STEREOSELECTIVE MONOREDUCTION OF BULKY 1,2-DICARBONYLS CATALYZED BY A BENZIL REDUCTASE FROM *Pichia glucozyma* (KRED1-Pglu)

Marco Rabuffetti[1,2*], Pietro Cannazza[2], Martina L. Contente[2], Andrea Pinto[2], Diego Romano[2], Pilar Hoyos[3], Andrés R. Alcántara[3], Ivano Eberini[4], Tommaso Laurenzi[4], Louise Gourlay[5], Flavio Di Pisa[5], Francesco Molinari[2]

[1] Department of Chemistry, University of Milan, via Golgi 19, 20133 Milan, Italy; [2] Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Via Mangiagalli 25, 20133 Milan, Italy; [3] Department of Chemistry in Pharmaceutical Sciences (QUICIFARM), Pharmacy Faculty, Complutense University, Plaza de Ramon y Cajal, s/n, 28040 Madrid, Spain; [4] Department of Pharmacological and Biomolecular Sciences (DiSFeB), University of Milan, Via Balzaretti 9, 20133 Milan, Italy; [5] Department of Biosciences, University of Milan, Via Celoria 26, 20133 Milan, Italy

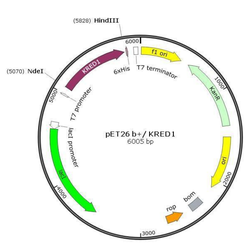
* contact: marco.rabuffetti1@unimi.it

BIOTRANS GRAZ I 2021

SUMMARY

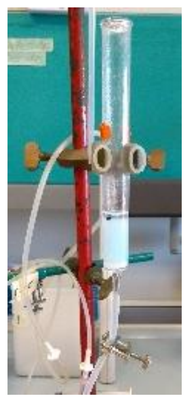
Enantiomerically enriched hydroxyketones are well-established intermediates for the synthesis of several bioactive compounds [1]. A NADPH-dependent benzil reductase from the non-conventional yeast *Pichia glucozyma* (KRED1-Pglu) [2] was over-expressed in *E. coli*, purified and exploited to catalyze the asymmetric monoreduction of bulky aromatic 1,2-dicarbonyl compounds. The cofactor was recycled by an enzyme-coupled system (glucose-glucose dehydrogenase (GDH) from *Bacillus megaterium*). The recombinant KRED1-Pglu showed a wide range of activity (24-97% conversion) and excellent stereoselectivity (*ee* ≥ 96% in all but one case). On the contrary, it proved either inactive or poorly active towards most 1,3- and 1,4-dicarbonyls. In order to understand this peculiar behavior, the enzyme was crystallized (1.77 Å resolution) and its active site was investigated to identify its recognition residues. QM/MM and classical calculations also allowed for a proposal of the catalytic mechanism [3].

KRED1-Pglu EXPRESSION AND PURIFICATION



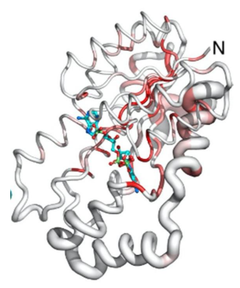
KRED1-Pglu plasmid

over-expression in *E. Coli* BL21(DE3)Star



purification by affinity chromatography (Ni^{II}-affinity resin)

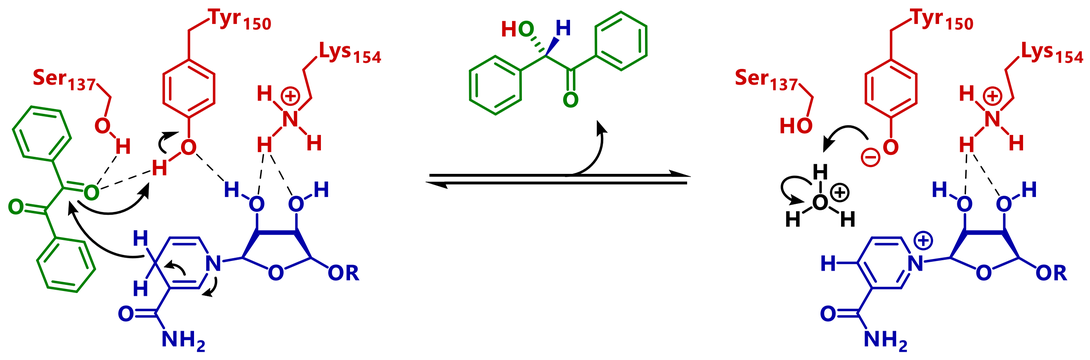
Substrate	k_{cat} (s^{-1})	K_m (mM)	k_{cat} / Km ($s^{-1}mM^{-1}$)
1b	4.04	0.62	6.45
1c	0.25	0.68	0.37
1d	0.02	0.33	0.06
1e	0.12	2.10	0.06
1f	0.14	0.82	0.17



KRED1-Pglu

15 mg pure protein/g cells
20-25 mU/mg specific activity

CRYSTAL STRUCTURE AND *IN SILICO* CALCULATIONS



Scheme 1. Proposed mechanism for the monoreduction of benzil (**1a**) catalyzed by KRED1-Pglu (QM/MM calculations)

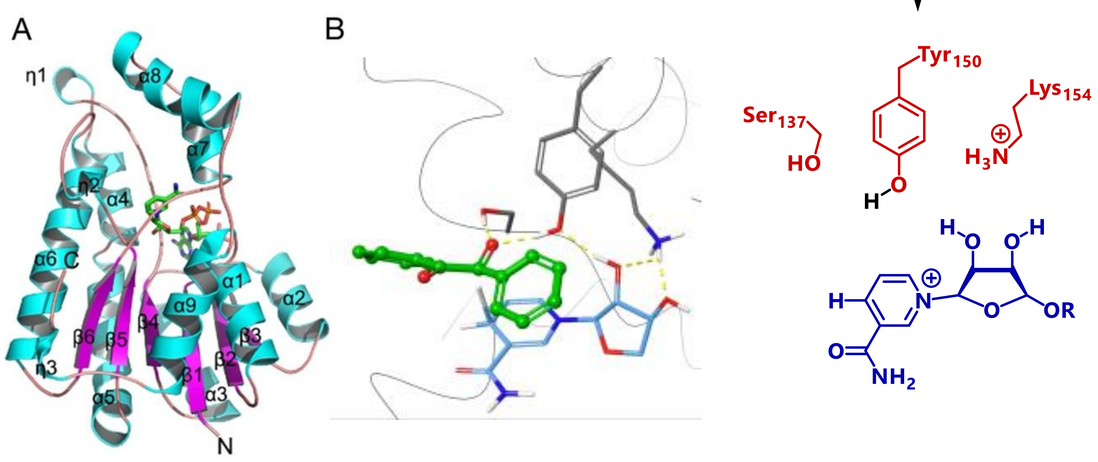
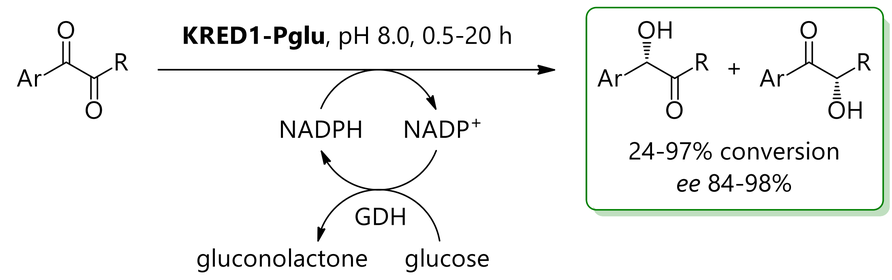


Figure 1. A) Secondary structure ribbon representation of the KRED1-Pglu monomer in complex with NADP⁺ (crystal structure at 1.77 Å resolution). **B**) Close up of the transition state corresponding to the reduction of benzil (**1a**) by QM/MM calculations

BIOTRANSFORMATIONS



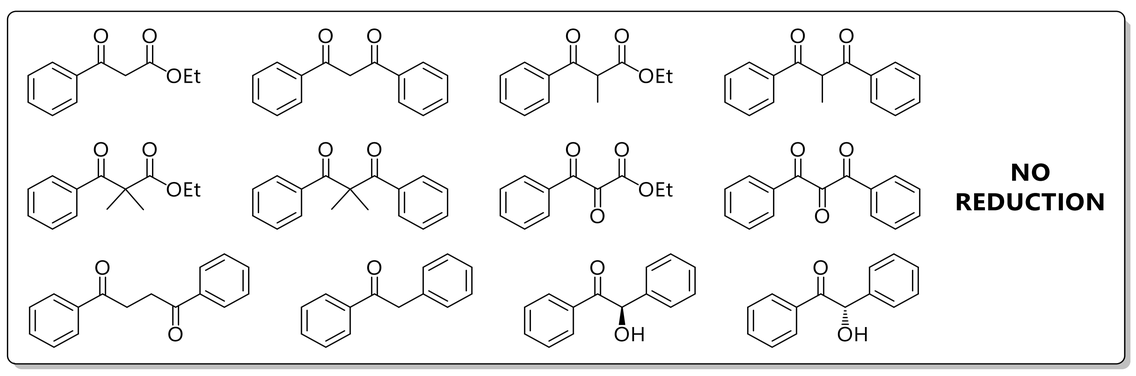
Substrate	Product	Relative Rate (%)[a]	Conv. (%)[b]	Time (h)
1a	**2a** (*ee* > 98%)[c]	100	97	2
1b	**2b** (*ee* > 98%)	134	>97	0.5
1c	**2c** (*ee* > 98%)	96	>97	2
1d	**2d** (*ee* > 98%) + **3d** (*ee* = 96%); **2d** / **3d** = 32 : 68 [b]	60	61	5
1e	**2e** (*ee* > 98%) + **3e** (*ee* > 98%); **2e** / **3e** = 45 : 55	76	96	3
1f	**2f** (*ee* > 98%)	120	96	0.5
1g	**2g** (*ee* > 98%)	30	40	5
1h	**2h** (*ee* = 87%) + **3h** (*ee* = 84%); **2h** / **3h** = 17 : 83	21	24	5
1i	**2i** (*ee* > 98%)	34	60	20

REACTIVE

POORLY REACTIVE

Table 1. KRED1-Pglu-catalyzed monoreduction of different dicarbonyls. Conditions: [substrate] = 5 mM (in 50 mM TRIS·HCl buffer, pH 8.0), KRED1-Pglu (20 mU/mL), GDH (1 U/mL), $NADP^+$ (0.1 mM, 2 mol%), D-(+)-glucose (20 mM, 4 equiv), DMSO (10% v/v).

[a] Determined by spectrophotometric assay; [b] Determined by achiral HPLC; [c] Determined by chiral HPLC.



NO REDUCTION

References

[1] G. Aullón, P. Romea, F. Urpí *Synthesis* **2017**, *49*, 484-503.

[2] a) M. L. Contente, I. Serra; M. Brambilla, I. Eberini, E. Giannazza, V. De Vitis, F. Molinari, P. Zambelli, D. Romano *Appl. Microbiol. Biotechnol.* **2016**, *100*, 193-201; b) M. L. Contente, F. Dall'Oglio, F. Annunziata, F. Molinari, M. Rabuffetti, D. Romano, L. Tamborini, D. Rother, A. Pinto *Catal. Lett.* **2020**, *150*, 1176-1185.

[3] M. Rabuffetti, P. Cannazza, M. L. Contente, A. Pinto, D. Romano, P. Hoyos, A. R. Alcántara, I. Eberini, T. Laurenzi, L. Gourlay, F. Di Pisa, F. Molinari *Bioorg. Chem.* **2021**, *108*, 104644.

UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI CHIMICA

DeFENS

Milano DiSFeB
Dipartimento di Scienze Farmacologiche e Biomolecolari

DBS Dipartimento di Bioscienze

UNIVERSIDAD COMPLUTENSE MADRID