Robust, reproducible, and economical phosphopeptide enrichment using calcium titanate

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Supporting Information

Table of contents

Figure S-1

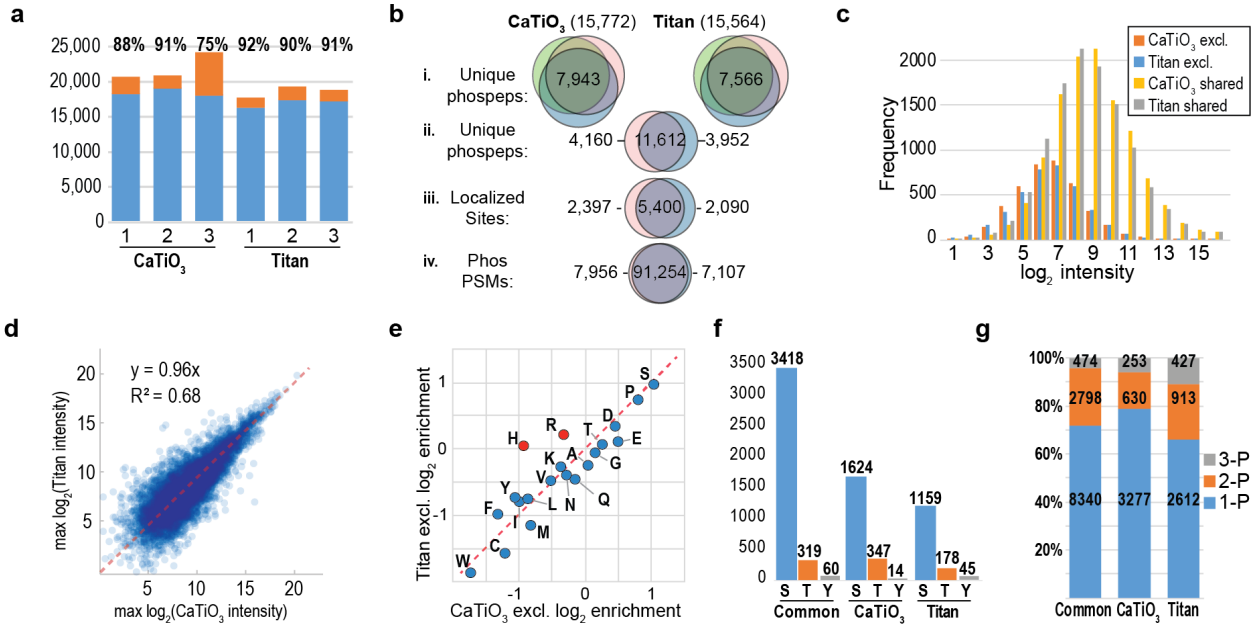

Fig S-1 | Benchmarking $CaTiO_3$ phosphopeptide enrichment against Titansphere TiO_2 enrichment in HeLa Cell extracts. a, $CaTiO_3$ and Titansphere provide comparable numbers of phosphopeptides with similar purity. The total number of spectra matched to phosphopeptides (blue) and non-phosphorylated (orange) peptides is shown. **b**, Overlap analysis of the unique sequences and sites identified by three trials of each method (i), overlap of unique phosphopeptides between the combined datasets for each (ii), overlap of confidently localized sites between methods (iii), and the overlap of all spectral matches between methods (iv). **c**, Commonly identified sequences are of higher intensity. The distribution of maximum log_2 signal intensities of phosphopeptides is plotted separately for shared and exclusive (excl.) phosphopeptides identified by each method. **d**, Peptides identified by both $CaTiO_3$ and Titansphere enrichment have similar intensities. For each unique sequence in common, the maximum signal-to-noise value for each method is plotted. **e**, Amino acid frequencies of $CaTiO_3$ and Titansphere exclusives phosphopeptides are plotted as the log_2 ratio of their frequencies relative to those found in a single 85 min analysis of unenriched Jurkat extract peptides. **f**, The distribution of Ser, Thr, and Tyr phosphorylations among confidently localized singly phosphorylated peptides exclusive to each method. **g**, Titansphere enrichment identifies more multiply phosphorylated peptides. The distributions of singly (1-P), doubly (2-P), and triply (3-P) phosphorylated peptides found with each method independently is shown along with the number of shared sites.

Table S-1. Peptide properties

A. Per Peptide properties (avg occurrences per sequence)

Cells	Sample	#	mc	length	mw	charged	acidic	basic	HIS	ARG	LYS	aromatic	aliphatic	small	tiny	polar	non-polar	K-D Hydro
Jurkat	NON-Phos	14612	0.31	12.5	1399	3.3	1.8	1.6	0.22	0.61	0.75	1.18	3.7	6.3	3.6	6.2	6.3	-0.32
HeLa	Shared	10213	0.78	18.4	1984	5.5	3.3	2.2	0.32	0.92	0.95	0.90	3.4	11.4	6.6	10.8	7.6	-1.01
HeLa	CaTiO3 Excl	3507	0.60	18.6	1981	5.0	3.1	1.9	0.24	0.82	0.84	0.84	3.6	11.7	7.2	10.7	8.0	-0.92
HeLa	Titan Excl	3111	1.14	19.0	2070	5.5	2.6	2.9	0.55	1.36	0.98	1.35	3.7	11.3	6.7	10.9	8.1	-0.93
Jurkat	Shared	9787	0.76	18.9	2029	5.7	3.6	2.2	0.33	0.89	0.94	0.91	3.5	11.6	6.7	11.1	7.7	-1.03
Jurkat	CaTiO3 Excl	2808	0.57	18.7	1998	5.1	3.2	1.9	0.24	0.80	0.82	0.86	3.5	11.7	7.2	10.8	7.9	-0.93
Jurkat	Titan Excl	2661	1.11	19.5	2127	5.8	3.0	2.8	0.54	1.30	1.00	1.30	3.7	11.6	6.8	11.3	8.2	-0.99

B. Feature frequency (above values normalize by length)

Cells	Sample	#	length	avg mass	charged	acidic	basic	HIS	ARG	LYS	aromatic	aliphatic	small	tiny	polar	non-polar
Jurkat	NON-Phos	14612	12.5	112.0	0.26	0.14	0.12	0.017	0.048	0.060	0.093	0.29	0.50	0.28	0.49	0.50
HeLa	Shared	10213	18.4	107.5	0.30	0.18	0.12	0.017	0.050	0.051	0.049	0.19	0.62	0.36	0.59	0.41
HeLa	CaTiO3 Excl	3507	18.6	106.4	0.27	0.16	0.10	0.013	0.044	0.045	0.045	0.19	0.63	0.39	0.57	0.43
HeLa	Titan Excl	3111	19.0	109.0	0.29	0.14	0.15	0.029	0.071	0.051	0.071	0.20	0.59	0.35	0.57	0.43
Jurkat	Shared	9787	18.9	107.5	0.30	0.19	0.11	0.018	0.047	0.050	0.048	0.18	0.62	0.36	0.59	0.41
Jurkat	CaTiO3 Excl	2808	18.7	106.7	0.27	0.17	0.10	0.013	0.043	0.044	0.046	0.19	0.62	0.38	0.58	0.42
Jurkat	Titan Excl	2661	19.5	108.9	0.30	0.15	0.15	0.028	0.066	0.051	0.066	0.19	0.60	0.35	0.58	0.42

Property	Description
MC	missed cleavages K.|R[^P]
Charged	[DEKHR]
Acidic	[DE]
Basic	[HKR]
HIS	H
ARG	R
LYS	K
Aromatic	[HFWY]
Aliphatic	[AILV]
Small	[ACDGNPSTV]
Tiny	[ACGST]
Polar	[DEHKNQRST]
Non-polar	[ACFGILMPVWY]
K-D Hydro	Kyte-Doolittle Hydrophobicity scale