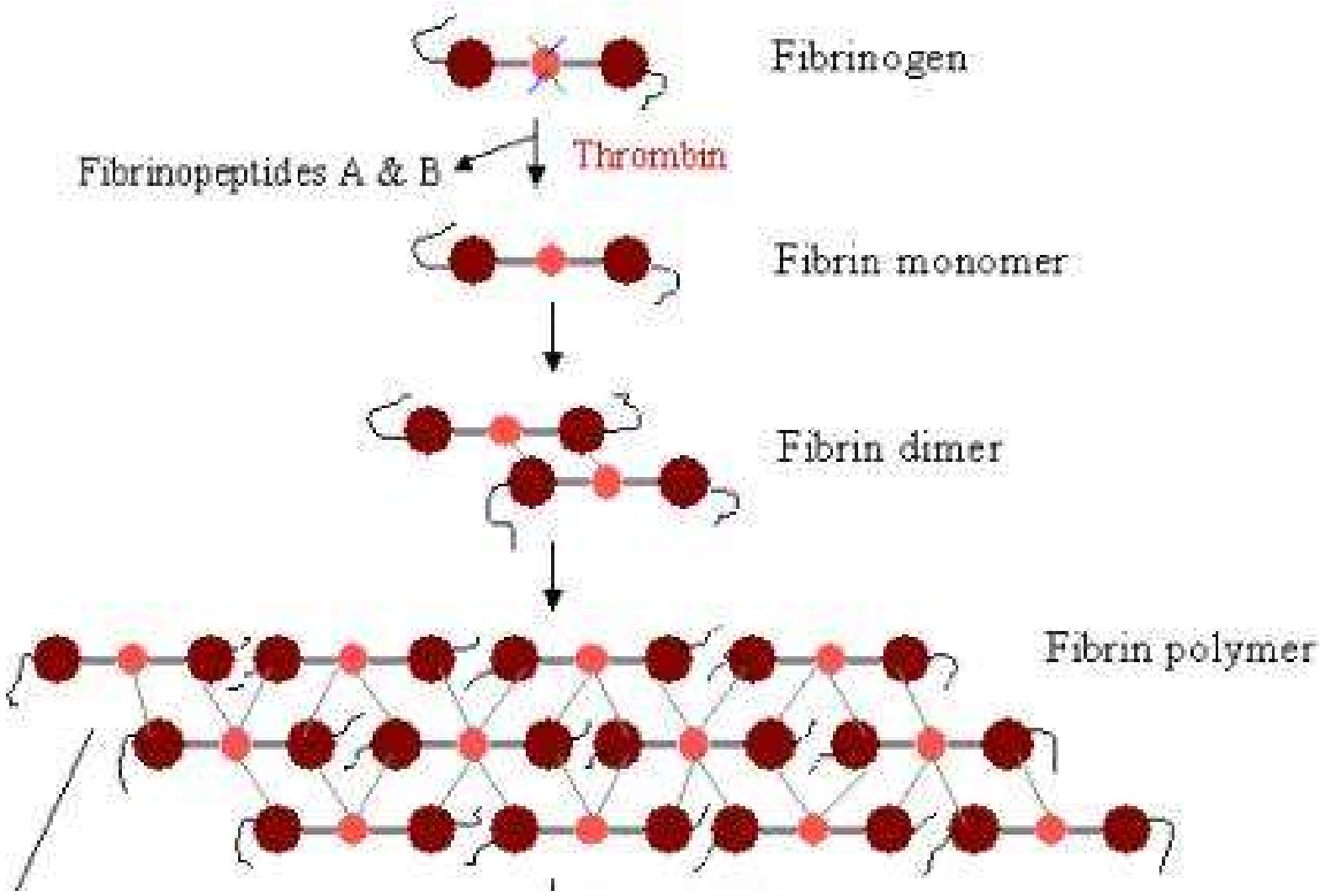
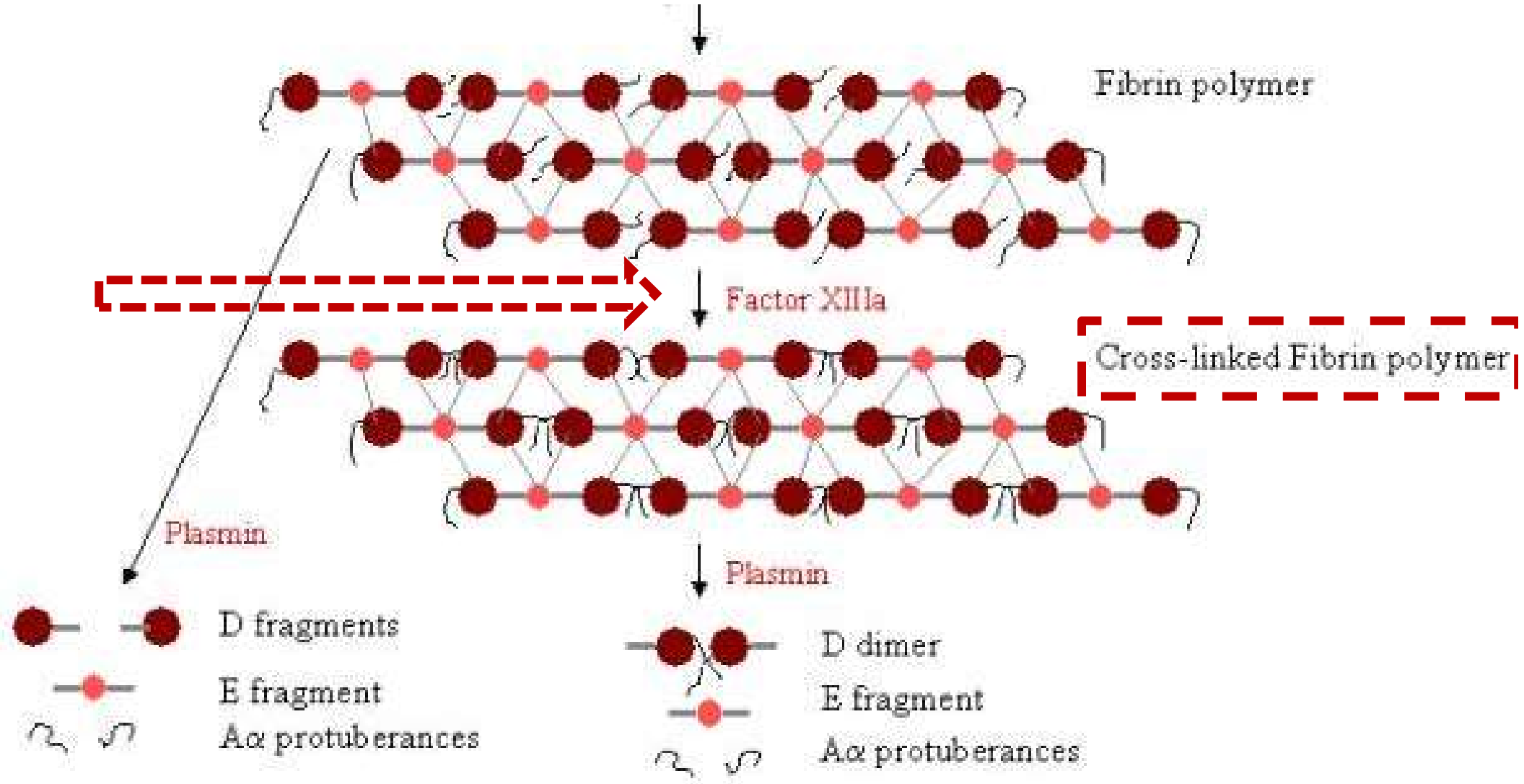
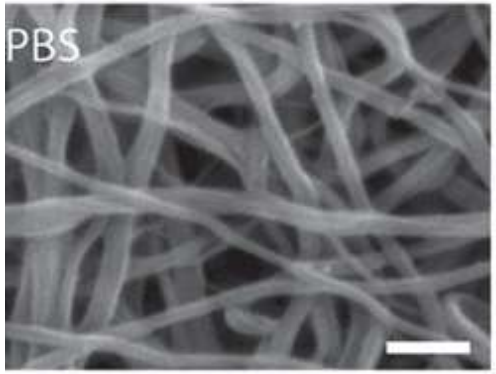
Parte finale coagulazione



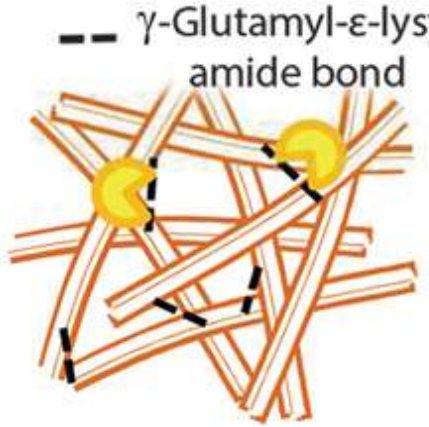
Parte finale coagulazione

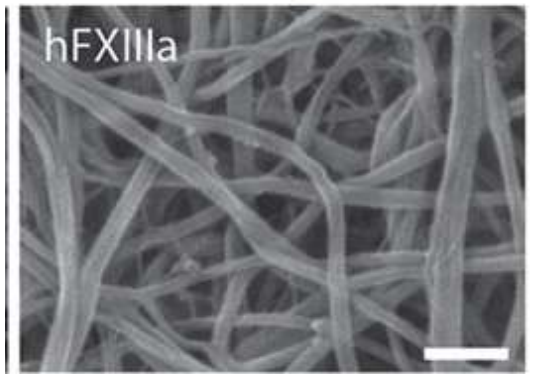


PBS

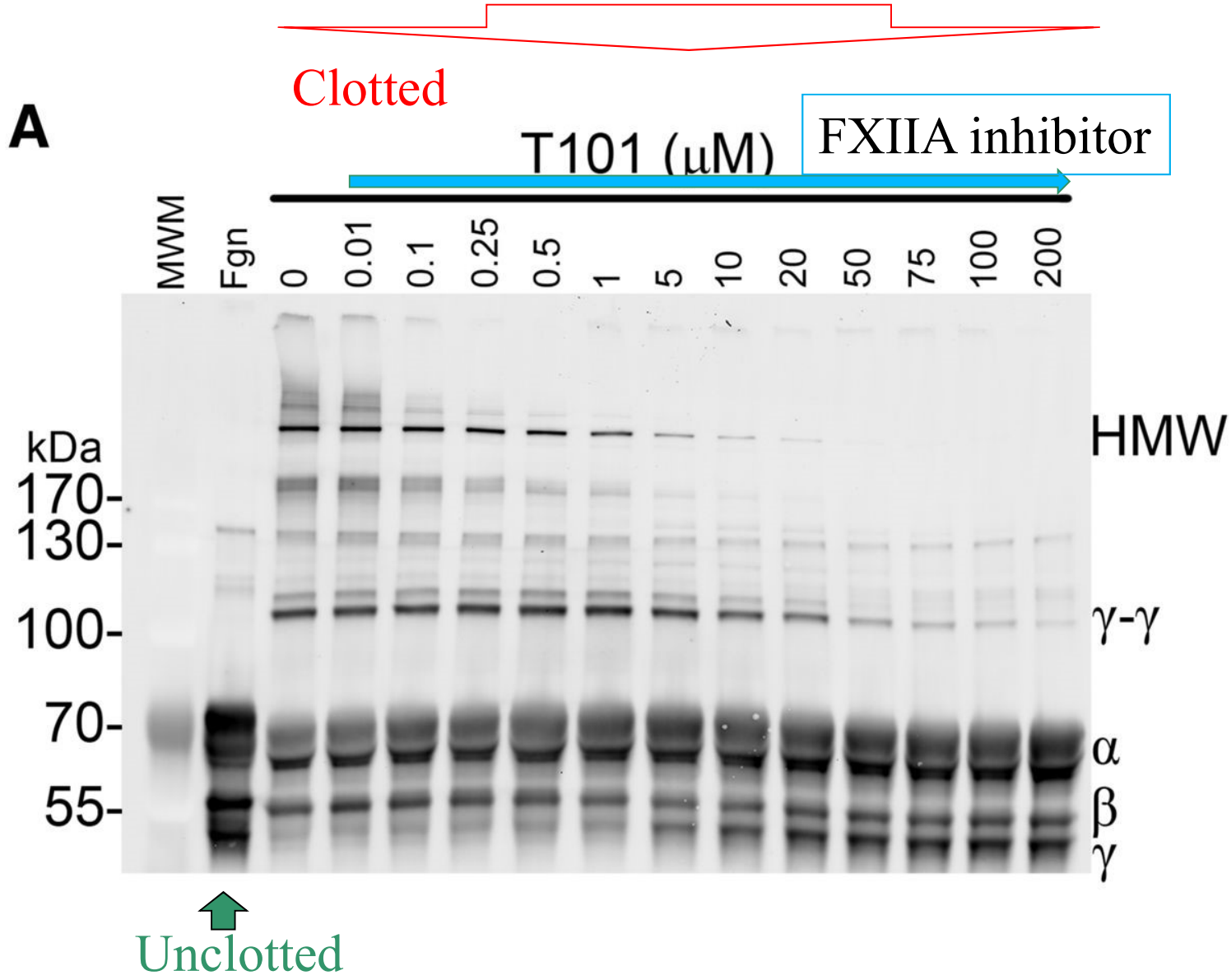


Factor XIIIa cross-linking





RBC retention is reduced at concentrations of T101 that inhibit α-chain crosslinking.



James R. Byrnes et al. Blood 2015;126:1940-1948

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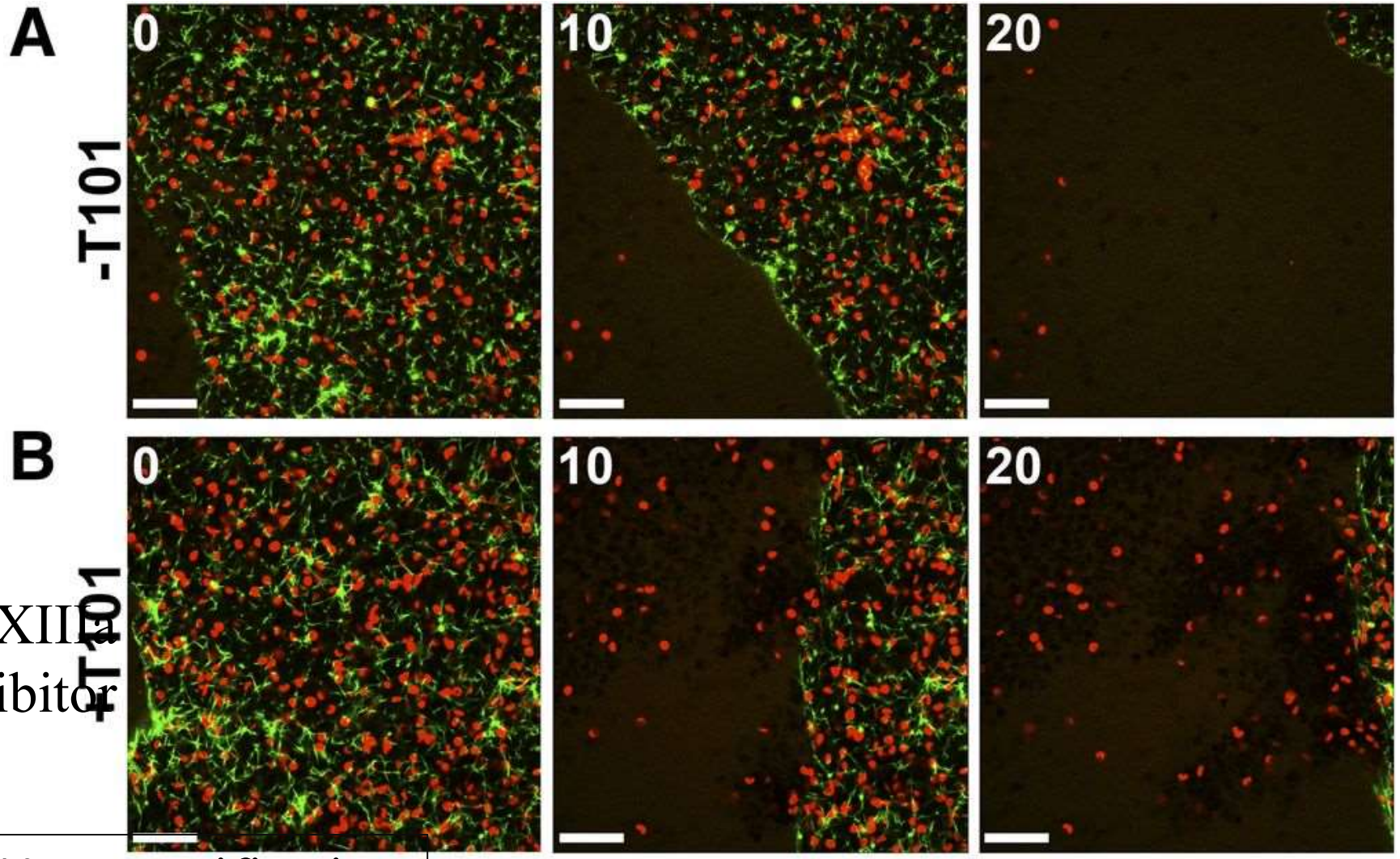
Clot formation and contraction in whole blood

RED octadecyl- rhodamine -labeled RBCs. GREEN -labeled fibrinogen,. Clot contraction Times (in seconds)



James R. Byrnes et al. Blood 2015;126:1940-1948

FXIIIa activity mantains RBCs within the clot during clot contraction.

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blood

Cross-linking reaction catalyzed by activated factor XIII.Activated factor XIII

first forms a thioester bond with a selected protein-bound glutamine residue, releasing ammonia.

Robert A. S. Ariëns et al. Blood 2002;100:743-754

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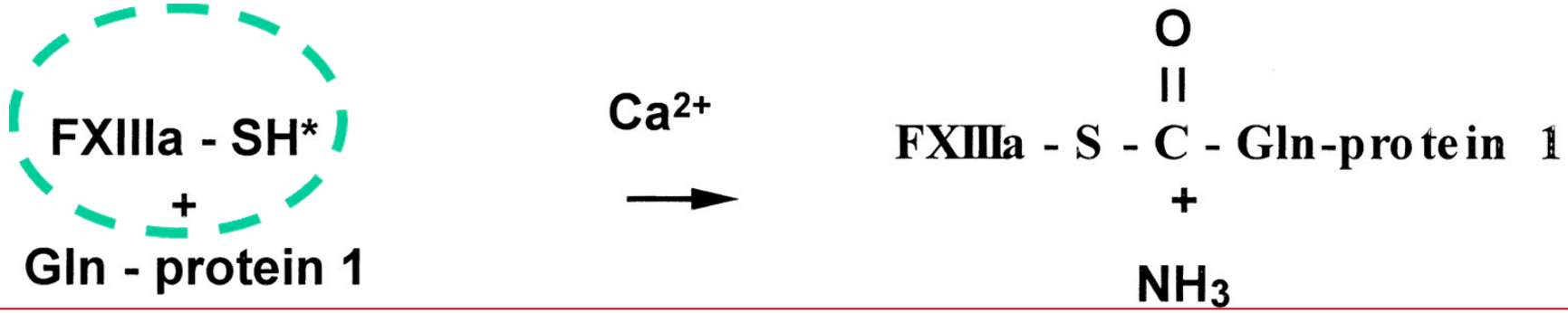
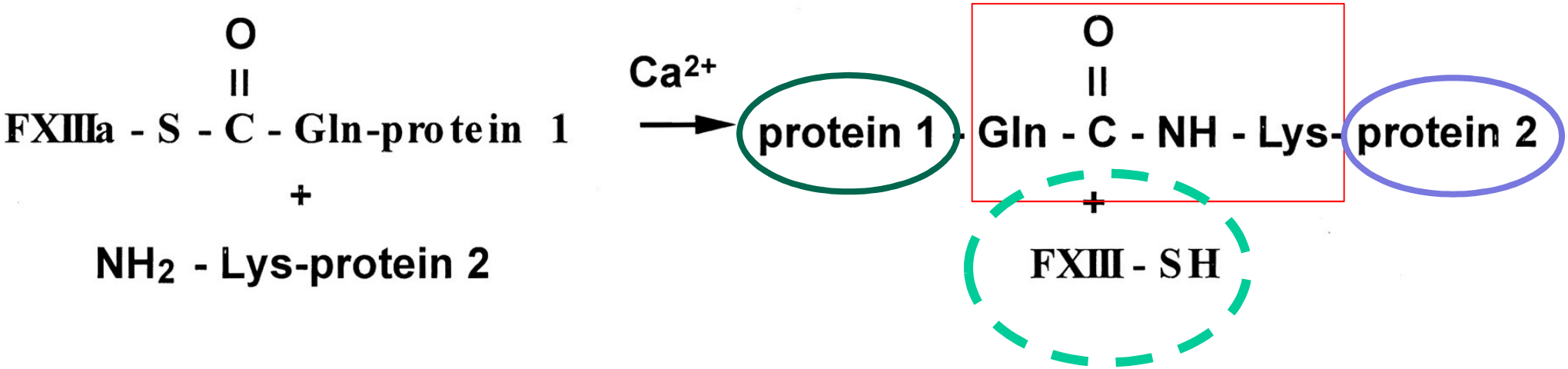
Cross-linking reaction catalyzed by activated factor XIII.Activated factor XIII first forms a thioester bond with a selected protein-bound glutamine residue, releasing ammonia, and …



the thioester intermediate then reacts with a primary amine from a protein-bound lysine residue resulting in an amide or isopeptide bond



Robert A. S. Ariëns et al. Blood 2002;100:743-754

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Table 1.

Factor XIII substrates

Substrate	Cross-linking site
Fibrin(ogen) γ-chain52–54	Gln398, Gln399, and Lys406
Fibrin(ogen) α-chain58–62	Gln221, Gln237, Gln328, Gln366, and 15 potential lysines from Lys208 to Lys606
α2-Antiplasmin67–69	Gln2
TAFI[150]	Gln2, Gln5, Gln292
PAI-2151 152	—
Fibronectin72 73	Gln3
Collagen72 80	—
Von Willebrand factor153 154	—
Vitronectin155 156	Gln93
Thrombospondin[157]	—
Factor V158 159	—
Actin160 161	—

Table 1.

Factor XIII substrates

Substrate	Substances with which it is cross-linked	Known or potential function
Fibrin(ogen) γ-chain52–54	Itself and α-chain	Clot stabilization
Fibrin(ogen) α-chain58–62	Itself and γ-chain	Clot stabilization
α2-Antiplasmin67–69	Lys303 fibrin α-chain	Resistance to fibrinolysis
TAFI[150]	Fibrin, itself	Resistance to fibrinolysis
PAI-2151 152	Lys148, Lys230, Lys413 fibrin α-chain	Resistance to fibrinolysis
Fibronectin72 73	Itself, fibrin, collagen	Migration of cells into the clot; wound healing
Collagen72 80	Fibronectin, fibrin	Stabilization of extracellular matrix
Von Willebrand factor153 154	Fibrin, collagen	Platelet adhesion to the clot
Vitronectin155 156	—	—
Thrombospondin[157]	Fibrin	—
Factor V158 159	Fibrin, platelets	Increased thrombin generation at the clot surface
Actin160 161	Fibrin	Clot retraction, stabilization of the platelet cytoskeleton

Coagulation factorXIIIa (FXIIIa) catalyzes cross-linking of Gln and Lys residues from many substrates during coagulation

? Identificare «tutti» i substrati del FXIIIa

A proteomic strategy based on a combination of

chromatographic separation

FXIIIa-specific labeling

High performance mass spectrometry.

Preparation of Plasma Samples

The plasma fraction was isolated after centrifugation at 950 rpm for 15 min.

EDTA was added to the plasma sample to a final concentration of 5 mM. Anticoagulant

The plasma was centrifuged at 13200 rpm and filtered through a 0.45-µm filter

Preparation of Plasma Samples

The plasma was centrifuged at 13200 rpm and filtered through a 0.45-µm filter

Filtered applied to a column containing the albumin-binding domain of protein G.

Cromatografia Affinita

Streptococcal protein G is a cell surface receptor protein with a multiple domain structure containing tandem repeats of **serum albumin-binding domains**

The albumin depleted flow through was collected and dialyzed against 40 mM Tris-HCl, 5 mM EDTA, pH 7.4 (buffer A),

Preparation of PROTEIN Plasma Samples 2

Applied to a 5-ml HiTrapQ column (GE Healthcare)

A strong anion exchange chromatography column for high-resolution, small-scale protein purification

The column was eluted using a linear gradient of NaCl flow rate of 2.5 ml/min.

Eluting fractions were monitored at 280 nm and pooled (five pools).

.

Preparation of PROTEIN Plasma Samples 3

Eluting fractions were monitored at 280 nm and pooled (five pools).

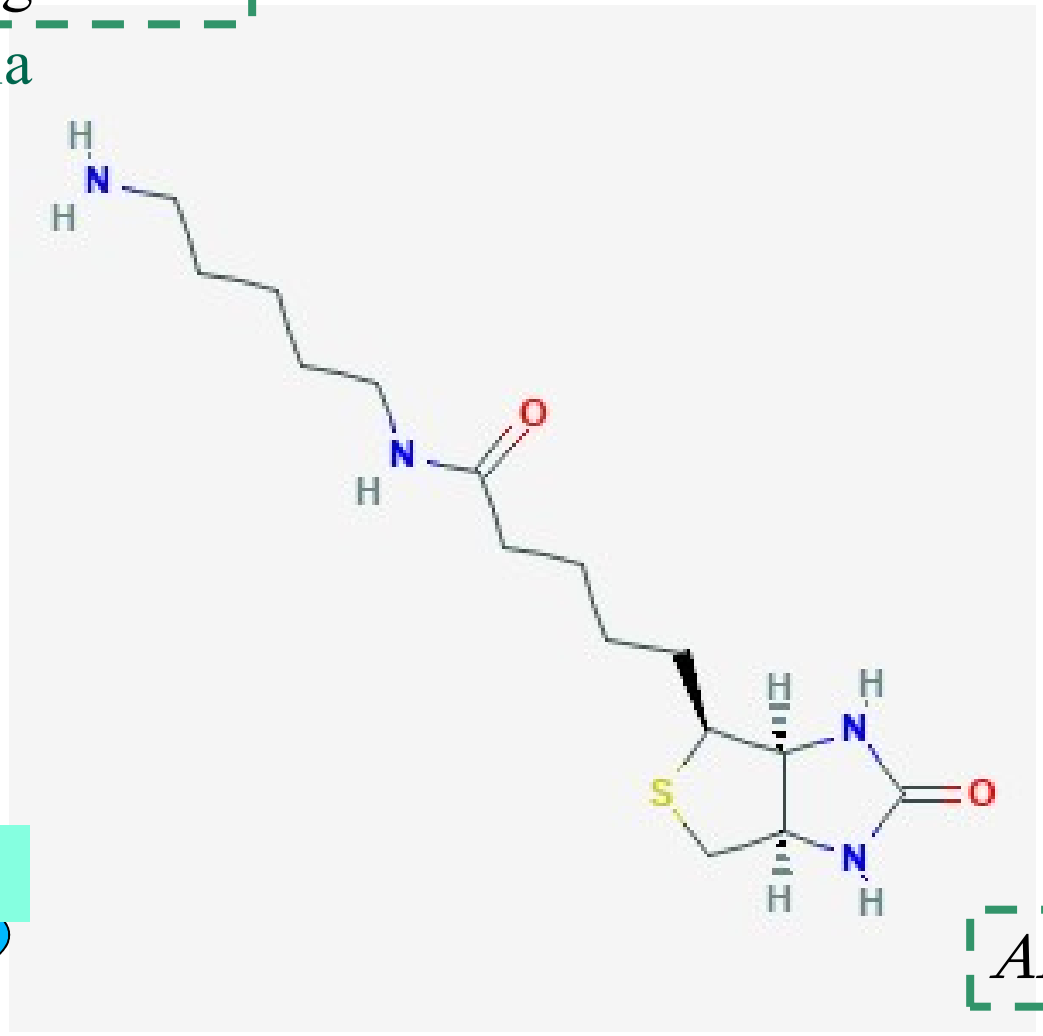
All pools were dialyzed into 20 mM Tris-HCl, 137 mM NaCl, pH 7.4, and concentrated using either Centriprep **centrifugal filters** (Millipore) or Amicon Ultra centrifugal filters (Millipore) (molecular weight **cutoff**, **10 kDa**).

By **SDS-PAGE**, the filtrate did not contain any proteins.

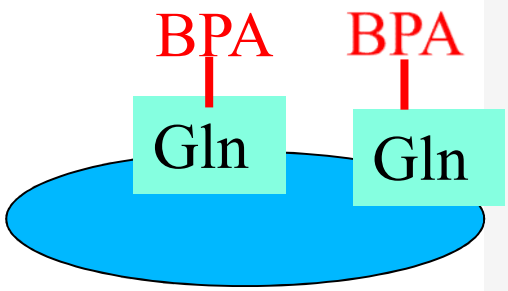
BPA (5-Biotinamido)PentylAmine

FXIIIa crosslinking to Gln

Ammina primaria



Biotina



Affinità Avidina

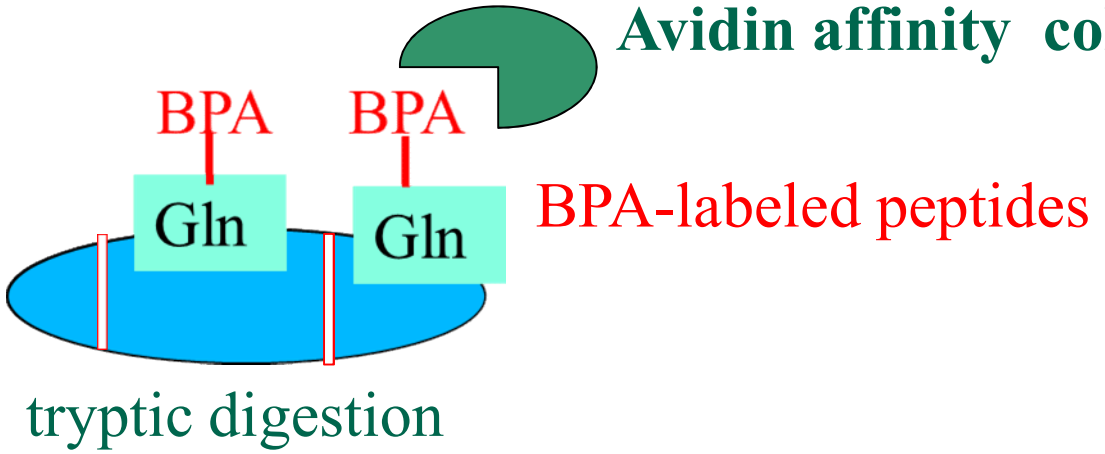
MARCATURA ENZIMATICA (FXIII) PROTEINE PLASMATICHE

MARCATURA ENZIMATICA (FXIII) PROTEINE PLASMATICHE

0. 3 mg of protein from each of the five HiTrapQ pools was incubated with FXIIIa in the presences of BPA (5-Biotinamido)PentylAmine) for 30, 60, and 180 min

Following tryptic digestion BPA-labeled peptides were obtained

Avidin affinity column - BPA-labeled peptides were eluted using 100 mM glycine, pH 2.8



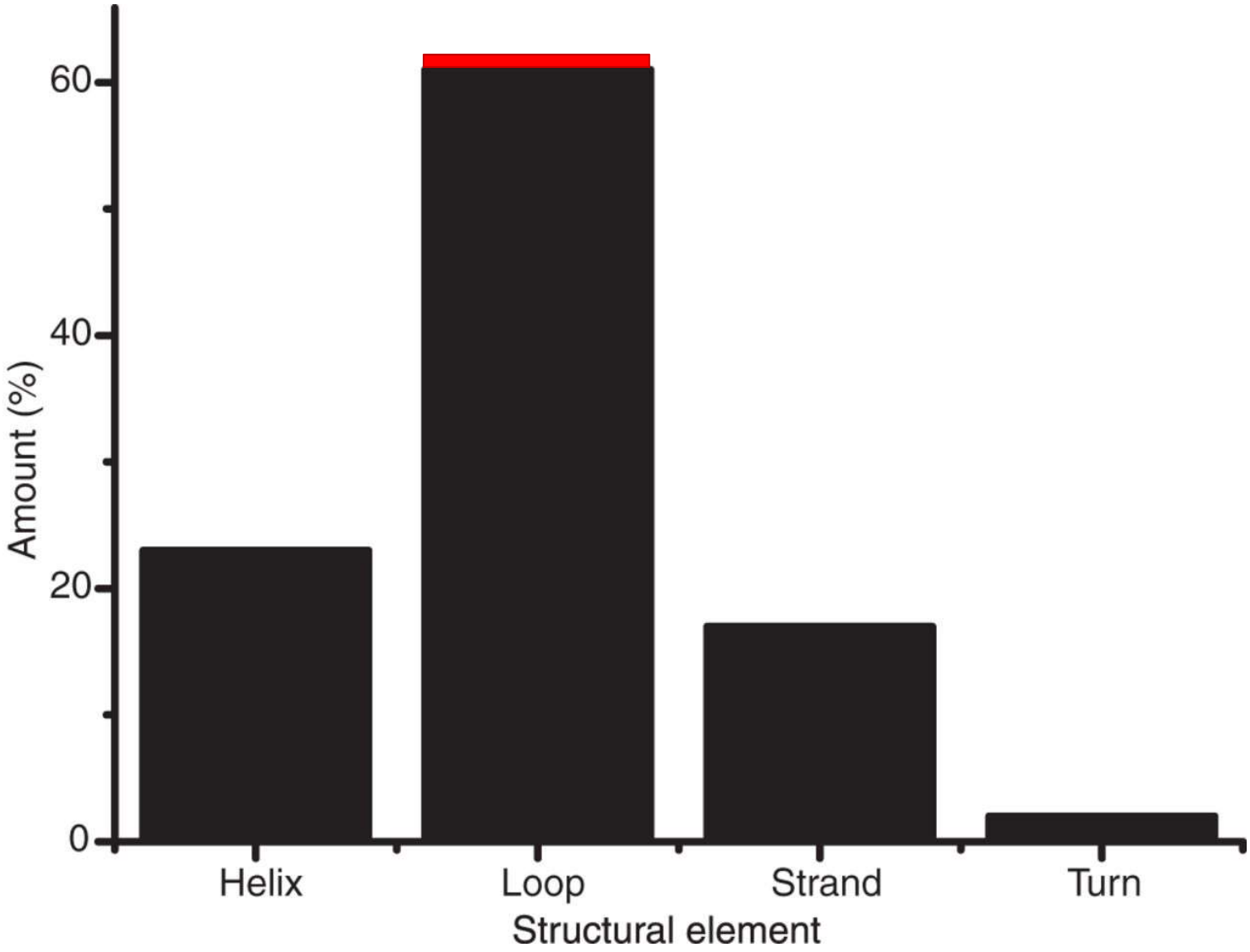
IDENTIFICAZIONE PEPTIDI

Identified by **LC-MS/MS** Spettrometria massa.

549 reactive Gln residues

PDB e UniProt database = identificazione peptidi e e proteine

Secondary structure localization of reactive Gln residues (n=389)



Camilla Lund Nikolajsen et al. J. Biol. Chem. 2014;289:6526-6534

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IDENTIFICAZIONE PEPTIDI

Identified by **LC-MS/MS** Spettrometria massa.

The identified substrates are listed according to the total number of spectral counts.

The number of spectral counts was used to evaluate the **level** of BPA incorporation **over time**

U for up-regulated or D for down-regulated. No regulation is indicated by N

	Accession number	Name	Sites	Spectral counts			Clot ID
				30 min	60 min	180 min	
1	P02671	Fibrinogen α-chain	8	U	N	N	×
2	P01023	α_2-Macroglobulin	15	U	U	N	×
3	P00488	Coagulation factor XIII A chain	8	U	U	N	×
4	P00747	Plasminogen	20	U	U	N	×
5	P00734	Prothrombin	11	U	U	N	×
6	P19823	Inter-α-trypsin inhibitor heavy chain H2	16	U	U	N	×
7	P06727	Apolipoprotein A-IV	18	U	U	U	×
8	P01024	Complement C3	27	U	U	N	×
9	P02787	Serotransferrin	10	U	U	U	×
10	P0C0L5	Complement C4-B	19	U	U	N	
11	P19827	Inter-α-trypsin inhibitor heavy chain H1	11	U	N	N	×
12	P10909	Clusterin	5	U	U	U	×
13	P02679	Fibrinogen γ-chain	5	U	N	N	×
14	P08697	α_2-Antiplasmin	11	U	U	U	×
15	P07360	Complement component	7	U	U	N	

Sites = number of reactive Gln residue

Clot ID indicates that the substrate was cross-linked to the plasma clot.

U for up-regulated/D for down-regulated.
No regulation is indicated by N if the change is less than 30%.

Verifica presenza peptidi nel coagulo

The plasma fraction was recalcified and allowed to clot for 2h at37°C.

To remove non covalently bound proteins, the clot was washed three times 1) 20mM Tris-HCl, 2 M NaCl, 2) 6 M guanidine HCl and 3) water.

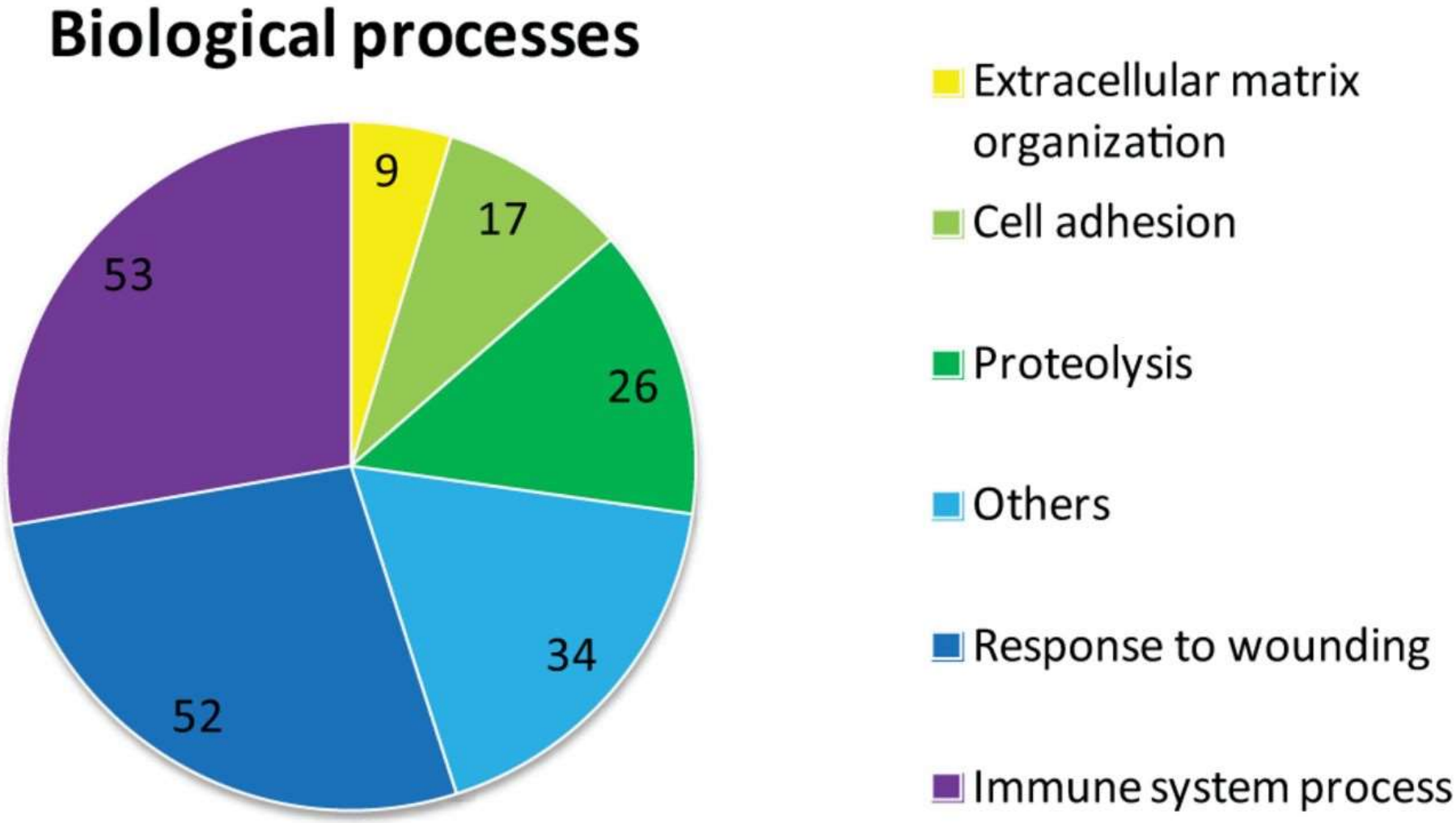
The sample was boiled in 0.1% SDS and separated by SDS-PAGE.

Covalently cross-linked proteins were retained in the stacking gel and could be collected after.

The sample was digested with trypsin for16h at 37°C.

The tryptic peptides were collected and micropurified, and were analyzed by mass spectrometry/ion chromatography

Gene Ontology summary of the identified FXIIIa substrates.



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