Supplemental Figures

Fig. S1A, B

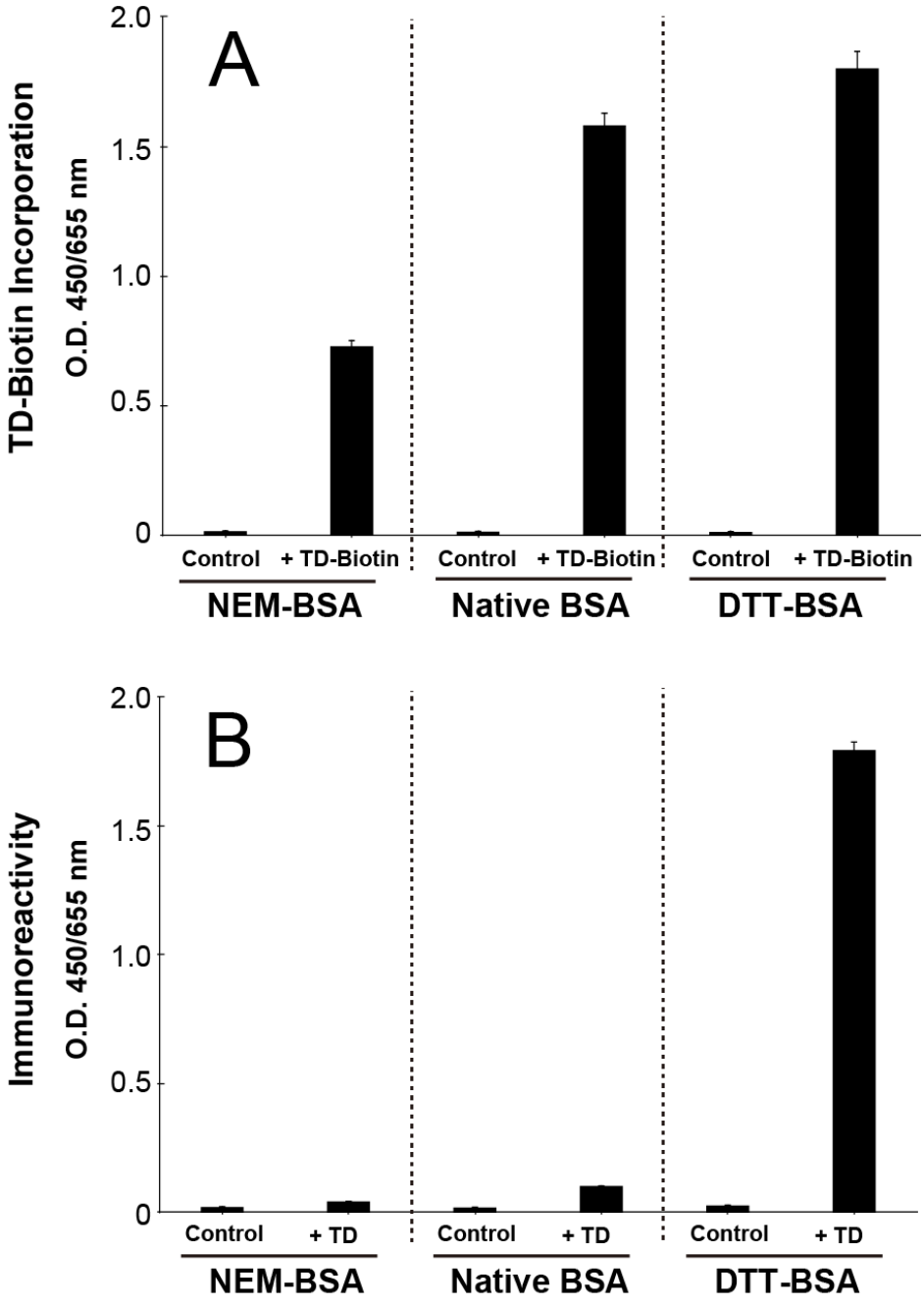

Fig. S2A, B

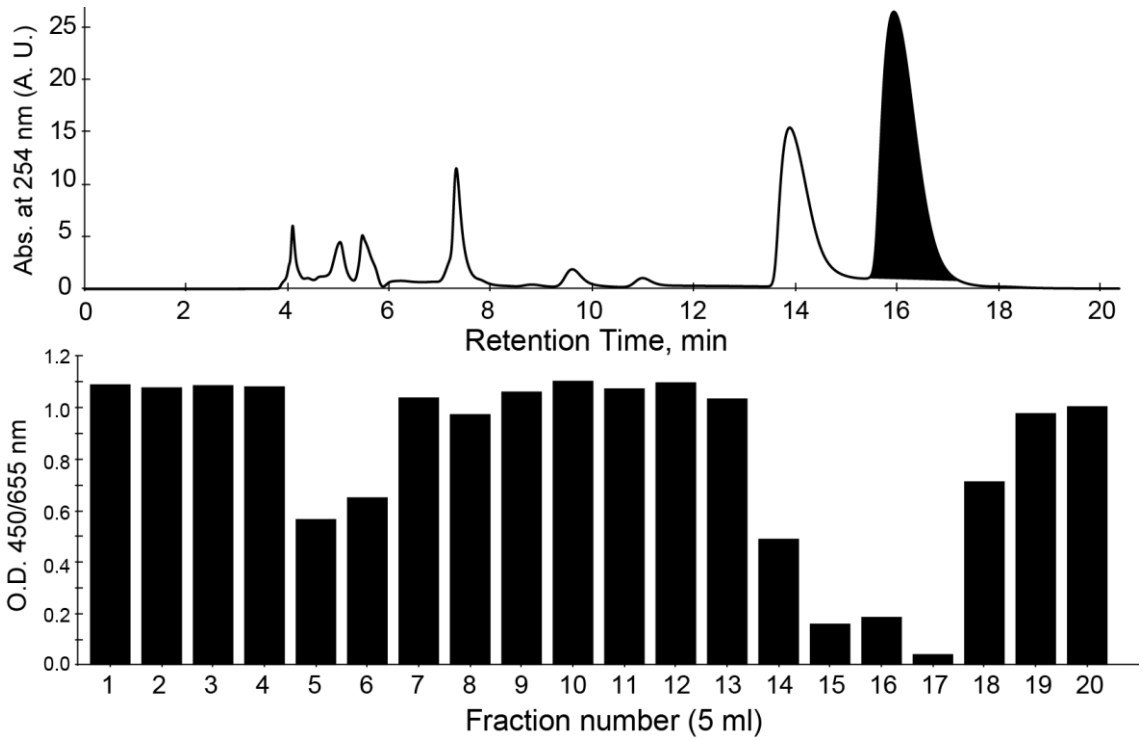
Abs. at 254 nm (A. U.)
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
18
20
Retention Time, min
O.D. 450/655 nm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Fraction number (5 ml)

Fig. S1A, B. Contribution of protein thiols to the adduction of the TD moiety to a protein molecule (A) and antigenicity by exposure to TD (B). N-ethylmaleimide-treated BSA (NEM-BSA) and dithiothreitol-treated-BSA (DTT-BSA) were prepared as described in Materials and Methods. TD and TD-Biotin conjugate were prepared by treatment of serotonin or serotonin-biotin conjugate with Fremy's reagent. Three types of BSA were exposed to TD or TD-Biotin in phosphate buffer for 15 min. As a control, a solvent vehicle was also prepared. The proteins were diluted and coated onto a microtiter plate. The generation of antigenicity was evaluated by using the novel antibody 1B7, followed by the reaction of anti-mouse labeled IgG-peroxidase. The conjugation of the TD moiety, evaluated by incorporation of the biotin moiety into the protein, was evaluated by treatment with streptavidin-peroxidase.

Fig. S2. Identification of the epitope from the reaction mixture of TD and NAC. NAC was incubated with synthetic TD in phosphate buffer. The reaction mixture was injected onto a reversed-phase HPLC column (Combi-RP, 20 × 100 mm) with 0.1% acetic acid in water/15% CH_3CN at a flow rate of 5 ml/min and fractionated into 5 ml portions. The elution was monitored by the absorbance at 254 nm (upper panel). Each fraction was evaporated to remove organic solvent and re-dissolved in PBS. The samples were analyzed by competitive ELISA (lower panel) as described in the Materials and Methods.