Adhesion-induced DNA naturation
Allahverdyan, A.E.; Gevorkian, Z.S.; Hu, C.-K.; Nieuwenhuizen, T.M.

Published in:
Physical Review Letters

DOI:
10.1103/PhysRevLett.96.098302

Citation for published version (APA):
Allahverdyan, A., Gevorkian, Z. S., Hu, C-K., & Nieuwenhuizen, T. M. (2006). Adhesion-induced DNA naturation. Physical Review Letters, 96(9), [098302]. DOI: 10.1103/PhysRevLett.96.098302

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Adhesion-Induced DNA Naturation

A.E. Allahverdyan,1,2 Zh. S. Gevorkian,1,3,4 Chin-Kun Hu,4 and Th. M. Nieuwenhuizen2

1Yerevan Physics Institute, Alikhanian Brothers Street 2, Yerevan 375036, Armenia
2Institute for Theoretical Physics, Valckenierstraat 65, 1018 XE Amsterdam, The Netherlands
3Institute of Radiophysics and Electronics, Ashtarak-2, 378410, Armenia
4Institute of Physics, Academia of Sinica, Nankang, Taipei 11529, Taiwan

(Received 31 July 2005; published 9 March 2006)

DNA adsorption and naturation is modeled via two interacting flexible homopolymers coupled to a solid surface. DNA denatures if the entropy gain for unbinding the two strands overcomes the loss of binding energy. When adsorbed to a surface, the entropy gain is smaller than in the bulk, leading to a stronger binding and, upon neglecting self-avoidance, absence of a denatured phase. Now consider conditions where the binding potentials are too weak for naturation, and the surface potential too weak to adsorb single strands. In a variational approach it is shown that their combined action may lead to a naturated adsorbed phase. Conditions for the absence of naturation and adsorption are derived too. The phase diagram is constructed qualitatively.

DOI: 10.1103/PhysRevLett.96.098302

PACS numbers: 82.35.Gh, 05.90.+m, 82.39.Pj

The structure of biopolymers is the key for understanding their biological functioning [1,2]. A known component of this structure is the double strand—two polymers lined up by hydrogen bonds—realized in DNA and responsible for stability of the genetic information.

We shall study the adsorption and surface (adhesion) induced naturation of a double-strand polymer with resolved motion of the separate strands. The adsorption of DNA onto surfaces is of interest for a number of applications such as design of substrates as carriers of genetically engineered vaccines, surface patterning, histone-DNA interactions, and electrophoresis [3]. DNA adsorption can be realized by different mechanisms [3]: covalent bonding, electrostatic attraction to a positively charged surface [4], hydrogen bonding, and hydrophobic forces.

The two strands are tightly connected to each other, the double strand is described via a single semiflexible chain [5–7]. Indeed, for the double-stranded DNA the corresponding persistence length \( l_p \) is about 100 nm [8], or 300 base pairs. However, this large value of \( l_p \) is more a result of “construction rigidity,” because for a single DNA strand \( l_p \) is much smaller, about 1 nm (2–3 base pairs) [9], while the length of the strand is 10–100 \( \mu \)m. The single DNA strand can thus be modeled as a flexible chain [5]. Adsorption of a (semi)flexible chain is now a classical problem in polymer physics [2,5,6].

If the motion of the single strands is resolved, i.e., when the interstrand hydrogen bonds are relatively weak, the polymer becomes a complex system with different, mutually balancing features. A realistic model of DNA should include stacking energy between two base pairs, the helical structure, self-avoidance, heterogeneity, etc. Such models do not seem to exist; there are, however, various models with different degrees of sophistication capturing at least some aspects of the problem [2,5–7,10–12].

Our model deals only with most basic features of the problem. We consider two flexible Gaussian chains interacting with each other and with a plane solid surface. Though neglecting some features of DNA (see below), our model predicts two mechanisms of adhesion-induced naturation. This model without surface was mentioned in [5] and studied in [10] in the context of DNA denaturation (melting); see also [1,2,11,12] for recent results.

Consider \( 2N \) coupled monomers with radius vectors \( \vec{r}_{1|k} \) and \( \vec{r}_{2|k} \) (\( k = 1, \ldots, N \)) and potential energy

\[
\sum_{k=1}^{N} \left[ U(\vec{r}_k) + \frac{2}{\alpha} \left( \vec{r}_a|k| - \vec{r}_a|k\!-\!1 \right)^2 + V(\vec{r}_a|k) \right],
\]

where \( \vec{r}_k = \vec{r}_{1|k} - \vec{r}_{2|k} \), \( U \) is the interstrand potential and \( V \) is the surface-monomer potential. The harmonic interaction with stiffness \( l \) is responsible for the linear structure of the polymers. The system embedded in an equilibrium thermal bath at temperature \( T = 1/\beta \) is a free energy of a single flexible chain (without self-interactions) is determined from an effective Schrödinger equation [2,5]. The considered two-strand situation is a direct generalization of this, with the two-particle equation being

\[
H = \sum_{\alpha = 1}^{2} \left[ -\frac{1}{2} \nabla_\alpha^2 + V(z_\alpha) \right] + U(r), \quad \partial_{r_{1\alpha}} = \partial_{r_{2\alpha}}. \quad (1)
\]

Here \( V = l \beta^2 V \), \( U = l \beta^2 U \), while \( \vec{r}_{1\alpha} = (x_{1\alpha}, y_{1\alpha}, z_{1\alpha}) \) are the position vectors of two quantum particles representing the strands and \( \vec{r} = \vec{r}_1 - \vec{r}_2 \) is the mutual position. We shall extensively use this quantum language. If there is a gap between the lowest two eigenvalues of \( H \), the ground-state wave function \( \Psi \) determines the monomer density as

\[
(n(\vec{r}_1, \vec{r}_2) = \Psi^2(\vec{r}_1, \vec{r}_2)).
\]

The eigenvalue \( E \) is the energy of the quantum pair, related to the free energy \( F \) of the polymer as \( E = l \beta^2 F/(2N) \).
The surface is described by an infinite potential wall,
\[ \Psi(r_1, r_2) = 0 \quad \text{if} \quad z_1 \leq 0 \quad \text{or} \quad z_2 \leq 0. \quad (2) \]

Both \( V(z) \) and \( U(r) \) are attractive, \( V \leq 0, U \leq 0 \), and short-ranged: \( \int_0^\infty dz V(z) \) and \( \int_0^\infty dr^2 U(r) \) are finite. When \( U = 0 \), the Hamiltonian \( H \) reduces to two uncoupled strands (or one-dimensional particles), each in the potential \( V(z) \). If \( V(z) \) is shallow enough, no bound (negative energy) state exists, while the second-order binding transition corresponds to adsorption of a single flexible polymer [2]. The physical order-parameter for this transition is \( 1/(z^2) \), which is finite (zero) in the adsorbed (desorbed) state. Denote by \( \mu \) the dimensionless coupling constant of \( V = \mu \tilde{V} \) such that (for \( U = 0 \)) the adsorption threshold is \( \mu_c = 1 \). Note that the adsorption of a single strand DNA is a part of the renaturaton via hybridization [1], a known method of genetic systematics.

Analogously, switching off both \( V(z) \) and the wall, we shall get a three-dimensional central-symmetric motion in the potential \( U(r) \) which again is not bound if \( U \) is shallow. This second-order unbinding transition with the order parameter \( 1/(r^2) \) corresponds to thermal denaturation (strand separation) of the double-strand polymer [1,10]. Writing likewise \( U = \lambda \tilde{U} \), \( \lambda \) is the dimensionless naturation strength. We take the naturation threshold in the bulk to be \( \lambda_c = 1 \). When the wall is included, i.e., (2) is imposed, the strands loose in the adsorbed phase part of the entropy needed to denature.

As seen from Eq. (1), in the \((x, y)\) plane \( H \) is both rotationally and translationally symmetric. Thus, for the low energy physics the only relevant variable coming from that plane is \( \rho = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2} \). The relevant wave function is thus \( \psi(z_1, z_2, \rho) \) and the reduced Hamiltonian \( H_\rho \) reads
\[ H_\rho = H_1 + H_2 - \rho^{-1} \partial_\rho \rho \partial_\rho \rho + U(\sqrt{\rho^2 + z^2}), \quad (3) \]
\[ H_{1,2} = -\frac{1}{2} \partial_{z_{1,2}}^2 + V(z_{1,2}), \quad z = z_1 - z_2. \quad (4) \]
There is no hope for an exact solution of this model, since it belongs to the class of three-body problems (the role of the third particle is being played by the surface; see also below). We shall estimate the lowest energy \( E_0 \) of \( H_\rho \) via a variational principle: \( E_0 \leq \langle \chi | H | \chi \rangle \), where \( \chi \) is a normalized trial function. Assume that \( V(z) \) (without \( U \)) has a lowest (negative) energy state with normalized wave function \( \phi(z_1, z_2) \); \( H_{1,2} \phi(z_{1,2}) = E \phi(z_{1,2}) \), with \( \phi(z_{1,2} \leq 0) = 0 \). We then make the ansatz
\[ \chi(\rho, z_1, z_2) = \omega(z_1, z_2) \xi(\rho), \quad \int_0^\infty d\rho \rho \xi^2(\rho) = 1, \quad (5) \]
where \( \omega(z_1, z_2) = \phi(z_1) \phi(z_2) \) and where \( \xi(\rho) \) is normalized. Varying \( \langle \chi | H | \chi \rangle \) with respect to \( \xi(\rho) \), we get an effective two-dimensional problem: \( -\rho^{-1} \partial_\rho (\rho \partial_\rho \xi) + U_\rho(\rho) \xi = (E - 2E \xi) \xi \), where by definition \( E \geq E_0 \), and where \( U_\rho(\rho) \) is an effective potential:
\[ U_\rho(\rho) = \int_0^\infty U(\sqrt{\rho^2 + z^2}) \frac{2}{a^n} dz_a \phi^2(z_a), \quad (6) \]
Like \( U, U_\rho \) is attractive and short ranged. It is well known that any (no matter how weak) attractive potential in two dimensions creates a bound state, though the localization length of this state is exponentially large for small energies [13]. Thus there is a normalizable function \( \xi(\rho) \) such that \( E_0 < E < 2E \xi < 0 \); i.e., there is an overall bound (double strand and localized next to the surface) state provided \( V(z) \) creates a bound state. In the present model strong enough surface potential prevents denaturation (melting) of the double strand. Upon taking into account the neglected features (helicity, self-avoidance, or stacking), the denaturation transition temperature in the adsorbed phase is enhanced but (presumably) finite.

Next to the threshold \( \mu_c = 1 \) of \( V(z) \), \( 2E \xi \equiv -\kappa^2 \) is small and the wave function is almost flat [13]: \( \phi(z) \approx \sqrt{2\kappa} e^{-\kappa z} \). Thus Eq. (6) implies that \( U_\rho = O(\kappa) \), so, naively, it predicts absence of binding at the threshold \( \kappa = 0 \) of \( V(z) \). But let us study the region where \( V(z) \) alone does not create any bound state, by employing the trial function (5), where now \( \xi(\rho) \) is a given normalized function (its form is irrelevant for us), while \( \omega(z_1, z_2) \) is found from the variational equation:
\[ [H_1 + H_2 + U_\rho(\rho)(|z_1 - z_2|) - E] \omega(z_1, z_2) = 0, \quad (7) \]
\[ U_\rho(\rho) = \int_0^\infty d\rho \rho \xi^2(\rho) U(\sqrt{\rho^2 + z^2}), \quad (8) \]
Equations (7) and (8) describe two one-dimensional particles interacting via short-range potential \( U_\rho = \lambda U_{\rho eff} \). We shall show that Eq. (7) predicts an overall binding—that is it predicts \( E < 0 \) and a localized normalizable wave function \( \omega(z_1, z_2) \)—at the threshold \( \kappa = 0 \) of the potential \( V(z) \). For a large \( \lambda \) this is expected, since two strongly coupled particles roughly behave as one with double mass in a double potential, which decreases the threshold value of \( V(z) \) by a factor of 4, i.e., \( \mu_c(\infty) = \frac{1}{4} \). Next we focus on small \( \lambda \). Though the energy \( 2E \xi \) is nearly zero, one can apply an ordinary perturbation expansion in \( \lambda \), since the suitable matrix elements of \( U_{\rho eff} \) appear to be small as well, grace to the small prefactor \( \kappa \) in the zero-order wave functions \( |00\rangle \approx \phi(z_1) \phi(z_2) \). Noting that \( \langle 00|U_{\rho eff}|00\rangle = O(\kappa) \) for \( \kappa \to 0 \) and thus negligible, we get at the second order [13]
\[ E = -\int_0^\infty d\kappa \frac{|\langle 00|U_{\rho eff}|\kappa\rangle|^2}{\epsilon_{\kappa} + \kappa^2}, \quad (9) \]
where the integration over the collective variable \( K \) involves all excited wave functions of the unperturbed two-particle system with wave vector \( K \) and energy \( \epsilon_{\kappa} \). There are three orthogonal families of these states:
\[ |0k\rangle = \phi(z_1) \xi_k(kz_2), \quad |k0\rangle = \xi_k(kz_1) \phi(z_2), \quad |k|k\rangle = \xi_k(kz_1) \xi_k(kz_2), \quad \text{where} \ \xi_k(z) \ \text{are the corresponding single-particle excited (continuous spectrum) wave func-} \]
tion of the potential \( V(z) \) with the wave number \( k \). For \( \kappa \to 0 \) we get, after some steps, the finite result

\[
\frac{E}{\delta^2} = -128 \int_0^\infty \frac{dk}{1 + k^2} \left[ \int_0^\infty du e^{-3\nu \delta_0(ku)} \right]^2 \nonumber \\
-2 \int_0^\infty \frac{dk_1dk_2}{k_1^2 + k_2^2} \left[ \int_0^\infty du e^{-\nu \delta_0(k_1u)\delta_0(k_2u)} \right]^2, \tag{10}
\]

where \( \delta = \int_0^\infty du U_{\text{eff}}(u) \). With \( \delta_0(ku) = \sqrt{\pi \sin(ku)} \), we get from (10) the numerical value \( E = -0.45\delta^2 \). Its order of magnitude can be checked from the exact relation

\[
\langle 00|U_{\text{eff}}|00\rangle = (E - 2E_V)|00\rangle, \quad 2E_V \equiv -\kappa^2. \tag{11}
\]

Assume that \( V(z) \) is close to its threshold (i.e., \( \kappa \to 1^+ \), or \( \kappa \to 0 \)) and that a weakly bound state exists with energy \( E = -p^2 \). Recall that \( |00\rangle \) is mainly a constant modulo a numerical factor which cancels from (11). Since all potentials in (7) are short range and thus negligible for large \( z_1 \) and \( z_2 \), we get \( \omega(z_1, z_2) = f(z_1, z_2) e^{-p(z_1 + z_2)} \), where \( f \to 1 \) for \( z_1, z_2 \to \infty \). This is put into (11) and for \( \kappa \to 0 \) and small \( p \) one gets indeed \( E \approx -\delta^2 \sim -\lambda^2 \).

For \( \mu < 1 \) and \( \lambda < 1 \) we are in the situation where neither \( V(z_1) + V(z_2) \) (the attractive wall alone) nor \( U \) alone can create a bound state. We thus conclude from (10) and (11) that the present approach does predict binding for \( \mu = 1 \) and for sufficiently small \( \lambda \). Since the ground state is supposed to be continuous in \( \lambda \) and \( \mu \), the very fact of having a negative energy at \( \mu = 1 \) and not very large \( \lambda \) implies that a naturated, adsorbed state will also exist for \( \mu_c(\lambda) < \mu < 1 \), where neither of the potentials \( V \) or \( U \) alone allows binding. The precise curve \( \mu_c(\lambda) \), where the ground-state energy is equal to its value at \( V = 0 \) (adsorption threshold), requires numerical analysis to be reported elsewhere [14]. We thus have found an example of so-called Borromean binding [15], where the involved potentials do not produce bound states separately, but their cumulative effect does so. We see from (5) that this unusual binding is connected to correlations between the \( z \) components of the particles and (separately) to correlations between their \( x \) and \( y \) components (in \( \rho \)).

No-binding conditions.—To complete the phase diagram, we employ a method suggested in [16]. Let us introduce a third fictive particle with mass \( M \) and the radius vector \( \vec{r}_3 = (x_3, y_3, z_3) \). This particle will substitute the surface: \( V(z_a) (\alpha = 1, 2) \) becomes \( V(|z_a - z_3|) \), and condition (2) is put at \( z_a = z_3 \) only. The modified problem with Hamiltonian \( H_M \) reduces to the original one when for \( M \to \infty, \vec{r}_3 \) becomes a fixed vector which can be chosen at the origin. We have

\[
H_M = -\frac{\vec{p}^2_3}{2M} + U(r) + \sum_{a=1}^2 \left[ -\frac{1}{2} \vec{p}_a^2 + V(|z_a - z_3|) \right].
\]

It is invariant when shifting the radius vectors \( \vec{r}_a \) (\( \alpha = 1, 2, 3 \)) over any fixed vector, while for a finite-particle quantum system, a symmetry of \( H_M \) implies the same symmetry for its ground-state wave function [13]. Thus,

\[
\Psi = \Psi(\vec{r}_1 - \vec{r}_2, \vec{r}_1 - \vec{r}_3, \vec{r}_2 - \vec{r}_3), \quad \sum_{a=1}^3 \vec{\partial}_a \Psi = 0. \tag{12}
\]

We now decompose \( H_M = H_0 + H_{12} + H_{13} + H_{23} \), where

\[
H_0 = -\frac{1}{2} \left[ a \vec{\partial}_3 + b \sum_{i=1}^2 \vec{\partial}_i \right] \sum_{a=1}^3 \vec{\partial}_a, \tag{13}
\]

\[
H_{13} = -c \left( \frac{1}{2} \vec{\partial}_3 - x \vec{\partial}_1 \right)^2 + V(|z_1 - z_3|), \tag{14}
\]

\[
H_{12} = -2d \left( \vec{\partial}_1 - \vec{\partial}_2 \right)^2 + U(|\vec{r}_1 - \vec{r}_2|), \tag{15}
\]

while \( H_{23} = H_{13}(1 \to 2) \). The coefficients \( a, b, c, \) and \( d \) can be read off directly: \( a = mM^{-1} - 2x^2/(1 + 2x)^2, b = d = 2x(1 + x)/(1 + 2x)^2, c = (1 + x)^2/(1 + 2x)^2 \). Since \( c \) and \( d \) are to be employed as effective masses, for the free parameter \( x \) we consider \( x \geq 0 \) (the regime \( x \leq 1 \) is of no help). Note that \( \langle \Psi|H_0|\Psi \rangle = 0 \) due to (12). Let us see when \( \langle \Psi|H_M|\Psi \rangle > 0 \), i.e., when a bound state is absent. Changing the variables—e.g., \( \vec{x}_1 = (1 + x)\vec{r}_1, \vec{x}_2 = (1 + x)\vec{r}_2 \) for \( H_{13} \)—we see that \( \langle \Psi|H_{13}|\Psi \rangle \) and \( \langle \Psi|H_{12}|\Psi \rangle \) are positive for, respectively, \( \mu \leq c(x) \) and \( \lambda \leq 2d(x) \). There is no binding under these conditions for any \( M \) including \( M \to \infty \) which returns to the original problem. By varying \( x \) from 0 to \( \infty \), we cover the domain

\[
0 \leq \mu \leq \frac{1}{4} (2 - \lambda + 2\sqrt{1 - \lambda}). \tag{16}
\]

This is the no-binding region \( a \) shown on Fig. 1. At \( \lambda = 1 \), one needs at least \( \mu_c = 0.25 \) to achieve binding, while at \( \mu = 1, \lambda = 0^+ \) suffices.

FIG. 1. Schematic phase diagram for the interstrand coupling \( \lambda \) versus the strand-surface coupling \( \mu \). The bold lines confine three thermodynamical phases. ND: Naturation and desorption. NA: Naturation and adsorption. DD: Desorption and denaturation. The critical naturation strength in the bulk is \( \lambda_c(1) = 1 \), for single strand adsorption it is \( \mu_c = 1 \). The following subregions are confined by normal lines. a: Domain described by the no-binding condition (16). b (bounded by the bold DD-NA line and two straight segments): Borromean naturation and adsorption. c: Adsorption and naturation due to overcritical coupling to the surface.
As shown on Fig. 1, there are three phases in the model: denatured and desorbed (DD), naturated and adsorbed (NA), and naturated and desorbed (ND). The denatured and adsorbed phase is present only for $U = 0$; recall the discussion below (6). The exact boundary between the DD and NA phases has to be convex: if $(\lambda_1, \mu_1)$ and $(\lambda_2, \mu_2)$ are in DD—i.e., $H(\lambda_1, \mu_1) > 0$ and $H(\lambda_2, \mu_2) > 0$—then the entire line joining these points is in DD, because $\lambda$ and $\mu$ enter $H$ linearly: $H(\lambda_1 + (1 - \epsilon)\lambda_2, \epsilon\mu_1 + (1 - \epsilon)\mu_2) \geq 0$, where $1 > \epsilon > 0$.

We already mentioned that for large $A$ the adsorption of naturated DNA will happen at $\mu_c = \frac{1}{2}$ due to doubling of the mass and potential. For $A$ approaching $\lambda_c = 1$ from above, the absorption is weaker and the entropy will increase so we expect that the potential $U$ will facilitate adsorption less, leading to the ND-NA curve with $\mu_c > \frac{1}{2}$; see Fig. 1. At $\lambda = 1$ it is expected to meet DD-NA at a point, that separates the three phases ND, NA, and DD. The DD-ND line is implied by the fact that in the desorbed phase the polymer is far from the wall.

Conclusion.—In conclusion, we modeled the surface adsorption of a double-stranded DNA. There are several situations where the description of this process via a single chain will not be adequate, and the two-chain modeling is needed. (1) The adsorption realized by the same hydrogen-bonding mechanism as the naturation [3]. Then in the vicinity of the (temperature or pH induced) melting transition also the binding to the surface will be small. Thus the motion of separate chains will be resolved. (2) The binding to hydrophobic surfaces (e.g., aldehyde-derivate glass) goes via partial melting which exposes the hydrophobic core of the helix and leads to the DNA-surface attraction [3]. Both naturation and adsorption are simultaneously weakened by increasing the pH [3]. (3) For homogeneous DNA at normal conditions ($\text{pH} = 7$ and NaCl concentration of 0.15 M) the thermal melting occurs at temperatures $67^\circ C$ and $110^\circ C$ for A-T and C-G unbinding, respectively [1,2]. This temperature can be radically decreased by increasing the pH factor, i.e., by decreasing the concentration of free protons in the solvent, since the negatively charged phosphate groups on each strand are not screened any more by protons and strongly repel each other [1]. Because of the same reason, for the DNA adsorption on a positively charged surface [4], the increase of the pH will increase the electrostatic attraction to the surface. Thus weakly naturated and weakly adsorbed states may be produced by controlling the pH and the surface charge.

Our model describes DNA strands as flexible homopolymers. Many features of real DNA are put aside in this way: stacking, self-avoidance, helicity, heterogeneity of the base sequence, dependence of the surface-strand interaction on the denaturation degree (in the naturated state the bases do not participate in the surface-strand interaction), etc. However, the model can still be useful in clarifying the mechanisms of DNA adsorption or naturation. We saw that a sufficiently strongly attracting surface prevents thermal denaturation (melting) of the double strand, because effectively the problem reduces to binding in two dimensions, where any attractive potential creates a bound state. In practice this implies that in the presence of the surface the melting temperature increases. It was shown that the attractive surface and interstrand coupling together can create bound states (i.e., double stranded and adsorbed to the surface), even when neither of these interactions alone is capable of creating bound states. This unusual type of binding is related to finely correlated motion of the strands. Such Borromean states, where binding is due to the cumulative effect of several potentials, first appeared in nuclear physics [15,16], but their detection in that field is difficult. Their observation in polymer physics might be easier, since the involved scales are mesoscopic. Our results led to the schematic phase diagram which, as we argued, may be checked experimentally.

Thanks to Y. Mamasakhlisov for discussions. A. E. A. and Zh. S. G. were supported by FOM/NWO and A. E. A. also by the CRDF Grant No. ARF2-2647-YE-05. C. K. H. was supported by NSC (Taipei) Grants No. NSC 93-2112-M 001-027 and No. NSC 94-2119-M-002-001, and Academia Sinica (Taipei) Grant No. AS-92-TP-A09.