X-Ray Diffraction Evidence for Cysteine-Tyrosine Crosslinks

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Introduction

Posttranslational modifications of tyrosine sidechains are of interest because they extend biological functions while remaining attached to the protein backbone. The modifications highlighted in the box below give the protein the ability to do reversible one-electron redox chemistry, similar to metal ion cofactors. The cysteine-tyrosine crosslink that is formed by bonding the epsilon carbon of the tyrosine molecule with the sulfur gamma of the cysteine.

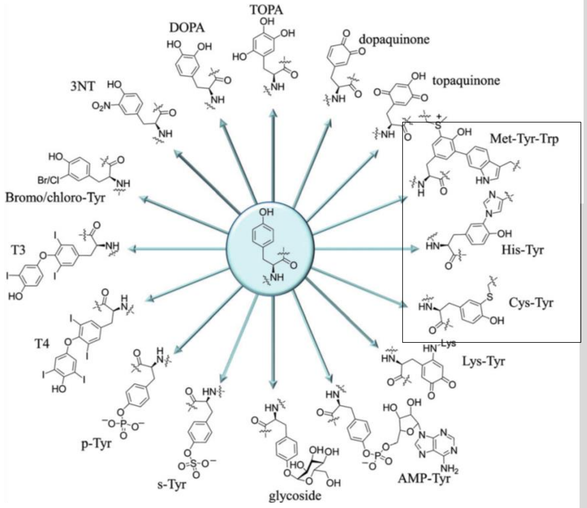

Figure 1. Posttranslational modifications of tyrosine. The modifications highlighted in the box are the focus of our research.

Objective

Our goal this summer was to develop a quantitative method for identifying the presence of a cysteine-tyrosine crosslink from raw crystallographic data in the protein data bank.

Method

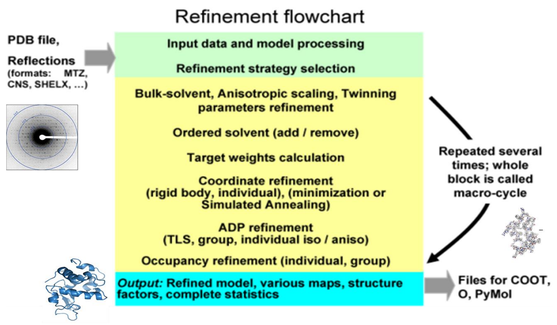

Figure 2. Process by which raw crystallography data is refined. Phenix Phaser first converts reflections to maps of electron density and an initial "guess" at the atom positions in the protein. Phenix Refinement modifies the atomic positions to better reflect the crystallographic data with different strategies. Each round of refinement is examined and modified by visual inspection using molecular graphics programs such as Coot or PyMol.

Each refinement was run twice; with no parameters for the C-S bond forming cysteine-tyrosine and one with C-S bond parameters (below). In order to easily identify new cysteine-tyrosine crosslinks, we were looking for quantifiable differences in refinements with and without C-S bond parameters. These differences could serve as metric to discover additional structures.

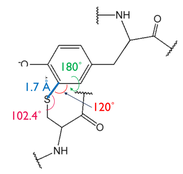

Figure 4. C-S bond refinement parameters that were applied to identify additional cysteine-tyrosine crosslinks.

MolProbity Test Case

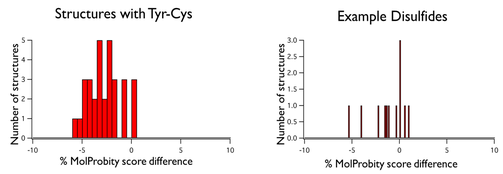

Figure 5. MolProbity scores account for atomic clashes and improper protein backbone and sidechain stereochemistries. Decreases in MolProbity suggest the refinements with Tyr-Cys better fit the raw crystallographic data.

Clash Score Test Case

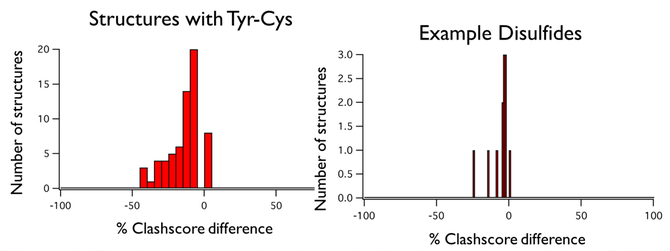

Figure 6. The figures show the change in Clashscore from without C-S bond parameters (no crosslink) to with parameters (crosslink). An obvious decrease in Clashscore is observed for cysteine-tyrosine (Tyr-Cys) and disulfides. While there was a quantifiable change, as expected since these structures included Tyr-Cys or disulfides, the decrease indicated atomic clashes went away upon bond formation. We realized any atomic contact (like C-S bond) less than 2.2 Å was not calculated in the Clashscore calculation, giving an unreliable metric.

Clashscore Compared to MolProbity

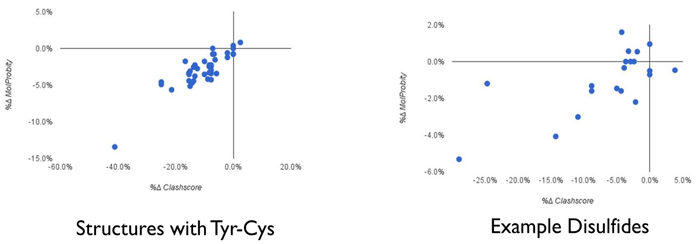

Figure 7. Comparing Clashscore and MolProbity changes showed a strong positive correlation. This suggested that the decreased MolProbity scores were dominated by the Clashscore.

Known Cys-Tyr Crosslinks

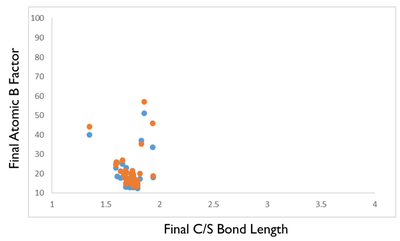

Figure 8. Comparison of the refined C-S bond length with the how well each atom in C-S bond (Tyr C_e blue, Cys S_g orange) fits the crystallographic data (B factor) provided a metric for Tyr-Cys formation.

New Tyr-Cys

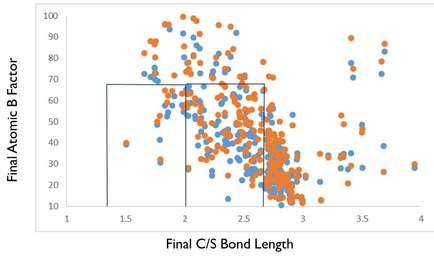

Figure 9. The metric described in Figure 8 was then applied to 300 proteins from the protein databank with tyrosine and cysteine sidechains close to each other. The structures in the box on the far left (refined C-S bond < 2 Å, B factor < 70) could have a Tyr-Cys. The box on the right shows more potential structures (C-S bond < 2.6 Å, B factor < 70) less likely, but potentially, containing a Tyr-Cys.

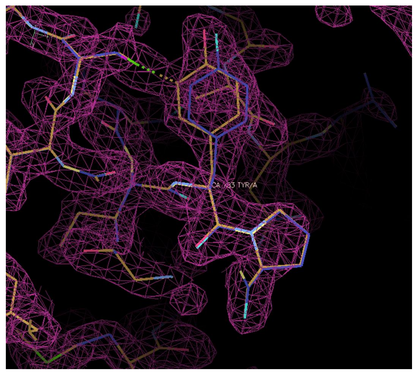

Figure 10. UBE2G2, a ubiquitin ligase, was identified with a Tyr-Cys crosslink (shown in the dashed line) from this work. These structures were in the left box in Figure 9.

Acknowledgements

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