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 His$_6$ 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 $^{15}NH_4Cl$ and/or $^{13}C_6$-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 × *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 His$_6$ tag was cleaved by TEV protease. The digestion reaction was loaded onto the HisTrap column. The protein was 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 °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 ^{1}H, ^{15}N, and ^{13}C resonances was achieved by 3D triple resonance through-bond scalar correlation experiments (CBCACONH, HNCACB, HBHA(CO)NH, HNCO, HN(CA)CO, HCCH-TOCSY, and ^{15}N-TOCSY-HSQC). 3D ^{13}C-separated NOESY and ^{15}N-separated NOESY experiments were obtained using the mixing time of 120 ms. Residual $^{1}D_{NH}$ 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 $^{1}H-^{15}N$ HSQC spectrum (1). ^{15}N-R_1 and ^{15}N-R_2 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 R_2 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. $^{1}H-^{15}N$ HSQC spectra were recorded with 0.2 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.

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3. Delaglio F, et al. (1995) NMRPipe: A multidimensional spectral processing system based on UNIX pipes. *J Biomol NMR* 6(3):277–293.
4. 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.
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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 μM, 200 μM, 150 μM, 50 μM, and 10 μM, and the elution concentration measured by the peak height were 103 μM, 50 μM, 40 μM, 12 μM, and 3 μM, respectively.

Fig. S2. ^{1}H–^{15}N HSQC spectra of ^{15}N-IAA17$_{III–IV}$ (*A*), ^{15}N-IAA17$_{M1}$ (*B*), and ^{15}N-IAA17$_{M2}$ (*C*) in 10 mM sodium phosphate, pH 7.4, at 25 °C. Protein concentration was 0.3 mM for each sample.

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.

Fig. S4. Plots of relaxation parameters of backbone amide groups of $IAA17_{M2}$. ^{15}N R_1 relaxation (*A*), ^{15}N R_2 relaxation (*B*), and $^{1}H-^{15}N$ 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-$IAA17_{M2}(\Delta 159-169)$ (red) in 10 mM sodium phosphate, pH 7.4, at 25 °C. The missing amide resonances in ^{15}N-$IAA17_{M2}(\Delta 159-169)$ due to the truncation of the α1′ 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 $IAA17_{M2}$(Δ159–169) (*A*), and $ARF5_{M1}$ and $IAA17_{M2}$(Δ159–169) (*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 μM. The calculation was carried out using 102.9 μM of IAA_{III-IV} as a total protein concentration and 6.6 μM 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.