W O R L D W I D E

wwPDB

PROTEIN DATA BANK

Full wwPDB NMR Structure Validation Report (i)

May 28, 2020 – 11:41 pm BST

PDB ID	:	2MAK
Title	:	Solution structure of the STIM1 CC1-CC2 homodimer in complex with two Orai1 C-terminal domains.
Authors	:	Stathopulos, P.B.; Ikura, M.
Deposited on	:	2013-07-12

This is a Full wwPDB NMR Structure Validation Report for a publicly released PDB entry.

We welcome your comments at *validation@mail.wwpdb.org*
A user guide is available at
https://www.wwpdb.org/validation/2017/NMRValidationReportHelp
with specific help available everywhere you see the (i) symbol.

The following versions of software and data (see references (i)) were used in the production of this report:

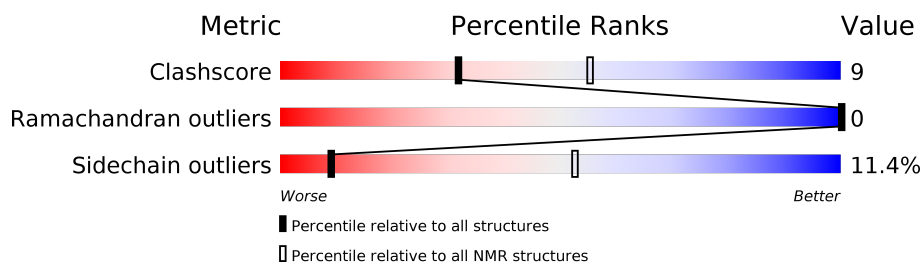|		
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	2.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.11

1 Overall quality at a glance ⓘ

The following experimental techniques were used to determine the structure:
SOLUTION NMR

The overall completeness of chemical shifts assignment is 42%.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.

Metric	Percentile Ranks	Value
Clashscore		9
Ramachandran outliers		0
Sidechain outliers		11.4%

Worse ⬤ Better

■ Percentile relative to all structures

▯ Percentile relative to all NMR structures

Metric	Whole archive (#Entries)	NMR archive (#Entries)
Clashscore	158937	12864
Ramachandran outliers	154571	11451
Sidechain outliers	154315	11428

The table below summarises the geometric issues observed across the polymeric chains and their fit to the experimental data. The red, orange, yellow and green segments indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria. A cyan segment indicates the fraction of residues that are not part of the well-defined cores, and a grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions <=5%

Mol	Chain	Length	Quality of chain
1	A	82	61% 20% • 18%
1	C	82	62% 17% • 18%
2	B	23	65% 26% 9%
2	D	23	61% 26% 13%

2 Ensemble composition and analysis (i)

This entry contains 20 models. Model 3 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative, based on the following criterion: *lowest energy*.

The following residues are included in the computation of the global validation metrics.

Well-defined (core) protein residues			
Well-defined core	Residue range (total)	Backbone RMSD (Å)	Medoid model
1	A:316-A:382, B:0-B:0, B:272-B:291, C:316-C:382, D:0-D:0, D:272-D:290 (175)	0.31	3

Ill-defined regions of proteins are excluded from the global statistics.

Ligands and non-protein polymers are included in the analysis.

The models can be grouped into 5 clusters and 3 single-model clusters were found.

Cluster number	Models
1	1, 2, 3, 4, 8, 12, 15
2	10, 18, 20
3	5, 9, 19
4	11, 14
5	16, 17
Single-model clusters	6; 7; 13

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 3454 atoms, of which 1714 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Stromal interaction molecule 1.

Mol	Chain	Residues	Atoms						Trace
1	A	82	Total	C	H	N	O	S	0
			1372	428	689	123	131	1	
1	C	82	Total	C	H	N	O	S	0
			1372	428	689	123	131	1	

There are 12 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-5	GLY	-	EXPRESSION TAG	UNP Q13586
A	-4	SER	-	EXPRESSION TAG	UNP Q13586
A	-3	HIS	-	EXPRESSION TAG	UNP Q13586
A	-2	MET	-	EXPRESSION TAG	UNP Q13586
A	-1	ALA	-	EXPRESSION TAG	UNP Q13586
A	0	SER	-	EXPRESSION TAG	UNP Q13586
C	-5	GLY	-	EXPRESSION TAG	UNP Q13586
C	-4	SER	-	EXPRESSION TAG	UNP Q13586
C	-3	HIS	-	EXPRESSION TAG	UNP Q13586
C	-2	MET	-	EXPRESSION TAG	UNP Q13586
C	-1	ALA	-	EXPRESSION TAG	UNP Q13586
C	0	SER	-	EXPRESSION TAG	UNP Q13586

- Molecule 2 is a protein called Calcium release-activated calcium channel protein 1.

Mol	Chain	Residues	Atoms					Trace
2	B	23	Total	C	H	N	O	0
			355	111	168	36	40	
2	D	23	Total	C	H	N	O	0
			355	111	168	36	40	

There are 4 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	-1	GLY	-	EXPRESSION TAG	UNP Q96D31
B	0	SER	-	EXPRESSION TAG	UNP Q96D31
D	-1	GLY	-	EXPRESSION TAG	UNP Q96D31
D	0	SER	-	EXPRESSION TAG	UNP Q96D31

# 4 Residue-property plots (i)

4.1 Average score per residue in the NMR ensemble

These plots are provided for all protein, RNA and DNA chains in the entry. The first graphic is the same as shown in the summary in section 1 of this report. The second graphic shows the sequence where residues are colour-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. Stretches of 2 or more consecutive residues without any outliers are shown as green connectors. Residues which are classified as ill-defined in the NMR ensemble, are shown in cyan with an underline colour-coded according to the previous scheme. Residues which were present in the experimental sample, but not modelled in the final structure are shown in grey.
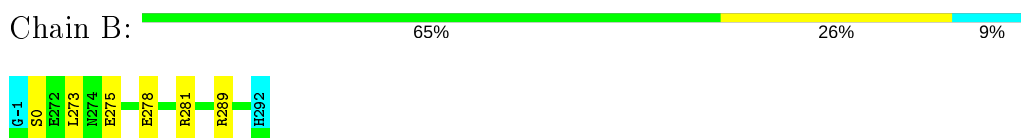
• Molecule 1: Stromal interaction molecule 1
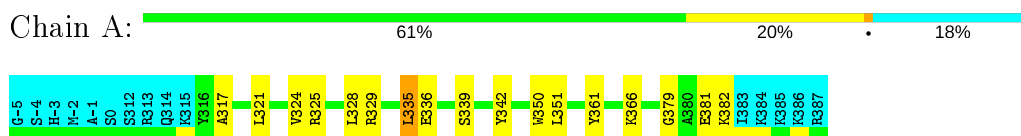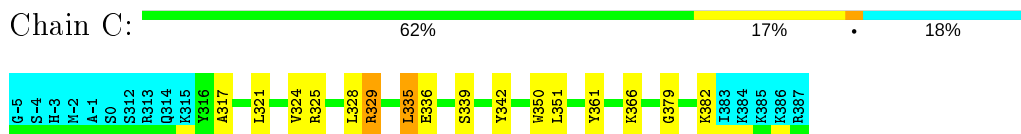
Chain A:

• Molecule 1: Stromal interaction molecule 1

Chain C:

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

• Molecule 2: Calcium release-activated calcium channel protein 1
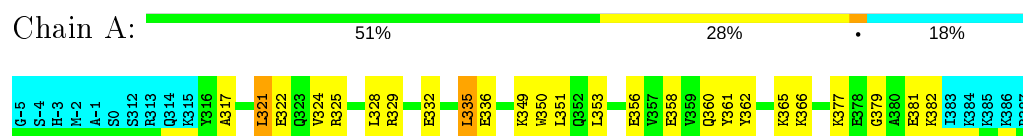
Chain D:

4.2 Scores per residue for each member of the ensemble
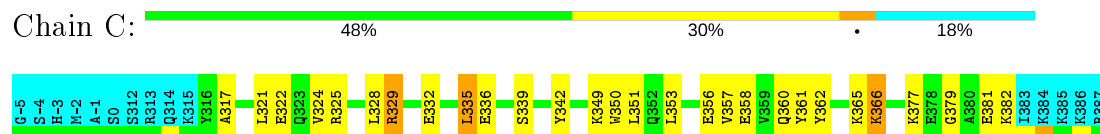
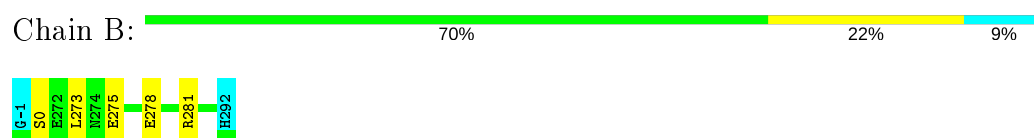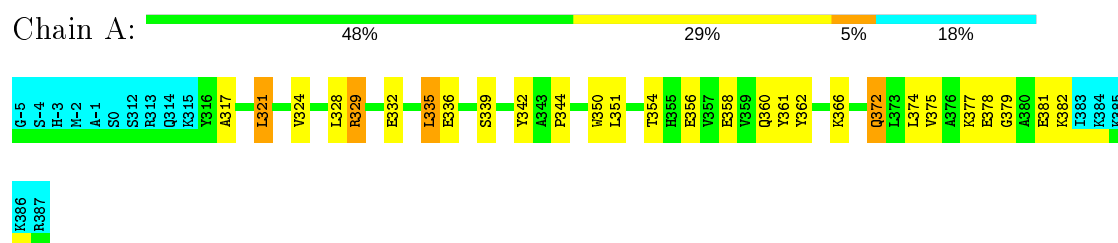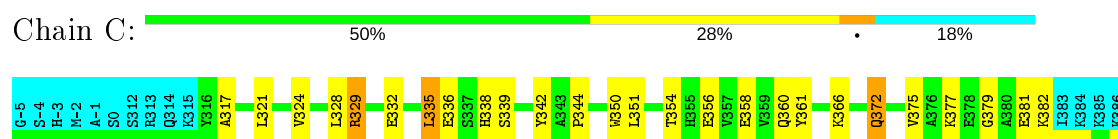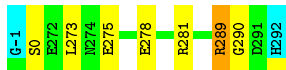Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

• Molecule 1: Stromal interaction molecule 1

Chain A:

51%   28%   •   18%

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 L321 E322 Q323 V324 R325 L328 R329 E332 E336 L335 K349 W350 L351 Q352 L353 E356 V357 E358 V359 Q360 Y361 Y362 K365 K366 K377 E378 G379 A380 E381 K382 I383 K384 K385 K386 R387

• Molecule 1: Stromal interaction molecule 1

Chain C:

48%   30%   •   18%

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 L321 E322 Q323 V324 R325 L328 R329 E332 L335 E336 S339 Y342 K349 W350 L351 Q352 L353 E356 V357 E358 V359 Q360 Y361 Y362 K365 K366 K377 E378 G379 A380 E381 K382 I383 K384 K385 K386 R387

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

70%   22%   9%

G-1 S0 E272 L273 N274 E275 E278 R281 H292

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

57%   30%   13%

G-1 S0 E272 L273 N274 E275 E278 R281 R289 G290 D291 H292

4.2.2 Score per residue for model 2

• Molecule 1: Stromal interaction molecule 1

Chain A:

48%   29%   5%   18%

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 L321 V324 L328 R329 E332 L335 E336 S339 Y342 A343 P344 W350 L351 T354 H355 E356 V357 E358 V359 Q360 Y361 Y362 K366 Q372 L373 L374 V375 A376 K377 E378 G379 A380 E381 K382 I383 K384 K385

K386 R387

• Molecule 1: Stromal interaction molecule 1

Chain C:

50%   28%   •   18%

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 L321 V324 L328 R329 E332 L335 E336 S337 H338 S339 Y342 A343 P344 W350 L351 T354 H355 E356 V357 E358 V359 Q360 Y361 K366 Q372 V375 A376 K377 E378 G379 A380 E381 K382 I383 K384 K385 K386

R387

● Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

61% 26% • 9%

G-1 S0 E272 L273 N274 E275 E278 R281 R289 G290 D291 H292

● Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

52% 30% • 13%

G-1 S0 E272 L273 N274 E275 E278 R281 Q285 R289 G290 D291 H292

4.2.3 Score per residue for model 3 (medoid)

● Molecule 1: Stromal interaction molecule 1

Chain A:

54% 24% • 18%

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 E318 L321 V324 R325 L328 R329 E332 L335 E336 S339 Y342 W350 L351 Q360 Y361 Y362 N363 I364 K365 K366 K377 A380 E381 K382 I383 K384 K385 K386 R387

● Molecule 1: Stromal interaction molecule 1

Chain C:

55% 24% • 18%

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 E318 L321 V324 R325 L328 R329 K330 A331 E332 L335 E336 S339 Y342 W350 L351 Y361 Y362 N363 I364 K365 K366 L374 E378 K382 I383 K384 K385 K386 R387

● Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

65% 26% 9%

G-1 S0 E272 L273 N274 E275 L286 R289 G290 D291 H292

● Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

74% 13% 13%

G-1 L273 L286 G290 D291 H292

4.2.4 Score per residue for model 4

• Molecule 1: Stromal interaction molecule 1

Chain A:

• Molecule 1: Stromal interaction molecule 1

Chain C:

• Molecule 2: Calcium release-activated calcium channel protein 1
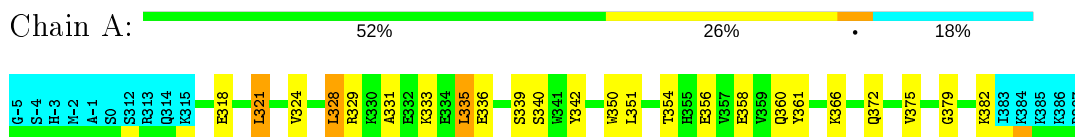
Chain B:

• Molecule 2: Calcium release-activated calcium channel protein 1
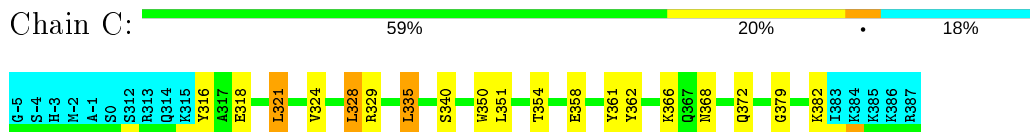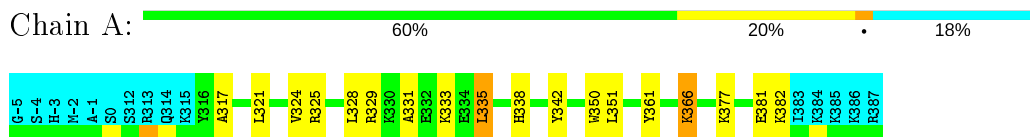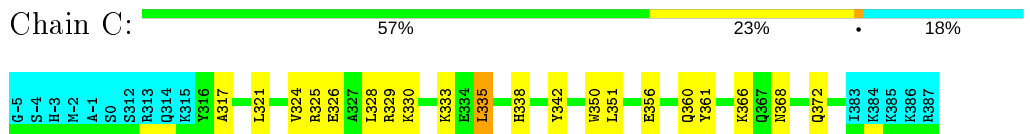
Chain D:

4.2.5 Score per residue for model 5

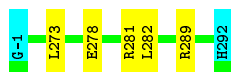• Molecule 1: Stromal interaction molecule 1

Chain A:

• Molecule 1: Stromal interaction molecule 1

Chain C:

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

G-1 L273 E278 R281 L282 R289 H292

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

70% 17% 13%

G-1 L273 E278 R281 R289 G290 D291 H292

4.2.6 Score per residue for model 6

• Molecule 1: Stromal interaction molecule 1

Chain A:

49% 30% • 18%

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 E318 L321 V324 R325 L328 R329 E332 K333 E334 L335 E336 Y342 A343 P344 W350 L351 T354 H355 E356 V357 E358 V359 Q360 Y361 K366 Q372 V375 A376 K377 G378 G379 A380 E381 K382 I383 K384 K385 K386

R387

• Molecule 1: Stromal interaction molecule 1

Chain C:

54% 26% • 18%

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 E318 L321 V324 R325 L328 R329 E332 K333 E334 L335 E336 W350 L351 T354 H355 E356 V357 E358 V359 Q360 Y361 K366 K377 G378 G379 A380 E381 K382 I383 K384 K385 K386 R387

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

57% 30% • 9%

G-1 S0 E272 L273 E278 R281 L282 Q285 R289 G290 D291 H292

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

48% 39% 13%

G-1 S0 E272 L273 N274 E275 E278 R281 L282 Q285 R289 G290 D291 H292

4.2.7 Score per residue for model 7

• Molecule 1: Stromal interaction molecule 1

Chain A:

55% 23% • 18%

- Molecule 1: Stromal interaction molecule 1

Chain C:

- Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

- Molecule 2: Calcium release-activated calcium channel protein 1
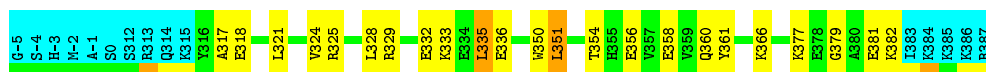
Chain D:

4.2.8 Score per residue for model 8
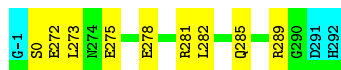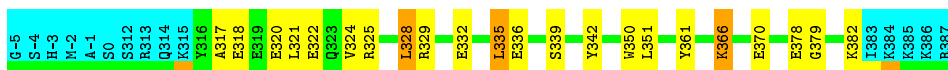
- Molecule 1: Stromal interaction molecule 1

Chain A:

- Molecule 1: Stromal interaction molecule 1

Chain C:

- Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

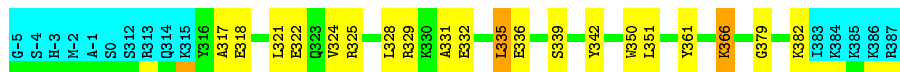- Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

4.2.9 Score per residue for model 9

• Molecule 1: Stromal interaction molecule 1

Chain A:

• Molecule 1: Stromal interaction molecule 1

Chain C:

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

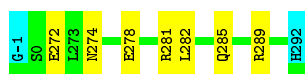
4.2.10 Score per residue for model 10

• Molecule 1: Stromal interaction molecule 1

Chain A:
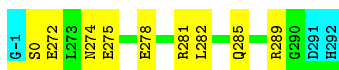

• Molecule 1: Stromal interaction molecule 1

Chain C:

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

4.2.11 Score per residue for model 11

• Molecule 1: Stromal interaction molecule 1

Chain A:

• Molecule 1: Stromal interaction molecule 1

Chain C:

• Molecule 2: Calcium release-activated calcium channel protein 1
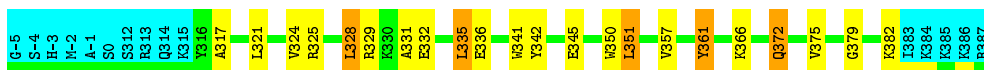
Chain B:

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

4.2.12 Score per residue for model 12

• Molecule 1: Stromal interaction molecule 1

Chain A:

- Molecule 1: Stromal interaction molecule 1

Chain C:

- Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

- Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

4.2.13 Score per residue for model 13

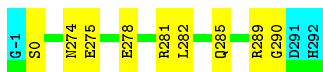- Molecule 1: Stromal interaction molecule 1

Chain A:

- Molecule 1: Stromal interaction molecule 1

Chain C:

- Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

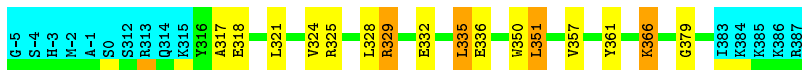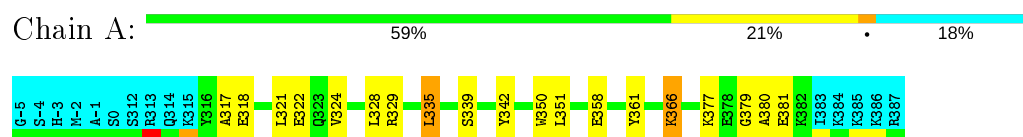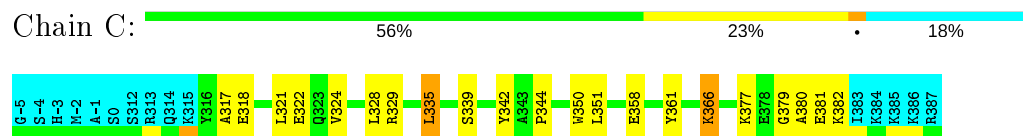- Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

G-1 S0 E272 L273 N274 E275 E278 R281 L286 D291 H292

4.2.14 Score per residue for model 14

• Molecule 1: Stromal interaction molecule 1

Chain A:

| | 52% | | 24% | • • | 18% |

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 E318 L321 V324 R325 E326 A327 L328 R329 K330 A331 E332 K333 E334 L335 E336 S339 Y342 W350 L351 T354 Y361 K365 K366 K377 E381 K382 I383 K384 K385 K386 R387

• Molecule 1: Stromal interaction molecule 1

Chain C:

| | 59% | 18% | • • | 18% |

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 E318 L321 V324 R325 E326 A327 L328 R329 L335 E345 W350 L351 T354 Y361 K365 K366 K377 E381 K382 I383 K384 K385 K386 R387

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

| | 65% | | 26% | 9% |

G-1 S0 E272 L273 N274 E275 E278 R281 L286 H292

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

| | 65% | | 22% | 13% |

G-1 L273 E278 R281 L282 L286 D291 H292

4.2.15 Score per residue for model 15

• Molecule 1: Stromal interaction molecule 1

Chain A:

| | 54% | 23% | 5% | 18% |

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 E320 L321 V324 L328 R329 E332 L335 E336 S339 Y342 W350 L351 H355 R356 V357 Y361 Y362 K366 L374 E378 G379 K382 I383 K384 K385 K386 R387

• Molecule 1: Stromal interaction molecule 1

Chain C:

| | 54% | 23% | 5% | 18% |

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 E318 L321 V324 R325 L328 R329 E332 L335 E336 S339 Y342 W350 L351 E356 Q360 Y361 Y362 K366 L374 E378 G379 K382 I383 K384 K385 K386 R387

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

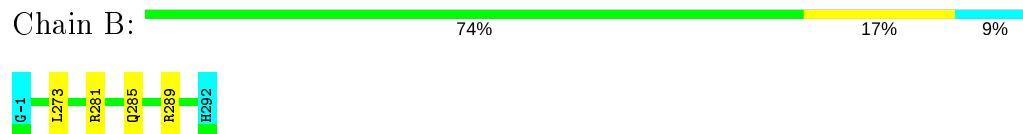### 4.2.16 Score per residue for model 16

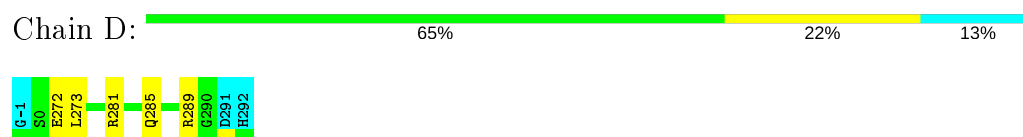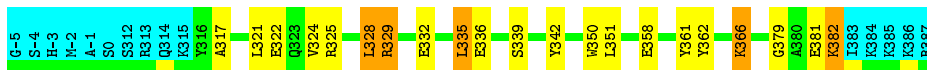• Molecule 1: Stromal interaction molecule 1

Chain A:

• Molecule 1: Stromal interaction molecule 1

Chain C:

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

4.2.17 Score per residue for model 17

• Molecule 1: Stromal interaction molecule 1
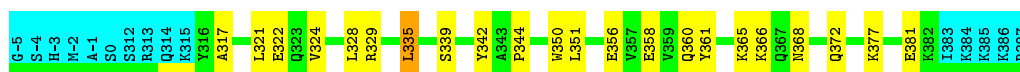
Chain A:

- Molecule 1: Stromal interaction molecule 1

Chain C:

- Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

- Molecule 2: Calcium release-activated calcium channel protein 1
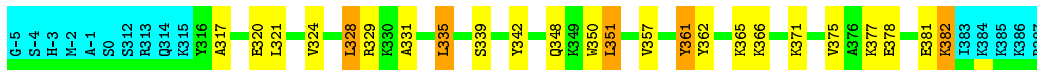
Chain D:

4.2.18 Score per residue for model 18

- Molecule 1: Stromal interaction molecule 1
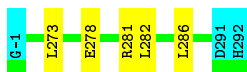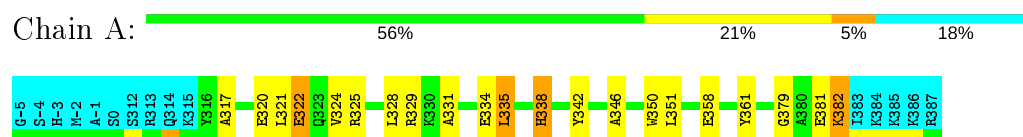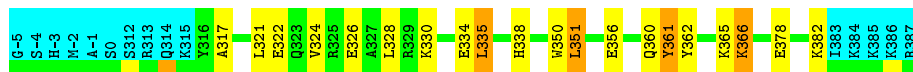
Chain A:

- Molecule 1: Stromal interaction molecule 1

Chain C:

- Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

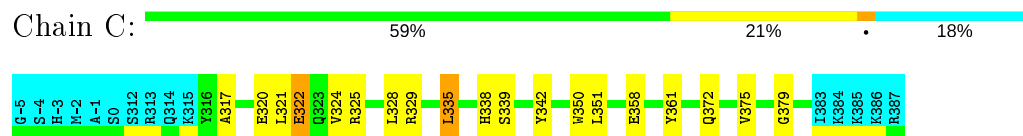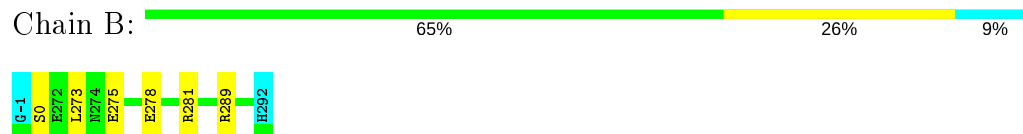- Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

G-1 S0 E272 L273 N274 E275 E278 R281 Q285 L286 R289 G290 D291 H292
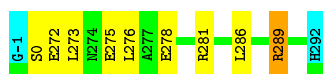
4.2.19 Score per residue for model 19

• Molecule 1: Stromal interaction molecule 1
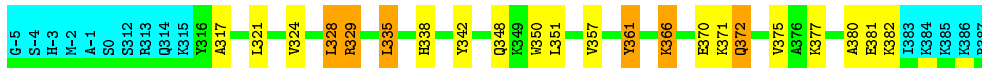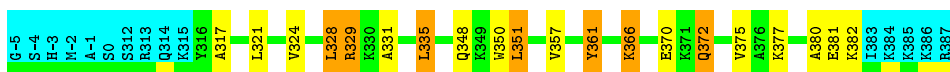
Chain A:　　　55%　　　20%　　7%　　18%

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 L321 V324 L328 R329 L335 H338 Y342 Q348 K349 W350 L351 V357 Y361 K366 E370 K371 Q372 V375 A376 K377 A380 E381 K382 I383 K384 K385 K386 R387

• Molecule 1: Stromal interaction molecule 1

Chain C:　　　57%　　　16%　　9%　　18%

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 L321 V324 L328 R329 K330 A331 L335 Q348 K349 W350 L351 V357 Y361 K366 E370 K371 Q372 V375 A376 K377 A380 E381 K382 I383 K384 K385 K386 R387

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:　　　70%　　　17%　　•　　9%

G-1 L273 E278 R281 L286 R289 H292

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:　　　65%　　　22%　　13%

G-1 L273 E278 R281 L286 R289 G290 D291 H292

4.2.20 Score per residue for model 20

• Molecule 1: Stromal interaction molecule 1

Chain A:　　　59%　　　20%　　•　　18%

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 E318 E319 E320 L321 E322 Q323 V324 R325 B326 A327 L328 R329 K330 L335 P344 L347 W350 L351 V357 Y361 K366 G379 I383 K384 K385 K386 R387

• Molecule 1: Stromal interaction molecule 1

Chain C:　　　59%　　　18%　　5%　　18%

G-5 S-4 H-3 M-2 A-1 S0 S312 R313 Q314 K315 Y316 A317 L321 V324 R325 E326 A327 L328 R329 K330 L335 S339 Y342 A343 P344 L347 W350 L351 V357 Y361 K366 E370 I383 K384 K385 K386 R387

• Molecule 2: Calcium release-activated calcium channel protein 1

Chain B:

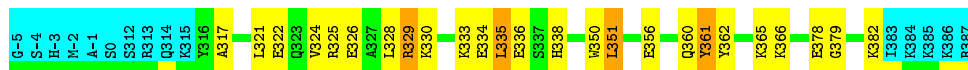• Molecule 2: Calcium release-activated calcium channel protein 1

Chain D:

5 Refinement protocol and experimental data overview ⓘ

The models were refined using the following method: *simulated annealing.*

Of the 200 calculated structures, 20 were deposited, based on the following criterion: *structures with the lowest energy.*

The following table shows the software used for structure solution, optimisation and refinement.

Software name	Classification	Version
TALOS	geometry optimization	
CYANA	structure solution	3.0
CNS	refinement	
CNS	geometry optimization	

The following table shows chemical shift validation statistics as aggregates over all chemical shift files. Detailed validation can be found in section 7 of this report.

Chemical shift file(s)	input_cs.cif
Number of chemical shift lists	2
Total number of shifts	1245
Number of shifts mapped to atoms	1245
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	42%

No validations of the models with respect to experimental NMR restraints is performed at this time.

6 Model quality (i)

6.1 Standard geometry (i)

There are no covalent bond-length or bond-angle outliers.

Chiral center outliers are detected by calculating the chiral volume of a chiral center and verifying if the center is modelled as a planar moiety or with the opposite hand. A planarity outlier is detected by checking planarity of atoms in a peptide group, atoms in a mainchain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	Chirality	Planarity
1	C	0.0±0.0	0.1±0.4
1	A	0.0±0.0	0.1±0.3
2	B	0.0±0.0	0.1±0.4
2	D	0.0±0.0	0.1±0.4
All	All	0	11

There are no bond-length outliers.

There are no bond-angle outliers.

There are no chirality outliers.

All unique planar outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Group	Models (Total)
1	C	329	ARG	Sidechain	3
2	B	289	ARG	Sidechain	3
2	D	289	ARG	Sidechain	3
1	A	329	ARG	Sidechain	2

6.2 Too-close contacts (i)

In the following table, the Non-H and H(model) columns list the number of non-hydrogen atoms and hydrogen atoms in each chain respectively. The H(added) column lists the number of hydrogen atoms added and optimized by MolProbity. The Clashes column lists the number of clashes averaged over the ensemble.

Mol	Chain	Non-H	H(model)	H(added)	Clashes
1	A	562	551	551	13±3
1	C	562	551	551	12±3
2	B	172	156	156	3±1
2	D	164	152	152	3±1
All	All	29200	28200	28200	545

The all-atom clashscore is defined as the number of clashes found per 1000 atoms (including hydrogen atoms). The all-atom clashscore for this structure is 9.

All unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:358:GLU:HA	1:A:361:TYR:CE2	0.74	2.18	12	7
1:C:358:GLU:HA	1:C:361:TYR:CE2	0.73	2.19	17	7
1:A:335:LEU:HG	1:A:350:TRP:CG	0.66	2.25	7	20
1:A:317:ALA:O	1:A:321:LEU:HG	0.64	1.92	7	19
1:C:372:GLN:HA	1:C:375:VAL:HG12	0.63	1.69	19	4
1:C:317:ALA:O	1:C:321:LEU:HG	0.62	1.93	9	19
1:A:320:GLU:HG3	1:C:324:VAL:HG23	0.62	1.72	7	1
1:C:335:LEU:HG	1:C:350:TRP:CG	0.61	2.30	17	20
2:D:0:SER:O	2:D:275:GLU:HG2	0.59	1.98	10	15
1:A:326:GLU:O	1:A:330:LYS:HB2	0.59	1.98	18	3
1:A:377:LYS:O	1:A:381:GLU:HG2	0.59	1.98	14	10
1:A:372:GLN:HA	1:A:375:VAL:HG12	0.58	1.74	9	5
1:C:332:GLU:O	1:C:336:GLU:HG2	0.58	1.98	12	10
1:A:366:LYS:O	1:A:370:GLU:HG2	0.57	1.98	7	3
2:B:277:ALA:O	2:B:281:ARG:HG2	0.57	1.99	4	1
2:B:0:SER:O	2:B:275:GLU:HG2	0.57	2.00	18	12
1:C:326:GLU:O	1:C:330:LYS:HB2	0.57	1.98	18	3
1:C:349:LYS:O	1:C:353:LEU:HG	0.57	1.99	1	1
1:C:339:SER:HA	1:C:342:TYR:CE1	0.56	2.35	2	12
1:A:329:ARG:HD2	1:A:329:ARG:N	0.56	2.15	14	3
1:C:324:VAL:O	1:C:328:LEU:HB3	0.56	2.00	14	20
1:A:324:VAL:O	1:A:328:LEU:HB3	0.56	2.00	14	20
1:C:377:LYS:O	1:C:381:GLU:HG2	0.56	2.00	14	7
2:D:277:ALA:O	2:D:281:ARG:HG2	0.56	2.00	4	1
1:C:329:ARG:N	1:C:329:ARG:HD2	0.56	2.16	14	3
1:A:332:GLU:O	1:A:336:GLU:HG2	0.56	2.00	12	9
1:A:324:VAL:HG11	1:C:324:VAL:HG21	0.56	1.77	7	1
2:B:285:GLN:O	2:B:289:ARG:HB2	0.55	2.02	12	3
1:A:349:LYS:O	1:A:353:LEU:HG	0.55	2.02	1	1
1:C:362:TYR:O	1:C:366:LYS:HD2	0.54	2.02	18	6
1:C:371:LYS:O	1:C:375:VAL:HG23	0.54	2.02	13	1
1:C:366:LYS:O	1:C:370:GLU:HG2	0.54	2.03	7	3
1:A:321:LEU:HD13	1:C:357:VAL:HG21	0.53	1.79	9	5
1:A:339:SER:HA	1:A:342:TYR:CE1	0.53	2.38	3	11
2:B:278:GLU:O	2:B:282:LEU:HG	0.53	2.04	17	5
1:C:378:GLU:O	1:C:382:LYS:HD3	0.53	2.04	18	1
1:A:329:ARG:NE	1:C:361:TYR:HB2	0.52	2.20	19	2
2:B:278:GLU:O	2:B:281:ARG:HG2	0.52	2.04	2	14

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:357:VAL:HG21	1:C:321:LEU:HD13	0.52	1.80	9	4
2:D:278:GLU:O	2:D:281:ARG:HG2	0.52	2.05	2	13
2:D:278:GLU:O	2:D:282:LEU:HG	0.51	2.04	8	6
1:C:378:GLU:O	1:C:382:LYS:HG3	0.51	2.05	7	1
1:A:361:TYR:HB2	1:C:329:ARG:NE	0.51	2.20	10	1
1:A:333:LYS:HA	1:A:336:GLU:OE2	0.51	2.05	14	1
1:C:362:TYR:CZ	1:C:366:LYS:HD3	0.51	2.40	3	1
1:C:366:LYS:HE2	1:C:366:LYS:HA	0.50	1.82	7	4
1:A:357:VAL:HG13	1:C:325:ARG:HB2	0.50	1.83	10	2
1:A:378:GLU:HB3	1:A:382:LYS:HE2	0.50	1.82	7	1
1:A:335:LEU:HG	1:A:350:TRP:CD2	0.50	2.42	20	15
1:A:324:VAL:HG21	1:C:324:VAL:HG11	0.50	1.83	7	1
1:A:361:TYR:HB2	1:C:329:ARG:HE	0.50	1.66	10	1
1:A:329:ARG:HE	1:C:361:TYR:HB2	0.50	1.66	19	2
1:A:340:SER:HA	2:B:290:GLY:CA	0.50	2.36	4	1
1:A:366:LYS:HA	1:A:366:LYS:HE2	0.50	1.84	19	3
1:A:351:LEU:HG	2:B:281:ARG:HB3	0.50	1.83	9	3
1:A:319:GLU:O	1:A:323:GLN:HG2	0.50	2.07	20	1
2:B:289:ARG:HD3	2:B:291:ASP:O	0.49	2.07	12	1
1:A:374:LEU:O	1:A:378:GLU:HG2	0.49	2.07	2	1
1:A:338:HIS:HB3	1:A:342:TYR:CE2	0.49	2.43	5	2
1:C:338:HIS:HB3	1:C:342:TYR:CE2	0.49	2.43	5	1
1:A:362:TYR:O	1:A:366:LYS:HD2	0.49	2.06	3	6
1:A:362:TYR:CZ	1:A:366:LYS:HD3	0.49	2.42	3	1
1:A:325:ARG:HB2	1:C:357:VAL:HG13	0.49	1.83	10	1
2:D:285:GLN:O	2:D:289:ARG:HB2	0.49	2.06	12	3
2:D:0:SER:O	2:D:274:ASN:HB3	0.49	2.07	10	1
2:B:281:ARG:O	2:B:285:GLN:HG2	0.49	2.08	11	3
1:A:361:TYR:O	1:A:365:LYS:HG2	0.49	2.08	13	3
1:A:329:ARG:HD3	1:C:361:TYR:HB2	0.48	1.85	2	1
1:A:371:LYS:O	1:A:375:VAL:HG23	0.48	2.08	13	1
1:A:356:GLU:O	1:A:360:GLN:HG2	0.48	2.09	6	7
1:C:335:LEU:HG	1:C:350:TRP:CD1	0.48	2.43	7	1
1:C:333:LYS:O	1:C:336:GLU:HG2	0.48	2.09	17	1
1:C:356:GLU:O	1:C:360:GLN:HG2	0.48	2.09	6	7
1:C:322:GLU:O	1:C:325:ARG:HB3	0.48	2.09	8	5
1:A:329:ARG:HH11	1:A:329:ARG:HG3	0.48	1.69	14	1
1:C:379:GLY:O	1:C:382:LYS:HB3	0.48	2.09	9	1
2:B:0:SER:O	2:B:274:ASN:HB3	0.48	2.09	10	1
1:A:342:TYR:O	2:B:290:GLY:HA3	0.47	2.08	3	4
1:A:324:VAL:HG23	1:C:320:GLU:HG3	0.47	1.85	7	1

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:379:GLY:O	1:A:382:LYS:HB3	0.47	2.08	9	2
1:C:351:LEU:HG	2:D:281:ARG:HB3	0.47	1.86	9	5
1:C:361:TYR:O	1:C:365:LYS:HG2	0.47	2.09	13	3
1:A:322:GLU:O	1:A:325:ARG:HB3	0.47	2.09	8	3
1:A:333:LYS:HA	1:A:336:GLU:CD	0.47	2.29	4	1
1:C:366:LYS:HA	1:C:366:LYS:HE2	0.47	1.85	11	1
1:C:329:ARG:HG3	1:C:329:ARG:HH11	0.47	1.69	14	1
1:A:361:TYR:HB2	1:C:329:ARG:HD3	0.46	1.86	9	5
1:A:368:ASN:O	1:A:372:GLN:HG3	0.46	2.10	17	1
1:A:324:VAL:HG11	1:C:324:VAL:HG11	0.46	1.87	4	1
1:A:354:THR:O	1:A:358:GLU:HB2	0.46	2.10	6	1
1:C:368:ASN:O	1:C:372:GLN:HG2	0.46	2.11	4	2
1:C:342:TYR:O	2:D:290:GLY:HA3	0.46	2.11	3	4
1:A:334:GLU:O	1:A:338:HIS:HB2	0.46	2.11	18	2
1:C:340:SER:HA	2:D:290:GLY:CA	0.46	2.41	4	1
1:C:329:ARG:HD2	1:C:329:ARG:N	0.46	2.26	9	2
1:A:365:LYS:HD2	1:C:329:ARG:CZ	0.46	2.40	12	1
1:C:374:LEU:O	1:C:378:GLU:HG2	0.45	2.10	7	2
1:A:351:LEU:HG	2:B:281:ARG:CB	0.45	2.42	14	3
1:C:348:GLN:OE1	1:C:348:GLN:HA	0.45	2.11	13	1
1:A:366:LYS:HE2	1:A:366:LYS:HA	0.45	1.88	11	2
1:A:379:GLY:HA3	2:D:287:ASP:OD2	0.45	2.10	20	1
1:A:358:GLU:HA	1:A:361:TYR:CD2	0.45	2.46	17	4
1:A:321:LEU:HD12	1:A:322:GLU:N	0.45	2.27	8	5
1:C:381:GLU:HG3	1:C:382:LYS:HD3	0.45	1.88	12	1
1:C:351:LEU:HG	2:D:281:ARG:CB	0.45	2.41	14	5
1:C:361:TYR:CD1	1:C:362:TYR:N	0.45	2.85	4	2
1:A:357:VAL:HG22	1:C:325:ARG:NH2	0.44	2.27	20	1
1:C:358:GLU:HA	1:C:361:TYR:CD2	0.44	2.47	4	4
1:A:354:THR:HA	1:C:321:LEU:HD21	0.44	1.89	14	2
1:C:366:LYS:HA	1:C:366:LYS:CE	0.44	2.42	19	2
1:C:354:THR:O	1:C:358:GLU:HB2	0.44	2.12	6	1
2:B:281:ARG:HG3	2:B:282:LEU:N	0.44	2.28	4	1
2:D:281:ARG:HG3	2:D:282:LEU:N	0.44	2.28	4	1
1:C:366:LYS:CE	1:C:366:LYS:HA	0.43	2.43	7	1
1:C:374:LEU:O	1:C:378:GLU:HG3	0.43	2.12	15	1
1:A:331:ALA:O	1:A:335:LEU:HB2	0.43	2.13	13	4
1:A:378:GLU:HA	1:A:381:GLU:OE2	0.43	2.14	12	1
1:A:329:ARG:NH2	1:C:365:LYS:HE3	0.43	2.28	14	1
2:B:272:GLU:OE2	2:B:276:LEU:HD12	0.43	2.14	18	1
1:A:316:TYR:O	1:A:320:GLU:HG2	0.43	2.14	15	1

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:329:ARG:CZ	1:C:365:LYS:HE3	0.43	2.44	17	1
1:A:329:ARG:HH12	1:A:332:GLU:CD	0.43	2.17	14	1
1:A:335:LEU:HG	1:A:350:TRP:CD1	0.43	2.49	7	1
1:A:378:GLU:O	1:A:382:LYS:HG3	0.43	2.13	7	2
1:A:344:PRO:HB3	2:B:289:ARG:HG2	0.42	1.90	2	3
1:A:321:LEU:HD21	1:C:354:THR:HA	0.42	1.91	14	3
1:C:344:PRO:HB3	2:D:289:ARG:HG2	0.42	1.90	20	3
2:B:287:ASP:OD2	1:C:379:GLY:HA3	0.42	2.14	4	1
1:A:329:ARG:N	1:A:329:ARG:HD2	0.42	2.29	9	1
1:C:325:ARG:O	1:C:329:ARG:HB2	0.42	2.13	20	1
1:C:331:ALA:O	1:C:335:LEU:HB2	0.42	2.14	9	5
1:A:365:LYS:HE3	1:C:329:ARG:NH2	0.42	2.30	14	2
1:A:329:ARG:HG2	1:C:364:ILE:HG21	0.42	1.92	3	1
1:C:334:GLU:O	1:C:338:HIS:HB2	0.42	2.15	18	1
2:D:281:ARG:O	2:D:285:GLN:HG2	0.42	2.14	11	3
1:A:360:GLN:O	1:A:364:ILE:HG13	0.42	2.15	3	1
1:A:331:ALA:HB2	1:C:316:TYR:CE1	0.42	2.49	4	1
1:A:374:LEU:O	1:A:378:GLU:HG3	0.42	2.15	15	1
1:A:348:GLN:OE1	1:A:348:GLN:HA	0.42	2.15	13	1
1:A:342:TYR:CE1	1:A:346:ALA:HB3	0.42	2.50	16	1
1:A:381:GLU:HG3	1:A:382:LYS:HD3	0.42	1.92	16	1
1:A:321:LEU:HD23	1:C:328:LEU:HD13	0.42	1.92	17	1
1:A:366:LYS:CE	1:A:366:LYS:HA	0.42	2.45	19	1
1:C:338:HIS:CD2	1:C:342:TYR:HE2	0.42	2.33	2	1
1:A:366:LYS:HA	1:A:366:LYS:CE	0.42	2.45	7	1
1:A:325:ARG:O	1:A:329:ARG:HB2	0.42	2.15	20	1
2:B:272:GLU:HA	2:B:275:GLU:CG	0.41	2.45	3	1
1:C:339:SER:HA	1:C:342:TYR:CZ	0.41	2.50	8	1
1:C:333:LYS:HA	1:C:336:GLU:OE2	0.41	2.16	18	1
1:A:361:TYR:CD1	1:A:362:TYR:N	0.41	2.88	2	1
1:C:321:LEU:HD12	1:C:322:GLU:N	0.41	2.29	7	5
1:A:325:ARG:NH2	1:C:357:VAL:HG22	0.41	2.31	20	1
1:C:361:TYR:CD1	1:C:361:TYR:C	0.41	2.94	18	1
1:A:329:ARG:HG3	1:A:329:ARG:HH11	0.41	1.75	3	1
1:C:378:GLU:O	1:C:382:LYS:HE3	0.41	2.16	13	1
1:A:355:HIS:NE2	2:B:274:ASN:HA	0.41	2.31	15	1
1:A:354:THR:HA	1:C:321:LEU:CD2	0.40	2.47	2	1
1:A:321:LEU:CD2	1:C:354:THR:HA	0.40	2.46	2	2
1:A:372:GLN:HA	1:A:375:VAL:CG1	0.40	2.47	6	1

6.3 Torsion angles (i)

6.3.1 Protein backbone (i)

In the following table, the Percentiles column shows the percent Ramachandran outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all NMR entries. The Analysed column shows the number of residues for which the backbone conformation was analysed and the total number of residues.

Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
1	A	67/82 (82%)	66±1 (99±1%)	1±1 (1±1%)	0±0 (0±0%)	100	100
1	C	67/82 (82%)	66±1 (99±1%)	1±1 (1±1%)	0±0 (0±0%)	100	100
2	B	21/23 (91%)	20±1 (96±3%)	1±1 (4±3%)	0±0 (0±0%)	100	100
2	D	20/23 (87%)	19±1 (95±3%)	1±1 (5±3%)	0±0 (0±0%)	100	100
All	All	3500/4200 (83%)	3434 (98%)	66 (2%)	0 (0%)	100	100

There are no Ramachandran outliers.

6.3.2 Protein sidechains (i)

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all NMR entries. The Analysed column shows the number of residues for which the sidechain conformation was analysed and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
1	A	58/71 (82%)	52±2 (89±3%)	7±2 (11±3%)	9	53
1	C	58/71 (82%)	52±1 (89±3%)	6±1 (11±3%)	9	53
2	B	18/19 (95%)	16±1 (88±4%)	2±1 (12±4%)	8	51
2	D	17/19 (89%)	15±1 (88±5%)	2±1 (12±5%)	8	50
All	All	3020/3600 (84%)	2676 (89%)	344 (11%)	9	52

All 49 unique residues with a non-rotameric sidechain are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
1	C	335	LEU	20
1	A	335	LEU	20
1	C	351	LEU	20
1	A	351	LEU	20

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Mol	Chain	Res	Type	Models (Total)
2	D	273	LEU	18
2	B	273	LEU	18
1	C	329	ARG	18
1	C	366	LYS	17
1	A	366	LYS	17
1	A	329	ARG	16
1	C	361	TYR	13
1	A	382	LYS	13
1	A	361	TYR	13
1	C	382	LYS	12
2	B	289	ARG	10
1	C	328	LEU	7
2	D	272	GLU	7
1	A	328	LEU	7
2	B	286	LEU	6
2	D	286	LEU	6
1	C	325	ARG	6
1	A	325	ARG	6
2	D	289	ARG	6
1	A	321	LEU	4
2	B	274	ASN	4
1	A	372	GLN	3
2	D	285	GLN	3
1	C	372	GLN	3
2	B	285	GLN	3
1	C	321	LEU	2
1	A	333	LYS	2
1	A	320	GLU	2
2	B	272	GLU	2
1	C	333	LYS	2
2	D	274	ASN	2
1	C	320	GLU	2
1	C	326	GLU	2
1	A	326	GLU	1
1	A	347	LEU	1
1	C	347	LEU	1
1	A	322	GLU	1
1	A	371	LYS	1
1	C	338	HIS	1
1	A	345	GLU	1
1	C	348	GLN	1
1	A	338	HIS	1

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Mol	Chain	Res	Type	Models (Total)
1	C	322	GLU	1
1	C	345	GLU	1
1	A	348	GLN	1

6.3.3　RNA (i)

There are no RNA molecules in this entry.

6.4　Non-standard residues in protein, DNA, RNA chains (i)

There are no non-standard protein/DNA/RNA residues in this entry.

6.5　Carbohydrates (i)

There are no carbohydrates in this entry.

6.6　Ligand geometry (i)

There are no ligands in this entry.

6.7　Other polymers (i)

There are no such molecules in this entry.

6.8　Polymer linkage issues (i)

There are no chain breaks in this entry.

7 Chemical shift validation (i)

The completeness of assignment taking into account all chemical shift lists is 42% for the well-defined parts and 41% for the entire structure.

7.1 Chemical shift list 1

File name: input_cs.cif

Chemical shift list name: *assigned_chem_shift_list_1*

7.1.1 Bookkeeping (i)

The following table shows the results of parsing the chemical shift list and reports the number of nuclei with statistically unusual chemical shifts.

Total number of shifts	1010
Number of shifts mapped to atoms	1010
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	0

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}\text{C}_\alpha$	82	-0.36 ± 0.11	None needed (< 0.5 ppm)
$^{13}\text{C}_\beta$	80	0.28 ± 0.03	None needed (< 0.5 ppm)
$^{13}\text{C}'$	77	-0.29 ± 0.08	None needed (< 0.5 ppm)
^{15}N	77	0.08 ± 0.16	None needed (< 0.5 ppm)

7.1.3 Completeness of resonance assignments (i)

The following table shows the completeness of the chemical shift assignments for the well-defined regions of the structure. The overall completeness is 33%, i.e. 762 atoms were assigned a chemical shift out of a possible 2287. 12 out of 32 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^1H	^{13}C	^{15}N
Backbone	329/871 (38%)	132/348 (38%)	132/350 (38%)	65/173 (38%)
Sidechain	395/1244 (32%)	247/728 (34%)	148/454 (33%)	0/62 (0%)

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	Total	**^{1}H**	**^{13}C**	**^{15}N**
Aromatic	38/172 (22%)	19/90 (21%)	17/72 (24%)	2/10 (20%)
Overall	762/2287 (33%)	398/1166 (34%)	297/876 (34%)	67/245 (27%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 34%, i.e. 928 atoms were assigned a chemical shift out of a possible 2758. 12 out of 32 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

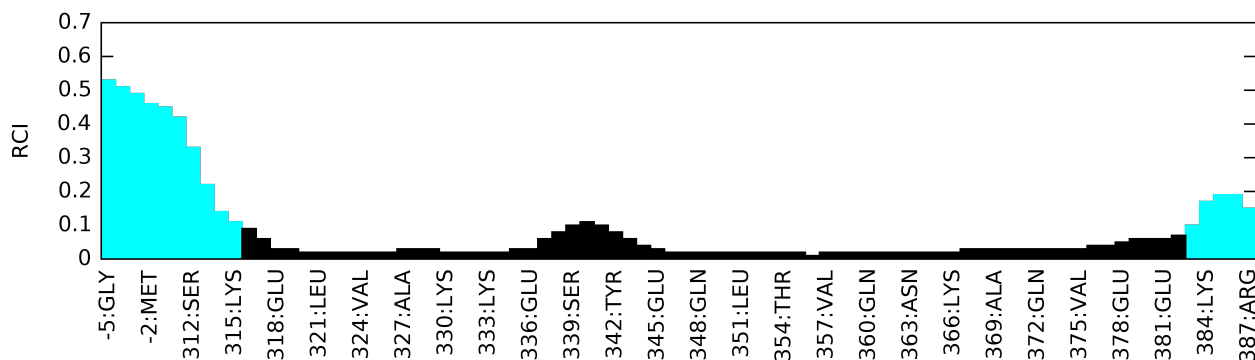|	**Total**	**^{1}H**	**^{13}C**	**^{15}N**
Backbone	395/1046 (38%)	159/418 (38%)	159/420 (38%)	77/208 (37%)
Sidechain	495/1512 (33%)	312/892 (35%)	183/536 (34%)	0/84 (0%)
Aromatic	38/200 (19%)	19/106 (18%)	17/80 (21%)	2/14 (14%)
Overall	928/2758 (34%)	490/1416 (35%)	359/1036 (35%)	79/306 (26%)

7.1.4 Statistically unusual chemical shifts ⓘ

There are no statistically unusual chemical shifts.

7.1.5 Random Coil Index (RCI) plots ⓘ

The image below reports *random coil index* values for the protein chains in the structure. The height of each bar gives a probability of a given residue to be disordered, as predicted from the available chemical shifts and the amino acid sequence. A value above 0.2 is an indication of significant predicted disorder. The colour of the bar shows whether the residue is in the well-defined core (black) or in the ill-defined residue ranges (cyan), as described in section 2 on ensemble composition.

Random coil index (RCI) for chain A:

7.2 Chemical shift list 2

File name: input_cs.cif

Chemical shift list name: *assigned_chem_shift_list_2*

7.2.1 Bookkeeping (i)

The following table shows the results of parsing the chemical shift list and reports the number of nuclei with statistically unusual chemical shifts.

Total number of shifts	235
Number of shifts mapped to atoms	235
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	0

7.2.2 Chemical shift referencing (i)

No chemical shift referencing corrections were calculated (not enough data).

7.2.3 Completeness of resonance assignments (i)

The following table shows the completeness of the chemical shift assignments for the well-defined regions of the structure. The overall completeness is 9%, i.e. 202 atoms were assigned a chemical shift out of a possible 2287. 4 out of 32 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	84/871 (10%)	42/348 (12%)	21/350 (6%)	21/173 (12%)
Sidechain	118/1244 (9%)	74/728 (10%)	41/454 (9%)	3/62 (5%)
Aromatic	0/172 (0%)	0/90 (0%)	0/72 (0%)	0/10 (0%)
Overall	202/2287 (9%)	116/1166 (10%)	62/876 (7%)	24/245 (10%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 8%, i.e. 212 atoms were assigned a chemical shift out of a possible 2758. 4 out of 32 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	91/1046 (9%)	45/418 (11%)	23/420 (5%)	23/208 (11%)
Sidechain	121/1512 (8%)	76/892 (9%)	42/536 (8%)	3/84 (4%)
Aromatic	0/200 (0%)	0/106 (0%)	0/80 (0%)	0/14 (0%)
Overall	212/2758 (8%)	121/1416 (9%)	65/1036 (6%)	26/306 (8%)

7.2.4 Statistically unusual chemical shifts (i)

There are no statistically unusual chemical shifts.

7.2.5 Random Coil Index (RCI) plots (i)

The image below reports *random coil index* values for the protein chains in the structure. The height of each bar gives a probability of a given residue to be disordered, as predicted from the available chemical shifts and the amino acid sequence. A value above 0.2 is an indication of significant predicted disorder. The colour of the bar shows whether the residue is in the well-defined core (black) or in the ill-defined residue ranges (cyan), as described in section 2 on ensemble composition.

Random coil index (RCI) for chain B: