Supplementary Material for
“Dissecting Electrostatic Screening, Specific Ion Binding, and Ligand Binding in an Energetic Model for Glycine Riboswitch Folding”

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Na$^+$ titration: Hydroxyl radical footprinting protection pattern

Fig. S1. Solvent exposure probed by hydroxyl radical cleavage for residues 47-150 of the VCI-II tandem aptamer as a function of increasing Na$^+$ concentration. Data were normalized such that the amount of cleavage at 0 mM added Na$^+$ (on top of the background from 50 mM Na-MOPS buffer) corresponds to zero. Blue regions show increasing protections from cleavage, and red regions show increasing cleavage, following the colorbar below. Data represent averaged values from at least three repeat measurements. The 20 mM Mg$^{2+}$ comparisons were obtained from hydroxyl radical footprinting conducted for different residue ranges (Lipfert et al. 2007). With increasing Na$^+$, in the absence of glycine, the final protection pattern at high concentrations ( >1000 mM) of Na$^+$ is comparable to that at 20 mM Mg$^{2+}$ in the absence of glycine (Lipfert et al. 2007). Noticeably, there are protections around residues 62-64, 82-84, 93-94, and 123-124, even though there appear to be some small structural differences. For details on data analysis, see main text.
**Na\textsuperscript{+} titration: Hill fits of the hydroxyl radical footprinting data**

Fig. S2. Relative protections from hydroxyl radical cleavage of the VCI-II glycine riboswitch as a function of Na\textsuperscript{+} in the absence of glycine. Protections (symbols) are scaled such that full protections at high salt are normalized to one, while protections in 50 mM Na-MOPS buffer only are zero (see Methods). Data were fitted to the Hill equation (solid lines) with two variable parameters (Hill coefficient and Hill midpoint; Equation 1 in the main text). Panels A-D show the footprinting data for different residue group regions of the riboswitch where protections from hydroxyl radical cleavage are observed; the corresponding residues are shown in the figure inserts. The protected regions shown correspond to aptamer I of the VCI-II riboswitch. Regions in aptamer II (except residues 140-150) were not probed in the 5' labeling scheme used. Data shown in A-D correspond to averages and standard deviations from at least three measurements. The fitted Hill coefficient and Hill midpoint values are summarized in Table S1.
**Na\(^+\) titration: Fitted parameters for the hydroxyl radical footprinting data**

<table>
<thead>
<tr>
<th>Region</th>
<th>Residues</th>
<th>Hill coefficient ((m_{1, Na}))</th>
<th>Hill midpoint ((K_{1, Na}, \text{mM}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>62-64</td>
<td>1.7 ± 0.4</td>
<td>250 ± 100</td>
</tr>
<tr>
<td>B</td>
<td>82-84</td>
<td>2.8 ± 0.4</td>
<td>160 ± 30</td>
</tr>
<tr>
<td>C</td>
<td>93-94</td>
<td>5.0 ± 0.6</td>
<td>130 ± 22</td>
</tr>
<tr>
<td>D</td>
<td>123-125</td>
<td>3.7 ± 1.4</td>
<td>240 ± 110</td>
</tr>
</tbody>
</table>

\(\langle m_{1, Na} \rangle = 3.3 ± 1.4\) \(\langle K_{1, Na} \rangle = 190 ± 60\) mM Na\(^+\).

Table S1. Hill coefficients and midpoints for the hydroxyl radical footprinting data as a function of Na\(^+\) concentration in the absence of glycine shown in Figure S2. The regions refer to the sets of residues as illustrated in Figure S2. Midpoint values were averaged over the different residues from each region. Averaging over all transitions, the Hill coefficient is \(\langle m_{1, Na} \rangle = 3.3 ± 1.4\) and the Hill midpoint is \(\langle K_{1, Na} \rangle = 190 ± 60\) mM Na\(^+\). For details of the footprinting experiments and analysis see the main text.
Sr$^{2+}$ titration: Hydroxyl radical footprinting protection pattern

Fig. S3. Solvent exposure probed by hydroxyl radical cleavage for residues 47-150 of the VCI-II tandem aptamer as a function of different Sr$^{2+}$ concentrations in the absence of glycine. Data were normalized such that the amount of cleavage at 0 mM Sr$^{2+}$ corresponds to zero. The final protection pattern at high concentrations (>100 mM) of Sr$^{2+}$ is comparable to that at 20 mM Mg$^{2+}$ in the absence of glycine (Lipfert et al. 2007), see for example the strong protections at residues 62/62 and 83-85 and weaker protections around residues ~95 and ~124. Nonetheless, there are small differences in the protection patterns which are difficult to quantitatively distinguish given the signal to noise of these hydroxyl radical cleavage experiments. Data represents averaged values from at least three repeat measurements. The 20 mM Mg$^{2+}$ comparison was obtained from hydroxyl radical footprinting conducted for different residue ranges (Lipfert et al. 2007).
Sr\(^{2+}\) titration: Hill fits of the hydroxyl radical footprinting data

Fig. S4. Relative protections from hydroxyl radical cleavage of the VCI-II glycine riboswitch as a function of Sr\(^{2+}\) in the absence of glycine. Protections (symbols) are scaled such that full protections at high salt are normalized to one, while protections in the absence of Sr\(^{2+}\) are zero. Data were fitted to the Hill equation (solid lines) with two variable parameters (Hill coefficient and Hill midpoint). Panels A and B show the footprinting data for different residue group regions of the riboswitch where protections from hydroxyl radical cleavage are observed; the corresponding residues are shown in the figure inserts. The protected regions shown correspond to aptamer I of the VCI-II riboswitch. Regions in aptamer II (except residues 140-150) were not probed in the 5'-labeling scheme used. Data shown in A and B correspond to averages and standard deviations from at least three measurements. The Hill coefficient and Hill midpoint values are summarized in Table S2. Residues 93 and 94 exhibit discernable protections from cleavage with increasing Sr\(^{2+}\) concentration, however, the signal-to-noise is much lower than for the residue groups 62/63 and 83-85. Fitting to the weaker set of protections for residues 93 and 94 yields a Hill coefficient of 1.3 ± 1 and a Hill midpoint of 0.88 ± 0.81 mM Sr\(^{2+}\).
Sr\(^{2+}\) titration: Fitted parameters for the hydroxyl radical footprinting data

<table>
<thead>
<tr>
<th>Region</th>
<th>Residues</th>
<th>Hill coefficient ((m_{1,Sr}))</th>
<th>Hill midpoint ((K_{1,Sr}, \text{mM}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>62-63</td>
<td>1.3 ± 0.4</td>
<td>0.32 ± 0.14</td>
</tr>
<tr>
<td>B</td>
<td>83-85</td>
<td>1.1 ± 0.2</td>
<td>0.11 ± 0.02</td>
</tr>
</tbody>
</table>

Table S2. Hill coefficients and midpoints for the hydroxyl radical footprinting data as a function of Sr\(^{2+}\) concentration in the absence of glycine shown in Figure S4. The regions refer to the sets of residues as illustrated in Figure S4. Midpoint values were averaged over the different residues from each region. Averaging over all transitions, the Hill coefficient is \(<m_{1,Sr}> = 1.2 ± 0.2\) and the Hill midpoint is \(<K_{1,Sr}> = 0.22 ± 0.15 \text{ mM Sr}^{2+}\). For details of the footprinting experiments and analysis see the main text.
Mg$^{2+}$ titration in the presence of 2 M NaCl and 10 mM glycine: Hydroxyl radical footprinting

Fig. S5. Example of a gel image of the hydroxyl radical footprinting data as a function of
Mg$^{2+}$ in the presence of 2 M NaCl and 10 mM glycine. Lanes from left to right are: U (untreated, no reaction), T1 (T1 digest), 2 M NaCl and 0, 0.01, 0.1, 1, 2, 5, 10, 20, 100, 200 µM Mg$^{2+}$ and T1 (T1 digest, repeated and loaded with more counts). The T1 digest ladder is annotated on the left and right of the figure. Footprinting data were analyzed from the gel images using the peak fitting routine in the software SAFA (Das et al. 2005). The data were then normalized and standardized, following the method of Takamot et al. (Takamoto et al. 2004). Independent repeat measurements were performed and averaged. Protections from cleavage are clearly visible in the gel image. Here, increase in protections with increasing Mg$^{2+}$ in regions around 63-65, 73-75, 81-82, 125-128 (aptamer I) and 146-147 (aptamer II) are observed, as indicated by the vertical black bars on the right. Protection further in the aptamer II region (residues >150) can also be qualitatively seen, but since data higher up in the gel are of lower resolution and hence harder to quantify, residues above 150 were not quantitatively analyzed.
**Mg\textsuperscript{2+} titration in the presence of 2 M NaCl and 10 mM glycine:**

Hill fits of the hydroxyl radical footprinting data

![Graphs A to E showing relative protections from hydroxyl radical cleavage of the VCI-II glycine riboswitch as a function of Mg\textsuperscript{2+} in the presence of 10 mM glycine, and a background of 2 M NaCl. Protections (symbols) are scaled such that full protection at 20 mM Mg\textsuperscript{2+} are normalized to one, while protections in the absence of Mg\textsuperscript{2+} are zero. Data were fitted to the Hill equation (solid lines) with two variable parameters (Hill coefficient and Hill midpoint). Panels A-E show the footprinting data for different residue group regions of the riboswitch where protections from hydroxyl radical cleavage are observed; the corresponding residues are shown in the figure inserts. The protected regions shown correspond to aptamer I of the VCI-II riboswitch, except for residue region 146-147, which corresponds to aptamer II. Data shown in A-E correspond to averages and standard deviations from at least three measurements. The Hill coefficient and Hill midpoint values are summarized in Table S3.**
Mg\textsuperscript{2+} titration in the presence of 2 M NaCl and 10 mM glycine:
Fitted parameters for the hydroxyl radical footprinting data

<table>
<thead>
<tr>
<th>Region</th>
<th>Residues</th>
<th>Hill coefficient ( (m_{2, \text{Mg}}) )</th>
<th>Hill midpoint ( (K_{2, \text{Mg}}, \text{mM}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>63-65</td>
<td>3.4 ± 1.1</td>
<td>2.5 ± 0.8</td>
</tr>
<tr>
<td>B</td>
<td>73-75</td>
<td>3.4 ± 0.9</td>
<td>2.9 ± 0.7</td>
</tr>
<tr>
<td>C</td>
<td>81-82</td>
<td>3 ± 2</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>D</td>
<td>125-128</td>
<td>2.4 ± 1.5</td>
<td>2.8 ± 1.7</td>
</tr>
<tr>
<td>E</td>
<td>146-147</td>
<td>2.1 ± 0.7</td>
<td>1.8 ± 0.5</td>
</tr>
</tbody>
</table>

Table S3. Hill coefficients and midpoints for the hydroxyl radical footprinting data as a function of Mg\textsuperscript{2+} concentration in the presence of 10 mM glycine and a background of 2 M NaCl shown in Figure S6. The regions refer to the sets of residues as illustrated in Figure S6. Midpoint values were averaged over the different residues from each region. Averaging over all transitions, the Hill coefficient is \( \langle m_{2, \text{Mg}} \rangle = 2.8 ± 1.3 \) and the Hill midpoint is \( \langle K_{2, \text{Mg}} \rangle = 2.8 ± 1.1 \) mM Mg\textsuperscript{2+}. For details of the footprinting experiments and analysis see the main text.
Hydroxyl radical footprinting: Residue level comparisons

Fig. S7. Identical to figure 2 of the main text, but with data scaled by the mean cleavage intensity of each ionic condition lane. This serves to illustrate that our conclusions do not depend heavily on the standardized residues chosen. (See main text for details.) Panel A: The profiles in the absence of glycine, under different ionic conditions: 100 mM Mg$^{2+}$ (green); 100 mM Ca$^{2+}$ (orange); 100 mM Sr$^{2+}$ (brown); 2 M Na$^{+}$ (black). Panel B: The profiles in the presence of glycine, under different ionic conditions: 100 mM Mg$^{2+}$ (red); 100 mM Ca$^{2+}$ (orange); 100 mM Sr$^{2+}$ (brown); 2 M Na$^{+}$ (black). Panel C: In 2 M Na$^{+}$, in the absence (black, dashed) and presence (black, solid) of 10 mM glycine. Panel D: In 100 mM Sr$^{2+}$, in the absence (brown, dashed) and presence (brown, solid) of 10 mM glycine.
Supplementary References

