Sir2 proteins, or sirtuins, are a family of enzymes that catalyze NAD\(^+\)-dependent deacetylation reactions and can also process ribosyltransferase, demalonylase, and desuccinylase activities. More than 40 crystal structures of sirtuins have been determined, alone or in various liganded forms. These high-resolution architectural details lay the foundation for understanding the molecular mechanisms of catalysis, regulation, substrate specificity, and inhibition of sirtuins. In this minireview, we summarize these structural features and discuss their implications for understanding sirtuin function.

Sirtuins, also known as Sir2 (silent information regulator 2) proteins, are NAD\(^+\)-dependent deacetylases but may have other related activities and are broadly conserved in all three kingdoms of life. Bacteria and archaea typically contain one or two sirtuin proteins that target DNA regulatory proteins and metabolic enzymes such as the chromatin protein Alba (1) and acetyl-CoA synthetase (2), whereas eukaryotes typically contain multiple sirtuins with more diverse protein targets (3). Yeast has five sirtuins, including the Sir2p founding member and Hst1–4. Mammals have seven sirtuins (SIRT1–7), of which SIRT1 is the most extensively studied. SIRT1 targets a broad range of substrates, including p53, FOXO, PGC1a, UCP2, liver X receptor, and others, and therefore is implicated in a variety of biological functions such as cell survival, apoptosis, and stress resistance (reviewed in Refs. 4 and 5).

Sirtuins employ a conserved catalytic core domain to catalyze deacetylation by transferring the acetyl group from the acetyllysine N\(_\alpha\) of proteins to NAD\(^+\) (6, 7), forming 2'-O-acetyl-ADP-ribose and free nicotinamide products (8, 9). The nicotinamide product is also a noncompetitive inhibitor of sirtuin activity at a slightly reduced rate (26).

Several sirtuins can also accommodate a thioacetyllysine peptide as substrate and form a stalled S-alkylamidate intermediate, a covalent conjugate of both substrates. Based on this observation, peptide inhibitors containing thioacetyllysine have been developed and show IC\(_{50}\) values in the micromolar to submicromolar range (reviewed in Ref. 13). Besides these mechanism-based inhibitors, many other sirtuin inhibitors such as sirtinol, Ro-318220, suramin, and EX-527 have been developed (reviewed in Ref. 14). Putative sirtuin activators have also been reported in the literature (15–17), although the effects of these activators have been shown to be substrate- and/or assay-dependent in vitro (18–21).

It appears that not all sirtuins carry out deacetylation as their primary activity. For example, several mammalian sirtuins, including SIRT4, SIRT5, and SIRT7, have very weak or no detectable deacetylase activity (22), SIRT6 has both ADP-ribosyltransferase and deacetylase activities (23), and SIRT5 has been shown to be a more active demalonylase and desuccinylase than deacetylase (24, 25). Thermotoga maritima Sir2 exhibits deacetylase activity but also harbors depropionylation activity at a slightly reduced rate (26).

Despite the many known sirtuin substrates (3), no consensus sequences or substrate determinants have been identified. Hst2 deacylates acetyllysine within unstructured regions of proteins, displaying conformational rather than sequence specificity (27). In addition, more detailed enzymatic studies of yeast Sir2 and Hst2 and human SIRT2 and SIRT1 show certain peptide substrate preferences but also suggest that sirtuins discriminate their substrates in a local context-based fashion with no sequence consensus (19, 28). Notably, many sirtuins, especially those from mammals, contain variable N- and C-terminal regions that flank the conserved catalytic core, and these regions may perform protein-specific functions and possibly also confer substrate specificity (29).

To help understand the molecular basis for catalysis, substrate binding specificity, and inhibition of sirtuins, several sirtuin structures have been determined alone and in several liganded forms (Table 1). These include sirtuins from the archaeon Archaeoglobus fulgidus (Sir2A1 and Sir2A2) (30–34), the bacterium T. maritima (Sir2Tm) (26, 34–37), and Escherichia coli (CobB) (38); Hst2 from the yeast Saccharomyces cerevisiae (39–42); and several human sirtuins (SIRT2 (43), SIRT3 (44), SIRT5 (24, 45), and SIRT6 (46)). Most of these structures are in complex with different ligands, including substrates, reaction intermediates and their analogs, and inhibitors (nicotinamide and suramin). These structures, together with complementary biochemical data, have provided valuable insights into overall sirtuin structure, catalytic mechanism, substrate specificity, and inhibition.

**Overall Structure**

Primary sequence alignment of sirtuins shows that they share a highly conserved catalytic core and N- and C-terminal segments with divergent lengths and sequences. X-ray crystal structures of nine sirtuin members have been determined, and all of these structures contain only the core domain, except for an Hst2 structure, which contains the full-length protein (Table 1). The overall structures of these core domains are highly similar, displaying a conserved large Rossmann fold domain for NAD\(^+\) binding and a more variant small domain that contains a zinc-binding ribbon module and a helical module with three or four helices (Fig. 1a). The two modules are tethered to the
Rossmann fold through four linking loops, two for each module. The four linking loops form a cleft between the small and large domains, within which most of the residues are highly conserved. The acetyllysine and NAD\(^+\) insert from opposite sides into a hydrophobic tunnel within the cleft, where catalysis occurs (Fig. 1b).

The zinc ion does not directly participate in deacetylation because it is too remote from the active site. Instead, it plays an essential structural role for integrity of the catalytic core domain. Generally, four cysteine residues coordinate the zinc ion in a tetrahedral geometry, and the zinc plays a role in maintaining the stability of the structure by holding the three \(\alpha\)-strands together (Fig. 1a). Removal of the zinc through mutation of the coordinating cysteine or using chelating reagent abolishes the deacetylase activity due to partial collapse of the structure, and subsequent supplementation of zinc restores activity (47).

The large domain is a classical Rossmann fold for NAD\(^+\) to bind. It consists of a central \(\beta\)-sheet with six parallel \(\alpha\)-strands sandwiched by several \(\alpha\)-helices on each side (30). The small domain packs against one-half of the Rossmann fold and forms a cleft in between that is perpendicular to the Rossmann fold \(\beta\)-sheet. As oriented in Fig. 1, the four linking loops participate to form the side wall of the cleft, with the small domain for the ceiling and the large domain for the floor (Fig. 1a).

The elongated NAD\(^+\) molecule inserts its nicotinamide ribose moiety into the cleft, lays the pyrophosphate group along the edge of the cleft.
the β-sheet in a positively charged groove, and places the adenine base in a pocket remote from the cleft (Fig. 1b). The orientation of NAD⁺ here is inverted compared with most Rossmann fold-containing enzymes, where the adenine base of NAD⁺ binds to the C-terminal half, and the nicotinamide group of NAD⁺ binds to the N-terminal half of the β-sheet (48).

**Binding of Substrates**

*NAD⁺*—The NAD⁺-binding region is divided into three sites: site A for adenine binding, site B for nicotinamide ribose binding, and site C for nicotinamide moiety binding (Fig. 1b). In site A, the adenine base sits in a partially hydrophobic pocket. A highly conserved Asn-248 (Hst2 numbering unless indicated otherwise) makes hydrogen bond interactions with the 3'-OH and/or 2'-OH group of the adenine ribose (Fig. 2a). The β7-α9 loop within the Rossmann fold interacts with the pyrophosphate group through several hydrogen bonds from Gly-223, Thr-224, and Ser-225 (Fig. 2a). The nicotinamide ribose binds to the enzyme at site B, proximal to the acetyllysine substrate-binding tunnel (Fig. 1b). The conformation of the nicotinamide

![Figure 2](https://example.com/figure2.png)

**Figure 2. Binding of sirtuin substrates.** a–d, binding of NAD⁺. a, site A. Residues represented in blue stick form hydrogen bonds (dashed lines) with NAD⁺. The adenine base sits in a hydrophobic pocket shown in surface representation. yHst2, yeast Hst2; PDB, Protein Data Bank. b, sites B and C. NAD⁺ forms hydrogen bonds with Ile-117, Asp-118, and acetyllysine. Gly-32, Ala-33, Gly-34, Ile-41, and Phe-44 make van der Waals interactions with NAD⁺. Asn-116 forms a hydrogen bond with an ordered water molecule (w; blue sphere). c, sites B and C represented in the Sir2Tm-NAD⁺-acetyllysine complex. The nicotinamide ribose is rotated ~90° compared with b, d, different conformations of the cofactor-binding loop. Hst2 bound to carba-NAD⁺ (purple) is superimposed on Hst2 bound to ADPR (blue), showing the different positions of Phe-44. e and f, binding of acetyllysine-containing p53 peptide bound to Sir2Tm. e, the acetyllysine peptide (light green) forms a β-staple with sirtuin residues (dark green). Only the backbone of the residues is shown except for where side chain interactions are made: Lys-381 (1 position) forms hydrogen bonds with Asn-165 and Gly-163, and Met-384 (2 position) makes van der Waals interactions with Phe-162 and Val-193. f, the acetyllysine side chain is buried in a hydrophobic tunnel and makes van der Waals interactions with sirtuin residues (blue stick). Val-160 also forms a hydrogen bond with the Ne of acetyllysine. g, succinyllysine (green) forms hydrogen bonds with SIRT5 residues (blue stick) and NAD⁺.
ribose varies among structures and even varies among different protomers within the same crystal lattice (33, 34). In one conformation, the nicotinamide moiety binds to site C (Fig. 1b), allowing both the nicotinamide ribose and nicotinamide moiety to interact with invariant key residues (Fig. 2b). This conformation has been referred to as a “productive binding” mode in the literature, as it is thought to represent the binding conformation of NAD$^+$ that would allow nucleophilic attack on the nicotinamide ribose for catalysis (33). As no products actually form in this conformation, we will refer to this conformation as the “active conformation” instead. In this conformation, the nicotinamide moiety binds to the C pocket and makes van der Waals interactions with a Gly-Ala-Gly motif and Ile-41, Phe-44, and Asn-116. The carboxamide makes hydrogen bond interactions with the backbone nitrogen of Ile-117 and the side chain of Asp-118. These interactions constrain NAD$^+$ in a conformation destabilized from its ground state such that the coplanarity of the nicotinamide moiety with the glycosidic bond is strained by $\sim$90° and the positive charge is buried in a hydrophobic environment.

The nicotinamide ribose ring in site B is observed to adopt slightly different conformations in two representative structures. In the ternary structure of Hst2 bound to a non-hydrolyzable NAD$^+$ analog, carba-NAD$^+$, and an acetyllysine-containing H4 peptide, the nicotinamide ribose ring (cyclopentane ring in this case) is coplanar with the acetyl group of acetyllysine. The 2′- and 3′-hydroxyls of carba-NAD$^+$ also form hydrogen bonds with the acetyllysine carbonyl oxygen, and the C1′ atom of the ribose ring is located 4.8 Å away from acetyllysine (Fig. 2b) (41). In this conformation, acetyllysine is not able to mount a nucleophilic attack at the C1′ atom. Instead, a nearby residue, Asn-116, is proposed to mount a nucleophilic attack mediated by an ordered water molecule to break the glycosidic bond. In contrast, in the structure of Sir2Tm bound to an acetyllysine-containing peptide and NAD$^+$ (36) or of Sir2A2 bound to a PEG ion and NAD$^+$ (34), the nicotinamide ribose ring is rotated by $\sim$30° around the glycosidic bond relative to its conformation in the Hst2 ternary complex (Fig. 2c). This conformation orients the $\alpha$-face of NAD$^+$ toward the acetyllysine, thereby exposing the C1′ atom of the ribose ring for direct nucleophilic attack from the carbonyl oxygen of acetyllysine. The two putative active conformations of the nicotinamide ribose ring in site B clearly have different implications in the mode of catalysis because the former and latter would argue for an S$_\text{N}$1 or S$_\text{N}$2 type of mechanism, respectively. Whether one of both mechanisms might be relevant is still an open question.

Most of the enzyme does not undergo significant conformational change upon NAD$^+$ binding, except for the cofactor-binding loop (Fig. 1a). The cofactor-binding loop (Gly-32–Thr-49 in Hst2), also referred as the flexible loop, is one of the four linking loops. This loop is disordered in unliganded sirtuin structures, as reflected by having high B factors or no definable electron density. Upon ligand binding, this loop becomes ordered and adopts multiple conformations dependent on the identity of the bound ligand. Several residues (Ala-33, Gly-34, Asp-43, and Phe-44) of the loop participate to form the C pocket and make extensive interactions with NAD$^+$. The different conformations of the cofactor-binding loop appear to be defined by its proximity to solvent and the location of Phe-44 in the loop (Fig. 2, b–d). When the enzyme is bound to NAD$^+$, the benzene ring of Phe-44 (Phe-33 in Sir2Tm and Phe-35 in Sir2A2) is located $\sim$4 Å from the nicotinamide moiety, making a π-stacking interaction (Fig. 2, b and c). When the C pocket is not occupied by the nicotinamide moiety, Phe-44 occupies the C pocket and thereby precludes NAD$^+$ binding in the active conformation. In this new position, the benzene ring of Phe-44 makes a stacking interaction with the nicotinamide ribose ring. There are two exceptions to this observation, one with Hst2 bound to ADP-(hydroxymethyl)pyrrolidinediol (HPD)$^2$ (42), a molecule that is designed to mimic a proposed oxocarbenium intermediate after nicotinamide cleavage, and the other one with Hst2 co-crystallized with ADP-ribose (ADPR; Protein Data Bank code 1SZD) (41). In both of these cases, the C pocket is filled with water molecules, and the cofactor-binding loop adopts a conformation almost identical to that of Hst2 when bound to NAD$^+$. Taken together, the dynamics of the cofactor-binding loop and the position of Phe-44 in particular appear to play a role in catalysis, specifically in protecting the reaction intermediate from hydrolysis and/or facilitating the release of nicotinamide product.

Acetyllysine—Several sirtuin structures have acetyllysine-containing peptides bound, with some of these structures also containing NAD$^+$ or its analogs. The peptide substrate forms a $\beta$-strand-like interaction with two adjacent loops, the $\beta$8-$\alpha$9 loop within the Rossmann fold and the $\beta$6-$\alpha$8 loop, which links the two domains (Fig. 2e, left). The peptide binding to the linking loop induces a shift in the loop toward the large domain and consequently a rigid body rotation of the small domain relative to the large domain (31, 41). The amino acids of the peptide flanking the acetyllysine make mainly backbone interactions with the enzyme (Fig. 2e, right). Exceptions to this observation are the amino acid immediately N-terminal (−1 position) and the second amino acid C-terminal (+2 position) to the cognate acetyllysine that also make side chain interactions, which include hydrogen bonds with Asn-165 and Gly-163, as well as van der Waals interaction with Phe-162 and Val-193 (Sir2Tm numbering), respectively. Varying the side chain interactions by mutating the residue at the −1 position of the peptide and the non-conserved Asn-165 of the enzyme has a significant effect on the substrate binding affinity, suggesting a role for substrate discrimination by these residues (35). The target acetyllysine side chain inserts into a tunnel that is formed by hydrophobic residues (160VFFG163 in Sir2Tm) from the $\beta$6-$\alpha$8 loop (Fig. 2f). The amide nitrogen of the acetyllysine makes hydrogen bond interactions with the backbone of Val-160. In the ternary structure, the acetyllysine also interacts with the proposed active conformations of NAD$^+$ as described above.

2 The abbreviations used are: HPD, (hydroxymethyl)pyrrolidinediol; ADPR, ADP-ribose; DADMe, 5′-$\text{O}$(R)-[(R)-[(3R,4R)-1-(3-carboxybenzyl)-4-hydroxypropylidin-3-yl][methoxy][hydroxy][phosphoryl][methyl][hydroxy][phosphoryl][adenosine].

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greater variability, especially among eukaryotic sirtuins. These variations may confer different activities that are observed for some sirtuin proteins such as SIRT5, which has been shown to be a weak deacetylase but an effective lysine desuccinylase and demalonylase (24, 25). In the ternary structure of SIRT5 with NAD$^+$ and a succinyllysine-containing peptide, the aliphatic chain of the succinyllysine makes similar contacts with the surrounding conserved residues. The carboxylate group of succinyllysine forms three hydrogen bonds with the side chains of Arg-105 and Tyr-102, two non-conserved residues located at the back of the cleft on a helix from the small domain (Fig. 2g). Mutation of these two residues separately abolishes the desuccinylation activity, supporting their key roles in substrate binding (24). Although other sirtuins lack these two residues, Sir2Af1 and CobB do harbor the corresponding residues at the same locations and would therefore be predicted to accommodate succinyllysine binding. Thus, it is possible that Sir2Af1 and CobB could also process desuccinylation activity like SIRT5, although both of these enzymes appear to have robust deacetylase activity (7, 30, 49).

**Binding of Inhibitors**

**Nicotinamide**—Sirtuin deacetylases couple the deacetylation reaction with the cleavage of NAD$^+$, which generates free nicotinamide. On the other hand, nicotinamide is also a noncompetitive inhibitor of sirtuins and inhibits deacetylation through a base-exchange mechanism by reacting with a reaction intermediate to reform NAD$^+$ (9, 11). A binding site for inhibitory nicotinamide is of interest because compounds targeting this site could be designed to occlude free nicotinamide binding and therefore serve as sirtuin activators. In efforts to uncover this binding site, several sirtuins have been co-crystallized with nicotinamide. In the structures of Sir2Af2-NAD$^+$-nicotinamide and Sir2Tm-acetylated peptide-nicotinamide (34), nicotinamide is observed to bind to the conserved C pocket, which is also where the nicotinamide moiety of NAD$^+$ would bind in the active conformation (Fig. 1b) (34). In this conformation, the carboxamide group of nicotinamide is anchored to the C pocket, whereas its pyrimidine ring adopts slightly different conformations, each forming a π-stacking interaction with Phe-35 of the cofactor-binding loop and the nicotinamide ribose ring in Sir2Af2 (Fig. 3a), thus taking on a similar conformation as the nicotinamide moiety of NAD$^+$ in an active conformation. Consistent with the mutually exclusive binding of nicotinamide and NAD$^+$, only inactive NAD$^+$ or ADPR binding conformations are observed in the presence of nicotinamide in Sir2Af2 structures. The carboxamide group is anchored by two hydrogen bonds with the side chain of a conserved aspartic acid (Asp-103, Sir2Af2 numbering) and the backbone amino group of a conserved isoleucine (Ile-102). The carboxamide also makes van der Waals interactions with the side chains of Asn-101 and Ile-102. These interactions are very similar to those made by the nicotinamide group of NAD$^+$ bound in the active conformation. It appears that the free nicotinamide adopts a favorable conformation in which the pyrimidine ring is planar with the carboxamide, whereas the nicotinamide group of bound NAD$^+$ is in a strained conformation.

**Suramin**—Suramin is a sirtuin inhibitor that competes with both NAD$^+$ and acetyllysine substrates. In the binary structure of SIRT5 and suramin, the symmetric inhibitor links two SIRT5 molecules to form a dimer, which is also observed in solution (Fig. 3b) (45). The trisulfonylnaphthyl group of suramin binds to the NAD$^+$-binding sites B and C, and several of the benzene rings bind in the peptide-binding site but not to the acetyllysine tunnel. These binding sites explain how suramin competes with both NAD$^+$ and acetyllysine substrates to inhibit catalysis by SIRT5. Suramin is a large, chemically multifunctional compound with poor sirtuin selectivity for inhibition. Nonetheless, the SIRT5-suramin structure provides important molecular

**FIGURE 3.** Inhibitor binding to sirtuins and NAD$^+$ analog structures. a, nicotinamide (NAM) bound to the C pocket. Nicotinamide forms hydrogen bonds with Ile-102 and Asp-103 (blue stick; Sir2Af2) and π-stacking interactions with Phe-35 (purple), b, suramin (yellow sticks) bound to SIRT5. A dimer of SIRT5 binds to the symmetric suramin, and only one SIRT5 is shown for clarity. The succinyllysine-containing peptide and NAD$^+$ (aqua lines; Protein Data Bank code 3RY) are superimposed to show that suramin competes with both substrates for sirtuin binding, c, structures of NAD$^+$ and its analogs in Corey-Pauling-Koltun colors. Yellow, carbon; dark yellow, sulfur; red, oxygen; blue, nitrogen; orange, phosphate. The AMP moiety is identical among the molecules and is not shown. The replacements from authentic molecules are indicated by red arrows.
Insights that may be useful in designing more selective sirtuin inhibitors.

Implications for Catalysis

Catalysis by sirtuins involves the binding of NAD\(^+\) and acetyllysine substrates; cleavage of the glycosidic bond; acetyl transfer; and formation of O-acetyl-ADPR, nicotinamide, and deacetylated lysine products. Sirtuin structures determined in complex with various substrates, reaction intermediates/transition state mimics, and products together provide important molecular information underlying catalysis. The initial reaction of NAD\(^+\) glycosidic bond cleavage has been proposed to proceed through either an \(S_N1\)-like mechanism, as supported by the structure of Hst2 bound to carba-NAD\(^+\) (Fig. 2b) (41), or an \(S_N2\)-like mechanism, as supported by the structure of Sir2Tm bound to NAD\(^+\) and an acetyllysine-containing peptide (Fig. 2c) (36). The dissociative \(S_N1\)-like mechanism is also supported by two intermediate structures, one bound to DADMe-NAD\(^+\) (37), a molecule that mimics the dissociative transition state of glycosidic bond cleavage, and another bound to ADP-HPD (42), a positively charged oxocarbenium ion intermediate-like molecule (Fig. 3c).

Following nicotinamide cleavage, acetyl transfer leads to the formation of an O-alkylamidate intermediate, a covalent conjugate of both substrates. All of the sirtuin structures are in agreement that a conserved histidine (His-135 in Hst2) acts as a general base to deprotonate one of the ribose oxygens to facilitate the formation of this intermediate (Fig. 2, b and c). The structure of Sir2Tm with S-alkylamidate, a long-lived intermediate mimicking the O-alkylamidate, reveals that His-116 is in position to directly deprotonate the 2'-hydroxyl to form the proposed 1',2'-bicyclic intermediate (37).

When both substrates were mixed with Hst2 or Sir2Af1, one of the reaction products, 2'-O-acetyl-ADPR was captured in the crystal structure, thus providing direct evidence for the product of sirtuin deacetylation (32, 40), which was also independently confirmed by mass spectrometry and NMR (8, 9, 51). Comparison of the conformations of the NAD\(^+\) substrates, intermediates, and products shows that most of the NAD\(^+\) molecule remains relatively stationary throughout catalysis. One exception to this is the flip of the nicotinamide ribose ring upon nicotinamide cleavage, as demonstrated by comparing the structures of Hst2 bound to carba-NAD\(^+\) and ADPR (40, 41).

Although the conclusions drawn about the mechanism of catalysis that are derived from the structures described above are likely to be largely correct, it should be noted that the analogs employed are not authentic reaction substrates or intermediates but instead are the best available mimics (Fig. 3c). For example, carba-NAD\(^+\) has a non-reactive cyclopentane ring replacing the nicotinamide ribose ring, which may affect its orientation when bound to sirtuins (41); DADMe-NAD\(^+\) contains an extra carbon between the ribose ring and nicotinamide moiety, which may push the ribose closer to acetyllysine (37); the S-alkylamidate analog has a longer C-S bond than C-O bond, which makes 2'-hydroxyl attack less efficient and may explain the longer half-life of the S-alkylamidate (37); and ADP-HPD has shown weaker binding compared with NAD\(^+\), implying that it may not be a perfect mimic of the reaction intermediate (42). Therefore, some of the molecular details associated with catalysis may have to be revised in the future. Nonetheless, the complex structures with these analogs have provided important insights into the catalytic mechanism. A more detailed discussion of the sirtuin catalytic mechanism is provided by Deniz and co-workers (53) in one of the accompanying minireviews in this series.

Conclusions and Future Prospects

The sirtuin catalytic core domain structures all contain a large Rossmann fold domain, a small zinc-binding domain, and a cleft between the domains that form the binding sites for both substrates for catalysis. Sirtuin structures with various substrates, intermediates/transition state mimics, and products have provided important information on NAD\(^+\) and acetyllysine substrate binding and catalysis, as well as information on how sirtuin dynamics facilitates each of these processes. Nonetheless, many details of the reaction mechanism are still not clear.

Emerging data have revealed that several sirtuins employ their divergent N- and C-terminal regions that flank the catalytic core for autoregulation. The full-length Hst2 structure reveals a homotrimer, which also occurs in solution and is mediated by the N- and C-terminal segments (39). In this homotrimer, the N-terminal segment from one Hst2 subunit occupies the acetyllysine substrate-binding site of another subunit, and a C-terminal helix partially overlaps with the NAD\(^+\) binding site of still another subunit, thereby locking Hst2 in an inactive homotrimeric conformation. A more recent biochemical study of SIRT1 reveals that the N- and C-terminal segments promote catalysis, although the molecular basis for this is unknown (52). Structures of other sirtuins that contain flanking N- and C-terminal segments may reveal other autoregulatory functions, particularly in the mammalian sirtuins that have the most divergent N- and C-terminal regions.

SIRT5 contains weak deacetylase activity but more potent demalonylase and desuccinylase activities, which was first predicted from the structure of SIRT5 bound to a thioacetyllysine-containing peptide that suggested that the larger substrates might be accommodated. This hypothesis was later confirmed in solution and by an x-ray crystal structure of SIRT5 bound to a succinyllysine-containing peptide (24). SIRT4–7 also have very weak deacetylase activity, suggesting that their true acetyllysine substrates have not yet been identified or that they may also preferentially process different modifications. As with SIRT5, structures of these other sirtuin enzymes might provide important insights into their biologically relevant substrates.

The SIRT5-suramin complex structure is the only sirtuin structure bound to a synthetic sirtuin inhibitor (45). This structural information is very important but also very limiting, considering that many other sirtuin inhibitors (and putative activators) have been identified, many of which have greater sirtuin selectivity (14). Structures of other sirtuin-effector complexes will clearly be of significant value, given their potential therapeutic applications.
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