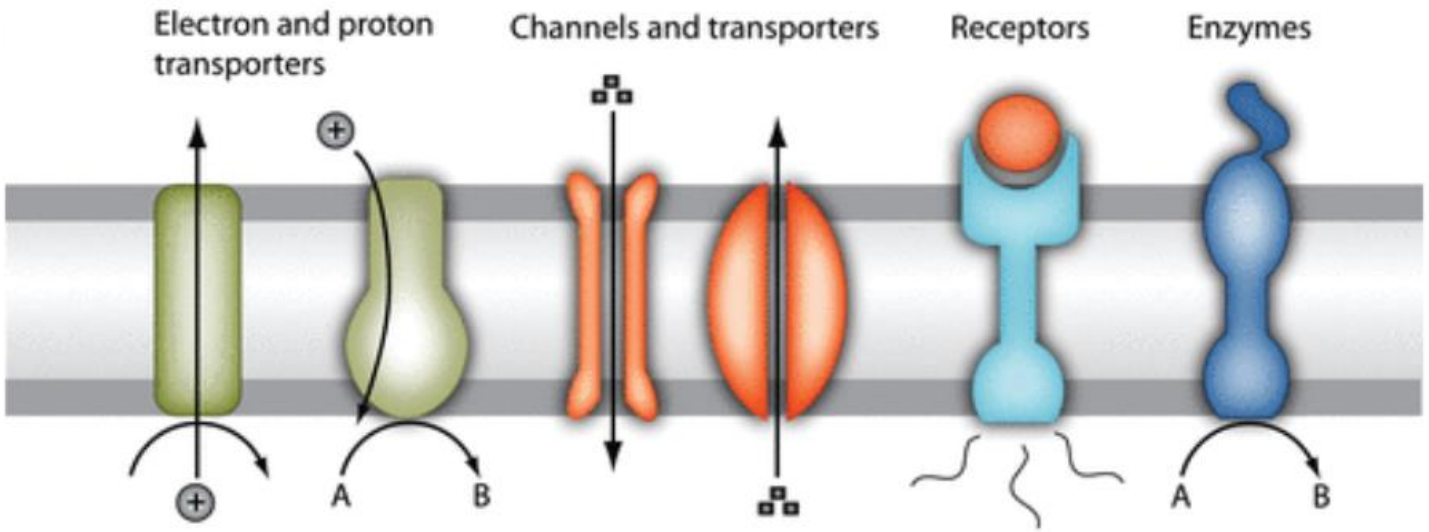
# REGULACIJA MEMBRANSKIH PROTEINOV S ŠEDAZAMI

Biološke membrane (prof. dr. Igor Križaj)

2024/25

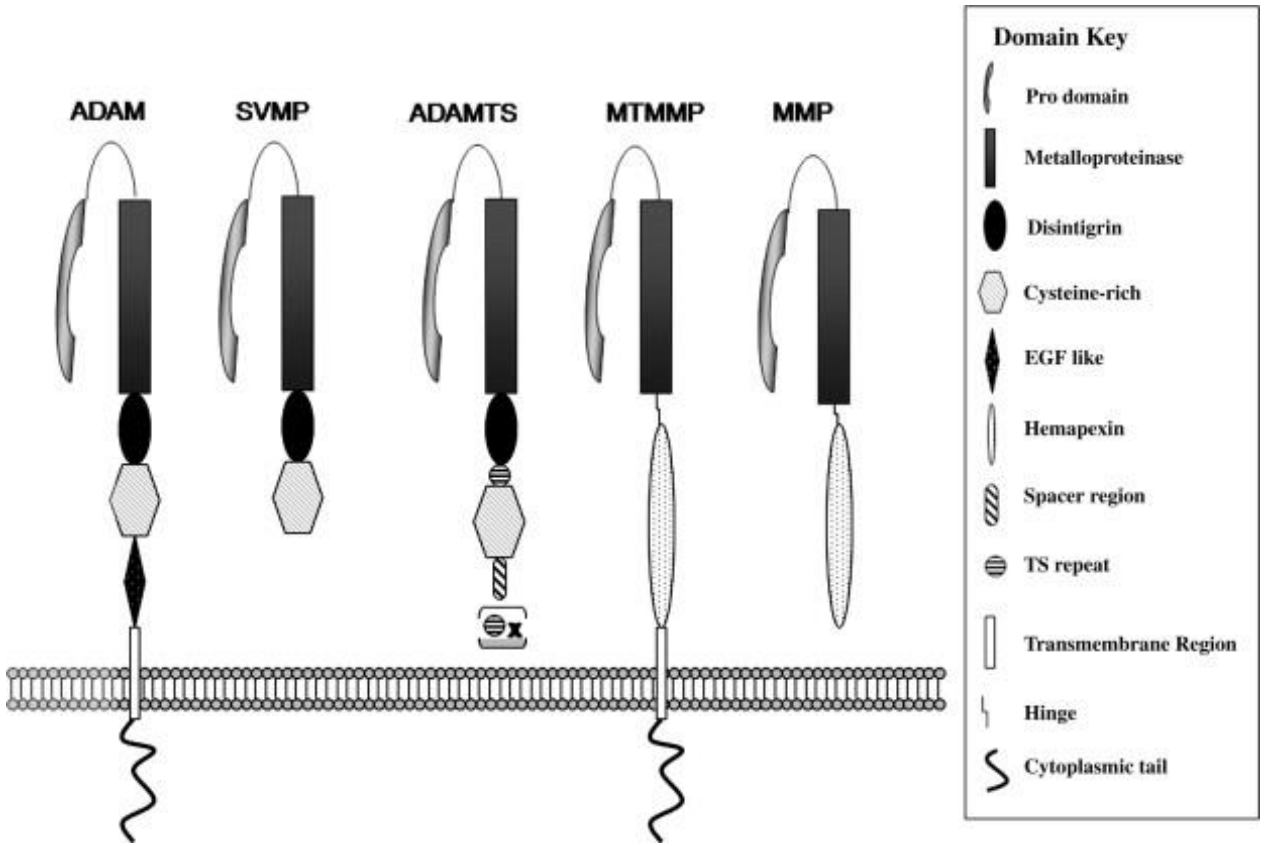
Tinkara Butara, Petja Premrl, Gaja Starc, Pia Špehar in Zarja Weingerl

# UVOD




*An Introduction to Membrane Proteins | Journal of Proteome Research* https://pubs.acs.org/doi/10.1021/pr200145a.

- Periferni in integralni proteini
- Receptorji, transportni proteini, celična adhezija, membranski encimi
- Šedaze → odcepljajo ektodomene
- Metaloproteaze / aspartatne proteaze




*D. R. Edwards, M. M. Handsley, C. J. Pennington: The ADAM metalloproteinases. Mol. Aspects Med.* ***2008****, 29, 258–289.*

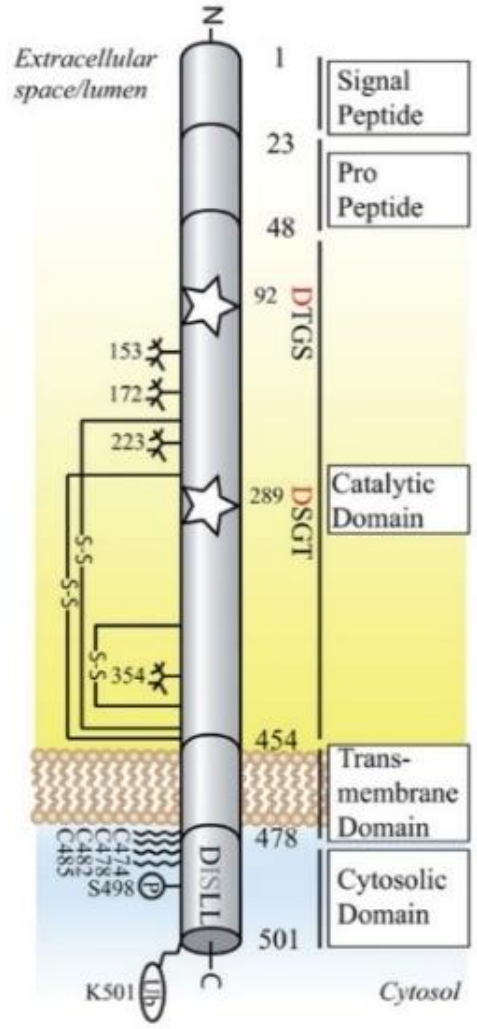
# ŠEDAZE (METALOPROTEAZE)

- Metcinkini
- MMP

(metaloproteaze matriksa)

- ADAM

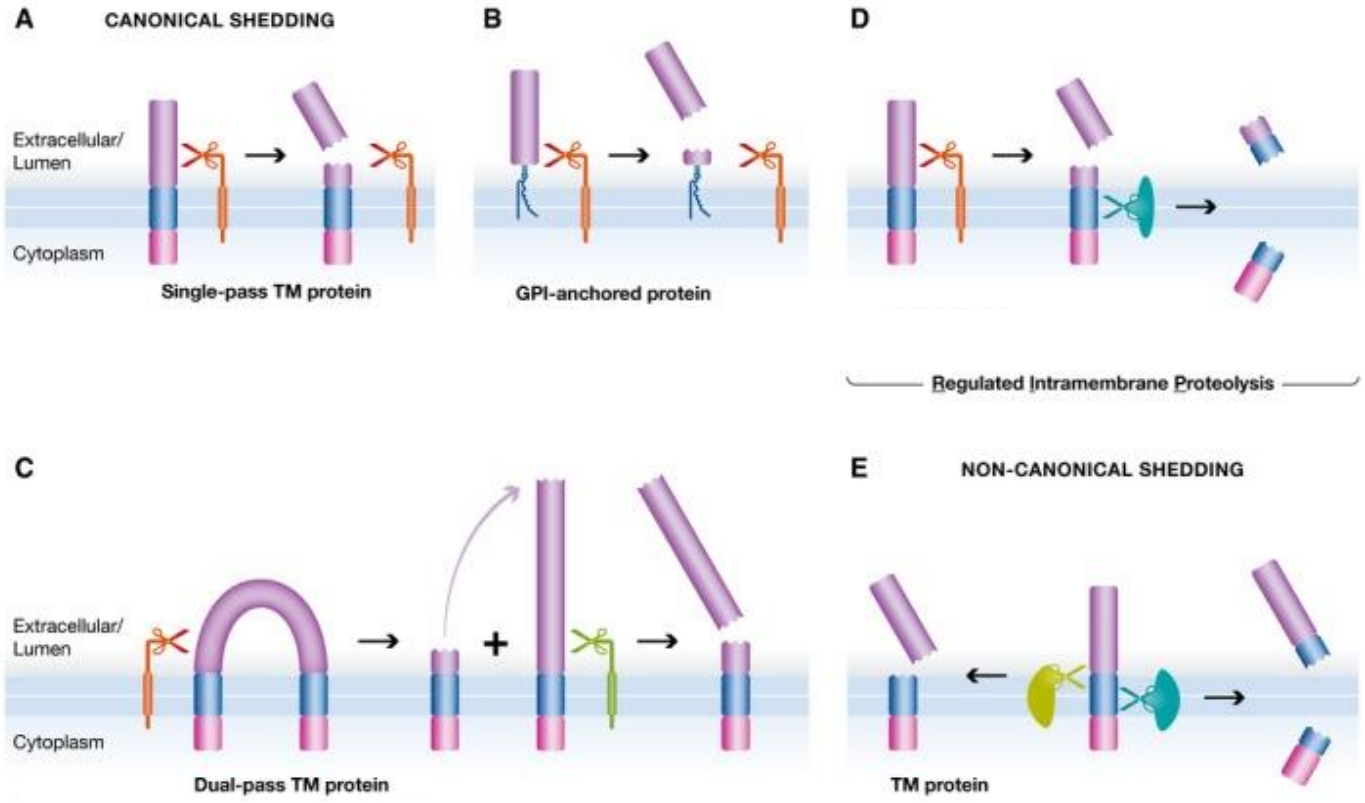
(ang. *a disintegrin and metalloprotease*)




*B. Dislich, S. F. Lichtenthaler: The Membrane-Bound Aspartyl Protease BACE1: Molecular and Functional Properties in Alzheimer's Disease and Beyond. Front. Physiol.* ***2012****, 3.*

# ŠEDAZE (ASPARTATNE PROTEAZE)

- BACE (ang. *β-site APP cleaving enzyme*)
  - Podobna struktura
  - Aktivno mesto: DTGS oz. DSGT




# MEHANIZMI DELOVANJA ŠEDAZ

## Kanonične šedaze

*'Full-time' šedaze*

- **ADAM**
- **BACE**
- **S1P** (proteaza mesta 1) – Ser proteaza
- **Meprin β** - metaloproteaza
- **MMP**

## Nekanonične šedaze

*'Full-time' šedaze*

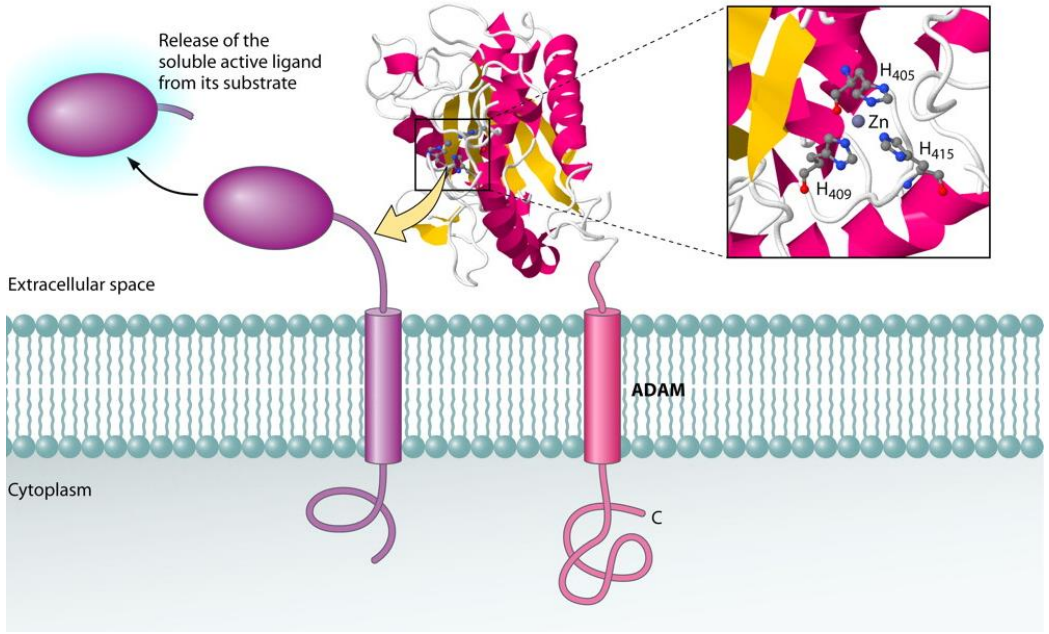
- **Romboidi**
- **SPPL3** *(ang. SPP-like 3)* – Asp proteaza
- **γ-sekretaza**
- **SPP** (peptidaza signalnih peptidov) – Asp proteaza

*S. F. Lichtenthaler, M. K. Lemberg, R. Fluhrer: Proteolytic ectodomain shedding of membrane proteins in mammals—hardware, concepts, and recent developments. EMBO J. 2018, 37*

# Proteaze ADAM

*(ang. a disintegrin and metalloprotease)*

- 'full-time' kanonične šedaze
- Metaloproteaze (metcinkini)
  - Ohranjen katalitični motiv **HE**XX**H**XX**G**XX**H**
  - Ohranjen Met

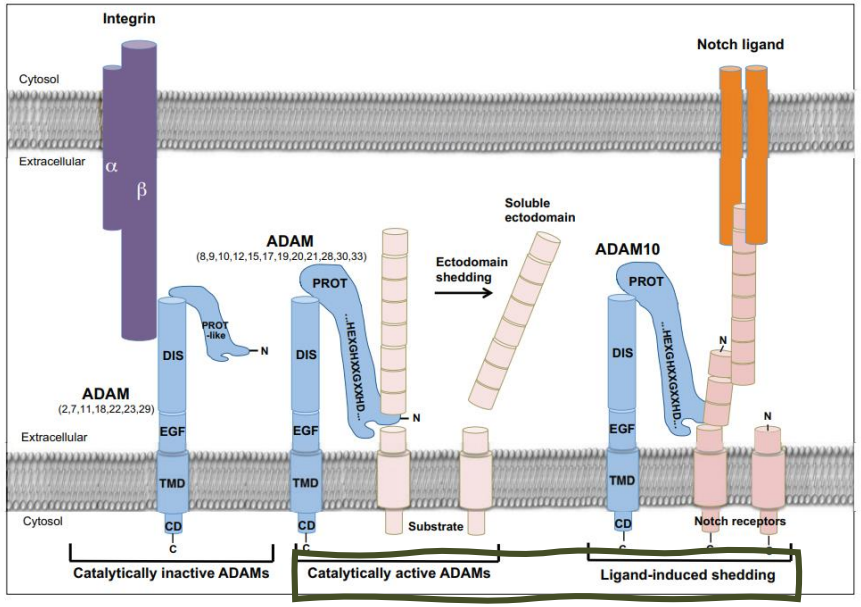



*R. E. Dalbey, P. Wang, J. M. van Dijl: Membrane Proteases in the Bacterial Protein Secretion and Quality Control Pathway. Microbiol. Mol. Biol. Rev.* ***2012****, 7*

- Za specifičnost so ključna mesta S3, S1 in **S1'**

  **ADAM10**
  - S1' žep: globok in hidrofoben
  - P1' substrata: velik hidrofoben ostanek

  **ADAM17**
  - S1' žep: plitvejši in dodatno omejen z Ala in Val
  - P1' substrata: majhen in hidrofoben ostanek

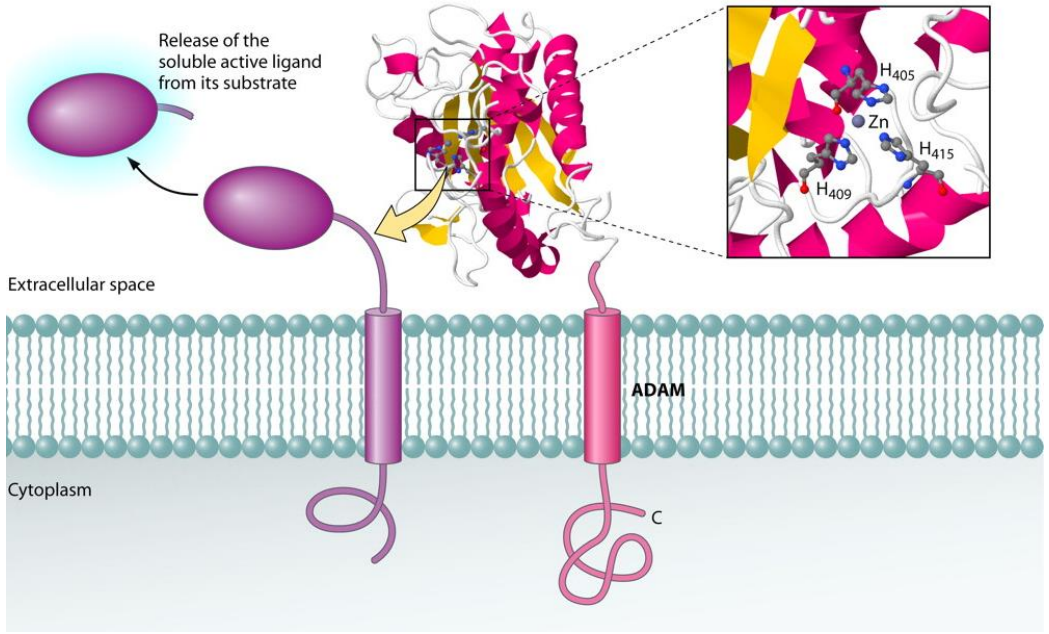



*S. Weber, P. Saftig: Ectodomain shedding and ADAMs in development. Development* ***2012****, 139*

# Proteaze ADAM

*(ang. a disintegrin and metalloprotease)*

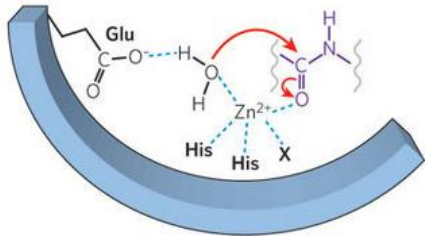
- ‘full-time’ kanonične šedaze
- Metaloproteaze (metcinkini)
  - Ohranjen katalitični motiv **HE**XX**H**XX**G**XX**H**
  - Ohranjen Met



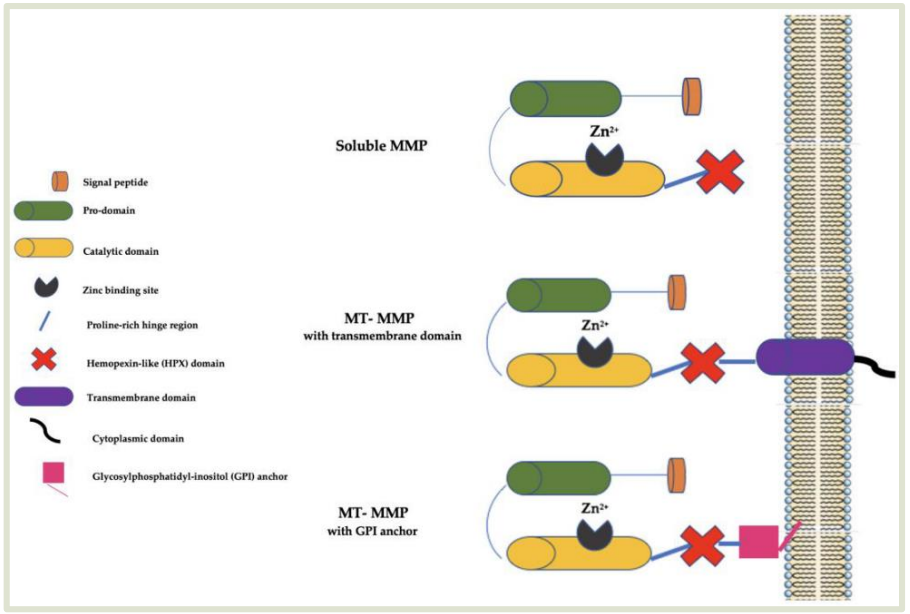

*R. E. Dalbey, P. Wang, J. M. van Dijl: Membrane Proteases in the Bacterial Protein Secretion and Quality Control Pathway. Microbiol. Mol. Biol. Rev.* ***2012****, 7*

# Metaloproteaze matriksa (MMP)

- ‘part-time’ kanonične šedaze
- Metaloproteaze (metcinkini)



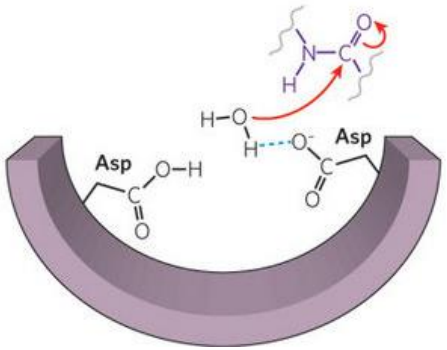

  - Topni (MMP-7, MMP-9)
  - Membraski ali GPI-sidrani proteini (MMP-14, MMP-16, MMP-24)



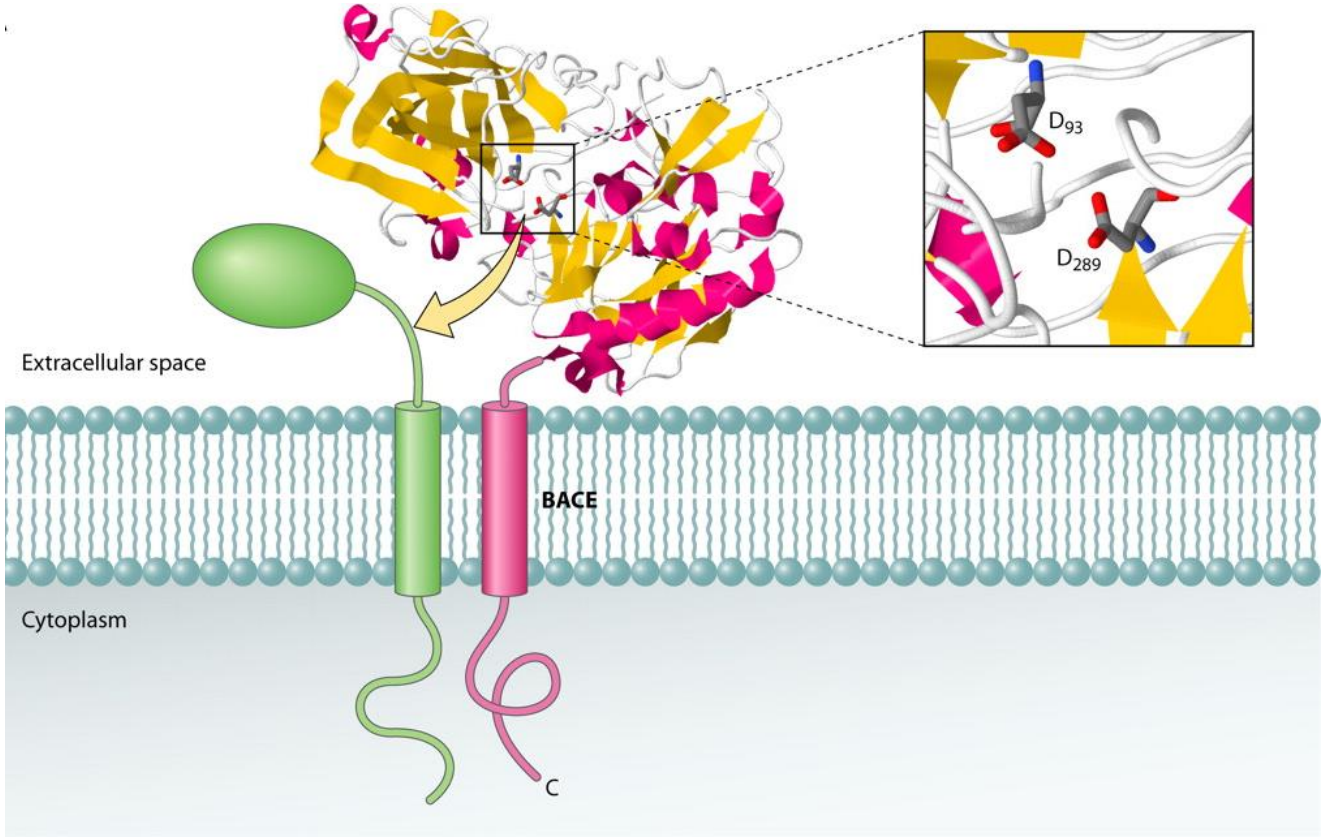

*D. Costa, idr.: Metalloproteinases between History, Health, Disease, and the Complex Dimension of Social Determinants of Health. J. Vasc. Dis. 2023, 2*

# **Proteaze BACE** (ang. beta-site APP cleaving enzyme)

- ‘full-time’ kanonične šedaze
- Asp proteaze



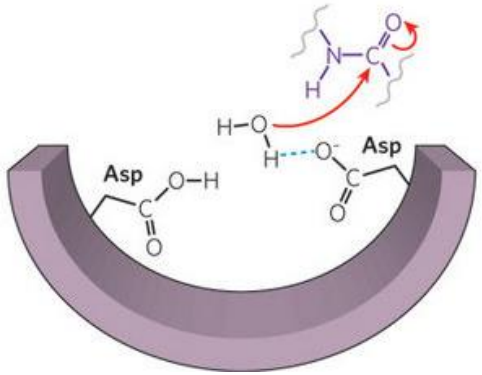

- **BACE1 in 2**
  - β-sekretazi
  - Integralna membranska proteina tipa I
  - Raznoliki substrati, denimo APP



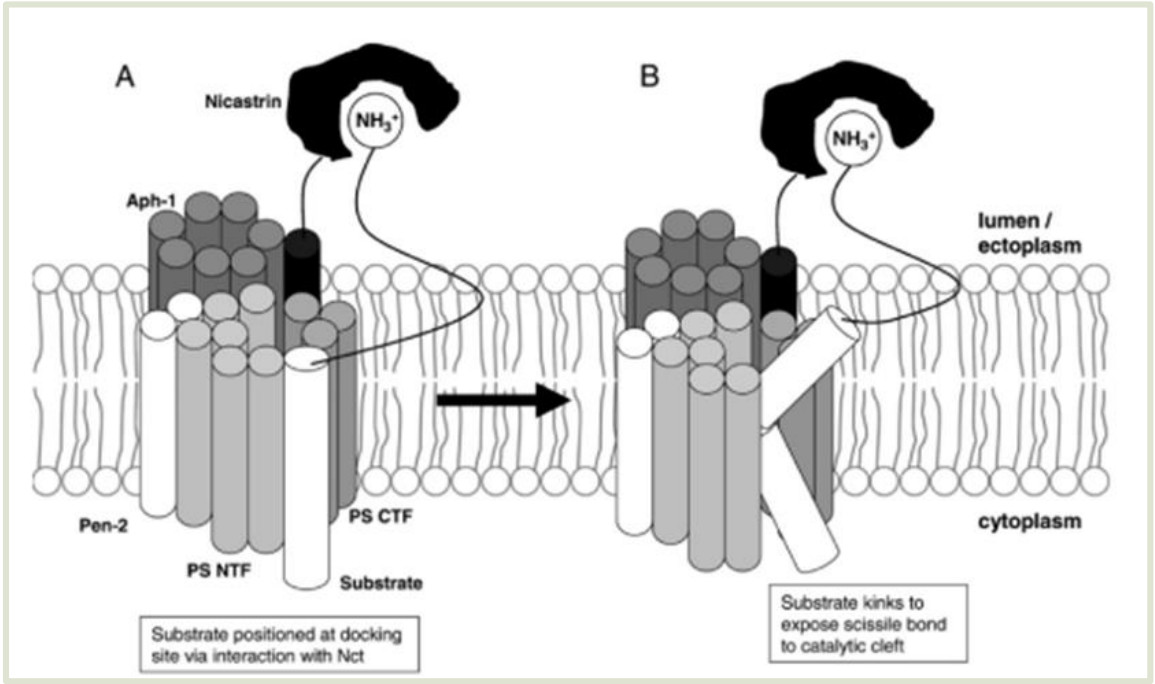

*R. E. Dalbey, P. Wang, J. M. van Dijl: Membrane Proteases in the Bacterial Protein Secretion and Quality Control Pathway. Microbiol. Mol. Biol. Rev.* ***2012****, 7*

# γ-sekretaza

- ‘part-time’ nekanonična šedaza
- Asp proteaza




- Transmembranski protein iz več podenot
  - **Presenilin 1 ali 2**
  - Nikastrin
  - Aph-1 *(ang. anterior pharynx-defective 1)*
  - Pen-2 *(ang. presenilin enchancer 2 )*
- Substrat
  - Membranski protein tipa I
  - Ektodomena krajša od ~50 aminokislin
    - Predhodnja cepitev
    - BCMA (antigen zorenja B-celic)

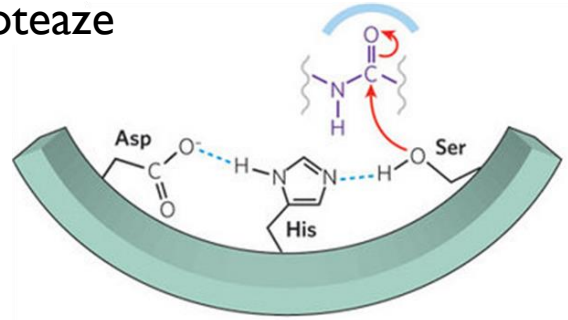



*A. J. Beel, C. R. Sanders: Substrate specificity of γ-secretase and other intramembrane proteases. Cell. Mol. Life Sci.* ***2008****, 65*

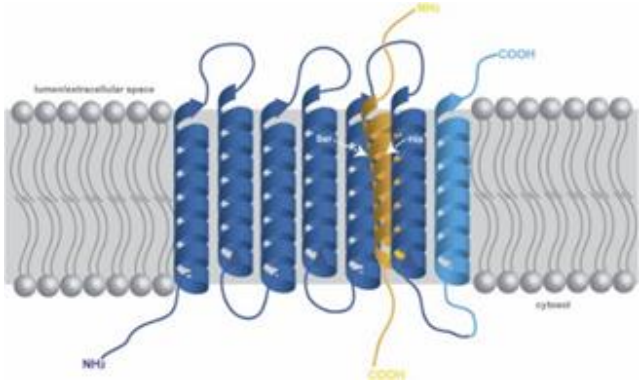
# Romboidi

## Splošne značilnosti

- ‘Full-time’ nekanonične šedaze
- Ser proteaze

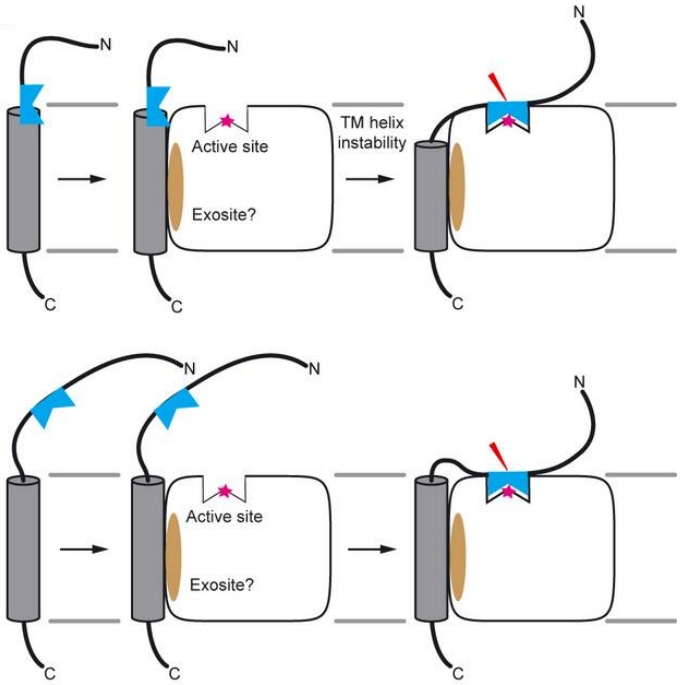



- Substrat: membranski protein tipa I



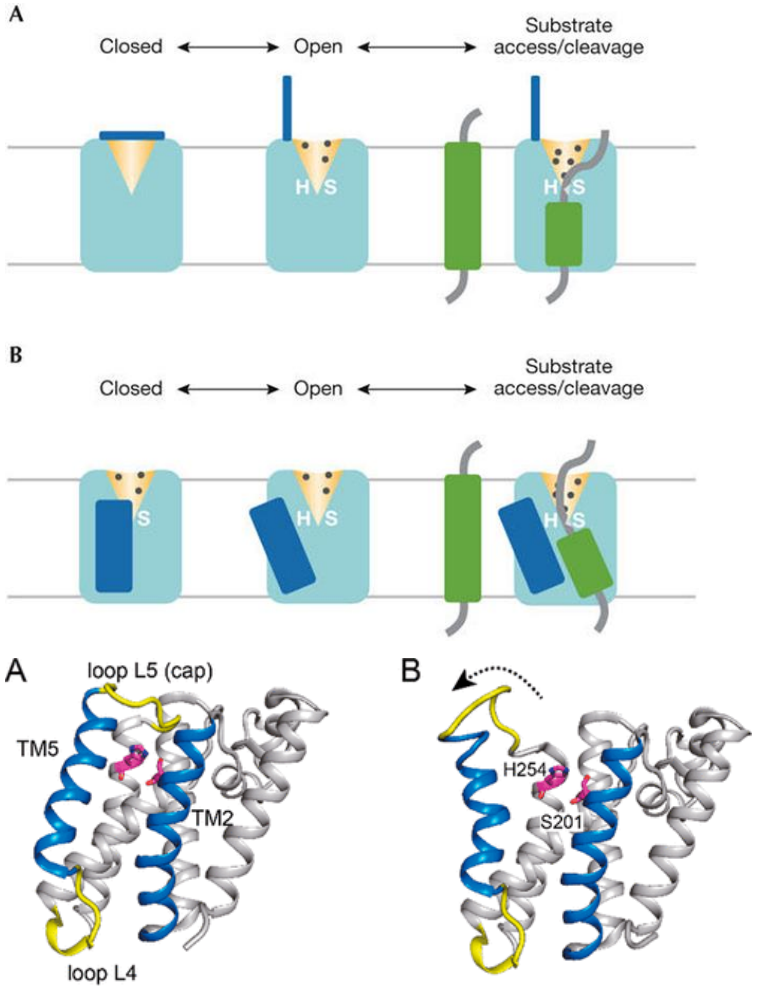
*V. L. Lastun, idr.: Substrates and physiological functions of secretase rhomboid proteases. Semin. Cell Dev. Biol. 2016, 60*

## Prepoznava substrata




*K. Strisovsky: Structural and mechanistic principles of intramembrane proteolysis – lessons from rhomboids. FEBS J. 2013, 280*

## Mehanizem



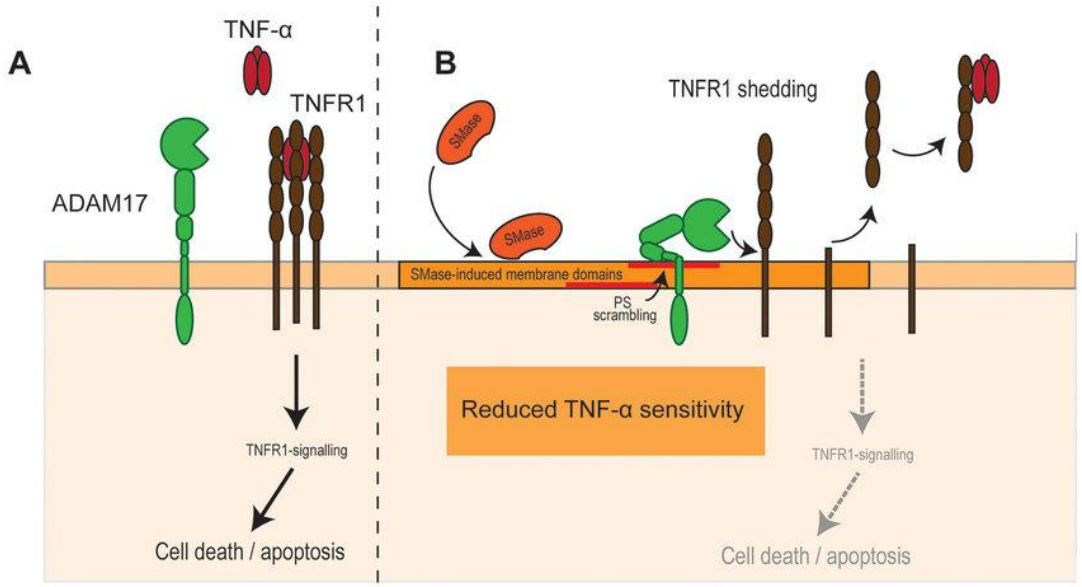

*C. Shi, idr.: Structure and Dynamics of the Rhomboid Protease GlpG in Liposomes Studied by Solid-State NMR. J. Am. Chem. Soc. 2019, 141*

# FIZIOLOGIJA

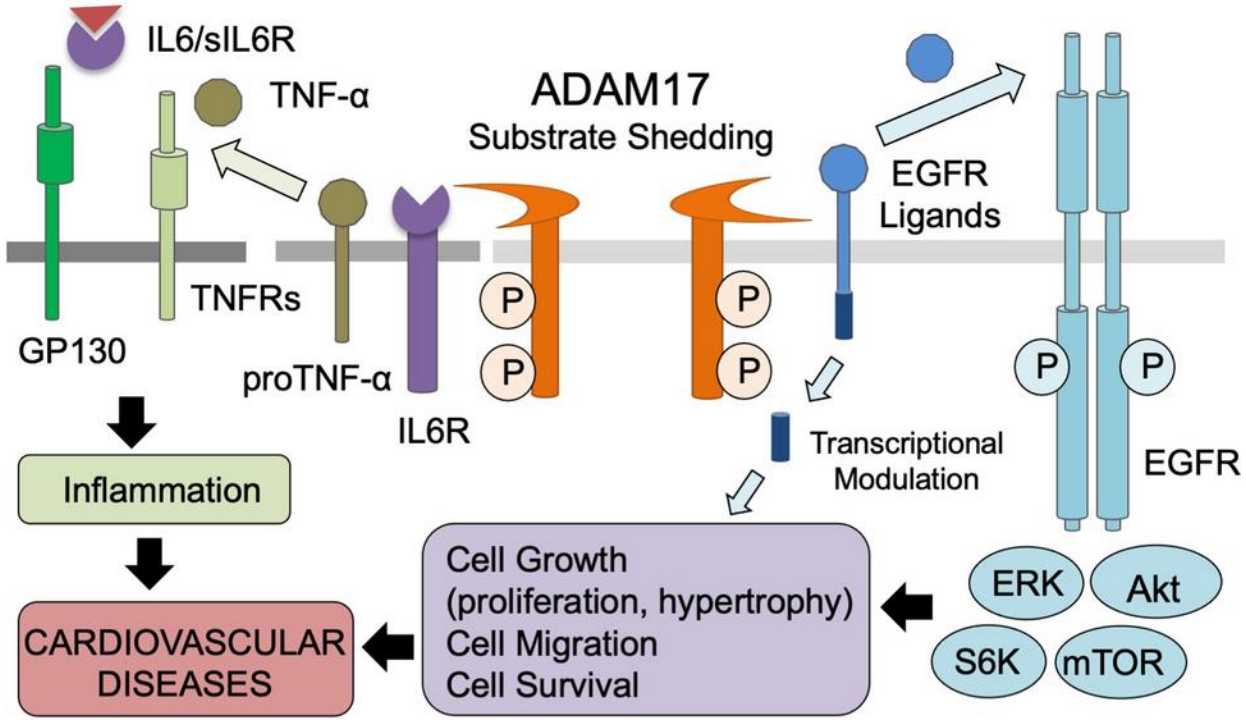
razvoj tkiv
angiogeneza
uravnavanje homeostaze
vnetni odziv
diferenciacija celic
komunikacija med celicami
apoptoza
...

## → ADAM17 (TACE):

- Pretvorba citokina **TNFα** iz netopne v topno obliko
  - TNFα = pro-vnetni citokin
- Uravnavanje aktivnosti receptorjev TNFα
- Aktivnost je regulirana in inducirana kot odziv na vnetje ali poškodbo
  - Aktivacija s pomočjo kinaz (PLK2, MAPK ali PKC), ki fosforilirajo citoplazemski repek ADAM17



Cepitev **receptorjev za interlevkine** (IL-1RII, IL-6R) → Nastane topna oblika receptorja, ki omogoča trans-signalizacijo!

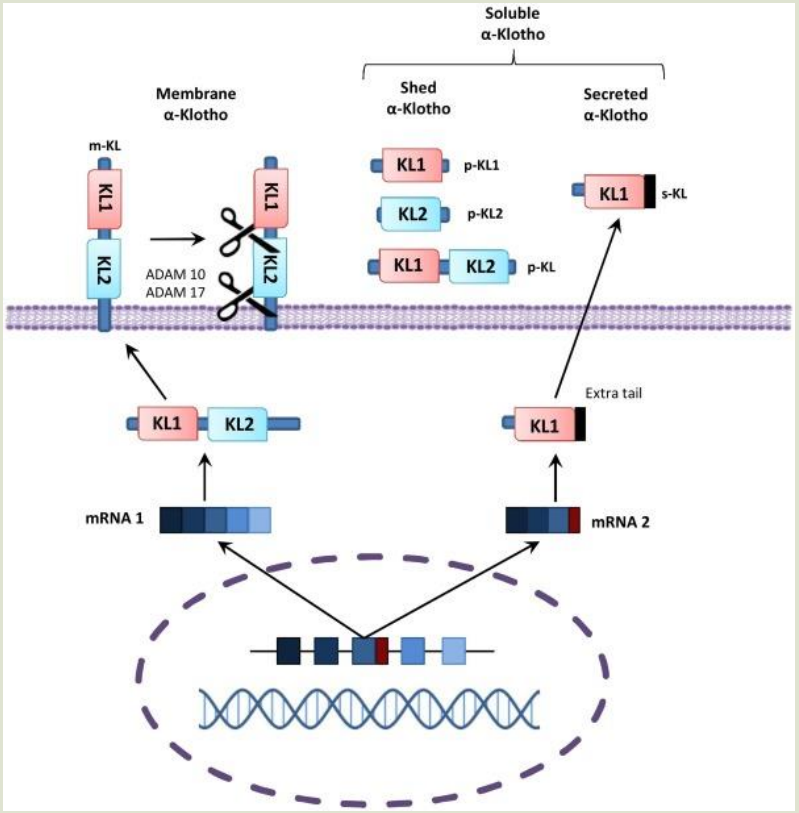



Cepitev **TGFα** → motnje v razvoju epitelija pri miškah z izbitim genom za ADAM17

## → ADAM10:

- ADAM17 se izraža inducibilno, ADAM10 konstitutivno
- Cepi preko 100 transmembranskih proteinov → **α-sekretaza**

- ADAM10 in ADAM17 cepita **protein Klotho**
- Cepitev povečana ob prisotnosti inzulina → sproži povečano izločanje proteaz na površino celice

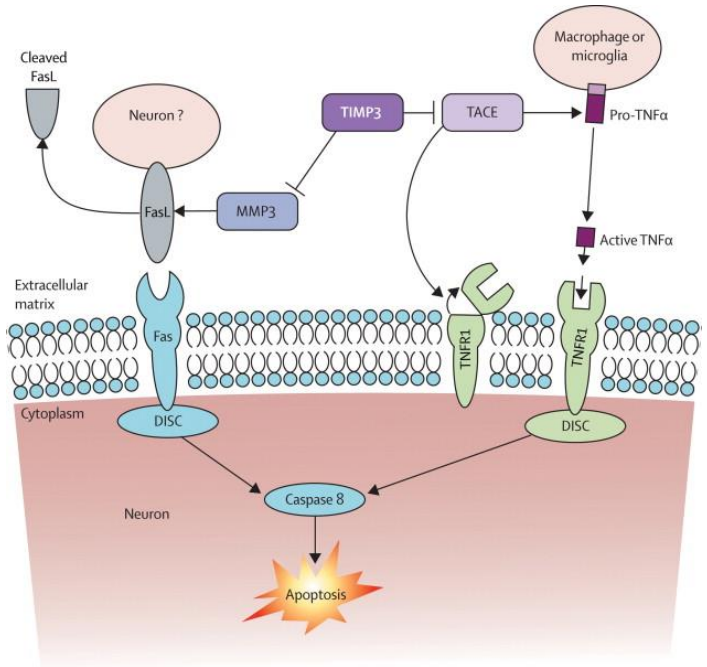


## → MMP-9:

- Cepi α-verigo receptorja za IL-2
- Sodeluje pri angiogenezi; cepi VEGF
- Cepi ostale signalne molekule, kot na primer IL-1β, TGF-β…

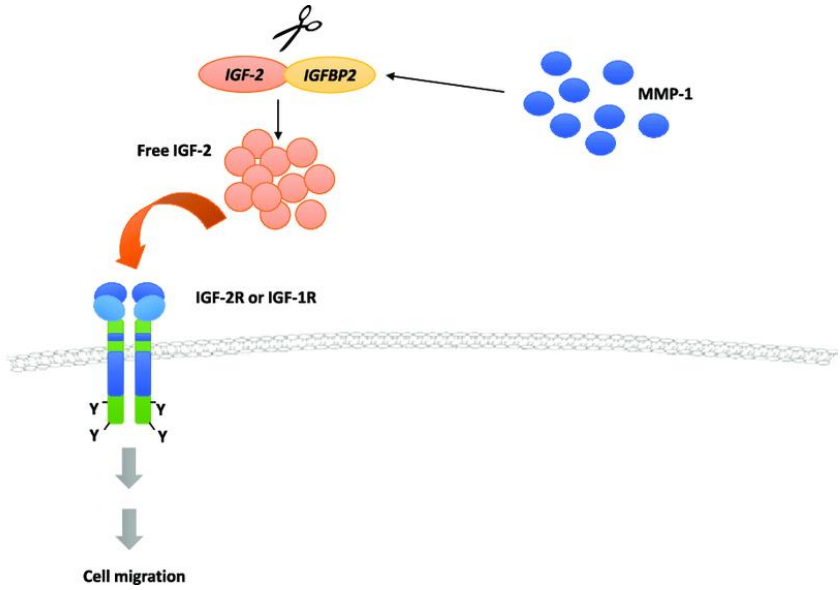
## → MMP-3:

- Cepi substrate, kot so ligand Fas, E-kadherin, PAI-1…



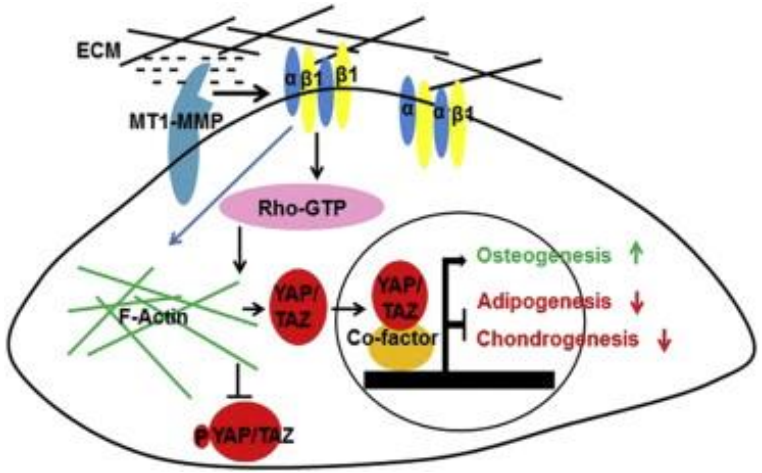

## → MMP-1:

- Cepi IGFBP-3, IGFBP-5, L-selektin…
- Cepi pro-vnetne citokine (IL-1β)



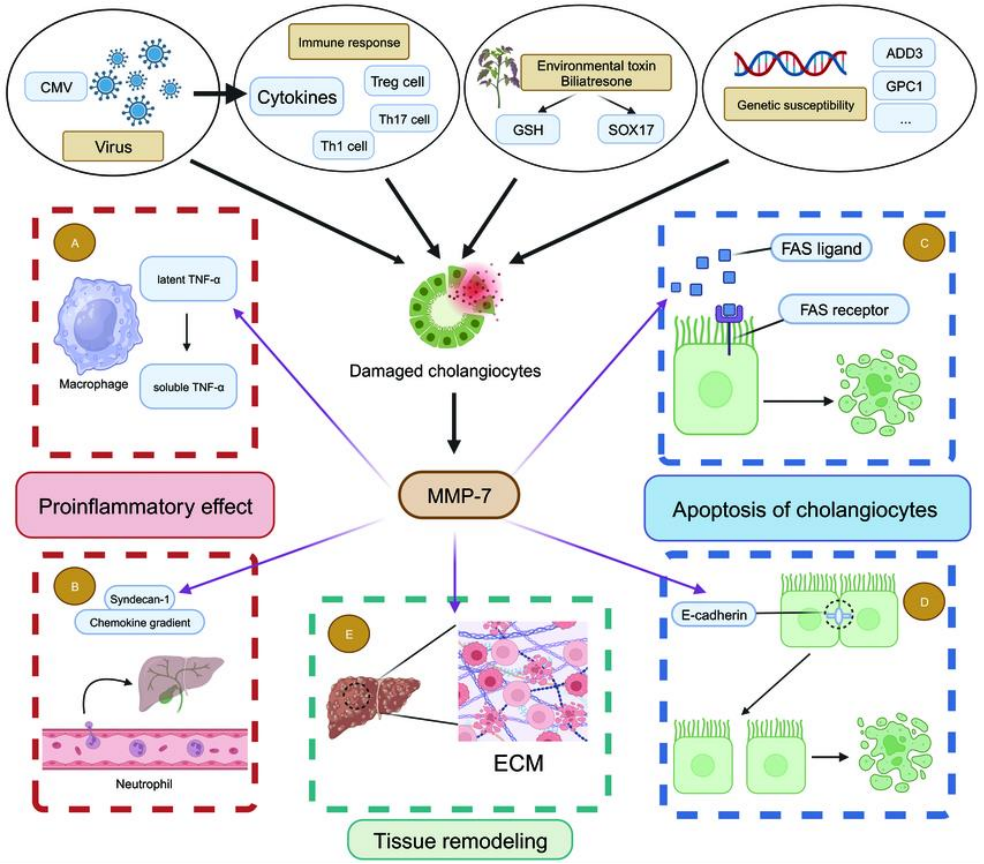
## → MMP-14:

- Cepi membranske proteine, kot so E-kadherin, N-kadherin, integrini, receptor za hialuronan, RANKL…
- Sodeluje pri angiogenezi; cepi VEGF-A




## → MMP-7:

- Cepi membranske proteine, kot so TNFα, Fas ligand, E-kadherin in integrin β4
- Konstitutivno izražanje v mukoznem epiteliju → aktivira pro-α-defenzine

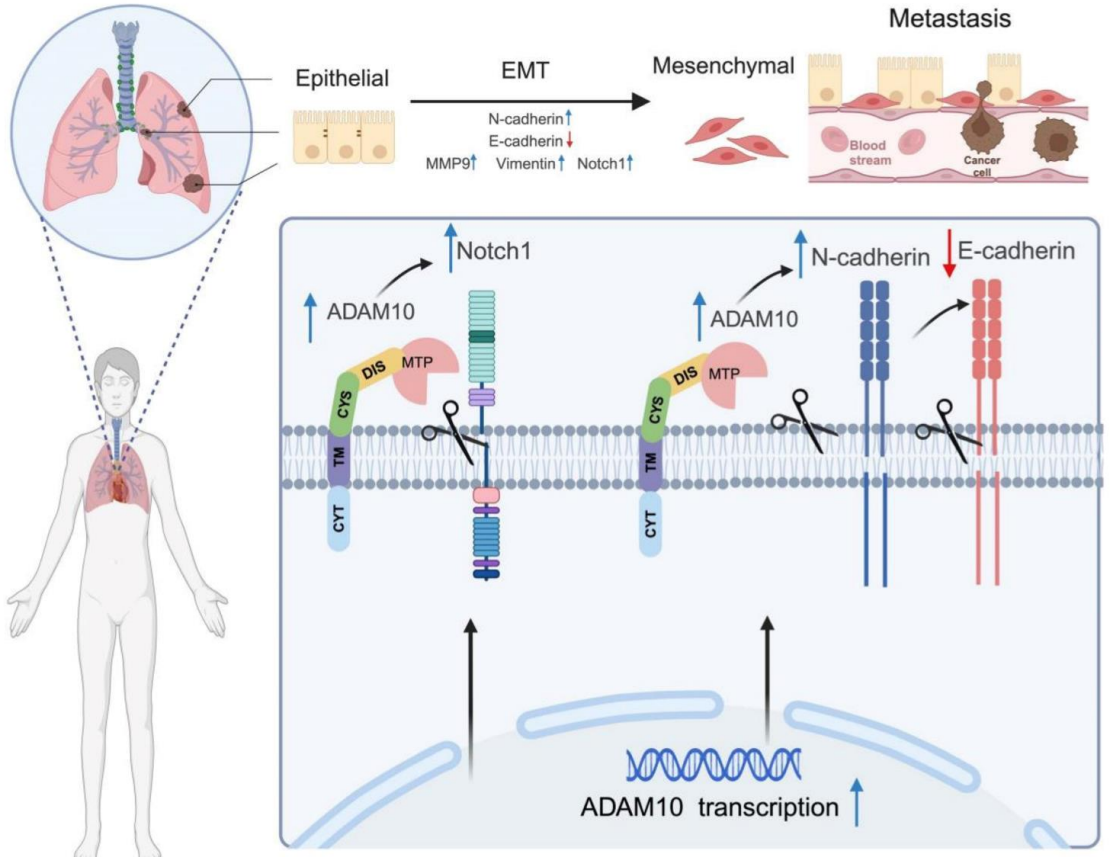


# PATOLOGIJA IN ZDRAVLJENJE

- **ADAM10 in 17**: razvoj raka, nevroloških in avtoimunih bolezni, diabetesa, alergij ter vnetij.
- **β- in γ-sekretaza**: nevrodegenerativne bolezni.
- **MMP-3 in MMP-7**: rak

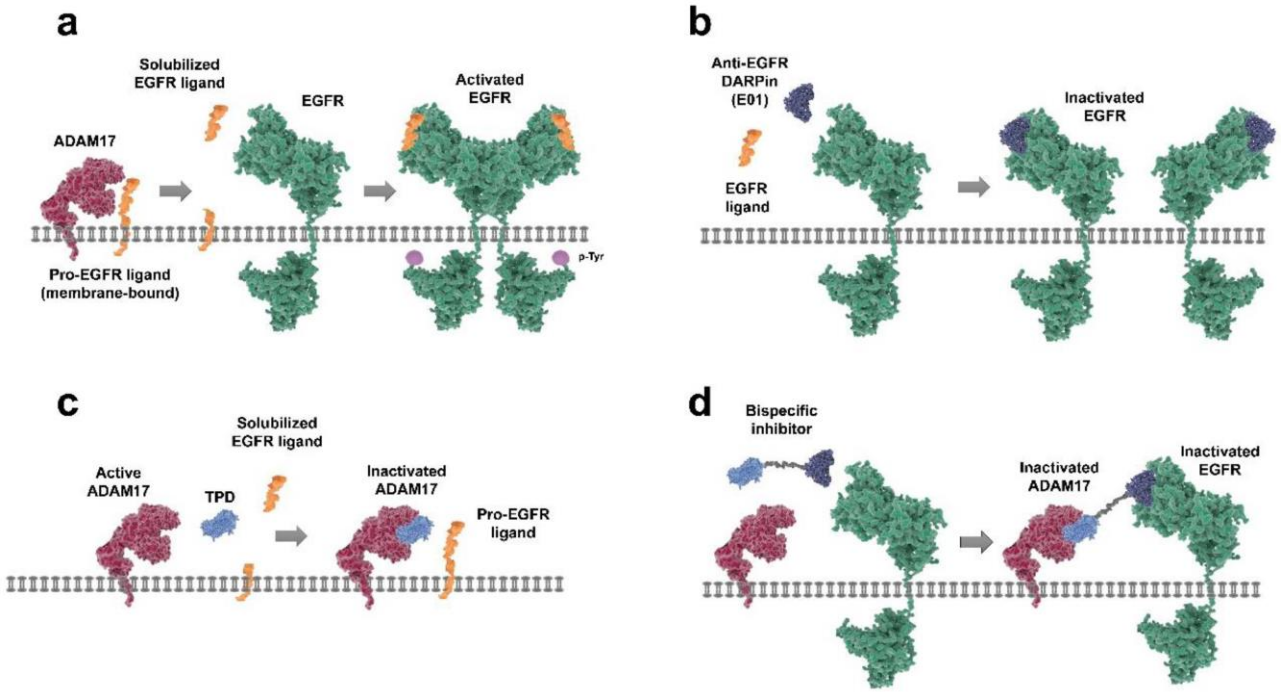
# ADAM10

Cepi adhezijske molekule, kot so N-, E- in VE-kadherin, kar spremeni celično adhezijo, migracijo ter sproži β-kateninsko signalizacijo.




W. Zhang, et al., Journal of Cancer 2025, 16, 1736–1746.

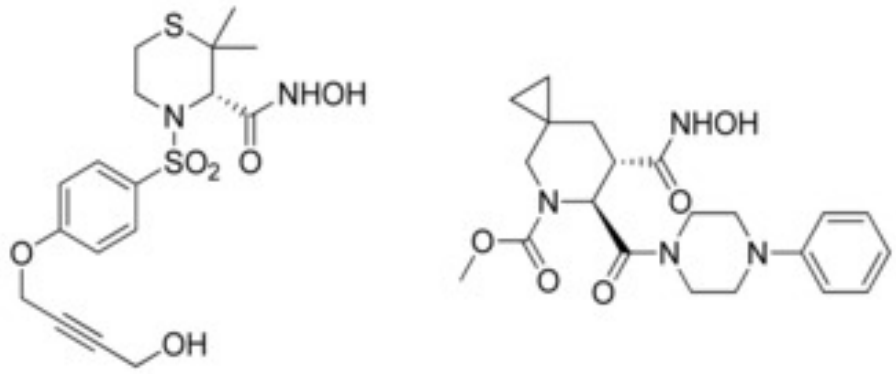
# ADAM17




A. Soto-Gamez, et. al., Cancers 2020, 12, 411.

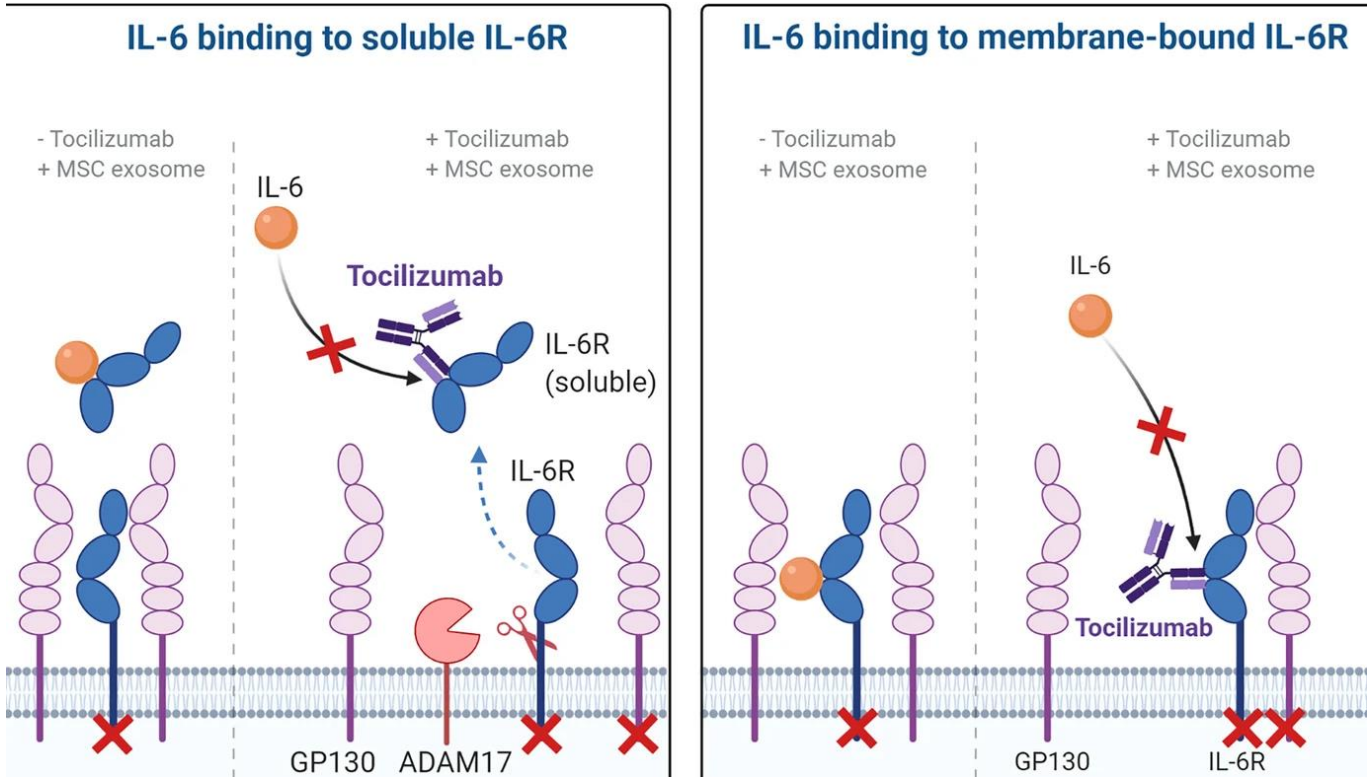
Sprošča TGFα in s tem aktivira EGFR-signalizacijo, kar ima pomembno vlogo tako pri razvoju raka kot pri razvoju avtoimunskih boleznih.

# INHIBITORJI ADAM17




**Apratastat** **INCB7839**

Maretzky & Reiss, *Molecules*, 2021, 26(4), 944.

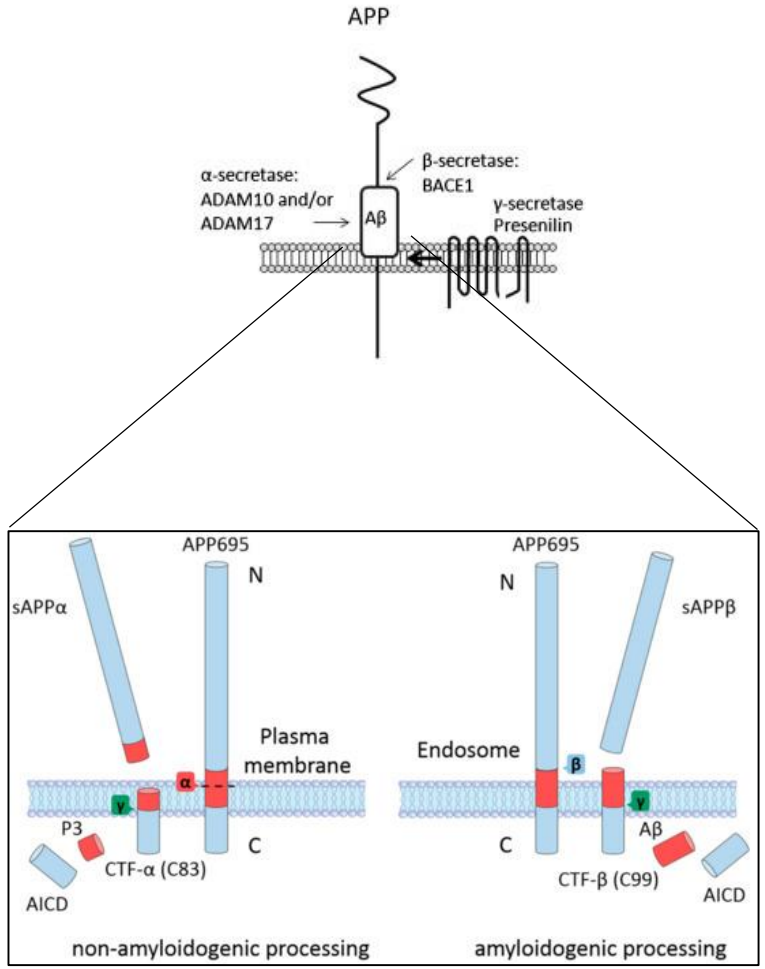



Zhou et al., 2022, *Stem Cell Reviews and Reports,* 2919–2935.

# SEKRETAZE

- **α-sekretaze** (ADAM9, 10 in 17) → usmerja APP v ne-amiloidogeno pot
- **β-sekretaza** (BACE1) → usmerja APP v amiloidogeno pot
- **γ-sekretaza** → mutacije v genih povzročijo usmerjanje v amiloidogeno pot

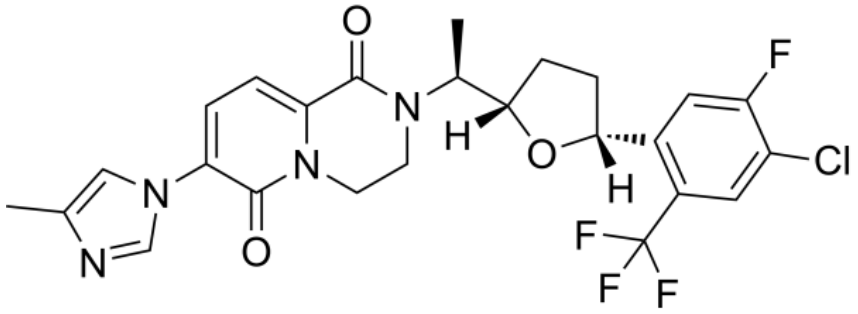
D. R. Edwards, et al., Molecular Aspects of Medicine 2008, 29, 258–289.




T. Zhang, et al., International Journal of Molecular Sciences 2020, 21, 209.

# INHIBITORJI GAMA-SEKRETAZ

- Nespecifični inhibitorji γ-sekretaz
- selektivni inhibitorji posameznih kompleksov γ-sekretaze
- γ-sekretazni modulatorji
- stabilizatorji encimskih kompleksov




PF-06648671

MedChemExpress. (n.d.). *PF-06648671*.

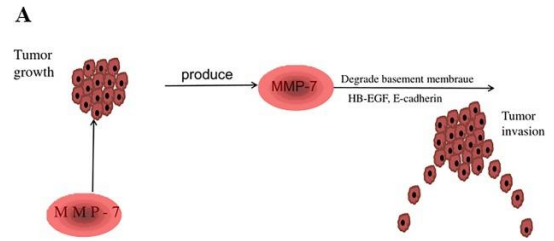
# MMP-7 IN 3

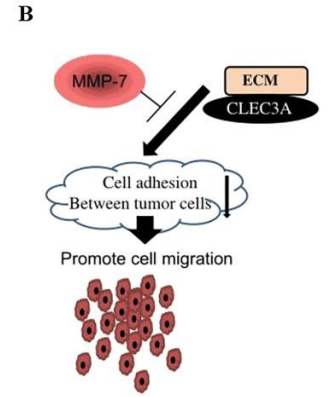
## MMP-7

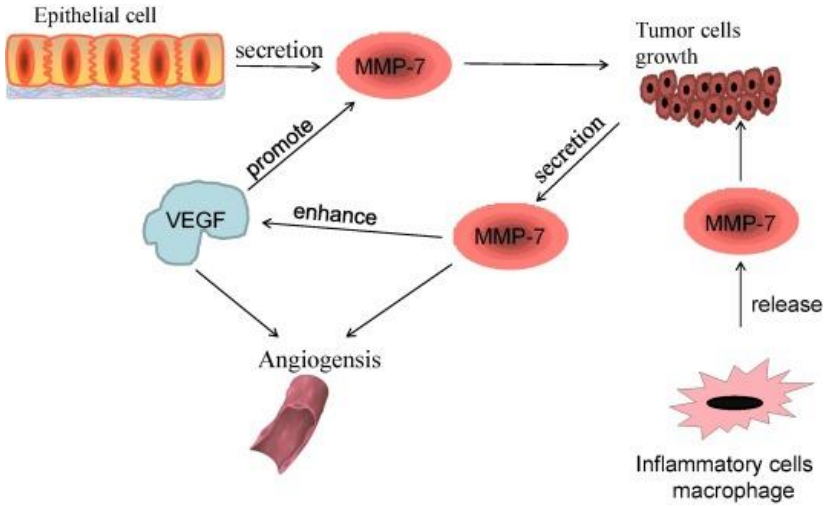
- Razgrajuje ECM → olajša invazijo tumorskih celic
- Aktivira druge MMP
- Cepi ektodomeno proHB-EGF → spodbuja proliferacijo in zavira apoptozo
- Cepi ektodomeno liganda Fas → sFas proži apoptozo v sosednjih celicah

## MMP-7 & MMP-3

- Cepita E-kadherin → nastane topna oblika → zavira celične stike, spodbuja migracijo
- Cepita vezavne proteine za inzulinu podoben rastni faktor (IGFBP) skupaj z MMP-19 → povečana razpoložljivost IGF → rast in preživetje



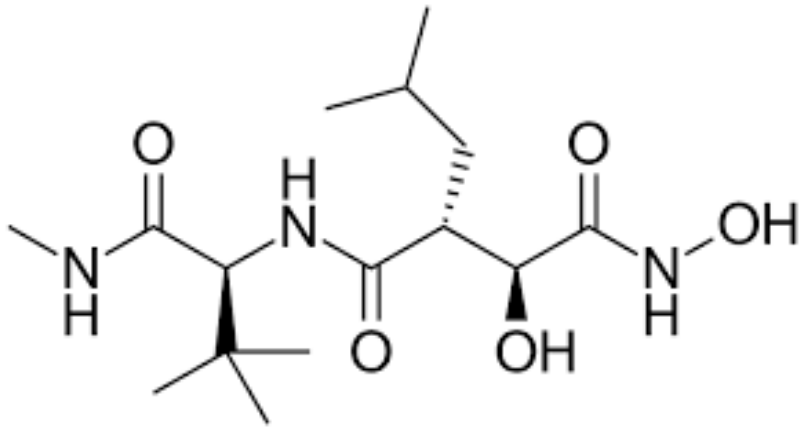




H.-Y. Liao, et al., Clinical Biochemistry 2021, 92, 9–18.

# INHIBITORJI MMP-7

- Povečano izražanje MMP-7 pri raku želodca, trebušne slinavke in mehurja
- Majne molekule, peptidi, protitelesa
- Ciljna vezava inhibitorja v aktivno mesto ali na druge dele encima
- Kelacija cinkovega iona
- Problem specifičnosti
- Marimastat = širokospektralni inhibitor MMP (III. faza kliničnih študij)




marimastat

*Marimastat chemical structure,* Wikipedia.

# VIRI IN LITERATURA


[1] L. E. Hedin, K. Illergård, A. Elofsson: An Introduction to Membrane Proteins. *J. Proteome Res.* **2011**, *10*, 3324–3331.
[2] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter: Membrane Proteins. V *Molecular Biology of the Cell. 4th edition*; Garland Science, 2002.
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