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The impact of the coverage of aptamers on a nanoparticle on the binding equilibrium and kinetics between aptamer and protein

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Characterisations of the AuNPs and AuNPs-aptamer conjugate

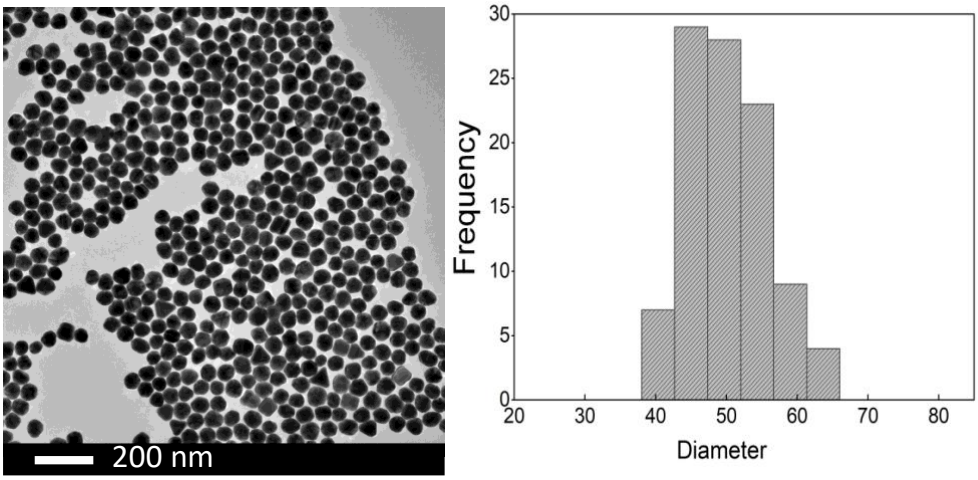

Figure S1. (Left) TEM image of the AuNPs. (Right) Histogram of the particles size. The average particle size is 49 ± 7 nm.

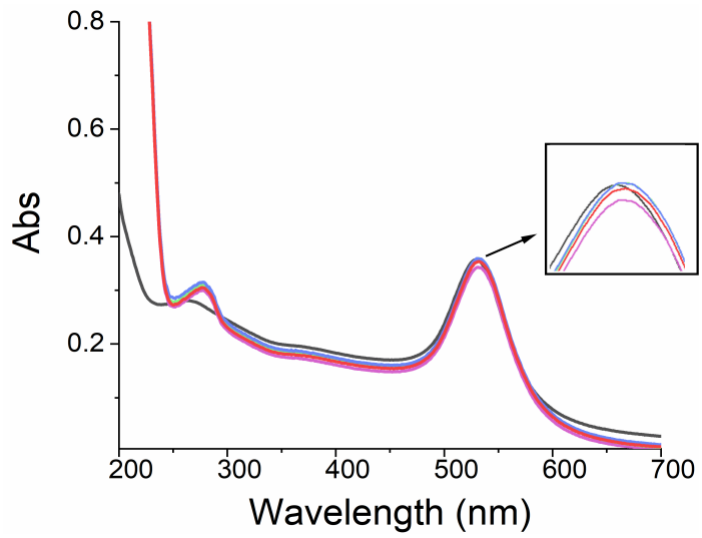

Figure S2 UV-Vis spectra of AuNPs (black line), AuNPs-aptamer conjugate with T_{10}/ aptamer = 7:1 (purple line), AuNPs-aptamer conjugate with T_{10}/aptamer = 3:1 (green line), AuNPs-aptamer conjugate with T_{10}/aptamer = 1:1 (blue line), and AuNPs-aptamer conjugate with only aptamer (red line). The position of the plasmon resonance peak is 531 nm for AuNPs and 533 nm for AuNPs-aptamer conjugate.

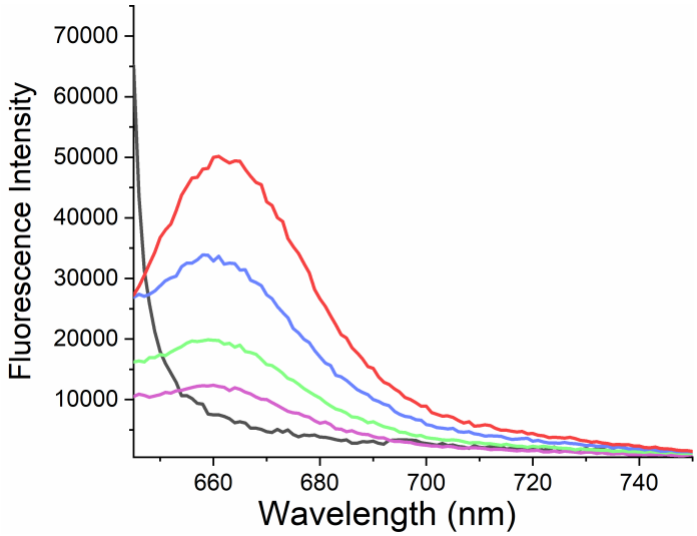

Figure S3 Fluorescence spectra of AuNPs (black line), AuNPs-aptamer conjugate with T_{10}/ aptamer = 7:1 (purple line), AuNPs-aptamer conjugate with T_{10}/aptamer = 3:1 (green line), AuNPs-aptamer conjugate with T_{10}/aptamer = 1:1 (blue line), and AuNPs-aptamer conjugate with only aptamer (red line).

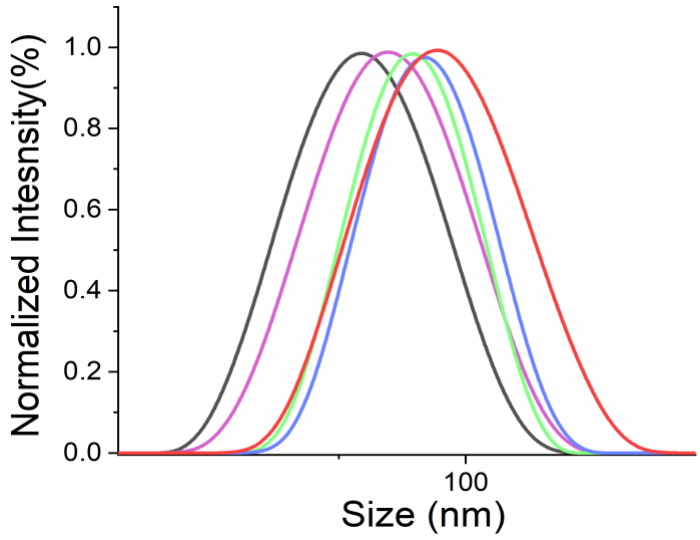

Figure S4 DLS intensity size distribution of AuNPs (black line), AuNPs-aptamer conjugate with T_{10}/ aptamer = 7:1 (purple line), AuNPs-aptamer conjugate with T_{10}/aptamer = 3:1 (green line), AuNPs-aptamer conjugate with T_{10}/aptamer = 1:1 (blue line), and AuNPs-aptamer conjugate with only aptamer (red line).

Table S1. Size, polydispersity Index (PDI) and average area occupied by an aptamer for various surface coverages. The value at saturation (258 aptamers per particle) can be used to find the footprint of the aptamer in its hairpin conformation: 29.2 nm^2.

	Intensity Mean (nm)	**PDI**	**Average area occupied by an aptamer on the surface (nm^2)**
AuNPs	61.0 ± 21.28	0.210	N.A.
Conjugate with 9.6 aptamers per particle	77.96 ± 29.84	0.125	787.4
Conjugate with 22.3 aptamers per particle	84.21 ± 27.75	0.105	338.2
Conjugate with 46.1 aptamers per particle	94.58 ± 38.04	0.167	163.6
Conjugate with 258 aptamers per particle	106.7 ± 31.15	0.160	29.2

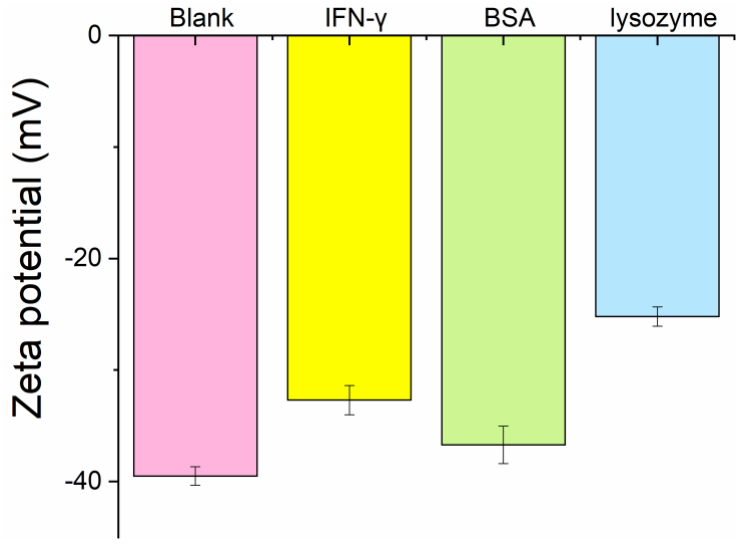

Figure S5 Zeta potential of AuNPs-aptamer conjugate with 22.3 aptamers alone and after incubation with IFN-γ, BSA and lysozyme.

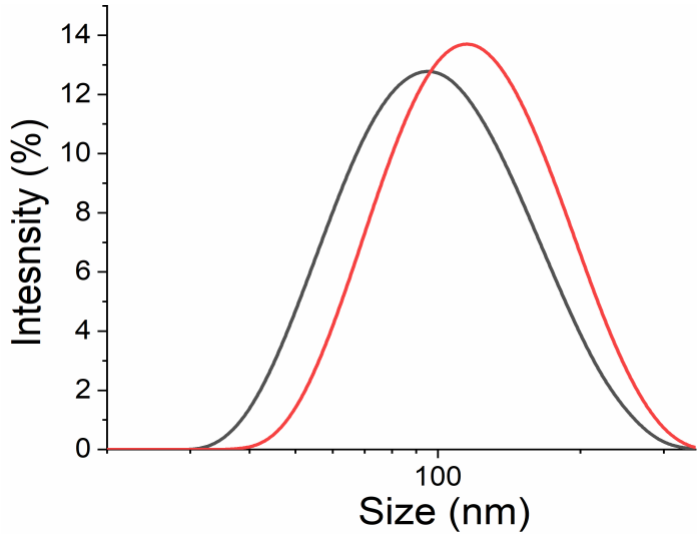

Figure S6. DLS intensity size distribution of AuNPs-aptamer conjugate with 258 aptamers alone and after incubation with 87 nM of IFN-γ. The size is 107.1± 35.23 nm for the conjugate without the target and 123 ± 41.37 nm when bound to IFN-γ.

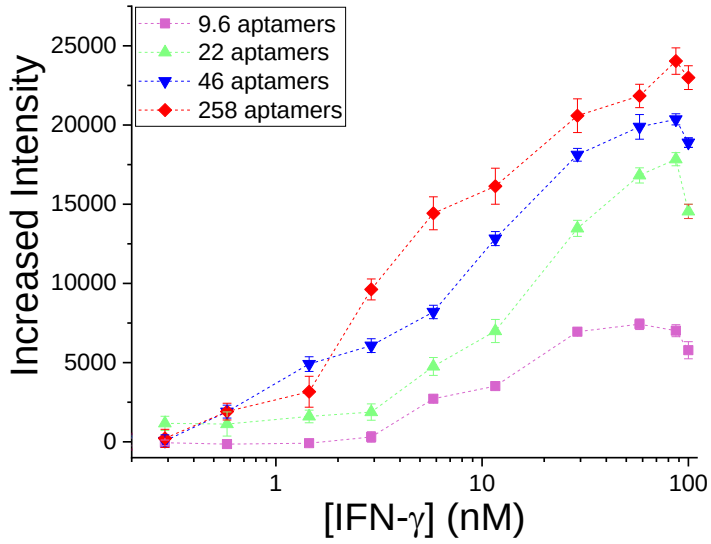

Figure S7. Increase of fluorescence intensity of AuNPs-aptamer conjugate with different coverage of aptamer after capturing IFN-γ.

Equilibrium analysis

Experimentally, a binding isotherm can be constructed by titrating a fixed concentration of a fluorescently tagged species (AuNPs-aptamer conjugate) with its binding partner (IFN-γ) that causes the fluorescence to change. It is the concentration of the unlabelled species that is varied because in this way any change in fluorescence is due to the binding. From such data, the equilibrium dissociation constant can be obtained by fitting the data with a suitable model.

The relationship between fluorescence and concentration of nanoparticles at the equilibrium is given again by equation like S2:

$$F = \alpha_A[A]_{eq} + \alpha_{AP}[AP]_{eq} \quad \text{(S1)}$$

With $[A]_0 = [A]_{eq} + [AP]_{eq}$. When no IFN-γ has been added, $[AP]_{eq} = 0$, $[A]_0 = [A]_{eq}$ and the fluorescence is:

$$F = F_0 = \alpha_A[A]_0 \quad \text{(S2)}$$

At saturation, 100% of the aptamers have reacted, therefore $[A]_{eq} = 0$ and $[A]_0 = [AP]_{eq}$:

$$F = F_{sat} = \alpha_{AP}[AP]_{eq} \quad \text{(S3)}$$

Therefore, the fluorescence F can be written as:

$$F = \frac{F_0}{[A]_0}[A]_{eq} + \frac{F_{sat}}{[A]_0}[AP]_{eq} \quad \text{(S4)}$$

$$F = \frac{F_0}{[A]_0}([A]_0 - [AP]_{eq}) + \frac{F_{sat}}{[A]_0}[AP]_{eq} \quad \text{(S5)}$$

$$[A]_0(F - F_0) = (F_{sat} - F_0)[AP]_{eq} \quad \text{(S6)}$$

$$\frac{[AP]_{eq}}{[A]_0} = \frac{(F - F_0)}{(F_{sat} - F_0)} \quad \text{(S7)}$$

Where $\frac{[AP]_{eq}}{[A]_0}$ is the 'bound fraction' θ.

The Hill-Langmuir equation used to fit the data requires the equilibrium concentrations of IFN-γ. Such concentrations were calculated from the mass balance equation of IFN-γ:

$$C_{INF\gamma} = [INF\gamma]_{eq} + [AP]_{eq} \quad \text{(S8)}$$

$$[INF\gamma]_{eq} = C_{INF\gamma} - [AP]_{eq} = C_{INF\gamma} - \left([A]_0 \frac{(F - F_0)}{(F_{sat} - F_0)}\right) \quad \text{(S9)}$$

$$\frac{(F - F_0)}{(F_{sat} - F_0)} = \frac{C_{INF\gamma} - \left([A]_0 \frac{(F - F_0)}{(F_{sat} - F_0)}\right)}{K_D' + C_{INF\gamma} - \left([A]_0 \frac{(F - F_0)}{(F_{sat} - F_0)}\right)} \quad \text{(S10)}$$

Equation S10 was used to fit the thermodynamic data.

Kinetic analysis

Analytical solution of a reversible second-order rate equation

The binding between the aptamer-coated nanoparticle and IFN-γ is a second-order reversible reaction:

$$\text{AuNPaptamer} + \text{IFN-}\gamma \underset{K_D}{\rightleftarrows} \text{AuNPaptamer–IFN-}\gamma$$

The rate equation is:

$$-\frac{d[AuNPaptamer]}{dt} = k_{on}[AuNPaptamer][IFN-\gamma] - k_{off}[AuNPaptamer-IFN-\gamma] \quad \text{(S11)}$$

Let us call Φ_A the fluorescence of aptamers without any IFN-γ at a concentration of 1 unit, and Φ_{AP} the fluorescence of aptamer bounded to IFN at a concentration equal to 1 unit. The fluorescence of the sample is given by:

$$F = \Phi_A[A] + \Phi_{AP}[AP] \quad \text{(S12)}$$

Where [A] = [AuNPaptamer] and [AP] = [AuNPaptamer–INFγ]. At t=0 all the aptamers are without any IFN, as a consequence [AP]=0 and $[A]=[A]_0$. As a consequence:

$$\Phi_A = F_0/[A]_0 \quad \text{(S13)}$$

$$F = F_0\frac{[A]}{[A]_0} + \Phi_{AP}[AP] \quad \text{(S14)}$$

At equilibrium:

$$F_{eq} = F_0\frac{[A]_{eq}}{[A]_0} + \Phi_{AP}[AP]_{eq} \quad \text{(S15)}$$

Thus Φ_{AP} can be written:

$$\Phi_{AP} = \frac{F_{eq}-F_0\frac{[A]_{eq}}{[A]_0}}{[AP]_{eq}} = \frac{F_{eq}}{[AP]_{eq}} - F_0\frac{[A]_0-[AP]_{eq}}{[AP]_{eq}[A]_0} = \frac{F_0}{[A]_0} + \frac{F_{eq}-F_0}{[AP]_{eq}} \quad \text{(S16)}$$

And the fluorescence becomes:

$$F = F_0\frac{[A]}{[A]_0} + \frac{F_0}{[A]_0}[AP] + \frac{F_{eq}-F_0}{[AP]_{eq}}[AP] = F_0\frac{[A]_0-[AP]}{[A]_0} + \frac{F_0}{[A]_0}[AP] + \frac{F_{eq}-F_0}{[AP]_{eq}}[AP] \quad \text{(S17)}$$

$$F = F_0 + \frac{F_{eq}-F_0}{[AP]_{eq}}[AP] \quad \text{(S18)}$$

Kinetic law integration:

$$\frac{d}{dt}[AP] = k_{on}[A][P] - k_{off}[AP] = k_{on}([A]_0 - [AP])([P]_0 - [AP]) - k_{off}[AP] \quad \text{(S19)}$$

$$\frac{d[AP]}{k_{on}[AP]^2-[AP]\left(k_{on}([A]_0+[P]_0)+k_{off}\right)+k_{on}[A]_0[P]_0} = dt \quad \text{(S20)}$$

$$\frac{d[AP]}{k_{on}([AP]-S_1)([AP]-S_2)} = dt \quad \text{(S21)}$$

With S_1 and S_2 the [AP] values solutions of:

$$k_{on}[AP]^2 - [AP]\left(k_{on}([A]_0 + [P]_0) + k_{off}\right) + k_{on}[A]_0[P]_0 = 0 \quad \text{(S22)}$$

Let us introduce Δ such as:

$$\Delta = \left(k_{on}([A]_0 + [P]_0) + k_{off}\right)^2 - 4{k_{on}}^2[A]_0[P]_0 \quad \text{(S23)}$$

It can be seen that Δ cannot be negative, therefore S_1 and S_2 are reals numbers.

$$S_1 = \frac{k_{on}([A]_0+[P]_0)+k_{off}-\sqrt{\Delta}}{2k_{on}} \quad \text{(S24)}$$

$$S_2 = \frac{k_{on}([A]_0+[P]_0)+k_{off}+\sqrt{\Delta}}{2k_{on}} \quad \text{(S25)}$$

Because $[AP]_{eq}$ cannot be above $[A]_0$ and $[P]_0$, the expression of $[AP]_{eq}$ can be written:

$$[AP]_{eq} = S_1 = \frac{k_{on}([A]_0+[P]_0)+k_{off}-\sqrt{\Delta}}{2k_{on}} \quad \text{(S26)}$$

As a consequence, we can write:

$$\frac{d[AP]}{([AP]-S_1)([AP]-S_2)} = \left(\frac{a}{([AP]-S_1)} + \frac{b}{([AP]-S_2)}\right) d[AP] = k_{on}dt \quad \text{(S27)}$$

Let us find a and b:

$$\frac{d[AP]}{([AP]-S_1)([AP]-S_2)} = \frac{[AP](a+b)-aS_2-bS_1}{([AP]-S_1)([AP]-S_2)} d[AP] \quad \text{(S28)}$$

By identification:

$$(a+b) = 0 \quad \text{(S29)}$$

Thus:

$$a = -b \quad \text{(S30)}$$

$$-aS_2 - bS_1 = bS_2 - bS_1 = 1 \quad \text{(S31)}$$

$$b = \frac{1}{S_2-S_1} \quad \text{(S32)}$$

$$\frac{d[AP]}{([AP]-S_1)([AP]-S_2)} = \left(\frac{\frac{1}{S_1-S_2}}{([AP]-S_1)} + \frac{\frac{1}{S_2-S_1}}{([AP]-S_2)}\right) d[AP] = k_{on}dt \quad \text{(S33)}$$

$$\left(\frac{1}{([AP]-S_2)} - \frac{1}{([AP]-S_1)}\right) d[AP] = (S_2-S_1)k_{on}dt \quad \text{(S34)}$$

Which can be integrate:

$$\int_0^{[AP]} \left(\frac{1}{([AP]-S_2)} - \frac{1}{([AP]-S_1)}\right) d[AP] = \int_0^t (S_2-S_1)k_{on}dt \quad \text{(S35)}$$

$$\left[Ln\left(\frac{[AP]-S_2}{[AP]-S_1}\right)\right]_0^{[AP]} = [(S_2-S_1)k_{on}t]_0^t = (S_2-S_1)k_{on}t \quad \text{(S36)}$$

$$Ln\left(\frac{[AP]-S_2}{[AP]-S_1}\right) - Ln\left(\frac{S_2}{S_1}\right) = (S_2-S_1)k_{on}t \quad \text{(S37)}$$

$$\frac{[AP]-S_2}{[AP]-S_1} = \frac{S_2}{S_1} Exp^{(S_2-S_1)k_{on}t} = f(t) \quad \text{(S38)}$$

With $f(t) = \frac{S_2}{S_1} Exp^{(S_2-S_1)k_{on}t}$

$$[AP] - S_2 = f(t)([AP] - S_1) \quad \text{(S39)}$$

$$[AP]\big(1 - f(t)\big) = S_2 - f(t)\, S_1 \quad \text{(S40)}$$

$$[AP] = \frac{S_2 - f(t)\, S_1}{1-f(t)} = \frac{S_2\left(1-Exp^{(S_2-S_1)k_{on}t}\right)}{1-\frac{S_2}{S_1}Exp^{(S_2-S_1)k_{on}t}} = \frac{1-Exp^{\sqrt{\Delta}t}}{\frac{1}{S_2}-\frac{1}{S_1}Exp^{\sqrt{\Delta}t}} \quad \text{(S41)}$$

Thus, the fluorescence can be written:

$$F = F_0 + \frac{F_{eq}-F_0}{[AP]_{eq}}[AP] = F_0 + \frac{F_{eq}-F_0}{S_1}\frac{1-Exp^{\sqrt{\Delta}t}}{\frac{1}{S_2}-\frac{1}{S_1}Exp^{\sqrt{\Delta}t}} = F_0 + \big(F_{eq} - F_0\big)\frac{1-Exp^{\sqrt{\Delta}t}}{\frac{S_1}{S_2}-Exp^{\sqrt{\Delta}t}} \quad \text{(S42)}$$

Equation S42 was then used on Origin Pro to fit the kinetic data.

The fitting function for the reversible second order rate equation can be downloaded at the link: https://github.com/Chemistry-fitting-functions/Chemistry-fitting-functions

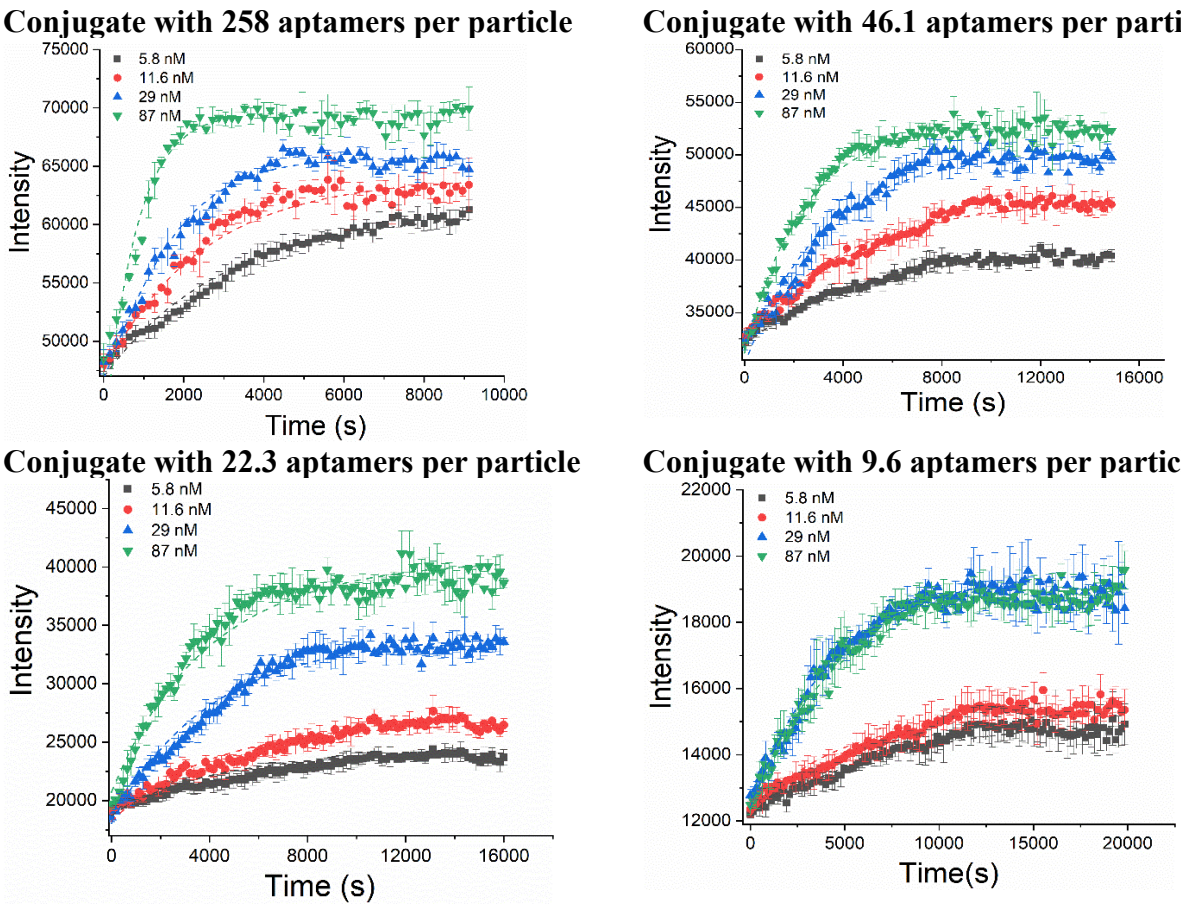

Figure S8. Kinetic analyses of IFN-γ captured by AuNPs-aptamer conjugate with different surface coverages (from 9.6 to 2.58 × 10^2 aptamers per particle). This set of experiments has been performed with concentration of IFN-γ varying from 5.8 nM to 87 nM. The intensity has been fitted to exponential functions and fittings are represented by dash lines.