Supporting Information

Foit et al. 10.1073/pnas.1222458110

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CLUSTAL 2.1 multiple sequence alignment

gi_226939674_ref_YP_002794747_      -------MKTRSATVLAALALALGAGMAHAAD--------------AAGK 29
gi|3660012|pdb|1BG8|A               ----------------------------AD----------------AQKA 6
gi|324115328|gb|EGC09292.1|         -------MKKAISILLGGLLLMPVISNAAE----------------TQKA 27
gi|365848927|ref|ZP_09389398.1      -------MKKVTGLILGSLLLIPALS----------------------QA 21
gi|339763595|gb|AEJ99815.1|         --------MKVKSLVFGAIGALFLTS--------------------AAHA 22
gi|317494458|ref|ZP_07952872.1      --------MNKKTLLISMVCLMGFSA--------------------IGHA 22
gi|183600608|ref|ZP_02962101.1      ----------MKKYFIVGIIAASLTSLSAM----------------AEDK 24
gi|268591600|ref|ZP_06125821.1      ----------MKKILFASIIATSLMTTSAF----------------AAT- 23
gi|354594842|ref|ZP_09012879.1      -------------------------MAAAN----------------AAEK 9
gi|187479095|ref|YP_787120.1|       ----------MKKAMLAVTMLG-LASLAG------------------AAH 21
gi|293604620|ref|ZP_06687022.1      MLIPHFGEIEMKKRIFTLALVA-MAAVSG------------------AAN 31
gi|187479269|ref|YP_787294.1|       ----------MKKIIISLSIAAATLSFSG------------------TAL 22
gi|323139382|ref|ZP_08074433.1      ----------MKLRYFAFVACLLAAQGA-------------------VAD 21
gi|323139170|ref|ZP_08074226.1      ----------MKTLYLAAAVAAVFATTA-------------------QAD 21
gi|226939974|ref|YP_002795047.      ---------MQRSLALSGLALALFATMS-------------------VAG 22
gi|225628791|ref|ZP_03786825.1      ---------MIKALFNKNTALAAVAILALSGGAMAES-------AKTHKT 34
gi|188584311|ref|YP_001927756.      ----------MRTGFLKTHLLPAIAVVLSAGLVQAETSG--GKSADKHQE 38
gi|357939260|gb|AET92817.1|         -------MKFSKS--IAAAMCVLVIGASG-----------------VARA 24
gi|238023991|ref|YP_002908223.      -------MKKITMWYMAAALAGIATANVA-----------------FAQT 26
gi|326314934|ref|YP_004232606.      ----MRVLARLSVLTLSSLAAAAAMAQTPSPAAPSAAATNPAAATAPAPA 46

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gi|324115328|gb|EGC09292.1|         ADNQKPVNSWTCEDFLALDATFQPTAVGFAEALNKKDKPEDAV-MDVQGI 76
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gi|183600608|ref|ZP_02962101.1      KNDTKPLAEWTCEEFLAIDDNFYPTAVGFGELLTKKDKVEDAV-LDVDGI 73
gi|268591600|ref|ZP_06125821.1      --DAKPVSQWTCEDFLAIDDAFYPTAIGAAEIITQKGKVEDPT-LDISGI 70
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gi|323139382|ref|ZP_08074433.1      T--KKPVSKWTCEEFLAVESDFQPKVVYFATAKERPARPT---------- 59
gi|323139170|ref|ZP_08074226.1      T--KKPVSKWTCEEFLAVEDQFQPKVIYWSSAQGKGGKPTGFV-DIEG-A 67
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gi|225628791|ref|ZP_03786825.1      DMAKKKVSELTCEDFNGLEESFKPTVVGWVVGFNKKGKEEDAV-IDVDGI 83
gi|188584311|ref|YP_001927756.      KLASKPLEKVTCEEFNGFEDTFKPKVVAWAAGYKQGQKKPDAVAIDIAGV 88
gi|357939260|gb|AET92817.1|         AEQKITPAKMTCADFVQVDDAYKPALVYWVAGVDKLGVTGTET-TVVDTM 73
gi|238023991|ref|YP_002908223.      AAKTISPAKMVCADFETLDDAYKPAVIYWATGVDKLGVRETDQ-ITIDTA 75
gi|326314934|ref|YP_004232606.      AKARKPLVKTTCSDYVGMDETIKPKFIYYAVGHTQGGKKEAVF--EEDAI 94
                                               * ::  .:    *  :

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gi|317494458|ref|ZP_07952872.1      EKVIPLVIEACKQNPKESFAQKVKSEWKKVKKDM------------ 105
gi|183600608|ref|ZP_02962101.1      QTLTPVVIDACKKDTKANFVEQVKNAKK------------------ 101
gi|268591600|ref|ZP_06125821.1      ATSTPLIVEACEKAPKESFIQKVEAHLKKM---------------- 100
gi|354594842|ref|ZP_09012879.1      AQVVPVLVQECKKSPKDNFVNKVKTNLTDKK--------------- 89
gi|187479095|ref|YP_787120.1|       EKIKPSLLTYCKENPKIALRDALDN----VRK-------------- 98
gi|293604620|ref|ZP_06687022.1      TKVKPEVLTYCKENPKIALRDALVQSWGKVKK-------------- 112
gi|187479269|ref|YP_787294.1|       AQITPTLVTYCQENPKVALTDALAQTKANAANTTSKTPPTSSTTAK 117
gi|323139382|ref|ZP_08074433.1      --------------PS---WT------------------------- 63
gi|323139170|ref|ZP_08074226.1      EKVVPMVIDDCKKAPQESYWSKLKAAWKKVEADAKALEKKM----- 108
gi|226939974|ref|YP_002795047.      LKVTPMVVTECQKDRKASFWSKLKTTWQHIEQKM------------ 105
gi|225628791|ref|ZP_03786825.1      ETVTP-CHHRSLQAGAESIVLEKG---------------------- 106
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gi|238023991|ref|YP_002908223.      HPVAEQVTEECKATPKVKIVDKIRMMAKAGKLSIYKRN-------- 113
gi|326314934|ref|YP_004232606.      EKIKPELDQYCSVHLTKSAYAKVMASSMASEGTAAHGHSKAK---- 136
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Fig. S1. ClustalW (1) sequence alignment of HdeA sequences. For *Escherichia coli* HdeA (Protein Data Bank ID code 1BG8), only the protein sequence reflecting the mature protein is shown. All other protein sequences contain their respective signal sequences.

1. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22(22):4673–4680.

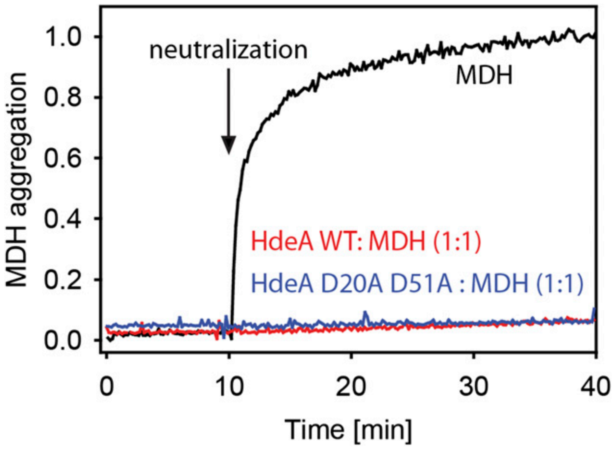

Fig. S2. Chaperone activity of WT HdeA and HdeA D20A D51A variant after neutralization. Guanidine denatured malate dehydrogenase (MDH) was diluted in aggregation buffer (pH 2.2) to a final concentration of 4 μM and incubated for 30 min. MDH aggregation was induced by neutralization with 2 M K_2HPO_4 and measured by monitoring light scattering at 350 nm in the presence or absence of various HdeA variants. Indicated is the extent of aggregation 30 min after neutralization, normalized to the aggregation of MDH in the absence of HdeA.

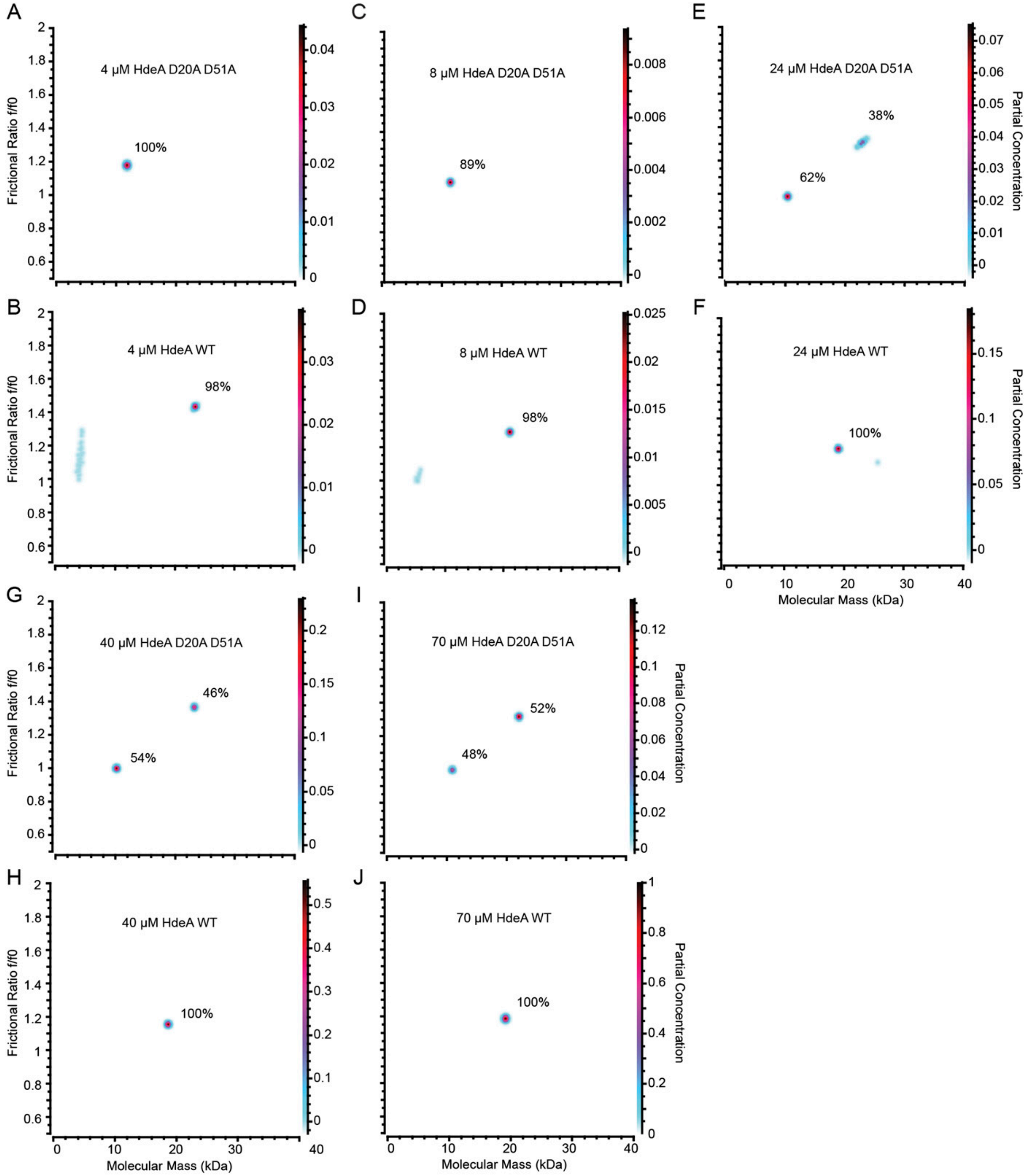

Fig. S3. Analytical ultracentrifugation sedimentation velocity analysis of WT HdeA (*B*, *D*, *F*, *H*, and *J*) and HdeA D20A D51A (*A*, *C*, *E*, *G*, and *I*) at pH 5. The molecular mass of WT HdeA is 9.7 kDa (monomer) and 19.5 kDa (dimer). Fractions of the monomeric and dimeric species are indicated. The K_d of dimerization for HdeA D20A D51A at pH 5 is in the range of 30 μM. (*C*) In addition to a species of about 10 kDa, a species of high molecular mass (about 90 kDa) was detected (11%), but no species corresponding to the HdeA dimer was detected.

Table S1. Predicted molecular mass and frictional ratios for putative dimeric species of different HdeA variants at different concentrations

	HdeA WT		HdeA D20A D51A	
Protein concentration, μM	Apparent molecular mass, kDa	Frictional ratio, f/f0	Apparent molecular mass, kDa	Frictional ratio, f/f0
4	23.33	1.43	n.a.	n.a.
8	21.16	1.29	n.a.	n.a.
17	19.50	1.20	21.80	1.29
24	19.00	1.18	22.91	1.35
40	18.61	1.16	23.20	1.37
70	19.10	1.19	22.08	1.32

The apparent molecular mass and the frictional ratios at pH 5 for HdeA WT and HdeA D20A D51A, respectively, were determined using analytical ultracentrifugation. Due to low signal extensity, the fractional ratio and molecular mass are approximate for protein concentrations of 4 and 8 μM. n.a., not applicable.