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ZEROS AND FACTORS OF POLYNOMIALS WITH POSITIVE COEFFICIENTS AND PROTEIN-LIGAND BINDING

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1. Introduction. Many physiological processes involve the interaction of ligands with biological macromolecules particularly proteins. The regulation of the actions of enzymes, hormones and ions is an important example as is the transport of oxygen by hemoglobin in the blood. A protein macromolecule will have a number of sites at which the ligand can become bound and interact with the protein. This general process is knows as protein-ligand binding. The experimental study of this process consists in determining by titration the amount of ligand which is bound per mole of protein as a function of ligand activity such as oxygen pressure. This results in a so-called binding function which can be represented as a binding curve. Characteristics of the interaction process for various proteins and ligands can then be described in terms of the properties of the binding functions and curves.

Many mathematical models have been developed to describe and interpret this binding process. Models are based on the kinetics of conformational changes in the molecule during its interaction with the ligand and on the symmetries and structure of the subunits of the molecule [7]. A fundamental tool in this analysis is the binding polynomial introduced by Wyman [11]. If the molecule has n binding sites (which generally is four in the case of hemoglobin), then the binding polynomial has degree n and represents a distribution function of the $n + 1$ possible species of the molecule having from zero to n sites bound. The coefficients of the polynomial can be determined from observed equilibrium data and are the overall equilibrium constants for the chemical reaction which fills j binding sites. If a_x represents ligand activity, then the binding polynomial can be written in the form $P(a_x) = 1 + \beta_1 a_x + \beta_2 a_x^2 + \cdots + \beta_n a_x^n$ where $\beta_j > 0$. The binding polynomial can also be written using stepwise equilibrium constants K_j for the stepwise reaction from $j - 1$ to j sites bound. In this form the usual representation is $P(a_x) = 1 + K_1 a_x + K_1 K_2 a_x^2 + \cdots + (K_1 K_2 \cdots K_n) a_x^n$. The relation between the binding polynomial and the saturation function is given by

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$$y = a_x P'(a_x)/nP(a_x). \tag{1}$$

The numerator represents the number of sites which are bound and the denominator the total number of sites so that y gives the fraction of sites bound or saturated as a function of a_x. This value is zero when $a_x = 0$ and is asymptotic to one as a_x increases without bound. Two basic curve shapes are shown in Figure 1. Curves which have a sigmoidal shape as in (a) indicate an enhancement in filling subsequent sites after some sites are filled initially. This phenomenon is termed "positive cooperativity" and is a characteristic shown generally in hemoglobin-oxygen binding. Curves which have a hyperbolic shape as in (b) often indicate an inhibition in filling subsequent sites and this is termed "negative or anti-cooperativity".

The development of measures of cooperativity has been an important part of the analysis of binding curves. Hill [5] introduced a particularly useful measure generally know as the Hill slope which is the slope of the

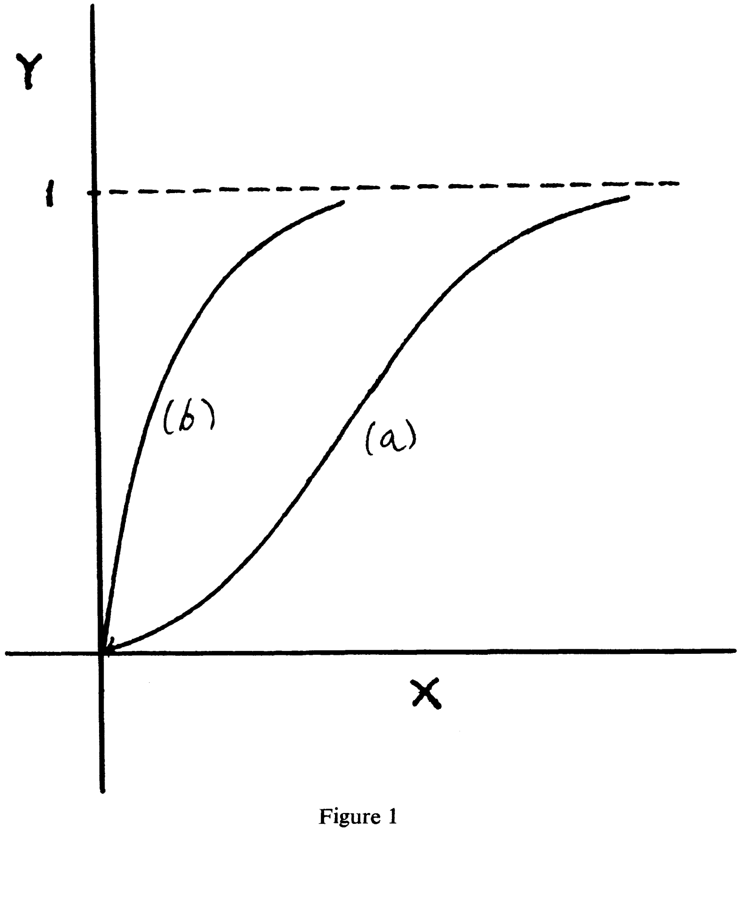

Figure 1

graph of $\log(y/(1-y))$ versus $\log a_x$. If a molecule consists of n identical but independent sites each with binding constant K, then its binding polynomial is $P(a_x) = (1 + Ka_x)^n$. The Hill slope is one for all values of a_x and the binding is statistically neutral with no site interaction. Thus the Hill slope can be used to indicate positive cooperativity when it is greater than one and negative cooperativity when it is less than one. It can be shown [2] that if the zeros of $P(a_x)$ are all real but not all equal, then the Hill slope is everywhere less than one. Therefore it is necessary for $P(a_x)$ to have non-real zeros for positive cooperativity to occur. The maximum possible value of the Hill slope is n and this occurs only in the theoretical case of all sites maximally linked when no intermediate species, having between zero and n sites bound, appear. The binding polynomial for this case is $1 + \beta_n a_x^n$. A change of variable of the form $a_x = cx$ will not alter these basic concepts and henceforth binding polynomials will be considered in the form $P(x)$, a function of x.

A hypothesis to explain cooperativity can be formulated in terms of linkages between two or more sites which produce conformational changes in the molecule when one site is filled which then enhance or inhibit filling other sites. If a binding polynomial of degree n can be factored into polynomials $P_1(x)$ and $P_2(x)$ of degrees n_1 and n_2, then a simple calculation using (1) shows that

$$y = \frac{1}{n}(n_1 y_1 + n_2 y_2) \tag{2}$$

where y_1 and y_2 are the saturation functions of $P_1(x)$ and $P_2(x)$. Thus it is natural to relate site linkages to the factorization of binding polynomials into polynomials with positive coefficients. The latter requirement arises from the need to interpret each factor as a binding polynomial for a subset of the binding sites. This hypothesis has been discussed extensively in the literature ([1], [2], [10]) and the purpose of this paper is to present some results about the zeros of polynomials with positive coefficients and the factorization of such polynomials into polynomials of the same type and to interpret irreducible positive factorizations in terms of site linkages.

2. Preliminary Results.

DEFINITION. A positive polynomial is a real polynomial whose leading and constant coefficients are positive and whose remaining coefficients are non-negative.

DEFINITION. A positive factorization of a polynomial is a non-trivial factorization in which each factor is a positive polynomial.

DEFINITION. A p-irreducible polynomial is a positive polynomial which does not admit a positive factorization.

The following assertions either are obvious or are immediate consequences of standard results.

i) A positive polynomial cannot have a positive real zero.

ii) A positive polynomial of odd degree must have a negative real zero.

iii) A positive polynomial cannot have a factor of the form $x^2 - bx - c$ with b and c positive.

iv) A positive polynomial of even degree cannot have all of its zeros with positive real parts.

v) The product of two or more quadratic factors of the form $x^2 - bx + c$ with b and c positive cannot be a positive polynomial.

An important result of Cowling and Thron [3] obtained as a special case of a result of Obreschkoff [9] is

THEOREM A. *A positive polynomial of degree n does not vanish for any value z with* $|\arg z| < \pi/n$ *with the exception of polynomials of the form* $ax^n + b$ *in which case there are two zeros with* $|\arg z| = \pi/n$.

3. Hurwtiz polynomials. An important class of positive polynomials consists of those whose zeros all lie in the half-plane $\operatorname{Re} z < 0$. Such a polynomial is called a Hurwitz or stable polynomial, the latter term arising from the property of stability in the solutions of linear differential equations describing physical systems. Routh in 1875 and Hurwitz in 1895 developed algorithms for determining the number of zeros of a polynomial in the right and left half-planes of the complex plane. The Routh-Hurwitz theorem provides a criterions for stability arising from the requirement that the number of zeros in the right half-plane be zero [9]. While all positive polynomials of degree two are Hurwitzian, the same is not true for polynomials of higher degree since for example

$$x^3 + x^2 + 2x + 8 = (x + 2)(x^2 - x + 4).$$

Binding polynomials which are Hurwitzian are significant because they can be factored uniquely into positive linear and p-irreducible quadratic factors. Acording to the hypothesis then, the protein will have a number of independent sites corresponding to the linear factors and will have the remaining sites linked in pairs corresponding to the p-irreducible quadratic factors. The algebraic problem reduces therefore to that of determining the factorization of non-Hurwitzian positive polynomials into p-irreducible polynomials. Of particular interest are binding polynomials which are p-irreducible since this implies that all sites are linked. As noted previously maximum cooperativity occurs for $1 + x^n$ which is p-irreducible so that there is a natural relationship between p-irreducibility and strong positive cooperativity.

4. (n = 3). Consider the positive cubic $P(x) = x^3 + a_1x^2 + a_2x + a_3$.

By the Routh-Hurwitz criterion, $P(x)$ will be stable if and only if $a_1a_2 > a_3$. In this case $P(x)$ can be factored either into three linear factors or a linear factor and a p-irreducible quadratic factor. If $a_1a_2 = a_3$, then $P(x) = (x + a_1)(x^2 + a_2)$ even though it is not stable.

Now assume the zeros of $P(x)$ to be $-r$, $\alpha \pm \beta i$ with $r > 0$ and $\alpha > 0$ so that $P(x) = (x + r)(x^2 - 2\alpha x + \alpha^2 + \beta^2)$. Since $P(x)$ is a positive polynomial, we have $r - 2\alpha \geqq 0$ and $\alpha^2 + \beta^2 - 2r\alpha \geqq 0$ or

(3) $$0 < \alpha \leqq r/2 \text{ and } (\alpha - r)^2 + \beta^2 \geqq r^2.$$

Thus we have

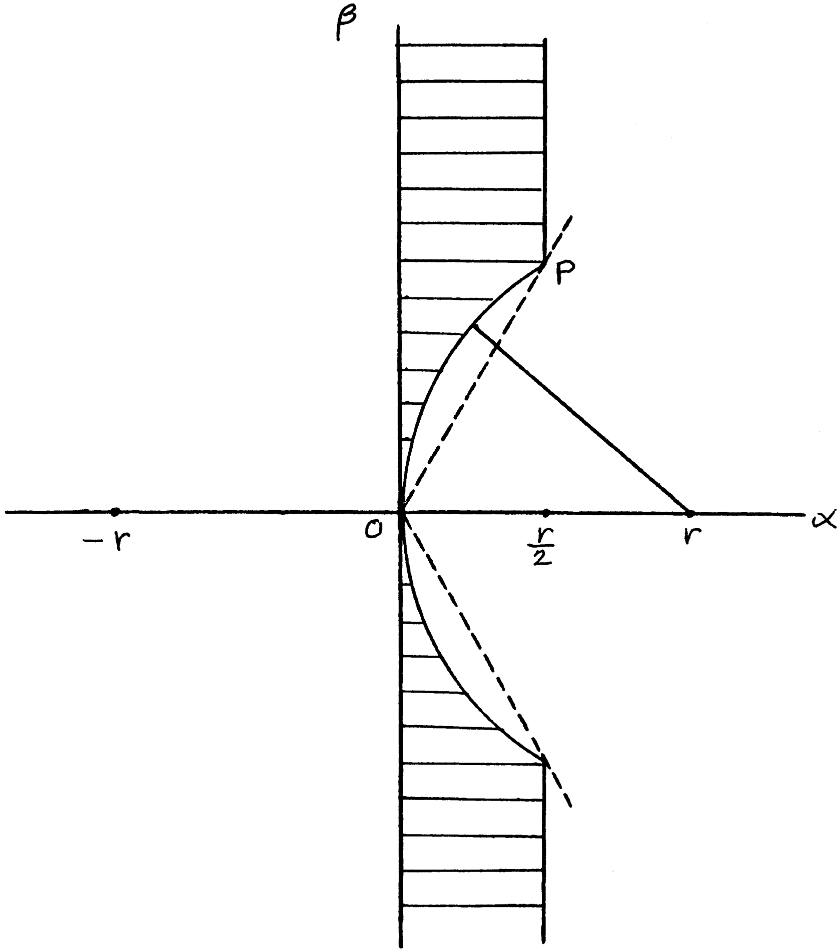

Figure 2

THEOREM 1. *A positive cubic is p-irreducible if and only if its zeros satisfy* (3).

The region of the complex plane defined by (3) is shown in Figure 2. The inclination of the line OP is $\pi/3$ in accord with Theorem A. Such polynomials represent positive cooperativity in proteins with three positively-linked sites.

5. (n = 4). Consider the positive quartic $P(x) = x^4 + a_1x^3 + a_2x^2 + a_3x + a_4$. By the Routh-Hurwitz criterion, $P(x)$ will be stable if and only if $a_1a_2a_3 > a_1^2a_4 + a_3^2$. In this case $P(x)$ has a unique positive factorization

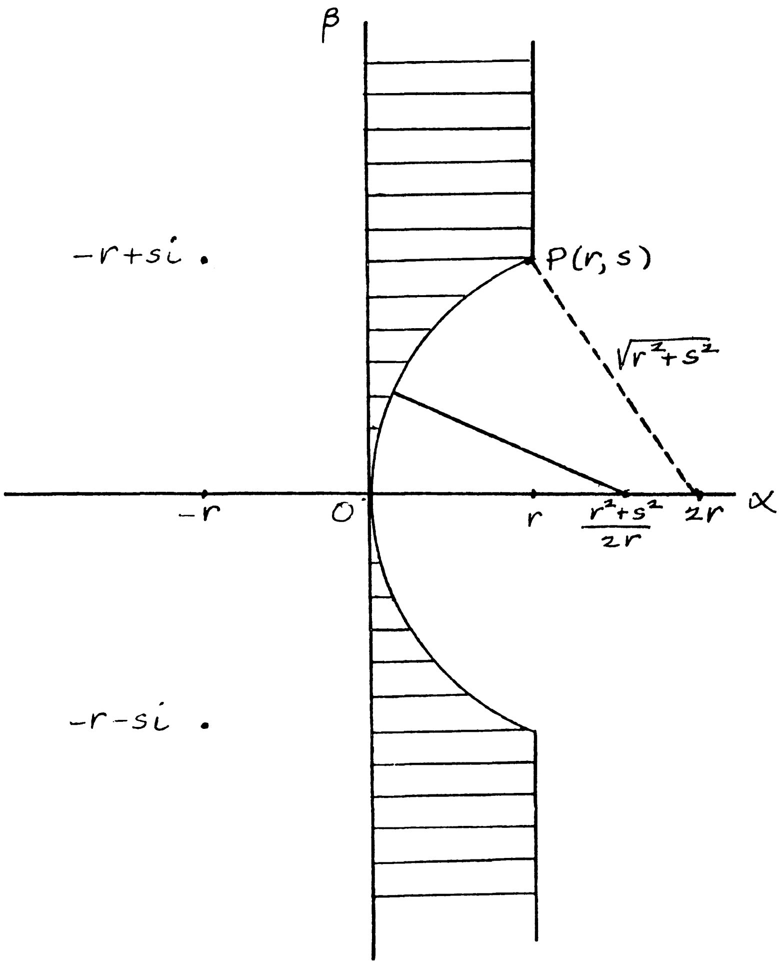

Figure 3

into linear and p-irreducible quadratic factors. If equality holds in this expression, then $P(x)$ has a factor of the form $x^2 + a$ and the same conclusion follows even though $P(x)$ is not stable.

Now assume that $P(x)$ is not stable and has two pairs of non-real zeros $-r \pm si$ and $\alpha \pm \beta i$ with $r > 0$ and $\alpha > 0$ so that $P(x) = (x^2 - 2rx +$

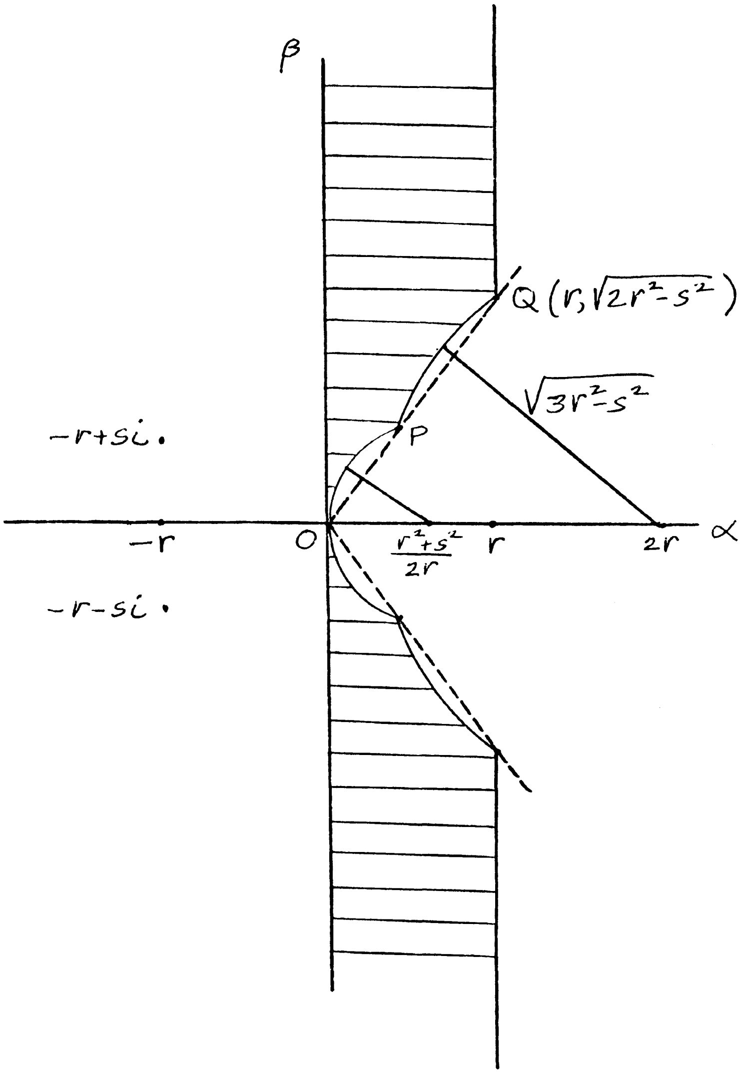

Figure 4

$r^2 + s^2)(x^2 + 2\alpha x + \alpha^2 + \beta^2)$. Since $P(x)$ is a positive polynomial, we must simultaneously have

$$(4)\qquad \begin{gathered} 0 < \alpha \leqq r \\ (\alpha - 2r)^2 + \beta^2 \geqq 3r^2 - s^2 \\ \left(\alpha - \frac{r^2 + s^2}{2r}\right)^2 + \beta^2 \geqq \left(\frac{r^2 + s^2}{2r}\right)^2. \end{gathered}$$

We now have

THEOREM 2. *A positive quartic with no real zeros is p-irreducible if and only if its zeros satisfy* (4).

If $r \leqq s$, then the second inequality of (4) is redundant since $3r^2 - s^2 \leqq r^2 + s^2$ and the region for this case is shown in Figure 3. The region for $r > s$ is shown in Figure 4. Such polynomials represent positive cooperativity in proteins having four positively-linked sites.

In Figure 4, 0, P and Q are collinear and the inclination of OPQ is $\tan^{-1}(\sqrt{2r^2 - s^2}/r)$. The inclination of OP in Figure 3 is $\tan^{-1}(s/r)$. Each has a minimum of $\pi/4$ when $r = s$ in accord with Theorem A.

Now assume that $P(x)$ has two real zeros $-r_1$ and $-r_2$ and two non-real zeros $\alpha \pm \beta i$ with $r_1 > 0$, $r_2 > 0$, $\alpha > 0$ so that $P(x) = (x + r_1)(x + r_2)(x^2 - 2\alpha x + \alpha^2 + \beta^2)$. Since $P(x)$ is a positive polynomial, we must have simultaneously

$$(5)\qquad \begin{gathered} 0 < \alpha \leqq \frac{1}{2}(r_1 + r_2) \\ [\alpha - (r_1 + r_2)]^2 + \beta^2 \geqq r_1^2 + r_1 r_2 + r_2^2 \\ [\alpha - r_1 r_2/(r_1 + r_2)]^2 + \beta^2 \geqq [r_1 r_2/(r_1 + r_2)]^2. \end{gathered}$$

It is possible however for either or both linear factors to combine with the quadratic factor to produce a positive cubic if (3) is satisfied. Thus $P(x)$ can be p-irreducible or can have one or two positive factorizations. Such polynomials represent positive cooperativity in proteins having four sites positively linked or having one independent site and three sites positively linked in either one or two combinations. Thus we have

THEOREM 3. *A positive quartic with two real and two non-real zeros is p-irreducible if and only if its zeros satisfy* (5) *but do not satisfy* (3) *for either real zero.*

The shaded regions in Figure 5 show the locations of the non-real zeros which give a $P(x)$ which is p-irreducible. The regions marked I and II show locations which give a $P(x)$ with one or two different p-irreducible factorizations.

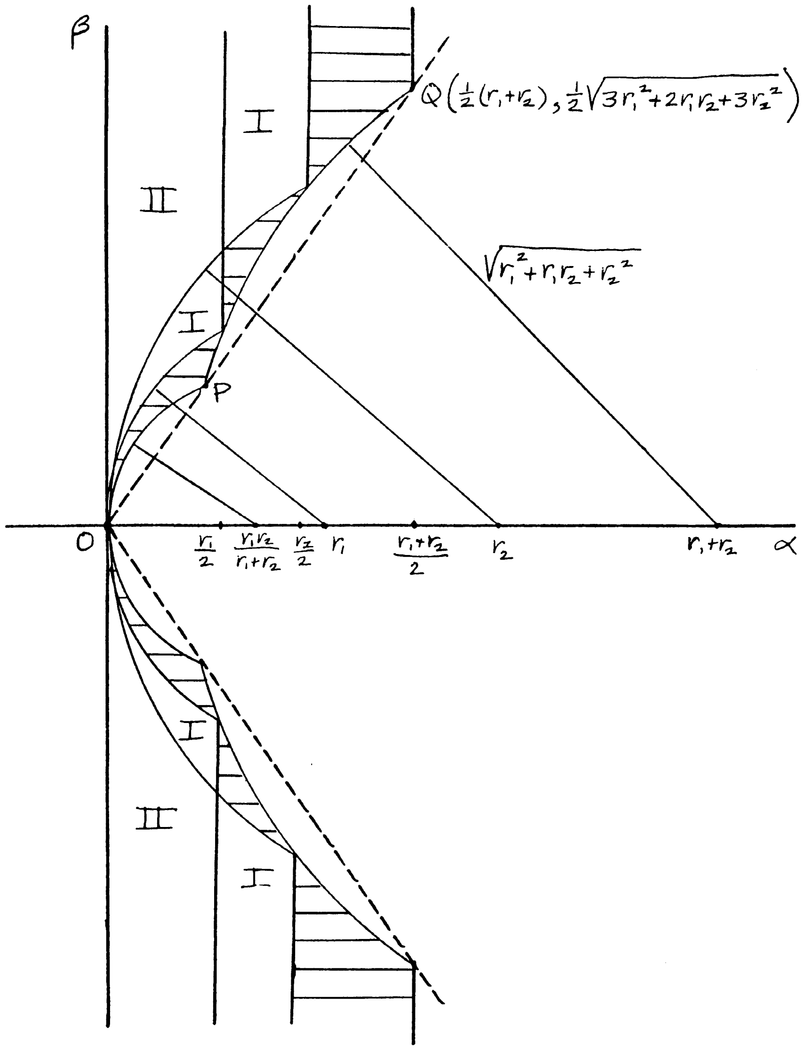

Figure 5

Once again O, P, and Q are collinear and the inclination of OPQ is $\tan^{-1}[(3r_1^2 + 2r_1r_2 + 3r_2^2)^{1/2}/(r_1 + r_2)]$. This quotient has a minimum of $\sqrt{2}$ when $r_1 = r_2$ and we have the

COROLLARY. *A positive quartic with real zeros does not vanish in* $|\arg z| < \tan^{-1} \sqrt{2}$.

6. Applications. The results of the previous section can be applied to experimental data obtained by Imai [6] for stripped native human hemoglobin. The values obtained for overall equilibrium constants are $\beta_1 = .456$, $\beta_2 = .113$, $\beta_3 = .088$, $\beta_4 = .089$. The zeros of the binding polynomial with these coefficients are $.95 \pm 1.169i$ and $-1.44 \pm .96i$ so that $P(x)$ is both non-Hurwitzian and p-irreducible. Thus all four sites are linked and positive cooperativity occurs everywhere. Maximum Hill slope is 2.51.

Another application can be made to the MWC model [8] which has been used extensively for molecules having certain structural properties

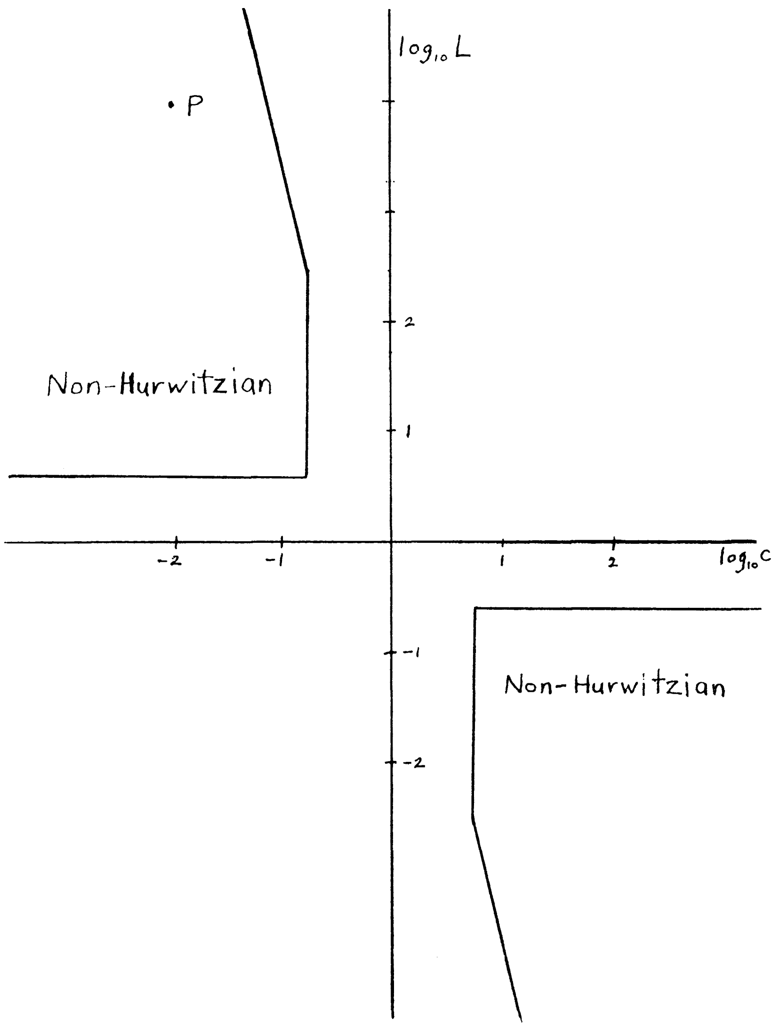

Figure 6

including hemoglobin. With the assumptions made in this model, the binding polynomial can be written as $N(x) = L(1 + cx)^n + (1 + x)^n$ where L and c are certain parameters. If $L = t^n$, then $N(x) = 0$ is equivalent to

$$\text{(6)} \qquad \frac{t(1 + cx)}{1 + x} = \omega \quad \text{or} \quad x = \frac{\omega - t}{-\omega + ct}$$

where $\omega^n = -1$. Thus the zeros of $N(x)$ are found by evaluating (6) for the n-th roots of -1. Consider the bilinear mapping

$$\text{(7)} \qquad w = \frac{z - t}{-z + ct}, \qquad t(c - 1) \neq 0$$

which is one-to-one and maps the unit circle onto another circle with center on the real axis. $N(x)$ will be Hurwitzian if the n-th roots of -1 are all mapped into the left half-plane. The zeros of $N(x)$ are all non-real except for a single real zero if n is odd. Thus the molecule has at most one independent binding site and the remainder form some pattern of linkages. For $n = 4$, $N(x)$ is Hurwitzian for all values of L if $3 - 2\sqrt{2} < c < 3 + 2\sqrt{2}$ and for values of $L^{1/4}$ not lying between $\sqrt{2}(c+1 \pm \sqrt{c^2 - 6c + 1})/4c$ otherwise. Approximations to these bounds for L are 4 and $1/(4c^4)$ if c is small and $4/c^4$ and $1/4$ if c is large. These regions are shown in Figure 6. If $N(x)$ is Hurwitzian, then there are two pairs of linked sites. If it is not, then it is p-irredicible and all four sites are linked. Also shown in Figure 6 is point P corresponding to $c = .01$ and $L = 10{,}000$ which are approximate values to those used in the MWC model for hemoglobin. P lies in a non-Hurwitzian region which is an indication of positive cooperativity.

7. Concluding remarks. The preceding results can be applied to any binding polynomial whose degree does not exceed four and this covers a large variety of classes of proteins including hemoglobin. This approach can be applied to polynomials of higher degrees by associating a pattern of positively linked sites with a factorization of the binding polynomial into p-irreducible factors. One generalization will be presented here which also has mathematical interest. Consider a binding polynomial $P(x)$ with k real zeros and a single pair of non-real zeros. The usual interpretation of site linkages applies if $P(x)$ is Hurwitzian. If $P(x)$ is non-Hurwitzian, then any number of sites from 3 to $k + 2$ may be positively linked with the latter occurring when $P(x)$ is p-irreducible. Assume therefore that $P(x)$ has zeros $-r_1, -r_2, \ldots, -r_k, \alpha \pm \beta i$ with $r_i > 0$ and $\alpha > 0$. Since $P(x)$ is a positive polynomial, we must have simultaneously

$$(8)\qquad \begin{gathered} 0 < \alpha \leqq \frac{1}{2}\sigma_1 \\ \left(\alpha - \frac{\sigma_i}{\sigma_{i-1}}\right)^2 + \beta^2 \geqq \left(\frac{\sigma_i}{\sigma_{i-1}}\right)^2 - \frac{\sigma_{i+1}}{\sigma_{i-1}}, \qquad i = 1, 2, \ldots, k \end{gathered}$$

where σ_i is the i-th elementary symmetric function of $r_1, \ldots, r_k$, $\sigma_0 = 1$ and $\sigma_{k+1} = 0$. As before we expect the boundary to consist of a sequence of circular arcs from the origin to a vertical line but it is necessary to employ a number of properties of the elementary symmetric functions before reaching this conclusion.

Let $C_1, C_2, \ldots, C_k$ denote the respective circles in the $\alpha\beta$-plane defined by (8) using equal signs. Let $(q_i, 0)$ denote the center and ρ_i the radius of C_i. It is well known that $\sigma_i^2 > \sigma_{i-1}\sigma_{i+1}$ so that

$$(9)\qquad q_k < q_{k-1} < \cdots < q_2 < q_1, \text{ and } \rho_i^2 > 0.$$

We show first of all that C_1 intersects $\alpha = (1/2)\sigma_1$. The center of C_1 is $q_1 = \sigma_1$ so that the condition for intersection is $\rho_1 > q_1 - (1/2)\sigma_1 = (1/2)\sigma_1$ or $\sigma_1^2 - \sigma_2 > (1/4)\sigma_1^2$ or $\sigma_1^2 > 4/3\ \sigma_2$. But

$$\sigma_1^2 = \sum_1^k r_i^2 + 2\sigma_2 > 2\sigma_2 > \frac{4}{3}\sigma_2$$

so that C_1 does intersect $\alpha = (1/2)\sigma_1$.

The points of intersection (α_i, β_i) of C_i and C_{i+1} for $i = 1, 2, \ldots, k-1$ are given by

$$(10)\qquad \begin{gathered} \alpha_i = \frac{\sigma_i\sigma_{i+1} - \sigma_{i-1}\sigma_{i+2}}{2(\sigma_i^2 - \sigma_{i-1}\sigma_{i+1})} \\ \beta_i^2 = \frac{3(\sigma_{i-1}\sigma_{1+2} + \sigma_i\sigma_{i+1})^2 - 4(\sigma_{i-1}\sigma_{i+1}^3 + \sigma_i^3\sigma_{i+2} + \sigma_{i-1}^2\sigma_{i+2}^2)}{(\sigma_i^2 - \sigma_{i-1}\sigma_{i+1})^2} \end{gathered}$$

so that there will be real points of intersection if the numerator of β_i^2 is non-negative. We will prove the

LEMMA. *If $r_1, r_2, \ldots, r_k$ are positive real numbers and $\sigma_1, \sigma_2, \ldots, \sigma_k$ are their elementary symmetric functions, then the numerator of β_i^2 in* (8) *is non-negative.*

PROOF. A theorem of Malo and I. Schur [9] says that if the zeros of the polynomials $a_0 + a_1x + \cdots + a_nx^n$ and $b_0 + b_1x + \cdots + b_nx^n$ are all real and if those of the latter are all of the same sign, then the zeros of $a_0b_0 + a_1b_1x + \cdots + a_nb_nx^n$ are all real. The theorem applies to the polynomials

$$\prod_{1}^{k} (r_i + x) = \sigma_k + \sigma_{k-1}\, x + \cdots + x^k$$

$$(1 + x)^k = 1 + \binom{k}{k-1} x + \cdots + x^k$$

so that

$$\sigma_k + \binom{k}{k-1} \sigma_{k-1} x + \cdots + \binom{k}{k-i} \sigma_{k-1} x^i + \cdots + x^k$$

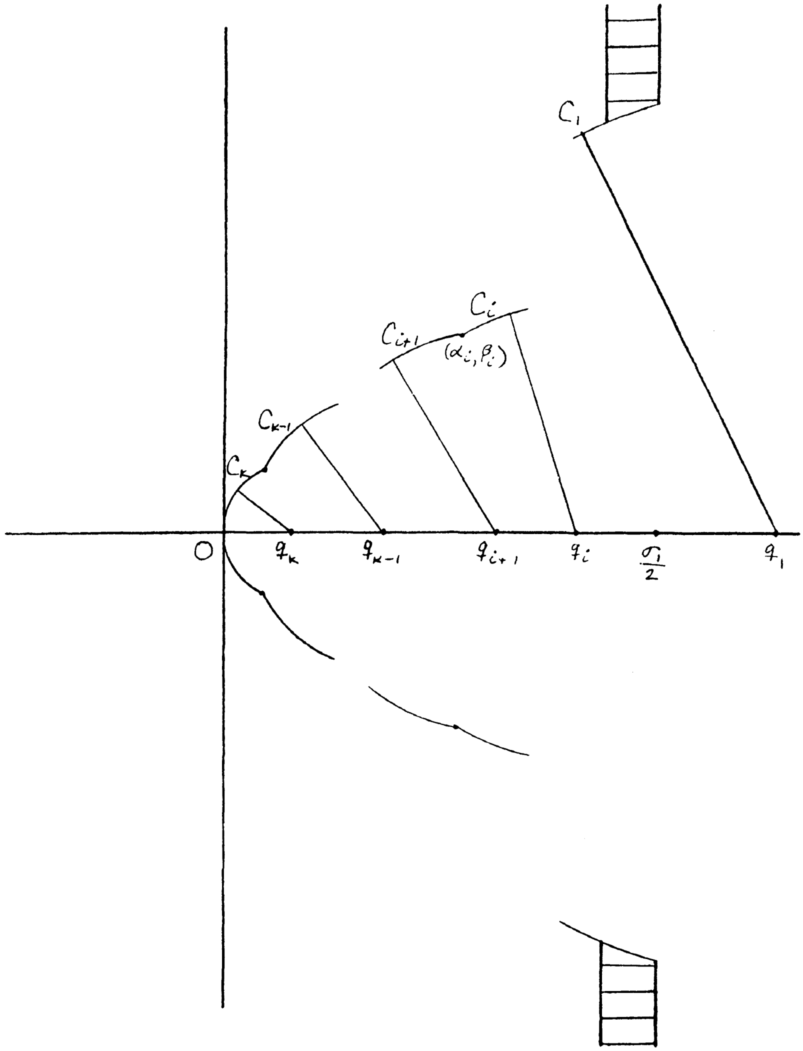

Figure 7

has k real zeros. If a homogeneous polynomial of degree k in x and y is formed form this polynomial, then any of its partial derivatives will have all real zeros x/y by repeated applications of Rolle's Theorem [**4**]. The various partial derivatives of order $k - 3$ are $\sigma_{i-1}x^3 + 3\sigma_i x^2 y + 3\sigma_{i+1}xy^2 + \sigma_{i+2}y^3$ which must have three real zeros. Therefore each discriminant must be non-negative which gives the desired result. (Applying this method to the first polynomial will give the same result for $\bar{\sigma}_i = \sigma_i/\binom{k}{i}$ instead of σ_i.)

Thus C_i and C_{i+1} have real points of intersection and the intersection of the regions defined by (8) is bounded by a sequence of circular arcs extending from the origin to $\alpha = (1/2)\sigma_1$ as shown in Figure 7.

If $\alpha \pm \beta i$ lie outside C_1 and $(1/2)(\sigma_1 - r_1) < \alpha \leqq (1/2)\sigma_1$ where r_1 is the smallest r_i, then $P(x)$ is p-irreducible and we have

THEOREM 4. *There are p-irreducible polynomials having any set of negative real numbers among its zeros.*

There are several unanswered question about the details of the boundary including whether the sequences $\{\alpha_i\}$ and $\{\rho_i\}$ are monotonic. The former is a monotonic sequence if

$$\sigma_{i+1}^3 + \sigma_i^2\sigma_{i+3} + \sigma_{i-1}\sigma_{i+2}^2 > \sigma_{i+1}(2\sigma_i\sigma_{i+2} + \sigma_{i-1}\sigma_{i+3})$$

and the latter if

$$\sigma_i^2(\sigma_i^2 - \sigma_{i-1}\sigma_{i+1}) > \sigma_{i-1}^2(\sigma_{i+1}^2 - \sigma_i\sigma_{i+2}).$$

It is easy to conjecture that both are true in general since they hold in the special case $r_i = 1$, $i = 1, 2, \ldots, k$ in which $\sigma_i = \binom{k}{i}$.

It is also of interest to examine the size of the angular sector of the zero-free region of the complex plane in this special case. Let M_i be the square of the slope of the line through the origin and the point of intersection of C_i and C_{i+1}. From (10) we obtain

$$M_i = \frac{\beta_i^2}{\alpha_i^2} = \frac{k+2}{(i+1)(k-i)}, \quad i = 1, 2, \ldots, k-1$$

and it is evident that the points of intersection are no longer collinear for $k > 2$. The size of the sector will be determined by the minimum of the M_i which is $4/k$ for k even and $4(k+2)/(k+1)^2$ for k odd. It is also easy to conjecture that the same result holds regardless of the values of the k real zeros.

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