Supplementary Experimental Procedures

Identification of a Hypochlorite-Specific Transcription Factor from *Escherichia coli*

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Generation of strains and expression plasmids–All strains used in this study are listed in Table S1. The expression plasmid pJC2 for the production of C-terminal His_6-tagged YjiE (YjiE-His) was obtained by cloning the PCR-amplified *yjiE* gene (using the primer Fw *yjiE* and Rv C-term His-Tag *yjiE*; primer sequences are given in Table S2) via *Nde*I/*BamH*I into pET11a. The expression plasmid pKMG20 for the production of YjiE with thrombin-cleavable N-terminal His_6-tag (His-T-YjiE) was generated using the primer Fw *yjiE* and Rv *yjiE* and cloning the *Nde*I/*BamH*I-digested PCR fragment into pET28a. For the production of C-terminally flash-tagged YjiE (YjiE-flash), the expression plasmids pAD1 and pAD2 were generated using the primer Fw *yjiE* and Rv *yjiE*-flash and cloning the *Nde*I/*Hind*III-digested or *Nde*I/*BamH*I-digested PCR fragment into pJW2 (pAD1) or into pET28a (pAD2). Strains JC15, KMG89, and AD3 were obtained by transforming BL21(DE3) gold cells with pJC2, pKMG20, or pAD2, respectively. Plasmid pKMG09 was generated by cloning the PCR-amplified *yjiE* gene into pJW2 (Winter et al., 2005) using the primer Fw *yjiE* and Rv *yjiE*. The *yjiE* deletion in C600 (KMG214) was generated according to Datsenko and Wanner (2000) (Datsenko and Wanner, 2000) using the primer Fw del *yjiE* and Rv del *yjiE*. KMG214 was transformed with pKMG09 to generate KMG229 and with pAD1 to yield AD2. The correct sequence of all generated plasmids was verified by DNA sequencing.

Transcription profiles–Strains C600 (WT, $yjiE^+$) and KMG214 ($yjiE^-$) at an OD_{600} of 0.5 were treated with HOCl (2 mM final concentration, 5 min). HOCl was quenched by the addition of 5-fold concentrated LB medium, cells harvested, and the cell pellet frozen in liquid nitrogen and stored at -80°C. The isolation of RNA and gene expression analyses was performed at MFT Services (Tübingen, Germany) as follows. Total RNA isolation was performed via phenol extraction and RNeasy Midi Kit (Qiagen) according to the manufacturer's instructions. Biotinylated cDNA was prepared after fragmentation according to the standard Affymetrix protocol from 3 µg total RNA (Prokaryotic Sample and Array Processing, Technical Manual 701030 Rev. 5, 2004, Affymetrix). 3 µg of cDNA were hybridized for 16 hours at 50° C on the *E. coli* Genome 2.0 GeneChip array. GeneChips were washed and stained in the Affymetrix Fluidics Station 450 and then scanned using the Affymetrix GeneChip Scanner 3000 7G. A probe level summary was determined using the Affymetrix™ GeneChip Operating Software using the MAS5 algorithm. Normalization of raw data was performed by the Array Assist™ Software 10.0 (Agilent), applying a GC-RMA (Robust Multichip Average) algorithm. Transcripts with a minimum change in expression level of 2-fold in at least two of the three replicates were considered significantly up- or down-regulated. Gene information and function were obtained from EcoCyc.

Sucrose gradient centrifugation– Sucrose gradients were generated by successively layering 750 µl of 0 %, 10 %, 20 %, 30 %, 40 %, and 50 % sucrose (in 10 mM NaH_2PO_4 pH7.5, 150 mM NaCl) in 5 ml centrifuge tubes (Beckman) and allowed to equilibrate at 4°C for 24 h. Gradients were overlaid with *in vivo* FlAsH-labeled lysates, centrifuged (78,000 g, 4°C for 16 h, Optima MAX-E ultracentrifuge with MLS50 rotor (Beckman Coulter)), fractionated, and fractions separated by SDS-PAGE. YjiE-flash*-containing fractions were visualized by fluorescence (Typhoon Imager 9200 (GE)). Other proteins were

visualized by Coomassie stain or identified by Western blot using specific antibodies produced in rabbit (GroES, DnaK, GroEL) or mass spectrometry as described before (Rudolph et al., 2010). Based on the known oligomerization state of the identified proteins (according to www.ecocyc.org), the molecular weight of YjiE-flash was estimated. The identified proteins and their apparent molecular weight were GroES (73 kDa, heptamer), DnaK (69kDa, monomer), TktA (144 kDa, dimer), GapA (142 kDa, tetramer), PurL (141 kDa, monomer), RNA polymerase core enzyme (378 kDa, heteroteramer $[RpoA]_2$ [RpoB] [RpoC]), GroEL (803 kDa, 14-mer), and SucB (1056 kDa, 24-mer).

Calculation of the YjiE-DNA-complex stoichiometry–50 nM AF488-labeled 158 bp *yjiE* DNA was titrated with YjiE concentrations ranging from 0.5-3 µM and mixtures were subjected to sedimentation velocity aUC analysis. The data sets were fitted using the 2DSA/GA-MC method, the "continuous c(S) with bimodal f/f0" model of SEDFIT and the "hybrid global continuous distribution and global discrete species" model of SEDPHAT to obtain the hydrodynamic parameters for the protein-DNA-complex. The fits returned S-values of 8.72 to 8.77 and a f/f0 of 1.29 to 1.33 yielding a buoyant mass of 34.8 kDa. The buoyant mass (Mb) is linked to the molecular mass (M) by equation (1),

$$Mb = M (1 - \bar{v} \rho) \qquad (1)$$

where $\bar{v}$ denotes the partial specific volume of the solute and ρ the density of the solvent (SEDNTERP). Using $\bar{v}_{YjiE} = 0.73$ and $\bar{v}_{DNA} = 0.55$, we calculated the $\bar{v}_{complex}$ for a variety of complexes consisting of 2-6 molecules YjiE (35 kDa) having one molecule of DNA (52 kDa) bound. Only the YjiE-Dimer-DNA complex with a $\bar{v}_{complex} = 0.6533$ matched the experimentally determined buoyant mass.

Generation of single-copy yjiE::lacZ reporter fusions and determination of β-Galactosidase activity–To construct the translational *yjiE::lacZ* reporter fusion, a PCR fragment starting at 304 bp upstream and ending 22 bp downstream of the translational start site of *yjiE* (thus containing the putative *yjiE* promoter region) was generated using chromosomal MG1655 DNA as template and the primer pair Z12-Fw304yjiE22 and Z13-Rv304yjiE22, which contain a *BamH*I and *Hind*III restriction site, respectively. The fragment was cloned into pJL28 (Lucht *et al.*, 1994). The reporter fusion was transferred to the att(λ) location of the chromosome of JG39 (C600 *lacZ::*Kan) and JG59 (C600 *lacZ::*Kan *yjiE::*Cm) via phage λRS74 (Simons *et al.*, 1987). Single lysogeny was tested by a PCR approach (Powell *et al.*, 1994). β-galactosidase activity was assayed by the use of o-nitrophenyl-β-D-galactopyranoside as substrate and is reported as micromoles of o-nitrophenol per minute per milligram of cellular protein (Miller, 1972). Experiments were performed in triplicates and data are presented as mean ± SD.

Circulardichroism measurements– Far-UV CD-Spectra were recorded using a Jasco-J-715 spectrometer. YjiE (ca. 14 µM) was either left untreated or incubated with a 5-fold molar excess of HOCl for 30 min at 25°C. Afterwards, HOCl was quenched by the addition of methionine. CD-Spectra were recorded between 195 und 260 nm with 0.1 mg/ml YjiE at 20°C with 20 nm/min. 16 spectra were accumulated. Melting curves were generated at 222 nm between 20°C and 80°C at a heating rate of 20°C/h.

3D-Reconstructions of YjiE oligomers–As mainly top-views of YjiE oligomers have been observed, due to preferential adsorption on the carbon support film, the 3D-structures have been approximated by projecting the 2D class averages of the top-views in horizontal direction. The height of the projection was chosen such that the volume of the 3D-reconstructions was in full agreement with the oligomeric masses.

Hydrodynamic simulations–Sedimentation coefficients of YjiE oligomers were estimated by hydrodynamic simulations conducted within the software package HYDROMIC (García de la Torre et al., 2001) using the above 3D reconstructions as input.

SUPPLEMENTAL REFERENCE LIST

Datsenko, K. A. and Wanner, B. L. (2000) Proc. Natl. Acad. Sci. USA 97, 6640-6645
García de la Torre, J., Llorca, O., Carrascosa, J. L., and Valpuesta, J. M. (2001) Eur. Biophys. J. 30, 457-462
Lucht, J. M. , Dersch, P. , Kempf, B., and Bremer, E. (1994) J. Biol. Chem. 269, 6578–6586
McHugh, J. P., Rodriguez-Quinones, F., Abdul-Tehrani, H., Svistunenko, D. A., Poole, R. K., Cooper, C. E., and Andrews, S. C. (2003) J. Biol. Chem. 278, 29478-29486
Miller, J. H. (1972) Experiments in Molecular Genetics. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory
Powell, B .S. , Court, D. L. , Nakamura, Y. , Rivas, M. P., and Turnbough, C. L. Jr. (1994) Nucleic Acids Res. 22, 5765–5766
Rudolph, B., Gebendorfer, K. M., Buchner, J., and Winter, J. (2010). J. Biol. Chem. 285, 19029-19034
Simons, R. W., Houman, F., and Kleckner, N. (1987) Gene 53, 85–96
Winter, J., Linke, K., Jatzek, A., and Jakob, U. (2005). Mol. Cell 17, 381-392

Supplementary Figure 1. A. Surface representations of reconstructed 3D-structures of YjiE. Given are the corresponding oligomeric masses and sedimentation coefficients, as obtained by hydrodynamic simulations (20°C, viscosity η=0.012P). B. YjiE-flash is specifically FlAsH-labeled in lysates. Sedimentation velocity analysis of 6 µM lysates that either expressed (black line) or did not express *yjiE-flash* (gray line). Lysates were labeled with FlAsH after cell lysis. Please note that the lysate lacking *yjiE-flash* expression showed no significant fluorescence. C. Lysates containing YjiE-flash that was FlAsH-labeled prior to cell lysis were analyzed by sucrose gradient centrifugation. YjiE-flash* was visualized using a fluorescence scanner and only the YjiE-flash* containing portion of the gel is shown (Inset). Gradient fractions are numbered and indicated. According to the known oligomerization state and molecular weight of the identified proteins in the fractions (see methods), the molecular weight of the YjiE oligomer was calculated to 140–380 kDa. The circles represent the molecular weight of the identified proteins and the line represents the regression line. D. Competition of AF488-labeled *yjiE* DNA with unlabeled *yjiE* DNA monitored by FA. Fluorescence of 10 nM AF488 DNA alone (1), after addition of 1 µM YjiE (2) and after addition of 100 nM unlabeled DNA (3). Samples were analyzed at 20°C.

Supplementary Figure 2. Translational fusions of the putative *yjiE* promoter region with *lacZ*. JG140 (C600 Δ*lacZ*; wild-type) and JG141 (C600 Δ*lacZ* Δ*yjiE*) cells were grown at 37°C in LB medium and were left untreated or supplemented with HOCl as indicated during exponential growth phase. Aliquots were removed after 15 min and *yjiE* expression was analyzed by determining β-galactosidase activity of the *yjiE::lacZ* reporter fusion.

Supplementary Figure 3. A. Far-UV-CD-Spectra of purified, untreated YjiE (filled circles) and YjiE treated with a 5-fold molar excess of HOCl (open circles). Spectra were measured with 0.1 mg/ml YjiE in YjiE storage buffer at 20°C. 16 spectra were accumulated. B. Melting curves of YjiE (black line) and YjiE treated with a 5-fold molar excess of HOCl (gray line) at 222 nm measured between 20°C and 80°C with a heating rate of 20°C/h. C. Typical TEM class average of YjiE treated with a 10-fold molar excess of HOCl and negatively stained with uranyl acetate (scale bar: 10 nm). D. Sedimentation velocity analysis of 12 µM YjiE-HOCl in storage buffer. During sedimentation the absorbance at 280 nm was monitored.

Gebendorfer et al., Table S1. Strains used in this study.

strain name	relevant genotype, plasmid	source / reference
C600	*thr leu thi lac4 rpsL supE* (F^-)	*E. coli* stock center
BL21(DE3) gold	F^- *ompT hsdS(rB- mB-) dcm*$^+$ *Tatr gal λ(DE3) endA Hte*	Stratagene
JC15	BL21 (DE3) gold pJC2	This study
KMG89	BL21 (DE3) gold pKMG20	This study
KMG214	C600 *yjiE::*Cm	This study
KMG229	KMG214 pKMG09	This study
AD2	KMG214 pAD1	This study
AD3	KMG39 pAD2	This study
JG39	C600 *lacZ::*Kan	This study
JG59	C600 *lacZ::*Kan *yjiE::*Cm	This study
JG140	JG39 *yjiE::lacZ*	This study
JG141	JG59 *yjiE::lacZ*	This study

Gebendorfer et al., Table S2. Primer used in this study.

Primer name	**Sequence (5' to 3')**
Fw *yjiE*	ggaattccatatggatgactgtggtgcgattttg
Rv C-term His-Tag *yjiE*	cccaagcttggatcctcagtgatggtgatggtgatggctaagcacaatctccagctc
Rv *yjiE*	cccaagcttggatcctcagctaagcacaatctccagctc
Rv *yjiE*-flash	cccaagctttcaacagcagcccggacagcagctaagcacaatctccagctc
Fw del *yjiE*	gacagagtaaaacgtaatggatgactgtggttgtgtaggctggagctgcttc
Rv del *yjiE*	cgacgctggagaatgtcagctaagcacaatcatatgaatatcctccttag
Fw -106bp *yjiE*	gacggcaaagcctgcgtgaa
Rv +52 bp *yjiE*	cataaagccatttggtttcaat
Fw -106 bp *metN*	attgatttagacgtctggatgccttaac
Rv +52 bp *metN*	tggtgcgggtgccctgg
Fw -106 bp *cydA*	tcacatggtatgatgaaagtgttcaaac
Rv +52 bp *cydA*	tcgcggtcaaggcaaactgtaag
TufB3-Fw	gtctaaagaaaagtttgaacgtacaaaaccg
TufB301-Rv	cgtccatctgcgcagcaccggtgatc
YjiE3-Fw	ggatgactgtggtgcgattttgcataatattg
YjiE301-Rv	gttgcgcgtaatcgctgccgccacg
MetN3-Fw	gataaaactttcgaatatcaccaaagtgttcc
MetN301-Rv	caaaaacagtacgcgaagagagcagg
CysH3-Fw	gtccaaactcgatctaaacgccctgaac
CysH301-Rv	ggttgagcttgagtttgtccgttaactg
MetB5-Fw	cgcgtaaacaggccaccatcgcagtgc
MetB303-Rv	gtagcagtcgtgcggcgcaaccagc
fecD3-Fw	gaaaattgcgctggttattttcatcaccc
fecD301-Rv	gtagagcccccacagaggccaggc
Z12-Fw304yjiE22	cgggatccgaaaccgtgcaggtgctggtc
Z13-Rv304yjiE22	cccaagcttgcaaaatcgcaccacagtcatc

Gebendorfer et al., Table S3. Up-regualted genes – Microarray analysis of *yjiE*$^+$ and *yjiE*$^-$ cells upon HOCl stress. Given are genes that are up-regulated more than two-fold in one or both strains in response to HOCl (ratio >2 in *yjiE*$^+$ cells and <2 in *yjiE*$^-$ cells and *vice versa*, ratio between both strains >2). Gene information and function were obtained from EcoCyc. Footnote denotes members of the [1]Fur regulon (McHugh et al., 2003).

Gene	Function	pathway	x-fold change in:	
			yjiE$^+$	*yjiE*$^-$
acpD	azoreductase	metabolism	106	415
alr	alanine racemase 1	metabolism	0.9	2.1
alx	putative transport protein	transport	1.1	2.4
avtA	valine--pyruvate transaminase	metabolism	1.7	3.5
bhsA	hypothetical protein	unknown	102	293
clpA	Clp protease ATP-binding subunit	protein homeostasis	1.6	5.6
clpP	Clp protease proteolytic subunit	protein homeostasis	1.8	5.6
cysH	3'-phospho-adenylylsulfate reductase	metabolism	6.4	1.8
cysK	cysteine synthase A	metabolism	32	123
cysN	sulfate adenylyltransferase, subunit 1	metabolism	10	27
cysP	thiosulfate transporter subunit	metabolism	107	232
cysU	sulfate/thiosulfate transporter subunit	metabolism	7.8	27
cysW	sulfate/thiosulfate transporter permease subunit	metabolism	6.6	13
dadX	alanine racemase 2, PLP-binding	metabolism	6.0	13
erpA	essential respiratory protein A	metabolism	1.3	3.5
fumC	fumarate hydratase	metabolism	1.7	4.0
glpE	thiosulfate sulfurtransferase	metabolism	5.5	11
glpG	intramembrane serine protease	protein homeostasis	1.3	3.7
glpQ	glycerophosphodiester phosphodiesterase	metabolism	1.6	3.4
hemF	coproporphyrinogen III oxidase	metabolism	1.0	2.8
hemH	ferrochelatase	metabolism	4.3	21
iaaA	L-asparaginase	metabolism	2.3	5.9
ibpB	heat shock chaperone	protein homeostasis	3.2	10
ileS	isoleucyl-tRNA synthetase	metabolism	1.0	2.5
ilvE	branched-chain amino acid aminotransferase	metabolism	2.3	18
ilvM	acetolactate synthase 2 regulatory subunit	metabolism	15	37
inaA	pH-inducible protein involved in stress response	stress	1.5	3.0
iscS	cysteine desulfurase	metabolism	4.0	6.2
ivbL	putative glutamine synthetase	metabolism	1.2	5.9
kefF	regulator of KefC-mediated potassium transport	metabolism	3.9	17
leuA	2-isopropylmalate synthase	metabolism	4.8	18
lldD	L-lactate dehydrogenase	metabolism	1.4	4.0
lptG	conserved inner membrane protein	unknown	0.8	2.3
marR	DNA-binding transcriptional repressor	regulation	133	325
metB	O-succinylhomoserine lyase	metabolism	3.4	0.9
metK	methionine adenosyltransferase	metabolism	2.8	1.5
metN	comp. of methionine uptake ABC permease	metabolism	6.7	1.2
nemA	N-ethylmaleimide reductase	metabolism	10	40
oxyS	ncRNA	stress	6.1	21
panD	aspartate alpha-decarboxylase	metabolism	1.7	3.6
pheL	pheA gene leader peptide	metabolism	1.1	4.2
proB	gamma-glutamyl kinase	metabolism	1.8	3.8
puuD	gamma-Glu-GABA hydrolase	metabolism	1.1	3.7

qorB	putative oxidoreductase	unknown	28	56
recA	recombinase A	DNA related	0.7	2.6
rlmE	23S rRNA methyltransferase	RNA related	1.9	4.1
sbp	sulfate transporter subunit	metabolism	45	320
sdhC	succinate dehydrogenase cytochrome b556 subunit	metabolism	4.2	12
sufA [1]	Fe-S transport protein in Fe-S cluster assembly	metabolism	1.5	3.3
tatD	DNase	DNA related	1.6	3.9
thrL	thr operon leader peptide	metabolism	1.5	5.2
tnaC	tna operon leader peptide	metabolism	2.2	1.1
trxA	thioredoxin 1	metabolism	0.6	2.0
trxC	thioredoxin 2	metabolism	69	143
xthA	exonuclease III	DNA related	1.1	3.7
yaaA	conserved protein	unknown	1.8	15
ybeZ	putative ATP-binding protein	unknown	1.4	3.5
yceA	conserved protein	unknown	1.4	3.2
yebA	predicted peptidase	unknown	2.6	6.5
yedY	reductase	metabolism	6.5	2.2
yeeD	conserved protein	unknown	40	92
yeeE	putative transport system permease protein	unknown	93	327
yhaK	predicted pirin-related protein	unknown	92	261
yheL	sulfur transfer complex subunit TusB	metabolism	1.5	2.3
yhhL	conserved inner membrane protein	unknown	1.0	2.2
yhhW	hypothetical protein	unknown	191	451
yhiI	predicted HlyD family secretion protein	unknown	0.8	3.1
yijE	predicted permease	unknown	1.1	11
yjeI	conserved protein	unknown	2.0	5.0
yjfO	conserved protein	stress	2.2	1.2
yjgH	predicted mRNA endoribonuclease	unknown	125	255
ykgA	predicted DNA-binding transcriptional regulator	unknown	2.3	1.0
ypfH	predicted hydrolase	unknown	11	27
yqjF	predicted quinol oxidase subunit	unknown	221	1255

Gebendorfer et al., Table S4. Down-regualted genes – Microarray analysis of *yjiE*$^+$ and *yjiE*$^-$ cells upon HOCl stress. Given are in response to HOCl down-regulated genes (ratio >2 in *yjiE*$^+$ cells and <2 in *yjiE*$^-$ cells and *vice versa*, ratio between both strains >2). Footnote denotes members of the [1]Fur regulon (McHugh et al., 2003). Expression changes of less than 2-fold are shown with gray backgrounds and were included because the corresponding genes are part of an operon where some members show more than 2-fold changes.

Gene	Function	pathway	x-fold change in:	
			yjiE$^+$	*yjiE*$^-$
adhE	alcohol dehydrogenase	metabolism	25	10
cydA	cytochrome bd-I terminal oxidase subunit I	metabolism	39	13
cydB	cytochrome d terminal oxidase subunit II	metabolism	19	9.8
entC [1]	isochorismate synthase 1	Fe acquisition	8.4	2.7
entH [1]	thioesterase in enterobactin biosynthesis	Fe acquisition	4.4	2.1
fabB	β-ketoacyl-ACP synthases I	metabolism	3.1	1.3
fecA [1]	ferric citrate outer membrane transporter	Fe acquisition	4.4	2.3
fecB [1]	iron-dicitrate transporter subunit	Fe acquisition	5.4	2.8
fecC [1]	comp. of iron dicitrate ABC transporter	Fe acquisition	5.2	2.4
fecD [1]	comp. of iron dicitrate ABC transporter	Fe acquisition	5.3	1.3
fecE [1]	comp. of iron dicitrate ABC transporter	Fe acquisition	2.8	1.4
fecR [1]	regulator for *fec* operon	Fe acquisition	11	5.0
fepC [1]	iron-enterobactin transporter ATP-binding protein	Fe acquisition	3.5	2.2
fepD [1]	iron-enterobactin transporter membrane protein	Fe acquisition	4.1	2.4
flgG	flagellar basal-body rod protein	motility	4.4	2.0
fmt	methionyl-tRNA formyltransferase	translation	2.0	0.5
fruA	comp. of fructose PTS transporter	transport	7.2	2.6
infA	protein chain initiation factor IF-1	translation	10	4.4
ispA	geranyl / farnesyl diphosphate synthase	metabolism	2.7	0.6
ksgA	16S rRNA dimethyltransferase	RNA related	4.1	1.8
lolA	periplasmic lipoprotein chaperone	metabolism	2.5	1.0
lpxK	tetraacyldisaccharide 4'-kinase	metabolism	3.0	1.0
manY	comp. of mannose PTS permease	transport	2.7	1.3
manZ	comp. of mannose PTS permease	transport	2.4	0.8
mdtD	transporter	transport	1.1	2.9
oppB [1]	comp. of murein tripeptide ABC transporter	transport	3.8	1.8
oppD [1]	comp. of murein tripeptide ABC transporter	transport	5.5	2.2
pdxA	4-hydroxy-L-threonine phosphate dehydrogenase	metabolism	5.5	2.6
pflB	pyruvate formate-lyase	metabolism	4.2	1.6
potC	comp. of putrescine ABC transporter	transport	2.7	1.2
proP	proline/glycine betaine transporter	transport	6.9	2.4
proV	comp. of proline ABC transporter	transport	9.4	3.2
proW	comp. of proline ABC transporter	transport	4.5	2.3
purA	adenylosuccinate synthetase	metabolism	4.1	1.9
rne	ribonuclease E	RNA related	4.8	1.8
rnpA	RNase P protein comp.	RNA related	5.1	2.0
rseP	zinc protease	proteolysis	2.1	1.0
rsmB	16S rRNA methyltransferase	RNA related	2.1	0.7
rttR	small RNA	regulation	33	12
ryhB [1]	small RNA involved in iron homeostasis	Fe homeostasis	10	2.4
thyA	thymidylate synthase	metabolism	2.4	1.2
tonB [1]	uptake of iron	Fe acquisition	3.6	2.0

truB	tRNA pseudouridine 55 synthase	RNA related	3.7	1.2
ybgT	small membrane protein	unknown	4.5	2.0
yceD	conserved protein	unknown	2.6	1.2
yicC	predicted protein	unknown	2.5	1.1
yigZ	predicted elongation factor	unknown	2.2	1.0
yncE [1]	conserved protein; probable role in Fe acquisition	Fe acquisition	4.3	2.1

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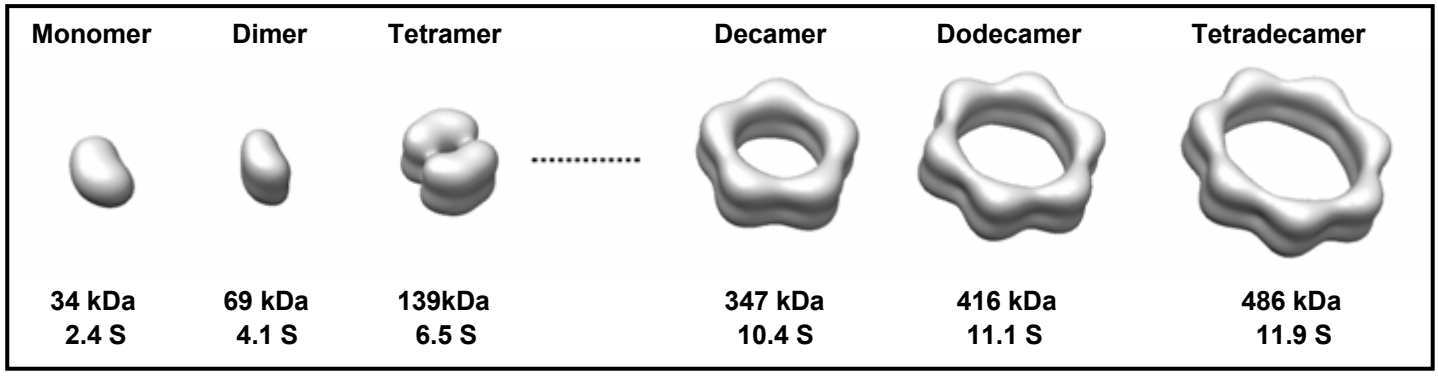
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Tetramer
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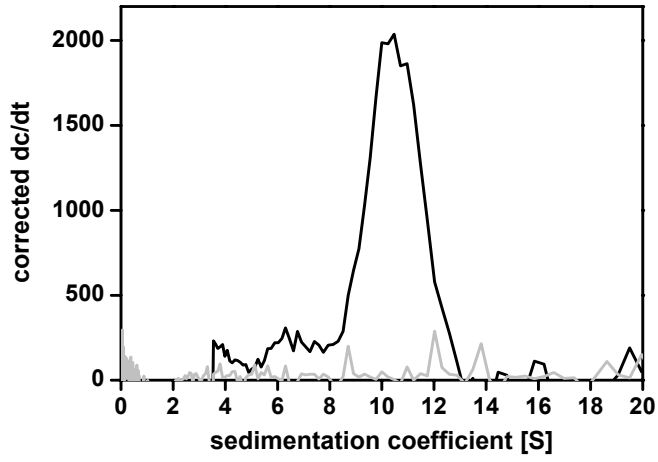
11.9 S

B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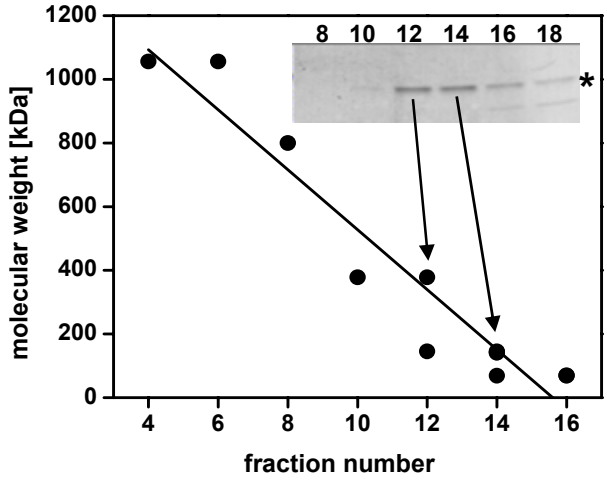
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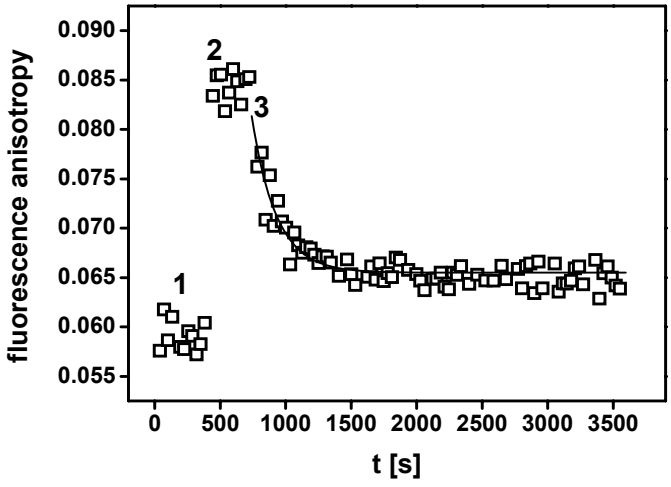
fluorescence anisotropy
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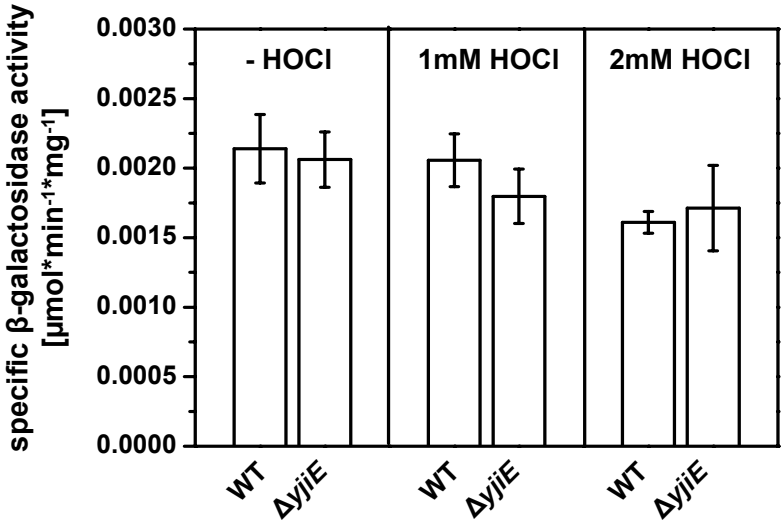
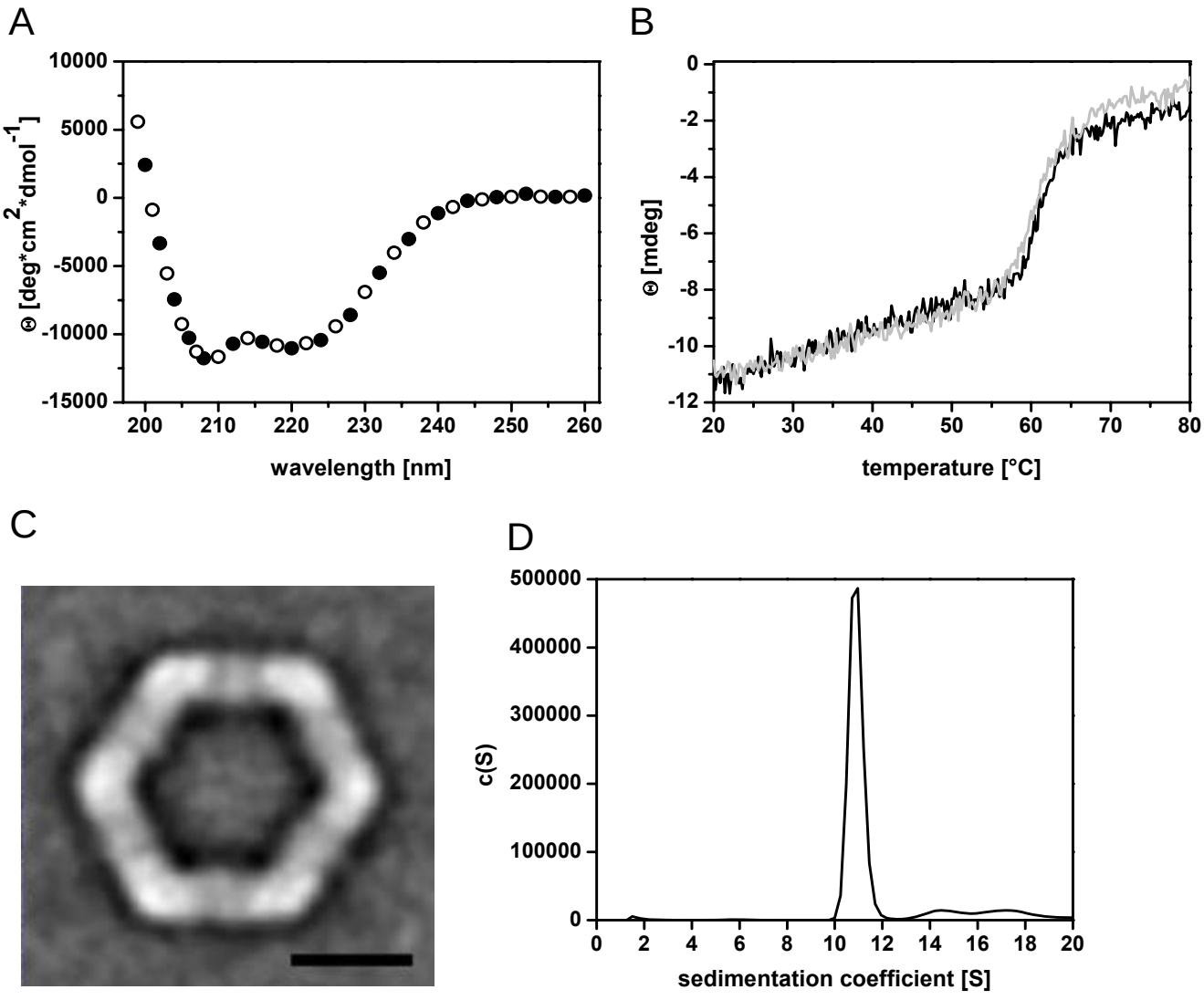
2
3


specific β-galactosidase activity
[µmol*min-1*mg-1]
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
- HOCl
1mM HOCl
2mM HOCl
WT
ΔyjiE
WT
ΔyjiE
WT
ΔyjiE


A
10000
5000
0
-5000
-10000
-15000
200
210
220
230
240
250
260
wavelength [nm]
Θ [deg*cm2*dmol-1]
B
0
-2
-4
-6
-8
-10
-12
20
30
40
50
60
70
80
temperature [°C]
Θ [mdeg]
C
D
500000
400000
300000
200000
100000
0
0
2
4
6
8
10
12
14
16
18
20
sedimentation coefficient [S]
c(S)