Large or charged ones also cause and
modulation – needed for voltage
inactivated conformation of riluzole
channels. 

Results 1: Pulsed illumination – conformation-specific covalent binding:

Central – weak block + weakness modulation

After washout

Central – weak block + weakness modulation

Normalized curves show the extent of modulation

Summary of antibody data

<table>
<thead>
<tr>
<th>WT</th>
<th>WT 90%</th>
<th>WT F1579, BSA-90%</th>
<th>WT, F1579, BSA-90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>low affinity block</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>high affinity block</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Results 2: Ultrafast solution exchange:

WT

WT

The pulse train (20 Hz), was repeated every 1 ms. Each time 0.1 ms at 100 MS (less than twice time)

Results 3: Monitoring changes in gating kinetics:

WT

WT

WT

F1579A

F1579A

Conclusions:

Results 1 section:

• We provided experimental evidence for two distinct binding sites at sodium channels. 
  Using covalently bound azido-riluzole we could exclude the effect of state-dependent dynamic association/dissociation.
  • The F1579 B5 has high affinity, and is responsible for the most of modulation
  • For certain drugs (especially small neutral ones) binding causes non-blocking modulation
  • For large or charged molecules binding causes block and modulation
  • An alternative moderate affinity binding site exists.
  • It is only accessible at inactivated conformation.
  • It causes minimal modulation in the case of azido-riluzole

Results 2 section:

• The most plausible mechanism for the action of riluzole is non-blocking modulation
• The ability of riluzole to cause modulation is abolished in the F1579 B5 mutant
• Riluzole is able to reach its binding site with a time constant of ~ 5 ms, but its effect is only manifested in modulation, not block
• Like azido-riluzole, it is also able to bind to a moderate affinity non-F1579 B5

Results 3 section:

• We are able to assess major properties of gating at 1 resolution
• Drug onset & offset kinetics on the time scale of seconds, as well as drug effects on gating kinetics on the time scale of milliseconds can be followed
• Different mechanisms of inhibition can be identified
• Interaction with F1579 can be tested
• The example of trazodone: Modulation is possible at a non-F1579 B5 as well

Background:

Hints of two kinds of inactivated state inhibition in the literature:

Methods:

• WT and F1579A mutant (mNav1.4 expressing CHO cells)
• Membrane Patch Clamp – Ultrasonic solution exchange
• Phosphorimaging: Imaging cAMP

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