## Supporting Information Available

Table S1. Backbone atoms in TM 5 touching water in the channel[a]

| Residue | Atom #[b] | Type |
|---|---|---|
| A213 | 1215 | N |
| | 1216 | CA |
| | 1218 | O |
| G215 | 1228 | N |
| | 1229 | CA |
| | 1230 | C |
| | 1231 | O |
| V216 | 1232 | N |
| | 1233 | CA |

[a]Analyzed with CASTp. [b]Atom number in the VAChT homology model.

Table S2. Dihedral angles ± RMS deviation in wild-type peptide

| Residue[a] | Dihedral angle | Value ° | RMS deviation ° |
|---|---|---|---|
| V216 | Phi (φ) | -65.555291 | 6.936921 |
| | Psi (ψ) | -43.138613 | 5.401761 |
| **L218** | Phi (φ) | -63.021921 | 6.339639 |
| | Psi (ψ) | -40.494594 | 6.386321 |
| **F220** | Phi (φ) | -63.517805 | 6.506374 |
| | Psi (ψ) | -43.892326 | 6.161458 |
| **S222** | Phi (φ) | -63.536205 | 6.353860 |
| | Psi (ψ) | -44.633001 | 6.054075 |
| **G224** | Phi (φ) | -64.103921 | 10.277491 |
| | Psi (ψ) | -33.447507 | 10.277491 |
| S225 | Phi (φ) | -68.750466 | 9.486298 |
| | Psi (ψ) | -36.103063 | 9.953188 |
| **L226** | Phi (φ) | -71.203591 | 11.781274 |
| | Psi (ψ) | -37.703837 | 10.356511 |
| V227 | Phi (φ) | -86.242893 | 11.997084 |
| | Psi (ψ) | -15.758688 | 14.054386 |
| **A228** | Phi (φ) | -63.513318 | 8.086073 |
| | Psi (ψ) | -44.367642 | 6.578547 |
| **P229** | Phi (φ) | -58.933661 | 9.769089 |

| | | | |
|---|---|---|---|
| | Psi (ψ) | -44.742296 | 8.307269 |
| **P230** | Phi (φ) | -65.272793 | 12.508464 |
| | Psi (ψ) | -39.525251 | 10.306190 |
| **F231** | Phi (φ) | -67.010451 | 8.410105 |
| | Psi (ψ) | -39.428020 | 7.912051 |
| **G232** | Phi (φ) | -65.293337 | 10.982078 |
| | Psi (ψ) | -34.308075 | 10.480118 |

[a]Boldface residues are invariant in nature.

Table S3. Dihedral angles ± RMS deviation in control peptides

| | | P229A-P230A | | | P229L-P230L | |
|---|---|---|---|---|---|---|
| Dihedral angle | Residue[a] | Value° | RMS deviation° | Residue[a] | Value° | RMS deviation° |
| Phi (φ) | V216 | -66.151705 | 7.143601 | V216 | -66.598757 | 7.366858 |
| Psi (ψ) | | -43.214935 | 5.571947 | | -41.675228 | 6.751400 |
| Phi (φ) | **L218** | -62.764346 | 6.310832 | **L218** | -62.894785 | 6.269384 |
| Psi (ψ) | | -41.264346 | 6.241227 | | -41.053425 | 6.205606 |
| Phi (φ) | **F220** | -63.510504 | 6.673013 | **F220** | -63.325874 | 6.497053 |
| Psi (ψ) | | -44.207536 | 6.212745 | | -43.718384 | 6.220137 |
| Phi (φ) | **S222** | -63.548513 | 6.362209 | **S222** | -63.440762 | 6.365096 |
| Psi (ψ) | | -45.227481 | 5.980474 | | -45.257702 | 6.048495 |
| Phi (φ) | **G224** | -64.908555 | 9.547635 | **G224** | -65.156737 | 9.432021 |
| Psi (ψ) | | -33.063923 | 10.260615 | | -33.821134 | 9.887177 |
| Phi (φ) | S225 | -65.700480 | 7.750358 | S225 | -65.829463 | 7.494770 |
| Psi (ψ) | | -39.778997 | 7.041728 | | -40.331762 | 6.913162 |
| Phi (φ) | **L226** | -65.657632 | 7.074075 | **L226** | -65.025562 | 7.024684 |
| Psi (ψ) | | -42.692424 | 6.330534 | | -42.556793 | 6.325052 |
| Phi (φ) | V227 | -64.712411 | 6.719386 | V227 | -64.215348 | 6.623397 |
| Psi (ψ) | | -42.800727 | 6.212694 | | -42.437325 | 6.028240 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Phi (φ) | **A228** | -63.208339 | 6.478427 | **A228** | -62.932937 | 6.400929 |
| Psi (ψ) | | -41.291354 | 6.517124 | | -41.091253 | 6.662350 |
| Phi (φ) | A229 | -64.687637 | 6.852738 | L229 | -64.296512 | 6.846522 |
| Psi (ψ) | | -42.198087 | 6.635754 | | -43.143305 | 6.506577 |
| Phi (φ) | A230 | -64.996407 | 6.993623 | L230 | -64.400831 | 7.173638 |
| Psi (ψ) | | -44.715800 | 6.514797 | | -45.783443 | 6.329465 |
| Phi (φ) | **F231** | -66.654670 | 8.392653 | **F231** | -66.018624 | 7.928432 |
| Psi (ψ) | | -38.862924 | 8.426826 | | -39.028099 | 8.062677 |
| Phi (φ) | **G232** | -63.136109 | 11.037018 | **G232** | -62.687294 | 10.852893 |
| Psi (ψ) | | -39.198949 | 10.594343 | | -39.487706 | 10.061940 |

[a]Boldface residues are invariant in nature.

**Figure S1:** Kink (κ) *versus* wobble (ω) angles at residue 227 in control peptide. See Figure 5 for the definitions of kink and wobble. MD trajectories from 3.6 ns to 7.8 ns for the control peptide (Table S3) that substitutes alanine residues for P229 and P230 were saved every 200 fs. Every fifth structure was extracted, and kink (κ) and wobble (ω) angles were calculated for each structure using the Simulaid program (*36*) with residue 227 as the kink. The histogram for kink and wobble coordinates (4200 dots) was plotted using the program KaleidaGraph. Kink is dramatically reduced (~ 0° to 30°), which allows wobble much more latitude (~ 30° to 210° ) than for wild-type peptide (Figure 6).