Pharmacology: the thrombolytic agents

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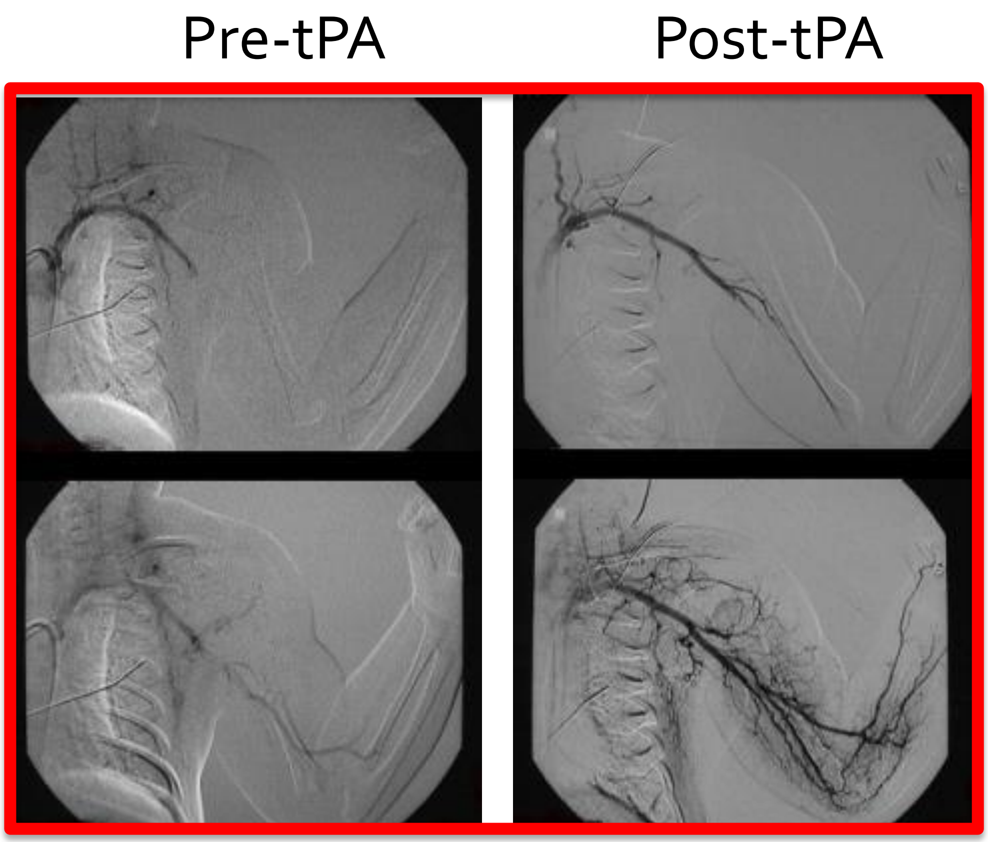
Fibrinolysis

- Physiology and normal mechanisms

- Therapeutics and fibrinolysis



Haemostatic Plug Formation

Response to injury to endothelial cell lining

Formation of an unstable platelet plug

platelet adhesion

platelet aggregation

Limits blood loss + provides surface for coagulation

⇓

Stabilisation of the plug with fibrin

blood coagulation

Stops blood loss

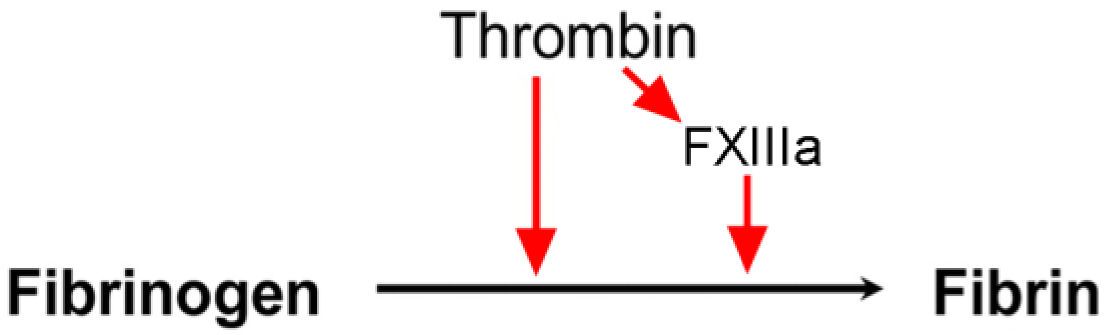
⇓

Vessel repair and dissolution of clot

Cell migration/proliferation & fibrinolysis

Restores vessel integrity

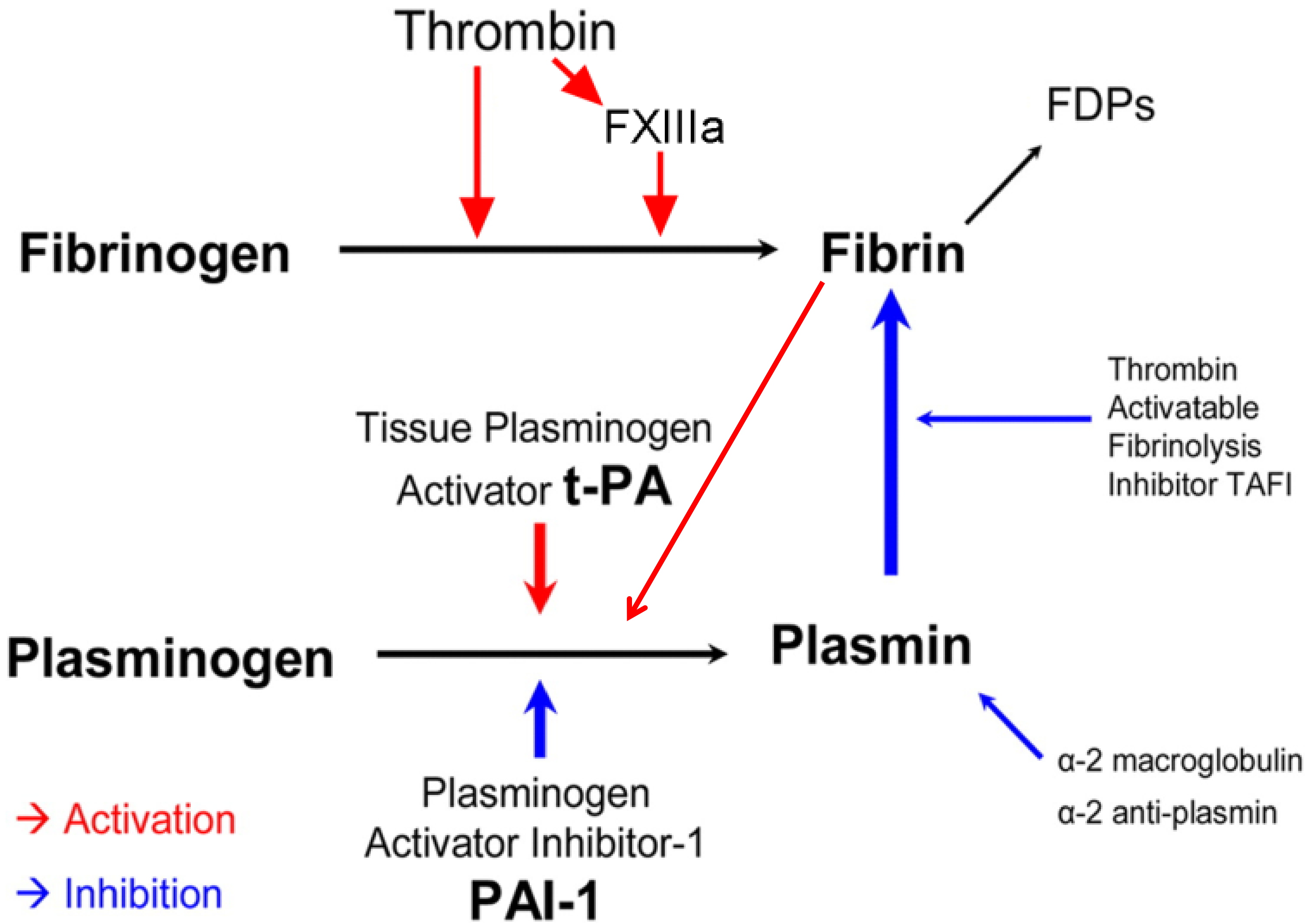
Fibrin formation



→ Activation

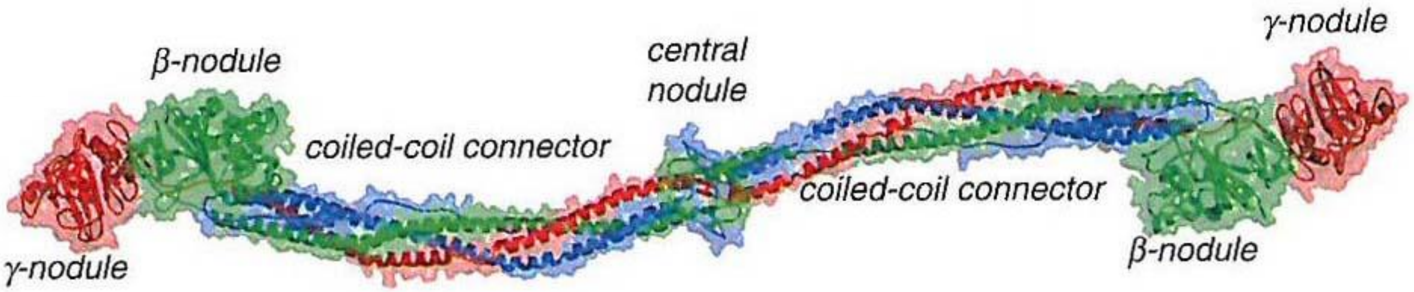
→ Inhibition

Fibrinolysis

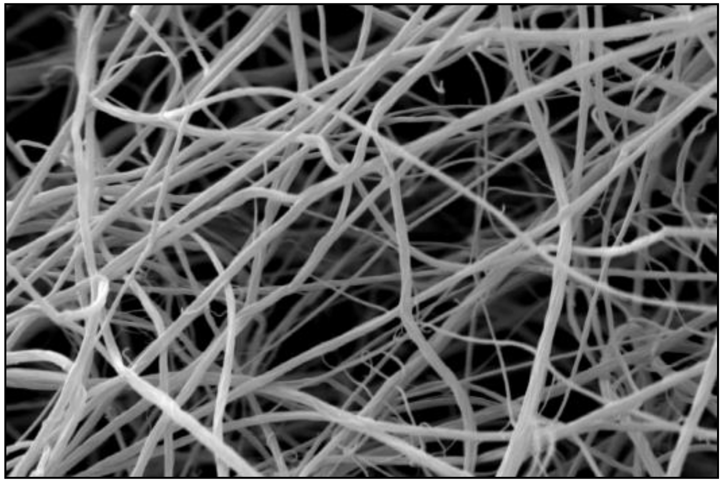


Fibrinogen (FGN)

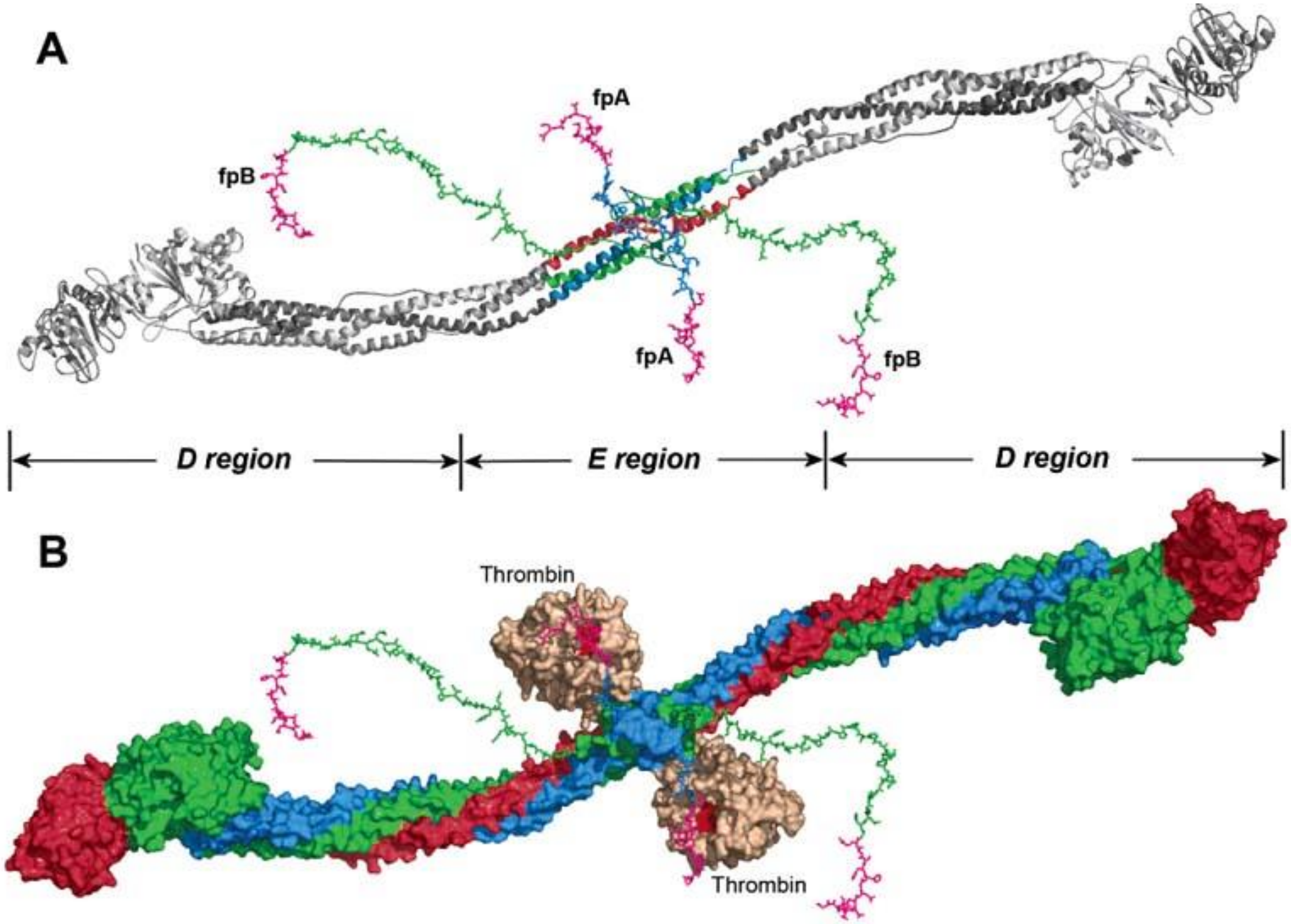
- 340kDa glycoprotein
- 6 polypeptide chains: 2α, 2β, 2γ



- Functions
  - precursor of insoluble fibrin
  - platelet to platelet adhesion



Cleavage of fibrinopeptides by thrombin



FpA is cleaved before FpB

Pechik et al 2006

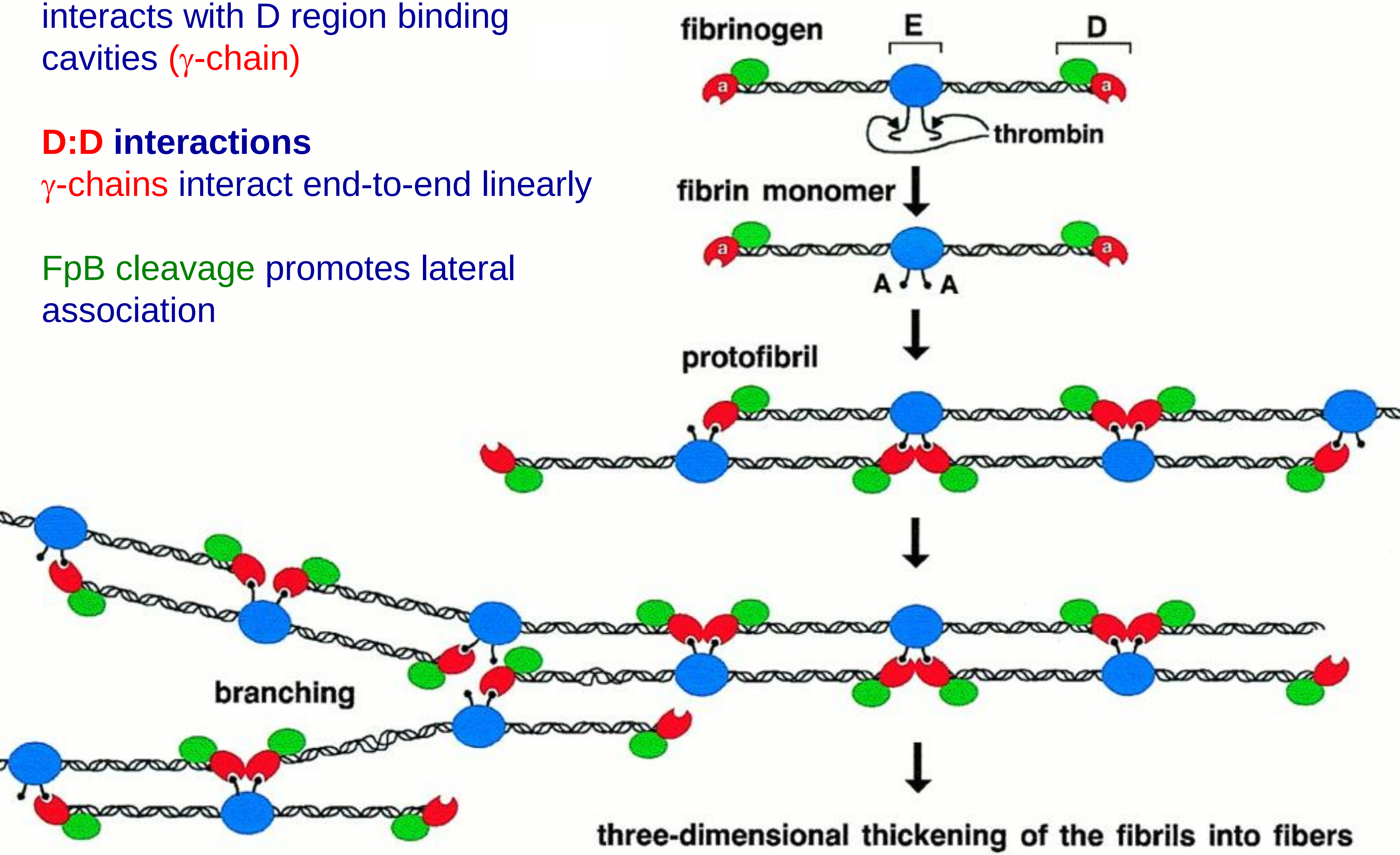
E:D interactions

E region (α-chain) spontaneously interacts with D region binding cavities (γ-chain)

D:D interactions

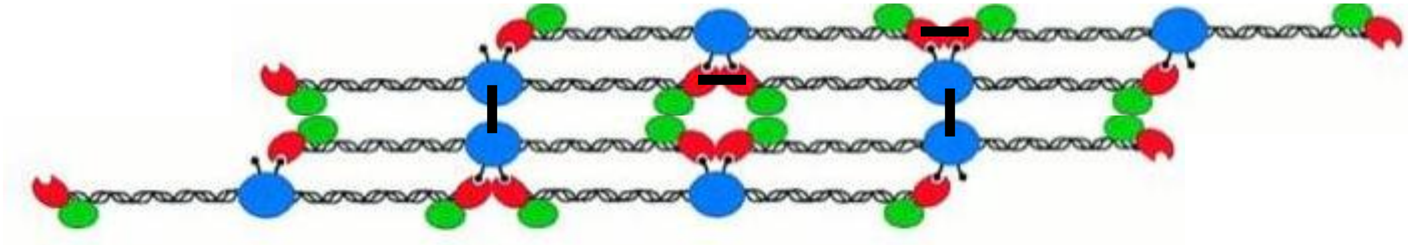
γ-chains interact end-to-end linearly

FpB cleavage promotes lateral association

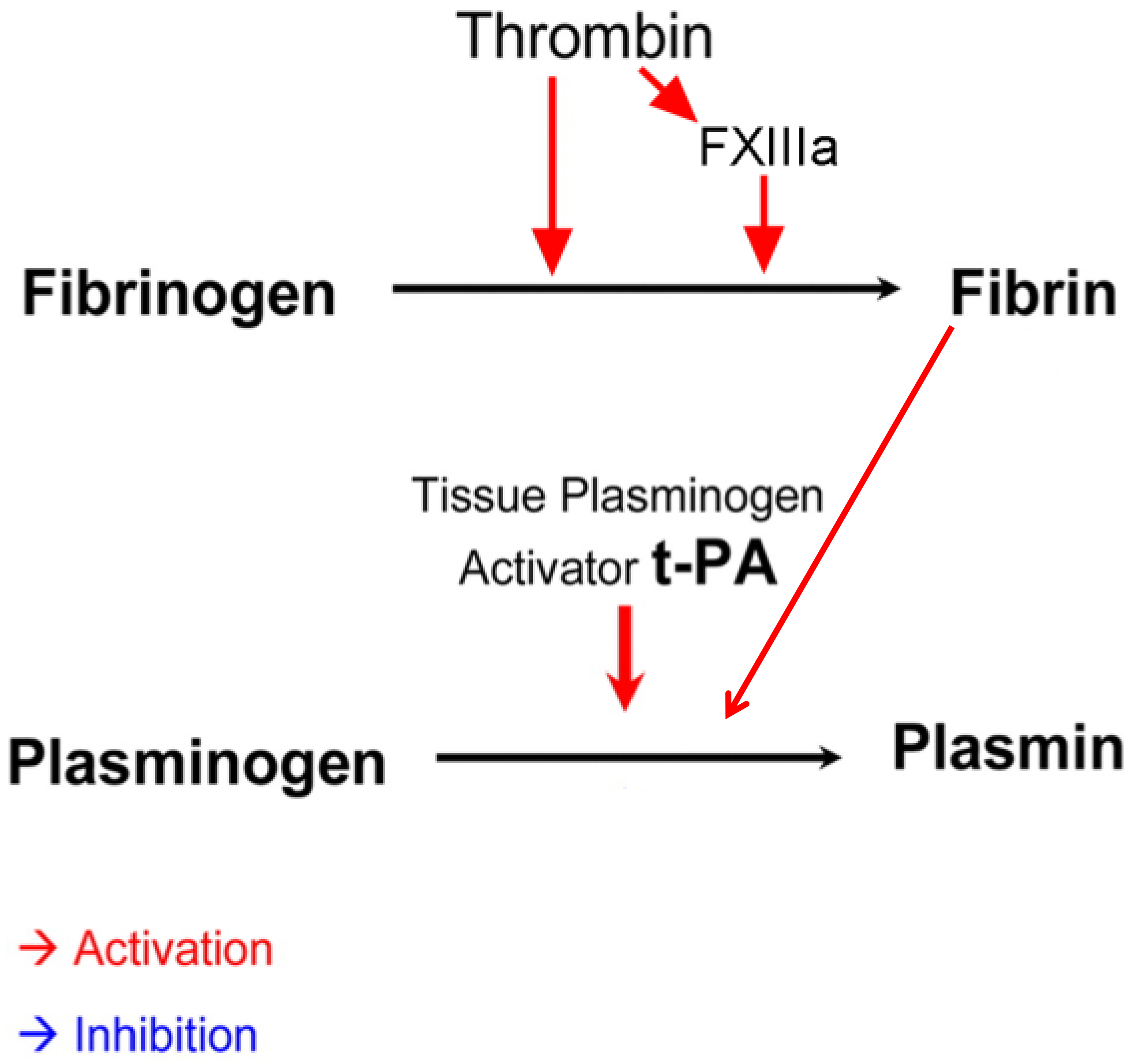


Cross-linking of fibrin

- Fibrin fibres are cross-linked by FXIIIa
- FXIIIa is a transglutaminase, activated by thrombin on fibrin surface
- FXIIIa catalyses the formation of covalent bonds between lysine (Lys) and glutamine (Gln) residues
- Acts to reinforce fibrin fibrils
- Firstly γ-γ cross-links formed
- Subsequently α-chain globular domains cross-link laterally



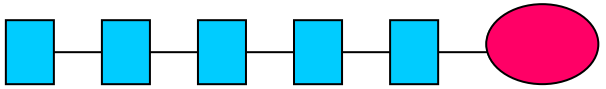
Fibrinolysis



Fibrinolytic zymogen-proteases

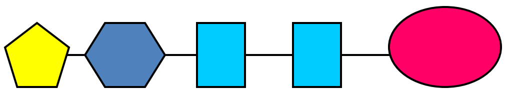
Plasminogen

- synthesized in liver, most abundant protease zymogen
- must be activated to plasmin
- plasmin has very broad substrate specificity
- plasminogen binds fibrin via lysine-binding kringle modules



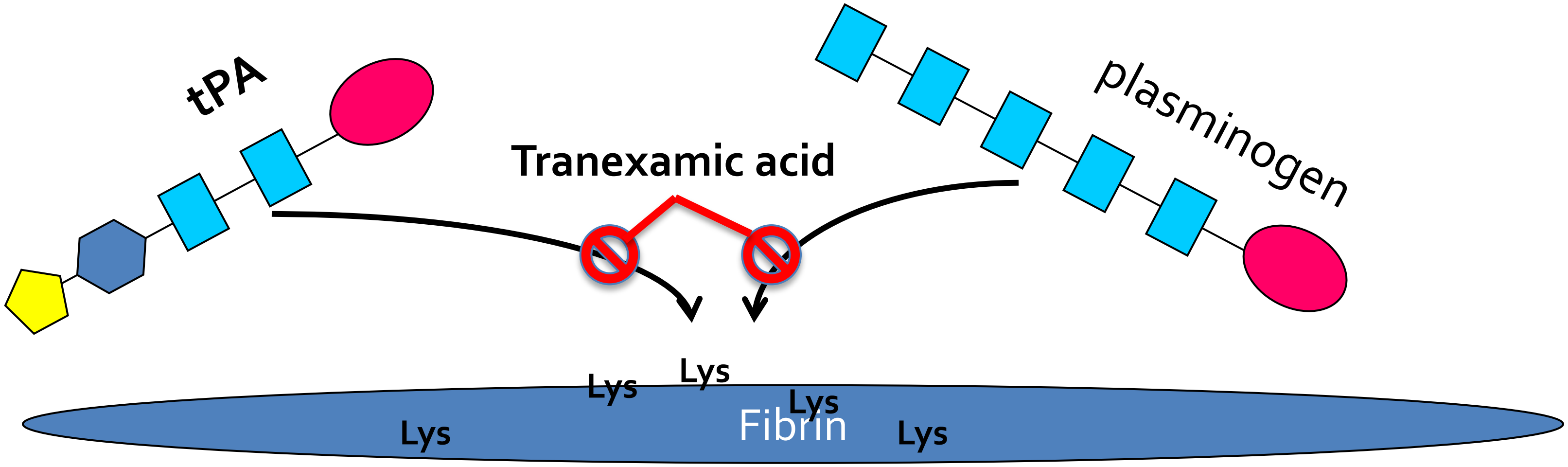
tPA, tissue plasminogen activator

- expressed by endothelial cells
- “active zymogen”
- activity stimulated by fibrin
- Low plasma conc. 3-6 ng/ml
- Released acutely during
    - Strenuous physical exercise
    - Vascular injury
    - Infusion of vasopressin

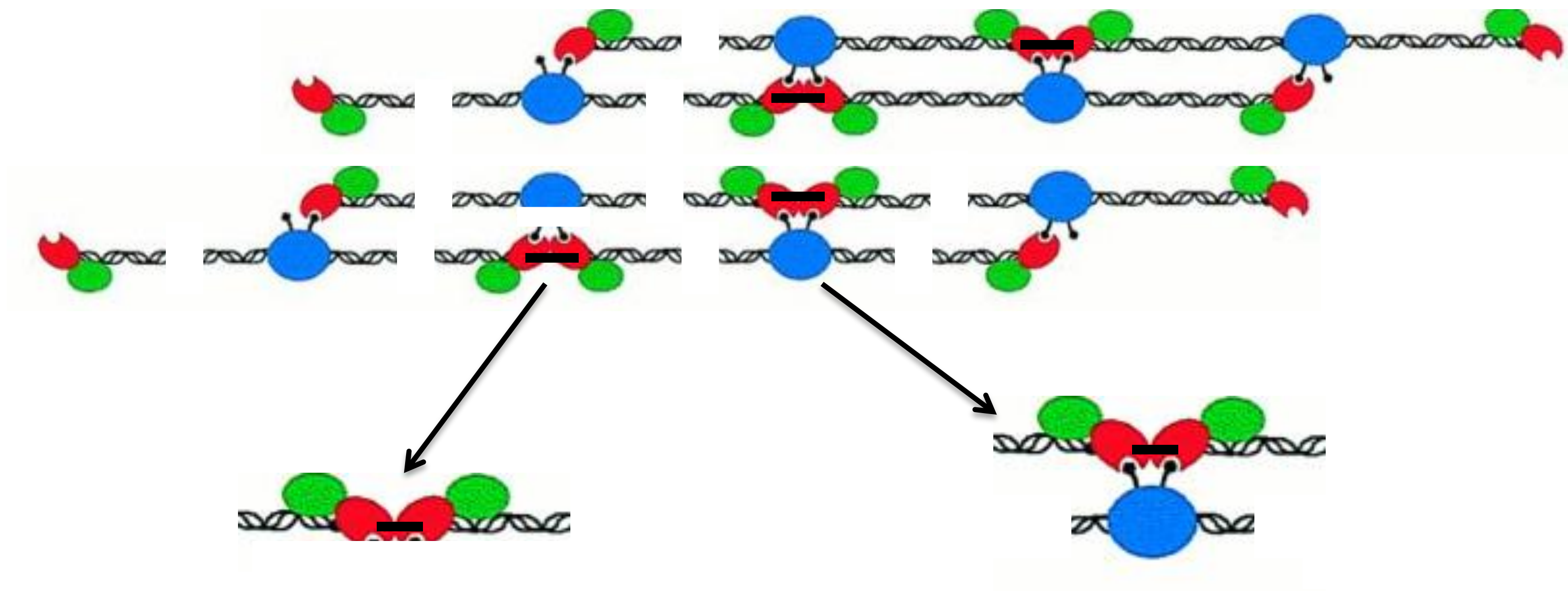


Fibrin stimulates fibrinolysis

- Plasminogen binds to lysines in fibrin via Kringle domains
- tPA binds to lysines in fibrin via Kringle domain and finger/Fn3 domain
- → enhances plasmin generation ≈ x1600
- Tranexamic acid (lysine analogue) is an anti-fibrinolytic agent that competes with fibrin for Plg & tPA



Characteristic degradation products of cross-linked fibrin



D-D (D-dimer)

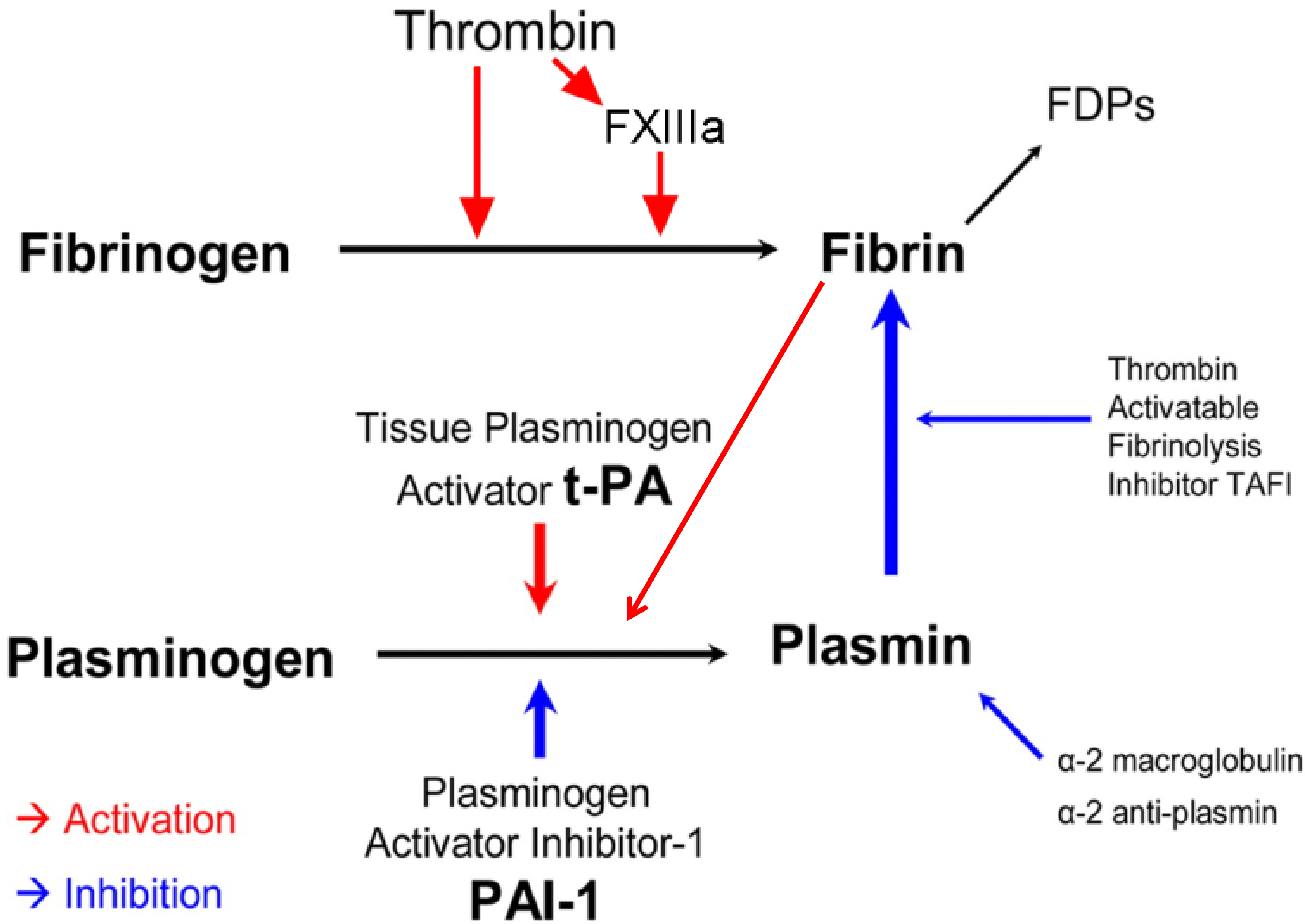
D-D-E (D-dimer E)

5-6 major plasmin cleavage sites
But 50-60 can be cleaved in total

D-dimer is measured in patients with DVT/PE and DIC

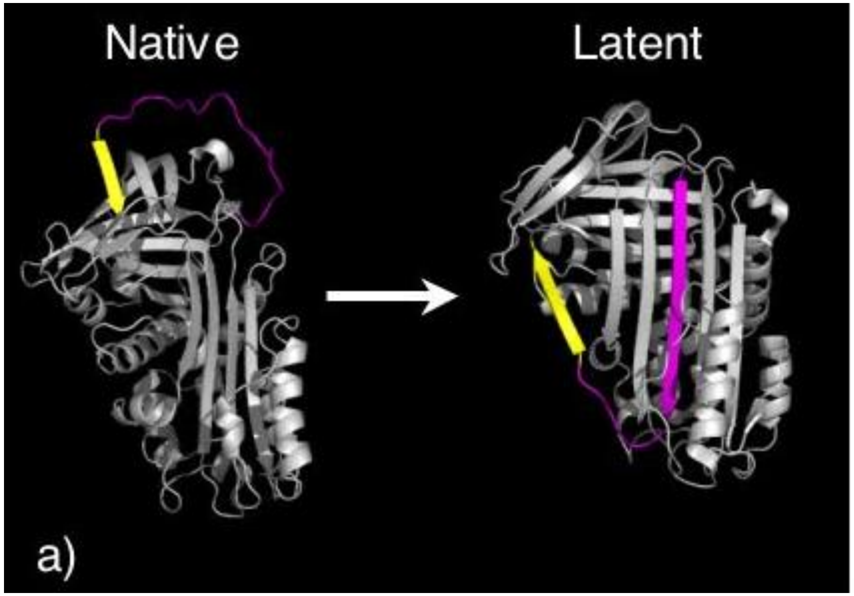
REGULATION OF FIBRINOLYSIS

Fibrinolysis



Regulation of fibrinolysis: PAI-1

Inhibitor of tPA



PAI-1 (plasminogen activator inhibitor 1), 52 kDa

Produced by endothelium

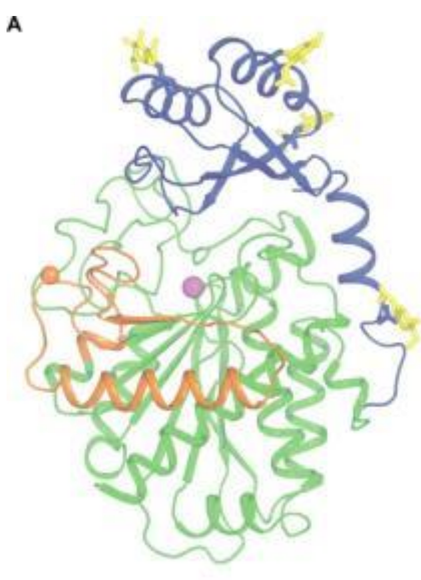
Converts spontaneously into latent form

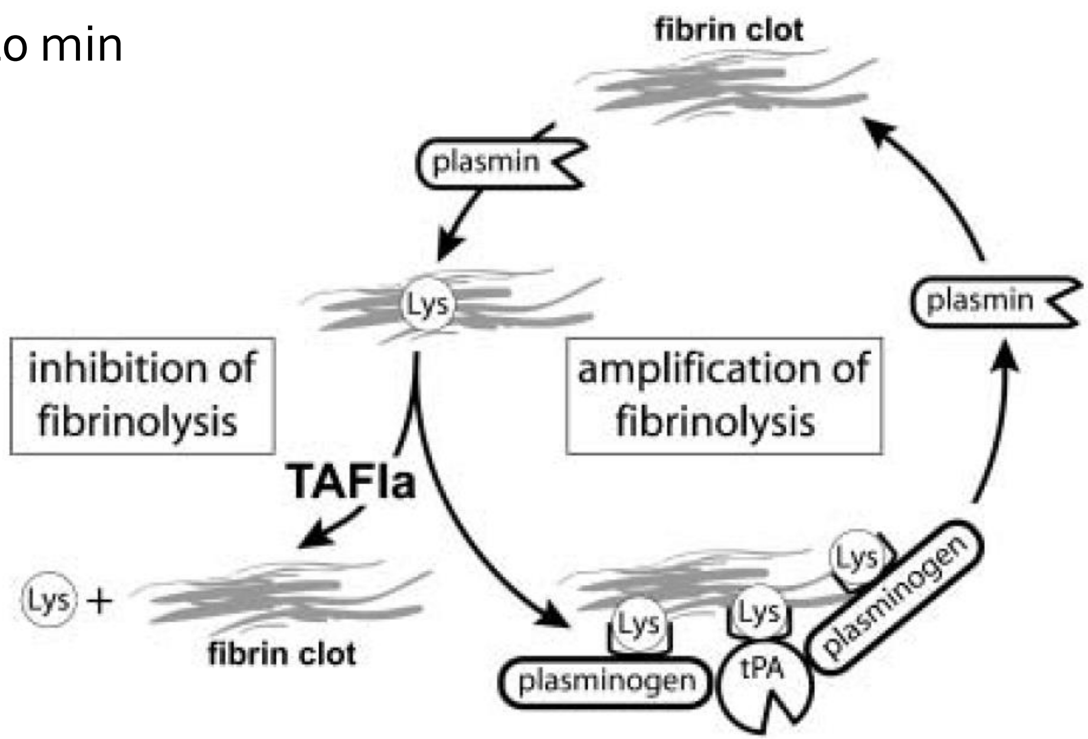
Member of Serpin family, inhibits tPA and uPA

Regulation of fibrinolysis: TAFI

TAFI (Thrombin Activatable Fibrinolysis Inhibitor), 401 AA

- Procarboxypeptidase
- Cleaves C-terminal lysine residues on fibrin
- Inhibits plasminogen and tPA binding
- Activated by TM-thrombin complex
- Half-life of activated TAFI 10 min



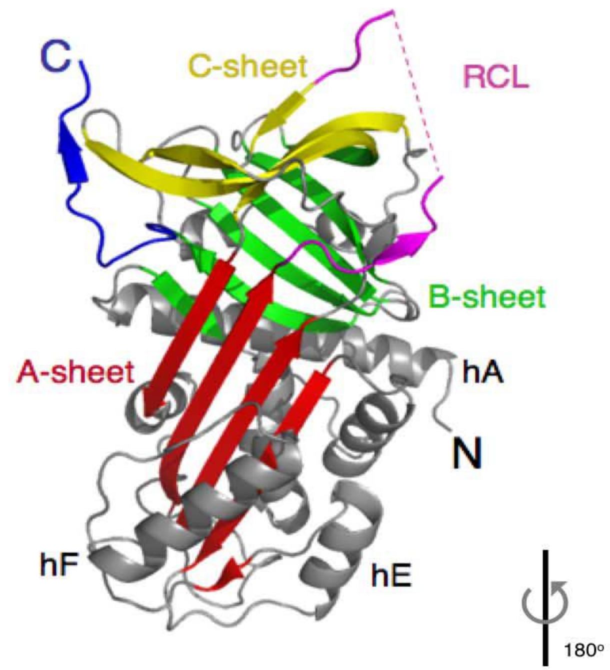


Marx PF. Blood 2008; 112: 2803

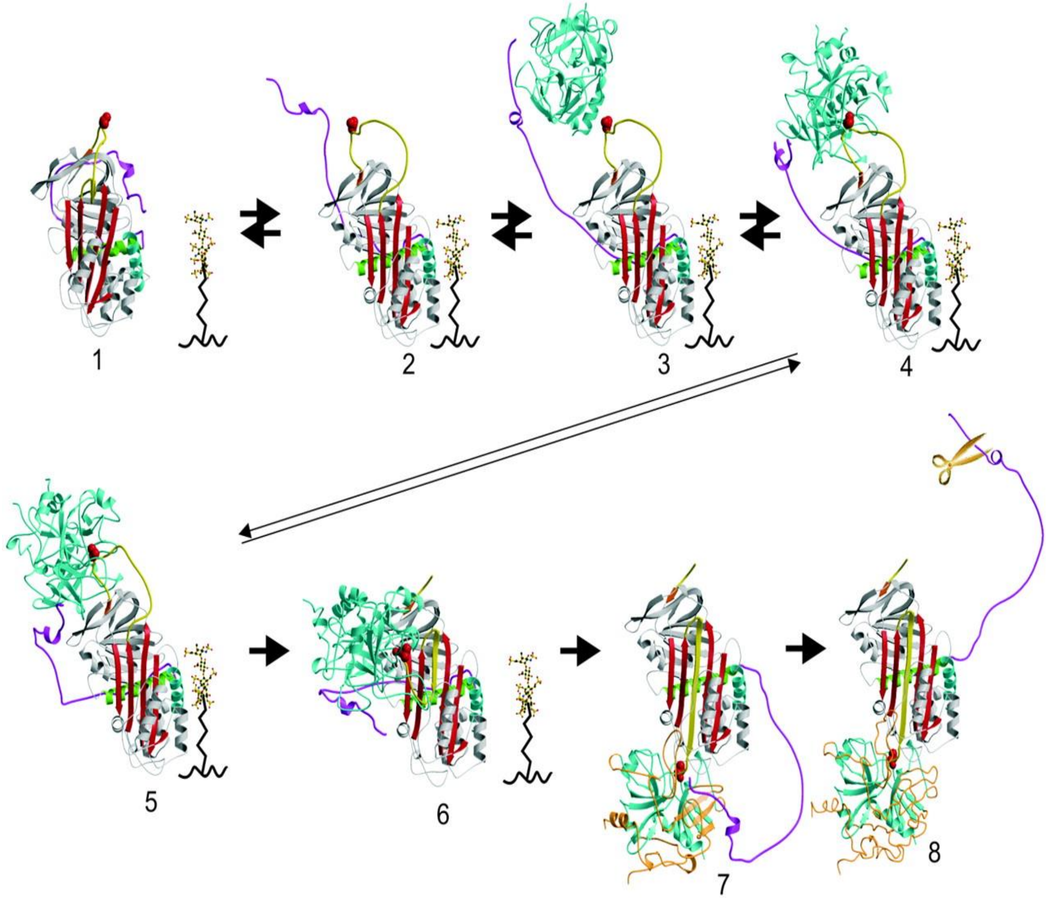
Bouma BN. Ann Med 2006; 38: 378

Regulation of fibrinolysis: a2-antiplasmin

Inhibitor of plasmin



Mechanism of inhibition



Baglin T. P. et.al. PNAS 2002;99:11079-11084

α2-antiplasmin

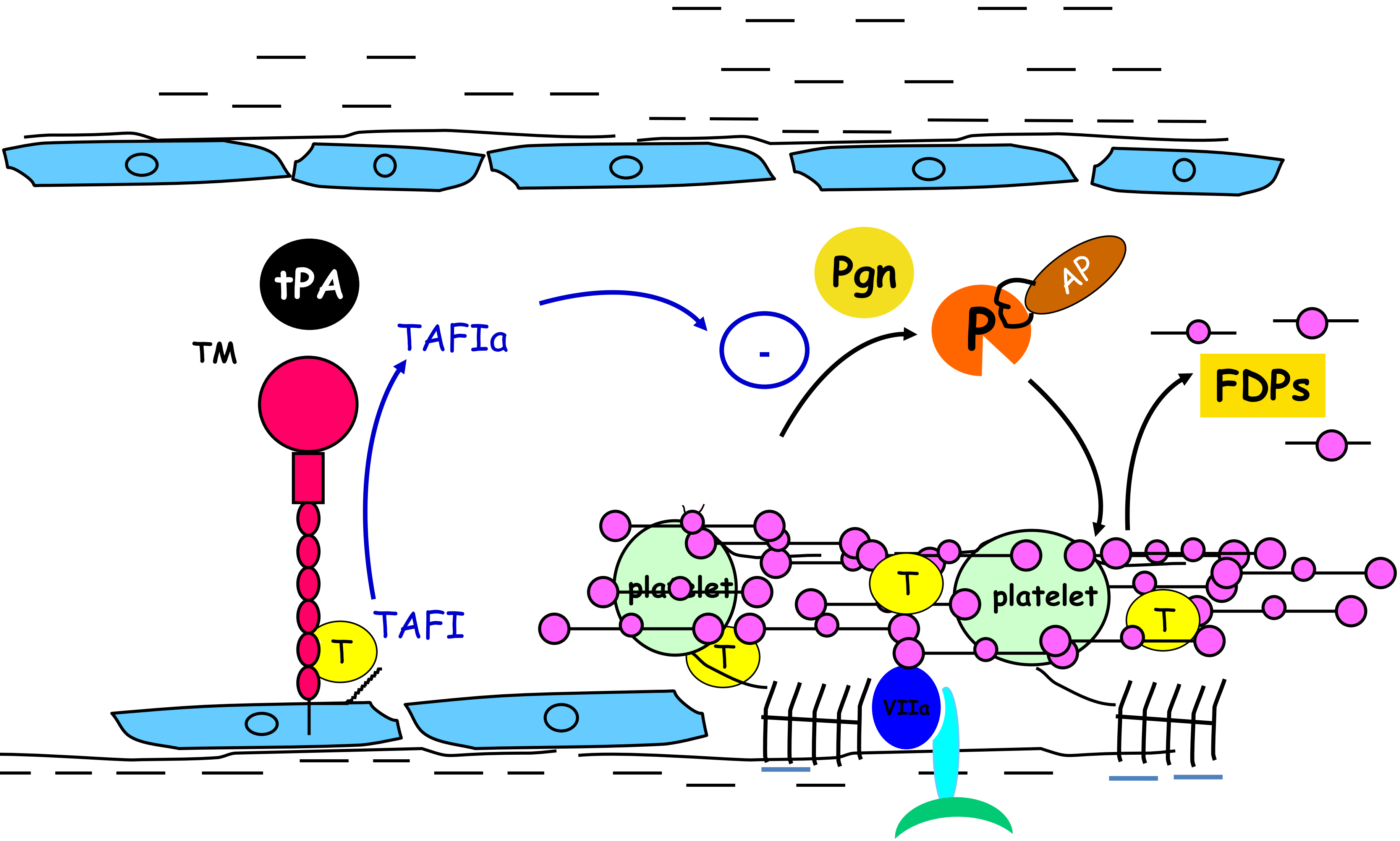
70 kDa, produced by liver

Gets cross-linked to fibrin by FXIIIa via Q14

Half life of plasmin in plasma is ~0.1 secs!!

Summary on fibrinolysis

- Initiated by the release of tPA by endothelium
- tPA converts plasminogen into plasmin which will cleave fibrin in several sites
- Regulated at every step by PAI-1, α2-AP and TAFI



Fibrinolysis

THE UROKINASE SYSTEM

Urokinase (uPA) - uPAR

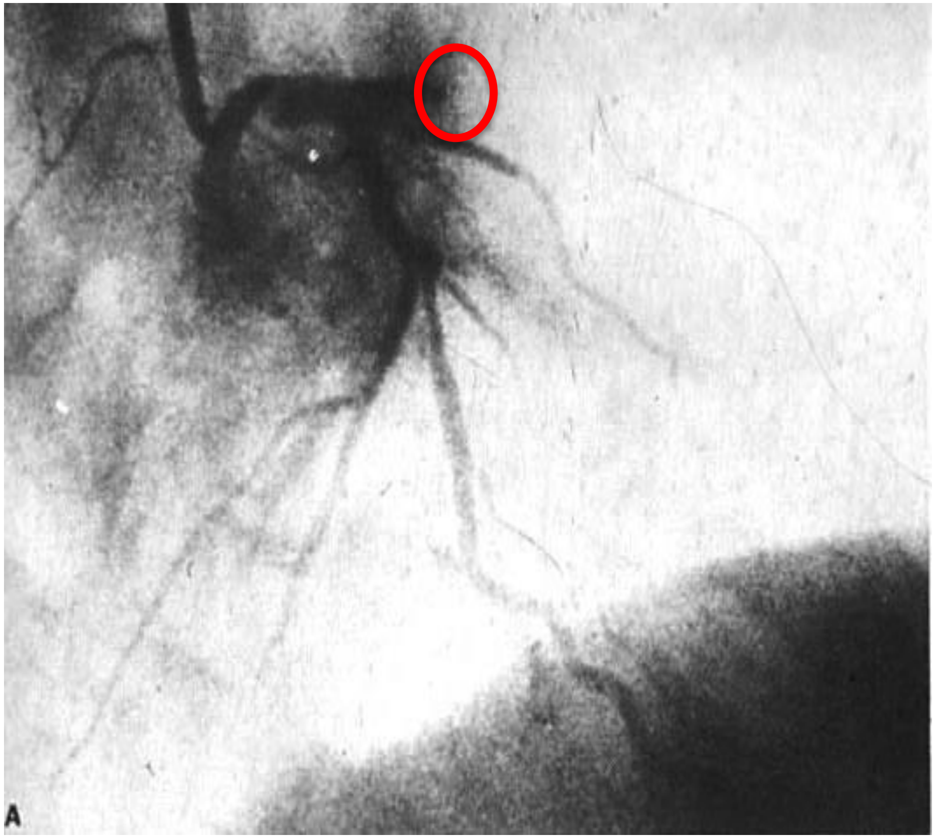
- Produced by monocytes, macrophages and urinary epithelium
- Cell based fibrinolytic system
  - uPAR – GPI linked protein on EC and monocytes
  - Pro-uPA is activated by binding to uPAR
- Many effects (mainly extravascular system)
  - Fibrin cleavage
  - TGF activation
  - Tissue growth, invasion, angiogenesis

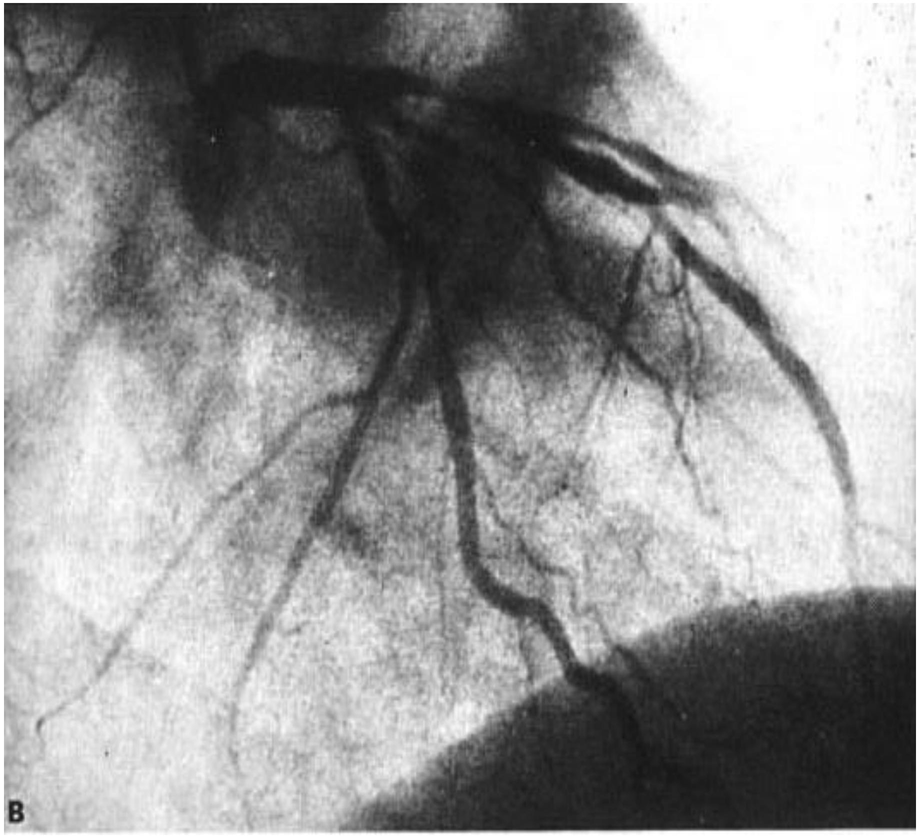
- Compared to tPA, uPA has lower affinity for plasmin and doesn't require fibrin as a co-factor

THERAPEUTIC FIBRINOLYSIS

Thrombolysis for treatment of AMI

before **after**

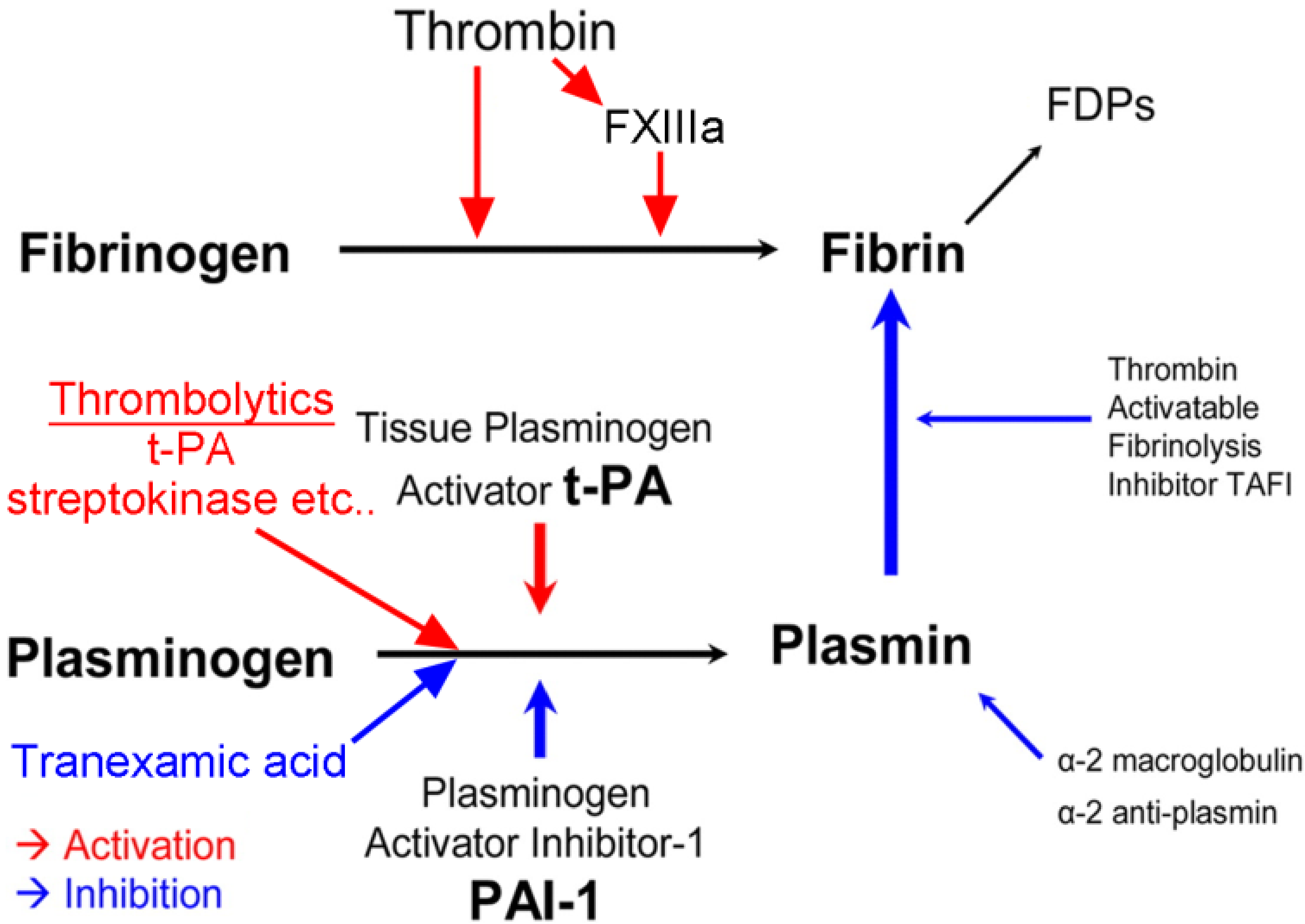




The major clinical use now is for

1) Ischaemic stroke
2) VTE (massive PE, CDT for extensive DVT)

Fibrinolytic therapies



Therapeutic fibrinolytic agents

Name	Protein	MW (kDa)	1/2-life (mins)	Fibrin selectivity	PAI inhibition	
Alteplase[1,2,3]	rtPA (CHO)	70	3 - 8	++	Yes	
Monteplase	rtPA (CHO)	68	23	++	Yes	Reduced clearance mutant
Tenectoplase[1]	rtPA (CHO)	70	11 - 20	+++	No	Increase fibrin selectivity & reduced PAI inhibition mutant
Reteplase[1]	r truncated tPA (e.coli)	39	14 - 18	+	Yes	Reduced clearance, but reduced fibrin selectivity
Lanoteplase	r truncated tPA (CHO)	54	23 - 37	+	Yes	Markedly reduced clearance mutant & normal fibrin selectivity
Pamiteplase	r truncated tPA (CHO)	58	30 - 47	++	Yes	Markedly reduced clearance mutant, but also reduced fibrin selectivity
Microplasmin	SP domain of plasmin	28	4 secs		-	Direct thrombolytic, short half-life limits systemic effects
Streptokinase[1,2,4]	SK (streptococcus protein)	47	20	-	No	Bacterial protein - non-proteolytic activation of Plg, no fibrin selectivity, immune response can occur
Desmoteplase	Vampire bat PA	42	180	++++	Relatively resistant	Enhanced fibrin selectivity and v long half life
Staphylokinase	SAK (S.aureus enzyme)	15	4 - 20	+++	No	Good fibrin selectivity and reduced plasmin inhibition on fibrin, immunogenic
Abbokinase[1,2]	ruPA (CHO)	31	12	-	Yes	uPA lacks fibrin specificity

Licensed in the UK for 1) MI, 2) PE, 3) IS, 4) DVT

Therapeutic fibrinolytics 2

- Alteplase
  - Recombinant single-chain tPA
  - 'Clot selective'
    - Activity enhanced in presence of fibrin ie higher ratio of activity for fibrin-bound plasminogen v plasma plasminogen
  - Not antigenic
  - Half-life 5-8 minutes
  - For stroke: 0.9mg/kg per kg body weight (max 90mg); IV infusion over 90 mins with 10% of total dose administered as initial IV bolus (1-2mins)

Therapeutic fibrinolytics 1

- Streptokinase
  - Non-enzymatic protein, MW 47kDa
  - Extracted from beta-haemolytic streptococci
  - Complexes with plasminogen→ conformational change, increasing enzymatic activity
  - Antigenic
    - Action reduced by anti-streptococcal antibodies already present (previous streptokinase 5days-1year, or recent streptococcal infection)
    - Anaphylactic reactions unusual
  - Half-life 23 mins
  - Infusion: 60mins for MI, longer for VTE
  - Hypotension

Other plasminogen activators used in thrombolysis

- Urokinase
  - uPA or urokinase type plasminogen activator
  - Originally found in urine; present in extracellular matrix
  - Binds to UPAR on cells enhancing its activity – cell specific plasminogen conversion
  - Prepared from cultures of HEK cells
  - Not antigenic
  - Loading dose over 10 minutes, then infusion
  - NB recombinant urokinase plasminogen activator also available
- Reteplase
  - Retavase, Rapilysin
  - Recombinant non-glycosylated shorter tPA (357/527) made in E coli
  - Longer halflife than alteplase of 13-16 min.
  - Lower fibrin binding than tPA
- Tenecteplase
  - Recombinant full-length, modified tPA made in CHO cells
  - Thr103Asp, Asp117Glu, 296-299Ala
  - Higher fibrin specificity than tPA
  - Resistant to inhibition by PAI-1

Limitations of therapeutic fibrinolysis 1

- Bleeding ~5% (0.5-0.7% risk ICH)
- Contraindications include
  - Past history of ICH
  - Major surgery within 14 days
  - History GI/GU haemorrhage last 3 weeks
  - Arterial puncture at a non-compressible site within last week
  - Uncontrolled HTN (>185/110)
  - Concomitant anticoagulant (eg SPC states INR <1.3 pre-alteplase, local/national guidelines on DOACs)

Limitations of therapeutic fibrinolysis 2

- Neurotoxicity
- Off-target substrates
  - FVa, FVIII
  - fibronectin, collagen, laminin, prekallikrein,TFPI, VWF, TSP, proMMP's, procarboxypeptidase B
- Limited therapeutic window (3 - 4.5hrs)

Bleeding post fibrinolysis

- tPA very short half life, but can generate hyperplasminemia
- Effects on FV and fibrinogen are more prolonged (can take 24-36 hrs to recover fibrinogen)
- Suspect bleeding if:
  - Intracranial haemorrhage
    - Increased neurological deficit, deteriorating GCS
    - New or increasing headache
    - Acute HTN
    - Nausea and vomiting
  - Extracranial haemorrhage
    - Hypotension
    - New local symptoms or signs

Bleeding post fibrinolysis - actions

- Stop tPA infusion

- Request
  - Bloods
    - Full blood count
    - Coagulation screen
    - Fibrinogen, FDPs
    - Ensure Group and Save in place
  - CT head if concern of ICH

- Treat with replacement
  - Tranexamic acid 1g IV
  - FFP
  - Cryoprecipitate / fibrinogen concentrate

Suggested reading

- Blood Reviews 2015, 29: 17-24. Fibrinolysis and the control of blood coagulation, Chaplin and Hajjar.

- JTH 2015, 13:S290-296. Treatment of acute ischaemic stroke: from fibrinolysis to neurointervention, Jacquin and Van Adel.

- Product characteristics:
  – https://www.medicines.org.uk/emc/

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