Monomer/Oligomer Quasi-racemic Protein Crystallography

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Supplementary Information

1. general information

A. Materials

2-Chlorotrityl Chloride Resin and Rink Amide-AM Resin were purchased from Tianjin Nankai Hecheng Science & Technology Co., Ltd (China). Fmoc-amino acids, Fmoc-D-amino acid and 1-hydroxy-7-azabenzotriazole (HOAt) were purchased from CSBio (Shanghai) Ltd, GL Biochem (Shanghai) Ltd. And BO MAI JIETECHNOLOGY CO, LTD. 4-mercaptophenylacetic acid (MPAA) was purchased from Alfa Aesar. Triisopropyldilsne (TIPS), N,N-Dimethylformamide (DMF), acetic acid (HPLC grade), thioanisole, trifluoroacetic acid (TFA, HPLC grade) and phenylsilane were purchased from J&K Chemical Ltd. (Shanghai). Ethyl cyanoglyoxylate-2-oxime, N,N-diisopropyl-carbodiimide (DIC), 1,2-ethanedithiol and N,N-diisopropylethylamine (DIEPA) were purchased from adamas-beta. Dithiothreitol (DTT) was purchased from Aladdin (Shanghai, China). Acetonitrile (HPLC grade) was purchase from J. T. Baker. $Na_2HPO_4 \cdot 12H_2O$, guanidine hydrochloride (Gn·HCl), and ether was purchase from Sinopharm Chemical Reagent Co., Ltd. Dichloromethane (DCM) and sodium nitrite ($NaNO_2$) were purchased from Beijing Chemical Industry Group CO., LTD. Glycyl 1-(2,4-dimethoxyphenyl)-2-mercaptoethyl auxiliary and Fmoc-N-(2,4-dimethoxybenzyl) glycine were purchased from Nantong peptide Biotech LTD (China). Liberty blue (automated microwave peptide synthesizer) was purchased from CEM corporation.

B. HPLC, Mass spectrometry and FPLC

Analytical RP-HPLC (SHIMADZU) was used to monitor the purity of crude peptide, chemical reaction progress and the purity of product with an analytical column (welch C18, 30 min, flow rate 1ml/min) at 214 nm and 254 nm. Semi-preparative HPLC (SHIMADZU) was used to separate and purify the crude peptide and reaction product with a semi preparative column (Grace Vydac C18, flow rate 5ml/min and welch XB-C18, flow rate 7ml/min) at 214 nm and 254 nm. Mobile

phase buffer A is acetonitrile (0.1% TFA) and Mobile phase buffer B deionized distilled water (0.1% TFA). Both buffers were sonicated for 30 min.

Crude peptide and reaction product were characterized by normal ESI mass spectra on LC/MS 2020 (SHIMADZU). And ultimate product was characterized by high-resolution ESI mass spectra on SYNAPTTM G2-Si HDMS.

Lyophilized polyubiquitin chains were dissolved in water contained 6 mol/L Gn HCl, 0.1 mM Na_2HPO_4, pH 3.0. Polyubiquitin chains were purified by AKTA (GE Healthcare Life Science) with Mono S cation exchange chromatography column and Superdex 75 column. Every injection was monitored at 280 nm and 214 nm.

2. Experimental figure

A. HPLC Chromatography and mass spectra

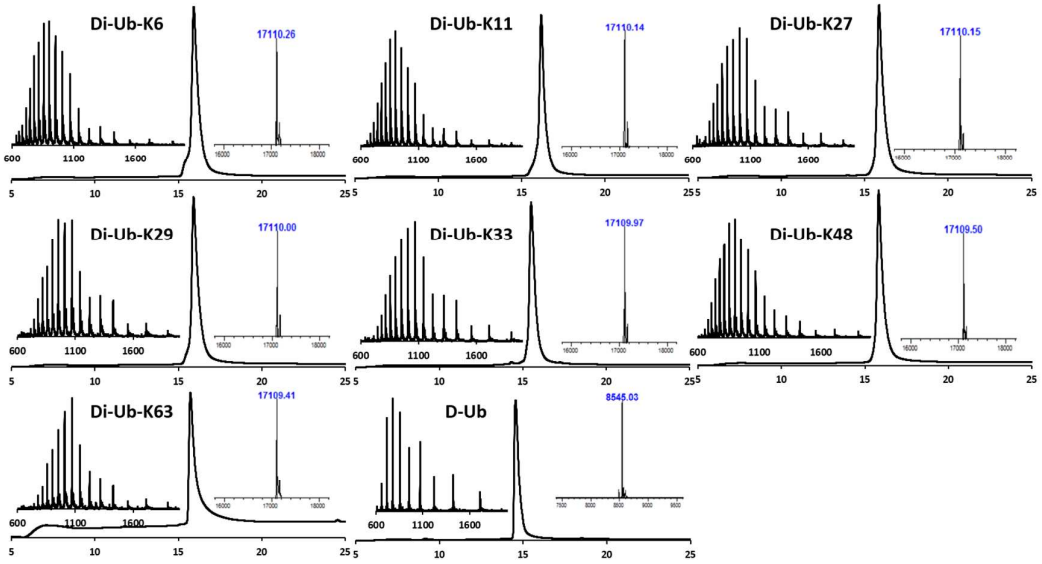

Figure S1. Analytical HPLC chromatograms of purified di-Ubs and D-Ub. Inset Electrospray ionization mass spectrum (**di-Ub-K6**: obs. 17110.3 Da, calc. 17110.6 Da; **di-Ub-K11**: obs. 17110.1 Da calc. 17110.6 Da; **di-Ub-K27**: obs. 17110.2 Da, calc. 17110.6 Da; **di-Ub-K29**: obs. 17110.0 Da, calc. 17110.6 Da; **di-Ub-K33**: obs. 17110.0 Da, calc. 17110.6 Da; **di-Ub-K48**: obs. 17109.5 Da, calc. 17110.6 Da; **di-Ub-K63**: obs. 17109.4 Da, calc. 17110.6 Da; **D-Ub**: obs. 8545.0 Da, calc. 8545.8 Da).

B Characterization of synthetic peptide segments of K11/K63-branched tri-Ub

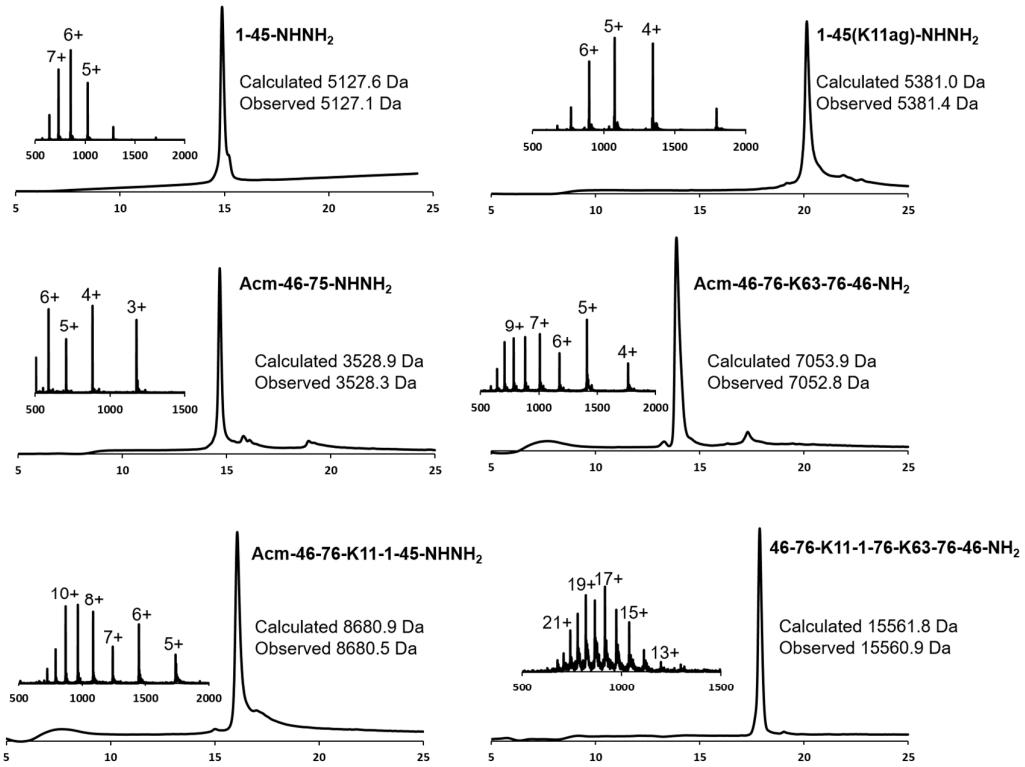

Figure S2. Analytical HPLC chromatograms of peptide segments of 11/63-branched tri-Ub. Inset Electrospray ionization mass spectrum (**1-45-NHNH$_2$**: obs. 5127.1 Da, calc. 5127.6 Da; **1-45(K11ag)-NHNH$_2$**: obs. 5381.4 Da calc. 5381.0 Da; **Acm-46-75-NHNH$_2$**: obs. 3528.3 Da, calc. 3528.9 Da; **Acm-46-76-K63-76-46-NH$_2$**: obs. 7052.8 Da, calc. 7053.9 Da; **Acm-46-76-K11-1-45-NHNH$_2$**: obs. 8680.5 Da, calc. 8680.9 Da; **46-76-K11-1-76-K63-76-46-NH$_2$**: obs. 15560.9 Da, calc. 15561.8 Da).

C. Crystals of di-ubiquitins

Figure S3. Di-ubiquitins crystals.

D. Number of crystallization conditions

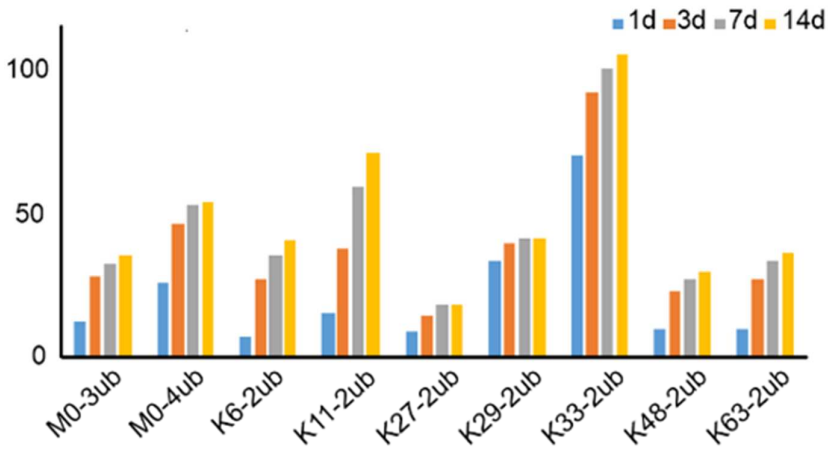

Figure S4. Number of crystallization conditions.

E. Static light scattering (SLS) and analytical ultracentrifugation (AUC) experiments

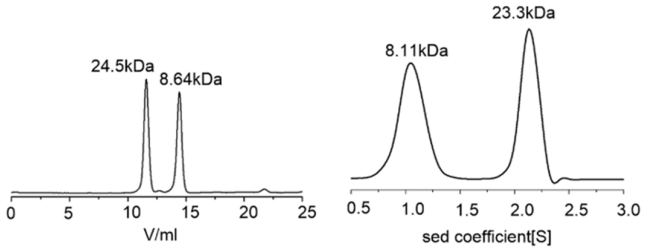

Figure S5. No interaction was found between linear tri-Ub and D-mono-Ub in the solution through static light scattering and analytical ultracentrifugation.

Supplementary Table 1 Data collection and refinement statistics

Crystal	K6-linked di-Ub	K11-linked di-Ub
Data collection		
Space group	P1	P1
Unit cell	24.730, 26.380, 45.120; 98.07,93.85,106.54	26.523, 26.733, 43.768; 75.17, 76.00, 80.96
Resolution(Å)	44~1.16 (1.20~1.16)	26~1.73 (1.8~1.73)
R_{merge}	0.066(0.749)	0.061(0.176)
I /σI	11.25(1.74)	8.43(2.41)
Completeness (%)	62.8(8.6)	68.2(5.4)
Redundancy	3.54(3.09)	1.8(1.3)
No. reflections	23459	7976
Wilson B-factor ($Å^2$)	10.0	13.8
Refinement		
R_{work} / R_{free}	0.1912/0.2285	0.1868/0.2355
No. atoms		
Protein	1194	1164
Water	191	165
R.m.s. deviations		
Bond lengths (Å)	0.005	0.007
Bond angles (°)	1.014	1.084
Ramachandran plot statistics (%)		
Most favourable	100%	100%
Disallowed	0%	0%
Crystallization condition	0.2M Magnesium chloride hexahydrate, 20%PEG3350 pH5.9	0.2M Li2SO4, 0.1M tris 8.5, 30%PEG4000

Crystal	K27-linked di-Ub	K29-linked di-Ub
Data collection		
Space group	P1	P2
Unit cell	38.240, 38.260, 48.000; 98.15, 98.15, 109.67	28.027, 46.497, 43.157; 90.00, 97.74, 90.00
Resolution(Å)	35~1.15 (1.19~1.15)	46~1.98 (2.05~1.98)
R_{merge}	0.034(0.604)	0.117(0.382)
I /σI	11.42(1.21)	7.31(1.40)
Completeness (%)	78.6(47.0)	78.8(44.6)
Redundancy	1.93(1.90)	2.5(1.5)
No. reflections	67778	5292
Wilson B-factor ($Å^2$)	11.6	28.0
Refinement		
R_{work} / R_{free}	0.2021/0.2215	0.2451/0.3297
No. atoms		
Protein	2404	1153
Water	340	54
R.m.s. deviations		
Bond lengths (Å)	0.006	0.019
Bond angles (°)	0.980	1.847
Ramachandran plot statistics (%)		
Most favourable	98%	96%
Disallowed	1%	0%
Crystallization condition	0.2M Magnesium acetate tetrahydrate, 0.1M Sodium cacodylate trihydrate pH6.5, 20%PEG 8000	0.2MPotassium sulfate 20%PEG3350

Crystal	K33-linked di-Ub	K48-linked di-Ub
Data collection		
Space group	P1	P1
Unit cell	26.436, 29.200, 44.232; 83.14, 86.82, 71.18	27.969, 40.834, 52.395; 98.469, 101.190, 105.937
Resolution(Å)	27~1.95 (2.02~1.95)	38~1.59 (1.65~1.59)
R_{merge}	0.076(0.291)	0.089(1.086)
I /σI	16.00(3.61)	4.00(0.64)
Completeness (%)	93.2(70.5)	79.9(67.3)
Redundancy	3.4(2.9)	1.69(1.62)
No. reflections	8490	23422
Wilson B-factor ($Å^2$)	22.1	17.6
Refinement		
R_{work} / R_{free}	0.2359/0.2713	0.2642/0.3106
No. atoms		
Protein	1183	2407
Water	139	221
R.m.s. deviations		
Bond lengths (Å)	0.009	0.004
Bond angles (°)	1.095	0.783
Ramachandran plot statistics (%)		
Most favourable	96%	99%
Disallowed	1%	0%
Crystallization condition	0.2M Magnesium sulfate heptahydrate, 20%PEG 3350	0.1M Sodium citrate tribasic dehydrate pH5.6, 20% 2-Propanol, 20%PEG 4000

Crystal	K63-linked di-Ub	K11/K63-branched tri-Ub
Data collection		
Space group	$P2_12_12$	$P2_12_12_1$
Unit cell	40.456, 79.268, 35.819; 90.000, 90.000, 90.000	50.073, 52.911, 58.230; 90.00, 90.00, 90.00
Resolution(Å)	36~1.55 (1.61~1.55)	31~1.84 (1.91~1.84)
R_{merge}	0.076(2.126)	0.124(0.490)
I /σI	11.07(0.57)	8.36(0.65)
Completeness (%)	98.9(98.4)	77.1(10.6)
Redundancy	6.20(5.58)	3.2(1.7)
No. reflections	17087	10779
Wilson B-factor ($Å^2$)	25.6	24.8
Refinement		
R_{work} / R_{free}	0.2490/0.2528	0.2208/0.2452
No. atoms		
Protein	1197	1179
Water	99	131
R.m.s. deviations		
Bond lengths (Å)	0.006	0.003
Bond angles (°)	0.999	0.753
Ramachandran plot statistics (%)		
Most favourable	99%	99%
Disallowed	1%	0%
Crystallization condition	0.1M TRIS hydrochloride pH8.5, 2.0M Ammonium phosphate monobasic	0.2M $MgSO_4$, 20%PEG3350, 4mM CdCl2，pH6.0

Crystal	linear tri-Ub	linear tetra-Ub
Data collection		
Space group	P1	$P2_1$
Unit cell	30.955, 31.123, 32.180;	29.436, 56.462, 38.532;
	71.54, 77.42, 89.43	90.000, 90.915, 90.000
Resolution(Å)	30~1.80 (1.87~1.80)	24~2.21 (2.29~2.21)
R_{merge}	0.207(0.426)	0.150(0.288)
I /σI	4.2(2.4)	7.73(1.36)
Completeness (%)	74.7(14.5)	56.2(7.1)
Redundancy	1.7(1.3)	3.5(1.4)
No. reflections	6648	3575
Wilson B-factor ($Å^2$)	6.5	29.2
Refinement		
R_{work} / R_{free}	0. 2258/0.3148	0.2220/ 0.3222
No. atoms		
Protein	1175	1160
Water	105	35
R.m.s. deviations		
Bond lengths (Å)	0.008	0.012
Bond angles (°)	1.116	1.337
Ramachandran plot statistics (%)		
Most favourable	99%	96%
Disallowed	0%	1%
Crystallization condition	0.2M MgSO4, 20%PEG3350, 4mM CdCl2 ，pH6.0	0.2M Sodium acetate trihydrate, 20%PEG3350, pH8.0