Supplementary information

Highly Efficient Abiotic Assay Formats for Methyl Parathion – MINA as Alternative to ELISA

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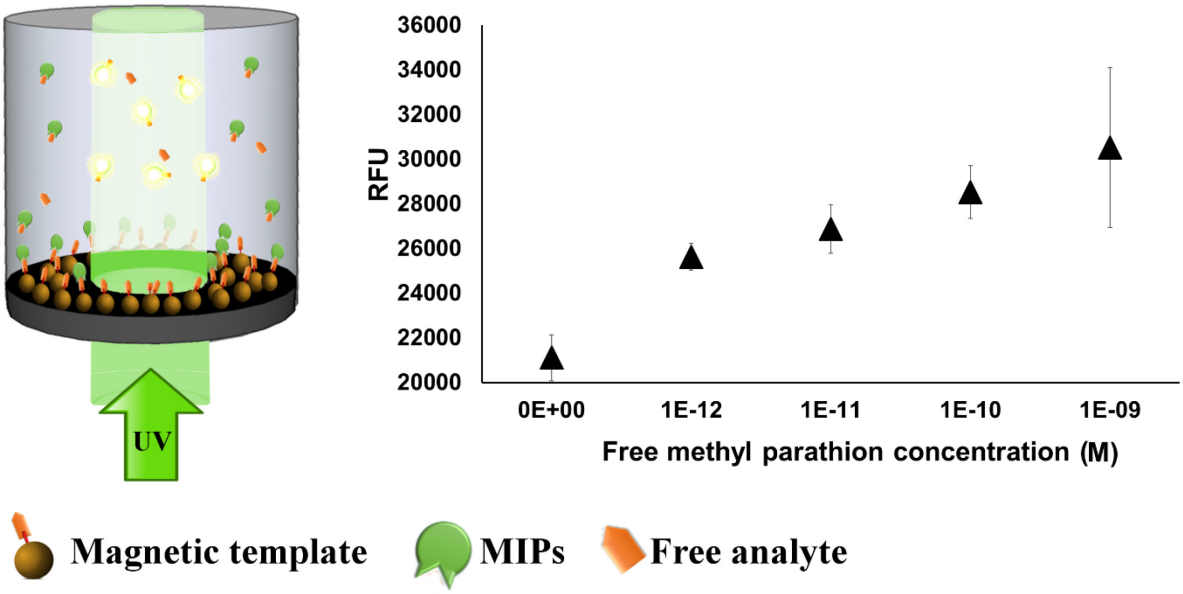
Enzyme-linked immunosorbent assay (ELISA) is a widely used standard method for sensitive detection of analytes of environmental, clinical or biotechnological interest. However, ELISA has clear drawbacks related to the use of relatively unstable antibodies and enzyme conjugates and need in several steps such as washing of non-bound conjugates and adding dye reagents. Herein we introduce a new completely abiotic assay where antibodies and enzymes are replaced with fluorescent molecularly imprinted polymer nanoparticles (nanoMIPs) and target-conjugated magnetic nanoparticles, which acted as both reporter probes and binding agents. The components of the molecularly imprinted polymer nanoparticles assay (MINA) are assembled in microtiter plates fitted with magnetic inserts. We have compared performance of new magnetic assay with MIP-based ELISA for the detection of methyl parathion (MP). Both assays have shown high sensitivity toward allowing detection of MP at picomolar concentrations without any cross-reactivity against chlorpyriphos and fenthion. The fully abiotic assays were also proven to detect analyte in real samples such as tap water and milk. Unlike ELISA-based systems the novel assay required no washing steps or addition of enzyme substrates, making it more user-friendly and suitable for high throughput screening.



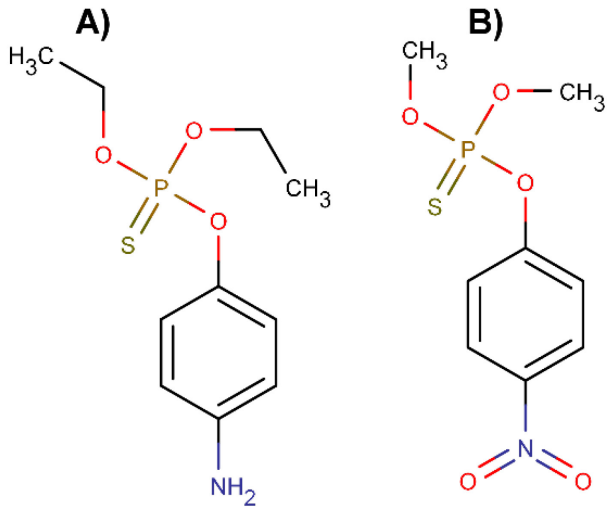


Figure S1. Structure of the template and analyte, amino parathion (A) and methyl parathion (B) respectively.

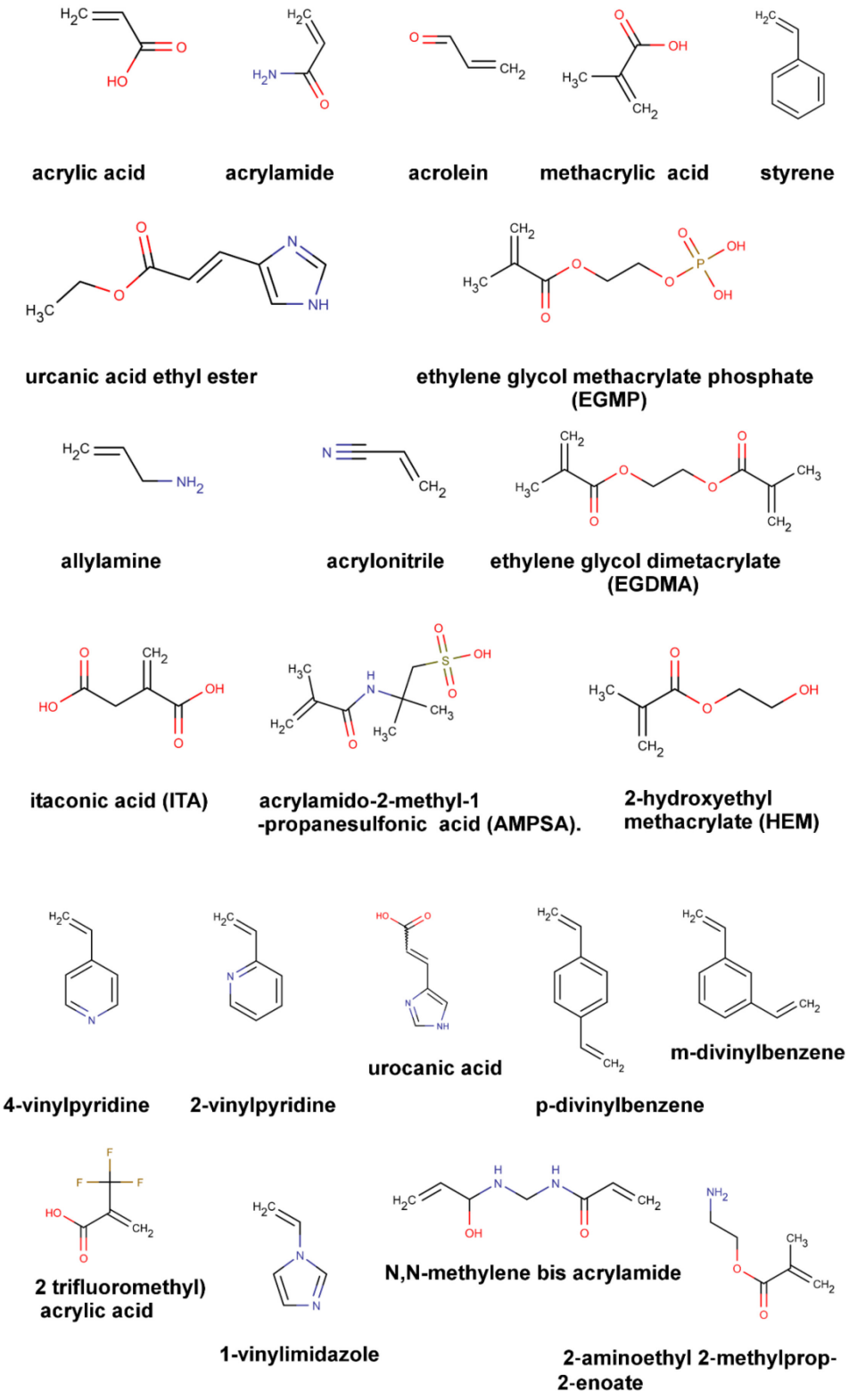


Figure S2. Database consisting of 28 (charged and uncharged) commonly used functional monomers used for screening in computational modelling.

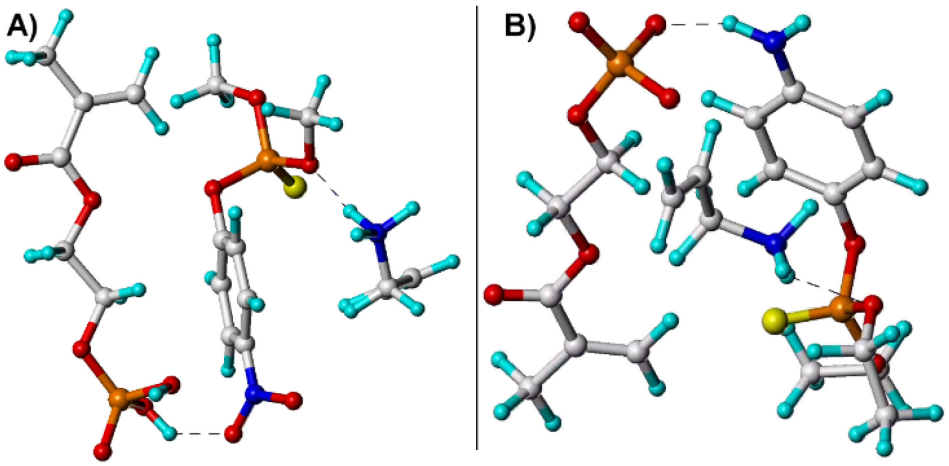


Figure S3. The binding between the chosen functional monomers and the analyte (A) and the template (B).

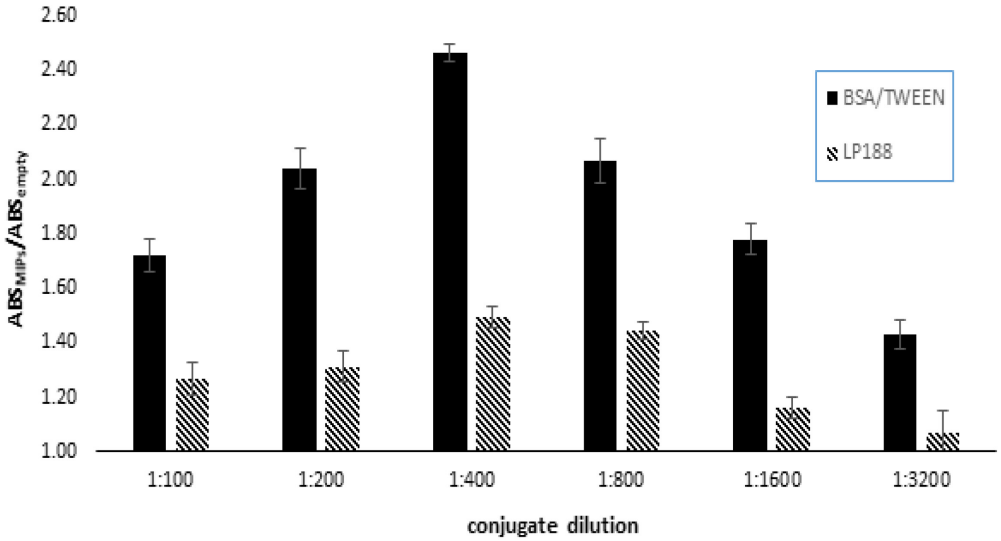


Figure S4. Optimization of conjugate dilutions and two different types of blocking solutions: BSA/Tween 20 and Kolliphor® P188.

Table S1. Repeatability of the over-all method and intra-/inter- assay CV for the magnetic assay in real samples:

	Tap water	Skimmed Milk
limits of detection, pM	5.2	4.1
limits of quantification, pM	15.8	14.9
repeatability of the entire method, %	2.4	3.5
intra-assay coefficient of variation (CV), %	3.7	4.2
inter-assay coefficient of variation (CV), %	4.1	4.5