CBC | CHICAGO BIOMEDICAL CONSORTIUM

CBC Catalyst Award Proposal Title Page

Title of the proposal *(no more than 100 characters including white space)*

Characterization of Cannabidiol and Congeneric Cannabinoids as Inducers of Interferons

Name, degree, title, institution, and contact information including the email address of each PI

PI #1 Marsha Rosner, Ph.D., Professor, University of Chicago; mrosner@uchicago.edu (Contact person)
PI #2 Guido Pauli, Ph.D., Professor, University of Illinois at Chicago; gfp@uic.edu

(Use this space for additional PIs)

Do you have any current or pending grant applications that potentially overlap with this application? Check ONE:

YES ☐ **NO** ☐✓

If YES, please identify them.

(ENTER THE OVERLAP HERE)

Explain the overlap in the Biosketch section.

Does the proposed research involve animal subjects? **Check ONE:** **YES** ☐ **NO** ☐✓

Does the proposed research involve human subjects? **Check ONE:** **YES** ☐ **NO** ☐✓

Does the proposed research involve embryonic stem cells? **Check ONE:** **YES** ☐ **NO** ☐✓

(Note: if the data entered exceeds one page, it is acceptable to submit it as two pages.)

Lay Summary. Cannabis is a growing industry. Two major types of cannabis plants or products exist: marijuana containing cannabinoids with >0.3% THC, the psychoactive ingredient; vs. hemp, with <0.3% THC and dominated by other cannabinoids such as cannabidiol (CBD). Unlike marijuana and THC, hemp cannabinoids are largely unregulated yet widely sold as 'pure compounds' or 'hemp preparations'. Unfortunately, these materials effectively lack quality control, and little understanding exists of the >300 other cannabinoids in hemp products. Despite its widespread use for pain and emotional distress, little is known about CBD's mechanism of action. Our team recently showed that CBD inhibits SARS-CoV-2 by activating interferons, antiviral factors in our immune system that play important roles in other diseases such as cancer. We now propose to develop processes to purify and quality control hemp cannabinoids, determine how CBD activates interferons, and identify cannabinoids that more effectively induce interferons and block SARS-CoV-2.

Risk/Reward. The results of this study are high risk/high reward at various levels. If successful, they will provide a basis for targeted cannabinoid purification and quality control across the country, potentially impacting the entire industry. Second, these studies could provide the basis for understanding one major function of CBD (congeners) as an activator of the innate immune system via interferon production. As the literature about CBD is confusing and often contradictory, increasing our understanding of its *specific, COVID-relevant* action on a molecular level will enable us to determine whether hemp cannabinoids can become a useful (preventive) therapy and/or exacerbate disease states such as COVID or cancer in a context-dependent manner. Third, CBD is relatively lipophilic and treatment involves ingestion of large volumes of oil-based preparations, making it challenging to dispense at therapeutic concentrations. Thus, the characterization of congeneric compounds with increased potency would increase our understanding of its structure-function relationships and potentially identify candidates with greater efficacy and reduced drug delivery challenges. Although these are all potentially exciting outcomes, we need additional supporting data to be competitive for funding from government agencies such as NIH or foundations.

DESCRIPTION OF PROPOSED RESEARCH

Specific Aims. As the use of CBD and other cannabinoids from hemp is on an upward trajectory, it is imperative that we find ways of establishing purity, quality control, and contents of study materials as well as understanding their mechanism of action and efficacy as therapeutic agents. A recent interdisciplinary study led by Rosner established[1] that CBD inhibits SARS-CoV-2 replication in cultured cells and in mice; patients taking a medically prescribed solution of CBD for the treatment of epilepsy tested positive for COVID-19 at lower rates than matched patients not taking CBD. In this study,[1] the **Pauli Lab** isolated and/or analyzed all compounds tested from both commercial sources and hemp extracts by a high precision quantitative NMR (qNMR) technique pioneered by this group. The **Rosner Lab** identified CBD generation of interferons as the main mechanism of SARS-CoV-2 inhibition. Leveraging these findings to increase the safety and efficacy of CBD and related cannabinoids use by the public, we plan to: **[Aim 1]** Source high-quality cannabinoids and improve methodology for their purification and quality control; **[Aim 2]** Identify key mechanisms by which CBD and congeneric cannabinoids activate antiviral interferon signaling; and **[Aim 3]** Determine the ability of CBD homologues to more effectively induce interferons and block SARS-CoV-2 infection.

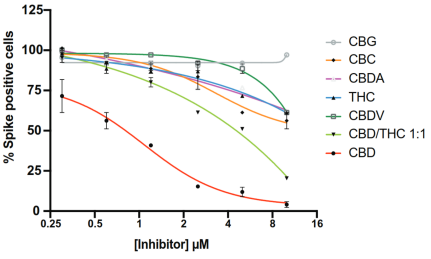

Fig.1 CBD but not other cannabinoids effectively inhibits SARS-CoV-2 replication. Note the combination of CBD and THC drastically increases IC50, highlighting the relevance of understanding molecular mechanism of action and of quality control efforts.

Background & Preliminary Results. To support interdisciplinary drug discovery from natural products (NP), the UIC group has spearheaded two enabling technologies: countercurrent separation (CCS) for NP purification; as well as qualitative and quantitative NMR (qNMR) for advanced characterization. Our recent cross-disciplinary study demonstrated that CBD (not Δ_9-THC) inhibits SARS-CoV-2 replication with an EC_{50} of ~1µM (**Fig. 1**). Analysis of

CBD by qNMR showed compounds from different sources were >97% pure (**Fig. 2**). One primary mechanism was interferon signaling, as supported by RNAseq (not shown) and anti-interferon antibodies (**Fig. 3**).[1]

METHODS

Aim 1. Source high-quality cannabinoids and improve methodology for their purification and quality control. The investigated cannabinoids will be sourced by two means: (i) naturally via purification from hemp, with full chemical and purity characterization of the isolates; (ii) commercial congeneric cannabinoids, such as those in Aim 3, subject to the same control measures as the isolates. For sourcing and characterization, we will use our advanced countercurrent separation methodologies (loss-free/high-resolution;[2,3] adaptable to cannabinoids via GUESS methodology;[4] scalable and capable of generating DESIGNER/knock-out extracts[5]). Characterization of the pharmacological agents will employ our sophisticated NMR methodologies (quantum-mechanical ^{1}H spin analysis [HifSA], for qualitative fingerprinting and rapid ID;[6,7] qNMR assays for absolute quantitation with metrological quality) to avoid hard to obtain *identical* reference materials. For purification, native supercritical-fluid CO_2 extract will be sourced from a licensed industry (Hopsteiner, Yakima, WA) and bulk decarboxylated by mild acid-heat transformation to the acid-free cannabinoids. Utilizing 2-phase solvent system design and loading study data,[2] cannabinoid-enriched extract (20g) will be subject to extractor-type centrifugal partition chromatography (CPC) for the selective fractionation of hundreds of cannabinoids into 5-7 polarity bands, yielding ~2-3g of 1° fractions. Subsequent polarity-matched orthogonal solvent systems will resolve them into 2° fractions (>100 mg each), from which at least 5 mg of individual cannabinoids will be purified via repeated RP-8/-18/-CN prep-HPLC. To enhance purification specificity and target certain classes such as CBD analogues, the 1° and 2° fractions will be subject to HR-LCMS and NMR analysis. This enables assignment of cannabinoid types to 1° polarity bands, helps design orthogonal 2° fractionation methods, and provides initial compound ID data. Detailed structural analysis will draw from our broad experience with phytochemical analysis and use HRMS, advanced 1D/2D NMR spectroscopy, and purity characterization with qNMR methods. To provide reference points for biological strength assays in Aims 2+3, all isolates will be subject to 100% qHNMR analysis, and select materials will be evaluated via metrology-level analysis absolute quantitation (IC-qHNMR). To establish a new interface between targeted isolation, quality control of cannabinoid for Aims 2+3, and future translation to industrial hemp products, isolate profiling will utilize HifSA methodology[6] (CT toolset, NMR Solutions, Kuopio, Finland). This approach also connects the structural features of the new isolates with our prior work on CBD and select analogues[1] and, therefore, enables advanced qNMR assays for cannabinoid quality control. Given our vast experience in this area, we do not anticipate major problems in achieving these goals.

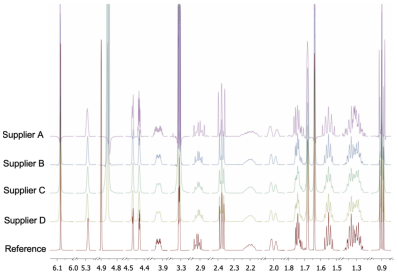

Fig.2 HifSA Fingerprint qNMR analysis of CBD from different suppliers showed ID congruence, but differences in their purity.

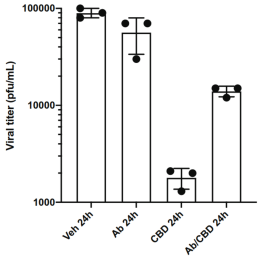

Fig. 3 Human IFN-γ antibody and human type I IFN neutralizing Ab mixture partially rescues SARS-CoV-2 infection after 2.5 μM CBD treatment in A549-ACE2 cells. Virus was measured by plaque assay.

Aim 2. Identify key mechanisms by which CBD activates antiviral interferon signaling. Preliminary studies based on CRISPR-Cas9 knockouts (KO) of NRF2, a CBD-activated transcription factor,[8] and MAVS, a key player in interferon production,[9] in lung A549 carcinoma cells showed that loss of either of these genes decreased interferon signaling genes (ISGs) by ~70% (data not shown). These findings, along with previous studies of interferon activation pathways, led to the proposed scheme for CBD activation of interferon signaling (Fig. 4).[10] Initially, the Rosner lab will repeat the NRF2 and MAVS KO studies. We will then determine whether the mechanism by which CBD activates NRF2 is protein stabilization, synthesis or mRNA transcription. If CBD blocks degradation by ubiquitylation, we will test E3 ligases that degrade NRF2.[11,12] We will then test if CBD acts via

RIG-I and/or MDA5, two upstream activators of MAVs via RIG-I-like Receptors (RLRs).[13] As MAVS-containing complexes signal to kinases including TBK1, we will test TBK1 inhibitors on ISG transcription (e.g., ISG15, IFIT3, data not shown). We will then determine whether IRF3 and/or IRF7 lead to type I interferon transcription using CRISPR Cas9 KO and western blotting. We will also use protein arrays to characterize interferon proteins and other immune factors. Finally, we will KO IFNAR1 or IFNAR2 receptors to identify the one(s) that signal to ISGs. Finally, cells lacking key players in the interferon pathway will be tested for CBD inhibition of SARS-CoV-2 replication using the HTRL BSL3 core facility. We anticipate that loss of these genes will prevent CBD from effectively suppressing viral production. The Rosner lab has extensive experience in signaling and does not anticipate problems with these studies. If needed, alternative intermediates will be identified by (i) transcripts and proteins induced by CBD via RNAseq and mass spectrometry, respectively, and (ii) affinity chromatography/mass spectrometry of protein components in NRF2, MAVS, and/or TBK1 complexes.

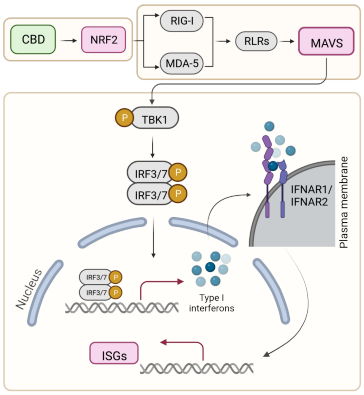

Fig. 4 Proposed mechanism of CBD activation of type-I IFN and subsequent ISG induction. Red squares highlight pathway nodes supported by knockout cell line experiments (NRF2, MAVS) and qPCR (ISGs).

Aim 3. Determine the ability of CBD homologues to more effectively induce interferons and block SARS-CoV-2 infection.

We showed that CBDV, which differs from CBD by 2 fewer aliphatic tail carbons, was ineffective at inhibiting SARS-CoV-2 replication, suggesting CBD specifically interacts with one or more proteins in the cell rather than a nonspecific effect. To determine the constraints on CBD structure-function, we will test the ability of highly related CBD homologues (Fig. 5) to induce ISG transcription and inhibit SARS-CoV-2 replication. We will also monitor toxicity to ensure that the loss of virus is not due to nonspecific cell death. We anticipate that small perturbations in aliphatic chain length (e.g. +/- 1C) might be effective, but regioisomers might not work if a specific CBD-receptor interaction is needed. Once we have identified active and inactive homologues, further directions will focus on the use of the compounds through crosslinking or other methods to identify the direct receptor for CBD responsible for interferon signaling.

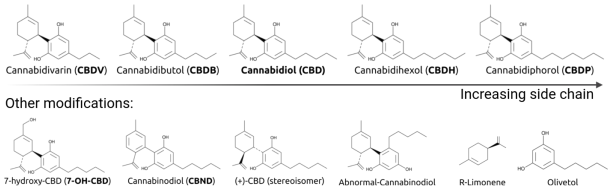

Fig. 5 Array of CBD structurally related compounds that will be investigated. Modifications include shorter and longer aliphatic chain, alternative arrangements of CBD atoms, and CBD metabolites.

Nature of Institutional Collaboration: In Aim 1, Dr. Pauli will develop/scale up his methodologies for compound sourcing and characterization; he will also produce and validate all intervention materials for Dr. Rosner's Aim 2 (CBD mechanistic studies) and Aim 3 (functional activities of CBD homologues). This collaboration is highly complementary and enables us to link rigorously defined natural products to mechanistically defined biological outcomes.

Criteria for Measuring Success: 1) High-purity and well-characterized analogues as well as novel compounds from hemp extracts; 2) Foundation of qNMR data and HifSA profiles for the establishment of a more general quality control for cannabinoids; 3) Identification of key intermediates in the interferon signaling pathway that, when knocked out, prevent CBD from acting as an antiviral agent; 4) Identification of at least one CBD homologue that has efficacy at inducing interferon signaling and blocking SARS-CoV-2 replication at a dose that is at least half that of CBD without causing toxicity.

Long-Term Funding Plan Beyond Catalyst Award: If successful, these studies will put us in a strong position to compete for NIH funding at the level of multi-PI R01s or P01s to investigate the action of CBD and new cannabinoids not only toward SARS-CoV-2 but also other viruses and cancers as well as other diseases that involve the innate immune host response.

References

(1) Nguyen, L. C.; Yang, D.; Nicolaescu, V.; Best, T. J.; Gula, H.; Saxena, D.; Gabbard, J. D.; Chen, S.-N.; Ohtsuki, T.; Friesen, J. B.; et al. *Sci Adv* **2022**, *8*, eabi6110. DOI:10.1126/sciadv.abi6110.

(2) Ohtsuki, T.; Friesen, J. B.; Chen, S.-N.; McAlpine, J. B.; Pauli, G. F. *J. Nat. Prod.* **2022**, *85*, 634–646. DOI:10.1021/acs.jnatprod.1c00976.

(3) Friesen, J. B.; McAlpine, J. B.; Chen, S.-N.; Pauli, G. F. *J. Nat. Prod.* **2015**, *78*, 1765–1796. DOI:10.1021/np501065h.

(4) Liu, Y.; Friesen, J. B.; Klein, L. L.; McAlpine, J. B.; Lankin, D. C.; Pauli, G. F.; Chen, S.-N. *J. Chromatogr. A* **2015**, *1426*, 248–251. DOI:10.1016/j.chroma.2015.11.046.

(5) Yu, Y.; Simmler, C.; Kuhn, P.; Poulev, A.; Raskin, I.; Ribnicky, D.; Floyd, Z. E.; Pauli, G. F. *J. Nat. Prod.* **2019**, *82*, 3321–3329. DOI:10.1021/acs.jnatprod.9b00548.

(6) Achanta, P. S.; Jaki, B. U.; McAlpine, J. B.; Friesen, J. B.; Niemitz, M.; Chen, S.-N.; Pauli, G. F. *J. Pharm. Biomed. Anal.* **2021**, *192*, 113601. DOI:10.1016/j.jpba.2020.113601.

(7) Napolitano, J. G.; Simmler, C.; McAlpine, J. B.; Lankin, D. C.; Chen, S.-N.; Pauli, G. F. *J. Nat. Prod.* **2015**, *78*, 658–665. DOI:10.1021/np5008203.

(8) Atalay Ekiner, S.; Gęgotek, A.; Skrzydlewska, E. *Redox Biol* **2022**, *57*, 102489. DOI:10.1016/j.redox.2022.102489.

(9) Chen, Y.; Shi, Y.; Wu, J.; Qi, N. *Front. Microbiol.* **2021**, *12*, 744348. DOI:10.3389/fmicb.2021.744348.

(10) Mazewski, C.; Perez, R. E.; Fish, E. N.; Platanias, L. C. *Front. Immunol.* **2020**, *11*, 606456. DOI:10.3389/fimmu.2020.606456.

(11) Chowdhry, S.; Zhang, Y.; McMahon, M.; Sutherland, C.; Cuadrado, A.; Hayes, J. D. *Oncogene* **2013**, *32*, 3765–3781. DOI:10.1038/onc.2012.388.

(12) Mikac, S.; Rychłowski, M.; Dziadosz, A.; Szabelska-Beresewicz, A.; Fahraeus, R.; Hupp, T.; Sznarkowska, A. *Antioxidants (Basel)* **2021**, *10*. DOI:10.3390/antiox10050786.

(13) Ren, Z.; Ding, T.; Zuo, Z.; Xu, Z.; Deng, J.; Wei, Z. *Front. Immunol.* **2020**, *11*, 1030. DOI:10.3389/fimmu.2020.01030.

Program Director/Principal Investigator (Last, First, Middle): Pauli, Guido, Frank

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH
	04/01/23	03/31/24

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Guido F. Pauli	PD/PI		0.09	0.03	195,213	1,952	694	2,646
Shaonong Chen	Co-I		1.35	0.38	97,124	14,569	5,178	19,747
To Be Named	Postdoc	6			48,000	24,000	8,530	32,530
SUBTOTALS →						40,521	14,402	54,923

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)*	
Phytochemical supplies and chemicals	4,577
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	
Spectroscopy user fees & NMR cryogens	3,000

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	0
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 62,500
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	0
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 62,500

Program Director/Principal Investigator (Last, First, Middle): Pauli, Guido, Frank

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	54,923	54,923			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	4,577	4,577			
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	3,000	3,000			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS	0	0			
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	62,500	62,500			
F&A CONSORTIUM/ CONTRACTUAL COSTS	0	0			
TOTAL DIRECT COSTS	62,500	62,500			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 125,000

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Personnel

Guido Pauli, Ph.D. Pharm.D. – Principal Investigator (0.09 academic, 0.03 summer months; 1% annualized effort). Dr. Pauli will oversee the natural products-related aspects of the project and work closely with the University of Chicago PI, Dr. Rosner, on providing general guidance to the program. Dr. Pauli will guide the design of the cannabinoid phytochemistry efforts including purification, chromatographic design, and spectroscopic experiments and participate in data analysis. He will also hold regular research team meetings, including meetings of the entire team coordinated with the Dr. Rosner, oversee the documentation of natural products research data, and manage data sharing and public dissemination.

Shao-Nong Chen, Ph.D. – Co-Investigator (1.35 academic, 0.38 summer months; 15% annualized effort). Dr. Chen will bring in his extensive expertise in spectroscopic and chromatographic analysis of plant-derived natural products. He will support the sourcing of commercially available cannabinoids and their spectroscopic and purity analysis. He will also help design the countercurrent and LC methods, will perform cross-platform experiments associated with the analysis of fractions and compounds, and lead the design of the advanced

(budget justification continuation page)

phytochemical methods for the polarity-band fractionation. Evolving from his AOAC training, Dr. Chen will support Dr. Pauli in the provision of the hemp extract and the coordination of the biological evaluation of the natural products with our University of Chicago collaborators in this program.

To Be Named, Ph.D. – Postdoctoral Research Associate (Dr. Pauli's group) (6 calendar months). The TBN postdoc will be involved in all aspects of the phytochemical analyses including sample preparation, documentation, performance of experiments, and data interpretation. He/she will actively participate in phytochemical method development, instrument maintenance and supervision, and compile electronic reports that interface with the public dissemination of research results.

The institutional base salary of Dr. Pauli exceeds the Federal Executive Level II salary limitation thus his salary and fringe request were calculated using the current, pro-rated federal cap.

UIC's projected FY24 fringe benefit rate is 35.54% for academic professionals.
The Chicago Medical Consortium does not allow Indirect Cost.

SUPPLIES
Miscellaneous biochemical supplies include: chemicals, chromatographic sorbents and columns, phytochemical supplies reference compounds, NMR solvents. ($4,577 per year)

OTHER EXPENSES
Equipment user fees: Spectroscopy fees/operation/cryogen costs [NMR, MS, ECD] ($3,000 per year)

Program Director/Principal Investigator (Last, First, Middle): Rosner, Marsha R.

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 04/01/23 THROUGH 03/31/24

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Marsha Rosner	PD/PI	0.06	0.06	0	330,123	1,651	418	2,069
Leticia Stock	co-I	6	6	0	60,372	30,186	7,637	37,823
SUBTOTALS →						31,837	8,055	39,892

CONSULTANT COSTS HTRL BSL3 core facility at Argonne National Laboratory	5,000
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* Tissue culture including media, serum, culture dishes, pipettes (10,010) Reagents for PCR, CRISPR, , western blotting, other routine lab supplies (7,000)	17,010
TRAVEL Travel to meeting for postdoc	500
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)* Publication costs	250

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 62,652
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 62,652

Program Director/Principal Investigator (Last, First, Middle): Rosner, Marsha R.

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	39,892	41,088			
CONSULTANT COSTS	5,000	5,000			
EQUIPMENT					
SUPPLIES	17,010	15,510			
TRAVEL	500	500			
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	250	250			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	62,652	62,348			
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	62,652	62,348			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 125,000

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Overall budget: We are proposing a budget of $125,000/year for 2 years to screen 15 patients/yr.

Personnel:

1) Marsha Rosner, Ph.D., co-PI (0.5% effort). Dr. Rosner has extensive expertise in signal transduction mechanisms. She formed and lead the team that showed CBD inhibits SARS-CoV-2 replication. Her lab also published a recent paper on the role of the ER stress response in the propagation of several coronaviruses including SARS-CoV-2 (Nguyen et al., mBio, 2022). She will supervise all of the work done at the University of Chicago including characterization of the CBD activation pathway leading to interferon signaling, and the characterization of CBD-related compounds as well as final data analysis and presentation related to Specific Aims 2 and 3.

For rest of Budget Justification, see continuing page 11

Rosner Budget Justification (Cont.)

2) Leticia Stock, Ph.D., co-I. (0% effort). Dr. Stock has been a postdoctoral fellow in the laboratory of Dr. Rosner for over a year. She was a coauthor on the Science Advances paper demonstrating that CBD suppresses SARS-CoV-2 replication. Dr. Stock has done the preliminary experiments knocking out NRF2 and MAVS in lung carcinoma cells, and showed the loss of transcriptional induction of interferon signaling proteins as a consequence. She has also been testing the efficacy of CBD related cannabinoids and has the background and experience to carry out Aims 2 and 3.

Supplies:

Tissue culture: we will be growing A549 cells to carry out all the assays needed to test the signaling pathways outlined in Aim 2. These include media, serum, plates, and pipettes,

Reagents for PCR, CRISPR, western blotting and other routine lab supplies such as gloves will be needed to do the studies described in Aim 2 and 3.

Howard Taylor Ricketts Laboratory (HRTL) located at Argonne: we will need to test replication of SARS-CoV-2 using this core facility that assesses both viral titer and spike protein to monitor viral replication as well as toxicity. We will test the effect of knockout of genes in the interferon pathway on the efficacy of CBD to inhibit the virus (Aim 2). In addition, we will test the efficacy of CBD related cannabinoids to inhibit the virus (Aim 3. We have used this facility previously in our Science Advances paper. This is a fee for service facility.

Travel: We request $500 per year for Dr. Stock to present her results at a meeting.

Publication: We request $250 per year toward publication costs. We anticipate this research will produce at least two publications.

BIOGRAPHICAL SKETCH

NAME:	Pauli, Guido F.
eRA COMMONS USER NAME:	PAULIG
POSITION TITLE:	Norman R. Farnsworth Professor of Pharmacognosy (Pharm. Sciences) Director Pharmacognosy Institute, UIC College of Pharmacy

EDUCATION/TRAINING:

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Philipps University, Marburg (Germany)	MS	12/1987	Pharmacy
Philipps University, Marburg (Germany)	PHMD	12/1988	Pharmacy
Heinrich Heine University, Düsseldorf (Germany)	PHD	05/1993	Pharmacognosy
Louisiana State University, Baton Rouge, LA	Post-doc	07/1995	Nat. Prod. Chemistry
GWL Hansen's Disease Center, Baton Rouge, LA	Post-doc	07/1995	Microbiology

A. Personal Statement

Project Specific Statement. Chemical and biological analysis of natural products in cross-disciplinary programs is a core expertise of my research. Notable for the proposed project is my expertise in the analysis of complex mixtures and their rigorous spectroscopic characterization. This includes familiarity with and development of new approaches in preparative separation and chromatography (unique experience with countercurrent separation methods, to be used in this program), as well as nuclear magnetic resonance (NMR; 1D/2D), mass spectrometry (MS; LC/GC-MS) and other spectroscopic methods (ECD, IR) that are state-of-the-art in the dereplication and *ab initio* structural analysis. My laboratory and research environment are very well-equipped to successfully work with small to large quantities of natural product samples, and my team has advanced capabilities for the acquisition, off-line processing and interpretation of the spectroscopic data. I have 30+ years of experience with all major classes of natural products, have pioneered the use of quantitative NMR and the advancement of modern countercurrent chromatography for purity assessment and analysis of natural products, respectively. My collaboration with Dr. Rosner started in 2020 and has evolved rapidly into a synergistic, trans-disciplinary collaboration exploring the mechanistic targets of *Cannabis sativa* phytoconstituents for antiviral (anti-SARS-Cov2 properties of CBD; *Sci.Adv.* 8, eabi6110, 2022) and cancer related biological endpoints

Project Specific Publications that highlight my experience and qualifications for this project follow.

Four publications most relevant to the current application [*denotes corresponding author]

Documenting experience and qualification in multi-disciplinary NP research programs and Cannabis research

Nguyen L, Yang D, Nicolaescu V, Best T, Gula H, Saxena D, Gabbard J, Chen SN, Ohtsuki T, Friesen JB, Drayman N, Mohamed A, Dann C, Silva D, Robinson-Mailman L, Valdespino A, Stock L, Suárez A, Jones K, Demarco J, Severson W, Millis JW, Dickinson B, Tay S, Oakes S, **Pauli GF**, Palmer K, The National COVID Cohort Collaborative Consortium, Meltzer D, Randall G, Rich Rosner M. Cannabidiol Inhibits SARS-CoV-2 Replication through Induction of the Host ER Stress and Innate Immune Responses. *Science Adv. 8, eabi6110 (2022)*; Open Access.

Ohtsuki T, Friesen JB, Chen SN, McAlpine J, **Pauli GF**. Selective Preparation and High Dynamic-Range Analysis of Cannabinoids in "CBD Oil" and Other *Cannabis sativa* Preparations. *J. Nat. Prod.* 85, in press (2022); PMC8957589.

Documenting innovative methodology in natural products research

Nishizaki Y, Lankin D, Chen SN, **Pauli GF**. Accurate and Precise External Calibration Enhances the Versatility of Quantitative NMR (qNMR). *Anal. Chem.* 93, 2733-2741 (2021). PMCID PMC8046598

Friesen JB, Liu Y, Chen SN, McAlpine J, **Pauli GF**. Selective Depletion and Enrichment of Constituents in "Curcumin" and Other *Curcuma longa* Preparations. *J. Nat. Prod,* 82, 621-630, 2019; Open Access.

Ongoing & recently completed projects relevant to be highlighted

U19 AI142735 (Fotouhi, PI)	03/01/19 – 02/29/24	0.90 academic
Global Alliance for TB Drug Development (NIH flow-through)	Role: Co-PL fv	0.25 summer
1R01 DE028194-01A1 (Bedran-Russo, PI)	07/01/19 – 06/30/24	

National Institute of Health — Role: Co-I
"Dentin Biomodification for Optimization of Bioadhesive Dental Restorations"

P50 AT00155 (Pauli, PI) — 9/1/15 – 6/30/22 (end 2nd NCE)
NIH/NCCIH/ODS — Role: Director, Project 1 and Core B Leader
Botanical Dietary Supplements for Women's Health

U41 AT008706 (Pauli, PI) — 09/01/15 – 06/30/20
NIH/NCCIH (ODS) — Role: Director/PD
Center for Natural Product Technologies

B. Positions and Honors

Positions and Employment

2019–present Distinguished Professor, University of Illinois at Chicago, Chicago (IL)
2017–present Director, Pharmacognosy Institute (formerly Program for Collaborative Research in the Pharmaceutical Sciences [PCRPS]), incl. the UIC Center for Botanical Dietary Supplements Research, and NAPRALERT
2012–present Professor (PSCI, formerly MCP), UIC College of Pharmacy, Chicago (IL)
2012–present Associate Director and Research Professor, Institute for Tuberculosis Research (ITR)

2012–2019 Head of the Graduate Program in Pharmacognosy
2012–2017 Co-Director, UIC Center for Botanical Dietary Supplements Research, and
2009–2012 Director of Graduate Studies in Pharmacognosy2008–2012 Associate Professor (MCP, Med. Chem. & Pharmacognosy) Department of Medicinal Chemistry and Pharmacognosy and Research Associate Professor (ITR, Inst. TB Research), College of Pharmacy, University of Illinois, Chicago (IL)
2002–2008 Assistant Professor, Department of Medicinal Chemistry and Pharmacognosy, MCP, College of Pharmacy, University of Illinois (UIC), Chicago (IL)
2001–2008 Research Assistant Professor, Institute for Tuberculosis Research (ITR), College of Pharmacy, University of Illinois, Chicago (IL)
1995–2001 Junior Professor, Institute of Pharmaceutical Biology and Phytochemistry, WWU Westfälische Wilhelms–University, Münster (Germany)

Other Experience and Professional Memberships

2014–present Chair, NCCIH/NIH Special Emphasis Panels (ZAT1 PK32, ZAT1 SM45, ZAT1AJT03, ZAT1 SM50, ZAT1 SH01)
2010-present Chair/Member, United States Pharmacopoeia (USP), Dietary Supplements Expert Committee
2006–present Member, NCCIH/NIH Basic Science and Special Emphasis Panels (ZAT1 DB22, ZAT1 DB25, ZAT1 DB26, ZAT1 SM23, ZAT1 SM24, ZAT1 SM25, ZAT1 HS19, ZAT1 PS08)
2004–present Chair (2006, 2015) & IAC Member, Int'l Network for Countercurrent Chromatography (INCCC, CCC20xx Conferences)
1999–present FAPA, Specialization in Pharmaceutical Analysis, Board of Pharmacists (Germany)
1995–present Member, Phytochemical Society of Europe (PSE)
1992–present Member, American Society of Pharmacognosy (ASP)
1988–present Licensed Pharmacist, Board of Pharmacists (Germany)
1991–2001 Member, Board of Examiners in Pharmacy (Germany): HHU Düsseldorf (1991-1994); WWU, Münster (1995-2001)
2013-2019 Adjunct Professor and Bagui Scholar, Guangxi Institute of Botany, Guilin (China)
2014 Member, AAAS peer review panel
2011 Visiting Professorships: 2011 IMB/NRC, Halifax (Canada); 2011 Novartis, Basel (Switzerland);
2009+2010 Member, NIH/CSR reviewer panels for RC-1 and RC-4 ARRA applications
2008 Member, NIAID/NIH Biodefense and Emerging Infectious Diseases U01 Panel (ZAI1-MMTM)
2006–2014 Treasurer and Assistant Treasurer, American Society of Pharmacognosy (ASP)
2006–2009 Chair (2008-2009) and Member, NACCAM/NIH Product Integrity Working Group (PIWG)

Honors

2022 Norman R. Farnsworth Research Excellence Award, American Botanical Council (ABC)
2019 Distinguished Professor, University of Illinois at Chicago
2018/2019 Varro E. Tyler Award, American Society of Pharmacognosy (ASP)

2017 Norman R. Farnsworth Professorship in Pharmacognosy, UIC College of Pharmacy
2014 University Scholar, University of Illinois at Chicago, Chicago, IL
2014 Bagui Scholar, Guangxi Institute of Botany, Guilin (China)
1995 Egon Stahl Award in Bronze, German Society for Medicinal Plant Research
1994 Best Ph.D. Thesis Award, Heinrich Heine-University, Düsseldorf (Germany)

C. Contribution to Science

Overview. My biomedical research expertise is at the interface of secondary metabolome and natural products (NPs), the assessment of their biological activity, as well as spectroscopic and chromatographic methodology. My long-term research objective is to enhance the understanding of the chemical composition and complex biological effects of NPs from/in biological systems. Focusing on human health, my research contributes to interdisciplinary studies in natural health products, drug discovery, and dietary supplements. Studies involve a diversity of organisms from higher plants to bryophytes to microbes, and develop/utilize new methodologies in spectroscopy, chromatography, *in vitro* bioassays, and computational analysis. I am actively engaged in the education of the next generation of pharmacognosists as mentor of Ph.D. students (15 finished, 2 ongoing) and postdocs (20+ to date); hosted international students/scholars (7 to date) and visiting professors (6 to date). As NIH awardee, I have been PI/PD/PL/Co-I of 20+ major projects since 2001. I am highly committed to academic publishing (**260** papers, 80 in the last 5 yrs), board and peer-review function for scientific journals, as well as service on expert (USP, NCI PDQ; chair and member) and review panels (NIH; chair, and reviewer).

Pauli GF*, Friesen JB, Gödecke T, Farnsworth NR, Glodny B. Occurrence of progesterone and related animal steroids in two higher plants. *J. Nat. Prod.*, 73, 338-345, 2010; PMCID: No PMCID [n/a].

Bisson J, McAlpine JB, Friesen JB, Chen S-N, Graham J, **Pauli GF**. Can Invalid Bioactives Undermine Natural Product-Based Drug Discovery? *J. Med. Chem.*, 59, 1671-1690, 2016; PMCID: PMC4791574.

Nelson KM, Dahlin JL, Bisson J, Graham J, **Pauli GF**, Walters MA. The Essential Medicinal Chemistry of Curcumin. *J. Med. Chem.*, 60, 1620-1637, 2017; PMCID: PMC5346970.

Nguyen L, Yang D, Nicolaescu V, Best T, Gula H, Saxena D, Gabbard J, Chen SN, Ohtsuki T, Friesen JB, Drayman N, Mohamed A, Dann C, Silva D, Robinson-Mailman L, Valdespino A, Stock L, Suárez A, Jones K, Demarco J, Severson W, Millis JW, Dickinson B, Tay S, Oakes S, **Pauli GF**, Palmer K, The National COVID Cohort Collaborative Consortium, Meltzer D, Randall G, Rich Rosner M. Cannabidiol Inhibits SARS-CoV-2 Replication through Induction of the Host ER Stress and Innate Immune Responses. *Science Adv. 8, eabi6110 (2022)*; PMCID in process.

Natural Health Products & Dietary Supplements. A major collaborative and multi-disciplinary effort involves research in the UIC/NIH Botanical Research Center (BRC; P50), where I lead the Botanical Integrity Core B as well as Project 1, focusing on the metabolomic standardization of botanicals. Core B pioneered the establishment of Botanical Integrity Dossiers (BPIDs), in which botanical raw materials, extracts, and purified phytoconstituents are characterized using a variety of orthogonal pharmacognostic assays (ranging from microscopy to UHPLC to qNMR). In addition to the bioassay-guided characterization of numerous bioactive metabolites from widely used botanical dietary supplements (e.g., black cohosh, hops, dang gui, licorice), we have developed biochemometrics as an innovative means of identifying new biomarkers. Serving collaborative projects at the chemistry/biology interface, we have introduced the concept of Residual Complexity (RC; static and dynamic) as an important aspect of bioactive natural products and have pioneered (quantitative) Purity-Activity Relationships ([q]PAR) as a new means of assessing RC in natural products materials and biological test systems. Using newly established methods such as qNMR (see below), we have developed metabolomic (multi-target) standardization for botanical intervention materials for detailed mechanistic research (in vitro and in vivo) and translational studies (three completed Phase I and II clinical trials).

Geller SE*, Shulman LP, van Breemen RB, Banuvar S, Epstein G, Hedayat S, Nikolic D, Krause EC, Piersen CE, Bolton JL, **Pauli GF**, Farnsworth NR. Safety and efficacy of black cohosh and red clover for the management of vasomotor symptoms: a randomized controlled trial. *Menopause*, 16, 1156-1166, 2009; PMCID: PMC2783540.

Simmler C, Anderson JR, Gauthier L, Lankin DC, McAlpine JB, Chen S-N, **Pauli GF**. Metabolite Profiling and Classification of DNA-Authenticated Licorice Botanicals. *J. Nat. Prod.*, 78, 2007-2022, 2015; PMCID PMC4553119.

Friesen JB, Liu Y, Chen SN, McAlpine J, **Pauli GF**. Selective Depletion and Enrichment of Constituents in "Curcumin" and Other *Curcuma longa* Preparations. *J. Nat. Prod*, 82, 621-630, 2019; PMCID in process (OA)

Yu Y, **Pauli GF**, Huang L, Gan LS, van Breemen F, Li D, McAlpine J, Lankin D, Chen SN. Classification of Flavonoid Metabolomes via Data Mining and Quantification of Hydroxyl NMR Signals. *Anal. Chem.* *92*, 4954-4962 (2020); PMCID in process.

Anti-TB Drug Discovery. The second major research effort of my group is dedicated to the discovery of new anti-TB drug leads from natural products. To this end, my research has contributed to various aspects of the discovery process: new biological test methods (low-oxygen recovery assay [LORA], TLC bioautography), utilization of a wide variety of phyla as natural sources (plants/ethnobotanicals, fungi, actinomycetes), metabolomic and diagnostic analyses (gaseous contact assay, advanced in vivo TBSA assay), and new methods for the targeted search of lead compounds (biochemometrics; PAR by qNMR) and the assessment of synergy (essential oils vs. XDR-TB). Discovered lead compounds with high potency low ug/mL activity and promising selectivity are micromolide, indigoids, ilamicin, hytramicin, and ecumicin. The tridecapeptide, ecumicin, has a new molecular target (ClpC1), a very favorable activity profile (selectivity index >500), has been shown to be efficacious in an animal model, and is currently in further pre-clinical development (PK, formulation). Performed as part of my appointment at UIC's Institute for Tuberculosis Research, pre-clinicl translation of our cyclopetide lead compounds into early-stage clinical trials is currently supported by a multi-site U19 award from NIAID.

Cho SH, Warit S, Wan B, Hwang CH, **Pauli GF**, Franzblau SG*. Low-oxygen-recovery assay for high-throughput screening of compounds against nonreplicating *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.*, 51, 1380-1385, 2007; PMCID: PMC1855511.

Gao W, Kim JY, Chen SN, Cho SH, Choi J, Jaki BU, Jin YY, Lankin DC, Lee JE, Lee SY, McAlpine JB, Napolitano JG, Franzblau SG, Suh JW, **Pauli GF**. Discovery and characterization of the tuberculosis drug lead ecumicin. *Org. Lett.*, 16, 6044-6047, 2014; PMCID: n/a.

Choules M; Klein L, Lankin D, McAlpine J, Cho SH, Cheng J, Lee H, Suh JW, Jaki B, Franzblau S, **Pauli GF** Residual Complexity Does Impact Organic Chemistry and Drug Discovery: The Case of Rufomyazine and Rufomycin. *J. Org. Chem.* 83, 6664-6672, 2018, PMCID PMC6006449.

Wolf N, Lee H, Zagal D, Nam JW, Oh DC, Lee H, Suh JW, **Pauli GF**, Cho S, Abad-Zapatero C. Structure of ClpC1-NTD in complex with the anti-TB natural product ecumicin reveals unique binding interactions. **Error! Hyperlink reference not valid.** *D 76*, 458-471 (2020); PMCID in process.

Innovative Bioanalytical Methodology. Serving all major areas of our interdisciplinary research, we have pioneered the numerous bioanalytical approaches that represent enabling technologies for new insights and enhance discovery. Particular expertise for these contributions involves the following platform technologies: nuclear magnetic resonance (NMR), in particular quantitative NMR (qNMR) 2D NMR barcoding; advanced models for data evaluation such as ^{1}H iterative Full Spin Analysis (HiFSA) in NMR; various new methods for qHNMR including acquisition and evaluation; countercurrent separation (CCS) technology, in particular the widely used GUESS concept for the rapid establishment of separation conditions, the K-based Reciprocal Symmetry (ReS) method for EECC chromatograms, and the concept of developing DESIGNER materials of natural products, such as Knock-out and knock-in extracts that enable unique biological studies.

Qiu F, McAlpine JB, Lankin DC, Burton I, Karakach T, Chen S-N, **Pauli GF***. 2D NMR barcoding and differential analysis of complex mixtures for chemical identification: the *Actaea* triterpenes. *Anal. Chem.*, 86, 3964-3972, 2014; PMCID: PMC4004191.

Pauli GF, Pro SM, Chadwick LR, Burdick T, Pro L, Friedl W, Novak N, Maltby J, Qiu F, Friesen JB. Real-Time Volumetric Phase Monitoring: Advancing Chemical Analysis by Countercurrent Separation. *Anal. Chem.*, 87, 7418-7425, 2015; PMCID PMC4509944.

Phansalkar R, Simmler C, Bisson J, Chen S-N, Lankin DC, McAlpine JB, Niemitz M, **Pauli GF**. Evolution of Quantitative Measures in NMR: Quantum Mechanical qHNMR Advances Chemical Standardization of a Red Clover (*Trifolium pratense*) Extract. *J. Nat. Prod.*, 80, 634-647, 2017; PMCID: PMC5368683 .

Phansalkar R, Nam JW, Chen SN, McAlpine J, Leme A, Aydin B, Bedran-Russo A, **Pauli GF**. Centrifugal partition chromatography enables selective enrichment of trimeric and tetrameric proanthocyanidins for biomaterial development. *J. Chrom. A*, 1535, 55-62, 2018; PMCID PMC5801063.

Biomodifying Agents for Restorative Dentistry. A third field of interdisciplinary study seeks to establish new intervention materials that utilize plant polyphenols as highly selective biomodifiers of dentin. This led to the identification of oligomeric proanthocyanidins from certain plant source (e.g., *Pinus*, *Cinnamomum*, *Camellia*), which are capable of enhancing the biomechanical properties of dentin, e.g. by increasing its strength by up to 10-fold and by augmenting its resistance to biodegradation. Phytochemical expertise contributed to this program involves the purification of bioactive principles in free phenolic form, their characterization by advanced spectroscopic methods (e.g., HiFSA NMR), and the production and standardization of special fractions with promising biological profiles, for the development of clinical intervention materials. (see above for latest papers)

Vidal CMP, Leme AA, Aguiar TR, Phansalkar R, Nam JW, Bisson J, McAlpine JB, Chen SN, **Pauli GF**, Bedran-Russo A. Mimicking the hierarchical functions of dentin collagen cross-links with plant derived phenols and phenolic acids. *Langmuir*, 30, 14887-14893, 2014; PMCID: in PMC4437200.

Nam J-W, Phansalkar RS, Lankin DC, Bisson J, McAlpine JB, Leme AA, Vidal CMP, Ramirez B, Niemitz M, Bedran-Russo A, Chen S-N, **Pauli GF**. Subtle Chemical Shifts Explain the NMR Fingerprints of Oligomeric Proanthocyanidins with High Dentin Biomodification Potency. *J. Org. Chem.*, 80, 7495-7507, 2015; PMCID PMC4537297.

Nam J-W, Phansalkar RS, Lankin DC, McAlpine JB, Leme-Kraus AA, Vidal CMP, Gan L-S, Bedran-Russo A, Chen S-N, **Pauli GF**. Absolute Configuration of Native Oligomeric Proanthocyanidins with Dentin Biomodification Potency. *J. Org. Chem.*, 82, 1316-1329, 2017; PMCID: PMC5451120.

Zhou B, Alania Y, Reis M, Phansalkar R, Nam JW, McAlpine J, Chen SN, Bedran-Russo A, **Pauli GF**. Rare A-Type, Spiro-Type, and Highly Oligomeric Proanthocyanidins from *Pinus massoniana*. *Org. Lett.* *22*, 5304-5308 (2020); PMCID in process.

Complete List of Publications. The following repositories list all my **260 publications** (avg. 16/yr for the last five years): **NCBI SciENcv** http://www.ncbi.nlm.nih.gov/myncbi/guido.pauli.1/cv/44276/**, NCBI MyBibliography** http://www.ncbi.nlm.nih.gov/sites/myncbi/guido.pauli.1/bibliograpahy/43336107/public/?sort=date&direction=ascending**,** and on my **University of Illinois at Chicago webpage** at go.uic.edu/gfpapers.

BIOGRAPHICAL SKETCH

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

NAME: Rosner, Marsha R.

eRA COMMONS USER NAME (credential, e.g., agency login): mrosner

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Harvard University	AB	06/1972	Biochemistry
Massachusetts Institute of Technology	PhD	06/1978	Biochemistry
Massachusetts Institute of Technology	Postdoc	09/1981	Biochemistry
Massachusetts Institute of Technology	Instructor	09/1982	Biochemistry

A. Personal Statement

I have extensive experience for over 30 years studying signaling pathways and networks, as well as purifying proteins and characterizing them. Throughout my career, I have had a record of successful and productive research projects in the field of signal transduction that are highly relevant to cancer. On an administrative level, I was founder and first Chair of the University of Chicago Committee on Cancer Biology (graduate program), Chair of the Ben May Department for Cancer Research for 13 years and Deputy Director of the University of Chicago Comprehensive Cancer Center (UCCCC) during this period. Associated with this role, I have been and remain a member of the Executive Committee of the UCCCC.

The current focus of my laboratory is to understand fundamental signaling mechanisms leading to the generation of tumor cells and their progression to metastatic disease, particularly in triple-negative breast cancer that lacks targeted therapies. We use systems level approaches including activity-based proteomics, RNAseq, ChIPseq, and mass spectrometry as well as computational, molecular, biophysical, cellular and mouse model-based methodologies to identify and characterize key regulators of tumor growth and metastasis. As an additional tool, we have utilized a specific physiological suppressor of metastasis, Raf Kinase Inhibitory Protein (RKIP or PEBP1), and a downstream target of RKIP in cells, BACH1, to identify both molecular and cellular mediators of metastasis.

Our recent studies have shown that regulators of metastasis control multiple processes within the tumor cell microenvironment including metabolism, redox state, extracellular matrix, and recruitment and programming of tumor-associated macrophages. These factors also direct extracellular vesicles (exosomes) secreted by tumor cells to reprogram other cells in the body toward a pro-metastatic phenotype. Correlating omic-generated data from these studies with clinical data from cancer patients led to the identification of novel signaling modules that we used to build gene signatures that predict the metastatic potential of a tumor. More recently, our studies have led us to potential therapeutic treatments based on the concept of targeting key regulators of tumorigenesis, mimicking the action of metastasis suppressors such as RKIP or reprogramming signaling networks in cells to sensitize tumors to therapeutic agents such as metabolic inhibitors. Finally, during the pandemic, we utilized our knowledge of host stress response pathways to identify therapeutics that inhibit SARS-CoV-2 replication through activation of the unfolded protein response and interferon pathways.

I have also had considerable experience mentoring numerous graduate students, postdoctoral scholars and clinical researchers who have gone on to successful careers in academia, industry or government. To date I have mentored 19 graduate students who received their Ph.D.s and 42 postdoctoral researchers. As the first woman department Chair in the Division of Biological Sciences at the University of Chicago, I have also been a strong advocate for promoting women and diversity in science.

Ongoing and recently completed projects that I would like to highlight include:

1R01CA266643-01A1
Rosner (PI)
9/1/22 – 8/31/27
Regulation of Tumor Oxygenation by BACH1 in Breast Cancer

DOD, W81XWH2110120
Rosner (PI)
3/1/21 – 2/28/24
Targeting Cancer Stem Cells in Triple-Negative Breast Cancer

CCT Searle Funds, Chicago Biomedical Consortium, C-092
Rosner (PI)
3/1/19 – 2/28/23 (Rosner project completed by 11/18/2022)
A Novel Diagnostic Biomarker for Breast Cancer

1RO1GM121735-04
Rosner (PI)
9/30/17 – 8/31/22
Regulation of RKIP Function

1RO1CA184494-04
Rosner (PI)
3/19/15 – 2/28/21
Tumor-stromal interactions as targets of tumor metastasis suppressors

Citations:

1. Lee, J., Yun, J., Yeung, K., Bevilacqua, Balázsi, G., and **Rosner, M.R.**, BACH1 and RKIP participate in a Bistable Network that affects Progression to Metastasis in Breast Cancer, *PNAS,* 21;111(3):E364-73 (2014). doi: 10.1073/pnas. Epub 2014.
2. Lee, J., Yesilkanal, A., Wynne, J., Frankenberger, C. Liu, J., Yan, J., Elbaz, M., Rabe, D.C., Rustandy, F.D., Tiwari, P., Grossman, E., Hart, P.C., Kang, C., Sanderson, S.M., Andrade, J., Nomura, D.K., Bonini, M., Locasale, J.W. and **Rosner, M.R.,** Effective breast cancer combination therapy targeting BACH1 and mitochondrial metabolism. *Nature*, *568*:254-258 (2019). DOI: 10.1038/s41586-019-1005-x.
3. Yesilkanal, A., Yang, D., Valdespino, A., Tiwari, P., Sabino, A.U., Nguyen, L., Lee, J., Xie, X.-H., Sun, S., Dann, C., Robinson-Mailman, L., Steinberg, E., Stuhlmiller, T., Frankenberger, C., Goldsmith, E., Johnson, J.L., Ramos, A.F., **Rosner, M.R**., Limited inhibition of multiple nodes in a driver network blocks metastasis. *eLife*, (2021) ;10:e59696. DOI: https://doi.org/10.7554/eLife.59696
4. Nugyen, L.C., Yang, D., Nicolaescu, V., Best, T.J., Gula H., Saxena, D., Gabbard, J.D., Chen, S.-N., Ohtsuki, T., Friesen, J.B., Drayman, N., Mohamed, A., Dann, C., Silva, D., Robinson-Mallman, L., Valdespino, A., Stock, L., Suarez, E., Jones, K.A., Azizi, S.-A., Demarco, J.K., Severson, W.E., Anderson, C. D., Millis, J. M., Dickson, B. C., Tay S., Oakes, S.A., Pauli, G.F., Palmer, K.E., The National COVID Cohort Collaborative Consortium, Meltzer, D., Randall, G. and **Rosner, M.R**. Cannabidiol Inhibits SARS-CoV-2 Replication through Induction of the Host ER stress and innate immune responses. *Sci Adv.* (2022) 8: eabi6110.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2009 - Present Member, Committee on Genetics, Genomics and Systems Biology, University of Chicago, Chicago IL

2007 - Present Fellow, Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL
2000 - Present Charles B. Huggins Professor, Ben May Department for Cancer Research, University of Chicago, Chicago, IL
1994 - Present Professor, Ben May Department for Cancer Research, Department of Neurobiology, Pharmacology, and Physiology, Cmtes. on Cancer Biology, Cell Physiology, and Developmental Biology, and the Cancer Research Center, University of Chicago, Chicago, IL

Other Experience and Professional Memberships

2009 - 2013 Member, Tumor Cell Biology (TCB), Study Section, NIH
2007 - 2009 TCB Study Section, NIH (ad hoc)
2007 - 2008 Reviewer, Austrian Science Foundation Hearing of the Doctoral Program "Molecular Mechanisms in Cell Signaling"
2001 - 2006 Member, Editorial Board, ASBMB, Journal Biological Chemistry

1999 - Present Member, Board of Trustees, IMSA
1997 - Present Member, Editorial Board, Gene Therapy and Molecular Biology
1997 - 2001 Member, Biochemistry Study Section, NIH (Chair 2000-2001)
1994 - 2005 Member, Children's Memorial Institute for Education and Research Scientific Advisory Committee
1994 - 1997 Member, Editorial Board, Molecular Endocrinology
1993 - 1997 Member, Biochemistry and Endocrinology Reviw Committee, Member, American Cancer Society
1986 International Life Sciences Institute Research Foundation Award

Honors and Awards

2019 Notable Women in Science, Crain's Chicago Business
2015 Fellow, Institute for Molecular Engineering, University of Chicago
2014 Fellow, American Association for the Advancement of Science (AAAS)
2011 Gerald N Wogan Prize Lecture, Massachusetts Institute of Technology
2007 - Present Member, Faculty of 1000 Medicine
2001 Quantrell Award for Excellence in Undergraduate Teaching
1999 - Present Fellow, Institute of Medicine of Chicago
1991 Quantrell Award for Excellence in Undergraduate Teaching, (Cell Biology)
1986 International Life Sciences Institute Research Foundation Award
1978 -1980 American Cancer Society Postdoctoral Fellowship
1975 -1977 Inst. National Research Service Award
1975 -1977 Inst. National Research Service Award
1973 -1975 Sloan Research Traineeship (Biophysics)
1972 -1973 MIT Endowed Fellowship

C. Contributions to Science

1. Our lab is among the founders of the field of signal transduction. We were among the earliest groups demonstrating the importance of kinase signaling cascades in the action of factors that promote cell growth. In particular, we showed that Protein Kinase C, through tumor promoters, is able to modulate the action of growth factors by regulating their receptor activity. These studies demonstrated the role of kinase networks in regulating the action of signal responders such as the epidermal growth factor receptor, and laid the foundation for understanding how cells respond to multiple environmental cues.

a. Friedman, B., Frackelton, A.R., Ross, A.H., Connors, J.M., Fujiki, H., Sugimura, T. and **Rosner, M.R.** Tumor promoters block tyrosine-specific phosphorylation of the epidermal growth factor receptor. *Proc. Natl. Acad. Sci.* USA **81**:3034-3038 (1984).
b. McCaffrey, P.G., Friedman, B. and **Rosner, M.R.** Diacylglycerol modulates binding and phosphorylation of the epidermal growth factor receptor. *J. Biol. Chem.* **259**:12502-12507 (1984).
c. **Rosner, M.R.**, McCaffrey, P.G., Friedman, B. and Foulkes, J.G. "Modulation of Growth Factor Action by Tumor Promoters and C Kinase" in *Cancer Cells*, Vol. **3** (Growth Factors and Transformation), eds.

Feramisco, J., Ozanne, B. and Stiles, C., Cold Spring Harbor Laboratory: Cold Spring Harbor, pp. 347-351 (1985).

2. We published one of the original papers characterizing MAP kinase (later called ERK), characterized key ERK regulatory networks in breast cancer and proposed novel approaches to treatment. One of the major families of intracellular kinases that regulates cell growth as well as most other physiological processes is the MAP kinase family. Our work showed that a kinase that phosphorylates the epidermal growth factor (EGF) receptor that we purified and characterized was in fact MAP kinase (later termed ERK kinase after cloning). Our work showed that MAP/ERK kinase is activated by the EGF receptor and functions as a downstream mediator. This pathway is now one of the most conserved signaling pathways utilized by growing cells. We also showed that epigenetic regulators such as HMGA2 that are downstream of ERK signaling pathways regulate breast cancer growth and metastasis and targeting ERK-regulated transcription factors can lead to metabolic reprogramming and increased sensitivity to therapeutic agents. Most recently, we have proposed and tested the intriguing concept that combined, low dose inhibition of multiple kinase signaling networks simultaneously is a more effective strategy overall to inhibit metastasis in models of breast cancer. This work models and establishes a pharmacological means to phenocopy the actions of RKIP, a metastasis suppressor that targets multiple stress kinase signaling networks. Overall, this is a new and interesting idea that runs counter to current combination therapy ideas and therefore has promise to be quite impactful.

a. Takishima, K., Griswold-Prenner, I., Ingebritsen, T. and **Rosner, M.R**.: Epidermal growth factor (EGF) receptor T669 peptide kinase from 3T3-L1 cells is an EGF-stimulated "MAP" kinase. *PNAS*, **88**:2520-2524 (1991).
b. Sun, M., Song, C-X., Huang, H., Frankenberger, C.A., Sankarasharma, D., Gomes, S., Chen, P., Chen, J., Chada, K., He, C., and **Rosner, M.R.**, An HMGA2/TET1/HOXA9 Signaling Pathway Regulates Breast Cancer Growth and Metastasis, *PNAS,* **110**(24)*:9920. (2013)* PMC3683728
c. Lee, J., Yesilkanal, A., Wynne, J., Frankenberger, C. Liu, J., Yan, J., Elbaz, M., Rabe, D.C., Rustandy, F.D., Tiwari, P., Grossman, E., Hart, P.C., Kang, C., Sanderson, S.M., Andrade, J., Nomura, D.K., Bonini, M., Locasale, J.W. and **Rosner, M.R.,** Effective breast cancer combination therapy targeting BACH1 and mitochondrial metabolism, *Nature*, *568*:254-258 (2019). DOI: 10.1038/s41586-019-1005-x.
d. Yesilkanal, A., Yang, D., Valdespino, A., Tiwari, P., Sabino, A.U., Nguyen, L., Lee, J., Xie, X.-H., Sun, S., Dann, C., Robinson-Mailman, L., Steinberg, E., Stuhlmiller, T., Frankenberger, C., Goldsmith, E., Johnson, J.L., Ramos, A.F., **Rosner, M.R**., Limited inhibition of multiple nodes in a driver network blocks metastasis. *eLife*, (2021) ;10:e59696. DOI: https://doi.org/10.7554/eLife.59696

3. We identified and characterized novel members of the MAP kinase family: Although the members of the ERK family that are most widely known are ERK1 and ERK2, there are other family members that are evolutionarily conserved and also play key roles in transmitting signals within the cells. These include ERK5, which also is activated by an upstream kinase, MEK5. However, there are other family members that are constitutively activated and regulated by proteolytic degradation. Among these are ERK7 and ERK8, which regulate steroid receptor signaling and whose expression is tightly controlled. Because of their low expression and rapid turnover, these kinases were a major challenge to clone and characterize.

a. Abe M.K, Kuo, W.-L, Hershenson, M.B and **Rosner M.R.** Extracellular signal-regulated kinase 7 (ERK7), a novel ERK with a C-terminal domain that regulates its activity, its cellular localization, and cell growth. *Mol. Cell Biol*. **19**:1301-1312 (1999).
b. Abe, M.K., Kahle, K.T., Orth, K., Dixon, J.E. and **Rosner, M.R.** ERK7 is an Autoactivated Member of the MAP Kinase family. *J. Biol. Chem.*, **276** (24). 21272-21279. (2001)
c. Abe, M.K., Saelzler, M.P., Espinosa III, R., Kahle, K.T., Hershenson, M.B., LeBeau, M.M., and **Rosner, M.R**., ERK8, a New Member of the MAP Kinase Family. *J. Biol. Chem.,* **277** (19) 16733-43. (2002)
d. Kuo, W.-L, Duke, C., Abe, M., Kaplan, E., Gomes, S. and **Rosner, M.R.** ERK7 Expression and Kinase Activity is Regulated by the Ubiquitin-Proteosome Pathway, *J. Biol. Chem*., 279(22), 23073-81. (2004)

4. We characterized a novel regulator of MAP kinase on a both molecular and cellular level: Raf Kinase Inhibitory Protein (RKIP). Raf Kinase Inhibitory Protein (RKIP), also termed Phosphatidylethanolamine binding protein1 (PEBP1), is an important regulator of kinase signaling in cells. RKIP represents a new class of signaling modulators that maintain the balance or homeostasis of biological systems. We and others have shown that

depletion or inactivation of RKIP, a metastasis suppressor, results in disruption of normal cellular stasis and can lead to chromosomal abnormalities and metastatic cancer. We also showed that RKIP plays a key role in regulating the cell cycle through the spindle checkpoint. Our recent studies have utilized RKIP as a tool to elucidate novel pathways that regulate metastasis of triple negative breast cancer. We are the only group that has utilized cellular, molecular and biophysical approaches to characterize the novel molecular mechanism by which RKIP acts as a switch to regulates key signaling pathways (MAP kinase and Protein Kinase A). A major focus of my laboratory relates to understanding the precise mechanism by which RKIP responds to the cellular environment. Our goal is to reactivate or mimic RKIP anti-metastatic activity in order to enable us to regulate this process and control metastatic progression in cancer. See papers under personal statement as well as:

a. Eves, E.M., Shapiro, P., Naik, K., Klein, U.R., and **Rosner, M.R**., Raf kinase inhibitory protein regulates aurora B kinase and the spindle checkpoint. *Molecular Cell*, **23**, 561-574. (2006)
b. Dangi-Garimella, S., Yun, J., Newman, M., Hammond, S. M., Eves, E.M., Minn, A.J. and **Rosner, M.R**. Raf Kinase Inhibitory Protein suppresses a metastasis signaling cascade involving LIN28 and *let-7, EMBO J*. **28**(4), 347-58. (2009) PMCID: PMC2646152
c. Yun, J., Frankenberger, C.A., Kuo, W.-L., Boelens, M.C., Eves, E.M., Cheng, N., Liang, H., Li, W.-H., Ishwaran, H., Minn, A.J., and **Rosner, M.R**., Signaling Pathway for RKIP and Let-7 Regulates and Predicts Metastatic Breast Cancer*, EMBO J,* **30(**21):4500-14, (2011) PMC3230370. *Cell Cycle Features – Invited*.
d. Skinner, J.j., Wang, S., Lee, J. Ong, C., Sommese, R., Sivaramakrishnan, S., Koelmel, W., Hirschbeck, M., Schindelin, H., Kisker, C., Lorenz, K., Sosnick, T., and **Rosner M.R**. A conserved salt bridge competition triggered by phosphorylation regulates the protein interactome. *PNAS*, **114:** 13453 (2017).

5.We have proposed novel perspectives on how cancer evolves. One of the major challenges to treatment of cancer is the evolutionary nature of the process that leads to tumor cell heterogeneity and eventual drug resistance. The mechanisms leading to such heterogeneity have largely been attributed to genetic and epigenetic processes. However, we have recently published studies that demonstrate a role for either nonheritable or heritable but nongenetic processes in establishing phenotypic heterogeneity that eventually enables cancer cells to adapt to stressful environments and survive drug treatment. In addition, we have demonstrated how metastasis suppressors in tumor cells can reprogram the microenvironment in ways that generate feedback networks that reinforce heterogeneity.

a. Frank, S.A., and **Rosner, M.R**., Nonheritable Cellular Variability Accelerates the Evolutionary Processes of Cancer. *PLoS Biology*, **10**(4):e1001296. Epub. (2012) PMCID: PMC3317895
b. Lee, J., Yun, J., Yeung, K., Bevilacqua, Balázsi, G., and **Rosner, M.R.**, BACH1 and RKIP participate in a Bistable Network that affects Progression to Metastasis in Breast Cancer, *PNAS,* 111(3):E364-73 (2014). PMCID: PMC4096871
c. Frankenberger, C., Rabe, D., Bainer R., Sankarasharma D., Chada K., Krausz, T., Gilad Y., Becker L., and **Rosner, M.R**., Metastasis suppressors regulate the tumor microenvironment by blocking recruitment of pro-metastatic tumor-associated macrophages, *Can. Res*., 75(19):4063-73 (2015). PMID: 26238785
d. Tiwari, P., Blank, A., Cui, C., Schoenfelt, K. Q., Zhou, G., Xu, Y., Khramtsova, G., Olopade, F., Shah, A. M., Khan, S. A., **Rosner, M. R.*,** Becker, L.* Metabolically activated adipose tissue macrophages link obesity to triple-negative breast cancer. *J Exp Med*, (2019).Epub 2019/05/06 (*co-corresponding)

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