Table S1. Sequences of primers for this study

Primer name	Primer sequence (5’-3’)
Expression and purification	
*Bpe*GReg Xa top	ATCGAGGGAAGGTTGAAGCCTTCGCCTGAAATC
Histidine Xa top	CACCACCACCACCACCACATCGAGGGAAGG
NdeI histidine top	AACATATGCACCACCACCACCACCAC
*Bpe*GReg BamHI bot	AAGGATCCCTAGTGCTGGCCGGACTG
*Av*GReg NdeI top	AACATATGTCGAACAGAGCAGGATC
*Av*GReg HindIII bot	AAAAGCTTGCCATCCAGCTTCCGGGC
*Cv*GReg NdeI top	AACATATGAAACAGACCGATCAAAC
*Cv*GReg NotI bot	AAGCGGCCGCGCGCCCGACTACCGGCGC
Expression in S. typhimurium	
*Bpe*GReg SacI top	AAGAGCTCTTGAAGCCTTCGCCTGAAATC
*Bpe*GReg BamHI bot	AAGGATCCCTAGTGCTGGCCGGACTG
*Av*GReg SacI top	AAGAGCTCATGTCGAACAGAGCAGGATC
*Av*GReg BamHI bot	AAGGATCCTCAGCCATCCAGCTTCCG
*Cv*GReg EcoRI top	AAGAATTCATGAAACAGACCGATCAAACC
*Cv*GReg SacI bot	AAGAGCTCCTAGCGCCCGACTACCGG
Site-directed mutagenesis	
*Bpe*GReg H225A-F	CGGCCTGTGGTTCATCGCCAAGGCGGCGCACGCC
*Bpe*GReg H225A-R	GGCGTGCGCCGCCTTGGCGATGAACCACAGGCCG
Construction of B. pertussis knockout strain	
*Bpe*GReg 289-888 HindIII top	AAAAGCTTGAAGTGCACGCGCGCATC
*Bpe*GReg 289-888 BamHI bot	AAGGATCCGCGCCCCGACTCCAGGTTG
*Bpe*GReg upstream region-F	CTGTGGACCACCCCCCTG
pFUS2-R	GAGAATTCGGTTACGCAA

Supplementary Figure Legends

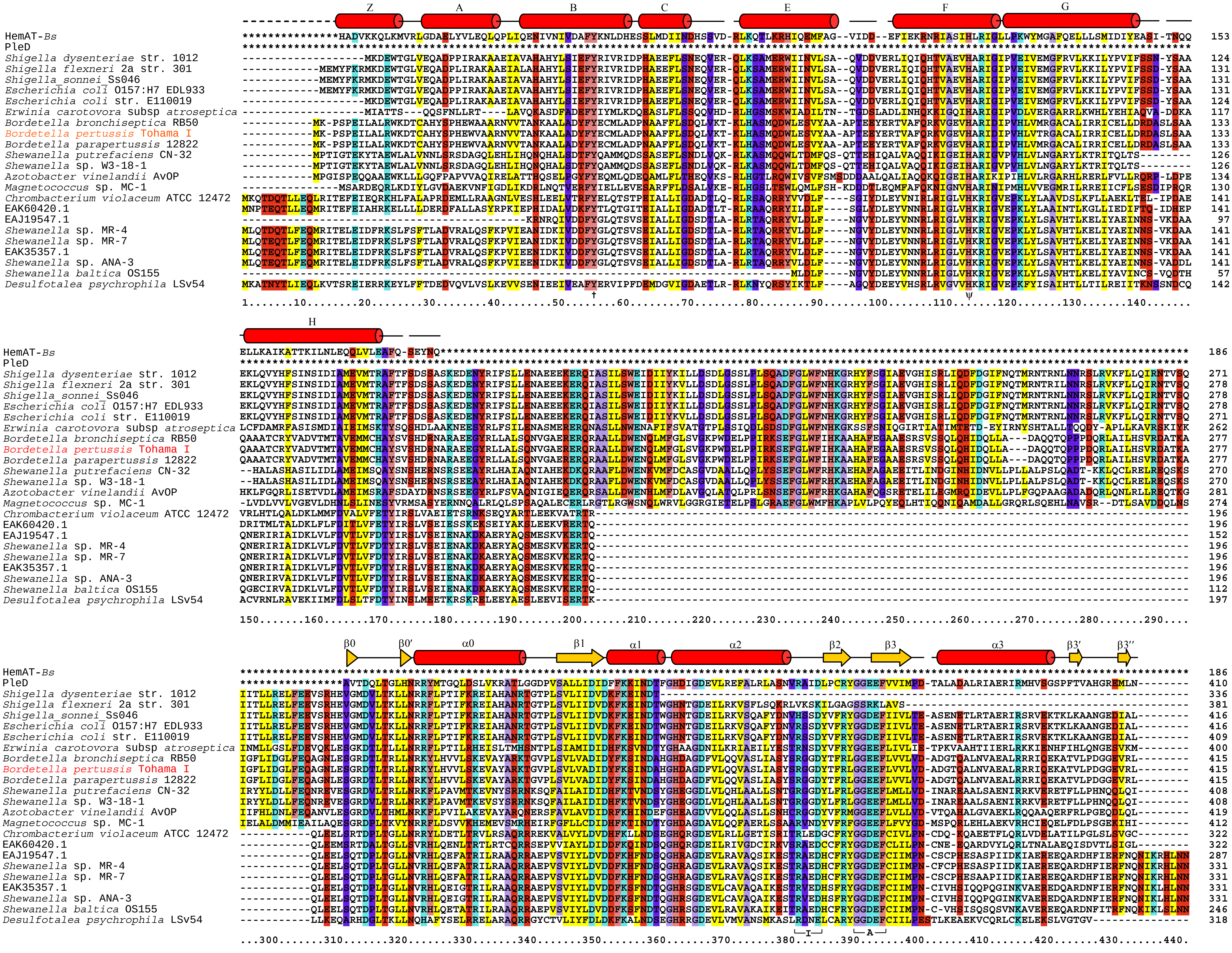
Figure S1. Multiple alignments of the globin-coupled diguanylate cyclases. The globin domain extends from the Z-helix to the H-helix, and the DGC domain from the β0 strand to β5strand. The secondary structures of the GCS globin domain of HemAT-*Bs* and the DGC domain of PleD have been included for reference. In addition to the B10 tyrosine (ψ) and proximal histidine (†) of the globin domain, the active, *A*, and inhibitory, *I*, sites of the DGC domain are indicated below the sequences. The three GCSs beginning with EAK or EAJ are from environmental samples taken from the Sargasso Sea. Two GCS sequences that do not possess the DGC motif originate from unfinished whole-genome shotgun sequences from TIGR (*Shigella dysenteriae*) and JGI (*Shewanella baltica*). The *Shigella flexneri* sequence comes from the completed genome. To simplify the presentation of sequences, the portions of the reference sequences, HemAT-*Bs* and PleD, that are unrelated to the globin or DGC domains were replaced with an asterisk (*). Gaps and ends of sequences are indicated with a hyphen (-) and period (.), respectively. The illustrated color code represents a 90% consensus among sequences: charged (DEHKR) in blue; aromatic (FHWY) in pink; polar (DEHKNQRSTY) in red; tiny (AGS) in light purple; small (ACDGNPSTV) in dark purple; and hydrophobic (ACLIVMHYFW) in yellow.

Figure S2. Absorption spectra of *Bpe*GReg with ferric heme bound to various ligands. (a) Ferric (Fe^{III}) *Bpe*GReg in met- (dashed line) and the imidazole-bound (solid line) forms. (b) Ferric (Fe^{III}) *Bpe*GReg in met- (dashed line) and the cyanomet-bound (solid line) forms. All measurements were done at 25°C and pH 7.0 in 100 mM sodium phosphate buffers.

Figure S3. Association of oxygen to ferrous *Bpe*GReg at 25°C and pH 7.0. (a) The association rate for binding of O_2 to deoxy-*Bpe*GReg was determined from examinations of O_2 rebinding to deoxy protein produced from laser-flash photolysis of the O_2-bound form. Each K_{obs} represents the average of six measurements of the rate of *Bpe*GReg association at a given concentration of O_2. (b) The kinetic traces for binding of *Bpe*GReg to 64, 256, and 1024 µM O_2 (red), along with the single-exponential fits that yielded the K_{obs} values (black).

Figure S4. Absorption spectrum of met-*Bpe*GReg H225A. Met-*Bpe*GReg H225A shows the same absorption spectrum as met-*Bpe*GReg, indicating that *Bpe*GReg H225A binds heme. All measurements were done at 25°C and pH 8.0 in 50 mM sodium phosphate buffers.

Figure S5. Characterization of *Av*GReg from *A. vinelandii* and *Cv*GReg from *C. violaceum*. (a) SDS-PAGE of purified *Av*GReg and *Cv*GReg. Lane 1, molecular weight marker; lane 2, *Av*$GReg_{6x\text{-}His}$; lane 3, *Cv*$GReg_{6x\text{-}His}$. Absorption spectrum of (b) *Av*GReg and (c) *Cv*GReg. Solid line, oxygen-bound form; dotted line, deoxy-form; dashed line, oxy-form. (d-f) Phenotypes of *S. typhimurium* cells expressing *Av*GReg and *Cv*GReg. (d) rdar morphotype development on Congo red plates. (e) Swimming motility on 0.3% tryptone agar plates. (f) Biofilm formation in liquid culture.



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HemAT-Bs                             ***************************************************************************************************************************************************
PleD                                 -------------------MKDEWTGLVEQADPPIRAKAAEIAVAHAHYLSIEFYRIVRIDPHAEEFLSNEQVER-QLKSAMERWIINVLSA--QVDDVERLIQIQHTVAEVHARIGIPVEIVEMGFRVLKKILYPVIFSSN-YSAA   124
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HemAT-Bs                             ***************HADVKKQLKMVRLGDAELYVLEQLQPLIQENIVNIVDAFYKNLDHESSLMDIINDHSSVD-RLKQTLKRHIQEMFAG--VIDD--EFIEKRNRIASIHLRIGLLPKWYMGAFQELLLSMIDIYEASI-TNQQ   153
PleD                                 ***************************************************************************************************************************************************
Shigella dysenteriae str. 1012       -------------------MKDEWTGLVEQADPPIRAKAAEIAVAHAHYLSIEFYRIVRIDPHAEEFLSNEQVER-QLKSAMERWIINVLSA--QVDDVERLIQIQHTVAEVHARIGIPVEIVEMGFRVLKKILYPVIFSSN-YSAA   124
Shigella flexneri 2a str. 301        ------------MEMYFKRMKDEWTGLVEQADPLIRAKAAEIALAHAHYLSIEFYRIVRIDPHAEEFLSNEQVER-QLKSAMERWIINVLSA--QVDDVERLIQIQHTVAEVHARIGIPVEIVEMGFRVLKKILYPVIFSSD-YSAA   131
Shigella_sonnei_Ss046                ------------MEMYFKRMKDEWTGLVEQADPLIRAKAAEIAVAHAHYLSIEFYRIVRIDPHAEEFLSNEQVER-QLKSAMERWIINVLSA--QVDDVERLIQIQHTVAEVHARIGIPVEIVEMGFRVLKKILYPVIFSSD-YSAA   131
Escherichia coli O157:H7 EDL933      ------------MEMYFKRMKDEWTGLVEQADPPIRAKAAEIAVAHAHYLSIEFYRIVRIDPHAEEFLSNEQVER-QLKSAMERWIINVLSA--QVDDVERLIQIQHTVAEVHARIGIPVEIVEMGFRVLKKILYPVIFSSD-YSAA   131
Escherichia coli str. E110019        -------------------MKDEWTGLVEQADPLIRAKAAEIAVAHAHYLSIEFYRIVRIDPHAEEFLSNEQVER-QLKSAMERWIINVLSA--QVDDVERLIQIQHTVAEVHARIGIPVEIVEMGFRVLKKILYPVIFSSD-YSAA   124
Erwinia carotovora subsp atroseptica -------------------MIATTS---QQSFNLLRT----LAVQKASDFADEFYIYMLKDQEASLFLSSQQVHD-RLHGSMSKWIADILTN--TGDSLADLINHQKKIGQIHARIGIPVDLVERGARRLKWHLYEHIAQVA-DDKA   117
Bordetella bronchiseptica RB50       -----------MK-PSPEILALRWKDTCAHYSPHEWAAARNVVTANKAALADYFYECMLADPNAAFFLSDQLVKT-KLHASMQDWLESVYAA-APTEEYERTVAFQRKVGEVHARIDIPVHLVMRGACALIRRICELLDRDASLSAA   133
Bordetella pertussis Tohama I        -----------MK-PSPEILALRWKDTCAHYSPHEWVAARNVVTANKAALADYFYECMLADPNAAFFLSDQLVKT-KLHASMQDWLESVYAA-APTEEYERTVAFQRKVGEVHARIDIPVHLVTRGACALIRRICELLDRDASLSAA   133
Bordetella parapertussis 12822       -----------MK-PSPEILALRWKDTCAHYSPHEWVAARNVVTANKAALADYFYECMLADPNAAFFLSDQLVKT-KLHASMQDWLESVYAA-APTEEYERTVAFQRKVGEVHARIDIPVHLVMRGACALIRRICELLDRDASLSAA   133
Shewanella putrefaciens CN-32        -----------MPTIGTEKYTAEWLALVNNLSRSDAGQLEHLIHQNQHALSDTFYQAMMQDSSASEFLSNDLVQK-RLKASMQQWLTDMFQS-QTTEHIQALVAQQIKIGEIHARIGVPVHLVLNGARYLKTRITQLTS--------   126
Shewanella sp. W3-18-1               -----------MPTIGTEKYTAEWLALVNNLSRSDAGQLEHLIHQNQHALSDTFYQAMMQDSSASEFLSNDLVQK-RLKASMQQWLTDMFQS-QTTEHIQALVAQQIKIGEIHARIGVPVHLVLNGARYLKTRITQLTS--------   126
Azotobacter vinelandii AvOP          -----------MPGISPEQQAAEWKLLLGQFPAPVVAQIRELATTHQSELPGYFYEQMLQDEQAMLFLTHEQVKS-RLHGTLRQWIVSVFSMSDDDAALQALIAQQKQIGEIHARIKIPIHLVLRGARHLRERLFVLLRQRP-LDPE   134
Magnetococcus sp. MC-1               ---------------MSARDEQRLKDIYLGVDAEKVNFIGDLIKDRLNQTVERFYIELLEVESARFFLDSALVKE-RLHGSLTEWLQMLFSH-KDDDTLEQMFAFQKNIGNVHARINIPMHLVVEGMRILRREIICFLSESDIPRQR   130
Chromobacterium violaceum ATCC 12472 MKQTDQTLLEQLRITEFEIEQRKHLFALAPRDEMLLRAAGNLVESHLEELVTRFYELQTSTPEIALLIGDADTLQ-RLRSAQRRYVVDLF----SGIYDLEYVNIRLRIGLVHKRIGVEPKLYLAAVDSLKFLLAEKLTEL-IPDAE   141
EAK60420.1                           MNPTEQTLLEQMRITEFEIAHRKELLLLDERDFALLASYRPKIEPHIDALVDKFYTLQTGITEIALLIGDADTLT-RLRAAQRRYILDLF----SGLYDLEYVNNRLRIGLVHKRIGVEPKLYLAAINTLKGLLIEDIFTQ-IDHEP   141
EAJ19547.1                           --------------------------------------------KRNRQIVDDFYGLQTSVSEIALLIGDSDTLA-RLRTAQRRYVLDLF----NGVYDLEYVNNRLRIGLVHKRIGVEPKLYLSAVHTLKELIYAEINNS-VKDAA    97
Shewanella sp. MR-4                  MLQTDQTLFEQMRITELEIDFRKSLFSFTLADVRALQSFKPVIEANIDKIVDDFYGLQTSVSEIALLIGDSDTLA-RLRTAQRRYVLDLF----NGVYDLEYVNNRLRIGLVHKRIGVEPKLYLSAVHTLKELIYAEINNS-VKDAA   141
Shewanella sp. MR-7                  MLQTEQTLFEQMRITELEIDFRKSLFSFTLADVRALQSFKPVIEANIDKIVDDFYGLQTSVSEIALLIGDSDTLA-RLRTAQRRYVLDLF----NGVYDLEYVNNRLRIGLVHKRIGVEPKLYLSAVHTLKELIYAEINNS-VKDAA   141
EAK35357.1                           MLQTEQTLFEQMRITELEIDFRKSLFSFTLADVRALQSFKPVIEENIDKIVDDFYGLQTSVSEIALLIGDSDTLA-RLRTAQRRYVLDLF----NGVYDLEYVNNRLRIGLVHKRIGVEPKLYLSAVHTLKELIYAEINNS-VKDAA   141
Shewanella sp. ANA-3                 MLQTEQTLFEQMRITELEIDFRKSLFSFTLADVRALQSFKPVIEENIDKIVDDFYGLQTSVSEIALLIGDSDTLA-RLRTAQRRYVLDLF----NGVYDLEYVNNRLRIGLVHKRIGVEPKLYLSAVHTLKELIYAEINNS-VADDL   141
Shewanella baltica OS155             -------------------------------------------------------------------------------------MLDLF----NGVYDLEYVNNRLRIGLVHKRIGVEPKLYLSAVHTLKELIYAVINCS-VQDTH    57
Desulfotalea psychrophila LSv54      MKATNYTLIEQLKVTSREIERRKEYLFFTDEDVQVLVSLKEVVSENIEEIVEAFYERVIPFDEMDGVIGDAETLR-RLKNYQRSYIKTLF----AGQYDEEYVHSRLRVGVVHKRIGVEPKFYISAIHTLLTILREIITKNSSNDCQ   142
                                                                                           †                                                         ψ
                                     1........10........20........30........40........50........60........70........80........90........100.......110.......120.......130.......140.....

HemAT-Bs                             ELLKAIKATTKILNLEQQLVLEAFQ-SEYNQ********************************************************************************************************************   186
PleD                                 ***************************************************************************************************************************************************
Shigella dysenteriae str. 1012       EKLQVYHFSINSIDIAMEVMTRAFTFSDSSASKEDENYRIFSLLENAEEEKERQIASILSWEIDIIYKILLDSDLGSSLPLSQADFGLWFNHKGRHYFSGIAEVGHISRLIQDFDGIFNQTMRNTRNLNNRSLRVKFLLQIRNTVSQ   271
Shigella flexneri 2a str. 301        EKLQVYHFSINSIDIAMEVMTRAFTFSDSSASKEDENYRIFSLLENAEEEKERQIASILSWEIDIIYKVLLDSDLGSSLPLSQADFGLWFNHKGRHYFSGIAEVGHISRLIQDFDGIFNQTMRNTRILNNRSLRVKFLLQIRNTVSQ   278
Shigella_sonnei_Ss046                EKLQVYHFSINSIDIAMEVMTRAFTFSDSSASKEDENYRIFSLLENAEEEKERQIASILSWEIDIIYKILLDSDLGSSLPLSQADFGLWFNHKGRHYFSGIAEVGHISRLIQDFDGIFNQTMRNTRNLNNRSLRVKFLLQIRNTVSQ   278
Escherichia coli O157:H7 EDL933      EKLQVYHFSINSIDIAMEVMTRAFTFSDSSASKEDENYRIFSLLENAEEEKERQIASILSWEIDIIYKILLDSDLGSSLPLSQADFGLWFNHKGRHYFSGIAEVGHISRLIQDFDGIFNQTMRNTRNLNNRSLRVKFLLQIRNTVSQ   278
Escherichia coli str. E110019        EKLQVYHFSINSIDIAMEVMTRAFTFSDSSASKEDENYRIFSLLENAEEEKERQIASILSWEIDIIYKVLLDSDLGSSLPLSQADFGLWFNHKGRHYFSGIAEVGHISRLIQDFDGIFNQTMRNTRNLNNRSLRVKFLLQIRNTVSQ   271
Erwinia carotovora subsp atroseptica LCFDAMRFASISMDIAIEIMSKTYSQSHDLAAKNEESYRLFSILENASMERERQNASLLNWENAFIFSVATGTPLSSIQDLSDSDFGLWFNHKGKSSFSNIQGIRTIATIMTETD-EYIRNYSHTALLTQQDY-APLLKAVRSKIYK   262
Bordetella bronchiseptica RB50       QAAATCRYVADVTMTAVEMMCHAYSVSHDRNARAEEGYRLLALSQNVGAERERQRAALLDWENQLMFGLSVGKPWDELPPIRKSEFGLWFIHKAAHAFEGAAESRSVSSQLQHIDQLLA---DAQQTQPPPDQRLAILHSVRDATKA   277
Bordetella pertussis Tohama I        QAAATCRYVADVTMTAVEMMCHAYSVSHDRNARAEEGYRLLALSQNVGAERERQRAALLDWENQLMFGLSVGKPWDELPPIRKSEFGLWFIHKAAHAFEGAAESRSVSSQLQHIDQLLA---DAQQTQPPPDQRLAILHSVRDATKA   277
Bordetella parapertussis 12822       QAAATCRYVADVTMTAVEMMCHAYSVSHDRNARAEEGYRLLALSQNVGAERERQRAALLDWENQLMFGLSVGKPWDELPPIRKSEFGLWFIHKAAHAFEGAAESRSVSSQLQHIDQLLA---DAQQTQPPPDQRLAILHSVRDATKA   277
Shewanella putrefaciens CN-32        --HALASHASILIDLAMEIMSQAYSNSHERNSRSEEAYRLHAIAQNIAHEKDKQRAAFLDWENKVMFDCASGVDAALLQPLYSSEFGLWFRHKAEHAFAGAEEITLINDGINHIDNVLLPLLALPSLQADT-KKLQCLRELREQSKS   270
Shewanella sp. W3-18-1               --HALASHASILIDLAMEIMSQAYSNSHERNSRSEEAYRLHAIAQNIAHEKDKQRAAFLDWENKVMFDCASGVDAALLQPLYSSEFGLWFRHKAEHAFAGAEEITLINDGINHIDNVLLPLLALPSLQADT-KKLQCLRELREQSKS   270
Azotobacter vinelandii AvOP          HKLFGQRLISETVDLAMEIMSRAFSDAYDRNSRSEEGYRLFSVAQNIGIEQERQRSALLDWENHLMFDLAVGQLATQLPRLSNSEFGLWFRHKGAHAFQGSRETELILEGMRQIDEVLLPLFGQPAAGADADQRLQNLRLLREQTKG   281
Magnetococcus sp. MC-1               -LVDLVVLVGEVLDHNLSLINESYVRMSASYERNNQALRLQLSPSAQALECERLRGTLRGWSNQLWRVLGGRGIETELPSLGRAEFGLWMFHKAPLVLPQYEQLHTIQQNIQAMDALLGRQRLSQEHLNAVSR--DTLSAVDDQLNS   274
Chromobacterium violaceum ATCC 12472 VRLHTLQALDKLMMFDVALVFETYIRSLVAEIETSRNKSEQYARTLEEKVATRTR--------------------------------------------------------------------------------------------   196
EAK60420.1                           DRITMLTALDKLFLFDITLVFETYIRSLVSEIESSKEKSEIYAKSLEEKVRERTQ--------------------------------------------------------------------------------------------   196
EAJ19547.1                           QNERIRIAIDKLVLFDVTLVFDTYIRSLVSEIENAKDKAERYAQSMESKVKERTQ--------------------------------------------------------------------------------------------   152
Shewanella sp. MR-4                  QNERIRIAIDKLVLFDVTLVFDTYIRSLVSEIENAKDKAERYAQSMESKVKERTQ--------------------------------------------------------------------------------------------   196
Shewanella sp. MR-7                  QNERIRIAIDKLVLFDVTLVFDTYIRSLVSEIENAKDKAERYAQSMESKVKERTQ--------------------------------------------------------------------------------------------   196
EAK35357.1                           QNERIRIAIDKLVLFDVTLVFDTYIRSLVSEIENAKDKAERYAQSMESKVKERTQ--------------------------------------------------------------------------------------------   196
Shewanella sp. ANA-3                 QNERIRVAIDKLVLFDVTLVFDTYIRSLVSEIENAKDKAERYAQSMESKVKERTQ--------------------------------------------------------------------------------------------   196
Shewanella baltica OS155             QGECIRVAIDKLVLFDVTLVFDTYIRSLVSEIENAKDKAEKYAQSMESKVKERTQ--------------------------------------------------------------------------------------------   112
Desulfotalea psychrophila LSv54      ACVRNLRAVEKIIMFDLSLTFDTYINSLMEETKRSKRELEEYAESLEEVISERTK--------------------------------------------------------------------------------------------   197

                                     150.......160.......170.......180.......190.......200.......210.......220.......230.......240.......250.......260.......270.......280.......290....

HemAT-Bs                             ***************************************************************************************************************************************************   186
PleD                                 ****************AVTDQLTGLHNRRYMTGQLDSLVKRATLGGDPVSALLIDIDFFKKINDTFGHDIGDEVLREFALRLASNVRAIDLPCRYGGEEFVVIMPD-TALADALRIAERIRMHVSGSPFTVAHGREMLN--------   410
Shigella dysenteriae str. 1012       IITLLRELFEEVSRHEVGMDVLTKLLNRRFLPTIFKREIAHANRTGTPLSVLIIDVDKFKEINDT----------------------------------------------------------------------------------   336
Shigella flexneri 2a str. 301        IITLLRELFEEVSRHEVGMDVLTKLLNRRFLPTIFKREIAHANRTGTPLSVLIIDVDKFKEINDTWGHNTGDEILRKVSFLSQKRLVKSKILGAGSSRKLAVS--------------------------------------------   381
Shigella_sonnei_Ss046                IITLLRELFEEVSRHEVGMDVLTKLLNRRFLPTIFKREIAHANRTGTPLSVLIIDVDKFKEINDTWGHNTGDEILRKVSQAFYDNVHSSDYVFRYGGDEFIIVLTE-ASENETLRTAERIRSRVEKTKLKAANGEDIAL--------   416
Escherichia coli O157:H7 EDL933      IITLLRELFEEVSRHEVGMDVLTKLLNRRFLPTIFKREIAHANRTGTPLSVLIIDVDKFKEINDTWGHNTGDEILRKVSQAFYDNVRSSDYVFRYGGDEFIIVLTE-ASENETLRTAERIRSRVEKTKLKAANGEDIAL--------   416
Escherichia coli str. E110019        IITLLRELFEEVSRHEVGMDVLTKLLNRRFLPTIFKREIAHANRTGTPLSVLIIDVDKFKEINDTWGHNTGDEILRKVSQAFYDNVRSSDYVFRYGGDEFIIVLTE-ASENETLRTAERIRSRVEKTKLKAANGEDIAL--------   409
Erwinia carotovora subsp atroseptica INMLLGSLFDEVQKLESGKDTLTLLLNRRFLPTILRHEISLTMHSNTPLSIAMIDIDHFKSVNDTYGHAAGDNILKRIAEILYESTRNSDYVFRYGGEEFLIVLIE-TPKVAAHTIIERLRKKIENHFIHLQNGESVKM--------   400
Bordetella bronchiseptica RB50       IGFLIDGLFEQAGNLESGRDTLTRLLNRKYLHVVLSKEVAYARKTGVPLSVLVADIDYFKSINDAHGHDAGDFVLQQVASLIASYSRGGDYTFRLGGEEFLVVLVD-ADGTQALNVAEALRRRIQEKATVLPDGGEVRL--------   415
Bordetella pertussis Tohama I        IGFLIDGLFEQAGNLESGRDTLTRLLNRKYLHVVLSKEVAYARKTGVPLSVLVADIDYFKSINDAHGHDAGDFVLQQVASLIASYSRGGDYTFRLGGEEFLVVLVD-ADGTQALNVAEALRRRIQEKATVLPDGGEVRL--------   415
Bordetella parapertussis 12822       IGFLIDGLFEQAGNLESGRDTLTRLLNRKYLHVVLSKEVAYARKTGVPLSVLVADIDYFKSINDAHGHDAGDFVLQQVASLIASYSRGGDYTFRLGGEEFLVVLVD-ADGTQALNVAEALRRRIQEKATVLPDGGEVRL--------   415
Shewanella putrefaciens CN-32        IRYYLDLLFEQNREVESGRDVLTRLLNRKFLPAVMTKEVNYSRRNKSQFAILAIDIDHFKTVNDSYGHEGGDLVLQHLAALLSNTGRGGDYLFRLGGEEFLMLLVD-INAREAALSAENIRRKVERETFLLPHNQQLQI--------   408
Shewanella sp. W3-18-1               IRYYLDLLFEQNREVESGRDVLTRLLNRKFLPAVMTKEVSYSRRNKSQFAILAIDIDHFKTVNDSYGHEGGDLVLQHLAALLSNTGRGGDYLFRLGGEEFLMLLVD-INAREAALSAENIRRKVERETFLLPHNQQLQI--------   408
Azotobacter vinelandii AvOP          IIFHLDNLFEQANVLESGRDVLTHMLNRKFLPVILAKEVAYARQNERSFAVLAVDIDRFKHINDEFGHEAGDVVLQQLASLLSNHCRGGDYVFRMGGEEFLVLLVD-VTQAHALGVAEKLRQQAAQERFRLPGEQDLQL--------   419
Magnetococcus sp. MC-1               IELALDMMIEAILAQESGRDPLTKVYNRRFLDSVVKHEMEVSMRHEIRFGFLLCDIDHFKTINDTYGHDAGDAVLQQFAERLSSAARVNDYVFRFGGEEFLFVYGD-MSPQRLEHLAEKVRHCIEQELFDLPSGEKIHI--------   412
Chromobacterium violaceum ATCC 12472 -----------QLEELSRTDPLTGLLNRRYLDETLTRVLRSAQRRREKVALVYLDVDHFKLLNDSEGHQRGDEVLRLLGETISRITRLEDGCFRYGGDEFCIVLPN-CDQ-KQAEETFLQRLVDELATILPGLSLSVGC--------   322
EAK60420.1                           -----------QLEEMSRTDALTGLLSVRHLQENLTRTLRTCQRRSEPVVIAYLDIDDFKQINDSQGHQRGDEILRIVGDCIRKVSRAEDCCFRYGGDEFCLILPN-CNE-EQARDVYLQRLTNALAEQISDVTLSIGL--------   322
EAJ19547.1                           -----------QLEELSQTDPLTGLLNVRHLQEFATRILRAAQRRAEPVSVIYLDVDDFKHFNDSQGHRAGDEVLRAVAQSIKESTRAEDHCFRYGGDEFCIIMPN-CSCPHESASPIIDKIAEREEQARDHFIERFNQNIKRHLNN   287
Shewanella sp. MR-4                  -----------QLEELSQTDPLTGLLNVRHLQEFATRILRAAQRRAEPVSVIYLDVDDFKHFNDSQGHRAGDEVLRAVAQSIKESTRAEDHCFRYGGDEFCIIMPN-CFCPHESASPIIDKIAEREEQARDHFIERFNQNIKRHLNN   331
Shewanella sp. MR-7                  -----------QLEELSQTDPLTGLLNVRHLQEFATRILRAAQRRAEPVSVIYLDVDDFKHFNDSQGHRAGDEVLRAVAQSIKESTRAEDHCFRYGGDEFCIIMPN-CSCPHESAAPIIDKIAEREEQARDHFIERFNQNIKRHLNN   331
EAK35357.1                           -----------QLEELSQTDPLTGLLNVRHLQEIGTRILRAAQRRAEPVSVIYLDVDDFKSFNDTQGHRSGDEVLCAVAQAIKESTRVEDHSFRYGGDEFCIIMPN-CIVHSIQQPQGINKVAEREDQARDNFIERFNQNIKLHLNN   331
Shewanella sp. ANA-3                 -----------QLEELSQTDPLTGLLNVRHLQEIGTRILRAAQRRAEPVSVIYLDVDDFKSFNDTQGHRSGDEVLCAVAQAIKESTRVEDHSFRYGGDEFCIIMPN-CIVHSIQQPQGINKVAEREDQARDNFIERFNQNIKLHLNN   331
Shewanella baltica OS155             -----------QLEELSQTDPLTGLLNVRHLQETATRILRAAQRRAEPVSVIYLDVDDFKSFNDTQGHRSGDEVLRAVAKAIKEITRAEDHCFRYGGDEFCIIMPN-C-VHSISQSQSVNKAVEREEQARDNFITRFNQKIKLSLNN   246
Desulfotalea psychrophila LSv54      -----------LLKEQARHDGLTKLLNQHAFYSELRRELARAQRRGYCTVLIYFDLDKFKALNDSEGHRRGDEVLVMVANSMKASLRDNELCARYGGDEFCIILPESTLKEAEKVCQRLCKELEKSLVGTGV---------------   318
                                                                                                                          └─I─┘     └─A─┘
                                     ...300.......310.......320.......330.......340.......350.......360.......370.......380.......390.......400.......410.......420.......430.......440.
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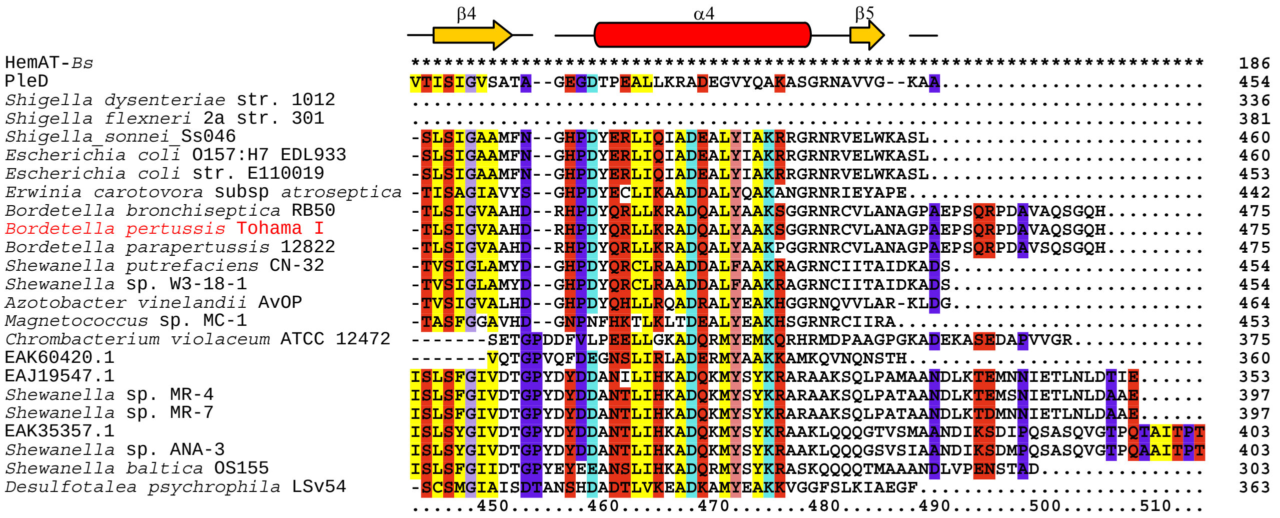

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HemAT-Bs                                   ************************************************************************   186
PleD                                       VTISIGVSATA--GEGDTPEALLKRADEGVYQAKASGRNAVVG--KAA........................   454
Shigella dysenteriae str. 1012             ........................................................................   336
Shigella flexneri 2a str. 301              ........................................................................   381
Shigella_sonnei_Ss046                      -SLSIGAAMFN--GHPDYERLIQIADEALYIAKRRGRNRVELWKASL.........................   460
Escherichia coli O157:H7 EDL933            -SLSIGAAMFN--GHPDYERLIQIADEALYIAKRRGRNRVELWKASL.........................   460
Escherichia coli str. E110019              -SLSIGAAMFN--GHPDYERLIQIADEALYIAKRRGRNRVELWKASL.........................   453
Erwinia carotovora subsp atroseptica       -TISAGIAVYS--GHPDYECLIKAADDALYQAKANGRNRIEYAPE...........................   442
Bordetella bronchiseptica RB50             -TLSIGVAAHD--RHPDYQRLLKRADQALYAAKSGGRNRCVLANAGPAEPSQRPDAVAQSGQH.........   475
Bordetella pertussis Tohama I              -TLSIGVAAHD--RHPDYQRLLKRADQALYAAKSGGRNRCVLANAGPAEPSQRPDAVAQSGQH.........   475
Bordetella parapertussis 12822             -TLSIGVAAHD--RHPDYQRLLKRADQALYAAKPGGRNRCVLANAGPAEPSQRPDAVSQSGQH.........   475
Shewanella putrefaciens CN-32              -TVSIGLAMYD--GHPDYQRCLRAADDALFAAKRAGRNCIITAIDKADS.......................   454
Shewanella sp. W3-18-1                     -TVSIGLAMYD--GHPDYQRCLRAADDALFAAKRAGRNCIITAIDKADS.......................   454
Azotobacter vinelandii AvOP                -TVSIGVALHD--GHPDYQHLLRQADRALYEAKHGGRNQVVLAR-KLDG.......................   464
Magnetococcus sp. MC-1                     -TASFGGAVHD--GNPNFHKTLKLTDEALYEAKHSGRNRCIIRA............................   453
Chrombacterium violaceum ATCC 12472        -------SETGPDDFVLPEELLGKADQRMYEMKQRHRMDPAAGPGKADEKASEDAPVVGR............   375
EAK60420.1                                 -------VQTGPVQFDEGNSLIRLADERMYAAKKAMKQVNQNSTH...........................   360
EAJ19547.1                                 ISLSFGIVDTGPYDYDDANILIHKADQKMYSYKRARAAKSQLPAMAANDLKTEMNNIETLNLDTIE......   353
Shewanella sp. MR-4                        ISLSFGIVDTGPYDYDDANTLIHKADQKMYSYKRARAAKSQLPATAANDLKTEMSNIETLNLDAAE......   397
Shewanella sp. MR-7                        ISLSFGIVDTGPYDYDDANTLIHKADQKMYSYKRARAAKSQLPATAANDLKTDMNNIETLNLDAAE......   397
EAK35357.1                                 ISLSYGIVDTGPYDYDDANTLIHKADQKMYSYKRAAKLQQQGTVSMAANDIKSDIPQSASQVGTPQTAITPT   403
Shewanella sp. ANA-3                       ISLSYGIVDTGPYDYDDANTLIHKADQKMYSYKRAAKLQQQGSVSIAANDIKSDMPQSASQVGTPQAAITPT   403
Shewanella baltica OS155                   ISLSFGIIDTGPYEYEEANSLIHKADQRMYSYKRASKQQQQTMAAANDLVPENSTAD...............   303
Desulfotalea psychrophila LSv54            -SCSMGIAISDTANSHDADTLVKEADKAMYEAKKVGGFSLKIAEGF..........................   363
                                           ......450.......460.......470.......480.......490.......500.......510...
```

Fig. S1

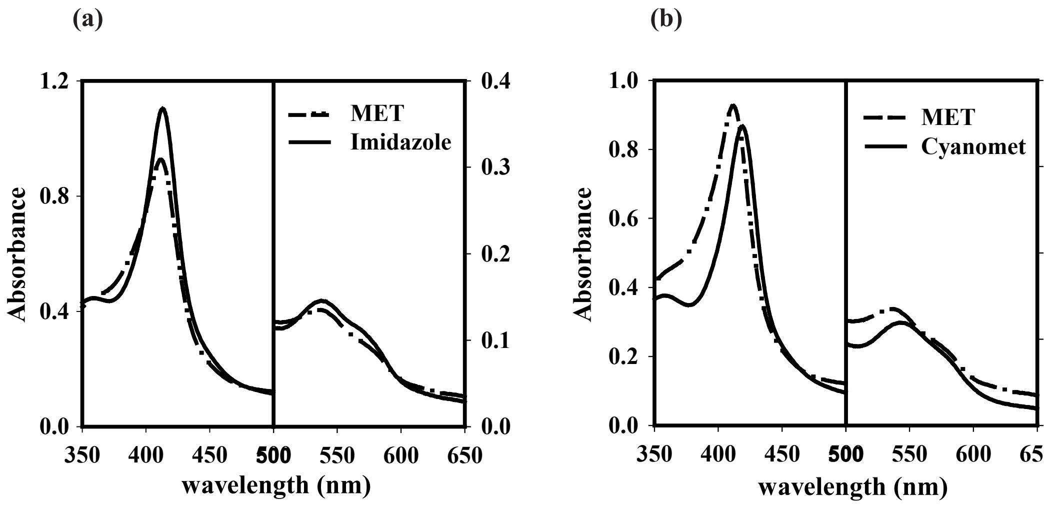

(a)
1.2
0.8
0.4
0.0
0.4
0.3
0.2
0.1
0.0
Absorbance
MET
Imidazole
350
400
450
500
550
600
650
wavelength (nm)
(b)
1.0
0.8
0.6
0.4
0.2
0.0
0.4
0.3
0.2
0.1
0.0
Absorbance
MET
Cyanomet
350
400
450
500
550
600
650
wavelength (nm)

Fig. S2

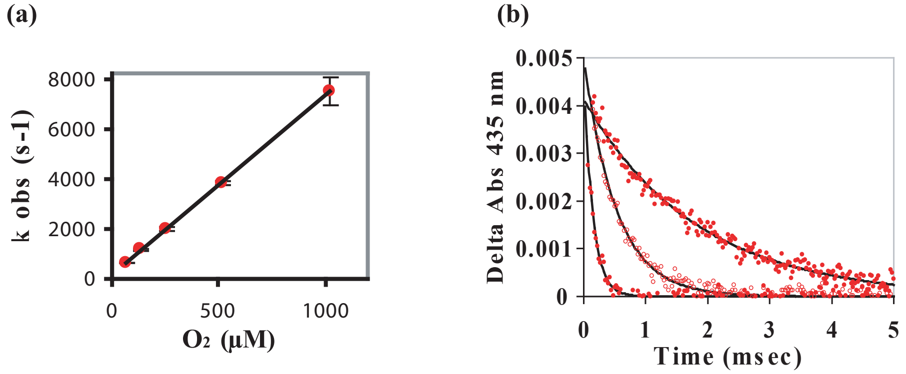
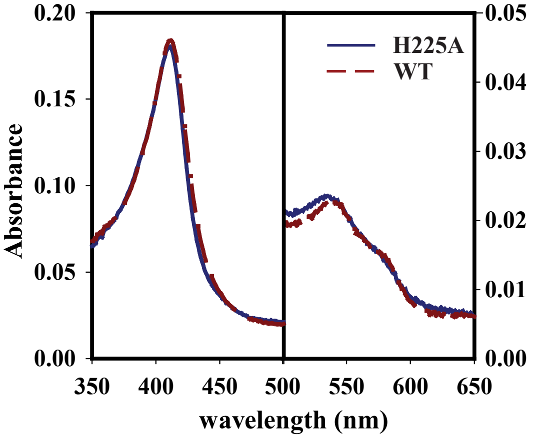

(a)
8000
6000
4000
2000
0
k obs (s-1)
0
500
1000
O₂ (µM)
(b)
0.005
0.004
0.003
0.002
0.001
0
Delta Abs 435 nm
0
1
2
3
4
5
Time (msec)

Fig. S3



0.20
0.15
0.10
0.05
0.00
Absorbance
0.05
0.04
0.03
0.02
0.01
0.00
H225A
WT
350
400
450
500
550
600
650
wavelength (nm)

Fig. S4

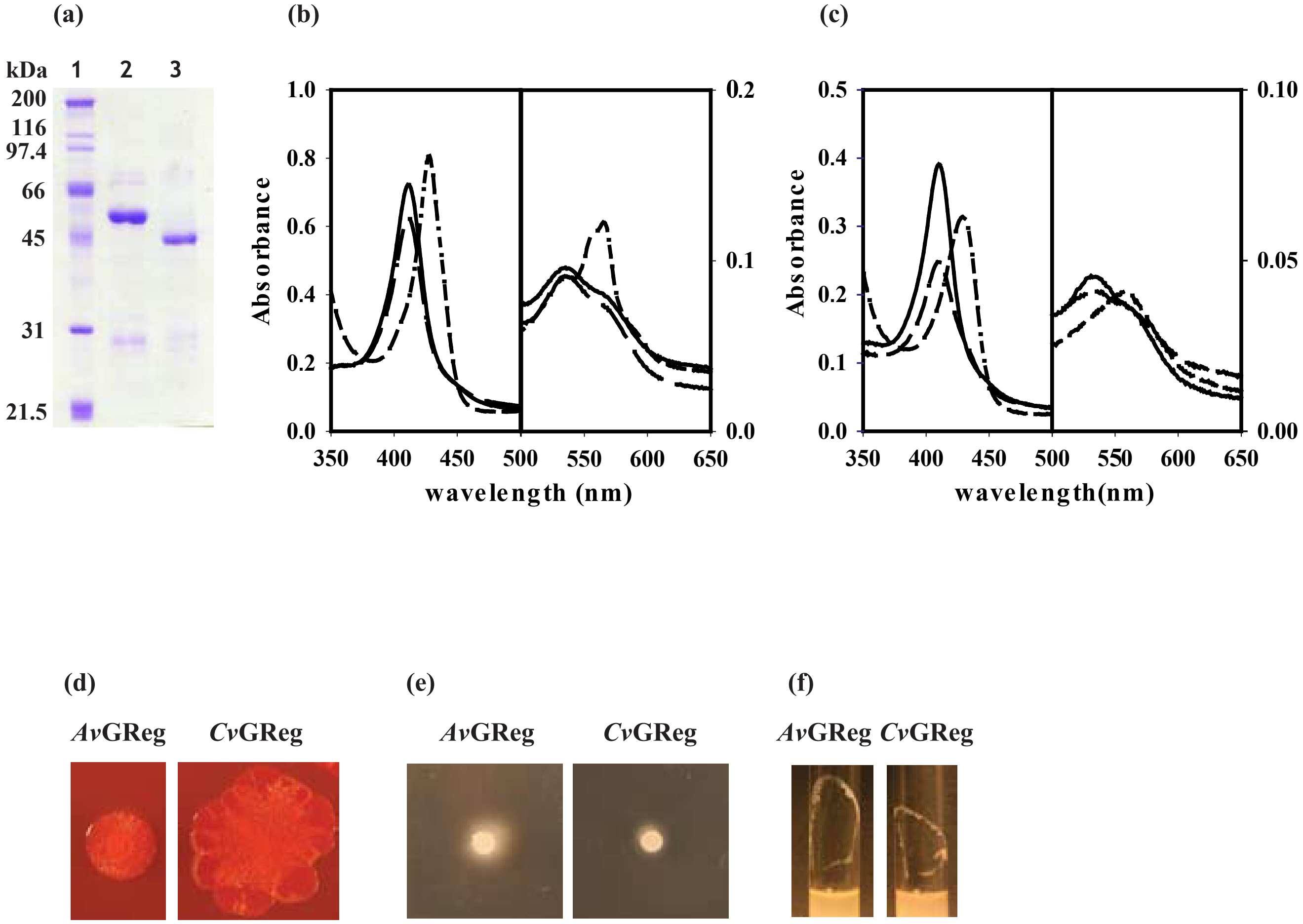

(a)
kDa
1
2
3
200
116
97.4
66
45
31
21.5
(b)
1.0
0.8
0.6
0.4
0.2
0.0
0.2
0.1
0.0
Absorbance
350
400
450
500
550
600
650
wavelength (nm)
(c)
0.5
0.4
0.3
0.2
0.1
0.0
0.10
0.05
0.00
Absorbance
350
400
450
500
550
600
650
wavelength(nm)
(d)
*Av*GReg
*Cv*GReg
(e)
*Av*GReg
*Cv*GReg
(f)
*Av*GReg
*Cv*GReg

Fig. S5