Supporting Information

Rapidly and Precisely Cross-Linked Enzymes Using Bio-Orthogonal Chemistry from Cell Lysate for the Synthesis of (S)-1-(2,6-Dichloro-3-fluorophenyl) Ethanol

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Table S1 Primers required for construction of akr-pZE21

Primer	Sequence (5'→3')	Restriction-enzyme cutting sites	Tm (°C)
AKR-pZE21-F	CGG**GGTACC***ATGCTGTACAAAGAACTGGGCCGTA*	KpnI	60
AKR-pZE21-R	CCC**AAGCTT**TTAGTGGTGGTGGTGGTGGTG *ACCCAGACTATCC*	HindIII	68

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

Table S2 Primers for AKR sites mutations

Primer	Sequence (5'→3')	Tm (°C)
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 Calculated and Detected Molecular Weights of wild type AKR and AKR mutants

Enzyme	calculated mass (Da)	detected mass (Da)
WT-AKR	32190.0	32191.5
AKR three-point mutant	32300.1	32301.5
AKR five-point mutant	32385.1	32386.6

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

Enzyme	30		40		50		60		E_a (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)	
AKR five-point mutant	7.09±0.12	9.77±0.20	8.15±0.16	8.50±0.18	9.61±0.19	7.21±0.18	11.62±0.13	5.97±0.13	13.8±0.4
AKR three-point mutant	6.74±0.11	10.28±0.21	7.62±0.21	9.09±0.22	9.30±0.21	7.45±0.16	11.10±0.31	6.24±0.15	14.2±0.4
Free WT-AKR	6.01±0.10	11.53±0.31	7.50±0.18	9.24±0.25	9.10±0.22	7.62±0.11	10.52±0.21	6.59±0.14	15.7±0.3
AKR three-point RP-CLEs	2.14±0.06	32.38±0.61	2.55±0.41	27.18±0.51	3.01±0.10	23.02±0.45	4.10±0.09	16.90±0.36	17.7±0.5
AKR five-point RP-CLEs	1.30±0.03	53.31±1.01	1.77±0.04	39.15±0.81	2.04±0.05	33.97±0.75	2.59±0.06	26.76±0.51	18.6±0.3

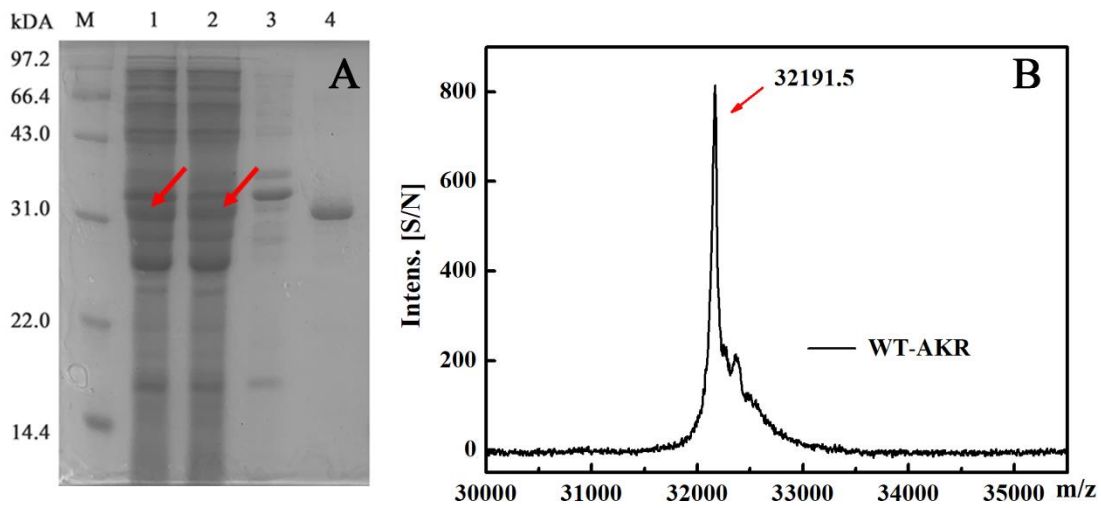

Figure S1 SDS-PAGE analysis (A) and MALDI-TOF of WT-AKR (B)

(Lane M: protein marker; lane 1: culture supernatant of WT-AKR; lane 2: cellular soluble fraction of WT-AKR-; lane 3: cellular insoluble fraction of WT-AKR; lane 4: purified WT-AKR)

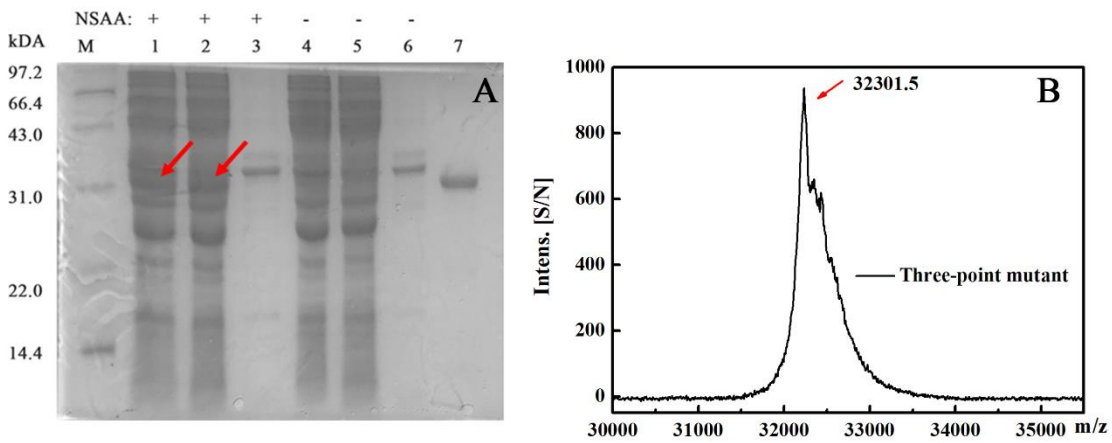

Figure S2 SDS-PAGE analysis (A) and MALDI-TOF of AKR three-point mutant(B)

(Lane M: protein marker; lane 1: culture supernatant of AKR three-point mutant + pAzF; lane 2: cellular soluble fraction of AKR three-point mutant + pAzF; lane 3: cellular insoluble fraction of AKR three-point mutant + pAzF; lane 4: culture supernatant of AKR three-point mutant - pAzF; lane 5: cellular soluble fraction of AKR three-point mutant - pAzF; lane 6: cellular insoluble fraction of AKR three-point mutant - pAzF; and lane 7: AKR purified three-point mutant + pAzF)

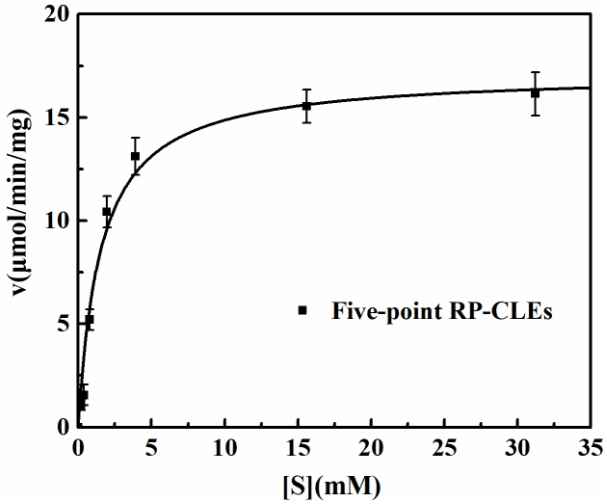

Figure S3 substrate saturation plots of AKR five-point RP-CLEs

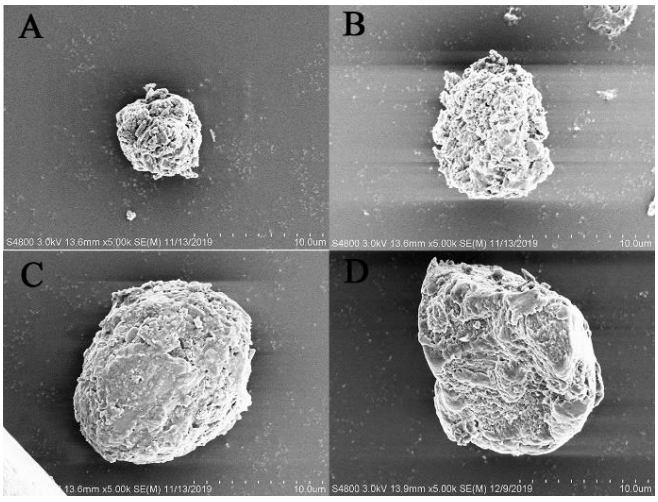

Figure S4 SEM of AKR RP-CLEs at different times

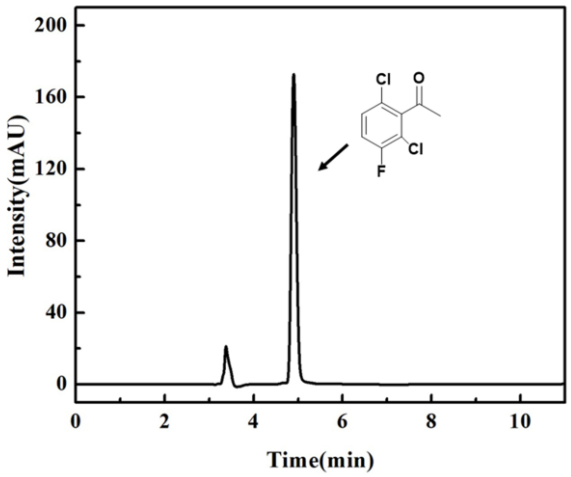

Figure S5 HPLC of 2,6-dichloro-3-fluoroacetophenone

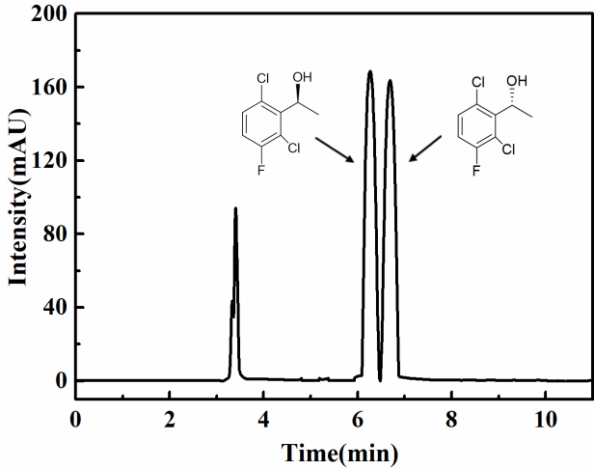

Figure S6 HPLC analysis of (±)-1-(2,6-dichloro-3-fluorophenyl) ethanol

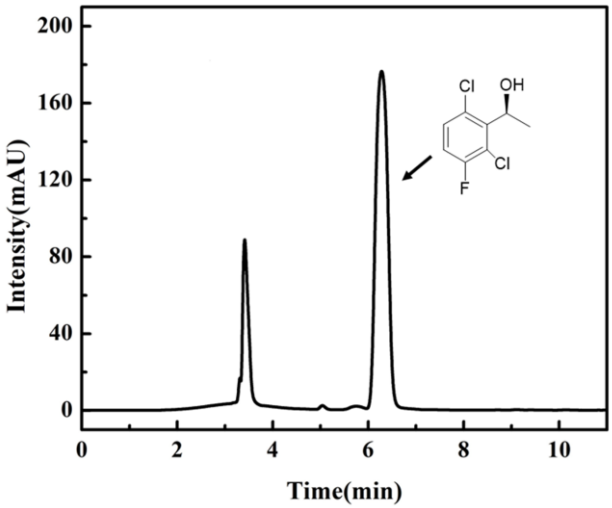

Figure S7 HPLC analysis of the synthesized (S)-1-(2,6-dichloro-3-fluorophenyl) ethanol