Antibodies and Recombinant Proteins

Human DPP4 rat monoclonal IgG2A antibody (Catalog #MAB1180) was used for capture, biotinylated human DPP-4 polyclonal goat IgG antibody (Catalog # BAF1180) was used for detection; and mouse myeloma cell line, NS0-derived human recombinant DPP-4 (Catalog #1180-SE-010) was used as standard. Human MME goat polyclonal IgG antibody (Catalog #MAB1182) was used for capture; biotinylated human MME goat polyclonal IgG antibody was used for detection (Catalog #BAF1182), and Chinese Hamster ovary cell line, CHO-derived human recombinant MME (Catalog#1182-ZNC-010) was used as standard. Human TIMP1 mouse monoclonal IgG2B antibody (Catalog #MAB970, clone #63515) was used for capture; biotinylated human TIMP1 goat polyclonal IgG antibody (Catalog # BAF970) was used for detection; and mouse myeloma cell line, NS0-derived human recombinant TIMP1 (Catalog #970-TM-010) was used as standard. Human tPA mouse monoclonal antibody (Catalog # ab82249) used for capture, biotinylated human tPA rabbit polyclonal IgG antibody (Catalog #ab28208) used for detection, and Chinese Hamster ovary cell line, CHO-derived human recombinant tPA protein (Catalog #ab92637) used as standard were purchased from Abcam (Cambridge, MA).

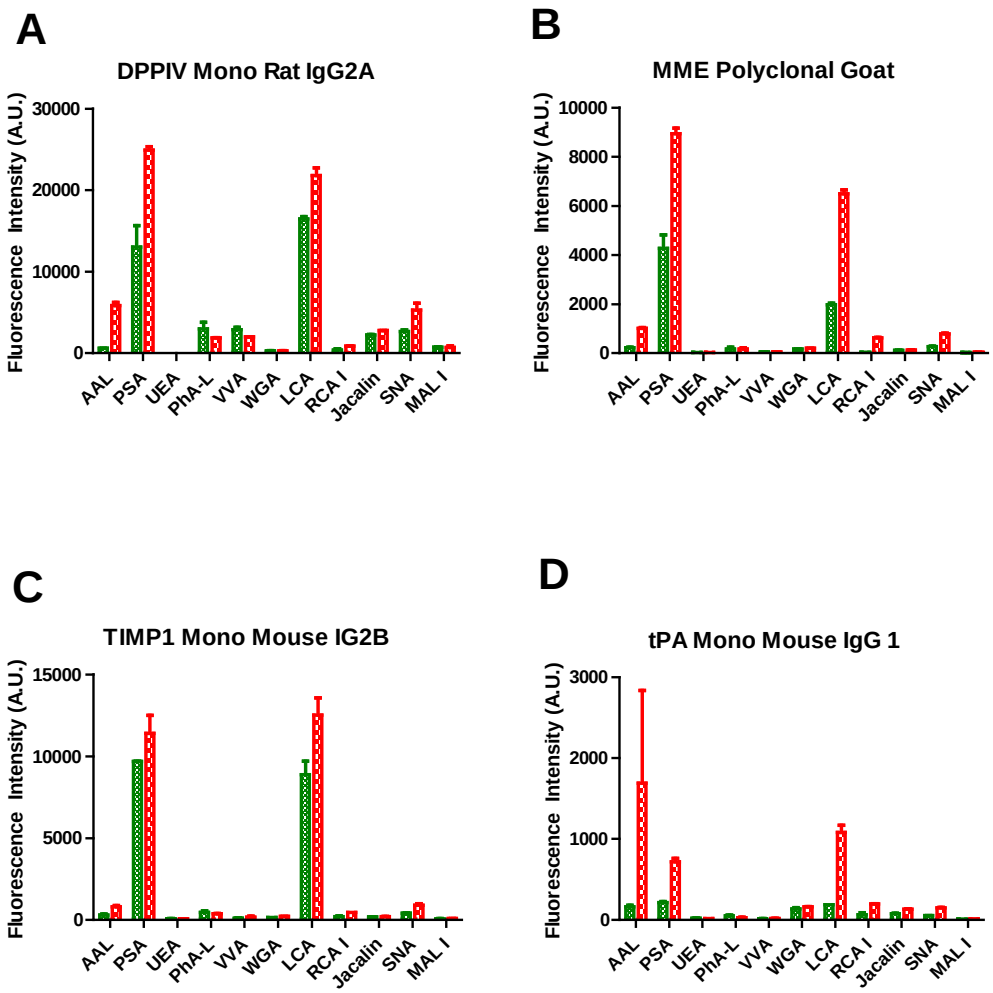
TIMP-1 Immunoassay

After coupling, beads were counted and validated to ensure binding of capture antibody to the beads using biotinylated goat anti-mouse IgG antibodies (Sigma-Aldrich, St. Louis, MO) before they were stored in the storage buffer at 4°C. BioRad Cytokine Assay Kit was used for development of TIMP-1 immunoassay and TIMP-1 LISAs. For TIMP-1 immunoassay, 2500 coupled beads were incubated with 50 µL of a sample diluted in the Sample Diluent (provided in the Cytokine Assay Kit). After 1-hour incubation at room temperature, the beads were washed and incubated with 25 µL of 2 µg/mL biotinylated TIMP-1 detection antibody diluted in the

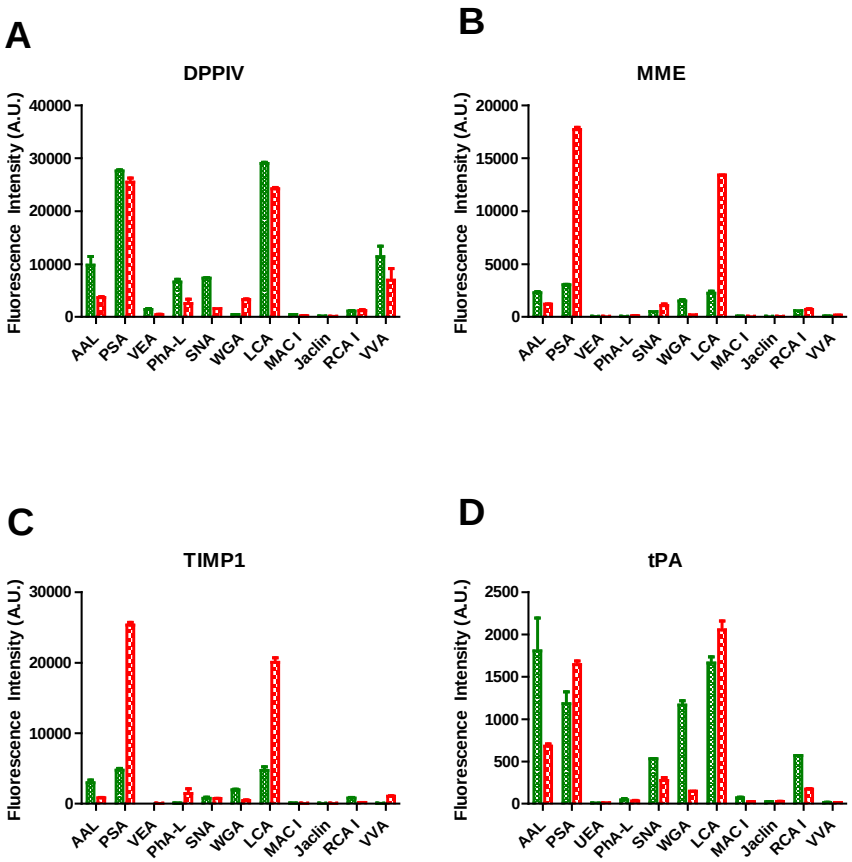
Detection Antibody Diluent (provided in the Cyotkine Assay Kit) at room temperature for 30 minutes. Then the beads were washed again and incubated with 50 µL of 2 µg/mL streptavidin-phycoerytherin diluted in the Assay Buffer at room temperature for 10 minutes before analysis using the Bioplex 200 System. For TIMP-1 LISAs, 2µg/mL biotinylated TIMP-1 detection antibody used in the immunoassay was replaced with 20 µg/mL of biotinylated UEA, PHA-L, AAL, or VVA.

Analyses of the Prostate Tissue Specimens by the Multiplex Integrated System

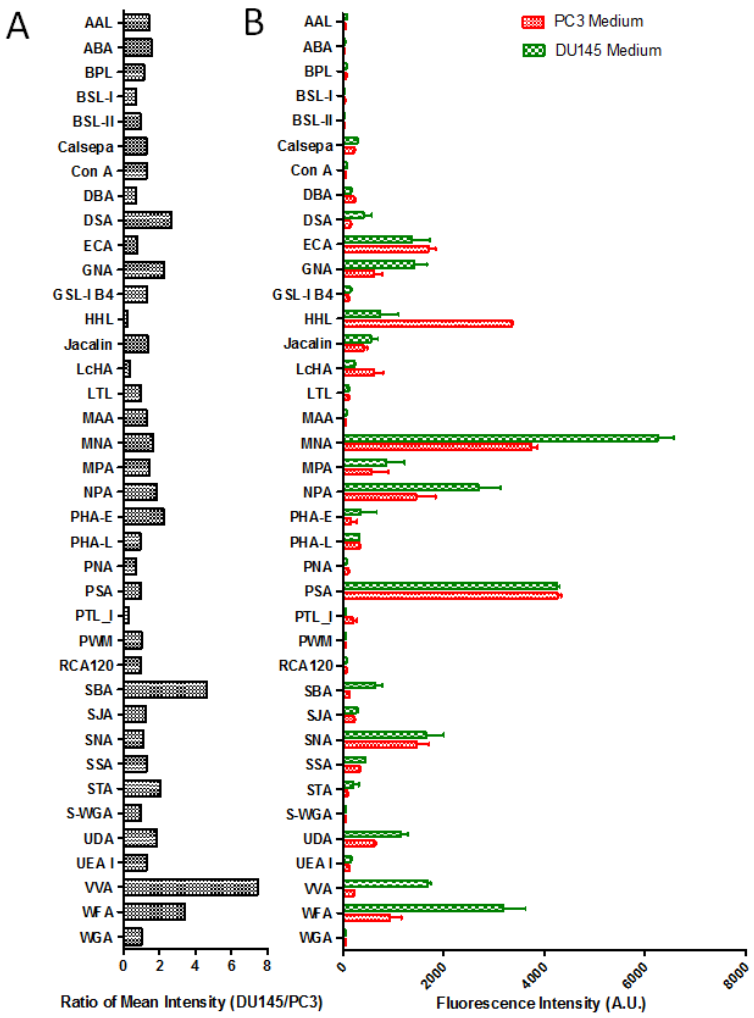
Fifteen micro liters of the prostate tissue specimens were further diluted 25 times using PBS + 1% BSA buffer to 375 µL prior to analyses. For detection of proteins, 50 µL of diluted specimens were incubated with the mixture of the coupled beads for 1 hour at room temperature. After incubation, the beads were washed and incubated with the mixture of detection antibodies diluted in the Detection Antibody Diluent at room temperature for 30 minutes. Then the beads were washed again and incubated with 50 µL of 2 µg/mL streptavidin-phycoerytherin diluted in the Assay Buffer at room temperature for 10 minutes before analysis using the Bioplex 200 System. For detection of glycans, the mixture of detection antibodies used in the multiplex immunoassay was replaced with 20 µg/mL of biotinylated UEA or PHA-L. Duplicate measurement was performed for each specimen.



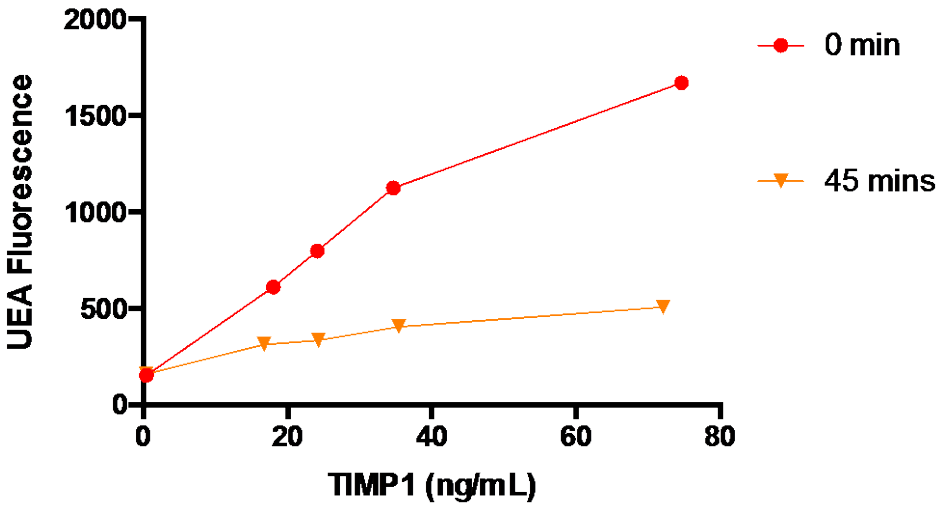
Supplemental Data Figure 1. Comparisons of background fluorescence signals detected by lectins before (red bars) and after (green bars) PNGase F treatment of the magnetic beads coupled with capture antibody of DPP-4 (A), MME(B), TIMP-1 (C), and tPA (D). For the PNGase F, the magnetic beads were incubated in the 1XG7 reaction buffer with PNGase F (New England BioLabs Inc, Ipswich, MA) at 37ºC for 1 hour.



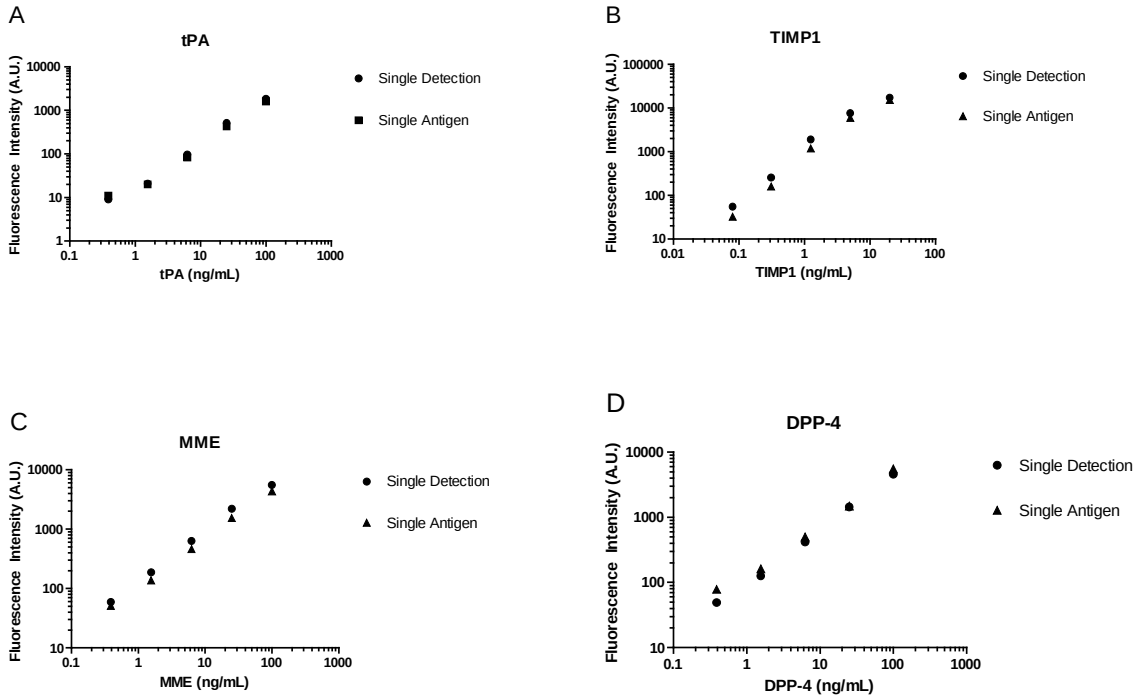
Supplemental Data Figure 2. Comparisons of background fluorescence signals detected by lectins before (red bars) and after (green bars) oxidation and dipeptide coupling treatment of the magnetic beads coupled with capture antibody of DPP-4 (A), MME(B), TIMP-1 (C), and tPA (D). For the oxidation and dipeptide coupling treatment, the beads were incubated with 10 µM sodium periodate (BioRad, Hercules, CA) in the oxidation buffer (150 mM sodium acetate, pH 5.5) in the dark at 4ºC for 30 minutes. Then the beads were washed and incubated with 1 mM MPBH (4-(4-N-Maleimidophenyl) butyric acid hydrazide-HCl) (Thermo Fisher Scientific, Rockford, IL) in the oxidation buffer at room temperature for 2 hours before incubation with 1 mM Cys-Gly dipeptide (Sigma-Aldrich, St Louis, MO) in the PBS + 0.1% Tween buffer overnight.



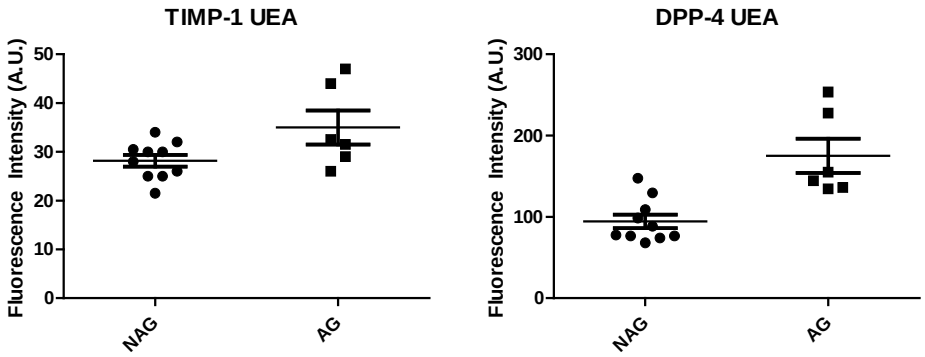
Supplemental Data Figure 3. Lectin profiles of TIMP-1 from PC3 and DU145 cell culture medium assessed by lectin microarray: (A) the ratios of mean fluorescence intensity for all the lectins (DU145/PC3) and (B) the mean fluorescence intensity for all the lectins.



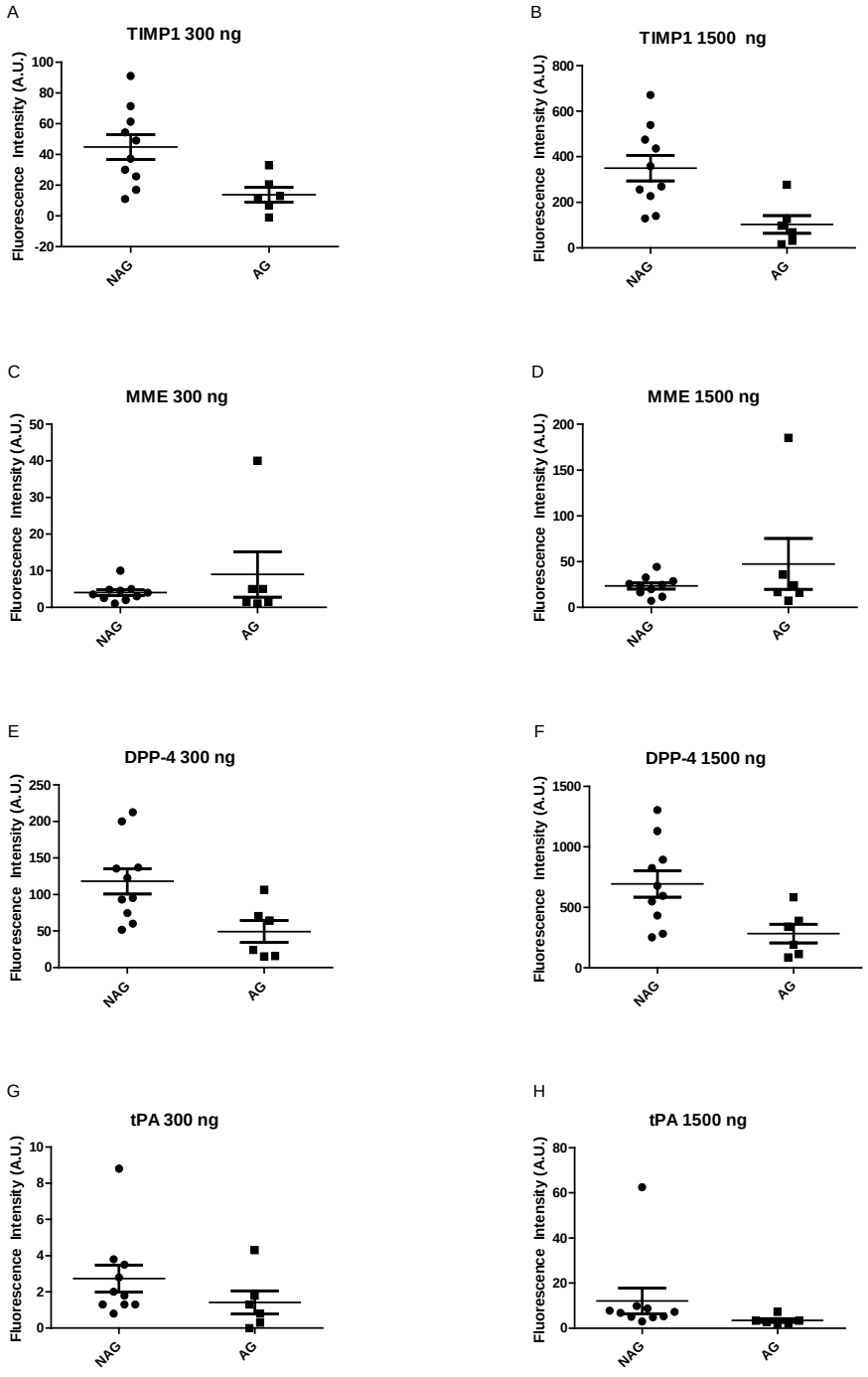
Supplemental Data Figure 4. Forty-five minute treatment decreased the fluorescence signals of TIMP1 measured by the UEA LISA compared to the controls (0 minute treatment) at various concentrations. Four microliter of the recombinant TIMP1 (10 µg/mL prepared in PBS + 1% BSA buffer) was mixed with 4 µL of α1-2 fucosidase (Catalog# P0724, New England Biolabs, Ipswich, MA). The mixture (8 µL) was then diluted 5 times in the 32 µL of the Reaction buffer (50 mM Sodium Citrate, 100mM Sodium Chloride, pH 6.0). For the 45 minutes treatment group, the mixture was incubated on shaker at 37°C for 45 minutes before its being mixed into serum. Presence of large quantities of glycoproteins in serum would stop α1-2 fucosidase's enzymatic digestion of the TIMP1. For the 0 minute treatment group, the mixture of TIMP1 and α1-2 fucosidase was mixed into serum without the 37°C incubation.



Supplemental Data Figure 5. Dose response curves of the single-antigen and single-detection cross-reactivity studies for tPA (A), TIMP-1(B), MME(C), and DPP-4(D).



Supplemental Data Figure 6. Measurement of fucosylated TIMP-1 and DPP-4 by the multiplex UEA LISAs in 10 non-aggressive cancer and 6 aggressive cancer tissues.



Supplemental Data Figure 7. Comparisons of the multiplex system in testing 300 nanograms and 1500 nanograms of 10 non-aggressive and 6 aggressive prostate cancer tissues.

	DPPIV	TIMP1	tPA	MME
N	16	16	6	4
Slope (95% CI)	0.93 (0.76 to 1.09)	1.05 (0.94 to1.16)	0.87 (0.86 to 0.88)	1.30 (1.21 to 1.39)
Y-intercept (95% CI)	-0.6 (-12.6 to 11.5)	-1.1 (-11.4 to 9.3)	1.4 (-1.1 to 3.8)	-5.5 (-30.3 to 19.3)
R^2	0.91	0.97	1.00	1.00

Supplemental Data Table 1. Linear regression analyses of the multiplex immunoassays and single immunoassays for DPP-4, TIMP-1, tPA and MME in serum.

	TIMP1				DPPIV		tPA
Sensitivity	**UEA**	**AAL**	**PHA-L**	**VVA**	**UEA**	**AAL**	**AAL**
Single	34 ± 2	72 ± 9	1.0 ± 0.1	23.0 ± 0.3	25.3 ± 0.1	136 ± 22	38 ± 14
Multiplex	43 ± 3	101 ± 14	1.4 ± 0.2	26 ± 1	30.4 ± 0.3	164 ± 24	43 ± 15
Statistically different?	No	No	No	No	No	No	No

Supplemental Data Table 2. Comparison of the sensitivity of the single and multiplex LISAs for measurement of glycan structures on recombinant TIMP-1, DPP-4, and tPA.