Supporting Information

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SI Materials and Methods

Protein Production. Residues 1–78 of Sgt2 (Sgt2_NT) and 70–152 of Get5 (Get5_UBL) from *S. cerevisiae* and 1–74 of human Ubl4a were amplified from plasmids using the following primers:

gacgacgacaagatgtcagcatcaaaagaagaaattgctgccc;
gaggagaagcccggttacgattctggaactcttgacgcagaat;
gacgacgacaagatggacaacgccgccgtccactta;
gaggagaagcccggttacggattcggcttaatcatgactgtgat;
gacgacgacaagatgcagctgacggtgaaggcg; and
gaggagaagcccggttacaggggtttgaccactaggttg.

They were inserted via ligation-independent Ek/LIC cloning into pET-46 vectors. Site-directed mutagenesis was performed using QuikChange (Stratagene).

NMR Structure Determination of the Sgt2_NT Dimer in the Free State. NMR structure determination of free Sgt2_NT dimer performed with ARIA2.1 (1) using a protocol adapted from that previously reported for a homodimeric system (2). Comparison of the 3D, ^{13}C-separated, (^{13}C, ^{15}N-*F1*)–reject filtered NOESY experiment (3) with the 3D ^{13}C-separated NOESY-HMQC permitted NOEs to be assigned as intra- or intermolecular, whereas those observed in the 3D ^{15}N-separated NOESY were allowed to be either. NOE restraints were applied to one monomer only, with the second monomer being refined under noncrystallographic symmetry restraints.

TALOS-derived dihedrals and hydrogen bonds in regions of regular secondary structure based on amide exchange rates and initial structures were applied to both monomers. Specific differences from the above-reported protocol were as follows: high temperature, first cooling, and second cooling refine steps: 15,000, 10,000, and 20,000, respectively; dihedral force constants (high temperature, cool 1, and cool 2 stages): 15, 40, and 300 Kcal/mol; noncrystallographic symmetry restraints force constant: 100 Kcal/mol. Packing to ensure retention of monomer proximity was used throughout, with packing force constants of 15, 10, and 5 Kcal/mol for the high temperature, first cooling, and second cooling steps, respectively. Network anchoring with default parameters was used in iterations 0–4. The final ensemble comprised 100 structures from which the lowest energy 20 were subjected to water refinement.

Structure Calculation for Sgt2_NT/Get5_UBL Complex. The chemical shift perturbation studies delineated clear interaction surface areas in the Sgt2_NT/Get5_UBL complex. These data in concert with several measured assignable intercomplex NOEs made this complex highly suitable for structure calculation using the HADDOCK approach. For the calculation, the crystal structure of Get5_UBL and the lowest energy NMR structure from the family of Sgt2_NT dimer structures were used. Ambiguous Interaction Restraints were implemented according to the standard protocol; specifically, from the chemical shift perturbation, 27 amino acid residues in Sgt2_NT and 31 in Get5_UBL were identified to have weighted chemical shift changes significantly greater than the average: 0.201 ppm for Sgt2_NT and 0.577 ppm for Get5_UBL were chosen as suitable cutoff values. After filtering for a relative solvent accessibility greater than 50%, as calculated using the program Naccess, 12 residues in Sgt2_NT (18, 40, 41, 42, 45, 48, 49, 52, 56, 63, 71, and 81) and 16 in Get5_UBL (29, 33, 55, 61, 64, 68, 69, 70, 73, 75, 79, 82, 83, 89, 95, and 97) were identified as active residues. Residues juxtaposed to these that have a relative solvent accessibility greater than 50% were termed "passive residues." These included nine further residues in Sgt2_NT (14, 37, 41, 61, 62, 66, 67, 70, and 84) and 10 in Get5_UBL (30, 31, 53, 54, 59, 77, 80, 84, 96, and 97). A number of intermolecular NOEs were identified from filtered NOESY experiments using two samples comprising either unlabeled Sgt2_NT and ^{15}N ^{13}C-Get5_UBL or vice versa. Due to differences in the free and bound state chemical shifts, only four of these could be assigned without a high degree of ambiguity and were included as explicit distance restraints to guide docking. Automatic selection of interfacial residues (i.e., those that are allowed to move during refinement) was invoked.

One thousand initial complex structures were generated by rigid body energy minimization, and the best 200 by total energy were selected for torsion angle dynamics and subsequent Cartesian dynamics in an explicit water solvent. Default scaling for energy terms was applied. Following the standard benchmarked protocol, cluster analysis of the water-refined structures yielded two clear ensembles with the lowest HADDOCK score. One of these could be discounted immediately due to violating the measured NOEs, leaving an ensemble of 13 structures. The 10 lowest energy structures from this were chosen to represent the complex and have been deposited under PDB accession no. 4ASW.

1. Rieping W, et al. (2007) ARIA2: Automated NOE assignment and data integration in NMR structure calculation. *Bioinformatics* 23(3):381–382.
2. Lingel A, Simon B, Izaurralde E, Sattler M (2005) The structure of the flock house virus B2 protein, a viral suppressor of RNA interference, shows a novel mode of double-stranded RNA recognition. *EMBO Rep* 6(12):1149–1155.
3. Zwahlen C, et al. (1997) Methods for measurement of intermolecular NOEs by multinuclear NMR Spectroscopy: Application to a bacteriophage λ N-peptide/boxB RNA complex. *J Am Chem Soc* 119(29):6711–6721.

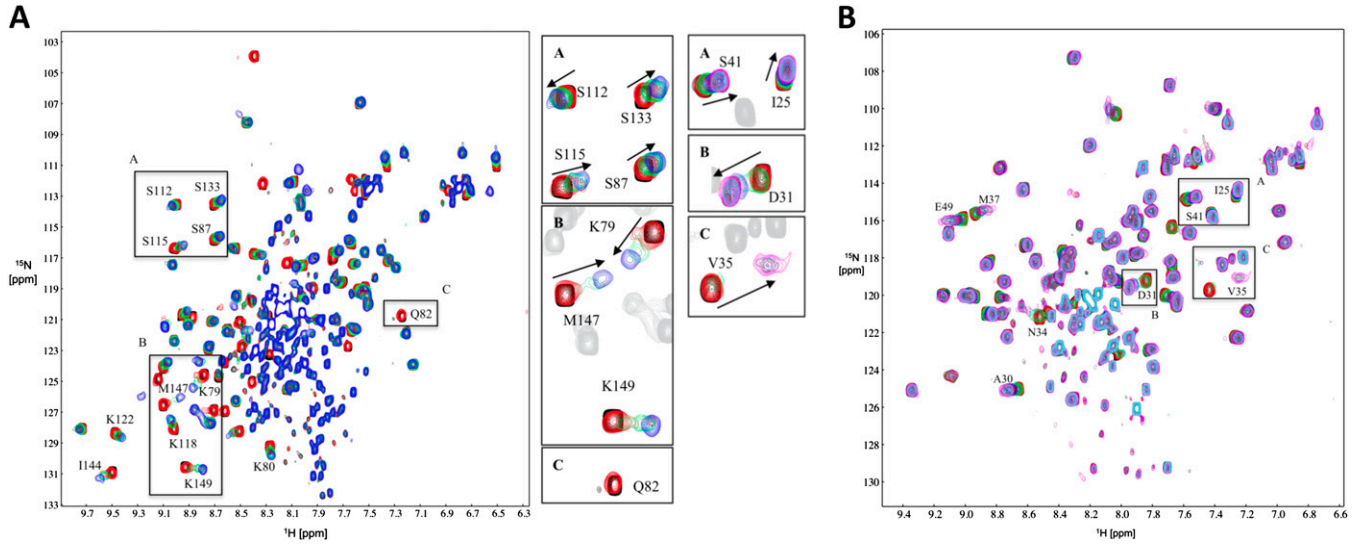

Fig. S1. ^{1}H-^{15}N HSQC spectra of (*A*) ^{15}*N*-labeled Sgt2_NT with increasing additions (black, 0; red, 0.25; green, 0.5; blue, 1 molar equivalents) of unlabeled Get5_UBL and (*B*) ^{15}*N*-labeled Get5_UBL with increasing additions (black, 0; red, 0.12; green, 0.36; blue, 0.6; purple, 0.84; cyan, 1 molar equivalent) of unlabeled Sgt2_NT. In both cases, the unlabeled binding partner was added until no further peak shifts occurred. Expanded boxes indicate residues displaying (*A*) fast, (*B*) intermediate, and (*C*) slow exchange properties.

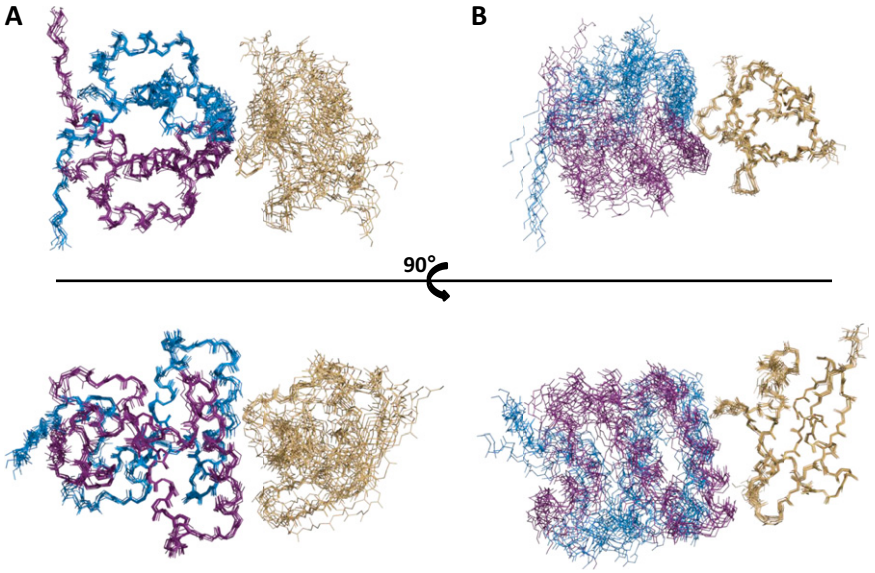

Fig. S2. Lowest energy ensemble of Sgt2_NT (blue/violet)/Get5_UBL (gold) complex structures generated by HADDOCK from chemical shift perturbation data and intermolecular NOEs: (*A*) ensemble aligned to Sgt2_NT dimer and (*B*) ensemble aligned to Get5_UBL structure.

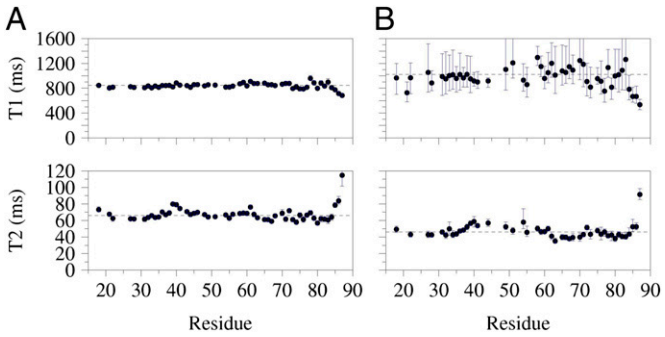

Fig. S3. Trimmed mean T_1 and T_2 relaxation data for (*A*) free Sgt2_NT dimer and (*B*) Sgt2_NT/Get5_UBL complex. The estimated rotational correlation times are 11 and 15 ns for the free dimer and full complex, respectively, which supports the presence of a complex comprising one dimer of Sgt2_NT and a single-bound copy of Get5_UBL.

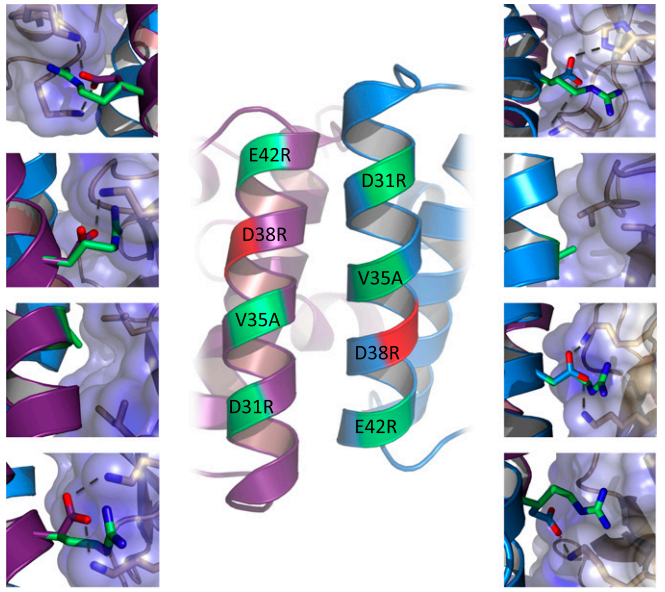

Fig. S4. Interactions in the Sgt2_NT/Get5_UBL complex probed by site-directed mutagenesis. (*Center*) Binding surface of Sgt2_NT with the position of key residues indicated. Sgt2 is shown in ribbon representation with the two monomers colored magenta and cyan. (*Left* and *Right*) Expanded views of the contacts made in each of the residues named in the middle panel and the result of mutation. Sgt2 is colored as in the middle panel and shown in ribbon and stick representation. Get5 is shown with semitransparent surface representation with interacting residues shown as sticks.

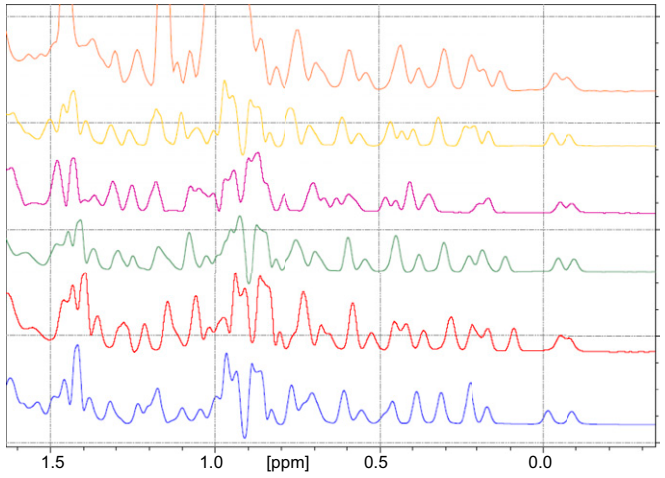

Fig. S5. Methyl regions of 1D NMR spectra indicating structure conservation in Sgt2_NT mutants. Orange, WT; yellow, V35A; purple, D38R; green, E42R; red, F16Y; blue, D31R.

Table S1. Summary of Sgt2_NT dimer solution structural statistics.

	Ensemble (20 structures)
Distance and dihedral restraints	
NOEs	
Intraresidue	974
Sequential	512
Medium range	416
Long range	414
Intermonomer	244
Total unambiguous	2,560
Ambiguous	1,092
Total NOE-derived	3,652
Hydrogen bond restraints*	58 (29 H-bonds)
Dihedral angles ($\varphi + \psi$)	102 (51 residues)
Structural statistics	
Ramachandran Plot (%)†	
Most favored	90.1
Additionally allowed	8.8
Generously allowed	0.4
Disallowed	0.6
RMSD from experimental restraints	
Distances (Å)	0.011 ± 0.001
Dihedrals (°)	0.132 ± 0.124
RMSD from idealized geometry	
Bonds (Å)	0.0029 ± 0.0001
Angles (°)	0.412 ± 0.0013
Impropers (°)	1.096 ± 0.0086
NOE violations	
> 0.5 Å	0 ± 0
> 0.3 Å	0.3 ± 0.7
> 0.1 Å	11 ± 2.9
Structural precision	
RMSD from average structure (Å)	
Backbone (all/2ndary structure)	0.99 ± 0.17/0.40 ± 0.07
Heavyatom (all/2ndary structure)	0.85 ± 0.06/1.46 ± 0.20

*Applied to each monomer.
†From Procheck-NMR.

Table S2. Summary of Get5 _UBL data collection and refinement statistics

X-ray source	Diamond I02
Data processing	Mosflm/Scala
Space group	P 3_2 2 1
Unit-cell parameters	
	a = b = 47.190 Å
	c = 78.410 Å
	$\alpha = \beta = 90.00°$
	$\gamma = 120.00°$
Wavelength/Å	0.97950
Resolution/Å	40.9–1.78 (1.88–1.78)
Measured reflections	78,172 (11,244)
Unique reflections	10,128 (1,441)
Mn(I/sd)	20.8 (5.13)
Completeness (%)	99.8 (100.0)
Multiplicity	7.72 (7.80)
Rmeas (%)	0.06 (0.37)
Solvent content (%)	57
R_{work}/R_{free} (%)	20.1/24.8
Protein atoms	894
Solvent atoms	131
RMSD from ideal	
Bond lengths (Å)	0.024
Bond angles (°)	1.987
Average B factor (Å^2)	24.1
Ramachandran favored region (%)	98.72
Ramachandran outlier region (%)	0

Table S3. ITC results for Sgt2_NT mutants binding to Get5_UBD

Construct	K_a (mol^{-1})	ΔH (kCal/mol)	ΔS (cal/mol/Δeg)	N	K_d (μM)	Relative affinity loss
WT	9.2×10^6	−12.7	−8.7	1.1	0.11	1
F16Y	6.5×10^6	−11	−5.1	1.4	0.15	1
D31R	2.5×10^5	−3.7	+12.4	0.8	4.0	36×
V35A	2.5×10^5	−13.2	+13.7	1.3	4.0	36×
D38R	ND*	ND	ND	ND	ND	>100×[†]
E42R	6×10^5	−13.4	−17.6	0.8	1.7	15×

*Not detected by ITC.
[†]Lower limit for the loss of affinity.