Experimental protocol:
1. HEK cells are used as either cultured wild-type of transfected with Shaker and GFP
2. HEK cell is whole-cell voltage clamped ($R_{seal} \geq 1\Omega$, $R_s < 10\Omega$)
3. AFM lever is engaged onto the cell membrane ($0.05nN \leq F_h \leq 1nN$)
4. Stimulation via a voltage ladder, recording current ($I$), lever displacement ($nm$)

Signal processing:
On-line PDT voltage
1. band-filtered (0.01Hz $\rightarrow$ 500Hz or 1kHz)
2. amplified 100x
3. averaged (3-10x)

Off-line
1. For a voltage step, steady-state lever displacement = $<\text{step height}> - <\text{baseline height}>$ (Due to mechanical noise baseline is averaged over 5msec, step height averaged over 2-5msec after steady-state current is reached)
2. Fractional displacement is $\text{Step}/(\text{max displacement})$

For wtHEK cells, the voltage-induced displacements (VD) are as we have seen before (Zheng et al., 2001). Displacement is linear with voltage. About 1Å/20mV at 0.2nN $F_h$. For cells transfected with voltage-independent Ach receptors, movement is also linear and amplitude is the same.
For HEK cells transfected with a voltage-gated shaker potassium channel, we observe strongly non-linear (Boltzmann $n^4$) voltage-dependent conductance.

Voltage-induced displacements are also non-linear, with the non-linearity at the point of channel activation. Below (left) is a set of typical VD experiments for HEK transfected with shaker. We see the non-linearity at all forces examined. For a particular $F_h$, the rising portion of the VD curve overlaps the data from wtHEK cells (below right).

The level of post-turn sloping varies from experiment to experiment.
The currents are not current-dependent. For wtHEK cells the displacement is roughly centered around zero current (below left). On the other hand, for Shaker transfected HEKs, the displacement is independent of current amplitude (below right).

To rule out voltage clamp artifacts we replaced bulk of K+ with NMDG (intracellular solution 1mM K+). At peak current, Rs ~ 5% total resistance. Curvature remains (below left) and overlaps the data for 140mM K+.

We have also recorded in symmetric K+ solutions (140mM K+ in and out). Voltage-dependent current records for these experiments are right-shifted (below left). Curvature is again present and overlaps data from (Kin/NaClout) (below right).
Shaker channel opening can be separated into two distinct parts: 1) voltage-dependent sensing (gating currents), 2) voltage-independent channel opening. Our data suggests that the curvature in the VD is due to channel opening and not voltage-sensing.

For a wtShaker channel voltage-dependent and voltage-independent events are close on the voltage and time axes. We have tested a mutant of this channel (V369I I372L or “IL”) that separates channel’s voltage sensing and channel opening (figure below – IL mutant is similar to ILT, but less right shifted). IL channel senses normally but opens at about 0mV.

In our results we see that the non-linear behavior is right-shifted to the point of channel activation.