# SUPPORTING INFORMATION

## The hapten-branched polyethylenimine (PEI) as a new antigen affinity ligand to purify antibodies with high efficiency and specificity

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**KEYWORDS:** Enrofloxacin, polyethylenimine (PEI), affinity purification, sensitivity, specificity.

This **Supporting Information** file includes solutions and buffers, results of NMR, the BLI method and plate layout.

## *1.Solutions and buffers*

**Buffers in column purification section:**

Coupling buffer A: 8.4 g $NaHCO_3$, 29.22 g NaCl, dissolved in 1 L distilled $H_2O$, adjusted pH to 7.4.

Coupling buffer B: 8.4 g $NaHCO_3$, 29.22 g NaCl, dissolved in 1 L distilled $H_2O$, adjusted pH to 7.0.

Blocking buffer: 12.11 g Tris, 1 L $H_2O$, adjusted pH to 8.0 by HCl.

Washing buffer A: 8.2 g NaAc, 29.22 g NaCl, 1 L $H_2O$, adjusted pH to 4.0 by HAc.

Washing buffer B: Blocking buffer containing 29.22 g NaCl, adjusted pH to 8.0 by HCl.

Elution Buffer: 7.5 g glycine, 1 L $H_2O$, adjusted pH to 2.5 by HCl.

Neutralizing buffer:12.11 g Tris, 1 L $H_2O$, adjusted pH to 8.5 by HCl.

**Buffers in ELISA section:**

Coating buffer: Carbonate buffered saline (CBS), 1.59 g $Na_2CO_3$, 2.93 g $NaHCO_3$, dissolved in 1 L distilled $H_2O$, filtered by 0.45 μm filter membrane, adjusted pH to 9.6.

Dilution buffer: Phosphate buffered saline (PBS), 40 g NaCl, 1 g KCl, 14.5 g $Na_2HPO_4 \cdot 12\ H_2O$, 1 g $KH_2PO_4$, 5 L $H_2O$, filtered by 0.45 μm filter membrane, adjusted pH to 7.4.

Washing buffer: PBST, PBS containing 0.05% (v/v) Tween-20.

Blocking buffer: PBST containing 5% (w/v) skim milk powder.

## *2.The results of NMR*

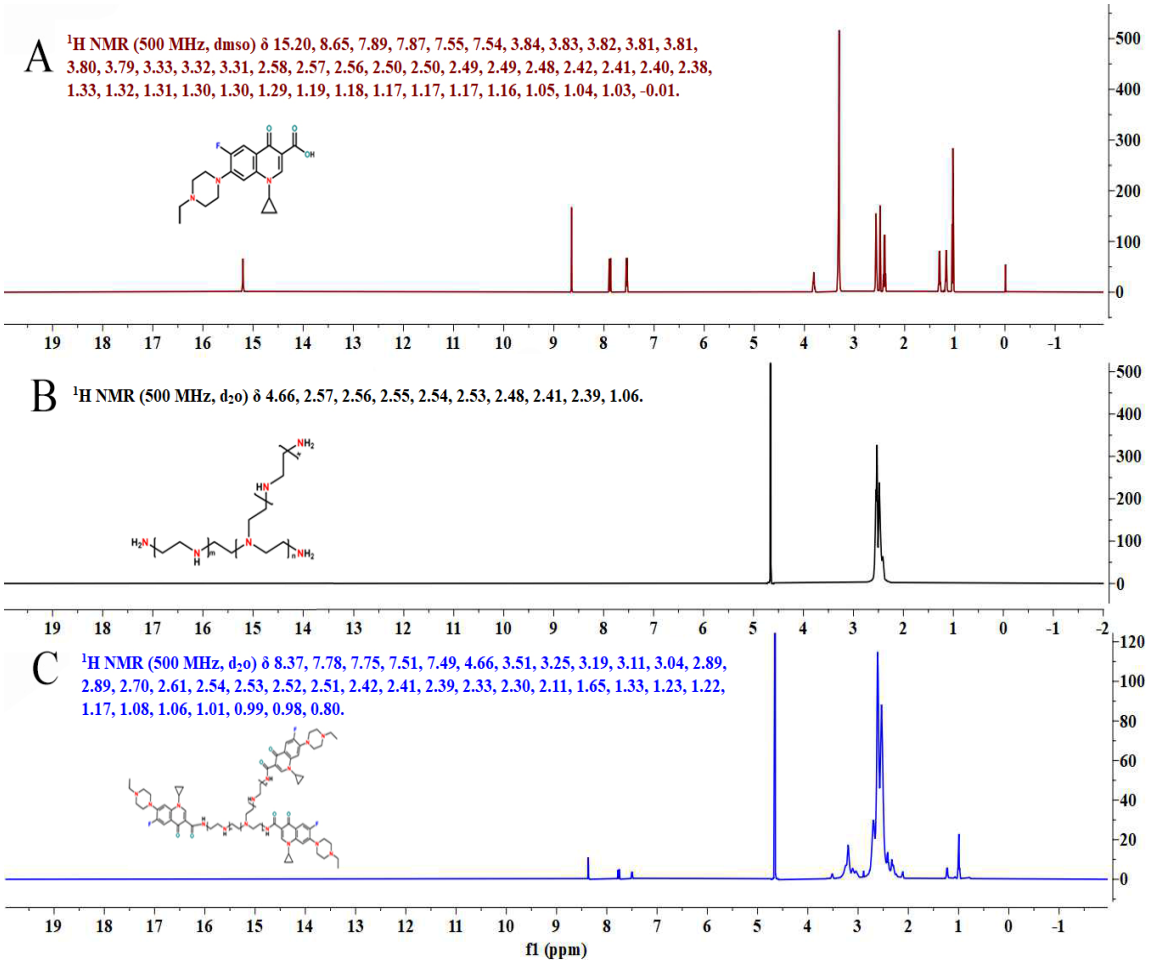


**Figure S1.** The $^{1}$H NMR results of ENR, PEI and ENR-PEI. (A) was the $^{1}$H NMR of the ENR. (B) was the $^{1}$H NMR of the PEI. (C) was the $^{1}$H NMR of the ENR-PEI.

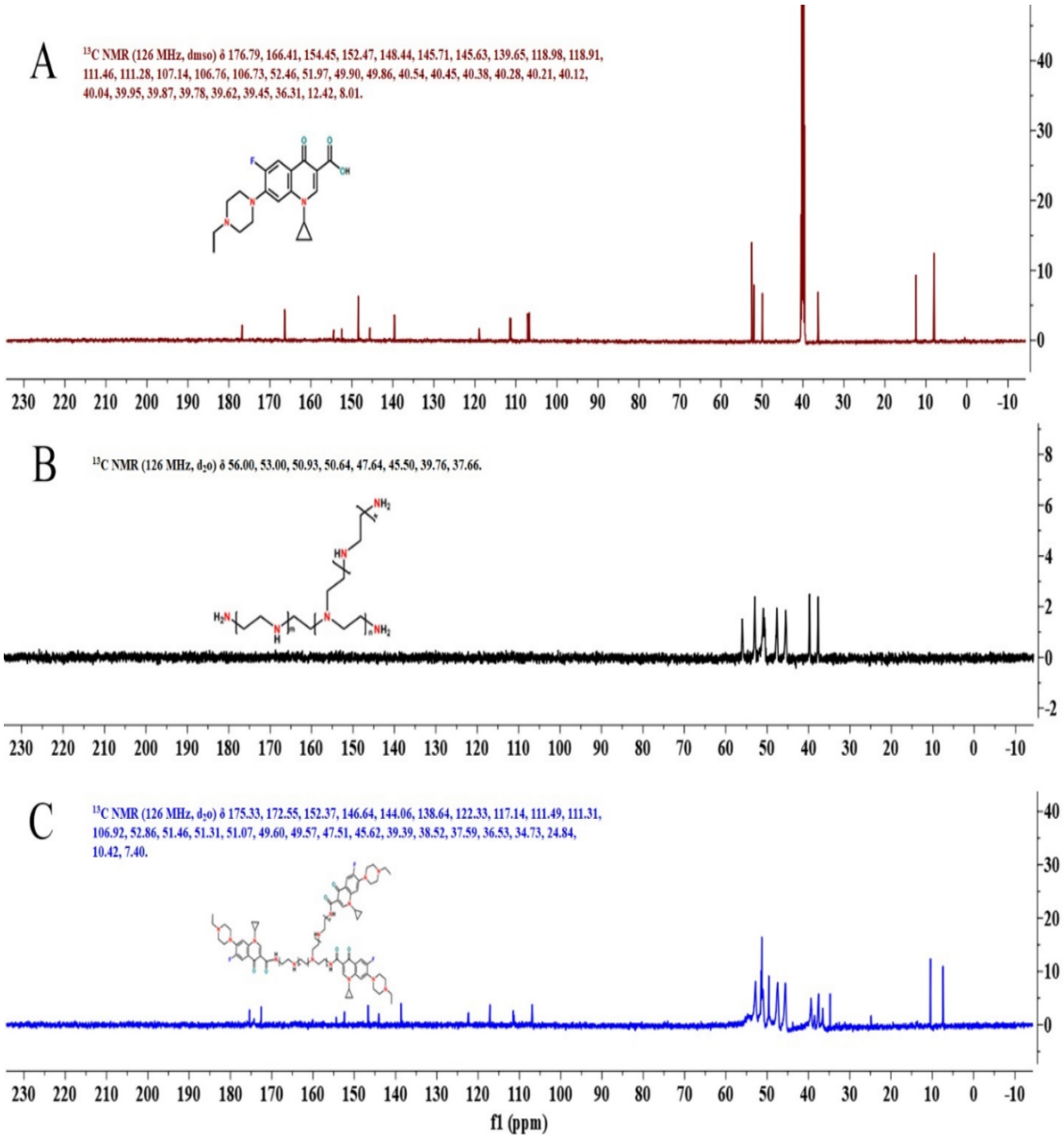


**Figure S2.** The $^{13}C$ NMR results of ENR, PEI and ENR-PEI. (A) was the $^{13}C$ NMR of the ENR. (B) was the $^{13}C$ NMR of the PEI. (C) was the $^{13}C$ NMR of the ENR-PEI.

## *3. Determination of unspecific binding of purified antibody with the carrier by BLI*

To evaluate the specificity of purified antibody, BLI with an Octet® RED96 System was used to determine the combination of purified antibody with OVA and BSA both by ENR-PEI affinity column and protein A to simulate ELISA process. The OVA, BSA were biotinylated according to the instruction of Biotinylation Kit. As shown in **Figure**

**S3** the assay procedure includes five steps: (1) baseline (1 min); (2) loading (3 min); (3) baseline (3 min); (4) association (3 min); (5) baseline (5 min). All the solutions were 0.02% Tween-20 in PBS. The response data obtained from the reaction surface were normalized by subtracting the signal simultaneously acquired from the blank surface to eliminate unspecific binding and buffer-induced interferometry spectrum shift.

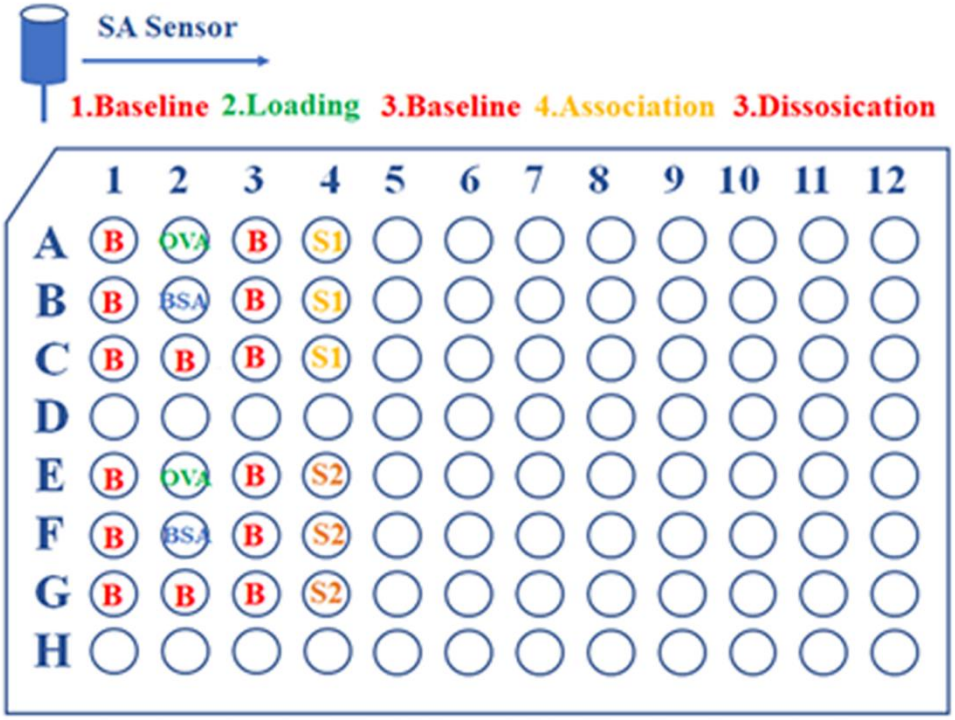


**Figure S3.** BLI technology assay principle process and plate layout. B was Buffer, S1 was the purified antibody by protein A, S2 was the purified antibody by ENR-PEI affinity column.