Supplementary materials

Specific Interaction of Platinated DNA and Proteins by Surface Plasmon Resonance Imaging

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The specific analysis process of "initial rate analysis" [1, 2]

The "initial rate analysis" method from Edwards and Leatherbarrow was adopted for the kinetic analysis which has been successfully applied to analyzing experimental binding data generated by optical biosensors and used to analyze the interaction between immune molecules for SPR. It can ignore the impact of space mass transfer resistance for different biosensors. Edwards and Leatherbarrow assumed that the kinetic interactions of binding events consisted of two phases including a faster initial linear phase and a slower second nonlinear phase between an immobilized probe and an analyte. In a SPR-based system, both these phases are recorded in real time on a SPR sensorgram. By linear regression, the initial binding rates of the linear region in the binding sensorgrams for each analyte concentration (c_A) can be obtained. Then, a plot of the binding rate *(dR/dt)* vs c_A results in a straight line, the k_a can be obtained from its slope. In the dissociation phase, the response depending on time decreases exponentially with k_d. From the plot of $ln(R_0/R_t)$ vs time (t), the slope corresponds to k_d. The K_d is then calculated by using the calculated values of k_a and k_d. The specific analysis process of the initial rate analysis method we adopted is described below.

The rate of formation product at time t could be written as the pseudo first-order kinetic reaction equation as Eq. (1):

$$\frac{dc_{PA}}{dt} = k_a c_P c_A - k_d c_{PA} \quad (1)$$

where c_{PA} is the concentration at time t, the subscript P, A and PA are on behalf of probes, analytes and their compounds respectively. We suppose that the total concentration of probe on the sensor chip surface is c_{P0} (the maximum recognition capacity), so $c_P = c_{P0} - c_{PA}$. The SPR signal R is proportional to the formation of the PA complexes on the surface and the maximum signal R_{max} will be proportional to the concentration of active probe on the surface, i.e. $R_{max} = Nc_{P0}$， $R = Nc_{PA}$ (N is the proportional coefficient). Substituting $c_P = c_{P0} - c_{PA}$ into Eq. (1), the equation becomes:

$$\frac{dR}{dt} = k_a R_{\max} c_A - (k_d + k_a c_A) R \quad (2)$$

rearranging Eq. (2), we got follows:

$$\frac{1}{k_a R_{\max} c_A - (k_d + k_a c_A) R} dR = dt$$

$$\frac{d\left[k_a R_{\max} c_A - (k_d + k_a c_A) R\right]}{k_a R_{\max} c_A - (k_d + k_a c_A) R} = -(k_d + k_a c_A) dt$$

Then, integrating the above equation, we got follows:

$$\ln\left[k_a R_{\max} c_A - (k_d + k_a c_A) R_t\right] - \ln\left[k_a R_{\max} c_A - (k_d + k_a c_A) R_0\right] = -(k_d + k_a c_A)(t - t_0)$$

when $t_0 = 0$, $R_0 = 0$, the above equation can be simplified into:

$$\frac{k_a R_{\max} c_A - (k_d + k_a c_A) R_t}{k_a R_{\max} c_A} = e^{-(k_d + k_a c_A) t}$$

for $K_d = k_d / k_a$, we got follows:

$$R_t = \frac{R_{\max} c_A}{K_d + c_A}\left(1 - e^{-(k_d + k_a c_A) t}\right) \quad (3)$$

For the "initial rate analysis" method, the entire binding data could be described by the integrated form of the rate equation under pseudo-first-order reaction conditions as Eq. (4):

$$R_t = (R_{eq} - R_0)(1 - e^{-k_a t}) + R_0 \quad (4)$$

where R_0 is the initial instrument response, R_t is the response at time t, R_{eq} is the equilibrium state response. But it has been noted that the data are often ill-described by Eq. (4) sometimes and are better fitted with addition of a second exponential term as Eq. (5):

$$R_t = A(1 - e^{-k_a t}) + B(1 - e^{-k'_{dif} t}) + R_0 \quad (5)$$

Where R_t varies with two apparent association rate constants (k_a and k'_{dif}). The faster rate (k_a) is found to most closely correspond to the rate constant for the interaction in solution. The slower process has been generally accepted to be a property of the biosensor method and provides little or no information on the intrinsic bio-molecular interaction. Eq. (5) is an empirical solution to the analysis of biosensor data and there is no firm theoretical basis to justify the use of second association rate constant to now. The derivation of the integrated rate equation for the biosensors could be described as Eq. (3) like above. The term $R_{max} c_A / (K_d + c_A)$ is equivalent to the term ($R_{eq} - R_0$) in Eq. (4). Differentiating Eq. (3) with respect to time t gives follows:

$$\frac{dR}{dt} = \frac{R_{max} c_A}{K_d + c_A}\left(k_d + k_a c_A\right) e^{-(k_d + k_a c_A) t} = k_a R_{max} c_A e^{-(k_d + k_a c_A) t} \quad (6)$$

Eq. (6) could be simplified to Eq. (7) at time $t = 0$, we got follows:

$$\frac{dR}{dt} = \frac{R_{\max} c_A}{Kd + c_A}(k_a c_A + k_d) = R_{\max} c_A k_a \quad (7)$$

The k_d could be derived by application of Eq. (8) to the dissociation data and R_∞ is the final response.

$$R_t = (R_0 - R_\infty)e^{-k_d t} + R_\infty \quad (8)$$

Here, the interaction between PC4 and *trans*PtTz-DNA2 was taken as an example. The initial rate for each concentration of protein analyte can be obtained from the first 60 seconds of the SPR dynamic curve. The initial rate (that is the slope in Fig S1 (A)) can be obtained through linear fitting by Origin software. Then a plot of the initial rate *(dR/dt)* vs c_A can be obtained and k_a can be obtained from the slope of the fitted line (Fig S1 (B)). The k_d can be obtained from the rate equation through R_{max}, all of the equations were represented as above. K_d of every group can be calculated by reproducing the process described above.

References

1. P. R. Edwards and R. J. Leatherbarrow, *Anal. Biochem.*, 1997, **246**, 1-6.
2. S. Hoebel, D. Vornicescu, M. Bauer, D. Fischer, M. Keusgen and A. Aigner, *Anal. Chem.*, 2014, **86**, 6827-6835.

Fig S1 The initial rate analysis of the interaction between PC4 and *trans*PtTz-DNA2

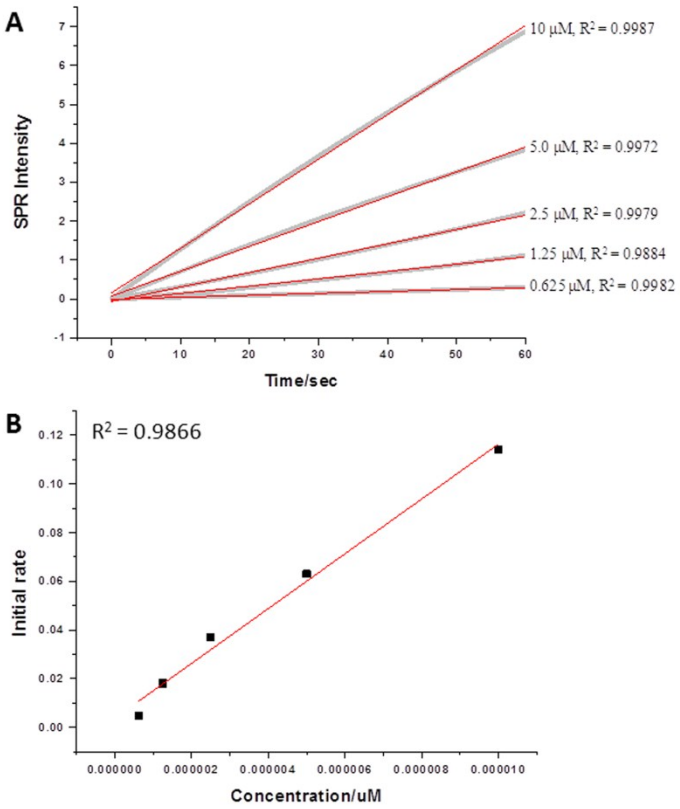

Fig. S1 (A) SPR dynamic curve of interaction between PC4 and *trans*PtTz-DNA2 (grey curve) and its linear fitting (red curve) within the first 60 seconds. The concentration of PC4 is 0.625, 1.25, 2.5, 5.0 and 10.0 μM respectively. (B) The initial rate *vs* concentration of PC4 and its linear fitting.

Fig S2 Choice of blocking solution

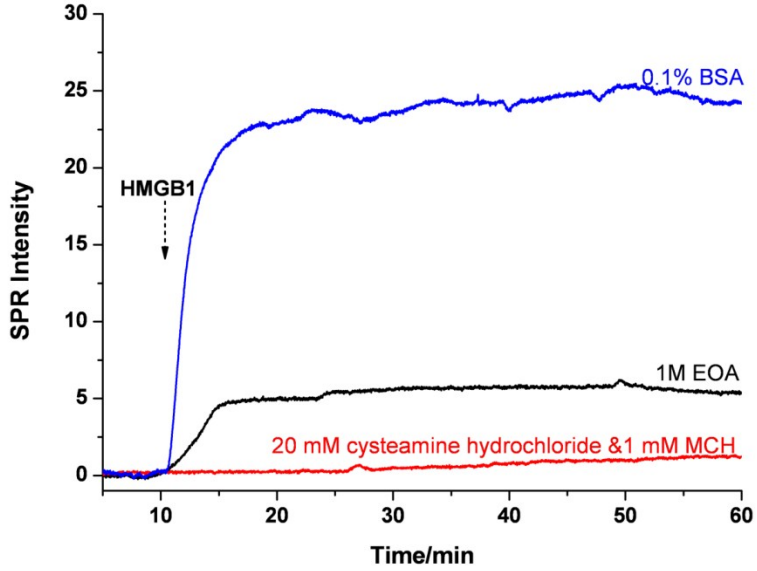

Fig. S2 SPRi sensorgram of non-specific adsorption of HMGB1 (10 μM) on three different blocking surface modified by 0.1% bovine serum albumin (BSA); 1 M ethanolamine (EOA) and a solution containing 20 mM cysteamine hydrochloride and 1mM 6-mercapto-1-hexanol (MCH).

Fig S3 Regeneration of chips

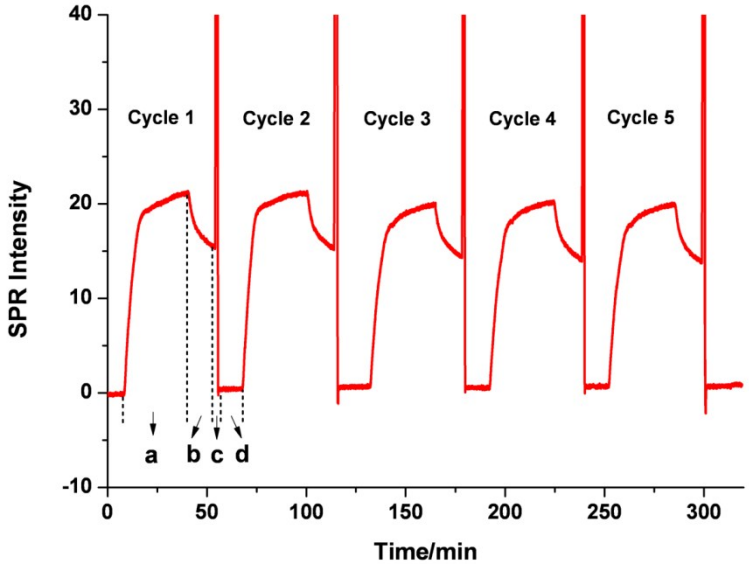

Fig. S3 SPRi sensorgram of five successive binding-regenerating cycles measured from the recognitions of PC4 with *trans*PtTz-DNA2 on the same chip. Each cycle includes 4 steps: (a) incubation of the chip with PC4 solution; (b) wash of the chip surface with TME_{Tween} buffer; (c) removal of the PC4 molecules by flashing the chip surface with 2 M NaCl; and (d) regeneration of the chip surface with TME_{Tween} buffer.

Fig S4 Interaction with other proteins

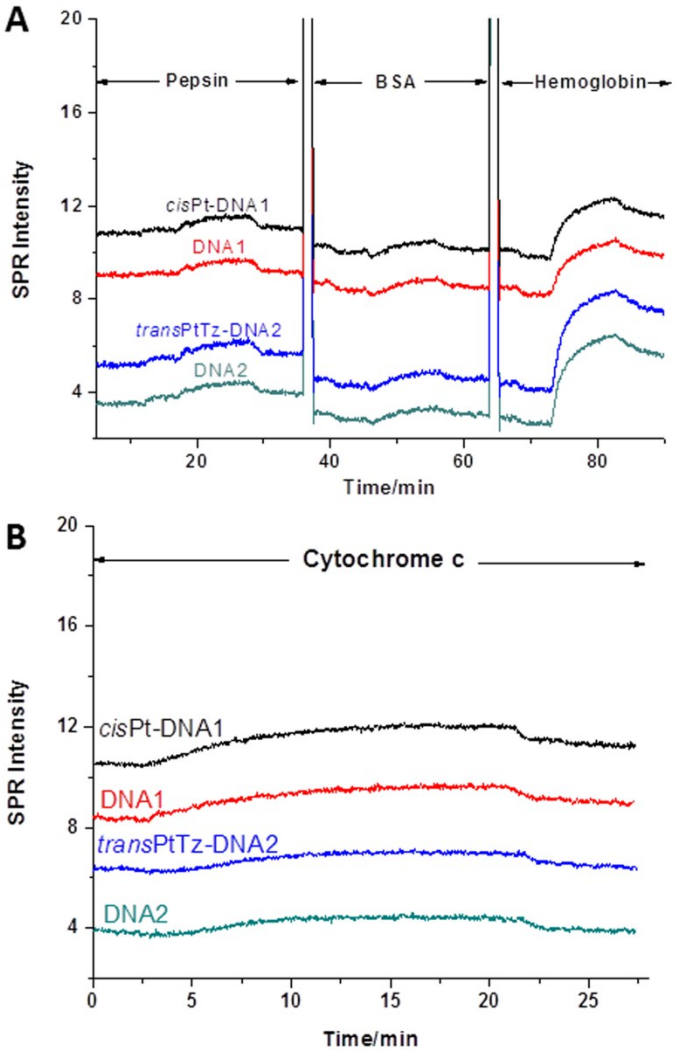

Fig. S4 SPRi sensorgram of interactions between four kinds of DNAs and pepsin, BSA, hemoglobin (A) and cytochrome c (B) respectively. The protein concentration was 10 μM respectively. The same DNA chip was used and regenerated by 2 M NaCl to remove the bound proteins from the sensor chip surface at the end of each measurement in (A) and another new chip was used in (B).

Table S1 Gibbs free energy change (Δ*G*) at various temperatures

Table S1. Values of Δ*G* at various temperatures obtained from SPRi experiments (The error demonstrated the standard deviation from three sensor chips)

Protein	DNA	Δ*G* [kJ/mol]				
		15 °C	20 °C	30 °C	37 °C	42 °C
HMGB1	cisplatin-DNA1	-29.79 ± 0.71	-30.24 ± 0.12	-31.16 ± 0.28	-32.33 ± 0.23	-32.48 ± 0.43
	DNA1	-26.52 ± 0.59	-26.22 ± 0.88	-27.58 ± 0.46	-28.77 ± 0.58	-28.76 ± 0.39
	trans-PtTz-DNA2	-26.59 ± 0.43	-26.85 ± 0.36	-27.57 ± 0.60	-28.23 ± 0.55	-28.46 ± 0.52
	DNA2	-26.28 ± 0.54	-26.52 ± 0.53	-27.21 ± 0.59	-27.88 ± 0.52	-28.30 ± 0.70
PC4	cisplatin-DNA1	-35.11 ± 0.24	-35.78 ± 0.14	-36.96 ± 0.22	-37.42 ± 0.14	-37.83 ± 0.29
	DNA1	-33.28 ± 0.07	-33.92 ± 0.05	-35.05 ± 0.08	-35.82 ± 0.03	-36.42 ± 0.07
	trans-PtTz-DNA2	-35.30 ± 0.37	-35.96 ± 0.24	-37.31 ± 0.22	-38.09 ± 0.15	-37.22 ± 0.27
	DNA2	-33.30 ± 0.15	-34.11 ± 0.08	-35.16 ± 0.06	-35.96 ± 0.08	-36.48 ± 0.03