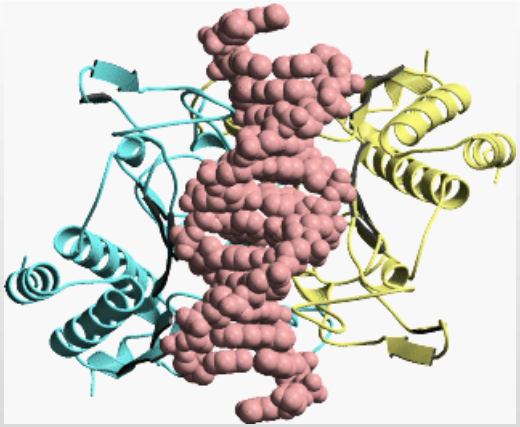
Background - Reversible Associations

Reversible Systems:



Self-association, hetero-association, or multiple reactions ($A + A + B <=> A_2B$)

The concentration in one component affects the concentration of another

Reactions can be fast (diffusion controlled) or slow (kinetically limited)

...and they observe mass action laws

Background - Reversible Associations

Reversible Reaction:

$$\sum_{i=1}^{n} M = M_n$$

Equilibrium Constant:

$$K_a = \frac{[M_n]}{[M]^n} \qquad K_d = \frac{[M]^n}{[M_n]}$$

Kinetics:

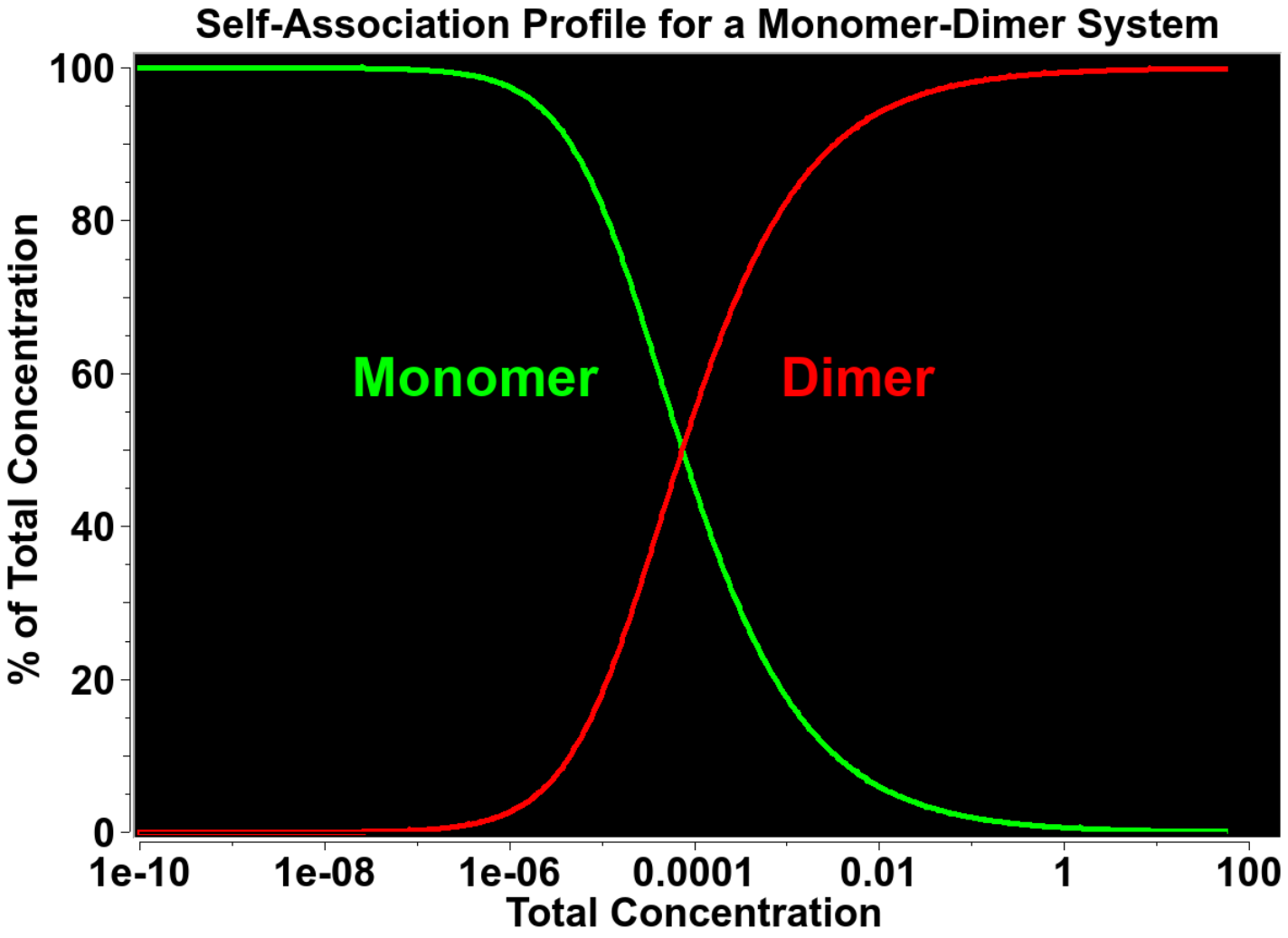
$$K_a = \frac{k_{on}}{k_{off}}$$

$$[M] + [M_n] = C_{total}$$

Solve polynomial:

$$[M] + K_A[M]^n - C_{total} = 0$$

Background - Reversible Associations



A Model for Reversible Reactions

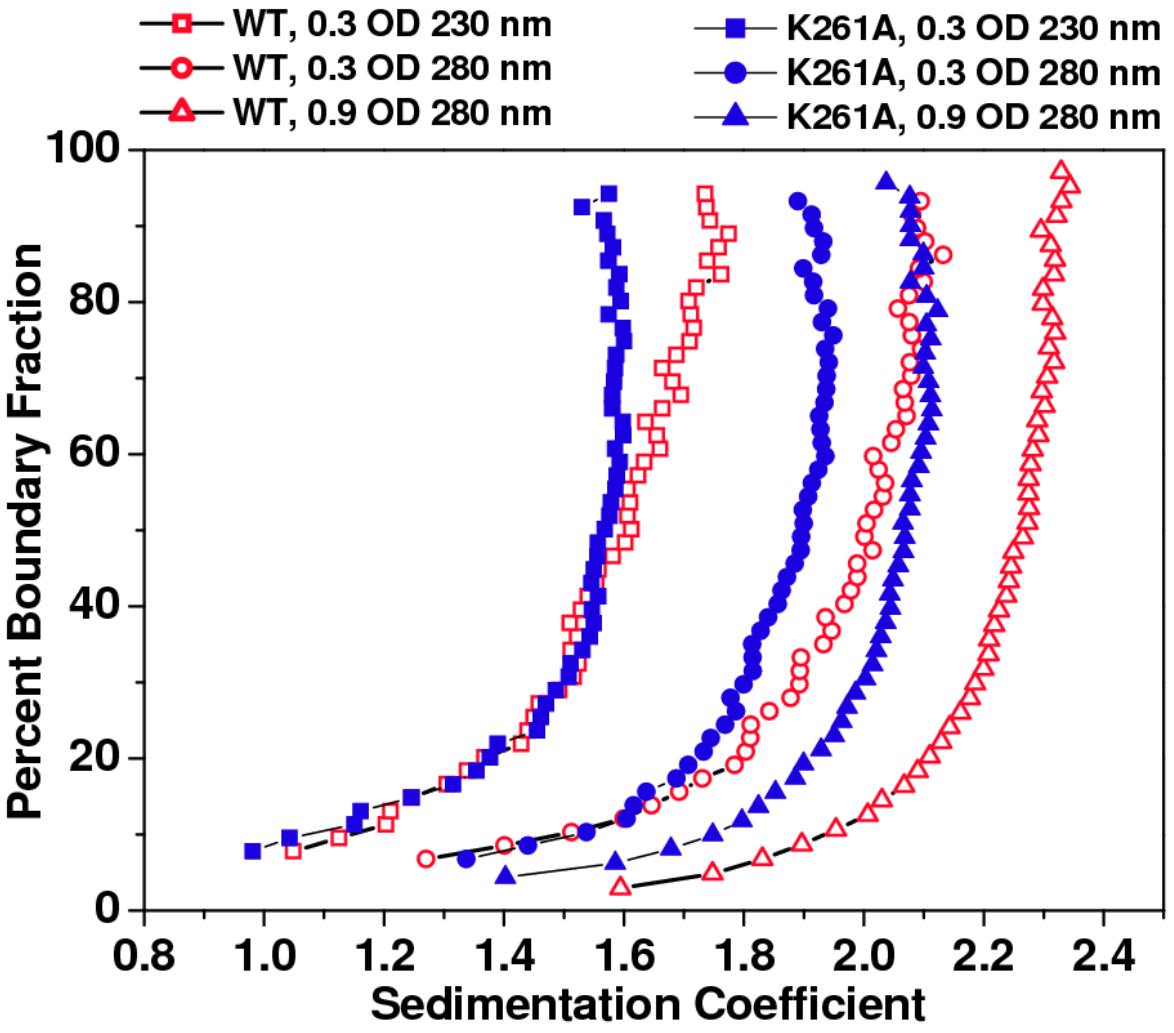
In the gradient, the *weight-average* sedimentation coefficient and the *gradient-average* diffusion coefficient are observed:

$$\bar{s} = \frac{\sum_{j=1}^{m} s_j C_j}{C_T} = \frac{\sum_{j=1}^{m} s_j K_j C_1^j}{C_T} \qquad \bar{D} = \frac{\sum_{j=1}^{m} D_j (\partial C_j / \partial r)}{\sum_{j=1}^{m} (\partial C_j / \partial r)} = \frac{\sum_{j=1}^{m} j D_j K_j C_1^{j-1}}{\sum_{j=1}^{m} j K_j C_1^{j-1}}$$

Claverie, J.-M., Dreux, H., and R. Cohen (1975). Sedimentation of Generalized Systems of Interacting Particles. I. Solutions of Systems of Complete Lamm Equations. Biopolymers 14:1685-1700

Todd GP, Haschemeyer RH. General solution to the inverse problem of the differential equation of the ultracentrifuge. Proc Natl Acad Sci U S A. 1981 78(11):6739-43.

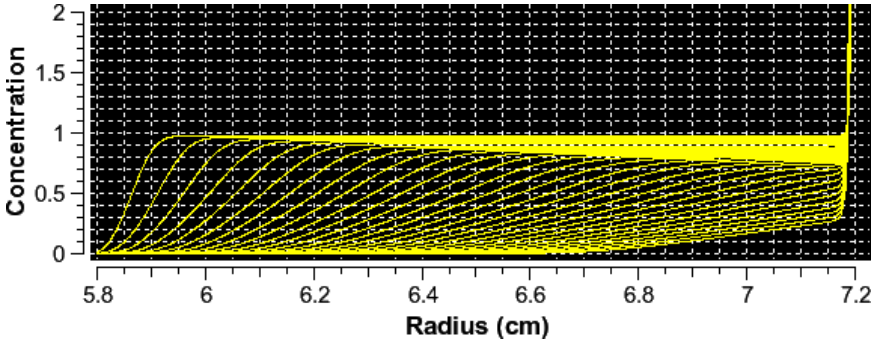
Monomer-Dimer Interface Mutation Analysis



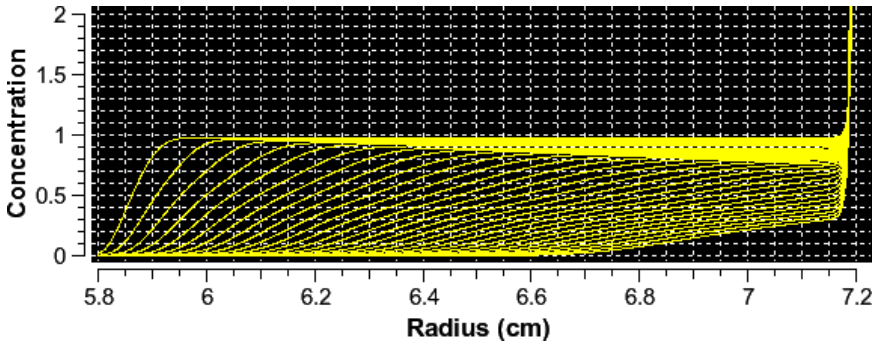
Models for Reacting Systems:

Monomer – Trimer Equilibrium, Monomer MW = 50 kDa

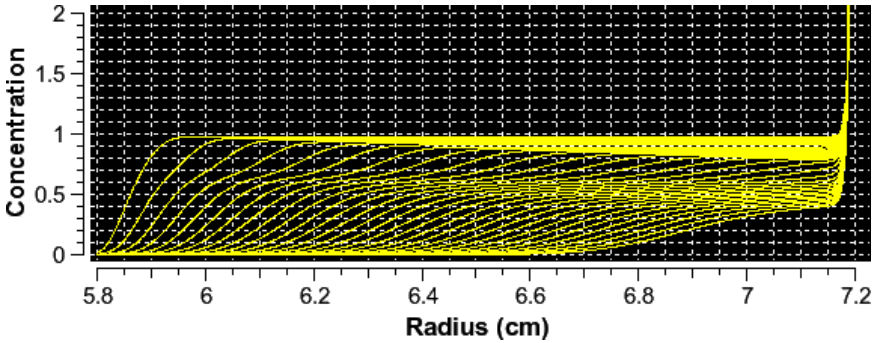
K_{off} = 1.0/sec



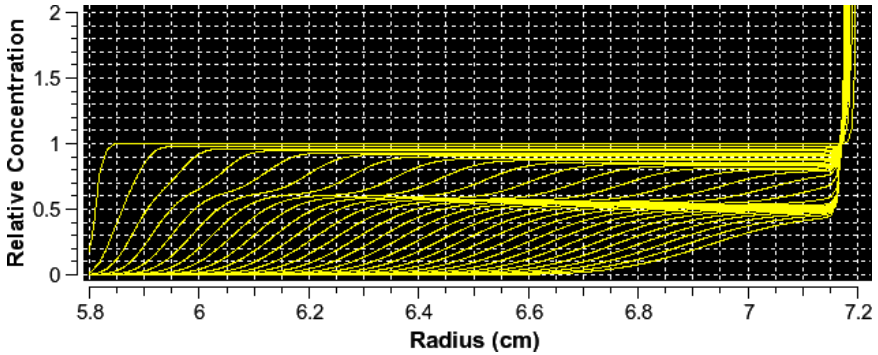
K_{off} = 1.0 x 10^{-3}/sec



K_{off} = 1.0 x 10^{-4}/sec



non-interacting



Models for Reacting Systems:

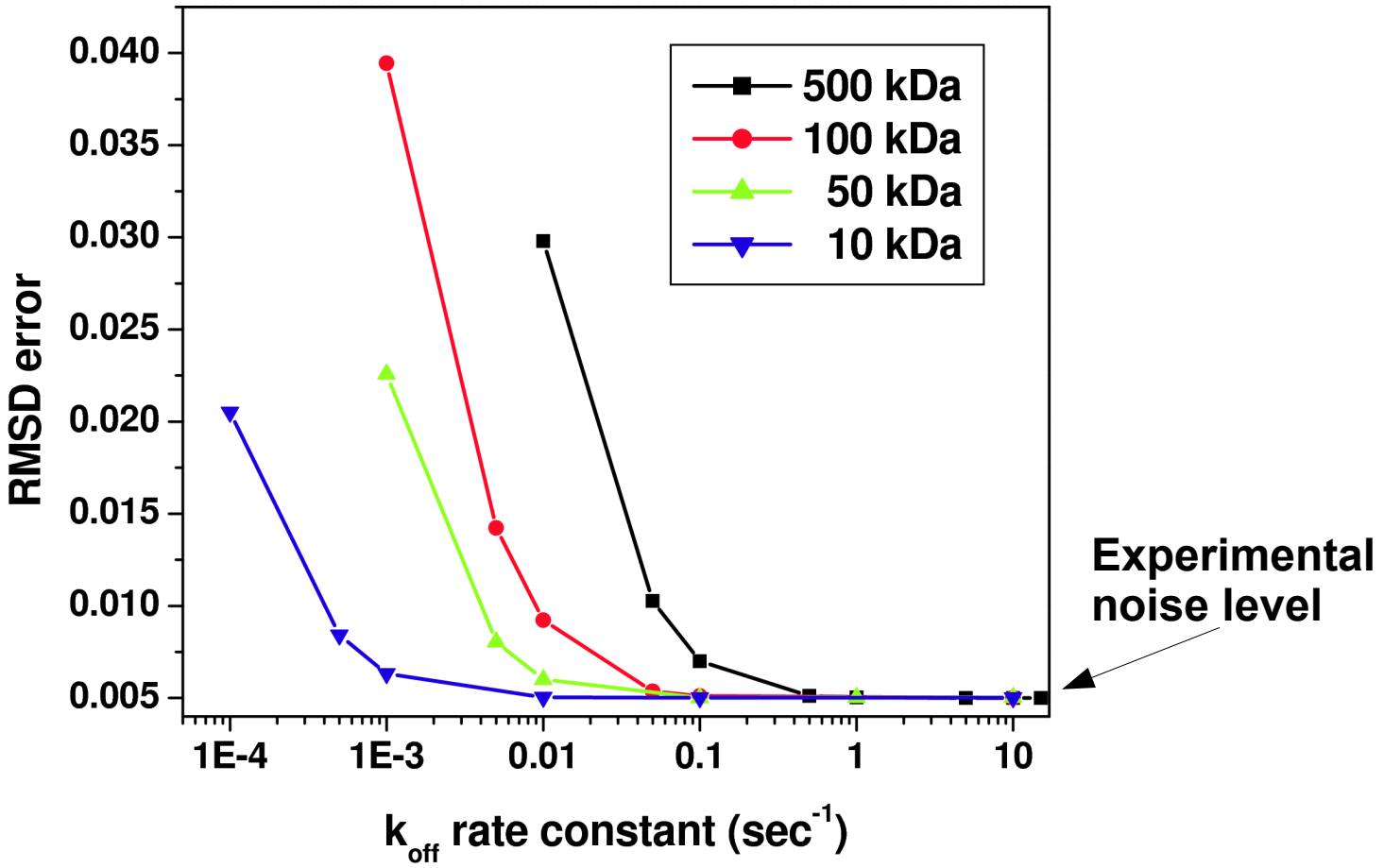
The magnitude of the measurable off-rate depends on rotor speed and sedimentation coefficient:

$$s \sim \frac{M}{f}$$

Faster rotor speed, higher molecular weight and globular shape will favor the measurement of faster rate constants.

Models for Reacting Systems:

Range of measurable k_{off} rate constants for different MW



Genetic Algorithm Optimization:

Genetic Algorithms (also called evolutionary programming) provide a stochastic optimization method

Holland J, Adaption in Natural and Artificial Systems, 1975, U. of Michigan Press

Based on nature – evolutionary paradigm

Mutation, recombination, deletion, insertion, crossover operators

Random number generators are used to manipulate operators

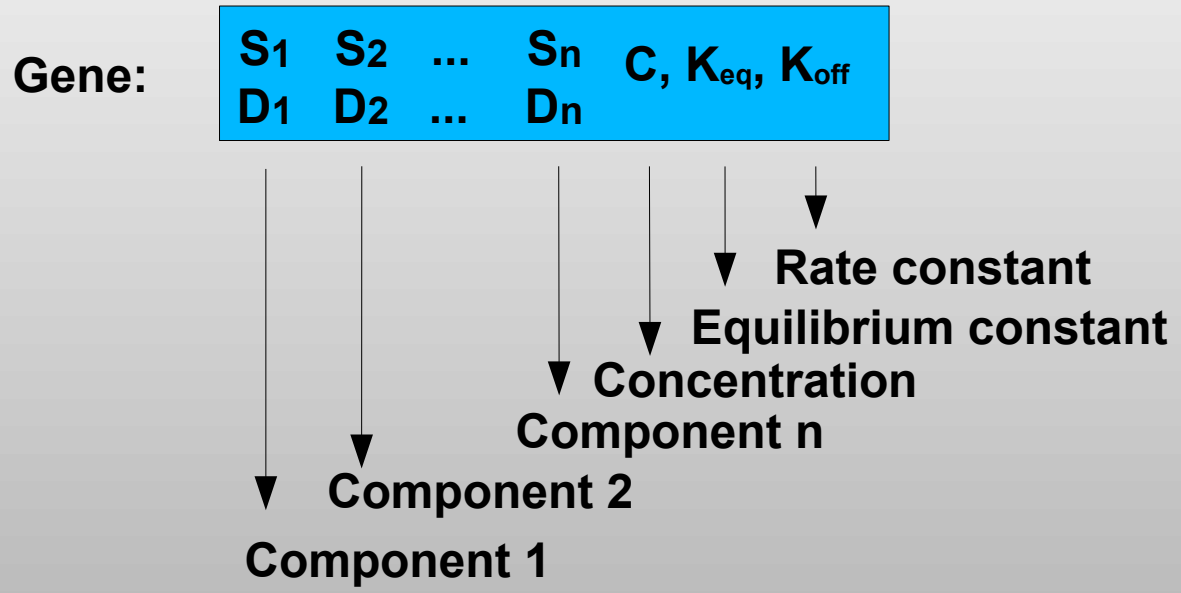
Generational Model – survival of the fittest (...fitting function)

Generation → iterations, genes → parameter strings, bases → s, D, K_d, k_{off}

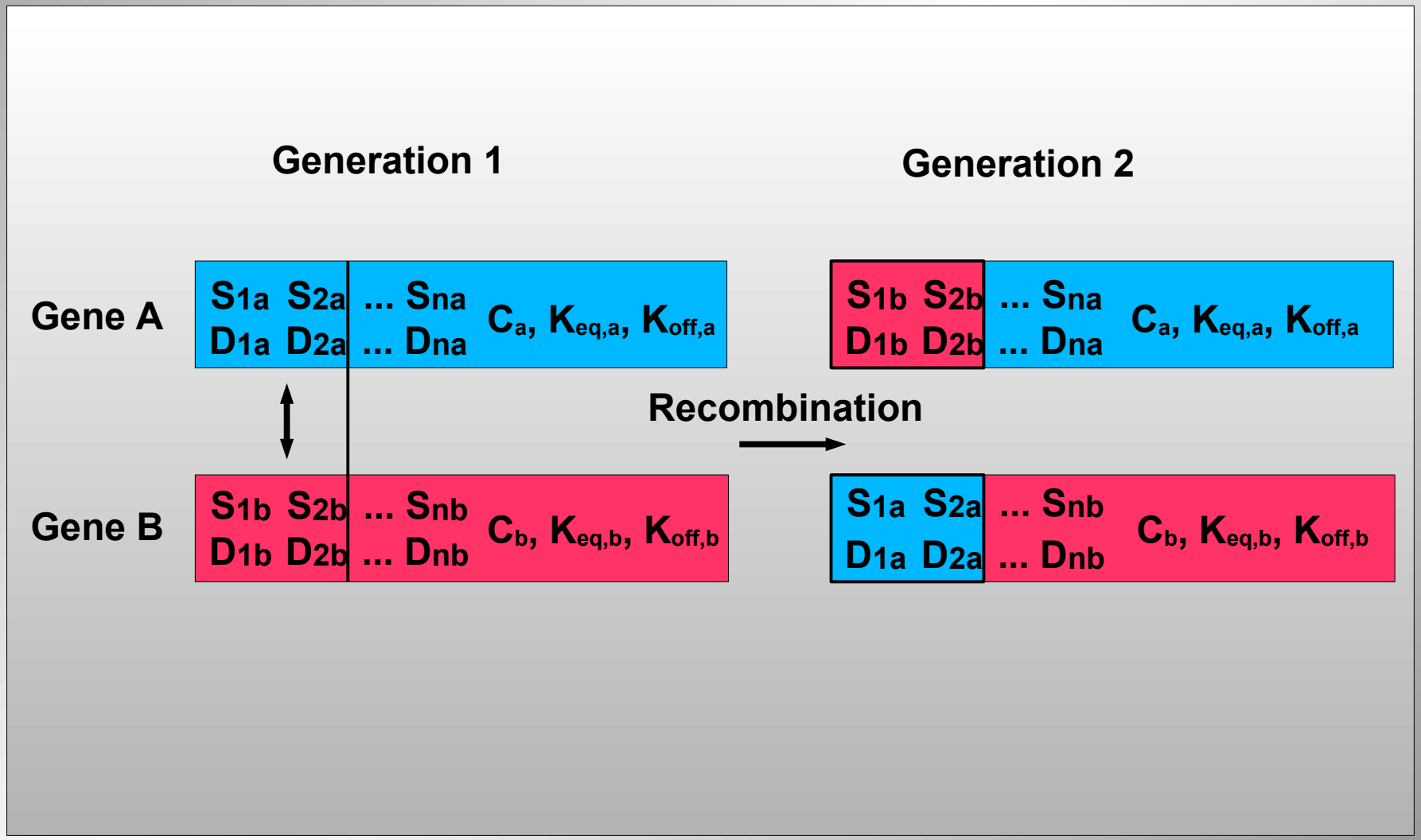
Use a Monte Carlo analysis to determine confidence level from noisy data

Genetic Algorithm Optimization:

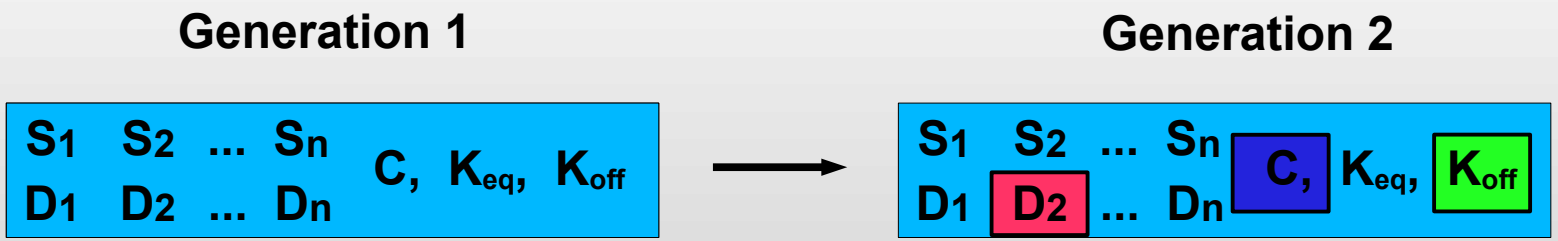
Genes are strings of parameters, each gene consists of a pair of corresponding sedimentation and diffusion coefficients, loading concentration, equilibrium constants and rate constants.



Crossover/Recombination



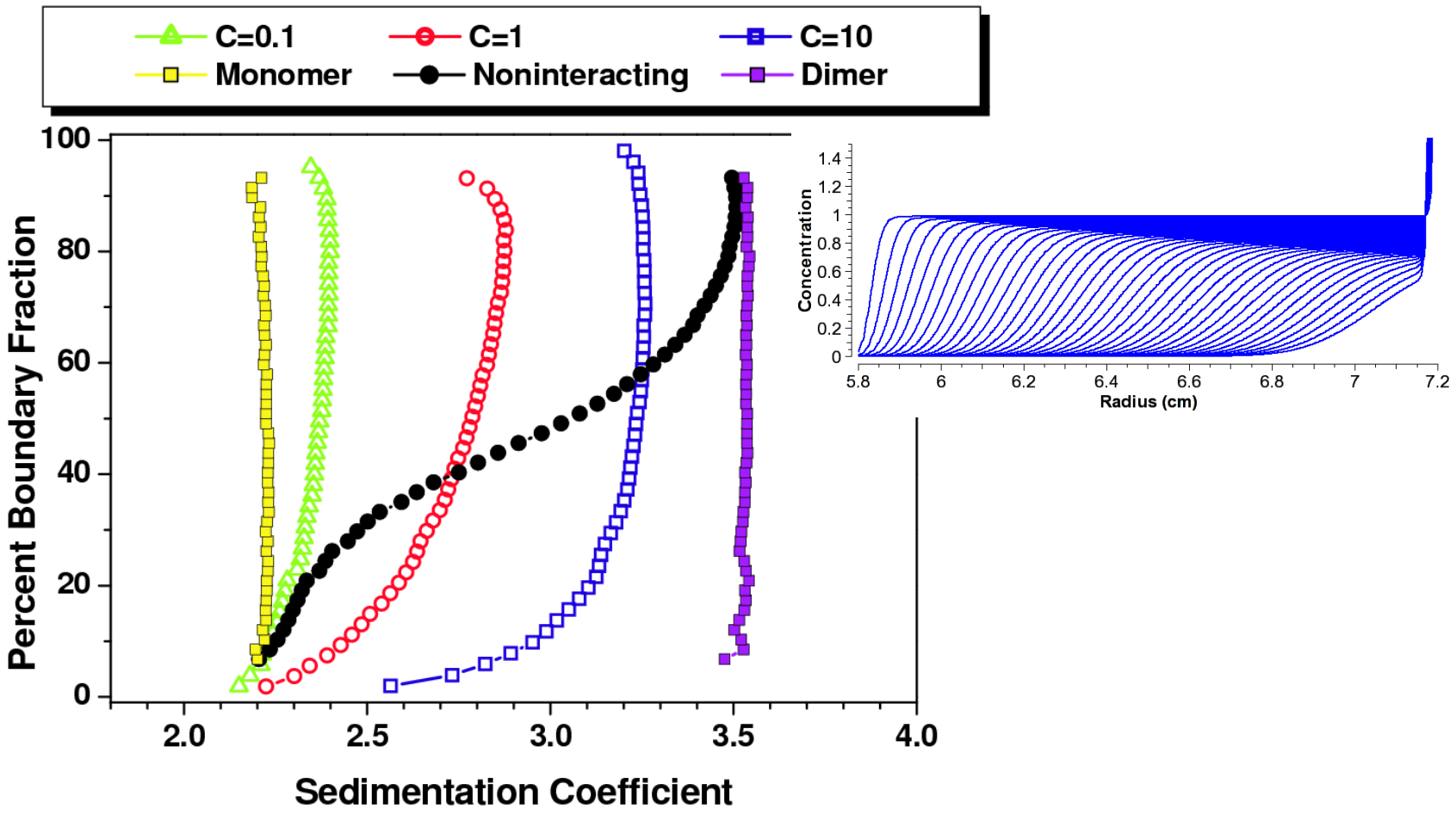
Mutation



Mutation Event
(within linear constraints)
maintaining molecular weight, shape constraints

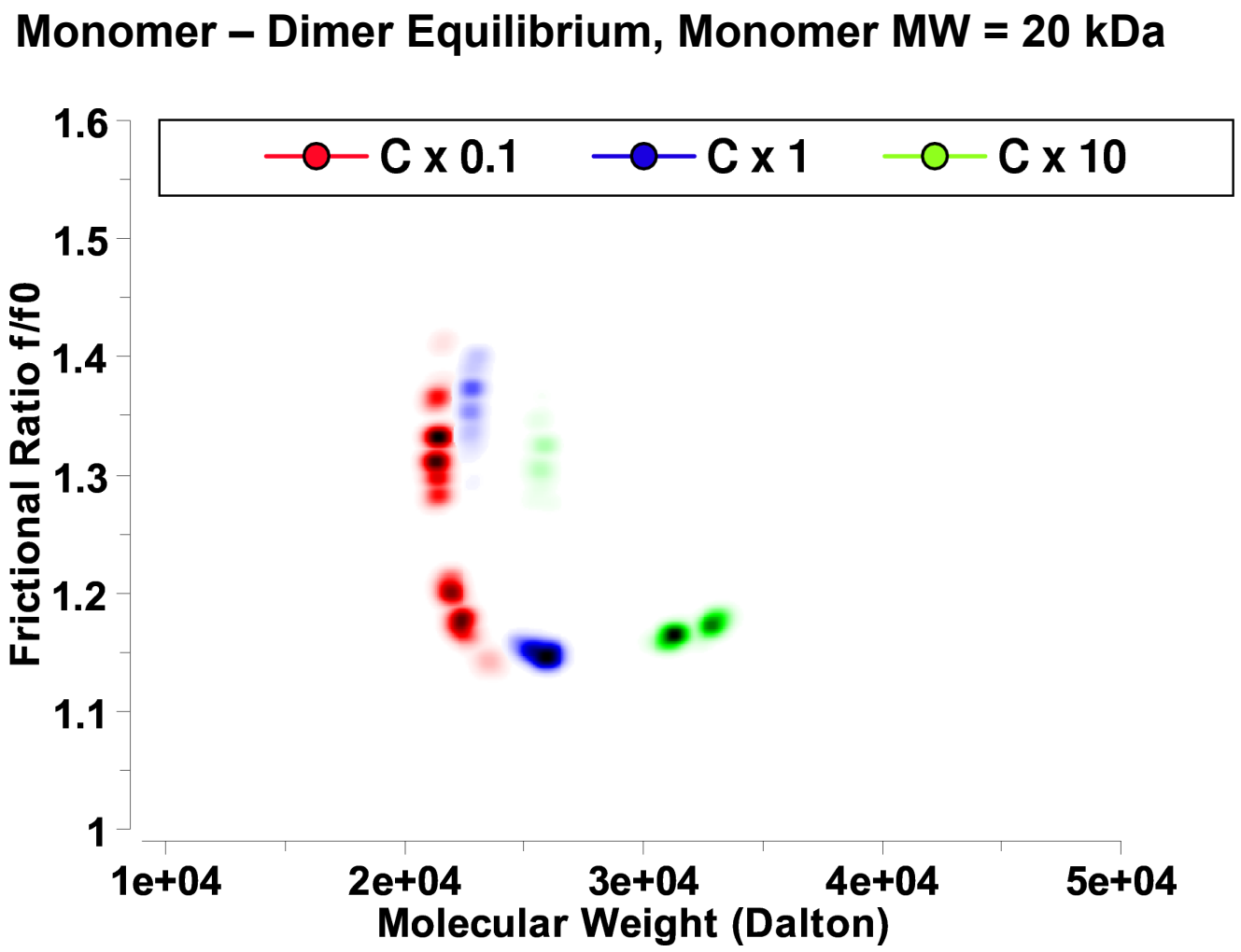
Diagnostics: van Holde – Weischet Analysis

Example 1: Simulated Monomer – Dimer Equilibrium

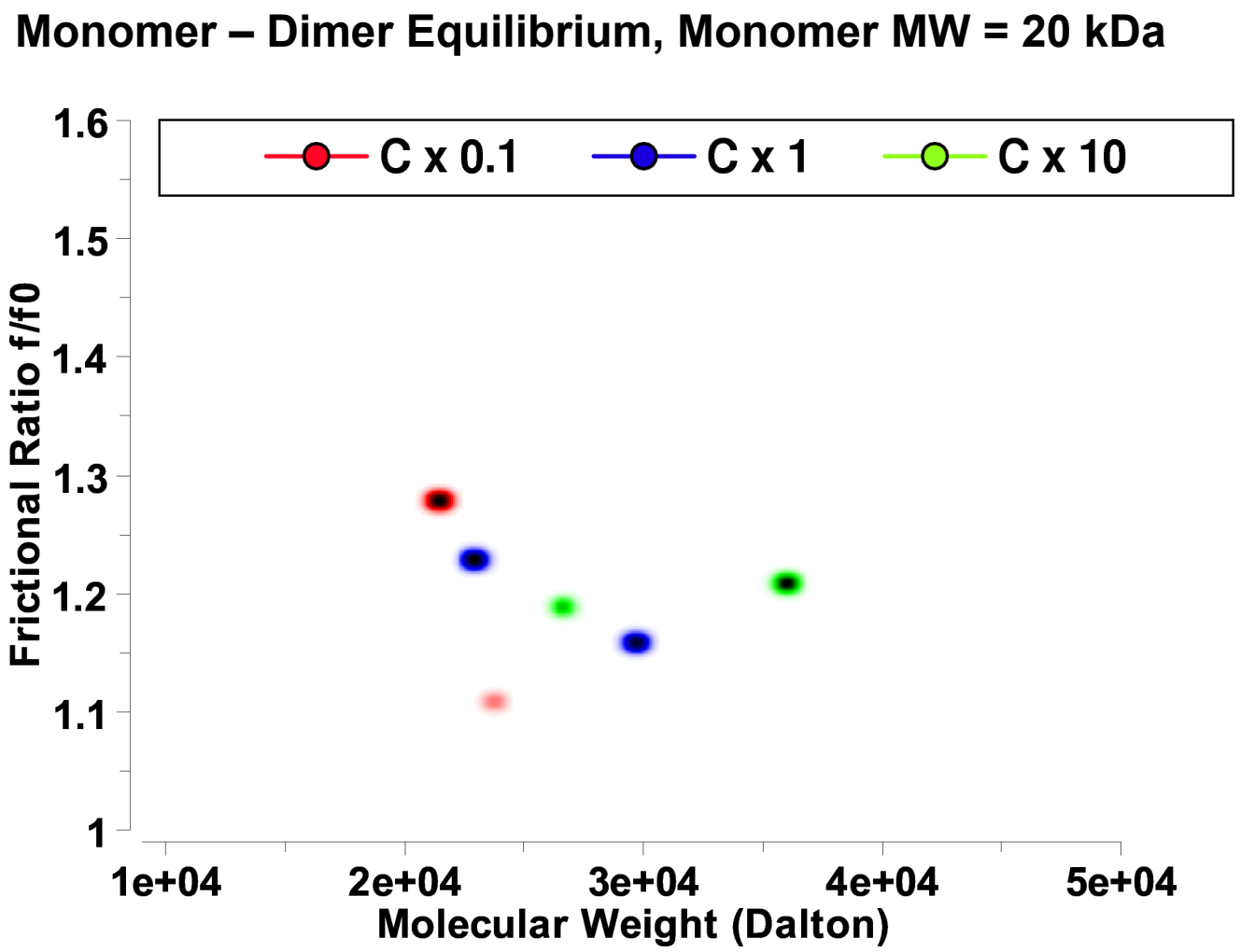


Monomer MW = 20 kDa, K_d = C x 1, k_{off}= 1x10^{-3}/sec, f/f0 = 1.25 (both)

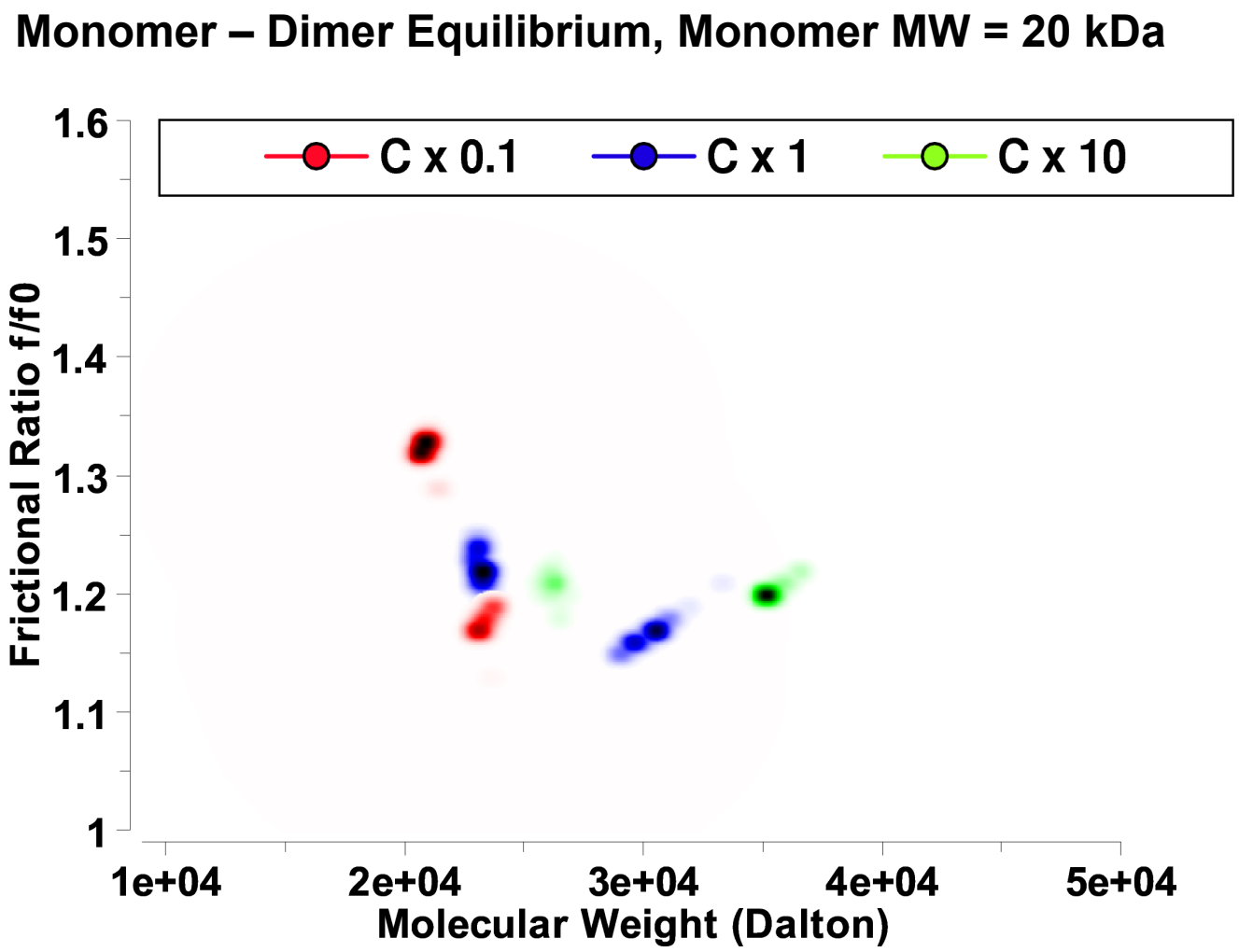
2DSA Monte Carlo Analysis



Genetic Algorithm Analysis



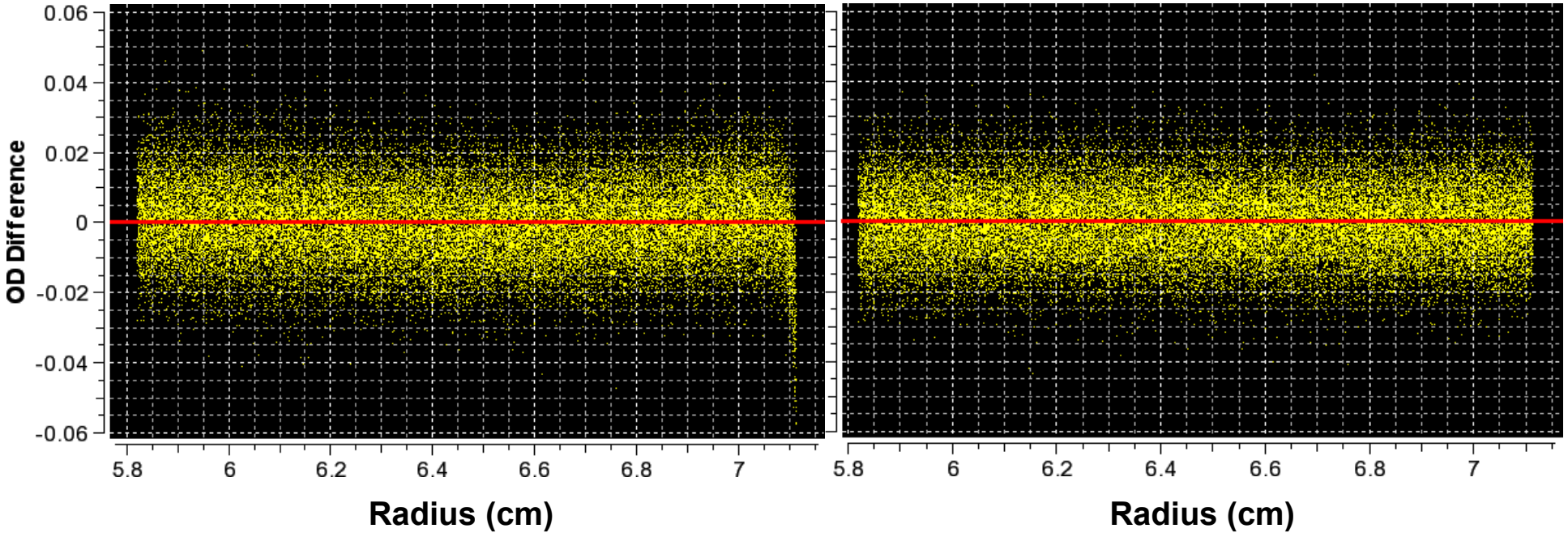
Genetic Algorithm Monte Carlo Analysis



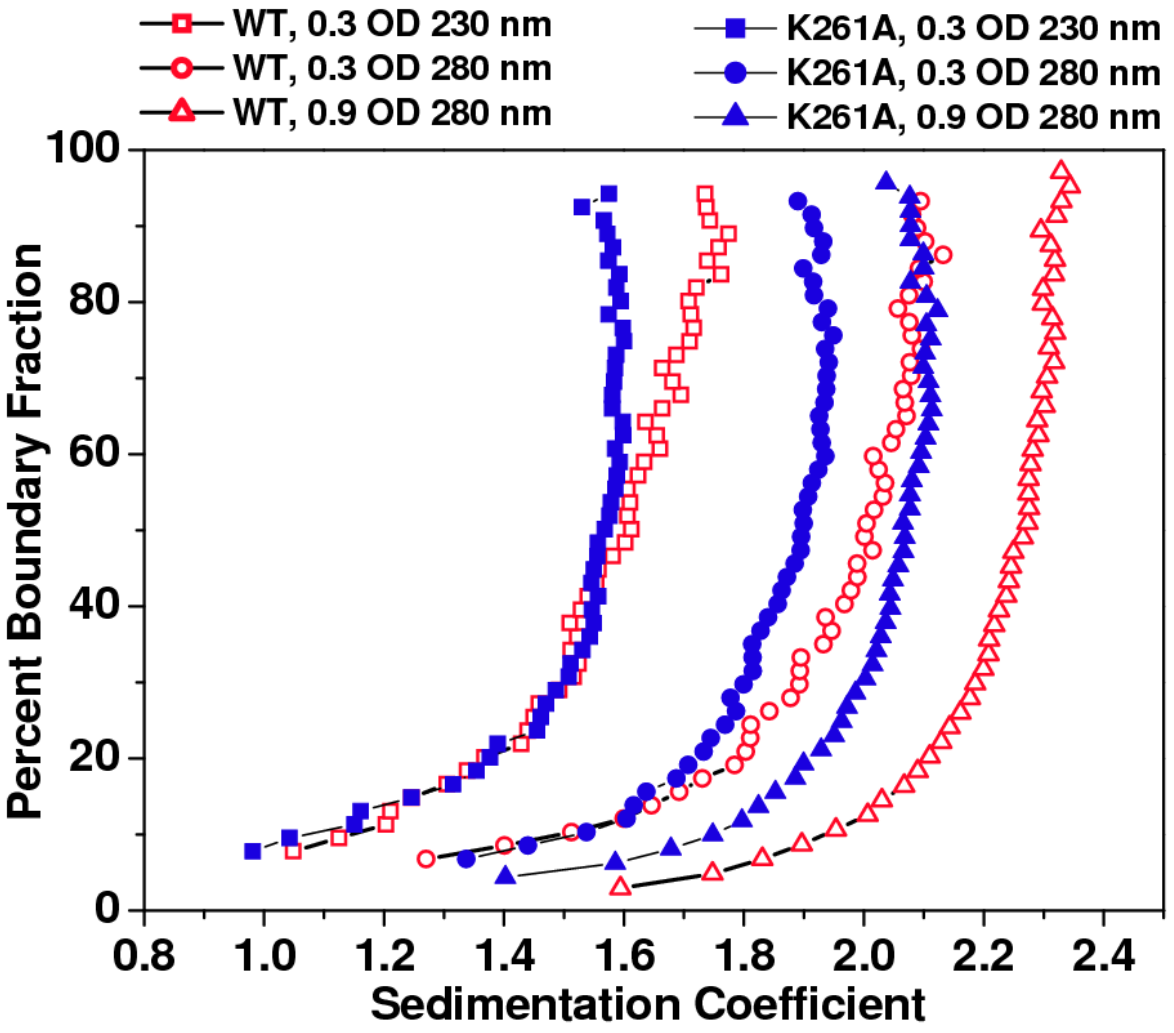
UltraScan Model Builder for Reacting Systems - Dimer:

GA-MC Non-Interacting Fit

GA Reversible Model Fit



Monomer-Dimer Interface Mutation Analysis



Monomer-Dimer Interface Mutation Analysis Velocity Results

Parameter:	wildtype, 0.3 OD	K261A, 0.3 OD
K_d (μM)	10.4 (9.62, 11.4)	17.1 (15.9, 18.4)
k_{off} (x 10^{-5} sec^{-1})	72.7 (26.5, 118.9)	84.0 (46.4, 121.6)
f/f_o (monomer)	1.31 (1.28, 1.34)	1.33 (1.32, 1.35)
f/f_o (dimer)	1.35 (1.33, 1.37)	1.43 (1.42, 1.45)

SV fitting results for C-RING1B wildtype and K261A mutant to a reversible monomer-dimer equilibrium model that allows for the presence of a contaminant. Values in parentheses represent 95% confidence intervals.