



University
of Victoria

Graduate Studies

Notice of the Final Oral Examination
for the Degree of Master of Science

of

CHARLOTTE DEWAR

BSc (University of Victoria, 2017)

“Antibody-free Affinity Enrichment for Global Methyllysine Discovery”

Department of Chemistry

Wednesday, October 30, 2019

1:00 P.M.

Elliott Building

Room 230

Supervisory Committee:

Dr. Fraser Hof, Department of Chemistry, University of Victoria (Supervisor)

Dr. Jeremy Wulff, Department of Chemistry, UVic (Member)

External Examiner:

Dr. Stephanie Willerth, Department of Mechanical Engineering, UVic

Chair of Oral Examination:

Dr. Richard Pickard, Department of English, UVic

Abstract

Lysine methylation is a post-translational modification that regulates a large array of functionally diverse processes that are vital for cellular function. The role of methylation is best characterized on histone proteins due to their high concentration in the cell, but alongside histone modifications, lower abundance non-histone methylation is emerging as a prevalent and functionally diverse regulator of cellular processes. The direct biological impact of non-histone lysine methylation is less well understood because of their low detection efficiency. The dynamic concentration range of the proteome masks their signal during proteomic analysis which impedes the detection of these low abundance methylated proteins. Increasing the concentration of proteins bearing methylation is required for improved discovery. This requires enriching the post-translational modification with a capturing reagent prior to analysis.

This thesis details an optimized method for using the supramolecular host *p*-sulfonatocalix[4]arene as a stationary phase methyllysine enrichment reagent for real-life cell-extracted proteins. Prior to the optimizations described in this thesis, cell-derived peptide extracts would not retain within the early generation upper-rim modified calixarene column. But with the new protocols detailed in this thesis, proteins extracted from both cultured prostate cancer cells and industrially sourced brewer's yeast were successfully retained by a lower-rim modified calixarene column. Thousands of methylated proteins with diverse functions and cellular localization were discovered using this method. Detection of low abundance methylated proteins will help discover the full methylome, which in turn, will help delineate its biological function.