

**Profilin binding to sub-micellar concentration of polyphosphoinositides PI(4,5)P<sub>2</sub> and PI(3,4,5)P<sub>3</sub>**  
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Profilin is a small (12-14 kDa) actin binding protein which promotes filament turnover. Profilin is also involved in the signalling pathway linking the receptors in the cell membrane to the microfilament system within the cell. Profilin is thought to play critical roles in this signalling pathway through its interaction with phosphatidylinositol 4,5-bisphosphate [PI(4,5)P<sub>2</sub>] and phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P<sub>3</sub>] (Lu *et al.*, 1996). So far, profilin's interaction with polyphosphoinositides (PPI) has only been studied in micelles or small vesicles. Profilin binds with high affinity to small clusters of PI(4,5)P<sub>2</sub> molecules. The binding stoichiometry of PI(4,5)P<sub>2</sub> to profilin ranges from 5:1 to 10:1 (Goldschmidt-Clermont *et al.*, 1991). In the cell, PPI lipids are not structured as they are in micelles or small vesicles, therefore their interaction with profilin might be quite different. In this work, we investigated the interactions of profilin with sub-micellar concentrations of PI(4,5)P<sub>2</sub> and PI(3,4,5)P<sub>3</sub>. We determined the relevant association/dissociation constant by fluorescence anisotropy when sub-micellar concentrations of fluorescently labelled PPI lipids bind to profilin. We show that the association/dissociation constant of profilin with sub-micellar concentrations of PPI lipids is significantly different to that of profilin with micelles or small vesicles. We also show that profilin binds more strongly to sub-micellar concentrations of PI(3,4,5)P<sub>3</sub> than to sub-micellar concentrations of PI(4,5)P<sub>2</sub>.

Goldschmidt-Clermont, P.J., Kim, J.W., Machesky, L.M., Rhee, S.G. & Pollard, T.D. (1991) *Science* 251, 1231-3.

Lu, P.J., Shieh, W.R., Rhee, S.G., Yin, H.L. & Chen, C.S. (1996) *Biochemistry* 35, 14027-34.