Supporting information for

**Kinetic characterization of over 100 glycoside hydrolase mutants enables the discovery of structural features correlated with kinetic constants**

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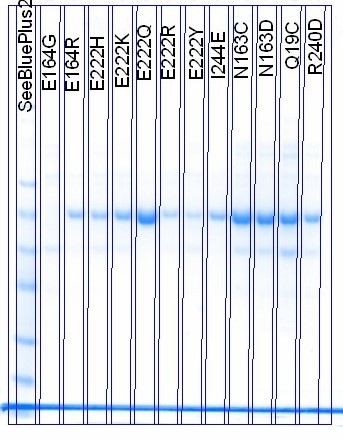
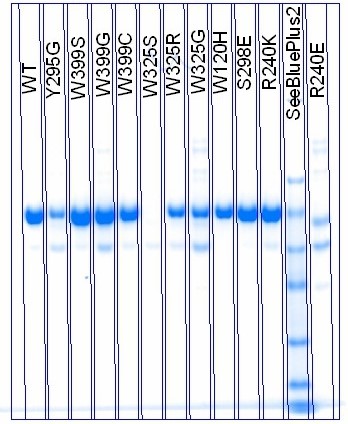
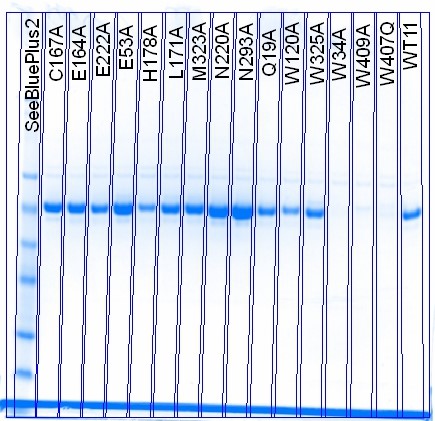
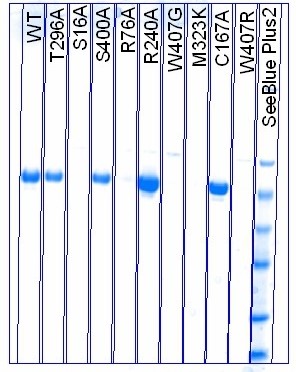
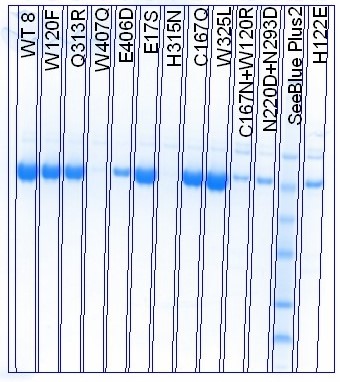
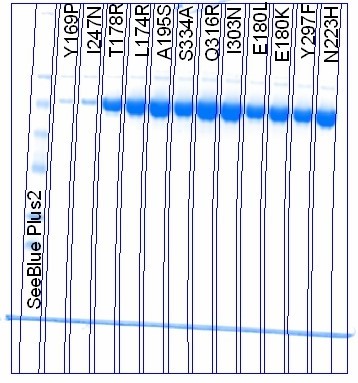
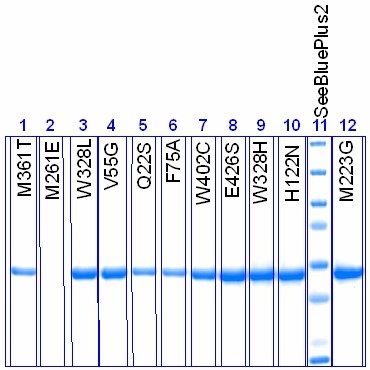
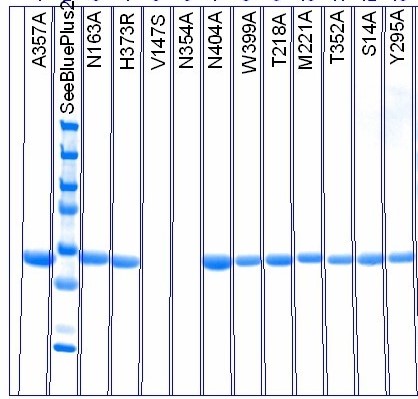
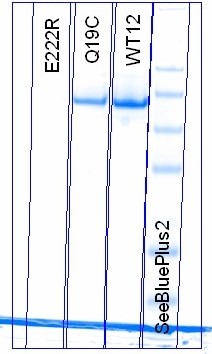
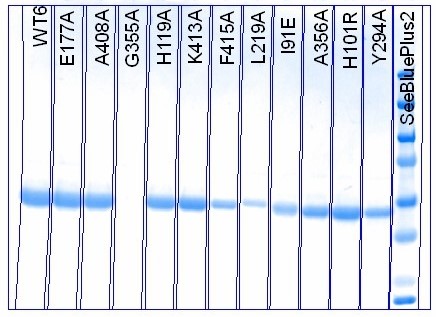
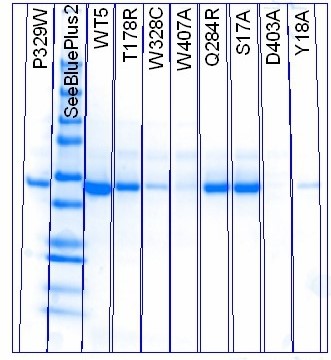
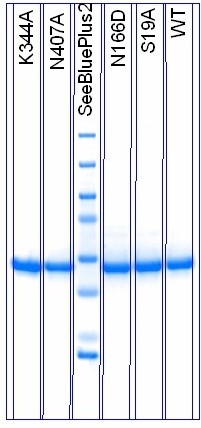
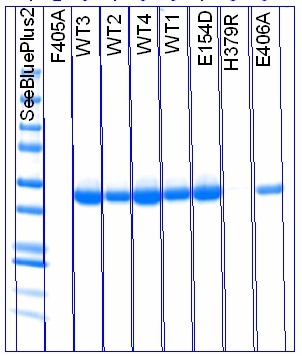
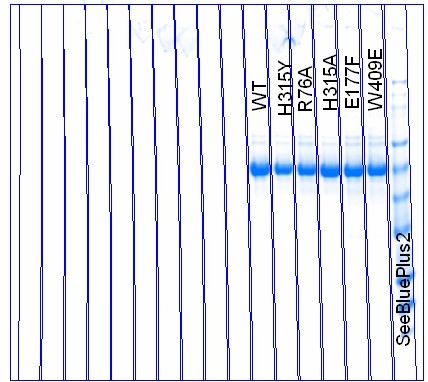
Supplemental materials and methods

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Mutant** | **Protein yield** | **SDS-PAGE** | **KM** | ***k*cat** | **KI** | ***k*cat/KM** |
|  | **mg/mL** |  | **mM** | **min-1** | **mM** | **M-1min-1** |
| BglB | 1.2 |  | 5.00 ± 0.2 | 880 ± 10 |  | 176,000 ± 8000 |
| S14A | 0.6 |  | 8.25 ± 1.02 | 320 ± 11 |  | 38,823 ± 4,972 |
| S16A | 0.83 |  | 14.01 ± 0.40 | 154 ± 1 |  | 10,997 ± 331 |
| E17S | 1.01 |  | 7.32 ± 0.38 | 641 ± 9 |  | 87,596 ± 4,719 |
| S17A | 0.65 |  | 18.45 ± 3.72 | 848 ± 76 |  | 45,978 ± 10,135 |
| Y18A | 0.17 |  | 31.55 ± 3.61 | 197 ± 9 |  | 6,230 ± 773 |
| Q19A | 0.26 |  |  |  |  | 11 ± 3 |
| Q19C | 0.4 |  |  |  |  | < 10 |
| Q19S | 0.43 |  |  |  |  | 13 ± 3 |
| W34A | 0.1 | NDE |  |  |  |  |
| V52G | 0.97 |  | 8.25 ± 0.54 | 687 ± 13 |  | 83,371 ± 5,707 |
| F75A | 0.44 |  | 5.47 ± 0.28 | 613 ± 8 |  | 112,224 ± 6,000 |
| R76A | 0.1 | NDE |  |  |  |  |
| I91E | 0.49 |  | 6.71 ± 0.79 | 846 ± 35 |  | 126,071 ± 15,714 |
| H101R | 1.03 |  | 10.62 ± 0.53 | 1059 ± 16 |  | 99,708 ± 5,225 |
| H119A | 1.21 |  | 15.10 ± 3.36 | 143 ± 11 |  | 9,483 ± 2,222 |
| H119E | 0.25 | NDE |  |  |  |  |
| H119N | 1.02 |  | 23.22 ± 2.20 | 2 ± 0 |  | 82 ± 8 |
| W120A | 0.16 |  |  |  |  |  |
| W120F | 0.78 |  | 16.08 ± 2.07 | 472 ± 21 |  | 29,334 ± 3,980 |
| W120H | 1 |  | 89.18 ± 4.31 | 84 ± 2 |  | 943 ± 53 |
| V147S | 0.23 |  | 6.45 ± 0.62 | 5 ± 0 |  | 706 ± 70 |
| E154D | 1.42 |  | 3.46 ± 0.76 | 878 ± 47 |  | 254,004 ± 57,175 |
| N163A | 0.74 |  | 11.95 ± 0.91 | 7 ± 0 |  | 558 ± 44 |
| N163C | 1.1 |  | 5.42 ± 0.32 | 26 ± 0 |  | 4,766 ± 291 |
| N163D | 1.05 |  | 15.19 ± 1.41 | 12 ± 0 |  | 789 ± 77 |
| E164A | 0.42 |  | 1.01 ± 0.17 | 0 ± 0 |  | 190 ± 33 |
| Y166P | 0.18 |  | 2.50 ± 0.45 | 27 ± 1 | 94.95 ± 10.18 | 10,596 ± 1,981 |
| C167A | 0.48 |  | 14.56 ± 1.27 | 479 ± 14 |  | 32,884 ± 3,026 |
| C167Q | 0.94 |  | 4.92 ± 0.19 | 504 ± 6 | 590.71 ± 86.56 | 102,415 ± 4,149 |
| L171A | 0.38 |  | 11.09 ± 0.42 | 807 ± 9 |  | 72,719 ± 2,851 |
| L171R | 1.06 |  | 3.36 ± 0.23 | 403 ± 7 |  | 120,146 ± 8,506 |
| T175R | 0.86 |  | 3.59 ± 0.15 | 801 ± 8 |  | 223,033 ± 9,663 |
| E177A | 0.96 |  | 5.98 ± 0.22 | 986 ± 10 |  | 164,804 ± 6,408 |
| E177K | 0.95 |  | 6.19 ± 0.30 | 555 ± 7 | 362.94 ± 36.97 | 89,609 ± 4,493 |
| E177L | 0.77 |  | 7.48 ± 0.36 | 670 ± 10 |  | 89,478 ± 4,555 |
| H178A | 0.25 |  | 7.67 ± 0.73 | 113 ± 3 | 173.34 ± 42.79 | 14,697 ± 1,463 |
| A192S | 1.17 |  | 5.09 ± 0.18 | 946 ± 10 |  | 185,848 ± 6,994 |
| T218A | 0.98 |  | 6.51 ± 0.94 | 464 ± 18 |  | 71,280 ± 10,669 |
| L219A | 0.47 |  | 7.87 ± 0.60 | 199 ± 5 |  | 25,262 ± 2,010 |
| N220A | 0.61 |  | 10.27 ± 0.68 | 405 ± 8 |  | 39,425 ± 2,745 |
| N220H | 1.12 |  | 5.14 ± 0.21 | 123 ± 1 |  | 23,874 ± 1,031 |
| M221A | 0.73 |  | 6.25 ± 0.60 | 547 ± 15 |  | 87,554 ± 8,701 |
| E222A | 0.29 |  | 0.63 ± 0.15 | 90 ± 4 | 95.24 ± 13.70 | 143,604 ± 36,130 |
| E222H | 0.7 |  | 8.54 ± 0.53 | 160 ± 3 |  | 18,695 ± 1,212 |
| E222K | 0.5 |  | 7.22 ± 0.75 | 108 ± 3 |  | 14,955 ± 1,618 |
| E222Q | 1.3 |  | 12.16 ± 0.65 | 668 ± 11 |  | 54,923 ± 3,084 |
| E222R | 0.15 |  | 2.48 ± 0.44 | 42 ± 2 |  | 17,098 ± 3,148 |
| E222Y | 0.7 |  | 18.43 ± 3.14 | 12 ± 1 |  | 636 ± 116 |
| M223G | 0.88 |  | 19.21 ± 2.91 | 154 ± 9 |  | 7,998 ± 1,302 |
| R240A | 1.11 |  | 19.46 ± 1.17 | 11011 ± 258 |  | 565,763 ± 36,384 |
| R240D | 0.8 |  | 10.82 ± 0.47 | 282 ± 4 |  | 26,093 ± 1,196 |
| R240K | 1.4 |  | 17.67 ± 3.32 | 898 ± 59 |  | 50,829 ± 10,102 |
| I244E | 0.6 |  | 5.97 ± 1.04 | 497 ± 23 |  | 83,137 ± 14,963 |
| I244N | 0.21 |  | 2.15 ± 0.13 | 271 ± 4 |  | 126,176 ± 7,795 |
| M261E | 0.11 |  |  |  |  | 702 ± 73 |
| Q284R | 0.52 |  | 9.68 ± 1.35 | 370 ± 15 |  | 38,182 ± 5,550 |
| N293A | 0.68 |  | 9.67 ± 0.44 | 13 ± 0 |  | 1,313 ± 63 |
| Y294A | 0.59 |  | 4.98 ± 0.17 | 166 ± 2 |  | 33,260 ± 1,180 |
| Y294F | 0.73 |  | 5.99 ± 0.32 | 735 ± 11 |  | 122,751 ± 6,883 |
| Y295A | 0.69 |  |  |  |  | < 10 |
| Y295G | 0.77 |  |  |  |  | < 10 |
| T296A | 0.39 |  | 11.05 ± 0.77 | 109 ± 2 | 142.75 ± 28.67 | 9,904 ± 722 |
| S298E | 1.1 |  | 5.28 ± 0.05 | 809 ± 2 |  | 153,264 ± 1,391 |
| I300N | 1.51 |  | 4.48 ± 0.32 | 693 ± 13 |  | 154,732 ± 11,520 |
| Q313R | 1.07 |  | 3.58 ± 0.51 | 689 ± 24 |  | 192,373 ± 28,109 |
| H315N | 0.08 | NDE |  |  |  |  |
| M323A | 0.35 |  | 9.34 ± 0.80 | 416 ± 11 | 126.29 ± 25.47 | 44,477 ± 3,991 |
| M323K | 0.05 | NDE |  |  |  |  |
| W325A | 0.26 |  | 1.61 ± 0.23 | 29 ± 1 | 171.02 ± 19.85 | 18,243 ± 2,607 |
| W325C | 0.22 |  | 4.18 ± 0.53 | 10 ± 0 | 159.19 ± 34.38 | 2,503 ± 327 |
| W325H | 1.12 |  | 3.08 ± 0.43 | 35 ± 1 | 143.45 ± 25.04 | 11,358 ± 1,645 |
| W325L | 1.08 |  | 5.74 ± 0.35 | 109 ± 2 |  | 18,909 ± 1,198 |
| P329W | 0.47 | NDE |  |  |  |  |
| S331A | 0.89 |  | 4.34 ± 0.11 | 817 ± 5 |  | 188,306 ± 5,055 |
| K341A | 0.92 |  | 5.46 ± 0.33 | 1046 ± 17 |  | 191,689 ± 12,041 |
| T352A | 0.7 |  | 14.26 ± 1.76 | 60 ± 2 |  | 4,174 ± 541 |
| E353A | 0.56 |  |  |  |  | < 10 |
| N354A | 0.34 |  | 5.38 ± 0.67 | 3 ± 0 |  | 547 ± 70 |
| G355A | 0.17 |  |  |  |  | 13 ± 2 |
| M358T | 0.62 |  | 4.83 ± 0.48 | 436 ± 11 |  | 90,241 ± 9,225 |
| H373R | 1.19 |  | 6.31 ± 0.30 | 707 ± 9 |  | 112,169 ± 5,512 |
| H379R | 0.14 |  | 6.24 ± 0.84 | 2 ± 0 |  | 380 ± 53 |
| W399A | 0.96 |  | 16.65 ± 2.52 | 0 ± 0 |  | 14 ± 2 |
| W399C | 0.93 |  | 70.33 ± 5.89 | 3 ± 0 |  | 39 ± 4 |
| W399G | 1.27 |  |  |  |  | < 10 |
| W399S | 1.5 |  |  |  |  | < 10 |
| S400A | 0.41 |  | 3.22 ± 0.22 | 531 ± 9 |  | 164,795 ± 11,833 |
| D403A | 0.12 | NDE |  |  |  |  |
| N404A | 1.41 |  | 9.42 ± 0.42 | 4 ± 0 |  | 393 ± 18 |
| F405A | 0.11 | NDE |  |  |  |  |
| E406A | 0.57 |  |  |  |  | < 10 |
| E406D | 0.27 |  | 34.13 ± 2.57 | 39 ± 1 |  | 1,146 ± 94 |
| N407A | 1.12 |  | 11.09 ± 0.64 | 50 ± 1 |  | 4,464 ± 273 |
| W407A | 0.12 | NDE |  |  |  |  |
| W407G | 0.05 | NDE |  |  |  |  |
| W407Q | 0.1 | NDE |  |  |  |  |
| W407R | 0.15 | NDE |  |  |  |  |
| W409A | 0.1 | NDE |  |  |  |  |
| K413A | 1.11 |  | 2.92 ± 0.48 | 835 ± 33 |  | 285,858 ± 48,589 |
| F415A | 0.53 |  | 16.63 ± 4.00 | 1 ± 0 |  | 80 ± 20 |
| E423S | 1.08 |  | 6.60 ± 0.42 | 646 ± 12 | 317.35 ± 65.22 | 97,777 ± 6,431 |

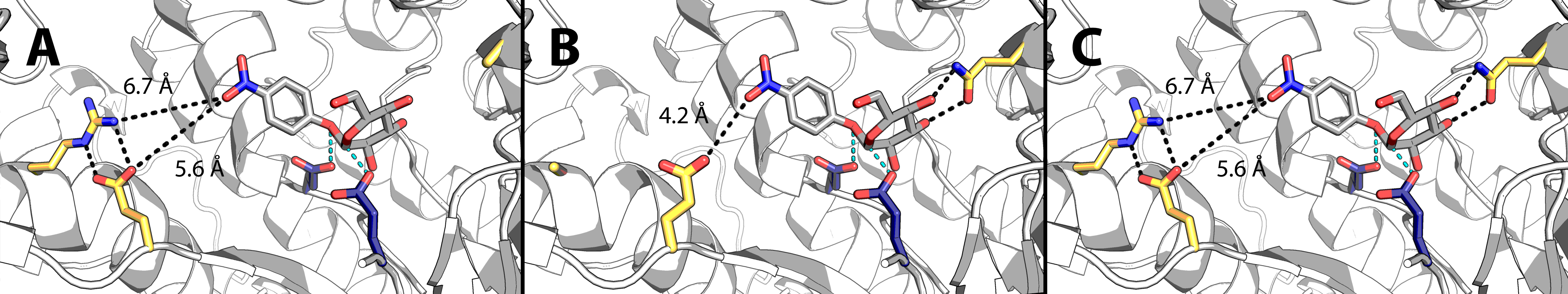
**Supplementary Table 1: List of 104 computationally designed mutants.** Included are columns (1) the mutation (2) protein yield as assessed by ratio of aborbance at 260 and 280 nm (3) expression assessed by SDS-PAGE (4, 5, 6, 7) kinetic constants and nonlinear regression analysis for each of kcat, KM, KI, and kcat/KM.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Feature** | **PCC (Kcat/KM）** | **SRC (Kcat/KM）** | **PCC (1/KM)** | **SRC (1/KM)** | **PCC (Kcat)** | **SRC (Kcat)** |
| all\_cst | 0.182 | 0.116 | 0.039 | 0.071 | 0.140 | 0.120 |
| fa\_rep | 0.289 | 0.268 | 0.253 | 0.155 | 0.064 | 0.084 |
| hbond\_sc | -0.352 | -0.352 | -0.248 | -0.297 | -0.309 | -0.266 |
| SR\_1\_all\_cst | -0.061 | -0.039 | -0.148 | -0.114 | -0.163 | -0.119 |
| SR\_1\_burunsat\_pm | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| SR\_1\_fa\_rep | 0.195 | 0.180 | 0.193 | 0.309 | 0.155 | 0.042 |
| SR\_1\_hbond\_pm | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| SR\_1\_hbond\_sc | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| SR\_1\_nlpstat\_pm | 0.142 | 0.087 | 0.263 | 0.317 | 0.007 | 0.055 |
| SR\_1\_pstat\_pm | 0.164 | 0.096 | 0.050 | 0.064 | -0.131 | -0.069 |
| SR\_1\_total\_score | -0.078 | 0.093 | -0.092 | -0.050 | -0.081 | 0.024 |
| SR\_2\_all\_cst | 0.039 | -0.064 | -0.089 | -0.059 | -0.005 | -0.096 |
| SR\_2\_burunsat\_pm | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| SR\_2\_fa\_rep | 0.074 | 0.168 | 0.258 | 0.168 | 0.011 | 0.014 |
| SR\_2\_hbond\_pm | 0.087 | 0.092 | 0.162 | 0.191 | 0.149 | 0.116 |
| SR\_2\_hbond\_sc | -0.149 | -0.235 | -0.142 | -0.229 | -0.126 | -0.138 |
| SR\_2\_nlpstat\_pm | 0.168 | 0.142 | 0.031 | 0.038 | 0.080 | 0.092 |
| SR\_2\_pstat\_pm | 0.102 | 0.070 | -0.042 | -0.017 | -0.023 | 0.013 |
| SR\_2\_total\_score | 0.071 | 0.002 | -0.078 | -0.079 | -0.055 | -0.134 |
| SR\_3\_all\_cst | -0.061 | -0.039 | -0.148 | -0.114 | -0.163 | -0.119 |
| SR\_3\_burunsat\_pm | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| SR\_3\_fa\_rep | 0.195 | 0.180 | 0.193 | 0.309 | 0.155 | 0.042 |
| SR\_3\_hbond\_pm | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| SR\_3\_hbond\_sc | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| SR\_3\_nlpstat\_pm | 0.142 | 0.087 | 0.263 | 0.317 | 0.007 | 0.055 |
| SR\_3\_pstat\_pm | 0.164 | 0.096 | 0.050 | 0.064 | -0.131 | -0.069 |
| SR\_3\_total\_score | -0.078 | 0.093 | -0.092 | -0.050 | -0.081 | 0.024 |
| SR\_4\_all\_cst | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| SR\_4\_burunsat\_pm | 0.123 | 0.135 | 0.089 | 0.078 | 0.067 | 0.138 |
| SR\_4\_fa\_rep | -0.118 | -0.024 | -0.235 | -0.212 | -0.153 | -0.103 |
| SR\_4\_hbond\_pm | -0.197 | -0.206 | 0.046 | 0.069 | -0.211 | -0.182 |
| SR\_4\_hbond\_sc | 0.299 | 0.092 | -0.009 | -0.063 | 0.058 | 0.061 |
| SR\_4\_nlpstat\_pm | 0.067 | 0.174 | 0.160 | 0.244 | -0.007 | -0.015 |
| SR\_4\_pstat\_pm | 0.040 | 0.098 | 0.105 | 0.152 | -0.025 | -0.072 |
| SR\_4\_total\_score | 0.096 | 0.045 | 0.193 | -0.121 | -0.181 | -0.076 |
| SR\_5\_all\_cst | 0.128 | 0.074 | -0.091 | -0.048 | -0.062 | -0.082 |
| SR\_5\_burunsat\_pm | 0.055 | 0.052 | 0.061 | -0.031 | 0.136 | 0.099 |
| SR\_5\_dsasa\_1\_2 | 0.235 | 0.189 | 0.037 | 0.147 | 0.215 | 0.201 |
| SR\_5\_fa\_rep | -0.012 | -0.064 | 0.165 | -0.019 | 0.058 | -0.030 |
| SR\_5\_hbond\_pm | 0.514 | 0.410 | 0.267 | 0.334 | 0.267 | 0.146 |
| SR\_5\_hbond\_sc | -0.524 | -0.447 | -0.266 | -0.341 | -0.273 | -0.194 |
| SR\_5\_interf\_E\_1\_2 | -0.461 | -0.469 | -0.237 | -0.231 | -0.266 | -0.278 |
| SR\_5\_total\_score | -0.462 | -0.471 | -0.237 | -0.230 | -0.267 | -0.279 |
| tot\_burunsat\_pm | -0.078 | -0.101 | -0.036 | -0.066 | 0.154 | 0.056 |
| tot\_hbond\_pm | 0.419 | 0.396 | 0.291 | 0.380 | 0.315 | 0.323 |
| tot\_NLconts\_pm | 0.570 | 0.548 | 0.246 | 0.238 | 0.432 | 0.421 |
| tot\_nlpstat\_pm | 0.334 | 0.313 | 0.223 | 0.164 | 0.277 | 0.277 |
| tot\_nlsurfaceE\_pm | -0.285 | -0.284 | -0.193 | -0.276 | -0.176 | -0.111 |
| tot\_pstat\_pm | 0.306 | 0.209 | 0.118 | -0.009 | 0.221 | 0.161 |
| tot\_seq\_recovery | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| tot\_total\_charge | 0.051 | 0.053 | 0.199 | 0.153 | 0.103 | 0.123 |
| tot\_total\_neg\_charges | -0.068 | -0.063 | -0.148 | -0.090 | -0.044 | -0.034 |
| tot\_total\_pos\_charges | 0.010 | 0.014 | 0.177 | 0.209 | 0.133 | 0.129 |
| total\_score | -0.340 | -0.396 | -0.059 | -0.014 | -0.320 | -0.362 |

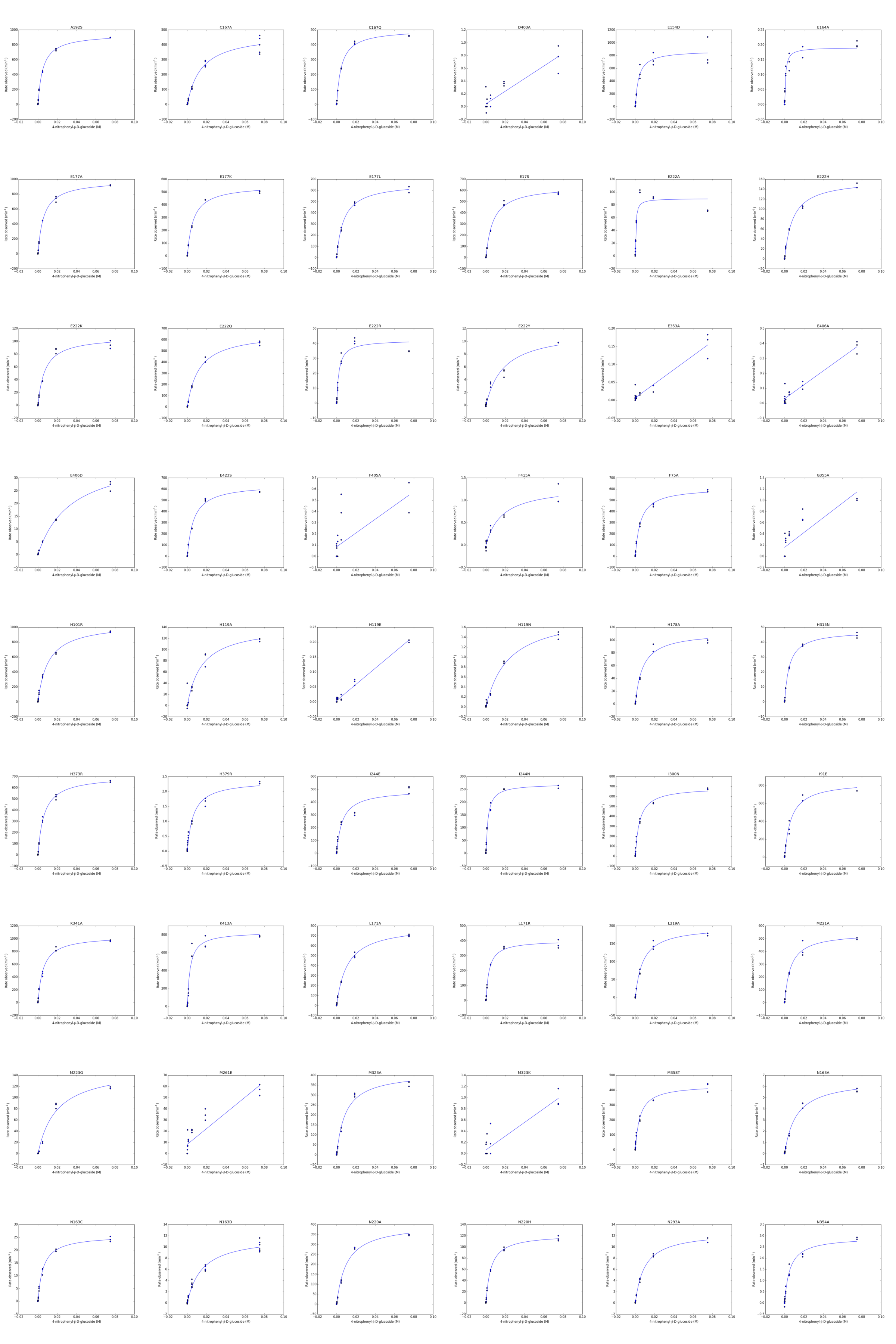
**Supplemental Table 2. Correlations between individual structural features and each of kcat, KM, and kcat/KM.** PCC and SRC values for each individual structural feature, given by Rosetta short name. For explanation of each short name, see main text and [doi 10.1371/journal.pone.0019230] .

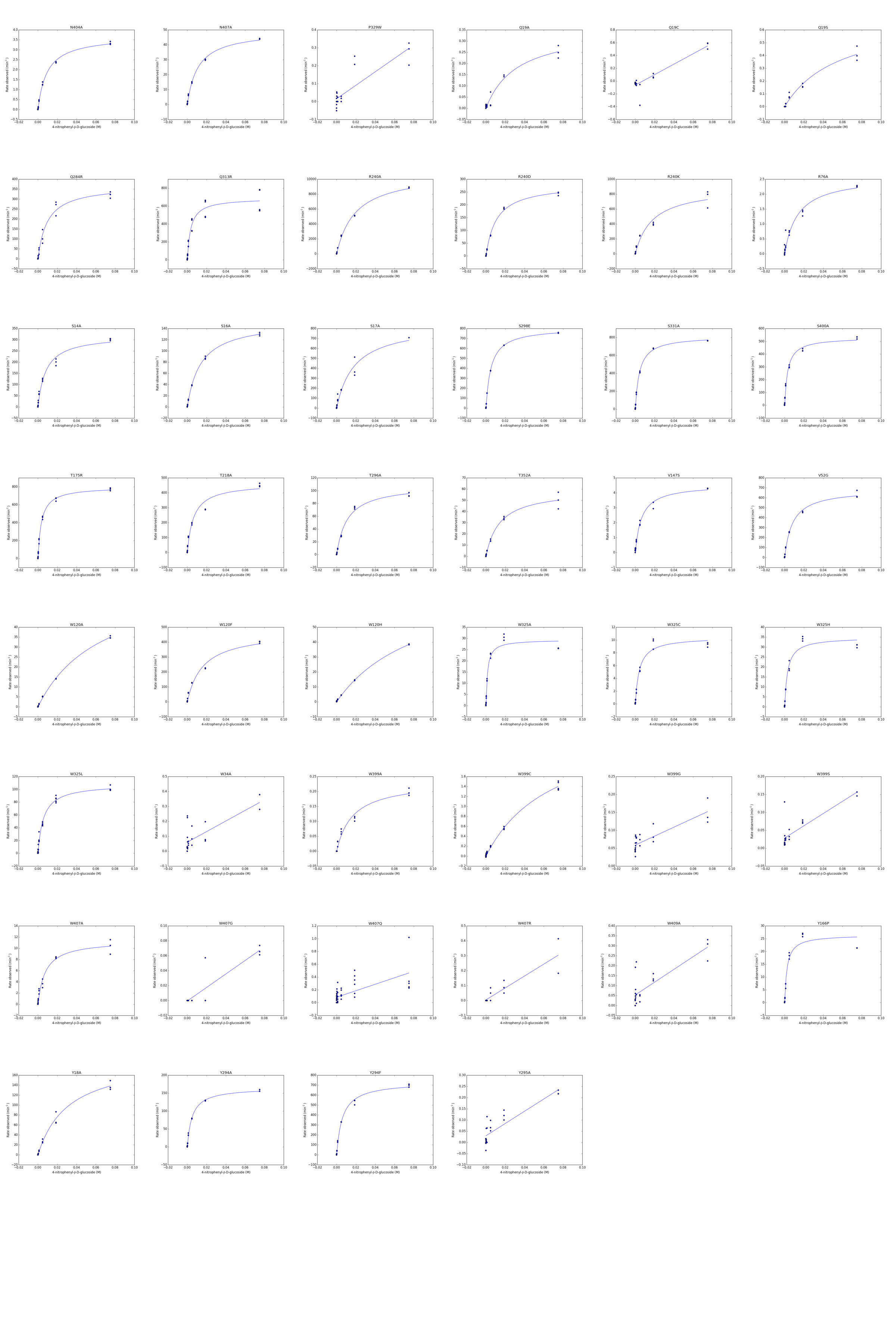


**Supplemental Figure 1: SDS-PAGE gel images for all mutants.** SDS-PAGE gels showing greater than 90% purity for all samples. Protein ladder used was SeeBlue® Plus2 Pre-stained Protein Standard (Life Technologies). Yields were also confirmed by comparing with visual gel yields.

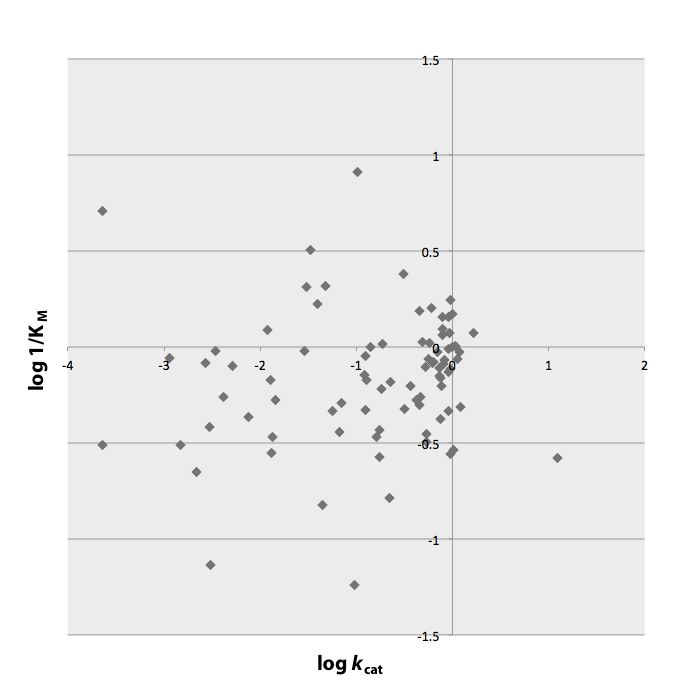
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**Supplementary Figure 2: Models of the active sites of mutants Q19A, R240A, and wild type BglB.** The lowest energy of 100 models for each mutant is depicted. The estimated distances and predicted hydrogen bonding between the substrate, *p*-nitrophenyl-ß-D-glucoside, and the BglB molecule are indicated by either black or blue dotted lines, the former being non-catalytic and the latter being catalytic bonds. Mutation of the arginine at position 240 to an alanine is predicted to allow for new conformers of the glutamate at position 222 that interact with the substrate. Mutation of the glutamine at position 19 to an alanine removes two hydrogen bonds to the substrate.





**Supplemental Figure 3.** Diagnostic plots showing Michaelis-Menten, Michaelis-Menten with substrate inhibition, or linear fit for each of 102 mutants.



**Supplemental Figure 4.** Plot of log kcat versus log 1/KM, relative to wild type BglB, showing the statistical independence of kcat and KM.

**Supplemental materials and methods**

Mutants were designed using the Foldit, a graphical user interface to the Rosetta Molecular Modeling Suite. Mutants were chosen based on proximity to the active site as well as Foldit’s predicted energy. Mutations within 12 Å of the active site, and those that did not increase the total system energy by more than 5 Rosetta energy units, were selected for experimental characterization. No other limitations were placed on designed mutations.

A sequence coding for BglB (Uniprot P22505) was codon-optimized for *Escherichia coli* and manufactured as a DNA String by Life Technologies. Using Gibson assembly, gene was inserted between the NdeI and XhoI sites of pET29b, adding a C-terminal His tag onto the protein sequence. Kunkel mutagenesis was used to create site-specific mutations, and all plasmids were sequence-verified.

For protein production, 20 μL of chemically-competent *Escherichia coli* BL21(DE3) (Novagen) were transformed on ice with 1 μL of plasmid in buffer at a concentration of 90 to 130 ng/μL. The competent cell-plasmid mixture was temperature shocked to induce plasmid uptake by heating at 42°C for one minute and then chilling on ice for one minute. Cells were recovered in 200 μL Terrific Broth (TB) media at 37 °C for one hour. They were then plated onto an LB agarose plate containing 50 mg/mL kanamycin, and incubated for 24 hours at 37 °C.

For each mutant, a 50 mL Falcon tube containing 5 mL TB with 50 mg/mL kanamycin was inoculated with one colony from a kanamycin selection plate. Tubes were covered with breathable seals and incubated with shaking for 24 hours at 37 °C.

Growth cultures were pelleted by centrifugation at 4700 RPM for 10 minutes and the supernatant was discarded. The cell pellet was resuspended in 5 mL of induction medium (TB with 1 mM isopropyl-β-D-thiogalactopyranoside and 50 mg/mL kanamycin). The tubes were covered again with breathable seals and incubated with shaking at 18 °C for 24 hours.

The 5 mL expression culture was pelleted by centrifugation at 4700 for 10 minutes and the supernatant was discarded. The resulting pellet was suspended in 500 μL wash buffer (50 mM HEPES, 150 mM sodium chloride, 15 mM imidazole, pH 7.50) and lysed with BugBuster protein extraction reagent (Millipore) and 1 mg lysozyme, 0.1 mg DNase, and 0.1 mg phenylmethylsulfonyl fluoride per sample.

After 20 min, lysate was centrifuged at 14,700 RPM for ten minutes. The supernatant was loaded on to protein purification columns (BioSpin 732-6008) prepared with 100 μL of 50% Ni-NTA resin slurry. After equilibration with 500 μL wash buffer, two 500 μL aliquots of supernatant were added to the columns. Six rounds of 500 μL wash buffer were then allowed to drip through the columns. Resulting protein micro-columns were then transferred to 2 mL tubes for elution. Protein was eluted in 2x100 μL elution buffer (50 mM HEPES, 150 mM sodium chloride, and 25 mM EDTA, pH 7.50). A brief centrifugation at 4000 RPM ensured all protein was collected.

Protein yield was then determined via ratio of absorbance at 260 and 280 nm and SDS-PAGE.

Each enzyme variant was assayed in triplicate at 8 substrate concentrations ranging from 0 to 75 mM. Diluted protein solution was dispensed in 25 μL aliquots into a 96-well plate (Corning Costar #3885). Separately, in another plate, 100 uL elution buffer with 8 different concentrations of pNPG (1 per row) were prepared. The assay was initiated by multi-channel pipetting 75 μL substrate from each row of the substrate plate into the corresponding row of the assay plate. The absorbance at 420 nm was monitored every minute for 60 minutes to determine the rate of the reaction.

Unless otherwise noted, all supplies were purchased from Sigma-Aldrich.

**Prediction and feature selection via Elastic net**

A regularized linear regression model, Elastic Net (EN), was chosen to fit the dataset of the kinetic constants, each constant fitted independently.1 Comparing to ordinary least squares regression, an EN model is able to make a prediction and select the most informative feature set simultaneously as  and  penalties are applied to the regression weights. The weight of each structural feature is estimated as



Where:

: the intercept;

: the weight of structural feature i in the regression model;

p: the number of structural features generated by the BglB model;

: the kinetic constant (the dependent variable to be predicted);

: structural features generated by the BglB model (the independent variables);

,: parameters tuning the constraints on the weights.

Since the structural feature were measured in different ranges and units, we first normalized all the features to be zero-centered with variance being one by subtracting the mean and dividing by the variance of the feature value. All the features are on the same scale to compare their contribution to the kinetic constants after the normalization. The tuning parameters,  are determined one by one via stratified 10-fold cross validation by searching a grid of and. Each round of cross validation generated a linear regression model. In order to build a more generalized model, cross validation was run 1,000 times with a different part of the dataset each time. The final prediction of a mutant’s kinetic constant was an average of all the predictions during the 1,000 rounds of training. The average number of non-zero weights when predicting *k*cat/KM, *k*cat and KM were 9, 8 and 10 respectively. The top features were chosen and listed in table 1 with their averaged weights among all the models (9 for *k*cat/KM, 8 for *k*cat, 10 for KM)

**Stratified 10-fold cross validation**

Stratified 10-fold cross validation was implemented to validate the EN model.2 Specifically, all the mutants were first ranked according to the experimentally-measured value of the kinetic constant to be predicted and every 10 adjacent datapoints were randomly marked with an index using integers from 1 to 10 without duplication. Finally, all the datapoints with the same index were grouped together, resulting in ten folds. Since the datapoints in each folds comes from different level of the dataset, this guarantees every fold is a good representative of the dataset. In order to build a robust prediction model, the cross validation was run 1,000 times, the dataset split into training set and testing set differently each time.

**Supplemental references**

1. Zou, H.; Hastie, T., Regularization and variable selection via the elastic net. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* **2005,** *67* (2), 301-320.

2. Kohavi, R. In *A study of cross-validation and bootstrap for accuracy estimation and model selection*, Ijcai, 1995; pp 1137-1145.