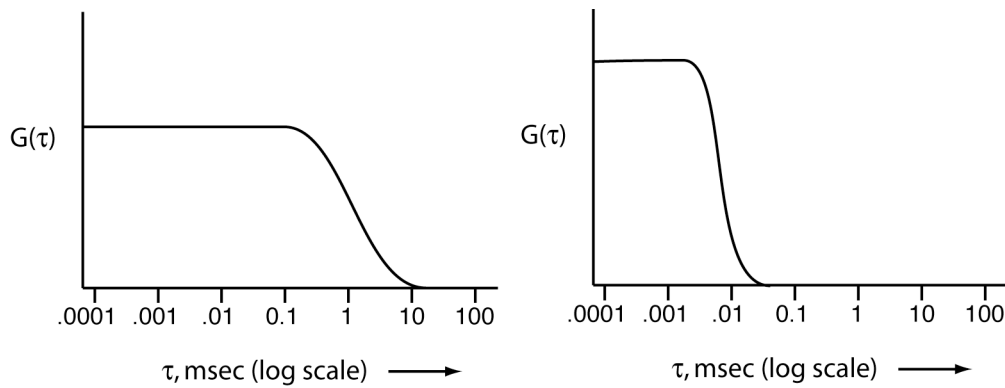


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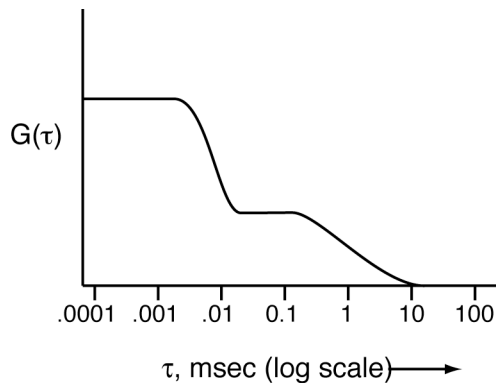
Study Questions on Fluorescence Correlation Spectroscopy

1. Two samples are analyzed by fluorescence correlation spectroscopy. One sample contains an antibiotic peptide (molecular weight roughly 1200 Da) labeled with a covalently attached green-fluorescent probe group. The other sample contains lipid vesicles (with a diameter of roughly 200 nm) that are labeled with a red-fluorescent lipid probe.

(a) The following autocorrelation functions $G(t)$ are obtained for the two samples. Which would be expected for which sample?



(b) A third sample is analyzed in which the peptide and the lipid vesicles are mixed. The following autocorrelation function is obtained monitoring the peptide (green) fluorescence:

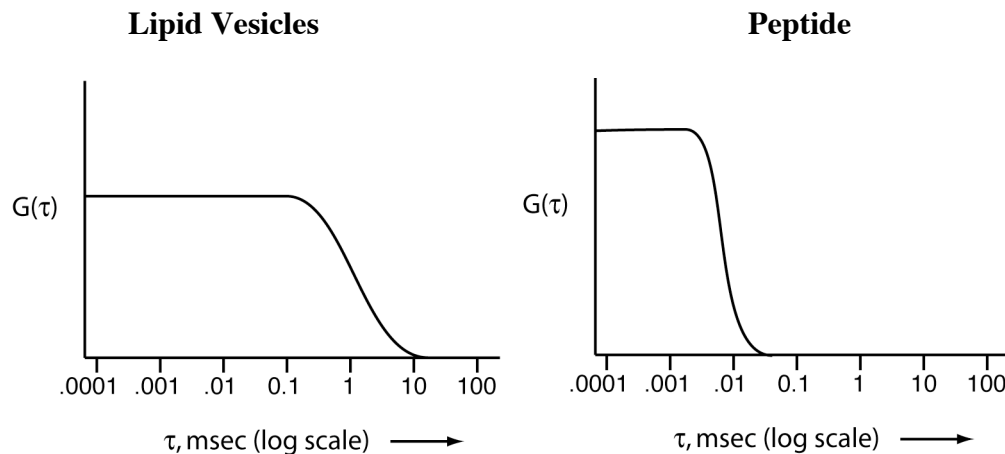


What do these results indicate? Explain your answer.

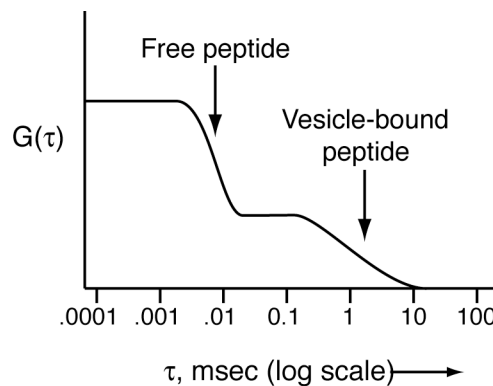
Answers - BIOC 404 FCS Study Questions

1 (a). The small peptide will have a much larger diffusion coefficient than the much larger liposomes. In a fluorescence correlation experiment, the fluctuations in the intensity of fluorescence due to (random) diffusion of fluorescent particles into and out of the small confocal volume become faster as the diffusion coefficient increases. We thus expect these fluctuations to be much faster (i.e., to occur on a much shorter time scale) for the peptide than for the lipid vesicles.

Now recall that the position of the inflection point in a plot of the autocorrelation function $G(\tau)$ gives a rough indication of the time scale ($\propto 1/(\text{rate})$) of the fluctuations just discussed. The plot on the right in this question indicates much faster fluctuations (occurring on a much shorter/faster time scale) than does the plot on the left. The plots can thus be assigned as follows:



(b) Here there are two inflection points in the plot of $G(\tau)$ vs. τ , one similar to that observed above for the free peptide and the second similar to that observed for the lipid vesicles. The simplest explanation is that the peptide is present in two forms: free and vesicle-bound:



We can determine the relative proportions of free and bound peptide from such results, although this should be done by appropriate curve-fitting and not just by visual inspection.