Electronic Supplementary Material (ESI) for Analyst.

Ion mobility coupled to native mass spectrometry as a relevant tool to investigate ligand-induced small conformational changes

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The electronic supplementary material file “Stojko et al SI.docx” contains six figures:

Denatured and native mass spectra of *apo*PDF1B (Fig. S1); Effects of ligand concentration and incubation time on individual PDF1B:ligand native MS profiles (Fig. S2); Real-time native MS monitoring of indirect competition experiments initiated from 1:1 PDF1B:actinonin and PDF1B:*6b* complexes (Fig. S3); Relative proportions of different species during indirect competition experiments initiated from 1:1 PDF1B:actinonin and PDF1B:*6b* complexes (Fig. S4); Comparison of the X-ray structures of *apo*- and *holo*PDF1B (Fig. S5); and collision-induced unfolding fingerprints analysis of different 1:1 PDF1B:ligand complexes (Fig. S6).

Supplementary material

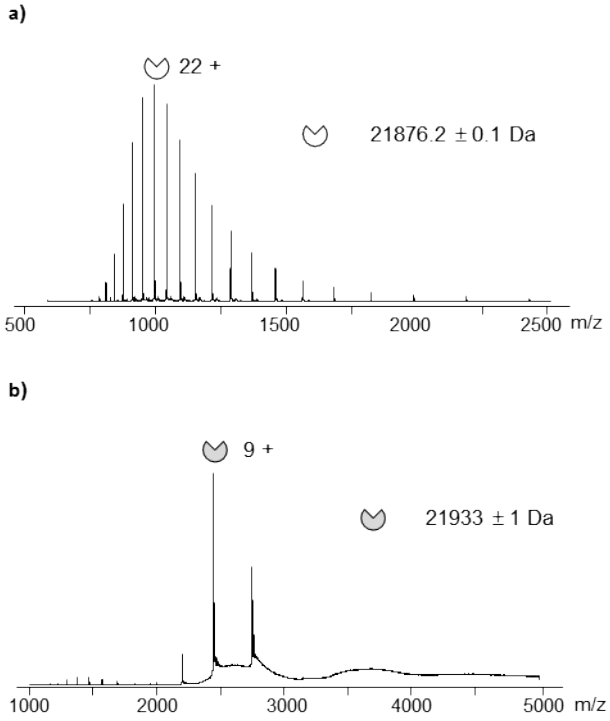

Fig. S1 (a) Denatured and (b) native mass spectra of *apo*PDF1B.

For ESI-MS performed in classical denaturing conditions (2-5 μM in a H_2O/CH_3CN – 1/1 – mixture acidified with 1% HCOOH), noncovalent interactions between PDF1B and the metal ion are disrupted. Proteins are denatured (which accounts for a broad charge state distribution with a high number of charges) and PDF1B mass is measured. In native conditions ($AcONH_4$ 100 mM pH 7.5), the quaternary structure of PDF1B along with the interaction with the metal ion are retained, which accounts for a narrow charge state distribution with fewer charges.

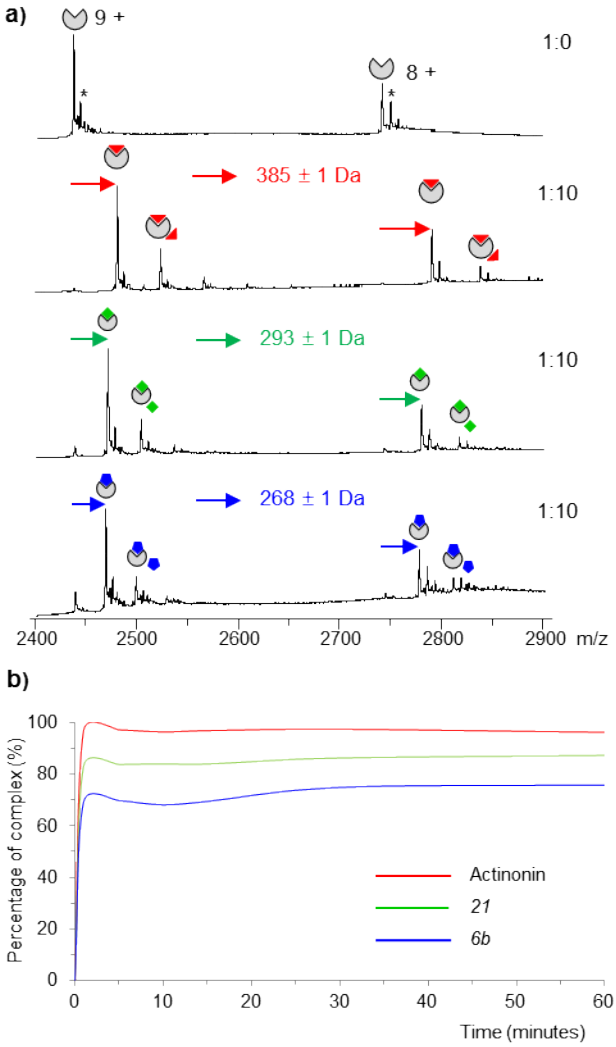

Fig. S2 (a) Native mass spectrometry analysis of individual 1:10 PDF1B:ligand mixtures after 10 min incubation and (b) population of the 1:1 PDF1B:ligand complexes as a function of time in the three 1:2 mixtures.

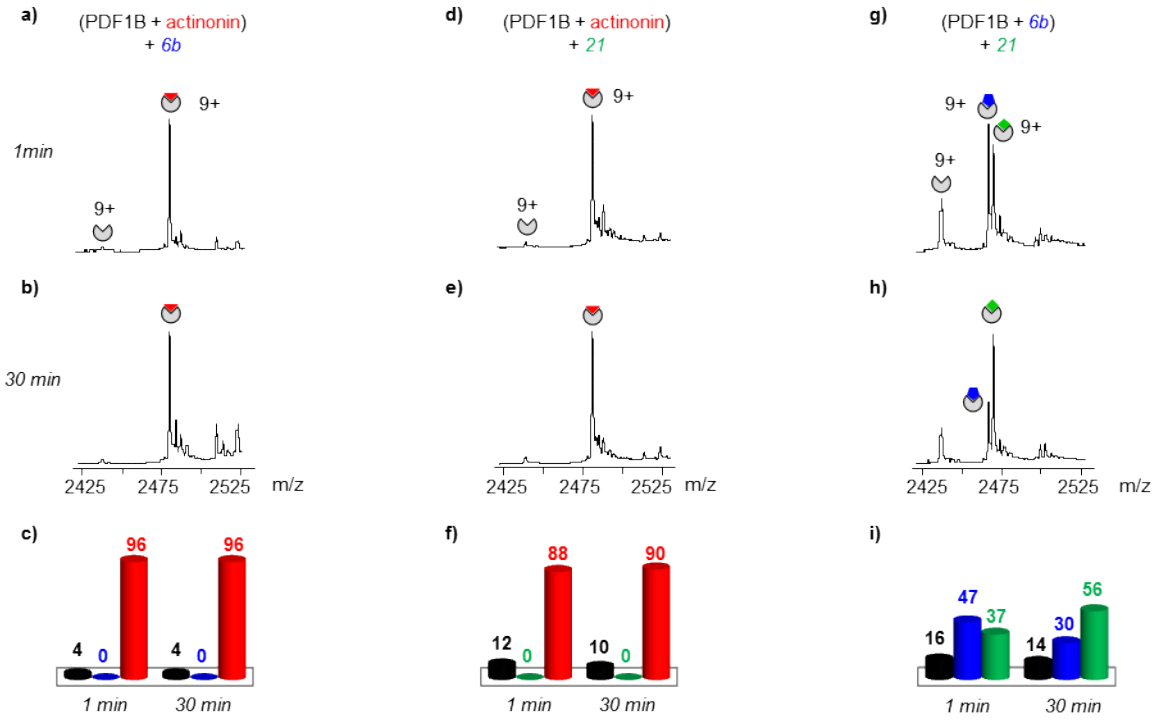

Fig. S3 Indirect competition experiments monitored by real-time native mass spectrometry (MS). (a, b, d, e, g, h) Native mass spectra (9+ charge state) of PDF1B incubated with (a, b) actinonin and then compound *6b*, (d, e) actinonin and then compound *21*, and (g, h) *6b* and then *21*, (a, d, g) 1 min and (b, e, h) 30 min after adding the second ligand. Relative proportions, as determined by native MS, of the different species present 1 and 30 min after adding the second ligand in sequential incubations of (c) actinonin and then *6b*, (f) actinonin and then *21*, and (i) *6b* and then *21*.

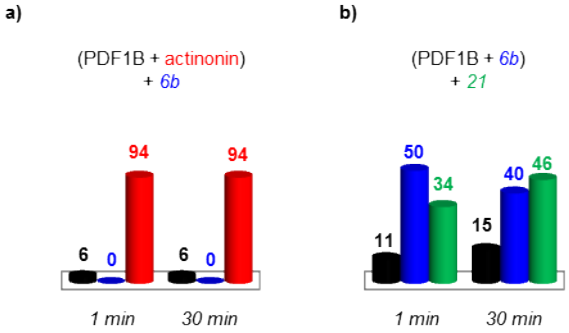

Fig. S4 Indirect sequential competition experiments monitored by real-time ion-mobility mass spectrometry. Relative proportions, calculated from the arrival time distribution intensities of the 9+ and 8+ charge states, of *apo*PDF1B (black), PDF1B:actinonin (red), PDF1B:*21* (green) and PDF1B:*6b* (blue) 1 and 30 min after adding the second ligand in sequential incubations of (a) actinonin and then *6b* and (b) *6b* and then *21*.

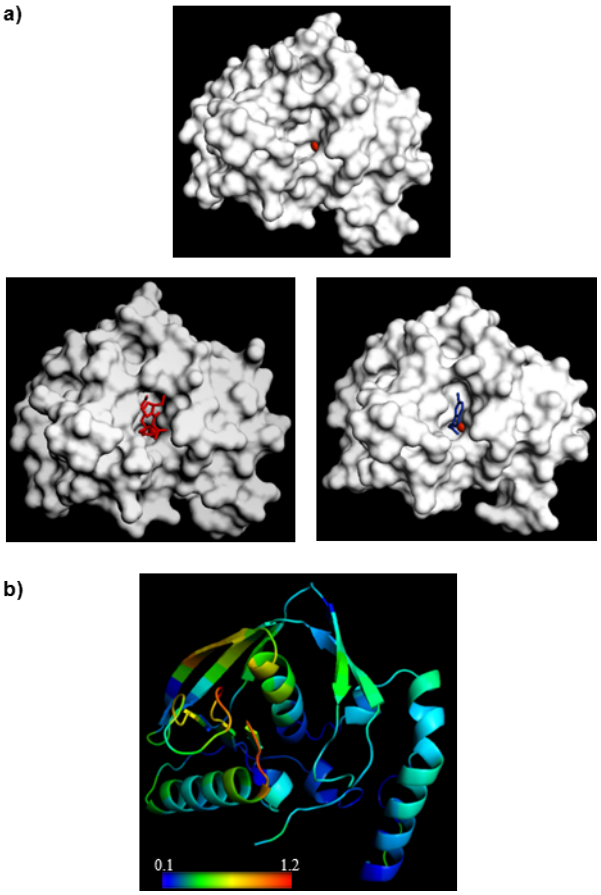

Fig. S5 Comparison of the X-ray structures of *apo*PDF1B and crystallized 1:1 PDF1B:ligand complexes. (a) Pymol[1] representations of *apo*PDF1B (PDB: 3PN2, top), PDF1B:actinonin (PDB: 3M6P, bottom left) and PDF1B:*6b* (PDB: 3O3J, bottom right) in the same orientation. (b) *apo*PDF1B and PDF1B:actinonin structures aligned along the alpha carbons using the software VMD[2]. The color scheme indicates the deviation (in Å) between alpha carbons in the two structures.

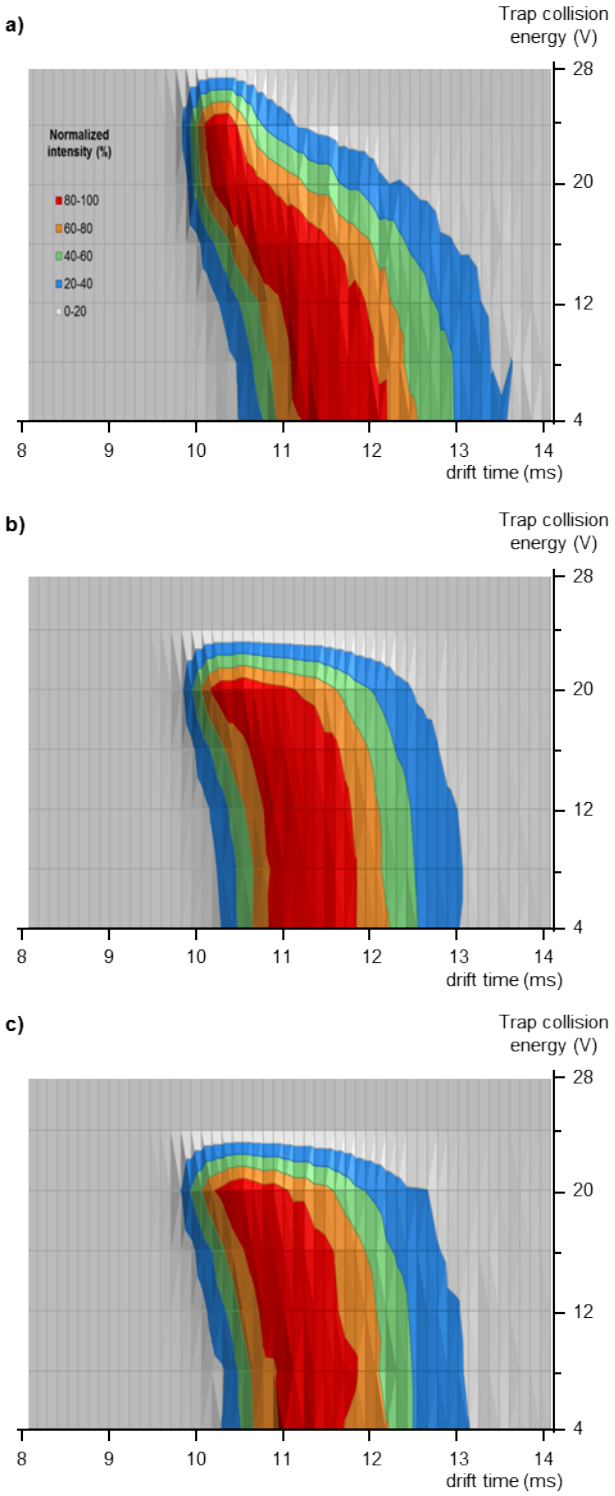

Fig. S6 Collision induced unfolding fingerprints (arrival drift time distributions recorded at increasing trap collision energies) of the 9+ charge states associated with 1:1 complexes of (a) PDF1B:actinonin, (b) PDF1B:*21* and (c) PDF1B:*6b*.

References:

1. DeLano Scientific, San Carlos, CA, USA.
2. W. Humphrey, A. Dalke and K. Schulten, *J. Mol. Graph.*, 1996, **14**, 33-38.