applied biosciences

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Supplementary Materials: A Simple Biosensor Based on Streptavidin-HRP for the Detection of Bacteria Exploiting HRPs Molecular Surface Properties

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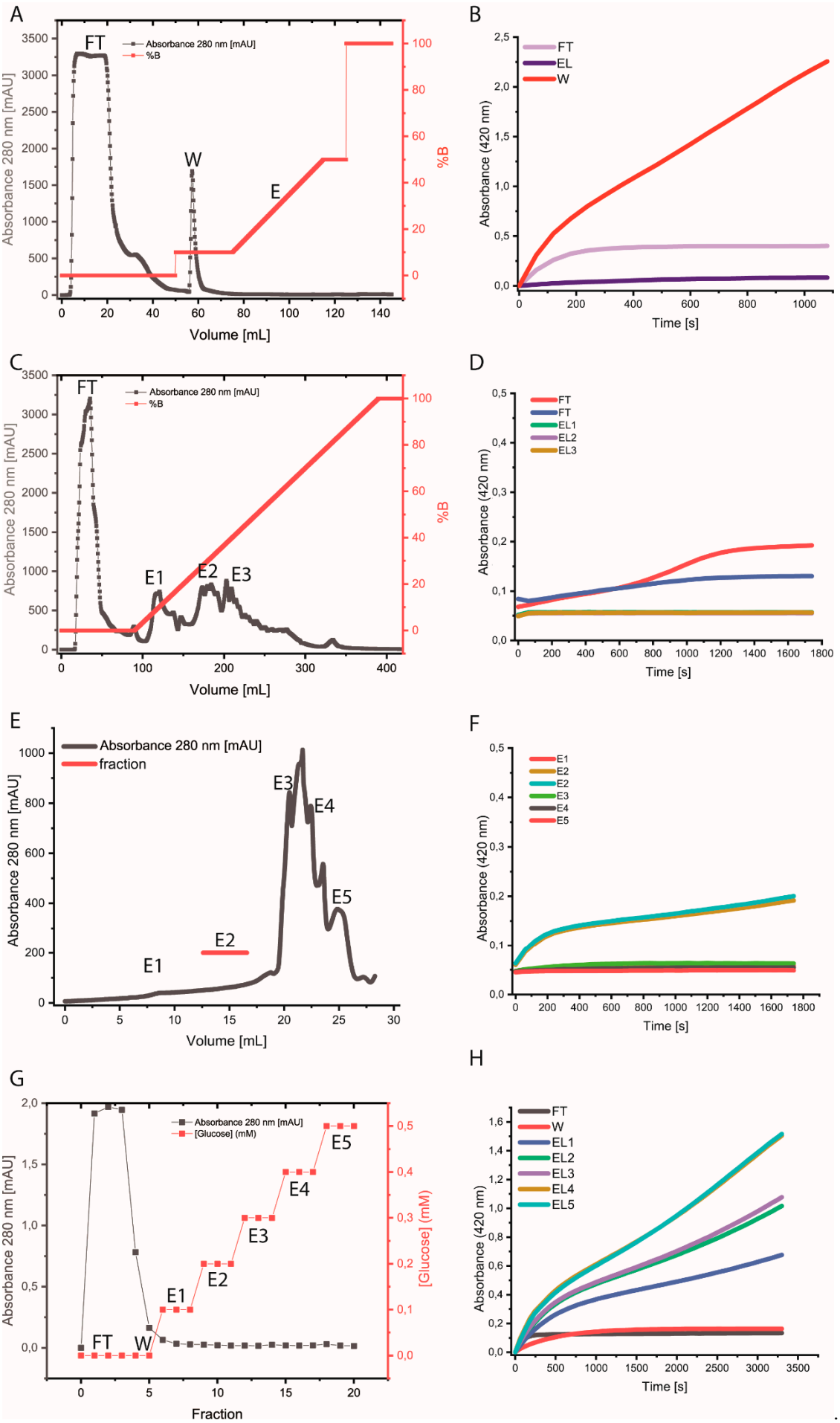
A
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Absorbance 280 nm [mAU]
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Figure S1. Purification of HRP from *P. pastoris.* Chromatograms of different purification methods and the corresponding HRP activity of the fractions are shown.HRP activity was determined by following the formation of the ABTS oxidation product by measuring absorbance. (A and B) Ni-NTA affinity chromatography. HRP supernatant was applied to a HisTrap HP column and eluted with increasing concentrations of imidazole (up to 1 M). Interestingly, HRP is not binding to the column as the flow-through (FT) and wash (W) fractions show peroxidase activity. While no proteins were found in the elution (E) fractions. Although HRP contained a His_6-tag, it was not binding to the column. (C and D) Anion exchange chromatography. Non-tagged HRP was applied on the HiPrepQ HP 16/10 column and a gradient with 1 M NaCl buffer was applied. Also, HRP has an pI of 5.9 it was not binding to the column as peroxidase activity was only observed in the flow-through (FT) fractions and all elution peaks (E1, E2 and E3) showed no activity. (E and F) Size exclusion chromatography. First, non-tagged HRP was precipitated with 80 % ammonium sulphate. The re-suspended HRP was applied on a Superdex 200 Increase 16/10 column. Several fractions were collected and tested for peroxidase activity. HRP did not elute as one peak, but as a shoulder spread over several mililiter (E2).(G and H) Concanvalin A affinity chromatography. Non-tagged HRP was loaded on the Con-A Sepharose® 4B resin and eluted with increasing concentrations of methyl-α-D-glucopyranoside (0.5 M). The glycosylated HRP bound to the column as little to no activity was observed in the flow-through and wash fractions. However, it did not eluted a certain methyl-α-D-glucopyranoside concetration, but was found in various fractions, according to the activity measurement.

Purification method	
Ni-NTA affinity chromatography	~ 0.2 mg
Anion exchange chromatography	~ 0 mg
Size exclusion chromatography	~ 0.2 mg
Concanvalin A chromatography	~ 0.5 mg

Table S1. Protein amount resulting from 100 mL *P. Pastoris* supernatant after purification with different methods.

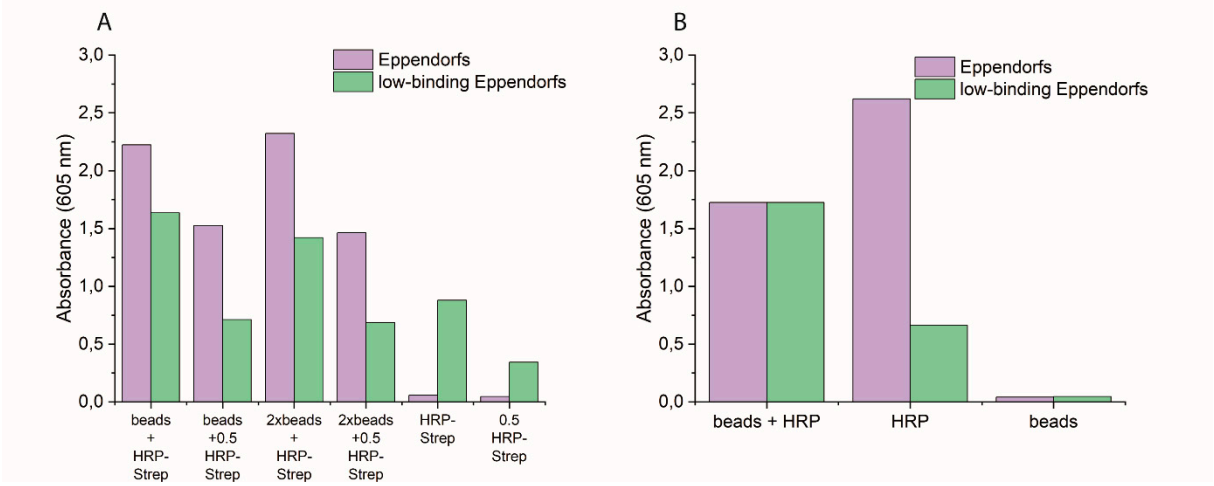

Figure S2. (A) Effect of different SMP and HRP-Strep concentrations tested in normal and protein low-binding Eppendorf tubes. (B) Interaction of HRP to magnetic beads in normal or protein low-binding Eppendorf tubes.

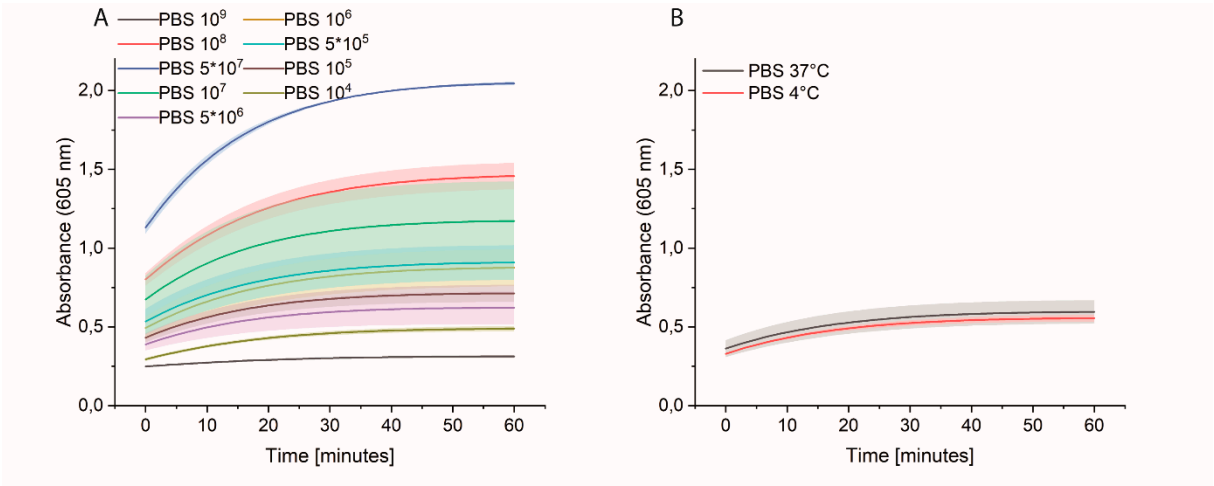

Figure S3. Effect of different *E. coli* BL21 (DE3) concentrations on the interaction of HRP-Strep with bacteria in Eppendorf tubes (A) pre-incubation of bacteria in PBS (B) control without bacteria in PBS.

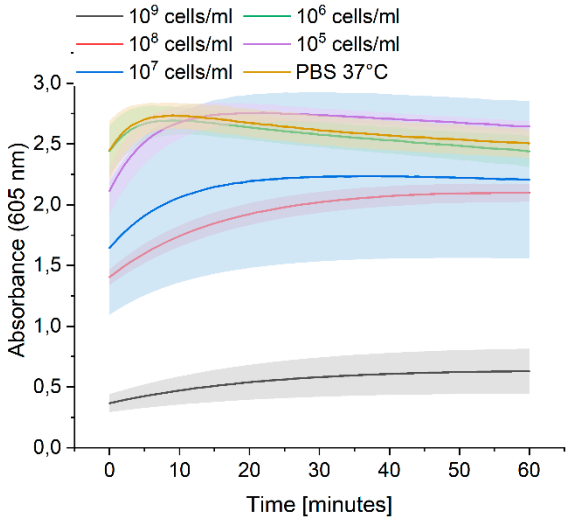

Figure S4. Effect of different *E. coli* BL21 (DE3) concentrations on the interaction of HRP with bacteria in Eppendorf tubes.

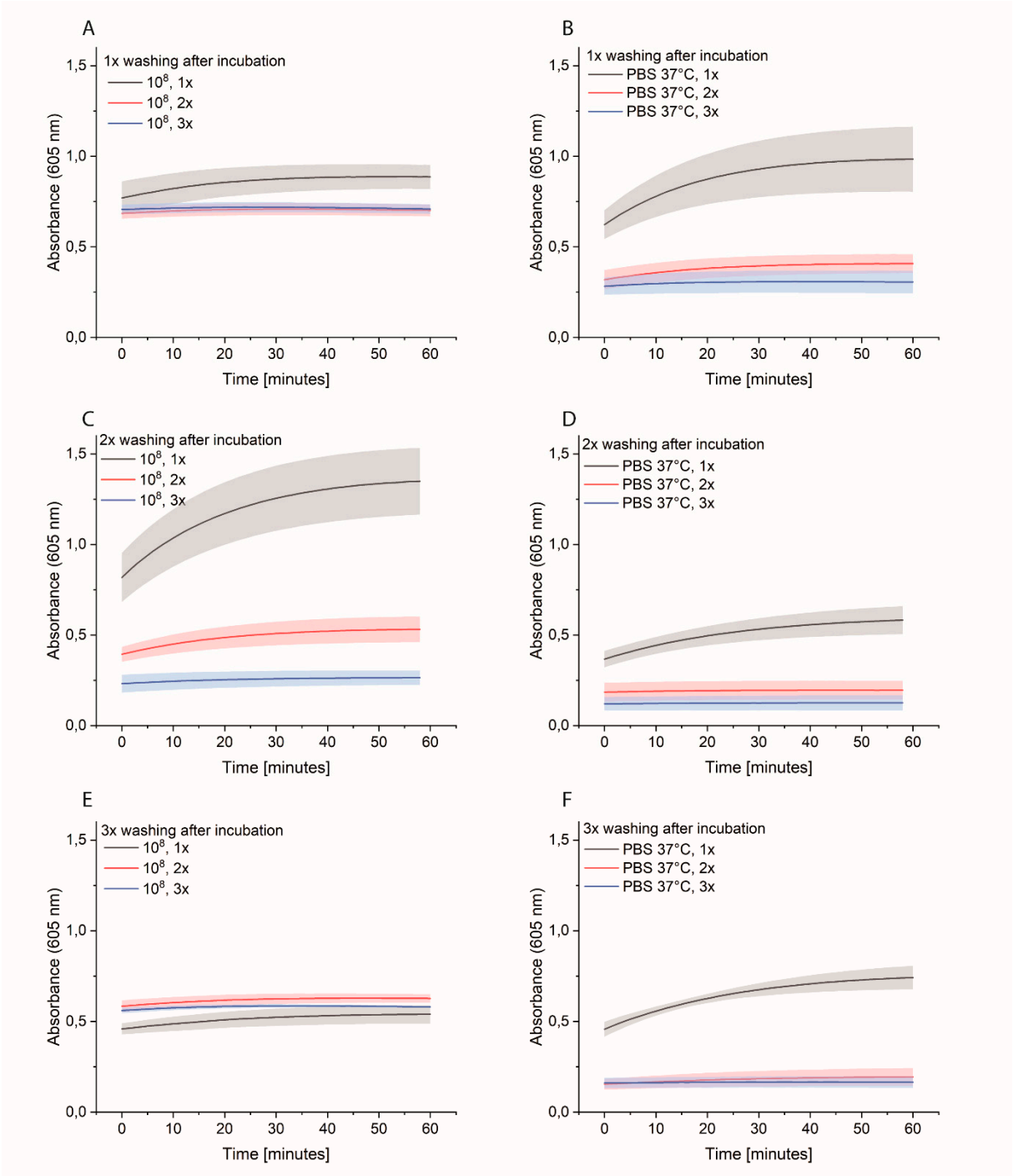

Figure S5. Effect of different washing steps on HRP-Strep binding tested in Eppendorf tubes. (A and B) After 3-hour incubation step, *E. coli* Bl21 (DE3) were washed once, washing step after HRP-Strep incubation was varied from 1x to 3x 1 mL PBS. (C and D) After 3-hour incubation step, bacteria were washed twice, washing step after HRP-Strep incubation was varied from 1x to 3x 1 mL PBS. (E and F) After 3-hour incubation step, bacteria were washed three times, washing step after HRP-Strep incubation was varied from 1x to 3x 1 mL PBS.

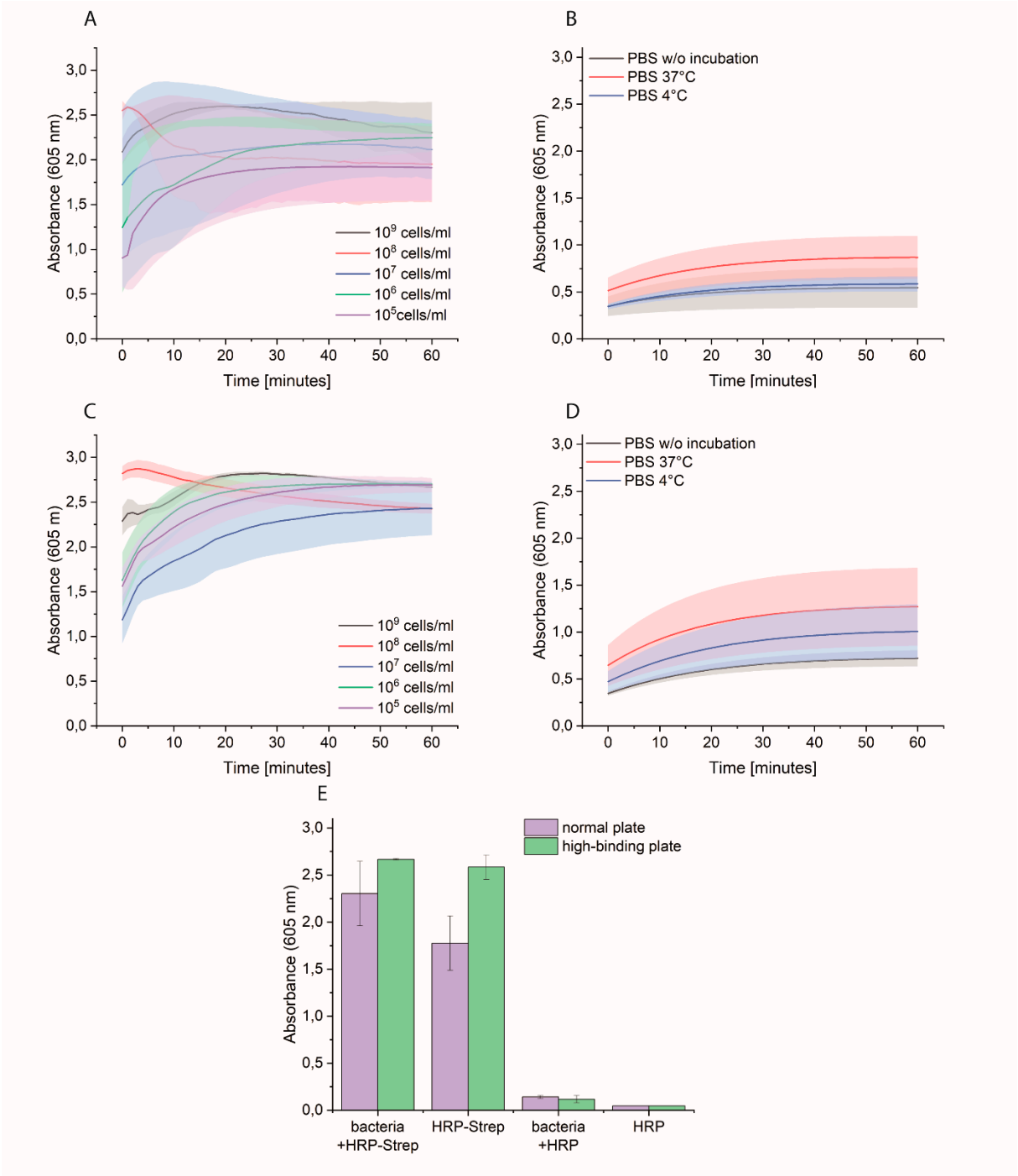

Figure S6. Effect of different *E. coli* BL21 (DE3) concentrations on the interaction of HRP-Strep with bacteria. A) *E. coli* in different 96-well plates (A and C) experiment performed in standard 96 well plates. (B and D) experiment performed in high-binding 96 well plates. (E) comparison of normal and high-binding plates. .

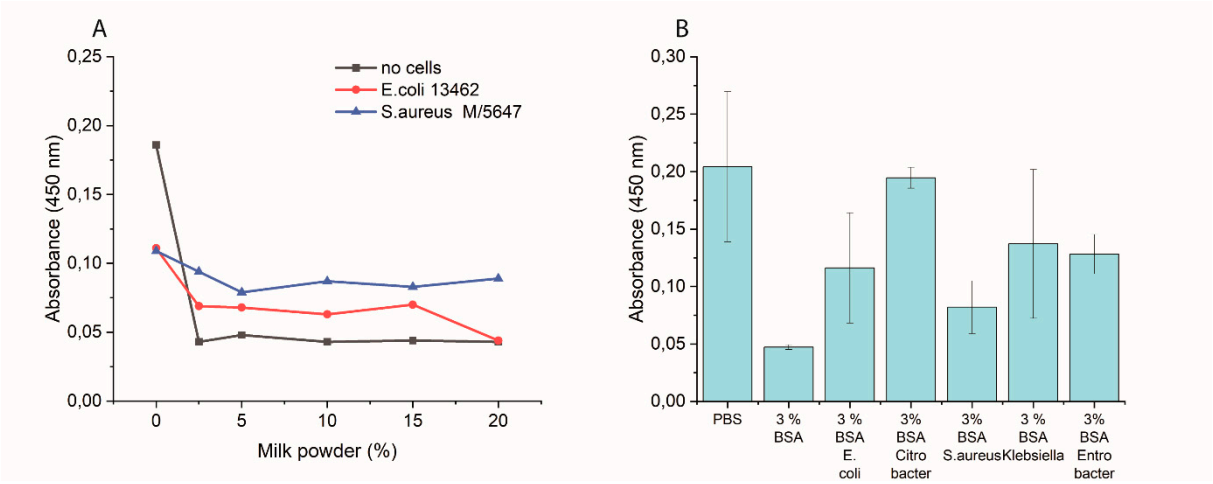

Figure S7. (A) Effect of different milk powder concentrations on the binding behavior of HRP-Strep to the high-binding 96-well plate in presence of E. coli 13462 or S. aureus M/5647 cells immobilized at OD_{600} 0.5. (B) HRP-Strep binding to different bacterial strains in high-binding 96 well microplates blocked with 3 % BSA.

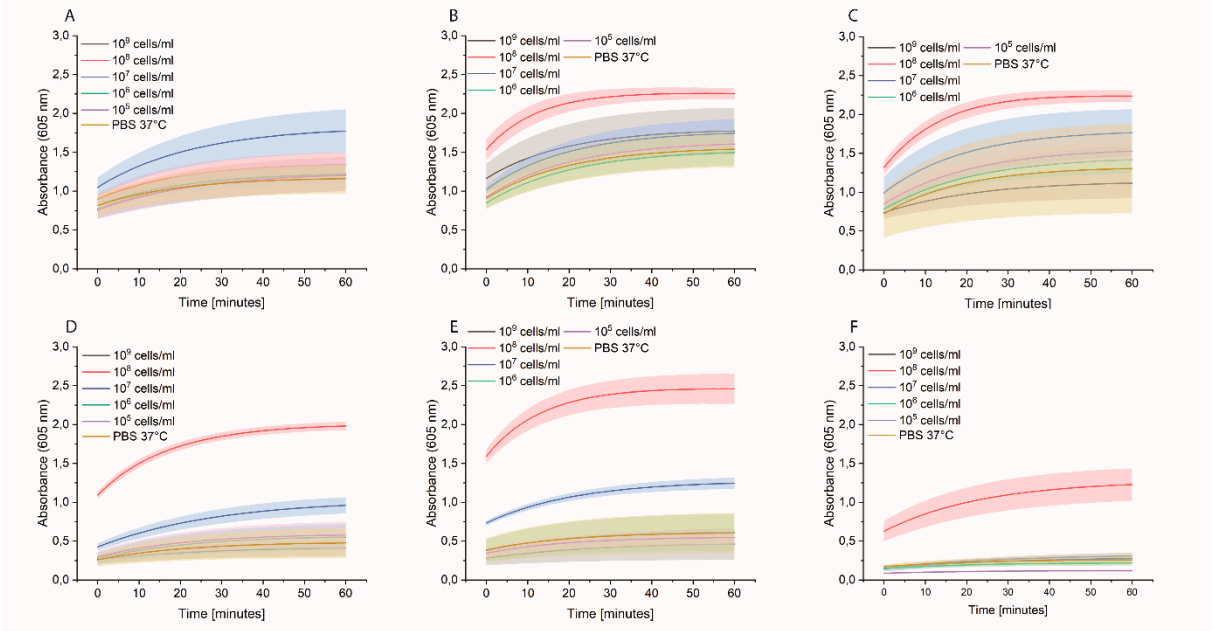

Figure S8. HRP-Strep binding to different bacterial strains using the nonmodified method with less washing steps and longer incubation steps described in the experimental section, tested in Eppendorf tubes. (A) *E. coli* M/11407-3. (B) *K. pneumoniae* DSM 3104. (C) *Salmonella* entericia ATCC 19430 (D) *Enterococcus durans* DSM 20633. (E) *Citrobacter freundii* 73489 (F) *Enterobacter cloacae* DSM 30054.