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**Mechanism of NAD<sup>+</sup>-dependent Deacetylation by Sir2 Enzymes.** Cynthia Wolberger<sup>1,2</sup>, Jef Boeke<sup>3</sup>, José Avalos<sup>1</sup>, <sup>1</sup>Dept. of Biophysics & Biophysical Chemistry, <sup>2</sup>Howard Hughes Medical Institute, <sup>3</sup>Dept. of Molecular Biology and Genetics, Johns Hopkins Univ. School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205.

Sir2 proteins comprise a distinct family of enzymes that carry out a unique and critical reaction in biology: the deacetylation of N $\epsilon$ -acetyl-lysine residues in an NAD<sup>+</sup>-dependent manner. The NAD<sup>+</sup> is cleaved during the deacetylation reaction, yielding nicotinamide, deacetylated peptide and O-acetyl ADP-ribose products. Sir2 proteins, also known as sirtuins, are widely conserved throughout evolution and play key roles in transcriptional regulation, DNA repair, chromosomal stability and lifespan regulation. Most eukaryotic genomes encode 4-7 sirtuin family members that presumably have distinct substrates and cellular roles. Targets for Sir2 proteins that have been identified include the N-terminal tails of histones H3 and H4 in yeast, the chromatin scaffold protein ALBA in archaea, tubulin and the regulatory C-terminal tail of p53 in humans. To gain insights into the mechanism of this unusual reaction, we determined structures of a sirtuin from *Archaeoglobus fulgidus*, Sir2-Af2, with bound peptide, with NAD<sup>+</sup>, and with ADP-ribose, as well as of the apoenzyme. The structures reveal a significant amount of conformational flexibility that is critical to enzyme function and regulation. A comparison of the enzyme bound to different substrates suggests that binding of the acetylated substrate is required for correct positioning of the NAD<sup>+</sup> cofactor. The structures allow us to propose a structure-based mechanism for the deacetylation reaction. In addition, the structures provide insights into how nicotinamide exchange, a side reaction catalyzed by Sir2 enzymes, may regulate the activity of sirtuins in the cell.