Supporting information
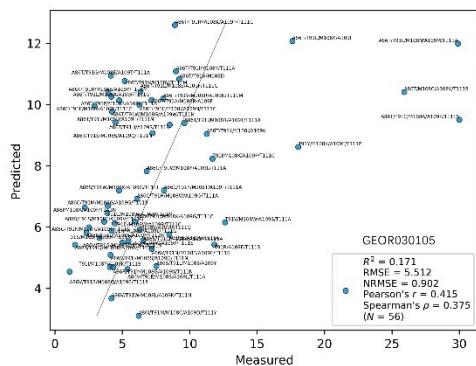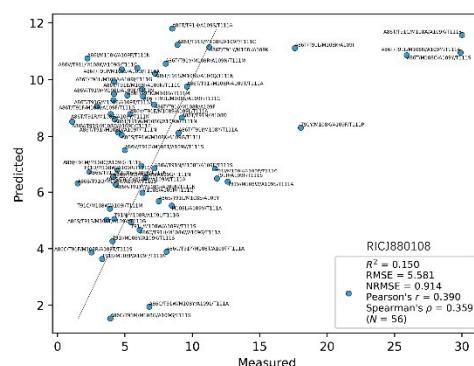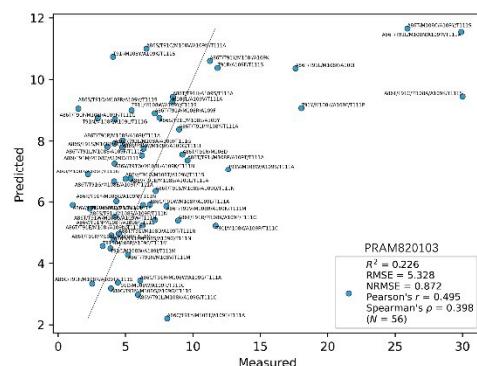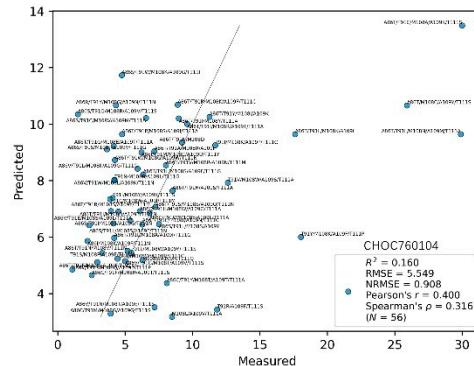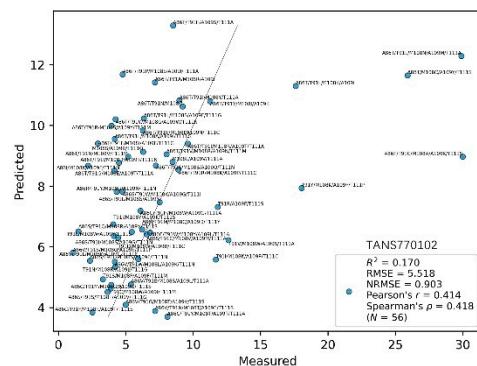
Figure S1. This set of figures is a fitting diagram generated by PyPEF. The Spearman coefficient of TANS770102 is 0.418, which proves that the evaluation of the five points using this model has a certain correlation.
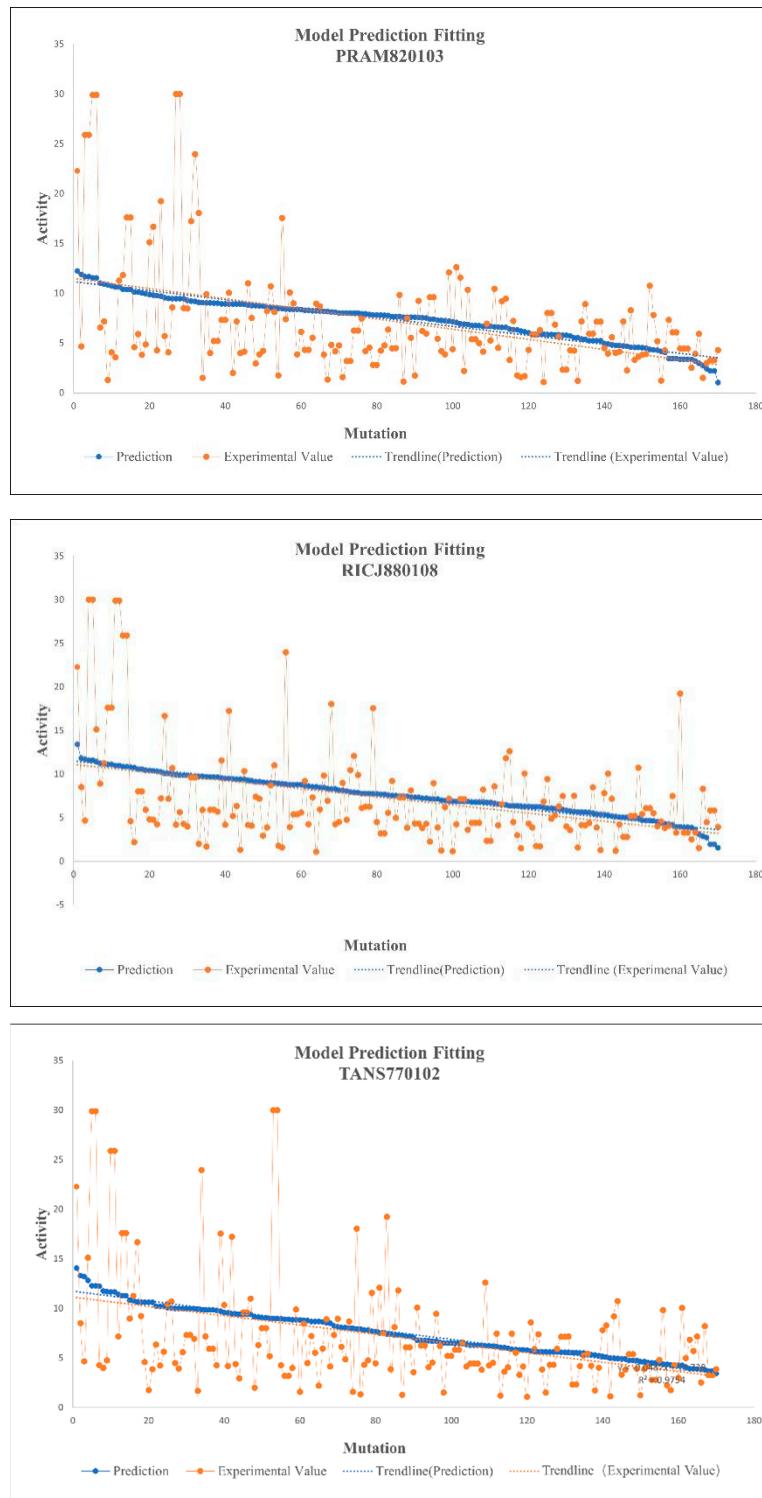
Figure S2. The above figure shows the fitting of 165 variants using three models. The three models meet the criteria for activity changes in trend. The yellow dashed line represents a trend composed of 165 variants, while the blue line represents the predicted results of the model. The two have unity.
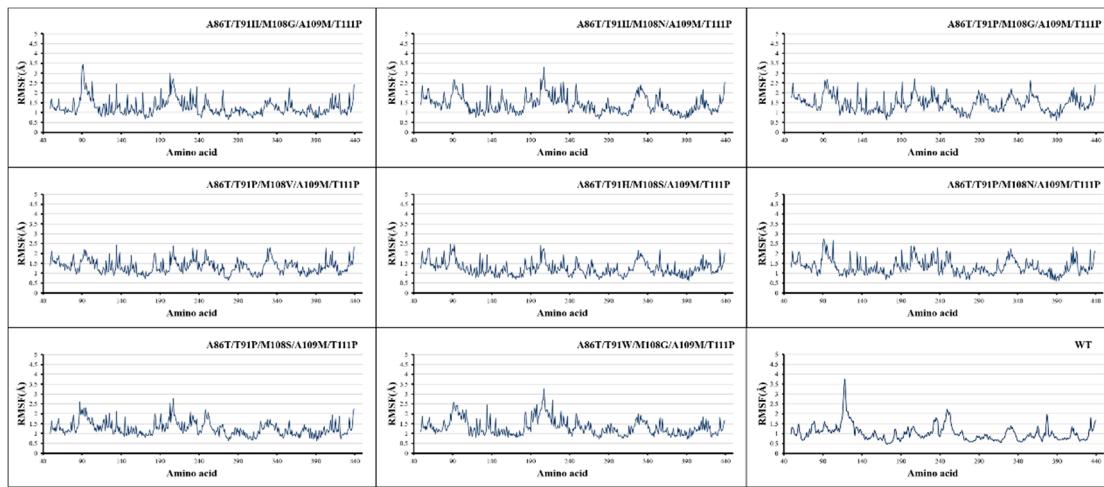
Figure S3. This image shows the positional changes of 8 variants of RMSF and wild-type RMSF. The peak of wild-type RMSF occurs at amino acids 90 to 140, which is consistent with the influence range of the five mutation sites.

Table S1. Abbreviation.

Abbreviation	Full Name
AAindex	Amino Acid Index
AI	Artificial Intelligence
DCA	Direct coupling analysis
MD	Molecular Dynamics Simulation
ML	Machine Learning
MLP	Multilayer Perceptron
OneHot	One-hot Encoding
PLS	Partial Least Squares
PyPEF	Pythonic Protein Engineering Framework
RMSF	Root Mean Square Fluctuation
RF	Random Forest
SVM	Support Vector Machine
SVR	Support Vector Regression

Table S2. This chart shows the RMSF of the Loop. The RMSF of the positions with the highest steric hindrance decreased. The overall RMSF of variants 05(A86T/T91P/M108V/A109M/T111P) and 07(A86T/T91H/M108S/A109M/T111P) showed a significant decrease. It can be inferred that the substrate throughput has been improved. The second variant could not be modeled because it was unstable.

Amino Acid	WT	01	03	04	05	06	07	08	09
77	1.47	1.02	1.55	1.4	1.37	1.45	1.49	1.14	1.26
78	1.5	1.11	1.5	1.39	1.63	1.5	1.45	1.58	1.4
79	1.63	1.74	2.06	2.02	1.96	2.15	1.64	1.92	1.69
80	1.29	1.45	1.67	1.64	1.43	1.55	1.44	1.34	1.41
81	1.04	0.93	1.47	1.24	1.21	1.29	1.32	1.07	1.18
82	1.08	0.86	1.29	1.1	1.04	1.16	1.02	1.03	1.13
83	1.07	1.01	1.18	1.09	0.94	1.27	0.86	1.19	1.24
84	1.21	1.18	1.35	1.27	1.2	1.37	1.11	1.29	1.39
85	1.01	1.29	1.47	1.31	1.26	1.45	1.19	1.37	1.4
86	1.08	1.34	1.19	1.11	1.04	1.85	0.95	1.71	1.48
87	1.1	1.77	1.48	1.32	1.27	2.47	1.22	2.61	1.99
88	1.15	1.61	1.84	1.45	1.69	1.92	1.72	1.98	1.63
89	1.11	2.03	1.91	1.89	1.59	2.13	1.66	2.02	2.08
90	1.15	3.21	1.94	2.01	1.63	2.25	2.47	2.23	2.08
91	1.41	3.44	2.6	2.15	1.77	2.09	2.74	1.94	2.59
92	1.61	3.01	2.68	2.63	2.03	2.45	2.55	2.27	2.46
93	1.44	2.39	2.31	2.47	2.22	1.97	2.29	2.29	2.3
94	1.34	2.17	2.18	2.21	1.88	1.48	1.92	1.88	2.14
95	1.39	2.5	2.36	2.68	2.15	1.79	2.46	2.3	2.44
105	1.23	1.71	1.64	1.86	1.7	1.16	1.6	1.49	2.04
106	1.17	1.25	1.43	1.44	1.23	1.03	1.24	1.17	1.57
107	1.48	1.25	1.46	1.68	1.52	1.24	1.36	1.28	2.2
108	1.27	1.25	1.56	2.33	1.49	1.37	1.15	1.09	1.73
109	1.15	1.25	1.26	1.66	1.75	1.3	1.18	1.32	1.63
110	1.27	1.28	1.48	1.46	1.22	1.09	1.17	1.45	1.44
111	1.09	0.94	0.89	0.84	1.1	0.91	0.86	1.12	0.87
112	1.32	1.33	1.25	1.07	1.16	1	0.93	1.41	0.91
113	1.54	1.14	0.95	1.12	0.98	1.43	0.92	1.2	1.35
114	1.67	0.88	1.01	1.37	1.03	1.15	0.96	1.39	1.18
115	1.87	0.8	0.96	1.02	0.92	0.87	0.86	1.12	0.85
116	2.64	1.01	1.21	1.38	1.02	0.98	0.95	1.29	1.05
117	3.35	1.44	1.03	1.62	1.3	1.04	1.18	1.64	1.23
118	3.76	1.44	1.18	1.77	1.23	1.28	1.24	1.72	1.3
119	3.06	1.07	1.03	1.34	1.14	1.07	1.08	1.42	1.11
Mean value	1.52	1.53	1.54	1.60	1.41	1.49	1.42	1.57	1.58