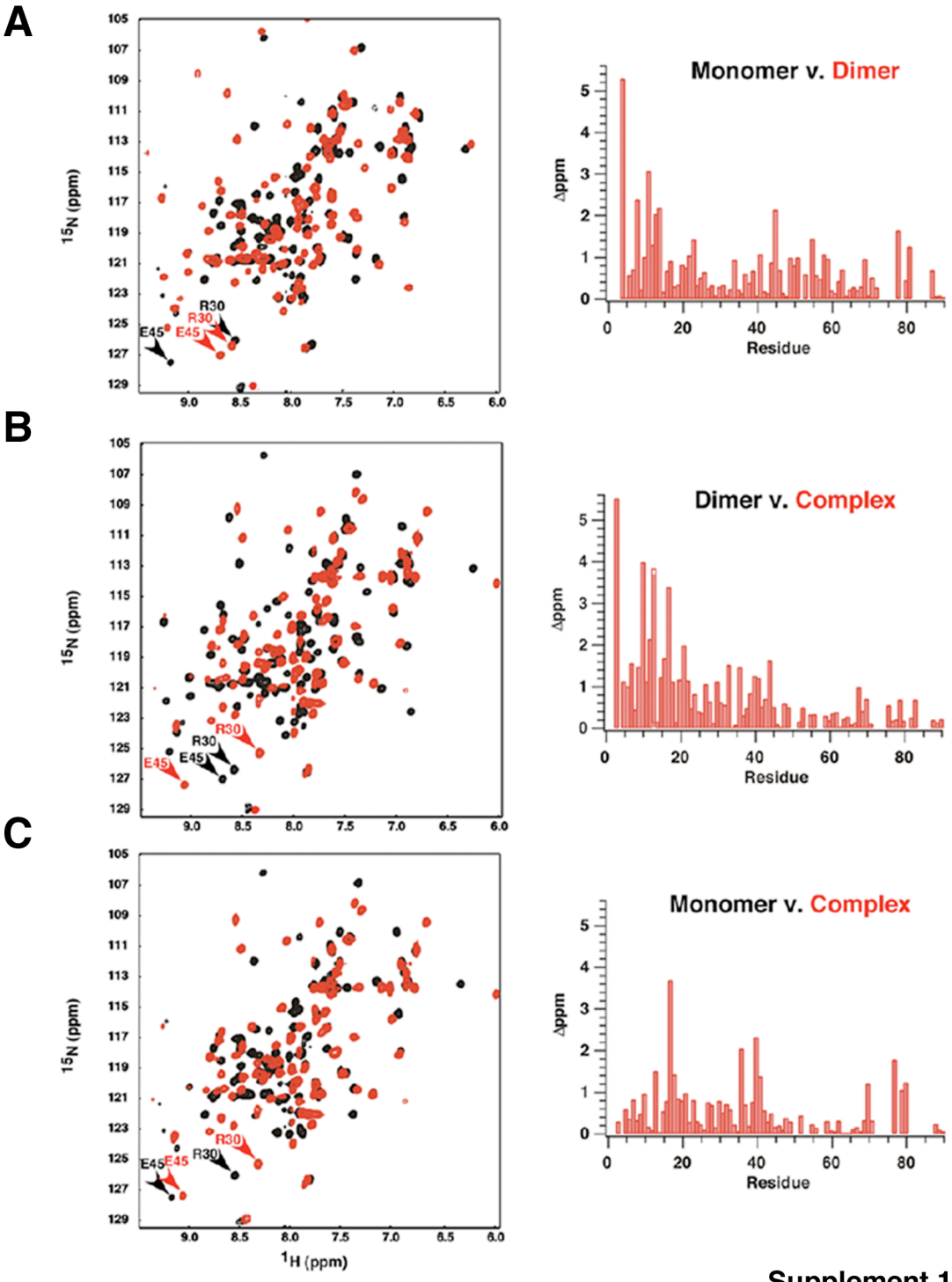
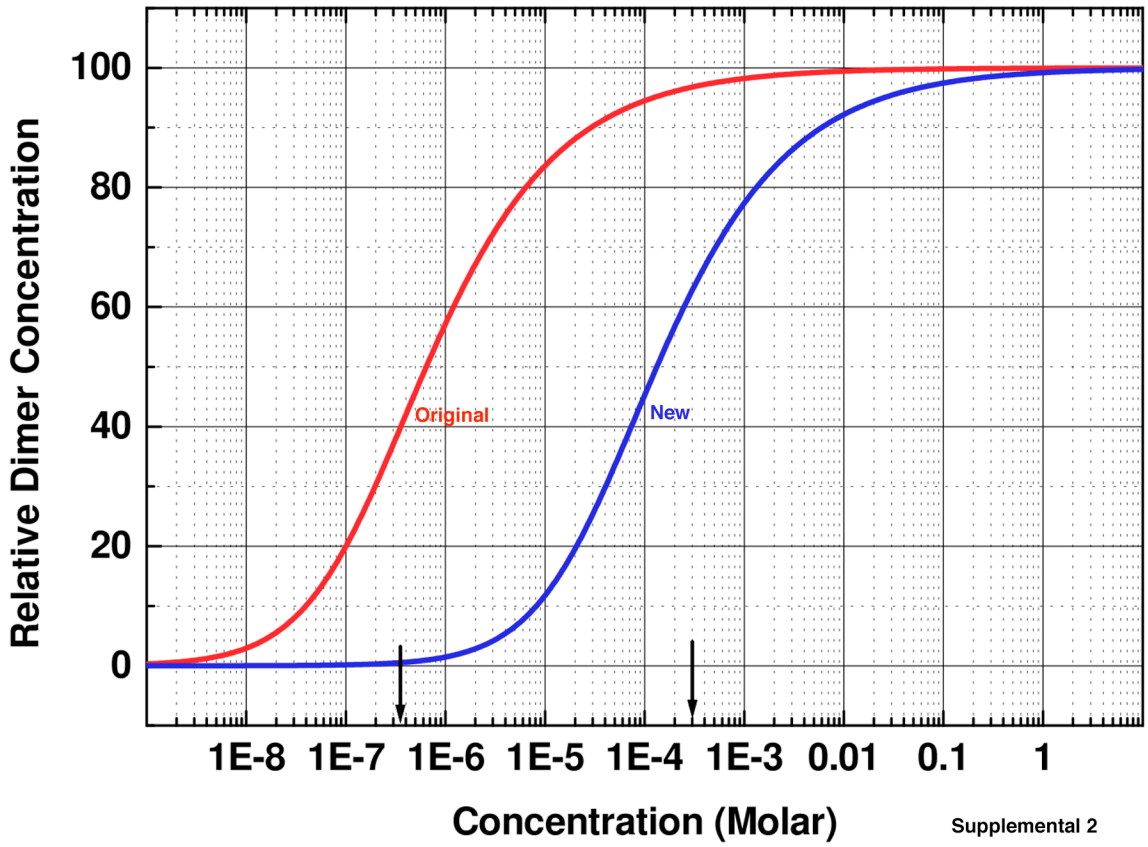
Supplementary Figure 1: Comparison of NMR spectra of AsiA dimer, AsiA monomer and the AsiA/EcSR4 complex. ^{1}H-^{15}N HSQC spectra of AsiA in its monomer, dimer and EcSR4 bound conformation and histograms of chemical shift changes between indicated pairs of spectra. Red and black define an overlay of spectra (left panel) and the spectral difference shown as a histogram as a function of residue number (right panel). Comparison of AsiA monomer vs. dimer (a) to the spectral overlay of AsiA dimer v. AsiA/EcSR4 complex (b) reveals that AsiA in the dimer state is as different spectrally from the complex as it is from monomer. By contrast, the spectral overlay of AsiA monomer v. AsiA/EcSR4 complex (c) reveals that the monomer is spectrally very similar to the complex, indicating that the monomer is most likely the functional conformation of the protein. A spectral signature of the monomer in the free and bound states is shown by arrowheads in each panel, representing the positions of R30 and E45 in each state of the protein. The closely paired arrowheads of the same color in (a, red) and (b, black) represent the positions of these peaks in AsiA dimer, while the distantly separated arrowheads of the same color in (a), (b) and (c) represent the position of these peaks in the monomer and complex. As is clear in (c), the pattern of these peaks is unique to AsiA monomer, both in the free (a and b) and EcSR4 bound states (c).

Supplementary Figure 2: Relative concentration of dimer for the original preparation of AsiA (Lambert *et al.*, 2001, 2003) and the new preparation of AsiA derived from cleavage of a His_6-affinity tag. Sedimentation equilibrium experiments on AsiA were performed with a Beckman Optima XL-I. Equilibrium and Monte Carlo analyses were performed with UltraScan version 6.2. Hydrodynamic corrections for buffer conditions were made according to data published by Laue *et al.* (1992) as implemented in UltraScan (Demeler, 2004). The partial specific volume of AsiA was estimated according to the method by Cohn and Edsall (1943). All samples were analyzed in a buffer containing 137 mM NaCl, 2.7 mM KCl, 2 mM CHAPS and 12 mM phosphate buffer, pH 7. Sedimentation equilibrium experiments were performed at 4°C. The protein sample was spun in a double-sector aluminum centerpiece in the AN-60-TI rotor, and scans were collected at equilibrium at 30, 37.5, 45, 52.5 and 60 krpm using the Rayleigh interference optics. Data ranging between 0-35 fringes (corresponding to a protein concentration

of about 0-1 mM) were obtained in the experiment. The curves illustrate the relative dimer concentration of AsiA derived from these two preparations as a function of the molar concentration of protein. The data for the new preparation of AsiA were globally fit to a reversible self-associating monomer-dimer equilibrium model using Monte-Carlo methods (Lambert *et al.*, 2001, 2003; Demeler, 2004) which results in a calculated molecular weight of 10,980 ± 580 Daltons for the monomer and a equilibrium dissociation constant of 305 ± 157 μM for the monomer-dimer equilibrium (blue curve). This is compared to the monomer-dimer equilibrium curve for the relative concentration of dimeric AsiA in the original preparation of the protein (red curve) (Lambert *et al.*, 2001, 2003). Arrows indicate the concentration limits measured in the equilibrium experiment.



Supplement 1


Relative Dimer Concentration
100
80
60
40
20
0
Original
New
1E-8
1E-7
1E-6
1E-5
1E-4
1E-3
0.01
0.1
1
Concentration (Molar)
Supplemental 2