Supporting information

Binding Mechanism of Model Charged Dye Carboxyfluorescein to Hyaluronan/Polylysine Multilayers

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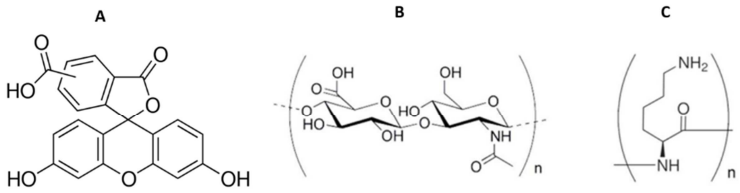


Figure S1. Molecular structure of CF (A), HA (B), and PLL (C).

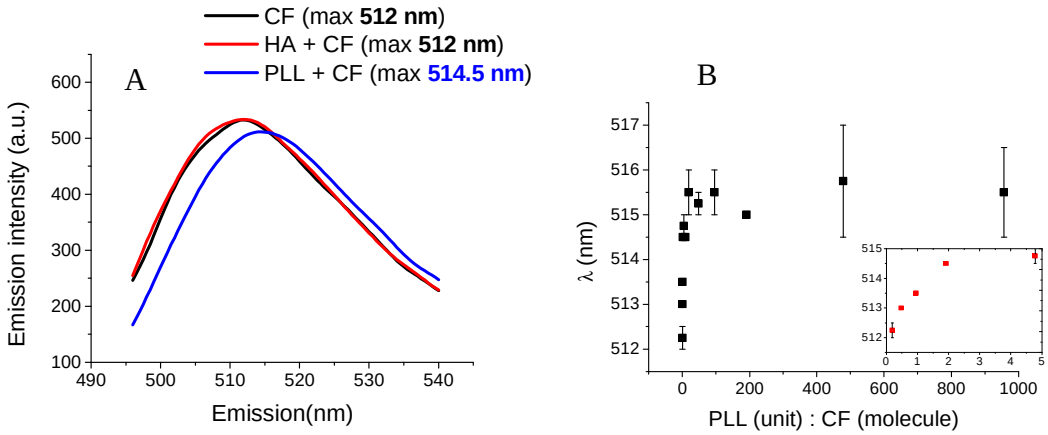


Figure S2. A - Fluorescence emission spectra of 20 µM CF in Tris-buffer (black) and in the buffer containing 0.5 mg/mL HA (red) or 0.5 mg/mL PLL (blue). B - Maxima of fluorescence emission spectra of 5 µM CF in Tris-buffer containing PLL at different PLL:CF molar charge ratio.

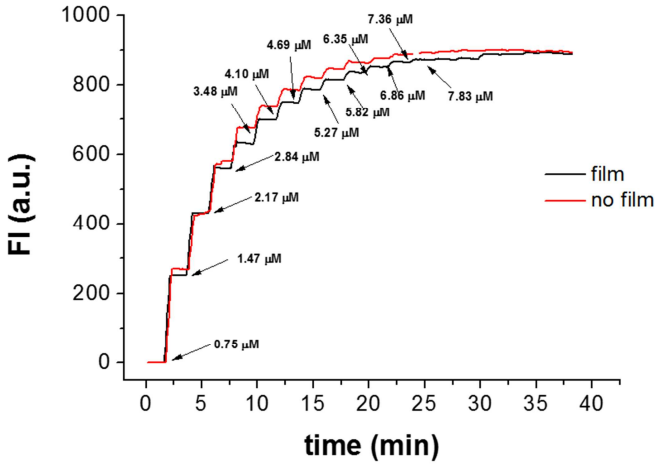
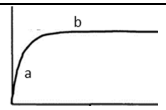
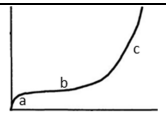
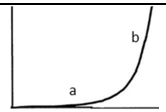
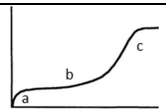
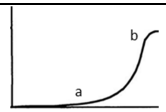
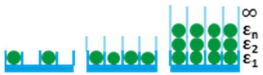
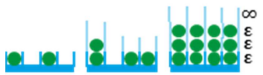


Figure S3. Fluorescence registered from the $(HA/PLL)_{24}$ multilayers deposited on a cover slip as a function of time when CF was added to the multilayers step by step with continuous stirring the solution around the multilayers. For more details see experimental section. The arrows indicate time points at which concentration of CF in the solution was increased to the designated value, assuming that no adsorption of CF took place (control sample – the uncoated cover slip, red curve). The difference in concentrations between supernatant and the control sample at each step was used to calculate the amount stored in the film and construct the adsorption isotherm (Fig. 5).

Table S1. Summary on 6 adsorption models[1] used for the mathematical treatment of the isotherm of adsorption of CF into HA/PLL multilayer film. Corresponding mathematical equations as well as fitted parameters are described in Table 1.

Model	Basic assumptions	
Langmuir	Monoadsorption: CF molecules are anchored to free NH_2 groups of PLL	- Isotherm may be derived from statistical thermodynamics; - CF molecules occupy free NH_2 groups of PLL which are homogeneously distributed inside HA/PLL film; once a dye molecule occupies a site, no additional adsorption can occur there; - No CF-CF interactions in the film - Model describes adsorption in a wide range of concentrations
Freundlich	Polyadsorption: Multiple CF molecules can be adsorbed to each amino group and/or each adsorbed CF provides a site for the adsorption of the molecule in the layer above it	- Empirical model; - CF molecules occupy NH_2 groups of PLL and each adsorbed CF provides a site for the adsorption of the molecule in the layer above it (heterogeneous distribution of surface energy systems). Depending on the value of n, the model describes the case of competitive adsorption of CF molecules ($n<1$) or cooperative adsorption ($n>1$); $n=1$ – no CF-CF interaction; - Model failed at high concentration because it does not predict a saturation limit
Langmuir–Freundlich		- Empirical, generalization of Langmuir and Freundlich isotherms; - Similarly to Freundlich model describes cooperative/competitive adsorption ($n = 1$ represents no interacting site, $0<n<1$ a positive cooperativity and $n>1$ a negative cooperativity, i.e. competition); - Model describes adsorption in a wide range of concentrations, predicts saturation limit
Temkin		- Empirical model; - CF molecules occupy uniformly distributed NH_2 groups of PLL and provide new sites for the adsorption of CF molecule in the layer above, but due to CF-CF repulsion the binding energy decreases linearly with the sorption coverage; - Model is applicable for the intermediate concentration range and failed at low and high concentrations
Sigmoidal Langmuir		- Empirically generalized Langmuir isotherm; - Describes cooperative adsorption of CF molecules, parameter S reflects the strength of cooperation; - Model describes adsorption in a wide range of concentrations
BET		- Isotherm may be derived from statistical thermodynamics; - CF molecules occupy uniformly distributed NH_2 groups of PLL and provide new sites for the adsorption of CF molecule in the layer above. Depending on parameters, BET isotherm describes different adsorption mechanism (for more details, see Table S1); - Model describes adsorption in a wide range of concentrations (isotherm types I, IV, V) or failed at high concentration because it does not predict a saturation limit (isotherm types II and II)

Table S2. The summary on 5 types of BET isotherms[2] adopted for the adsorption of CF molecules into HA/PLL multilayer film. In the schemes, green round represent CF molecules, blue line illustrate PLL chain. Blue lines indicate the cites for the adsorption.

BET Isotherm Type	**Type I**	**Type II**	**Type III**	**Type IV**	**Type V**
Type of adsorption	Monoadsorption: PLL free amino groups in HA/PLL film act as receptor cites. Each receptor cite in HA/PLL film can be empty or occupied by one CF molecule. No CF-CF interaction is assumed	Polyadsorption: multiple CF molecules can be adsorbed to each receptor cite in HA/PLL film site or each adsorbed CF molecule provides a new site for the adsorption of the molecule in the layer above it			
Representative curve					
Schematic illustration of adsorption mechanism					
Description of adsorption mechanism; n - number of adsorbed molecules per cite	First CF molecules are anchored to free amino groups of PLL, n<1 (this corresponds to zone a, each amino group is occupied by one CF molecule or empty). At high concentration of CF (zone b) n=1 (each amino group is occupied by one CF molecule)	Zones a and b are the same as for Type I isotherm: CF molecules are anchored to amino groups. When the first layer of NH_2-anchored CF being formed (zone b), the second level of CF is adsorbed to CF molecules stored in the film (CF-CF interaction). The model does not predict saturation limit, i.e. at high concentration (zone c) $n\rightarrow\infty$: each receptor cite is occupied by an infinity of CF molecules	At low concentration each amino group is occupied by one or multiple CF molecules or empty (n<1, zone a). At high concentration (zone b) $n\rightarrow\infty$ (each receptor cite is occupied by an infinity of CF molecules), this is the same as zone c for Type II isotherm (no saturation is predicted)	Intermediate case between Types I and II Zones a and b are the same as for Type II isotherm: CF molecules are anchored to amino groups via PLL-CF interaction. At high concentration (zone c) more CF molecules are adsorbed to CF in the film (CF-CF interaction) but in contrary with Type II isotherm, value of n is finite (each receptor cite is occupied by a limited # of CF molecules)	Intermediate case between Types I and III Zone a is the same as for Type III isotherm. In contrary with Type III isotherm, at high concentration (zone b), value of n is finite (each receptor cite is occupied by a limited number of CF molecules)
Adsorption energy level (ε)	All receptor cites in HA/PLL film are equivalent, i.e. all CF molecules are adsorbed with the same energy (-ε)	All receptor cites within each level are equivalent. This means that the first CF molecules are anchored to amino groups of PLL with energy $-\varepsilon_1$, the second layer of CF molecules which are anchored to CF molecules stored in the film is absorbed with energy $-\varepsilon_2$, etc. $\varepsilon_1>\varepsilon_2>\ldots>\varepsilon_n$	All receptor cites in HA/PLL film (both, amino groups of PLL and all layers of CF molecules in the film) are equivalent, i.e. all CF molecules are adsorbed with the same/closed values of energy (-ε)	The same as Type II	The same as Type III

References

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2. Khalfaoui, M.; Knani, S.; Hachicha, M. A.; Ben Lamine, A., New Theoretical Expressions for the Five Adsorption Type Isotherms Classified by BET Based on Statistical Physics Treatment. *J. Colloid Interface Sci.* **2003,** 263, 350-356.