Fig. S3. The interplay between calcium ions, magnesium ions and ionic strength on PIP$_2$-F binding to gelsolin. In the absence of divalent cations, the anisotropy of PIP$_2$-F (0.5 µM) increased with increasing gelsolin concentration and this interaction was potassium ion dependent. The $K_d$s calculated from the binding curves fitted with Eq. 2 showed diminishing affinities with increasing potassium ion concentrations (blue triangles). Inclusion of 1 mM MgCl$_2$ slightly weakened the affinity of PIP$_2$-F for gelsolin (magenta squares) at 100 mM and 120 mM KCl (K, potassium ions, KM, potassium and magnesium ions). (B) Anisotropy of PIP$_2$-F (0.5 µM) in the presence of gelsolin (5 µM) as a function of KCl or NaCl concentration. Data were fitted with simple sigmoidal curves. The decrease in anisotropy indicates that PIP$_2$-F is dissociated from gelsolin by increasing potassium or sodium ion concentrations, with the half-effective concentrations of $141.2 \pm 3.0$ mM and $143.3 \pm 1.6$ mM, respectively. This indicates the effect of KCl on gelsolin:PIP$_2$-F binding is largely ionic and nonspecific.