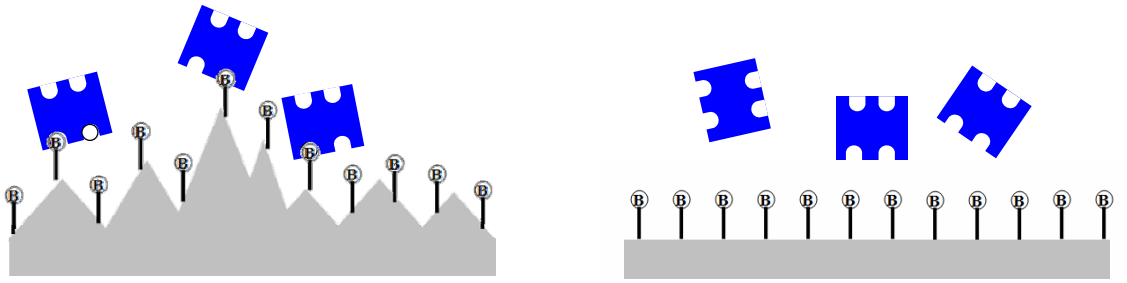
Supplementary Figures



(a) (b)

Figure S1: Schematic of 57 % surface coverage of biotin immobilized on (a) a rough surface, (b) a planar surface. Steric crowding effects do not inhibit all SA binding on the rough surface at this biotin concentration. Dimensions not to scale.

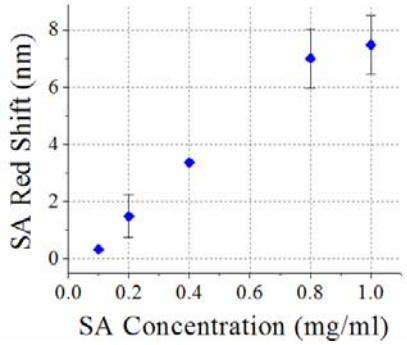


Figure S2: SA binding to a fixed surface coverage of biotin (Biotin-LC-LC-NHS=0.33 mg ml^{-1}). SA binding is observed to begin to saturate at ~0.8 mg ml^{-1}.

				Conc. to cover PSi surface area (Theory)		*Optimal Solution Conc. (Experimental)*	
Molecule	***radius, r*** *(nm)*	***molecular weight*** *(Da)*	***Area, A*** *(cm2)*	*(nmol)*	*(mg/ml)*	*(nmol)*	*mg/ml*
biotin-LC-LC-NHS	0.3	669.75	3.83E-15	15.3	0.685	11.2	0.5
SA	2.5	53,000	1.96E-13	0.22	0.78	0.198	0.07
B-Ab	5.6	150,000	9.85E-13	0.044	0.4399	0.007	0.07

Figure S3: Geometric Model Approximations of Molecule Concentrations. Geometric model based on area approximations used to quantify theoretical maximum amount of bound molecules to form a monolayer on internal surface area of PSi device (SA = 26.1cm^2)[26]. Concentrations (conc.) based on 15µl solution volume. All optimal solution concentrations determined by optimization of linking chemistry (discussed in section 3.1 of paper) are less than maximum amount calculated by geometric approximations. This data supports the idea that all molecules applied to sensor surface at each optimal value have enough surface area to bind.

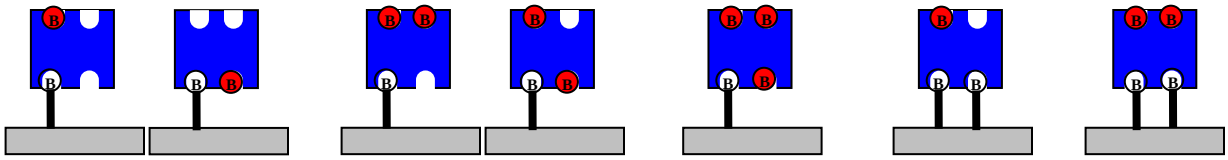


Figure S4: Schematic of SA bound to biotin-LC-LC-NHS (SA-biotin*) with varying number of biotin-binding sites preblocked with biotin in solution. Illustrations of SA-biotin* complex via single link with (a)1 biotin-binding site blocked with biotin, (b)2 biotin-binding sites blocked with biotin, (c)3 biotin-binding sites blocked with biotin. Illustrations of SA-biotin* complex via two links with (d)1 biotin-binding site blocked with biotin, (e)2 biotin-binding sites blocked with biotin.

Binding Behavior of Preblocked SA to Surface-Linked Biotin

The amount of SA bound to surface-linked biotin was examined as a function of the number of preblocked biotin-binding sites (PB-BBS) on the SA molecule for two different values of biotin surface coverage (10 % and 57 %). Results plotted in Figure S5 indicate that for both values of biotin coverage the amount of SA that binds decreases as the number of PB-BBS increases as expected. The effect is more pronounced at a higher (57 %) biotin coverage where SA binding begins to substantially decrease (~42 % of maximum) with 2 PB-BBS (Figure S5 (b)) and continues to fall below 20 % of the maximum with 3 PB-BBS. For lower biotin surface coverage (10%) the SA binding

decrease is delayed and only falls to below 80 % the maximum when 3 biotin-binding sites are blocked. A negative control proved true for both values of biotin surface coverage, where zero red shift was observed when all 4 PB-BBS were blocked.

(a) Low (10%) Biotin Surface Coverage (b) Medium (50%) Biotin Surface Coverage

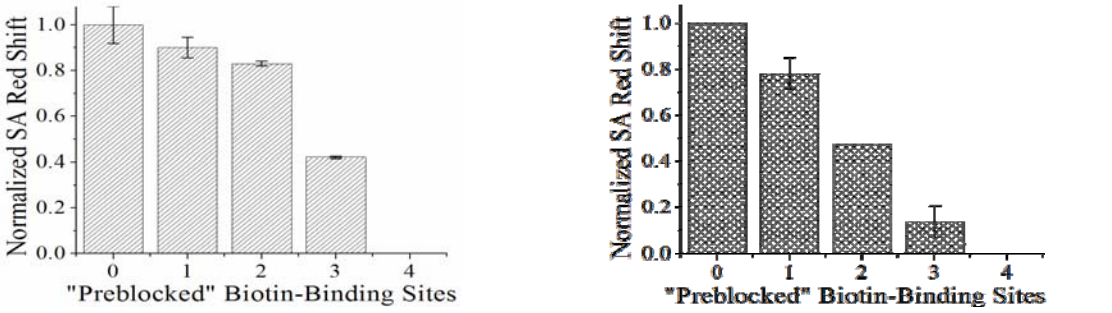


Figure S5: Bar graphs of normalized SA red shift as a function of the number of biotin-binding sites pre-blocked with biotin: (a) Low (10%) biotin surface coverage. Normalized data derived from experiments employing 3 different streptavidin concentrations (0.1, 0.2, 0.5 mg ml^{-1}). (b) Medium (57%) biotin surface coverage. Normalized data derived from experiments employing a SA concentration of 0.1mg ml^{-1}. (a) - (b) All concentrations refer to solution concentrations applied to sensor.

This data supports the SA-biotin linking model proposed by Pérez-Luna et al.[13] and Jung et al.[12] on planar surfaces. The more dramatic decrease in SA binding at 57 % biotin surface coverage (where steric crowding initiates on PSi) is consistent with SA binding via 2 links to the surface-linked biotin. With 2 PB-BBS, the SA can only bind to the surface-linked biotin in one configuration in which the PB-BBSs occur on the same side of the SA molecule (Refer to Figure S4). Statistically this has a probability of occurring 33 % of the time. When binding via 1 link (with 2 PB-BBS) the SA could bind in two possible configurations (PB-BBS on same side, 1 PB-BBS on each side of the SA molecule); therefore, the SA would have a much greater probability of binding (as seen in Figure S5(a) for 10 % biotin surface coverage). Hence, the behavior of SA binding shown in Figure S5 is also consistent with the model proposed by Pérez-Luna et al.[13] where at lower (10 %) biotin surface coverage SA attaches via 1 link to the surface bound biotin.

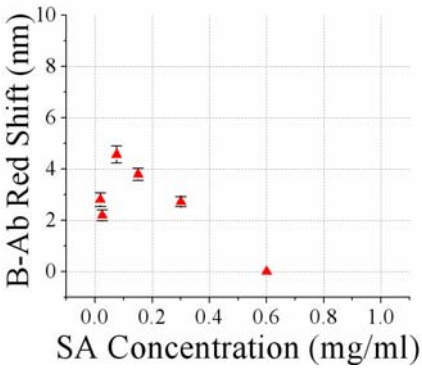


Figure S6: B-Ab binding to "Preblocked" SA (1 PB-BBS) on low (10%) biotin surface coverage. ([biotin-LC-LC-NHS]=0.1 mg ml^{-1}, [B-Ab]=0.5mg/ml)

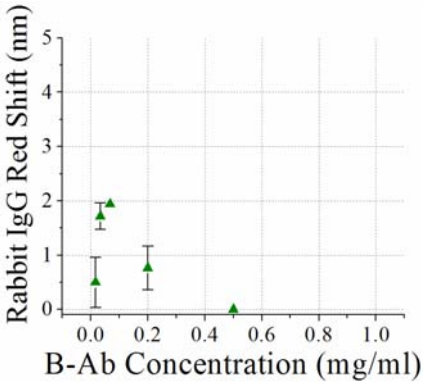


Figure S7: Target (Rabbit IgG) detection using "preblocked" SA (2 PB-BBS) on low (10%) biotin surface coverage. ([biotin-LC-LC-NHS]=0.1 mg ml^{-1}, [preblocked SA]=0.3 mg ml^{-1}) The SA concentration was chosen based on the peak value in Figure 5(d) (maximum binding of B-Ab ~4.8 nm)

Confirmation of "Bottom Up" Methodology

A question arises as to whether the "bottom-up" method does configure the layers to achieve maximum target detection sensitivity. The first check is shown in Figure 5 where the receptor (B-Ab) binding was examined as a function of biotin and SA surface coverage. It can be seen that the maximum binding of B-Ab does occur for biotin surface coverage of 57 % with a maximum red shift of approximately 8.6 nm (Fig 5(b)). This optimal biotin concentration corresponds to that found by the "bottom-up" method when the binding of streptavidin was analyzed as a function of surface-linked biotin concentration (Fig 3(b)). A second check was performed by analyzing the binding of target protein (rabbit IgG=1 mg ml^{-1}) as a function of SA concentration. The biotin-LC-LC-NHS was kept constant at the optimal 0.5 mg ml^{-1} and the B-Ab receptor concentration was kept constant at the optimal 0.07 mg ml^{-1}. The maximum binding of target occurred at the same optimal value of SA as was determined by the "bottom up" method which was 0.07 mg ml^{-1} (Fig S8).

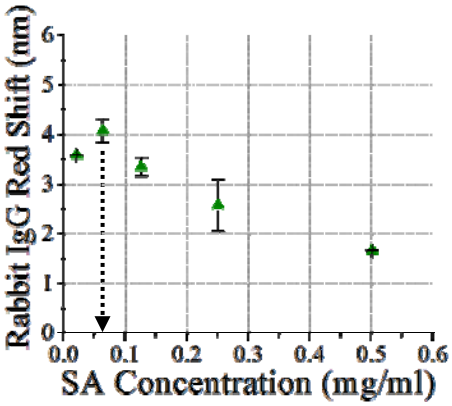


Figure S8: Binding of Target Rabbit IgG as a function of SA Concentration. ([Biotin-LC-LC-NHS] = 0.5 mg ml^{-1} , [B-Ab] = 0.07 mg ml^{-1})