



Figure S2. Differential Toluidine Blue staining capability of IP₈ and IP₆.

To ascertain the relative efficiency of staining of IP₈ and IP₆ by Toluidine blue serial amounts of IP₈ from 1nmol (A) to 16nmol (E) were incubated in the presence of 1M Perchloric acid in 20 μ l (sample from A' to E') for 30 min at 90^oC. Untreated (from A to E) and acid.treated (from A' to E') samples were resolved on 35% PAGE. To avoid loss of material during the neutralization step, acid.treated samples were directly loaded on the gel causing a slight retardation in migration in these lanes (as shown by the different migration of Bromophenol blue (BBF) between treated and untreated samples). Once stained with Toluidine blue, the gel was analysed with ImageJ software. Densitometry analysis enabled each pair of samples (treated and untreated) to be plotted on a graph. The areas of the peaks in these graphs correspond to the relative staining of the IP₆ and IP₈ bands on the gel. Depicted are the analyses of samples D-D' and E-E'. Dividing the densitometry derived values for untreated IP₈ by those for the acid-generated IP₆ indicates the difference in staining efficiency of the two molecules by Toluidine blue. On average IP₈ is stained 1.27 \pm 0.08 (\pm SD) better than IP₆. A virtually identical result was obtained from a second, independent experiment also run in quintuplicate. The experimentally calculated value of 1.27 is in good accordance with the theoretical value of 1.33 reflecting the presence of eight phosphates groups in IP₈ rather than the six in IP₆ (8/6 = 1.33).