Supporting Information

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Title of the submitted article

Dioxin-binding pentapeptide for use in a high sensitivity on-bead detection assay

Abstract

This supporting information includes two figures. Figure S-1 indicates the screening strategy and the schematic of the dioxin detection on resin beads used in this study. Figure S-2 shows the structures of side chains of natural and nonnatural amino acids used in the one-amino acid-substituted library. Figure S-3 shows the dioxin detection abilities of the amino acid substituted derivatives not shown in the text. Figure S-4 shows the results of dioxin detection abilities using dye-changed conjugates from NBD. Figure S-5 shows the results of dioxin detection abilities using the peptide synthesized on the three kinds of resins.

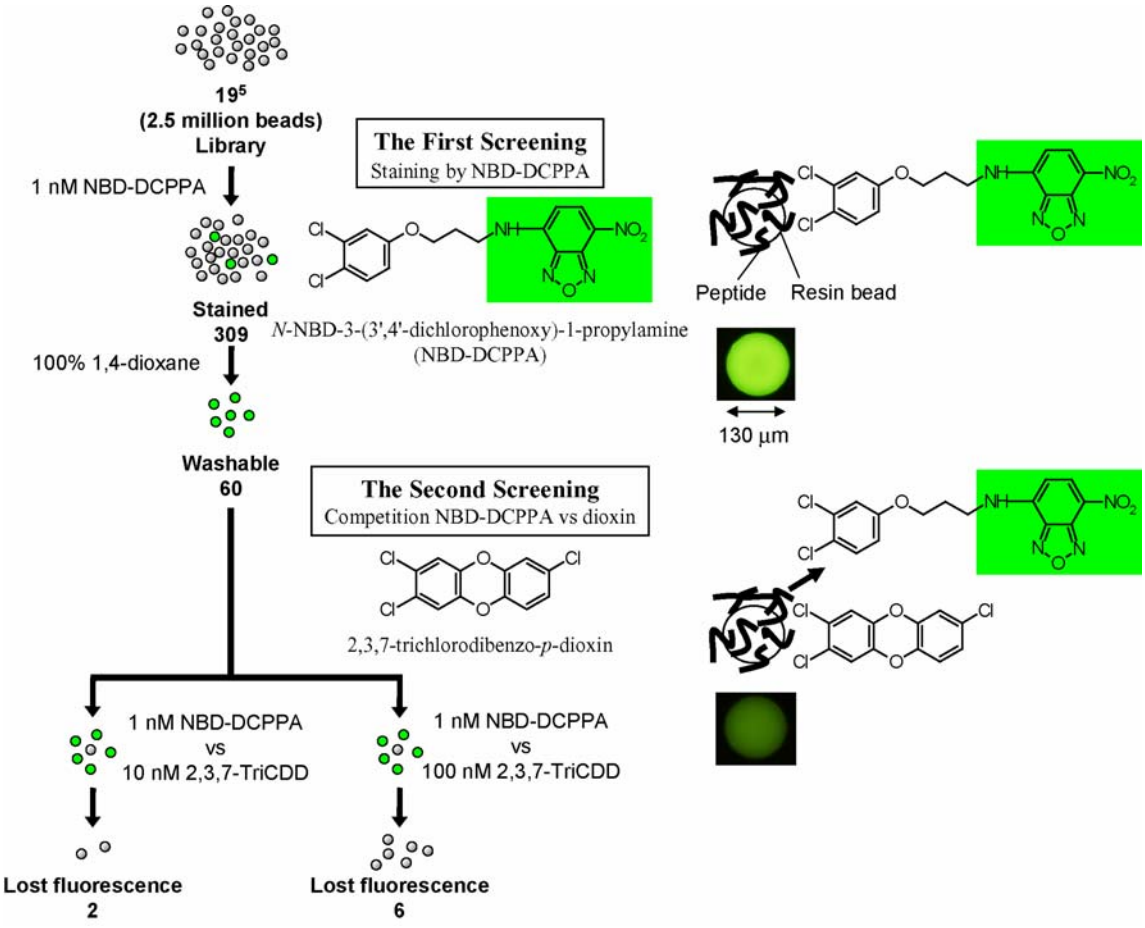

Figure S-1. Screening strategy and fluorescence microscopic dioxin detection on beads using competitive binding of the NBD-DCPPA.

The library screening was performed in 10 mM phosphate buffer (pH 7.4) containing 20 % 1,4-dioxane. The NBD-DCPPAs (*N*-NBD-3-(3′,4′-dichlorophenoxyl)-1-propylamine) and dioxins are bound competitively to the DB2 peptide beads. The fluorescence intensity on the bead decreases with increase of dioxin concentration. The bright bead was stained under conditions containing only 1 nM NBD-DCPPA and the dark bead was stained under competitive conditions against 100 nM 2,3,7-TriCDD

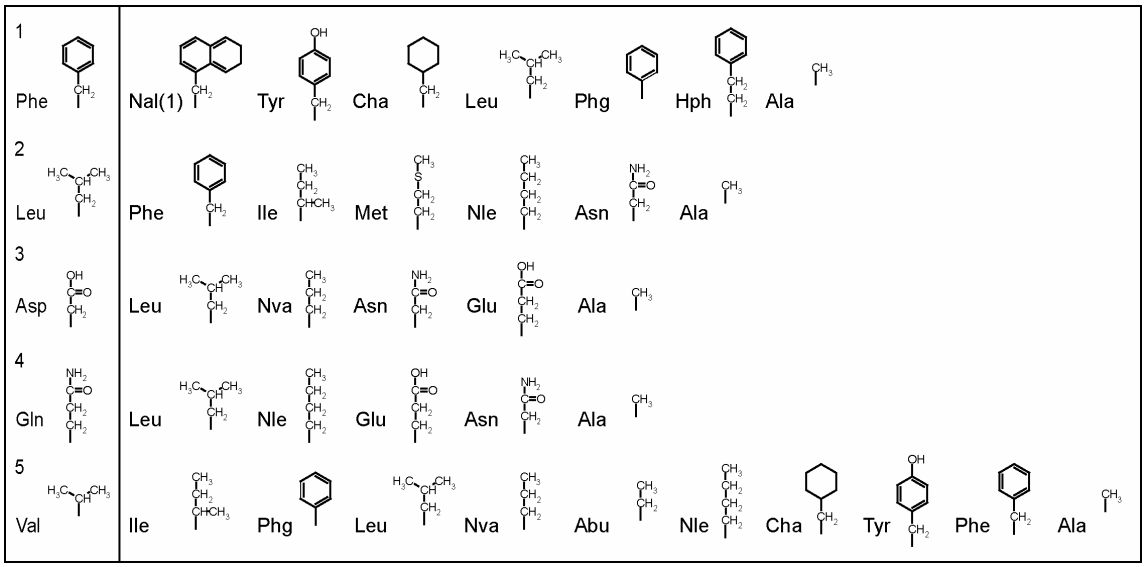

Figure S-2. Structures of the side chains of the substitution amino acids. The structures of the side chains of DB2 are shown in the left column and the corresponding substitutes are shown on the same line in the right column.

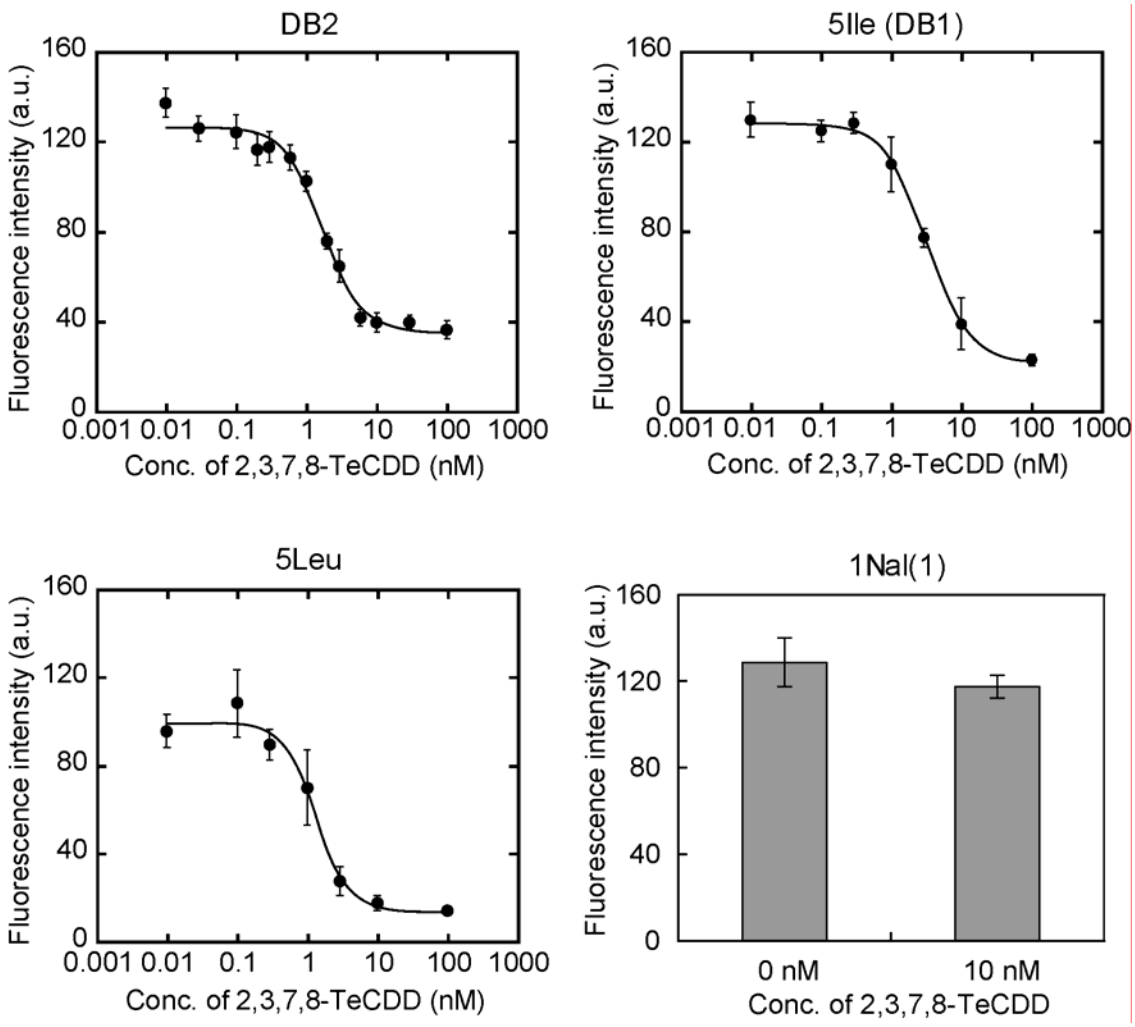

Figure S-3. Calibration curves of competitive binding of 2,3,7,8-TeCDD using the derivative peptides. Each derivative peptide beads were incubated in 1 ml of 10 mM phosphate buffer containing 4 nM NBD-DCPPA and various concentrations of 2,3,7,8-TeCDD under 30 % 1,4-dioxane condition.

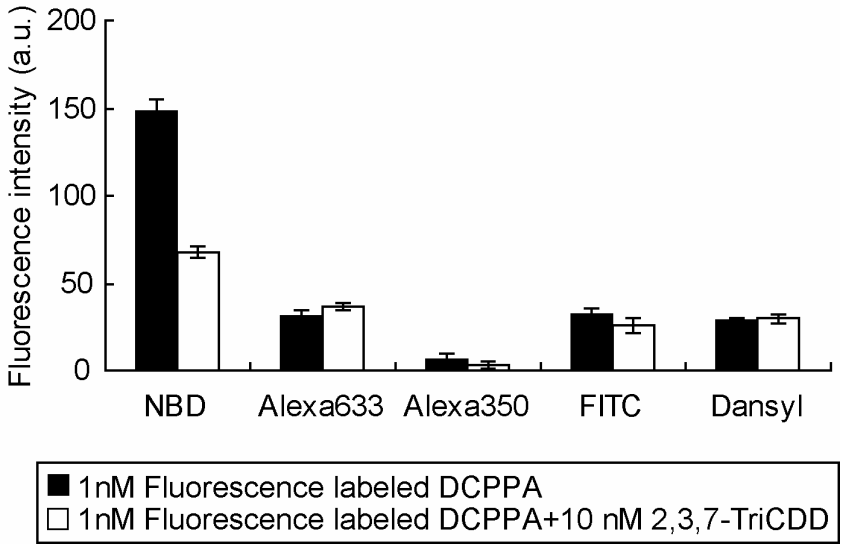

Figure S-4. Effect of change of fluorescent dye on the conjugate. Four kinds of fluorescence labeled DCPPA were synthesized using different fluorescent dyes. DB2 peptide beads were incubated in 1 ml of 10 mM phosphate buffer containing 1 nM fluorescence labeled DCPPA with or without 10 nM of 2,3,7,8-TeCDD under 20 % 1,4-dioxane condition. Only NBD-DCPPA showed competitive binding to 2,3,7-TriCDD to the peptide beads.

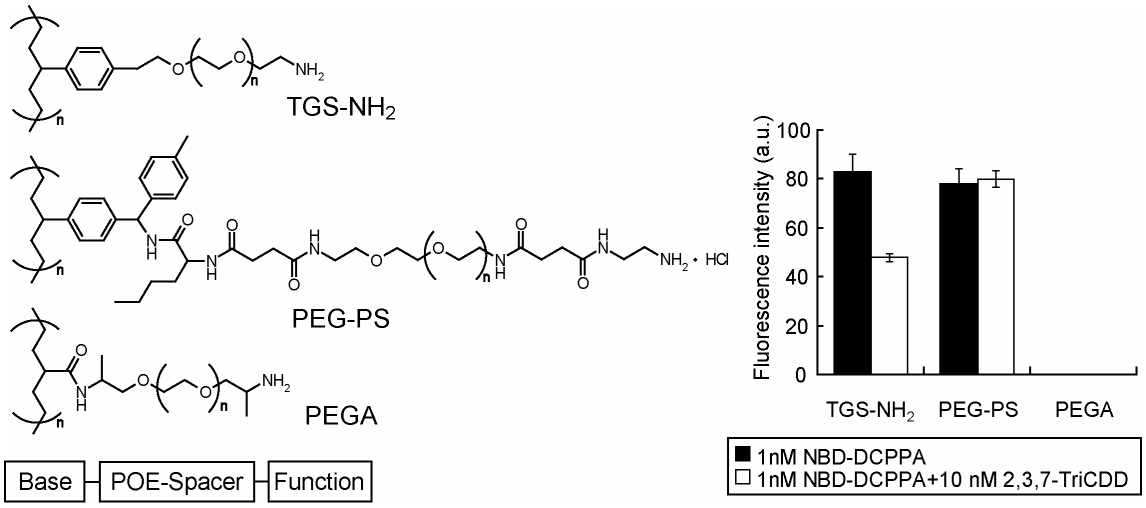

Figure S-5. Effect of change of polymer structure of resin or spacer. DB2 peptide synthesized on resins with different polymer structures or different spacers were prepared. DB2 peptide beads were incubated in 1 ml of 10 mM phosphate buffer containing 1 nM fluorescence labeled DCPPA with or without 10 nM of 2,3,7,8-TeCDD under 20 % 1,4-dioxane condition. Only the peptide synthesized on TGS-NH_2 resin showed dioxin binding ability.