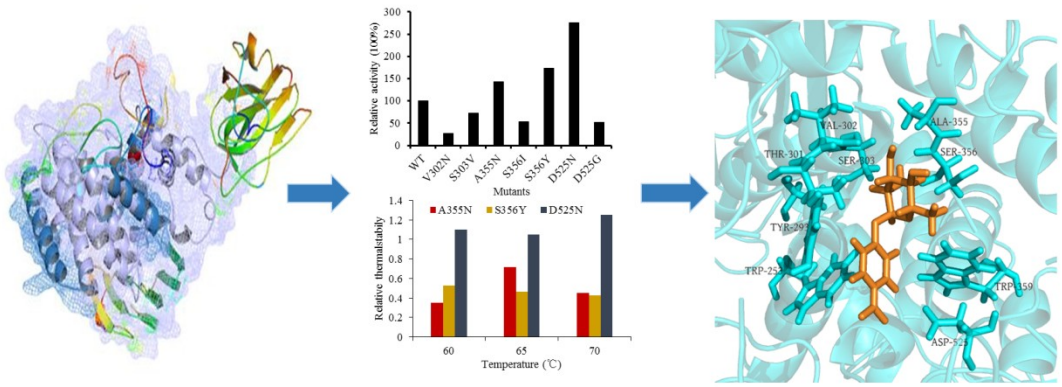
Figures

An effective computational-screening strategy for simultaneously improving both catalytic activity and thermostability of α-L-rhamnosidase

Graphical Abstract



A double-screening strategy that combines molecular docking and conservation site comparison, is proposed to improve the enzyme activity and thermostability of a α-L-rhamnosidase

A double-screening strategy is proposed to fish potential mutants with both improved enzymatic activity and thermostability.

The key of the success of the double-screening strategy lies in the fact that the mutation site was selected to be within the range of 5 Å of the substrate binding sites, which brings only local conformational change.

Figure captions

Fig.1 Computer-assisted screening of mutant residues. (A) Overall view of docking results of α-L-rhamosidase r-Rha1 bound to *p*NPR, *p*NPR is shown as blue CPK model, proteins is shown as cartoon/surface model, blue surface represents positively charged residues, and red surface represents negatively charged residues. (B) Binding pocket view of α-L-rhamosidase r-Rha1 bound to *p*NPR; (C) The mutate energy of virtual saturation mutations of binding pocket residues.

Fig.2 Enzymatic properties of α-L-rhamosidase r-Rha1. (A) Effects of relative activity of WT r-Rha1 and its mutants. (B) Optimum temperature and thermostability in different temperature of wild type r-Rha1 (WT) and its mutants. (a) The optimum temperature of WT and its mutants. (b) 60ºC thermostability of WT and its mutants. (c) 65ºC thermostability of WT and its mutants. (d) 70ºC thermostability of WT and its mutants. (C) The ratio of the half-life of the mutant to the wild type at different temperatures.

Fig. 3 Interaction analysis of the wild type r-Rha1 (WT) and its mutants. A,C,E is the interaction analysis diagram of the WT and *p*NPR; B is the interaction analysis diagram of the A355N and *p*NPR; D is the interaction analysis diagram of the S356Y and *p*NPR; E B is the interaction analysis diagram of the D525N and *p*NPR, the hydrogen bonds are shown as yellow dashed lines.

Fig.1

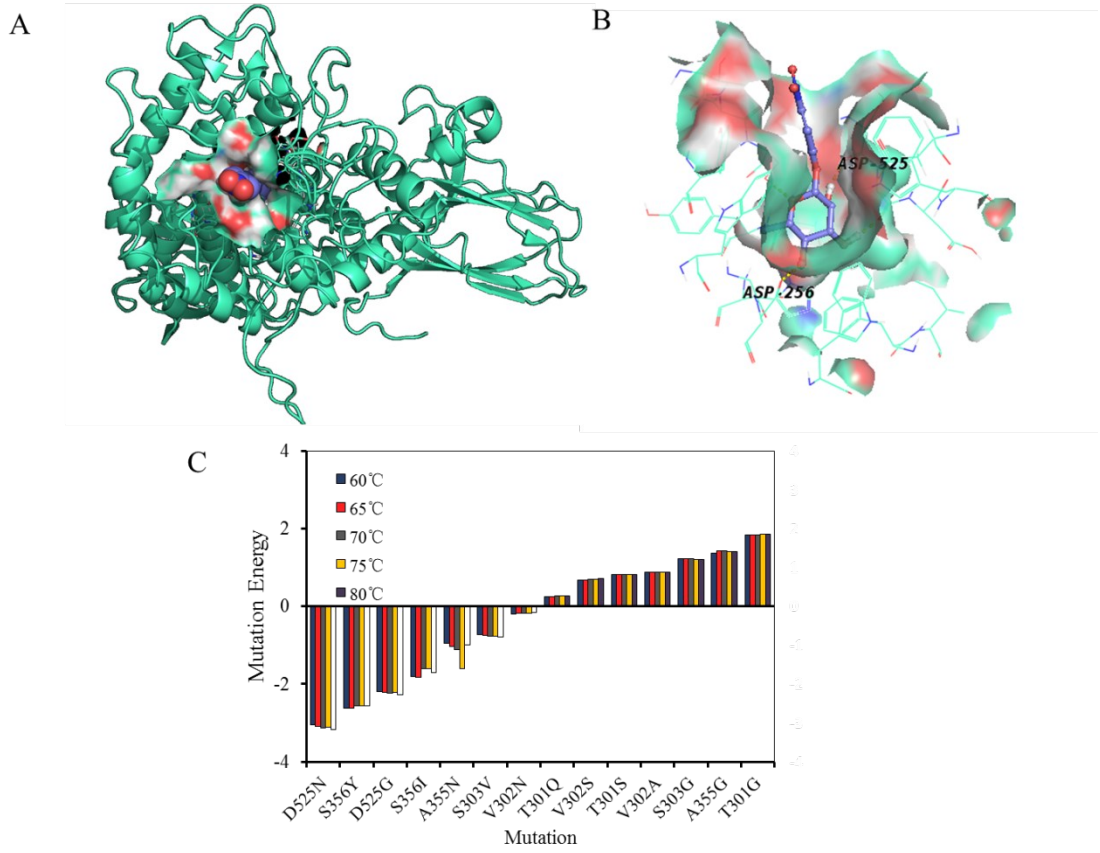

A
B
ASP-525
ASP-256
C
60℃
65℃
70℃
75℃
80℃
Mutation Energy
4
2
0
-2
-4
D525N
S356Y
D525G
S356I
A355N
S303V
V302N
T301Q
V302S
T301S
V302A
S303G
A355G
T301G
Mutation

Fig.2

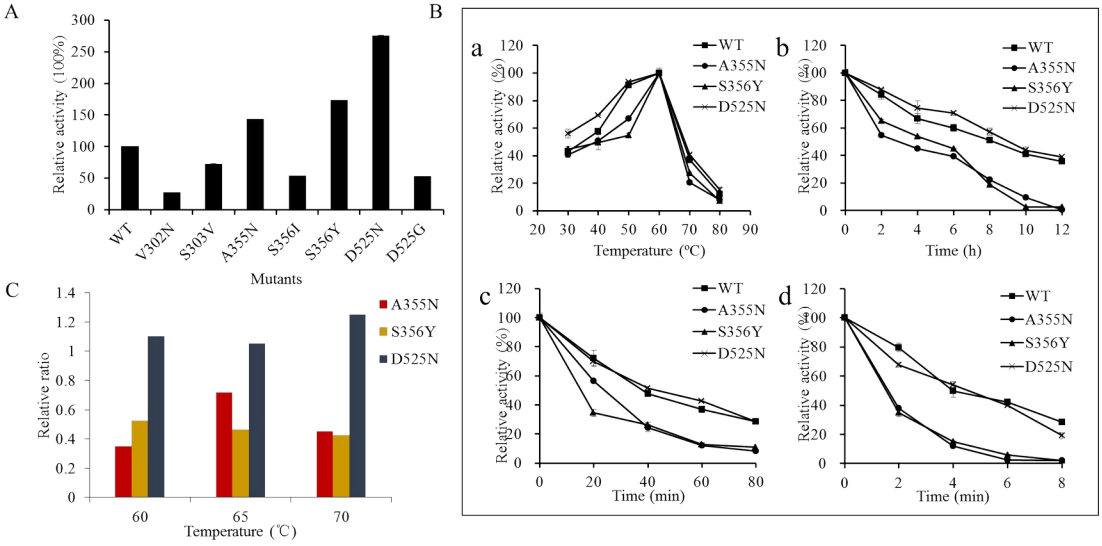
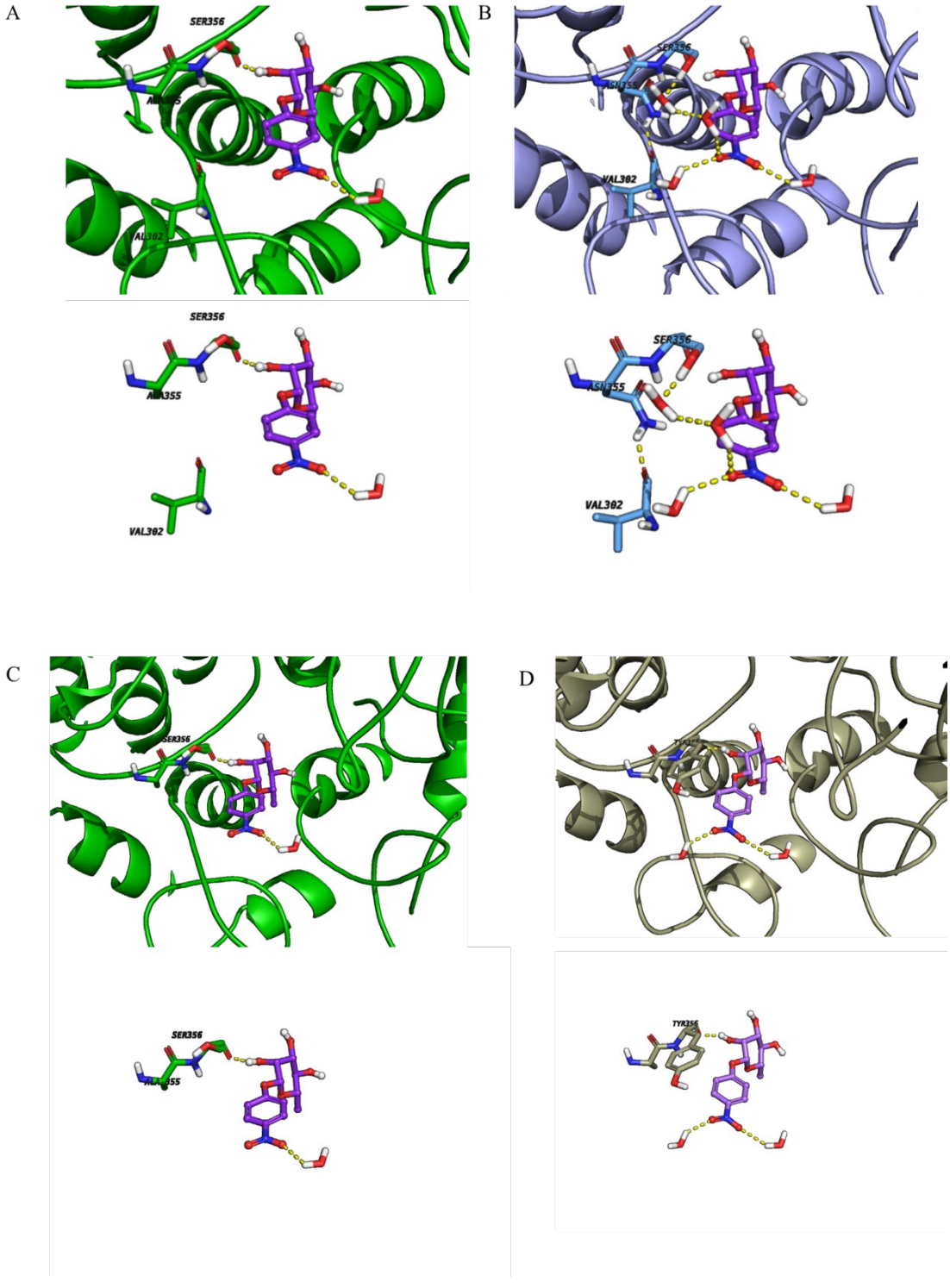
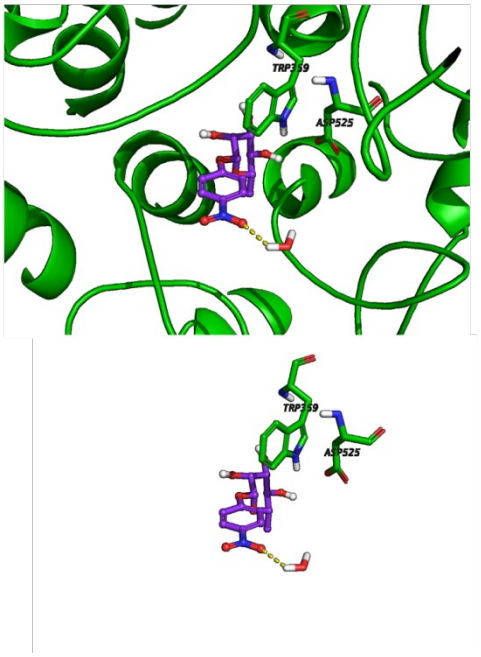

A
Relative activity (100%)
300
250
200
150
100
50
0
WT
V302N
S303V
A355N
S356I
S356Y
D525N
D525G
Mutants
B
a
Relative activity (%)
Temperature (°C)
b
Time (h)
c
Time (min)
d
Time (min)
WT
A355N
S356Y
D525N
C
Relative ratio
1.4
1.2
1
0.8
0.6
0.4
0.2
0
60
65
70
Temperature (℃)
A355N
S356Y
D525N

Fig.3


A
SER356
VAL302
SER356
VAL302
B
VAL302
SER356
VAL302
C
SER356
SER356
D
TYR356

E



F