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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 August 2007 (16.08.2007)

PCT

(10) International Publication Number
**WO 2007/090873 A3**

(51) **International Patent Classification:**
***C12Q 1/00*** (2006.01) *C12N 9/06* (2006.01)

(21) **International Application Number:**
PCT/EP2007/051232

**(22) International Filing Date:** 8 February 2007 (08.02.2007)

**(25) Filing Language:** English

**(26) Publication Language:** English

**(30) Priority Data:**
06250703.3 9 February 2006 (09.02.2006) EP

(71) **Applicant** *(for all designated States except US):* **Leiden University** [NL/NL]; Faculty of Science, Einsteinweg 55, NL-233CC Leiden (NL).

**(72) Inventors; and**
**(75) Inventors/Applicants** *(for US only):* **CANTERS, Gerard W.** [NL/NL]; Leiden University, Faculty of Science, Einsteinweg 55, NL-233CC Leiden (NL). **WIJMA, Hein Jakob** [NL/US]; Duke University Medical Centre, Department of Biochemistry, Durham, North Carolina 27710 (US).

**(74) Agent: JONES, Helen Marjorie Meredith;** Broadgate House, 7 Eldon Street, London Greater London EC2M 7LH (GB).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, **BR,** BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FT, GB, GD, GE, GH, GM, GT, HN, **HR,** HU, **ID,** IL, IN, IS, **JP,** KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW

**(84) Designated States** *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report*
— *with amended claims*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

**(88) Date of publication of the international search report:**
4 October 2007

**Date of publication of the amended claims:** 3 January 2008

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**(54) Title:** OXIDOREDUCTASES AND PROCESSES UTILISING SUCH ENZYMES

**(57) Abstract:** In Cu-containing nitrite reductase from Alcaligenes faecalis S-6 the axial methionine ligand of the typel site was replaced (M150G) to make the copper atom accessible to external ligands that might affect the enzyme's catalytic activity. The type-1 site optical spectrum of M150G (A460/A600 = 0.71) differs significantly from that of the native nitrite reductase (A460/A600 = 1.3). The reduction potential of the type-1 site of nitrite reductase M150G (EM = 312 - 5 mV versus hydrogen) is higher than that of the native enzyme (EM = 213 - 5 mV). M150G has a lower catalytic activity (kcat = 133 - 6 s-1) than the wild-type nitrite reductase (kcat = 416 - 10 - s 1). The binding of external ligands to M150G restores spectral properties, reduction potential (EM < 225 mV), and catalytic activity (kcat = 374 - 28 s-1). Also the M150H (A460/A600 = 7.7, EM = 104 - 5 mV, kcat = 0.099 - 0.006 s-1) and M150T (A460/A600 = 0.085, EM = 340 - 5 mV, kcat = 126 - 2 s -1) variants were characterized to compare their properties with those of M150G. Crystal structures show that the ligands act as allosteric effectors by displacing Met62 which moves to bind to the Cu in the position emptied by the M150G mutation. The reconstituted type-1 site has an otherwise unaltered geometry. The observation that a rearranged ligand can introduce allosteric control in a redox enzyme suggests potential for structural and functional flexibility of copper-containing redox sites.

WO 2007/090873 A3

# AMENDED CLAIMS

## received by the International Bureau on 25 September 2007 (25.'9.07)

23. A sensor according to claim 22 in which the separate aliquots each contain the same sample suspected of containing a solute molecule, whereby a profile of enzyme activity is determined to identify the solute.

24. A sensor according to any of claims 18 to 23 in which the or each electron transfer protein is as defined in any of claims 5 to 8.

25. A sensor according to any of claims 18 to 24 in which the substrate is nitrite.

26. A sensor according to any of claims 18 to 24 in which the solute molecule is selected from metabolites, such as creatinine, cholesterol, drugs, hormones, sugars, fatty acids and peptides, alcohols, imidazoles, a cetamide and dialkylsulphides.

27. Apparatus comprising a sensor according to any of claims 18 to 26, a counter electrode an electrical circuit connected to the electrodes and current voltage or resistance measuring device in the circuit.