



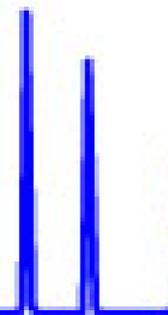
Zirconium Dioxide and Titanium Dioxide for the Enrichment of Phosphorylated Peptides for MS Analysis

PITTCON 2008

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Outline

- Abstract
- Chemistry
- Product Specifications and Capacity
- Applications
- Conclusion



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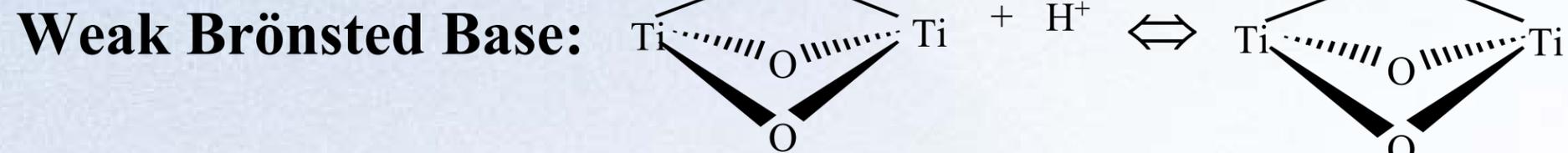
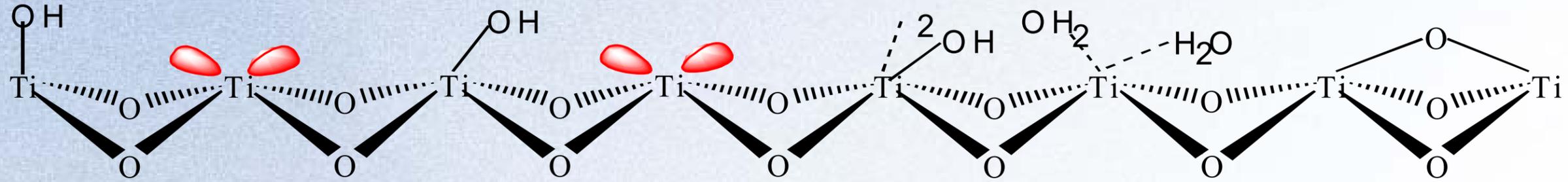
Abstract

Historically, researchers have been unable to fully realize the benefits of mass spectrometry as an analysis method for phosphopeptides because isolation of the molecules from nonphosphorylated peptides is frequently required before examination of the complex samples can proceed. Previously, immobilized metal affinity chromatography (IMAC) was the most widely utilized technique for phosphopeptide enrichment by mass spectroscopy. IMAC methods can vary widely in effectiveness depending on the type of metal ion and loading/elution procedure. The technique also uses valuable research time for the required metal ion loading and washing steps and is difficult to incorporate into an on-line application. As non-specific binding of non phosphorylated peptides further hampers the technique, researchers using mass spectroscopy needed a more specific on-line technique for isolating the phosphopeptides in order to fully realize the time saving benefits of mass spectroscopy. Recent innovative research highlights the unparalleled selectivity and ease of use that are fast making titanium dioxide and zirconium dioxide particles the new standard for enrichment of phosphorylated peptides for MS analysis. This poster profiles the use and benefits of these revolutionary materials for phosphopeptide enrichment.

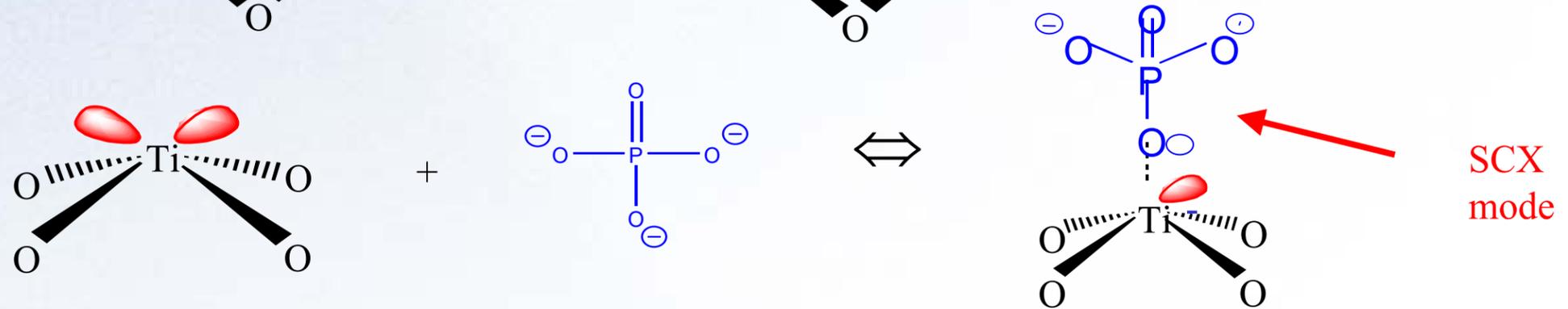


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Surface Chemistry of Titania-Based Supports for HPLC



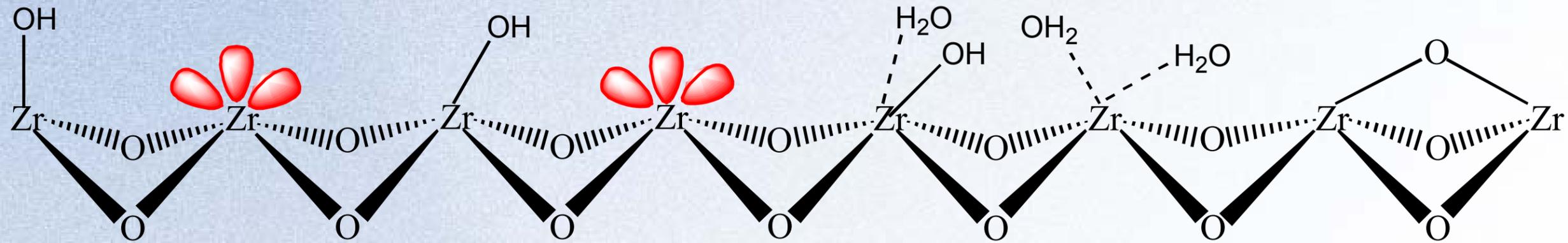
Lewis Acid:





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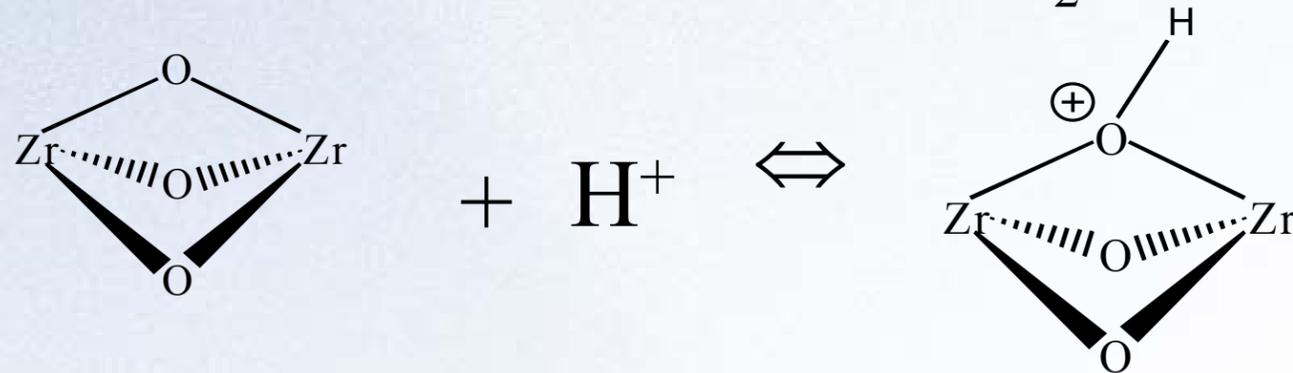
Surface Chemistry of Zirconia-Based Supports for HPLC



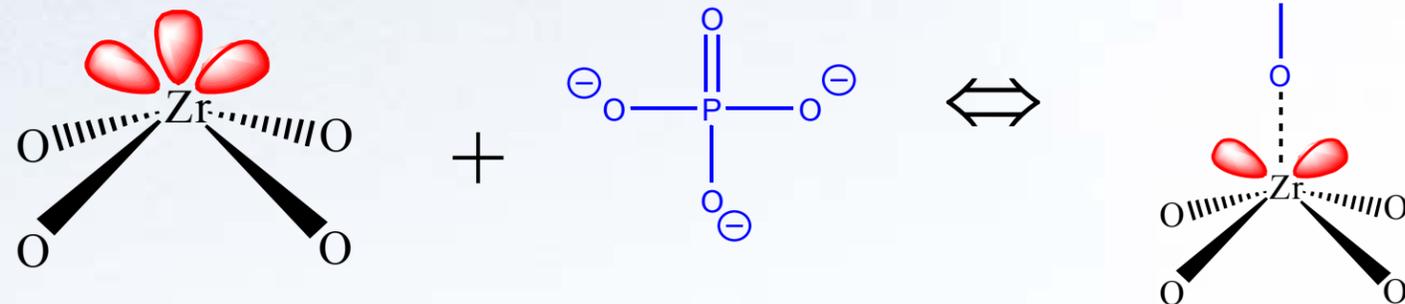
Brönsted Acid:



Brönsted Base:



Lewis Acid:



Unique surface chemistry of Titania and Zirconia allows for multi-modal preferential binding of Lewis Basic molecule such as phosphopeptides



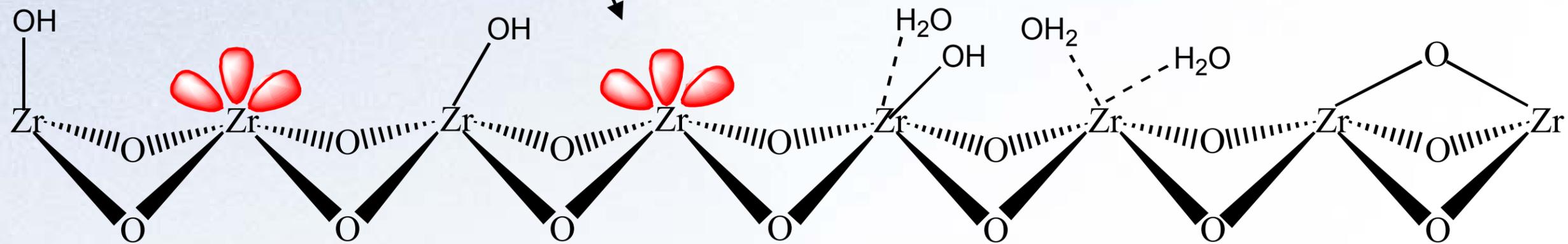
Unique Selectivity

Multi-modal Interactions

Hydrophobic/Hydrophilic Interactions

Lewis Base (Phosphate) on Phosphopeptides interact with Lewis Acid sites

Ion-Exchange





Unparalleled Stability

Robust materials allow for increased method stability

Particle Stability

- pH range 1-14
- Allows for Full Range of Mobile Phase and Solvent Options
- Temperature Range up to 200C

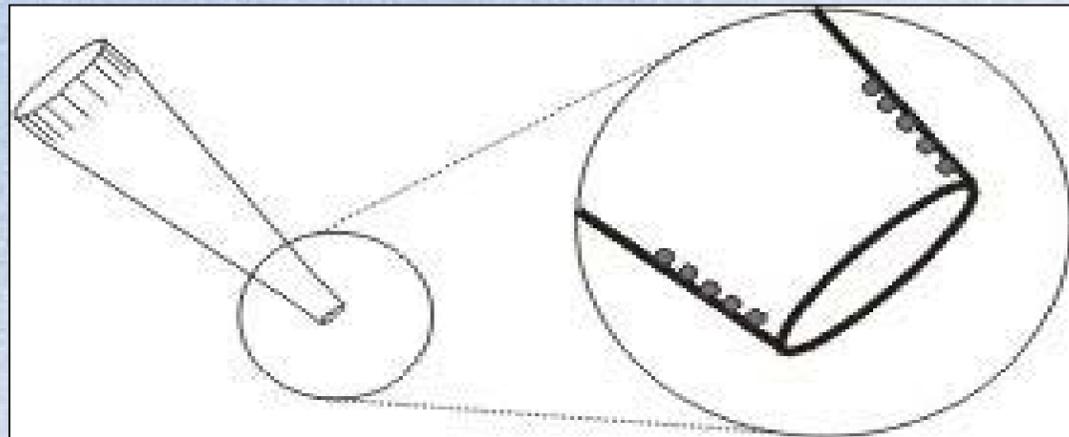
Tip Materials

- Polypropylene
- pH Range 0-14



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NuTip™ Overview



A revolutionary new SPE cartridge in which the chromatography material is embedded in the inner surface of a pipette tip. This maximizes the surface area in contact with the sample. The lack of polymers or glue for embedding the material, avoids potential problems with contamination or permeability.

- Faster sample preparation with minimal sample loss
- No contamination from the supporting matrix
- Sample volumes as small as 0.1 μl
- Available in volumes of: 0.1-10 μl and 10-200 μl



NuTip™ Capacities



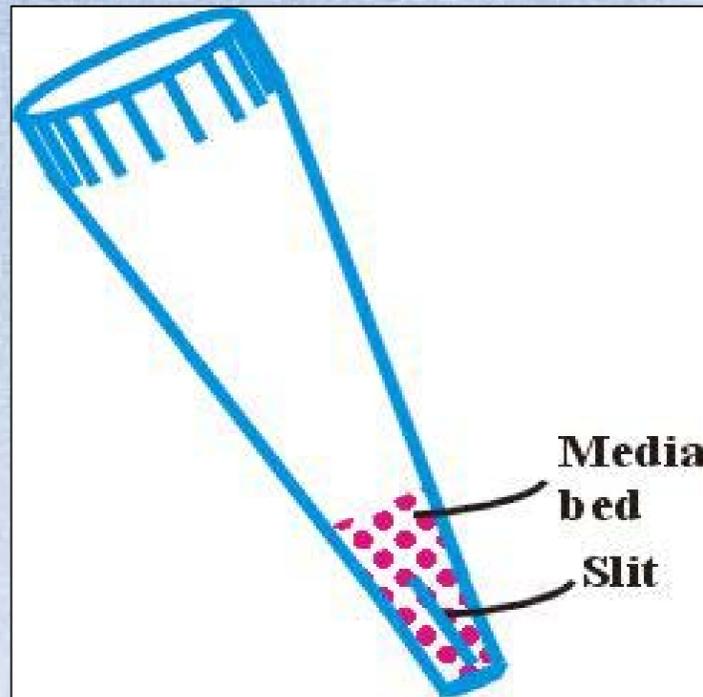
All capacities are estimates based on the standard enrichment protocol with mono-phosphorylated peptides.

Particle	Sample Capacity	Estimated Phosphopeptide Capacity	Sample Capacity	Estimated Phosphopeptide Capacity
<i>Zirconium dioxide</i>	0.1-10 μ l	1 μ g	10-200 μ l	2 μ g
<i>Titanium dioxide</i>	0.1-10 μ l	1 μ g	10-200 μ l	2 μ g



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TopTip™ Overview



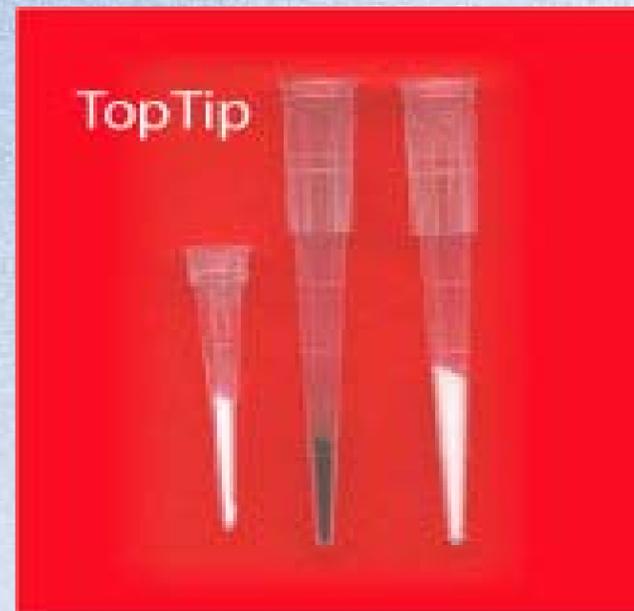
This is a unique concept in solid phase extraction (SPE). Top Tip is a pipette tip with a fine slit at the bottom (slit width: 1-2 μm which permits liquid to pass through but retains the chromatographic material (20-30 μm) in the tip. This also eliminates the need for a filter and, thus, dead volume. Top Tip contains just your desired chromatography material and nothing else and is excellent for working with small samples.

Revolutionary SPE Micropipette Tips:

- Faster sample preparation with minimal sample loss
- No contamination from the supporting matrix
- Sample volumes as small as 1 μl
- Available in volumes of: 1-10 μl , 10-200 μl , 100-1000 μl



TopTip™ Capacities



All capacities are estimates based on the standard enrichment protocol with mono-phosphorylated peptides.

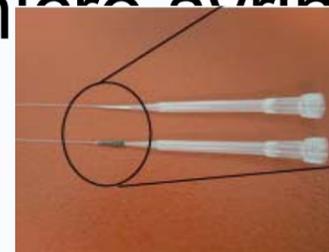
Particle	Sample Capacity	Estimated Phosphopeptide Capacity	Sample Capacity	Estimated Phosphopeptide Capacity	Sample Capacity	Estimated Phosphopeptide Capacity
<i>Zirconium dioxide</i>	1-10µl	100 µg	10-200 µl	200 µg	100-1000 µl	500 µg
<i>Titanium dioxide</i>	1-10µl	100 µg	10-200 µl	200 µg	100-1000 µl	500 µg



Bulk Material & Other Products

Additional phosphopeptide enrichment formats available:

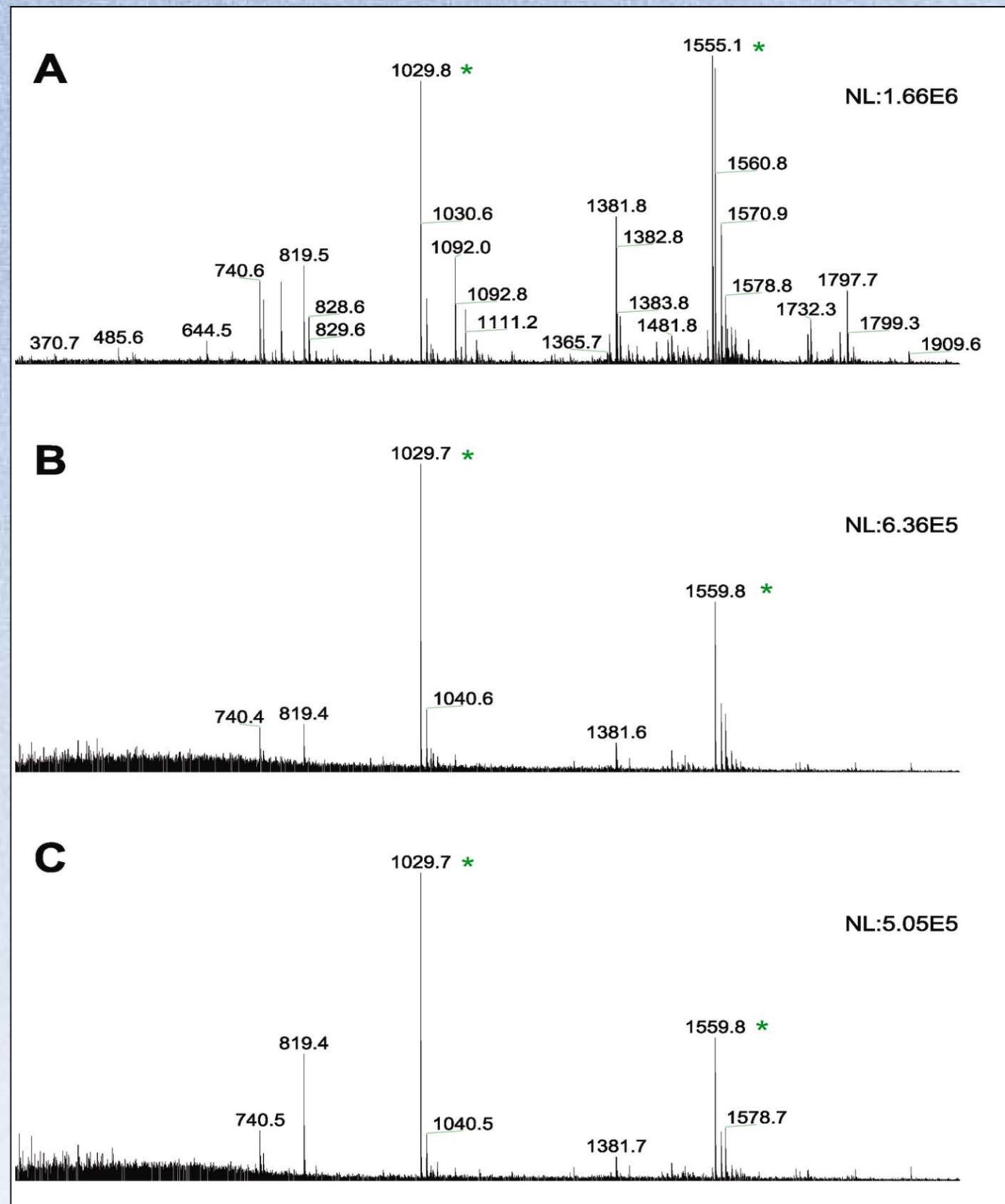
- Bulk Material-Zirconia and Titania in various particle and pore sizes
- Lab-in-a-Plate™ SPE plate
- Lab-in-a-Film™ Microtiter Film
- Lab-in-a-Filter Plate™
- LC-Fiber – sample prep capillary tubing
- SyringeTip - revolutionary new micro syringe tip
- Micro Gel-loader Tip Columns
- MALDI-PEN™
- PEEK Trap columns





General Enrichment Protocol

- Product:** Glygen TiO₂ & ZrO₂ NuTip™ (part # NT1TIO & NT1ZRO)
- Conditioning:** Tips conditioned with 5 aspiration/expulsion (A/E) cycles of HPLC grade water
- Loading:** 10 µL of sample loaded in 10 A/E cycles
- Wash:** 10 µL of HPLC grade water for 10 A/E cycles
- Elution:** 2 µL of 50/50 50mM NH₄HCO₃/50mM TEA
- Post Elution:** Addition of 2 µL of a 50mM TEA in methanol solution followed by immediate mixing and centrifugation.
- Detection:** All samples were analyzed via ESI-MS in negative-ion mode.



An overnight tryptic β -casein digest was performed and the sample was then diluted with a 0.1% formic acid solution to generate a 1 pmol/ μL solution. The enrichment procedure outlined previously was applied.

Figure 1. A) Spectrum of β -casein without Enrichment B) Spectrum of β -casein after purification by TiO_2 tip C) Spectrum of β -casein after purification by ZrO_2 tip



Discussion

Figure one clearly demonstrates both Zirconia and Titania preferentially enrich phosphopeptides. The results obtained on both TiO_2 and ZrO_2 compare favorably with traditional techniques, successfully enriching the phosphopeptides and thus greatly improving the signal-to-noise ratio for phosphopeptide analysis. Interestingly the materials are complementary, each preferentially enriching different types of phosphopeptides, thus allowing for 20-30% increase in coverage.



Conclusions

Zirconia and Titania based phosphopeptide enrichments allows for:

- Ease of Use
- Robust Materials
- Versatile Formats
- Increased Specificity of Phosphopeptide Binding
- Complementary Selectivity between Zirconia and Titania

For more information visit us at PITTCON Booth #2702