Supplementary note 1 Voltage-dependence of the Fv effect

When we considered the inhibitory effects of the Fv on channels at specific voltages, the normalized inhibition \( f_{ac} \) reflected the fraction of channels \( f_{ac} \times N \), whose VSDs were bound by Fv in the incubation period, and can be expressed by:

\[
 f_{ac} \times N = N \times \int_{0}^{T} Q_{o}(V) \times k_{on} \times [Fv] \, dt
\]

(S1)

Here, \( N \) represents the total number of channels, \( T \) the duration of Fv incubation, \( Q_{o}(V) \) the probability of the VSDs switching to the Fv-accessible conformation at specific voltage \( V \), and \( k_{on} \times [Fv] \) the apparent ON-rate of Fv (see below and we assume that there is only one significant “up” conformation). Under the assumption that the elementary ON-rate \( (k_{on}) \) of Fv does not vary much once the VSDs are “up”, the only voltage-dependent term is \( Q_{o}(V) \). Because the conformational switch of the VSDs is relatively fast (millisecond), the measured \( Q_{o}(V) \) is time-averaged in duration \( T \), which is similar to the averaged \( Q(V) \) measured in a 100 ms pulse. When \( [Fv] \) and \( T \) are constant and selected properly so that \( f_{ac} \) varies from 0 to 1, normalized \( f_{ac} \) closely reflects the \( Q_{o}(V) \) and its valence approximates that of the \( Q-V \) curve of the VSD. The measured \( Q_{o}(V) \) likely reflects only the \( C_{0} \leftrightarrow C_{1} \) switch (Fig. 1c) because the Fv binds to \( C_{1} \) as well. Comparing our measurements (Fig. 1b) with those from the Shaker channel\(^{37,38,10} \) indicates that similar to the Shaker, all KvAP VSDs switch to the “up” conformation before the pore domain gains a substantial open probability.

Supplementary note 2 Kinetic analysis of the KvAP in the presence of low [Fv]

We assumed that when a channel is bound with one Fv molecule, its kinetic behavior should change because the Fv-binding is reversible. From our data, we know that channels with all four VSDs bound to Fv molecules are inhibited. We expect that using low concentration of Fv would
lead to fewer than four Fv molecules bound to one channel, and the gating property of the channel would change. To measure the activation constants, the ionic current of a test pulse was normalized against the averaged current in the last 20 ms of the segment of the test voltage in the pulse. At time 0, 1.0 μg/ml Fv was perfused into the recording chamber while the bilayer was held at -80 mV. The recordings with the test pulses delivered right before (black, as control) and after (red) Fv-addition were normalized and compared (Fig. 1d). A single exponential function was used to fit the current traces from 50-70 ms and gave rise to the activation kinetic constants ($k_{+1,Fv}$ and $k_{+1,noFv}$). The ratios of $k_{+1,Fv} / k_{+1,noFv}$ from four independent bilayers were averaged, and presented in Fig. 1e.

To obtain the deactivation constants, currents from the deactivation segments in the test pulses were normalized against the average current in the last 10 ms of the +80 mV (activation) segment. Single exponential function was used to fit the deactivation current at -80 mV (Fig. 1d). The obtained deactivation constant ($k_{-1,Fv}$) in the presence of Fv was divided by that without Fv ($k_{-1,noFv}$), and showed in Fig. 1e.

The inactivation kinetics was measured with a long pulse (2000 ms at +80 mV) in order to make sure that the inactivation was complete. The current was normalized against the peak current (Fig. 1d). Single exponential fitting of the inactivation phase of the currents generated the time constants. The obtained inactivation constant in the presence of Fv ($k_{+2,Fv}$) was normalized against $k_{+2,noFv}$. Data from four different membranes were averaged and showed in Fig. 1e.

The recovery rate from inactivation was measured with paired pulses that had different intervals between them. The first long pulse (2000 ms at +80 mV, the inactivation pulse) was delivered to make sure that complete inactivation was achieved. After different intervals at -80
mV (from 1.0 to 120 seconds), a test pulse (100 ms at 80 mV) was delivered to measure the fraction of channels that have recovered. After each pair of pulses, the membrane was held at -80 mV for 2-5 minutes before the next paired pulses were delivered. The peak current from the second test pulse was normalized against the first inactivation pulse (black and red traces in the inset of Fig. 1d) and plotted as a functional of the interval between the paired pulses (black and red dots in the absence and presence of 1.0 μg/ml Fv respectively in Fig. 1d). Single exponential fitting of the data yielded the recovery rate constants from the inactivated state (k−3, no Fv and k−3, Fv).

The ratios of k−3, Fv / k−3, no Fv from four different sets of experiments were averaged and presented in Fig. 1e.

In order to evaluate the thermodynamic effect of Fv-binding, we combined a conventional gating scheme for the Shaker potassium channel and a recent parsimonious gating model for KvAP to propose a tentative gating scheme in Fig. 1c. At depolarizing voltages, the steady-state inactivation (k+3) is much slower than the activation (k+1) because upon strong depolarization almost all channels open fairly quickly. The return rate (k−2) from the inactivated state to the open state (I → O) has to be very slow (assumed to be almost zero at 80 mV) because a long depolarization pulse inactivates the channels completely. Presumably the structure of Kv1.2/2.1 chimera reflects an inactivated state of the channel. But it remains unclear whether the “up” conformations of the voltage sensors are the same in C1, Cx, O and I states. Currently we are assuming that the “up” conformations in these states are rather similar, if not exactly the same. Inclusion of the slow Cx ↔ I step not only accounts for the steady-state inactivation as discussed by Schmidt et al, but also will likely provide better fittings to all processes than the parsimonious model presented by the same authors. Using this gating scheme,
our data argue that both the faster activation to the open state and the slower recovery rate from
the inactivated state (Fig. 1e) contribute to the stabilized inactivated state by Fv-binding.

Supplementary note 3  Characterization of the Fv binding to channels in lipid bilayers

The dose-response was measured at different concentrations of Fv. Stable recordings from a
bilayer were first obtained for 15-20 minutes. At time 0, Fv was added while the bilayer was held
at -80 mV. Test pulses (100 ms at +80 mV from a holding potential of -80 mV) were delivered
every two minutes. The recordings were continued until no further change in the current
amplitude was observed. For each condition, the averaged current from the last 50 ms of each
recording (the +80 mV segment) was normalized against the average of the five traces before
time 0. Results from multiple experiments were averaged, and plotted as a function of time
(Supplementary Figure S2a). The error bars were s.d. (n > 2) or variation range (n = 2) from
repeated experiments with Fv concentrations of 0.017 μg/ml, 0.17 μg/ml, 0.40 μg/ml, 0.80 μg/ml,
and 1.7 μg/ml. For each plot, an exponential function, \( I = e^{-\frac{t}{\tau}} + C \), was used to fit the data
points from time 0 to 18th minute. The time constants were used to calculate the apparent ON-
rates (\( k_{\text{app}} = 1/\tau, \text{s}^{-1} \)). The apparent ON-rate was plotted against the Fv concentration in
Supplementary Figure S2b, and was fitted with a polynomial function, \( k_{\text{app}} = k_{\text{ON}} [Fv]^n \).
Best least-squares fitting (blue line in Supplementary Figure S2b) yielded \( n = 1.0 \) and \( k_{\text{ON}} = 1.2 \)
\( \times 10^5 \text{ s}^{-1}\text{M}^{-1} \). The steady-state inhibition (\( 1.0 - C \) from the exponential fitting) was plotted against
the Fv concentration (Supplementary Figure S2c). A typical washout of Fv from the recording
chamber was showed in Supplementary Figure S2d.
Supplementary Note 4  The phage action on KvAP channels in a lipid bilayer

Because each phage contains more than one copy of peptides (5 copies in our experiment), we could use the following scheme to represent the interaction of one phage with multiple binding sites in channels.

\[ \phi_D + Ch \leftrightarrow \phi_D.Ch + Ch \leftrightarrow \phi_D.Ch2 + Ch \leftrightarrow ... \leftrightarrow \phi_D.ChN \]  \hspace{1cm} (S2)

Under the assumption that there is long enough time for each elementary step to reach equilibrium and using \( K_1, K_2, ..., K_N \) to represent the equilibrium constants for these steps, we obtain the following set of equations.

\[
\begin{align*}
[\phi_D] [Ch] &= K_1 [\phi_D.Ch] \\
[\phi_D.Ch][Ch] &= K_2 [\phi_D.Ch2] \\
& \ldots \\
[\phi_D.Ch(N-1)][Ch] &= K_N [\phi_D.ChN] \\
[Ch] + [\phi_D.Ch] + 2 [\phi_D.Ch2] + \ldots + N [\phi_D.ChN] &= C_0 \\
\end{align*}
\]

(total channels)

where \([Ch]\) denotes the concentration of free-diffusing channels in the membrane.

It is easy to show that

\[
\sum_{i=1}^{n} \frac{[\phi_D][Ch]^i}{\prod_{j=1}^{i} K_j} + [Ch] = C_0
\]  \hspace{1cm} (S3)

Because of the necessity for all four voltage sensors to be in the ‘up’ position for each channel to open, and considering the multiple copies of peptides on each phage, we can assume that all channels bound to individual phages are inhibited, and then the normalized inhibition (I\%) would be:

\[
I\% = \frac{\sum_{i=1}^{N} \frac{[\phi_D][Ch]^i}{\prod_{j=1}^{i} K_j} }{C_0}
\]  \hspace{1cm} (S4)
Now at EC50, \( [Ch] = C_0/2 \), 50% of the channels are inhibited and \( [\phi_D] = EC50 \). We have

\[
I\%_6 = \sum_{i=1}^{N} \frac{EC50 \left( \frac{C_0}{2} \right)^i}{\Pi_{j=1}^{i} K_j} = \sum_{i=1}^{N} \frac{EC50}{\Pi_{j=1}^{i} \frac{2K_j}{C_0}} i = 0.50
\]

(S5)

Using equation S4 to fit the data in Figure 5a would be complicated. If we assume that the OFF-rate of a phage is very slow due to its multiple copies of peptides and the multiple binding sites on each channel, and that the encountering of one channel with one phage would probably be enough to keep the channel inhibited, we expect that the dose-response of \( \phi_D \) follows roughly a linear relation:

\[
I\% = k_{app} [\phi_D]
\]

The data points in Figure 5a can indeed be fitted with a linear function reasonably well (not shown), giving \( k_{app} \approx 2.2 \times 10^7 \) M\(^{-1}\). It suggests that the two assumptions we made are approximately right.

In addition, due to the multivalent nature of both the phage and the channel, we would expect that adding the phages could, to certain extent, induce lateral clustering of the channels in a lipid bilayer, which might further affect channel function.
Supplementary Figure S1  Chemical Structures of lipids used in the experiments

POPE and POPG have the same acyl chains. DOPC, DOTAP and DOGS all contain double oleoyl chains and all form stable fluidic bilayers. Cryo-EM examination of DOTAP and DOGS vesicles revealed that their bilayers have similar thickness to that of DOPC vesicles. Binary phase diagrams of DOPC/Cholesterol reported in literature\(^5\) show that there is no obvious phase separation in the membrane of DOPC/Cholesterol when the mol % of cholesterol is less than 40%.
Supplementary Figure S2  Fv is highly specific for the “up” conformation of KvAP VSD

a. Inhibition of channel activity at different Fv concentrations. Test pulses were delivered every two minutes, and the currents were normalized against the average from 5 traces right before time 0. For each Fv concentration, data from four independent experiments were averaged, and the errors bars were s.d. n=4. Single exponential fitting of current decrease (continuous lines) yielded the time constants (τ, plotted in b) and the steady state inhibition (normalized inhibition, plotted in c) at each Fv concentration.

b. The time constants obtained in panel a were used to generate the apparent on-rate (1/τ; black dots) - [Fv] plot. The best polynomial fitting to the data is linear (blue line), yielding an elementary kon of 1.2 x 10^5 s⁻¹M⁻¹.

c. Dose-response of Fv-inhibition. The steady-state inhibition of the channel activity was estimated from the normalized current reduction at different Fv concentrations and plotted here. A Michaelis-Menten equation was used to fit the data (black line), giving an estimated EC50 of 0.65 μg/ml. The measurement at the lowest concentration of Fv (17 ng/ml with 0.5 mg/ml BSA) had a significant error bar due to the small amount of Fv protein in perfusion.

d. Slow unbinding of Fv from channels held at -80 mV. With 2.0 μg/ml Fv added to the extracellular side, the channel activity was almost completely inhibited after 20 minutes. Washout by perfusion was very slow, ~70% recovery after 26 minutes (after that the membrane was broken).
Supplementary Figure S3  Binding of fluorescent Fv to channels in PE/PG vesicles.

a. Fluorescent spectra of FvA61C-Alexa 488; excitation (red) and emission (blue) peaks at 495 and 517 nm respectively.

b. Specific binding of Fv-Alexa 488 to KvAP in PE/PG vesicles. Vesicles (0.20 mg/ml protein) were incubated with Fv-Alexa 488 for five minutes, centrifuged at 250,000 x g for 45 min. The pellet was resuspended in 50 mM β-OG or 20 mM DM solution for fluorescence measurement. Blue line was derived from fitting with a Langmuir isothermal function. Underneath the plot shows 10% of the vesicles from ultracentrifugation in 12% SDS-PAGE (six pairs of samples with MW markers in between).
Supplementary Figure S4  Reconstitution keeps almost all channel proteins in membranes.

Fractions from the flotation of channel vesicles in Ficoll 400 gradients were analyzed by Coomassie-blue stained SDS-PAGE. The channels in PE/PG vesicles were found at the 10-15% interface and those in DOGS and DOTAP vesicles ran in the 10% layer and very close to the 0-10% demarcation. Fractions 0, 9 and 18 were controls before floatation. Fractions 1-8, 10-17, and 19-26 were 140 μl fractions taken from the top to the bottom of three gradients respectively, and 20 μl were used for SDS-PAGE assay. Due to the binding of SDS to DOTAP, 20 mM DM was added to channels in DOTAP vesicles before the addition of the SDS gel buffer, which rescued 20-50% of the total protein, and was the main reason that the bands in SDS-PAGE of DOTAP vesicles were fainter than those of PE/PG vesicles.
Supplementary Figure S5  Dimerization of wild-type KvAP at cys247

Channels reconstituted in PE/PG vesicles were reduced with 5.0 mM DTT for 30 minutes at room temperature, and spun at 200,000 x g for 45 minutes to remove DTT. The vesicles were then resuspended in a DTT-free buffer with(+) or without (-) 5.0 mM oxidized glutathione (GSSG), and incubated for 30 minutes before 20 mM DM was added and the samples were mixed with non-reducing SDS-PAGE buffer. Almost all wild-type KvAP were cross-linked dimers after such treatment.
Supplementary Figure S6  Screen of phage-displayed peptides against KvAP in vesicles

After the first run starting with the original library, the phages were amplified, titered, and used for the next biopanning. The positive-selections against the KvAP channels in DOTAP and DOGS vesicles were carried out in odd and even runs respectively. After 10 runs, the amplified phages were started to be tested on channels in lipid bilayers at 100 nM from both the intracellular and extracellular sides. After 14 runs, 40 colonies were selected for sequencing, and only gave rise to four different clones. ~50% of the colonies were phage $\phi_D$. 
Supplementary Reference

61 Schmidt, D., Cross, S. R. & MacKinnon, R. A gating model for the archeal voltage-dependent K(+) channel KvAP in DPhPC and POPE:POPG decane lipid bilayers. *J Mol Biol* **390**, 902-912, doi:10.1016/j.jmb.2009.05.062 [doi] (2009).