Preparation of human dihydroorotate dehydrogenase for interaction studies with lipid bilayers

Supplementary material

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Table S1. Expression conditions resulting in the highest volumetric and specific DHODH activities in the lysates of TUNER(DE3) *E. coli* cells for different constructs. Cell lysates were prepared by resuspending cell pellets in Assay Buffer and disrupting the cell suspension using a VibraCell ultrasonicator (20% amplitude, 3 s per pulse, 3×10 pulses). Enzymatic activity and protein concentrations were determined as outlined in Material and Methods.

Protein construct	OD ₆₀₀ at induction	Temperature (°C)	IPTG (µM)	Media	Activity (U/L culture)	Specific activity (U/mg)
His- <i>Ec</i> DHODH	0.6	18	100	TB	7633	4.36
DHODH-His	0.8	18	20	TB	3734	1.60
His- ∆29DHODH	0.6	18	100	TB	2229	1.16
His- ∆10DHODH	0.6	18	100	TB	250	0.13
Δ29DHODH- His	0.6	18	1000	TB	1092	0.42

Compound	Molecular Weight	Volume	Density	Area per lipid molecule (A_m)
	$g mol^{-1}$	Å ³	$\mathrm{g~cm}^{-3}$	\AA^2
POPC	760	1243 ¹	1.016	64.2 ²
TOCL	1502	2 395 ¹	1.041	129.8 ³
Q ₁₀	863.4	1576.5 ⁴	0.909	N.C.
∆29DHODH	39 780	26 858 ⁵	1.241 ⁶	1520 ⁵
<i>Ec</i> DHODH	36 775	26 858 ⁵	1.1856	1520 ⁵

Table S2. Physical properties used for QCM-D calculations.

N.C. = Not calculated.

¹Calculated from component volumes according to Armen *et al.* 1998 ^[1].

²From Kucerka *et al.* 2011^[2]

³From Pan *et al.* 2015 ^[3].

⁴Assuming a quinone headgroup and 10 isoprene units.

⁵Assuming a spherical protein with diameter = 44 Å.

⁶Assuming a hydration of 50%.

DHODH A29DHODH Ecdhodh	MAWRHLKKRAQDAVIILGGGGLLFASYLMATGDERFYAEHLMPTLQGLLDPESAHRLAVR GMATGDERFYAEHLMPTLQGLLDPESAHRLAVR YY-PFV-RKALFQLDPERAHEFTF- :* .: . **** **.::.	60 33 24
DHODH ∆29DHODH EcDHODH	FTSLGLLPRARFQDSDMLEVRVLGHKFRNPVGIAAGFDKHGEAVDGLYKMGFGFVFTSLGLLPRARFQDSDMLEVRVLGHKFRNPVGIAAGFDKHGEAVDGLYKMGFGFV-QQLRRITGTPFEALVRQKVPAKPVNCMGLTFKNPLGLAAGLDKDGECIDALGAMGFGSI.*::*.*::*:: </td <td>115 88 83</td>	115 88 83
DHODH ∆29DHODH EcDHODH	EIGSVTPKPQEGNPRPRVFRLPEDQAVINRYGFNSHGLSVVEHRLRARQQKQAKLTEDGL EIGSVTPKPQEGNPRPRVFRLPEDQAVINRYGFNSHGLSVVEHRLRARQQKQAKLTEDGL EIGTVTPRPQPGNDKPRLFRLVDAEGLINRMGFNNLGVDNLVENVKKAHYDG ***:***:** ** :**:*** : :::*** ***. *:. :::	175 148 135
DHODH ∆29DHODH EcDHODH	PLGVNLGKNKTSVDAAEDYAEGVRVLGPLADYLVVNVSSPNTAGLRSLQGKAELRRLL PLGVNLGKNKTSVDAAEDYAEGVRVLGPLADYLVVNVSSPNTAGLRSLQGKAELRRLL VLGINIGKNKDTPVEQGKDDYLICMEKIYAYAGYIAINISSPNTPGLRTLQYGEALDDLL **:*:**** : :.:** :.: *.*::***** ***:** * **	233 206 195
DHODH ∆29DHODH EcDHODH	TKVLQERDGLRRVHRPAVLVKIAPDLTSQDKEDIASVVKELGIDGLIVTNTTVSRPAG TKVLQERDGLRRVHRPAVLVKIAPDLTSQDKEDIASVVKELGIDGLIVTNTTVSRPAG TAIKNKQNDLQAMHHKYVPIAVKIAPDLSEEELIQVADSLVRHNIDGVIATNTTLDRSLV * : ::::.*: :*: : *******:::: ::*. :***:*.***:.*	291 264 255
DHODH ∆29DHODH EcDHODH	LQGALRSETGGLSGKPLRDLSTQTIREMYALTQGRVPIIGVGGVSSGQDALEKIRAGASL LQGALRSETGGLSGKPLRDLSTQTIREMYALTQGRVPIIGVGGVSSGQDALEKIRAGASL QGMKNCDQTGGLSGRPLQLKSTEIIRRLSLELNGRLPIIGVGGIDSVIAAREKIAAGASL .:******: **: :**: :**: :**:***********	351 324 315
DHODH ∆29DHODH EcDHODH	VQLYTALTFWGPPVVGKVKRELEALLKEQGFGGVTDAIGADHRREFPGENLYFQ VQLYTALTFWGPPVVGKVKRELEALLKEQGFGGVTDAIGADHRR VQIYSGFIFKGPPLIKEIVTHI	405 368 337

Figure S1. Multiple sequence alignment of DHODH, $\Delta 29$ DHODH and *Ec*DHODH. The alignment was done with CLUSTAL OMEGA(1.2.4)^[4]. In the DHODH sequence, the mitochondrial signal and transmembrane segment that determines both its import and correct insertion into the inner mitochondrial membrane^[5] are marked in yellow and blue, respectively. The $\alpha 1-\alpha 2$ micro domain connecting the N-terminus of DHODH to its catalytic domain, which is found in nearly all family 2 DHODH, is shown in green.^[6]. Remaining residues due to the TEV-cleavage site in DHODH-His, His- $\Delta 29$ DHODH and His-*Ec*DHODH are marked in grey.



Fig S2. QCM-D frequency (Δ f) and dissipation (Δ D) traces for the interaction between *Ec*DHODH (0.4 mg/mL) and lipid bilayers consisting of (A) 100 mol% POPC. (B) 90 mol% POPC + 10% Q₁₀. (C) 90 mol% POPC + 10% TOCL. (D) 80 mol% POPC + 10% Q₁₀ + 10% TOCL. Frequency traces are shown in blue, dissipation traces are shown in red. Asterisks (*) indicate addition of the lipid vesicles. Arrows indicate addition of the protein. Rinses with Milli-Q water and Buffer (10 mM Tris-HCl, 100 mM NaCl, pH 7.4) are indicated in the figures.

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