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Supplemental Data

Promiscuous Partitioning of a Covalent Intermediate Common in the Pentein Superfamily

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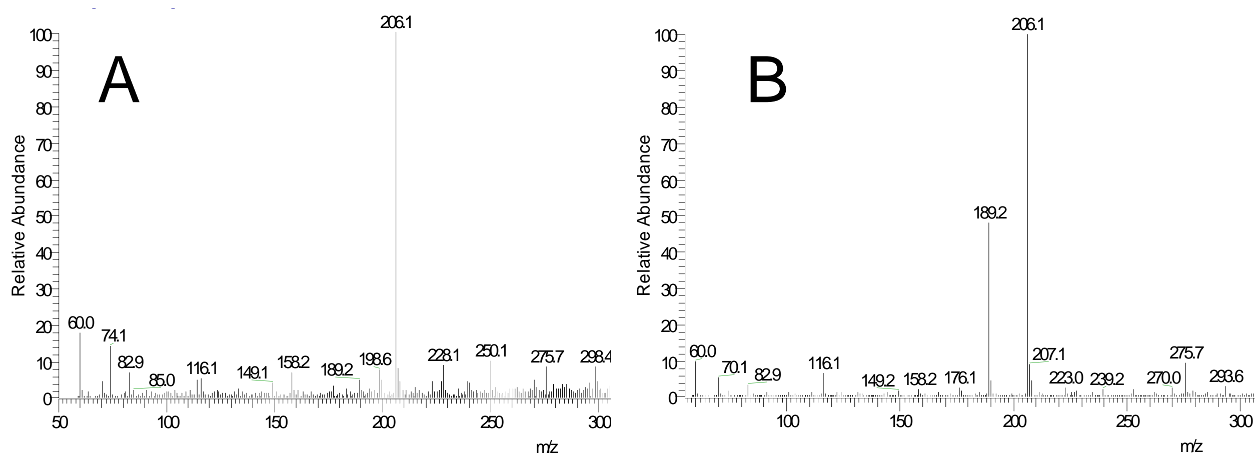

Figure S1. ESI-MS of Chemical Rescue Experiments
Comparison of mixtures containing SMTC and methylamine (A) with mixtures containing SMTC, methylamine, and H162G DDAH (B) show a new peak at 189 Da, matching that expected for N^{ω}-methyl-L-arginine (MH^+_{calc} = 189 Da). The calculated mass of SMTC is MH^+_{calc} = 206 Da and L-citrulline is MH^+_{calc} = 176. Reaction conditions were the same as that described in Figure S2. Relative abundance is indicated on the Y-axis as percentages.

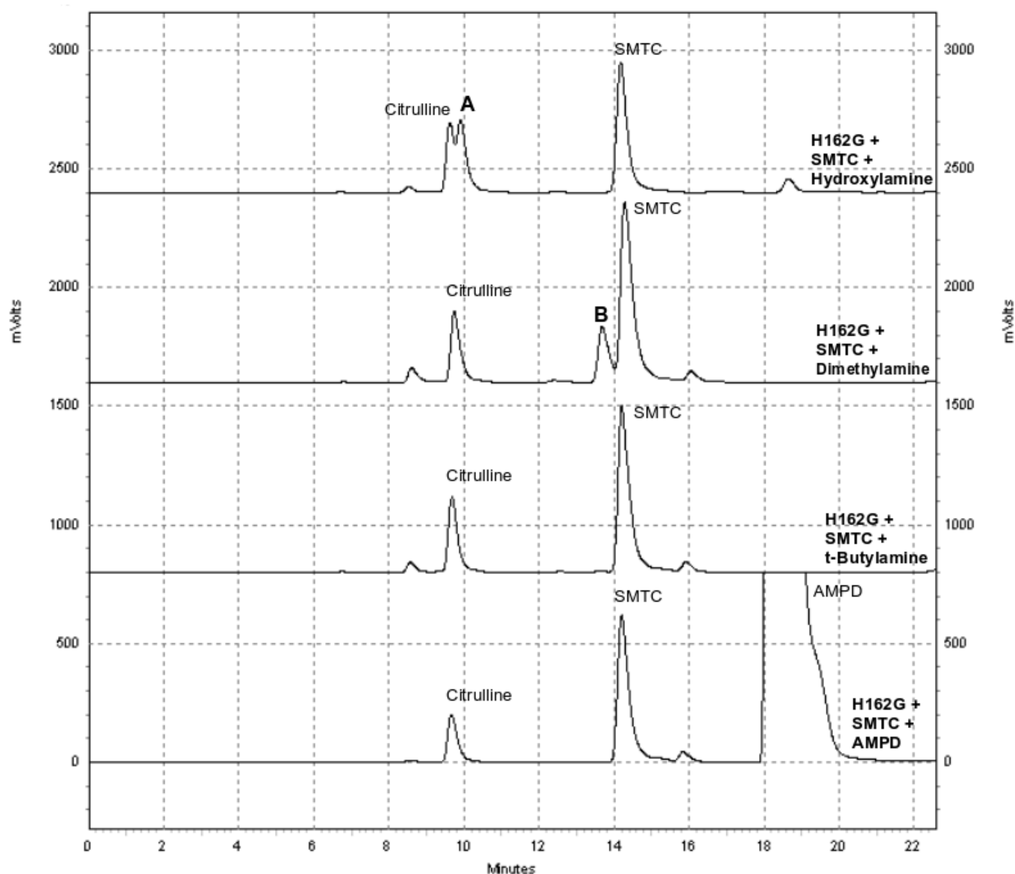

Figure S2. HPLC Product Analysis of Chemical Rescue Experiments
Reaction products produced by various amines were characterized by HPLC. Briefly, either hydroxylamine (100 mM, top trace), dimethylamine (100 mM, second trace), *t*-butylamine (100 mM, third trace) or 2-amino-2-methyl-1,3-propanediol (AMPD, 100 mM, bottom trace) was incubated with H162G DDAH (18 μM) and *S*-methyl-L-thiocitrulline (SMTC, 6 mM) for four hours. The incubation mixtures were prepared as before except that each amine was used as the reaction buffer and was adjusted to the final pH (9.5) using acetic acid. Rescue by hydroxylamine produced a new peak (A) with a retention time (9.9 min) that matches that of an N^{ω}-hydroxy-L-arginine standard (9.9 min). Rescue by dimethylamine produced a new peak (B) with a retention time (13.7 min) that matches that of an asymmetric N^{ω},N^{ω}-dimethyl-L-arginine standard (13.7 min). No new product peaks were obvious in the chromatograms of the slower rescue reagents *t*-butylamine or AMPD. Separate incubation mixtures using different buffers (see Experimental Procedures) were used to quantify (Knipp and Vasak, 2000) increased rates of citrulline production in the presence of 100 mM of methylamine (1.2-fold increase), dimethylamine (2.2-fold), *t*-butylamine (17-fold), AMPD (4-fold), and hydroxylamine (56-fold) in comparison to control reactions that contain H162G DDAH (18 μM) and SMTC (6 mM) but omit the amine reagent.

Knipp, M., and Vasak, M. (2000). A colorimetric 96-well microtiter plate assay for the determination of enzymatically formed citrulline. Anal. Biochem. 286, 257–264.

B

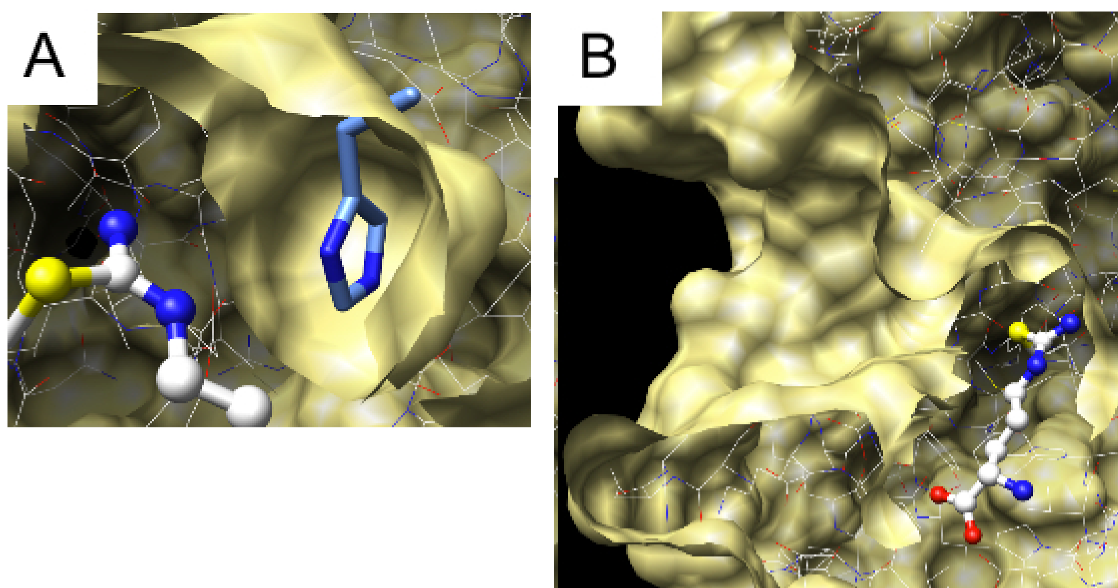

Figure S3. Protein Cavities
A) The surface (1.4 Å probe radius) of the SMTC-H162G structure is shown in tan. The citrulline-C249S structure (Murray-Rust et al., 2001) is superimposed, but only the His162 sidechain is shown (in blue). The imidazole of His162 fits well into the cavity created by the H162G mutation. B) A second, funnel shaped channel extends out from the covalent adduct (shown as a ball-and-stick representation) toward bulk solvent, and presumably provides access for the chemical rescue reagents.