

SUPPLEMENTAL DATA

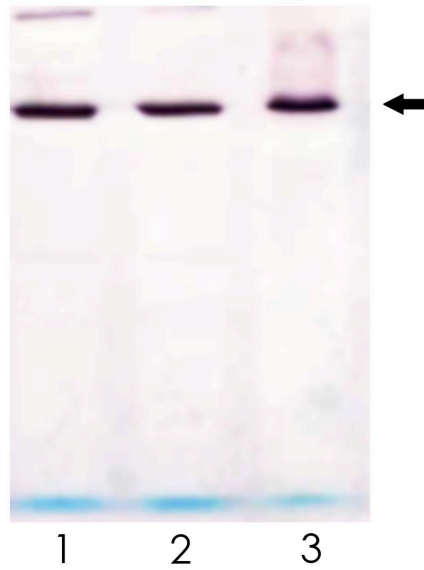
Structure and catalytic mechanism of 3-ketosteroid- Δ^4 -(5 α)-dehydrogenase (Δ^4 -(5 α)-KSTD)
from *Rhodococcus jostii* RHA1*

**Niels van Oosterwijk^{1,2}, Jan Knol^{1,3}, Lubbert Dijkhuizen³, Robert van der Geize³ and
Bauke W. Dijkstra²**

*²Laboratory of Biophysical Chemistry and ³Department of Microbiology, Groningen
Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen,
Nijenborgh 7, 9747 AG Groningen, The Netherlands*

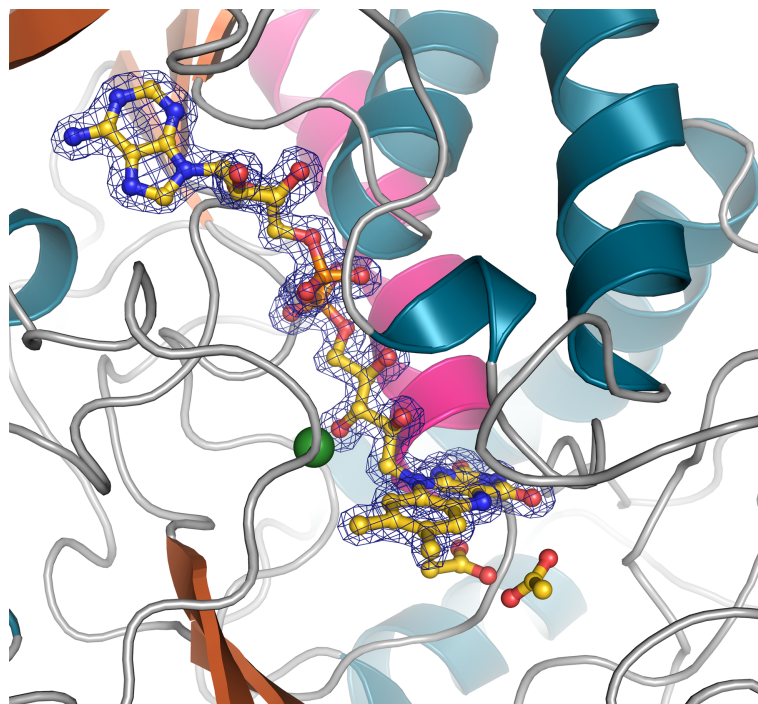
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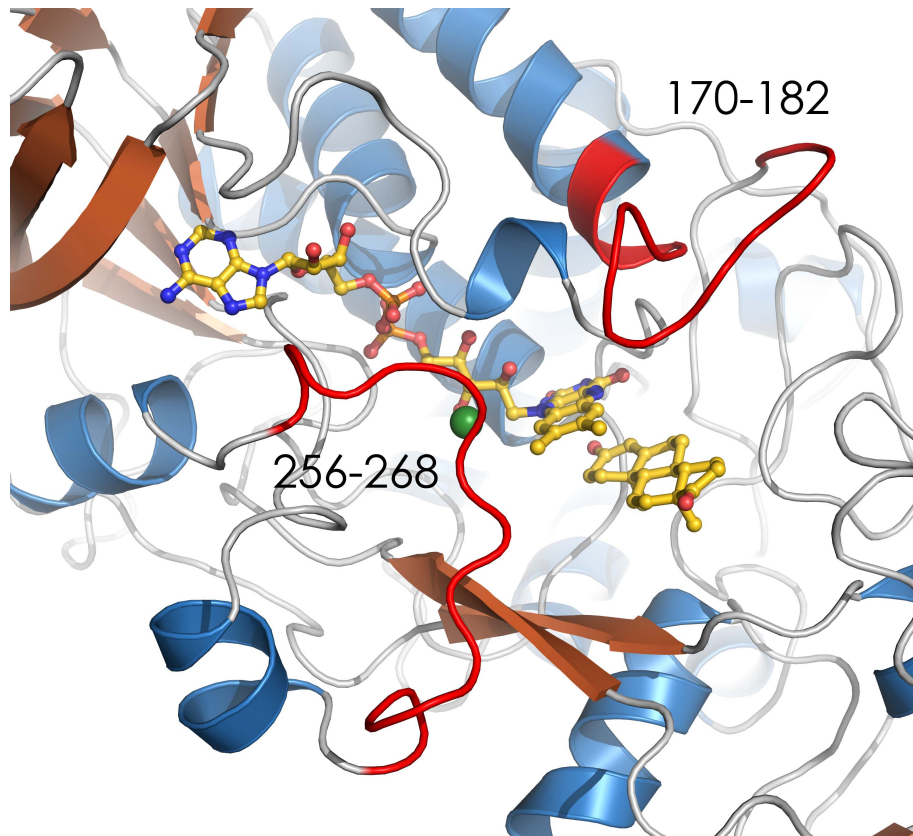
Supplemental Figure 1

Activity-stained native PAGE gel of Δ^4 -(5 α)-KSTDs from *R. erythropolis* SQ1 (lane 1), *R. rhodochrous* DSM43269 (lane 2) and *R. jostii* RHA1 (lane 3). Δ^4 -(5 α)-KSTD activity was visualized (black arrow) by incubating a native PAGE gel loaded with cell-free extracts (30-50 μ g of protein) in a Tris-HCl buffer containing the electron acceptors phenazine methosulphate and nitro blue tetrazolium, as well as the substrate 1-(5 α)-androstene-3,17-dione (1-(5 α)-AD).



Supplemental Figure 2

FAD binding in $\Delta 4-(5\alpha)$ -KSTD. The electron density around the FAD is shown at 1σ . The C-terminal helix, which is thought to provide a helix dipole moment important for the reaction, is shown in pink. The FAD and acetate molecules are yellow and the chloride ion is shown in green.



Supplemental Figure 3

Two loops (residues 170-182 and 256-268, shown in red) close to the active site. Both show less well-defined electron density, which suggests flexibility of these loops. The FAD and 4AD are shown in yellow and the chloride ion in green.

Supplemental Table I – Sequences of sense primers for site-directed mutagenesis
The mutated nucleotides and corresponding amino acids are printed in bold.

Mutation	Primer										
W136A	tgg	ggc	gaa	ccc	gga	gcg	gaa	ccc	ccg	ttc	gac
	W	G	E	P	G	A	E	P	P	F	D
W136F	gg	ggc	gaa	ccc	gga	ttc	gaa	ccc	ccg	ttc	gac
		G	E	P	G	F	E	P	P	F	D
Y319F	gtt	ccg	gag	gac	acc	ttc	tcc	ggg	cgg	atc	gg
	V	P	E	D	T	F	S	G	R	I	
Y466A	gtg	tgc	gcg	ggc	ggc	gcc	gcc	agc	ggc	acc	tcg
	V	C	A	G	G	A	A	S	G	T	S
Y466F	gtg	tgc	gcg	ggc	ggc	ttc	gcc	agc	ggc	acc	tcg
	V	C	A	G	G	F	A	S	G	T	S
S468T	gcg	ggc	ggc	tac	gcc	acc	ggc	acc	tcg	ctc	gg
	A	G	G	Y	A	T	G	T	S	L	