Supplemental Figures

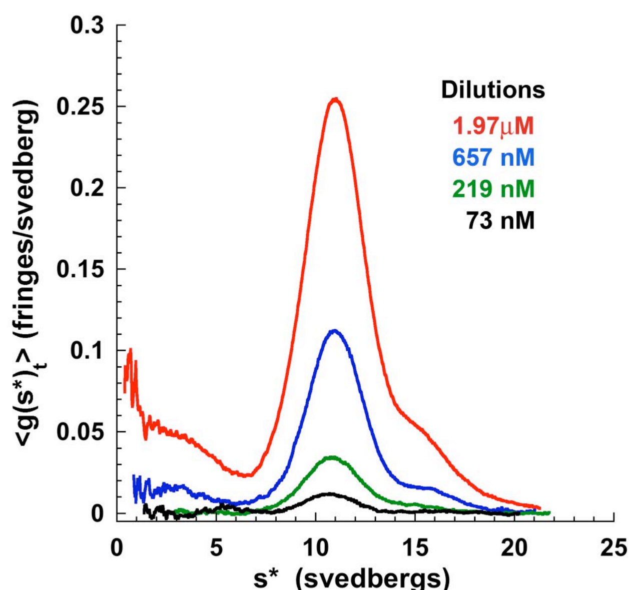

Figure S1. Actin-WCA binds Arp2/3 complex with higher affinity than WCA alone. The graph shows a dilution series AUC experiment of Arp2/3 complex with bound actin-WCA. The peak of the complex at ~11 S is the predominant species at all concentrations down to 73 nM, suggesting that the dissociation constant (K_d) is at or lower than this value. Note that WCA alone binds Arp2/3 complex with lower affinity (K_d=2 μM) (Panchal et al., 2003). The sample of Arp2/3 complex used in this experiment displays a shoulder at higher S values, which is an early indication of the formation an Arp2/3 complex dimer by internal crosslinking in the absence of reducing agents (see also Figure S2).

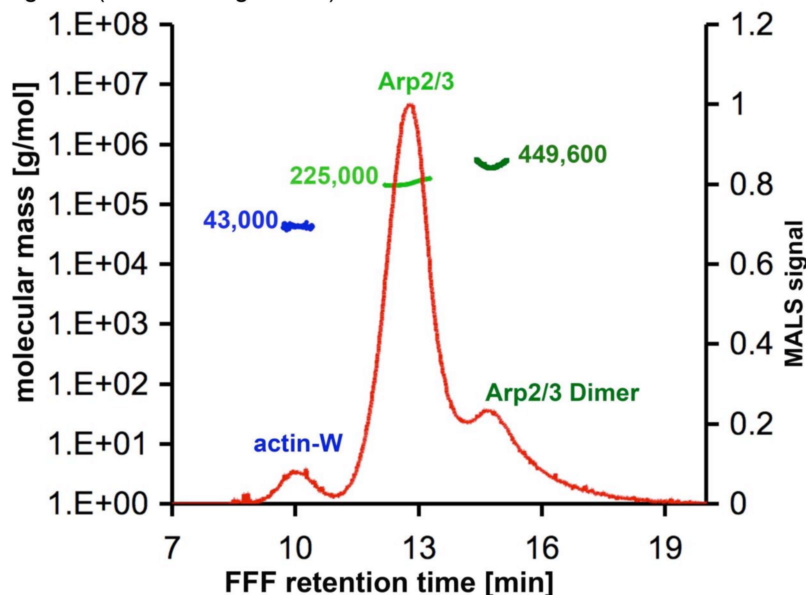

Figure S2. Actin-W does not bind Arp2/3 complex. Actin cross-linked with the W motif of N-WASP (amino acids 425-448) and Arp2/3 complex run separately on FFF-MALS, demonstrating a lack of interaction (curve corresponds to light scattering signal). This relatively older sample of Arp2/3 complex (kept on ice for ~20 days) also illustrates how in the absence of a reducing agent Arp2/3 complex dimerizes over time by intermolecular cross-linking (peak with molecular mass 449,600 Da). The use of reducing agents is precluded in this case by the need to preserve the crosslinked actin-W complex (or actin-WCA).

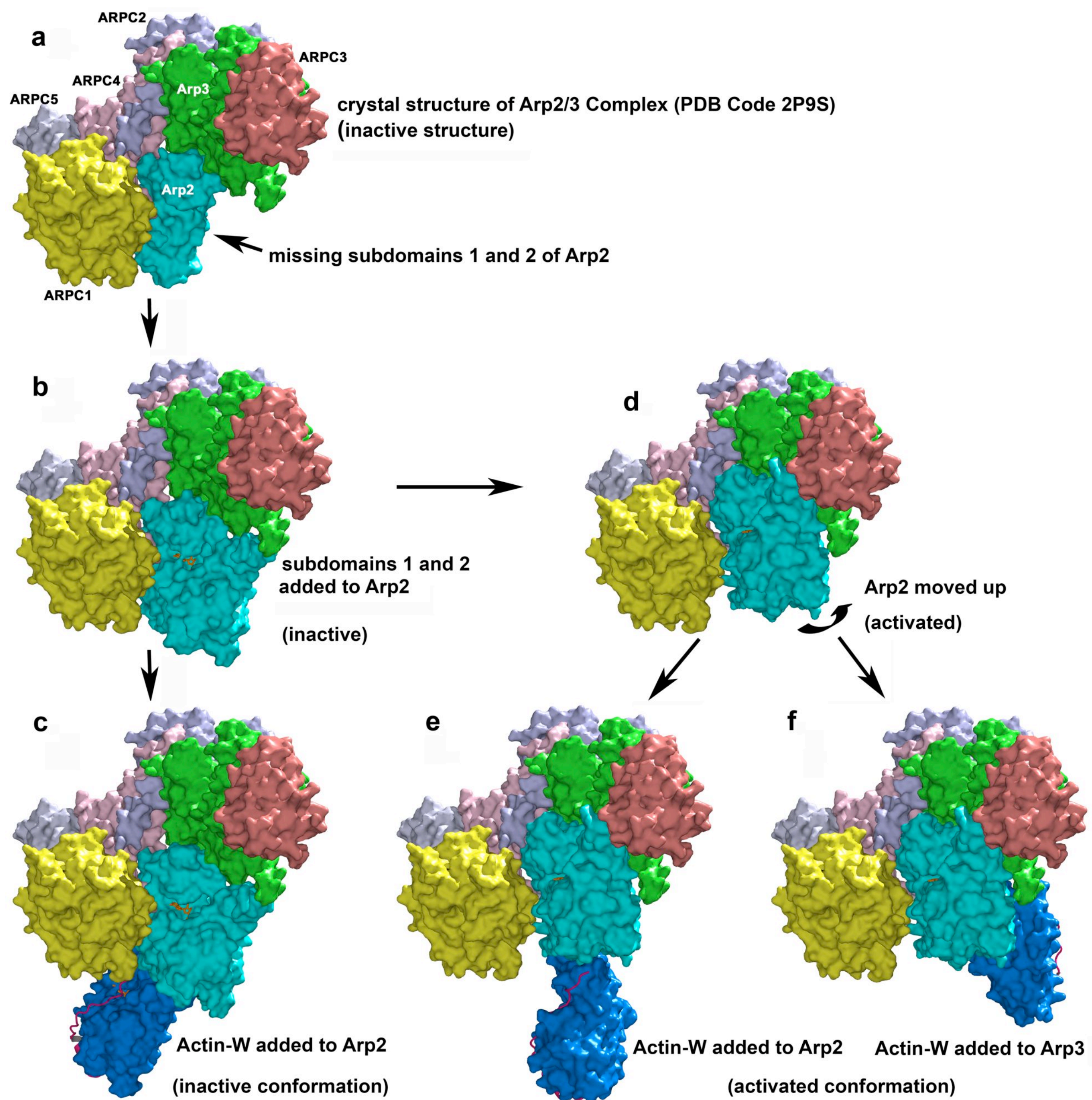

Figure S3. Models of Arp2/3 complex activation considered in this study.
(a) Crystal structure of inactive Arp2/3 complex with bound ATP (2P9S), which lacks subdomains 1 and 2 of Arp2 (Nolen and Pollard, 2007).
(b) Subdomains 1 and 2 of Arp2 were added to the crystal structure by analogy with actin.
(c) The crystal structure of actin-W (Chereau et al., 2005) was added at the barbed end of Arp2 according to the arrangement of actin subunits in the filament (Holmes et al., 1990).
(d) Activation of Arp2/3 complex by moving Arp2 alone next to Arp3, according to the actin filament.
(e) Addition of the structure of actin-W at the barbed end of Arp2 (best fit to the scattering data).
(f) Addition of actin-W at the barbed end of Arp3 (worse fit to the scattering data). Color scheme: W motif, red; actin, blue; Arp2, cyan; Arp3, green, ARPC1, yellow; ARPC2, purple; ARPC3, maroon; ARPC4, pink; ARPC5, gray (as indicated in part a).

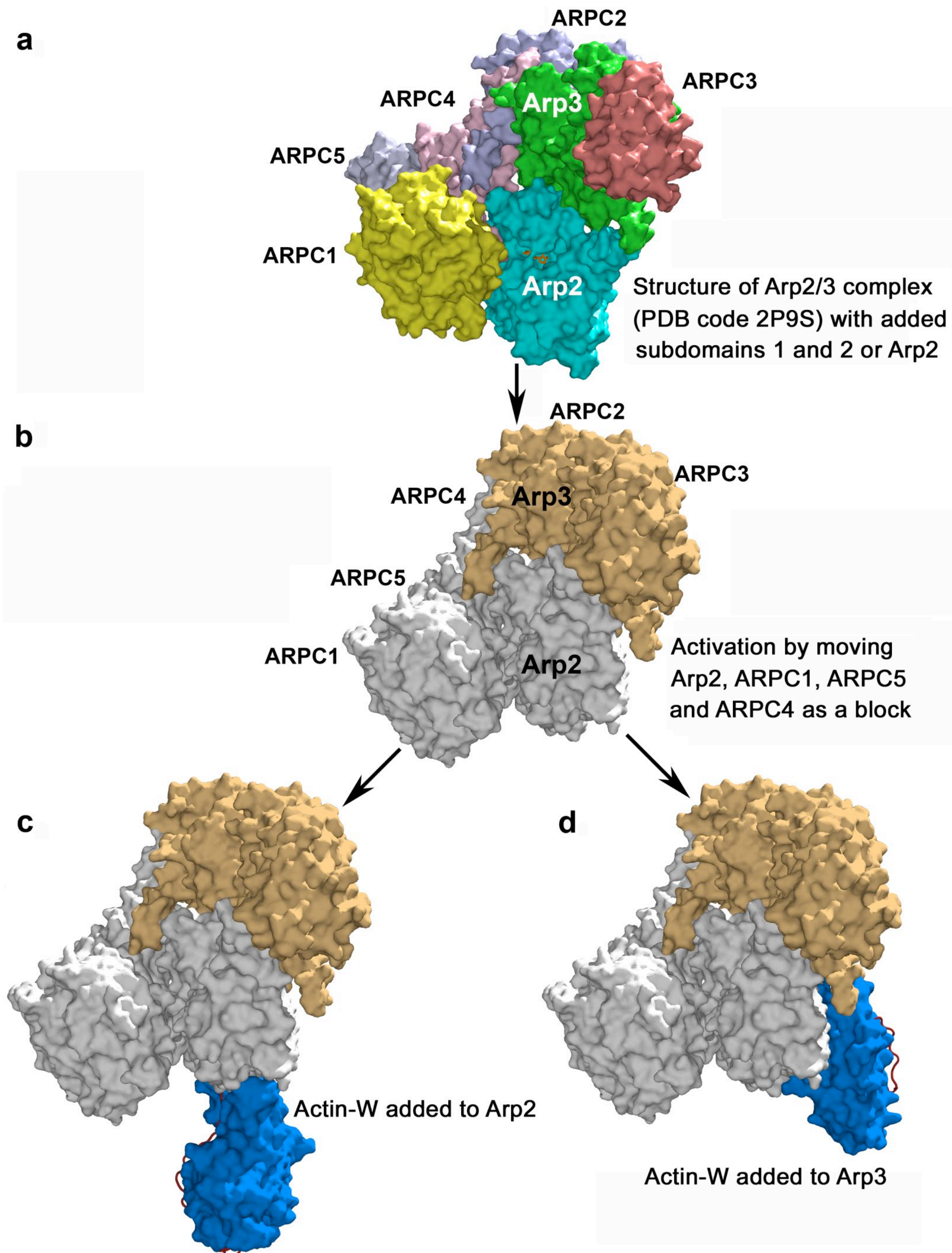

Figure S4. Activation of Arp2/3 complex by moving subunits Arp2, ARPC1, ARPC4 and ARPC5 as a group.
(a) Crystal structure of inactive Arp2/3 complex with bound ATP (2P9S) to which subdomains 1 and 2 of Arp2 were added by analogy with actin.
(b) Realignment of subunit Arp2 into a filament-like conformation with Arp3 by moving Arp2, ARPC1, ARPC4 and ARPC5 (gray) with respect to Arp3, ARPC2 and ARPC3 (orange).
(c-d) The crystal structure of actin-W (Chereau et al., 2005) was added at the barbed end of Arp2 or Arp3. Note that at low resolution these two models look very similar to the models in which only Arp2 was moved (Figures S3e and S3f).

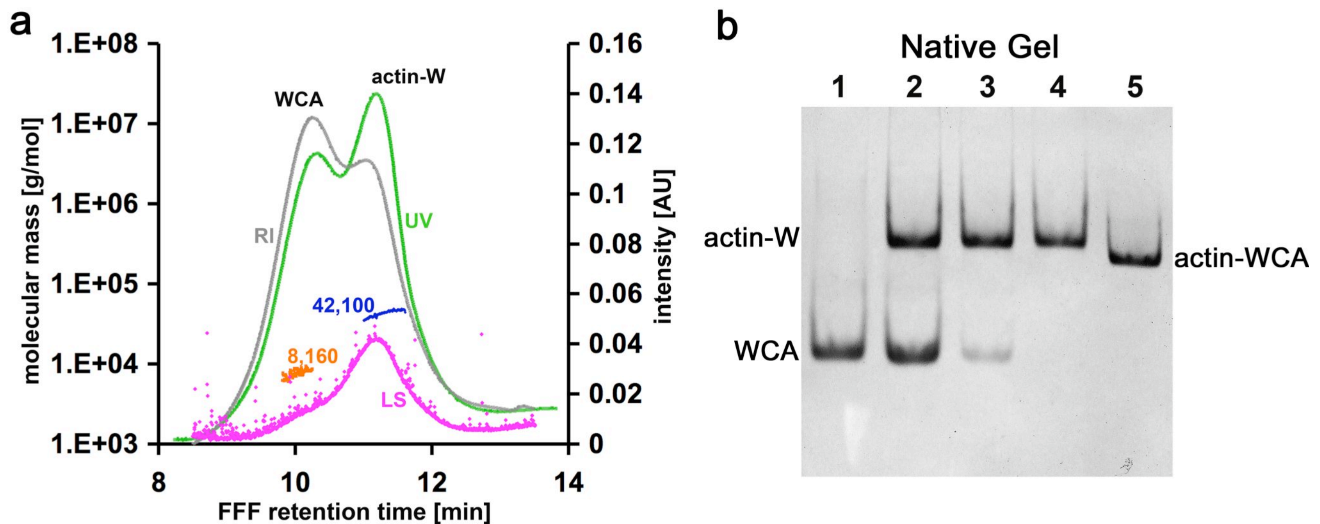

Figure S5. Cross-linked actin-W does not bind WCA. (a) Actin-W (25 μM) and WCA (10-fold molar excess) run separately on FFF-MALS, demonstrating a lack of interaction. The curves of all three detectors are shown: light scattering detector (LS, pink), ultraviolet detector (UV, green) and refractive index detector (RI, gray). The molecular masses of the separate species determined by light scattering (shown with each peak) correspond closely to their expected values from sequence (7,880 and 44,700 Da). (b) In a native non-reducing gel, where proteins are separated according to their mass/charge ratio, WCA (lane 1) runs much faster than actin-WCA (lane 5), which in turn runs faster than actin-W (lane 4). WCA in 6 or 25 molar excesses (lanes 3 and 2, respectively) runs separately from actin-W, which also proves a lack of interaction. Together these results demonstrate that the only binding site for WCA on the actin monomer is the hydrophobic cleft between subdomains 1 and 3, which in this case is permanently occupied by the cross-linked W motif of N-WASP. The C motif has been shown to bind to actin (Kelly et al., 2006), which is in agreement with our own observations (data not shown), but this interaction most likely takes place through the hydrophobic cleft in actin. This cleft in ATP-bound actin has a preference for W motifs (Chereau et al., 2005), but appears to also bind the related C motif (see Figure 5b). We believe that the natural binding site of the C motif, however, is the cleft in Arp2 (see Figure 5a).

References

Chereau, D., Kerff, F., Graceffa, P., Grabarek, Z., Langsetmo, K., and Dominguez, R. (2005). Actin-bound structures of Wiskott-Aldrich syndrome protein (WASP)-homology domain 2 and the implications for filament assembly. Proc Natl Acad Sci U S A *102*, 16644-16649.

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