Spectophotomeric Activity Assays for AtzA, AtzB, and AtzC. Activity assays were all performed in the 50 mM HEPES , 100 mM NaCl, 5% glycerol (HNG) at pH 7.6. AtzA was incubated beforehand in 200 uM $FeSO_4$, and 1.5 mM DTT at room temperature for half an hour. AtzB and AtzC were incubated in 100 uM $ZnCl_2$ at room temperature for half an hour. Preparation of the substrate buffers required various techniques. The atrazine stock was made by adding a concentrated solution of it in DMSO to the HNG buffer and leaving it to stir overnight. Note that the low solubility of atrazine meant the highest concentration that could be made in an aqueous buffer was around 153 uM. The hydroxyatrazine stock was made using a 10% hydroxyatrazine suspension in methanol which was added to the HNG buffer, which was heated for 3 hours while stirring then left to stir overnight. The maximum concentration of hydroxyatrazine in aqueous solution was 60 uM. The absorbance of atrazine was measured at 262 nm, the absorbance of hydroxyatrazine was measured at 246 nm, and the absorbance of the n-isopropylammelide was measured at 240 nm. Note that the atrazine also absorbs at 246 nm and atrazine and n-ispropylammelide also absorb at 240 nm, making spectophotomeric analysis of the entire reaction pathway difficult. Reactions were initiated by adding the enzyme and measuring absorbances with a Tecan infinite 200 PRO from an half area 96-well transparent UV-star Greiner plate.

Precipitation of Cyanuric Acid with Melamine. A solution of 2.5 mg/ml melamine in water was made. This was added to the sample aliquot in a cuvette in a 1:1 ratio for a total volume of 500 ul. Cyanuric acid standards were prepared by preparing a 10% suspension in methanol which was dissolved in DI water overnight. The cuvette was then mixed and incubated for 2-5 minutes after which a spectrophotometer measured the absorbance at 600 nm of the melamine cyanurate precipitate.