Supporting Information

Assessment of Binding Affinity between Drugs and Human Serum Albumin using Nanoporous Anodic Alumina Photonic Crystals

Mahdieh Nemati[1], Abel Santos[1*],Cheryl Suwen Law[1] and Dusan Losic[1*]

[1]School of Chemical Engineering, The University of Adelaide, Engineering North Building, 5005 Adelaide, Australia

***E-Mail:** abel.santos@adelaide.edu.au ; dusan.losic@adelaide.edu.au

S.1 Summary of Sensing Parameters for NAA-RFs

Table S1 summarizes the different sensing parameters (i.e. sensitivity – *S*, low limit of detection – *LLoD* and linearity – R^2) obtained for the different NAA-RF structures used in our study (**Table 1**) for each of the sensing parameters (i.e. $\Delta\lambda_{Peak}$ and ΔOT_{eff}).

Table S1. Summary of the sensing parameters obtained for the different NAA-RFs by measuring $\Delta\lambda_{Peak}$ and ΔOT_{eff} by RIfS.

Sensing Parameter $\Delta\lambda_{Peak}$

NAA-RF Structure	***S*** (nm mM^{-1})	***LLoD*** (mM)	R^2
NAA-$RF_{650-0.14}$	0.37 ± 0.03	0.28 ± 0.01	0.997
NAA-$RF_{650-0.28}$	0.52 ± 0.01	0.23 ± 0.02	0.998
NAA-$RF_{650-0.42}$	0.56 ± 0.01	0.39 ± 0.02	0.994
NAA-$RF_{700-0.14}$	0.42 ± 0.01	0.33 ± 0.03	0.996
NAA-$RF_{700-0.28}$	0.38 ± 0.01	0.51 ± 0.01	0.991
NAA-$RF_{700-0.42}$	0.33 ± 0.01	0.44 ± 0.02	0.993
NAA-$RF_{750-0.14}$	0.52 ± 0.02	0.52 ± 0.03	0.990
NAA-$RF_{750-0.28}$	0.39 ± 0.01	0.29 ± 0.01	0.997
NAA-$RF_{750-0.42}$	0.66 ± 0.03	0.65 ± 0.02	0.985

Sensing Parameter ΔOT_{eff}

NAA-RF Structure	***S*** (nm mM^{-1})	***LLoD*** (mM)	R^2
NAA-$RF_{650-0.14}$	45.7 ± 0.8	0.20 ± 0.01	0.998
NAA-$RF_{650-0.28}$	49.9 ± 2.2	0.48 ± 0.02	0.992
NAA-$RF_{650-0.42}$	74.3 ± 3.3	0.48 ± 0.01	0.992
NAA-$RF_{700-0.14}$	82.6 ± 8.3	1.10 ± 0.03	0.960
NAA-$RF_{700-0.28}$	67.0 ± 2.8	0.45 ± 0.01	0.993
NAA-$RF_{700-0.42}$	38.5 ± 5.2	1.47 ± 0.02	0.931
NAA-$RF_{750-0.14}$	91.1 ± 3.4	0.41 ± 0.03	0.994
NAA-$RF_{750-0.28}$	53.6 ± 1.4	0.29 ± 0.02	0.997
NAA-$RF_{750-0.42}$	82.2 ± 3.8	0.51 ± 0.01	0.991

S.2 ANOVA Calculations

The ANOVA table shown in the manuscript (**Table 2**) was calculated from the equations shown in **Table S2**, where SS is the sum of squares of the corresponding source, DF denotes the degree of freedom of such source, MS corresponds to the mean square of the corresponding source, F_0 is the test statistic of that source, a and b denote the total number of levels corresponding to T_P and J_{Offset}, respectively, and n is the total number of replications. The strategy for testing the hypotheses H_0, H_1 and H_2 was to compare the value of F_0 calculated from the ANOVA table to the value of the F-distribution for a significance level of 95% (i.e. 0.05) with the corresponding value of DF(Source) and DF(Error) (i.e. $F_{(0.05;\ DF(Source);\ DF(Error))}$). In this way, the tested null hypotheses (i.e. H_0, H_1 and H_2) associated with each case enumerated in section 2.3 (i.e. cases i, ii and iii) were rejected when:

i) H_0: $F_{0\text{-}Tp} \geq F_{(0.05;\ DF(Tp);\ DF(Error))}$

ii) H_1: $F_{0\text{-}Joffset} \geq F_{(0.05;\ DF(Joffset);\ DF(Error))}$

iii) H_2: $F_{0\text{-}Tp\cdot Joffset} \geq F_{(0.05;\ DF(Tp\cdot Joffset);\ DF(Error))}$

Table S2. Summary of the equations used to calculate the different ANOVA parameters.

Source	SS	DF	MS	F_0
T_P	$SS_{R_v} = \frac{1}{bn}\sum_{i=1}^{a} y_{i..}^2 - \frac{y_{...}^2}{abn}$	$a-1$	$MS_{R_v} = \frac{SS_{R_v}}{a-1}$	$F_{0-R_v} = \frac{MS_{R_v}}{MS_E}$
J_{Offset}	$SS_{V_{HA}} = \frac{1}{an}\sum_{j=1}^{b} y_{.j.}^2 - \frac{y_{...}^2}{abn}$	$b-1$	$MS_{V_{HA}} = \frac{SS_{V_{HA}}}{b-1}$	$F_{0-V_{HA}} = \frac{MS_{V_{HA}}}{MS_E}$
$T_P \cdot J_{Offset}$	$SS_{R_v \cdot V_{HA}} = \left(\frac{1}{n}\sum_{i=1}^{a}\sum_{j=1}^{b} y_{ij.}^2 - \frac{y_{...}^2}{abn}\right) - SS_{R_v} - SS_{V_{HA}}$	$(a-1)(b-1)$	$MS_{R_v \cdot V_{HA}} = \frac{SS_{R_v \cdot V_{HA}}}{(a-1)\cdot(b-1)}$	$F_{0-R_v \cdot V_{HA}} = \frac{MS_{R_v \cdot V_{HA}}}{MS_E}$
Error	$SS_E = SS_T - \left(\frac{1}{n}\sum_{i=1}^{a}\sum_{j=1}^{b} y_{ij.}^2 - \frac{y_{...}^2}{abn}\right)$	$ab(n-1)$	$MS_E = \frac{SS_E}{ab(n-1)}$	
Total	$SS_T = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \frac{y_{...}^2}{abn}$	$abn-1$		