Human sirtuins: structures and flexibility

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Abstract

In recent years, sirtuins (SIRT), members of histone deacetylases (HDACs) class III, have been found to modulate cellular processes related to the development of human aging-related pathologies (*i.e.* cancer, neurodegeneration, metabolic disorders). Several crystallographic structures and computational studies have shed light into their catalytic mechanism of action, identifying also the structural elements for the design of selective drug candidates. In this review, we first aim at summarizing the structural features characterizing human SIRT. We then describe the observed mass and one-off movements related to conformational changes upon SIRT-mediated recognition events. Such information will be useful not only for rationalizing the design of new SIRT modulators, but also for improving the comprehension of SIRT-related biological roles.

Key-words

Sirtuins, conformational changes, protein-protein interactions, modulators, structure-based drug design

Abbreviations

ECS, extended C-site; HDACs, histone deacetylases; SIRT, sirtuins; 3D, three-dimensional.

1. Introduction

In 1987, Rine *et al.*, identified four proteins responsible for the silencing of the Hidden MAT *loci* of *Saccharomyces cerevisiae*, implicated in the mating process of the yeast (Rine and Herskowitz, 1987). These four proteins, called silent information regulatory (Sir) proteins, have been found to bind to the nucleosome through interactions with histone proteins H3 and H4, and to repress gene expression (Johnson *et al.*, 1990; Murphy *et al.*, 2003; Cubizolles *et al.*, 2006; Ehrentraut *et al.*, 2011; Oppikofer *et al.*, 2013). Sir proteins have aroused great hope when it was found that they may participate in extending the yeast life span (Lin *et al.*, 2004). As Sir homologous proteins have been found in many organisms including mammals, this opened a new hope for the development of novel therapeutic treatments. The human genome encodes for seven Sir-like proteins, commonly called sirtuins (SIRTs, **Fig. 1**) (Frye, 1999; Frye, 2000). Phylogenetically, they belong to the class III histone deacetylase (HDAC) family (Gray and Ekström, 2001). In contrast to class I, II and IV HDACs, which are all zinc-dependent enzymes, SIRTs have been found to be nicotinamide adenine dinucleotide (NAD⁺) dependent enzymes able to generate 1-O-acetyl-ADP-ribose as a by-product (Imai *et al.*, 2000; Tanner *et al.*, 2000). As members of the HDAC family, SIRTs possess deacetylase activity. However, other catalytic activities have been reported for certain isoforms. For example, SIRT4 has been found to have low deacetylase activity, and a main ADP ribosyltransferase one (Ahuja *et al.*, 2007). SIRT5 has been found to prefer succinyl, malonyl and glutaryl lysine substrate with low deacetylase activity reported (Du *et al.*, 2011; Peng *et al.*, 2011). And regarding SIRT2, which main activity is the deacetylation of lysine substrate, it has been reported that like SIRT6 to also possess demyristoylation activity (Teng *et al.*, 2015). The SIRT cellular localization is isoform-dependent, and can be subjected to compartment shuttling during particular cell cycle phases, also in response to diverse cellular stress (Michishita *et al.*, 2005; Vaquero and Reinberg, 2009; Bosch-Presegue and Vaquero, 2014). Whereas SIRT1, SIRT6, and SIRT7 are the isoforms mainly found in the nucleus, where they can exert their activity by regulating DNA stability, gene expression, chromatin structure maintenance and/or cell-cycle progression (Michishita *et al.*, 2005), SIRT2 has been found to co-localize and deacetylate α -tubulin in the cytoplasm. This suggests that SIRT2 plays a role in maintaining cellular architecture and division. Interestingly, SIRT2 was found to be shuttled into the nucleus of the cell during mitosis

where it promotes G2/M transition through the deacetylation of histone H4K16 (North *et al.*, 2003). SIRT3, SIRT4, and SIRT5 are mitochondrial SIRTs that mainly interact with non-histone proteins on which they exert their catalytic activity (Onyango *et al.*, 2002; Michishita *et al.*, 2005). Diseases associated with SIRT-related cellular mechanisms (e.g. cancer, neurodegeneration, and metabolic disorders) have received considerable attention in research in recent decades due to their impact on the healthcare systems (Mariotto *et al.*, 2011). For this reason, SIRTs are now considered promising therapeutic targets. For reviews on SIRT-related cellular mechanisms, and on the recent advances in the discovery of chemical modulators of SIRTs, please refer to (Villalba *et al.*, 2012; Anderson *et al.*, 2014; Carafa *et al.*, 2016; Mellini *et al.*, 2015).

In this manuscript, a summary of the structural features characterizing human SIRT proteins is reported. We then focus our attention on the massive movements characterizing SIRTs, induced by the binding of biological partners. Residues responsible of one-off movements in SIRT binding sites, exerting a fundamental role in molecular recognition, are also identified. We conclude by highlighting how this structural data can be useful for the design of new selective and potent SIRT modulators, and which are the missing structural data that are required to fully understand SIRT-related biological roles.

2. Common structural features of human SIRT proteins

Structurally, human SIRT proteins share a common catalytic core of around 250 amino-acids, with N- and C-terminal regions of variable length (**Fig. 1**). Those regions seem to be able to acquire different conformational states, also upon post-translational modifications, that may explain their ability to bind multiple partners, and to exert multiple roles in cells (North and Verdin, 2007; Pandithage *et al.*, 2008; Grob *et al.*, 2009; Costantini *et al.*, 2013) (**Fig. S1**). The importance of the terminal regions was also highlighted by the work from Kang *et al.*, in which they demonstrated the essential role of 25 residues (from Gln641 to Val665) localised at the C-terminal region of SIRT1 for catalytic activity modulation. This peptide sequence is in fact able to interact with the core domain, inducing conformational changes that increase the affinity of SIRT1 for its substrate (Kang *et al.*, 2011).

Structural information regarding the behaviour of those regions is lacking for the other SIRT isoforms so far.

SIRT2 was the first human isoform to be crystallised in 2001 by Finnin *et al.*, and provided a useful insight into the organisation of the SIRT catalytic core (**Fig. 2A**) (Finnin *et al.*, 2001). The SIRT catalytic core is organised as follow: i) a large domain characterized by an inverted Rossmann fold, typical of an NAD(H)/NADP(H) binding enzyme (Bellamacina, 1996; Finnin *et al.*, 2001), which consists of six β -strands, organised in a parallel β -sheet, and of six α -helices packing against the β -sheet; ii) a small domain composed of two modules, one with four α -helices, called helical module, and the other one with a structural zinc ion, tetra-coordinated by four Cys residues, called zinc module (Finnin *et al.*, 2001). A binding site accommodating both acetylated substrates and NAD⁺ is found at the interface of the two domains, linked to each other by two flexible loops (L1 and L2). L3 also takes part in the formation of the binding site, and participates in stabilizing the binding of acetylated substrates (**Fig. 2A, Fig. 2B**). This binding site is composed by a NAD⁺ binding pocket, and by a lysine channel (**Fig. 2A**). The NAD⁺ binding pocket is characterised by three sub-pockets. The ADP-ribose moiety binds to the sub-pocket A. Nicotinamide-ribose accommodates in the B sub-pocket while, during catalysis, it occupies the C sub-pocket, characterised by the TQNXD sequence (**Fig. S2**), allowing the transfer of the acetyl group from the Lys residue of a protein substrate to the ribose of NAD⁺. The lysine channel, able to accommodate acetylated substrates, consists of a narrow channel adjacent to the NAD⁺ binding pocket (B sub-pocket, **Fig. 2A**).

To date, 62 crystals of human SIRTs catalytic core have been reported in the Protein Data Bank (PDB) (<http://www.rcsb.org/>, accessed: June 17th 2016), providing insight into the different stages of SIRT catalytic cycles. As yet, no structures have been elucidated for SIRT4 and SIRT7 isoforms. Details are reported in **Table 1**. Unfortunately, until now, a structure-based strategy for the discovery of SIRT isoform-selective modulators has been difficult to set up due to the high structural similarity among SIRT catalytic cores.

3. Human SIRTs are flexible proteins undergoing massive and punctual conformational changes

3.1. Substrate binding induces a massive rearrangement of the SIRT catalytic core domain

It has been observed that SIRT proteins are able to adopt different conformational states. Moniot *et al.* first described how SIRT2 can switch from an apo conformation (open) to a productive conformation (closed) upon substrate and NAD⁺ binding (Moniot *et al.*, 2013). Substrate binding results in a 25° rotation of the small domain compared to the Rossmann fold, leading to the rearrangement of the L1-L3 loops (**Fig. 2B**):

- L1 is reoriented in order to interact with ADP-ribose and therefore to enclose the C sub-pocket of the NAD⁺ binding site. This is accompanied by a 45° reorientation, compared to the apo conformation, of the α -helix of L1, along its unwinding. Such conformational change has been previously reported to occur upon NAD⁺ binding by Sakkiah *et al.*, using molecular dynamics simulation of docking complexes obtained from the apo SIRT2 isoform (Sakkiah *et al.*, 2012; Sakkiah, S. *et al.*, 2013a; Sakkiah, S. *et al.*, 2013b).
- L2 moves close to the NAD⁺ binding loop L1, creating a channel able to interact with the acetylated lysine substrates in the pocket.
- L3, found in the large domain, undergoes conformational changes interacting with ADP-ribose as a result of the side chain reorientations of two conserved residues (Val266 and Ser263, **Fig. 5**). This conformational change also helps in stabilizing the acetylated substrates into the lysine channel.

Similar behaviour upon substrate and/or NAD⁺ binding has been described in the literature for SIRT1, SIRT3, and SIRT5 isoforms (Jin *et al.*, 2009; Zhou *et al.*, 2012; Zhao *et al.*, 2013).

Currently, no structural information is available for SIRT4, and no assumptions concerning the flexibility of its catalytic pocket can be done since this enzyme exhibits better ADP-ribosyl transferase rather than deacetylase activity (Ahuja *et al.*, 2007). L1-L3 sequences are highlighted in **Fig. S2**.

3.2. SIRT6: elements of structural specificity

Pan *et al.* reported the lack of the helical module characterizing the small domain of SIRT6, which was present in the other SIRT isoforms (**Fig. 2C**). This particular feature was proposed to explain the observed rigidity of L1 in SIRT6, even in the absence of a NAD⁺ molecule (Pan *et al.*, 2011). Indeed,

the zinc-binding domain of SIRT6, being unable to interact with the helical module, leads to a more flaring small domain that is positioned in an open conformation through direct interaction with the large domain, tilting at an angle of 45° compared to the Rossmann fold. Pan *et al.* also reported the presence of a highly flexible loop, connecting zinc-binding Cys residues (**Fig. 2C**), but did not provide any information about its effect on SIRT6 activity. The zinc-binding domain is thought to modulate substrate-specific binding (Zhao *et al.*, 2004). It can be hypothesised that such a loop may play a role in this specific recognition. Even though a crystallographic structure has not been resolved yet, a similar behaviour for SIRT7 was proposed, considering the lack of the helix bundle in the small domain (Pan *et al.*, 2011).

With regard to L2, Jiang *et al.* observed in SIRT6 the same movements as in the other isoforms, upon long-chain fatty acyl lysine substrate binding (Jiang *et al.*, 2013). However, Pan *et al.* described a difference in backbone orientation of the Trp186 residue compared to the Phe residue conserved in other SIRT isoforms (**Fig S2**). This difference was proposed as an explanation for the decreased deacetylase activity observed in SIRT6: the substrate cannot interact efficiently with the protein due to the orientation of Trp186.

3.3. Loops modulate isoform-specific protein-protein interactions

As previously mentioned, the organisation of the SIRT catalytic core is relatively well conserved among all members of the SIRT family (**Fig. 1A**). However, there are zones, out of the catalytic pocket, that may be involved in the specific recognition of biological partners. Moniot *et al.* reported the presence in SIRT2 of a distinctive flexible loop in the Rossmann fold, composed by 15 residues (from Ser293 to Met307) (Kang *et al.*, 2011; Moniot *et al.*, 2013) (**Fig. 3A**).

In the apo conformation of SIRT2, the loop appears to be very well organized (yellow loop, **Fig. 3A**) forming an α -helix structure. In the productive conformation, upon substrate and NAD⁺ binding, this loop appeared to be completely disorganised (dark green loop, **Fig. 3A**), with the loss of the α -helix structure. The β -factor analysis for this loop indicates, surprisingly, a great stability in both conformational states. Moniot *et al.* therefore proposed a protein-protein interaction mediating role for this loop, based on the fact that it is known to be responsible for the artificial crystal dimerization of

SIRT2 apo conformation. Moreover, in the crystallographic productive conformation, this loop was found to mediate the interaction with other chains present in the asymmetric unit through a Leu residue (Leu297) that may mimic the acetyl chain of substrates (Moniot *et al.*, 2013).

In SIRT5, Schuetz *et al.* described a loop (from Ala183 to Leu204) inserted in the zinc-binding domain overlaying the helix bundle (magenta loop, **Fig. 3B**). This loop, also found in SIRT4 (from Ala194 to Val217), was proposed to mediate protein-protein interaction and substrate-specific binding (Zhao *et al.*, 2004; Schuetz *et al.*, 2007).

3.4. Allosteric pockets modulate SIRT catalytic activity

Allosteric activation is a mechanism mediated by an effector molecule that aims at increasing the protein enzymatic activity. The effector molecule binds to a pocket distinct from the catalytic one, and triggers a conformational change in the protein structure, enabling better binding of the substrate, and hence better activity. The discovery of SIRT1 activation by resveratrol (Howitz *et al.*, 2003) was linked to the beneficial effects of this compound in type 2 diabetes (Milne *et al.*, 2007). In 2015, Cao *et al.* have managed to crystallise SIRT1 protein in complex with resveratrol and a coumarin-bound peptide acting as substrate (PDB code 5BTR, **Table 1** (Cao *et al.*, 2015)). It appears that three resveratrol molecules bind SIRT1 at the entry of the substrate channel. Of those three molecules, two were found to be essential for mediating the interaction between the SIRT1 catalytic core domain and the extended N-terminal domain typical of SIRT1 (**Fig. 4**). Such interaction allows tighter binding of the co-crystallized SIRT1 peptide substrate, and promotes SIRT1 catalytic activity. Other crystals of SIRT proteins in a complex with a fluorophore-bound peptide and with resveratrol derivatives have been published previously (**Table 1**). Different behaviours were reported, depending on the isoform and on the molecule studied. The work from Cao *et al.* supports the idea of an artificial activation of SIRT1 by resveratrol. It seems that the effect of resveratrol depends on the presence of the coumarin moiety of the peptide bonded to SIRT1. Cao *et al.*, by testing the native peptide without coumarin or with an aromatic residue close to the acetylated lysine, did not observe any activation of SIRT1 *in vitro* (Cao *et al.*, 2015).

The first X-ray structure of SIRT2 in a complex with the potent and selective SIRT2 drug-like inhibitor SirReal2 was reported. The basis for its high isoform selectivity was proposed to be a ligand-induced structural rearrangement of the active site, exploiting an adjacent, allosteric binding pocket (Rumpf. *et al.*, 2015b). In fact, upon binding of the SirReal2 molecule in the extended C-site (ECS) composed of the C sub-pocket and of a hydrophobic adjacent area formed by several Phe residues (Phe96, Phe119, Phe190, Phe131, Phe143, Phe234, and Phe235), SIRT2 was found to be locked in an open conformation (**Fig. 5**). Such a conformation allows the formation of an allosteric pocket behind the ECS, which Rumpf *et al.* reported as selectivity pocket. This pocket is formed by two loops (from Lys136 to Lys144; from Gly188 to Tyr191) resulting from a 7.2 Å rearrangement of a hinge region than connects the Rossmann fold domain with the zinc binding domain (**Fig. 5A**) (Rumpf. *et al.*, 2015b). Recently, the structural study of a series of indole derivatives in complex with SIRT2 identified the implication of this hinge region in complex stabilization (Rumpf *et al.*, 2015a). In particular, two indole molecules (EX-243) were crystallized in the SIRT2 catalytic site, one of them occupying the selectivity pocket. In this case, one of the loops from the hinge region (from Lys136 to Lys144) was observed to go through a 6 Å shift from the apo conformation (**Fig 5B**). Interestingly, this selectivity pocket was also able to accommodate long acylated chains such as those characterizing myristoylated substrates (**Fig. 5C**), explaining the preference of SIRT2 for such peptides characterized by long acylated chains (Feldman *et al.*, 2015).

4. One-off movements in human SIRT proteins: the gatekeepers

In addition to loop and domain movements, punctual movements, referred to single amino acids, have been reported in the literature for SIRT proteins. Shifts in side chain orientations actually act as gate keepers, unmasking concealed sub-pockets, and thus influencing the binding of ligands and/or biological partners. Several works described the essential role of a conserved Phe residue from L1 in both SIRT deacetylase and inhibition mechanisms (**Fig. S2**) (Jin *et al.*, 2009; Zhou *et al.*, 2012; Moniot *et al.*, 2013). This Phe residue when NAD⁺ is present, can adopt a perpendicular side chain conformation with regard to the ribose/nicotinamide moiety, allowing water molecules to enter into the C sub-pocket, and enabling the release of nicotinamide when cleaved during the reaction. By

contrast, when only ADP-ribose is present in the pocket, the Phe side chain adopts a parallel conformation compared to the ribose moiety, preventing water molecules from entering the C sub-pocket, therefore disabling the base-exchange reaction that causes NAD⁺ regeneration and SIRT inhibition by nicotinamide (**Fig. 6A**). The role of Phe was also reported very recently by both Gertz *et al.* and Zhao *et al.* (Gertz *et al.*, 2013; Zhao *et al.*, 2013). Phe273, interacting with EX-527 in SIRT1, and with other indole derivative molecules in SIRT3 (Phe157), participated in further stabilizing the complexes, thus favouring the inhibition mechanisms of those molecules. The specific conformation adopted by this residue traps the inhibitors in the C sub-pocket, preventing the release of nicotinamide (Gertz *et al.*, 2013; Zhao *et al.*, 2013).

It is known that the main catalytic activity of SIRTs is deacetylation. However, Du *et al.* reported that SIRT5 deacetylation activity is very low, compared to the capability of this isoform to de-succinylate and de-malonylate substrates (Du *et al.*, 2011). This specificity towards substrates having chemical groups other than acetyl groups can be correlated with the presence of two residues, Tyr102 and Arg105, not conserved in other isoforms (**Fig. S2**), and able to create specific polar contacts with succinylate and de-malonylate substrates (**Fig. 6B**). In 2012, Fischer *et al.* showed that insensitivity of SIRT5 to nicotinamide inhibition was the result of Arg105: this residue, pointing towards the C sub-pocket, prevents the positioning of nicotinamide in the C sub-pocket (Fischer *et al.*, 2012).

5. Conclusion

Through the removal of acetyl groups from specific lysine residues and/or through protein-protein interactions, SIRTs are considered as emerging therapeutic targets able to change function and sub-cellular localization of protein substrates, influencing signaling pathways mainly related to cancer, neurodegeneration, and metabolic diseases. At the time of our data collection, approximately 60 crystallographic structures of human SIRTs, excluding isoforms 4 and 7, were available (**Table 1**). From their analysis, it appeared that SIRTs are very flexible proteins, undergoing from punctual to massive conformational changes, most of them linked to interactions with molecular partners. The majority of the available SIRT structures revealed information on the catalytic site. Nevertheless, the high structural conservation among catalytic structures has limited the development of isoform-selective modulators. Such compounds are necessary not only for understanding isoform-specific

biological functions, but also for reducing off-target effects of future therapeutics. In our review, we have reported that the flexibility of the catalytic pocket relies, at least in part, on the behavior of L1-L3 loops, that was different in SIRT6 compared to other isoforms. Thus, targeting those loops may lead to locked conformations with a specific effect (increase/decrease) on SIRT catalytic activity. Very recent works on SIRT2 have shed light on catalytic isoform selectivity, by discovering molecules able to bind exclusive sub-pockets whose formation is driven by ligand-induced conformational arrangements. It was also reported that the targeting of allosteric pockets in SIRT1 may be source of selective catalytic modulation. From these observations, it is clear that structural and computational (e.g. molecular dynamics simulation) studies on SIRTs, focusing on protein plasticity, are necessary to identify unexplored structural regions of isoform selectivity.

Indeed, structural information on protein-protein complexes can provide crucial clues about SIRT-mediated biological mechanisms, and also ideas for the development of new drugs. Despite the lack of crystallographic data of protein-protein complexes involving SIRTs (**Table 1**), their zinc binding module has been proposed to mediate such interactions. Investigations on this topic should be now a top priority, first for identifying SIRT protein partners targeting this region, then for determining hotspots for the design of protein-protein disruptors or stabilizers. Further biochemical and conformational works on SIRT N- and C-terminal domains are also required. As they have been proven to exert a role in regulating SIRT activity, they may be considered as alternative targets for SIRT therapeutic regulation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at XXXX.

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Figure captions

Fig.1. Schematic representation (topology) of the seven human SIRT isoforms. N-terminal, catalytic, and C-terminal domains are represented in light blue, brown, and light green, respectively. The amino acid (aa) length of each domain is also reported.

Fig.2. Overall three-dimensional (3D) structure of SIRTs, and details on the structural organisation of the SIRT catalytic domain. SIRT2 structure is taken as an example (A). Flexible loops that undergo massive changes upon substrate binding are highlighted according to the legend (B). Structural comparison between SIRT2 and SIRT6: the helical module in SIRT6 is missing (C). The loop inserted in the SIRT6 zinc-binding module is coloured in red, and the myristoylated peptide co-crystallised with SIRT6 is represented in black capped sticks. Proteins are represented by ribbons, coloured according to the legend. Catalytic sub-pocket areas are represented as coloured surfaces. Images are generated using MOE 2014.09 (Chemical Computing Group, Inc., Montreal, Canada).

Fig.3. SIRT2 (A) and SIRT5 (B) loops involved in isoform-specific protein-protein interactions. Proteins are represented by ribbons. Loops are coloured as described in the text. Helical and zinc binding modules of each isoform are coloured in brown and blue, respectively. The large domain of each protein is coloured in light grey. Images are generated using MOE 2014.09 (Chemical Computing Group, Inc., Montreal, Canada).

Fig. 4. Overall structure of SIRT1 in complex with a coumarin-bound peptide and resveratrol molecules (PDB code 5BTR). The large domain, the zinc binding, and the helical modules of SIRT1 are represented by grey, blue, and brown ribbons, respectively. The same colour code is used for molecular surfaces. The N-terminal domain of SIRT1 implicated in allosteric regulation is represented by light pink ribbons. Peptide bound to a coumarin fluorophore is represented by magenta line and balls. Resveratrol molecules 1, 2, and 3 are reported in capped sticks and coloured in cyan, yellow, and dark green, respectively. Amino acids important for interactions with resveratrol molecules are represented by line and balls, and coloured according to SIRT1 domains colour code. Images are generated using MOE 2014.09 (Chemical Computing Group, Inc., Montreal, Canada).

Fig. 5. Overall SIRT2 structure, with a focus on hinge region movements formed upon the binding of SirReal2 (A), EX-243 (B), and of the myristoylated peptide substrate (C). Proteins are represented by ribbons and coloured according to the legend. Inhibitors and substrates are represented by ball and lines and coloured according to the legend. Amino acids belonging to the ECS site and to the selectivity pocket (from Lys133 to Lys144; from Gly188 to Tyr191) are represented by capped sticks and line and balls, respectively. Images are generated using MOE 2014.09 (Chemical Computing Group, Inc., Montreal, Canada).

Fig. 6. Phe96 acts as a gate keeper in SIRT2 (A). When ADP-ribose, represented by magenta purple line and balls, interacts with SIRT2, Phe96 is able to trap nicotinamide (yellow line and balls) in the C sub-pocket. Tyr102 and Arg105 are responsible for succinylate and de-malonylate activities in SIRT5 (B). C sub-pocket and a NAD⁺ molecule are highlighted in the pocket. Images are generated using MOE 2014.09 (Chemical Computing Group, Inc., Montreal, Canada).

Tables

Table 1 Review of human SIRT structures reported in the PDB (<http://www.rcsb.org/>, accessed: June 17th, 2016).

SIRT isoform	PDB code	Ligand	Resolution	Reference
SIRT1	4I5I	NAD ⁺ 6S-2-chloro-5,6,7,8,9,10- hexahydrocyclohepta[b]indole-6-carboxamide	2.50 Å	(Zhao <i>et al.</i> , 2013)
	4IF6	ADP-ribose	2.25 Å	NA
	4KXQ	ADP-ribose	1.85 Å	(Davenport <i>et al.</i> , 2014)
	4IG9	-	2.64 Å	
	5BTR	Resveratrol AMC-containing peptide	3.20 Å	(Cao <i>et al.</i> , 2015)
	4ZZH	(4S)-N-[3-(1,3-oxazol-5-yl)phenyl]-7-[3-(trifluoromethyl)phenyl]-3,4-dihydro-1,4-methanopyrido[2,3-b][1,4]diazepine-5(2H)-carboxamide	3.10 Å	(Dai <i>et al.</i> , 2015)
	4ZZI	4-(4-{2-[(methylsulfonyl)amino]ethyl}piperidin-1-yl)thieno[3,2-d]pyrimidine-6-carboxamide	2.73 Å	
	4ZZJ	(3S)-1,3-dimethyl-N-[3-(1,3-oxazol-5-yl)phenyl]-6-[3-(trifluoromethyl)phenyl]-2,3-dihydropyrido[2,3-b]pyrazine-4(1H)-carboxamide	2.74 Å	
SIRT2	1J8F	-	1.70 Å	(Finnin <i>et al.</i> , 2001)
	3ZGV	NAD ⁺	2.27 Å	(Moniot <i>et al.</i> , 2013)
	3ZGO	-	1.63 Å	
	4L3O	Macrocyclic peptide (S2iL5)	2.52 Å	(Yamagata <i>et al.</i> , 2014)
	4RMG	2-[(4,6-dimethylpyrimidin-2-yl)sulfanyl]-N-[5-(naphthalen-1-ylmethyl)-1,3-thiazol-2-yl]acetamide (Sir2real)	1.88 Å	(Rumpf,T. <i>et al.</i> , 2015b)
	4RMH	Sir2real Ac-Lys-H3 peptide	1.42 Å	
	4RMI	N-(5-benzyl-1,3-thiazol-2-yl)-2-[(4,6-dimethylpyrimidin-2-yl)sulfanyl]acetamide (Sir1real)	1.45 Å	
	4RMJ	ADP-ribose Nicotinamide	1.87 Å	
	4R8M	Thiomristoyl peptide	2.10 Å	(Teng <i>et al.</i> , 2015)
	5D7O	ADP-ribose	1.63 Å	(Rumpf,T. <i>et al.</i> , 2015a)
	5D7P	ADP-ribose EX-243	1.76 Å	
	5D7Q	ADP-ribose (6S)-2-chloro-5,6,7,8,9,10- hexahydrocyclohepta[b]indole-6-carboxamide	2.01 Å	
	4Y6L	H3K9 myristoylated	1.6 Å	(Feldman <i>et al.</i> , 2015)
	4Y6O	TNF-αK20 myristoylated	1.6 Å	
	4Y6Q	2-O-myristoyl-ADP-ribose	1.9 Å	
	4X3O	2-O-myristoyl-ADP-ribose	1.5 Å	NA

SIRT3	4X3P	Carba-NAD ⁺ Tridecanethial	1.8 Å	
	5DY4	NAD ⁺ N-{5-[(7-bromonaphthalen-1-yl)methyl]-1,3-thiazol-2-yl}-2-[(4,6-dimethylpyrimidin-2-yl)sulfanyl]acetamide	1.77 Å	(Schiedel, M. <i>et al.</i> , 2016a)
	5DY5	N-(5-{3-[(1-benzyl-1H-1,2,3-triazol-4-yl)methoxy]benzyl}-1,3-thiazol-2-yl)-2-[(4,6-dimethylpyrimidin-2-yl)sulfanyl]acetamide	1.95 Å	(Schiedel, M. <i>et al.</i> , 2016b)
	3GLR	AceCS2 peptide	1.80 Å	(Jin <i>et al.</i> , 2009)
	3GLS	-	2.70 Å	
	3GLT	ADP ribose bound to AceCS2 peptide with thioacetyl lysine	2.10 Å	
	3GLU	AceCS2 peptide	2.50 Å	
	4FVT	Carba-NAD ⁺ Ac-ACS peptide	2.47 Å	(Szczepankiewicz <i>et al.</i> , 2012)
	4HD8	Fluor-de-Lys peptide Piceatannol	2.30 Å	(Gertz <i>et al.</i> , 2012)
	4FZ3	p53 peptide coupled to 4-amino-7-methylcoumarin	2.10 Å	(Wu <i>et al.</i> , 2013)
	4JSR	N-{2-[1-(6-carbamoylthieno[3,2-d]pyrimidin-4-yl)piperidin-4-yl]ethyl}-N'-ethylthiophene-2,5-dicarboxamide	1.70 Å	(Disch <i>et al.</i> , 2013)
	4JT8	4-(4-{2-[(2,2-dimethylpropanoyl)amino]ethyl}piperidin-1-yl)thieno[3,2-d]pyrimidine-6-carboxamide	2.26 Å	
	4JT9	4-(4-{2-[(methylsulfonyl)amino]ethyl}piperidin-1-yl)thieno[3,2-d]pyrimidine-6-carboxamide	2.24 Å	
	4BN4	ADP-ribose	1.30 Å	(Nguyen, G.T. <i>et al.</i> , 2013b)
	4BN5	Carba-NAD ⁺ SRT1720	3.25 Å	
	4BV3	NAD ⁺ EX-527 ADP-ribose	2.00 Å	
	4BVB	ADP-ribose EX-527	2.00 Å	
	4BVE	Thioalkylimidate	2.05 Å	(Gertz <i>et al.</i> , 2013)
	4BVF	Thioalkylimidate EX-527	2.70 Å	
	4BVG	ADP-ribose EX-527	2.50 Å	
	4BVH	2'-O-acetyl-ADP-ribose EX-527	1.90 Å	
	4C78	Bromo-resveratrol ACS2 peptide	2.00 Å	(Nguyen, G.T. <i>et al.</i> , 2013a)
	4C7B	Bromo-resveratrol Fluor de Lys peptide	2.10 Å	
	4V1C	-	2.95 Å	(Bao <i>et al.</i> , 2014)
	4O8Z	(2-butyl-1-benzofuran-3-yl){4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl}methanone	2.00 Å	To be published

	5D7N	-	1.83 Å	(Rumpf, T. <i>et al.</i> , 2015a)
SIRT5	2B4Y	ADP-ribose 4-(2-Hydroxyethyl)-1-piperazine ethansulfonic acid	1.90 Å	NA
	2NYR	Suramin	2.06 Å	(Schuetz <i>et al.</i> , 2007)
	3RIG	Thioacetylated lysine peptide	2.00 Å	(Du <i>et al.</i> , 2011)
	3RIY	NAD ⁺ Succinyl lysine peptide	1.55 Å	
	4F4U	Succinyl lysine peptide	2.00 Å	(Zhou <i>et al.</i> , 2012)
	4F56	ADP ribose bound to thiosuccinyl peptide	1.70 Å	
	4GC1	Succinyl-IDH2 Carba-NAD	1.94 Å	(Szczepankiewicz <i>et al.</i> , 2012)
	4HDA	Acetylated peptide Resveratrol	2.60 Å	(Gertz <i>et al.</i> , 2012)
SIRT6	3K35	ADP-ribose	2.00 Å	(Pan <i>et al.</i> , 2011)
	3PKI	ADP-ribose	2.04 Å	
	3PKJ	2'-N-Acetyl-ADP-ribose	2.12 Å	
	3ZG6	ADP-ribose N6-myristoyl lysine peptide	2.20 Å	(Jiang <i>et al.</i> , 2013)

Figure1_revised

SIRT2

PDB code 1JF8 (green loop, apo-form)

PDB code 3ZGV (yellow loop, productive form)

SIRT5

PDB code 3RIY

Figure4_revised