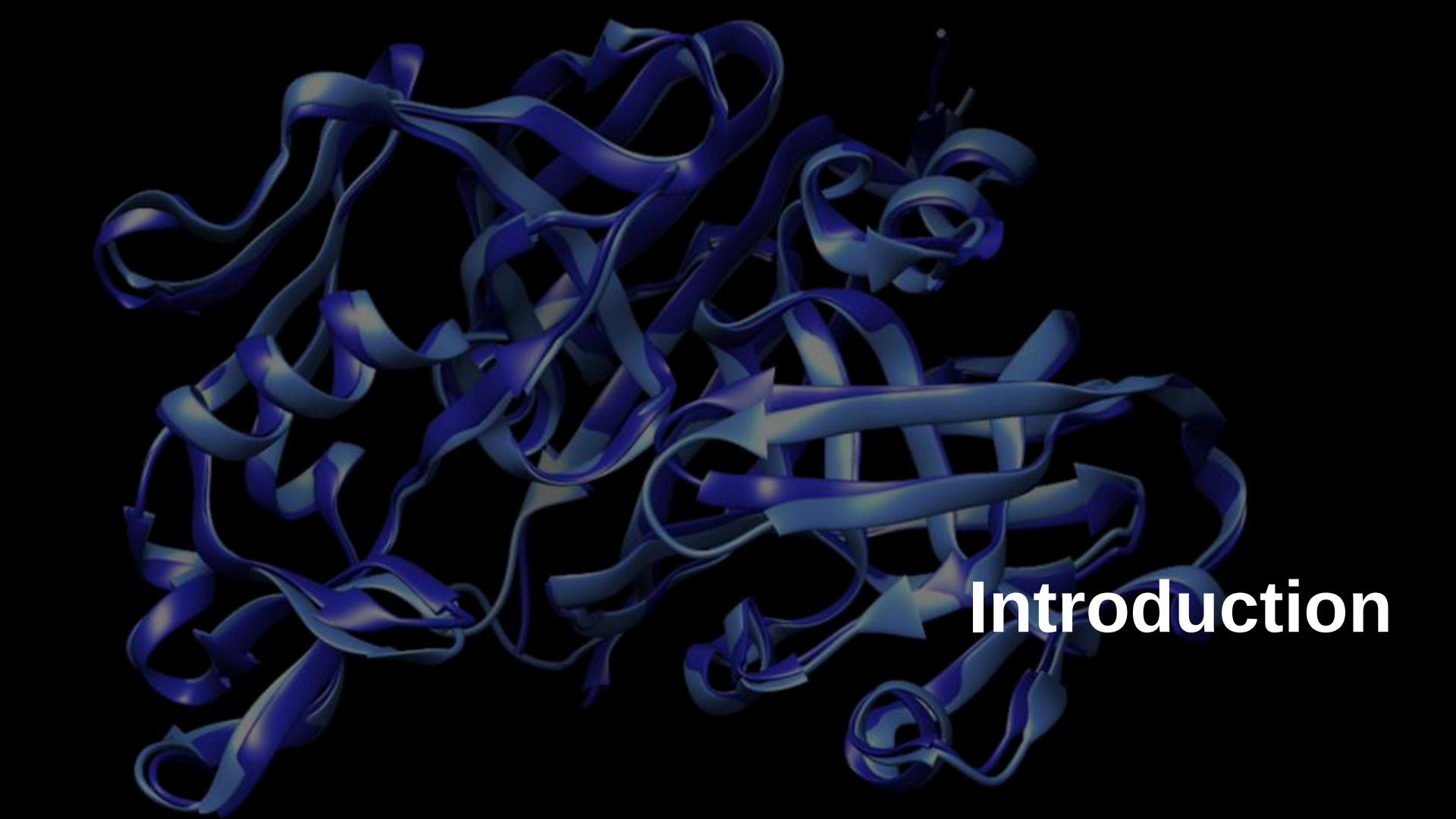
ENDOPEPTIDASES

Renin

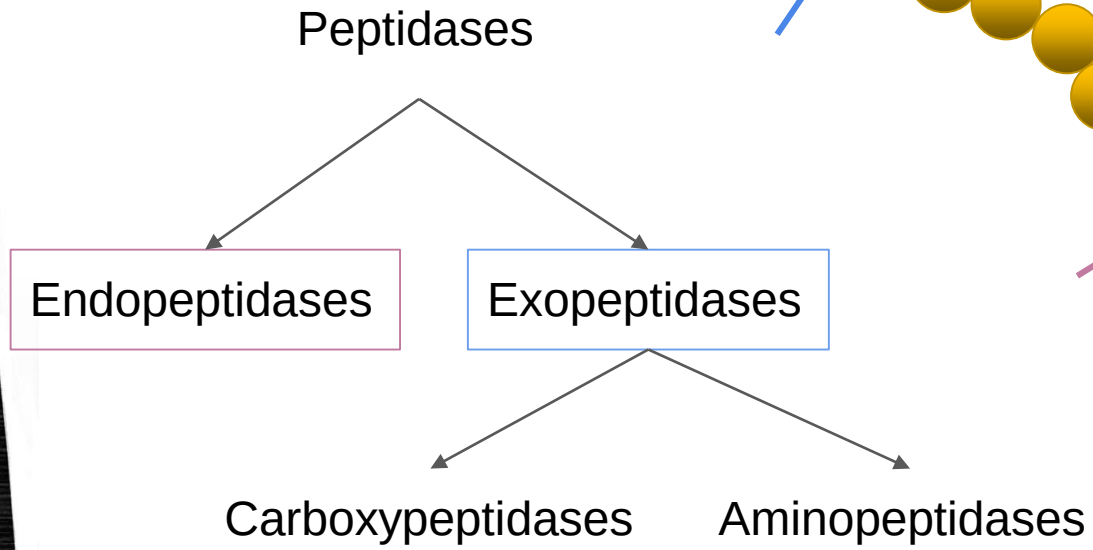
Susana Güerri, Ada Melo, Marta Moral, Alba Segarra
Structural Biology, 2019-2020

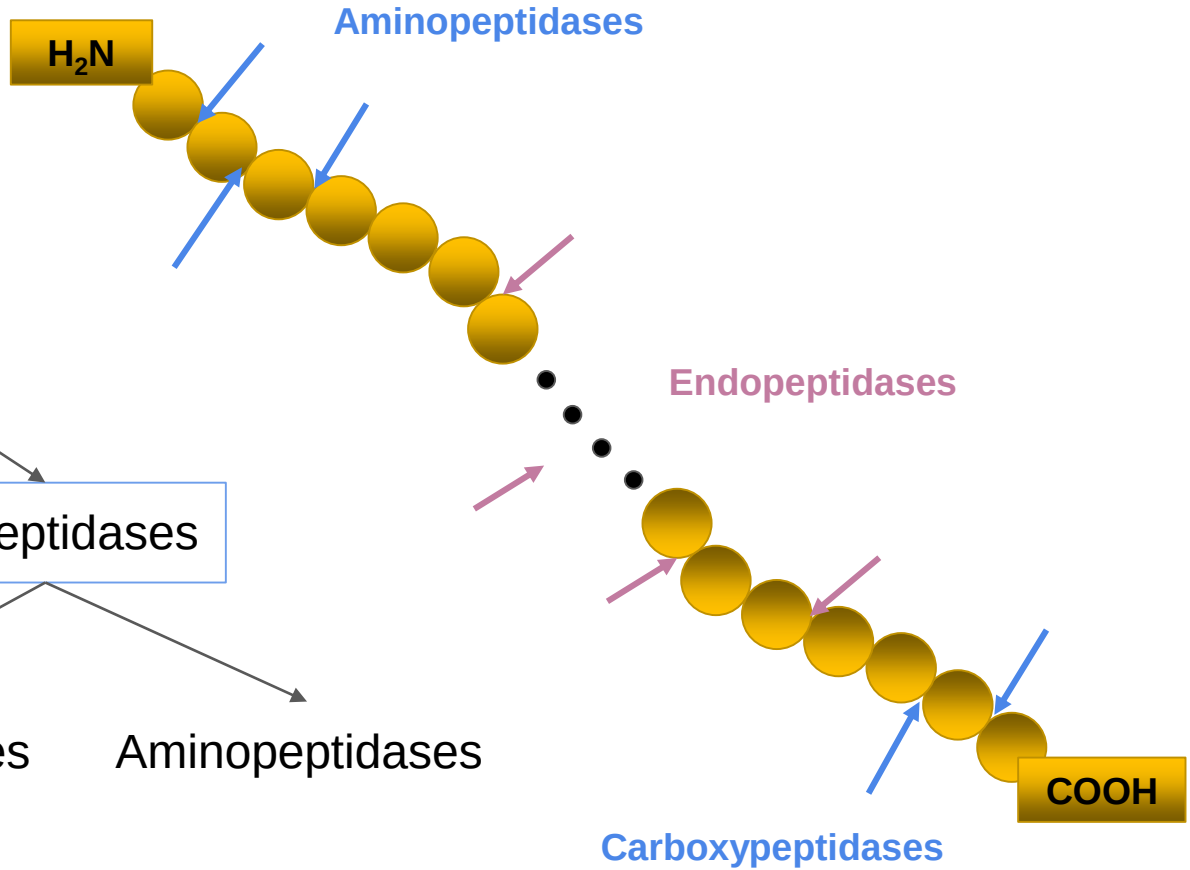
Index



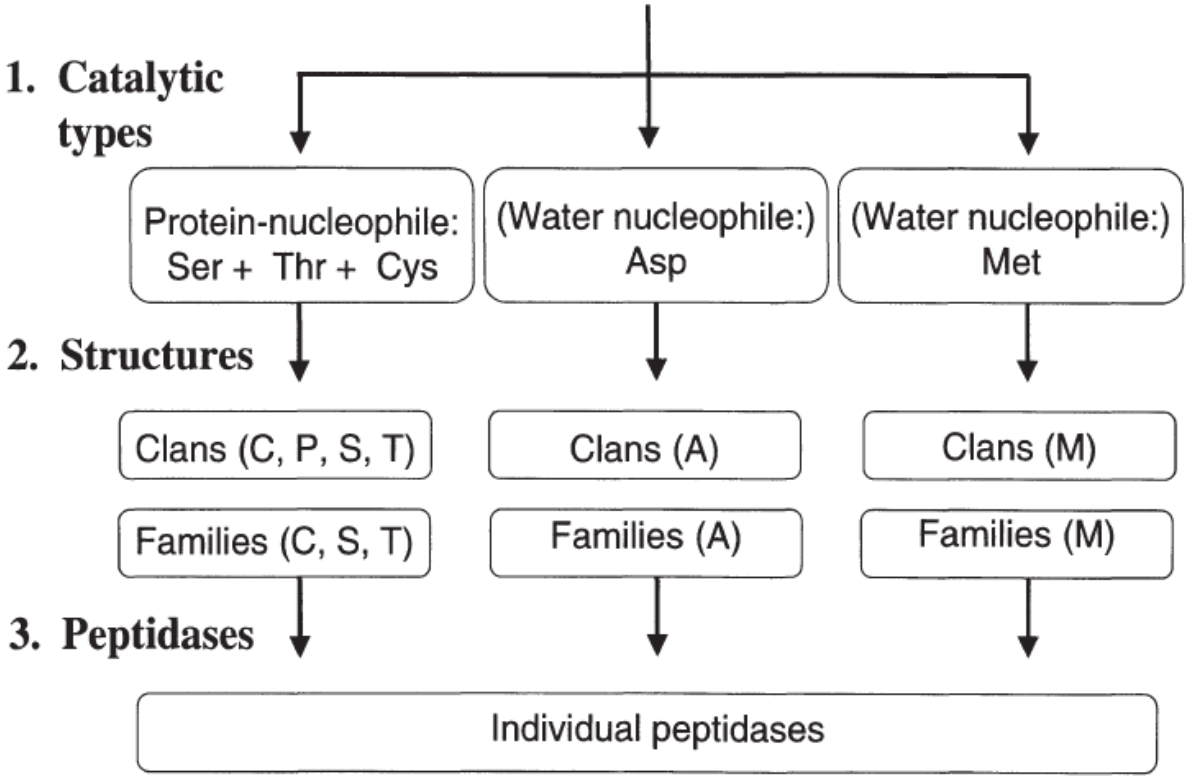
Introduction

Peptidases


Peptidases
Endopeptidases
Exopeptidases
Carboxypeptidases
Aminopeptidases

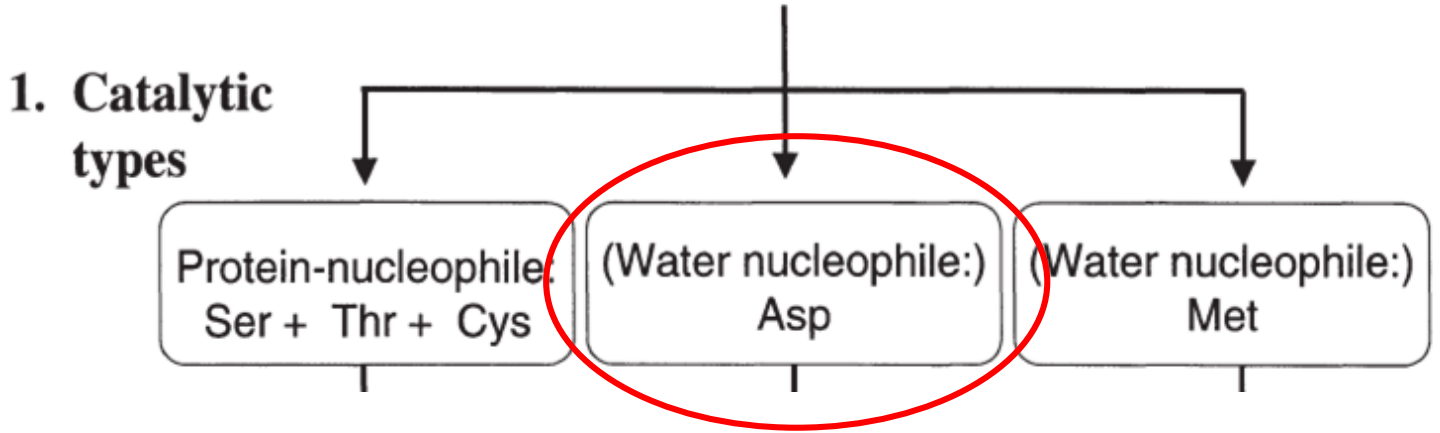

H2N
Aminopeptidases
Endopeptidases
COOH
Carboxypeptidases

Peptidases: Classification



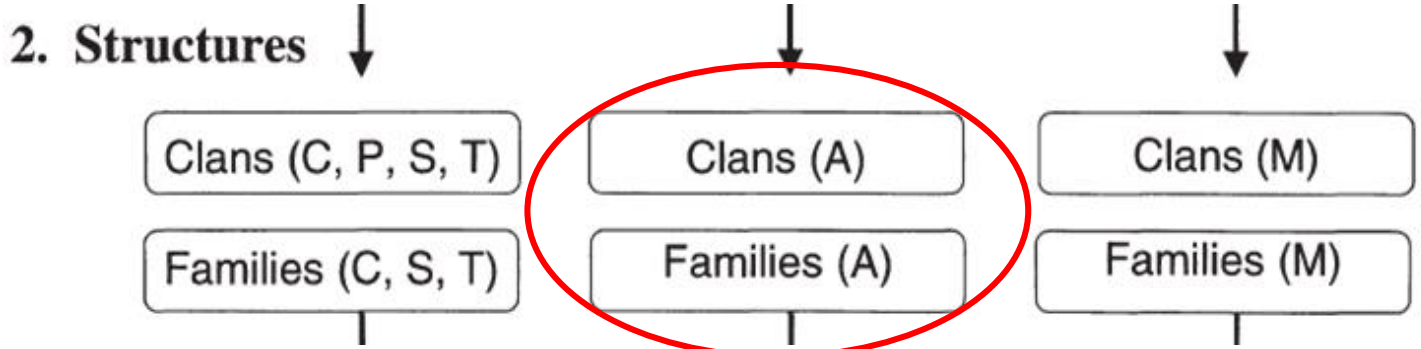
1. Catalytic types

- According to Enzyme Commission (EC) numbers classification.



2. Structures

- MEROPS Classification



3. Individual peptidases

Endopeptidases

Serin endopeptidases

Metalo endopeptidases

Aspartic endopeptidases

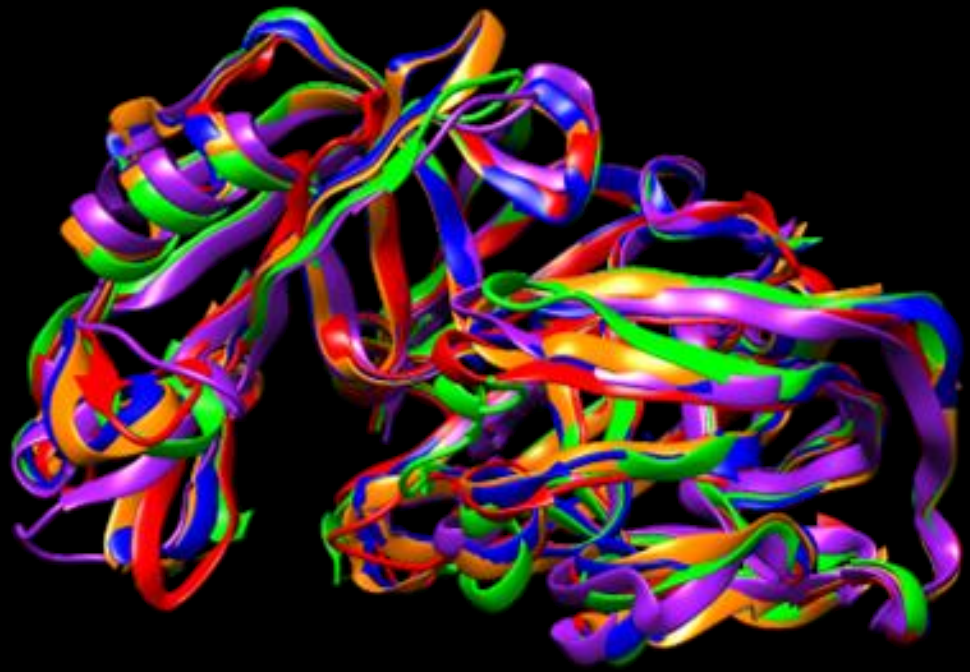
Cysteine endopeptidases

Unknown mechanism endopeptidases

Aspartic Peptidases

- Protozoa, Viruses, Plants, Vertebrates.
- Two Aspartic Acids (D) are directly responsible for the catalytic activity.
  - Water molecule.
  - Transient tetrahedral intermediate.
- Synthesized as proenzymes → cleavage of the pro-segment.

Superimposition of Aspartic Peptidases



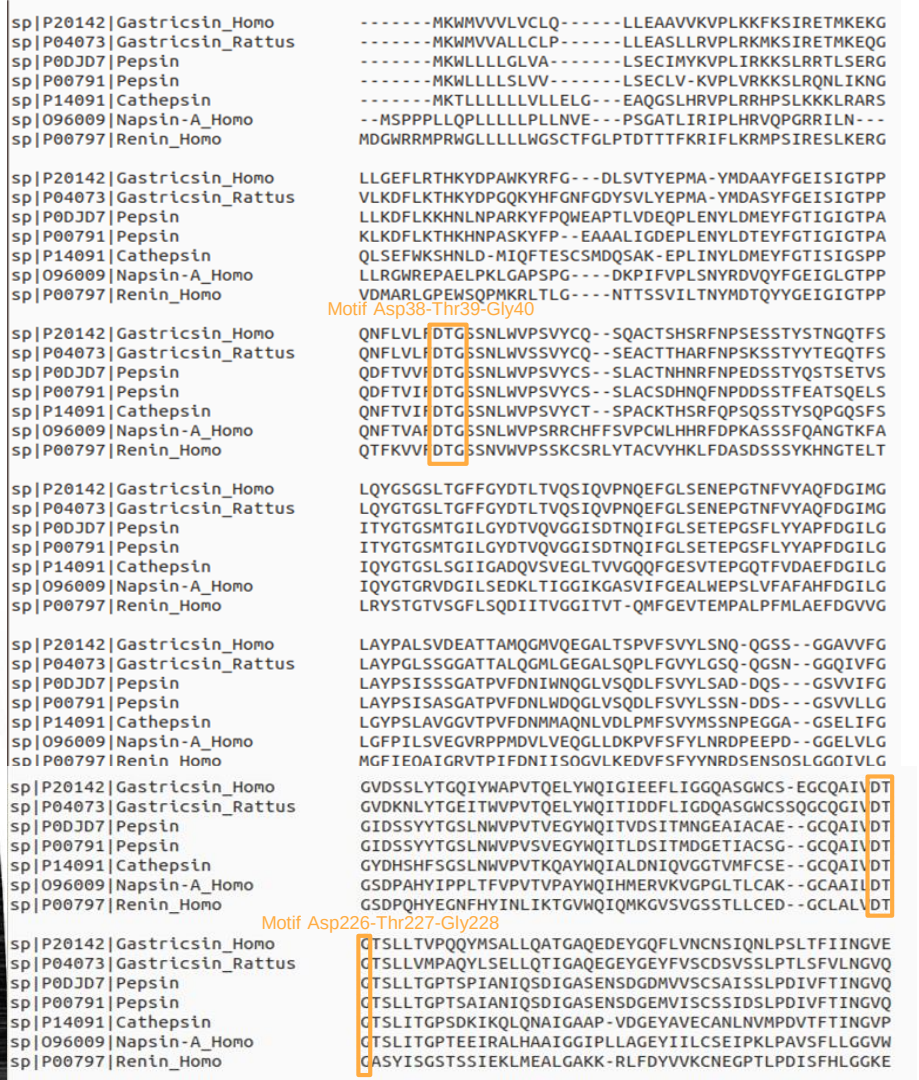
1rne - Renin (Human)

1tzs - Cathepsin E (Human)

4aa8 - Chymosin (Bovine)

1flh - Uropepsin (Human)

4pep - Pepsin (porcine)

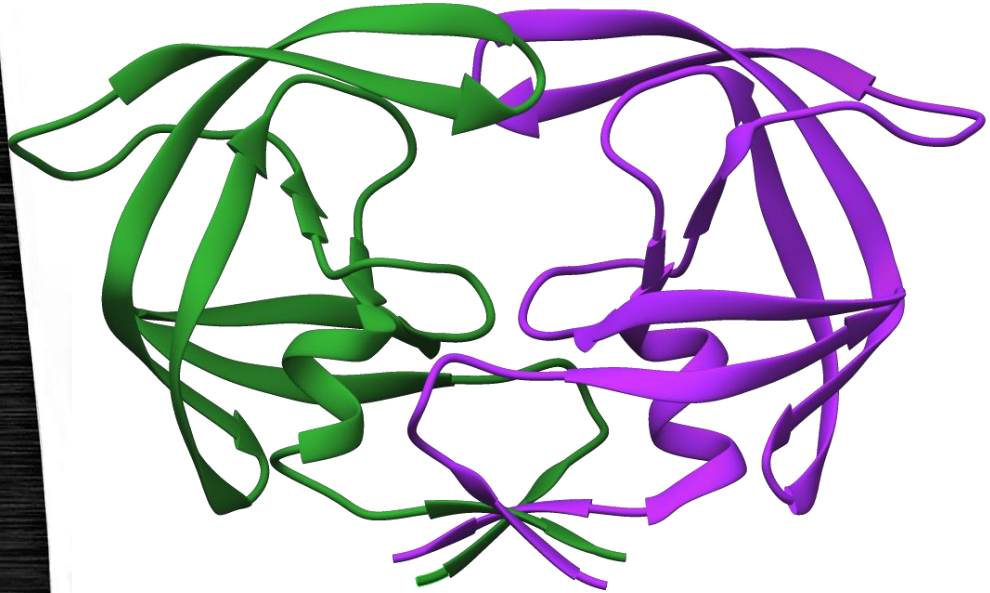


Residues conserved in Aspartic Peptidases

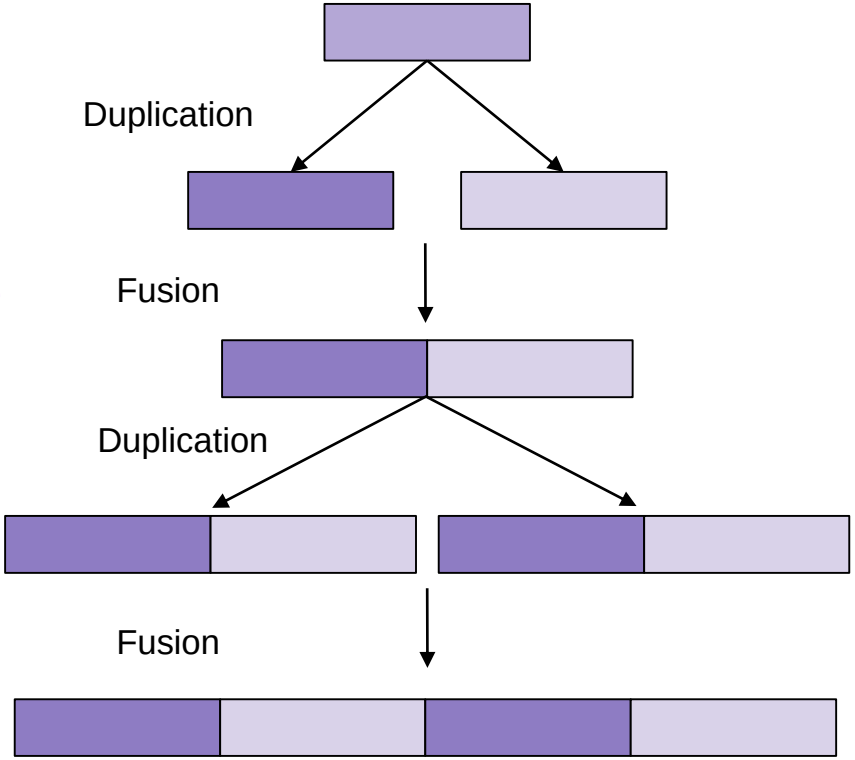
2 Asp-Thr/Ser-Gly motifs forming the catalytic active site

It suggests gene duplication

Gene duplication



Crystal structure of HIV-1 PROTEASE



Residues conserved in Aspartic Peptidases

2 Asp-Thr/Ser-Gly motifs

The hydrogen bond network Trp45-Tyr83-water (W2)-Ser 41-Asp38 is conserved

Residues conserved in Aspartic Peptidases

Motif Asp226-Thr227-Gly228

```
sp|P20142|Gastricsin_Homo        GVDSSLYTGQIYWAPVTQELYWQIGIEEFLIGGQASGWCS-EGCQAIVDT
sp|P04073|Gastricsin_Rattus      GVDKNLYTGEITWVPVTQELYWQITIDDFLIGDQASGWCSSQGCQGIVDT
sp|P0DJD7|Pepsin                 GIDSSYYTGSLNWVPVTVEGYWQITVDSITMNGEAIACAE--GCQAIVDT
sp|P00791|Pepsin                 GIDSSYYTGSLNWVPVSVEGYWQITLDSITMDGETIACSG--GCQAIVDT
sp|P14091|Cathepsin              GYDHSHFSGSLNWVPVTKQAYWQIALDNIQVGGTVMFCSE--GCQAIVDT
sp|O96009|Napsin-A_Homo          GSDPAHYIPPLTFVPVTVPAYWQIHMERVKVGPGLTLCAK--GCAAILDT
sp|P00797|Renin_Homo             GSDPQHYEGNFHYINLIKTGVWQIQMKGVSVGSSTLLCED--GCLALVDT

sp|P20142|Gastricsin_Homo        GTSLLTVPQQYMSALLQATGAQEDEYGQFLVNCNSIQNLPSLTFIINGVE
sp|P04073|Gastricsin_Rattus      GTSLLVMPAQYLSELLQTIGAQEGEYGEYFVSCDSVSSLPTLSFVLNGVQ
sp|P0DJD7|Pepsin                 GTSLLTGPTSPIANIQSDIGASENSDGDMVVSCSAISSLPDIVFTINGVQ
sp|P00791|Pepsin                 GTSLLTGPTSAIANIQSDIGASENSDGEMVISCSSIDSLPDIVFTINGVQ
sp|P14091|Cathepsin              GTSLITGPSDKIKQLQNAIGAAP-VDGEYAVECANLNVMPDVTFTINGVP
sp|O96009|Napsin-A_Homo          GTSLITGPTEEIRALHAAIGGIPLLAGEYIILCSEIPKLPAVSFLLGGVW
sp|P00797|Renin_Homo             GASYISGSTSSIEKLMEALGAKK-RLFDYVVKCNEGPTLPDISFHLGGKE
```

Thr229

2 Asp-Thr/Ser-Gly motifs

The hydrogen bond network Trp45-Tyr83-water (W2)-Ser 41-Asp38 is present

Threonine 229 is present in all aspartic proteinases except renins, where it is replace by Alanine (in human) or Serine (in mouse)

Residues conserved in all Aspartic Peptidases

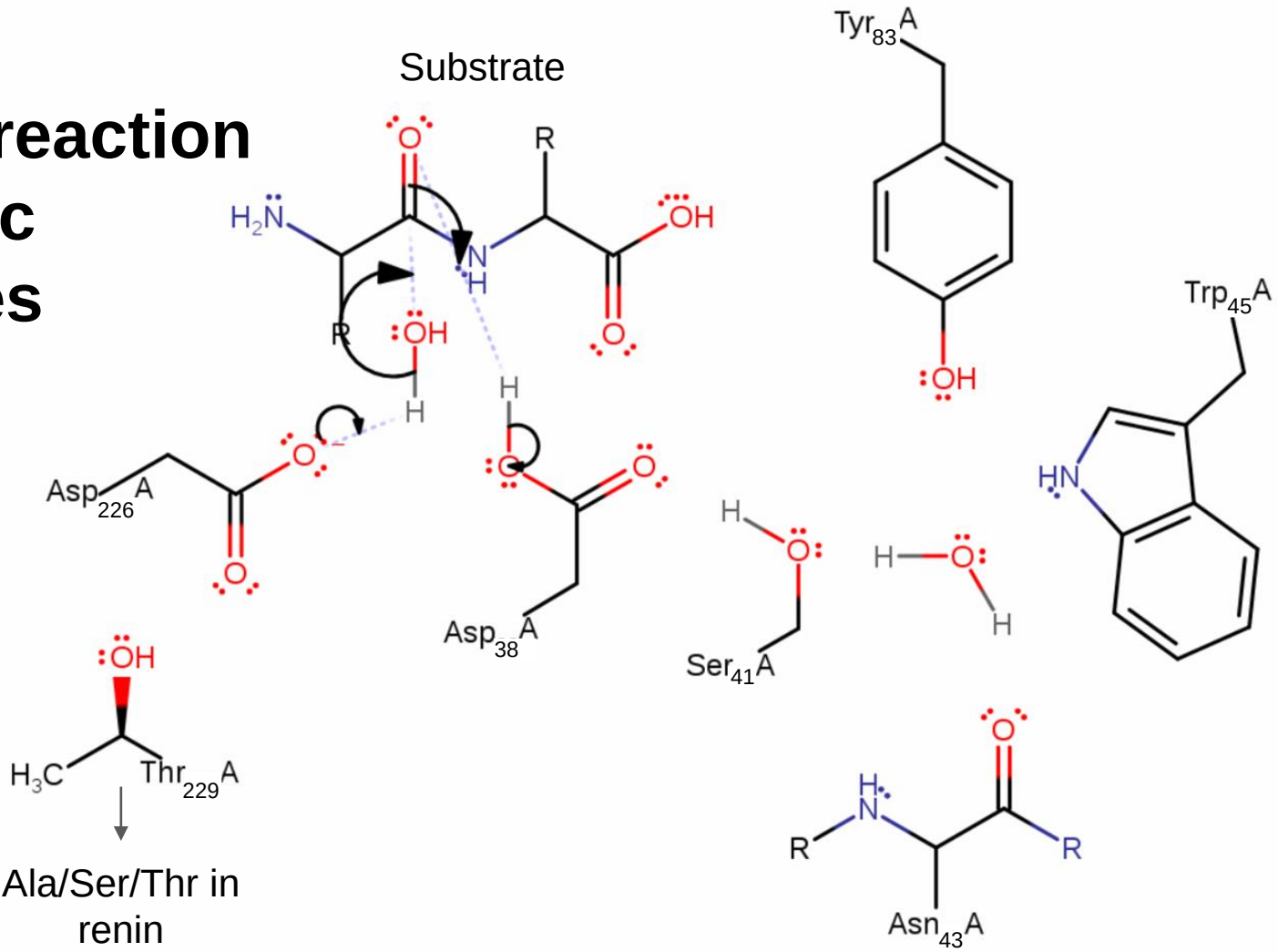
```
sp|P20142|Gastricsin_Homo       -------MKWMVVVLVCLQ------LLEAAVVKVPLKKFKSIRETMKEKG
sp|P04073|Gastricsin_Rattus     -------MKWMVVALLCLP------LLEASLLRVPLRKMKSIRETMKEQG
sp|P0DJD7|Pepsin                -------MKWLLLLGLVA-------LSECIMYKVPLIRKKSLRRTLSERG
sp|P00791|Pepsin                -------MKWLLLLSLVV-------LSECLV-KVPLVRKKSLRQNLIKNG
sp|P14091|Cathepsin             -------MKTLLLLLLVLLELG---EAQGSLHRVPLRRHPSLKKKLRARS
sp|O96009|Napsin-A_Homo         --MSPPPLLQPLLLLLPLLNVE---PSGATLIRIPLHRVQPGRRILN---
sp|P00797|Renin_Homo            MDGWRRMPRWGLLLLLWGSCTFGLPTDTTTFKRIFLKRMPSIRESLKERG

sp|P20142|Gastricsin_Homo       LLGEFLRTHKYDPAWKYRFG---DLSVTYEPMA-YMDAAYFGEISIGTPP
sp|P04073|Gastricsin_Rattus     VLKDFLKTHKYDPGQKYHFGNFGDYSVLYEPMA-YMDASYFGEISIGTPP
sp|P0DJD7|Pepsin                LLKDFLKKHNLNPARKYFPQWEAPTLVDEQPLENYLDMEYFGTIGIGTPA
sp|P00791|Pepsin                KLKDFLKTHKHNPASKYFP--EAAALIGDEPLENYLDTEYFGTIGIGTPA
sp|P14091|Cathepsin             QLSEFWKSHNLD-MIQFTESCSMDQSAK-EPLINYLDMEYFGTISIGSPP
sp|O96009|Napsin-A_Homo         LLRGWREPAELPKLGAPSPG----DKPIFVPLSNYRDVQYFGEIGLGTPP
sp|P00797|Renin_Homo            VDMARLGPEWSQPMKRLTLG----NTTSSVILTNYMDTQYYGEIGIGTPP

sp|P20142|Gastricsin_Homo       QNFLVLFDTGSSNLWVPSVYCQ--SQACTSHSRFNPSESSTYSTNGQTFS
sp|P04073|Gastricsin_Rattus     QNFLVLFDTGSSNLWVSSVYCQ--SEACTTHARFNPSKSSTYYTEGQTFS
sp|P0DJD7|Pepsin                QDFTVVFDTGSSNLWVPSVYCS--SLACTNHNRFNPEDSSTYQSTSETVS
sp|P00791|Pepsin                QDFTVIFDTGSSNLWVPSVYCS--SLACSDHNQFNPDDSSTFEATSQELS
sp|P14091|Cathepsin             QNFTVIFDTGSSNLWVPSVYCT--SPACKTHSRFQPSQSSTYSQPGQSFS
sp|O96009|Napsin-A_Homo         QNFTVAFDTGSSNLWVPSRRCHFFSVPCWLHHRFDPKASSSFQANGTKFA
sp|P00797|Renin_Homo            QTFKVVFDTGSSNVWVPSSKCSRLYTACVYHKLFDASDSSSYKHNGTELT

sp|P20142|Gastricsin_Homo       LQYGSGSLTGFFGYDTLTVQSIQVPNQEFGLSENEPGTNFVYAQFDGIMG
sp|P04073|Gastricsin_Rattus     LQYGTGSLTGFFGYDTLTVQSIQVPNQEFGLSENEPGTNFVYAQFDGIMG
sp|P0DJD7|Pepsin                ITYGTGSMTGILGYDTVQVGGISDTNQIFGLSETEPGSFLYYAPFDGILG
sp|P00791|Pepsin                ITYGTGSMTGILGYDTVQVGGISDTNQIFGLSETEPGSFLYYAPFDGILG
sp|P14091|Cathepsin             IQYGTGSLSGIIGADQVSVEGLTVVGQQFGESVTEPGQTFVDAEFDGILG
sp|O96009|Napsin-A_Homo         IQYGTGRVDGILSEDKLTIGGIKGASVIFGEALWEPSLVFAFAHFDGILG
sp|P00797|Renin_Homo            LRYSIGTVSGFLSQDIITVGGITVT-QMFGEVTEMPALPFMLAEFDGVVG

sp|P20142|Gastricsin_Homo       LAYPALSVDEATTAMQGMVQEGALTSPVFSVYLSNQ-QGSS--GGAVVFG
sp|P04073|Gastricsin_Rattus     LAYPGLSSGGATTALQGMLGEGALSQPLFGVYLGSQ-QGSN--GGQIVFG
sp|P0DJD7|Pepsin                LAYPSISSSGATPVFDNIWNQGLVSQDLFSVYLSAD-DQS---GSVVIFG
sp|P00791|Pepsin                LAYPSISASGATPVFDNLWDQGLVSQDLFSVYLSSN-DDS---GSVVLLG
sp|P14091|Cathepsin             LGYPSLAVGGVTPVFDNMMAQNLVDLPMFSVYMSSNPEGGA--GSELIFG
sp|O96009|Napsin-A_Homo         LGFPILSVEGVRPPMDVLVEQGLLDKPVFSFYLNRDPEEPD--GGELVLG
sp|P00797|Renin_Homo            MGFIEQAIGRVTPIFDNIISQGVLKEDVFSFYYNRDSENSQSLGGQIVLG
```

2 Asp-Thr/Ser-Gly motifs

The hydrogen bond network Trp45-Tyr83-water (W2)-Ser 41-Asp38 is present

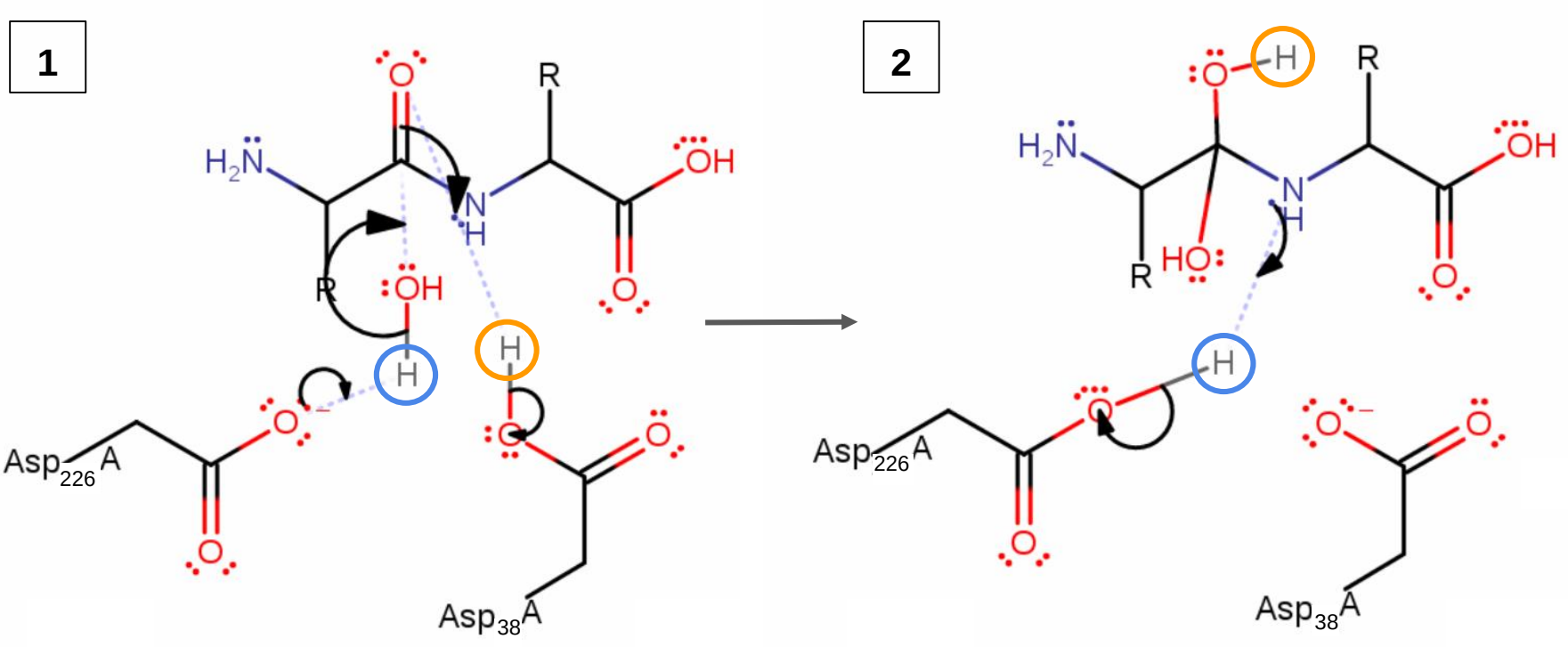
Flap region of renin

Catalytic reaction in Aspartic Peptidases



Target protein

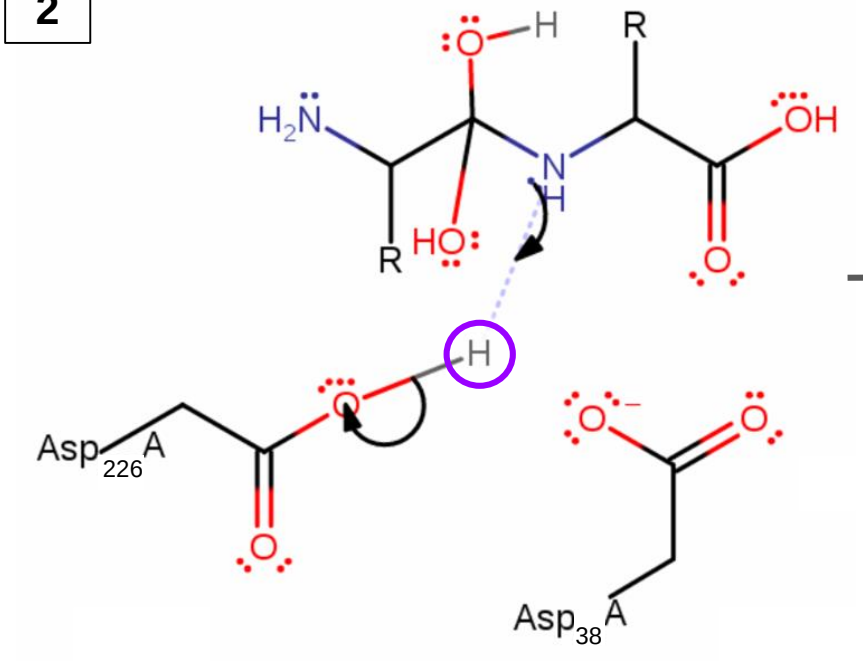
Target protein



Target protein

Target protein

2



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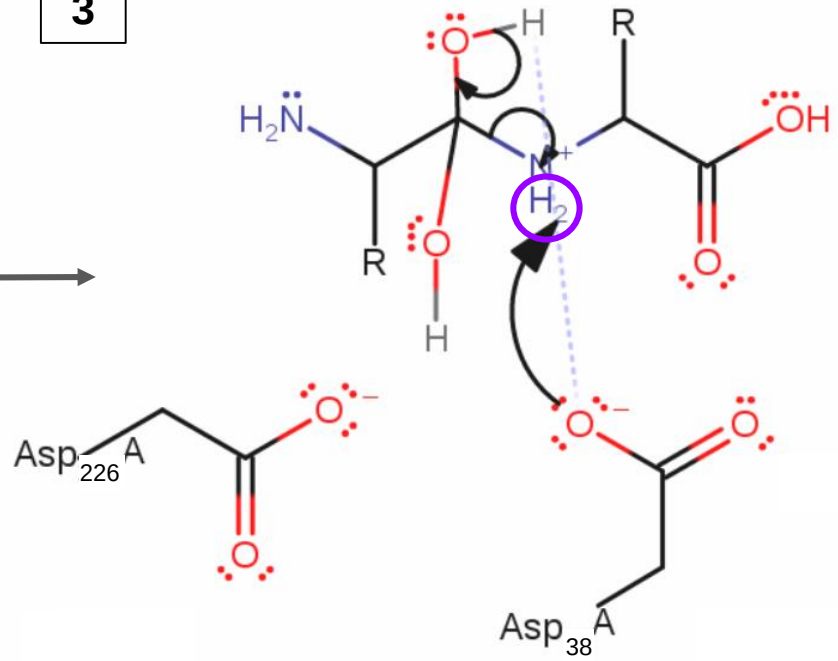
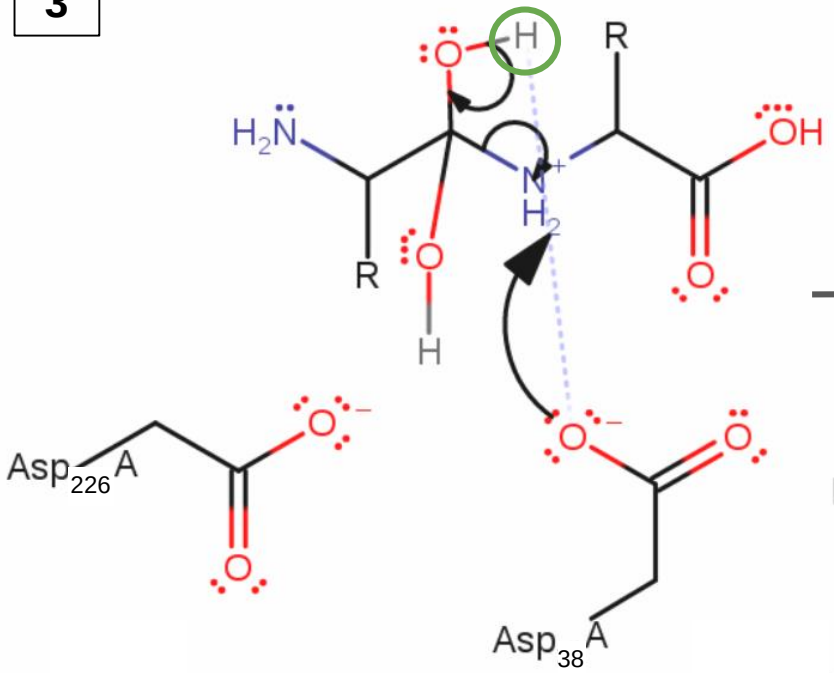


Product 2

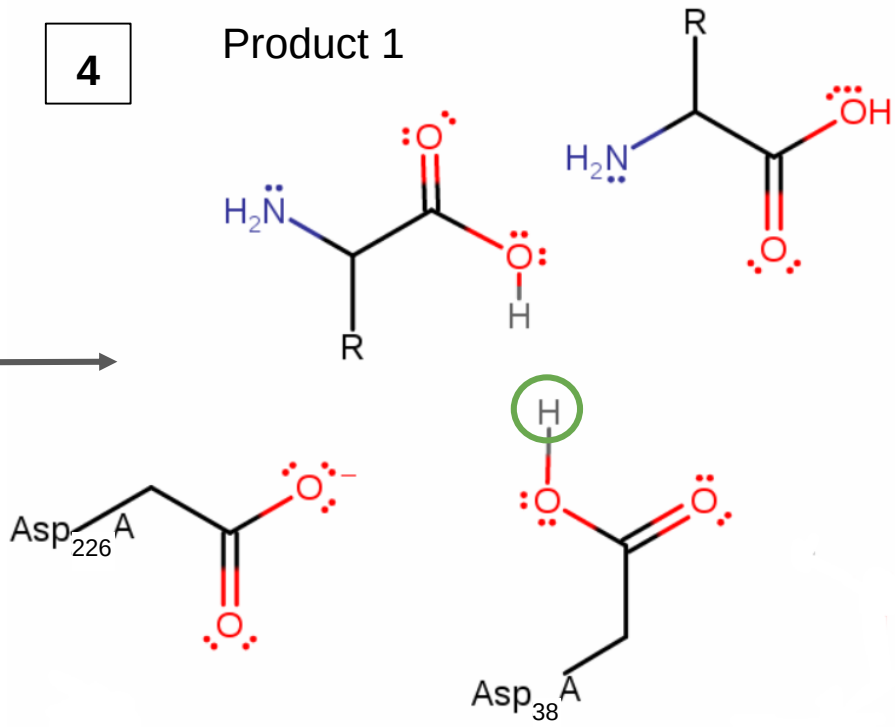
Target protein

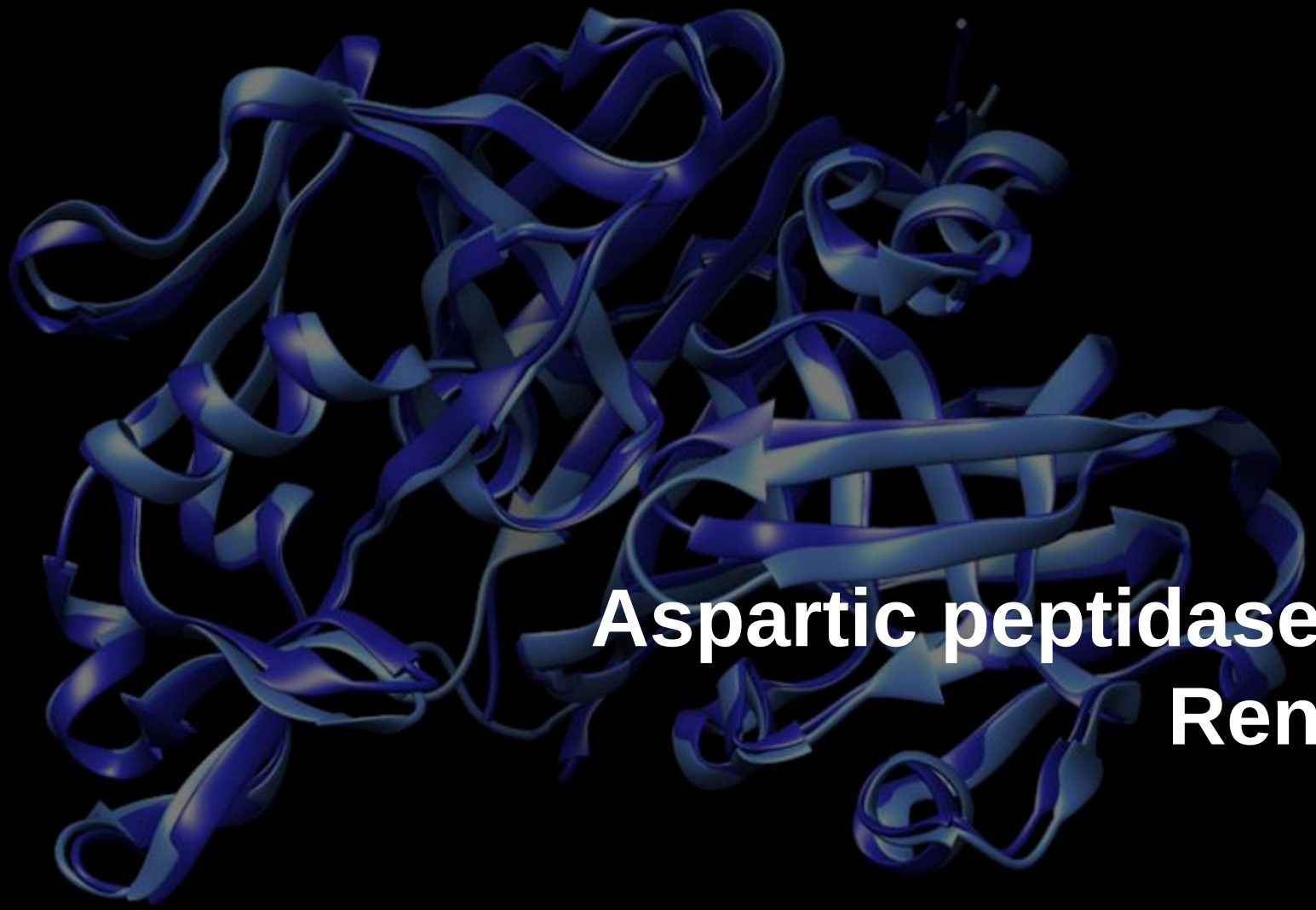
3



4

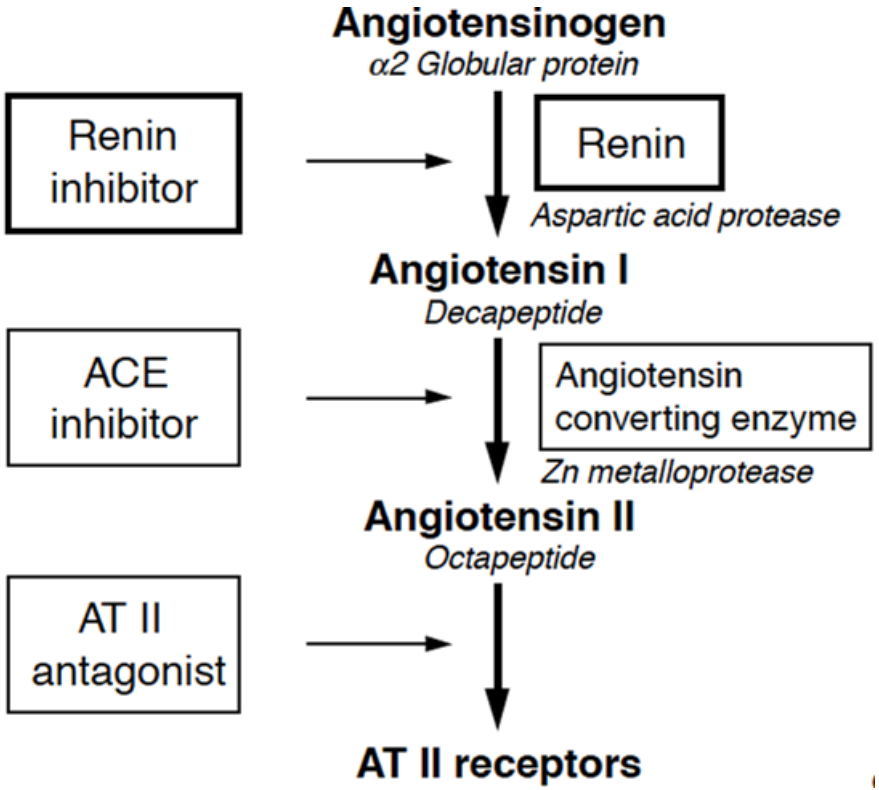
Product 1





Aspartic peptidases: Renin

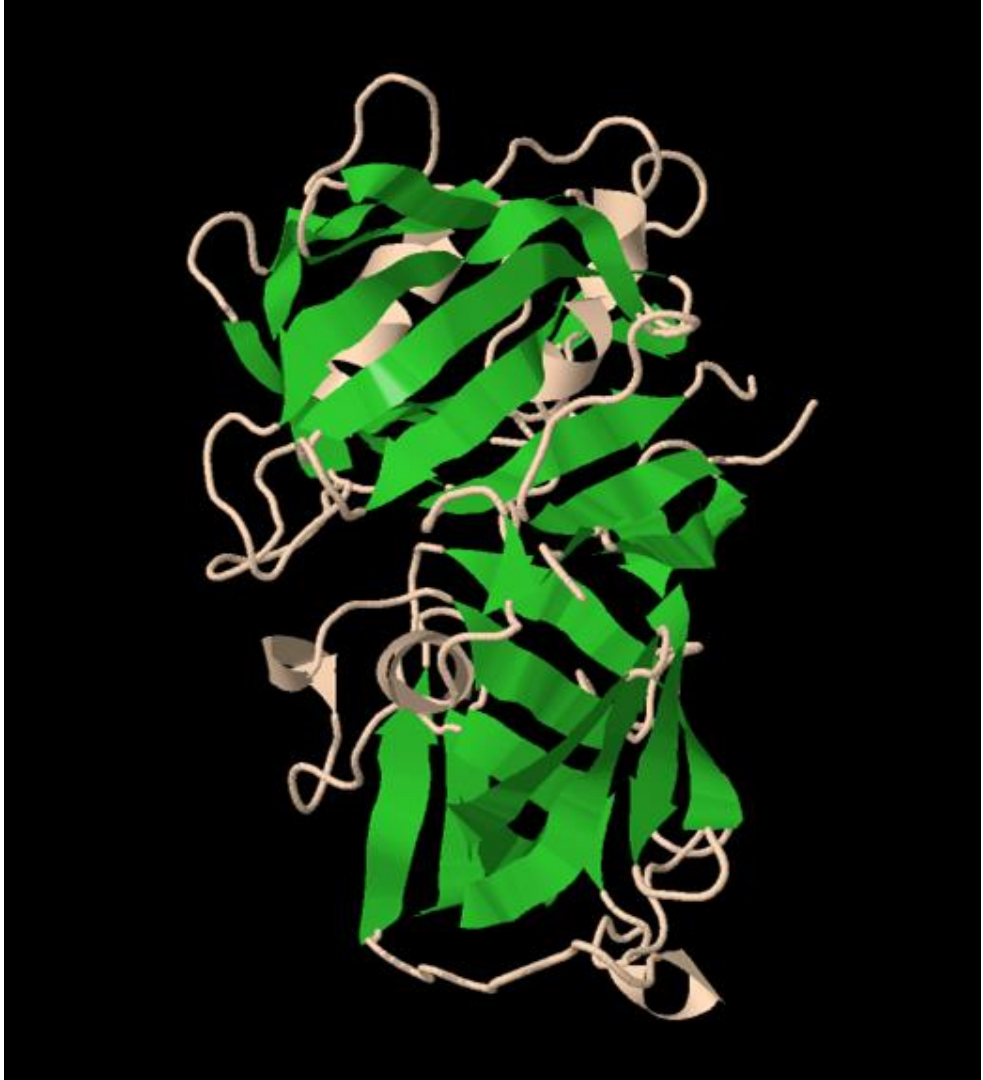
Physiological importance of renin



Aliskiren: the first renin inhibitor for clinical treatment. 2008, Jensen C.

Renin: structure

SCOP Classification: All-beta



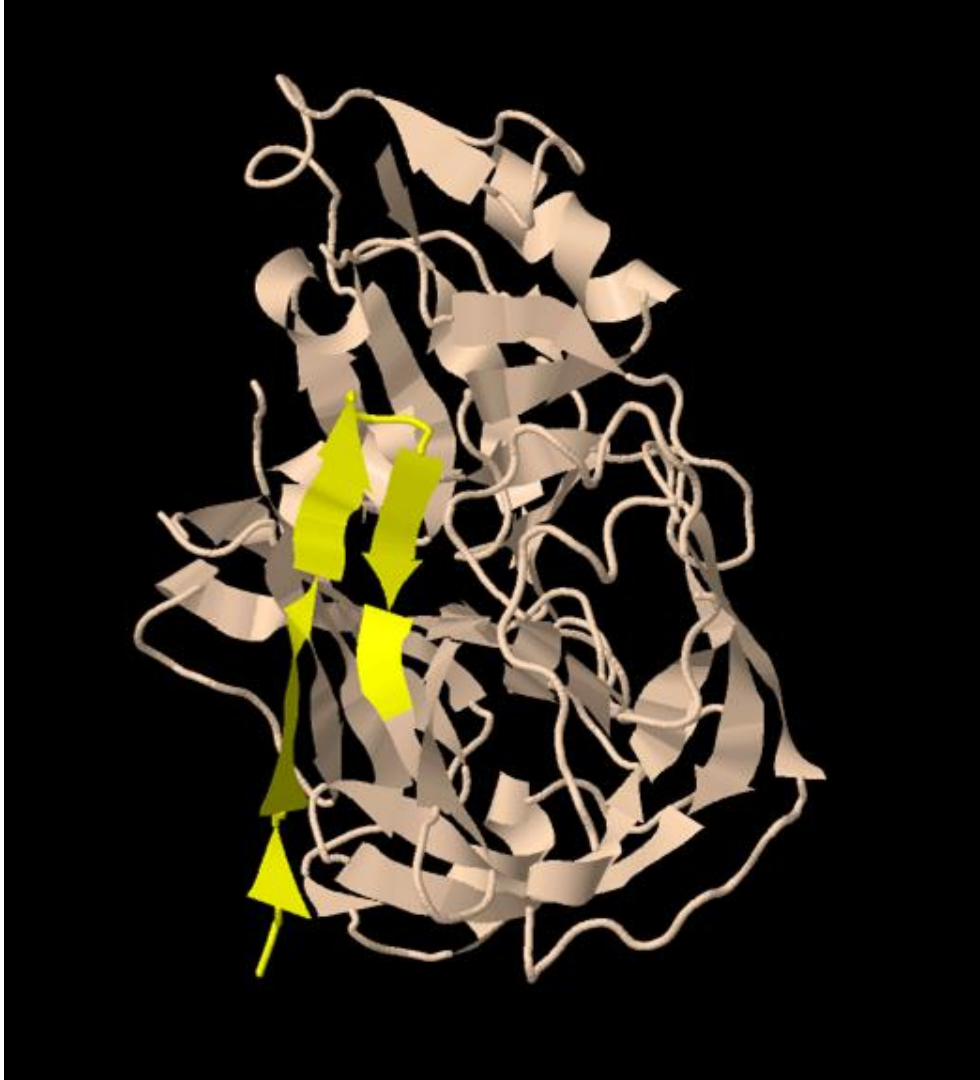
Renin: structure

Pro-renin: 406 aa

- **1-23: signal peptide**
- 24-66: propeptide

Renin: 340 aa

- 29 antiparallel β sheets
- 4 α helix
- 2 3_{10} helix
- 18 turns
- 3 disulfide bonds



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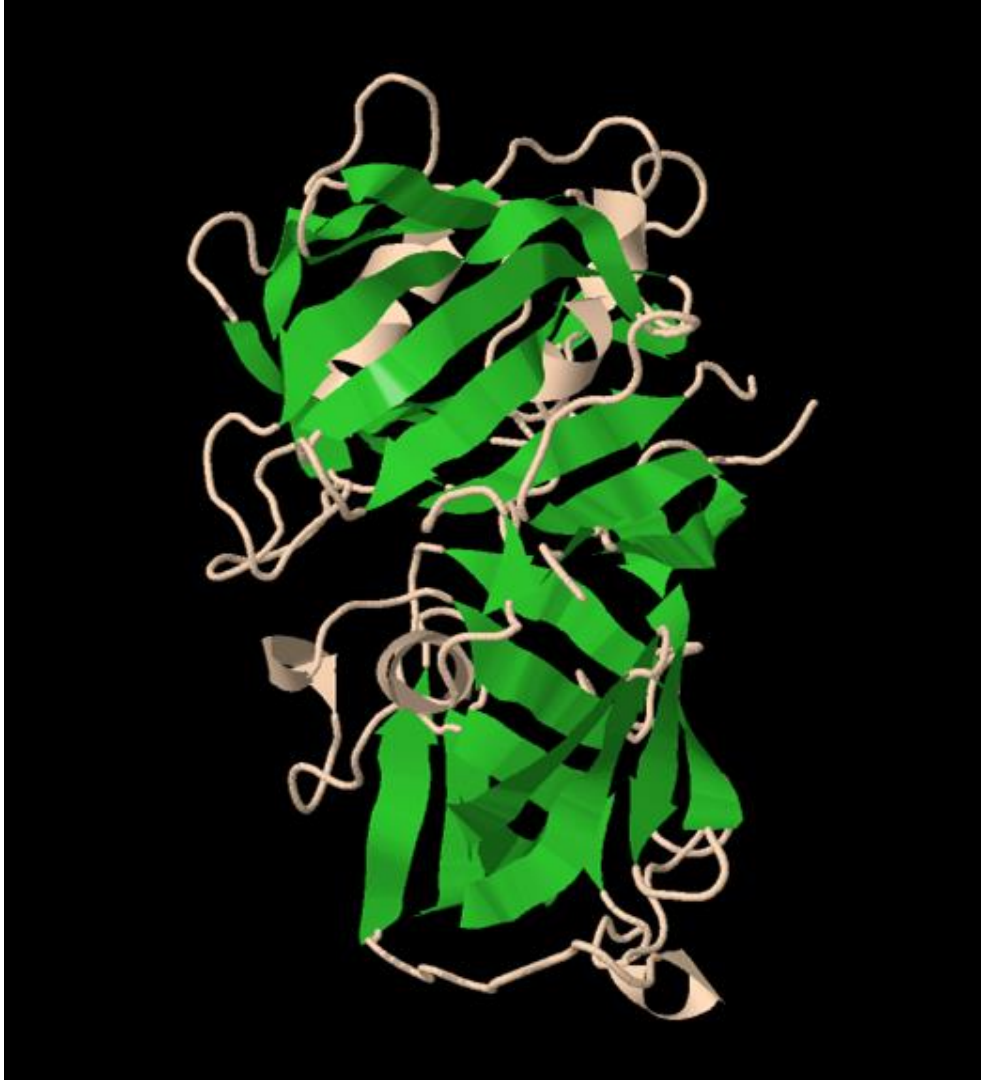

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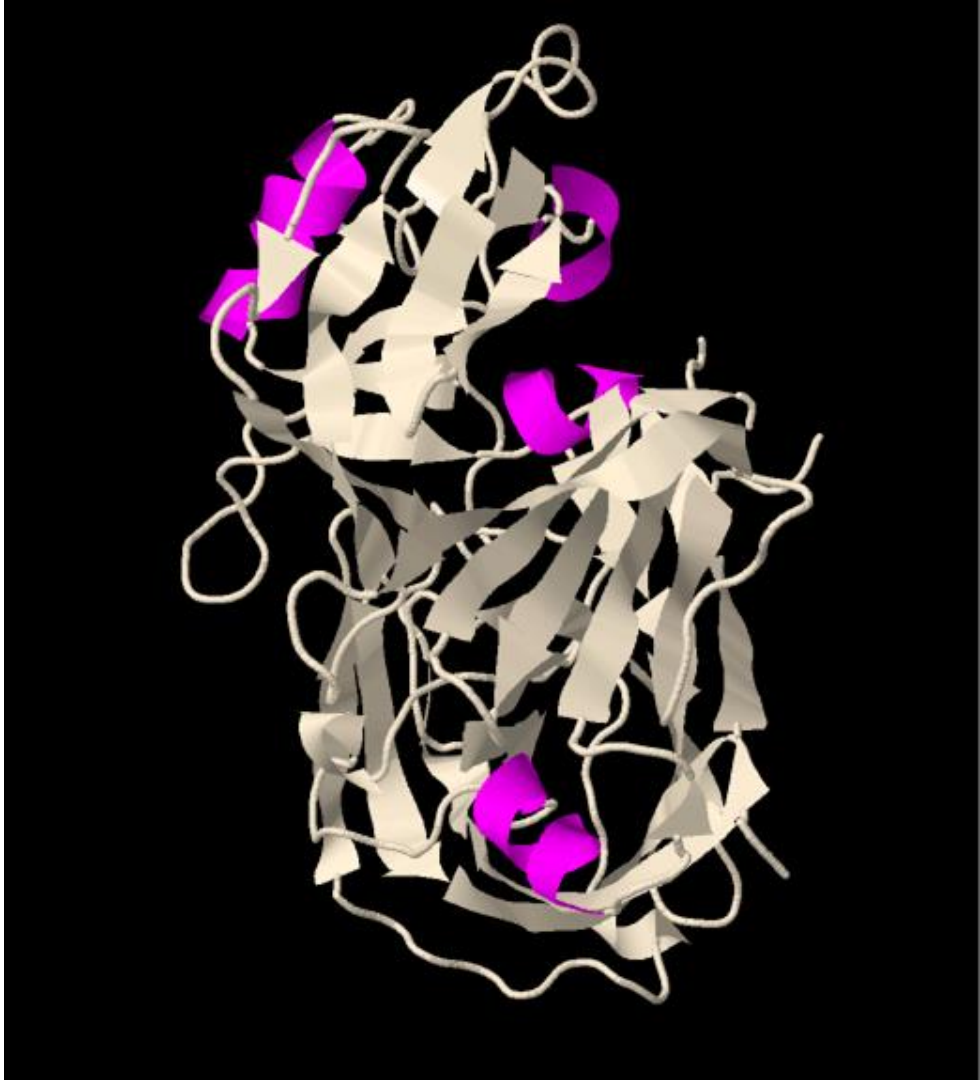

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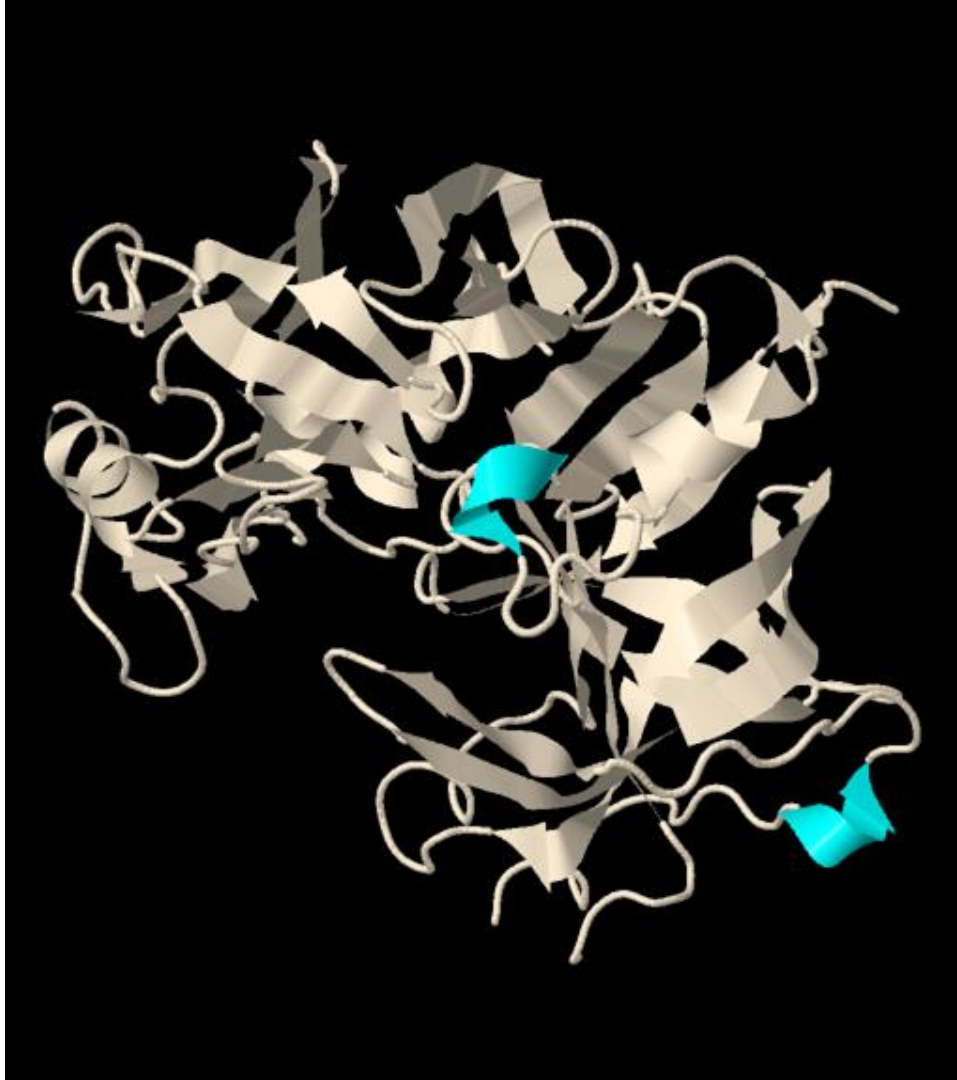

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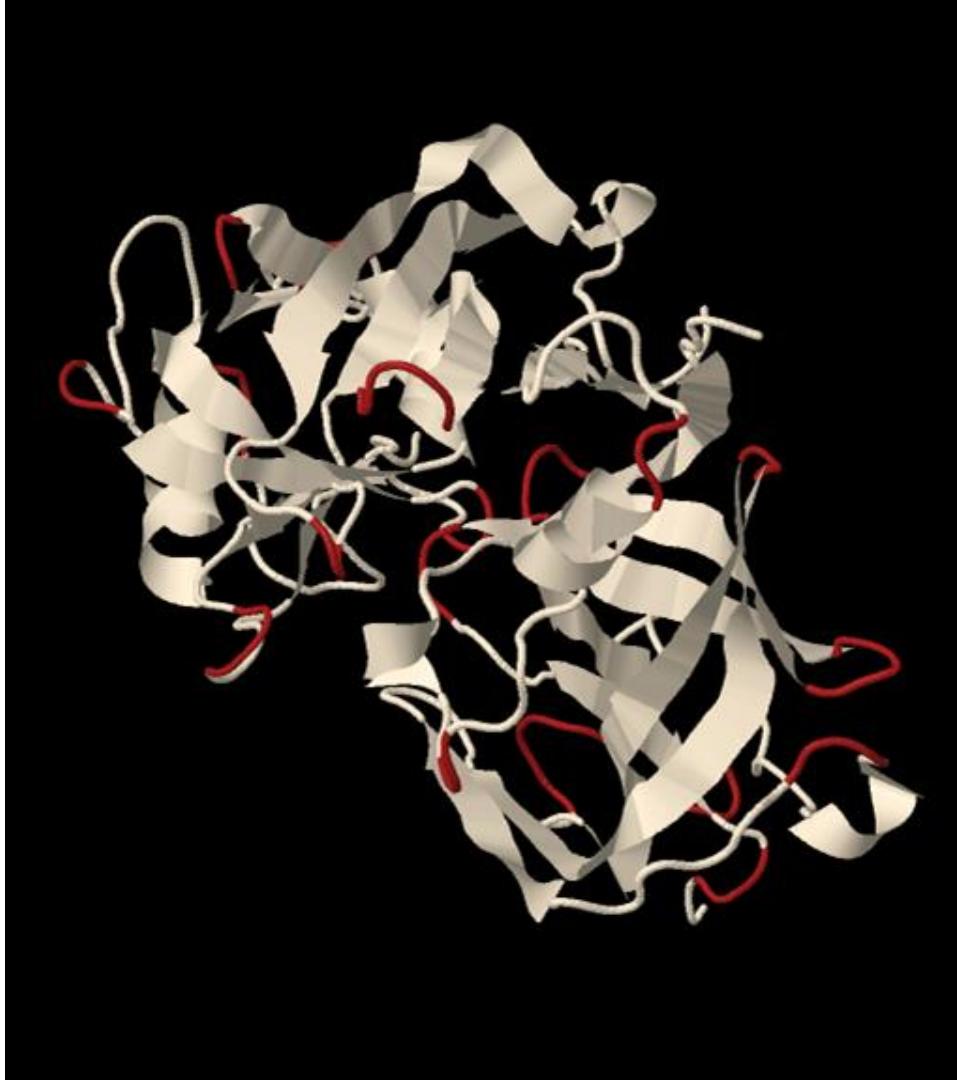

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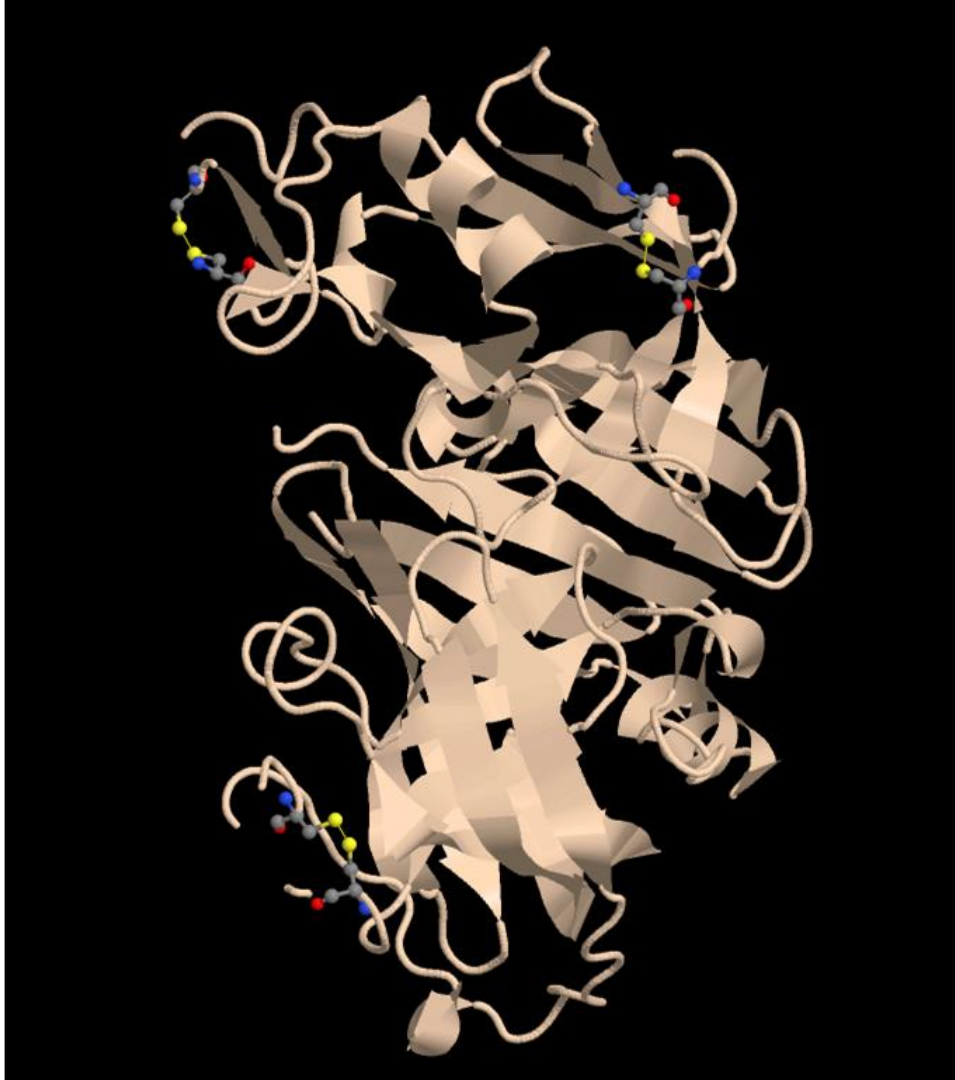
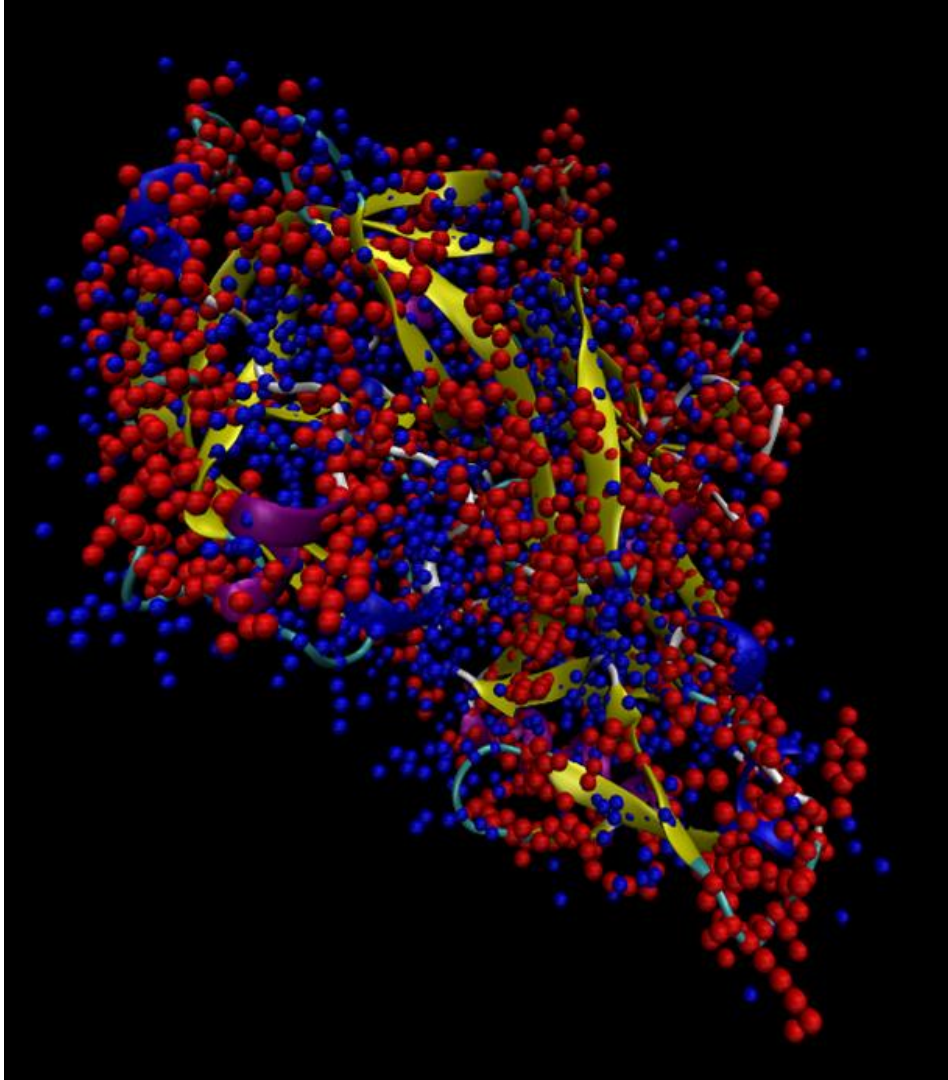
- 4 α helix
- 2 3_{10} helix
- 18 turns
- **3 disulfide bonds**
  - Cys51 - Cys58
  - Cys217 - Cys221
  - Cys259 - Cys296



Renin: structure

- **Hydrophobic residues**: mainly inside.

- **Hydrophilic residues**: mainly outside.



Prorenin

- Cathepsin B cuts ~40 aa from **prorenin** to form **renin** itself

```
Renin           ----------------------------------------LTLGNTTSSVILTNYMD
Prorenin        LPTDTTTFKRIFLKRMPSIRESLKERGVDMARLGPEWSQPMKRLTLGNTTSSVILTNYMD
                                                         *****************

Renin           TQYYGEIGIGTPPQTFKVVFDTGSSNVWVPSSKCSRLYTACVYHKLFDASDSSSYKHNGT
Prorenin        TQYYGEIGIGTPPQTFKVVFDTGSSNVWVPSSKCSRLYTACVYHKLFDASDSSSYKHNGT
                ************************************************************

Renin           ELTLRYSTGTVSGFLSQDIITVGGITVTQMFGEVTEMPALPFMLAEFDGVVGMGFIEQAI
Prorenin        ELTLRYSTGTVSGFLSQDIITVGGITVTQMFGEVTEMPALPFMLAEFDGVVGMGFIEQAI
                ************************************************************
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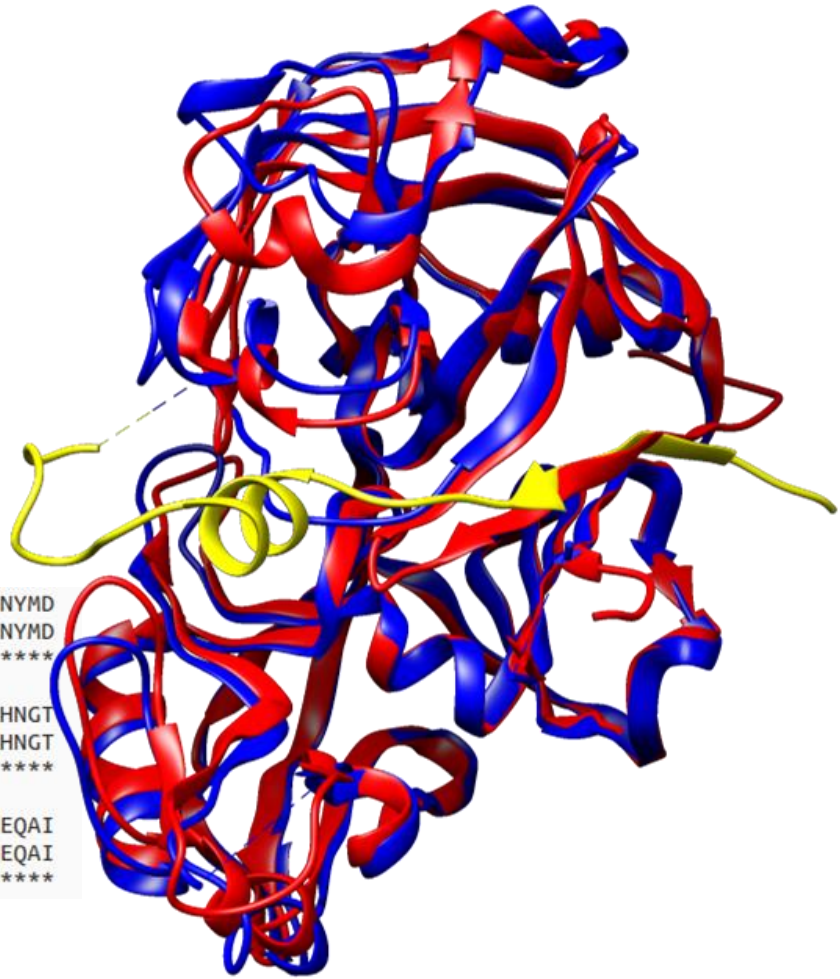
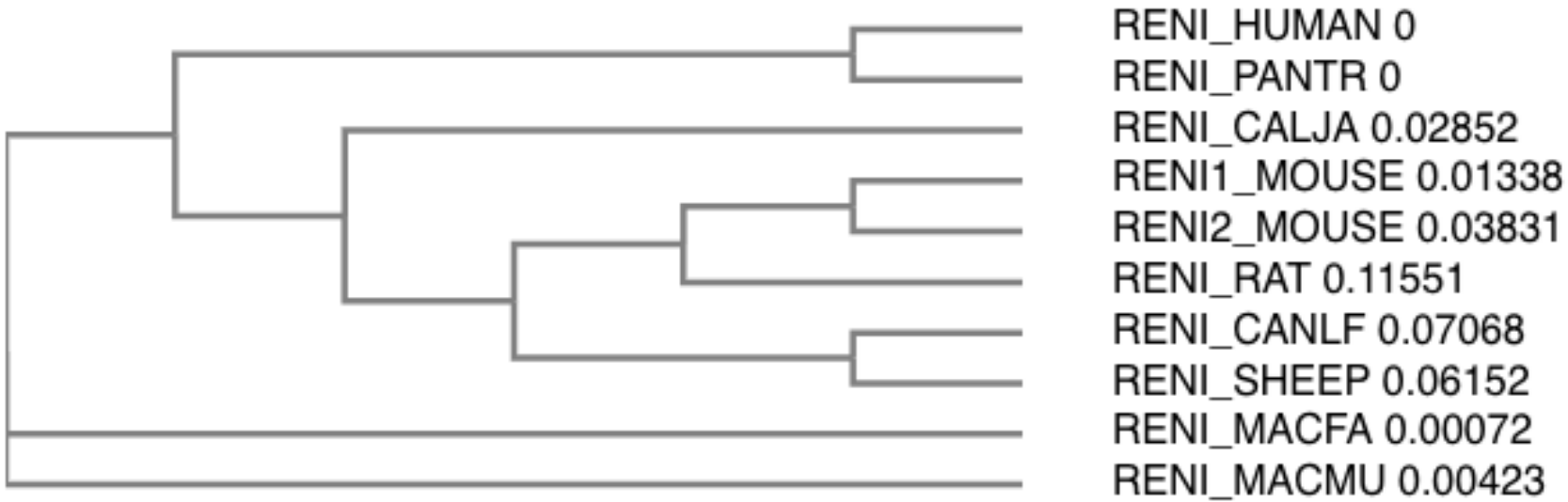


Renin: phylogenetic tree



Human renin does not need acidic environment for its catalytic activity

Ala/Ser/Thr in Renin

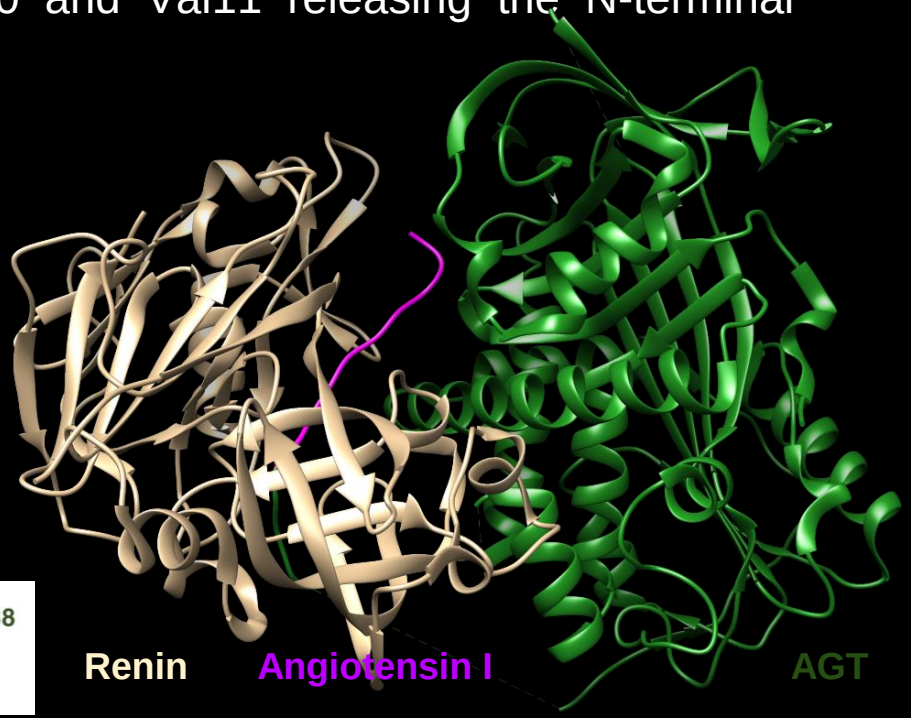
```
RENI_HUMAN      QIVLGGSDPQHYEGNFHYINLIKTGVWQIQMKGVSVGSSTLLCEDGCLALVDTGASYISG
RENI_PANTR      QIVLGGSDPQHYEGNFHYINLIKTGVWQIQMKGVSVGSSTLLCEDGCLALVDTGASYISG
RENI_MACFA      QIVLGGSDPQHYEGNFHYINLIKTGVWQIQMKGVSVGSSTLLCEDGCLALVDTGASYISG
RENI_MACMU      QIVLGGSDPQHYEGNFHYINLIKTGVWQIPMKGVSVGSSTLLCEDGCLALVDTGASYISG
RENI_CALJA      QIVLGGSDPQHYEGNFHYINLIRTGLWQIPMKGVSVGSSTLLCEDGCLALVDTGASYISG
RENI1_MOUSE     EVVLGGSDPQHYQGNFHYVSISKTDSWQITMKGVSVGSSTLLCEEGCAVVVDTGSSFISA
RENI2_MOUSE     EVVLGGSDPEHYQGDFHYVSLSKTDSWQITMKGVSVGSSTLLCEEGCEVVVDTGSSFISA
RENI_RAT        EVVLGGSDPQHYQGNFHYVSISKAGSWQITMKGVSVGPATLLCEEGCMAVVDTGTSYISG
RENI_CANLF      EVVLGGSDPQYYQGNFHYVSISKTGSWQIKMKGVSVRSATLVCEEGCMVVVDTGASYISG
RENI_SHEEP      EIVLGGSDPQYYQENFHYVSISKPGSWQIRMKGVSVRSTTLLCEEGCMVVVDTGASYISG
```

Renin-mediated angiotensinogen (AGT) cleavage

Renin-mediated angiotensinogen (AGT) cleavage

- Renin cleaves AGT between Leu10 and Val11 releasing the N-terminal angiotensin I peptide.

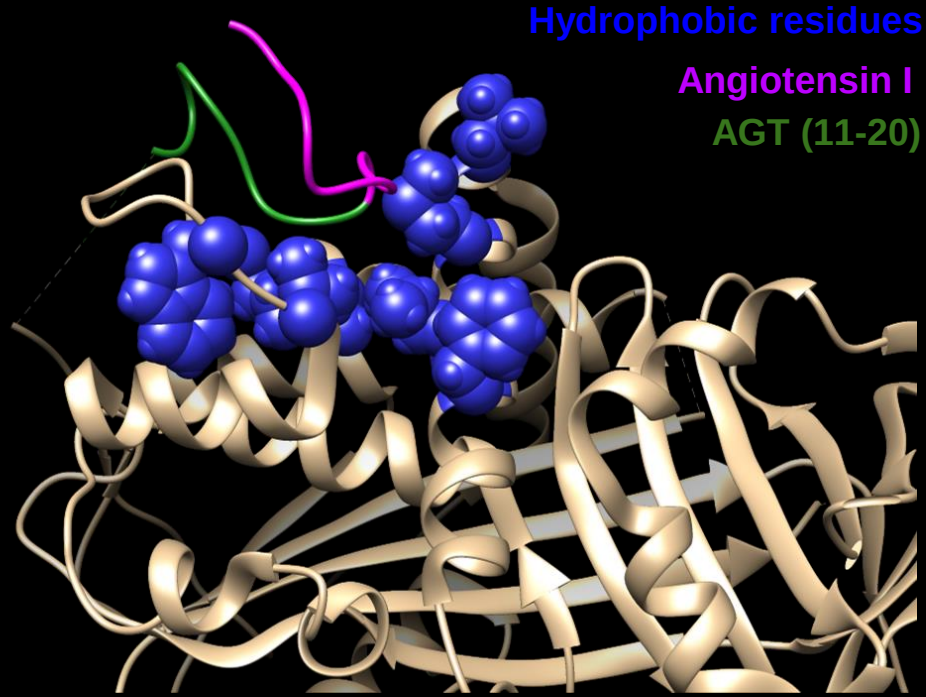
- AGT adopts the typical serpin (serine protease inhibitor) framework.



The N-terminal tail is sequestered in native AGT

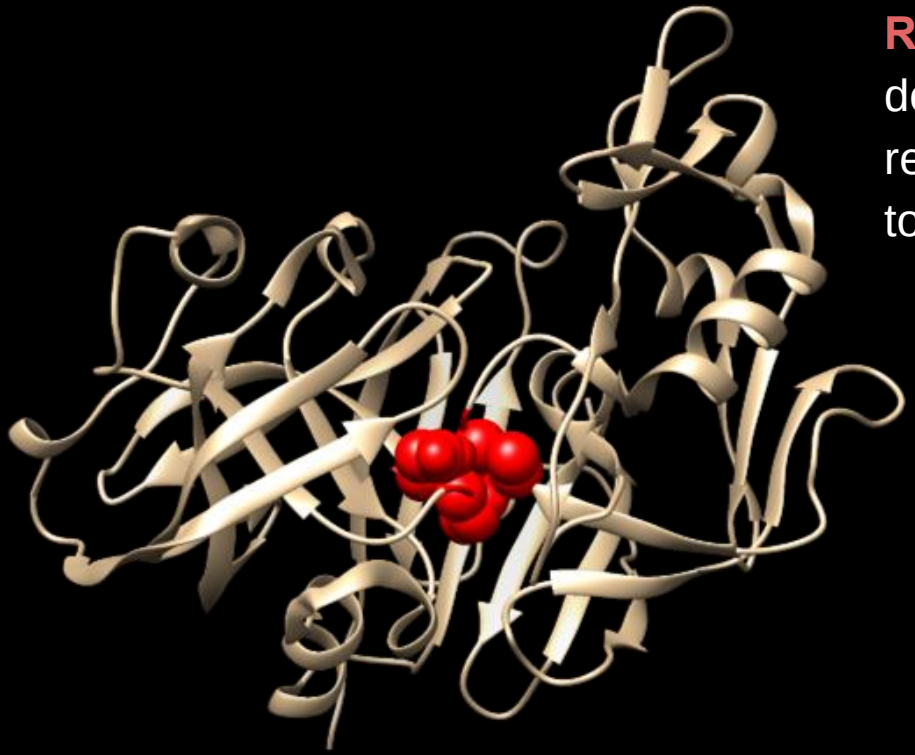
The scissile bond (Leu10–Val11) is buried in the hydrophobic cavity in the native AGT, protecting it from nonspecific cleavage.

AGT has to undergo conformational changes for the scissile bond to move out to bind renin.



Crystal structure of native human glycosylated AGT.

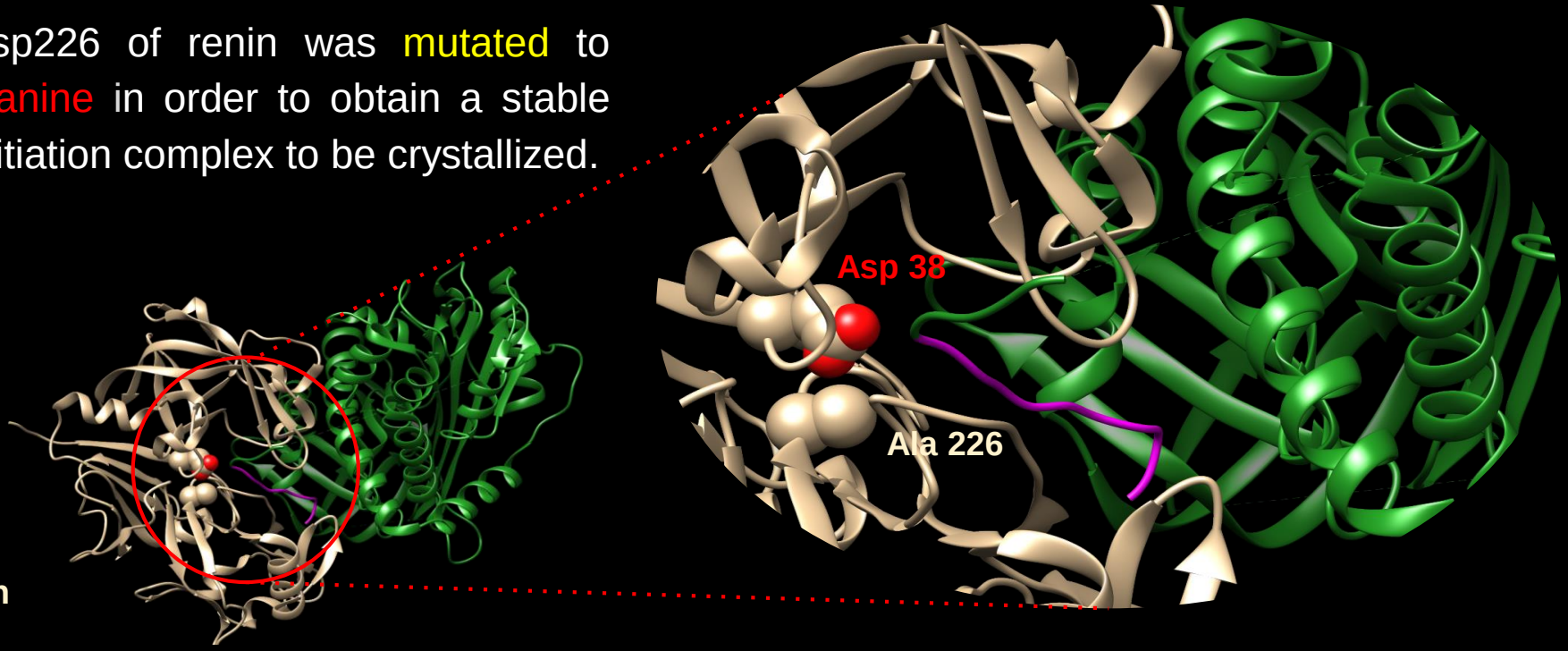
Renin-mediated angiotensinogen (AGT) cleavage



Renin's Active Site: Junction of two similar domains, each containing an aspartic acid residue (Asp38 and Asp226 in human renin) to form a catalytic dyad.

Binding interactions between renin and AGT: Renin active site

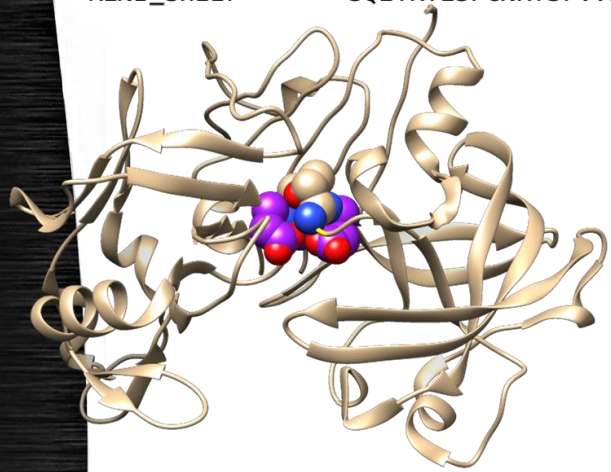
Asp226 of renin was mutated to alanine in order to obtain a stable initiation complex to be crystallized.



Hydrogen bond network conserved among renins

Asp38-Thr39-Gly 40

```
RENI_HUMAN       SQPMKRLTLGNTTSSVILTNYMDTQYYGEIGIGTPPQTFKVVFDTGSSNVWVPSSKCSRL
RENI_PANTR       SQPMKRLTLGNTTSSVILTNYMDTQYYGEIGIGTPPQTFKVVFDTGSSNVWVPSSKCSRL
RENI_MACFA       SQPMKRLALGNTTSSVILTNYMDTQYYGEIGIGTPPQTFKVVFDTGSSNVWVPSSKCSRL
RENI_MACMU       SQPMKRLALGNTTSSVILTNYMDTQYYGEIGIGTPPQTFKVVFDTGSSNVWVPSSKCSRL
RENI_CALJA       -----RMALVNITSSVILTNYMDTQYYGEIGIGTPPQTFKVVFDTGSSNVWVPSSKCSRL
RENI1_MOUSE      GVFTKRPSLTNLTSPVVLTNYLNTQYYGEIGIGTPPQTFKVIFDTGSANLWVPSTKCSRL
RENI2_MOUSE      DVFTKRSSLTDLISPVVLTNYLNSQYYGEIGIGTPPQTFKVIFDTGSANLWVPSTKCSRL
RENI_RAT         GEFIKKSSFTNVTSPVVLTNYLDTQYYGEIGIGTPSQTFKVIFDTGSANLWVPSTKCGPL
RENI_CANLF       NQFTKRLSSGNSTSPVVLTNYLDTQYYGEIGIGTPPQTFKVVFDTGSANLWVPSTRCSPL
RENI_SHEEP       SQLTKTLSFGNRTSPVVLTNYLDTQYYGEIGIGTPPQTFKVIFDTGSANLWVPSTKCSPL
```

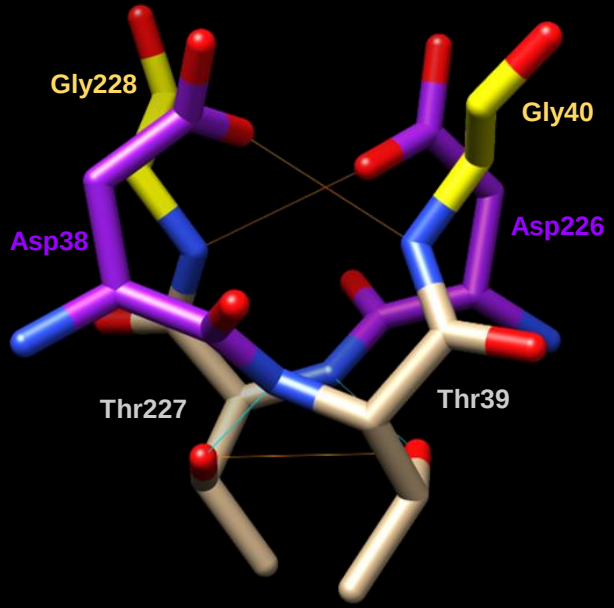


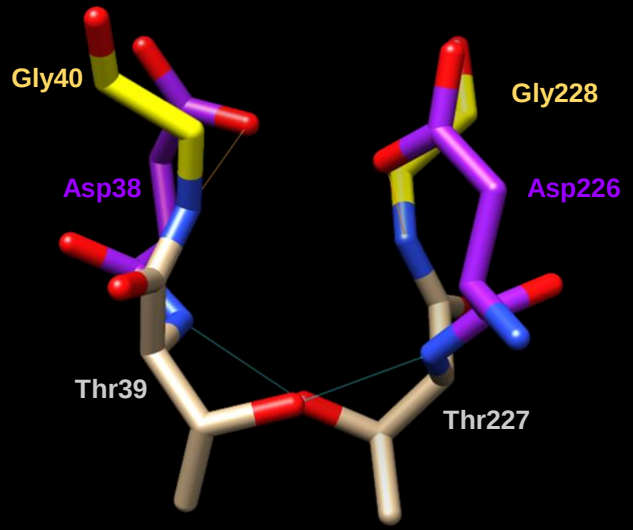
Asp226-Thr227-Gly228

```
RENI_HUMAN       QIVLGGSDPQHYEGNFHYINLIKTGVWQIQMKGVSVGSSTLLCEDGCLALVDTGASYISG
RENI_PANTR       QIVLGGSDPQHYEGNFHYINLIKTGVWQIQMKGVSVGSSTLLCEDGCLALVDTGASYISG
RENI_MACFA       QIVLGGSDPQHYEGNFHYINLIKTGVWQIQMKGVSVGSSTLLCEDGCLALVDTGASYISG
RENI_MACMU       QIVLGGSDPQHYEGNFHYINLIKTGVWQIPMKGVSVGSSTLLCEDGCLALVDTGASYISG
RENI_CALJA       QIVLGGSDPQHYEGNFHYINLIRTGLWQIPMKGVSVGSSTLLCEDGCLALVDTGASYISG
RENI1_MOUSE      EVVLGGSDPQHYQGNFHYVSISKTDSWQITMKGVSVGSSTLLCEEGCAVVVDTGSSFISA
RENI2_MOUSE      EVVLGGSDPEHYQGDFHYVSLSKTDSWQITMKGVSVGSSTLLCEEGCEVVVDTGSSFISA
RENI_RAT         EVVLGGSDPQHYQGNFHYVSISKAGSWQITMKGVSVGPATLLCEEGCMAVVDTGTSYISG
RENI_CANLF       EVVLGGSDPQYYQGNFHYVSISKTGSWQIKMKGVSVRSATLVCEEGCMVVVDTGASYISG
RENI_SHEEP       EIVLGGSDPQYYQENFHYVSISKPGSWQIRMKGVSVRSTTLLCEEGCMVVVDTGASYISG
```

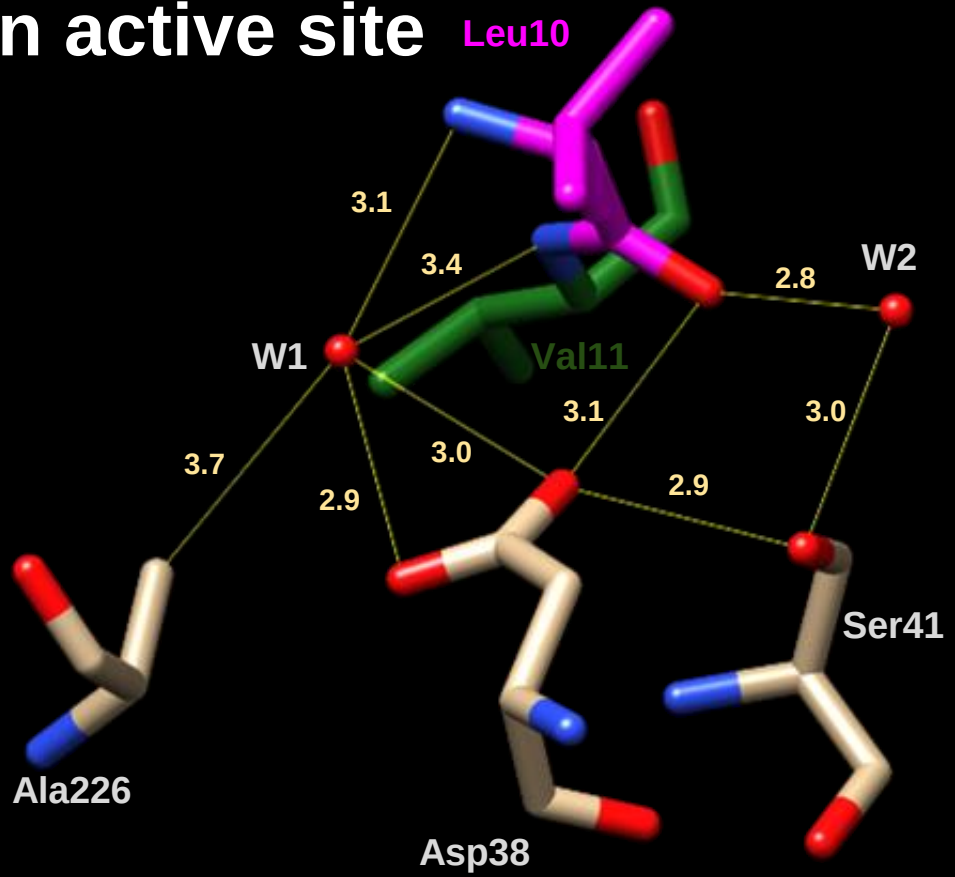
Fireman's grip: Asp-Thr-Gly motif

3D structure of motif Asp-Thr-Gly: conserved in Aspartic proteases and retroviral proteases.





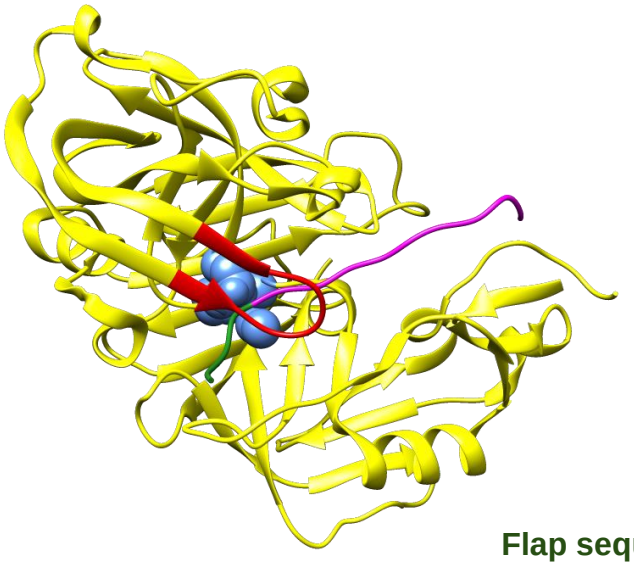
Renin active site

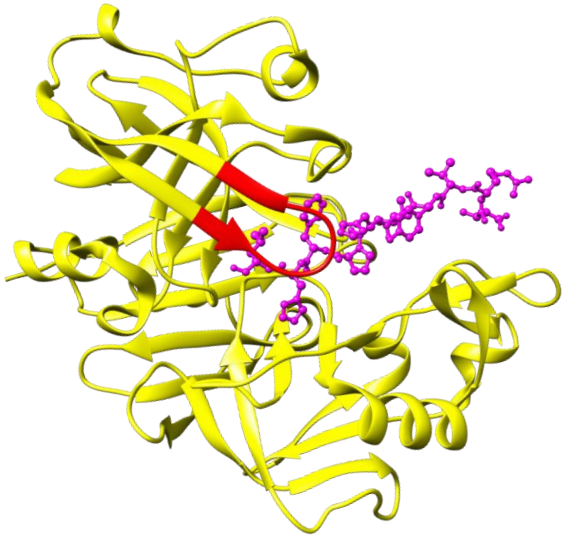


Atoms	Distances (Å)
Ala226-W1	3.7
Asp38-W1	2.9
Asp38-W1	3.0
Val11-W1	3.4
Leu10-W1	3.1
Asp38-Leu10	3.1
Ser41-Asp38	2.9
Ser41-W2	3.0
Leu10-W2	2.8

Conserved hydrogen bond network
Trp45-Tyr83-W2-Ser41-Asp38

Hydrogen bond network connecting the flap



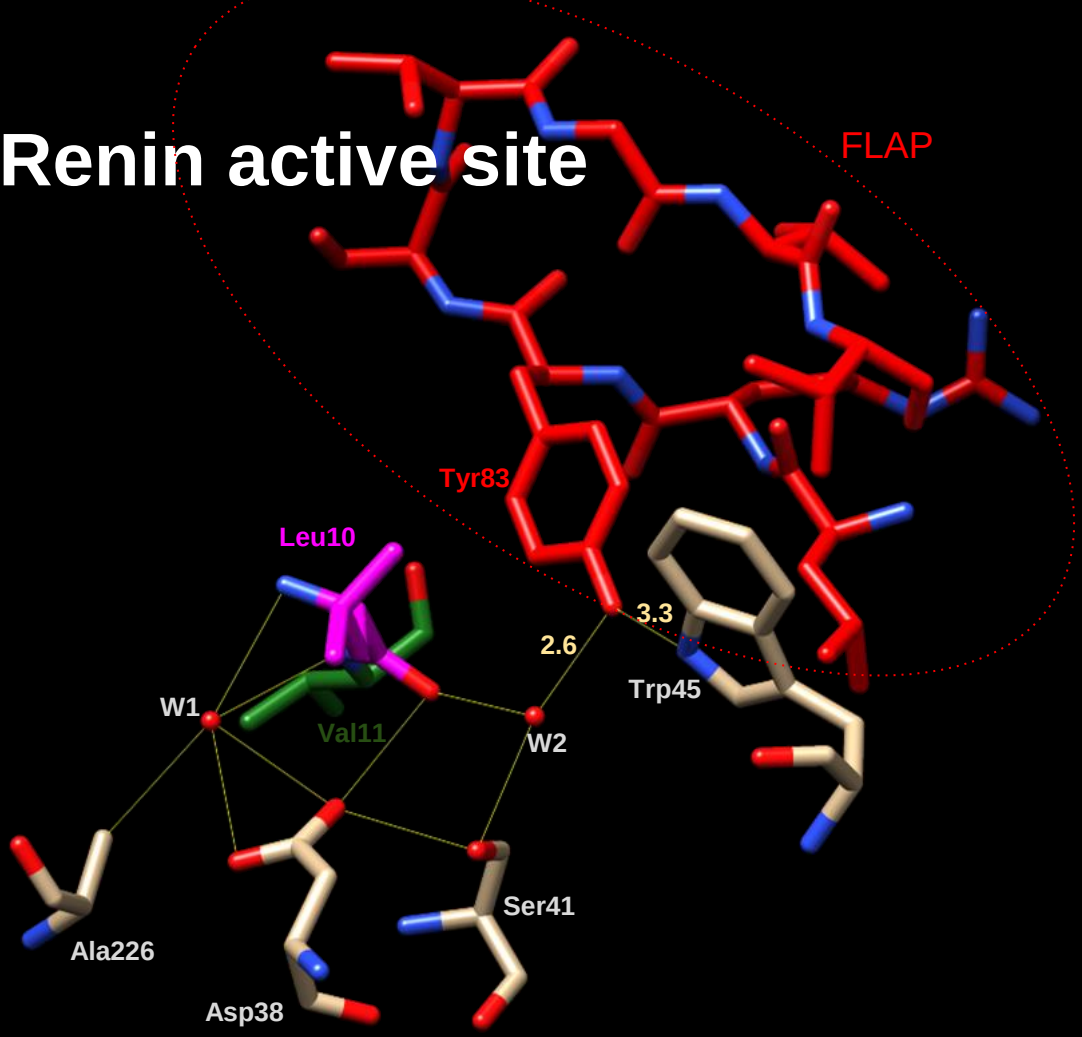


Flap sequence

```
RENI_HUMAN      YTACVYHKLFDASDSSSYKHNGTELTLRYSTGTVSGFLSQDIITVGGITVTQMFGEVTEM
RENI_PANTR      YTACVYHKLFDASDSSSYKHNGTELTLRYSTGTVSGFLSQDIITVGGITVTQMFGEVTEM
RENI_MACFA      YTACVYHKLFDASDSSSYKHNGTELTLRYSTGTVSGFLSQDIITVGGITVTQMFGEVTEM
RENI_MACMU      YTACVYHKLFDASDSSSYKHNGTELTLRYSTGTVSGFLSQDIITVGGITVTQMFGEVTEM
RENI_CALJA      YTACVYHKLFDASDSSSYKHNGTELTLRYSTGTVSGFLSQDVITVGGITVTQTFGEVTEM
RENI1_MOUSE     YLACGIHSLYESSDSSSYMENGSDFTIHYGSGRVKGFLSQDSVTVGGITVTQTFGEVTEL
RENI2_MOUSE     YLACGIHSLYESSDSSSYMENGDDFTIHYGSGRVKGFLSQDSVTVGGITVTQTFGEVTEL
RENI_RAT        YTACEIHNLYDSSESSSYMENGTEFTIHYGSGKVKGFLSQDVVTVGGIIVTQTFGEVTEL
RENI_CANLF      YTACEIHCLYDSSESSSYMENGTTFTIRYGSGKVKGFLSQDMVTVGGITVTQTFGEVTEL
RENI_SHEEP      YTACEIHSLYDSLESSSYVENGTEFTIYYGSGKVKGFLSQDLVTVGGITVTQTFGEVTEL
```

The Flap sequence is not conserved in all renins nor the aspartic proteases

Renin active site

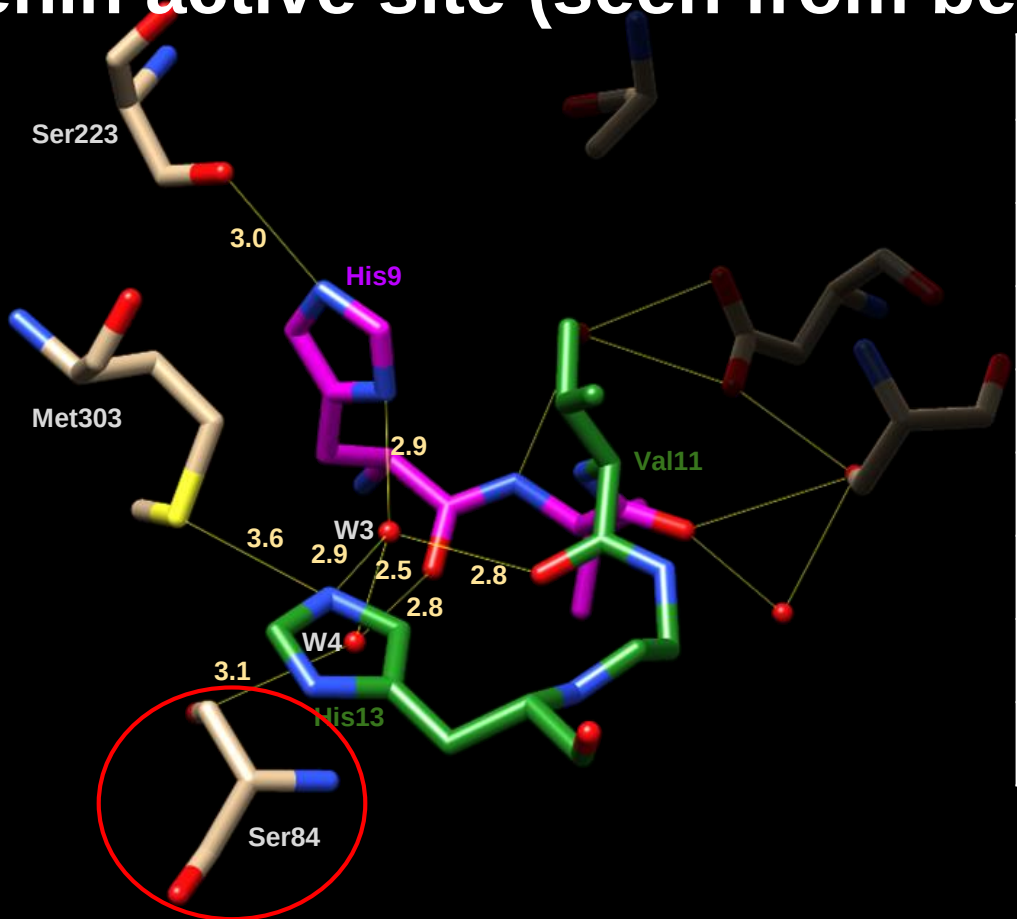


Atoms	Distances (Å)
Tyr83-W2	2.6
Trp45-Tyr83	3.3

The hydrogen bond network connects the flap to the catalytic site.

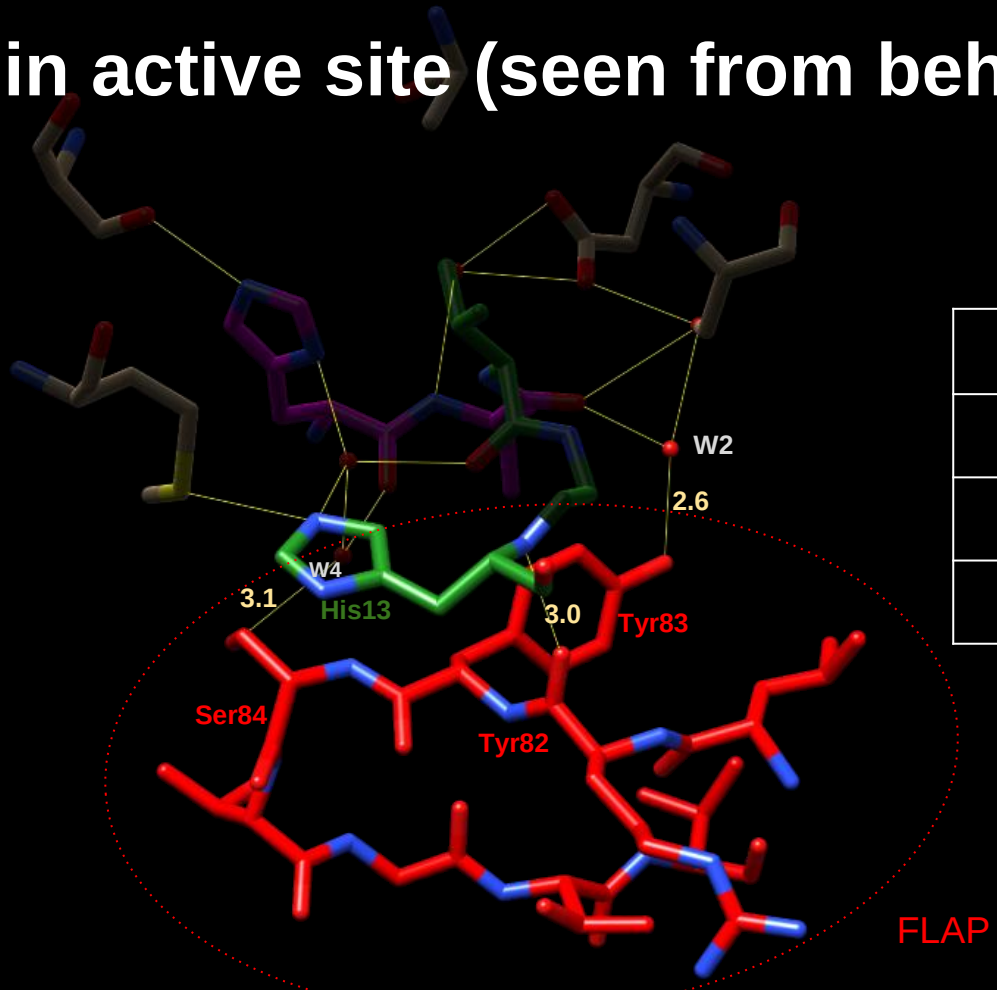
Hydrogen bond network Trp45-Tyr83-W2-Ser41-Asp38

Renin active site (seen from behind)



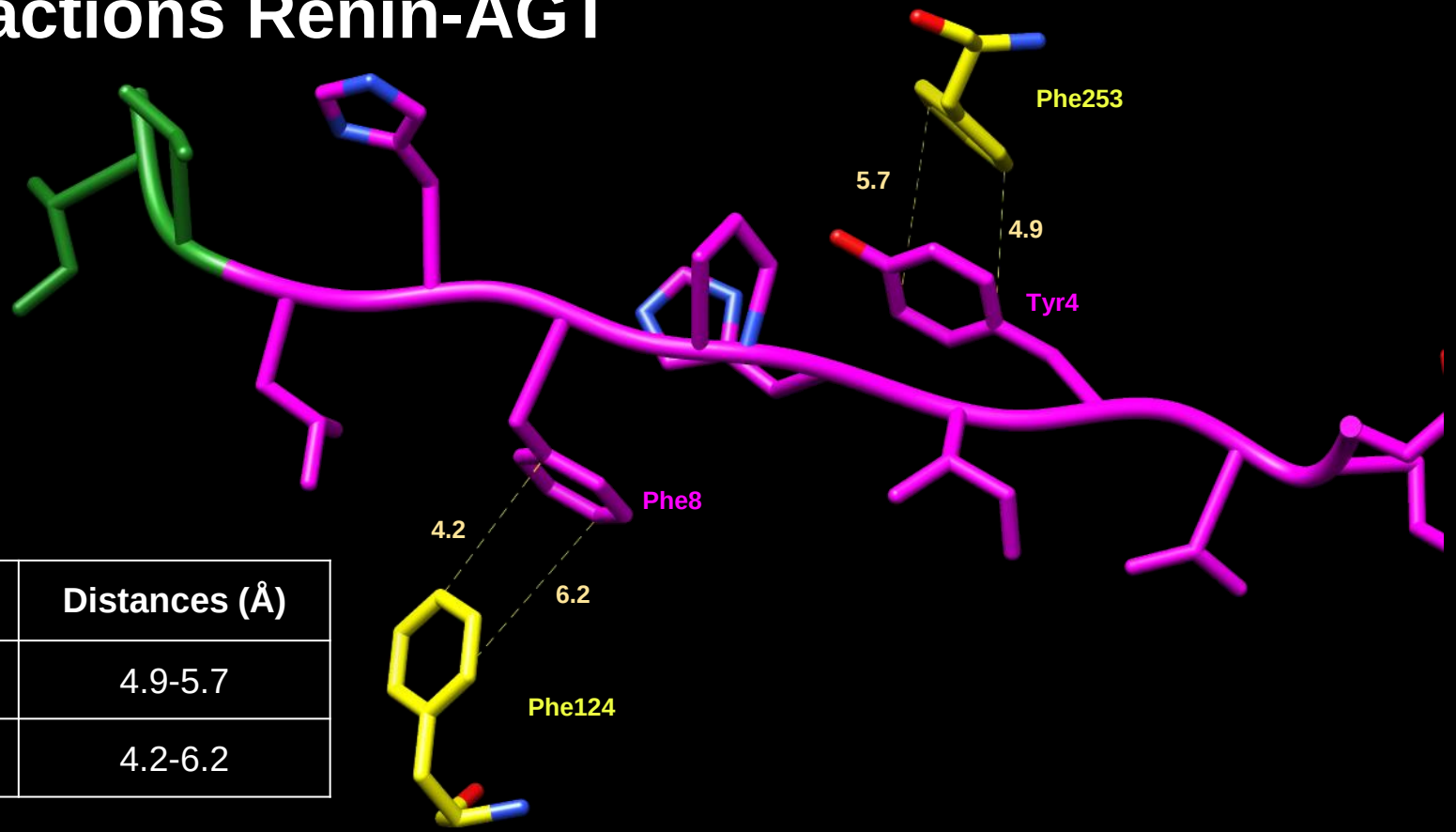
Atoms	Distances (Å)
Ser233-His9	3.0
His9-W3	2.9
Met303-His13	3.6
His13-W3	2.9
W3-W4	2.5
Ser84-W4	**3.1**
His9-W4	2.8
Val11-W3	2.8

Renin active site (seen from behind)



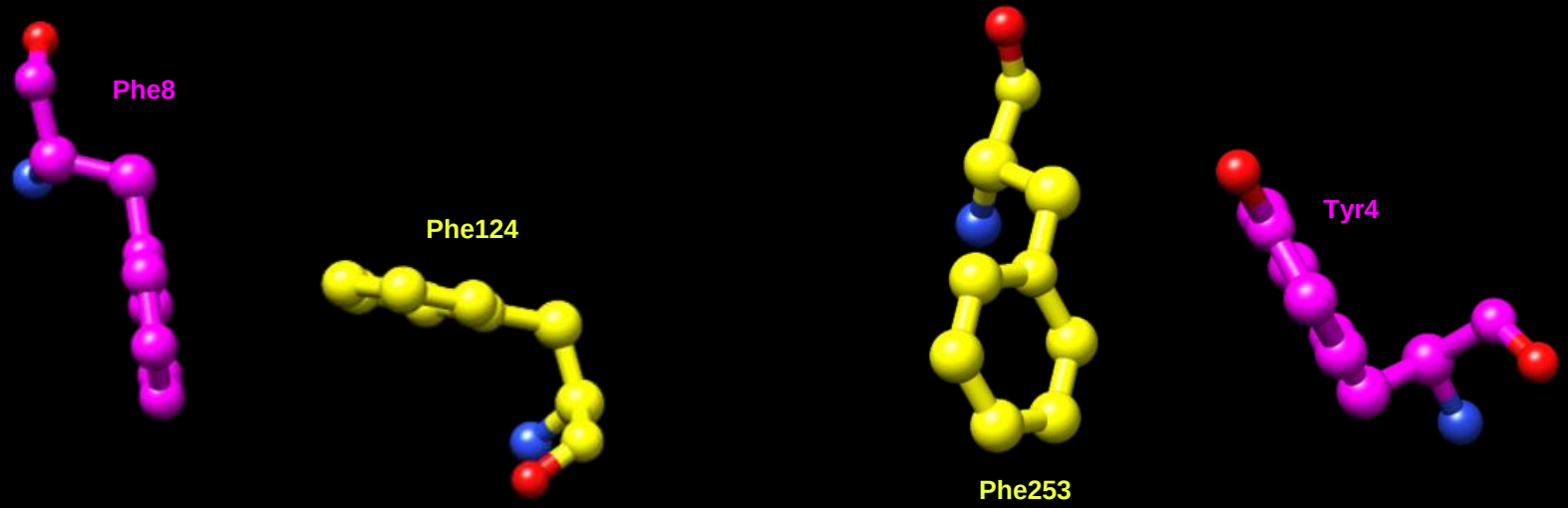
Atoms	Distances (Å)
Ser84-W4	3.1
Tyr83-W2	2.6
Arg82-His13	3.0

Pi interactions Renin-AGT



Atoms	Distances (Å)
Phe253-Tyr4	4.9-5.7
Phe124-Phe8	4.2-6.2

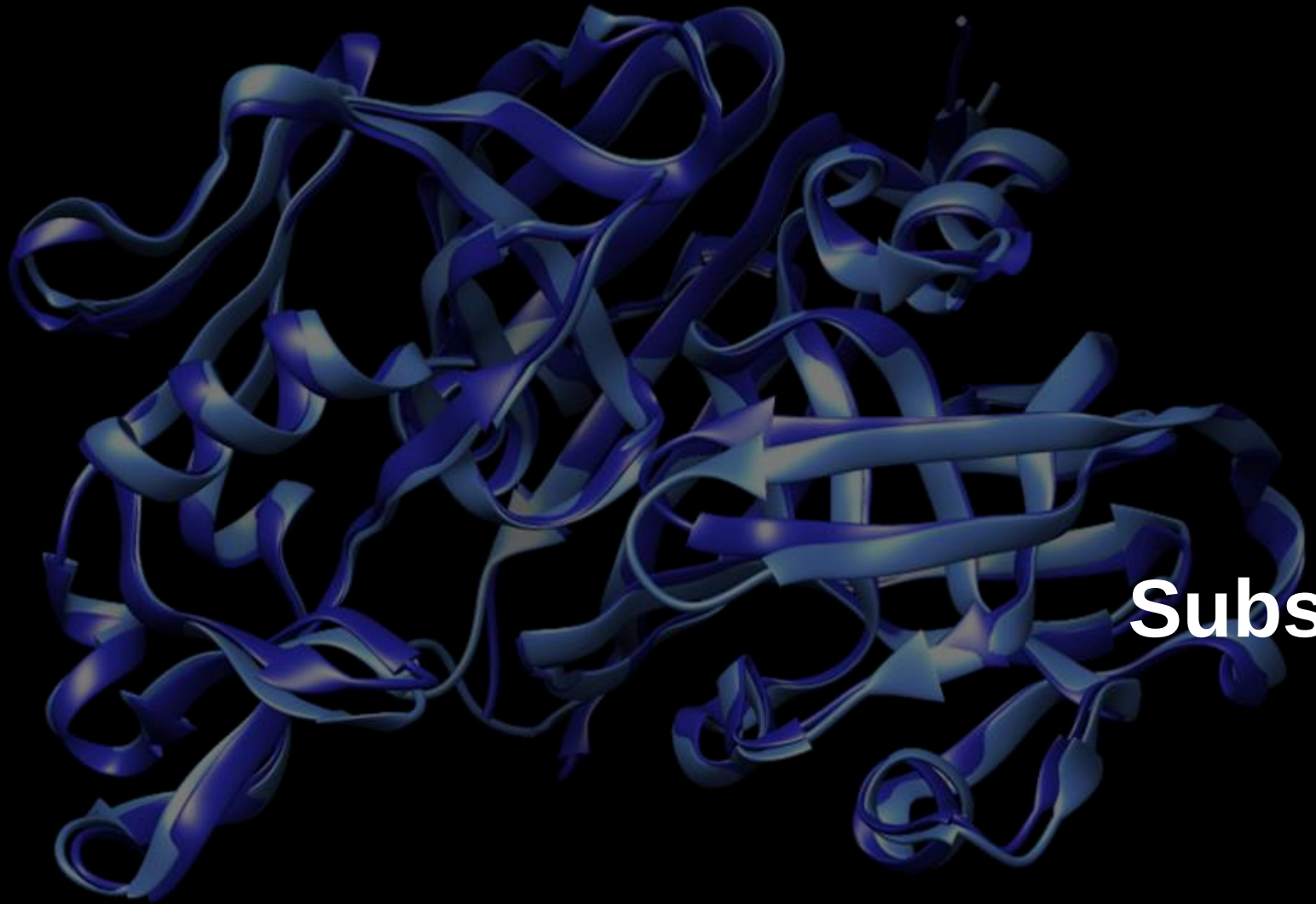
Pi interactions Renin-AGT



Atoms	Distances (Å)
Phe253-Tyr4	4.9-5.7
Phe124-Phe8	4.2-6.2

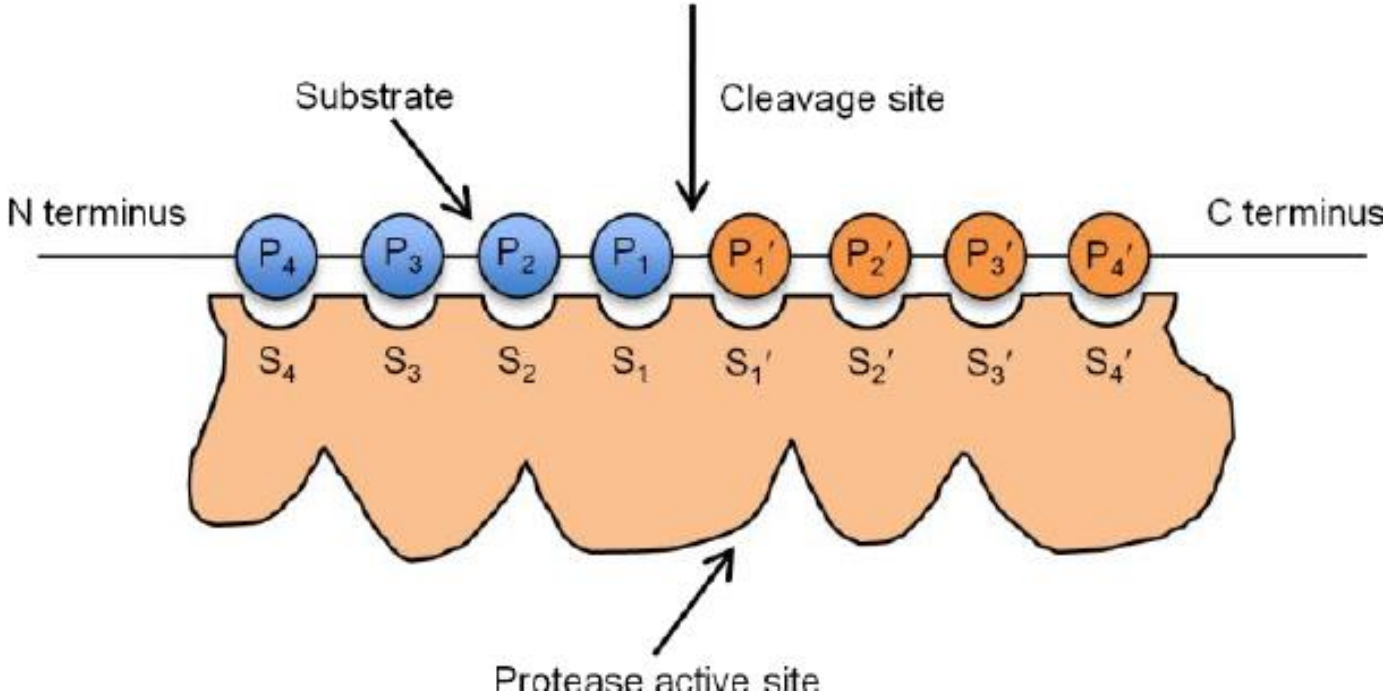
Renin

AGT



Subsites

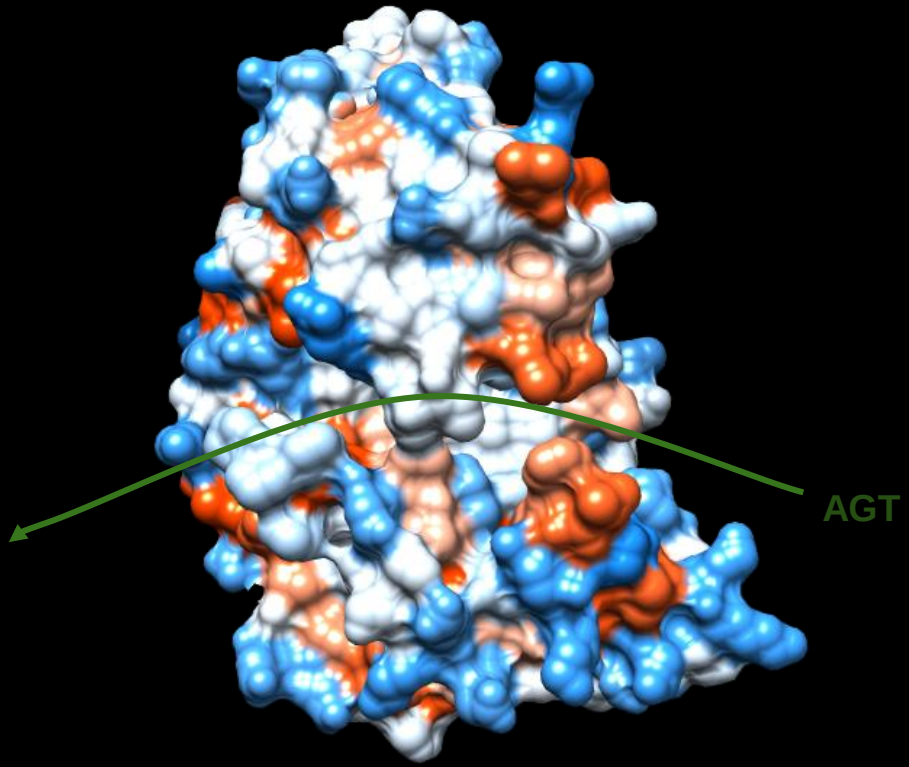
Nomenclature of Schechter and Berger for subsites



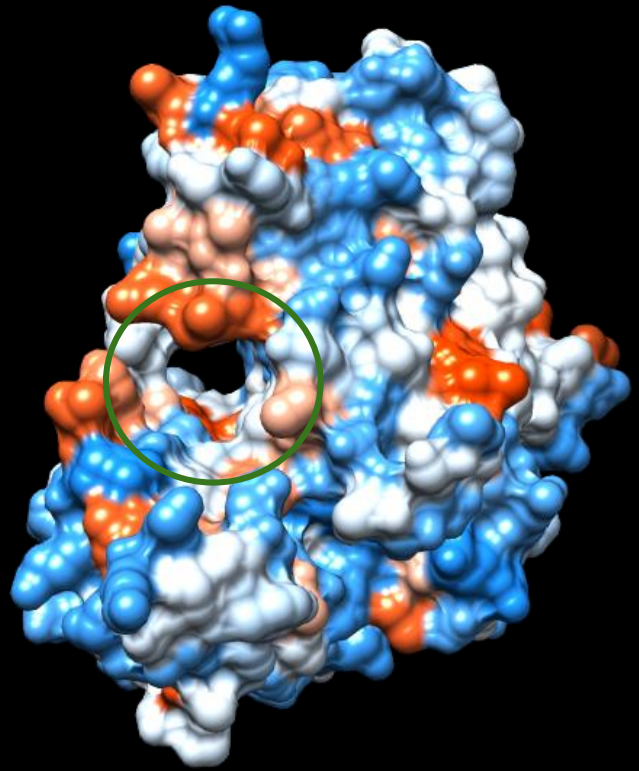
Drugs, enzymes and Receptors of the Renin-Angiotensin. 2000, Husain A.

Renin surface

Hydrophilic Hydrophobic

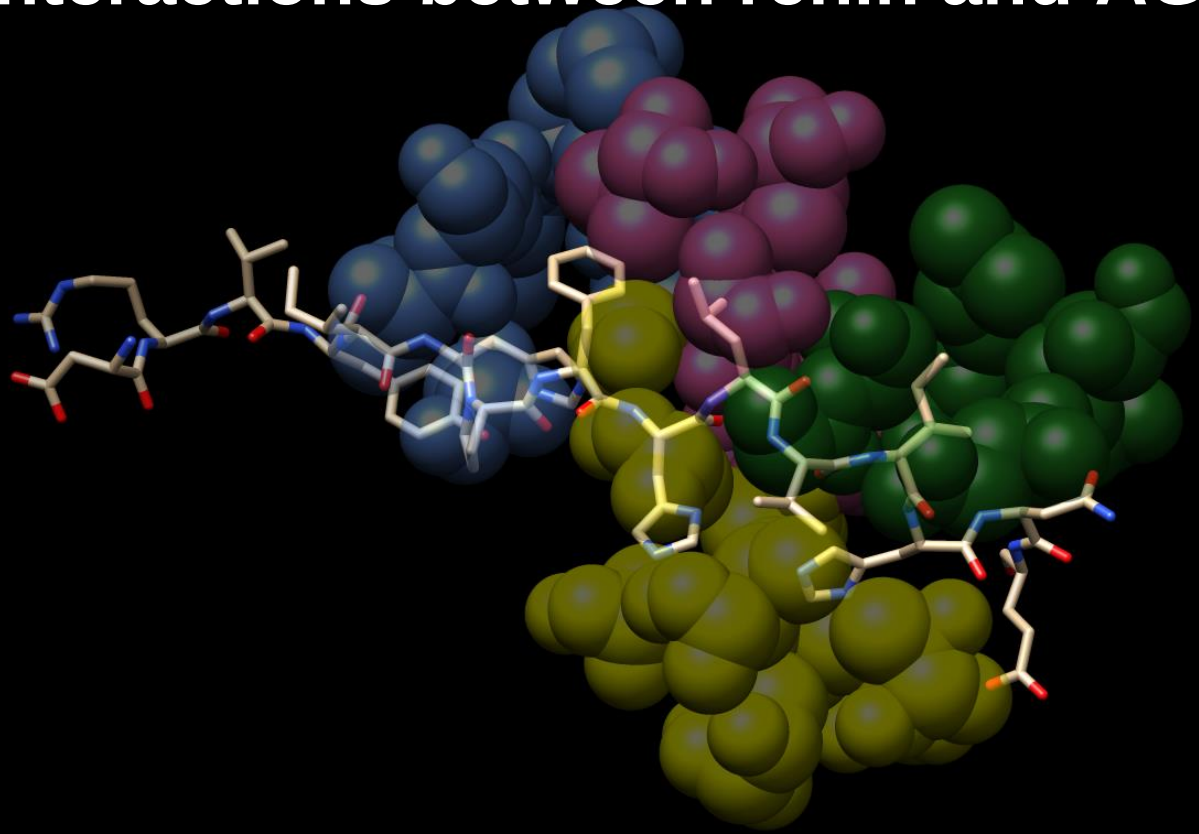


Frontal view



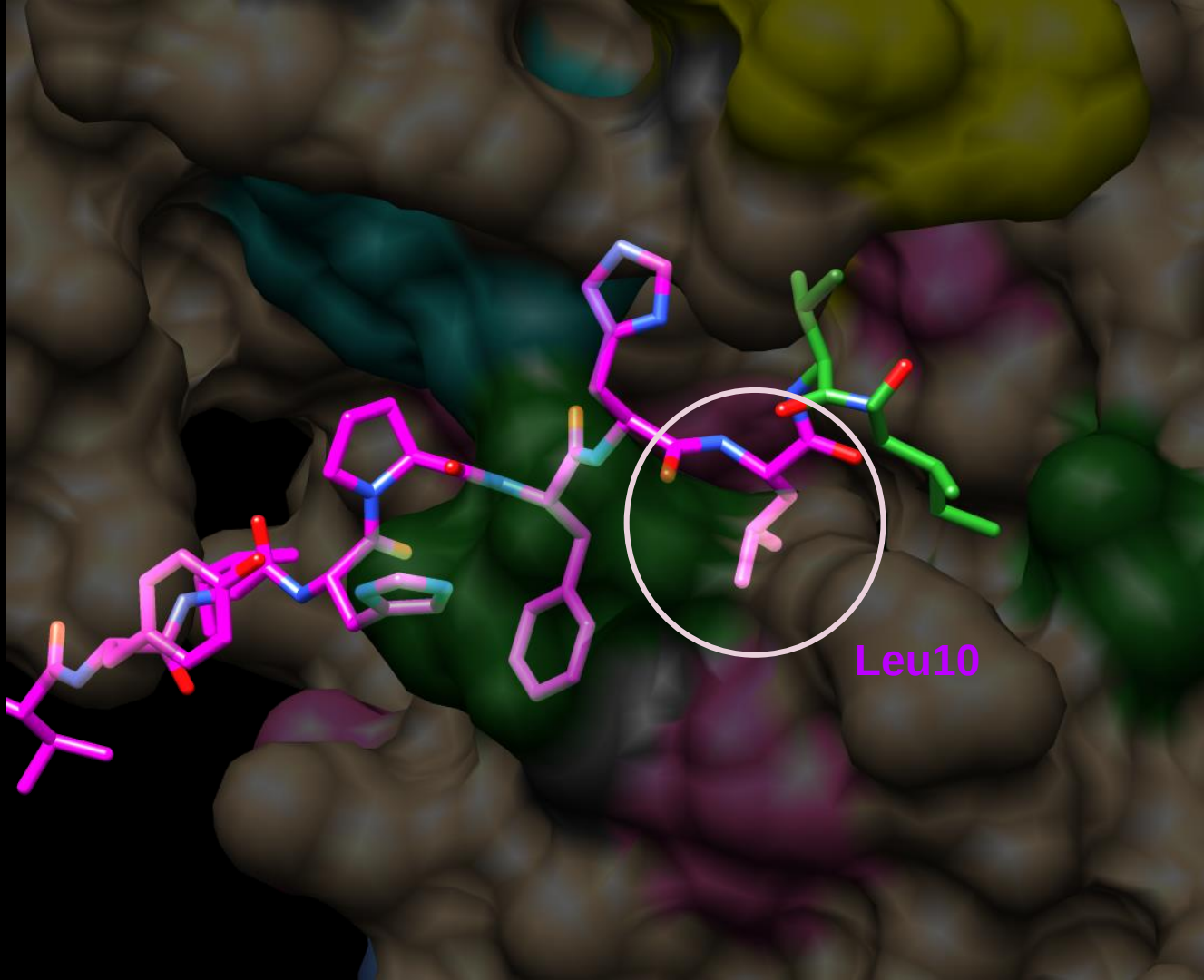
Lateral view

Binding interactions between renin and AGT



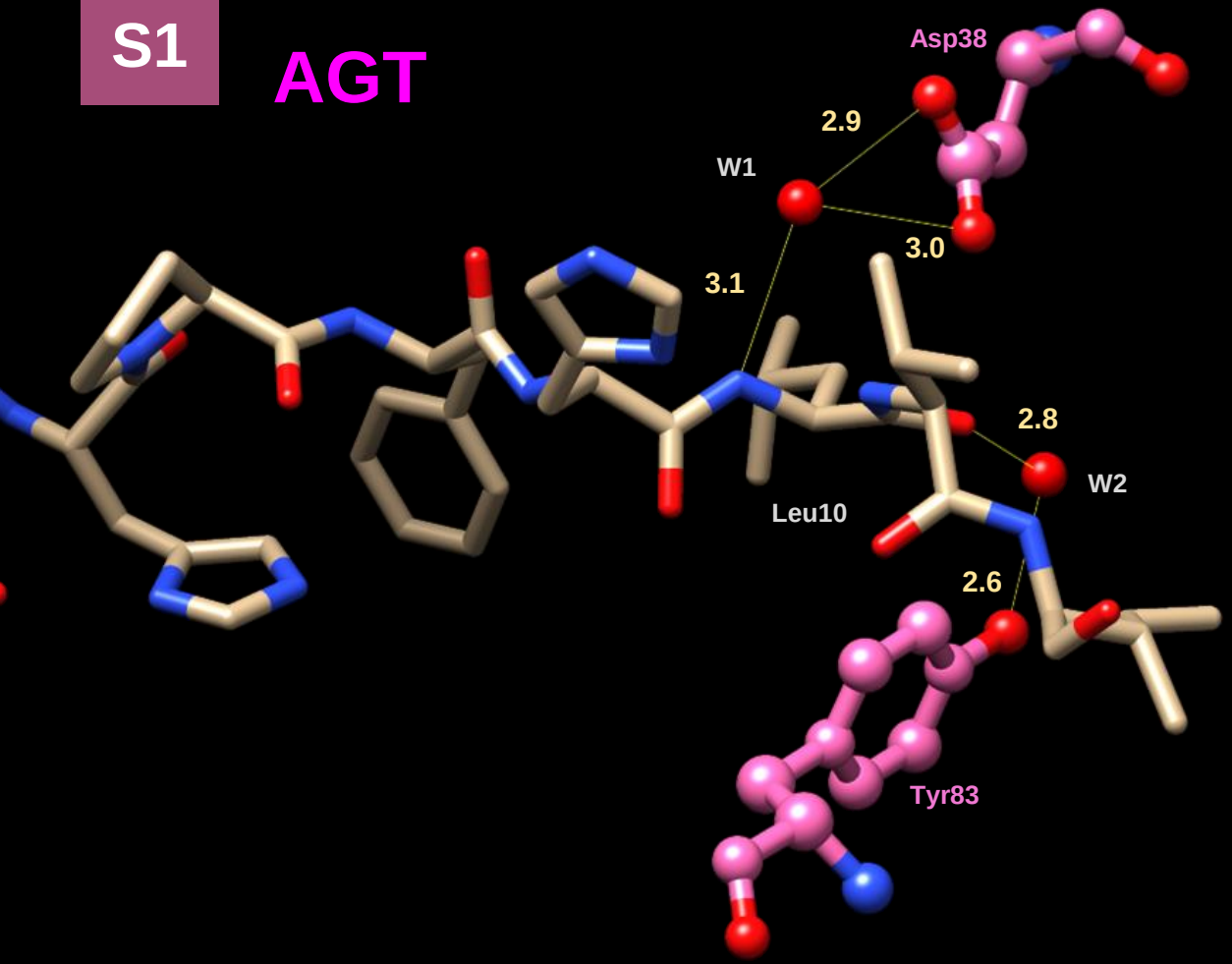
S1 AGT

S1 is occupied by an hydrophobic residue of P1, a Leucine.



S1

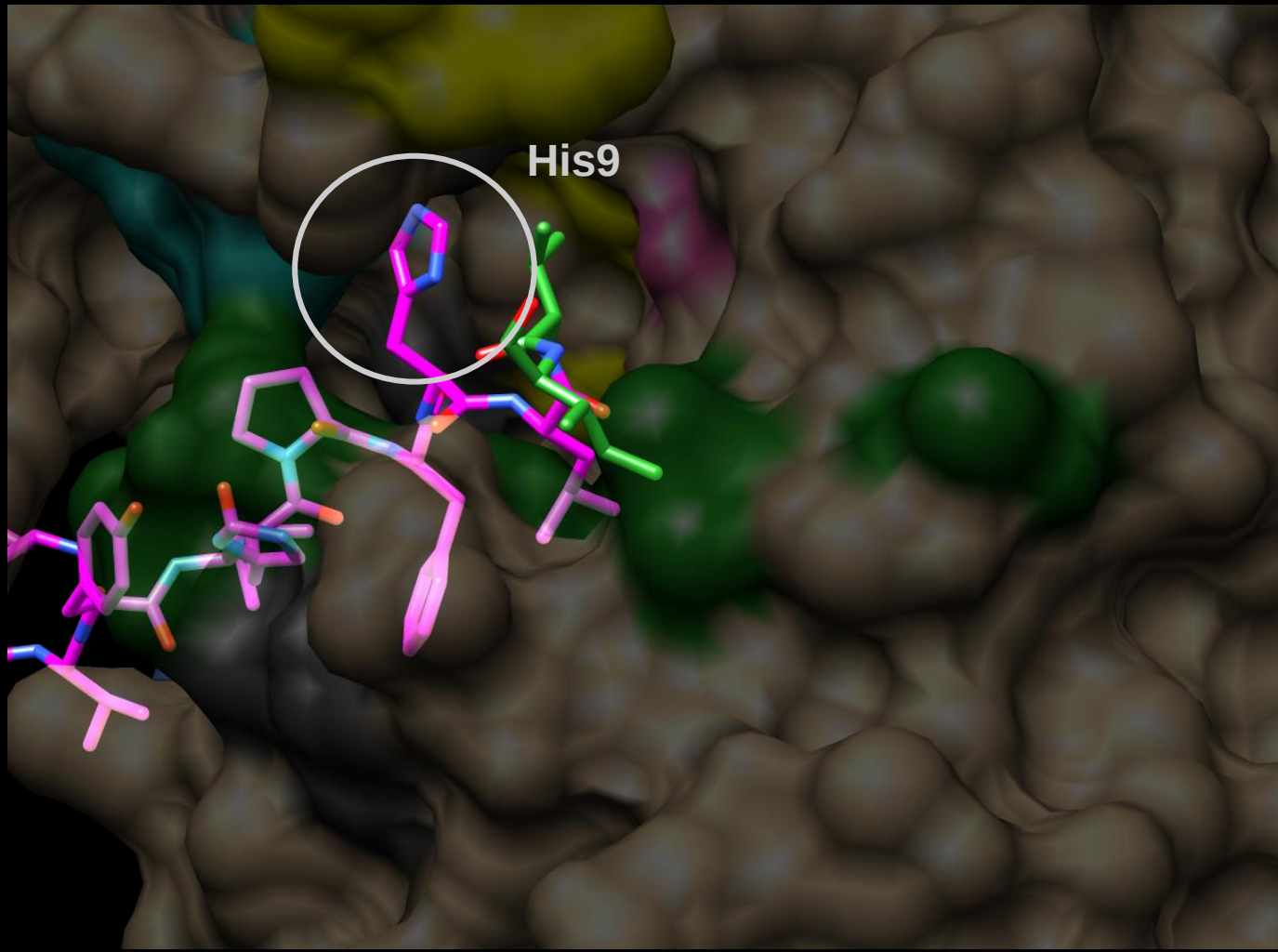
AGT



Atoms	Distances (Å)
Asp38-W1	2.9
Asp38-W1	3.0
Leu10-W1	3.1
Asp38-Leu10	3.1
Leu10-W2	2.8
Tyr83-W2	2.6

S2 AGT

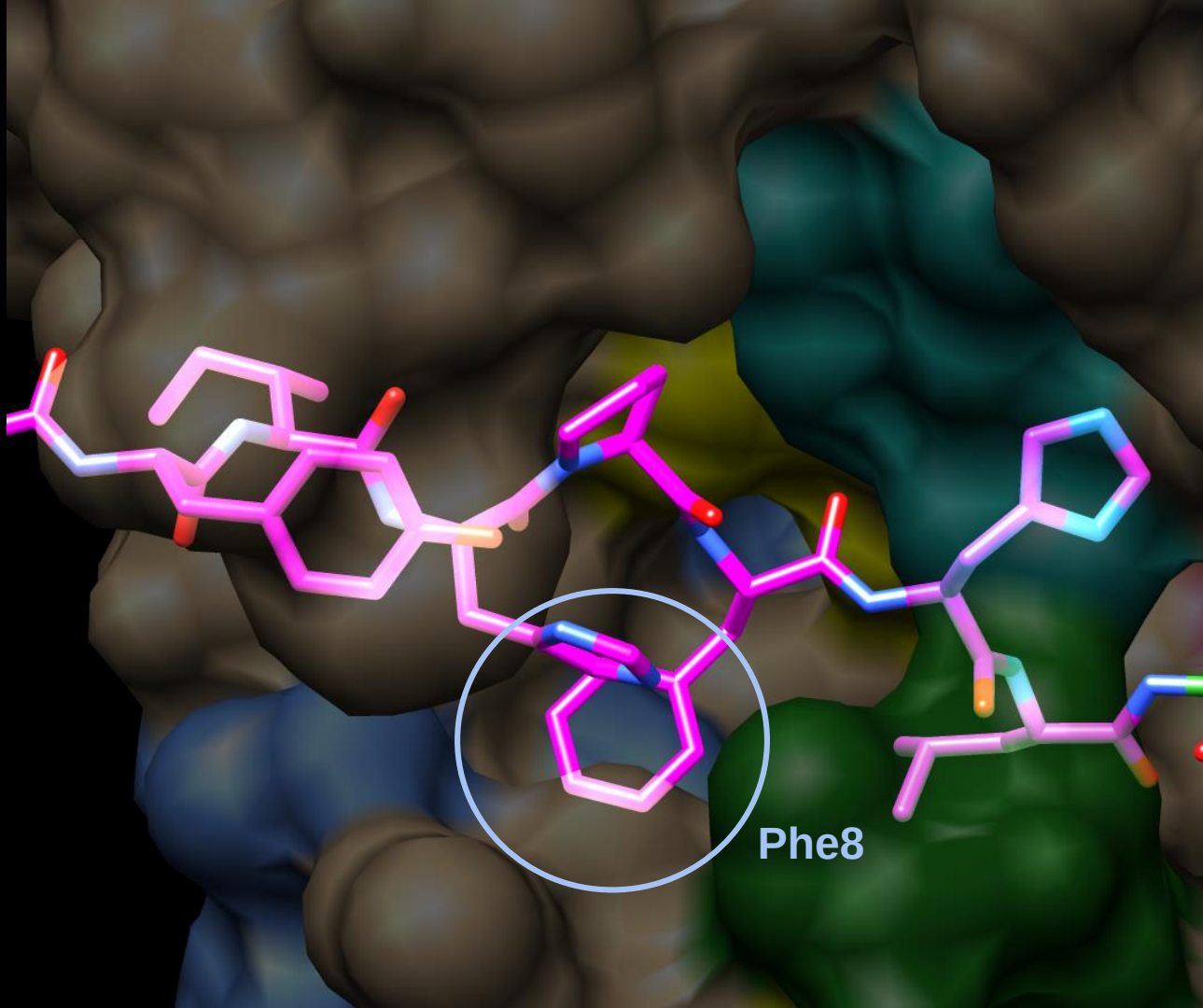
S2 is important for the binding AGT-Renin.



S3 AGT

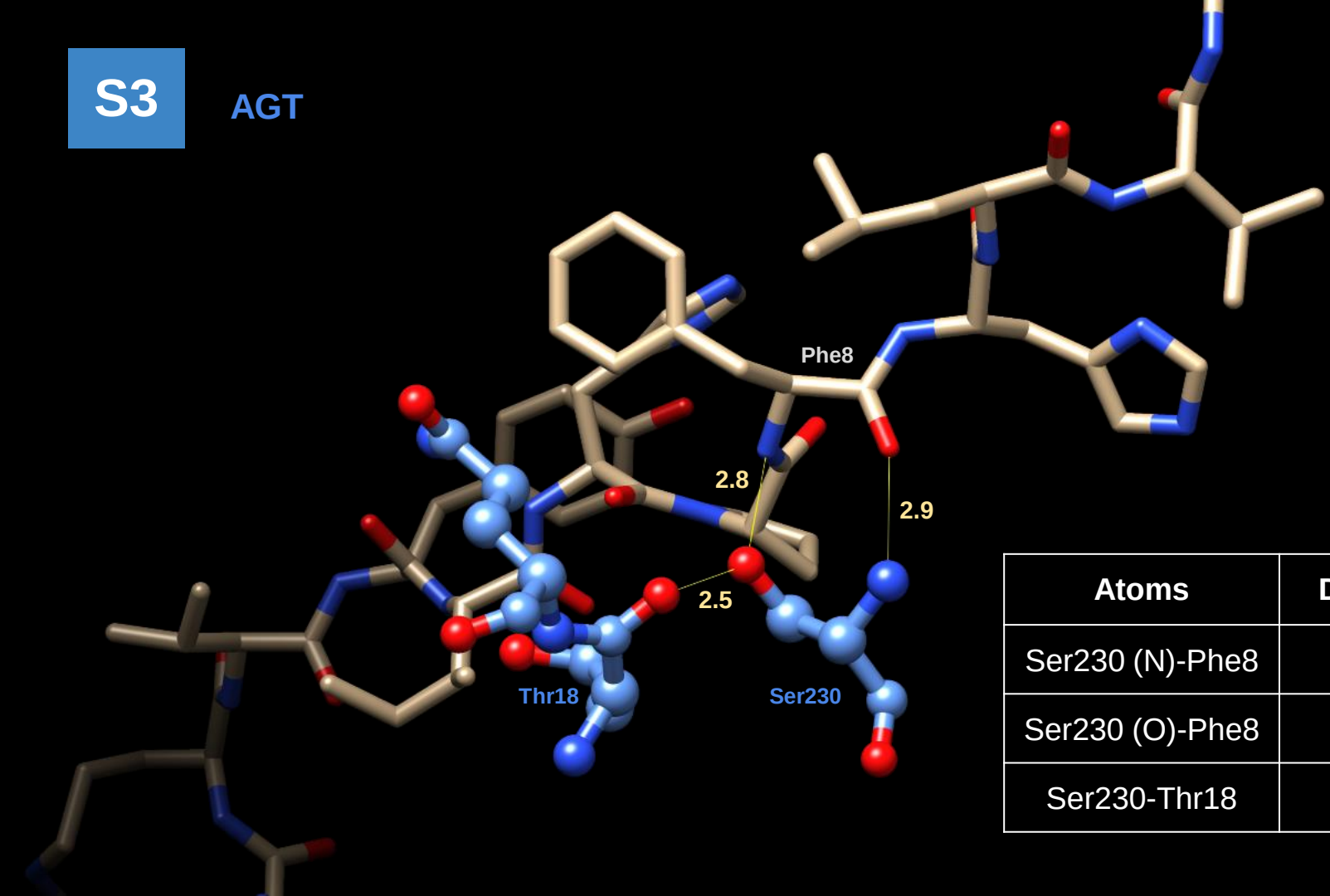
S3 has preference for hydrophobic aromatic residues.

Very important for binding.



S3

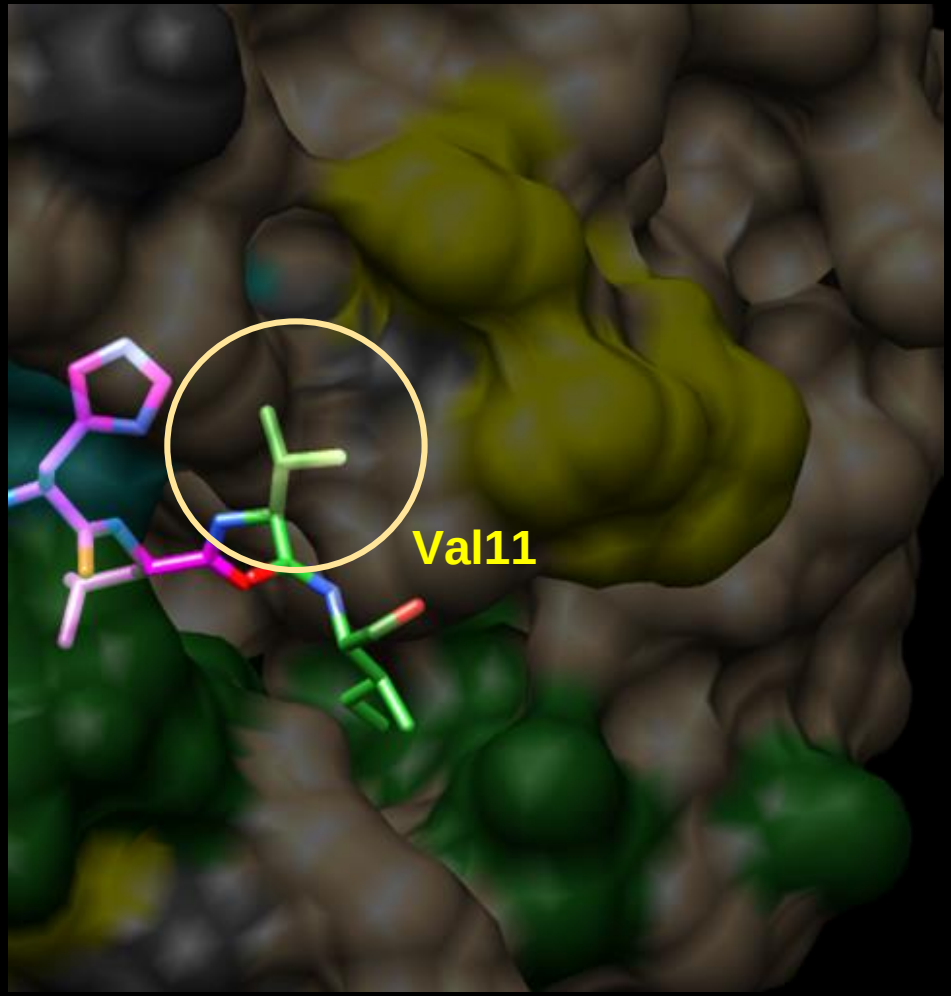
AGT



Atoms	Distances (Å)
Ser230 (N)-Phe8	2.9
Ser230 (O)-Phe8	2.8
Ser230-Thr18	2.5

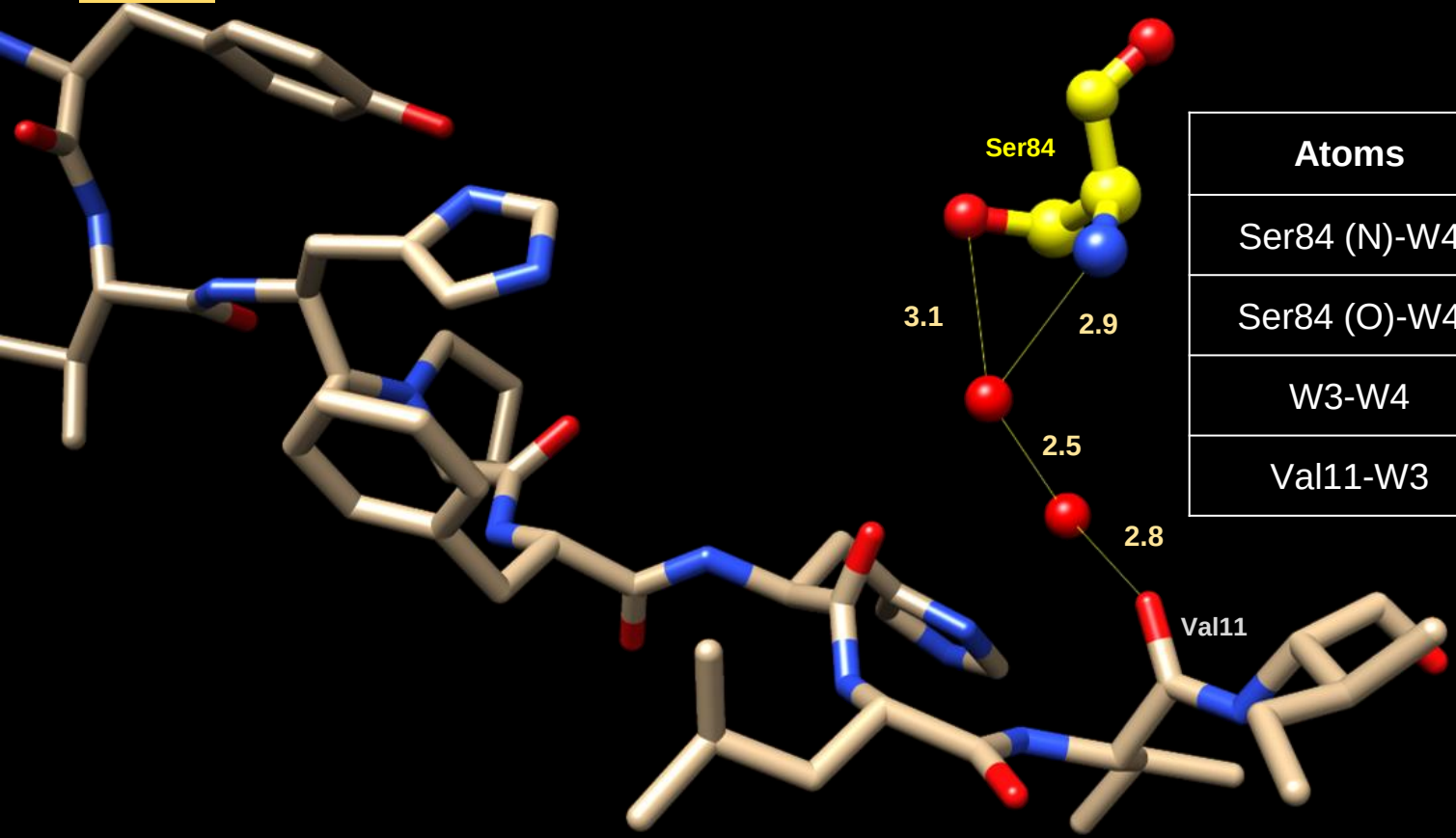
S1’ AGT

S1’ is critical for the binding AGT-Renin.



S1’

AGT

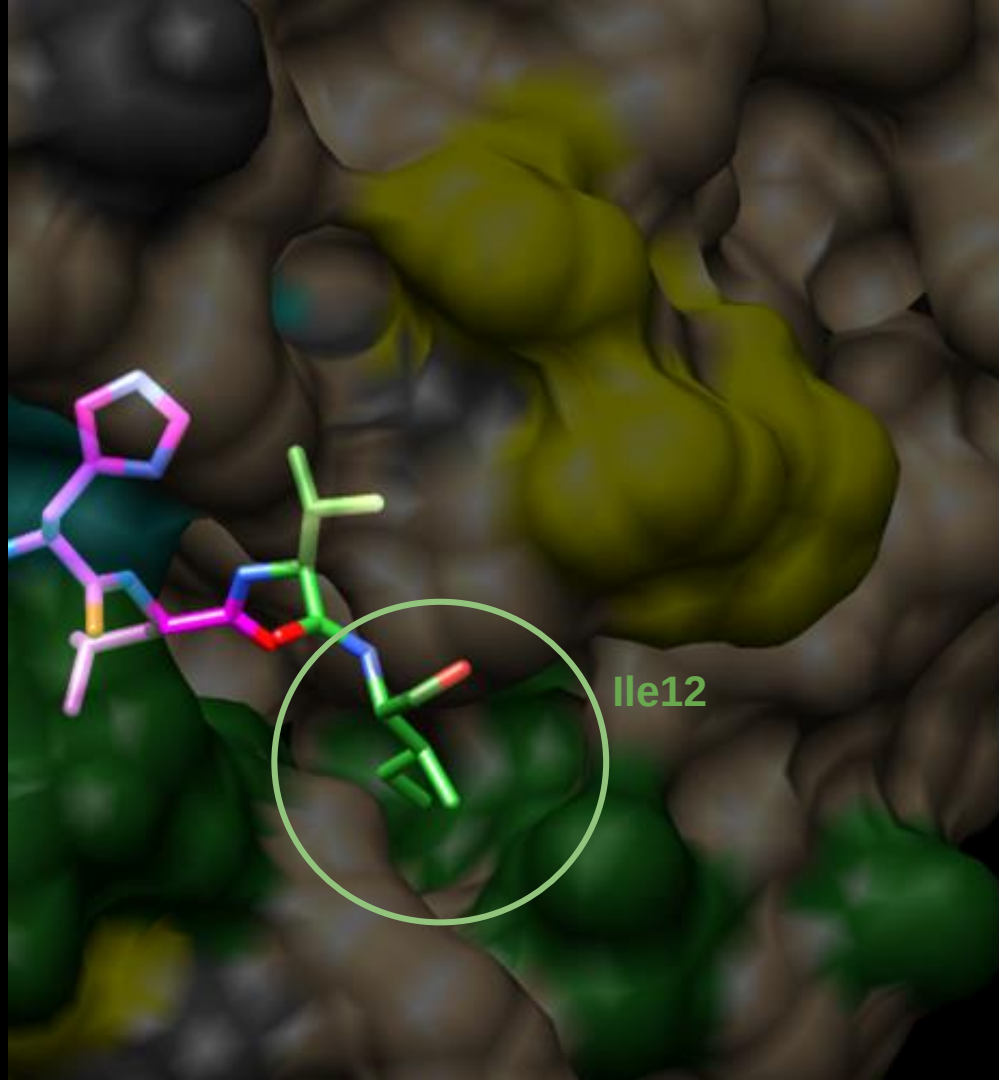


Atoms	Distances (Å)
Ser84 (N)-W4	2.9
Ser84 (O)-W4	3.1
W3-W4	2.5
Val11-W3	2.8

S2’

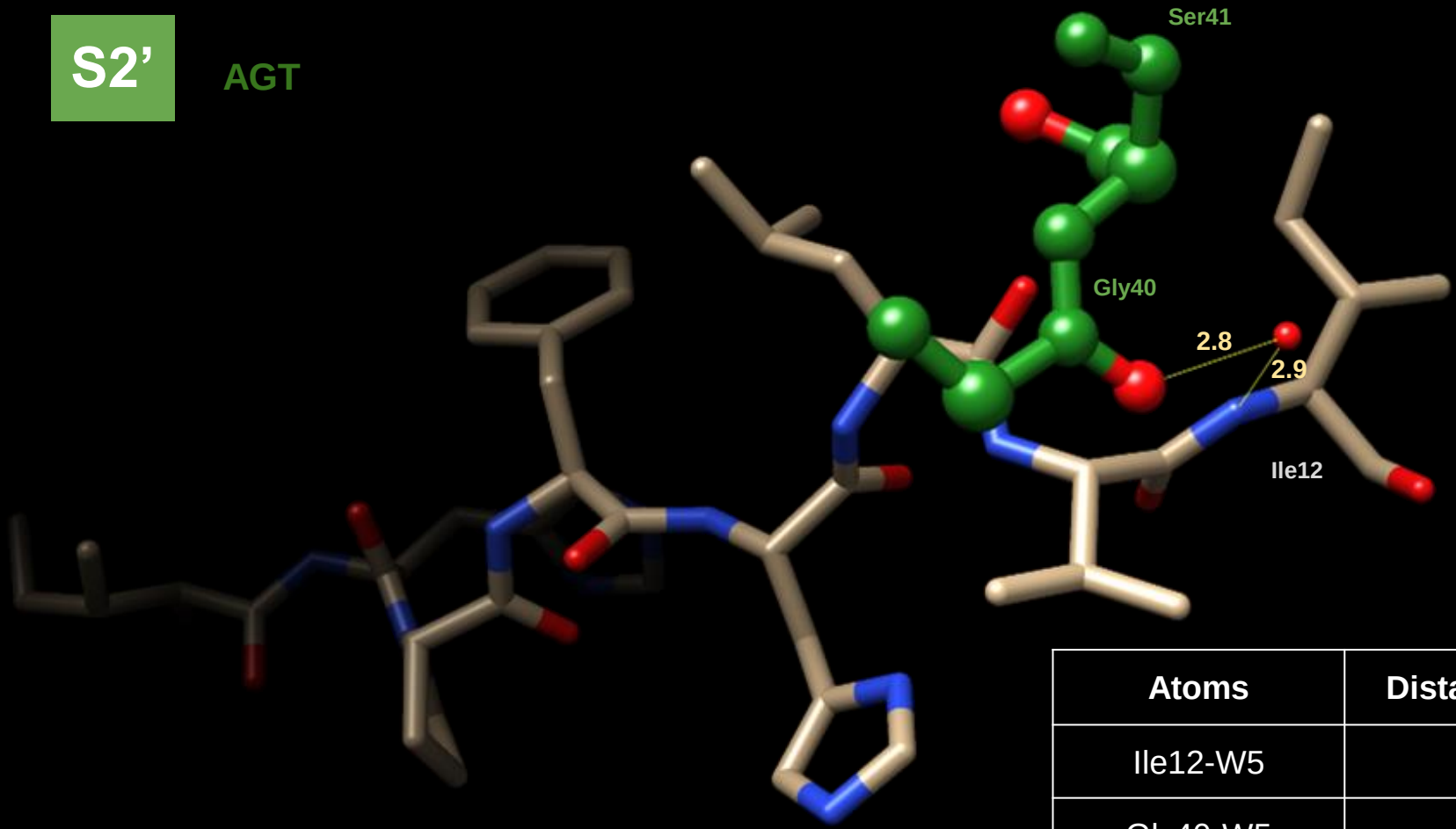
AGT

S2’ is a polar subsite. It is occupied by an Isoleucine.

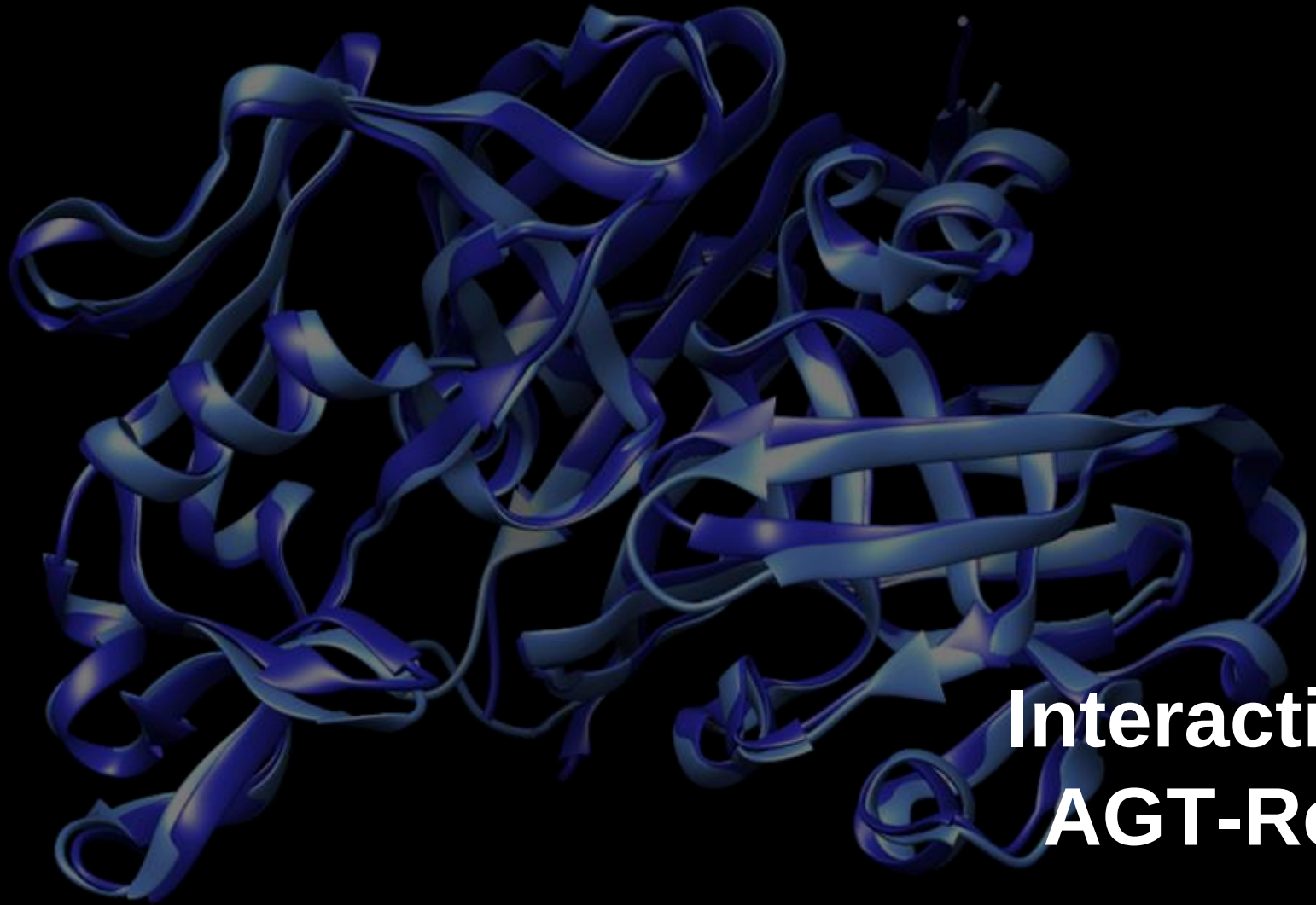


S2’

AGT



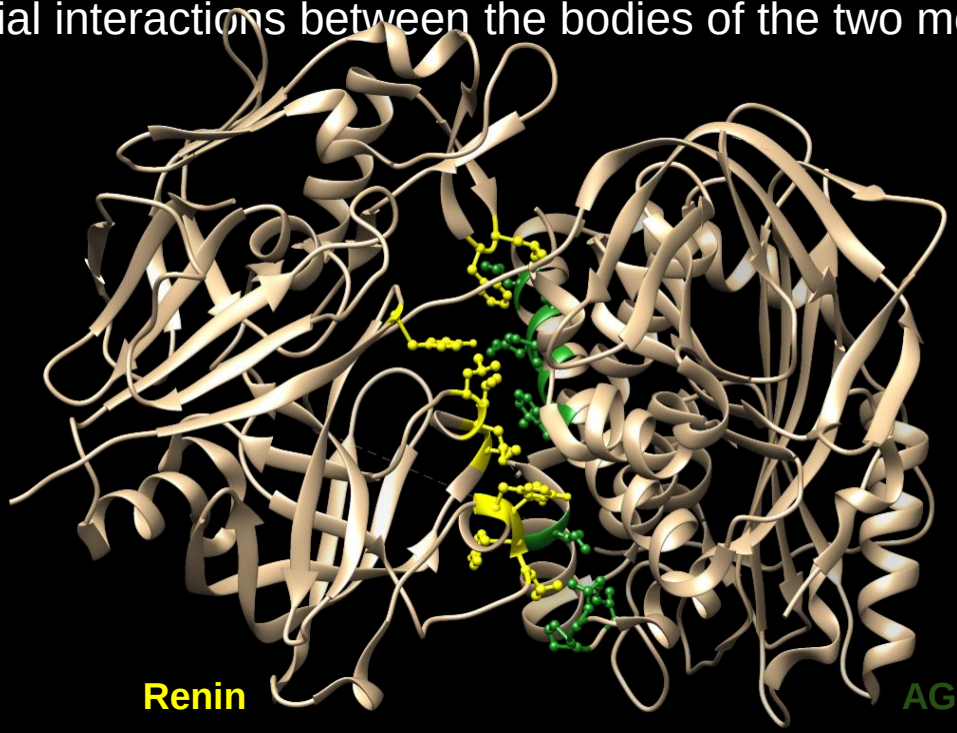
Atoms	Distances (Å)
Ile12-W5	2.9
Gly40-W5	2.8



Interactions AGT-Renin

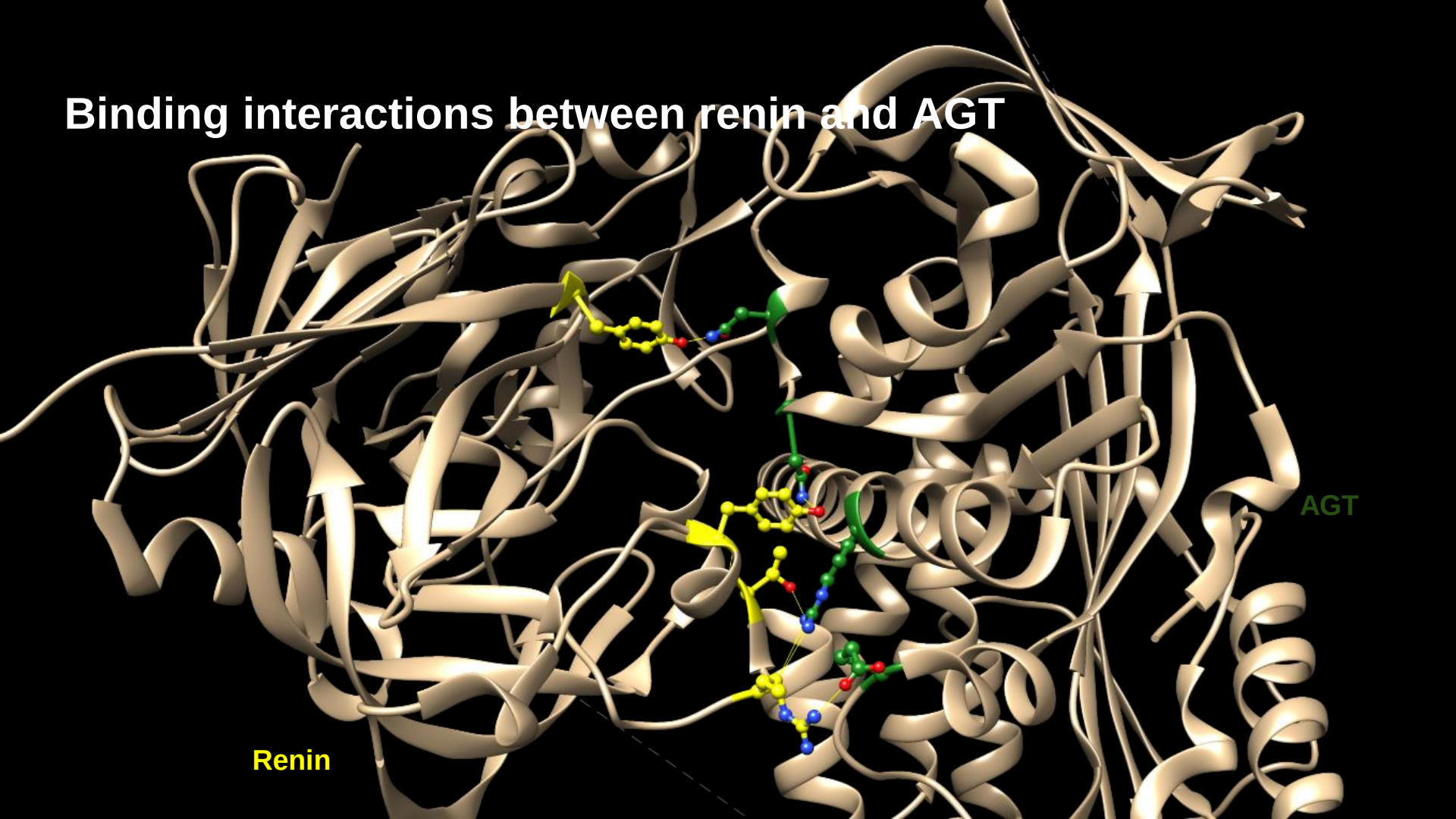
Hydrophobic interactions between renin and AGT

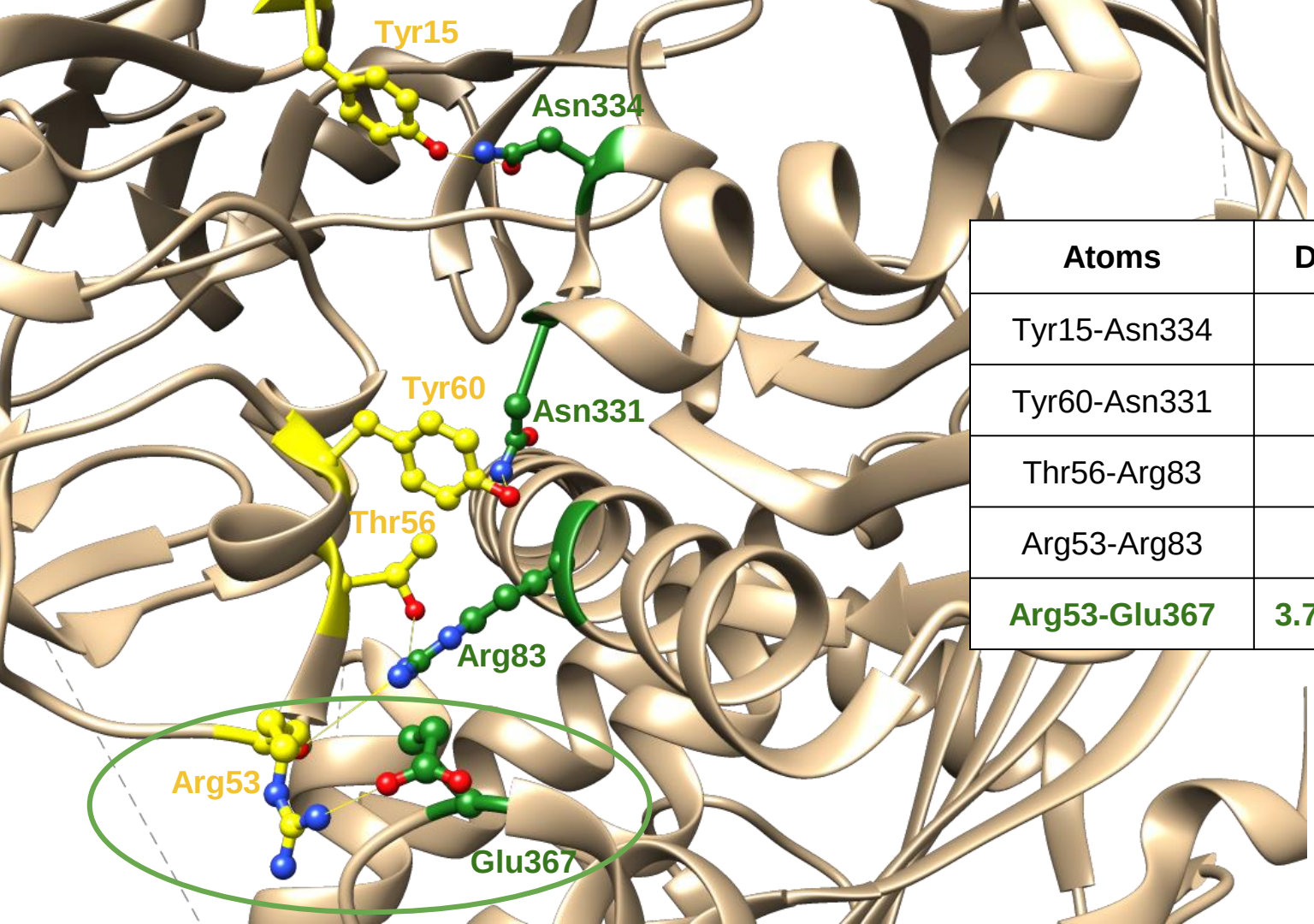
The substantial interactions between the bodies of the two molecules are mainly hydrophobic.



Hydrophobic residues from both proteins are facing each other.

Binding interactions between renin and AGT



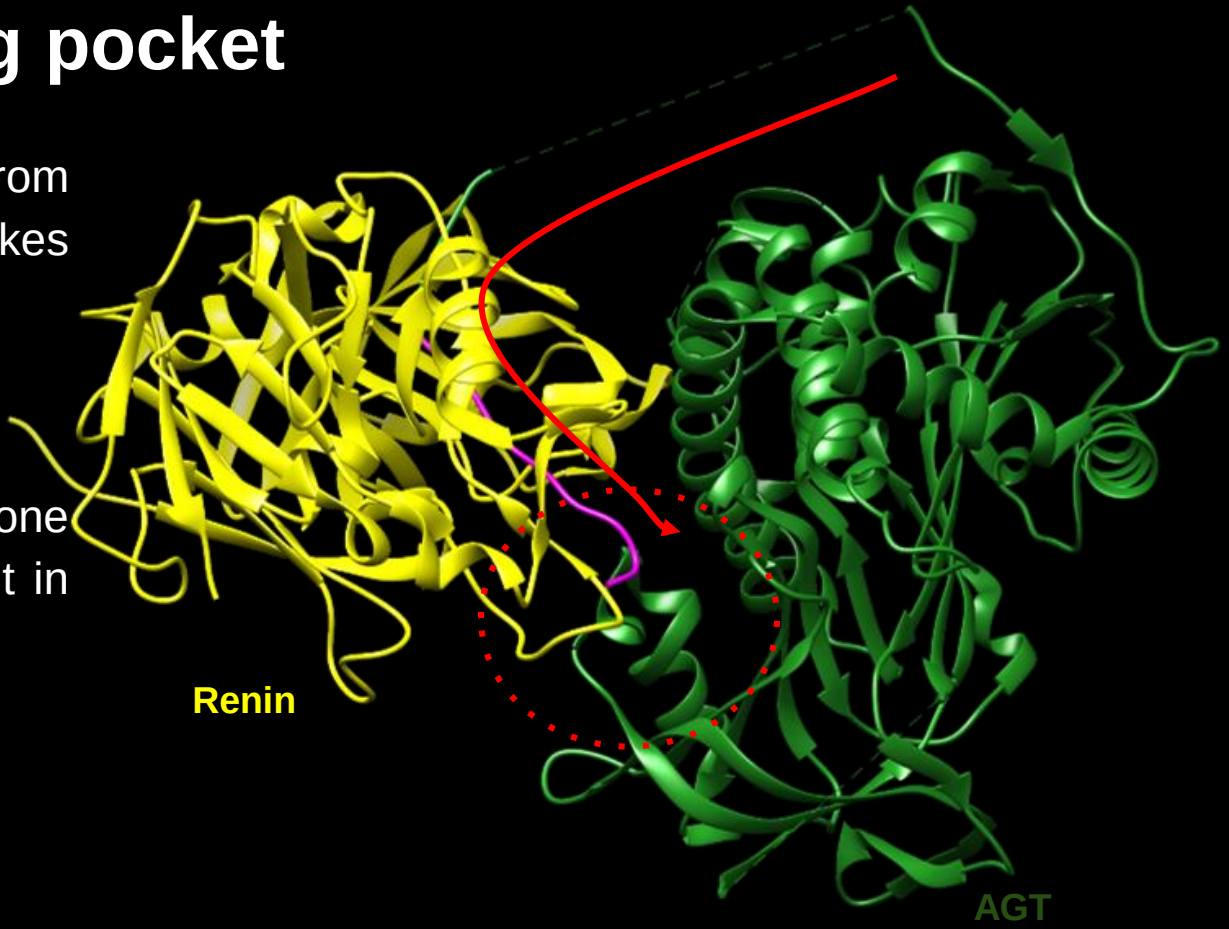


Atoms	Distances (Å)
Tyr15-Asn334	2.9
Tyr60-Asn331	3.7
Thr56-Arg83	3.4
Arg53-Arg83	2.9
Arg53-Glu367	**3.7 (Salt Bridge)**

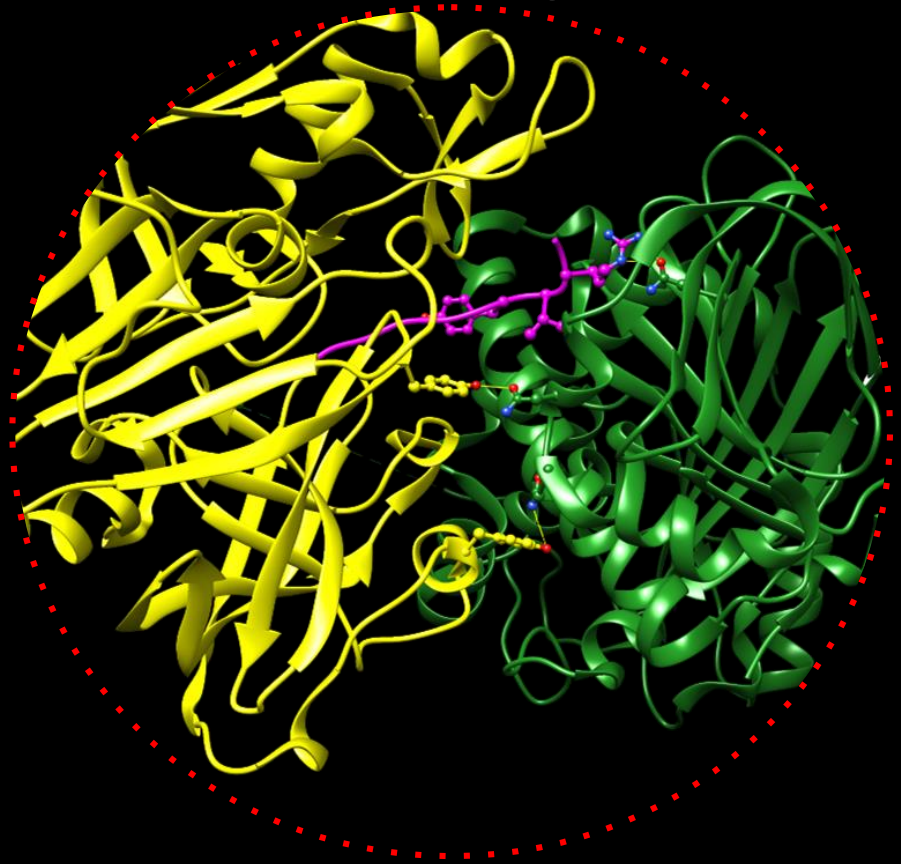
Hormone-binding pocket

AGT N-terminus emerges from the renin active cleft and pokes into a surface cavity of AGT.

Such cavity is called hormone binding pocket and is present in other serpins.



Hormone-binding pocket

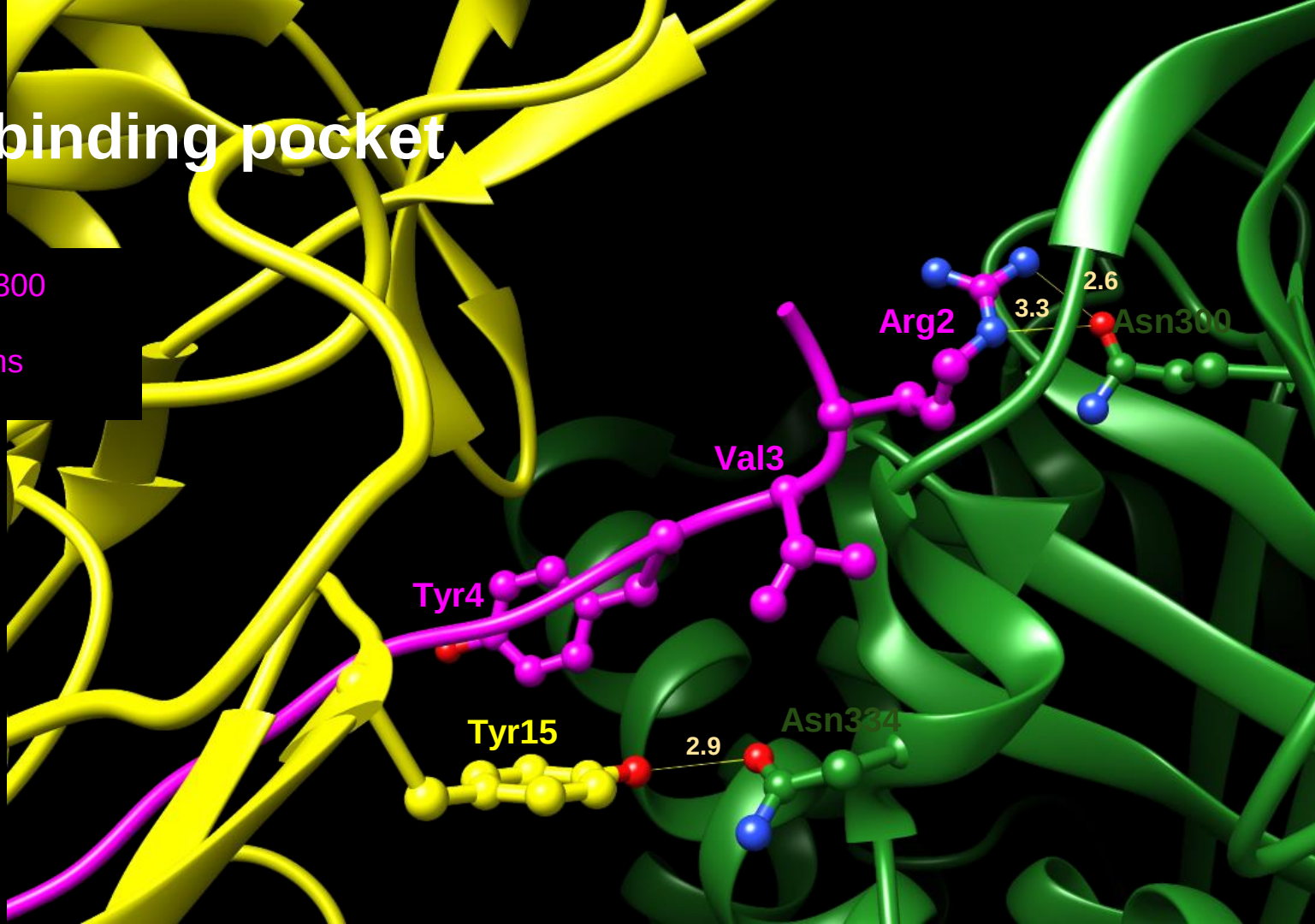


“Tail-in-mouth mechanism”

Insertion of the N-terminus into the hormone binding pocket is needed to stabilize the complex

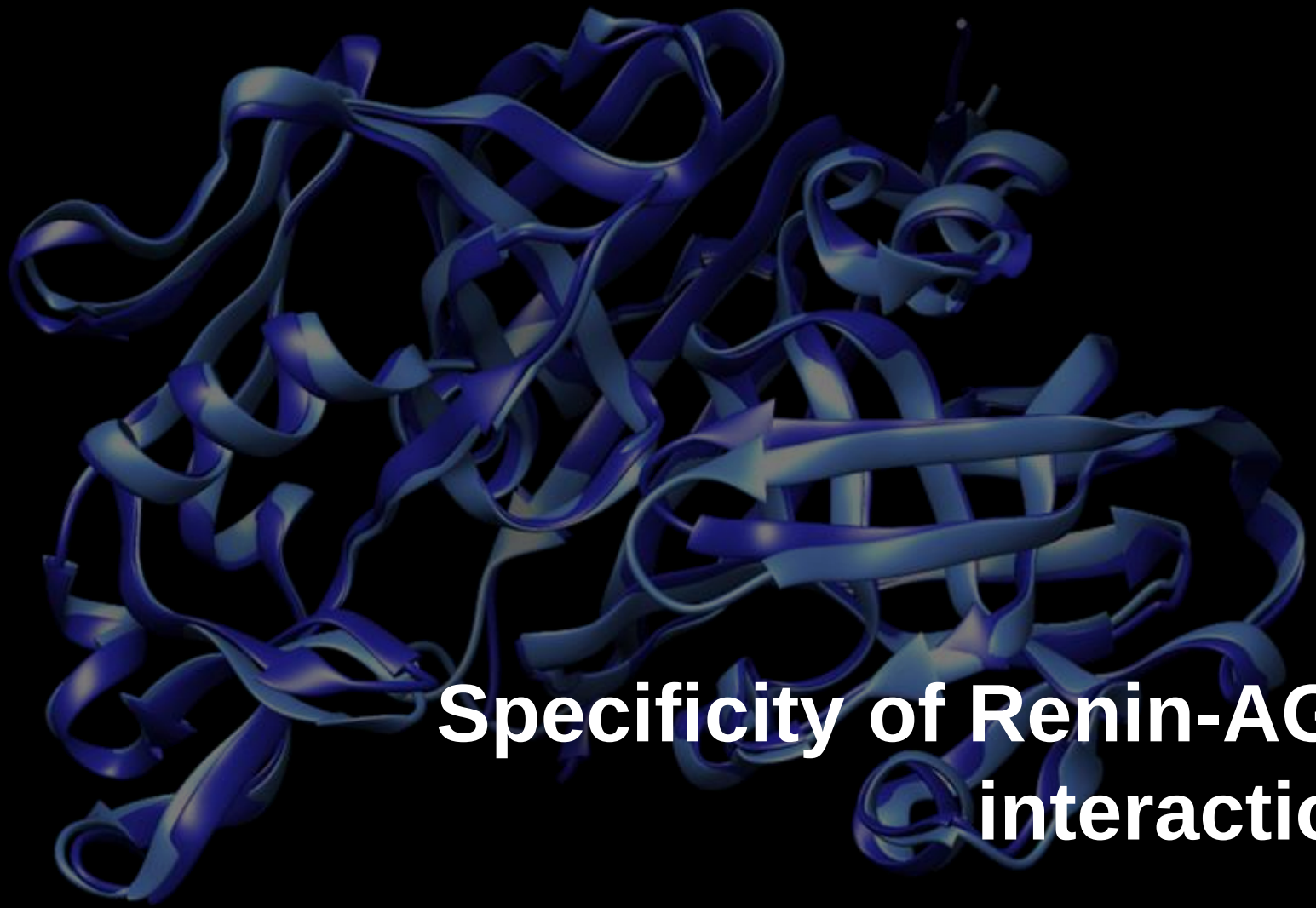
Hormone-binding pocket

Arg2 is buried by Gln300
Val3 and Tyr4 form
hydropobic interactions



Hormone-binding pocket

A possible therapeutic application



Specificity of Renin-AGT interaction

His-Pro-Phe motif in AGT

```
sp|P01019|ANGT_Homo_sapiens          MRKRAPQSEMAPAGVSLRATILCLLAWAGLAAGDRVYIHPFHLVIHNEST
sp|Q9GLP6|ANGT_Gorilla_gorilla       MRKRAPQSEMAPAGMSLRATILCLLAWAGLAAGDRVYIHPFHLVIHNEST
sp|Q9GLN8|ANGT_Pan_troglodytes       MRKRAPQSEMAPAGVSLRATILCLVAWAGLAAGDRVYIHPFHLVIHNEST
sp|Q9TSZ0|ANGT_Callithrix_jacc       MQKRVPQSEMPPASMSLRVTILCLLAWAGLAAGDRVYIHPFHLVIHNEST
sp|P20757|ANGT_Ovis_aries            ---------MAPAGLSLGATILCLLAWAGLAAGDRVYIHPFHLLVHSKSN
sp|P01015|ANGT_Rattus_norvegic       ---------MTPTGAGLKATIFCILTWVSLTAGDRVYIHPFHLLYYSKST
sp|P11859|ANGT_Mus_musculus          ---------MTPTGAGLKATIFCILTWVSLTAGDRVYIHPFHLLYHNKST
                                           *.*:. .* .**:*:::*..*:**********: :.:*.
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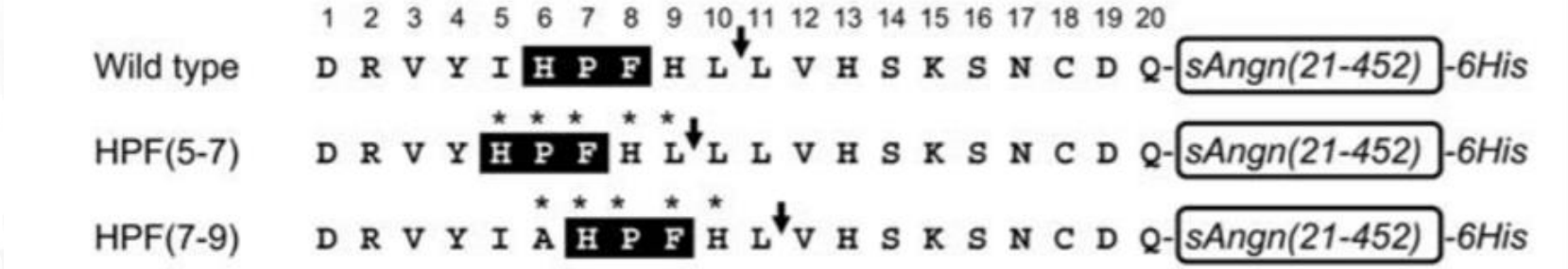
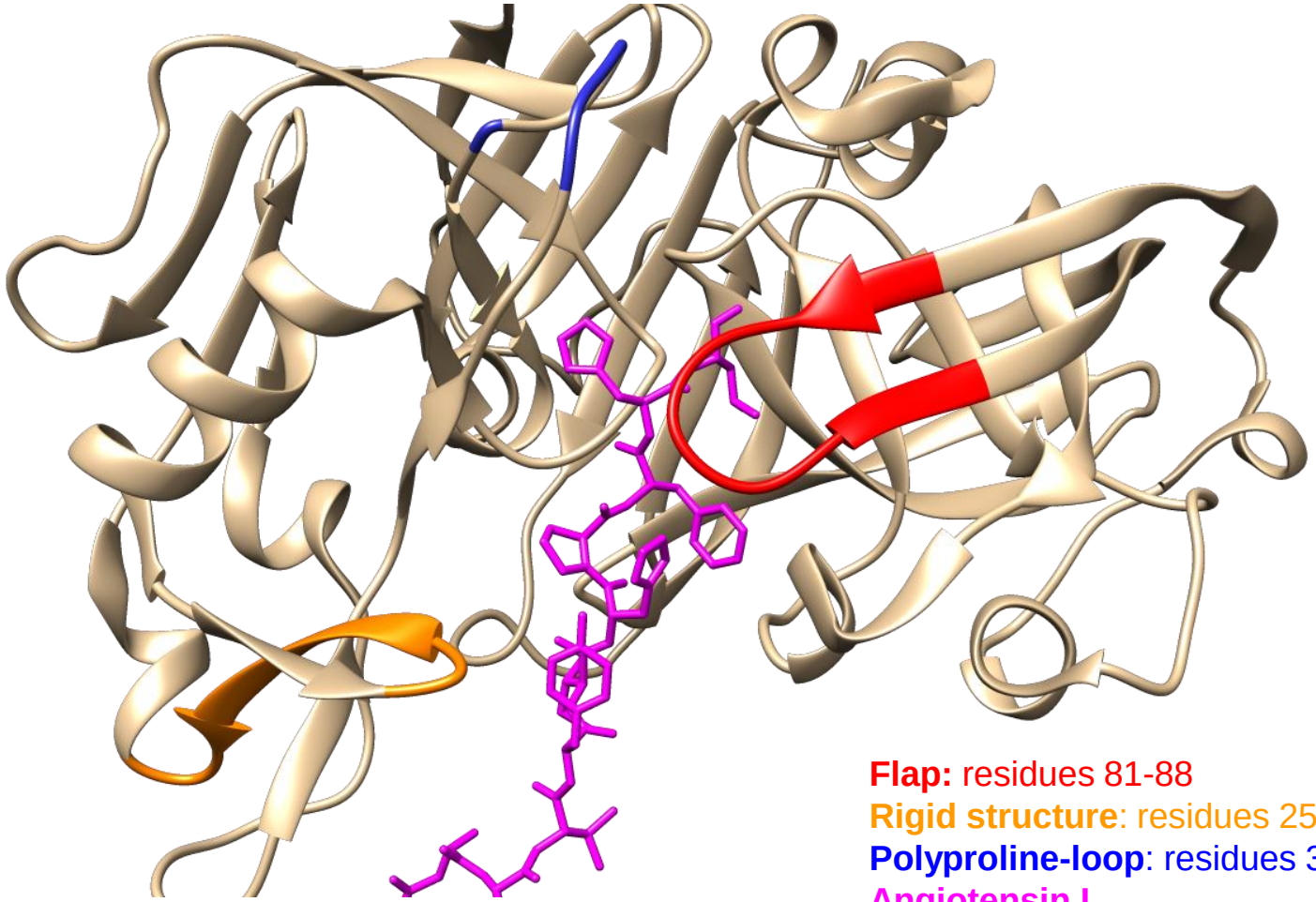


The His-Pro-Phe motif of angiotensinogen is a crucial determinant of the substrate specificity of renin. 2007, Nakagawa T.

Poly-prolines provide an effective mean of constructing well defined pockets from loops that would otherwise be more flexible

```
sp|P20142|Gastricsin_Homo       FPLPPSSYILSN----NGYCTVGVEPTYLSSQNGQPLWILGDVFLRSYYS
sp|P04073|Gastricsin_Rattus     FPLSPSSYIIQE----DNFCMVGLESISLTSESGQPLWILGDVFLRSYYA
sp|P0DJD7|Pepsin                YPVPPSAYILQS----EGSCISGFQGMNLPTESGE-LWILGDVFIRQYFT
sp|P00791|Pepsin                YPLSPSAYILQD----DDSCTSGFEGMDVPTSSGE-LWILGDVFIRQYYT
sp|P14091|Cathepsin             YTLSPTAYTLLDFVDGMQFCSSGFQGLDIHPPAGP-LWILGDVFIRQFYS
sp|O96009|Napsin-A_Homo         FNLTAHDYVIQTTRNGVRLCLSGFQALDVPPPAGP-FWILGDVFLGTYVA
sp|P00797|Renin_Homo            YTLTSADYVFQESYSSKKLCTLAIHAMDIPPPTGP-TWALGATFIRKFYT
                                : :..  * :        *  ...   : .  *   * ** .*:  : :
```

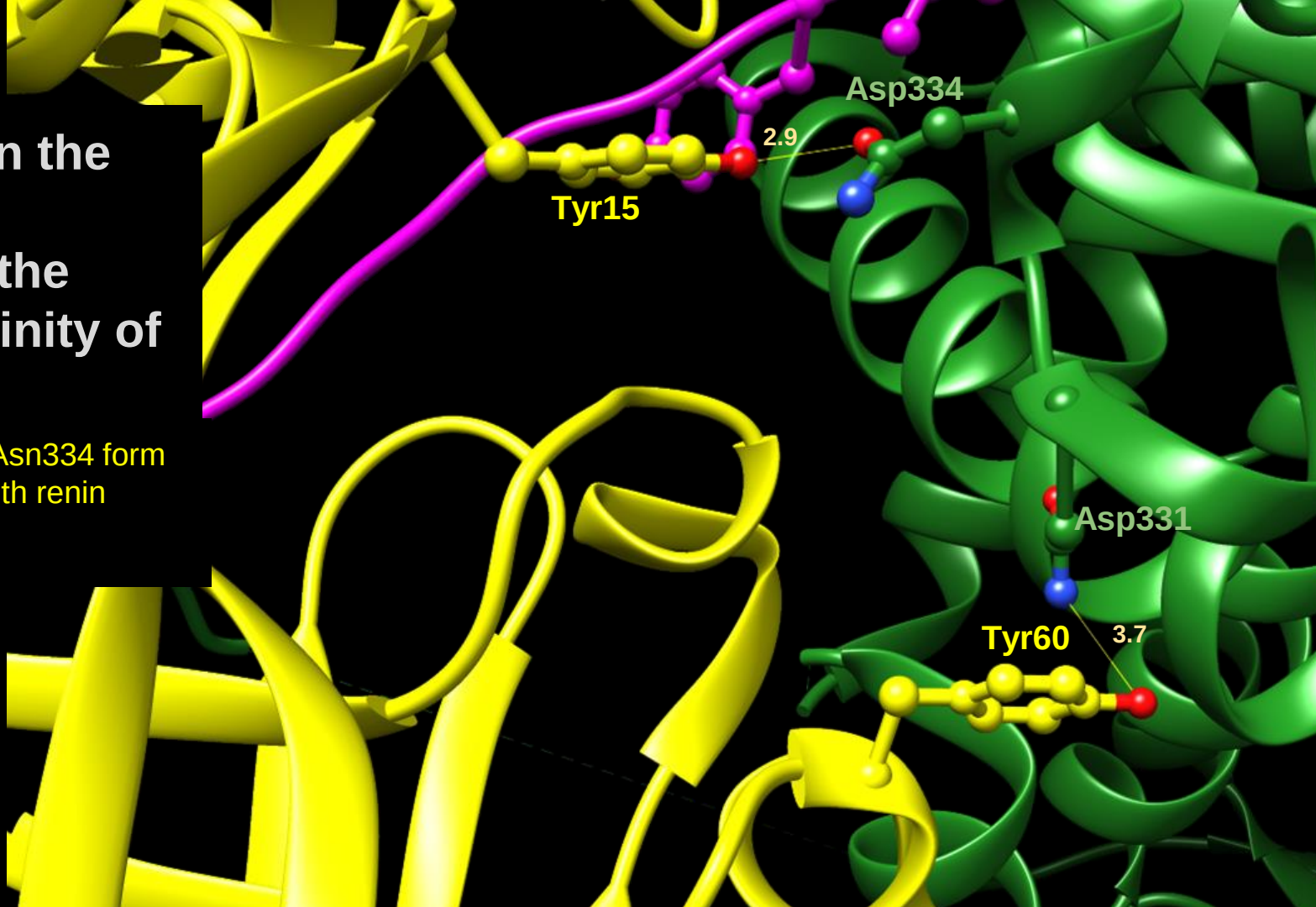
Pro306, Pro307, Pro308 and Pro311

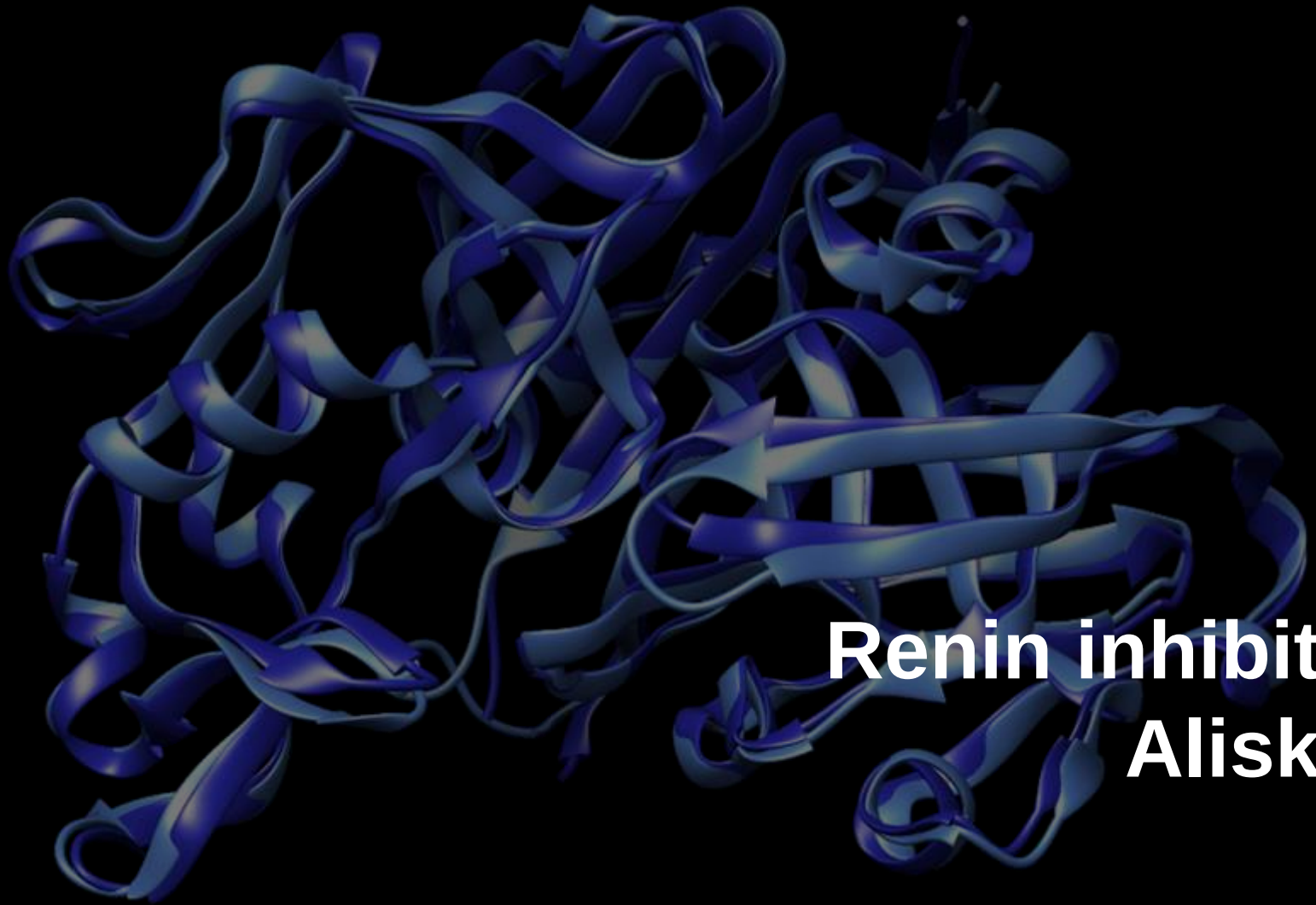


Flap: residues 81-88
Rigid structure: residues 252-260
Polyproline-loop: residues 306-308, 311
Angiotensin I

Residues in the interface determine the binding affinity of renin

Both Asn331 and Asn334 form hydrogen bonds with renin (Tyr15 and Tyr60)





Renin inhibition: Aliskiren

Aliskiren

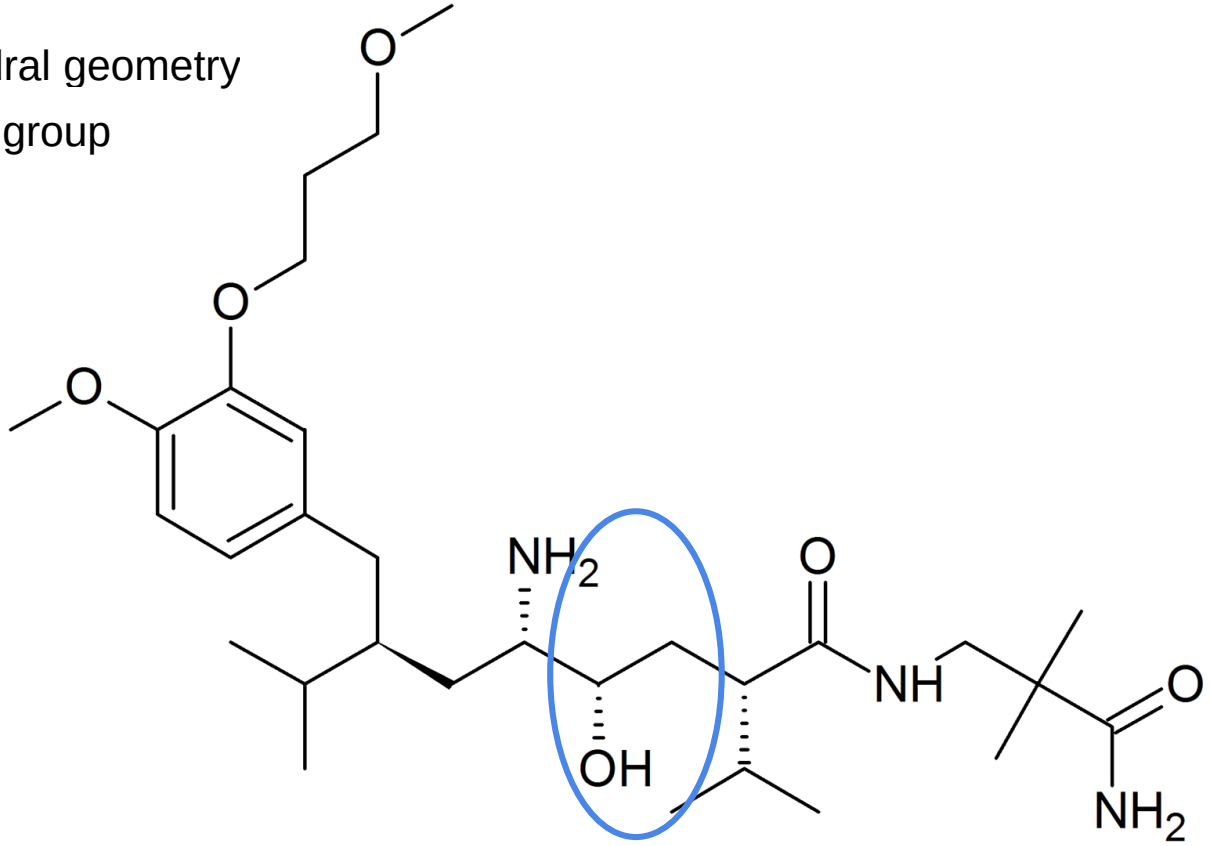
- Non-peptide inhibitor
- First orally active renin inhibitor approved by FDA in 2007
- Good pharmacological profile:
  - Metabolically stable and half-life of >40h
  - No adverse interactions with a range of drugs that are likely to be co-prescribed

- Stability and efficacy due to:
  - Mimics the transition state tetramer
  - Hydrophobic interactions with renin subsites and hydrogen bonding
  - Only one peptide bond

Transition state analogue

✓ Tetrahedral geometry

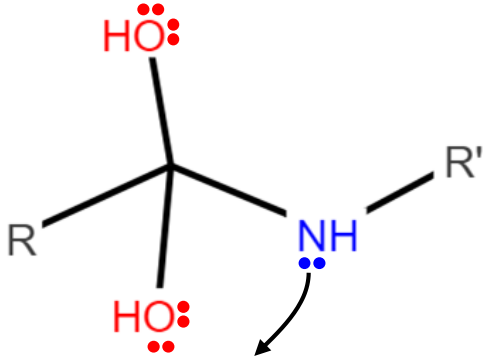
✗ Leaving group



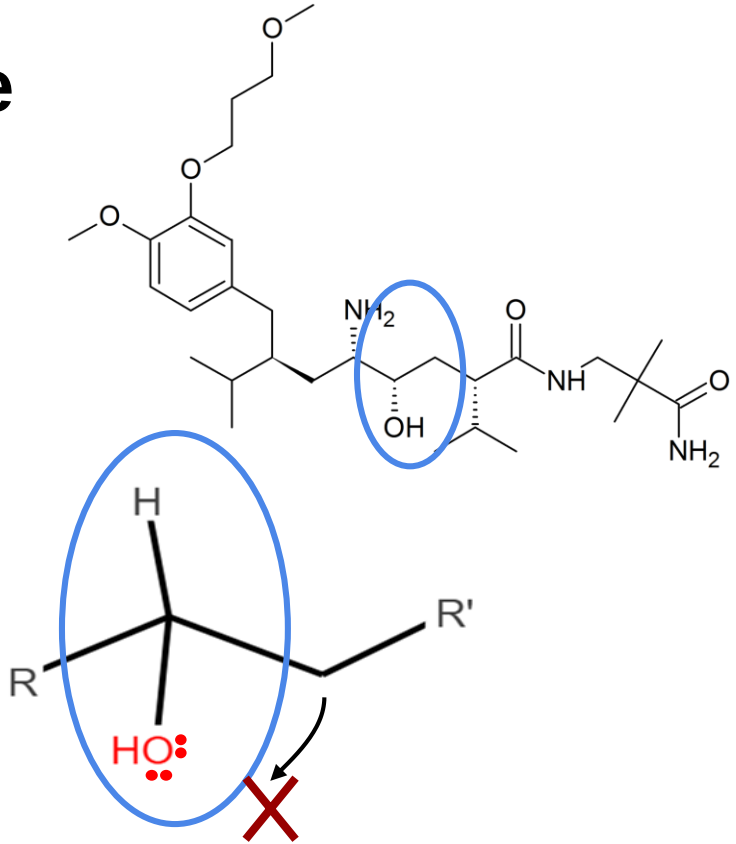
Transition state analogue

✓ Tetrahedral geometry

✗ Leaving group



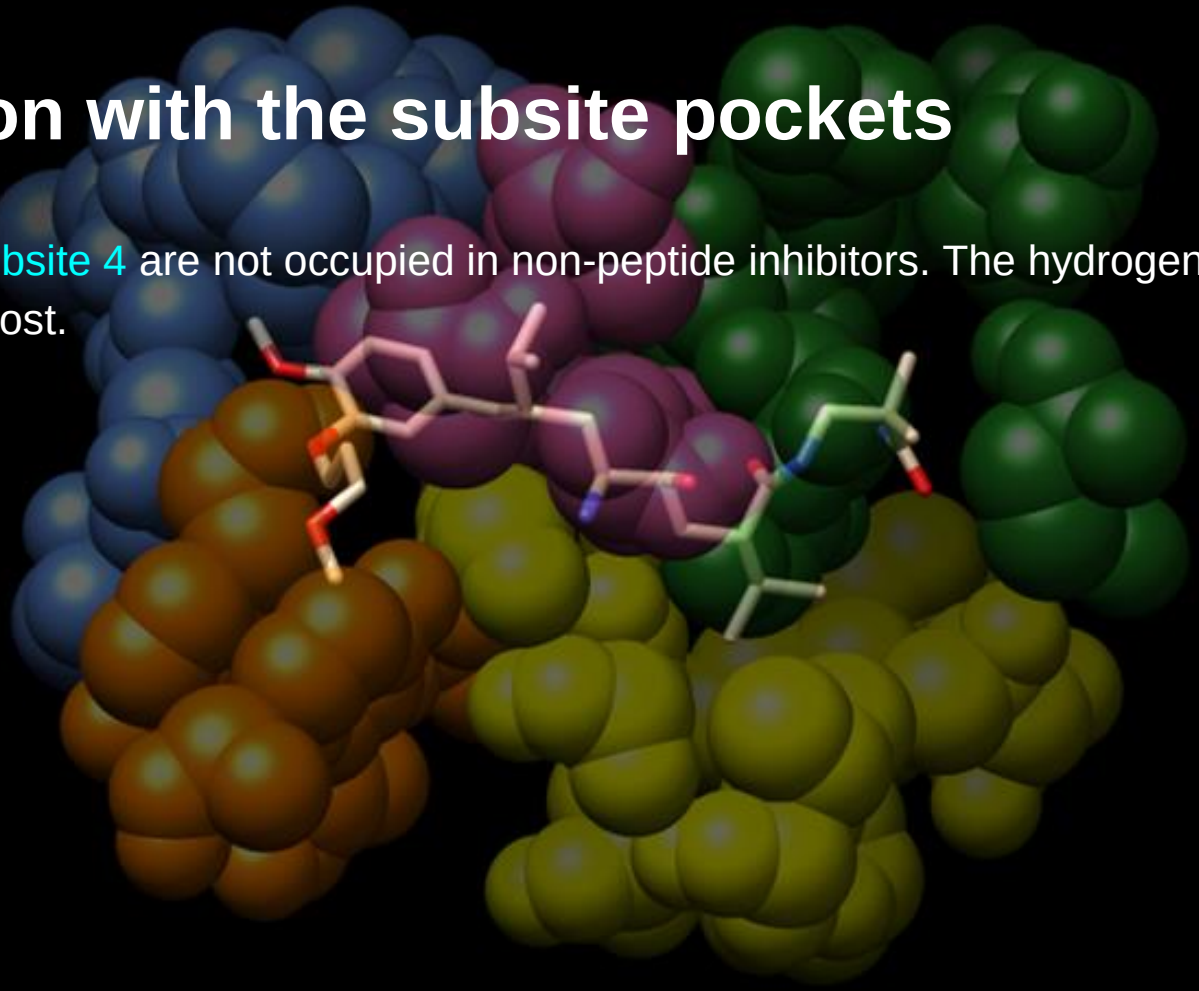
AGT transition state



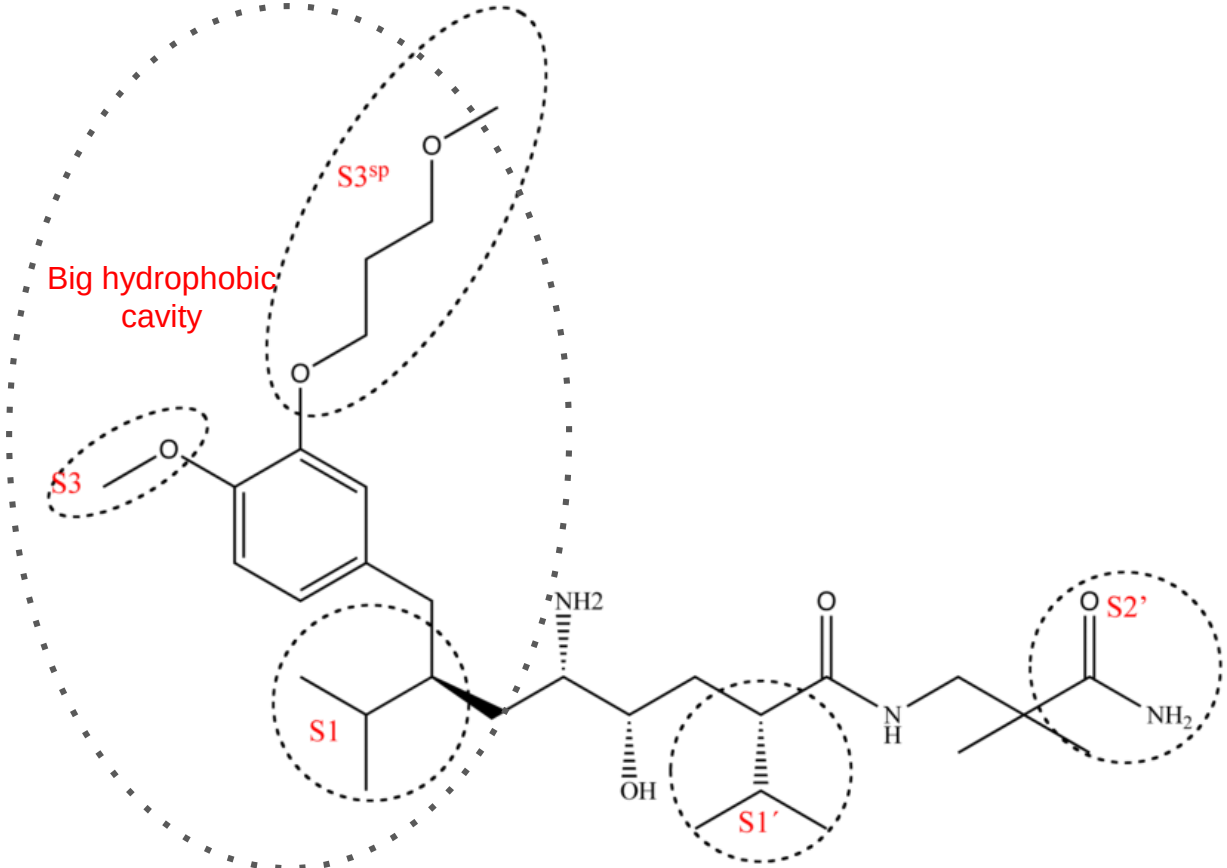
Transition state inhibitor

Interaction with the subsite pockets

Subsite 2 and subsite 4 are not occupied in non-peptide inhibitors. The hydrogen network present there is lost.



Interaction with the subsite pockets

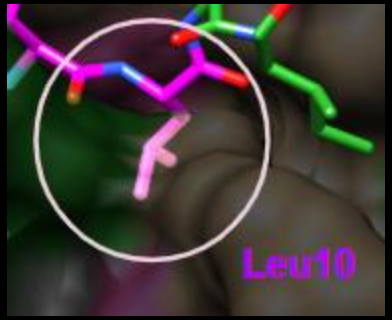


S1

ALISKIREN

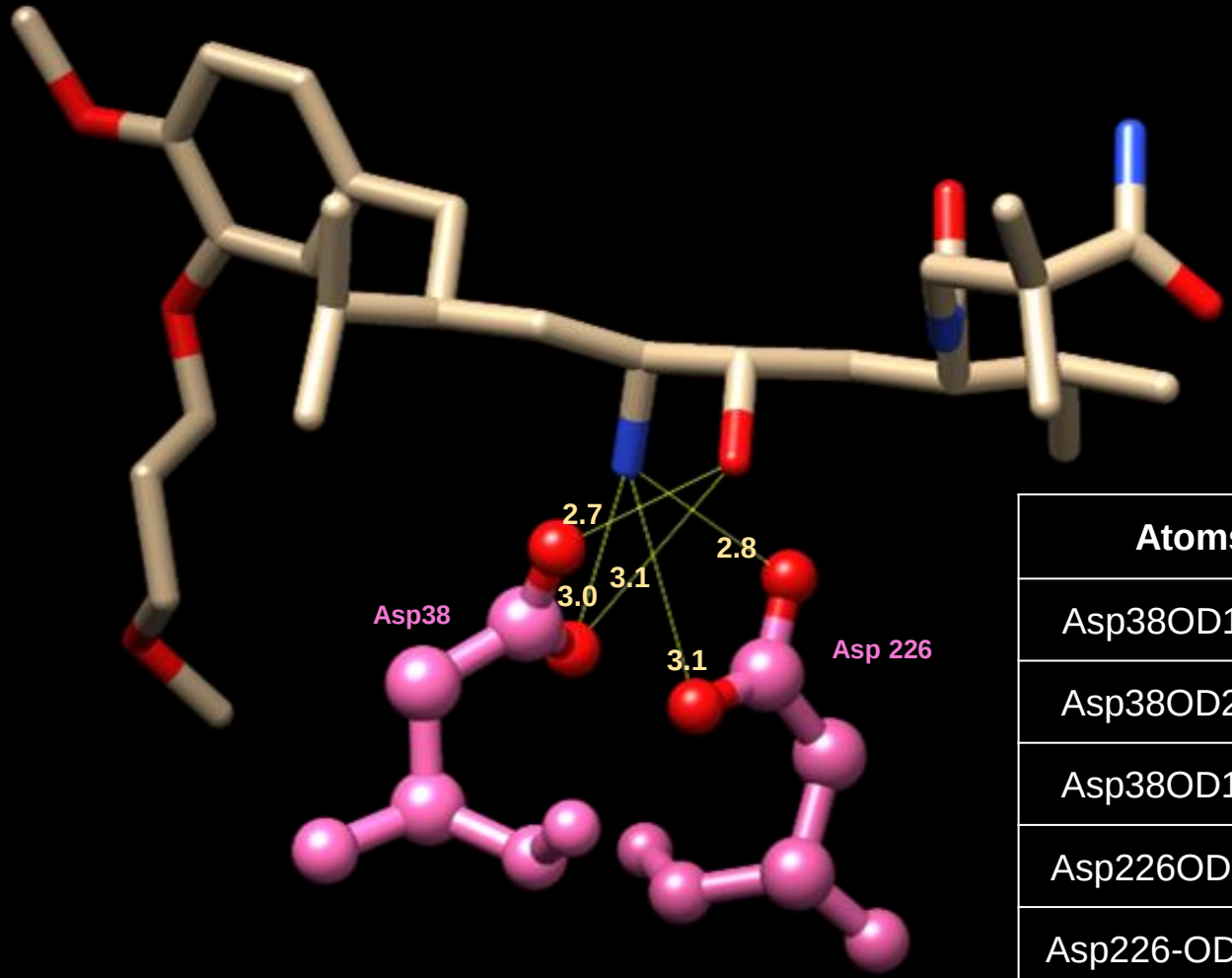
S1 is occupied by an hydrophobic structure of P1, an isopropyl moiety

It mimics leucine 10 in AGT



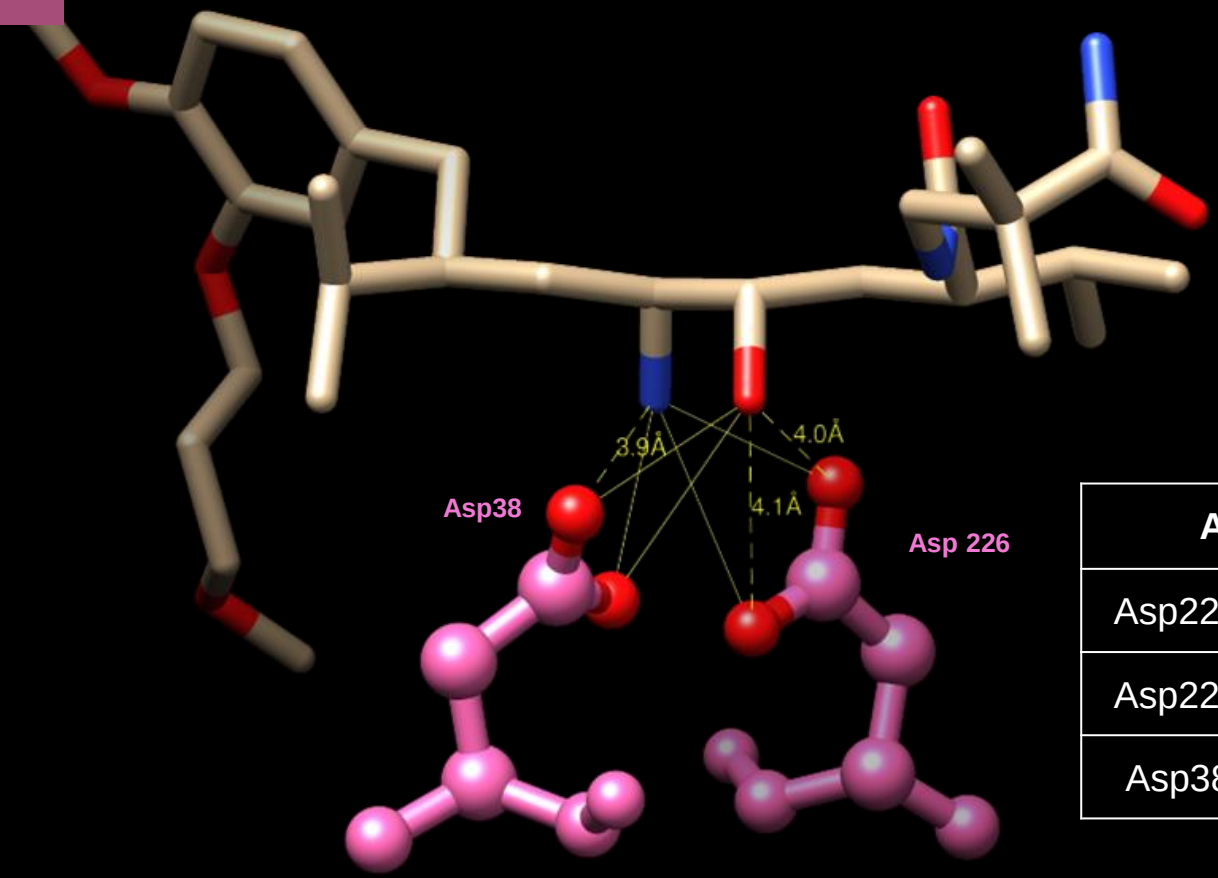
The ring linker and the C-terminal were removed to be able to see the isopropyl moiety

S1



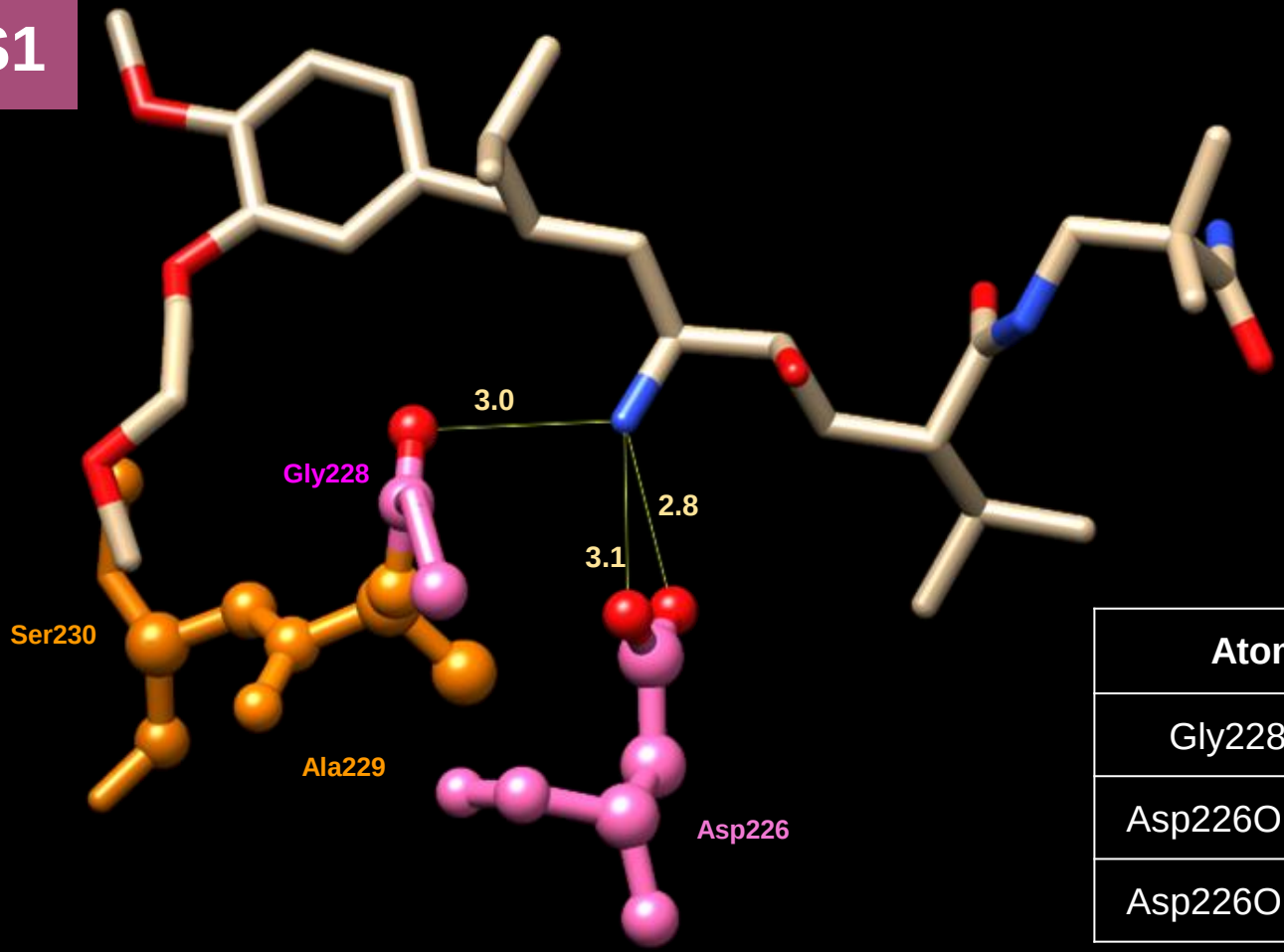
Atoms	Distances (Å)
Asp38OD1-N22	3.0
Asp38OD2-O24	2.7
Asp38OD1-O24	3.1
Asp226OD1-O24	2.8
Asp226-OD2-N22	3.1

S1



Atoms	Distances (Å)
Asp226OD1-O24	4,0
Asp226OD2-O24	4,1
Asp38OD2-N22	3.9

S1



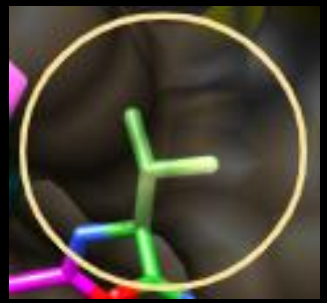
Atoms	Distances (Å)
Gly228-N22	3.0
Asp226OD2-N22	3.1
Asp226OD1-N22	2.8

S1’

ALISKIREN

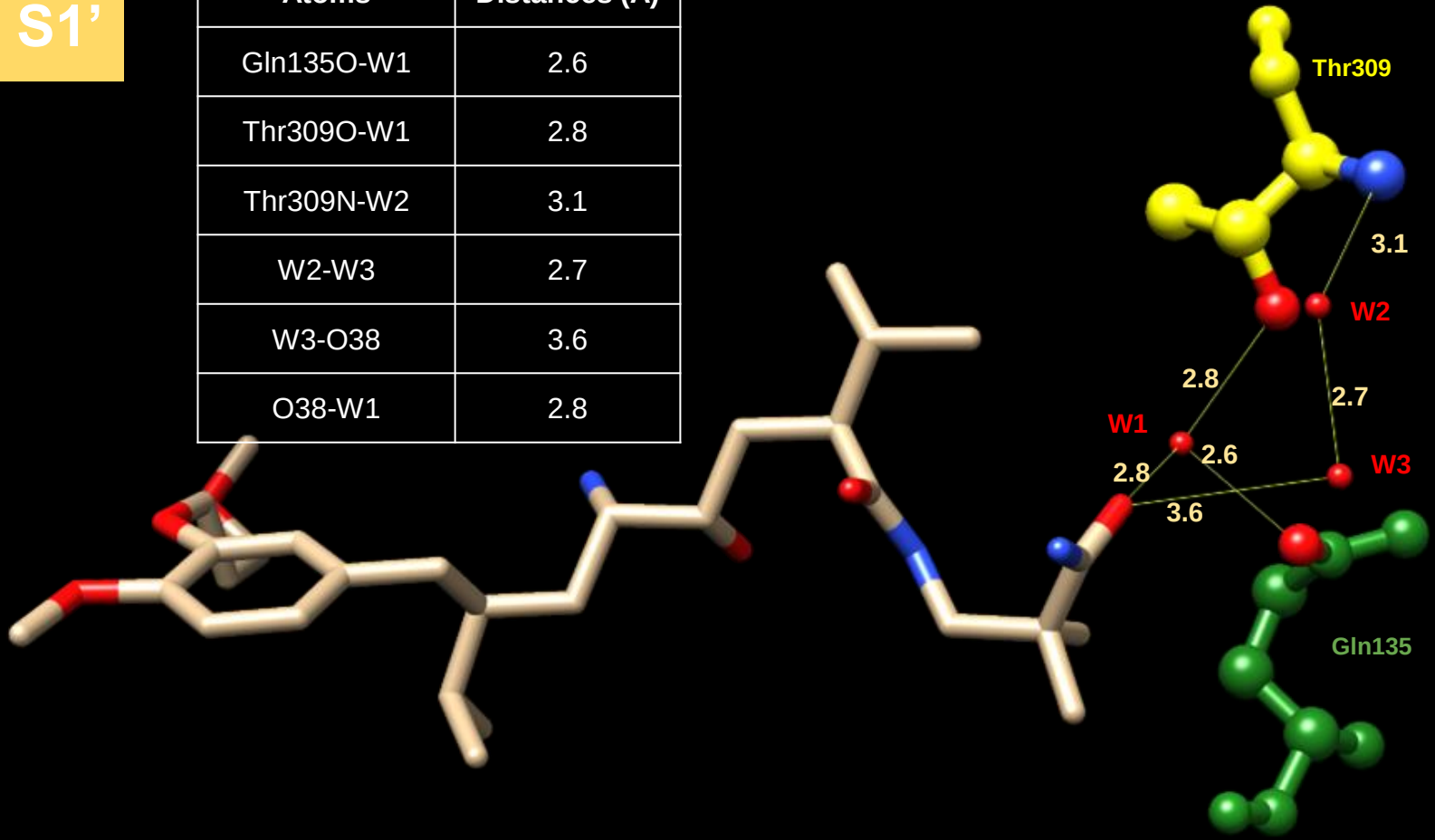
S1’ is partially filled with an isopropyl group

It mimics Val11 in AGT



S1’

Atoms	Distances (Å)
Gln135O-W1	2.6
Thr309O-W1	2.8
Thr309N-W2	3.1
W2-W3	2.7
W3-O38	3.6
O38-W1	2.8

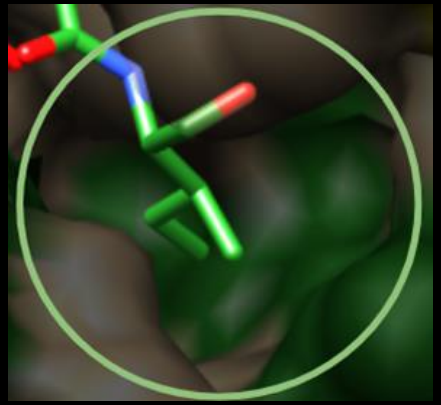


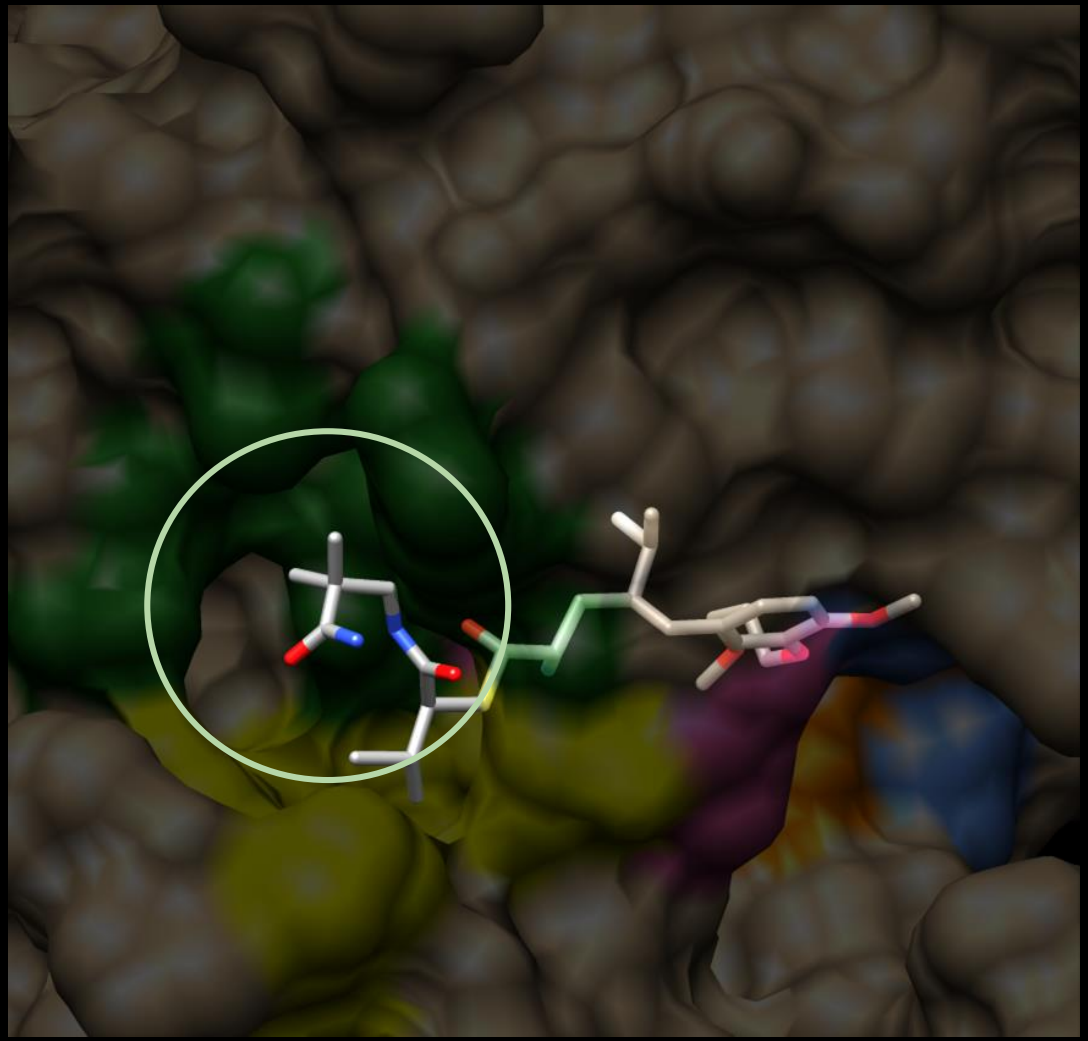
S2’

ALISKIREN

S2’ has some hydrophobic and hydrophilic residues, hiding the carboxamide and the dimetil moieties

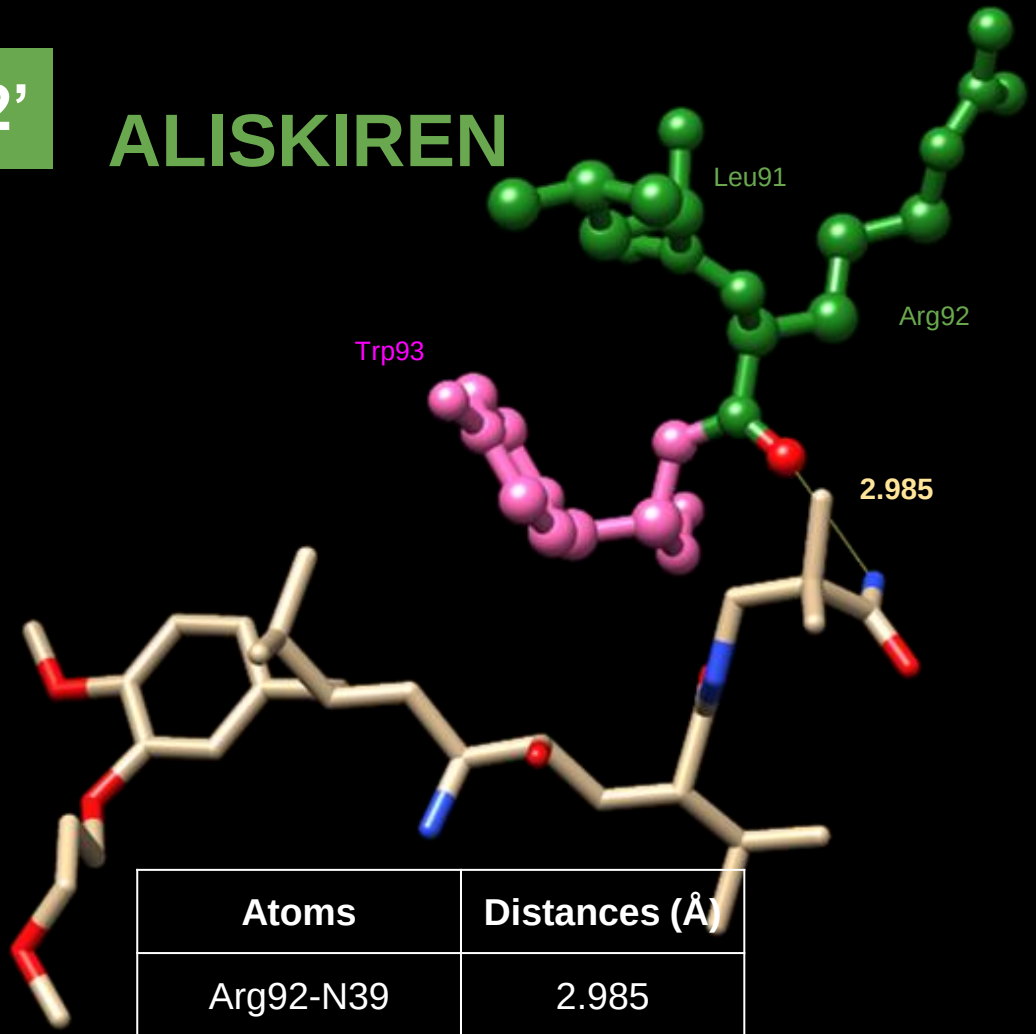
The carboxamide moiety mimics isoleucine 12 in AGT



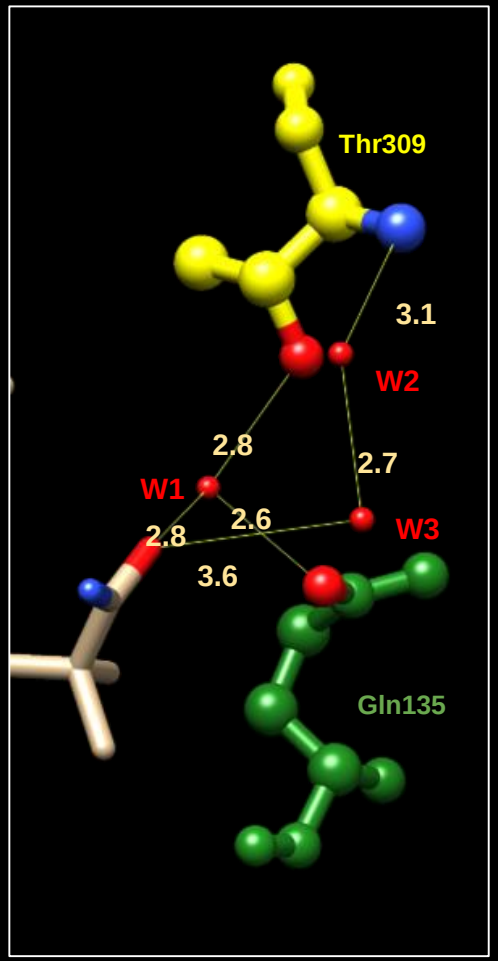


S2’

ALISKIREN

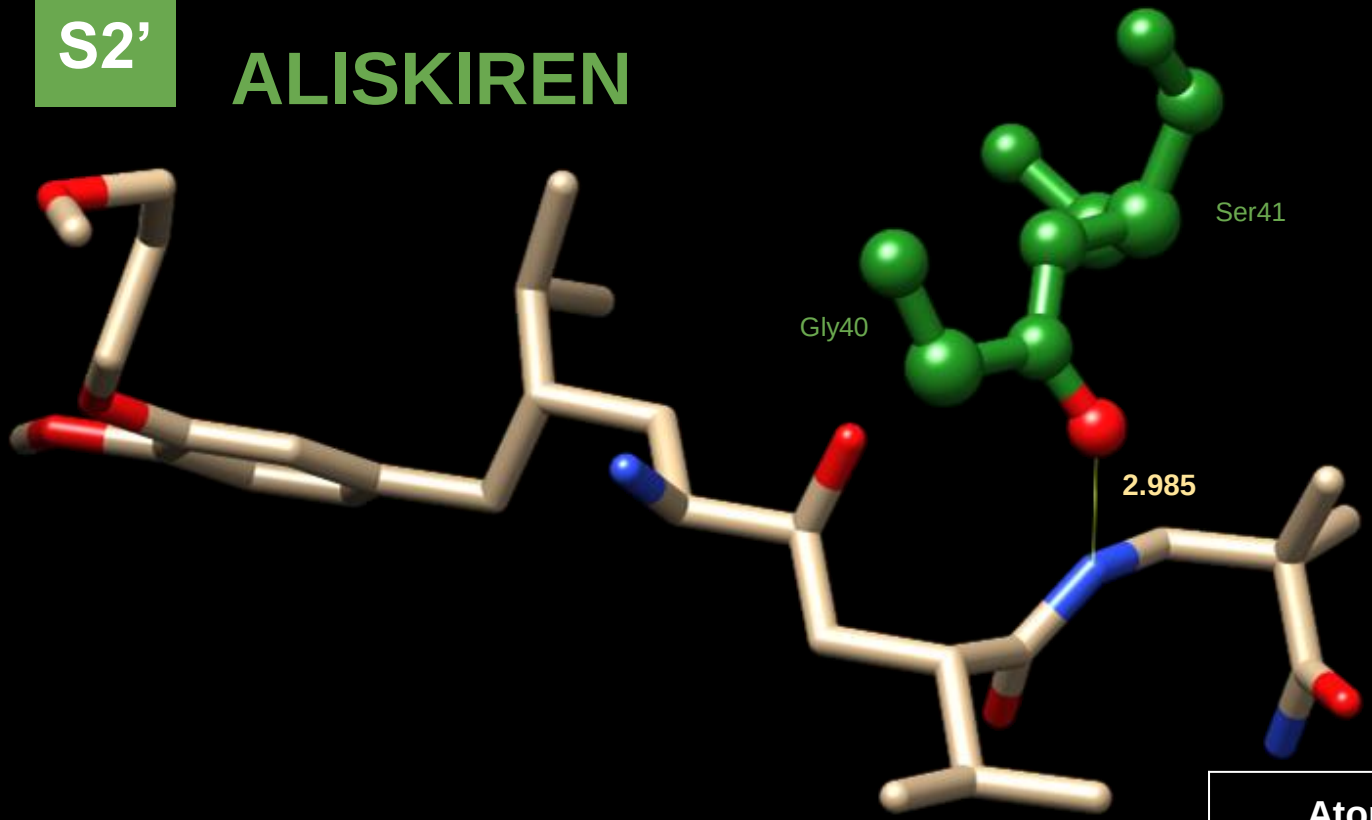


Atoms	Distances (Å)
Arg92-N39	2.985



S2’

ALISKIREN

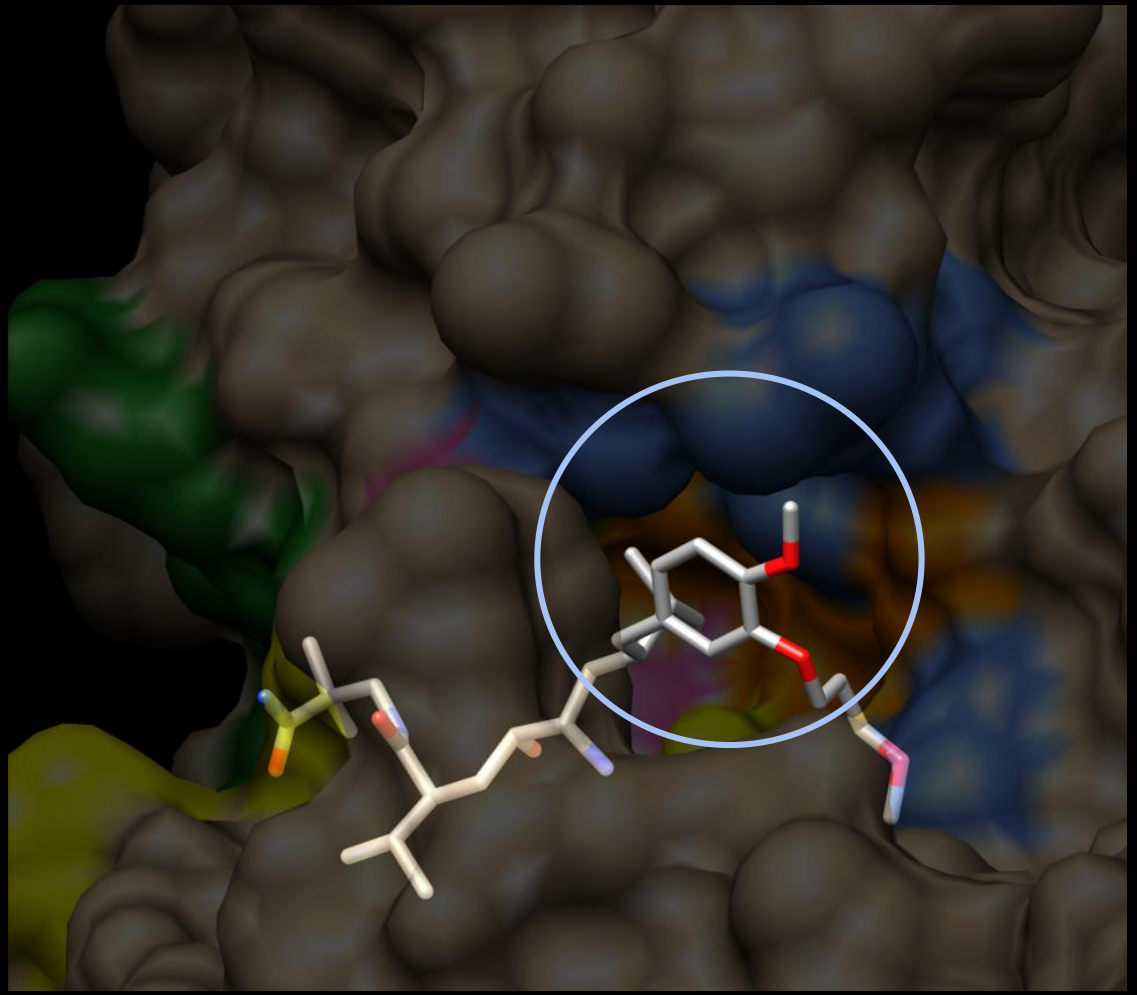


Atoms	Distances (Å)
Gly40-N32	2.985

S3 ALISKIREN

S3 has preference for hydrophobic aromatic residues. However, polar methoxy also sits in this binding site.

The phenyl ring linker between S1 and S3 mimics Phe 8

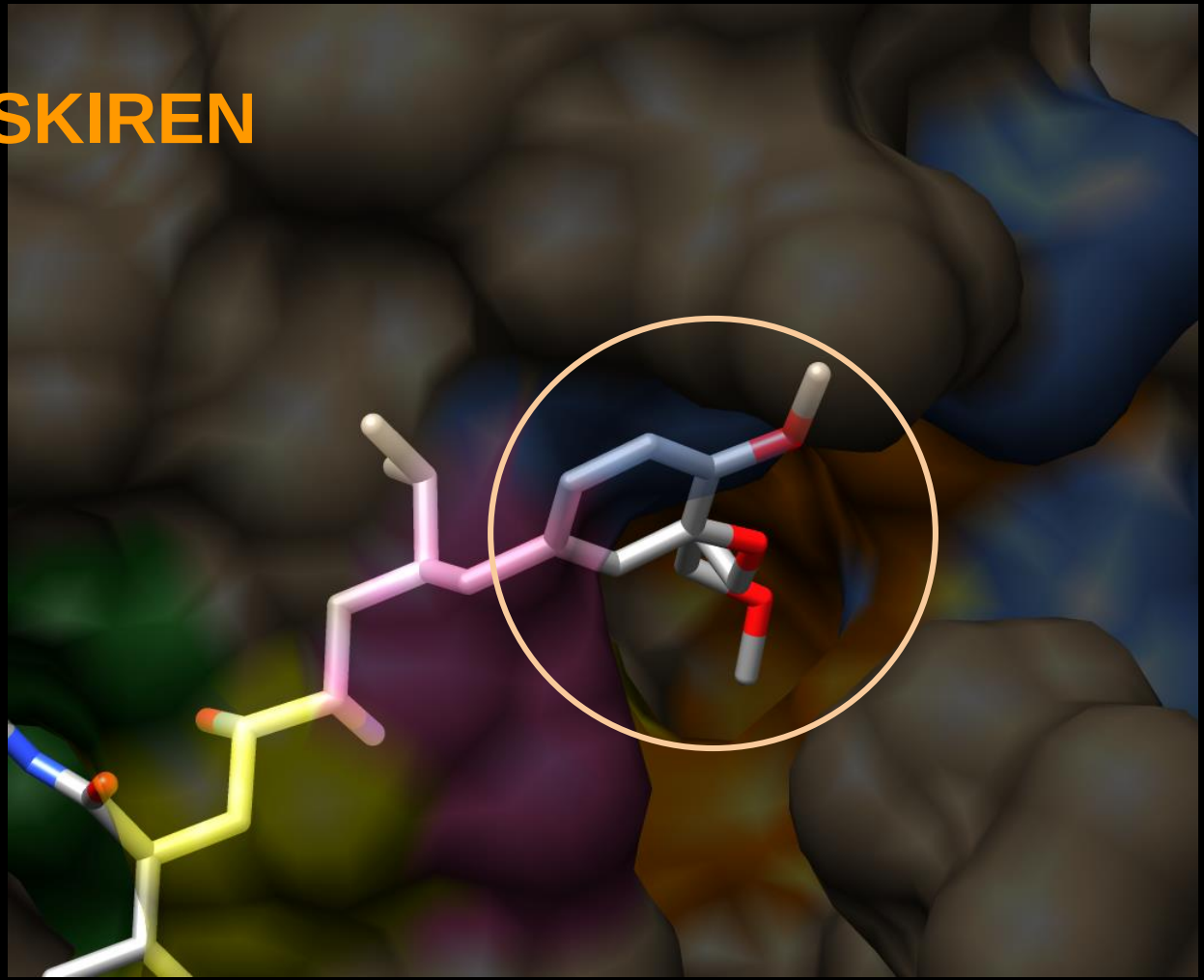


S3sp

ALISKIREN

The subpocket cannot be reached by AGT nor peptide inhibitors

Exploiting it can make the inhibitor very stable

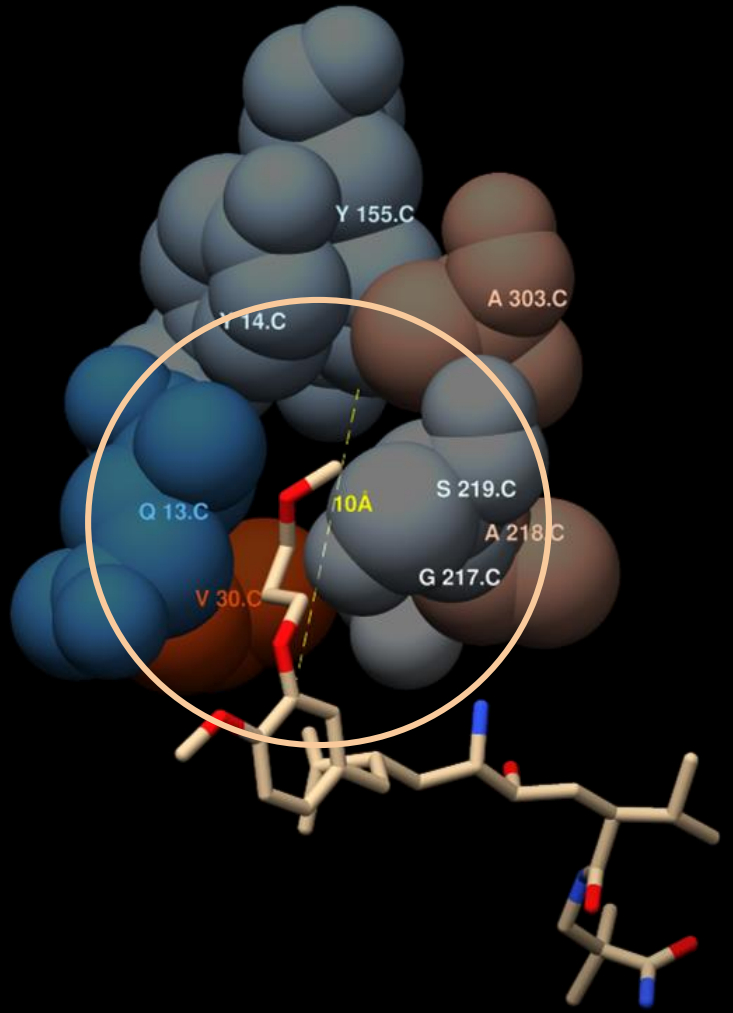


S3sp

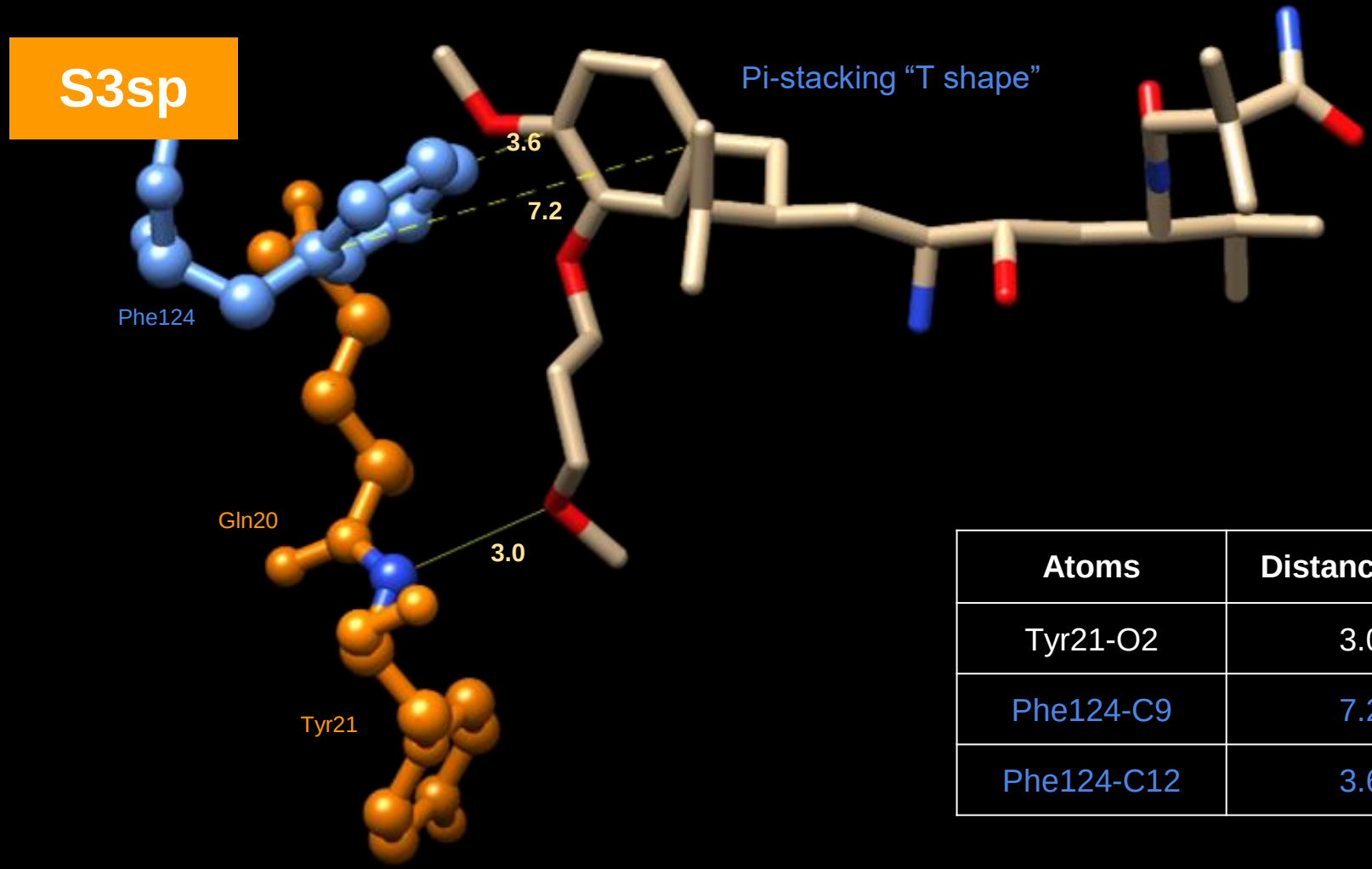
ALISKIREN

The subpocket cannot be reached by AGT nor peptide inhibitors

Exploiting it can make the inhibitor very stable

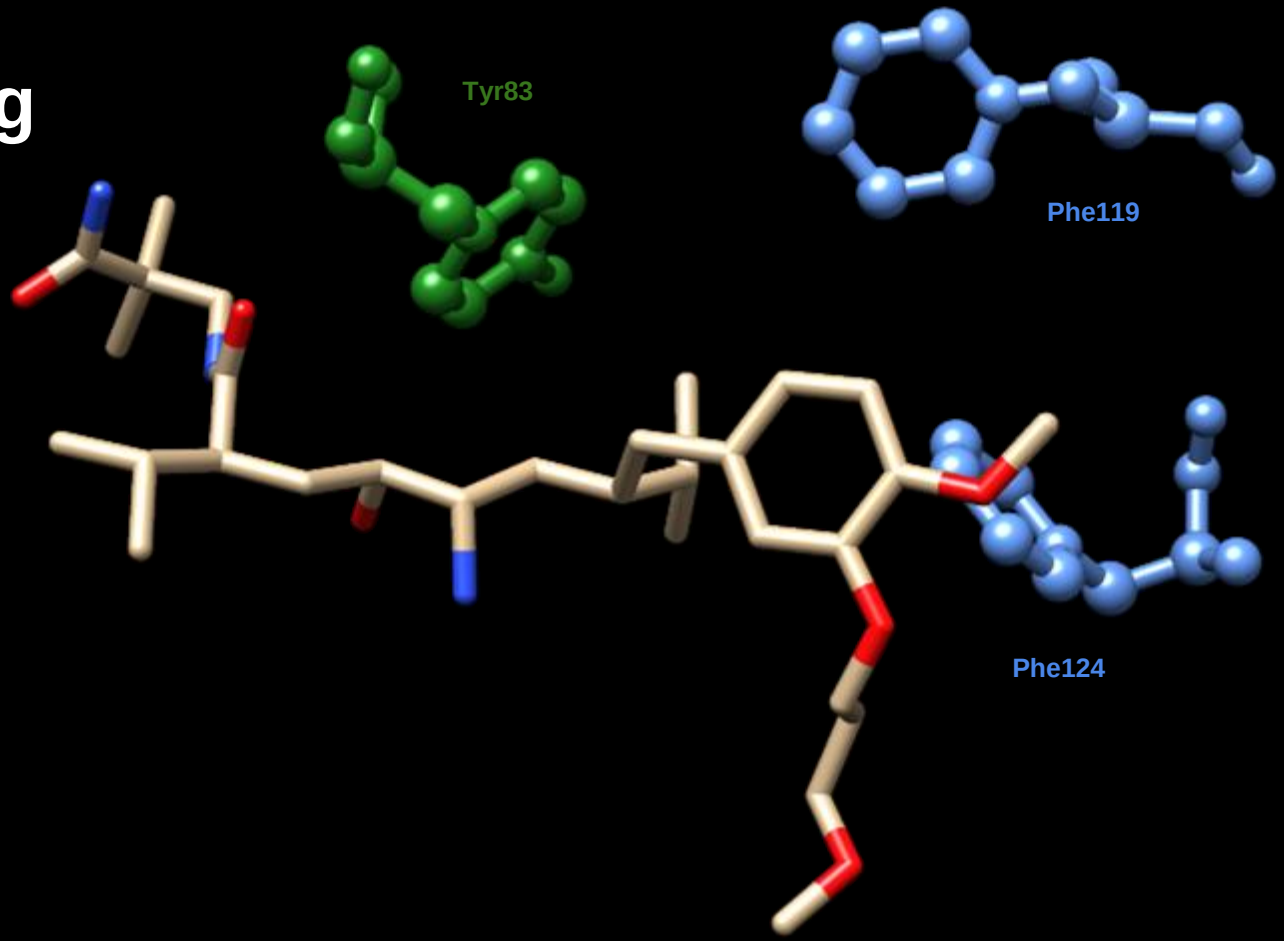


S3sp



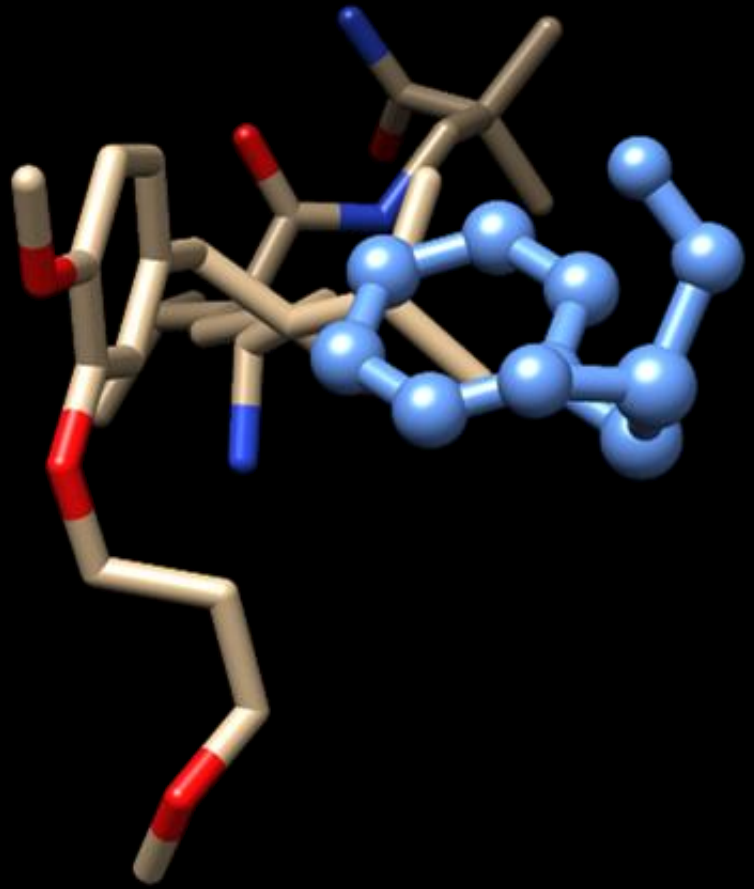
Atoms	Distances (Å)
Tyr21-O2	3.0
Phe124-C9	7.2
Phe124-C12	3.6

Pi stacking



Pi stacking

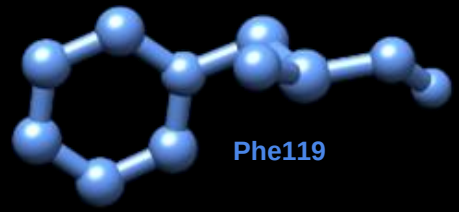
T-shape pi stacking

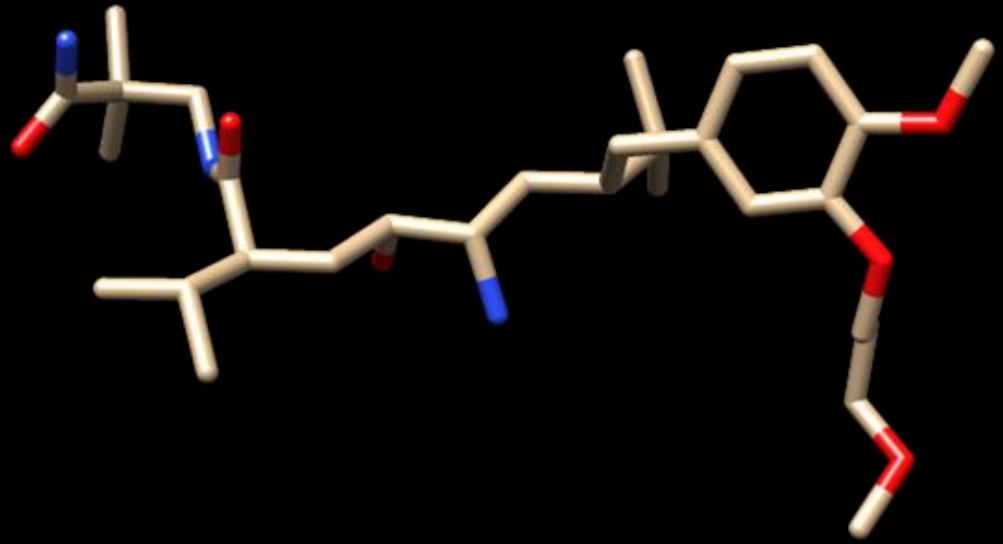


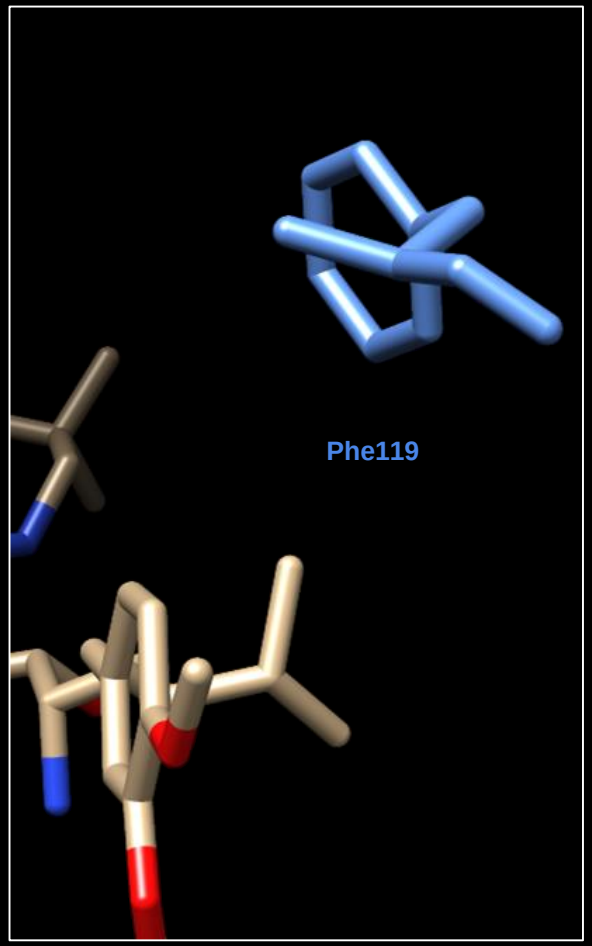
Phe124

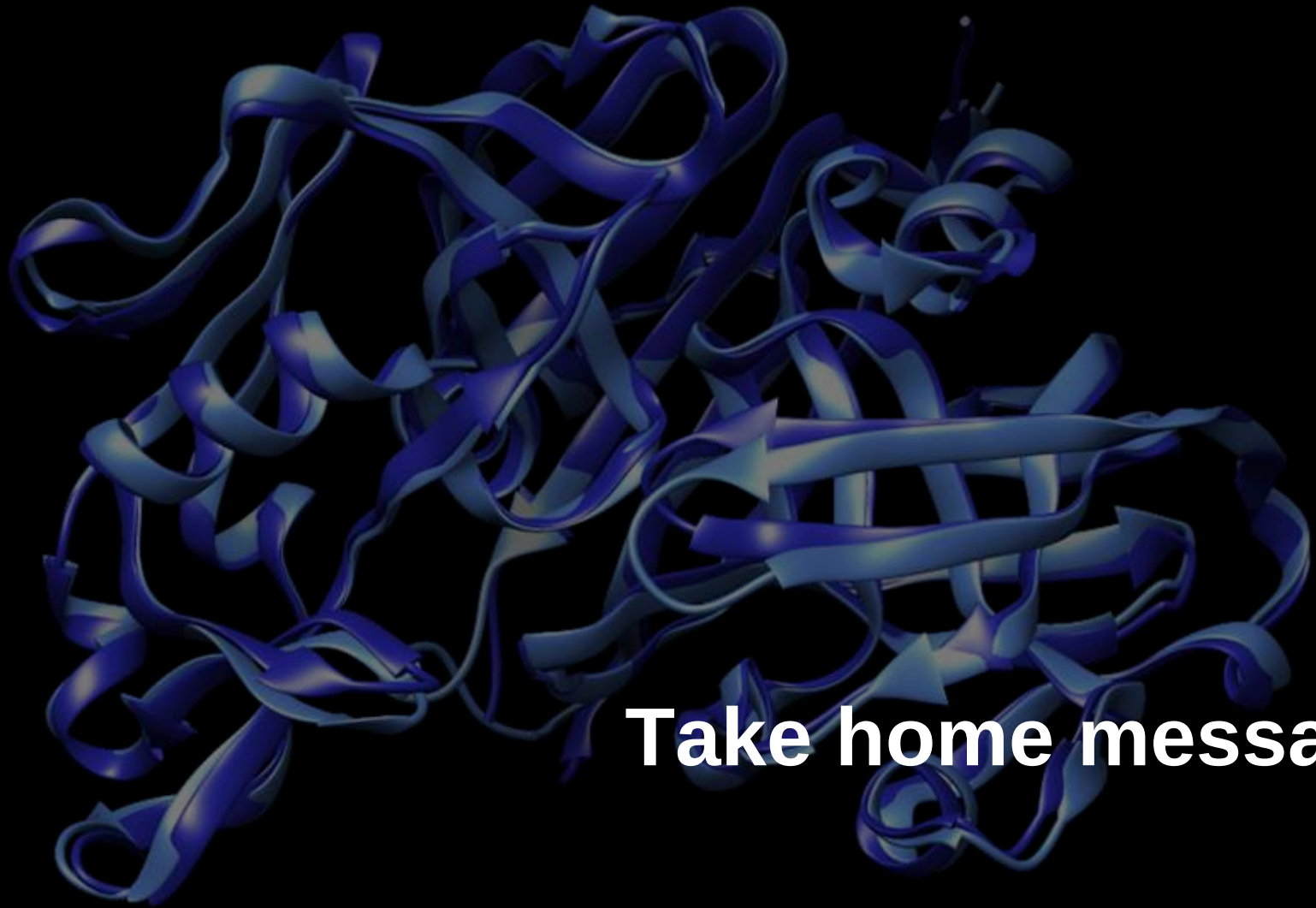
Pi stacking

Displaced
pi stacking









Take home messages

Take home messages

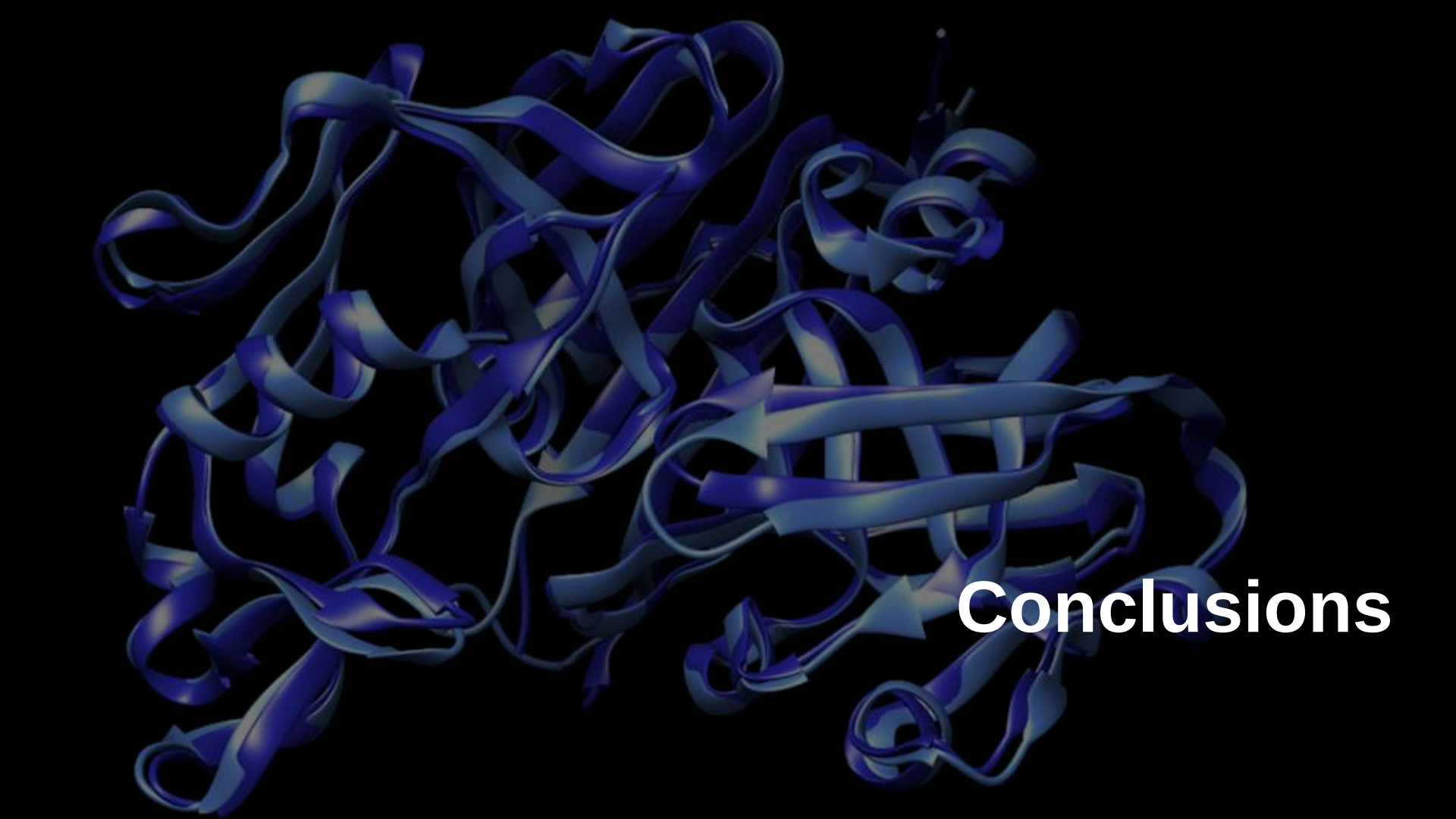
- Aspartic peptidases have two aspartic acids on its active site (which is the junction of two similar domains) that are directly responsible for the catalytic activity of the protein. A water molecule has an essential role in the catalytic reaction.

- Renin is an aspartic peptidase which cleaves AGT between Leu10 and Val11 releasing the N-terminal angiotensin I peptide.

- The hydrogen bond network located in the active site is the setting where it takes place the catalytic reaction.

Take home messages

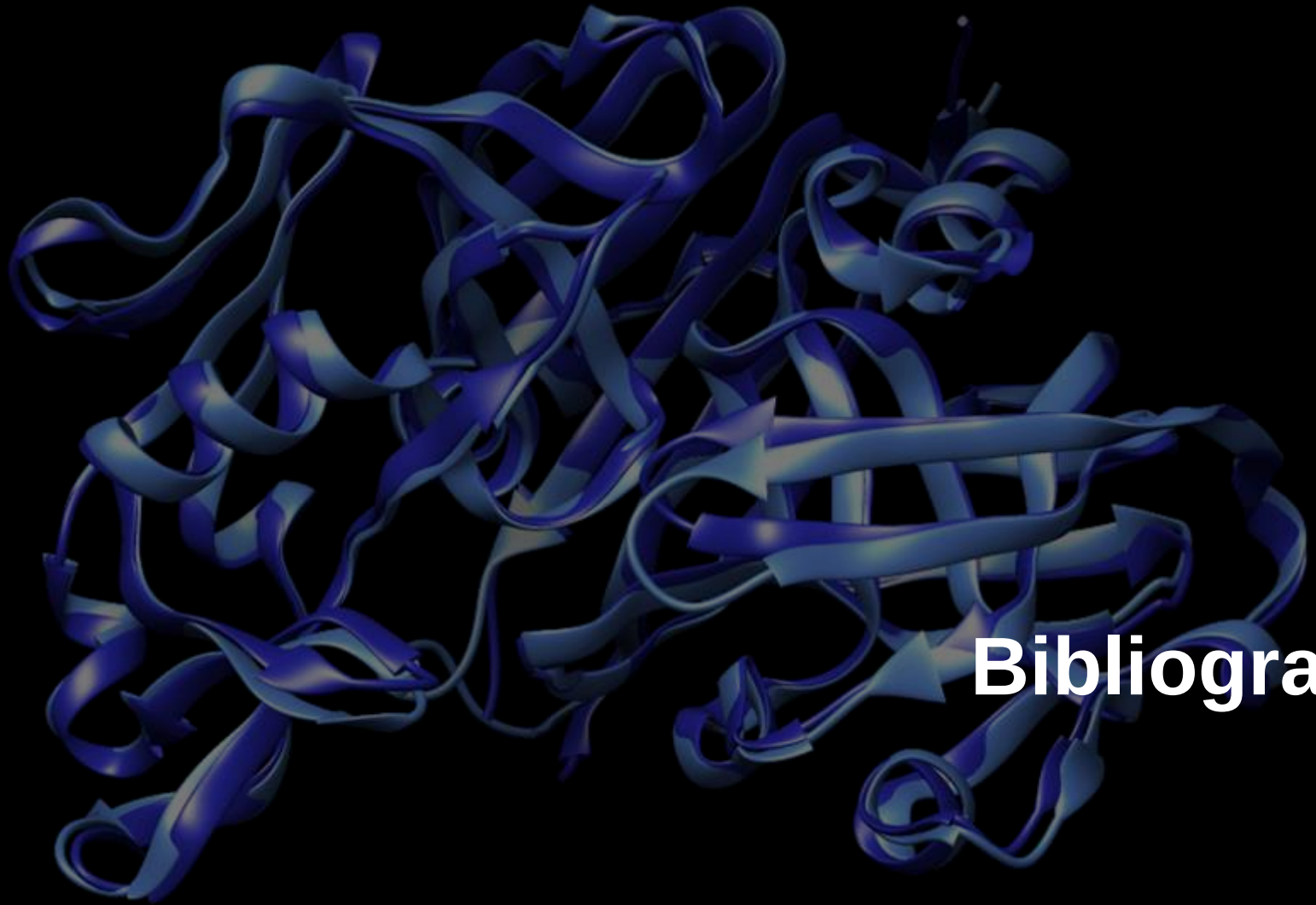
- AGT has to undergo conformational changes in order to expose the N-terminal tail allowing renin to cleave it.
- Insertion of the N-terminus into the hormone binding pocket is needed to stabilize the complex.
- Aliskiren non-peptide inhibitor of renin.
- It is a transition-state analogue because it mimics the transition state of the catalytic reaction but has no leaving group in order to proceed with it.



Conclusions

Conclusions

- Renin plays an essential role in the control of blood pressure.
- People taking ACE-inhibitors can overexpress renin as a compensation mechanism, which makes it important to find renin-inhibitors.
- The understanding of the protein structure and its interactions with angiotensinogen is essential to develop new inhibitors.
- An alternative potential target would be to block the angiotensinogen hormone-binding pocket.



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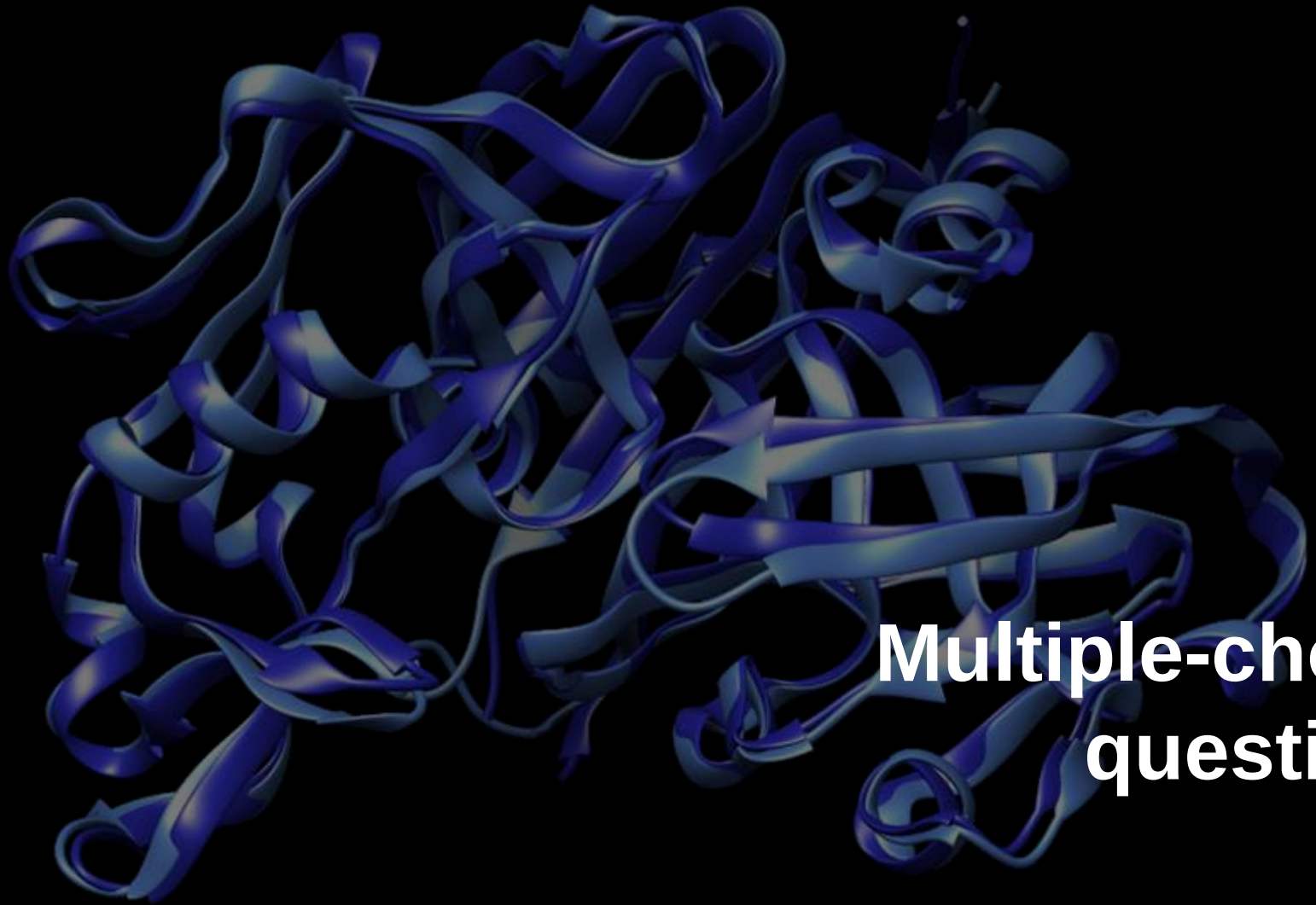

Multiple-choice questions

MULTIPLE CHOICE QUESTIONS

1. **The reasons why aliskiren is very stable and effective in inhibiting renin are:**
    a. It is a non-peptide inhibitor, which means that it is more difficult for proteases to break it than peptide inhibitors.
    b. It is a transition state analogue.
    c. a and b are correct.
    d. The multiple hydrogen bonds with renin residues and the binding to renin specificity subsites makes it more stable.
    e. a, b and d are correct.
2. **In reference to aliskiren:**
    a. It is a non-peptide inhibitor
    b. It is a peptide inhibitor
    c. a and b are correct
    d. Its function is inhibiting the angiotensinogen
    e. a, b and d are correct

MULTIPLE CHOICE QUESTIONS

1. **In reference to the activity of aspartic proteases in an acidic environment:**
   a. The presence of a specific Thr in Aspartic proteases conferes them this activity.
   b. Renin does not cleave in an acidic environment.
   c. a and b are correct.
   d. Renin is the only Aspartic protease that can cleave in an acidic environment and a neutral environment.
   e. a, b and d are correct.
2. **About the active site of renin, which statement is correct?**
   a. There is an important salt bridge network that is conserved in all renins.
   b. The presence of the Fireman's grip is characteristic not only in renin but also in other aspartic proteases.
   c. a and b are correct.
   d. It is composed of three Aspartic acids that play an important role in the catalytic reaction.
   e. a, b and d are correct.
3. **The cleavage of Angiotensinogen by renin ...**
   a. Is highly specific and can be explained by different reasons.
   b. Is performed between the positions 10 and 11 of AGT.
   c. a and b are correct
   d. Releases the N-terminal part of Angiotensinogen, which is known as Angiotensin I and has its physiological effects in blood pressure.
   e. a, b and d are correct.

MULTIPLE CHOICE QUESTIONS

1. **The hormone-binding pocket of Angiotensinogen:**
    a. It plays an essential role in the stabilization of the complex AGT-renin.
    b. It can be a potential target to inhibit the formation of Angiotensin I.
    c. a and b are correct.
    d. Aliskiren is a hormone-binding pocket inhibitor.
    e. a, b and d are correct.
2. **Which residues are essential in the catalytic site of renin?**
    a. Two aspartic acids.
    b. A water molecule.
    c. a and b are correct.
    d. Only one aspartic acid and one water molecule.
    e. a, b and d are correct.
3. **How is renin classified in SCOP?**
    a. All alpha proteins.
    b. All beta proteins.
    c. Alpha and beta proteins (a/b).
    d. Alpha and beta proteins (a+b).
    e. Membrane and cell surface proteins.

MULTIPLE CHOICE QUESTIONS

1. **Which is/are some of the different theories that explain renin specificity for angiotensinogen?**

   1- The presence of a Histidine-Proline-Phenylalanine motif in AGT.

   2- There are important residues in renin AGT interface that are not highly conserved

   3- Poly-proline provides a way to construct well defined pockets that would otherwise be more flexible.

   4- This interaction is not highly specific, renin has many other substrates.

   a) 1, 2 and 3
   b) 1 and 3
   c) 2 and 4
   d) 4
   e) 1, 2, 3 and 4

2. **Regarding general characteristics of peptidases, which sentence is NOT correct?**

   a) Peptidases include endopeptidases, which perform their action in inner regions of the polypeptide chain, and exopeptidases, which act in both extremes.
   b) There is a 3 level-classification of peptidases: catalytic type, structure and individual peptidases.
   c) It is thought that a gene duplication process took place in aspartic peptidases.
   d) Aspartic proteases are synthetized as active enzymes.
   e) Peptidases are involved in many different process in the organism.


THANK YOU FOR YOUR
ATTENTION