Supporting Information

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SI Materials and Methods

Sample Preparation. IAA17_{III-IV} (G109-L217) and ARF5_{III-IV} (T789-G885) were cloned into a pET28a vector (Merck Millipore) with an N-terminal His6 tag. Mutations were introduced to generate monomeric proteins: K114M for IAA17_{M1}, D183N/ D187N for IAA17_{M2}, K797M for ARF5_{M1}, and D847N/D851N ARF5_{M2}. IAA17_{M2} was used for structure calculation and dynamics using MMR spectroscopy. Cysteine residues were further mutated into alanine or serine residues for calorimetry to avoid the use of reducing agents, such that the C203A mutation was introduced into IAA17_{III-IV}, and C825S/C866S/C869S into ARF5_{III-IV}. Site-directed mutagenesis was performed using the QuikChange Kit (Agilent Technology), and the new constructs were verified by DNA sequencing. The plasmids were introduced into Escherichia coli strain BL21-CodonPlus(DE3)-RIL (Agilent Technology) for expression. Transformed cells were grown in Luria Bertani or minimal media (with ¹⁵NH₄Cl and/or ${\rm ^{13}C_6\mathchar`-glucose}$ as the sole nitrogen or carbon sources, respectively). Protein expression was induced by 1 mM isopropyl-D-thiogalactopyranoside at an A_{600} of 0.6~0.8, and the cells were harvested by centrifugation after 5 h of induction. The pellet were resuspended in 50 mL (per liter of culture) of 20 mM Tris, pH 7.4, 200 mM NaCl, 2 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, lysed using Emulsiflex C3 (Avestin), and centrifuged at $25,000 \times g$ for 20 min. The supernatant fraction was loaded onto a HisTrap HP column (GE Healthcare), and the fusion protein was eluted with a 100-mL gradient of imidazole (15-500 mM). Fractions containing the protein were identified by SDS-polyacrylamide gel electrophoresis. The fusion protein was then dialyzed against 20 mM Tris, pH 8.0, 100 mM NaCl, and 2 mM β-mercaptoethanol, and the His₆ tag was cleaved by TEV protease. The digestion reaction was loaded onto the HisTrap column. The protein was

- Bax A, Kontaxis G, Tjandra N (2001) Dipolar couplings in macromolecular structure determination. *Methods Enzymol* 339:127–174.
- Farrow NA, Zhang O, Forman-Kay JD, Kay LE (1994) A heteronuclear correlation experiment for simultaneous determination of 15N longitudinal decay and chemical exchange rates of systems in slow equilibrium. J Biomol NMR 4(5):727–734.
- Delaglio F, et al. (1995) NMRPipe: A multidimensional spectral processing system based on UNIX pipes. J Biomol NMR 6(3):277–293.

further purified by size exclusion chromatography using a HiLoad Superdex 75 column (GE Healthcare) and then by anion exchange chromatography using a monoQ column (GE Healthcare). All protein samples were finally dialyzed against 10 mM sodium phosphate, pH 7.4, and 10 mM DTT.

NMR Spectroscopy. NMR spectra were recorded at 25 $^\circ\!\mathrm{C}$ on Bruker 600-, 700-, 800-, 900-MHz spectrometers equipped with a z-shielded gradient triple resonance probe. The NMR sample contained 1 mM ${}^{13}C$, ${}^{15}N$ -IAA17_{M2} in 10 mM sodium phosphate, pH 7.4, and 10 mM DTT. Sequential and side chain assignments of ¹H, ¹⁵N, and ¹³C resonances was achieved by 3D triple resonance through-bond scalar correlation experiments (CBCACONH, HNCACB, HBHA(CO)NH, HNCO, HN(CA)CO, HCCH-TOCSY, and ¹⁵N-TOCSY-HSQC). 3D ¹³C-separated NOESY and ¹⁵Nseparated NOESY experiments were obtained using the mixing time of 120 ms. Residual ¹D_{NH} dipolar couplings were obtained by taking the difference in the J splitting values measured in oriented (6.5% neutral gel alignment medium) and isotropic (water) media using 2D in-phase/antiphase ¹H-¹⁵N HSQC spectrum (1). ${}^{15}N$ -R₁ and ${}^{15}N$ -R₂ relaxation, and ${}^{1}H$ - ${}^{15}N$ heteronuclear NOE measurements were carried out using pulse schemes described previously (2). Delays of 10, 20, 50, 100, 400, 800, 1200, 1500 ms were used for the R_1 relaxation measurement, and 17.0, 33.9, 50.9, 67.8, 101.8, 118.7, 152.6, 203.5 ms were used for the R₂ relaxation measurement. NMR spectra were processed using the NMRPipe program (3), and analyzed using PIPP (4) and NMRView (5) programs. NMR titration experiments were recorded at 25 °C on a Bruker 600 MHz spectrometer. ¹H-¹⁵N HSQC spectra were recorded with $0.2 \text{ mM}^{15}N$ -IAA_{M1} or ${}^{15}N$ -IAA_{M2} titrating stoichiometrically with the partner proteins, and changes in the backbone amide chemical shifts were measured.

- Garrett DS, Powers R, Gronenborn AM, Clore GM (1991) A common sense approach to peak picking in two-, three-, and four-dimensional spectra using automatic computer analysis of contour diagrams. J Magn Reson 95(1):214–220.
- Johnson BA, Blevins RA (1994) NMR View: A computer program for the visualization and analysis of NMR data. J Biomol NMR 4(5):603–614.



Fig. S1. Size exclusion chromatograms using a Superdex 75 10/300 GL column (GE Healthcare) for IAA17_{III-IV} at varying concentrations (A) and IAA17_{M1} and IAA17_{M2} (B). The injection concentrations of IAA17_{III-IV} were 400 μ M, 200 μ M, 150 μ M, 50 μ M, and 10 μ M, and the elution concentration measured by the peak height were 103 μ M, 50 μ M, 40 μ M, 12 μ M, and 3 μ M, respectively.



Fig. 52. ¹H-¹⁵N HSQC spectra of ¹⁵N-IAA17_{III-IV} (A), ¹⁵N-IAA17_{M1} (B), and ¹⁵N-IAA17_{M2} (C) in 10 mM sodium phosphate, pH 7.4, at 25 °C. Protein concentration was 0.3 mM for each sample.

	β1	β 2	α1			α1	•	β3	(34	α2	β5	α3	
				MM	۱۸	\mathcal{M}	ΛΛΛΛ				\mathcal{M}	\ <u> </u>		
TAA17			RMYKSYDEL	SNAL SNMES	SETMGKHG	GEEGMIDEMNER	KI MDI VNSWD	YVPSYF	OKDGD		PWPMEVD	CKRIRI	MKGSDATGI	APRAMEK-CKSRA
170(17)	110	120	130	140	150	160	170	180		190	200)	210	220
IAA17	AAFV <mark>K</mark> VSMDG	APYLRKIDL	RMYKSYDEL	SNALSNMFS	SFTMGKHG	GEEGMIDFMNER	KLMDLVNSWD	YVPSYE	KDGD	WMLVGDV	PWPMFVD1	CKRLRL	MKGSDAIGL	APRAMEK-CKSRA
IAA7	AGLV <mark>K</mark> VSMDG	APYLRKVDL	KMYKSYQDL	SDALAKMES	SFTMGNY-	GAQGMIDFMNES	KLMNLLNSSE	YVPSYE) KDG D	WMLVGDV	PWEMFVES	CKRLRI	1KGSEAVGL	APRAMEKYCKNRS
IAA14	VAFV <mark>K</mark> VSMDG	APYLRKVDL	KMYTSYKDL	SDALAKMFS	SFTMGSY-	GAQGMIDFMNES	KVMDLLNSSE	YVPSYE) KDG D	WMLVGDV	PWPMFVES	CKRLRI	MKGSEAIGL	APRAMEKF-KNRS
IAA16	VAYV <mark>K</mark> VSMDG	APYLRKIDL	KLYKTYQDL	SNALSKMFS	SSFTIGNY-	GPQGMKDFMNESI	KLIDLLNGSD	YVPTYE	okdgd	WMLVGDV	PWEMFVDS	CKRIRI	MKGSEAIGL	APRALEK-CKNRS
IAA27	CLYVKVSMEG	APYLRKIDL	KTYKSYLEL	SSALEKMFS	SCFTIGQFGSH	GGCG-RDGLNES	RLTDLLRGSE	YVVTYE	OKDSD	WMLVGDV	PWEMFICS	CKKLRI	MKSSEAIGL	APRVMEK-CRSRN
IAA8	VLFVKVSMDG	APYLRKVDL	RIYISYQQL	SALEKMES		GAQG-RERMSEI	KLKDLLHGSE	FVLIYE	KDGD	WMLVGDV	PWEIFIEI	CQKLKI	1KGSDSIGL	APGAVEK-SKNKERV
1449	ALFVKVSMDG					GAAG-KUMLSEII		EVETVE			PWEMFID		MKGCDAIGL	AAAPRAMEK-SKMRA
ΤΔΔΟ				KALENNE-	KVMIGE VC	EREG		EVPTYE	KDGD		PWDMESSS	CKBIBI	INGSEAFTA	DSSI
TAA3		APYIRKIDI	SCYKGYSEL	KALEVME-	KESVGE-YE	ERDG -	YKGSD	EVPTYE	KDGD	WHL TGDV	PWEMETCT	CKRIRI	IKGSEAKGI	6C6V
IAA4	GNYVKVSMDG	APYLRKIDL	TMYKOYPELI	1KSLENMF-	KESVGEYE	EREG	YKGSD	FVPTYE	KDGD	WMLVGDV	PWEMEVSS	CKRLRI	1KGSEVKGL	GCGGL
IAA5	YVKVSVDG	AAFLRKIDL	EMYKCYQDL	ASALQILFO	CYINFDDTLK	ESEC		-VPIYE	KDGD	WMLAGDV	PWEMFLGS	CKRLRI	MKRSCNRG-	
IAA6	IGYV <mark>K</mark> VSMDG	VPYMRKIDL	GSSNSYINL	/TVLENLFG	GCLGIGVA-KE	GKKC	E	YIIIYE	KDRD	WMLVGDV	PWQMFKES	CKRLRI	/KRSDATGF	GLQQD
IAA19	LGYV <mark>K</mark> VSMDG	VPYLRKMDL	GSSQGYDDL	AFALDKLFG	GFRGIGVALKD	GDNC	E	YVTIYE) KDG <mark>D</mark>	WMLAGDV	PWGMFLES	CKRLRI	1KRSDATGF	GLQPRGVDE
IAA15	RKYV <mark>K</mark> VALDG	AAYLRKVDL	GMYDCYGQL	FTALENMF-		QGIITIC	RVTELERKGE	FVATYE) KDG D	LMLVGDV	PWMMFVES	CKRMRL	MKTGDAIGL	
IAA10	SML V <mark>K</mark> VTMDG	VIIGRKVDL	NALDSYAAL	EKTLDLMFF	QIPSPVTRSN	TQGY-KTIKETC	TSKLLDGSSE	YIITYQ	KDGD	WMLVGDV	PWQMFLGS	VTRLRI	1KTSIGAGV	GK
IAA11	SMFVKVTMDG	IPIGRKIDL	NAHKCYESL	SNTLEEMFL	KPKLGSR	TLET-DGHMETP	VKILPDGSSG	LVLTYE	DKEGD	WMLVGDV	PWGMFIGS	VRRLRI	MKTSEATGK	AQMIL
IAA12	LGFVKVNMDG	VGIGRKVDM	RAHSSYENL	AQILEEMFF	GMIGII	CREKVKI	PLRLLDGSSD	FVLIYE	KEGD	WMLVGDV	PWRMFINS	VKRLRI	1GTSEASGL	APRRQEQKDRQRNNPV
1AA13								FVLIYE	NECD		PWRMELINS		MKISEANGL	AARNQEPNERQRKQPV
TAATO	SEVWKVNMEG			TOTI DEMEN		991E					PWEMELST			
IAA26	GMEVKINMDG	VPIGRKIDL	NAYNSYFOL	SEVVDKLER		DGOGFEKP		ETI TYE	NEGD	KMI VGDV	PWOMEVS	VKRLRV	IKSSEISSA	
IAA28	ELYVKINMEG	VPIGRKVNL	SAYNNYOOL	SHAVDOLF-	SKKDS-		WDLNRO	YTLVYE	DTEGD	KVLVGDV	PWEMFVST	VKRLHVI	KTSHAFSL	SPRKHGKE
IAA30	SFYVKVNMEG	VPIGRKIDL	LSLNGYHDL	ITTLDYMFN	ASILWAEEED	MC	SEKS	HVLTYA	KEGD	WMMVGDV	PWEMFLSS	VRRLKIS	SRA-YHY	
IAA31	SLFV <mark>K</mark> VYMEG	VPIGRKLDL	CVFSGYESL	LENLSHMFD	DTSII	-CGN	RDRKH	HVLTYE) KDG <mark>D</mark>	WMMVGDI	PWDMFLET	VRRLKI	TRP-ERY	
IAA29	SMYV <mark>K</mark> VKMDG	VAIARKVDI	KLFNSYESL	TNSLITMFT	EYEDCDREDT	N		YTFTFQ	GKEG <mark>D</mark>	WLLRGDV	TWKIFAES	VHRISI	IRDRPCAYT	RCLF
IAA32	– – YV <mark>K</mark> VNLDG	LVVGRKVCL	VDQGAYATL	ALQLNDMFG	GMQTVSGLRLF	QTES	E	FSLVYR	REGI	WRNVGDV	PWKEFVES	VDRMRI	ARRNDALLP	F
IAA34	YV <mark>K</mark> VTMDG	VVGRKVCVL	DHGSYSTLA	HQLEDMFGM	IQSVSGLRLFQ	MES	EF	CLLVYR	DEEGL	WRNAGDV	PWNEFIES	VERLRI	FRRNDAVLP	F
IAA33	VTVVLEG	RSICQRISL	DKHGSYQSL	ASALRQMEV	/DGADSTDDLD	LSNA	IPG	HLIAYE	DMEND	LLLAGDL	TWKDFVR	AKRIRII	_PVKGNTRQ	VKRNE
ARFI	RSCIKVHMQG	SAVGRAIDL		TNDIEKLER	VIKGELLES-II	KKWQ		VVYI			PWNEFCGP		I PEEVKKL	
ARF9 ADE11		TAVCRAVDL		INDIEVTLP Indievtler	TECELS-D-K			IVFI			PWPEFCNP		VOREEVRRI	
ARE18	RSRTKVOMOG	TAVGRAVDL	TELKSYDEL		TOGOLI - A - RI	DKWT			DEGD		PWNEECKN	AKKIFI	YSSDEVKKM	TTKIKISSSI -ENE
ARF12	RTCTKVOMOG	VTIGRAVDI			TKGOLOTR	NOWF		TAFT	SDFD	KMI VGDD	PWPEECNM	VKKIFI	OKRR	RISSE ENE
ARF22	RTCTKVOMOG	VTIERAVDL	SVLNGYDOL	ILELEELFD	DLKGOLOTR	NOWE		IAFT	SDDD	KMLVGDD	PWPEFCNM	IVKKILI	KRGGOKLE	V0
ARF15	RTCTKVQMQG	VTIGRAVDL	SVLNGYDQL	ILELEKLFD	LKGQLQTR	NQWK		IIFT	SDED	EMLVGDD	PWPEFCNM	IVKRIYI(2KRR	
ARF20	RTCTKVQMQG	VTIGRAVDL	SVLNGYDQL	ILELEKLFD	DLKGQLQTR	NQWK		IAFT	SDGY	EMLVGDD	PWPEFCKM	IVKKILIY	SKEEVKNL	KSSKSLSS
ARF21	RTCT <mark>K</mark> VQMQG	VTIGRAVDL	SVLNGYDQL	ILELEKLFD	DIKGQLQTR	NQWK		IAFT) S D G <mark>Y</mark>	EMLVGDD	PWPEFCKM	IVKKILIY	YSKEEVKNL	KSSKSLSS
ARF14	RTCT <mark>K</mark> VQMQG	VTIGRAVDL	SVLNGYDQL	ILELEKLFD	DLKGQLQAR	NQWE		IAFT	NEED	KMLVGED	PWPEFCNM	IVKKIFI	YSKEEVKNL	KSRKSLSS
ARF2	RSCTKVHKQG	IALGRSVDL	SKFQNYEEL	VAELDRLFE	FNGELMAP-K	KDWL		IVYT	DEEND	MMLVGDD	PWQEFCCM	IVRKIFI	TKEEVRKM	NPGTLSCRSE-EEA
AKF4	RICIKVHKUG	SUVGRAIDL	SKLNGYDDL			KGWK		ILYI	JSENU VECD		PWHDFCN	WRITE	I KEEVENA	NDDNKSCLEQAALMMEASKSS
ARES		S-FCPSIDT	I SEKUTEELI SVESSVHEII	VONTECULE		SCW0				VIIICOD	PWPEEVS			CKRCIELINSARSSNNUDVIR
ARES	KNEVKVYKSG	S-VGRSIDT	SRESSYHEL	SEEL GKMEA	ATEGILEDPIR	SGWQ			KEND	TILLGDD	PWESEVNN	IVWAIKII		GDHGFGSGGIFP
ARF7	RTYTKVOKRG	S-VGRSIDV	NRYRGYDFU	RHDLARMEG	TEGOLEDPOT	SDWK		LVYV	HEND	ILLVGDD	PWEEFVNO	VOSIKT	SSAEVOOM	SLDGNFAGVP
ARF19	RTYTKVOKRG	S-VGRSIDV	TRYSGYDEL	RHDLARMFO	SIEGQLEDPLT	SDWK		LVYT	HEND	ILLVGDD	PWEEFVNC	VQNIKI	SSVEVQOM	SLDGDLAAIP
ARF10	TGHCKVFMES	EDVGRTLDL	SVIGSYQEL	YRKLAEMFH	IIEERSD	LLTH		VVYR	ANGV	IKRIGDE	PFSDFMKA	TKRLTI	KMDIGGDNV	RKTWITGIRTGENGIDASTKT
ARF16	TGHC <mark>K</mark> VFMES	DDVGRTLDL	SVLGSYEEL	SRKLSDMFG	SIKK-SE	MLSS		VLYR	ASGA	IKYAGNE	PFSEFLKT	ARRLTI		LTEQ

Fig. S3. Multiple sequence alignment of *A. thaliana* IAA and ARF family proteins against IAA17_{III-IV} using the program ClustalW. Lys114, Arg124, Lys125, Arg205, and Arg207 at the positive surface are highlighted by blue boxes, and Asp183, Asp185, Asp187, Asp193 at the negative surface are highlighted by red boxes in the IAA17 sequence at the top. In the sequence alignment, highly conserved Lys114 at the positive surface, and Asp183 and Asp187 at the negative surface are shaded in blue and red. Sequences of ARF3, ARF13, and ARF17 are less conserved and not included in the alignment.

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Fig. S4. Plots of relaxation parameters of backbone amide groups of IAA17_{M2}. ¹⁵N R₁ relaxation (A), ¹⁵N R₂ relaxation (B), and ¹H–¹⁵N heteronuclear NOE data (C) are shown as a function of residue number. The secondary structures of IAA17_{M2} are shown on top in a schematic representation.



Fig. S5. Superimposed ${}^{1}H-{}^{15}N$ HSQC spectra of ${}^{15}N$ -IAA17_{M2} (black) and ${}^{15}N$ -IAA17_{M2}(Δ 159–169) (red) in 10 mM sodium phosphate, pH 7.4, at 25 °C. The missing amide resonances in ${}^{15}N$ -IAA17_{M2}(Δ 159–169) due to the truncation of the α 1' helix are annotated with residue names and numbers. Amide resonances with chemical shift changes are also annotated, in italics.



Fig. S6. Raw ITC data (*Upper*) and integrated heats of injection (*Lower*) for the titration between IAA17_{M1} and IAA17_{M2}(Δ 159–169) (*A*), and ARF5_{M1} and IAA17_{M2}(Δ 159–169) (*B*). In the bottom panels, squares are the experimental data, and solid lines represent the least-squares best fit curves derived from a simple one-site binding model.



Fig. S7. Size exclusion chromatograms using a Superdex 75 10/300 GL column (GE Healthcare) for ARF5_{M1} (blue), ARF5_{M2} (red), and the complex between ARF5_{M1} and ARF5_{M2} (black).

Table S1. Population of the monomer and oligomers of IAA_{III-IV} obtained by the monomer-oligomer equilibrium model

Number of participating species	Monomer	Dimer	Trimer	Tetramer	Pentamer	Hexamer	Heptamer	Octamer	Nonamer	Decamer	Calculated average subunit numbers	Average subunit numbers from SEC
2	28.1%	71.9%									1.72	3.74
3	21.4%	31.7%	46.9%								2.25	3.74
4	19.9%	23.0%	26.5%	30.7%							2.60	3.74
5	19.6%	19.8%	20.0%	20.2%	20.4%						3.02	3.74
6	19.7%	18.4%	17.2%	16.0%	14.9%	13.9%					3.30	3.74
7	20.0%	17.7%	15.7%	13.9%	12.3%	10.9%	9.6%				3.52	3.74
8	20.3%	17.3%	14.8%	12.7%	10.8%	9.3%	7.9%	6.8%			3.70	3.74
9	20.6%	17.2%	14.3%	12.0%	10.0%	8.3%	7.0%	5.8%	4.8%		3.85	3.74
10	20.9%	17.1%	14.0%	11.5%	9.4%	7.7%	6.3%	5.2%	4.3%	3.5%	3.96	3.74

The calculated average subunit numbers from the model and experimental average subunit numbers from SEC are presented. The peak height concentration of IAA_{III-IV} in SEC was 102.9 μ M. The calculation was carried out using 102.9 μ M of IAA_{III-IV} as a total protein concentration and 6.6 μ M as an equilibrium dissociation constant. The calculation predicts that the equilibria between monomer and oligomers up to an octamer can reproduce the experimental oligomer size.