The Need for Speed. Targeted Library Screening Yields Minimalist Enzymes for Chem-Bio Decontamination

The need for speed has never been more necessary than for the warfighter exposed to a toxic nerve agent. Speed counts in applying a countermeasure and describing how well that decontaminating system (a patch, a wipe, a cream, etc.) neutralizes the threat. The faster the agent can be neutralized, the faster the warfighter can return to their mission.

Speed is also critical to life as many of the most important reactions in biology are inherently slow and must utilize enzymes to accelerate them to a point of becoming useful. For example, the ambient temperature half-lives for hydrolysis of proteins and DNA are 400 and 1,000,000 years, respectively. Enzymes must consequently accelerate chemical reactions by factors of 10^7-10^19 to bring them into the useful range.

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A Giant Nano Leap Forward in Pursuit of Clean Water

Can researchers engineer fuel free micro and nano motors that target, isolate, separate, and eliminate chemical agents encountered in environmental matrices and unprocessed body fluids? Indeed they can.

Presently, the decontamination of chemical warfare agent contaminated assets presents logistical challenges for military units. This is due to limitations on the volume of decontaminant available and the need to redirect warfighters to perform physically demanding decontamination operations that include scrubbing or brushing procedures while in Mission Oriented Protective Posture (MOPP) gear. However, thanks to work by the Defense Threat Reduction Agency (DTRA), this might not be a problem in the future.

Work managed by Dr. Brian Pate of DTRA’s Chemical and Biological Technologies Department, and including principal investigator Dr. Joseph Wang in the Department of Nanobioelectronics at the University of California, San Diego (UCSD), recently met a major programmatic milestone by demonstrating that self-propelled activated carbon-based micromotors rapidly remove toxic substances in water matrices.

The UCSD research team's approach toward the design and control of micromotor surface chemistry and motion might soon provide warfighters the additional capability to rapidly obtain complex, unprocessed samples of interest (for example, “pond scum”) and transport them to multiple sensors simultaneously for subsequent analysis, treatment, or elimination.

A newly published article in the journal Small titled, “Self-Propelled Activated Carbon Janus Micromotors for Efficient Water Purification,” details the remarkable decontamination efficiency that these self-propelled, activated carbon-based Janus micromotors demonstrated in the rapid removal of heavy metals, nitroaromatic explosives, organophosphorous nerve agents and azo-dye compounds in water.

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These dramatic accelerations underpin a long held chemical-biological decontamination goal of repurposing enzymes suitably enhanced to detoxify (non-natural) nerve agents, as high-speed tools to fit into the decontamination system needed to mitigate threats to the warfighter. Achieving this goal, however, has been hampered by the fragility and complexity of enzymes, features that hinder efforts to methodically improve activity.

In addition to accelerating reactions, enzymes must meet many additional criteria that are not relevant to decontamination efforts, such as being innocuous neighbors, having function that can be regulated, and being formed from biologically available materials. Biomimetic catalysts hold great promise in providing the efficiency of enzymes without the limitations.

But how does one find a needle in the haystack that is practically in the infinite “structure-space?” Researchers for DTRA CB/JSTO have developed a methodology that combs libraries of peptides that have been structurally biased to display catalytic activity. Since only peptides that display their side chains in active site-like structures will likely be good catalysts, focusing on these arrangements solves the problem of too many to screen. This discovery will be used to develop catalysts with properties ranging from broad-spectrum activity towards multiple nerve agent threats to highly targeted activity towards particularly problematic threats. Applications ranging from decontamination to prophylaxis are also envisioned.

This basic research effort, managed by Dr. Ilya Elashvili of DTRA CB/JSTO and a team led by Drs. Michel Gagné and Marcey Waters at the University of North Carolina at Chapel Hill, sought a roadmap for the synthesis of super-active, but small, easily scalable, and robust peptide-based catalysts for nerve agent degrading-related reactions.

The researchers discovered a family of small peptide structures that display catalytic rate accelerations in excess of 100,000,000 over background. These results set a record for biomimetic catalysis, and have appeared in a series of papers including the Journal of the American Chemical Society, entitled “A Catalyst Selection Protocol That Identifies Biomimetic Motifs From β-Hairpin Libraries.” This undertaking sets the groundwork for utilizing such small, robust, and inexpensive catalysts for nerve agent countermeasure efforts.

The team discovered these catalysts by developing and combining two innovations that emerged from several key insights into enzyme-like methods of catalysis. First, the researchers only utilized peptide fragments that adopt specific secondary structures, including the beta-hairpin or the alpha helix.

Figure 1. a) The basis of the reactive tagging scheme focuses on the intermediacy of a covalent intermediate in the nucleophilic mechanism for transesterification. In the absence of a nucleophile this intermediate deprotonates and generates a tagged peptide, which colorimetrically identifies those structures (in a solid phase library) able to most rapidly generate an acylated intermediate. Since the k2 step is typically faster than k1, selecting for compounds able to most quickly generate the covalent intermediate usually also finds compounds with rapid deacylation steps. b) and c) represent two of the top catalytic hits, each displaying rate accelerations of $2.4 \times 10^8$ and $6.5 \times 10^7$, respectively. In each case a biomimetic non-covalent network of H-bonds activates the histidine for addition to the ester reactant. Not shown are a series of cross-strand H-bonds that help fold the peptide into a beta-hairpin structure.
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From relatively small but insightfully “designed” libraries of 100 to 1,000 peptides emerged a set of incredibly fast catalysts. Beta-hairpins were particularly effective as they position the side chains on one face of the hairpin into an active site-like arrangement that enables synergistic interactions to accelerate catalytic reactions. In this way, the previously unmanageable peptide space is reduced to a highly focused library of compounds that can be screened on a polymer bead where each bead contains one unique peptide.

The second tool selects the top catalyst candidates from the library of beads with a tagging scheme that ingeniously colorizes only those library members (i.e., beads) capable of reacting with the colored reagent of interest. At the root of this tool is the realization that the trailing step of a two step nucleophilic acyl transfer mechanism is usually fast, and one should search for compounds that covalently modify, or ‘tag’ the peptide as this action represents a proxy for step one of the mechanism. This approach should find catalysts that rapidly accelerate both steps of the mechanism. The bead colorizing assay thus searches the library for compounds or beads that colorize by the same mechanism used for the first step of catalysis.

A common feature among the high activity catalysts was the presence of a histidine amino acid paired with one or more amino acid side chains to create dyads, triads and tetrads of amino acid side-chains that interact non-covalently with the histidine. Each non-covalent interaction enhances the nucleophilicity of the histidine, a key element in catalyzing the reactions of interest. Most intriguing was the observation that despite the small size of the catalysts and the non-biological medium in which they operate, the best catalysts recapitulated the catalytic functionality used by enzymes to enhance the activity of active site histidine residues. Although the mechanistic role of an active site histidine in enzymology is usually as a base, and only occasionally as a nucleophile, Gagné and Waters have found that their catalysts operate by a nucleophilic histidine mechanism.

An important feature of this work is the applicability of the approach to the discovery of catalysts for other reactions. For example, the researchers plan to identify catalysts for phosphate ester hydrolysis and determine if these mechanistic features are transferable, or if there is a different preferred mechanism for this class of substrates. This can be accomplished by modifying the screening dye to one that is based on a close simulant for a P-based nerve agent. Like the carbonyl case, the guiding hypothesis is that in a nucleophilic mechanism, it is the first step that is typically turnover limiting, and this is the step that should be screened for when searching for new catalysts for P-ester decontamination.

One significant benefit to searching for small molecule peptide biomimetic catalysts is the structural simplicity of the discovered catalysts (relative to an enzyme), which enables the catalysts to be synthesized by straightforward chemical means and have both natural and unnatural amino acid components incorporated. Both features will enable catalysts to be optimized with high stabilities for operating under harsh conditions.

In addition to developing a rapid and effective way of discovering fast-reacting minimalist peptides for carbonyl esterolysis, this research also illuminates a pathway for discovering simple, but fast acting catalysts for nerve agent decomposition. Time will tell if this approach will solve the long standing problem of enzyme fragility and cost, but initial studies suggest a promising new ‘arrow in the quiver’ for decontamination scientists looking to keep warfighters safe.

In Pursuit of Clean Water...

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The bubble-propelled micromotors rely on the asymmetric deposition of a catalytic platinum patch on the surface of the activated carbon microspheres to act as nanoadsorbents. Additionally, this rough microsphere surface provides a highly catalytic layer, which generates effective bubble evolution and micromotor propulsion speeds of more than 500 μm/s. The corresponding fluid dynamics and mixing afford this highly efficient moving adsorption microsphere the ability to accelerate water purification. This research holds considerable promise for new, rapid, cost-effective defense biomedical decontamination/remediation applications due to higher efficiencies and shorter clean up times, and it could significantly minimize consumable needs and other logistical burdens.

Contributions to the Chemical and Biological Defense Program by this research team thus far include: successful efforts in the multi analyte separation and detection of a biological simulant, demonstration of motor-accelerated degradation of chemical warfare agents, development of an efficient micromotor propulsion system for use in a variety of real-life unprocessed environments, and the engineering of self-destroying motors that have an autonomous cargo release capability.

These activities are leading to fundamentally new concepts for sensing, isolating, and identifying biological targets and for detoxifying chem-bio threats.

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Staying Cool: Lifting the Thermal Burden on Warfighters

New technology in uniforms could have warfighters, and their uniforms, breathing easier. Climate, physical exertion, chemical and biological protective clothing add up to a thermal burden that often exceeds human physiological tolerance limits. Exhaustion from heat strain continues to hinder the warfighters’ abilities to complete a mission in a chemical-biological compromised environment.

Decades of ongoing work to reduce incidents of heat stress and strain have brought forth a plethora of new protective garment materials, the most optimal to date being selectively permeable membrane materials. But there are still tradeoffs between the ability to capture toxic molecules while keeping the wearer cool.

Thermal burden might soon be a thing of the past due to efforts by a Defense Threat Reduction Agency team managed by the Chemical and Biological Technologies Department’s Dr. Brian Pate and principal investigator Dr. Alexander Neimark from Rutgers University. This Interfacial Dynamics and Reactivity Program reports on new findings predicting unexpected relationships between morphology and water transport (garment breathability) in future barrier materials.

Furthermore, researchers on this team recently met another program milestone in their establishment of a fundamental model capability to predict the thermodynamic and water transport properties of hydrated Nafion polyelectrolyte membranes. These Nafion materials are now being evaluated as protective materials for warfighters. Additionally, Nafion and related materials have received considerable recent attention as proton conductors for proton exchange membrane fuel cells.

Technical details regarding how this recent work developed the capability to predict unexpected dependence of mass transport on polymer volume fraction within a three-dimensional network structure are found in The Journal of Physical Chemistry B article entitled, “Self-Assembly in Nafion Membranes upon Hydration: Water Mobility and Adsorption Isotherms.”

This research effort shows promise for future development of self-decontaminating protective barrier materials and fuel cells.

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Nafion has unique properties due to nano-segregation and selective permeability. Image created for the Department of Defense by Dr. Alexander Neimark, Rutgers University and courtesy of the American Chemical Society.
The Defense Threat Reduction Agency’s (DTRA) Research and Development (J9) Directorate, Chemical and Biological (CB) Technologies Department, serves as the Joint Science and Technology Office for Chemical and Biological Defense. This publication highlights the organization’s accomplishments to protect warfighters and citizens through the innovative application of science and technology research.