Supplemental Information

G172D Mature Structure Folds Similarly to the Wild-Type GA Hydrolase, related to Figure 2

Comparing to previously reported GA wild-type structure (PDB code 2GAW), the G172D mature enzyme has an essentially identical structure, with an rmsd of 0.64 Å between these two structures for all main-chain atoms (Supplemental Figure S2). There is only a small shift (~ 1.8 Å) of an α-helix (residues 126-136). However, this shift does not appear to be due to the G172D mutation since it is more than 12 Å away and without any direct contact with the mutated residue. It is worth noting that the current crystal belongs to the triclinic P1 space group, whereas the wild-type GA structure was determined in the monoclinic $P2_1$ space group (Cui et al., 1999). Thus, similar to the comparison with the G172D precursor in the main text, the small shift of this helix is likely to result from differences in crystal contacts. As pointed out in the main text (Figure 1C), hydrolysis activity of glycoasparagine appears to correlate with the amount of autocleaved G172D protein. Hence, even though with a lower hydrolysis activity than the wild-type enzyme, the G172D mutant is still capable of hydrolyzing substrate once it is autocleaved into the mature form.

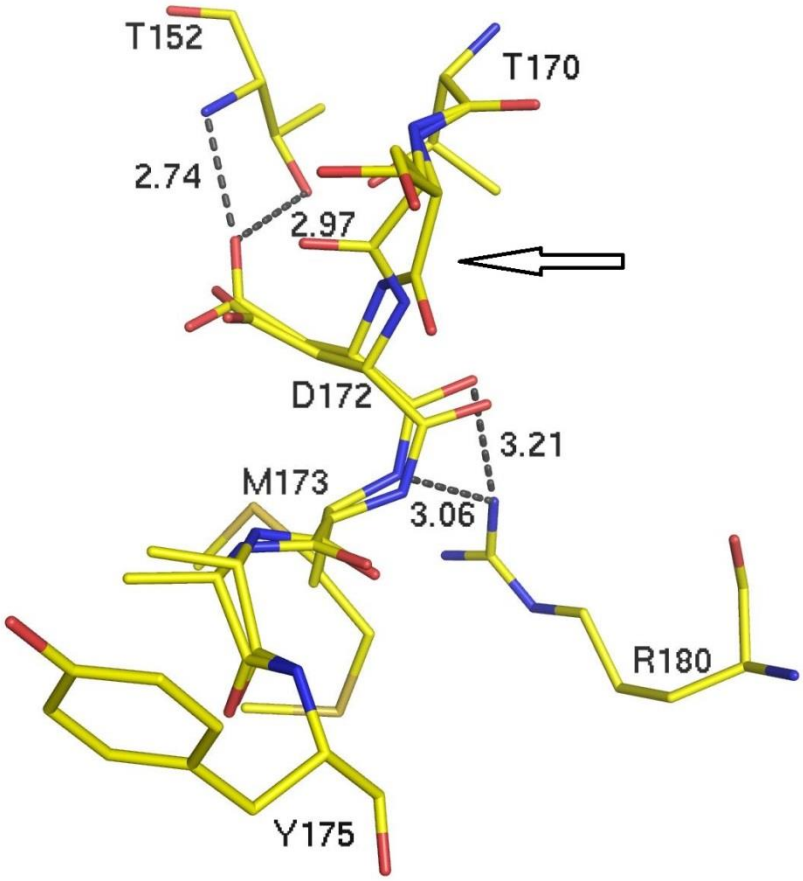

Figure S1, related to Figure 2. Main-chain conformational difference between the G172D precursor and its autocleaved Form.

Bonds are shown by atom type: yellow for carbon atoms, blue for nitrogen atoms, red for oxygen atoms. A 3 Å deviation in a loop's main-chain atoms, at residues 171-172, is highlighted by an open arrow.

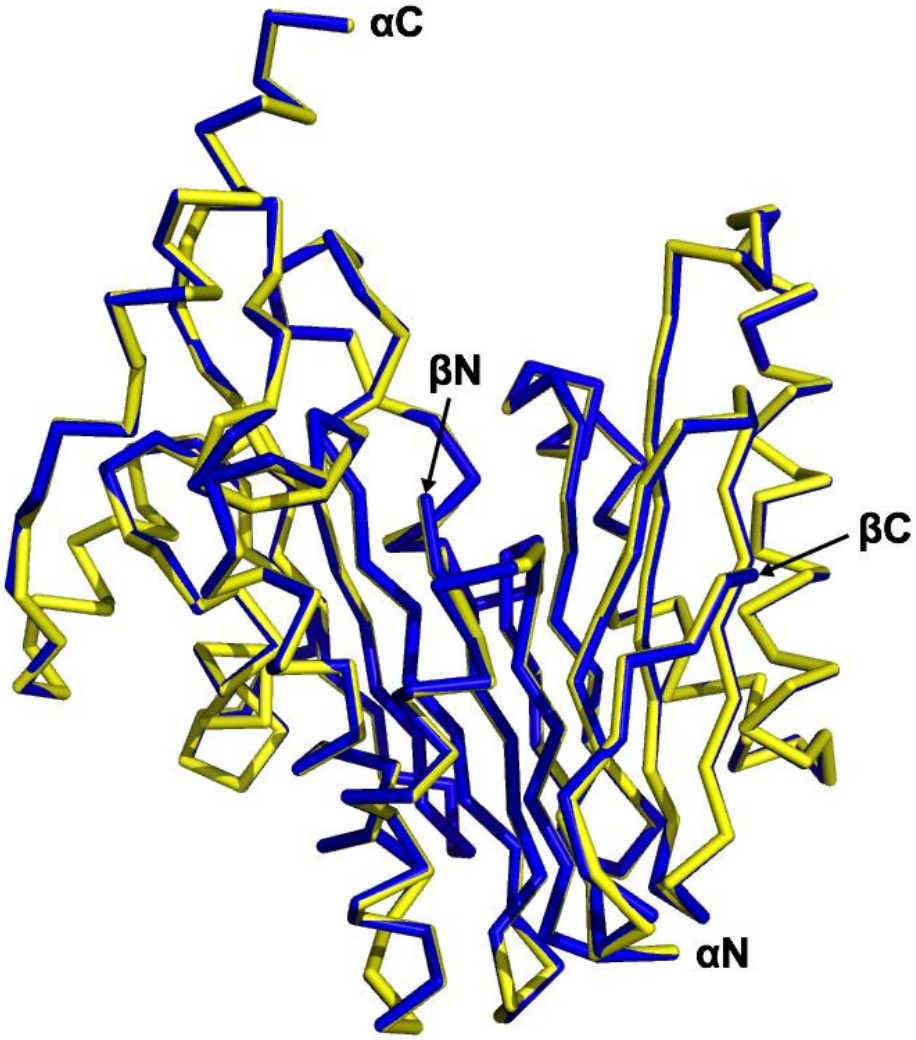

Figure S2, related to Figure 2. G172D mature structure folds similarly to the wild-type GA hydrolase.

C-α traces are shown as yellow and blue for the G172D mature form and the wild-type GA, respectively. Termini of the α and β subunits are also indicated.

Supplemental Experimental Procedures

Hydrolase Activity Assay. Hydrolase activity was assayed by a method modified from a previously described approach (Liu et al., 1998). Purified protein samples were incubated at 37°C to initiate an *in vitro* autoproteolysis in a buffer solution of 100mM Tri, pH 7.5, 50 mM NaCl, 10 mM EDTA. At various time points, 50μl of autoprocessed protein samples were removed to add Aspartic Acid β-(p-nitroanilide) to a final concentration of 1.5 mM in a 100μl reaction mixture, which was then incubated at 37°C for 1 hour. The release of p-nitroaniline was subsequently monitored at 405nm using Spectramax-M2 spectrophotometer.