

Aggregation and cooperative binding of Streptavidin with biotinylated Immunoglobulin G

Jörg Ackermann, Wolfgang Erker and Natasa Kukoc-Zivojnov

Abstract

The interaction of streptavidin with biotinylated human Immunoglobulin G is studied by single molecule fluorescence fluctuations experiments. Utilizing flUIT's Accurate Stochastic Fluorescence Spectroscopy offers new insight into the binding characteristics of this system. A concerted aggregation of labeled streptavidin and biotinylated Immunoglobulin G to large complexes was observed. The question of cooperative binding is tackled by computing the Hill constant. The obtained value of $n_H = 1.67 \pm 0.09$ is well above one and, hence, indicates cooperative binding.

Introduction

Streptavidin (STV) is tetrameric protein with molecular mass of 52.8 kD from the bacterium *Streptomyces avidinii* and has four biotin binding sites with an extremely strong affinity. Although the binding of biotinylated IgG (around 150 kD) to Streptavidin may not have the same affinity as free biotin to STV, this pair is widely used as a standard system in molecular biology, therapeutics and diagnostics.

Materials and method

Atto532-STV conjugate (from Atto-Tec GmbH) was mixed with biotinylated monoclonal mouse anti-human IgG (IgG-Biotin, Invitrogen) in PBS buffer. Samples with constant concentrations of Atto532-STV and varying concentrations of Biotin-IgG were prepared. They were measured in the ConSense Analyzer with laser excitation at 532 nm and 160 μ W. The arrival of single photons from the confocal optical set-up were recorded with high temporal resolution for 30 s and then analyzed with the Virtual Lab software. Three measurements were performed for each sample.

Results and discussion

The binding curve of labeled STV to biotinylated IgG is shown in Figure 1. Upon binding the total fluorescence intensity increased by more than one order of magnitude. Because the fluorophore concentration was held constant, binding was connected with a strong increase of the fluorescence quantum yield.

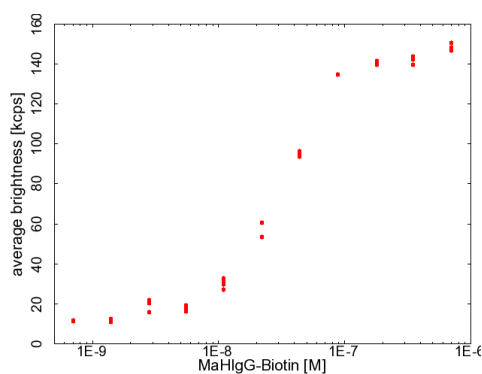


Fig.1 Binding curve of labeled STV with biotinylated IgG. The concentration of streptavidin was held constant (7.32 nM) whereas the concentration of biotinylated IgG was varied, see x-axis. Upon binding the intensity (y-axis) of the fluorescence light increased significantly.

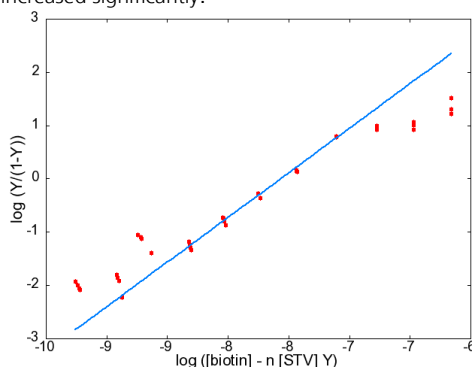
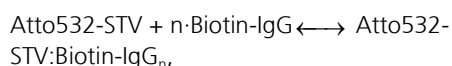


Fig. 2: A typical Hill plot for the binding of Atto532-STV to biotinylated IgG with a straight line in the region where the binding occurs. The slope of the fitted straight line gives a Hill coefficient of $n_H = 1.67 \pm 0.09$ which is significantly greater than one and, hence, indicates cooperative binding.

A perfect cooperative binding is represented by mass action equilibrium



In which no complex with fewer than n molecules of IgG exists. Assuming such simple two-state binding model leads directly to the well-known Hill plot and the determination of the Hill constant n_H

A value of n_H identical to the number of binding sides of STV, i.e. four, would proof perfect cooperative binding, whereas a value of one (or below) stands for non-cooperative binding. The Hill plot for the data from Figure 1 is shown in Figure 2. The obtained value of 1.69 strongly suggest a (while not perfect) cooperative binding. Y is the fraction saturation of the labeled STV.

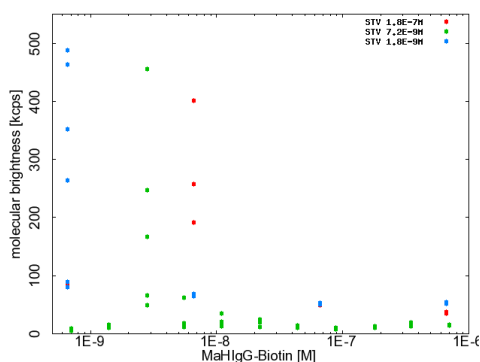


Fig. 3: Plot of the molecular brightness ϵ versus concentration of biotinylated IgG for various concentration of Atto532-STV. Typically a maximum in ϵ appears at a concentration ratio Atto532-STV:IgG of around 2.8:1.

A different insight into the binding mechanism was obtained by inspection of the molecular brightness ϵ appearing during binding, see Figure 3. The molecular brightness ϵ is the maximal fluorescence of a single particle of a kind and reflects a variety of molecular parameters, such as the number and the quantum yield of the fluorophore attached to the particle. Such a particle may consist of several aggregated biomolecules. Epsilon is one of the central parameters of the ASFS method.

The plot is dominated by a distinct peak in ϵ at a certain concentration of biotin-IgG. The concentration at which such high values for ϵ appear depends on the initial concentration of labeled STV.

Decreasing the concentration of labeled STV shifts the position of the maximum to lower values. Typically the maximum in ϵ appears at a concentration ratio labeled STV:IgG of 2.8:1.

The observation of species with very high molecular brightness indicates the emergence of complexes containing a large number of labeled STV. Since each molecule of biotinylated IgG is tagged with several biotins, the IgG is able to bind several labeled STV molecules. Therefore, we attribute the peaks to aggregates with high molar mass and, consequently, a large number of fluorophores.

Since all experiments with different labeled STV concentrations exhibit aggregate peaks, it can be assumed that the dissociation constant of this interaction is well below the range studied here.

The analysis of diffusion times shows that the high molecular brightness attributed to aggregates is correlated with higher diffusion times.

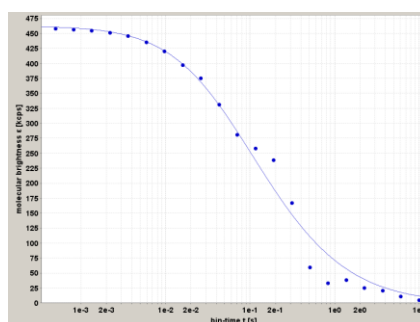


Fig. 4: Molecular brightness versus bin time for aggregated STV + Biotin-IgG. The decay curve is dominated by the typical diffusion times (30-100 ms) of aggregates. The diffusion times for labeled STV saturated with Biotin-IgG and free Atto532-STV are 9 ms and 0.6 ms respectively.

Combining the results based on brightness, epsilon and diffusion time, mixing of labeled STV with Biotin-IgG leads to the formation of aggregates whose sizes vary dramatically with the mixing stoichiometry.