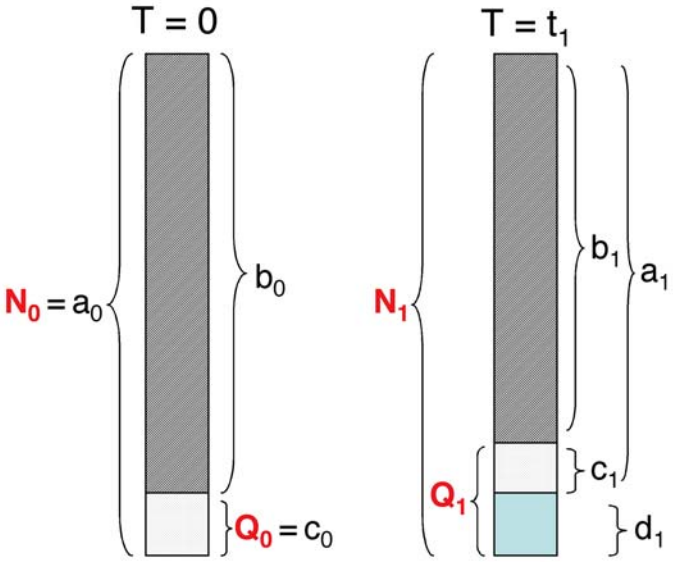


= quenchable surface (b)

= residual surface (c)

= internalized (d)

$$b_1 = a_1 - c_1 = N_1 - Q_1 \qquad (1)$$

$$a_1 \left(1 - \frac{c_1}{a_1}\right) = N_1 - Q_1$$

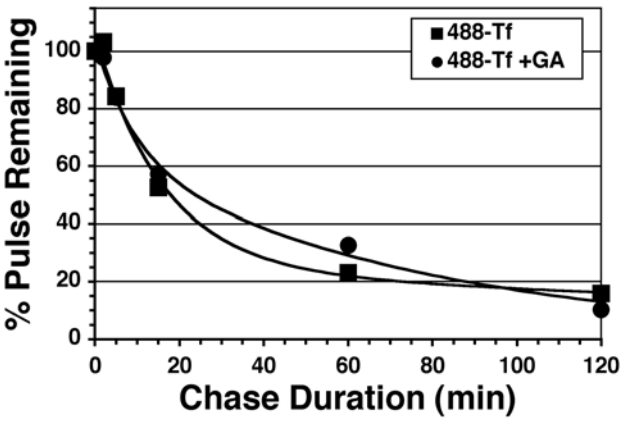
$$a_1 = \frac{(N_1 - Q_1)}{\left(1 - \frac{c_1}{a_1}\right)} \qquad (2)$$

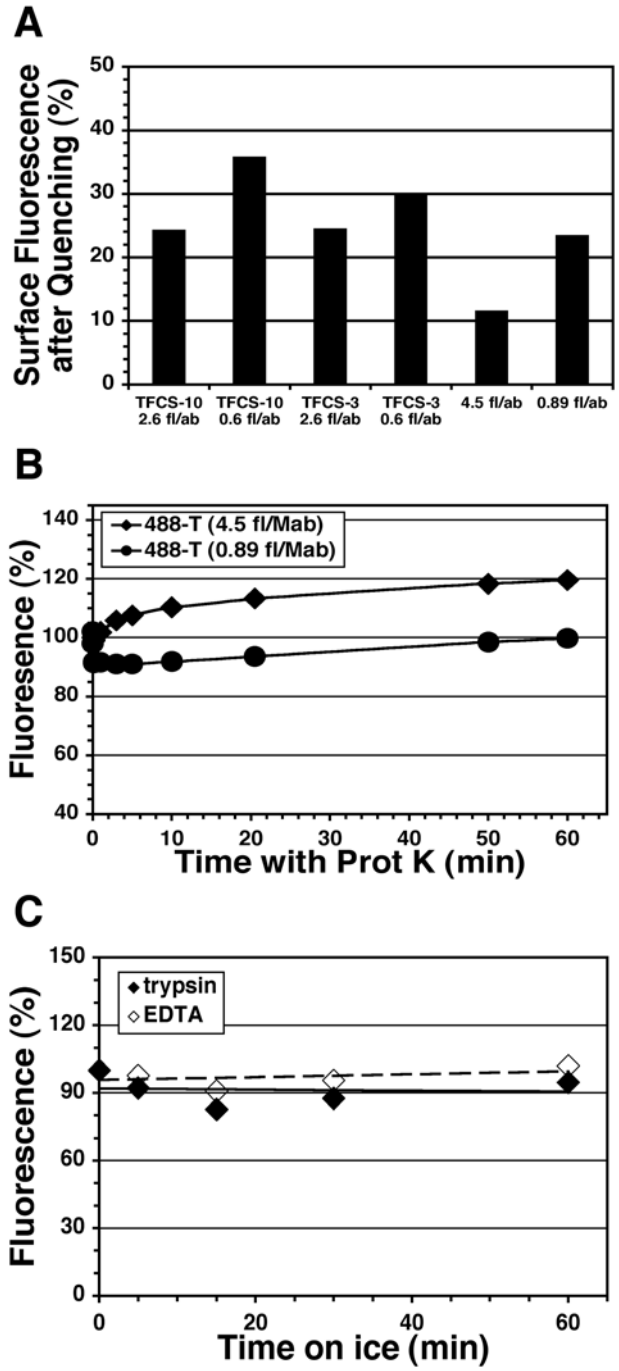
assume $\frac{c_1}{a_1} = \frac{c_0}{a_0} = \frac{Q_0}{N_0}$

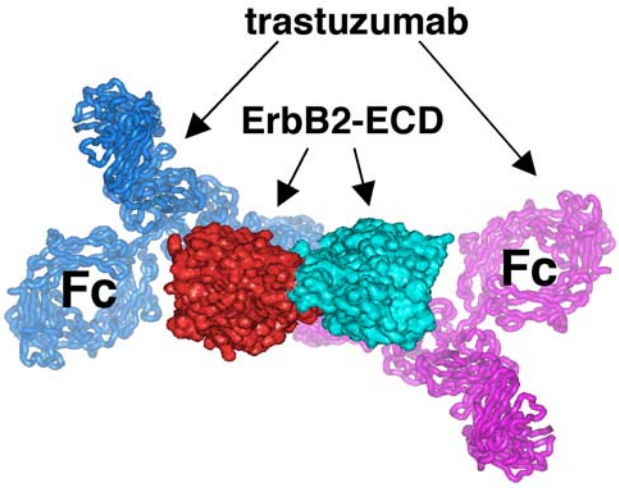
$$a_1 = \frac{N_1 - Q_1}{\left(1 - \frac{Q_0}{N_0}\right)} \qquad (3)$$

$$\frac{d_1}{N_1} = \frac{N_1 - a_1}{N_1} = 1 - \left(\frac{N_1 - Q_1}{N_1 - \left(\frac{N_1 Q_0}{N_0}\right)}\right) \qquad (4)$$

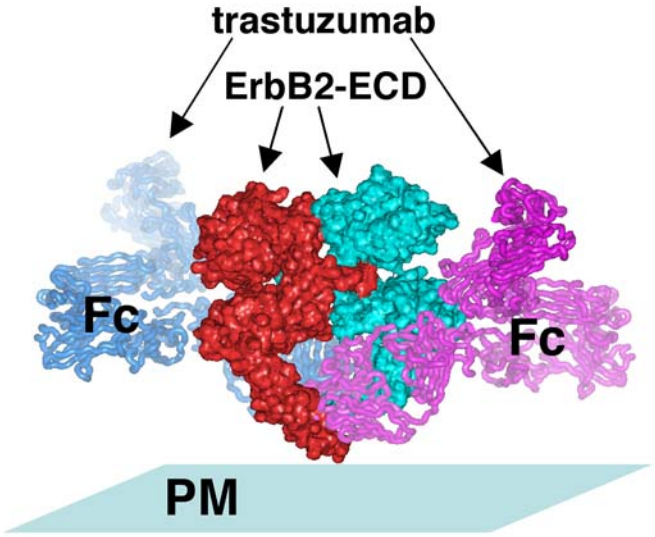
$$\text{fraction internalized} = \left(\frac{d_1}{N}\right) = \left(\frac{Q_1 - Q_0}{N - Q_0}\right) \qquad (5)$$


% Pulse Remaining
100
80
60
40
20
0
0
20
40
60
80
100
120
Chase Duration (min)
488-Tf
488-Tf +GA


A
Surface Fluorescence after Quenching (%)
50
40
30
20
10
0
TFCS-10 2.6 fl/ab
TFCS-10 0.6 fl/ab
TFCS-3 2.6 fl/ab
TFCS-3 0.6 fl/ab
4.5 fl/ab
0.89 fl/ab
B
Fluorescence (%)
140
120
100
80
60
40
488-T (4.5 fl/Mab)
488-T (0.89 fl/Mab)
0
10
20
30
40
50
60
Time with Prot K (min)
C
Fluorescence (%)
150
120
90
60
30
0
trypsin
EDTA
0
20
40
60
Time on ice (min)



Top View



Lateral View