

# Binary Bacterial Toxins

## C2- and VIP-Toxin

**Michael Leuber**

Biotechnology

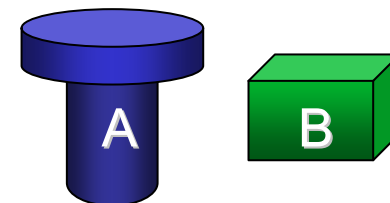
University Würzburg

## Bacterial Toxins

- (1) Porin-like toxins
- (2) Toxins that bind to or modify existing ion channels
- (3) Toxins with intracellular target that form a channel and translocate a second component, i.e. binary toxins

## Binary Toxins

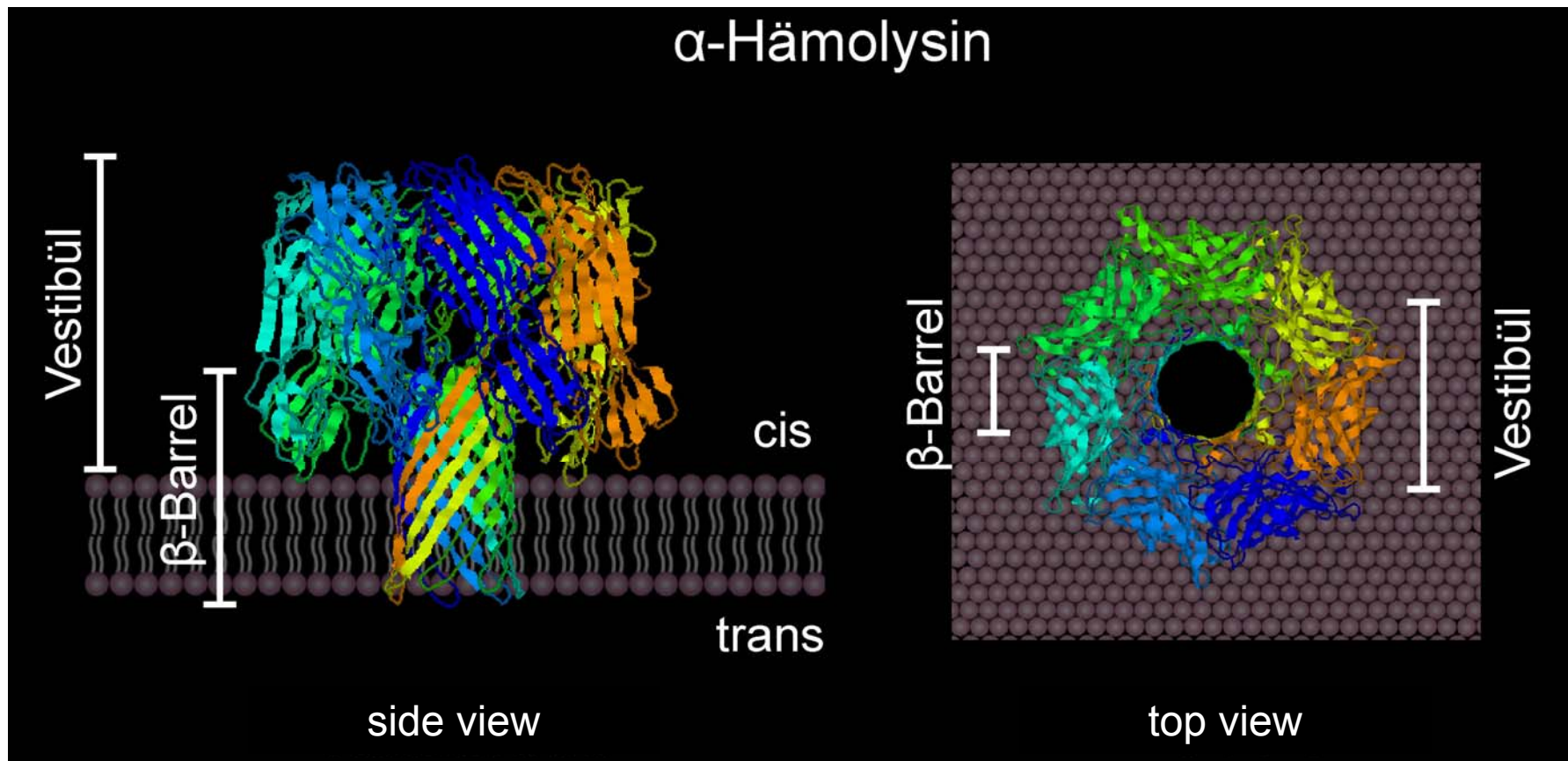
- The toxin contains both enzyme (A) and transport function (B), for example adenylate cyclase toxin (ACT, CyaA) of *Bordetella pertussis*.
- Toxin and transport function are secreted separately, e.g. C2- and Anthrax toxin



## Binary Toxins – Overview

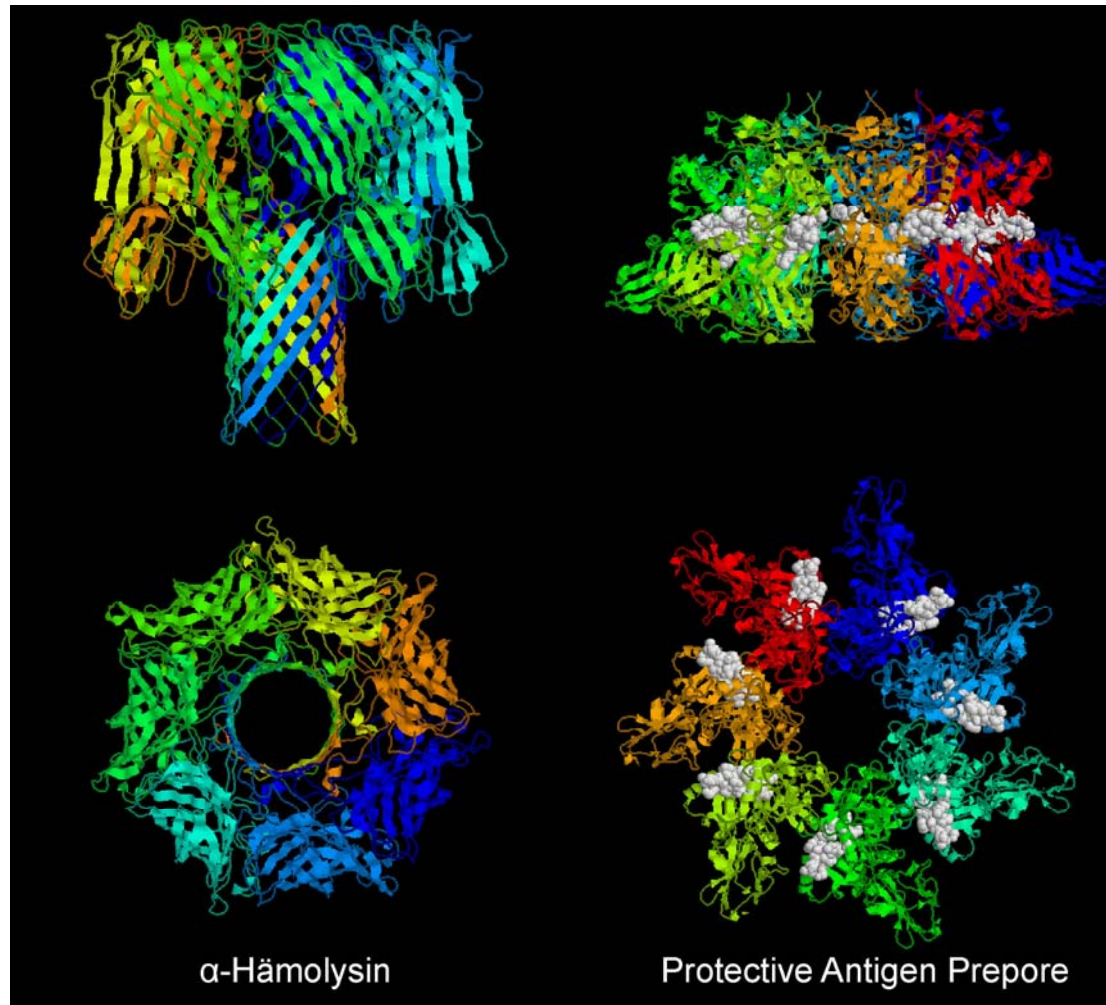
- C2-Toxin\* *Clostridium botulinum* (A; B)
- Iota-Toxin *Clostridium perfringens* (A; B)
- Toxin A/B *Clostridium difficile* (A-B)
- Epsilon-Toxin *Clostridium perfringens* (A?; B)
- Anthrax Toxin *Bacillus anthracis* (A1, A2; B)
- VIP-Toxin\* *Bacillus thuringiensis* (A; B)  
*Bacillus cereus* (A; B)
- CyaA (ACT) *Bordetella pertussis* (A-B)

# Binary Toxins – Our Working Model



Adapted from: Song *et al.*, 1996

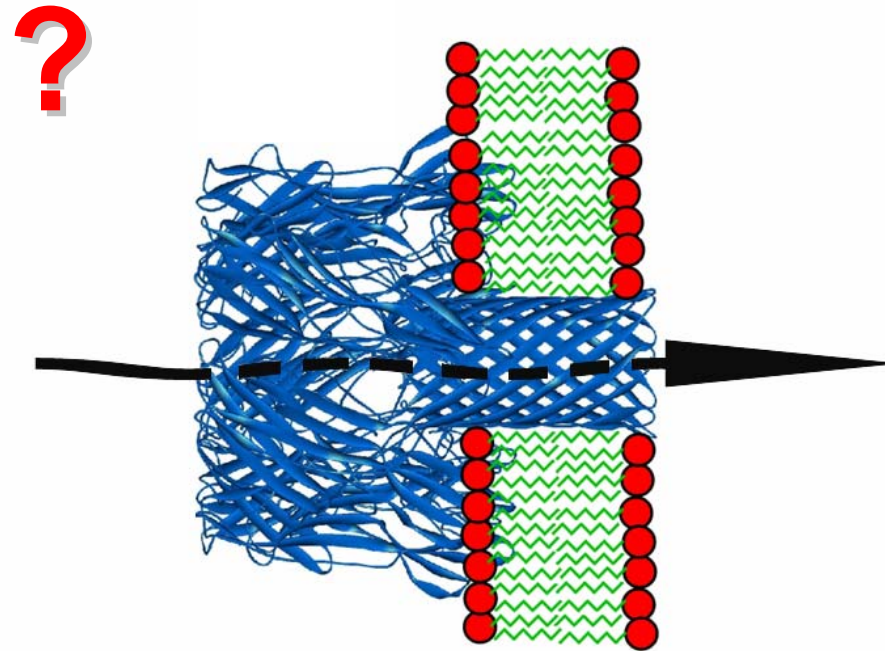
## Binary Toxins – Our Working Model



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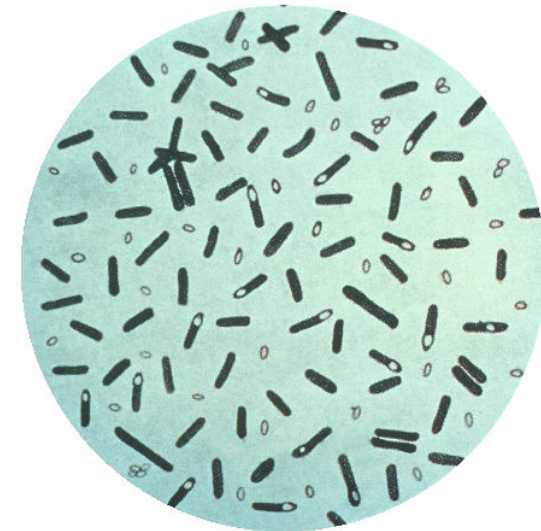
Enzymatic component (A) to  
be delivered into target cells



Heptameric binding  
component (B) inserted in  
artificial bilayer membrane

## C2-Toxin of *Clostridium botulinum*

- First recognized and isolated in 1896 by Emile van Ermengem
- Gram-positive, spore forming, rod-shaped, anaerobic soil bacteria
- The spores can survive in most environments and are hard to kill
- *C. botulinum* produces various types of extremely potent neurotoxins (BoNT). Beside these neurotoxins, certain strains produce the exotoxin C2 and the exoenzyme C3

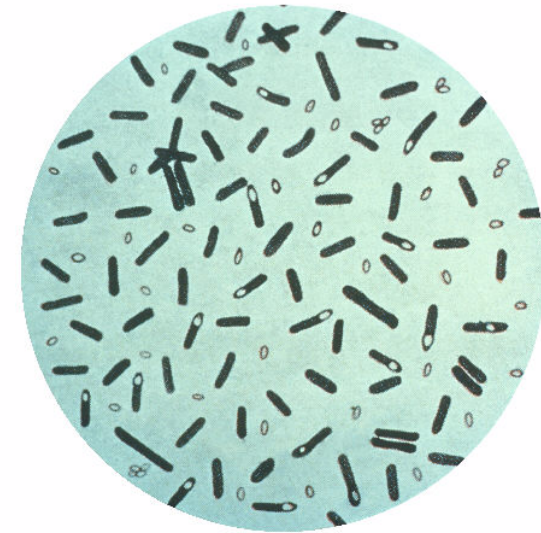


Center for Disease Control and Prevention,  
Department of Health and Human Services US



## C2-Toxin of *Clostridium botulinum*

- C2-toxin seems not to be involved in botulism but also acts lethal when applied to animals (LD<sub>50</sub> (i.v.) for mice is less than 50 fmol)
- Main effect after i.v. application is a decrease in vascular barrier functions of the endothelium, resulting in decreased blood pressure and edema

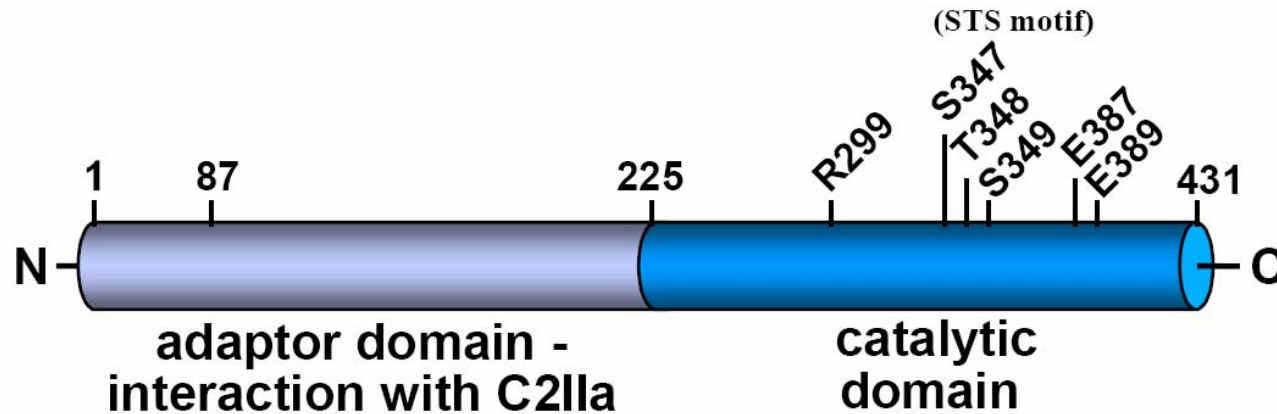


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# C2-Toxin

## ➤ Enzymatic component: C2I

- 50 kDa protein, 2 domains, ADP-ribosyltransferase activity

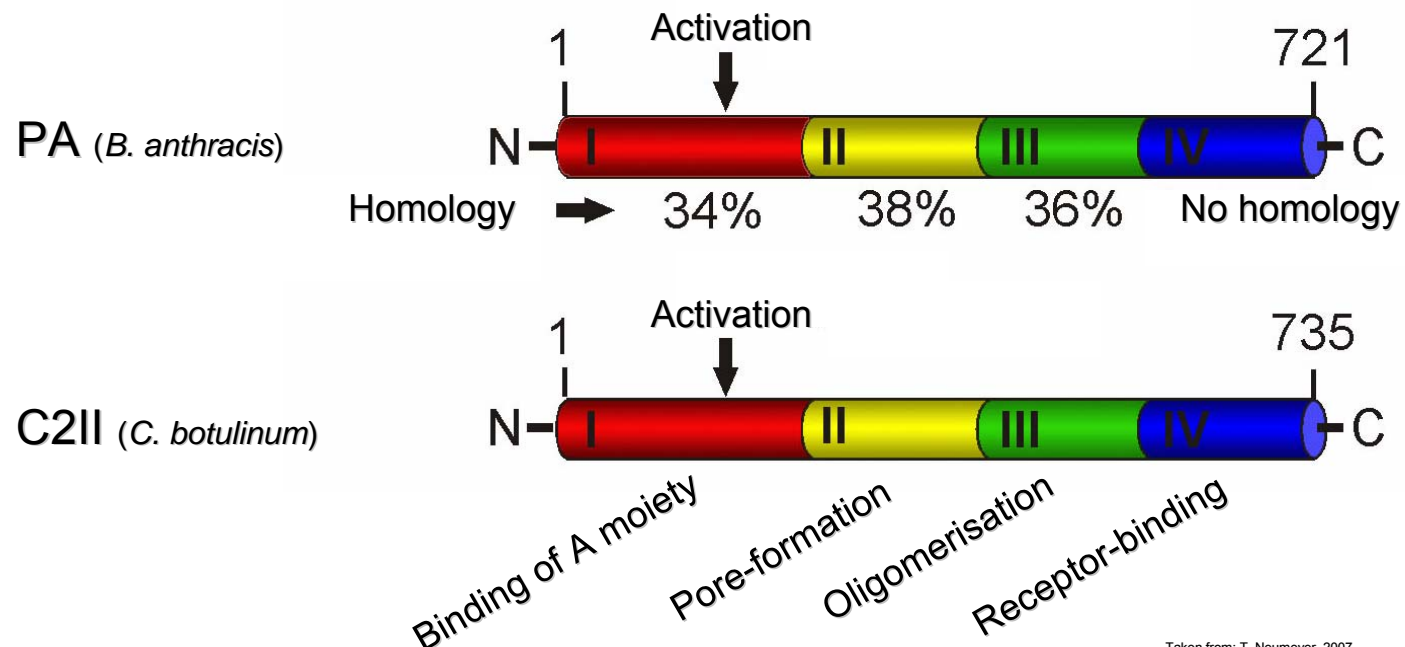


Akories & Barth, 2004

## C2-Toxin

### ➤ Binding component: C2II

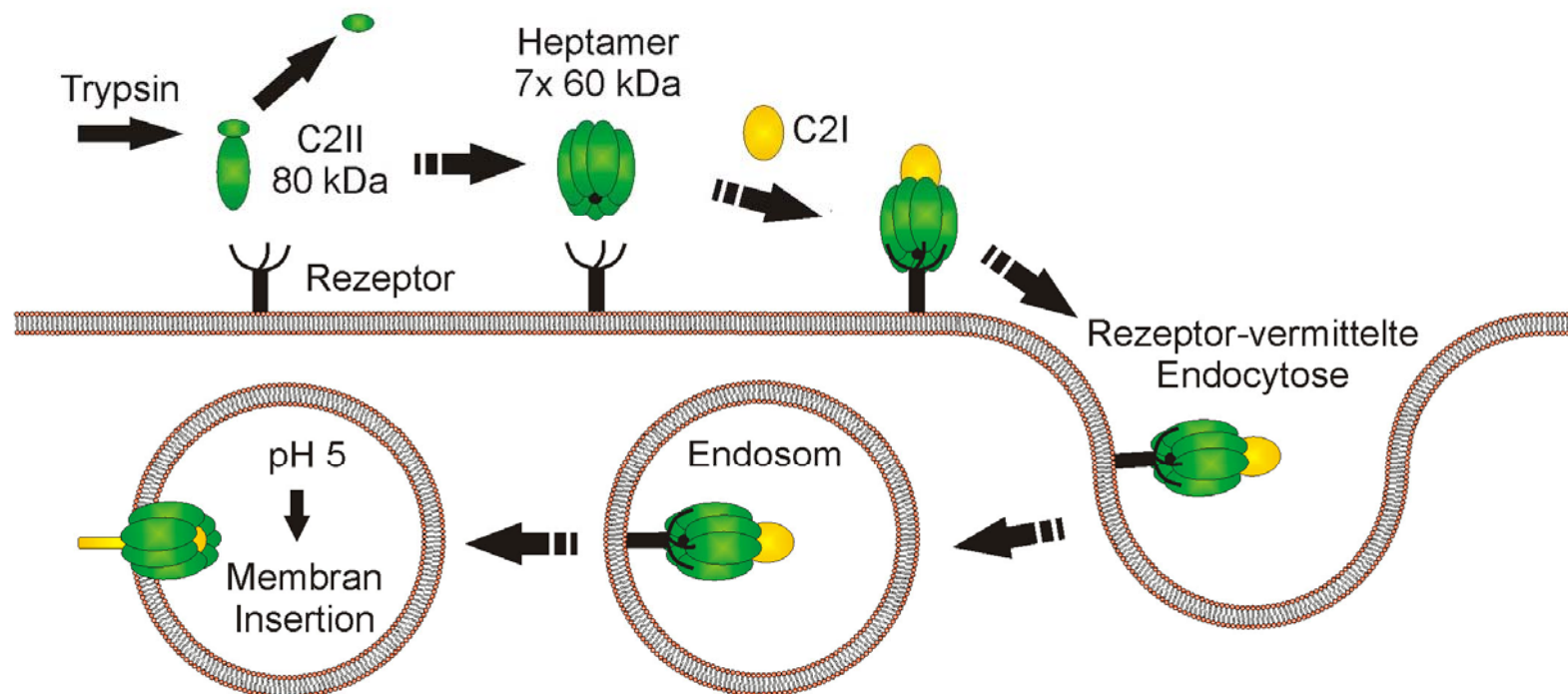
- 80 kDa protein, 4 domains, distinct homology to other binary toxins' binding components, such as PA, Vip1Ac, Iota b, and to  $\beta$ -barrel PFTs like  $\alpha$ -Hemolysin.



Taken from: T. Neumeyer, 2007

## C2-Toxin

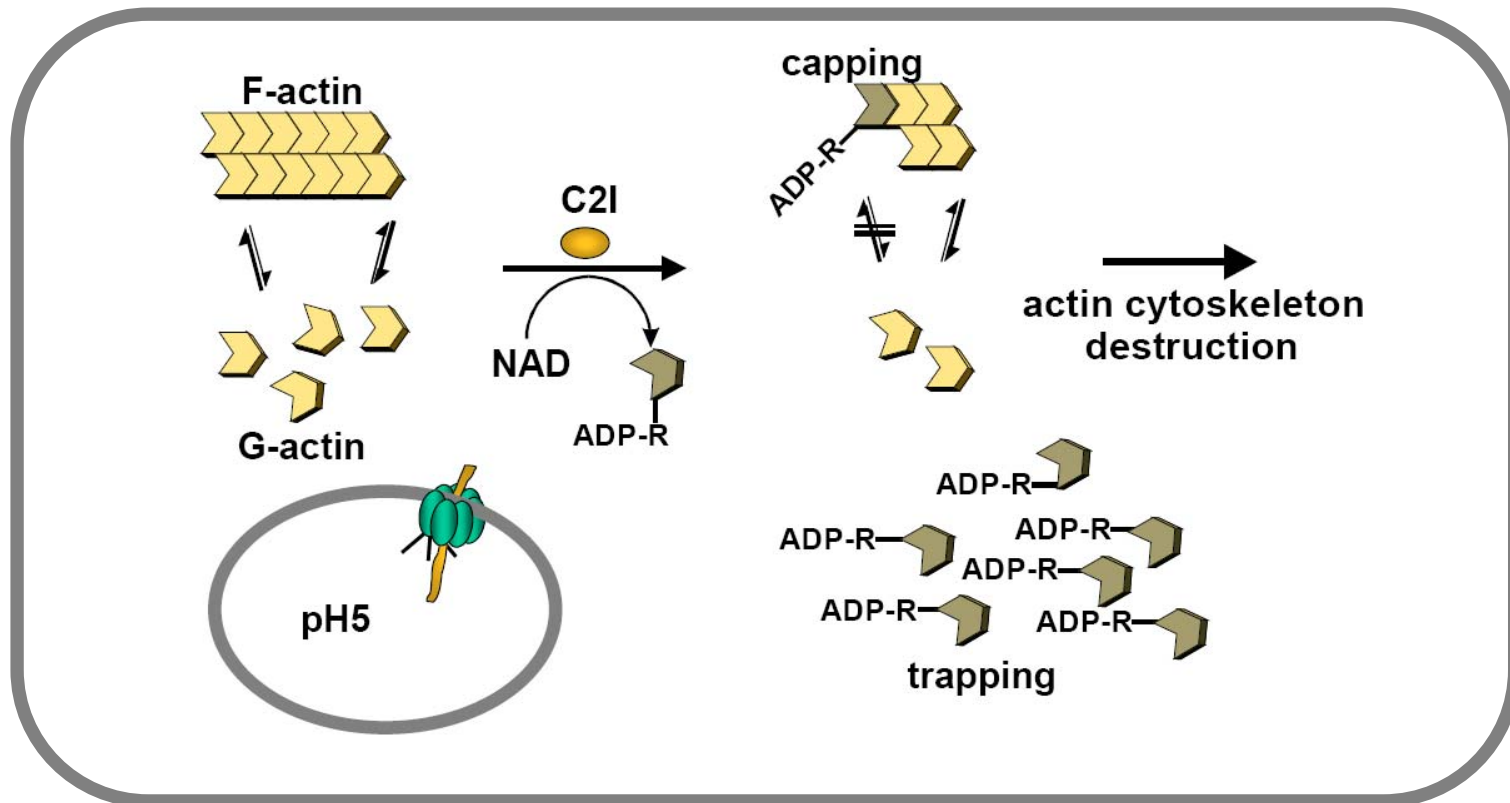
### ➤ Intoxication – binding and internalization



Taken from: T. Neumeyer, 2007

## C2-Toxin

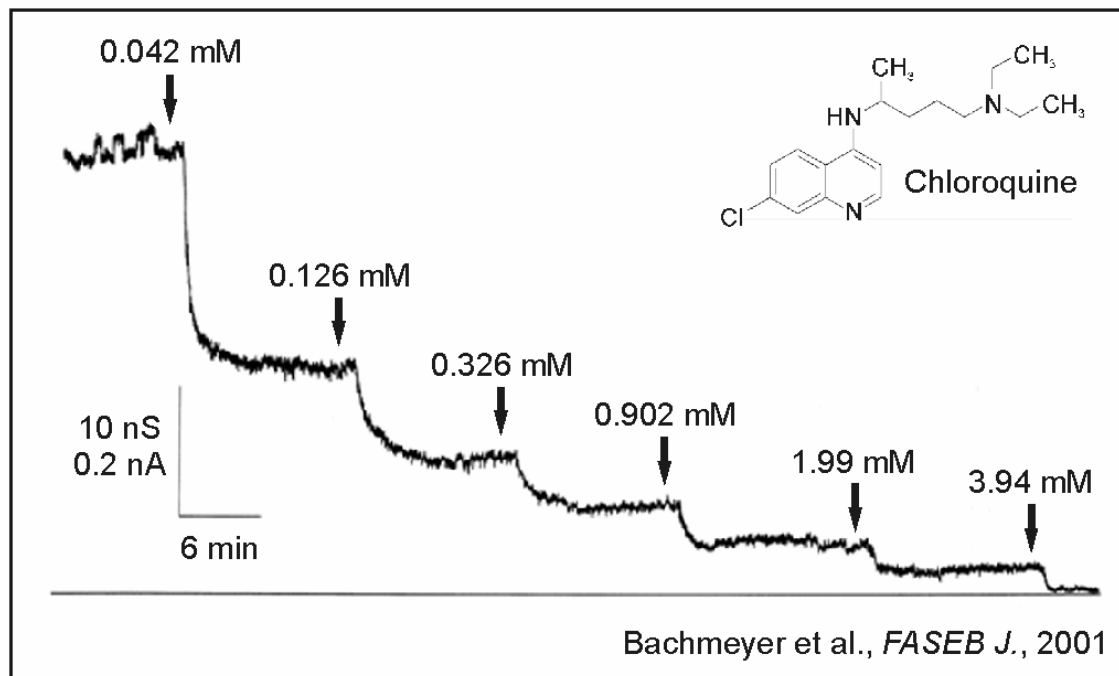
- Intoxication – enzymatic reaction of C2I



Akories & Barth, 2004

## C2-Toxin

- Inhibition of intoxication by chloroquine

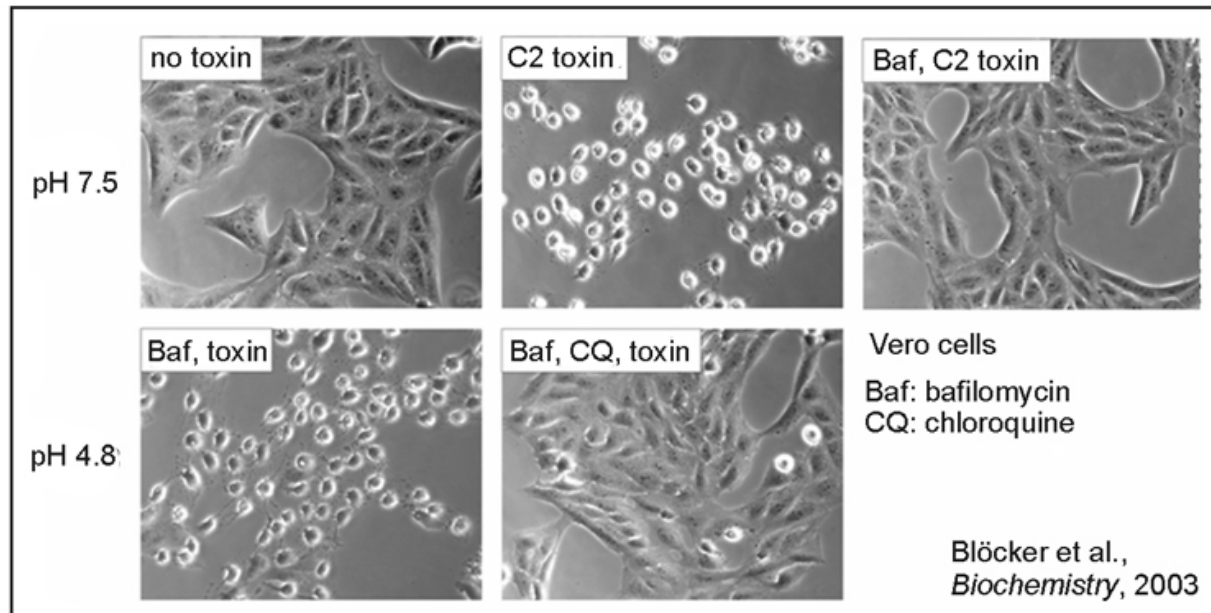


Titration experiments with chloroquine and related compounds resulted in a decrease of membrane conductance.

$$K_S = 44 \mu\text{M}$$

## C2-Toxin

- Inhibition of intoxication by chloroquine



Chloroquine is able to prevent Vero cells from toxin induced cell rounding and cell death.

$K_S = 23 \mu\text{M}$  (% inhibition)

## C2-Toxin

### ➤ Biophysical properties

- Single channel conductance depends on ionic strength of salt solution (single channel conductance in 150mM KCl = 40 pS).
- Channels are cation-selective ( $P_{\text{cation}}/P_{\text{anion}} = 11$ )
- Membrane inserted channels bind 4-aminoquinolines, e.g. chloroquine, which blocks the channel. This binding also is ionic strength dependent.
- Chloroquine is twofold positive charged



Negatively charged amino acids are involved in binding and contribute to biophysical properties.



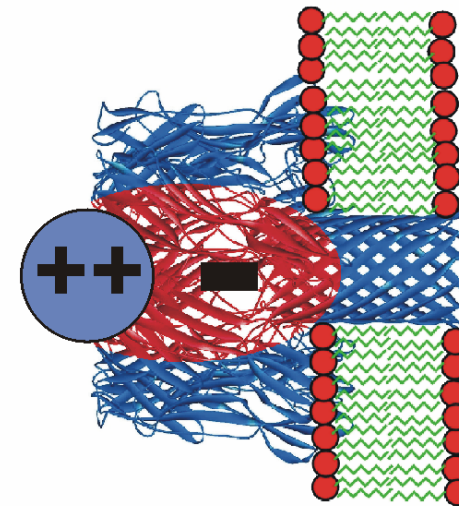
## C2-Toxin

### ➤ Biophysical properties

- Furthermore binding is asymmetric with respect to the side of addition (binding resulting from addition of chloroquine to the cis side is much stronger).



Binding site is localized inside the vestibule on the cis side of the mushroom-shaped heptamer.



# C2-Toxin

## ➤ Sequence comparison – site-directed mutagenesis

```

AYPxVxVxMExxILSKNExxSx-SxxSxxSxxxxxxxxTxxxxVxA--GxxxxxxxxGFxxGxSxSYxHSxxx-xTVxxDxxxSxxNTx-----xxxNTAxx
272 AFPSVNVSMExKVLILSPNENLSN-SVESHSSTNWSYTNTEGASVEA--GIG---PKGISFGVSVNYQHSE----TVAQEWGTSTGNNTS-----QFNTASA 355
272 AFPSINVDMRKMILSKDSNLSN-SAEAHSNNSYTYANSEGASIEA--GFG---PKGFSFGVVSANYQHTE----TVGSDWGNSSKSNTE-----QFNSSASA 355
256 AYPVVGVMENLIISTNEHASS-DQGKTVSRATTNSKTDANTVGV--SISAGYQNGFTGNITTSYSHTTDN-STAVQDSNGESWNTG-----LSINKGES 346
258 AYPVIVHVDMENIILSKNEDQSTQNTDSQTRTISKNTSISRHTTSEVHGNAEVHASFFDIGGSVSAGFSNSNSSTVAIDHSLSLAGERTWAETMGLNTADT 357
263 AYPVIVGVQMERLVVSKSEITITGDSTKS-MSKSTSHSSININTVGAEVSGSLQLAGGIFPVFMSMA--SANYSHTWQNTSTVDDTTGSEFSQGLSINTGES 359
  
```

● E272 ● E280

▲ transmembrane domain ▲

● D341 ● E346  
● D342

```

AYLNANVRYxNTGTAPIYxVxPTTSxVL-xxxTxATIKAKxNxxxxLxPGxxYFxxKGxxPIALNTMDDFNSxPITxNYxQLxxLxNxKxxxxLxTxQxxG
356 GYLNANVRYNNVGTGAIYDVKPTTSFVL--NNDTIATITAKSNSTALNISPGESYFPPKKGQNGIAITSMDDFNHSHPIITLNKKQVDNLLNKPMMLETNQTGD 454
356 GYLNANVHYNNVGTGGIYDAQPTTSFIL--QDSTIATITAKSNATALSIPSGDRYP-ASKEGISLKTMDFNHSHPIITLNKPQLDAVLNNEVIKINTDQTDG 453
347 AYINANVRYNTGTAPMYKVTPTTNLVL--DGETLATIKAQDNQIGNNLSPNETYFPPKGLSPLALNTMDQFNARLIPINYDQLKKLDSGKQIKLETTQVSG 445
358 ARLNANIRYVNTGTAPIYNVLPPTSLVLGKNQTLATIKAKENQLSQILAPNNYFPPSKNLAPIALNAQDDFSSTPITMNYNQFLELEKTKQLRLDQVYG 457
360 AYINPNIRYNTGTAPVYNVTPTTTIVI--DKQSVATIKGQESLIGDYLNPPGGTYPIIGEPALNTMDQESSRLIPINYNQLKSIDNGGTVMLSISQFTG 458
  
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● E399

● D426 ● F428 ●

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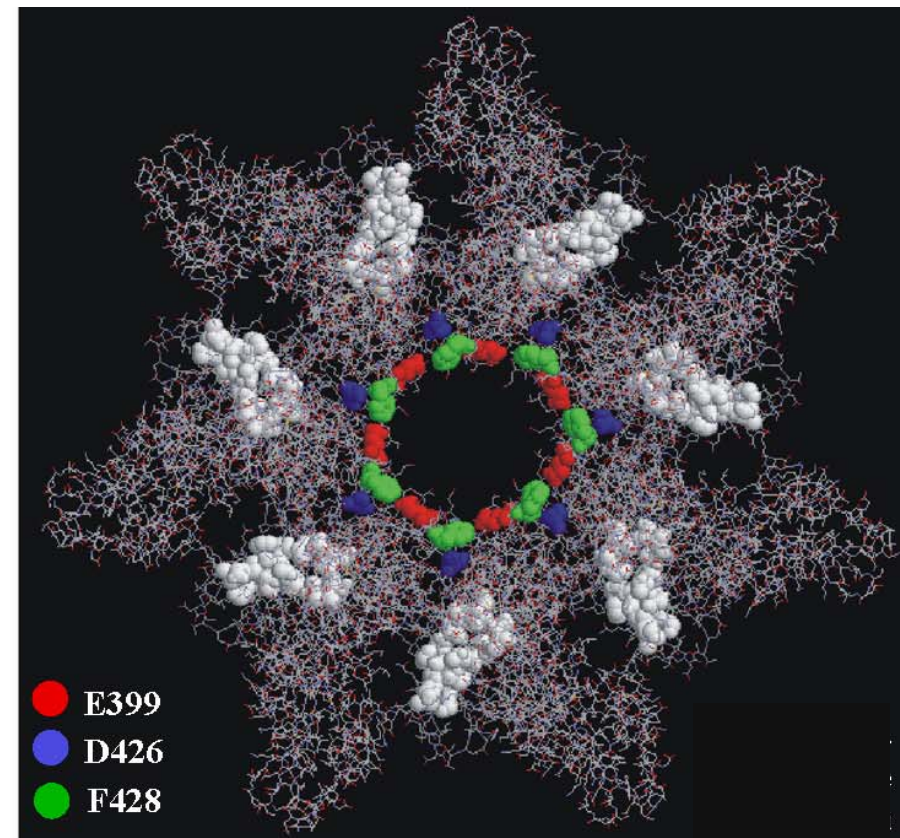
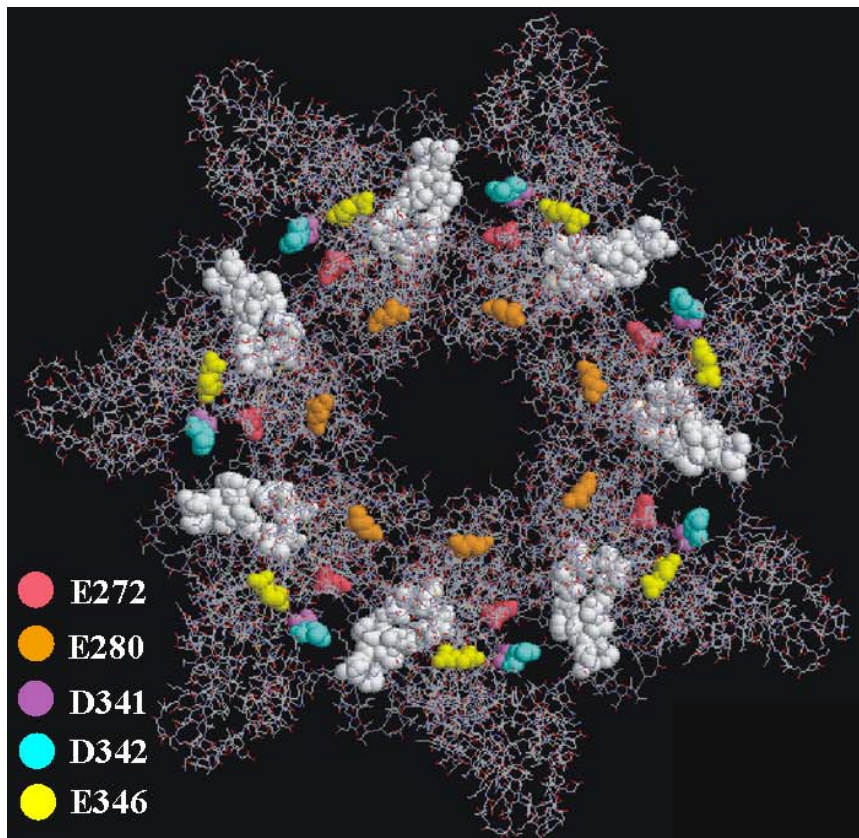
NYxxxN-xxGxxxTx-GxxWSxxIxQIKxxT
455 VYKIKD-THGNIVT--GGEWNGVIQQIKAKT 482
454 RYGIIG-VDGKAEI--GDRWSPIDEIKGRT 481
445 NYGTKN-SQGQIITE-GNSWSNYISQIDSVS 473
458 NIATYNFENGRVRVDTGSNWSEVLPQIQETT 487
459 NFAKYN-SNGNLVTD-GNNWGPYLGTIKSTT 486
  
```

Consensus

VIP1 Aa	<i>B. cereus</i>
VIP1 Ac	<i>B. thuringiensis</i>
Iota b	<i>C. perfringens</i>
PA	<i>B. anthracis</i>
C2	<i>C. botulinum</i>

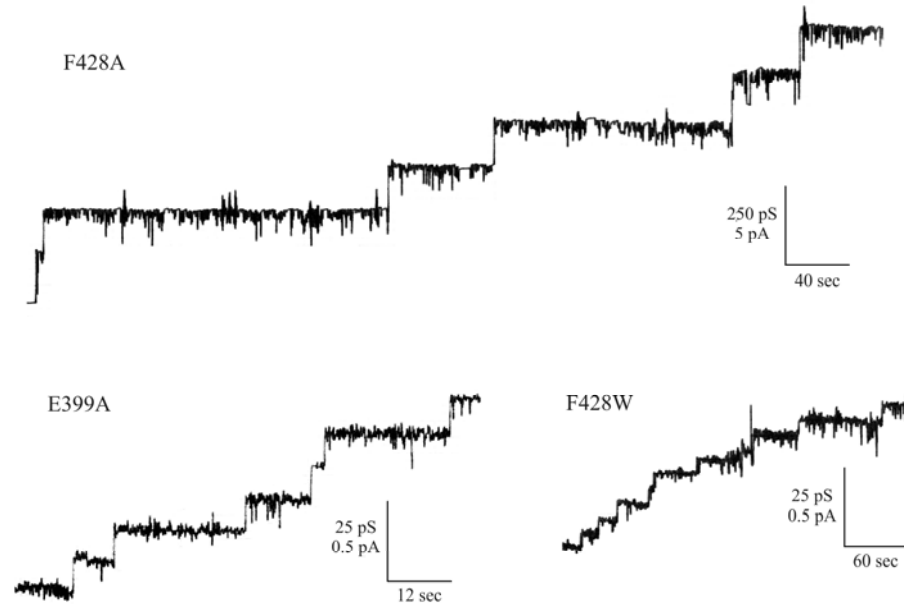
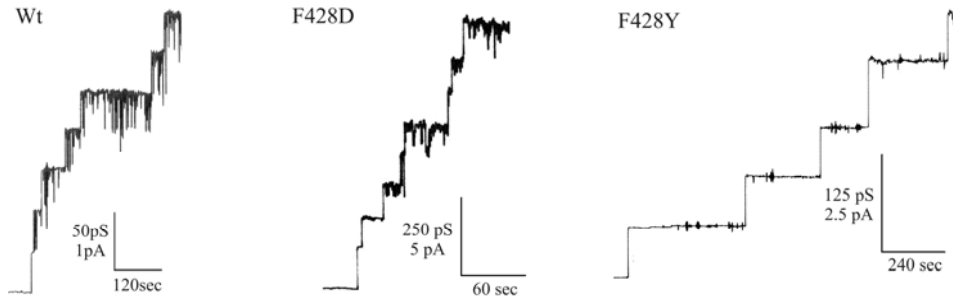
## C2-Toxin

- Position of the mutated amino acids inside the prepore



Taken from: T. Neumeyer, 2007

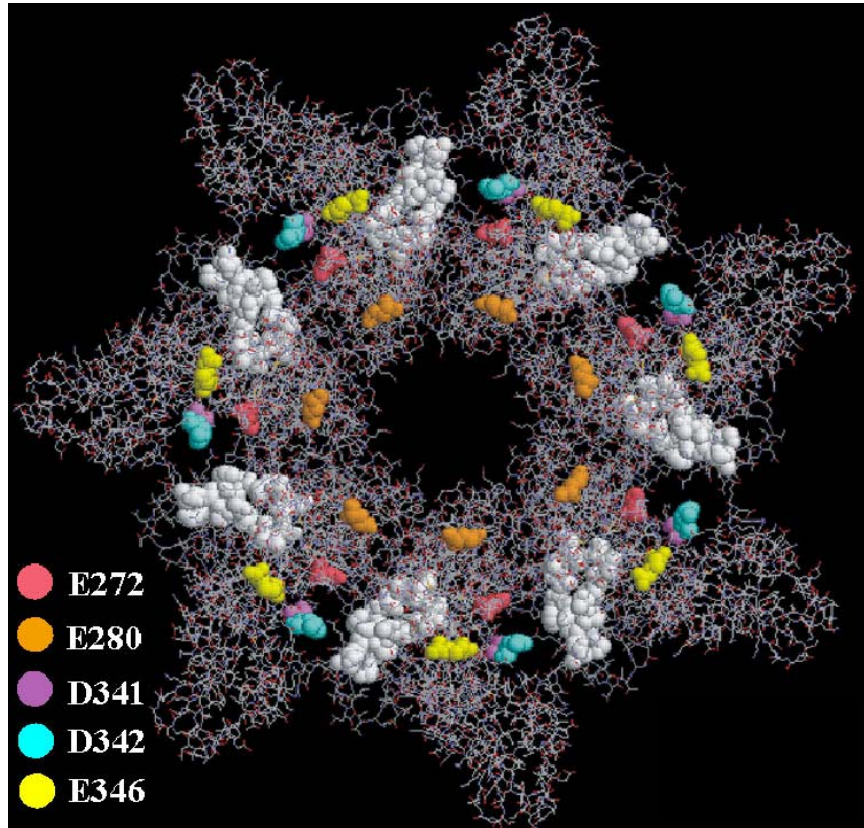
→ Single channel conductance of the C2II-mutants



	KCl-Konzentration [M]	0,15	1,0
Einzelkanalleitfähigkeit G [pS]	Wildtyp	40	130
	E272A	35	100
	E280C	45	100
	D341A	35	n.b.
	D342C	40	100
	E346A	30	125
	D341A E346A	25	100

	KCl-Konzentration [M]	0,15	1,0
Einzelkanalleitfähigkeit G [pS]	Wildtyp	40	130
	E399A	13 ± 2	80 ± 11
	D426A	20 ± 8	100 ± 28
	F428A	140 ± 18	500
	F428D	110 ± 22	600
	F428Y	60	210
	F428W	5 ± 1	n.b.
	E399A D426A	14 ± 2	n.b.
	E399A F428A	60	n.b.
	D426A F428A	40	370 ± 44
	E399A D426A F428A	30 ± 4	330 ± 55

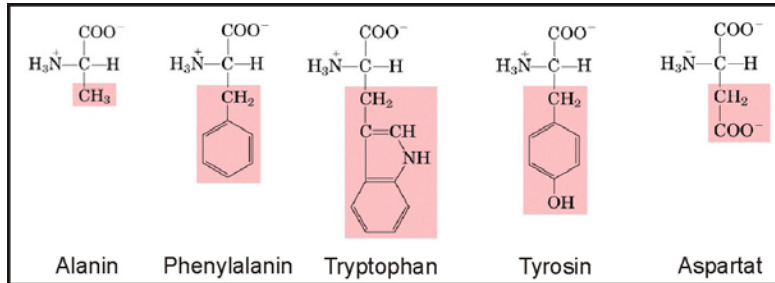
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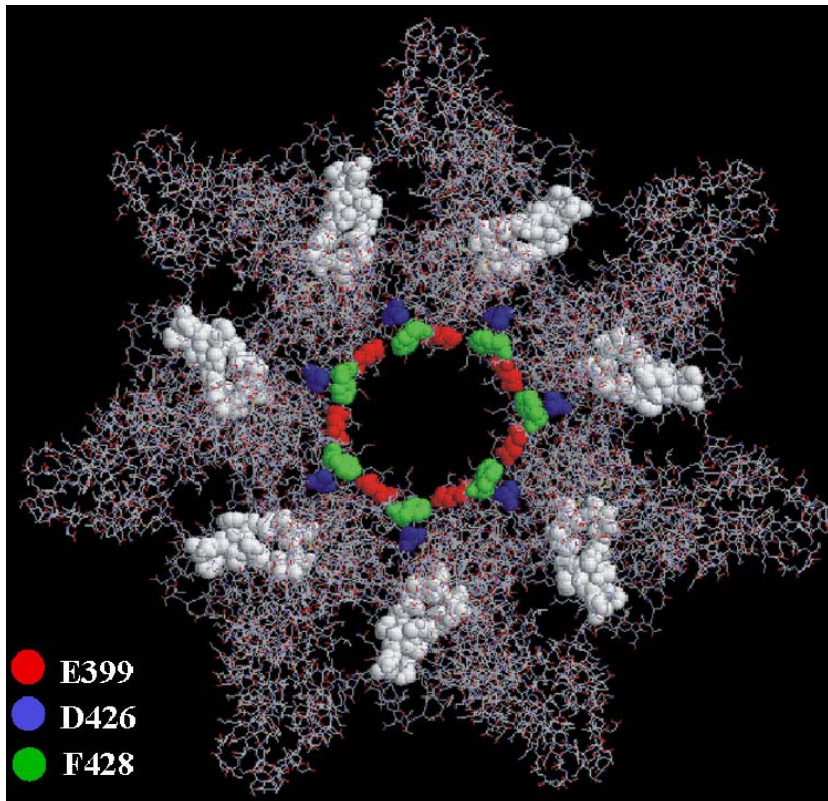
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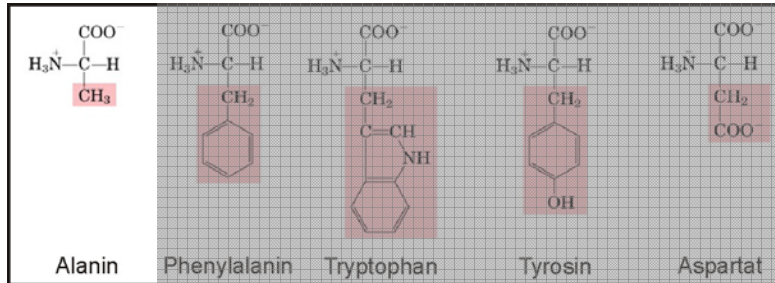


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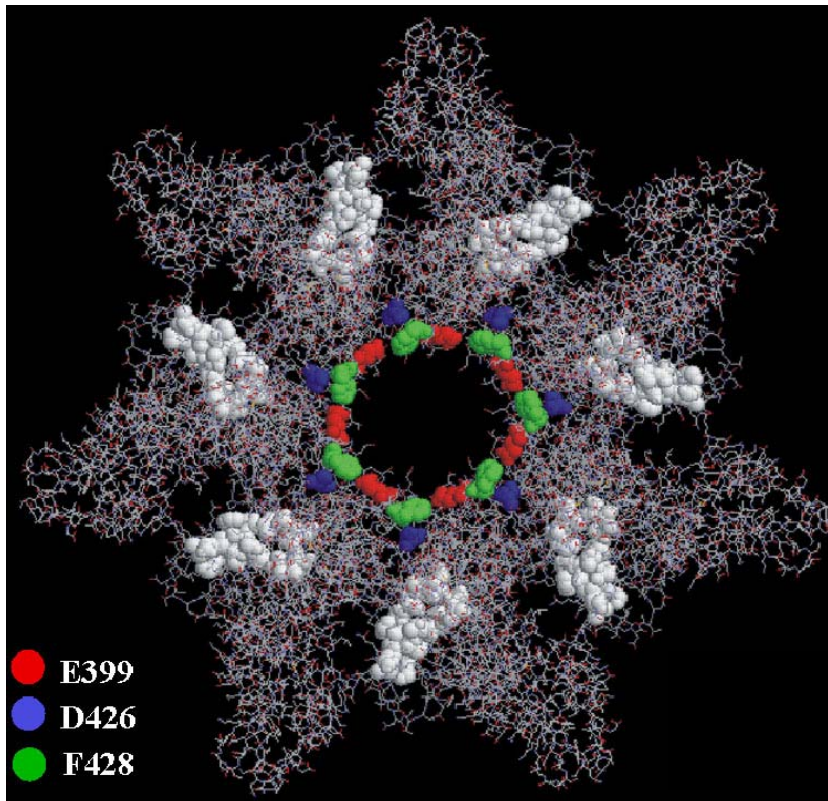


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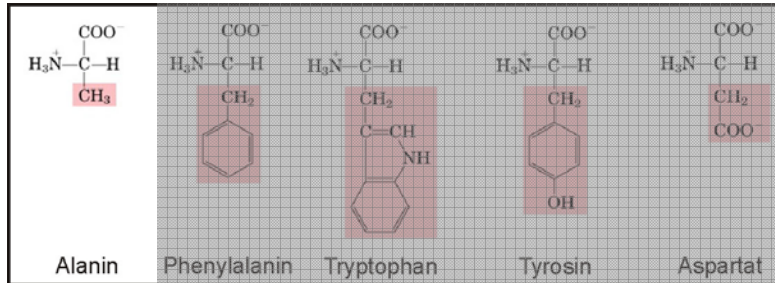


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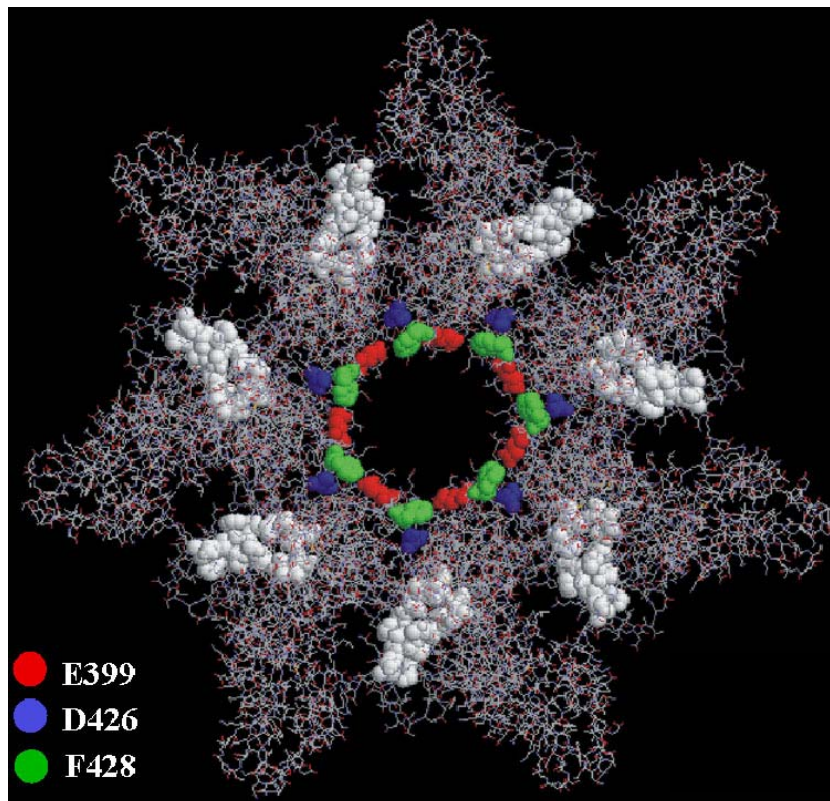


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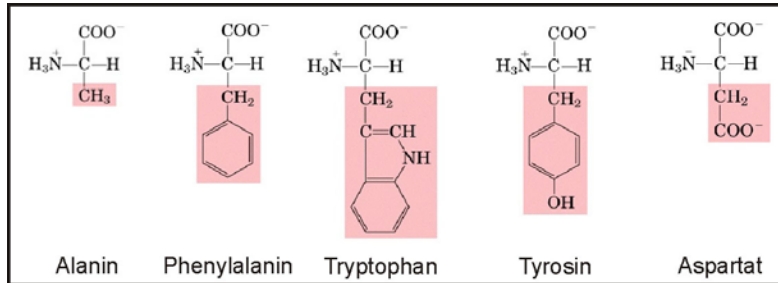
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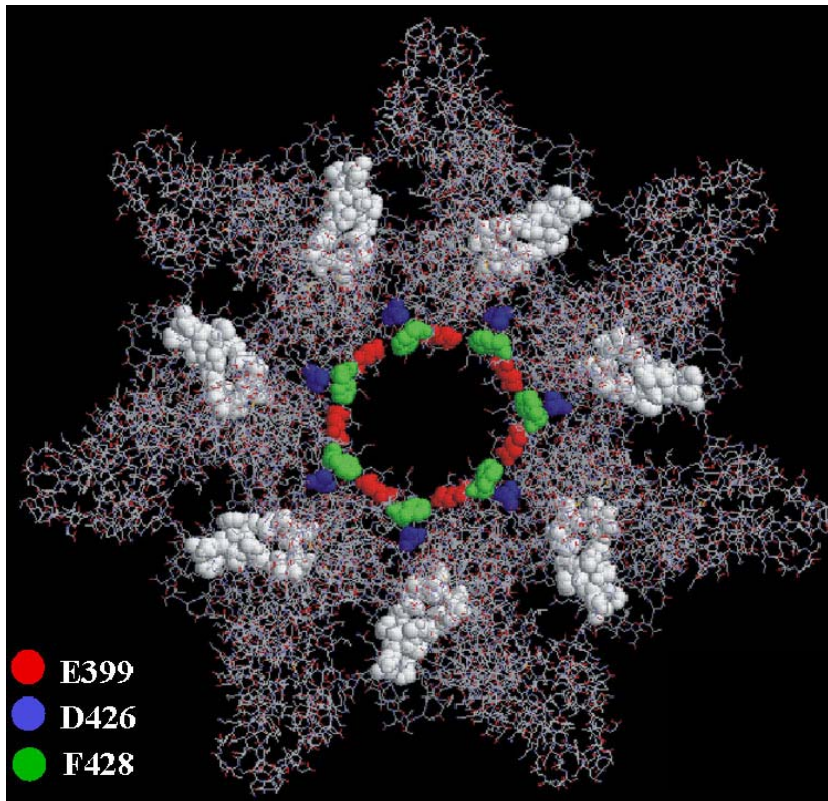
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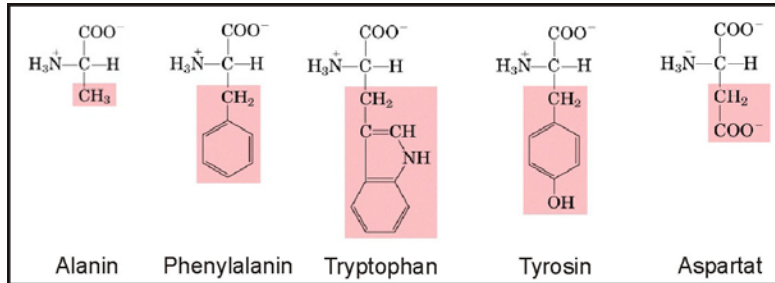


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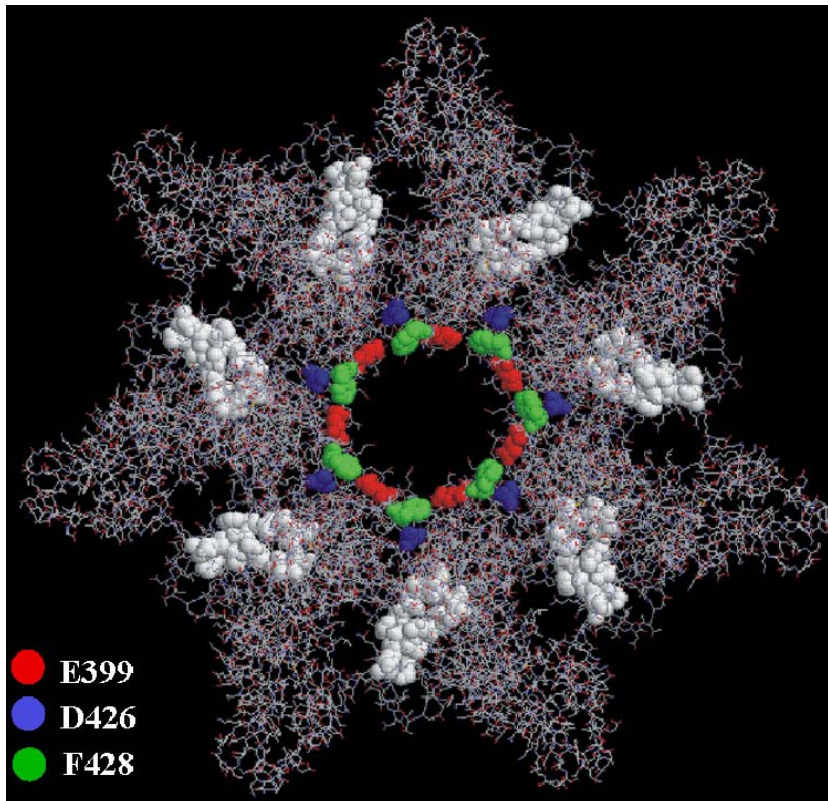


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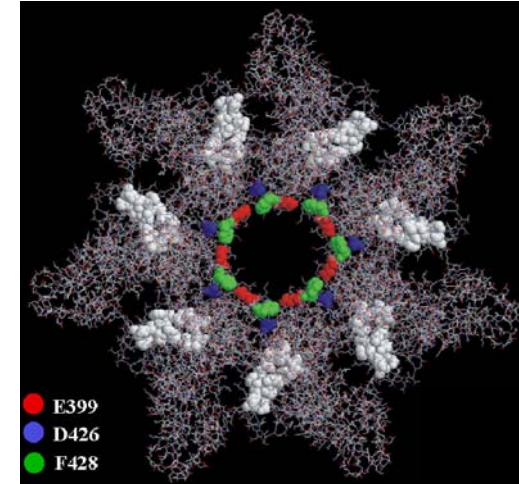
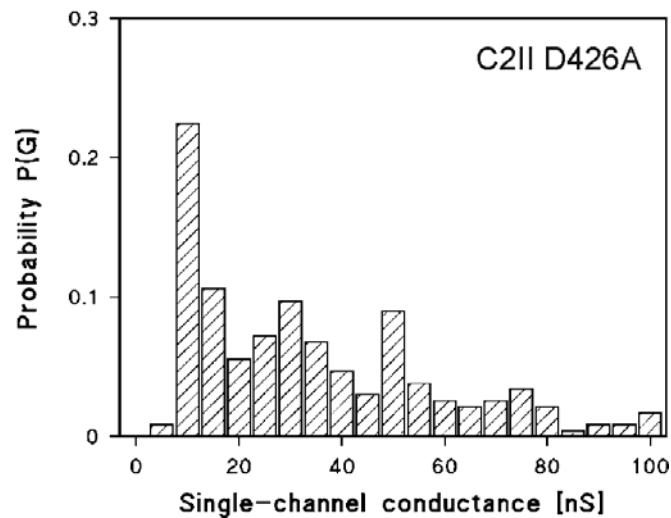
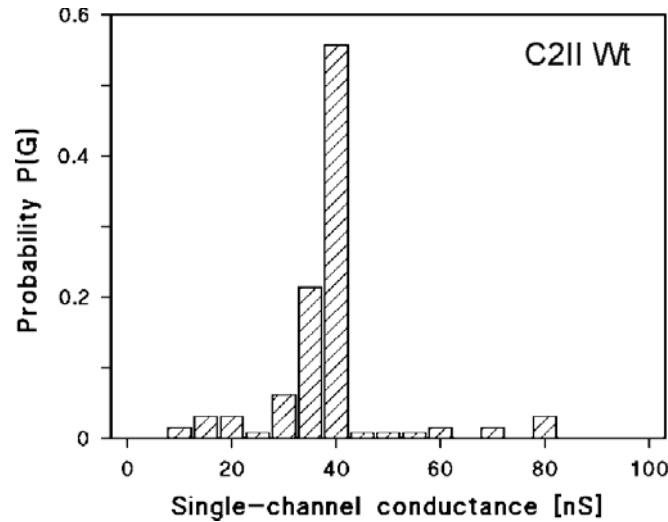


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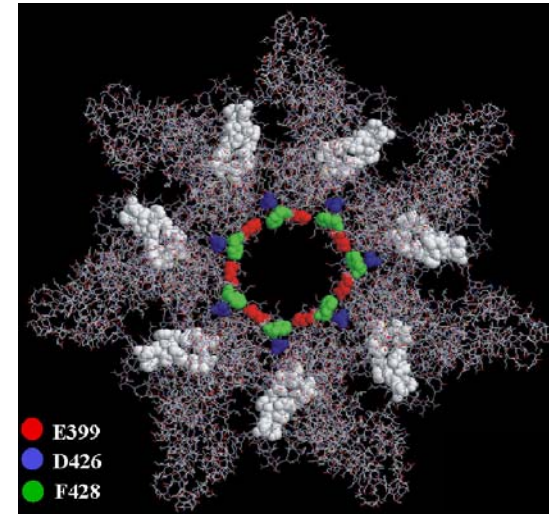
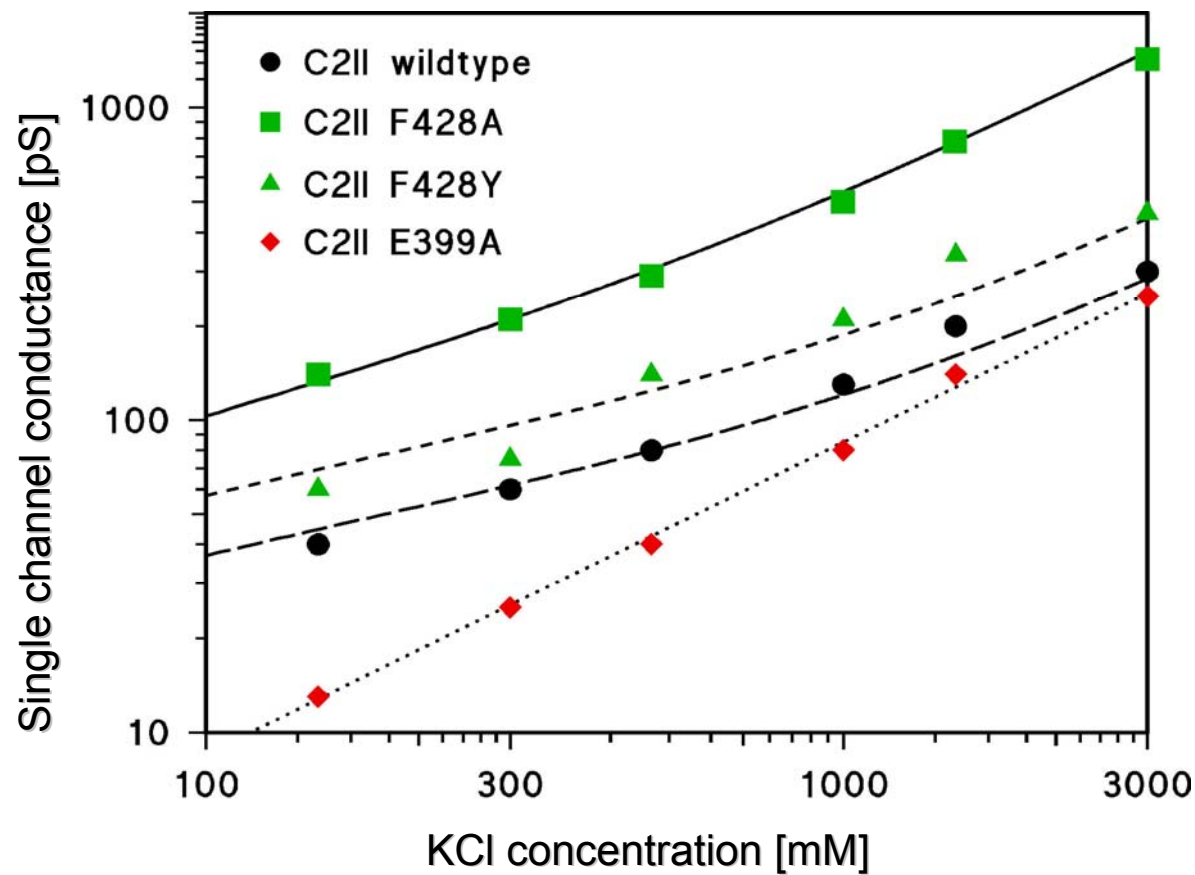
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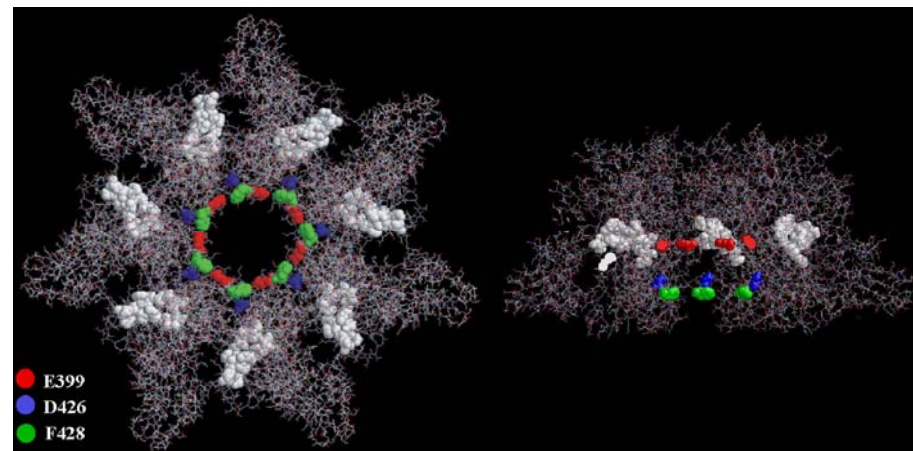
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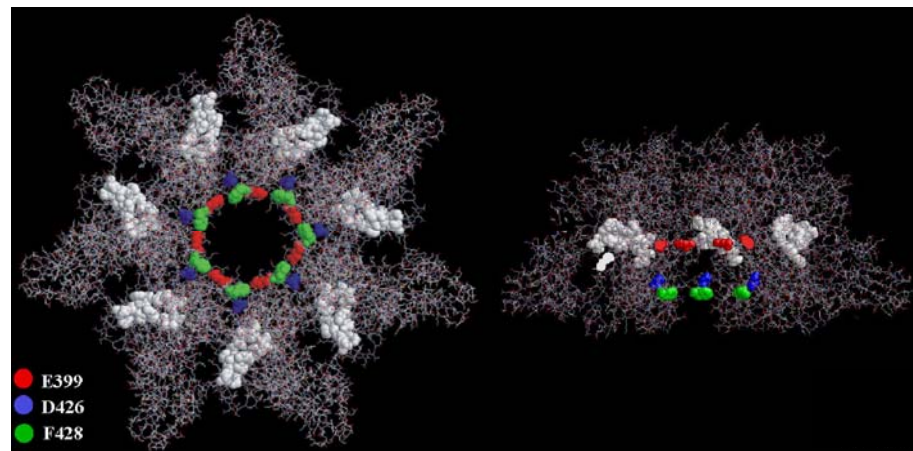
→ Selectivity of the C2II-mutants

C2II		Zero-current membrane potential $V_m$ (mV)	Selectivity $P_c/P_a$
Wildtyp		-	11
E399A	●	19	5,6
D426A	●	13	3,0
F428A	●	20	7,0
E399A F428A	●●	19	5,7
D426A F428A	●●	16	4,0
E399A D426A F428A		12	2,5



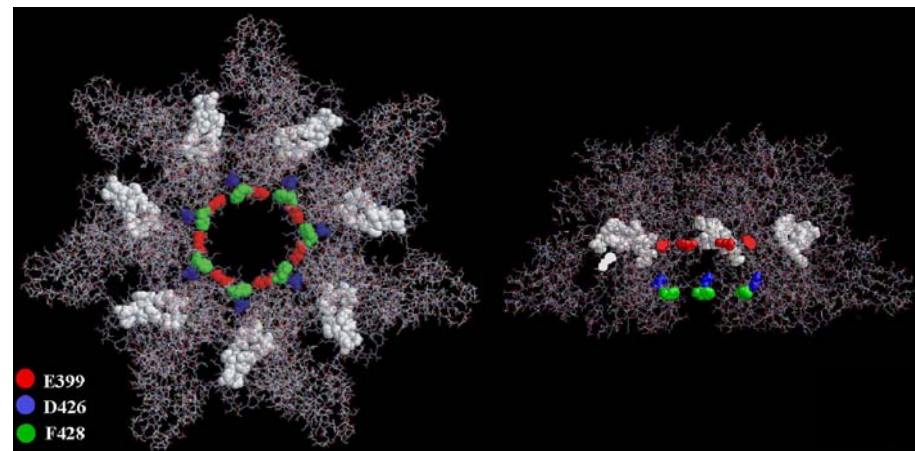
→ Selectivity of the C2II-mutants

C2II	Zero-current membrane potential $V_m$ (mV)	Selectivity $P_c/P_a$
Wildtyp	-	11
E399A <span style="color: red;">●</span>	19	5,6
D426A <span style="color: blue;">●</span>	13	3,0
F428A <span style="color: green;">●</span>	20	7,0
E399A F428A <span style="color: red;">●</span> <span style="color: green;">●</span>	19	5,7
D426A F428A <span style="color: blue;">●</span> <span style="color: green;">●</span>	16	4,0
E399A D426A F428A	12	2,5

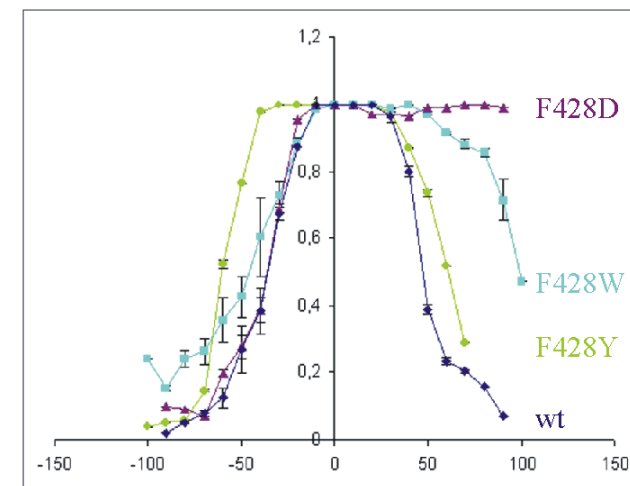
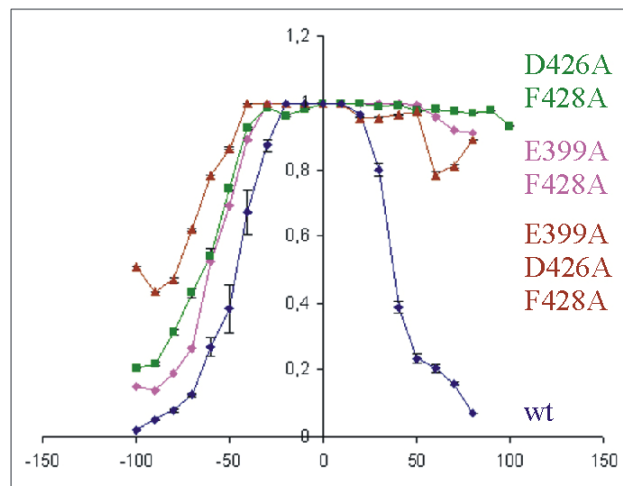
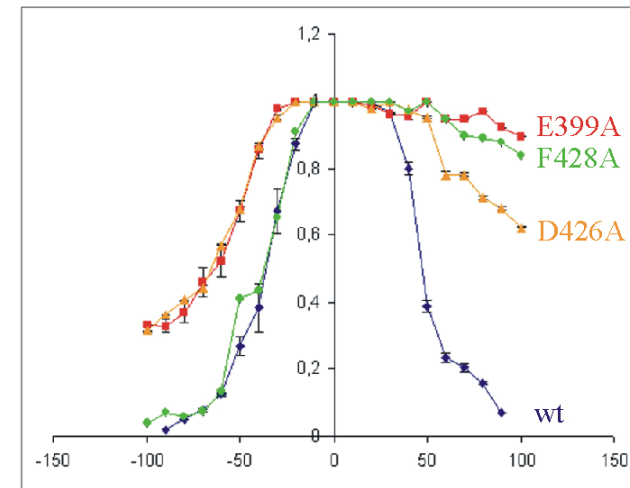
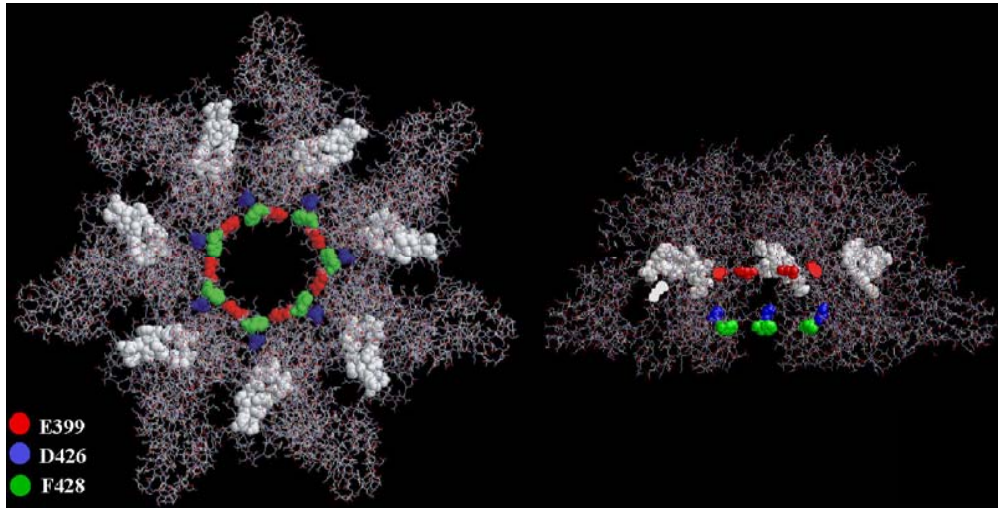


→ Selectivity of the C2II-mutants

C2II	Zero-current membrane potential $V_m$ (mV)	Selectivity $P_c/P_a$
Wildtyp	-	11
E399A ●	19	5,6
D426A ●	13	3,0
F428A ●	20	7,0
E399A F428A ●●	19	5,7
D426A F428A ●●	16	4,0
E399A D426A F428A .	12	2,5

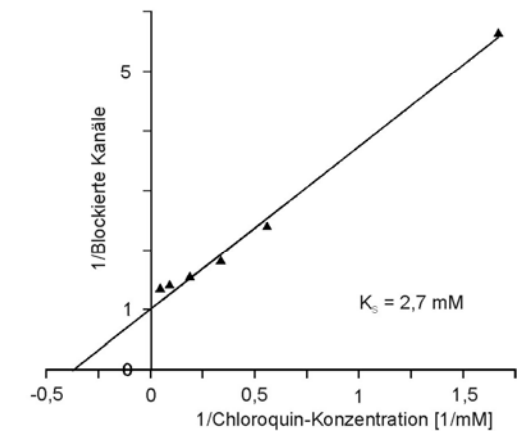
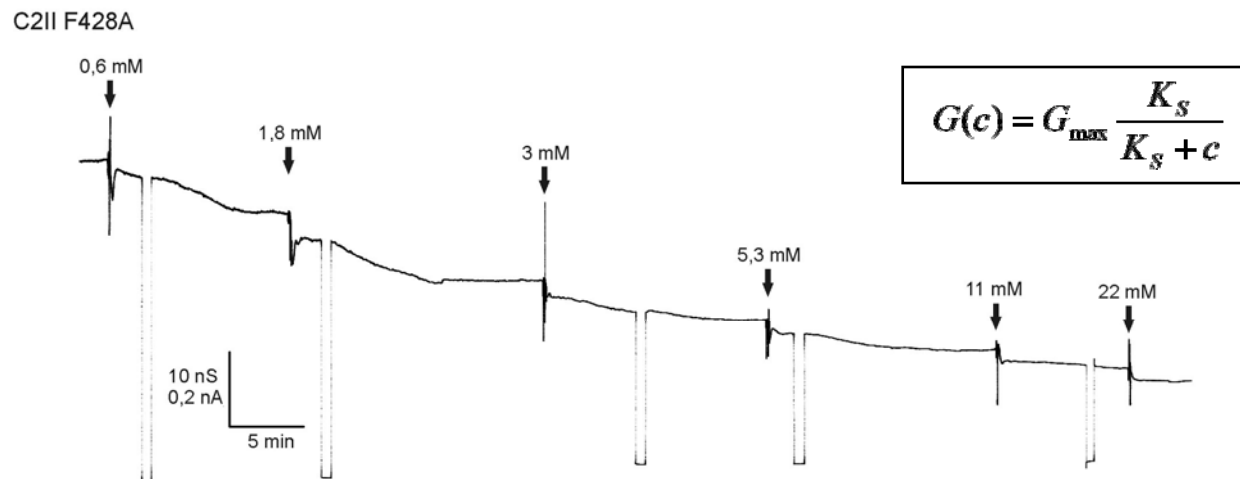
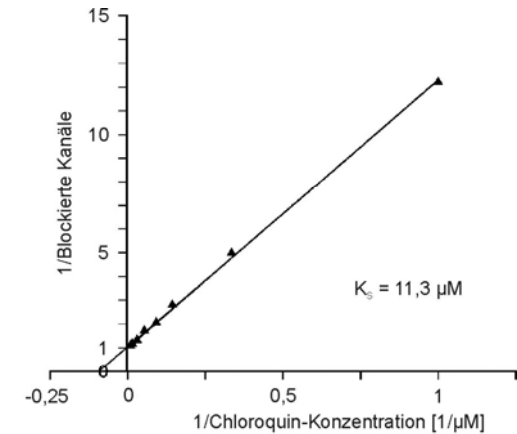
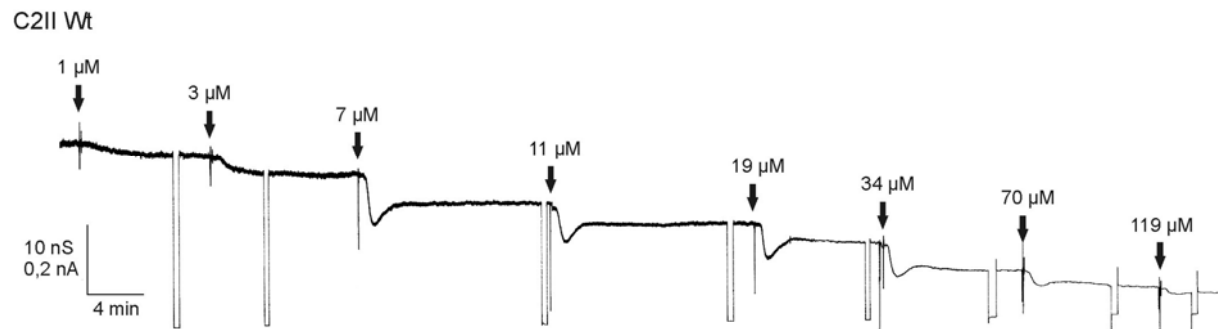


→ Voltage-dependence of the C2II-mutants



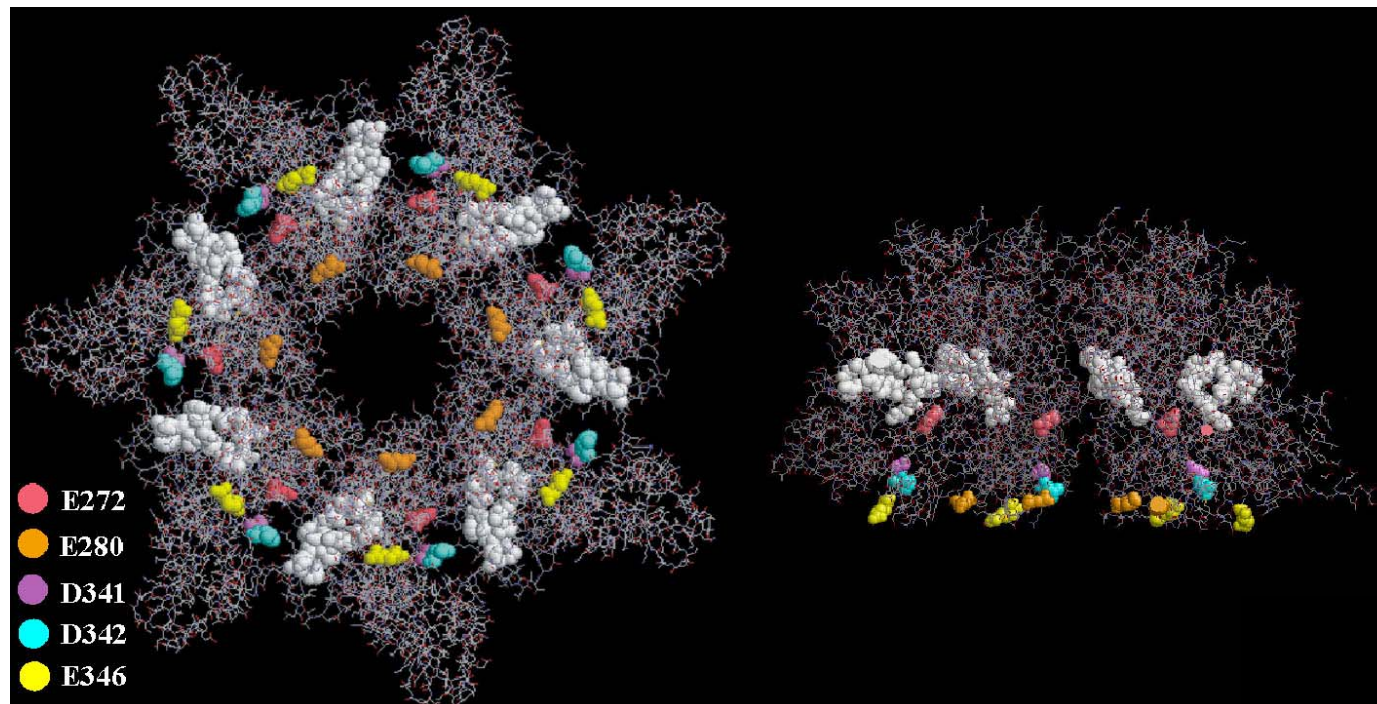


→ Half-saturation concentrations of 4-aminoquinolone-binding



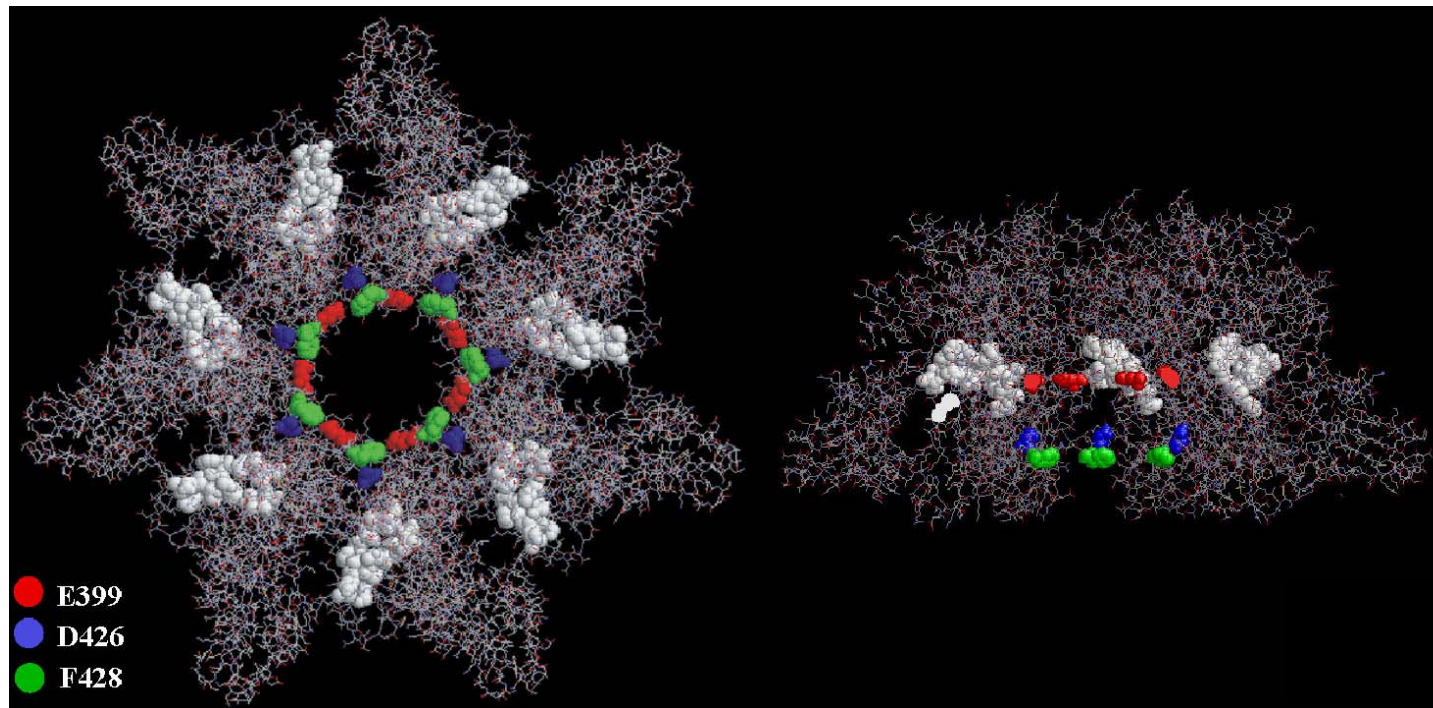
→ Half-saturation concentrations of 4-aminoquinolone-binding

	Wt	E272A	E280C	D341A	D342C	E346A	D341A E346A
C2II		●	●	●	●	●	
$K_s$ [ $\mu$ M] in 150 mM KCl							
Chloroquin beidseitig	10	5	12	13	5	3	3



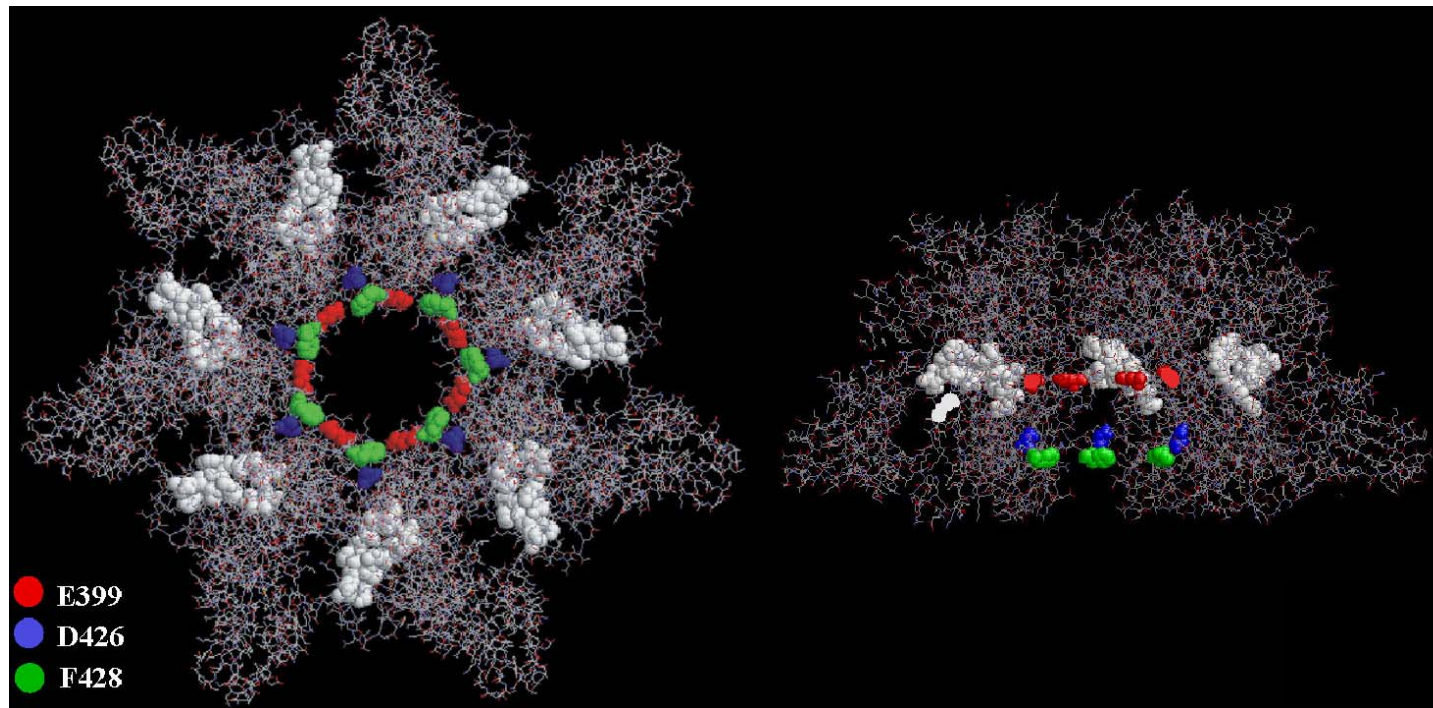
→ Half-saturation concentrations of 4-aminoquinolone-binding

	Wt	E399A	D426A	F428A	F428D	F428Y	F428W	E399A D426A	D426A F428A	E399A D426A F428A
C2II			●	●						
$K_s$ [ $\mu$ M] in 150 mM KCl										
Chloroquin beidseitig	10	250	2500	3700	3400	240	170	3700	1900	2800



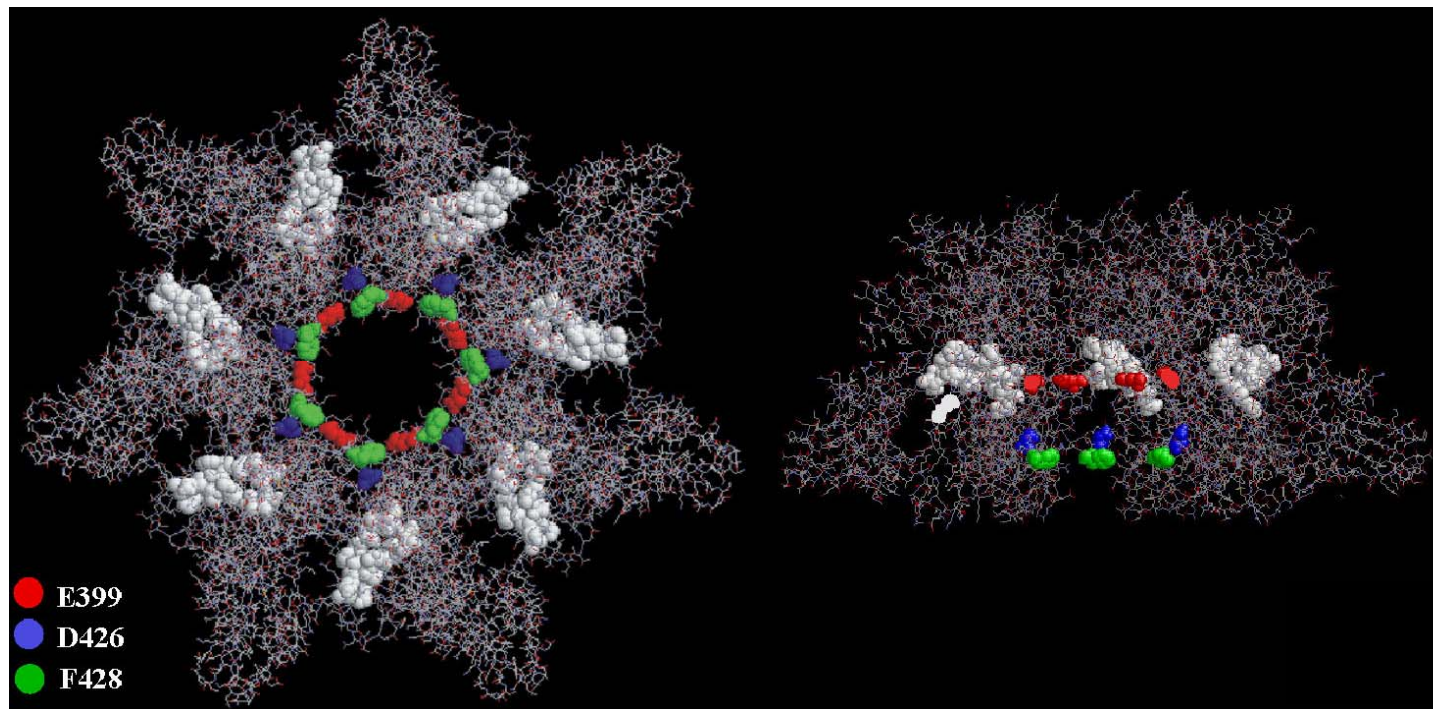
→ Half-saturation concentrations of 4-aminoquinolone-binding

	Wt	E399A	D426A	F428A	F428D	F428Y	F428W	E399A D426A	D426A F428A	E399A D426A F428A
C2II			●	●	●					
$K_s$ [ $\mu$ M] in 150 mM KCl										
Chloroquin beidseitig	10	250	2500	3700	3400	240	170	3700	1900	2800



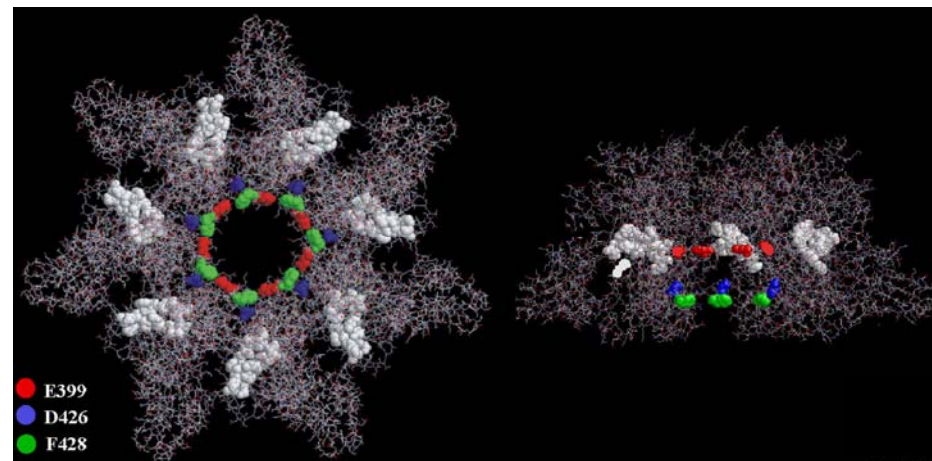
→ Half-saturation concentrations of 4-aminoquinolone-binding

	Wt	E399A	D426A	F428A	F428D	F428Y	F428W	E399A D426A	D426A F428A	E399A D426A F428A
C2II		●				●	●			
$K_s$ [ $\mu$ M] in 150 mM KCl										
Chloroquin beidseitig	10	250	2500	3700	3400	240	170	3700	1900	2800



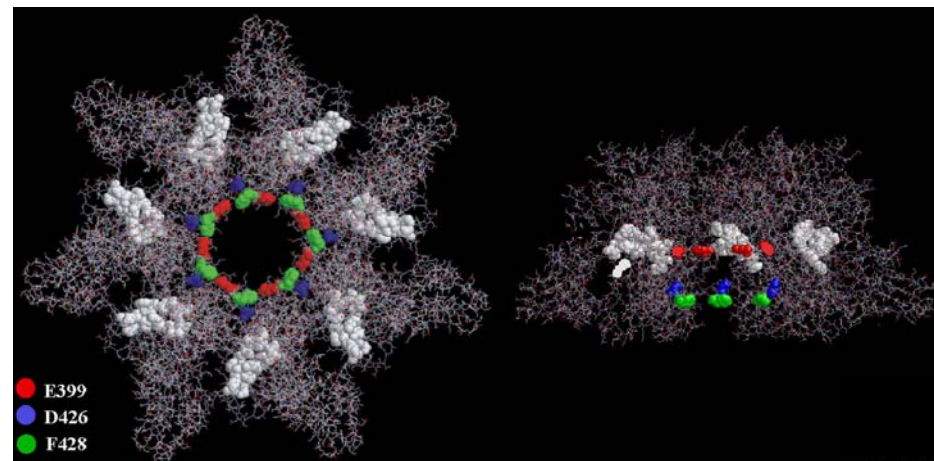
→ Half-saturation concentrations of 4-aminoquinolone-binding

	Wt	E399A	D426A	F428A	F428D	F428Y	F428W	E399A D426A	D426A F428A	E399A D426A F428A
C2II										
<b>K<sub>s</sub> [μM] in 150 mM KCl</b>										
Chloroquin beidseitig	10	250	2500	3700	3400	240	170	3700	1900	2800
Chloroquin cis	10	460	8300	3400	4500	320	180	10800	6600	5700
Chloroquin trans	180	1300	5300	6700	4600	2900	3700	5500	5900	5200



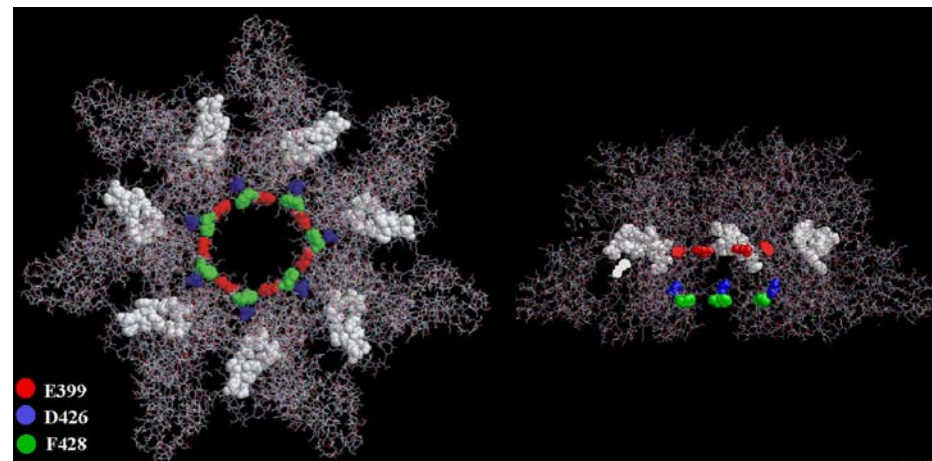
→ Half-saturation concentrations of 4-aminoquinolone-binding

	Wt	E399A	D426A	F428A	F428D	F428Y	F428W	E399A D426A	D426A F428A	E399A D426A F428A
C2II			●	●	●			●●	●●	
<b>K<sub>s</sub> [μM] in 150 mM KCl</b>										
Chloroquin beidseitig	10	250	2500	3700	3400	240	170	3700	1900	2800
Chloroquin cis	10	460	8300	3400	4500	320	180	10800	6600	5700
Chloroquin trans	180	1300	5300	6700	4600	2900	3700	5500	5900	5200



→ Half-saturation concentrations of 4-aminoquinolone-binding

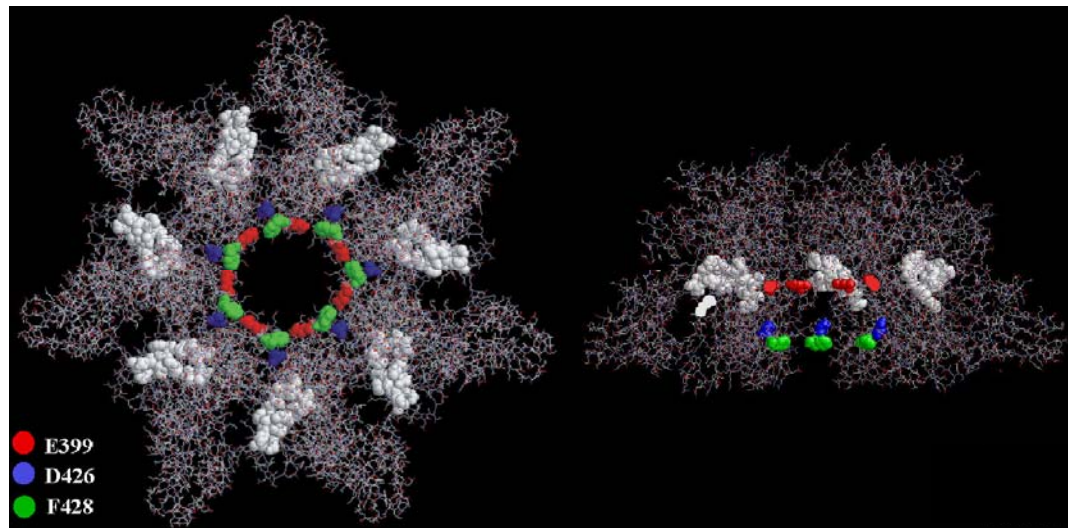
	Wt	E399A	D426A	F428A	F428D	F428Y	F428W	E399A D426A	D426A F428A	E399A D426A F428A
C2II						●	●			
<b>K<sub>s</sub> [μM] in 150 mM KCl</b>										
Chloroquin beidseitig	10	250	2500	3700	3400	240	170	3700	1900	2800
Chloroquin cis	10	460	8300	3400	4500	320	180	10800	6600	5700
Chloroquin trans	180	1300	5300	6700	4600	2900	3700	5500	5900	5200





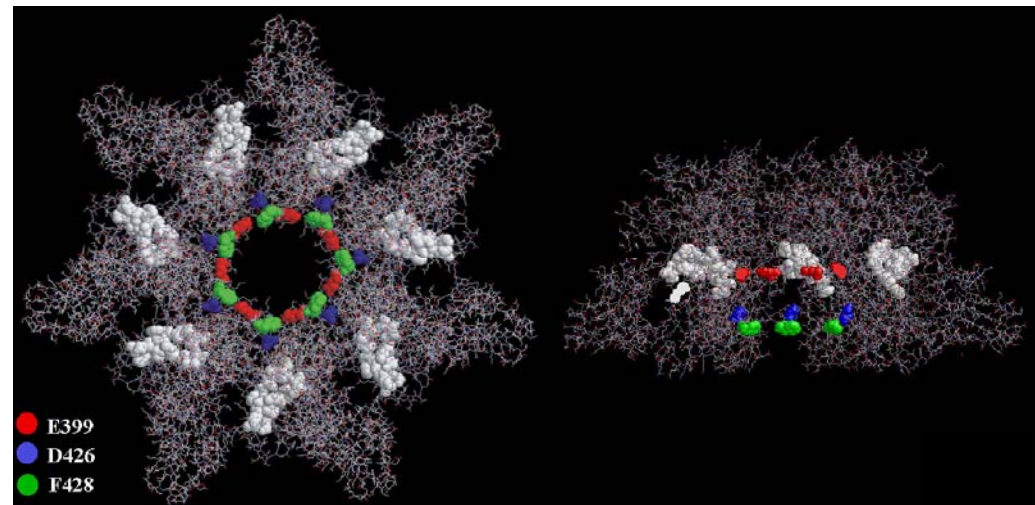
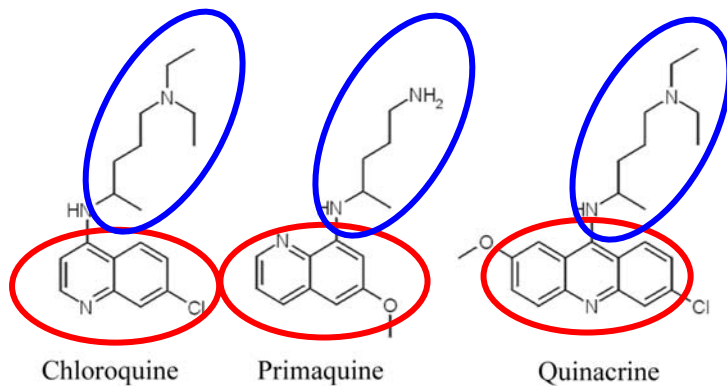
→ Half-saturation concentrations of 4-aminoquinolone-binding

	Wt	E399A ●	D426A	F428A ●	F428D ●	F428Y ●	F428W	E399A D426A	D426A F428A	E399A D426A F428A
C2II										
<b>K<sub>s</sub> [μM] in 150 mM KCl</b>										
Chloroquin beidseitig	10	250	2500	3700	3400	240	170	3700	1900	2800
<b>K<sub>s</sub> [μM] in 1M KCl</b>										
Chloroquin beidseitig	45	110	n.m.	29500	22700	2600	n.m.	n.m.	n.m.	n.m.

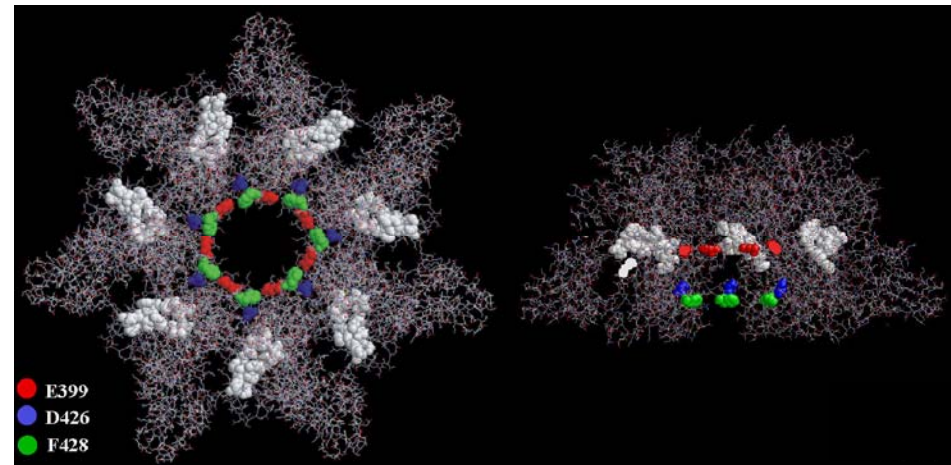


→ Half-saturation concentrations of 4-aminoquinolone-binding

	Wt	E399A ●	D426A ●	F428A ●	F428D ●	F428Y ●	F428W ●	E399A D426A ●●	D426A F428A ●●	E399A D426A F428A
<b>C2II</b>										
<b>K<sub>s</sub> [μM] in 150 mM KCl</b>										
<b>Chloroquin beidseitig</b>	10	250	2500	3700	3400	240	170	3700	1900	2800
<b>Quinacrin beidseitig</b>	1,15	100	2300	440	70	70	230	1200	1900	n.m.
<b>Primaquin beidseitig</b>	90	250	n.m.	930	120	420	680	1800	10600	n.m.



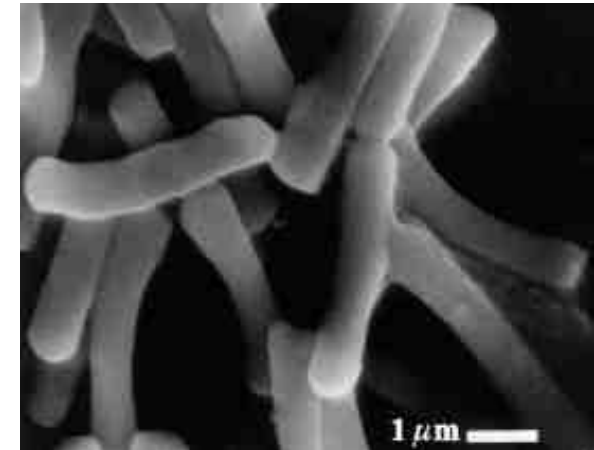
→ Summary



Impact on:	● E399A	● D426A	● F428A	● F428Y	● F428W	● F428D
SDS-stability of oligomers	↓	-	-	-	-	-
Membrane activity	-	↓	-	-	-	-
Single channel conductance	↓↓	↓↓	↑↑↑	↑↑	↓↓↓	↑↑
Ionic-strength-dependence of single channel conductance	+	+	-	-		
Selectivity	↓↓↓	↓↓↓	↓↓			
Voltage-dependence	+	+	+	-	-	
Half-saturation-constants (4-aminoquinolone)	↑↑	↑↑↑	↑↑↑	↑↑	↑↑	↑↑↑
Ionic-strength-dependence of 4-aminoquinolone-binding	+		-	-		-

## VIP-Toxins of *Bacillus thuringiensis* (and *B. cereus*)

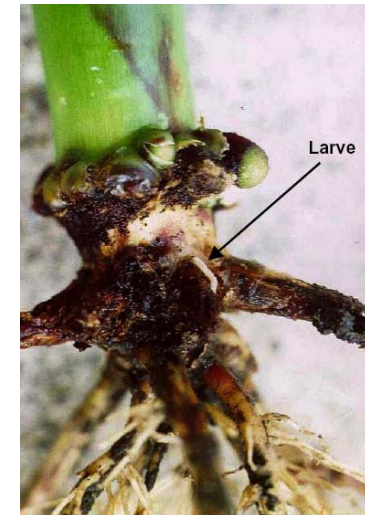
- *Bacillus thuringiensis* was first discovered in flour silos in Thuringia (German federal state) in 1911
- Gram-positive, spore forming, rod-shaped, soil bacteria
- Belongs to the family of *Bacillaceae*, closely related to *Bacillus cereus* and *Bacillus anthracis*
- Pathogen for flour moths (*Ephesia kuehniella*)



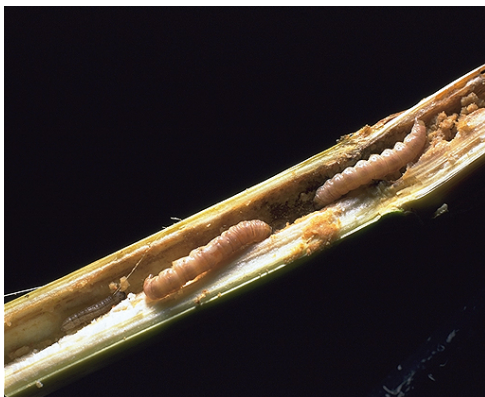
Andrup et al., 1996

## VIP-Toxins of *Bacillus thuringiensis*

- *Bacillus thuringiensis* is well known for its insecticidal  $\delta$ -endotoxins, targeting different corn pests, such as the European corn borer (*Ostrinia nubilalis*) and the Western corn rootworm (*Diabrotica virgifera virgifera* LeConte)



<http://www.bba.de/veroeff/popwiss/diabrotica.pdf>



<http://www.syngenta.com>



<http://www.syngenta.com>

## VIP-Toxins of *Bacillus thuringiensis*

### ➤ $\delta$ -endotoxins

- Parasporal crystals, produced during sporulation phase of the bacteria
- Pore-forming proteins, that target the insects' midgut cells and lyse them
- Already used in transgenic corn since 1996



<http://helios.bto.ed.ac.uk/bto/microbes/bt.htm>

- ### ➤ *Bacillus thuringiensis* is also capable of producing insecticidal proteins during its vegetative growth phase

→ Vegetative Insecticidal Proteins → VIP-Toxins

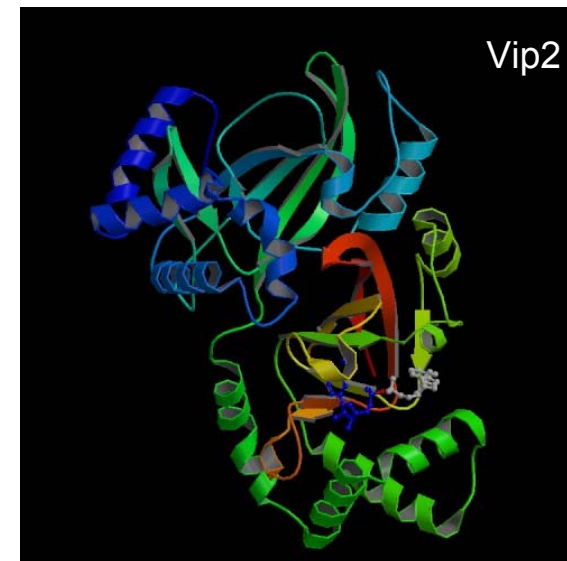
# VIP-Toxin

## ➤ Binding component: Vip1Ac

- 96 kDa precursor protein, chymotrypsin cleavage leads to active monomers (66 kDa) that can form oligomers
- Highly homologous to C2II, PA and other  $\beta$ -barrel PFTs

## ➤ Enzymatic component: Vip2Ac

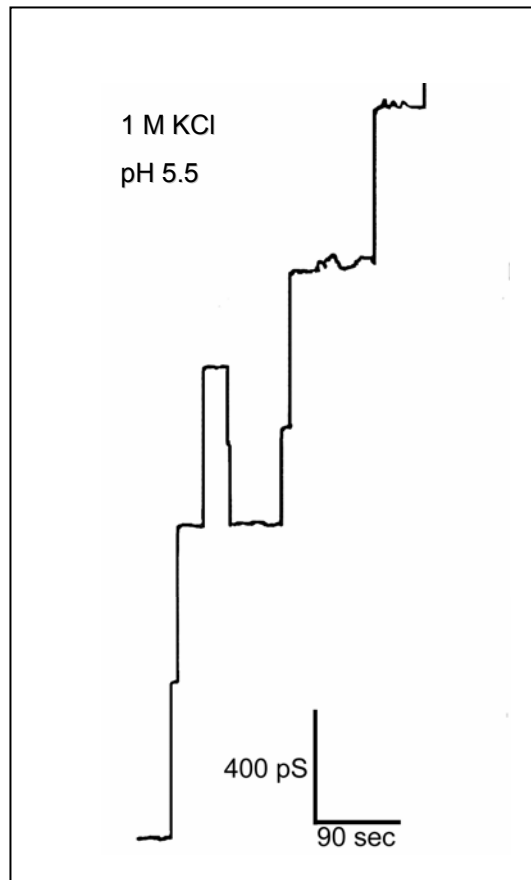
- 46 kDa protein, 2 domains, ADP-ribosyltransferase activity
- Highly homologous to C2I and Ia (*C. perfringens*)
- ~90 % homology to Vip2 from *B. cereus* AB78



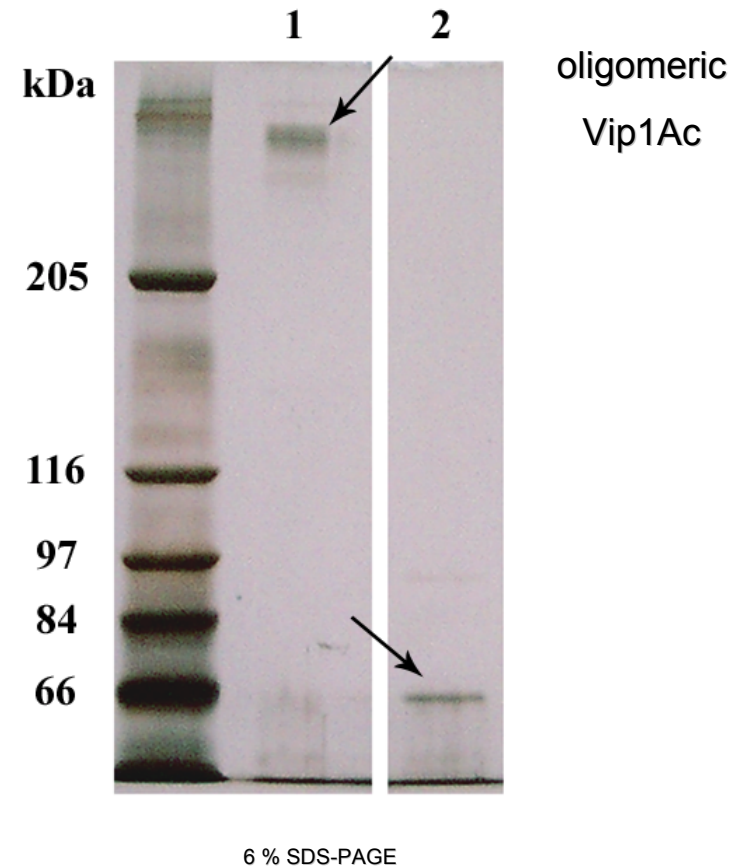
Han et al, 1999

# VIP-Toxin

## ➤ Binding component: Vip1Ac



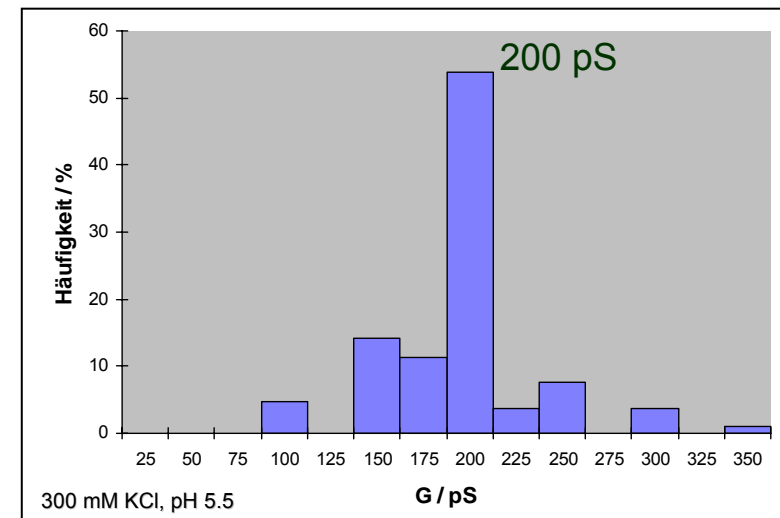
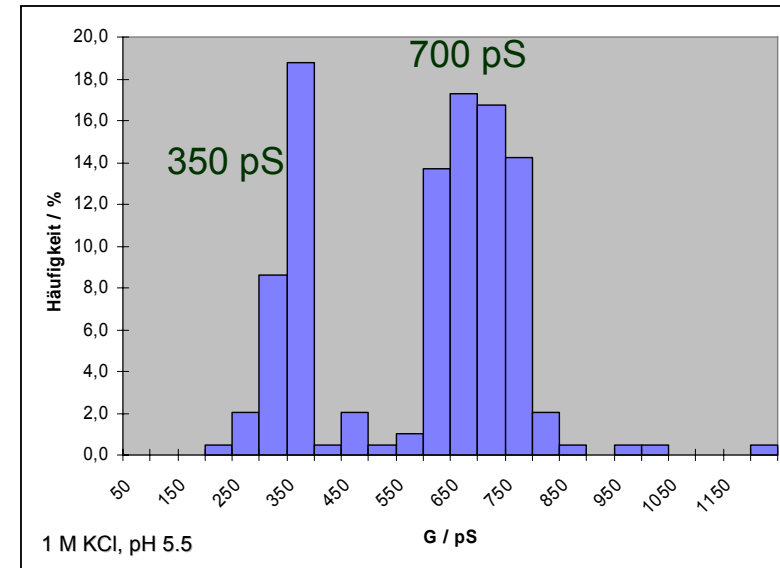
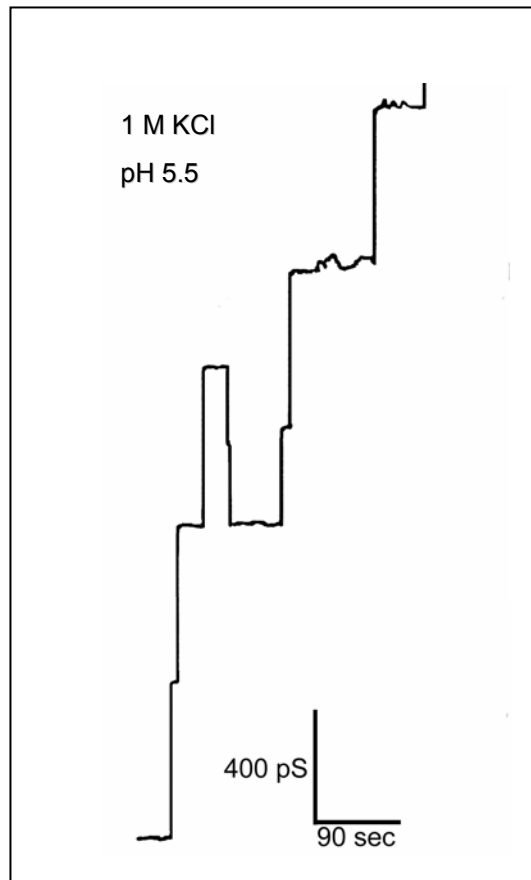
- |   |   |
|---|---|
| 1 | <i>B. thuringiensis</i> gel-eluted Vip1Ac, native |
| 2 | <i>B. thuringiensis</i> gel-eluted Vip1Ac, boiled |





# VIP-Toxin

- Binding component: Vip1Ac



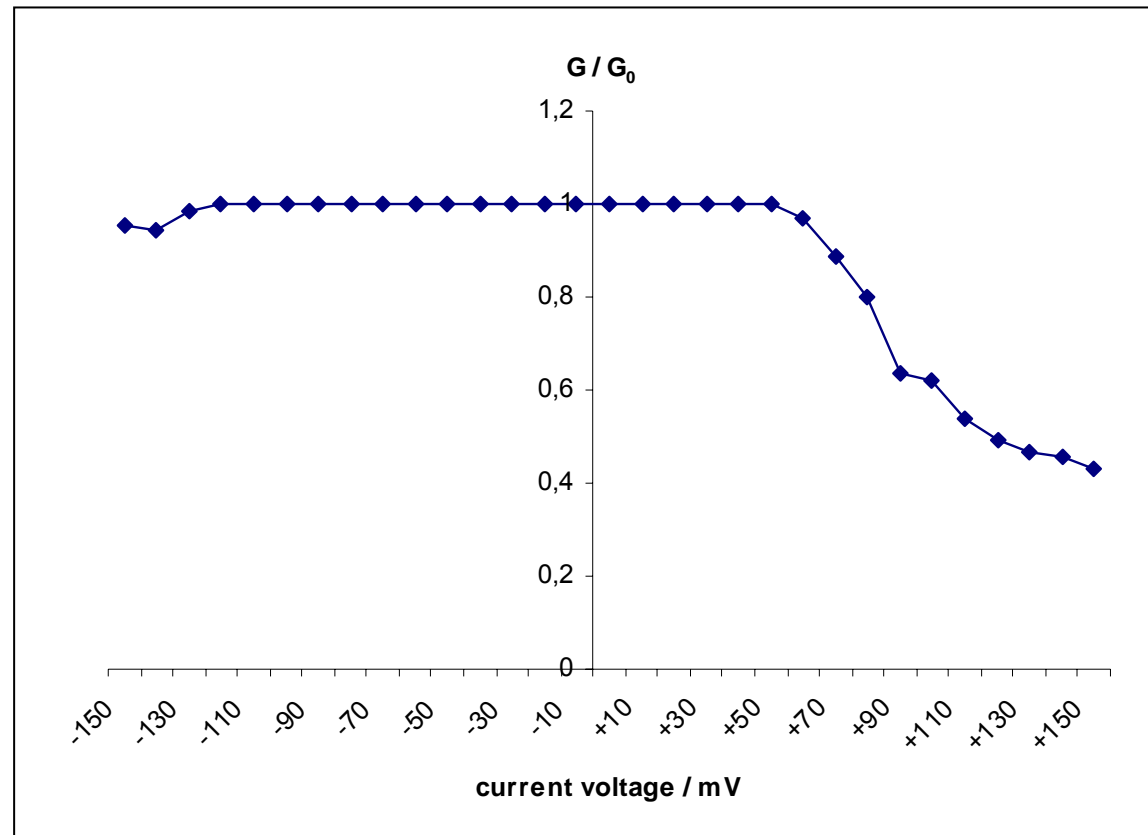
## VIP-Toxin

- Vip1Ac single channel conductance

<b>Electrolyte</b>	<b>Conductance state 1</b>	<b>Conductance state 2</b>
<b>50 mM KCl</b>		<b>75 pS</b>
<b>150 mM KCl</b>		<b>150 pS</b>
<b>300 mM KCl</b>		<b>200 pS</b>
<b>500 mM KCl</b>	<b>160 pS</b>	<b>300 pS</b>
<b>1 M KCl</b>	<b>350 pS</b>	<b>700 pS</b>
<b>3 M KCl</b>	<b>960 pS</b>	<b>1900 pS</b>
<b>1 M LiCl</b>	<b>160 pS</b>	<b>300 pS</b>
<b>1 M KF</b>	<b>220 pS</b>	<b>400 pS</b>

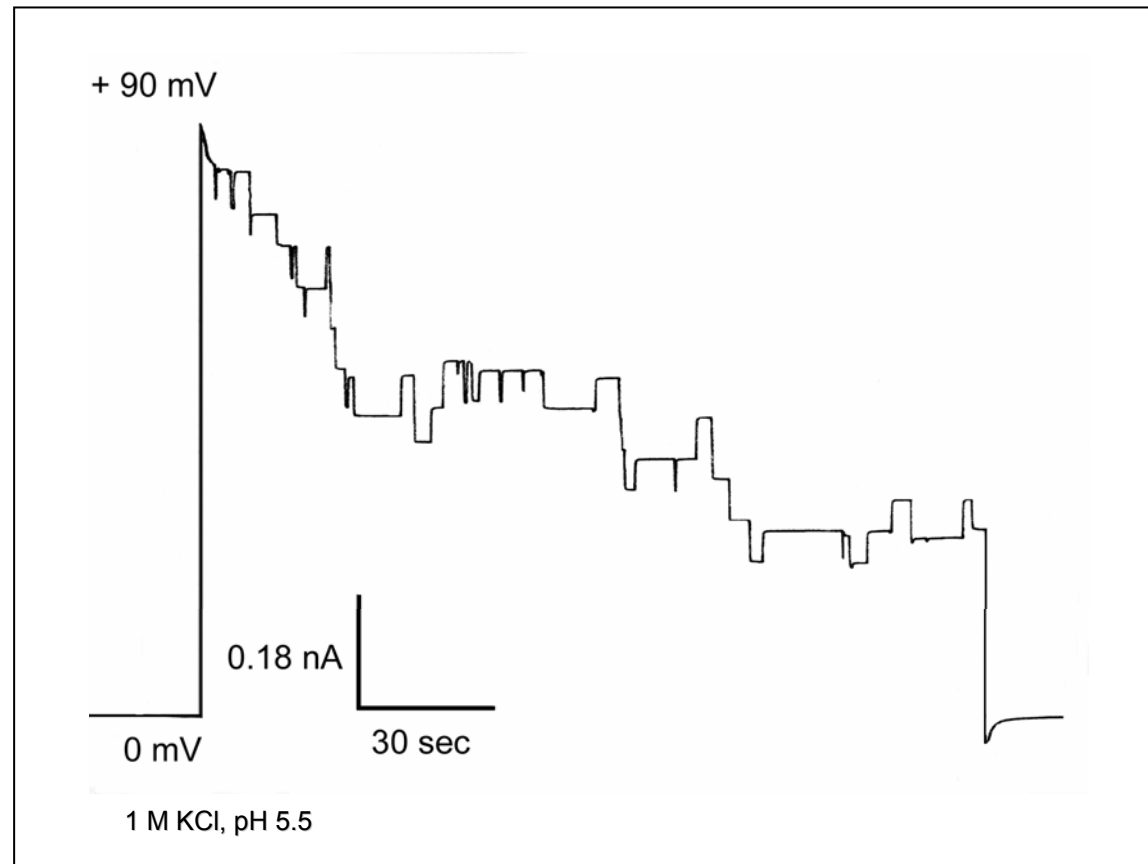
# VIP-Toxin

➤ Vip1Ac voltage-dependence



# VIP-Toxin

- Vip1Ac voltage-dependence



## VIP-Toxin – why so interesting?

- Anion-selective ( $P_c/P_a = 0.36$ ) channels in artificial lipid bilayers with two conductance states at higher salt concentrations  
 ≠ other known binary toxins, e.g. C2- and Anthrax-toxin
- Asymmetric, voltage dependent closure of the channel at high positive voltages applied to the cis side  
 ≠ other known binary toxins, e.g. C2- and Anthrax-toxin
- No binding of the enzymatic component Vip2Ac & chloroquine  
 ≠ other known binary toxins, e.g. C2- and Anthrax-toxin



Different mode of interaction despite the high homology to other binary toxins ?

# Binary Bacterial Toxins

## C2- and VIP-Toxin

**Thank you for your attention !**

**Michael Leuber**

Biotechnology

University Würzburg

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