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The Effects of Histone and Glycosaminoglycans on Human Factor Xa and Antithrombin III Interactions

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THE EFFECTS OF HISTONE AND GLYCOSAMINOGLYCANS ON HUMAN FACTOR Xa AND ANTITHROMBIN III INTERACTIONS

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HONORS PROJECT

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Abstract

Heparin and protamine have been found neutralize each other such that the anticoagulant effects of both compounds are not observed. In the present study, the effects of histone and glycosaminoglycans on the formation and degradation of the 1°Xaα-ATIII complex to the 3°Xaβ-ATIII complex are explored. In the presence of histone, there is a dramatic drop in the levels of 1°Xa-ATIII complexes and an increase in the levels of 3°Xa-ATIII complexes, measured relative to the control, indicating the effect of histone to promote the degradation of the 1°Xa-ATIII complexes. Heparin alone moderately reduces levels of the 1°Xa-ATIII complexes and the 3°Xaα-ATIII complex, while having no appreciable effect on the 3°Xaβ-ATIII complex. When mixed, histone and heparin return the levels of the 1°Xa-ATIII complexes to near control levels while slightly decreasing the levels of 3°Xa-ATIII complexes, indicating an inhibition of the histone effect by heparin. In the presence of chondroitin sulfate A, histone slightly lowers the 1°Xa-ATIII complexes while having no appreciable effect on the 3°Xa-ATIII complexes, indicating a slight inhibition of the histone effect, although it does not have as great an inhibitory effect as heparin does. Dermatan sulfate (CSB) and chondroitin sulfate C, when individually mixed with histone, increase levels of the 1°Xa-ATIII complexes while decreasing the levels of 3°Xa-ATIII complexes, indicating that both compounds inhibit degradation of the 1°Xa-ATIII complexes. Additionally, the data suggest that dermatan sulfate and chondroitin sulfate C exhibit a higher inhibitory effect on histone than heparin.

Introduction

Blood coagulation, or hemostasis, occurs naturally in living beings. It involves a complex set of chemical reactions that activate various coagulant factors to form thrombin.[1] Thrombin, once active in the blood, can in turn catalyze the active fibrinogen, a protein occurring naturally in the blood, and forms cross-linked fibrin. This "mesh-like" fibrin network is what turns into the blood clot. If left completely alone, thrombin would cascade all the blood in the body into clots. Luckily, various anticoagulation factors exist to prevent this. These factors include antithrombin III (ATIII), heparin and chondroitin sulfates A, B and C.

The blood coagulation cascade can take place in two ways: the intrinsic, or interior, pathway and the extrinsic, or exterior, pathway.[2] The intrinsic pathway occurs with some sort of interior perturbance, such as a physical abnormality or bacterial infiltration. It requires activation of Human Factors VIII, IX, X, XI, and XII. The intrinsic pathway initiates when kininogen, prekallikrein, and Human Factors XI and XII come into contact with a negatively charged surface. Prekallikrein becomes activated into kallikrein, then kallikrein will activate Human Factor XII to XIIa, which in turn will activate Human Factor XI to XIa. Human Factor XIIa will also hydrolyze additional prekallikrein into kallikrein and will cause the cascade to start over. Meanwhile, Human Factor XIa is free to cause the activation of subsequent species. In the presence of the Ca^{2+} ion, Human Factor XIa will activate Human Factor IX into Human Factor IXa. Human Factor IXa will activate Human Factor X into Human Factor Xa. This cascade is shown in Figure 1.

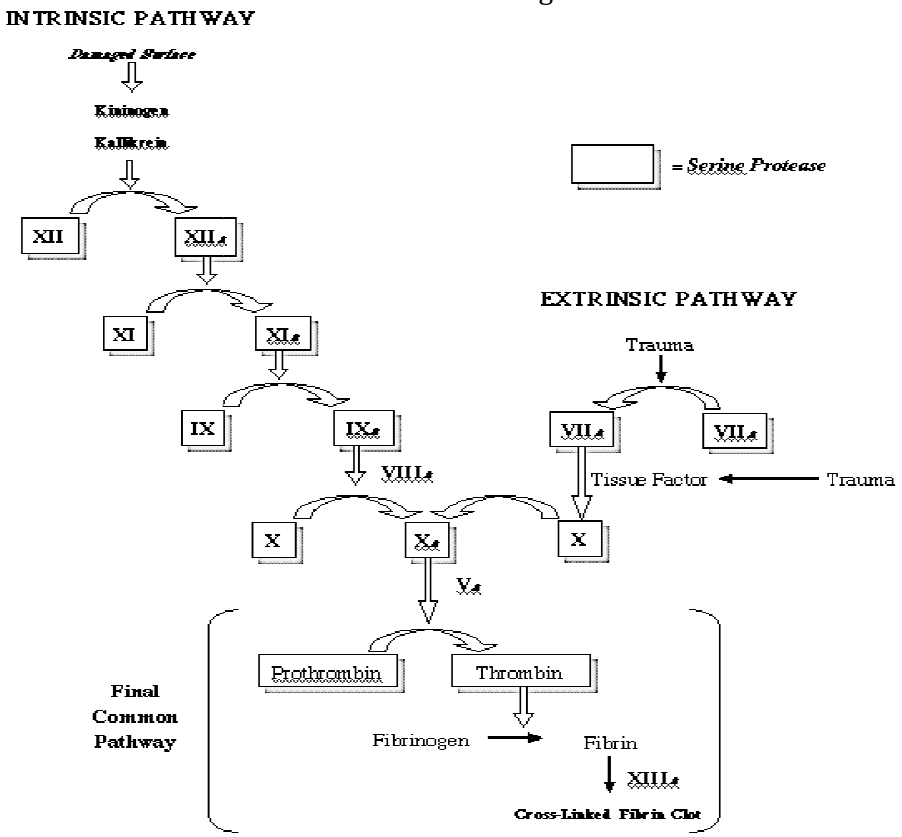


Figure 1: Intrinsic and extrinsic blood coagulation cascade.[3]

The extrinsic pathway occurs when an outside perturbance, such as in injury, is observed.[2] When a tissue injury is sustained, a release of Tissue Factor (Human Factor III) occurs. Tissue Factor by itself is not enough to activate Human Factor X, but must also rely on Human Factor VIIa. Human Factor VII can be activated by Human Factor Xa and Thrombin. This cascade is also shown in Figure 1.

The two pathways converge at the activation of Human Factor X. This is the reason for Human Factor X being of particular interest. Once active, Human Factor Xa can activate

prothrombin (Human Factor II) into thrombin (Human Factor IIa).[2] Thrombin can then activate Fibrinogen into Fibrin, forming the cross-linked system that becomes the blood clot.

Antithrombin III (ATIII) works to inhibit thrombin from forming a fibrin clot by forming complexes with activated Human Factors.[2] Various compounds will strengthen or reduce ATIII anticoagulant power. Numerous studies have shown that acetaldehyde used along with glycosaminoglycans have prolonged clotting time.[4,5,6] Additionally, it has been shown that acetaldehyde can limite the Human Factor Xa activation of prothrombin to thrombin.[7] It is desirable to explore whether or not histone will have the same effects on Xa-ATIII complex formation as acetaldehyde, in conjunction with ATIII.

In order to evaluate whether or not histone will have any effect on complex formation, a way to separate the complexes is necessary. Gel electrophoresis will provide a way to systematically separate proteins according to weight and will be used in this study. Gel electrophoresis works by running an electric current through a buffer solution.[8] Electrophoresis separates compounds based on the compounds' charge to size ratio. Smaller complexes move faster through the gel and larger complexes move slower through the gel. Staining and destaining will provide a way to see the complexes and a way to evaluate the data contained in the gel. In this study, the bands that will be seen correspond to the following complexes, from top to bottom:

1. 1° Xaα-ATIII
2. 1° Xaβ-ATIII
3. 2° Xaα-ATIII
4. 2° Xaβ-ATIII
5. 3° Xaα-ATIII
6. 3° Xaβ-ATIII
7. ATIII-M
8. ATIII
9. Xaα
10. Xaβ
11. Xaγ
12. Solvent front

1° Xaα-ATIII is the first complex formed when Xa and ATIII come into contact with each other. 1° Xaα-ATIII will evolve into 1° Xaβ-ATIII by cleavage of a bond. Then, another bond will get cleaved to form 2° Xaα-ATIII and so on until 3° Xaβ-ATIII is formed. Compounds that cause a decrease in the 1° Xaα-ATIII complex and an increase in the amount of 3° Xaβ-ATIII will be considered procoagulant, as they will promote the evolution of the complexes. Compounds that have the converse effect will be considered anticoagulant. ATIII, Xaα, Xaβ, and Xaγ are all leftover reactants that did not form complexes. Xaγ is not expected to be found in every lane in every gel.

Materials and Methods

Haematologic Technologies, Incorporated (Essex Junction, VT, USA) provided the Human Antithrombin III (HCATIII-0120, Lot BB0419-1 MG, 7.2 mg/mL) and the Human Factor Xa (HCXA-0060, Lot BB0927-0.1 MG, 9.2 mg/mL). Worthington Chemical Company (Lakewood, NJ, USA) provided histone (2544, Lot 31D12695). Sigma Chemical Company, (Louis, MO, USA) provided the heparin (H-3393, Lot 69H1151 Grade I-A from porcine intestinal mucosa), chondroitin sulfate A (C-8529, Lot 55H0306, 70% from bovine trachea), chondroitin sulfate B (C-3788, Lot 94H0656, 90% from porcine intestinal mucosa) and chondroitin sulfate C (C-4384, Lot 107H1029, 90% from shark cartilage).

Routine slab electrophoresis was performed using resolving and stacking gels appropriate for Human Factor Xa, as shown below.

I. Resolving Gel
   i. 4.8 mL deionized-H_2O
   ii. 2.5 mL 1.5 M Tris (pH 8.8)
   iii. 2.5 mL Acrylamide stock solution
   iv. 100 µL 10% SDS solution
   v. 50 µL 10% APS solution
   vi. 10 µL tetramethylethylenediamine (TEMED)
      a. Combine i-iv above and pour into filtering flask
      b. Add APS and degas with water vacuum for 15-60 seconds
      c. Add TEMED and pipet solution into gel molds until ¾ full, drop isobutyl alcohol onto top to remove bubbles/smooth surface
      d. Wait at least 15 minutes to set
      e. Pour off isobutyl alcohol and rinse with deionized-H_2O

II. Stacking Gel
   i. 3.0 mL deionized-H_2O
   ii. 1.25 mL 0.5 M Tris (pH 6.8)
   iii. 0.65 mL acrylamide stock solution
   iv. 100 µL 10% SDS solution
   v. 25 µL 10% APS solution
   vi. 5 µL TEMED
      a. Combine all reagents and pipet onto top of resolving gel; insert lane combs
      b. Wait at least 25 minutes for gel to set
      c. Remove lane combs and rinse with deionized-H_2O

The lanes used in the experiment are represented in Table 1. Solutions of heparin, histone and chondroitin sulfates A, B, and C were made to contain 1 µg compound for every 2 µL Tris/NaCl pH 8 solution. Molecular weight marker (MWM) was made to contain 0.26 µg/µL Histone in Tris. The lanes were prepared as shown below.

Lane	Contents
1	5 µL MWM (0.26 µg/µL Histone in Tris)
2	2 µL (1.94 µg) Xa
3	2 µL (2.32 µg) ATIII
4	2 µL (2.32 µg) ATIII + 2 µL (1.94 µg) Xa
5	(2 µL (1 µg) His + 2 µL (2.32 µg) ATIII)$_{30'}$ + 2 µL (1.94 µg) Xa
6	(2 µL (1 µg) Hep + 2 µL (2.32 µg) ATIII)$_{30'}$ + 2 µL (1.94 µg) Xa
7	2 µL (1 µg) His + (2 µL (1 µg) Hep + 2 µL (2.32 µg) ATIII)$_{30'}$ + 2 µL (1.94 µg) Xa
8	(2 µL (1 µg) His + 2 µL (1 µg) CSA + 2 µL (2.32 µg) ATIII)$_{30'}$ + 2 µL (1.94 µg) Xa
9	(2 µL (1 µg) His + 2 µL (1 µg) CSB + 2 µL (2.32 µg) ATIII)$_{30'}$ + 2 µL (1.94 µg) Xa
10	(2 µL (1 µg) His + 2 µL (1 µg) CSC + 2 µL (2.32 µg) ATIII)$_{30'}$ + 2 µL (1.94 µg) Xa

Lanes 5-10 were incubated for 30 minutes before Human Factor Xa was added. After the Xa was added, all the lanes were incubated for another 30 minutes. After the final incubation, 5 μL of 5x running buffer was added to each lane and the lanes were boiled for 2 minutes and 5 μL of lane contents were added to each lane once cooled. A solution of 1x running buffer was made from 10x running buffer by dilution.

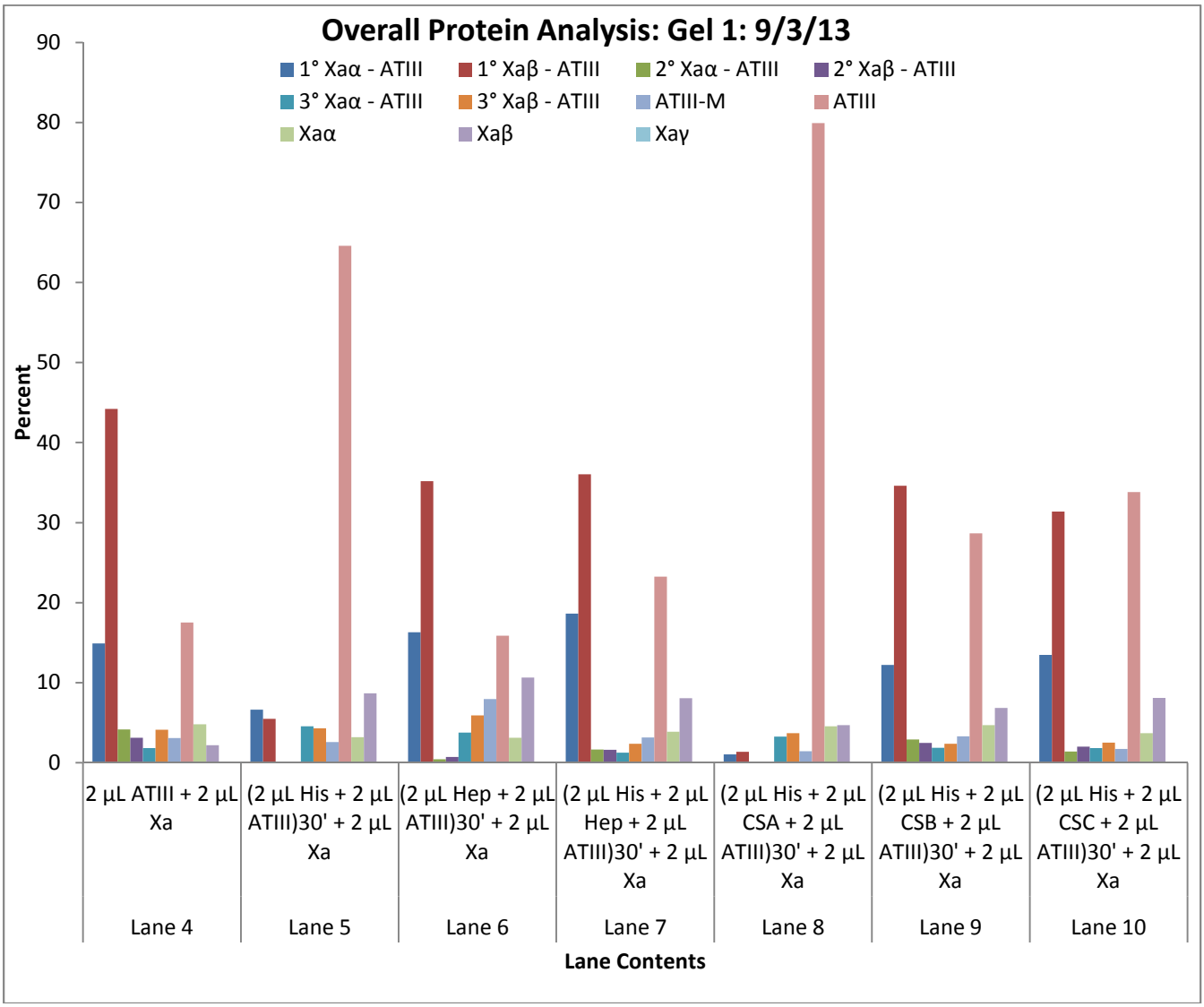
Gels were stained using a solution of Coomassie Brilliant Blue. The gels were then destained by using a solution of water, methanol, and acetic acid. Finally, the gels were placed in equilibrium solution, a mixture of methanol, glycerol, and water. The procedures for making all solutions needed during the experimentation process can be found in Appendix A.

Pictures of the gels were collected and data was extrapolated from the photos using ImageJ software. The image type was changed to 8-bit, the background was subtracted, and the brightness and contrast was adjusted so that all bands could be clearly seen. The gels were examined to get the percent abundance of each complex in each lane. The data was analyzed using Excel and interpreted using a t-test (paired two-sample for means). All lanes were compared to both the control lane (ATIII + Xa) and the histone lane (His + ATIII + Xa), however the comparison to histone only serves as a means of measuring how effective additional compounds were at inhibiting the histone effect and are not discussed quantitatively, as is the comparison to control. The results of the data analysis were compiled into a table which shows the mean, standard error of the mean, standard deviation, and one- and two-tail p values for each complex. Because of the nature of the p values, only the two-tail p values under 0.1 are considered when assessing statistical significance in reporting complex values.

Results

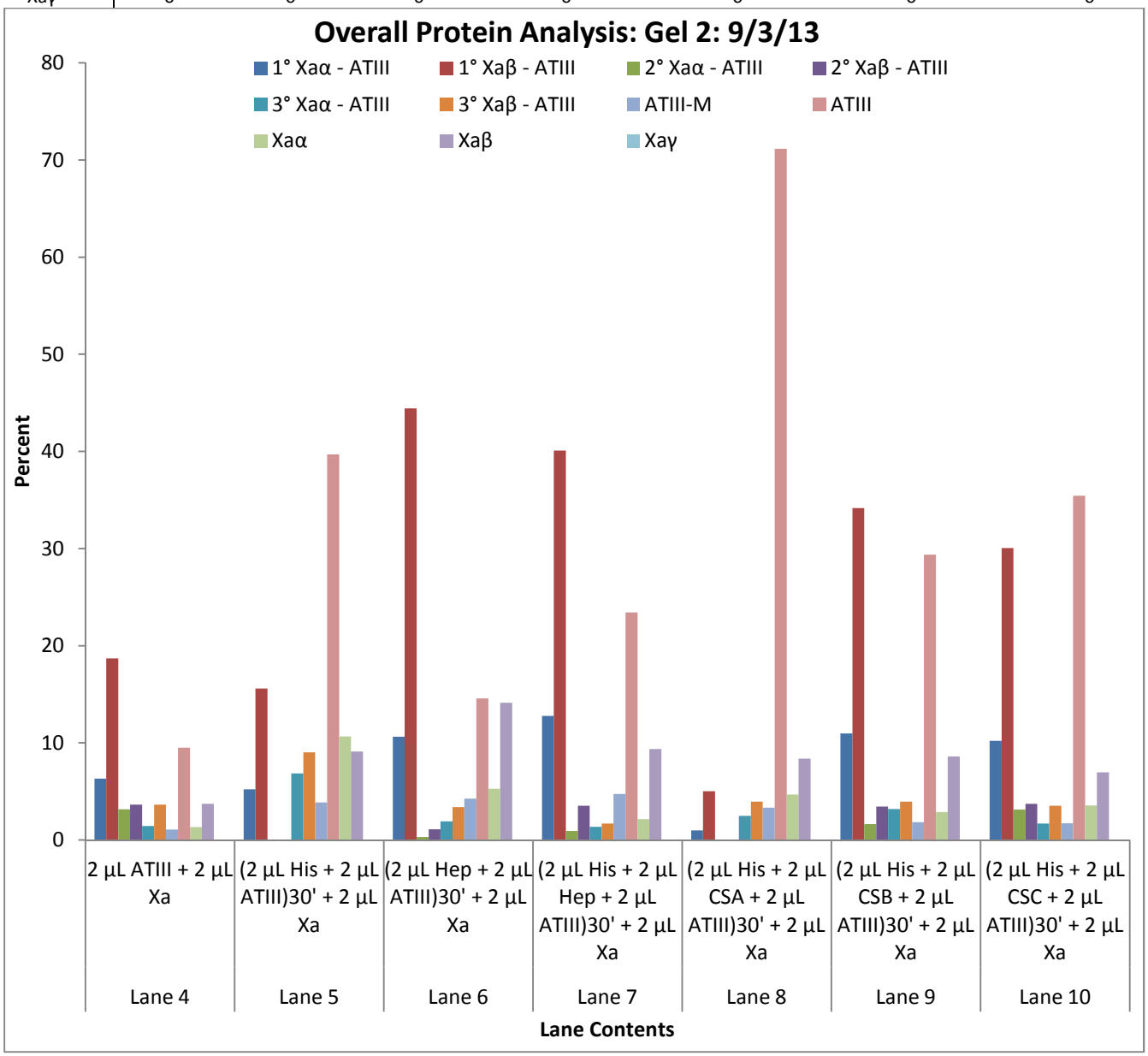
Overall protein analysis: Gel 1, 9/3/2013

Gel 1	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9	Lane 10
	2 µL ATIII + 2 µL Xa	(2 µL His + 2 µL $ATIII)_{30'}$ + 2 µL Xa	(2 µL Hep + 2 µL $ATIII)_{30'}$ + 2 µL Xa	(2 µL His + 2 µL Hep + 2 µL $ATIII)_{30'}$ + 2 µL Xa	(2 µL His + 2 µL CSA + 2 µL $ATIII)_{30'}$ + 2 µL Xa	(2 µL His + 2 µL CSB + 2 µL $ATIII)_{30'}$ + 2 µL Xa	(2 µL His + 2 µL CSC + 2 µL $ATIII)_{30'}$ + 2 µL Xa
1° Xaα - ATIII	14.91	6.64	16.317	18.648	1.032	12.207	13.473
1° Xaβ - ATIII	44.199	5.486	35.185	36.035	1.384	34.604	31.379
2° Xaα - ATIII	4.164	0	0.443	1.671	0	2.897	1.413
2° Xaβ - ATIII	3.129	0	0.718	1.626	0	2.481	2.012
3° Xaα - ATIII	1.832	4.549	3.78	1.261	3.263	1.885	1.83
3° Xaβ - ATIII	4.139	4.292	5.934	2.392	3.715	2.381	2.524
ATIII-M	3.085	2.573	7.952	3.152	1.429	3.323	1.73
ATIII	17.538	64.59	15.889	23.264	79.93	28.657	33.831
Xaα	4.822	3.196	3.133	3.868	4.542	4.711	3.689
Xaβ	2.181	8.674	10.649	8.084	4.705	6.854	8.119
Xaγ	0	0	0	0	0	0	0



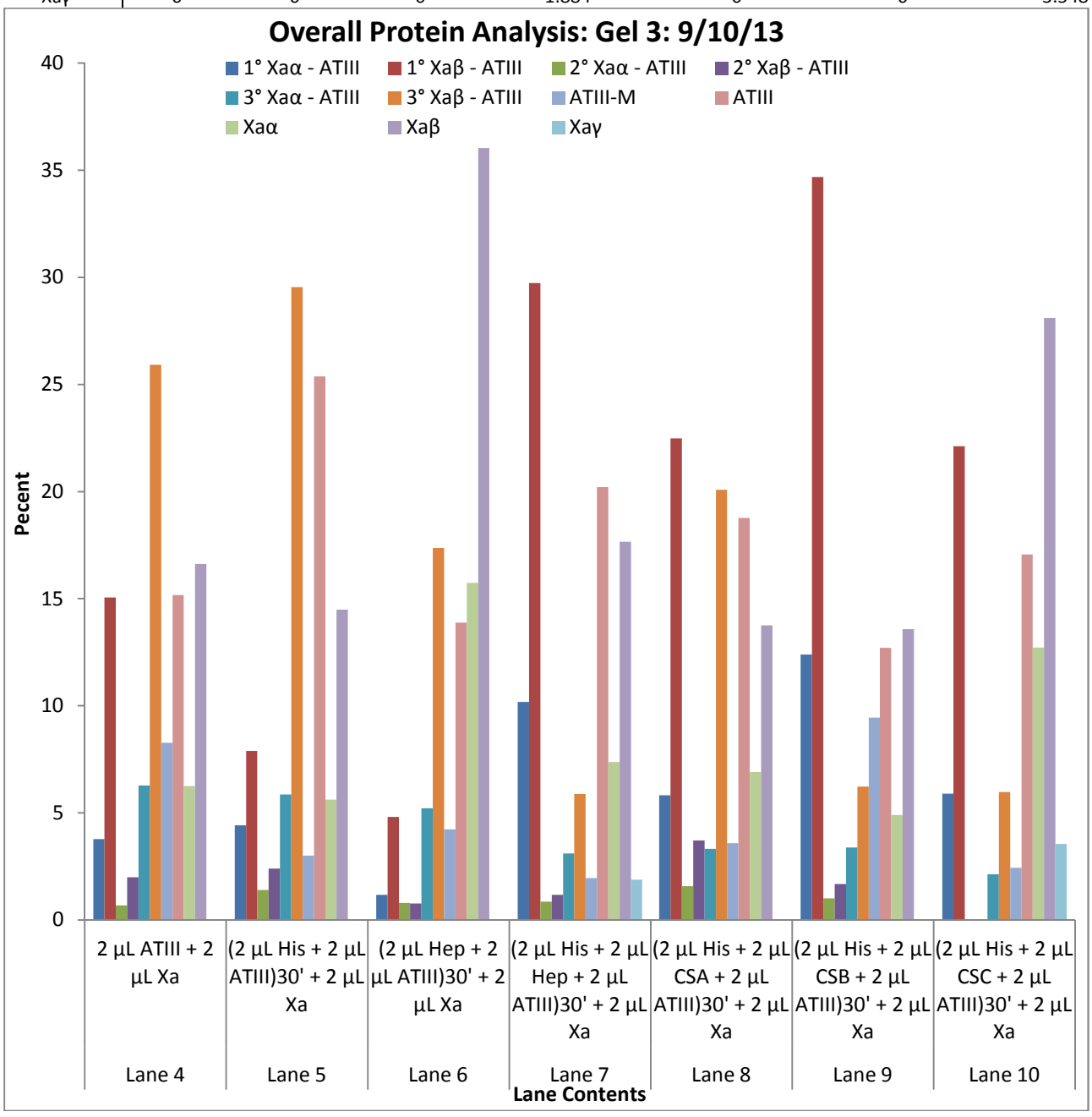
Overall protein analysis: Gel 2, 9/3/2013

Gel 2	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL $ATIII)_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL $ATIII)_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL $ATIII)_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL $ATIII)_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL $ATIII)_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL $ATIII)_{30'}$ + 2 µL Xa
1° Xaα - ATIII	6.329	5.218	10.618	12.779	0.998	10.965	10.208
1° Xaβ - ATIII	18.691	15.602	44.427	40.102	5.009	34.16	30.046
2° Xaα - ATIII	3.146	0	0.301	0.923	0	1.639	3.128
2° Xaβ - ATIII	3.638	0	1.105	3.513	0	3.427	3.724
3° Xaα - ATIII	1.45	6.855	1.929	1.341	2.48	3.185	1.7
3° Xaβ - ATIII	3.642	9.017	3.376	1.686	3.955	3.951	3.535
ATIII-M	1.062	3.872	4.267	4.731	3.338	1.827	1.715
ATIII	9.516	39.682	14.569	23.431	71.15	29.374	35.427
Xaα	1.316	10.647	5.281	2.136	4.685	2.883	3.543
Xaβ	3.735	9.107	14.128	9.358	8.385	8.588	6.975
Xaγ	0	0	0	0	0	0	0



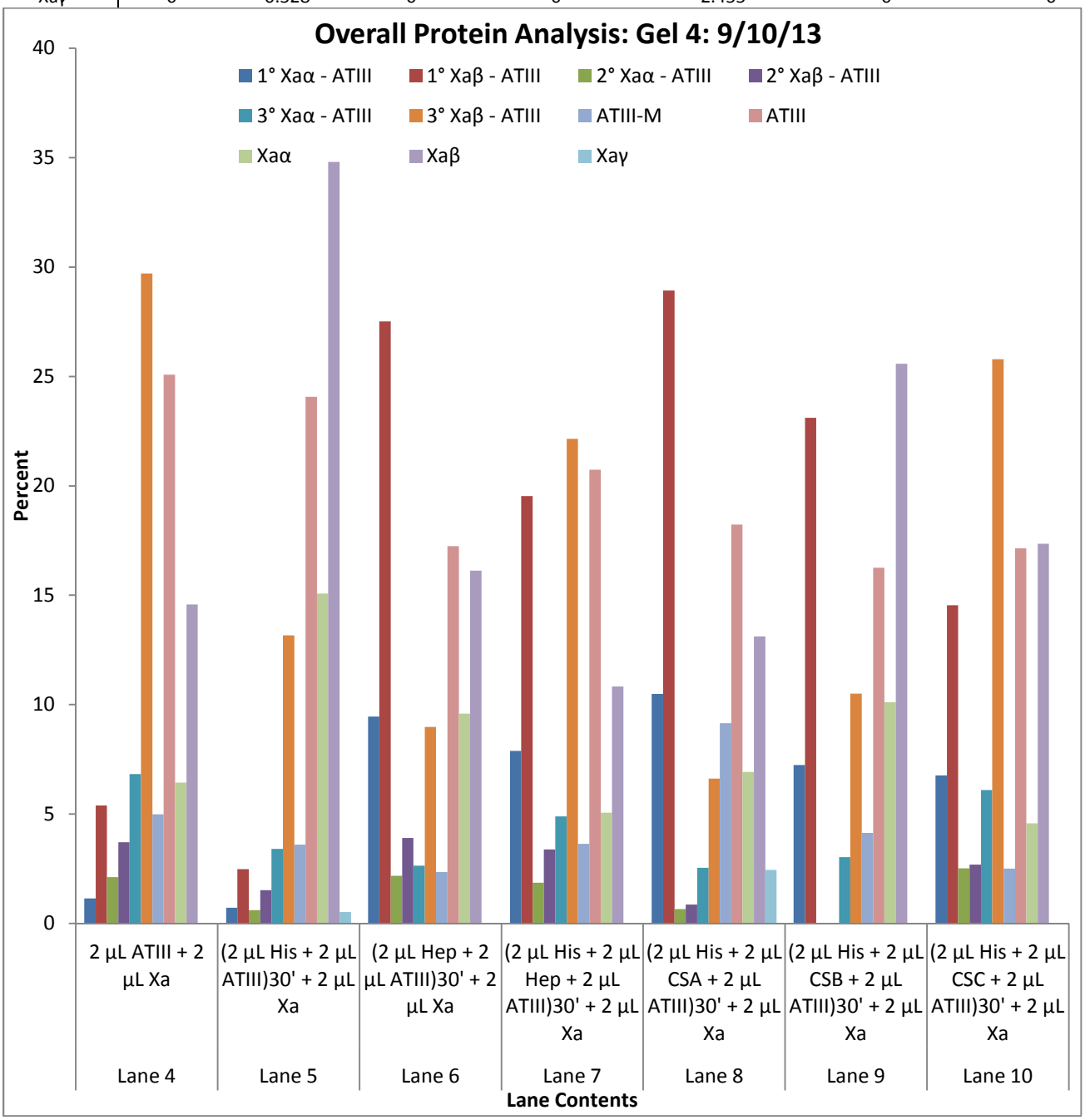
Overall protein analysis: Gel 3, 9/10/2013

Gel 3	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1° Xaα - ATIII	3.77	4.421	1.177	10.18	5.823	12.385	5.898
1° Xaβ - ATIII	15.061	7.888	4.81	29.731	22.481	34.685	22.117
2° Xaα - ATIII	0.673	1.397	0.797	0.855	1.575	1.013	0
2° Xaβ - ATIII	1.995	2.393	0.765	1.172	3.706	1.68	0
3° Xaα - ATIII	6.274	5.854	5.21	3.105	3.321	3.388	2.134
3° Xaβ - ATIII	25.925	29.55	17.366	5.882	20.083	6.222	5.975
ATIII-M	8.268	3.006	4.226	1.956	3.579	9.445	2.433
ATIII	15.164	25.378	13.874	20.209	18.775	12.701	17.065
Xaα	6.247	5.621	15.732	7.371	6.906	4.904	12.719
Xaβ	16.622	14.492	36.043	17.656	13.75	13.577	28.109
Xaγ	0	0	0	1.884	0	0	3.548



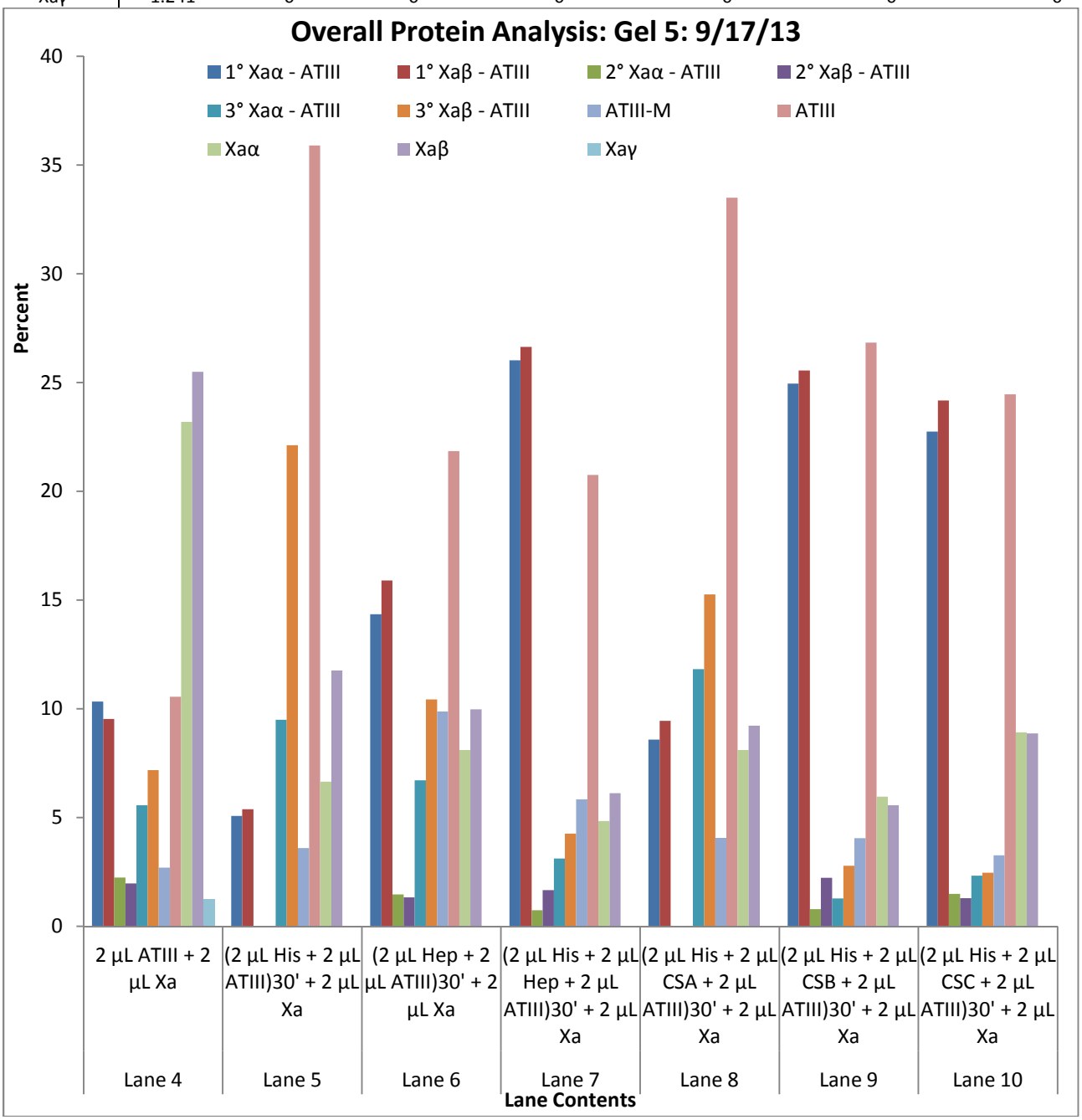
Overall protein analysis: Gel 4, 9/10/2013

Gel 4	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1° Xaα - ATIII	1.147	0.724	9.454	7.889	10.488	7.243	6.774
1° Xaβ - ATIII	5.397	2.481	27.508	19.537	28.922	23.11	14.544
2° Xaα - ATIII	2.124	0.617	2.188	1.865	0.66	0	2.518
2° Xaβ - ATIII	3.714	1.522	3.905	3.387	0.873	0	2.692
3° Xaα - ATIII	6.827	3.407	2.65	4.895	2.55	3.038	6.096
3° Xaβ - ATIII	29.697	13.17	8.986	22.146	6.622	10.509	25.785
ATIII-M	4.988	3.602	2.35	3.644	9.153	4.146	2.516
ATIII	25.082	24.065	17.244	20.735	18.232	16.256	17.143
Xaα	6.447	15.082	9.587	5.063	6.926	10.115	4.577
Xaβ	14.578	34.803	16.127	10.838	13.119	25.584	17.355
Xaγ	0	0.528	0	0	2.455	0	0



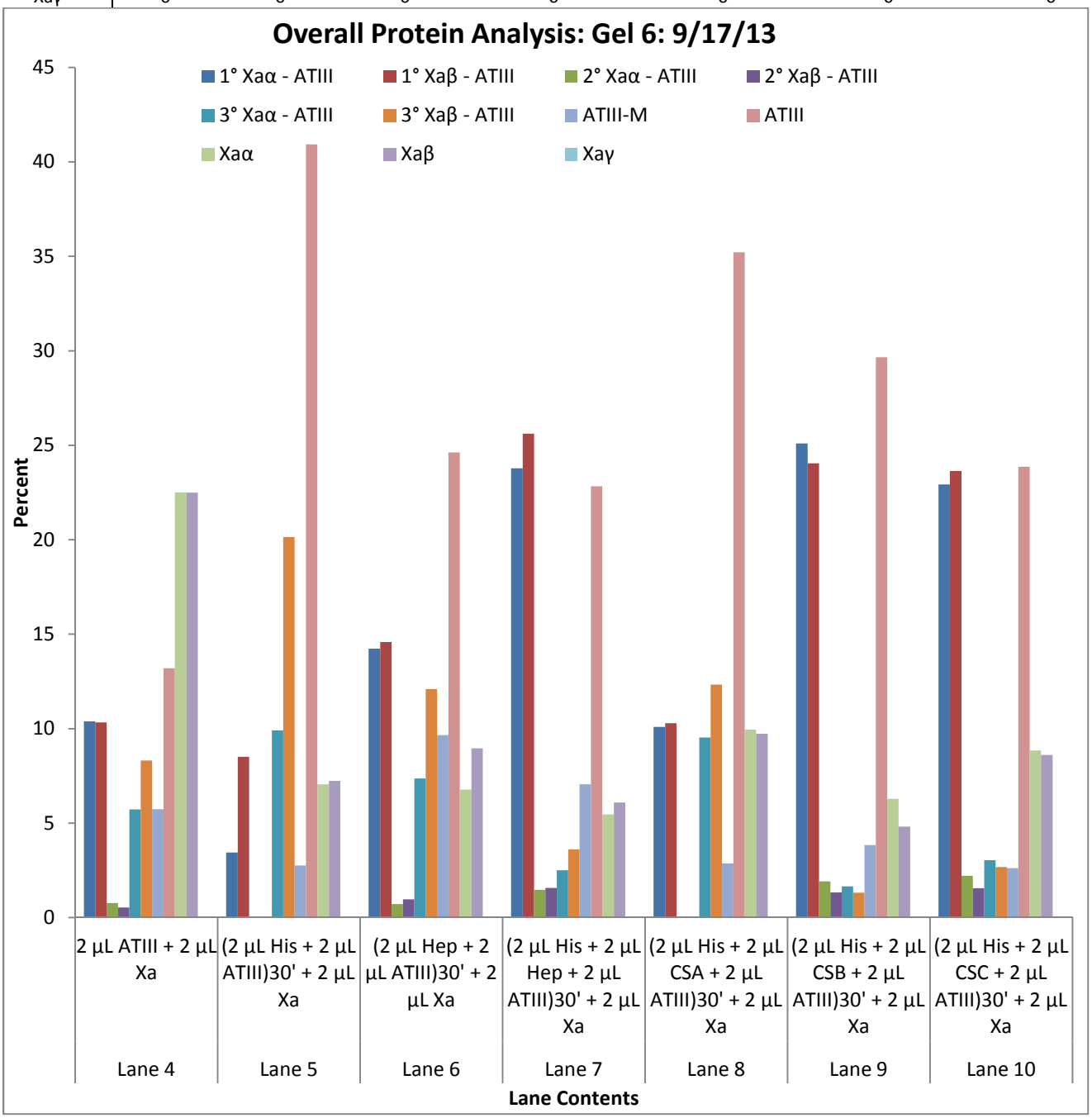
Overall protein analysis: Gel 5, 9/17/2013

Gel 5	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1° Xaα - ATIII	10.332	5.08	14.352	26.028	8.59	24.951	22.744
1° Xaβ - ATIII	9.537	5.378	15.903	26.639	9.442	25.555	24.181
2° Xaα - ATIII	2.241	0	1.468	0.737	0	0.784	1.488
2° Xaβ - ATIII	1.972	0	1.335	1.663	0	2.225	1.289
3° Xaα - ATIII	5.561	9.497	6.715	3.12	11.824	1.279	2.33
3° Xaβ - ATIII	7.183	22.125	10.435	4.261	15.254	2.786	2.463
ATIII-M	2.694	3.599	9.875	5.836	4.06	4.054	3.268
ATIII	10.559	35.905	21.843	20.757	33.496	26.833	24.456
Xaα	23.185	6.653	8.103	4.843	8.107	5.967	8.913
Xaβ	25.494	11.763	9.971	6.117	9.227	5.565	8.869
Xaγ	1.241	0	0	0	0	0	0



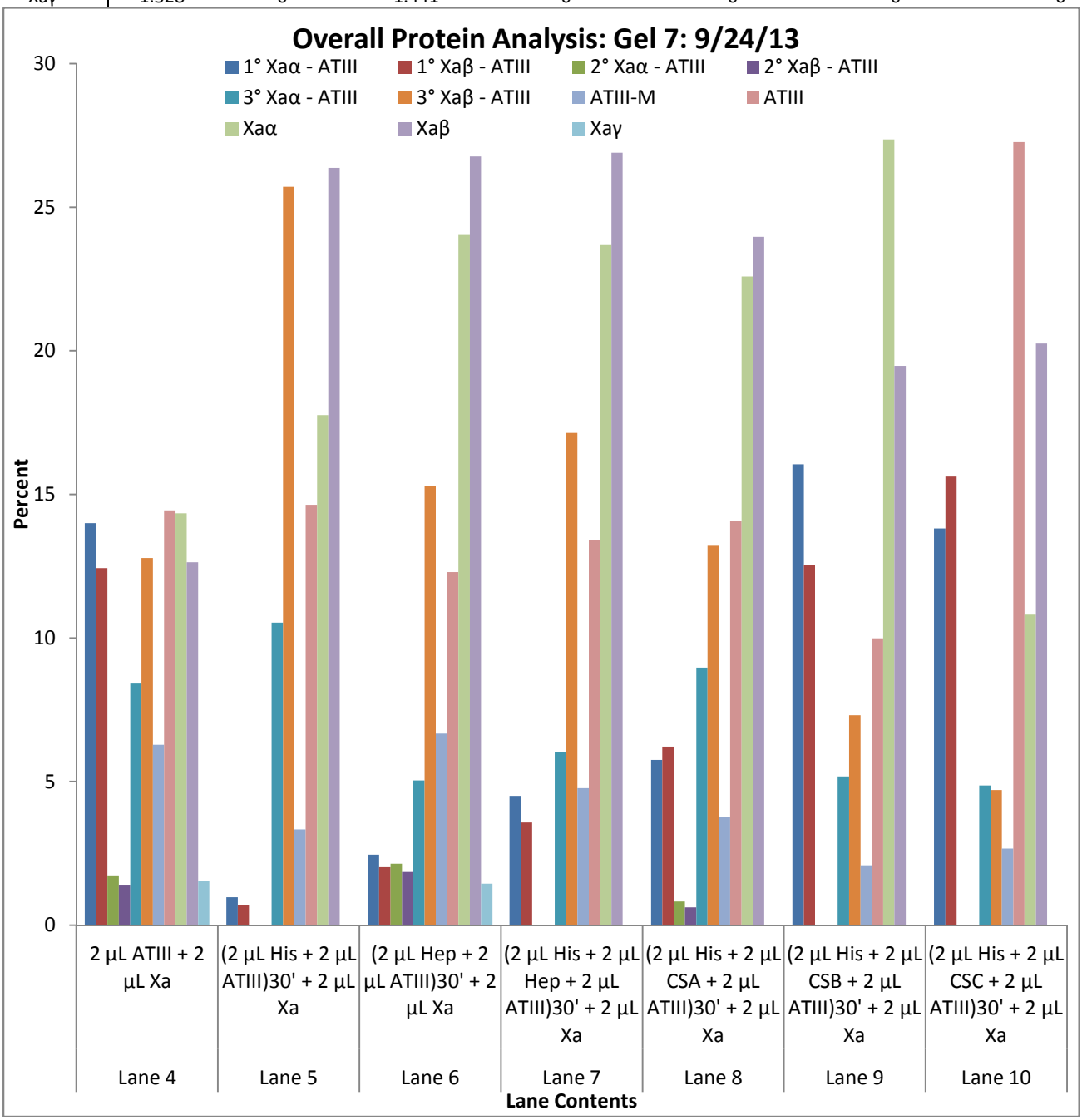
Overall protein analysis: Gel 6, 9/17/2013

Gel 6	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1° Xaα - ATIII	10.387	3.449	14.233	23.784	10.089	25.092	22.927
1° Xaβ - ATIII	10.325	8.514	14.585	25.614	10.284	24.05	23.635
2° Xaα - ATIII	0.773	0	0.718	1.476	0	1.926	2.21
2° Xaβ - ATIII	0.531	0	0.97	1.572	0	1.329	1.56
3° Xaα - ATIII	5.722	9.918	7.368	2.512	9.532	1.662	3.046
3° Xaβ - ATIII	8.313	20.143	12.101	3.613	12.328	1.325	2.682
ATIII-M	5.744	2.766	9.664	7.052	2.873	3.838	2.626
ATIII	13.205	40.918	24.625	22.833	35.211	29.662	23.863
Xaα	22.511	7.055	6.778	5.461	9.952	6.293	8.844
Xaβ	22.491	7.237	8.956	6.085	9.731	4.822	8.608
Xaγ	0	0	0	0	0	0	0



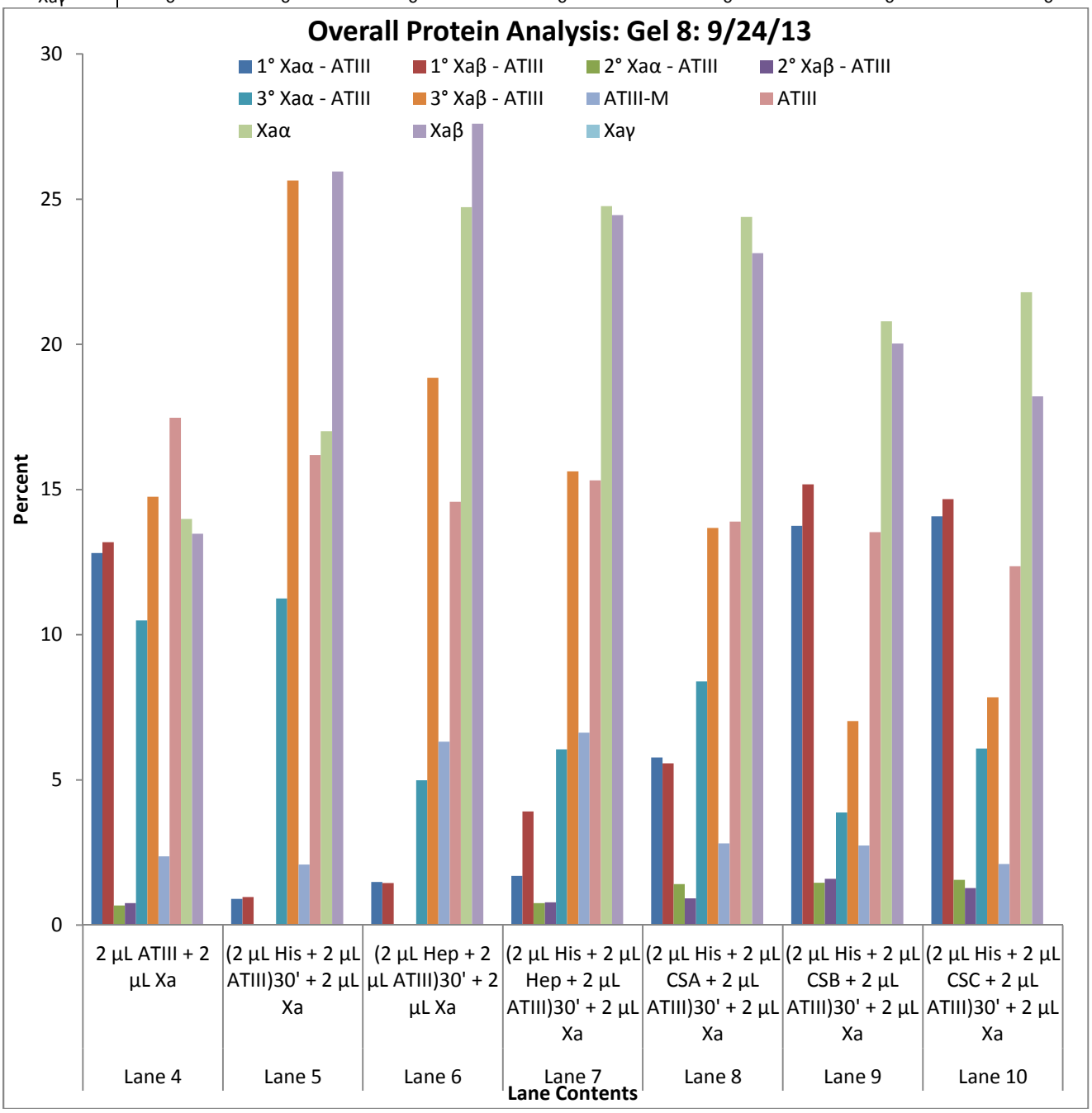
Overall protein analysis: Gel 7, 9/24/2013

Gel 7	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1° Xaα - ATIII	14	0.967	2.456	4.505	5.749	16.044	13.816
1° Xaβ - ATIII	12.438	0.683	2.022	3.571	6.214	12.548	15.621
2° Xaα - ATIII	1.728	0	2.142	0	0.826	0	0
2° Xaβ - ATIII	1.404	0	1.849	0	0.621	0	0
3° Xaα - ATIII	8.41	10.538	5.044	6.009	8.965	5.181	4.865
3° Xaβ - ATIII	12.788	25.711	15.276	17.141	13.21	7.312	4.704
ATIII-M	6.284	3.33	6.674	4.771	3.781	2.087	2.663
ATIII	14.442	14.635	12.294	13.422	14.068	9.99	27.272
Xaα	14.342	17.764	24.031	23.686	22.594	27.365	10.808
Xaβ	12.636	26.372	26.771	26.895	23.971	19.472	20.251
Xaγ	1.528	0	1.441	0	0	0	0



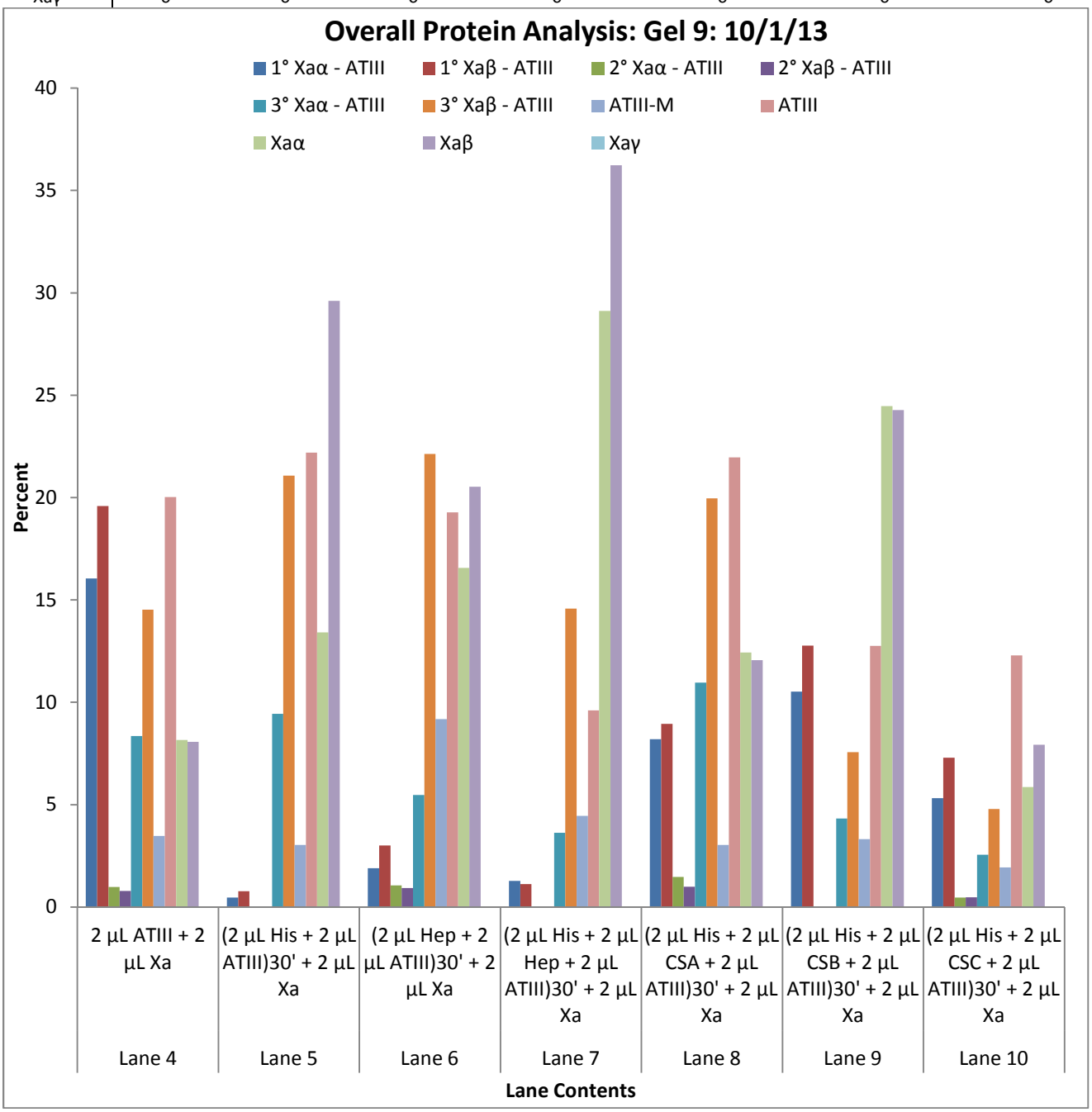
Overall protein analysis: Gel 8, 9/24/2013

Gel 8	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1° Xaα - ATIII	12.816	0.901	1.489	1.701	5.774	13.754	14.083
1° Xaβ - ATIII	13.188	0.967	1.451	3.915	5.574	15.178	14.675
2° Xaα - ATIII	0.676	0	0	0.761	1.413	1.463	1.558
2° Xaβ - ATIII	0.76	0	0	0.783	0.925	1.595	1.275
3° Xaα - ATIII	10.496	11.25	4.988	6.055	8.389	3.876	6.086
3° Xaβ - ATIII	14.755	25.642	18.851	15.626	13.675	7.031	7.847
ATIII-M	2.371	2.085	6.318	6.631	2.82	2.744	2.11
ATIII	17.47	16.192	14.579	15.313	13.894	13.533	12.363
Xaα	13.99	17.012	24.725	24.758	24.389	20.798	21.791
Xaβ	13.477	25.949	27.599	24.456	23.146	20.028	18.213
Xaγ	0	0	0	0	0	0	0



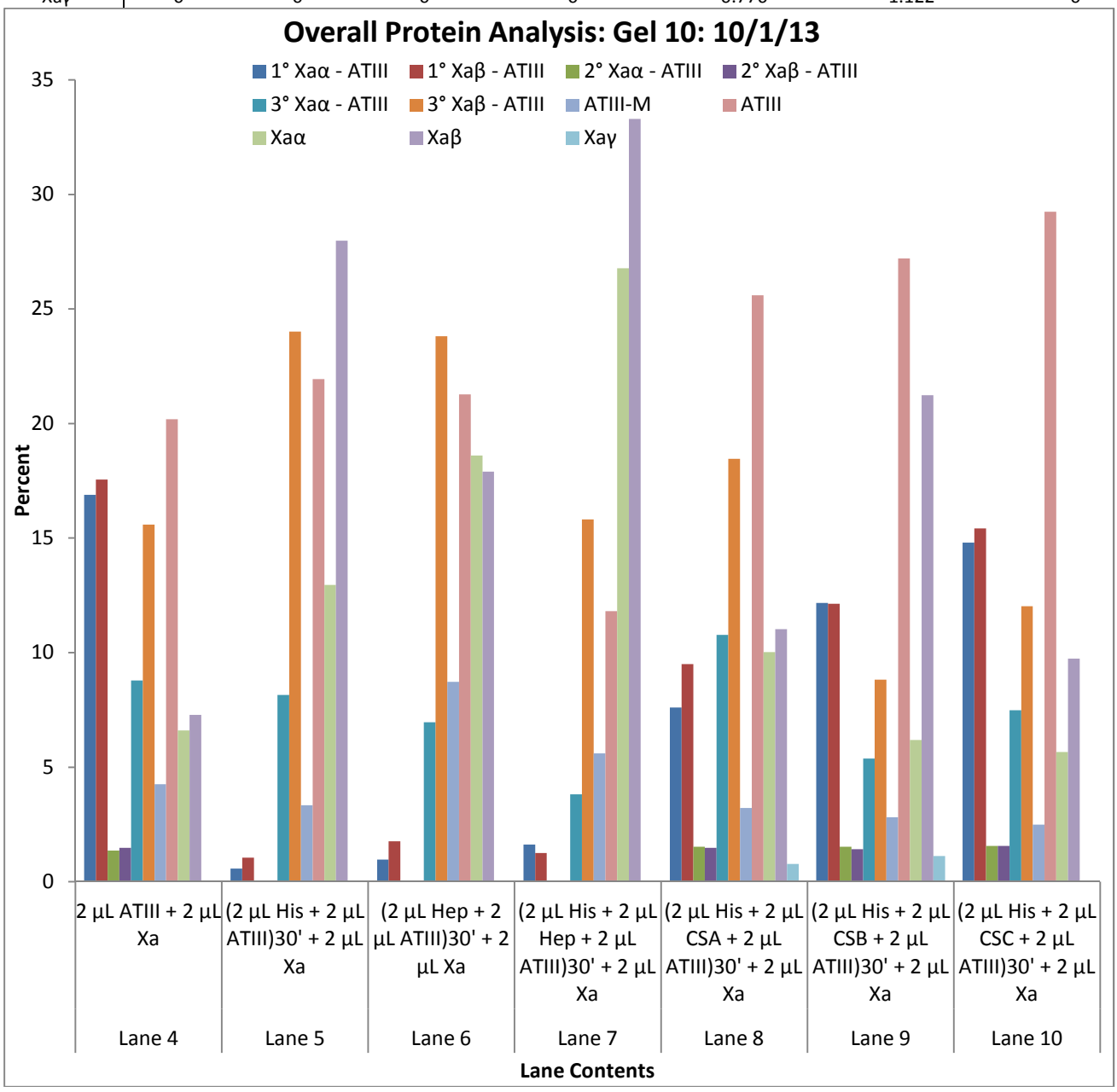
Overall protein analysis: Gel 9, 10/1/2013

Gel 9	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1° Xaα - ATIII	16.053	0.464	1.89	1.278	8.192	10.52	5.314
1° Xaβ - ATIII	19.589	0.773	3.003	1.119	8.951	12.771	7.291
2° Xaα - ATIII	0.973	0	1.051	0	1.468	0	0.46
2° Xaβ - ATIII	0.783	0	0.92	0	0.99	0	0.48
3° Xaα - ATIII	8.349	9.438	5.469	3.62	10.964	4.319	2.55
3° Xaβ - ATIII	14.526	21.076	22.126	14.571	19.959	7.562	4.786
ATIII-M	3.475	3.027	9.172	4.452	3.028	3.318	1.929
ATIII	20.024	22.199	19.28	9.605	21.966	12.757	12.293
Xaα	8.161	13.41	16.566	29.121	12.432	24.473	5.861
Xaβ	8.067	29.612	20.523	36.234	12.052	24.28	7.919
Xaγ	0	0	0	0	0	0	0



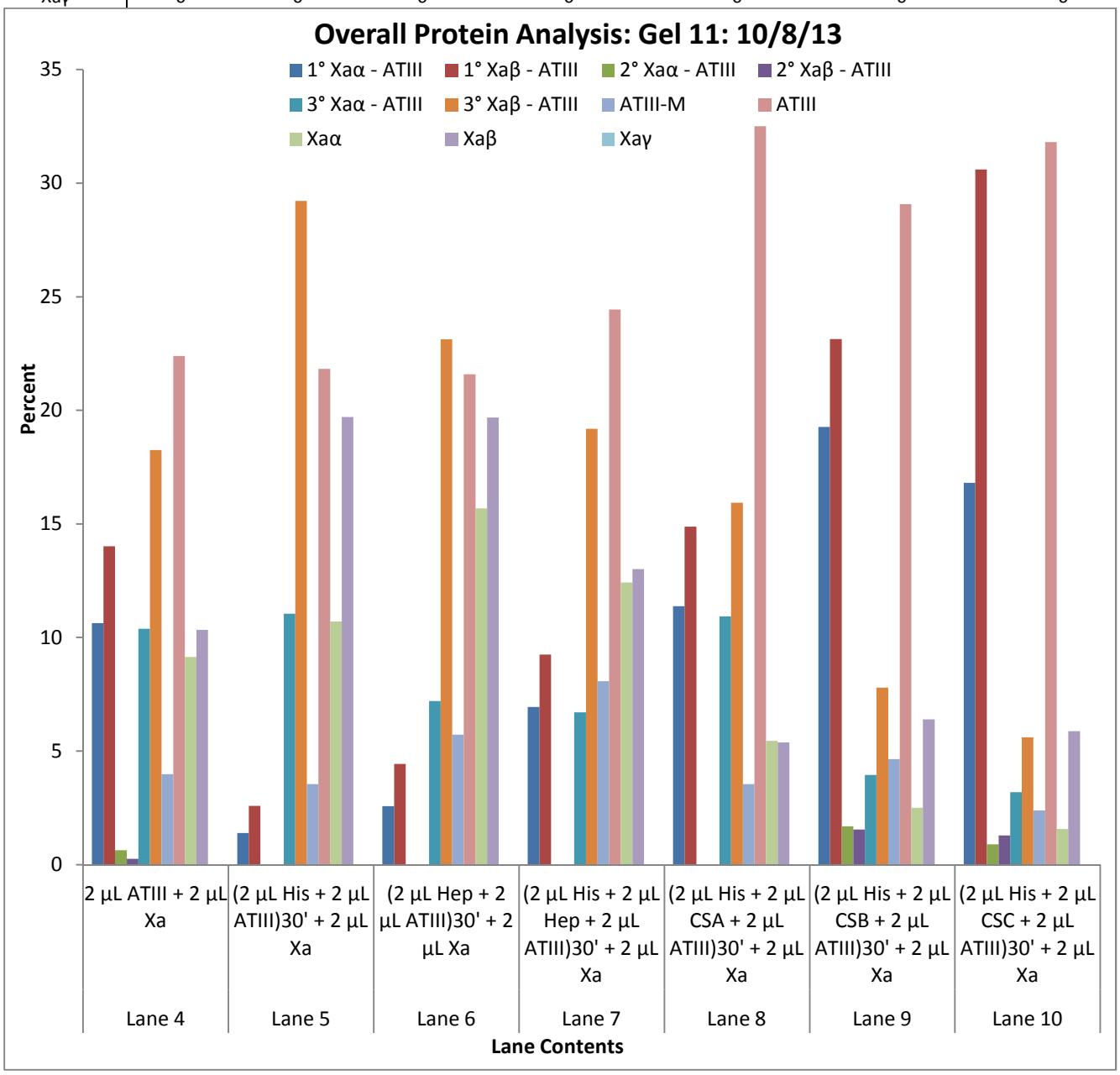
Overall protein analysis: Gel 10, 10/1/2013

Gel 10	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9	Lane 10
	2 µL ATIII + 2 µL Xa	(2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	(2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	(2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	(2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	(2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	(2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1° Xaα - ATIII	16.89	0.577	0.963	1.626	7.609	12.165	14.796
1° Xaβ - ATIII	17.559	1.047	1.767	1.256	9.501	12.129	15.42
2° Xaα - ATIII	1.365	0	0	0	1.532	1.526	1.566
2° Xaβ - ATIII	1.479	0	0	0	1.475	1.42	1.566
3° Xaα - ATIII	8.789	8.156	6.966	3.817	10.78	5.375	7.486
3° Xaβ - ATIII	15.593	24.018	23.815	15.816	18.458	8.825	12.028
ATIII-M	4.257	3.337	8.719	5.605	3.214	2.813	2.494
ATIII	20.184	21.938	21.271	11.816	25.603	27.206	29.243
Xaα	6.601	12.95	18.598	26.773	10.029	6.181	5.666
Xaβ	7.283	27.978	17.9	33.292	11.025	21.238	9.733
Xaγ	0	0	0	0	0.776	1.122	0



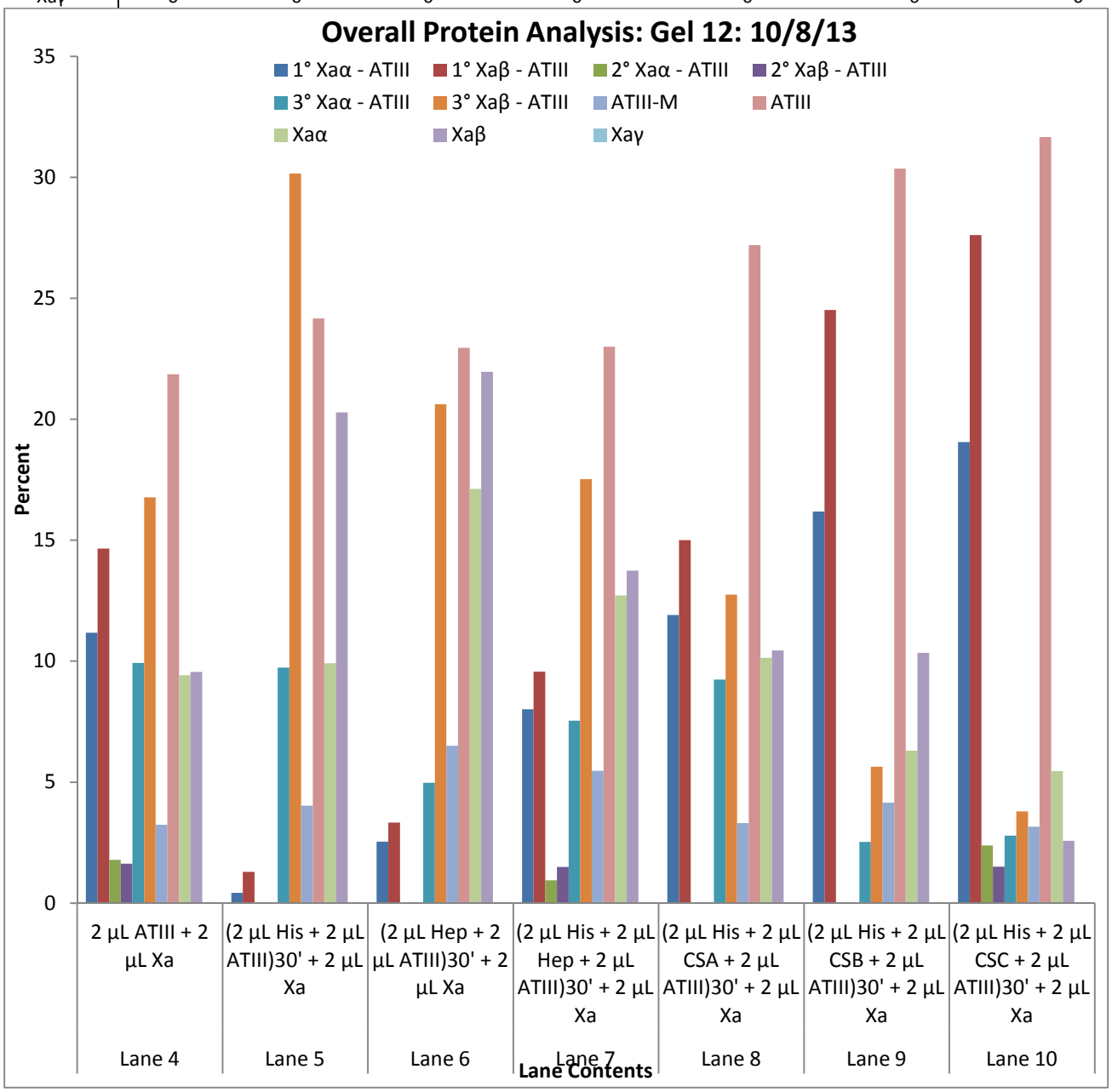
Overall protein analysis: Gel 11, 10/8/2013

Gel 11	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9	Lane 10
	2 µL ATIII + 2 µL Xa	(2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	(2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	(2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	(2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	(2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	(2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1° Xaα - ATIII	10.627	1.388	2.572	6.938	11.382	19.273	16.805
1° Xaβ - ATIII	14.009	2.584	4.431	9.242	14.883	23.134	30.599
2° Xaα - ATIII	0.634	0	0	0	0	1.691	0.893
2° Xaβ - ATIII	0.255	0	0	0	0	1.549	1.281
3° Xaα - ATIII	10.387	11.044	7.204	6.7	10.925	3.945	3.184
3° Xaβ - ATIII	18.249	29.213	23.13	19.187	15.932	7.787	5.602
ATIII-M	3.978	3.543	5.72	8.072	3.538	4.643	2.38
ATIII	22.389	21.823	21.582	24.439	32.511	29.081	31.814
Xaα	9.136	10.704	15.678	12.412	5.446	2.505	1.569
Xaβ	10.335	19.701	19.682	13.009	5.383	6.391	5.873
Xaγ	0	0	0	0	0	0	0



Overall protein analysis: Gel 12, 10/8/2013

Gel 12	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1° Xaα - ATIII	11.174	0.426	2.541	8.01	11.906	16.181	19.056
1° Xaβ - ATIII	14.648	1.295	3.328	9.569	15.005	24.517	27.614
2° Xaα - ATIII	1.782	0	0	0.943	0	0	2.388
2° Xaβ - ATIII	1.628	0	0	1.495	0	0	1.5
3° Xaα - ATIII	9.924	9.736	4.977	7.541	9.243	2.525	2.784
3° Xaβ - ATIII	16.77	30.156	20.624	17.524	12.754	5.64	3.794
ATIII-M	3.242	4.027	6.499	5.463	3.306	4.148	3.164
ATIII	21.859	24.161	22.953	23	27.196	30.353	31.662
Xaα	9.418	9.912	17.123	12.717	10.144	6.297	5.459
Xaβ	9.555	20.286	21.955	13.739	10.446	10.338	2.579
Xaγ	0	0	0	0	0	0	0



Data analysis for the 1° Xaα – ATIII complex across all gels

Gel	Complex	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1	1° Xaα - ATIII	14.91	6.64	16.317	18.648	1.032	12.207	13.473
2	1° Xaα - ATIII	6.329	5.218	10.618	12.779	0.998	10.965	10.208
3	1° Xaα - ATIII	3.77	4.421	1.177	10.18	5.823	12.385	5.898
4	1° Xaα - ATIII	1.147	0.724	9.454	7.889	10.488	7.243	6.774
5	1° Xaα - ATIII	10.332	5.08	14.352	26.028	8.59	24.951	22.744
6	1° Xaα - ATIII	10.387	3.449	14.233	23.784	10.089	25.092	22.927
7	1° Xaα - ATIII	14	0.967	2.456	4.505	5.749	16.044	13.816
8	1° Xaα - ATIII	12.816	0.901	1.489	1.701	5.774	13.754	14.083
9	1° Xaα - ATIII	16.053	0.464	1.89	1.278	8.192	10.52	5.314
10	1° Xaα - ATIII	16.89	0.577	0.963	1.626	7.609	12.165	14.796
11	1° Xaα - ATIII	10.627	1.388	2.572	6.938	11.382	19.273	16.805
12	1° Xaα - ATIII	11.174	0.426	2.541	8.01	11.906	16.181	19.056

t-test table for 1° Xaα – ATIII complex across all gels as compared to the control lane, ATIII + Xa

	ATIII + Xa	*(His + ATIII) + Xa*
Mean	10.70291667	2.52125
Variance	23.53781008	5.201023477
Observations	12	12
Std. Error of Mean	0.404298175	0.190047938
Std. Deviation	4.851578102	2.280575251
t Stat	4.923507037	
P(T<=t) one-tail	0.000227162	
t Critical one-tail	1.795884814	
P(T<=t) two-tail	0.000454324	
t Critical two-tail	2.200985159	

	ATIII + Xa	*(His + CSA + ATIII) + Xa*
Mean	10.70291667	7.302666667
Variance	23.53781008	13.11761988
Observations	12	12
Std. Error of Mean	0.404298175	0.301818791
Std. Deviation	4.851578102	3.62182549
t Stat	1.854868433	
P(T<=t) one-tail	0.045296526	
t Critical one-tail	1.795884814	
P(T<=t) two-tail	0.090593051	
t Critical two-tail	2.200985159	

	ATIII + Xa	*(Hep + ATIII) + Xa*
Mean	10.70291667	6.505166667
Variance	23.53781008	36.02928379
Observations	12	12
Std. Error of Mean	0.404298175	0.500203318
Std. Deviation	4.851578102	6.00243982
t Stat	1.737085927	
P(T<=t) one-tail	0.05512619	
t Critical one-tail	1.795884814	
P(T<=t) two-tail	0.110252379	
t Critical two-tail	2.200985159	

	ATIII + Xa	*(His + CSB + ATIII) + Xa*
Mean	10.70291667	15.065
Variance	23.53781008	31.11953455
Observations	12	12
Std. Error of Mean	0.404298175	0.464874046
Std. Deviation	4.851578102	5.578488554
t Stat	-2.242135794	
P(T<=t) one-tail	0.023263414	
t Critical one-tail	1.795884814	
P(T<=t) two-tail	0.046526828	
t Critical two-tail	2.200985159	

	ATIII + Xa	*(His + Hep + ATIII) + Xa*
Mean	10.70291667	10.2805
Variance	23.53781008	71.95929755
Observations	12	12
Std. Error of Mean	0.404298175	0.706906885
Std. Deviation	4.851578102	8.48288262
t Stat	0.137026639	
P(T<=t) one-tail	0.446742797	
t Critical one-tail	1.795884814	
P(T<=t) two-tail	0.893485593	
t Critical two-tail	2.200985159	

	ATIII + Xa	*(His + CSC + ATIII) + Xa*
Mean	10.70291667	13.8245
Variance	23.53781008	36.16780482
Observations	12	12
Std. Error of Mean	0.404298175	0.501163956
Std. Deviation	4.851578102	6.013967477
t Stat	-1.65548463	
P(T<=t) one-tail	0.063023131	
t Critical one-tail	1.795884814	
P(T<=t) two-tail	0.126046262	
t Critical two-tail	2.200985159	

t-test table for 1° Xaα – ATIII complex across all gels as compared to the histone lane, (His + ATIII) + Xa

	(His + ATIII) + Xa	*ATIII + Xa*
Mean	2.52125	10.70291667
Variance	5.201023477	23.53781008
Observations	12	12
Std. Error of Mean	0.190047938	0.404298175
Std. Deviation	2.280575251	4.851578102
t Stat	-4.923507037	
P(T<=t) one-tail	0.000227162	
t Critical one-tail	1.795884814	
P(T<=t) two-tail	0.000454324	
t Critical two-tail	2.200985159	

	(His + ATIII) + Xa	*(His + CSA + ATIII) + Xa*
Mean	2.52125	7.302666667
Variance	5.201023477	13.11761988
Observations	12	12
Std. Error of Mean	0.190047938	0.301818791
Std. Deviation	2.280575251	3.62182549
t Stat	-3.089900446	
P(T<=t) one-tail	0.005143947	
t Critical one-tail	1.795884814	
P(T<=t) two-tail	0.010287893	
t Critical two-tail	2.200985159	

	(His + ATIII) + Xa	*(Hep + ATIII) + Xa*
Mean	2.52125	6.505166667
Variance	5.201023477	36.02928379
Observations	12	12
Std. Error of Mean	0.190047938	0.500203318
Std. Deviation	2.280575251	6.00243982
t Stat	-3.005276057	
P(T<=t) one-tail	0.005983233	
t Critical one-tail	1.795884814	
P(T<=t) two-tail	0.011966467	
t Critical two-tail	2.200985159	

	(His + ATIII) + Xa	*(His + CSB + ATIII) + Xa*
Mean	2.52125	15.065
Variance	5.201023477	31.11953455
Observations	12	12
Std. Error of Mean	0.190047938	0.464874046
Std. Deviation	2.280575251	5.578488554
t Stat	-7.803397267	
P(T<=t) one-tail	4.13459E-06	
t Critical one-tail	1.795884814	
P(T<=t) two-tail	8.26918E-06	
t Critical two-tail	2.200985159	

	(His + ATIII) + Xa	*(His + Hep + ATIII) + Xa*
Mean	2.52125	10.2805
Variance	5.201023477	71.95929755
Observations	12	12
Std. Error of Mean	0.190047938	0.706906885
Std. Deviation	2.280575251	8.48288262
t Stat	-3.913256431	
P(T<=t) one-tail	0.00121037	
t Critical one-tail	1.795884814	
P(T<=t) two-tail	0.00242074	
t Critical two-tail	2.200985159	

	(His + ATIII) + Xa	*(His + CSC + ATIII) + Xa*
Mean	2.52125	13.8245
Variance	5.201023477	36.16780482
Observations	12	12
Std. Error of Mean	0.190047938	0.501163956
Std. Deviation	2.280575251	6.013967477
t Stat	-6.352280523	
P(T<=t) one-tail	2.71387E-05	
t Critical one-tail	1.795884814	
P(T<=t) two-tail	5.42774E-05	
t Critical two-tail	2.200985159	

Data analysis for the 1° Xaβ – ATIII complex across all gels

Gel	Complex	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1	1° Xaβ - ATIII	44.199	5.486	35.185	36.035	1.384	34.604	31.379
2	1° Xaβ - ATIII	18.691	15.602	44.427	40.102	5.009	34.16	30.046
3	1° Xaβ - ATIII	15.061	7.888	4.81	29.731	22.481	34.685	22.117
4	1° Xaβ - ATIII	5.397	2.481	27.508	19.537	28.922	23.11	14.544
5	1° Xaβ - ATIII	9.537	5.378	15.903	26.639	9.442	25.555	24.181
6	1° Xaβ - ATIII	10.325	8.514	14.585	25.614	10.284	24.05	23.635
7	1° Xaβ - ATIII	12.438	0.683	2.022	3.571	6.214	12.548	15.621
8	1° Xaβ - ATIII	13.188	0.967	1.451	3.915	5.574	15.178	14.675
9	1° Xaβ - ATIII	19.589	0.773	3.003	1.119	8.951	12.771	7.291
10	1° Xaβ - ATIII	17.559	1.047	1.767	1.256	9.501	12.129	15.42
11	1° Xaβ - ATIII	14.009	2.584	4.431	9.242	14.883	23.134	30.599
12	1° Xaβ - ATIII	14.648	1.295	3.328	9.569	15.005	24.517	27.614

t-test table for 1° Xaβ – ATIII complex across all gels as compared to the control lane, ATIII + Xa

	ATIII + Xa	*(His + ATIII) + Xa*
Mean	16.22008333	4.3915
Variance	93.71124917	20.30288318
Observations	12	12
Std. Error of Mean	0.806704756	0.37548934
Std. Deviation	9.680457075	4.505872078
t Stat	4.037713086	
P(T<=t) one-tail	0.000978046	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.001956093	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSA + ATIII) + Xa*
Mean	16.22008333	11.47083333
Variance	93.71124917	61.05101652
Observations	12	12
Std. Error of Mean	0.806704756	0.651126249
Std. Deviation	9.680457075	7.813514991
t Stat	1.0632036	
P(T<=t) one-tail	0.15523666	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.31047332	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(Hep + ATIII) + Xa*
Mean	16.22008333	13.20166667
Variance	93.71124917	219.5301679
Observations	12	12
Std. Error of Mean	0.806704756	1.234712539
Std. Deviation	9.680457075	14.81655047
t Stat	0.726664755	
P(T<=t) one-tail	0.241304449	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.482608899	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSB + ATIII) + Xa*
Mean	16.22008333	23.03675
Variance	93.71124917	72.62930675
Observations	12	12
Std. Error of Mean	0.806704756	0.710190246
Std. Deviation	9.680457075	8.522282954
t Stat	-2.281691922	
P(T<=t) one-tail	0.021703463	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.043406926	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + Hep + ATIII) + Xa*
Mean	16.22008333	17.19416667
Variance	93.71124917	199.8407778
Observations	12	12
Std. Error of Mean	0.806704756	1.178042096
Std. Deviation	9.680457075	14.13650515
t Stat	-0.234627677	
P(T<=t) one-tail	0.409403933	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.818807866	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSC + ATIII) + Xa*
Mean	16.22008333	21.42683333
Variance	93.71124917	61.02320652
Observations	12	12
Std. Error of Mean	0.806704756	0.650977932
Std. Deviation	9.680457075	7.81173518
t Stat	-1.799970982	
P(T<=t) one-tail	0.049660351	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.099320702	
t Critical two-tail	2.20098516	

t-test table for 1° Xaβ – ATIII complex across all gels as compared to the histone lane, (His + ATIII) + Xa

	(His + ATIII) + Xa	*ATIII + Xa*
Mean	4.3915	16.22008333
Variance	20.30288318	93.71124917
Observations	12	12
Std. Error of Mean	0.37548934	0.806704756
Std. Deviation	4.505872078	9.680457075
t Stat	-4.037713086	
P(T<=t) one-tail	0.000978046	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.001956093	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSA + ATIII) + Xa*
Mean	4.3915	11.47083333
Variance	20.30288318	61.05101652
Observations	12	12
Std. Error of Mean	0.37548934	0.651126249
Std. Deviation	4.505872078	7.813514991
t Stat	-2.58662567	
P(T<=t) one-tail	0.012644941	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.025289882	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(Hep + ATIII) + Xa*
Mean	4.3915	13.20166667
Variance	20.30288318	219.5301679
Observations	12	12
Std. Error of Mean	0.37548934	1.234712539
Std. Deviation	4.505872078	14.81655047
t Stat	-2.546490739	
P(T<=t) one-tail	0.013582142	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.027164284	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSB + ATIII) + Xa*
Mean	4.3915	23.03675
Variance	20.30288318	72.62930675
Observations	12	12
Std. Error of Mean	0.37548934	0.710190246
Std. Deviation	4.505872078	8.522282954
t Stat	-10.93624968	
P(T<=t) one-tail	1.50099E-07	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	3.00198E-07	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + Hep + ATIII) + Xa*
Mean	4.3915	17.19416667
Variance	20.30288318	199.8407778
Observations	12	12
Std. Error of Mean	0.37548934	1.178042096
Std. Deviation	4.505872078	14.13650515
t Stat	-4.230262344	
P(T<=t) one-tail	0.000705897	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.001411795	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSC + ATIII) + Xa*
Mean	4.3915	21.42683333
Variance	20.30288318	61.02320652
Observations	12	12
Std. Error of Mean	0.37548934	0.650977932
Std. Deviation	4.505872078	7.81173518
t Stat	-9.081485468	
P(T<=t) one-tail	9.59526E-07	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	1.91905E-06	
t Critical two-tail	2.20098516	

Data analysis for 2° Xaα – ATIII complex across all gels

Gel	Complex	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1	2° Xaα - ATIII	4.164	0	0.443	1.671	0	2.897	1.413
2	2° Xaα - ATIII	3.146	0	0.301	0.923	0	1.639	3.128
3	2° Xaα - ATIII	0.673	1.397	0.797	0.855	1.575	1.013	0
4	2° Xaα - ATIII	2.124	0.617	2.188	1.865	0.66	0	2.518
5	2° Xaα - ATIII	2.241	0	1.468	0.737	0	0.784	1.488
6	2° Xaα - ATIII	0.773	0	0.718	1.476	0	1.926	2.21
7	2° Xaα - ATIII	1.728	0	2.142	0	0.826	0	0
8	2° Xaα - ATIII	0.676	0	0	0.761	1.413	1.463	1.558
9	2° Xaα - ATIII	0.973	0	1.051	0	1.468	0	0.46
10	2° Xaα - ATIII	1.365	0	0	0	1.532	1.526	1.566
11	2° Xaα - ATIII	0.634	0	0	0	0	1.691	0.893
12	2° Xaα - ATIII	1.782	0	0	0.943	0	0	2.388

t-test table for 2° Xaα – ATIII complex across all gels as compared to the control lane, ATIII + Xa

	ATIII + Xa	*(His + ATIII) + Xa*
Mean	1.689916667	0.167833333
Variance	1.216427356	0.181298333
Observations	12	12
Std. Error of Mean	0.091909805	0.035482618
Std. Deviation	1.102917656	0.42579142
t Stat	4.15888616	
P(T<=t) one-tail	0.000796172	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.001592344	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSA + ATIII) + Xa*
Mean	1.689916667	0.622833333
Variance	1.216427356	0.494732879
Observations	12	12
Std. Error of Mean	0.091909805	0.058614375
Std. Deviation	1.102917656	0.703372504
t Stat	2.344012387	
P(T<=t) one-tail	0.019448019	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.038896037	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(Hep + ATIII) + Xa*
Mean	1.689916667	0.759
Variance	1.216427356	0.650885818
Observations	12	12
Std. Error of Mean	0.091909805	0.067231246
Std. Deviation	1.102917656	0.806774949
t Stat	2.504583785	
P(T<=t) one-tail	0.014633466	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.029266933	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSB + ATIII) + Xa*
Mean	1.689916667	1.07825
Variance	1.216427356	0.891480023
Observations	12	12
Std. Error of Mean	0.091909805	0.07868185
Std. Deviation	1.102917656	0.944182198
t Stat	1.738679036	
P(T<=t) one-tail	0.054981272	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.109962544	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + Hep + ATIII) + Xa*
Mean	1.689916667	0.76925
Variance	1.216427356	0.449326205
Observations	12	12
Std. Error of Mean	0.091909805	0.055859832
Std. Deviation	1.102917656	0.670317988
t Stat	3.226046001	
P(T<=t) one-tail	0.004035895	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.00807179	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSC + ATIII) + Xa*
Mean	1.689916667	1.4685
Variance	1.216427356	0.993902455
Observations	12	12
Std. Error of Mean	0.091909805	0.08307888
Std. Deviation	1.102917656	0.996946566
t Stat	0.663001442	
P(T<=t) one-tail	0.260490332	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.520980664	
t Critical two-tail	2.20098516	

t-test table for 2° Xaα – ATIII complex across all gels as compared to the histone lane, (His + ATIII) + Xa

	(His + ATIII) + Xa	*ATIII + Xa*
Mean	0.167833333	1.689916667
Variance	0.181298333	1.216427356
Observations	12	12
Std. Error of Mean	0.035482618	0.091909805
Std. Deviation	0.42579142	1.102917656
t Stat	-4.15888616	
P(T<=t) one-tail	0.000796172	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.001592344	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSA + ATIII) + Xa*
Mean	0.167833333	0.622833333
Variance	0.181298333	0.494732879
Observations	12	12
Std. Error of Mean	0.035482618	0.058614375
Std. Deviation	0.42579142	0.703372504
t Stat	-2.40359896	
P(T<=t) one-tail	0.017504442	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.035008885	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(Hep + ATIII) + Xa*
Mean	0.167833333	0.759
Variance	0.181298333	0.650885818
Observations	12	12
Std. Error of Mean	0.035482618	0.067231246
Std. Deviation	0.42579142	0.806774949
t Stat	-2.516506136	
P(T<=t) one-tail	0.014326511	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.028653023	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSB + ATIII) + Xa*
Mean	0.167833333	1.07825
Variance	0.181298333	0.891480023
Observations	12	12
Std. Error of Mean	0.035482618	0.07868185
Std. Deviation	0.42579142	0.944182198
t Stat	-2.8666995	
P(T<=t) one-tail	0.007665891	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.015331783	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + Hep + ATIII) + Xa*
Mean	0.167833333	0.76925
Variance	0.181298333	0.449326205
Observations	12	12
Std. Error of Mean	0.035482618	0.055859832
Std. Deviation	0.42579142	0.670317988
t Stat	-2.988706945	
P(T<=t) one-tail	0.006163077	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.012326155	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSC + ATIII) + Xa*
Mean	0.167833333	1.4685
Variance	0.181298333	0.993902455
Observations	12	12
Std. Error of Mean	0.035482618	0.08307888
Std. Deviation	0.42579142	0.996946566
t Stat	-3.767194296	
P(T<=t) one-tail	0.001557759	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.003115517	
t Critical two-tail	2.20098516	

Data analysis for 2° Xaβ – ATIII complex across all gels

Gel	Complex	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1	2° Xaβ - ATIII	3.129	0	0.718	1.626	0	2.481	2.012
2	2° Xaβ - ATIII	3.638	0	1.105	3.513	0	3.427	3.724
3	2° Xaβ - ATIII	1.995	2.393	0.765	1.172	3.706	1.68	0
4	2° Xaβ - ATIII	3.714	1.522	3.905	3.387	0.873	0	2.692
5	2° Xaβ - ATIII	1.972	0	1.335	1.663	0	2.225	1.289
6	2° Xaβ - ATIII	0.531	0	0.97	1.572	0	1.329	1.56
7	2° Xaβ - ATIII	1.404	0	1.849	0	0.621	0	0
8	2° Xaβ - ATIII	0.76	0	0	0.783	0.925	1.595	1.275
9	2° Xaβ - ATIII	0.783	0	0.92	0	0.99	0	0.48
10	2° Xaβ - ATIII	1.479	0	0	0	1.475	1.42	1.566
11	2° Xaβ - ATIII	0.255	0	0	0	0	1.549	1.281
12	2° Xaβ - ATIII	1.628	0	0	1.495	0	0	1.5

t-test table for 2° Xaβ – ATIII complex across all gels as compared to the control lane, ATIII + Xa

	ATIII + Xa	*(His + ATIII) + Xa*
Mean	1.774	0.32625
Variance	1.388190364	0.615060386
Observations	12	12
Std. Error of Mean	0.098184575	0.065354821
Std. Deviation	1.178214906	0.784257857
t Stat	4.284351785	
P(T<=t) one-tail	0.000644645	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.001289289	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSA + ATIII) + Xa*
Mean	1.774	0.715833333
Variance	1.388190364	1.158595242
Observations	12	12
Std. Error of Mean	0.098184575	0.089698385
Std. Deviation	1.178214906	1.076380622
t Stat	2.291111144	
P(T<=t) one-tail	0.021347096	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.042694191	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(Hep + ATIII) + Xa*
Mean	1.774	0.963916667
Variance	1.388190364	1.219045538
Observations	12	12
Std. Error of Mean	0.098184575	0.092008663
Std. Deviation	1.178214906	1.104103952
t Stat	2.664704893	
P(T<=t) one-tail	0.011000539	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.022001078	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSB + ATIII) + Xa*
Mean	1.774	1.308833333
Variance	1.388190364	1.25838597
Observations	12	12
Std. Error of Mean	0.098184575	0.093481503
Std. Deviation	1.178214906	1.121778039
t Stat	1.192903508	
P(T<=t) one-tail	0.129001493	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.258002986	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + Hep + ATIII) + Xa*
Mean	1.774	1.267583333
Variance	1.388190364	1.512186811
Observations	12	12
Std. Error of Mean	0.098184575	0.102475838
Std. Deviation	1.178214906	1.229710051
t Stat	2.372232899	
P(T<=t) one-tail	0.018502796	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.037005592	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSC + ATIII) + Xa*
Mean	1.774	1.44825
Variance	1.388190364	1.117137295
Observations	12	12
Std. Error of Mean	0.098184575	0.08807893
Std. Deviation	1.178214906	1.056947158
t Stat	1.187287365	
P(T<=t) one-tail	0.130060476	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.260120951	
t Critical two-tail	2.20098516	

t-test table for 2° Xaβ – ATIII complex across all gels as compared to the histone lane, (His + ATIII) + Xa

	(His + ATIII) + Xa	*ATIII + Xa*
Mean	0.32625	1.774
Variance	0.615060386	1.388190364
Observations	12	12
Std. Error of Mean	0.065354821	0.098184575
Std. Deviation	0.784257857	1.178214906
t Stat	-4.284351785	
P(T<=t) one-tail	0.000644645	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.001289289	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSA + ATIII) + Xa*
Mean	0.32625	0.715833333
Variance	0.615060386	1.158595242
Observations	12	12
Std. Error of Mean	0.065354821	0.089698385
Std. Deviation	0.784257857	1.076380622
t Stat	-2.060045553	
P(T<=t) one-tail	0.031930182	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.063860364	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(Hep + ATIII) + Xa*
Mean	0.32625	0.963916667
Variance	0.615060386	1.219045538
Observations	12	12
Std. Error of Mean	0.065354821	0.092008663
Std. Deviation	0.784257857	1.104103952
t Stat	-2.099687726	
P(T<=t) one-tail	0.029816275	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.05963255	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSB + ATIII) + Xa*
Mean	0.32625	1.308833333
Variance	0.615060386	1.25838597
Observations	12	12
Std. Error of Mean	0.065354821	0.093481503
Std. Deviation	0.784257857	1.121778039
t Stat	-2.363417572	
P(T<=t) one-tail	0.018793177	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.037586354	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + Hep + ATIII) + Xa*
Mean	0.32625	1.267583333
Variance	0.615060386	1.512186811
Observations	12	12
Std. Error of Mean	0.065354821	0.102475838
Std. Deviation	0.784257857	1.229710051
t Stat	-2.592298135	
P(T<=t) one-tail	0.012517719	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.025035438	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSC + ATIII) + Xa*
Mean	0.32625	1.44825
Variance	0.615060386	1.117137295
Observations	12	12
Std. Error of Mean	0.065354821	0.08807893
Std. Deviation	0.784257857	1.056947158
t Stat	-2.73594896	
P(T<=t) one-tail	0.009685616	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.019371232	
t Critical two-tail	2.20098516	

Data analysis for 3° Xaα – ATIII complex across all gels

Gel	Complex	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1	3° Xaα - ATIII	1.832	4.549	3.78	1.261	3.263	1.885	1.83
2	3° Xaα - ATIII	1.45	6.855	1.929	1.341	2.48	3.185	1.7
3	3° Xaα - ATIII	6.274	5.854	5.21	3.105	3.321	3.388	2.134
4	3° Xaα - ATIII	6.827	3.407	2.65	4.895	2.55	3.038	6.096
5	3° Xaα - ATIII	5.561	9.497	6.715	3.12	11.824	1.279	2.33
6	3° Xaα - ATIII	5.722	9.918	7.368	2.512	9.532	1.662	3.046
7	3° Xaα - ATIII	8.41	10.538	5.044	6.009	8.965	5.181	4.865
8	3° Xaα - ATIII	10.496	11.25	4.988	6.055	8.389	3.876	6.086
9	3° Xaα - ATIII	8.349	9.438	5.469	3.62	10.964	4.319	2.55
10	3° Xaα - ATIII	8.789	8.156	6.966	3.817	10.78	5.375	7.486
11	3° Xaα - ATIII	10.387	11.044	7.204	6.7	10.925	3.945	3.184
12	3° Xaα - ATIII	9.924	9.736	4.977	7.541	9.243	2.525	2.784

t-test table for 3° Xaα – ATIII complex across all gels as compared to the control lane, ATIII + Xa

	ATIII + Xa	*(His + ATIII) + Xa*
Mean	7.00175	8.3535
Variance	9.183970932	6.792484818
Observations	12	12
Std. Error of Mean	0.252542226	0.217186633
Std. Deviation	3.030506712	2.606239593
t Stat	-1.900306903	
P(T<=t) one-tail	0.041953421	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.083906842	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSA + ATIII) + Xa*
Mean	7.00175	7.686333333
Variance	9.183970932	13.45376224
Observations	12	12
Std. Error of Mean	0.252542226	0.305661421
Std. Deviation	3.030506712	3.667937055
t Stat	-0.808911282	
P(T<=t) one-tail	0.217856626	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.435713253	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(Hep + ATIII) + Xa*
Mean	7.00175	5.191666667
Variance	9.183970932	3.049018061
Observations	12	12
Std. Error of Mean	0.252542226	0.145511981
Std. Deviation	3.030506712	1.746143769
t Stat	2.397867262	
P(T<=t) one-tail	0.017682887	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.035365774	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSB + ATIII) + Xa*
Mean	7.00175	3.304833333
Variance	9.183970932	1.735419606
Observations	12	12
Std. Error of Mean	0.252542226	0.109779438
Std. Deviation	3.030506712	1.317353258
t Stat	4.90768679	
P(T<=t) one-tail	0.000232944	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.000465889	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + Hep + ATIII) + Xa*
Mean	7.00175	4.164666667
Variance	9.183970932	4.282117333
Observations	12	12
Std. Error of Mean	0.252542226	0.172443979
Std. Deviation	3.030506712	2.069327749
t Stat	6.43833114	
P(T<=t) one-tail	2.40945E-05	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	4.8189E-05	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSC + ATIII) + Xa*
Mean	7.00175	3.67425
Variance	9.183970932	3.799869114
Observations	12	12
Std. Error of Mean	0.252542226	0.162443775
Std. Deviation	3.030506712	1.949325297
t Stat	4.48451915	
P(T<=t) one-tail	0.000462244	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.000924488	
t Critical two-tail	2.20098516	

t-test table for 3° Xaα – ATIII complex across all gels as compared to the histone lane, (His + ATIII) + Xa

	(His + ATIII) + Xa	*ATIII + Xa*
Mean	8.3535	7.00175
Variance	6.792484818	9.183970932
Observations	12	12
Std. Error of Mean	0.217186633	0.252542226
Std. Deviation	2.606239593	3.030506712
t Stat	1.900306903	
P(T<=t) one-tail	0.041953421	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.083906842	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSA + ATIII) + Xa*
Mean	8.3535	7.686333333
Variance	6.792484818	13.45376224
Observations	12	12
Std. Error of Mean	0.217186633	0.305661421
Std. Deviation	2.606239593	3.667937055
t Stat	1.102947054	
P(T<=t) one-tail	0.146797721	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.293595442	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(Hep + ATIII) + Xa*
Mean	8.3535	5.191666667
Variance	6.792484818	3.049018061
Observations	12	12
Std. Error of Mean	0.217186633	0.145511981
Std. Deviation	2.606239593	1.746143769
t Stat	5.471497339	
P(T<=t) one-tail	9.72081E-05	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.000194416	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSB + ATIII) + Xa*
Mean	8.3535	3.304833333
Variance	6.792484818	1.735419606
Observations	12	12
Std. Error of Mean	0.217186633	0.109779438
Std. Deviation	2.606239593	1.317353258
t Stat	6.652835927	
P(T<=t) one-tail	1.79869E-05	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	3.59738E-05	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + Hep + ATIII) + Xa*
Mean	8.3535	4.164666667
Variance	6.792484818	4.282117333
Observations	12	12
Std. Error of Mean	0.217186633	0.172443979
Std. Deviation	2.606239593	2.069327749
t Stat	6.222924105	
P(T<=t) one-tail	3.25141E-05	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	6.50282E-05	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSC + ATIII) + Xa*
Mean	8.3535	3.67425
Variance	6.792484818	3.799869114
Observations	12	12
Std. Error of Mean	0.217186633	0.162443775
Std. Deviation	2.606239593	1.949325297
t Stat	5.179464185	
P(T<=t) one-tail	0.000152002	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.000304004	
t Critical two-tail	2.20098516	

Data analysis for 3° Xaβ – ATIII complex across all gels

Gel	Complex	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1	3° Xaβ - ATIII	4.139	4.292	5.934	2.392	3.715	2.381	2.524
2	3° Xaβ - ATIII	3.642	9.017	3.376	1.686	3.955	3.951	3.535
3	3° Xaβ - ATIII	25.925	29.55	17.366	5.882	20.083	6.222	5.975
4	3° Xaβ - ATIII	29.697	13.17	8.986	22.146	6.622	10.509	25.785
5	3° Xaβ - ATIII	7.183	22.125	10.435	4.261	15.254	2.786	2.463
6	3° Xaβ - ATIII	8.313	20.143	12.101	3.613	12.328	1.325	2.682
7	3° Xaβ - ATIII	12.788	25.711	15.276	17.141	13.21	7.312	4.704
8	3° Xaβ - ATIII	14.755	25.642	18.851	15.626	13.675	7.031	7.847
9	3° Xaβ - ATIII	14.526	21.076	22.126	14.571	19.959	7.562	4.786
10	3° Xaβ - ATIII	15.593	24.018	23.815	15.816	18.458	8.825	12.028
11	3° Xaβ - ATIII	18.249	29.213	23.13	19.187	15.932	7.787	5.602
12	3° Xaβ - ATIII	16.77	30.156	20.624	17.524	12.754	5.64	3.794

t-test table for 3° Xaβ – ATIII complex across all gels as compared to the control lane, ATIII + Xa

	ATIII + Xa	*(His + ATIII) + Xa*
Mean	14.29833333	21.17608333
Variance	63.76859442	69.3573139
Observations	12	12
Std. Error of Mean	0.665460338	0.694008655
Std. Deviation	7.985524054	8.32810386
t Stat	-2.777184243	
P(T<=t) one-tail	0.008997151	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.017994302	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSA + ATIII) + Xa*
Mean	14.29833333	12.99541667
Variance	63.76859442	31.97974372
Observations	12	12
Std. Error of Mean	0.665460338	0.620959627
Std. Deviation	7.985524054	7.451515528
t Stat	0.570569545	
P(T<=t) one-tail	0.289879934	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.579759869	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(Hep + ATIII) + Xa*
Mean	14.29833333	15.16833333
Variance	63.76859442	48.09963915
Observations	12	12
Std. Error of Mean	0.665460338	0.577949195
Std. Deviation	7.985524054	6.935390339
t Stat	-0.375564516	
P(T<=t) one-tail	0.357191119	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.714382237	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSB + ATIII) + Xa*
Mean	14.29833333	5.94425
Variance	63.76859442	7.850163114
Observations	12	12
Std. Error of Mean	0.665460338	0.233484521
Std. Deviation	7.985524054	2.801814254
t Stat	4.754506089	
P(T<=t) one-tail	0.000297676	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.000595351	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + Hep + ATIII) + Xa*
Mean	14.29833333	11.65375
Variance	63.76859442	55.52508366
Observations	12	12
Std. Error of Mean	0.665460338	0.620959627
Std. Deviation	7.985524054	7.451515528
t Stat	1.459271671	
P(T<=t) one-tail	0.086224741	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.172449481	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSC + ATIII) + Xa*
Mean	14.29833333	6.810416667
Variance	63.76859442	43.01548063
Observations	12	12
Std. Error of Mean	0.665460338	0.546551567
Std. Deviation	7.985524054	6.558618805
t Stat	4.624598006	
P(T<=t) one-tail	0.000367423	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.000734845	
t Critical two-tail	2.20098516	

t-test table for 3° Xaβ – ATIII complex across all gels as compared to the histone lane, (His + ATIII) + Xa

	(His + ATIII) + Xa	*ATIII + Xa*
Mean	21.17608333	14.29833333
Variance	69.3573139	63.76859442
Observations	12	12
Std. Error of Mean	0.694008655	0.665460338
Std. Deviation	8.32810386	7.985524054
t Stat	2.777184243	
P(T<=t) one-tail	0.008997151	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.017994302	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSA + ATIII) + Xa*
Mean	21.17608333	12.99541667
Variance	69.3573139	31.97974372
Observations	12	12
Std. Error of Mean	0.694008655	0.620959627
Std. Deviation	8.32810386	7.451515528
t Stat	5.672661325	
P(T<=t) one-tail	7.19481E-05	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.000143896	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(Hep + ATIII) + Xa*
Mean	21.17608333	15.16833333
Variance	69.3573139	48.09963915
Observations	12	12
Std. Error of Mean	0.694008655	0.577949195
Std. Deviation	8.32810386	6.935390339
t Stat	4.342077266	
P(T<=t) one-tail	0.000585368	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.001170737	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSB + ATIII) + Xa*
Mean	21.17608333	5.94425
Variance	69.3573139	7.850163114
Observations	12	12
Std. Error of Mean	0.694008655	0.233484521
Std. Deviation	8.32810386	2.801814254
t Stat	6.695923629	
P(T<=t) one-tail	1.69733E-05	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	3.39466E-05	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + Hep + ATIII) + Xa*
Mean	21.17608333	11.65375
Variance	69.3573139	55.52508366
Observations	12	12
Std. Error of Mean	0.694008655	0.620959627
Std. Deviation	8.32810386	7.451515528
t Stat	4.00606807	
P(T<=t) one-tail	0.001032334	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.002064667	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSC + ATIII) + Xa*
Mean	21.17608333	6.810416667
Variance	69.3573139	43.01548063
Observations	12	12
Std. Error of Mean	0.694008655	0.546551567
Std. Deviation	8.32810386	6.558618805
t Stat	4.438750432	
P(T<=t) one-tail	0.000498537	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.000997074	
t Critical two-tail	2.20098516	

Data analysis for ATIII-M complex across all gels

Gel	Complex	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1	ATIII-M	3.085	2.573	7.952	3.152	1.429	3.323	1.73
2	ATIII-M	1.062	3.872	4.267	4.731	3.338	1.827	1.715
3	ATIII-M	8.268	3.006	4.226	1.956	3.579	9.445	2.433
4	ATIII-M	4.988	3.602	2.35	3.644	9.153	4.146	2.516
5	ATIII-M	2.694	3.599	9.875	5.836	4.06	4.054	3.268
6	ATIII-M	5.744	2.766	9.664	7.052	2.873	3.838	2.626
7	ATIII-M	6.284	3.33	6.674	4.771	3.781	2.087	2.663
8	ATIII-M	2.371	2.085	6.318	6.631	2.82	2.744	2.11
9	ATIII-M	3.475	3.027	9.172	4.452	3.028	3.318	1.929
10	ATIII-M	4.257	3.337	8.719	5.605	3.214	2.813	2.494
11	ATIII-M	3.978	3.543	5.72	8.072	3.538	4.643	2.38
12	ATIII-M	3.242	4.027	6.499	5.463	3.306	4.148	3.164

t-test table for ATIII-M complex across all gels as compared to the control lane, ATIII + Xa

	ATIII + Xa	*(His + ATIII) + Xa*
Mean	4.120666667	3.230583333
Variance	3.820045697	0.317335174
Observations	12	12
Std. Error of Mean	0.162874477	0.046943759
Std. Deviation	1.954493719	0.563325105
t Stat	1.466762615	
P(T<=t) one-tail	0.08521945	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.170438899	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSA + ATIII) + Xa*
Mean	4.120666667	3.676583333
Variance	3.820045697	3.408682265
Observations	12	12
Std. Error of Mean	0.162874477	0.142968511
Std. Deviation	1.954493719	1.715622131
t Stat	0.643060526	
P(T<=t) one-tail	0.266680223	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.533360446	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(Hep + ATIII) + Xa*
Mean	4.120666667	6.786333333
Variance	3.820045697	5.694654061
Observations	12	12
Std. Error of Mean	0.162874477	0.198862286
Std. Deviation	1.954493719	2.386347431
t Stat	-2.788128227	
P(T<=t) one-tail	0.008822756	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.017645513	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSB + ATIII) + Xa*
Mean	4.120666667	3.8655
Variance	3.820045697	3.837111545
Observations	12	12
Std. Error of Mean	0.162874477	0.163237888
Std. Deviation	1.954493719	1.958854651
t Stat	0.548530682	
P(T<=t) one-tail	0.297142731	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.594285462	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + Hep + ATIII) + Xa*
Mean	4.120666667	5.11375
Variance	3.820045697	2.943359295
Observations	12	12
Std. Error of Mean	0.162874477	0.142968511
Std. Deviation	1.954493719	1.715622131
t Stat	-1.142313107	
P(T<=t) one-tail	0.138789482	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.277578965	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSC + ATIII) + Xa*
Mean	4.120666667	2.419
Variance	3.820045697	0.245534545
Observations	12	12
Std. Error of Mean	0.162874477	0.041292869
Std. Deviation	1.954493719	0.495514425
t Stat	3.132155925	
P(T<=t) one-tail	0.004770453	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.009540907	
t Critical two-tail	2.20098516	

t-test table for ATIII-M complex across all gels as compared to the histone lane, (His + ATIII) + Xa

	(His + ATIII) + Xa	*ATIII + Xa*
Mean	3.230583333	4.120666667
Variance	0.317335174	3.820045697
Observations	12	12
Std. Error of Mean	0.046943759	0.162874477
Std. Deviation	0.563325105	1.954493719
t Stat	-1.466762615	
P(T<=t) one-tail	0.08521945	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.170438899	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSA + ATIII) + Xa*
Mean	3.230583333	3.676583333
Variance	0.317335174	3.408682265
Observations	12	12
Std. Error of Mean	0.046943759	0.142968511
Std. Deviation	0.563325105	1.715622131
t Stat	-0.907940547	
P(T<=t) one-tail	0.191684951	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.383369901	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(Hep + ATIII) + Xa*
Mean	3.230583333	6.786333333
Variance	0.317335174	5.694654061
Observations	12	12
Std. Error of Mean	0.046943759	0.198862286
Std. Deviation	0.563325105	2.386347431
t Stat	-4.746643702	
P(T<=t) one-tail	0.000301472	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.000602945	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSB + ATIII) + Xa*
Mean	3.230583333	3.8655
Variance	0.317335174	3.837111545
Observations	12	12
Std. Error of Mean	0.046943759	0.163237888
Std. Deviation	0.563325105	1.958854651
t Stat	-1.068781888	
P(T<=t) one-tail	0.154030559	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.308061117	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + Hep + ATIII) + Xa*
Mean	3.230583333	5.11375
Variance	0.317335174	2.943359295
Observations	12	12
Std. Error of Mean	0.046943759	0.142968511
Std. Deviation	0.563325105	1.715622131
t Stat	-3.631984063	
P(T<=t) one-tail	0.001971473	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.003942945	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSC + ATIII) + Xa*
Mean	3.230583333	2.419
Variance	0.317335174	0.245534545
Observations	12	12
Std. Error of Mean	0.046943759	0.041292869
Std. Deviation	0.563325105	0.495514425
t Stat	4.94519301	
P(T<=t) one-tail	0.000219481	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.000438961	
t Critical two-tail	2.20098516	

Data analysis for ATIII band across all gels

Gel	Complex	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1	ATIII	17.538	64.59	15.889	23.264	79.93	28.657	33.831
2	ATIII	9.516	39.682	14.569	23.431	71.15	29.374	35.427
3	ATIII	15.164	25.378	13.874	20.209	18.775	12.701	17.065
4	ATIII	25.082	24.065	17.244	20.735	18.232	16.256	17.143
5	ATIII	10.559	35.905	21.843	20.757	33.496	26.833	24.456
6	ATIII	13.205	40.918	24.625	22.833	35.211	29.662	23.863
7	ATIII	14.442	14.635	12.294	13.422	14.068	9.99	27.272
8	ATIII	17.47	16.192	14.579	15.313	13.894	13.533	12.363
9	ATIII	20.024	22.199	19.28	9.605	21.966	12.757	12.293
10	ATIII	20.184	21.938	21.271	11.816	25.603	27.206	29.243
11	ATIII	22.389	21.823	21.582	24.439	32.511	29.081	31.814
12	ATIII	21.859	24.161	22.953	23	27.196	30.353	31.662

t-test table for ATIII band across all gels as compared to the control lane, ATIII + Xa

	ATIII + Xa	*(His + ATIII) + Xa*
Mean	17.286	29.2905
Variance	23.50856109	195.3072894
Observations	12	12
Std. Error of Mean	0.404046899	1.164603203
Std. Deviation	4.848562786	13.97523844
t Stat	-2.549959367	
P(T<=t) one-tail	0.013498521	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.026997043	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSA + ATIII) + Xa*
Mean	17.286	32.66933333
Variance	23.50856109	455.9967693
Observations	12	12
Std. Error of Mean	0.404046899	1.779506738
Std. Deviation	4.848562786	21.35408086
t Stat	-2.263107268	
P(T<=t) one-tail	0.022423441	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.044846881	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(Hep + ATIII) + Xa*
Mean	17.286	18.33358333
Variance	23.50856109	16.82817136
Observations	12	12
Std. Error of Mean	0.404046899	0.341851285
Std. Deviation	4.848562786	4.10221542
t Stat	-0.641073105	
P(T<=t) one-tail	0.267301766	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.534603533	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSB + ATIII) + Xa*
Mean	17.286	22.20025
Variance	23.50856109	68.01074893
Observations	12	12
Std. Error of Mean	0.404046899	0.687238581
Std. Deviation	4.848562786	8.246862975
t Stat	-1.700658988	
P(T<=t) one-tail	0.058534842	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.117069685	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + Hep + ATIII) + Xa*
Mean	17.286	19.06866667
Variance	23.50856109	26.37967042
Observations	12	12
Std. Error of Mean	0.404046899	0.428009528
Std. Deviation	4.848562786	5.136114331
t Stat	-0.823144403	
P(T<=t) one-tail	0.21395542	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.427910841	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSC + ATIII) + Xa*
Mean	17.286	24.70266667
Variance	23.50856109	67.91571588
Observations	12	12
Std. Error of Mean	0.404046899	0.686758266
Std. Deviation	4.848562786	8.241099191
t Stat	-2.493754377	
P(T<=t) one-tail	0.014917856	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.029835711	
t Critical two-tail	2.20098516	

t-test table for ATIII band across all gels as compared to the histone lane, (His + ATIII) + Xa

	(His + ATIII) + Xa	*ATIII + Xa*
Mean	29.2905	17.286
Variance	195.3072894	23.50856109
Observations	12	12
Std. Error of Mean	1.164603203	0.404046899
Std. Deviation	13.97523844	4.848562786
t Stat	2.549959367	
P(T<=t) one-tail	0.013498521	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.026997043	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSA + ATIII) + Xa*
Mean	29.2905	32.66933333
Variance	195.3072894	455.9967693
Observations	12	12
Std. Error of Mean	1.164603203	1.779506738
Std. Deviation	13.97523844	21.35408086
t Stat	-1.057685733	
P(T<=t) one-tail	0.15643668	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.31287336	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(Hep + ATIII) + Xa*
Mean	29.2905	18.33358333
Variance	195.3072894	16.82817136
Observations	12	12
Std. Error of Mean	1.164603203	0.341851285
Std. Deviation	13.97523844	4.10221542
t Stat	2.67030362	
P(T<=t) one-tail	0.010891096	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.021782192	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSB + ATIII) + Xa*
Mean	29.2905	22.20025
Variance	195.3072894	68.01074893
Observations	12	12
Std. Error of Mean	1.164603203	0.687238581
Std. Deviation	13.97523844	8.246862975
t Stat	2.130882464	
P(T<=t) one-tail	0.028246476	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.056492952	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + Hep + ATIII) + Xa*
Mean	29.2905	19.06866667
Variance	195.3072894	26.37967042
Observations	12	12
Std. Error of Mean	1.164603203	0.428009528
Std. Deviation	13.97523844	5.136114331
t Stat	2.950846441	
P(T<=t) one-tail	0.006594708	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.013189416	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSC + ATIII) + Xa*
Mean	29.2905	24.70266667
Variance	195.3072894	67.91571588
Observations	12	12
Std. Error of Mean	1.164603203	0.686758266
Std. Deviation	13.97523844	8.241099191
t Stat	1.266345074	
P(T<=t) one-tail	0.115774517	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.231549034	
t Critical two-tail	2.20098516	

Data analysis for free Xaα across all gels

Gel	Complex	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1	Xaα	4.822	3.196	3.133	3.868	4.542	4.711	3.689
2	Xaα	1.316	10.647	5.281	2.136	4.685	2.883	3.543
3	Xaα	6.247	5.621	15.732	7.371	6.906	4.904	12.719
4	Xaα	6.447	15.082	9.587	5.063	6.926	10.115	4.577
5	Xaα	23.185	6.653	8.103	4.843	8.107	5.967	8.913
6	Xaα	22.511	7.055	6.778	5.461	9.952	6.293	8.844
7	Xaα	14.342	17.764	24.031	23.686	22.594	27.365	10.808
8	Xaα	13.99	17.012	24.725	24.758	24.389	20.798	21.791
9	Xaα	8.161	13.41	16.566	29.121	12.432	24.473	5.861
10	Xaα	6.601	12.95	18.598	26.773	10.029	6.181	5.666
11	Xaα	9.136	10.704	15.678	12.412	5.446	2.505	1.569
12	Xaα	9.418	9.912	17.123	12.717	10.144	6.297	5.459

t-test table for free Xaα across all gels as compared to the control lane, ATIII + Xa

	ATIII + Xa	*(His + ATIII) + Xa*
Mean	10.51466667	10.83383333
Variance	46.08332079	21.22993524
Observations	12	12
Std. Error of Mean	0.565705808	0.383966282
Std. Deviation	6.788469694	4.607595386
t Stat	-0.132401759	
P(T<=t) one-tail	0.448528699	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.897057398	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSA + ATIII) + Xa*
Mean	10.51466667	10.51266667
Variance	46.08332079	42.7109457
Observations	12	12
Std. Error of Mean	0.565705808	0.544613431
Std. Deviation	6.788469694	6.535361176
t Stat	0.000924667	
P(T<=t) one-tail	0.499639389	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.999278779	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(Hep + ATIII) + Xa*
Mean	10.51466667	13.77791667
Variance	46.08332079	50.80622208
Observations	12	12
Std. Error of Mean	0.565705808	0.593987362
Std. Deviation	6.788469694	7.127848349
t Stat	-1.190692194	
P(T<=t) one-tail	0.129417643	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.258835286	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSB + ATIII) + Xa*
Mean	10.51466667	10.20766667
Variance	46.08332079	76.92516697
Observations	12	12
Std. Error of Mean	0.565705808	0.730891612
Std. Deviation	6.788469694	8.770699343
t Stat	0.105772844	
P(T<=t) one-tail	0.458833405	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.917666811	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + Hep + ATIII) + Xa*
Mean	10.51466667	13.18408333
Variance	46.08332079	101.9561112
Observations	12	12
Std. Error of Mean	0.565705808	0.841444324
Std. Deviation	6.788469694	10.09733188
t Stat	-0.76257487	
P(T<=t) one-tail	0.230878382	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.461756763	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSC + ATIII) + Xa*
Mean	10.51466667	7.786583333
Variance	46.08332079	29.94078808
Observations	12	12
Std. Error of Mean	0.565705808	0.455984802
Std. Deviation	6.788469694	5.471817622
t Stat	1.394431449	
P(T<=t) one-tail	0.095355138	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.190710276	
t Critical two-tail	2.20098516	

t-test table for free Xaα across all gels as compared to the histone lane, (His + ATIII) + Xa

	(His + ATIII) + Xa	*ATIII + Xa*
Mean	10.83383333	10.51466667
Variance	21.22993524	46.08332079
Observations	12	12
Std. Error of Mean	0.383966282	0.565705808
Std. Deviation	4.607595386	6.788469694
t Stat	0.132401759	
P(T<=t) one-tail	0.448528699	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.897057398	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSA + ATIII) + Xa*
Mean	10.83383333	10.51266667
Variance	21.22993524	42.7109457
Observations	12	12
Std. Error of Mean	0.383966282	0.544613431
Std. Deviation	4.607595386	6.535361176
t Stat	0.243446245	
P(T<=t) one-tail	0.406070057	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.812140114	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(Hep + ATIII) + Xa*
Mean	10.83383333	13.77791667
Variance	21.22993524	50.80622208
Observations	12	12
Std. Error of Mean	0.383966282	0.593987362
Std. Deviation	4.607595386	7.127848349
t Stat	-2.033231895	
P(T<=t) one-tail	0.033439101	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.066878203	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSB + ATIII) + Xa*
Mean	10.83383333	10.20766667
Variance	21.22993524	76.92516697
Observations	12	12
Std. Error of Mean	0.383966282	0.730891612
Std. Deviation	4.607595386	8.770699343
t Stat	0.343308903	
P(T<=t) one-tail	0.368918881	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.737837762	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + Hep + ATIII) + Xa*
Mean	10.83383333	13.18408333
Variance	21.22993524	101.9561112
Observations	12	12
Std. Error of Mean	0.383966282	0.841444324
Std. Deviation	4.607595386	10.09733188
t Stat	-1.051357584	
P(T<=t) one-tail	0.157821482	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.315642964	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSC + ATIII) + Xa*
Mean	10.83383333	7.786583333
Variance	21.22993524	29.94078808
Observations	12	12
Std. Error of Mean	0.383966282	0.455984802
Std. Deviation	4.607595386	5.471817622
t Stat	1.766320305	
P(T<=t) one-tail	0.052520936	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.105041871	
t Critical two-tail	2.20098516	

Data analysis for free Xaβ across all gels

Gel	Complex	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1	Xaβ	2.181	8.674	10.649	8.084	4.705	6.854	8.119
2	Xaβ	3.735	9.107	14.128	9.358	8.385	8.588	6.975
3	Xaβ	16.622	14.492	36.043	17.656	13.75	13.577	28.109
4	Xaβ	14.578	34.803	16.127	10.838	13.119	25.584	17.355
5	Xaβ	25.494	11.763	9.971	6.117	9.227	5.565	8.869
6	Xaβ	22.491	7.237	8.956	6.085	9.731	4.822	8.608
7	Xaβ	12.636	26.372	26.771	26.895	23.971	19.472	20.251
8	Xaβ	13.477	25.949	27.599	24.456	23.146	20.028	18.213
9	Xaβ	8.067	29.612	20.523	36.234	12.052	24.28	7.919
10	Xaβ	7.283	27.978	17.9	33.292	11.025	21.238	9.733
11	Xaβ	10.335	19.701	19.682	13.009	5.383	6.391	5.873
12	Xaβ	9.555	20.286	21.955	13.739	10.446	10.338	2.579

t-test table for free Xaβ across all gels as compared to the control lane, ATIII + Xa

	ATIII + Xa	*(His + ATIII) + Xa*
Mean	12.2045	19.6645
Variance	48.49400736	87.09067482
Observations	12	12
Std. Error of Mean	0.580313657	0.777686539
Std. Deviation	6.963763879	9.332238468
t Stat	-2.092586714	
P(T<=t) one-tail	0.030184962	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.060369925	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSA + ATIII) + Xa*
Mean	12.2045	12.07833333
Variance	48.49400736	36.1868417
Observations	12	12
Std. Error of Mean	0.580313657	0.501295833
Std. Deviation	6.963763879	6.015549991
t Stat	0.053186411	
P(T<=t) one-tail	0.479268507	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.958537014	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(Hep + ATIII) + Xa*
Mean	12.2045	19.192
Variance	48.49400736	65.56489927
Observations	12	12
Std. Error of Mean	0.580313657	0.67476796
Std. Deviation	6.963763879	8.097215526
t Stat	-2.221638664	
P(T<=t) one-tail	0.024113466	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.048226932	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSB + ATIII) + Xa*
Mean	12.2045	13.89475
Variance	48.49400736	60.51170548
Observations	12	12
Std. Error of Mean	0.580313657	0.648243918
Std. Deviation	6.963763879	7.778927013
t Stat	-0.516379857	
P(T<=t) one-tail	0.307905488	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.615810977	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + Hep + ATIII) + Xa*
Mean	12.2045	17.14691667
Variance	48.49400736	111.7322621
Observations	12	12
Std. Error of Mean	0.580313657	0.880862354
Std. Deviation	6.963763879	10.57034825
t Stat	-1.196746593	
P(T<=t) one-tail	0.128280777	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.256561553	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSC + ATIII) + Xa*
Mean	12.2045	11.88358333
Variance	48.49400736	54.94586117
Observations	12	12
Std. Error of Mean	0.580313657	0.617712296
Std. Deviation	6.963763879	7.41254755
t Stat	0.129683618	
P(T<=t) one-tail	0.449578871	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.899157742	
t Critical two-tail	2.20098516	

t-test table for free Xaβ across all gels as compared to the histone lane, (His + ATIII) + Xa

	(His + ATIII) + Xa	*ATIII + Xa*
Mean	19.6645	12.2045
Variance	87.09067482	48.49400736
Observations	12	12
Std. Error of Mean	0.777686539	0.580313657
Std. Deviation	9.332238468	6.963763879
t Stat	2.092586714	
P(T<=t) one-tail	0.030184962	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.060369925	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSA + ATIII) + Xa*
Mean	19.6645	12.07833333
Variance	87.09067482	36.1868417
Observations	12	12
Std. Error of Mean	0.777686539	0.501295833
Std. Deviation	9.332238468	6.015549991
t Stat	3.247564235	
P(T<=t) one-tail	0.003884395	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.00776879	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(Hep + ATIII) + Xa*
Mean	19.6645	19.192
Variance	87.09067482	65.56489927
Observations	12	12
Std. Error of Mean	0.777686539	0.67476796
Std. Deviation	9.332238468	8.097215526
t Stat	0.168684779	
P(T<=t) one-tail	0.434553169	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.869106337	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSB + ATIII) + Xa*
Mean	19.6645	13.89475
Variance	87.09067482	60.51170548
Observations	12	12
Std. Error of Mean	0.777686539	0.648243918
Std. Deviation	9.332238468	7.778927013
t Stat	5.132376617	
P(T<=t) one-tail	0.000163548	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.000327096	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + Hep + ATIII) + Xa*
Mean	19.6645	17.14691667
Variance	87.09067482	111.7322621
Observations	12	12
Std. Error of Mean	0.777686539	0.880862354
Std. Deviation	9.332238468	10.57034825
t Stat	1.090047196	
P(T<=t) one-tail	0.149497598	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.298995195	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSC + ATIII) + Xa*
Mean	19.6645	11.88358333
Variance	87.09067482	54.94586117
Observations	12	12
Std. Error of Mean	0.777686539	0.617712296
Std. Deviation	9.332238468	7.41254755
t Stat	2.598036317	
P(T<=t) one-tail	0.012390306	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.024780612	
t Critical two-tail	2.20098516	

Data analysis for free Xaγ across all gels

Gel	Complex	Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1	Xaγ	0	0	0	0	0	0	0
2	Xaγ	0	0	0	0	0	0	0
3	Xaγ	0	0	0	1.884	0	0	3.548
4	Xaγ	0	0.528	0	0	2.455	0	0
5	Xaγ	1.241	0	0	0	0	0	0
6	Xaγ	0	0	0	0	0	0	0
7	Xaγ	1.528	0	1.441	0	0	0	0
8	Xaγ	0	0	0	0	0	0	0
9	Xaγ	0	0	0	0	0	0	0
10	Xaγ	0	0	0	0	0.776	1.122	0
11	Xaγ	0	0	0	0	0	0	0
12	Xaγ	0	0	0	0	0	0	0

t-test table for free Xaγ across all gels as compared to the control lane, ATIII + Xa

	ATIII + Xa	*(His + ATIII) + Xa*
Mean	0.23075	0.044
Variance	0.294174386	0.023232
Observations	12	12
Std. Error of Mean	0.045198204	0.012701706
Std. Deviation	0.542378453	0.152420471
t Stat	1.110181543	
P(T<=t) one-tail	0.145299989	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.290599977	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSA + ATIII) + Xa*
Mean	0.23075	0.26925
Variance	0.294174386	0.523568568
Observations	12	12
Std. Error of Mean	0.045198204	0.060298365
Std. Deviation	0.542378453	0.723580381
t Stat	-0.136595688	
P(T<=t) one-tail	0.446909157	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.893818314	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(Hep + ATIII) + Xa*
Mean	0.23075	0.120083333
Variance	0.294174386	0.173040083
Observations	12	12
Std. Error of Mean	0.045198204	0.034665072
Std. Deviation	0.542378453	0.415980869
t Stat	1.074319868	
P(T<=t) one-tail	0.152840181	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.305680362	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSB + ATIII) + Xa*
Mean	0.23075	0.0935
Variance	0.294174386	0.104907
Observations	12	12
Std. Error of Mean	0.045198204	0.026991125
Std. Deviation	0.542378453	0.323893501
t Stat	0.711803948	
P(T<=t) one-tail	0.245703374	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.491406748	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + Hep + ATIII) + Xa*
Mean	0.23075	0.157
Variance	0.294174386	0.295788
Observations	12	12
Std. Error of Mean	0.045198204	0.045321996
Std. Deviation	0.542378453	0.543863954
t Stat	0.312347509	
P(T<=t) one-tail	0.380308846	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.760617693	
t Critical two-tail	2.20098516	

	ATIII + Xa	*(His + CSC + ATIII) + Xa*
Mean	0.23075	0.295666667
Variance	0.294174386	1.049025333
Observations	12	12
Std. Error of Mean	0.045198204	0.085351615
Std. Deviation	0.542378453	1.024219378
t Stat	-0.184100301	
P(T<=t) one-tail	0.428642362	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.857284724	
t Critical two-tail	2.20098516	

t-test table for free Xaγ across all gels as compared to the histone lane, (His + ATIII) + Xa

	(His + ATIII) + Xa	*ATIII + Xa*
Mean	0.044	0.23075
Variance	0.023232	0.294174386
Observations	12	12
Std. Error of Mean	0.012701706	0.045198204
Std. Deviation	0.152420471	0.542378453
t Stat	-1.110181543	
P(T<=t) one-tail	0.145299989	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.290599977	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSA + ATIII) + Xa*
Mean	0.044	0.26925
Variance	0.023232	0.523568568
Observations	12	12
Std. Error of Mean	0.012701706	0.060298365
Std. Deviation	0.152420471	0.723580381
t Stat	-1.344190205	
P(T<=t) one-tail	0.10297642	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.20595284	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(Hep + ATIII) + Xa*
Mean	0.044	0.120083333
Variance	0.023232	0.173040083
Observations	12	12
Std. Error of Mean	0.012701706	0.034665072
Std. Deviation	0.152420471	0.415980869
t Stat	-0.57817232	
P(T<=t) one-tail	0.287396665	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.57479333	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSB + ATIII) + Xa*
Mean	0.044	0.0935
Variance	0.023232	0.104907
Observations	12	12
Std. Error of Mean	0.012701706	0.026991125
Std. Deviation	0.152420471	0.323893501
t Stat	-0.463077135	
P(T<=t) one-tail	0.326168013	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.652336026	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + Hep + ATIII) + Xa*
Mean	0.044	0.157
Variance	0.023232	0.295788
Observations	12	12
Std. Error of Mean	0.012701706	0.045321996
Std. Deviation	0.152420471	0.543863954
t Stat	-0.677229751	
P(T<=t) one-tail	0.256125747	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.512251493	
t Critical two-tail	2.20098516	

	(His + ATIII) + Xa	*(His + CSC + ATIII) + Xa*
Mean	0.044	0.295666667
Variance	0.023232	1.049025333
Observations	12	12
Std. Error of Mean	0.012701706	0.085351615
Std. Deviation	0.152420471	1.024219378
t Stat	-0.830985433	
P(T<=t) one-tail	0.211826179	
t Critical one-tail	1.795884819	
P(T<=t) two-tail	0.423652359	
t Critical two-tail	2.20098516	

On the following pages, there are tables showing the mean, standard error of the mean, standard deviation, and one- and two-tail P values for all complexes across all lanes as compared to the control lane and to the histone lane. Highlighted P values indicate statistical significance.

Compared to ATIII + Xa (Lane 4)

Complex		Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1° Xaα - ATIII	Mean	10.7029167	2.52125	6.50516667	10.2805	7.30266667	15.065	13.8245
	Std. Error of Mean	0.40429818	0.19004794	0.50020332	0.706906885	0.30181879	0.46487405	0.50116396
	Std. Deviation	4.8515781	2.28057525	6.00243982	8.48288262	3.62182549	5.57848855	6.01396748
	P(T<=t) one-tail	n/a	0.00022716	0.05512619	0.446742797	0.04529653	0.02326341	0.06302313
	P(T<=t) two-tail	n/a	0.00045432	0.11025238	0.893485593	0.09059305	0.04652683	0.12604626
1° Xaβ - ATIII	Mean	16.2200833	4.3915	13.2016667	17.19416667	11.4708333	23.03675	21.4268333
	Std. Error of Mean	0.80670476	0.37548934	1.23471254	1.178042096	0.65112625	0.71019025	0.65097793
	Std. Deviation	9.68045707	4.50587208	14.8165505	14.13650515	7.81351499	8.52228295	7.81173518
	P(T<=t) one-tail	n/a	0.00097805	0.24130445	0.409403933	0.15523666	0.02170346	0.04966035
	P(T<=t) two-tail	n/a	0.00195609	0.4826089	0.818807866	0.31047332	0.04340693	0.0993207
2° Xaα - ATIII	Mean	1.68991667	0.16783333	0.759	0.76925	0.62283333	1.07825	1.4685
	Std. Error of Mean	0.0919098	0.03548262	0.06723125	0.055859832	0.05861438	0.07868185	0.08307888
	Std. Deviation	1.10291766	0.42579142	0.80677495	0.670317988	0.7033725	0.9441822	0.99694657
	P(T<=t) one-tail	n/a	0.00079617	0.01463347	0.004035895	0.01944802	0.05498127	0.26049033
	P(T<=t) two-tail	n/a	0.00159234	0.02926693	0.00807179	0.03889604	0.10996254	0.52098066
2° Xaβ - ATIII	Mean	1.774	0.32625	0.96391667	1.267583333	0.71583333	1.30883333	1.44825
	Std. Error of Mean	0.09818458	0.06535482	0.09200866	0.102475838	0.08969839	0.0934815	0.08807893
	Std. Deviation	1.17821491	0.78425786	1.10410395	1.229710051	1.07638062	1.12177804	1.05694716
	P(T<=t) one-tail	n/a	0.00064464	0.01100054	0.018502796	0.0213471	0.12900149	0.13006048
	P(T<=t) two-tail	n/a	0.00128929	0.02200108	0.037005592	0.04269419	0.25800299	0.26012095
3° Xaα - ATIII	Mean	7.00175	8.3535	5.19166667	4.164666667	7.68633333	3.30483333	3.67425
	Std. Error of Mean	0.25254223	0.21718663	0.14551198	0.172443979	0.30566142	0.10977944	0.16244377
	Std. Deviation	3.03050671	2.60623959	1.74614377	2.069327749	3.66793706	1.31735326	1.9493253
	P(T<=t) one-tail	n/a	0.04195342	0.01768289	2.40945E-05	0.21785663	0.00023294	0.00046224
	P(T<=t) two-tail	n/a	0.08390684	0.03536577	4.8189E-05	0.43571325	0.00046589	0.00092449
3° Xaβ - ATIII	Mean	14.2983333	21.1760833	15.1683333	11.65375	12.9954167	5.94425	6.81041667
	Std. Error of Mean	0.66546034	0.69400865	0.57794919	0.620959627	0.62095963	0.23348452	0.54655157
	Std. Deviation	7.98552405	8.32810386	6.93539034	7.451515528	7.45151553	2.80181425	6.5586188
	P(T<=t) one-tail	n/a	0.00899715	0.35719112	0.086224741	0.28987993	0.00029768	0.00036742
	P(T<=t) two-tail	n/a	0.0179943	0.71438224	0.172449481	0.57975987	0.00059535	0.00073485
ATIII-M	Mean	4.12066667	3.23058333	6.78633333	5.11375	3.67658333	3.8655	2.419
	Std. Error of Mean	0.16287448	0.04694376	0.19886229	0.142968511	0.14296851	0.16323789	0.04129287
	Std. Deviation	1.95449372	0.56332511	2.38634743	1.715622131	1.71562213	1.95885465	0.49551443
	P(T<=t) one-tail	n/a	0.08521945	0.00882276	0.138789482	0.26668022	0.29714273	0.00477045
	P(T<=t) two-tail	n/a	0.1704389	0.01764551	0.277578965	0.53336045	0.59428546	0.00954091
ATIII	Mean	17.286	29.2905	18.3335833	19.06866667	32.6693333	22.20025	24.7026667
	Std. Error of Mean	0.4040469	1.1646032	0.34185129	0.428009528	1.77950674	0.68723858	0.68675827
	Std. Deviation	4.84856279	13.9752384	4.10221542	5.136114331	21.3540809	8.24686298	8.24109919
	P(T<=t) one-tail	n/a	0.01349852	0.26730177	0.21395542	0.02242344	0.05853484	0.01491786
	P(T<=t) two-tail	n/a	0.02699704	0.53460353	0.427910841	0.04484688	0.11706968	0.02983571
Xaα	Mean	10.5146667	10.8338333	13.7779167	13.18408333	10.5126667	10.2076667	7.78658333
	Std. Error of Mean	0.56570581	0.38396628	0.59398736	0.841444324	0.54461343	0.73089161	0.4559848
	Std. Deviation	6.78846969	4.60759539	7.12784835	10.09733188	6.53536118	8.77069934	5.47181762
	P(T<=t) one-tail	n/a	0.4485287	0.12941764	0.230878382	0.49963939	0.45883341	0.09535514
	P(T<=t) two-tail	n/a	0.8970574	0.25883529	0.461756763	0.99927878	0.91766681	0.19071028
Xaβ	Mean	12.2045	19.6645	19.192	17.14691667	12.0783333	13.89475	11.8835833
	Std. Error of Mean	0.58031366	0.77768654	0.67476796	0.880862354	0.50129583	0.64824392	0.6177123
	Std. Deviation	6.96376388	9.33223847	8.09721553	10.57034825	6.01554999	7.77892701	7.41254755
	P(T<=t) one-tail	n/a	0.03018496	0.02411347	0.128280777	0.47926851	0.30790549	0.44957887
	P(T<=t) two-tail	n/a	0.06036992	0.04822693	0.256561553	0.95853701	0.61581098	0.89915774
Xaγ	Mean	0.23075	0.044	0.12008333	0.157	0.26925	0.0935	0.29566667
	Std. Error of Mean	0.0451982	0.01270171	0.03466507	0.045321996	0.06029837	0.02699113	0.08535161
	Std. Deviation	0.54237845	0.15242047	0.41598087	0.543863954	0.72358038	0.3238935	1.02421938
	P(T<=t) one-tail	n/a	0.14529999	0.15284018	0.380308846	0.44690916	0.24570337	0.42864236
	P(T<=t) two-tail	n/a	0.29059998	0.30568036	0.760617693	0.89381831	0.49140675	0.85728472

Compared to (His + ATIII) + Xa (Lane 5)

Complex		Lane 4 2 µL ATIII + 2 µL Xa	Lane 5 (2 µL His + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 6 (2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 7 (2 µL His + 2 µL Hep + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 8 (2 µL His + 2 µL CSA + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 9 (2 µL His + 2 µL CSB + 2 µL ATIII)$_{30'}$ + 2 µL Xa	Lane 10 (2 µL His + 2 µL CSC + 2 µL ATIII)$_{30'}$ + 2 µL Xa
1° Xaα - ATIII	Mean	10.7029167	2.52125	6.505166667	10.2805	7.302666667	15.065	13.8245
	Std. Error of Mean	0.40429818	0.19004794	0.500203318	0.706906885	0.301818791	0.464874046	0.501163956
	Std. Deviation	4.8515781	2.28057525	6.00243982	8.48288262	3.62182549	5.578488554	6.013967477
	P(T<=t) one-tail	0.00022716	n/a	0.005983233	0.00121037	0.005143947	4.13459E-06	2.71387E-05
	P(T<=t) two-tail	0.00045432	n/a	0.011966467	0.00242074	0.010287893	8.26918E-06	5.42774E-05
1° Xaβ - ATIII	Mean	16.2200833	4.3915	13.20166667	17.19416667	11.47083333	23.03675	21.42683333
	Std. Error of Mean	0.80670476	0.37548934	1.234712539	1.178042096	0.651126249	0.710190246	0.650977932
	Std. Deviation	9.68045707	4.50587208	14.81655047	14.13650515	7.813514991	8.522282954	7.81173518
	P(T<=t) one-tail	0.00097805	n/a	0.013582142	0.000705897	0.012644941	1.50099E-07	9.59526E-07
	P(T<=t) two-tail	0.00195609	n/a	0.027164284	0.001411795	0.025289882	3.00198E-07	1.91905E-06
2° Xaα - ATIII	Mean	1.68991667	0.16783333	0.759	0.76925	0.622833333	1.07825	1.4685
	Std. Error of Mean	0.0919098	0.03548262	0.067231246	0.055859832	0.058614375	0.07868185	0.08307888
	Std. Deviation	1.10291766	0.42579142	0.806774949	0.670317988	0.703372504	0.944182198	0.996946566
	P(T<=t) one-tail	0.00079617	n/a	0.014326511	0.006163077	0.017504442	0.007665891	0.001557759
	P(T<=t) two-tail	0.00159234	n/a	0.028653023	0.012326155	0.035008885	0.015331783	0.003115517
2° Xaβ - ATIII	Mean	1.774	0.32625	0.963916667	1.267583333	0.715833333	1.308833333	1.44825
	Std. Error of Mean	0.09818458	0.06535482	0.092008663	0.102475838	0.089698385	0.093481503	0.08807893
	Std. Deviation	1.17821491	0.78425786	1.104103952	1.229710051	1.076380622	1.121778039	1.056947158
	P(T<=t) one-tail	0.00064464	n/a	0.029816275	0.012517719	0.031930182	0.018793177	0.009685616
	P(T<=t) two-tail	0.00128929	n/a	0.05963255	0.025035438	0.063860364	0.037586354	0.019371232
3° Xaα - ATIII	Mean	7.00175	8.3535	5.191666667	4.164666667	7.686333333	3.304833333	3.67425
	Std. Error of Mean	0.25254223	0.21718663	0.145511981	0.172443979	0.305661421	0.109779438	0.162443775
	Std. Deviation	3.03050671	2.60623959	1.746143769	2.069327749	3.667937055	1.317353258	1.949325297
	P(T<=t) one-tail	0.04195342	n/a	9.72081E-05	3.25141E-05	0.146797721	1.79869E-05	0.000152002
	P(T<=t) two-tail	0.08390684	n/a	0.000194416	6.50282E-05	0.293595442	3.59738E-05	0.000304004
3° Xaβ - ATIII	Mean	14.2983333	21.1760833	15.16833333	11.65375	12.99541667	5.94425	6.810416667
	Std. Error of Mean	0.66546034	0.69400865	0.577949195	0.620959627	0.620959627	0.233484521	0.546551567
	Std. Deviation	7.98552405	8.32810386	6.935390339	7.451515528	7.451515528	2.801814254	6.558618805
	P(T<=t) one-tail	0.00899715	n/a	0.000585368	0.001032334	7.19481E-05	1.69733E-05	0.000498537
	P(T<=t) two-tail	0.0179943	n/a	0.001170737	0.002064667	0.000143896	3.39466E-05	0.000997074
ATIII-M	Mean	4.12066667	3.23058333	6.786333333	5.11375	3.676583333	3.8655	2.419
	Std. Error of Mean	0.16287448	0.04694376	0.198862286	0.142968511	0.142968511	0.163237888	0.041292869
	Std. Deviation	1.95449372	0.56332511	2.386347431	1.715622131	1.715622131	1.958854651	0.495514425
	P(T<=t) one-tail	0.08521945	n/a	0.000301472	0.001971473	0.191684951	0.154030559	0.000219481
	P(T<=t) two-tail	0.1704389	n/a	0.000602945	0.003942945	0.383369901	0.308061117	0.000438961
ATIII	Mean	17.286	29.2905	18.33358333	19.06866667	32.66933333	22.20025	24.70266667
	Std. Error of Mean	0.4040469	1.1646032	0.341851285	0.428009528	1.779506738	0.687238581	0.686758266
	Std. Deviation	4.84856279	13.9752384	4.10221542	5.136114331	21.35408086	8.246862975	8.241099191
	P(T<=t) one-tail	0.01349852	n/a	0.010891096	0.006594708	0.15643668	0.028246476	0.115774517
	P(T<=t) two-tail	0.02699704	n/a	0.021782192	0.013189416	0.31287336	0.056492952	0.231549034
Xaα	Mean	10.5146667	10.8338333	13.77791667	13.18408333	10.51266667	10.20766667	7.786583333
	Std. Error of Mean	0.56570581	0.38396628	0.593987362	0.841444324	0.544613431	0.730891612	0.455984802
	Std. Deviation	6.78846969	4.60759539	7.127848349	10.09733188	6.535361176	8.770699343	5.471817622
	P(T<=t) one-tail	0.4485287	n/a	0.033439101	0.157821482	0.406070057	0.368918881	0.052520936
	P(T<=t) two-tail	0.8970574	n/a	0.066878203	0.315642964	0.812140114	0.737837762	0.105041871
Xaβ	Mean	12.2045	19.6645	19.192	17.14691667	12.07833333	13.89475	11.88358333
	Std. Error of Mean	0.58031366	0.77768654	0.67476796	0.880862354	0.501295833	0.648243918	0.617712296
	Std. Deviation	6.96376388	9.33223847	8.097215526	10.57034825	6.015549991	7.778927013	7.41254755
	P(T<=t) one-tail	0.03018496	n/a	0.434553169	0.149497598	0.003884395	0.000163548	0.012390306
	P(T<=t) two-tail	0.06036992	n/a	0.869106337	0.298995195	0.00776879	0.000327096	0.024780612
Xaγ	Mean	0.23075	0.044	0.120083333	0.157	0.26925	0.0935	0.295666667
	Std. Error of Mean	0.0451982	0.01270171	0.034665072	0.045321996	0.060298365	0.026991125	0.085351615
	Std. Deviation	0.54237845	0.15242047	0.415980869	0.543863954	0.723580381	0.323893501	1.024219378
	P(T<=t) one-tail	0.14529999	n/a	0.287396665	0.256125747	0.10297642	0.326168013	0.211826179
	P(T<=t) two-tail	0.29059998	n/a	0.57479333	0.512251493	0.20595284	0.652336026	0.423652359

Discussion and Conclusion

Control bands for the 1° Xaα-ATIII and 1° Xaβ-ATIII complexes were found to be 10.7±0.404 DU and 16.2±0.807 DU, respectively, and 3° Xaα-ATIII and 3° Xaβ-ATIII complexes were found to be 7.00±0.253 DU and 14.3±0.665 DU, respectively. In the presence of histone, there was a statistically significant drop in the 1° Xaα-ATIII and 1° Xaβ-ATIII complexes to 2.52±0.190 DU (p=0.000454) and 4.39±0.375 DU (p=0.00196), respectively, and a statistically significant increase in the 3° Xaα-ATIII and 3° Xaβ-ATIII complexes to 8.35±0.217 DU (p=0.0839) and 21.2±0.694 DU (p=0.0180), respectively. Heparin produces a non-statistically significant drop in the 1° Xaα-ATIII and 1° Xaβ-ATIII complexes to 6.51±0.500 DU (p=0.110) and 13.2±1.23 DU (p=0.483), respectively, and a statistically significant drop in the 3° Xaα-ATIII complex to 5.19±0.146 DU (p=0.0354) and a non-statistically significant increase in the 3° Xaβ-ATIII complex to 15.2±0.578 DU (p=0.714). When mixed, histone and heparin effectively neutralize each other to produce a non-statistically significant drop in the 1° Xaα-ATIII complex to 10.3±0.707 DU (p=0.893) and a non-statistically significant increase in the 1° Xaβ-ATIII complex 17.2±1.18 DU (p=0.819), and statistically significant drop in the 3° Xaα-ATIII complex to 4.16±0.172 DU (p=0.0000412) and a non-statistically significant drop in the 3° Xaβ-ATIII complex to 11.7±0.621 DU (p=0.172). When mixed with histone, chondroitin sulfate A cannot neutralize the histone effect in the 1° complexes and produces a statistically significant drop in the 1° Xaα-ATIII complex to 7.30±0.302 DU (p=0.0906) and a non-statistically significant drop in the 1° Xaβ-ATIII complex to 11.5±0.651 DU (p=0.310), and is able to neutralize the histone effect in the 3° complexes to produce a non-statistically significant increase in 3° Xaα-ATIII complex to 7.69±0.306 DU (p=0.436) and a non-statistically significant drop in the 3° Xaβ-ATIII complex to 13.0±0.621 DU (p=0.580). Chondroitin sulfate B (dermatan sulfate), when mixed with histone, produced statistically significant increases in the 1° Xaα-ATIII and 1° Xaβ-ATIII complexes to 15.1±0.465 DU (p=0.0465) and 23.0±0.710 DU (p=0.0434), respectively, and a statistically significant drop in the 3° Xaα-ATIII and 3° Xaβ-ATIII complexes to 3.30±0.110 DU (p=0.000466) and 5.94±0.233 DU (p=0.000735), respectively. Chondroitin sulfate C, when mixed with histone, produced a non-statistically significant increase in the 1° Xaα-ATIII complex to 13.8±0.501 DU (p=0.126) and a statistically significant increase in the 1° Xaβ-ATIII complex to 21.4±0.651 DU (p=0.0993), and statistically significant drops in the 3° Xaα-ATIII and 3° Xaβ-ATIII complexes to 3.67±0.110 DU (p=0.000924) and 6.81±0.547 DU (p=0.000735), respectively. These data suggest that, when used with together, histone and heparin decrease the degradation of the 1° Xa-ATIII complexes, as observed by a drop in the levels of the 3° Xa-ATIII complexes, possibly by inhibition of free FXa. Additionally, chondroitin sulfates A and C and dermatan sulfate combined with histone, decrease the degradation of the 1° Xa-ATIII complexes, by reduction of the levels of the 3° Xaβ-ATIII complex, however, the level of degradation is much lower than when histone is used with heparin.

References

[1]Kavanagh, C.; Shaw, S.; Webster, C.R.L. Coagulation in hepatobiliary disease. *J. Vet. Emer. Crit. Care* [Online] **2011,** *21,* 589-604. Academic Search Complete. http://0-web.ebscohost.com.maurice .bgsu.edu/ehost/pdfviewer/pdfviewer?vid=4&sid=9fa1c27e-36be-4c3b-9e8b-ccfa740e8d5e%40 sessionmgr115&hid=121 (accessed April 16, 2013).

[2]Blood Coagulation. *The Medical Biochemistry Page* [Online] **2013.** http://themedicalbiochemistrypage. org/blood-coagulation.php (accessed April 16, 2013).

[3]Miller, K. The Evolution of Vertebrate Blood Clotting. http://www.millerandlevine.com/km/evol/DI/clot/Clotting.html (accessed April 16, 2013).

[4]Brecher, A.S.; Adamu, M.T. The effect of glycosaminoglycans with acetaldehyde on the activation of prothrombin. *Can. J. Physiol. Pharmacol.* [Online] **2005,** *83,* 431-438. Academic Search Complete. http://0-web.ebscohost.com.maurice.bgsu.edu/ehost/detail?vid=4&sid=cad91588-102d-47d6-ab54-8e2721632b71%40sessionmgr198&hid=121&bdata=JnNpdGU9ZWhvc3QtbGl2ZSZzY29wZT1zaXRl#db=a9h&AN=17187022 (accessed April 16, 2013).

[5]Brecher, A.S.; Hommema, E.L. The influence of acetaldehyde and glycosaminoglycans upon Factor X_a and Factor X-deficient plasma. *Can. J. Physiol. Pharmacol.* [Online] **2002,** *80,* 879-886. Academic Search Complete. http://0-web.ebscohost.com.maurice.bgsu.edu/ehost/detail?vid=5&sid=cad9 1588-102d-47d6-ab54-8e2721632b71%40sessionmgr198&hid=121&bdata=JnNpdGU9ZWhvc3Q tbGl2ZSZzY29wZT1zaXRl#db=a9h&AN=10349275 (accessed April 16, 2013).

[6]Brecher, A.S.; Adamu, M.T. Coagulation Protein Function: Enhancement of the Anticoagulant Effect of Acetaldehyde by Sulfated Glycosaminoglycans. *Digestive Diseases and Sciences* **2011,** *46,* 2033-2042.

[7]Brecher, A.S.; Koterba, A.P.; Basista, M.H. Coagulation Protein Function IV: Effect of Acetaldehyde Upon Factor X and Factor Xa, the proteins at the Gateway to the Common Coagulation Pathway. *Alcohol* **1996**, *13*, 539-545.

[8]Skoog, D.A.; Holler, F.J.; Crouch, S.R. Capillary Electrophoresis, Capillary Electrochromatography, and Field-Flow Fractionation. *Principles of Instrumental Methods*, 6th ed.; Brooks/Cole: Belmont, 2007; 867-883.

Appendix A: Reagents needed for electrophoresis experiment: SDS-PAGE Reagents

I. 0.5M Tris/HCL pH 6.8:
   i. 6.055 g Trizma base in 50 mL deionized-H_2O
   ii. Add 0.1 M HCl until pH 6.8 is reached
      – Dilute to 100 mL; store at 4°C

II. 1.5M Tris/HCL pH 8.8:
   i. 36.33 g Trizma base and fill to 150 mL with deionized-H_2O
   ii. Add 0.1 M HCl, drop-wise, to pH 8.8
      – Dilute to 200 mL; store at 4°C

III. 5X Sample Buffer
   i. 21.5 mL glycerol
   ii. 15 mL of 0.5 M Tris/HCL stock solution pH 6.8
   iii. 10 mL 10% SDS solution
   iv. 3.5 mL of 1% Bromophenol Blue(BPB) (prepared in MeOH~ 1 g BPB in 100 mL MeOH)
      – Total volume will be 50 mL

IV. 10% APS (Ammonium Persulfate):
   i. 0.1 g ammonium persulfate into 1 mL deionized-H_2O
      – Store at -20°C in 200 µL aliquots; prepare monthly for fresh solution

V. 10% SDS Solution (w/v):
   i. 10 g Sodium Lauryl Sulfate (Sodium Dodecyl sulfate, SDS) into 75 mL deionized-H_2O
      – Dilute to 100 mL in volumetric Flask

VI. 10X Running Buffer:
   i. 30.3 g Trizma Base
   ii. 144 g Glycine
   iii. 10 g SDS
      – Bring to 1000 mL with deionized-H_2O, mix well but do not adjust the pH; store at 4°C
      – If a precipitate forms, bring to room temperature before diluting with deionized-H_2O before use

VII. Acrylamide Stock Solution
   i. 29.2 g acrylamide
   ii. 0.8 g N'N'-methylene-bis-acrylamide
      – Dilute to 100 mL; filter via vacuum filtration with Whatman #1 filter paper
      – Store in a brown bottle or in the dark

VIII. Deionized-H_2O Saturated Isobutyl Alcohol
   i. Wash isobutyl alcohol three times with deionized-H_2O in a separatory funnel and extract
      – This solution must be stored in the dark

IX. Destain:
   i. 100 mL ACS reagent grade methanol
   ii. 100 mL glacial acetic acid
      – Dilute to 1000 mL with deionized-H_2O

X. Equilibrium Solution A (30% Methanol, 3% Glycerol)
   i. 150 mL methanol
   ii. 15 mL glycerol
      – Dilute to 500 mL with deionized-H_2O

XI. Resolving Gel
   i. 4.8 mL deionized-H_2O
   ii. 2.5 mL 1.5 M Tris (pH 8.8)
   iii. 2.5 mL Acrylamide stock solution
   iv. 100 µL 10% SDS solution
   v. 50 µL 10% APS solution
   vi. 10 µL tetramethylethylenediamine (TEMED)
      – Combine i-iv above and pour into filtering flask
      – Add APS and degas with water vacuum for 15-60 seconds
      – Add TEMED and pipet solution into gel molds until ¾ full, drop isobutyl alcohol onto top to remove bubbles/smooth surface
      – Wait at least 15 minutes to set
      – Pour off isobutyl alcohol and rinse with deionized-H_2O

XII. Stacking Gel
   i. 3.0 mL deionized-H_2O
   ii. 1.25 mL 0.5 M Tris (pH 6.8)
   iii. 0.65 mL acrylamide stock solution
   iv. 100 µL 10% SDS solution
   v. 25 µL 10% APS solution
   vi. 5 µL TEMED
      – Combine all reagents and pipet onto top of resolving gel; insert lane combs
      – Wait at least 25 minutes for gel to set

XIII. Stain Solution
   i. 0.5 g Coomassie Brilliant Blue
   ii. 250 mL of ACS reagent-grade methanol
   iii. 200 mL deionized-H_2O
   iv. 50 mL glacial acetic acid
      – Filter via vacuum filtration using Whatman #1 filter paper

XIV. Tris/NaCl Buffer pH 8.0:
   i. 0.224 g Trizma base
   ii. 0.325 g reagent-grade NaCl
      – Dilute to 100 mL with deionized-H_2O; mix well and do not adjust pH
      – Store at 4°C