Supporting information

For

‘Indirect Optical Analysis of a Dynamic Chemical System’

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1. Synthesis and characterization of compounds 1B and 2A.

Aldehyde 1 or 2 was dissolved in MeOH and 1 equivalent of hydrazide was added to the solution. The reaction mixture was stirred at 40°C for 8 hours and evaporated to dryness.

**Hydrazone 1B**

![Chemical structure of 1B]

**Yield:** 93%

**$^1$H-NMR** (250 MHz, CDCl$_3$) $\delta$ (ppm): 8.79 (s, 1H), 8.16 (d, 1H, $J=7.71$ Hz), 7.78 (s, 1H), 7.46-7.08 (m, 4H), 6.69 (q, 1H), 3.32 (m, 8H), 2.32 (dd, 1H), 1.99 (s, 2H), 1.66 (m, 8H), 1.37 (m, 8H), 1.16 (dt, 3H, $J=13.4$ Hz, $J=27.3$ Hz), 0.96 (t, 12H, $J=7.1$ Hz)

**$^{13}$C-NMR** (63 MHz, CDCl$_3$) $\delta$ (ppm): 164.8, 157.5, 153.9 (d, $J_P-C=7.8$ Hz), 147.4, 147.1, 132.6, 127.6, 126.9 (d, $J_P-C=4.6$ Hz), 124.4, 122.3 (d, $J_P-C=2.7$ Hz), 116.9, 113.3, 59.5, 24.8, 22.9, 20.7, 13.9, 8.1.

**$^{31}$P-NMR** (62%) (122 MHz, CDCl$_3$) proton decoupled $\delta$ (ppm): 28.2.

**ESI-MS** CH$_3$OH, (m/z): 321.06 [M-H]

**Hydrazone 2A**

![Chemical structure of 2A]

**Yield:** 91%

**Rf:** 0.5 (ethyl acetate);

**$^1$H-NMR first isomer (80%),** (250 MHz, CDCl$_3$) $\delta$ (ppm): 7.79 (d, 1H, $J=8.18$ Hz), 7.58-7.51 (m, 2H), 7.41-7.32 (m, 3H), 7.16-6.86 (m, 2H), 4.69 (s, 2H), 3.39 (s, 9H);

**$^1$H-NMR second isomer (20%),** (250 MHz, CDCl$_3$) $\delta$ (ppm): 8.45 (d, 1H, $J=8.18$ Hz), 7.58-7.51 (m, 2H), 7.41-7.32 (m, 3H), 7.16-6.86 (m, 2H), 4.29 (s, 2H), 3.39 (s, 9H);

**$^{13}$C-NMR first isomer (80%),** (63 MHz, CDCl$_3$) $\delta$ (ppm): 175.6, 151.5, 137.5, 130.6, 130.1, 129.7, 128.3, 125.9, 48.7, 40.1;

**$^{13}$C-NMR second isomer (20%),** (63 MHz, CDCl$_3$) $\delta$ (ppm): 175.6, 148.2, 137.5, 130.6, 130.1, 129.7, 128.3, 125.8, 48.7, 40.1;

**ESI-MS (+):** CH$_3$OH, (m/z): 246.3 [M$^+$]
2. UV/Vis calibration curve for hydrazone 2B.

Ten samples of 2B were individually weighted and dissolved in quantities of the same solvent mixture as used for the competition experiments (MeOH : DMSO : CH₃CN = 70 : 15 : 15) sufficient to obtain concentrations ranging from 10 to 70 µM.

UV-Vis calibration curve for 2B

A = 0.0266 [2B] + 0.033 (R² = 0.9911) with [2B] in µM.

<table>
<thead>
<tr>
<th>[2B] µM</th>
<th>10.0</th>
<th>15.0</th>
<th>20.0</th>
<th>25.0</th>
<th>30.0</th>
<th>35.0</th>
<th>40.0</th>
<th>50.0</th>
<th>60.0</th>
<th>70.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.268</td>
<td>0.365</td>
<td>0.579</td>
<td>0.731</td>
<td>0.885</td>
<td>1.022</td>
<td>1.084</td>
<td>1.323</td>
<td>1.690</td>
<td>1.836</td>
</tr>
</tbody>
</table>

Calibration Curve 2B

A = 0.0266[2B] + 0.033
R² = 0.9911
3. Concentrations obtained from direct ($^1$H NMR) and indirect (UV/Vis) measurements (Figure 3).

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>A$_{348}$</td>
<td>0.9281</td>
<td>0.7669</td>
<td>0.9418</td>
<td>0.7758</td>
<td>1.0267</td>
<td></td>
</tr>
<tr>
<td>[2B] mM UV-Vis</td>
<td>3.37</td>
<td>2.76</td>
<td>3.42</td>
<td>2.79</td>
<td>3.74</td>
<td></td>
</tr>
<tr>
<td>[2X] mM NMR</td>
<td>3.42</td>
<td>2.79</td>
<td>3.43</td>
<td>2.72</td>
<td>3.79</td>
<td></td>
</tr>
</tbody>
</table>
4. Scientist Model used for the theoretical simulation

// MicroMath Scientist Model File
IndVars: H10, H20
DepVars: ampl, ratio, AH1, AH2, A, H1, H2
Params: K, af, A0

AH1=K*AF*A*H1
AH2=K*A*H2
A=A0-AH1-AH2
H1=H10-AH1
H2=H20-AH2
ampl=AH1/(AH1+AH2)*100
ratio=AH1/AH2

// boundaries
0<A<A0
0<H1<H10
0<H2<H20
0<AH1<A0
0<AH2<A0
***

In the model $H1$ and $H2$ represent hydrazides A and B; $A$ represents aldehyde 1. The thermodynamic stability of 1A is affected by an amplification factor AF.
5. Calculations of the mixture compositions as reported in Figure 5C.

The separate competition experiments leading towards the data given in Figure 3 were performed using 5 mM of aldehyde 1, 5 mM of hydrazide B and 5 mM of hydrazide X (X = A, C – F). Scavenging using aldehyde 2 (10 mM) quantitatively converts the ‘left-over’ amount of hydrazide B into aldehyde 2B, the concentration of which can be determined from a comparison of the absorbance at 348 nm with a calibration curve (SI: page 4). The concentrations of all remaining compounds (1B, 1X, and X) of the equilibrium can then be calculated using mass balances. The equilibrium constants for the equilibrium 1B + X <=> 1X + B can then be calculated according to:

\[ K_{\text{equilibrium}} = \frac{[1X][B]}{[1B][X]} \]

The results of these calculations for each hydrazide X are:

<table>
<thead>
<tr>
<th>X</th>
<th>A\text{348 nm}</th>
<th>[2B]=[B] mM</th>
<th>[1B] =5-[B] mM</th>
<th>[1X] = 5-[1B] mM</th>
<th>[X]=5-[1X] mM</th>
<th>K_{\text{equi, X}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.9281</td>
<td>3.37</td>
<td>1.63</td>
<td>3.37</td>
<td>1.63</td>
<td>4.27</td>
</tr>
<tr>
<td>C</td>
<td>0.7669</td>
<td>2.76</td>
<td>2.23</td>
<td>2.76</td>
<td>2.23</td>
<td>1.52</td>
</tr>
<tr>
<td>D</td>
<td>0.9418</td>
<td>3.42</td>
<td>1.58</td>
<td>3.42</td>
<td>1.58</td>
<td>4.69</td>
</tr>
<tr>
<td>E</td>
<td>0.7758</td>
<td>2.79</td>
<td>2.21</td>
<td>2.79</td>
<td>2.21</td>
<td>1.59</td>
</tr>
<tr>
<td>F</td>
<td>1.0267</td>
<td>3.74</td>
<td>1.26</td>
<td>3.74</td>
<td>1.26</td>
<td>8.81</td>
</tr>
</tbody>
</table>

These equilibrium constants relate the thermodynamic stability of all hydrazones 1X to the reference hydrazone 1B. Subsequently, mixtures were simulated using the experimental initial conditions ([1] =5 mM, [B]=5 mM and all other hydrazides (except for 1) at 1 mM. The enriched hydrazide has an initial concentration of 3 mM. Simulations were performed using the freeware program Hyperquad Simulation and Speciation (http://www.hyperquad.co.uk/hyss.htm) by Peter Gans imposing the following equilibria:

\[ 1 + B <=> 1B \] (with an arbitrary \( K_{\text{equilib}} = 10^6 \) based on the experimental observation that this equilibrium is completely shifted to the right)

\[ 1 + A <=> 1A \] \( K_{\text{equil,A}} = 4.27\times10^6 \)

\[ 1 + C <=> 1C \] \( K_{\text{equil,C}} = 1.52\times10^6 \)

\[ 1 + D <=> 1D \] \( K_{\text{equil,D}} = 4.69\times10^6 \)

\[ 1 + E <=> 1E \] \( K_{\text{equil,E}} = 1.59\times10^6 \)

\[ 1 + F <=> 1F \] \( K_{\text{equil,F}} = 8.81\times10^6 \)

Concentrations of B at thermodynamic equilibrium were calculated for each enriched mixture and converted into expected absorbance values and compared to the experimental values (see following table). These values are plotted in Figure 5c in the manuscript.
<table>
<thead>
<tr>
<th>X</th>
<th>[B] mM mixture</th>
<th>[B] µM 100x dilution&lt;sup&gt;a&lt;/sup&gt;</th>
<th>$A_{348\ nm}$ calculated&lt;sup&gt;b&lt;/sup&gt;</th>
<th>$A_{348\ nm}$ experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.77</td>
<td>37.7</td>
<td>1.035</td>
<td>1.027</td>
</tr>
<tr>
<td>C</td>
<td>3.58</td>
<td>35.8</td>
<td>0.984</td>
<td>0.968</td>
</tr>
<tr>
<td>D</td>
<td>3.79</td>
<td>37.9</td>
<td>1.040</td>
<td>1.024</td>
</tr>
<tr>
<td>E</td>
<td>3.58</td>
<td>35.8</td>
<td>0.986</td>
<td>0.986</td>
</tr>
<tr>
<td>F</td>
<td>3.89</td>
<td>38.9</td>
<td>1.067</td>
<td>1.073</td>
</tr>
</tbody>
</table>

<sup>a</sup> UV/Vis concentration after 100x dilution.  
<sup>b</sup> from calibration curve (SI page 3).