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Supplemental Material for"

Informing Efforts to Develop Nitroreductase for Amine Production

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1. Contents

2. Tables

2.1. Table S1. Compounds used to calibrate E° calculations, and resulting calibrated calculated E° values, with ChemSpider ID numbers and measured E° values.[1]

Calibration Compound	ChemSpider[2]	Uncalibrated Computed E° [3]	Measured E° [1] (mV vs. NHE)	Calculated $E°_c$ [4] (Calibrated)
nitrobenzene	7138	-486	-486	-483
4-chloronitrobenzene	21106020	-231	-450	-428
4-nitroacetophenone	21106581	81	-356	-361
trinitrotoluene	8073	561	-253	-259
3-methyl nitrobenzene	21106146	-556	-475	-498
4-methyl nitrobenzene	13863774	-649	-500	-518
3-chloronitrobenzene	21106013	-188	-405	-419
1,4-dinitrobenzene	7211	551	-257	-261
2,4-dinitrotoluene	8150	41	-397	-370
3-nitroacetophenone	21106145	-185	-437	-419
Experimental Compound				
7 3-nitrofurazone	4566720		-270 [5]	-349
Experimental Compound		**π system** (# atoms)	**Log(P)**	
1 nitrobenzene	7138	9	0.32	-483
3 4-nitrobenzene sulfonamide	21360	12	-1.17	-342
4 3-trifluoromethyl nitrobenzene	7108	9	1.24	-392
5 3-nitrophthalimide	11286	14	-0.96	-334
6 1-nitronaphthalene	6588	13	1.32	-427
7 3-nitrofurazone	4566720	14	-2.62	-349
8 4-nitro-1,8-naphthalic anhydride	73216	18	0.72	-170
9 BTZ043	24747357	15	2.01	-259 [6]
10 1,3-dinitrobenzene	7172	12	-1.39	-339
12 2,4,6-trinitrotoluene	8073	15	-2.61	-259
13 3,5-dinitrobenzotrifluoride	109682	12	-0.47	

[1] Measured values were obtained from [1, 2]. Plots are provided as Supplemental Figure S2.

[2] Information and links on each compound can be retrieved by entering the ChemSpider number as the basis of a search at http://www.chemspider.com/Default.aspx

[3] *Ab-initio* computations were used to produce 1-electron *relative* E°s using the functional ωB97X-D and the basis set 6-311+G** as described in methods.

[4] Values calibrated to experiment using the linear dependence in Supplemental Figure S2(**a**): Calibrated = (Computed + 379)*4.667.

[5] The measured E° for 3-nitrofurazone at 7.45, which corresponds to $\sim NO_2 + 2e^- + 2H^+ \rightarrow \sim NO_2 + H_2O$ [3].

[6] Computations of E° retained the full aromatic system and attached amine but replaced the aliphatic extension with two Hs.

2.2. Table S2. Substrates used for kinetic analysis, with ChemSpider ID numbers and Hammett *para* constants (σ). Plots are provided as Supplemental Figure S4.

Compound	Chem-Spider[1a]	Hammett σ para [2]	*St*NfsB		*Ms*PnbA	
			k_{cat} (s^{-1})	k_{cat}/K_M ($s^{-1}.mM^{-1}$)	k_{cat} (s^{-1})	k_{cat}/K_m ($s^{-1}.mM^{-1}$)
nitrobenzene	7138	0	-	1.7 ± 0.1	3.4 ± 0.4	1.2 ± 0.3
4-nitrobenzoate	3620348	0	5.8 ± 0.3	5.1 ± 0.6	-	-
4-chloronitrobenzene	21106020	0.227	-	5.2 ± 0.2	4.5 ± 0.5	11 ± 4
methyl-4-nitrobenzoate	11586	0.45	-	84.5 ± 6.8	9.0 ± 0.3	75 ± 7
4-nitroacetophenone	21106581	0.5	166 ± 25	167 ± 54	-	-
4-nitrobenzenesulfonamide	21360	0.6	182 ± 11	108 ± 20	17.2 ± 0.9	91 ± 13
4-nitrobenzonitrile	11593	0.66	290 ± 13	238 ± 22	16.0 ± 1.0	111 ± 22
methyl 4-nitrobenzenesulfonate	21173	0.9	275 ± 22	373 ± 90	19.1 ± 0.8	165 ± 18

[1] Information and links on each compound can be retrieved by entering the ChemSpider number as the basis of a search at http://www.chemspider.com/Default.aspx

[2] [4]

66 2.3. Table S3. Surface area (per monomer) buried in dimer interface.

Subgroup	**Instance** (PDB ID)	**Buried Area** (Å^2)[1]	**Statistics** (Å^2)
NfsB	5J8D	4907, 5225	Avg = **4600** ± 400
	3HZN	4137	
	1YKI	4681, 4849	
	1VFR	4135	
	3OF4	4321	
	2HAY	4708	
NfsA	1F5V	5751	Avg = **5500** ± 300
	2BKJ	4892	
	3N2S	5599, 5593	
	3EOF	5787	
PnbA	2WZW	5014	Avg = **5080** ± 70
	3GR3	5146	
Frm2	2IFA	4050, 3957, 3806	Avg = **3900** ± 100
HUB	3E39	4168	Avg = **4200** ± 100
	3E10	4372	
	4DN2	4172	

67 [1] Average of areas buried of each of two participating monomers, multiple values correspond
68 to area buried in AB dimer, CD dimer (if present), EF dimer (if present). Calculated in Chimera [5].

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71 2.4. Table S4. List of the pdb files and amino acid sequences used in the Figures and the sequence
72 alignment in Figure S9.

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Accession code[1] (ID in MSA)[4]	**Species source and annotated name**	Subgroup[2], Amine production and ref.
Cons_NfsB	*NONE* consensus sequence for NfsB from SFLD[3]	
5J8D Ent_5J8D	*Enterobacter cloacae* NR UniProtKB AC: Q01234	NfsB
1YKI Ecoli_1YKI	*Escherichia coli* NfnB UniProtKB AC: P38489	NfsB
1VFR Vfisch_1VFR	*Vibrio fischeri* major NAD(P):FMN oxidoreductase UniProtKB AC: P46072	NfsB
3QDL Helicobac_3QDL	*Helicobacter pylori* RdxA UniProtKB AC: O25608	NfsB
3OF4 Idiomar_3OF4	*Idiomarina loihiensis* L2TR Nitroreductase/dihydropteridine reductase UniProtKB AC: Q5R179	NfsB
3HZN Styph_3HZN	**Salmonella typhinurium *dihydropteridine reductase* *UniProtKB AC: P15888***	***NfsB AMINE (this work)***
2HAY Spyo_2HAY	*Streptococcus pyogenes* M1 GAS Putative NAD(P)H-Flavin Oxidoreductase UniProtKB AC: Q9A120	NfsB
3BEM Bsubt_3BEM	*Bacillus subtilis* putative nitroreductase YdfN (2632848) UniProtKB AC: P96692	MhqN
4QLX Lplant_4QLX	*Lactobacillus plantarum* enone reductase UniProtKB AC: U6C5W9	MhqN
3GBH Sepider_3GBH	*Staphylococcus epidermidis* ATCC 12228 putative NAD(P)H:FMN oxidoreductase (SE1966) UniProtKB AC: A0A0H2VHN8	MhqN
3GE6 Esibir_3GE6	*Exiguobacterium sibiricum* 255-15 putative nitroreductase EXIG_2970 UniProtKB AC: B1YG32	MhqN
AAP09971 AMINE_Bcer3024	*Bacillus cereus* ATCC 14579 NAD(P)H nitroreductase called BC_3024 and YdgI_Bc	MhqN, AMINE inferred [6]
3GAG Smutants_3GAG	*Streptococcus mutans* nitroreductase-like protein (smu.346) UniProtKB AC: Q8DVW4	MhqN
2B67 Spneum_2B67	*Streptococcus pneumoniae* TIGR4 nitroreductase family protein UniProtKB AC: A0A0H2UP38	MhqN
Cons_NfsA	*NONE*	

	consensus sequence for NfsA from SFLD	
1F5V Ecoli_1F5V	*Escherichia coli* NfsA UniProtKB AC: P17117	NfsA
2BKJ Vharv_2BKJ	*Vibrio harveyi* Flavin reductase UniProtKB AC: Q56691	NfsA
3N2S Bsubt_3N2S	*Bacillus subtilis* NfrA1 nitroreductase UniProtKB AC: P39605	NfsA AMINE [7]
3EOF Bfrag_3EOF	Bacteroides fragilis Oxidoreductase (YP_213212.1) UniProtKB AC: Q5L9C9	NfsA
KXV33494 AMINE_Gox0834	*Gluconobacter oxydans* Nitroreductase Gox0834	NfsA AMINE [8]
AAP08598 AMINE_Bcer1619	*Bacillus cereus* ATCC 14579 Oxygen-insensitive NADPH nitroreductase called BC_1619 or YfkO_Bc	NfsA, AMINE inferred [6]
010964037 AMINE_CaceNitA	*Clostridium acetobutylicum* 824 NitA WP_010964037 NADPH-dependent oxidoreductase	NfsA AMINE [9]
Cons_PnbA	*NONE* consensus sequence for PnbA from SFLD	
2WZW AMINE_Msmg_2WZW	*Mycobacterium smegmatis* Nitroreductase NfnB UniProtKB AC: A0R6D0	PnbA, AMINE [10]
3GR3 Bhen_3GR3	*Bartonella henselae* str. houston-1 PnbA UniProtKB AC: A0A0H3M323	PnbA
WP_031019703.1 Strep	*Streptomyces* sp. NRRL WC-3795 Nitroreductase	PnbA
WP_055507769.1 Saur	*Streptomyces aurantiacus* Oxidoreductase	PnbA
WP_080719519.1 Coryn	*Corynebacterium jeikeium* Nitroreductase	PnbA
WP_014983339.1 Nocar	*Nocardia brasiliensis* Oxidoreductase	PnbA
WP_043519713.1 Kpneu	*Klebsiella pneumoniae* Nitroreductase	PnbA
WP_057084594.1 Dicke	*Dickeya solani* Nitroreductase	PnbA
WP_076001261.1 Halio	*Halioglobus pacificus* Nitroreductase	PnbA

KPQ07647.1 Rhodo	*Rhodobacteraceae bacterium* HLUCCA12 Nitroreductase	PnbA
ONF96818.1 Sphin	*Sphingomonas* sp. G39 Coenzyme F420:L-glutamate ligase	PnbA
WP_014992242.1 Actino	*Actinobacillus suis* Nitroreductase	PnbA
Cons_Frm2	*NONE* consensus sequence for Frm2 from SFLD	
2IFA Smut_2IFA	*Streptococcus mutans* Putative nitroreductase UniProtKB AC: Q8DW21	Frm2
4URP Scere_4URP	*Saccharomyces cerevisiae* Nitroreductase UniProtKB AC: P37261	Frm2
2WQF Llact_2WQF	*Lactococcus lactis* Nitroreductase CinD UniProtKB AC: Q9CED0	Frm2
1YWQ Bcer_1YWQ	*Bacillus cereus* ATCC 14579 Nitroreductase family member UniProtKB AC: Q81EW9	Frm2
Cons_HUB	*NONE* consensus sequence for HUB from SFLD	
3E39 Desulf_3E39	*Desulfovibrio desulfuricans* putative nitroreductase dde_0787 UniProtKB AC: Q314Q8	HUB
3E10 Cace_3E10	*Clostridium acetobutylicum* putative NADH oxidase NP_348178.1 UniProtKB AC: Q97IT9	HUB
WP_010966820 AMINE_CaceNitB	*Clostridium acetobutylicum* NitB nitroreductase	HUB AMINE [9]
3KWK Bthet_3KWK	*Bacteroides thetaiotaomicron* putative nitroreductase NP_809094.1 UniProtKB AC: Q8ABC9	HUB
3GE5 Pging_3GE5	*Porphyromonas gingivalis* w83 Putative NAD(P)H:FMN oxidoreductase UniProtKB AC: Q7MX99	HUB
3G14 Cnov_3G14	*Clostridium novyi* NT Nitroreductase family protein YP_877874.1 UniProtKB AC: A0PZS2	HUB
4DN2	*Geobacter metallireducens* GS-15 putative	HUB

Gmetal_4DN2	nitroreductase UniProtKB AC: Q39RS1	
3GFA Cdiff_3GFA	*Clostridium difficile* 630 putative nitroreductase YP_001089721.1 UniProtKB AC: Q17ZU8	HUB
Tther_1NOX	*Thermus thermophilus* NADH oxidase UniProtKB AC: Q60049	HUB
3EO8 Cdiff_3EO8	*Clostridium difficile* 630 BluB-like flavoprotein YP_001089088 UniProtKB AC: Q182R2	HUB
Mtub_4XOM Mtubr_4XOM	*Mycobacterium tuberculosis* C-terminal domain of CoenzymeF420:L-glutamate ligase UniProtKB AC: P9WP79	FbiB
4EO3 Tmar_4EO3	*Thermotoga maritima* Peroxireductase nitroreductase fusion enzyme. UniProtKB AC: Q9WYL7	TdsD
2FRE Agro_2FRE	*Agrobacterium tumefaciens* (fabrum) Oxidoreductase UniProtKB AC: A9CKT4	TdsD
AFW02496 Gox	*Gluconobacter oxydans* H24 Nitroreductase	TdsD
3GB5 Mmus_3GB5	*Mus musculus* iodotyrosine deiodinase IYD UniProtKB AC: Q9DCX8	Iyd
4TTC Hsap_4TTC	*Homo sapiens* iodotyrosine deiodinase IYD UniProtKB AC: Q6PHW0	Iyd
3EK3 Bfrag_3EK3	*Bacteroides fragilis* NCTC 9343 Nitroreductase YP_211706.1 UniProtKB AC: Q5LDN3	Unknown 2
3PXV Dhafn_3PXV	*Desulfitobacterium hafniense* DCB-2 Nitroreductase UniProtKB AC: B8FRE0	Unknown 2
2ISJ Smel_2ISJ	*Sinorhizobium meliloti* BluB Flavin conversion to 5,6-dimethylbenzimidazole. UniProtKB AC: Q92PC8	BluB
3K6H Agro_3K6H	*Agrobacterium tumefaciens* Str. C58 (fabrum) Nitroreductase UniProtKB AC: A9CIP6	Unknown 1

[1] PDB IDs are provided if applicable. For sequences not associated with a structure the NCBI accession number is provided and in parenthesis the abbreviation used in the Multiple Sequence Alignment. Accession codes can be associated with further information by using them as the basis for a search at https://www.ncbi.nlm.nih.gov/home/proteins/ courtesy of the National Center for Biotechnology Information, U.S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 20894 USA

80 [2] Subgroups are those of Akiva/Copp [11]. Enzymes for which we found literature reports of
81 amine production are so indicated with the reference.

82 [3] SFLD is the Structure-Function Linkage Database [12].

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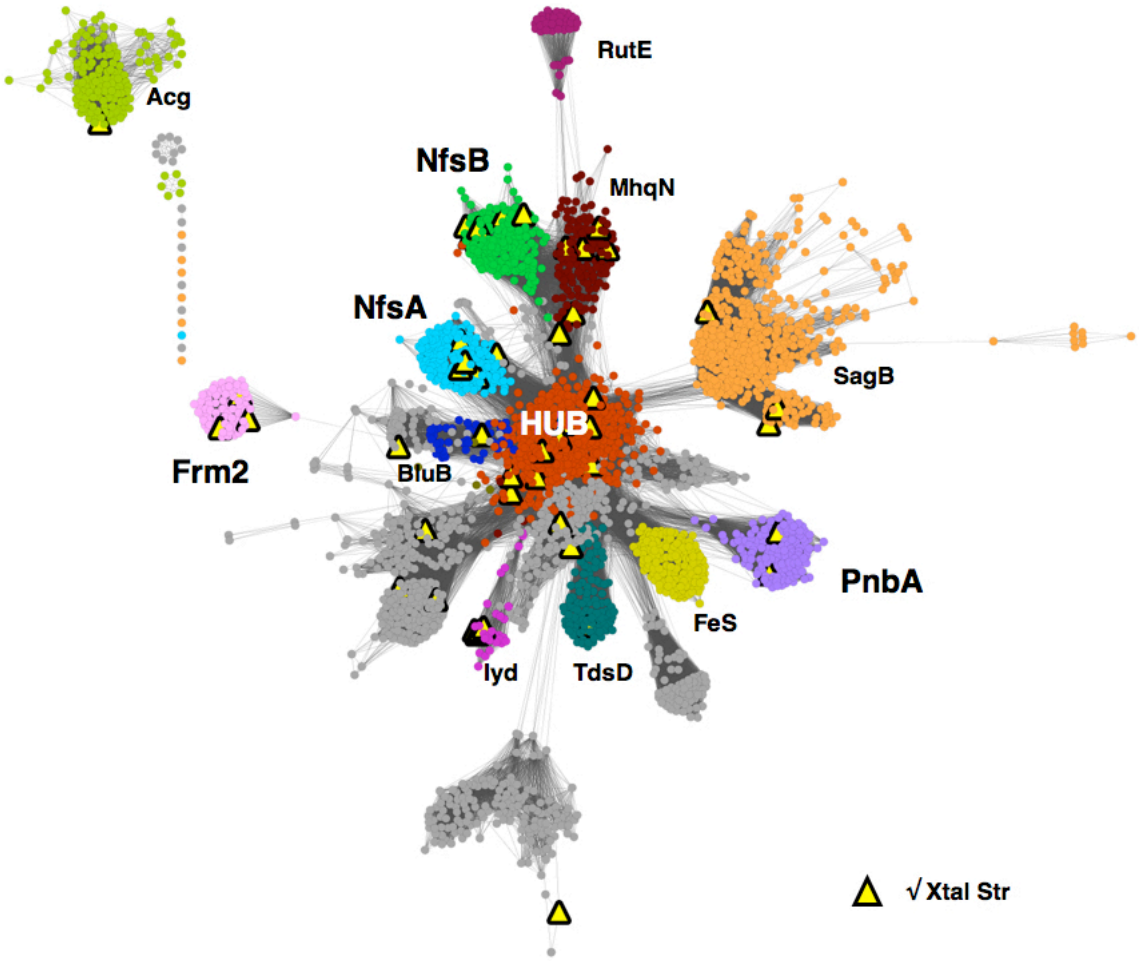
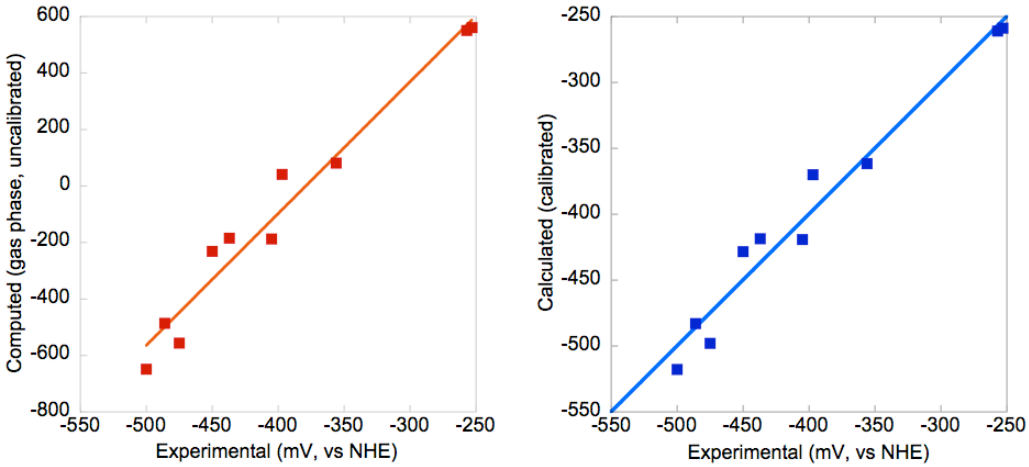
3. Figures



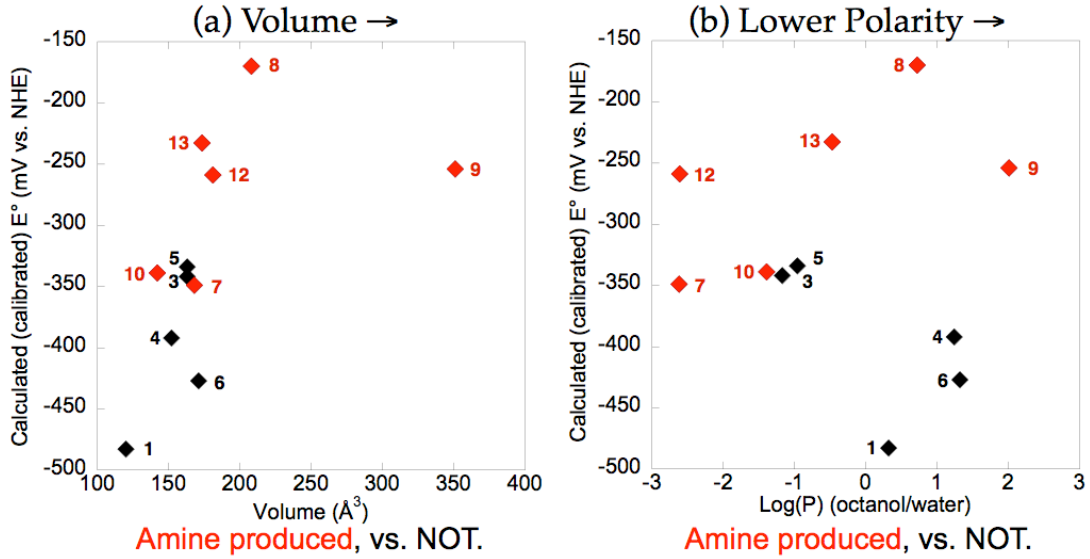
3.1. Figure S1. Sequence similarity network of the different subgroups of the nitroreductase superfamily coloured similarly to Akiva, Copp et al [11] but with unknown subgroups in grey. Small differences in the distribution of the subgroups have resulted from the use of a different software but the topology of the network is unchanged (compare with Figure 2 of [11]). Each small coloured or grey dot is a node of amino acid sequences sharing 50% or higher identity. Any notes sharing homology scoring higher than e^{-16} with another node is connected to it with a line. Those nodes that include a solved crystal structure are depicted as yellow triangles with heavy borders. In each subgroup with ours colored and subgroups containing amine producers identified. Otherwise each node is coloured according to the subgroup to which it is assigned. The colours replicate those of Akiva/Copp except where needed to permit use of the same colours in structural figures. Thus rust replaces the red of Akiva/Copp in order to allow CPK to be used (red = oxygen); bright blue replaces light teal for NfsA, the pale mauve (PnbA) and rose (Frm2) of Akiva/Copp have also been rendered in more saturated colours, to improve structural figures. For the origins of subgroup names, see Akiva, Copp et al. [11].



(a) (b)

3.2. Figure S2. (**a**) Calibration of calculated E° values *vs*. experimental values and (**b**) resulting correspondence between calibrated calculations and experiment yielding MAD = 14 mV. *Ab-initio* computations were used to produce 1-electron *relative* E°s using the functional ωB97X-D and the basis set 6-311+G** as described in methods. These computed values of E° show a linear trend vs. experimental values with $R^2 = 0.96$ and Comp = (Exp + 379)*4.67 where 'Comp' is the value from *ab initio* gas-phase calculation in mV. The 379 mV systematic offset and the multiplier of 4.67 both likely arise from our use of gas-phase calculations and a modest level of theory whereas the measured values derive from water (for another example see [1, 13]). Thus we emphasize that the *ab-initio* calculations at our level of theory in gas phase do not themselves yield values that replicate experiment in water. They do however produce a tight and linear correlation with all 10 experimental values that were most pertinent to the class of molecules and reaction under study, and therefore permit estimations of anticipated E°s where they are not available from experiment. Thus we treated the straight-line correlation as a calibration curve, and applied the calibration to obtain calibrated values Cal = (Comp/4.67)-379 to produce calculated 1-e E°s that are calibrated against experiment. This compromise has allowed us to apply the method to larger molecules than would have been accessible without access to specialized resources. The success of the calculated results can be assessed by comparing the plot vs. experimental data (dark blue squares) with an Y=X line. The mean absolute deviation (MAD) of the calculated values from the line is 14 mV.

Compounds used are tabulated in Supplemental Table S2 with measured values obtained from [1, 2].



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130 3.3. Figure S3. (**a**) Dependence of amine formation on molecular volume and calculated E° or (**b**) on
131 log(P) and calculated E°. Volume and log(P) were calculated in Spartan based on CPK atomic
132 volumes and an estimated octanol/water partition coefficient calculated by the method of Ghose and
133 Crippen. While larger molecules (right hand side of panel (a)) seem slightly more prone to
134 conversion to amines, molecular polarity does not appear to correlate with propensity to form
135 amines (**b**). In both panels the vertical axis is calculated E°, which correlates with formation of
136 amine product.

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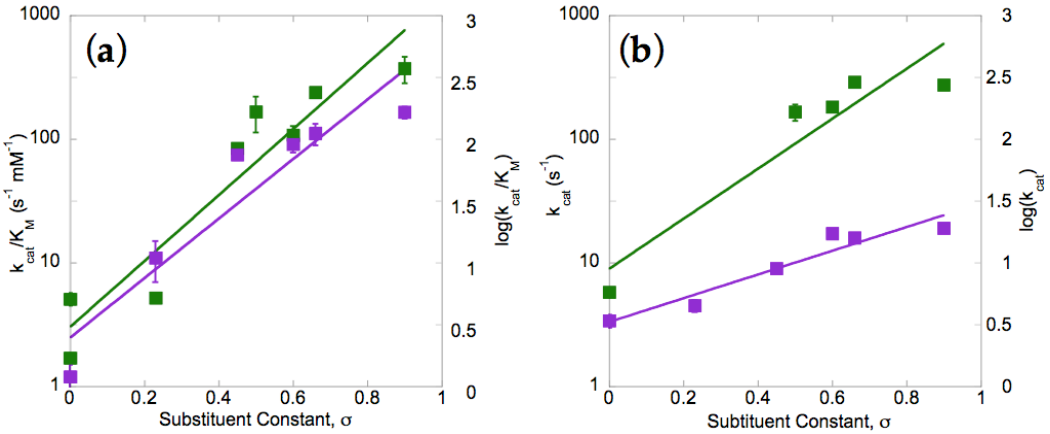
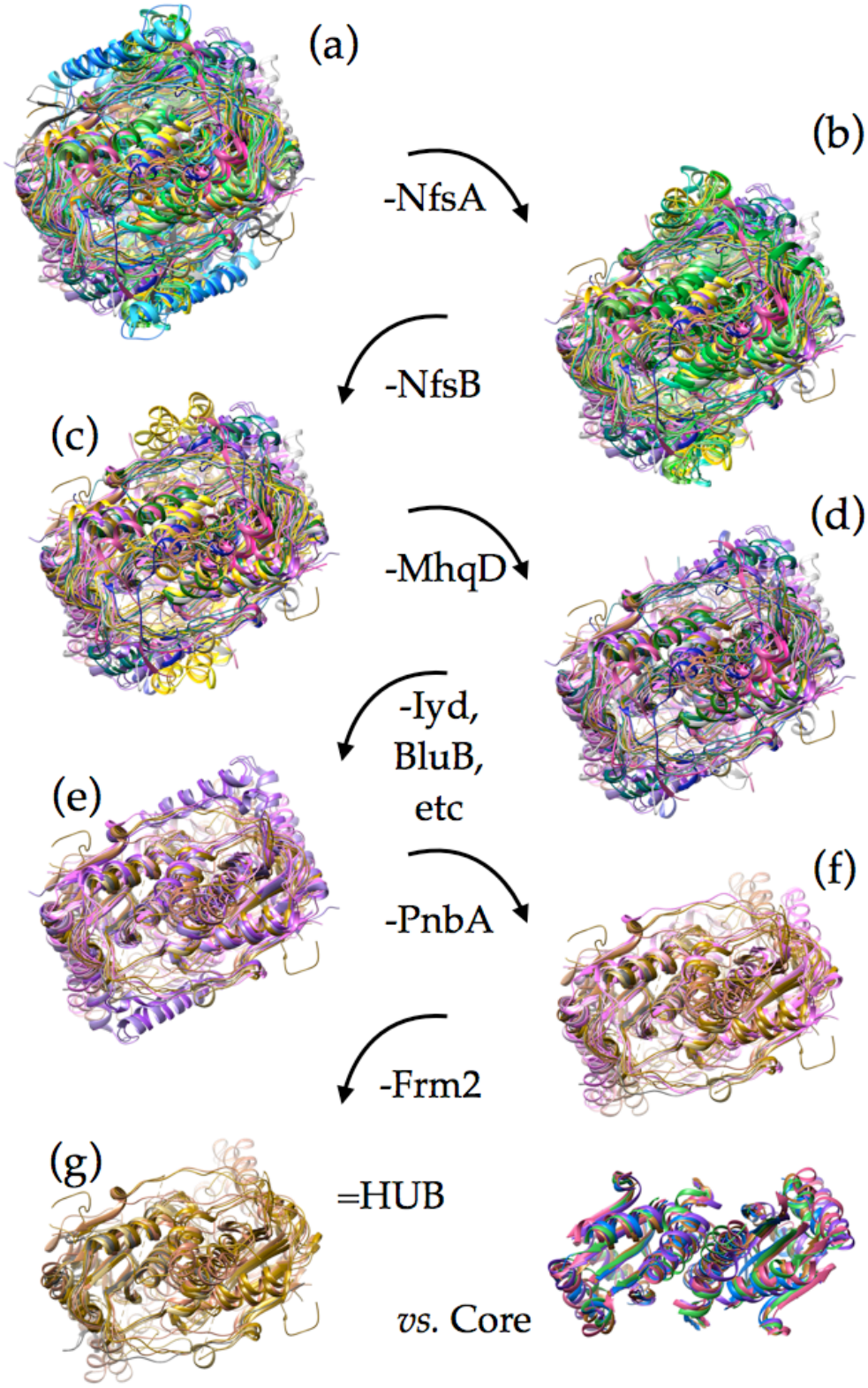


3.4. Figure S4. Plots of enzymatic reaction rates showing the errors evaluated via triplicate measurements for *St*NfsB (green) and *Ms*PnbA (purple). Data are plotted with respect to sensitivity to Hammett para constant σ [4]. (**a**) shows dependence of the second-order rate constant and (**b**) shows that of the first-order rate constant. (**b**) here is the same as panel (**b**) of Figure 3, but is reproduced here to permit direct comparison with panel (**a**). Note that error bars are shown but rarely exceed the size of the symbol. Note also that these data are not normalized to the rate constant of the unsubstituted parent compound but presented as measured to permit direct comparison between the rates produced by the two different enzymes. Log(k_{cat}/K_M) data are fit with lines, with for *St*NfsB a slope of 2.7± 0.4 and an intercept of 0.5 ± 0.2 (R^2=0.89), and for *Ms*PnbA a slope of 2.4 ± 0.4 and intercept of 0.4 ± 0.2 (R^2=0.88). Log(k_{cat}) data are fit with lines, with for *St*NfsB a slope of 2.0 ± 0.4 and an intercept of 0.9 ± 0.3 (R^2=0.88), and for *Ms*PnbA a slope of 1.0 ± 0.1 and intercept of 0.5 ± 0.08 (R^2=0.92). Values are tabulated in Supplementary Table S1.



3.5. Figure S5. View of steps in which successive subgroups can be identified and removed from the remaining large collection of NR-related enzyme structures. Panel A is the overlay of 36 structures shown in the manuscript, in B the NfsA structures have been removed, in C the NfsB structures have additionally been removed, D further lacks the structures of MhqD subgroup

161 members, and so forth. Finally only the structures of HUB subgroup members remain. PDB
162 accession numbers of all the structures are in Supplementary Table S4.

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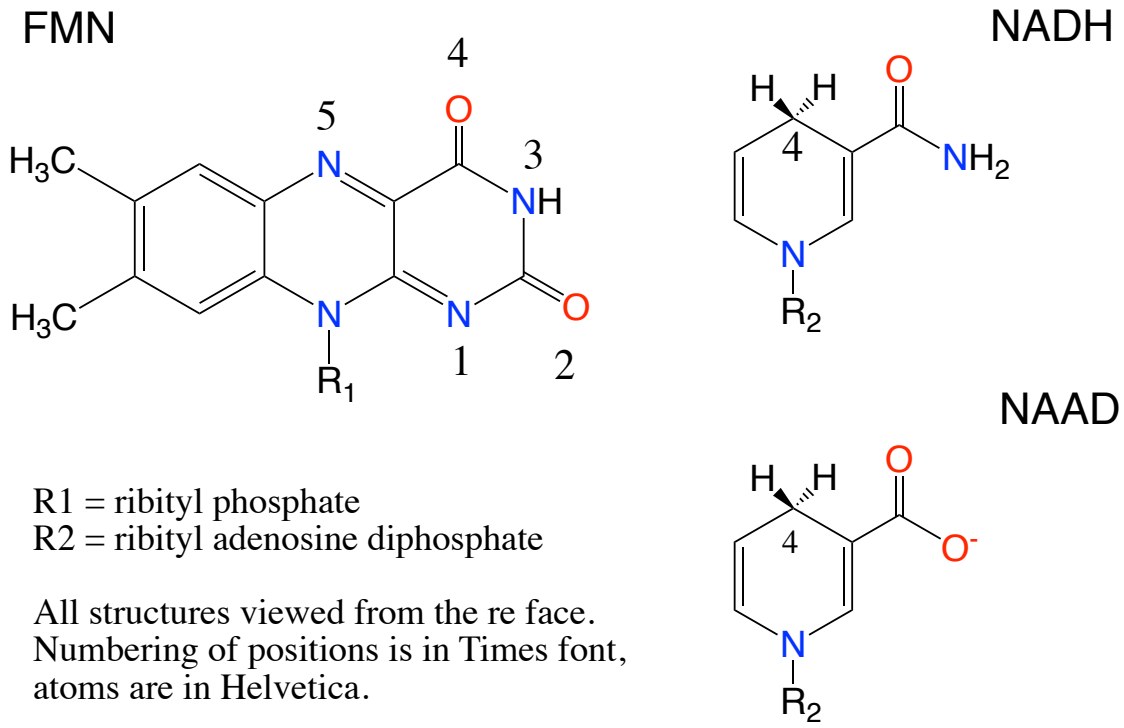

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165 3.6. Figure S6. Position numbering for flavin, nicotinamide ring of NADH and analogous nicotinic
166 acid ring of the nicotinic acid adenine dinucleotide used as a model for NADH in crystallography of
167 *Ent*NfsB.

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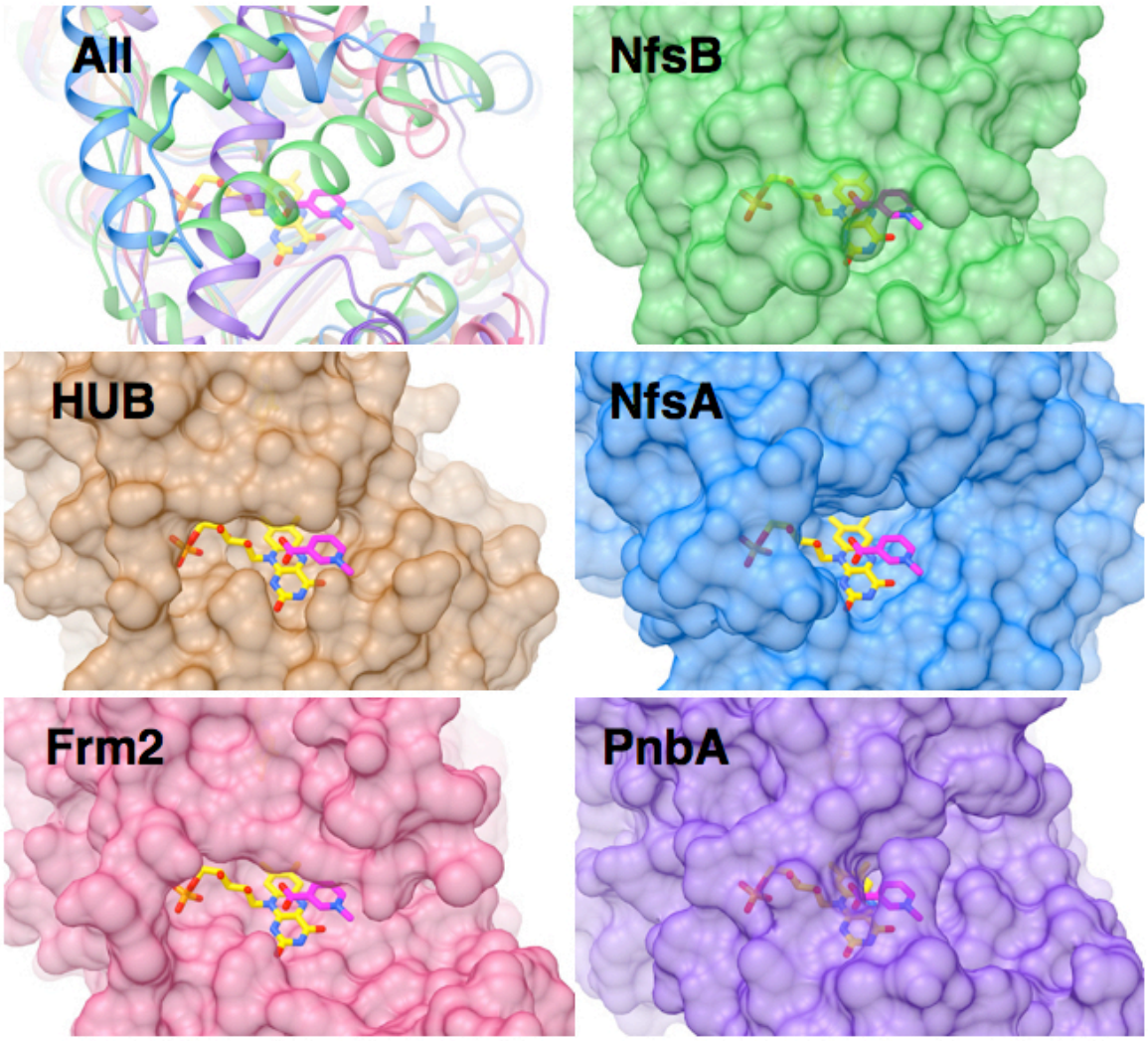
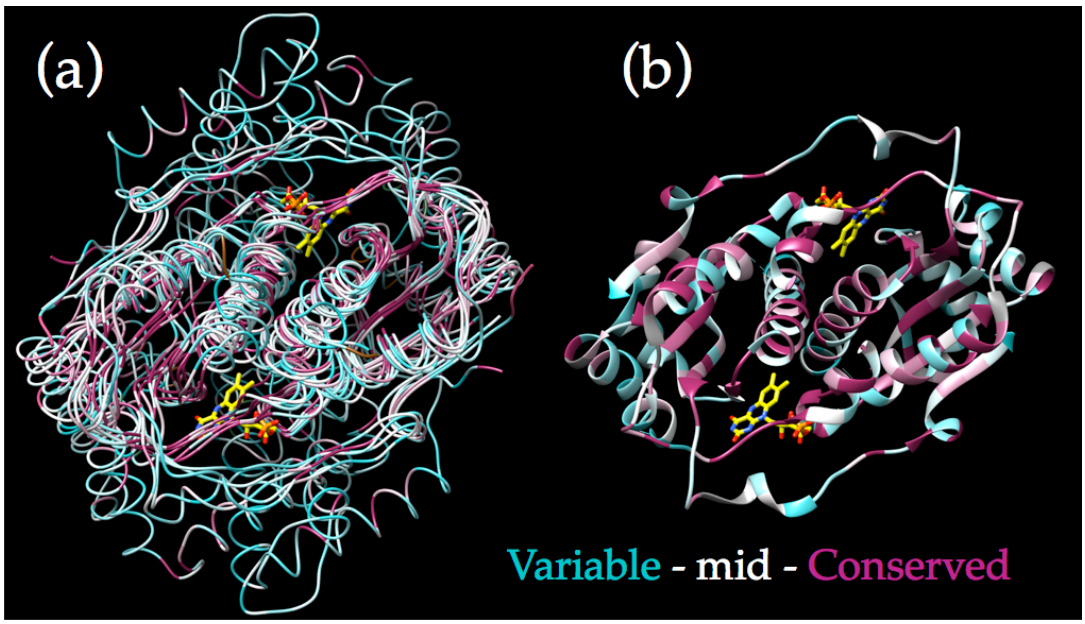


3.7. Figure S7. Comparison of the shapes and sizes of binding sites provided by NRs representing each of the four groups. Overlay of the ribbon structures of *Ms*PnbA (purple, 2WZV), *St*NfsB (green, 5J8D), *E. coli* NfsA (blue, 1F5V), *Smut* Frm2 (pink, 2IFA), *Desulf* HUB (brown, 3E39). In each case one of the fine structures' surface is shown at 50% transparency, and the ribbons of all structures are shown for reference in the top left panel. The FMN and the nicotinic acid ring of bound NAAD of 5J8D are shown in all cases to provide markers for the active site. NAAD is nicotinic acid adenine dinucleotide, and analog of NADH (Supplemental Figure S6).



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180 3.8. Figure S8. Comparison of (**a**) distribution of conservation within each of the subgroups - each
181 depicted as a cord, with (**b**) conservation between subgroups depicted on core sequence only
182 (ribbons). Subgroups included are NsfA, NsfB, PnbA, Frm2, HUB. Conservation at each residue
183 was provided from multiple sequence alignment of each subgroup provided by the Structure
184 Function Linkage Database [12].

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189 3.9. Figure S9. Following 5 pages:

190 Alignment of the crystallographic and supporting other amino acid sequences showing amine
191 producers. The representative sequence from each subgroup is labeled on a coloured background
192 with the theme that has been used throughout: NfsB (Ent_5J8D, green), NfsA (Ecoli_1F5V, blue),
193 Frm2 (Smut_2IFA, pink), PnbA (AMINE_Msmg_2WZW, purple) and HUB (Desulf_3E39, rust). In
194 the alignment, secondary structure is enclosed in yellow boxes (helices) and green (beta strands).

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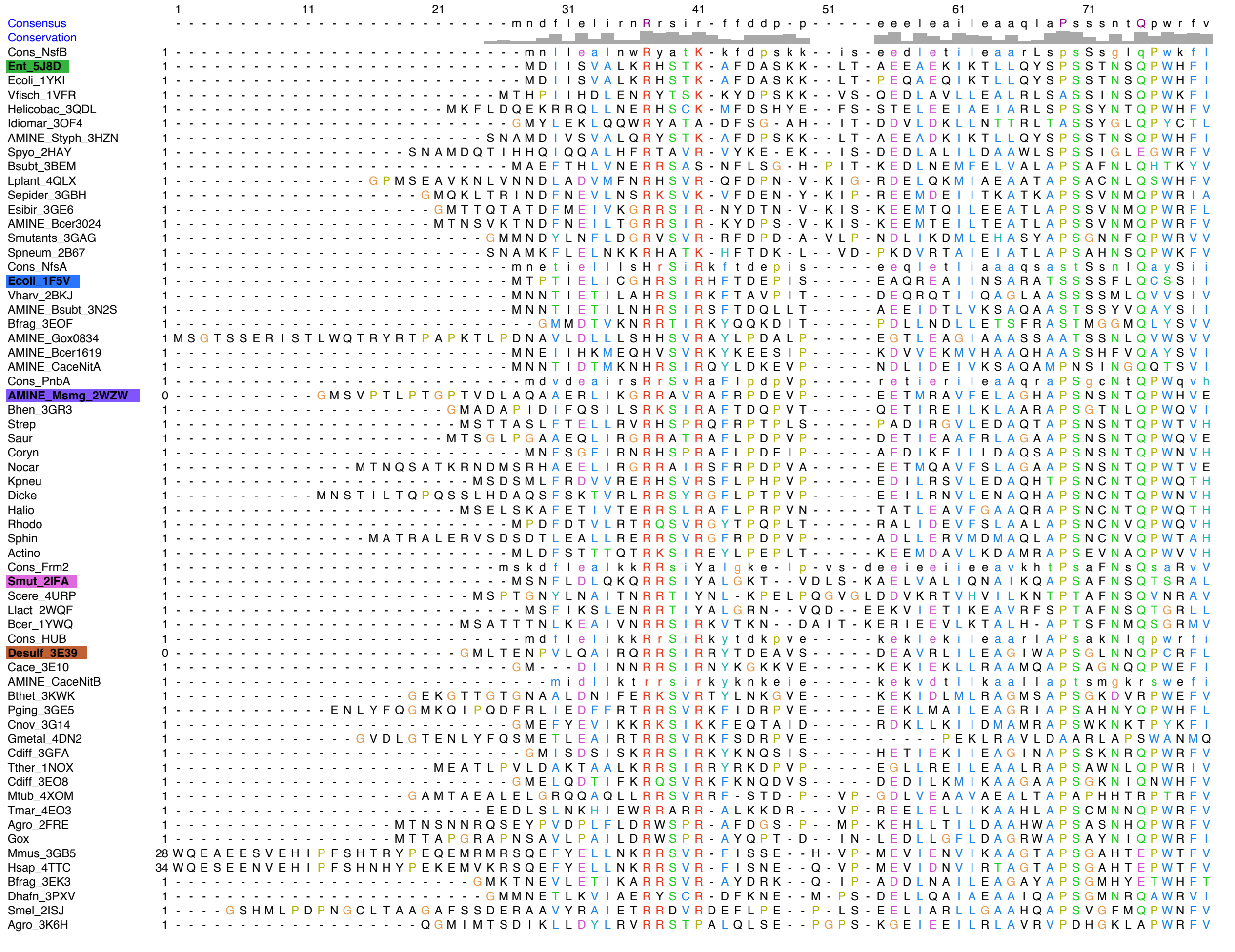
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11
21
31
41
51
61
71
Consensus
Conservation
Cons_NsfB
Ent_5J8D
Ecoli_1YKI
Vfisch_1VFR
Helicobac_3QDL
Idiomar_3OF4
AMINE_Styph_3HZN
Spyo_2HAY
Bsubt_3BEM
Lplant_4QLX
Sepider_3GBH
Esibir_3GE6
AMINE_Bcer3024
Smutants_3GAG
Spneum_2B67
Cons_NfsA
Ecoli_1F5V
Vharv_2BKJ
AMINE_Bsubt_3N2S
Bfrag_3EOF
AMINE_Gox0834
AMINE_Bcer1619
AMINE_CaceNitA
Cons_PnbA
AMINE_Msmg_2WZW
Bhen_3GR3
Strep
Saur
Coryn
Nocar
Kpneu
Dicke
Halio
Rhodo
Sphin
Actino
Cons_Frm2
Smut_2IFA
Scere_4URP
Llact_2WQF
Bcer_1YWQ
Cons_HUB
Desulf_3E39
Cace_3E10
AMINE_CaceNitB
Bthet_3KWK
Pging_3GE5
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Gmetal_4DN2
Cdiff_3GFA
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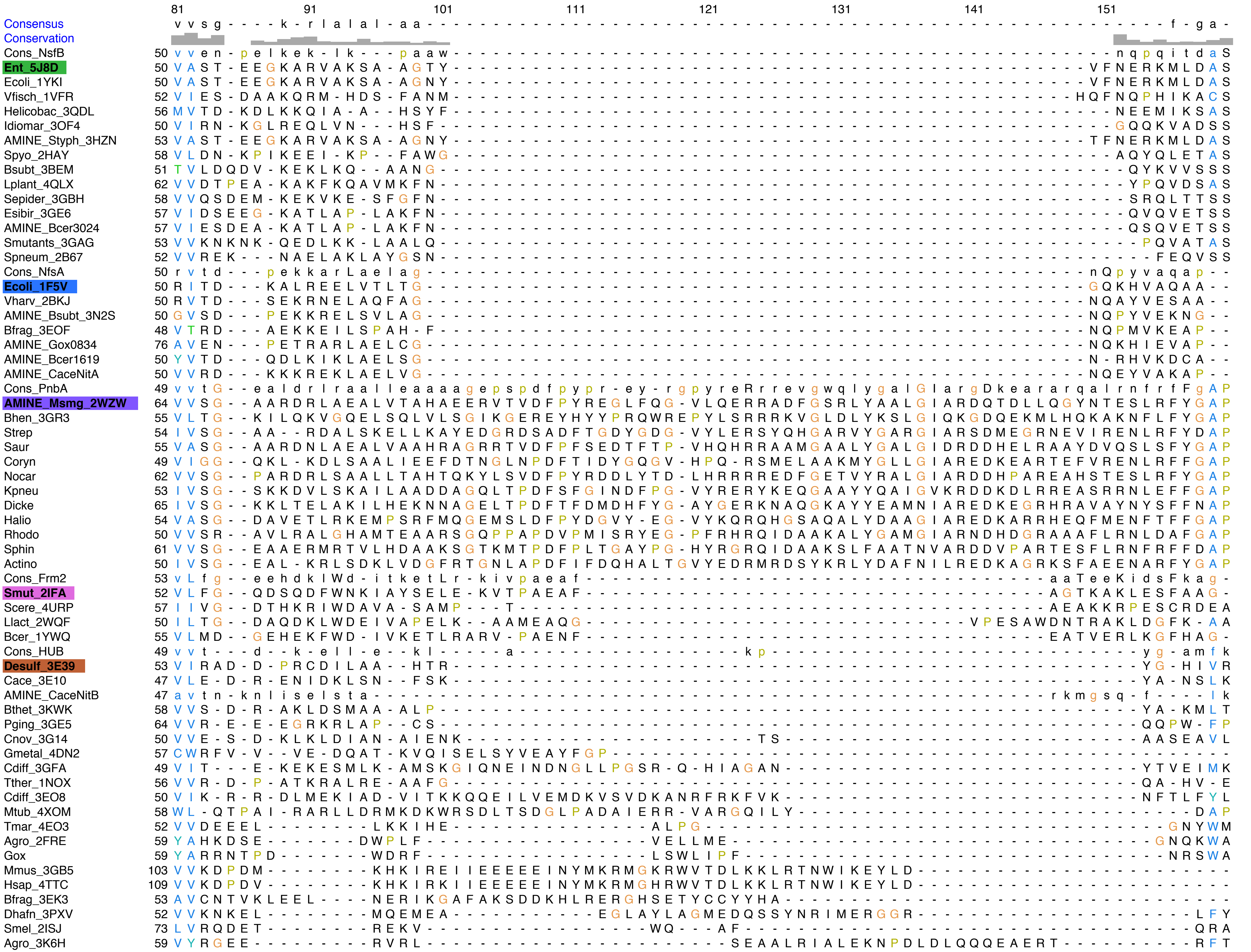
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Conservation
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Helicobac_3QDL
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AMINE_Msmg_2WZW
Bhen_3GR3
Strep
Saur
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Dicke
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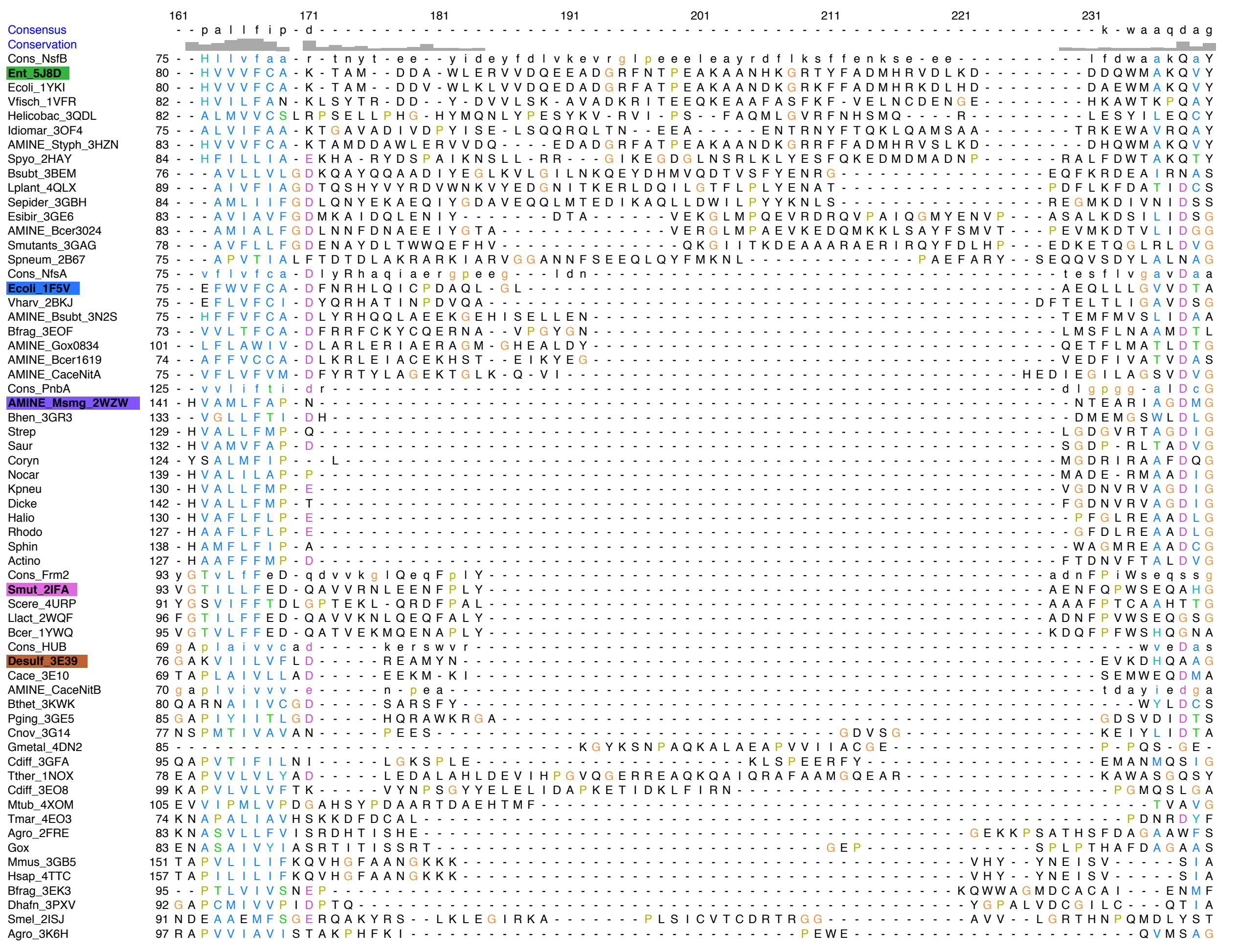
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Bsubt_3BEM
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Tmar_4EO3
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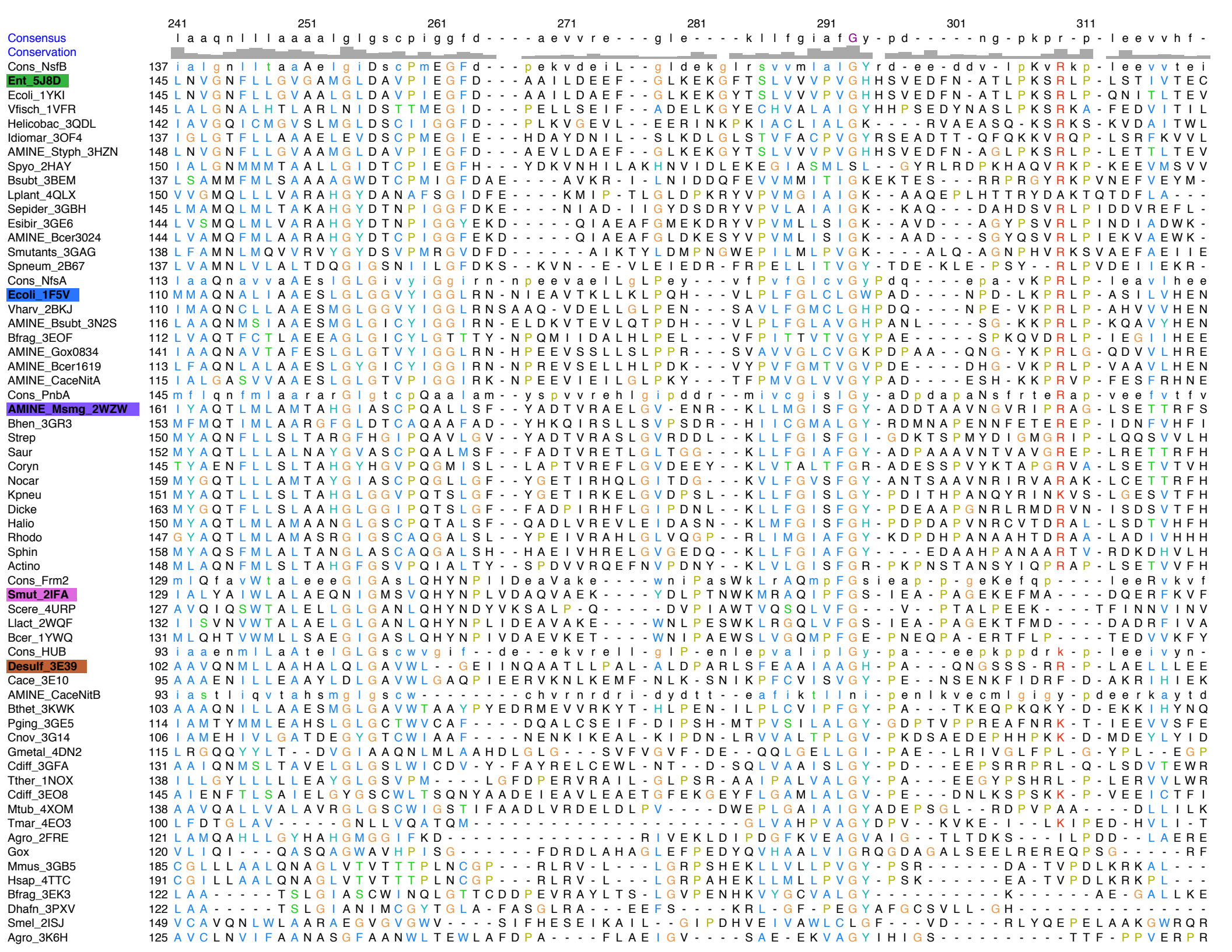
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Sepider_3GBH
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Vharv_2BKJ
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Strep
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Cdiff_3EO8
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Tmar_4EO3
Agro_2FRE
Gox
Mmus_3GB5
Hsap_4TTC
Bfrag_3EK3
Dhafn_3PXV
Smel_2ISJ
Agro_3K6H

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Consensus                  -----------------------------------------------------------------------
Conservation
Cons_NsfB             207  -----------------------------------------------------------------------
Ent_5J8D              217  -----------------------------------------------------------------------
Ecoli_1YKI            217  -----------------------------------------------------------------------
Vfisch_1VFR           218  -----------------------------------------------------------------------
Helicobac_3QDL        210  -----------------------------------------------------------------------
Idiomar_3OF4          209  -----------------------------------------------------------------------
AMINE_Styph_3HZN      220  -----------------------------------------------------------------------
Spyo_2HAY             224  K----------------------------------------------------------------------
Bsubt_3BEM            206  -----------------------------------------------------------------------
Lplant_4QLX           219  -----------------------------------------------------------------------
Sepider_3GBH          213  -----------------------------------------------------------------------
Esibir_3GE6           212  -----------------------------------------------------------------------
AMINE_Bcer3024        212  -----------------------------------------------------------------------
Smutants_3GAG         206  -----------------------------------------------------------------------
Spneum_2B67           204  -----------------------------------------------------------------------
Cons_NfsA             183  tY---tde----ealaaYdetlseyyqertsnqr--ttwseqiaarlskegr----phlleflkkqGfllr
Ecoli_1F5V            180  SYQPLD-----KGALAQYDEQLAEYYLTRGSNNRR-DTWSDHIRRTIIKESR----PFILDYLHKQGWATR
Vharv_2BKJ            180  QYQELN-----LDDIQSY-QTMQAYYASRTSNQKL-STWSQEVTGKLAGESRSK--GLAKR----------
AMINE_Bsubt_3N2S      186  TYNVNTDD-F-RHTMNTYDKTISDYYRERTNGKRE-ETWSDQILNFMKQKPR----TYLNDYVKEKGFNKN
Bfrag_3EOF            182  SYHDYTAEDINRLYAYKESLPENKLFIEENQKETLPQVFT-DVRYTKKDN-EFMSENLLK-VLRRQGFMD-
AMINE_Gox0834         213  RY--STENEA-KGIAAY-DRIADDYQKEQGLTSRV---WSETVAKRVDSFKGLSGRHVIRTVLHRLGFPLR
AMINE_Bcer1619        183  GYDEQKYDE----LLNEYDETMNAYYKERSSNKKN-VTWTESMSSFMSKE-KR---MHMKEFLSEKGLNKK
AMINE_CaceNitA        185  SYDIKAVED----SINVYEQ-MNKYLKEIGRAEKE-INW-STFTSTIYQ------SVYYKQGLKTK-----
Cons_PnbA             219  g----------------------------------------------------------------------
AMINE_Msmg_2WZW       234  R----------------------------------------------------------------------
Bhen_3GR3             227  KSYP-------------------------------------------------------------------
Strep                 224  DTPGVLDEQ--------------------------------------------------------------
Saur                  225  R----------------------------------------------------------------------
Coryn                 219  GIEGLGL----------------------------------------------------------------
Nocar                 232  T----------------------------------------------------------------------
Kpneu                 225  Q----------------------------------------------------------------------
Dicke                 237  N----------------------------------------------------------------------
Halio                 224  D----------------------------------------------------------------------
Rhodo                 221  GDT--------------------------------------------------------------------
Sphin                 229  G----------------------------------------------------------------------
Actino                222  E----------------------------------------------------------------------
Cons_Frm2             199  k----------------------------------------------------------------------
Smut_2IFA             199  GDLE-------------------------------------------------------------------
Scere_4URP            192  YH---------------------------------------------------------------------
Llact_2WQF            202  K----------------------------------------------------------------------
Bcer_1YWQ             200  -----------------------------------------------------------------------
Cons_HUB              159  -----------------------------------------------------------------------
Desulf_3E39           172  PFPQPE-----------------------------------------------------------------
Cace_3E10             168  Y----------------------------------------------------------------------
AMINE_CaceNitB        160  edlalnklhyd-kysk-------------------------------------------------------
Bthet_3KWK            175  Y----------------------------------------------------------------------
Pging_3GE5            185  KL---------------------------------------------------------------------
Cnov_3G14             177  KWGTSFMESNVKILEKN------------------------------------------------------
Gmetal_4DN2           180  KAGPSRKPLDEIVHYGKYQA---------------------------------------------------
Cdiff_3GFA            198  -----------------------------------------------------------------------
Tther_1NOX            205  -----------------------------------------------------------------------
Cdiff_3EO8            219  K----------------------------------------------------------------------
Mtub_4XOM             207  -----------------------------------------------------------------------
Tmar_4EO3             147  LIAVGYLGDESELSEKHRELERSERVRKELSEIVRWNL---------------------------------
Agro_2FRE             179  VPSKRVPLADVAFEGRFTGKAD-------------------------------------------------
Gox                   186  PLA-EIAFEGRFRSNE-------------------------------------------------------
Mmus_3GB5             247  -DQIMVTV---------------------------------------------------------------
Hsap_4TTC             253  -DQIMVTV---------------------------------------------------------------
Bfrag_3EK3            179  KTVK----AGTITIVE-------------------------------------------------------
Dhafn_3PXV            171  ---A-NTTKPPHVPD----KDKITYVE--------------------------------------------
Smel_2ISJ             216  L-----------PLEDLVFEEGWGVR---------------------------------------------
Agro_3K6H             185  P-----------ELADVVTWVGDV-----------------------------------------------
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197

198 **4. References**

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