Supporting information for:

Engineering Transcriptional Regulator Effector Specificity using Computational Design and In Vitro Rapid Prototyping: Developing a Vanillin Sensor

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Table S1: **Initial qacR mutants chosen through computationally guided design** List of the amino acid mutations by position of the initial set of qacR mutants selected. Low energy sequences from different optimization runs were analyzed and a set of 10 mutants was selected for testing.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Amino Acid Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>qacR-wt</td>
<td>F L E E W S T E Y Q I F M L E A N N T</td>
</tr>
<tr>
<td>mutant1</td>
<td>A W Q L Y S T Q Y M Q S Q Y Q A M Q M</td>
</tr>
<tr>
<td>mutant2</td>
<td>A W Q L Y S T Q Y Q Q F Q Y Q A M L M</td>
</tr>
<tr>
<td>mutant3</td>
<td>A W Q L Y S T Q Y Q I S Q Y Q A M L M</td>
</tr>
<tr>
<td>mutant4</td>
<td>A W Q L Y S T Q Y M Q S Q Y Q A M Q M</td>
</tr>
<tr>
<td>mutant5</td>
<td>A W E E Y S T Q Y M Q S Q Y Q A N N T</td>
</tr>
<tr>
<td>mutant6</td>
<td>A W E E Y S T Q Y Q Q F Q Y Q A N N T</td>
</tr>
<tr>
<td>mutant7</td>
<td>A W E E Y S T Q Y Q I S Q Y Q A N N T</td>
</tr>
<tr>
<td>mutant8</td>
<td>A W E E Y S T Q Y M Q S Q Y Q A N N T</td>
</tr>
<tr>
<td>mutant9</td>
<td>A W Q L W S T Q Y M Q S Q Y Q A M Q M</td>
</tr>
<tr>
<td>mutant10</td>
<td>A W E E W S T Q Y Q I F M Y Q A N N T</td>
</tr>
</tbody>
</table>

Table S2: **QacR mutants tested** List of the amino acid mutations by position of the second set of qacR mutants tested in the second TX-TL screen. The amino acid identities of the wild-type protein for the positions considered are also shown.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Amino Acid Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>qacR-wt</td>
<td>F L E E W S T E Y Q I F M L E A N N T</td>
</tr>
<tr>
<td>qacR1</td>
<td>F L Q L Y S T Q Y M Q S Q Y Q A M Q M</td>
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<td>qacR2</td>
<td>F L Q L Y S T Q Y Q Q F Q Y Q A M L M</td>
</tr>
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<td>qacR3</td>
<td>F L Q L Y S T Q Y Q I S Q Y Q A M L M</td>
</tr>
<tr>
<td>qacR4</td>
<td>F L Q L Y S T Q Y M Q S Q Y Q A M Q M</td>
</tr>
<tr>
<td>qacR5</td>
<td>F L E E Y S T Q Y M Q S Q Y Q A N N T</td>
</tr>
<tr>
<td>qacR6</td>
<td>F L E E Y S T Q Y Q Q F Q Y Q A N N T</td>
</tr>
<tr>
<td>qacR7</td>
<td>F L E E Y S T Q Y Q I S Q Y Q A N N T</td>
</tr>
<tr>
<td>qacR8</td>
<td>F L Q L W S T Q Y M Q S Q Y Q A M Q M</td>
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<td>qacR9</td>
<td>A W Q L Y S T Q Y M Q S Q L Q A M Q M</td>
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<td>qacR10</td>
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<td>qacR11</td>
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<td>qacR13</td>
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<td>qacR16</td>
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<td>qacR17</td>
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### Table S3: OD600 of Cells at 4 ng/mL aTc

<table>
<thead>
<tr>
<th>vanillin (mM)</th>
<th>GFP</th>
<th>wild-type</th>
<th>qacR2-1</th>
<th>qacR5-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>0.456</td>
<td>0.44</td>
<td>0.75</td>
<td>0.72</td>
</tr>
<tr>
<td>500</td>
<td>0.62</td>
<td>0.63</td>
<td>0.78</td>
<td>0.77</td>
</tr>
<tr>
<td>250</td>
<td>0.718</td>
<td>0.75</td>
<td>0.82</td>
<td>0.83</td>
</tr>
<tr>
<td>125</td>
<td>0.775</td>
<td>0.75</td>
<td>0.82</td>
<td>0.81</td>
</tr>
<tr>
<td>62.5</td>
<td>0.783</td>
<td>0.81</td>
<td>0.84</td>
<td>0.86</td>
</tr>
<tr>
<td>31.25</td>
<td>0.823</td>
<td>0.83</td>
<td>0.84</td>
<td>0.85</td>
</tr>
<tr>
<td>0</td>
<td>0.855</td>
<td>0.86</td>
<td>0.85</td>
<td>0.86</td>
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<tr>
<td>no aTc</td>
<td>0.866</td>
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<td>0.88</td>
<td>0.9</td>
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### Table S4: OD600 of Cells at 4 ng/mL aTc

<table>
<thead>
<tr>
<th>vanillin (mM)</th>
<th>GFP</th>
<th>wild-type</th>
<th>qacR2-1</th>
<th>qacR5-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>0.52</td>
<td>0.49</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>500</td>
<td>0.63</td>
<td>0.64</td>
<td>0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>250</td>
<td>0.78</td>
<td>0.74</td>
<td>0.75</td>
<td>0.76</td>
</tr>
<tr>
<td>125</td>
<td>0.76</td>
<td>0.78</td>
<td>0.74</td>
<td>0.77</td>
</tr>
<tr>
<td>62.5</td>
<td>0.84</td>
<td>0.81</td>
<td>0.75</td>
<td>0.77</td>
</tr>
<tr>
<td>31.25</td>
<td>0.79</td>
<td>0.82</td>
<td>0.75</td>
<td>0.74</td>
</tr>
<tr>
<td>0</td>
<td>0.84</td>
<td>0.86</td>
<td>0.78</td>
<td>0.8</td>
</tr>
<tr>
<td>no aTc</td>
<td>0.82</td>
<td>0.85</td>
<td>0.64</td>
<td>0.85</td>
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</tbody>
</table>

### Table S5: OD600 of Cells at 8 ng/mL aTc

<table>
<thead>
<tr>
<th>vanillin (mM)</th>
<th>GFP</th>
<th>wild-type</th>
<th>qacR2-1</th>
<th>qacR5-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>0.46</td>
<td>0.46</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>500</td>
<td>0.67</td>
<td>0.64</td>
<td>0.27</td>
<td>0.24</td>
</tr>
<tr>
<td>250</td>
<td>0.72</td>
<td>0.72</td>
<td>0.11</td>
<td>0.12</td>
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<td>125</td>
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<td>0.77</td>
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<td>0.09</td>
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<tr>
<td>62.5</td>
<td>0.87</td>
<td>0.82</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>31.25</td>
<td>0.81</td>
<td>0.83</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>0</td>
<td>0.84</td>
<td>0.83</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>no aTc</td>
<td>0.8</td>
<td>0.79</td>
<td>0.84</td>
<td>0.86</td>
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</table>

### Table S6: OD600 of Cells at 12 ng/mL aTc

<table>
<thead>
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<th>vanillin (mM)</th>
<th>GFP</th>
<th>wild-type</th>
<th>qacR2-1</th>
<th>qacR5-1</th>
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</thead>
<tbody>
<tr>
<td>1000</td>
<td>0.48</td>
<td>0.45</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>500</td>
<td>0.69</td>
<td>0.67</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>250</td>
<td>0.76</td>
<td>0.76</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>125</td>
<td>0.82</td>
<td>0.79</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>62.5</td>
<td>0.81</td>
<td>0.82</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>31.25</td>
<td>0.82</td>
<td>0.84</td>
<td>0.04</td>
<td>0.04</td>
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<tr>
<td>0</td>
<td>0.84</td>
<td>0.87</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>no aTc</td>
<td>0.82</td>
<td>0.81</td>
<td>0.84</td>
<td>0.84</td>
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</table>
Table S7: Design Positions for CPD

<table>
<thead>
<tr>
<th>Residue ID</th>
<th>Wild-Type Identity</th>
<th>Allowed Amino Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>F</td>
<td>FWYILMA</td>
</tr>
<tr>
<td>54</td>
<td>L</td>
<td>STNQYW F</td>
</tr>
<tr>
<td>57</td>
<td>E</td>
<td>STNQYW F</td>
</tr>
<tr>
<td>58</td>
<td>E</td>
<td>WFYMLIQNST</td>
</tr>
<tr>
<td>61</td>
<td>W</td>
<td>FWY</td>
</tr>
<tr>
<td>86</td>
<td>S</td>
<td>FWYILM</td>
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<tr>
<td>89</td>
<td>T</td>
<td>FWYILMQNSVT</td>
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<tr>
<td>90</td>
<td>E</td>
<td>FWYILMQNSTV</td>
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<tr>
<td>162</td>
<td>T</td>
<td>WFYMLVIQNST</td>
</tr>
</tbody>
</table>
Figure S1: **Initial qacR induction test.** Fluorescence of cells encoding wild-type qacR was compared in the presence and absence of berberine, a native qacR inducer. While we observed repression upon the addition of qacR, we did not observe induction when berberine was added.
Figure S2: **Steric clashes from computational model.** We looked at a computational model (red) of the DNA-bound qacR structure that was three mutations away from a qacR mutant that was previously shown to be functional. The wild-type residues from the structure are shown in cyan. These mutations could have been causing steric clashes in the DNA-bound state. We observed a potential steric clash between the tryptophan (position 54) and tyrosine (position 119) mutations. Based on this model we grouped two of the mutations together and reverted them to their wild-type identity F50A/L54W (a) and Y119L (b), to relieve this potential clash.

Figure S3: **Timetrace of qacR mutant screen.** GFP fluorescence time traces for TX-TL reactions set up to screen for qacR mutants that were sensitive to vanillin. Reactions contained DNA encoding a qacR variant, T7 RNA polymerase, a fluorescent reporter and either water, dequilinium or vanillin.
Figure S4: **Vanillin dosage response curves.** Cells expressing GFP without any repressor were used as a control to normalize for differences in fluorescence due to aTc and vanillin levels. Fluorescence was measured for different inducer concentrations. Mutants display an increased sensitivity to vanillin concentrations for most of the range of aTc concentrations tested.