Supplementary Information

Lyophilization protects [FeFe]-hydrogenases against O_2-induced H-cluster degradation

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This file includes:

Supplementary Table S1: ***Comparison of HYDA1 protein purification yields***

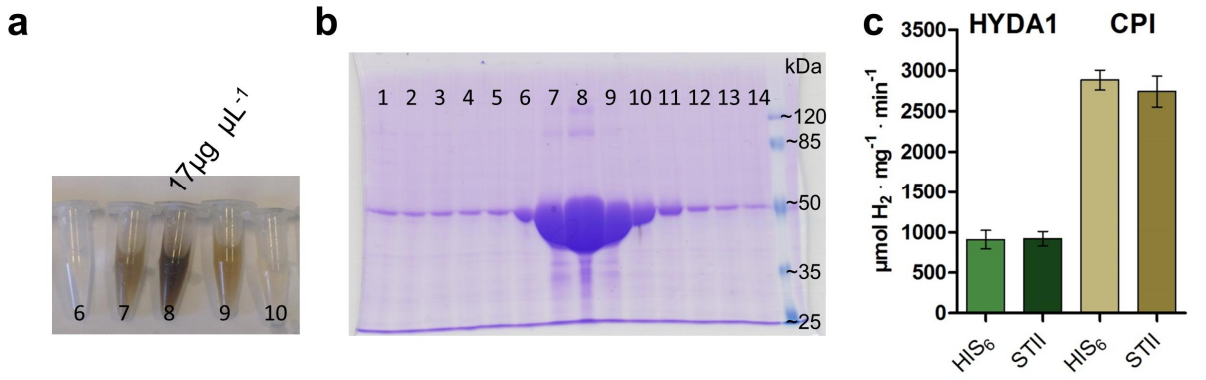
Supplementary Figure S1: ***Purification and activity measurements of HIS_6- and STII-tagged HYDA1 and CPI***

Supplementary References

Supplementary Table S1. Comparison of HYDA1 protein purification yields.[a]

Expression host	Cluster occupation	Purification tag	Yield [mg L^{-1}]	Spec. activity [µmol H_2 · mg^{-1} · min^{-1}]	Reference
C. reinhardtii	[4FeH] + [2FeH]	w/o	$3.2 \cdot 10^{-4}$	360	Roessler and Lien, 1984
C. reinhardtii	[4FeH] + [2FeH]	w/o	0.07	935	Happe and Naber, 1993
E. coli	[4FeH] + [2FeH]	STII	0.9	150	King et al, 2006
S. oneidensis	[4FeH] + [2FeH]	STII	0.45	700	Sybirna et al, 2008
C. acetobutylicum	[4FeH] + [2FeH]	STII	1.0	625	v. Abendroth et al, 2008
E. coli	[4FeH] + [2FeH]	STII	30	641	Kuchenreuther et al, 2010
E. coli	[4FeH] + [2FeH]	HIS_6	45	660	This work
E. coli	[4FeH] [4FeH] + [$2FeH_{adt}$]	HIS_6	90	0 950	This work

[a]Specific activities in the present work were determined using the sodium-dithionite-driven hydrogen-production-assay with methyl viologen as an artificial electron mediator. Activities in ref.[1] were determined using 10 µM of ferredoxin for electron shuttling instead and the total reported yield had a purity of approximately 40%. HYDA1[4FeH] was *in vitro* maturated with $2Fe_{adt}$ prior to activity determination[2].



Supplementary Figure S1. Purification and activity measurements of HIS_6- and STII-tagged HYDA1 and CPI. A Ni-NTA gravity-flow column with a volume of 4 mL was used to purify 45 mg of HYDA1[4FeH]-HIS_6 from 0.5 L of *E. coli* culture. (A) Photograph of the main [FeFe]-hydrogenase elution fractions (6-10), the dark-brown fraction 8 with a volume of 1.1 mL has a concentration of 17 µg protein μL^{-1} (340 µM). (B) The Coomassie-stained SDS-PAGE (10%) shows one significant protein band at a molecular weight of 50 kDa. Each lane (1-14) was loaded with 5 µL of protein eluate. (C) Comparison of the specific activities of Strep-tag II (STII) and HIS_6-tag purified hydrogenase proteins HYDA1[4FeH] and CPI[4FeH] were *in vitro* activated with $2Fe_{adt}^{2}$ prior to the activity assay. 1 µg HYDA1 or 0.5 µg CPI was used in the activity assays, each data point represents the mean of 3 measurements, error bars give standard deviations.

Supplementary references

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