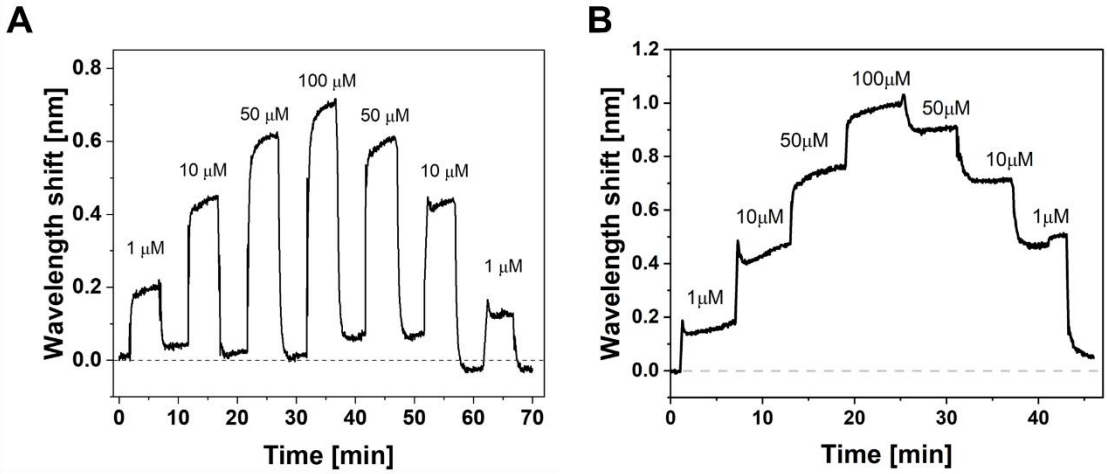
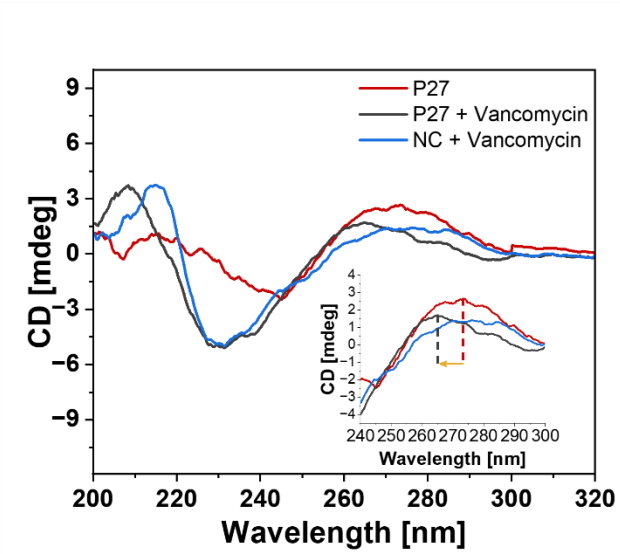
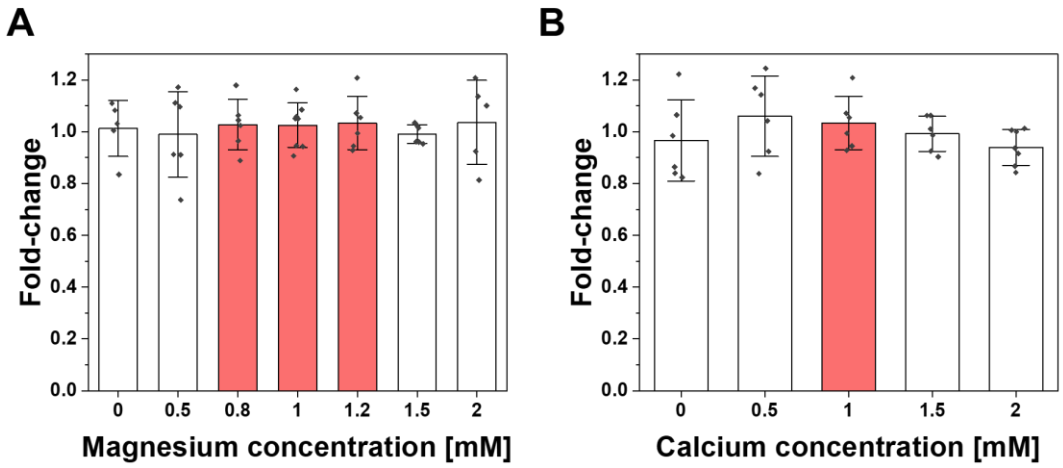
SUPPLEMENTARY INFORMATION



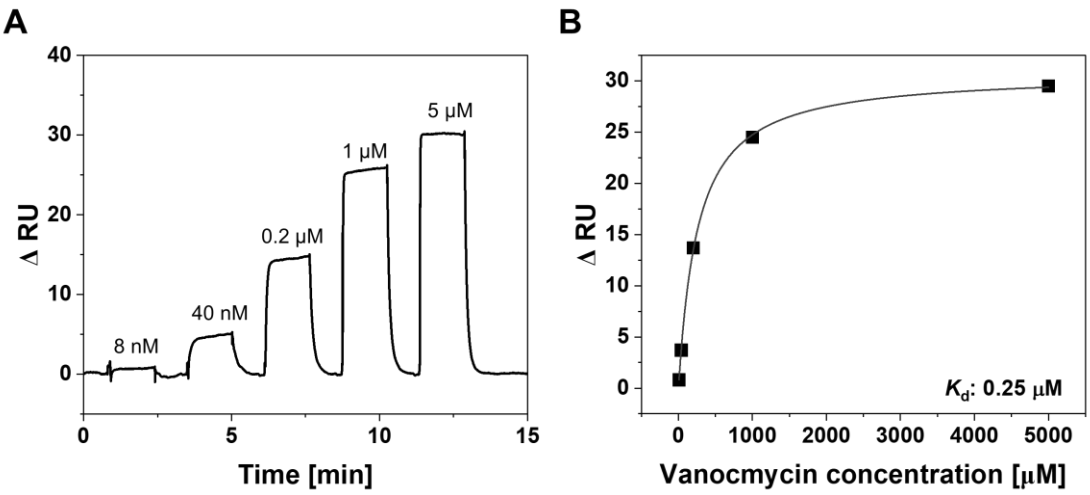
Supplementary Figure 1. Real-time monitoring of vancomycin using P27 split-aptamer. Sensorgrams showing SPR responses to different vancomycin concentrations, (A) each followed by a buffer wash step or (B) gradual increase and decrease without wash steps. The graph demonstrates the sensor's sensitivity and reversibility across a range of vancomycin concentrations.



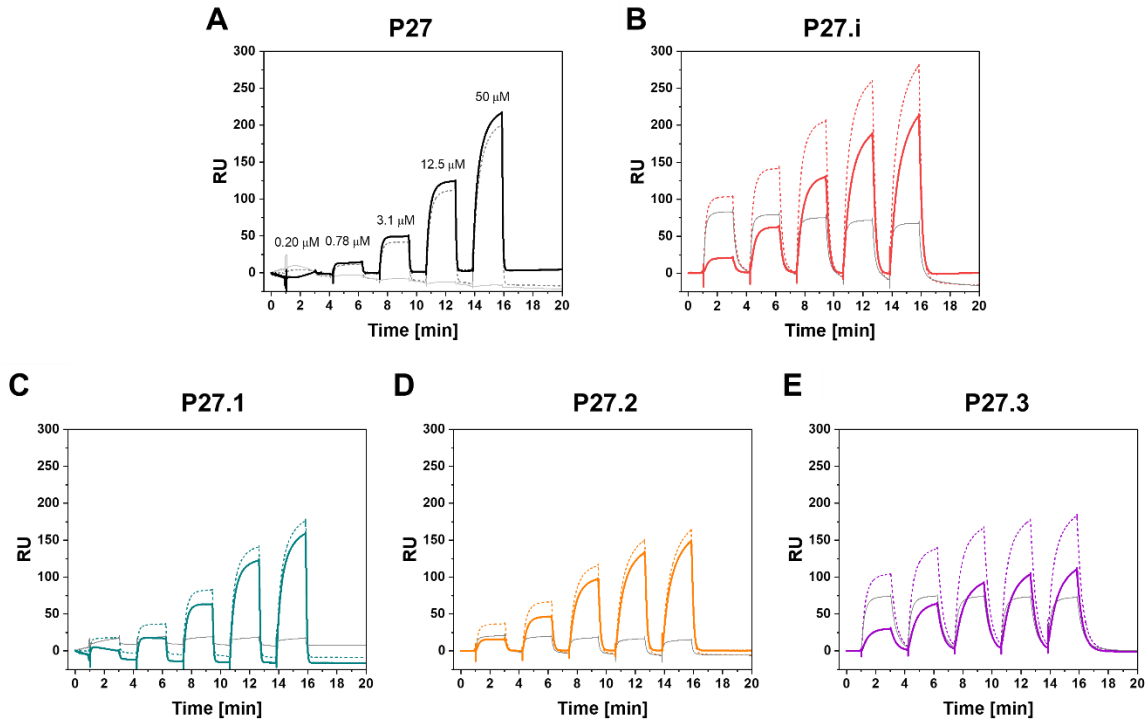
Supplementary Figure 2. Circular dichroism (CD) evaluation of split-aptamer structure upon vancomycin binding. CD spectra of P27 split-aptamer in the absence (red) and presence (black) of vancomycin. A similar peak shift in the 250–290 nm region is observed (yellow dashed lines and arrow) as reported for the full original aptamer.[6] Vertical dashed lines indicate the peak shift positions. NC (blue) represents the negative control using a randomized aptamer sequence, demonstrating no significant spectral change upon vancomycin addition. This confirms the specificity of the vancomycin-induced structural change in the P27 split-aptamer.



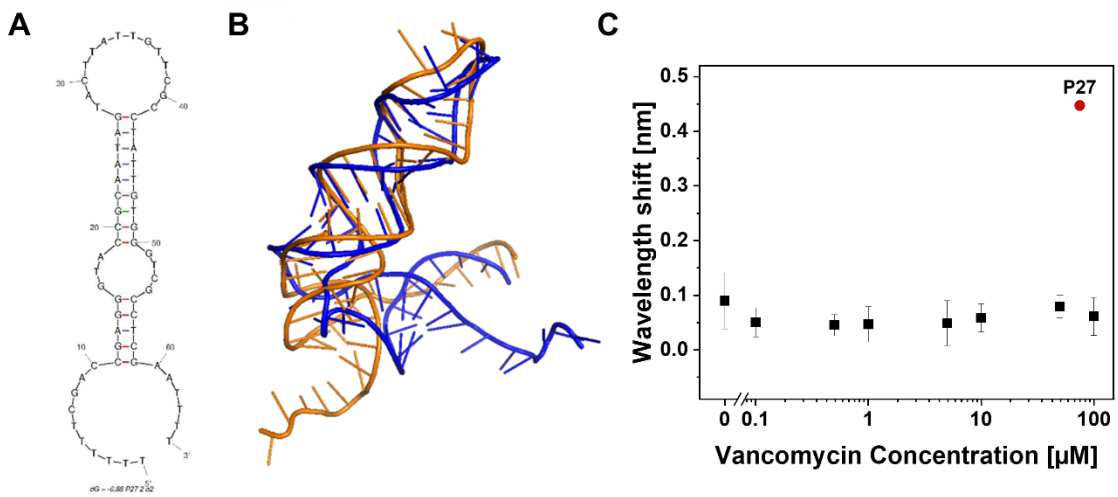
Supplementary Figure 3. Normalized response of the aptamer pair to 50 µM vancomycin in aCSF with varying concentrations of (A) magnesium and (B) calcium. Values are normalized to the response at 1.2 mM magnesium and 1 mM calcium, respectively. Highlighted areas represent the physiologically reported ranges. Error bars represent standard deviation.



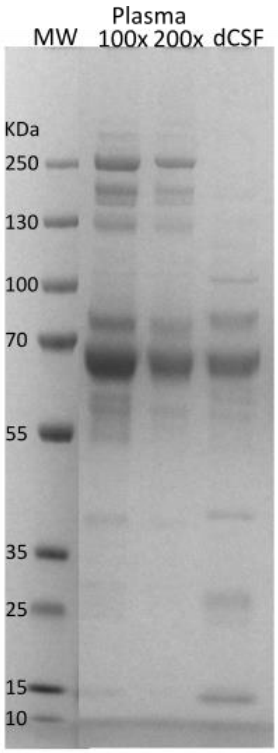
Supplementary Figure 4. Vancomycin sensing using the full-length aptamer under flow conditions in Biacore T200. (A) Full sensorgram showing SPR responses to increasing vancomycin concentrations. (B) Corresponding dose-response curve fitted with the Hill equation. The apparent K_d derived from this fit is 0.25 µM.



Supplementary Figure 5. Full sensorgrams from Biacore T200 flow experiments for split-aptamer variants. (A) P27, (B) P27.i, (C) P27.1, (D) P27.2, and (E) P27.3. Each graph shows two cycles: reference cycle without vancomycin (grey line), cycle with varying vancomycin concentrations (colored-dashed lines), and the subtracted response (solid-colored lines). Vancomycin concentrations, labeled in (A), are consistent across all experiments.



Supplementary Figure 6. (A) mfold prediction of the secondary structure for the loss-of-function modified aptamer pair (P27.2.o2), represented as a full-length molecule. (B) 3D visualization comparing the P27.2.o2 (orange) and P27 pair with 6-thymine extension (blue). PyMOL was used for the 3D visualization, while RNAfold and RNAcomposer were used to predict the secondary structure. Note that this is an RNA representation of a DNA molecule. C) Dose-response of the modified aptamer pair at various vancomycin concentrations performed at steady-state. The red dot indicates a single measurement using the P27 and 75 µM of vancomycin for comparison.



Supplementary Figure 7. Protein profile comparison of rat plasma and dog CSF. SDS-PAGE analysis proves that the 200-fold diluted rat plasma exhibits a protein profile similar to that of dog CSF, validating its use as a CSF surrogate in long-term stability studies. Lanes show: (1) molecular weight marker, (2) rat plasma diluted 100x in aCSF, (3) rat plasma diluted 200x in aCSF, and (4) undiluted dog CSF.

SUPPLEMENTARY TABLES

Supplementary Table 1. DNA sequences of aptamers and their variants used in this study. The table includes: full-length original aptamer, split-aptamer segments (P27, P27.i, P27.1, P27.2, P27.3), and negative control sequences. Modifications such as biotinylation, base substitutions, and stem extensions are indicated. All sequences are listed in 5' to 3' orientation. Nucleotide positions are numbered according to the original full-length aptamer sequence.

Aptamer strand name	**Aptamer sequence**
Full-length aptamer	5BioTEG CGACC GAGGG TACCG CAATA GTACT TATTG TTCGC CTATT GTGGG TCGGG TCG
P16 biotinylated immobilized strand	5BioTEG CGACC GAGGG TACCG C
P16 free flowing strand	AATA GTACT TATTG TTCGC CTATT GTGGG TCGGG TCG
P18 biotinylated immobilized strand	5BioTEG CGACC GAGGG TACCG CAA
P18 free flowing strand	TA GTACT TATTG TTCGC CTATT GTGGG TCGGG TCG
P27 biotinylated immobilized strand	5BioTEG CGACC GAGGG TACCG CAATA GTACT TA
P27-6T spacer biotinylated immobilized strand	5Biosg **TTTTTT** CGACC GAGGG TACCG CAATA GTACT TA
P27 free flowing strand	TTG TTCGC CTATT GTGGG TCGGG TCG
P27.i free flowing strand	TTG TTCGC CTATT G**C**GGG TCGGG TCG
P27.1 free flowing strand	TTG TTCGC CTATT GTGGG TCGGG TCG **A**TTTTT
P27.2 free flowing strand	TTG TTCGC CTATT GTGGG TCGGG TCG **AA**TTTT
P27.3 free flowing strand	TTG TTCGC CTATT GTGGG TCGGG TCG **AAA**TTT
P27.2.o2 free flowing strand	TTG TTCGC CTATT GTGGG TCG**CC** TCG AATTTT
Negative control (CD experiment)	AAAAAAAAAA GGG AGT CAA GAA C