**Supplementary Information**

# Using environment-sensitive tetramethylated thiophene-BODIPY fluorophore in DNA probes for studying effector-induced conformational changes of protein-DNA complexes

Markéta Šoltysová[a#], Pedro Güixens-Gallardo[a#], Irena Sieglová[a], Anna Soldánová[a], Veronika Krejčiříková[a], Milan Fábry[a], Jiří Brynda[a], Petro Khoroshyy[a], Michal Hocek[a,b *], Pavlína Řezáčová[a,*]

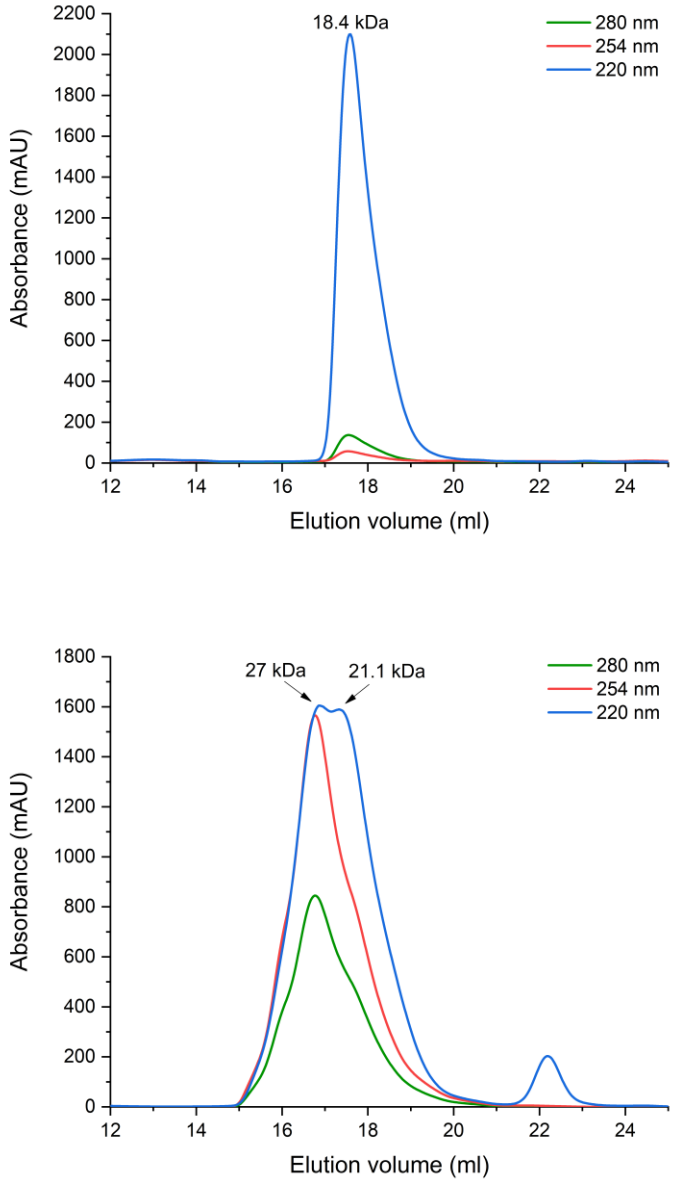



**Figure S1**: Analytical size-exclusion chromatography of (A) LutR-DBD (33.2 µM) and (B) LutR-DBD in the complex with a 15 bp long DNA operator (16.6 µM) variant. The apparent molecular weights of eluted components are indicated in the plots and were calculated based on the calibration equation logMW = −0.2362x + 8.4183.

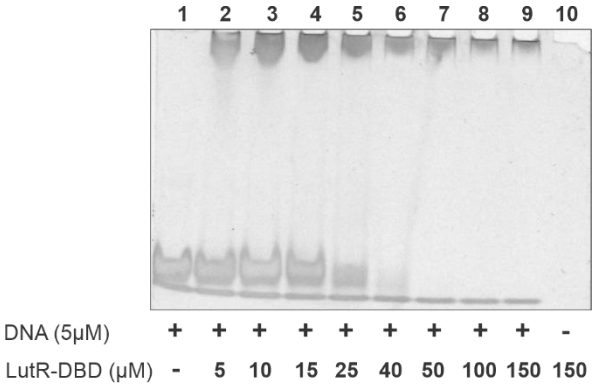



**Figure S2.** Electromobility shift assay of LutR-DBD (residues 2 – 78) and 15 bp DNA containing the inverted repeat (5′-GGTCATCAGATGACC-3′) in the 15% (w/v) polyacrylamide gel.

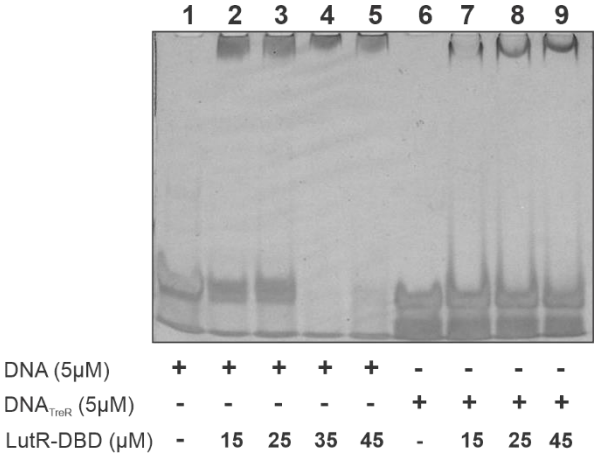



**Figure S3.** Electromobility shift assay of LutR-DBD (residues 2 – 78) and 15 bp DNA (DNA) containing the inverted repeat (5′-GGTCATCAGATGACC-3′) and an unrelated DNA operator of another protein from the GntR family, TreR, used as a negative control. The assay was run in the 17% (w/v) polyacrylamide gel.

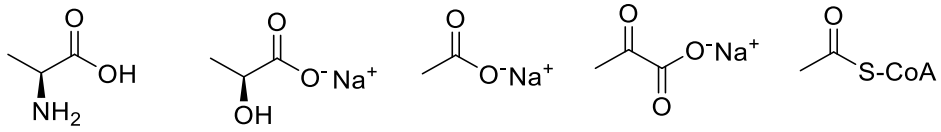
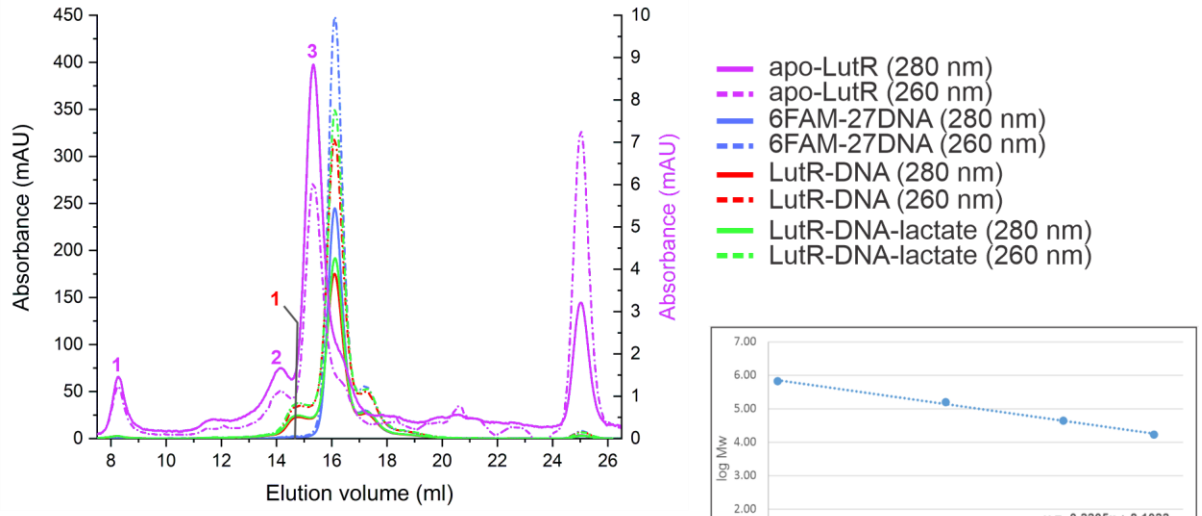



**Figure S4.** Formulas of metabolites tested for their effector activity.




| Sample | apo-LutR | | | LutR-DNA |
|---|---|---|---|---|
| Peak | 1 | 2 | 3 | 1 |
| Theoretical $MW_{theor}$ | 29.59 kDa/59.18 kDa (monomer/dimer) | | | 75.74 kDa (dimer+DNA) |
| Elution volume | 8.27 ml | 14.12 ml | 15.33 ml | 14.81 ml |
| Apparent $MW_{app}$ [a] | 1,900 kDa | 97.44 kDa | 52.72 kDa | 68.64 kDa |
| Assembly [b] | aggregate | trimer (3.3) | dimer (0.9) | dimer+DNA (0.9) |

[a] $MW_{app} = 10^{(-0.2205 \times Ve + 8.1022)}$

[b] Assembly = $MW_{app}/MW_{theor}$

**Figure S5.** Size-exclusion chromatography analysis of LutR (pink), single 5'-6FAM labelled 27DNA (blue), and the mixtures of LutR-DNA (red) and LutR-DNA-L-lactate (green). Overlays of chromatograms at 280 and 260 nm are shown. For the low signal of the LutR apo-protein with respect to DNA and the mixtures, the second y axis (pink) was added. Elution volumes and calculations of the apparent molecular weights ($MW_{app}$) and assemblies of all the apo-LutR's peaks and the most relevant peak of the mixtures are indicated in the table. The calibration of the Superdex 200 10/300 GL column is presented on the right.

**Table S1: Crystal data and diffraction data collection and refinement statistics**

| | **LutR-DBD** |
|---|---|
| **Data collection statistics** | |
| Space group | *P*222 |
| Cell parameters (Å; °) | 28.31, 38.37, 66.71; 90, 90, 90 |
| Wavelength (Å) | 0.9797 |
| Resolution (Å) | 50.0–1.46 (1.55–1.46) [a] |
| Number of unique reflections | 24387 (3958) |
| Multiplicity | 6.76 (6.55) |
| Completeness (%) | 100.0 (100.0) |
| $R_{meas}$ [b] | 7.3 (338.8) |
| $CC_{(1/2)}$ [c] | 99.9 (31.9) |
| Average I/δ(I) | 12.26 (0.49) |
| Wilson B ($Å^2$) | 33.47 |
| **Refinement statistics** | |
| Resolution range (Å) | 33.38–1.46 |
| No. of reflections in working set | 13,187 |
| No. of reflections in test set | 660 |
| R value [d] | 0.20 |
| $R_{free}$ value [e] | 0.24 |
| RMSD bond length (Å) | 0.0093 |
| RMSD angle (°) | 1.572 |
| Number of atoms in AU | |
| Protein | 639 |
| Water | 81 |
| Sodium ions | 2 |
| Mean B value ($Å^2$) | 43.25 |
| Ramachandran plot statistics | |
| Residues in favored regions (%) | 96.0 |
| Residues in allowed regions (%) | 4.00 |
| PDB code | 8PQM |

[a] The data in parentheses refer to the highest-resolution shell.

[b] $R_{meas}$ – redundancy-independent R factor [63].

[c] $CC_{(1/2)}$ is the correlation coefficient between random half-datasets; from its value the Pearson correlation coefficient of the true level of signal can be calculated as follows: $CC = \sqrt{2\,CC_{1/2}/1 + CC_{1/2}}$. [64]

[d] R value = $||F_o| - |F_c|| / |F_o|$, where $F_o$ and $F_c$ are the observed and calculated structure factors, respectively.

[e] $R_{free}$ is equivalent to the R value but is calculated for 5% of reflections chosen at random and omitted from the refinement process [65].

[f] as determined by Molprobity [59,60].

**Table S2**. List of oligonucleotides used

| Oligonucleotide | Sequence 5′→ 3′ [(a)] | Length |
|---|---|---|
| $prim^{LutR1C}$ | CAGAATAATGGGTCAGTAGTC | 21-mer |
| $prim^{LutR1C}$-Cy5 [(b)] | CAGAATAATGGGTCAGTAGTC | 21-mer |
| $prim^{LutR}$ | CAGAATAATGGGTC | 14-mer |
| $prim^{LutR}$-Cy5 [(b)] | CAGAATAATGGGTC | 14-mer |
| $temp^{5PLutR}$ [(c)] | CTAGTAGACTACTGACCCATTATTCTG | 27-mer |

[a] Primer sequences in the template are underlined.
[b] Cyanine-5 (Cy5) used for oligonucleotide labeling at the 5′ end.
[c] Template phosphorylated at the 5′ end

**Table S3**. Mean fluorescence lifetime of 27 bp long DNA (300 nM) bearing one ($1C^{TBdp}$) or two ($2C^{TBdp}$) modifications and titrated with LutR or BSA.

| | Protein concentration (μM) | Mean lifetime of 27DNA_$1C^{TBdp}$ | | Mean lifetime of 27DNA_$2C^{TBdp}$ | |
|---|---|---|---|---|---|
| | | τ (ns) | ±SD | τ (ns) | ±SD |
| LutR | 0 | 2.43 | 0.04 | 2.28 | 0.08 |
| | 1.5 | 3.9 | 0.05 | 3.38 | 0.20 |
| | 3.0 | 4.11 | 0.05 | 4.63 | 0.08 |
| | 6.0 | 4.33 | 0.11 | 4.90 | 0.02 |
| | 12.0 | 4.53 | 0.07 | 4.92 | 0.03 |
| LutR + effector* | 12.0 | 2.71 | 0.18 | 3.14 | 0.19 |
| BSA | 1.5 | 2.64 | – | 2.35 | – |
| | 3.0 | 2.59 | – | 2.54 | – |
| | 6.0 | 2.92 | – | 2.80 | – |
| | 12.0 | 3.04 | – | 2.71 | – |

*Sodium L-lactate was added to give a final concentration of 2.5 mM.