WP2 Synthesis of IMAC Sorbent for Phosphopeptide Enrichment

Technical Flowchart

WP2.1. Synthesis and characterization of Immobilized Metal Affinity Chromatography (IMAC) sorbents in microsphere form

Synthesis:

- **Mold Material;** poly(methacrylic acid-co-ethylene dimethacrylate), poly(MAA-co-EDMA) based microspheres.
- **Adsorption of the metal oxide precurso**r**;** tetraethyl orthosilicate (TEOS) dispersion medium.
- **Immobilization of Ti(IV) ions on SiO2 microspheres;** polyethyleneimine (PEI)
- **Immobilization of the metal ion into the microspheres;** 20% HCl solution and TiCl4 solution.

Characterisation:

- **Size** and **surface morphology;** by Scanning Electron Microscopy (SEM).
- **Surface composition**, **distribution of the atoms on the surface** and **valence levels;** by using X-ray Photoelectron Spectroscopy (XPS) and Energy Dispersive Spectroscopy (EDS) mapping.
- **Surface area** and **pore size** properties; by the nitrogen (N2) adsorption-desorption method (according to the Brunauer-Emmet-Teller (BET) model).

WP2.2. Phosphopeptide enrichment in reference standard protein and synovial fluid

Tryptic digestion (with NH4HCO3).

- β-casein (control).
- Synovial Joint Fluid (experimental).
- Enrichment for; SiO2@PEI@Ti(IV) and SiO2@PDA@Ti(IV) microspheres.

WP2.3. Investigation of phosphopeptide isolation performance of microspheres using MALDI-TOF mass spectrometry

- MALDI-TOF mass spectrometry (Ultraflextreme, Bruker Daltonics).
  -in positive ion and high resolution (reflectron) mode using DHB or HCCA matrix.

WP2.4. Investigation of phospholipid isolation performance of microspheres with Q-TOF LC/MS

- The phospholipid isolation performance of microspheres will be demonstrated via LC-MS/QTOF.