Interaction of Fluorescent Probes with Sirtuin Proteins

James Fusco[1], Brian P. Weiser[1]

[1]*Department of Molecular Biology, Rowan University School of Osteopathic Medicine, Stratford, NJ*

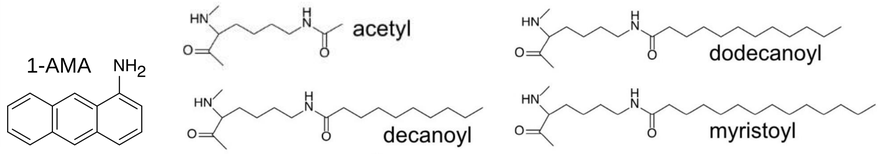
Abstract

Sirtuins are a class of proteins belonging to the Sir2 (Silencing information regulator 2) family of NAD+ dependent protein lysine deacylases. Different Isoforms (SIRT1-SIRT7) differ in their specific deacylase activity and cellular location. They have roles in DNA repair, glucose metabolism, and cellular proliferation which make them highly desirable targets for carcinoma therapeutics. We previously used 1-aminoanthracene's (AMA) fluorescent properties when bound with SIRT2 (Kd of 37 µM) to develop a high-throughput screen to identify novel ligands that inhibit SIRT2's enzymatic activities. We hope to reveal other potential probes for future high-throughput screening with all the sitruin isotopes. 1-AMA's fluorescence along with fluorescent labeled peptides "Cy3-PEG4-H4K16(myr)" and "FAM-PEG4-H4K16(myr)" were used in binding assays to determine their affinities with SIRT2, SIRT3, and SIRT6. Further, we determined 1-AMA's ability to bind sirtuin isoforms when they were equilibrated with 100 µM of various acyl-peptides. 1-AMA displays weak binding to SIRT3 and SIRT6 when compared to SIRT2. FAM-PEG4-H4K16(myr) binds SIRT2 with a Kd of 7nM which is much higher than its interaction with SIRT3 and SIRT6 (Kd of 6 µM and 2 µM, respectively). Cy3-PEG4-H4K16(myr) binds as expected SIRT2,3,6 although its affinity for SIRT6 changes minimally with the addition of ADP-ribose, which suggests Cy3 may facilitate binding in the absence of SIRT6's co-factor. Future work will test additional probes with the other sirtuin proteins and establish their competency to be utilized for high-throughput screening.

Methods

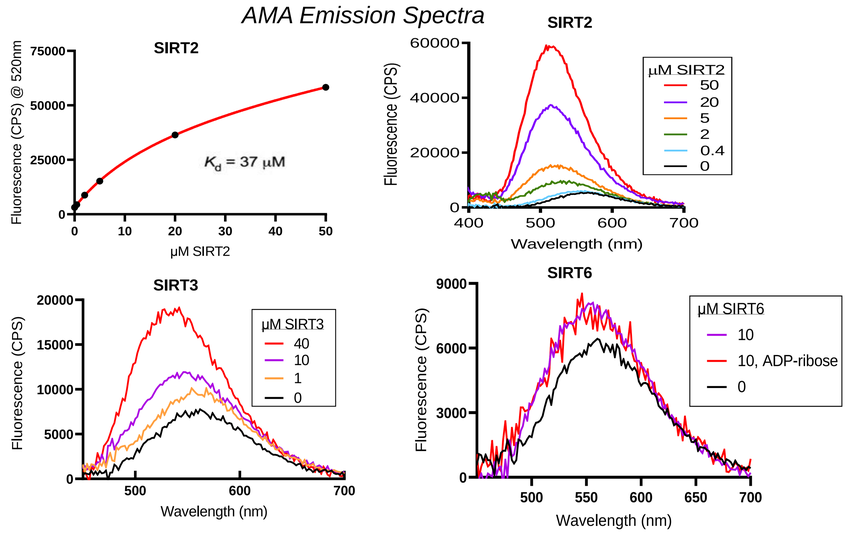
Binding assays with recombinant human SIRT2, SIRT3, and SIRT6 proteins were studied using a Horiba Fluoromax-4 instrument. All assays were performed at 23 degrees Celsius in a buffer of PBS using a quartz microcuvette (3mm path length) with an assay volume of 200 µL. To measure sirtuin's interaction with AMA, the protein was diluted to the appropriate concentration and scanned with the fluorometer (λ_{ex} = 410 nm, and λ_{em} = 420 – 700 nm). Slit width for excitation was 4 nm and emission slit width was 8 nm. 1 µL of AMA was added such that the final concentration of AMA was 100 nM. The sample was gently mixed by pipetting and the fluorescent scan was repeated. Background spectra of the protein-only sample was subtracted from the sample containing 1-AMA and counts per second (CPS) fluorescence intensities were plotted versus sirtuin concentration. A similar procedure measured the interaction of each sirtuin with Cy3-PEG4-H4K16(myr) except peptide concentration was 50 nm and λ_{ex} / λ_{em} was 535nm / 550 – 570. Sirtuin's interaction with FAM-PEG4-H4K16(myr) was measured either with anisotropy or CPS with λ_{ex} = 495 nm and λ_{em} = 520 nm, and the peptide concentration was also 50 nM. Scans with fluorescent peptides had excitation and emission slit widths of 2 and 4, respectively. The two labeled peptides are 14 amino acid peptides whose sequence is derived from histone H4 with lysine 16 myristoylated. The N-terminus of the peptides is modified with a FAM or Cy3 group.

Chemical Structures of AMA and Lysine Acyl Modifications

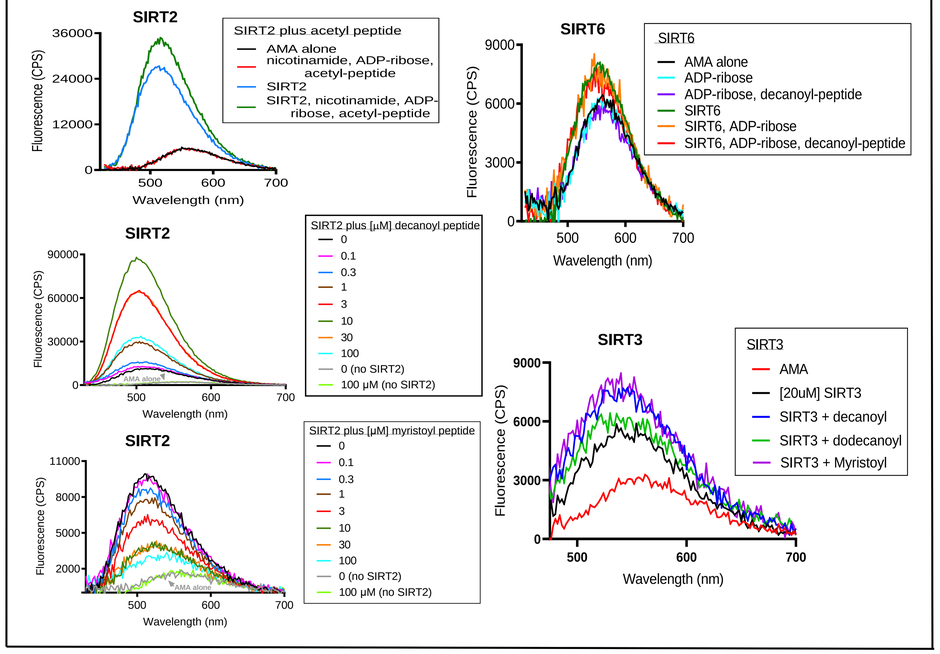


Interaction of SIRT2, SIRT3, and SIRT6 with AMA

AMA Emission Spectra

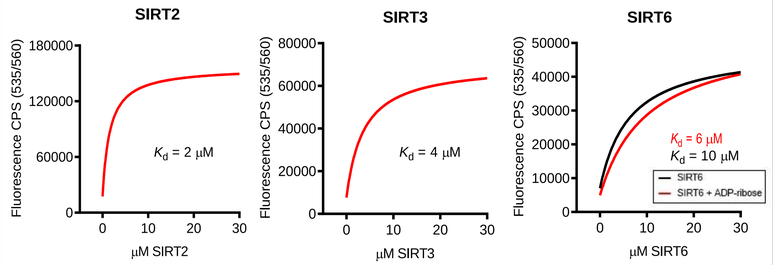


AMA Emission Spectra with Acyl-peptide bound to sirtuins



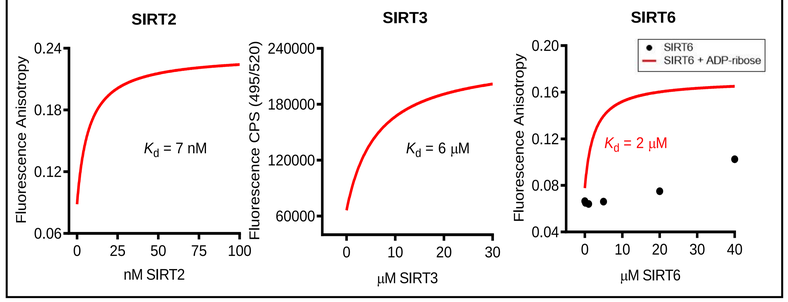
Sirtuin Interaction with Cy3-Labeled Peptide

Cy3-PEG4-H4K16(myr) Binding isotherms based on emission intensity



Sirtuin Interaction with FAM-Labeled Peptide

FAM-PEG4-H4K16(myr) Binding isotherms



Future Work and Conclusions

This work represents an early step to enhance and diversify high-throughput screening tools to identify novel ligands that may affect sirtuin activity.

Future work will test the specificity of fluorescent probes and ligands on other sirtuin isoforms. This will help identify novel compounds for potential drug development and to elucidate sirtuin enzyme function.

Acknowledgements

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References

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