

## Numerical problems in fluorescence quenching

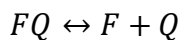
1. The table contains lifetime data N-methylacridinium iodide (N-MEAI) quenching by guanosine-5'-monophosphate (GMP). A. Assuming that the quenching is dynamic determine the quenching rate constant,  $k_q$ . B. Assuming that the quenching has both dynamic and static components the data can be fit to the following equation:

$$\frac{\Phi_0}{\Phi} = (1 + \tau_0 k_q [Q])(1 + K_a [Q])$$

where  $K_a$  is the association binding constant of the quencher to the fluorophore. Use this equation to estimate the values of  $k_q$  and  $K$ . C. Show that the above equation can be derived from the result:

$$\Phi = f\Phi_0$$

where  $f$  is the fraction of *unbound* fluorophores and  $K_d$  is the corresponding dissociation equilibrium constant. Calling the fluorophore, F, and the quencher, Q, the reaction is:



and

$$K_d = \frac{[F][Q]}{[FQ]}$$

The data obtained using TCSPC are given in the table below.

[GMP] ( $10^{-3}$ M)	$\tau$ (ns)
0	34.8
1	30.9
2	27.8
3	25
4	22.5
6	18.8
8	15.7

2. Given the intensity data in the table below, determine the optimal concentration for a fluorescence measurement. Specifically, that optimum refers to the maximum concentration possible that does not suffer from internal absorption.

[A] ( $10^{-7}$ M)	I (counts)
1.2	5203
2.4	10104
4.8	20340
9.2	39980
18.4	77034
36.8	125502

73.4	221053
146.8	355035