System Design

The seesaw gate is formed by an input (I), one GO gate that bears an output strand on top of a gate strand, and fuel (F). Each strand (input, gate or fuel) has three parts: feet, extension, the active part. The feet ensures that each strand attaches to a specific staple of the origami. The extension (T5 or T15 sequence) provides with some additional flexibility. The active part of these strands corresponds to the sequences of the bulk experiment. The choice of the feet is dictated by the requirement that each strand attaches to a specific point in the origami. The ends of staple strands are arranged in a stretched hexagonal lattice. Therefore, each of these points is a possible place to attach the strands of the circuit. Fig. 1 (main text) shows a possible arrangement: the input strand is surrounded by four GO gates, each of which is close to a fuel. This arrangement was chosen to reflect the catalytic behaviour of seesaw gates.

If all the gates and fuels are identical, only three types of feet need to be designed. The design should be done in such a way that feet are as orthogonal as possible: each feet binds
to its complementary and not to the other feet. Another geometrical requirement is that
the active part of the strands (gates, input and fuel) should be able to interact one with each
other. We assumed a length of 5 nm (15 bases) for the feet when they are hybridized with
their complementary. The approximate distance between two contiguous attachment points
being 5.5nm, 5nm feet length is enough for gates to interact.

An annealed sampling method was used (in combination with NUPACK\textsuperscript{1}) to determine a
set of three orthogonal feet. The resulting sequences, as well as the free energy of interaction
between each of them and their complementary is given in the following table.

<table>
<thead>
<tr>
<th>Feet</th>
<th>complementary</th>
<th>free energy (Kcal/Mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TACAAACAACAAACA</td>
<td>TGTTTGTTGTTTGTGA</td>
<td>-21.176</td>
</tr>
<tr>
<td>TACAAACAACAAACA</td>
<td>TATTACATTCACTCA</td>
<td>-2.898</td>
</tr>
<tr>
<td>TACAAACAACAAACA</td>
<td>TAAAGCGAAAAATA</td>
<td>-4.105</td>
</tr>
<tr>
<td>TGAGTGAATGTAATA</td>
<td>TATTACATTCACTCA</td>
<td>-19.972</td>
</tr>
<tr>
<td>TGAGTGAATGTAATA</td>
<td>TAAAGCGAAAAATA</td>
<td>-3.225</td>
</tr>
<tr>
<td>TATTTTTTCGCTTTTA</td>
<td>TAAAGCGAAAAATA</td>
<td>-20.44</td>
</tr>
</tbody>
</table>

The sequences of the different strands are as follows (5' to 3'):

beacon: 6FAM TCTG CAGATGAGCAT CAGA BHQ

output: TGCTCATCTCATACAACATCTACA

input: \textbf{CATACAACATCTACA TCACA} (TTTTT) TGTTTGTTTGTTTGTGA

fuel: TAAAGCGAAAAATA (TTT) \textbf{CATCT CATACAACATCTACA}

fuel + 1: TAAAGCGAAAAATA (TTT) \textbf{CATCT CATACAACATCTACAT}

gate: TATTACATTCACTCA (TTTTT) \textbf{TGTGA TGTAGATGTTTGTATG AGATG}

input protection: \textit{TGTGA TGTAGATGTTTGTATG} TATTTCTTTTA

input activator: TAAAGAAATA CATACAACATCTACA T

fuel protection: TGTGAAAAGT TGTAGATGTTTGTATG AGATG

fuel activator: TCATACAACATCTACAACACTTT TCACA
$I_{act}^5$: TAAAGAAATA CATACAACATCTACA TCACA

The linker (TTTTT for the input, TTT for the fuel) is inside parentheses. It is replaced by a T$_{15}$ sequence in the T15 experiments. The active part of the gates is in bold characters, the toeholds (or their complementary) appear in italic. Note that the activator strands only include the first nucleotide of the sequence complementary to the respective toehold. The $I_{act}^5$ contains the full toehold.

**Gel assay**

A fluoro-labelled output strand was employed in order to verify the correct assembly between the gate-output system and the origami platform. For this purpose, the duplex gate-output was formed using an output sequence labelled at the 5-end with the organic dye FAM. The origami platform was then prepared applying the standard protocol previously described. After the corresponding filtration, the agarose gel confirmed the presence of one single origami species (see figure 1 right). In addition, the fluorescence spectrum of the origami solution corresponded to the organic FAM dye (see figure 1 left), which confirmed the presence of the gate-output system correctly bound to the origami.

Gel staining: agarose gel electrophoresis was carried out on 1% agarose gel and running buffer (TBE 0.5X). The loaded samples were prepared mixing the corresponding sample (2.5 µL filtered origami 15 nM and 20 µL filtrate solution) with 1 µL Midori Green DNA stain and 1.7 µL BioLabs loading gel. A BioLabs ladder (0.5 to 1.5 k-bp) was used as a marker. After electrophoresis, the gel was visualized by UV with a DNR Bio-imaging, MF-ChemiBIS 3.2 system. Fluorescence spectra measurements: fluorescence spectra were recorded on Tecan spectrofluorometer using an excitation wavelength of 490 nm. The excitation and emission slit width was 5 nm, the step size 1 nm and integration time 20 µs.
Figure 1: Fluorescence emission spectra of the origami solution containing a FAM-labelled output strand (left). Agarose gel of the origami solution and the residual filtrate (right).
Figure 2: AFM imaging (peak force in liquid) of LAC circuits tethered to DNA origamis, deposited on mica. (a) Overall view. Rectangular origamis have some propensity to stick in an upside down way. Each origami is ‘decorated’ by a protrusion of 30-40 nm diameter, that corresponds in size and position to a LAC circuit. Bar corresponds to 100 nm. (b) A detailed view with a section (c) showing the measured height along the segment in (b). The two vertical dashed lines in (c) correspond to the two crosses in (b).
Figure 3: Fitting of the beacon response: fluorescence observed after the addition of 2pmol of output. In red, fit of the beacon response by the model $\text{O} + \text{b} \xrightleftharpoons[k_{\text{rep}}]{{q_{\text{rep}}}} \text{f}$, with $k_{\text{rep}} = 9.4 \times 10^4 M^{-1}s^{-1}$, $q_{\text{rep}} = 0.09s^{-1}$. 
Fitting of the LAC response

The fitting of the LAC response was done by numerically integrating the set of kinetic equations corresponding to the reactions:

\[
\begin{align*}
\text{p(} \lambda_4 \text{) + I}_{\text{act}} & \xrightarrow{k_{\text{ac}}} \lambda_4 + pI_{\text{act}} \\
\lambda_4 & \xrightarrow{k} \lambda_3 + O \\
O + b & \xrightarrow{q_{\text{rep}} f} \text{f}
\end{align*}
\]

and the following values for the kinetic constants: \(k_{\text{rep}} = 9.4 \times 10^4 M^{-1}s^{-1}, q_{\text{rep}} = 0.09 s^{-1}, k_{\text{ac}} = 5 \times 10^4 M^{-1}s^{-1}\). The constant \(k\), that governs the internal dynamics of the LAC was then varied (Figure S4) to obtain an estimate of its value. Due to the limitations of the reporting system, only a lower bound \(k > 0.5 s^{-1}\) can be given.

Similarly, when both I and F are activated, the system can be modelled as above and also considering the reactions \(\lambda_n \xrightarrow{k} \lambda_{n-1} + O\), for \(n = 3, 2, 1\). The same fitting procedure shows the previous estimate can be refined to \(k > 5 s^{-1}\). Figure 5 also shows that some slow trends due to slow leaky, blunt-end reactions, not present in the model, are indeed present in the experiment.

References

Figure 4: Fluorescence observed when only activating the input gate. Raw data (squares) were fitted with the model, and different values of $k$: $5 \, \text{s}^{-1}$ (green), $0.5 \, \text{s}^{-1}$ (red), $0.05 \, \text{s}^{-1}$ (blue).
Figure 5: Fluorescence observed when activating the input and fuel gates. Raw data (squares) were fitted with the model, and different values of $k$: 5 s$^{-1}$ (green), 0.5 s$^{-1}$ (red), 50 s$^{-1}$ (blue).