRSVEFARDNLYNAVYEKPEHVEFNPVPE
16	MYNKRFVLNYARFRTFPHRSYSRQYTVNNTKVKLPGNYFYYGILGLFLISASNFACDR YYRNDSMLLKNSKVITPEEVARHNKRDDCWVVINDSVYNLTDFIDSHPGGKNVIVANS GKDVTKLFEPIHAPNVLEKYLQPKMRLGTLSKPMPPELVCDVYAPGETAEELNSKIKLR ASLPALNEISNIYDFEYLASKILSKQAWAYYSSGSDDEISLRENHNAYHRIFFNPRVLVD VSEIDTSTTIFGKKQDVPFYASATALCKLGNPLEGEKDITRGCGQGPTKIPQMVSTLAS CSPSEISSSKIDNNQSLWFQLYLNHDRNLTNLLVKEVEKLGYTAIFVTVDAPTFGKREK DAKLKFLKDQEGSAKIMKDKPSSDEEAGASRALSKFIDPSVTWNDIAELKKLTTLPIIIKG VQRKEDILKAAEIGCQGVVISNHGGRQLDFAKAPIEVLAESMDILKERKLDKTFNVFIDG GIRRGTDILKALCLGAKGIGLGRPFLYANSCYGKDGVQKTIDILKYELEMSMRLLGVTSI DELDESFLDLSSIKNRTVLVPKDTLSEMVYNPSKLIEILPKE

17	MLKIKPLLRISRVPEAATLRTSRTRLNTARSYSSAVGKSKSFQQHSGKRTQSWTTLSV GTILAAAGSMAYLNWSNDQIGNDPKLDMNKQKISPAEVAKHNSADDCWVVINGYVYD LTRFIPNHPGGSDVIKYNAGRDVTAIFEPLHAPNVIDKYIAPEKKLGPLQGSMPPELVCP PYAPGETKEDIARKEQLKSLLPPLNNIINLYDFEYLASQTLTKQAWAYYSSGANDEVSH RENHNAYHRIFFKPKILVDVSKVDLSTDMLGSHVDVPFYVSATALCKLGNPLEGEKDIA RGCGQGITKVPQMISTLASCSPEEIISAAPSDEQIQWYQLYVNSDRKITDDLVKNVEKL GVKALFVTVDAPSLGQREKDMKLKFSNSKAGPKAMKKTDVEESKGASRALSKFIDPSL TWKDIEELKSKTNLPIVIKGVQRTEDVIKAAEVGVSGVVLSNHGGRQLDFSRAPIEVLAE AMPALEERKLKDKLEVYIDGGVRRGTDILKALCLGAKGVGLGRPFLYANSCYGRDGVE KAIEILRDEVEMSMRLLGVASIAELKPELLDLSTLKARTVAVPNDVLYNEVYESPTLTDF NDA
18	MFKYKPLLRMSRSSEAAMLRTSKTRLNTVRSYRSAVTKSKSFEQSSRKRTQSWTALSI GAILATAGSMVCLNWYNGQIDNEPKLDMNKQKISPAEVAKHNKADDCWVVINGYVYD LTRFMPNHPGGPDVIKFNAGRDVTAIFEPLHAPNVIDKYIAPEKKLGPLQGSMPPELVC PPYAPGETKEDIARKEQLKSLLPPLSSIINLYDFEYLASQILTKQAWAYYSSGANDEVTH RENHNAYHRIFFKPKILVDVSKVDVSTDMLGSRVDVPFYVSATALCKLGNPLEGEKDIA RGCGQGLTKVPQMISTLASCSPEEIIGAAPSNRQIQWYQLYVNSDRKITDDLVKNVEKL GVKALFVTVDAPSLGQREKDMKLKFSNSKAGPKAMKKTNVEESQGASRALSKFIDPTL TWKDIEELKSKTKLPIVIKGVQRTEDVIKAAEIGVSGVVLSNHGGRQLDFSRAPIEVLAE TMPILEKRNLKGKLEVYVDGGVRRGTDILKALCLGAKGVGLGRPFLYANSCYGRDGVE KAIEILRDEVEMSMRLLGVNSIAELKPDLLDLSTLKARSVAVPNDVLYNEVYEGPTLTEF EDA
19	MLKYKPLLKISKNCEAAILRASKTRLNTIRAYGSTVPKSKSFEQDSRKRTQSWTALRVG AILAATSSVAYLNWHNGQIDNEPKLDMNKQKISPAEVAKHNKPDDCWVVINGYVYDLT RFLPNHPGGQDVIKFNAGKDVTAIFEPLHAPNVIDKYIAPEKKLGPLQGSMPPELVCPP YAPGETKEDIARKEQLKSLLPPLDNIINLYDFEYLASQTLTKQAWAYYSSGANDEVTHR ENHNAYHRIFFKPRILVDVRKVDISTDMLGSHVDVPFYVSATALCKLGNPLEGEKDVAR GCGQGVTKVPQMISTLASCSPEEIIEAAPSDKQIQWYQLYVNSDRKITDDLVKNVEKLG VKALFVTVDAPSLGQREKDMKLKFSNTKAGPKAMKKTNVEESQGASRALSKFIDPSLT WKDIEELKKKTKLPIVIKGVQRTEDVIKAAEIGVSGVVLSNHGGRQLDFSRAPIEVLAET MPILEQRNLKDKLEVFVDGGVRRGTDVLKALCLGAKGVGLGRPFLYANSCYGRNGVE KAIEILRDEIEMSMRLLGVTSIAELKPDLLDLSTLKARTVGVPNDVLYNEVYEGPTLTEFE DA
20	MLKYKPLLKISRNCGAAILRSSKIRLNTIRAYGSVVSESKSFEQGSRKRTQSWTALSVG TILAAASSMAYLNWCNGQIDNEPKLDMNKQKISPAEVAKHNKPDDCWVVINGYVYDLT RFLPNHPGGQDVIKFNAGKDVTAIFEPLHAPNVIDKYIAPEKKLGPLQGSMPPELVCPP YAPGETKEDIARKEQLKSLLPPLDNIINLYDFEYLASQILTKQAWAYYSSGANDEVTHRE NHNAYHRIFFKPKILVDVRKVDISTDMLGSHVDVPFYVSATALCKLGNPLEGEKDVARG CGQGVTKVPQMISTLASCSPEEIIGAAPSDKQIQWYQLYVNSDRKITDDLVKNVEKLGV KALFVTVDAPSLGQREKDMKLKFSNSKAGPKAMKKTDVEESQGASRALSKFIDPSLT WKDIEELKSKTKLPIVIKGVQRTEDVIKAAEIGVSGVVLSNHGGRQLDFSRAPIEVLAET MPILEKRNLKDKLEVFVDGGVRRGTDVLKALCLGAKGVGLGRPFLYANSCYGRNGVE KAIEILRDEIEMSMRLLGVTSIAELKPDLLDLSTLKARTVGVPNDILYNEVYEGPTLTEFQ DA
21	MIRNNITALKVVRQPLKFKNLSRCISTSQISLKNKGYKNYQYGKNYKTTTIVASSALSTT LLAYLYTNNNINNISNDVSIEQLSKKSLKIITPQEVIKHNSKDDCWVVINDIVYDLTFFIKK HPGGQDVIVGNAGKDVSNIFLPLHPLDTIEKFIPKDKIIGVINEPMPADYICPKYAPGETP EEIAEKERLKTLLPSIGAISNLYDFEYLASHILSKQAWAYYSSAADDEVSLRENHSAYHR IFFKPKVLVDVSEIDLSTEFFGQKSDAPFYATAAALGKLGNPAEGEKDITRGVGQGSTK VPQMVSTLASCSIDEVMGARVSENQPIWFQLYVNSDRKITNDLVKHVEELGVKALFVT VDAPALGHREKDEKVKFSANQKESTNMLKEAKVDADADGASRALSKFIDPSLSWKDII ELKKLTKLPIIIKGVQRSEDVIKAAEIGCQGVVISNHGGRQLDFSRAPIEVLAESKPELEK RNLDKNFDIFIDGGVRRGTDILKALCLGAKGVGLGRPFIYANSCYGAAGVQRAVDILRE ELEMSMRLLGVTSVKDLNEDLLDLSALKYRSVSVPHDELSQSVYKHLAFTEFRPDDAQ

22	MQRYIFKTPHSRVLRSLPRHAINSTTKRGIVSTRSSLNNQFFNSERQRKSYKWFWSIG LTVGLTAAIADNLHNKKDIYNDAKFDSSKPKISPSEVIKHNTPEDCWVVIDGYVYDLTNF IALHPGGPDIIKTNAGKDVTAIFDPIHPPDAIEKYIKPEQHIGPLDGKLDAEYICPPYAPGE TPDDIARKAALRARLPPLSSIMNLYDFEYLASQILSKQAWAYYSSASDDEVSYRENHNA YHRIFFNPKVLVDVSKVDTSTEMLGHKVDVPFYVTATALCKLGNPKEGEKDIARGCGQ GPNKTPQMISTLASCSVDEIVNAAPSKDQVIWYQLYVNSDRKITENLIKHVEDLGVKAIF VTVDAPSLGSREKDKKVKFNNTMSGPKSMKKSDVGESEGAAQTLSKFIDPSLSWQDI KILRKKTKLPIVIKGVQRVQDVVKAAEIGCNGVVLSNHGGRQLDFARAPIEVLAETMPVL KEKKLDKNFEVFVDGGVRRGTDVIKALCLGASGVGLGRPFLYANSCYGKDETKNISEF P
23	MQRYIFKTPHSRVLRSLPRHAINSTTKRGIVSSRSSLNNQFFNSERQRKSYKWFWSIG LTVGLTAAIADNLHNKKDIYNDAKFDSSKPKISPSEVIKHNTPEDCWVVIDGYVYDLTNF IALHPGGPDIIKTNAGKDVTAIFDPIHPPDAIEKYIKPEQHVGPLDGKLDAEYICPPYAPG ETPDDIARKAALRARLPPLSSIMNLYDFEYLASQILSKQAWAYYSSASDDEVSYRENHN AYHRIFFNPKVLVDVSKVDTSTEMLGHKVDVPFYVTATALCKLGNPKEGEKDIARGCG QGPNKTPQMISTLASCSVDEIVNAAPSKDQVIWYQLYVNSDRKITENLIKHVEDLGVKAI FVTVDAPSLGSREKDKKVKFNNTMSGPKSMKKSDVGESEGAAQTLSKFIDPSLSWQD IKILRKKTKLPIVIKGVQRVQDVVKAAEIGCNGVVLSNHGGRQLDFARAPIEVLAETMPV LKEKKLDKNFEVFVDGGVRRGTDVIKALCLGASGVGLGRPFLYANSCYGKDGVQKAID LLKTEIEMNMRLLGVTSIKDMNPELLDLSSLHGRTVNVPKDSLYVNVYNKPELAEFLDD ASD
24	MRSAVRVFGKNRVSGLRFASTSSKSPIHKSSATPRFGRSAKSDYSEFSFGKKVTTGG AFLVAGSVGIALISHFNDSVENGAKVDMTKPKVSPTEVAKHSSPKDCWVVIEGYVYNL TDFISAHPGGPAIIENNAGKDVTKIFAPIHAPDVIEKYIAPENRIGPLDGTMPDDLVCAAL TPGETPEDVARKEQLRQSLPDIDSLVNIYDFEFLASQILTKQAWSYYSSAADDEVTHRE NHAAYHRIFFKPRILVNVKEVDTSTTMLGEKVGVPFYVSATALCKLGNPKEGEKDIARG CGESDVKPVQMISTLASCSLQEIVEAAPSKEQIQWFQLYVNSDRKITEDLIKNVEKLGL KAIFVTVDAPSLGNREKDAKVKFTNSNGAKAMEKSKVKESKGASRALSSFIDPALNWD DIIEFKKKTKLPIVIKGVQCVEDVLKAAEIGVAGVVLSNHGGRQLDFSRAPIEVLAETMP VLREKKLDDKIEIFIDGGVRRGTDILKALCLGAKGVGLGRPFLYSNSCYGKEGVKKAIEL LKDELEMSMRLLGVTSIDQLSEKYLDLSTIHGRTVNVPRDNLYREVYVPHEPTQFLEE
25	MFRQSVLHPAFKRAFDRGRWRALSHSAKQVGARHVTRPSGNIALLTSFSVITYALCSN REKSFIKNDLVRDLKRSSICASEVLLHNKEDDCWVVIDDFVYDLTNFINQHPGGPAVIKF NAGKDVTALFSALHAPDVIEKFIPDSDRLGPLEGHMPSEHVCPPPATGESPEDIQRKE ELRHALPDLDSLMNLYDFENLASQILSNMAWAYYSSAADDEFTHRENHAIYHRVFFKP RVLVDVTEVDLSTEMLGSKVSVPFYASATALVKLGNPKEGEKDIARGCGQGEYKCAQ MISTFASCSLREIVEAAPSKEQTQWLQLYVNTNRSATENLLKEAESLGLKAIFVTVDTP ASGRREKDMKIKFSSKNAAPNAKSAKGGSARGASQALAGFIDPSLTWEDITDLKEKTH LPIVVKGVQCVEDILKAVEIGVDGVVISNHGGRQLDFARAPLEVLAEAMPILREQRLDD KLEVFIDGGVRRGTDILKALCLGAKGVGIGRPFLYANSCYGKDGVEKAINMLAEEVECS MRLLGAKTIKDLRPELLDLSGVKNRYIELPGDSLYQAVYQKPEPARFVER
26	MLRPQLIKSILGSSRLSSKGLSHSSLRLFAGSNKRLLSTLKKTTKSKTYSQNSLIALGISI TAIASIYSYNQSQNYLSADVPKWSDIELTPEIVSQHNKKDDCWVVIKGQVYDLTDFLTS HPGGQKVILRYAGKDATKIFVPIHPPDSIDKFIPPEKHLGPLIGEFEEEVEELSEEEIDRL ERIERKPPLSQMINLHDFETIARQILPPPALAYYCSAADDEVTLRENHNAYHRIFFNPKIL VDVKNIDLTTEFFGDKTTAPFYISATALAKLGNPEGEVDIARGAGREGIHQMISTLASCS FDEIADARVEGQNQWYQLYVNADRSITEKAVRHAEERGMKGLFITVDAPSLGRREKD MKMKFEADSSVQSDDDEVDRSQGAARAISSFIDPSLSWKDIGFIQSITKMPIVIKGVQR KEDVFLAIEHGLQGVVLSNHGGRQLDYTRAPVEVLAEVMPELRAKGLDKKIEIYIDGGV RRGTDVLKALCLGAKGVGLGRPFLYANSSYGDKGVQRAIQLLKDELEMNMRLLGVTKI EDLGPELLDTRNIYNRSVPVAKDYLYEQNYGRMAGAAFRPGIED

27	MRSAVRVFRKNRFPTGLRLSSTASSARQGIALSVGKYNKYNKNGTNGSWKSSRKSTT YGGAFLVASAVAIAFSSQITQSLENGVKVDMGKPSVSPAEVVKHSSPDDCWVVIDGYV YNLTEFIGAHPGGPAIIKNNAGKDVTKIFAPIHAPDVIEKYIAPKNRLGPLEGTMPEDRIC AALTPGETAEDVARKEQLRQLLPDVNNLTNLYDFEFLASQILTKQAWSYYSSAADDEV TQRENHAAYHRIFFKPRILVNVKEVDTSTTMLGEKVGVPFYVSATALCKLGNPQEGEK DIARGCGESDAKPVQMISTLASCSLQEIVEAAPSKEQIQWFQLYVNSDRKITDDLVKNA EKLGLKAIFVTVDAPSLGNREKDAKVKFTNSNGAKAMEKSNVDESKGASRALSSFIDP ALNWDDIVELKTKTKLPIVIKGVQCVEDVLKAAEVGAAGVVLSNHGGRQLDFSRAPIEV LAETMPALREKKLDDKLEVFIDGGVRRGTDILKALCLGAKGVGLGRPFLYSNSCYGKE GVKKAIELLKDELEMSMRLLGVTSIDQLSEKYLDLSTIHGRTVSVPRDNIYRDVYVAQE PSQFRED
28	MRSAARVINKSCSGSALSRRCLRKSSLSMSMRYLSTSNIGVRKGFNGQGKSSNKTML FLAAGASAVAGIGLLSQFSDSLQNATKEELNKPKVSPLEVAKHSSPDDCWVVIDGFVY NLTEFISAHPGGPAIIENNAGKDVTAIFGPIHAPDVIEKYIAPENRIGPLDGKMPDDLICAP LTPGETPEDVARKEELRQNMPDLDSLVNIYDFEFLASQILTKQAWSYYSSAADDEVTH RENHAAYHRIFFKPRILVNVKEVDTSTTMLGEKVGVPFYVSATALCKLGNPKEGEKDIA RGCGESDVKPIQMISTLASCSLQEIVEAAPSKDQIQWFQLYVNSDRKITEELIKNVEKLG LKAIFVTVDAPSLGNREKDAKVKFTNKDSSAKAMEKSNVKESKGASRALSTFIDPALC WDDIVTLKSKTKLPIVIKGVQCVEDVLKAAEIGAAGVVLSNHGGRQLDFSRAPIEVLAET MPILKEKKLDDKIEIFIDGGVRRGTDILKALCLGAKGVGLGRPFLYANSCYGKEGVKKAI ELLKDELEMSMRLLGVTSIDQLSEKYLDLSTLHGRTVSVPRDNLYNGVYVPHEPTDFK EN
29	MLSSRIPGVASKRLLQLARQQQGNAVVGAAVRSRLRASSYSTQSRSTRRNGTRLVTG AAAVATVLLVSQLYQIENESAQQQPLLPGAPTKSISPSEVAKHNTPDDCWVVIEGYVY NLTEFIPTHPGGPMVIKSNAGKDVTAIFKPLHAKGTIEKFVKKEWFIGPLSEPMPAELVC PEYTPGETREEIVLKERLRESLPPLANIVNLYDFEKLASKILSNQAWAYYSSGSDDEISL RENHNAYHRIFFKPKVLVDVSKVDTRTKMLGSQTDVPFYVTATALMKLGNPQGGEMDI AKGCGATDVRVPQMISTLASCSIDEIADAKVHDDQIQWYQLYVNSDRKVTKELIQHVEA LGLKALFVTVDAPSLGHREKDLKIKFSTMQSGPELMQSKPEHKDAGAEKGASRALSKF IDPALSWNDIVEFKKHTKLPIVIKGVQRAEDVVKAAELGLAGVVISNHGGRQLDFSRSP VEVLAESVPELEKRGLRSDNFQLFIDGGVRRGTDILKALCLGANGVGLGRPFIYANSCY GKEGVAKAISLLTDELEMSMRLLGVTSIDQLGPELLDLSALRNRNVSVAHDNLYNSVYK SPTLAQFIDEDGDLDDI
30	MHLLDMLFRWKPVWQLSKVTVRSLRADAGFNGKRSLSGKTRDNKVWFHFPMSKNR RMAIALLIAAITAASTVEPFGGHLDNGTKVDMSKPAINPEEVAKHNSPGDCWVVIDGYV YDLSEFAPVHPGGPTIIKTNAGKDVSAIFDPLHAPDAIEKFIDPSKRLGPLDGSMPEELV CAPFSPGETAEDVKRKLELRARLPPLEAITNLYDFEFLASQILTKQAWAYYSSGADDEI SMRENHSAYHRIFFKPKVLINVAKVDTKTEMLGVPVDLPFYVSATALCKLGNPAEGEK DIARGCGSGEKKIPQMVSTLASCSLEEVVDAGKEDQVRWFQLYVNEDRGVVDQLISN AEKLGFKGIFVTVDAPGLGNREKDSKVKFSGSSSGPNTVKKEDRDGGQENGESSGAS KFLSKFIDPSLDWDDLVEMKKKTKLPIVVKGVQRVEDIVKAAEVGANGVVLSNHGGRQ LDFSRSPIEVLAEAHPVLEERNFKDFDVFVDGGIRRGTDVVKALCLGAKGVGLGRPFL YANSAYGKEGVQKAIDILNFEIEMTMRFLGVTSIKQLGPEFLDTSNIKSRSVQVPKDYLY DKAYVSSDLINFAPPLEDEGGTTTPEDG
31	MQALGRRSIAFRACKQFKFTKISKRALSTSNFTKSRNYYNNNSKNLRNSLLLITSASAL SYYAWNQLNNQSSSDYISNDNGQPSKKKPISPDEVAKHNKPDDCWVVINGYVYDVTF FIPNHPGGEDVIKANAGKDVTAMFAPLHAKGTLEKNIPEDKKLGPLSKPMPKKLVCPPY APGESPYEIMTKQKLRDNMPPLGNVLNLYDFERLASKILTNQAWAYYSSGADDEITYR ENHNAYHRIFFKPHILVDVEHVNLKTTMLGEKTDVPFYVSATALCKLGNPEGGEVDIAK GCGSTSYSVPQMISTLASCSLDEVAEAKVNDKQLQWFQLYVNSDRKITRNLIKHAEEL GLKAIFVTVDAPSLGNREKDQKIKFTTQSSGGPKILQKKEDEKKEKNSDGASKALSKFI DPSLSWEDIAKLREITKLPIVIKGVQRAEDAVRAAQMGCQGVVLSNHGGRQLDFSRAPI EVLAETMPILKHHGLDKNFDVFVDGGIRRGTDILKALCLGATGVGLGRPFLYANSCYG QQGVAHAIDIITKELEMSMRLLGVSKIEDLNPGFLDLTSLHSRSVSVARDSLYENSYKE PQLAKFLIDDDD

32	MLGVSRQCEVTGRKLFSGKVLSKNFKYLSNRKYSLATTKNGAKYESKKFNKAQLMAIA GAGLIATTTLLYSNFNSEIQNESKLAPKRVIDPSEVARHNTPADCWIVINGVVYDLTSFIP VHPGGADIIKSNAGKDVTAIFEPIHAPGVIEKYLPPKCRIGTLKKPMPAELTCDPYTPGE SKDDIIKKLELRNNLPHLDSIINIYDFEKLASKILSNQAWAYYSSGADDEISYRDNHSAYR RIFFKPRILRDVSSVDVKTTMLGSKVDVPFYVSATALCKLGNPKEGEKDIARGCGQGD TKVPQMISTLASCSVDEIVDAAPSKDQIAWYQVYVNSDRNITRDMIKHVEKLGIKALFIT VDAPSLGRREKDMKIKFSGSDQGAKVMKEPLKKVEKKDDGEMSKGASTTLSKFIDPSL TWDDVVKMRKWTKLPIVIKGVQSVEDVVKAAELGVDGVVLSNHGGRQLDYSRPPIEV LAETVPVLKEKHLDGKLELFVDGGVRRGTDVIKALCLGAKGVGLGRPFLYSNSCYGKD GVEKTIELLKTEIEMSMRLLGVTSIDQLTPELLDTTNLKSRTVNVSKDVLFDTVYRNPTL AAFQNGYDDDE
33	MLKQSQKILIKSFNYSNKRIINNSKIGFKNQLFRNYTTNSIKNSINNNNNNANKFNTFLSII TILGIGLTISSSSIISNDSSSSKKNAKKLISVEEFVKHNKPDDCWVVIHGNVYDMTDFIPK HPGGRAPITINAGKDVTDIFTPIHPPGVIEQYLPKDKFLGKIDGDAPKPEVVLDDDEIERL DNVDNKPSLNKIFNLHDFEYVAKSILPKNAWAYYSSGSDDEIIMRENHYAYQRIYFRPRI LEKVGSIDLSTEMLGIKTSVPFYITATALAKLGHPEGEVAIAKAAGKEDVIQMISTFSSCS LDECAEASIEGQSQWYQLYVNTERKVAFDLVKKAENLGMKGIFITVDAPCLGNREKDR KMKFTEDTSIGIGESAERDSGAAAALSSFIDPNLCWDDIKEIKSRSNIPIVIKGVQRVEDV LKAAEYGVNGVVLSNHGGRQLDFAPAPVQLLAEVMPILKRKNLDKNFDVFVDGGVRR GTDILKALCLGAKGVGIGRPILYSLSGYGEEGVRKAIHILKDELELDMRLLGAKNIGELN PNFVDTRNLIGRYAPDSLYGQVYTPLDFVKFKNE
34	MLGGIRSKFVRNLVTNGARASHRPQGRLLSGSKRYLATHRVAKDQKFVYPLVVGALG LGAVAASALHWGSVIDNGPVKDPTKKPIKPEEVSRHCTPEDCWVVIDGYVYDLTDFID VHPGGRAIIEANAGKDVSKVFDPLHAPDVIEKYIAPEKRLGPLDGQMPEDRITPALTPG ETPEDVAEKERLRQALPDLDSLINLYDFEYLASQILTKQAWCYYSSAAEDEFTHRENHA AYHRIFFKPRVLVDVRQVDTSTEMLGQKVDVPFYVSATALVKLGNPQEGEKDIARGCG EGSVKCPQMISTLASCSLQEIVEAAPSKEQIQWFQLYVNADRSITDKLISDVERLGLKAI FVTVDAPSLGRREKDMQVKFSSKSAPQRMKNTSDARGASRALSTFIDPSLTWDDIIEF KKKTKLPIVIKGIQRVEDVLKAAEIGVDGVVLSNHGGRQLDFARPPIEVLAETVPILKEKH LDDKLEIFIDGGVRRGTDILKALCLGAKGVGLGRPFLYANSCYGKAGVERAVQMLTEE VEYAMRLLGVNSIKELNPELLDLSSLGSRSVGFPEDFLYKGVYQKAQTAQFKDTD
35	MLFRSKPIWQLSRTTARSLRAGINSKRSLAGNARSNNVLSRFSVSRNRNWATALLVTA VAAASTVKLSAGPLDNGPKLDMSKPAISPDEVAKHNSPEDCWVVIDGYVYDLSEFAPV HPGGPVIIKTNAGKDVSAIFDPLHAPDAIEKFIDPSKRLGPLEGSMPEELVCAPFAPGET PDDVKRKTELRARLPPLEAITNLYDFEFLASQILTKQAWAYYSSGADDEITMRENHSAY HRIFFKPKVLVNVSKVDTKTEMLGVPVDLPFYVSATALCKLGNPAEGEKDIARGCGSG GKKIPQMVSTLASCSLEEVANVGKEDQIRWFQLYMNEDRGIVDQMVSNAEKLGYKGL FITVDAPGLGNREKDAKVKFSGSTSGPNASKKSEGKEKGKQNGESSGASRYLSKFIDP SFDWDDLLEMKKKTKLPIVIKGVQTVEDIVKAAESGISGVVISNHGGRQLDFSRSPIEVL AEAQPILKERNFENFDVFVDGGIRRGTDVIKALCLGAKGVGLGRPFLYANSCYGKEGV QKAIDILNFELEMSMRLLGVTSIKQLGPELLDTSSIKSRSVQVPKDYLYDKAYRSSDLIE FMPPPEDEASTGDE
36	MFKSQLRTATARSSFRSLASKLNPQRFNSSKTPLLNATRGSNRSKNSLIALAISLSAVS SSYYLYQKDKFISADVPHWKDIELTPEIVSQHNKKDDLWVVLNGQVYDLTDFLPNHPG GQKIIIRYAGKDATKIFVPIHPPDTIEKFIPPEKHLGPLVGEFEQEEEELSDEEIDRLERIE RKPPLSQMINLHDFETIARQILPPPALAYYCSAADDEVTLRENHNAYHRIFFNPKILIDVK DVDISTEFFGEKTSAPFYISATALAKLGHPEGEVAIAKGAGREDVVQMISTLASCSFDEI ADARIPGQQQWYQLYVNADRSITEKAVRHAEERGMKGLFITVDAPSLGRREKDMKMK FEADSDVQGDDEDIDRSQGASRALSSFIDPSLSWKDIAFIKSITKMPIVIKGVQRKEDVL LAAEHGLQGVVLSNHGGRQLDYTRAPVEVLAEVMPILKERGLDQKIDIFVDGGVRRGT DVLKALCLGAKGVGLGRPFLYAMSSYGDKGVTKAIQLLKDEIEMNMRLLGVNKIEELTP ELLDTRSIHNRAVPVAKDYLYEQNYQRMSGAEFRPGIED

37	MFHSTSLRVSRAIRASKVIHSSPLRGPRSKVRQLTTASIKSAHNNKLLPALGFLGASASI AALLLNNTEPFIRLDAPTQGPLYTPDDVSAHNTKDDLWVVLNGKVYDLTEFLPNHPGG QRIILKYAGKDASKIFNPIHPPDAIDKYLPPEKCIGTLDGEFPEEEEVLSDEELLRLDRVD RKPPLSKIMNLHDFEAVARQILPPGALAYYSSAADDEITLRENHNAYHRIFFNPKVLVDV SKVDITTEFLGNETSAPFYITATALAKLGHPDGEVSIARGAGKEDITQMISTLASCSFDQI ADARVKDTQAQWYQLYVSEDRELSKKQLKHCEERGMKGIFITVDAPSLGRREKDMKL KFEGNADVQSDDDIDRSQGAAVALSSFIDSSLTWKDITEIRKWTKLPIVIKGVQRKEDVL LALEHGCDGVVLSNHGGRQLDFSRAPVEVLAEVMPILREKGVDKKFEVYVDGGVRRG TDVLKALCLGAKGVGLGRPFLYANSAYGEKGVRRAIQLLKSELIMNMRLLGVTKIEDLT PELLDTTTIHQRTVSVPADYLYKENYLHMQGVEFNPELEE
38	MFNSRYVFQLSRLTLRKRPFDGISVRRMSTRHFVSNSKIPRFQTRNLLFYTTLTAASLS SFYLYSDIDGPCTISNEAITTRLILPEEVAKHKTPDDCWVVINGYVYDITSFIMSHPGGPD VLETNAGKDVTAIFVPLHAPGVLEKYLPRELKLGKLQGSMPEELVCPPYAPGETKEDIM RKENLRANLPPLESILNLYDFERLASLILSNQAWAYYSSAADDEITLRENHLAYHRLFFK PRVLVDVSNIDLKTEMLGEPTEVPFYVTATALCKLGNPNEGEKDIARGCGLAHKLVPQ MLSTLASCSPDEVAEAKVKSEQLQWYQLYINSDRKITRNLVKHVEELGYKAIFVTVDAP LMGSREKDLKIKFSTTKQGPKIMKETEKLEDTEKVTHGASQALSEFIDPSITWKDIKELQ KITKLPIVIKGIQRREDVIKAAEVGCSGVVLSNHGGRQLDFSRAPIEVLAETMPELKKRG LDQHFDVFIDGGIRRGTDIIKALCLGAKGVGLGRPFLYANSCYGKEGVAKAIELLTKEM ETSMKLLGAQSVKDLNESFLDLSALHQKSVNVPIDSLYNDVYKAPTTIEVE
39	MEPKLDMNKQKISPAEVAKHNKPDDCWVVINGYVYDLTRFLPNHPGGQDVIKFNAGK DVTAIFEPLHAPNVIDKYIAPEKKLGPLQGSMPPELVCPPYAPGETKEDIARKEQLKSLL PPLDNIINLYDFEYLASQTLTKQAWAYYSSGANDEVTHRENHNAYHRIFFKPKILVDVR KVDISTDMLGSHVDVPFYVSATALCKLGNPLEGEKDVARGCGQGVTKVPQMISTLASC SPEEIIEAAPSDKQIQWYQLYVNSDRKITDDLVKNVEKLGVKALFVTVDAPSLGQREKD MKLKFSNTKAGPKAMKKTNVEESQGASRALSKFIDPSLTWKDIEELKKKTKLPIVIKGV QRTEDVIKAAEIGVSGVVLSNHGGRQLDFSRAPIEVLAETMPILEQRNLKDKLEVFVDG GVRRGTDVLKALCLGAKGVGLGRPFLYANSCYGRNGVEKAIEILRDEIEMSMRLLGVT SIAELKPDLLDLSTLKARTVGVPNDVLYNEVYEGPTLTEFEDA
40	MFRSAAQRLRSVGKNGRLTRLVHTTRAQAKMSIRNATIIGFGAGAITSAYYLSQLQQQ SPILLESPSVIGELTPEVVSKHNKRDDLWIVLNGKVYDLTDFLPNHPGGQKVLLKLAGK DASKIFNPIHPPDAIEKFLPPEKYLGPLKPPLSQIINLHDFETVARQILPPAALAYYSSAAD DEVSLRENHNAYHRIFFNPKILVDVKEVDITTEFFGNKTSAPFYASATALAKLGHPDGEL SIARGCGKEDIVQMISTLASCSFDEIADARVKDDQQQWFQLYVNSDRKLMEKQVKHAE ERGMKGLFITVDAPSLGRREKDMKMKFDDDADVLEDSDDNIDRSQGASRALSSFIDPS LSWKDIGHIRSLTKMPIVIKGVQRKEDVLLAVDHGCQGVVLSNHGGRQLDFSRAPVEV LAEVMPELRAKGLDKNFEVYVDGGIRRGTDVLKALCLGAKGVGLGRVFLYANSAYGE NGVRKACQLLKDELEMNMRLLGVTKIEDLKPELLDLRSLPNRTVAVAQDNLYSNNYQP MTSVEFKPEQD
41	MRSPFSYTAIFTVFATLTYTMGQYRDATFIKNDAAFDLKRQGVSASEVIRHNRKDDCW VVIDGYVYDVTAFINQHPGGSAVIRGNAGKDVSAIFSALHPPDVIQKYIHESQRLGPLK GQMPSDLICPPPPTGETPDDIQRKEELRQALPDLDSLMNLYDFEYIASQILSNQAWAYY SSASDDEFTYRENHAAYHRIFFKPRVLVNVKDVDISTEMLGFKVSVPFYVSATALMKLG NPAEGEKDIARGCGQGKHKCPQMISTFASCSLKEIVEAAPSKEQLQWLQLYVSTDRSA TESLLREAEDLGLKAIFLTVDTPVSGRREKDMKLKFSSSNAAHNAEPAKARSSRGASQ TLASFIDPTLTWEEVAELKTKTNLPVVIKGVQCVEDVLKAAEIGVDGVVISNHGGRQLD FSRAPLEVLEETMPVLRRNNLDDKLEVFLDGGVRRGTDILKALCLGAKGVGLGRPFLY ANSCYGKEGVEKAIDMLAEELQCSMKLLGAKSIKELGPELLDTTGVKNRFIESPGDSLY NAVYQKPDFAHFVEGHKP

42	MFRSNFIRNSRSAFKQFSKRSYSWRSVSTSPRLAKHATSALAIGLSSAIVLSVTSSIISN DIKYEDKLFPEPKLIPVDEFLKHNKPDDCWVVLNGWVYDMTEFKEIHPGGRNVIIRNAG KDATKIFTPIHPPDAIEKFLPAEKIIGKLDGIIEQEEEEDPDELERQERISNIPSLNQVYNIA DFENIAKQILPKGAWAYYSSGADDEITLRENHYAYHRVFFRPRILVDVSEIDLSTTMLG QKTSAPFYISATALARLGHPDGEVAIAKAAGREDIIQMISTLASASLDEITEVATEDQKQ WFQLYVNADRNIAYKMVANAESKKSIKGIFITVDAPSLGNREKDKKIKFEGDTDVDLED NADRASGASRALSSFIDTALTWKDIDVIKQKTKLPIVIKGIQRAEDALKAAEHGVDAIVLS NHGGRQLDFAPAPLQLLSDIMPVLREKKLDGKMEVFVDGGVRRGTDVLKALCLGATG VGIGRPVLYSMSAYGEAGVTKAIQLLKDELEMDMRLLGVTKISDLGPEFVDTRGLIGVH AKDMLYDNLYAPLEPVKFKDE
43	MFARSTRTVAQVVRKAPSRSLLARRGISGLHIQRNGLPKNRHFITGAVALASLSTLLWI SDRPTIDNGTVKDSTKKSIDPKEVEKHNAPDDCWVVIDGYVYDLSDFIPSHPGGPAVIR ANAGKDVSAIFDPLHAPDVIEKYIAPEKRLGPLEGQMREELICPPFAPGDSVDEMAEKQ QLRAELPPLEHISNIYDFEYLASQILSKQAWAYYSSGSDDEVTMRENHSAYHRIFFKPK VLVDVANVDTSTEFLGLPVDVPFYVSATALCKLGNPKEGEKDIARGCGSGPKKVVQMI STLASCSLKEIIEAAPSKDQIQWFQLYVNSDRKACDELLAEAEKLGAKAIFVTVDAPSLG NREKDAKVKFSADKSGPEAMERTKERKQKAKEVEEQGASRALSKFIDPSLSWNDVVE MKKKTKLPIVIKGVQRAEDVLKAAELGIDGVVLSNHGGRQLDFSRSPVEVLAEVAPMIK EKNLDKDFQIFVDGGIRRGTDILKALCLGAKGVGLGRPFLYANSTYGKEGVEKLIERLS FELEMDMRLLGVNSVKELGPELLDLTALKSRSVEVPKDYLYNQAYKKASFTDFET
44	MSGRLMLQGYRSSIKATVFAARNLRPLQASRKLSSILPKVGSEKSQSSRKILIKGGLVIG IAVAATTATIWSQSLASPISNDDERKVTPEELAKHNSGTDCWVAINGKVYDLTEFLPQH PGGRNVILKRAGKDASKVFNPIHPPDAISKFLPADKFVGVLDGELPEEEEVLTDAEIER QERIANKPPLSSMFNVYDFEYVAQNILDEAAWAYYSSAADDEITLRENHFAYHKVFFR PRILVDVTNIELETEMLGIKTSAPFYISATALAKLGHPEGEVGIAKGAGRGDIIQMISTLAS CSLDETVAAAKEGQSQWFQLYVNSDREVAYNMIKHCEELGIKGIFVTVDAPSLGNREK DRRMKFTEDTDVDLSGDGKTEVNRSNGAAAALSSFIDTAVTWKDIQEFKRRTNLPIVIK GVQRTEDVILAAEHGVDGVVLSNHGGRQLDGAPPALQVLAECMPVLRQRGLDKKLEV FVDGGIRRGTDIMKALCLGAKGVGLGRPFLYANSAYGPDGVEKAIDILKNELIMNMRLL GVTKISDLSPEFVDTRPLFGLTANDRLFNNNYLDIEFPKFKDE
45	MFRIRLQRGCLLRTNQTIVRRQLRPFTTNIRTVNAQRVVGRLGFVAGIAIFSAGLYTFEK RKFASVIKNDVRQDSKKPGILPSEVARHDKDNDCWVVINGFVYDLTEFIGAHPGGPAII QANAGKDVSAIFSAFHAPDVIEKYLPEEKCLGPLDGHMQPNLVSPALAPGETPEDIERK EELRQALPELDSLINLYDFEYLASQILTKQAWTYYSSGSEDEFAHRENHAAYHRIFFKP RVLVNVGEIDTSTVMLGHDVSVPFYVSATALVKLGNPEEGEKDIARGCGQGEFKCPQ MISNFASCTLKETVEAAPSKDQLQWLQVYVNTNKSITENLLKEAEKLGLKAIFITVDAPC LGRREKDIKLKFTSDNNPQSIKKTKLESSIGASRALSSFQDAALTWDDVVELKSKTNLPI VIKGVQCVEDVLKAVEIGASGVVLSNHGGRQLDFSRAPIEVLAETMPILKERHLDDKLEI FIDGGVRRGTDILKALCLGAKGVGLGRPFLYANSCYGRDGVEKAIDMLTEEIQYSMRLL GVKSINELRPELLDLSGIKNRSVDFPQDSLYKSVYEKPEHAQFVERD
46	MRRRLITSLVKKGNFQSKRSLHLTNNNLYISSSILKKHPNTGNIPGIQSIENAYKRSYHT YLYMSLTGISFYSLAIIILNNSIHDRHIDNDSKMELDPVKSKTPITPNELMKHNTPEDCWV VINNQVYDLTTFIQVHPGGPNIIRSNAGKDVTAIFNPLHPPNTIETMLPKQCYVGPLEGG LPKELICNPYTPGESMEDIRRKIILKNHLPPLESIINLYDFEKLASKILSNQAWAYYSSGA NDEISYRENHSAYTRIFFKPKVLVDVSKVDLSTEMLGSKIEVPFYATATALCKLGNPEG GEMDIARGCGQGLIKVPQMISTLASCSVEEIVGAAPSKDQIQWYQVYINSDRNVTRHMI KKVEDLGVKALFVTVDAPFMGAREKDLKIKFSNSSQGPKVMTSKAKDDINKSIVNKKEE AAGDVTEGASRTLSKFIDPSLTWKDIIELKKWTKLPIVIKGVQRVEDVVKAAEIGVDGVV LSNHGGRQLDFSRPPVELLAESVPILKEKKLDDKLELYVDGGIRRGTDVLKALCLGAKG VGLGRPFLYANSCYGKDGVQKAIDILKDEIEMSMRLLGVTSIKELNEDLLEISNLKSRSV NVSKDVLFEDLYQNPTYADFSSVEPAE

47	MFKSQLRTATARSSFRSLASKLNPQRFNSSKTPLLNATRGSNRSKNSLIALAISLSAVS SSYYLYQKDKFISADVPHWKDIELTPEIVSQHNKKDDLWVVLNGQVYDLTDFLPNHPG GQKIIIRYAGKDATKIFVPIHPPDTIEKFIPPEKHLGPLVGEFEQEEEELSDEEIDRLERIE RKPPLSQMINLHDFETIARQILPPPALAYYCSAADDEVTLRENHNAYHRIFFNPKILIDVK DVDISTEFFGEKTSAPFYISATALAKLGHPEGEVAIAKGAGREDVVQMISTLASCSFDEI ADARIPGQQQWYQLYVNADRSITEKAVRHAEERGMKGLFITVDAPSLGRREKDMKMK FEADSDVQGDDEDIDRSQGASRALSSFIDPSLSWKDIAFIKSITKMPIVIKGVQRKEDVL LAAEHGLQGVVLSNHGGRQLDYTRAPVEVLAEVMPILKERGLDQKIDIFVDGGVRRGT DVLKALCLGAKGVGLGRPFLYAMSSYGDKGVTKAIQLLKDEIEMNMRLLGVNKIEELTP ELLDTRSIHNRAVPVAKDYLYEQNYQRMSGAEFRPGIED
48	MFHSTSLRVSRAIRASKVIHSSPLRGPRSKVRQLTTASIKSAHNNKLLPALGFLGASASI AALLLNNTEPFIRLDAPTQGPLYTPDDVSAHNTKDDLWVVLNGKVYDLTEFLPNHPGG QRIILKYAGKDASKIFNPIHPPDAIDKYLPPEKCIGTLDGEFPEEEEVLSDEELLRLDRVD RKPPLSKIMNLHDFEAVARQILPPGALAYYSSAADDEITLRENHNAYHRIFFNPKVLVDV SKVDITTEFLGNETSAPFYITATALAKLGHPDGEVSIARGAGKEDITQMISTLASCSFDQI ADARVKDTQAQWYQLYVSEDRELSKKQLKHCEERGMKGIFITVDAPSLGRREKDMKL KFEGNADVQSDDDIDRSQGAAVALSSFIDSSLTWKDITEIRKWTKLPIVIKGVQRKEDVL LALEHGCDGVVLSNHGGRQLDFSRAPVEVLAEVMPILREKGVDKKFEVYVDGGVRRG TDVLKALCLGAKGVGLGRPFLYANSAYGEKGVRRAIQLLKSELIMNMRLLGVTKIEDLT PELLDTTTIHQRTVSVPADYLYKENYLHMQGVEFNPELEE
49	MLGVSRQCEVTGRKLFSGKVLSKNFKYLSNRKYSLATTKNGAKYESKKFNKAQLMAIA GAGLIATTTLLYSNFNSEIQNESKLAPKRVIDPSEVARHNTPADCWIVINGVVYDLTSFIP VHPGGADIIKSNAGKDVTAIFEPIHAPGVIEKYLPPKCRIGTLKKPMPAELTCDPYTPGE SKDDIIKKLELRNNLPHLDSIINIYDFEKLASKILSNQAWAYYSSGADDEISYRDNHSAYR RIFFKPRILRDVSSVDVKTTMLGSKVDVPFYVSATALCKLGNPKEGEKDIARGCGQGD TKVPQMISTLASCSVDEIVDAAPSKDQIAWYQVYVNSDRNITRDMIKHVEKLGIKALFIT VDAPSLGRREKDMKIKFSGSDQGAKVMKEPLKKVEKKDDGEMSKGASTTLSKFIDPSL TWDDVVKMRKWTKLPIVIKGVQSVEDVVKAAELGVDGVVLSNHGGRQLDYSRPPIEV LAETVPVLKEKHLDGKLELFVDGGVRRGTDVIKALCLGAKGVGLGRPFLYSNSCYGKD GVEKTIELLKTEIEMSMRLLGVTSIDQLTPELLDTTNLKSRTVNVSKDVLFDTVYRNPTL AAFQNGYDDDE
50	MYKRLISKKVYLNTFNSKANFTGKYLSAPKCSFNCIRGYSKFNGNYNVHSAKKYDNNN NNNNSRFSLFAAVAATVITVGTTAIITSNKSDASFLSNTSSVLNKPPIDPKEVVKHNKPD DCWIVINNVVYDLTDFIAKHPGGPDIIQSNAGKDVSAIFNPLHASDVIDKYIKPECQLGPL KEPLPESYICPPLTPGETAEDVARKAELRDKLPPLDSLINLYDFEYLASQILTKQAWGYY SSAADDEVTYRENHAAYHRIFFKPRILINVKECDLSTTMLGTKMDLPFYVSATALCKLG NPSEGEKDIARGCGMGEFNLTQMISTLASCSLKEIVEAKVNDKQTQWFQLYVNADRKI TDNLIKNVEELGLKAIFVTVDAPSLGRREKDMKIKFDPSKTPEITNEDKSHKKQQNTRG ASKALSTFIDPSLTWNDVIDIKKKTKLPVVIKGVQCTADILKAAEIGVDGVVLSNHGGRQ LDFSRAPIEVLAEAMPILKEKGLDKKLEVYIDGGVRRGTDVLKALCLGAKGVGLGRPFL YANSCYGKDGVNRLKEMLADEIEMNMRLLGVTKISDLKPEYLDMISLHARSVNVPKDN LYQEVYIPSTTVEFLDE

SEQ ID NO:10 to 38 and SEQ ID NO:40 to 46 are from Saccharomycetes. SEQ ID NO: 39 is from WO2022258733A1. SEQ ID NO:47 is from *Wickerhamomyces anomalus*. SEQ ID NO:48 is from *Cyberlindnera fabianii*. SEQ ID NO:49 is from *Naumovozyma castellii*. SEQ ID NO:50 is from *Saccharomycodes ludwigii*.

The enzymatic activity of a cellobiose dehydrogenase, or a flavocytochrome b2, or a variant thereof can be determined by a cytochrome c assay assessing the enzymatic

activity from the colorimetric reduction of cytochrome c (CytC) at 30°C and 550 nm, *e.g.* as previously described by Diêp Lê et al. (Diêp Lê et al. 2009); molar extinction coefficient=20 mM−1 cm−1. The assay mixture is buffered at pH 7.4 with 11 mM potassium phosphate, 137 mM NaCl, 3 mM KCl and contains 10 mM lactose or lactate, depending on the enzyme used, 20 µM CytC, which acts as a terminal electron acceptor and specifically detects the activity of the whole enzyme as a product of all partial electron transfers (flavin and haem domain). The CytC assay thereby provides a measure of the efficiency of the intramolecular electron transfer (IET) between both domains and to external electron acceptors as an indication of the enzyme's response on electrodes. One unit of enzymatic activity is defined as the amount of enzyme that oxidizes 1 µmol of lactate per min under the assay conditions. The reaction stoichiometry of lactate: CytC is 1 : 2, since two electrons are gained per lactate molecule and transferred individually to 2 molecules CytC. For the detection of activity with other substrates, lactate can be exchanged for other compounds. The cytochrome c assay can be used for determining the direct electron transfer (DET) capability of an enzyme.

The term "**functional variant**" or "**functionally active variant**" also includes naturally occurring allelic variants, as well as mutants, or any other non-naturally occurring variants. As is known in the art, an allelic variant, or also referred to as homologue, is an alternate form of a nucleic acid or peptide that is characterized as having a substitution, deletion, or addition of one or more nucleotides or amino acids that does essentially not alter the biological function of the nucleic acid or polypeptide. Specifically, a functional variant may comprise a substitution, deletion and/or addition of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid residues, or a combination thereof. Specifically, substitutions, deletions and/or additions may be conservative modifications. Specifically, substitutions, deletions and/or additions do not decrease the enzyme's specific activity. Specifically, a functionally active variant of the enzyme described herein comprises specific enzymatic activity towards a substrate or analyte of at least 1 U/mg, as determined by the respective assay as described herein.

Specifically, a functional variant as described herein comprises no more than or up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25,26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 amino acid substitutions, deletions and/or additions. Specifically, these modifications may be conservative modifications. Specifically, these modifications do not decrease the enzyme's specific activity. Specifically, a functionally active variant as described herein comprises up to

15, preferably up to 10 or up to 5, amino acid substitutions, deletions and/or additions. Specifically, these modifications may be conservative modifications. Specifically, these modifications do not decrease the enzyme's specific activity.

Specifically, a functionally active variant described herein comprises at least 40, 50, 60, 70, 80, 85, 90, 95 or 100% or even more of the enzymatic activity of the respective wild type enzyme.

Functional variants may be obtained by sequence alterations in the polypeptide or the nucleotide sequence e.g., by one or more point mutations, wherein the sequence alterations retain or improve a feature of the enzyme, such as its stability or activity for example. Such sequence alterations can include, but are not limited to, (conservative) substitutions, additions, deletions, mutations, and insertions. Conservative substitutions are those that take place within a family of amino acids that are related in their side chains and chemical properties. Examples of such families are amino acids with basic side chains, with acidic side chains, with non-polar aliphatic side chains, with non-polar aromatic side chains, with uncharged polar side chains, with small side chains, with large side chains etc.

A point mutation is particularly understood as the engineering of a polynucleotide that results in the expression of an amino acid sequence that differs from the non-engineered amino acid sequence in the substitution, or exchange, deletion, or insertion of one or more single (non-consecutive) or doublets of amino acids for different amino acids.

The term "**sequence identity**" as used herein is understood as the relatedness between two amino acid sequences or between two nucleotide sequences and described by the degree of sequence identity or sequence complementarity. The sequence identity of a variant, homologue, or orthologue as compared to a parent nucleotide or amino acid sequence indicates the degree of identity of two or more sequences. Two or more amino acid sequences may have the same or conserved amino acid residues at a corresponding position, to a certain degree, up to 100%. Two or more nucleotide sequences may have the same or conserved base pairs at a corresponding position, to a certain degree, up to 100%.

Sequence similarity searching is an effective and reliable strategy for identifying homologs with excess (*e.g.*, at least 50%) sequence identity. Sequence similarity search tools frequently used are *e.g.*, BLAST, FASTA, and HMMER.

Sequence similarity searches can identify such homologous proteins or polynucleotides by detecting excess similarity, and statistically significant similarity that reflects common ancestry. Homologues may encompass orthologues, which are herein understood as the same protein in different organisms, *e.g.*, variants of such protein in different organisms or species.

To determine the % complementarity of two complementary sequences, one of the two sequences needs to be converted to its complementary sequence before the % complementarity can then be calculated as the % identity between the first sequence and the second converted sequences using the above-mentioned algorithm.

"Percent (%) identity" with respect to an amino acid sequence, homologs and orthologues described herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific polypeptide sequence, after aligning the sequence and introducing gaps, if necessary, and not considering any conservative substitutions as part of the sequence identity. Those skilled in the art can determine appropriate parameters for the alignment, including any algorithms needed to achieve the highest scoring alignment over the full length of the sequences being compared. In case of percentages determined for sequence identities, it is possible that arithmetical decimal places may result which are not possible with regard to full nucleotides or amino acids. In this case, the percentages shall be rounded up to whole nucleotides or amino acids.

For purposes described herein, the sequence identity between two amino acid sequences is determined using standard methods, *e.g.* using the NCBI BLAST program version 2.2.29 (Jan-06-2014) or online using the multiple sequence alignment tool EMBL-EBI Clustal Omega (Sievers, F. et al. (2011)).

"Percent (%) identity" with respect to a nucleotide sequence *e.g.*, of a nucleic acid molecule or a part thereof, in particular a coding DNA sequence, is defined as the percentage of nucleotides in a candidate DNA sequence that is identical with the nucleotides in the DNA sequence, after aligning the sequence and introducing gaps, if necessary, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent nucleotide sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software. Those skilled in the art can determine appropriate parameters for the alignment, including any algorithms needed to achieve the highest scoring alignment over the full length of the sequences being compared.

Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting examples of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, MAFFT based algorithms: multiple alignment using fast fourier transform, algorithms based on the Burrows-Wheeler Transform (e.g., the Burrows Wheeler Aligner), Clustal W, Clustal X, BLAT, Novoalign (Novocraft Technologies; available at novocraft.com), ELAND (Illumina, San Diego, CA), SOAP (available at soap.genomies.org.cn), and Maq (available at maq.sourceforge.net).

In a structure alignment the maximal set of corresponding pairs of amino acid residues that gives a good structural match when the structures are overlaid, i.e., superposed, is identified. Thereby, the positions of the protein's backbone C-alpha atoms and/or location of secondary structural elements are considered in this alignment. Tools for performing a structure alignment are available, e.g., the protein data bank provides a tool for pairwise structure alignment. Specifically, structure superposition is also a tool for determining corresponding amino acid positions in different enzymes. Structure superposition can be performed using the Molecular Graphics System PyMOL, (Schrödinger) using the command "align".

According to one embodiment of the invention, in the mixture described herein the ratio of enzyme:carbon particle suspension is in the range of 1:1 to 1:10 (v/v). Specifically, in the mixture described herein the ratio of enzyme:carbon particle suspension is in the range of 1:1 to 1:15 (v/v), 1:1 to 1:10 (v/v), 1:1 to 1:9 (v/v), 1:1 to 1:8 (v/v), 1:1 to 1:7 (v/v), 1:1 to 1:6 (v/v), 1:1 to 1:5 (v/v), 1:1 to 1:4 (v/v), 1:2 to 1:10 (v/v), 1:2 to 1:9 (v/v), 1:2 to 1:8 (v/v), 1:2 to 1:7 (v/v), 1:2 to 1:6 (v/v), 1:2 to 1:5 (v/v), 1:2 to 1:4 (v/v), 1:3 to 1:10 (v/v), 1:3 to 1:9 (v/v), 1:3 to 1:8 (v/v), 1:3 to 1:7 (v/v), 1:3 to 1:6 (v/v), 1:3 to 1:5 (v/v), or 1:3 to 1:4 (v/v). More specifically, in the mixture described herein the ratio of enzyme:carbon particle suspension is 1:4 (v:v).

According to one embodiment of the invention, the enzyme is in solution prior to preparing the mixture.

According to a specific embodiment, the concentration of enzyme in solution is in the range of 1-50 mg/ml, 1-40 mg/ml, 1-30 mg/ml, 1-25 mg/ml, 1-20 mg/ml, 10-50 mg/ml, 10-40 mg/ml, or 10-30 mg/ml. Thereby, the concentration of enzyme in solution may be adjusted according to the enzyme activity of the enzyme used.

According to a specific embodiment, the solution in which the enzyme is dissolved prior to preparing the mixture is an aqueous solution. Specifically, the enzyme is dissolved in a buffered aqueous solution.

According to one embodiment, applying of the mixture comprising enzyme:carbon particle suspension described herein to a conductive surface of an electrode can be performed by any method suitable for applying such a mixture.

The term "**drying**" as used herein refers to the removal of the liquid part of the mixture by e.g., evaporation. Thereby, the solid part of the mixture remains at the conductive surface of the electrode.

According to one embodiment, drying is performed after application of the enzyme:carbon particle suspension by incubation at an elevated temperature until the surface of the electrode is dry.

According to a specific embodiment, drying is performed by incubation at a temperature in the range of 40 to 70 °C. Specifically, drying is performed at a temperature in the range of 50 to 70, or 55 to 65 °C. More specifically, drying is performed at 60 °C.

According to a specific embodiment, drying is performed by incubation at 60 °C for 1 hour.

According to one embodiment, an electrode comprising an analyte sensing layer is described herein, wherein said analyte sensing layer comprises carbon black, a cationic polymer, and an enzyme. Specifically, the cationic polymer is non-conducting.

According to one embodiment, an electrode comprising an analyte sensing layer is described herein, wherein said analyte sensing layer is a single layer comprising carbon black, a cationic polymer, and an enzyme. Specifically, the cationic polymer is non-conducting.

The term "single layer" as used herein refers to a layer in which all three components carbon black, a non-conducting cationic polymer, and an enzyme are present as one layer, originating from one solution containing all three components.

According to one embodiment, an electrode is described herein, wherein said electrode is prepared by the method of manufacturing an electrode as described herein.

According to one embodiment of the invention, the electrode described herein is used for the determination of an analyte.

According to one embodiment of the invention, the electrode may be used as single electrode or as a stack of electrodes of e.g., 2, 3, 4, 5, or more electrodes.

According to one embodiment of the invention, for the determination of an analyte by electrochemical means, the electrode described herein is contacted with the sample. This contact between electrode and sample can be performed by any approach which brings the electrode and the sample in contact in order that the enzyme is allowed to react with the analyte or with the sample suspected to contain the analyte.

The term "**determining**" as used herein refers to detecting and/or quantifying an analyte such as lactate. The term "**detecting**" refers to the general determination if analyte is present. Detection does not require the exact quantification of analyte but rather provides the user of the method with the information if e.g., the analyte is present with a concentration above a certain threshold. These thresholds are to be adapted to the respective application and sample. The term "**quantifying**" refers to the determination of the concentration or amount of an analyte. Quantification may refer to the determination of an exact amount of an analyte or may alternatively refer to a semi-quantitative determination of an analyte e.g., if the amount of the analyte in a sample is in a certain range. Such a range may be a concentration range suitable for the respective purpose of the determination of the analyte.

According to one embodiment, the **sample** may be any material for which determining the presence of an analyte is relevant or of interest. In particular, the sample is a human or animal sample, specifically any one of body fluid, interstitial fluid, blood, blood plasma, blood serum, dermal fluid, urine, tears, sweat, saliva, skin, flesh, tissue, eyeballs, cornea, and gastric fluid. Alternatively, the sample is a food or beverage sample, specifically any one of milk, dairy product, and non-dairy milk alternative products. Examples of dairy products are whey and cheese. An example of a non-dairy milk alternative products is an oat drink. If solid products are analyzed, the analyte of interest, may be extracted or the solid product may be fluidized, such as by dissolving.

According to one embodiment of the invention, determining of analyte is performed electrochemically.

The term "**electrochemically**" as used herein refers to the usage of an electrochemical biosensor based on the measurement of biological binding event-dependent changes in conductance, resistance, or capacitance of the biosensor surface. In such an electrochemical biosensor, one of the electrodes is immobilized with a biological recognition molecule. The contact of the analyte to the biological recognition molecule triggers a change in the electrical properties due to oxidation and reduction reactions taking place as a result of biological interaction activity, thus providing the

sensor signal. Electrochemical biosensors rely mostly on enzyme-catalyzed reactions to produce current/potential difference which is then detected.

Electrochemical biosensors can be impedimetric, potentiometric, or amperometric. In an amperometric biosensor, a biochemical signal is transduced into a quantifiable amperometric signal.

As described in Rocchitta G. et al. (2016) amperometric biosensors are commonly divided into three main generations depending on the electron transfer method used for the measurement of the biochemical reaction or the degree of separation of the biosensor components (transducer, enzyme, mediators, and cofactors). First-generation biosensors measure the concentration of analytes and/or products of enzymatic reactions that diffuse to the transducer surface and generate an electrical response. They are also called mediatorless amperometric biosensors. Commonly, oxidases are used in first-generation biosensors. Oxidases need molecular oxygen as a second substrate so the oxidase-based biosensors are oxygen dependent. Second-generation biosensors require an electron mediator for the transfer of electrons obtained from enzymatic reactions to the transducer surface and thereby generate an electrical response. In third-generation biosensors, direct electron transfer is enabled between the redox-active biomolecule i.e., the enzyme, and the electrode surface.

According to one embodiment of the invention, the electrode described herein is part of a biosensor. Thereby, a specific use of the electrodes of the invention is in the provision of a biosensor, more specifically a third-generation biosensor using direct electron transfer properties to detect an analyte and/or to measure the analyte concentration. The biosensor may be suitable for use at acidic, neutral, or alkaline pH. The biosensor may be suitable for use at room temperature or at body temperature. Specifically, the biosensor may be suitable for the detection and/or quantification at 4°C, 10°C, 15 °C, 20 °C, 21 °C, 22 °C, 23 °C, 24 °C, 25 °C, 26 °C, 27 °C, 28 °C, 29 °C, 30 °C, 31 °C, 32 °C, 33 °C, 34 °C, 35 °C, 36 °C, 37 °C, 38 °C, 39 °C, 40 °C, 41 °C, 42 °C, 43 °C, 44 °C, 45 °C, or higher.

According to another embodiment, the biosensor may have one or more electrodes comprising the enzyme as described herein as working electrode. One or more other electrodes may be included such as one or more counter electrodes, one or more reference electrodes and/or one or more counter/reference electrodes.

The particular configuration of the biosensor may depend on the use for which the biosensor is intended and the conditions under which it will operate.

In a specific embodiment of the present invention, the biosensor may be a single use biosensor for the detection of analyte. Thereby, the biosensor may be a biosensor strip.

According to one embodiment of the present invention, the enzymes described herein may be recombinantly expressed by methods commonly known in the art. For example, the enzymes described herein may be expressed using standard methods for cloning, transformation, and recombinant production in suitable host organisms e.g., in *Escherichia coli* or in *Pichia pastoris*.

The examples described herein are illustrative of the present invention and are not intended to be limitations thereon. Many modifications and variations may be made to the techniques described and illustrated herein without departing from scope of the invention.

EXAMPLES

Example 1: Performance of carbon/polycation/enzyme ink on various electrode materials

Materials and methods:

Gold electrodes type "AUTE100AgCl" were obtained from Zensor R&D co., Ltd, carbon, platinum, and gold paste electrodes types "DRP-C110", DRP-C550 and DRP-C220AT were obtained from DropSens/Metrohm. All electrodes contained an Ag/AgCl reference electrode and a counter electrode of the same material as the working electrode. To prepare the carbon ink, 50 mg of graphitized mesoporous carbon black (Sigma 699624) was dispersed in a 4 ml aqueous solution of 0.2% PEI using a probe sonicator for 2 min at 0.4 W/cm^3. Particle suspension was characterized by dynamic light scattering (DLS) methods using a Zetasizer nano system (Malvern Panalytical Ltd).

80 µl of the decanted carbon/PEI ink was mixed with 20 µl of a direct electron transfer type glucose sensitive cellobiose dehydrogenase (CDH having SEQ ID NO:1, dissolved at 15 mg/ml dissolved in 1 mM phosphate buffer, pH 7.4). All electrodes were modified with 0.28 µl enzyme/carbon/PEI ink per mm^2 of working electrode area. Electrodes were dried for 1 h at 60°C afterwards. Electrodes were measured and mounted in a horizontal way and a drop of 100 µl 20 mM glucose (in 50 mM PBS, pH 7.4) was added to the electrodes. Chronoamperometry was measured at 0 V vs Ag/AgCl

for 60 s. Catalytic currents were evaluated from t=50-55s. Absolute catalytic currents were related to the working electrode area to obtain current densities J. Three electrodes per enzyme type were measured.

CDH enzymes such as CDH having SEQ ID NO:1 are e.g. disclosed in EP2636733A1 and such enzymes can be produced according to EP2636733A1.

SEQ ID NO:1 (CDH):

MKLLSRVGATALAATLSLKQCAAQMTEGTYTHEATGITFKTWTPSDGSTFTFG
LALPGDALTNDATEYIGLLRCQITDPSSPGYCGISHGQSGQMTQALLLVAWASEDVVY
TSFRYATGYTLPELYTGDAKLTQIASSVSGDSFEVLFRCENCFSWDQNGATGSVSTS
NGALVLGYAASKSGLTGATCPDTAEFGFHNNGFGQWGAVLEGATSDSYEEWAQLA
TITPPTTCDGNGPGDKVCVPAPEDTYDYIVVGAGAGGITVADKLSEAGHKVLLIEKGP
PSTGLWNGTMKPEWLEGTDLTRFDVPGLYNQIWVDSAGIACTDTDQMAGCVLGGGT
AVNAGLWWKPHPADWDDNFPHGWKSSDLADATERVFSRIPGTWHPSQDGKLYRQ
EGFEVISQGLANAGWREVDANQEPSEKNRTYSHSVFMFSGGERGGPLATYLASAAQ
RSNFNLWVNTSVRRAIRTGPRVSGVELECLADGGFNGTVNLKEGGGVIFSAGAFGSA
KLLLRSGIGPEDQLEIVASSKDGETFISKNDWIKLPVGHNLIDHLNTDLIITHPDVVFYDF
YAAWDNPITEDKEAYLNSRSGILAQAAPNIGPLMWEEVTPSDGITRQFQWTCRVEGD
SSKTNSTHAMTLSQYLGRGVVSRGRMGITSGLTTTVAEHPYLHNDGDLEAVIQGIQN
VVDALSQVPDLEWVLPPPNTTVEEYVNSLIVSPANRRANHWMGTAKMGLDDGRSGG
SAVVDLNTKVYGTDNLFVVDASIFPGMSTGNPSAMIVIVAEQAAQRILSLRY

Results:

The described preparation methods of the carbon/polycation ink results in a monodisperse suspension with particle diameter below 1000 nm as shown in the DLS results (Fig. 1).

The addition of glucose yields catalytic currents for all tested electrode materials which were modified with carbon/polycation/enzyme ink, see Fig. 2A. Using enzyme without the carbon/polycation ink yields negligible currents (Fig. 2B). Currents originate from the oxidation of glucose by the enzyme which in turn is re-oxidized directly by the electrode surface (direct electron transfer). On all tested electrode materials, the same current densities are obtained after adding 20 mM glucose. This indicates that the underlying base electrode material does not impact sensor performance and that the carbon/polycation/enzyme ink works universally on all tested electrode materials.

Example 2: Performance of various enzymes with the carbon/polycation ink

Materials and methods:

Gold electrodes type "AUTE100AgCl" were obtained from Zensor R&D co., Ltd.. The electrodes contained an Ag/AgCl reference electrode and a counter gold electrode. To prepare the carbon ink, 50 mg of graphitized mesoporous carbon black (Sigma 699624) was dispersed in a 4 ml aqueous solution of 0.2% polyethylenimine (PEI) using a probe sonicator for 2 min at 0.4 W/cm^3. 80 µl of the decanted carbon/polycation ink was mixed with 20 µl of enzyme. Direct electron transfer enzymes were either a glucose sensitive cellobiose dehydrogenase (CDH having SEQ ID NO:1, dissolved at 15 mg/ml dissolved in 1 mM phosphate buffer, pH 7.4) or a lactate sensitive dehydrogenase (FCb2 having SEQ ID NO:2, dissolved at 10 mg/ml in 100 mM potassium phosphate buffer pH 7.0).

FCb2 enzymes such as FCb2 having SEQ ID NO:2 are e.g. disclosed in WO2022/258733A1 and such enzymes can be produced according to WO2022/258733A1.

SEQ ID NO:2 (FCb2):

MEPKLDMNKQKISPAEVAKHNKPDDCWVVINGYVYDLTRFLPNHPGGQDVIK
FNAGKDVTAIFEPLHAPNVIDKYIAPEKKLGPLQGSMPPELVCPPYAPGETKEDIARKE
QLKSLLPPLDNIINLYDFEYLASQTLTKQAWAYYSSGANDEVTHRENHNAYHRIFFKP
KILVDVRKVDISTDMLGSHVDVPFYVSATALCKLGNPLEGEKDVARGCGQGVTKVPQ
MISTLASCSPEEIIEAAPSDKQIQWYQLYVNSDRKITDDLVKNVEKLGVKALFVTVDAP
SLGQREKDMKLKFSNTKAGPKAMKKTNVEESQGASRALSKFIDPSLTWKDIEELKKK
TKLPIVIKGVQRTEDVIKAAEIGVSGVVLSNHGGRQLDFSRAPIEVLAETMPILEQRNLK
DKLEVFVDGGVRRGTDVLKALCLGAKGVGLGRPFLYANSCYGRNGVEKAIEILRDEIE
MSMRLLGVTSIAELKPDLLDLSTLKARTVGVPNDVLYNEVYEGPTLTEFEDA

The working electrodes were modified with 2 µl of the enzyme/carbon/polycation ink. Electrodes were dried for 1 h at 60°C afterwards. Electrodes were measured and mounted in a horizontal way and 100 µl of buffer was added and chronoamperometry was started. Buffers were 50 mM PBS, pH 7.4 for the glucose and lactate oxidizing enzymes. Chronoamperometry was measured at 0 V (for glucose) and 0.2 V (for lactate) vs Ag/AgCl for 15 min. The drop of buffer was removed and increasing concentrations of 100 µl of substrates (glucose and lactate dissolved in the respective buffer) were added successively after the previous drop was removed. 1 min after each substrate

addition the catalytic current was read and related to the working electrode area of 7.1 mm^2 to obtain current densities J. Three electrodes per enzyme type were measured.

Results:

Both tested direct electron transfer type enzymes yield clear catalytic currents increasing with increased substrate concentrations, see Fig. 3. Currents originate from direct electron transfer as no mediators are present and the applied potentials are low. Thus, the carbon/polycation ink universally works for various direct electron transfer enzymes. Multiple substrate additions are possible without a wash-off of the sensing layer.

Example 3: Performance of carbon/polycation/enzyme ink for carbon dispersed using various polycations

Materials and methods:

Gold electrodes type "AUTE100AgCl" were obtained from Zensor R&D co., Ltd.. The electrodes contained an Ag/AgCl reference electrode and a counter gold electrode. To prepare the carbon inks, 50 mg of graphitized mesoporous carbon black (Sigma 699624) was dispersed in a 4 ml solution of polycation using a probe sonicator for 2 min at 0.4 W/cm^3. The polycations were aqueous solutions of either 0.4% w/v PDADMAC or 0.4% w/v DEAE or 0.2% w/v PEI or 1 % w/v polylysine. Thus, the ratio of carbon black:polycation is 3.125:1 for PDADMAC, 3.125:1 for DEAE, 6.25:1 for PEI, and 1.25:1 for polylysine in the prepared carbon inks.

80 µl of the decanted carbon/polycation ink was mixed with 20 µl of the glucose sensitive direct electron transfer enzyme cellobiose dehydrogenase (CDH having SEQ ID NO:1, dissolved at 15 mg/ml dissolved in 1 mM phosphate buffer, pH 7.4).

The working electrodes were modified with 2 µl of enzyme/carbon/polycation ink. Electrodes were dried for 1 h at 60°C afterwards. Electrodes were measured and mounted in a horizontal way and a drop of 100 µl of 20 mM glucose (in 50 mM PBS, pH 7.4) was added to the electrodes. Chronoamperometry was measured at 0 V vs Ag/AgCl for 60 s. Catalytic currents were evaluated from t=50-55s. Absolute catalytic currents were related to the working electrode area to obtain current densities J. Three electrodes per enzyme type were measured.

Results:

All tested polycations yielded deagglomerated carbon black solutions. Optimum polycation concentrations to obtain best deagglomeration results differed depending on

the polycation. Sensors modified with inks containing carbon black dispersed by the various polycations PEI, DEAE, PDADMAC or polylysine mixed with glucose oxidizing enzyme all responded similarly when glucose was added, see Fig. 4. The obtained catalytic currents originate from direct electron transfer as no mediators are present and the applied potential of 0 V is low.

Example 4: Performance of various carbon black types dispersed by polycation in an enzyme ink

Materials and methods:

Gold electrodes type "AUTE100AgCl" were obtained from Zensor R&D co., Ltd.. The electrodes contained an Ag/AgCl reference electrode and a counter gold electrode. To prepare the carbon inks, 50 mg of various carbon black types were dispersed each in a 4 ml aqueous solution of 0.2% PEI using a probe sonicator for 2 min at 0.4 W/cm^3. The tested carbon black types were "graphitized mesoporous carbon black with a specific surface area of 50-100 m^2/g (Sigma 699624); "Ketjenblack EC300J" from Lion Specialty Chemicals Co., Ltd., "Super P" from Imerys S.A., "Conductex SC Ultra" and "Conductex K Ultra" from BIRLA carbon.

80 µl of the decanted carbon/polycation ink was mixed with 20 µl of a direct electron transfer type glucose sensitive cellobiose dehydrogenase (CDH having SEQ ID NO:1, dissolved at 15 mg/ml dissolved in 1 mM phosphate buffer, pH 7.4).

The working electrodes were modified with 2 µl of carbon/polycation/enzyme ink. Electrodes were dried for 1 h at 60°C afterwards. Electrodes were measured and mounted in a horizontal way and a drop of 100 µl of 20 mM glucose (in 50 mM PBS, pH 7.4) was added to the electrodes. Chronoamperometry was measured at 0 V vs Ag/AgCl for 60 s. Catalytic currents were evaluated from t=50-55s. Absolute catalytic currents were related to the working electrode area to obtain current densities J. Three electrodes per enzyme type were measured.

Results:

All tested carbon black types dispersed well in the polycation.

Sensors modified with the various carbon/polycation/enzyme inks yielded similar catalytic current responses for 20 mM glucose independent on the used type of carbon black, see Fig. 5. The obtained catalytic currents originate from direct electron transfer as no mediators are present and the applied potential of 0 V is low.

Example 5: Shelf life of sensors under rapid aging conditions

Materials and methods:

Gold electrodes type "AUTE100AgCl" were obtained from Zensor R&D co., Ltd.. The electrodes contained an Ag/AgCl reference electrode and a counter gold electrode. To prepare the carbon inks, 50 mg of graphitized mesoporous carbon black (Sigma 699624) was dispersed in a 4 ml solution of polycation using a probe sonicator for 2 min at 0.4 W/cm^3. The polycation was an aqueous solution of either 0.4% w/v DEAE or 0.2% w/v PEI.

80 µl of the decanted carbon/polycation ink was mixed with 20 µl of the glucose sensitive direct electron transfer enzyme cellobiose dehydrogenase (CDH having SEQ ID NO:1, dissolved at 15 mg/ml dissolved in 1 mM phosphate buffer, pH 7.4).

The working electrodes were modified with 2 µl of enzyme/carbon/polycation ink. Electrodes were dried for 1 h at 60°C afterwards. Electrodes were packed into air and humidity tight aluminum bags together with 2 grains of as silica desiccant. Aluminum bags were heat sealed and sealed bags containing the electrodes and silica were stored at 40±0.5°C and 60±0.5°C in an oven. After 2, 7 and 15 days a set of stored electrodes was measured and discarded afterwards. Day 0 electrodes were measured immediately without packing them into aluminum bags.

Electrodes were measured and mounted in a horizontal way and a drop of 100 µl of 20 mM glucose (in 50 mM PBS, pH 7.4) was added to the electrodes. Chronoamperometry was measured at 0 V vs Ag/AgCl for 60 s. Catalytic currents were evaluated from t=50-55s. Absolute catalytic currents were related to the working electrode area to obtain current densities J. Three electrodes per day and storage temperature were measured.

Results:

See Fig. 6. At 40°C electrodes loose ~20% of the initial currents within the first data point, but are then stable for at least 14 days.

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Rocchitta, G., et al. (2016). Enzyme Biosensors for Biomedical Applications: Strategies for Safeguarding Analytical Performances in Biological Fluids. *Sensors (Basel, Switzerland)*, *16*(6), 780. https://doi.org/10.3390/s16060780

Shimizu, H.; Tsugawa, W. Glucose Monitoring by Direct Electron Transfer Needle-Type Miniaturized Electrode. *Electrochemistry* 2012, *80* (5), 375–378.

Sievers, F. et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol. 7, 539 (2011)

CLAIMS

1. A method for manufacturing an enzyme electrode, wherein said method comprises the steps of:

i. preparing a carbon particle suspension comprising carbon black and a non-conducting cationic polymer;

ii. preparing a mixture comprising an enzyme and the carbon particle suspension;

iii. applying the mixture to a conductive surface of an electrode; and

iv. drying the mixture applied to the conductive surface of the electrode.

2. The method of claim 1, wherein the carbon black is graphitized carbon black.

3. The method of claim 1 or 2, wherein the non-conducting cationic polymer is polyethyleneimine (PEI), diethylaminoethyl dextran (DEAE), polylysine, or polydiallyldimethylammonium chloride (PDADMAC).

4. The method of any one of claims 1 to 3, wherein in the carbon particle suspension the ratio of carbon black:non-conducting cationic polymer is in the range of 10:1 to 1:10.

5. The method of any one of claims 1 to 4, wherein the carbon particle suspension further comprises water.

6. The method of claim 5, wherein the carbon particle suspension has a concentration in the range of 1 to 10 % (w/v).

7. The method of any one of claims 1 to 6, wherein the carbon particle suspension is prepared by a sonication treatment.

8. The method of claim 7, wherein a probe sonicator is used for sonication treatment.

9. The method of claim 7 or 8, wherein the sonication treatment is performed for at least 60 seconds.

10. The method of any one of claims 7 to 9, wherein the sonication treatment is performed at a power density of 0.4 W/cm^3.

11. The method of any one of claims 7 to 10, wherein the sonication treatment is an ultrasonic treatment.

12. The method of any one of claims 1 to 11, wherein agglomerates are removed prior to preparing the mixture comprising the carbon particle suspension and the enzyme.

13. The method of any one of claims 1 to 12, wherein the conductive surface is gold, platinum, or carbon.

14. The method of any one of claims 1 to 13, wherein the enzyme is a direct electron transfer (DET) enzyme.

15. The method of claim 14, wherein the DET enzyme is DET enzyme having analyte oxidizing activity.

16. The method of claim 15, wherein the analyte oxidizing activity is glucose oxidizing activity, lactose oxidizing activity, or lactate oxidizing activity.

17. The method of any one of claims 14 to 16, wherein the DET enzyme is cellobiose dehydrogenase (CDH), flavocytochrome b2 (FCb2), or a functional variant thereof.

18. The method of any one of claims 1 to 17, wherein in the mixture the ratio of enzyme:carbon particle suspension is 1:4.

19. An enzyme electrode comprising an analyte sensing layer, wherein said analyte sensing layer is a single layer comprising carbon black, a non-conducting cationic polymer, and an enzyme.

20. The enzyme electrode of claim 19, wherein carbon black is graphitized carbon black.

21. The enzyme electrode of claim 19 or 20, wherein the non-conducting cationic polymer is polyethyleneimine (PEI), diethylaminoethyl dextran (DEAE), polylysine, or polydiallyldimethylammonium chloride (PDADAMC).

22. The enzyme electrode of any one of claims 19 to 21, wherein the enzyme is a direct electron transfer (DET) enzyme.

23. The enzyme electrode of claim 22, wherein the DET enzyme is DET enzyme having analyte oxidizing activity.

24. The enzyme electrode of claim 23, wherein the analyte oxidizing activity is glucose oxidizing activity, lactose oxidizing activity, or lactate oxidizing activity.

25. The enzyme electrode of any one of claims of any one of claims 22 to 24, wherein the DET enzyme is cellobiose dehydrogenase (CDH), flavocytochrome b2 (FCb2), or a functional variant thereof.

26. The enzyme electrode of any one of claims 19 to 25, wherein the electrode is a gold, platinum, or carbon electrode.

27. An enzyme electrode produced by the method of any one of claims 1 to 18.

FIGURES

Fig. 1

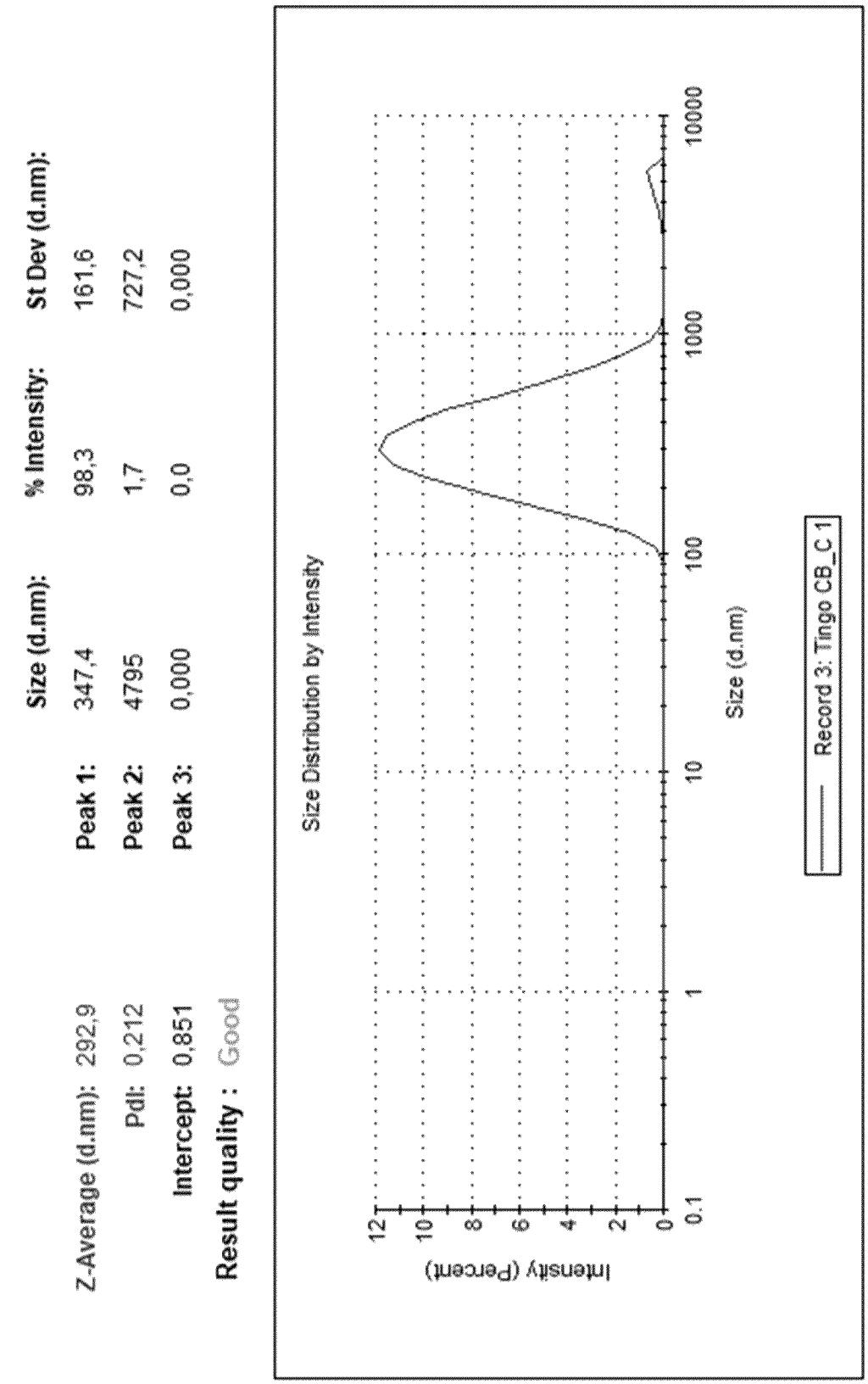
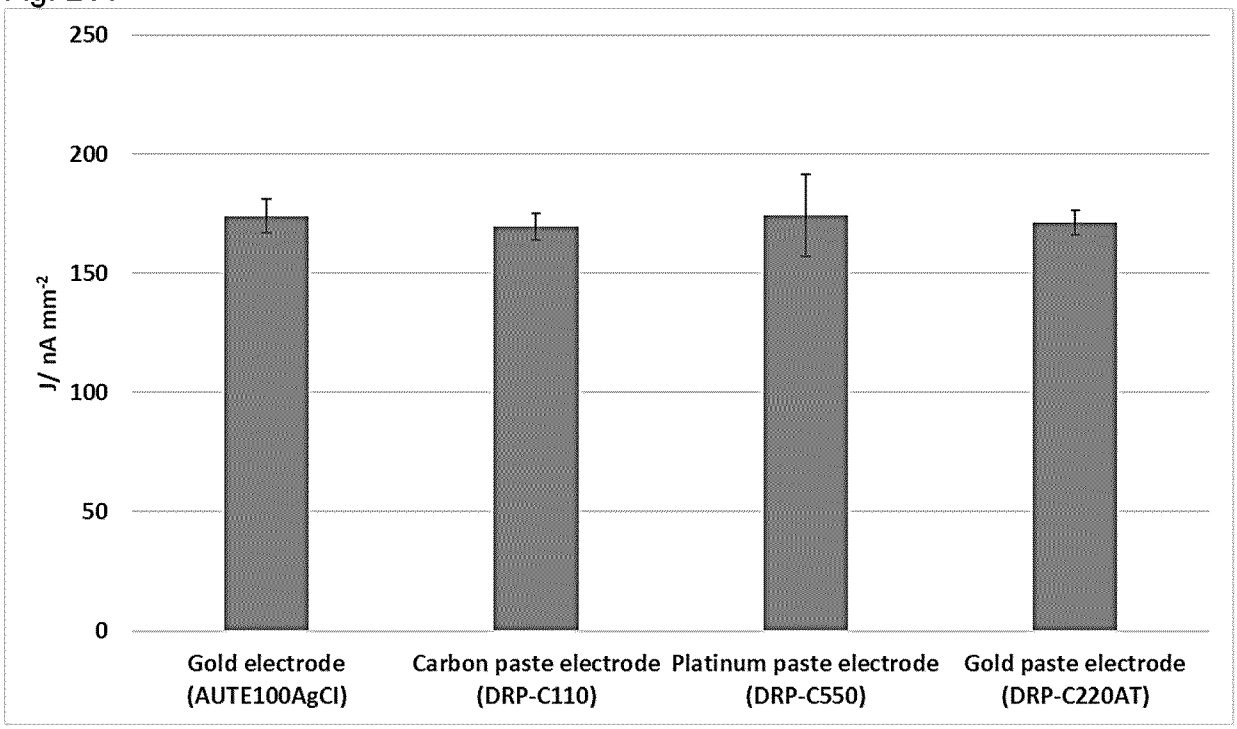


Fig. 2 A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200
150
100
50
0
J/ nA mm-2
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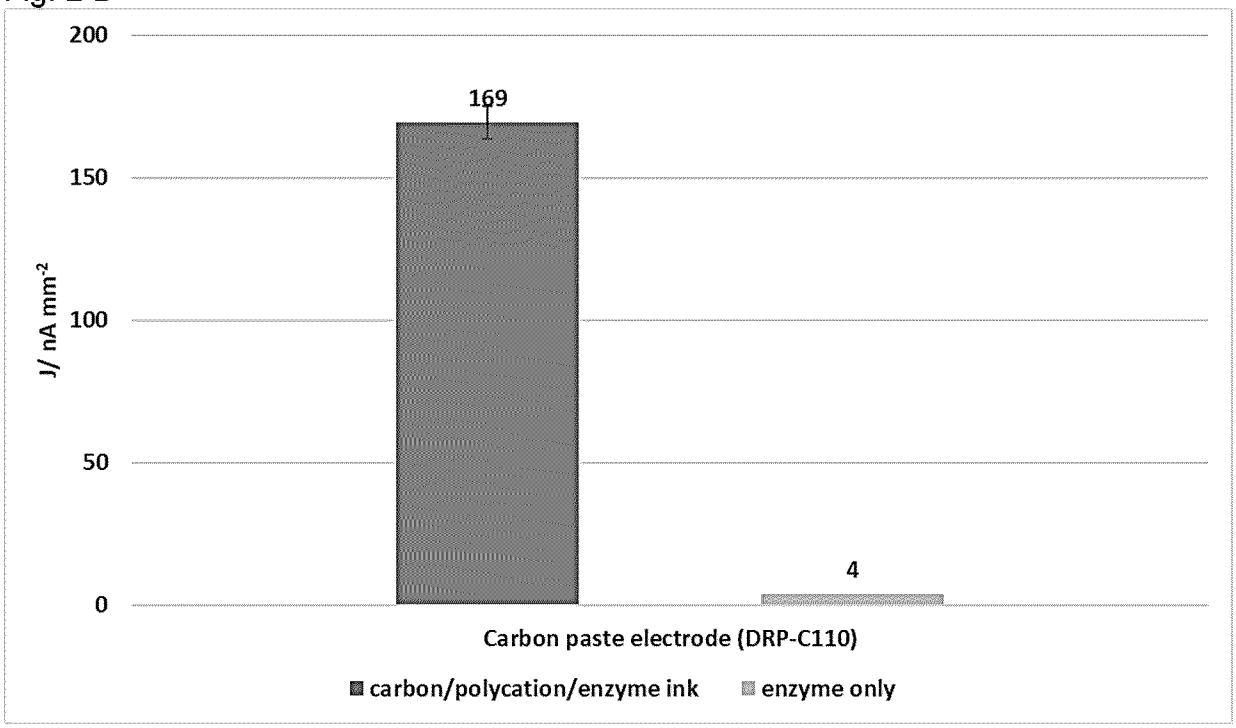
Carbon paste electrode (DRP-C110)
Platinum paste electrode (DRP-C550)
Gold paste electrode (DRP-C220AT)

Fig. 2 B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100
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J/ nA mm-2
169
4
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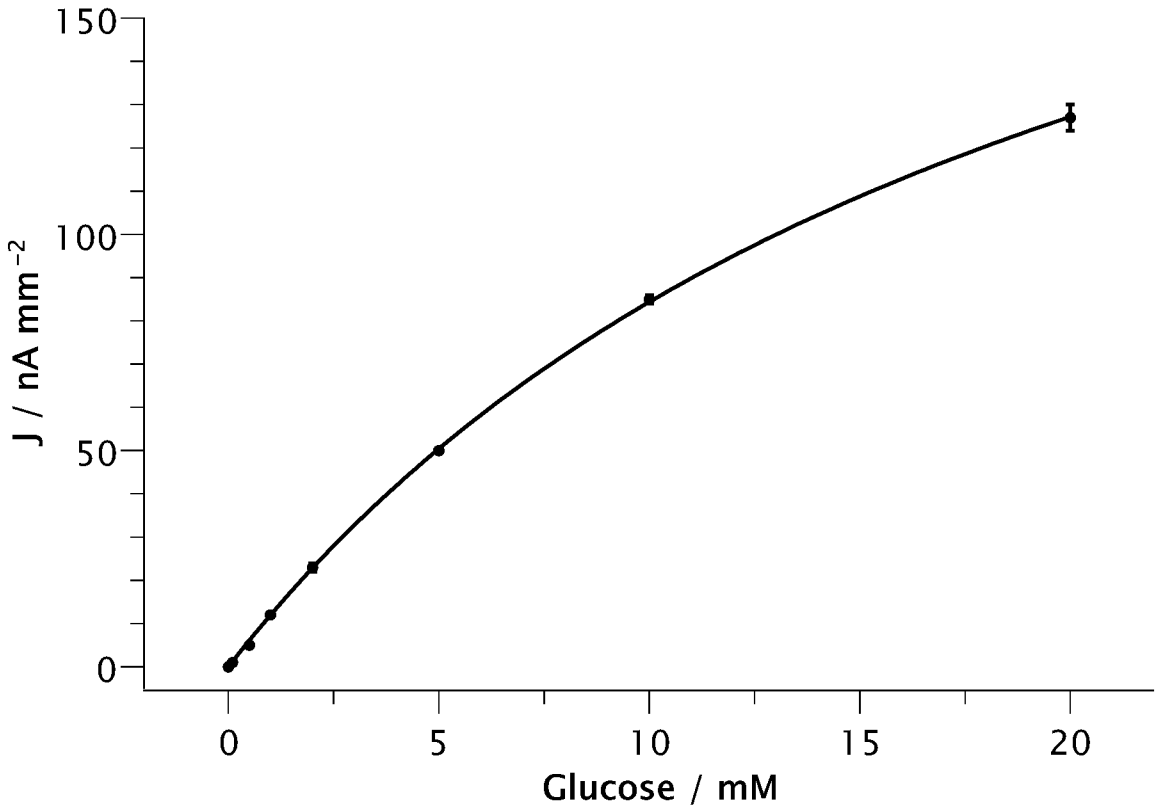
carbon/polycation/enzyme ink
enzyme only

Fig. 3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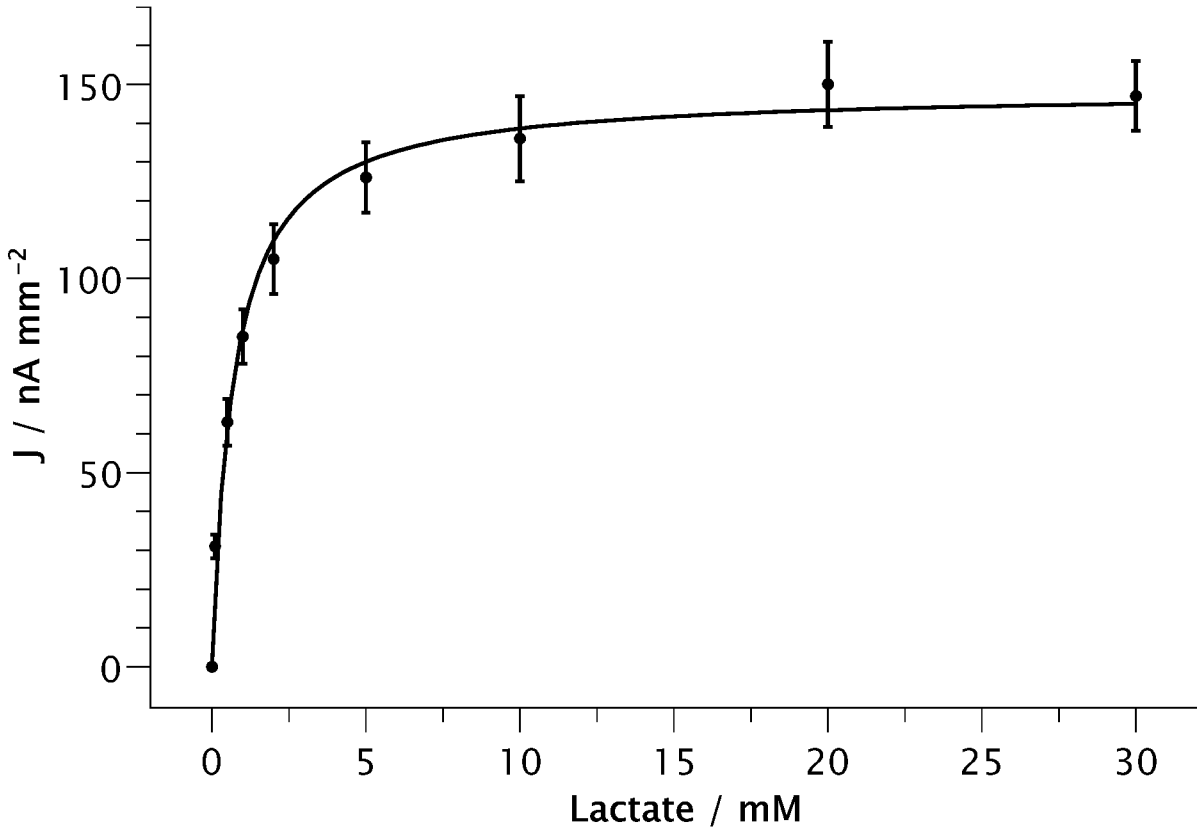
50
0
J / nA mm^{-2}
0
5
10
15
20
Glucose / mM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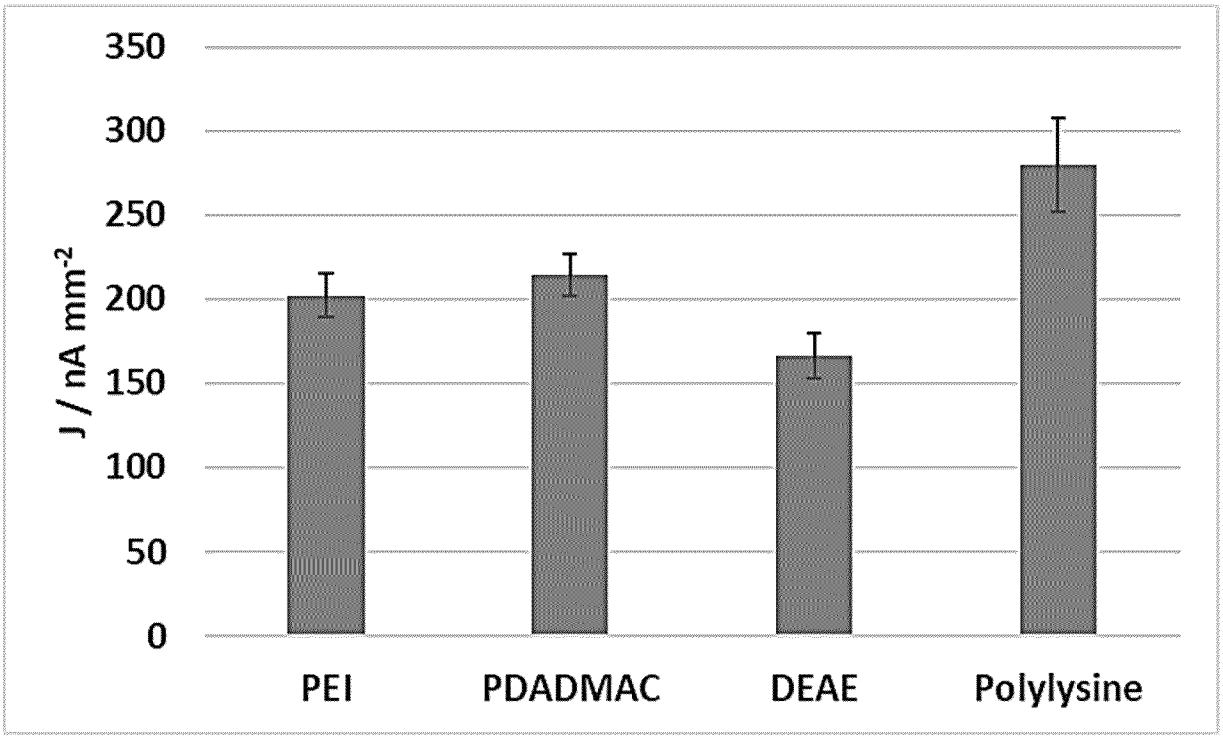
Lactate / mM

Fig. 4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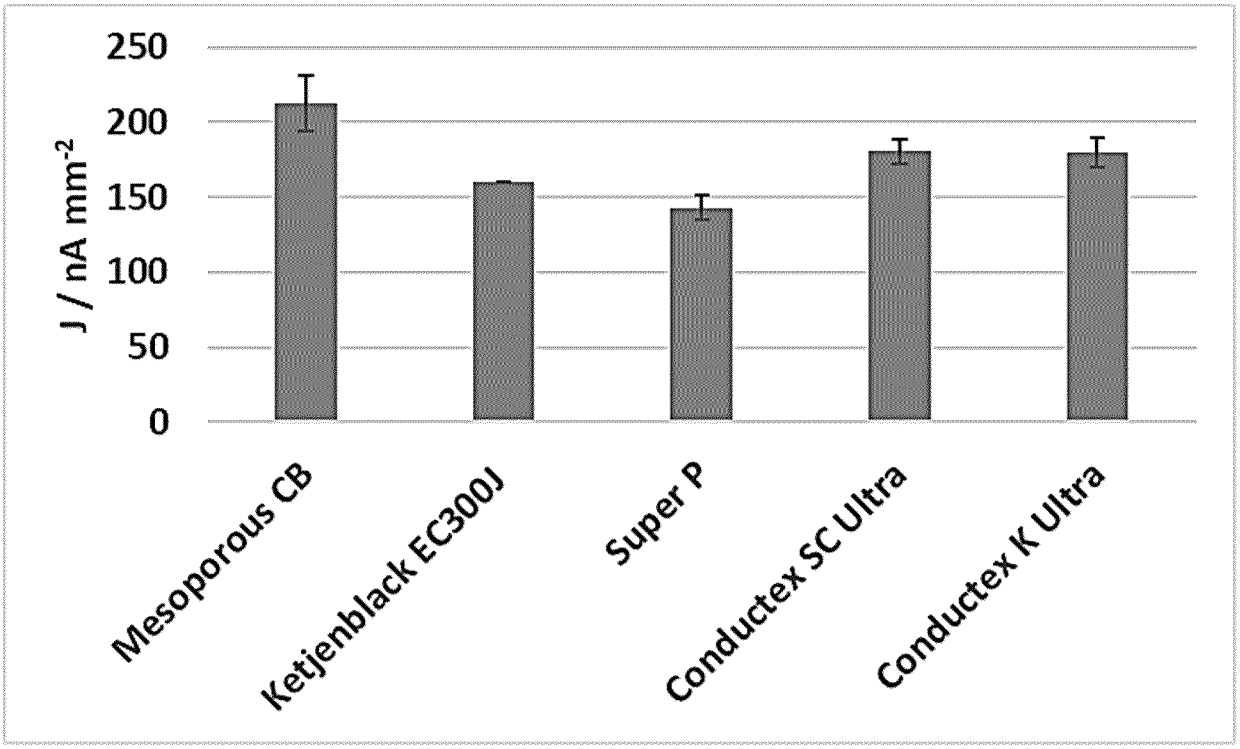
PDADMAC
DEAE
Polylysine

Fig. 5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Super P
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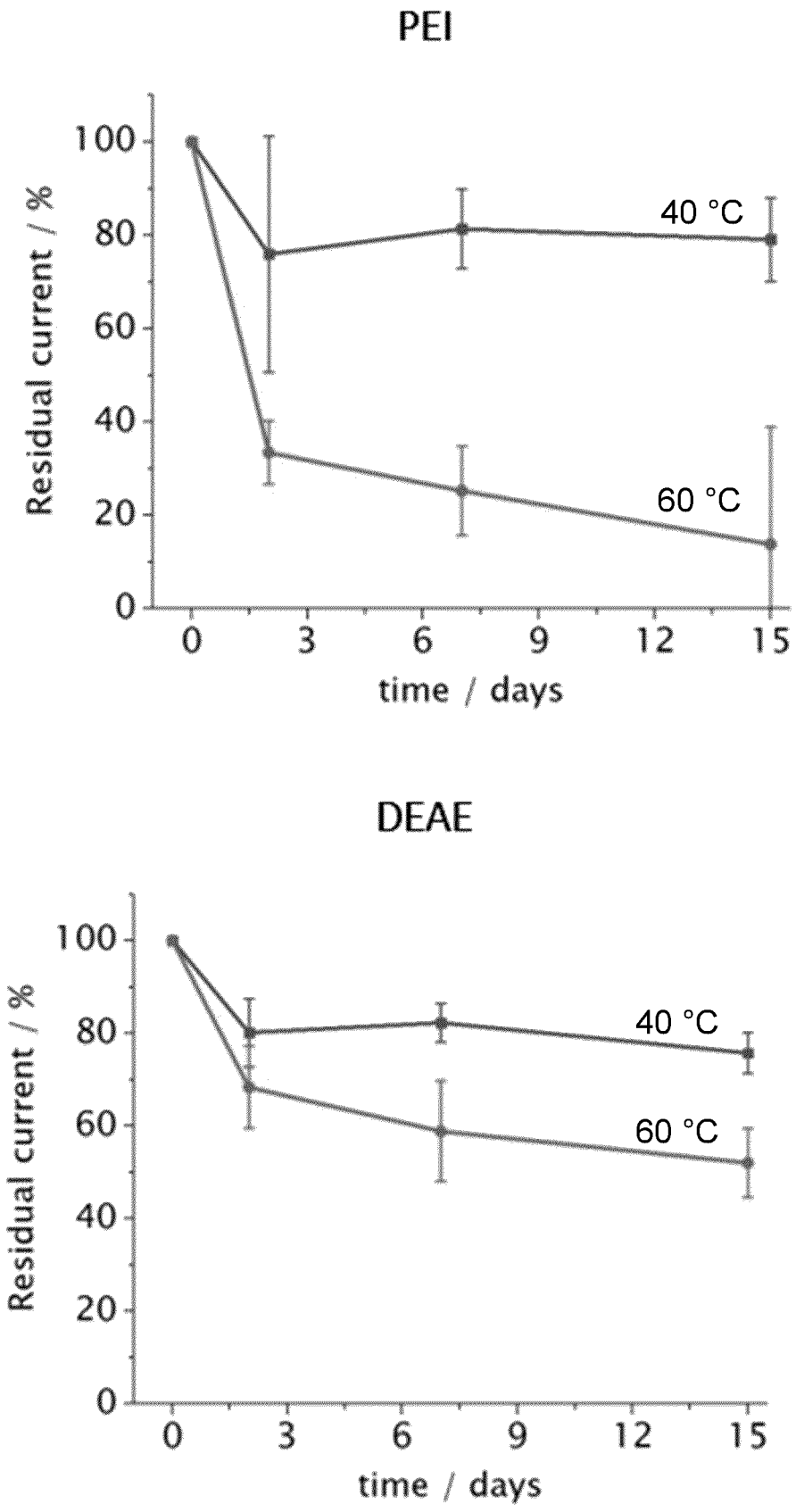
Conductex K Ultra

Fig 6


PEI
Residual current / %
100
80
60
40
20
0
40 °C
60 °C
0
3
6
9
12
15
time / days
DEAE
Residual current / %
100
80
60
40
20
0
40 °C
60 °C
0
3
6
9
12
15
time / days

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2024/067660

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/067660

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N27/327 C12Q1/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IBÁÑEZ-REDÍN GISELA ET AL: "Screen-printed electrodes modified with carbon black and polyelectrolyte films for determination of cancer marker carbohydrate antigen 19-9", MICROCHIMICA ACTA, SPRINGER VIENNA, VIENNA, vol. 187, no. 7, 1 July 2020 (2020-07-01), XP037180242, ISSN: 0026-3672, DOI: 10.1007/S00604-020-04404-6 [retrieved on 2020-07-01]	19-21,26
A	abstract section Chemical and reagents section Electrodes modification section Preparation of CB-polyelectrolyte electrochemical immunosensors page 417, right-hand column, line 4	1-18,27

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 September 2024	25/09/2024

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2

Form PCT/ISA/210 (second sheet) (April 2005)

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International application No
PCT/EP2024/067660

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