Supplementary Information

Affimer-based impedimetric biosensors for fibroblast growth factor receptor 3 (FGFR3): a novel tool for detection and surveillance of recurrent bladder cancer

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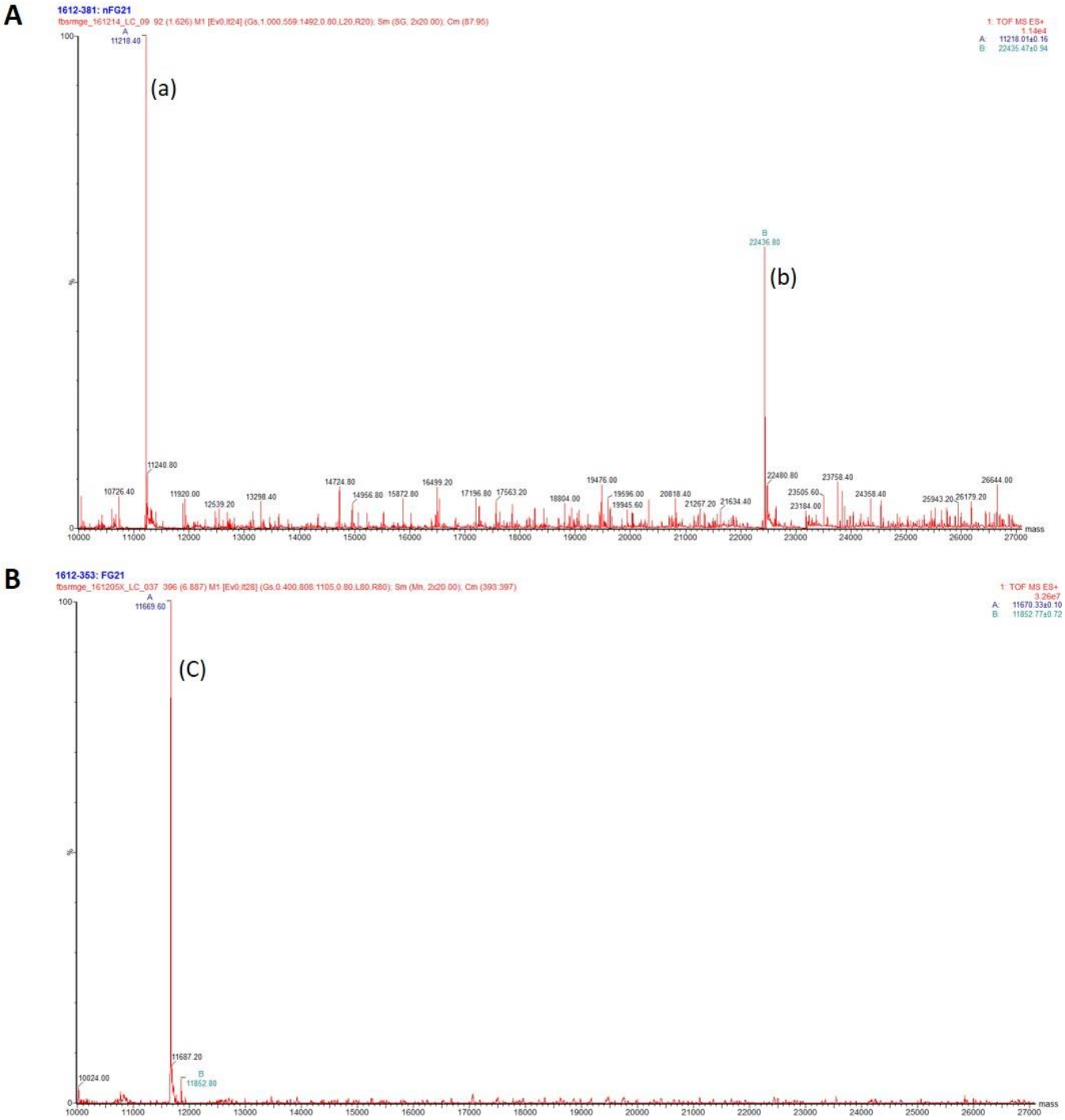


Figure S1 LC-MS spectra showing the successful biotinylation of FGFR3-21 Affimer proteins. (A) non-biotinylated FGFR3-21 Affimers showed two distinct peaks, which represent (a) monomeric forms and (b) dimeric forms. (B) biotinylated FGFR3-21 Affimers showed a peak representing (c) biotinylated monomeric forms.

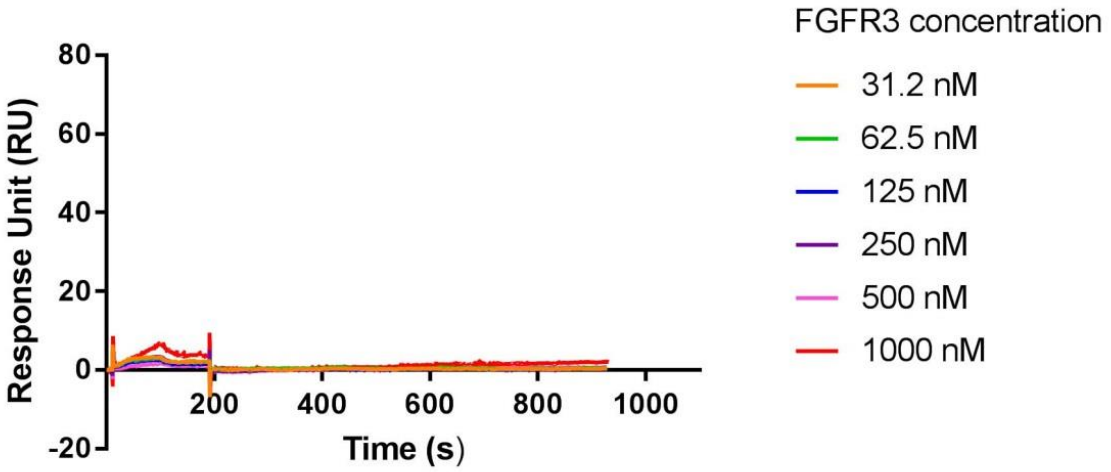


Figure S2 SPR sensorgram of FGFR3-8 Affimer interaction with FGFR3 protein. The FGFR3-8 Affimers were immobilised onto a streptavidin-coated sensor chip before being challenged by FGFR3 at concentrations from 0 to 1000 nM. In this case, the association phase was run for 180 s, followed by 900 s of the dissociation phase.

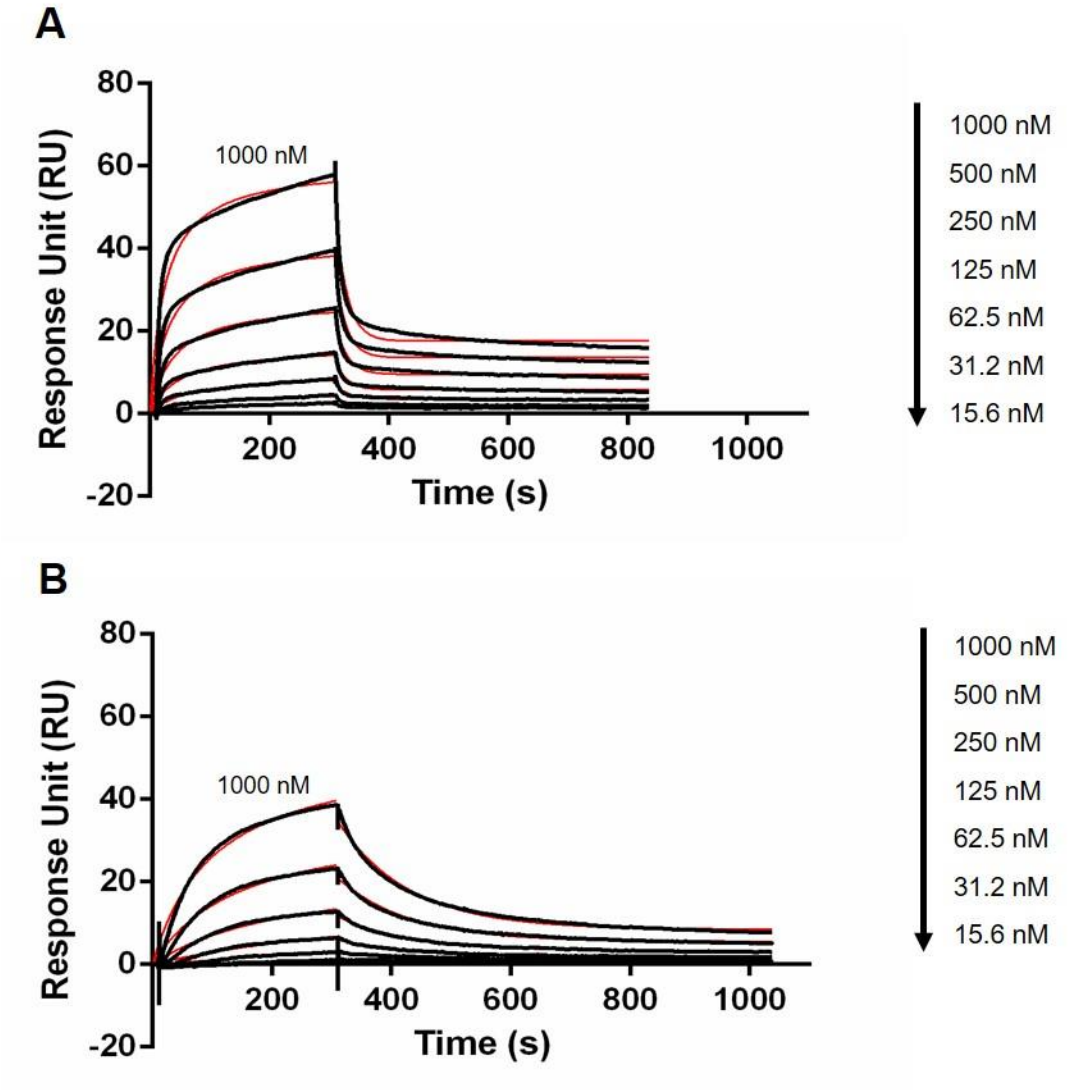


Figure S3 SPR data fitting with a one-site specific model. (A), fitting for FGFR3-14 Affimer and (B), for FGFR3-21 Affimer. The black lines represent experimental data from SPR whilst the red lines show the fitting results which are overlaid. The data from association phase were fitted with a one-site specific binding model whereas the data from dissociation phase was analysed using a one-phase decay exponential model.

Table S1 Fitting SPR data from a one-site binding model. Parameters were determined from the data in Figure S3.

Affimer clones	FGFR3-14	FGFR3-21
$\mathbf{k_{on}}$ **(M^{-1} s^{-1})**	1.60×10^{8}	4.49×10^{8}
$\mathbf{k_{off}}$ **(s^{-1})**	5.24×10^{-2}	8.31×10^{-3}
$\mathbf{K_D}$ **(M)**	3.27×10^{-10}	1.85×10^{-11}
$\mathbf{R^2}$	0.955	0.976
$\boldsymbol{\chi^2}$	1.610	1.071

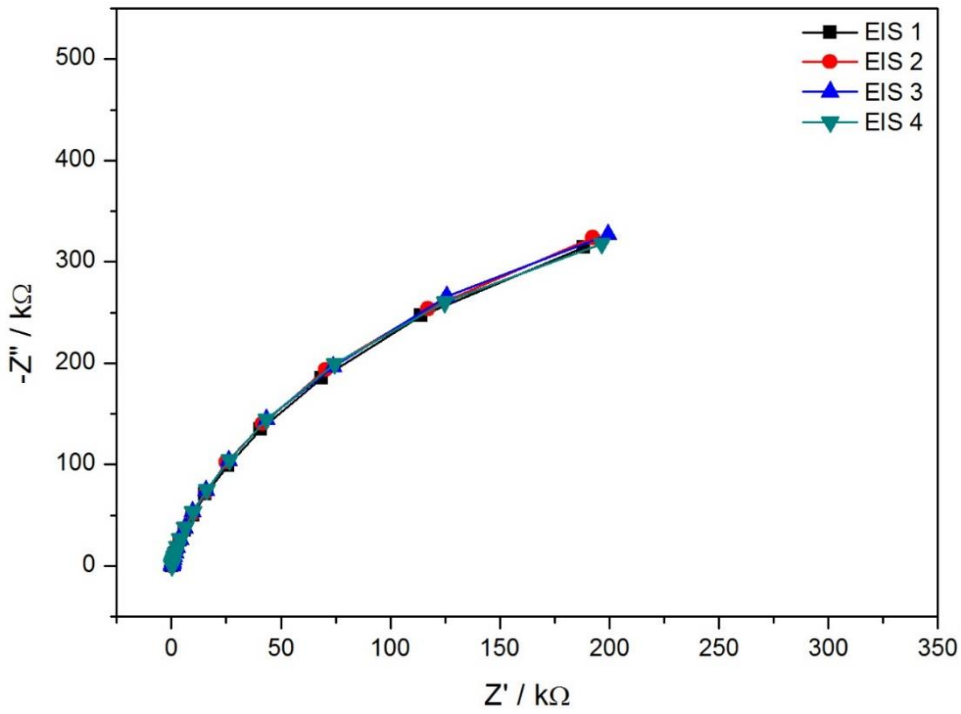
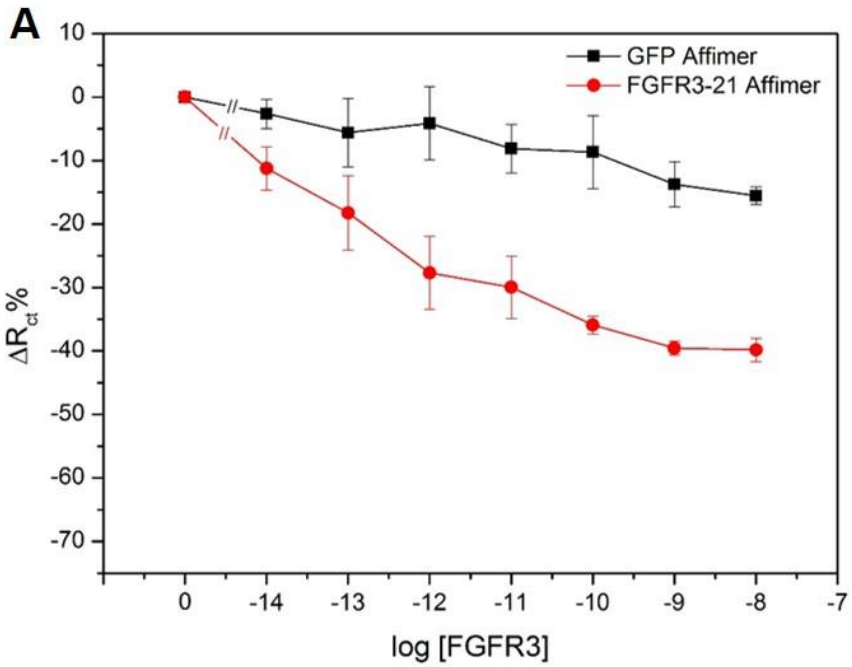
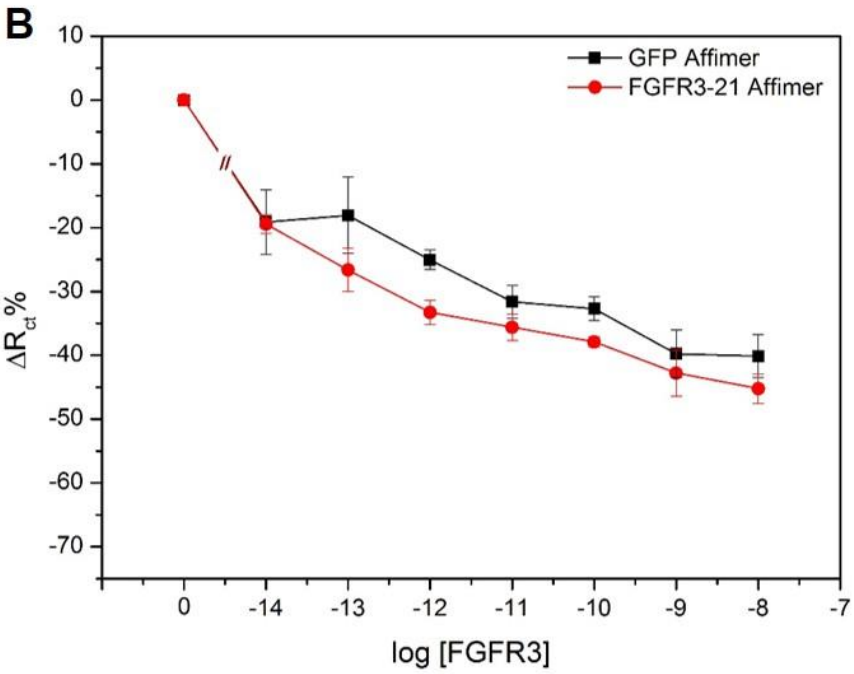


Figure S4 Nyquist plots showing the stability of baseline before a sensor chip was tested with FGFR3 protein. Four consecutive impedance measurements were performed after the sensor was incubated in 100 mM PBS pH 7.2 for 1 h. The measurement was performed in a solution of 100 mM PBS pH 7.2 containing 10 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ over a range of frequencies from 2.5 kHz to 250 mHz.





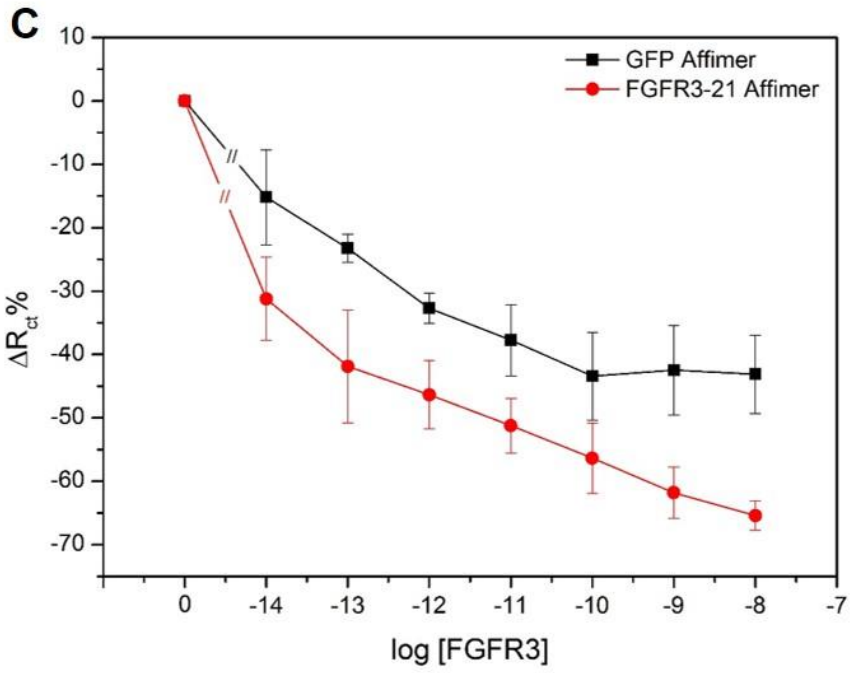
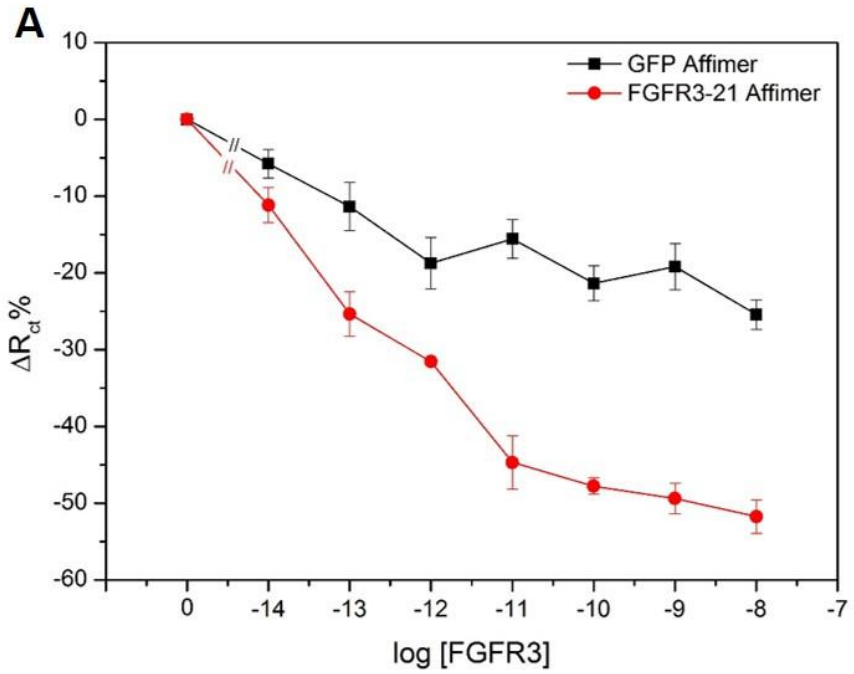
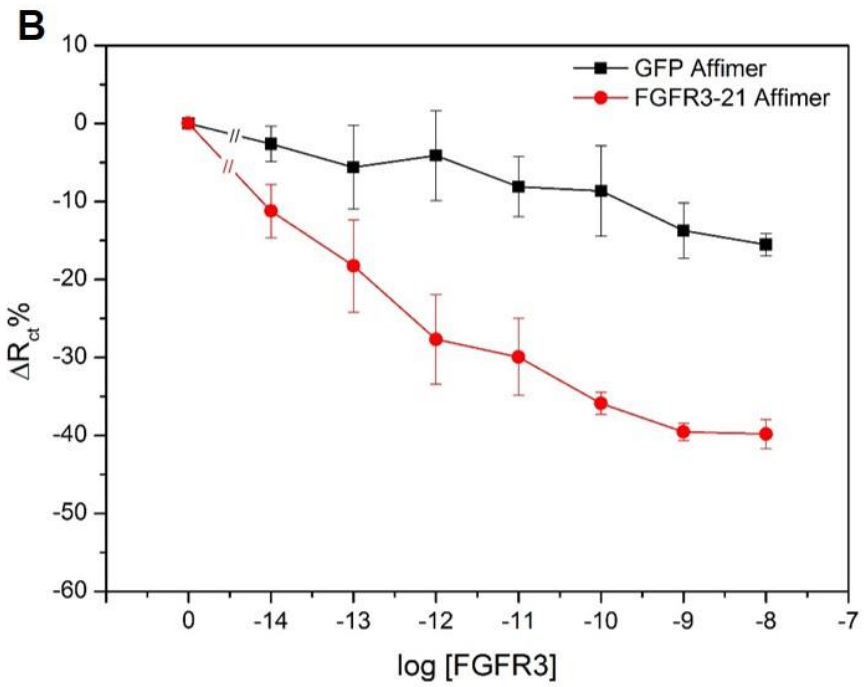


Figure S5 Calibration curves of the Affimer-based impedimetric biosensors for detecting FGFR3 in PBS. Three blocking agents, (A), 6.7 µM BSA; (B), 2x casein blocking buffer from Sigma-Aldrich and (C), 0.2 mg/ml sodium caseinate, were tested for minimising non-specific binding effects. The EIS was performed in 10 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ solution. Data are means ± SEM (n=3).





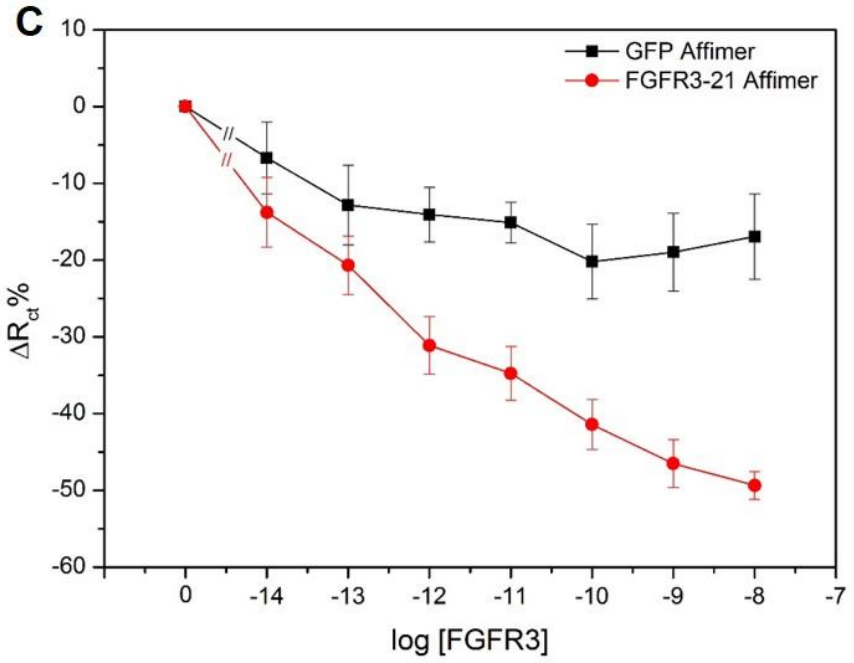


Figure S6 Calibration curves of the Affimer-based impedimetric biosensors for detecting FGFR3 in PBS. The sensors were blocked with 6.7 μM BSA prior to Affimer attachment. Three concentration of Affimers, (A), 0.3 μM; (B), 1 μM and (C), 3 μM, were tested for the optimal condition. The EIS was performed in 10 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ solution. Data are means ± SEM (n=3).