Supplementary Information

Multifaceted SlyD from *Helicobacter pylori*: implication in [NiFe] hydrogenase maturation

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Circular dichroism

About 10 μM of proteins were freshly prepared in 10 mM potassium phosphate buffer containing 50 mM $(NH_4)_2SO_4$ at pH 7.4. CD experiments were carried out on a JASCO J-815 circular dichroism spectrometer using a quartz cuvette with a path length of 1 mm at room temperature and scanned from 190 to 260 nm at the speed of 50 nm/min with a data interval of 0.2 nm. Each spectrum was averaged from five scans with baseline subtracted. The secondary structure fractions were estimated by the CDNN software package [1]. For thermal denaturation, 2 μM of proteins were used in a 10-mm quartz cuvette with heating and cooling rate of 1 ℃/min.

PPIase activity

RCM-T1 was used for determination of PPIase activity as previously described [2] with some modification. *E. coli* strain DH5*α* cells harboring pT1LE (a modified plasmid of pA2T1-1 [3], kindly granted by U. Hahn, Universität Hamburg) was used with site-directed mutagenesis (generating pT1LE-S54G/P55N) for expressing (S54G, P55N)-RNase T1. (S54G, P55N)-RNase T1 was expressed and purified accordingly [3]. RCM-T1 was prepared as previously described [4]. Progress of refolding of 0.3 μM RCM-T1 in the absence and presence of *Hp*SlyD and its variants at ambient temperature (20 ℃) was followed by the increase of fluorescence at 320 nm after excitation at 268 nm on a Hitachi F-7000 fluorescence spectrophotometer.

Chaperone activity

DTT-induced insulin aggregation assay was performed to determine the chaperone activity according to a previously described method [5, 6] with minor modification. The insulin solution was diluted to a final concentration of 45 μM in 50 mM Na_2HPO_4, 100 mM NaCl (pH 7.5) and mixed with *Hp*SlyD and its variants at 25 ℃. The aggregation process was monitored by the increase in scattered light at 400 nm on a Hitachi F-7000 fluorescence spectrophotometer.

Table S1 Primers and plasmids used in this study

(A) Primers used in this study

Name	**Sequence (5'-3')**	**Enzyme**
HpSlyD-for	GGAATTCCATATGCAAAACCATGATTTAGAG	NdeI
HpSlyD-rev	CGGAATTCCTACCCATGCGAACATGAGCA	EcoRI
HpSlyDΔC-rev	CGGAATTCCTATTCTTCTTCGCTCACTTCCCT	EcoRI
ΔIF-1	GTGGGATGATGCCTGGGTGCCCAGTGGCCCCATAAGCTTCCTCTGG	
ΔIF-2	GCACCCAGGCATCATCCCACCACATGCCACTTTAGCGTTTCGTTTCAAGGT	
MalE-SPless	GGAATTCCATATGAAAATCGAAGAAGGTAAA	NdeI
MalE-Xa	CGGAATTCTGAAATCCTTCCCTCGAT	EcoRI
HydA-SP-up	CGGAATTCATGTTCTACGATGAAA	EcoRI
HydA-SP-down	CCGCTCGAGCGCCTTCAAAGTCAAG	XhoI

(B) Plasmids used and constructed in this study

Name	**Purpose**	**Source**
pET32-HpSlyD	Express HpSlyD	This study
pET32-HpSlyDΔC	Express HpSlyDΔC	This study
pET32-HpSlyD ΔIF	Express HpSlyDΔIF	This study
pET32-HpSlyD ΔIFΔC	Express HpSlyDΔIFΔC	This study
pETMalE	Intermediate plasmid for constructing pETMalEHis	This study
pETMalEHis	Express ΦMalE::H6	This study
pETMalE-HydASP	Express ΦMalE::HydASP-H6	This study
pET32a(+)	Expression plasmid vector	Novagen
pMAL-p2x	Amplification of a signal peptide-less MBP	NEB

Table S2 Thermal stability parameters and PPIase activities of *Hp*SlyD and its variants

(A) PPIase activity data obtained from the refolding of RCM-T1 at 20 ℃

	k_{cat}/K_m (M^{-1} s^{-1})	%*
*Hp*SlyD	$1.17 \pm 0.05 \times 10^6$	100
*Hp*SlyDΔC	$0.94 \pm 0.05 \times 10^6$	80
*Hp*SlyDΔIF	$4.4 \pm 0.3 \times 10^3$	0.4
*Hp*SlyDΔIFΔC	$1.2 \pm 0.3 \times 10^3$	0.1

* %: the percentage of remaining PPIase activity (k_{cat}/K_m) of variants compared with wild-type *Hp*SlyD

(B) Inhibition of PPIase activity *Hp*SlyD by two immunosuppressive agents at different concentrations ‡

Conc. (μM)	1	10
FK506	97%	60%
Rapamycin	82%	24%

‡ Compared with k_{app} of 20 nM *Hp*SlyD

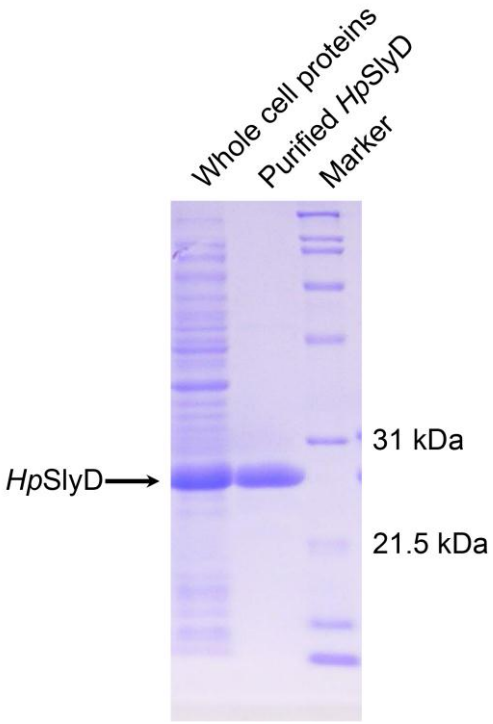

Fig. S1 Expression and purification of *Hp*SlyD

*Hp*SlyD expressed in KMl603 *E. coli* cells harboring pET32-HpSlyD was purified into homogeneity by a three-step procedure. *Hp*SlyD shows a larger apparent molecular mass of about 25 kDa than theoretical value of 20 kDa.

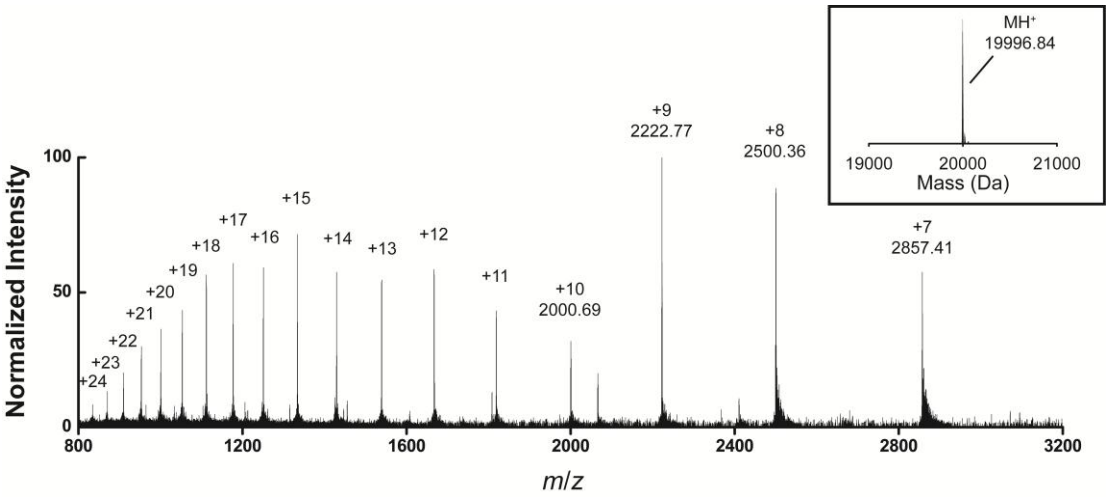

Fig. S2 The determination of the molecular weight of *Hp*SlyD by ESI-MS

The mass spectra of about 50 μM *Hp*SlyD in 10 mM ammonium acetate, pH6.4 were obtained on a LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, USA) equipped with an electrospray source in positive mode. Mass range scanned from *m*/*z* 200-4000 Da at resolution of 60000 with total accumulation time of 2 to 3 minutes. The instrument parameters are as follow: ion spray voltage 4.0 kV, sheath gas (N_2) flow rate at 6 arbitrary units, ion source temperature 275 ℃. The spectra were deconvoluted with the raw data range from *m*/*z* 800-3200 Da, with the threshold of resolution of 40000 and signal to noise ratio of 10. For the peaks of *Hp*SlyD with *m*/*z* > 2000, both charges and m/z are labeled, otherwise, only charges are labeled. The molecular weight (MH^+) was obtained from the deconvoluted result as shown in inset.

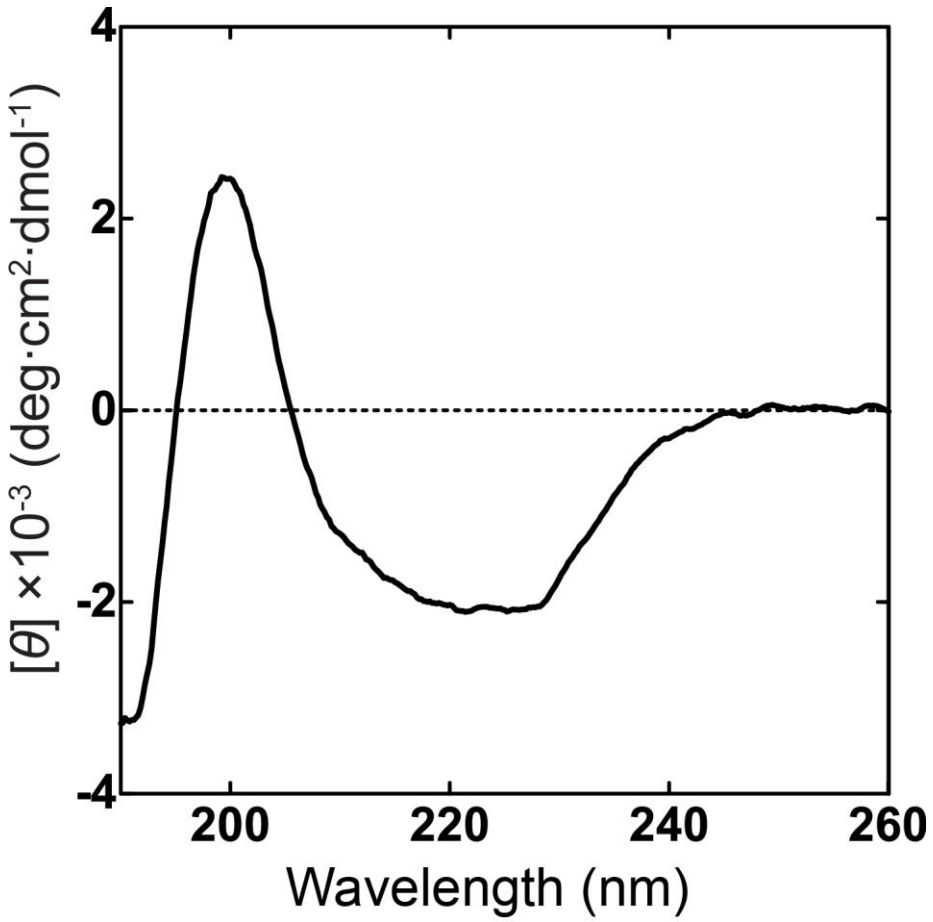

Fig. S3 CD spectrum of 10 μM *Hp*SlyD in 10 mM potassium phosphate, 50 mM $(NH_4)_2SO_4$ at pH 7.4

The spectrum was obtained from the average of five scans and smoothed using Savitzky–Golay method with 25 points of window on a Spectra Manager II software of JASCO.

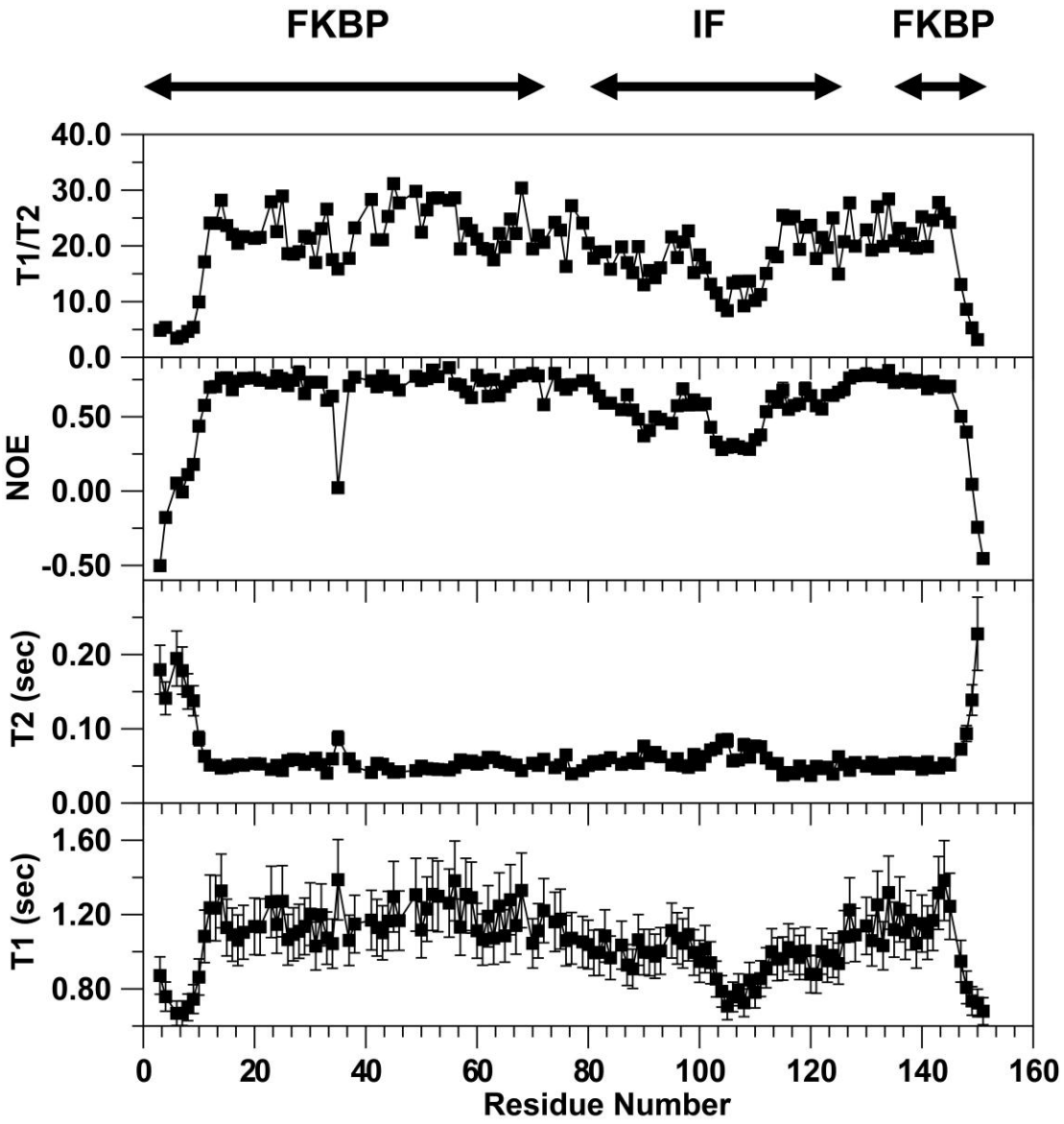

Fig. S4 ^{15}N backbone amide relaxation parameters of *Hp*SlyDΔC

The values of T_1, T_2 and proton-irradiated NOE for individual residues are shown as a function of residue number in the protein sequence.

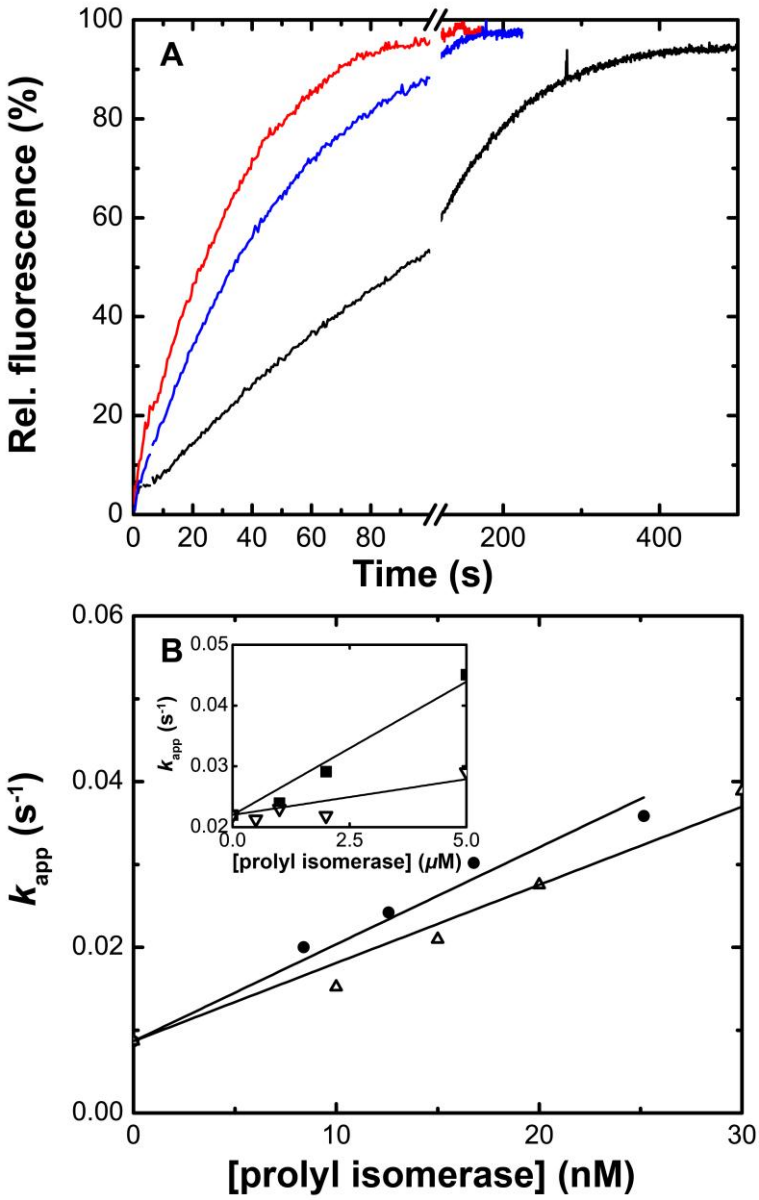

Fig. S5 PPIase activities of *Hp*SlyD and its variants

(**a**) The progress curves of refolding of 0.3 µM RCM-T1 at 20 ℃ in the absence (black) and presence of 20 nM *Hp*SlyD (red) and 15 nM *Hp*SlyDΔC (blue). (**b**) Catalytic efficiencies of refolding 0.3 μM RCM-T1. The measured rate constants k_{app} of RCM-T1 folding are shown as function of *Hp*SlyD (●), HpSlyDΔC (△), *Hp*SlyDΔIF (■) and *Hp*SlyDΔIFΔC (▽). The k_{cat}/K_m values obtained from the slopes are listed in Table S2a.

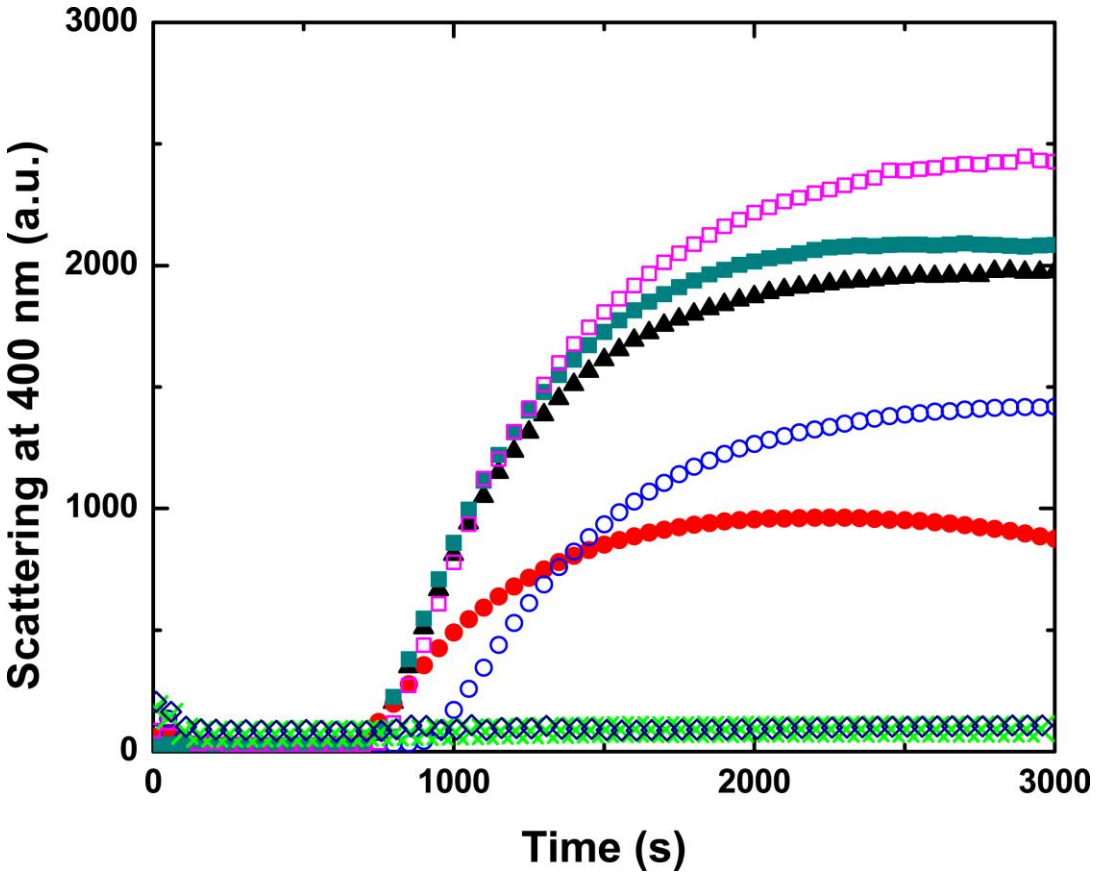

Fig. S6 Chaperone properties of *Hp*SlyD and its variants

The chaperone activities were demonstrated using DTT-induced insulin aggregation assay at 25 ℃. The aggregation of insulin was monitored by the increase in light scattering at 400 nm. The progress curves of 45 μM insulin in the absence of *Hp*SlyD (▲) and presence of 20 μM of *Hp*SlyD (●), *Hp*SlyDΔC (○), *Hp*SlyDΔIF (■) and *Hp*SlyDΔIFΔC (□), and 45 μM of *Hp*SlyD (×) and *Hp*SlyDΔC (◇).

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