Supporting Information

Stable immobilization of aldehyde ketone reductase mutants containing non-natural amino acids on an epoxy resin via strain-promoted alkyne-azide cycloaddition

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Table S1 Primers required for construction of AKR-pZE21

Primer	Sequence (5'→3')	Restriction-enzyme cutting sites	Tm (℃)
AKR-pZE21-F	*CGG**GGTACC**ATGCTGTACAAAGAACTGGGCCGTA*	KpnI	60
AKR-pZE21-R	*CCC**AAGCTT**TTAGTGGTGGTGGTGGTGGTG ACCCAGACTATCC*	HindIII	44

Italic, conserved base; Bold, restriction site; Underlined, his-tag label

Table S2 Primers for AKR sites mutations

Primer	Sequence (5'→3')	Tm (℃)
AKR-110Y-pZE21-F	*CGTTTAGATACCGATTAGGTGGATCTGTATCTG*	57
AKR-110Y-pZE21-R	*CAGATACAGATCCACCTAATCGGTATCTAAACG*	57
AKR-114Y-pZE21-F	*GATTATGTGGATCTGTAGCTGATTCATTGGCCG*	60
AKR-114Y-pZE21-R	*CGGCCAATGAATCAGCTACAGATCCACATAATC*	59
AKR-143Y-pZE21-F	*CAGGGCTTAATTCGCTAGATTGGTGTGAGTAAT*	61
AKR-143Y-pZE21-R	*ATTACTCACACCAATCTAGCGAATTAAGCCCTG*	59
AKR-162Q-pZE21-F	*GCCATTTCTAAATCATAGGAACCGATTGTTTGT*	59
AKR-162Q -pZE21-R	*ACAAACAATCGGTTCCTATGATTTAGAAATGGC*	59
AKR-189Q -pZE21-F	*TTACTGGAATTTTGTTAGAAAAATGGCGTGACC*	59
AKR-189Q -pZE21-R	*GGTCACGCCATTTTTCTAACAAAATTCCAGTAA*	58

Italic, conserved base ; Underlined, mutation site

Table S3 Expression and activity of free AKR preparations

Enzyme	Purified enzyme (mg/L)	Free enzyme activity (U/mg)	Immobilized enzyme activity (U/mg)
AKR- pZE21-BL21	180.00±0.03	1.070±0.001	1.090±0.002
AKR-110Y- pZE21-MG1655	169.00±0.02	0.940±0.005	1.050±0.005
AKR-114Y- pZE21-MG1655	182.00±0.05	1.080±0.008	1.240±0.007
AKR-143Y- pZE21-MG1655	165.00±0.01	0.950±0.002	1.100±0.006
AKR-162Q- pZE21-MG1655	158.00±0.04	0.920±0.007	1.020±0.004
AKR-189Q- pZE21-MG1655	175.00±0.03	0.980±0.008	1.120±0.003
AKR-N114Y-189Q-pZE21-MG1655	120.00±0.04	0.900±0.006	1.010±0.005
AKR-N114Y-189Q-143Y -pZE21-MG1655	100.00±0.02	0.870±0.005	0.990±0.002
AKR-N114Y-189Q-143Y-110Y-162Q-pZE21-MG1655	82.00±0.05	0.800±0.004	0.850±0.006

Table S4 Denaturation rate constant (K_D, t^{-1}), half-life ($t_{1/2}$, h) and reaction activation energy (E_a, KJ·moL^{-1}) of AKR preparations at different temperatures

Enzyme	30		40		50		60		***Ea*** (KJ·moL^{-1})
	K_D ×10^{-2} (h^{-1})	$t_{1/2}$ (h)	K_D ×10^{-2} (h^{-1})	$t_{1/2}$ (h)	K_D ×10^{-2} (h^{-1})	$t_{1/2}$ (h)	K_D ×10^{-2} (h^{-1})	$t_{1/2}$ (h)	
Free AKR	7.80	8.88	8.84	7.84	10.19	6.80	11.18	6.20	10.27
Wild type immobilization	4.39	15.79	4.78	14.50	5.81	11.93	7.27	9.53	14.26
One-point immobilization	2.50	27.72	2.77	25.02	4.20	16.31	4.92	14.09	20.61.
Three-point immobilization	1.80	38.50	2.39	29.00	3.12	22.21	3.79	18.28	21.03
Five-point immobilization	0.65	106.62	0.9	77.00	1.27	54.57	1.54	45.00	24.67

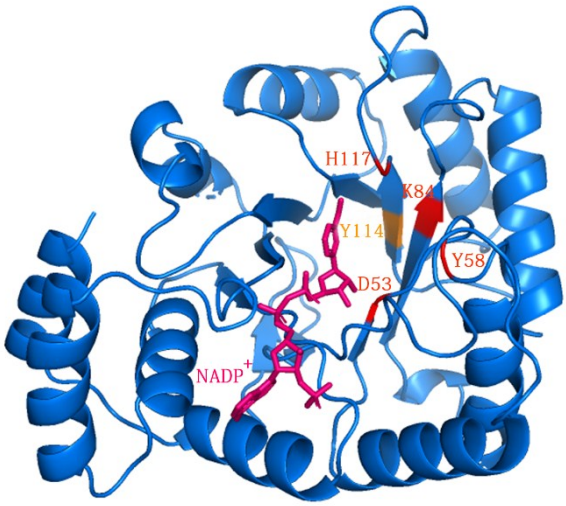

Figure S1. The structure of a one-point mutation site for the insertion of the aldehyde ketone reductase of pAzF. The orange mark represents the enzyme mutation site, the red mark represents the active site[1] ,the pink short line structure is the active center of the enzyme NADPH, (PDB ID: 5dan.1; resolution, 2.0 Å).

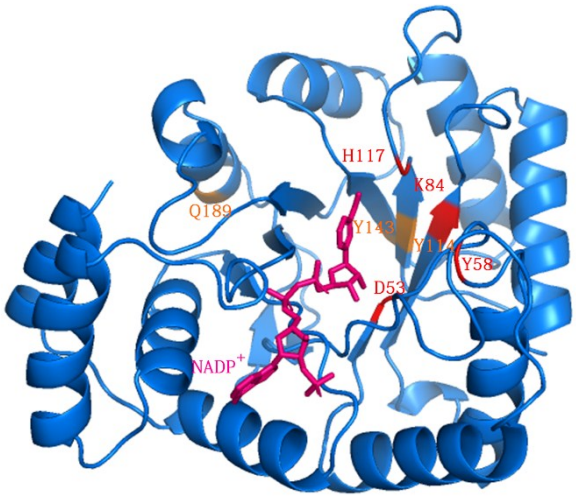

Figure S2. The structure of a three-point mutation site for the insertion of the aldehyde ketone reductase of pAzF. The orange mark represents the enzyme mutation site, the red mark represents the active site ,the pink short line structure is the active center of the enzyme NADPH, (PDB ID:

5dan.1; resolution, 2.0 Å).

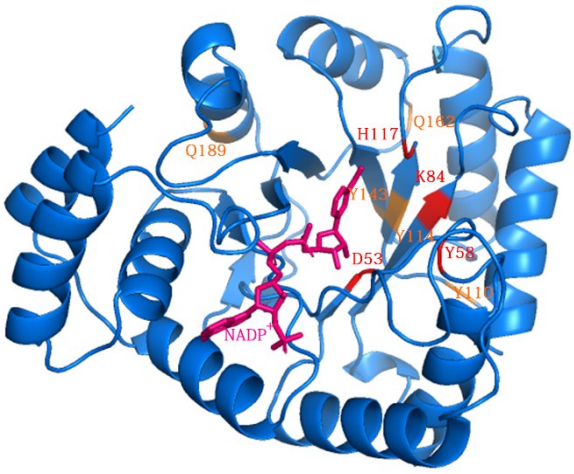

Figure S3. The structure of a five-point mutation site for the insertion of the aldehyde ketone reductase of pAzF, The orange mark represents the enzyme mutation site, the red mark represents the active site ,the pink short line structure is the active center of the enzyme NADPH, (PDB ID: 5dan.1; resolution, 2.0 Å).

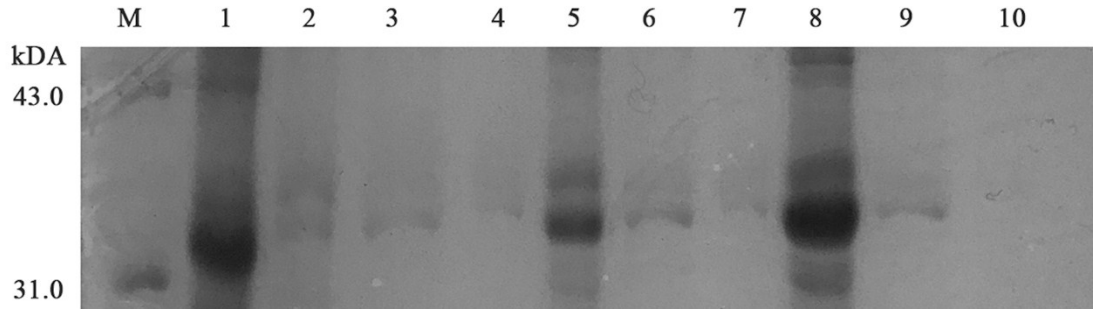

Figure S4. SDS-PAGE photograph of supernatant and elution in the precise one-point immobilization of AKR-114Y using different amino acid modified resins (Lane M, protein marker; Lane 1, pre-immobilization crude enzyme; Lane 2, supernatant from immobilization; Lane 3, elution of first time from the immobilized enzyme using 2M NaCl aqueous; Lane 4, elution of second time from the immobilized enzyme using 2M NaCl aqueous. Three groups, lane 2, 3, 4/5, 6, 7/8, 9, 10, were for the resin modified using lysine, glutamate, glycine.)

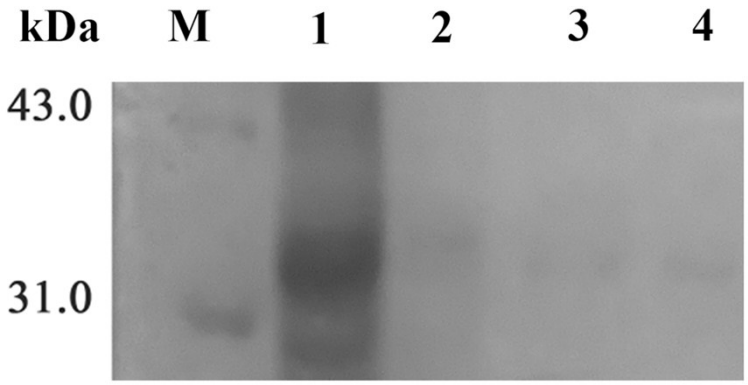

Figure S5. SDS-PAGE photograph of supernatant and elution in the precise two-point immobilization of AKR mutant using lysine-modified resins (Lane M, protein marker; Lane 1, pre-immobilization crude enzyme; Lane 2, supernatant from immobilization; Lane 3, elution of first time from the immobilized enzyme using 2M NaCl aqueous; Lane 4, elution of second time from the immobilized enzyme using 2M NaCl aqueous.)

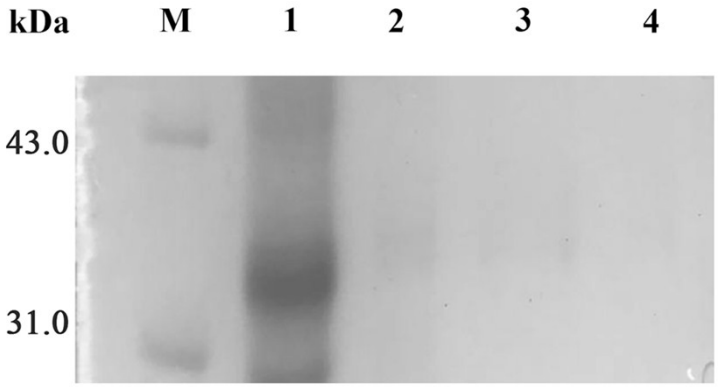

Figure S6. SDS-PAGE photograph of supernatant and elution in the precise five-point immobilization of AKR mutant using lysine-modified resins (Lane M, protein marker; Lane 1, pre-immobilization crude enzyme; Lane 2, supernatant from immobilization; Lane 3, elution of first time from the immobilized enzyme using 2M NaCl aqueous solution; Lane 4, elution of second time from the immobilized enzyme using 2M NaCl aqueous solution.)

Reference

1. H. Hou, R. Y. Li, X. Y. Wang, Z. Yuan, X. M. Liu, Z. M. Chen and X. L. Xu, *Acta Crystallogr F*, 2015, **71**, 847-855.