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US Army Research Laboratory

Protein Catalyzed Capture (PCC) Agent Workshop: August 29, 2018

by Matthew B Coppock and Dimitra Stratis-Cullum

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14. ABSTRACT
This report describes the proceedings and outcomes of a workshop sponsored by the US Army Research Laboratory on August 29, 2018. Bringing together Army entities and joint Department of Defense assets interested in antibody alternatives to achieve reliable biodetection and monitoring in fully operational conditions, including diagnostic and therapeutic applications, the workshop provided an in-depth overview of an emerging synthetic biorecognition technology called protein catalyzed capture agents. Each represented organization provided information on their specific sensing/diagnostic needs and made suggestions for possible future research directions. There was a focus on real-time Soldier health and performance monitoring, as well as environmental sensing of biothreats.

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protein catalyzed capture agent, biodetection, performance monitoring, environmental monitoring

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Contents

Executive Summary

On August 29, 2018, a protein catalyzed capture (PCC) technology workshop was held at the Adelphi Laboratory Center, bringing together key Army entities and joint Department of Defense assets interested in antibody alternatives to achieve reliable biodetection and monitoring in fully operational conditions, including diagnostic and therapeutic applications. Goals of the workshop included:

1) Provide visibility of PCC technology to Army entities and joint assets
2) Identify technological knowledge gaps for antibody alternative applications
3) Gather input to help shape future research direction (e.g., new targets, performance metrics, assay platforms, etc.)
4) Lay groundwork and relationships for future discussions
5) Work toward establishing transition partnerships

The workshop was held in an interactive format with scientific presentations as well as discussion sessions. The single-day meeting included a morning session of four presentations providing an in-depth background and applications of PCC technology, a working lunch of five presentations by Army stakeholders to help understand current/future programs and antibody alternative needs, and an afternoon open discussion session on possible future research directions.

PCC research at the US Army Research Laboratory is currently on its second 3-year Institute of Collaborative Biotechnologies University Affiliated Research Center funding cycle, and has been additionally supported with a 1-year Defense Threat Reduction Agency program. Over this period of time, the technology has matured capture reagents from TRL-2 to TRL-4, including successful integration into multiple assay platforms for ruggedized biological sensing.

PCC technology advantages include the following:

- Peptide-based capture reagent development – epitope and full antigen targeting
- 1/40th the size of monoclonal antibodies
- High affinity: tunable K_D between μM and pM
- High selectivity
- Rapid development time: approximately 2 weeks

- Thermally stable: e.g., cyclic PCC >99% active after 1-h heat at 90° C solution
- Biologically stable: Nonnatural amino acid integration (e.g., D-amino acids)
- Manufacturability: Robotic methods; limited batch-to-batch variability
- Adaptability
    - Reagents are highly modular
    - Flexibility in target screening for difficult analytes
    - Easily integrated into assays

A representative from five of the Army organizations provided a brief overview of their current and future programs, along with current technological gaps for these programs that PCC technology could address. There were multiple needs identified including the following:

1) Broader biomarker class sets including protein and peptide targets for Soldier health and performance.
2) Capture agent panels against identified biomarkers for multiplex monitoring.
3) Pairs of reagents (capture and detection) against biothreats recognized by the Centers for Disease Control and Prevention for integration into assays such as lateral flow platforms.
4) Capture agents against full organisms such as biothreats, *E. Coli*, algal blooms, and so on, for environmental monitoring.
5) Capture agents against biomarkers indicating chemical warfare agent exposure for detection and diagnostics.
6) Identification and integration of a wearable platform for real-time multiplex biomarker monitoring.

PCC technology undoubtedly is capable of addressing many of the technological gaps in terms of reagent stability, manufacturability, and adaptability for Army sensing and diagnostic applications. Based on the discussions from the workshop, future directions of the PCC technology will include the following:

1) **Near-term:** Rapid development of protein biomarker receptor panels, such as against multiple cytokines, for Soldier performance monitoring or

biological threats for far-forward detection. The simultaneous development of a panel of receptors will not only reduce the timeline and cost of production, but will also greatly reduce cross-reactivity between receptors, a common issue in multiplex assays.

2) **Mid-term:** Development of reagents against full organisms, such as *E. Coli* and algal bloom particles, which could also have an impact in food and water safety.

3) **Far-term:** Aid in the development of a wearable sensor platform to address the biomarker knowledge gap in training environments to enhance Soldier lethality, as well as potential development of receptors for small molecule detection since they seem to be the most common biomarkers currently being assessed by multiple Army entities.

1. Introduction

The complexity of future armed conflict will require specially equipped Army forces to sustain peak performance in inhospitable conditions. To win in such multifaceted operational environments, the Army will need to be more adaptive, more expeditionary, and have a near-zero logistical demand to optimize squad execution through real-time health and performance monitoring, while simultaneously sensing current and emerging environmental threats. As a result, there is a need for the rapid development and production of biorecognition receptors capable of superior performance in multi-domain environments.

Protein catalyzed capture (PCC) agent technology is an emerging capability for the fast development, production, and integration of biorecognition elements into point-of-need assays/therapies and continuous monitoring sensor platforms. PCCs are ruggedized, peptide-based replacements for antibodies that address critical gaps in adaptability, manufacturability, and stability of bioreceptors and capture reagents. With binding performance comparable to, and often exceeding, monoclonal antibodies, PCCs have the added benefits of a rapid development time (~2 weeks), superior biological, chemical, and thermal stabilities,[1-4] epitope targeting capabilities,[2, 5-7] and limited batch-to-batch variabilities.

The goal of the PCC workshop on August 29, 2018 was to bring together key Army entities and joint Department of Defense assets interested in antibody alternatives to achieve reliable biodetection and monitoring in fully operational conditions, including diagnostic and therapeutic applications, to discuss and identify key technological knowledge gaps. The gathered input will help shape the future of PCC research (e.g., targets, performance metrics, and so on). The workshop also laid the groundwork for establishing transition partnerships and future discussions on far-forward sensing and diagnostics. The workshop was held in an interactive format, with scientific presentations as well as discussion sessions. The single-day meeting included a morning session of four presentations providing an in-depth background and applications of PCC technology, a working lunch of five presentations by Army stakeholders to help understand current and future programs and antibody alternative needs, and an afternoon open discussion session on possible future research directions. Presentations from the workshop are included in Appendixes A–I.

2. Results

2.1 Protein Catalyzed Capture (PCC) Agent Overview

Professor James Heath (Institute of Systems Biology), Dr Heather Agnew (IndiMolecular), and Dr Matthew Coppock (US Army Research Laboratory [ARL]) provided an in-depth overview of the PCC development process including relevant applications and targeting strategies to proteins of interest.

PCCs are peptide-based receptors that can be developed to bind to specific epitopes of specific proteins using standardized *in situ* click chemistry. The technology screens molecular architectures of linear or, more commonly, cyclic peptides, containing a 5-mer variable region resulting in a 2-million element library attached to Tentagel resin. Incubating the library with the full target or a 10–15 amino acid length peptide fragment from the full target results in triazole formation through *in situ* click chemistry to only a select few library members in a specific geometry to the target. Therefore, the screening is for a reaction product, not just target binding. Multiple epitopes, for instance, can be targeted and the resulting peptide candidates can be tethered to exploit cooperativity, resulting in a reagent with strong affinity (pM) and high selectivity for the target. Over 25 PCCs have been developed for a range of targets with tunable affinities of μM to pM to many different protein targets. High throughput production optimization allows development of a high performing PCC to occur in as little as 15 days and computational modeling has advanced to aid in the understanding of PCC-protein interactions. PCCs are highly modular, resulting in tailorable characteristics like biological stability, physical stability (thermal, pH, etc.), cell penetration capabilities, novel labeling, increased affinity, and increased selectivity. The flexibility of the targeting strategies and maturation strategies is capable of creating reagents against hard-to-target protein analytes.

2.2 Army Sensing and Diagnostic Needs

A representative from each Army entity discussed their organizational needs in regard to current and future programs: Dr John Player (US Army Natick Soldier Research, Development, and Engineering Center [NSRDEC]), Dr Roy Vigneulle (US Army Medical Research and Materiel Command [MRMC]), Dr Randy Hofmann (Edgewood Chemical Biological Center [ECBC]), Dr Keri Donohue (US Army Engineer Research and Development Center [ERDC]), and Dr Benedict Capacio (US Army Medical Research Institute of Chemical Defense [AMRICD]).

2.2.1 NSRDEC: Human Performance Monitoring

NSRDEC performed a multi-domain human performance study called the Monitoring and Assessing Soldier Tactical Readiness and Effectiveness (MASTR-E) Program. The study investigates collective lethality at the individual and small unit level, including biomechanics, load carriage, performance nutrition, and recovery times. In the 3-day investigation, quantification of small-molecule metabolic products in urine and saliva was conducted. These biomarkers are known to be indicators of stress, fatigue, and activity level. The samples were collected and preserved, and will be analyzed by mass spectrometry for presence of the small molecules/metabolites. The small-molecule/metabolite biomarkers were chosen as initial targets partly because of their ease of detection by mass spectrometry, but it is recognized that it will be necessary to switch to a different, more portable platform to achieve real-time monitoring. Outside of small molecules, there is also a significant interest in expanding the study to more complex biomarkers like proteins.

Need: The MASTR-E program is a start toward wearable monitoring for real-time detection of biomarkers on the sensored Soldier. It is currently unknown if any of the analyzed biomarkers have a signature correlating to specific performance metrics, so a massive need is identifying specific biomarkers to monitor. Further, a broader biomarker class set is needed, to include protein and peptide targets that can be uniquely targeted using the PCC technology. Ideally, the real-time sensing of many biomarkers will be multiplexed to allow straightforward determination of performance. Therefore, there is a need for panels of receptors that could be integrated into sensors against potential performance biomarkers that are both highly manufacturable and exhibit little cross reaction between targets. Because it only takes 2 weeks or less to develop a new PCC reagent to new biomarker targets, the sensing platforms can be iteratively updated, providing a unique tool for maximizing training effectiveness.

2.2.2 ECBC: Biothreat Detection

The ECBC mission research and development includes biothreat detection capabilities, such as BLINDSPOT technology, that combines multiplex detection strategies with lateral flow assays for rapid threat detection resulting in signatures easily analyzed through cell phone readers. The concept of spotting polyclonal or monoclonal antibodies on nitrocellulose as opposed to line deposition aids in the sensitivity of binding as the sample flows down the membrane to the farther detection points. There is a clear balance between the strength of binding by an antibody pair and the concentration at which the sample is introduced to the assay. Biothreats such as botulinum neurotoxin, ricin, Staphylococcal areus enterotoxin

B, and other Centers for Disease Control and Prevention (CDC) select biothreat agents are the main analytes of interest for these assays.

Need: There is a need for stronger binding reagent pairs against most CDC-select biothreats that can be developed rapidly and more inexpensively (~$1M for development of an individual antibody). While some of these priority targets are proteins (e.g., botulinum toxin), full virus/organism detection is also a top priority for these assays. A key and unique advantage of the PCC technology approach would be to not only develop pairs of antibody reagents, but to use the up-front knowledge of both the sample platform and other target/reagent materials to eliminate cross-reactivity and maximize compatible and efficient integration.

2.2.3 MRMC: Health Wearables Integration

The Military Operational Medicine Research Program is developing effective countermeasures against stressors to maximize health, performance, and fitness. An aspect of this program requires real-time monitoring of physiological and psychological biomarkers in wearable platforms, as well as the detection of environmental targets like toxic industrial chemicals and toxic industrial materials. For example, the Health Readiness and Performance System (HRAPS) concept is an integrated system of sensors that communicate accurate, real-time, actionable information about health, readiness, and performance to both Service Members and unit leaders. Ideally, an HRAPS readiness score after complete Soldier analysis can enhance readiness, reduce injuries, increase force health protection, and optimize performance. Identified use cases include land navigation training, airborne assault, and sensitive site exploitation (chemical, biological, radiological, nuclear, and explosive [CBRNE]). There is also a current focus on Soldier hydration monitoring.

Need: MRMC is in need of identified biomarkers relevant to Soldier health aspects of interest that could then be monitored through wearable biosensors. For example, there is interest in predetermining if a Soldier can resist acute mountain sickness at high altitudes in a predeployment test, which would significantly improve squad assembly. These general screens could also greatly benefit training in other stressful environments. Again, receptor panels for these biomarkers will be necessary and eventually identified biomarkers could potentially overlap with the biomarkers determined through NSRDEC work.

2.2.4 ERDC: Environmental Monitoring

ERDC proposed aero-sensing capabilities through antibody or antibody alternative binding through a porous construct that could potentially be attached to an unmanned aerial vehicle. This capability would allow far-forward monitoring and

detection of potential CBRNE threats outside the immediate vicinity of a Soldier or squad. It is apparent that a synthetic capture agent will need to be integrated into such a system for stability purposes. Potential targets could include biologicals larger than proteins such as toxic algal bloom particles.

Need: This application will require capture reagents with high thermal stability, chemical stability, and biological stability against important environmental targets such as algal blooms. The capture agents will also need to be easily integrated into porous constructs and active in minimally aqueous conditions.

2.2.5 AMRICD: Medical Countermeasures and Diagnostics

Medical AMRICD is working toward superior medical countermeasures and diagnostic capabilities against nerve agents, vesicant agents, pulmonary agents, cyanide, and toxins. Initially, a simpler positive/negative readout can significantly help for Soldier quarantine. A focus on small molecule detection has made it difficult to find a receptor technology capable of addressing the detection and countermeasures for many of these analytes of interest.

Need: There are needs ranging from exposure detection to diagnostics to forensics in the low ng/mL range. For example, capture agents are needed against chemical warfare agent (CWA) biomarkers such as human serum albumin adducts or peptides as a result of CWA exposure for diagnostic purposes. A large majority of the receptor development needed is for small molecule recognition such as anti-opioid, metabolite, or agent breakdown product. All are needed for both lab-based and field-forward assays.

3. Conclusions and Future Directions

Key scientists across the Army enterprise are now more aware of the capability of PCC technology and overall interests, research, and potential applications for antibody alternatives were discussed by the representative organizations. Discussions on wearable technology for improved Soldier performance, health, and environmental monitoring will continue. This community was also made aware of research facilities and capabilities available for collaboration at both Walter Reed National Military Medical Center (WRNMMC) and Uniformed Services University of Health Sciences (USUHS), which will be investigated further.

In regard to health and performance monitoring, which became a large focus of discussion, it is still unclear which biomarkers will be the most beneficial to monitor in terms of maintaining a high performing and ready force. NSRDEC is at the beginning stages of identifying important performance biomarkers by focusing

on eight small molecule markers associated with stress, fatigue, and so on, through the MASTR-E program. Additional follow-on studies will need to be performed to categorize a more significant breadth of biomarkers and the lack of biomarker knowledge hinders the readiness of synthetic capture agents. Furthermore, with the end goal of real-time monitoring occurring on a Soldier-equipped wearable platform, the current analysis method of mass spectrometry will not suffice. However, a preferred wearable platform has not been identified, limiting the design capabilities upfront for PCC development. However, after the discussion it is apparent that the simultaneous sensing of multiple biomarkers in a multiplex format will be imperative to truly understand a Soldier's performance in real time and the PCC technology can be that key enabler as a means to that end.

PCC technology undoubtedly is capable of addressing many of the technological gaps in terms of reagent stability, manufacturability, and adaptability for Army sensing and diagnostic applications. Through the gathered input from this workshop, near-term objectives will consist of the rapid development of protein biomarker receptor panels, such as against the cytokines, for Soldier performance monitoring or biological threats for far-forward detection. The modularity of the technology will allow a rapid, integrated development approach to ensure no cross reactivity between reagents of similar classes of targets, a common challenge for antibody-based multiplex libraries. Midterm goals include the development of reagents against full organisms, such as *E. Coli* and algal bloom particles, which could also have an impact in food and water safety. PCCs for nerve agent adducts are another possibility and could exploit the single-point mutation discrimination capabilities of the technology. More far-term objectives could include the development of receptors for small molecule detection since they seem to be the most common biomarkers currently being assessed by multiple Army entities. Since the PCC technology has focused efforts strictly on protein capture, successful development of capture agents for small molecules will likely require a significant effort. As a whole, PCC is an enabling technology as we work toward the development of advanced wearable sensors to address the biomarker training performance knowledge gap for the sensored Soldier and enhanced Soldier lethality.

4. Workshop Participants

AMRICD	Franz Frye, Shae Kasten, Benedict Capacio, Kimberly Frock
ARL	Matthew Coppock, Dimitra Stratis-Cullum, James Sumner, Deborah Sarkes, Sanchao Liu, Paul Pellegrino, Justin Bickford, Mikella Farrell, Ellen Holthoff, Romeo Del Rosario
DARPA	Tyler Stukenbroeker
DTRA	Revell Phillips
ECBC	Randy Hofmann, Shanmuga Sozhamannan, Michael Retford
ERDC	Keri Donohue
ICB	David Gay, Robert Kokoska
IndiMolecular	Heather Agnew, Bert Lai
Institute of Systems Biology	James Heath
MRMC	Roy Vigneulle
NSRDEC	John Player, Michael Wiederoder
USUHS	Bruce Doll, Rafaela Nita
WRNMMC	Olcay Jones

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Appendix A. PCC Overview

Protein Catalyzed Capture Agents (PCCs)

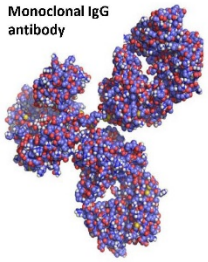
First reported by the Heath & Sharpless groups, Angew. Chemie. 2009 (Heather Agnew was 1st author).

Licensed into Integrated Diagnostics in 2010.

Supported in ARL 6.1 research programs and the National Cancer Institute since 2011

Co-developed with ARL collaborators via 2 consecutive 6.2 programs since 2014

Indi Molecular (spun out of Integrated Diagnostics) in 2015; focused on PCC development.



Monoclonal antibodies (mAbs) THE GOOD

- The gold standard technology for protein detection.
- Can achieve picoM affinities, but 10-100 nM more typical
- Can be engineered for specific applications
  - Humanized (drug development)
  - increased stability
  - minibodies and diabodies (reduced molecular weight)

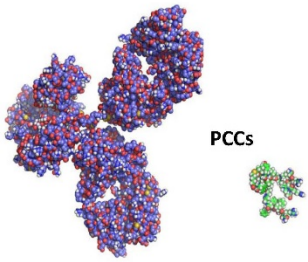
  lends control over in vivo circulation times, tissue penetration
- Can be labeled with fluorophores and similar tags
- Can be targeted at specific epitopes of specific proteins

  enables many drugs and also enables sandwich immunoassay design

Monoclonal Antibodies (mAbs) THE BAD

- Expensive to purchase, expensive to develop
- Scaling to milligram quantities is challenging
- Engineering for specific applications is not straightforward
- mAbs are not exact chemical structures – can exhibit batch-to-batch variability

  adds significant challenges to multiplex assays
- mAbs often require a refrigeration chain ***(limits practical applications)***
- Selectivity is challenging to quantify
- not cell penetrant ***(limits drug applications)***

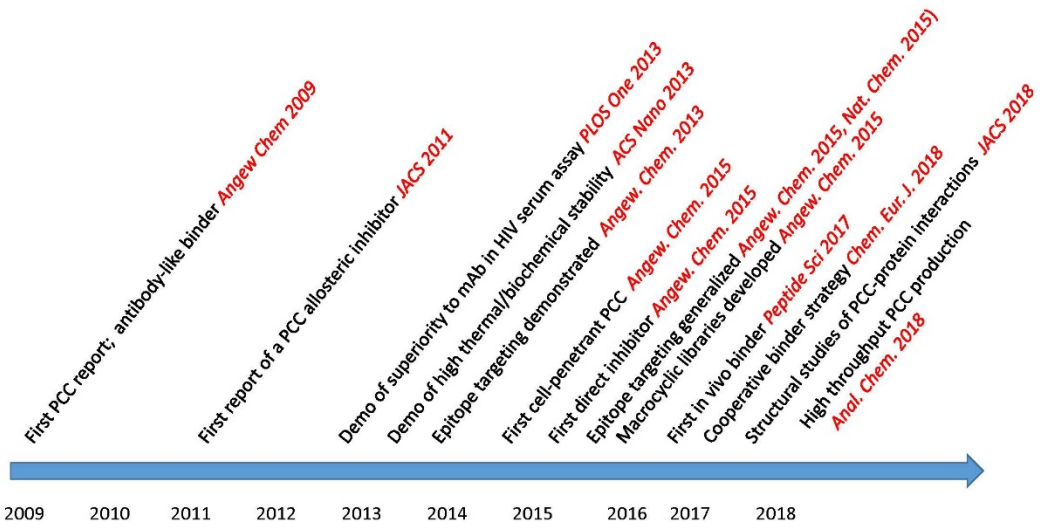


PCCs THE GOOD

- Can achieve picoM affinities, but 10-100 nM more typical
- Can be engineered for specific applications
  - Physical stability or biomolecular stability
  - Cell penetration
  - In vivo clearance
- Built with a label for a biotin or fluorophore or other tag
- Can be targeted at specific epitopes of specific proteins
- Rapid development through single generation screen (15 days)
- scaling to more than milligram quantities is not challenging
- Exact chemical structures
- No refrigeration chain needed
- PCCs around 1/40th the weight of mAbs

PCCs THE BAD

- Slow to engineer for optimized properties (but faster than for mAbs)
- Selectivity can be high, but hard to guarantee. May require engineering (true for mAbs also)



I will discuss 6 aspects of PCC Agents

The design rationale for PCC molecular architecture

What 'epitope targeting' means, and why it is of high value

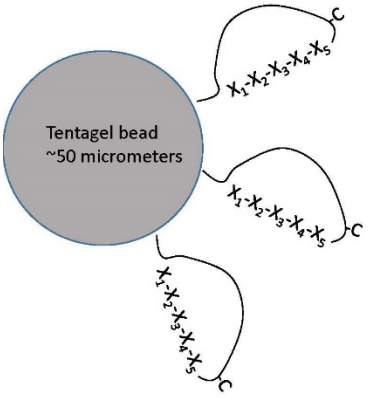
The strategy in which PCCs are identified from a single generation screen

How picoM binding affinity PCCs are routinely obtained

High throughput production of PCCs

How PCCs are engineered for specific applications

PCC One-Bead-one-compound peptide libraries



Many copies of an individual peptide on each bead

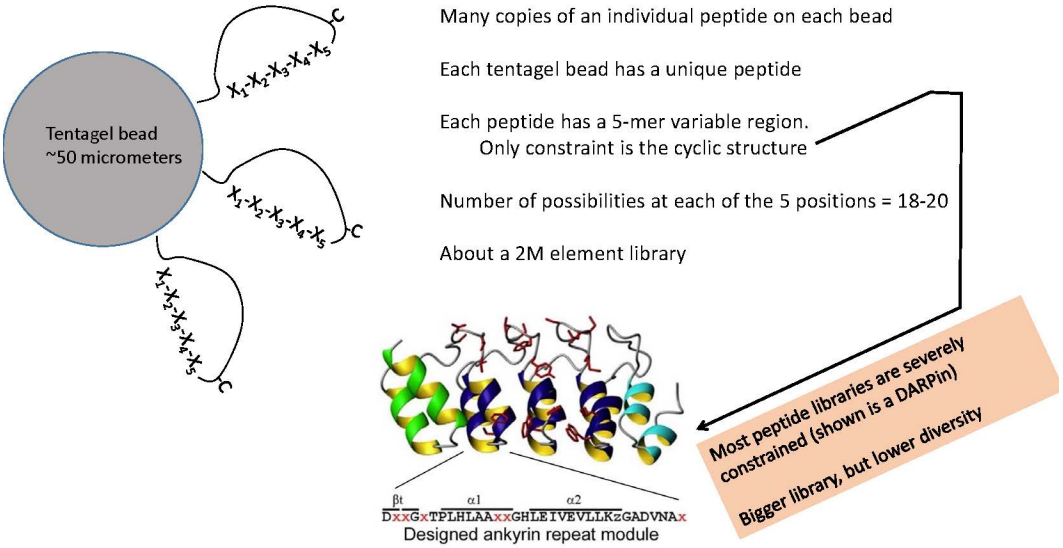
Each tentagel bead has a unique peptide

Each peptide has a 5-mer variable region.
Only constraint is the cyclic structure

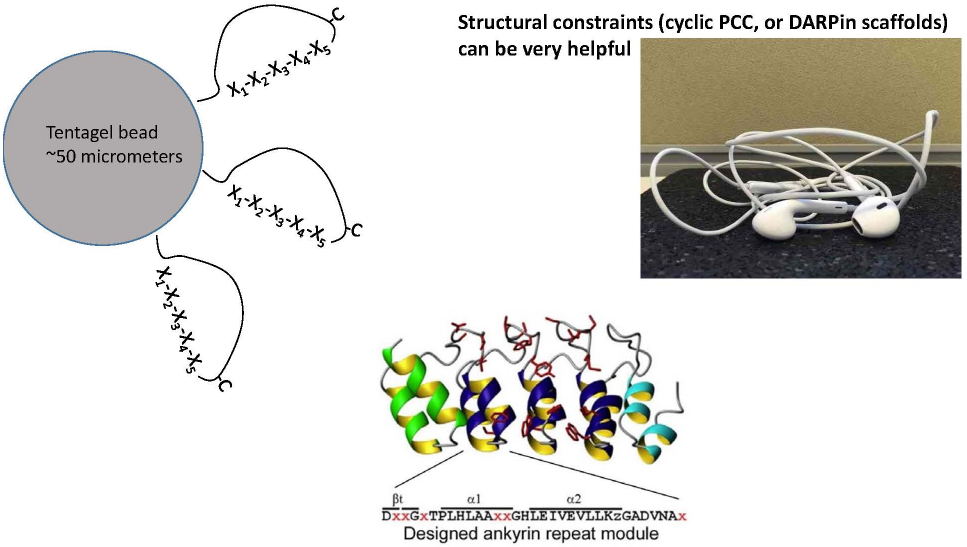
Number of possibilities at each of the 5 positions = 18-20

About a 2M element library

PCC One-Bead-one-compound peptide libraries

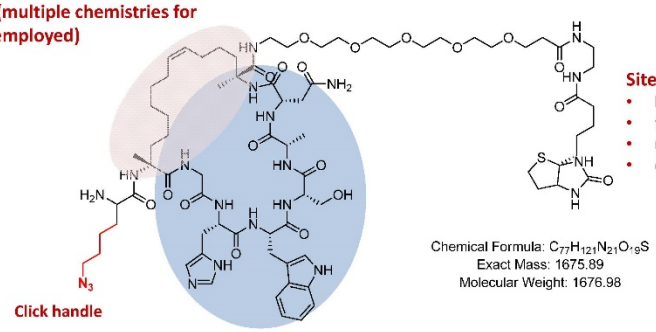


PCC One-Bead-one-compound peptide libraries



Cyclic peptide libraries for in situ click screening

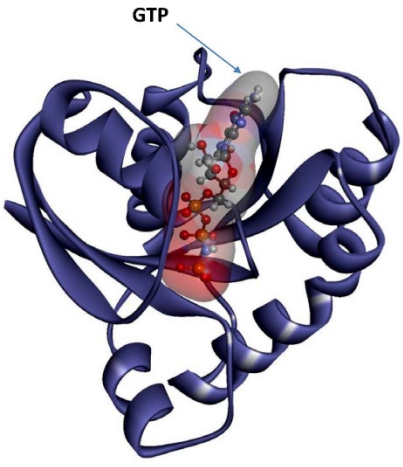
Constant region (multiple chemistries for ring closure are employed)



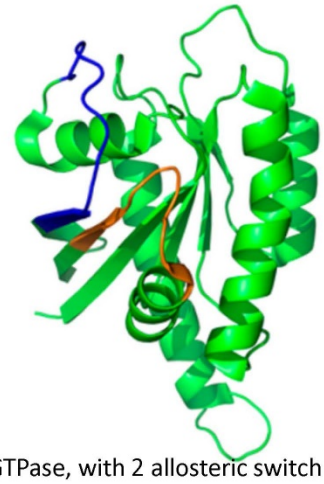
- 5-mer variable region
- About a 2 M element library
- A chemically synthesized library can include artificial and non-natural amino acids

Libraries can be sequenced via Edman degradation (slow) or mass spectrometry (fast)

The value of epitope targeting

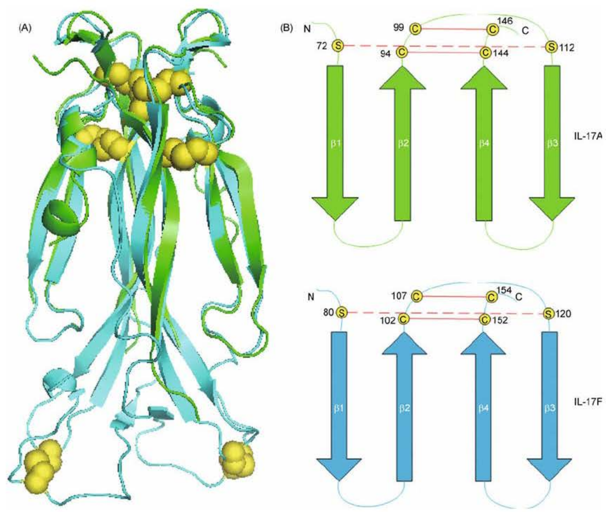


A GTPase (enzyme)



The same GTPase, with 2 allosteric switch epitopes highlighted. PCCs targeted there are more valuable

The value of epitope targeting

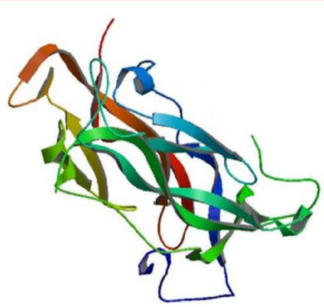


Two very proteins that are closely related in sequence and in structure

But quite different in function

IL17F and IL17A

The value of epitope targeting



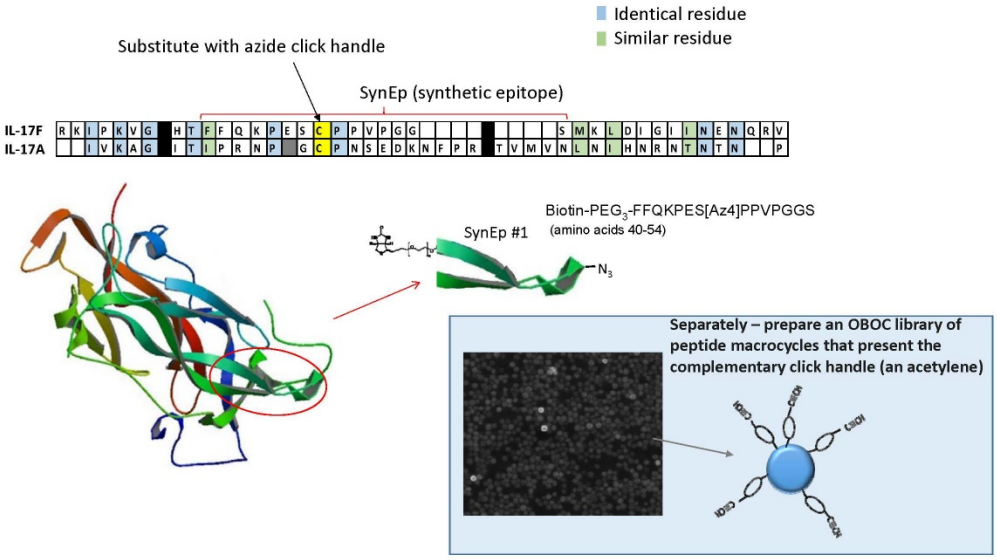
- Identical residue
- Similar residue

Sequence-wise, these two isoforms are also very similar
The sequence below highlights where they are most different

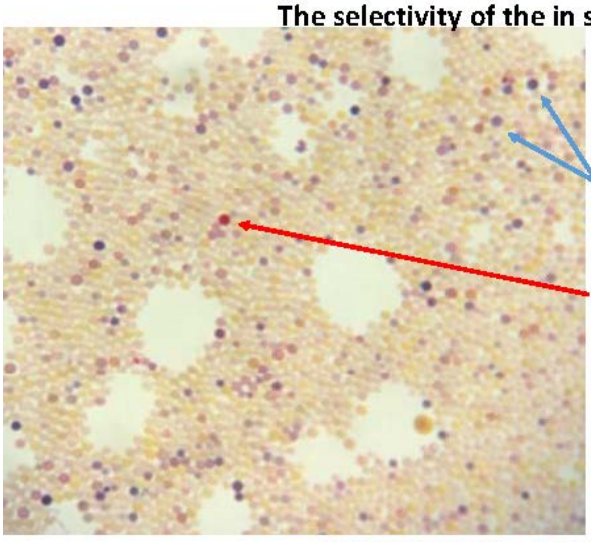
IL-17F	R	K	I	P	K	V	G		H	T	F	F	Q	K	P	E
IL-17A			I	V	K	A	G		I	T	I	P	R	N	P	

IL-17F	S	C	P	P	V	P	G	G								
IL-17A	G	C	P	N	S	E	D	K	N	F	P	R		T	V	M

IL-17F		S	M	K	L	D	I	G	I	I	N	E	N	Q	R	V
IL-17A	V	N	L	N	I	H	N	R	N	T	N	T	N			P



The selectivity of the in situ click screen



Clear bead – doesn't bind to anything

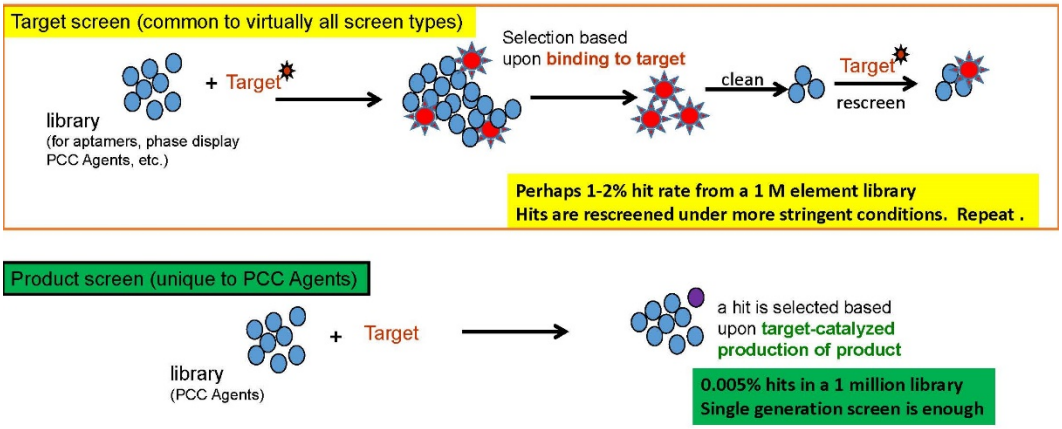
Blue beads – on-bead compound is non-selective, binds to interferents in serum

Red Bead - on-bead compound selectively promoted in situ click reaction to the synthetic epitope peptide (SynEp)

Any other color: non-selective; ignore

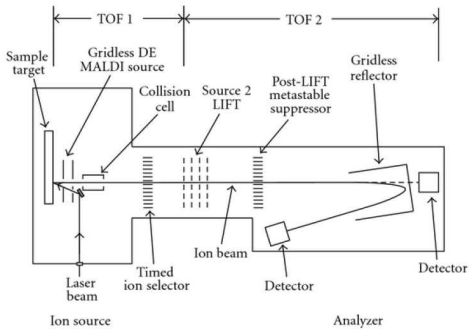
This screen of 500,000 compounds yielded 3 hits

PCC Screens: screen for reaction product, not target binding



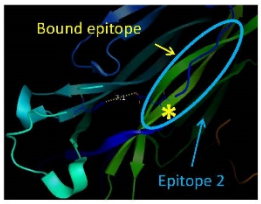
Agnew, et al., Angew. Chem. 2009 Millward, et al., JACS, 2011

- Pluck out the hit beads
- Sequence by Mass Spectrometry
  bead peptides are optimized for sequencing
- Synthesize using automated peptide synthesizer
  synthetic protocols well-established
- Test for affinity, selectivity, stability, etc.

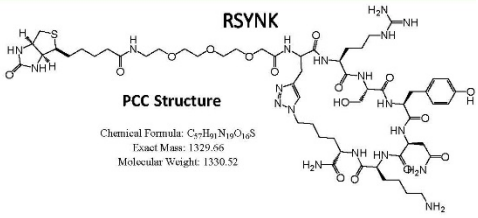


PCC against IL-17f epitope 2

IL-17F epitope 2 (a.a. 60-69):
GI[I→Az4]NENQRVS



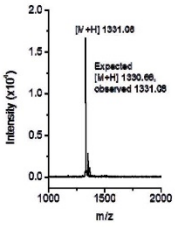
RSYNK



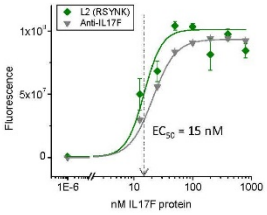
PCC Structure

Chemical Formula: $C_{57}H_{91}N_{19}O_{16}S$
Exact Mass: 1329.66
Molecular Weight: 1330.52

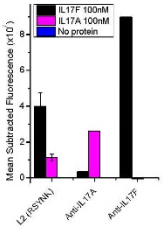
Mass Analysis



Affinity



Selectivity



Determining Epitope Orientation on IL-17F: does PCC bind to c-terminal or n-terminal side of epitope?

Synthetic epitope (SynEp)

Biotin-PEG_3-FFQKPESCPPVPGGS (amino acids 40-54)

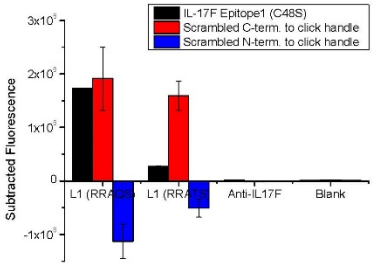
Modified epitopes

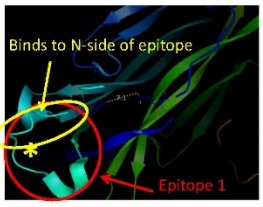
N-blanked
Synthetic epitope (SynEp)

Biotin-PEG_3-GGGGGGCPPVPGGS
or N-scrambled

C-blanked
Synthetic epitope (SynEp)

Biotin-PEG_3-FFQKPESCGGGGGG
or C-scrambled





Epitope targeted PCCs have been constructed against these (and more) targets

protein target		kD or EC50	Note
PfLDH		20 nM	
PxLDH		1.7 μM	linear
PfHRP2	multiple epitopes	500 pM - 200 nM	
L1R		875 nM	
IL17A	multiple epitopes	10 nM range	
IL17F	multiple epitopes	50 nM range	
p-Akt2		4 μM	
Akt2		122 nM	
Akt2 E17K		100 nM	
BONT	multiple epitopes	200 pM	biligand
SOD1		1 μM	
KRAS		1 μM	
IL6	multiple epitopes	10 nM range	
CD8	multiple epitopes	10 nM range	
and others...			

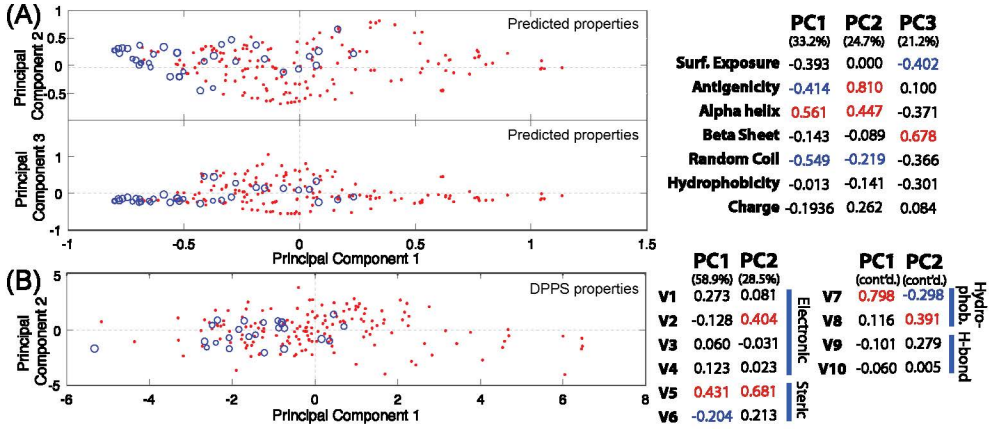
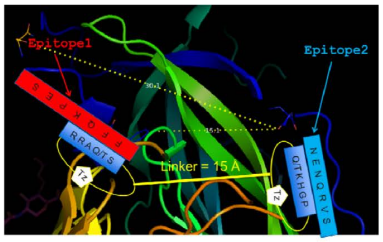
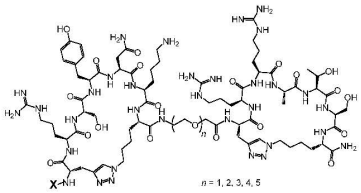


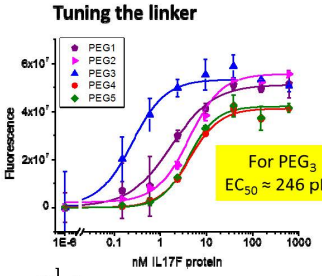
Figure 2. PCA analyses of epitope data from **(A,B)** predicted properties and **(C)** DPPS-modified properties. Data from PCC epitopes are plotted in blue circles, the sizes of which correspond to the relative affinities (IC_{50} or K_D) of the PCC to the full-length protein. The red dots reflect the properties of antigens obtained from the IEDB free antigen database for the same proteins targeted by PCC. The target proteins, target epitopes and affinities are listed in Table S1. Predicted properties included in this PCA analyses are surface hydrophobicity, antigenicity, hydropathy, and charge, which were calculated as an average for each epitope and normalized before being input into PCA analyses.

Biligands: Targeting two adjacent epitopes and the optimally linking

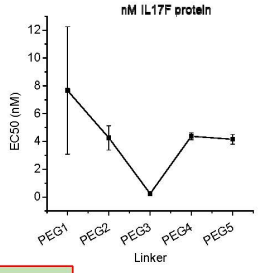


In IL-17F, the distance between FFQKPES (in Epitope1) and IL-17F Epitope2 is **~15 Å**





Linker lengths:
PEG_1 = 8.8 Å
PEG_2 = 13.2 Å
PEG_3 = 17.6 Å
PEG_4 = 22 Å
PEG_5 = 26.4 Å



picoM K_D cooperative PCCs similarly achieved against IL-17A, CD8, BoNT

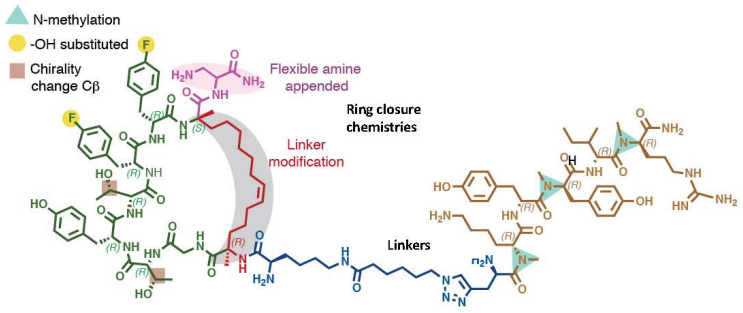
High throughput production of PCCs

	2 yrs ago	Platform Development	today
Epitope Synthesis	5 days	Automated peptide synthesizer and HPLC-mass spec	2 days
Screening	20 days	Automated screening steps	5 days
Sequencing	5 days	Encoded Cyclic Peptide Library	1 day
Peptide Synthesis & Purification	20 days	Automated peptide synthesizer and HPLC-mass spec	3 days
Characterization & Validation	25 days	High Throughput Assays	4 days
Total	**75 days**		**15 days**

Can yield multiple PCCs against different epitopes of a target in the 15 day period

Can likely be cut to 10 days. Further efficiencies will require full automation of the process.

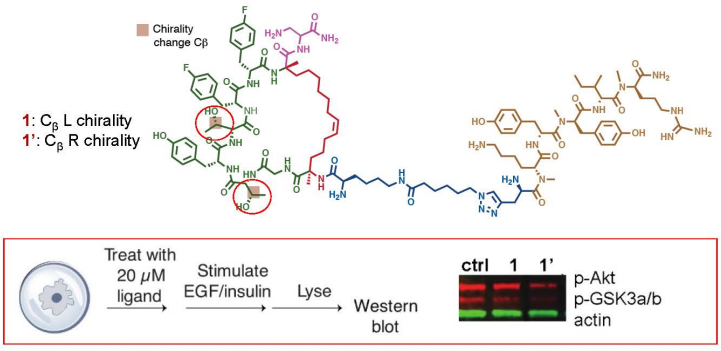
Engineering a PCC for Specific Purposes



- Biological (protease) stability (many demonstrations)
- Physical (thermal, pH, etc.) stability (>90^{0}C stability shown)
- Cell penetration (demonstrated for 3 PCCs)
- Novel labels for detection (radiometric, fluorophores, etc.) (many demonstrations)
- Increased affinity
- Increased selectivity

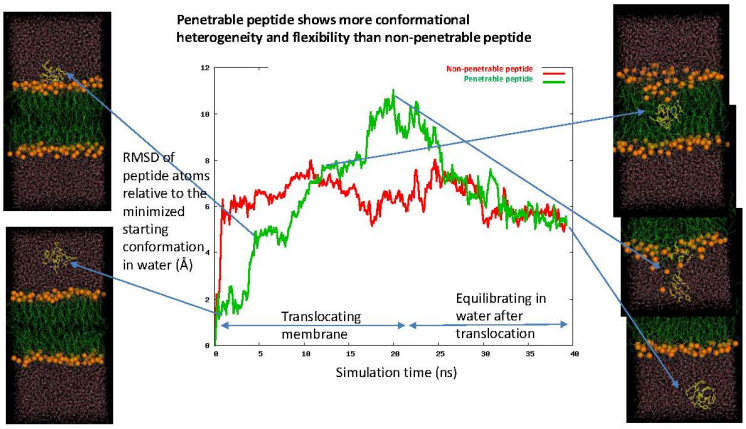
Best cell penetrant anti-pSer474 Akt2 PCCs

Switching these two threonines from L to R chirality promotes cell penetration



Also, flow cytometry data, microscopy imaging, etc., confirms cell penetration of 1'

24



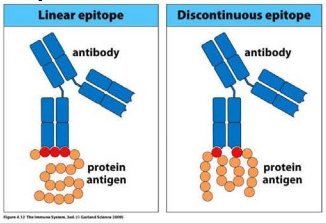
Summary

PCCs can be developed to bind to specific epitopes of specific proteins using a standardized methodology ***(very general (>25 examples))***

By targeting two discontinuous epitopes, cooperative binders with picoM level affinities can be achieved

(reasonably general)

Cooperative PCC biligands resemble common (discontinuous) B-cell epitopes



PCCs can be engineered for cell penetration, selectivity, stability, affinity, etc.

Near term:

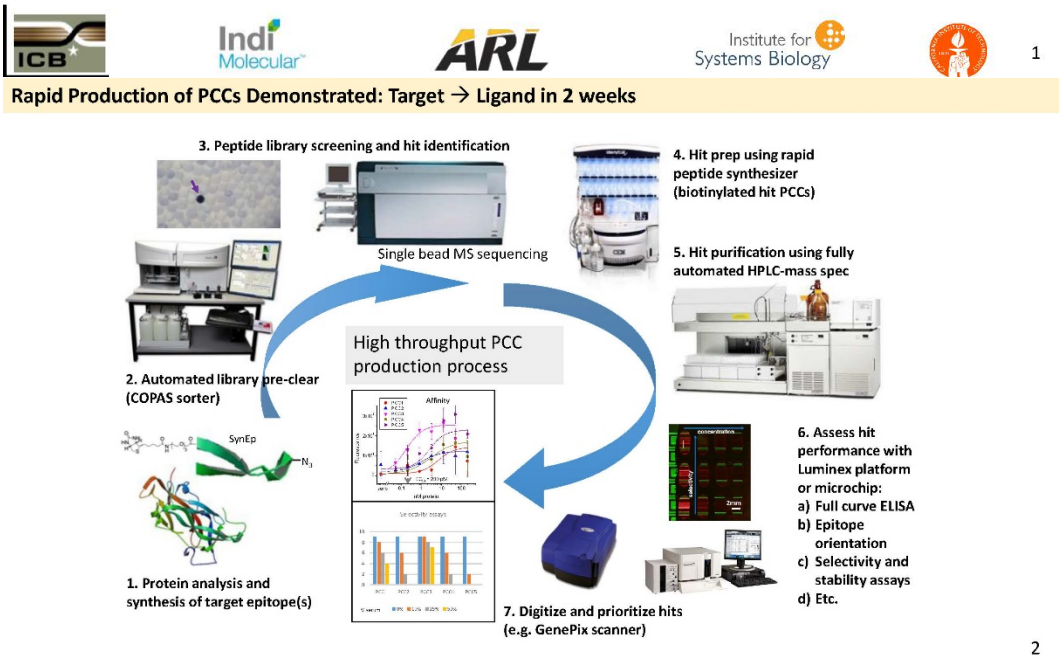
- **Panels for multiplex protein detection in challenging environments**
- **various in vivo and in vitro molecular probe applications**
- **increase automation of PCC production**

Appendix B. PCC Rapid Production

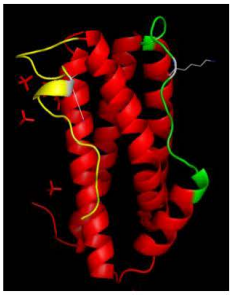
Rapid PCC Production

Heather Agnew, Indi Molecular
hagnew@indimolecular.com

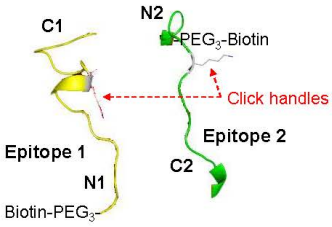
August 29, 2018



Protein Analysis and Epitope Synthesis — Target: Interleukin-6 (IL-6)



IL-6 protein structure visualized in PyMOL (PDB ID: 1ALU).



After the epitopes are designed, they are rapidly synthesized using the microwave peptide synthesizer and purified with the HPLC.

Azide click handles are substituted at a centrally located residue. Thus, screening could yield two sets of epitope-targeted macrocycles – one set pointing towards the N-terminus and another set pointing towards the C-terminus of each epitope.

CEM Liberty 1 microwave peptide synthesizer

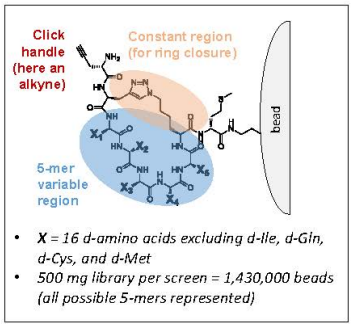
Shimadzu prep HPLC

Epitope 1: NLNLPKMAEK[D→Az4]G[C→S]FQSGFN (61-79)
Epitope 2: KAKNLD[A→Az4]ITTPDPT (129-142)

Automated peptide synthesizer and LC-MS shortens this step from 5 to 2 days.

3

Macrocycle Library Synthesis and Automated Pre-clear Screen

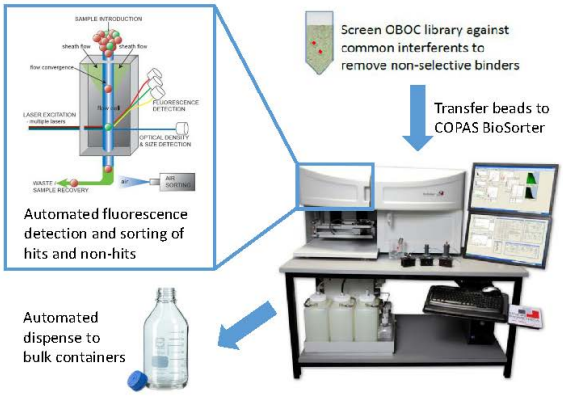


- *X = 16 d-amino acids excluding d-Ile, d-Gln, d-Cys, and d-Met*
- *500 mg library per screen = 1,430,000 beads (all possible 5-mers represented)*

AAPPTec Titan 357 split-mix peptide synthesizer

Library is synthesized on the split-mix peptide synthesizer on 10 g scale.

One batch of library can support the generation of PCCs against up to 20 different target proteins.



Automated bead sorter shortens screening process from 20 to 5 days.

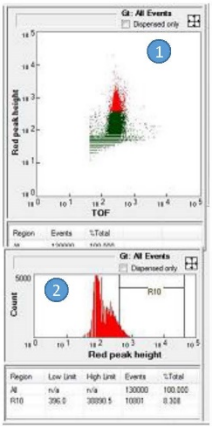
4

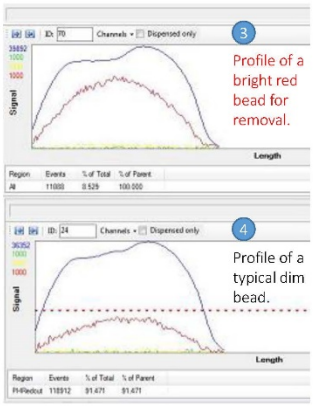
Automated Library Pre-clear Screen of a Bead Library — Target: IL-6

Dot plot shows the distribution of red signal by object size (TOF). Red dots are the brightest beads to be eliminated.

Distribution of beads *vs.* the red signal. R10 signifies the region where the beads are discarded.

Tech transfer of the sorting methods from ARL and Caltech/ISB to Indi Molecular.



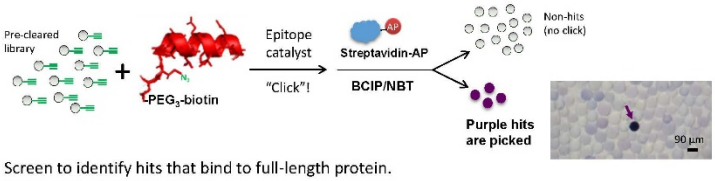


5

Multi-step Screens Yield Hits at Epitope and Protein Levels

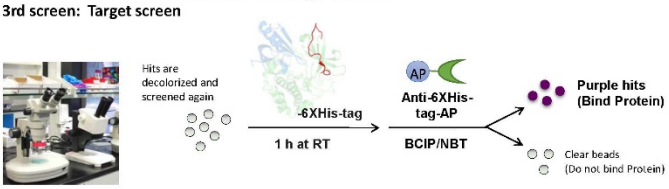
Obtain hits from *in situ* click chemistry between the synthetic epitope (here an azide) and pre-cleared library of cyclic peptides (here an alkyne).

2nd screen: Product screen



Screen to identify hits that bind to full-length protein.

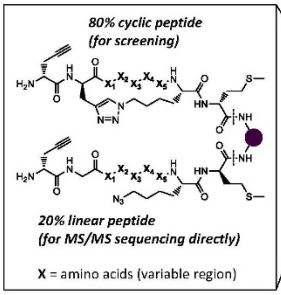
3rd screen: Target screen



Leica StereoZoom

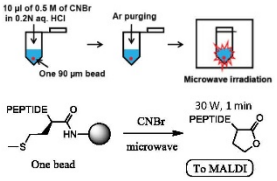
6

Encoded Cyclic Peptide Library (ECPL)



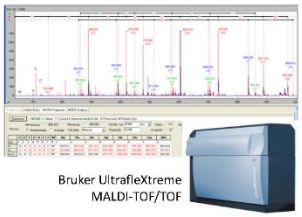
Pick hits and decolorize

Single bead cleavage



Lee et al., *J. Comb. Chem.* **2008**, *10*, 807-809.

MALDI-TOF/TOF peptide sequencing



Bruker UltrafleXtreme MALDI-TOF/TOF

Household microwave

SpeedVac concentrator

ECPL has 80% of a cyclic peptide and 20% of the corresponding linear peptide.

After releasing hit peptides from a single bead, the linear peptide is sequenced by MS/MS.

ECPL shortens sequencing process from 5 days to 1 day.

7

Hit Peptide Synthesis/Purification and Performance Assays — Target: IL-6

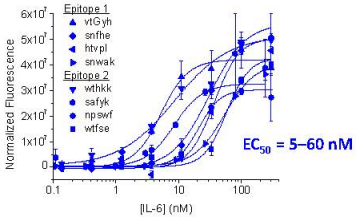
Candidates synthesized in parallel on Titan

Peptide purification by Waters LC-MS

Multiwell plate ELISA

Automated peptide synthesizer and LC-MS shortens this step from 20 to 3 days.

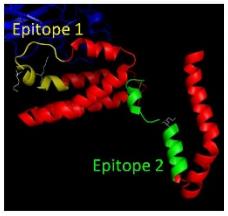
- PCCs developed against two regions of IL-6 for paired reagent development.
- PCC macrocycles show binding affinities (EC_{50}) ≤ 100 nM for IL-6 protein.



8

Demonstration of 2-week Timeline for PCC Macrocycle Development — Target: IL-10

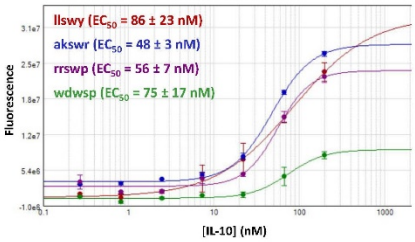
- Interleukin-10 (IL-10) is an immunosuppressive cytokine that is produced by regulatory T cells.



IL-10 protein

Epitope 1a:
AFSRV[K→Az4]TFFQMKDQL (29-47)
Epitope 1b:
RVTFFQM[K→Az4]DQLDNLL (29-47)
Epitope 2:
HRFLP[C→S]ENKS[K →Az4]AVEQVKNAFN (109-129)

Affinity: IL-10 ELISA



IL-10 PCC Development Timeline

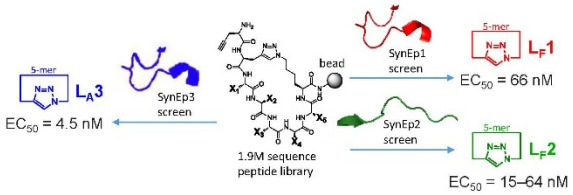
Date	Task/topic	Day #
February 2, 2018	Design of Target Epitopes	Day 1
February 7 & 8, 2018	Epitope Synthesis	Day 2 & 3
February 10, 2018	Epitope Cleavage & Purification	Day 4
February 20, 2018	Setup Library Pre-clear	Day 1
February 20-22, 2018	Automated Library Pre-clear	Day 1-3
February 26, 2018	In situ Click Screen	Day 5
February 27, 2018	Target Screen	Day 6
February 28, 2018	MS/MS Sequencing	Day 7
March 5 & 12, 2018	Hits Synthesis	Day 8 & 9
March 10 & 14, 2018	Hits Workup	Day 9 & 10
March 15, 2018	Hits Purification	Day 11
March 21, 2018	Stock Preparation	Day 12
March 26 & 29, 2018	ELISA Validation of Hits	Day 13 & 14

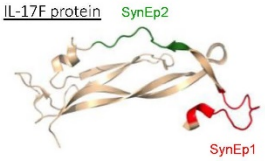
Structure visualized in PyMOL (PDB ID: 1J7V).

9

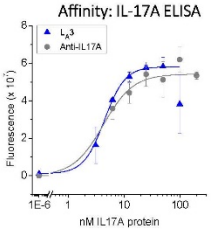
Example of Isoform-Selective Binding by PCCs — Target: IL-17, Isoforms A and F

- Interleukin-17 (IL-17) is an inflammation-associated interleukin
- Developed PCC macrocycles to discriminate between closely related isoforms: IL-17A (1 epitope) *vs.* IL-17F (2 epitopes)

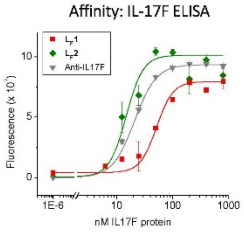




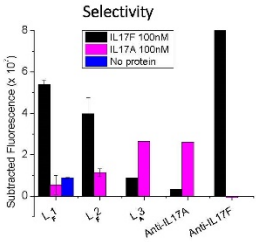
Affinity: IL-17A ELISA



Affinity: IL-17F ELISA



Selectivity

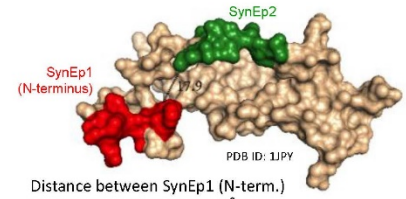


Lai, et al., *Chem. Eur. J.* 2018, 24(15): 3760-3767.

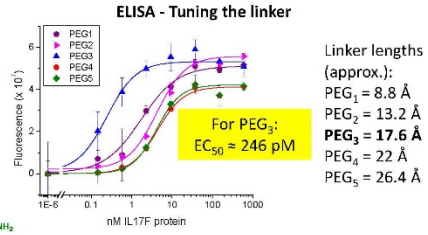
10

Example of Cooperative Binding by PCCs **Target: IL-17F**

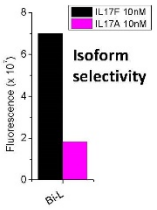
- Applied chemical cooperativity principles to yield picomolar (pM) specific binders against IL-17F

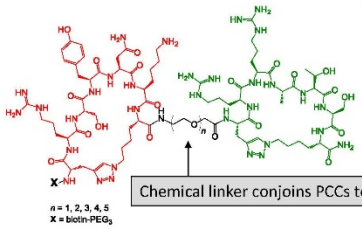


Distance between SynEp1 (N-term.) and SynEp2 in IL-17F is **~18 Å**



Linker lengths (approx.):
PEG_1 = 8.8 Å
PEG_2 = 13.2 Å
PEG_3 = 17.6 Å
PEG_4 = 22 Å
PEG_5 = 26.4 Å





- Targeting two distinct regions of IL-17F provided a model for investigation of cooperative binding when two macrocycles are covalently coupled

Chemical linker conjoins PCCs to force cooperativity

Lai, et al., *Chem. Eur. J.* 2018, 24(15): 3760-3767.

11

Appendix C. ARL PCC Applications

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U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

Army PCC Efforts

Matthew B. Coppock, Ph.D.

Chemist

Biotechnology Branch

UNCLASSIFIED

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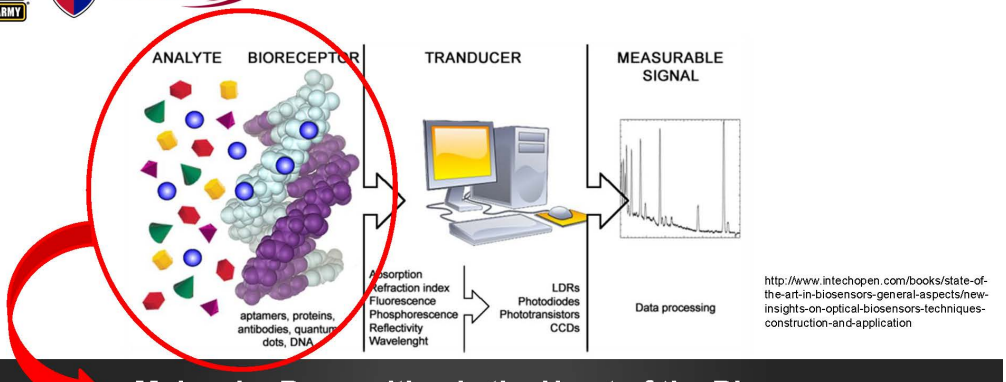
BIOSENSING FOR THE SOLDIER



http://www.intechopen.com/books/state-of-the-art-in-biosensors-general-aspects/new-insights-on-optical-biosensors-techniques-construction-and-application

Molecular Recognition is the Heart of the Biosensor

Meeting requirements for:

- **Robust functionality under environmental extremes**
- **Suitability for new and emerging threats**
- **Capability for rapid development and production**

WILL PROVIDE

Technology to support:

- **Water and food defense**
- **Individual soldier protection**
- **Collective protection**
- **Soldier Health Monitoring**

UNCLASSIFIED 2

RESEARCH OBJECTIVES

Fulfill the need for alternative antibodies by addressing critical gaps in adaptability, manufacturability, and stability

CURRENT

Laboratory- Controlled Environments

Either Limited to Lab or Limited shelf-life, single target, hand held indicators

NEED

- **Development time**
- **Adaptability**
- **Manufacturability**
- **Thermal stability**
- **Biological/Chemical stability**
- **Assay Integration**

FUTURE

Ubiquitous Biosensing

Surveillance and monitoring across platforms and environments, on-demand and point-of need adaptation

ARL: PCC HISTORY AND INFRASTRUCTURE

- ICB (6.2) – "PCCs for Improving BioDetection Assays" 2012 – 2015; $2.1M
  – "High Throughput Platform" 2016 - early 2020; $2.1M
- DTRA – "Affinity Development and Evaluation Against L1R" 2012 – 2013; $150K

- Spent 7 weeks over 2 trips in Heath lab at CalTech
- Core Team: Dimitra Stratis-Cullum (team leader), Maggie Hurley (computational modeling), Deborah Sarkes (protein production)
- Instrumentation
  - Peptide Synthesis – Titan 357
    – Biotage Alstra
  - Shimadzu Prep HPLC
  - Freeze Dryer
  - Shimadzu Protein Sequencer
  - COPAS 500 sorter
  - Luminex 200
  - Biotage T200 SPR

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ARMY EFFORTS

Fulfill the need for alternative antibodies by addressing critical gaps in adaptability, manufacturability, and stability

Targets: Toxins, Biothreats, Performance and Health Biomarkers

Targeting Strategies	– IL-17F, IL-6, IL-10, UCH-L1 – Chikungunya (CHIKV) E2 – Alphavirus nsP1
Maturation Strategies	– Anthrax Protective Antigen (PA), Botulinum Neurotoxin (BoNT) – Vaccinia L1R – CHIKV E2
Computational Modeling	– Vaccinia L1R – CHIKV E2
Thermal Stability	– PA – Vaccinia L1R – CHIK E2
Assay Integration	– PA – CHIKV E2 – UCH-L1

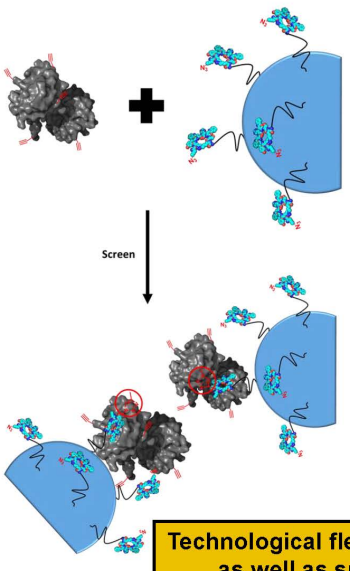
USAMMDA

UNCLASSIFIED

TARGETING STRATEGIES

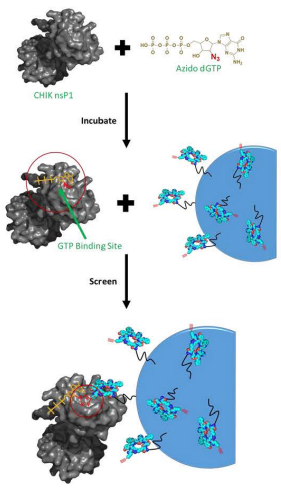
CHIKV E2

"Polyclonal" Screen Strategy

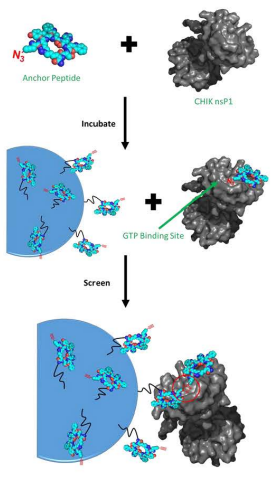


Alphavirus nsP1

CHIKV nsP1 Anchor Screen



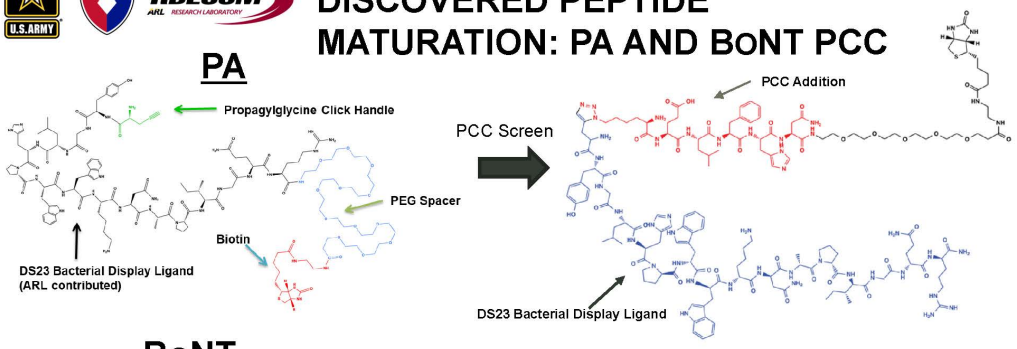
GTP Inhibitor Screen



Technological flexibility for broadly targeting unknown analytes, as well as specific regions for "hard to target" analytes

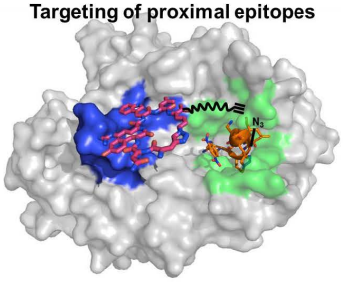
DISCOVERED PEPTIDE MATURATION: PA AND BoNT PCC

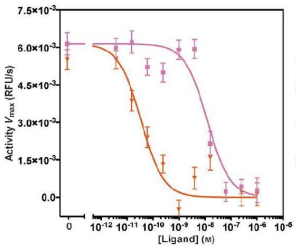
PA

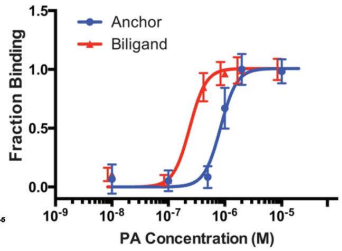


BoNT

Targeting of proximal epitopes



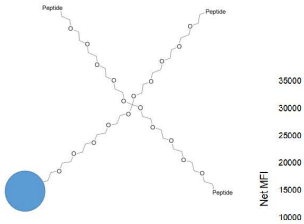


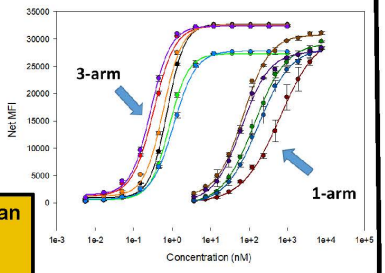


Maturation of previously discovered peptides improves binding affinity and inhibition

MULTI-VALENT MATURATION: CHIKV E2 AND L1R PCC

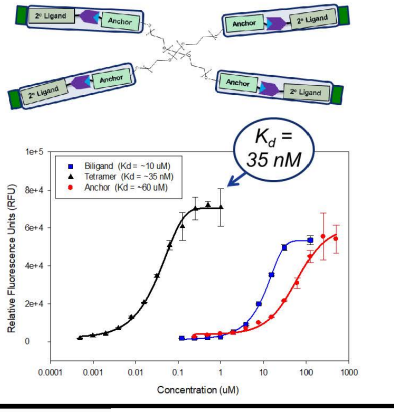
CHIKV E2



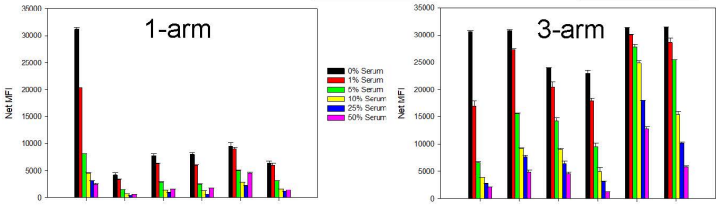


Multi-valent constructs can lead to > 2 orders of magnitude affinity and significant selectivity improvements

L1R

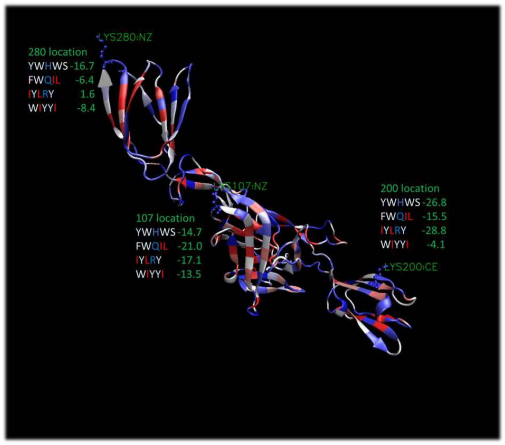


50 nM E2



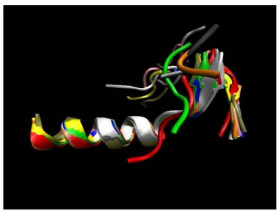
COMPUTATIONAL MODELING

CHIKV E2

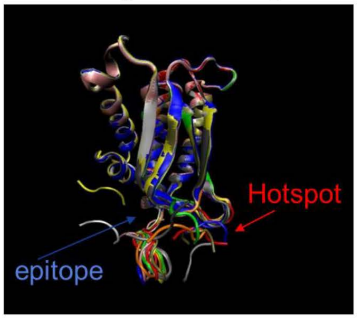


L1R

Epitope Docking



Antigen Docking

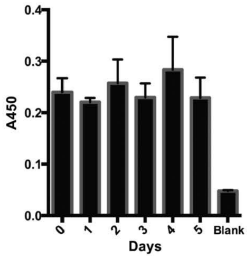


- **Computational studies aid in reagent development, helping to understand binding characteristics of the candidates (e.g. binding hotspots)**
- **Molecular dynamics and docking**
- **Total incorporation of non-naturals and 'click' handle**

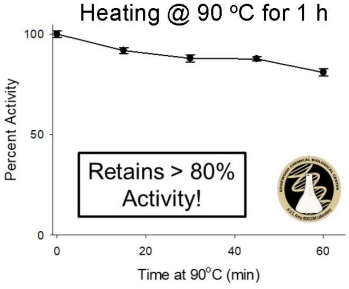
THERMAL STABILITY

PA PCC

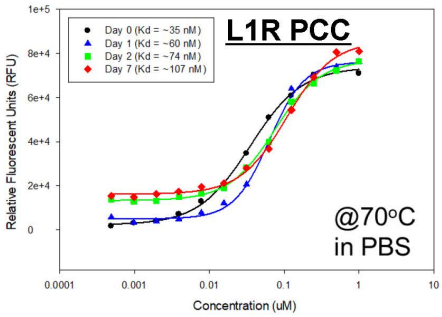
Powder Storage @ 65 °C



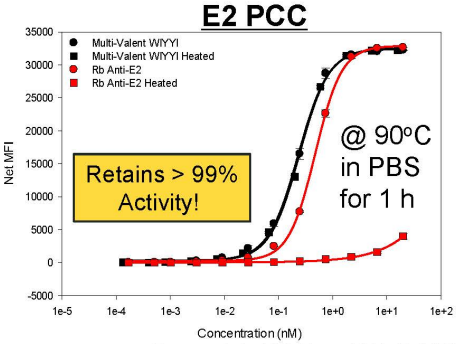
Heating @ 90 °C for 1 h



L1R PCC



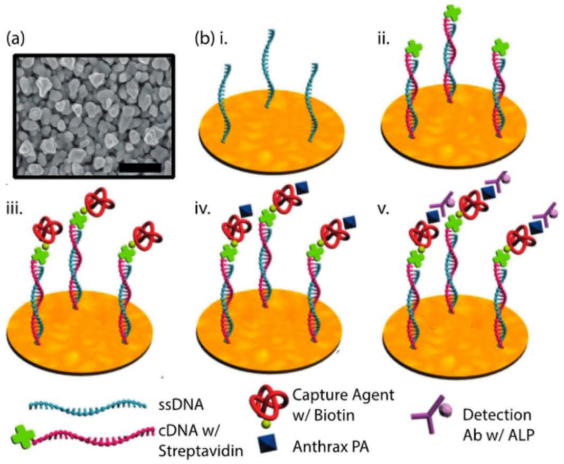
E2 PCC

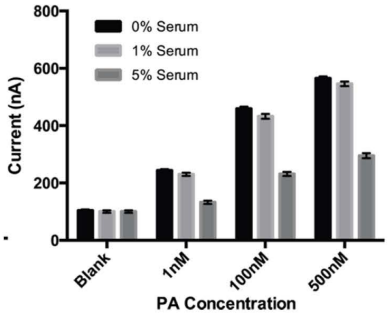


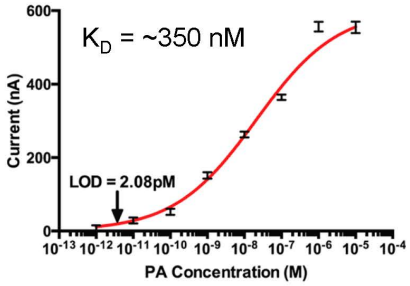
Farrow et al. *ACS Nano*, 2013, 7, 9452
Coppock et al., *Peptide Science*, 2017, *108*, e22934

UNCLASSIFIED

ASSAY INTEGRATION: PA PCC





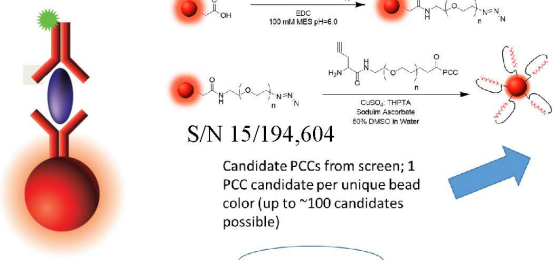


- Integration can follow common methods
- Dissociation constant doesn't completely define limit of detection in an assay

Farrow et al. *ACS Nano*, 2013, 7, 9452

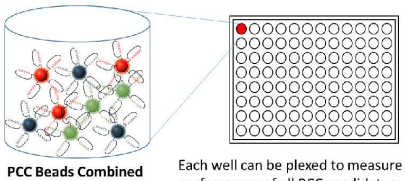
UNCLASSIFIED

ASSAY INTEGRATION: UCH-L1 PCC



S/N 15/194,604

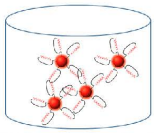
Candidate PCCs from screen; 1 PCC candidate per unique bead color (up to ~100 candidates possible)



PCC Beads Combined in 96-well plate

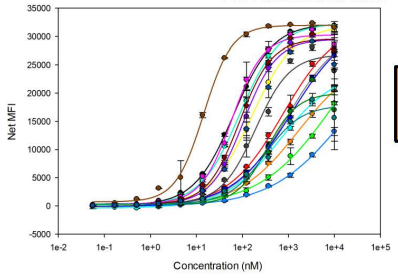
Each well can be plexed to measure performance of all PCC candidates under a specific condition

Epitope 1: EC50 = 59 ± 5 nM
Epitope 2: EC50 = 14 ± 0.8 nM
Epitope 3: EC50 = 87 ± 4 nM
Epitope 4: EC50 = 125 ± 17 nM



PCC Functionalized Beads

All experiments are analyzed on a Luminex 200 instrument



- Commercial platform integration
- Rapid characterization of candidates

Coppock et al., *Methods*, 2019, invited.

USAMMDA

FUTURE

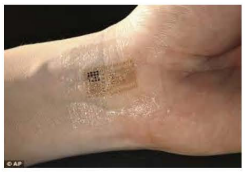
Fulfill the need for alternative antibodies by addressing critical gaps in adaptability, manufacturability, and stability

Far forward environmental monitoring

Advanced sensing concepts- Wearable-soldier health/performance, low cost-disposable

Interleukin panel for multiplex monitoring

IL-17F, IL-6, IL-10, TNF-α



Medical Diagnostics and Therapeutics

Appendix D. Additional PCC Applications

Additional PCC Applications

Heather Agnew, Indi Molecular
hagnew@indimolecular.com

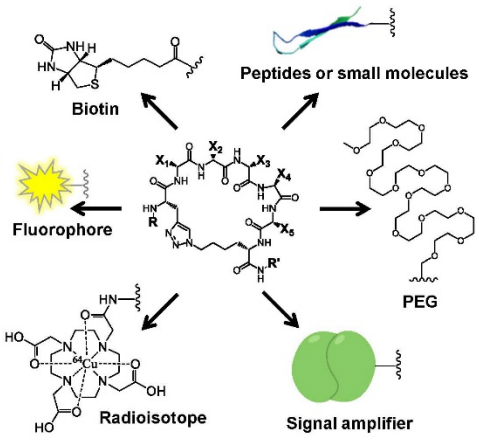
August 29, 2018

Simple Chemistry & Manufacturing for Dx, Imaging and Rx

PCCs can be structurally diverse

PCCs are modular: labels (**R, R'**) to permit adaptation into any *in vitro* assay, *in vitro* and *in vivo* imaging, etc.

Modularity also enables med. chem. iterations to improve performance (protease stability, cell penetration, solubility, etc.)

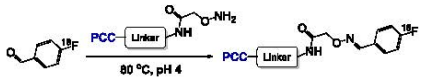


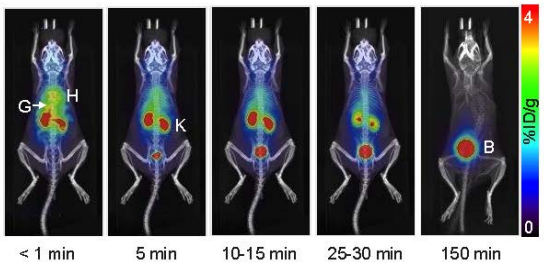
2

PET Imaging of PCCs in Normal Mouse: Small Molecule Clearance Characteristics

^{18}F – Anti-human immune cell-targeted PCC macrocycle delivered *i.v.*

^{18}F labeled using standard 4-[^{18}F]fluorobenzaldehyde chemistry at clinical PET imaging site





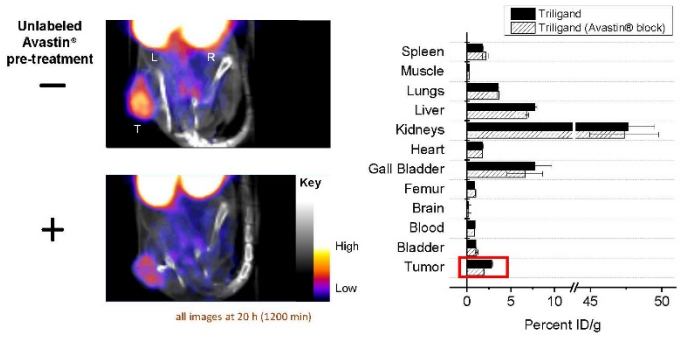
- Normal mouse; no target expressed
- Initial cardiac localization at 1 min
- Kidney clearance to the bladder
- At 150 min peptide is mostly in bladder

K, kidney; B, bladder, H, heart; G, gall bladder

3

PET Imaging Feasibility: PCC <VEGF> in Human Tumor Xenograft in Immunodeficient Mouse

Imaging *i.p.* ^{64}Cu-DOTA-labeled PCC at VEGF-expressing tumor xenograft



Pre-treatment with unlabeled Avastin® antibody decreases intensity of PCC signal consistent with competition for the same targeted VEGF epitope *in vivo*

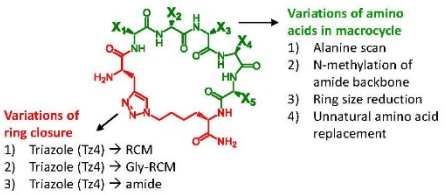
T, tumor; L, left kidney, R, right kidney

Coppock et al., *Biopolymers (Pept Sci).* 2017; 108(2): e22934.

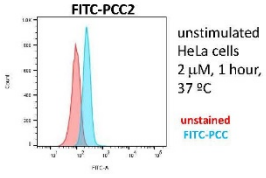
4

PCC Design Considerations for Intracellular Targeting

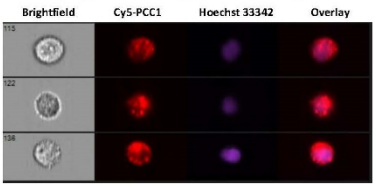
PCC modifications for increasing cell penetration



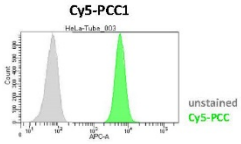
FITC flow experiment



ImageStream imaging flow cytometry



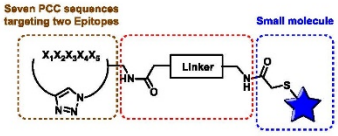
Cy5 flow experiment

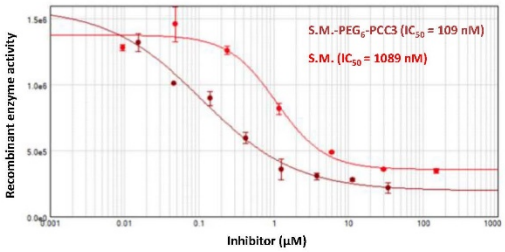


5

Heterobiligand Inhibitor against Intracellular Proprietary Target

- PCC macrocycles selected against epitopes adjacent to the active site of target
- Heterobiligands created by attaching a small molecule inhibitor to epitope-targeted PCC macrocycle using a chemical linker



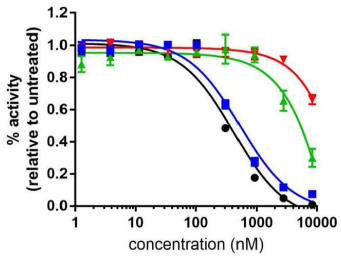


- **Heterobiligand inhibitor is 10 X more potent than small molecule alone**
  - Molecular docking studies predict that PEG_6 is the best linker to bridge the active site and the PCC binding site

S.M. = small molecule inhibitor.

6

Heterobiligand Inhibition of Intracellular Enzyme Activity in HeLa cells



140c: S.M.-PEG_6-PCC3(NMe)
136h: S.M.-BA-PEG_5-PCC3
136j: S.M.-BA-$(Val)_3$-PCC3
S.M.

- **N-methylated heterobiligands show both cellular uptake and intracellular enzyme inhibition in HeLa cells**
- Additional medicinal chemistry variants are under development to maximize the cell penetration and inhibition

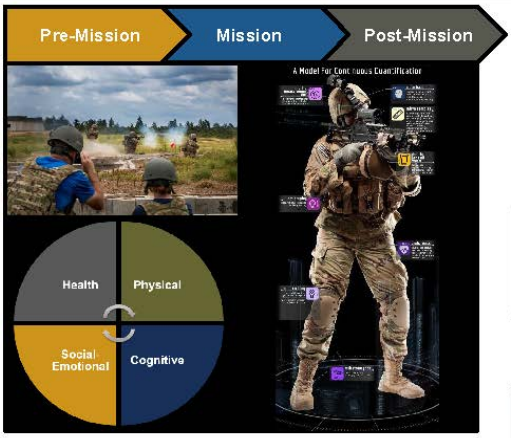
ID#	Heterobiligand	Inhibition HeLa cells IC50 nM
140c	S.M.-PEG_6-PCC3(NMe)	533.8
136h	S.M.-BA-PEG_5-PCC3	36109
136j	S.M.-BA-$(Val)_3$-PCC3	no curve fit
	S.M.	417.1

S.M. = small molecule inhibitor; NMe = N-methylated; BA = beta-alanine; Val = valine.

7

Appendix E. NSRDEC MASTR-E Program

Monitoring and Assessing Soldier Tactical Readiness & Effectiveness (MASTR-E)



Key Contributors

- NSRDEC (in addition to SPOD): Office of the Chief Scientist, Combat Feeding, G3-5 Office, Soldier Squad Integration, HQ R&D Detachment, and members of the Electronics and Calibrations Team (Field Strength Box, External Power Supply)
- NSRDEC SPOD Leads: Dr. Erika Hussey (Cognitive Science & Applications Team) and Dr. John Ramsay (Biomechanics Team
- USARIEM

Purpose

Scientific advances in the quantification of human performance (via wearable sensors, cutting-edge assessment tools, and biosamples) present an opportunity to obtain accurate data to make evidence-based decisions regarding Soldier and Small Unit readiness. This study will identify the human dimension measurements that reliably account for sustained dismount Soldier and small unit performance for fundamental warfighting task, specifically shoot and move.

Products

1. Collective lethality measurement
Best available research grade measurement to the field for individual & small unit.
Results to date: 450 metrics; 46 complete data sets; first draft of Methods manuscript due out in one month

2. Down selected human dimension "X factors"
Identify which measurements across cognitive, physical, social/emotional, and health domains predict warfighting task performance.

3. Characterized recovery
Informed subsequent mission planning based on "x-factor" measurements.

TAKE HOME MESSAGE

This is the first study of its kind to investigate "collective lethality" at the individual & small unit level. This study will establish initial relationships on what human measurements correlate to "shoot", and "move" warfighting tasks.

It is the culmination of over two years of engaging with FORSCOM partner units where Soldiers and scientists/engineers are partners in the science of close combat.

UNCLASSIFIED

Appendix F. MRMC Wearables Integration across DOD

Dismounted Service Member Wearables DoD Integration Strategy

Military Operational Medicine Research Program (MOMRP)

Dr Roy Vigneulle
US Army Medical Research & Materiel Command
August 29, 2018

DoD Wearables Initiative Background

Disclaimer

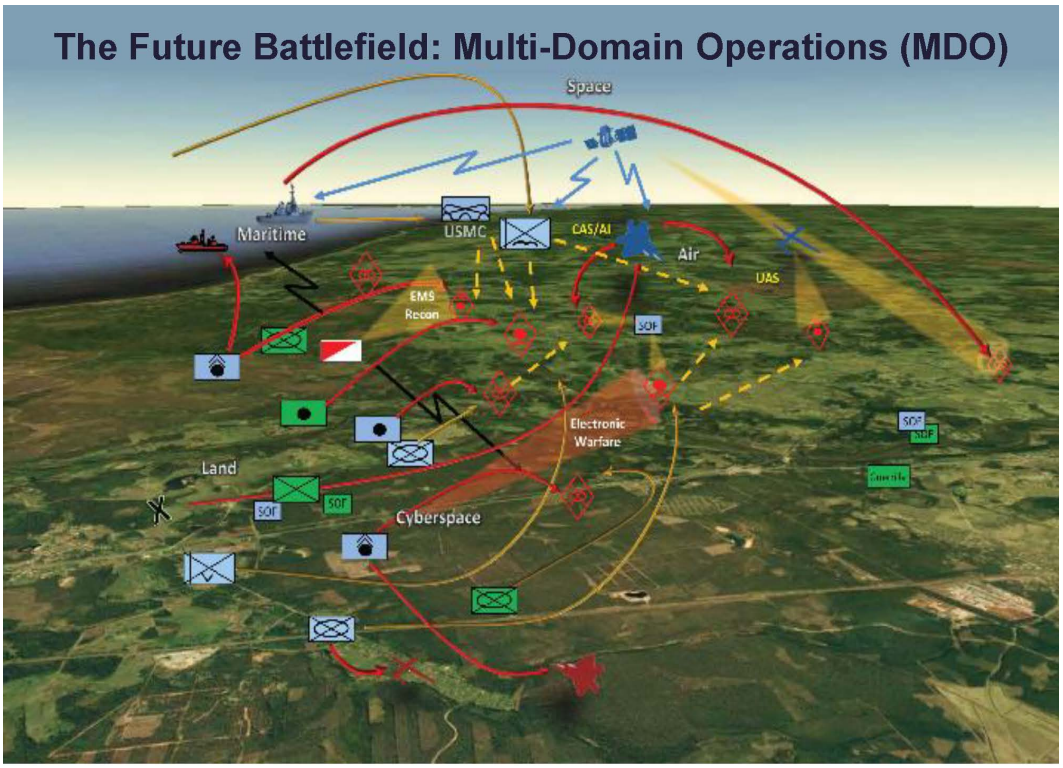
The authors of this presentation have nothing to disclose.

All opinions are those of the authors and do not reflect the official position of the Army, the DHA or the DoD.

Outline

» Background
» Army Priorities and Key Drivers for Wearables
» HRAPS Link
» DoD Wearables Roadmap Strategy
» Capability Integration Pathway Forward
» Wearables Initiative Integration Solutions

The Future Battlefield: Multi-Domain Operations (MDO)




The Future Battlefield: Dense Urban Environments


The Future Battlefield: Subterranean Operations

Key Drivers for the Wearables

» Big 6 Modernization Priorities:

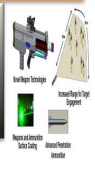
1. Long Range Precision Fires
2. Next Generation Combat Vehicle*
3. Future Vertical Lift*
4. Network
5. Air and Missile Defense
6. Soldier Lethality*

* Areas in which medical research has equities

Soldier Lethality

Next Generation Squad Weapon*

-NG Family of Ammo
-Precision Munition Squad Optic Performance Enhancement
-Target Acquisition Sensors
-Next Generation Squad Weapon Technology



Soldier Protection*

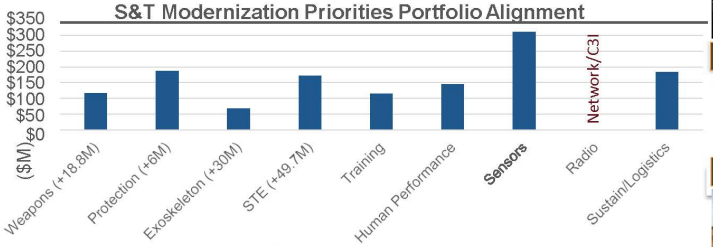
-Body Armor
- Integrated Headborne Systems
- Soldier Signature Management
-Extreme Austere Environmental Protection

Exoskeleton*

-Advancement of Technologies for Logistics & Infantry Tasks
-Research for Advanced Development: Human-machine Interface
- Single & Multi-Joint Exo Systems

Synthetic Training Environment*

-One-World Terrain Point of Need
- Common Synthetic Environment
- Data Management Tools



Human Performance

-Decision Making and Communication
- Enhanced Mobility and Lethality
- Performance Nutrition

Sensors

-Advanced Soldier Sensors/ Display for Dismounts
-Asymmetric Vision & Decide Faster Integrated Capability Demos

Soldier Lethality $1.298B* over POM 19-23

Approximation, not subject to FOIA

Training

-Tactical Combat Casualty Care
-Full Spectrum Combat Training Simulation
- Multi-Domain Battle Training Simulations
- Responsive Training Simulations for Commanders

Sustain/Logistics

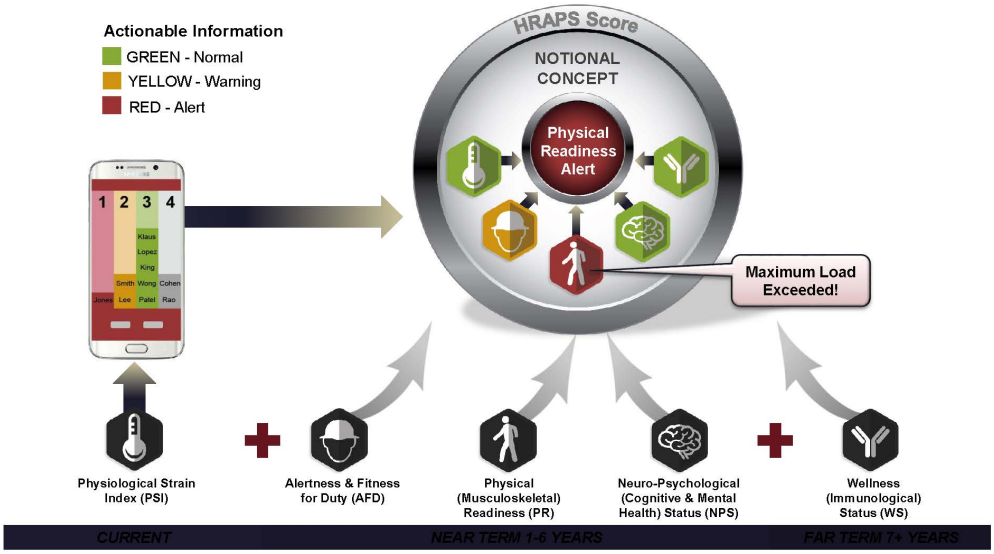
-Cargo Aerial Delivery
- Expeditionary Mission Command Platforms
- Individual H_2O Filtration
- Personnel Airdrop
- Power and Energy
- Combat Rations

Radio

- Automation and Intelligence
- Resiliency
- Situational Understanding

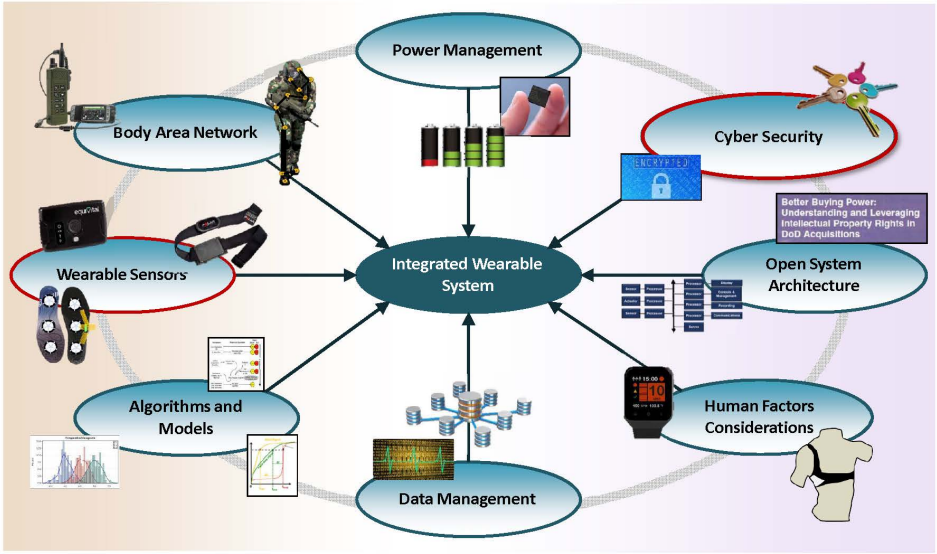
HRAPS Concept

HRAPS is an integrated system of wearable sensors that provides Commanders with actionable information to improve performance and mitigate non-battle injuries during operations.

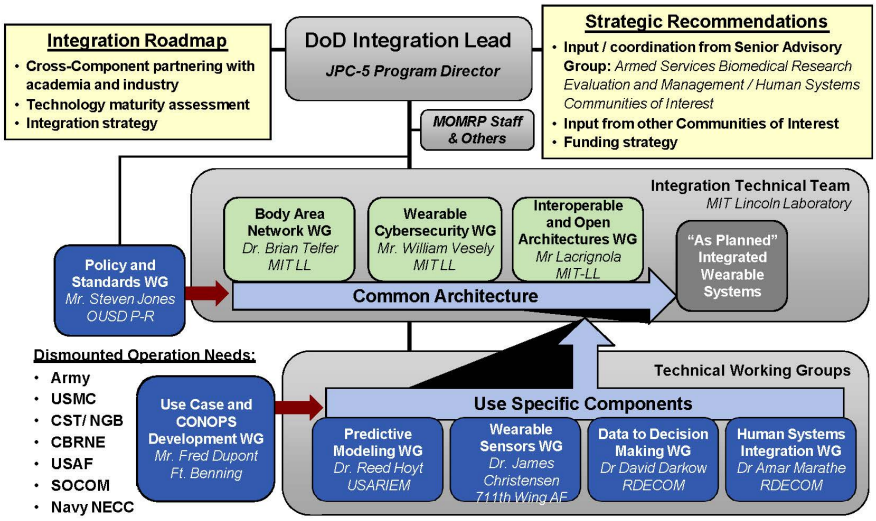


Dr. Roy Vigneulle roy.m.vigneulle.ctr@mail.mil

Health Readiness and Performance Systems Engineering Approach

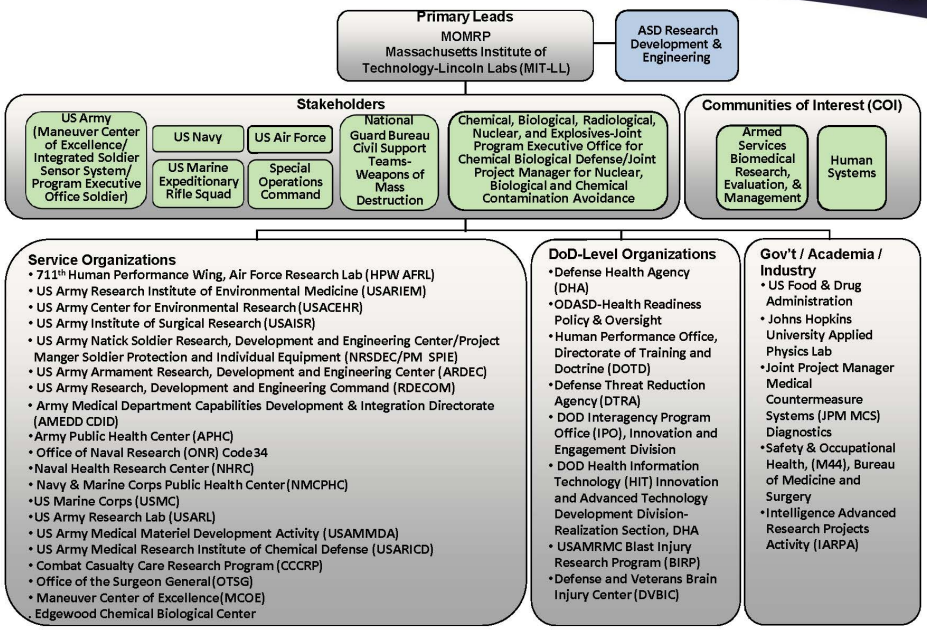


DoD Wearables Roadmap Strategy

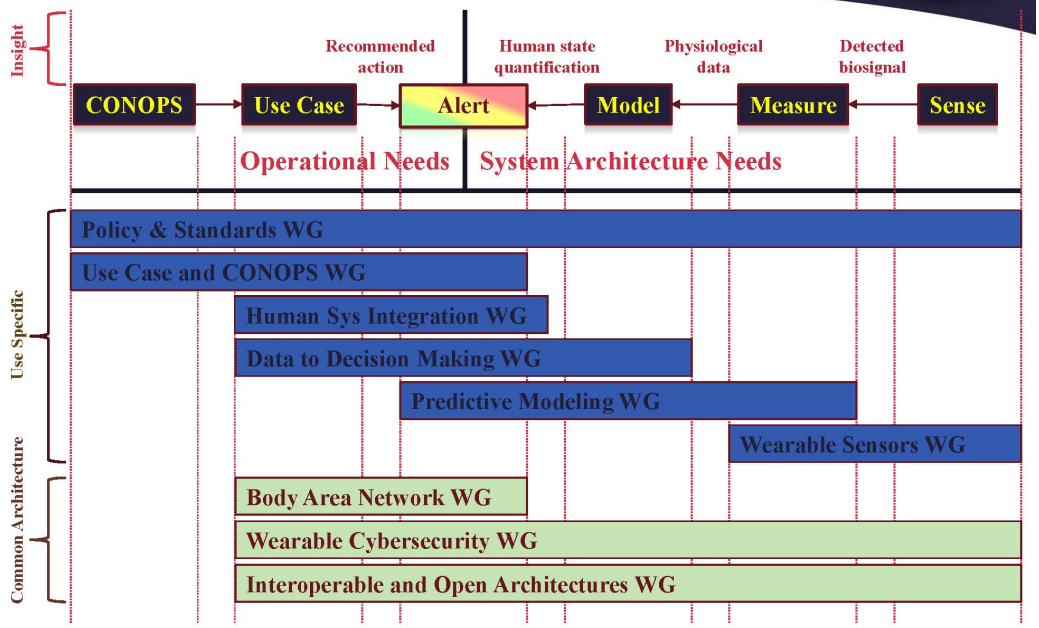


Way ahead to be able to Assess real-time physiological, cognitive, psychological health status in order to provide actionable information to Service members and leaders in all environments, to include MDO

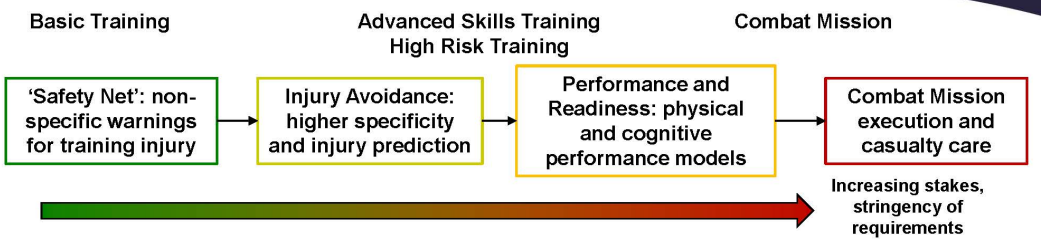
Wearables Initiative Partners



Deriving Needs from Working Group Inputs



Use Cases and Phased Implementation



- Early deployment of 'partial' capabilities (i.e., not yet battlefield capable) in training provides significant benefits
  - Reduction of injury risk in training
  - Routine, large scale data collection speeds pace of model development
  - Ability to test / refine decision support in increasingly realistic environments
  - Allows combat commanders to gain experience with new capabilities and 'pull' them into the operational domain
- Interoperability and clear definition of functional interfaces is key to ensuring smooth evolution of design as new devices and concepts become available

CONOP / Use Cases

Overarching CONOP: Multi-Domain Operations / Joint Forced Entry

Use Case 1: Land Navigation Training

- Relevant Service-wide
- Solo operation with limited safety monitoring
- Significant physical risk due to environmental extremes
- Significant alertness risk due to day / night duration

Use Case 2: Airborne Assault

- Relevant DoD-wide and internationally
- Significant physical risk of becoming combat casualty
- Ad hoc fighting units formed during ground operations

Use Case 3: Sensitive Site Exploitation (CBRNE)

- Additional risk elements not found in other scenarios: protective encapsulation, TIC/TIM exposure monitoring
- Use of organic sensors for man-machine teaming (Human Systems Integration)

Wearables Biosensing Alert Maturity

Findings Roll-up from Sensors, Models, D2DM WGs

Actionable Alerts	Sense	Model	Decide	Limitations
Heat stress				Needs compensation, use-specific clarification of index to action
Cold stress				Confounded core temp sensing
Musculoskeletal injury				Limited predictive models
Agility				Limited predictive models
Hypoxia				Challenges in collecting meaningful data
Dehydration				No physiological sensing modality; limited predictive
Exhaustion / metabolic				Cost models limited; alert states poorly defined
Training recovery				Proprietary commercial products not validated
Diminished cognition / judgment				Alert states poorly defined
Alertness				Current metrics require intervention; limited predictive models
Emotional instability				Lacking sensing modalities, alert states poorly defined
Infection / bioagent				Primate models only; alert states poorly defined
Stopped activity				Simple 'Are you OK?' based on location / movement
Chemical exposure				Primate models only; alert states poorly defined

Sufficiently validated for routine use

Further development needed

Further research needed

Actionable information must be clearly understood by small unit leaders, and relevant to current operational conditions and mission requirements

Capability Integration Path Forward

- To become an operational system suitable for broad military operational use, each component must mature to meet the following criteria:
  - Actionable information clearly understood by small unit leaders, and relevant to current operational conditions and mission requirements
  - Science-based predictive models thoroughly vetted for the range of expected operational conditions
  - Field-hardened wearable sensors with sufficient accuracy, user acceptance and ability to integrate with operational gear
- Expect advances in low-SWaP sensor technologies, signal processing, and data analytics could make alerting goals achievable. Meaningful alerts require well-defined use-specific performance metric.
- Different operational needs may require different models and robust data sets with common data elements

Adoption of a well-coordinated prioritized development strategy is essential

Wearables Initiative Strategy Solutions

- Leveraging commercial investments in biosensing is an opportunity, but commercial and DoD needs do not perfectly align

  Solution: Establish processes to prioritize and coordinate government investment to ensure that DoD-unique needs are met

- Flexible, expedient ways to develop integrated biosensing systems are required to take advantage of technology advances at reasonable cost

  Solution: Adopt technology-agnostic open architecture approach to integrate sensing that fits operational needs defined by user communities and does not interfere with other mission gear

- Requirements for DoD integrated biosensing continue to evolve

  Solution: Prototype technology and test with Warfighters in training environments to obtain objective data while providing leave-behind safety capability

Intra-Service and cross-Service coordination is essential

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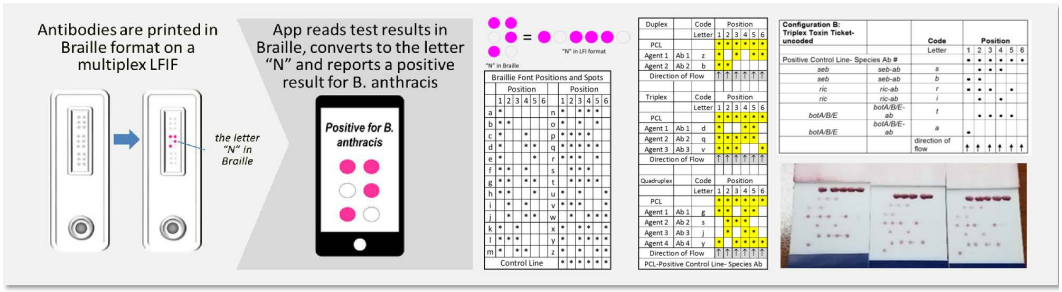
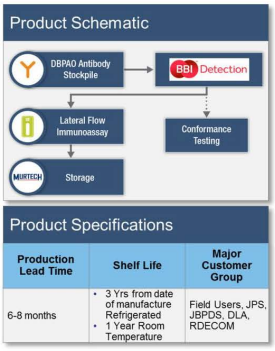
Appendix G. ECBC BlindSpot

BLINDSPOT:
A NOVEL MULTIPLEX LATERAL FLOW IMMUNOASSAY FORMAT FOR RAPID DETECTION POTENTIALLY USING A CELL PHONE APP

Randy Hofmann, PhD – Excet Inc./ECBC

Shanmuga Sozhamannan, PhD – Tauri/DBPAO





- ❑ Easy to Use
- ❑ Multiplex capability:
  - Good for when dealing with reduced sample volumes
  - Reduces costs
  - Currently can evaluate up to 6 samples, potential for more
- ❑ Cell phone App development

Approved for Public Release; Distribution Unlimited

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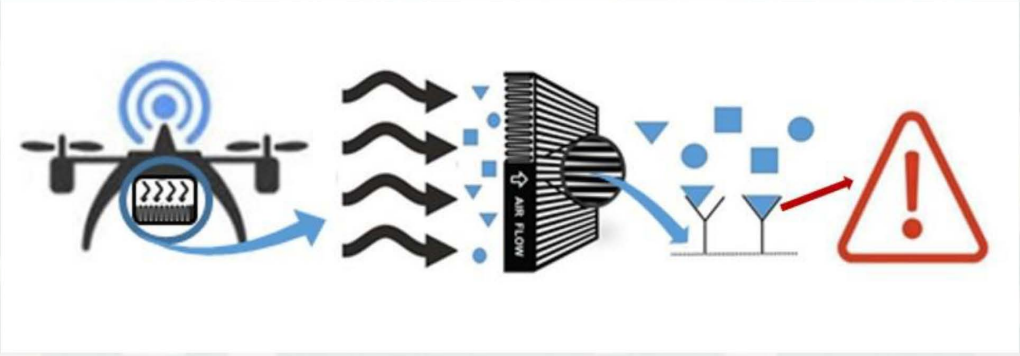
Appendix H. USACE Environmental Monitoring

ERDC Need: Aero Sensing



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Appendix I. AMRICD Countermeasures and Diagnostics

Application of PCC to Detection of CWA Biomarkers

Exposure Detection | Diagnostic | Forensic

- Human serum albumin- tyrosine-411 adducts
  - Discriminate agent or generic +/- for exposure
- Peptide biomarkers resulting from exposure
- Small molecule recognition - anti-opioid/metabolite or agent breakdown product

Scope: both lab based and field forward POC

List of Symbols, Abbreviations, and Acronyms

AMRICD	US Army Medical Research Institute of Chemical Defense
ARL	US Army Research Laboratory
CBRNE	chemical, biological, radiological, nuclear, and explosive
CDC	Centers for Disease Control and Prevention
CWA	chemical warfare agent
DARPA	Defense Advanced Research Projects Agency
DTRA	Defense Threat Reduction Agency
ECBC	Edgewood Chemical Biological Center
ERDC	US Army Engineer Research and Development Center
HRAPS	Health Readiness and Performance System
ICB	Institute for Collaborative Biotechnologies
MASTR-E	Monitoring and Assessing Soldier Tactical Readiness and Effectiveness
MRMC	US Army Medical Research and Materiel Command
NSRDEC	US Army Natick Soldier Research, Development, and Engineering Center
PCC	protein catalyzed capture
USUHS	Uniformed Services University of Health Sciences
WRNMMC	Walter Reed National Military Medical Center

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2 (PDF) DIR ARL IMAL HRA RECORDS MGMT RDRL DCL TECH LIB

1 (PDF) GOVT PRINTG OFC A MALHOTRA

1 (PDF) INSTITUTE OF SYSTEMS BIOLOGY J HEATH

2 (PDF) INDIMOLECULAR H AGNEW B LAI

2 (PDF) INSTITUTE OF COLLABORATIVE BIOTECHNOLOGIES D GAY R KOKOSKA

5 (PDF) NSRDEC J PLAYER M WIEDERODER J MAGNONE S MCGRAW E BRACK

4 (PDF) ECBC R HOFMANN S SOZHAMANNAN S BOOMALL M RETFORD

9 (PDF) MRMC R VIGNEULLE K SCHMID K CAUDLE D JACKSON J MCKNIGHT CR STEELE V DIVITO J KOONTZ K FULLERTON

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