

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

(19) World Intellectual Property
Organization

International Bureau

(43) International Publication Date
12 August 2021 (12.08.2021)



(10) International Publication Number
WO 2021/156200 A1

(51) International Patent Classification:
C12N 9/02 (2006.01) C12P 7/22 (2006.01)

(21) International Application Number:
PCT/EP2021/052295

(22) International Filing Date:
01 February 2021 (01.02.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
20155122.3 03 February 2020 (03.02.2020) EP

(71) Applicants: **BAYER AKTIENGESELLSCHAFT**
[DE/DE]; Kaiser-Wilhelm-Allee 1, 51373 Leverkusen
(DE). **BAYER PHARMA AKTIENGESELLSCHAFT**
[DE/DE]; Müllerstr. 178, 13353 Berlin (DE).

(72) Inventors: **KENSCH, Oliver**; Brunostr.125, 50259 Pul-
heim (DE). **THEDE, Kai**; Stargarder Str. 62, 10437 Berlin
(DE). **HELFRICH, Petra**; Im Wolfsgarten 17, 14612
Falkensee (DE). **SKALDEN, Lilly**; Medebacher Weg 22,
13507 Berlin (DE). **ZORN, Ludwig**; Osianderweg 45a,
13509 Berlin (DE). **TRENNER, Sabine**; Geibelstr. 47,
12305 Berlin (DE). **BURMEISTER, Jens**; Eidechsenweg
41, 51375 Leverkusen (DE). **KRETSCHMANN, Nils**;
Geschwister-Scholl-Str. 27, 40789 Monheim am Rhein
(DE). **RICHTER, Florian**; Glasstr. 37, 50823 Köln (DE).
COCO, Wayne; Am Forsthaus 27, 50259 Pulheim (DE).
LUDWIG, Marcus; Knipprather Str. 136, 40789 Mon-
heim am Rhein (DE). **BULUT, Dalia**; Am Schlagbaum
15, 51399 Burscheid (DE). **BERENDES, Frank**; Goeben-
str. 38, 48151 Münster (DE). **PILLING, Jens**; Schulstr.
9, 44289 Dortmund (DE). **WAGNER, Jakob**; Eichenweg
19, 16727 Oberkrämer OT Schwante (DE). **LINHOFF,**
Ruben; Hackländerstr. 32, 50825 Köln (DE).

(74) Agent: **BIP PATENTS**; Alfred-Nobel-Str. 10, 40789 Mon-
heim am Rhein NRW (DE).

(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, CZ, DE, DJ, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN,
KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD,
ME, MG, MK, MN, 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, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, 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, 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).

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a
patent (Rule 4.17(ii))

Published:

— with international search report (Art. 21(3))
— before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))
— with sequence listing part of description (Rule 5.2(a))

(54) Title: P450 BM3 MONOOXYGENASE VARIANTS FOR C19-HYDROXYLATION OF STEROIDS

(57) Abstract: The present invention relates to novel recombinant *Bacillus megaterium* cytochrome P450-monoxygenase (P450-BM3) variants for the C19 hydroxylation of steroids and derivatives thereof or for improved BM3 protein expression. In particular, the present invention also relates to methods and processes using P450-BM3 variants for the production of estrone and estradiol. The invention further relates to nucleotide sequences, constructs and vectors for the expression of these P450-BM3 variants

WO 2021/156200 A1