Protein dynamics revealed by tuning relative humidity

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Proteins are elemental to most molecular functions inside cells, conducting a wide array of activities, from the binding of antibodies to antigens over enzyme catalysis to molecular signaling. A molecular understanding of these processes is essential for creating, for example, improved enzymes for biotechnological processes or new or improved drugs.

These functions, however, are not governed by the static structures of proteins; rather, they are driven by protein dynamics - movements that span a range of time- and length-scales. This dynamic nature is essential for the specificity and efficiency of molecular processes, allowing proteins to adopt multiple conformations, respond to environmental cues, and adjust their activities accordingly. X-ray crystallography can yield highly detailed pictures of protein structures, but gives typically only limited insight into the dynamics and conformational plasticity of proteins. Nevertheless, proteins are functional inside crystals as they are, for example, still able to switch conformations of chromophores upon light activation or turnover substrates that have been soaked into crystals. However not all proteins are receptible to these means of inducing structural changes. In contrast other more general environmental factors such as temperature, pH or relative humidity can affect the structure of proteins. To probe the effect of these factors on proteins, we have built a temperature- and humidity-control setup that extends the functionality of the Roadrunner fixed-target sample delivery system. To showcase its effectiveness, we collected room-temperature, small wedge data from the antibiotic resistance protein FosAKP while changing the relative humidity in fine steps. In a first analysis we identified a clear switch in the unit cell of the crystals. In a next step solving the corresponding structures revealed a conformational change in the active site of the protein leading to a more open conformation. Finally, we used serial synchrotron crystallography to improve the resolution of the structural changes at the various humidity levels. Previous studies already suggested the high flexibility of this site.

Here we show that careful adjustment of relative humidity can be used to induce structural changes in proteins inside crystals and that these changes can reveal the underlying conformational flexibility of proteins and, ultimately, help to better understand their function.