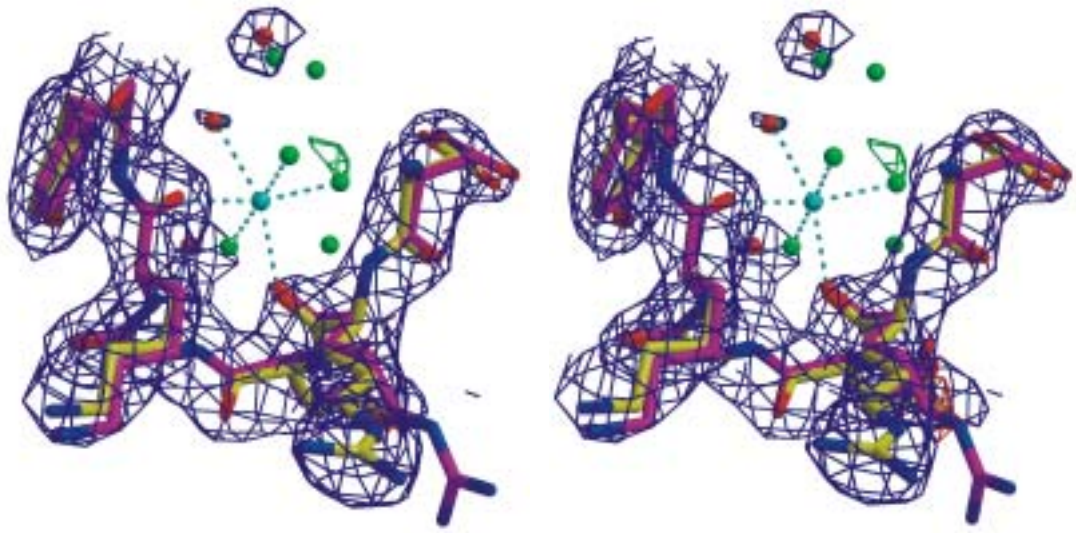
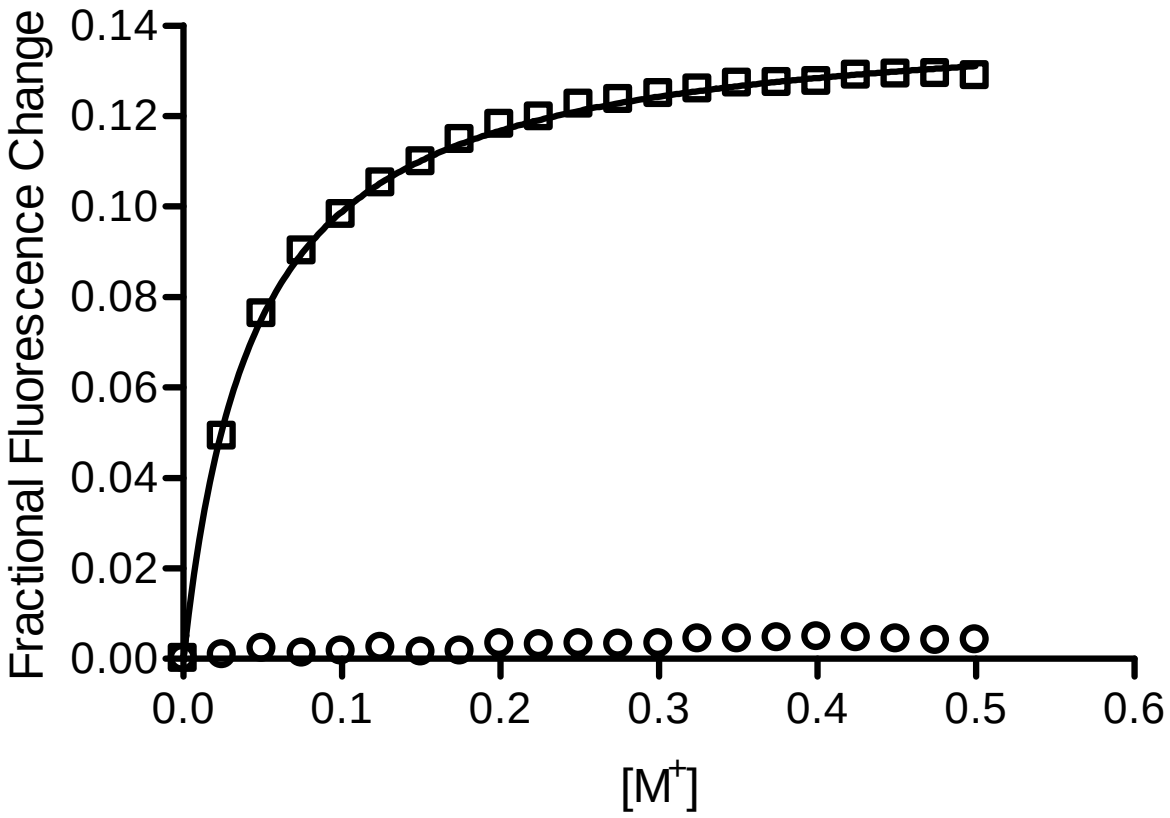


Supplementary Figure 1S. A crystal structure of thrombin in 360mM Li^+ shows no evidence of Li^+ coordination. Li^+ has been reported to bind in the crystallographically defined Na^+ binding site, but our recent structure of S195A thrombin in complex with antithrombin and heparin (1TB6, yellow with red waters) revealed only three water molecules in the Na^+ binding region, with no density corresponding to a monovalent cation or three of the four coordinating water molecules (in spite of the presence of 360mM Li^+). The Na^+ binding loop, however, was found to be in a similar conformation to that of Na^+-bound thrombin (1JOU, monomer AB, magenta with green waters and cyan Na^+). A stereo view of the electron density (2Fo-Fc, blue, contoured at 1σ; and Fo-Fc, contoured at 3σ, green, and -3σ, red) for the Na^+ binding region (Asp221 to Tyr225, and solvent atoms) shows no evidence of Li^+ occupying the position of coordinated Na^+.



Supplementary Figure 2S. Li^+ does not bind to thrombin under crystallisation conditions. Crystals were grown in 125mM MgAcetate, 10mM Tris, pH 7.0 at 21°C. To determine whether Li^+ binds to thrombin under these conditions, we conducted a titration of 800mM LiCl, 10mM Tris, 0.1% PEG8000, pH 7.0 into 2ml of 125mM $MgCl_2$, 10mM Tris, 0.1% PEG8000, pH 7.0, with both solutions containing 200nM thrombin (plasma derived human thrombin from Haematologic Technologies, Vermont). Titrations were carried out at 21°C using the automatic titration utility of the Bio-Kine software on a stopped flow instrument fitted with a titration attachment (MOS-450, SFM-300, BioLogic, Grenoble, France). An excitation wavelength of 280nm and an emission cut-off filter of 305nm were used. After each addition, the solution was allowed to mix for 45 seconds followed by the removal of the volume added and a reading of fluorescence intensity for 20 seconds. Thus the concentration of thrombin and the volume in the cuvet did not vary over the course of the titration. Data obtained for Na^+ binding (K_d=44.3±0.6mM with a maximal fluorescence enhancement of 14%) correspond well to previously published values in the presence of choline chloride (squares), while no intrinsic fluorescence enhancement was observed upon titration of Li^+ (circles).