# From Volcanoes to the Bench **Advantages of Novel Hyperthermoacidic Archaeal Proteases for Proteomics Workflows**

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#### **One-Step 30-Minute Sample Preparation**



proteins and blocks disulfide reformation. No Chaotropes or IAA needed for rapid sample preparation.



### Novel ID's and New Sequence and PTM's



HTA-Proteases provide improved coverage for specific protein classes when combined with trypsin digest data. (A) Scatter plot of protein coverage with trypsin alone vs. trypsin in combination with HTA-Protease data. Proteins with <20% coverage improvement are in grey, proteins with >20% coverage improvement are colored by classThe dashed line indicates the 20% improvement boundary. (B) Bar plots showing coverage contributions by trypsin (red), CB14057 (blue), and CB23726 (green) for representative proteins from each listed class.

[1] M.C. McCabe, V. Gejji, A. Barnebey, G. Suizdak, L.T. Hoang, T. Pham, K.Y. Larson, A.J. Saviola, S.M. Yannone, K.C. Hansen, From volcanoes to the bench: Advantages of novel hyperthermoacidic archaeal proteases for proteomics workflows, J Proteomics (2023) 104992. [2] S.M. Yannone, J.O. Fuss, A. Barnebey, Methods for rapidly digesting biopolymers with ultrastable enzymes for mass spectrometry-based analyses, in: USPTO (Ed.) Cinder Biological, Inc., US, 2017.

## Deep *De Novo* IgG Sequence Coverage



# Simple Histone Coverage from Lysates



Commercially available K562 whole-cell lysate HTA-protease digestion with no chemistry or manipulation. Data publicly available on PRIDE.

#### **Coverage for 'Difficult' Target Proteins**



# New ID's, Hormones, & PTM's in Blood



Yannone SM, Tuteja V, Goleva O, Leung DYM, Stotland A, Keoseyan AJ, Hendricks NG, Van Eyk JE, Kreimer S. **Blood to Biomarker Quantitation in Under One Hour** with Rapid Proteomics using a Hyperthermoacidic Protease. bioRxiv [Preprint]. 2024 Jun











Proteins				Krakat	oa (167)	
	Trypsin (331)					
					47	
		257	74			
					46	
<b>─</b> ←						
			↓ ↓	>3 p >90°	% of sam	nles
			%Samr	oles	Ave. Inte	ensity
	Gene	#Pre.	Plasma	Serum	Plasma	Serum
	IGLC2	75	100%	100%	1E+06	1E+06
	COL4A1	23	100%	100%	2E+06	2E+06
	AHNAKZ	19	100%	100%	2E+05 7E+05	2E+05
	SOX3	16	100%	100%	1E+05	2E+05
	MUC5AC	13	100%	100%	1E+06	1E+06
	CREBBP	13	100%	100%	3E+05	4E+05
	IGLC3	13	100%	100%	3E+05	4E+05
		11	100%	100%	3E+05	3E+05
	DOCK9	9	100%	100%	4F+05	5E+05
	MYH8	8	100%	100%	1E+06	2E+06
	MUC6	8	100%	100%	5E+05	7E+05
	DEPDC5	7	100%	100%	1E+06	1E+06
	MEGF8	7	100%	100%	3E+05	4E+05
		7	98% 98%	100%	4E+06 6E+05	4E+06
	PRPF8	6	100%	100%	6E+06	6E+06
	KALRN	6	100%	100%	6E+05	1E+06
	FAM83B	6	100%	100%	5E+05	6E+05
	SAP130	6	100%	100%	4E+05	4E+05
	SLC2A13	6	100% 100%	100%	2E+05	3E+05
	DSCAM	6	100%	100%	9E+04	1E+05
	AGFG1	6	100%	100%	8E+04	9E+04
_	IGKV2-28	6	100%	100%	6E+04	5E+04
	ROBO3	6	98%	100%	2E+05	3E+05
	NFAT5	6	96% 06%	100%	3E+05	4E+05
	KLF18	6	90% 94%	96%	2E+04	2E+04
	FUT8	5	100%	100%	3E+06	2E+06
-	SOS2	5	100%	100%	1E+06	9E+05
	NIBAN3	5	100%	100%	4E+05	5E+05
		5	100%	100%	3E+05	3E+05
	PLFKHG4B	5	100%	98%	2E+05	2E+05
	ZIC2	5	100%	100%	2E+05	2E+05
	HDHD5	5	100%	100%	1E+05	1E+05
	ADGRG6	5	98%	100%	6E+05	5E+05
	MYPN	5	96%	100%	3E+06	3E+06
	STK30 SNUPN	4 4	100%	100% 72%	2E+06	1F+06
05 1.E+06 1.E+07	ACAD8	4	100%	92%	3E+05	3E+05
tensity Krakatoa Serum	GRM6	4	94%	94%	1E+05	7E+04
Trypsin Serum	GPRASP2	4	94%	100%	5E+04	6E+04



