



Supporting Information

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Glyphosate–Exonuclease Interactions: Reduced Enzymatic Activity as a Route to Glyphosate Biosensing

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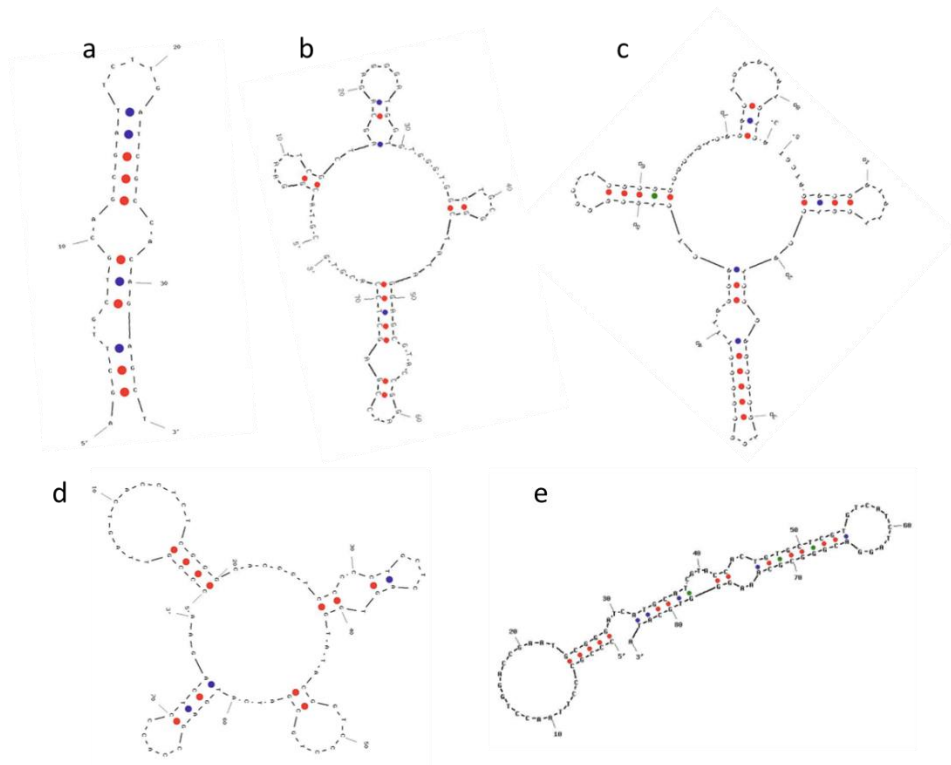


Figure S1. Conformations of (a) GLY1, (b) GLY2, (c) GLY3, (d) SCR GLY2 and (e) SCR GLY3 in their specific interaction buffer (Integrated DNA Technologies website).

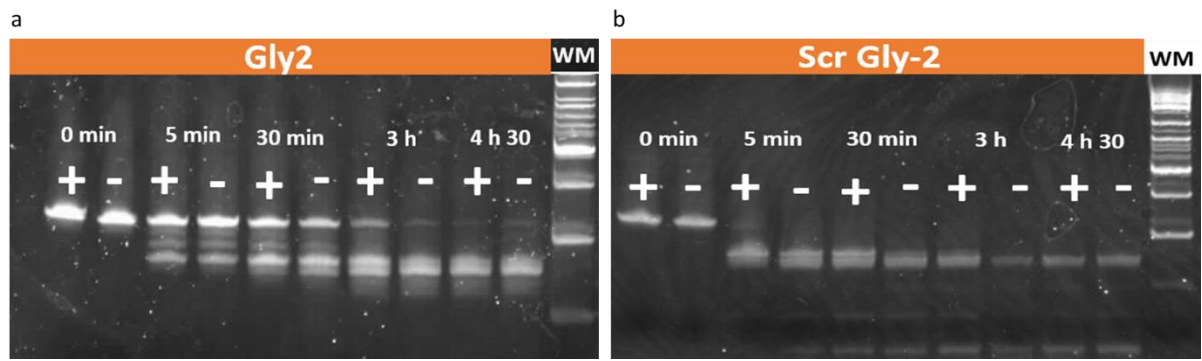


Figure S2. Digestion of (a) candidate GLY2 and (b) SCR GLY2 at $1 \mu\text{M}$ by T5 Exo ($0.2 \text{ U } \mu\text{L}^{-1}$) at room temperature in GLY2 buffer after incubation for 30 min at room temperature with (+) or without (-) glyphosate (1 mM).

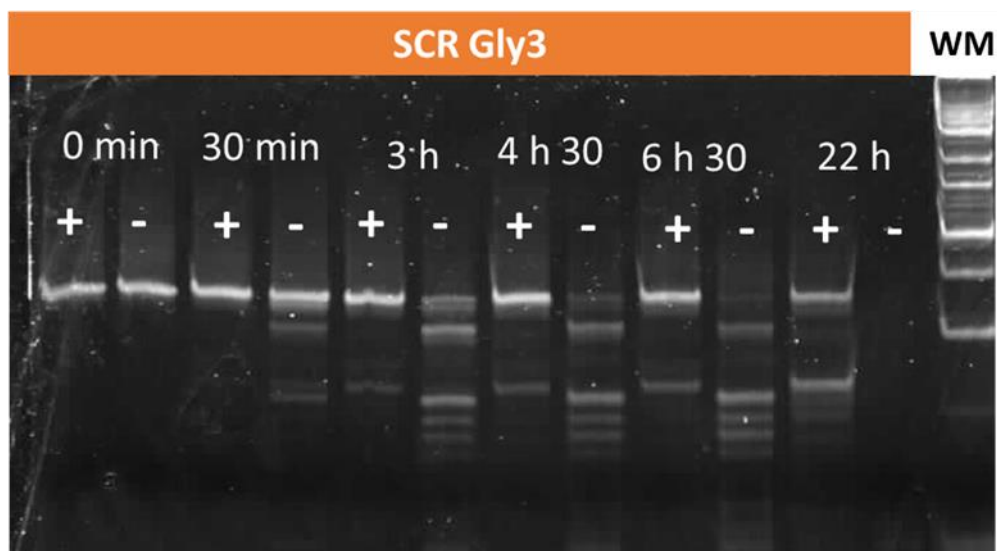


Figure S3. Digestion of SCR GLY3 at $1 \mu\text{M}$ by T5 Exo ($0.2 \text{ U } \mu\text{L}^{-1}$) at room temperature in GLY3 buffer after incubation for 2 hours at $37 \text{ }^\circ\text{C}$ at room temperature with (+) or without (-) glyhosate (1 mM).

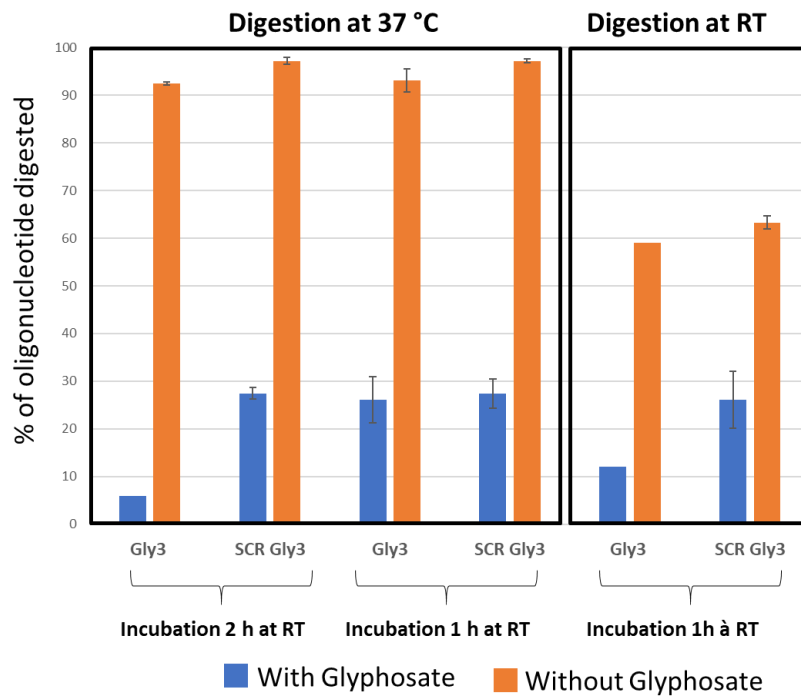


Figure S4. Digestion of GLY3 and SCR GLY3 (1 μ M each) by both enzymes, T5 Exo and Exo I, in GLY3 buffer with or without glyphosate (1 mM) and at different conditions of incubation and digestion.

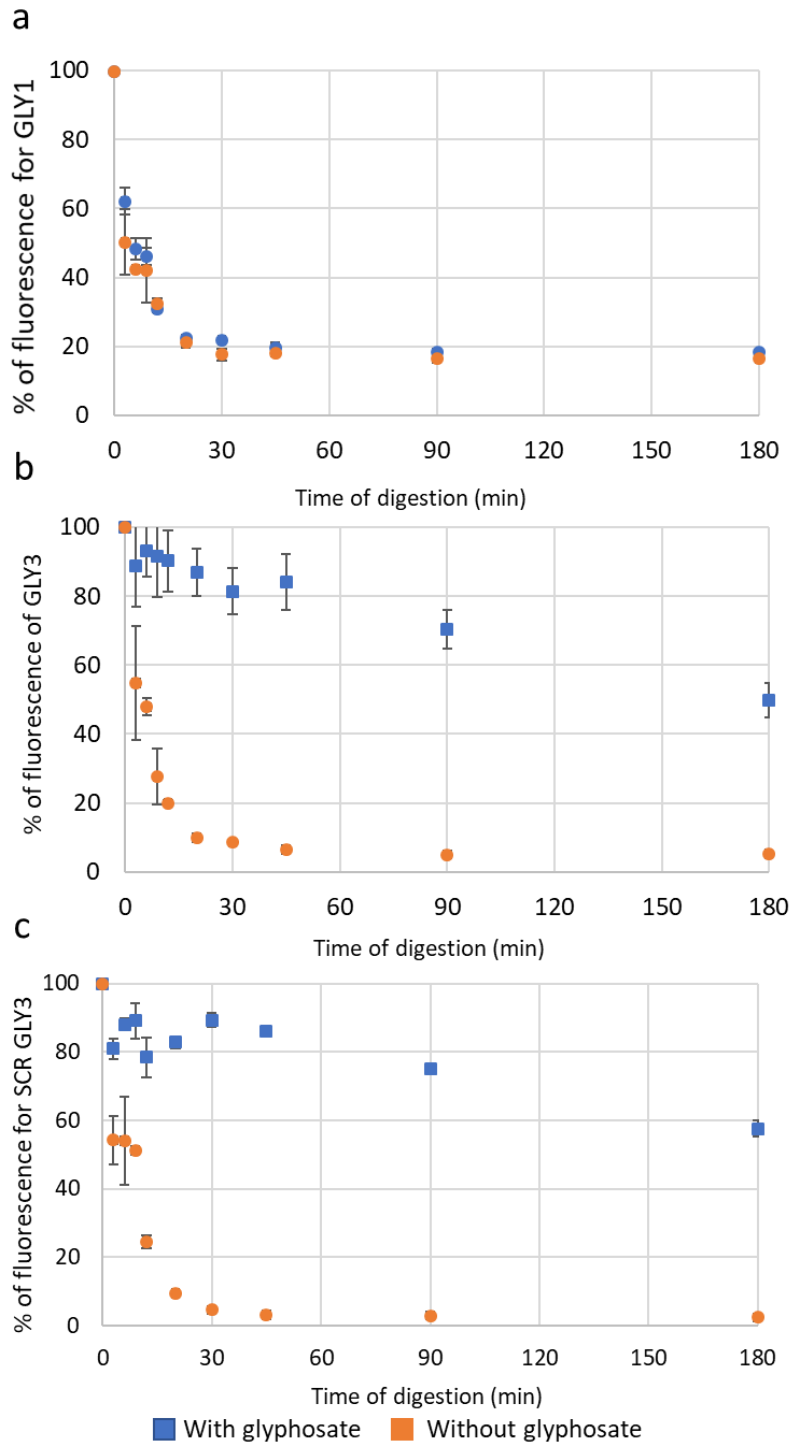


Figure S5. Digestion kinetics of GLY1 candidate (a), GLY3 candidate (b), and the SCR GLY3 (c), at 1 μ M each by the two enzymes, T5 Exo and Exo I, at 37 $^{\circ}$ C in GLY1 and GLY3 buffers successively after 2 hours of incubation at 37 $^{\circ}$ C with or without glyphosate (1 mM).

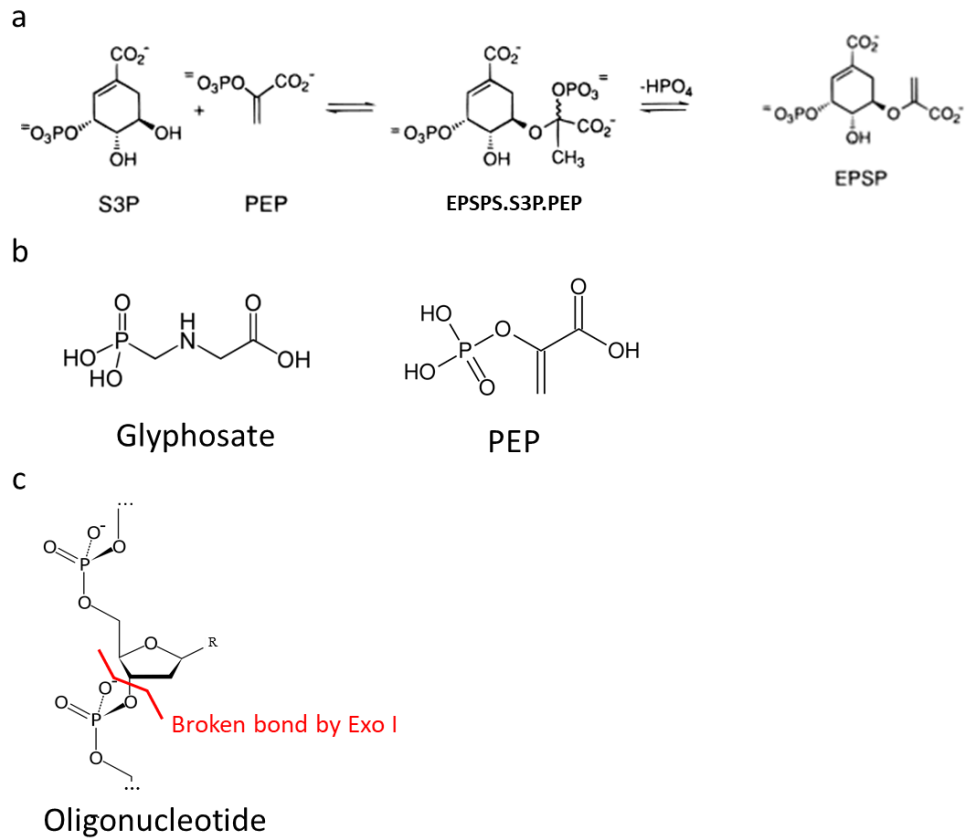


Figure S6. (a) Reaction catalyzed by EPSP synthase (EPSPS). (b) Structural similarity between glyphosate and PEP involved in the EPSPS inhibition mechanism. (c) Exo I activity: phosphodiester bond breakage^[57].

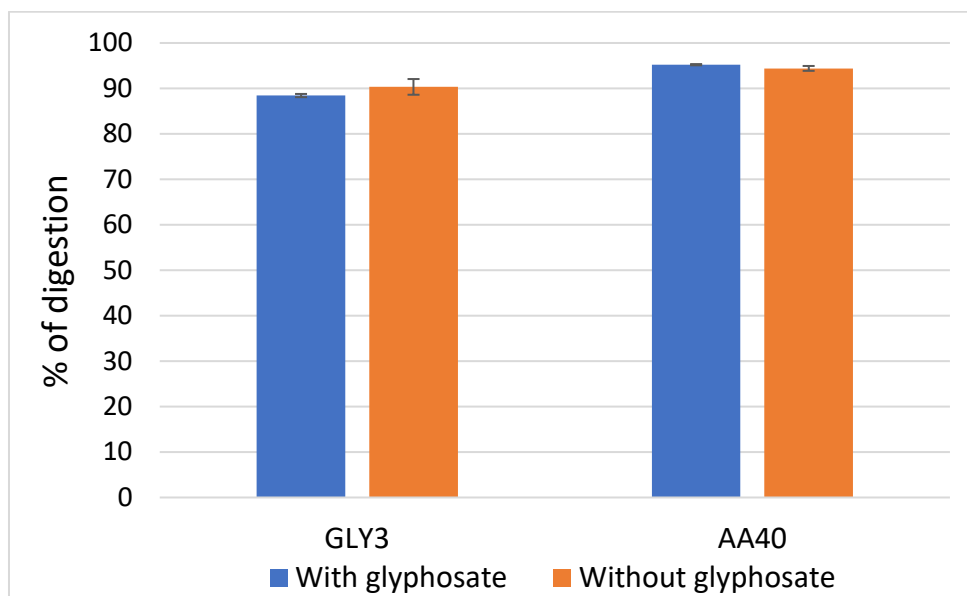


Figure S7. Digestion of GLY3 and AA40 library sequences at 1 μ M by both enzymes, T5 Exo and Exo I, at 37 °C for 30 min after 2 hours of incubation at 37 °C with or without glyphosate (1 mM) in HEPES buffer.

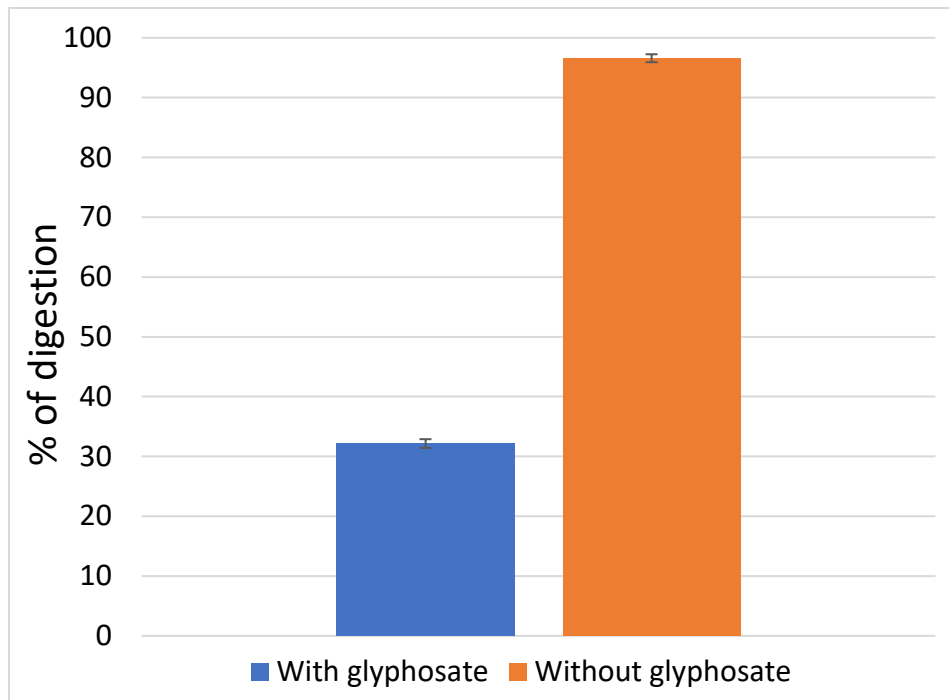


Figure S8. Digestion of GLY3 (1 μ M) by both enzymes, T5 Exo and Exo I, at 37 $^{\circ}$ C for 30 min after 2 hours of incubation at 37 $^{\circ}$ C with or without glyphosate (1 mM) in GLY3 buffer containing 5 mM Mg^{2+} cations.

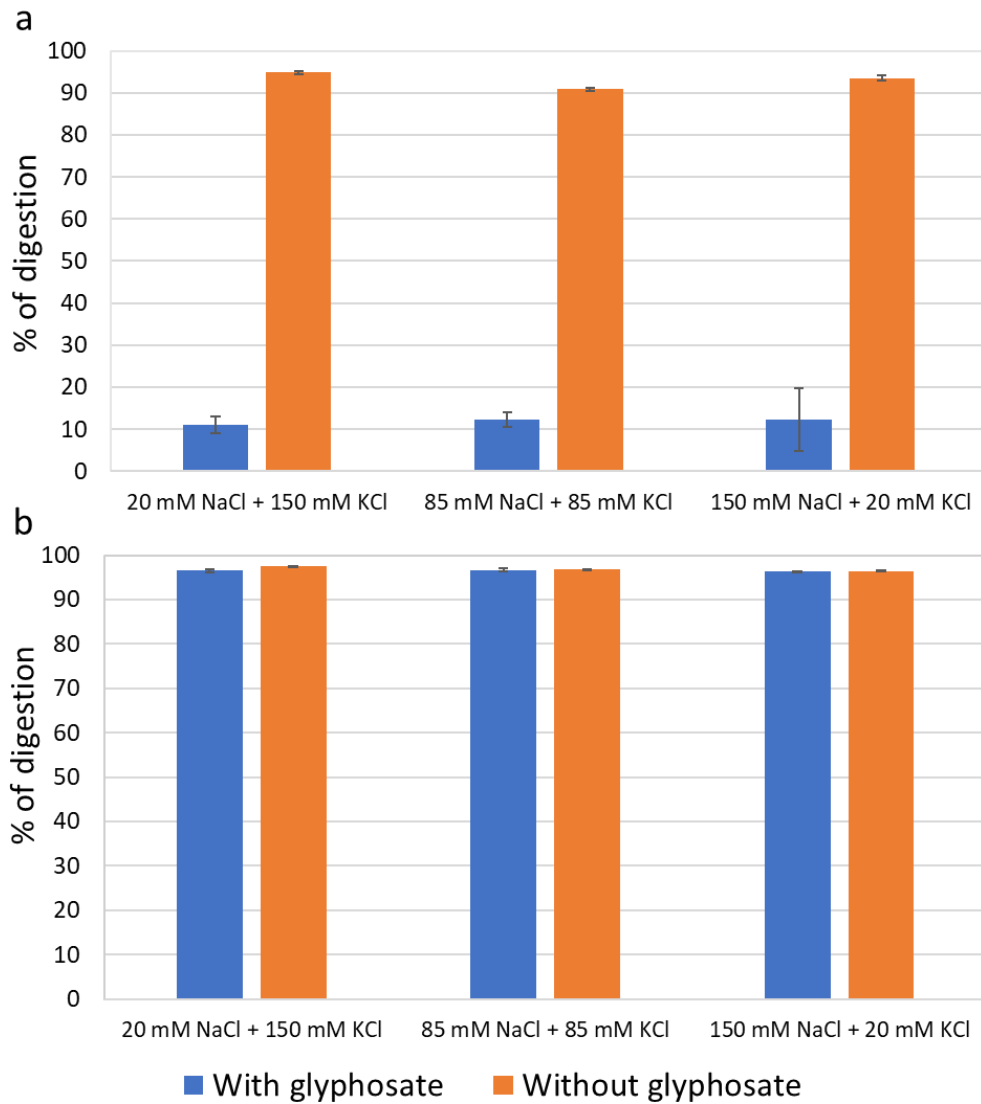


Figure S9. Digestion of GLY3 (1 μ M) by both enzymes, T5 Exo and Exo I, for 30 min at 37 $^{\circ}$ C after 2 hours of incubation at 37 $^{\circ}$ C with or without glyphosate (1 mM) in (a) GLY3 buffer, and (b) HEPES buffer with different $[K^+]/[Na^+]$ ratios.

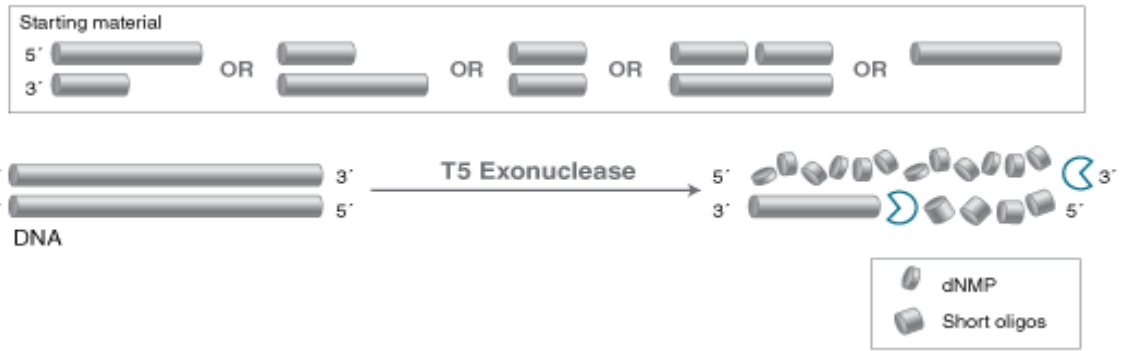


Figure S10. Mechanism of operation of the T5 Exo enzyme (England Biolabs).

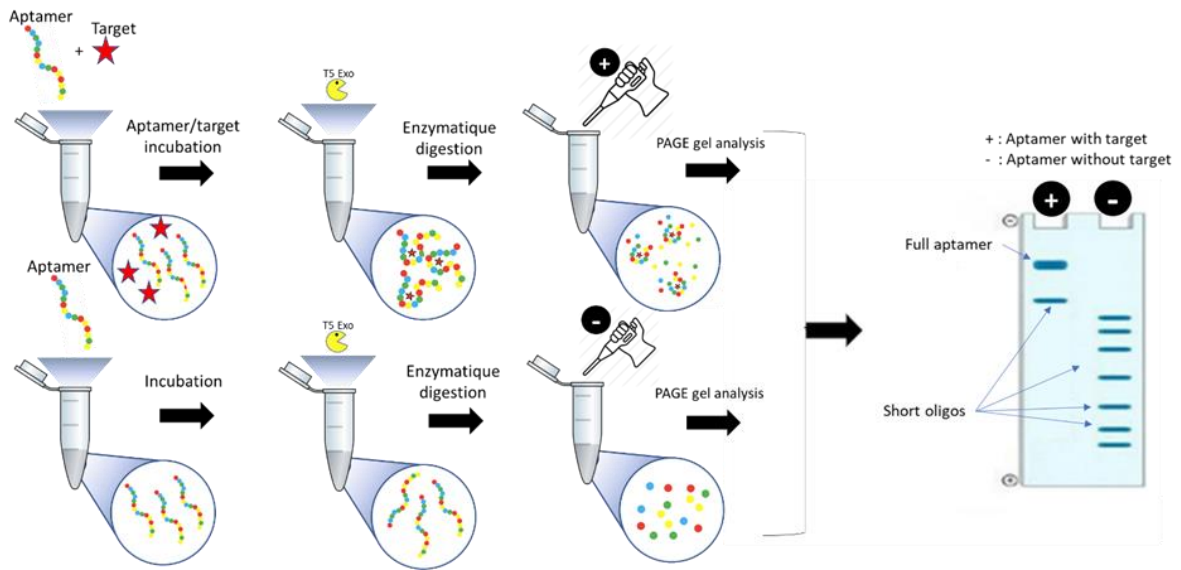


Figure S11. Principle of T5 Exo digestion followed by PAGE to assess an aptamer's interaction with its target.

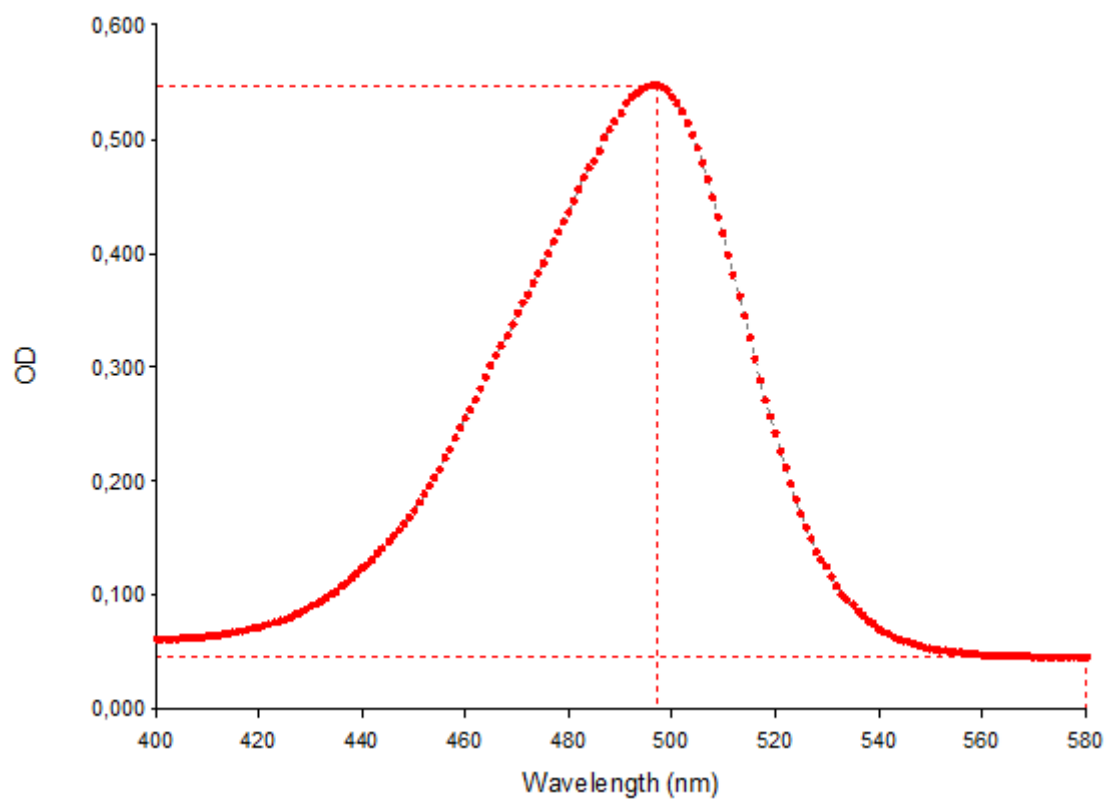


Figure S12. Absorbance spectrum of 10X SYBR Gold ($\lambda_{\max} = 497$ nm).

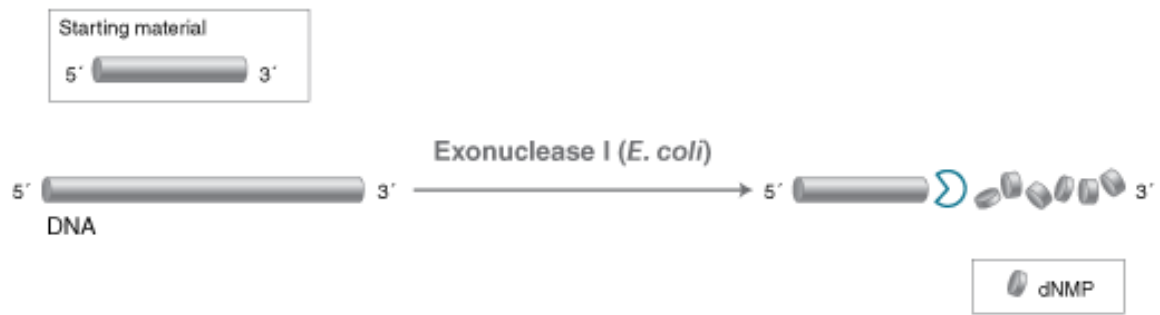


Figure S13. Mechanism of operation of the Exo I enzyme (New England Biolabs).

Denaturing PAGE preparation:

Polyacrylamide 20%, TBE 0.5X, 7 M urea:

- 1 liter acrylamide/bisacrylamide 40%, 19/1 (v/v) [20% final]
- 200 mL TBE 10X [0.5X final]
- 840.84 g urea (MW = 60.06 g/mol) [7 M final]
- Mix in a 2 L beaker with a stirrer and heat to facilitate dissolution (caution: do not exceed 55 °C!)
- Once the solution is clear, adjust the volume to 2 L with H₂O

Cut a whatman paper of the same diameter as the sinter, then filter the solution on the sinter + the whatman paper thanks to the spouted flask and the vacuum pump. Then pour the solution into 2 brown bottles and store at 4 °C.

PAGE plate (15%):

- 15 mL of 0.5X TBE is added to 49 mL of 20% acrylamide (in 0.5X TBE and 7 M Urea), the whole is mixed.
- 650 µL of 10% APS are added to the reaction volume.
- The whole is mixed in a 50 mL tube then 65 µL of TEMED is added to the volume reaction.
- The whole is mixed and poured directly into the gel plates.

Table S1. Different biosensors developed against glyphosate pesticide.

Pesticide	Detection technique	Biomolecule or nanocomposite	Limit of detection (LOD)	Validity for real samples	References
Glyphosate	Fluorometric assay	Aptamer & antibody	0.01 mg L ⁻¹	Phosphate-buffered saline (PBS)	Lee et al. (2010) ^[1]
Trichlorfon, glyphosate and malathion	Fluorometric assay	MNPs/complementary DNA/FAM-aptamer	72.2 ng L ⁻¹ , 88.8 ng L ⁻¹ and 195.37 ng L ⁻¹	Lettuce and carrot	Jiang et al., (2020) ^[2]
Glyphosate	SERS	Aptamer	0.338 ng L ⁻¹	Soil	Liu et al., (2021) ^[3]
Glyphosate	Luminescent assay	Aptamer	72.2 ng L ⁻¹	Lettuce and carrot	Chen et al., (2020) ^[4]
Isocarbophos, omethoate and glyphosate	Colorimetric assay	Aptamer	Isocarbophos: 0.47 µg L ⁻¹ Omethoate: 0.35 µg L ⁻¹	Buffer	Liu et al., (2020) ^[5]
Glyphosate	Potentiometric assay	Urease	0.5 mg L ⁻¹	Tap water	Vaghela et al. (2018) ^[6]
Glyphosate	Fluorometric assay	Antibody	0.021 µg L ⁻¹	Water and soil	Gonzalez-Martinez et al. (2005) ^[7]
Glyphosate	Chronoamperometric assay	Antibody	5 ng L ⁻¹	Commercial beer	Betazzi et al. (2018) ^[8]
Glyphosate	Fluorometric assay	Enzyme	16.9 mg L ⁻¹	Drinking water	The present work

Table S2. DNA oligonucleotide sequences used in this work as negative controls

Oligonucleotide	Sequence
SCR GLY2	5'-CCC-GTT-AGT-CAC-CTC-TCG-GGC-ACG-GTC-CCC-CTG-CTC-AGT-GGT-ATA-CGG-TCC-CTG-CGA-TCATGA-GCC-ACC-CTC-AGA-A-3'
SCR GLY3	5'-TGC-TAG-ACG-ATA-TTC-GTC-CAT-CCG-AGC-CCG-TGG-CGG-GCT-TTA-GGA-CTC-TGC-GGG-CTT-CGCGGC-GCT-GTC-AGA-CTG-AAT-ATG-TCA-3'
ATZ1 ^[9]	5'-TGT-ACC-GTC-TGA-GCG-ATT-CGT-ACG-AAC-GGC-TTT-GTA-CTG-TTT-GCA-CTG-GCG-GAT-TTA-GCCAGT-CAG-TGT-TAA-GGA-GTG-C-3'
ATZ2 ^[10]	5'-TAC-TGT-TTG-CAC-TGG-CGG-ATT-TAG-CCA-GTC-AGT-G-3'
AA40 library	5'-AGC-CTG-TTG-TGA-GCC-TCC-TGT-CGA-ANN-NNN-NNN-NNN-NNN-NNN-NNN-NNN-NNN-NNN-NNN-NNN-NTT-TGA-GCG-TTT-ATT-CTT-GTC-TCC-C-3'
Arsenic aptamer ^[11]	5'-GGTAATACGACTCACTATAGGGAGATAACCAGCTTATTCAATTTACAGAACAAACCAACGTCGCTCCGGGTACTTCTTCATCGAGATAGTAAGTGCAATCT-3'
Thrombin aptamer ^[12]	5'-CCAACGGTTGGTGTGGTTGG-3'
ssDNA	5'CGGAATCAGTGAATGCTTATACATCCG-3'

Table S3. Composition of the interaction buffers used with their corresponding aptamer

Aptamer	Interaction buffer composition
GLY1	10 mM Tris-HCl, pH 7.2, 150 mM NaCl, 122.5 mM MgCl ₂ (6H ₂ O), and 10 mM KCl
GLY2	20 mM Tris-HCl, pH 7.6, 100 mM NaCl, 2 mM MgCl ₂ (6H ₂ O), 5 mM KCl, and 1 mM CaCl ₂ (4H ₂ O)
GLY3	20 mM Tris-HCl, pH 7.2, 150 mM NaCl, 1 mM MgCl ₂ (6H ₂ O), and 20 mM KCl

Table S4. Incubation conditions of glyphosate with aptamers

Aptamer	Target	Incubation conditions with target
GLY1	Glyphosate	2 hours at 37 °C under gentle stirring in GLY1 buffer
GLY2	Glyphosate	30 minutes at room temperature under gentle stirring in GLY2 buffer
GLY3	Glyphosate	2 hours at 37 °C under slight gentle in GLY3 buffer

Table S5. Calculation of the molar concentration of 10X SYBR Gold

λ_{max} (nm)	Absorbance (n = 3)	Optical path (cm)	Extinction coefficient ($\text{M}^{-1} \text{cm}^{-1}$)	[SYBR Gold] (μM)
497	0.494 +/- 0.008	0.7	57000 ^[13]	12.38

References

1. H. U. Lee *et al.*, *J. Agric. Food Chem.* **2010**, *58*, 12096.
2. M. Jiang, C. Chen, J. He, H. Zhang, Z. Xu, *Food Chemistry* **2020**, *307*, 125534.
3. Q. Liu, R. Zhang, B. Yu, A. Liang, Z. Jiang, *Sensors and Actuators B: Chemical* **2021**, *344*, 130288.
4. F. Chen, G. Li, H. Liu, C.-H. Leung, D.-L. Ma, *Sensors and Actuators B: Chemical* **2020**, *320*, 128393.
5. D.-L. Liu *et al.*, *Journal of Nanoscience and Nanotechnology* **2020**, *20*, 2114.
6. C. Vaghela, M. Kulkarni, S. Haram, R. Aiyer, M. Karve, *International Journal of Biological Macromolecules* **2018**, *108*, 32.
7. M. Á. González-Martínez *et al.*, *Anal. Chem.* **2005**, *77*, 4219.
8. F. Bettazzi, A. Romero Natale, E. Torres, I. Palchetti, *Sensors* **2018**, *18*, 2965.
9. R. M. Williams, C. L. Crihfield, S. Gattu, L. A. Holland, L. J. Sooter, *International Journal of Molecular Sciences* **2014**, *15*, 14332.
10. M. Roueinfar, K. M. Abraham, K. L. Hong, *ACS Omega* **2019**, *4*, 16201.
11. M. Kim *et al.*, *Environ. Sci. Technol.* **2009**, *43*, 9335.
12. M. Mir, M. Vreeke, I. Katakis, *Electrochemistry Communications* **2006**, *8*, 505.
13. P. J. Kolbeck *et al.*, *Nucleic Acids Research* **2021**, *49*, 5143.