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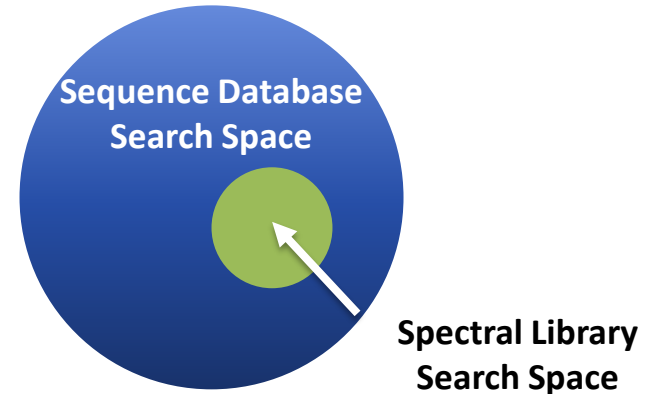
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Human Hair Keratin Extraction and Genetically Variant Peptide Detection

Meghan C. Burke, Zheng Zhang, William E. Wallace,
Yuxue Liang, Sergey Sheetlin, Yuri A. Mirokhin,
Dmitrii V. Tchekovskoi & Stephen E. Stein

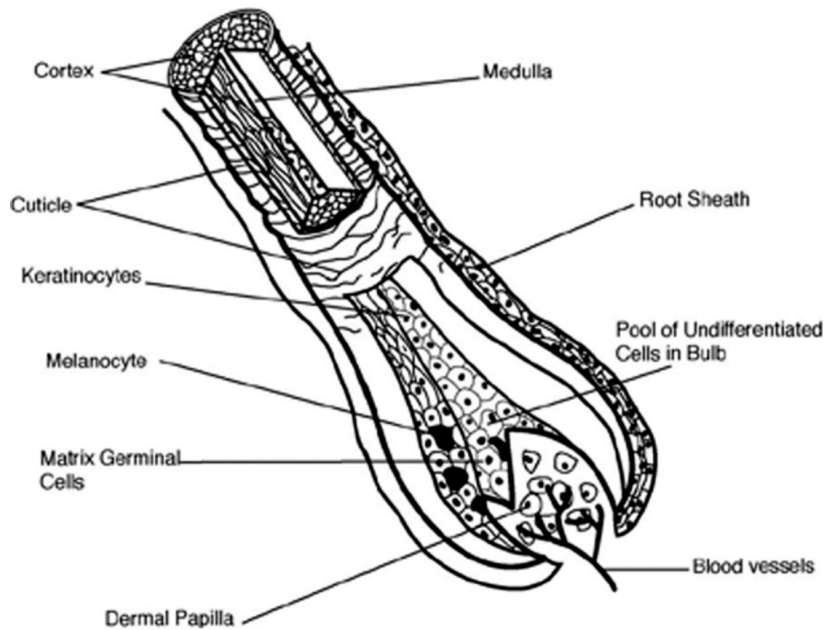
Human Hair-derived Genetically Variant Peptides (GVPs)

- Why hair?
 - *Chemically stable, easily obtained*
- Why protein?
 - *Chemically more stable than DNA*
- Why peptides?
 - *Advantageous for complex mixtures and limited sample*
 - *Ionize and fragment well in a mass spectrometer*
- Why GVPs?
 - *Can provide inheritance information in the absence of high quality DNA*

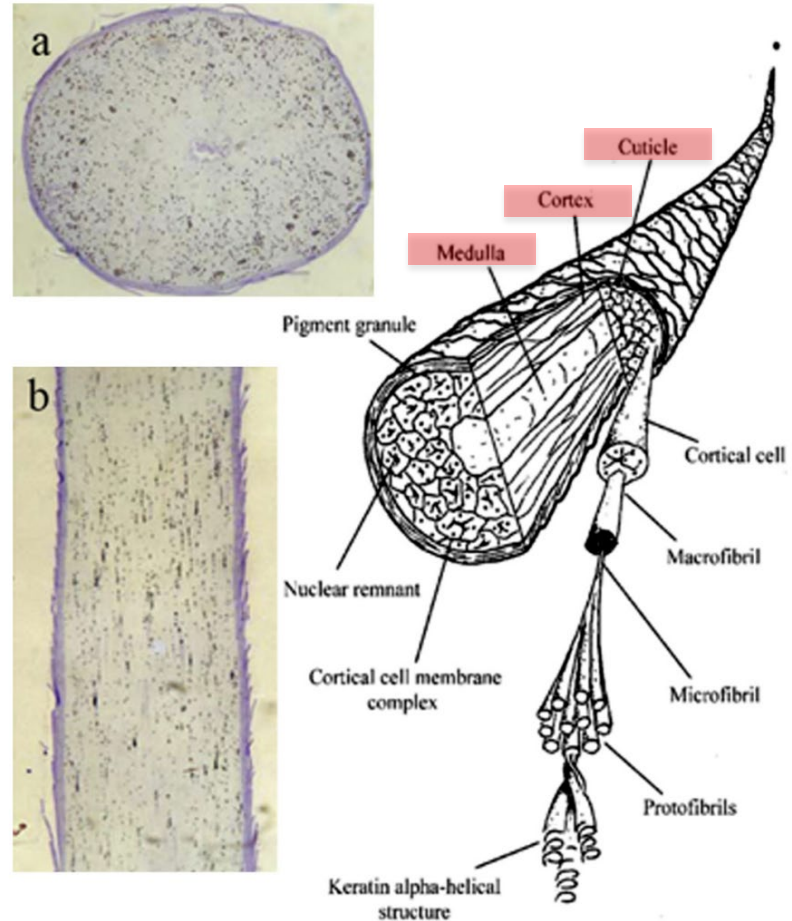


Human Hair Structure

Hair Root



Hair Shaft



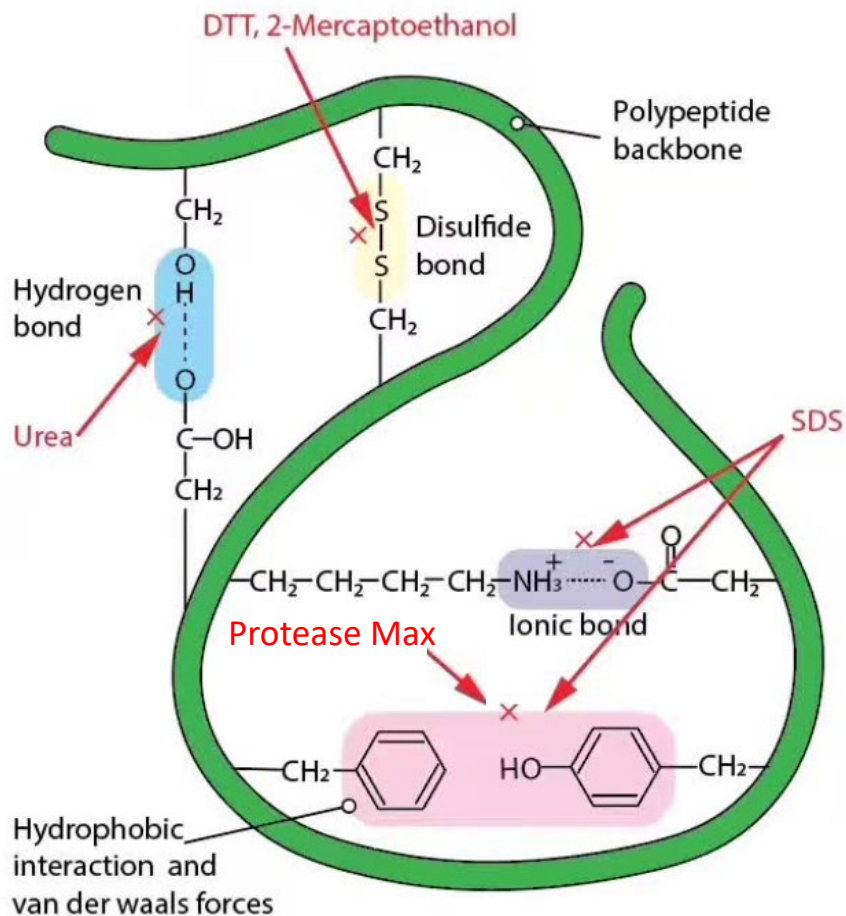
Bengtsson, C. F.; Olsen, M. E.; Brandt, L. Ø.; Bertelsen, M. F.; Willerslev, E.; Tobin, D. J.; Wilson, A. S.; Gilbert, M. T. P., DNA from keratinous tissue. *Annals of Anatomy - Anatomischer Anzeiger* **2012**, *194* (1), 17-25.



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Protein Extraction From Human Hair



<https://kimberlybiochemist.wordpress.com>

[1] Urea & Protease Max¹

[2] NaOH & Sodium Dodecyl Sulfate (SDS)²

– Repeat extraction 3x

[3] Single Step Method (SDS Alone)

¹Parker GJ, Leppert T, Anex DS, Hilmer JK, Matsunami N, et al. (2016) PLOS ONE 11(9): e0160653.

²Wong, S. Y., Lee, C. C., Ashrafzadeh, A., Junit, S. M., Abraham, N., & Hashim, O. H. (2016). PLOS ONE , 11(10), e0164993.

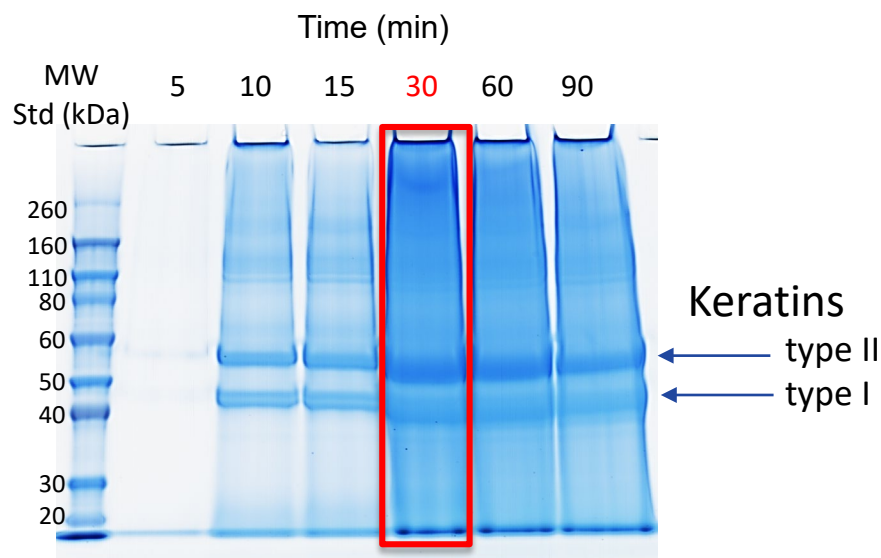


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Optimization of Single Step Method Protein Extraction

Time course study to optimize the heating time



Time(min)	Scanned Intensity	
	Type I	Type II
5	1582	3511
10	31593	34084
15	33281	36510
30	45417	53283
60	44089	51787
90	39515	45820

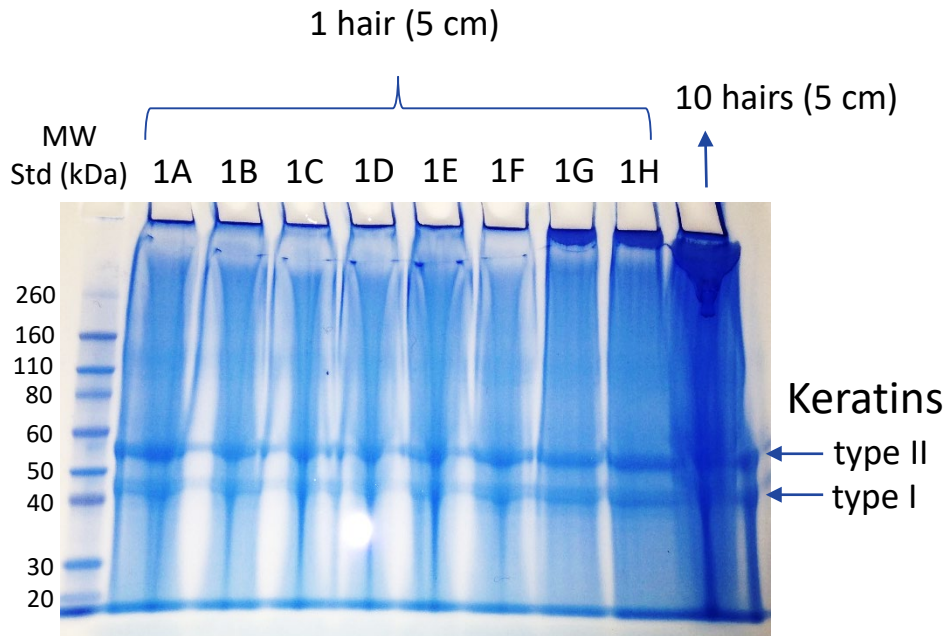


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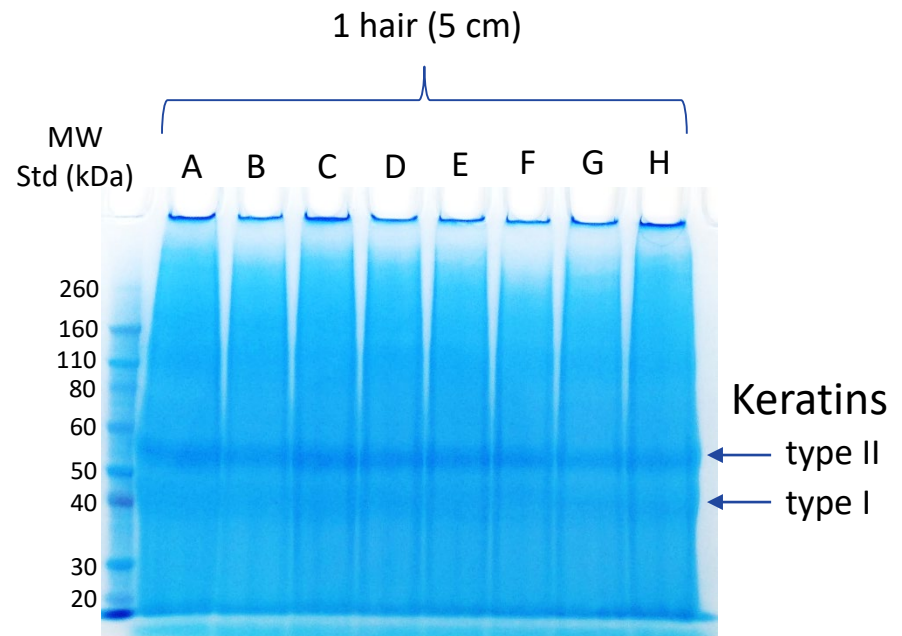
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Reproducibility of Extraction Methods

NaOH & SDS Extraction Method



Single Step Extraction Method

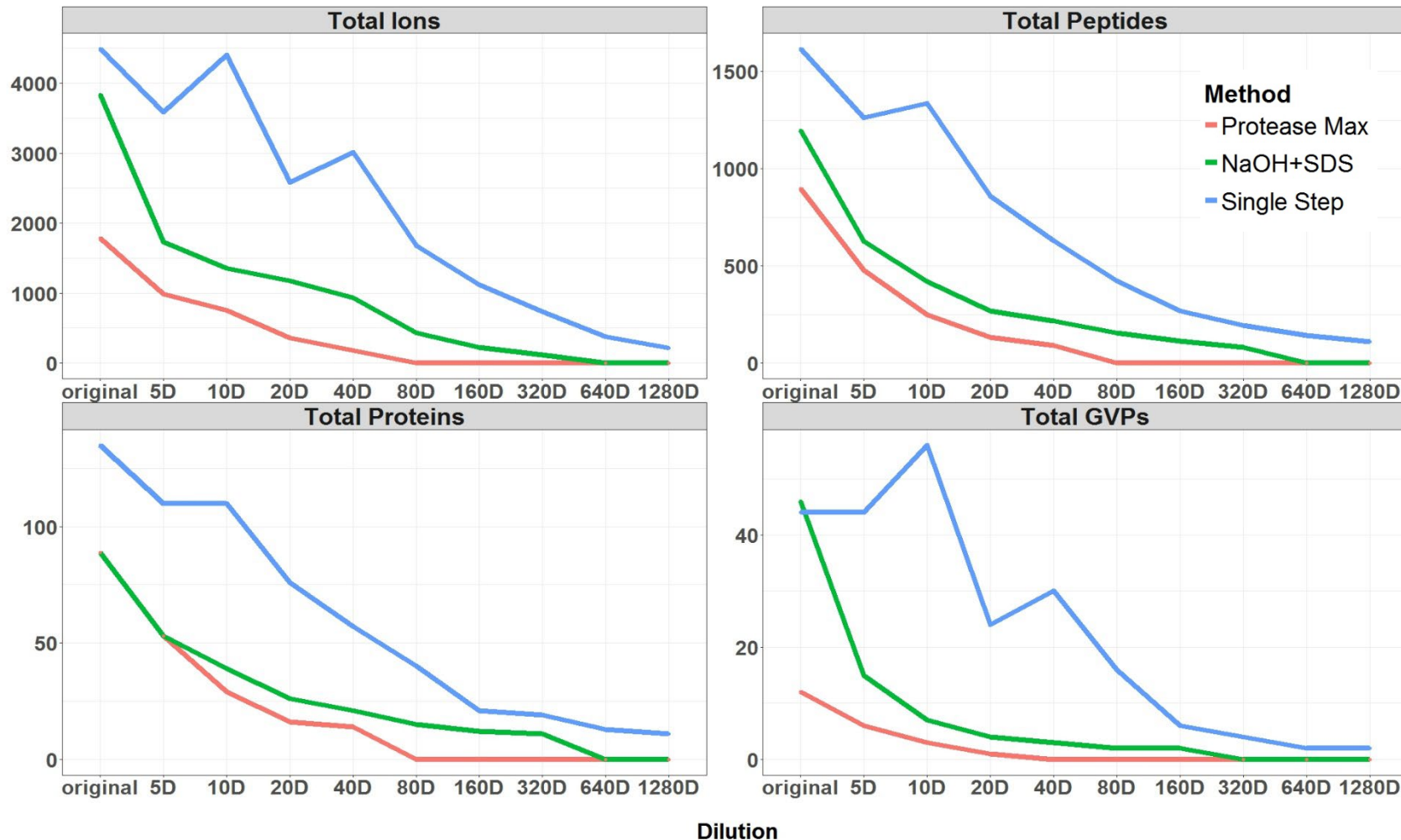


Summary:

Single step extraction method is simpler and more reliable regarding the sample preparation



Sensitivity Across Three Extraction Methods



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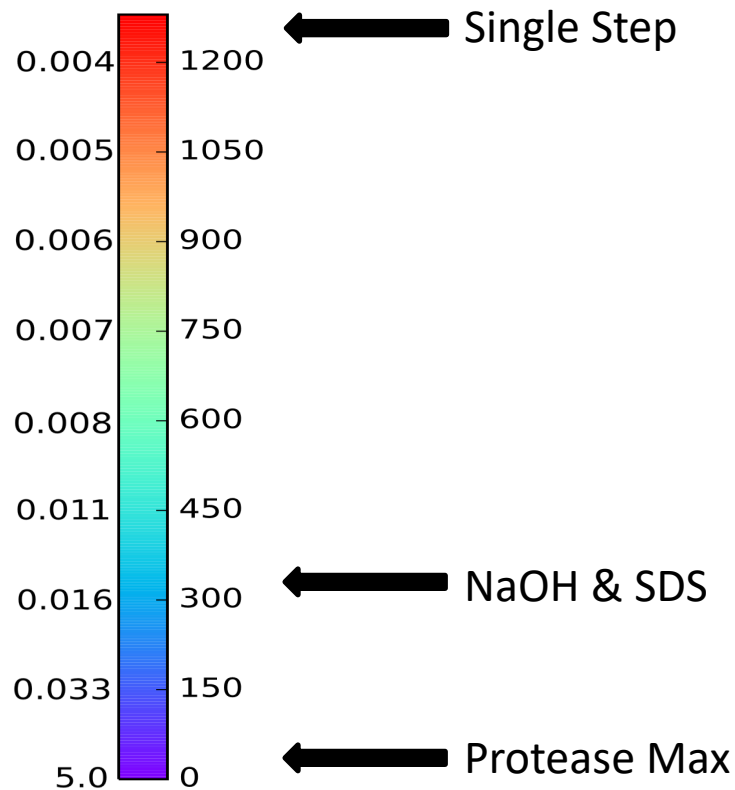
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Sensitivity Across Three Extraction Methods

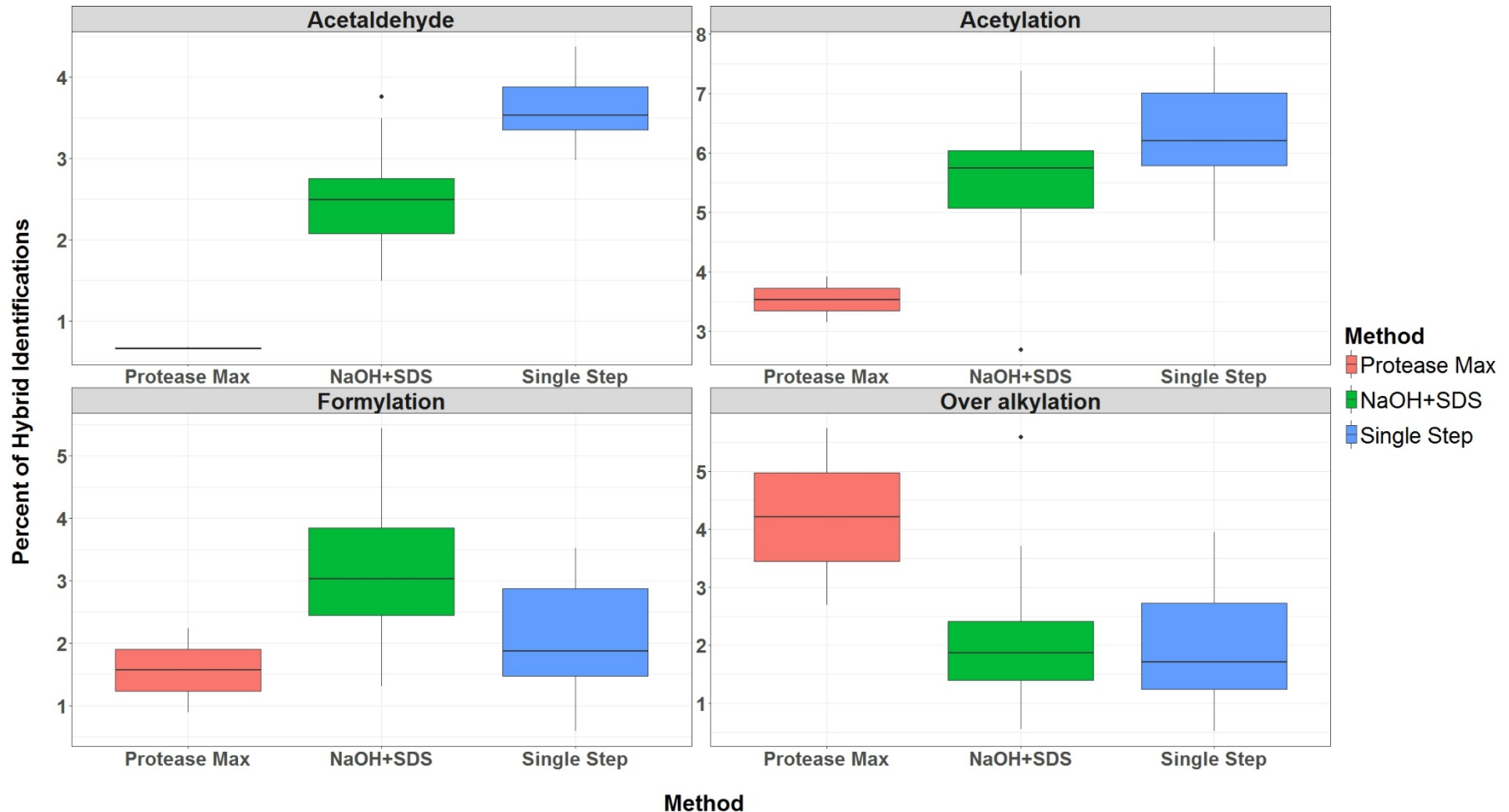
Methods

Convert Serial Dilution to Hair Length Equivalent

Hair Length (cm) Dilution Fold

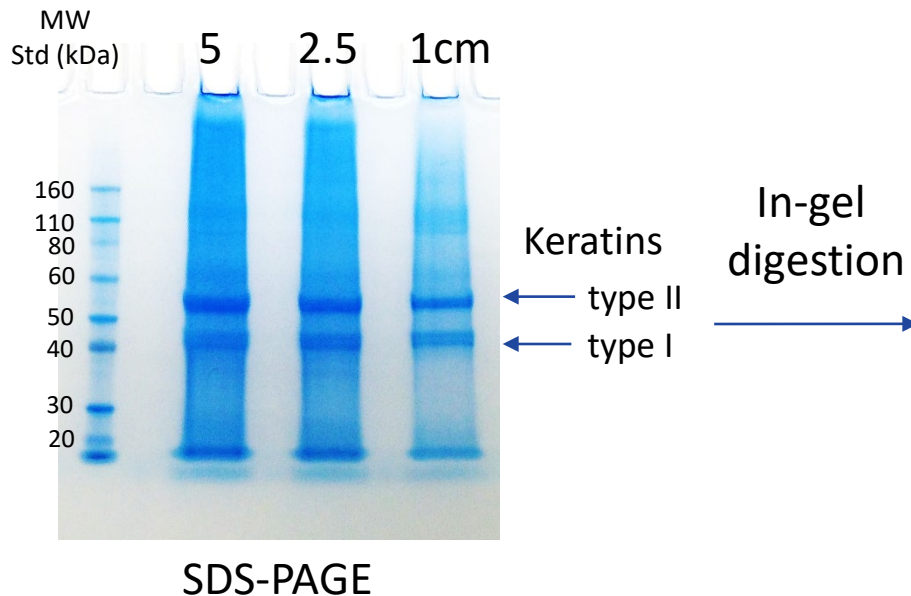


Comparison of Experimentally Introduced Modifications



Single Step Extraction

Reduction of Length of Human Hair



Hair Length	Proteome		Hair Keratins	
	Proteins	Peptides	Proteins	Peptides
5 cm	194	2224	15	740
2.5 cm	119	1578	14	663
1 cm	113	1546	14	561

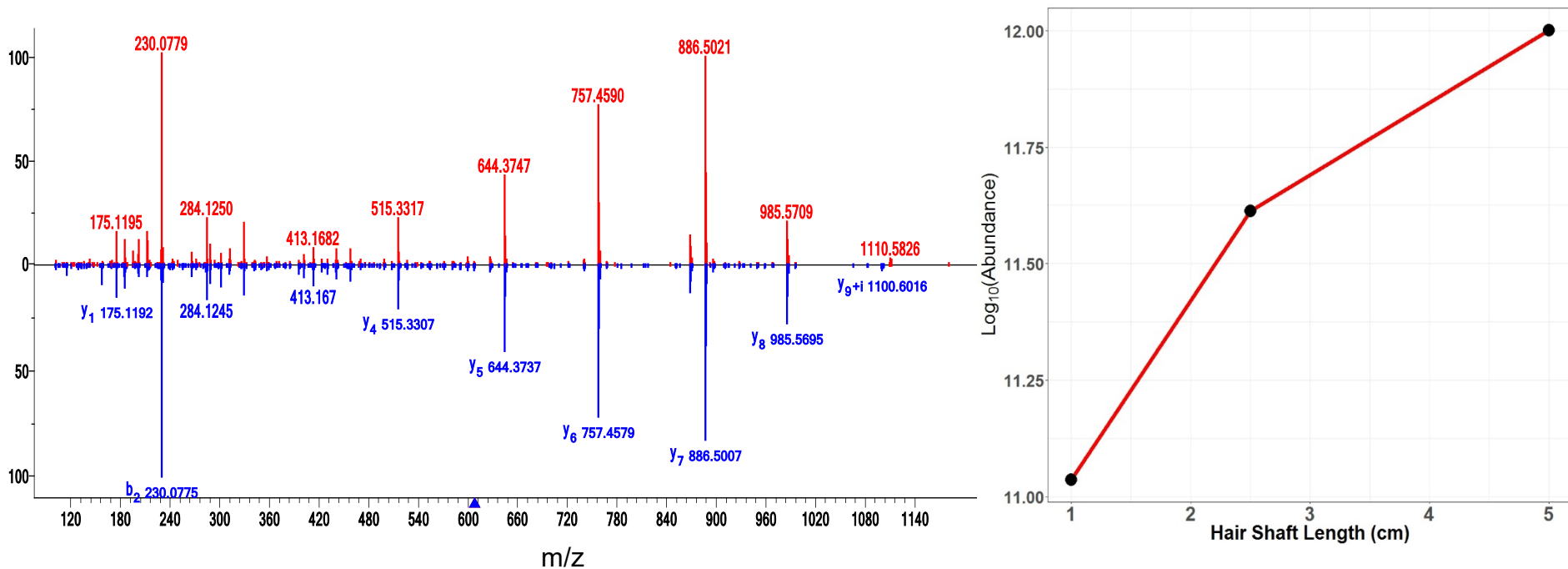
Summary:

- Similar results were obtained from 5-cm and shorter hair samples
 - Starting material can be as little as 1-cm hair shaft



Mass Spectral Library Identification of Keratin-derived GVP

DNAELENLIR, Ala82→Val, Gene=KRT31



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Summary

- The NIST single step protein extraction method provides best depth of coverage of hair proteome
- Identification of artifactual modifications reduces false positive GVP identifications
- Previously published GVP-containing peptides have been curated in a spectral library that will be publicly available

Contact:

meghan.burke@nist.gov



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