How fast do proteins break apart with light?

Proteins are microscopic machines that perform diverse functions in nature from transporting materials, to carrying out chemical reactions in the cell, to transmitting signals throughout the body. To do these tasks, the long protein chains must wrap up into a certain structure, which they are amazingly able to do in the blink of an eye. However, ultraviolet (UV) radiation is known to damage the functional structure of proteins and is responsible for diseases including cataract formation. Specific amino acids, or protein building blocks, are able to absorb this UV light. Upon absorption, they can transfer an electron to nearby disulfide bonds formed between cysteine (Cys) amino acids, causing them to break. By breaking these key bonds, the protein begins to unravel, and very reactive Cys radicals are created which can promote side-reactions and protein aggregation or clumping. Although this UV light-induced damage process has been studied extensively in small molecules, not much is known about how fast this process occurs in proteins or the exact mechanism involved. Therefore, we set out to learn about the kinetics and products of this event.

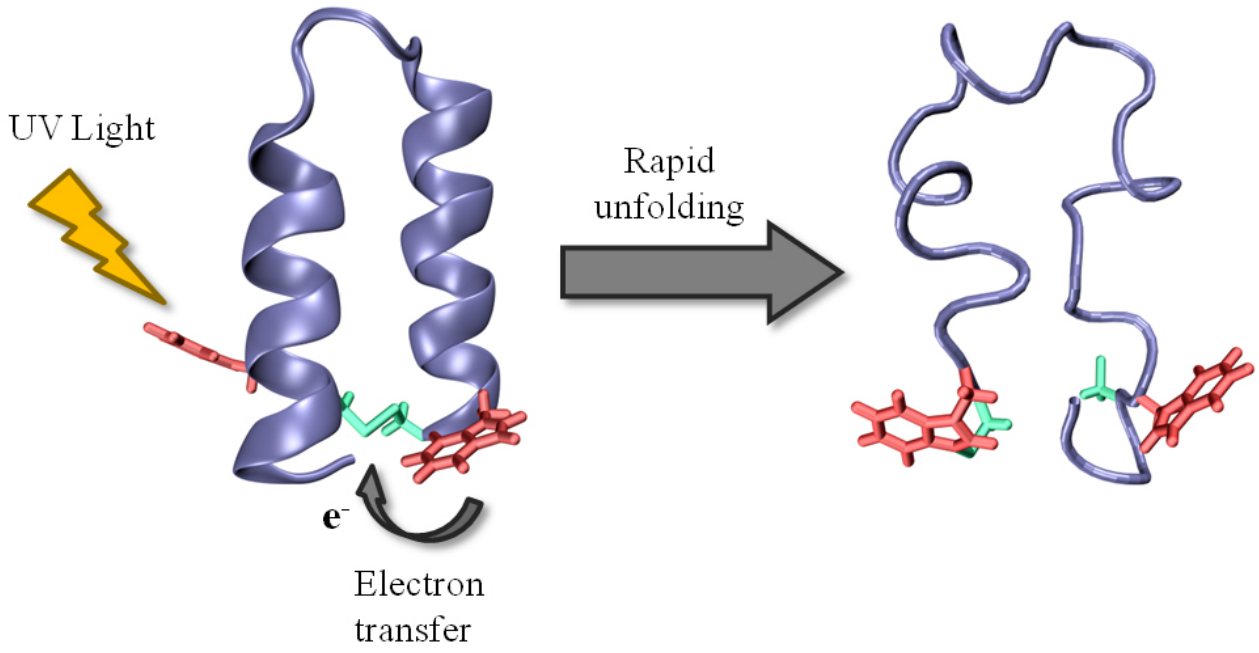

Fig. 1. Graphical representation of the disulfide bond breakage reaction in Z34C by UV light.

In order to study this process, we need to use a method that can access nanosecond and microsecond timescales and is sensitive to changes in the protein structure. Transient infrared (IR) spectroscopy is a useful technique in this regard. Performing a transient IR measurement is similar to taking a series of pictures using the continuous shooting mode of a digital camera, where the UV

light acts as a trigger to initiate the reaction (to start the shooting) and the IR light continuously provides feedback about the progress of the reaction.

In this study, we use a small protein called Z34C, which forms a helix-turn-helix structure stabilized by a disulfide bond near the ends (Fig. 1). By illuminating two UV-absorbing amino acids, i.e., tryptophan (Trp), near this bond with UV light, we found that this disulfide bond breaks apart in approximately 2 microseconds, and the protein immediately unravels. Based on this and other studies, we presented a mechanism to explain this reaction, which involves transfer of an electron from the Trp amino acid directly to the disulfide bond (Fig. 1). Lastly, we found that one of the Cys residues forms a radical which goes on to react with the Trp amino acid next to it. Over extended irradiation time, however, the protein molecules tend to form large clumps. Although this disulfide breakage reaction is usually detrimental in nature, these results suggest that it is possible to use this reaction in a beneficial manner, for example, to control protein structure and function by light.

Rachel M. Abaskharon *and* ***Feng Gai***
Department of Chemistry, University of Pennsylvania, Philadelphia, PA, USA

Publication

Direct measurement of the tryptophan-mediated photocleavage kinetics of a protein disulfide bond.
Abaskharon RM, Gai F
Phys Chem Chem Phys. 2016 Apr 14