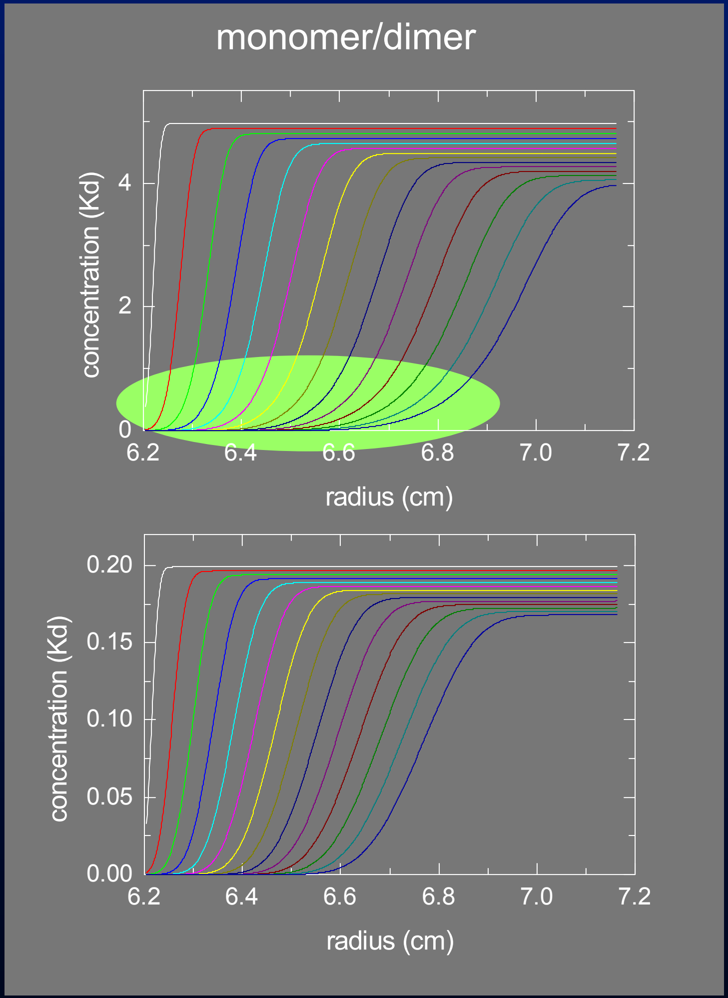
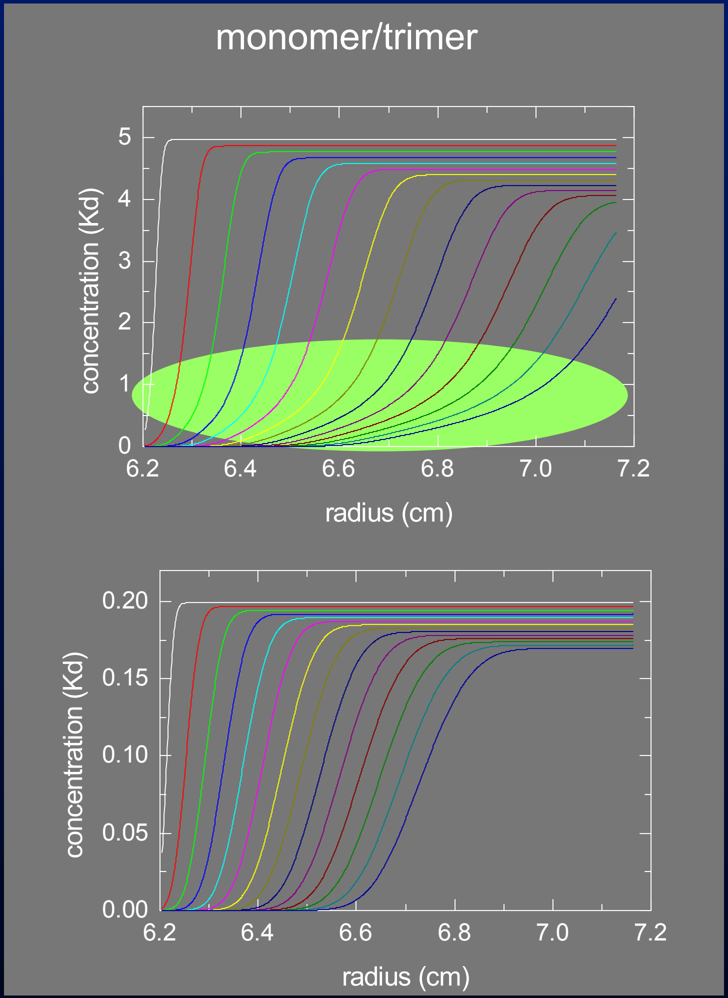
Sedimentation Velocity Analysis of Interacting Systems using c(s)

Peter Schuck

simulated sedimentation of rapidly self-association systems



monomer/dimer



monomer/trimer

→ large amount of data

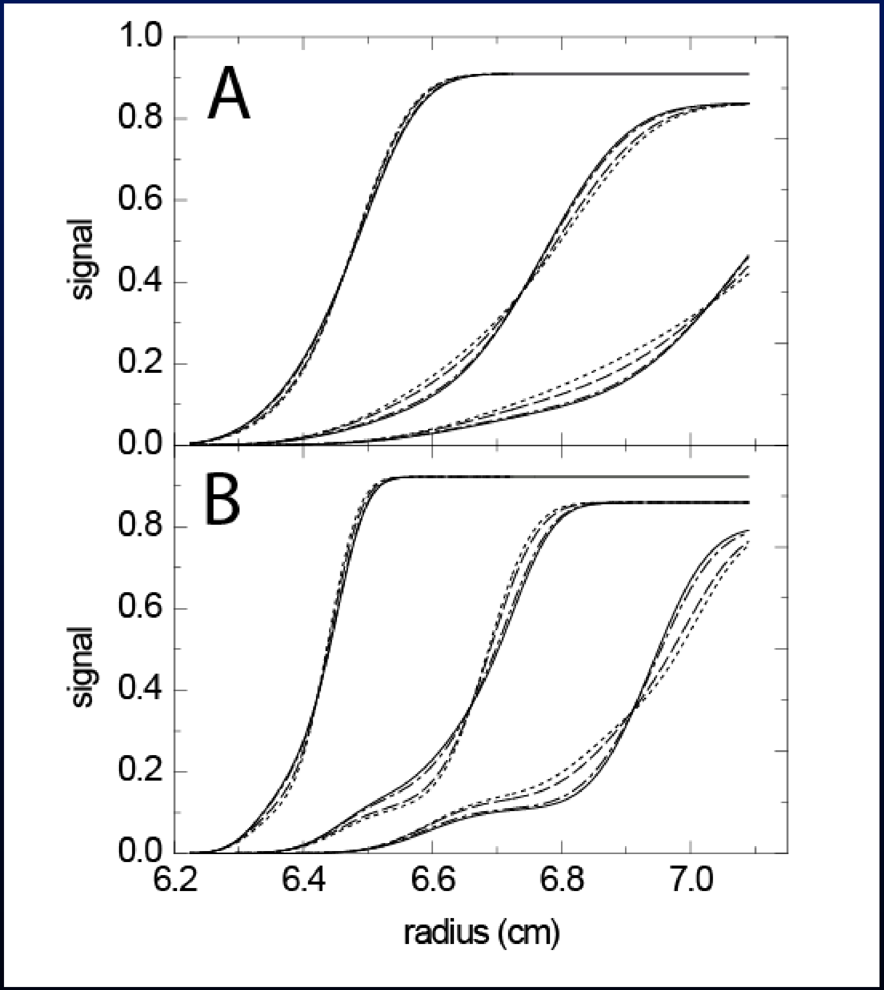
→ for fast reactions: provides information on complete isotherm of $s_w(c)$ for range of $c < c_{load}$

Boundary shapes for a heterogeneous reaction with different binding kinetics
➔ effect of protein size

A+B←→AB, concentration equimolar at 3K_D
with koff = 1e-2, 1e-3, 1e-4, 1e-5

A = 30 kDa, B = 50 kDa

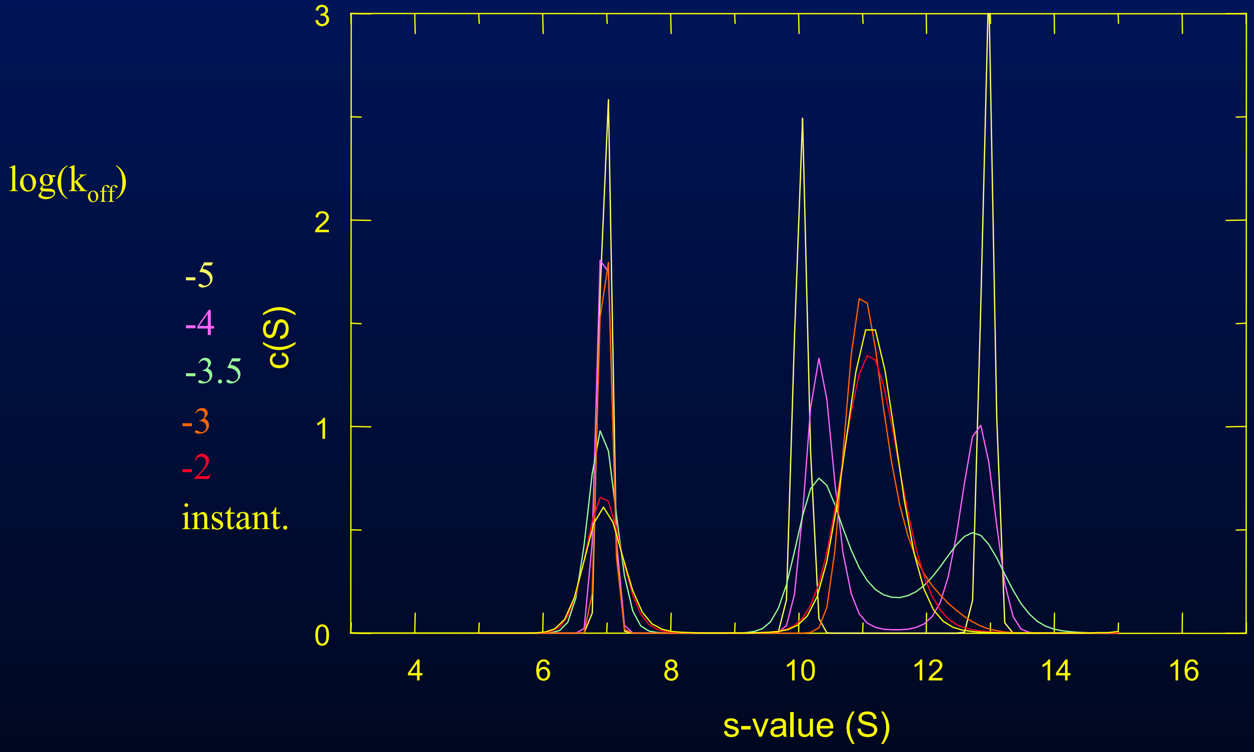
A = 90 kDa, B = 150 kDa



bimodal boundary for 2component mixture

smaller size diminishes characteristic features from reaction kinetics

Boundary shapes for a heterogeneous reaction with different binding kinetics
➔ c(s) curves from slow to fast reactions



c(s) curves can be used to diagnose kinetic regime

J. Dam, C.A. Velikovsky, R.A. Mariuzza, C. Urbanke, P. Schuck (2005) Sedimenetation velocity analysis of heterogeneous protein-protein interactions: Lamm equation modeling and sedimentation coefficient distributions c(s). Biophysical Journal 89:619-634

Boundary shapes for a heterogeneous reaction with different binding kinetics
➔ limitations

→diffusion does reduce information on kinetics, reaction scheme, therefore **small proteins will be more difficult**

→ interpretation of boundary broadening is exquisitely sensitive to everything that made already the determination of molar mass difficult: **(micro)-heterogeneity, multiple conformations, glycosylation**

→ sensitivity only over a narrow range of time-constants, given by the sedimentation time: **best for ~ 10^{-4} - 10^{-3} /sec**

→ describing kinetic models possible, but depend on parameters that are frequently **ill-defined by the experiment**

→ **need less detailed models, which permit to quantitatively analyze interactions in SV based on the strength of SV: separation in mixtures**

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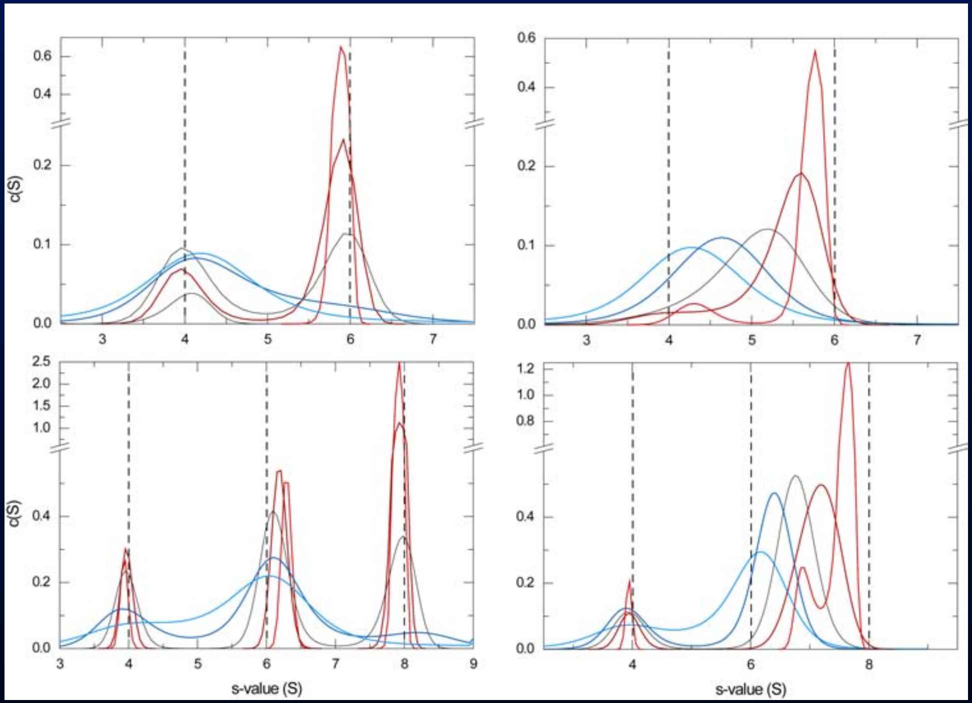
c(s) curves from analysis of self-association and hetero-association

slow (0.00005/sec)

fast (0.005/sec)

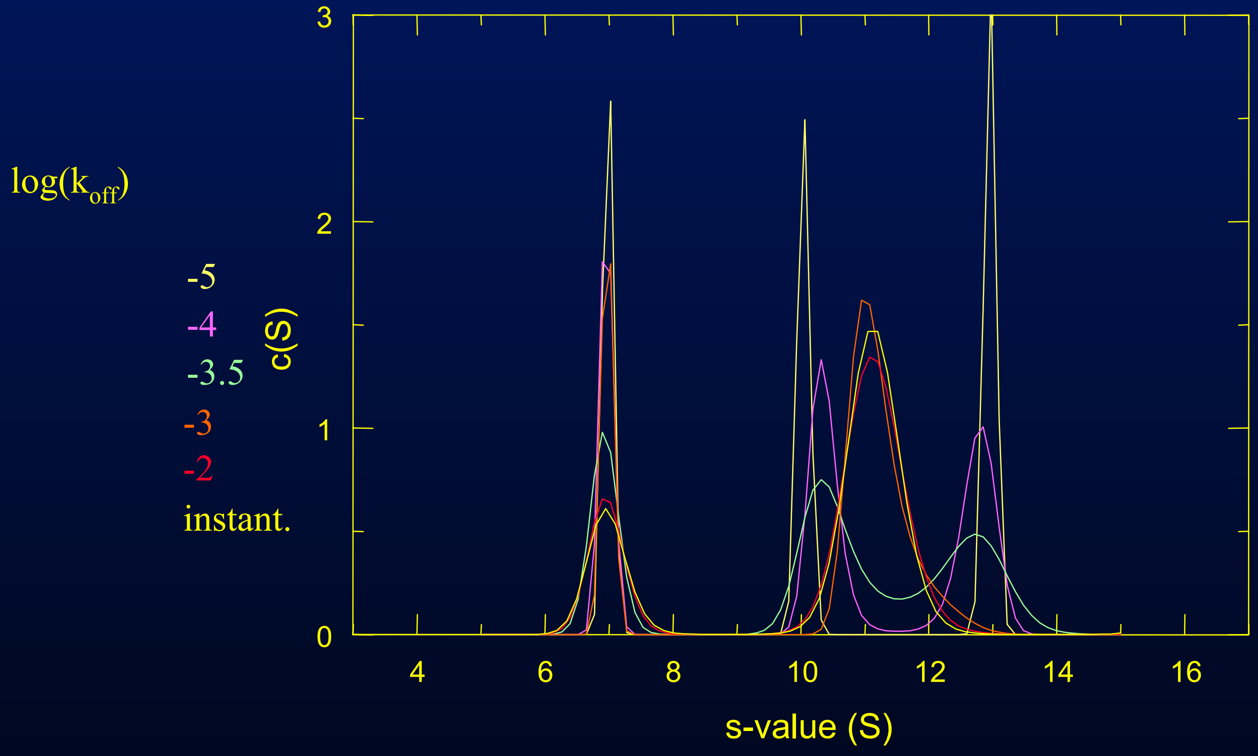
self-association

hetero-association



⇒ c(s) analysis of reaction boundary may result in broad distribution
⇒ rms error can go up
⇒ apparent f/f_0 may be smaller than real f/f_0 (sometimes < 1)
⇒ for slow systems, c(s) gives peak positions of species
⇒ for fast systems, c(s) gives undisturbed boundary and approximations of asymptotic reaction boundary

Boundary shapes for a heterogeneous reaction with different binding kinetics
➔ c(s) curves from slow to fast reactions



c(s) curves can be used to diagnose kinetic regime

c(s) shows either populations of species, or something in between
→ how does this work?
→peak s-values do not coincide well with species s-values – why?
→ **what do these curves mean, and how can they be used quantitatively?**

‘Constant Bath’ Approximation:

Krauss, Pingoud, Boehme, Riesner, Peters, Maass. Eur. J. Biochem. (1975)

Example: A + B ←→ AB

$$\boxed{\begin{aligned}
\frac{\partial a}{\partial t} &= D_a\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial a}{\partial r}\right)\right] - s_a\omega^2\frac{1}{r}\frac{\partial}{\partial r}\left(r^2 a\right) + q_a \\
\frac{\partial b}{\partial t} &= D_b\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial b}{\partial r}\right)\right] - s_b\omega^2\frac{1}{r}\frac{\partial}{\partial r}\left(r^2 b\right) + q_b \\
\frac{\partial c}{\partial t} &= D_c\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial c}{\partial r}\right)\right] - s_c\omega^2\frac{1}{r}\frac{\partial}{\partial r}\left(r^2 c\right) + q_c
\end{aligned}}$$

system of Lamm equations

coupled with reaction fluxes

$q_a = q_b = -q_c = k_{on}ab - k_{off}c$

$K_D = k_{on}/k_{off}$ ($K_D = ab/c$ for instant. reaction)

what if A was small and would not exhibit a spatial gradient? $\partial a/\partial r = 0$ fast reaction → $\partial c/\partial r = Ka\,\partial b/\partial r$

add Lamm eq. for b + c

$$\frac{\partial b}{\partial t} + \frac{\partial c}{\partial t} = \left(D_b + KaD_c\right)\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial b}{\partial r}\right)\right] - \left(s_b + Kas_c\right)\omega^2\frac{1}{r}\frac{\partial}{\partial r}\left(r^2 b\right) + q_b + q_c$$

introduce new variable for b+c $\beta = b + c$

$$\frac{\partial \beta}{\partial t} = D^*\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial \beta}{\partial r}\right)\right] - s^*\omega^2\frac{1}{r}\frac{\partial}{\partial r}\left(r^2\beta\right)$$

➔ non-interacting single-species Lamm equation

$$D^* = \frac{D_b + KaD_c}{1 + Ka}, \quad s^* = \frac{s_b + Kas_c}{1 + Ka}$$

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‘Constant Bath’ Approximation:

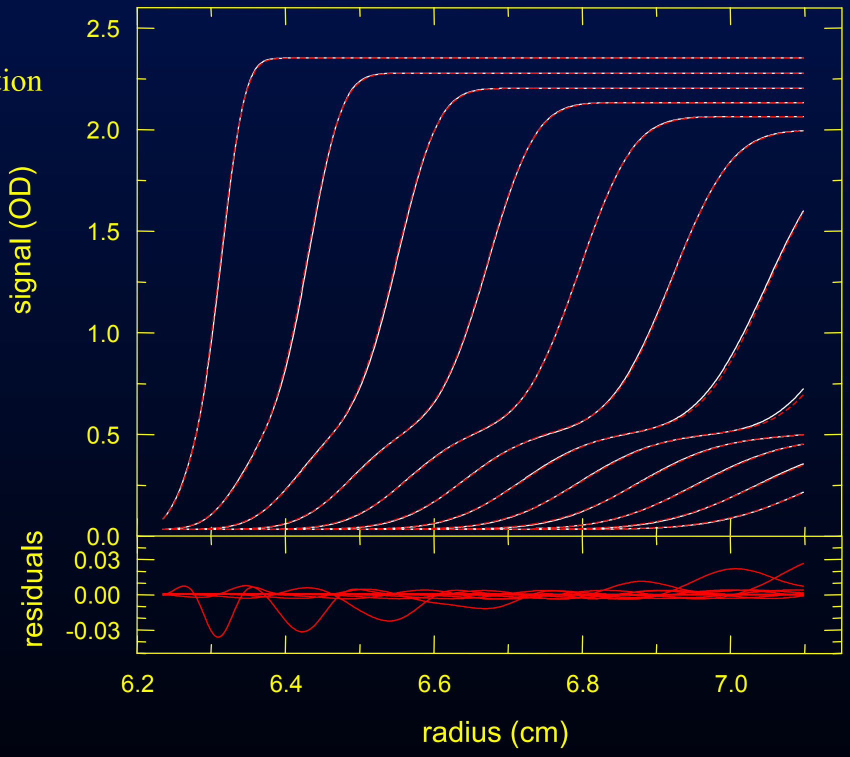
- cannot be strictly fulfilled, since A, B, and C are always in mass action law equilibrium
  → there **will** be a gradient of A.

→ how large is this gradient?
→ can this be a realistic and useful limiting case for protein-protein interactions?

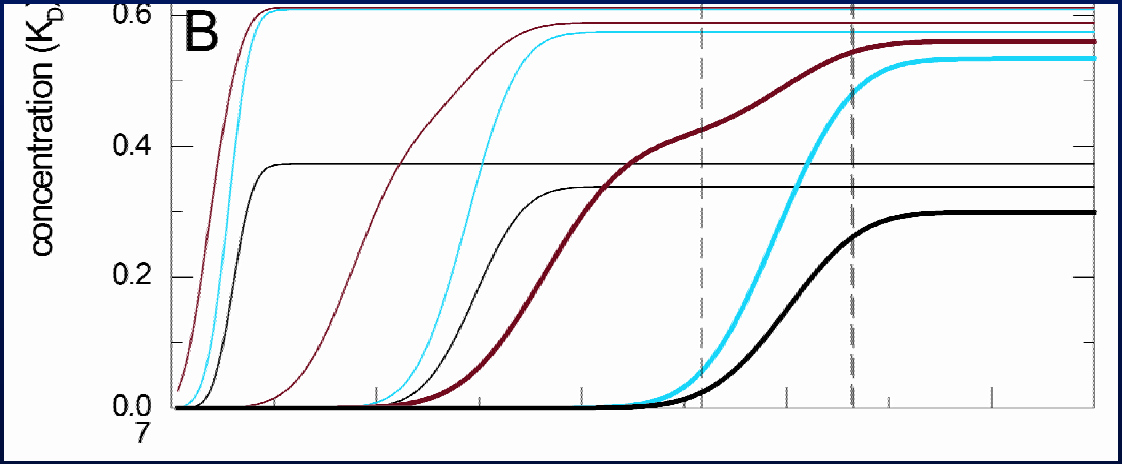
address this questions using
Lamm equation solutions for instantaneous reaction
of proteins A = 100 kDa, B = 200 kDa
$s_A = 7$ S, $s_B = 10$ S, $s_{AB} = 13$ S
equimolar concentration at Kd = 10 uM

two boundaries:
- free A at 7 S
- reaction boundary

fit with 2 species model:
$s_1 = 7.02$ S (expect 7.0 S)
$s_2 = 11.15$ S ($s^* = 11.14$ S)
$M_A = 83.4$ kDa (– 17 %)
$M_B = 198$ kDa (– 25%)
rmsd = 0.0043



→ diffusion coefficients quantitatively not very precise, but s-values very good
→ excellent description – **why**?



→ indeed the first boundary is of free A
→ there is free A in the fast boundary
→ there is some gradient of free A
→ however: free A changes from 76 to 97 % where free B goes from 10 to 90% ➔ mass action law causes the fractional occupation of B (i.e. C/(B+C)) to vary only between 30 to 35% → this means that the gradient in A does not lead to a big change in the 'pseudo-species B+C' constituting the fast boundary in the the constant bath theory. Therefore, overall description good.
(the dispersion in s caused by the finite range of C/(B+C) does show up in a slight increase in D; how to treat the dispersion in terms of range of s-values will be topic of Gilbert-Jenkins theory later)

→ the big result here is that, in a first approximation, diffusion of the reaction boundary is very similar to that of a single species.
→ consistent s-values of the fast boundary with s* from 'constant bath' theory

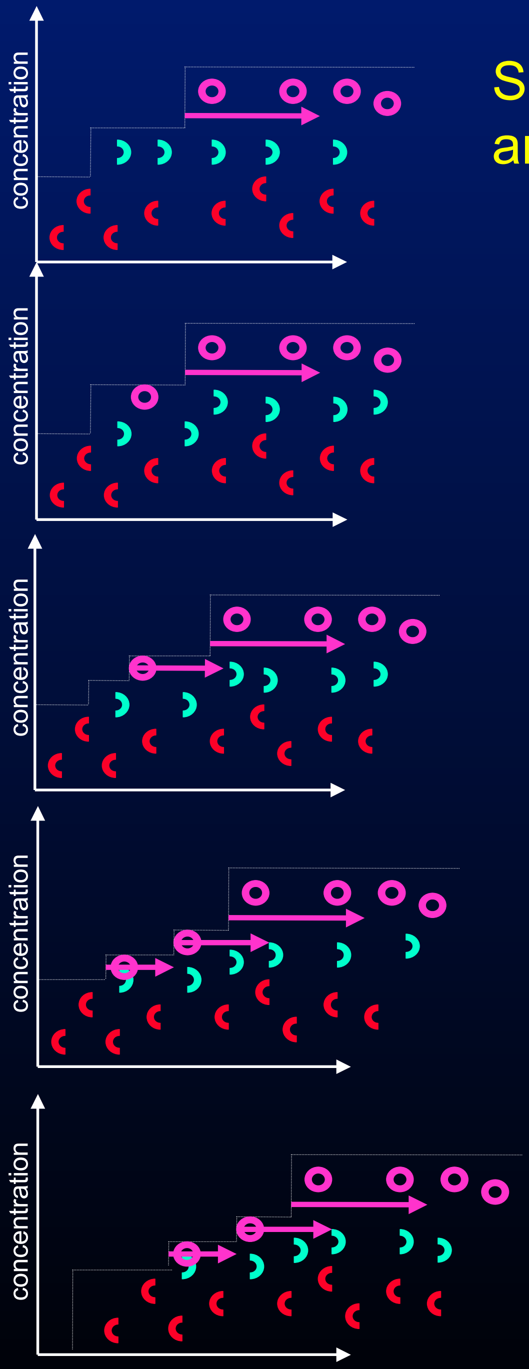
Schematics of diffusion-free sedimentation of an rapidly reacting system

( ) ○

$A + B \Leftrightarrow AB$

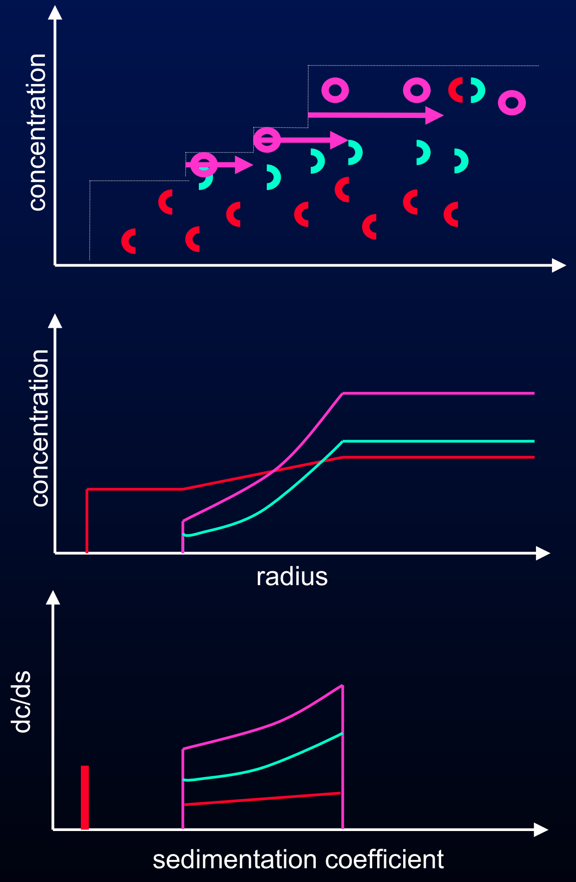
with

$s_{(} < s_{)} < s_{\circ}$

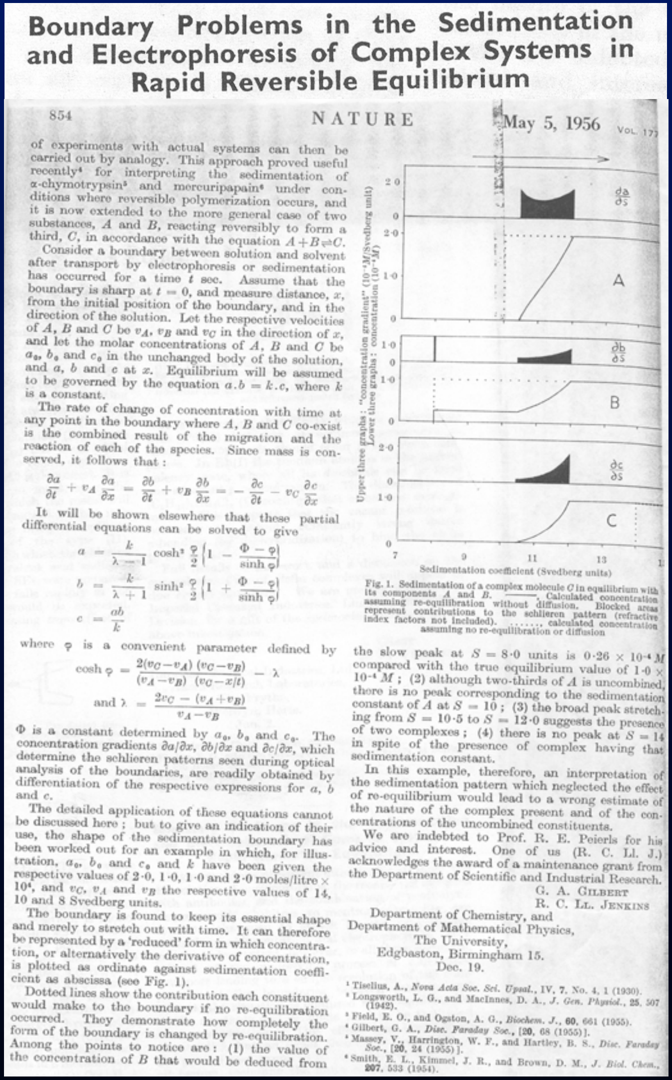


mass action law: $ab = cK_D$

→ time-average $\tau_a \tau_b = \tau_c K_D$



Gilbert & Jenkins, Nature, 1956



Boundary Problems in the Sedimentation and Electrophoresis of Complex Systems in Rapid Reversible Equilibrium

854 NATURE May 5, 1956 Vol. 177

of experiments with actual systems can then be carried out by analogy. This approach proved useful recently[4] for interpreting the sedimentation of α-chymotrypsin[5] and mercuripapain[6] under conditions where reversible polymerization occurs, and it is now extended to the more general case of two substances, A and B, reacting reversibly to form a third, C, in accordance with the equation $A+B \rightleftharpoons C$.

Consider a boundary between solution and solvent after transport by electrophoresis or sedimentation has occurred for a time t sec. Assume that the boundary is sharp at $t = 0$, and measure distance, x, from the initial position of the boundary, and in the direction of the solution. Let the respective velocities of A, B and C be v_A, v_B and v_C in the direction of x, and let the molar concentrations of A, B and C be a_0, b_0 and c_0 in the unchanged body of the solution, and a, b and c at x. Equilibrium will be assumed to be governed by the equation $a.b = k.c$, where k is a constant.

The rate of change of concentration with time at any point in the boundary where A, B and C co-exist is the combined result of the migration and the reaction of each of the species. Since mass is conserved, it follows that:

$$\frac{\partial a}{\partial t} + v_A \frac{\partial a}{\partial x} = \frac{\partial b}{\partial t} + v_B \frac{\partial b}{\partial x} = -\frac{\partial c}{\partial t} - v_C \frac{\partial c}{\partial x}$$

It will be shown elsewhere that these partial differential equations can be solved to give

$$a = \frac{k}{\lambda - 1} \cosh^2 \frac{\varphi}{2} \left\{1 - \frac{\Phi - \varphi}{\sinh \varphi}\right\}$$

$$b = \frac{k}{\lambda + 1} \sinh^2 \frac{\varphi}{2} \left\{1 + \frac{\Phi - \varphi}{\sinh \varphi}\right\}$$

$$c = \frac{ab}{k}$$

where φ is a convenient parameter defined by

$$\cosh \varphi = \frac{2(v_C - v_A)\,(v_C - v_B)}{(v_A - v_B)\,(v_C - x/t)} - \lambda$$

$$\text{and } \lambda = \frac{2v_C - (v_A + v_B)}{v_A - v_B}$$

Φ is a constant determined by a_0, b_0 and c_0. The concentration gradients $\partial a/\partial x$, $\partial b/\partial x$ and $\partial c/\partial x$, which determine the schlieren patterns seen during optical analysis of the boundaries, are readily obtained by differentiation of the respective expressions for a, b and c.

The detailed application of these equations cannot be discussed here; but to give an indication of their use, the shape of the sedimentation boundary has been worked out for an example in which, for illustration, a_0, b_0 and c_0 and k have been given the respective values of 2·0, 1·0, 1·0 and 2·0 moles/litre × 10^4, and v_C, v_A and v_B the respective values of 14, 10 and 8 Svedberg units.

The boundary is found to keep its essential shape and merely to stretch out with time. It can therefore be represented by a 'reduced' form in which concentration, or alternatively the derivative of concentration, is plotted as ordinate against sedimentation coefficient as abscissa (see Fig. 1).

Dotted lines show the contribution each constituent would make to the boundary if no re-equilibration occurred. They demonstrate how completely the form of the boundary is changed by re-equilibration. Among the points to notice are: (1) the value of the concentration of B that would be deduced from the slow peak at $S = 8{\cdot}0$ units is $0{\cdot}26 \times 10^{-4}\,M$ compared with the true equilibrium value of $1{\cdot}0 \times 10^{-4}\,M$; (2) although two-thirds of A is uncombined, there is no peak corresponding to the sedimentation constant of A at $S = 10$; (3) the broad peak stretching from $S = 10{\cdot}5$ to $S = 12{\cdot}0$ suggests the presence of two complexes; (4) there is no peak at $S = 14$ in spite of the presence of complex having that sedimentation constant.

Fig. 1. Sedimentation of a complex molecule C in equilibrium with its components A and B. ———, Calculated concentration assuming re-equilibration without diffusion. Blocked areas represent contributions to the schlieren pattern (refractive index factors not included). ...., calculated concentration assuming no re-equilibration or diffusion

In this example, therefore, an interpretation of the sedimentation pattern which neglected the effect of re-equilibrium would lead to a wrong estimate of the nature of the complex present and of the concentrations of the uncombined constituents.

We are indebted to Prof. R. E. Peierls for his advice and interest. One of us (R. C. Ll. J.) acknowledges the award of a maintenance grant from the Department of Scientific and Industrial Research.

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linear geometry and radial-independent force

$$\frac{\partial c_i}{\partial t} = D_i \frac{\partial^2 c_i}{\partial x^2} - v_i \frac{\partial c_i}{\partial x} + j_i$$

The reaction fluxes j_i follow mass conservation with $j_A = j_B = -j_C = j$, and it is assumed that all species are in instantaneous equilibrium following mass action law

$$c_A c_B K = c_C$$

A change of variables from spatial and time coordinates x and t to the velocity $v = x/t$ and the inverse time $w = 1/t$

$$(v - v_i)\frac{\partial c_i}{\partial v} + w\left(\frac{\partial c_i}{\partial w} + D_i \frac{\partial^2 c_i}{\partial v^2}\right) = -\frac{j}{w}$$

the limit of infinite time ($w \to 0$)

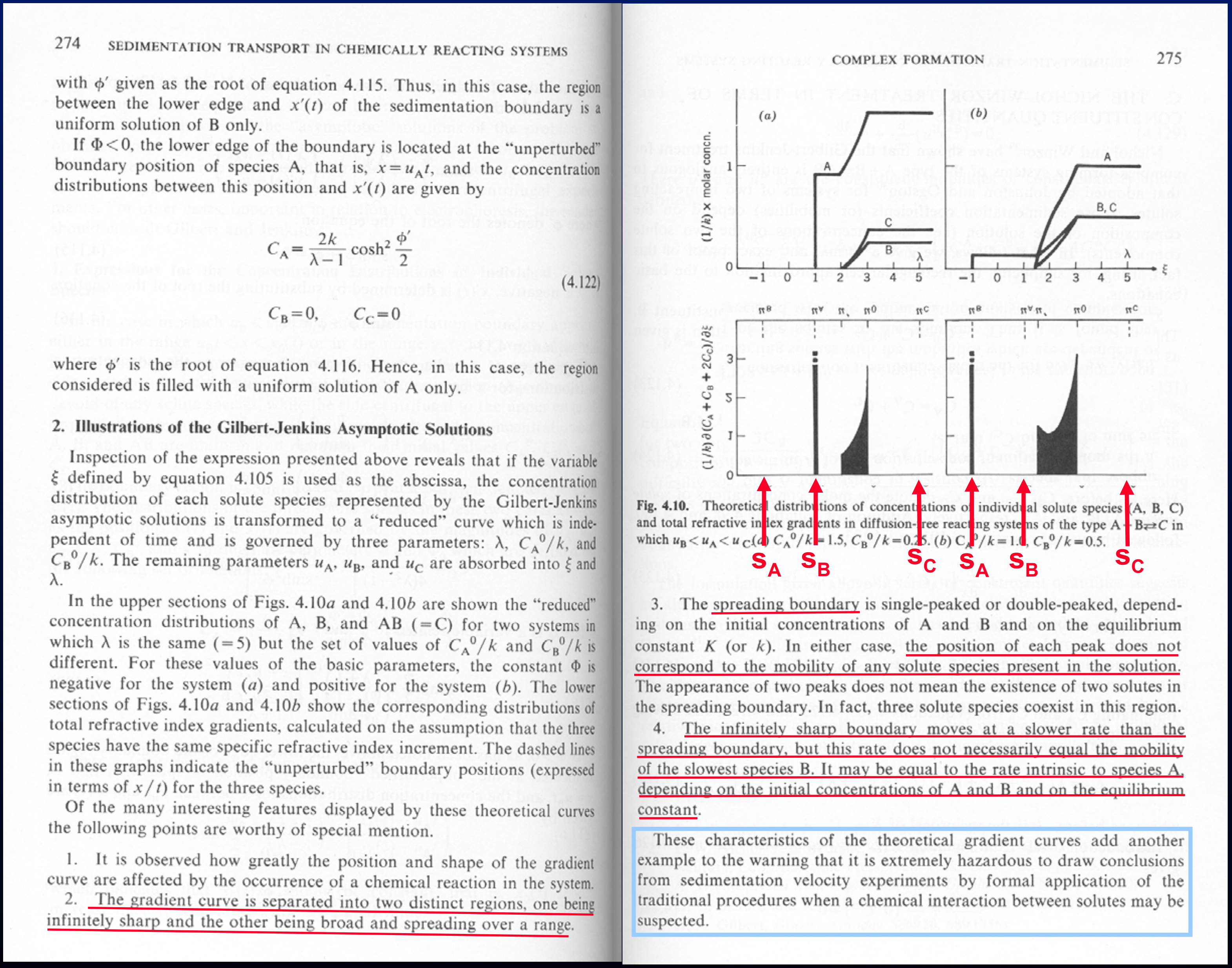
$$(v - v_a)\frac{\partial c_A}{\partial v} = (v - v_b)\frac{\partial c_B}{\partial v} = -(v - v_c)\frac{\partial c_C}{\partial v}$$

which can be solved for $c_A(v)$, $c_B(v)$, $c_C(v)$

→ **stable boundaries** with **asymptotic Schlieren patterns *ds/dv***

j → 0 with w → 0 → "**no net reaction at infinite time**"
(Gilbert & Jenkins, Proc. Royal Soc. London A, 1959)

summarized by Fujita (1975)



274 SEDIMENTATION TRANSPORT IN CHEMICALLY REACTING SYSTEMS

with ϕ' given as the root of equation 4.115. Thus, in this case, the region between the lower edge and $x'(t)$ of the sedimentation boundary is a uniform solution of B only.

If $\Phi < 0$, the lower edge of the boundary is located at the "unperturbed" boundary position of species A, that is, $x = u_A t$, and the concentration distributions between this position and $x'(t)$ are given by

$$C_A = \frac{2k}{\lambda - 1} \cosh^2 \frac{\phi'}{2} \qquad (4.122)$$

$$C_B = 0, \qquad C_C = 0$$

where ϕ' is the root of equation 4.116. Hence, in this case, the region considered is filled with a uniform solution of A only.

2. Illustrations of the Gilbert-Jenkins Asymptotic Solutions

Inspection of the expression presented above reveals that if the variable ξ defined by equation 4.105 is used as the abscissa, the concentration distribution of each solute species represented by the Gilbert-Jenkins asymptotic solutions is transformed to a "reduced" curve which is independent of time and is governed by three parameters: λ, $C_A{}^0/k$, and $C_B{}^0/k$. The remaining parameters u_A, u_B, and u_C are absorbed into ξ and λ.

In the upper sections of Figs. 4.10*a* and 4.10*b* are shown the "reduced" concentration distributions of A, B, and AB (=C) for two systems in which λ is the same (=5) but the set of values of $C_A{}^0/k$ and $C_B{}^0/k$ is different. For these values of the basic parameters, the constant Φ is negative for the system (*a*) and positive for the system (*b*). The lower sections of Figs. 4.10*a* and 4.10*b* show the corresponding distributions of total refractive index gradients, calculated on the assumption that the three species have the same specific refractive index increment. The dashed lines in these graphs indicate the "unperturbed" boundary positions (expressed in terms of x/t) for the three species.

Of the many interesting features displayed by these theoretical curves the following points are worthy of special mention.

1. It is observed how greatly the position and shape of the gradient curve are affected by the occurrence of a chemical reaction in the system.

2. The gradient curve is separated into two distinct regions, one being infinitely sharp and the other being broad and spreading over a range.

COMPLEX FORMATION 275

Fig. 4.10. Theoretical distributions of concentrations of individual solute species (A, B, C) and total refractive index gradients in diffusion-free reacting systems of the type $A + B \rightleftarrows C$ in which $u_B < u_A < u_C$ (*a*) $C_A{}^0/k = 1.5$, $C_B{}^0/k = 0.25$. (*b*) $C_A{}^0/k = 1.0$, $C_B{}^0/k = 0.5$.

3. The spreading boundary is single-peaked or double-peaked, depending on the initial concentrations of A and B and on the equilibrium constant K (or k). In either case, the position of each peak does not correspond to the mobility of any solute species present in the solution. The appearance of two peaks does not mean the existence of two solutes in the spreading boundary. In fact, three solute species coexist in this region.

4. The infinitely sharp boundary moves at a slower rate than the spreading boundary, but this rate does not necessarily equal the mobility of the slowest species B. It may be equal to the rate intrinsic to species A, depending on the initial concentrations of A and B and on the equilibrium constant.

These characteristics of the theoretical gradient curves add another example to the warning that it is extremely hazardous to draw conclusions from sedimentation velocity experiments by formal application of the traditional procedures when a chemical interaction between solutes may be suspected.

quantitative application in AUC has been largely dormant:
difficulty in measuring asymptotic boundary; computational limitation at the time

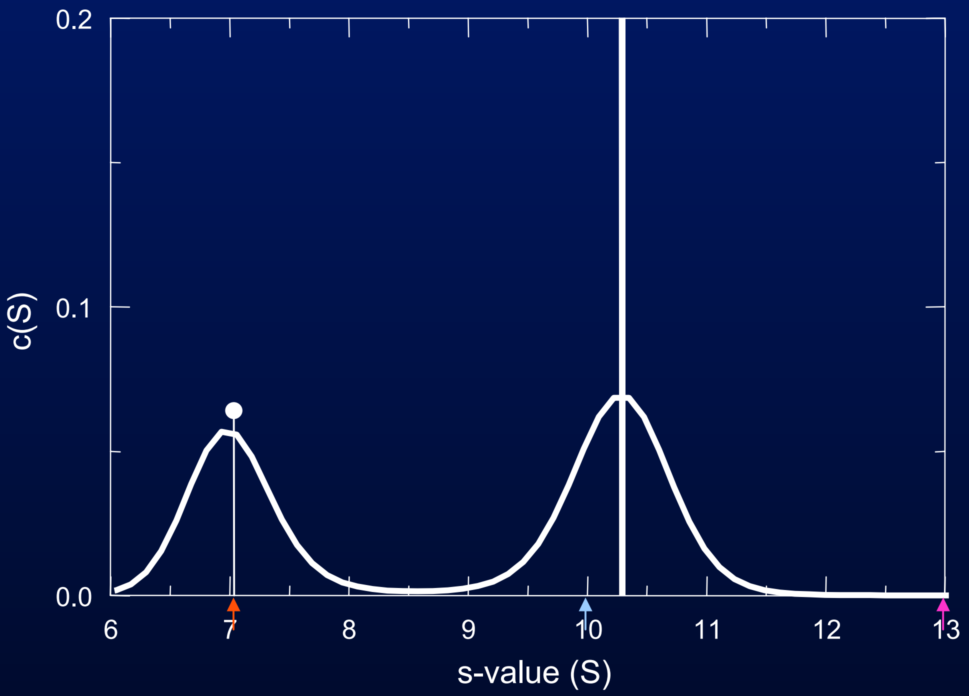
→ c(s) approximately deconvolutes diffusion from reaction boundaries → $D \approx 0$

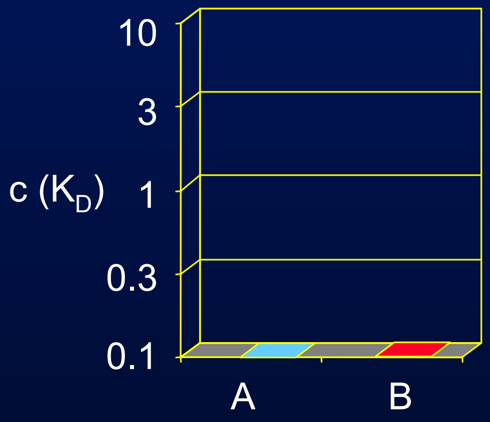
→ Gilbert-Jenkins theory predicts diffusion-free asymptotic boundaries at $D = 0$

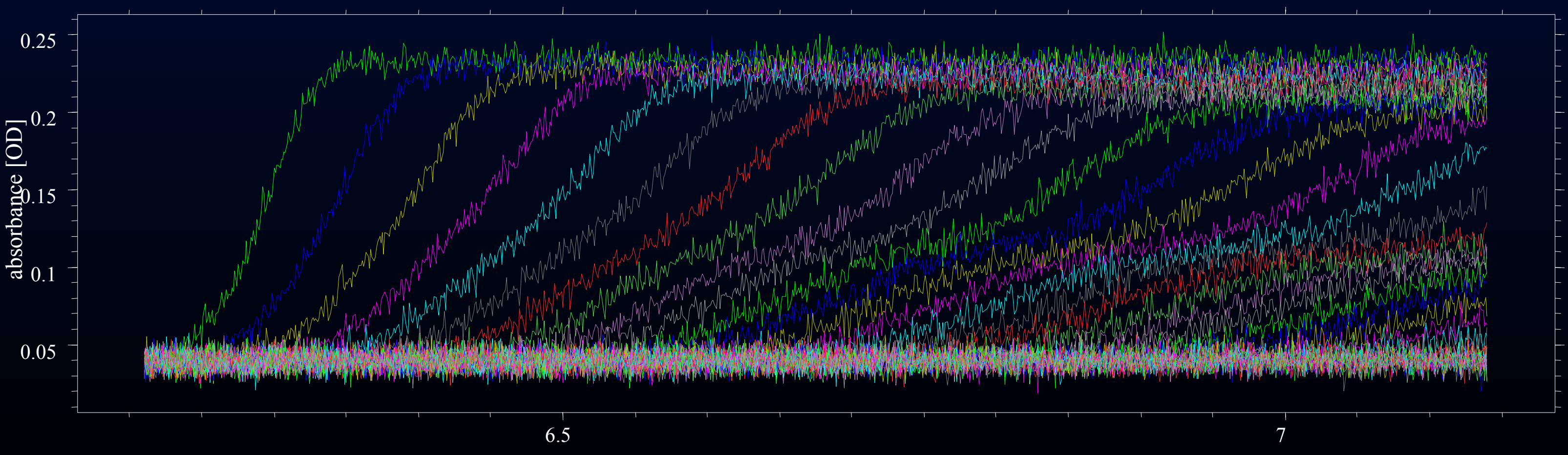
→ should be equivalent !

J. Dam, P. Schuck (2005) Sedimentation velocity analysis of heterogeneous protein-protein interactions: Sedimentation coefficient distributions c(s) and asymptotic boundary profiles from Gilbert-Jenkins theory. Biophysical Journal 89:651-666

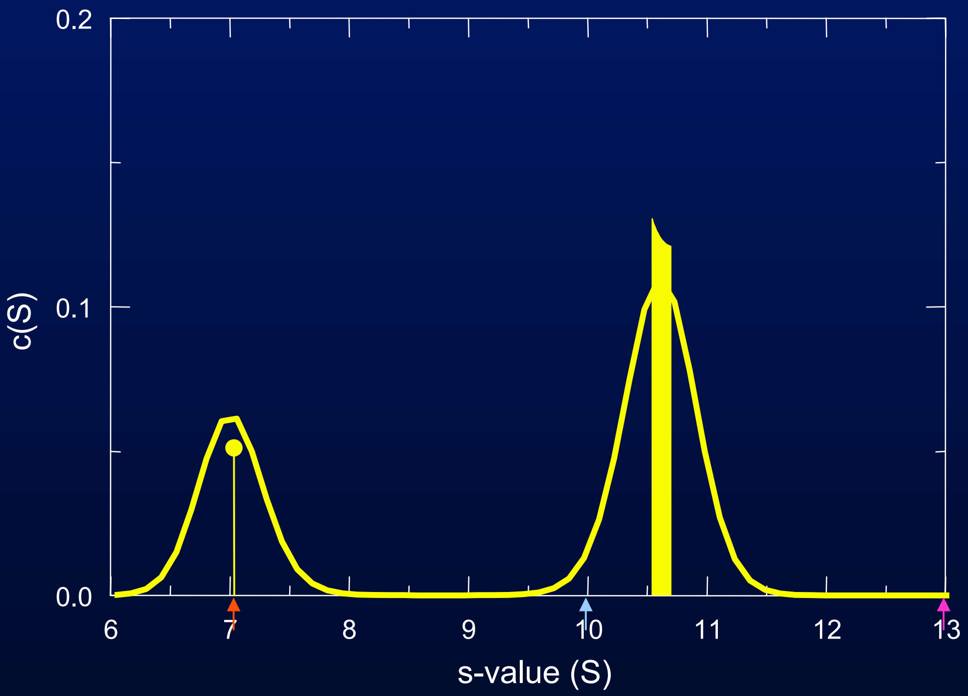
equimolar mixture

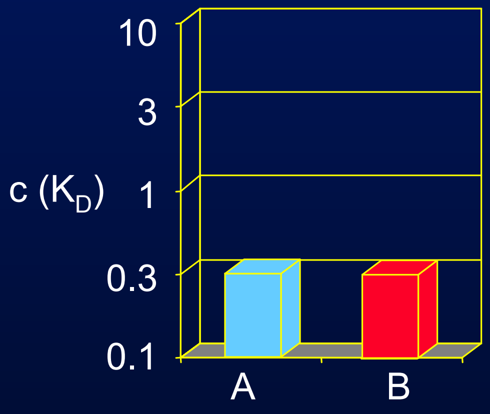


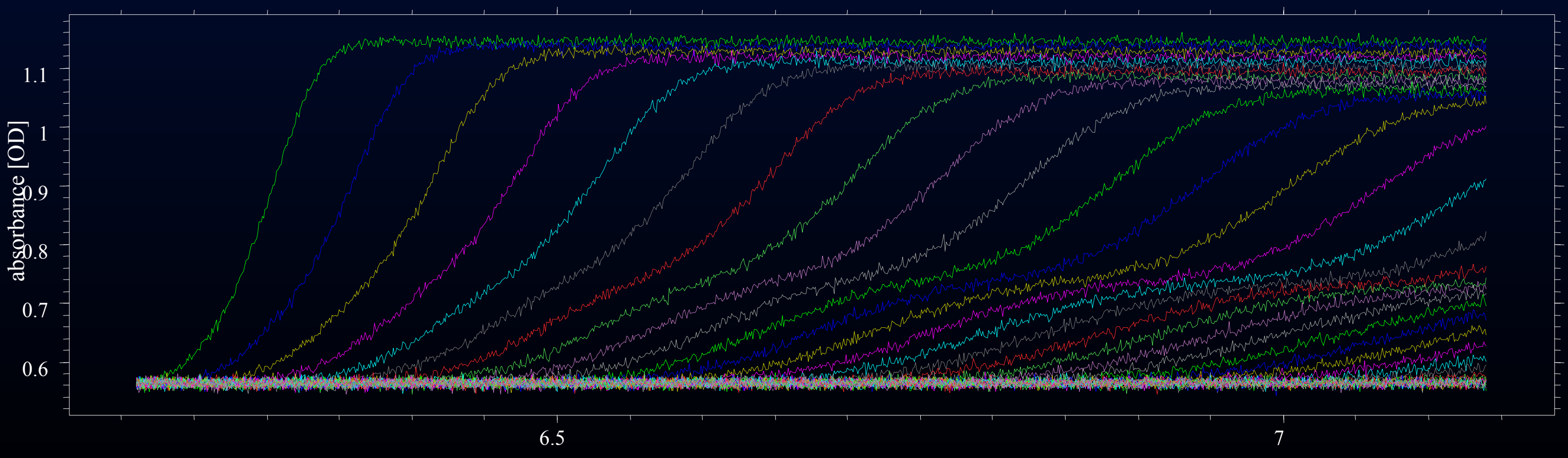




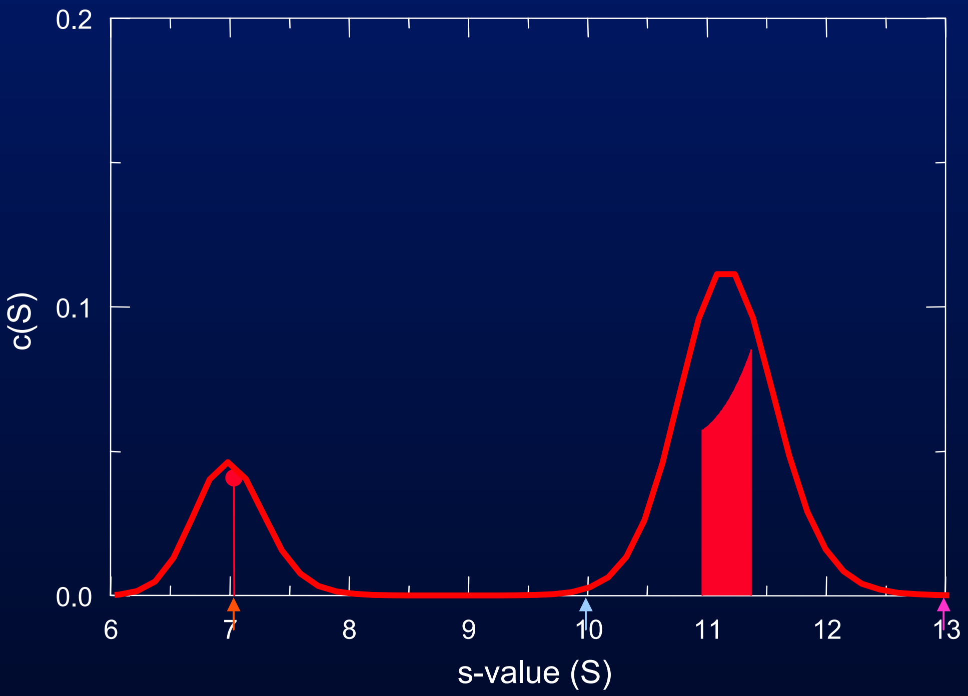
equimolar mixture

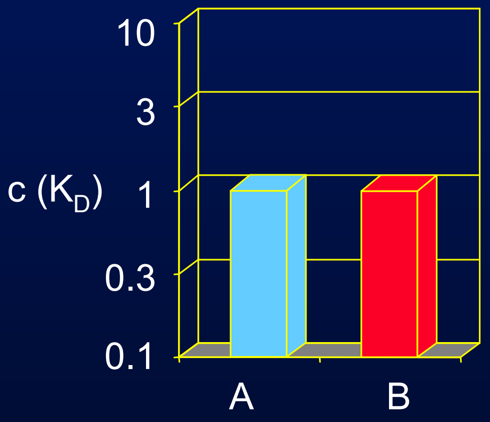


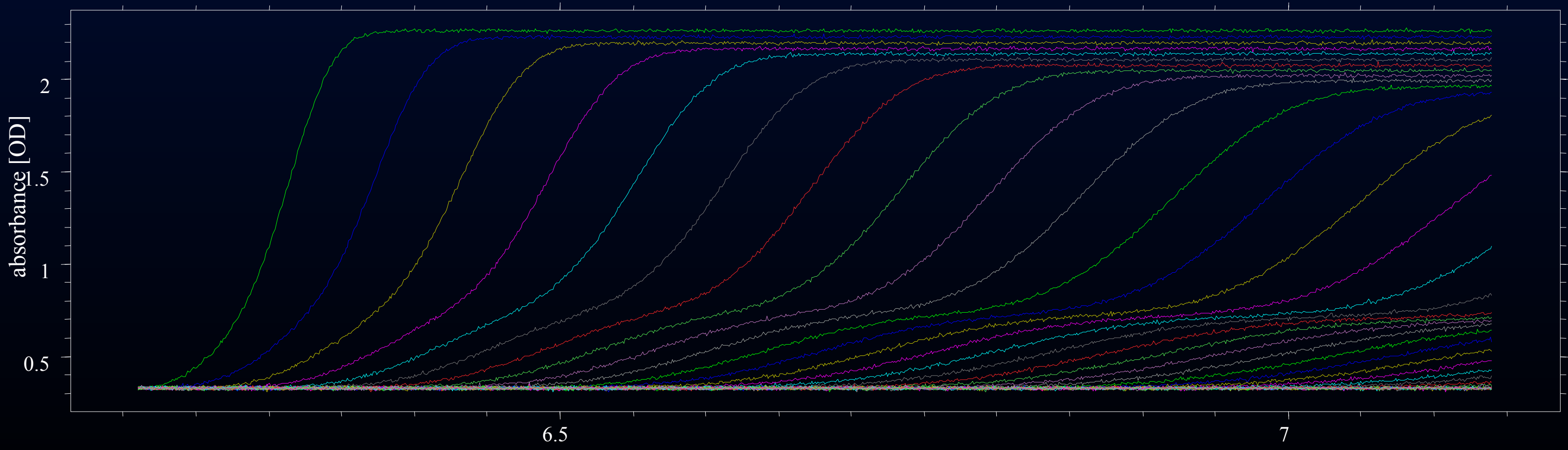




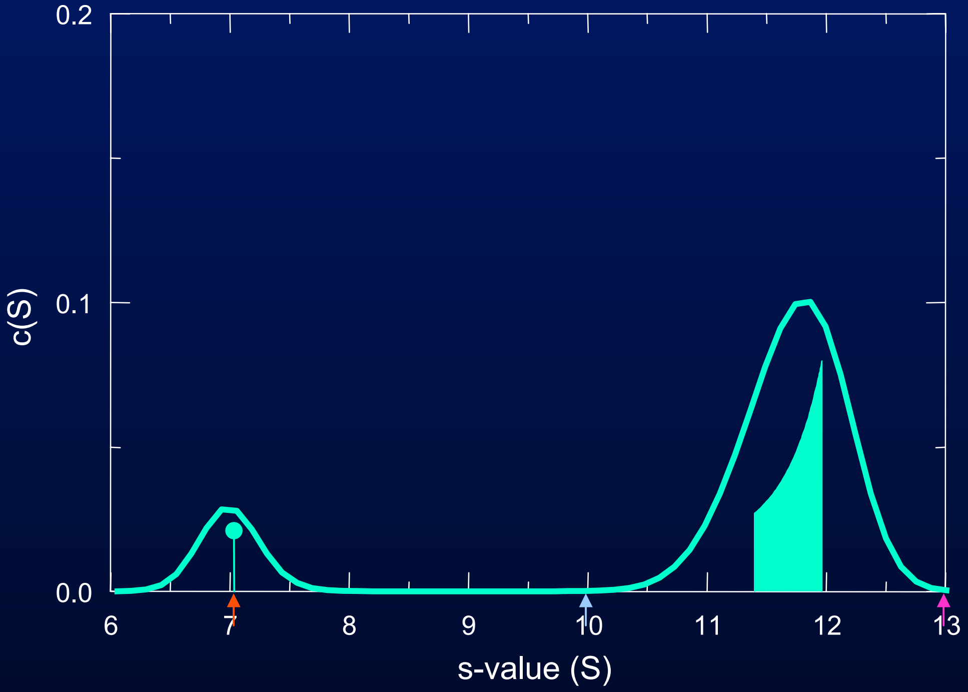
equimolar mixture

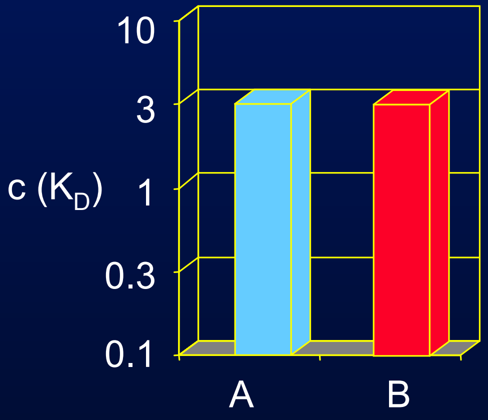


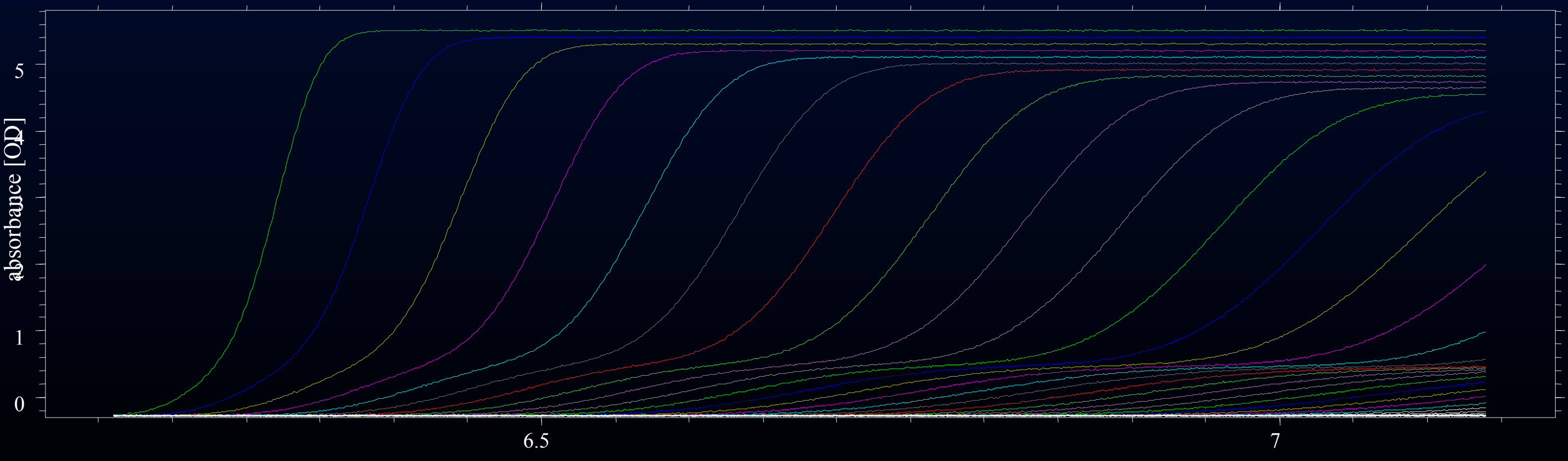




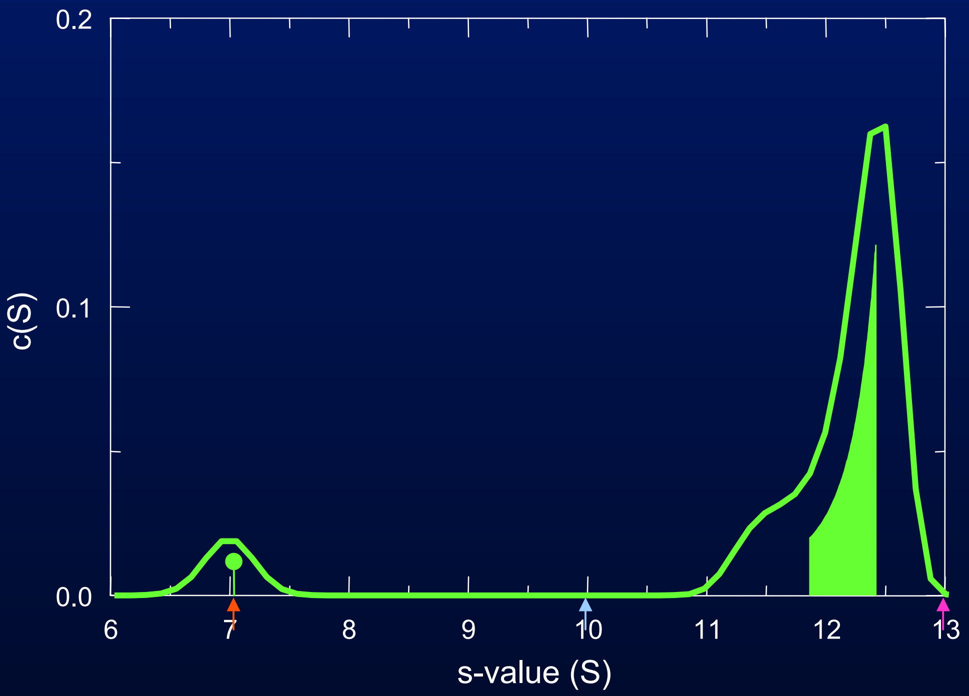
equimolar mixture

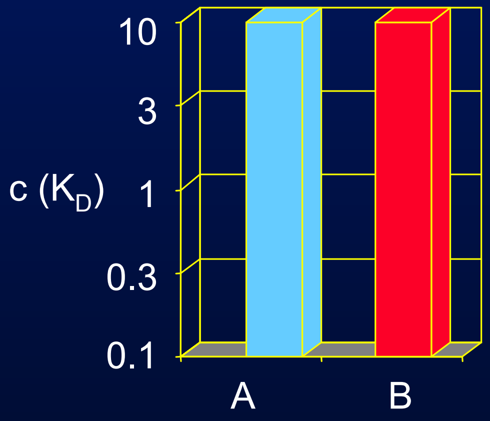


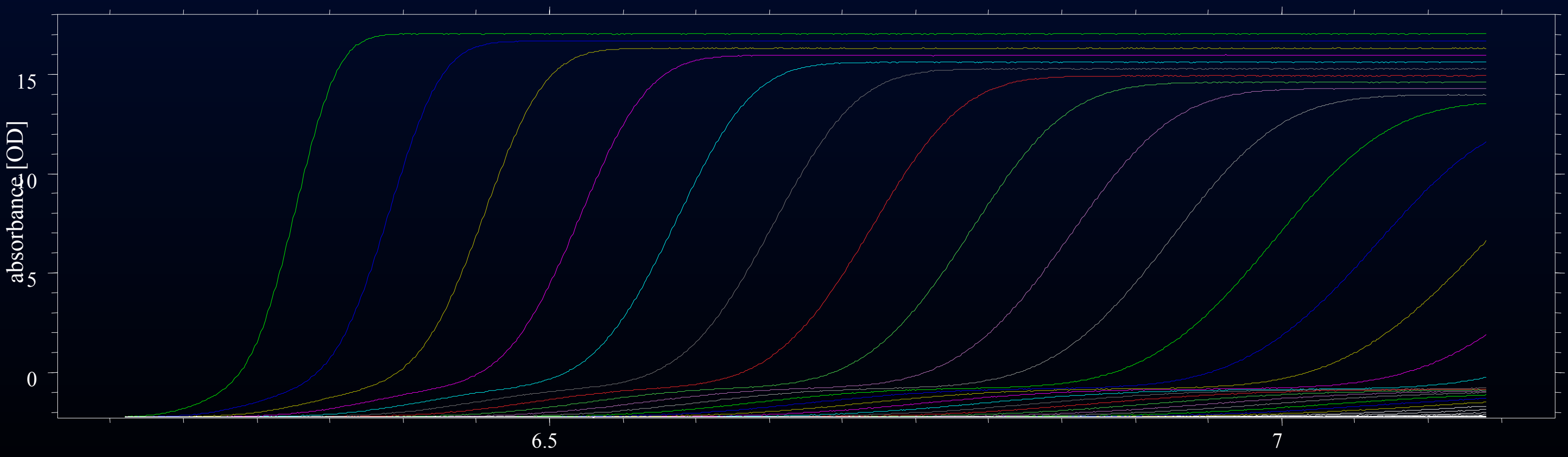




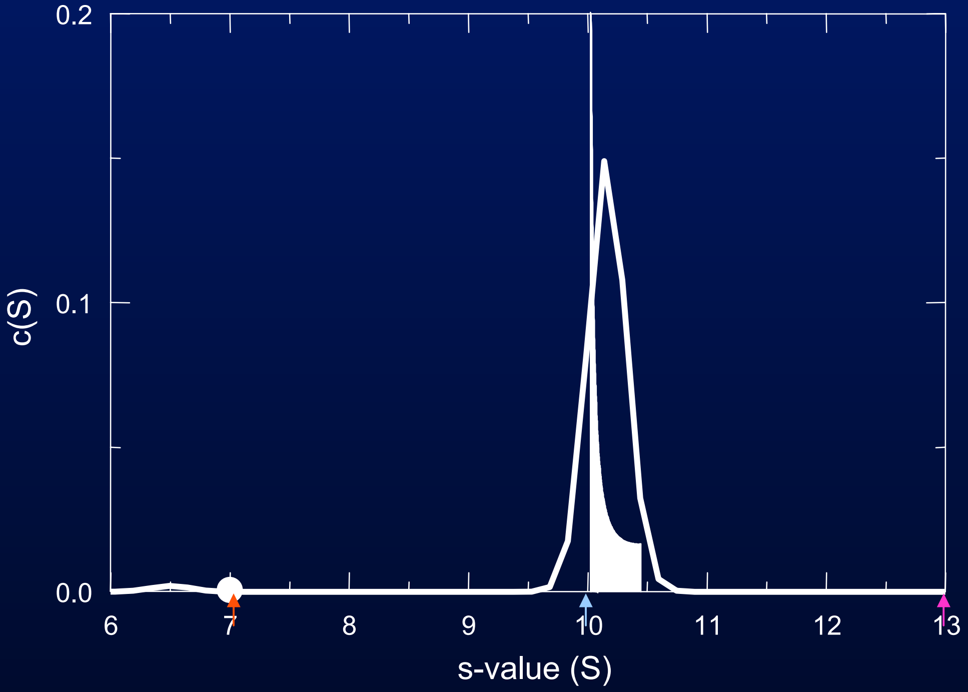
equimolar mixture

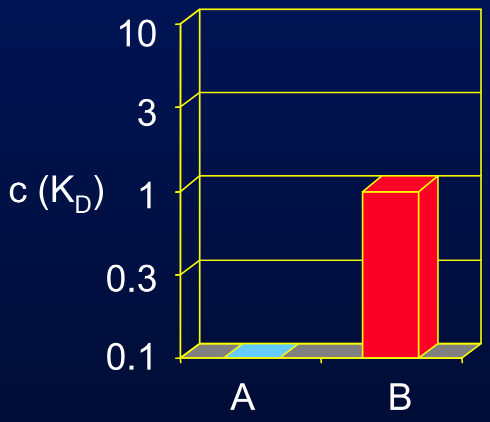


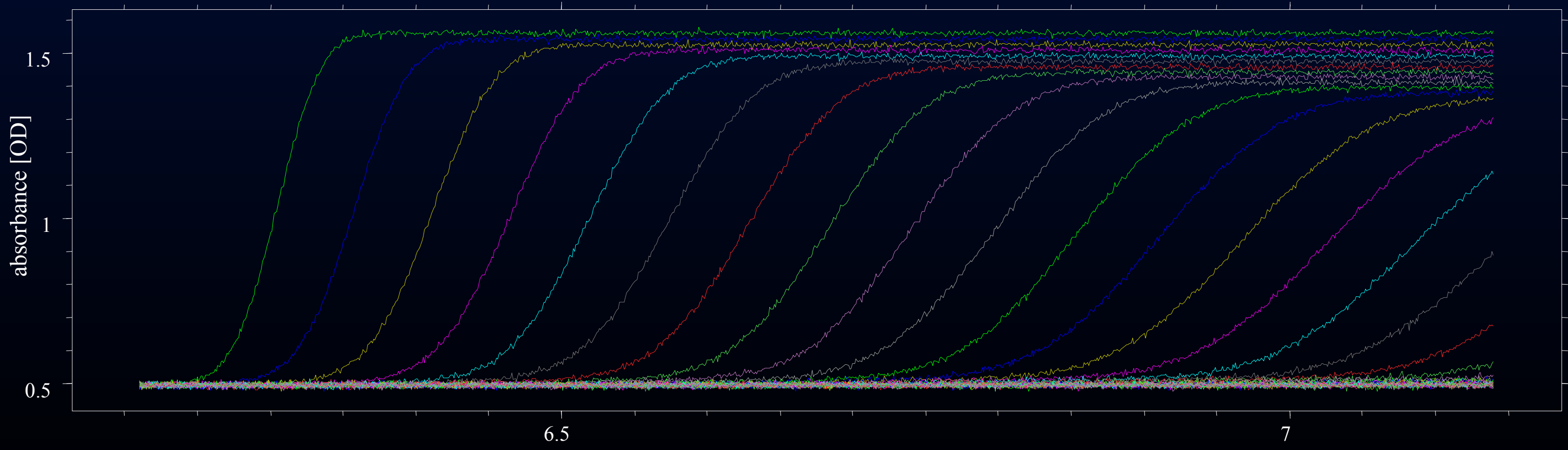




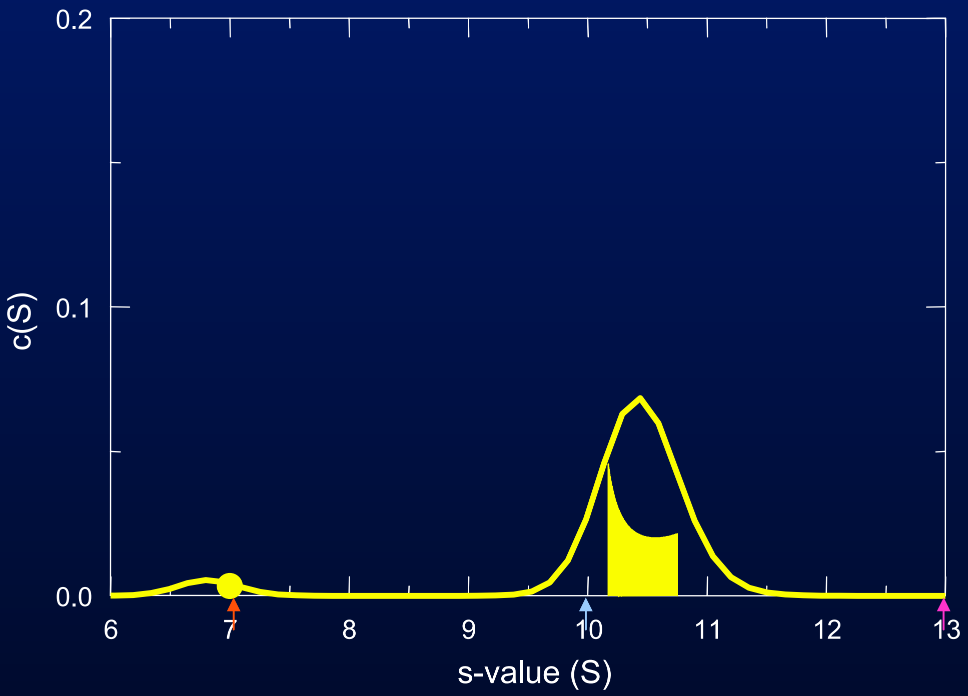
larger species constant at K_D

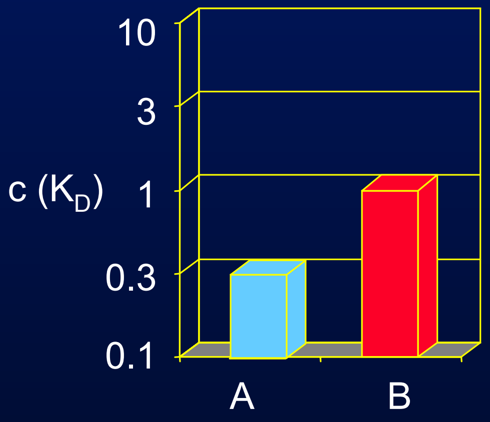


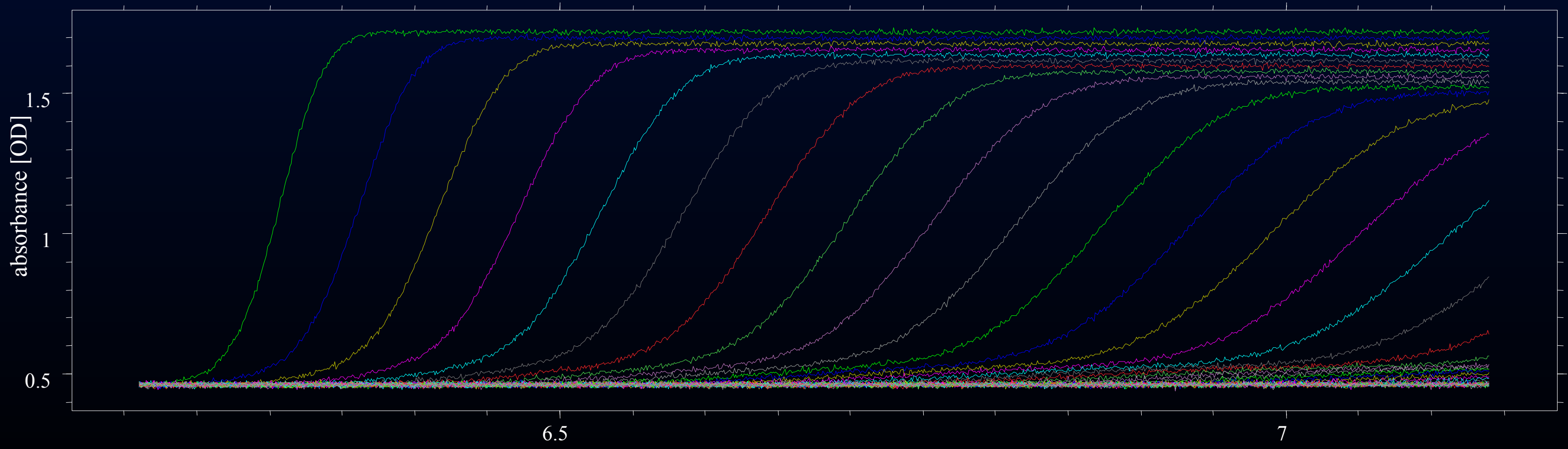




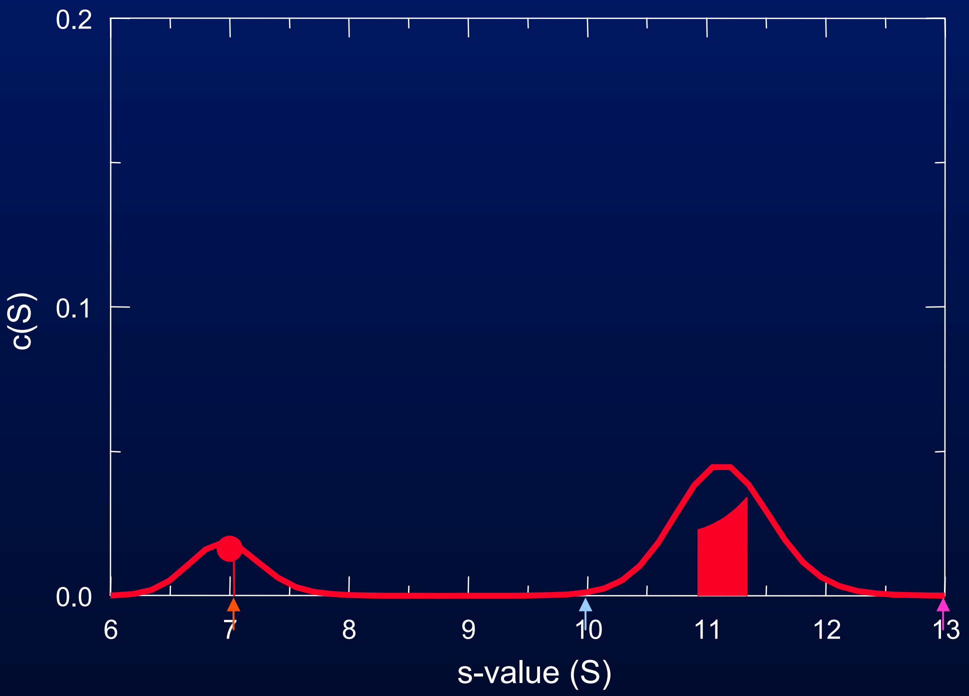
larger species constant at K_D

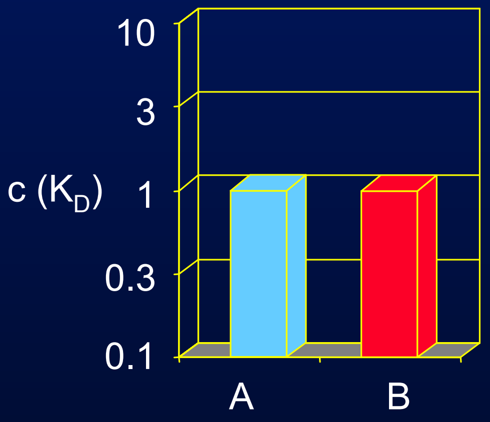


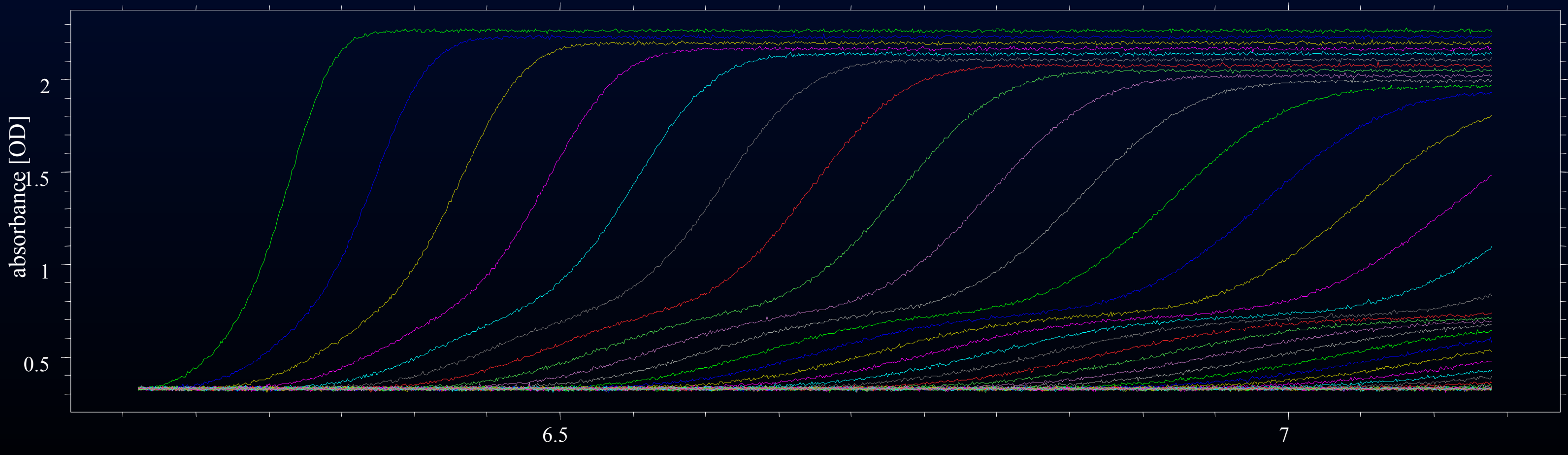




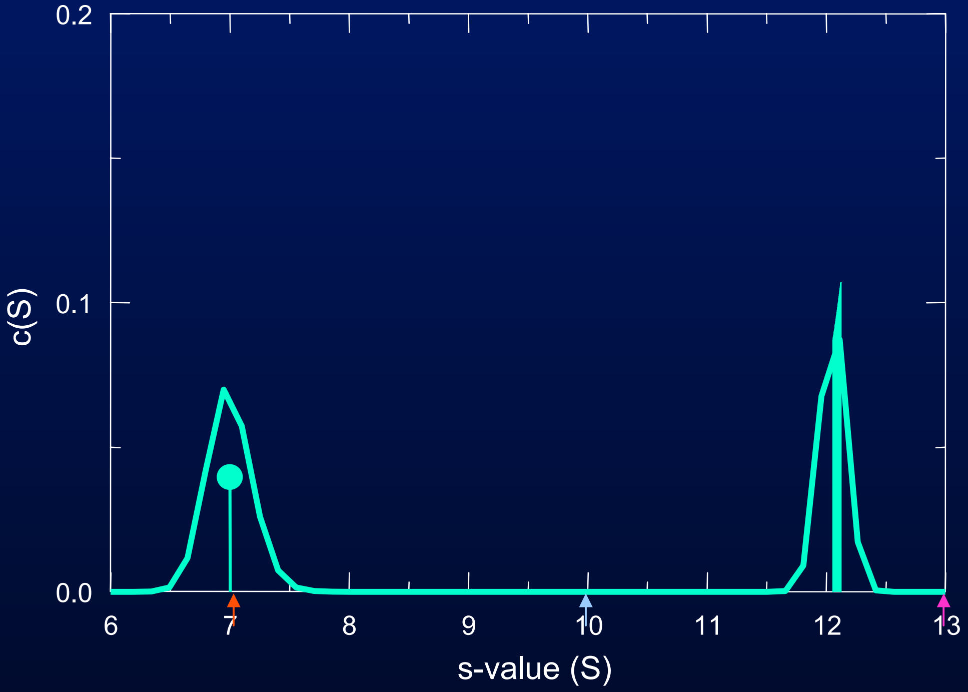
larger species constant at K_D

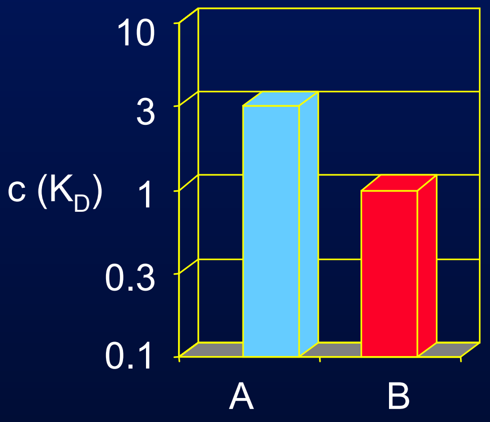


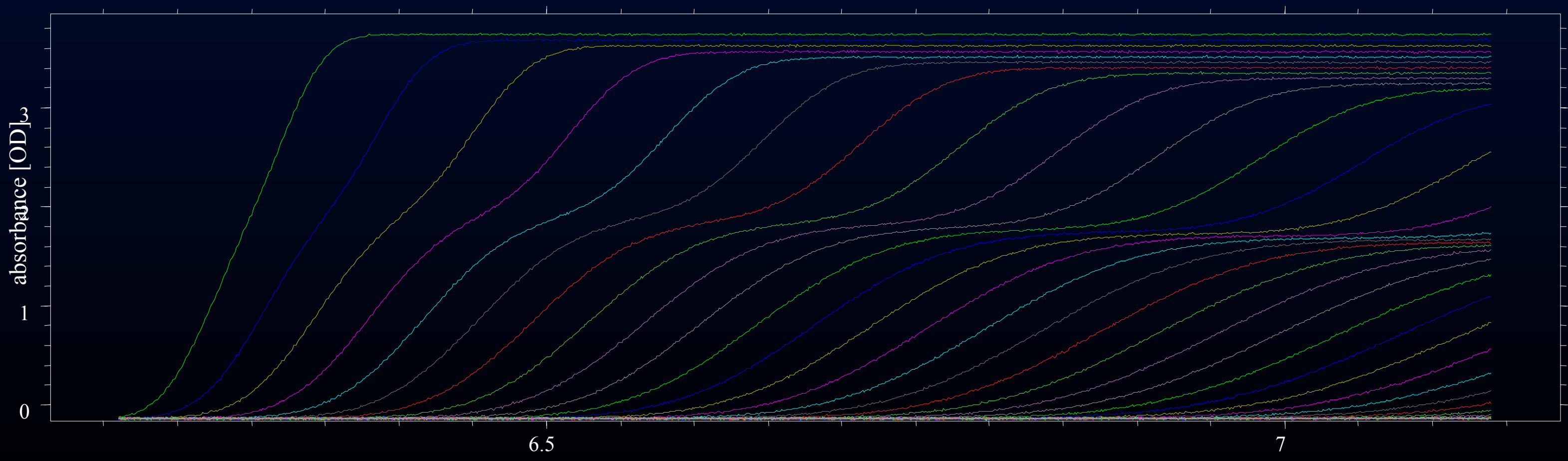




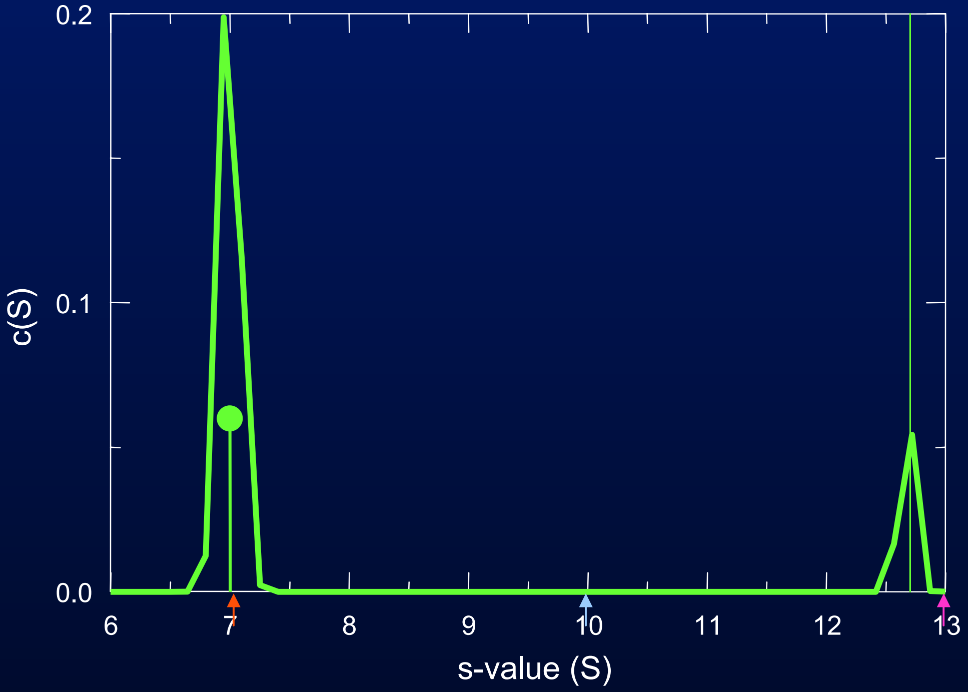
larger species constant at K_D

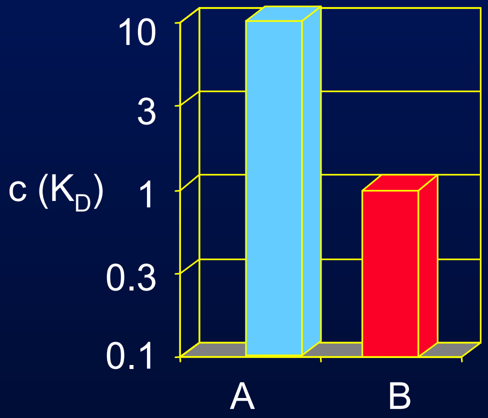


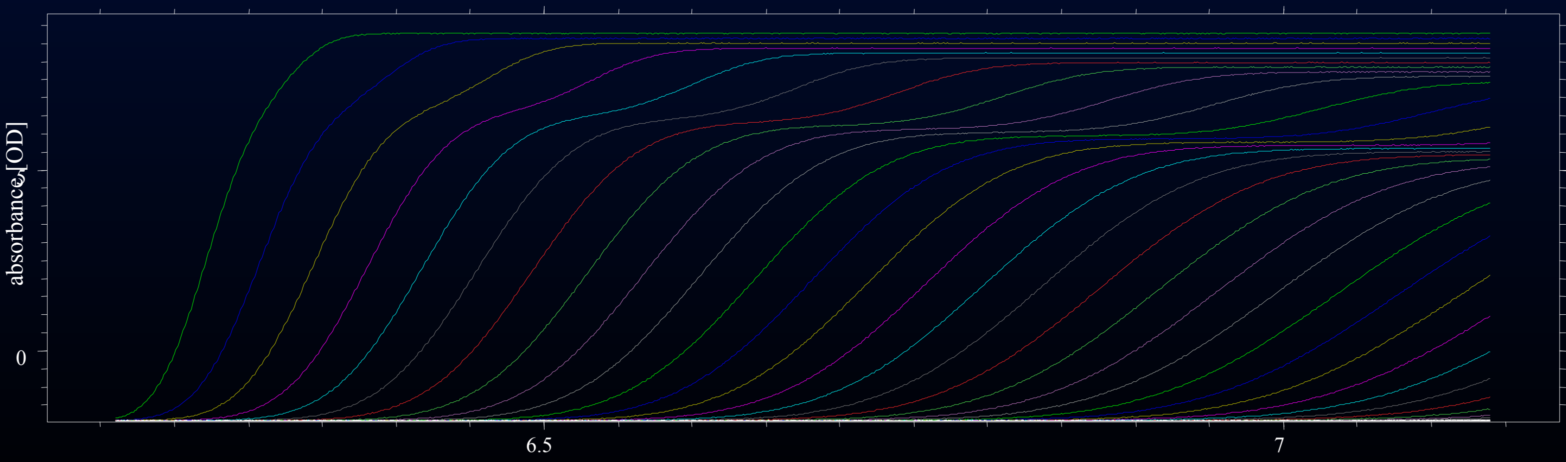




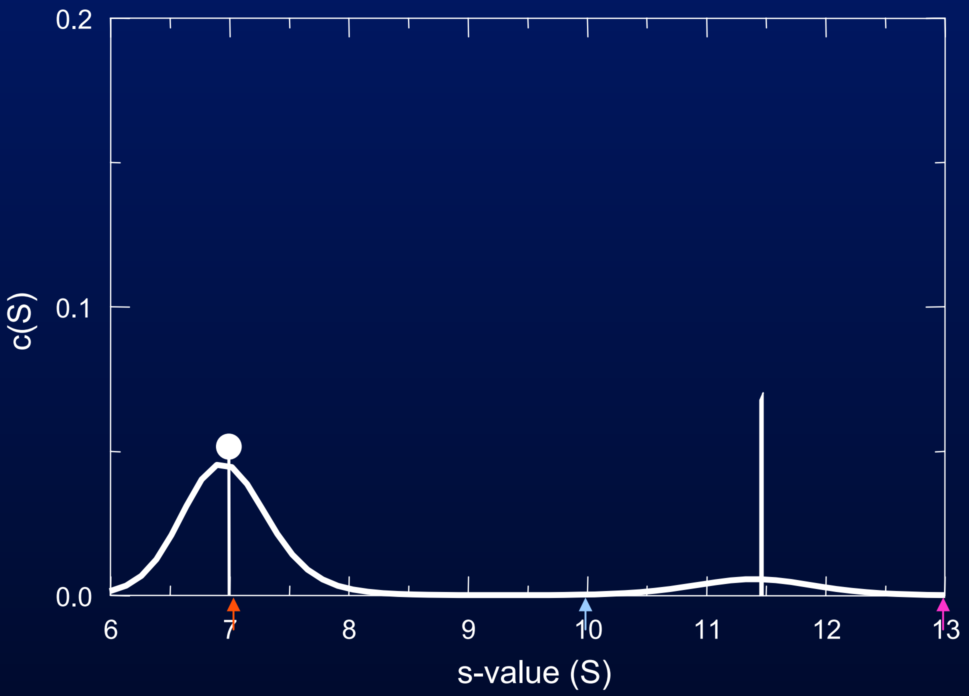
larger species constant at K_D

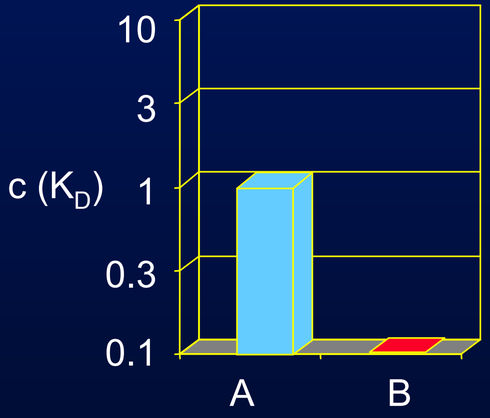


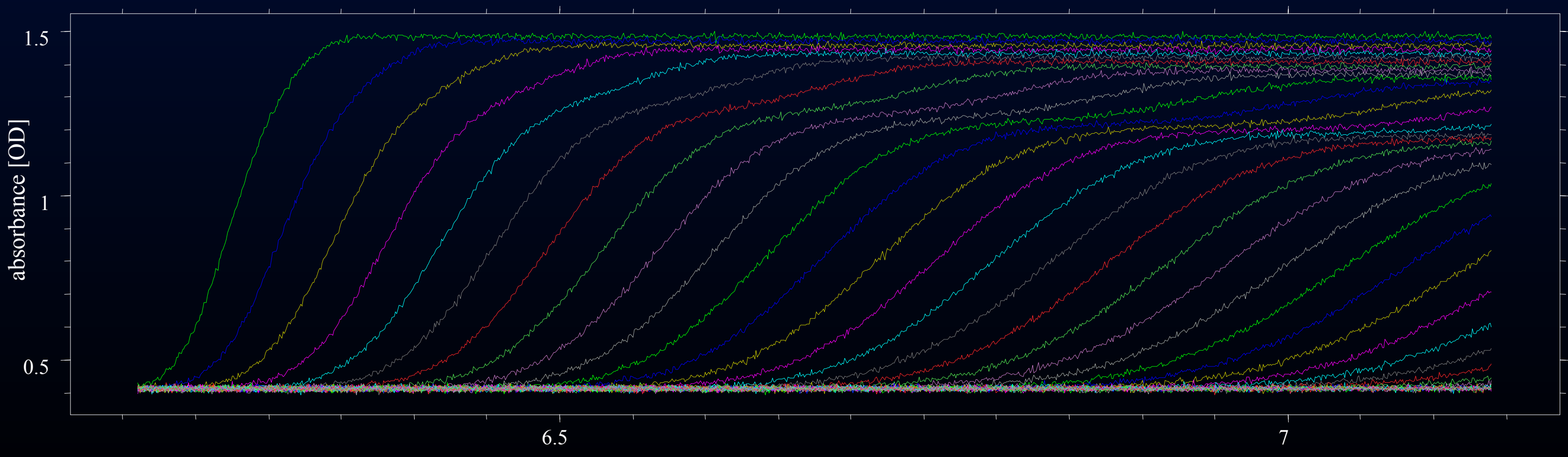




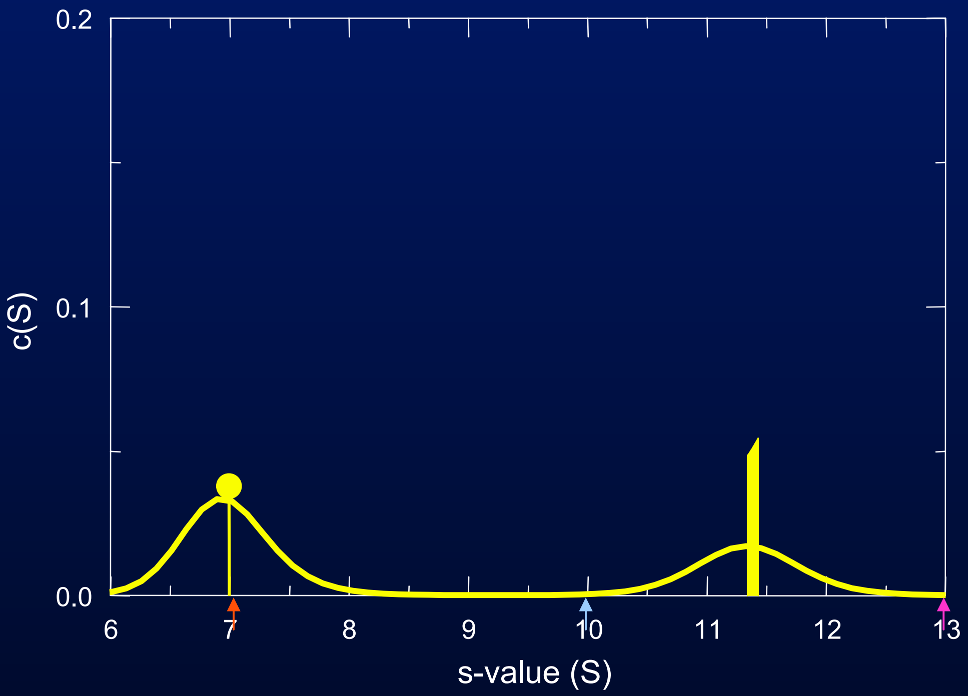
smaller species constant at K_D

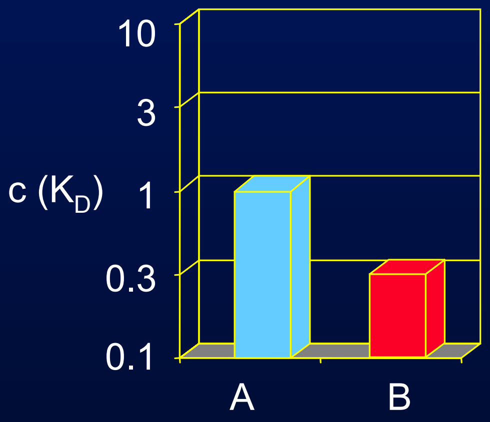


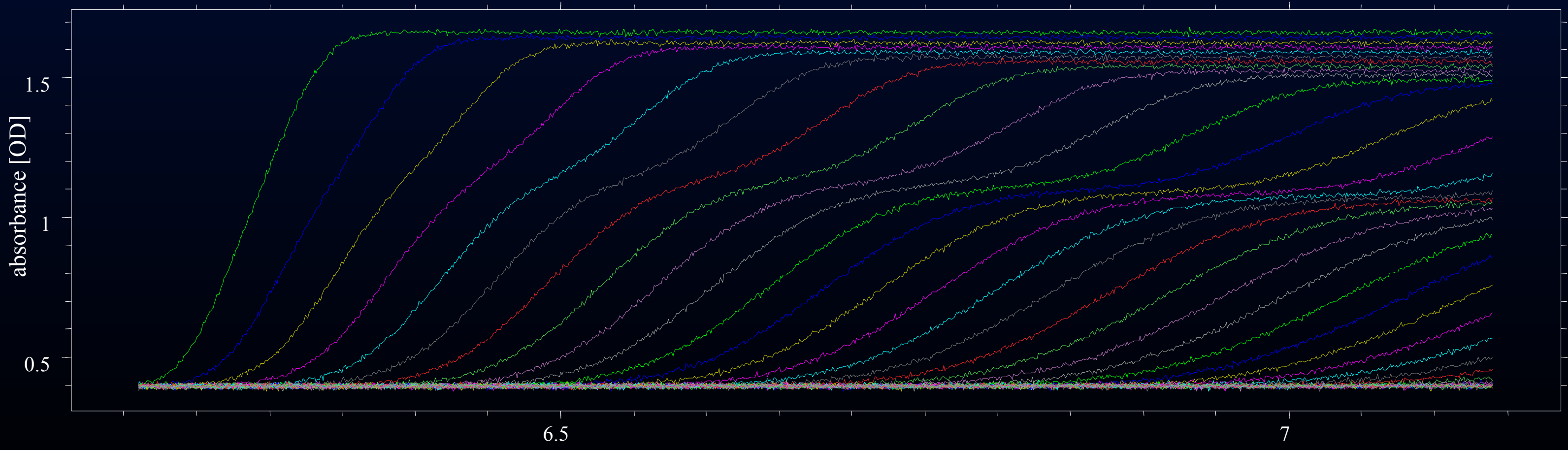




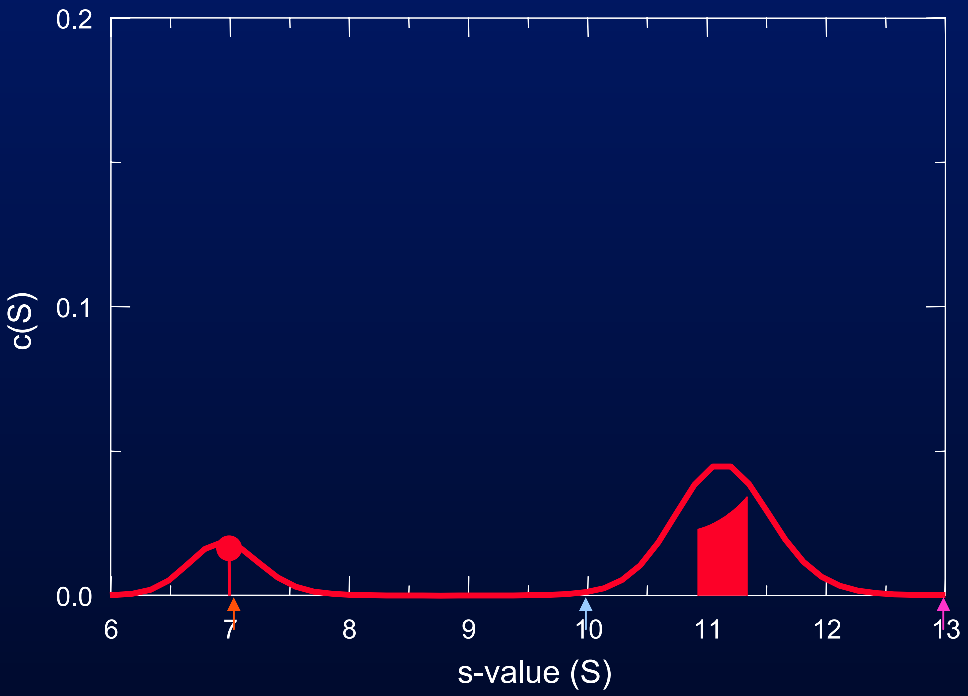
smaller species constant at K_D

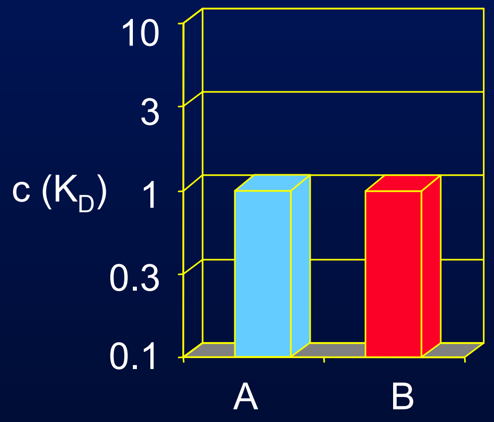


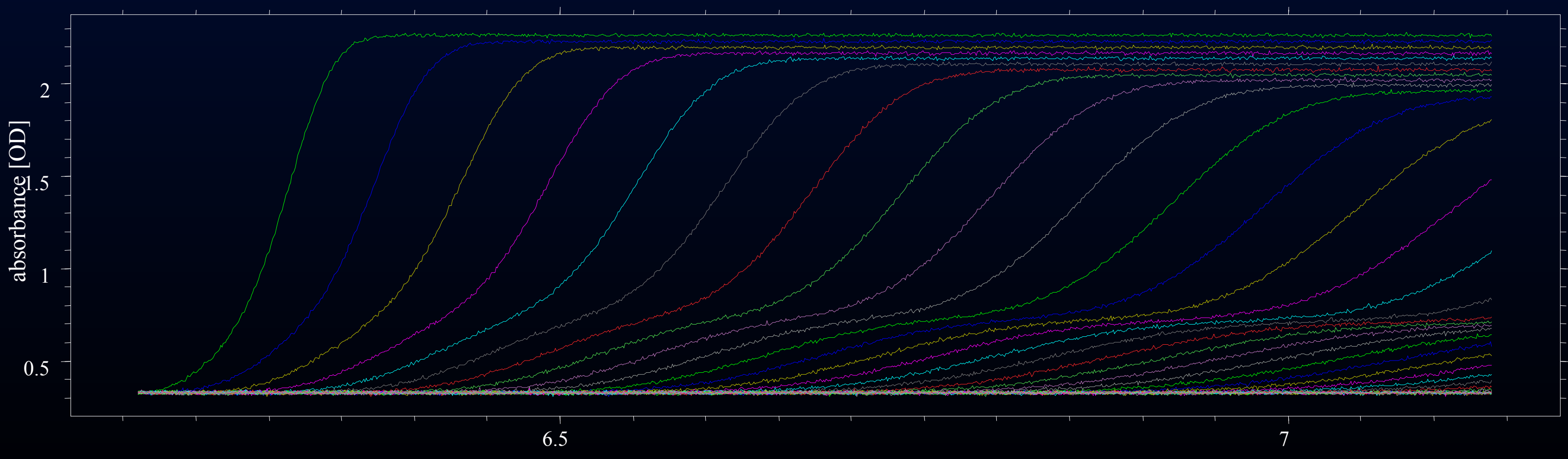




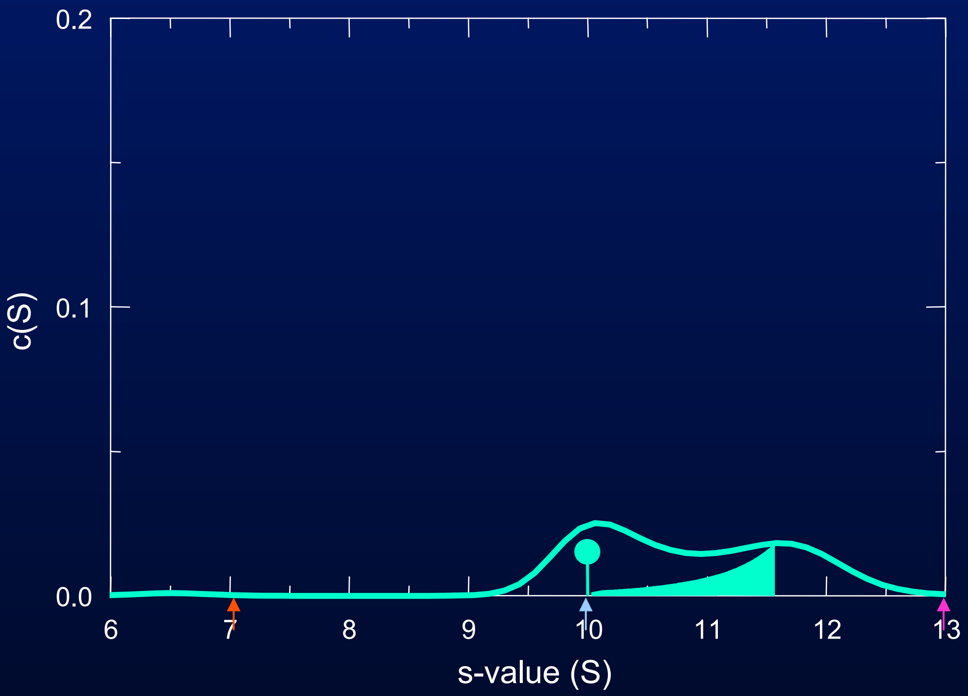
smaller species constant at K_D

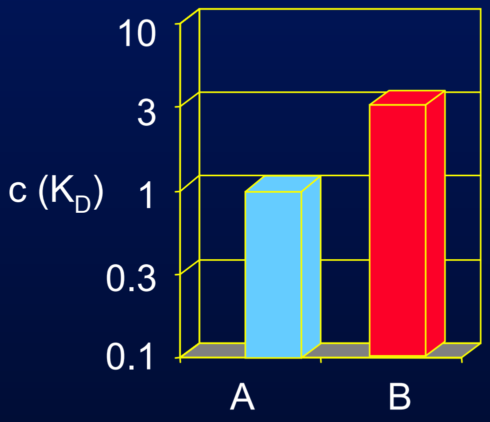


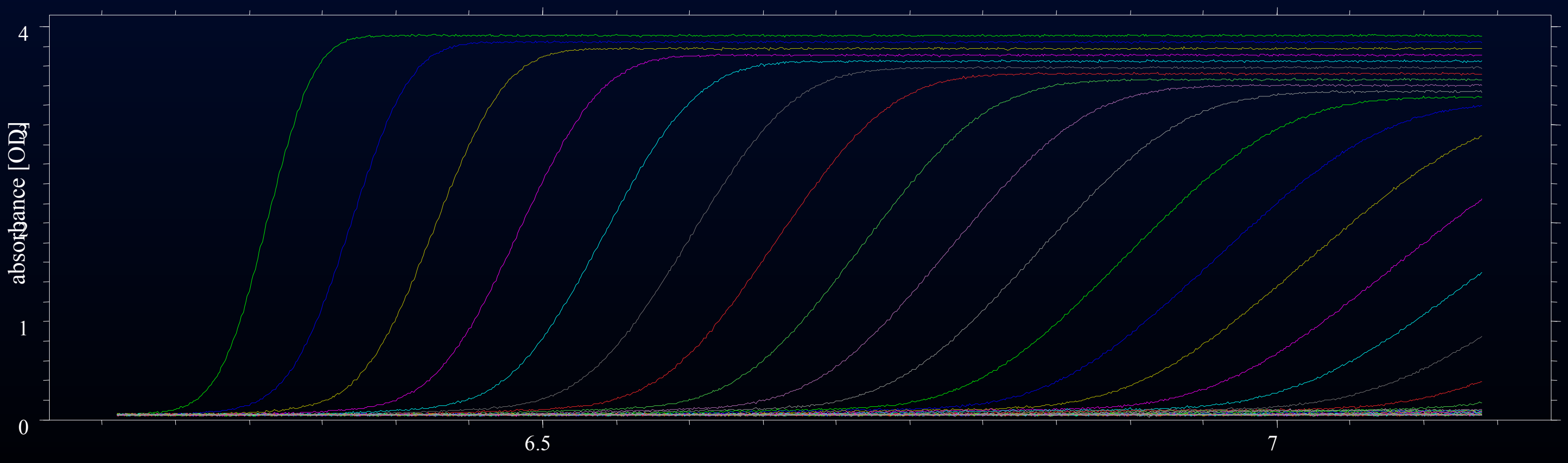




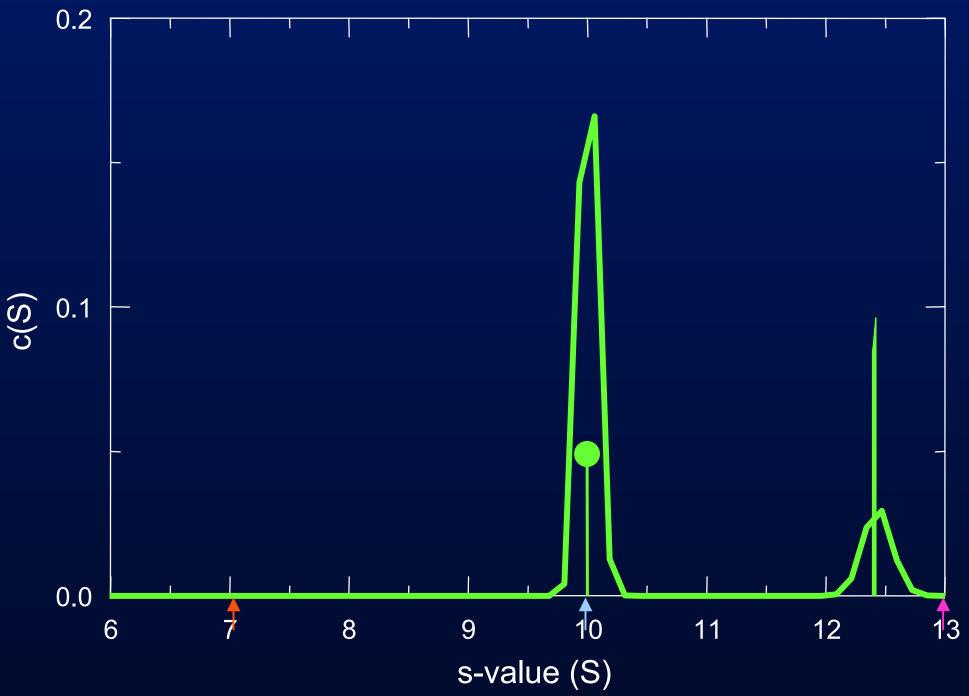
smaller species constant at K_D

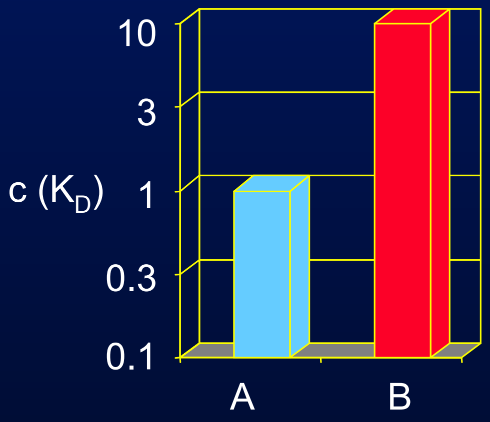


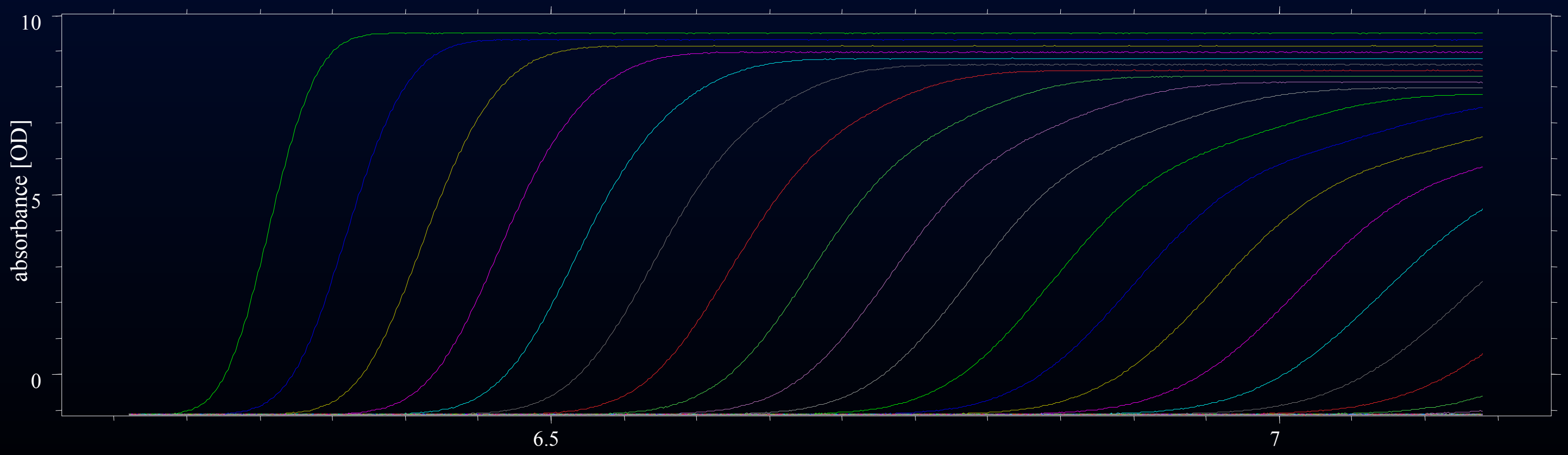




smaller species constant at K_D







→ pretty consistent, enough to convince conceptually OK
→ but not 100% identical (maximum entropy regularization, linear Lamm equation model)

how to utilize that quantitatively?

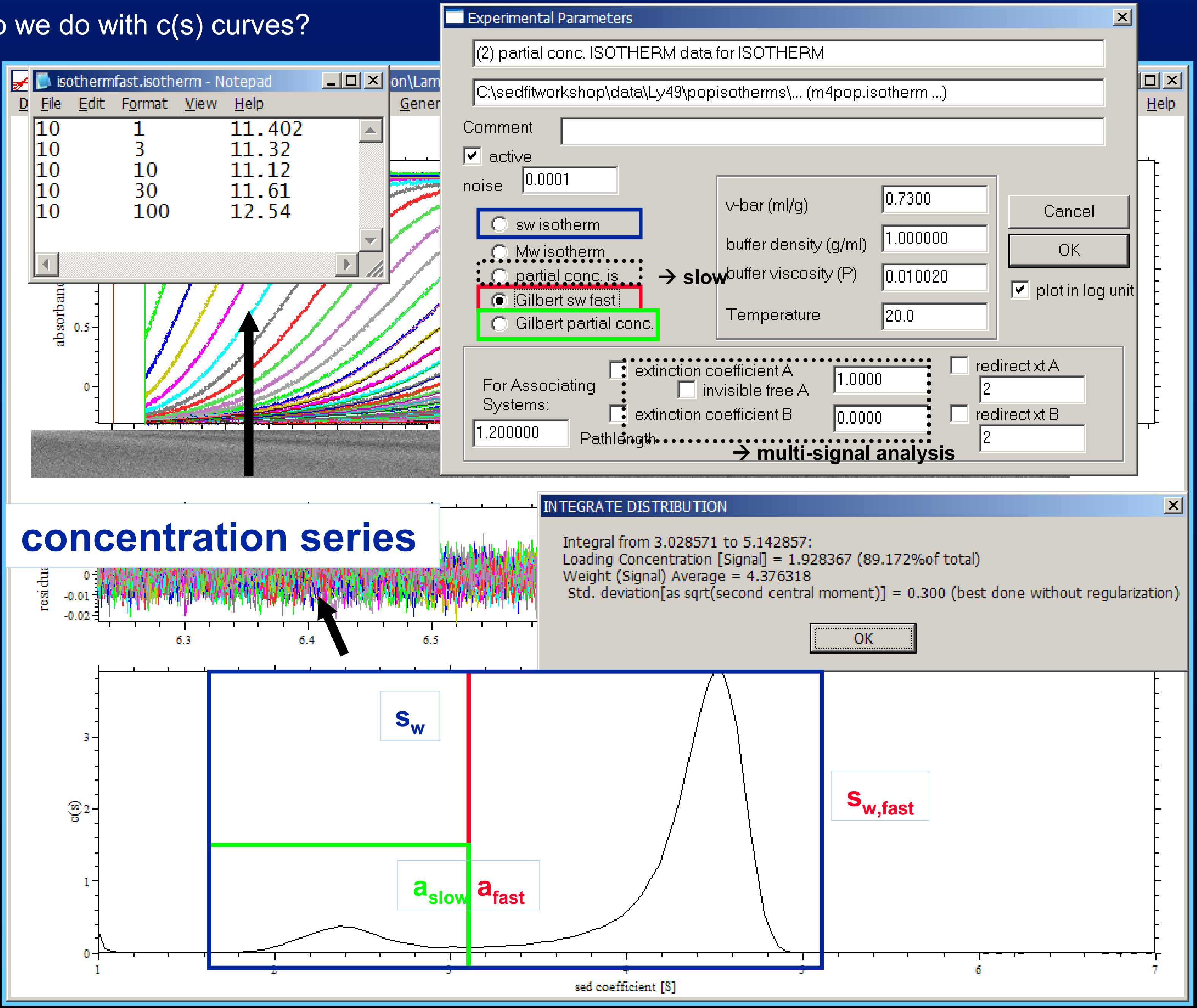
➔ INTEGRATE weight-average s-value of of boundary components, and amplitudes
➔ compare with theoretical values from GJT, also integrated

corresponds to a BOUNDARY SHAPE analysis **without** requirements of Lamm equation modeling

- in global model with weight-average
- in global model with different signals/wavelengths

J. Dam, P. Schuck (2005) Sedimentation velocity analysis of heterogeneous protein-protein interactions: Sedimentation coefficient distributions c(s) and asymptotic boundary profiles from Gilbert-Jenkins theory. Biophysical Journal 89:651-666

what do we do with c(s) curves?



isothermfast.isotherm - Notepad

```
10        1       11.402
10        3       11.32
10        10      11.12
10        30      11.61
10        100     12.54
```

Experimental Parameters

(2) partial conc. ISOTHERM data for ISOTHERM

C:\sedfitworkshop\data\Ly49\popisotherms\... (m4pop.isotherm ...)

Comment

☑ active

noise 0.0001

○ sw isotherm

○ Mw isotherm

○ partial conc. is

● Gilbert sw fast

○ Gilbert partial conc.

→ slow

v-bar (ml/g) 0.7300

buffer density (g/ml) 1.000000

buffer viscosity (P) 0.010020

Temperature 20.0

☑ plot in log unit

For Associating Systems:

☐ extinction coefficient A 1.0000

☐ invisible free A

☐ extinction coefficient B 0.0000

1.200000 Pathlength

☐ redirect xt A 2

☐ redirect xt B 2

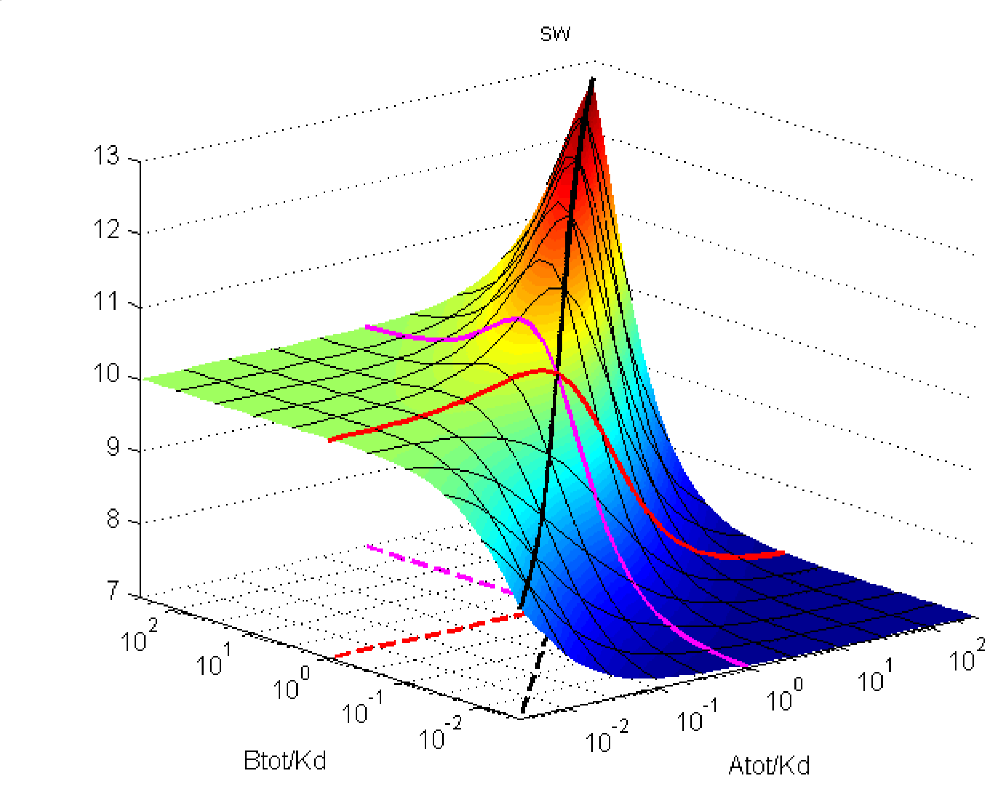
→ multi-signal analysis

concentration series

INTEGRATE DISTRIBUTION

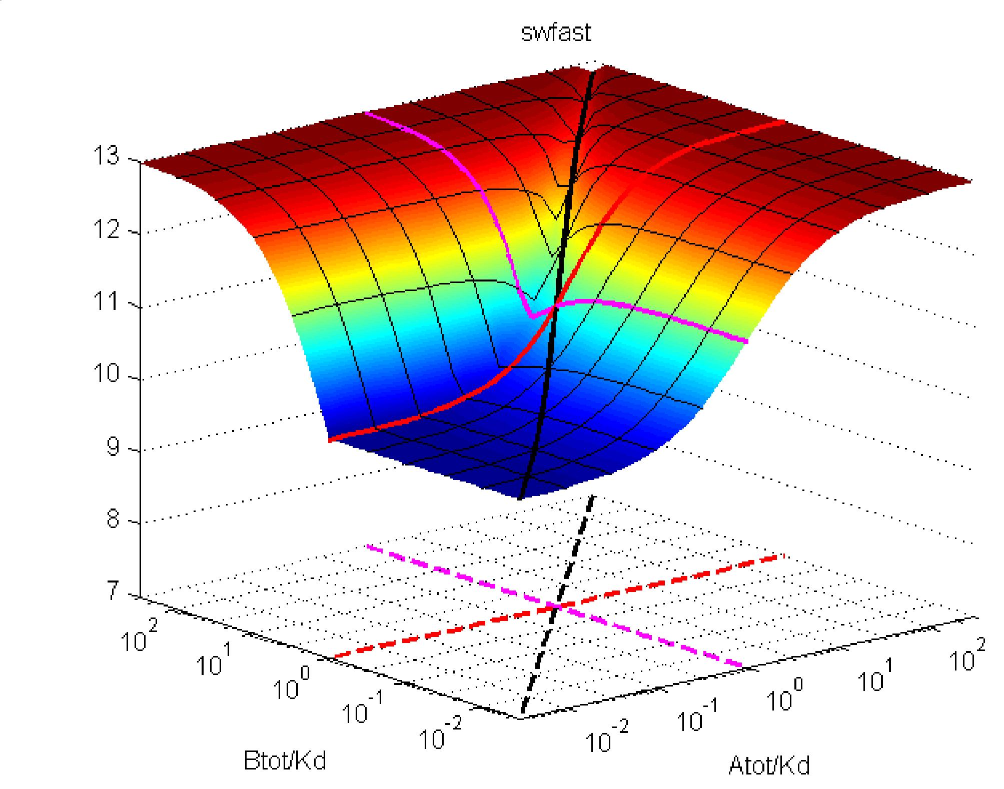
```
Integral from 3.028571 to 5.142857:
Loading Concentration [Signal] = 1.928367 (89.172%of total)
Weight (Signal) Average = 4.376318
 Std. deviation[as sqrt(second central moment)] = 0.300 (best done without regularization)
```

THEORY - what to expect? - sw is well-known



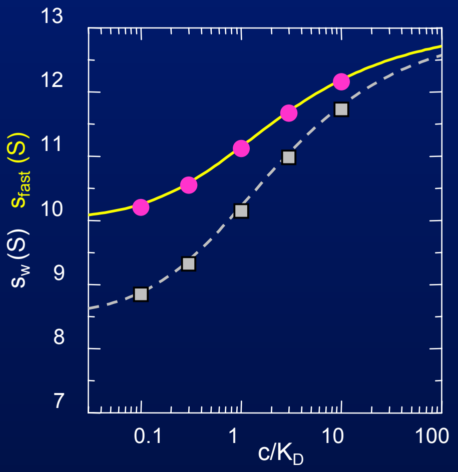
s_w approaches s_{AB} only for precise stoichiometry, otherwise far away

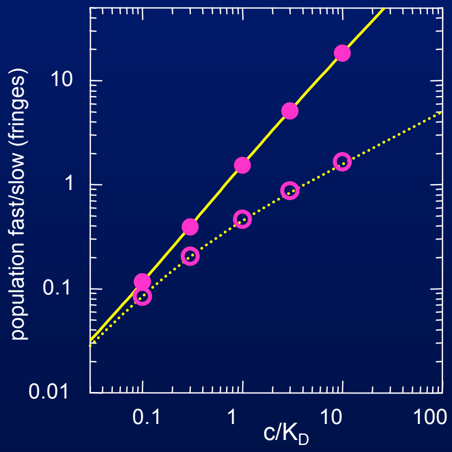
THEORY - this is s_{fast} from GJT



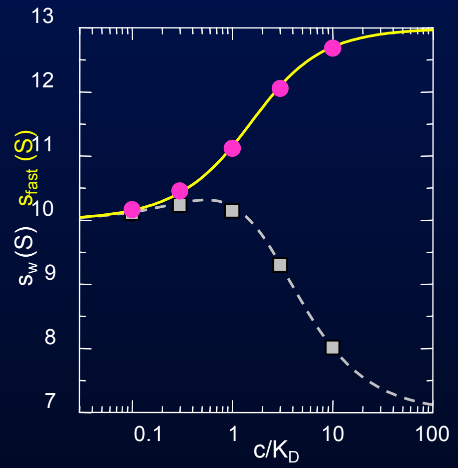
s_{fast} approaches s_{AB} in any case with c_A or c_B >> K_D

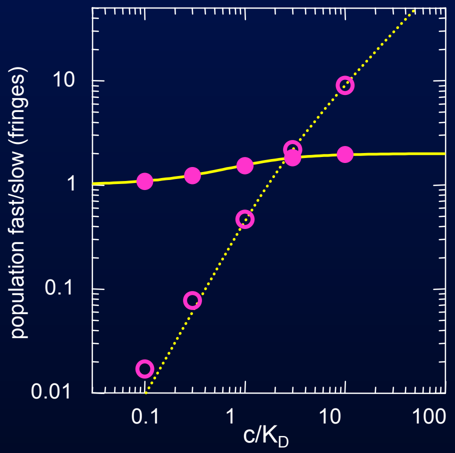
equimolar



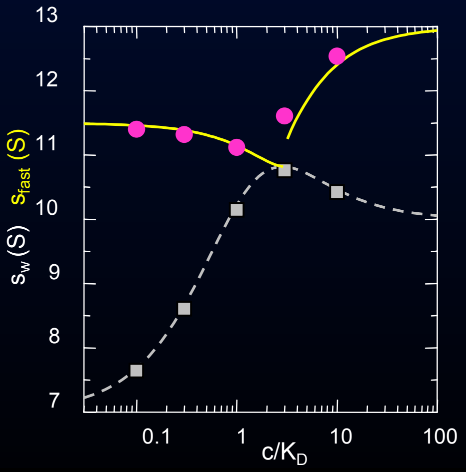


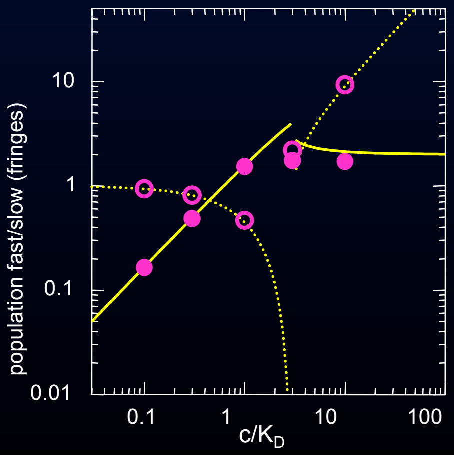
fix b = K_D





fix a = K_D



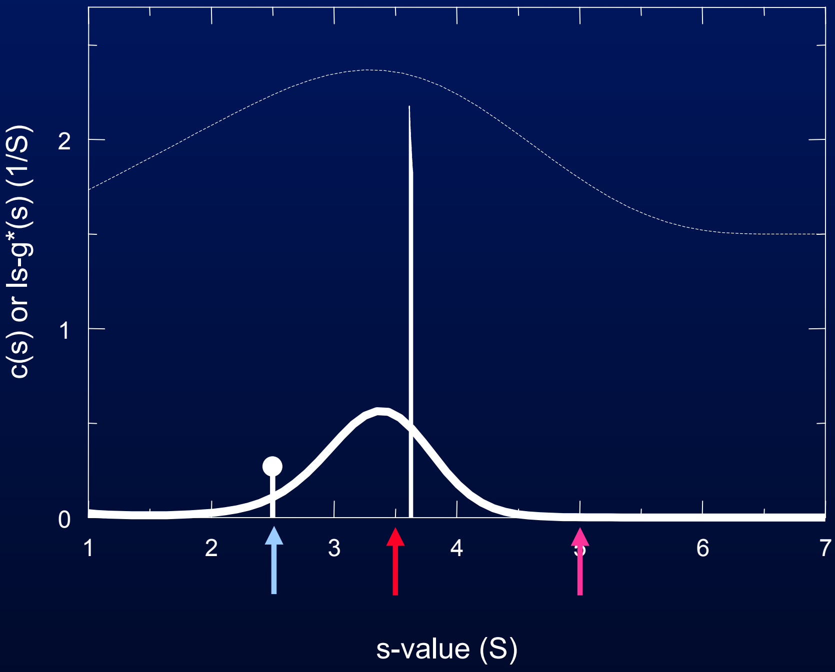


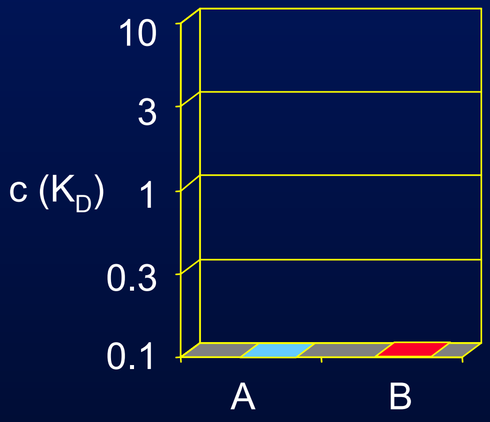
So far, examples have shown clearly separate boundaries.

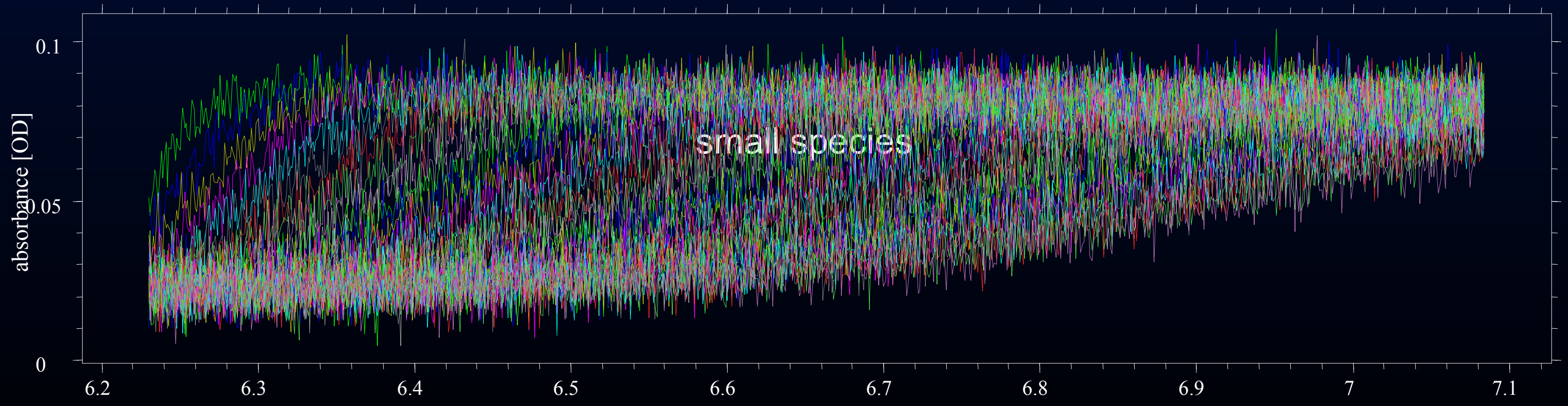
→ how does it work for a small species with high D?

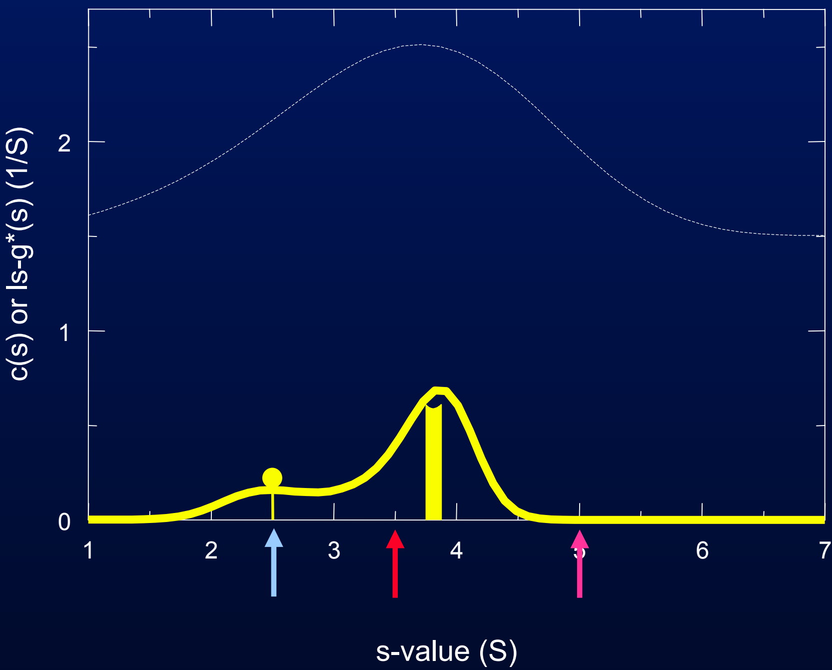
… real test for deconvolution of diffusion in c(s)

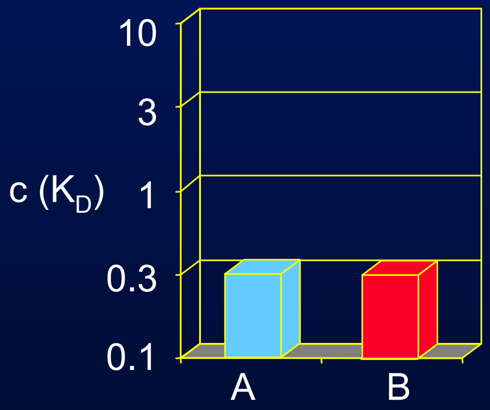
A (25 kDa, 2.5 S) + B (40kDa, 3.5 S) ⇔ AB (5 S), k_{off} = 0.01/sec

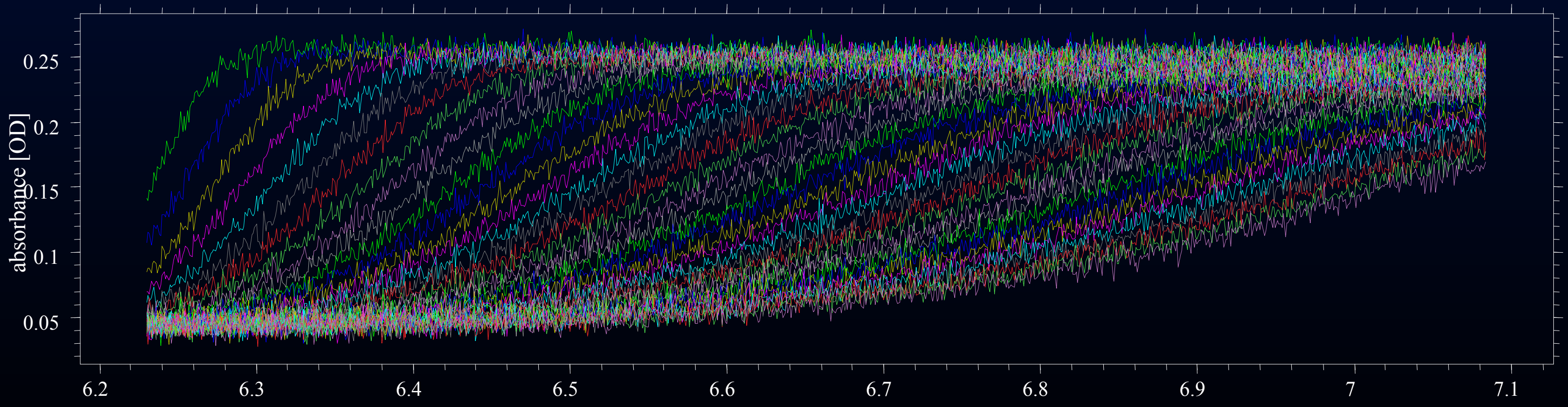


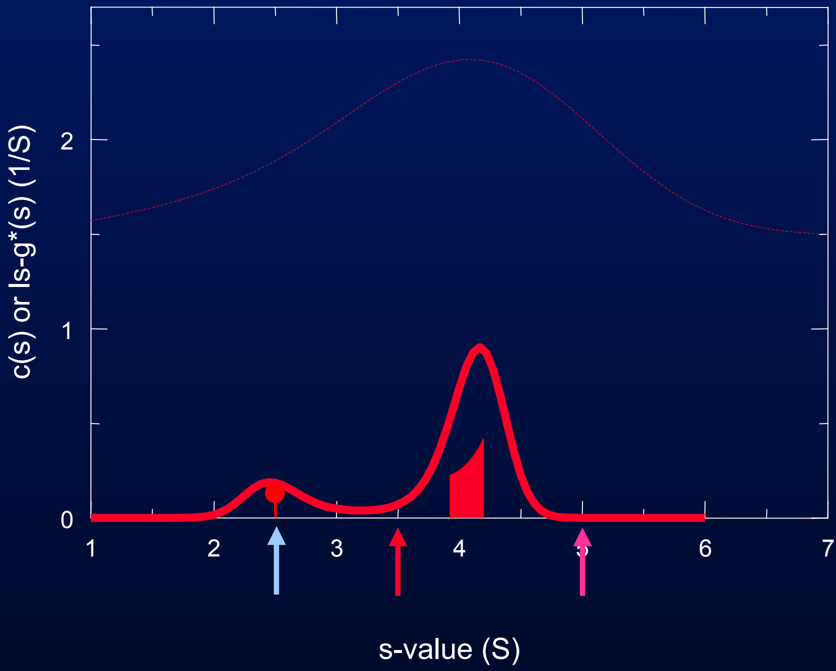


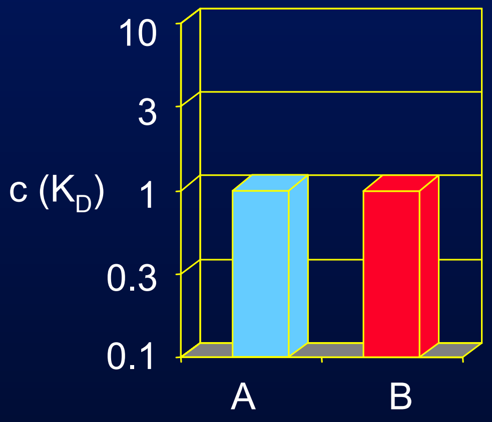


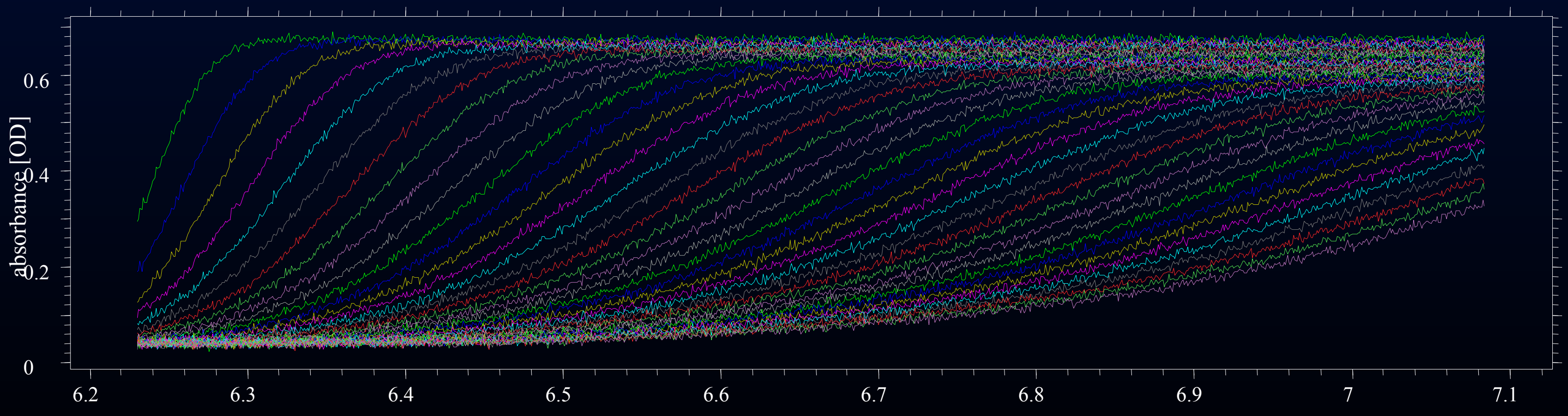

c(s) or ls-g*(s) (1/S)
0
1
2
1
2
3
4
5
6
7
s-value (S)

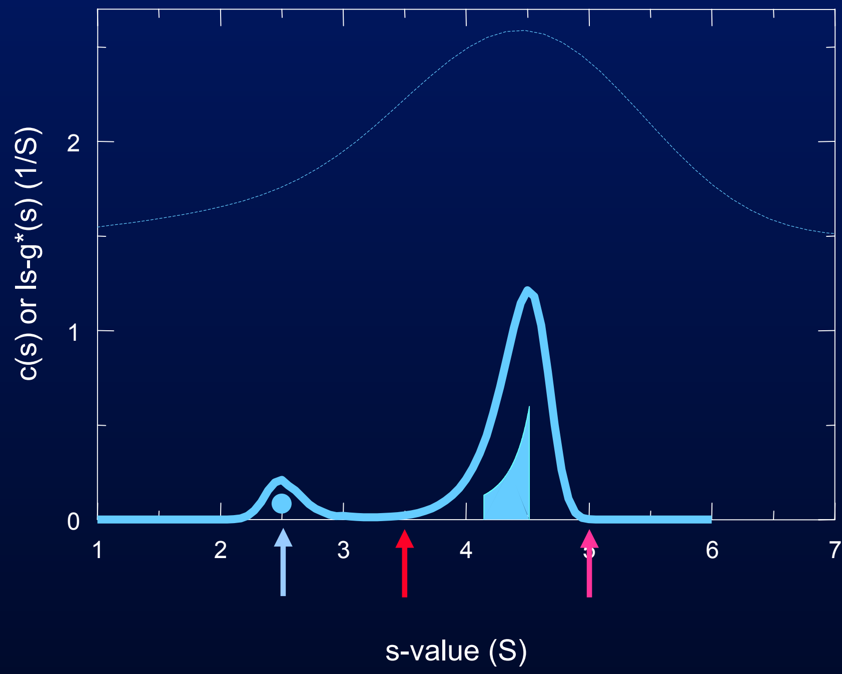

c (K_D)
10
3
1
0.3
0.1
A
B

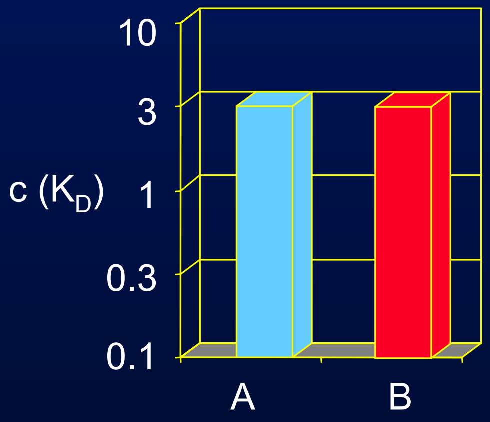

absorbance [OD]
0.05
0.1
0.15
0.2
0.25
6.2
6.3
6.4
6.5
6.6
6.7
6.8
6.9
7
7.1

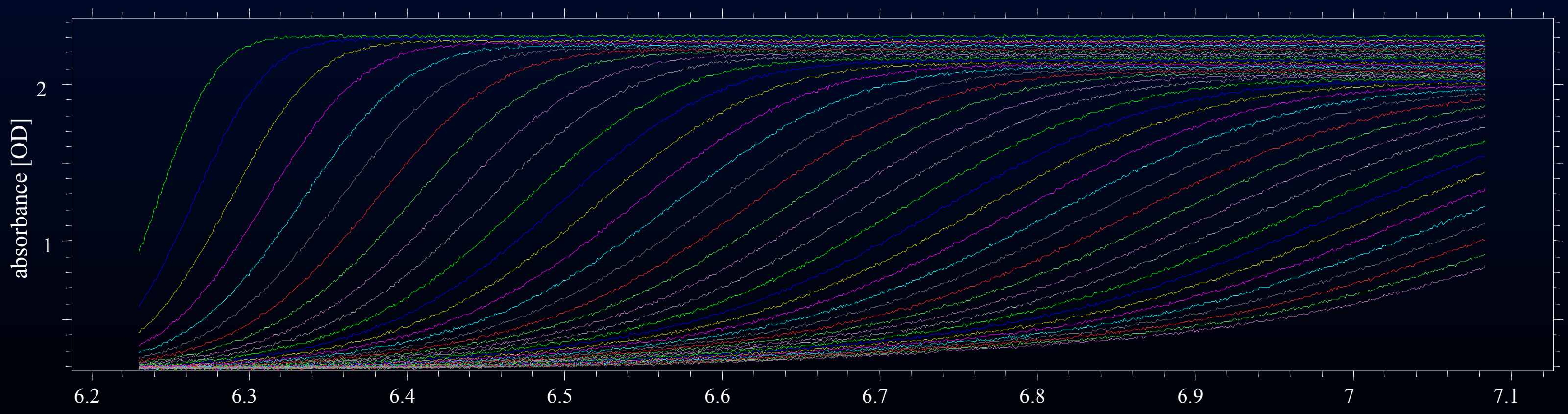

c(s) or ls-g*(s) (1/S)
0
1
2
1
2
3
4
5
6
7
s-value (S)

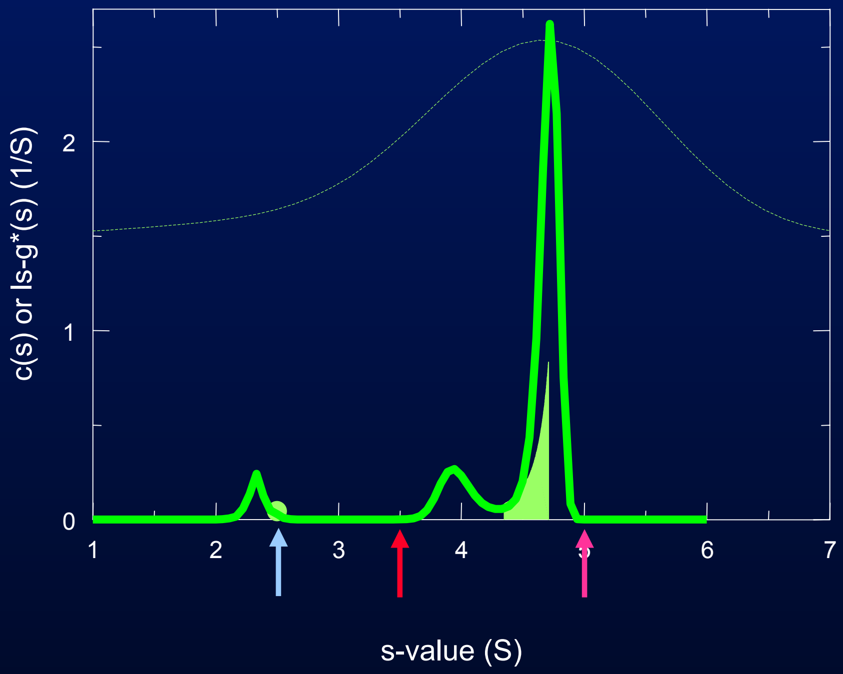

c (K_D)
10
3
1
0.3
0.1
A
B

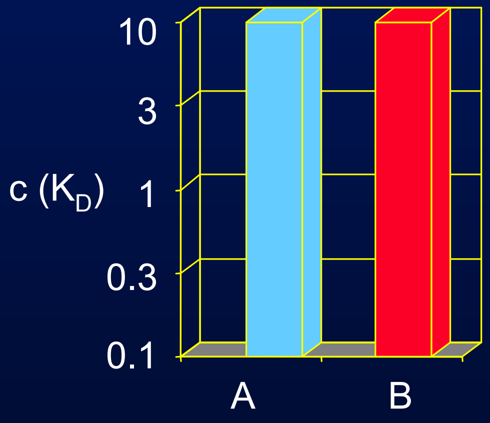

absorbance [OD]
0
0.2
0.4
0.6
6.2
6.3
6.4
6.5
6.6
6.7
6.8
6.9
7
7.1

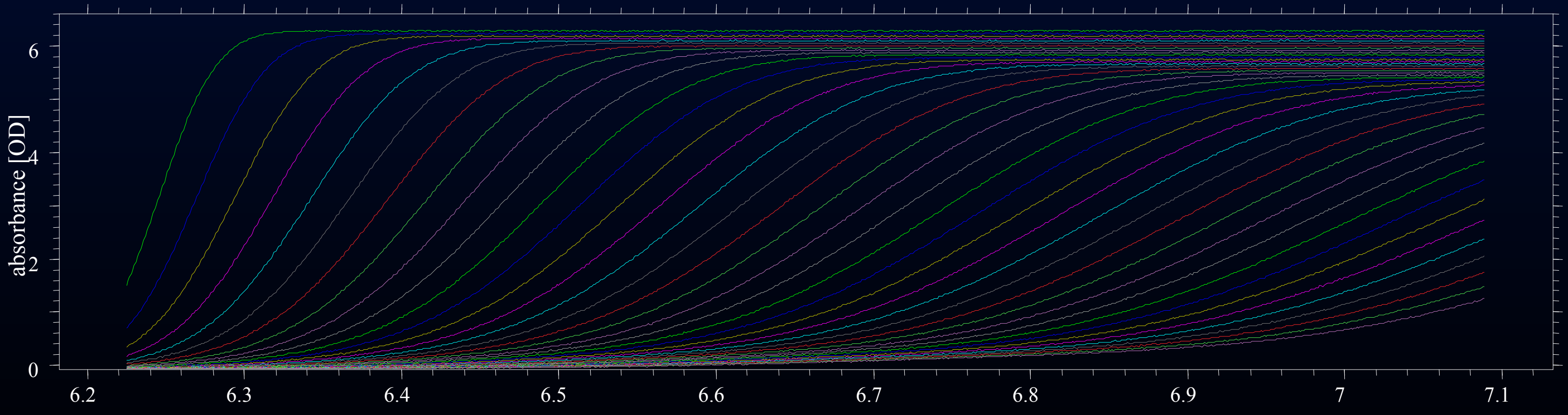

c(s) or ls-g*(s) (1/S)
0
1
2
1
2
3
4
6
7
s-value (S)

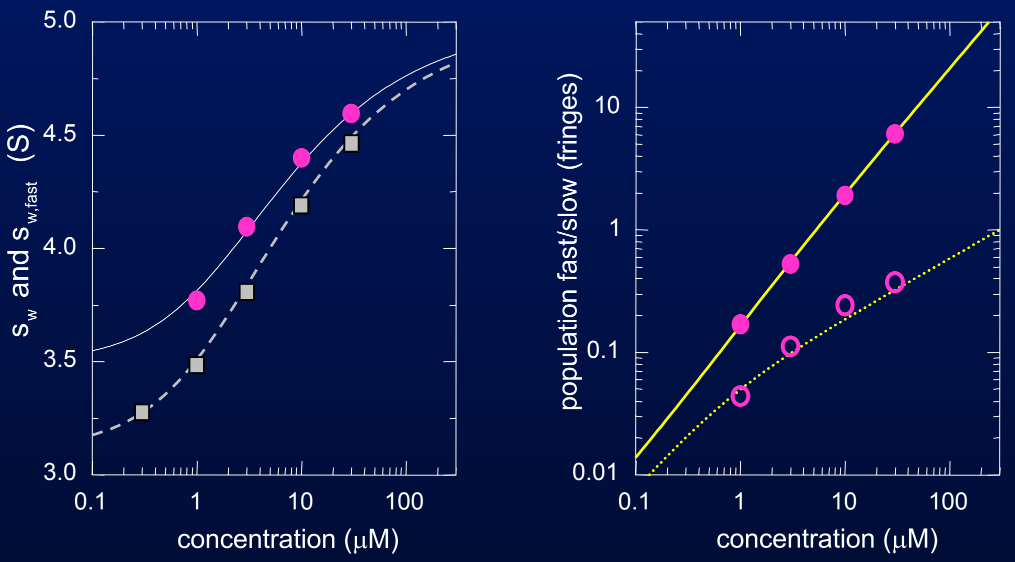

c (K$_D$)
10
3
1
0.3
0.1
A
B


absorbance [OD]
1
2
6.2
6.3
6.4
6.5
6.6
6.7
6.8
6.9
7
7.1


c(s) or ls-g*(s) (1/S)
0
1
2
1
2
3
4
5
6
7
s-value (S)


c (K$_D$)
10
3
1
0.3
0.1
A
B

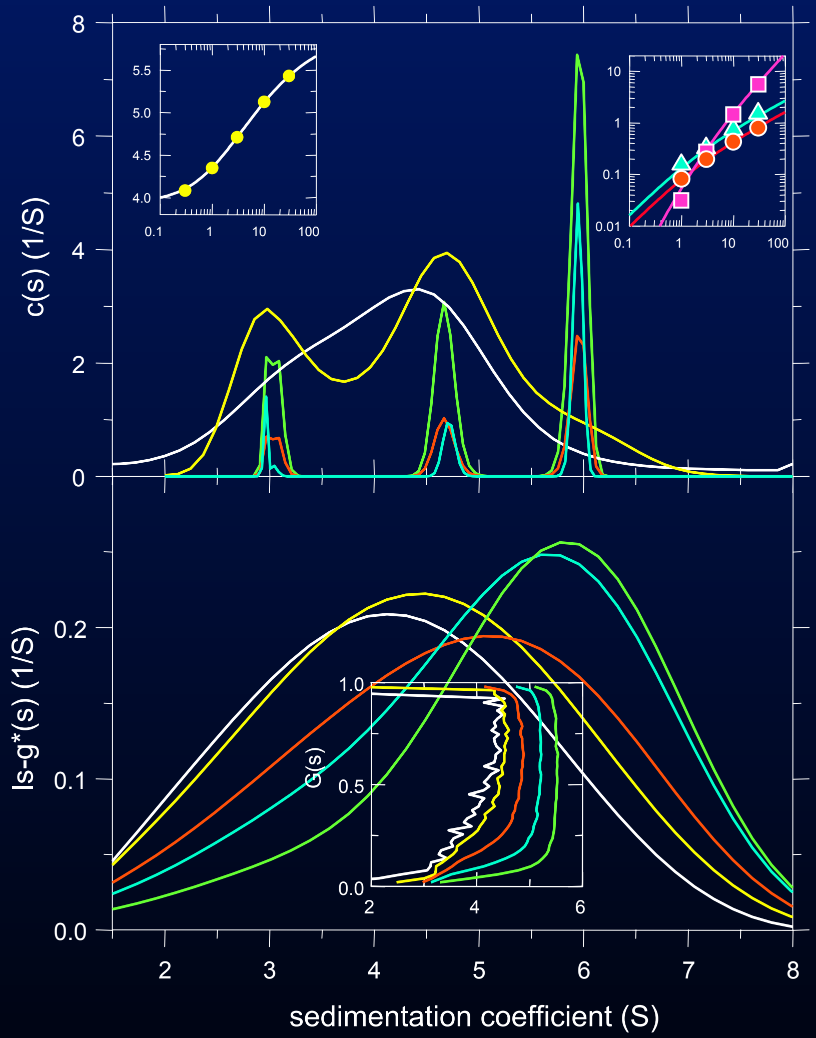

absorbance [OD]
0
2
4
6
6.2
6.3
6.4
6.5
6.6
6.7
6.8
6.9
7
7.1


5.0
4.5
4.0
3.5
3.0
s_w and $s_{w,fast}$ (S)
0.1
1
10
100
concentration (μM)
10
1
0.1
0.01
population fast/slow (fringes)
0.1
1
10
100
concentration (μM)

slow

$k_{off} = 3.2\times10^{-5}$/sec

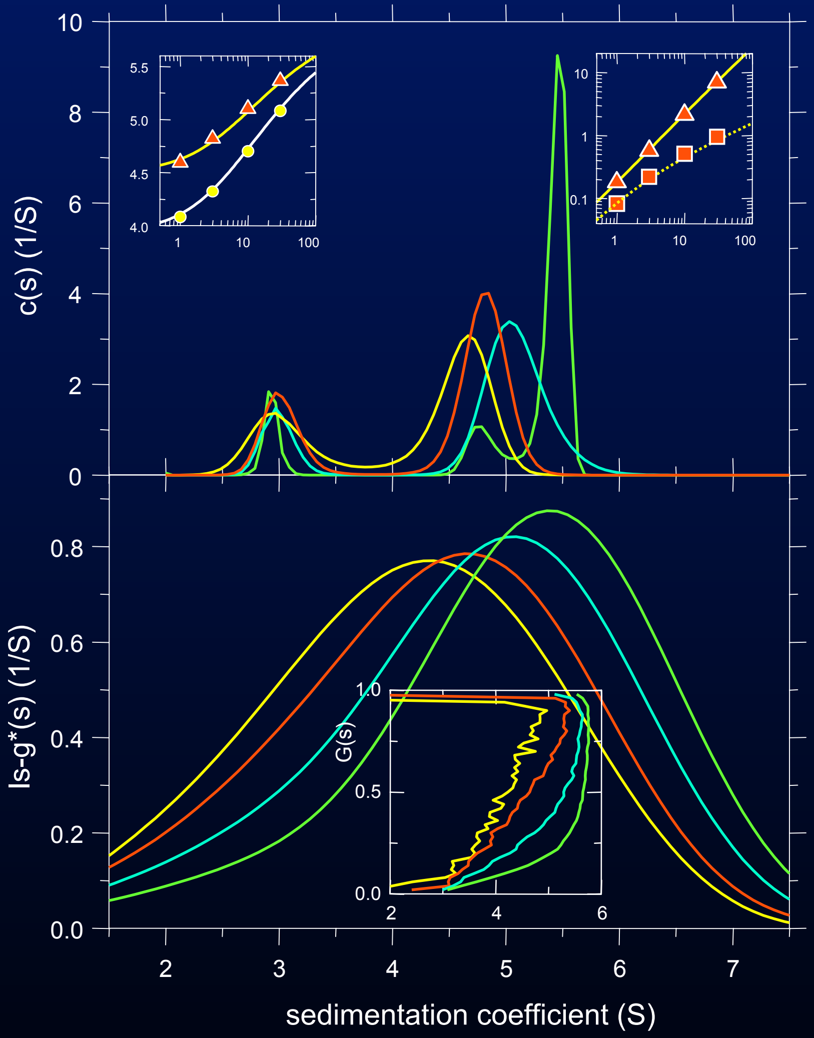
$K_d = 3.3\ \mu M$,
c = 0.3, 1, 3, 10, 30 µM



fast

$k_{off} = 0.01$/sec

Kd = 10 µM,
c = 1, 3, 10, 30 µM



Summary of c(s) analysis of reacting systems:

- c(s) approximately deconvolutes diffusion from reaction boundaries
- c(s) distributions are approximations of the asymptotic boundary from Gilbert-Jenkins theory
- Gilbert-Jenkins modeling of c(s) can be quantitatively used to model isotherms of reaction boundary, in addition to $s_w(c)$
- higher confidence in K_D and $s_{complex}$
- characteristic information on stoichiometry
- takes advantage of bimodal boundary structure, without need for detailed Lamm eq. modeling (can of course be used, afterwards, too, using parameter estimates)

J. Dam, P. Schuck (2005) Sedimentation velocity analysis of heterogeneous protein-protein interactions: Sedimentation coefficient distributions c(s) and asymptotic boundary profiles from Gilbert-Jenkins theory. Biophysical Journal 89:651-666

Summary of c(s) based analysis methods for reacting systems

second-moment method: mass balance

a) integrate all c(s) peaks at once

b) model weight-average (signal-average) $s_w(c)$ with isotherm model

for fast reactions: bi-modal boundary according to Gilbert-Jenkins theory

a) integrate fast and slow c(s) peaks

b) model signal-average s(c) of fast boundary with isotherm model

c) model amplitudes of fast and slow boundary with isotherm model

for slow reactions: baseline-separated peaks reveal species populations

a) integrate c(s) peaks individually

b) species population isotherm

for heterogeneous interactions: global multi-signal analysis for multi-component c(s)

a) for determining stoichiometry of extended associations

b) requires either $k_{off} < 10^{-4}$/sec or c > 3fold K_D

- no detailed boundary shape info used
- can deal with impurities outside range of interacting system
- useful to build model (stoichiometry, K_D , $s_{complex}$)
- concentration series → global modeling of isotherms

isotherm analysis

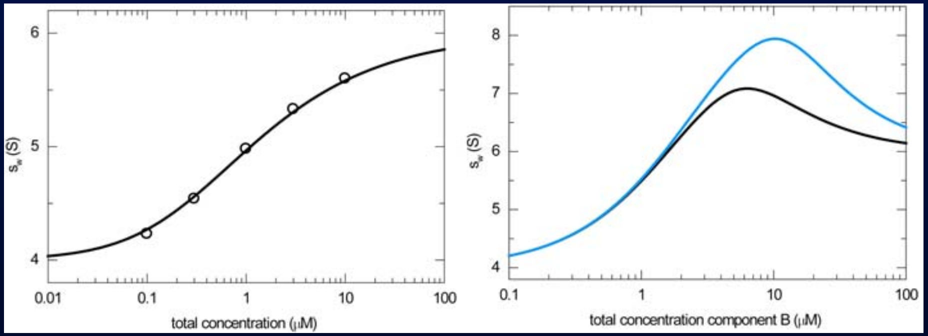
weight-average s-value

$$s_w(c^{tot}) = \frac{1}{c_{tot}} \sum_i c_i s_i$$

$$s_w^l(c_A^{tot}, c_B^{tot}) = \frac{e_A c_A^{free} s_A + e_B c_B^{free} s_B + (e_A + e_B) K c_A^{free} c_B^{free} s_{AB}}{e_A c_A^{tot} + e_B c_B^{tot}}$$

$$c_A^{tot} = c_A^{free} + K c_A^{free} c_B^{free}$$

$$c_B^{tot} = c_B^{free} + K c_A^{free} c_B^{free}$$



equimolar concentrations

titration configuration

- only mass balance consideration, no boundary shape
- slow and fast
- s_w(c) for self-association/hetero-association
- stoichiometry for hetero-association
- K_D, limiting s-value
- $s_{complex}$ hydrodynamic shape?

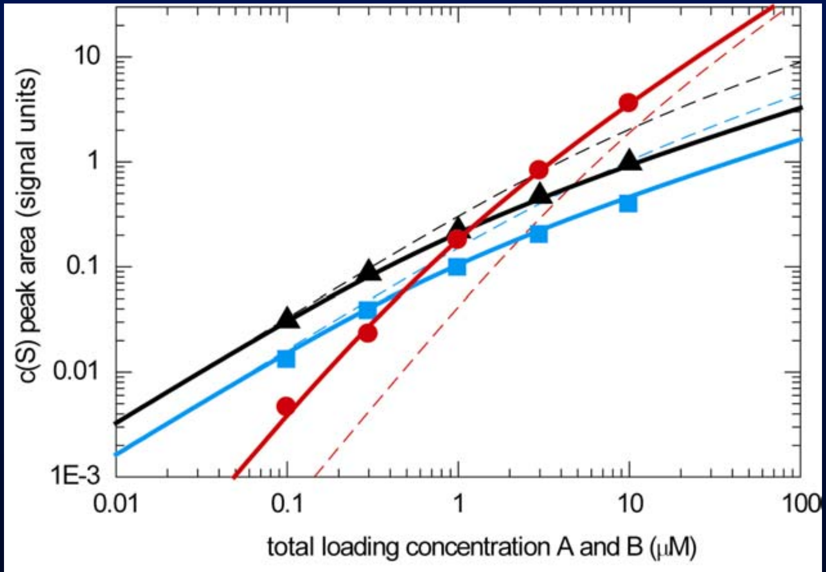
isotherm analysis

population isotherm

$$c_A^{free}(c_A^{tot}, c_B^{tot}), \quad c_B^{free}(c_A^{tot}, c_B^{tot}), \quad c_{AB}(c_A^{tot}, c_B^{tot})$$

$$c_A^{tot} = c_A^{free} + K c_A^{free} c_B^{free}$$

$$c_B^{tot} = c_B^{free} + K c_A^{free} c_B^{free}$$



- for $k_{off} < 10^{-4}$/sec
- exploit boundary shape to distinguish species
- for self-association/hetero-association
- K_D

isotherm analysis

Gilbert-Jenkins theory-based isotherms

$$c_{undisturbed}(c_A^{tot}, c_B^{tot}), \quad c_{reaction}(c_A^{tot}, c_B^{tot}), \quad s_{w,reaction}(c_A^{tot}, c_B^{tot})$$

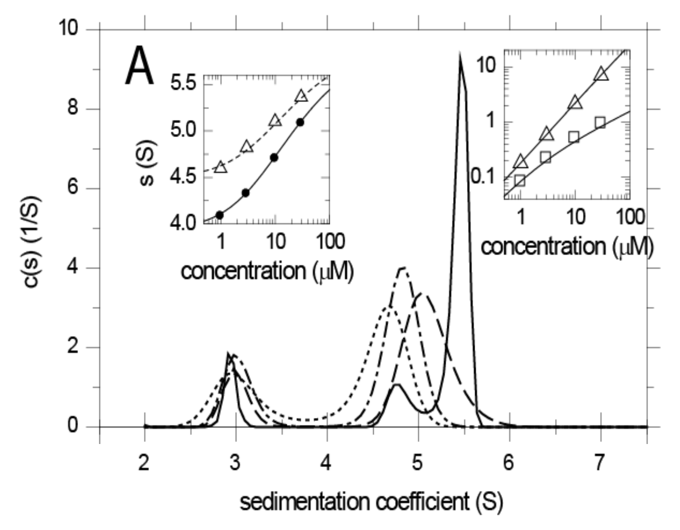
model based on

a) 'constant bath theory' → diffusion of reaction boundary approximately like non-interacting species

b) c(s) can approximately deconvolute diffusion from reaction boundary

c) prediction from Gilbert-Jenkins theory on asymptotic boundary (D = 0)

d) c(s) $\approx$ asymptotic boundary



- for $k_{off} > 10^{-3}$/sec
- exploit bimodal nature of boundary
- for hetero-association
- K_D
- $s_{complex}$ (hydrodynamic shape)