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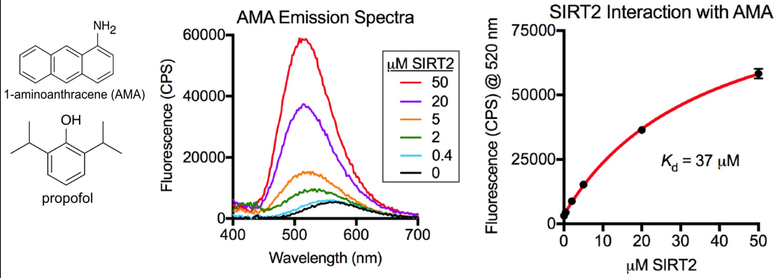
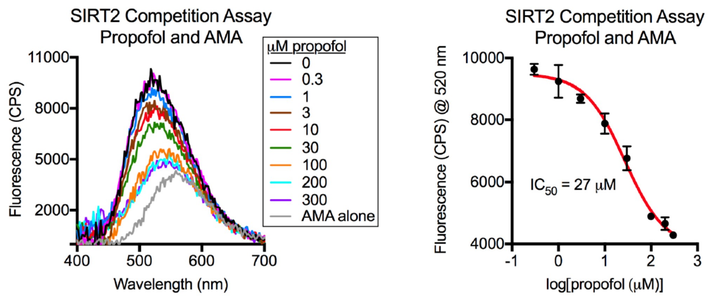
Abstract

Sirtuin isoform 2 (SIRT2) is an enzyme that catalyzes the removal of acyl groups from lysine residues. SIRT2's catalytic domain has a hydrophobic tunnel where its substrate acyl groups bind. Here, we report that the fluorescent probe 1-aminoanthracene (AMA) binds within SIRT2's hydrophobic tunnel in a substrate-dependent manner. AMA's interaction with SIRT2 was characterized by its enhanced fluorescence upon protein binding (>10-fold). AMA interacted weakly with SIRT2 alone in solution (K_d = 37 μM). However, when SIRT2 was equilibrated with a decanoylated peptide substrate, AMA's affinity for SIRT2 was enhanced ~10-fold (K_d = 4μM). The peptide's decanoyl chain and AMA co-occupied SIRT2's hydrophobic tunnel when bound to the protein. In contrast, binding of AMA to SIRT2 was competitive with a myristoylated substrate whose longer acyl chain occluded the entire tunnel. AMA competitively inhibited SIRT2 demyristoylase activity with an IC_{50} of 21 μM, which was significantly more potent than its inhibition of other deacylase activities. Finally, binding and structural analysis suggests that the AMA binding site in SIRT2's hydrophobic tunnel was structurally stabilized when SIRT2 interacted with a decanoylated or 4-oxononanoylated substrate, but AMA's binding site was less stable when SIRT2 was bound to an acetylated substrate. Our use of AMA to explore changes in SIRT2's hydrophobic tunnel that are induced by interactions with specific acylated substrates has implications for developing ligands that modulate SIRT2's substrate specificity.

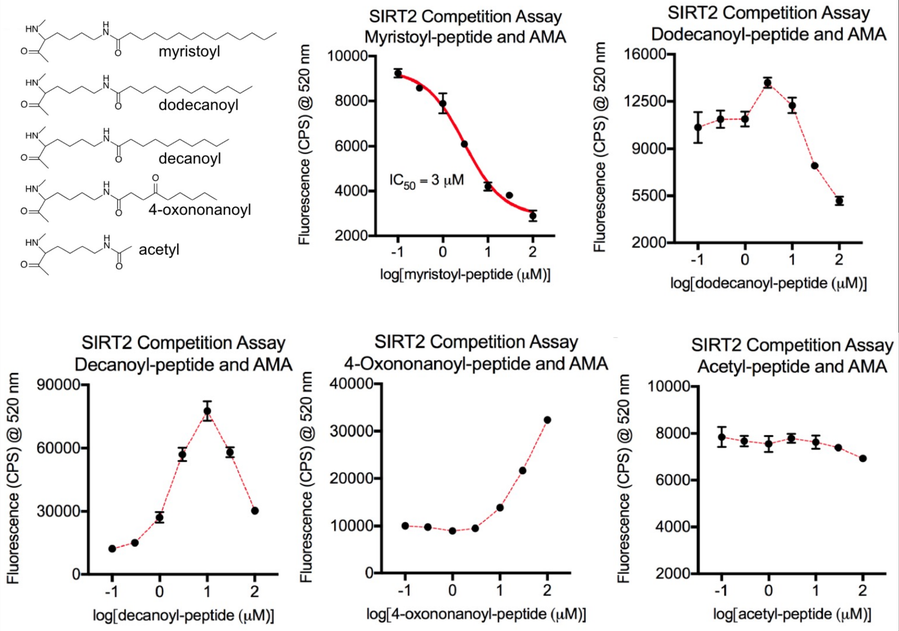
1-AMA Binds SIRT2



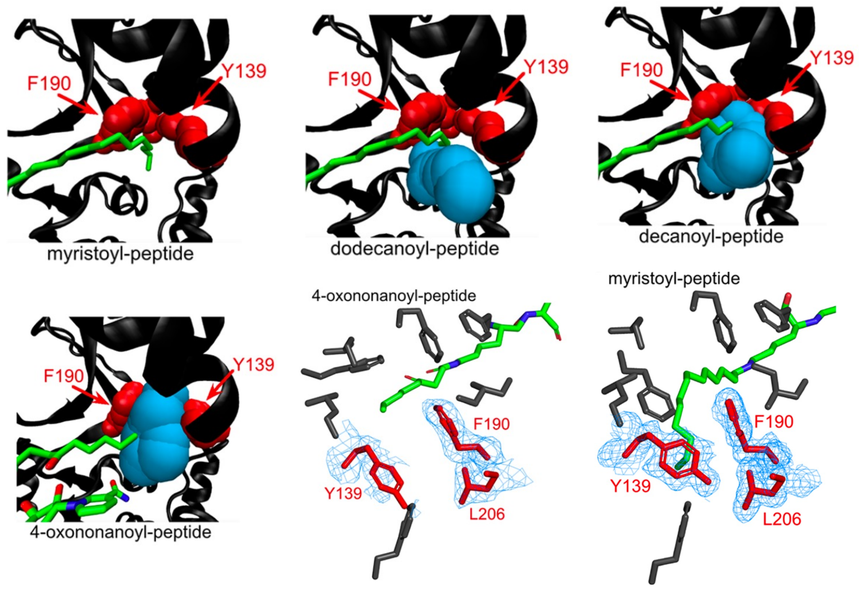
1-AMA Binds the Propofol Site



Acylated Peptides interacts with SIRT2 and AMA

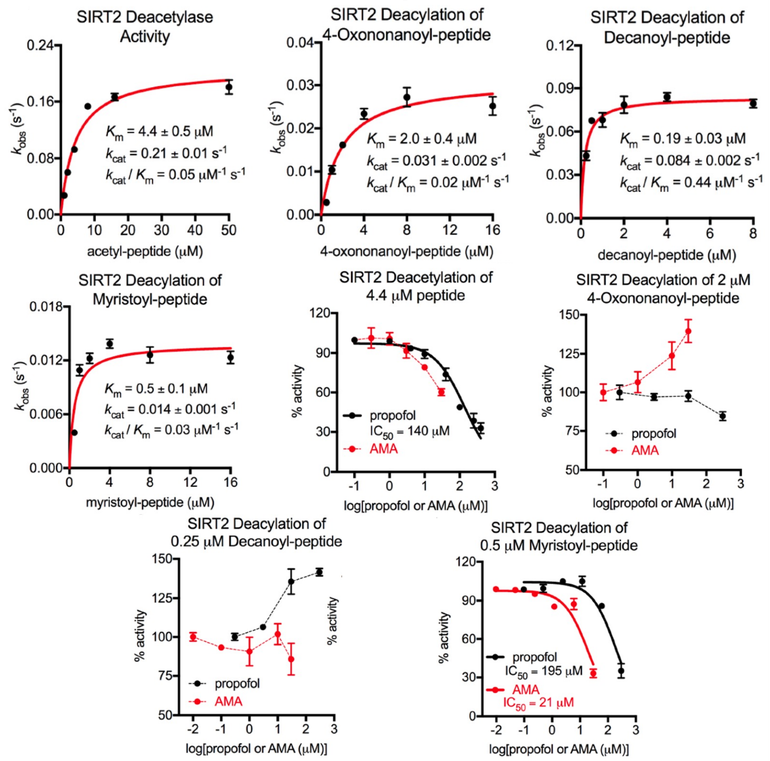


Acylated Peptides' Binding Site on SIRT2



Green sticks represent acylated peptides. Red spheres represent the sites where the residues interact with the substrate. Blue spheres represent the pocket where AMA and propofol binds to on SIRT2.

Modulated SIRT2's Deacylase Activity with AMA and Propofol



Conclusions

Using AMA's fluorescent propertites, It should now be possible to understand how the shape of the hydrophobic tunnel changes with different acyl chains bound, and to design or modify ligands specific for each pocket. Molecules that alter select deacylase activities of SIRT2 will be important to understand the role of the diverse lysine modifications that occur in the cell and this may suggest diseases that can be treated with substrate-dependent SIRT2 modulators that have reduced toxicity compared to nonspecific SIRT2 ligands.

Acknowledgements

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References

1. Bi, D., Yang, J., Hong, JY., Parikh, P., Hinds, N., Infanti, J., Lin, H., Weiser, B.,: Substrate-Dependent Modulation of SIRT2 by a Fluorescent Probe, 1-Aminoanthracene. *American Chemical Society: Biochemistry (2020) doi:10.1021/acs.biochem.0c00564.*