POTENTIAL ENERGY

The Critical Role of Non-catalytic Cysteine Residues in Proteins

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Dr. Jean Bertoldo received his MSc degree in biotechnology (2010) and PhD degree in biochemistry (2013) at the Federal University of Santa Catarina, Brazil, under the supervision of Prof. Dr. Hernán Terenzi. He was then awarded a prestigious fellowship from the Brazilian National Council for Scientific and Technological Development to pursue post-doctoral training at the University of Cambridge in Dr. Gonçalo Bernardes's research group. After a successful research collaboration that yielded two high-impact publications, Dr. Bertoldo moved to Martin Luther University of Halle-Wittenberg, Germany, where he is a junior research associate in the Faculty of Medicine.

I discovered my passion for science when I was 13 years old; there is no doubt that the work and influence of my science teacher sparked this passion. Years later, I found myself finishing a BSc in the biological sciences. Before I realized I wanted to be a scientist, I was fascinated by human biology, its function, its complexities, and the intricate chemical, molecular, and cellular mechanisms that give rise to higher organisms such as humans. Along with my fascination for human biology came the realization of the terrible diseases that affect it, especially infectious diseases, a particular burden in the world's poorest countries. Thus, as a PhD student I knew that in order to find a treatment for these diseases, I would have to study the molecular biology behind them. That was when I understood the importance of basic science and found my field: protein biochemistry.

Of all the proteins, I have always been intrigued by cysteine-containing proteins. Interestingly, the appearance of cysteine in the genetic code is rather late,[1] and given the codons used for its expression (UGC and UGU), its expected percentage in proteins is 3.28%. However, cysteine typically occurs in the human proteome less than 2.2%,[2] which indicates an evolutionary pressure against its utilization. This selective pressure might be due to cysteine's unique set of chemical properties, which make it critical for catalytic reactions but also vulnerable to oxidative damage. In my first year as a postdoc working with Prof. Hernán Terenzi, I already had an idea of how cysteines could affect protein activity, but still a key question remained unanswered: could cysteine residues still play a similar role in prokaryotic proteins, given that they represent only 0.5% of amino acid content?[2] Furthermore, could cysteine residues, or cysteine-containing structural motifs, be validated for selective drug design in proteins of pathogenic bacteria? With these questions in mind, I ventured into choosing the right protocols and methodologies to try to answer them. At this point, I was working with the protein tyrosine phosphatase A (PtpA) from *Mycobacterium tuberculosis*, the infamous tuberculosis-causing pathogen. PtpA is a validated target for tuberculosis treatment and one of the most studied proteins from this bacterium; however, many important virulence mechanisms remain elusive. One of the intriguing things about this protein is its cysteine content of 1.8%, which contrasts with the overall percentage of 0.5% for prokaryotic proteins. Despite the fact that it is a cysteine phosphatase, the remaining two non-catalytic cysteines had not been studied or assigned any function. Prof. Hernán Terenzi's group then showed the *in vitro* S-nitrosylation of the non-catalytic Cys53 and its effect on PtpA's activity.[3] This result then pointed me to another question: would it be possible for this non-catalytic cysteine to have a key cellular function?

I was still struggling with these questions when I was asked to give a talk about the results I obtained from using a different mutagenesis approach. It turned out that Prof. Gonçalo Bernardes from the University of Cambridge would attend this meeting. Prof. Bernardes had developed a pioneering chemical mutagenesis approach[4] to selectively modify single cysteine residues in proteins, and I was testing his compounds on PtpA. After a very productive talk, he invited me to join his group. I then moved to Cambridge to work with him in the Department of Chemistry. In this issue of *Chem*,[5] we demonstrated the results of this pioneering approach. At first, we aimed to test the selectivity of chemical mutagenesis in proteins with multiple naturally occurring cysteines. By using increasing concentrations of the Cys-to-Dha-converting compound α,α′-dibromo-adipyl(bis)amide, we were able to modify first one and then all three cysteines residues in PtpA. Our first question was addressed—we showed that it is indeed possible to selectively modify a single cysteine residue in a protein with multiple reactive

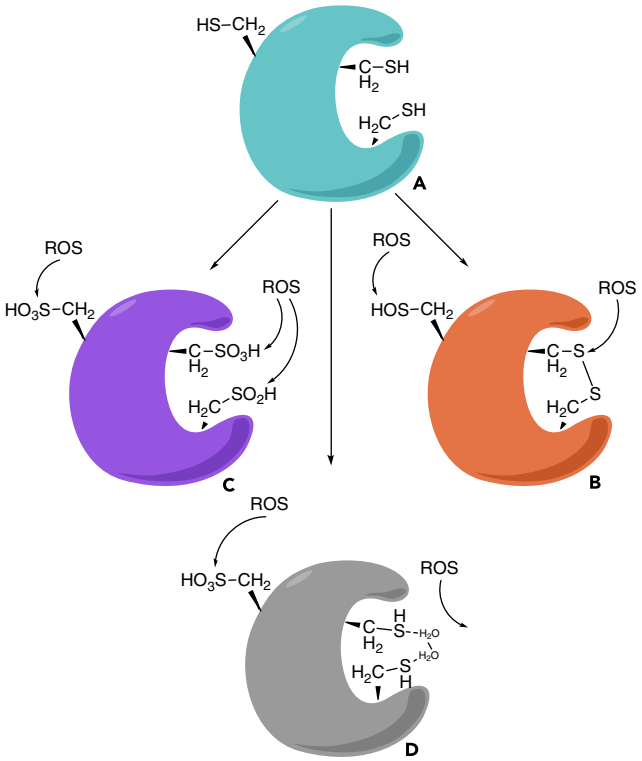

Figure 1. Schematics of the Proposed Water-Bridged Cysteine-Cysteine Redox Regulation Mechanism in Protein Tyrosine Phosphatases

Cysteine phosphatases (A) can prevent catalytic overoxidation (C) by forming a disulfide bridge with a backdoor non-catalytic cysteine in close proximity (B) and thus becoming reversibly inactivated. Non-catalytic cysteines (D) act synergistically as oxidative scavengers while a water-bridged motif consisting of a catalytic cysteine and a backdoor cysteine protects the catalytic pocket, allowing the enzyme to remain active under stress conditions.

cysteines. We then proceeded to identify which of the three cysteines was preferentially modified, and once again Cys53 emerged. The structural analyses revealed that Cys53 was exposed on the surface; thus, the explanation for the selective modification lay in the facile access granted by the amino acid position. Nonetheless, we also showed that for the YopH phosphatase from *Yersinia enterocolitica*, the preferentially modified cysteine was buried in a structural motif. With this in mind, we still had to explain the absence of stoichiometric correlation in the chemical modifications, and we found that after several computational, biochemical, and biophysical experiments, a water-bridged Cys-to-Cys structural motif was taking place in PtpA's catalytic pocket to mediate its accessibility. Amazed by this finding, we then hypothesized the likelihood of the conservation status of this new structural motif and experimentally showed its existence in another bacterial phosphatase. In addition, our findings also led us to propose a new redox-state mechanism contrasting with the well-established disulfide paradigm, given that Cys53 had proved to be playing an oxidative scavenger role by preventing the overoxidation of the catalytic Cys11, and Cys16 acted as backdoor cysteine for the water-bridged Cys-to-Cys motif (Figure 1). These findings not only shift the field of anti-tuberculosis drug design by providing an applicable platform for assessing native protein dynamics but also show how reactive non-catalytic cysteine residues can work synergistically to protect proteins against oxidative damage. It also shows that once expressed either in the surface or buried, non-catalytic cysteine residues play a critical role and can therefore be validated as drug-oriented targets.

At last, I would like to express my sincere gratitude for and acknowledge the time I spent working with Prof. Hernán Terenzi in Brazil and especially Dr. Gonçalo Bernardes at the University of Cambridge; both experiences had a tremendous impact on my career. They also boosted my passion for science and my willingness to go even further to always ask yet another question and to understand failure as a "try again" and not a "give up." As for my perspectives, I hope to be able to establish my own group and provide the same creative and thought-provoking environment for my students as I experienced in the Terenzi and Bernardes laboratories.

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