SUPPORTING INFORMATION

**Title:** The Advantage of Covalent Capture in the Combinatorial Screening of a Dynamic Library for the Detection of Weak Interactions

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**Ref. No.:** O200901516
Library equilibration studies

**Figure S1.** Time course of the equilibration of the library starting from $\text{P1B}_3$ (left) and $\text{P1A}_3$ (right).
Simulations

Simulations were performed with Hyperquad Simulation and Speciation version 3.2.24 available as freeware at http://www.hyperquad.co.uk/hyss.htm.

As example, the calculated mixture composition under experimental conditions as discussed in the manuscript was obtained imposing the following equilibria and constants (binding constants were imposed taking $P_1B_3$ as the reference $K_{app} = 1 \times 10^8$ imposing quantitative imine formation as observed experimentally). Additional binding constants were imposed based on the experimentally determined ratio.

$$P_1 + 3A \rightleftharpoons P_1A_3 \quad (K_{app} = 4.59 \times 10^8)$$

$$P_1 + 2A + B \rightleftharpoons P_1A_2B_1 \quad (K_{app} = 9.39 \times 10^8)$$

$$P_1 + A + 2B \rightleftharpoons P_1A_1B_2 \quad (K_{app} = 5.31 \times 10^8)$$

$$P_1 + 3B \rightleftharpoons P_1B_3 \quad (K_{app} = 1 \times 10^8)$$

And the complexes with target $T$

$$P_1 + 2A + B + T \rightleftharpoons P_1A_2B_1T \quad (K_{app} = 4.69 \times 10^{10} \text{ assuming an additional binding constant of } 50 \text{ M}^{-1} \text{ between } P_1A_2B_1 \text{ and } T)$$

$$P_1 + A + 2B + T \rightleftharpoons P_1A_1B_2T \quad (K_{app} = 3.18 \times 10^{11} \text{ assuming an additional binding constant of } 600 \text{ M}^{-1} \text{ between } P_1A_1B_2 \text{ and } T)$$

$$P_1 + 3B + T \rightleftharpoons P_1B_3T \quad (K_{app} = 2.4 \times 10^{11} \text{ assuming an additional binding constant of } 2700 \text{ M}^{-1} \text{ between } P_1B_3 \text{ and } T)$$

Conditions used for the simulations

$$[P_1] = 0.01 \text{ M}$$

$$[A] = [B] = 0.044 \text{ M}$$

The concentrations were calculated in the presence and absence of $T = 0.01 \text{ M}$ yielding an amplification factor of 3.1 for the concentration of receptor $P_1B_3$ upon the addition of $T$.

In a similar manner the other simulations were performed using the parameters as indicated in the manuscript.
Binding studies of receptor P1B₃ with different anions

All binding isotherms were fitted to a 1:1 model using Micromath Scientist for Windows, version 2.01. For monoanions no different stochiometries of binding are reasonable due the very low binding constants. For the di- and trianions also 1:2 models were implemented. Their efficacy in fitting the binding isotherms is discussed.

a) Benzoic acid, sodium salt

![Graph showing chemical shift vs concentration of anion for Benzoic acid, sodium salt]

Confidence Intervals:

<table>
<thead>
<tr>
<th>Parameter Name</th>
<th>Estimate Value</th>
<th>Standard Deviation</th>
<th>95% Range (Univar)</th>
<th>95% Range (S-Plane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>42.9</td>
<td>8.3</td>
<td>21.5, 64.3</td>
<td>9.3, 76.4</td>
</tr>
<tr>
<td>SA</td>
<td>8.909</td>
<td>0.001</td>
<td>8.907, 8.910</td>
<td>8.906, 8.911</td>
</tr>
<tr>
<td>SAB</td>
<td>8.848</td>
<td>0.006</td>
<td>8.833, 8.863</td>
<td>8.825, 8.871</td>
</tr>
</tbody>
</table>

b) Bis-p-nitrophenyl phosphate, sodium salt

![Graph showing chemical shift vs concentration of anion for Bis-p-nitrophenyl phosphate, sodium salt]

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<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>60.7</td>
<td>9.2</td>
<td>37.1, 84.3</td>
<td>23.7, 97.7</td>
</tr>
<tr>
<td>SA</td>
<td>8.908</td>
<td>0.000</td>
<td>8.906, 8.909</td>
<td>8.906, 8.910</td>
</tr>
<tr>
<td>SAB</td>
<td>8.829</td>
<td>0.006</td>
<td>8.813, 8.845</td>
<td>8.804, 8.854</td>
</tr>
</tbody>
</table>
c) 1,3-benzene dicarboxylate, disodium salt

Results from fitting to a 1:1 model.

Fitting the data to a 1:2 model (assuming complex formation between the scaffold and two anions with identical binding constants) was performed in two ways:

1) iterating the binding constant, the chemical shift of the 1:1 complex (SAB), and the chemical shift of the 1:2 complex (SABB)

Confidence Intervals:

Parameter Name : K
Estimate Value = 680.5896
Standard Deviation = 40.70643
95% Range (Univar) = 575.9504 785.2288
95% Range (S-Plane) = 516.606 844.5731

Parameter Name : SAB
Estimate Value = 8.531
Standard Deviation = 0.048
95% Range (Univar) = 8.414 8.648
95% Range (S-Plane) = 8.378 8.684

The first method gives a binding constant more than 10 fold higher than previously determined for the 1:1 complex with an unacceptable large standard deviation.

The second method gives a chemical shift for the 1:1 complex AB (8.531), which is lower than the chemical shift of A (8.909), whereas the calculated chemical shift for the 1:2 complex ABB (9.089) is higher.
d) \(p\)-Nitrophenylphosphate, disodium salt

Results from fitting to a 1:1 model.

Fitting the data to a 1:2 model (assuming complex formation between the scaffold and two anions with identical binding constants) was performed in two ways:

1) iterating the binding constant, the chemical shift of the 1:1 complex (\(S_{AB}\)), and the chemical shift of the 1:2 complex (\(S_{ABB}\))

Confidence Intervals:

<table>
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<tr>
<th>Parameter Name</th>
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<th>95% Range (S-Plane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K)</td>
<td>658</td>
<td>203</td>
<td>-160 - 1477</td>
<td>-160 - 1477</td>
</tr>
<tr>
<td>(S_{AB})</td>
<td>8,910</td>
<td>0,002</td>
<td>8,895 - 8,915</td>
<td>8,902 - 8,917</td>
</tr>
<tr>
<td>(S_{ABB})</td>
<td>8,870</td>
<td>0,005</td>
<td>8,868 - 8,888</td>
<td>8,873 - 8,877</td>
</tr>
</tbody>
</table>

2) fixing the binding constant to the known value of the 1:1 complex (61 M\(^{-1}\)) and iterating the chemical shift of the 1:1 complex (AB), and the chemical shift of the 1:2 complex (ABB)

Confidence Intervals:

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<th>95% Range (S-Plane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S_{AB})</td>
<td>8,870</td>
<td>0,005</td>
<td>8,858 - 8,881</td>
<td>8,880 - 8,888</td>
</tr>
<tr>
<td>(S_{ABB})</td>
<td>8,948</td>
<td>0,021</td>
<td>8,896 - 9,001</td>
<td>8,917</td>
</tr>
</tbody>
</table>

The first method gives a binding constant 10 fold higher than previously determined for the 1:1 complex with an unacceptable large standard deviation.

The second method gives a chemical shift for the 1:1 complex AB (8.750), which is lower than the chemical shift of A (8.909), whereas the calculated chemical shift for the 1:2 complex ABB (8.948) is higher.
c) 1,3,5-benzene tricarboxylate, trisodium salt (2.9x10^{-3} M)

Results from fitting to a 1:1 model.

Fitting the data to a 1:2 model (assuming complex formation between the scaffold, one anion with a single bond (KM; AB) and one anion with two bonds (KD; ABB) was performed in two ways:

1) Imposing the binding constants known from the previous titrations (KM = 43 M^{-1}; KD = 681 M^{-1}) and the chemical shift of the platform, iterating the chemical shift of the complexes AB (SAB) and ABB (SABB)

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<th>95% Range (S-Plane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAB</td>
<td>8.883</td>
<td>0.012</td>
<td>8.852</td>
<td>8.915</td>
</tr>
<tr>
<td>SABB</td>
<td>8.793</td>
<td>0.002</td>
<td>8.787</td>
<td>8.802</td>
</tr>
</tbody>
</table>

2) fixing the chemical shift of complex AB on 8.848 ppm as determined previously and iterating on KM, KD, and the chemical shift of ABB.

No acceptable fit was obtained

The first method gives a chemical shift for the AB complex which is much different from the chemical shift obtained for the benzoic acid complex (7.944 vs 8.848). Additionally, a chemical shift for the 1:1 complex ABB is obtained which is intermediate between the platform A (8.909), and the complex AB (8.883).