# Dummy-template imprinted bovine serum albumin for extraction of zearalenone

## Supplementary Information

**Table S1** Molecularly imprinted polymers for zearalenone extraction

| *Monomer*, Cross-linking agent | Template | Support | Detection method | LOD | Linear range | Recovery | Adsorption capacity | Ref. |
|---|---|---|---|---|---|---|---|---|
| *MMA*, EDMA | 3–CCA, naringenin | HA | HPLC–FLD | $1.32\,\mu g\,kg^{-1}$ | – | 70.09–101.88 % | – | [1] |
| *APTES*, TEOS | Coumafene | MNP | HPLC–FLD | $0.4\,ng\,kg^{-1}$ | – | 90.56–99.96 % | $8.71\,mg\,g^{-1}$ | [2] |
| *Dopamine*, – | Quercetin | MNP | HPLC–FLD | $0.68\,ng\,mL^{-1}$ | $1–20\,ng\,mL^{-1}$ | 93.8–101.0 % | $1.49\,mg\,g^{-1}$ | [3] |
| *MMA*, EDMA | Coumafene | MNP | HPLC–FLD | $2.5\,ng\,mL^{-1}$ | $10–200\,ng\,mL^{-1}$ | 74.95–88.41 % | $6.07\,mg\,g^{-1}$ | [4] |
| *EAMA*, EDMA | S2, CDBH | Silica beads | HPLC–FLD, HPLC–MS/MS | $5.2\,\mu g\,kg^{-1}$ | – | 94–108 % | – | [5] |

List of acronyms: **3–CCA** — Coumarin-3-carboxylic acid; **APTES** — 3-Aminopropyl triethoxysilane; **CDHB** — Cyclododecyl 2,4-dihydroxybenzoate; **EAMA** — N-(2-aminoethyl) methacrylamide (EAMA); **EDMA** — Ethylene glycol dimethacrylate; **HA** — Hydroxyapatite; **HPLC–FLD** — High performance liquid chromatography with fluorescence detection; **MMA** — Methacrylic acid; **MNP** — Magnetic nanoparticles; **S2** — 3,8,9-trihydroxy-6H-dibenzo[b,d]pyran-6-one; **TEOS** — Tetraethyl orthosilicate.

[1] doi.org/10.1007/s00216-020-02610-y — Application of surface-imprinted polymers supported by hydroxyapatite in the extraction of zearalenone in various cereals

[2] doi.org/10.1007/s00216-020-02729-y — Preparation of magnetic molecularly imprinted polymers for the identification of zearalenone in grains

[3] doi.org/10.1080/00032719.2021.1931268 — Preconcentration and Determination of Zearalenone in Corn Oil by a One-Step Prepared Polydopamine-Based Magnetic Molecularly Imprinted Polymer (MIP) with High-Performance Liquid Chromatography with Fluorescence (HPLC-FLD) Detection

[4] doi.org/10.1016/j.foodchem.2019.125696 — Synthesis and application of magnetic-surfaced pseudo molecularly imprinted polymers for zearalenone pretreatment in cereal samples

[5] doi.org/10.1016/j.foodchem.2023.135538 — Simultaneous determination of zearalenone and alternariol mycotoxins in oil samples using mixed molecularly imprinted polymer beads

**Table S2** Samples for cells experiments. All stock solutions are prepared in PBS (7.4, 10 mM)

| **Sample name** | **Cross-linked agent** | **Concentration**, mg $mL^{-1}$ | Fig. number |
|---|---|---|---|
| nIP before purification | GA, Genipin, EDC/NHS | 1 | S2 |
| nIP after purification | GA, Genipin, EDC/NHS | 1 | S2 |
| IPs before purification | GA | 1 | S10 |
| IPs after purification | GA | 1 | S10 |
| nIP before purification | GA | 1 | S10 |
| nIP after purification | GA | 1 | S10 |

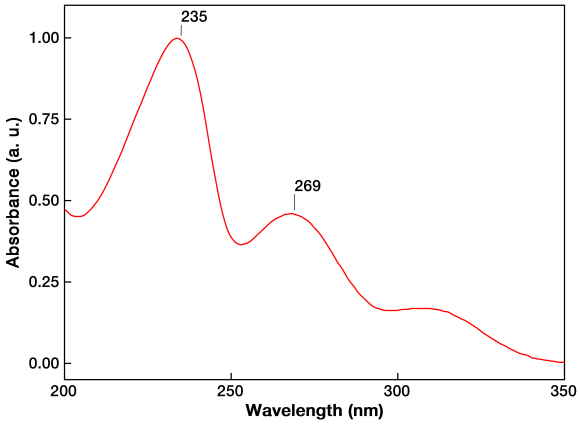


**Fig. S1** ZEN UV Spectrum

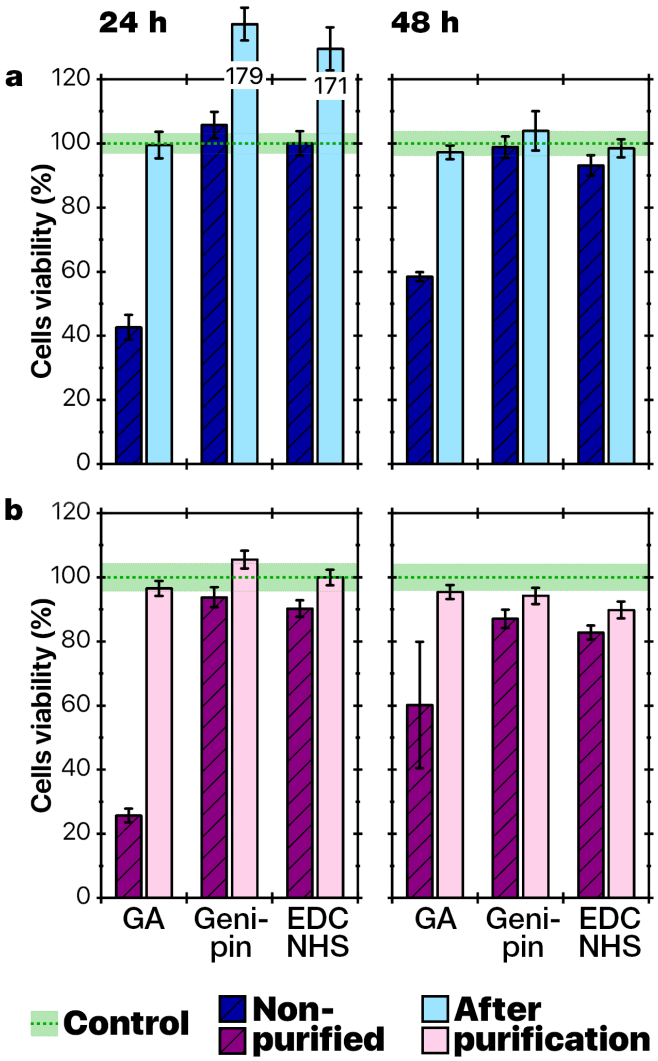


**Fig. S2** Effect of cross-linking agent type and purification nIP on cell viability of (a) 4T1 cells and (b) L929 cells

## S1 Optimising the step of adding templates

The synthesis was optimized by selecting initial pH values of 1.0, 2.0 and 3.0 using orthophosphoric and citric acids, and initial pH values of 7.4 using PBS.

The remaining steps were consistent with the described protocol for the synthesis of IP. Optimization of the nIP synthesis was not conducted, and a protocol identical to the best IPs synthesis was chosen.

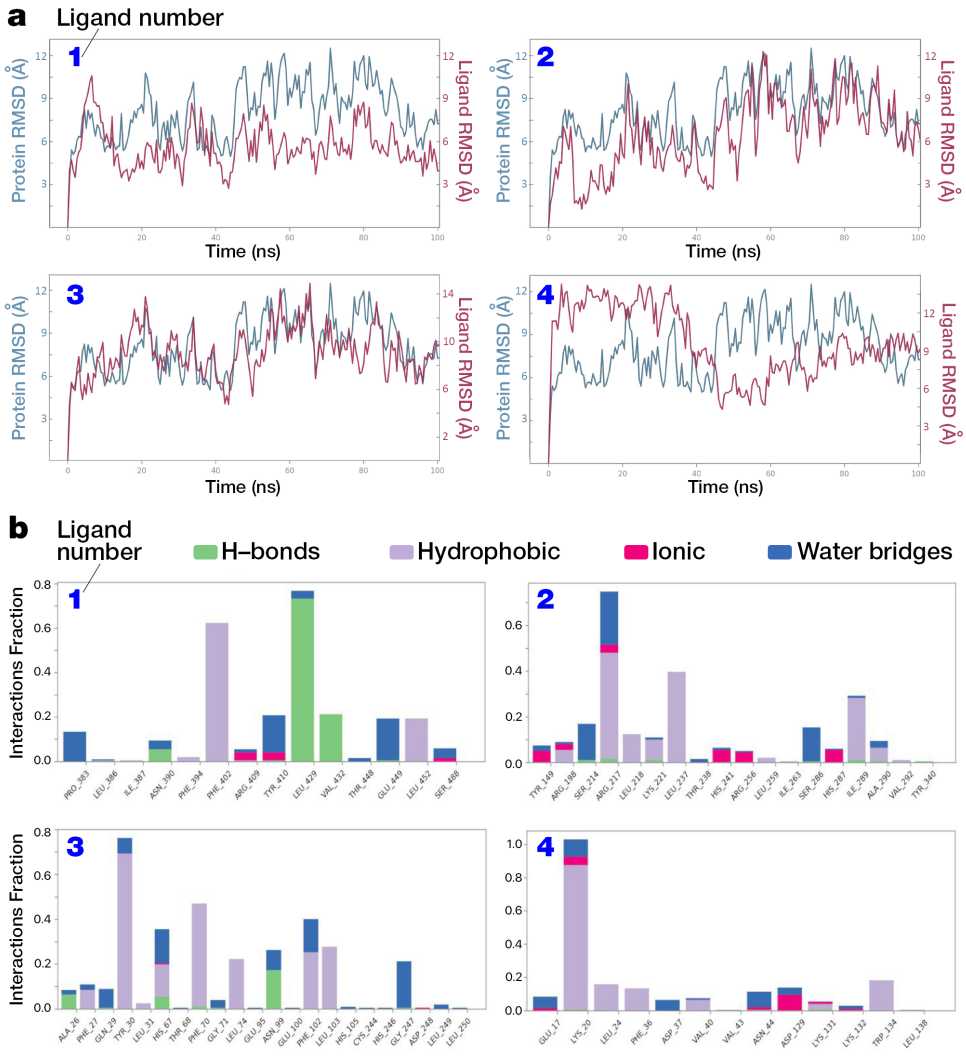


**Fig. S3** Results of molecular modelling of BSA–4–HC complex at pH 3.0: (a) system RMSD; (b) protein–ligand interactions percentage

## BSA–4–HC binding studies

The number of binding sites of BSA with 4–HC decreases when the pH value decreases from 7.4 to 3.0 (Fig. S5), and their location also changes. The choice of these acidity values is consistent with the basic values used in IPs synthesis (Table S3). The most commonly used pH value is 3.0; however, Ghulam Murtaza *et al.*[1] used pH 7.4.

[1] doi.org/10.1016/j.aca.2020.04.018 — Glycated albumin based photonic crystal sensors for detection of lipopolysaccharides and discrimination of Gram-negative bacteria

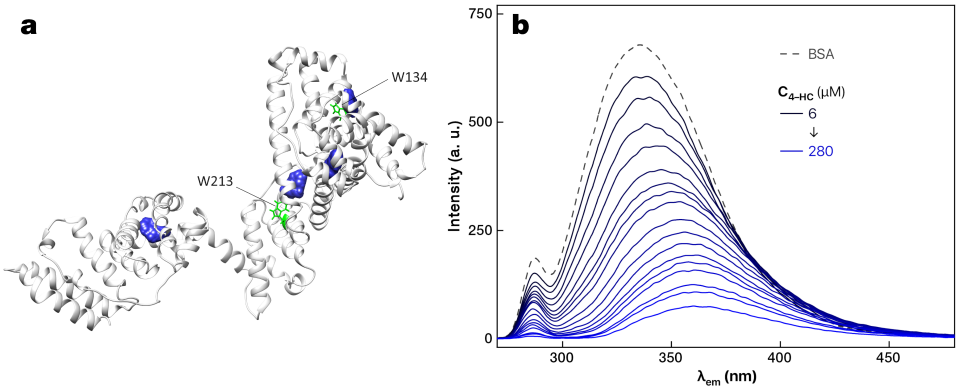


**Fig. S4** (a) BSA quenching in the presence of 4–HC (6–280 μM), pH 3; (b) Results of molecular modelling of BSA–4–HC complex at pH 3.0, location of 4–HC in the supposed binding site near tryptophan (green)

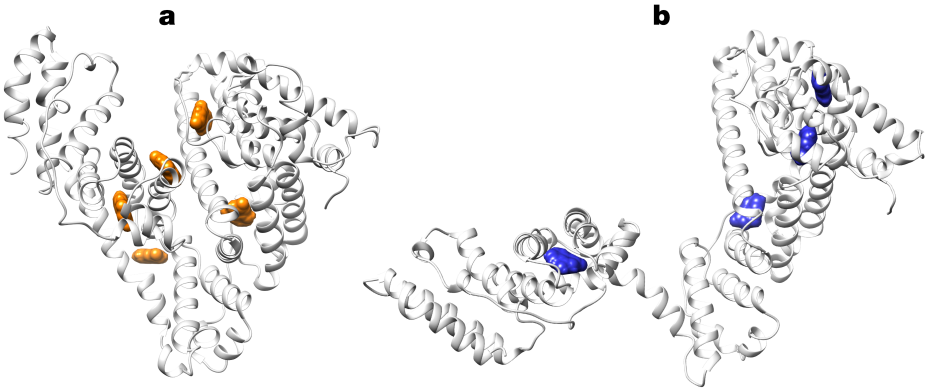


**Fig. S5** Results of molecular modelling of a complex of full-size BSA and molecules of 4–HC at (a) pH 7.4 and (b) pH 3.0: template molecules are located in the potential binding sites

## S2 Optimizing the step of removal templates

To select the optimal eluent for 4–HC removal, 4 mL of the IPs BSA sample, synthesized using the standard protocol, was mixed with 4 mL of one of the following buffer solutions (10 mM): citrate (pH 3.0), glycine–HCl (pH 2.2), acetate (pH 5.0), PBS (pH 7.4), Tris–glycine (pH 8.5), or carbonate–bicarbonate (pH 10.2). The mixtures were then thoroughly vortexed and concentrated using a Biodialyzer 30 000 MWCO (3600 rcf, 4 °C), until reaching a final volume of 4 mL. The registration of three-dimensional fluorescence spectra and the purification procedure were repeated until the characteristic fluorescence spectrum of the protein solution appeared and the characteristic maximum of the template disappeared — Fig. 4, S9.

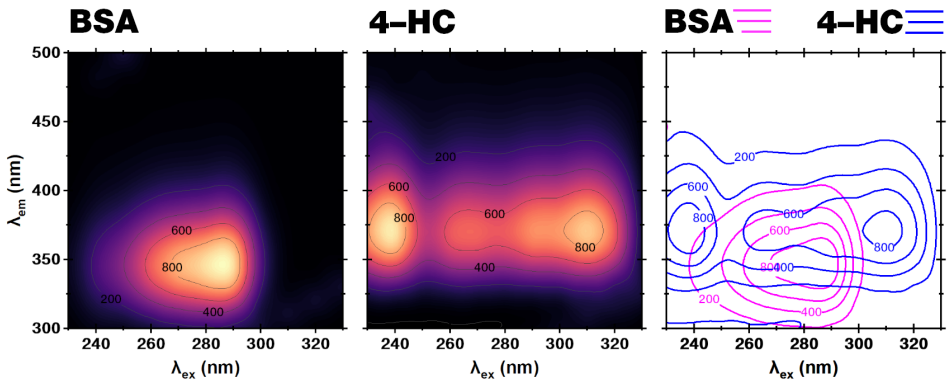


**Fig. S6** Heatmaps for BSA, 4–HC and overlay heatmaps of both substances

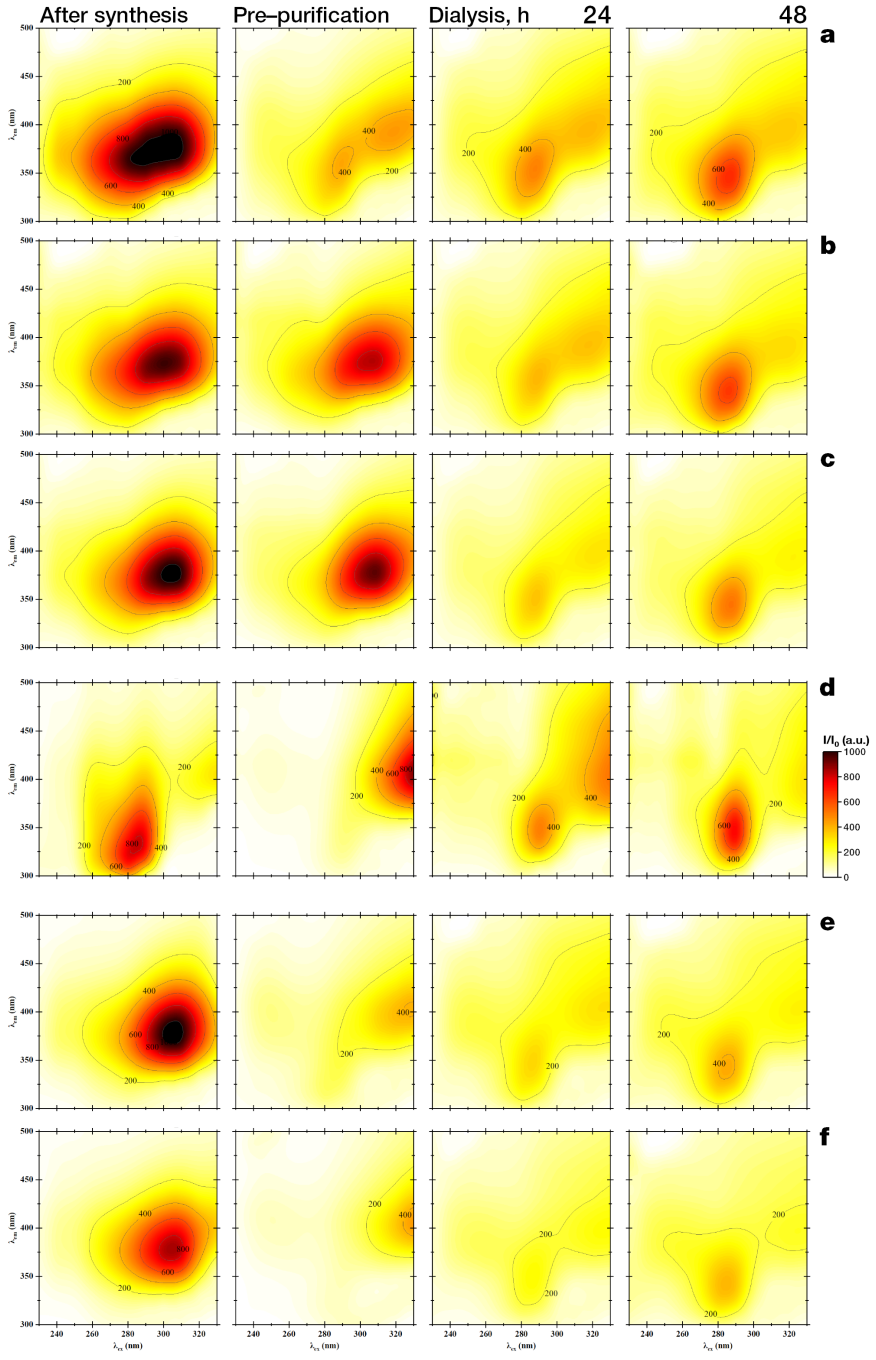


**Fig. S7** Heatmaps for IPs (HCl, 4–HC) pH 3.0 (a), pH 2.0 (b), pH 1.0 (c) and nIP (d) during purification

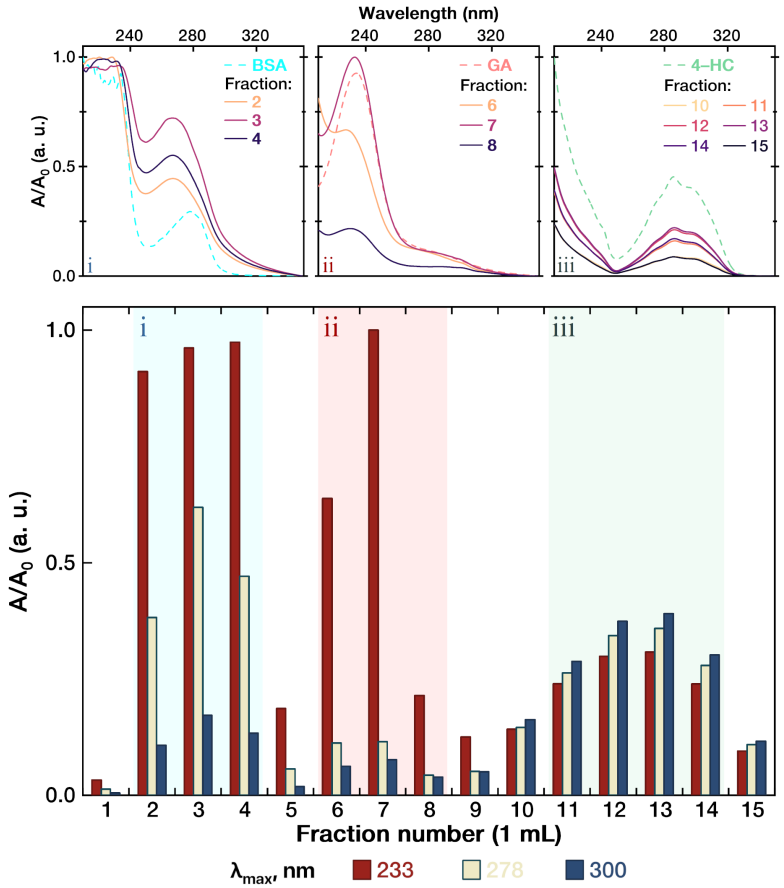


**Fig. S8** Chromatogram of the IP-BSA pre-purification. Only fractions with a characteristic maximum are indicated: 2…4 — IPs BSA, 6…7 — GA, 12…15 — 4–HC

**Table S3** Basic conditions for IPs synthesis in various papers

| Protein | Mass, mg | Solvent; IT, min | Template molecule, $pH_T$ | V, µL | Concentration of template | IT, min | Synthesis time | Template removal conditions | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Egg albumin | 1 | n/a; 60 | GSH–2DNP, 3.0 | – | 5 mg | 60 | 12 h | 50 mM phosphate buffer, 48 h | [1] |
| OVA or BSA | 1 | MQ; 10 | Aflatoxin B1, 3.0 | 100 | 200 µg $mL^{-1}$ | 10 | Overnight | 10 mM potassium phosphate buffer, 48 h | [2] |
| BSA or OVA | 1 | CiB; 10 | ZEN, 3 | – | 120 µg | 10 | Overnight | 10 mM PBS, 48 h | [3] |
| BSA | 1 | CiB; 10 | ZEN + DON, 3.0 | – | 120 µg | 10 | Overnight | 10 mM PBS, 48 h | [4] |
| GoX | 1 | n/a; 60 | OVA, 3 | 100 | 0.2 mg $mL^{-1}$ | 60 | Overnight | 10 mM PBS, 24 h, gel filtration | [5] |
| OVA or *et al.* | 1 | DW; 10 | Kwa, 3 | 100 | 0.2 mg $mL^{-1}$ | 10 | Overnight | n/a PBS + 5% glycerol v/v, 48 h | [6] |
| GoX | 1 | MQ; 10 | ZEN or DT, 3.0 | 100 | 640 µM | 10 | 12 h | 0.1 M PBS, 24 h | [7] |
| G–alb | 10 | PBS; n/a | LPS, 7.4 | – | 50 mg | 120 | n/a | Tris–EDTA buffer, pH 8.0, 55 °C, 10 min | [8] |
| BSA | 1 | MQ; 10 | 4HC or ZEN, 2.0 | 100 | 1.21 mM | 20 | 18 h | 10 mM PBS, pH 7.4, 1 h | [9] |

List of acronyms: **BSA** — Bovine serum albumin; **CiB** — Citrate buffer (pH 4); **DT** — Dummy-templates: 4–hydroxycoumarin or coumarin or quercetin; **DON** — Deoxynivalenol; **DW** — Distilled water; ***et al.*** — BSA, human serum albumin, γ-globulin from human serum, mouse serum albumin, avidin from egg white, thyroglobulin from bovine thyroid, MAb 2H2, MAb 1D2; **G–alb** — Glycated albumin; **GoX** — Glucose oxidase; **GSH–2DNP** — N,S-bis-2,4-dinitrobenzyl-glutathione; **IT** — Incubation time; **Kwa** — Kwakhurin; **LPS** — *E. coli* lipopolysaccharides; **MQ** — Milli–Q water; **OVA** — Ovalbumin; **PBS** — Phosphate buffer saline; **$pH_T$** — pH of template addition; **ZEN** — Zearalenone

[1] doi.org/10.1016/S0003-2670(03)00763-3 — Bioimprinted Protein Exhibits Glutathione Peroxidase Activity

[2] doi.org/10.1016/j.btre.2016.05.006 — Bioimprinting as a Tool for the Detection of Aflatoxin B1 Using a Capacitive Biosensor

[3] doi.org/10.1016/j.aca.2018.07.062 — Imprinted Proteins as a Receptor for Detection of Zearalenone

[4] doi.org/10.1016/j.talanta.2018.09.042 —Bioimprinting for Multiplex Luminescent Detection of Deoxynivalenol and Zearalenone

[5] doi.org/10.1007/s00216-022-04009-3 — Imprinted Proteins for Determination of Ovalbumin

[6] doi.org/10.3390/biom12081064 — Bioimprinting as a Receptor for Detection of Kwakhurin

[7] doi.org/10.1134/S1061934823090101 — Selective Adsorbents Based on Imprinted Glucose Oxidase

[8] doi.org/10.1016/j.aca.2020.04.018 — Glycated Albumin Based Photonic Crystal Sensors for Detection of Lipopolysaccharides and Discrimination of Gram-negative Bacteria

[9] This Work

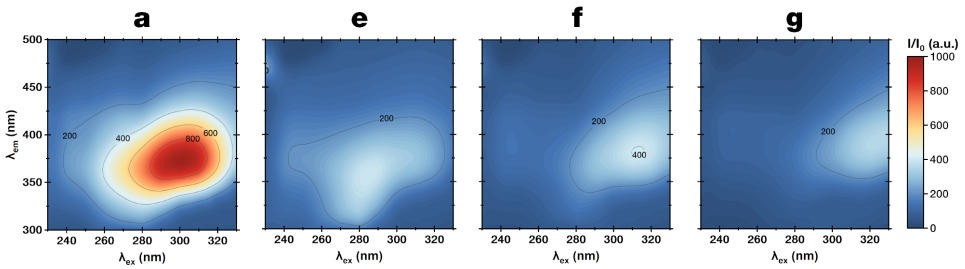


**Fig. S9** Heatmaps of the IP–4–HC system for (a) post–synthesis sample and samples after the final step of purification with different eluents (V = 8 mL): (e) acetate buffer solution (pH 4.94; 0.1 M); (f) Tris–glycine buffer solution (pH 8.5; 0.1 M); (g) carbonate–bicarbonate buffer (pH 10.2; 0.1 M). Other samples—Fig. 4

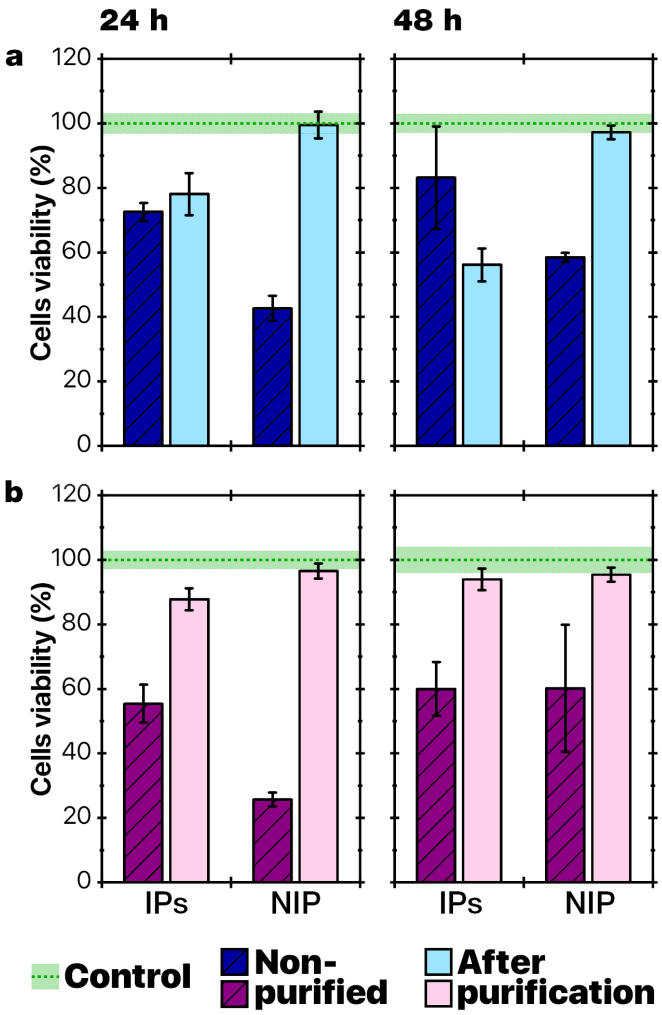


**Fig. S10** Effect of purification IPs and nIP on cell viability of (a) 4T1 cells and (b) L929 cells

## S3 IPs Immobilization

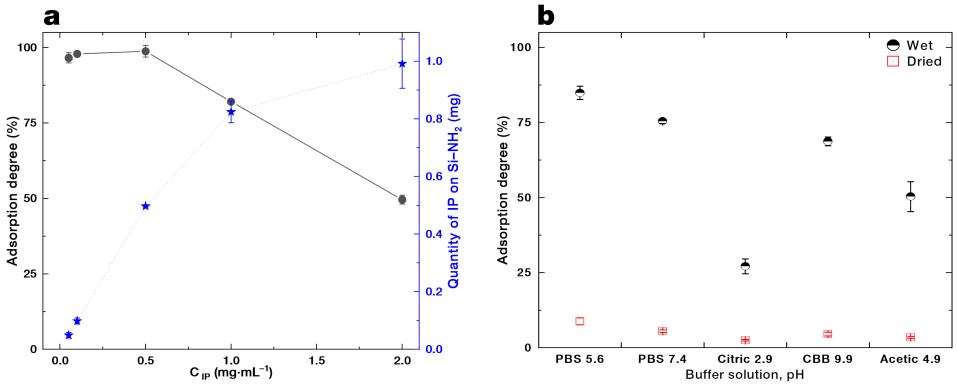


**Fig. S11** (a) Effect of pH on adsorption degree of IPs during immobilization on dried and wet Si−$HN_2$ particles; (b) Effect of IPs concentration on adsorption degree and amount of immobilized protein on wet Si−$HN_2$ particles particles

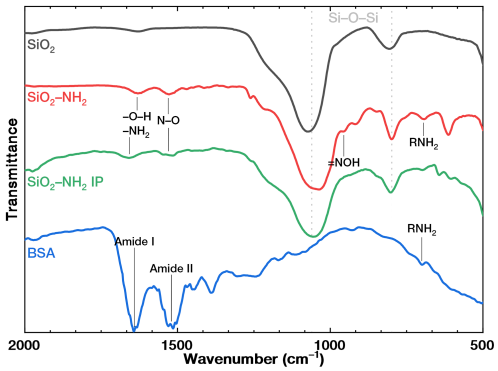


**Fig. S12** ATR spectra of BSA (blue), silica NPs (black), aminated silica NPs (red) and IP–BIS (green)

## S4 Extraction of zearalenone

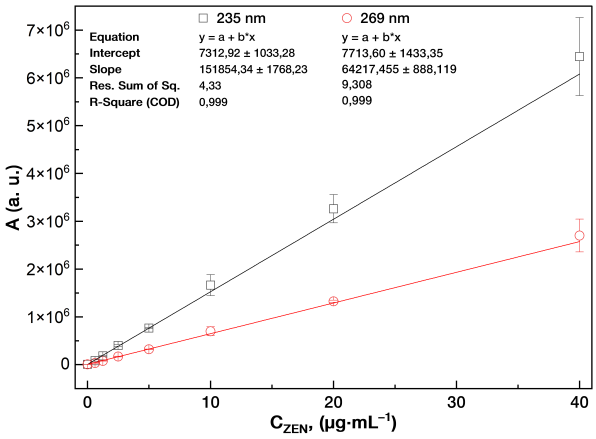


**Fig. S13** Calibration curves for extract from a real sample spiked with ZEN