Investigating Epigenetic Regulation of Histone Proteins

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BACKGROUND

Chromatin fibre consists of DNA coiling around nucleosomes which made of an octamer of histone proteins. Epigenetic enzymes regulate gene expression via introduction, recognition or removal of (**P**ost**T**ranslational **M**odifications) **PTM**'s on histone tails. Abnormal PTM patterns due to enzyme dysregulation are linked to various diseases.

Sirt1 is an NAD+-dependent histone deacetylase (HDAC). Deacetylation leads to change in chromatin structure and regulation of gene expression (repression of gene expression). Contributes to activation of stress response pathways, namely during autophagy and DNA repair. Associated with some cancers, neurodegenerative disorders, cardiovascular diseases, and metabolic disorders.

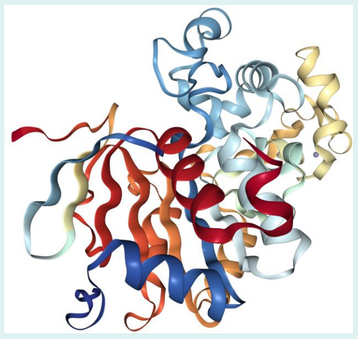


Figure 1: Sirt1 protein structure [1].
PDB: 4IG9

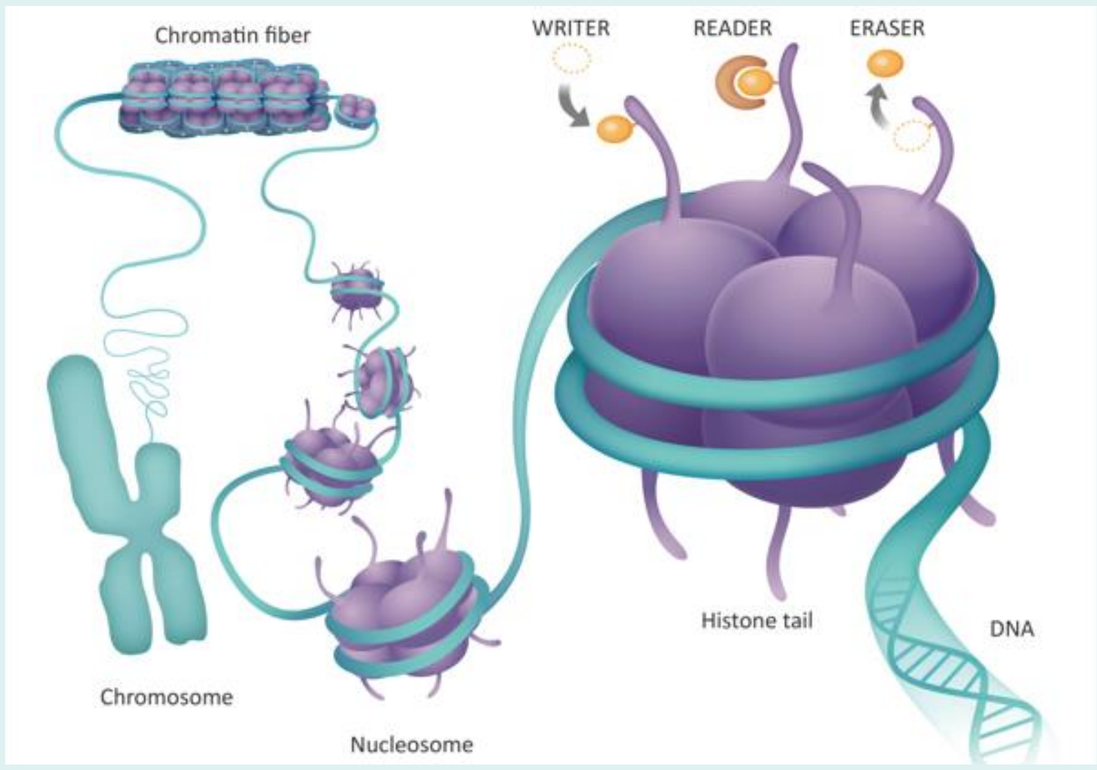


Figure 2: Action area of epigenetic enzymes [2].

- **Aims:** Development of a high-throughput method of analysing interaction between unnatural PTM's and epigenetic enzymes.
- **Applications:** learning about substrate specificity of the enzyme could potentially lead to new therapeutic discoveries (e.g. aid with design of inhibitors).

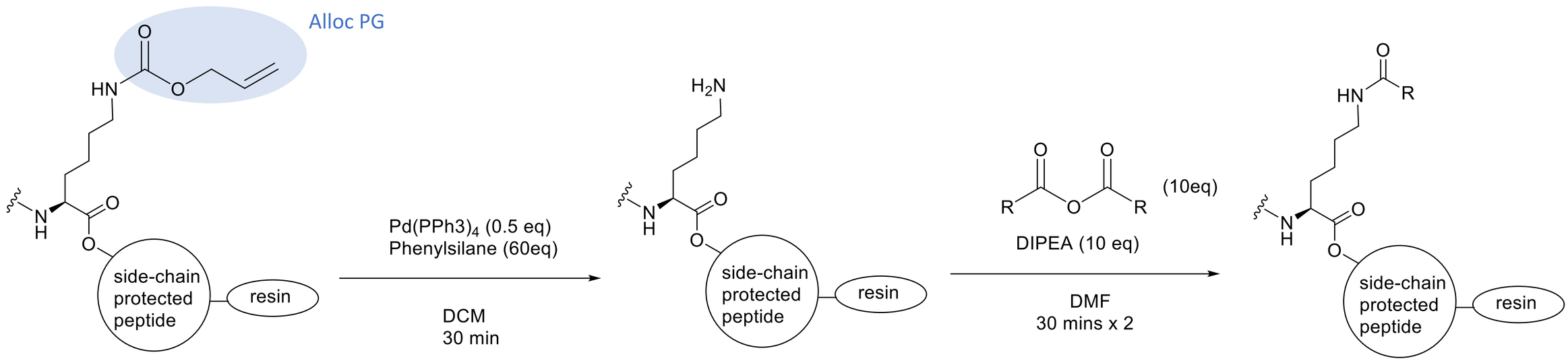


Figure above: Left to right: Alloc deprotection, modification of free Lysine with various anhydrides

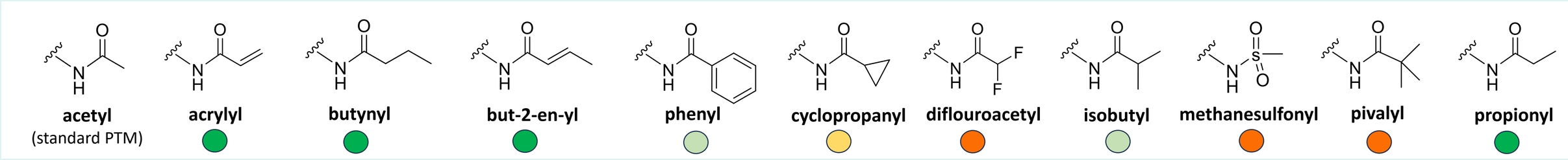


Figure above: Various PTM's (R-groups) synthesized as a result of reaction of free lysin with anhydrides.

RESULTS & DISCUSSION

- Successful synthesis of peptides with the modified lysine through orthogonal protecting group (PG) – Alloc
- Successful protein expression – Sirt1
- Assay testing using MALDI-TOF – deacetylation was confirmed by formation of the unmodified lysine H3 (1-21) product, as quantified by mass spectrometry, indicating the peptide was a substrate of Sirt1 (especially important due to crude nature of it – no HPLC purification).
- Novel potential substrates were discovered, known were verified.

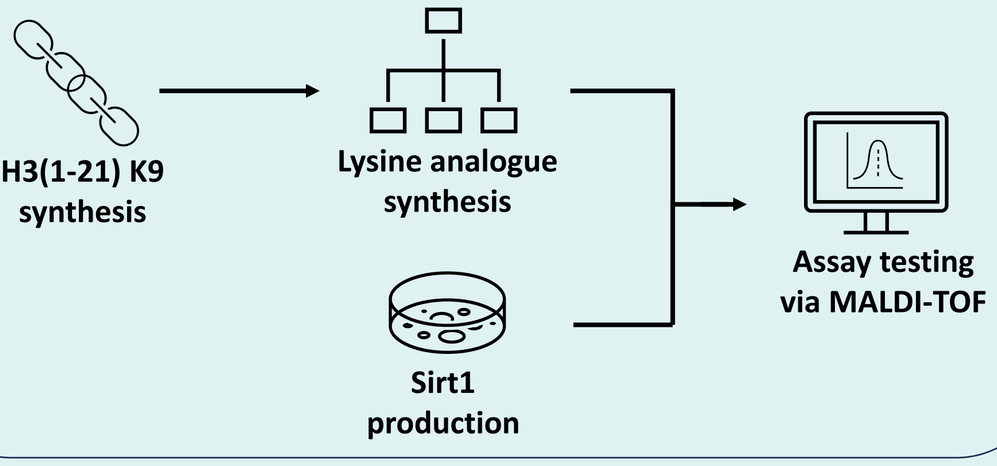
H3(1-21) sequence: ARTKQTARK*STGGKAPRKQLA-*NH*$_2$ where K* is modified

peptide	K9 modification	SIRT1 activity
H3(1-21)	free lysine (purified)	
	acetyl (purified)	
	acetyl	
	acrylyl	
	butynyl	
	but-2-enyl	
	phenyl	
	cyclopropanyl	
	difluoroacetyl	
	isobutynyl	
	methanesulfonyl	
	pivalyl	
	propionyl	

key:

color	note
	complete/near complete removal
	major removal
	partial removal
	no removal/minimal removal

SUMMARY



References:
1. Sinobiological, https://www.sinobiologial.com/resource/sirt1/proteins, (accessed July 2023).
2. Oryzon, https://www.oryzon.com/en/epigenetics/epigenetics, (accessed July 2023).

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