MECHANISM OF LIGAND BINDING TO POLYMERS

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From an energetic view the origin of binding can be enthalpic, entropic, or both. From the kinetics viewpoint the process of binding between two molecules can be diffusion or activation controlled. For the formation of the weakly bound encounter pair one expects a diffusion controlled process. The corresponding very fast relaxation rate $1/\tau 1$ does not produce an optical extinction change at $\lambda = 500$ nm. The formation of the isolated bound nucleation ligand is found to be activation controlled. Much of the activation free enthalpy for the nucleation reaction may be of entropic origin, probably caused by a particular folding and alignment requirements of the polymer and activated solvation states of the ligand before binding. The difference in rate constants for nucleation and growth is not very large. The activation can be viewed as a pre-equilibrium between the native folding state and a local conformation state comprising a stretch of monomers and their cytidilic side chains. In the growth reaction the requirement of desolvation of cooperatively bound molecules will include activated (partially desolvated) states of the ligands. The lifetime of an encounter complex is limited. If the ligand does not find an activated region on the polymer it may dissociate again instead of binding. A region may heal or become stabilized by the binding of a first ligand (nucleation), but this does not lower the activation energy required for binding a second neighbor. The presence of stacks of bound ligands will delay the rate at which the ligand in an encounter complex finds a reactive nucleation or growth binding site.

Key words: polymers; ligand binding; energetics of binding; non-ionic binding.

INTRODUCTION

1.1. **The system.** We consider a system in which the total initial concentration of ligands A is C_{A0} and the initial concentration of polymer chains P_n is $C_{Pn} = C_{P0}/n$ (in this text we use the MKSA system of units. Concentrations are expressed in $mol.m^{-3}$. Concentration values in the common chemical unit $mollit^{-1}$ must be converted to MKSA unit $molm^{-3}$ by multiplying with 1000.) The value of n is the average number of monomeric units in a chain and C_{P0} is the total initial concentration of monomeric units in the polymer solution. A binding position u on the chain may involve more than one monomeric unit. The number of binding positions per monomeric unit is taken into account by the factor $g \leq 1$. A polymer chain P_n of n monomers contains at most gn binding positions u for the ligand. The maximal initial concentration of binding positions for all chains in the system is $C_{U0} = gC_{P0}$. The binding of a ligand requires the formation of an intermediate encounter complex between the ligand and a polymer chain, followed by the reaction of the ligand with one of the binding positions. The kinetic model of binding reactions developed in the following applies to the experimentally investigated reaction between polycyticidilic acid (PolyC) and the ligand Violamycin BI.

1.2. **Binding Sites.** A binding site on the polymer belongs to two topologically different categories of binding position sequences *uuu* resp. *uua* on the polymer chain, leading to bound states uA_1u, resp. $uA_l u$, ($l \geq 2$). *uuu* represents a free

binding position u neighbored on each side by another free binding position. *uua* represents a free binding position u neighbored on one side by a free binding position, on the other side by a ligand-occupied position. A third category of free binding positions *aua*, neighbored on both sides by a bound ligand, is not considered. It may become important when the initial concentration of ligands C_{A0} greatly exceeds the initial concentration of polymers C_{P0} (expressed as monomeric units).

Binding positions *uuu* are called *nucleation positions*. If the ligand of the encounter complex binds to a binding site on the chain that is neighboring an already bound ligand this is characterized as a *growth reaction*. Binding positions *uua* are called *growth positions*.

Besides binding to the polymer the ligand may associate[1] to form dimers. The presence of dimerized ligands A_2 will be accounted for in the calculation of equilibrium concentrations but will be neglected in the derivation of the kinetic equations for the reaction mechanism of binding to the polymer. It is known that at large excess of monomeric units in polyC chain relative to the concentration of ligands the latter bind as isolated dimers as well as isolated monomers on the polymer chain. This occurs at ratios of $p = C_{P0}/C_{A0}$ far beyond those at which the kinetic experiments discussed in the following have been carried out. Dimers are assumed to be always in a fast equilibrium with the free unbound ligand. The relaxation amplitude of the dimerization reaction contributes only a small part to the amplitudes of the other relaxation processes.

Dimer formation is mainly driven by the entropy increase when water molecules from the boundary between the polar solvent and the non-polar aromatic chromophore of the monomeric molecule are released from the contact surface between the non-polar parts of neighbouring ligand molecules in the dimer. This leads to increased entropy of translational and orientational degrees of freedom. In the dimer the ionic charges, both located at the same side of the anthracylic chromophore of the ligand, oppose the entropic binding forces.

This favors an antiparallel orientation of the anthracylinic chromophores in the dimer. In the presence of the oppositely charged polymer chain the repulsive effect of the ligand charges on the dimer equilibrium are neutralized. The parallel orientation of the chromophores of neighboring

ligands stacked on a polymer chain is now favored. The same entropic forces (often called *hydrophobic forces*) leading to dimerization also favors the cooperative binding of chromophoric ligands to the polymer. The name hydrophobic forces indicate an apparent attractive force between surfaces of non-polar molecules. In reality the surfaces are pushed together by polar forces between the molecules of the solvent.

Binding reaction of a monomeric ligand with binding sites on a polymeric chain differ from normal bimolecular reactions because binding sites on a polymer are not homogeneously distributed in the solvent, but bundled together as linear arrangements on the polymer molecule. The number of available binding sites on a polymer chain is gn minus the number of binding sites already bound to a ligand.

1.2.1. Ligand States. The following symbols are used for characterizing the varying states of a ligand, determined by its environment. The symbol A is used for a non-bound ligand, irrespective of its environment. C_A is the concentration of unbound ligands, C_{Af} is the concentration of free unbound ligands that are not in the immediate vicinity of a polymer chain. The symbol A_P designates an unbound ligand that has entered the immediate vicinity, the encounter volume of a polymer chain P_n, but still is not bound to one of its binding sites. The ligand and the polymer chain form an encounter complex, or encounter pair A_P. We have:

$$C_A = C_{A_f} + C_{A_P}$$

Polymer-bound ligand states are characterized by symbols uA_1u (for an isolated bound ligand) and uA_lu; $l > 2$ for a stack of neighboring ligands of length l. The stability constant for isolated bound ligands measured by titration is:

$$K_{nuc} = \overline{C}_{uA1u} / (\overline{C}_{uuu} \overline{C}_{A_f})$$

Overlined concentration values refer to equilibrium values. The stability constant for stacked ligands is expressed by a cooperativity factor q:

$$K_{grow} = qK_{nuc} = \frac{\overline{C}_{u A_l u}}{\overline{C}_{uA_l u} \overline{C}_{A_f}}$$

For the binding of Violamycin BI to PolyC the

stability constants have been experimentally determined from measured optical properties of sets of initial concentrations C_{A0} and C_{P0} and involve the unknown free ligand concentration $\overline{C}_{A_f}$. The optical extinction coefficient of an isolated bound ligand uA_1u does not differ from that of the free ligand since the excited state electronic orbitals of the isolated bound ligand have quite different energies compared to those of the cytidilic site chain of the polymer. But for cooperatively bound ligands uA_lu, $l \geq 1$ orbital interacions between the aromatic parts of neighboring ligands decrease the extinction coefficient and change the fluorescence properties. The ratio of the two stability constants is:

$$ q = \frac{\overline{C}_{uA_2u}}{\overline{C}_{A_f}\overline{C}_{uA_1u}} \times \frac{\overline{C}_{A_f}\overline{C}_{uuu}}{\overline{C}_{uA_1u}} = \frac{\overline{C}_{uA_2u}\overline{C}_{uuu}}{\overline{C}^2_{uA_1u}} $$

Since A_f and uA_1u have the same extinction coefficient at the wavelength $\lambda = 500$ nm, the procedure involves the titration of a fixed concentration C_{A0} with increasing amounts of C_{P0}. At low ratio of $p = \dfrac{C_{P_0}}{C_{A_0}}$ and sufficient affinity most binding positions on the polymer chain will be occupied by a ligand, mostly bound as stacks (saturation of the binding positions). At very high values of p a large fraction of the ligands are bound on isolated sites. Since A_f and uA_1u have the same extinction coefficient at the wavelength $\lambda = 500$nm, the procedure involves the titration of a fixed concentration C_{A0} with increasing amounts of C_{P0}. At very high values of p a large fraction of the ligands are bound on isolated sites.

Analysis of the measured function $E_p^{500}/E_{p=0}^{500}$ at different experimental values of p allows the determination of the quantities g, q, $Knuc$. E^{500} is the *optical extinction* of the solution at the wavelength $\lambda = 500$nm. E^{500}/C_{A0} is the apparent

extinction coefficient at a given initial concentration C_{A0} of the ligand. With $E_{Amon} = E_{uA1u}$, $E_{Ast} = E_{uA2u}$ and E_{Adim} as the respective extinction coefficients of monomeric dissolved and isolated bound ligand, cooperatively bound ligand pairs and longer stacks of cooperatively bound ligands, and dissolved ligand dimers, one has:

$$ E_{A_{app}} = E_{A_{mon}}(C_A + C_{uA_lu}) + E_{A_{st}}\sum_l lC_{uA_lu} + E_{A_{dim}}2C_{A_{dim}} $$

Expressions will be simplified by defining

$$ C_{A_{stackd}} = \sum_l lC_{uA_lu} $$

For the binding of Violamycin BI to PolyC the stability constants have been experimentally determined from measured optical properties of sets of initial concentration C_{A0} and C_{P0} and involve the unknown free ligand concentration $\overline{C}_{Af}$.

The experimental procedures for determining the equilibrium parameters for this system have been described previously [2].

The experimental values for the nucleation equilibrium constant K_{nuc} and the cooperativity factor q at 298K and ionic strength 0.025M corresponding to the present system for which also kinetic relaxation data have been determined and which will been used in numerical calculations are:

$$ K_{nuc} = 70 \text{ M}^{-1}; $$
$$ q = 100; $$
$$ I = 0; 025\text{M}; $$
$$ T = 298 \text{ K} $$

1.3. **Equilibrium concentrations of bound states.** The equilibrium product of isolated bound ligands is:

$$ K_{nuc} = \frac{\overline{C}_{uA_1u}}{\overline{C}_{uuu}\overline{C}_{A_f}} $$

For stacked ligands one has:

$$K_{st} = qK_{nuc} = \frac{\overline{C}_{u A_{k+1} u}}{\overline{C}_{u A_k u} \overline{C}_{A_f}}$$

Using the following formula for infinite series:

(1)
$$\sum_{m=0}^{m=\infty} s^m = 1/(1-s)$$

(2)
$$\sum_{m=0}^{m=\infty} m s^m = s/(1-s)^2$$

expressions (with $s = qK_{nuc}\overline{C}_{A_f}$ and under the condition $s < 1$) for the equilibrium concentrations $\overline{C}_{stacks}$ and $\overline{C}_{A_{bound}}$ in terms of $\overline{C}_{uA_1 u}$ are obtained:

(3) $\quad \overline{C}_{stacks} = \overline{C}_{uA_2 u} + \overline{C}_{uA_3 u} + \overline{C}_{uA_4 u} + ... = \overline{C}_{uA_2 u} \sum_{m=0}^{m=\infty} (qK_{nuc}\overline{C}_{A_f})^m$

(4)
$$= \overline{C}_{ua_1 u} \frac{qK_{nuc}\overline{C}_{A_f}}{1 - qK_{nuc}\overline{C}_{A_f}}$$

(5)
$$= \overline{C}_{ua_1 u} = \frac{s}{1-s}$$

The equilibrium concentration $\overline{C}_{A_{bound}}$ is

(6) $\overline{C}_{uA_1 u} + 2\overline{C}_{uA_2 u} + 3\overline{C}_{uA_3 u} + 4\overline{C}_{uA_4 u} + ... = \overline{C}_{uA_1 u} \sum_{m=0}^{m=\infty} (m+1)(qK_{nuc}\overline{C}_{A_f})^m)$

(7)
$$\overline{C}_{A_{bound}} = \overline{C}_{uA_1 u} \left(\frac{s}{(1-s)^2} + \frac{1}{1-s} \right)$$

(8)
$$= \overline{C}_{uA_1 u} \frac{1}{(1-s)^2}$$

For $s < 1$ the cooperatively bound stacks have decreasing lengths. For $s \geq 1$ the series equation is not applicable. Bound stacks of increasing length are then formed. The distinct behavior by selection of the total concentration of C_{A0} in titration experiments with increasing values of the ratio p allows the experimental determination of the values q and qK_{nuc}. The quantity s used here is identical with s in the theoretical treatment of the binding equilibria by G. Schwarz.[3]

1.3.1. Encounter pairs as intermediates. The binding of a ligand to either category of binding sites occurs in two steps. The first step is a 3-dimensional diffusion process leading to an encounter complex (encounter pair A_P) between a ligand and a polymer chain.

The actual concentration of non-bound ligands in the system is C_A, the concentration of polymer chains is C_{P0}/n. The concentration of polymer chains does not change by the formation of encounter complexes C_{AP}, but the concentrations of non-bound ligands $C_A = C_{Af} + C_{AP}$ must be separated in free non-bound ligands C_{Af} and encounter complexes C_{AP}. The concentration of encounter-complexes C_{AP} is in dynamic equilibrium with the free non-bound ligand concentration C_{Af} and the polymer chain concentration $C_{P0/n}$:

$$K_{A_P} = \frac{\overline{C}_{A_P}}{\overline{C}_{A_f} C_{P_{0/n}}}$$

C_{A_P} is the part of ligand molecules that are at an unspecific position within an encounter distance d_{enc} of a polymer chain, where they are subject to mutual electrostatic interactions between the electric charges on the ligand and the polymer. The encounter equilibrium product K_{A_P} is proportional to the encounter volume surrounding the polymer chains, but it is also dependent on electrostatic forces between charges on the ligand and on the chain (see section 5).

Note. The encounter equilibrium product. The product $K_c = \prod \overline{C}_i^{\upsilon_i}$ for a reaction equilibrium between chemical components i with signed stoechiometric coefficients υ_i defines the equilibrium constant of the transformation. The value of K_c is usually not constant, however, except in ideal systems where other interactions between the components i are not present. But in the case of reactions between charged species this assumption does not hold. We therefore will use the term equilibrium product for $\prod \overline{C}_i^{\upsilon_i}$ .In thermodynamic relations the equilibrium constant appears in the form

$$\ln K = -\Delta G / RT = \ln \prod \gamma_i^{\upsilon_i} \overline{C}_i^{\upsilon_i}$$

Here concentration values C_i must be interpreted as non-dimensional numerical values relative to a standard concentration, e.g. 1mol.m^{-3} since the logarithm function is only applicable to numbers. The dependency of the equilibrium on other parameters of the composition is expressed by the introduction of dimensionless activity coefficients γ_i multiplying the concentration values C_i.

The encounter complex ligand A_P can move inside the encounter volume and get in close contact (but not yet bound) with one of the uncovered binding sites of the chain, or it can dissociate again. It may also bind to the nucleation resp. growth binding site with which it is in contact with specific rate constants

$$k_{R_{nuc}}^* = k_R^{*0} \exp- E_{nuc}^{act} / kT \quad \text{resp.}$$

$$k_{R_{grow}}^* = k_R^{*0} \exp- E_{grow}^{act} / kT \; .$$

k_R^{*0} is a site-independent unimolecular rate constant (dimension s^{-1}) for transformation of a contact pair into a bound state, while $\mathbf{E}_{nuc}^{act} \geq 0$ resp. $\mathbf{E}_{grow}^{act} \geq 0$ are positive activation free enthalpies related to changes in the ligand and chain configuration that make binding possible. The asterisk in the rate constant symbol indicates that this rate constant applies to reaction rates of contact ligands within the encounter volume. The formation of a bound state uA_1u involves the reaction:

$$A_{P_n} + uuu \leftrightarrow uA_1u$$
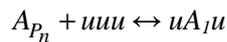
uuu is one of the nucleation sites on the polymer chain of length n that contains the reactive encounter ligand A_{Pn}.

If none of the binding sites on a polymer chain is occupied by a ligand the number of available binding sites on that chain is *ng*. If the chain is partly covered by bound ligands the number of available uncovered binding sites (irrespective of

their nature) is: $B_u = ng \text{x} (1 - \frac{C_{A_{bound}}}{C_{U_0}})$. The probability that any of these is a nucleation site is $\frac{C_{uuu}}{C_{U_0}}$. The number of uncovered *nucleation* sites on the chain is then:

$$B_u = ng \text{ x } (1 - \frac{C_{A_{bound}}}{C_{U_0}}) \text{x} \frac{C_{uuu}}{C_{U_0}} = B_u \frac{C_{uuu}}{C_{U_0}}$$

The number of growth site is:

$$B_u \frac{C_{uuu}}{C_{U_0}} = 2 B_u \frac{C_{uA_lu} + C_{stacks}}{C_{U_0}}$$

since a bound uA_1u ligand or a stack of neighboring ligands offer 2 growth positions at their ends. The transition rate of encounter ligands with their facing nucleation site to the bound state is $k_{R_{nuc}}^*$. The rate of formation of bound states uA_1u is then:

$$dC_{uA_lu} / dt = k^*R_{nuc} C_{A_P} \text{x} B_u \frac{C_{uuu}}{C_{U_0}}$$

The growth rate at the growth site is:

$$dC_{uA_{l+1}u} / dt = 2 k_{R_{grow}}^* C_{A_P} \text{x} B_u \frac{C_{uA_lu}}{C_{U_0}}$$

Dissociation rate constants are obtained from microscopic reversibility:

$$k_{D_{nuc}}^* = k_{R_{nuc}}^* B_u \frac{\overline{C}_{uuu}}{C_{U_0}} \frac{\overline{C}_{A_P}}{\overline{C}_{uA_lu}}$$

and

$$k_{D_{grow}}^* = k_{R_{nuc}}^* B_u \frac{2\overline{C}_{uA_lu} + C_{stacks}}{C_{U_0}} \frac{\overline{C}_{A_P}}{2\overline{C}_{stacks}}$$

1.4. Encounter pair equilibrium.

1.4.1. The entropic part. The entropic part of the encounter equilibrium product comes from the fact that the partners for this equilibrium must share a common volume. The ligand looses transitional degrees of freedom when it is

constrained to the encounter volume surrounding a polymer chain. For a neutral ligand the probability p_{enc} that it is in the encounter volume of a single chain is $p_{enc} = v_{enc}/V_{system}$. The entropy loss for a single ligand molecule is $-k \ln \dfrac{1}{p_{enc}} = k \ln p_{enc}$. The standard molar entropy change for N_A ligand molecules and N_A chains in unit system volume is $\Delta S_{enc}^0 = kN_A \ln(N_A v_{enc} / V_{system}) = -R \ln V_{enc}^0$ where the standard V_{system} is $1m^3$. $V_{enc}^0 = N_A v_{enc} / V_{system}$ is the standard molar encounter volume expressed as fraction of the system volume. This negative standard entropy change is always present even if the probability p_{enc} is modified by electrostatic interactions of the encounter partners.

1.4.2. The enthalpic part. The ligand part of the encounter pair carries on its molecular backbone two alkyl ammonium cation charges $-N(CH_3)_2H^+$ separated by about $12.0 \times 10^{-10}m$ when the charge carrying glycosyl residues are extended outward. This configuration of the non-bound ligand is slightly stabilized by the mutual repulsive forces, but in this configuration the repulsive energy is only about $RT/2$. The average separation will be less due to the positive entropy of different possible orientations of their attachment groups and to the screening effect of counterions and ionic strength of the medium.

The dissolved poly-cytidylic acid chain is characterized by a negative surface charge resulting from charged PO^- in the phosphodiester units that link the riboses of the polymer chain. Their average distance is only $6.0 \times 10^{-10}m$. The mutual repulsion energy of the phosphate ions stretches the polymer chain to a thin rodlike structure because the repulsive force between neighbor and next neighbor charges is additive. Again, the stretching force is weakened by counterion and ionic strength effects, allowing the chain to take spiral-like configurations. In the encounter complex the electrostatic potential of the ligand charges is lowered by the vicinity of the opposite surface charges on the polymer chain, enhancing the stability of the encounter complex by an electrostatic enthalpic contribution ΔH_{enc}^0.

The enthalpic contribution by electrostatic forces depends on the average distance between ligand and polymer charges within the encounter volume. It is influenced by the presence of other mobile charged entities (e.g. counter ions or added salt ions) contributing to ionic strength I in the system.

Note. For the contact encounter state there is a distance mismatch between that of neighboring fixed anionic charges on the polymer and the more mobile cationic charges on the ligand. We neglect a small enthalpic contribution $\Delta H_{exch} = \Delta H^0_{contact} - \Delta H^0_{enc}$ which is difficult to evaluate because assumptions on the effective charge distribution on the polymer must be made.

The thermodynamic expression:

$$K = -\frac{\Delta G}{RT} = \exp\left(-\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R}\right) = \left(\exp -\frac{\Delta H^0}{R}\right) \times \left(\exp \frac{\Delta S^0}{R}\right)$$

where:

$$K = \frac{\Pi \text{equilibrium product concentrations}}{\Pi \text{equilibrium reactant concentrations}}$$

The entropic and enthalpic influences on an equilibrium product can be written as the product of two factors:

$$K = K^h \times K^s$$
$$K^s = \exp(\Delta S^0 / R) = \exp(R \ln(N_A v_{enc} / V_{system}) / R) = \exp(\ln V_{enc}^0) = V^0$$

V_{enc}^0 is the standard molar encounter volume fraction, or the encounter volume of one mol of chains relative to the standard system volume of $1\ m^3$. With the result of section 5.1.2 the electrostatic work

performed by one mol of positively charged ligands and negatively charged polymer chains coming to a distance d_{enc} is:

$$K_{A_P}^h = \exp- \frac{N_A \, m^{eff}(z) e_0^2}{4\pi \in_0 \cdot \in} \frac{1}{kT} x \frac{\exp-(\frac{d_{enc}\sqrt{I}}{3.041 \times 10^{-10}})}{d_{enc}}$$

The factor $m_z^{eff} = z_A z_{P_n}^{eff}$ is the signed product of the effective valencies (numbers) of the ionic charges on the encounter partners A and P_n. $e_0 = 1.6022 \times 10^{-19}$ *amp.s* is the elementary charge of an electron, $\epsilon_0 = 8.954 \times 10^{-12}$ *amp.s.volt^{-1}m^{-1}* is the electrical permittivity of free space, ϵ is the dielectric constant of the medium (the permittivity of the medium relative to that of free space). I is the *ionic strength* (a measure of charge concentration) of the medium, conventionally defined as $I = \frac{1}{2}\sum_i c_i z_i^2$. The index i encompasses all ionic species in the medium, z_i their valencies and c_i their concentrations, expresses by convention in *mol/lit*, so that I is also measured by this unit. Multiplication with 10^3 is required to use I in MKSA context. This multiplication has been included in the numerical factor 3.041×10^{-10} as well as a factor 2, compensating the factor ½ in the conventional definition of I. The factor 3.041×10^{-10} has dimension *length* x *(mol/lit)$^{1/2}$*.

The factor z_{Pn} in m_z is an effective value that does not correspond to n times the number of phosphate monomers of the chain. The equation belongs to a theoretically well defined equivalent spherically distribution of ionized chain monomers, counterions and foreign ions that would correspond to the same amount of electrostatic work as for the true average non-symmetric distribution.

With an empirical value for m_z^{eff}, found by least-square parameter fitting of measured relaxation times with the subsequently developed kinetic model, the use of this equation has the advantage that many unknown parameters of the real distribution are represented by a single parameter that has a simple interpretation. For a detailed discussion of the electrostatic equation see section 5. Encounter complexes play an important role in most chemical reaction mechanisms.

2. THE KINETIC MODEL OF LIGAND BINDING

2.1. **Rate of formation of encounter pairs.** The encounter rate to a given distance d_{enc} between two particles of different species is proportional to the product of their volume densities. Since each encounter produces an encounter pair, the production rate of encounter pairs is diffusion controlled. The bimolecular rate constant k_{Renc} of the diffusion controlled formation of encounter pairs has the dimension mol.s^{-1}m^{-3}. It depends on the encounter distance d_{enc}, on the diffusion constants and on the attractive or repulsive forces between the reacting partners. k_{Renc} is the production rate of encounter pairs (in molnumber per unit time and per unit volume) for normalized volume densities *(1 mol.m^{-3})* of the reaction partners. For reaction partners with diffusion coefficients μ_A and μ_{Pn} (in *m^2.s^{-1}*) and signed electrical valencies z_A, z_{Pn} the encounter rate constant can be derived as

$$k_{R_{enc}} = 4\pi N_A x(\mu_A + \mu_B)x \frac{r_{B_j}}{\exp\frac{z_A z_B r_{B_j}}{d_{enc}} - 1}$$

where

$$r_{B_j} = \frac{|e_A \cdot e_B|}{(4\pi \in_0 \in)xkT}$$

is the "Bjerrum distance" at which the energy of mutual attraction or repulsion is equal to kT.

$$k_{R_{enc}} = \frac{N_A \cdot z_A z_{P_n} e_0^2}{\in_0 \in \cdot kT} \frac{(\mu_A + \mu_{P_n})}{\exp\left(\dfrac{z_A z_{P_n} e_0^2}{4\pi \in_0 \in d_{enc} kT}\right) - 1}$$

The production rate of encounter complexes by diffusional encounter of free ligands at concentrations C_{Af} and polymer chains at concentration C_{P0}/n is:

$$dC_{A_P}^{+,diff} / dt = k_{R_{enc}} C_{A_f} C_{P_0} / n$$

The polymer chain is surrounded by its *encounter volume* v_{enc}. This is a cylindrical shell with inner diameter d_{chain}, outer diameter $d_{chain} + d_{ligand}$ and length $l_{chain} = nd_{pp}$ where d_{pp} is the distance between the monomeric phosphates of the polymeric chain. An estimate for the encounter distance is obtained from the diffusion volume of the polymer chain of length l_P. The diffusion volume V_P is taken equivalent to the volume of the cylindrical shell that encloses the polymer chain but its shape is assumed spherical instead of cylindrical. The encounter distance d_{enc} is the radius of the spherical diffusion volume:

$$d_{enc} = \sqrt[3]{\frac{3}{4\pi} V_P} \text{ with } V_P = \pi((d_{chain}/2 + d_{ligand})^2 - (d_{chain}/2)^2)nd_{pp}$$

When the diffusing ligand (arriving from the total space angle 4π) reaches the spherical surface of the diffusion volume it can enter the encounter volume and be-come an encounter complex A_P, which may find an unoccupied binding site on the chain with which it can form an encounter contact pair A_P, able to be transformed in a bound ligand.

2.2. **Rates of ligand binding to a binding site.** The encounter complex A_P has three alternative ways for disappearing:

1) by dissociation into a free ligand A_f with unimolecular rate $k_{Denc} C_{Ap}$,

2) by binding to a nucleation site with unimolecular rate

$$k_{R_{nuc}}^* C_{A_P} B_u \frac{C_{uuu}}{C_{U_0}}$$

3) by binding to a growth site with unimolecular rate

$$k_{R_{grow}}^* C_{A_P} B_u \frac{C_{uua}}{C_{U_0}}$$

where $\dfrac{C_{uuu}}{C_{U_0}}$ and $\dfrac{C_{uua}}{C_{U_0}} = 2\dfrac{\Sigma_{l \geq 1} C_{uA_l u}}{C_{U_0}}$ are probabilities that an uncovered binding site is a nucleation resp. a growth site. (For $l \geq 1$ every $uA_l u$ provides 2 uua growth sites.).

$B_u = ng\left(1 - \dfrac{C_{A_{bound}}}{C_{U_0}}\right)$ is the number of uncovered binding sites on a chain. The rate of formation of C_{uA1u} (nucleation) by reaction in an encounter volume is:

$$d^{+,1} C_{uA_1 u} / dt = k_{R_{nuc}}^* B_u \frac{C_{uuu}}{C_{U_0}} C_{A_P}$$

The rate of formation of $C_{uA_1 u}$ from dissociation of uA$_2$u in an encounter volume is:

$$d^{+,2} C_{uA_1 u} / dt = +2 k_{D_{grow}}^* C_{uA_2 u}$$

The rate of dissociation of $C_{uA_1 u}$ is:

$$d^{-,3} C_{uA_1 u} / dt = -k_{D_{nuc}}^* C_{uA_1 u}$$

The rate of reaction of $C_{uA_1 u}$ for formation of uA$_2$u is:

$$d^{-,4} C_{uA_1 u} / dt = -k_{R_{grow}}^* 2 B_u \frac{C_{uA_1 u}}{C_{U_0}} C_{A_P}$$

Summing these rates gives the rate of creation of $uA_1 u$:

$$dC_{uA_1u} / dt = k^*_{R_{nuc}} B_u \frac{C_{uuu}}{C_{U_0}} C_{A_P} + 2k^*_{D_{grow}} C_{uA_2u} - 2k^*_{D_{nuc}} C_{uA_1u} - 2k^*_{R_{grow}} B_u \frac{C_{uA_1u}}{C_{U_0}} C_{A_P}$$

The rate of creation of uA_2u is:

$$dC_{uA_2u} / dt = 2k^*_{R_{grow}} B_u \frac{C_{uA_1u}}{C_{U_0}} C_{A_P} + 2k^*_{D_{grow}} C_{uA_3u} - 2k^*_{D_{grow}} C_{uA_2u} - 2k^*_{R_{grow}} B_u \frac{C_{uA_2u}}{C_{U_0}} C_{A_P}$$

The rate of creation of uA_3u is:

$$dC_{uA_3u} / dt = 2k^*_{R_{grow}} B_u \frac{C_{uA_2u}}{C_{U_0}} C_{A_P} + 2k^*_{D_{grow}} C_{uA_4u} - 2k^*_{D_{grow}} C_{uA_3u} - 2k^*_{R_{grow}} B_u \frac{C_{uA_3u}}{C_{U_0}} C_{A_P}$$

The rate of creation of uA_4u is:

$$dC_{uA_4u} / dt = 2k^*_{R_{grow}} B_u \frac{C_{uA_3u}}{C_{U_0}} C_{A_P} + 2k^*_{D_{grow}} C_{uA_5u} - 2k^*_{D_{grow}} C_{uA_4u} - 2k^*_{R_{grow}} B_u \frac{C_{uA_4u}}{C_{U_0}} C_{A_P}$$

………

There is a unimolecular formation of encounter complexes A_P when a bound ligand A_{bound} dissociates from its binding site without leaving the encounter volume. This will occur with probability $(1 - p_D)$, where p_D is the probability that the created encounter ligand A_P leaves the encounter volume to become a free ligand A_{free}. p_D is equal to the dissociation rate of an encounter complex k_{Denc} divided by the sum of the rates of the different reaction pathways a ligand can take inside the encounter volume and thereby end the lifetime of the encounter complex.

$$p_D = \frac{k_{D_{enc}}}{k_{D_{enc}} + k^*_{R_{nuc}} B_u \frac{C_{uuu}}{C_{U_0}} + 2k^*_{R_{grow}} B_u \frac{(C_{uA_1u} + C_{stacks})}{C_{U_0}}}$$

$p_D < 1$ indicates that the encounter complex created by dissociation of a bound ligand can re-engage in binding to the same or a different binding site on the same chain without leaving the encounter complex.

The probabilities that the encounter complex ligand A_P binds to a nucleation resp. a growth site can be obtained in an analogous way:

$$p_{Nuc} = \frac{k^*_{R_{nuc}} B_u \frac{C_{uuu}}{C_{U_0}}}{k_{D_{enc}} + k^*_{R_{nuc}} B_u \frac{C_{uuu}}{C_{U_0}} + 2k^*_{R_{grow}} B_u \frac{(C_{uA_1u} + C_{stacks})}{C_{U_0}}}$$

$$p_{grow} = \frac{2k_{R_{grow}}^{*} B_u \dfrac{(C_{uA_1u} + C_{stacks})}{C_{U_0}}}{k_{D_{enc}} + k_{R_{nuc}}^{*} B_u \dfrac{C_{uuu}}{C_{U_0}} + 2k_{R_{grow}}^{*} B_u \dfrac{(C_{uA_1u} + C_{stacks})}{C_{U_0}}}$$

Because the reaction pathways in the encounter complex turn out to involve activated processes, in contrast to the dissociation of the encounter complex k_{Denc}, p_D is not very different from unity if the activation free enthalpies are the order of several kT. The consequence, however, is that there are two competing processes for the creation of an encounter pair. One is the bimolecular encounter process $k_{Renc} C_{Af} C_{P0} /n$. The other is the fraction $1 - p_D$ of unimolecular dissociation processes.

The created encounter ligand A_P may rebind with a different binding site within the encounter volume, or it may dissociate from the encounter volume to form a free non-bound ligand. The first unimolecular process provides for unimolecular transformations between isolated bound ligands and stacks (and for exchange of bound ligands between stacks of different lengths), but bypassing the intervention of the free ligand concentration C_{Af}. The second process provides for equilibration of free and bound ligand states.

This lets us foresee two main different processes that cooperate in establishing the equilibrium between the concentrations of the different species in the system:

1.   The equilibrium between bound and non-bound free ligand states requires the bimolecular reaction between free ligands and polymer chains via diffusional formation of encounter complexes.

2.   The equilibration between isolated and neighboring bound states can occur by exchange reactions within the encounter volume of a polymer chain.

For $l \geq 2$ the dissociation of a bound ligand at either one of the two growth positions at the end of the stack and rebinding to a nucleation position does not change the number of growth positions nor the number of bound ligands. It changes, however, the number of neighboring bound ligands by one. The same is true for moving an isolated bound ligand to a growth position. This is important for the optical properties of the system. The extinction coeffcient of neighboring bound

ligands may be different from that of isolated bound ligands or non-bound ligands.

Effective bimolecular rate constants $k_{R_{nuc}}^{eff}$ and unimolecular k_{Dnuc} are used in the expression for the experimentally determined the equilibrium product K_{nuc}

$$K_{nuc} = \frac{k_{R_{nuc}}^{eff}}{k_{D_{nuc}}}$$

$$K_{nuc} = \frac{C_{uA_lu}}{C_{uuu} C_{A_f}}$$

leading to

$$k_{R_{nuc}}^{eff} C_{uuu} C_{A_f} = k_{D_{nuc}} C_{uA_lu}$$

$k_{R_{nuc}}^{eff}$ is related to the encounter pair rate constant

$k_{R_{nuc}}^{*}$ by $k_{R_{nuc}}^{eff} = k_{R_{nuc}} \times p_{nuc}$

$k_{R_{nuc}}^{eff}$ depends on the covering of binding sites by both kinds of bound ligands. The dissociation rate constants are related by $k_{Dnuc} = p_D k_{Dnuc}^{*}$.

A similar treatment for obtaining the equilibrium constant qK_{Nuc} for cooperatively bound ligands is not possible. In fact, there is no way to express a dynamic equilibrium equation for cooperatively bound ligands in terms of only the dissociable ligands bound at the end of a stack because binding at a growth position does not increase the number of dissociable growth ligands, although it increases the number of cooperatively bound ligands in a stack.

An effective bimolecular rate constant $k_R{}^b_{grow}$ may, however, be defined by the bimolecular rate constant of encounter multiplied by the probability that the encounter results in growth binding:

$$k_{R_{grow}}^{eff} = k_{R_{enc}} \times p_{Grow}$$ but it cannot be used to derive qK_{Nuc}.

2.3. **Kinetic model equations.** After these preliminaries we can formulate the following set of kinetic equations that corresponds to the described mechanism:

$$dC_{A_f}/dt = (-k_{R_{enc}} C_{A_f} C_{P_0}/n + k_{D_{enc}} C_{A_P} + p_D k_{D_{nuc}}^* C_{uA_1u} + 2 p_D k_{D_{grow}}^* \times \sum_{l>1} C_{uA_1u})$$

$$dC_{A_P}/dt = [k_{R_{enc}} C_{A_f} C_{P_0}/n - k_{D_{enc}} C_{A_P} + (1 - p_D k_{D_{nuc}}^* C_{uA_1u} + 2k_{D_{grow}}^* \times \sum_{l>1} C_{uA_1u}) - k_{R_{nuc}}^* B_u \frac{C_{uuu}}{C_{U_0}} C_{A_P} -$$

$$2k_{R_{grow}}^* B_u \frac{C_{uA_1u} + C_{stacks}}{C_{U_0}} C_{A_P}]$$

$$dC_{uuu}/dt = (k_{D_{nuc}}^* C_{uA_1u} C_{P_0}/n + 2k_{D_{grow}}^* \sum_{l>1} C_{uA_1u} - k_{R_{nuc}}^* B_u \frac{C_{uuu}}{C_{U_0}} C_{A_P} - 2k_{R_{grow}}^* B_u \frac{C_{uA_1u} + C_{stacks}}{C_{U_0}} C_{A_P})$$

$$dC_{uA_1u}/dt = k_{R_{nuc}}^* B_u \frac{C_{uuu}}{C_{U_0}} C_{A_P} + 2k_{D_{grow}}^* C_{uA_2u} - k_{D_{nuc}}^* C_{uA_1u} - 2k_{R_{grow}}^* B_u \frac{C_{uA_1u}}{C_{U_0}} C_{A_P}$$

$$dC_{uA_2u}/dt = 2k_{R_{grow}}^* B_u \frac{C_{uA_1u}}{C_{U_0}} C_{A_P} + 2k_{D_{grow}}^* C_{uA_3u} - 2k_{Dgrow}^* C_{uA_2u} - 2k_{R_{grow}}^* B_u \frac{C_{uA_2u}}{C_{U_0}} C_{A_P}$$

$$dC_{uA_3u}/dt = 2k_{R_{grow}}^* B_u \frac{C_{uA_2u}}{C_{U_0}} C_{A_P} + 2k_{D_{grow}}^* C_{uA_4u} - 2k_{D_{grow}}^* C_{uA_3u} - 2k_{R_{grow}}^* B_u \frac{C_{uA_3u}}{C_{U_0}} C_{A_P}$$

....

Summing the equations dC_{uAlu}/dt for $l > 1$ and introducing the variables $\sum_{l>1} C_{uA_1u} = C_{stacks}$ and $\sum_{l \geq 1} C_{uA_l u} = C_{stacks} + C_{uA_1u}$ leads to:

$$d\sum_{i>1} C_{uA_iu}/dt = dC_{stacks}/dt = 2k_{R_{grow}}^* B_u \frac{C_{uA_1u}}{C_{U_0}} C_{A_P} + 2k_{D_{grow}}^* C_{uA_3u} - 2k_{D_{grow}}^* C_{uA_2u} - 2k_{R_{grow}}^* B_u \frac{C_{uA_2u}}{C_{U_0}} C_{A_P}$$

$$+ 2k_{R_{grow}}^* B_u \frac{C_{uA_2u}}{C_{U_0}} C_{A_P} + 2k_{D_{grow}}^* C_{uA_4u} - 2k_{D_{grow}}^* C_{uA_3u} - 2k_{R_{grow}}^* B_u \frac{C_{uA_3u}}{C_{U_0}} C_{A_P} + 2k_{R_{grow}}^* B_u \frac{C_{uA_3u}}{C_{U_0}} C_{A_P} + 2k_{D_{grow}}^* C_{uA_5u}$$

$$- 2k_{D_{grow}}^* C_{uA_4u} - 2k_{R_{grow}}^* B_u \frac{C_{uA_4u}}{C_{U_0}} C_{A_P}$$

....

or : $$dC_{stacks}/dt = 2k_{R_{grow}}^* B_u \frac{C_{uA_1u}}{C_{U_0}} C_{A_P} + 2k_{D_{grow}}^* \sum_{l>2} \frac{C_{uA_l u} - C_{uA_{(l-1)}u}}{C_{U_0}}$$

Since:
$$\sum_{l>2} (C_{uA_lu} - C_{uA_{(l-1)}u}) = \sum_{l>2} C_{uA_lu} - \sum_{l>1} C_{uA_lu} = (\sum_{l\geq 1} C_{uA_lu} - C_{uA_2u} - C_{uA_1u}) - (\sum_{l\geq 1} C_{uA_lu} - C_{uA_1u}) = -C_{uA_2u}$$

it follows:
$$d\sum_{l>1} C_{uA_1u}/dt = dC_{stacks}/dt = 2k_{R_{grow}}^* B_u \frac{C_{uA_1u}}{C_{U_0}} C_{A_P} - 2k_{D_{grow}}^* C_{uA_2u}$$

2.3.1. *Conservation conditions*. There exist conservation conditions for the number of ligands *A* and for the number of monomer units that can serve as binding positions of the different categories.

The initial total concentration of ligands in the system is C_{A_0}. The concentration of unbound ligands is $C_A = C_{A_f} + C_{A_P}$. The concentration of ligands appearing in encounter complex with the polymer is $C_{A_P} = (C_{A_f} \text{x} K_{A_P})(C_{P_0}/n)$. The concentration of bound ligands is $C_{A_{bound}} = \sum_{l \geq 1} 1$ x $C_{uA_l u}$. The concentration of isolated bound ligands is $C_{uA_1 u}$. The concentration of stacks (irrespective of their length) is $C_{stacks} = \sum_{l \geq 2} C_{uA_l u}$. The concentration of nucleation binding positions is C_{uuu}. The concentration of growth positions is $C_{uua} = 2(C_{uA_1 u} + C_{stacks})$

The conservation equation for *A* is:

$$C_{A_0} = C_{A_f} + C_{A_P} + C_{A_{bound}}.$$

The conservation of monomer units is given by:

$$C_{P_0} = (1/g)(C_{uuu} + C_{A_{bound}} + 2 \text{ x}$$
$$\sum_{l \geq 1} C_{uA_l u}) = (1/g)(C_{uuu} + C_{A_{bound}} + 2(C_{uA_l u} + C_{stacks}))$$

since every $uA_l u$ occupies l/g monomers and is flanked by 2 monomers belonging to 2 non-isolated binding positions.

2.3.2. *Linearization.* For linearizing the equation system equilibrium quantities $\overline{C}$ (.) and perturbation quantities x (.) $<<$ $\overline{C}$ (.) are introduced:

$$x_{A_f} = C_{A_f} - \overline{C}_{A_f}$$
$$x_{A_P} = C_{A_P} - \overline{C}_{A_P}$$
$$x_i = C_{uA_i u} - \overline{C}_{uA_i u}$$
$$x_{uuu} = C_{uuu} - \overline{C}_{uuu}$$
$$\sum_{l > 1} x_l = \sum_{l > 1} (C_{uA_l u} - \overline{C}_{uA_l u})$$

The conservation equations impose:

$$x_{A_P} = -x_{A_f} - x_{A_{bound}}, \ x_{A_{bound}} = -x_{uuu} - 2 \text{ x } x_{stacks}$$
-2 x x_1

Details of the derivation of the linearized kinetic equations are given in the Appendix.

2.3.3. *Matrix form of the linearized equations*. The result of the linearization may be written as a matrix equation:

$$\dot{x} = R \text{ x } x = \begin{bmatrix} \dot{x}_{A_f} \\ \dot{x}_{uuu} \\ \dot{x}_{uA_1 u} \\ \dot{x}_{A_{bound}} \end{bmatrix} = \begin{bmatrix} a_{11} & a_{12} & a_{13} & a_{14} \\ a_{21} & a_{22} & a_{23} & a_{24} \\ a_{31} & a_{32} & a_{33} & a_{34} \\ a_{41} & a_{42} & a_{43} & a_{44} \end{bmatrix} \text{ x } \begin{bmatrix} x_{A_f} \\ x_{uuu} \\ x_{uA_1 u} \\ x_{A_{bound}} \end{bmatrix}$$

$a_{11} \quad -k_{R_{enc}} \text{ x } (C_{P_0}/n) - k_{D_{enc}}$

$a_{12} \quad -p_D k^*_{D_{grow}}$

$a_{13} \quad +p_D(k^*_{D_{nuc}} - 2p_D k^*_{D_{grow}})$

$a_{14} \quad -k_{D_{enc}} - (p_D)k^*_{D_{grow}}$

a_{21} $\quad + k^*_{R_{nuc}}$ x $F\overline{C}_{uuu} + 2k^*_{R_{grow}}$ x $F(\overline{C}_{stacks} + \overline{C}_{uA_1u})$

a_{22} $\quad (-k^*_{R_{nuc}} + k^*_{R_{grow}})$ x $F\overline{C}_{A_P} - k^*_{D_{grow}}$

a_{23} $\quad + k^*_{D_{nuc}} - 2k^*_{D_{grow}}$

a_{24} $\quad + k^*_{R_{grow}}$ x $F(\overline{C}_{A_P} + 2\overline{C}_{uA_1u} + 2\overline{C}_{stacks}) + k^*_{R_{nuc}}$ x$F\overline{C}_{uuu} - k^*_{D_{grow}}$

a_{31} $\quad - k^*_{R_{nuc}}$ x $F\overline{C}_{uuu} + 2k^*_{R_{grow}}$ x $F\overline{C}_{uA_1u} + 2k^*_{D_{grow}}$ x $qK_{nuc}\overline{C}_{uA_1u}$

a_{32} $\quad + k^*_{R_{nuc}}$ x $F\overline{C}_{A_P}$

a_{33} $\quad - 2k^*_{R_{grow}}$ x $F\overline{C}_{A_P} - k^*_{D_{nuc}}$ x$(\overline{C}_a qK_{nuc})$

a_{34} $\quad - k^*_{R_{nuc}}$ x $F\overline{C}_{uuu} + 2k^*_{R_{grow}}$ x $\overline{C}_{uA_1u}$

a_{41} $\quad - 2k^*_{R_{grow}}$ x $F(\overline{C}_{uA_1u} + \overline{C}_{stacks}) + k^*_{R_{nuc}}$ x $F\overline{C}_{uuu}$

a_{42} $\quad - k^*_{R_{grow}}$ x $F\overline{C}_{A_P} - k^*_{D_{grow}} - k^*_{R_{nuc}}$ x $\overline{C}_{uuu}$

a_{43} $\quad + k^*_{D_{nuc}} + 2k^*_{D_{grow}}$

a_{44} $\quad + k^*_{R_{nuc}}$ x $F\overline{C}_{uuu} - k^*_{R_{grow}}$ x $F\overline{C}_{A_P} + 2\overline{C}_{uA_1u} + 2\overline{C}_{stacks} + k^*_{D_{grow}}$

Here the factor $F = \dfrac{B_u}{C_{U_0}}$

3. ITERATIVE CALCULATION OF SYSTEM EQUILIBRIUM AND RELAXATION BEHAVIOUR

3.1. **Simulation program.** A simulation computer program **VBIPolyC.exe** was developed for facilitating the interpretation of experimentally observed binding equilibria and relaxation times obtained for the system violamycin BI and poly(C).The iterative computer program is written in Microsofts Visual Studio C++ and may be executed on personal computers with Microsoft Windows operating systems.

It calculates the equilibrium concentrations of free $\overline{C}_{A_f}$, dimerized $\overline{C}_{dim}$, isolated bound ligand sites $\overline{C}_{uA_1u}$ and stacked bound ligands of increasing stack length $\overline{C}_{uA_u}$ from measured equilibrium products K_{dim}, K_{nuc} ,cooperativity factor q and initial concentrations C_{A_0} and C_{P_0}. The following parameters characterizing the system can be entered manually in the dialog-based user-interface of the program:
- equilibrium products K_{dim}, K_{nuc} and q

- initial concentration C_{A_0} and C_{P_0} , the average number n of monomers in a polymer chain, and the number of binding positions per monomer g and the ionic strength I in $mol.lit^{-1}$ of the solution of reactants.
- extinction coefficients for free, dimerized, isolated bound, neighboring bound ligands E_{VBI} ; E_{dim}; E_{mon};
  E_{stck} used for calculation of measurable optical density
- molecular diameters of the polymer chain P_{diam} and of the ligand L_{diam} (in $m.$).
- recombination rate constants

$$k_{R_{enc}}, k^*_{R_{nuc}}, k^*_{R_{grow}}$$

for the calculation of relaxation times. Dissociation rate constants also appearing in the matrix elements are calculated from the recombination values and corresponding equilibrium products. Default values for the parameters are provided.

The program function **Do Single** uses an iterative non-linear least-squares optimization

procedure (J.-G. Reich, C Curve Fitting and Modeling for scientists and Engineers, McGraw-Hill 1992) for calculating the equilibrium concentrations of the different ligand states. These and derived values for C_{stacks} and C_{Abound} are needed to build the kinetic matrix. The eigenvalues of the kinetic matrix represent the negative reciprocal relaxation times of the linearized kinetic equations. Since the kinetic matrix is real non-symmetric the algorithm for finding its eigenvalues proceeds as follows:

- the kinetic matrix is squared by multiplying it by the transposed matrix,

- the squared matrix is symmetric, its eigenvalues are determined using a modified Jacobi solution method introduced by H.F. Kaiser (The Computer Journal **15**, 271 (1972)).

The real eigenvalues of the squared matrix are the squared eigenvalues of the original matrix. Since the kinetic mechanism describes decaying normal-mode equilibrium deviations, the negative square roots must be selected to give the eigenvalues λ_i of the original kinetic matrix. The calculated relaxation times $\tau_i = -1/\lambda_i$ are given by the program as positive values. The four independent variables describing the kinetic equations lead to four relaxation times. They are not relaxation times belonging to each of the four independent variables, but belonging to sets of four linear combinations of the independent variables. Each eigenvalue of the matrix (corresponding to a relaxation time) transforms the matrix into a different eigenvector with four components. They are the coefficients of the relaxing linear combination of independent variables. The program does not attempt to calculate the eigenvector belonging to each eigenvalue because a reliable algorithm for this calculation with the given non-symmetric real matrix was not available.
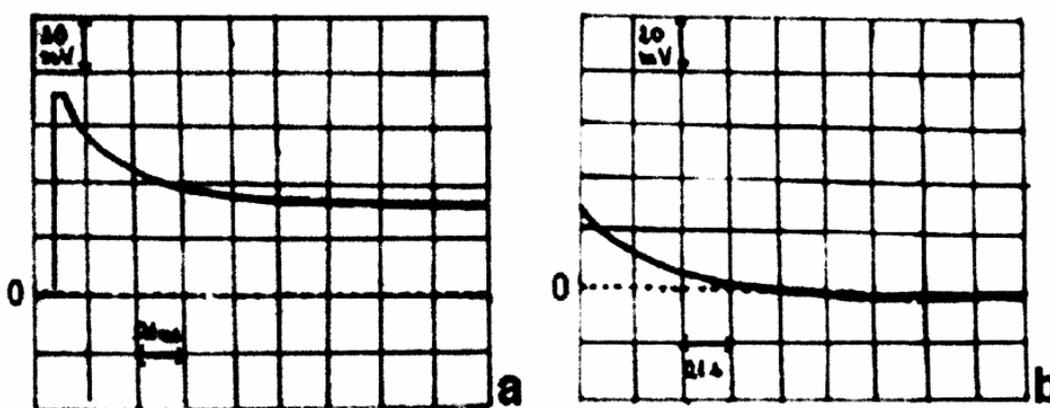

Fig. 1. Observed Relaxation Rates, $C_{A0} = 1.5 \times 10^{-4}$ M; $C_{P0} = 11.85 \times 10^{-4}$ (Results)

The function **Do Single** does a single calculation with the concentration values and the other parameters entered manually. Since some of these values are initial guesses of unknowns, their entered values do not necessarily correspond to the true parameter values (e.g. the length of the polymer chain or the reaction rate constants). The results of the calculation will then differ from those obtained by a direct measurement.

When a set of measured relaxation times at the entered concentrations is available the program function **fit kinetics** uses the iterative non-linear least squares algorithm to search for optimized values of the unknown parameters that will result in a best agreement of the results of the calculation of the relaxation times with the measured results. The parameters iteratively changed during this optimization are the unknown rate constants, the unknown length of the polymer chain and the unknown effective electric charge on the polymer.

The function **fit kinetics** uses a set of default measured relaxation times for a corresponding set of initial concentrations used in the measurement. It is possible to enter manually a different set of measurement results or to save and load it from a file. Other available program options are calculations of optical properties as a function of the concentration parameter

$$p = \frac{C_{P_0}}{C_{A_0}}$$

at given values of C_{P_0} or C_{A_0}. The results of the progam functions **p-Range at constant CVBI** and variable C_{A0} and C_{P0} at constant p are given as graphical displays (with different options for the measurable optical properties to be plotted) of concentration values and measurable optical extinction as a function of p. This is useful for simulating different titration experiments.

4.1 Relaxation times. Only two relaxation times τ_{fast} and τ_{slow} are experimentally observed for a system violamycin BI/Poly(C) with the following compositions at ionic strength $I = 0.025$ mol.lit^{-1}.

4. RESULTS AND DISCUSSION

Table 1

C_{A0} $mol.lit^{-1}$	C_{P0} $mol.lit^{-1}$	$1/\tau_{fast}$ s^{-1}	$1/\tau_{slow}$ s^{-1}
$1.5 \ 10^{-4}$	$11.58 \ 10^{-4}$	$1.86 10^4$	12
$1.0 \ 10^{-4}$	$7.91 \ 10^{-4}$	$1.42 10^4$	12
$9.0 \ 10^{-5}$	$7.11 \ 10^{-4}$	$1.29 10^4$	12
$7.5 \ 10^{-5}$	$5.93 \ 10^{-4}$	$1.06 10^4$	12
$7.0 \ 10^{-5}$	$5.53 \ 10^{-4}$	$1.02 10^4$	12

The kinetic model developed above is characterized by 4 relaxation times. Calculated $1/\tau$ values for the given concentrations and ionic strength, measured $K_{nuc} = 70$, measured q = 110 are:

Table 2

C_{A0} $mol.lit^{-1}$	C_{P0} $mol.lit^{-1}$	$1/\tau_1$ s^{-1}	$1/\tau_2$ s^{-1}	$1/\tau_3$ s^{-1}	$1/\tau_4$ s^{-1}
$1.5 \ 10^{-4}$	$11.58 \ 10^{-4}$	$2.11 \ 10^9$	$1.86 \ 10^4$	$1.76 \ 10^1$	$8.68 \ 10^0$
$1.0 \ 10^{-4}$	$7.91 \ 10^{-4}$	$1.57 \ 10^9$	$1.29 \ 10^4$	$2.16 \ 10^1$	$1.40 \ 10^1$
$9.0 \ 10^{-5}$	$7.11 \ 10^{-4}$	$1.43 \ 10^9$	$1.21 \ 10^4$	$1.88 \ 10^1$	$9.78 \ 10^0$
$7.5 \ 10^{-5}$	$5.93 \ 10^{-4}$	$1.25 \ 10^9$	$1.12 \ 10^4$	$2.12 \ 10^1$	$2.86 \ 10^0$
$7.0 \ 10^{-5}$	$5.53 \ 10^{-4}$	$1.20 \ 10^9$	$1.10 \ 10^4$	$2.21 \ 10^1$	$5.27 \ 10^0$

These calculated values are obtained with optimized chain length $n = 170$ and values for $k_{Rnuc} = 3652$ s^{-1}, $k^*_{Rgrow} = 1500$ s^{-1}.

The calculated $1/\tau_1$ corresponds to the encounter complex formation. This fast relaxation process is not experimentally observable since the free ligand and the encounter complex ligand do not differ in their optical properties.

The measured values for $1/\tau_{fast}$ are very well represented by the calculated $1/\tau_2$. The observed $1/\tau_{slow}$ is an average of the two slow calculated $1/\tau_3$ and $1/\tau_4$. The calculated values are obtained by least-squares optimization. The sum of squared

differences between calculated and measured reciprocal relaxation times is minimized by an iterative algorithm. The optimized parameters for the model are: polymer chain length, polymer charge, k^*_{Rnuc} and k^*_{Rgrow}. Dependent on chosen initial values they will somewhat differ with different runs of the optimization, but they remain reasonably within a range of ±50 percent of their average values.

The rate constants k^*_{Rnuc} and k^*_{Rgrow} have moderate values of the same order of magnitude, indicating similar but considerable activation free enthalpies. Under conditions of saturation of

binding sites τ_2 depends on k^*_{Rnuc} and k^*_{Rgrow}. τ_2 represents equilibration of C_{Af} with nucleation and growth binding sites, but establishing the equilibrium distribution of stack lengths occurs by slower processes.

Effective bimolecular rate constants for binding to a nucleation site resp. binding to a growth site are:

$$(9) \quad k_{R_{Nuc}} = 6.41 \times 10^7 \text{ s}^{-1} (mol/lit)^{-1}$$

$$(10) \quad k_{R_{Grow}} = 1.19 \times 10^6 \text{ s}^{-1} (mol/lit)^{-1}$$

These bimolecular rate constants are obtained by multiplication of the bimolecular encounter rate constant with the probability that the encounter results in binding to a nucleation resp. a growth site. The probabilities are:

$$(11) \quad p_{Nuc} = \frac{k^*_{R_{nuc}} \times F\overline{C}_{uuu}}{k_{D_{enc}} + k^*_{R_{nuc}} \times F\overline{C}_{uuu} + 2k^*_{R_{grow}} \times F(\overline{C}_{stacks} + \overline{C}_{uA_iu})}$$

$$(12) \quad p_{Grow} = \frac{2k^*_{R_{grow}} \times F(\overline{C}_{stacks} + \overline{C}_{uA_iu})}{k_{D_{enc}} + k^*_{R_{nuc}} \times F\overline{C}_{uuu} + 2k^*_{R_{grow}} \times F(\overline{C}_{stacks} + \overline{C}_{uA_iu})}$$

The two slowest relaxation times τ_3 and τ_4 represent coupled consecutive reactions system $A \leftrightarrow AP \leftrightarrow A_{bound}$ that establish the final equilibrium length of stacks while the free ligand concentration and that of nucleation and growth sites remain in equilibrium with changing length distribution. The true reaction system is a set of coupled consecutive reactions with a multitude of forms of A_{bound}. The theoretical kinetic model developed above describes the observed behavior very well.

An important result of the experiments and their interpretation in terms of a mechanism that explicitly considers the formation of encounter complexes preceding the binding reaction is that neither the nucleation reaction nor the growth reaction are diffusion controlled. The moderate values of k^*_{Rnuc} and k^*_{Rgrow}, or k_{RNuc} and k_{RGrow}, indicate that binding is an activated process, contrary to what one would expect for a pure electrostatic neutralization of simple oppositely charged reaction partners. The activation free enthalpies are the order of many *kJmol⁻¹*. The origin of the required large activation free enthalpies remains unclear as long as the molecular structure of the bound states is unknown. A large part of the activation enthalpy must be of entropic origin. Configuration changes of the polymer chain are obviously required for binding. The desolvation (release of solvating water molecules around the organic parts of the

ligands) that must take place before these parts can come in close enough contact to contribute to the stability of the next neighbor interaction of a cooperatively bound pair may also play a role. It is true that the enhanced stability results from the final gain of orientational degrees of freedom of the released water molecules, but this release in the case of rather large solvated surfaces of the organic moiety of the ligands do not occur in a single step. The activation free enthalpy may come partly from the required intermediary desolvated states, where the reacting molecules are not yet in close contact of their surfaces and temporary loss of hydrogen bonding energy and decreased electrostatic permittivity of their surrounding environment must be overcome.

5. ENERGETICS OF BINDING

5.1. Electrostatics.

5.1.1. *Electrostatic work and electrostatic potential*. The following recalls some fundamental concepts. Charles Augustin de Coulomb was the first to measure the mechanical force F between two electrical charges q and q' at a distance r as:

$$F = k \times \frac{q \cdot q'}{r^2}$$

He found that electrical charges are signed quantities. The factor k is the mechanical-

electrical coupling constant. The relation allows a definition of the unit of charge in terms of mechanical quantities. Setting $k = 1$ and measuring the force F in dyne, the distance in cm, we are dealing with the electrostatic cgs (esu) system of units. The cgs-unit of charge (one franklin) then repels an identical charge at a distance of 1 cm with a force of 1 dyne. The dimension of this unit of charge is $[L^{3/2}M^{1/2}T^{-1}]$.

The electrodynamics phenomena observed later by Andre-Marie Ampere with moving charges (charge flow, electrical current) led to the de definition of different variants of the cgs system (emu) for dealing with electromagnetic phenomena. It became obvious that the electric charge must be considered as a fundamental physical property of matter with its own dimension Q, next to the other fundamental physical quantities L, M, T. This introduced the MKSA system, in which the ampere (the unit of current flow) is the additional base unit, besides the meter-kilogram-second base of the SI system. The value of the electro-mechanical coupling constant k in vacuum, expressed in MKSA-units is then:

$$k = 1/4\pi\epsilon_0$$

$\epsilon_0 = 8.854 \ 10^{-12}$ amp.s/volt.m is the dielectric permittivity, a fundamental property of the free space that carries the field energy of the charge. Including the numerical factor $1/4\pi$ in k is done in order to simplify certain derived mathematical expressions.

The dielectric permittivity of material media is ϵ_0 multiplied by the dimensionless dielectric constant of the medium in which the charges are embedded. The product $\epsilon\epsilon_0$ is the dielectric permittivity in a material medium (ratio of dielectric displacement D in $amp.s.m^{-2}$ to electric field strength E in **volt.m**$^{-1}$). In water with a temperature of 298K (25 degree C) at one atmosphere pressure $\epsilon = 78.5$.

Changing the distance between the two charges from $r_{initial}$ or r_{final} involves the electrostatic work

$$(w(r) = \int_{r_{initial}}^{r_{final}} force \ dr):$$

$$w = \frac{q.q'}{4\pi(\epsilon_0\epsilon)} \int_{r_{final}}^{r_{initial}} \frac{1}{r^2} dr = -\frac{q.q'}{4\pi(\epsilon_0\epsilon)} \left(\frac{1}{r_{final}} - \frac{1}{r_{initial}}\right)$$

The work required to bring a positive unit of charge (1 amp.s) from infinity to the distance r of a charge q located at the origin of a coordinate system is a function of $1/r$:

$$w(r) = (1 \, amp.s) \times \frac{q}{4\pi(\epsilon_0\epsilon)} \frac{1}{r}$$

The sign of the central charge defines the sign of performed work w. The expression:

$$\psi(r) = \frac{q}{4\pi(\epsilon_0\epsilon)} \frac{1}{r}$$

in the work equation for the approaching unit charge measures the *electrical potential* $\psi(r)$ at the location r, in volt, created by the charge q at the origin. The product $q' \ \psi(r)$ measures (in *volt.amp.s* = joule), if positive, the work required for, if negative, the work performed by, the relative displacement r of a charge q' in the electrostatic field of a central charge q.

In general, the function $\psi(r, \theta, \varphi)$ measures the electrical potential created at the location (r, θ, φ) by a distribution of charges $\rho(x; y; z)$. The electrical potentials are additive; charges at

different positions in the system contribute additively to the potential at a given location.

The internal energy U and therefore the enthalpy $H = U + PV$ of the system with electrically charged particles now contain not only the kinetic and potential energy associated with mechanical degrees of freedom. U contains also the internal electric field energy (the electrostatic potential $\psi(r, \theta, \varphi)$, in volt, associated with the spatial distribution of the charges). Moving a charge q' (in amp.s) from a position where the potential is ψ_{r_a} volt to a position where it is ψ_{r_b} volt, requires the electrostatic (equivalent mechanical) work:

$$q' \times (\psi_{r_b} - \psi_{r_a})$$

in *volt.amp.s = joule*.

In the following, when discussing ionic interactions, we will often replace the symbol q, representing a charge of q amp.s, by the symbol $z_i e_0$ in which e_0 is the elementary charge of the electron $e_0 = 1.902.10^{-19} amp.s$, and the accompanying symbol z_i represents the sign and the valency (number of elementary charges) of an ionic charge i.

In a medium with many more than the two charges considered before, including also additional mobile ionic charges, resulting from a dissolved strong electrolyte salt present in the solvent, the work equation must be corrected. All charges are spatially distributed in a manner that is not completely random but that nevertheless guaranties electrical neutrality of the system.

The non-randomness leads to the existence of an average ion distribution (the ionic atmosphere) around any central ion, with an average charge density of opposite sign. The charge of a central ion is thereby effectively screened at long distance. The average electrical potential (potential energy of an electrical force field) around a central ion is lower than that of the unscreened case and the mechanical work performed by relative displacements of the charges is lower. This influences the chemical interactions between charged particles.

5.1.2. *Screening effect of charges, ionic atmosphere and ionic strength*. The excess density of the charges opposite to that of the central ion has a spherically symmetric Boltzmann distribution that depends on a parameter, the ionic strength I, which takes into account the concentration as well as the valency of all ionic species in the medium:

$$I = \frac{1}{2} \sum_i c_i z_i^2$$

c_i is the concentration of charges i, usually expressed in *mol/lit*, z_i the product of sign and valency. The distance r_j from the position of the maximum of the Boltzmann distribution to the origin (the position of the screened charge j) is inversely proportional to the square root of the ionic strength. r_j is known as the screening length or the average radius of the ionic atmosphere.

In order to be consistent with our use of SI-mksa electrostatic units r_j must be expressed in m and I in mol/m^3, equal to 1000x the value conventionally given in (*mol/lit*). r_j in m and I in *mol.lit^{-1}* are related by the equation:

$$r_j = \sqrt{\frac{\epsilon_0 \in k_B T}{N^A e_0^2 \cdot 2 \cdot 1000 \cdot I}} = \sqrt{\frac{\epsilon_0 \in k_B T}{2 \cdot 1000 \cdot N^A e_0^2}} x \frac{1}{\sqrt{I}} = \frac{3.041 \cdot 10^{-10}}{\sqrt{I}}$$

A derivation of this equation[4] (the equations given here follow the treatment of the ionic cloud by Arnold Eucken in his Lehrbuch der Chemischen Physik, Teilband II,2, Leipzig 1944) starts by postulating a Boltzman-distribution of the average number density $N_i(r)$ (in m^{-3} for MKSA units) a pointlike ion of the kind i in a spherically symmetric average electrical potential $\overline{\psi}_r$ in the vicinity of a positive central ion:

$$N_i(r) = N_{i,\infty,} \exp- \frac{z_i e_0 \overline{\psi}_r}{kT}$$

The average charge density $\overline{\rho}$ at a position with average potential $\overline{\psi}$ is then:

$$\overline{\rho}(r) = e_0 \sum_i z_i N_{i,\infty} \exp - \frac{z_i e_0 \overline{\psi}_r}{kT}$$

Taking only the linear term of the series expansion of the exponential function:

$$\overline{\rho}(r) = e_0 \sum_i z_i N_{i,\infty}(1 - \frac{z_i e_0 \overline{\psi}_r}{kT}) = e_0 \sum_i z_i N_{i,\infty} - \sum_i z_i^2 N_{i,\infty} \frac{e_0^2 \overline{\psi}_r}{kT}$$

Since the solution is electrically neutral the first term $e_0 \sum_i z_i N_{i,\infty}$ vanishes. There remains:

$$\overline{\rho}(r) = -\sum_i z_i^2 N_{i,\infty} \frac{e_0^2 \overline{\psi}_r}{kT}$$

For moderately dilute solutions the number densities at infinity may be expressed by the concentrations $N_{i,\infty} = N_A c_i$

For determining explicitly two unknowns $\rho(r)$ and $\psi(r)$, two equations relating them are required. A second relation between the average charge density $\overline{\rho}$ and the average potential $\overline{\psi}$ is given by the Poisson equation:

$$\Delta\overline{\psi} = divgrad\overline{\psi} = -\frac{\overline{\rho}}{\in_0 \in} = \overline{\psi} \frac{e_0^2}{\in_0 \in \, kT} N_A \sum_i z_i^2 c_i$$

Using polar coordinates this gives the second order differential equation:

$$\frac{d^2}{dr^2}(r \bullet \psi) = [\kappa]^2 r \cdot \psi$$

where the abbreviation:

$$[\kappa]^2 = \frac{e_0^2}{\in_0 \in \cdot kT} N_A \sum_i z_i^2 c_i$$

is introduced. Using $\varphi = r - \psi$ as the variable there follows the simple expression:

$$\frac{d^2}{dr^2}\varphi(r) = [\kappa]^2 \cdot \varphi$$

The general solution of this differential equation is

$$\varphi = A \cdot e^{+[\kappa]r} + B \cdot e^{-[\kappa]\,r}$$

leading to

$$\psi(r) = \frac{A}{r} \cdot e^{+[\kappa]r} + \frac{B}{r} \cdot e^{-[\kappa]\,r}$$

The integration constant A must be zero, because otherwise $\psi(r)$ should be infinite at infinite distance from the central ion j. When the radius of the central ion is small compared to the extension of the oppositely charged ionic atmosphere $\psi(r)$ in the limit $r \to 0$ must be equal to the potential of the central ion

$$\psi(r \to 0) = \frac{z_j e_0}{4\pi \in_0 \in \, r}$$

because more distant charges can not influence the potential in the immediate vicinity of the central ion. This requires $B = z_j \dfrac{e_0}{4\pi \in_0 e}$ for the second integration constant, giving

$$\overline{\psi}(r) = \frac{z_j e_0}{4\pi \in_0 \in \, r} e^{-[\kappa]\,r}$$

$\overline{\psi}(r)$ may be written as the sum of the two terms

$$\overline{\psi}(r) = \psi_{central} + \psi_{atmosphere}$$

Where $\psi_{central} = \dfrac{z_j e_0}{4\pi \in_0 \in \, r}$ is contributed by the potential of the central ion and

$\psi_{atmosphere} = -\dfrac{z_j e_0}{4\pi \in_0 \in r}(1 - e^{-[\kappa]r})$ is the contributed by the Boltzmann distribution of the ionic atmosphere.

From the Poisson equation $\dfrac{\rho}{\in_0 \in} = \Delta\psi = -[\kappa]^2\psi$ one obtains the charge distribution around the central ion: A spherical shell between r and $r + dr$ contains the charge

$$q(r)dr = 4\pi r^2 \rho(r)dr = -4\pi r^2 \frac{z_j e_0}{\in_0 \in r}([\kappa]^2)e^{-[\kappa]\,r} \cdot dr = -4\pi \frac{z_j e_0}{\in_0 \in}([\kappa]r)e^{-([\kappa]\,r)}d([\kappa]r)$$

The function $y = xe^{-x}$ with $dy = e^{-x}(1-x)$ has maximum at $x = 1$ so that $[\kappa]r = 1$ or $r = \dfrac{1}{[\kappa]}$ at the maximum

of charge distribution. $r_j = \dfrac{1}{[\kappa]}$ has the dimension *length* and is known as the *screening length or average*

radius of the ionic atmosphere. Taking the square root of $\dfrac{1}{[\kappa]^2}$ gives

$$r_j = \frac{1}{[\kappa]} = \sqrt{\frac{\in_0 \in \cdot kT}{e_0^2 N_A} \frac{1}{\sum_i z_i^2 c_i}}$$

Although in this derivation there appears nowhere a factor 2, it is the generally accepted definition of the

ionic strength $I = \dfrac{1}{2}\sum_i z_i^2 C_i$ in *mol.lit⁻¹*. The use of this definition in the equation for r_j requires the

introduction of a factor 2, compensating the factor ½ in the definition, and the factor 1000 for the change of units *mol.m⁻³* to *mol.lit⁻¹*:

$$r_j = \sqrt{\frac{\in_0 \in \cdot kT}{e_0^2 N_A \cdot 2 \cdot 1000}}\sqrt{\frac{1}{\frac{1}{2}\sum_i z_i^2 c_i}} = 3.041 \times 10^{-10}\frac{1}{\sqrt{I}}$$

Because this equation is obtained by taking only the linear term of the series expansion of the Boltzmann equation it is valid only for $I < 0.001$ *mol.lit* ⁻¹. The numerical factor 3.041×10^{-10} depends on the square root of the temperature. It has the dimension $m\sqrt{mol.lit^{-1}}$. One must be careful with the use of this numerical value when the effect of ionic strength is compared for different systems where observations may have been made at different temperatures. Several modifications of the first order Debye-Hückel approximation have been proposed in order to extend the validity to higher ionic concentrations. Taking into account the finite radius a of the central ion introduces a factor

$$\frac{1}{1+[\kappa]\,a}$$

in the expression for the integration constant B. Other extended Debye-Hückel theories use in addition higher order terms of the expansion of the Boltzmann equation. The concept of ionic association[5] in which associated ions with valencies z_i and z_j within distance

$$r < r_{min} = -\frac{z_i z_j e_0^2}{8\pi \in_0 \in \cdot kT}$$

behave as a weak electrolyte without, however, involving their electron shells in a chemical bond. r_{min} is called the *Bjerrum distance* at which the electrostatic energy of the approaching ions is equal to kT .

None of these extended theories is really satisfactory at high ionic strengths. In many applications it is useful to modify the first order Debye-Hückel equation by multiplication with

an empirical dependent factor:

$$r_j^{corr} = \sqrt{\frac{\in_0 \in \cdot kT}{e_0^2 N_A \cdot 2 \cdot 1000}} \frac{1}{\sqrt{I}} \text{ x } \frac{1}{1 + f^{emp}\sqrt{I}}$$

f^{emp} is called *kappafaktor* and must be obtained by numerically fitting the equation to observed ionic-strength-dependent properties. The equation for the electrical potential at distance r from the central ion in first order Debye-Hückel approximation may be written as

$$\overline{\psi}(r) = \frac{z_j e_0}{4\pi \in_0 \in r} \exp- \frac{r}{r_j}$$

and used for calculating the electrostatic energy w_{ij} of two charges z_j and z_i at the reciprocal distance $1/r$:

$$w_{ij}(r) = z_i e_0 \overline{\psi}(r) = \frac{z_j z_i e_0^2}{4\pi \in_0 \in r} \exp- \frac{r}{r_j}$$

We will use the value

$$r_j^{corr} = r_j \frac{1}{1 + f^{emp}\sqrt{I}}$$

in this equation to find the electrostatic enthalpy contribution ΔH^{el} the binding free enthalpy ΔG of a chemical association reaction between electrically charged reaction partners.

A fitting program **Equilibria** has been developed using the ionic strength dependence for distinguishing ionic and non-ionic binding forces in association or binding equilibria between molecules that carry charges but where the free enthalpy of the reaction includes contributions of non-ionic origin.

6.   ELECTROSTATIC LIGAND/POLYMER/SOLVENT INTERACTIONS.

6.1. **Standard enthalpies of components.** In the ligand/polymer system three categories of charges are present: the charges $-N(CH_3)_2\ H^+$

fixed on the mobile ligand molecules and their mobile OH^- countercharges, the charges $-PO^-$ fixed on the polymer chain and their mobile H^+ countercharges, and a third category of mobile ionic charges resulting from a dissolved strong electrolyte salt added to the aqueous solvent. The majority of charges is of the third category.

The electrostatic enthalpies of the ions of the added strong electrolyte are not explicitly included but only their ionic strength effect on the electrostatic enthalpy of the first two categories.

For dilute dissolved species the standard states are defined as individual solutions with properties extrapolated from infinite dilution in the selected solvent to the standard 1 molar concentration. In the MKSA system of units the usual chemical volume concentration 1molar = 1 *mol/lit* = 1000 *mol.m*$^{-3}$. The standard enthalpy includes the enthalpy of non-electrostatic as well as of electrostatic origin. Whereas the electrostatic part of the standard enthalpy of a charged species can with reasonable accuracy be calculated from the electrostatic interactions with other charged species in the system, a priori calculation of non-ionic enthalpies is much more difficult. It needs in most cases a quantum-mechanical approach. Non-ionic contributions to measured enthalpy values (e.g. from calorimetric data or other equilibrium determinations) can be evaluated by subtracting the calculated electrostatic parts.

For dilute dissolved electrically charged entities (here, for example a ligand molecule or a binding site) our definition of the standard enthalpy is the enthalpy of one mol of the pure entity (pure = with properties at in nite dilution in the given solvent with ionic strength only resulting from the added strong electrolyte), corrected for electrostatic enthalpies of its component charges and their interactions with the solvent at one-molar concentration.

6.1.1. *Standard ligand enthalpy*. The standard electrostatic enthalpy of ligand, dissolved in the aqueous solvent of ionic strength I, is the addition of several contributions:

1. The first one is N_A times the electrostatic enthalpy of the charges of the ligand ion (the electrostatic potential of a single pair of positive charges) at their fixed distance

$$d_{pair} = -N(CH_3)_2 H^+ ......^+ HN(CH_3)_2 - \approx 12.0 \text{x} 10^{-10} m.$$

in the ligand molecule, submerged in a solvent of dielectric constant $\epsilon_{solvent}$ and including the correction to their electrostatic potential for the ionic strength of the solvent.

The field energy of the charge $z_j e_0$ of simple ion, e.g. Mg^{++} at infinite dilution in a solvent of zero ionic strength consists only of its interaction with the solvent. It depends only on the dielectric constant of the solvent. By convention this energy is not considered part of the enthalpy of the ion, except in the case where the solubility in different solvents is discussed. In a solvent of ionic strength I the field energy is lowered by screening of the charge. This result in a negative enthalpy compared to a solution of zero ionic strength. The contribution of the ionic atmosphere to the potential was obtained as

$$\psi_{atm}(r) = \frac{z_j e_0}{4\pi \in_0 \in r}(1 - e^{-r/r_j}).$$

For small value of r using $(1 - e^{-r/r_j}) \approx r/r_j$ the expression becomes

$$\psi_{atm}(r \to 0) = -\frac{z_j e_0}{4\pi \in_0 \in r_j}$$

The electrostatic energy w_q of a charge q at the potential ψ is $q\psi$. We cannot simply obtain the energy of the central charge $z_j e_0$ by its multiplication with the potential $\psi_{atm}(0)$ because the central charge itself is origin of ψ_{atm}. We obtain the field energy of the central charge with its surrounding ion cloud by a charging procedure in which the central charge $z_j e_0$ is replaced by a charge $z_j \lambda e_0$ and its energy in the potential $\psi_{atm}(0)$ is

$$z_j \lambda e_0 \text{x} \frac{z_j e_0}{4\pi \in_0 \in r_j}.$$

By integrating over λ when it changes from 0 to 1 the molar screening energy for one mol of ions is obtained as:

$$N_A w_{centralion} = -\frac{N_A z_j^2 e_0^2}{4\pi \in_0 \in r_j}\int_0^1 \lambda^2 d\lambda = -\frac{N_A z_j^2 e_0^2}{4\pi \in_0 \in r_j}\frac{1}{3}$$

By this charging procedure the central charge and the potential generated at its position are simultaneously increased to their final values. Since temperature and volume are held constant during charging (changes in their values have not been explicitly accounted for) the dissipated energy $n_j N_A w_{ion}$ for a solution containing n_j screened ions becomes part of the internal energy of the system.

The difference between a simple divalent ion and the two monovalent ionic states at a fixed distance on the same ligand molecule does not affect the resulting screening energy since central ion size is neglected in the first order approximation. It would be accounted for in the empirical correction factor that corrects for this and other size influences.

2. The second contribution comes from the counterions and is equal to two times the electrostatic enthalpy of $2N_A$ mobile counterions OH^- at infinite dilution, corrected for the dielectric constant and the ionic strength of the solvent. The factor 2 is included because the standard state of the mobile ions is 1 molar. By definition the electrostatic enthalpy of an isolated charge, separated infinitely from all other charges, is zero. The electrostatic enthalpy at infinite dilution of a mobile counterion can only come from its interaction with the dipolar molecules of the solvent and with the average distributed opposite charge of the ionic atmosphere supplied by the ionic strength of the solvent. The first interaction is taken into account by the dielectric constant of the solvent; it does not contribute to the electrostatic ion enthalpy. The screening interaction of the OH^- ion is calculated by the above charging procedure. The sum of the ligand ion screening energy and that of two OH^- ions is the electrostatic standard enthalpy of one mol

dissolved ligand molecules.

3. The third contribution is the work required for bringing N_A ligands with $2N_A$ fixed charges and their $2N_A$ mobile counterions in a common volume of one liter. As in the case of an ideal gas, the volume compression work done on neutral non-interacting ligands does not change their enthalpy. The electrostatic work to bring the charged parts associated with ligands and counterions from infinite dilution to the one-molar standard state of the ligand is the opposite work of moving them to infinity, starting from the initial distance distribution at one-molar concentration. This is equivalent to removing from the ionic strength the contribution of ligand and counterion charges. The standard ligand enthalpy as a result of the interaction with screening ions is then given by:

$$H_{lig}^0 = -\frac{N_A(4+2)e_0^2}{4\pi \in_0 \in} \frac{\sqrt{I}}{3 \times 3.041 \times 10^{-10}}$$

where the ligand and counterion charges are not included in I. The factor $(4 + 2)$ accounts for n_j mol of divalent ligand ions ($z_{lig}^2 = 4$) and $2n_j$ mol of univalent OH^- $z_{OH}^2 = 1$)

Neglecting small entropy changes caused by the charging procedure, the system free enthalpy change by dissolving n_j moles of ligand is:

$$\Delta G_{sys}^{lig} = -n_j \frac{N_A(4+2)e_0^2}{4\pi \in_0 \in} \frac{\sqrt{I}}{3 \times 3.041 \times 10^{-10}}$$

In a reaction mixture the OH^- counterions of the ionized ligand will have been neutralized by H^+ counterions of the polymer at preparation of the mixture. Their contribution to a separate solution of ligands must not be included in the reaction enthalpy of formation of encounter complexes or bound states in a pre-prepared mixture of ligand and polymer chains. (This would not apply for calorimetric measurements where the heat of mixing must be included).

6.1.2. *Polymer chain enthalpy*. We measure the concentration of the polymer not in number of moles of chains in the standard volume, but in number of monomeric units of which the chains of average length n are composed. The standard

enthalpy of a polymer chain will therefore be defined as the enthalpy of hypothetical one molar solution of chains, each with the enthalpy of a chain at infinite dilution. With its n screened ionic phosphate ions the chain at infinite dilution may be considered as a droplet of extremely high concentration of n ions with $6 \times 10^{-10} m$ distances from each other in the chain volume. The screening of the ions will be completely different from the Debye-Hückel model considered before. For most of the ions screening as envisioned by Bjerrum will apply, in which many phosphates are in their neutral undissociated state. At infinite dilution the chain occupies a volume nearly equal to the encounter volume and carries an effective charge $z_{Pn}e^0$, much smaller than that of n ionizable phosphate groups. This charged chain will then still be surrounded by a cloud of screening ions with a prolate ellipsoidal instead of a spherical distribution. The one molar standard solution of polycytidine chains contains $N_A \times n$ nucleotides. A rather crude approximation for the standard enthalpy of the screened ionized chains would then be obtained by using the same approach as for dissolved ligand molecules but with the central charge equal to the effective chain charge.

The standard polymer chain enthalpy is expressed by an equation similar to that for the ligands, but the charge factor $N_A(nz_j^2)$ for n non-interacting ions with valencies z_j, as in the case of dissolved ligands, must be replaced by an effective charge number $N_A z_{P_n}^2$. The ionic strength

$$I = \frac{1}{2}\sum_i z_i c_i$$

will include the counterions. All corrections for non-shericity will be implicitly accounted for in the average chain charge:

$$H_{P_n}^0 = \frac{N_A z_{P_n}^2 e_0^2}{4\pi \in_0 \in} \frac{\sqrt{I}}{3 \times 3.041 \times 10^{-10}}$$

The unknown valency factor may be obtained by least-square fitting procedures for measured data (e.g. conductivities, binding constants) from systems of different concentrations.

6.1.3. *Encounter pair enthalpy*. The standard enthalpy of encounter complexes is then the

standard chain enthalpy according to this definition, but each chain associated at the encounter contact distance with an unbound ligand. The standard enthalpy of an encounter pair differs from that of the chain plus that of the free ligand by the addition of the field energy of the charged ligand and the charged chain in each others field at the encounter distance. The screening enthalpies of ligand and chain are not changed very much by the association so that ΔH^0 for the formation of the encounter pair (in which these reactant enthalpies are again subtracted) is practically identical with this added field energy. The electrostatic energy w_{ij} of two charges z_j and z_i at the reciprocal distance $1/r$ was obtained as:

$$w_{ij}(r) = z_i e_0 \overline{\Psi}(r) = \frac{z_j z_i e_0^2}{4\pi \in_0 \in\ r} \exp - \frac{r}{r_j}$$

This gives

$$\Delta H_{bond}^0 = N_A z_L z_B \frac{e_0^2}{4\pi \in_0 \in\ d_{bond}} \exp - \frac{d_{bond} \sqrt{I}}{3.041 \times 10^{-10}} + \frac{N_A(4)e_0^2}{4\pi \in_0 \in} \frac{\sqrt{I}}{3 \times 3.041 \times 10^{-10}}$$

z_B is the valency of the binding site. The last term arises from the disappearance of the screening energy of the ligand when it reacts with a binding site. With a bond distance 3.3×10^{-10} m and valencies $z_L = 2$ and $z_B = -2$ at $I = 0.025$ $lit.mol^{-1}$ this equation predicts $\Delta H^0 = -16.2 kJmol^{-1}$ and $K = 69.1$ $mol.lit^{-1}$. Although his corresponds to the experimentally observed value of K_{nuc} it is very improbable that d_{bond} can be as small as 3.3×10^{-10} m in view of the large discrepancy of the charge separations in the ligand and in the chain. Appreciable configuration changes of the chain would be required to obtain such a small bond distance d_{bond} and the non-ionic ΔG_0 of these configuration changes should also contribute to value of K_{nuc}. The electrostatic part of the standard enthalpy of one mol of growth bonds is similar to the enthalpy of a nucleation bond but the effective d_{bond} may be different because other configuration changes may be involved. The non-ionic interaction between neighboring ligands is mostly responsible for the cooperativity factor q. Non-ionic interactions will be discussed in later section

$$\Delta H^0 = N_A z_L z_{P_n} \frac{e_0^2}{4\pi \in_0 \in\ d_{enc}} \exp - \frac{d_{enc} \sqrt{I}}{3.041 \times 10^{-10}}$$

where z_L is the valency of the ligand and z_{Pn} the effective valency of the chain. This equation has been used in Section 1.4.2 to obtain an estimate for K_{AP}.

6.1.4. *Bound state enthalpies*. The bound ligand enthalpy includes contributions of electrostatic as well as contributions of non-electrostatic origin. The one molar standard solution of isolated bound ligands is represented by a solution of one mol of ligand, each bound to $1/g$ monomers on chains of length n. The electrostatic bond interactions with the polyelectrolyte backbone for one mol nucleation bonds are accounted for additively in the standard enthalpy but neighboring interaction enthalpies of growth bonds and the electrostatic work for screening the non-occupied binding sites are not included.

(cf. 6.3.1).

6.2. **Charge interaction influence on rate of encounter.** The rate constant of encounter of two neutral particles A and B moving at random in a viscous medium to a mutual encounter distance d_{enc} is

$$k_{R_{enc}} = 4\pi N_A \times (\mu_A + \mu_B) \times d_{enc}$$

This applies to spherical particles with diffusion coefficients $\mu_A = kT/f_A$ and $\mu_B = kT/f_B$ and friction coefficients according to Stokes law $f_i = 6\pi\eta r_i$, where r_i is the radius of the spherical particle i.

An estimate for the (diffusion controlled) encounter rate constant k_{Renc} for ligand-polymer chain encounter is obtained from the sum of diffusion coefficients $\mu_A + \mu_{Pn}$ and the encounter distance d_{enc} to the polymer chain P. The volume v_{chain} enclosing the polymer chain is the volume of a cylinder with radius $d_{Pn}/2$ and length $l_{Pn} = nd_{pp}$ where d_{pp} is the distance between the monomeric phosphates of the polymeric chain. d_{Pn} includes

the bound ligands. The diffusion volume V_{Pn} has the same volume as v_{chain} but has a spherical shape. The sum of the radius of the diffusion volume and the ligand radius $d_A/2$ approximates the average encounter distance d_{enc} when the diffusing ligand (arriving from the total space angle 4π) reaches the surface of the diffusion volume.

This leads to:

$$d_{enc} = \frac{d_A}{2} + \left(\frac{V_P}{(4/3)\pi}\right)^{1/3}$$

and

$$k_{R_{enc}} = 4\pi N_A \text{x} (\mu_A) \text{ x } d_{enc} \text{ x } 1000$$

This expression gives k_{Renc} in lit.mol^{-1}s^{-1} when $\mu_A + \mu_B$ is given in $m^2 s^{-1}$ and length quantities d_P; d_L; P_{len}; d_{enc} are given in m. For charged particles the effects of mutual attraction or repulsion play a role when the the electrostatic (force times distance) between the particles becomes comparable with their average kinetic energy kT. This occurs at *the Bjerrum distance:*

$$r_{B_j} = \frac{z_A z_{P_n} e_0^2}{(4\pi \in_0 \in) \text{ x } kT}$$

The rate constant for reaching the distance d_{enc} is modified by the factor

$$\frac{r_{B_j}/d_{enc}}{\exp \dfrac{r_{B_j}}{d_{enc}} - 1}$$

$$k_{R_{enc}} = 4\pi N_A \text{x} (\mu_A + \mu_B) \text{ x } \frac{r_{B_j}}{\exp \dfrac{r_{B_j}}{d_{enc}} - 1}$$

Since the encounter process is not accompanied by changes in the physical properties of the ligand nor the polymer the relaxation experiments do not directly show the encounter relaxation.

6.3. Electrostatics of ligand binding equilibria.

The total free enthalpy of reaction calculated from the measured equilibrium products at $I = 0$ is:

$$\Delta G^0_{nuc} = RTlnK_{nuc} = -15.5 \ kJ.mol^{-1}$$

$$\Delta G^0_{grow} = RTlnK_{grow} = -26.8 \ . \ kJ.mol^{-1}$$

At $I = 0.025$ mol/lit these values are:
$$\Delta G^0_{nuc} = RTlnK_{nuc} = -10.5 \ kJ.mol^{-1}$$

$$\Delta G^0_{grow} = RTlnK_{grow} = -21.9 \ kJ.mol^{-1}$$

These enthalpies include the electrostatic work for the formation of the encounter pair as well as the final electrostatic work of stable bond formation when the encounter pair transforms into the bound state. They also include any other non-ionic standard free enthalpy changes in ligand, polymer chain, counter ions and solvation upon binding

The reaction $2 -N(CH3)_2H^+ + 2 \ ^-OP-$ is not a neutralization like $H^+ + OH^-$, although balancing counterions may have neutralized this way when the reaction mixture was prepared. It might involve, if steric conditions allow, the formation of a pair of strongly polarized heteronuclear hydrogen bond $-N^+ - H - O^- P-$ and release of 4 or more H_2O molecules formerly solvating the ligand and polymer charges $-N(CH3)_2H^+$ and $^-OP-$. For this kind of binding the smallest final charge separation distance d_{bond} would be equivalent to the length of a heteronuclear intramolecular $N - H - O$ hydrogen bond. This would be between 1.0 and $1.2 m^{-10}$. The dielectric permittivity of the surrounding environment of this bond can be less than that of water because some water molecules are excluded by the surrounding organic part.

$$w_{bond} = N^A \frac{z_L z e_0^2}{4\pi \in \in_0} \text{ x } \int_\infty^{d_{bond}} r^{-2} dr \text{ x } 0.001 kj.mol^{-1}$$

$$w_{bond} = -N^A \frac{z_L z_B e_0^2}{4\pi \in_0 \in} \text{ x } \frac{\exp - \dfrac{d_{bond} \sqrt{I}}{3.057 \text{ x } 10^{-10}}}{d_{bond}}$$

z_L and z_B are the effective valencies. Even

assuming that the dielectric constant ($\epsilon = 78.5$ in water) remains unchanged during the structural transition from the encounter complex to the bound ligand the electrostatic work of bond formation with $d_{bond} = 1.2 \cdot 10^{-10}$ m is -57.9 $kjoule.mol^{-1}$ at $I = 0$ if we take the effective valencies $z_{ligand} = 2$ and $z_{bindingsite} = -2$. This is more than the $\Delta G^0_{formation} = 15.5$ $kjoule.mol^{-1} = \Delta H^0 - T\Delta S^0$ obtained from measurement of the equilibrium product at $I = 0$. Either the effective bond distance must be larger than $1.2m^{-10}$ or there must be a large non-ionic contribution to $\Delta G^0_{formation}$ or both.

The reaction between the ligand and the polymer chain $2 - N(CH3)_2H^+ + 2\ ^-OP -$ involves:
- the two ligand ions and two polymer ions making a bound state with negative (dependent on ionic strength) $\Delta_{H^0_{ionicdistancechange}}$
- neutralization of two pairs of screened counterions H^+ and OH^- with standard screening enthalpies

$$-\frac{N_A e_0^2 4\pi \in_0 \in [\kappa]}{3},$$

giving a negative $\Delta_{H^0_{counterionneutralization}} = -114.2$ $kJ.mol^{-1}$ at $I = 0$. The neutralization at finite I of the screened ions decreases $\Delta_{H^0_{neutralization}}$. The neutralization enthalpy would only appear in calorimetric measurements when the reaction mixture is prepared. It plays no role in optically measured binding constants in the reaction mixture.

Note. The calorimetrically measured heat of neutralization of H^+ with OH^- is $\Delta_{H0} = -57.1$ $kJ.mol^{-1}$ as measured from the reaction between HCl and $NaOH$. With $CH3COOH$ and $NaOH$ it is $\Delta H^0 = -55.9 kJ.mol^{-1}$. The difference is $+1.2$ $kJ.mol^{-1}$. This is because $CH3COOH$ is a weak acid that must firrst dissociate into H^+ and $CH3COO^-$ ions. This process is accompanied by the absorption of energy, the heat of dissociation of CH_3COOH in H_2O. Therefore, the overall liberated energy upon neutralization of acetic acid with $NaOH$ is somewhat less than 57.1 $kJ.mol^{-1}$. Conversely, the binding of an H^+ ion to a CH_3OO^- must have a reaction enthalpy Δ_{H0} of -1.2 $kJ.mol^{-1}$. Here the final distance between $H+$ and CH_3OO^- is almost zero (the bond is stil polar), therefore one would expect (as a result of the foregoing equations) that the electrostatic work of bringing the charges together must be considerably larger. Much of this work is absorbed, however, to change the configuration of the ionic $-COO^-$ quantum state, into the very different configuration of the neutral $-COOH$ radical. In the ionic state the negative charge is shared between the two oxygen atoms. The distances to the carbon atom and the energies of the binding orbitals are very different from the neutral molecule. One concludes that most of the work needed for separating the oppositely charged ions at the dissociation of CH_3COOH into ions is driven by the large energy difference between these configurations. In the case of the ionic dissociation of H_2O, or alcohols, there is not such a quantum mechanical difference between the configurations of the neutral molecule and the negative ion. This exemplifies the fact that the electrostatic work done by ion motion may not alone be responsible for bond formation but may be accompanied by other work of enthalpic or entropic nature due to atomic or molecular configuration changes in the reaction.

- configuration changes with Δ_{Hconf} and $T\,\Delta_{Sconf}$ of positive or negative signs. Only in rare cases are these configuration changes predictable. The configuration change may also involve the solvent, e.g. in the case of dimer association. Water molecules solvating non-polar parts of a molecule may be restricted in their orientational degrees of freedom. Upon association to a dimer this restriction is removed for water molecules that were solvating non-polar surfaces that are now in direct contact. Removing the restriction for the liberated water molecules creates a positive reaction free enthalpy that stabilizes the dimeric form. The contribution $-\Delta H^0_{ionicdistancechange}$ is recognizable by its dependence on ionic strength.

6.3.1. *Non-ionic contributions to ligand binding*. It is possible that the electrostatic work done by bringing together opposite charges is not converted completely into heat of reaction, but partially transformed into potential mechanical work, saved and stored in the force field of some non-randomly selected mechanical degrees of freedom of the product species. Then the electrostatic contribution $\Delta H^{0,el}$ is compensated by an opposite amount of enthalpy of mechanical origin, which must already be included in $\sum H^{0,non-el}_{products}$. If there is complete conversion of electrostatic work of binding in configurationally work the net electrostatic heat of reaction $\Delta H^{0,el}$ is zero. The ionic strength dependent attractive electrostatic forces between the opposite charges on ligand and polymer then still do contribute to the chemical binding but do not generate heat of reaction. The electrostatic work is transformed into work of structural mechanical forces present only in the bound state. The standard free enthalpy of the bound state must then already include the work of the structural forces. The partial electrical to mechanical conversion is not improbable in the present case, because binding of the ligand may be accompanied by changes in the solvation of the ligand and the polymer and changes in its folding conformation.

There may also exist non-ionic interactions between the bound ligand and the polymer that do not oppose, but favor the binding stability.

We write $-\Delta G^0 = RTlnK$ as a sum of contributions of different origin:

$$RT \ln K = -\Delta H^0_{ionic} - \Delta H^0_{non\text{-}ionic} + T\Delta S^0$$

$\Delta H^0_{total} = \Delta H^0_{ionic} + \Delta H^0_{non\text{-}ionic}$ is measurable as the heat of reaction. ΔH^0_{ionic} may be obtained from the foregoing theoretical equations of electrostatics, but knowledge of the bond distance and the equivalent valency factors is required. The bond distance is the distance between effective centers of charge on the ligand and its polymeric counterpart. $\Delta H^0_{non\text{-}ionic}$ can be obtained from the difference $\Delta H^0_{total} = \Delta H^0_{ionic}$. A priory calculations of ΔH^0_{ionic} are difficult: they require detailed knowledge of the structural configuration changes and corresponding quantum-mechanical energy differences. $\Delta G^0_{non\text{-}ionic}$ is the difference between measured ΔG^0 (from equilibrium data) and calculated ΔH^0_{ionic} (from electrostatics). It contains enthalpic and entropic contributions of non-electrostatic origin:

$$\Delta G^0_{non\text{-}ionic} = \Delta H^0_{non\text{-}ionic} - T\Delta S^0_{non\text{-}ionic.}$$

The entropy term is dominant in the present case.

7. NUMERIC CALCULATION OF NON-IONIC BINDING CONTRIBUTIONS

From a set of measured values of K_{nuc} and K_{grow} under different conditions of ionic strength I one can obtain the best FItting value of $\Delta G^0_{non\ ionic}$ to the theoretical model:

$$RTlnK(I)_{measured} = \Delta G^0_{non\ ionic} + \Delta H^0_{ionic}(I)_{calculated.}$$

A computer program **Equilibria** was developed for this purpose. It has been used for measured sets $K_{Nuc}(I)$ and $K_{Grow}(I)$ as a function of I.
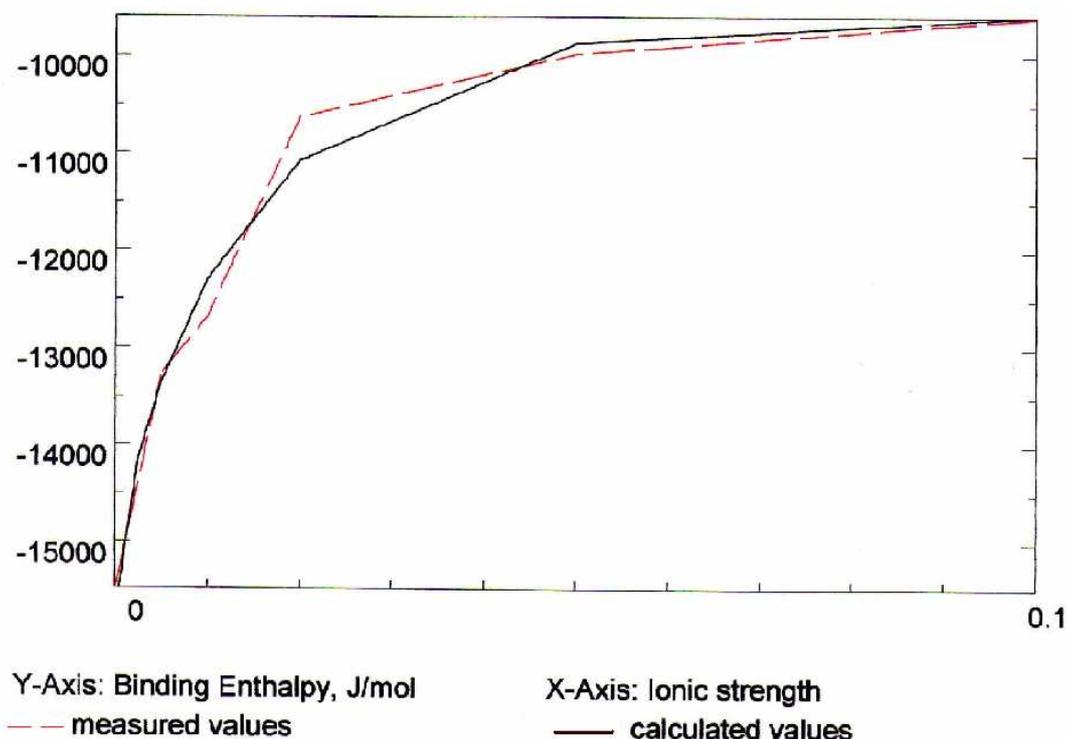

Fig. 2. Fit of K_{Nuc} with program Equilibria.

Table 3

Block	Ionic strength, M	K_eq M^{-1}
K_{nuc}	0.0000	515
K_{nuc}	0.0025	331
K_{nuc}	0.0050	211
K_{nuc}	0.0100	167
K_{nuc}	0.0200	73
K_{nuc}	0.0500	56
K_{nuc}	0.1000	48

The non-linear least-squares algorithm simultaneously optimizes the values of d_{bond}, the empirical correction factor f in

$$r_j^{corr} = \frac{r_j}{1 + f \cdot \sqrt{I}}$$

and the value of $\Delta G^0_{non\ ionic}$ for fixed $m(z) = -4$; $\varepsilon = 78.5$.

Table 4

I, M	K_{Nuc}, M^{-1}
0.0000	00515.0000
0.0025	00331.0000
0.0050	00211.0000
0.0100	00167.0000
0.0200	00073.0000
0.0500	00056.0000
0.1000	00048.0000

For K_{nuc}; $I = 0$ the results are:

$\Delta H^0_{ionic} = -6.15\ kJ.mol^{-1}$; $d_{bond} = 11.51\ 10^{-10}m$; $\Delta G^0_{non\ ionic} = -9.55\ kJ.mol^{-1}$; $\Delta G^0_{total} = -15.47\ kJ.mol^{-1}$; $f = +11.36$

Table 5

I, M	K_{Grow}, M^{-1}
0.0000	51500.0000
0.0025	36400.0000
0.0050	23200.0000
0.0100	18400.0000
0.0200	08000.0000
0.0500	06200.0000
0.1000	05300.0000

For K_{grow}, $I = 0$ the results are: $\Delta H^0_{ionic} = -6.03\ kJ.mol^{-1}$; $d_{bond} = 11.74\ 10^{-10}$ m

$\Delta G^0_{non\ ionic} = -21.18\ kJ.mol^{-1}$; $\Delta G^0_{total} = -26.88\ kJ.mol^{-1}$; $f = +10.23$

Although these values may have large error bounds because they are very much dependent on the chosen value of m(z) = $z_L z_B$ and ϵ, they indicate that the electrostatic part of binding energy is almost the same for nucleation and for growth, and that nucleation involves a substantial negative non-ionic energy of formation (larger than that of the electrostatic part) of the isolated bound ligand. The ionic part of binding enthalpy of the growth reaction is similar to that of nucleation. The growth reaction is supported by additional non-ionic binding.

The theoretical expression for ΔH^0_{ionic} used in these calculations did not distinguish between nucleation and growth binding since possible additional work from electrostatic forces resulting from interacting neighboring ligands was not considered. This and the considerable error bounds on measured values of $K(I)$ and calculated values of ΔH^0_{ionic} limits the numerical correctness but not the basis of the above conclusions. One may compare these results with those obtained for the dimerization equilibrium[2] from spectral data:
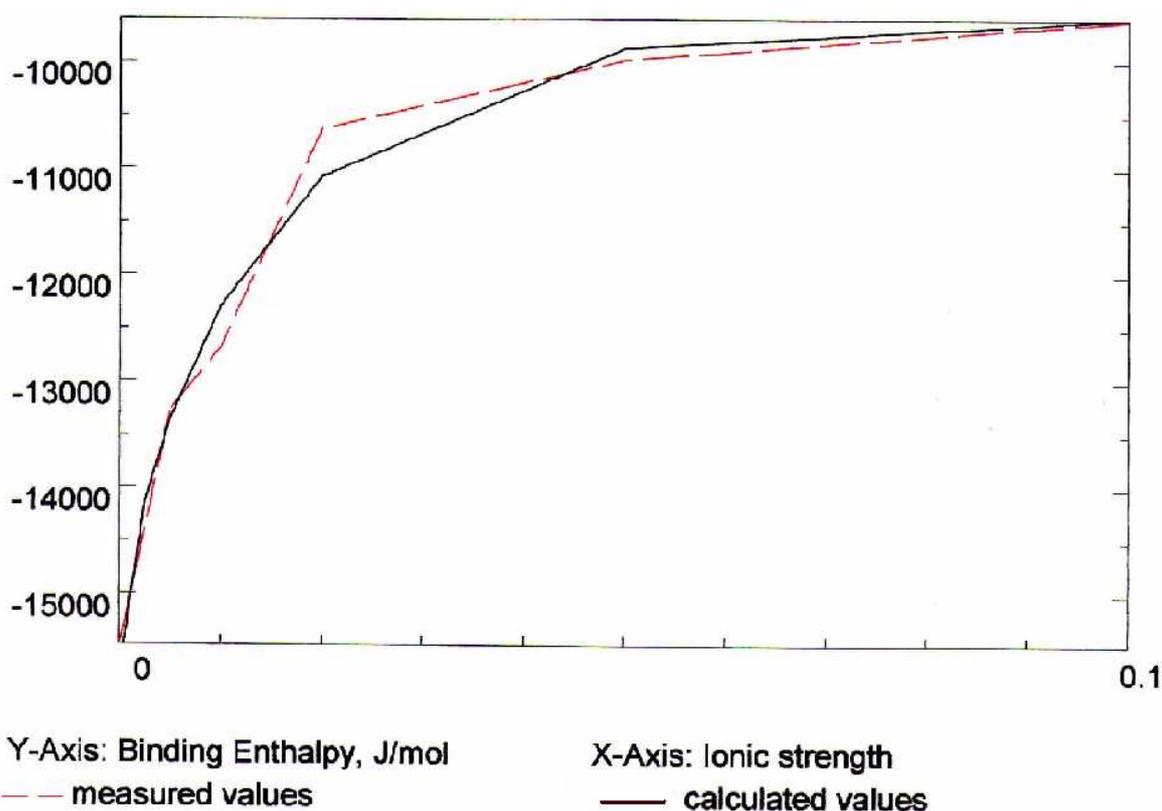

Y-Axis: Binding Enthalpy, J/mol   X-Axis: Ionic strength
– – measured values   —— calculated values

Fig. 3. Fit of K_{Dim} with program Equilibria.

Table 6

I, M	$K_{eq.,}$ M^{-1}	$K_{Dim,}$ M^{-1}
0.0200	732	732
0.0450	1499	1499
0.0700	3400	3400
0.1200	6150	6150
0.2200	9355	9366

gives fit parameters: $m_z = +4$; $\epsilon = 78.5$ $\Delta H^0_{ionic} = +6{:}98$ $kJ.mol^{-1}$; $d_{bond} = 6.19 \ 10^{-10} m$

$\Delta H^0_{\ non \ ionic} = -23.18 \ kJ.mol^{-1}$; $\Delta H^0_{total} = -16.34 \ kJ.mol^{-1}$; $f = +5.02$

Dimerization is driven by the non-ionic part of ΔH^0_{dimer}. The distance of the repulsive charge configuration is smaller than in the attractive configuration with the opposite charges of the polymer. These values are highly dependent on the selected values of the dielectric constant and the effective charge valencies. The quality of least-squares fitting the ionic strength dependencies of the equilibrium products to the model that introduces non-ionic contributions to the binding can be judged from the graphic representation of the results in figures 1. to 3.

CONCLUSIONS

From an energetic view the origin of binding can be enthalpic, entropic, or both. From the kinetics viewpoint the process of binding between two molecules can be diffusion or activation controlled. For the formation of the weakly bound encounter pair one expects a diffusion controlled process. The corresponding very fast relaxation rate $1/\tau_1$ does not produce an optical extinction change at $\lambda = 500$ nm.

The formation of the isolated bound nucleation ligand is found to be activation controlled. Much of the activation free enthalpy for the nucleation reaction may be of entropic origin, probably caused by a particular folding and alignment requirements of the polymer and activated solvation states of the ligand before binding.

The difference in rate constants for nucleation and growth is not very large. The activation can be viewed as a pre-equilibrium between the native folding state and a local conformation state comprising a stretch of monomers and their cytidilic side chains. In the growth reaction the requirement of desolvation of cooperatively bound molecules will include activated (partially desolvated) states of the ligands. The lifetime of an encounter complex is limited. If the ligand does not find an activated region on the polymer it may dissociate again instead of binding. A region may heal or become stabilized by the binding of a first ligand (nucleation), but this does not lower the activation energy required for binding a second neighbor. The presence of stacks of bound ligands will delay the rate at which the ligand in an encounter complex finds a reactive nucleation or growth binding site. The observed rate constants can be written as $k^*_{R_{nuc}} = K^{nuc}_{activation}k^0_R$ and $k^*_{R_{grow}} = K^{grow}_{activation}k^0_R$ where k^0_R is an intrinsic binding rate constant of the order of $10^8 - 10^9 s^{-1}$ and $K^{nuc}_{activation}$ resp. $K^{grow}_{activation}$ are equilibrium constants describing an equilibrium of 'reactive' ligands and 'reactive' binding positions relative to their native 'nonreactive' state. The activation process is required in both the forward and reverse binding process. It influences only the process rates but not the equilibria. If k^0_R is of the order k_{Renc}, which would be required for non-activated diffusion controlled binding, the lowering to k_{Rnuc}

shows K_{active} to be of the order $10^{-6} - 10^{-7}$, equivalent to an activation free enthalpy of up to $30 kJ.mol^{-1}$. This is an upper bound because part of the lowering is caused by the time needed by the ligand inside the encounter volume to find the appropriate binding site. The required thermal activation may be much smaller because a large part of the activation free enthalpy may be of entropic origin. The activated state must have a steric configuration that allows proximity of the two ligand charges to two polymer charges at a mutual distance dictated by their distance in the ligand and at the same time require particular steric conformations of ligand and polymer chain to allow the non-ionic contribution to the binding that it requires an activated desolvation process.

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Appendix A. Derivation of linearized kinetic equations

A.1. **The nonlinear equations.** The number of unoccupied positions on a chain B_u is equal to the chain length (expressed by the number of binding sites $n \cdot g$) multiplied by the total fraction of unoccupied sites: $B_u = ng(CU_0 - C_{Abound})/CU_0 = ng(1 - C_{Abound}/CU_0)$.

Of these, the fraction $B_u C_{uuu}/CU_0$ gives the number of available nucleation sites and $2B_u(C_{uA1} + C_{stacks})$ is the number of available growth sites on the polymer chain of an encounter complex.

Once it is formed the ligand in the encounter complex reacts with probability $p_n = (k^* R_{nuc}TAP)B_u C_{uuu}/CU_0$ resp. $(k^* R_{grow}TAP)2B_u(C_{uA1} + C_{stacks})$ with an available binding site or leaves the encounter complex with probability $kD_{enc}TAP$ $TAP = 1/(kD_{enc} + k^*R_{nuc}B_u C_{uuu}/CU_0 + k^*R_{grow}2B_u(C_{uA1} + C_{stacks})$ is the lifetime of an encounter complex. The non-linear system of kinetic equations is:

$$dC_{Af}/dt = -k_{Renc} C_{Af} C_{P0}/n + k_{Denc} C_{AP} + p_D k^*_{Dnuc} C_{uA1u} + 2p_D k^*_{Dgrow} x \Sigma_{l>1} C_{uAlu}$$

$$dC_{AP}/dt = +k_{Renc} C_{Af} C_{P0}/n - k_{Denc} C_{AP} + (1-p_D)k^*_{Dnuc} C_{uA1u} + 2(1-p_D)k^*_{Dgrow} x \Sigma_{l>1} C_{uAlu} - k^*_{Rnuc} FC_{uuu}C_{AP}$$
$$- 2k^*_{Rgrow} FC_{uA1u}C_{AP} - 2k^*_{Rgrow} FC_{stacks}C_{U0} C_{AP}.$$

$$dC_{uuu}/dt = +k^*_{Dnuc} C_{uA1u} + 2k^*_{Dgrow} \Sigma_{l>1} C_{uA1u} - k^*_{Rnuc} FC_{uuu}C_{AP} - 2k^*_{Rgrow}FC_{uA1u}C_{AP} - 2k^*_{Rgrow} C_{stacks}C_{U0}C_{AP}$$

$$dC_{uA1u}/dt = +k^*_{Rnuc} FC_{uuu}C_{AP} + 2k^*_{Dgrow} C_{uA2u} - k^*_{Dnuc} C_{uA1u} - 2k^*_{Rgrow}FC_{uA1u}C_{AP}$$

$$dC_{uA2u}/dt = +2k^*_{Rgrow} FC_{uA1u}C_{AP} + 2k^*_{Dgrow} C_{uA3u} - 2k^*_{Dgrow} C_{uA2u} - 2k^*_{Rgrow}FC_{uA2u}C_{AP}$$

$$dC_{uA3u}/dt = 2k^*_{Rgrow} FC_{uA2u}C_{AP} + 2k^*_{Dgrow} C_{uA4u} - 2k^*_{Dgrow}C_{uA3u} - 2k^*_{Rgrow} FC_{uA3u}C_{AP}$$

$$dC_{uA4u}/dt = 2k^*_{Rgrow} C_{uA3u}C_{AP} + 2k^*_{Dgrow} C_{uA5u} - 2k^*_{Dgrow} C_{uA4u} - 2k^*_{Rgrow}FC_{uA4u}C_{AP}$$

…

…

Summing the equations dC_{uA1u}/dt for $l > 1$ and introducing the variables $\Sigma l_{>1}C_{uAlu} = C_{stacks}$ and $\Sigma_{l \geqslant > 1}C_{uAlu} = C_{stacks} + C_{uAlu}$ leads to:

$d\Sigma_{i>1}C_{uAiu}/dt = dC_{stacks}/dt + 2k^*_{Rgrow}FC_{uA1u}C_{AP} + 2k^*_{Dgrow}C_{uA3u} - 2k^*_{Dgrow}C_{uA2u} - 2k^*_{Rgrow}FC_{uA2u}C_{AP}$

$+2k^*_{Rgrow}FC_{uA2u}C_{AP} +2k^*_{Dgrow}C_{uA4u} -2k^*_{Dgrow}C_{uA3u} -2k^*_{Rgrow}FC_{uA3u}C_{AP} +2k^*_{Rgrow}FC_{uA3u}C_{AP} +2k^*_{Dgrow}C_{uA5u}$

$-2k^*_{Dgrow}C_{uA4u} -2k^*_{Rgrow}FC_{uA4u}C_{AP}$

…

or
$dC_{stacks}/dt = +2k^*_{Rgrow}FC_{uA1u}C_{AP} +2k^*_{Dgrow}\Sigma_{l>2}F(C_{uAlu} - C_{uAl-1u})$

Since
$\Sigma_{l>2}(C_{uAlu} - C_{uAl-1u}) = \Sigma_{l>2}C_{uAlu} - \Sigma_{l>1}C_{uAlu} = (\Sigma_{l\geq1}C_{uAlu} - C_{uA2u} - C_{uA1u}) - (\Sigma_{l\geq1}C_{uAlu} - C_{uA1u}) = -C_{uA2u}$

it follows:

$d\Sigma_{l>1}C_{uAlu}/dt = dC_{stacks} = +2k^*_{Rgrow}FC_{uA1u}C_{AP} -2k^*_{Dgrow}C_{uA2u}$

A.1.1. The linearizing expressions. The linearized system of kinetic equations is:

$dC_{A_f}/dt = d\overline{C}_{A_f}/dt + dx_{A_f}/dt = -k_{R_{enc}}\overline{C}_{A_f}C_{P_0}/n + k_{D_{enc}}\overline{C}_{A_P} + p_D k^*_{D_{nuc}}\overline{C}_{uA_1u} + 2p_D k^*_{D_{grow}}\sum_{l>1}\overline{C}_{uA_lu}$

Since the first part $d\overline{C}_{A_f}/dt = 0$, there remains

$dx_{A_f}/dt = -k_{R_{enc}}C_{P_0}/nx_{A_f} + k_{D_{enc}}x_{A_P} + p_D(k^*_{D_{nuc}}x_{uA_1u} + 2p_D k^*_{D_{grow}})x_{stacks}$

$dC_{A_P}/dt = k_{R_{enc}}\overline{C}_A C_{P_0}/n - k_{D_{enc}}\overline{C}_{A_P} + (1 - p_D)k^*_{D_{nuc}}\overline{C}_{uA_1u} + 2(1 - p_D)k^*_{D_{grow}}\overline{C}_{stacks} - k^*_{R_{nuc}}F\overline{C}_{uuu}\overline{C}_{A_P}$

$-2k^*_{R_{grow}}F\overline{C}_{uA_1u}\overline{C}_{A_P} - 2k^*_{R_{grow}}F\overline{C}_{stacks}\overline{C}_{A_P}$

$dx_{A_P}/dt = k_{R_{enc}}C_{P_0}/n\,x_{A_f} - k_{D_{enc}}x_{A_P} + (1 - p_D)k^*_{D_{nuc}}x_{uA_1u} + 2(1 - p_D)k^*_{D_{grow}}x_{stacks} - k^*_{R_{enc}}F\overline{C}_{uuu}x_{A_P}$

$-k^*_{R_{enc}}F\overline{C}_{A_P}x_{uuu} - 2k^*_{R_{grow}}F(\overline{C}_{uA_1u}x_{A_P} - 2k^*_{R_{grow}}F\overline{C}_{stacks})x_{A_P} - 2k^*_{R_{grow}}F\overline{C}_{A_P}x_{uA_1u} - 2k^*_{R_{grow}}F\overline{C}_{A_P}x_{stacks}$

$dC_{uuu}/dt = k^*_{D_{nuc}}\overline{C}_{uA1u} + 2k^*_{D_{grow}}\overline{C}_{stacks} - k^*_{R_{nuc}}F\overline{C}_{uuu}\overline{C}_{A_P} - 2k^*_{R_{grow}}F(\overline{C}_{uA_1u}\overline{C}_{A_P} - 2k^*_{R_{grow}}F\overline{C}_{stacks})\overline{C}_{A_P}$

$dx_{uuu}/dt = k^*_{D_{nuc}}x_{ua_1u} + 2k^*_{D_{grow}}x_{stacks} - k^*_{R_{nuc}}F\overline{C}_{uuu}x_{A_P} - k^*_{R_{nuc}}F\overline{C}_{A_P}x_{uuu} - 2k^*_{R_{grow}}F\overline{C}_{uA_1u}x_{A_P} - 2k^*_{R_{grow}}F\overline{C}_{A_P}x_{uA_1u}$

$-2k^*_{R_{grow}}F\overline{C}_{stacks}x_{A_P} - 2k^*_{R_{grow}}F\overline{C}_{A_P}x_{stacks}$

$dC_{uA_1u}/dt = k^*_{R_{nuc}}F\overline{C}_{uuu}\overline{C}_{Ap} + 2k^*_{D_{grow}}\overline{C}_{uA_2u} - k^*_{D_{nuc}}\overline{C}_{uA_1u} - 2k^*_{R_{grow}}F\overline{C}_{uA1u}\overline{C}_{A_P}$

$dx_{uA_1u}/dt = k^*_{R_{nuc}}F\overline{C}_{uuu}x_{Ap} + k^*_{R_{nuc}}F\overline{C}_{Ap}x_{uuu} + 2k^*_{D_{grow}}x_{uA_2u} - k^*_{D_{nuc}}x_{uA_1u} - 2k^*_{R_{grow}}F\overline{C}_{uA_1u}x_{Ap} - 2k^*_{R_{grow}}F\overline{C}_{Ap}x_{uA_lu}$

$dC_{stacks}/dt = 2k^*_{R_{grow}}F\overline{C}_{uA_1u}\overline{C}_{Ap} - 2k^*_{D_{grow}}\overline{C}_{uA_2u}$

$dx_{stacks}/dt = 2k^*_{R_{grow}}F\overline{C}_{uA_1u}x_{Ap} + 2k^*_{R_{grow}}F\overline{C}_{Ap}x_{uA_1u} - 2k^*_{D_{grow}}x_{uA_2u}$

A.1.2. The linearizing equations. The linear system of kinetic equations is:

$dx_{A_f}/dt = -k_{Renc}C_{P_0}/nx_{A_f} + k_{D_{enc}}x_{A_P} + p_D k^*_{D_{enc}}x_{uA_1u} + 2p_D k^*_{D_{grow}}x_{stacks}$

$$dx_{Ap}/dt = k_{R_{enc}} C_{P_0}/nx_{Af} - k_{D_{enc}} x_{Ap} - k^*_{R_{nuc}} F\overline{C}_{uuu} x_{Ap} - k^*_{R_{nuc}} F\overline{C}_{Ap} x_{uuu} - 2k^*_{R_{grow}} F\overline{C}_{uA_1u} x_{Ap} - 2k^*_{R_{grow}} F\overline{C}_{Ap} x_{uA_1u}$$

$$- 2k^*_{R_{grow}} F\overline{C}_{stacks} x_{Ap} - 2k^*_{R_{grow}} F\overline{C}_{Ap} x_{stacks} + (1-pD)k^*_{D_{nuc}} x_{uA_1u} + 2k^*_{D_{grow}} x_{stacks}$$

$$dx_{uuu}/dt = k^*_{D_{nuc}} x_{uA_1u} - 2k^*_{R_{grow}} F\overline{C}_{uA_1u} x_{Ap} - 2k^*_{R_{grow}} F\overline{C}_{A_P} x_{uA_1u} - 2k^*_{R_{grow}} F\overline{C}_{stacks} x_{A_P} - 2k^*_{R_{grow}} F\overline{C}_{A_P} x_{stacks}$$

$$- k^*_{R_{nuc}} F\overline{C}_{uuu} x_{AP} - k^*_{R_{nuc}} F\overline{C}_{AP} x_{uuu} + 2k^*_{D_{grow}} x_{stacks}$$

$$dx_{uA1u}/dt = k^*_{R_{nuc}} F\overline{C}_{uuu} x_{A_P} + k^*_{R_{nuc}} F\overline{C}_{A_P} x_{uuu} - 2k^*_{grow} F\overline{C}_{uA_1u} x_{A_P} - 2k^*_{grow} F\overline{C}_{A_P} x_{uA_1u} + 2k^*_{D_{grow}} x_{uA_2u} - k^*_{D_{nuc}} x_{uA1u}$$

$$dx_{stacks}/dt = 2k^*_{R_{grow}} F\overline{C}_{uA_1u} x_{A_P} + 2k^*_{R_{grow}} F\overline{C}_{A_P} x_{uA_1u} - 2k^*_{D_{grow}} x_{uA_2u}$$

A.1.3. ***Selection of independent variables***. The summation of the variables x_l for $(l >1)$ has reduced to six the number of variables used to derive the kinetic equations. They are x_{Af} , x_{AP1}, x_{uuu}, x_{uA1u}, x_{uA2u}, and $x_{stacks} = \sum_{l>1} x_u A_l u$.. The rate of change of variable x_{uA2u} does not appear explicitly but is included in dx_{stacks}/dt. In view of the two conservation conditions the system dynamics is determined by only four independent variables. For solving the system we must make a convenient selection of four independent variables and express the other ones in terms of the selected independent ones.

Linearizing $\overline{C}_{uA_2u} = \overline{C}_{uA_1u} q K_{nuc} \overline{C}_{A_f}$ gives

$$x_{uA_2u} = \overline{C}_{uA_1u} q K_{nuc} x_{A_f} + \overline{C}_{A_f} q K_{nuc} x_{uA_1u}$$

An alternative substitution for x_{uA2u} is obtained from

$$\overline{C}_{stacks} = \frac{C_{uA_1u} q K_{nuc} C_{A_f}}{1 - q K_{nuc} C_{A_f}} = \frac{C_{uA_2u}}{1 - q K_{nuc} C_{A_f}}$$

$$\overline{C}_{uA_2u} = (1 - q K_{nuc} \overline{C}_{A_f}) \times \overline{C}_{stacks}$$

from which one gets the linearized expression:

$$x_{uA_2u} = (1 - q K_{nuc} \overline{C}_{A_f}) \times x_{stacks} - q K_{nuc} \overline{C}_{stacks} \times x_{A_f}$$

but this applies only if $q K_{nuc} \overline{C}_{A_f} < 1$.

The conservation equation

$$C_{A_{bound}} = C_{A_0} - C_{A_f} - C_{A_P}$$

can be used to replace $x_{AP} = -x_{Abound} - x_{A_f}$

The conservation of monomer units is given by:

$$C_{P_0} = (1/g)(C_{uuu} + C_{A_{bound}} + 2 \times \sum_{l \geq 1} C_{uA_l u}) = (1/g)(C_{uuu} + C_{A_{bound}} + 2(C_{uA1u} + C_{stacks}))$$

since each A_{bound} occupies one binding position and each stack and each uA_1u is flanked by two uua, which are not counted as uuu. This leads to the relation

$$x_{uuu} + x_{A_{bound}} + 2 \times x_1 + 2 \times x_{stacks} = 0$$

Stacks can be formed by $uA_1u + A_P \rightarrow uA_2u$, giving the creation of one A_{bound}, no loss of uua, loss of one ua_1u and increasing the number of stacks by one. The conservation equation would lead to: $x_{Abound} = +1$, $x_{stacks} = +1$; $x_1 = -1$ giving $(x_{uuu}) = -(x_{Abound}) - 2(x_{stacks}) - 2(x_{uA1u}) = -1 -2 +2 = -1$ thus the conservation condition requires that the binding of one A_P requires the loss of one uuu. A stack can also be formed by dissociating an A_P from $uA_k u$ and binding it to uA_1u: $uA_k u + uA_1u \rightarrow uA_2u + uA_{k-1}u$, giving $x_{Abound} = 0$, $x_1 = -1$, $x_{stacks} = +1$. But the dissociation $uA_1u \rightarrow uuu + A_P$ is accompanied by $x_1 = -1$; $x_{Abound} = -1$; $x_{uuu} = +3$; $x_{stacks} = 0$, since the two neighboring uua are transformed into uuu. According to the conservation condition: $+3(x_{uuu}) - 1(x_{Abound}) - 2(x_{uA1u}) = 0$. This allows the introduction of x_{Abound} as

independent variable.

$$dx_{A_{bound}} / dt = - dx_{uuu} / dt - 2dx_{stacks} / dt - 2dx_{uA_1u} / dt$$

$$dx_{A_{bound}} / dt = -k_{D_{nuc}}^* x_{uA_1u} + 2k_{R_{grow}}^* F\overline{C}_{uA_1u} x_{A_P} + 2k_{R_{grow}}^* F\overline{C}_{A_P} x_{uA_1u} + k_{R_{nuc}}^* F\overline{C}_{uuu} x_{A_P} + k_{R_{nuc}}^* F\overline{C}_{A_P} x_{uuu}$$

$$+ 2k_{R_{grow}}^* F\overline{C}_{stacks} x_{A_P} + 2k_{R_{grow}}^* F\overline{C}_{A_P} x_{stacks} - 2k_{D_{grow}}^* x_{stacks} - 4k_{R_{grow}}^* F\overline{C}_{uA_1u} x_{A_P} - 4k_{R_{grow}}^* F\overline{C}_{A_P} x_{uA_1u} + 4k_{D_{grow}}^* x_{uA_2u}$$

$$- 2k_{R_{nuc}}^* \overline{C}_{uuu} x_{A_P} - 2k_{R_{nuc}}^* F\overline{C}_{A_P} x_{uuu} + 4k_{R_{grow}}^* F\overline{C}_{uA_1u} x_{A_P} + 4k_{R_{grow}}^* F\overline{C}_{A_P} x_{uA_1u} - 4k_{D_{grow}}^* x_{uA_2u} + 2k_{D_{nuc}}^* x_{uA_1u}$$

After eliminating compensating terms this becomes:

$$dx_{A_{bound}} /dt = 2k_{R_{grow}}^* F\overline{C}_{uA_1u} x_{A_P} + 2k_{R_{grow}}^* F\overline{C}_{A_P} x_{uA_1u} + 2k_{R_{grow}}^* F\overline{C}_{stacks} x_{A_P} + 2k_{R_{grow}}^* F\overline{C}_{A_P} x_{stacks} - 2k_{D_{grow}}^* x_{stacks}$$

$$- k_{R_{nuc}}^* F\overline{C}_{uuu} x_{A_P} - k_{R_{nuc}}^* F\overline{C}_{A_P} x_{uuu} + k_{D_{nuc}}^* x_{uA_1u}$$

The system of linear kinetic differential equation now contains expressions for dx_{Af} /dt, dx_{AP} /dt, dx_{uuu} /dt, dx_{uA1u} /dt, dx_{stacks} /dt and $dx_{Abound} /dt =$ in terms of seven variables (x_{Af} ; x_{AP} ; x_{uuu}; x_{uA1u}; x_{uA2u}; x_{stacks}; x_{Abound}) of which only four are independent.

As independent variables we use the set (x_{Af} ; x_{uuu}; x_{uA1u}; x_{Abound}).

The rate equations dx_{AP} /dt and dx_{stacks}/dt may be omitted since they are implied by the remaining rate equations dx_{Af} /dt; dx_{uuu} /dt; dx_{uA1u} /dt; dx_{Abound} /dt.

Using the substitutions:

$$x_{uA_2u} = \overline{C}_{uA_1u} q K_{nuc} x_{A_f} + \overline{C}_{A_f} q K_{nuc} x_{uA_1u}$$

and

$$x_{stacks} = -(1/2) x_{uuu} - x_{uA_1u} - (1/2) x_{A_{bound}}$$

$$x_{A_P} = -x_{A_f} - x_{A_{bound}}$$

Results in:

$$dx_{A_f} / dt = -k_{R_{enc}} C_{P_0} / n \, x_{A_f} + k_{D_{enc}} x_{A_P} + p_D k_{D_{nuc}}^* x_{uA_1u} + 2 p_D k_{D_{grow}}^* x_{stacks}$$

Becomes

$$dx_{A_f} / dt = -k_{R_{enc}} C_{P_0} / n \, x_{A_f} - k_{D_{enc}} x_{A_f} - k_{D_{enc}} x_{A_{bound}} + p_D k_{D_{nuc}}^* x_{uA_1u} - p_D k_{D_{grow}}^* x_{uuu} - 2 p_D k_{D_{grow}}^* x_{uA_1u} - p_D k_{D_{grow}}^* x_{A_{bound}}$$

$$dx_{uuu} / dt = k_{D_{nuc}}^* x_{uA_1u} - 2k_{R_{grow}}^* F\overline{C}_{uA_1u} x_{A_P} - 2k_{R_{grow}}^* F\overline{C}_{A_P} x_{uA_1u} - k_{R_{nuc}}^* F\overline{C}_{uuu} x_{A_P} - k_{R_{nuc}}^* F\overline{C}_{A_P} x_{uuu} - 2k_{R_{grow}}^* F\overline{C}_{stacks} x_{A_P}$$

$$- 2k_{R_{grow}}^* F\overline{C}_{A_P} x_{stacks} + 2k_{D_{grow}}^* x_{stacks}$$

becomes

$$dx_{uuu} / dt = k_{D_{nuc}}^* x_{uA_1u} + 2k_{R_{grow}}^* F\overline{C}_{uA_1u} x_{A_f} + 2k_{R_{grow}}^* F\overline{C}_{uA_1u} x_{A_{bound}} - 2k_{R_{grow}}^* F\overline{C}_{A_P} x_{uA_1u} + k_{R_{nuc}}^* F\overline{C}_{uuu} x_{A_f} + k_{R_{nuc}}^* F\overline{C}_{uuu} x_{A_{bound}}$$

$$- 2k_{R_{grow}}^* F\overline{C}_{A_P} x_{A_{bound}} - k_{R_{nuc}}^* F\overline{C}_{A_P} x_{uuu} + 2k_{R_{grow}}^* F\overline{C}_{stacks} x_{A_f} + 2k_{R_{grow}}^* F\overline{C}_{stacks} x_{A_{bound}} + k_{R_{grow}}^* F\overline{C}_{A_P} x_{A_{bound}} + 2k_{R_{grow}}^* F\overline{C}_{A_P} x_{uA_1u}$$

$$+ k_{R_{grow}}^* F\overline{C}_{A_P} x_{uuu} - k_{D_{grow}}^* x_{uuu} - 2k_{D_{grow}}^* x_{uA_1u} - k_{D_{grow}}^* x_{A_{bound}}$$

$$dx_{uA_1u} / dt = k_{R_{nuc}}^* F\overline{C}_{uuu} x_{A_P} + k_{R_{nuc}}^* F\overline{C}_{A_P} x_{uuu} - 2k_{R_{grow}}^* F\overline{C}_{uA_1u} x_{A_P} - 2k_{R_{grow}}^* F\overline{C}_{A_P} x_{uA_1u} + 2k_{D_{grow}}^* x_{uA_2u} - k_{D_{nuc}}^* x_{uA_1u}$$

becomes

$$dx_{uA_1u} / dt = -k_{R_{nuc}}^* F\overline{C}_{uuu} x_{A_f} - k_{R_{nuc}}^* F\overline{C}_{uuu} x_{A_{bound}} + k_{R_{nuc}}^* F\overline{C}_{A_P} x_{uuu} + 2k_{R_{grow}}^* F\overline{C}_{uA_1u} x_{A_f} + 2k_{R_{grow}}^* F\overline{C}_{uA_1u} x_{A_{bound}}$$

$$- 2k_{R_{grow}}^* F\overline{C}_{A_P} x_{uA_1u} + 2k_{D_{grow}}^* F\overline{C}_{uA_1u} q K_{nuc} x_{A_f} + 2k_{D_{grow}}^* F\overline{C}_{A_f} K_{nuc} x_{uA_1u} - k_{D_{nuc}}^* x_{uA_1u}$$

$dx_{A_{bound}} / dt = 2k^*_{R_{grow}} F\overline{C}_{uA_1u} x_{A_P} + 2k^*_{R_{grow}} F\overline{C}_{A_P} x_{uA_1u} + 2k^*_{R_{grow}} F\overline{C}_{stacks} x_{A_P} + 2k^*_{R_{grow}} F\overline{C}_{A_P} x_{stacks} - 2k^*_{D_{grow}} x_{stacks}$
$- k^*_{R_{nuc}} F\overline{C}_{uuu} x_{A_P} - k^*_{R_{nuc}} F\overline{C}_{A_P} x_{uuu} + k^*_{D_{nuc}} x_{uA_1u}$

becomes

$dx_{A_{bound}} / dt = -2k^*_{R_{grow}} F\overline{C}_{uA_1u} x_{A_f} - 2k^*_{R_{grow}} F\overline{C}_{uA_1u} x_{A_{bound}} + 2k^*_{R_{grow}} F\overline{C}_{A_P} x_{uA_1u} - 2k^*_{R_{grow}} F\overline{C}_{stacks} x_{A_f} - 2k^*_{R_{grow}} F\overline{C}_{stacks} x_{A_{bound}}$
$- k^*_{R_{grow}} F\overline{C}_{A_P} x_{uuu} - 2k^*_{R_{grow}} F\overline{C}_{A_P} x_{uA_1u} - k^*_{R_{grow}} \overline{C}_{A_P} x_{A_{bound}} + k^*_{D_{grow}} x_{uuu} + 2k^*_{D_{grow}} x_{uA_1u} + k^*_{D_{grow}} x_{A_{bound}} + k^*_{R_{nuc}} F\overline{C}_{uuu} x_{A_f}$
$+ k^*_{R_{nuc}} F\overline{C}_{uuu} x_{A_{bound}} - k^*_{R_{nuc}} F\overline{C}_{A_P} x_{uuu} + k^*_{D_{nuc}} x_{uA_1u}$

For the relaxation matrix we get

$$\dot{x} = R \times x = \begin{bmatrix} \dot{\mathbf{x}}_{A_f} \\ \dot{\mathbf{x}}_{uuu} \\ \dot{\mathbf{x}}_{uA_1u} \\ \dot{\mathbf{x}}_{A_{bound}} \end{bmatrix} = \begin{bmatrix} a_{11} & a_{12} & a_{13} & a_{14} \\ a_{21} & a_{22} & a_{23} & a_{24} \\ a_{31} & a_{32} & a_{33} & a_{34} \\ a_{41} & a_{42} & a_{43} & a_{44} \end{bmatrix} \times \begin{bmatrix} x_{A_f} \\ x_{uuu} \\ x_{uA_1u} \\ x_{A_{bound}} \end{bmatrix}$$

$dx_{A_f} / dt = (-k_{R_{enc}} C_{P_0} / n - k_{D_{enc}}) x_{A_f}$
$- p_D k^*_{D_{grow}} x_{uuu}$
$(+ p_D k^*_{D_{nuc}} - 2 p_D k^*_{D_{grow}}) x_{uA_1u}$
$(-k_{D_{enc}} - p_D k^*_{D_{grow}}) x_{A_{bound}}$

$dx_{uuu}/dt = (+ 2k^*_{R_{grow}} F(\overline{C}_{uA_1u} + \overline{C}_{stacks}) + k^*_{R_{nuc}} F\overline{C}_{uuu}) x_{A_f}$
$((-k^*_{R_{nuc}} F + k^*_{R_{grow}}) F\overline{C}_{A_P} - k^*_{D_{grow}}) x_{uuu}$
$(+ k^*_{D_{nuc}} - 2k^*_{D_{grow}}) x_{uA_1u}$
$(+ k^*_{R_{grow}} F(\overline{C}_{A_P} + 2\overline{C}_{uA_1u} + 2\overline{C}_{stacks}) + k^*_{R_{nuc}} F\overline{C}_{uuu} - k^*_{D_{grow}}) x_{A_{bound}}$

$dx_{uA_1u} / dt = (-k^*_{R_{nuc}} F\overline{C}_{uuu} + 2k^*_{R_{grow}} F\overline{C}_{uA_1u} + 2k^*_{D_{grow}} \overline{C}_{uA_1u} qK_{nuc}) x_{A_f}$
$+ k^*_{R_{nuc}} F\overline{C}_{A_P} x_{uuu}$
$(-2k^*_{R_{grow}} F\overline{C}_{A_P} - k^*_{D_{nuc}} + 2k^*_{D_{grow}} \overline{C}_{A_f} qK_{nuc}) x_{uA_1u}$
$(-k^*_{R_{nuc}} F\overline{C}_{uuu} + 2k^*_{R_{grow}} F\overline{C}_{uA_1u}) x_{A_{bound}}$

$dx_{A_{bound}} / dt = (-2k^*_{R_{grow}} F\overline{C}_{uA_1u} - 2k^*_{R_{grow}} F\overline{C}_{stacks} + k^*_{R_{nuc}} F\overline{C}_{uuu}) x_{A_f}$
$(-k^*_{R_{grow}} F\overline{C}_{A_P} + k^*_{D_{grow}} - k^*_{R_{nuc}} FC_{A_P}) x_{uuu}$
$(+ 2k^*_{D_{grow}} + k^*_{D_{nuc}}) x_{uA_1u}$
$(+ k^*_{R_{nuc}} F\overline{C}_{uuu} - k^*_{R_{grow}} F(2\overline{C}_{uA_1u} + 2\overline{C}_{stacks} + \overline{C}_{A_P}) + k^*_{D_{grow}}) x_{A_{bound}}$

The elements of the kinetic matrix are:

$$a_{11} = (-k_{R_{enc}}C_{P_0}/n - k_{D_{enc}})$$

$$a_{12} = -p_D k^*_{D_{grow}}$$

$$a_{13} = (+p_D k^*_{D_{nuc}} - 2p_D k^*_{D_{grow}})$$

$$a_{14} = (-k_{D_{enc}} - p_D k^*_{D_{grow}})$$

$$a_{21} = (+2k^*_{R_{grow}}F(\overline{C}_{uA_1u} + \overline{C}_{stacks}) + 2k^*_{R_{nuc}}F\overline{C}_{uuu})$$

$$a_{22} = ((-k^*_{R_{nuc}}F + k^*_{R_{grow}})F\overline{C}_{A_P} - k^*_{D_{grow}})$$

$$a_{23} = (+k^*_{D_{nuc}} - 2k^*_{D_{grow}})$$

$$a_{24} = (+k^*_{R_{grow}}F(\overline{C}_{A_P} + 2\overline{C}_{uA_1u} + 2\overline{C}_{stacks}) + k^*_{R_{nuc}}F\overline{C}_{uuu} - k^*_{D_{grow}})$$

$$a_{31} = (-k^*_{R_{nuc}}F\overline{C}_{uuu} + 2k^*_{R_{grow}}F\overline{C}_{uA_1u} + 2k^*_{D_{grow}}\overline{C}_{uA_1u}qK_{nuc})$$

$$a_{32} = +k^*_{R_{nuc}}F\overline{C}_{A_P}$$

$$a_{33} = (-2k^*_{R_{grow}}F\overline{C}_{A_P} - k^*_{D_{nuc}} + 2k^*_{D_{grow}}\overline{C}_f qK_{nuc})$$

$$a_{34} = (-k^*_{R_{nuc}}F\overline{C}_{uuu} + 2k^*_{Rgrow}F\overline{C}_{uA_1u})$$

$$a_{41} = (-2k^*_{R_{grow}}F\overline{C}_{uA_1u} - 2k^*_{R_{grow}}F\overline{C}_{stacks} + k^*_{R_{nuc}}F\overline{C}_{uuu})$$

$$a_{42} = (-k^*_{R_{grow}}F\overline{C}_{A_P} + k^*_{D_{grow}} - k^*_{R_{nuc}}F\overline{C}_{A_P})$$

$$a_{43} = (+2k^*_{D_{grow}} + k^*_{D_{nuc}})$$

$$a_{44} = (-k^*_{R_{grow}}F(2\overline{C}_{uA_1u} + 2\overline{C}_{stacks} - \overline{C}_{A_P}) + k^*_{D_{grow}})$$