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-*Ec*DHODH	0.6	18	100	TB	7633	4.36
DHODH-His	0.8	18	20	TB	3734	1.60
His-Δ29DHODH	0.6	18	100	TB	2229	1.16
His-Δ10DHODH	0.6	18	100	TB	250	0.13
Δ29DHODH-His	0.6	18	1000	TB	1092	0.42

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

Compound	Molecular Weight g mol^{-1}	Volume Å^3	Density g cm^{-3}	Area per lipid molecule (A_m) Å^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.
Δ29DHODH	39 780	26 858[5]	1.241[6]	1520[5]
*Ec*DHODH	36 775	26 858[5]	1.185[6]	1520[5]

N.C. = Not calculated.

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

[2]From Kucerka *et al.* 2011[2]

[3]From Pan *et al.* 2015 [3].

[4]Assuming a quinone headgroup and 10 isoprene units.

[5]Assuming a spherical protein with diameter = 44 Å.

[6]Assuming a hydration of 50%.

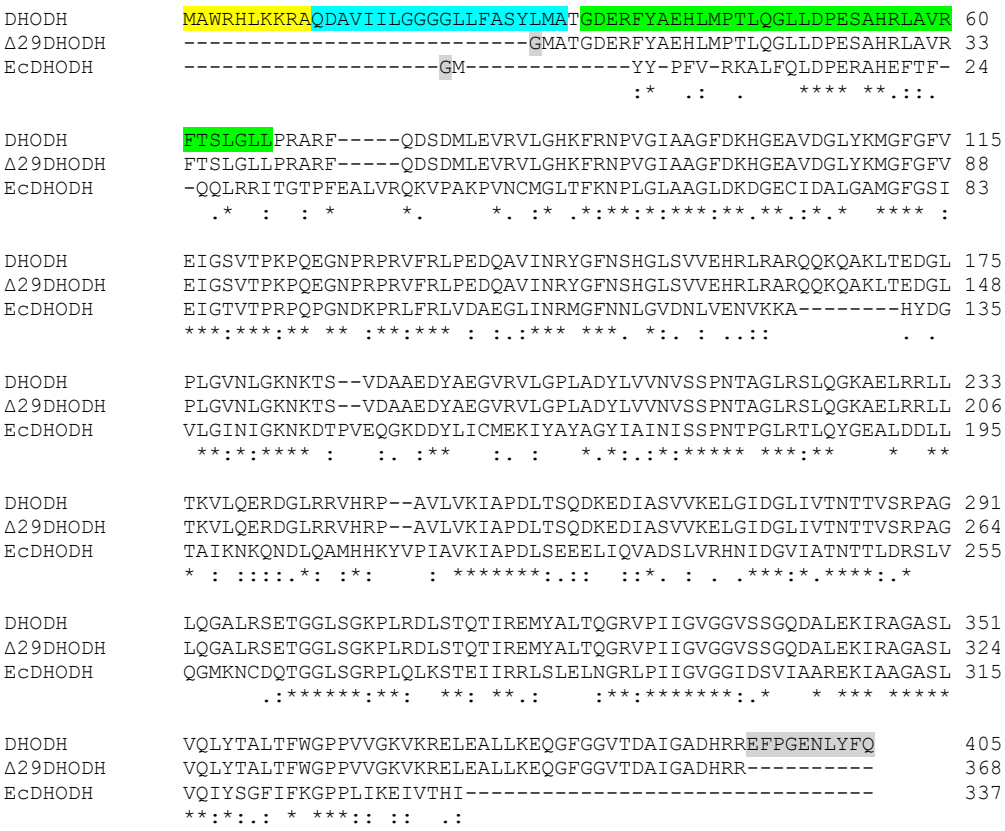

Figure S1. Multiple sequence alignment of DHODH, Δ29DHODH and *Ec*DHODH. The alignment was done with CLUSTAL OMEGA(1.2.4) [4]. In the DHODH sequence, the mitochondrial signal and transmembrane segment that determines both its import and correct insertion into the inner mitochondrial membrane [5] are marked in yellow and blue, respectively. The α1-α2 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-Δ29DHODH and His-*Ec*DHODH are marked in grey.

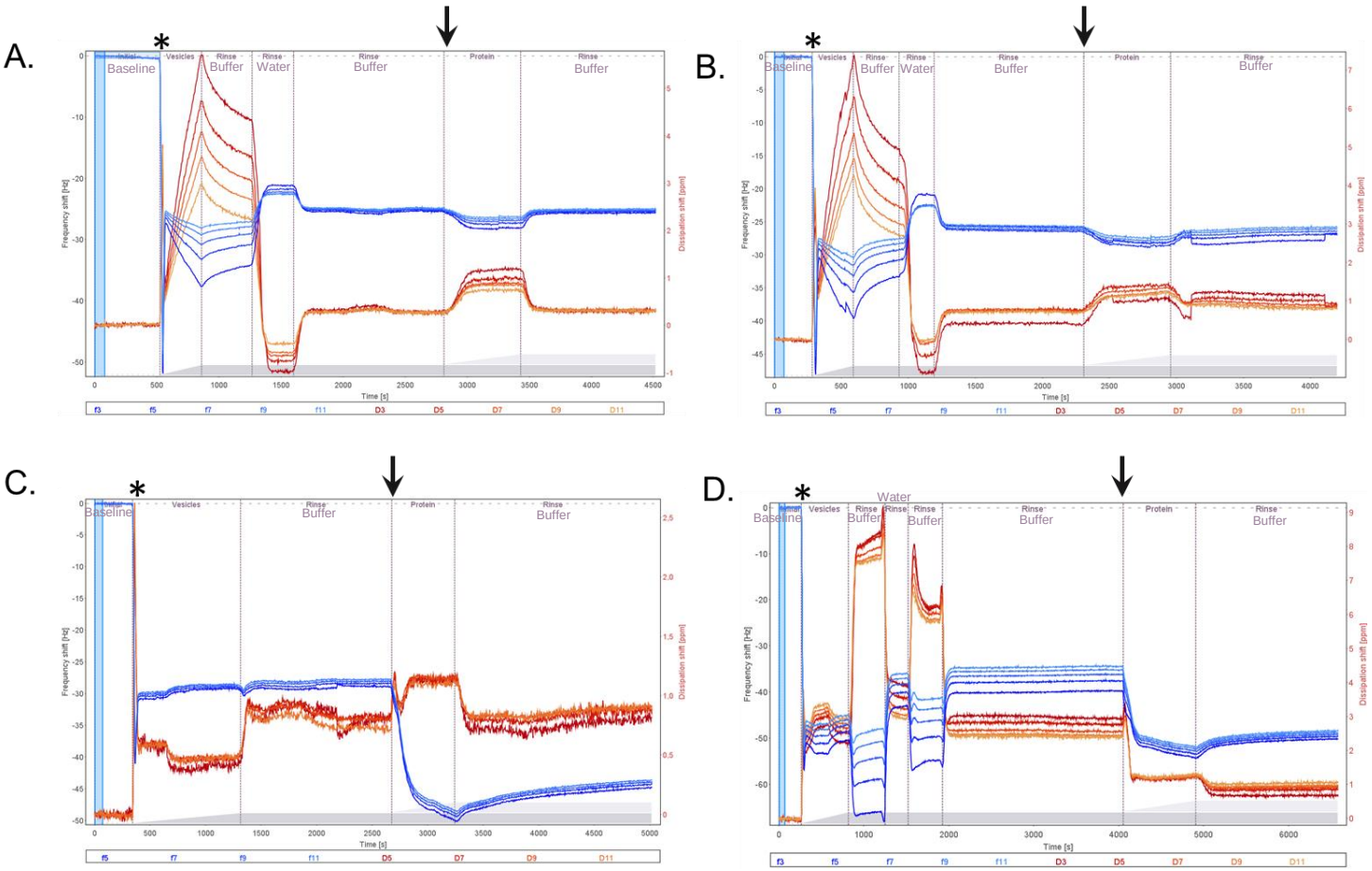

Fig S2. QCM-D frequency (Δf) and dissipation (ΔD) traces for the interaction between *Ec*DHODH (0.4 mg/mL) and lipid bilayers consisting of (A) 100 mol% POPC. (B) 90 mol% POPC + 10% Q_{10}. (C) 90 mol% POPC + 10% TOCL. (D) 80 mol% POPC + 10% Q_{10} + 10% TOCL. Frequency traces are shown in blue, dissipation 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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