Supplementary Table 1 Summary of sigma-scaled anomalous peak heights from methionine sulfur atoms and assigned iodine signals after refinement

Spy:Im7 E12I (crystal 1)		**Spy:Im7 E12I (crystal 2)**		**Spy:Im7 A13I**			
Median Methionine S	3.9	Median Methionine S	3.35	Median Methionine S	4		
Iodine(s)	4.8	Iodine(s)	3.8	Iodine(s)	4.1		
			3.7		4.1		
Spy:Im7 E14I		**Spy:Im7 Q17I**		**Spy:Im7 L19I (crystal 1)**		**Spy:Im7 L19I (crystal 2)**	
Median Methionine S	3.5	Median Methionine S	3.15	Median Methionine S	3.5	Median Methionine S	3.1
Iodine(s)	3.3	Iodine(s)	3.5	Iodine(s)	3.3	Iodine(s)	3.0
	4.7		3.4		6.1		3.4
	3.4		3.3		3.5		3.4
	4.4						3.0
	3.5						4.6
							3.8
Spy:Im7 E21I (crystal 1)		**Spy:Im7 E21I (crystal 2)**		**Spy:Im7 V27I**		**Spy:Im7 A28I**	
Median Methionine S	3.95	Median Methionine S	3.6	Median Methionine S	3.7	Median Methionine S	3.5
Iodine(s)	4.4	Iodine(s)	3.9	Iodine(s)	4.4	Iodine(s)	3.1
	3.8						4.4
	3.7						3.6
							3.3
							3.2
							4.1
							3.5

Supplementary Table 2 Additional crystallography statistics

	Spy:Im7 A28I	Spy:Im7 A28I	Spy:Im7 V27I	Spy:Im7 V27I
Data collection				
Wavelength (Å)	1.90749	0.88554	1.90749	0.88553
Space group	$P4_122$	$P4_122$	$P4_122$	$P4_122$
Cell dimensions				
a, *b*, *c* (Å)	43.08, 43,08, 259.8	43.2, 43.2, 260.6	42.6, 42.6, 257.1	42.6, 42.6, 256.8
α,*β*,*γ* (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	52.97-2.70(2.83-2.70)	30.62-2.31(2.39-2.31)	42.60-2.91(3.08-2.91)	28.53-2.59(2.71-2.59)
R_{merge} (%)	16.8(29.7)	11.8(23.1)	5.1(17.3)	5.0(14)
$I/\sigma(I)$	10.0(8.7)	14.7(9.0)	15.9(6.8)	22.6(10.5)
Completeness (%)	100(100)	100(100)	97.7(94.9)	95.2(87.2)
Redundancy	11.7(11.8)	13.4(13.2)	4.9(4.7)	7.4(7.7)

	Spy:Im7 E12I (crystal 2)	Spy:Im7 E12I (crystal 2)
Data collection		
Wavelength (Å)	1.90749	0.88553
Space group	$P4_122$	$P4_122$
Cell dimensions		
a, *b*, *c* (Å)	42.8, 42.8 257.9	42.9, 42.9, 258.8
α,*β*,*γ* (°)	90, 90, 90	90, 90, 90
Resolution (Å)	64.48-2.91(3.08-2.91)	29.53-2.59(2.71-2.59)
R_{merge} (%)	6.1(17)	5.5(19.6)
$I/\sigma(I)$	20.0(10.4)	17.8(6.9)
Completeness (%)	99.8(99.6)	99.7(99.3)
Redundancy	8.2(8.1)	6.0(5.5)

	Spy:Im7 A13I	Spy:Im7 A13I	Spy:Im7 E14I	Spy:Im7 E14I
Data collection				
Wavelength (Å)	1.90748	0.88554	1.90749	0.88553
Space group	$P4_122$	$P4_122$	$P4_122$	$P4_122$
Cell dimensions				
a, *b*, *c* (Å)	43.1, 43.1, 259.9	43.1, 43.1, 259.8	42.8, 42.8, 256.7	42.9, 42.9, 257.5
α,*β*,*γ* (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	51.97-2.80(2.95-2.80)	32.47-2.82(2.97-2.82)	64.19-2.91(3.08-2.91)	28.61-2.59(2.71-2.59)
R_{merge} (%)	5.3(11.5)	5.1(11.2)	6.4(26.8)	7.0(40.1)
$I/\sigma(I)$	30.6(18.5)	30.6(18.7)	18.7(6.6)	16.1(4.6)
Completeness (%)	99.9(100)	99.5(98.9)	95.2(89.6)	94.8(88.7)
Redundancy	12.6(12.6)	11.9(11.8)	8.0(8.4)	7.7(8.0)

	Spy:Im7 E12I (crystal 1)	Spy:Im7 E12I (crystal 1)	Spy:Im7 Q17I	Spy:Im7 Q17I
Data collection				
Wavelength (Å)	1.90749	0.88553	1.90748	0.88553
Space group	$P4_122$	$P4_122$	$P4_122$	$P4_122$
Cell dimensions				
a, *b*, *c* (Å)	42.7, 42.7, 257.5	42.7, 42.7, 257.8	42.7, 42.7, 257.3	42.7, 42.7, 257.6
α,*β*,*γ* (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	42.71-2.91(3.08-2.91)	29.41-2.59(2.71-2.59)	64.32-2.91(3.08-2.91)	28.47-2.59(2.71-2.59)
R_{merge} (%)	5.1(11.3)	6.2(16.5)	6.7(22.3)	5.7(38.4)
$I/\sigma(I)$	24.2(14.8)	18.8(10.2)	21.0(8.3)	14.4(3.6)
Completeness (%)	90.0(92.7)	88.7(90.6)	94.7(87.7)	93.4(87.0)
Redundancy	7.3(6.8)	7.7(7.1)	8.0(8.6)	5.3(5.4)

	Spy:Im7 L19I (crystal 1)	Spy:Im7 L19I (crystal 1)	Spy:Im7 L19I (crystal 2)	Spy:Im7 L19I (crystal 2)
Data collection				
Wavelength (Å)	1.90749	0.88553	1.90749	0.88553
Space group	$P4_122$	$P4_122$	$P4_122$	$P4_122$
Cell dimensions				
a, *b*, *c* (Å)	42.9, 42.9, 257.3	42.9, 42.9, 257.9	42.9, 42.9, 257.3	42.9, 42.9, 257.9
α,*β*,*γ* (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	64.32-2.91(3.08-2.91)	28.62-2.59(2.71-2.59)	64.32-2.91(3.08-2.91)	30.38-2.59(2.71-2.59)
R_{merge} (%)	7.0(16.9)	7.4(31.0)	7.7(26.1)	10.0(71.8)
$I/\sigma(I)$	19.1(10.2)	17.1(6.2)	16.7(7.1)	12.3(2.7)
Completeness (%)	100(100)	95.6(96.8)	100(100)	99.9(100)
Redundancy	8.5(8.5)	9.2(8.8)	8.4(8.6)	8.0(8.2)

	Spy:Im7 E21I (crystal 1)	Spy:Im7 E21I (crystal 1)	Spy:Im7 E21I (crystal 2)	Spy:Im7 E21I (crystal 2)
Data collection				
Wavelength (Å)	1.90749	0.88553	1.90749	0.88553
Space group	$P4_122$	$P4_122$	$P4_122$	$P4_122$
Cell dimensions				
a, *b*, *c* (Å)	42.7, 42.7, 256.8	42.7, 42.7, 257.6	42.8, 42.8, 257.6	42.8, 42.8, 258.2
α,β,γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	42.19-2.91(3.08-2.91)	30.28-2.59(2.71-2.59)	64.39-2.91(3.08-2.91)	32.27-2.59(2.71-2.59)
R_{merge} (%)	4.8(7.9)	4.7(15.2)	6.3(13.6)	6.6(15.9)
$I/\sigma(I)$	28.9(19.6)	25.6(12.6)	20.3(11.0)	19.4(10.1)
Completeness (%)	100(100)	99.9(100)	99.8(99.3)	99.9(100)
Redundancy	8.6(8.7)	8.8(8.9)	8.1(7.9)	8.6(8.5)

Supplementary Table 3 Strains and plasmids

Strain	Genotype or relevant characteristics	Source
SQ765	MG1655 (*F⁻ λ⁻ ilvG⁻ rfb-50 rph-1*), *ΔhsdR*	[19]
SQ1805	SQ765, pCDFTrc-ssIm7L53AI54A, pTrc-spy	[19]
SQ1809	SQ765, pCDFTrc-ssIm7L53AI54A	[19]
SQ2049	SQ765, pCDFTrc-ssIm7L53AI54A, pTrc-spy 33-130	This study
SQ2050	SQ765, pCDFTrc-ssIm7L53AI54A, pTrc-spy 30-130	This study
SQ2051	SQ765, pCDFTrc-ssIm7L53AI54A, pTrc-spy 27-130	This study
SQ2052	SQ765, pCDFTrc-ssIm7L53AI54A, pTrc-spy 24-130	This study
SQ2053	SQ765, pCDFTrc-ssIm7L53AI54A, pTrc-spy 21-130	This study

Plasmid	Relevant characteristics	Source
pTrc-spy	Spy cloned into pTrc99a derived vector pssTrx	[19]
pTrc-spy 33-130	Spy fragment (33-130) with native signal sequence cloned into pssTrx	This study
pTrc-spy 30-130	Spy fragment (30-130) with native signal sequence cloned into pssTrx	This study
pTrc-spy 27-130	Spy fragment (27-130) with native signal sequence cloned into pssTrx	This study
pTrc-spy 24-130	Spy fragment (24-130) with native signal sequence cloned into pssTrx	This study
pTrc-spy 21-130	Spy fragment (21-130) with native signal sequence cloned into pssTrx	This study