Electronic Supplementary Information (ESI) to

A bifunctional old yellow enzyme from *Penicillium roqueforti* is involved in the ergot alkaloid biosynthesis

Nina Gerhards[a] and Shu-Ming Li[a]

[a] Institut für Pharmazeutische Biologie und Biotechnologie, Philipps-Universität Marburg, Robert-Koch-Straße 4, D-35037 Marburg, Germany

To whom correspondence should be addressed: Prof. Shu-Ming Li, Telephone: +49-6421-28-22461; FAX: +49-6421-28-26678; E-mail: shuming.li@staff.uni-marburg.de

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Tables

Table S1 ^{1}H-NMR data for festuclavine

Proton	festuclavine isolated from the incubation mixture of chanoclavine-I with FgaDH$_{Pr}$, FgaOx3$_{Pr3}$ and FgaFS	festuclavine isolated from the incubation mixture of chanoclavine-I with ChaDH, FgaOx3$_{Pr3}$ and EasG
2	7.04 s	7.04 s
4α	2.93 m	2.93 m
4β	3.71 dd (14.1; 3.5)	3.71 dd (14.1; 3.5)
5	3.25 m	3.26 m
7α	3.59 brd (11.8)	3.59 brd (11.8)
7β	2.90 m	2.89 m
8	2.20 m	2.20 m
9α	2.83 brd (13.5)	2.83 brd (13.5)
9β	1.38 d (7.5)	1.38 d (7.5)
10	3.27 m	3.27 m
12	6.93 d (7.1)	6.93 d (7.1)
13	7.12 t (7.6)	7.12 t (7.6)
14	7.21 d (8.2)	7.21 d (8.2)
17	1.16 d (6.6)	1.15 d (6.6)
18	3.09 s	3.09 s

The spectra were taken at 500 MHz in CD_3OD whose signal was used as reference (3.31 ppm). The data correspond well to the NMR data for festuclavine published previously (Wallwey *et al*., *Org. Biomol. Chem.*, 2010, **8**, 3500-3508).

Table S2 ^{1}H-NMR data for chanoclavine-I aldehyde

Proton	chanoclavine-I aldehyde isolated from the incubation mixture of chanoclavine-I with	
	ChaDH and heat-denaturated FgaOx3$_{Pr3}$	**ChaDH and FgaOx3$_{Pr3}$**
1	8.01 brs	8.01 brs
2	6.98 brs	6.98 brs
4α	2.87 ddd (15.3; 7.4; 1.1)	2.86 ddd (15.3; 7.4; 1.1)
4β	3.28 ddd (15.3; 4.2; 1.1)	3.28 ddd (15.3; 4.2; 1.1)
5	3.13 m	3.12 m
7	9.49 s	9.49 s
9	6.50 dq (10.2; 1.1)	6.51 dq (10.2; 1.1)
10	4.31 dd (10.2; 6.9)	4.30 dd (10.2; 6.9)
12	6.72 d (7.1)	6.71 d (7.1)
13	7.15 dd (8.1; 7.1)	7.15 dd (8.1; 7.1)
14	7.25 d (6.7)	7.25 d (6.7)
17	2.00 d (1.1)	2.00 d (1.1)
18	2.53 s	2.52 s

The spectra were taken at 500 MHz in $CDCl_3$ whose signal was used as reference (7.26 ppm). The data correspond well to the NMR data for chanoclavine-I and chanoclavine-I aldehyde published previously (Wallwey *et al.*, *Microbiology*, 2012, **158**, 1634-1644).

Figures

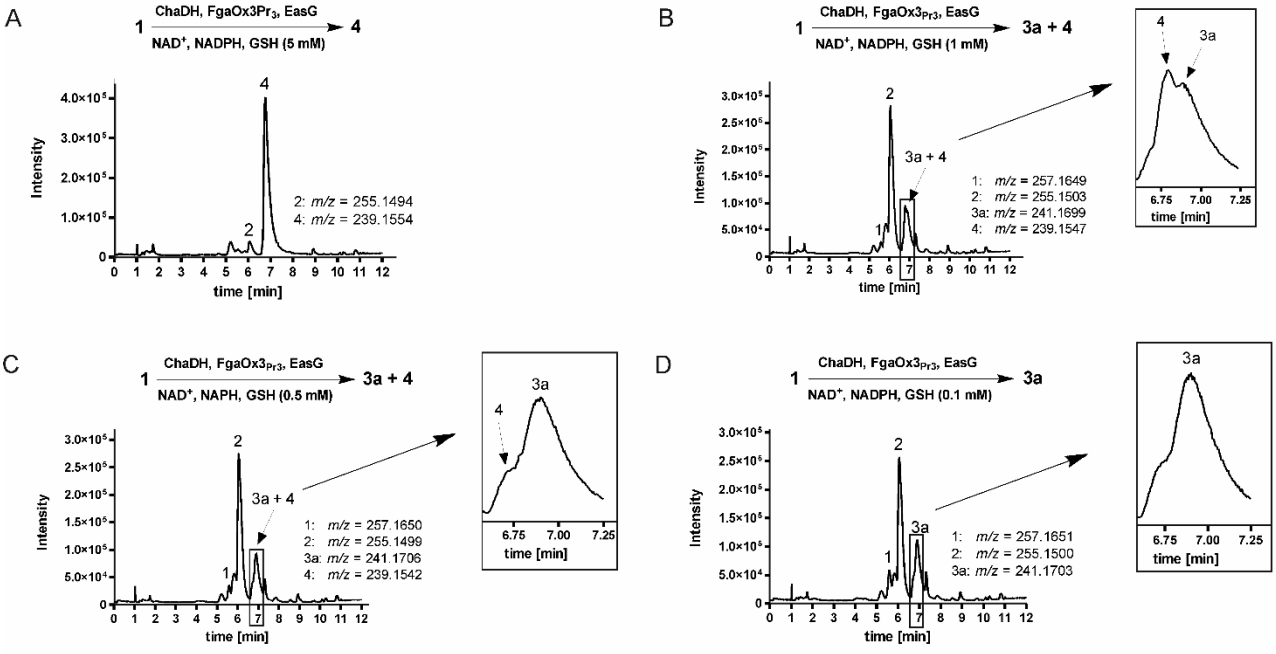

Figure S1 LC-MS analysis of the *in vitro* assays with ChaDH, FgaOx3$_{Pr3}$ and EasG. The reaction mixtures contained 1 mM chanoclavine-I, 10 µg enzymes, 5 mM of the respective cofactors and different concentrations of GSH and were incubated at 30°C for 16h.

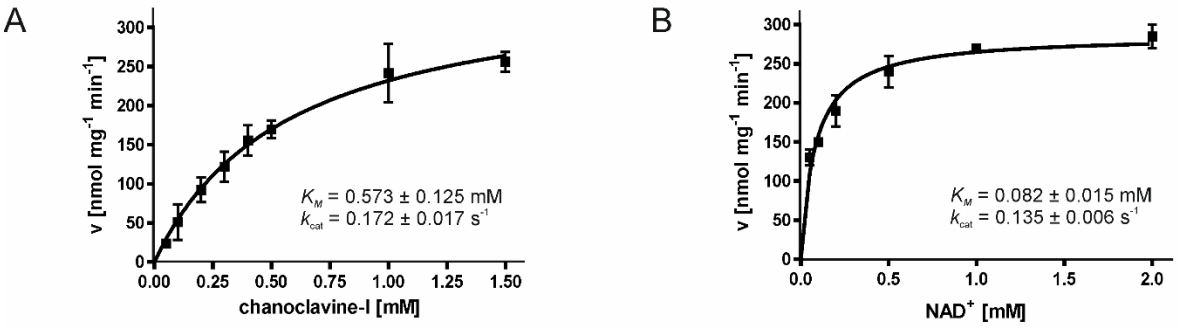

Figure S2 Determination of the kinetic parameters for FgaDH$_{Pr}$.

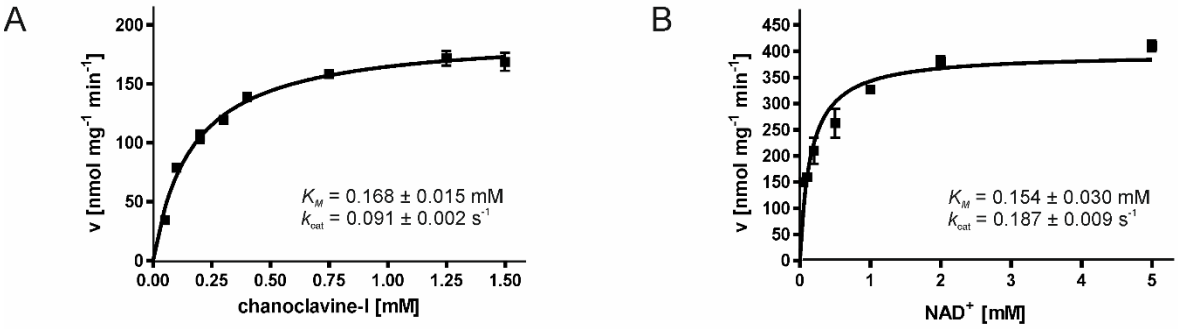

Figure S3 Determination of the kinetic parameters for FgaDH$_{Pr}$ in the presence of FgaOx3$_{Pr3}$.

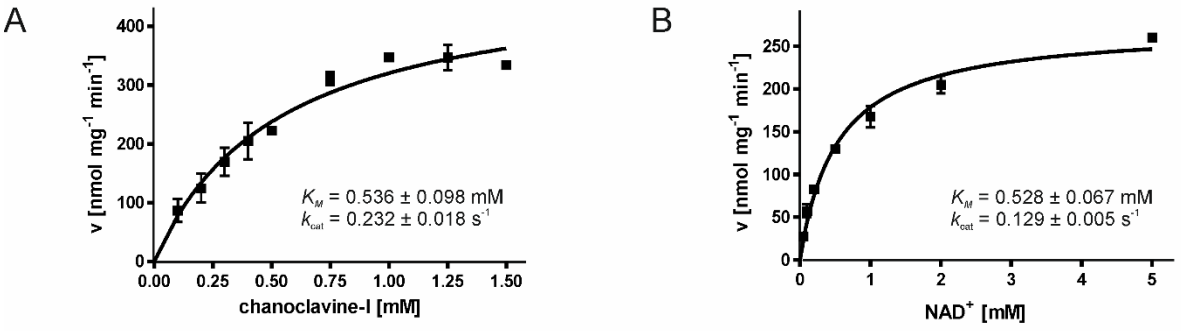

Figure S4 Determination of the kinetic parameters for $FgaDH_{Pca}$.

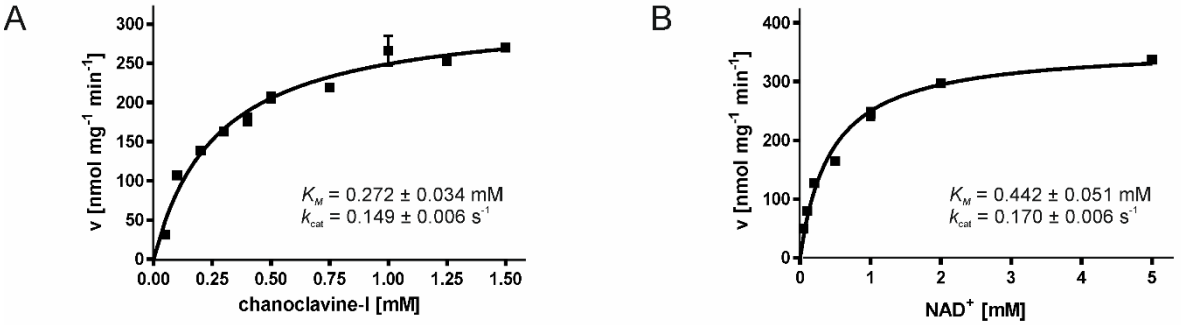

Figure S5 Determination of the kinetic parameters for $FgaDH_{Pca}$ in the presence of $FgaOx3_{Pr3}$.

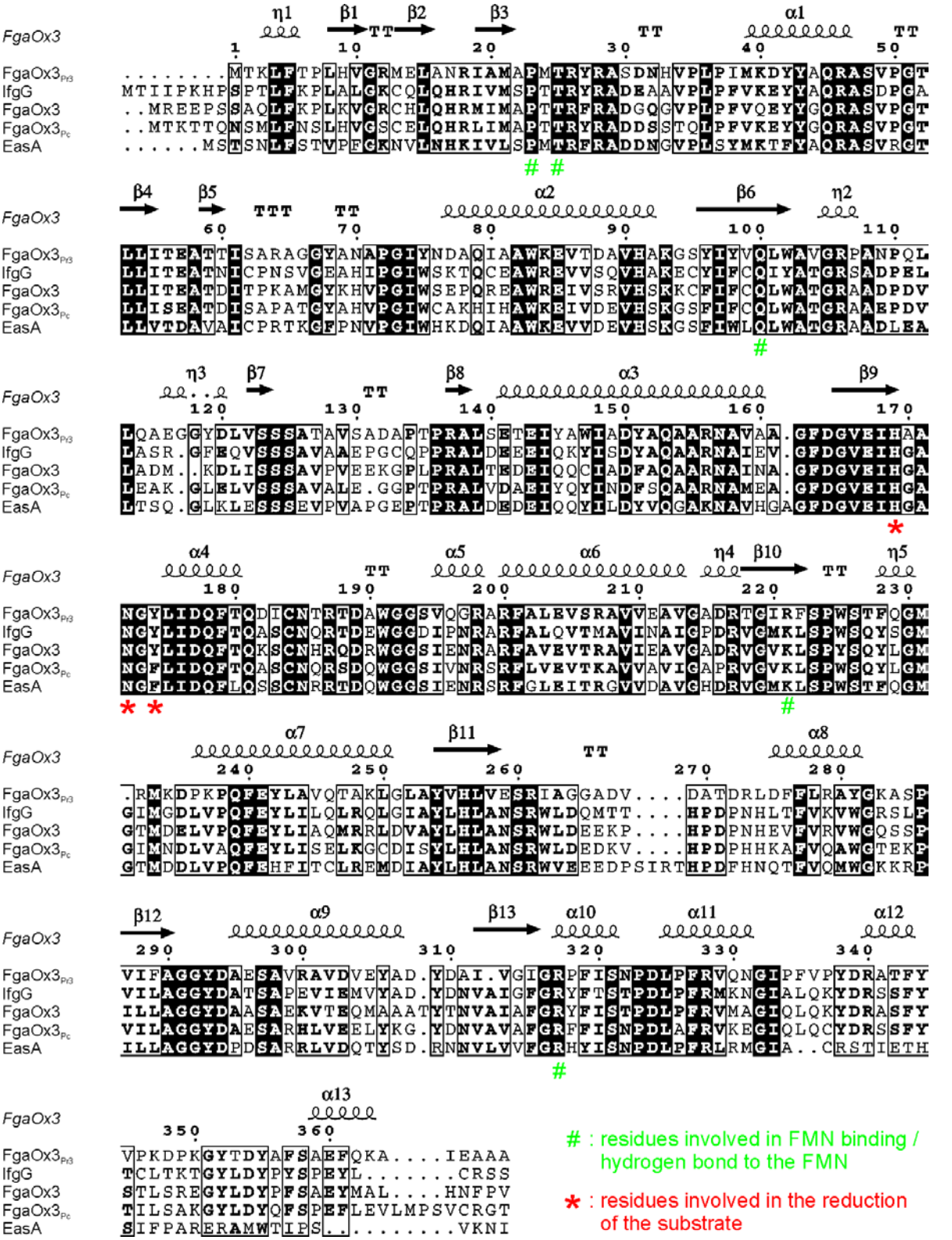

Figure S6 Structure-based sequence alignments of different OYEs involved in the ergot alkaloid biosynthesis. The protein sequences were taken from the NCBI database with the accession numbers EAL94095 (FgaOx3 from *A. fumigatus*), CDM30151 (IfgG from *P. roqueforti*), CDM33403 (FgaOx3$_{Pr3}$ from *P. roqueforti*), CAG28312 (EasA from *C. purpurea*) and AFM84626 (FgaOx3$_{PC}$ from *P. commune*).