

Time-Resolved Molecular Transport across Living Cell Membranes

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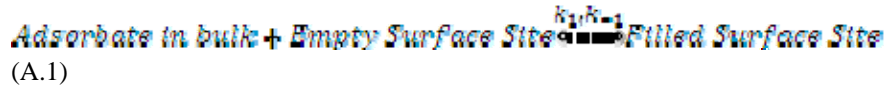
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SUPPORTING INFORMATION

Modified Langmuir Adsorption Model

The *Langmuir* adsorption model has three assumptions: adsorption is limited to a monolayer, the surface is divided into sites for adsorption with equal probability of adsorption to each site, and there are no inter-adsorbate interactions (1). Adsorption is then described as the “reaction” between the adsorbates in the bulk solution and those on the colloidal surface.



where k_1 and k_{-1} are the rate constants for adsorption and desorption, respectively. The kinetic equation describing the time dependent density of adsorbates is as follows:

$$\frac{dN}{dt} = k_1 \frac{(C - N)}{55.5} (N_{\max} - N) - k_{-1}N \quad (\text{A.2})$$

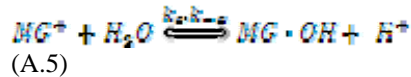
where N is the number of adsorbed molecules on the surface, N_{\max} is the maximum adsorption density, C is the total concentration of the adsorbate molecules, including both the molecules in the bulk and adsorbed on the surface. 55.5 is the molarity of water. At equilibrium, $\frac{dN}{dt} = 0$. Thus, the expression for surface coverage, θ , can be related to C , N_{\max} and the equilibrium constant K (defined as $K = k_1 / k_{-1}$) as:

$$\theta = \frac{N}{N_{\max}} = \frac{(C + N_{\max} + \frac{55.5}{K}) - \sqrt{(C + N_{\max} + \frac{55.5}{K})^2 - 4CN_{\max}}}{2N_{\max}} \quad (\text{A.3})$$

The model can be used to analyze the adsorption isotherm of malachite green (MG^+) adsorption at colloidal surfaces and K and N_{\max} can be obtained from the isotherm fitting. K can then be used to determine the adsorption free energy using $\Delta G = -RT \ln K$.

Kinetic Model of Malachite Green Transport

At $\text{pH}=7.4$, which is slightly above the reported pKa of MG^+ of 6.8 (2), only a fraction of MG molecules exist in cationic form. However, the resonantly enhanced Second Harmonic signal is dominated by the MG^+ species and thus, both equilibria involving MG in the system must be considered:



The kinetic equations are:

$$\frac{dN}{dt} = k_1 \frac{[\text{MG}^+]}{55.5} (N_{\max} - N) - k_{-1}N \quad (\text{A.6})$$

$$\frac{d[\text{MG}^+]}{dt} = -k_1 \frac{[\text{MG}^+]}{55.5} (N_{\max} - N) + k_{-1}N - k_2[\text{MG}^+] + k_{-2}[\text{MG} \cdot \text{OH}][\text{H}^+] \quad (\text{A.7})$$

where

$$C = [MG \cdot OH] + [MG^+] + N \quad (\text{A.8})$$

$[MG \cdot OH]$, $[MG^+]$ and N are the number of $MG \cdot OH$ in bulk, MG^+ cations in bulk, and MG^+ cations adsorbed on the cell membrane surface, respectively. k_a and k_{-a} are the ionization rate constants. The total adsorption/desorption and transport physical process can be expressed as:



where M , M_1 , and M_2 represent MG^+ molecules in the bulk solution outside the cell membrane, in the periplasmic space in between the two membranes, and in the cytoplasmic space inside the inner membrane, respectively. E_{1o} , E_{1i} , E_{2o} , and E_{2i} denote the empty adsorption sites on the outer surface of the outer membrane, on the inner surface of the outer membrane, on the outer surface of the cytoplasmic membrane, and on the inner surface of the cytoplasmic membrane, respectively. k_i / k_{-i} ($i=1, 3, 4$) are the adsorption/desorption rate constant on the respective cell membrane surfaces. k_2 and k_5 are the transport rate constant through the outer membrane and the cytoplasmic membrane, respectively. In the model, we assume equal forward/backward transport rate constant for both membranes. Also, due to the symmetry of the cytoplasmic membrane, it is also reasonable to assume equal maximum number densities for both sides of the cytoplasmic membrane. The physical model can be written-up as differential equations:

$$\frac{dM}{dt} = -k_1 \cdot M \cdot (N_{1o}^{max} - N_{1o}) + k_{-1} \cdot M_{H_2O} \cdot N_{1o} \quad (\text{A.15})$$

$$\frac{dN_{1o}}{dt} = k_1 \cdot M \cdot (N_{1o}^{max} - N_{1o}) - k_{-1} \cdot M_{H_2O} \cdot N_{1o} - k_2 \cdot (N_{1o} - N_{1i}) \quad (\text{A.16})$$

$$\frac{dN_{1i}}{dt} = k_2 \cdot (N_{1o} - N_{1i}) + k_3 \cdot M_1 \cdot (N_{1i}^{max} - N_{1i}) - k_{-3} \cdot M_{H_2O} \cdot N_{1i} \quad (\text{A.17})$$

$$\frac{dM_1}{dt} = -k_3 \cdot M_1 \cdot (N_{1i}^{max} - N_{1i}) + k_{-3} \cdot M_{H_2O} \cdot N_{1i} - k_4 \cdot M_1 \cdot (N_{2o}^{max} - N_{2o}) + k_{-4} \cdot M_{H_2O} \cdot N_{2o} \quad (\text{A.18})$$

$$\frac{dN_{2o}}{dt} = k_4 \cdot M_1 \cdot (N_{2o}^{max} - N_{2o}) - k_{-4} \cdot M_{H_2O} \cdot N_{2o} - k_5 \cdot (N_{2o} - N_{2i}) \quad (\text{A.19})$$

$$\frac{dN_{2i}}{dt} = k_5 \cdot (N_{2o} - N_{2i}) + k_4 \cdot M_2 \cdot (N_{2i}^{max} - N_{2i}) - k_{-4} \cdot M_{H_2O} \cdot N_{2i} \quad (\text{A.20})$$

$$\frac{dM_2}{dt} = -k_4 \cdot M_2 \cdot (N_{2i}^{max} - N_{2i}) + k_{-4} \cdot M_{H_2O} \cdot N_{2i} \quad (\text{A.21})$$

where N_{10}^{max} , N_{1f}^{max} , N_{20}^{max} , N_{2f}^{max} are the maximum number densities on all four surfaces and M_{H_2O} is the water molarity 55.5M. The kinetic model allows incremental determination of the surface coverages N_{10} , N_{1f} , N_{20} , N_{2f} as a function of time.

Finally, the SH intensity, I_{SH} , is related to these surface MG^+ ions as:

$$I_{SH} \propto (N_{10} - N_{1f} + N_{20} - N_{2f})^2 \quad (A.22)$$

The experimentally observed SH signal as a function of time can be fit through a nonlinear least-square program for the determination of the adsorption equilibrium constants $K_i = k_i / k_{-i}$ ($i=1, 3, 4$) ($K_{1i}=K_3$, $K_{2o=2i}=K_4$), the transport rate constants k_2 and k_5 , as well as the maximum surface coverage N_{10}^{max} , N_{1f}^{max} , and $N_{20}^{max}=N_{2f}^{max}$.

[1] Evans, D.F. and H. Wennerström, *The Colloidal Domain- where physics, chemistry, biology, and technology meet*. 2nd ed. 1999, New York: Wiley-VCH.

[2] Anthony-Barbier, A.M., P. Rumpf, and C. Viel, *Spectrophotometric study of ionization equilibriums of a diaminated triphenylcarbinol.*, in *Bulletin de la Societe Chimique de France* 1959. p. 1474-1481.