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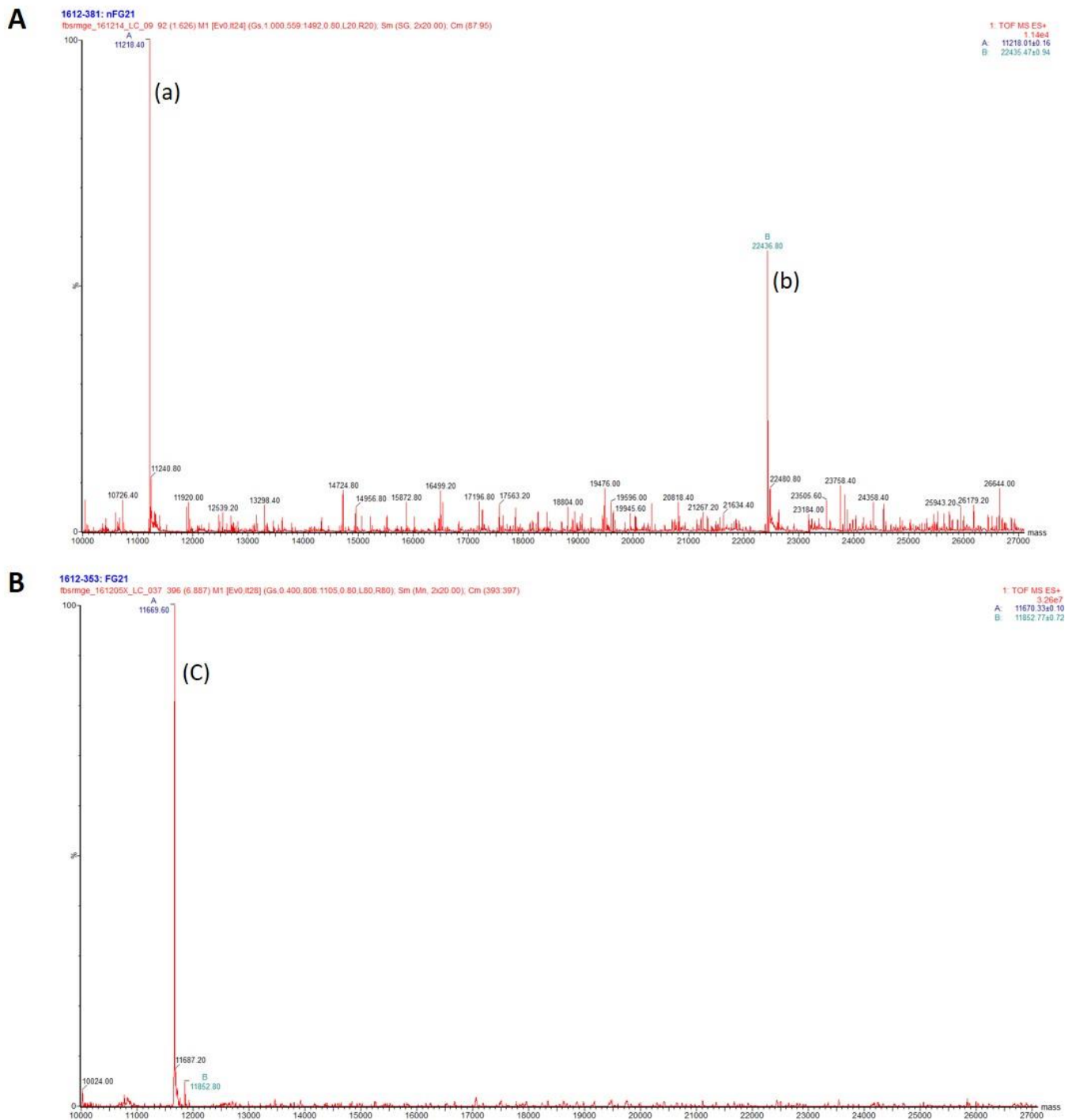
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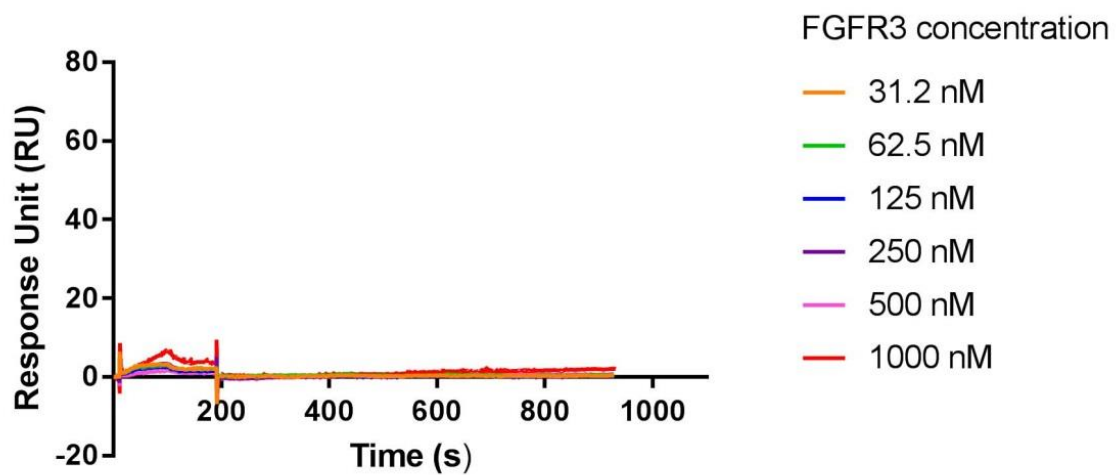
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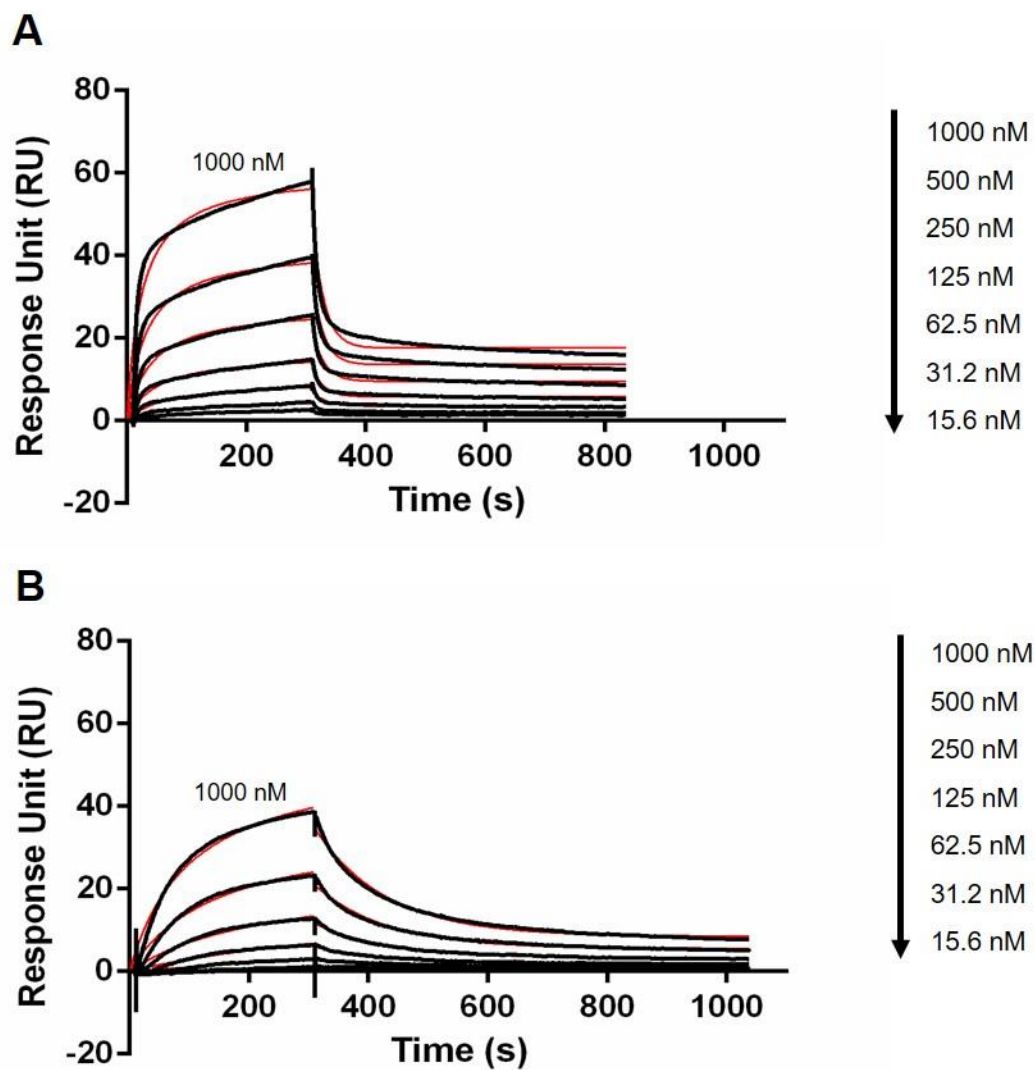
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**Figure S1** LC-MS spectra showing the successful biotinylation of FGFR3-21 Affimer proteins. (A) non-biotinylated FGFR3-21 Affimers showed two distinct peaks, which represent (a) monomeric forms and (b) dimeric forms. (B) biotinylated FGFR3-21 Affimers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



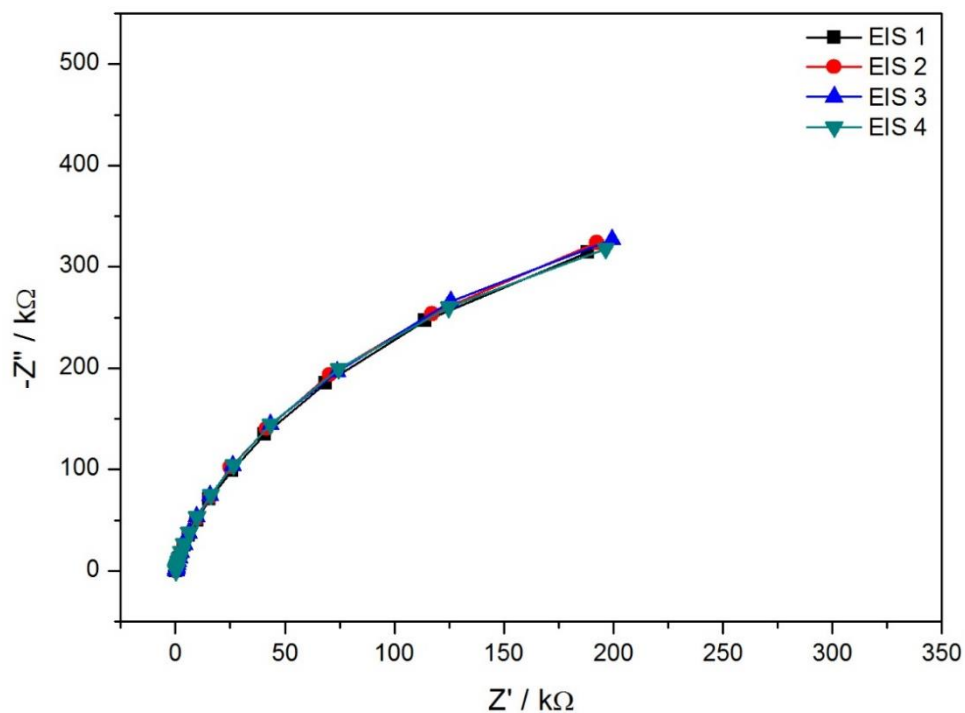
**Figure S2** SPR sensorgram of FGFR3-8 Affimer interaction with FGFR3 protein. The FGFR3-8 Affimers were immobilised onto a streptavidin-coated sensor chip before being challenged by FGFR3 at concentrations from 0 to 1000 nM. In this case, the association phase was run for 180 s, followed by 900 s of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



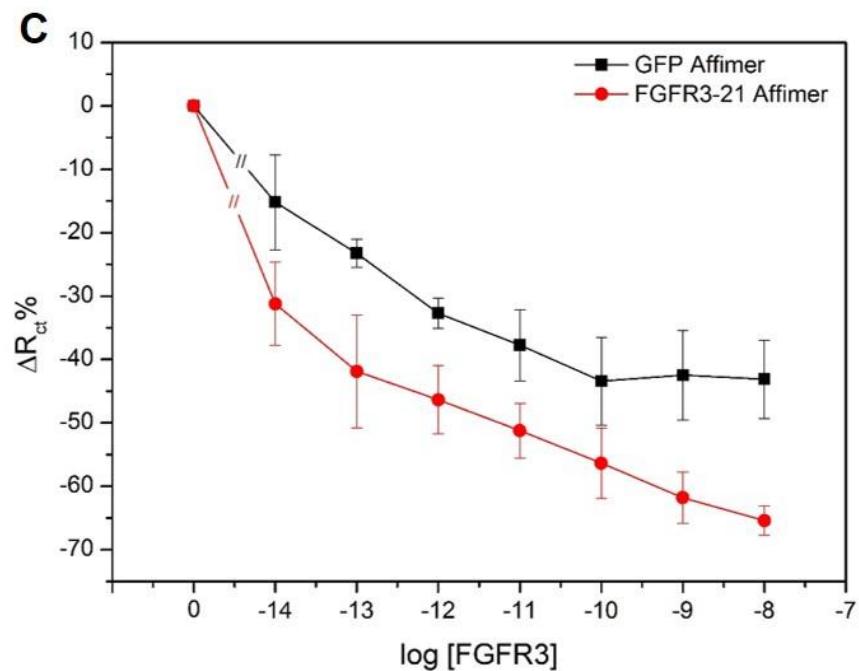
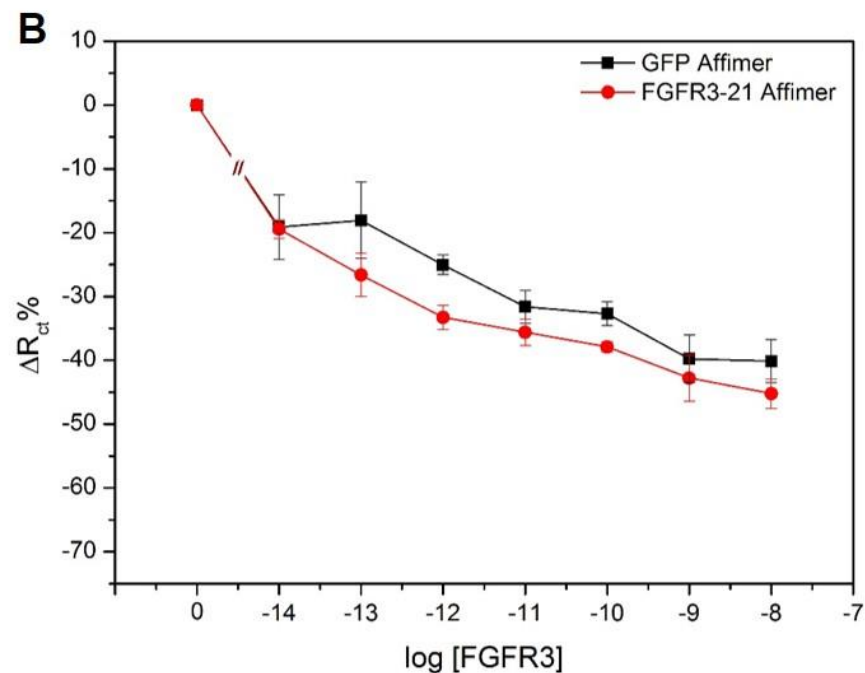
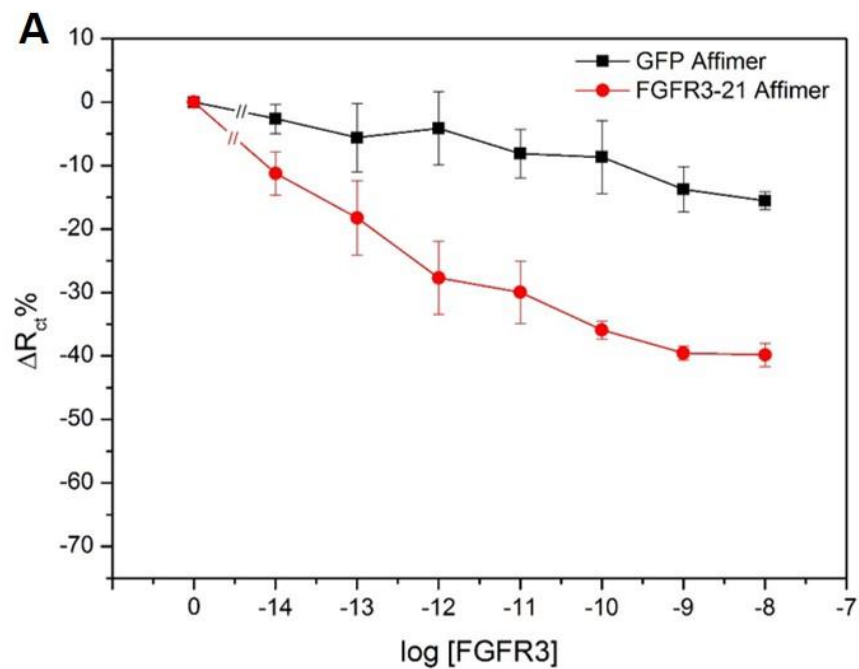
**Figure S3** SPR data fitting with a one-site specific model. (A), fitting for FGFR3-14 Affimer and (B), for FGFR3-21 Affimer. The black lines represent experimental data from SPR whilst the red lines show the fitting results which are overlaid. The data from association phase were fitted with a one-site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

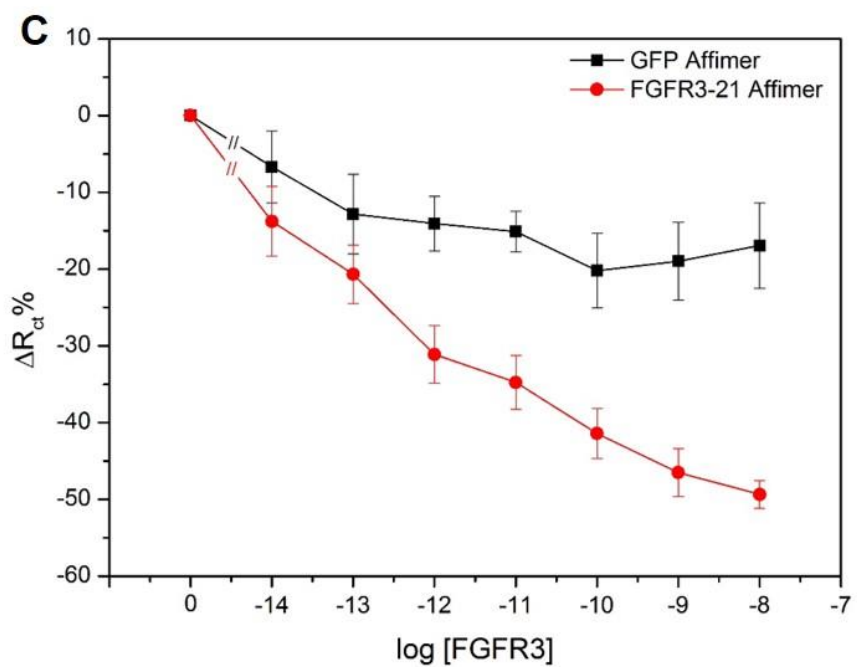
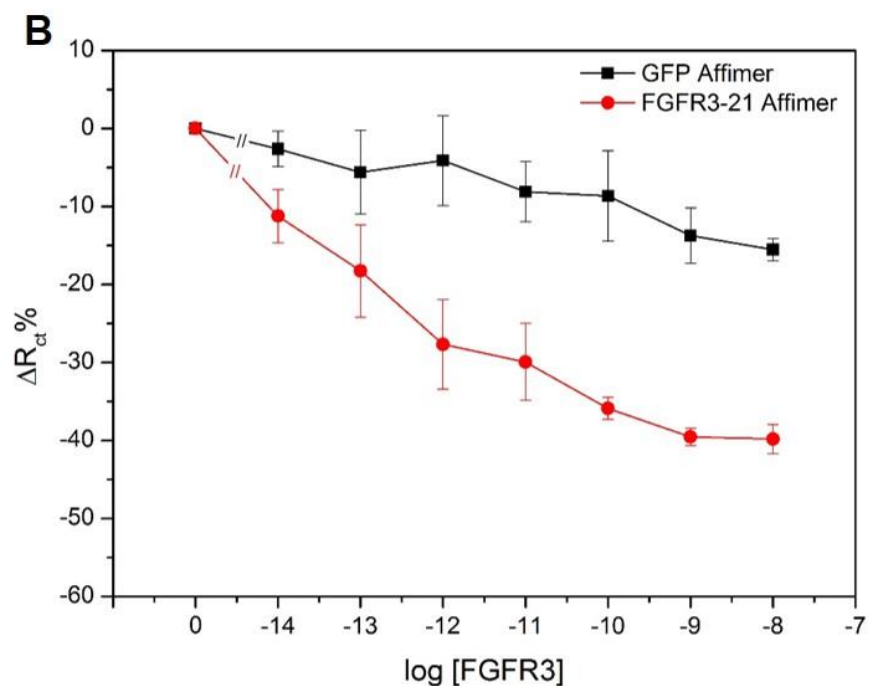
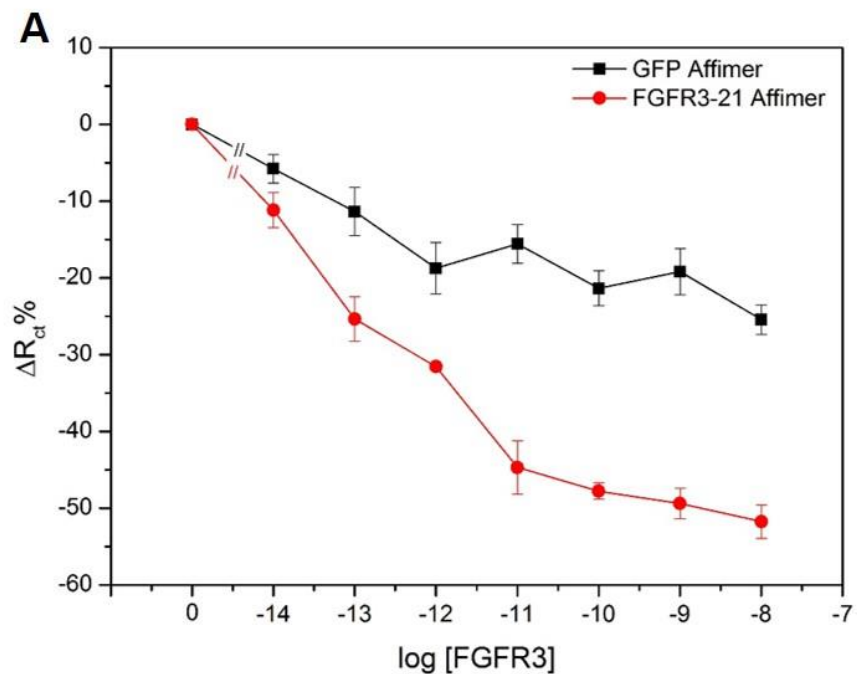
<b>Affimer clones</b>	<b>FGFR3-14</b>	<b>FGFR3-21</b>
<b><math>k_{on}</math> (<math>M^{-1} s^{-1}</math>)</b>	$1.60 \times 10^8$	$4.49 \times 10^8$
<b><math>k_{off}</math> (<math>s^{-1}</math>)</b>	$5.24 \times 10^{-2}$	$8.31 \times 10^{-3}$
<b><math>K_D</math> (M)</b>	$3.27 \times 10^{-10}$	$1.85 \times 10^{-11}$
<b><math>R^2</math></b>	0.955	0.976
<b><math>\chi^2</math></b>	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  $\mu\text{M}$  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  $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$  solution. Data are means  $\pm$  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  $\pm$  SEM (n=3).