Electronic Supplementary Material (ESI) for RSC Advances.

Genetic incorporation of D-amino acids into green fluorescent protein based on polysubstrate specificity

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Supplementary Table 1. MALDI-TOF-MS analysis of peptide fragments

Mutant	Calculated[a] (m/z)	Observed[b] (m/z)	Sequence
GFPuv	2062.8650	2062.9690	MGSSHHHHHHSQDPNSMSK[c]
S18^{D}F	2122.9010	2123.0957	MGSSHHHHHHSQDPNSM^{D}FK[c]
S18^{D}N	2073.8805	2073.9888	MGSSHHHHHHSQDPNSM^{D}NK[c]
S18^{D}M	2122.8679	2123.0171	MGSSHHHHHHSQDPNSM^{D}MK[c]
S18^{D}R	2003.8386	ND[d]	MGSSHHHHHHSQDPNSMR
	2115.9387	2115.9658	MGSSHHHHHHSQDPNSM^{D}RK[c]
S18^{D}P	2056.8903	2055.9924	MGSSHHHHHHSQDPNSM^{D}PK[c]
S18^{D}A	2177.9101	2176.4929	MGSSHHHHHHSQDPNSM^{D}AK[c]
S18^{D}I	2132.2548	2132.0412	MGSSHHHHHHSQDPNSM^{D}IK[c]
S18^{D}V	2223.3874	2224.1098	MGSSHHHHHHSQDPNSM^{D}VK[c]
Y66^{D}F	2383.8350	2383.1250	LPVPWPTLVTTFS^{D}FGVQCFSR[c]

a. Calculated mass for peptide fragment incorporated a D-amino acid.

b. Determined by MALDI-TOF-MS.

c. D-amino acid in the peptide fragment is underlined.

d. Undetectable.

Supplementary Table 2. Primers used for the mutagenesis

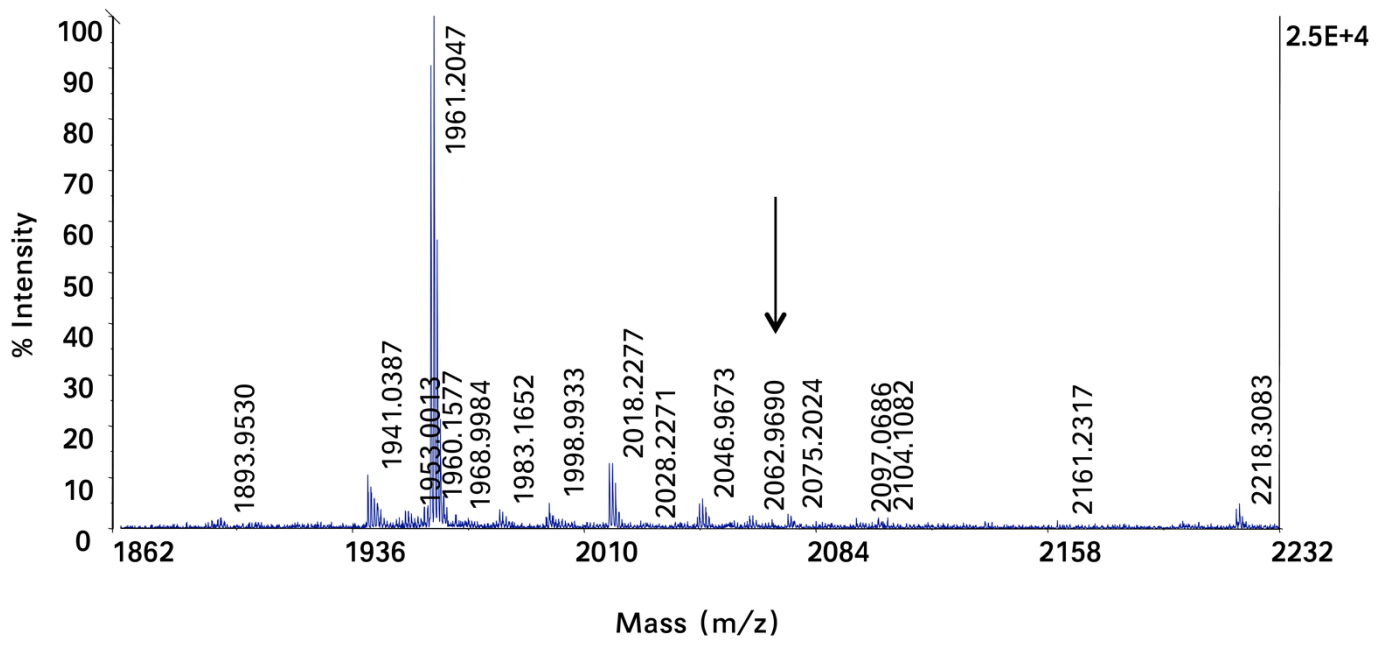
Primer	Nucleotide sequence (5'— 3')	Gene product
MGU101(F[a])	GAATTCGATGAGTAAAGGAGAAGAACTTTTC	GFPuv
MGU102(R[b])	AAGCTTATTTGTAGAGCTCATCCATGC	
MGU103(F[a])	GAATTCGATGTAGAAAGGAGAAGAACTTTTC	GFPuv-18TAG[c]
MGU104 (F[a])	ACTACTTTCTCTTTTGGTGTT	GFPuv-Y66F
MGU105 (R[b])	ACCAAAAGAGAAAGTAGTGACAAG	
MGU106 (F[a])	CTACTTTCGCCTAGGGTGTTCAAT	GFPuv-Y66TAG
MGU107 (R[b])	TTGAACACCCTAGGCGAAAGTAGT	
MGU110 (F[a])	CATATGTAACGCCGTTATACGTTGTT	pCNFRS with *Nde*I & *Pst*I
MGU111 (R[b])	GACGTCAAAAGCACGCAAACTCAATA	

a. Forward primer

b. Reverse primer

c. GFPuv-18TAG mutant gene was amplified using primer MGU103 as a forward primer and MGU102 as a reverse primer.

a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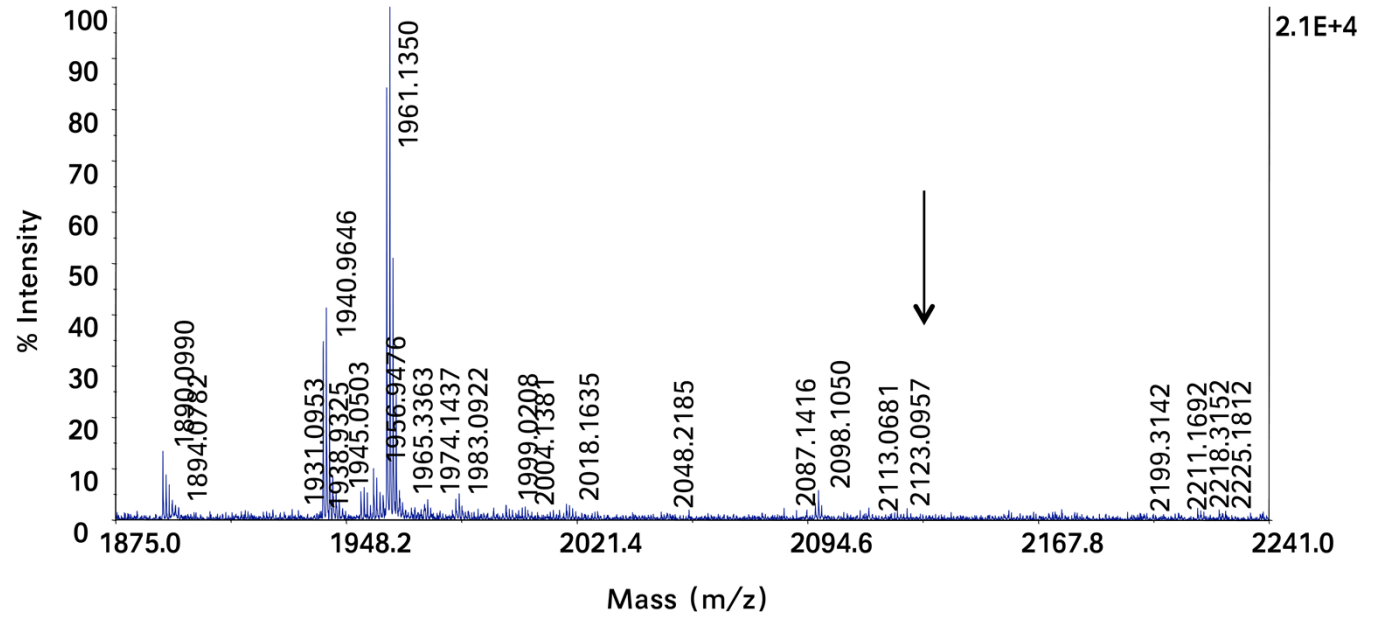
b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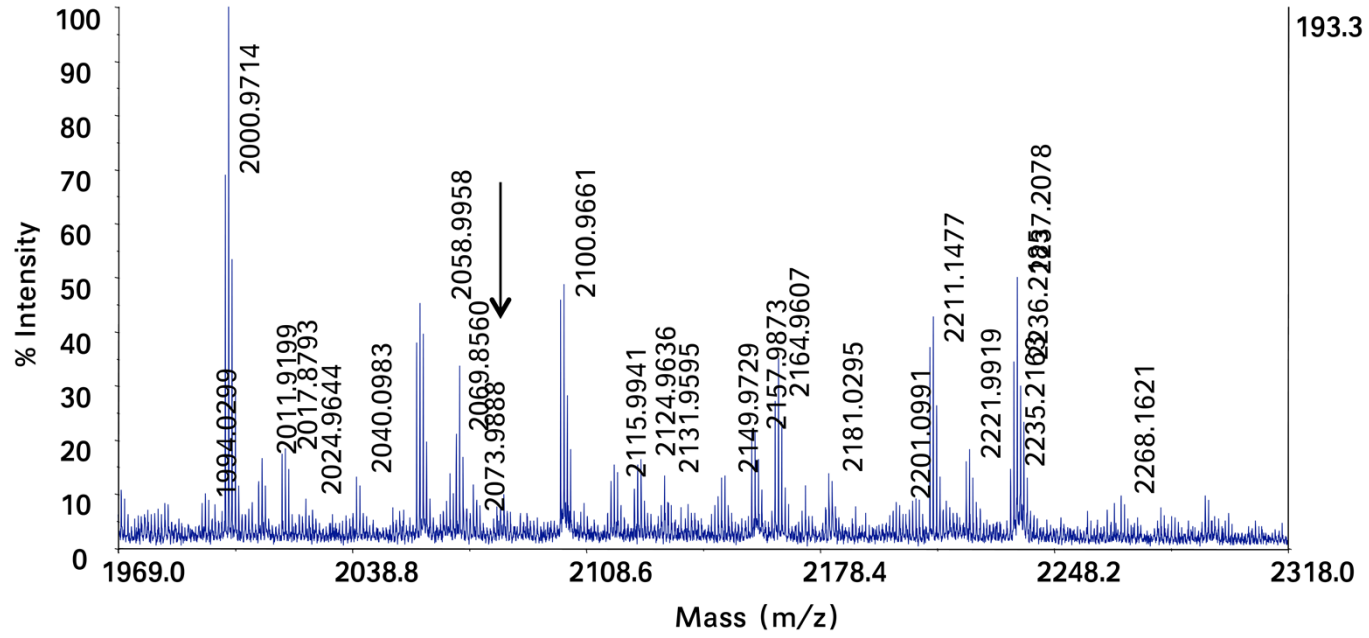
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Mass (m/z)

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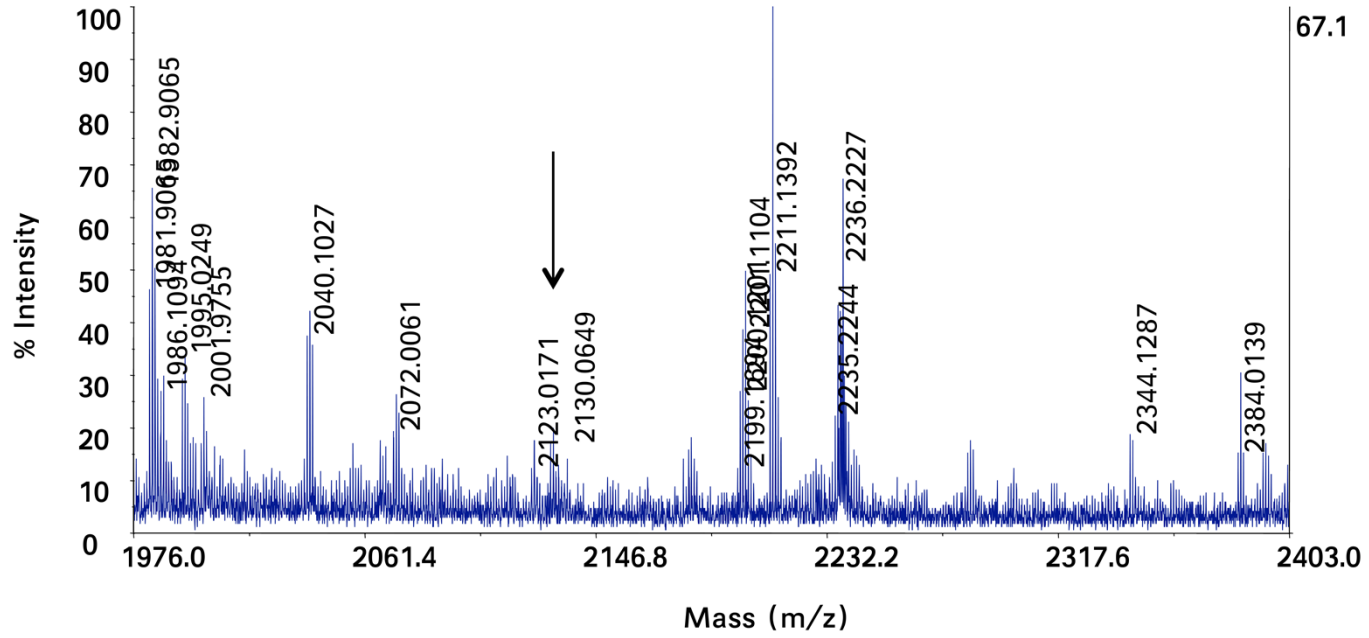
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Mass (m/z)

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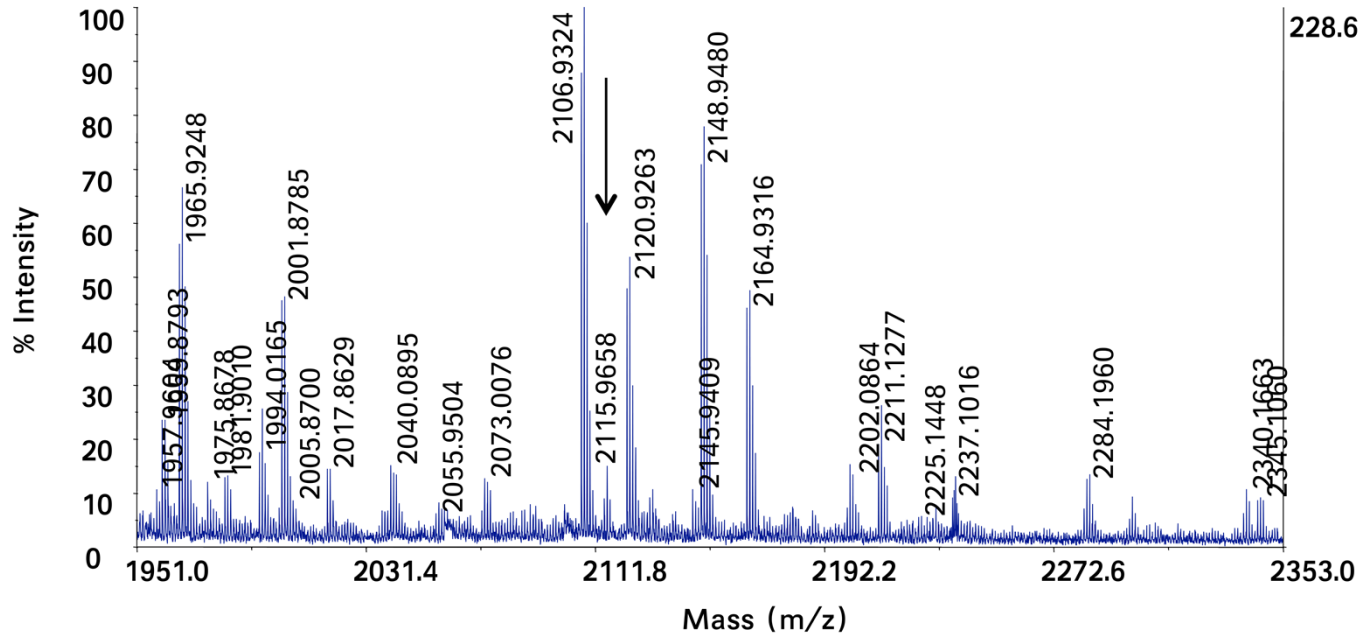
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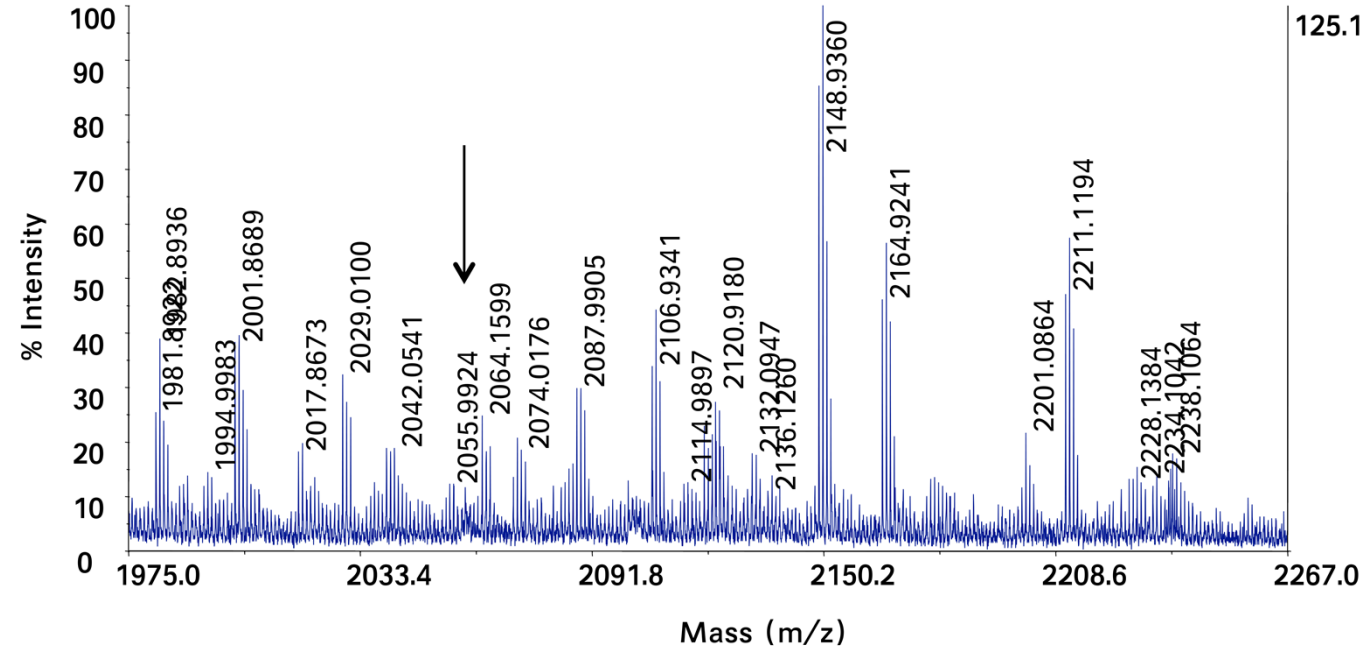
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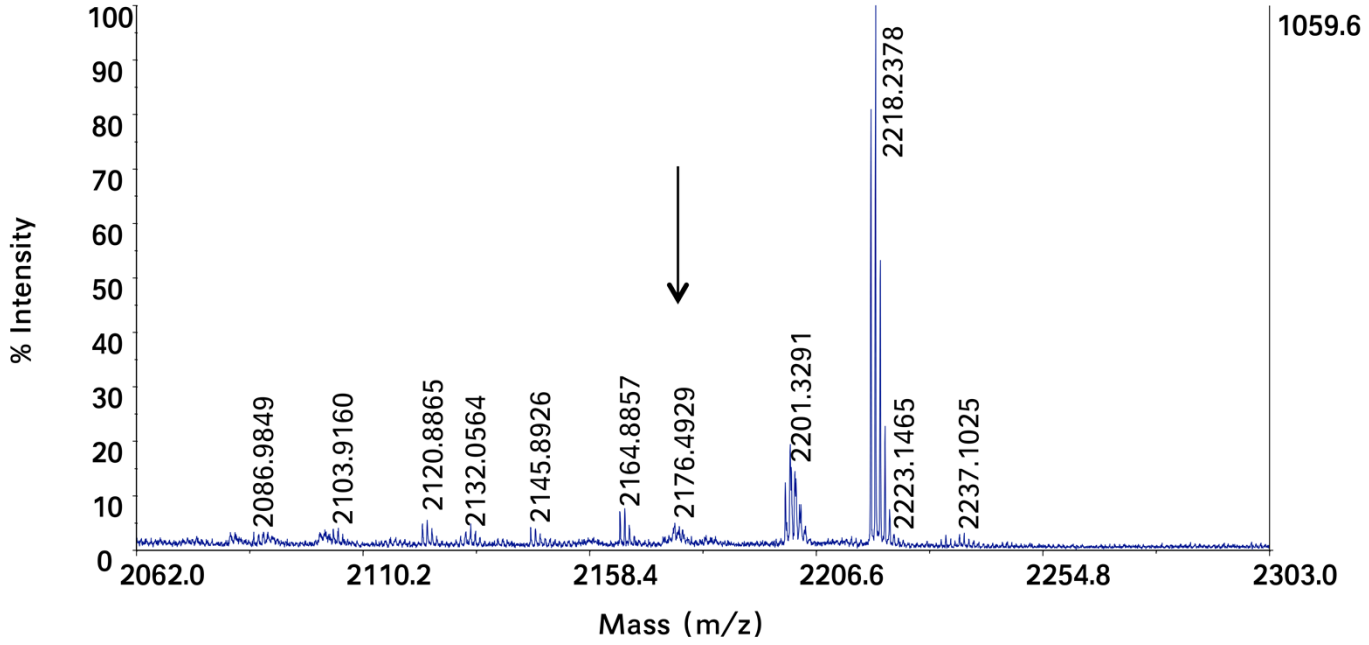
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Mass (m/z)

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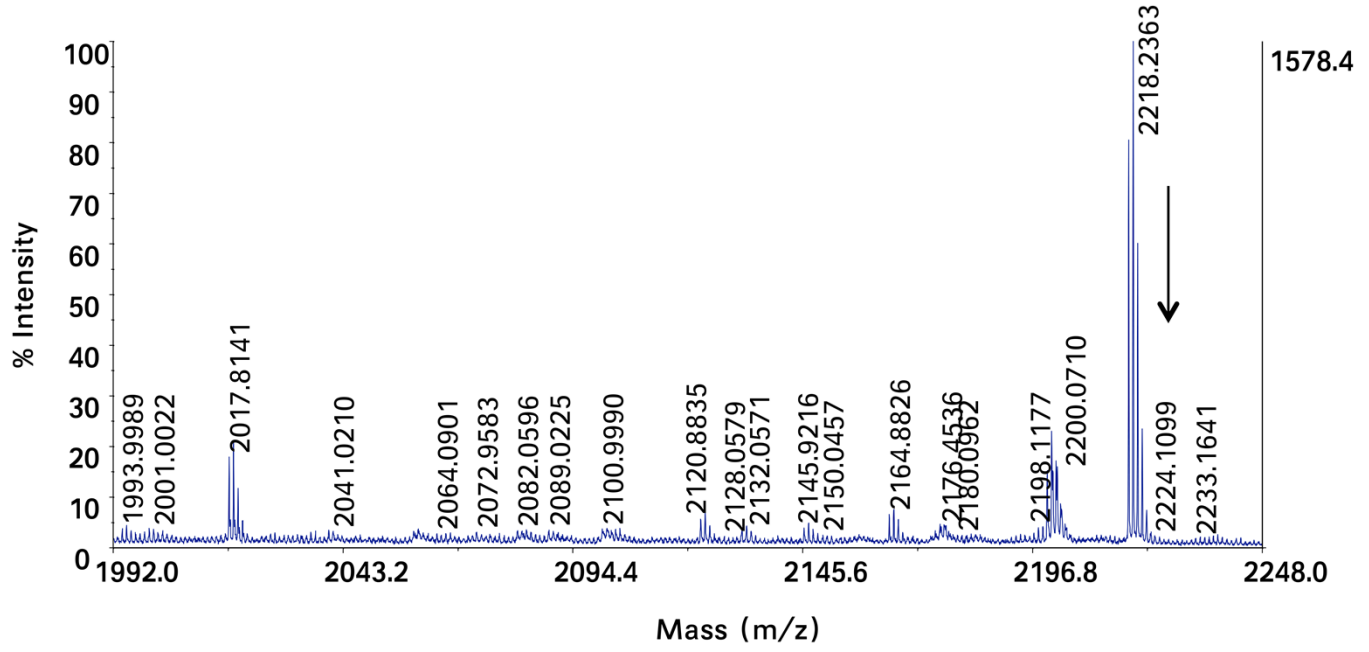
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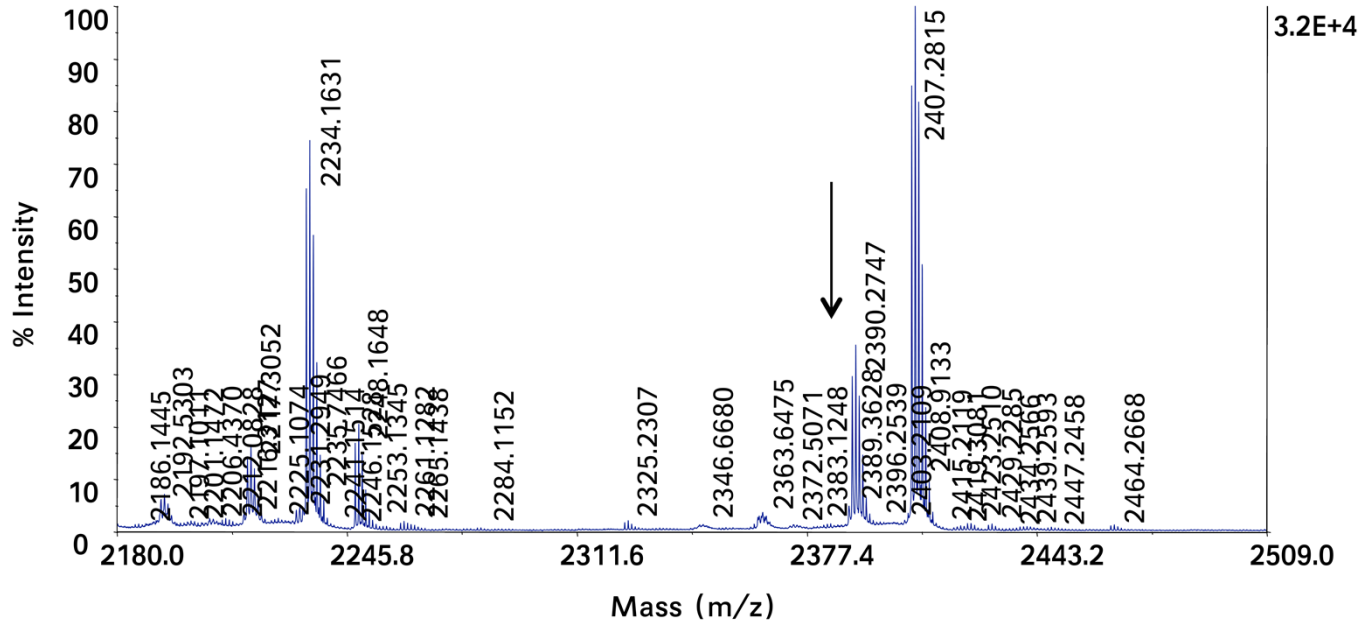
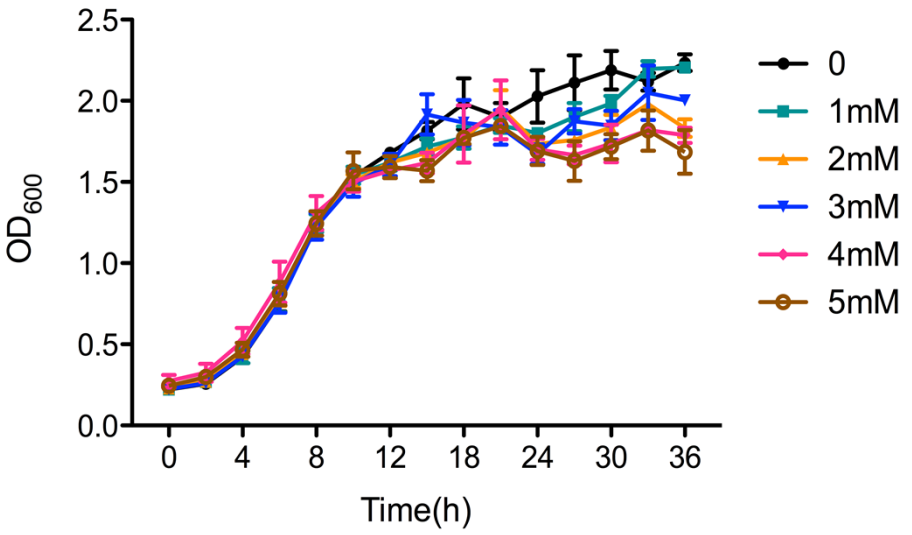
Mass (m/z)

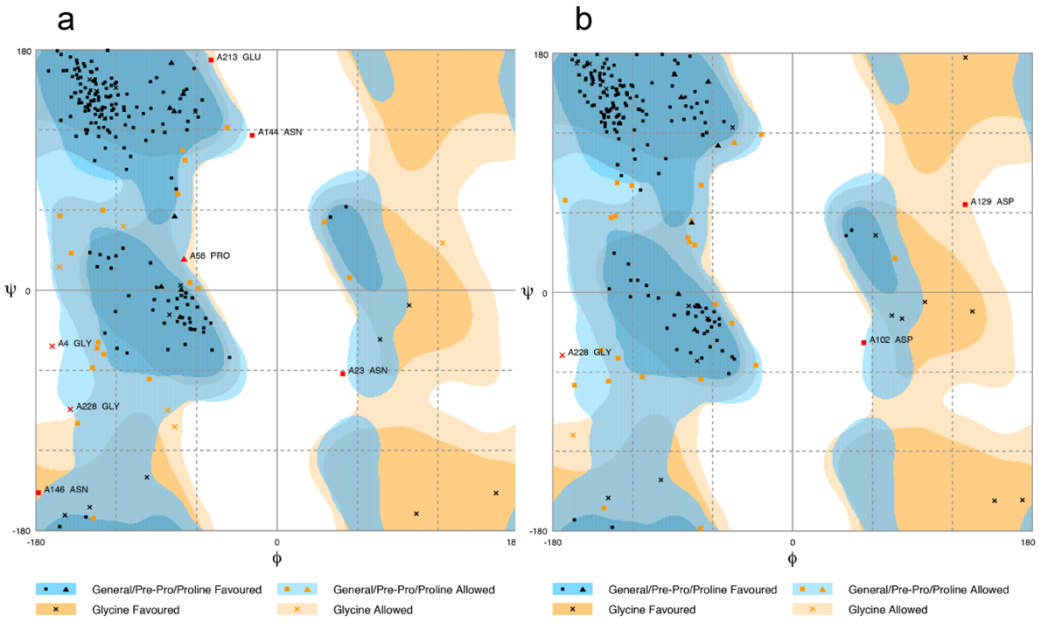
i



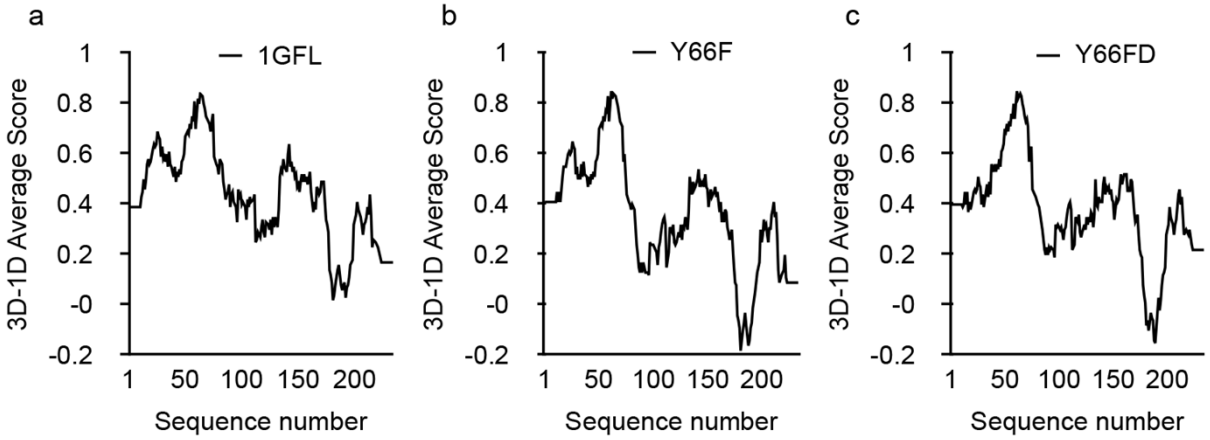
Supplementary Figure 1. MALDI-TOF/MS analysis of purified mutants containing D-amino acid at residue 18 after tryptic digestion. (**a**) Wild-type GFPuv; (**b**) GFPuv-S18^{D}F; (**c**) GFPuv-S18^{D}N; (**d**) GFPuv-S18^{D}M; (**e**) GFPuv-S18^{D}R; (**f**) GFPuv-S18^{D}P; (**g**) GFPuv-S18^{D}A; (**h**) GFPuv-S18^{D}V; (**i**) GFPuv-Y66^{D}F.



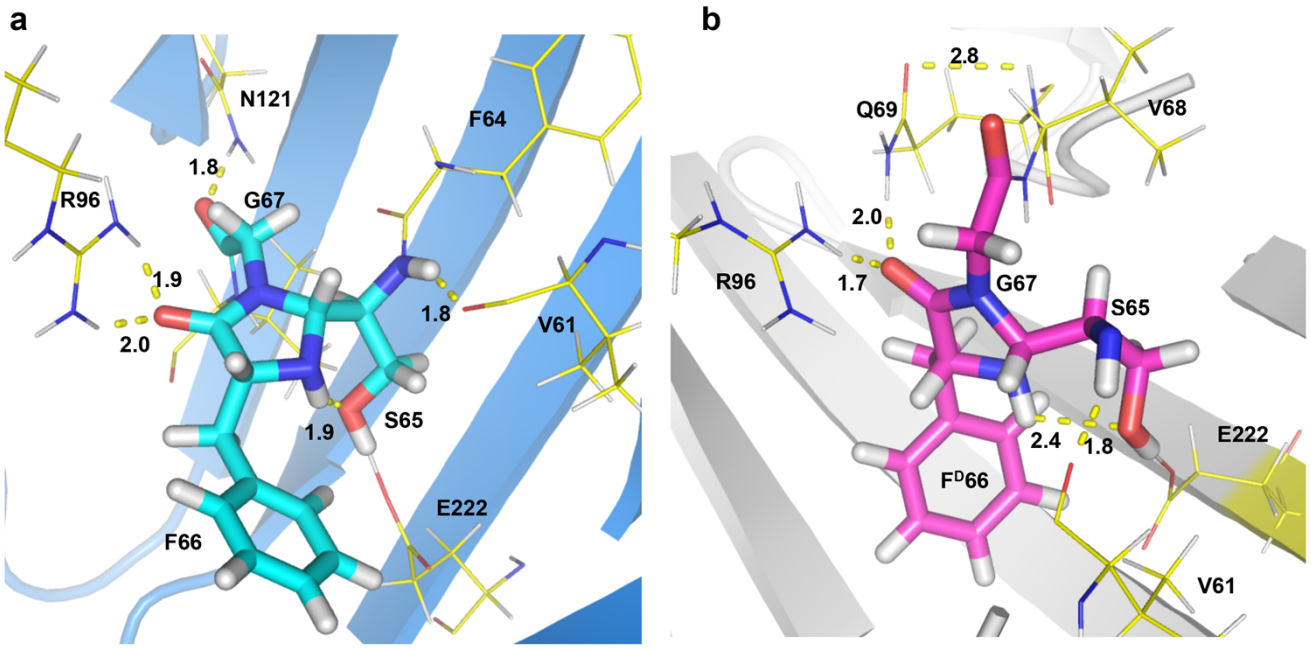
Supplementary Figure 2. Effects of D-Phe on the growth of engineered *E. coli* cells. The engineered *E. coli* containing pCNFRSII-tRNA and pET-GFPY66TAG grew in M9 media supplemented with 0, 1, 2, 3 4, 5 mM D-Phe, and then induced by 1 mM IPTG, 0.2% arabinose at 30℃ for 30 hours. The OD_{600} value of *E. coli* suspension was determined by spectrophotometer every 2 h over a period of 36 h (n=3).



Supplementary Figure 3. Ramachandran plots of GFPuv-Y66F and GFPuv-Y66^{D}F models. A: GFPuv-Y66F; GFPuv-Y66^{D}F.



Supplementary Figure 4. The VERIFY-3D scores of model structures. (a), Wild-type; (b), GFPuv-Y66F and (c) GFPuv-Y66^{D}F.



Supplementary Figure 5. Comparison of the interactions between the fluorophore and surrounding amino acids. Side chains are labeled with one-letter code for amino acid and the number of residue in yellow. Probable hydrogen bonds are shown as dotted lines labeled with the distance between the heteroatoms in angstroms. (**a**) Amino acid side chains in the immediate vicinity of the fluorophore forming residues S65-F66-G67 of GFP-Y66F mutant. Fluorophore is highlighted in cyan. (**b**) Amino acid side chains in the immediate vicinity of the fluorophore forming residues S65-^{D}F66-G67 of GFP-Y66^{D}F mutant. Fluorophore is highlighted in Magentas. Compare to GFPuv-Y66F, the hydrogen bond between C-O in Gly67 and -NH_2 in Asn121 in GFPuv-Y66^{D}F disappeared, and Gly67 turned to Val68 forming a hydrogen bond. Additionally, the carbonyl group in imidazolinone interacted with both Arg96 and Gln69 in GFPuv-Y66^{D}F, in which additional binding creates a more stable structure of fluorophore.