NCATS is pleased to offer this valuable resource of sample applications to the small business community. Seeing how successful applicants have presented their ideas brings a wholly new perspective to our own application preparation. In that spirit, with the gracious permission of successful grant applicants, NCATS and other Institutes at NIH provide below samples of funded applications, and summary statements, data or resource sharing plans, leadership plans and more.

Always follow your funding opportunity's instructions for application format. Although these applications demonstrate good grantsmanship, time has passed since these grant recipients applied. The samples may not reflect the latest format or rules.

PI: Personal Info	Title: Development of a Gene and Oligonucleotide Delivery System	
Received: 12/05/2014	Opportunity: PA-14-308	Council: 05/2015
Competition ID: FORMS-C	FOA Title: PLATFORM DELIVERY TECHNOLOGIES FOR NUCLEIC ACID THERAPEUTICS (R41/R42)	
1R41TR001338-01	Dual: EB	Accession Number: 3769420
IPF: 3719901	Organization: MOLECULAR EXPRESS, INC.	
Former Number:	Department:	
IRG/SRG: ZRG1 IMST-S (12)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: Itemized Cost	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Personal Info		PD/PI
		Co-Investigator
		Other (Specify)-Collaborator
		Other (Specify)-Collaborator

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*
❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED 2014-12-05

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: Proprietary Info

Legal Name*: Molecular Express, Inc.
Department:
Division: Proprietary Info
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:

Person to be contacted on matters involving this application
Prefix: Personal Info First Name*: Personal Info Middle Name: Last Name*: Personal Info Suffix: Personal Info
Position/Title: President & CEO
Street1*: Personal Info
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:
Phone Number*: Personal Info Fax Number: Personal Info Email: Personal Info

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** Proprietary Info

7. TYPE OF APPLICANT* R: Small Business

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*
● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Development of a Gene and Oligonucleotide Delivery System

12. PROPOSED PROJECT
Start Date* 07/01/2015
Ending Date* 06/30/2016

13. CONGRESSIONAL DISTRICTS OF APPLICANT
37th

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Personal Info First Name*: Personal Info Middle Name: Last Name*: Personal Info Suffix: Personal Info

Position/Title: President & CEO

Organization Name*: Molecular Express, Inc.

Department:

Division: Personal Info

Street1*:

Street2:

City*:

County:

State*:

Province:

Country*:

ZIP / Postal Code*:

Phone Number*: Personal Info Fax Number: Personal Info Email*: Personal Info

15. ESTIMATED PROJECT FUNDING

	Itemized Cost
a. Total Federal Funds Requested*	
b. Total Non-Federal Funds*	
c. Total Federal & Non-Federal Funds*	
d. Estimated Program Income*	

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Personal Info First Name*: Personal Info Middle Name: Last Name*: Personal Info Suffix:

Position/Title*: Secretary & COO

Organization Name*: Personal Info

Department:

Division:

Street1*:

Street2:

City*:

County:

State*:

Province:

Country*:

ZIP / Postal Code*: Personal Info

Phone Number*: Personal Info Fax Number: Personal Info Email*: Personal Info

Signature of Authorized Representative*

Personal Info

Date Signed*

12/05/2014

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name: 1235-CvrLtr EVLP STTR I Final 5-Dec-2014.pdf

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Molecular Express, Inc.
Duns Number: Proprietary Info
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:
Project/Performance Site Congressional District*: Proprietary Info

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Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of California Los Angeles
DUNS Number: Proprietary Info
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:
Project/Performance Site Congressional District*: Proprietary Info

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File Name

Additional Location(s)

Funding Opportunity Number: PA-14-308. Received Date: 2014-12-05T17:09:23.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ○ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	1236-PrjSum EVLP STTR I Final 5-Dec-2014.pdf
8. Project Narrative*	1237-PblcHlthRel EVLP STTR I Final 4-Dec-2014.pdf
9. Bibliography & References Cited	1238-Refs EVLP STTR I Final 5-Dec-2014.pdf
10.Facilities & Other Resources	1239-FacRes EVLP STTR I Final 5-Dec-2014.pdf
11.Equipment	1240-EqptRes EVLP STTR I Final 24-Nov-2014.pdf
12. Other Attachments	1241-Proprietary Info Registration Confirmation.pdf

Funding Opportunity Number: PA-14-308. Received Date: 2014-12-05T17:09:23.000-05:00

Project Summary

The knockdown of targeted genes by anti-sense oligonucleotides (ODNS) and genetc medicines (collectively, G-MEDS) holds promise for a variety of therapies. The delivery of effective quantities of ODNS to specific cells, however, has proved to be challenging. We propose here a novel approach to ODN delivery that involves *enveloped virus-like-particles (EVLPs)*. These delivery agents are prepared by the in vitro self-assembly of pure G-MEDS and pure viral capsid protein (CP) into virus-like nanoparticles particles (VLPs). The capsid protects the contents yet, as we have demonstrated, is capable of giving up its contents in the cytoplasm of mamalian cells. The particles, which are highly monodisperse, are then enveloped by lipid bilayers that can suppress the immunogenicity of the VLPs and are capable of being functionalized for targeting and uptake by mammalian cells of interest.

In preliminary experiments, we have demonstrated our ability to prepare EVLPs using the CP of the cowpea chlorotic mottle virus and a model antisense ODN for vascular endothelial growth factor (VEGF), a protein over-expressed in many cancer cells to stimulate angiogenesis a facilitate tumor survival under low-oxygen conditions. We propose to optimize the assembly and to functionalize the lipid bilayers with epidermal growth factor (EGF), which binds the EGF receptor that is over-expressed on cancer cells, especially those of breast cancer. The effectiveness of the EVLP will be demonstrated by assaying the reduction in the secretion of VEGF in cultured breast cancer cells.

Public Health Relevance

The development of the proposed system for delivering genetic medicines such as genes and oligonucleotides would be an important medical advance. Its development would facilitate the targeted delivery of genetic medicines to specific cells, tissues and organs, thus enabling the development of healthcare products which will exert a significant impact on the practice of medicine.

Facilities and Resources

Overview of the scientific environment

This STTR Phase I application benefits greatly from the collaboration between the Company and the University of California Los Angeles (UCLA) by providing access to extensive and well-developed facilities (laboratory bench space, specialized equipment, technical support, computer systems, and office space) at multiple sites. This rich environment provides the necessary sophisticated facilities and resources to successfully complete the Aims proposed in this application. As part of our ongoing collaboration, the investigative and commercialization team has already established effective lines of scientific communication/interaction between the investigators at the different sites, together with established procedures for scientific study coordination and data interchange. Thus, we are strongly positioned to take optimal advantage of all available facilities immediately upon funding. Specific facilities, resources, and equipment to be made available to the proposed project are detailed below at each physical location: Molecular Express, Inc. as the primary applicant organization, and UCLA as the collaborating institution.

Facilities and resources at Molecular Express

The R&D laboratory facilities for Molecular Express, Inc, recently re-located into a Square Footage building at Proprietary Info which is shared by the parent company, Molecular GPS Technologies, and sister companies, BioCerax and Clayton Chemical. This building houses support staff for typical office functions, accounts and project management, grant management, and personnel records.

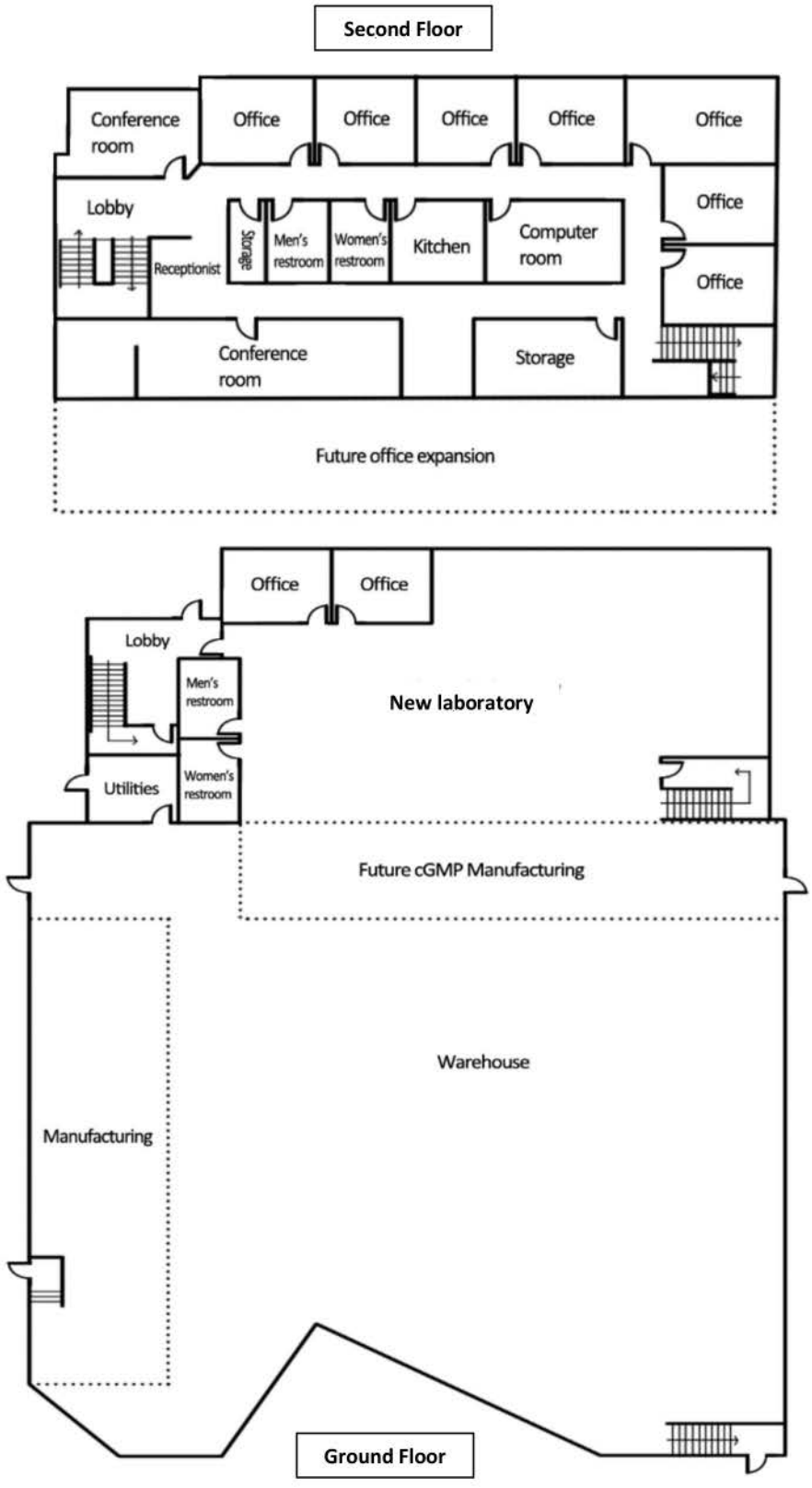
Newly renovated laboratory facilities available to conduct the studies proposed in this application include approximately Square Footage of wet bench space, Square Footage tissue culture space, and Square Footage of equipment space. Included in the overall facility is office and general work space including conference rooms, lunch areas and open warehouse space for future expansion (see schematic diagram of the facility below). Laboratory and technical facilities at Molecular Express fully support the R&D and production activities to be performed by the Company in this proposal, including recombinant protein expression and purification, liposomal formulation of biologically active molecules, QA/QC assay development, cytotoxicity testing, and product stability testing. Major lab equipment and facilities for protein expression and characterization are as follows: two climate controlled incubators with shaking platform, an oxygen generator, a GE Wave™ rocker with heat and oscillation control that supports 25 L fermentation runs and a M-110 microfluidizer with a dry-air compressor. Added equipment and facilities for purifying, formulating, and characterizing products at Molecular Express include: FPLC and HPLC chromatography systems (three analytical and one semi-preparative), a chromatography cabinet, a newly acquired Yamato spray dryer, a liposome extrusion apparatus, a light-scatter ultra-fine particle analyzer, an ultra-filtration apparatus, super-speed and ultra-speed centrifuges, a microfuge, a refrigerated table-top centrifuge, a roto-evaporator, sonicators (flow-through, probe, and bath), a UV spectrophotometer, a bio-safety cabinet, laminar flow and chemical hoods, electrophoresis equipment, a CO_2 incubator, an Axiovert inverted fluorescent microscope with digital camera, PCR equipment, a computer integrated Gel Documentation system, a compound microscope, and a full complement of freezers, refrigerators, and general lab equipment. A soft-wall clean room is present for the manufacture of products for pre-clinical or clinical use. Both lab and office space has a computer network providing software for word processing, relational databases, statistical programs, internet access, reference retrieval and Genebank/EMBL comparisons. All company facilities are secured by a card key entry system.

Molecular Express laboratory and administrative personnel have extensive industry and commercial experience and possess sufficient resources to conduct the proposed activities efficiently. Molecular Express

also has the appropriate combination of business expertise, personnel, and technical facilities necessary to plan and execute the most appropriate commercialization strategy during or following completion of the proposed activities.

Biohazards

The Molecular Express facilities are equipped to handle BSL1 and BSL2 microbial organisms. The Company itself does not conduct animal experiments in its facilities; it relies exclusively on its collaborators to conduct these studies. The Company does maintain standard operating procedures for the proper handling and disposal of biohazardous waste. Samples are stored in approved containers and disposed of through an outside contractor.



Molecular Express

Proprietary Info

December 2014

Facilities and resources at UCLA (Personal Info

Professors Personal Info and Personal Info have a Square Footage biochemistry laboratory, and a Square Footage plant growth room where they regularly grow, infect, and purify virus from cowpea plants, both on the 2nd floor of the Young Hall Chemistry building. The laboratory is equipped with basic equipment such as a chemical hood, a biological safety cabinet, centrifuges, 4°C, -20°C and -80°C freezers, a CO_2 incubator, ultracentrifuges, thermal cyclers, shakers, pH meters, gel electrophoresis apparatus, a liquid chromatograph, and a sonicator. Shared departmental facilities that are regularly used include a cold room, autoclaves and a Biochemistry Instrumentation Facility that includes imagers, a spectrofluorimeter, UV-Vis spectrophotometer, Agilent 2100 Bioanalyzer and an analytical ultracentrifuge.

There is also full access to the core facilities at the UCLA California Nanosystems Institute (CNSI) just across from Young Hall, which includes the Advanced Light Microscopy/Spectroscopy Facility (Wide-Field Fluorescence Imaging Microscopy, Confocal One- and Two-Photon Laser Scanning Microscopy, Total Internal Reflection Microscopy, FRET and FCS) and the Electron Imaging Center (Cryo-Electron Microscopy and Tomography, and Scanning Transmission Electron Microscopy). All of students in the Personal Info grouop receive the requisite training from the CNSI staff, and - accordingly - have regular and frequent hands on access to all of this equipment.

The Personal Info laboratory has exclusive access to two GPU-computing-capable computers; these Nvidia Tesla modules contain hundreds of processing cores which – together with CUDA parallel programming toolkits and the Open MM library – allow for up to 50-fold speedups of our simulations. There is also shared access to the UCLA central Hoffman2 computing cluster (with 256 processor cores) for non-GPU accelerated applications such as cryo-EM image analysis of RNA and RNA-capsid protein complexes.

Equipment

The extensive research equipment available for this SBIR research project at Molecular Express, Inc. and at the collaborating institution (UCLA) is detailed and discussed in the context of the overall Facilities and Resources section of the proposal. Please see the section on **Facilities and Resources** for specific equipment items.

U.S. Small Business Administration

SBIR.gov SBC Registration Control ID Form

Company Information

SBC Control ID	Proprietary Info				
Company Name	Molecular Express, Inc.				
Address	Proprietary Info				
City	Proprietary Info	**State**	Proprietary Info	**Zip**	Personal Info
TIN/EIN	Proprietary Info	**DUNS**	Proprietary Info		
Company URL	www.molecularexpress.com				
Number of Employees	# OF EMPLOYEES				
Is this SBC majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?	No				
What percentage (%) of the SBC is majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?	0%				

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Personal Info First Name*: Personal Info Middle Name Last Name*: Personal Info Suffix: Ph.D

Position/Title*: President & CEO
Organization Name*: Molecular Express, Inc.
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Personal Info

Phone Number*: Personal Info Fax Number: Personal Info E-Mail*: Personal Info

Credential, e.g., agency login: eRA Commons User Name

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1991

Attach Biographical Sketch*: File Name: 1245-NIH Bio Personal Info EVLP STTR I 4-Dec-2014.pdf

Attach Current & Pending Support:

Personal Info

PROFILE - Senior/Key Person

Prefix: First Name*: Personal Info Middle Name Personal Info Last Name*: Personal Info Suffix:

Personal Info

Position/Title*: Professor
Organization Name*: University of California Los Angeles
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Personal Info

Phone Number*: Personal Info Fax Number: E-Mail*: Personal Info

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: Personal Info Degree Year: Personal Info

Attach Biographical Sketch*: File Name: 1246-NIH Bio Personal Info EVLP STTR I 5-Dec-2014.pdf

Attach Current & Pending Support:

Funding Opportunity Number: PA-14-308 . Received Date: 2014-12-05T17:09:23.000-05:00

Contact PD/PI: Personal Info

PROFILE - Senior/Key Person

Prefix: Personal Info First Name*: Personal Info Middle Name: Personal Info Last Name*: Personal Info Suffix: Personal Info

Position/Title*: Director of Chemistry
Organization Name*: Molecular Express, Inc.
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Personal Info

Phone Number*: Personal Info Fax Number: Personal Info E-Mail*: Personal Info

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Personal Info Degree Year: Personal Info

Attach Biographical Sketch*: File Name: 1247-NIH Bio Personal Info EVLP STTR I 4-Dec-2014.pdf

Attach Current & Pending Support:

PROFILE - Senior/Key Person

Personal Info

Prefix: First Name*: Personal Info Middle Name: Personal Info Last Name*: Personal Info Suffix: Personal Info

Position/Title*: Professor
Organization Name*: University of California Los Angeles
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Personal Info

Phone Number*: Personal Info Fax Number: E-Mail*: Personal Info

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: Personal Info Degree Year: Personal Info

Attach Biographical Sketch*: File Name: 1248-NIH Bio Personal Info EVLP STTR I 5-Dec- 2014.pdf

Attach Current & Pending Support:

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Personal Info	President and CEO Molecular Express, Inc.
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of California Irvine	B.S.	1979	Biological Sciences
University of California Irvine	B.S.	1981	Phys Sciences (Chem)
University of California Los Angeles	Ph.D.	1991	Physical Chemistry

A. Personal Statement

As President and CEO of Molecular Express, Inc., I am delighted to assume the role of Principal Investigator for the proposed STTR Phase I application submitted in response to PA-14-308 – "Platform Delivery Technologies for Nucleic Acid Therapeutics" to develop a gene and oligonucleotide delivery vehicle that can be easily customized to address a particular medical need. I have an extensive background as a life scientist and entrepreneur with a focus on facilitating the translation of scientific discoveries from academia into the commercial sector. I am also an experienced biophysical chemist with liposome-based drug development expertise; my previous experience at Vestar/NeXstar/Gilead developing and commercializing approved liposome products such as Ves**C**an®, Am**B**isome® and Dauno**X**ome® will be of value to this project. As previously described in a review article (see reference 11 below), the four major barriers that needed to be overcome in order to develop an effective molecular delivery system for genetic medicines were outlined. For the last 15 years, I have been focused on addressing these barriers by developing highly efficient methods for encapsulating genetic medicines into lipid-based carriers, and engineering hydrophobic domain proteins, which can be fused to antigens or targeting ligands using standard recombinant techniques, that are water-soluble, yet stably incorporate into lipid bilayers. I have also designed lipid-based anchors that can provide even more functionality to liposomal carriers. In this STTR project, I will continue to collaborate with Personal Info Personal Info and Personal Info with whom we have been working on developing the EVLP technology for the past 15 years. While our progress has been hampered by a lack of funds that directly support our efforts to achieve the goal of designing and engineering the EVLP gene delivery system proposed in this application, we are optimistic that we can accelerate and possibly, even complete the development of the proposed EVLP delivery vehicle through PA-14-308.

B. Positions and Honors

Experience and Employment

1981-1982	Staff Chemist	Occidental Petroleum Corp., Irvine, CA
1982-1984	Analytical Chemist	IT Analytical Services, Cerritos, CA
1984-1986	Mgr, NMR Service Center	Vestar, Inc., Pasadena, CA
1986-1990	Independent Consultant	Vestar, Inc., Pasadena, CA Huntington Memorial Research Institutes, Pasadena, CA Carson Environmental Corporation, Los Angeles, CA
1991-1996	Director, Gene Delivery	NeXstar Pharmaceuticals, Inc., San Dimas, CA
1991-1993	Postdoctoral Scholar	University of California Los Angeles
1993-1996	Visiting Scientist	University of California Los Angeles
1996-present	President and CEO	Molecular Express, Inc., Los Angeles, CA
2000-present	Board of Directors	Southern California Biomedical Council
2001-2006	Editorial Board Member	Pharmaceutical Research

National Institutes of Health Grants and Contracts Review Panel Consultant Activities

2003	RFA-AI-02-026	Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics Immunotherapeutics and Diagnostics for Biodefense

2003	RFA-AI-02-031	Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases (RCE)
2004	NOT-AI-04-044	Biodefense Countermeasure Development: Project Bioshield (Chairman)
2004	RFP-NIAID-DMID-04-17	Development, Testing and Evaluation of Candidate Vaccines Against Plague
2004	RFP-NIAID-DMID-04-40	In Vitro and Animal Models for Emerging Infectious Diseases and Biodefense
2006	ZRG1 RES-A 10 B	Small Business: Respiratory Sciences
2007	RFP-NIAID-DMID-07-05	Development of a Third Generation Anthrax Vaccine
2008	ZRG1 CB-D (10)	Small Business: Intercellular Interactions
2008	RFA-AI-08-022	Regional Centers for Excellence in Biodefense and Emerging Infectious Diseases (Chairman, Vaccines sub-committee)
2009	ZRG1 CB-B (10) B	Small Business: Cell Biology
2009	RFA-AI-08-044	Sexually Transmitted Infections Cooperative Research Centers
2009	RFA-08-055	Innovative Approaches to Target Identification and Assay Development for Fungal Diagnosis
2009	NIH-RFA-OD-09-003	ARRA Challenge Grants (RC1): Panels 3 and 4
2009	ZRG1 IMST A-17	Small Business: Cell Biology and Instrumentation
2009	VMD	Vaccines Against Microbial Diseases
2010	VMD	Vaccines Against Microbial Diseases (regular member)
2010	RFA-AI-09-027	Partnerships for Biodefense Food- and Water-Borne Diseases (R01)
2010	ZRG1 IMM-G (12) B	Small Business: Microbial Vaccine Development
2010	RFA-EB-09-003	NIBIB Quantum Projects: Implementation Phase II (U01)
2010	ZAI1-QV-M-J1	Clinical Trial Planning and Implementation Grants
2011	VMD	Vaccines Against Microbial Diseases (regular member)
2012	VMD	Vaccines Against Microbial Diseases (regular member)
2012	ZAI1-FDS-M (C1)	Manufacture and Characterization Services for Vaccines and Other Biologics for Infectious Diseases (Chairman)
2012	ZAI1-JTS-M (M1)	NIAID Clinical Trial Planning and Implementation
2013	VMD	Vaccines Against Microbial Diseases (regular member)
2013	ZDA1-JXR-D (10) 1	Strategic Alliances for Medications Development to Treat Substance Use Disorders (R01)
2014	VMD	Vaccines Against Microbial Diseases (regular member)
2014	ZAI1-QV-I-C1	Adjuvant Discovery Program
2014	ZAI1-EC-M-C1	Development of Vaccine Formulations Effective Against NIAID Priority Pathogens
2014	PAR-13-146	NCI Exploratory/Developmental Research Grant Program
2014	PAR-14-007	NCI Small Grants Program for Cancer Research

C. Publications

Selected peer reviewed journal articles and book chapters

1. Ernst WA, Kim HJ, Tumpey TM, Jansen ADA, Tai W, Cramer DV, Adler-Moore JP, and **Fujii G.** (2006) *Protection against H1, H5, H6 and H9 influenza A infection with liposomal matrix 2 epitope vaccines.* Vaccine **24(24):**5158-5168. PMID: 16713037
2. **Fujii G**, Ernst W, Adler-Moore J. (2008) *The VesiVax System: A method for rapid vaccine development.* Frontiers in Bioscience. **13:**1968-1990. PMID: 17981684
3. Olson K, Macias P, Hutton S, Ernst WA, **Fujii G**, and Adler-Moore J.P. (2009) *Liposomal gD ectodomain (gD1-306) vaccine protects against HSV2 genital or rectal infection of female and male mice.* Vaccine **28(2):**548-560. PMID: 19835825
4. Adler-Moore J, Munoz M, Kim H, Romero J, Tumpey T, Zeng H, Petro C, Ernst W, Kosina S, Jimenez G, and **Fujii G**. (2011) *Characterization of the murine Th2 response to immunization with liposomal M2e influenza vaccine.* Vaccine. **29(27):** 4460-4468. Epub May 3, 2011 PMID: 21545821
5. Lockner JW, Ho, SO, McCague KC, Chiang SM, Do TQ, **Fujii G**, and Janda KD (2013). *Enhancing nicotine vaccine immunogenicity with liposomes.* Bioorganic & Medicinal Chemistry Letters **23**:975-978.
6. **Fujii G**, Horvath S, Woodward S, Eiserling F, and Eisenberg D. (1992) *A molecular model for membrane fusion based on solution studies of an amphiphilic peptide from HIV gp41.* Protein Science **1:**1454-1464. PMID: 1303764

7. **Fujii G**, Selsted M, and Eisenberg D. (1993) *Defensins promote fusion and lysis of negatively charged membranes.* Protein Science **2:**1301-1312. PMID: 8401215
8. Weakliem CL, **Fujii G**, Chang J-E, Ben-Shaul A, and Gelbart WM. (1995) *Effect of tension on pore formation in drug-containing vesicles.* J. Phys. Chem. **99:**7694-7697.
9. **Fujii G.** (1996) *Liposomal amphotericin B (AmBisome): Realization of the drug delivery concept.* Vesicles. M. Rosoff, ed. Marcel Dekker Inc., New York, New York.
10. **Fujii G**, Chang J-E, Coley T, and Steere B. (1997) *The formation of amphotericin B ion channels in lipid bilayers.* Biochemistry **36:**4959-4968. PMID: 9125518
11. **Fujii G.** (1999) *To fuse or not to fuse: The effects of electrostatic interactions, hydrophobic forces, and structural amphiphilicity on protein-mediated membrane destabilization.* Adv. Drug Del. Rev. **38:**257-277. PMID: 10837760
12. Wu G-D, Johnson E, **Fujii G,** Swensson J, Oakley O, Cramer DV. (1999) *Antibodies to Forssman Antigen do not induce rejection of mouse cardiac xenografts in rats.* Xenotransplantation **6:**90-97. PMID: 10431785
13. Swenson S, Costa F, Minea R, Sherwin R, Ernst W, **Fujii G**, Yang D. and Markland FS. (2004) *Intravenous liposomal delivery of the snake venom disintegrin contortrostatin limits breast cancer progression.* Mol. Cancer Ther. **3(4):**1-13. PMID: 15078994
14. Swenson S, Costa F, Ernst W, **Fujii G,** and Markland FS. (2005) *Contortrostatin, a snake venom disintegrin with anti-angiogenic and anti-tumor activity.* Pathophysiol. Haemost. Thromb. **34:**169-176. PMID: 16707922
15. Do, TQ, Moshkani S, Castillo P, Anunta S, Pogosyan A, Cheung A, Marbois B, Faull KF, Ernst W, Chiang SM, **Fujii G**, Clarke CF, Foster K, Porter E. (2008) *Lipids Including Cholesteryl Linoleate and Cholesteryl Arachidonate Contribute to the Inherent Antibacterial Activity of Human Nasal Fluid.* J. Immunol. **181(6):**4177-4187. PMID: 18768875

Patents

1. Eley C., Schmidt P., and **Fujii G.** *Delivery vehicles with amphiphile-associated active ingredient.* Canadian #1,319,614 (June 29, 1993); United States #5,320,906 (June 14, 1994).
2. **Fujii G**, Schmidt P, and Gamble R. *Composition and method of use for liposome encapsulated compounds for neutron capture tumor therapy.* Canadian #1,314,209 (March 9, 1993); European #1386146 (April 28, 1992); United States #5,328,678 (July 12, 1994).
3. **Fujii G**, and Huff L. *Method of Rejuvenating Rubber Printing Blankets.* United States #5,326,590 (July 5, 1994).
4. **Fujii G**, and Fujii DR. *Odorless fixing solution.* United States #5,932,400 (August 3, 1999).
5. PATENT PENDING
6. **Fujii G.** *Nail Polish Remover.* United States #7,074,746 B2 (July 11, 2006).
7. **Fujii G**, Szoka FC, Watson DS. *Methods and Compositions for Liposomal Formulations of Antigens and Uses Thereof.* United States #8,765,171 (July 1, 2014).
8. PATENT PENDING
9. PATENT PENDING

D. Research Support

Active Research Support

"*Targeted Dendritic Cell Activation through Multi-Adjuvant Liposomes*"
Role: Co-Investigator EFFORT NO COST EXTENSION
Agency: NIH/NIAID *Type:* SBIR (5R43AI094891-02) *Period:* 3/1/2012-2/28/2014
The aim of this Phase I SBIR project is demonstrate that a recombinant form of CD40L can be designed and engineered which targets the CD40 receptor on dendritic cells to improve the immune response to an antigen.

"*Formulation of TLR 3 and 7/8 Agonists in Conjugatable Adjuvant Lipid Vesicles*"
Role: Co-Investigator EFFORT NO COST EXTENSION

Agency: NIH/NIAID *Type:* SHIFT SBIR (5R43AI94770-02) *Period:* 8/1/2012 - 7/31/2014
The aim of this SBIR project is develop VesiVax® CALV formulations containing TLR3 and TLR7/8 agonists.

"*Development of a Novel SP-B Peptide Lung Surfactant*"
Role: Principal Investigator EFFORT
Agency: NIH/NHLBI *Type:* SBIR (5R44HL-080775-05) *Period:* 8/1/2012 - 7/31/2015
This project is focused on commercializing a synthetic lung surfactant product based on the development of the methods for chemical synthesis, formulation, and quality control of SP-B peptides dispersed in lipids.

"*Development of VesiVax Carbohydrate Conjugation Chemistry*"
Role: Principal Investigator EFFORT NO COST EXTENSION
Agency: NIH/NIAID *Type*: SBIR (1R43AI108120-01) *Period*: 8/1/2013 - 7/31/2014
This project is directed towards developing the VesiVax® carbohydrate conjugation chemistry that can be used to enhance the immune response to carbohydrate antigens.

"*Novel VLP Adjuvant Based on the VesiVax® System*"
Role: Principal Investigator EFFORT
Agency: NIH/NIAID *Type:* SBIR (1R43AI104073-01) *Period:* 8/1/2013 - 7/31/2015
This project is directed towards demonstrating that the VesiVax® Conjugatable Adjuvant Lipid Vesicle platform technology can be used to enhance the immune response to Virus-Like Particle antigens.

"*Engineering of a Novel CDN Nanoparticle Adjuvant Platform*"
Role: Principal Investigator EFFORT
Agency: NIH/NIAID *Type:* SBIR (1R43AI112132-01) *Period:* 6/20/2014 - 5/31/2015
This project is focused on developing VesiVax formulations containing cyclic dinucleotides as potent activators of STING.

Pending

N/A

Completed support in the last four years

HHSN261201100068C (PI) NIH/NCI, "*Viratrodes" Biosensors for the Detection of Circulating Tumor Cells and Cancer Biomarkers*". This SBIR Phase I contract aimed to develop a viratrode that would capture and detect circulating tumor cells.

R44AI066621 (PI), NIH/NIAID, "*Genital Herpes Vaccine Discovery*". This project was focused on developing a vaccine for prevention of genital herpes.

R43AI078654 (PI) NIH/NIAID, "*Surrogate Endpoints for Correlating Protective Immunity in Response to Influenza Vaccination*". This project was aimed at developing a universal vaccine for prevention of disease caused by type A strains of influenza.

U01AI074508 (PI), NIH/NIAID, "*Non-Viral Pandemic Influenza Vaccine Development*". This project proposes to develop a vaccine against pandemic strains of influenza using a novel liposomal technology.

R43AI074163 (PI), NIH/NIAID, "*Development of Virus Electrodes for Fungal Pathogen Detection*". This project studied the development of a viratrode for diagnosis of invasive aspergillosis.

R43AI075690 (PI), NIH/NIAID, "*Thermal Targeting of Drugs to Sites of Infection*". This project studied the development of thermally sensitive liposomes able to release their contents at sites of infection.

R43AI077119 (PI), NIH/NIAID, "*Liposomal Adjuvant for Vaccine Development*". This project studied the development of a conjugatable vaccine system that could be used by vaccine researchers to improve the immunogenicity of antigens.

R43AI069595 (PI), NIH/NIAID, "*Development of an Aspergillus Vaccine*". This project studied developing a vaccine against *Aspergillus fumigatus* using a novel liposomal technology.

R41CA126001 (Co-I), NIH/NCI, "*Development of a combination therapy for treating prostate cancer*". This project studied the effectiveness of a liposomally-entrapped disintegrin (vicrostatin) for treating prostate cancer.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.

Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Personal Info	Distinguished Professor of Chemistry and Biochemistry
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Harvard University	B.S.	1963-7	Chemistry and Physics
University of Chicago	M.S.	1967-8	Chemical Physics
University of Chicago	Ph.D.	1968-70	Chemical Physics
University of Paris-Orsay	postdoctoral	1970-1	Physical Chemistry
University of California, Berkeley	postdoctoral	1971-2	Physical Chemistry

A. Personal Statement

I am trained as a theorist and have worked since the late 1970s on a wide range of problems dealing with the statistical mechanics and structural properties of "complex fluids", including liquid crystals, surfactant solutions, phospholipid bilayers, polymers, and nanoparticle suspensions. In recognition of my many contributions in this area I was the recipient of the American Chemical Society "Liquids" Prize, in 2001. At about the same time, as a natural outgrowth of my work on the association of colloidal particles and their interaction with polyelectrolytes, I began to work on the self-assembly of viral capsid proteins and their packaging of viral genomes, providing the first thermodynamic theory of capsid icosahedral symmetry and making the first predictions of the pressure in double-stranded DNA viruses. In collaboration with my colleague Personal Info we initiated an experimental program to measure these pressures and their dependence on genome length and ambient salt conditions. Over the past several years we have concentrated our efforts on the *in vitro* reconstitutions of single-stranded RNA viruses and of a wide range of virus-like-particles, specifically of plant viral protein capsids self-assembled around anionic polymers (viral RNA or synthetic polyelectrolyte) surrounded in turn by phospholipid bilayer (viral envelope or liposomes). Our laboratory has achieved the first cryo-electron microscopy visualization of large (viral-size) RNAs in their native states, and has similarly obtained the first 3D reconstructions of these molecules via small-angle X-ray scattering. We have also investigated the nature of budding in alphaviruses, the mammalian viral descendants of the plant viruses on which we focus; most recently, we have succeeded in demonstrating the direct budding of preformed alphavirus nucleocapsids from uninfected cells expressing the viral membrane proteins. These results – and our general expertise with the *in vitro* syntheses of enveloped virus-like particles – provide much of the basis for our contribution to the present SBIR Fast Track collaborative proposal with Dr. Gary Fujii and his team at Molecular Express.

B. Positions and Honors

Employment

1972-1975 Assistant Professor of Chemistry, University of California, Berkeley
1975-1979 Associate Professor of Chemistry, University of California, Los Angeles
1979-1999 Professor of Chemistry, University of California, Los Angeles
1999-present Distinguished Professor of Chemistry and Biochemistry, University of California, Los Angeles

Other positions

1983 Exchange Professor, University of Paris-Orsay, France
1988 Brotherton Professor (Endowed Lectureship), University of Leeds, England
1999 Rothschild Professor, Institut Curie, Paris, France
2001-2004 Chair, Department of Chemistry and Biochemistry, University of California, Los Angeles

Honors

1970 NSF Postdoctoral Fellowship

1971	Miller Institute Fellowship at Berkeley
1974	Alfred P. Sloan Fellowship
1976	Camille and Henry Dreyfus Teacher-Scholar Award
1981	Glenn T. Seaborg Award (University of California, Los Angeles)
1986	Hanson-Dow Distinguished Teaching Award (University of California, Los Angeles)
1987	Fellow, American Physical Society
1988	H. N. McCoy Award (University of California, Los Angeles)
1991	Lennard-Jones Medal (British Royal Society)
1996	Luckman Distinguished Teaching Award
1998	J. S. Guggenheim Fellowship
2001	American Chemical Society "Liquids" (Hildebrand) Prize
2002	Bikerman Lecturer, Case Western Reserve University
2003	H. N. McCoy Award (University of California, Los Angeles)
2006	Laughlin Lecturer, Cornell University
2009	Elected to American Academy of Arts and Sciences
2010	Buhl Lecturer, Carnegie Mellon University

C. Selected peer-reviewed publications (from a total of 189, going back to 1969)

1. Yoffe, A. M., Prinsen, P., Gopal, A., Knobler, C. M., Gelbart, W.M. , Ben Shaul, A. "Predicting the Sizes of Large RNA Molecules," Proc. Natl. Acad. Sci. (USA) 2008, 105, 1633-8."183.

2. Hu, Y., Zandi, R., Anavitarte, A., Knobler, C. M., Gelbart, W. M., Packaging of a Polymer by a Viral Capsid: The Interplay Beyween Polymer Length and Capsid Size," 2008, Biophys. J., 94, 1428 – 36.

3. Knobler, C. M., Gelbart, W.M. "What Determines the Size of a Virus," in "Emerging Topics in Physical Virology," Ed. P. G. Stockley and R. Twarock, Imperial College Press, London 2010, p 185-216.

4. Cadena-Nava, R. D., Hu, Y., Garmann, R., Ng, B., Zelikin, A. N., Knobler, C. M. and Gelbart, W. M., "Exploiting Fluorescent Polymers to Probe the Self-Assembly of Virus-like Particles," 2011, J. Phys. Chem. B,115, 2386-91.

5. Snyder, J. E., Azizgolshani, O., Wu, B. , He, Y., Lee, A. C., Jose, J., Suter, D. M., Knobler, C. M., Gelbart, W. M. and Kuhn, R. J., "Rescue of Infectious Particles from Pre-Assembled Alphavirus Nucleocapsid Cores," 2011, J. Virol., 85, 5773-81.

6. Gopal, A., Zhou, Z. H., Knobler, C. M., and Gelbart, W. M., "Visualizing large RNA molecules in solution," 2012, RNA, 18, 284-99.

7. Cadena-Nava, R. D., Comas-Garcia,M. Garmann, R. F., Rao, A. L. N., Knobler, C. M., Gelbart, W. M., "Self-assembly of viral capsid protein and RNA molecules of different sizes: requirement for a specific high protein.RNA mass ratio," 2012, J. Virol., 86, 12271-82.

8. Comas-Garcia, M., Cadena-Nava, R. D. , Rao, A. L.N., Knobler, C. M., Gelbart, W. M. , "In Vitro Quantification of the Relative Packaging Efficiencies of Single-Stranded RNA Molecule by Viral Capsid Protein," 2012, J. Virol. 86, 12271-82.

9. Azizgolshani, O., Garmann, R. F., Cadena-Nava, R., Knobler, C. M., Gelbart, W. M., "Reconstituted Plant Viral Capsids Can Release Genes to Mammalian Cells," 2013 Virol. 441, 12 – 17.

10. Garmann, R. F., Comas-Garcia, M. Gopal, A., Knobler, C. M., Gelbart, W. M., "The Assembly Pathway of an Icosahedral Single-Stranded RNA Virus Depends on the Strength of Inter-Subunit Attractions," 2014, J. Mol. Biol., 426, 1050-60.

11. Vega-Acosta, J. R., Cadena-Nava, R. D., Gelbart, W. M., Knobler, C. M. , Ruiz-Garcia, J. "Electrophoretic Mobility and surface charge of the CCMV capsid, its capsid protein dimer and their relation to viral assembly", 2014, J. Phys. Chem. B,118, 1984-9.

12. Comas-Garcia, M., Garmann, R. F., Singaram, S. W., Ben-Shaul, A., Knobler, C. M., Gelbart, W. M. "Characterization of Viral Capsid Protein Self-Assembly Around Short Single-Stranded RNA," 2014, J. Phys. Chem. B, 118. 7510-9.

13. Garmann, R. F., Comas-Garcia, M., Koay, M. S. T., Cornelissen, J. J. L. M., Knobler, C. M., Gelbart, W. M., "The Role of Electrostatics in the Assembly Pathway of a Single-Stranded RNA Virus," 2014, J. Virol., 88, 10472-9.

14. Gopal, A., Egeciolglu, D. E., Yoffe, A. M., Ben-Shaul, A., Rao, A. L. N., Knobler, C. M., Gelbart, W. M. "Viral RNAs Are Unusually Compact," 2014, PLOS ONE, 9, e105875.

D. Research Support

Continuing Research Support

Private Source | Personal Info | 01/10/14 – 09/30/15

"Armored" Self-Replicating Genes as Delivery Systems for Early Pancreatic Cancer Detection by Magnetic Resonance Molecular Imaging

Completed Research Support

NSF CHE 1051507 Personal Info 01/01/11 – 12/31/13

Self-Assembly and Packaging of RNA and DNA in Viruses and Virus-like Particles

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Personal Info	Research Scientist
eRA COMMONS USER NAME	
eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Hawaii-Manoa	B.S.	2002	Chemistry
University of California-Los Angeles	Ph.D.	2008	Physical Chemistry

A. Personal Statement.

The main goal of this application is to develop a nanoparticulate oligonucleotide delivery system. During my appointment at Molecular Express, I have made direct contributions to the development and launch of the VesiVax® sulfhydryl-targeting CALV kit, and have worked with numerous collaborators to incorporate different antigens, adjuvants and targeting ligands into the liposomes. For this proposal, I will conjugate the epidermal growth factor to the EVLPs, and perform the *in vitro* assays to test the targeted ODN-loaded EVLPs. I believe that this project has great potential for efficient nucleic acid entrapment and delivery to targeted cells.

B. Positions and Honors.

Positions and Employment

2000-2001 *Research Assistant* with Professor Randy Larsen, Chemistry Department, University of Hawaii-Manoa
2001-2002 *Research Assistant* with Professor John Head, Chemistry Department, University of Hawaii-Manoa
2003-2006 *Teaching Assistant,* Department of Chemistry and Biochemistry, UCLA
2003-2008 *Graduate Student Researcher* with Professor Shimon Weiss, Department of Chemistry and Biochemistry, UCLA
2006-2007 *Research Associate*, Nesher Technologies, Inc.
2008-2014 *Research Scientist*, Molecular Express, Inc.
2014- Director, Chemistry, Molecular Express, Inc.

Honors

1998 CRC Freshman Award
2000 Colonel Geraldine Y.K. Tom Scholarship
2001 Scheuer-Iwamoto Scholarship
2002 Merck Award
2002 US Department of Education Graduate Assistance in Areas of National Need Fellowship

C. Selected peer-reviewed publications (in chronological order).

Kapanidis, A.N., Margeat, E., Laurence, T.A., Doose, S., **Ho, S.O.**, Mukhopadhyay, J., Kortkhonjia, E., Mekler, V., Ebright, R.H., and Weiss, S. (2005) Retention of transcription factor σ^{70} in transcription elongation: single-molecule analysis. *Molecular Cell* **20**: 347-356.

Lee, N.K., Kapanidis, A.N., Koh, H.R., Korlann, Y., **Ho, S.O.**, Kim, Y., Gassman, N., Kim, S.K., and Weiss, S. (2005) Three-color Alternating-Laser Excitation of single molecules: monitoring multiple interactions and distances. *Biophysical Journal* **88**:2939-2953.

Kapanidis, N., Margeat, E., **Ho, S.O.**, Kortkhonjia, E., Weiss, S., and Ebright, R.H. (2006). Initial transcription by RNA Polymerase proceeds through a DNA-scrunching mechanism. *Science* **314**: 1144-1147.

Kim, Y., **Ho, S.O.**, Gassman, N.R., Korlann, Y., Landrof, E.V., Collart, F., and Weiss, S. (2007). Efficient site-specific labeling of proteins via cysteines. *Bioconjugate Chemistry* **19**: 786-791.

Gassman, N.R., **Ho, S.O.**, Korlann, Y., Chiang, J., Wu, Y., Perry, L.J., Kim, Y., and Weiss, S. (2009). *In vivo* assembly and single-molecule characterization of the transcription machinery from *Shenwanell Oneidensis* MR-1. *Protein Expression and Purification* **65**:66-76.

Lockner, J.W., **Ho, S.O.**, Chiang, S, Do, T.Q., Fujii, G., Janda, K.D. (2013). Enchancing nicotine vaccine immunogenicity with liposomes. *Bioorganic & Medicinal Chemistry Letters* **23**:975-978.

D. Active Research Support

"*Formulation of TLR 3 and 7/8 Agonists in Conjugatable Adjuvant Lipid Vesicles*"
Role: Principle Investigator
Agency: NIH/NIAID Type: SHIFT SBIR (1R43AI-94770-01A1) Period: 8/1/2012 - 7/31/2014
The aim of this SBIR project is develop VesiVax® CALV formulations containing TLR3 and TLR7/8 agonists.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Personal Info	Research Professor of Chemistry
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
New York University	B.A.	1951-5	Chemistry
Pennsylvania State University		1955-9	Physical Chemistry
University of Leiden (Netherlands)	Ph.D.	1959-61	Molecular Physics
Ohio State University	Postdoctoral	1961-2	Physical Chemistry
California Institute of Technology	Postdoctoral	1962-4	Chemical Engineering

A. Personal Statement

My undergraduate degree is in Chemistry, I began graduate study in Physical Chemistry, received my doctorate in Molecular Physics, and was a post-doc in Chemistry and Chemical Engineering. This breadth of studies is reflected in my research, almost entirely experimental, which ranged from low-temperature calorimetry, intermolecular forces, thermodynamics of fluid mixtures, critical phenomena, and organic thin films at the air/water interface. It is this last topic, which I pursued for over a dozen years that I was recognized with the 2002 ACS Award in Colloid Chemistry. None of the changes of direction of my research has been as marked as the last, when a dozen years ago I joined my colleague Personal Info to investigate the pressure-induced ejection of dsDNA from bacteriophage. This first foray into biophysical chemistry rapidly grew to a full-fledged program of studies on the *in vitro* self-assembly of ssRNA viruses, work which has led us to examine the nature of the how the interplay between the interactions between RNA and viral protein and between the proteins can be controlled by the path of assembly, and which has provided us with the expertise to contribute significantly to the present collaboration with Personal Info and his team at Molecular Express.

B. Positions and Honors

Employment

1964-70 Assistant Professor of Chemistry, UCLA
1970-6 Associate Professor of Chemistry, UCLA
1976-2004 Professor of Chemistry, UCLA
2004- Research Professor of Chemistry, UCLA

Other Positions

1975-8 Vice-chair, Department of Chemistry and Biochemistry, UCLA
1984-7 , 1994-7 Chair, Department of Chemistry and Biochemistry, UCLA
1998-2005 Senior Editor, Journal of Physical Chemistry
2005 - 10 Associate Dean of Physical Sciences, UCLA

Honors

1957-8 Union Carbide Fellow
1958-9 Fulbright Scholar
1980, 2004 Herbert Newby McCoy Award
1983 Fellow, American Physical Society
1983 UCLA Alumni Association Distinguished Teaching Award
1990 Alexander von Humboldt Senior Award, University of Mainz
1997 UCLA College of Letters and Science Faculty Award
1998 Alexander von Humboldt Senior Award, Max Planck Institute, Potsdam
2000 Kolthoff Lecturer, University of Minnesota
2002 American Chemical Society Award in Colloid Chemistry

2009 Fellow, Royal Society of Chemistry
2009 Dickson Award
2014 Fellow , American Chemical Society

B. **Selected peer-reviewed publications**
(Publications selected from 197 peer-reviewed papers.)

1. Yoffe, A. M., Prinsen, P., Gopal, A., Knobler, C. M., Gelbart, W.M. , Ben Shaul, A. "Predicting the Sizes of Large RNA Molecules," Proc. Natl. Acad. Sci. (USA) 2008, 105, 1633-8."183.

2. Hu, Y., Zandi, R., Anavitarte, A., Knobler, C. M., Gelbart, W. M., Packaging of a Polymer by a Viral Capsid: The Interplay Beyween Polymer Length and Capsid Size," 2008, Biophys. J., 94, 1428 – 36.

3. Knobler, C. M., Gelbart, W.M. "What Determines the Size of a Virus," in "Emerging Topics in Physical Virology," Ed. P. G. Stockley and R. Twarock, Imperial College Press, London 2010, p 185-216.

4. Cadena-Nava, R. D., Hu, Y., Garmann, R., Ng, B., Zelikin, A. N., Knobler, C. M. and Gelbart, W. M., "Exploiting Fluorescent Polymers to Probe the Self-Assembly of Virus-like Particles," 2011, J. Phys. Chem. B,115, 2386-91.

5. Snyder, J. E., Azizgolshani, O., Wu, B. , He, Y., Lee, A. C., Jose, J., Suter, D. M., Knobler, C. M., Gelbart, W. M. and Kuhn, R. J., "Rescue of Infectious Particles from Pre-Assembled Alphavirus Nucleocapsid Cores," 2011, J. Virol., 85, 5773-81.

6. Gopal, A., Zhou, Z. H., Knobler, C. M., and Gelbart, W. M., "Visualizing large RNA molecules in solution," 2012, RNA, 18, 284-99.

7. Cadena-Nava, R. D., Comas-Garcia,M. Garmann, R. F., Rao, A. L. N., Knobler, C. M., Gelbart, W. M., "Self-assembly of viral capsid protein and RNA molecules of different sizes: requirement for a specific high protein.RNA mass ratio," 2012, J. Virol., 86, 12271-82.

8. Comas-Garcia, M., Cadena-Nava, R. D. , Rao, A. L.N., Knobler, C. M., Gelbart, W. M. , "In Vitro Quantification of the Relative Packaging Efficiencies of Single-Stranded RNA Molecule by Viral Capsid Protein," 2012, J. Virol. 86, 12271-82.

9. Azizgolshani, O., Garmann, R. F., Cadena-Nava, R., Knobler, C. M., Gelbart, W. M., "Reconstituted Plant Viral Capsids Can Release Genes to Mammalian Cells," 2013 Virol. 441, 12 – 17.

10. Garmann, R. F., Comas-Garcia, M. Gopal, A., Knobler, C. M., Gelbart, W. M., "The Assembly Pathway of an Icosahedral Single-Stranded RNA Virus Depends on the Strength of Inter-Subunit Attractions," 2014, J. Mol. Biol., 426, 1050-60.

11. Vega-Acosta, J. R., Cadena-Nava, R. D., Gelbart, W. M., Knobler, C. M. , Ruiz-Garcia, J. "Electrophoretic Mobility and surface charge of the CCMV capsid, its capsid protein dimer and their relation to viral assembly", 2014, J. Phys. Chem. B,118, 1984-9.

12. Comas-Garcia, M., Garmann, R. F., Singaram, S. W., Ben-Shaul, A., Knobler, C. M., Gelbart, W. M. "Characterization of Viral Capsid Protein Self-Assembly Around Short Single-Stranded RNA," 2014, J. Phys. Chem. B, 118. 7510-9.

13. Garmann, R. F., Comas-Garcia, M., Koay, M. S. T., Cornelissen, J. J. L. M., Knobler, C. M., Gelbart, W. M., "The Role of Electrostatics in the Assembly Pathway of a Single-Stranded RNA Virus," 2014, J. Virol., 88, 10472-9.

14. Gopal, A., Egeciolglu, D. E., Yoffe, A. M., Ben-Shaul, A., Rao, A. L. N., Knobler, C. M., Gelbart, W. M. "Viral RNAs Are Unusually Compact," 2014, PLOS ONE, 9, e105875.

C. Research Support

Continuing Research Support

Private Source (Personal Info) 01/10/14 – 09/30/15
"Armored" Self-Replicating Genes as Delivery Systems for Early Pancreatic Cancer Detection by Magnetic Resonance Molecular Imaging

Completed Research Support

NSF CHE 1051507 (Personal Info) 01/01/11 – 12/31/13
Self-Assembly and Packaging of RNA and DNA in Viruses and Virus-like Particles

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Molecular Express, Inc.

Start Date*: 07-01-2015 **End Date*:** 06-30-2016 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Personal Info				PD/PI		EFFORT			Itemized Cost		
2.		Personal Info				Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** Itemized Cost

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical	EFFORT			Itemized Cost		
# OF EMPLOYEES	R&D Management						
	Research Associate						

Total Number Other Personnel **Total Other Personnel** Itemized Cost

Total Salary, Wages and Fringe Benefits (A+B)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Molecular Express, Inc.

Start Date*: 07-01-2015 **End Date*:** 06-30-2016 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item **Funds Requested ($)***

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name:

D. Travel **Funds Requested ($)***

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions) Itemized Cost
2. Foreign Travel Costs

Total Travel Cost Itemized Cost

E. Participant/Trainee Support Costs **Funds Requested ($)***

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees **Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ● Project ○ Subaward/Consortium

Organization: Molecular Express, Inc.

Start Date*: 07-01-2015 **End Date*:** 06-30-2016 **Budget Period:** 1

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	Itemized Cost
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	Itemized Cost
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Outside vendor services and hypoxia chamber and assays	Itemized Cost
Total Other Direct Costs	

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	Itemized Cost

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . G&A		Itemized Cost	
		Total Indirect Costs	Itemized Cost

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	Itemized Cost

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1234-BdgJst EVLP STTR I RT Final 5-Dec-2014.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Molecular Express Budget Justification

Personnel

Personal Info is the CEO and scientific founder of Molecular Express and will serve as the Principal Investigator for this grant proposal. He is an experienced bio/physical chemist with expertise in the development and commercialization of life science products, and has made major contributions to the development of the use of protein/lipid complexes as drug delivery vehicles, and diagnostics. Personal Info will be responsible for final review of the technical reports and advise the research team as needed. He will also coordinate the administrative interactions between the Company and the NIH. It is anticipated that Personal Info will devote approximately calendar months effort) to this project. EFFORT

EFFORT Personal Info is the Director of Chemistry at Molecular Express. She is an experienced biophysical chemist. Personal Info will serve as the Co-Investigator and be directly responsible for conducting the EVLP-EGF conjugation chemistries and *in vitro* cell assays associated with this project. Personal Info will also manage the scientific aspects of the project and coordinate the technical interactions between Molecular Express and other organizations as necessary. We estimate that Personal Info will devote calendar months EFFORT effort) to this project. EFFORT

R&D Management. The R&D management support team for this project consists of Personal Info **and** Personal Info is the Director of Formulations at Molecular Express and will participate in the lipid wrapping studies proposed in Specific Aim 2. Personal Info is the Director of Analytical Chemistry and will analyze the components of the various lipid components EVLPs. We anticipate that our Directors will devote approximately a combined total of calendar months effort) to these activities. EFFORT

EFFORT **TBD - Research Associate.** Collectively, this individual will be responsible for the assisting Personal Info in the synthesis of the conjugatable ligands, conjugating the EGF ligand to the EVLPs, providing support for formulation, conducting the *in vitro* cellular assays and data analysis. We estimate that these activities will require approximately calendar months effort) time and effort to complete. EFFORT EFFORT

Supplies

It is anticipated that reagents, solvents, buffers, VEGF ELISA kits, antibodies to EGF and EGFR, and cell culture media supplies will be required for this project. We are requesting Itemized Cost for these supplies. Lipids, ODNs and chemical linkers will be required to formulate targeted and untargeted EVLPs. We are requesting Itemized Cost for these supplies.

Travel expenses

Travel funds are requested for the PI and/or Personal Info to attend a meeting with interested parties, the NIH and/or a national conference to present results to potential partners or to the scientific community. We are budgeting Itemized Cost for travel costs.

Subcontractual/Consortium Costs

Funds for a subcontract to the Personal Info laboratory at UCLA are requested.

Other Direct Costs

Funds are allocated to conduct tests through an outside vendor or at a local university using equipment that the Company currently does not have on-site. Funds are also requested for a hypoxia chamber with accessories to carry out the appropriate cellular cytotoxicity assays. We are budgeting Itemized Cost for the vendor services and Itemized Cost for the hypoxia chamber and assays.

Indirect Costs

Itemized Cost

An Indirect Cost rate of is requested.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		Itemized Cost
Section B, Other Personnel		Itemized Cost
Total Number Other Personnel	# OF EMPLOYEES	
Total Salary, Wages and Fringe Benefits (A+B)		Itemized Cost
Section C, Equipment		
Section D, Travel		Itemized Cost
1. Domestic	Itemized Cost	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		Itemized Cost
Section F, Other Direct Costs		
1. Materials and Supplies	Itemized Cost	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	Itemized Cost	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	Itemized Cost	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		Itemized Cost
Section H, Indirect Costs		Itemized Cost
Section I, Total Direct and Indirect Costs (G + H)		Itemized Cost
Section J, Fee		

Tracking Number: GRANT11797407 Funding Opportunity Number: PA-14-308 . Received Date: 2014-12-05T17:09:23.000-05:00

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California Los Angeles

Start Date*: 07-01-2015 **End Date*:** 06-30-2016 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Personal Info				PD/PI		EFFORT			Itemized Cost		

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person Itemized Cost

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
# OF EMPLOYEES	Graduate Students	EFFORT			Itemized Cost		
	Undergraduate Students						
	Secretarial/Clerical						
# OF EMPLOYEES	**Total Number Other Personnel**				**Total Other Personnel**		Itemized Cost

Total Salary, Wages and Fringe Benefits (A+B)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: University of California Los Angeles

Start Date*: 07-01-2015 **End Date*:** 06-30-2016 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	Itemized Cost
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	Itemized Cost

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: University of California Los Angeles

Start Date*: 07-01-2015 **End Date*:** 06-30-2016 **Budget Period:** 1

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	Itemized Cost
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	Itemized Cost
7. Alterations and Renovations	
8 . Microscopy Recharges	Itemized Cost
Total Other Direct Costs	Itemized Cost

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	Itemized Cost

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . F&A		Itemized Cost	
		Total Indirect Costs	Itemized Cost

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	Itemized Cost

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: 1242-BdgJst Personal Info
UCLA Proposal.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT11797407 Funding Opportunity Number: PA-14-308 , Received Date: 2014-12-05T17:09:23.000-05:00

Budget Justification

Personnel:

To Be Named, Graduate Student Researcher EFFORT effort), will be responsible for the in vitro assembly of virus-like particles (VLPs) in which the cowpea chlorotic mottle virus capsid protein encapsulates anti-sense oligonucleotides for gene knockdown under the direction of Personal Info and Personal Info

Fringe Benefits:

The fringe benefit rates were estimated using the composite rates agreed upon by the University of California, Office of the President. Benefit rates used in the proposal include:

Itemized Cost for undergraduate and graduate students, academic year
for undergraduate and graduate students, summer

Graduate Student Fee Remission of Itemized Cost per UCLA Graduate Division. We have projected a increase in costs in subsequent years.
Itemized Cost

Materials and Supplies:

Supplies such as buffer reagents, oligos, enzymes, transcription kits, pipeters, tips, Eppendorf tubes, size-exclusion column, and electron microscope grids.

Other (Microscopy Recharges):

For use of the electron microscopes in the California Nanosystems Institute facility to characterize the virus-like particles and enveloped virus-like particles that will be assembled.

Other direct costs:

The Technology Infrastructure Fee (TIF) is a consistently-applied direct charge assessed to each and every campus activity unit, regardless of funding source, including units identified as individual grant and contract awards. The TIF pays for campus communication services on the basis of a monthly accounting of actual usage data. These costs are charged as direct costs and are not recovered as indirect costs. Effective July 1, 2013, TIF rates are Itemized Cost per FTE per month.

Indirect costs:

Indirect costs calculated at Itemized Cost of MDTC Base per blanket agreement with Department of Health and Human Services dated April 27, 2011.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		Itemized Cost
Section B, Other Personnel	# OF EMPLOYEES	Itemized Cost
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		Itemized Cost
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		Itemized Cost
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance	Itemized Cost	
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		Itemized Cost
Section F, Other Direct Costs	Itemized Cost	
1. Materials and Supplies		
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	Itemized Cost	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations	Itemized Cost	
8. Other 1		
9. Other 2		
10. Other 3		Itemized Cost
Section G, Direct Costs (A thru F)		Itemized Cost
Section H, Indirect Costs		Itemized Cost
Section I, Total Direct and Indirect Costs (G + H)		
Section J, Fee		

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

Program Type (select only one)*

○ SBIR ● STTR ○ Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

SBIR/STTR Type (select only one)*

● Phase I ○ Phase II ○ Fast-Track (See agency-specific instructions to determine whether a particular agency participates in Fast-Track)

Questions 1-7 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* ● Yes ○ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* # OF EMPLOYEES

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ○ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov.* ● Yes ○ No

4. Will all research and development on the project be performed in its entirety in the United States?* ● Yes ○ No

If no, provide an explanation in an attached file. Explanation:*

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* ○ Yes ● No

If yes, insert the names of the other Federal agencies:*

6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ● Yes ○ No

7. Commercialization Plan: If you are submitting a Phase II or Phase I/Phase II Fast-Track Application, include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:*

Funding Opportunity Number: PA-14-308. Received Date: 2014-12-05T17:09:23.000-05:00

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.* ○ Yes ○ No

Attach File:*

9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ○ Yes ○ No

STTR-Specific Questions:

Questions 10 and 11 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 and 11 blank.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ● Yes ○ No

(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?* ● Yes ○ No

Funding Opportunity Number: PA-14-308. Received Date: 2014-12-05T17:09:23.000-05:00

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OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: Personal Info
Middle Name:
Last Name*: Personal Info
Suffix:

2. Human Subjects

Clinical Trial? ○ No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ○ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

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PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	1243-SpAms EVLP STTR I Final 5-Dec-2014.pdf
3. Research Strategy*	1244-ResStr EVLP STTR I Final 5-Dec-2014.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	
9. Select Agent Research	1249-Biohaz EVLP STTR Final 5-Dec-2014.pdf
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	1250-LOS Personal Info UCLA.pdf
13. Resource Sharing Plan(s)	
Appendix (if applicable)	
14. Appendix	

Development of a Gene and Oligonucleotide Delivery System

Specific Aims

Among the many problems that need to be overcome in order to fulfill the enormous promise of small interfering RNAs (siRNA) and antisense oligodeoxynucleotides (ODNs) for targeted gene knockdown, and of genes encoding proteins of medical relevance (collectively, gene-based medicines, G-MEDS), delivering effective quantities to specific cells has proven especially challenging. It is not surprising, then, that there is a huge literature on this subject [1], describing a wide variety of viral [2,3], lipid-based [4,5], and polymer [6] vectors for protecting, targeting and delivering many different G-MEDS. As an alternative approach to enable targeted gene delivery to mammalian cells, we propose to develop a novel method involving *enveloped virus-like-particles* (EVLPs). These systems are reconstituted *in vitro* in two steps: first, we self-assemble the G-MEDS into virus-like particles (VLPs) by mixing them with purified viral capsid protein (CP); and then we wrap the resulting VLPs in lipid bilayers that are capable of being functionalized for targeting of, and uptake by, mammalian cells of interest. In our on-going efforts, we have demonstrated our ability to prepare EVLPs using a model antisense ODN for vascular endothelial growth factor (VEGF) [7], a protein over-expressed in many cancer cells to stimulate angiogenesis and facilitate tumor survival under low-oxygen (hypoxia) conditions [8]. Viral CP can be produced in high yield in plants by agroinfiltration [9], a method that we have been able to apply to the CP of cowpea chlorotic mottle virus (CCMV), which we choose because it is arguably the most robust and effective for packaging of a wide range of RNA and DNA molecules [10]. Moreover, we have demonstrated that CCMV VLPs are capable of releasing their genetic content into the cytoplasm of mammalian cells [11]. Finally, we will functionalize the lipid bilayers with epidermal growth factor (EGF), which binds to the EGF receptor (EGFR) over-expressed on cancer cells – especially the breast cancer derived cell lines with which we choose to work in this study – thereby facilitating their targeting and uptake. **In summary, to demonstrate the utility of our EVLP technology, we will package an antisense G-MED that targets the expression of the Hypoxia-Inducible Factor 1α (HIF-1α) gene in a VLP formed spontaneously by mixing it with the purified CP of a particular plant virus, CCMV. Then, to suppress the immunogenicity of the VLP, and to facilitate its uptake by specific cells, we will wrap it *in vitro* in a lipid bilayer to which targeting ligands can be conjugated, thereby completing the synthesis of the EVLP. The effectiveness of this EVLP will be demonstrated by assaying the reduction in the secretion of VEGF in cultured breast cancer cells. Our Specific Aims are as follows:**

SPECIFIC AIM 1: *In vitro* reconstitution of VLPs with ODNs. In preliminary experiments, we have demonstrated that we can utilize CCMV CP to prepare *in vitro* VLPs containing an ODN – in particular, the antisense ODN for VEGF – as the first step in preparing EVLPs. Now, we will optimize the conditions of assembly by refining the pH, ionic strength, and the relative and absolute concentrations of the CPs and ODNs to maximize the yield and stability of the assembled VLPs, and show that this can be done as well for the antisense ODN for HIF-1α, an upstream target for VEGF knockdown.

SPECIFIC AIM 2: *In vitro* synthesis of EVLPs by wrapping of VLPs with a lipid bilayer. We have also demonstrated that we can prepare EVLPs in which the VLPs are wrapped by a bilayer containing a small fraction of cationic lipid. Being mindful of potential toxicities that may be caused by cationic lipids, we will further minimize the amount of cationic lipid needed to successfully wrap the VLPs. We will also test alternative cationic lipids which we synthesize in-house as well as different chain length phospholipids. Lastly, we will investigate lipid anchors that provide conjugatable moieties to couple our model targeting ligand, EGF, to facilitate binding and uptake by EGFR that is over-expressed on the plasma membranes of MDA-MB-231 and MDA-MB-468 breast cancer cells.

SPECIFIC AIM 3: Measurement of relative VEGF knockdown efficiencies of naked, VLP, and EVLP containing anti-HIF-1α antisense ODNs. Breast cancer cells (MDA-MB-231 and 468) will be grown in culture under normoxic and hypoxic conditions and transfected alternately with each of these four forms of anti-HIF-1α ODN (*i.e.*, naked, VLP, and targeted and untargeted EVLPs, *i.e.*, with and without EGF-conjugation), and the effect on VEGF production assayed. We will also assay for cellular toxicity of the VLPs and EVLPs. To assess targeted uptake of the EVLPs, fluorescently labeled EVLPs will be prepared, incubated with the cells and examined by fluorescence microscopy.

Research Strategy

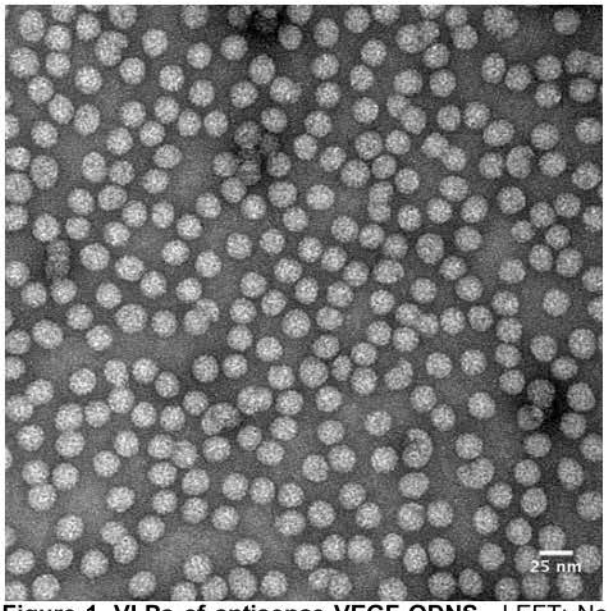
As mentioned above, considerable effort has been devoted to *in vitro* packaging of siRNAs and ODNs in *un*-enveloped VLPs. Most of this work has involved use of the CP of bacteriophage MS2 [2,3]. MS2 is particular in that its single-stranded (ss) RNA genome contains an especially strong "packaging sequence" [12,13] that is essential for binding its CP with high affinity and for inducing the conformational changes in the CP dimers necessary for capsid formation. Accordingly, this (19-nucleotide [nt]) sequence must be added to any nucleic acid (e.g., siRNA or ODN) that one wants to package *in vitro* with high efficiency. Viruses other than MS2 have also been employed for siRNA delivery, but – instead of *in vitro* reconstitution of the VLP – encapsidation is carried out in cells, as, for example, with adenovirus [14], or in cell extracts [15]. *In contrast, CCMV CP can package with 100% efficiency any ssRNA or ssDNA, independent of their sequence (and of their length, up to 4000 nts) [10]; no packaging signal needs to be added.* Moreover, the packaging is carried out *in vitro* with purified components. Another advantage of CCMV is that its capsids – unlike those of MS2 – have evolved to enter their host cells and bind to ribosomes, releasing their RNA content there. This is an important feature for ensuring that the siRNA or antisense ODN being introduced for gene knockdown will end up close to target mRNA molecules, namely, near ribosomal sites.

In addition, wrapping of our VLPs with a lipid bilayer provides an additional layer of protection for the nucleic acid cargo, as well as an additional platform for functionalization of the delivery system for specific uptake into the target cells, reduced immunogenicity by avoiding undesired accumulation in non-target cells. Virosomes – viral envelopes lacking an enclosed nucleocapsid – have been studied as siRNA delivery vehicles [16], but do not have the benefit of efficient packaging and stability. Thus, the realization of EVLPs involves a powerful combination of the respective advantages of VLPs and of lipid bilayer envelopes, and yet their synthesis has not yet been attempted *in vitro*. The closest efforts along these lines have been those by the group of Mukhopadhyay [17] and ours with the Kuhn group [18], in which EVLPs have been prepared by transfecting viral-glycoprotein-expressing cells with *in vitro* reconstituted nucleocapsids containing reporting genes.

SPECIFIC AIM 1: *In vitro* reconstitution of VLPs with ODNs.

***In vitro* self-assembly "reactions"**. In our preliminary studies, we used an antisense ODN for VEGF that is a 19-nt ssDNA ODN (5'-CACCCAAGACAGCAGAAAG-3') [7]. Because all of our earlier work with *in vitro* reconstitution of CCMV VLPs involved ssRNA molecules whose lengths ranged from 500nt to 12000nt, it is not clear that the protocols we optimized [10] for them would carry over to the more than 20 times shorter ssDNA ODNs. In our preliminary studies, we started with the assembly pathway and solution conditions developed by us earlier for the longer ssRNAs. Basically, this involves incubating at 4°C for 20 hr a >6:1 mass ratio of CP to nucleic acid in "assembly buffer" (50 mM NaCl, 10 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 50 mM Tris-HCl pH 7.2), followed by dialysis for 4 hr against "virus suspension buffer" (50 mM sodium acetate, 8 mM magnesium acetate, pH 4.5). The assembly products are then analyzed by agarose gel electrophoresis, and by negative stain electron microscopy. For example, gel results for the anti-VEGF ODN indicated that all of the ODNs are packaged for CP:ODN mass ratios larger than 6:1, with the corresponding EM images suggesting T=1 (i.e., 60 CPs) 20-nm diameter VLPs – see **Fig. 1**. Furthermore, from measured values of the ratio of the 260 to 280 nm absorption peaks in the UV spectrum of the purified VLPs, we estimate the average number of ODNs per VLP to be 20 ± 1. Thus, not only is 100% of the ODN packaged into VLPs, but there is a uniformly large number of ODNs per particle. We will examine two strategies for increasing the number of ODNs packaged: a) the addition to the assembly mixture of polyamine "condensing" agents such as spermine or spermidine (2-5 mM); and b) changes in the absolute and relative concentrations of ODN and CP with the aim of increasing the size and the ODN carrying capacity of the VLP to that of a 180 CP (T = 3) capsid (with a diameter of 28 nm, that of wild-type CCMV).

Note that in our Specific Aim 1 studies, we plan to use an anti-HIF-1α ODN (5'-TCGCAAGCATCCTGTA-3) that is currently being tested in the clinic [19]. The anti-HIF-1α ODN will be synthesized with either a complete phosphorothioate backbone or with phosphorothioates at the 5'- and 3'-ends. We expect that the two-step *in vitro* packaging requirements for this ODN to form VLPs, will be essentially identical to those for the VEGF antisense ODN described above, but this will be confirmed by systematic variation of pH (from 4.5 up to about 6) for the second dialysis step, to control the strength of CP-CP lateral interactions [20] and hence, the stability of the VLP. Also, because the anti-HIF-1α ODN is slightly shorter (and hence less charged) than the anti-VEGF ODN, we expect proportionally more copies of it to be packaged on average per VLP. (In Specific Aim 3, we will compare VEGF knockdown efficiencies for these two ODNs.)



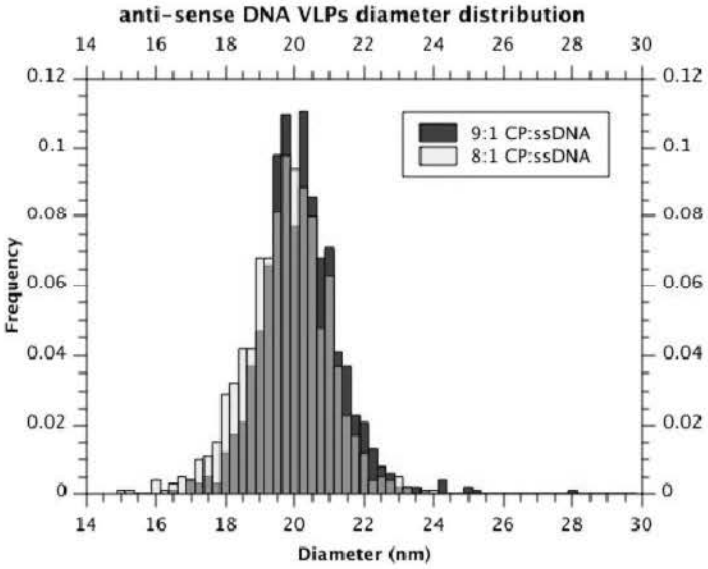


Figure 1. VLPs of antisense VEGF ODNS. LEFT: Negative-stain, 300,000 magnification, EM image of VLPs formed from *in vitro* self-assembly of a 9:1 CP:DNA mass ratio of purified CCMV CP and VEGF antisense ODN. RIGHT: Corresponding distribution of VLPs diameters for this 9:1 sample, and for an 8:1 sample.

SPECIFIC AIM 2: *In vitro* synthesis of EVLPs by wrapping of VLPs with a functionalized lipid bilayer.

It is significant that the charge on capsids assembled from CCMV CP is *independent* of the charge or nature of its contents; it is negative for pHs in excess of its isoelectric point (pH=3.7) [21], thereby enabling wrapping of the VLP by positively charged lipid bilayers. We will carry out wrapping of the VLPs by a special double ("extra")-hydration procedure that we have developed for this purpose. In our preliminary experiments, we introduce as our minority component (10%) a positively-charged lipid, so that the envelope membranes consist of neutral (DMPC) and cationic (DOTAP) lipids and cholesterol in an approximate mole ratio of 6:1:3, respectively. Proprietary Info

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Proprietary Info Small aggregates of capsids are also wrapped, but this is not a problem because these double and triple VLPs encapsulated in an EVLP are likely to be taken up by cells just as easily as are the canonical (i.e., single VLP) EVLPs.

Minimize the mole ratio of cationic lipid to the other lipids. Keeping in mind that cationic lipids may cause undesirable toxicities, our initial studies will focus on reducing the amount of cationic lipid relative to the other lipid components while still maintaining the ability to wrap ODN VLPs. As described above, our current lipid composition contains 10 mole% DOTAP. Accordingly, we will prepare lipid films with varying ratios of DOTAP (i.e., 10%, 9%, 8%, 7%, 6% and 5% DOTAP with a corresponding increase of the DMPC component) following our standard procedures. EVLPs will then be prepared and characterized by EM to ensure that the VLPs are completely wrapped. If the 5% DOTAP formulation still wraps VLPs efficiently, then we will test lower ratios; if not,

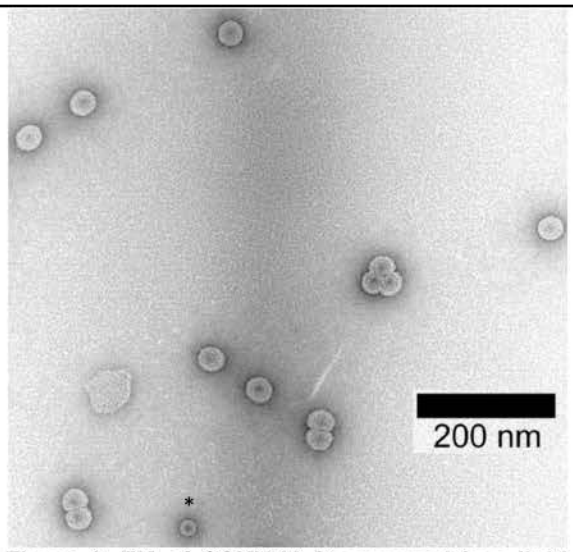

Figure 2. EM of CCMV VLPs wrapped in a lipid membrane. An unwrapped particle is denoted by the asterisk; others correspond to VLPs that are enveloped by lipid bilayer, either alone (in 6 instances here), or as pairs (2), or a triple (1).

then we may try further refining the minimal mole% (e.g., 0.1-0.5 mole% increments between, for example, 6%-7%). Once the minimal amount of cationic lipid required to efficiently wrap VLPs to form the EVLPs has been determined, we will test these formulations in the *in vitro* assays described below in Specific Aim 3.

Evaluate other cationic lipids. DOTAP has unsaturated oleyl chains which can cause a problem with respect to stability; the presence of the unsaturated double bonds makes the lipid susceptible to degradation by oxidation. To eliminate this as a potential problem for manufacturing shelf life and compromising *in vivo* pharmacological activity, we plan to test alternative cationic lipids by simply exchanging the DOTAP at the minimized mole ratio for alternative cationic lipids. One of the candidates that we will test is DMTAP which contains saturated myristoyl (C14) lipid chains. We will also test several lipid-PEG-amine derivatives that we have synthesized as described below (**Fig. 3**). For these studies, each candidate cationic lipid will simply replace the DOTAP component in the minimized wrapping formulation determined above and processed with VLPs to form EVLPs. Each sample will be characterized by EM and the *in vitro* assays.

Optimize the lipid composition. The current lipid wrapping procedure consists of relatively low transition temperature phospholipids and cholesterol. For *in vivo* applications, this may cause problems due to blood components, e.g., serum proteins associating with the lipids and "unwrapping" the EVLP before it reaches the desired target. To optimize the overall composition, we will test the ability of the wrapping procedure to be adapted to lipid compositions that may be more useful for *in vivo* applications. This will involve several sets of studies that consist of: a) testing phosphatidylcholines with longer chains (i.e., dipalmitoyl and distearoyl); b) changing the relative mole ratios of the cholesterol component (i.e., test lower mole ratios, e.g., 25%, 20%, 15%, 10%, 5%, and 0% while keeping the cationic lipid mole ratio at the minimal amount); c) adding up to 5 mole % PEG lipids and; d) incorporating a maleimide-based conjugatable molecule described below.

Conjugation of targeting ligands to lipid bilayers. In studies funded through R43AI077119 ("Liposomal Adjuvant for Vaccine Development"), we synthesized a series of lipid anchors with conjugatable moieties, such as maleimide, on one end (see top right, **Fig. 3**). The primary goal was to synthesize conjugatable lipid anchors that could be easily incorporated into liposomes and that had the appropriate length linker to be able to display a candidate ligand from the membrane surface. Although this application was originally intended to conjugate antigens to highly immunogenic liposomes (see marketing materials in the Appendix), we have more recently tested the possibility of conjugating protein ligands such as CD40L to target immune cells expressing the CD40 receptor from the cell surface (funded through R43AI094891, "Targeted Dendritic Cell Activation through Multi-Adjuvant Liposomes"). For the proposed studies, we will synthesize and purify several conjugatable lipid anchors following the methods developed by the Company, formulate them in liposomes and conjugate our model targeting ligand, EGF, to the liposomes.

Synthesis of lipid-linker-amines. Proprietary Info

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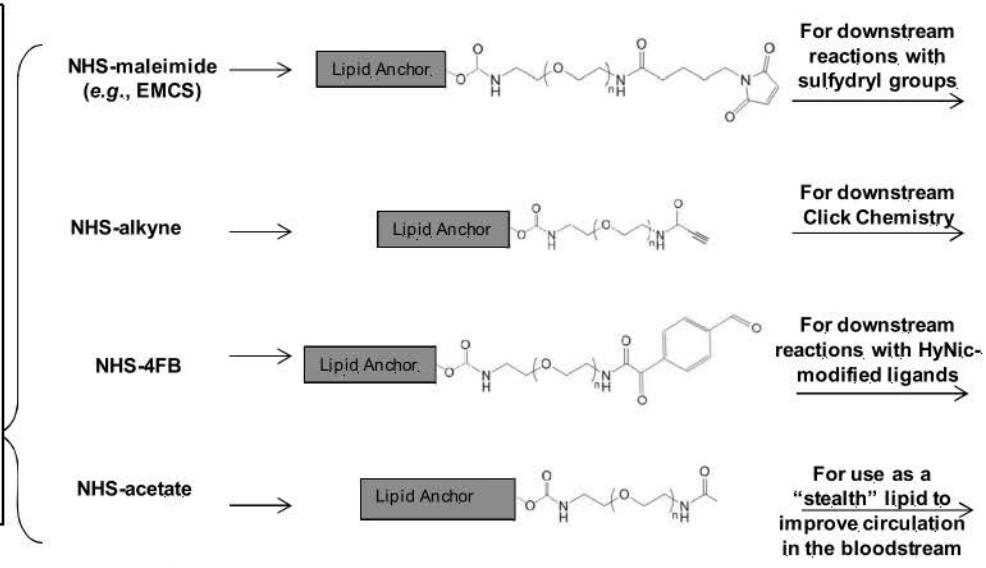

Figure 3. Schematic of available conjugation chemistries developed by Molecular Express.

mixture is loaded onto an Alltech® Alltima C18 column (5 µm, 250 x 22 mm; Grace, Deerfield, IL). Samples will be eluted with 0.0025% v/v acetic acid in methanol at a flow rate of 10.0 mL/min for 35 mins, and isopropanol for 25 mins at 8.0 mL/min. The eluate will be monitored by UV absorbance at 205 and 220 nm to determine which fractions contain the target compounds. The purity of each fraction will be confirmed by analytical RP-HPLC. Fractions containing pure lipid-PEG-amine will be pooled and dried under vacuum. Purified lipid-PEG-amine can be stored at 4°C in methanol (100 mg/mL). The purities of lipid-PEG-amines (see bottom left, **Fig. 3**) are typically higher than 98%, judging from the RP-HPLC chromatograms.

Synthesis of conjugatable moieties. The lipid-PEG-amines can then be functionalized with several different conjugatable moieties by Proprietary Info (**Fig. 3**). For these studies, we will couple a maleimide group to the lipid-PEG-amine Proprietary Info Proprietary Info Proprietary Info Proprietary Info The conversion is monitored by HPLC. The reaction is typically complete in ~30 mins. Excess Proprietary Info is removed by semi-prep HPLC, fractions containing cholesterol-maleimide are lyophilized and stored at -20°C until use. After coupling of the maleimide, EVLPs will be prepared with 1-2 mole% of the lipid-PEG-maleimide, using the wrapping protocol outlined in the beginning of this SPECIFIC AIM.

EGF as a model targeting ligand. In support of the proposed project, we have been conducting studies aimed at demonstrating our ability to design and engineer targeting ligands that are compatible with lipid bilayers. For this proposal, we will work with EGF as a targeting ligand. EGF recognizes the EGFR which is over-expressed on the surface of MDA-MB-231 and MDA-MB-468 cancer cell lines [23]. We have demonstrated the utility of these targeting ligands by conjugating them to filamentous phage as a scaffold and then assaying them for the ability to bind to cells expressing the appropriate receptor. (This work was done as part of SBIR contract HHSN261201100068c – "Viratrodes: Biosensors for the detection of tumor cells"). For example, M13 phage was conjugated separately to EGF and to the bifunctional maleimide-succinimide ligand EMCS, and ELISA was performed to quantify their respective binding affinities for EGFR-expressing MDA-MB-231 cells. Anti-M13 antibody conjugated to horseradish peroxidase was added to the wells, and incubated for 30 minutes at room temperature. 1-Step Ultra TMB-ELISA was added and the reaction stopped with an equal volume of 2 M sulfuric acid. The absorbance of each well at 450nm was measured by a Thermo Scientific Multiskan® Microplate Photometer. The results in **Fig. 4** show that the EGF-phage binds significantly better to the EGFR-expressing MDA-MB-231 cells than does EMCS-phage.

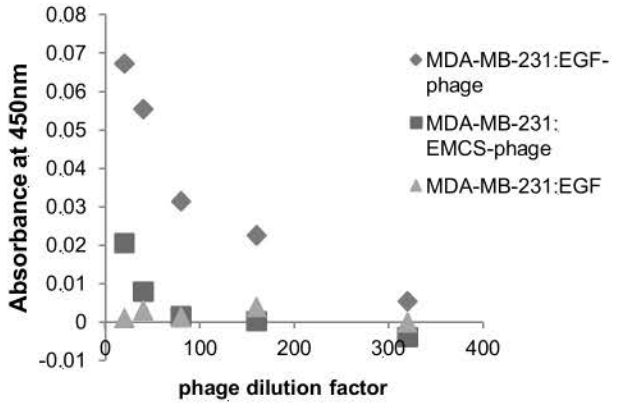

Figure 4. Demonstration of binding of EGF- and EMCS-conjugated M13 phage to cancer cells. MDA-MB-231 cells were incubated with either EGF-phage, EMCS-phage or EGF, as indicated. The cells were then incubated with a HRP-conjugated anti-M13 antibody, and the presence of HRP measured by absorbance at 450 nm, after TMB and sulfuric acid incubations.

Conjugation of EGF to conjugatable EVLPs. Conjugatable EVLPs will be dispersed in sterile 100 mM sodium phosphate buffer, pH 7.0. Lyophilized EGF protein (Reprokine Ltd.) will be reconstituted in the same phosphate buffer at 2 mg/mL and reduced with 1 mM 2-mercaptoethanol for 1 hour at room temperature. Excess 2-mercaptoethanol will be removed by passage through a desalting column and the fractions containing EGF will be collected and combined. The amount of reduced sulfydryl groups on the recovered EGF will be quantified by Ellman's assay. Then, one volume of the reduced EGF will be added to one volume of the EVLPs, and incubated for 1 hr at room temperature. The conjugation efficiency will be determined by HPLC. If necessary, the EVLPs will be buffer exchanged and concentrated by ultrafiltration in an Amicon device.

SPECIFIC AIM 3: Measurement of relative VEGF knockdown efficiencies of naked, VLP, and EVLP forms of VEGF antisense ODNs.

To evaluate the ability of our VLP and EVLP constructs to effectively deliver anti-HIF-1α ODNs to the relevant cells, we plan to use the breast cancer cell lines [23] mentioned earlier. We select these specific

breast cancer cells as a model system because they have been particularly well-studied with respect to EGFR expression [23], HIF-1α induction [22] and because of our in-house experience with them. This significantly reduces the risks associated with successful execution of all aspects of this project, whose focus is on developing and demonstrating a new, improved, G-MED delivery system (EVLPs) for targeted gene knockdown. The modified VLPs and EVLPs described in the first two SPECIFIC AIMS will be tested for their effectiveness in cell culture studies. The cytotoxicity, specificity, and efficacy of these ODN-containing constructs will be characterized for both cancer and non-cancer cells.

Cellular Cytotoxicity. Cancer cells (*e.g.,* MDA-MB-231, MDA-MB-468) and non-cancer cells (*e.g.,* VERO, CHO-S) will be cultured in media supplemented with 10% Fetal Bovine Serum (FBS) and 1X Penicillin-Streptomycin-Glutamine (PSG) at 37°C under normoxic (5% CO_2 , 20% O_2, 75% N_2) or hypoxic (5% CO_2, 0.1%-1% O_2, 94%-94.9% N_2) conditions in 96-well plates until subconfluent. ODN EVLPs, *i.e.*, containing the anti-HIF-1α 5'-TCGCAAGCATCCTGTA-3' ODN [22] or controls – *i.e.,* buffer only, EVLPs containing a similar but non-functional ODN such as 5'-TGGCAAGCATCCTGTA-3' [22], ODN-containing EVLPs without EGF conjugation, VLPs only, and ODNs only – will then be incubated with the cells for 24 hours at 37°C under the normoxic or hypoxic conditions. The effect of each sample on the viability of cells will be evaluated by the AlamarBlue® (Life Technologies) assay per the manufacturer's protocol, by measuring the fluorescence of each well using a Fluoroskan Ascent Microplate Reader (Thermo Scientific) with 544 nm excitation and 590 nm emission filters.

VEGF expression. Cancer cells and non-cancer cells will be cultured in 96-well plates until they are subconfluent, in their recommended media supplemented with 10% FBS and 1X PSG, at 37°C under the normoxic or hypoxic conditions described above. The medium is then removed and ODN EVLPs or the controls listed above will be diluted in fresh media and incubated with the cells for 24 hours at 37°C. The supernatant from each test sample will be harvested and the amount of VEGF analyzed with a VEGF ELISA Kit (Life Technologies). The cells from each well will also be harvested by trypsinization and counted. The VEGF expression levels will be calculated as pg of VEGF protein per mL of medium and per 10^5 cells.

Specificity of targeted EVLPs. The targeting capability of EGF functionalized EVLPs will be evaluated by incubating the cancer and non-cancer cells with targeted and untargeted ODN EVLPs as described above. Candidate ODN EVLPs will be prepared containing a fluorescently labeled lipid probe such as rhodamine-PE (560 nm excitation/580 nm emission; 0.1-0.2 mole%) [24, 25] and ODNs labeled at either the 5'- or 3'- end with a fluorescent probe such as fluorescein (495 nm excitation/520 nm emission). MDA-MB-231, MDA-MB-468 cells and CHO-S cells (negative control) will be cultured, harvested and blocked with 10% fetal bovine serum in PBS buffer. Fluorescently-labeled targeted and untargeted ODN EVLPs at a range of concentrations will be incubated with the cells for 30 minutes to 1 hour at room temperature. The cells will then be washed and the binding activities of the EVLPs will be evaluated by the number of cells with fluorescence and the fluorescent intensity at the end of the assay, under an inverted microscope fitted with an incident light illuminator and fluorescence filter sets for fluorescein and rhodamine.

Anticipated outcomes, risk factors, contingency plans

Through the execution of the activities proposed in Specific Aim 1, we expect to easily achieve ODN encapsidation efficiencies of >95% and possibly, >98-99%. We also expect to be able to identify the buffer conditions where the resulting VLPs are most stable and will be compatible with the wrapping and conjugation procedures to be developed in Specific Aim 2. We do not anticipate encountering any difficulties performing the studies proposed in Specific Aim 2 since the EVLP wrapping process parameters, the synthesis of the conjugatable molecules and the conjugation conditions to attach our model targeting ligand have been largely developed. While cationic lipids might still pose a toxicity issue, we anticipate that, by minimizing the mole ratio, we will be able to essentially eliminate any observable toxic effects to the cells. If this is still a problem, we have synthesized chol-amines that have a carbonate linkage between the PEG linker and the cholesterol. This linkage is easily hydrolyzed under acidic or basic conditions to generate cholesterol and the PEG amine. These molecules can be tested if necessary. The *in vitro* testing proposed in Specific Aim 3 should also be conducted without any problems since we continuously grow the MDA-MB-231 and MDA-MB-468 cell lines for our internal studies. As an alternative, we have produced recombinant versions of echistatin, a disintegrin isolated from the venom of the Southern Copperhead snake. Echistatin binds the αvβ3 integrin which is commonly displayed on the surface of cancer cells and, using the same conjugation and phage binding methods described above, we have demonstrated ligand-specific targeting to MDA-MB-435 cells. We can thus easily switch to this ligand and cell line if necessary. We would like to emphasize that the focus of this STTR Phase I proposal is not directed to specifically developing an ODN-based therapeutic for treating breast and other cancers, but to develop the EVLP technology using a model ODN (i.e., the anti-HIF1α antisense ODN)

and an *in vitro* system in which the targeting ligands and the cell lines are well-characterized and we have extensive experience working with them. It is possible that we will be able to observe untargeted or targeted uptake of the ODNs by the cancer cells, but still not observe anti-HIF-1α activity in terms of a reduction of VEGF expression. This would presumably be caused by inefficient delivery of the antisense ODN to the cytosol by the EVLP since Azizgolshani et al. (2013) showed that the CCMV VLPs will disassemble and release active genetic material if they reach the cellular cytoplasm [11]. Although not a part of the present proposal, we have been engineering proteins designed to promote fusion and delivery of drug molecules such as ODNs into cells. We anticipate that these proteins might be available by the time this project is initiated. If we do find that a targeted or untargeted EVLP ODN reduces VEGF production *in vitro*, then we can test those candidates in the breast cancer animal models established by Personal Info [23] at the University of Texas Austin with whom we have been working collaboratively on this and other projects.

Timeline

We expect to be conducting these studies on an iterative basis. Thus, all three Specific Aims are essentially going to be concurrently as each new VLP/EVLP construct is prepared. An estimated timeline is provided below.

Specific Aims	Q1			Q2			Q3			Q4		
SA 1: In vitro reconstitution of VLPs with ODNs												
SA 2; In vitro synthesis of EVLPs by wrapping of VLPs with a lipid bilayer												
SA 3: Assay of gene knockdown efficiencies and cellular cytotoxicity												

Biohazards

Molecular Express

The Molecular Express facilities are equipped to handle BSL1 and BSL2 microbial organisms. The Company itself does not conduct animal experiments in its facilities; it relies exclusively on its collaborators to conduct these studies. The Company does maintain standard operating procedures for the proper handling and disposal of biohazardous waste generated by its tissue culture and microbial fermentation activities. Samples are stored in approved containers and disposed of through an outside contractor.

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