Supporting Experimental Procedures and References

3-D Modeling of MSP Domain and Ankyrin Domain.

Modeling of the 3D-structure of the MSP and ankyrin domains of PAM1 was performed by a stepwise procedure starting from a template structure that was retrieved from PDB (protein databank) (Berman *et al.*, 2000) by searching with BLASTP (Altschul *et al.*, 1990). The templates chosen were the mouse MSP domain of the VAMP-associated protein (VAP) with the PDB-ID number 2CRI-A, and human ankyrin R with the PDB-ID number 1N11-A. The target and template sequences share 29% sequence identity and 50 % similarity in the MSP domain, and 33% an identity and 48% similarity in ankyrin domain. The template and target sequences were aligned using the MALIGN script. After manual examination for potential alignment errors, the automated comparative protein modelling program MODELLER7v7 was used to build the model (Sali and Blundell, 1993). In the first step of model building, distance and dihedral angle restraints on the target sequence were derived from its alignment with the template 3D-structure. The spatial restraints and the energy minimization steps were performed with the CHARMM22 force field program for proper stereochemistry of proteins. Optimization of the model was carried out by the molecular dynamics simulated annealing method, and the modeled structure with minimum objective function was taken and evaluated by PROCHECK (Laskowski *et al.*, 1993). The overall stereochemical quality of the protein was assessed by Ramachandran plot analysis (Ramachandran *et al.*, 1963). The 3D-model was visualized and illustrated using PyMOL (DeLano 2002).

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SUPPORTING MATERIAL

Supporting Movie 1

Dynamic tonosphere movement in a cell of a transgenic hairy root expressing ProPAM1:PAM1-GFP.

Same cell and same magnification as in Figure 6c. The cytoplasm forms a reticulate network of strands with low PAM1-GFP signal intensity, to which the brightly fluorescent tonospheres are attached. Tonospheres move rapidly along the cytoplasmic strands. Frames were acquired every 5.12 seconds. The entire movie covers 4 min 16s in real time.

See http://dx.doi.org/10.1111/j.1365-313X.2010.04341.x