Immuno-proteomics: A Novel Workflow for Autoantigen Identification

Impact PROTEOMICS

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Abstract

Autoimmune diseases affect >20 million people in the US today. Currently, disease-specific autoantibodies are thought to be the best biomarkers for diagnosis. Conventional immunoprecipitation methods have been used to identify autoantigens from the most common autoimmune diseases. However, these diseases account for only 6.5 million of the 20 million patients suffering from autoimmune diseases, leaving many without diagnoses until irreversible damage occurs. The remaining 13.5 million patients have >80 autoimmune disorders without well characterized autoantibodies. The state-of-the-art diagnostic test of these remaining diseases relies on gel electrophoresis of immunoprecipitated radiolabeled proteins, which cannot be identified by MS due to safety issues and the overwhelming presence of immunoglobulins.

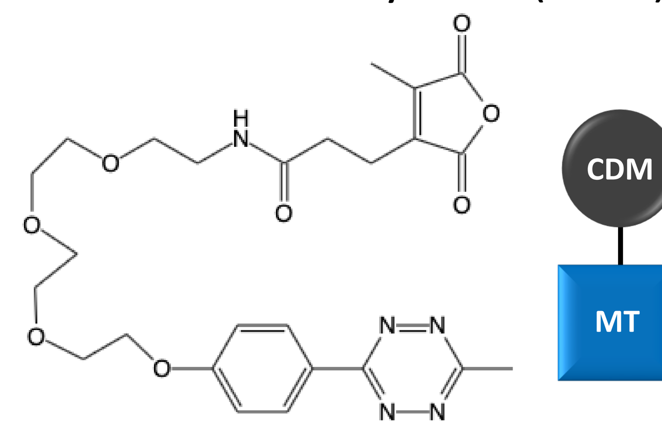
We have created an immunoprecipitation method that uses serum from patients with any autoimmune disorder to identify patient-specific autoantigen proteins. This method uses a reversible click chemistry tag, called ProMTag. One end of the ProMTag forms a reversible, covalent bond with protein by coupling to lysines and amino termini. The other end of the ProMTag can form an irreversible, covalent bond with a solid bead support via a click chemistry, methyltetrazine-TCO, pairing. In this study, the proteins of cell lysates that contain potential autoantigens were labeled with ProMTag. The ProMTagged-proteins were exposed to patient antibodies bound to Protein A beads, thus capturing the ProMTagged autoantigens. All proteins were released from the Protein A beads, including ProMTagged- autoantigens and untagged-antibodies. The ProMTagged-autoantigens were subsequently coupled to TCO beads, and the untagged-antibodies were washed away. The linkage between the ProMTag and autoantigens was then reversed, yielding autoantigen proteins with greatly reduced antibody contamination ready for MS analysis. MS analysis successfully identified autoantigens from patient serums with scleroderma or myositis/anti-synthetase syndrome. This autoimmune biomarker discovery method can accelerate sample testing for known autoantigens and facilitate rapid discovery of novel autoantigens for both diagnostic and predictive biomarkers.

ProMTag workflow key components

ProMTag Protein tagging

Reversible Protein Binding Reagent

Carboxyethyl-dialkyl-maleic Anhydride (CDM)



CDM

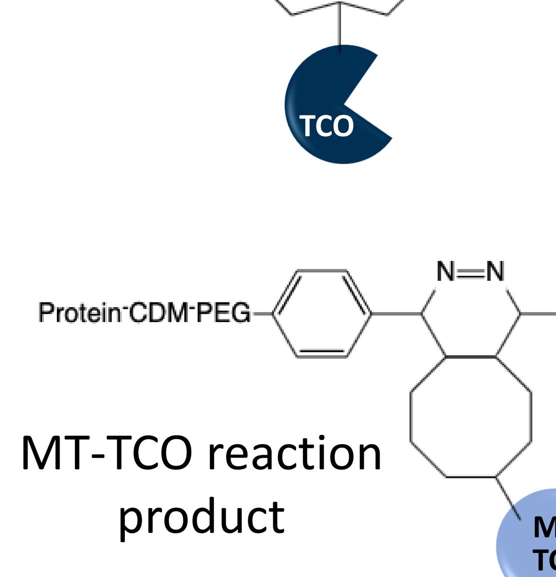
MT

Irreversible Bead Binding Reagent

Methyltetrazine (MT)

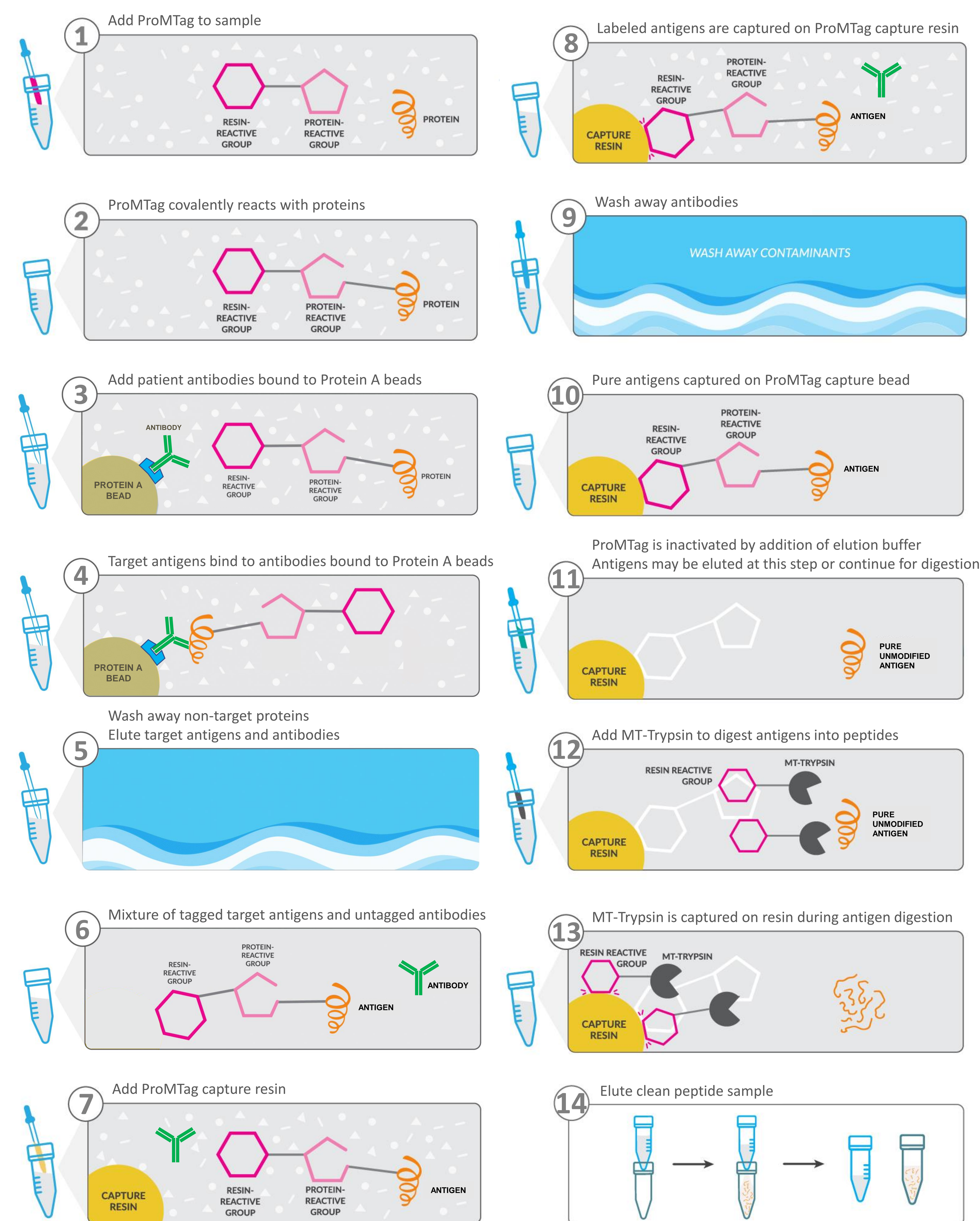
ProMTag Capture TCO-bead

Trans-cyclooctene (TCO)

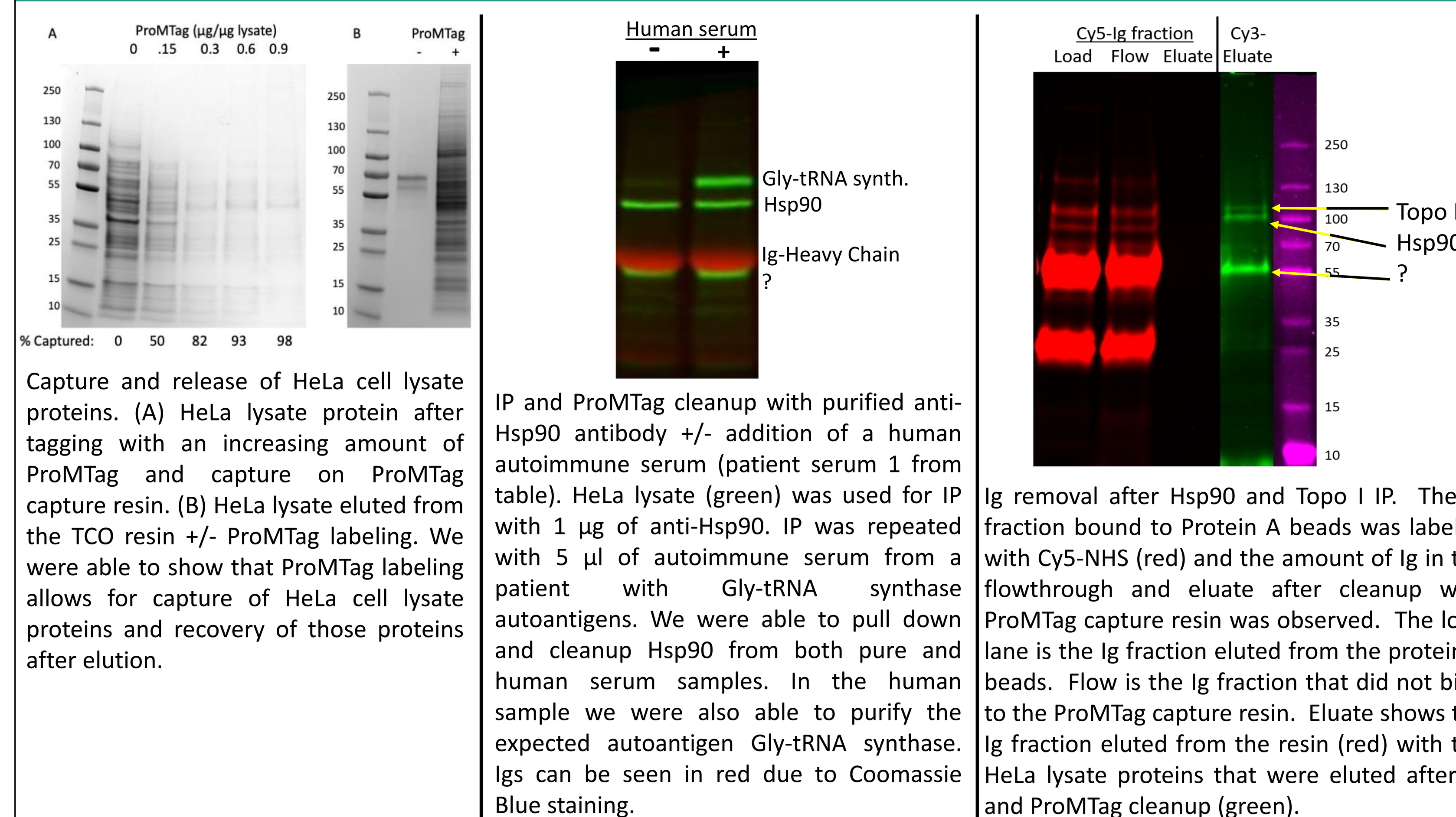


MT-TCO reaction product

ProMTag immunoprecipitation method for purification of autoantigens



Gel-based validation of the ProMTag immunoprecipitation workflow



Capture and release of HeLa cell lysate proteins. (A) HeLa lysate protein after tagging with an increasing amount of ProMTag and capture on ProMTag capture resin. (B) HeLa lysate eluted from the TCO resin +/- ProMTag labeling. We were able to show that ProMTag labeling allows for capture of HeLa cell lysate proteins and recovery of those proteins after elution.

IP and ProMTag cleanup with purified anti-Hsp90 antibody +/- addition of a human autoimmune serum (patient serum 1 from table). HeLa lysate (green) was used for IP with 1 µg of anti-Hsp90. IP was repeated with 5 µl of autoimmune serum from a patient with Gly-tRNA synthase autoantigens. We were able to pull down and cleanup Hsp90 from both pure and human serum samples. In the human sample we were also able to purify the expected autoantigen Gly-tRNA synthase. Igs can be seen in red due to Coomassie Blue staining.

Ig removal after Hsp90 and Topo I IP. The Ig fraction bound to Protein A beads was labeled with Cy5-NHS (red) and the amount of Ig in the flowthrough and eluate after cleanup with ProMTag capture resin was observed. The load lane is the Ig fraction eluted from the protein A beads. Flow is the Ig fraction that did not bind to the ProMTag capture resin. Eluate shows the Ig fraction eluted from the resin (red) with the HeLa lysate proteins that were eluted after IP and ProMTag cleanup (green).

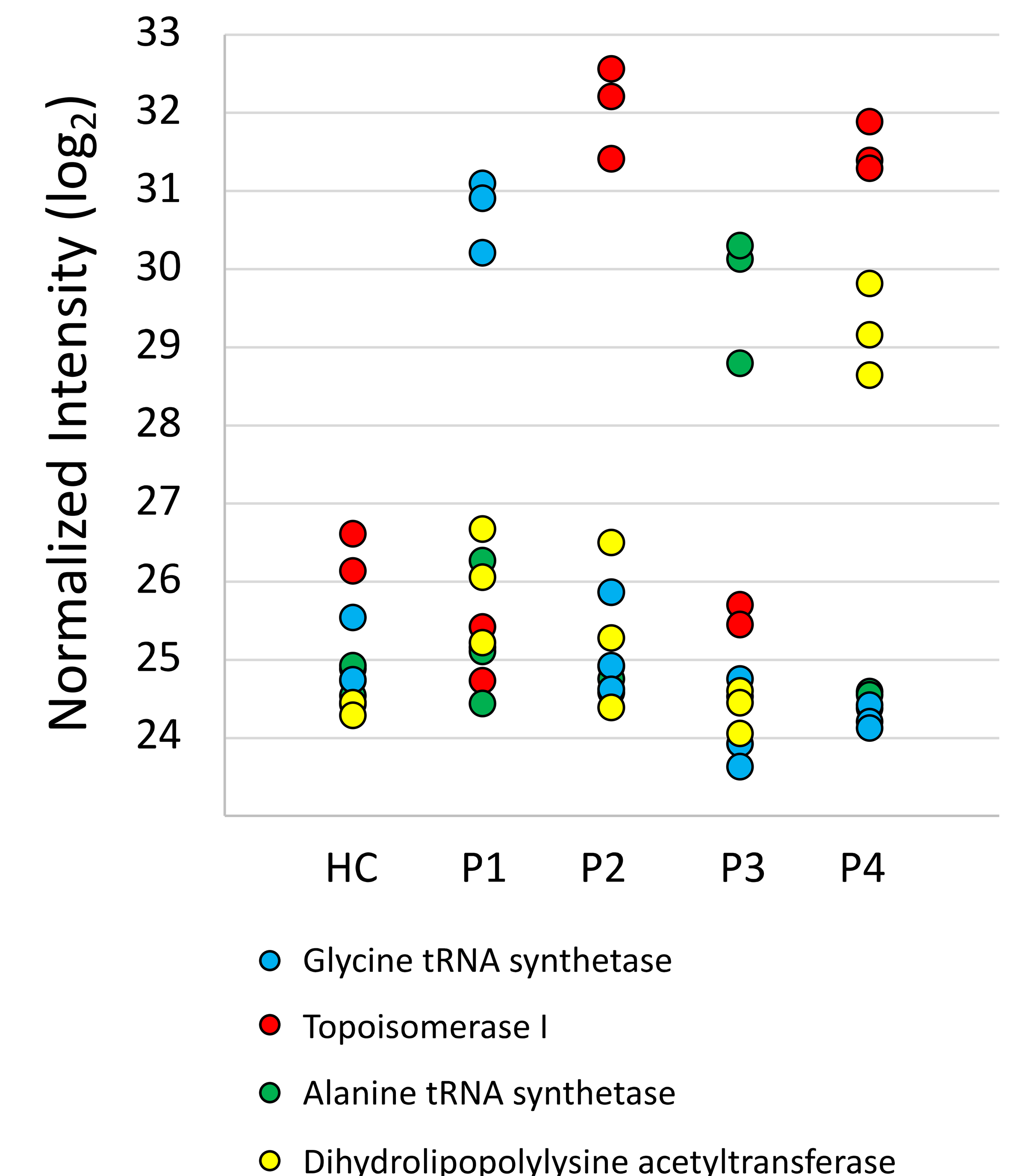
Control immunoprecipitation of K562 lysate with normal serum

Normal Patient Serum Control

Description	Log Prob.	Total Intensity	# of spectra	# of unique peptides	Coverage %
Albumin	447.63	1.72E+09	197	69	78.82
Complement C3	443.94	9.78E+08	211	85	54.66
Vimentin	338.95	1.05E+09	135	50	74.03
Apolipoprotein B-100	224.01	1.62E+08	53	46	14.42
CD5 antigen-like	164.28	4.40E+08	72	22	72.05
Alpha-2-macroglobulin	84.98	8.10E+07	24	17	25.44
Polyadenylate-binding protein 1	81.26	1.39E+08	27	15	30.66
Tubulin beta chain	69.21	4.70E+07	13	8	32.43
Actin, cytoplasmic 2	56.92	1.37E+08	22	14	52.00
Complement C4-B	42.44	1.41E+07	5	4	3.10

Mass spectrometry results of negative control identify background proteins that are in the sample after the ProMTag immunoprecipitation workflow.

Immunoprecipitation of K562 lysate with four characterized patient sera



Autoantibodies from patient sera with known autoantigens were immobilized on Protein A beads and used for immunoprecipitation of a ProMTag labeled protein lysate from K562 cells. P1 and P2 had myositis/anti-synthetase syndrome; P2 and P4 had scleroderma (anti-Topoisomerase I). Immunoprecipitated antigens were then sent for mass spectrometry identification. In all four tests, the correct autoantigen was identified in the mass spectrometry result. Notice that P4 had an additional, novel autoantigen.

Conclusions

The ProMTag immunoprecipitation method allowed for mass spectrometry analysis of autoantigens immediately after immunoprecipitation, something that previously could not be done because of antibody contamination. Additionally, we correctly identified all known antigens in clinically relevant patient samples.

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