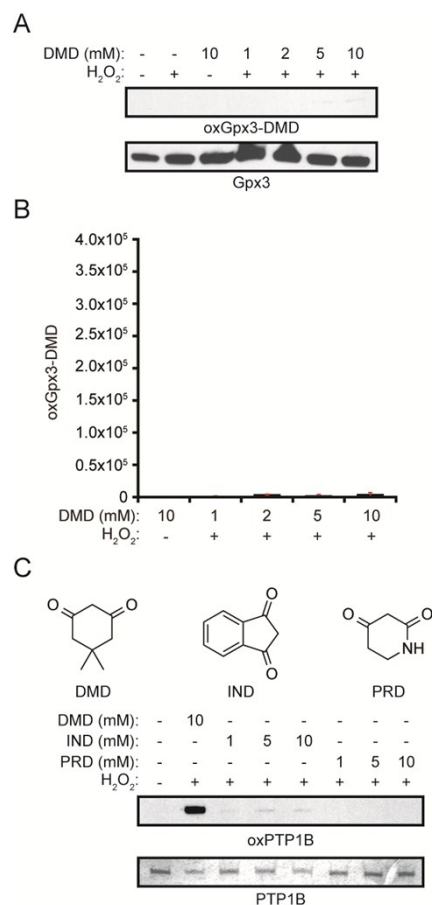


**An Immunochemical Approach to Detect Oxidized Protein Tyrosine Phosphatases
Using a Selective C-Nucleophile Tag**

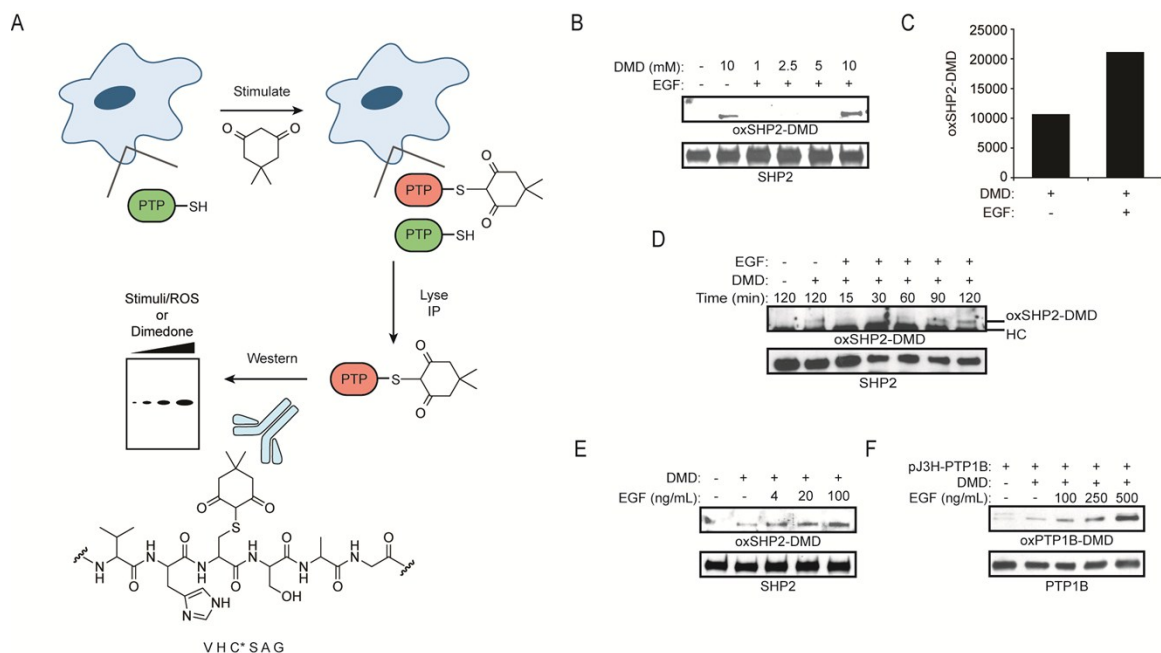
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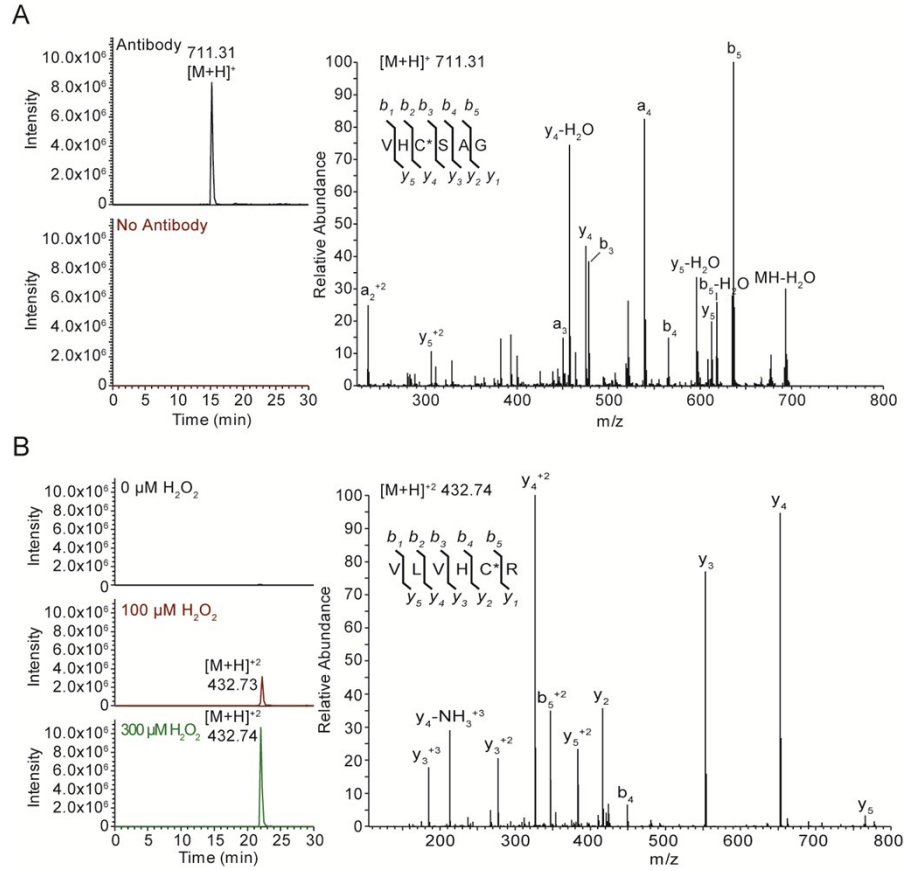
Email: kcarroll@scripps.edu



Supplemental Figure 1: Assessing the selectivity of the PTP-S-DMD antibody. (A) Recombinant Gpx3 (10 μ M) was treated with 1.5 equivalences (15 μ M) H₂O₂ in the presence of increasing concentrations of DMD. Subsequent Western blot analysis showed no detection of DMD-modified oxGpx3 suggesting that the antibody is selective for the conserved PTP epitope. (B) Densitometric analysis shows only background and no signal pertaining to DMD-modified oxGpx3. (C) PTP1B was treated with nucleophiles that are structurally similar to DMD but possess enhanced reactivity towards sulfenic acids. Western blot analysis showed no detection of oxPTP1B modified with these nucleophiles, suggesting that the antibody is selective for the DMD epitope.



Supplemental Figure 2: Facile detection of oxPTPs in cells. (A) A simplified approach to monitor oxidized phosphatases during growth factor dependent ROS production. (B) Detection of DMD-modified oxSHP2 in A431 cells. Titration of DMD into A431 cells after stimulation with 100 ng/mL EGF demonstrated robust labeling at 10 mM. (C) Densitometric analysis demonstrated a two-fold increase in oxSHP2 after stimulation with 100 ng/mL EGF. (D) Time-dependent analysis revealed enhanced oxSHP2 detection. (E) Growth factor mediated increases of oxPTPs. A dose-dependent increase of endogenous oxSHP2 was observed in A431 cells were stimulated with varying concentrations of EGF. (F) Dose-dependent increases in oxPTP1B was observed in HA-PTP1B transfected COS1 cells stimulated with EGF.



Supplemental Figure 3: Immunoaffinity enrichment of DMD-modified PTP peptides. (A) The hapten was enriched and verified by MS/MS only in the presence of the PTP-S-DMD antibody. (B) Immunoaffinity enrichment and subsequent MS/MS of DMD-modified PTP peptides after stimulation with varying concentrations of H_2O_2 displayed a dose-dependent increase of oxVHR and confirmed the peptide sequence.