Financial Report

Sponsor:	**CIVILIAN RES & DEL FOUNDATION**
Project Name:	**NFSAT MB 078-02/CRDF 12027**
Contract Number:	**AP2 # 3222**
Project Number:	**32066BV**
Award Period:	**1/1/03 - 01/22/05**
Report Period:	**1/1/03 - 01/22/05**

Invoices Issued:

Date	Amount
04/27/04	263.26
06/18/04	1,496.13
09/13/04	1,977.89
02/28/05	455.50
Total	$ 4,192.78

Payments Received:

Date	Amount
5/4/03	263.26
6/29/04	1,496.13
10/5/04	1,977.89
Total	$ 3,737.28

Expenditures by Category:	**BUDGET**	**Cumulative**
Personal Services	$ 4,000.00	$ 2,716.28
Fringe Benefits	-	-
Materials & Supplies	500.00	732.98
Tuition Remission		743.52
Equipment	-	-
Travel	-	-
Capital Outlay	-	-
Subcontracts - Non MTDC	-	-
Subcontracts - MTDC	-	-
Total Direct Costs	$ 4,500.00	$ 4,192.78
Indirect Costs	-	-
Total Costs	$ 4,500.00	$ 4,192.78

I certify that to the best of my knowledge and belief that the amounts above are correct and are in accordance with the contract.

Charles T. Duffy
Director, Grants & Contracts Accounting

March 10, 2005
Date

Section IV B: US Final Reconciliation and Confirmation

(to be completed by the US Principal Investigator only)

Project Award Number:

1. Please complete the following table detailing all costs incurred and funds received from CRDF for the project, and submit it, along with a final financial statement or final invoice, by the date specified in your project agreement.

Expense Category	Total Expenditures	Remaining Balance
US Student Support	$3,459.80	$540.20
US Materials & Services	$732.98	-$232.98
US Travel	$	$
Other [Enter Category Name Here]	$	$
TOTAL	$4,192.78	$307.22

CRDF reserves the right to request, in writing, the date of your last audit, as well as a recent copy of your organization's federal audit report (e.g., A-133), if available.

U.S. CIVILIAN RESEARCH & DEVELOPMENT FOUNDATION (CRDF)

2002 ARMENIAN-U.S. BILATERAL GRANTS PROGRAM
FINAL PROJECT REPORT

GENERAL INSTRUCTIONS

- The completed Final Project Report is due **within 30 days** of the termination date of the award.
- Please indicate your **AWARD NUMBER (format NFSAT- MB 078-02/CRDF- 12027)** on all submitted documents.
- The Final Project Report forms are available in English from the CRDF web site at **www.crdf.org** and in Armenian from the NFSAT web site at **www.nfsat.am**.
- The Final Project Reports must be submitted as follows:
  - **The US and Armenian Principal Investigators must jointly complete the Final Project Report in English and submit it as ONE electronic document attached to an e-mail message to CRDF (armenia@crdf.org).**
  - One Principal Investigator should send this email message to CRDF and copy ("cc") the other Principal Investigator to indicate that the Report has been jointly completed and approved by both Principal Investigators.
  - **The Armenian Principal Investigator must complete the corresponding Final Project Report in Armenian and submit a signed hard copy to NFSAT. In addition, the Armenian Principal Investigator should submit the Armenian report as ONE electronic document attached to an e-mail message to NFSAT (bgp3@nfsat.am).**
  - Microsoft Word (.doc) format is preferred. However, Adobe Acrobat (.pdf) or Rich-Text (.rtf) formats are also acceptable. If you are unable to submit the reports electronically, please contact CRDF or NFSAT.
  - For questions regarding the Final Project Report, please contact:

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- Information provided by the Principal Investigators in Section I (Public Summary) of the Project Report will be treated as public information and may be used by CRDF or NFSAT in publicly distributed materials. Individual responses provided in the other sections will not be made public without the permission of the Principal Investigators. Information identified as business-confidential will be held in strict confidence. *Please do not include business-confidential data in Section I of the Project Report.*
- **Both Principal Investigators must print, sign, and date a *Final Reconciliation and Certification Form* (sections III B and IV B below)** and submit this page as a scanned electronic file, by fax, or by mail. The Armenian Principal Investigator must submit his/her signed form to NFSAT. The US Principal Investigator is only required to submit this form to CRDF.

- Please note that under the CRDF Award General Conditions governing this award, the final payments for Individual Financial Support and Institutional Support will not be released until the completed Final Project Report and Final Financial Reports have been received and accepted by CRDF and NFSAT.

FINAL PROJECT REPORT CONTENTS

The Final Project Report consists of the following sections. Both the US Principal Investigator and the Armenian Principal Investigator must complete all sections jointly, except where noted. Detailed instructions for each section are described below.

Section I	**Public Summary**
Section II	**Technical Report**
Section III	**Armenian Team Report (*to be completed by the Armenian Principal Investigator only*)**
Section III A	**Armenian Team Data**
Section III B	**Armenian Final Reconciliation and Certification**
Section IV	**US Team Report (*to be completed by the US Principal investigator only*)**
Section IV A	**US Team Data**
Section IV B	**US Final Reconciliation and Certification**
Section V	**Bibliography of Project-Related Publications**
Section VI	**Conference Presentation List**
Section VII	**Supplemental Information (optional)**

2002 ARMENIAN-U.S. BILATERAL GRANTS PROGRAM
FINAL PROJECT REPORT

SECTION I: Public Summary

Award Number: NFSAT MB 078-02/CRDF 12027

INSTRUCTIONS

- **Contents:**

  1) Brief Statement of Major Accomplishment: In one or two sentences, please state succinctly the major accomplishment that your research achieved.

  2) Public Summary (English): The public summary should begin with a sentence that describes the project's original major goal(s) without restating the project title. The summary should then follow with findings and implications stated as concisely and informatively as possible, commenting as appropriate on the techniques or approaches used. Please indicate how your research results represent an advance in scientific knowledge and any potential social or commercial applications. The summary should be written from the point of view of a completed project, and should be self-contained and intelligible to a layperson. Please do not re-submit the proposal abstract. The public summary should be **200-300 words** in length.

- **Use:** Please note that CRDF or NFSAT may use the public summary in publicly distributed documents and other materials. **Please do not include proprietary or business-sensitive information.**

1) Brief statement of project's major accomplishment (Please summarize in one or two sentences what you consider to be the major accomplishment achieved during your research):

Properties of DNA that contains strongly stabilized sites and interstrand crosslinks caused by DNA chemical modification with metal–based antitumor compounds and some other molecules have been studied.

2) Public Summary (English)

DNA interstrand cross-links are usually formed due to bidentate covalent or coordination binding of antitumor compounds to nucleotides of different strands. However interstrand linkages can be also caused by any type of chemical modification that gives rise to a strong local stabilization of the double helix. This local stabilization makes DNA melting fully reversible and independent of strand concentration like ordinary covalent interstrand cross-links. The stabilization can be caused by all the types of chemical modifications (interstrand cross-links, intrastrand cross-links or monofunctional adducts).

Our study demonstrates that an increase in stability by 25 to 30 kcal in the free energy of a single base pair of the double helix is sufficient for this "cross-linking effect". For the situation where there is more then one stabilized site in a DNA duplex, a lower stabilization per site is sufficient for the "cross-linking effect" (18 - 20 kcal). A substantial increase in DNA stability was found in various experimental studies for several metal-based anti-tumor compounds. If ligand induced stabilization is distributed among several neighboring base pairs, a much lower minimum increase per stabilized base pair is sufficient to produce the cross-linking effect. Stretches of GC pairs more than 25bp in length inserted into poly(AT) DNA also exhibit properties of stabilizing interstrand cross-links. As an ordinary interstrand cross-link, a single strongly stabilized site makes a DNA's melting temperature independent of strand concentration.

10 new water-soluble porphyrins and metalloporphyrins, containing double bond in a peripheral substituent, have been synthesized. The Ag(II) containing metalloporphyrins demonstrate high antibacterial activity. Interaction of these porphyrins with DNA was studied by UV/visible spectrophotometry, fluorimetry and circular dichroism methods.

It was shown that, in some conditions, melting of DNA is hypersensitive to binding of platinum: both the melting temperature and interval of DNA appreciably increase at very low concentration (10^{-7}-10^{-9} M) of platinum. These changes of DNA melting parameters can be explained by changes in DNA topology, namely, by formation of pseudo-ring structures in linear DNA.

2002 ARMENIAN-U.S. BILATERAL GRANTS PROGRAM
FINAL PROJECT REPORT

SECTION II: Technical Report

Award Number: NFSAT MB 078-02/CRDF 12027

INSTRUCTIONS

- **Length:** The technical report should be **no more than 5 pages** in length.
- **Content:** The technical report should outline the goals of the original research project and provide a technical description of how these goals were or were not met, highlighting specific achievements. Please do not re-submit the project narrative from the original proposal.
- **Use:** From time to time, CRDF and NFSAT conduct reviews of completed grant projects for possible inclusion in publicity materials, for presentations at symposia, etc. In connection with this, CRDF and NFSAT occasionally ask expert reviewers from the original grant selection process to review the final technical reports to assist staff in selecting projects for possible feature in such activities. The CRDF and NFSAT do not use specific information (except as otherwise indicated in these Final Project Report instructions) about individual projects in publicity activities without the permission of both Principal Investigators.
- **Language:** The technical report must be submitted in English.

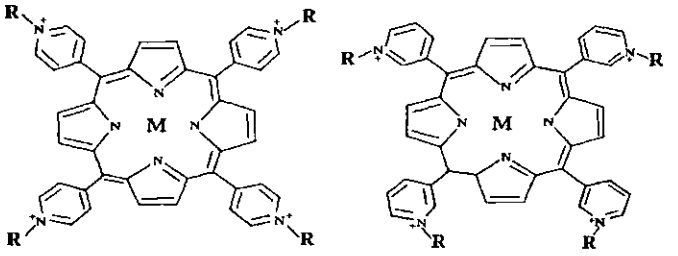
Technical Report

1. Synthesis of new meso-displaced pyridylporphyrins and their metal complexes that give rise to strong local stabilization of the DNA double helix. Study their cytotoxicity, binding to DNA, and influence on DNA structure and stability.

Many of the important biological and medically relevant properties of porphyrins are dependent on the type of peripheral substituent, their location on the periphery of porphyrin and the type of central metal. The three binding mode for porphyrin-DNA complex has been generally accepted, namely intercalation, outside self-stacking and outside random binding. The presence, absence and the type of central metal have an important factor for binding mode.

During Project implementation, new meso-tetra-(3N-allylpyridyl) porphyrin (TAlPyP(3)), its 4N- analogue meso-tetra-(4N-allylpyridyl) porphyrin (TAlPyP(4)) and their Zn-, Co-, Cu- and Ag– containing derivatives were synthesized (see figure 1).

Using NMR and absorption spectroscopy a structural analysis of these compounds has been carried out. It is checked up their cytotoxicity. The Ag(II) containing metalloporphyrins have high antibacterial activity.



R= CH_2-CH=CH_2 (Allyl)

M= 2H	TAlPyP(4)	M= 2H	TAlPyP(3)
M= Cu	CuTAlPyP(4)	M= Cu	CuTAlPyP(3)
M= Co	CoTAlPyP(4)	M= Co	CoTAlPyP(3)
M= Zn	ZnTAlPyP(4)	M= Zn	ZnTAlPyP(3)
M= Ag	AgTAlPyP(4)	M= Ag	AgTAlPyP(3)

Figure 1

Porphyrin complexes with DNA

By methods of UV/visible spectrophotometry, circular dichroism and fluorescence the interaction of these new porphyrins with calf thymus DNA was studied. The new spectrophotometer LAMBDA-800 with high response was used also for recording of differential absorption spectra of DNA-porphyrin complexes. The isotherms of absorption of these porphyries with DNA were constructed from which we can calculate the binding constant. The range of investigated relative concentrations of porphyrins counting on one base pairs of DNA was $0.01 < r < 1.0$. All these porphyrins essentially increase the melting temperature of DNA (T_m), for which some time it becomes impossible the melting curve registration. This increase of melting temperature is proportional to porphyrin's concentration and the type of porphyrin. For example, the melting process of DNA+ AgTAlPyP(4) complex begins only from 95 °C at $r > 0.3$.

The binding of Ag(II)- and Zn(II)- containing metalloporphyrins with DNA brings to the quenching of porphyrins' fluorescence, while for TAlPyP(4) or TAlPyP(3) the changes of fluorescence's spectra have more complicated character. On the curves of fluorescence's dependence on relative concentration of porphyrin the stoichiometry (r_{sat}) of saturated DNA+porphyrin complexes has been determined. And the Co(II)- and Cu(II)-containing metalloporphyrins have non fluorescence.

The analysis of induced CD spectra of complexes of *metal non containing and Ag(II)- and Cu(II)-containing metalloporphyrins* with DNA shows that these porphyrins prefer to interact with DNA mainly by the way of intercalation. However, at high relative concentrations of porphyrins the external mode of binding has taken place, too. At the same relative concentrations of porphyrins, there are more changes in CD spectra of DNA caused by AgTAlPy(4).

The site of peripheral substituent on pyridylic ring essentially influences the mechanism of porphyrin-DNA interaction. For (4N-alyllpyridyl) porphyrin it is energetically more preferable to intercalate into the structure of DNA, than (3N-alyllpyridyl) porphyrin. CD spectra in UV range (220-310nm) of DNA/porphyrin complexes show that addition of porphyrins give rise to the B-Z- or B-Ψ-like conformational changes for (4N-alyllpyridyl) porphyrin, while (3N-alyllpyridyl) porphyrin - B-C conformational change.

The induced CD spectra of complexes of *Zn(II)- and Co(II)-containing metalloporphyrins* with DNA show the external staking binding mode for these porphyrins. The absorption spectra of porphyrins during their titration with DNA show hypochromicity and a red shift of Soret band and, only for CoTAllPyP(4)-DNA complexes, a blue shift of spectra are observed. The changes in differential absorption spectra (at titration of DNA by porphyrins) are also observed. The results obtained from the differential absorption spectra of DNA/porphyrin complexes have demonstrated that the site of peripheral substituents of porphyrin influence on the efficiency of their interaction with DNA. This result is confirmed by CD method.

Porphyrin complexes with RNA duplexes.

The effect of peripheral substituent of porphyrins on RNA duplexes binding mode is investigated. The binding of new water-soluble *meso*-tetra-(4-oxyethylpyridyl)porphyrin (TOEPyP(4)), *meso*-tetra-(4N-allylpyridyl)porphyrin (TAlPyP(4)) and *meso*-tetra-(4N- metallylpyridyl) porphyrin (TMAlPyP(4)) to RNA duplexes has been studied. These porphyrins are different from well known TMPyP(4) by peripheral substituent: TOEPyP(4) – peripheral radical have OH-group, TAlPyP(4) - peripheral radical have double bond (allyl), MetAlPyP(4) - peripheral radical have branching and double bond (metallyl).

The target polymers were poly(rA)•poly(rU) and poly(rI)•poly(rC). The strong conservative induced CD spectra suggested that the porphyrins preferred outside self-stacking on the polynucleotide surface. The binding isotherms for all complexes were obtained from absorption and fluorescence titration experiments and the binding constants were calculated. The changes in enthalpy and entropy associated with complexation were determined by measuring the temperature dependence of the binding constant (i.e., the van't Hoff method). These data provide important new information about thermodynamic parameters of porphyrin/nucleic acid complexation.

The interaction of TAlPyP(4) and AgTAlPyP(4) with poly(rG)•poly(rC), poly (rI)•poly(rC) and poly(rA)•poly(rU) was studied also. It was shown that non-metalloporphyrins bind to poly(rG)•poly(rC) and poly (rI)•poly(rC) by two binding modes, preferring the intercalation to the external mode for poly(rG)•poly(rC). They bind to poly(rA)•poly(rU) preferentially in an outside manner. In contrast, Ag-containing metalloporphyrin bind with the studied RNA's via outside mode. By the intensity of the interaction with porphyrins the studied RNA's can be placed in following order: poly(rI)•poly(rC) ≥ poly(rA)•poly(rU) > poly(rG)•poly(rC).

2. Development of a thermodynamic theory for cross-linked DNA and for DNA locally stabilized by metal-based antitumor compounds.

Interstrand cross-links are usually formed by covalent or strong coordination bidentate binding of cross-linking agents to nucleotides of different DNA strands. Some of the compounds that form interstrand cross-links in DNA molecules are effective antitumor drugs. DNA interstrand cross-links can be highly lethal lesions because they can irreversibly halt DNA metabolic processes. In addition, interstrand cross-links are usually more stable against DNA repair systems in comparison with other types of DNA chemical modifications (intrastrand cross-links and monofunctional adducts). Correlation between cytotoxicity and ability to form DNA interstrand cross-links in vitro has been demonstrated for several cross-linking agents.

The difference in non-modified and crosslinked DNA is the following. In a non-modified DNA that is partially melted, the helical regions prevent full strand separation. Only after melting of all base pairs in the DNA chain, the strands will separate. Therefore the process of full strand separation is equivalent to the transition from a partially (or fully) helical state to the fully melted state. The separation transition is dependent on total strand concentration (C_t) and helix nucleation parameter (β). It is characterized by the fraction of fully melted molecules that is equal to $1-\vartheta_{ext}$ where ϑ_{ext} is the fraction of DNA molecules that contain at least one helical unit ($0 < \vartheta_{ext} < 1$). If $T \sim T_m$, i.e. $\vartheta \sim 0.5$ a lot of internal melted regions (i.e. melted regions bordered upon helical ones from both sides) arise in long DNA molecules. These regions form loops. At higher temperatures when ϑ_{ext} and $\vartheta << 1$, neither internal melted regions nor loops form.

In contrast to non-modified DNA, full strand separation is impossible in cross-linked DNA even after melting of all base pairs. As with a non-modified DNA, a cross-linked DNA does not form helical and internal melted regions at high temperature when ϑ_{ext} and $\vartheta \rightarrow 0$, but it forms $(\omega - 1)$ loops where ω is the number of interstrand cross-links in its chain. Cross-linking decreases the order of the reaction of full strand separation from 2 to 1 and makes T_m as well as melting behavior as a whole independent of DNA concentration. However, for crosslinked DNA, strand separation (ϑ_{ext}) is dependent on the helix initiation parameter, β, as for DNA without interstrand cross-links.

One can imagine another possible way of preventing strand separation without a covalent interstrand cross-link. If any chemical modification or irreversibly bound ligand causes a strong local stabilization of one (L = 1 bp) or several (L > 1 bp) neighboring base pairs, then full melting as well as full strand separation will not occur after melting of the non-modified base pairs. Such modifications might produce the same influence on melting behavior and biological effects as ordinary covalent interstrand cross-links. Because of the prohibition of full melting, this type of modification makes melting of the non-modified base pairs independent of helix nucleation parameter β in contrast to non-modified DNA as well as DNA with ordinary covalent interstrand cross-links. In this case, $\vartheta_{ext} \equiv 1$ until full melting of non-modified AT and GC base pairs. A computer modeling procedure is described below for evaluating the minimum value of local stabilization of a DNA double helix that is necessary to prohibit full melting and full strand separation.

An increase in the free energy of the helix-coil transition of modified base pairs causes local stabilization of the double helix. We suppose that a chemical modification gives sufficient stabilization for the prohibition of local and total melting as well as of strand separation if all the stabilized sites maintain helical base pairs after melting the non-modified AT and GC base pairs.

Interstrand cross-links give rise to three effects that influence stability of the double helix:

1. A single interstrand cross-link is sufficient to prevent full strand separation and decrease the order of reaction of strand dissociation from two to one. It makes melting independent of strand concentration and stabilizes the entire double helix. The effect is pronounced in the case of short DNA's less than 100 bp in length.

2. In addition to decreasing the order of reaction of strand dissociation, ω interstrand cross-links cause the formation of $(\omega - 1)$ loops in a fully melted state. Loop formation decreases the entropy of the partially melted and fully melted states increasing the stability of the double helix. This effect gives rise to a strong increase in the melting temperature if the average distance between neighboring cross-links is less 100 bp.

3. Besides these two effects interstrand cross-links can locally stabilize or destabilize the double helix.

Another way to prevent strand separation without covalently linking the two DNA strands can be caused by any chemical modification of DNA or irreversible ligand binding to DNA if it causes a sufficiently strong local stabilization of the double helix. This type of modification might demonstrate biological and thermodynamic effects that are similar to covalent interstrand cross-links. However ordinary interstrand cross-links generally give rise to a local distortion and local destabilization of the double helix and cannot prevent full DNA melting. The chemical modifications under consideration always give rise to a strong local stabilization and can be caused by any type of chemical modifications (monofunctional adducts, interstrand and intrastrand cross-links) as well as by very stable non-covalent irreversible ligand binding to DNA. Base pairs with all the types of chemical modifications (even with

interstrand cross-links) that do not cause strong local stabilization are melted in the same temperature interval as ordinary AT and GC base pairs. They only increase or decrease the overall stability of the double helix, but do not prohibit melting of the chemically modified base pairs. In contrast, modified strongly stabilized base pairs conserve the helicity after melting of ordinary AT and GC base pairs and prevent full melting as well as full strand separation.

Analytical expressions were derived and used to evaluate the thermodynamic properties of DNA with several different distributions of stable sites. It was found that, as an ordinary interstrand cross-link, a single strongly stabilized site makes a DNA's melting temperature (T_m) independent of strand concentration. However in contrast to a DNA with an interstrand cross-link, a strongly stabilized site makes the DNA's T_m independent of DNA length and equal to T_∞, the melting temperature of an infinite length DNA with the same GC-content and without a stabilized site. Moreover, at a temperature where more than 80% of base pairs are melted, the number of ordinary (non-modified) helical base pairs (n) is independent of both the DNA length and the location of the stabilized sites. For this condition, $n(T) = (2\omega\text{-}a)S/(1\text{-}S)$ and $S = \exp[\Delta S(T_\infty\text{-}T)/(RT)]$ where ω is the number of strongly stabilized sites in the DNA chain, a is the number of DNA ends that contain a stabilized site, and ΔS, T, and R are the base pair entropy change, the temperature, and the universal gas constant per mole. The above expression is valid for a temperature interval that corresponds to $n < 0.2N$ for $\omega = 1$, and $n < 0.1N$ for $\omega > 1$, where N is the number of ordinary base pairs in the DNA chain.

3. Study of the alteration of DNA structure and stability caused by newly synthesized compounds as well as known platinum compounds.

The helix-coil transition of DNA at complex-formation with antitumor drug *cis*-diaminedichlorplatinum(II) (cisplatin) and its medically inactive analogue *trans*-diaminedichlorplatinum(II) (transplatin) has been studied. The melting curves of complex of DNA's of various GC-content with low and ultra low relative concentration ($0.001 < r < 0.0001$) of cisplatin and transplatin are investigated. (r = [concentration of platinum] / [concentration of DNA base pairs]). Results have shown that melting of DNA is hypersensitive to low concentration of platinum: both the melting temperature and interval of all DNA's appreciably increase. The values of changes of melting parameters are in direct dependence on GC-content of DNA. The similar change of DNA melting parameters at very low concentration of platinum (when 1-5 molecules of platinum are on one DNA macromolecule) can be explained only by changes in topology of DNA macromolecule, namely, formation of pseudo-ring structures in linear DNA.

If our assumption about of formation of pseudo-ring structures from linear DNA at the presence of platinum is truly, the effect of change of melting parameters of DNA at ultra low concentration of platinum should depend on length of DNA chain. It should be weakened with reduction of length of a macromolecule. For check of this hypothesis, the research of melting of high-molecular ($\sim 10^5$ -10^6 bp) and fragmented (less than 10^3 bp) calf thymus DNA at this concentration of platinum was carried out. The comparative analysis of melting parameters of high-molecular and fragmented DNA's at the same concentration of platinum has shown that, for fragmented DNA, the changes of melting parameters are essentially less expressed than for high-molecular weight DNA.

The peculiarities of helix-coil transition of DNA at complex-formation with cisplatin have been investigated in acidic media. It was shown, that observed features of behavior of DNA melting interval via pH and melting temperature via pH at pH 2.8- 3.0 is possible to explain by formation of pseudo-ring structures in DNA at covalent linking of cisplatin with DNA.

4. Simulation of the influence on DNA stability and thermodynamic properties of interstrand cross-links and strongly stabilized sites using DNA melting theory. Determination of the minimum energy of local stabilization of a double helix that causes prohibition of local and total strand separation.

Let $\delta(\Delta F)$ be the change in free energy of a single chemically modified strongly stabilized site. Strand separation occurs only after melting of this stabilized site. For a given $\delta(\Delta F)$, existence of a temperature interval for which ϑ_{ext} is high enough ($0.99 < \vartheta_{ext} < 1$) and ϑ is only slightly higher than the fraction of stabilized base pairs (L/N) demonstrates that the $\delta(\Delta F)$ value is sufficient for a cross-linking effect.

For non-modified poly(AT) [$\delta(\Delta F) = 0$] as well as for $0 < \delta(\Delta F) < 9$ kcal per stabilized site, the differential melting curve, $\vartheta'_T(T)$, and the curve of strand separation, $\vartheta_{ext}(T)$, are located in the same temperature regions. If $\delta(\Delta F) > 11$ kcal, the differential melting curve and T_m are not changed significantly, but there is a strong increase in the fraction of non-dissociated DNA molecules, $\vartheta_{ext}(T)$. For $\delta(\Delta F) = 18$ kcal, there is a temperature interval in which the fraction of not fully dissociated molecules, $\vartheta_{ext}(T)$, is close to 1 but the fraction of helical base pairs, $\vartheta(T)$, as well as $\vartheta'_T(T)$ is close to zero. This implies that full melting and full strand separation do not occur after melting of ordinary (non-modified) base pairs.

Similar results were obtained for the heterogeneous DNA chain with a single stabilized site. T_m, $\vartheta_{ext}(T)$, and $\vartheta'_{\Gamma}(T)$ are not markedly changed during an increase in $\delta(\Delta F)$ from 0 to 12 kcal. Further increases in $\delta(\Delta F)$ give rise to a strong elevation of $\vartheta_{ext}(T)$ without a change in the differential melting curve. For $\delta(\Delta F) > 22$ kcal, there is a temperature interval ($90°C < T < 91°C$) in which the fraction of not fully melted DNA's, $\vartheta_{ext}(T)$, is close to 1 but the fraction of helical base pairs, $\vartheta(T)$, is close to zero.

If a DNA includes more than one strongly stabilized site ($\omega > 1$), melting of the non-modified base pairs at a temperature where <u>all</u> the stabilized sites remain helical gives rise to ω helical regions that form ω -1 loops. Therefore $n = \omega$ where n is the number of helical regions. This equality indicates the prohibition of local strand separation at the stabilized sites. If stabilized base pairs are absent or are not stable enough to prohibit local strand separation then $n \cong 0$ when the temperature is high enough.

Alteration of $n(T)$ and $\vartheta'_{\Gamma}(T)$ caused by an increase in the free energy of six separate base pairs ($\omega = 6$) at positions 1, 1000, 2000, 3000, 4000, 5000 has been considered. Poly AT without stabilized sites [$\delta(\Delta F) = 0$] melts from the ends and does not form loops. Therefore there is a single helical region ($n \approx 1$) before DNA melting ($T < T_m = 65°C$). At higher temperatures after ordinary base pairs are melted, $n = 0$. Elevation of $\delta(\Delta F)$ for each of the six base pairs from zero up to 9 kcal causes a small monotonous increase in the melting temperature (T_m) but does not give rise to loop formation (n is close to zero if $T > T_m$). Further elevation of $\delta(\Delta F)$ does not change T_m and differential melting curve but increases the number of helical regions (n) and loops at high temperatures from $n = 1$ up to $n = \omega = 6$. If $\delta(\Delta F) \geq 15$kcal, local and total strand separation do not take place for $65.8°C < T < 68°C$, although the degree of helicity is close to zero for this temperature interval.

The same cross-linking effect was demonstrated for a DNA with a random distribution of base pairs ($N =$ 5000 bp, $GC = 50\%$). As in the previous case, there are six stabilized base pairs ($\omega = 6$) at positions 1, 1000, 2000, 3000, 4000, 5000. In contrast to poly(AT), a heterogeneous DNA forms many internal melted regions in the temperature interval that corresponds to DNA melting. Their number tends to zero after melting of ordinary base pairs for a DNA without stabilized sites as well as for the case of $0 < \delta(\Delta F) < 12$ kcal. Alteration of $\delta(\Delta F)$ for each of these base pairs in this interval slightly increases T_m and strongly changes the differential melting curve (DMC). A further increase in $\delta(\Delta F)$ does not change DMC, but for a high enough temperature when almost all ordinary base pairs are melted, n tends to change from zero to the value that indicates prohibition of local and total strand separation ($n = \omega = 6$). If $\delta(\Delta F) \geq 18$kcal, local strand separation does not occur at the stabilized sites ($n(T) = \omega = 6$) although ϑ is close to zero ($90°C < T < 93°C$).

Using this approach, we have shown that an increase in stability by 25 to 30 kcal in the free energy of a single base pair of the double helix is sufficient for the "cross-linking effect" (i.e. conserving the helicity of this base pair and preventing strand separation after melting of ordinary base pairs). For the situation where there is more then one stabilized site in a DNA duplex (e.g., 1 stabilized site per 1000 bp), a lower stabilization per site is sufficient for the "cross-linking effect" (18 - 20 kcal). A substantial increase in DNA stability was found in various experimental studies for some metal-based anti-tumor compounds. These compounds give rise to the effect described above. If ligand induced stabilization is distributed among several neighboring base pairs, a much lower minimum increase per stabilized base pair is sufficient to produce the cross-linking effect. Therefore, the relatively weak non-covalent binding of histones or protamines that cover long regions of DNA (20-40bp) also causes this effect if the salt concentration of the solution is sufficiently low to cause strong local stabilization of the double helix. Stretches of GC pairs more than 25bp in length inserted into poly(AT) DNA also exhibit properties of stabilizing interstrand cross-links.

5. ***Development of a thermodynamic method for revealing if a metal-based compound causes a strong local stabilization in a DNA double helix.***

During the Project implementation, we have shown by computer modeling that melting temperature does not tends to infinity with infinite increase in energy of stabilized sites but it tends to saturation. For a given relative concentration of stabilized sites ($r_b = \omega/(2P)$), there is an upper limit in $\delta(T_m)$ increase during infinite increase in free energy of the helix-coil per stabilized site ($\delta(\Delta F)$). As was found, this temperature limit is dependent on distribution of stabilized sites along the DNA chain (random or uniform) and slightly dependent on GC-content as well as on distribution of AT and GC base pairs along DNA chain. A periodical distribution gives higher $\delta(T_m)$ than a random one. Chemical modifications that give rise to the "cross-linking effect" caused by strong local stabilization of the double helix must demonstrate $\delta(T_m)$ comparable a calculated one. Our calculations have shown that ordinary interstrand crosslinking gives rise to much lower $\delta(T_m)$.

We have carried out a search in literature of compounds that strongly increase the melting temperature. Four such compounds were found ($[\{trans\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_4NH_2]Cl_2$, $[Ru(phen)_2(OH_2)_2]^{2+}$,

$[\{cis\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_4NH_2]^{2+}$, $[\{cis\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_6NH_2]^{2+}$). They give rise to a very strong stabilization effect that is shown in the figure. All found compounds are metal-based complexes, and tree of them are effective cytostatics.

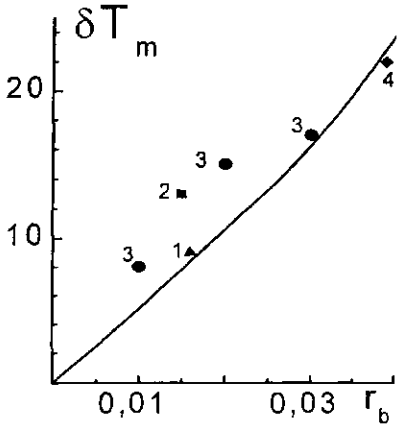


Figure 2. Dependence on r_b of the upper limit of the shift in melting temperature caused by strongly stabilize base pairs randomly located along DNA with random distribution of AT and GC (GC=42%).
Experimental data: 1 - $[\{trans\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_4NH_2]Cl_2$
2 - $[Ru(phen)_2(OH_2)_2]^{2+}$
3 - $[\{cis\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_4NH_2]^{2+}$
4- $[\{cis\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_6NH_2]^{2+}$

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SECTION III A: Armenian Team Data
(to be completed by the Armenian Principal Investigator only)

Award Number: NFSAT MB 078-02/CRDF 12027

A. Research Information

1. Scientific Results

a. Were the scientific and technical objectives of your original proposal accomplished?

Yes ☒ No ☐ The research objectives changed. ☐

b. If specific research objectives were *not* accomplished, please briefly describe the factors that impeded their successful completion (e.g., unanticipated research results, difficulty in communications, administrative or financial complications, etc.).

c. If specific research objectives were *changed*, please describe:

d. Please indicate the type of accomplishments achieved under your project (check all that apply):

- ☒ New theoretical results
- ☐ Elaboration of known topic
- ☒ New experimental results
- ☐ New techniques developed or improved
- ☐ Development of "know-how"
- ☐ Prototype development
- ☐ Patent Application
  - ☐ Pending
  - ☐ Received
- ☒ Publication of results in journal
- ☒ Conference presentations
- ☐ Other (please describe)

2. Collaborative Benefits

a. Describe the benefits of having conducted your research in collaboration with U.S. counterparts rather than independently.

- ☒ Exchange of ideas
- ☐ Access to new facilities
- ☒ Joint publications
- ☐ Access to new research methods
- ☐ Other (please describe)
- ☐ Complementary expertise in particular research area
- ☐ Access to new or previously unavailable information
- ☐ Access to new geographical research area
- ☒ Educational effect on young researchers/students

b. Describe any difficulties related to the collaborative nature of the effort.

- ☐ Language barriers
- ☐ Procuring equipment or supplies
- ☐ Other time commitments
- ☐ Travel/Visas
- ☐ Other (please describe)
- ☐ E-mail/Internet difficulties
- ☐ Paperwork
- ☐ Intellectual Property Rights issues
- ☐ Financial Issues

c. Will the collaboration with the US team continue? Yes ☒ No ☐

d. If "Yes", by which of the following means? (check all that apply)

- ☒ Future joint publications
- ☒ Exchange visits
- ☒ E-Mail contact
- ☒ New grant proposals
- ☐ Joint patents, copyrights, or trademarks
- ☐ Other (please describe)

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3. Additional Support

a. Have you submitted applications to any funding agencies for support of your collaborative research? Yes ☒ No ☐

b. If "Yes", please indicate which funding agencies you applied to for possible funding (check all that apply).

- ☒ ISTC/STCU
- ☐ INTAS
- ☒ NATO
- ☐ U.S. Department of Energy
- ☐ CRDF/NFSAT (Program:)
- ☒ Armenian Govt Agency/Ministry: Ministry Edu.& Sci.
- ☐ European Community 6th Framework Programme
- ☐ U.S. Department of Defense
- ☐ National Institutes of Health (NIH) (Please specify NIH institute:)
- ☐ For-Profit Company (Please identify:)
- ☐ Other: (Please identify:)

c. If you received any funding to continue your collaborative research, please identify the source(s) from which you have received this funding (check all that apply).

- ☒ ISTC/STCU
- ☐ INTAS
- ☒ NATO
- ☐ U.S. Department of Energy
- ☐ CRDF/NFSAT (Program:)
- ☒ Armenian Govt Agency/Ministry: Ministry Edu.& Sci .
- ☐ European Community 6th Framework Programme
- ☐ U.S. Department of Defense
- ☐ National Institutes of Health (NIH) (Please specify NIH institute:)
- ☐ For-Profit Company (Please identify:)
- ☐ Other: (Please identify:)

d. In the future, do you plan to apply for support for continuation of your collaborative research? Yes ☒ No ☐

e. If "Yes," please specify which funding source(s) you plan to apply to for support (check all that apply).

☒ ISTC/STCU
☐ INTAS
☒ NATO
☐ U.S. Department of Energy
☒ CRDF/NFSAT (Program:)
☒ Armenian Govt Agency/Ministry: Ministry Edu.& Sci.
☐ European Community 6th Framework Programme
☐ U.S. Department of Defense
☐ National Institutes of Health (NIH) (Please specify NIH institute:)
☐ For-Profit Company (Please identify:)
☐ Other: (Please identify:)

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4. **Technology Commercialization**

a. Are you pursuing commercial application of your research results? Yes ☐ No ☒

b. If "Yes," please check all that apply:

☐ Planning joint patent, copyright or trademark application
☐ Approved joint patent, copyright or trademark application
☐ Planning country-specific patent, copyright or trademark application
☐ Approved country-specific patent, copyright or trademark application
☐ Contract with for-profit company
☐ Prototype development
☐ Marketing
☐ Manufacturing
☐ Licensing
☐ Seeking venture capital investment
☐ Other: (please describe)

c. If "Yes" to question 4.a, please provide a paragraph with details about the above-checked plans:

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5. **Transition to Civilian Science**

a. Did your project include researchers who were formerly actively engaged in weapons research? Yes ☒ No ☐ *(if you check No, please skip to Question 6)*

b. Did this CRDF/NFSAT research project provide a productive means for engaging and retaining former weapons scientist(s) in civilian science? Yes ☒ No ☐

c. Please describe: ☐

d. Did any of the former weapons researchers on your team change institutional affiliation or country of residency during this project? Yes ☐ No ☒

e. If "Yes," please describe. Include details of whether the change was permanent or temporary. If temporary, how long was the visit?

f. Did the former weapons researchers on your team work on the project full-time? Yes ☒ No ☐

g. If "No," please describe their other research and professional activities during the grant period:

6. **Research Infrastructure**

a. How did you use technological information resources (such as the Internet, e-mail) to support your BGP project? (check all that apply)

- ☒ To obtain data or information
- ☒ To consult with co-investigator by e-mail
- ☒ To consult with other researchers working on the same or related topics by e-mail
- ☒ To identify future research collaborators
- ☒ To identify funding sources
- ☐ To promote/market the results of the research project
- ☒ To help educate student researchers
- ☒ To aid in the submission of additional collaborative research proposals and publications
- ☒ To submit conference abstracts or register for conferences
- ☒ To research or order materials and services
- ☐ Other (please describe)

b. Over the course of the award, did you or your laboratory/institute develop new linkages (international or in-country) with any of the following in order to carry out the research project (check all that apply)?

- ☒ Academy of Sciences Research institutions
- ☐ Government Research Institutions
- ☒ Armenian Universities
- ☒ Other Universities
- ☐ For-Profit Companies
- ☐ Other (please describe)

c. Please briefly identify and describe the institutional linkages developed (e.g., "developed arrangement to share access to research equipment with XXX Institute"):

d. Over the course of the award, did you have the opportunity to utilize equipment (for project-related purposes) at your U.S. collaborator's institution or other foreign or Armenian institutions?

Yes☒ No ☐

If "Yes", please describe:

1. Institute of Bioorganic Chemistry of Belarus National Academy of Sciences, Minsk, Belarus.
2. University of Toronto, Toronto, Canada.

B. Administrative Information

1. **Project Personnel**

a. List all members of your BGP research team (including subcontractors and those who worked on the project but did not receive individual financial support from CRDF and NFSAT) including name, date of birth, gender, project role, and affiliation (if different from Principal Investigator's institution). Please include and identify students. Please identify as "Former Weapons Researchers" those project participants who were formerly or are currently actively engaged in research at a current or former weapons laboratory or institution. *For those researchers only*, please indicate the type of weapons research by using the code list provided in the Appendix.

#	Name/Project Role/Institutional Affiliation	Date of Birth (MM/DD/YYYY)	Gender [M/F]	Student ?	Former Weapons Researcher?	Defense Code (see Appendix for code)
1	Samvel G. Haroutiunian, principal investigator, YSU	19/07/55	M	☐	☐	
2	Yeva B. Dalyan investigator, YSU	02/27/49	F	☐	☐	
3	Volodya I. Vardanyan, investigator, YSU	08/04/51	M	☐	☒	A1
4	Sergey Nersesyan, investigator, YSU	08/12/46	M	☐	☒	B4
5	Pogos Vardevanyan, investigator, YSU	01/11/53	M	☐	☐	
6	Ara A. Ghazaryan, investigator, Ph.D student, YSU	15/09/80	M	☒	☐	
7	Tigran S. Haroutunyan, Ph.D student, YSU	01/05/81	M	☒	☐	
8	Lusine Aloyan, Ph.D student, YSU	11/10/79	F	☒	☐	
9	Lusine Abgaryan, Master Student, YSU	17/08/83	F	☒	☐	
10	Dmitri Yu. Lando, IBOCh, Belarus	01/04/50	M	☐	☒	C4

b. Did any of the participating students complete a thesis in whole or in part based on research directly related to this CRDF and NFSAT-sponsored project? Yes ☒ No ☐

c. If "Yes", check all that apply: ☐ Doctoral/Candidat ☒ Masters ☒ Undergraduate

2. **Project-Related Travel**

a. How many Armenian team members traveled to the United States for project-related purposes during the term of the grant?	
b. Of these, how many were students?	
c. How many Armenian team members traveled to countries other than the U.S. for project-related purposes such as presenting BGP research results at an international conference?	3
d. Of these, how many were students?	1
e. How many Armenian team members left Armenia for six months or more during the grant period to take a position in a foreign laboratory or organization?	
f. Of these, how many were students?	

For all participants in Question "2.e" above, please provide the following information:

	Student?	Period of Time Abroad				Destination	
		< 1 year	1-2 years	> 2 years	Returned home?	USA	Other (please specify)
Team member 1	☐	☐	☐	☐	☐	☐	☐
Team member 2	☐	☐	☐	☐	☐	☐	☐
Team member 3	☐	☐	☐	☐	☐	☐	☐

3. Award Administration

a. Did you encounter any administrative difficulties during the course of the project?
Yes ☐ No ☒

b. If "Yes", please identify the type of problem encountered by checking the appropriate box below (check all that apply).

- ☐ Individual financial support payments
- ☐ Purchase of materials & services
- ☐ Institutional support payments
- ☐ Communication with NFSAT staff
- ☐ Other (please describe)
- ☐ Travel /Visa Issues
- ☐ Cost-share payments
- ☐ Communication with CRDF staff

c. Please comment on how these difficulties were addressed and/or resolved.

4. NFSAT Performance

On a scale of 1 to 5, rate the performance of NFSAT staff in administering your award. (If there are specific instances of poor performance or instances of excellent performance, please provide a brief explanation.)

Poor 1 ☐ 2 ☐ *Good* 3 ☐ 4 ☐ *Excellent* 5 ☒

Explanation: The Armenian team thanks NFSAT, CRDF, NATO, ISTC foundations for financing works which helped to establish important scientific contacts to the well known scientific-educational centers of USA, Canada and Europe. As a result the Armenian team has organized the International Symposium in Armenia ("Hydration and Thermodynamics of Molecular Recognition " (HTMR-2005), Tsakhkadzor, Armenia, March, 1-5, 2005 ") in which more than 70 scientists from more than 15 countries including USA, Canada, Germany, France, England, Japan will participate.

2002 ARMENIAN-U.S. BILATERAL GRANTS PROGRAM
FINAL PROJECT REPORT

SECTION III B: Armenian Final Reconciliation and Confirmation
(to be completed by the Armenian Principal Investigator only)

Award Number: NFSAT MB 078-02/CRDF 12027

The original hard copy of Section III B must be completed, signed and submitted to NFSAT.

1. **Total Amounts Received:**

Please indicate the total amount approved and received from NFSAT in support of this project.			
Expense Categories	**Approved Budget Amount (US$)**	**Amount Received from NFSAT (US$) (Including final payments for IFS and IS)**	**Remaining funds, (US$)**
Individual Financial Support	**$ 22880**	**$ 22880**	**$**
Materials and Services	**$ 49840**	**$ 49840**	**$**
Travel Expenses	**$**	**$**	**$**
Institutional Support	**$ 2280**	**$ 2280**	**$**
Subcontracts	**$**	**$**	**$**
TOTAL	**$ 75000**	**$ 75000**	**$**

2. **Institutional Support:** Please indicate how the institutional support component of the award was used to support the project.

Expense Type	Amount (US$)
Individual Financial Support	$
Materials and Services	$
Internet Connection/Access	$
Communications Costs (telephone, fax, etc.)	$ 200
Utilities (such as heat, electricity, etc.)	$ 240
Administrative/Clerical Support	$
Publications	$
Travel Expenses	$
Equipment maintenance/repair	$ 900
Facilities upgrades/repair	$ 940
Other - Please specify:	$
TOTAL	$ 2280

3. **Amount Received from Other Sources:** This refers to any monetary or material support you received as additional support for research relating to this project from sources not included in the amount awarded by CRDF and NFSAT in your original award documents. BGP funds from NFSAT *should not* be noted here. Please complete the table below for each source of support. Copy the table as necessary.

SOURCE: NATO Collaborative Linkage Grant # LST.CLG.979777		
Expense Category	Amt. in Local Currency	Amount in USD
Individual Financial Support		$
Materials & Services		$ 6000
Travel Expenses		$ 15000
Institutional Support		$
Subcontracts		$
Other Project Expenses		$
TOTAL		$ 21000

4. Did you and your team members receive **NFSAT funds** in full and with no taxes withheld?
   Yes ☒ No ☐

FINAL CERTIFICATION

We hereby certify that the information provided in this report is complete and accurate to the best of our knowledge and belief and that all work performed under this project supported by NFSAT funds was carried out in accordance with the Award General Conditions.

Signature of Armenian Principal Investigator — Date: 17/01/2005

Signature of Authorized Institutional Representative & Institutional Seal — Date: 26.01.05

The original hard copy of Section III B must be completed, signed, and submitted to NFSAT.

2002 ARMENIAN-U.S. BILATERAL GRANTS PROGRAM
FINAL PROJECT REPORT

SECTION IV A: US Team Data
(to be completed by the US Principal Investigator only)

Award Number: NFSAT MB 078-02/CRDF 12027

A. Research Information

1. Scientific Results

a. Were the scientific and technical objectives of your original BGP proposal accomplished?
Yes ☒ No ☐ The research objectives changed ☐

b. If specific research objectives were not accomplished, please describe the factors that impeded their successful completion (e.g., unanticipated research results, difficulty in communications, administrative or financial complications, etc.).

c. If specific research objectives were *changed*, please describe:

d. Please indicate the type of accomplishments achieved under your project (please check all that apply):

- ☒ New theoretical results
- ☐ Elaboration of known topic
- ☐ New experimental results
- ☐ New techniques developed or improved
- ☐ Prototype Development
- ☐ Development of "know-how"
- ☐ Patent Application
  - ☐ Pending
  - ☐ Received
- ☒ Publication of results in journal
- ☒ Conference Presentations
- ☐ Other (please describe)

2. Collaborative Benefits

a. Describe the benefits of having conducted your research in collaboration with Armenian counterparts rather than independently.

- ☒ Exchange of ideas
- ☐ Access to new facilities
- ☒ Joint publications
- ☐ Access to new research methods
- ☐ Other (please describe)
- ☒ Complementary expertise in particular research area
- ☐ Access to new or previously unavailable information
- ☐ Access to new geographical research area
- ☒ Educational effect on young researchers/students

b. Describe any difficulties related to the collaborative nature of the effort.

- ☐ Language barriers
- ☐ Procuring equipment or supplies
- ☐ Other time commitments
- ☐ Travel/Visas
- ☐ Other (please describe)
- ☐ E-mail/internet difficulties
- ☐ Paperwork
- ☐ Intellectual Property Rights issues
- ☐ Financial Issues

c. Will your collaboration with the Armenian team continue? Yes ☒ No ☐

d. If "Yes," by which of the following means? (please check all that apply)

- ☒ Future joint publications
- ☒ Exchange visits
- ☒ E-Mail contact
- ☒ New grant proposals
- ☐ Joint patents, copyrights, or trademarks
- ☐ Other (please describe)

---

3. Additional Support

a. Do you feel your work on this project helped to enable you or your institution to obtain support for continuation of your collaborative research from sources other than CRDF/NFSAT?
Yes ☐ No ☒

b. If "Yes," please check the sources below:

- ☐ National Institutes of Health (Please indicate institute:)
- ☐ National Science Foundation (Please indicate Program Area/Division:)
- ☐ NASA
- ☐ U.S Department of Energy
- ☐ U.S. Department of Agriculture
- ☐ U.S. Department of Defense
- ☐ Other:

c. In the future, do you plan to apply for support for continuation of this collaborative research from sources other than CRDF/NFSAT? Yes ☒ No ☐

If "Yes," list potential sources.

- ☐ National Institutes of Health (Please indicate institute:)
- ☒ National Science Foundation (Please indicate Program Area/Division: mole. biophysics)
- ☐ NASA
- ☐ U.S Department of Energy
- ☐ U.S. Department of Agriculture
- ☐ U.S. Department of Defense
- ☐ Other:

4. Technology Commercialization

a. Are you pursuing commercial application of your research results? Yes ☐ No ☒

b. If "Yes," please check all that apply:

- ☐ Planning joint patent application, copyright or trademark
- ☐ Approved joint patent application, copyright or trademark
- ☐ Planning country-specific patent application, copyright or trademark
- ☐ Approved country-specific patent application, copyright or trademark
- ☐ Contract with for-profit company
- ☐ Prototype development
- ☐ Marketing
- ☐ Manufacturing
- ☐ Obtaining private equity funds (Venture capital, angel investors, banks, etc.)
- ☐ Joint venture/strategic alliance formation
- ☐ Other: (please describe)

c. If "Yes" to question 4.a, please provide a paragraph with details about the above-checked plans:

B. Administrative Information

1. Project Personnel

a. List all U.S. members of your BGP research team including name, age, gender, and affiliation (if different from Principal Investigator's institution). Include and identify students, even if not paid.

#	Name/Institutional Affiliation	Date of Birth (MM/DD/YYYY) (Optional: For Statistical Purposes Only)	Gender [M/F]	Student?
1	**Roger M. Wartell**	**2241945**	**M**	☐
2	**Brooke Bourdelat-Parks**	**08-24-1973**	**F**	☒
3				☐
4				☐
5				☐
6				☐
7				☐
8				☐
9				☐
10				☐

b. Did any of the participating students complete a thesis in whole or in part based on research directly related to the CRDF-sponsored project? Yes ☒ No ☐

c. If "Yes", check all that apply: ☒ Doctoral ☐ Masters ☐ Undergraduate

2. **Project-Related Travel**

a. How many U.S. team members traveled to Armenia for project-related purposes during the term of the grant?	
b. Of these, how many were students?	
c. How many U.S. researchers traveled to countries besides Armenia for project-related purposes such as presenting BGP research results at an international conference?	
d. Of these, how many were students?	

3. Award Administration

a. Describe any administrative difficulties encountered during the course of the BGP grant.

☐ Graduate student stipend payments
☐ Purchase of materials and services
☐ Cost-share payments
☐ Travel/Visa Issues
☐ Communication with CRDF staff
☐ Other (please describe)

b. Please comment on how these difficulties were addressed and/or resolved.

4. CRDF Performance

On a scale of 1 to 5, rate the performance of CRDF staff in administering your award. (If there are specific instances of poor performance or instances of excellent performance, please provide a brief explanation.)

Poor *Good* *Excellent*
1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☒

Comments:

2002 ARMENIAN-U.S. BILATERAL GRANTS PROGRAM
FINAL PROJECT REPORT

SECTION IV B: US Final Reconciliation and Confirmation
(to be completed by the US Principal Investigator only)

Award Number: NFSAT MB 078-02/CRDF 12027

Section IV B must be completed, printed, signed, and submitted to CRDF as a scanned electronic file, by fax, or by mail.

FINANCIAL REPORTING

(1) The U.S. institution should submit a final financial report detailing all costs incurred and funds received from CRDF for the project. This report should be attached to this certification or may be submitted by mail to CRDF's Arlington Office along with item (2) below.

(2) Please provide a recent copy of your organization's federal audit report (e.g., A-133), if available.

FINAL CERTIFICATION
We hereby certify that the information provided in this report is complete and accurate to the best of our knowledge and belief and that all work performed under this project supported by CRDF funds was carried out in accordance with the Award General Conditions.

Signature of US Principal Investigator	Date
Signature of Authorized Institutional Representative	March 25, 2005 Date

Section III B must be completed, printed, signed, and submitted to CRDF as a scanned electronic file, by fax, or by mail.

2002 ARMENIAN-U.S. BILATERAL GRANTS PROGRAM
FINAL PROJECT REPORT

SECTION IV B: US Final Reconciliation and Confirmation
(to be completed by the US Principal Investigator only)

Award Number: NFSAT MB 078-02/CRDF 12027

Section IV B must be completed, printed, signed, and submitted to CRDF as a scanned electronic file, by fax, or by mail.

FINANCIAL REPORTING

(1) The U.S. institution should submit a final financial report detailing all costs incurred and funds received from CRDF for the project. This report should be attached to this certification or may be submitted by mail to CRDF's Arlington Office along with item (2) below.

(2) Please provide a recent copy of your organization's federal audit report (e.g., A-133), if available.

FINAL CERTIFICATION
We hereby certify that the information provided in this report is complete and accurate to the best of our knowledge and belief and that all work performed under this project supported by CRDF funds was carried out in accordance with the Award General Conditions.

Roger M Warthen — February 8, 2005
Signature of US Principal Investigator — Date

Lev Tsybeliov — March 25, 2005
Signature of Authorized Institutional Representative — Date

Section III B must be completed, printed, signed, and submitted to CRDF as a scanned electronic file, by fax, or by mail.

2002 ARMENIAN-U.S. BILATERAL GRANTS PROGRAM
FINAL PROJECT REPORT

SECTION V: Bibliography of Project-Related Publications

Award Number: NFSAT MB 078-02/CRDF 12027

1. Did you publish any project-related papers during the grant period?
   Yes ☒ No ☐

2. If "Yes," how many? 9
   Please list below, using the instructions provided

3. If "No," please provide a brief explanation and describe any future publication plans.

INSTRUCTIONS

- **Format:** Please use the following format to list publications:

  ➢ *For a journal or magazine article:*
  Author Name(s). "Article Title." Journal Name Volume (Year): Page Numbers. (Country of Publication)

  Journal/Article Example:
  Feldstein, M.M., I.M. Raigarodskii, A.L. Iordanskii, and J. Hadgraft. "Modeling of percutaneous drug transport in vitro using skin-imitating Carbosil membrane." Journal of Controlled Release 52 (1998): 25-40. (Netherlands)

  ➢ *For a book:*
  Author Name. Title. Place: Publisher, Copyright Year.

  Book Example:
  Ebbing, Darrell D. General Chemistry. Boston: Houghton Mifflin Company, 1996.

- Please do not abbreviate the titles of journals or other publications.
- Please do not include abstracts from conferences and conference proceedings. Such abstracts should be cited in Section VI, Conference Presentation List.
- If you include items that have been submitted for publication but have not yet been accepted for publication, please clearly mark these items as "submitted for publication."

BIBLIOGRAPHY OF PROJECT-RELATED PUBLICATIONS

1. Haroutiunian S., Vardanyan V., Dalyan Y.- The peculiarity of complexation of DNA macromolecule with low molecular ligands, - Scientific Reports of YSU, (2004), v.3, No. 205, p. 3-34. (Russian).

2. Arakelyan V.B., Haroutiunian S.G., Abgaryan H.H., Arakelyan A.V.,.Haroutunyan T.S – Ligand Binding With Continuous Modification of Binding Sites. -J.Biomol.Struct.& Dyn., (2004), v.22, No.2, p.245-251.

3. D. Y. Lando, A. S. Fridman and R. Wartell. Thermodynamics of DNA Containing Very Stable Chemically Modified Base Pairs. *J. Biomol. Struct. & Dynam.* (2003) 20(4) 533-546.

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7. Dalyan Y.B., Fridman A.S., Lando D.Y., Vardanyan V.I. "On the mechanism of increase of DNA stability after exposure to low dose of radiation and its decrease at high dose." Proceedings of The International Scientific Conference "Unification and Optimization of Radiation Monitoring on NPP Location Regions ("URM-2004"), sponsored by ISTC, Yerevan, Sept.22-26, 2004, p.30-39.

8. D. Y. Lando, A. S. Fridman, S. G. Haroutiunian, V. I. Vardanyan. "High temperature thermodynamics of deoxyribonucleic acid chemically modified with antitumor metal-based compounds". Proceedings of The International Scientific Conference "Molecular, Membrane and Cellular Basis of Functioning of Biological Systems" and of the VI Congress of Byelorussian Biophysical Society. (October 6-8, 2004, Minsk, Belarus). V.2, p. 74-76. (Russian).

9. E. N. Galyuk, D. Y. Lando, V. P. Egorova, S. G. Haroutiunian, V. I. Vardanyan. "About strengthening of DNA double helix destabilization by cisplatin in alkaline medium". Proceedings of The International Scientific Conference "Molecular, Membrane and Cellular Basis of Functioning of Biological Systems" and of the VI Congress of Byelorussian Biophysical Society. (October 6-8, 2004, Minsk, Belarus). V.2, p. 18-20. (Russian).

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SECTION VI: Conference Presentation List

Award Number: NFSAT MB 078-02/CRDF 12027

1. Did any members of your team give project-related conference presentations during the grant period?
   Yes ☒ No ☐

2. If "Yes," how many presentations? 13
   Please list below, using the instructions provided

3. If you do not have any project-related conference presentations to cite, please provide a brief explanation and describe any future plans for conference presentations:

INSTRUCTIONS

- **Format:** Please use the following format to list conference presentations:

  Presenter's Name(s). "Presentation Title, (Type of Presentation*), Conference/Workshop Name, Dates of Conference, Location of Conference.

  Example:

  Iordanskii, A. L. "Diffusion Modeling of the Propranol Drug Delivery from a Hydrophilic Transdermal Therapeutic System," (Oral Presentation), Third Spanish-Portuguese Conference on Controlled Drug Delivery, September 6-9, 1998, Lisbon, Portugal.

For "Type of Presentation," please indicate either "Oral Presentation," "Poster Presentation," or "Abstract."

LIST OF PROJECT-RELATED CONFERENCE PRESENTATIONS

1. Sargsyan S., Dalyan Y., Abgaryan H., Chalikian T., Haroutiunian S. "Melting of DNA Complexes with Cis-Diaminedichlorplatinum(II) at Acidic pH." (Poster Presentation) In Abstracts of HUPO 2nd Annual & IUBMB XIX World Congress, Molecular & Cellular Proteomics (2003) v. 2, No.9, p.971.
2. Dalyan Y., Karapetyan N., Haroutiunian S., Ananyan G., Aloyan L., Vardanyan V., Chalikian T. "Binding of New Meso-Tetra-(3N-Pyridil) Porphyrins to DNA." (Poster Presentation) In Abstracts of HUPO 2nd Annual & IUBMB XIX World Congress, Molecular & Cellular Proteomics (2003) v. 2, No.9, p.972.
3. Gyulkhandanyan, G., Ghambaryan, S., Aroutiunian, R., Hovhannisyam, G., Haroutiunian, S. "Cytotoxic and Genotoxic Effects of Action of Porphyrins and their Metal Complexes on Human Blood Lymphocytes." (Poster Presentation) In Abstracts of HUPO 2nd Annual & IUBMB XIX World Congress, Molecular & Cellular Proteomics (2003) v. 2, No.9, p.984.
4. Dalyan Y.B., Fridman A.S., Lando D.Y., Vardanyan V.I. "On the mechanism of increase of DNA stability after exposure to low dose of radiation and its decrease at high dose." (Oral Presentation) The International Conference "Unification and Optimization of Radiation Monitoring on NPP Location Regions, sponsored by ISTC, Yerevan, Sept.22-26, 2004, p.30-39.
5. D. Y. Lando, A. S. Fridman, S. G. Haroutiunian, V. I. Vardanyan. "High temperature thermodynamics of deoxyribonucleic acid chemically modified with antitumor metal-based compounds". (oral presentation). The International Scientific Conference "Molecular, Membrane and Cellular Basis of Functioning of Biological Systems" and of the VI Congress of Byelorussian Biophysical Society. (October 6-8, 2004, Minsk, Belarus). V.2, p. 74-76. (Russian).

6. E. N. Galyuk, D. Y. Lando, V. P. Egorova, S. G. Haroutiunian, V. I. Vardanyan. "About strengthening of DNA double helix destabilization by cisplatin in alkaline medium". (Poster presentation). The International Scientific Conference "Molecular, Membrane and Cellular Basis of Functioning of Biological Systems" and of the VI Congress of Byelorussian Biophysical Society. (October 6-8, 2004, Minsk, Belarus). V.2, p. 18-20. (Russian).
7. Ghazaryan A.A., Dalyan Y.B., Haroutiunian S.G., Chalikian T.V. "RNA binding by novel porphyrins: role of peripheral substituents." (Poster Presentation) Int. Symposium on Hydration and Thermodynamics of Molecular Recognition, Tsakhkadzor, Armenia, Mar. 1-5, 2005. (Submitted)
8. Ghazaryan A.A., Dalyan Y.B., Haroutiunian S.G., Chalikian T.V.- "Binding of TAlPyP(4) and AgTAlPyP(4) porphyrins to double helical RNAs." (Poster Presentation) Int. Symposium on Hydration and Thermodynamics of Molecular Recognition, Tsakhkadzor, Armenia, Mar. 1-5, 2005. (Submitted)
9. Ananyan G.V., Vardanyan V.I., Dalyan Y.B., Haroutiunian S.G. "Interaction of Co-contaning meso-tetra-(N-pyridyl) porphyrins with DNA." (Poster Presentation) Int. Symposium on Hydration and Thermodynamics of Molecular Recognition, Tsakhkadzor, Armenia, Mar. 1-5, 2005. (Submitted)
10. Aloyan L.R., Vardanyan V.I., Dalyan Y.B. "Interaction of Zn containing meso-tetra-(3N-pyridyl) porphyrins with DNA. Effect of peripheral substituent." (Poster Presentation) Int. Symposium on Hydration and Thermodynamics of Molecular Recognition, Tsakhkadzor, Armenia, Mar. 1-5, 2005. (Submitted)
11. Barkhudaryan V., Dalyan Y., Karamyan K., Ananyan G. "Viscometric investigation of interaction of some porphyrins with DNA." (Poster Presentation) Int. Symposium on Hydration and Thermodynamics of Molecular Recognition, Tsakhkadzor, Armenia, Mar. 1-5, 2005. (Submitted)
12. Lando D. Y., Fridman A. S., Haroutiunian S. G, Vardanyan V. I. "Helix-coil Transition of Crosslinked DNA. On the Two Types of Crosslinking Agents." Symposium on Hydration and Thermodynamics of Molecular Recognition, Tsakhkadzor, Armenia, Mar. 1-5, 2005. (Submitted)
13. Bourdelat-Parks, B. and Wartell, R.M. " Thermodynamics of DNA and RNA hairpins containing tandem mismatches." Biophysics J. 84, 142a, 2003. Biophysical Society Annual Meeting San Antonio, TX March 1-4, 2003.

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SUPPLEMENTAL INFORMATION (optional)

Award Number: NFSAT MB 078-02/CRDF 12027

The CRDF and NFSAT appreciate receiving supplemental information, such as **photographs, publicity articles, publication copies, Power Point presentations, or other materials.** Please send such materials directly to the CRDF and NFSAT contacts listed in the General Instructions on page 2.

If you submit photographs, please be sure to identify all persons pictured and indicate their roles in the BGP project. Please be aware that unless you indicate otherwise, CRDF and NFSAT reserve the right to use photographs and other materials above in publicly distributed CRDF and NFSAT documents.

Please check this box if you are submitting any photographs, publications, or other materials along with your final report.

☐

LIST OF SUPPLEMENTAL INFORMATION SUBMITTED

Thank you.

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APPENDIX
CODES FOR WEAPONS RESEARCH

INSTRUCTIONS

This code list is to be used in Section III, Armenian Team Data, to identify project participants who are currently or were formerly actively engaged in research at a current or former defense laboratory or institution. Please fill in the code listed below which corresponds most closely to the *primary* area of the participant's weapons experience.

CATEGORY A: MISSILE TECHNOLOGY EXPERTS	
CODE	**DESCRIPTION**
A1	Design, construction and performance of air, space, surface and underwater - launched missiles. Materials and technologies for these missiles. Production of engines, fuels, composites, integrated elements, radio-electronic equipment, different testing devices for missiles.
A2	Techniques for guidance and control of missiles from launching to impact. Includes optical guidance, television guidance, wire guidance, present and terminal guidance, internal guidance, command guidance, and homing guidance.
A3	Missile handling and launching, including transportation, storage, and preparation for launching. Air, space, surface and underwater launching and support equipment and technologies; Checkout equipment and procedures. Guided missile ranges.
A4	Techniques and systems for tracking missiles as defensive measures. Can be from surface installations or air and space-borne platforms.
CATEGORY B: CHEMICAL WEAPONS EXPERTS	
B1	Design and performance of missile warheads and rockets for delivery of chemical weapons.
B2	Materials, facilities and performance processes needed for the production of chemical weapon agents and their key precursors.
B3	Dissemination of chemical weapon agents.
B4	Basic knowledge on CW design and their effect on human system.
CATEGORY C: BIOLOGICAL WEAPONS EXPERTS	
C1	Design and performance of missile warheads and rockets for delivery of biological weapons.
C2	Biopolymer production related to biological warhead capabilities.
C3	Dissemination of biological weapon agents.
C4	Basic knowledge on BW design and their effect on human system.
CATEGORY D: NUCLEAR WEAPONS EXPERTS	
D1	Basic knowledge of Nuclear Weapons design, construction, characteristics and the effect on human system.
D2	Design, construction and performance of missile warheads for delivery of nuclear weapons.
D3	Design, construction and performance of the equipment and Components for Uranium and Plutonium separation.
D4	Design, construction and performance of the equipment connected with Heavy Water Production.
D5	Design, construction and performance of the equipment for Development of Detonators.
D6	Design, construction and performance of Explosive Substances and Related Equipment.
D7	Design, construction and performance of the equipment and Components for Nuclear Testing.
D8	Design, construction, performance and operation of production-type nuclear reactors for fissile and tritium-content materials production (breeding).
D9	Design, construction, performance of nuclear reactors and units for submarine and for military space program.
CATEGORY E: OTHER	
E1	Design, construction, and performance of powerful laser facilities for military applications.
E2	Design, construction and performance of accelerator facilities for military applications in space programs.
E3	Others

GEORGIA TECH RESEARCH CORPORATION

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