Supporting Information

Dissipative Synthetic DNA-Based Receptors for the Transient Loading and Release of Molecular Cargo

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anie_201801318_sm_misellaneous_information.pdf
Author Contributions

A.A. Conceptualization: Equal; Data curation: Equal; Writing – original draft: Supporting; Writing – review & editing: Supporting
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Reagents

Reagent-grade chemicals, including disodium phosphate, monosodium phosphate, tris-(2-carboxyethyl) phosphine hydrochloride, magnesium chloride were used without further purifications. RNase-H recombinant, Nb.BsmI recombinant and Nt.BsmAI recombinant (all from New England BioLabs) were used without further purifications. HPLC purified DNA and RNA - oligonucleotides and modified with 6-carboxyfluorescein (6-FAM) and a quencher (Black Hole Quencher 1, BHQ-1) were purchased from Biosearch Technologies (Risskov, Denmark) and from IBA (Gottingen, Germany) and employed without further purification.

Oligonucleotide sequences

The following DNA and RNA oligonucleotides modified and non-modified were used. All oligonucleotides were suspended to a final concentration of 100 µM in phosphate buffer 50 mM, pH 7 and stored at -20°C.

System 1: Clamp-like receptor (Figures 2, 4):
Clamp-like DNA-based receptor: 5’-TCT CTC CTT TCT CTT TGA ATG CTT TTC TTT CCT CTC T- 3’
DNA-cargo 9 mer: 5’-(FAM)-AGA GAG GAA (BHQ1)
RNA-fuel 5’-GAA GAA AAG CAU UCA AAG-3’
DNA-fuel 5’-GAA GAA AAG CAT TCA AAG-3’
Clamp-like DNA-based receptor (system 2, Figure 4): 5’-TTC CCT CTT CTT CCT CCT

**TTT AAT GAT TTT CAT** CC TTC TTC TCC CTT - 3’

DNA-cargo (system 2) 9 mer: 5’-(Quasar-670)-AAG AAG AGG -(BHQ2)

RNA-fuel (system 2) 5’-GAU GAA AAU CAU UAA AAG-3’

For the receptor sequence above the bold bases represent the portion complementary to the fuel, the underlined bases represent the cargo-binding site (through Watson-Crick bonds) and italics bases represent the triplex-forming portion.

**System 2: Hairpin-like receptor** (Figure 3):

Hairpin-like receptor: 5’-CT TTG AAT GCT TTT CTC T TTTCC TTG ATC GGC TGT TTA

**TT-(BHQ1)-** GGAAG AGG GTT CAT CAT CAA CTA - 3’

DNA-cargo 11 mer: 5’-(Quasar 570)-TAA ACA GCC GA-3’

RNA-fuel: 5’-AGA GAG AAA AGC AUU CAA - 3’

For the receptor sequence above the bold bases represent the stem-loop portion, the italics bases represent the portion complementary to the fuel and the underlined bases represent the cargo-binding site.

**Enzymes**

Each enzyme used in this work present different features and restriction site.

The RNase-H is an endoribonuclease that specifically hydrolyzes the phosphodiester bonds of RNA, if hybridized to DNA.

The Nb.BsmI is an endonuclease that cleaves only one DNA-strand on a duplex structure, binding its specific restriction site (the asterisk indicates the cut-point).

5’ … - GAATGCN - … 3’

3’ … - CTTAC*GN - … 5’
Also the Nt.BsmAI is an endonuclease that cleaves only one DNA-strand on a duplex structure, binding its specific restriction site (the asterisk indicates the cut-point).

5’ ... - GTCTCN*N- ... 3’
3’ ... - CAGAGNN- ... 5’

**Fluorescence measurements**

Fluorescence measurements (for binding curves and time-course experiments) were obtained using a Cary Eclipse Fluorometer (Varian), with an excitation at 495 nm (± 5 nm) and emission at 520 nm (± 10 nm) (for FAM labeled oligos) or an excitation wavelength of 544 nm (± 5 nm) and an emission one of 563 nm (± 5 nm) (for Quasar-570 labeled oligos).

**Native PAGE experiments**

Native PAGE experiments were obtained with 18 % polyacrylamide (29:1 acrylamide/bisacrylamide) in TAE 10x buffer (400 mM Tris, 200 mM acetic acid, 10 mM EDTA and 20 mM MgCl$_2$) pH 8.0. A volume of 50 µl of each sample was mixed with 5 µl of glycerol and then the mixture was added into the gel for the electrophoresis. The native PAGE was carried out in a Mini-PROTEAN Tetra cell electrophoresis unit (Bio-Rad) at room temperature, at a constant voltage of 90 V, using TAE 1x buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA and 2 mM MgCl$_2$) at pH 8.0 for 3 h and 45 minutes. After 30 minutes of staining in SYBR gold (Invitrogen) dissolved in a TAE 1x buffer at pH 8.0, the gel was scanned by a Gel Doc XR+ system (Bio-Rad).

**Model and curve fitting**

A set of differential equations describing the kinetic model shown in Figure S5 was implemented in Matlab R2017a and solved using ode23s function (the notation used is reported in Figure S6). The emission intensity profiles were fitted using lsqcurvefit function.
The optimized constants were $k_{d\_cargo}$, $k_{d\_fuel}$ and $k_{cat}$. The error associated to each optimized value have been evaluated using the nparci function. The experimental binding constants for DNA cargo and RNA fuel with the receptor (Figure S7) were employed. The average values determined for the two set of experiments (Figure 2c,d) are comparable, within the experimental error.

To assess the sensitivity of the kinetic trace to the optimized parameters, for each of them kinetic traces were simulated changing only one parameter in a range going from 10 times smaller to 10 times bigger, while leaving the other parameters unchanged. Figures S8, S9 and S10 show the corresponding simulations performed to assess the sensitivity of the model to changes in $k_{cat}$, $k_{d\_cargo}$ and $k_{d\_fuel}$, respectively.

To calculate the chemical affinities (Figure S11) the following formula has been used:

$$A = RT \ln K - RT \ln Q$$

Where K is the equilibrium constant of a certain reaction, and Q is its reaction quotient.

**Curve fitting**

*Model.* Both data sets were fitted according to the kinetic model shown in Figure S5, and the notation reported in Figure S6 was used.

The initial slope of the kinetic trace reports on the detachment of C from RC. This process did not show a marked dependence on the amount of F present in solution, therefore it is reasonably described as a first-order process. This may arise from the dissociation of RC, or from a fast interaction of F with RC, followed by a slower dissociation of C. Under the employed experimental conditions it is not possible to exclude either of the two, because the free and complexed receptor R and RC are in comparable amounts (ca. 40% and 60%, respectively), therefore both can interact with F. With the present data it is not possible to accurately distinguish between the two pathways, therefore, it has been
chosen to keep the model as simple as possible: the direct interaction of F with RC has not been modeled, and the rate of detachment should be better considered as an apparent detachment rate, that indirectly takes into account also the F-mediated detachment pathway.

The system is described by the following set of differential equations.

\[ \frac{dC}{dt} = k_{d\_cargo} \cdot RC - K_{a\_cargo} \cdot k_{d\_cargo} \cdot R \cdot C; \]
\[ \frac{dR}{dt} = k_{d\_cargo} \cdot RC - K_{a\_cargo} \cdot k_{d\_cargo} \cdot R \cdot C + k_{\text{cat}} \cdot RFE + k_{d\_fuel} \cdot RF - K_{a\_fuel} \cdot k_{d\_fuel} \cdot F \cdot R + k_{d\_waste} \cdot RW - k_{a\_waste} \cdot R \cdot W; \]
\[ \frac{dF}{dt} = -K_{a\_fuel} \cdot k_{d\_fuel} \cdot R \cdot F + k_{d\_fuel} \cdot RF; \]
\[ \frac{dRC}{dt} = -k_{d\_cargo} \cdot RC + K_{a\_cargo} \cdot k_{d\_cargo} \cdot R \cdot C; \]
\[ \frac{dRF}{dt} = K_{a\_fuel} \cdot k_{d\_fuel} \cdot R \cdot F - k_{d\_fuel} \cdot RF - k_{a\_enz} \cdot E \cdot RF + k_{d\_enz} \cdot RFE; \]
\[ \frac{dRFE}{dt} = k_{a\_enz} \cdot E \cdot RF - k_{d\_enz} \cdot RFE - k_{\text{cat}} \cdot RFE; \]
\[ \frac{dE}{dt} = -k_{a\_enz} \cdot E \cdot RF + k_{d\_enz} \cdot RFE + k_{\text{cat}} \cdot RFE; \]
\[ \frac{dW}{dt} = k_{\text{cat}} \cdot RFE - k_{a\_waste} \cdot R \cdot W + k_{d\_waste} \cdot RW; \]
\[ \frac{dRW}{dt} = k_{a\_waste} \cdot R \cdot W - k_{d\_waste} \cdot RW; \]

Data fitting. The binding constant between R and C was determined experimentally from a binding isotherm (titration of C with R, Figure S7, left), that was fitted according to a 1:1 binding model, to give an association constant \( K_{a\_cargo} = 1.32 \times 10^8 \text{ M}^{-1} \) (\( K_{d\_cargo} = 7 \pm 2 \times 10^{-9} \text{ M} \)). The binding constant between R and F was determined experimentally from a displacement experiment (titration of RC with F, Figure S7, right), that was fitted according to two competitive 1:1 binding equilibria, to give an association constant \( K_{a\_fuel} = 2.2 \times 10^9 \text{ M}^{-1} \) (\( K_{d\_fuel} = 4 \pm 1 \times 10^{10} \text{ M} \)). To be consistent with the concentration units (M) and for numerical reasons, both \( K_{a\_cargo} \) and \( K_{a\_fuel} \) were expressed and used in M\(^{-1}\) units (\( K_{a\_cargo} = 0.13 \times 10^{-9} \text{ M}^{-1} \) and \( K_{a\_fuel} = 2.2 \times 10^{-9} \text{ M}^{-1} \)).
The binding constant constraints were exploited imposing $k_{a\_cargo}$ and $k_{a\_fuel}$ equal respectively to $K_{a\_cargo} * k_{d\_cargo}$ and $K_{a\_fuel} * k_{d\_fuel}$ (the dissociation constants are optimized by the matlab routine, *vide infra*).

The enzyme concentration was modelled as an apparent M concentration, e.g. 30 U/mL of E are modelled as $30 \times 10^{-9}$ M E. The equilibrium constant for the enzyme association to RF was set to $100 \times 10^{-9}$ M$^{-1}$, to ensure > 95% binding of the enzyme to the substrate (enzyme saturation regime). This was done by fixing $k_{a\_enz} = 10 \times 10^{-9}$ M$^{-1}$ min$^{-1}$ and $k_{d\_enz} = 0.1$ min$^{-1}$.

The waste was modelled as a single entity, i.e. hydrolysis of one F strand produces 1 molecule of waste. An apparent binding of the waste to the receptor was considered when the final fluorescence plateau was not coincident with the starting fluorescent intensity. In order to do this, $k_{d\_waste}$ was fixed to 100 min$^{-1}$ and $k_{a\_waste}$ was set to the arbitrary value that makes the final plateau coincide with the experimental data. The choice of fixing $k_{d\_waste}$ instead of $k_{a\_waste}$ was made for numerical reason (i.e. fixing $k_{d\_waste}$ improves the capacity of the matlab routine to optimize the parameters). It was verified that qualitatively identical curves are obtained by setting $k_{a\_waste}$ to $10 \times 10^{-9}$ M$^{-1}$ min$^{-1}$, changing $k_{d\_waste}$ accordingly and using the previously optimized parameters.

The output fluorescent signal was modelled according to $F_{out} = F_{RC} * RC + F_{C} * C$, where $F_{RC}$ and $F_{C}$ are the “brightness” of RC and C under the employed experimental conditions. The brightness of RC and C were evaluated from the fluorescence intensity profiles. Under the employed experimental conditions, at time zero 61% of C is associated (in accordance to the equilibrium constant $K_{a\_cargo}$), whereas in the presence of $100 \times 10^{-9}$ M F, only 4% of C is associated; at higher F concentrations C was considered fully “free”. These association values were combined with the emission intensities recorded at time 0 and in plateau regions obtained just after the addition of the F, to afford the brightness of RC and C in each experiment. When the plateau values were not available, the average value was
used. In the case of the experiments at different RNA concentration, the value of the final plateau was taken as reference instead of the initial emission intensity because the final emission intensity is appreciably higher than the starting point. Initial concentrations of R, C and RC were set at $11.757 \times 10^{-9}$ M, $11.757 \times 10^{-9}$ M and $18.243 \times 10^{-9}$ M, respectively. The concentrations of F and E were set for each curve and the concentration of every other species was set to zero.

In summary, the parameters reported in Table S1 were identical in every optimization. The following parameters were simultaneously optimized for every experimental curve.

$k_{d\_cargo}$ (min$^{-1}$);

$k_{d\_fuel}$ (min$^{-1}$);

$k_{cat}$ (min$^{-1}$);

The parameters reported in Tables S2 and S3 were fixed for each experimental trace set according to the reported values.

The set of differential equations was implemented in Matlab R2017a and solved using ode23s function. The emission intensity profiles were fitted using lsqcurvefit function. Typical initial guesses for $k_{d\_cargo}$, $k_{d\_fuel}$ and $k_{cat}$ were 0.8, 0.4 and 0.2 min$^{-1}$ respectively. The error associated to each optimized value is the average of the errors obtained with nparci function.

The optimized values are reported in Tables S4 and S5.

The average values determined for the two set of experiments are comparable, within the experimental error. The mean values considering both experiments are:

$k_{d\_cargo} = 0.46 \pm 0.27$ min$^{-1}$;

$k_{d\_fuel} = 0.24 \pm 0.24$ min$^{-1}$;

$k_{cat} = 0.41 \pm 0.38$ min$^{-1}$;
Table S1. Fixed parameters. $C_{\text{start}}$ indicates the initial concentration of each associated component.

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Parameter fixed value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{a_{\text{cargo}}} \ (10^{9} \text{ M}^{-1})$</td>
<td>0.132</td>
</tr>
<tr>
<td>$K_{a_{\text{fuel}}} \ (10^{9} \text{ M}^{-1})$</td>
<td>2.2</td>
</tr>
<tr>
<td>$k_{a_{\text{enz}}} \ (10^{9} \text{ M min}^{-1})$</td>
<td>10</td>
</tr>
<tr>
<td>$k_{d_{\text{enz}}} \ (\text{min}^{-1})$</td>
<td>0.1</td>
</tr>
<tr>
<td>$C_{\text{start } C} \ (10^{-9} \text{ M})$</td>
<td>11.757</td>
</tr>
<tr>
<td>$C_{\text{start } R} \ (10^{-9} \text{ M})$</td>
<td>11.757</td>
</tr>
<tr>
<td>$C_{\text{start } RC} \ (10^{-9} \text{ M})$</td>
<td>18.243</td>
</tr>
<tr>
<td>$C_{\text{start } RF} \ (10^{-9} \text{ M})$</td>
<td>0</td>
</tr>
<tr>
<td>$C_{\text{start } RFE} \ (10^{-9} \text{ M})$</td>
<td>0</td>
</tr>
<tr>
<td>$C_{\text{start } W} \ (10^{-9} \text{ M})$</td>
<td>0</td>
</tr>
<tr>
<td>$C_{\text{start } RW} \ (10^{-9} \text{ M})$</td>
<td>0</td>
</tr>
</tbody>
</table>
Table S2. Values used to fit the curves collected at different Fuel (F) concentration.

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>F = 50</th>
<th>F = 70</th>
<th>F = 100</th>
<th>F = 150</th>
<th>F = 200</th>
<th>F = 250</th>
</tr>
</thead>
<tbody>
<tr>
<td>F ( (10^{-9} \text{ M}) )</td>
<td>50</td>
<td>70</td>
<td>100</td>
<td>150</td>
<td>200</td>
<td>250</td>
</tr>
<tr>
<td>E ( (10^{-9} \text{ M}) )</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>( k_{a, \text{waste}} ) ( (10^{-9} \text{ M}^{-1} \text{ min}^{-1}) )</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.29</td>
<td>0.80</td>
<td>1.8</td>
</tr>
<tr>
<td>( k_{d, \text{waste}} ) ( (10^{-9} \text{ M}^{-1}) )</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>( F_C ) (a.u. ( 10^{-9} \text{ M}^{-1}) )</td>
<td>2.413(^a)</td>
<td>2.413(^a)</td>
<td>2.413(^a)</td>
<td>2.413</td>
<td>2.412</td>
<td>2.413</td>
</tr>
<tr>
<td>( F_{RC} ) (a.u. ( 10^{-9} \text{ M}^{-1}) )</td>
<td>3.110</td>
<td>3.126</td>
<td>3.137</td>
<td>3.128(^b)</td>
<td>3.128(^b)</td>
<td>3.128(^b)</td>
</tr>
</tbody>
</table>

\(^a\): average of the last three values; \(^b\): average of the first three values.
Table S3. Values used to fit the curves collected at different Enzyme (E) concentration.

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>E = 5</th>
<th>E = 8</th>
<th>E = 10</th>
<th>E = 20</th>
<th>E = 30</th>
<th>E = 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>F ($10^{-9}$ M)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>E ($10^{-8}$ M)</td>
<td>5</td>
<td>8</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>$k_{a_waste}$ ($10^{-3}$ M$^{-1}$ min$^{-1}$)</td>
<td>0.43$^a$</td>
<td>0.43$^a$</td>
<td>0.65</td>
<td>0.39</td>
<td>0.38</td>
<td>0.30</td>
</tr>
<tr>
<td>$k_{d_waste}$ (min$^{-1}$)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>$F_C$ (a.u. $10^{-9}$ M$^{-1}$)</td>
<td>1.479</td>
<td>1.457</td>
<td>1.490</td>
<td>1.475$^a$</td>
<td>1.475$^b$</td>
<td>1.475$^b$</td>
</tr>
<tr>
<td>$F_{RC}$ (a.u. $10^{-9}$ M$^{-1}$)</td>
<td>2.476</td>
<td>2.482</td>
<td>2.472</td>
<td>2.462</td>
<td>2.48</td>
<td>2.475</td>
</tr>
</tbody>
</table>

$^a$: average of the last four values; $^b$: average of the first three values;
Table S4. Optimized values obtained for the curves collected at different Fuel (F) concentration.

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>F = 50</th>
<th>F = 70</th>
<th>F = 100</th>
<th>F = 150</th>
<th>F = 200</th>
<th>F = 250</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{d_cargo} ) (min(^{-1}))</td>
<td>0.713 ±0.578</td>
<td>0.376 ±0.074</td>
<td>0.460 ±0.061</td>
<td>0.976 ±0.037</td>
<td>0.610 ±0.031</td>
<td>0.739 ±0.048</td>
<td>0.65 ±0.22(^a)</td>
</tr>
<tr>
<td>error</td>
<td>±0.078</td>
<td>±0.061</td>
<td>±0.037</td>
<td>±0.031</td>
<td>±0.048</td>
<td>±0.22</td>
<td></td>
</tr>
<tr>
<td>( k_{d_fuel} ) (min(^{-1}))</td>
<td>0.085 ±0.047</td>
<td>0.137 ±0.067</td>
<td>0.091 ±0.019</td>
<td>0.248 ±0.037</td>
<td>0.180 ±0.011</td>
<td>0.256 ±0.014</td>
<td>0.17 ±0.08(^a)</td>
</tr>
<tr>
<td>error</td>
<td>±0.047</td>
<td>±0.067</td>
<td>±0.019</td>
<td>±0.037</td>
<td>±0.011</td>
<td>±0.014</td>
<td></td>
</tr>
<tr>
<td>( k_{cat} ) (min(^{-1}))</td>
<td>0.578 ±0.054</td>
<td>0.328 ±0.016</td>
<td>0.347 ±0.010</td>
<td>0.280 ±0.003</td>
<td>0.211 ±0.003</td>
<td>0.252 ±0.005</td>
<td>0.33 ±0.13(^a)</td>
</tr>
<tr>
<td>error</td>
<td>±0.054</td>
<td>±0.016</td>
<td>±0.010</td>
<td>±0.003</td>
<td>±0.003</td>
<td>±0.005</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\): this average error is the standard deviation associated to the different values, and not the average of the errors obtained with the nlparci function for each value.
Table S5. Optimized values obtained for the curves collected at different Enzyme (E) concentration.

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>E = 5</th>
<th>E = 8</th>
<th>E = 10</th>
<th>E = 20</th>
<th>E = 30</th>
<th>E = 50</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{d_cargo}$ (min$^{-1}$)</td>
<td>0.443</td>
<td>0.251</td>
<td>0.496</td>
<td>0.223</td>
<td>0.125</td>
<td>0.076</td>
<td>0.27</td>
</tr>
<tr>
<td>error</td>
<td>±0.083</td>
<td>±0.012</td>
<td>±0.001</td>
<td>±0.018</td>
<td>±0.008</td>
<td>±0.011</td>
<td>±0.17$^a$</td>
</tr>
<tr>
<td>$k_{d_fuel}$ (min$^{-1}$)</td>
<td>0.034</td>
<td>0.440</td>
<td>0.915</td>
<td>0.041</td>
<td>0.156</td>
<td>0.254</td>
<td>0.30</td>
</tr>
<tr>
<td>error</td>
<td>±0.018</td>
<td>±0.022</td>
<td>±0.001</td>
<td>±0.005</td>
<td>±0.036</td>
<td>±0.028</td>
<td>±0.33$^a$</td>
</tr>
<tr>
<td>$k_{cat}$ (min$^{-1}$)</td>
<td>0.106</td>
<td>0.142</td>
<td>0.261</td>
<td>0.333</td>
<td>0.586</td>
<td>1.540</td>
<td>0.50</td>
</tr>
<tr>
<td>error</td>
<td>±0.015</td>
<td>±0.001</td>
<td>±0.0003</td>
<td>±0.007</td>
<td>±0.021</td>
<td>±0.113</td>
<td>±0.54$^a$</td>
</tr>
</tbody>
</table>

$^a$: this average error is the standard deviation associated to the different values, and not the average of the errors obtained with the nlparci function for each value.
Figure S1. The stability of the receptor/cargo complex is not affected by the presence of RNase-H. We show here that the signal increase followed by the binding of the receptor to a solution containing the cargo does not change over a period of more than 45 minutes in the presence of RNase-H (25 U/mL). Experiments shown in this figure have been obtained in 10 mM Tris buffer + 3 mM MgCl₂ + 10 mM DTT, pH 7.0, 25°C in the presence of cargo (3 x 10⁻⁸ M) and receptor (3 x 10⁻⁸ M).
Figure S2. Release of the DNA cargo from the clamp-like receptor shown in Figure 2 is also demonstrated by native PAGE electrophoresis. A clear band corresponding to the DNA clamp-like receptor bound to the RNA fuel (5) is visible in the absence of the enzyme. In the presence of RNase-H (7) the band of the RNA fuel disappears and the band corresponding to the receptor/cargo complex is visible.
Figure S3. Dissipative release of the DNA cargo from the clamp-like receptor shown in Figure 2 is also demonstrated by native PAGE electrophoresis. The band corresponding to the RNA fuel gradually disappears with the increase in time-reaction in the presence of the enzyme.
**Figure S4.** Dissipative release of the cargo from the clamp-like receptor (Figure 2) is controlled by varying the RNase-H and the RNA fuel concentrations. Here are shown the $t_{1/2}$ values obtained under the conditions shown in Figure 2c (left) and Figure 2d (right).
Figure S5. Kinetic steps considered in the model. The notation employed for the cartoon representation is reported in Figure S6. The fuel hydrolysis has been considered as an irreversible step, whereas all the other reactions involved were considered reversible. Experimental data have been used - where available – to impose constraints on the kinetic constants. The fitting routine was used to optimize $k_{cat}$, $k_{d\_cargo}$ and $k_{d\_fuel}$.
Figure S6. Compact letter notation used to identify the species involved in the model and their cartoon representation. In the SI text this compact notation is used.
Figure S7. Left: binding curve for the receptor-cargo association reaction ([Cargo] = 3 x 10^{-8} M). Right: displacement curve obtained upon addition of RNA fuel to an equimolar mixture of receptor and cargo ([Receptor] = [Cargo] = 3 x 10^{-8} M). Solid curves represent the optimized fit.
Figure S8. Kinetic trace simulation upon varying $k_{\text{cat}}$. Starting conditions correspond to those of the curve collected at [Fuel] = $1.5 \times 10^{-7}$ M. The employed $k_{\text{cat}}$ (min$^{-1}$) values associated to each trace are also reported. The corresponding experimental data are also reported for comparison (black dots).
Figure S9. Kinetic trace simulation upon varying $k_{d\_cargo}$. Starting conditions correspond to those of the curve collected at [Fuel] = $1.5 \times 10^{-7}$ M. The employed $k_{d\_cargo}$ (min$^{-1}$) values associated to each trace are also reported. The corresponding experimental data are also reported for comparison (black dots).
Figure S10. Kinetic trace simulation upon varying $k_{d\text{-cargo}}$. Starting conditions correspond to those of the curve collected at [Fuel] = 1.5 x $10^{-7}$ M. The employed $k_{d\text{-cargo}}$ (min$^{-1}$) values associated to each trace are also reported. The corresponding experimental data are also reported for comparison (black dots).
**Figure S11.** Left axis: data (black dots) and optimal fit (black trace) associated to the fluorescence kinetic trace collected at [Fuel] = 1.5 \times 10^{-7} \text{ M}. Right axis: chemical affinity associated to the RF and RC association reactions. A positive affinity value corresponds to a chemical reaction progressing towards the associated components.
Figure S12. Because the recognition of the endonuclease to its substrate is highly specific, the dissipative behaviour is also highly enzyme-specific. Here we show that the dissipative release of the cargo from the clamp-like receptor (Figure 2) is only observed in the presence of the specific enzyme recognizing the fuel/receptor complex while no change is observed in the presence of a non-specific nuclease enzyme (Nt.BsmAI, 100 U/mL).
The same system described in Figure 2a can also be controlled by a DNA fuel strand (instead of RNA) and a DNA recognizing nuclease enzyme as the fuel consuming unit. As the fuel-consuming unit we employed here Nb.Bsml, a nicking endonuclease that cleaves only a specific sequence of one strand of DNA on a double-stranded DNA substrate. Such enzymatic activity is thus able to restore the DNA receptor ability to load again its DNA cargo.
Figure S14. Kinetic traces using the DNA clamp-like receptor shown in Figure S13 showing the reversible dissipative release of the cargo (5 x 10^{-8} M) from the receptor (3 x 10^{-8} M) after sequential addition of the DNA fuel strand (7 x 10^{-8} M) in the presence of Nb.Bsml (1000 U/mL). Experiments shown in this figure have been obtained in 10 mM Tris buffer + 3 mM MgCl₂, pH 6.5, 40°C.
Figure S15. DNA clamp-like receptor shown in Figure S13 (3 x 10^{-8} M) after addition of the DNA fuel strand (7 x 10^{-8} M) in the presence of a fixed concentration of cargo (5 x 10^{-8} M) and a different concentrations of Nb.Bsml (indicated in the figure). Experiments shown in this figure have been obtained in 10 mM Tris buffer + 3 mM MgCl₂, pH 6.5, 40°C.
Figure S16. Binding curves of receptor/cargo interactions for the hairpin-like receptor (Figure 3) in the absence (black) and presence (green) of the fuel strand (i.e. $10^{-5}$ M).
Figure S17. Kinetic traces showing the dissipative release of the cargo ($3 \times 10^{-8}$ M) from the hairpin-like receptor (Figure 3) ($10^{-8}$ M) after addition of different concentration of the fuel strand (indicated in the figure) and at a fixed concentration of RNase-H (25 U/mL).
**Figure S18.** Dissipative loading of the cargo from the hairpin-like receptor (Figure 3) is controlled by varying the RNase-H and the RNA fuel concentrations. Here are shown the $t_{1/2}$ values obtained under the conditions shown in Figure 3b (left) and Figure S17 (right).
Figure S19. Loading of the DNA cargo from the hairpin-like receptor shown in Figure 3 is also demonstrated by native PAGE electrophoresis. A clear band corresponding to the DNA hairpin-like receptor bound to the RNA fuel (4) is visible in the absence of the enzyme. In the presence of RNase-H (6) the band of the RNA fuel disappears and the band corresponding to the receptor/cargo complex is visible.
Because the recognition of the RNA fuel to its receptor is highly specific, the dissipative behaviour is only observed in the presence of the specific RNA fuel. No dissipative release of the cargo from the clamp-like receptor (Figure 2) is observed in the presence of a 2-base mismatch fuel strand. Kinetic traces showing the reversible dissipative release of the cargo (3 x 10^{-8} M) from the receptor (3 x 10^{-8} M) after sequential addition of the mismatch (2MM) and perfect match (PM) fuel strand (both at 10^{-7} M) in the presence of RNase-H (25 U/mL). Experiments shown in this figure have been obtained in 10 mM Tris buffer + 3 mM MgCl$_2$ + 10 mM DTT, pH 7.0, 25°C.