

Supplemental Table 2a. Crystallization, Data Collection and Refinement Statistics

	Rabenosyn₄₄₀₋₅₀₃ Rab4A Q67L	Rabenosyn₇₂₈₋₇₈₄ Rab22A Q64L	Rabenosyn₄₅₈₋₅₀₃
Crystallization			
Precipitation solution	20% PEG 4000, 200mM NH ₄ F, 50mM MES, pH 6.0	10% PEG 6000, 50mM Na acetate, pH 5.0	10% PEG 6000, 50mM Na acetate, pH 5.0
Seeding	none	Microseeded	Microseeded
Temperature (°C)	18	4	4
Data Collection			
X-ray source	RUH3R / MAR 300	NLSL X25	RUH3R / MAR 300
Resolution (Å)	20-1.90	20-1.30	20-1.50
Space group	P3 ₁ 21	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
Unit cell			
a, b, c (Å)	81.44, 81.44, 137.846	44.825, 55.515, 85.027	26.366, 42.236, 43.059
α, β, γ (°)	90, 90, 120	90, 90, 90	90, 90, 90
No. of mol. in AU	2	1	1
Completeness ^a	99.6 (98.9)	85.1 (48.7)	93.0 (79.5)
<I>/σ(I) ^a	27.3 (4.0)	54.1 (3.8)	19.5 (5.8)
R _{sym} ^{a, b}	0.054 (0.379)	0.038 (0.381)	0.070 (0.221)
Structure Determination and Refinement			
Search model	Rab4A	Rab22A	polyalanine-gp41 (PDB ID, 1AIK.pdb)
R Factor	0.223	0.169	0.195
R _{free}	0.258	0.208	0.209
RMS deviations			
Bond length (Å)	0.008	0.006	0.007
Bond angle (°)	1.302	1.110	0.893

^a Values in parentheses represent the highest resolution shell

$$^b R_{\text{sym}} = \frac{\sum_h \sum_j |I_j(h) - \langle I(h) \rangle|}{\sum_h \sum_j I_j(h)}$$

Supplemental Table 2b. Crystallization, Data Collection and Refinement Statistics

	Ypt1p GppNHp	Rab4A GppNHp	Rab6A GppNHp
Crystallization			
Precipitation solution	30% MPD, 10% glycerol, 100mM MES, pH 6.5	1.6M (NH ₄) ₂ SO ₄ , 20% Glycerol, 50mM MES, pH 6.0	20% PEG 6000, 300mM MgCl ₂ , 10% Glycerol, 50mM HEPES, pH 7.0
Seeding	none	none	none
Temperature (°C)	18	4	4
Data Collection			
X-ray source	RUH3R / MAR 300	RUH3R / MAR 300	RUH3R / MAR 300
Resolution (Å)	20-2.05	20-2.05	20-1.78
Space group	P2 ₁ 2 ₁ 2 ₁	P4 ₃ 2 ₁ 2	P2 ₁ 2 ₁ 2 ₁
Unit cell			
a, b, c (Å)	39.011, 56.930, 74.720	68.808, 68.808, 90.72	40.495, 68.072, 73.102
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90
No. of mol. in AU	1	1	1
Completeness ^a	99.2 (99.7)	98.9 (96.6)	99.4 (95.1)
<I>/σ(I) ^a	18.1 (4.7)	15.1 (4.1)	27.7 (6.2)
R _{sym} ^{a, b}	0.068 (0.293)	0.078 (0.282)	0.058 (0.282)
Structure Determination and Refinement			
Search model	polyalanine Rab3A	polyalanine Rab3A	polyalanine Rab3A
R Factor	0.192	0.133	0.215
R _{free}	0.252	0.182	0.249
RMS deviations			
Bond length (Å)	0.011	0.019	0.010
Bond angle (°)	1.414	1.807	1.287

^a Values in parentheses represent the highest resolution shell

^b $R_{\text{sym}} = \sum_h \sum_j |I_j(h) - \langle I(h) \rangle| / \sum_h \sum_j I_j(h)$

Supplemental Table 2c. Crystallization, Data Collection and Refinement Statistics

	Rab9 GppNHp	Rab11A GppNHp	Rab22A GppNHp
Crystallization			
Precipitation solution	20% PEG 6000, 200mM MgCl ₂ , 50mM MES, pH 6.0	1.4M NaCl, 20% Glycerol 50mM MES, pH 6.0	20% PEG 6000, 10% Glycerol 1M LiCl ₂ , 50mM MES, pH 6.0
Seeding	none	none	Microseeded
Temperature (°C)	4	4	4
Data Collection			
X-ray source	RUH3R / MAR 300	RUH3R / MAR 300	RUH3R / MAR 300
Resolution (Å)	20-1.85	20-2.0	20-1.90
Space group	P3 ₂ 21	I422	P4 ₃ 2 ₁ 2
Unit cell			
a, b, c (Å)	68.325, 68.325, 83.229	44.825, 55.515, 85.027	45.455, 45.455, 150.146
α, β, γ (°)	90, 90, 120	90, 90, 90	90, 90, 90
No. of mol. in AU	1	1	1
Completeness ^a	99.2 (98.5)	99.8 (97.8)	99.9 (99.2)
<I>/σ(I) ^a	26.2 (5.0)	31.6 (7.6)	33.9 (10.4)
R _{sym} ^{a, b}	0.06 (0.394)	0.058 (0.252)	0.086 (0.224)
Structure Determination and Refinement			
Search model	polyalanine Rab4A	polyalanine Rab5C	polyalanine Rab3A
R Factor	0.191	0.176	0.187
R _{free}	0.221	0.222	0.241
RMS deviations			
Bond length (Å)	0.008	0.007	0.006
Bond angle (°)	1.197	1.065	1.051

^a Values in parentheses represent the highest resolution shell

^b $R_{\text{sym}} = \sum_h \sum_j |I_j(h) - \langle I(h) \rangle| / \sum_h \sum_j I_j(h)$

Supplemental Table 2d. Crystallization, Data Collection and Refinement Statistics

	Rab21 GppNHp	Rab21 GppNHp	Rab33 GppNHp
Crystallization			
Precipitation solution	15% PEG 4000, 10% Glycerol, 200mM NaSCN, 50mM MES, pH 6.5	18% PEG 6000, 200mM LiSO ₄ , 50mM Tris, pH 8.0	10% PEG 4000, 10% Glycerol, 150mM LiSO ₄ , 50mM MES, pH 6.0
Seeding	Microseeded	Microseeded	none
Temperature (°C)	4	4	4
Data Collection			
X-ray source	RUH3R / MAR 300	RUH3R / MAR 300	RUH3R / MAR 300
Resolution (Å)	20-2.5	20-2.05	20-1.80
Space group	I2 ₁ 2 ₁ 2 ₁	C2	P3 ₂
Unit cell			
a, b, c (Å)	77.834, 99.719, 105.760	130.357, 36.567, 86.666	56.194, 56.194, 55.039
α, β, γ (°)	90, 90, 90	90, 113.404, 90	90, 90, 120
No. of mol. in AU	2	2	1
Completeness ^a	95.6 (99.2)	99.9 (99.6)	96.7 (84.2)
<I>/σ(I) ^a	21.2 (4.8)	15.8 (3.8)	29.0 (7.7)
R _{sym} ^{a, b}	0.067 (0.387)	0.080 (0.368)	0.057 (0.249)
Structure Determination and Refinement			
Search model	polyalanine Rab3A	polyalanine Rab3A	polyalanine Rab5C
R Factor	0.225	0.228	0.169
R _{free}	0.282	0.268	0.216
RMS deviations			
Bond length (Å)	0.006	0.009	0.021
Bond angle (°)	0.992	1.238	1.923

^a Values in parentheses represent the highest resolution shell

^b $R_{\text{sym}} = \frac{\sum_h \sum_j |I_j(h) - \langle I(h) \rangle|}{\sum_h \sum_j I_j(h)}$

Supplemental Table 2e. Crystallization, Data Collection and Refinement Statistics

	Rab21 Q53G GppNHp	Rab5C G55Q GppNHp
Crystallization		
Precipitation solution	20% PEG 4000, 200mM Mg(CH ₃ COO) ₂ , 50mM HEPES, pH 7.0	10% PEG 6000, 50mM MES, pH 6.0
Seeding	none	none
Temperature (°C)	4	4
Data Collection		
X-ray source	RUH3R / MAR 300	RUH3R / MAR 300
Resolution (Å)	20-1.8	20-1.80
Space group	C2	P2 ₁ 2 ₁ 2 ₁
Unit cell		
a, b, c (Å)	91.200, 77.425, 107.965	36.317, 64.008, 66.314
α, β, γ (°)	90.0, 89.8, 90.0	90, 90, 90
No. of mol. in AU	4	1
Completeness ^a	98.4 (97.6)	97.1 (73.4)
$\langle I \rangle / \sigma(I)$ ^a	14.4 (3.4)	16.0 (5.0)
R _{sym} ^{a, b}	0.064 (0.311)	0.058 (0.229)
Structure Determination and Refinement		
Search model	polyalanine Rab3A	polyalanine Rab5C
R Factor	0.172	0.161
R _{free}	0.218	0.209
RMS deviations		
Bond length (Å)	0.005	0.009
Bond angle (°)	1.025	1.147

^a Values in parentheses represent the highest resolution shell

^b $R_{\text{sym}} = \sum_h \sum_j |I_j(h) - \langle I(h) \rangle| / \sum_h \sum_j I_j(h)$

Supplemental Table 2f. Crystallization, Data Collection and Refinement Statistics

	Rab2A GDP	Rab5C GDP	Rab14 GDP
Crystallization			
Precipitation solution	15% PEG 4000, 400mM LiSO ₄ , 100mM Citrate, pH 5.5	10% PEG 4000, 200mM LiSO ₄ 50mM NaAcetate, pH 4.5	7% PEG 6000, 100mM CaCl ₂ 50mM HEPES, pH 7.0
Seeding	Microseeded	Microseeded	Microseeded
Temperature (°C)	18	18	18
Data Collection			
X-ray source	NLSL X25	RUH3R / MAR 300	RUH3R / MAR 300
Resolution (Å)	20-2.12	20-2.20	20-2.05
Space group	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2 ₁	P2 ₁
Unit cell			
a, b, c (Å)	98.773, 128.473, 68.236	38.401, 70.931, 137.610	43.065, 40.780, 47.678
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 108.446, 90
No. of mol. in AU	4	2	1
Completeness ^a	98.7 (99.2)	98.6 (97.3)	97.4 (84.9)
<I>/σ(I) ^a	32.9 (3.7)	17.0 (4.0)	16.1 (3.6)
R _{sym} ^{a, b}	0.069 (0.377)	0.088 (0.407)	0.122 (0.386)
Structure Determination and Refinement			
Search model	polyalanine Rab4A	polyalanine Rab3A	polyalanine Rab4A
R Factor	0.226	0.181	0.201
R _{free}	0.265	0.235	0.255
RMS deviations			
Bond length (Å)	0.006	0.008	0.008
Bond angle (°)	0.972	1.228	1.105

^a Values in parentheses represent the highest resolution shell

^b $R_{\text{sym}} = \frac{\sum_h \sum_j |I_j(h) - \langle I(h) \rangle|}{\sum_h \sum_j I_j(h)}$

Supplemental Table 2g. Crystallization, Data Collection and Refinement Statistics

	Rab21 GDP	Rab23 GDP	Rab23 GDP
Crystallization			
Precipitation solution	20% PEG 6000, 10% Glycerol, 200mM (NH ₄) ₂ SO ₄ , 50mM Na acetate, pH 4.5	27% PEG 6000, 200mM Citrate, 50mM MES, pH 6.0	15% PEG 6000, 50mM CH ₃ COONa 50mM NaAcetate, pH 5.0
Seeding	Microseeded	Microseeded	Microseeded
Temperature (°C)	4	4	4
Data Collection			
X-ray source	RUH3R / MAR 300	RUH3R / MAR 300	RUH3R / MAR 300
Resolution (Å)	20-2.30	20-2.06	20-1.90
Space group	C222 ₁	C222 ₁	P2 ₁ 2 ₁ 2 ₁
Unit cell			
a, b, c (Å)	36.295, 88.861, 118.030	67.010, 71.333, 65.684	40.281, 61.640, 63.850
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90
No. of mol. in AU	1	1	1
Completeness ^a	97.7 (96.2)	98.9 (89.2)	95.6 (79.4)
<I>/σ(I) ^a	17.4 (4.3)	16.4 (4.3)	24.5 (5.3)
R _{sym} ^{a, b}	0.159 (0.448)	0.097(0.306)	0.044 (0.268)
Structure Determination and Refinement			
Search model	polyalanine Rab21	polyalanine Rab3A	polyalanine Rab3A
R Factor	0.249	0.179	0.210
R _{free}	0.276	0.224	0.261
RMS deviations			
Bond length (Å)	0.008	0.009	0.011
Bond angle (°)	1.178	1.205	1.232

^a Values in parentheses represent the highest resolution shell

^b $R_{\text{sym}} = \frac{\sum_h \sum_j |I_j(h) - \langle I(h) \rangle|}{\sum_h \sum_j I_j(h)}$