International Symposium on Compound Design Technology

The significance of protein structure data set choices for *in-silico* drug discovery: Design of BACE1 inhibitors

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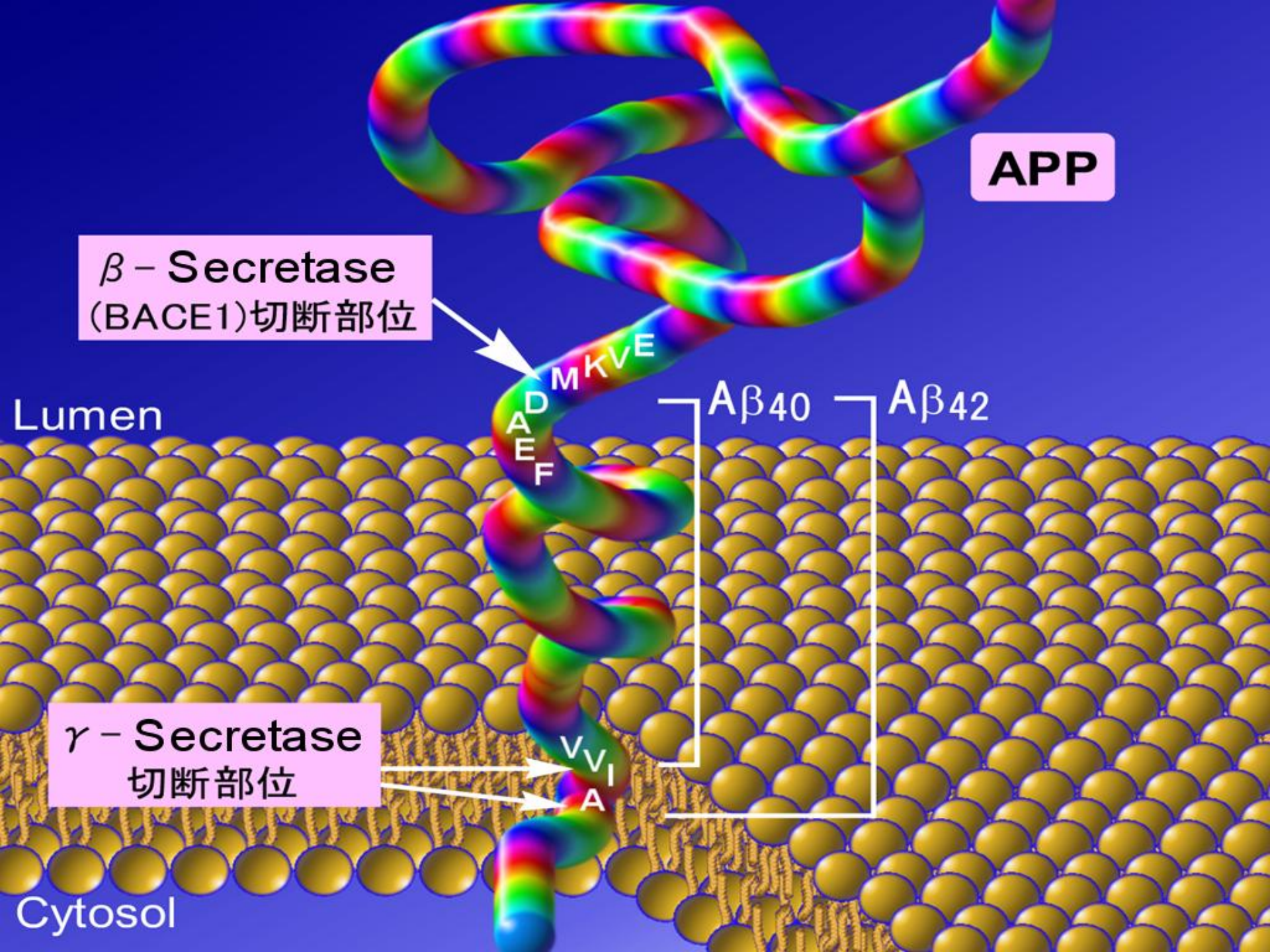
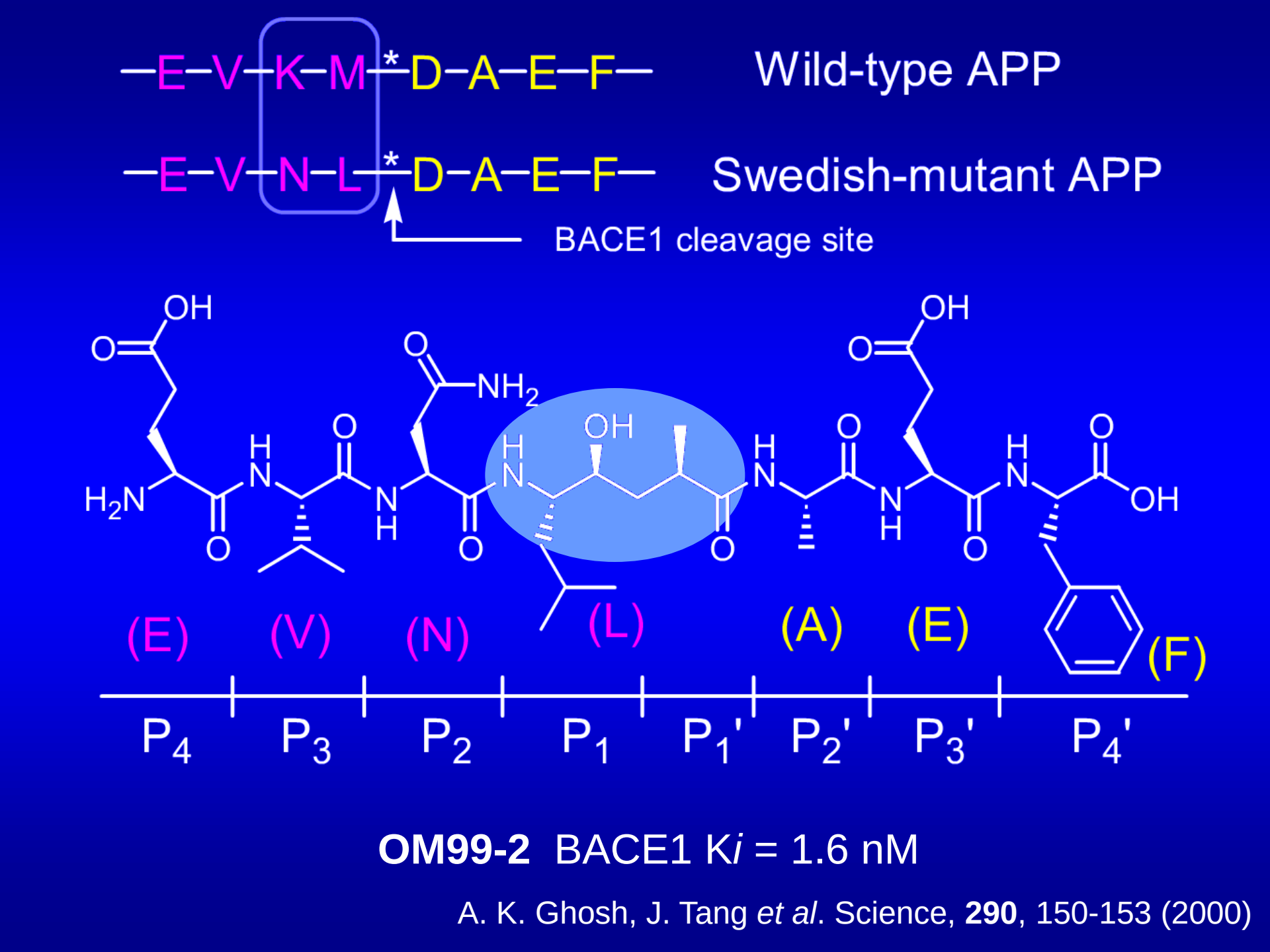
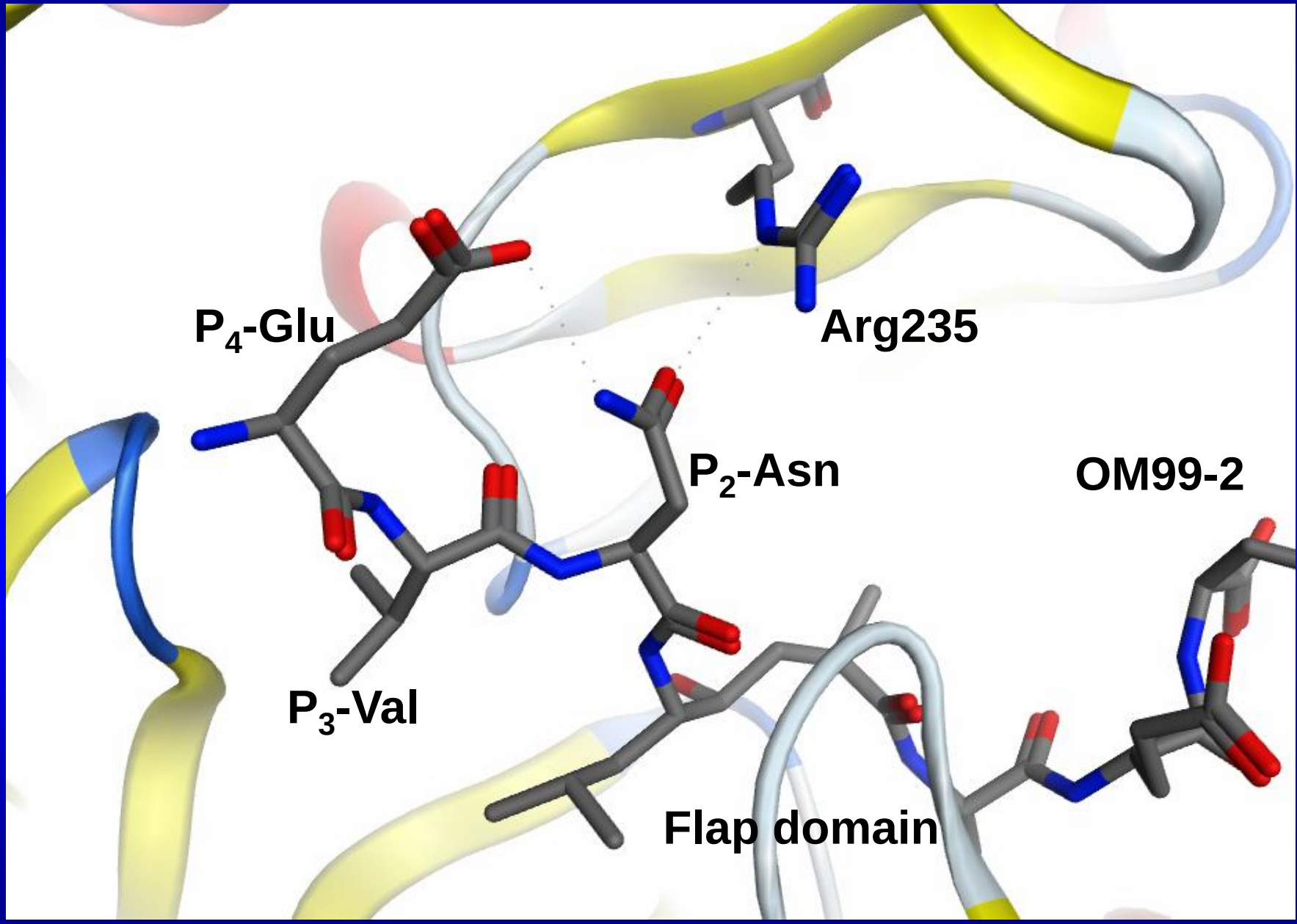

APP
β－Secretase
(BACE1)切断部位
M
K
V
E
D
A
E
F
Aβ40
Aβ42
γ－Secretase
切断部位
V
V
I
A
Lumen
Cytosol

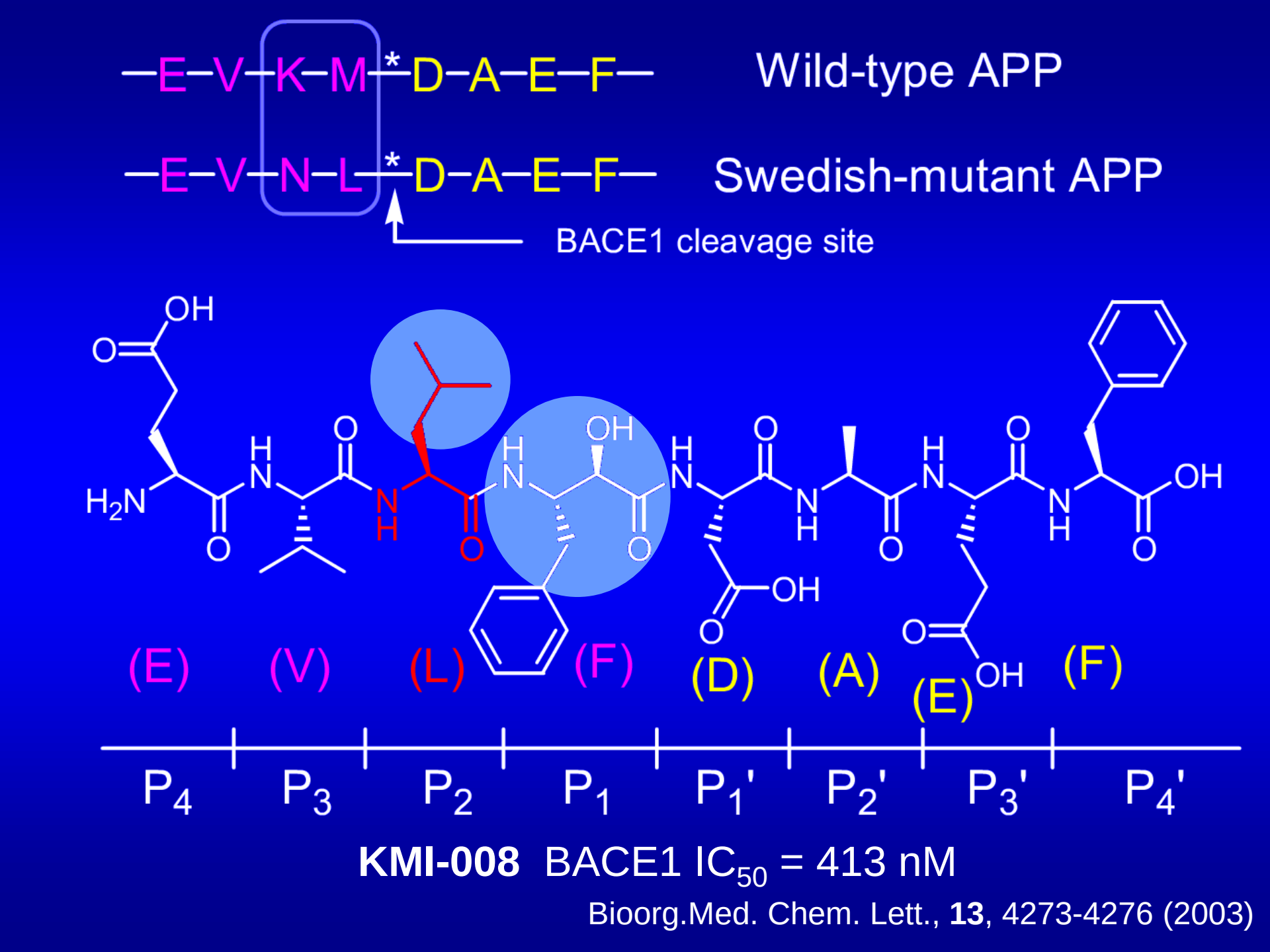


OM99-2 BACE1 K*i* = 1.6 nM

A. K. Ghosh, J. Tang *et al*. Science, **290**, 150-153 (2000)

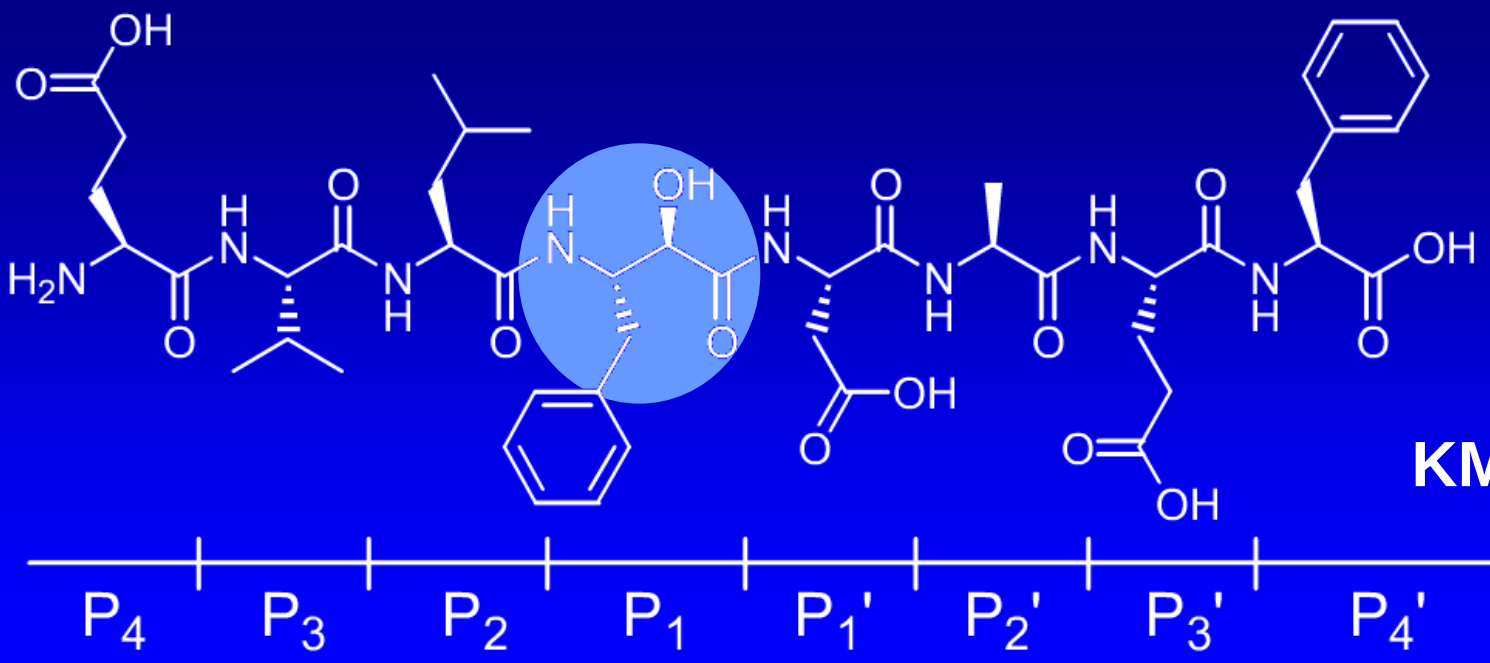
1FKN



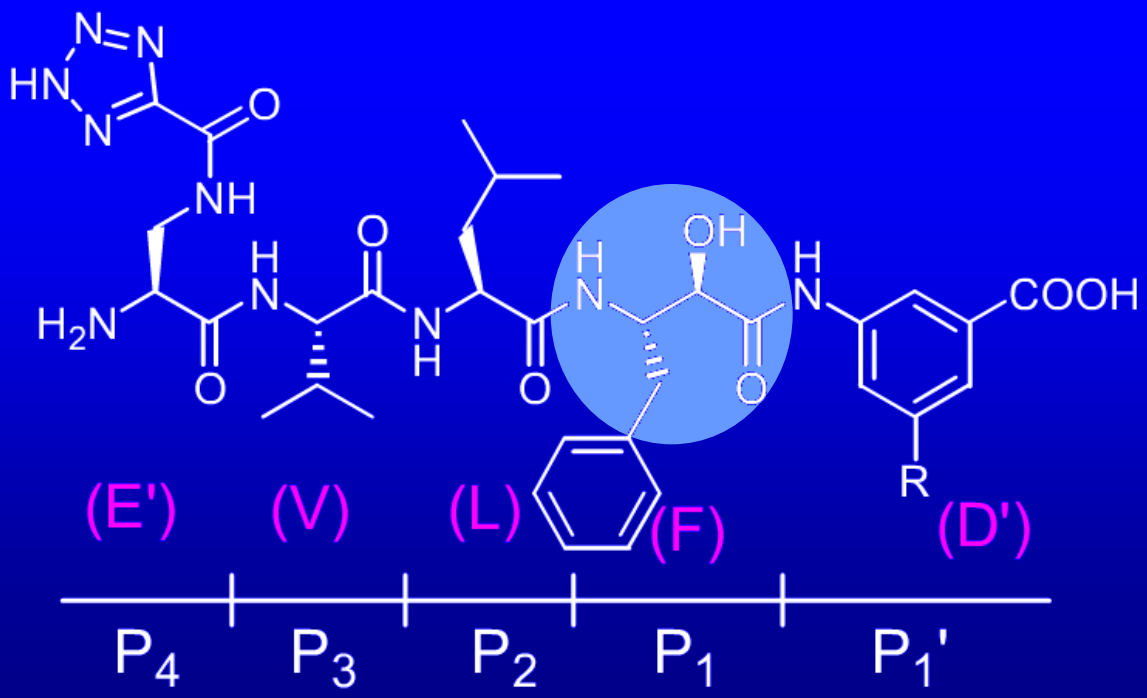


KMI-008 BACE1 IC_{50} = 413 nM

Bioorg.Med. Chem. Lett., **13**, 4273-4276 (2003)



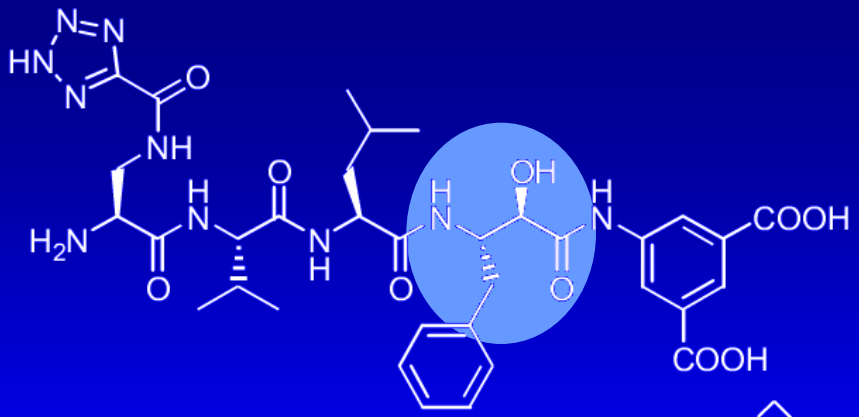
KMI-008



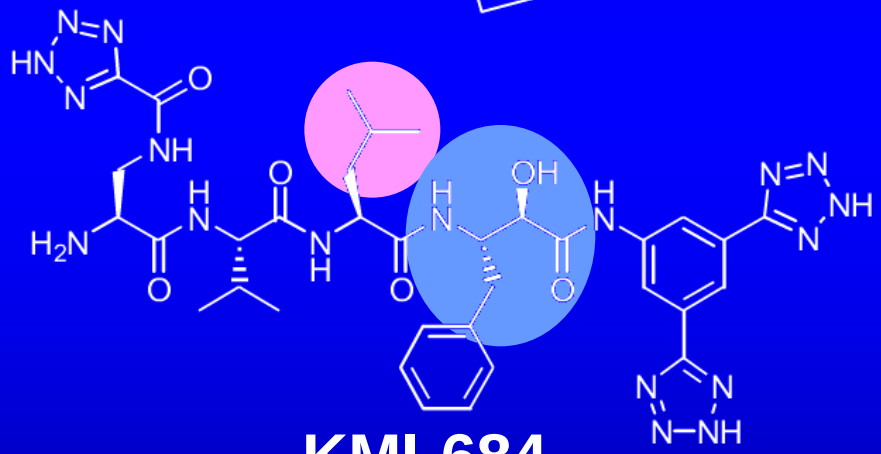
KMI-420 (R = -H)
BACE1 IC_{50} = 8.2 nM

KMI-429 (R = -COOH)
BACE1 IC_{50} = 3.9 nM

Bioorg.Med. Chem. Lett., **15**, 211-215 (2005)

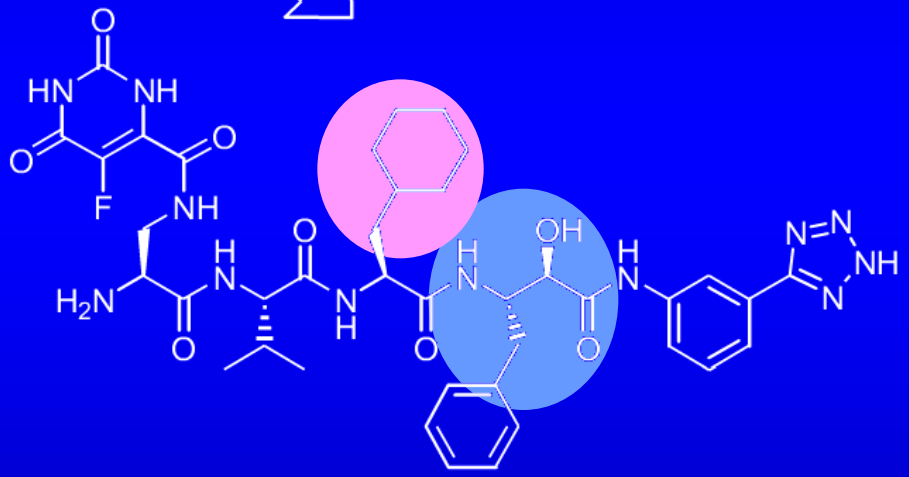


KMI-429



KMI-684

BACE1 IC_{50} = 1.2 nM

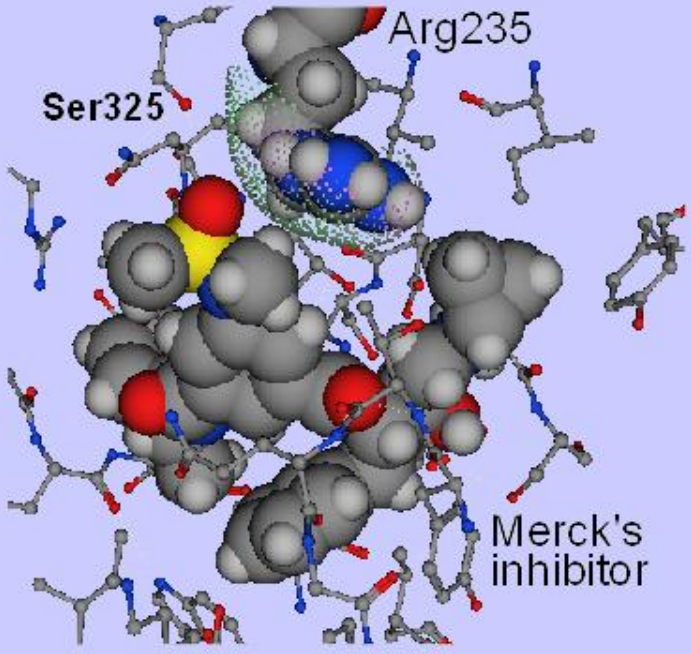


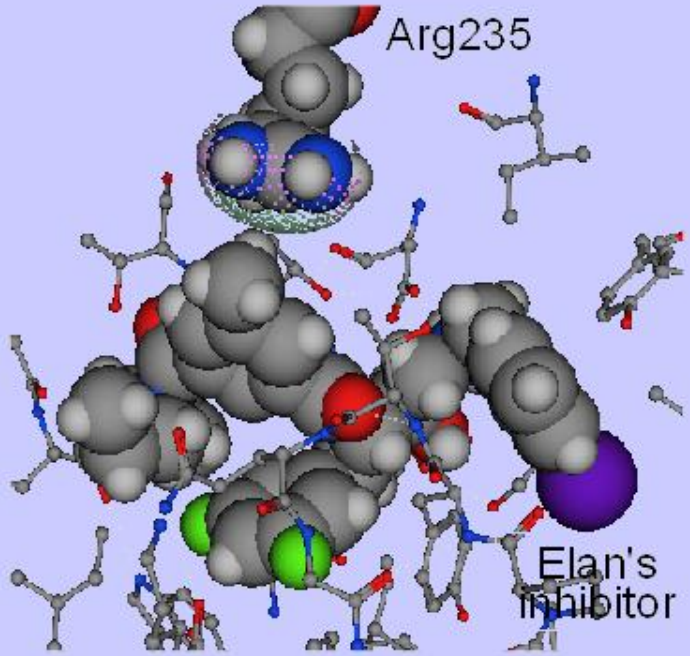
KMI-574

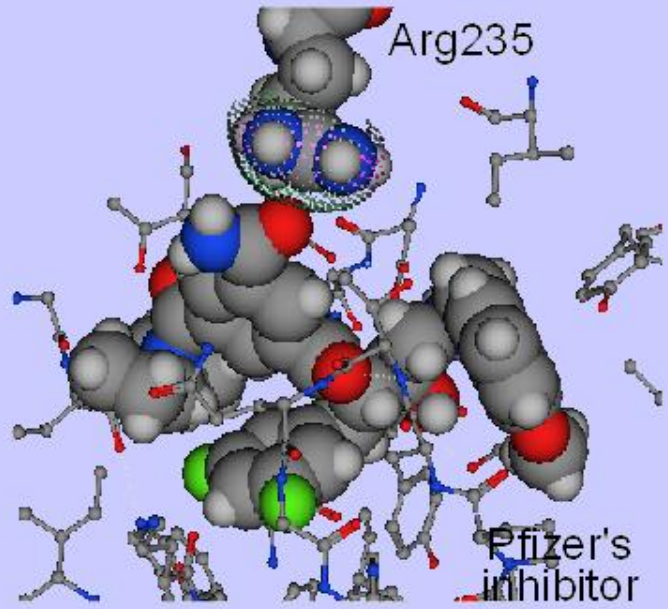
BACE1 IC_{50} = 5.6 nM

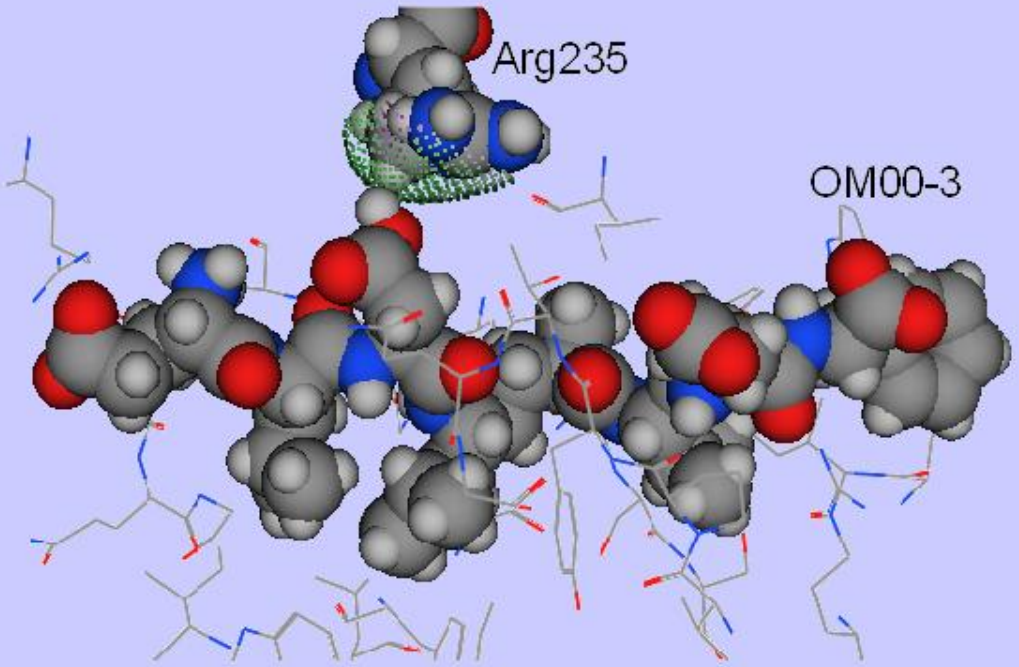
Y. Hamada *et al.* Bioorg. Med. Chem. Lett., **16**, 4354-4359 (2006)

Y. Hamada *et al.* Bioorg. Med. Chem. Lett., **16**, 1649-1653 (2008)


Arg235
Ser325
Merck's
inhibitor


Arg235
Elan's
inhibitor

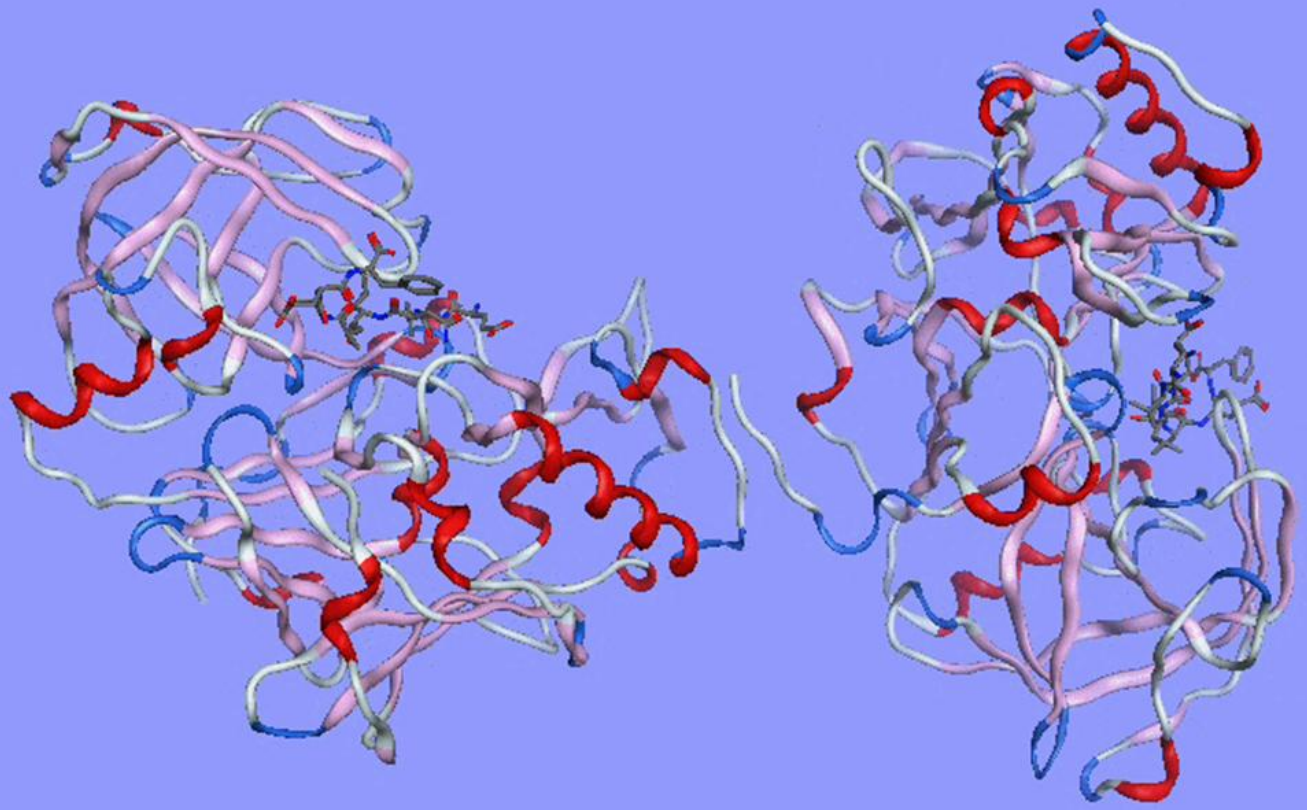

Arg235
Pfizer's
inhibitor


Arg235
OM00-3

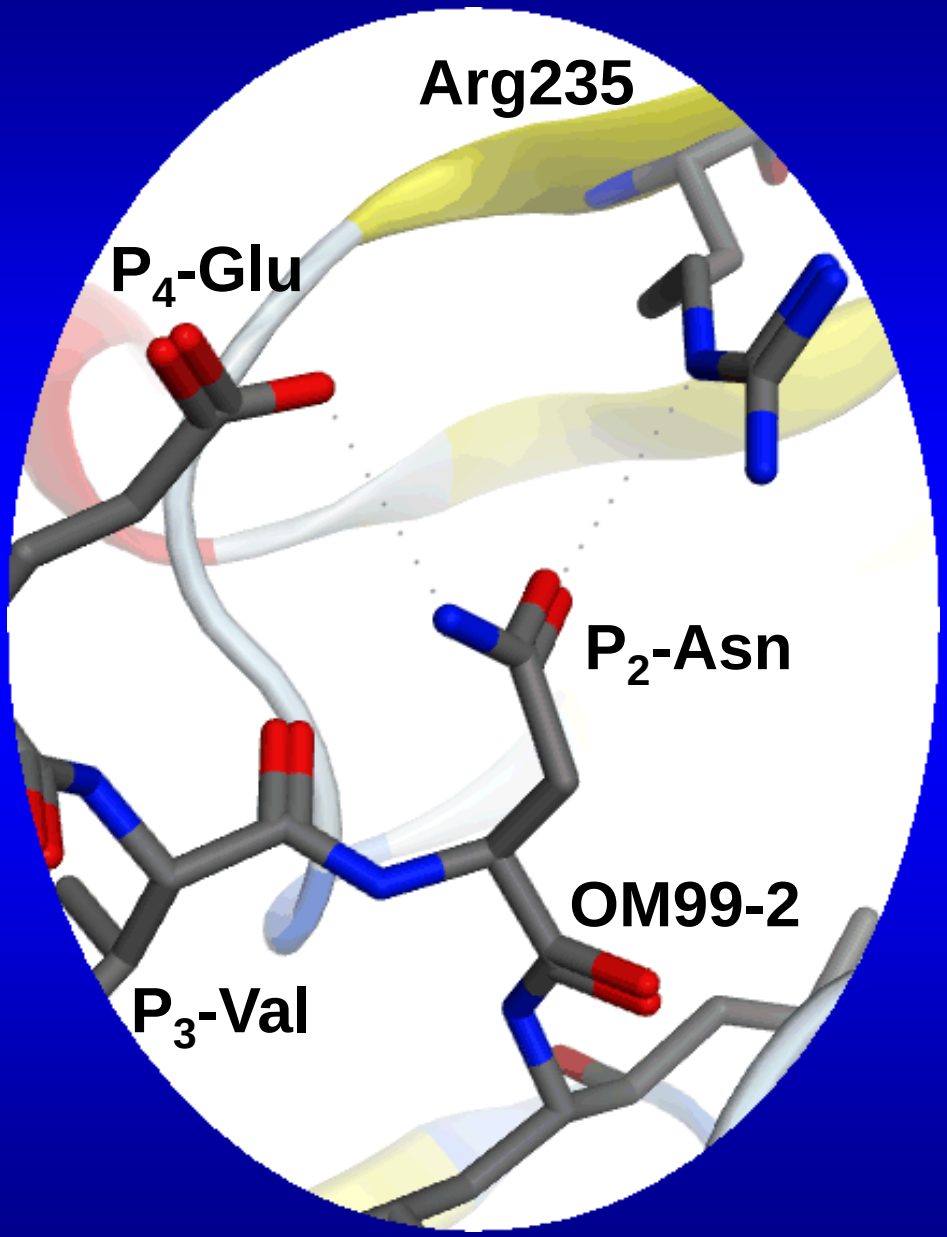
PDB ID	The distance (Å) from P_2 part				the closest P_2 atom to guanidino -plane
	N^a	N^b	N^c	plane*1	
2P83	3.2	2.9	2.9	2.7	=O ($CONH_2$)*2
2B8L	4.6 (3.7)	4.6 (3.7)	3.9 (2.8)	- (2.8)	*N*-methyl*3
2P8H	4.8 (3.8)	4.7 (3.7)	4.1 (2.8)	- (3.0)	*N*-methyl*3
2PH6	4.7 (3.8)	4.4 (3.4)	3.9 (2.8)	- (2.8)	*N*-methyl*3
20AH	4.1 (3.1)	4.2 (3.4)	3.5 (2.9)	- (2.8)	*N*-methyl*3
2QZL	4.5 (3.6)	4.5 (3.7)	3.7 (2.8)	- (2.8)	*N*-methyl*3
2P4J	4.4 (4.0)	3.6 (3.3)	3.5 (2.9)	- (2.7)	*N*-methyl*3
2IRZ	4.8 (3.9)	4.5 (3.5)	4.0 (2.9)	- (2.9)	*N*-methyl*3
2IS0	4.8 (3.9)	4.5 (3.5)	3.9 (2.8)	- (2.8)	*N*-methyl*3
2QK5	4.8 (3.9)	4.5 (3.7)	4.1 (3.0)	- (3.0)	*N*-methyl*3
2B8V	4.3 (3.4)	4.6 (3.6)	3.8 (2.7)	- (2.9)	*N*-methyl*3
1M4H	4.3 (4.1)	5.1 (3.4)	3.9 (3.2)	- (2.9)	-COOH (OM00-3)
2HM1	2.7	2.7	2.9	2.4	pyridine ring
1TQF	4.8	4.8	3.3	2.7	=O ($C_6H_5CH_2SO_2$)
1YM2	4.2	3.9	3.5	3.5	-S- (methionine)
2G94	4.3	3.4	4.3	3.3	=O ($CH_3SO_2CH_2$-)
2HIZ	2.9	2.6	3.1	2.5	methylene proton*4
1XS7	3.8	3.1	2.6	2.6	carbonyl (amide)

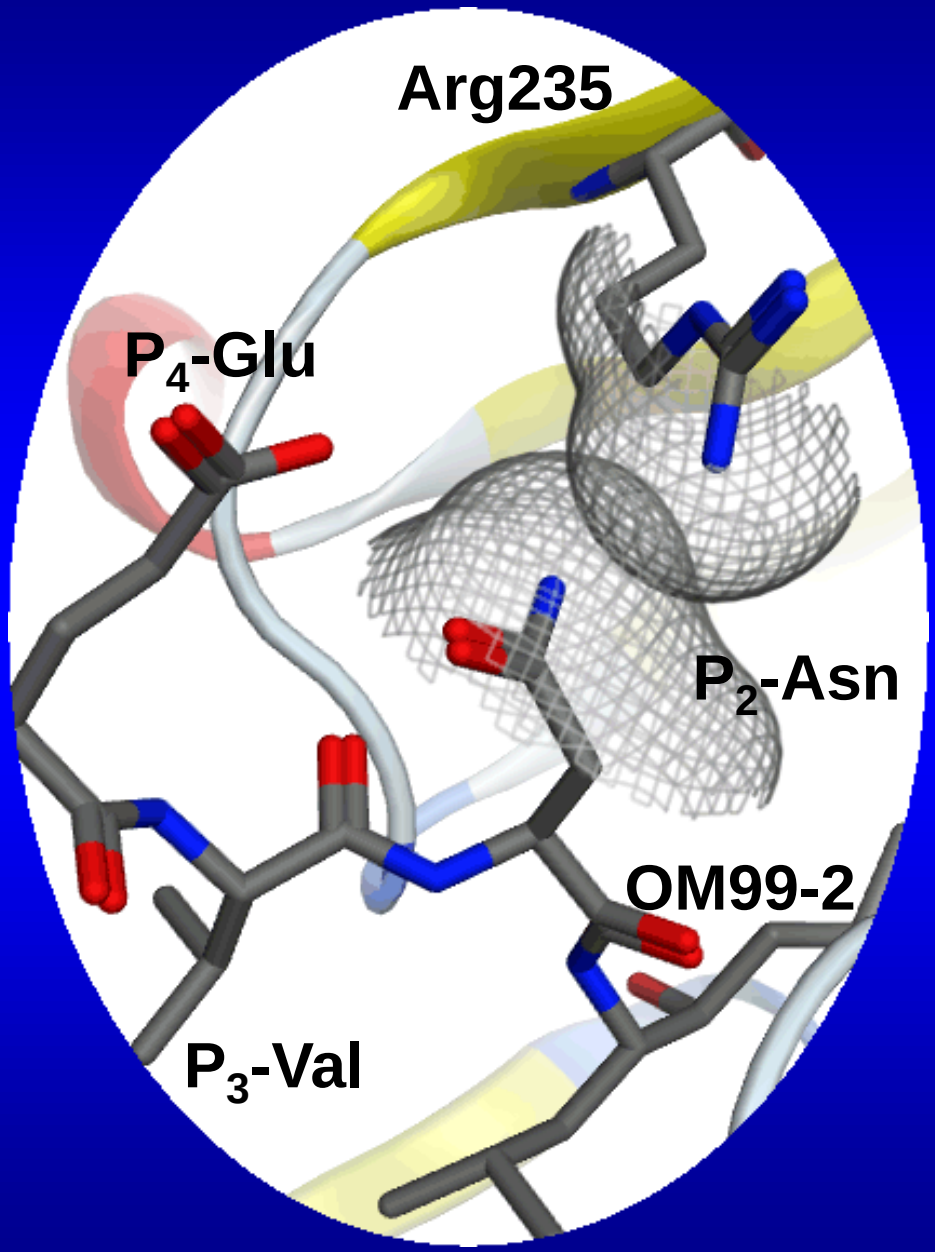
PDB ID	The distance (Å) from P_2 part				the closest P_2 atom to guanidino -plane
	N^a	N^b	N^c	plane*1	
2IQG	4.4 (3.6)	3.9 (3.2)	4.1 (2.8)	- (3.0)	methyl*5
2QK5	4.8 (3.8)	4.5 (3.6)	4.1 (3.1)	- (3.1)	methyl*5
2QP8	4.2 (3.1)	4.4 (3.6)	3.9 (3.0)	- (2.8)	methyl*5
3CIB	4.4 (3.4)	4.5 (3.6)	4.2 (3.1)	- (3.0)	methyl*5
3CIC	4.3 (3.3)	4.4 (3.5)	4.0 (3.0)	- (2.9)	methyl*5
3CID	4.2 (3.1)	4.4 (3.5)	4.1 (3.0)	- (2.9)	methyl*5
2QMD	4.2 (3.2)	4.4 (3.5)	4.0 (3.1)	- (2.9)	methyl*5
2QMF	4.2 (3.2)	4.3 (3.4)	3.9 (2.9)	- (2.8)	methyl*5
2QMG	4.0 (3.0)	4.1 (3.4)	3.7 (2.8)	- (2.7)	methyl*5
1W51	3.21	4.0	4.0	3.2	isophthalic ring*6
2FDP	4.9	3.7	3.9	3.1	isophthalic ring*6
2VIE	4.7	4.0	3.4	3.0	pyrrolidone ring
2VJ9	4.2	4.5	3.7	3.7	pyrrolidone ring
2VIZ	4.3	3.7	2.9	2.6	pyrrolidone ring
2VNM	4.9	4.2	3.6	3.3	butanesultan ring
2VIJ	4.9	4.3	3.7	3.6	butanesultan ring
2VNN	5.6 (4.6)	5.1 (3.4)	4.3 (3.4)	- (2.7)	*N*-methyl
2PH8	5.0 (4.1)	4.7 (3.7)	4.2 (3.5)	- (3.3)	*N*-methyl*3

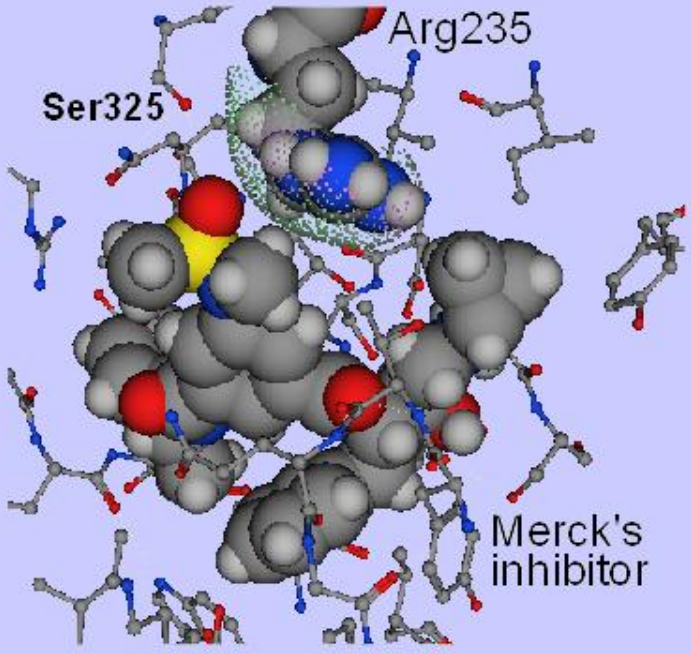
1FKN

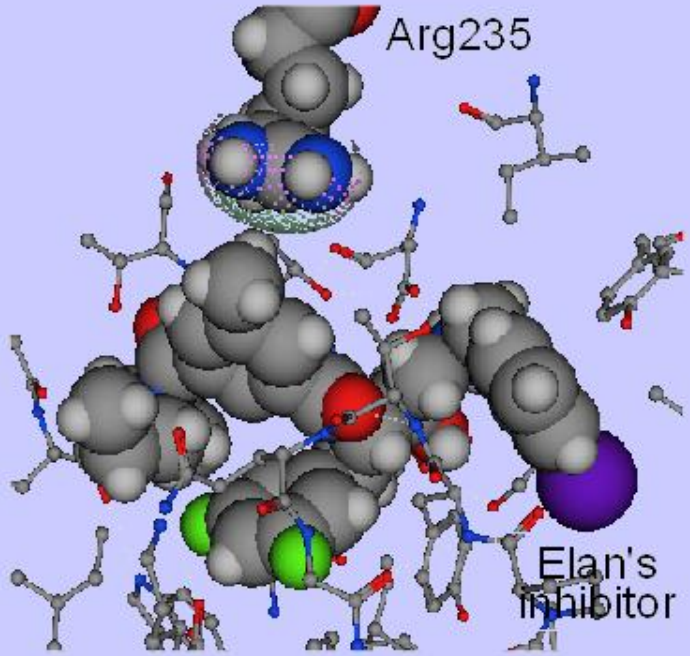


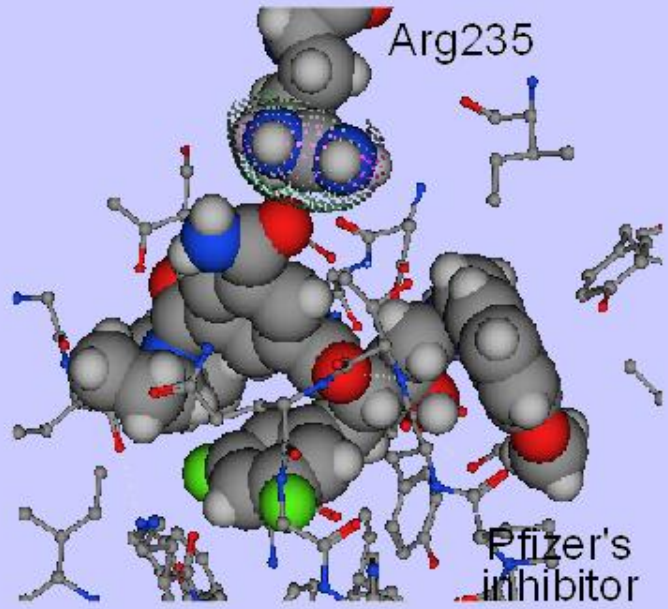
1FKN

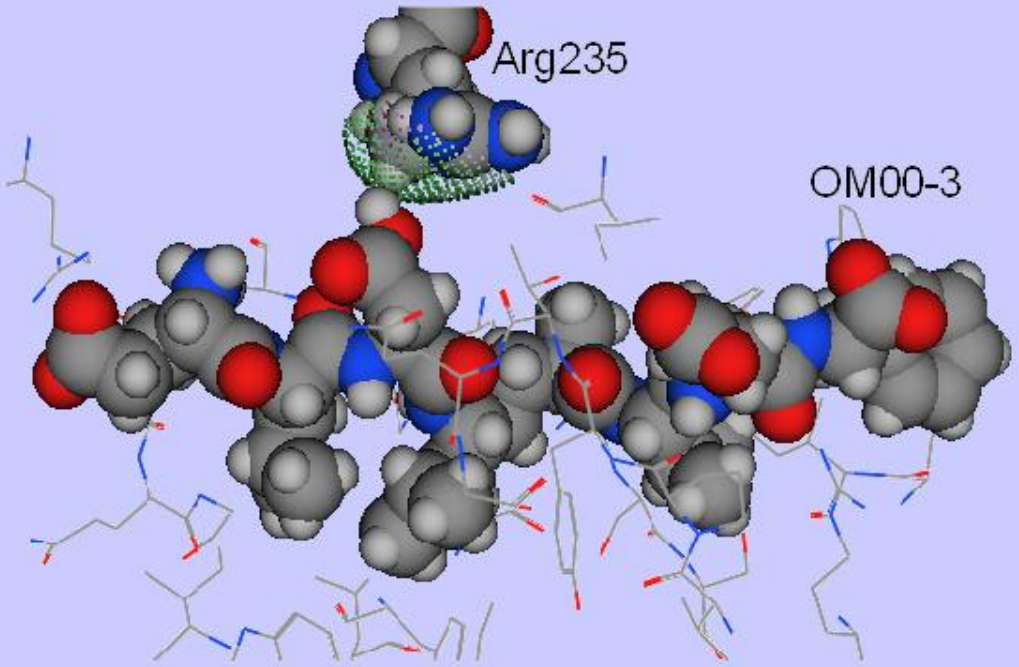

Arg235
P4-Glu
P2-Asn
OM99-2
P3-Val


Arg235
P4-Glu
P2-Asn
OM99-2
P3-Val


Arg235
Ser325
Merck's
inhibitor


Arg235
Elan's
inhibitor


Arg235
Pfizer's
inhibitor


Arg235
OM00-3

JOURNAL OF VIROLOGY, Jan. 2009, p. 673–686 Vol. 83, No. 2
0022-538X/09/$08.00+0 doi:10.1128/JVI.01967-08

An Arginine Switch in the Species B Adenovirus Knob Determines High-Affinity Engagement of Cellular Receptor CD46▽

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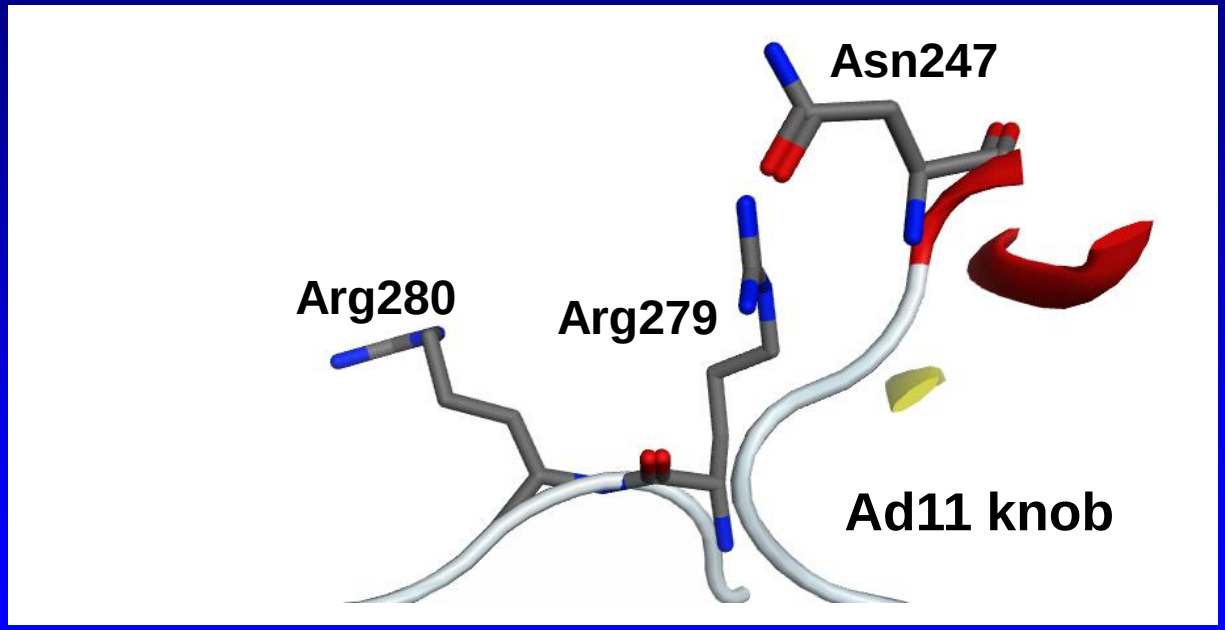
Interfaculty Institute for Biochemistry, University of Tübingen, D-72076 Tübingen, Germany[1]; Institute for Physical and Theoretical Chemistry, University of Tübingen, D-72076 Tübingen, Germany[2]; Division of Virology, Department of Clinical Microbiology, University of Umeå, SE-90185 Umeå, Sweden[3]; and Vanderbilt University School of Medicine, Nashville, Tennessee 37232[4]

Received 18 September 2008/Accepted 28 October 2008

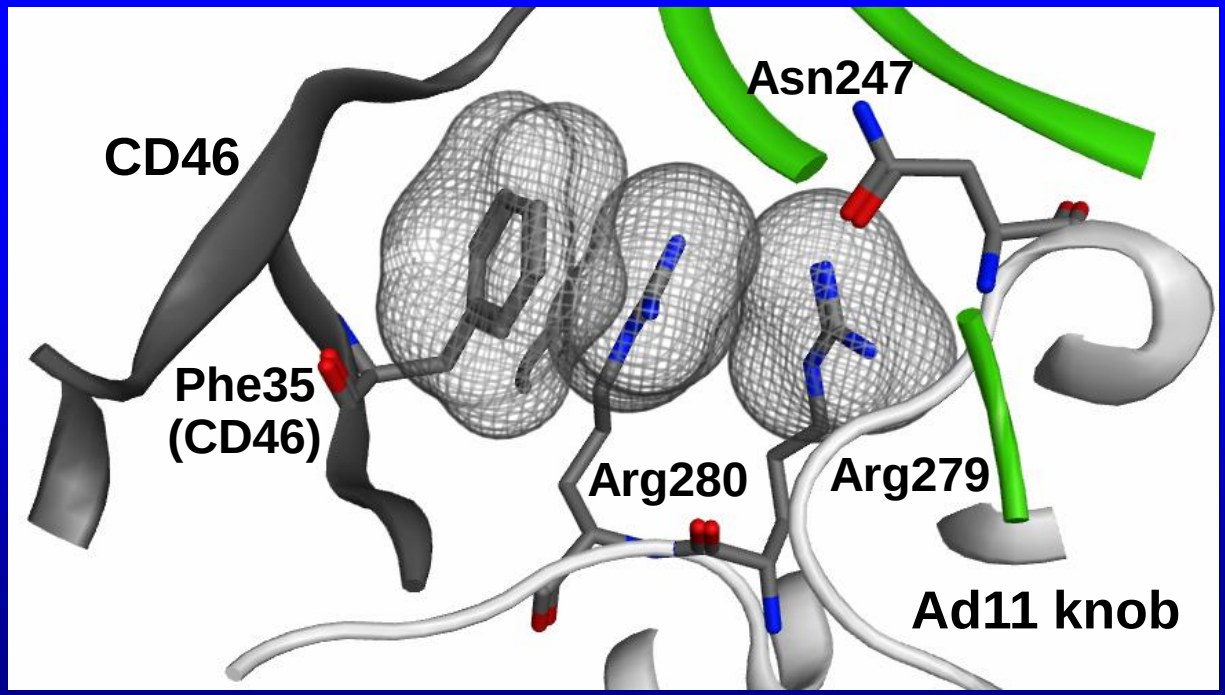
Adenoviruses (Ads) are icosahedral, nonenveloped viruses with a double-stranded DNA genome. The 51 known Ad serotypes exhibit profound variations in cell tropism and disease types. The number of observed Ad infections is steadily increasing, sometimes leading to fatal outcomes even in healthy individuals. Species B Ads can cause kidney infections, hemorrhagic cystitis, and severe respiratory infections, and most of them use the membrane cofactor protein CD46 as a cellular receptor. The crystal structure of the human Ad type 11 (Ad11) knob complexed with CD46 is known; however, the determinants of CD46 binding in related species B Ads remain unclear. We report here a structural and functional analysis of the Ad11 knob, as well as the Ad7 and Ad14 knobs, which are closely related in sequence to the Ad11 knob but have altered CD46-binding properties. The comparison of the structures of the three knobs, which we determined at very high resolution, provides a platform for understanding these differences and allows us to propose a mechanism for productive high-affinity engagement of CD46. At the center of this mechanism is an Ad knob arginine that needs to switch its orientation in order to engage CD46 with high affinity. Quantum chemical calculations showed that the CD46-binding affinity of Ad11 is significantly higher than that of Ad7. Thus, while Ad7 and Ad14 also bind CD46, the affinity and kinetics of these interactions suggest that these Ads are unlikely to use CD46 productively. The proposed mechanism is likely to determine the receptor usage of all CD46-binding Ads.

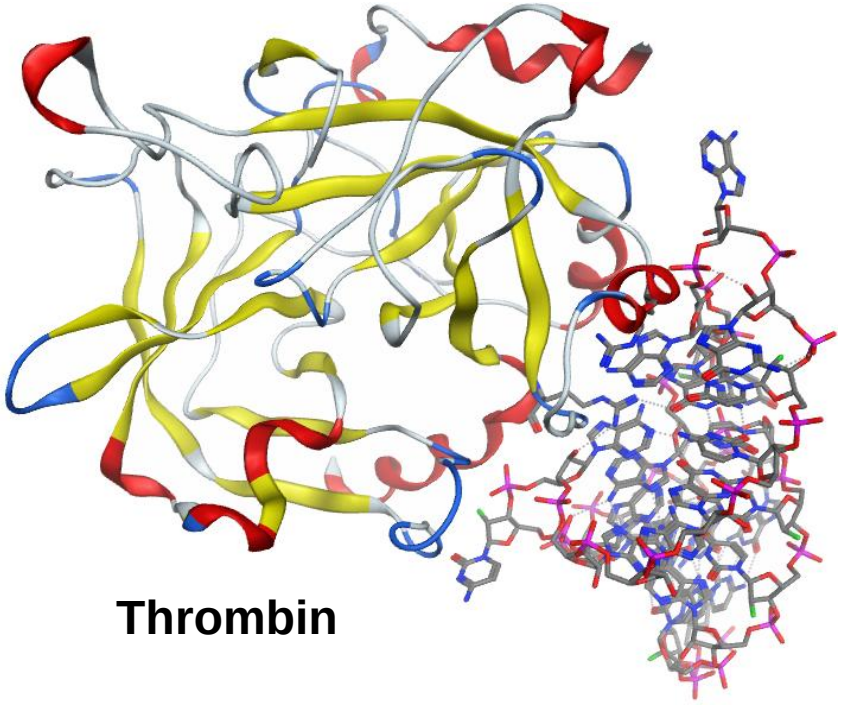
Ad11 knob



Ad11 knob

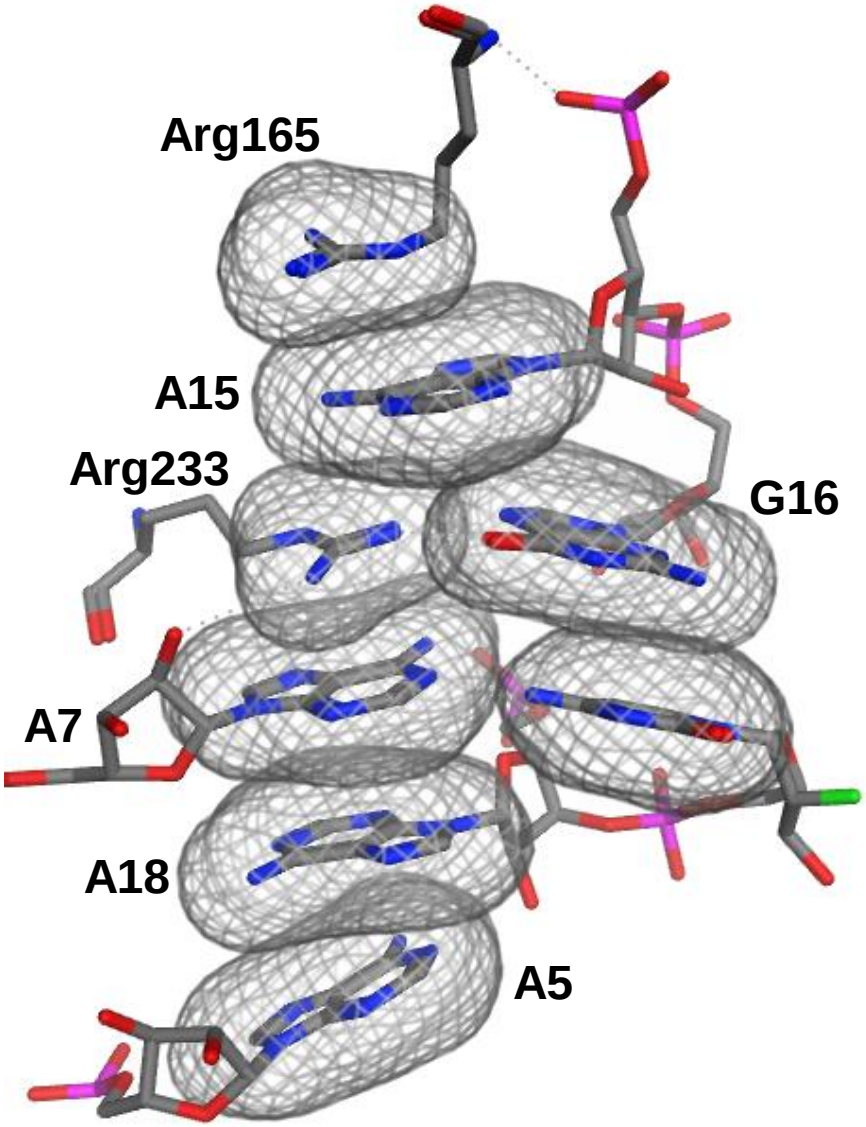
+ CD46





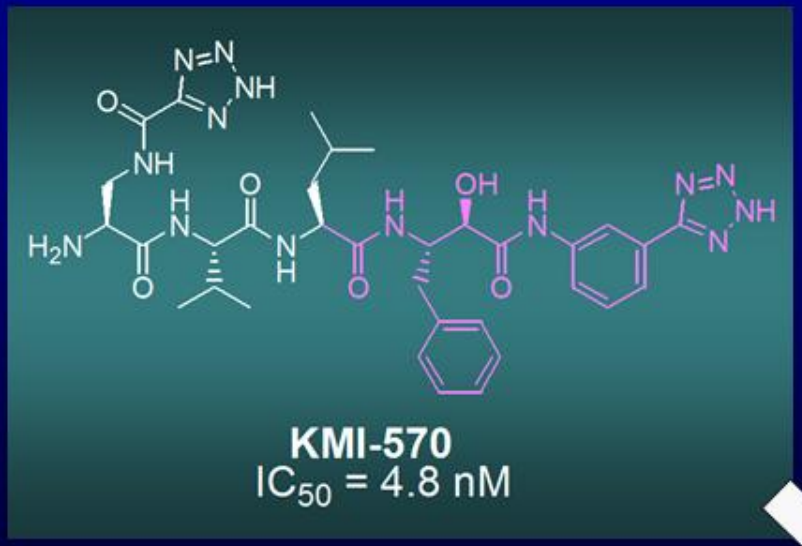
Thrombin

RNA aptamer

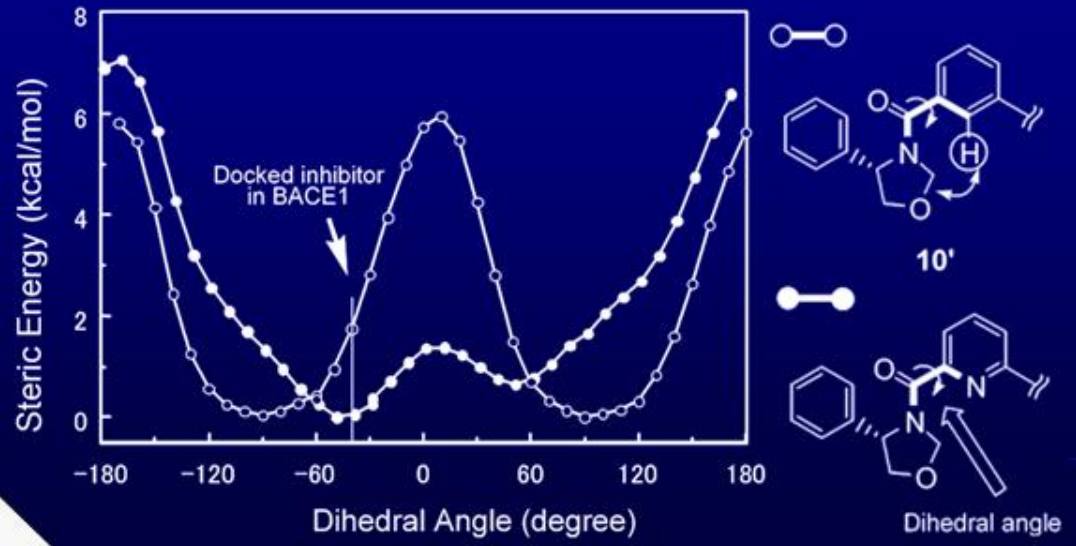


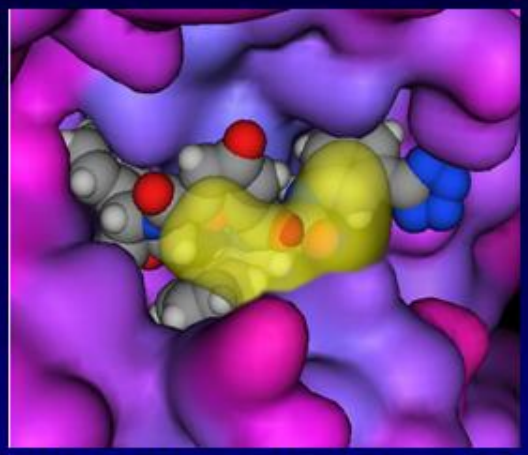
S. B. Long *et al*. RNA, **14**, 2504-2512 (2008)

Conformational structure-based design of BACE1 inhibitors

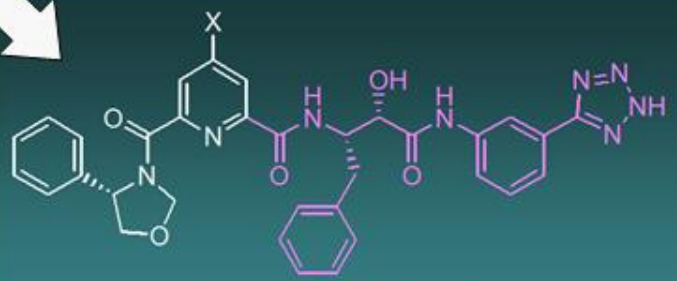


KMI-570
IC_{50} = 4.8 nM

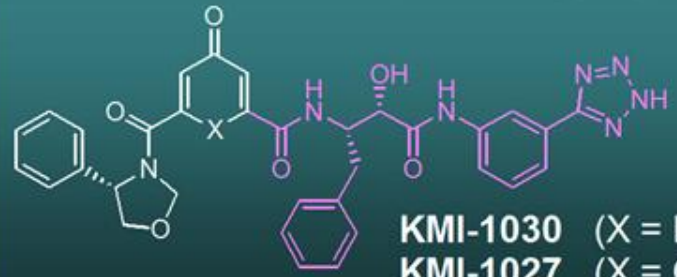




KMI-1027 docked in BACE1



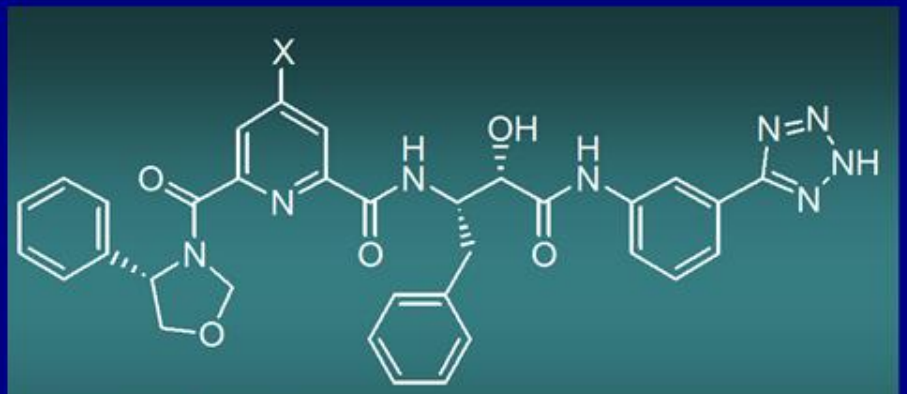
KMI-1023 (X = -H) IC_{50} = 140 nM
KMI-1036 (X = $-OSO_2CH_3$) IC_{50} = 96 nM



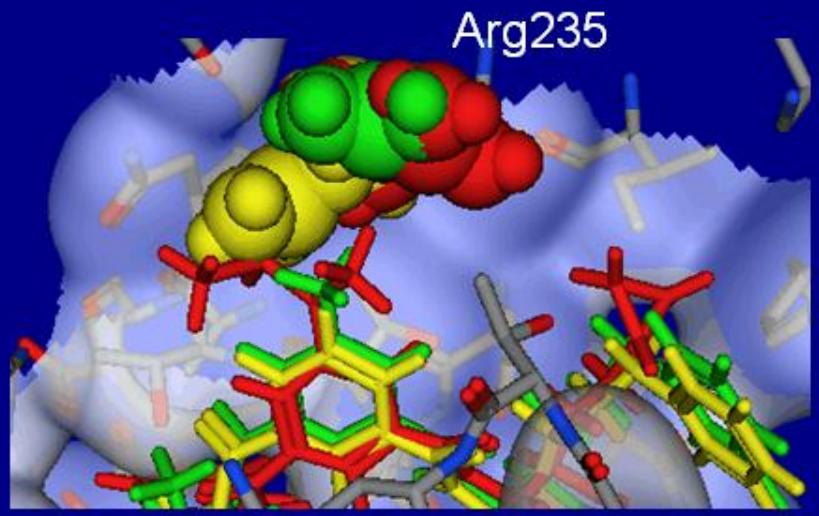
KMI-1030 (X = NH) IC_{50} = 360 nM
KMI-1027 (X = O) IC_{50} = 50 nM

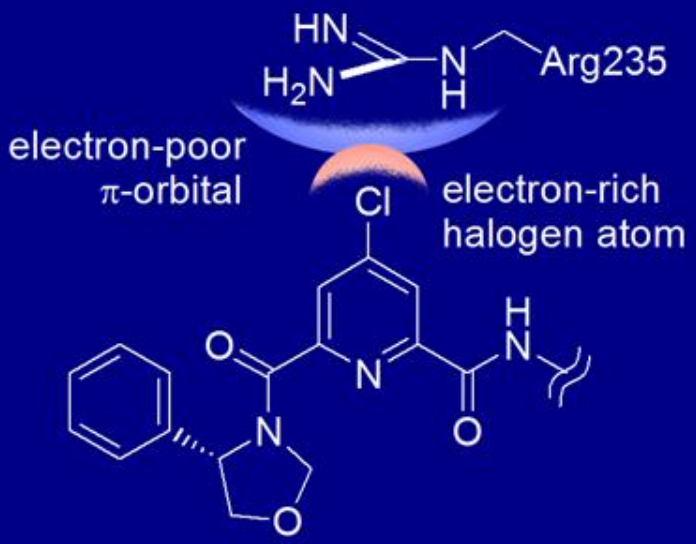
Y. Hamada *et al. Bioorg. Med. Chem. Lett.* **2008,** *18*, 1654-1658.

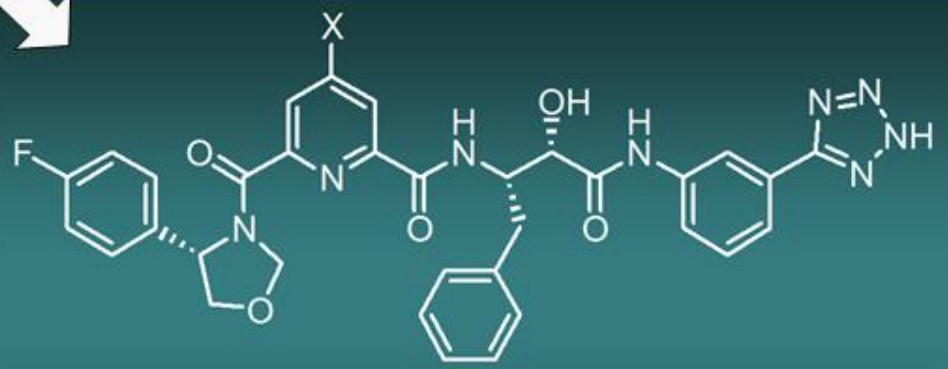
Non-peptidic BACE1 inhibitor, KMI-1303



KMI-1023 (X = -H) IC_{50} = 140 nM
KMI-1036 (X = $-OSO_2CH_3$) IC_{50} = 96 nM

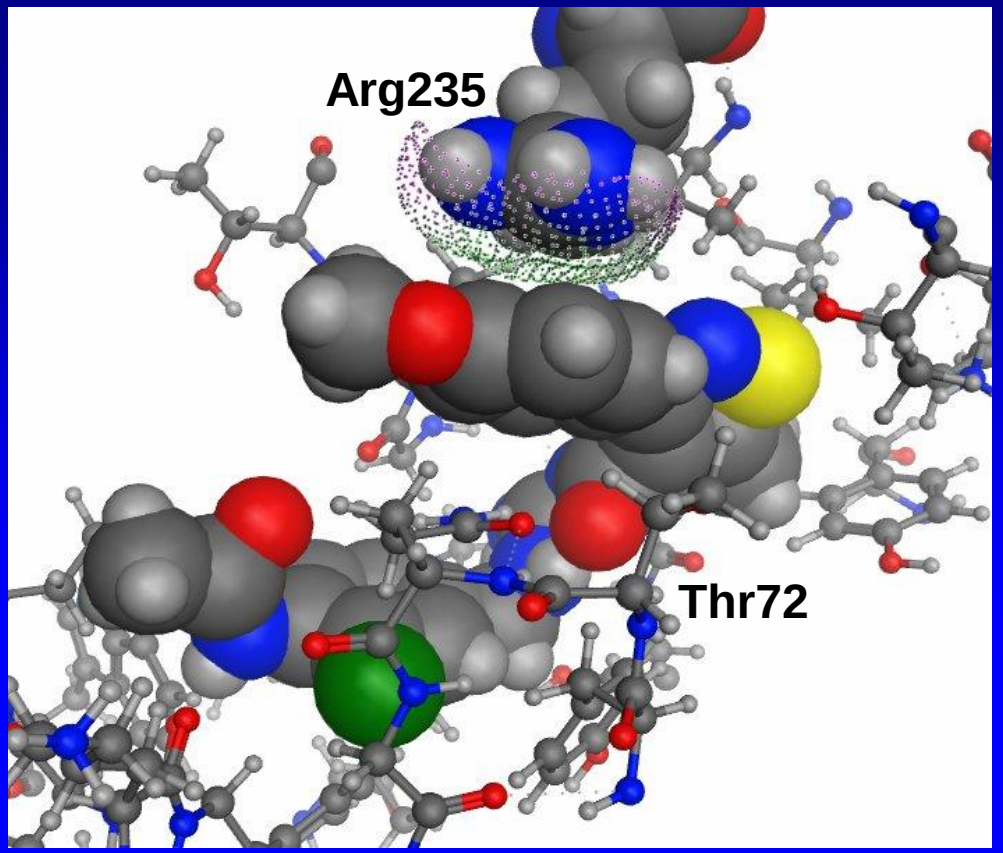


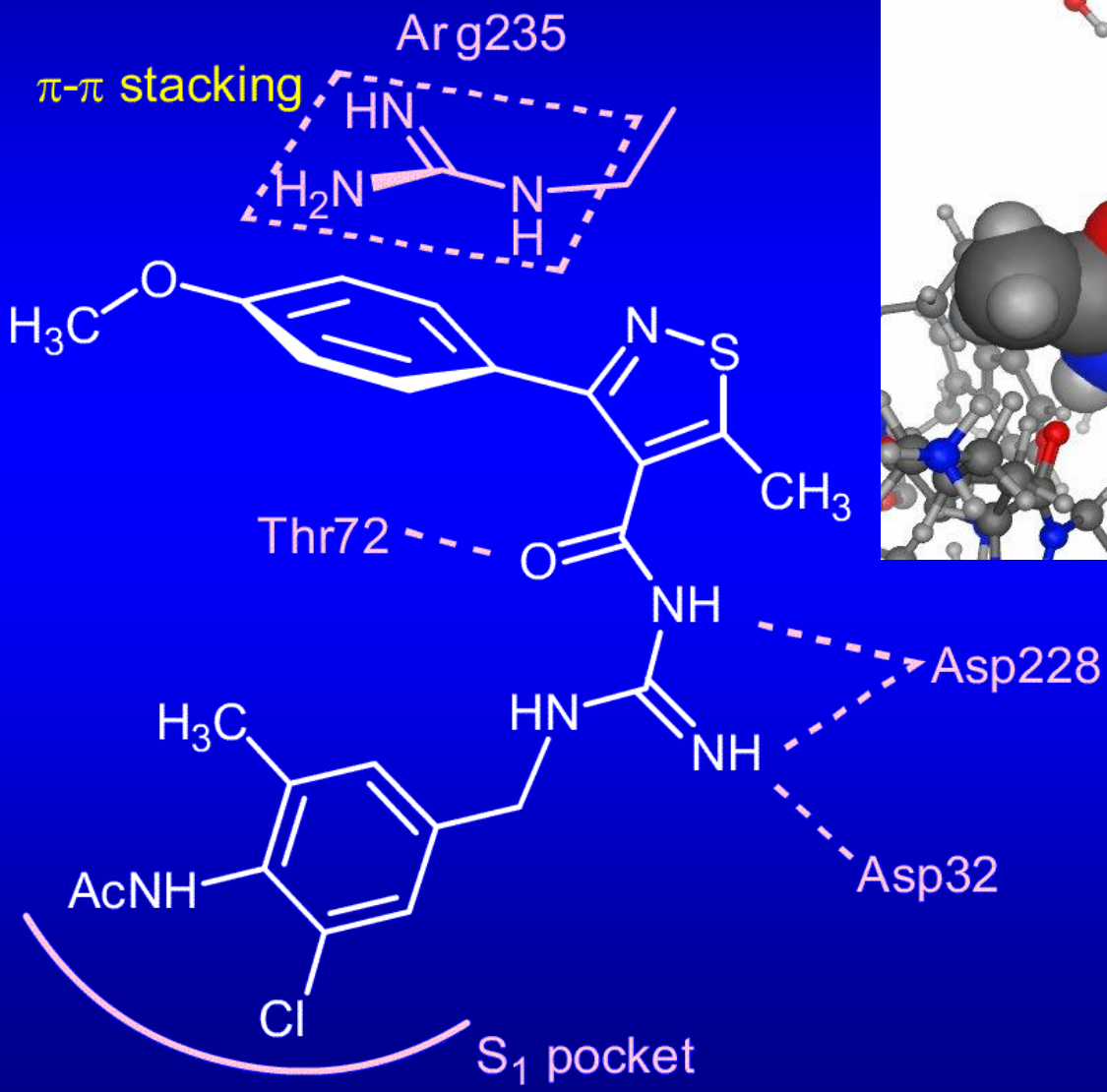




KMI-1283 (X = Cl) BACE1 IC_{50} = 13 nM
KMI-1303 (X = Br) BACE1 IC_{50} = 9 nM
KMI-1302 (X = I) BACE1 IC_{50} = 10 nM

Y. Hamada *et al. Bioorg. Med. Chem. Lett.* **2009**, *19*, 2435-2439.



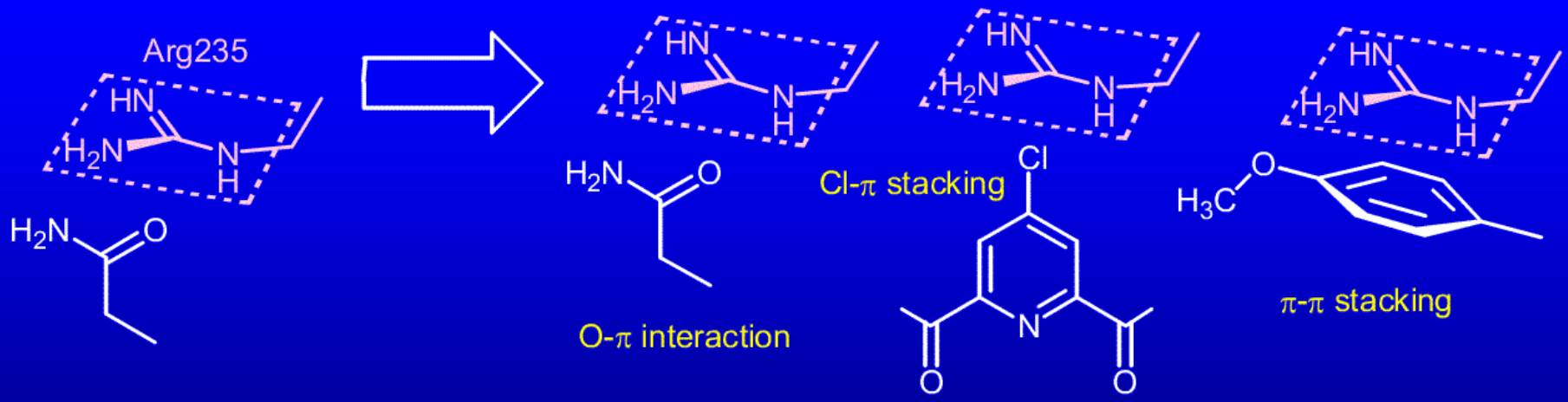


Samuel W. Gerrits et al. *J. Med Chem.* **2012**, *55*, 9208.

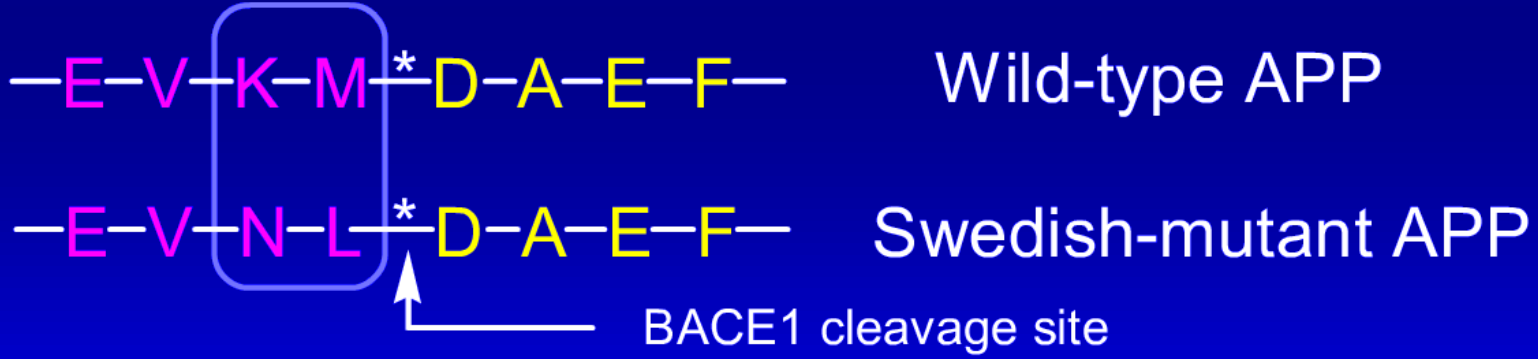
R	BACE1 Ki (μM)
-H	3.9
-F	2.2
$-OCH_3$	0.67

Amide bioisostere
Asn bioisostere

"Electron-donor bioisostere"

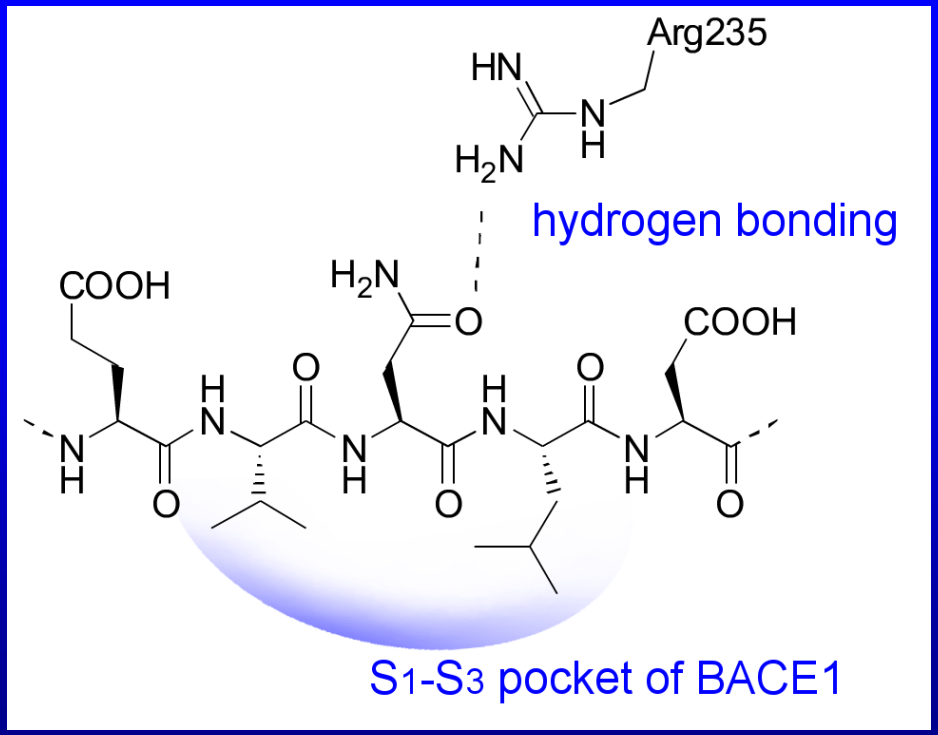


Y. Hamada *et al*.: **The application of bioisosteres in drug design for novel drug discovery: focusing on acid protease inhibitors**. *Expert Opinion on Drug Discovery*, **2012**, *7*, 903-922.



Swedish mutant -type substrate	*K*m = 9 μM	k_{cat} = 0.02 s^{-1}
wild-type substrate	*K*m = 7 μM	k_{cat} = 0.002 s^{-1}

Grüninger-Leitch, F. *et al.* (2002) *J. Biol. Chem.*, **277**, 4687-4693.



S1-S3 pocket of BACE1

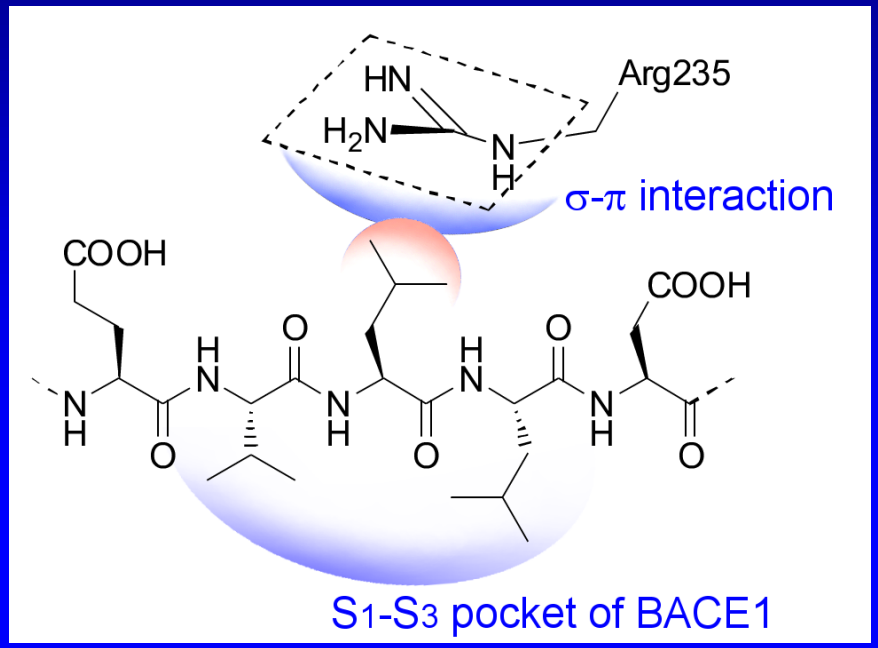
hydrogen bond interaction

Small *K*m value

⇒ High affinity for the active site of enzyme

Big k_{cat} value

⇒ Substrate is readily cleaved by enzyme



Peptides

possessing a P_2–Leu residue

σ-π interaction

Small *K*m value

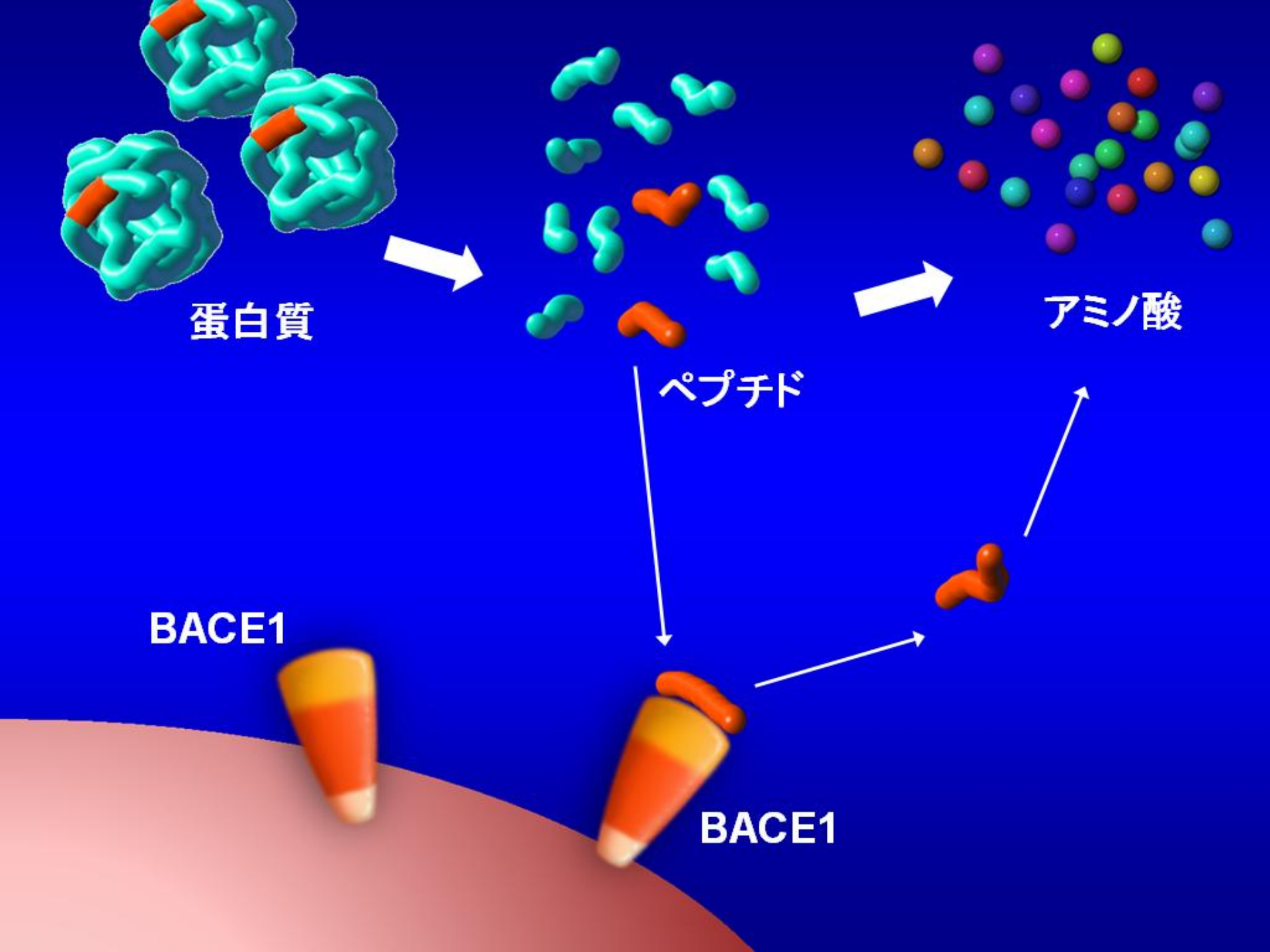
⇨ High affinity for the active site of enzyme

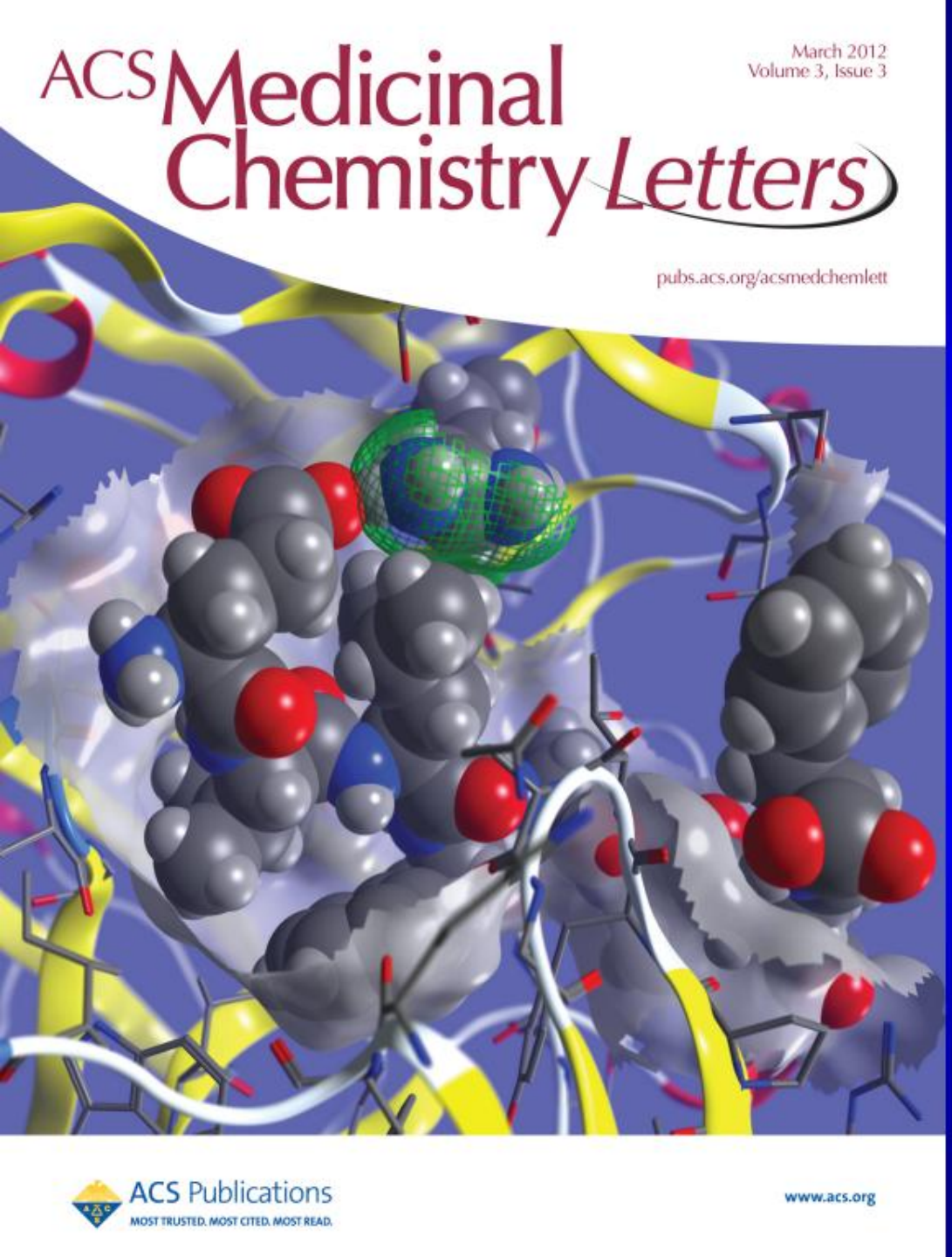
Vastly reduced k_{cat} value

⇨ Inhibitor ?

Peptides and BACE1 inhibitory activity

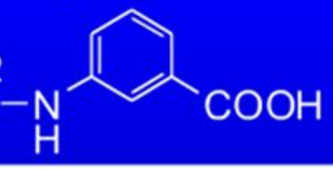
Compound	P_4	P_3	P_2	P_1	P_1'	P_2'	P_3'	P_4'	Inhibition % at 2 μM
KMI-1634	H-Glu - Val - Leu - Phe - Ser - Ala - Glu - Phe-OH								6
KMI-1638	H-Glu - Val - Leu - Phe-D-Ser -Ala - Glu - Phe-OH								28
KMI-1705	H-Glu - Val - Leu - Phe-D-Ser -OH								23
KMI-1708	H-Glu - Val - Leu - Phe-D-Asn -OH								29
KMI-1006	H-Glu - Val - Leu - Phe -OH								8
KMI-1855	H-Glu - Val - Asn - Phe -OH								<5


蛋白質
ペプチド
アミノ酸
BACE1
BACE1

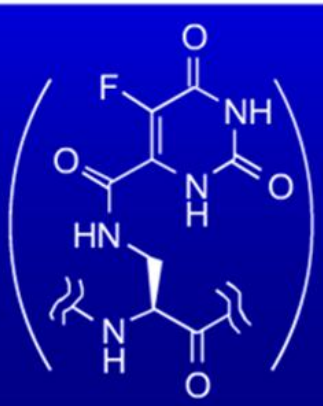


Y. Hamada, S. Ishiura, Y. Kiso: BACE1 Inhibitor Peptides: Can an Infinitely Small *k*cat Value Turn the Substrate of an Enzyme into Its Inhibitor? *ACS Med. Chem. Lett*, **3**, 193-197 (2012)

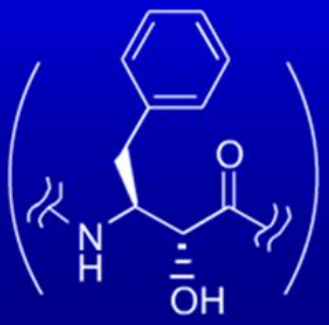
P_4-modified peptides and BACE1 inhibitory activity

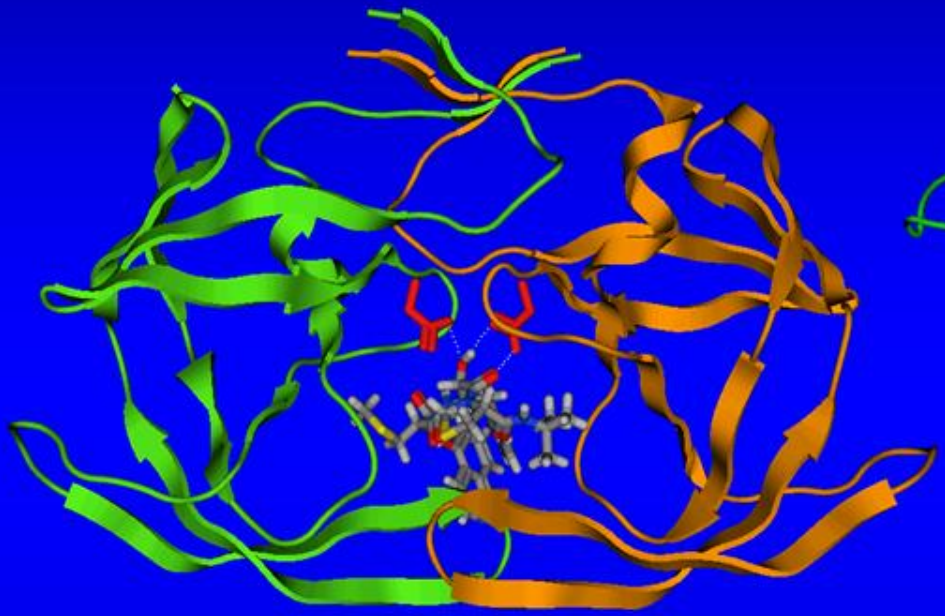
Compound	P_4	P_3	P_2	P_1	P_1'	Inhibition % at 2 μM
KMI-1791	H-DAP(5FO)[*1]	- Val -	Leu -	Phe -	Ser -OH	85
KMI-1706	H-DAP(5FO)	- Val -	Leu -	Phe-D-	Ser -OH	94
KMI-1792	H-DAP(5FO)	- Val -	Leu -	Phe -	Asn -OH	89
KMI-1709	H-DAP(5FO)	- Val -	Leu -	Phe-D-	Asn -OH	95
KMI-1858	H-DAP(5FO)	- Val -	Asn -	Phe -	Asn -OH	<5
KMI-1795	H-DAP(5FO)	- Val -	Leu -	Phe -	OH	90
KMI-1855	H-DAP(5FO)	- Val -	Asn -	Phe -	OH	<5
(KMI-446)	H-DAP(5FO)	- Val -	Leu -	Pns[*2]		99

[*1] DAP(5FO):
N^{β}-(5-fluoroorotyl)-L-2,3-diaminopropionic acid

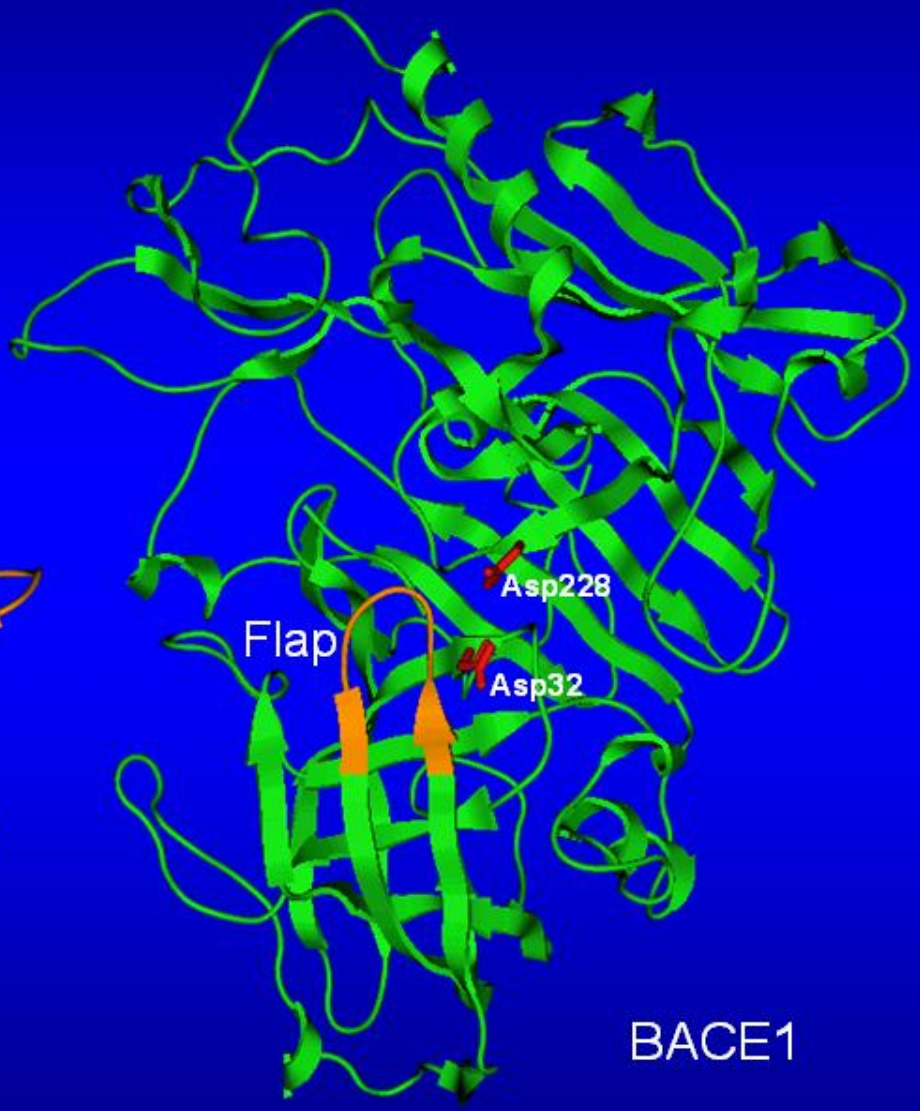


[*2] Pns:
a substrate transition-state analogue



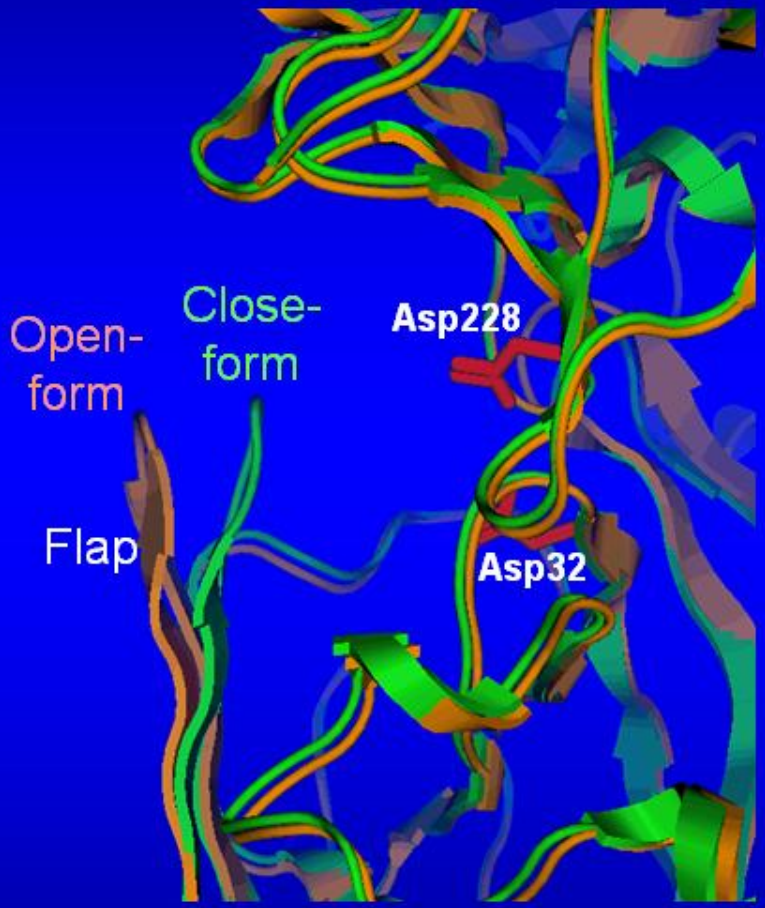


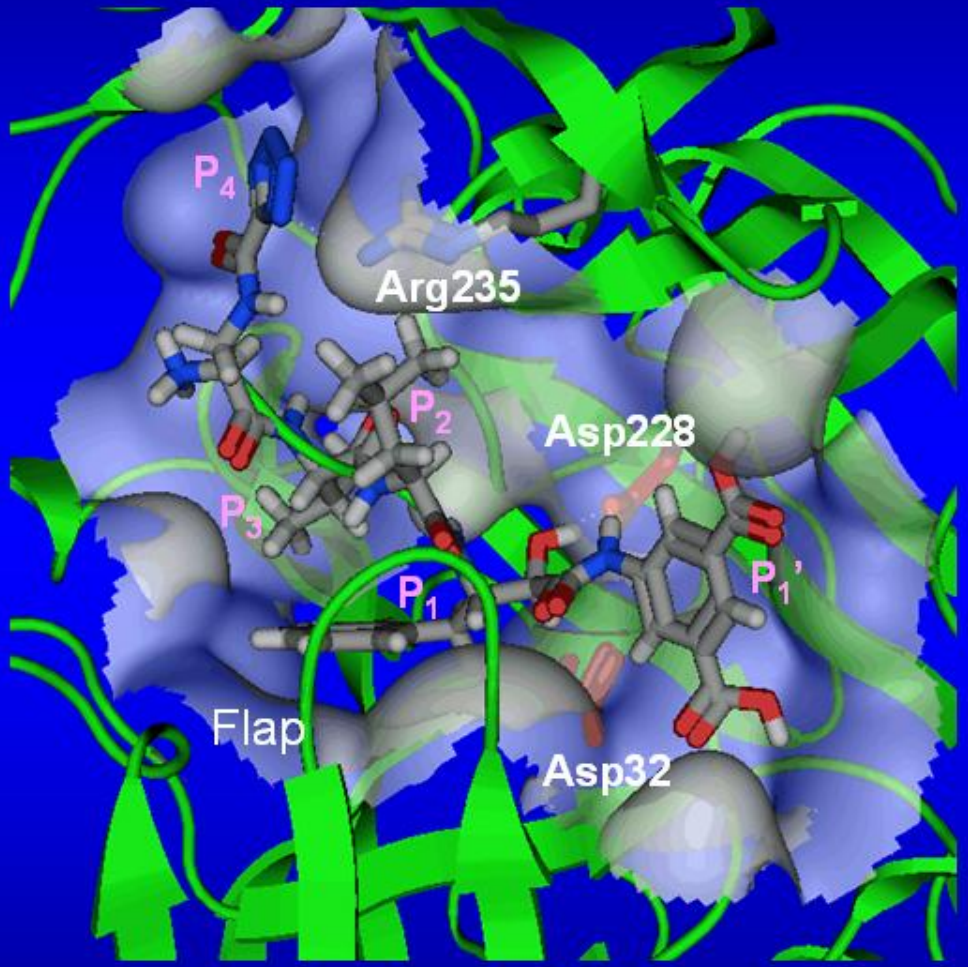
HIV-1 protease-KNI-272 complex



BACE1

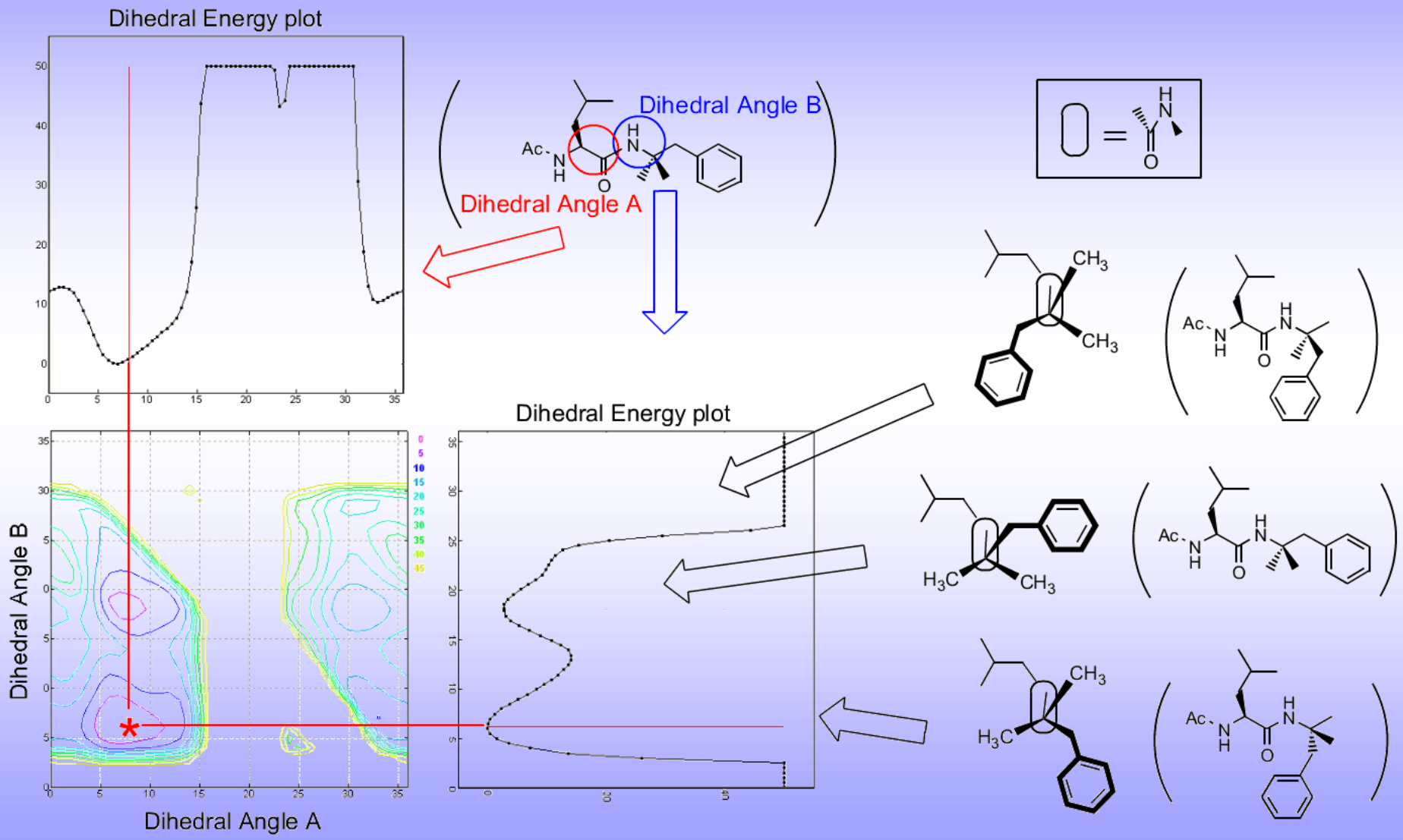
フラップとの相互作用の重要性



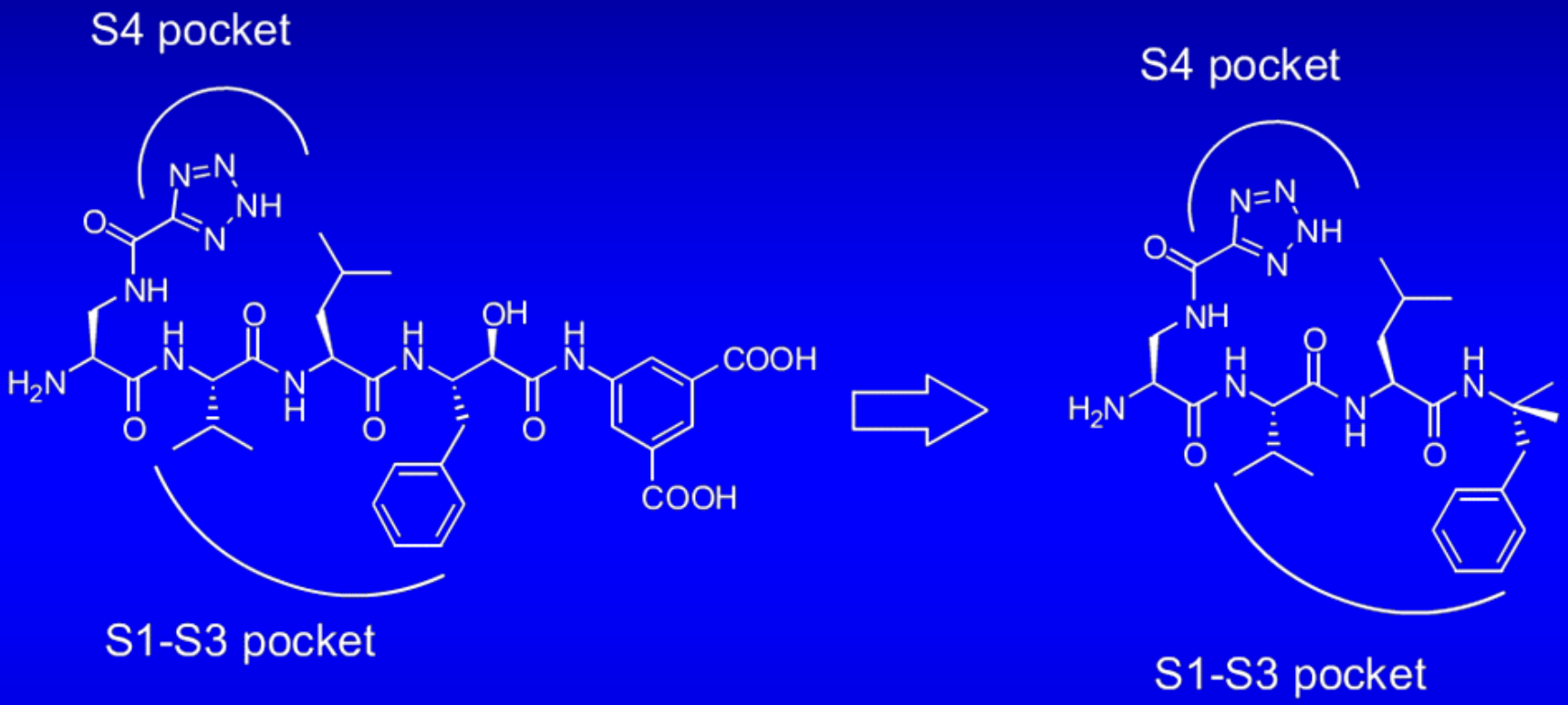


Y. Hamada, Y. Kiso. *Expert Opinion on Drug Discovery* **2009,** *4*, 391-416.

Trpeptidic BACE1 inhibitors devised by in-silico conformational structure-based design



トリペプチド型BACE1阻害剤の設計

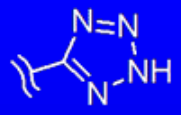
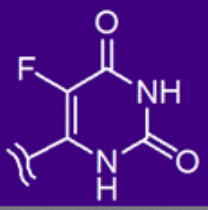
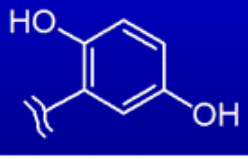


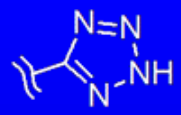
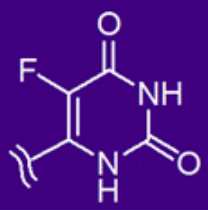
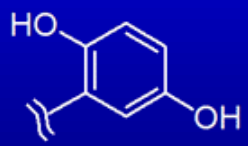
KMI-429

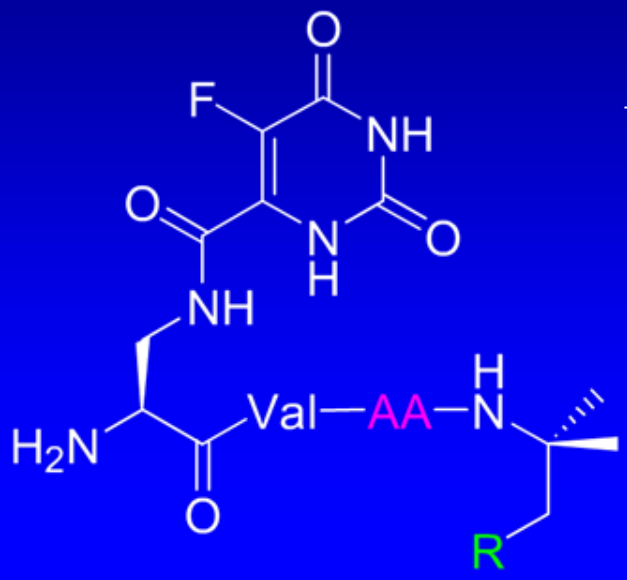
(100 % BACE1 inhibition at 2μM
IC_{50} = 3.9 nM)

Y. Hamada et al. *Bioorg. Med Chem. Lett.* **2012**, *22*, 1130

トリペプチド型BACE1阻害剤

Compd.	R	BACE1 inhibition % at 2μM
KMI-1564		70
KMI-1565		90
KMI-1566		77

Compd.	R	BACE1 inhibition % at 2μM
KMI-1607		76
KMI-1608		92
KMI-1609		80

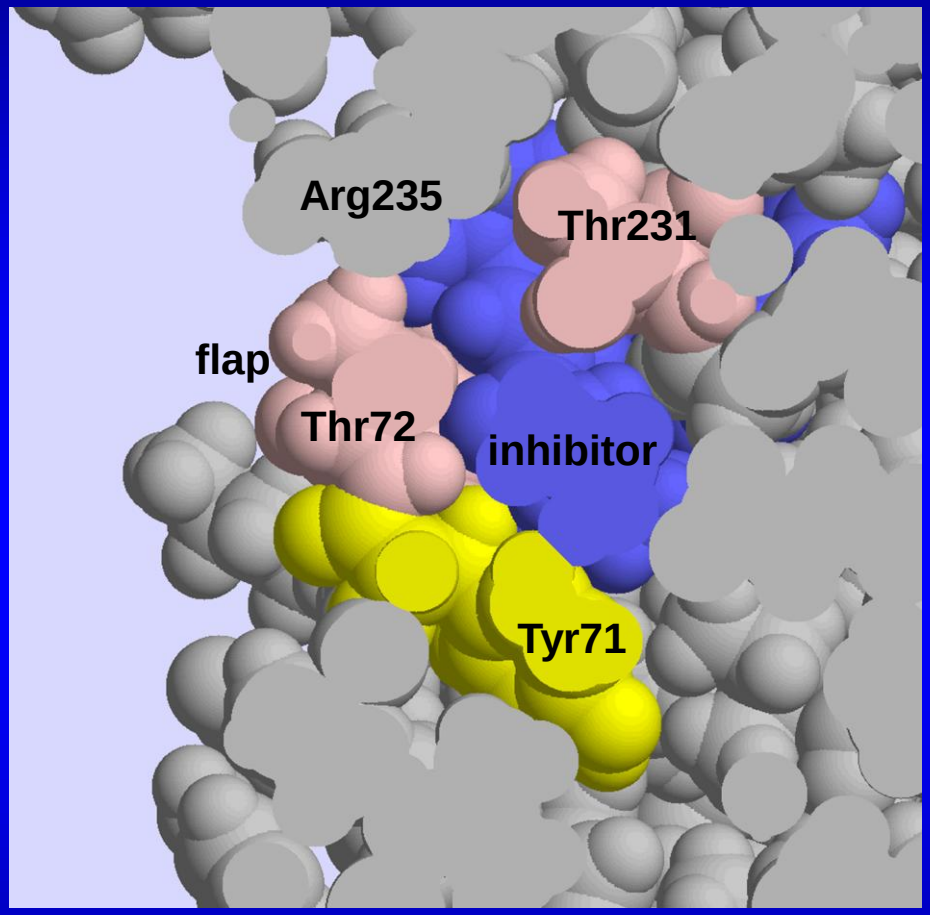


(IC_{50} = 50 nM)

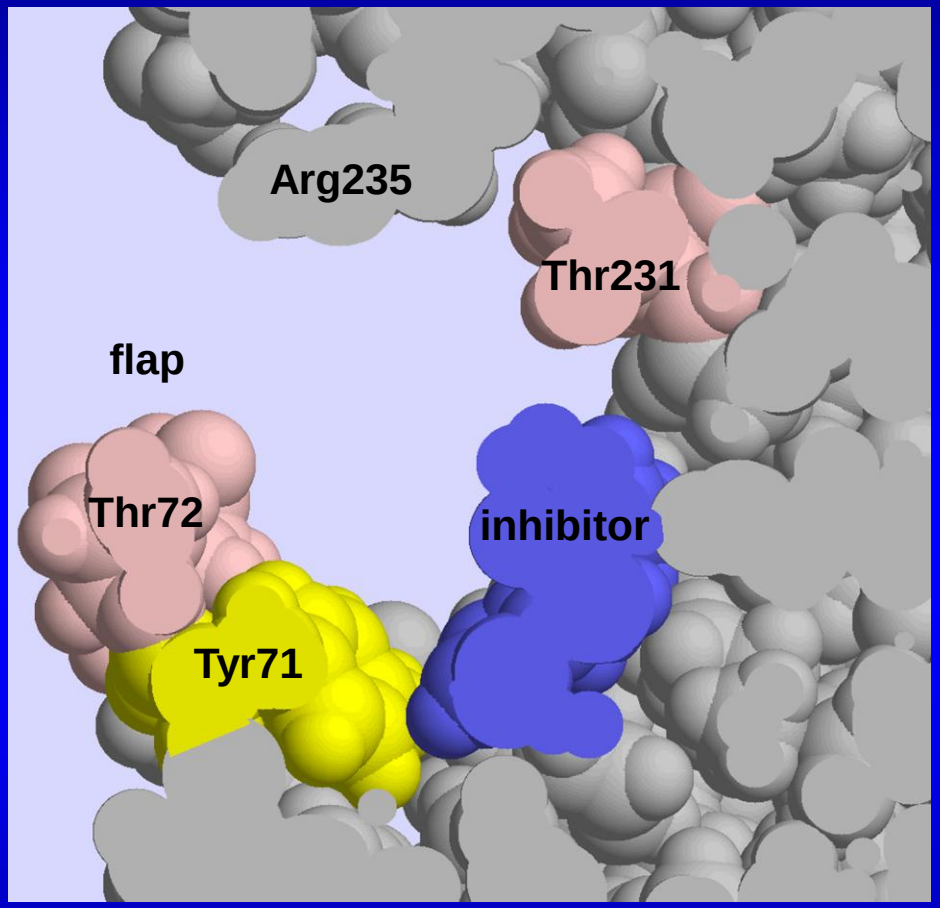
Compound (KMI-No)	AA	R	BACE1 inhibition % at 2 μM
KMI-1693	Leu	*p*-nitrophenyl	27
KMI-1717	Cha	*p*-nitrophenyl	89
KMI-1719	Leu	*p*-bromophenyl	85
KMI-1723	Cha	*p*-bromophenyl	92
KMI-1886	Leu	*p*-methylphenyl	86
KMI-1895	Cha	*p*-methylphenyl	88
KMI-1840	Leu	*o*-methylphenyl	91
KMI-1878	Cha	*o*-methylphenyl	84
KMI-1865	Leu	*p*-methoxyphenyl	83
KMI-1880	Cha	*p*-methoxyphenyl	85
KMI-1882	Leu	*m*-methoxyphenyl	84
KMI-1884	Cha	*m*-methoxyphenyl	77
KMI-1725	Leu	2,4-diiodophenyl	91
KMI-1777	Cha	2,4-diiodophenyl	96
KMI-1896	Leu	3,5-difluorophenyl	86
KMI-1898	Cha	3,5-difluorophenyl	91

Cha: cyclohexylalanine

Cutaway views of BACE1 inhibitor complexes

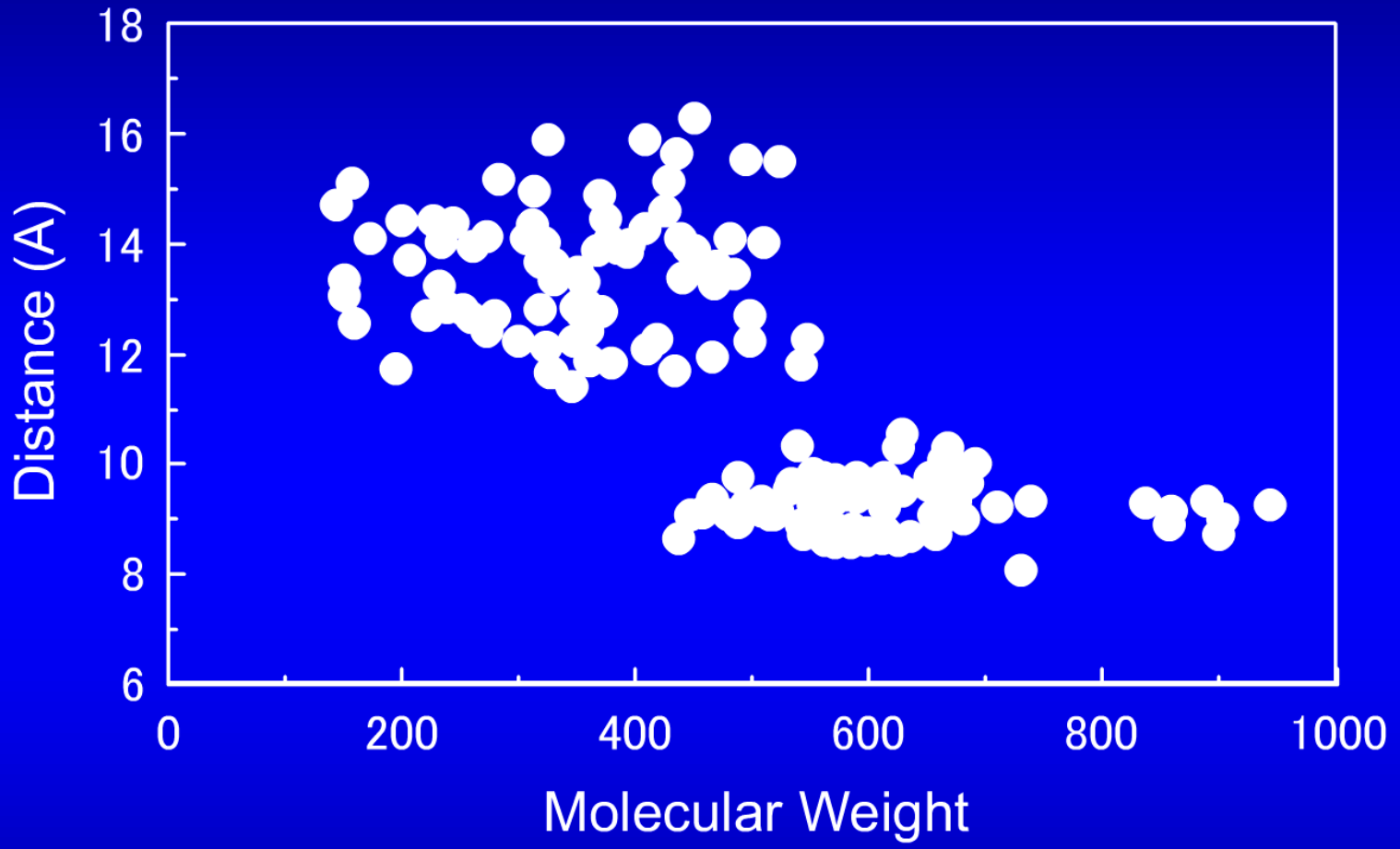


Closed flap form
(PDB ID: 2B8L)



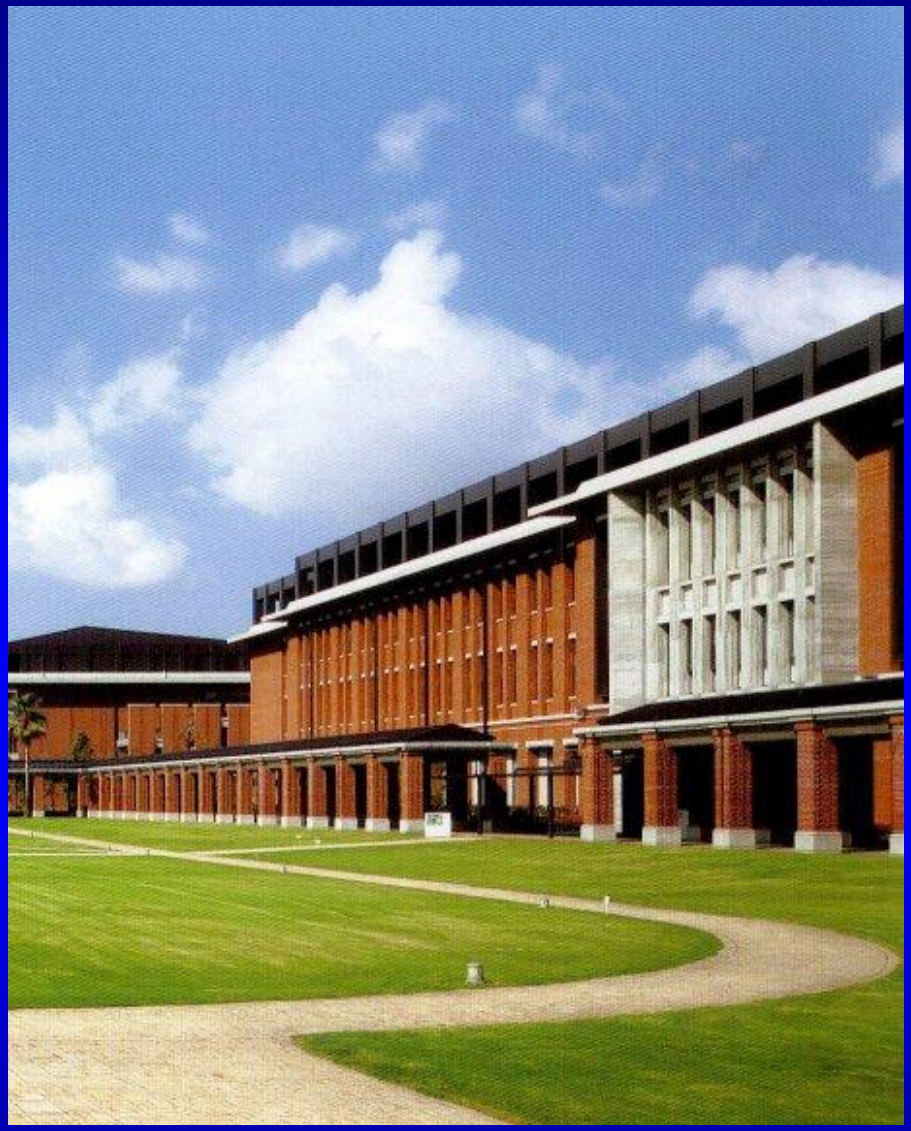
Opened flap form
(PDB ID: 2QU2)

The position of flap domain and MWs of inhibitors/ligands in X-ray crystal structures of BACE1-inhibitor complexes



The distances between α-carbons of Thr72 (flap side) and Thr231 (cleft side) and molecular weights of inhibitors/ligands docked in BACE1 were plotted in the scatter chart.

Y. Hamada *et al*.: Advances in the identification of β-secretase inhibitors. *Expert Opinion on Drug Discovery*, **2013**, *8*, 903-922.



薬学の未来をつくる
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