Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Heavy chain single domain antibodies to detect native human soluble epoxide hydrolase

Yongliang Cui, Dongyang Li, Christophe Morisseau, Jie-Xian Dong, Jun Yang, Debin Wan, Martín A. Rossotti, Shirley J. Gee, Gualberto G. González-Sapienza, Bruce D. Hammock

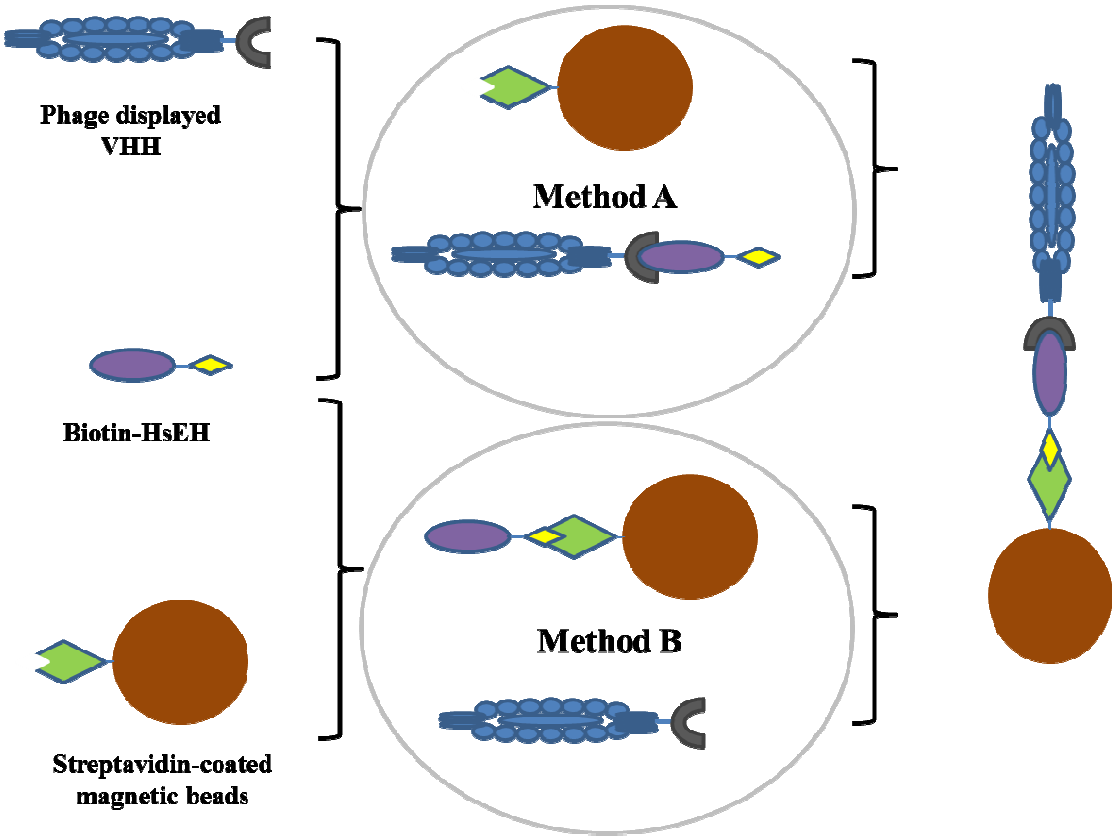

Fig. S1 Schematic illustration of selection methods **A** and **B**. For Method **A**, biotin-HsEH (10 µL, 16 µM) was incubated with phage displaying VHH for 1 h at 4 °C. Then prewashed streptavidin magnetic beads (10 µL) were added and incubated for 15 min at 4°C. For Method **B**, biotin-HsEH preincubated with streptavidin coated magnetic beads (10 µL, 16 µM) were added to the tube containing phage displaying VHH and incubated for 1 h at 4 °C. The tube was then placed on the magnetic particle separator for 15-30 seconds. The supernatant was removed. The pellet was washed with 300 µL of PBB five times

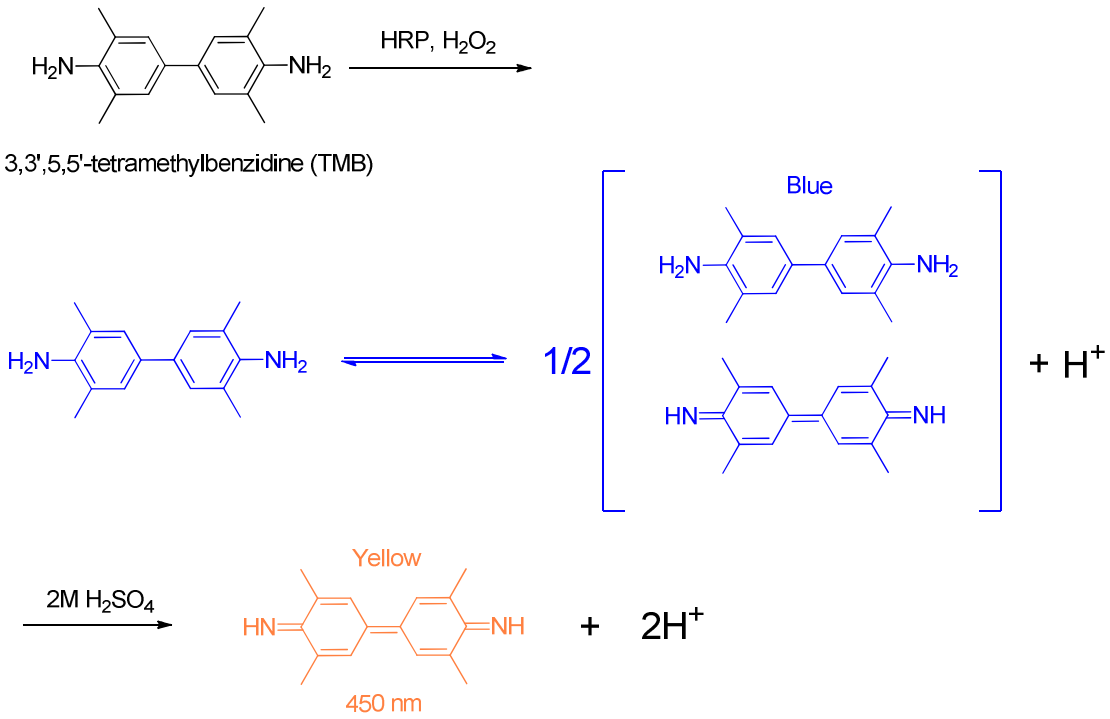

Fig. S2 The chemistry reaction of the color development.
3,3',5,5'-Tetramethylbenzidine (TMB) acts as a hydrogen donor for the reduction of hydrogen peroxide to water by horseradish peroxidase (HRP). The resulting diimine is blue in color. Addition of sulfuric acid turns the color to yellow and is read at 450 nm

Table S1 Sequence of primers used to construct the VHH phage library

Name	Sequence
VH1	CATGCCATGACTCGC*GGCCCAGGCGGCC*ATGGCCCAGGTGCAGCTGGTGCAGTCTGG
VH3	CATGCCATGACTCGC*GGCCCAGGCGGCC*ATGGCCGAGGTGCAGCTGGTGGAGTCTGG
VH4	CATGCCATGACTCGC*GGCCCAGGCGGCC*ATGGCCCAGGTGCAGCTGCAGGAGTCGGG
JH	CCACGATTCT*GGCCGGCCTGGCC*TGAGGAGACRGTGACCTGGGTCC

*Sfi*I restriction sites are in italic and underlined. Forward primers VH1, VH3 and VH4, Reverse primer JH