Supporting Information

Nanopeptamers for the Development of Small-Analyte Lateral Flow Tests with a Positive Readout

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Supporting Figures

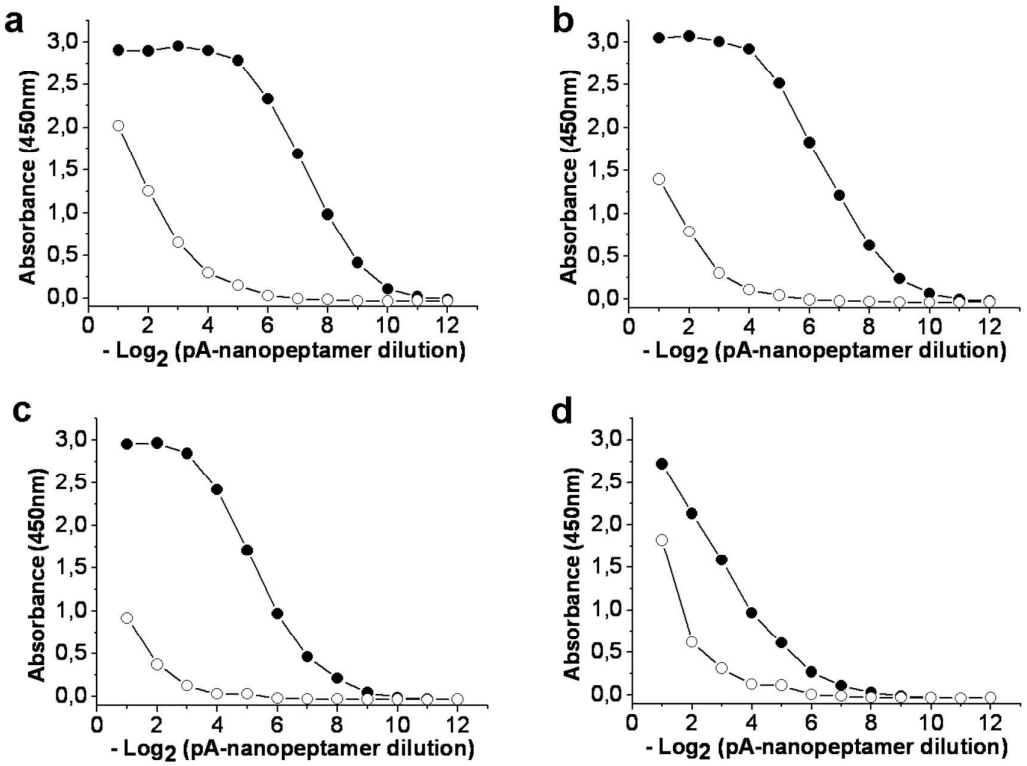

Figure S-1. Reactivity of the pA-Nanopeptamer against the molinate-antibody immunocomplex and the uncombined antibody. Plates were coated with 10 µg/mL of MoAb 14D7 (a), 5 µg/mL (b), 2.5 µg/mL (c), or 1.25 µg/mL (d) and incubated with two-fold serial dilutions of pA-Nanopeptamer in the presence (black circles) or absence (white circles) of molinate (100 ng/mL) using a starting concentration of Nanopeptamer concentration of 2.4 µg/mL. An approximate 50-fold molar excess of biotinylated peptide was used for these ELISAs.

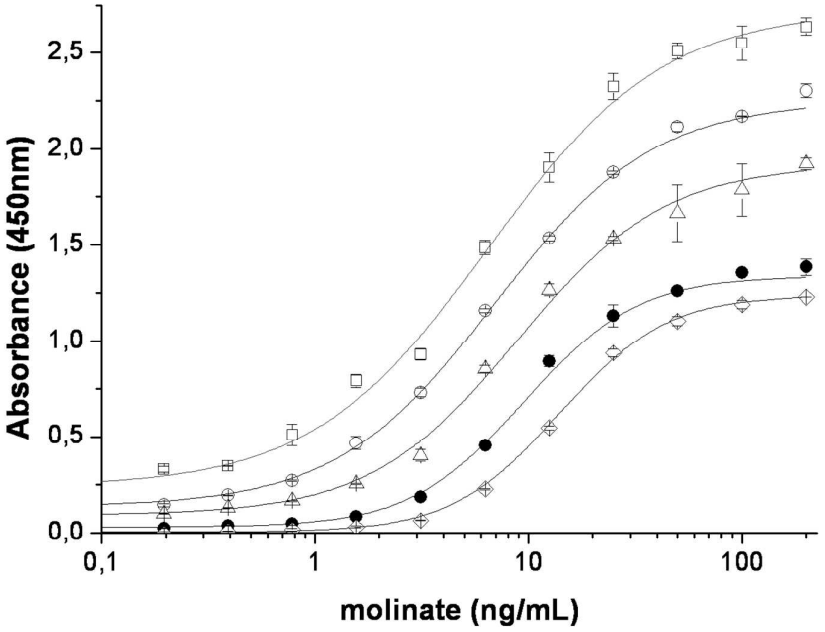

Figure S-2. Effect of the final concentration of pA-Nanopeptamer on the molinate noncompetitive ELISA. Microtiter plates coated with 10 µg/mL of MoAb 14D7 were incubated with two-fold serial dilutions of standard molinate and different concentrations of pA-Nanopeptamer. White squares (pA-Nanopeptamer, 6 µg/mL) SC_{50}= 4.0 ± 0.3 ng/mL; white circles (pA-Nanopeptamer, 3 µg/mL) SC_{50}= 3.7 ± 0.2 ng/mL; white triangles (pA-Nanopeptamer, 0.75 µg/mL) SC_{50}= 8.7 ± 0.4 ng/mL; black circles (pA-Nanopeptamer, 0.38 µg/mL) SC_{50}= 20.3 ± 1 ng/mL; white diamonds (pA-Nanopeptamer, 0.18 µg/mL) SC50= 32.4 ± 1 ng/mL. From these experiments a final concentration of 0.75 µg/mL pA saturated SPO-pA-Nanopeptamer was chosen for further experiments.

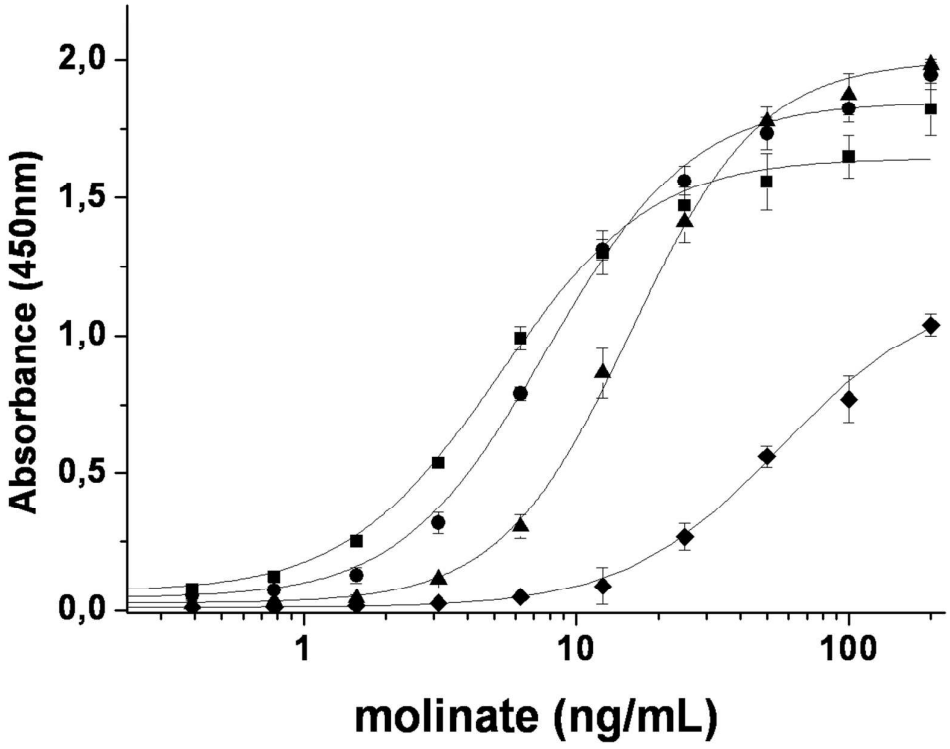

Figure S-3. Effect of pA to SPO ratio in pA-Nanopeptamer assay performance. Microtiter plates were coated with 10 µg/mL of MoAb 14D7. SPO was preincubated with the approximate following molar ratios of biotinylated peptide to SPO: 50 (squares, SC_{50}= 5.5 ± 0.3), 6.5 (circles, SC_{50}= 7.7 ± 0.3), 1.5 (triangles, SC_{50}= 15.8 ± 1.0) and 0.6 (diamonds, SC_{50}= 55.6 ± 3.42). Similar results were obtained with the p1M-Nanopeptamer (not shown).

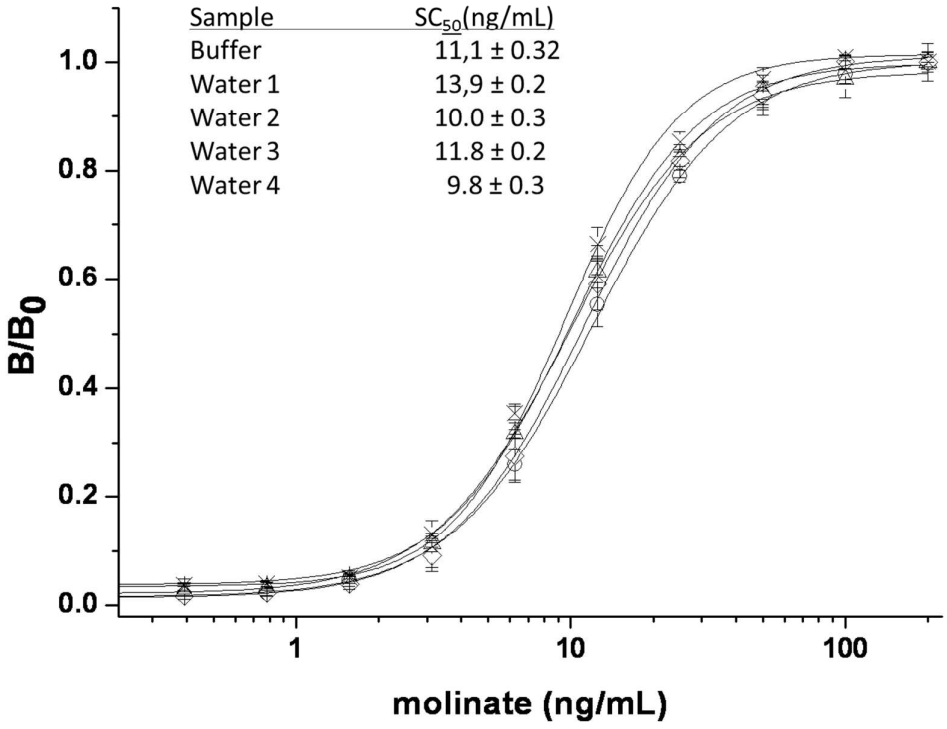

Figure S-4. **Matrix effect on the molinate pA-Nanopeptamer assay.** MoAb 14D7 (10 µg/mL) was used for coating and SPO (0.75 µg/mL) complexed with a 50 fold molar excess of pA (triangles) for detection. The test was performed with molinate dissolved in assay buffer (circles) or undiluted water from agricultural runoffs, squares, triangles, diamonds or crosses, for water samples 1-4, respectively

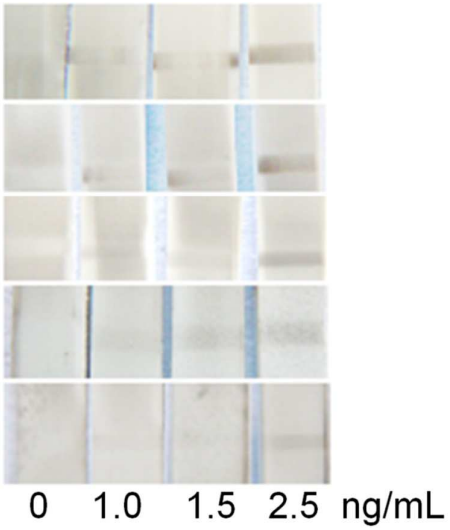

Figure S-5. Lateral-flow analysis of molinate in surface water samples using the avidin-pA Nanopeptamer labeled with carbon-black. Water samples from rivers and dams of Uruguay were spiked with various amounts of molinate mixed with 5% (v/v) of concentrated (10 x) PBS-T before the assay. Only the 0-2.5 ng/mL range is shown.

Supporting Table S-1. Strip densitometry measurements of molinate and clomazone Nanopeptamers Lateral-Flow Assays. A) and B), densitometry values corresponding to lateral-flow strips shown in figures 3 and 4, respectively

A		B	
Molinate (ng/mL)	Peak Area	Clomazone (ng/mL)	Peak Area
0	167.7	0	145.2
2.5	1087.3	2.5	565.6
5	2307.7	5	1571.3
10	3292.4	10	3875.7
25	3971.3	25	7375.3
200	8324.1	200	8058.3

Images were processed with the ImageJ software (NIH)