Supplementary Figure 1: SAXS measurements of Arp4.

A) Measured scattering curve of yArp4 after buffer correction. The measured molecular weight of the protein calculated from Intensity at zero angle in the Gunier-Plot (not shown) is $M_w=51$ kDa in good agreement with the determined $M_w$ from the Porod-volume of 55.6kDa (2.1mg/ml) and 56.5 kDa (4.7mg/ml). This indicates that Arp4 is monomeric in solution with a radius of gyration of $R_G=2.8$nm obtained from the slope in the Guinier-plot.

B) Kratky-Plot ($I*s^2$ vs. s) of Arp4 has the typical bell shaped form of a folded protein (see e.g. (Putnam et al, 2007)).
C) Calculated P(r)-distribution obtained using a maximum particle diameter of \( D_{\text{max}} = 9.7 \text{nm} \).

D) Comparison between the measured data (blue points) with the theoretical scattering curve of the yArp4-crystal structure calculated with CRYSOL (Svergun et al, 1995).

Supplementary Figure 2: SAXS measurements of Arp8.

A) Normalized scattering curves of yArp8 (blue) and its truncated version (red) lacking the first 255 amino acids. The shape already suggests that yArp8-truncated is more compact and globular.

B) Kratky-plots (\( I_s^2 \) vs \( s \)) for both proteins. The bell-shaped curves indicate that both yArp8 and yArp8-truncated are folded.

<table>
<thead>
<tr>
<th>Sample</th>
<th>conc. mg/ml</th>
<th>Mw from Porod-volume [kDa]</th>
<th>( R_g ) [nm]</th>
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<td>4.42</td>
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<td>(97.8)</td>
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<td>75.9</td>
<td>2.85</td>
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</table>
C) P(r)-distributions calculated using GNOM. Whereas full-length yArp8 seems to possess protrusions or elongated parts, the truncated yArp8 is clearly more compact.

D) Molecular weights and radii of gyration ($R_G$) determined from excluded volume (autoPorod, (Petoukhov et al, 2007)). The values in parentheses (yArp8-4.3 mg/ml) have somewhat lower data quality but also support that both yArp8 and yArp8-truncated are monomeric in solution.

Supplementary Figure 3: Sequence alignment of Arp8 with actin

Sequence alignment between *S.cerevisiae* Arp8 (top) and actin (bottom). Identical residues are shaded in red, similar residues in yellow. Note the long n-terminal tail of Arp8 that displays no homology with actin.
Supplementary Figure 4: Surface representation of Arp4 and actin

A) Surface representation of monomeric actin as found in the filament according to electron microscopy (pdb 3MFP) (Fujii et al); front view (left) and back view (right). The ATP nucleotide is depicted as sticks and the subdomains are numbered. In both orientations it becomes obvious that the nucleotide and its phosphate moieties are accessible to the surrounding solvent.

B) Surface representation of a front and back view of Arp4. The ATP nucleotide is depicted as sticks. Note that the nucleotide is much more shielded from the environment as compared to
actin. The back view illustrates that no opening to the solvent exists. It was chosen to compare Arp4 to actin monomers as found in the filament because actin mainly displays ATPase activity when it is assembled into filaments.
Supplementary Figure 5: Surface plasmon resonance data of Arp4 binding to actin

A) Arp4 was passed over a sensor chip containing immobilized actin in a concentration range of 1 – 60µM. Change in surface plasmon resonance was measured and the sensograms are depicted. 

B) The corresponding binding curve of Arp4 and actin can be used to estimate the $K_D$. Three independent measurements were performed and a $K_D$ of $1.6 \pm 0.8 \mu M$ was obtained.

Supplementary Figure 6: Pointed end elongation assay with Arp4.

20 nM actin filaments capped at the barbed end by 3 nM CapZ were used in polymerization assays to measure pointed end elongation. Increasing amounts of Arp4 inhibit pointed end elongation.
Supplementary Figure 7: Surface plasmon resonance data of Arp8 binding to actin

Arp8 was passed over a sensor chip containing immobilized actin in a concentration range of 0.01 – 10µM. Change in surface plasmon resonance was measured and the sensograms are depicted.
Supplemental methods:

Surface plasmon resonance experiments:

The experiments to test for the interaction between actin and Arp4 and Arp8 respectively were carried out on a Biacore X-100 machine (Biacore, GE Healthcare). About 300 resonance units of actin were immobilized on the surface of a CM5 chip (GE Healthcare) by amine coupling at a pH of 5.0. Binding experiments were performed in a buffer of 10 mM EPPS pH 8.0, 150 mM NaCl and 5 mM β-ME at a flow rate of 10 µl/min and 25°C. Functionality of the chip was tested by addition of DNaseI as a positive binding control and BSA as a negative control. Arp4 and Arp8 were passed over the chip in a concentration range of 1 – 60 µM and 0.01 – 10 µM respectively and allowed to bind for 120s. The changes in signal were measured and the sensograms recorded. The chips were regenerated after each successive round of binding by three alternate additions of running buffer containing 3 M NaCl and 0 M NaCl respectively. This regeneration procedure did not alter the ability of the immobilized actin to bind protein in subsequent cycles. Analysis of the data was performed using the BIAevaluation software supplied with the instrument. The steady-state binding response in the case of Arp4 was determined by averaging the response over 5s at the end of the injection and was corrected for background binding. Binding data were fitted to a 1:1 model.
Supplemental references


