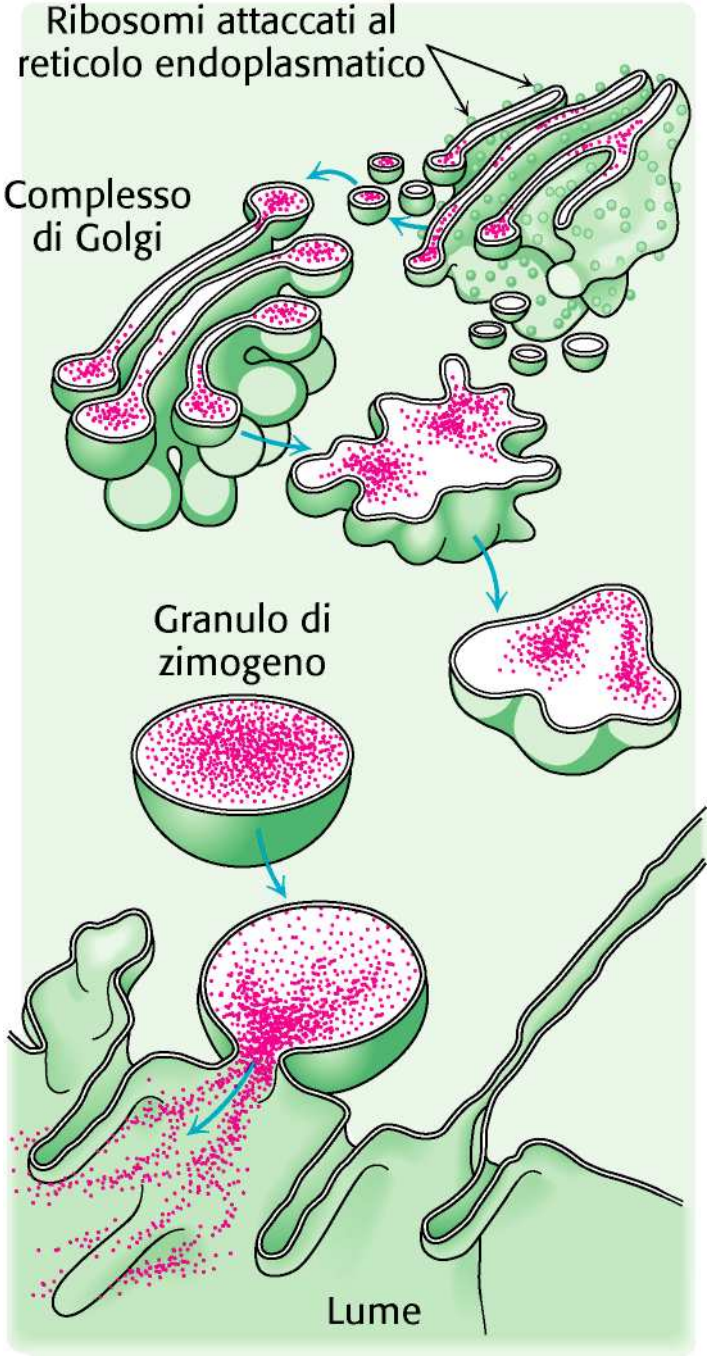
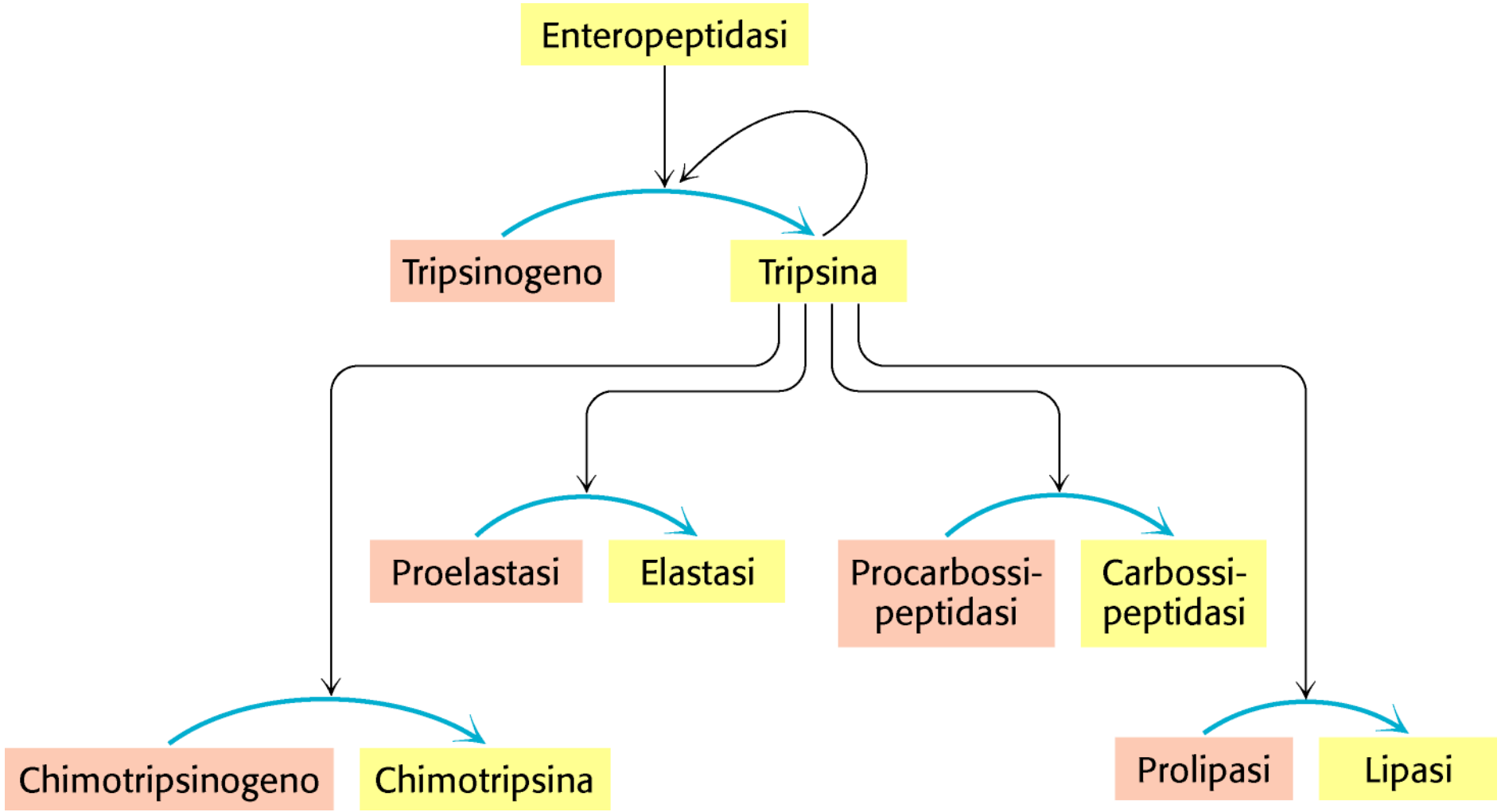
Meccanismi alla base dell’attivazione



Qual è il meccanismo che previene l'attività delle proteasi quando non ce n'è bisogno?

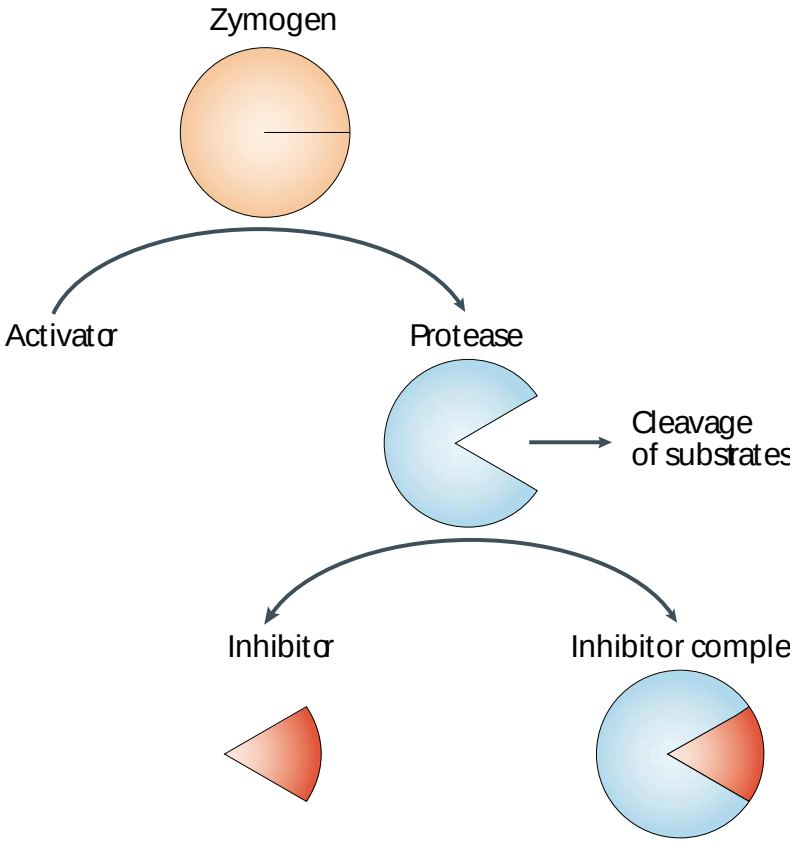

Enteropeptidasi
Tripsinogeno
Tripsina
Proelastasi
Elastasi
Procarbossi-
peptidasi
Carbossi-
peptidasi
Chimotripsinogeno
Chimotripsina
Prolipasi
Lipasi

Proteases Are Synthesized as Zymogens

Zymogen:

A proenzyme or inactive enzyme. It requires a biochemical change to reveal the active site for it to become an active enzyme.

Zymogens lack the structural attributes required for formation of the enzyme-substrate complex.



Why Synthesize as a Zymogen?

- **prevent unwanted protein degradation**
- **enable spatial and temporal regulation**
- **tightly controlled**

Serine Proteases: Mechanism of Control

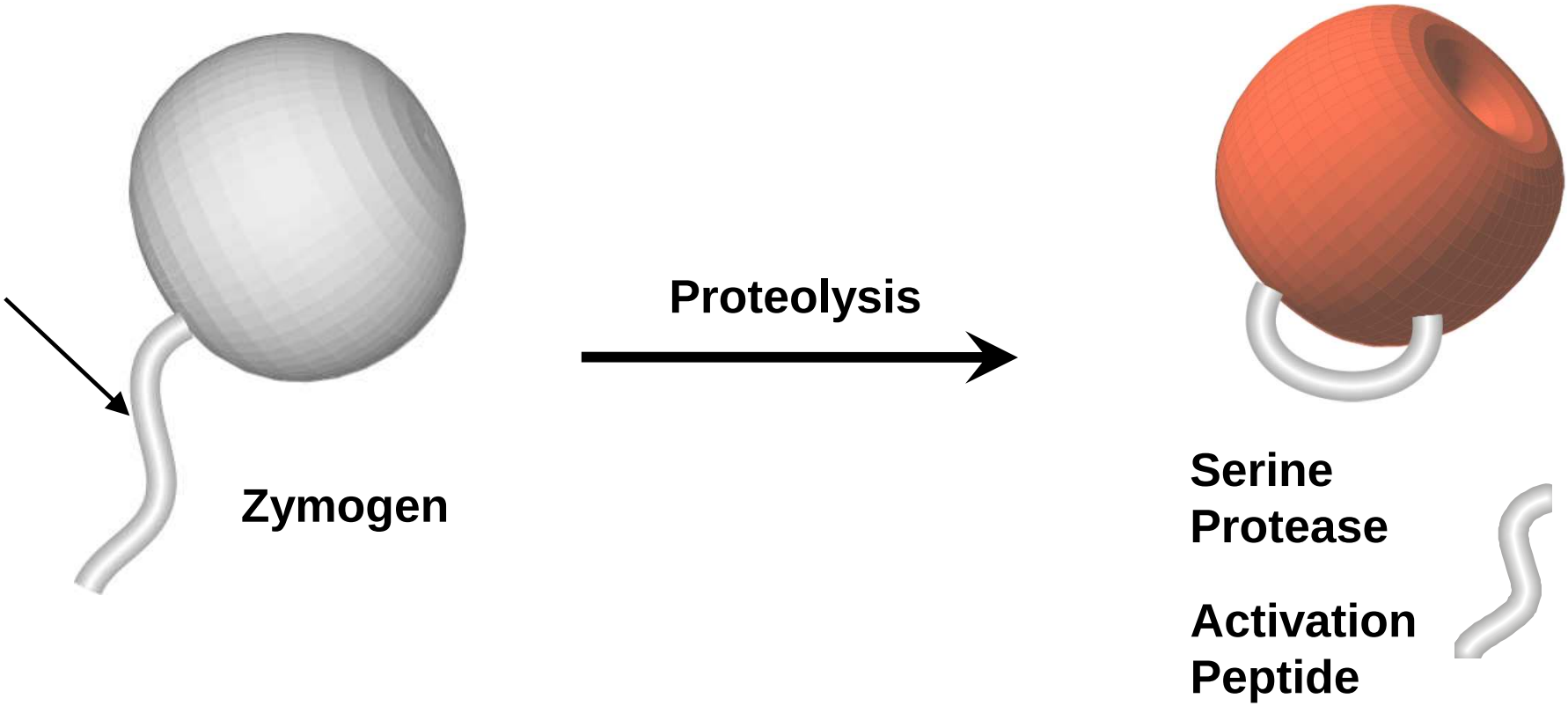


TABLE I

Sites cleaved in the activation of the human vitamin K dependent zymogens

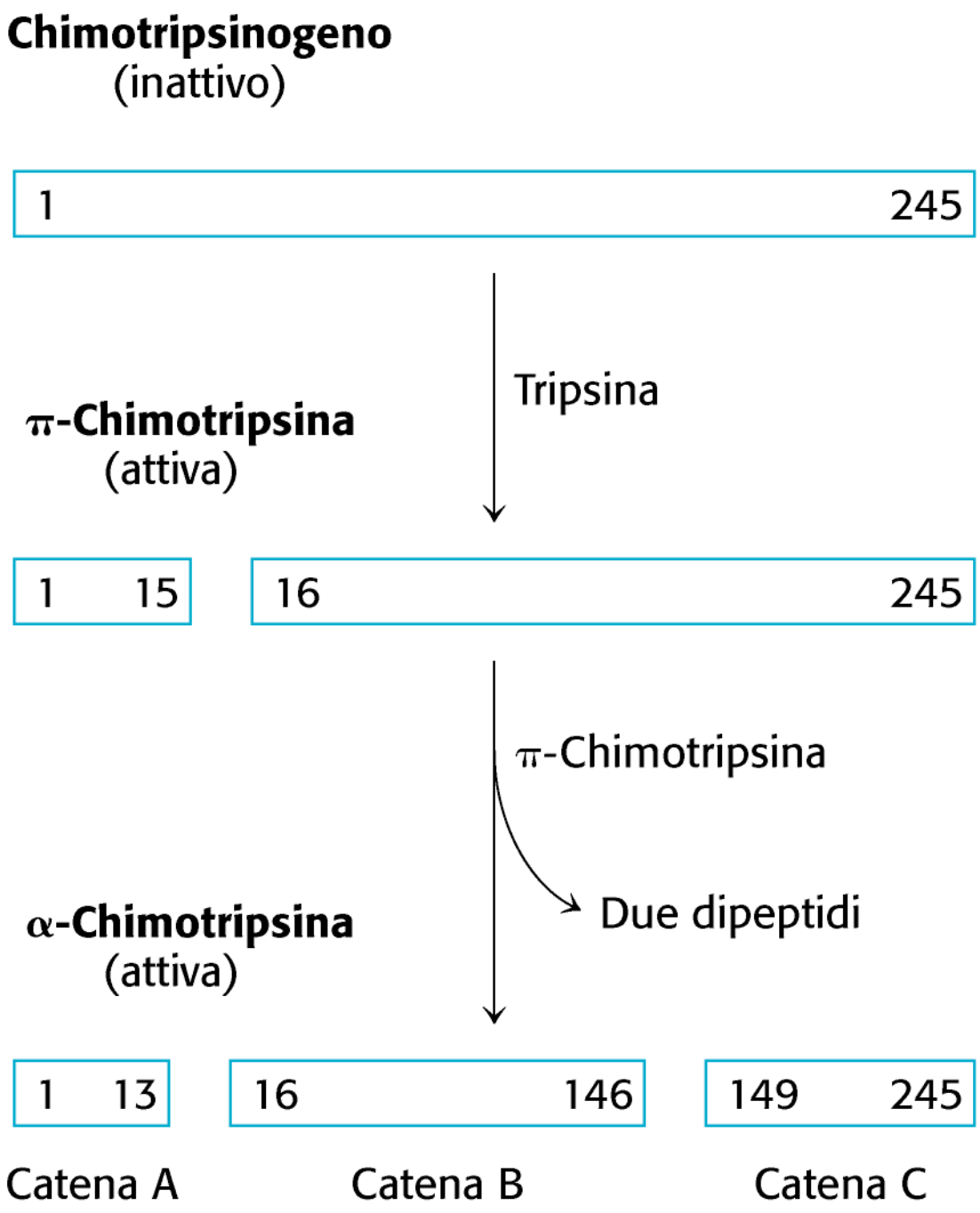
Enzyme	Substrate[b]	Cleavage Site Sequence[a]							
		P_4	P_3	P_2	P_1	↓	$P_{1'}$	$P_{2'}$	$P_{3'}$	$P_{4'}$
Xa/Va	II	I	E	G	R		T	A	T	S
	II (15-16)	I	D	G	R		I	V	E	G
VIIa/TF, IXa/VIIIa	X (15-16)	N	L	T	R		I	V	G	G
VIIa/TF, XIa	IX	K	L	T	R		A	E	A	V
	IX (15-16)	D	F	T	R		V	V	G	G
VIIa/TF, Xa	VII (15-16)	P	Q	G	R		I	V	G	G
IIa/TM	PC (15-16)	V	D	P	R		L	I	D	G

TABLE I

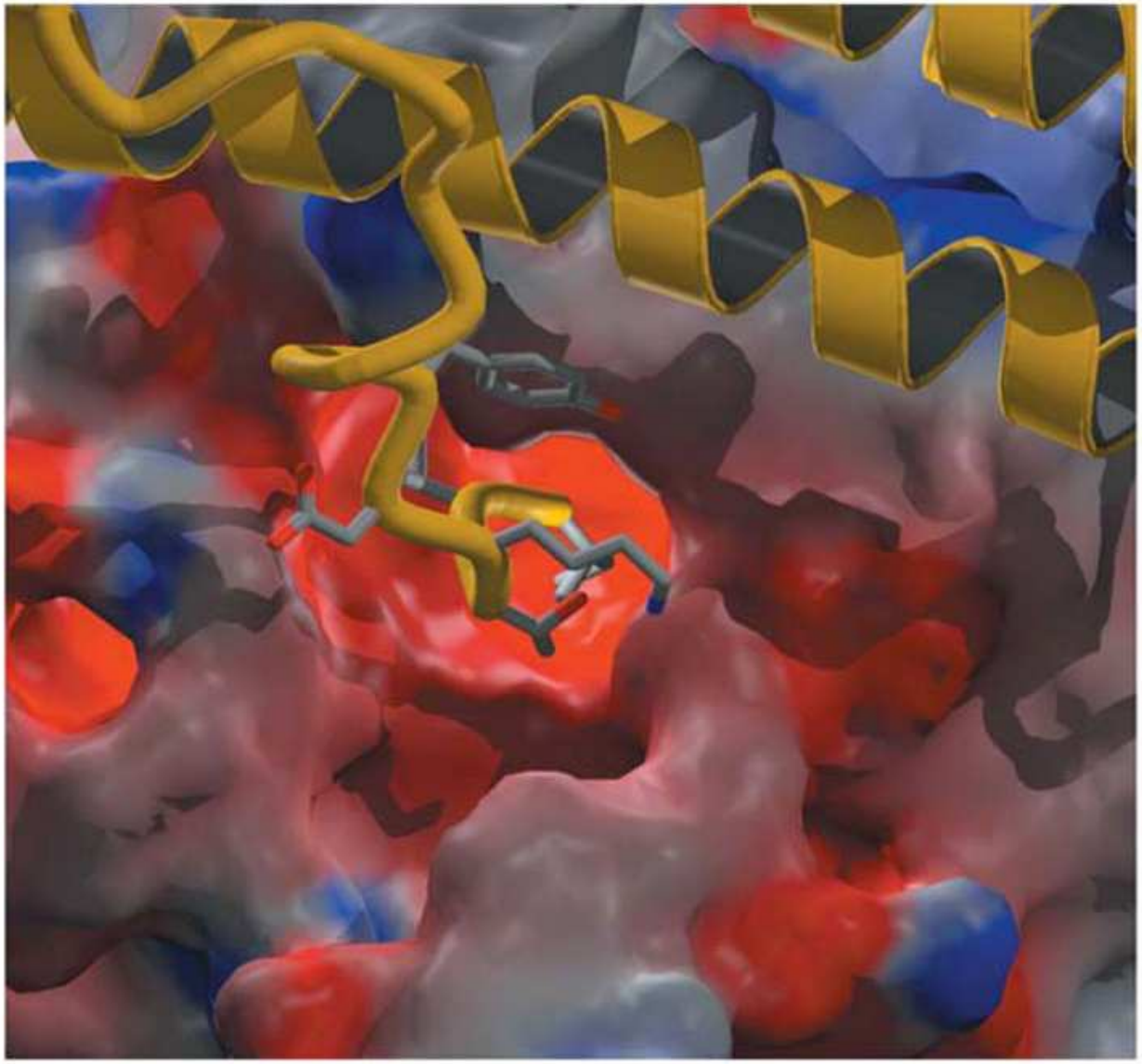
Sites cleaved in the activation of the human vitamin K dependent zymogens

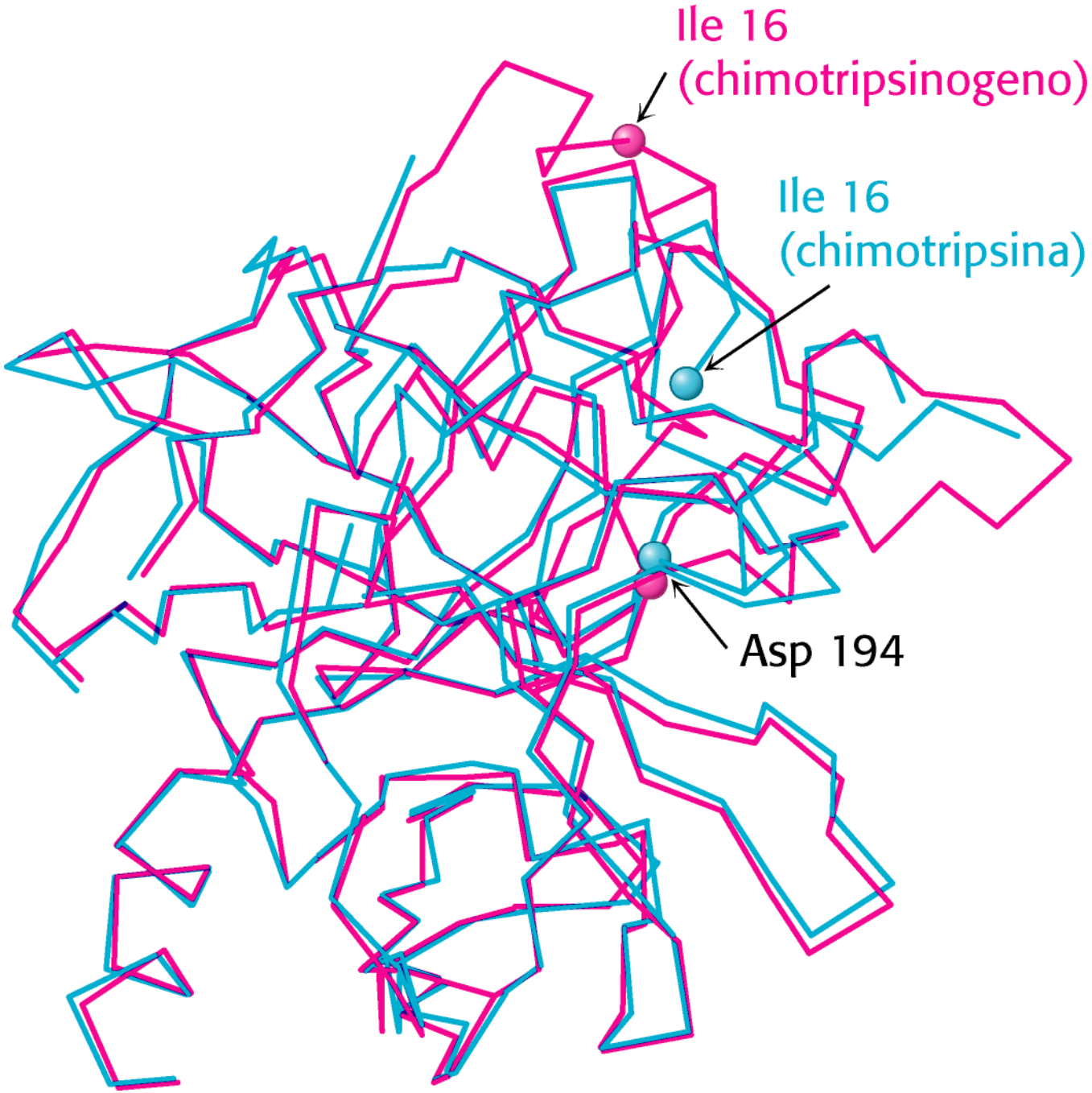
Enzyme	Substrate[b]	Cleavage Site Sequence[a] P_4	P_3	P_2	P_1	↓	$P_{1'}$	$P_{2'}$	$P_{3'}$	$P_{4'}$
Xa/Va	II	I	E	G	R		T	A	T	S
	II (15-16)	I	D	G	R		I	V	E	G
VIIa/TF, IXa/VIIIa	X (15-16)	N	L	T	R		I	V	G	G
VIIa/TF, XIa	IX	K	L	T	R		A	E	A	V
	IX (15-16)	D	F	T	R		V	V	G	G
VIIa/TF, Xa	VII (15-16)	P	Q	G	R		I	V	G	G
IIa/TM	PC (15-16)	V	D	P	R		L	I	D	G

New N-terminus


Chimotripsinogeno
(inattivo)
1
245
Tripsina
π-Chimotripsina
(attiva)
1
15
16
245
π-Chimotripsina
Due dipeptidi
α-Chimotripsina
(attiva)
1
13
16
146
149
245
Catena A
Catena B
Catena C

The Key Step: the salt bridge formation

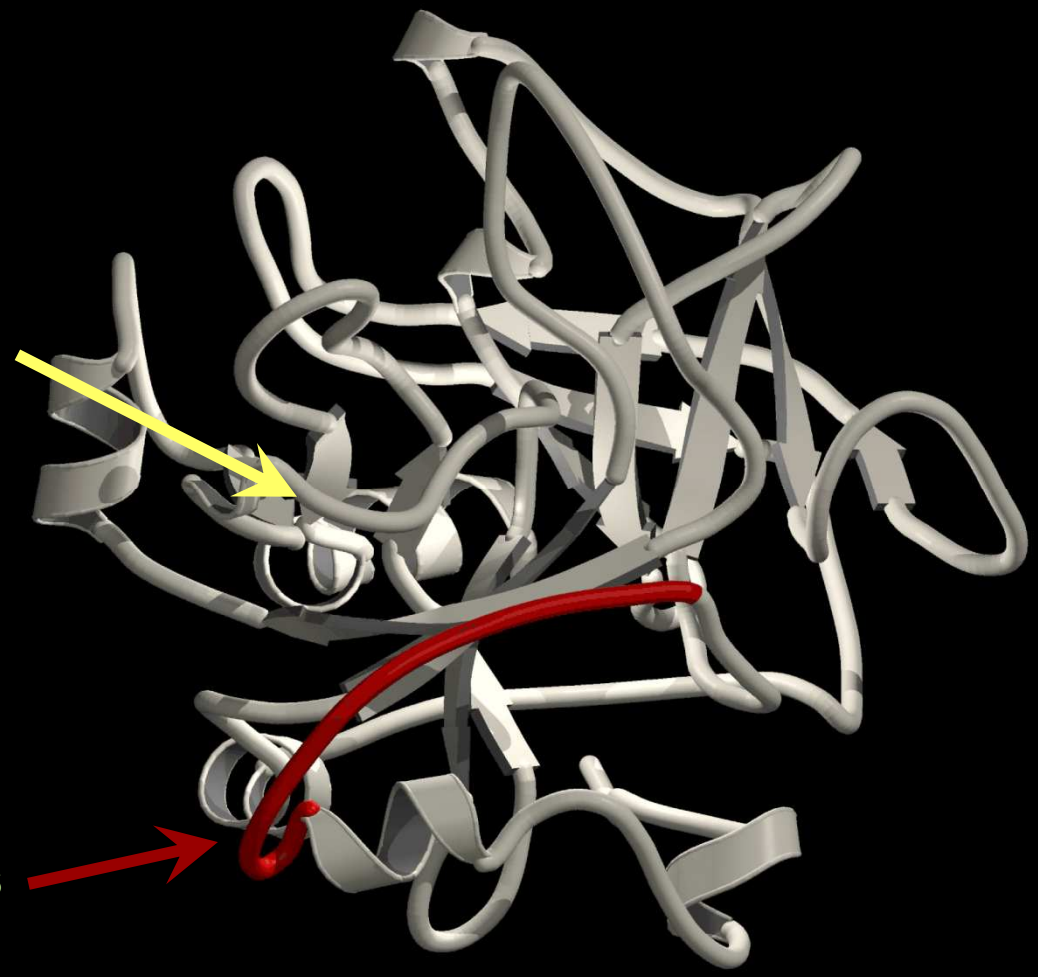



Ile 16
(chimotripsinogeno)
Ile 16
(chimotripsina)
Asp 194

RASMOL

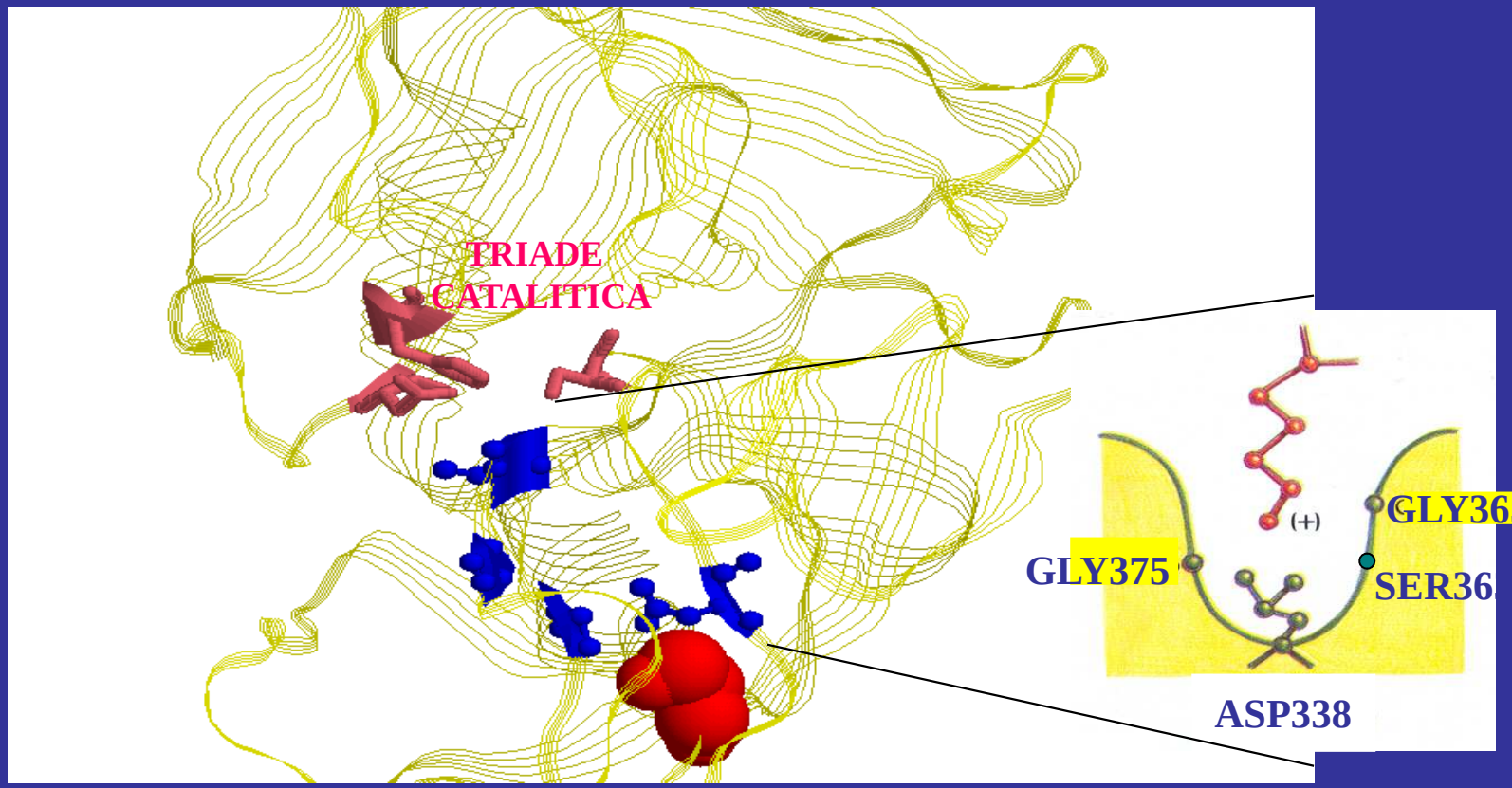
Serine Proteases: Conversion Pathway

- Cleavage between Arg^{15}-Ile^{16} → Exposure of new N-terminus
- New N-terminus (IVGG) forms salt bridge with Asp^{194}
- N-terminal insertion leads to a conformational change in the “activation domain”



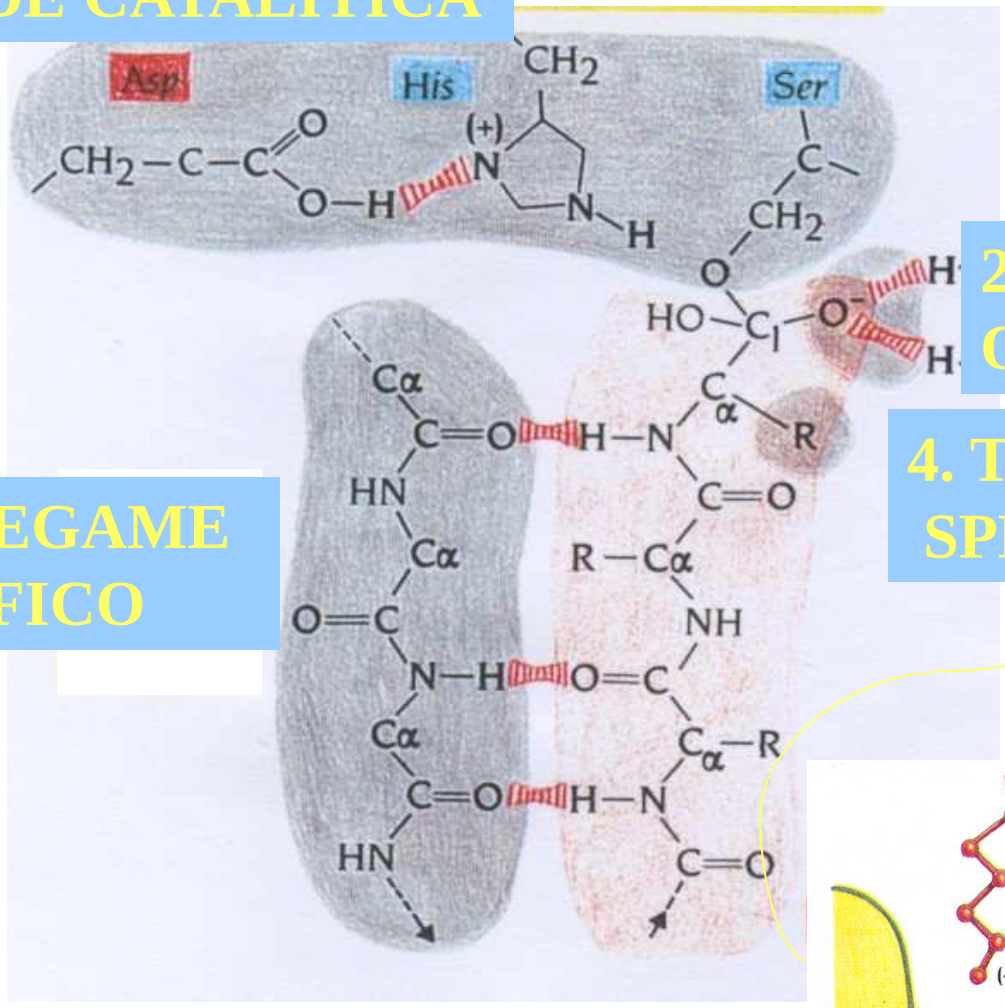
Courtesy of W. Bode,
Max Planck
Institute of Biochemistry

Il taglio nel sito di attivazione determina modificazioni conformazionali del dominio catalitico e la formazione del sito attivo della serin proteasi



CARATTERISTICHE PER LA CATALISI SERIN PROTEASICA

1. TRIADE CATALITICA

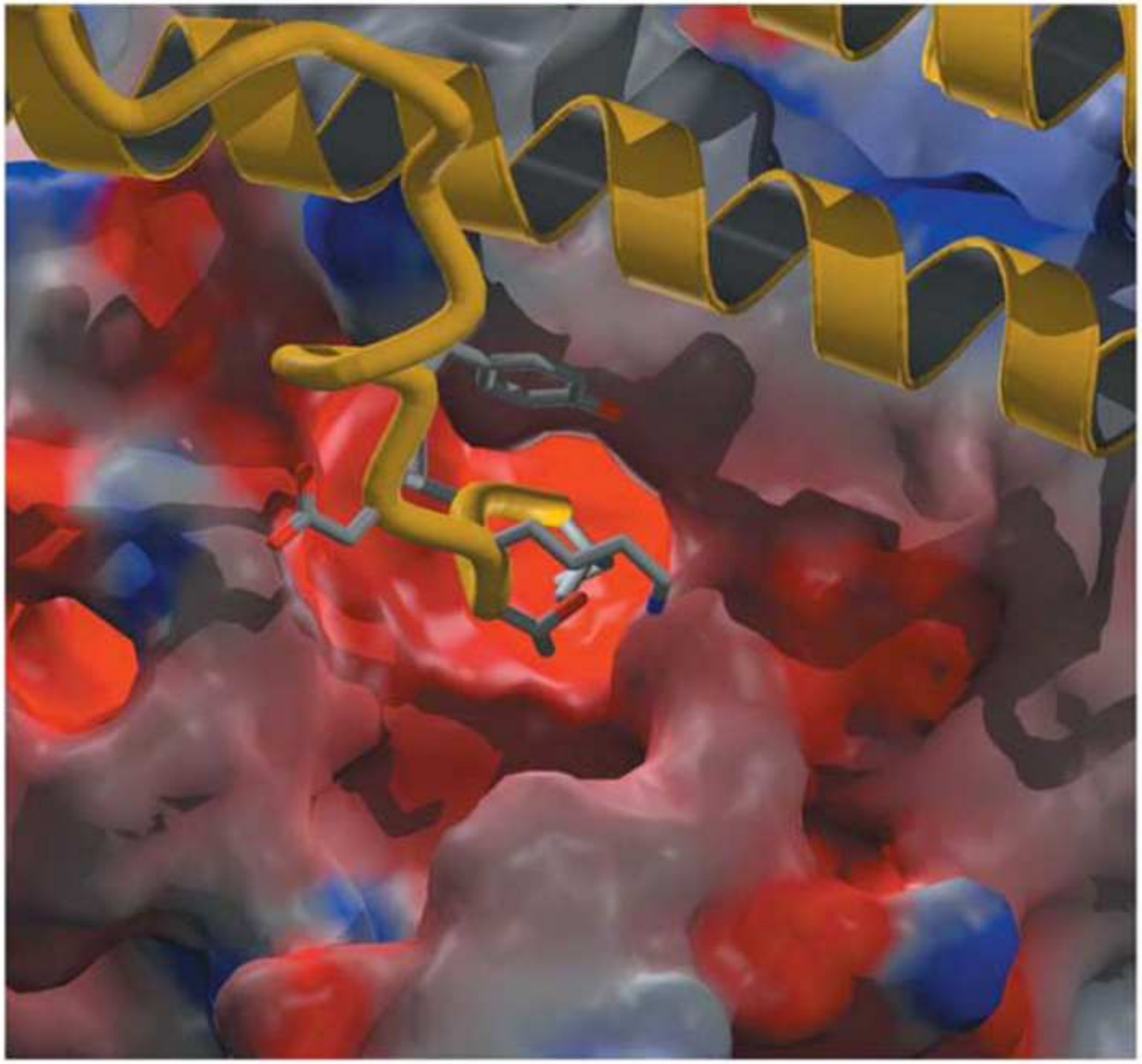


2. INCAVO OSSIANIONICO

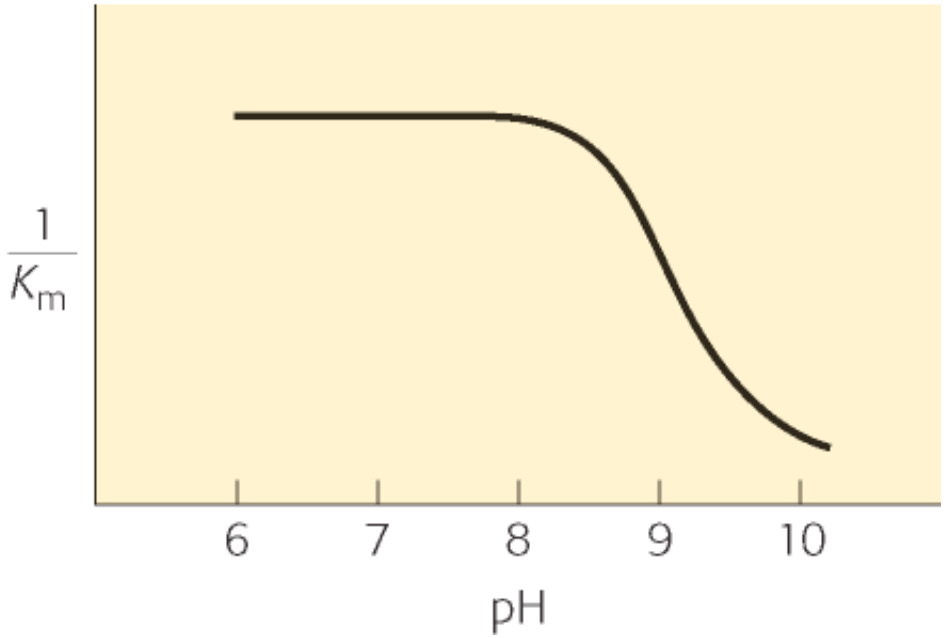
3. SITO DI LEGAME ASPECIFICO

4. TASCA DI SPECIFICITA'

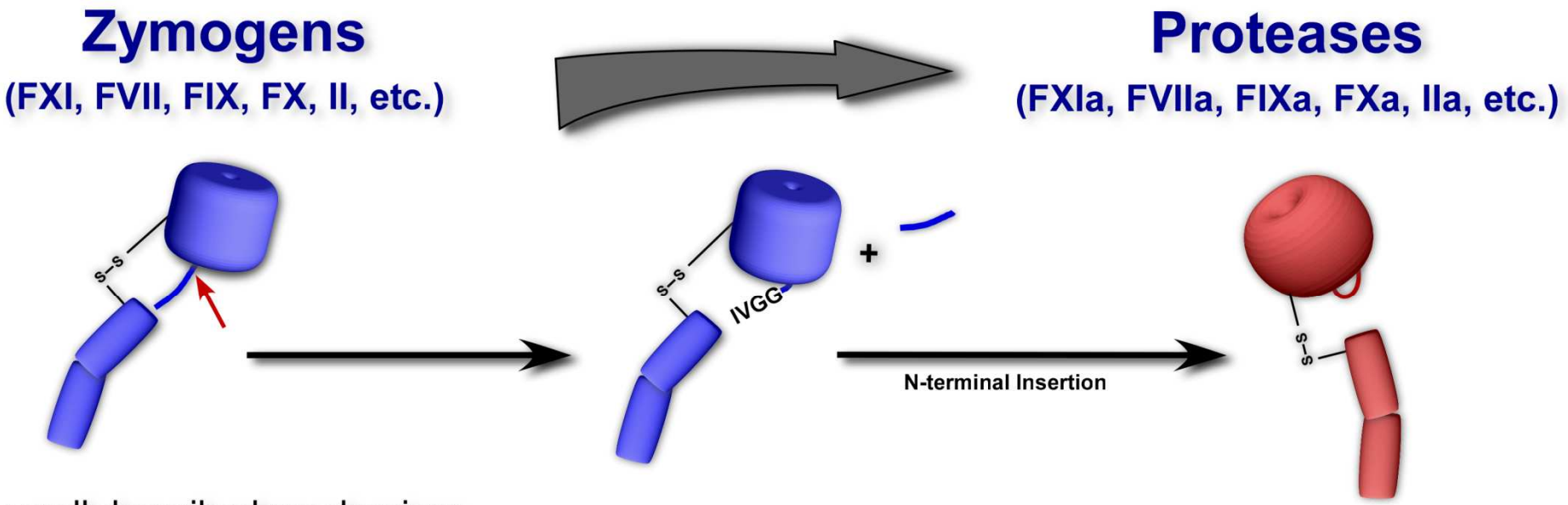
The Key Step: the salt bridge formation



Lo stato di ionizzazione dell'Ile16 influenza la formazione del ponte salino con Asp194 e quindi la Km dell'enzima



Zymogen Activation as an important way to control enzymatic activity

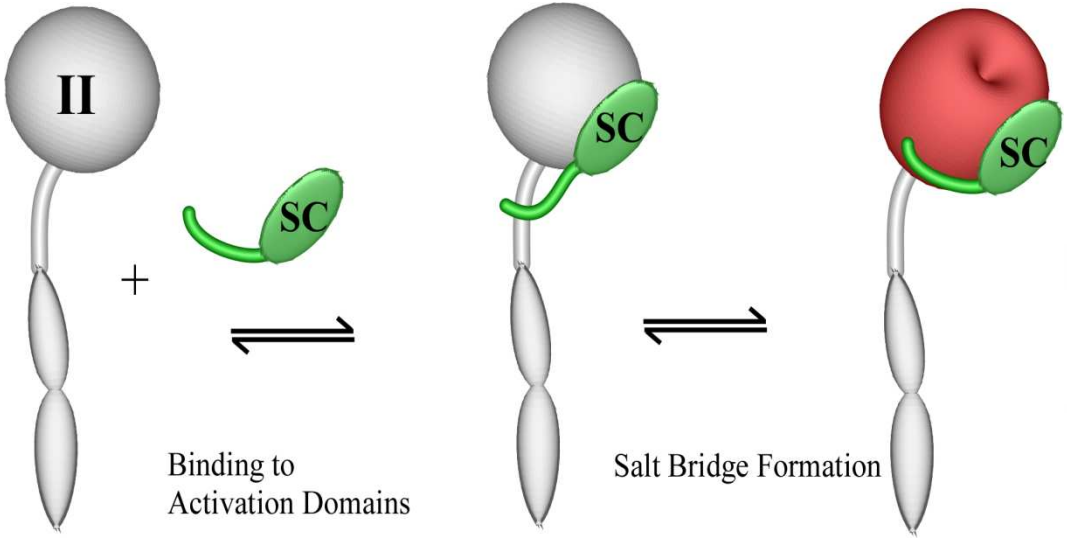


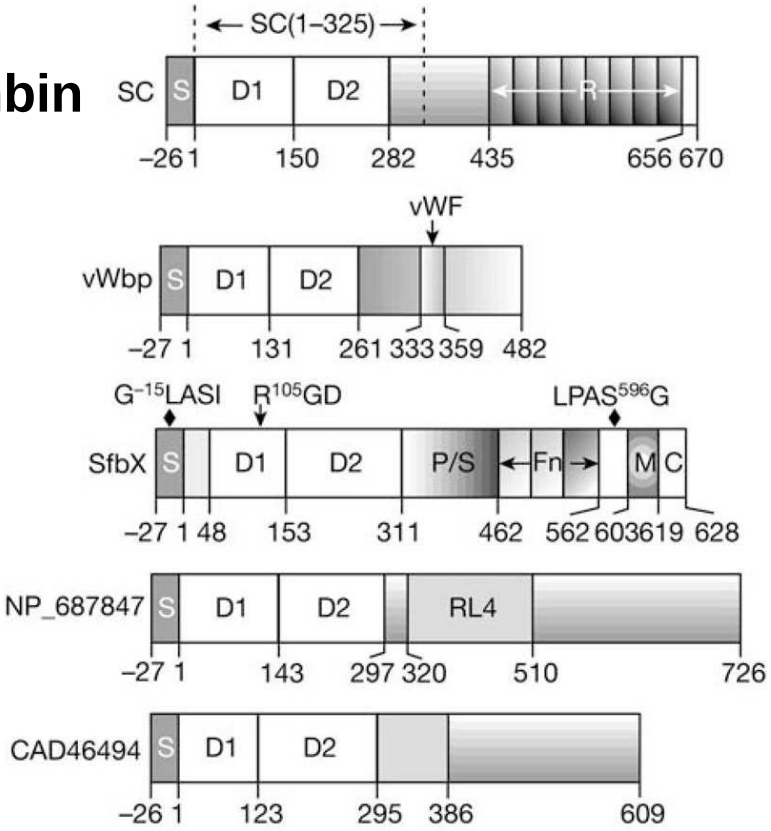
- well described mechanism
- specific conformational change

Allosteric Zymogen Activation:

Staphylocoagulase family of ZAAPs:

- non-proteolytic activators of zymogens
- found in gram-positive bacteria (e.g. staphylococcus)
- examples:
  - streptokinase—activates fibrinolysis
  - staphylocoagulase-activates prothrombin





Non-Proteolytic Activation of Prothrombin by Staphylocoagulase Support for the "Molecular Sexuality" Hypothesis