Lupus Anticoagulant and phospholipids

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Rationale

Clotting reactions take place at phospholipid surfaces and are dependent upon the nature of that surface

LACs interfere with such reactions at these surfaces

So:

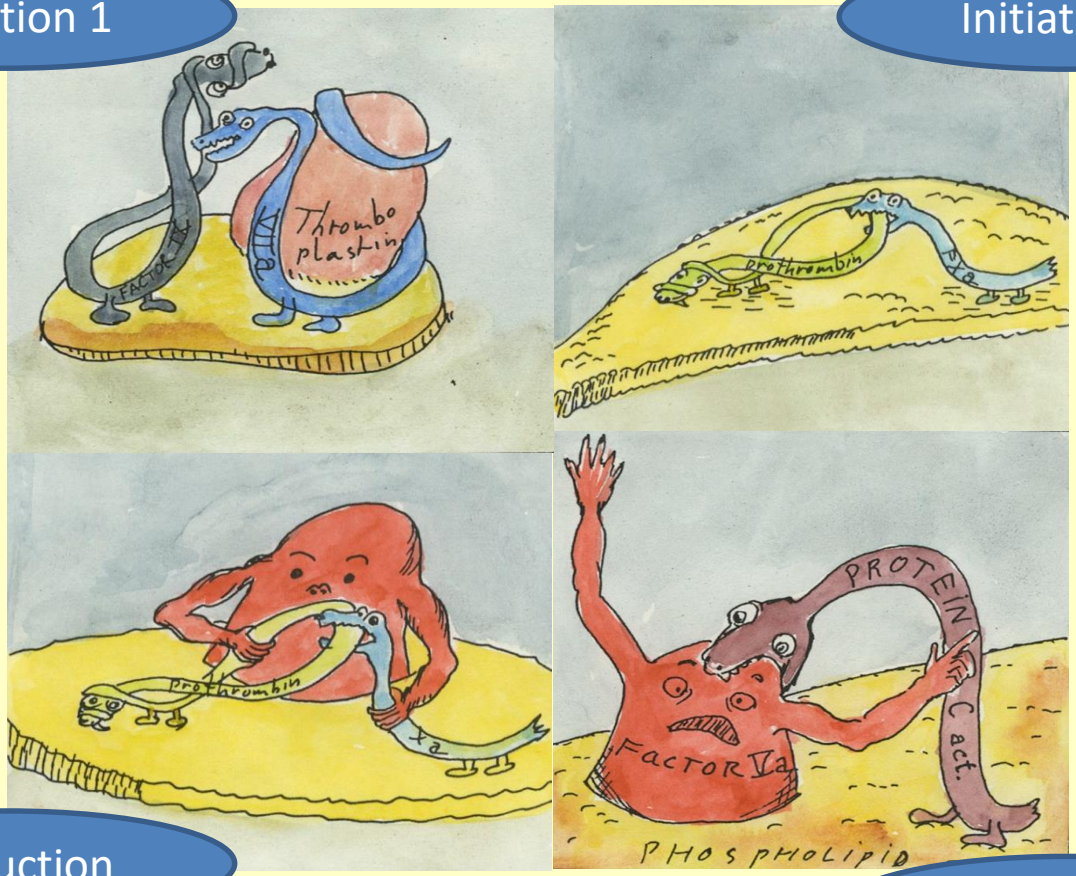
LAC effects are likely to be dependent upon the nature of these surfaces. (e.g. Hexagonal , Cardiolipin)

Practical importance: As long as the phospholipids are not well defined, results will be difficult to reproduce.

Clotting reactions at PL-interphases

Initiation 1

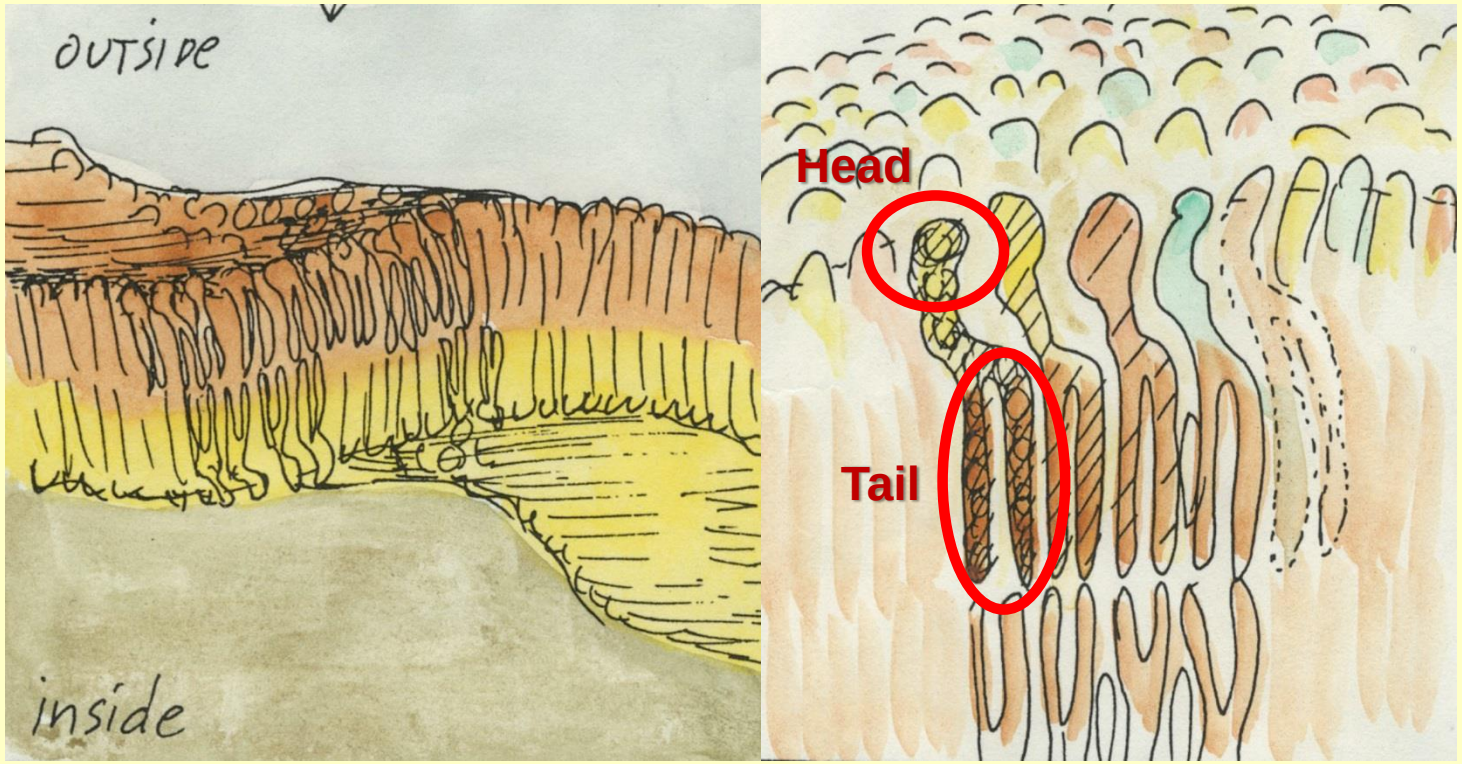
Initiation 2



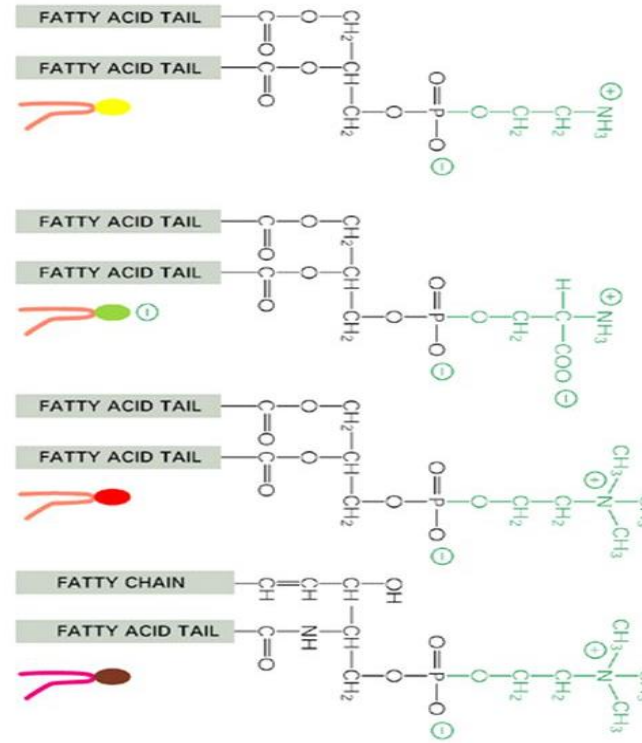
Production

Limitation

Phospholipid surfaces are variable both chemically and physically



Numerous possible Head groups

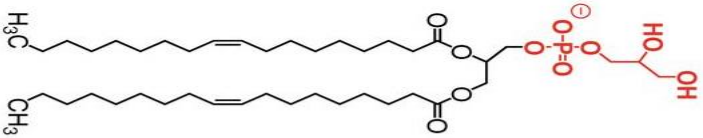


- Phosphatidylethanolamine

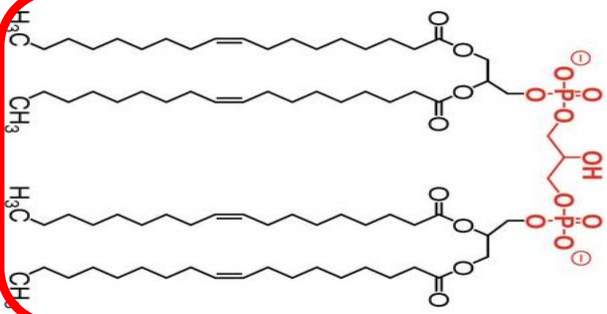
- Phosphatidylserine

- Phosphatidylcholine

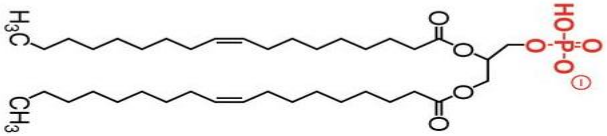
- Sphingomyeline



- Phosphatidylglycerol

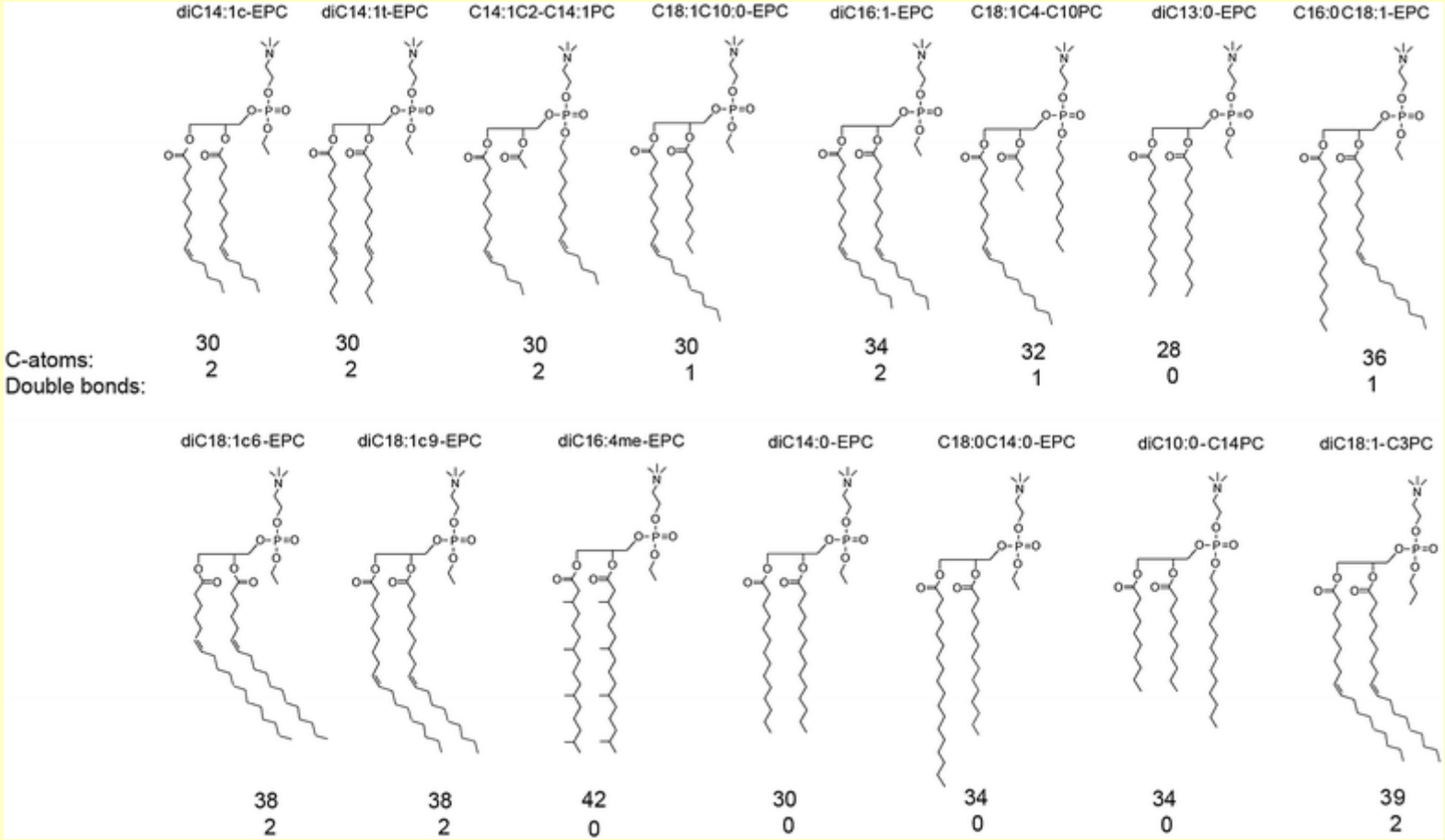


- Cardiolipin



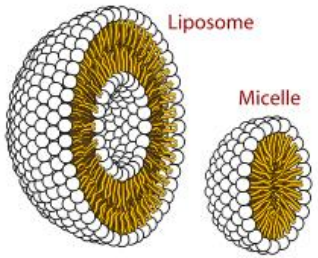
- Phosphatidic acid

Infinitely many possible tails



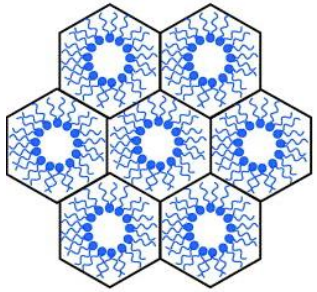
Various different Physical Forms

Usual



Hollandaise

Hexagonal 2



Mayonnaise

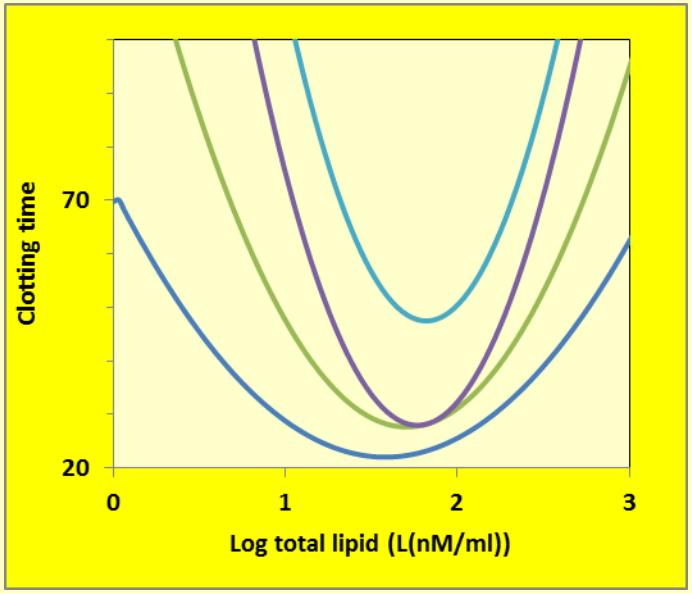
Old fashioned clotting times

Patient	Age	Diagnosis	APTT	APTT 1:1	KCT	KCT 1:1	RVV	RVV 1:1
R.A.	**30**	**SLE-TIA**	**46**	**35**	**186**	**168**	**52**	**46**
G.S.	33	PAT	36	36	98	96	60	49
C.L.	27	Hab-Ab DVT	46	37	178	126	73	46
D.S.	31	SLE	40		196			
M.S.	60	DVT	48	36	130	113	65	48
Z.R.	41	CerThr	35	34	109	111	63	
T.A.	**29**	**Hab-Ab SLE**	**75**	**66**	**110**	**97**	**41**	**38**
T.N.	36	Hab-Ab	34	30	127	100		
B.A.	56	SLE	60	43	140	121		
Control (10)			26-30		64-75		25-30	

Galli M, Béguin S, Lindhout T, Hemker HC: Inhibition of phospholipid and platelet dependent prothrombinase activity in the plasma of patients with lupus anticoagulants. Br J Haemat 1989, 72:549-55

Clotting times are extremely dependent upon

- Phospholipid concentration
- Head-group composition



Clotting times of mixtures of *dioleoylphosphatidylcholine* (PC) and *dipoleoylphosphatidylserine (PS)* *of different composition.*

100 mole% PS

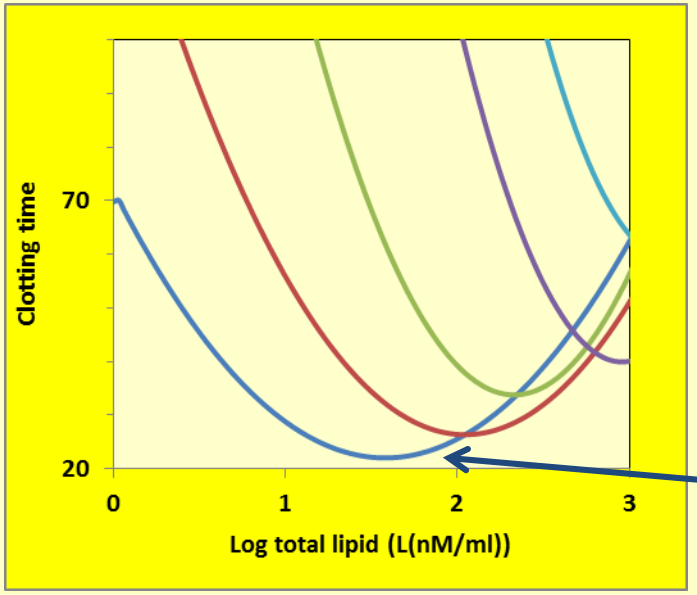
80 mole% PS

50 mole% PS

20 mole% PS

Tans G, van Zutphen H, Comfurius P, Hemker HC, Zwaal RF: Lipid phase transitions and procoagulant activity. Eur J Biochem 1979, 95:449-57

Clotting times are also extremely dependent upon membrane fluidity, i.e. admixture of Cholesterol



Clotting times of a 50:50 mixture of *dipalmitoylphosphatidylcholine* and *dipalmitoylphosphatidylserine*

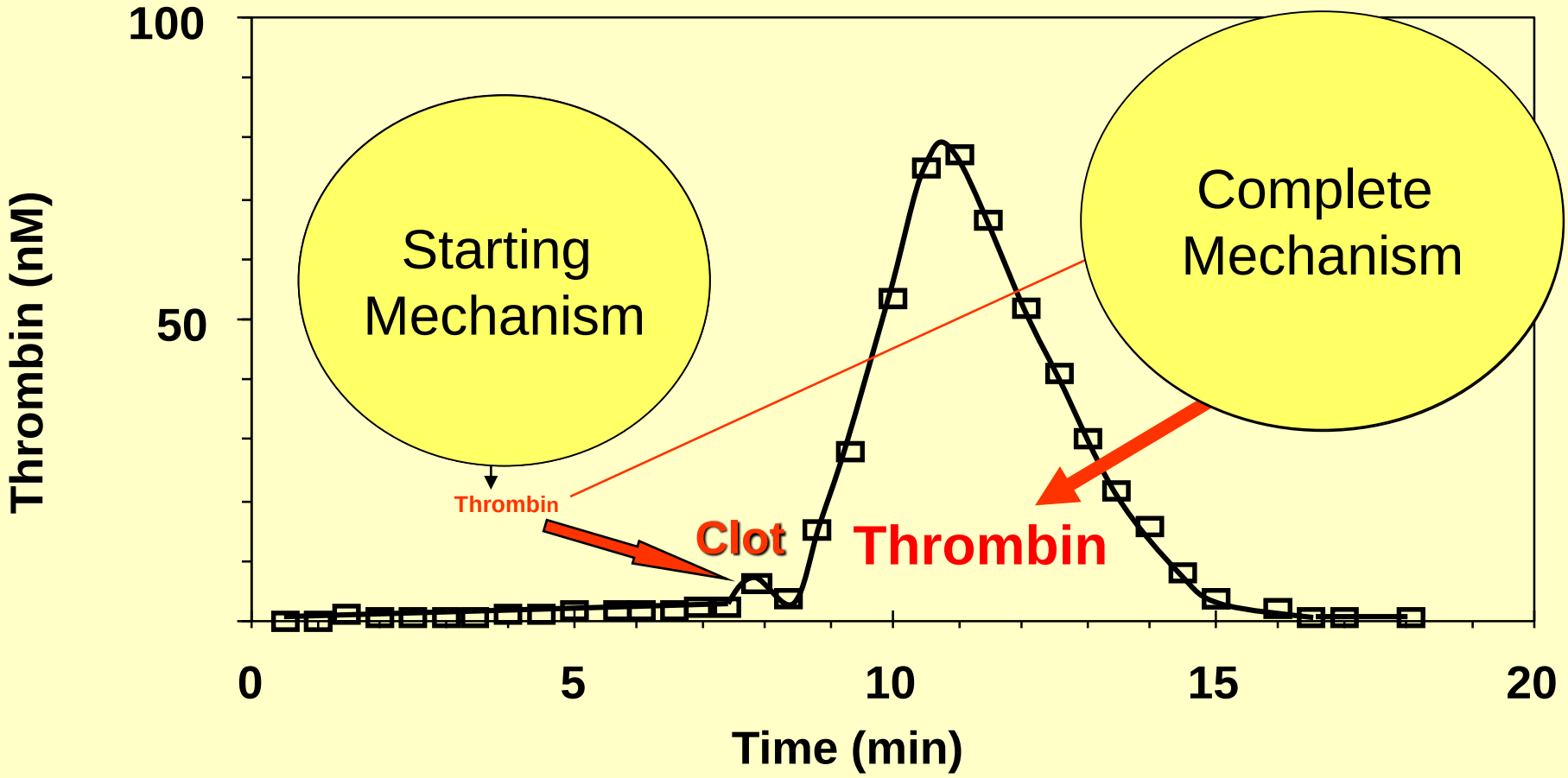
with different amounts of cholesterol:

None – 15 – 20 – 33 mole%

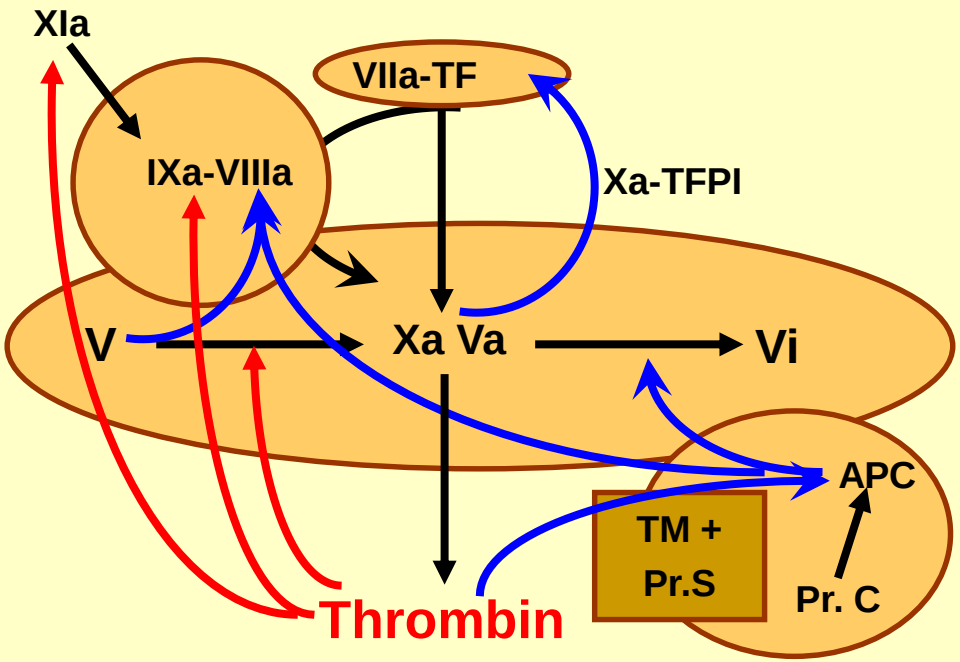
Clotting times of a 50:50 mixture of *dioleoylphosphatidylcholine* and *dipoleoylphosphatidylserine with 33 mole% cholesterol*

Tans G, van Zutphen H, Comfurius P, Hemker HC, Zwaal RF: Lipid phase transitions and procoagulant activity. Eur J Biochem 1979, 95:449-57

And then: Clotting times tell only half the story



membrane surfaces play a role at many different levels in the complete mechanism

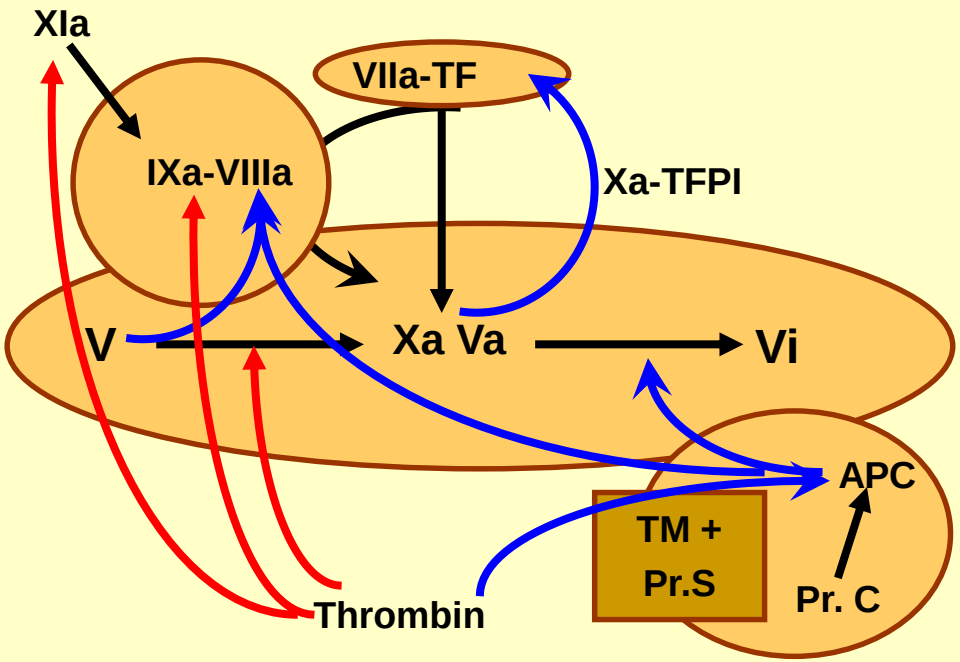


Nearest approach to their combined effect:

Thrombin generation ± APC or TM

Membrane sustained mechanisms are critically dependent upon:

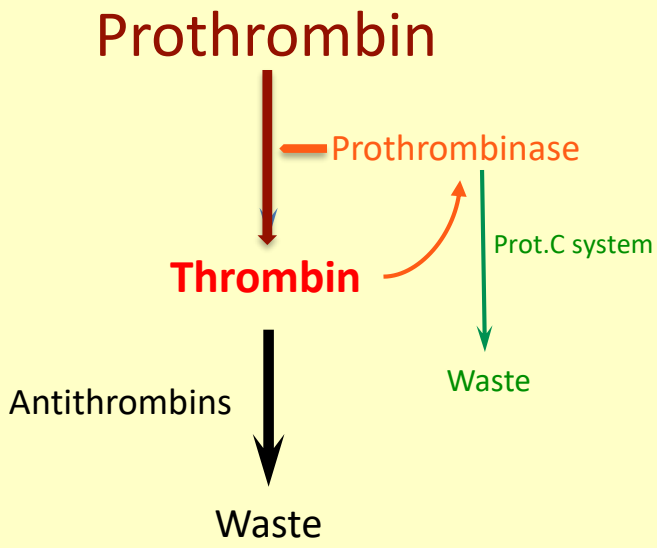
As a consequence
the effect of LAC
will be dependent as well



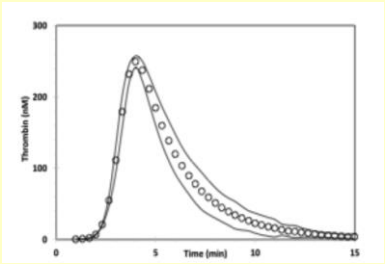
Composition of the membrane
(head groups)
Size of the membrane
(vesicles, micelles, macroscopic surface)
Fluidity of the membrane
(length of the tail, saturated unsaturated, cholesterol)

Part One: HOW ?

For understanding TG the minimal clotting scheme suffices



$$dP(t)/dt = -k_1 E(t) P(t)/(P(t) + Km)$$
$$dE(t)/dt = k_1 E(t) P(t)/(P(t) + Km) - k_2 E(t)$$
$$dT(t)/dt = k_2 E(t) - k_3 T(t)$$



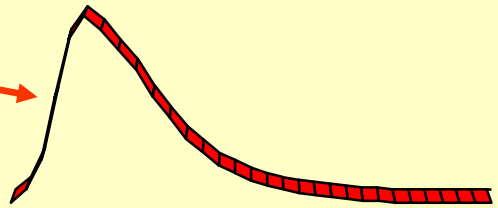
Exploring the limits of modelling thrombus formation. H.C. Hemker, S. Bloemen, P.W. Hemker
Physics of Life Reviews https://doi.org/10.1016/j.plrev.2018.06.010

Part One: HOW ?

Washbasin kinetics

Prothrombin

↓ Prothrombinase

Thrombin → 

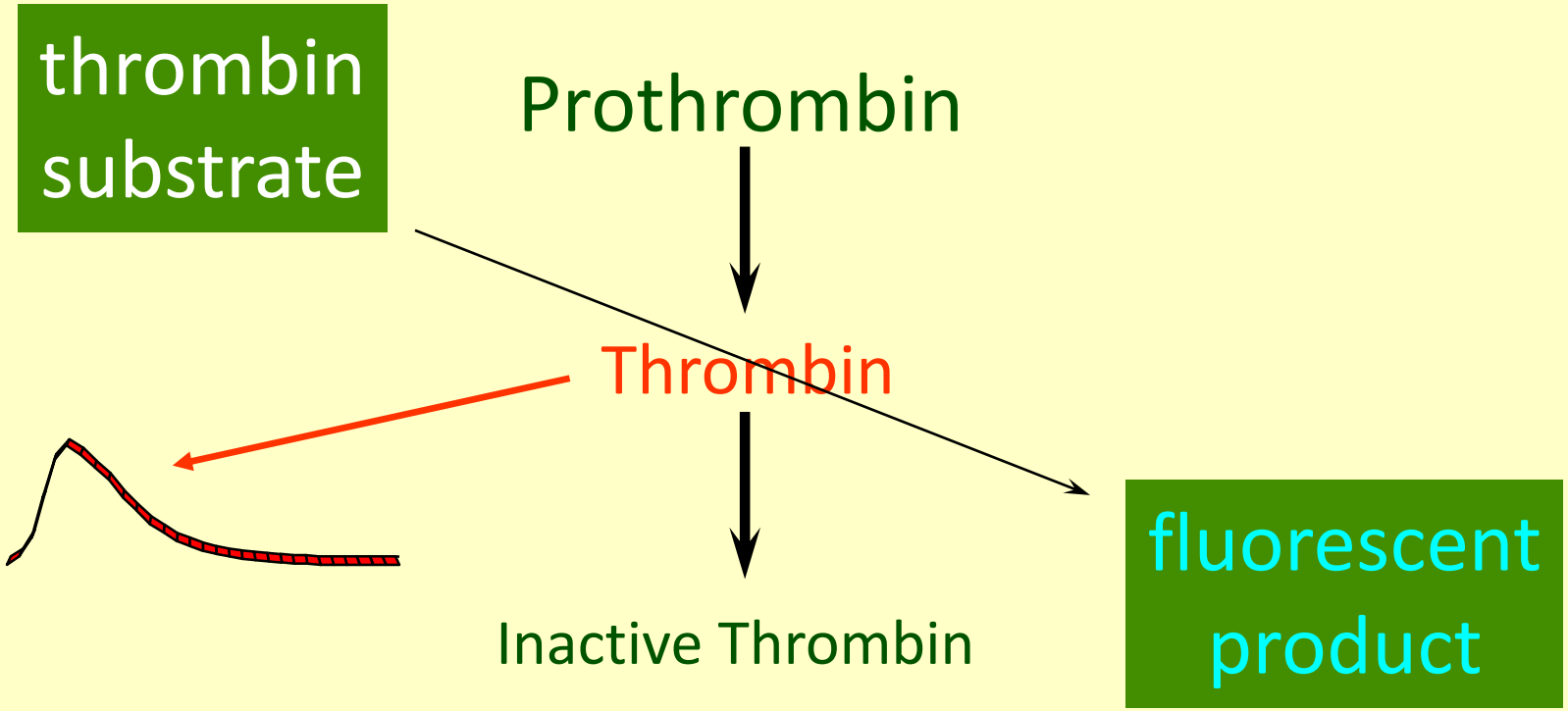
↓ Antithrombins

Inactive Thrombin

A dynamic equilibrium of
pro- and anticoagulant processes

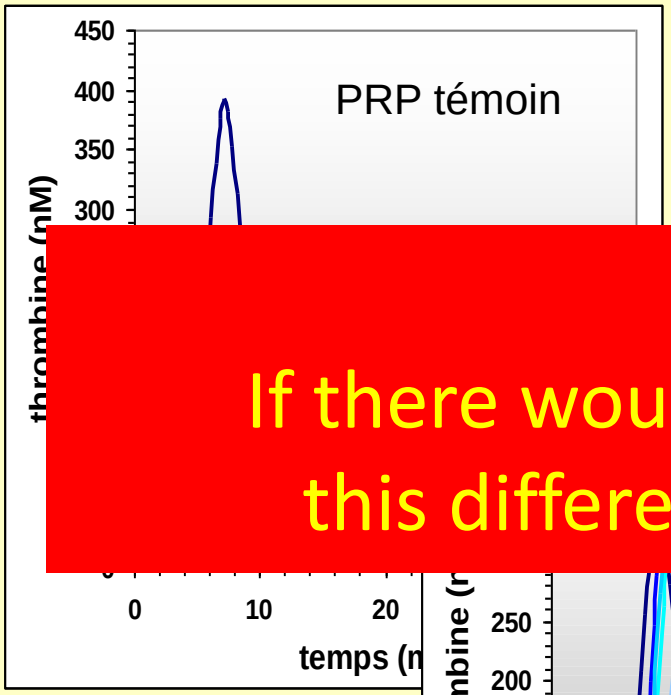
Part One: HOW ?

How to measure the level of thrombin?

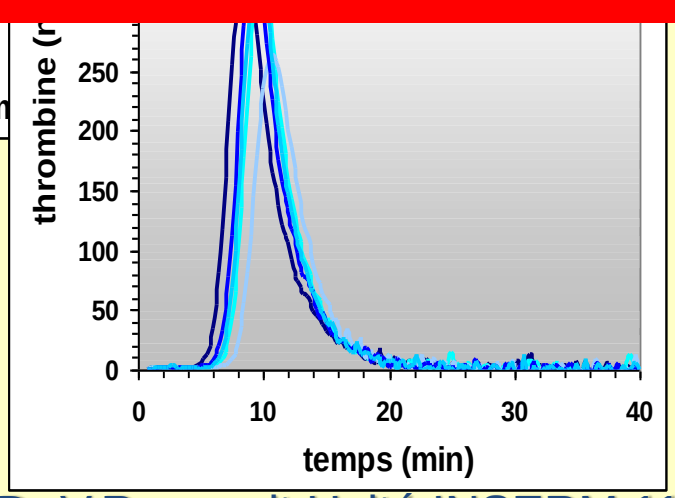


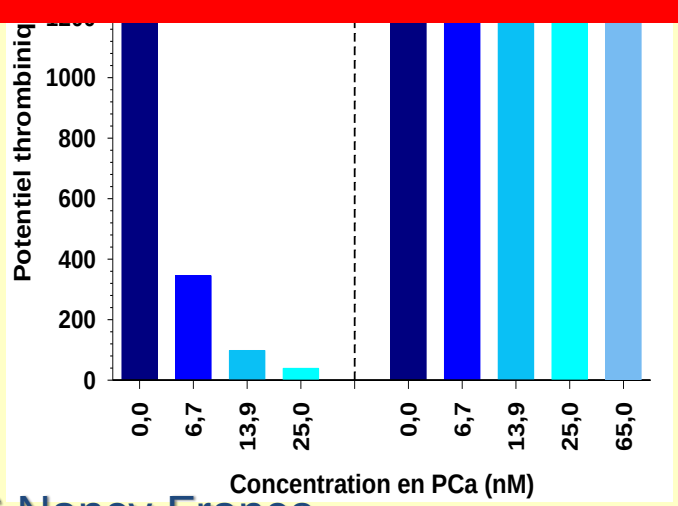
By its activity on a (fluorogenic) substrate

Just one example:
Prolongation of the lag time and resistance to APC



NB:
If there would be no PE in the membrane
this difference would not be observed





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Conclusion:

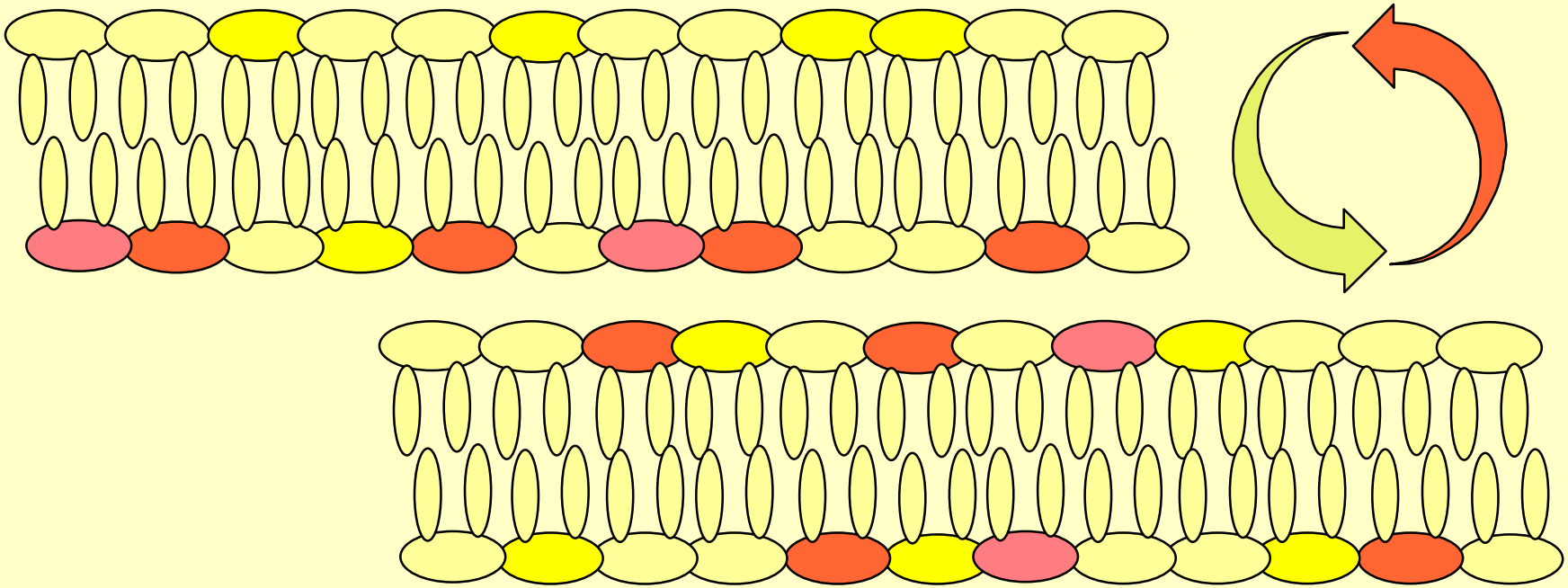
Either use exactly defined lipids

OR

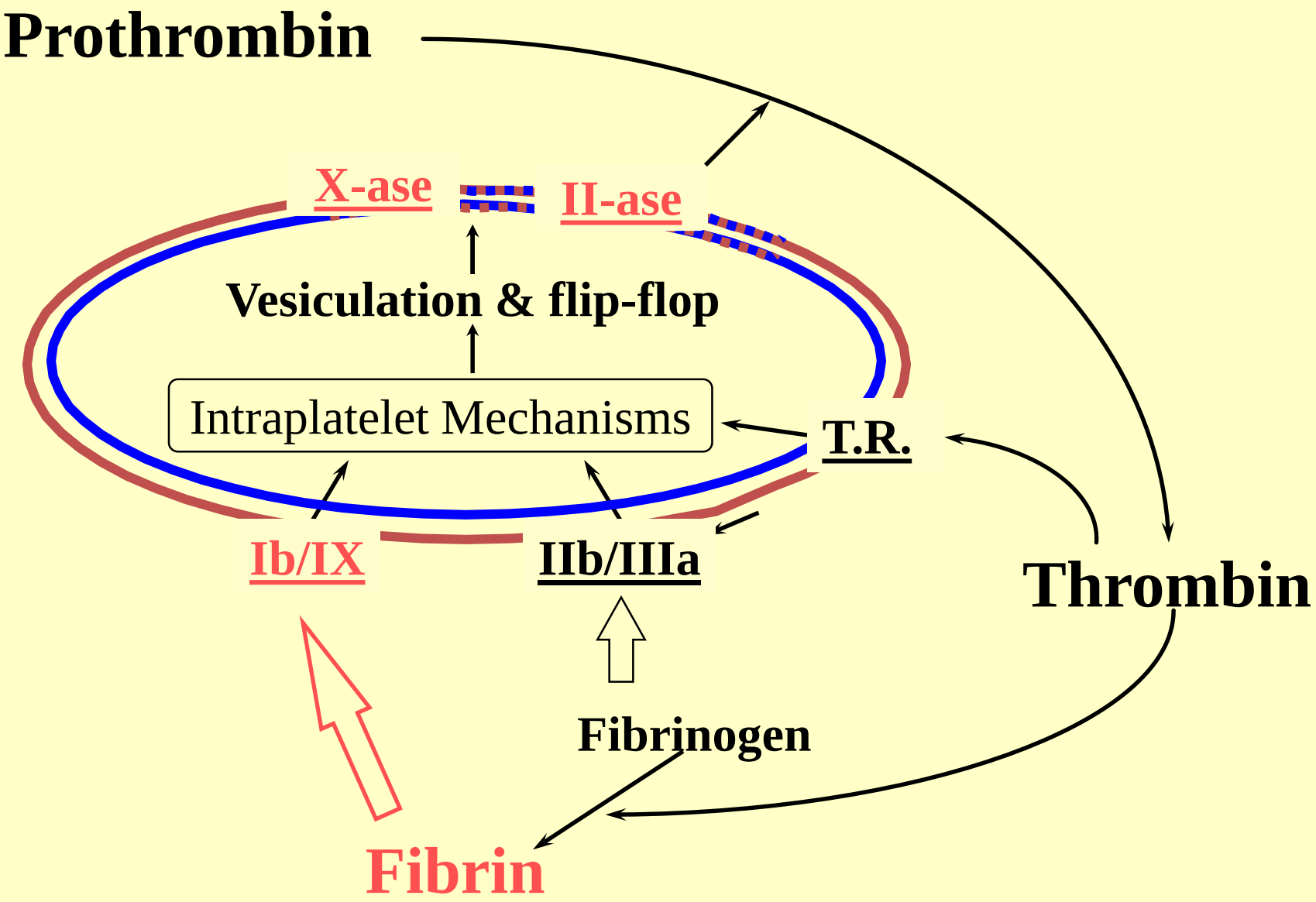
Use the natural phospholipids,

- Either platelets or platelet derived microparticles
- Either from normal donor or from the patient

Platelet Activation makes procoagulant PL (PPL) appear at the plasma side

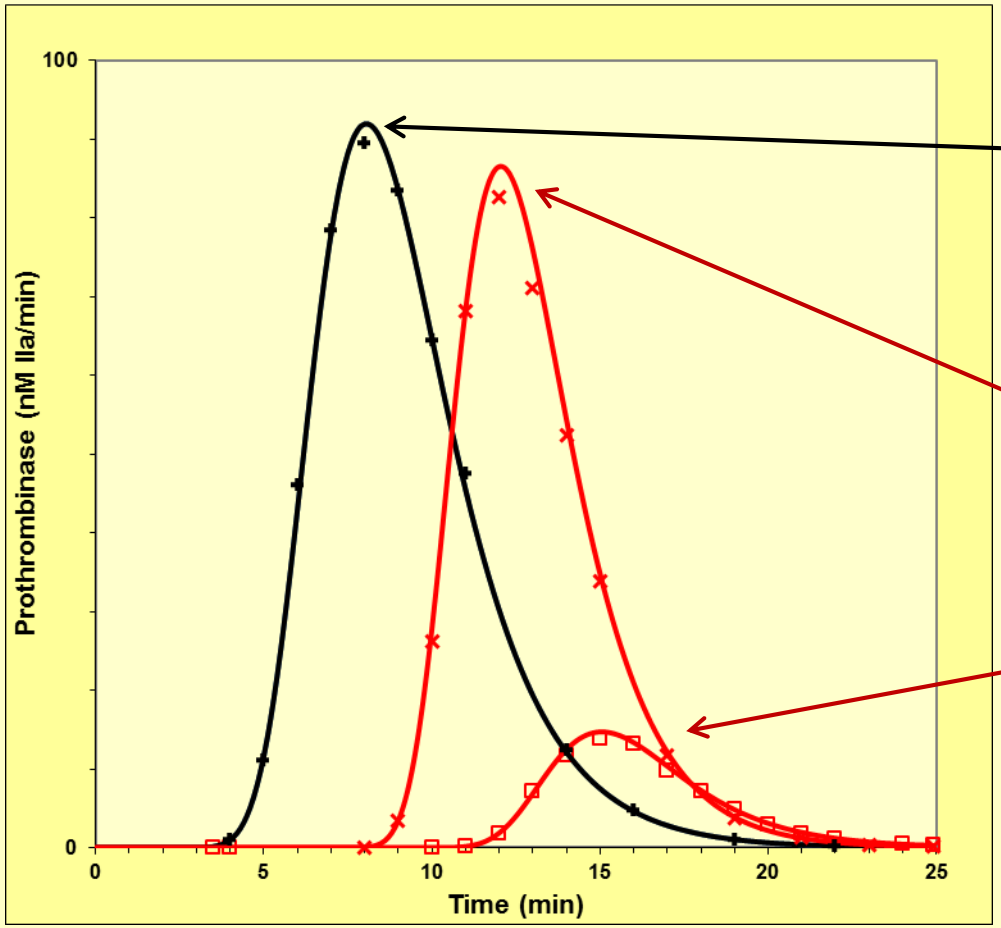


Flip-flop or Scrambling, (Bevers, Zwaal et al. 1980)



Flip-flop mechanisms (Béguin et al. 1992)

LA-IgG in normal PRP



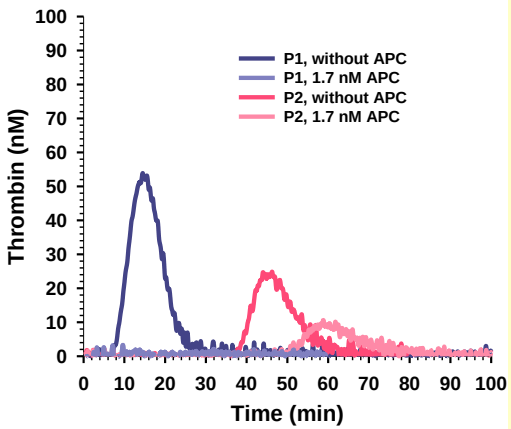
Normal IgG 10 mg/ml

IgG patient B.A. 0.1 mg/ml

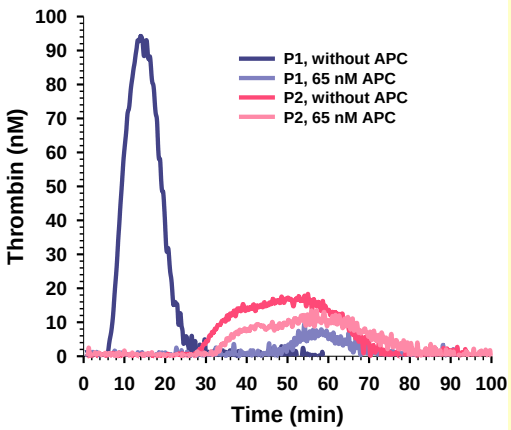
IgG patient B.A.10 mg/ml

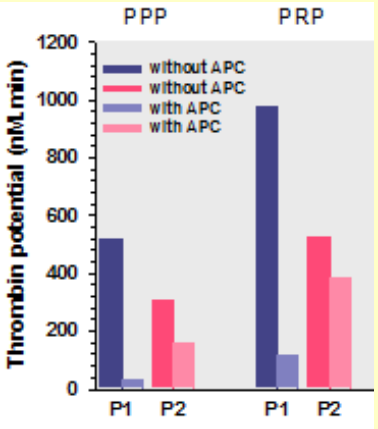
Patient	Age	Diagn.	APTT	APTT 1:1	KCT	KCT 1:1
B.A.	56	SLE	60	43	140	121

PPP



PRP





	Patient 1 (P1)	Patient 2 (P2)
underlying disease	SLE	LLD, APS
thrombosis	no	venous
LA	negative	positive
aCL IgG antibodies	negative	negative
aCL IgM antibodies	negative	positive (160 MPL)
anti-β_2GPI IgG antibodies	negative	positive (25 GPL)
anti-β_2GPI IgG antibodies	negative	positive (102 MPL)

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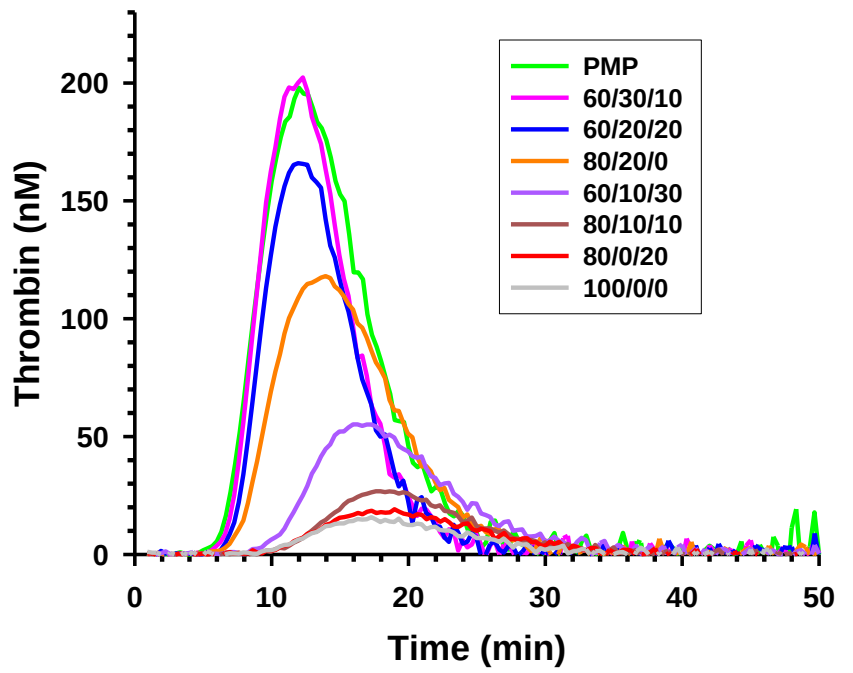
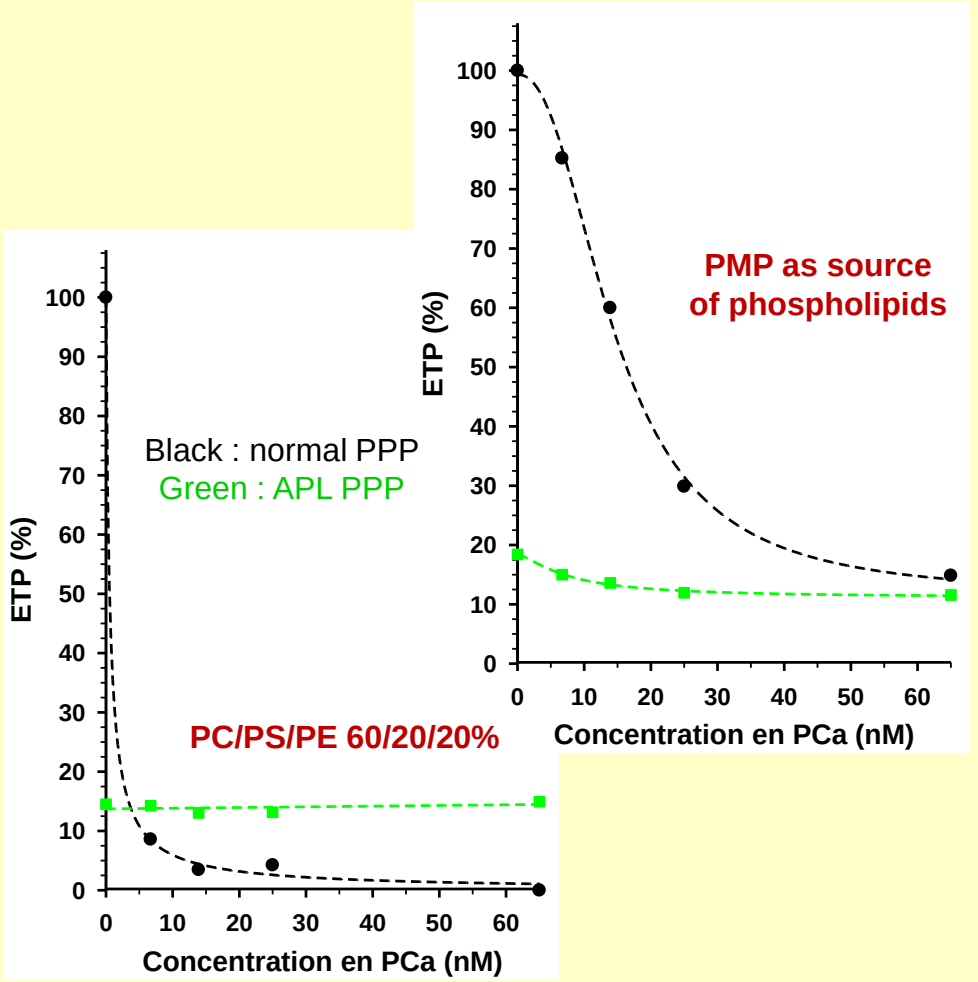
platelet-derived microparticles (PMP)

Thrombin generation in normal PPP + phospholipid vesicles of various compositions in PC/PS/PE or in (PMP)



Influence of the phospholipid source on the interference of APL with APC downregulation of thrombin generation



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Influence of the phospholipid source on the interference of APL with APC anticoagulant activity

(% Inhibition of thrombin activity)

	PRP	PPP/PMP_{coll}	PS/PC/PE
Control 1	59	65	96
Control 2	56	70	95
P 1	**37**	< 10	16
P2	**78**	98	100
P3	30	< 10	41
P4	25	30	80
P5	41	18	73

PPP/PMP_{coll}: PRP activated with 20 µg/mL collagen and then centrifuged at 1500 g for 10 min

Synthetic PL : PC/PS/PE (60/20/20%)

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Conclusion

- If you need reproducibility between experiments: use well defined phospholipid preparations (composition, physical form)
- If you need insight in what happens in a patient: use his own phospholipids, i.e. his platelets.
- If you want to know what is going on: study the influence of PL composition on the inhibitory action of lupus antibodies.

Thank you