

# Comparative Animal Mucomics: Inspiration for Functional Materials from Ubiquitous and Understudied Biopolymers

Antonio R. Cerullo, Tsoi Ying Lai, Bassem Allam, Alexander Baer, W. Jon P. Barnes, Zaidett Barrientos, Dimitri D. Deheyn, Douglas S. Fudge, John Gould, Matthew J. Harrington, Mandë Holford, Chia-Suei Hung, Gaurav Jain, Georg Mayer, Mónica Medina, Julian Monge-Nájera, Tanya Napolitano, Emmanuelle Pales Espinosa, Stephan Schmidt, Eric M. Thompson, and Adam B. Braunschweig\*



Cite This: *ACS Biomater. Sci. Eng.* 2020, 6, 5377–5398



Read Online

ACCESS |

 Metrics & More

 Article Recommendations

**ABSTRACT:** The functions of secreted animal mucus are remarkably diverse and include lubricants, wet adhesives, protective barriers, and mineralizing agents. Although present in all animals, many open questions related to the hierarchical architectures, material properties, and genetics of mucus remain. Here, we summarize what is known about secreted mucus structure, describe the work of research groups throughout the world who are investigating various animal mucus, and relate how these studies are revealing new mucus properties and the relationships between mucus hierarchical structure and hydrogel function. Finally, we call for a more systematic approach to studying animal mucus so that data sets can be compared, omics-style, to address unanswered questions in the emerging field of mucomics. One major result that we anticipate from these efforts is design rules for creating new materials that are inspired by the structures and functions of animal mucus.

**KEYWORDS:** *mucus, mucins, omics, functional materials, bioinspired materials, biomimetics*



## INTRODUCTION

Secreted mucus are heterogeneous hydrogels containing a large fraction of high molecular weight, highly glycosylated proteins called mucins.<sup>1–10</sup> Mucuses are ubiquitous in animals and appeared roughly 600 million years ago in metazoans.<sup>11</sup> Their physiological roles and material properties are remarkably diverse. Most higher animals express at least five individual mucin genes but in some cases can contain as many as 25,<sup>11</sup> suggesting their function as a fast-evolving and modular scaffold.<sup>11,12</sup> While all of these animals produce internal gels that line respiratory and digestive tracts, and frequently many more,<sup>13,14</sup> here we focus on mucus secreted outside the body that are associated with unique biological functions. For example, mucus acts in some animals as a lubricant<sup>15,16</sup> and in others as an adhesive;<sup>17</sup> it can direct hydration<sup>18</sup> or mineralization;<sup>19</sup> mucus has a prominent role in marine ecosystem energy cycling;<sup>20,21</sup> or can mediate predator–prey dynamics<sup>22</sup> and immune responses<sup>23,24</sup> where it additionally acts as a semipermeable barrier.<sup>25,26</sup> As such, the systematic evaluation of these abundant biopolymers could lead to the development of technical and biomedical applications. These include new, bioinspired glues for surgical implants,<sup>27</sup> coatings to mediate organic–inorganic interfaces in medical implants,<sup>28</sup> biocompatible lubricants,<sup>29</sup> composites for 3D bioprinting,<sup>30</sup> sustainable alternatives to industrial plastics,<sup>31</sup> antimicrobial and immune agents,<sup>32</sup> additives for

wound healing,<sup>33</sup> environmental remediation systems,<sup>34</sup> and many other useful ecologically derived materials and biomedically relevant compounds.

Despite their prevalence and utility, mucus have not been studied to the same extent as other biological materials such as cellulose,<sup>35–37</sup> nucleic acids,<sup>38,39</sup> and silk.<sup>40–42</sup> This is partly because of the complexity of their structure and uncertainty as to what seems to be the requirement for a sophisticated network of genes and proteins working together to make a functional hydrogel. Mucus is a hierarchical material in that interactions and structures at the Ångstrom, nanometer, and micrometer length scales contribute to its desirable macroscopic properties (Figure 1).<sup>43,44</sup> In addition, it is a heterogeneous material that encases other proteins, salts, and small molecules;<sup>45</sup> these additives affect mechanical properties and physiological function.<sup>46</sup> While individual mucin glycoproteins vary in their specific viscoelastic properties, mucins are known in general to provide boundary lubrication to

**Received:** May 13, 2020

**Accepted:** August 24, 2020

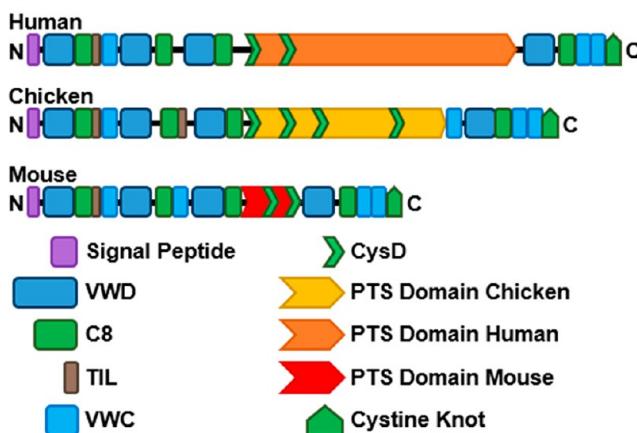
**Published:** August 24, 2020



A

### Human MUC2 Protein Sequence

B



**Figure 1.** (A) Sequence of human MUC2 protein. O-glycosylated PTS and VNTR domains (composed of 95.8 and 80.9% serine, threonine, and proline, respectively) are labeled in blue. N- and C-terminal D domains (composed of 8.6 and 10.5% cysteine, respectively) are labeled in green. Cysteines within the D domains are underlined. Region outlined in red indicates the sequence repeated 103 times within the tandem repeat domain, QXPTXTXIX<sup>54</sup>TTTVPPTPTGT, where X is a mutational hotspot. (B) MUC2 protein domain architectures from human, chicken, and mouse models. Image adapted from Jiang et al, 2013.<sup>54</sup> (C) Mucus structural hierarchy. Mucins are organized into glycosylated serine-, threonine-, and proline-rich domains (orange), CysD domains (green), and D domains (blue). Mucin glycoproteins form gel networks as a result of disulfide bridges and H-bonding networks.

biological surfaces, reducing friction in a manner similar to that of hydrophilic polymer brushes.<sup>16,47–50</sup> Additionally, the substances' adhesive and cohesive properties can vary with concentration,<sup>51</sup> and the rheological interactions of materials with mucus (mucoadhesion) is highly dependent on the nature of the substrate.<sup>52,53</sup> These multifaceted features make investigating structure at all length scales necessary to understand the origin and design principles that direct mucus functional properties and account for their diversity.

Although mucus has widely varying physical properties, all mucins have certain conserved features. Their molecular weights range typically from 5–10 MDa and can have individual chain lengths of nearly 14 000 amino acids.<sup>43,55,56</sup> Their sequences generally incorporate two major domains: (1) disulfide-forming cysteine-rich (D) domains at the termini which participate in the establishment of mucus gel networks<sup>1,10,57</sup> and (2) the proline-, threonine-, and serine-rich “mucin domain”, whose dense O-glycosylation accounts for

upward of 80% of mucin molecular weight (Figure 1A).<sup>9,58,59</sup> To date, 22 human mucin genes have been identified,<sup>60</sup> each with a unique set of glycoforms and expression profiles.<sup>61–64</sup> The majority of the mucin proteins encoded by these genes could be categorized as gel-forming secreted mucins (MUC2, SAC, SB, 6, 19), nongel-forming secreted mucins (MUC7, 8, 9), or transmembrane mucins (MUC1, 3A, 3B, 4, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22) based on their cellular localizations.<sup>65</sup> At the C-terminus of the protein, all gel-forming mucins contain a “cystine-knot domain” (CK domain), whose cystines are critical for end-to-end dimerization of mucin proteins via intermolecular disulfide-bond formation (Figure 1B).<sup>56,66</sup> In addition, all but MUC6 proteins also contain a von Willebrand Factor like C-domain (VWC) at the C-terminus, which is known to participate in protein complex formation.<sup>67–69</sup> In general, the N-terminal portions of gel-forming mucin proteins contain domains with high homology to von Willebrand Factor like D-domains (VWD). MUC2, SAC, and SB also contain a VWD domain at the C-terminal end. The N-terminal VWD domains, and in particular, the one referred to as the third VWD domain, play an important role in mediating trimerization of dimeric mucin molecules, inducing matrix formation and contributing to hydrogel formation.<sup>70</sup> Another unique feature of MUC2, MUCSAC, and MUCSB mucin proteins is the presence of hydrophobic cysteine-containing domains (CysD) between adjacent mucin domains. Each CysD domain contains multiple cysteines that form intramolecular disulfide bonds. Although at least one of the CysD domains in each mucin was predicted to be C-mannosylated, tissue culture experiments suggest that the mucin CysD domains are not C-mannosylated.<sup>71</sup> It has been suggested that the CysD domains also participate in multimerization of mucin proteins and stiffening of mucus gels (Figure 1C).<sup>71,72</sup>

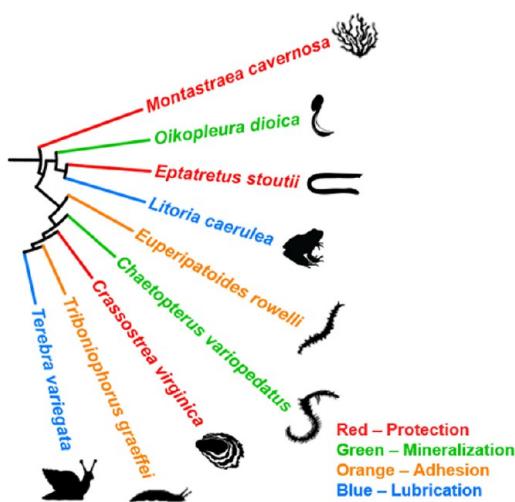
The central portion of secreted mucins constitutes the majority of the protein mass and is the site of heavy O-linked glycosylation. This region is typically comprised of two types of repeats: one that is rich in proline, threonine, and serine (PTS domain), and another having a variable number of tandem repeats (VNTR domain). The former has a high abundance of these three amino acids, while the latter contains these as well as glutamine, glycine, isoleucine, and valine in numerous short repeating sequences (Figure 1A). The number of repeats could range from tens to hundreds of repeating units. Regarding glycosylation, there is a high proportion of GalNAc residues directly O-linked to the peptide chain, and the average number of monosaccharides per glycan,  $n$ , is typically smaller ( $n \sim 3$ ) than cell-surface mucins, where  $n \sim 8$ .<sup>73–75</sup> In fact, these repeated domains are so heavily glycosylated that mucins are typically fixed in a linear conformation.<sup>76</sup> While the sequences of these repeats do not show strict conservation within and between individual mucin types, both PTS and tandem repeat domains are enriched in PTS amino acid residues. Both MUC2 and MUCSAC proteins also contain an autocatalytic proteolytic cleavage site near the C-terminus with the sequence of GDPH (glycine-aspartate-proline-histidine). The presence of this proteolytic cleavage site in the less glycosylated region of the protein suggests that the mucin gel matrix is destabilized by proteases.

Nongel-forming secreted mucins, MUC7, MUC8, and MUC9, do not appear to contain CK domains at the C-terminus. Based on available sequences, the nongel-forming mucins are smaller proteins of less than 1000 amino acids.

MUC7 is the most studied of these three mucins.<sup>77–83</sup> Similar to the gel-forming secreted mucins, the majority of the protein is comprised of tandem repeats: sites of heavily O-linked glycosylation. The N-terminal region of MUC7 also contains two cysteine residues. Two histatin-like domains are found at the N-terminal portion of the protein. Histatins are histidine-rich peptides frequently found in human saliva<sup>84</sup> and, given that the N-terminal peptides of MUC7 exhibit antimicrobial and antifungal activities, possibly play a biological role as part of the immune response.<sup>81,85</sup>

Detailed knowledge of the mucin gene family in other metazoans is currently unavailable partly because of the lack of complete genome sequences from key animal lineages. Lang and coworkers reported that the genome of the amphibian, *Xenopus tropicalis* (western clawed frog), contains 26 gel-forming mucin genes corresponding to MUC2, MUC5, and MUC19 orthologs.<sup>86</sup> While one of the major features of the human MUC2 and MUC5 proteins is the presence of CysD domains interspersed in the mucin domain, 15 *X. tropicalis* mucin proteins do not contain CysD domain. Furthermore, some of the MUC5 orthologs do not have the fourth VWD domain at the C-terminus, which is present in all mammalian and sauropsid MUC2 and MUC5 proteins. Based on limited genome sequence of *Danio rerio* (zebrafish), 11 gel-forming mucin genes were identified that are considered to be human MUC2, MUC5, and MUC19 orthologs. Using available genome and protein sequences, Lang and colleagues also identified putative gel-forming mucin genes in invertebrates, including members of Lophotrochozoa and Arthropoda clades, and even in lower metazoans such as the comb jelly *Pleurobrachia bachei* (sea gooseberry).<sup>86</sup> Many of these mucins contain the characteristic VWD and PTS domains,<sup>86</sup> including the well-characterized pig proteins MUCSAC and MUCSB. MUCSAC and MUCSB have very similar domain features. The major differences between the two proteins are the arrangement of PTS domains and tandem repeats, as well as intermixture of the CysD repeats in the mucin domain. Differential presentation of these domains in mucin proteins could influence the macrostructure of mucus. Immunohistochemical and lectin staining of airway epithelium in *Sus scrofa domesticus* (pig) revealed that MUCSAC mucins are secreted from the epithelial goblet cells, while MUCSB mucins are produced by the mucous cells in the submucosal glands. Nonetheless, mucus formed by these mucin proteins exhibited different structures, where MUCSAC forms mucus sheets and threads of 1 to 4  $\mu\text{m}$  in diameter, while MUCSB constitutes thick mucus strands of 5 to 50  $\mu\text{m}$  in diameter.<sup>87</sup> Although both MUCSAC and MUCSB are gel-forming mucins, the *in vivo* structural differences suggest that these proteins might play a role in influencing the final assembly and, potentially, composition of mucus. For example, the altered expression of MUCSAC and MUCSB in humans is implicated in asthma pathology.<sup>88</sup>

Many open questions remain regarding the evolutionary history of animal mucus. Mucins appeared early in multicellular organisms and are present in virtually all metazoans (Figure 2), leading to many theories on their origins. For example, some argue that mucin diversity arose as a result of gene duplication,<sup>11</sup> while others suggest mucins evolved from domain shuffling.<sup>89</sup> Both events quite possibly occurred to arrive at current genetic diversity, however it is difficult to determine the precise trajectory of mucin evolution, and to what extent gene duplication and domain shuffling may



**Figure 2.** Phylogenetic tree of the species described in this paper. Clades are colored according to the species' mucus function that is discussed herein, although it should be noted that each of these animals produces multiple mucuses with diverse functions and properties.

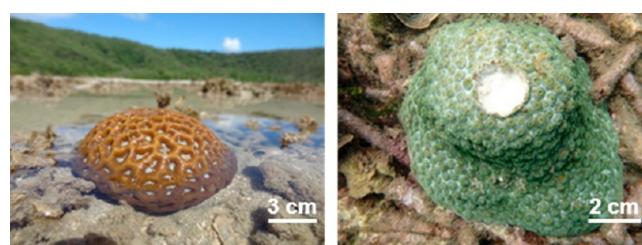
have played a role. In addition, mucin-like proteins have been found to function in *Saccharomyces cerevisiae* (yeast) cell adhesion, further complicating mucin evolutionary history.<sup>90</sup> Finally, studies into O-glycomes of *Salmo salar* (Atlantic salmon) revealed structural variation in skin mucin glycans between populations from different geographical regions, indicating differential glycosylation within a single species.<sup>91</sup> Thus, there are many unidentified drivers of mucin evolution that require further exploration in order to understand the genetic and functional diversity of modern mucuses.

Testing these hypotheses requires genetic analysis at multiple levels. Integrated investigation of mucus genomics, transcriptomics, proteomics, and glycomics could allow researchers to better understand the timing and nature of events that generated each mucin gene. Further, these analyses may reveal that synergistic interactions of peptide sequence and glycan composition are the driving forces behind mucus behavior, so methods are emerging to determine mucus structures at all levels. For example, quantifying the amount of mucin glycan and protein components is an instrumental first step. Experimentally, this comes from gel permeation chromatographic (molecular weight),<sup>92</sup> mass spectroscopic (glycan identification),<sup>93,94</sup> and transcriptomic/proteomic (sequencing)<sup>95</sup> analyses. In addition, rheology of natural mucus characterizes the viscoelastic material properties of mucus hydrogels.<sup>8</sup> However, even in concert, these methods are limited in that sequences of the repeat domains, glycan structures, and absolute configurations remain difficult to determine. As a result, the data reported on animal mucin structures vary widely and are difficult to compare. This lack of standardization makes structure–activity relationships elusive, thus obscuring our understanding of how different structural motifs lead to different properties.

Research into animal mucus and the questions being asked are as diverse as the mucus itself, and by understanding key factors contributing to mucin functional diversity, the versatility of mucus could be exploited in highly tunable advanced materials. Examples of secretions actively studied and the properties investigated include tissue-dependent proteomic immunology in oysters,<sup>96</sup> biomechanics and biochemistry of

slime in velvet worms,<sup>97</sup> venom transport in marine snails,<sup>98</sup> predator traps in slugs,<sup>99</sup> wet adhesion in tree frogs,<sup>100</sup> particle capturing networks in tunicates,<sup>21</sup> UV protection in corals,<sup>101</sup> bioluminescence<sup>102</sup> and tube construction<sup>103</sup> in marine worms, and fibrous network formation in hagfish slime.<sup>104</sup> Our goal here is to highlight recent advances by the groups who study these animal mucuses, and in doing so, illustrate the wide diversity of approaches used to investigate these fascinating matrixes. We thereby bring attention to the small but growing research field of comparative animal mucomics: the comparative study of mucus molecular structures, physical properties, and functions of mucuses across metazoans. The following sections are ordered by grouping the discussed organisms according to both evolutionary, ecological, and mucus material similarities.

**Cnidaria: Coral Mucus.** The Medina laboratory at Pennsylvania State University, United States, applies multiomics approaches to investigate the interactions between corals and their microbiomes. Reef-building corals are sessile animals that secrete vast amounts of mucus that perform many roles in coral biology, including UV protection, calcification creating a physical barrier to prevent desiccation, and as a means to overcome sediment load (Figure 3).<sup>20,105,106</sup> Additionally,



**Figure 3.** White, viscous mucus is found over the surface of entire coral colonies. Examples of the *Goniastrea aspera* (left) and *Montastraea cavernosa* (right) corals. The *M. cavernosa* colony has been cored for mucus microbiome-processing of the corals.

mucus is a key component of a coral's innate immune system,<sup>107</sup> where a symbiotic microbial community linked to the release of antimicrobial compounds assists as a first barrier of defense against predation.<sup>108</sup> Coral mucus often traps organic matter that, once released into the water column, acts as a conduit of nutrient recycling and is therefore essential in marine biogeochemical cycles.<sup>20,109,110</sup> The microbial actors supporting the movement of nutrients across the benthos are currently poorly characterized and deserve further study. Some of the recent interest in coral mucus microbiomes stems from the increased incidence of microbe-induced diseases.<sup>111</sup> Ecological factors such as shifts in herbivore communities, increased nutrient pollution, and bleaching affect coral mucus microbial communities, rendering them more vulnerable to pathogens.<sup>112–114</sup> Coral mucus microbiomes show specificity to some level of coevolution with particular hosts.<sup>115</sup> Additionally, coral mucus carbohydrate composition has been found to vary across species.<sup>116</sup> Understanding coral mucus has therefore important implications for ensuring the survival of tropical reef ecosystems for which corals are foundational species.

Mucins are critical components of coral mucus, and these proteins serve multiple functions.<sup>117</sup> Coral mucins and mucin-like proteins have been reported to have differential expression along a colony in *Acropora* species<sup>118,119</sup> hinting that these

glycoproteins may have a role in skeletal organization.<sup>120</sup> Studies into *A. digitifera* (staghorn coral) revealed that Mucin4-like protein, which is involved in skeletal matrix formation, shares multiple functional domains with human MUC4 and an *Aiptasia* (sea anemone) protein, as well as high sequence identity with *A. millepora* (staghorn coral) mucin-like protein.<sup>89,121</sup> In the Caribbean species *A. palmata* (elkhorn coral) and *A. cervicornis* (staghorn coral), MUCSAC was found to span three divergent genomic intervals, which could underlie differences in colony mucus composition.<sup>120</sup> Co-option and domain shuffling of mucins may have allowed this dual role in both protection (against desiccation, predators, and pathogens) and biominerilization.<sup>89</sup> Recent findings also suggest that some proteins, such as the transcription factor NF- $\kappa$ B, may regulate mucin expression in corals as part of their innate immune response.<sup>120</sup>

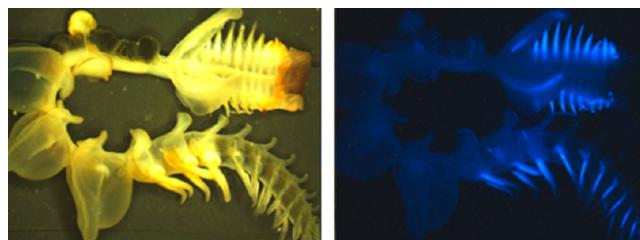
During a multiday heat stress experiment with the Caribbean corals *Pseudodiploria clivosa* and *Orbicella faveolata*, a mucin-like protein shows opposite differential gene expression in these two species. *P. clivosa* is a heat-sensitive species that shows downregulation of this protein in response to increasing temperature, while *O. faveolata*, a thermally tolerant species, upregulates the protein.<sup>122</sup> Also observed in these experiments is that another putative mucin, MUC3A, is upregulated in both species.<sup>122</sup> After acute thermal stress, *P. clivosa* shuts down many functions, including the expression of genes involved in the early immune response such as those which code for mucins. However, upregulation of MUC3A suggests again multiple possible roles for these glycoproteins. A search for these putative mucins in the genomes of three species encompassing the genus *Orbicella* complex (*O. faveolata*, *O. annularis*, and *O. franksi*) revealed multiple MUC3A paralogs in each lineage (M. Medina, unpublished). Mucins have also been recently reported as having a role in tissue regeneration after lesion in the Caribbean coral *Montastraea cavernosa*.<sup>123</sup> While the integumentary mucin-like protein increased in abundance 2–4 weeks after the lesion, mucin-5B showed a decrease in abundance, illustrating how poorly understood the role of mucins is in coral physiology. Coral mucus production is threatened as a result of decreasing coral cover worldwide. Given the critical role in nutrient cycling and host defense, better understanding of the makeup, structure, and function of coral mucus is paramount.

The establishment of related model organisms such as the jellyfish, *Cassiopea xamachana* (upside-down jellyfish), facilitates controlled experimentation to study cnidarian biology.<sup>124</sup> The recent discovery of motile stinging cell structures called cassiosomes that are released into the water column within *C. xamachana* mucus seem to play an important role in prey capture.<sup>125</sup> Cassiosomes are also found in other related jellyfish belonging to the taxon Rhizostomeae.<sup>125</sup> Characterizing the mucus microbiome of *C. xamachana* and its cassiosomes will enable further investigation of cnidarian mucus and its role in tropical marine environments. Characterization of the varied properties of coral and jellyfish mucus will lead to inspiration of similarly functioning materials, as these organisms naturally create hydrating agents, UV barriers, and microbe-regulating gels that function in tandem to allow corals to thrive in ever-changing conditions.

**Annelida: Tube Worm Mucus.** The Deheyn lab at the Scripps Institution of Oceanography at the University of California, San Diego, United States, specializes in the biochemistry of light production in marine organisms,

particularly polychaete marine worms that secrete a glowing mucus. Such organisms have long been reported by explorers and scientists alike,<sup>126</sup> yet with few rigorous studies detailing the biochemistry of light production.<sup>127</sup> This is a consequence of the difficulty in acquiring sufficient quantities of the precious secretion in water.<sup>128</sup> The Deheyn group studies two types of luminous mucus: the secretion of *Odontosyllis* worms in open water during reproduction, and one secreted by the seafloor-dwelling tube worm belonging to *Chaetopterus*. *Odontosyllis* species are also referred to as “fireworms” (not to be confused with the evolutionarily unrelated venomous “bearded fireworm”)<sup>129</sup> because these organisms launch an underwater “firework” display of glowing mucus as part of their mating ritual.<sup>127,129–131</sup> Fireworms produce bursts of this glowing mucus in the water column at very specific times of the solar and lunar cycles.<sup>132,133</sup> These flares are made of fireworm-secreted mucus that the female emitters release together with egg gametes, so that males waiting on the seafloor can easily locate potential fertilization sites. The males then quickly swim to these glowing puffs of light to release sperm within them.<sup>134</sup> In addition to being highly visible to mates, the mucus is more viscous than the surrounding seawater and therefore concentrates the gametes by limiting diffusion, increasing the chance of fertilization. The luminous mucus also provides a protective environment that persists long enough to enable the critical first steps of reproduction.

The *Chaetopterus* worm (Figure 4), in contrast, is sedentary and benthic, meaning that it lives on the seafloor where it



**Figure 4.** Tube worm *Chaetopterus dewysee* shown in white light (left panel) and exhibiting bioluminescence inside the appendages of the body that will be secreted as a glowing mucus (right panel).

makes a tube likely composed of a solidified mucus.<sup>103</sup> The tube consists of smooth concentric sheets of a woven, fiber-like material, leading to the organism's nickname: the “parchment tube worm”.<sup>103,135–137</sup> The tube itself has fascinating material properties, including rubber-like flexibility in water, and glass-like behavior when dehydrated.<sup>137</sup> Furthermore, the mechanical properties are unaffected by temperature changes from  $-50$  to  $200$   $^{\circ}$ C, and each layer of the tube is constructed from a parallel array of fibers that is oriented  $45^{\circ}$  from the main angular direction of the juxtaposing layer.<sup>103,137</sup> Being built in such an organized fashion provides the tube some anisotropy, increasing angular resistance to pulling or pressure forces, a trait found in well-engineered pipes.<sup>138</sup>

Intriguingly, this same organism also produces an adhesive luminescent mucus that glows bright blue (Figure 4).<sup>102,139,140</sup> This adhesive mucus is spat by the animal and could adhere to attacking predators, suggesting that this mucus may have evolved for antipredatory functions. The stickiness of this mucus is clearly related to its chemical composition, which is made of various glycoproteins.<sup>141</sup> The mucus shows ferning patterns when drying, suggesting it contains mucins; however,

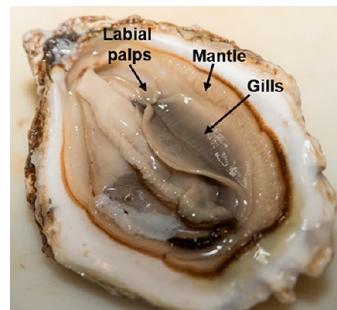
there are no reported peptide sequences of *Chaetopterus* mucus proteins currently available.<sup>142</sup> We also know that this mucus presents rheological and microrheological properties similar to other mucuses,<sup>140,143</sup> but with unique properties related to the content of ferritin/flavin complex, iron, and an unknown secreted chromophore. With regards to mucus function, any assailant of the worm is tagged with a visible glowing mark, likely making hunters more vulnerable to their own predators for extended periods of time.<sup>141,144</sup> This mucus results in a remote and prolonged defense using a unique light-producing system fueled by a highly performant ferritin, which is exceptional among luminous marine organisms. The Deheyn group has identified that the ferritin can build redox potential by coupling oxidoreduction reactions of iron with flavins,<sup>102,141</sup> which releases electrons to power the unidentified luminescent chromophore. Tube worm ferritin shares high sequence identity with human ferritin, but exhibits redox properties with nearly an order of magnitude increase in catalytic efficiency.<sup>142</sup>

Future efforts to understand the structures and properties of these tube worm mucuses will require interdisciplinary efforts that bring together researchers from ecology, biochemistry, and material science. Active studies into these substances focus on the hierarchical nature of the mucuses, which exhibit distinct functionality dependent on the scale in question of the material.<sup>135,136,143,145,146</sup> Further investigation into the genomics of these organisms' secretions will improve our understanding of mucin evolutionary history, providing insight as to if mucus, like bioluminescence, is a convergent phenomenon.<sup>147</sup> Comparative studies of secretions produced by other annelids will shed light on the underlying molecular basis of the material properties of marine worm mucus.<sup>148</sup> Questions currently being addressed in these studies include: How do structures of varying mucuses fluctuate to change properties so dramatically? And, do the structures of their mucins fundamentally differ? Or do intermolecular interactions between the various components of the mucuses drive function in these animals? Investigation into the molecular nature of the solidifying and luminescent tube worm mucuses will be the next step in answering these questions, with the aim of leading scientists toward leveraging these systems to develop novel materials that can act as both environmentally responsive sensors and biological cements. Tube worm-inspired materials can be used as self-patching surfaces, glues in marine environments, and contact-reporting dyes.

**Mollusca: Bivalve Mollusk Mucus.** Faculty at the Marine Animal Disease Laboratory (MADL) at Stony Brook University (Allam and Pales Espinosa), United States, investigate bivalve immunology, with a particular emphasis on how these organisms use differential protein expression to regulate interactions with environmental particulates and microbes. With about 200 000 described (and likely many more undescribed) extant species, the Mollusca is the second largest major bilaterian group after the arthropods.<sup>149</sup> Gastropods and bivalves represent the largest two subtaxa, encompassing 98% of the known living molluscan species.<sup>150</sup> Mollusks, and suspension-feeding bivalves in particular (e.g., clams, oysters and mussels), also represent an important source of food and valuable goods (shells, pearls) around the world, with over 17 million tons captured or produced from seas or inland waters worldwide, accounting for over 30 billion USD in economic activity annually.<sup>151</sup> In addition to their economic value, suspension-feeding bivalves are among the most

important foundation species in coastal waters as they build habitats for other species, remove phytoplankton, and transfer energy to the benthos.<sup>152</sup> The biology, ecology, and economic importance of bivalve mollusks makes them ideal candidates for investigations targeting critical basic and applied research questions, including those pertaining to health and industry.<sup>153</sup> The soft tissue of these animals is covered with copious mucus secretions that have a role in multiple functions, including protection from biological and environmental stressors, as well as mediation of interactions with waterborne microbes.<sup>154–157</sup> The importance of mucus in molluscan biology is well-reflected in the energy allocated to mucus production, sometimes exceeding 15% of energy gained from food.<sup>158</sup> Their mucosal tissues are also readily accessible both for *in vivo* observation<sup>159</sup> and sampling,<sup>160</sup> making them ideal candidates for the investigation of mucosal processes.

These animals have the intriguing ability to sort their food from a complex mix of particles suspended in water by using the mucus as a semipermeable filter. To do so, they pump and circulate water in the shell (pallial) cavity, then use mucus covering their feeding organs (gills, labial palps) to capture and selectively transport food particles to be ingested or rejected using a "conveyor belt" made with mucus (Figure 5).<sup>161–164</sup>



**Figure 5.** American oyster *Crassostrea virginica* with the right shell removed. Major mucus-producing tissues are noted. Viscous mucus covers all tissues inside the oyster.

Particles rejected before reaching the mouth are embedded in mucus and expelled back to the environment as masses of mucoid substances entangling live unwanted cells, debris, and abiotic material of low nutritional value. Those directed to the mouth are ingested in a cohesive mucus string. How these animals discriminate and sort food particles has been the subject of dozens of studies since the early 1900s, although the underlying mechanisms remained elusive until recently, as the past decade revealed the critical role of mucus in all of these processes.<sup>164–167</sup>

Recent joint investigations between the Pales Espinosa and Allam laboratories combining proteomics, transcriptomics, and reverse genetics showed that mucus covering the feeding organs is not a mere carrier for particles but that specific interactions take place between mucus and food particles. The researchers demonstrated that mucus covering the feeding organs of oysters and mussels contains sugar-binding proteins (i.e., lectins) that differentially bind microalga cell surface carbohydrates, triggering selection with a preference for glucose and mannose residues.<sup>165–168</sup> Pales Espinosa and coworkers also demonstrated that mucosal lectins are necessary for food particle selection in the oyster, *Crassostrea virginica*.<sup>164</sup> Some of the most important open research questions regarding these processes include: Do mucosal

lectins mediate an efficient particle-sorting mechanism that is common across all suspension feeders? What is the nature of the interactions between bivalve lectins and mucins? And how do these animals control mucus characteristics to regulate food uptake?

The primary role of mucosal immunity in maintaining animal health is now well-recognized in vertebrates.<sup>169</sup> The net created by cross-linked glycoproteins contained in mucus traps microorganisms before reaching the soft tissues. In addition to representing an efficient physical barrier, mucus matrixes contain various cells and bioactive molecules and have gained prominence in the last few decades as an essential component of the innate and acquired immune system. Suspension-feeding bivalves are excellent model organisms for investigating host–microbe interactions at mucosal interfaces, in part, given the extraordinarily large number of microbes (~25 million microbes/second) they encounter via their water filtering activities.<sup>170</sup> In these animals, the mucus layer covering soft molluscan tissues is the first host factor encountered by waterborne, soft tissue-attaching microbes regardless of whether it leads to predation, mutualism, commensalism or parasitism.<sup>171–175</sup> Therefore, the outcome of interactions between waterborne microbes and pallial mucus can determine the success or failure of these associations.

In this context, MADL researchers investigate the role of mucosal interfaces in bivalve innate immunity and defense against pathogens. Investigations on *C. virginica* (Atlantic oyster) showed significant regulation of the proliferation and virulence of the alveolate parasite *Perkinsus marinus* following exposure to host mucus.<sup>96,175,176</sup> While mucus collected from oyster pallial organs enhanced the proliferation of the parasite, mucus collected from the digestive gland was inhibitory. Interestingly, pallial mucus of the noncompatible host *C. gigas* (Pacific oyster) was strongly inhibitory suggesting that host specificity of *P. marinus* may begin in the mucus.<sup>176</sup> The exact regulatory nature of the mucosal molecules and how these factors are regulated in response to environmental or pathologic stress remain to be determined. Additionally, comparative investigations into each species' mucus could provide insights into the structure–property relationships in microbiome-regulating materials.

Bivalves are an excellent system for understanding the role of mucosal microbial communities in animal health given the interplay between mutualistic, commensal, and pathogenic microbes at mucosal interfaces. A growing body of evidence highlights the role of mucosal microbiomes in regulating host resistance to infection either directly through microbe–microbe interactions (e.g., “non-host-derived immunity”)<sup>177</sup> or indirectly via immune stimulation and maturation.<sup>178</sup> One such example is the presence of IgGFc-binding proteins in *C. virginica* mucus,<sup>179</sup> a human IgGFc-binding protein found on mucosal surfaces (FcγBP) contains a mucin-like cysteine-rich domain as well as an amino acid motif conserved in MUC2.<sup>180</sup> How mucus interacts with microbes (whether beneficial, commensal, or harmful) and how changes in mucus physicochemical characteristics (either caused by disease, by other microbes, or by natural cycles) affect microbial homeostasis at mucosal surfaces are among the many questions that still need to be addressed, and doing so could lead to better disease management strategies and improvements in state-of-the-art biomimetic materials. These studies raise fascinating questions around host–microbe crosstalk and feedback controls, and studies into the molecular nature of

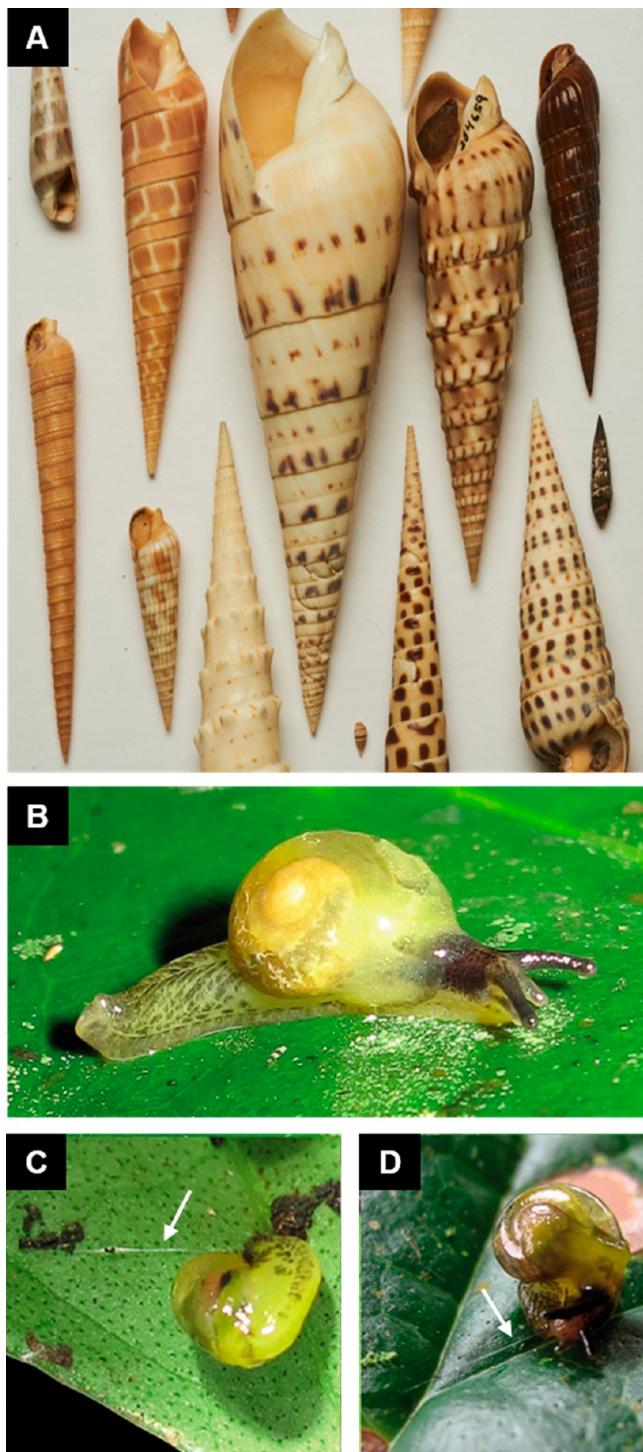
bivalve mucus may lead to powerful insights in the development of barrier technologies. Advances in this area can bring about new technologies in terms of bacteria- or particle-selecting filters for commercial and research applications as well as microfluidics mobility agents.

### Mollusca, Gastropoda: Marine and Terrestrial Snail Mucus.

The Holford laboratory at Hunter College, City University of New York, United States, examines the evolution and the potential therapeutic applications of marine snail mucus and venom peptides from Conoideans, while the Barrientos group at Universidad Estatal a Distancia, Costa Rica, specializes in the ecology of tropical land snails. Snails secrete a variety of mucoses for strong adhesion to both dry and wet surfaces, as a potent lubricating and hydrating agent, and also as a protective barrier.<sup>181</sup> Snail mucus protects their skin against cuts, bacteria, and UV radiation through a combination of antimicrobial peptides and glycoproteins.<sup>181</sup> Most of the mucins found in gastropods exhibit excellent antimicrobial activity against various microorganisms.<sup>98</sup> Snail mucus is an attractive area of study because of the increased focus on developing alternative treatments for antibiotic-resistant bacteria.<sup>98</sup> Additionally, the development of new technologies allows these mucins to be examined at the level of detail required to use their designs in future functional materials.<sup>181</sup>

Conoidea, made up of Conidae, Terebridae, and Turridae snails, includes species that produce and secrete very complex venoms, with thousands of unique toxin and mucin peptides (Figure 6A).<sup>182,183</sup> The venom peptide toxins found in conoideans have evolved over millions of years to rapidly and effectively disrupt macromolecular functions in their prey by manipulating important physiologically relevant drug targets such as G-protein coupled receptors, ion channels, enzymes, receptors and transporters.<sup>182,184,185</sup> The first conoidean commercially available therapeutic, ziconotide (Prialt), is a nonaddictive, nonopiod analgesic peptide, isolated from the venom of *Conus magus* (magical cone snail) that is used to treat chronic pain in cancer patients.<sup>186</sup> Ziconotide opened the floodgates for the therapeutic development of snail venom peptides, however, an equally promising, but less explored avenue is the potential application of conoidean snail mucins. The Holford lab has leveraged omics technologies (genomics, transcriptomics, and proteomics) to advance the discovery of venom peptides from previously neglected venomous animals such as terebrids.<sup>182,185</sup> The group currently seeks to apply this general omics approach to identify conoidean mucus and investigate if there is an evolutionary pattern that can be used to determine mucin molecular function.

The Barrientos laboratory specializes in the ecology of tropical land snails and is currently focused on investigating *Tikoconus costarricanus*, a tiny snail that lives in Costa Rica's forests (Figure 6B).<sup>187</sup> This recently described snail species has a “caudal gland” on the dorsal side of the foot, and the snail uses mucus secreted from this gland to hang upside down during aestivation to avoid dehydration under leaf cover (Figure 6C). When abandoning the inverted position, a thread of mucus is formed between the caudal gland and the leaf surface (Figure 6D). In some cases, the thread becomes so tough that the snail cannot break it through tensile force alone, and it must use its mouth to break the tether. It is possible that this land snail, like many others, produces several types of mucus that function in surface adhesion, locomotion, lubrication, and hydration.<sup>181</sup> The lab's efforts aim to answer



**Figure 6.** Marine and terrestrial snails. (A) Assortment of shells from terebrid marine snails. Image credit Robert Clark, National Geographic. (B) *Tikoconus costarricanus* sliding on mucus. (C) Same species aestivating in a bat-like position, hanging from the underside of a leaf with the assistance of adhesive mucus. White arrow points to mucus strand. (D) Snail using the radular teeth to cut the mucus thread between the caudal gland and the leaf. White arrow points to mucus strand.

questions related to the molecular composition of and differences between the snail's multiple mucuses, the ability of the caudal mucus to be drawn into tensile fibers, and the reversibility of the mucus's phase transitions.

As mentioned above, mucuses from snails are relatively unexplored. There are several areas in which discovery-driven research targeted on identifying genetic phenotypes that elucidate functional activity of mucins would lead to transdisciplinary breakthroughs. For example, the increase of antibiotic-resistant bacteria is a growing threat that requires the use of new therapeutics and mucins are a resource for finding potentially new antimicrobial compounds.<sup>98</sup> Additionally, a recent study of several Giant African snail genomes (*Achatina* spp.) identified 99 mucin genes in *A. immaculata* and 71 in *A. fulica* that may have roles in immunity, water retention, and wound healing, underscoring the need to investigate gastropod mucus further.<sup>188</sup> By applying a systems-wide omics approach to the discovery and characterization of mucins across the tree of life, we can establish a repository of information for how genes have evolved over time and how functionalization and novelty arise. This information is at the heart of scientific questions ranging from evolutionary biology to cellular physiology. The Holford and Barrientos laboratories have only scratched the surface of snail mucin and peptide discovery, and it is astonishing to consider that in the secretions of a marine or terrestrial snails we can find answers to how and why venoms and mucuses evolved; treatments for infections,<sup>189,190</sup> cancer,<sup>191,192</sup> pain,<sup>193,194</sup> and a host of other human diseases and disorders; and inspiration for adhesive, lubricating, and tensile materials.

**Mollusca, Gastropoda: Red Triangle Slug Mucus.** The team of Gould at the University of Newcastle, Australia investigates the behavior, evolution, and natural history of Australia's diverse and unique wildlife.<sup>195,196</sup> The red triangle slug, *Triboniophorus graeffei*,<sup>197</sup> is Australia's largest and arguably most striking terrestrial slug.<sup>198,199</sup> While the body of the slug is ghostly white, the base (or foot) is skirted by a thin band of intense vermillion, with a triangular mantle that is also skirted by the same intense pigmentation which gives this species its name. Like many of the Australian terrestrial mollusks, most aspects of *T. graeffei*'s ecology remain poorly described, which is surprising given that it can be found in forest systems up and down the east coast of the continent. This could perhaps be attributed to its elusive nature, as it is often only observed during rainy periods, when it comes out of hiding to feed on algae growing on the exterior of smooth-barked eucalypts. Gould's team has discovered that *T. graeffei* produces an adhesive mucus when disturbed.<sup>99</sup>

The discovery of *T. graeffei*'s extraordinary secretory ability was made by chance while conducting fieldwork in the Watagan Mountains in New South Wales. On one particular night of fieldwork, an adult red-eyed green tree frog, *Litoria chloris*, was found immobile on the side of a fallen eucalyptus branch in close proximity to a large *T. graeffei* specimen (Figure 7). On closer inspection, the ventral skin surfaces of the frog were found to be adhered to the branch and surrounded by mucus, with the toe pads and webbing of the front and back legs adhered to each other and to the branch as well. Given the close proximity of the frog to the slug, the Gould team speculated that it had become ensnared in the slug's mucus secretions. They subsequently tested this hypothesis by examining the secretions of multiple *T. graeffei* specimens under laboratory conditions and found that an adhesive was secreted in regions of dorsum that were mechanically stimulated, providing evidence that the frog was indeed trapped in *T. graeffei* mucus.



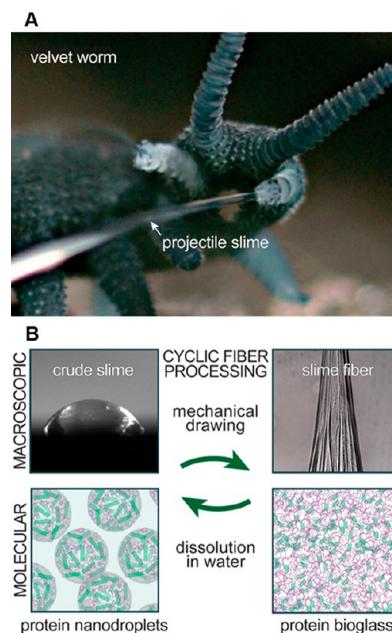
**Figure 7.** Adult *Litoria chloris* frog adhered to a eucalyptus branch in close proximity to a *Triboniophorus graeffei* slug (light gray mass, lower right). The white arrow points to the slug's adhesive mucus.

Mechanical stimulation of the *T. graeffei* dorsum results in contractions in the immediate area and the rapid development of mucus droplets, which then coalesce and spread out over the surrounding surface. Upon secretion, this mucus is initially wet and translucent, but rapidly forms a thick, sticky, and opaque mass. It has been proposed that this adhesive mucus is a defense against predation, causing predators to stick to themselves or their immediate surroundings and thereby preventing them from successfully finalizing an attack. This adaptive function of the mucus has been observed in the field under natural conditions (as stated above), with a potential predator being found adhered to surrounding vegetation upon contact with the mucus, and for an extended period of time. Given these findings, it is likely that this adhesive serves to incapacitate predators, possibly to allow the slug to make its getaway. However, the aforementioned data is the first reporting of the red triangle slug's adhesive mucus,<sup>99</sup> and thus investigations into the underlying molecular composition are still needed.

The production of defense adhesives has been recorded for at least two unrelated species of terrestrial slugs.<sup>200–202</sup> However, it has not been recorded among Australian forms. While the natural predators of *T. graeffei* remain unknown, amphibians have been reported to predate on slugs,<sup>203,204</sup> suggesting that the aforementioned observations in the wild are the first showing the antipredatory function of this mucus for slugs. What continues to remain a mystery is the mechanism that allows individual slugs to remain unadhered to their own secretions upon their release, as opposed to predators which appear to become quickly trapped and possibly for days. A strong bioadhesive is a valuable form of defense, particularly for slugs which are slow moving and lacking any protective shell. Interestingly, this study by Gould and coworkers<sup>99</sup> indicates that the adhesive property of *T. graeffei* defense mucus is reactivated upon hydration, making it potentially useful in the development of biological glues. A precedent for the economic value of these properties of slug mucus has been set by the recent development of a biological adhesive based on studies of the secretions in a different species, *Arion subfuscus*.<sup>205</sup> Interestingly, it has been suggested this model organism produces glues based on double network hydrogels,<sup>202,206–208</sup> consisting of distinct stiff and deformable polymer networks linked by sulfated polysaccharides and divalent metal ions.<sup>209,210</sup> Because the biochemical makeup of mucus varies between species,<sup>211,212</sup> there is an exciting opportunity to exploit the specific adhesive and water-activated

properties of *T. graeffei*'s defense mucus to create new bioinspired materials.

**Onychophora: Velvet Worm Slime.** The Mayer (University of Kassel, Germany), Schmidt (Heinrich Heine University Düsseldorf, Germany), Harrington (McGill University, Canada), and Monge-Nájera (Universidad de Costa Rica, Costa Rica) groups study the biochemical and biomechanical properties of the adhesive slime launched from onychophorans, or velvet worms (Figure 8, top).



**Figure 8.** (A) *Euperipatoides rowelli* during slime ejection. (B) Fiber formation in velvet worm prey capture slime. Cyclic fiber processing is triggered by mechanical impact at macroscopic and molecular levels. At the macroscale, the crude slime instantly forms a stiff fiber via compressing and drawing. In water, the fiber returns back into the fluid slime state. At the molecular level,  $\beta$ -sheet containing proteins self-assemble into nanodroplets which aggregate into a gel-like state by shear forces. When further stressed, the nanodroplets disrupt and the stored proteins unfold and cross-link into the stiff and glassy fiber. Fibrillated proteins dissolve in water and reassemble back into the storage nanodroplets (via coacervation). Images adapted with permission from ref 228. Copyright 2019 American Chemical Society.

Onychophorans comprise a phylogenetically ancient group of soft-bodied, terrestrial invertebrates that originated 600–540 million years ago.<sup>213,214</sup> Approximately 200 extant species of both major velvet worm subgroups, the Peripatidae and the Peripatopsidae, have been described, and they mainly live in temperate and tropical forests of the southern hemisphere.<sup>215,216</sup> Within their humid microhabitats—mostly decaying wood and leaf litter—they implement a fascinating strategy for capturing prey and defending themselves against predation. The velvet worms shoot out a mucus-like slime (Figure 8, top) onto their prey to entrap it before consuming it.<sup>22,217–226</sup> This projectile glue is strong enough to immobilize even the powerful legs of a cricket.<sup>22</sup> To achieve this feat, velvet worm slime exhibits several remarkable qualities. When the fluid slime is mechanically stimulated (e.g., via compression, agitation, or shearing), it converts immediately into a gel, which is adhesive even in humid environments and underwater. In this activated state, the slime can be rapidly drawn into load-bearing fibers. These stiff fibers are

presumably adapted to resist escape attempts of trapped insects. Remarkably, this mechanoresponsive transformation process is fully reversible as the drawn material reverts to the original fluid state when dissolved in water, from which new fibers can be generated through drawing (Figure 8, bottom).<sup>97,216,223,227–234</sup> Onychophoran slime proteins exhibit some structural distinctions from mucin, as protein secondary structure and divalent ions are more prominent in this slime.

The Monge-Nájera group has studied this exotic creature for nearly 40 years, and has made significant contributions to understanding of onychophoran natural history, behavior, and biomechanics, having discovered many species of velvet worm.<sup>235–237</sup> Recently, his group elucidated the mechanics of the velvet worm's oscillatory slime ballistics,<sup>218</sup> and also found that the adhesive slime is used as a food source for young worms.<sup>238</sup> Working collaboratively the groups of Mayer, Schmidt, and Harrington have investigated the slime of the Australian velvet worm *Euperipatoides rowelli* to reveal the physical and biochemical principles behind its reversible fiber formation. An initial multiscale, structural and compositional investigation provided the first clear evidence of the molecular and nanoscale origins of fibers assembly.<sup>216</sup> The slime is comprised of nearly monodisperse protein-based nanodroplets (diameter ~100 nm), which appear to be stabilized by noncovalent electrostatic interactions between charged domains of the dominant protein building blocks that likely possess  $\beta$ -crystalline structure,<sup>229</sup> and positively charged divalent cations in the slime (e.g.,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ).<sup>228</sup> Electrostatic repulsion between the nanodroplets, which carry a weak positive surface charge, prevents the premature aggregation of proteins into a gel-like network and keeps the slime in the fluid storage state. However, when agitated, nanodroplets are forced into contact and the nanodroplets aggregate, forming an activated gel phase, which can then be further transformed into stiff fibers when drawn and dried. Unlike many other natural fibers, velvet worm fiber proteins do not exhibit a preferred orientation along the fiber axis and are linked only by noncovalent interactions. This accounts for the reversibility of the fiber formation process—fibers can be dissolved in water and regenerated by drawing.<sup>97,216</sup>

Biomechanical and physicochemical analyses of the last 20 years have provided wide-ranging insights into the properties and complex functionalities of velvet worm prey capture slime.<sup>97,216,227,228,230,231</sup> However, there are a number of open questions, which must be answered to understand the molecular principles in this material required for transferring the lessons of velvet worm slime into synthetic systems. For example, only a small fraction of the total number of slime proteins has been identified and characterized. To understand the function of the proteins and their potential role in fiber formation, complete sequences and post-translational modifications of the key proteins implicated in the process are required. Additionally, the potential functions of lipid, carbohydrate, and ion components of the slime need to be further assessed. Thus far, structure–function analyses were primarily performed only on a single velvet worm species: the peripatopsid, *E. rowelli*. A comparison between representatives from both major onychophoran subgroups, the Peripatidae and the Peripatopsidae, will be highly relevant to discover the entire range of fiber formation strategies of the velvet worms. These efforts will allow us to mimic the material in a simplified synthetic model that could be used as reversible surgical adhesives and structural materials in ionic environments. In

addition, the analysis of slime ejection<sup>218</sup> could be used as inspiration for oscillatory microfluidics systems.

**Chordata, Tunicata: Appendicularian Mucus.** The Thompson laboratory at the University of Bergen, Norway, studies the molecular ecology of tunicates (or urochordates), the marine organisms comprising the closest living relatives to vertebrates.<sup>238</sup> Tunicates (comprised of Ascidiacea, Appendicularia, and Thalicea) are animals partially or completely enclosed in either mucus “houses” (Appendicularians) or “tunics” (Ascidiaceans, Thaliceans). The filter-feeding house secreted by appendicularians (also called larvaceans) is among the most complex extracellular structures constructed by any organism.<sup>240–242</sup> These structures feature complexity in their architecture, consisting of physically and functionally distinct inner and outer layers, and can extend to lengths of over one meter, nearly 100 times the length of the animal itself (Figure 9). The resulting so-called house allows appendicularians to



**Figure 9.** Tunicate *Oikopleura dioica* (in yellow at center) in its mucus-coated filter-feeding house. The house captures floating organic matter, and filter sets concentrate particles toward the animal's mouth in the center of the image.

exploit a wide range of food particles, including nanoplankton and submicrometer colloids, establishing them as important, abundant components of marine zooplankton communities. The Thompson group focuses on *Oikopleura dioica* (Figure 9), a coastal marine appendicularian with a pan-global distribution. This species is noted for rapid expansion of population size in response to algal blooms and, to maintain sufficient filtration rates, it synthesizes an entirely new house (15% of its total body carbon) every 3–4 h. These discarded filter-feeding houses are a major component of marine snow and have significant, sometimes dominant, roles in vertical carbon flux cycles.<sup>243</sup>

The oikopleurid house is built on a scaffold of cellulose microfibrils<sup>244,245</sup> and associated house proteins (oikosins).<sup>240</sup>

Oikosins generally lack identity with known proteins but do share architectural similarities with mucins. Phylogenetic analyses indicate that a single lateral gene transfer event from a prokaryote at the base of the lineage conferred cellulose biosynthetic capacity in tunicates.<sup>244</sup> Despite the common tunicate strategy of extracellular mucus filter-feeding structures, the Thompson group has shown that the proteome of the *Oikopleura* house has little in common with the proteome of the sister group, the ascidian, *Ciona* tunics. Of the now 80 identified oikosins, about half lack domain modules or similarity to known proteins, suggesting *de novo* appearance in appendicularians.

The oikoplastic epithelium, a monolayer of cells covering the trunk of the animal, is responsible for secretion of the house. Expression patterns revealed that individual oikosins are produced from specific fields of cells within the oikoplastic epithelium, but in some cases migrate up to at least 20 cell diameters in extracellular space to combine in defined house structures. Among the oikosins, Oikosin1 has 13 repeats of a Cys-domain, a subunit of repeating sequences, also present in some vertebrate mucins. One such repeat of this Cys-domain is also found in human cartilage intermediate layer protein (CILP), but no evidence of this domain in any other invertebrate species has been found. Oikosin1 is produced in an intermediate zone between the anterior and posterior mesh zones of the food-concentrating filter. In this respect, the weak sequence homology with human CILP is intriguing, as CILP is found only in cartilage in an intermediate layer between collagen mats.<sup>246</sup> The high concentration of CILP in rib cartilage compared to low levels in tracheal cartilage has been interpreted to suggest that compressive load is a factor in controlling the tissue distribution of this protein. The fact that CILP is restricted to an interterritorial zone indicates that this protein has a structural rather than regulatory role, and it is known that the expression of CILP is increased during the early stages of osteoarthritis.<sup>247</sup> In this context, Oikosin1 may be an interesting example of recruitment of the Cys-domain found in mucins for related structural purposes in very different functional settings. Further characterization of the mucin-homologous region of Oikosin1 will bolster mucin structure–function analysis, strengthening the design rules generated for biomimetic materials.

Though all tunicates employ extracellular matrixes founded on a cellulose scaffold, they have evolved quite different protein compositions that build large multiplexed structures. Though tunicates employ a common cellulose building block, they have been innovative in incorporating various structural domains into original extracellular proteins for specific architectural solutions in the three main urochordate lineages. The *Oikopleura* house offers a tractable model to investigate how proteins evolved in different eras. In this system, roughly 100 distinct proteins have combined and diversified to create a complex extracellular structure essential to filter feeding. Studying these proteins will help to better understand the architecture in similar mineralized biosystems, such as those in corals and jellyfish. Additionally, the *de novo* appearance of the numerous oikosins provides a large unexplored space to investigate the structural basis of these unique mucus proteins. Greater understanding of tunicate houses can lead to the development of underwater filtration systems to improve the longevity of aquatic vehicles and machinery.

**Chordata, Cyclostomata: Hagfish Mucus.** The Fudge Laboratory at Chapman University, United States, studies

hagfishes in contexts ranging from their ecology and evolution, to their cellular and organismal behavior, to the biochemical properties of their natural defenses. Hagfishes are marine, bottom-dwelling animals known for their burrowing behavior,<sup>248</sup> their recycling of organic matter in the world's oceans,<sup>249</sup> and, most strikingly, their ability to secrete enormous amounts of slime when they are provoked (Figure 10).<sup>104,250,251</sup> There are currently 82 hagfish species known



**Figure 10.** Pacific hagfish, *Eptatretus stoutii* (left panel), and hagfish defensive slime (right panel).

worldwide of which 48 species fall within the taxon *Eptatretus*.<sup>252</sup> One of the most widely studied species, the Pacific hagfish (*E. stoutii*), is found at depths of 15–800 m and is distributed in Pacific waters stretching from Mexico to southern Alaska (Figure 10, left).

Hagfish slime is secreted as a defense mechanism to discourage attacks from gill-breathing predators, such as other fishes.<sup>253,254</sup> When a hagfish is attacked, approximately 100 mg of white, viscous exudate is ejected from several of its numerous slime glands.<sup>104</sup> This amount of exudate, after mixing with seawater, is capable of forming about one liter of slime in 100–400 ms (Figure 10, right).<sup>104</sup> This exudate contains two main components: skeins and mucous vesicles.<sup>255,256</sup> Each skein is an intricately coiled bundle of a silk-like thread consisting of intermediate filament family cytoskeletal proteins.<sup>257,258</sup> When deployed in seawater, skein unraveling occurs alongside mucous vesicles. These vesicles contain mucous glycoproteins, which, working synergistically with the unraveled threads, provide remarkable strength to the slime network.<sup>259</sup> Vesicle deployment involves swelling of condensed glycoproteins and their transformation into a vast mucous network that interpenetrates the network of slime threads from the skeins.<sup>260</sup>

One way of understanding the large volumes of slime that hagfishes can produce is by recognizing that the slime is remarkably dilute, with the dry weight of mucus and thread components being only 15 and 20 mg mL<sup>-1</sup>, respectively.<sup>104</sup> The dilute nature of hagfish slime can be further understood as a consequence of the fact that the slime does not bind seawater as much as it traps it, which is supported by the fact that substantial volumes of seawater drain out of the slime when it is subjected to a pressure gradient, e.g. when it is pulled out of water into air.<sup>104</sup>

The glycoproteins that make up the mucous component of the slime remain mostly uncharacterized. Salo et al. showed that the mucous fraction of *E. stoutii* slime contains 77% protein, 12% carbohydrate, 6% sulfate, and 5% lipid on a dry weight basis.<sup>259</sup> The amino acid composition revealed some

resemblance to mucin glycoproteins but also differed in many aspects such as proline, sulfate, and carbohydrate content in addition to the ratio of neutral to amino sugars. Characterizing the glycoproteins (i.e., the protein backbone and the attached glycans) that make up the mucous component of hagfish slime will shed further light on the biophysics of mucus vesicle deployment, the interactions between slime threads and mucus, and the rules that govern mucus glycoprotein properties in other organisms.

The Fudge Laboratory continues to investigate hagfish slime, with recent work focusing on the biophysics of slime deployment, molecular mechanisms of slime production, and biomimetic applications. Hagfish slime differs from other mucus secretions in that it is reinforced with high-aspect ratio silk-like fibers, which imbue the slime with unique biomechanical properties and make it fiendishly effective at clogging the gills of would-be fish predators. In the past few years, the group has begun producing materials possessing physical properties that resemble hagfish slime. They anticipate that hagfish slime mimics, once produced, could have uses in a diverse array of consumer and industrial products and applications, including naval defense and absorbent technologies.

**Tetrapoda, Lissamphibia: Tree Frog Mucus.** The Barnes group at the University of Glasgow, Scotland, studies the physicochemical basis of tree frog adhesion and uses their discoveries to guide the design of biomimetic materials. Tree frogs are mainly found in the tropics and are well-adapted to living in trees.<sup>261</sup> Their main adaptation for climbing is their possession of adhesive toe pads at the end of each digit, and pad-like structures (subarticular tubercles) located more proximally on the ventral surface of the digits. Like all frogs they respire through their skin despite possessing lungs. This means that their skin must be kept moist to allow gaseous exchange, something that is achieved by mucus secreted by subdermal mucus glands.<sup>262</sup> Mucus also plays an important role in adhesion, in that there is a mucus-filled joint between the toe pad and the structure (leaf, branch) to which the frog is adhering.<sup>263</sup> Here, capillary forces, generated by the meniscus surrounding each toe pad (and subarticular tubercle), are thought to play an important role in the tree frog's adhesive mechanism, allowing them to climb inclined and vertical surfaces (Figure 11).<sup>264</sup> Additionally, the Barnes Lab's experiments show that such a mechanism (known as "wet adhesion") allows frogs to climb overhanging surfaces, but, unlike geckos, tree frogs are not able to "walk on the ceiling".<sup>265</sup>

In a recent paper, Langowski and coworkers show that the ventral digital mucus glands, whose ducts end in the toe pads, form distinct clusters that differ in their morphology from regular anuran mucus glands.<sup>266</sup> However, a chemical analysis, based mainly on cryo-histochemical techniques, failed to identify clear-cut differences between ventral and other mucus glands and between the chemistry of the mucus from climbing and nonclimbing frog species. Interestingly, recent work on the chemistry of the mucus has shown that tree frogs can, for instance, exert capillary forces that allow adhesion to the surfaces of hydrophobic leaves. This is because their mucus contains molecules such as carboxylic acids that act as surfactants, lowering contact angles to levels where capillarity can occur (<90°).<sup>267</sup>

Adhesion in climbing animals is dynamic. It must be reversible but strong enough to support the weight of the



**Figure 11.** Australian green tree frog, *Litoria caerulea*, adhering to a vertical glass rod. Images adapted with permission from ref 263. Copyright 2018 Journal of Experimental Biology.

animal.<sup>268</sup> Indeed effective climbing requires friction as well as adhesion.<sup>265</sup> There must also be mechanisms for self-cleaning and adhering only when required.<sup>269,270</sup> Understanding such mechanisms involves precise measurement of the forces generated by single toe pads. This involved the design and construction of miniature two and three-dimensional force transducers.<sup>271</sup> Studies of toe pad structure and physical properties involving both electron microscopy techniques and microindentation are also essential.<sup>100,272</sup> Tree frog adhesion is complicated, and many questions remain unresolved. These include a better understanding of how toe pads actually adhere. In addition to capillary forces, there is good evidence for involvement of viscosity-dependent hydrodynamic forces and possibly van der Waals forces, since Barnes's recent research shows that contact between the tips of the nanopillars that cover each epithelial cell becomes extremely close as tree frogs, tilted on a microscope stage, adjust their pads to prevent sliding or falling.

Because frogs stick to wet surfaces and can repeatedly stick and detach their sticky pads every time they take a step, there is potential for using tree frog adhesion as inspiration for new adhesives that can stick reversibly to wet surfaces and possess the ability to self-clean, so that they do not degrade with use. Improved wet weather tires, nonslip footwear, plasters for surgery that are able to adhere to tissue, holding devices for neurosurgery, and MEMS devices are other obvious examples of the many uses to which these toe pad analogues might be applied.

**Conclusions and Outlook.** In highlighting this selection of animal mucus research, we find that many unresolved

questions and challenges persist that must be addressed so that the full potential of mucus can be mimicked in bioinspired materials. Specific unresolved questions are: (i) Do mucins with similar functions have similar structures and shared phylogenetic histories, or are they the result of convergent evolution? (ii) What are the differences between vertebrate and invertebrate mucins? (iii) How do the mucin peptide and glycan components each contribute to the material behavior of the mucus? (iv) When animals produce multiple mucoses with distinct functions, do these mucoses contain similar mucin proteins? (v) How do nonmucin additives of the hydrogels contribute to their properties?

Studying mucus by conventional molecular biological techniques faces many challenges. For example, recombinant expression of synthetic mucin proteins that retain natural function has been difficult as a result of several factors inherent to the mucins, including the size of the mucin protein backbone, the lack of understanding in the function of different mucin protein domains, the complexity of the polysaccharide structures, and the challenge of introducing glycan domains into the recombinant protein.<sup>273–290</sup> Furthermore, many mucins have variable mucin domain lengths arising from alternative splice variants, adding the difficulty of characterizing each isoform.<sup>291–294</sup> Considering the factors above, the number of mucin genes present in typical animal genomes, and the complex tissue expression patterns of mucin proteins, it is challenging to decipher the roles of different domain features in mucin function from typical genomics and proteomics analyses alone. To address these unresolved issues, the researchers in the newly established Comparative Animal Muomics Project (CAMP) have adopted a collaborative approach that combines field work with experimental and computational methods. The various research groups will make the best effort to ensure that data sets are compatible and collect similar information, so that the salient properties of the mucoses can be more easily compared.

To address issues of data consistency, an omics-style approach that compares different mucus samples at multiple hierarchical levels across both the central dogma of biology and length scales is needed. Data-driven genomics, transcriptomics, proteomics, and metabolomics methods are highly effective strategies to quantitatively organize and analyze large, multidimensional data sets to answer the complex biological questions listed above. Models like the Consortium for Functional Glycomics,<sup>295</sup> National Center for Biotechnology Information,<sup>296</sup> the Protein Data Bank,<sup>297</sup> and the Omics Database Generator<sup>298</sup> set rigorous standards for data collection, organization, and analysis to be universally accessible and practical. Therefore, we suggest that adopting a similar “muomics” approach is essential to answering the aforementioned questions about mucus structure–function relationships. This muomics approach will compile gene and protein sequences, transcriptomic data, glycomics profiles, molecular weights of the mucins, the additives that exist in a mucus sample, and the material properties of the hydrogel. These data and integrative analyses, information on our mucus sample library, a list of CAMP members, publications, and means of being involved are available on our CAMP Web site, reachable at [muomics.org](http://muomics.org).

Our aim in establishing CAMP is to understand the roles of diverse mucins in nature and tease apart structure–activity relationships that can guide the design of synthetic mucus mimics. These bioinspired analogues could be used to replace

current materials that serve as adhesives, lubricants, structural materials, barriers, and semipermeable membranes. Although several important papers have shown that the advantageous properties of mucoses can be emulated with synthetics,<sup>27,299–313</sup> the field of synthetic mucus is currently in its infancy. We hope that the efforts of CAMP and others already working to understand the structures and properties of mucoses<sup>18,314–320</sup> will support efforts to design biomimetic materials that seek to emulate the remarkable properties of these secretions found throughout the animal kingdom.

## ■ AUTHOR INFORMATION

### Corresponding Author

**Adam B. Braunschweig** — *The PhD Program in Biochemistry, The Advanced Science Research Center, and The PhD Program in Chemistry, Graduate Center of the City University of New York, New York, New York 10016, United States; Department of Chemistry and Biochemistry, Hunter College, New York, New York 10065, United States; [orcid.org/0000-0003-0344-3029](https://orcid.org/0000-0003-0344-3029); Email: [abraunschweig@gc.cuny.edu](mailto:abraunschweig@gc.cuny.edu)*

### Authors

**Antonio R. Cerullo** — *The PhD Program in Biochemistry and The Advanced Science Research Center, Graduate Center of the City University of New York, New York, New York 10016, United States; Department of Chemistry and Biochemistry, Hunter College, New York, New York 10065, United States*

**Tsoi Ying Lai** — *The Advanced Science Research Center, Graduate Center of the City University of New York, New York, New York 10031, United States*

**Bassem Allam** — *School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York 11794-5000, United States*

**Alexander Baer** — *Department of Zoology, Institute of Biology, University of Kassel, 34132 Kassel, Germany*

**W. Jon P. Barnes** — *Centre for Cell Engineering, Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.*

**Zaidett Barrientos** — *Laboratorio de Ecología Urbana, Universidad Estatal a Distancia, San José 474-2050, Costa Rica*

**Dimitri D. Deheyn** — *Marine Biology Research Division-0202, Scripps Institute of Oceanography, UCSD, La Jolla, California 92093, United States*

**Douglas S. Fudge** — *Schmid College of Science and Technology, Chapman University, Orange, California 92866, United States; [orcid.org/0000-0001-9569-1374](https://orcid.org/0000-0001-9569-1374)*

**John Gould** — *School of Environmental and Life Sciences, University of Newcastle, Callaghan, New South Wales 2308, Australia*

**Matthew J. Harrington** — *Department of Chemistry, McGill University, Montreal, Quebec H3A 0B8, Canada; [orcid.org/0000-0003-1417-9251](https://orcid.org/0000-0003-1417-9251)*

**Mandé Holford** — *The PhD Program in Biochemistry, The PhD Program in Chemistry, and The PhD Program in Biology, Graduate Center of the City University of New York, New York, New York 10016, United States; Department of Chemistry and Biochemistry, Hunter College, New York, New York 10065, United States; Department of Invertebrate Zoology, The American Museum of Natural History, New York, New York 10024, United States*

**Chia-Suei Hung** — *Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson Air Force Base, Dayton, Ohio 45433, United States*

**Gaurav Jain** — Schmid College of Science and Technology, Chapman University, Orange, California 92866, United States

**Georg Mayer** — Department of Zoology, Institute of Biology, University of Kassel, 34132 Kassel, Germany

**Mónica Medina** — Department of Biology, Pennsylvania State University, University Park, Pennsylvania 16802, United States

**Julian Monge-Nájera** — Laboratorio de Ecología Urbana, Universidad Estatal a Distancia, San José 474-2050, Costa Rica

**Tanya Napolitano** — The PhD Program in Biochemistry, Graduate Center of the City University of New York, New York, New York 10016, United States; Department of Chemistry and Biochemistry, Hunter College, New York, New York 10065, United States

**Emmanuelle Pales Espinosa** — School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York 11794-5000, United States

**Stephan Schmidt** — Institute of Organic and Macromolecular Chemistry, Heinrich-Heine-Universität Düsseldorf, 40225 Düsseldorf, Germany; [orcid.org/0000-0002-4357-304X](https://orcid.org/0000-0002-4357-304X)

**Eric M. Thompson** — Sars Centre for Marine Molecular Biology, 5020 Bergen, Norway; Department of Biological Sciences, University of Bergen, N-5006 Bergen, Norway

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsbiomaterials.0c00713>

## Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

A.R.C. acknowledges support from a CUNY Science Scholarship and a CUNY Llewellyn Fellowship. B.A. and E.P.E. are supported by the National Science Foundation (Projects IOS 1050596 and IOS 1656753). G.M. and A.B. are supported by the German Research Foundation (DFG: MA 4147/7-1 and MA 4147/7-2). W.J.P.B. acknowledges collaborators Dr. Thomas Endlein (MPI for Polymerforschung; Mainz, Germany), Prof. Stas Gorb (Kiel, Germany), and Dr. Walter Federle (Cambridge, UK) as well as funding from DFG (Deutsche Forschungsgemeinschaft), and Royal Society of London. Z.B. acknowledges support from Vicerrectoría de Investigación, Universidad Estatal a Distancia, Costa Rica. D.F. acknowledges support from the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery (RGPIN-2016-04967) and Accelerator (RGPAS-2016-493149) grants and support from the National Science Foundation NSF (Project IOS 1755397). M.J.H. acknowledges support from the Natural Sciences and Engineering Research Council of Canada (NSERC Discovery Grant RGPIN-2018-05243). M.H. acknowledges research grants by the Camille & Henry Dreyfus Foundation and the National Institutes of Health (NIH-NIMHD grant 8-G-12-MD007599). M.M. was funded by the US National Science Foundation (NSF OCE 1442206 and OCE 1642311). J.M.N. acknowledges the support of El Laboratorio de Ecología Urbana de Costa Rica. T.N. acknowledges support from the CUNY Graduate Center Graduate Fellowship program. E.M.T. acknowledges support by the Norwegian Research Council (17541/S10-FUGE and 133335/V40). The Air Force Office of Scientific Research is acknowledged for support by D.D.D. (FA9550-17-0189) and A.B.B. (FA9550-19-1-0220).

## REFERENCES

- (1) Bansil, R.; Turner, B. S. Mucin Structure, Aggregation, Physiological Functions and Biomedical Applications. *Curr. Opin. Colloid Interface Sci.* **2006**, *11* (2–3), 164–170.
- (2) Home, E. XXV. Some Account of the Nests of the Java Swallow, and of the Glands that Secrete the Mucus of Which They are Composed. *Philosophical Transactions of the Royal Society of London* **1817**, *107*, 332–338.
- (3) Hammarsten, O. Ueber das Mucin der Submaxillardrüse. *Zeitschrift für Physiologische Chemie* **1888**, *12* (1–2), 163–195.
- (4) Reid, E. W. Mucin Granules of Myxine. *J. Physiol.* **1893**, *14* (4–5), 340.
- (5) Waller, A. LVIII. Microscopic Observation on the Perforation of the Capillaries by the Corpuscles of the Blood, and on the Origin of Mucus and Pus-Globules. *London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science* **1846**, *29* (195), 397–405.
- (6) Darwin, C. Experiments. *Medical and Physical Journal* **1800**, *4* (17), 50.
- (7) Ravi, V.; Kesavan, K.; Sandhya, S.; Rajagopal, S. Antibacterial Activity of the Mucus of Mudskipper *Boleophthalmus boddarti*. *Pallas* **1770**, *11*–14.
- (8) Celli, J. P.; Turner, B. S.; Afdhal, N. H.; Ewoldt, R. H.; McKinley, G. H.; Bansil, R.; Erramilli, S. Rheology of Gastric Mucin Exhibits a pH-dependent Sol–Gel Transition. *Biomacromolecules* **2007**, *8* (5), 1580–1586.
- (9) Guzman-Aranguez, A.; Argüeso, P. Structure and Biological Roles of Mucin-Type O-Glycans at the Ocular Surface. *Ocular Surface* **2010**, *8* (1), 8–17.
- (10) Perez-Vilar, J.; Hill, R. L. Mucin Family of Glycoproteins. In *Encyclopedia of Biological Chemistry*, Lennarz, W. J.; Lane, M. D., Eds.; Elsevier: New York, 2004; pp 758–764.
- (11) Lang, T.; Hansson, G. C.; Samuelsson, T. Gel-Forming Mucins Appeared Early in Metazoan Evolution. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104* (41), 16209–16214.
- (12) Desseyn, J.-L.; Aubert, J.-P.; Porchet, N.; Laine, A. Evolution of the Large Secreted Gel-Forming Mucins. *Mol. Biol. Evol.* **2000**, *17* (8), 1175–1184.
- (13) Becher, N.; Waldorf, K. A.; Hein, M.; Uldbjerg, N. The cervical mucus plug: structured review of the literature. *Acta Obstet. Gynecol. Scand.* **2009**, *88* (5), 502–513.
- (14) Reverter, M.; Tapissier-Bontemps, N.; Lecchini, D.; Banaigs, B.; Sasal, P. Biological and ecological roles of external fish mucus: a review. *Fishes* **2018**, *3* (4), 41.
- (15) Marczynski, M.; Balzer, B. N.; Jiang, K.; Lutz, T. M.; Crouzier, T.; Lieleg, O. Charged glycan residues critically contribute to the adsorption and lubricity of mucins. *Colloids Surf., B* **2020**, *187*, 110614.
- (16) Coles, J. M.; Chang, D. P.; Zauscher, S. Molecular mechanisms of aqueous boundary lubrication by mucinous glycoproteins. *Curr. Opin. Colloid Interface Sci.* **2010**, *15* (6), 406–416.
- (17) Ewoldt, R. H.; Clasen, C.; Hosoi, A. E.; McKinley, G. H. Rheological Fingerprinting of Gastropod Pedal Mucus and Synthetic Complex Fluids for Biomimicking Adhesive Locomotion. *Soft Matter* **2007**, *3* (5), 634–643.
- (18) Crouzier, T.; Boettcher, K.; Geonnotti, A. R.; Kavanaugh, N. L.; Hirsch, J. B.; Ribbeck, K.; Lieleg, O. Modulating Mucin Hydration and Lubrication by Deglycosylation and Polyethylene Glycol Binding. *Adv. Mater. Interfaces* **2015**, *2* (18), 1500308.
- (19) Marin, F.; Corstjens, P.; De Gaulejac, B.; de Vrind-De Jong, E.; Westbroek, P. Mucins and Molluscan Calcification Molecular Characterization of Mucoperlin, a Novel Mucin-like Protein From the Nacreous Shell Layer of the Fan Mussel *Pinna nobilis* (Bivalvia, Pteriomorpha). *J. Biol. Chem.* **2000**, *275* (27), 20667–20675.
- (20) Bythell, J. C.; Wild, C. Biology and Ecology of Coral Mucus Release. *J. Exp. Mar. Biol. Ecol.* **2011**, *408* (1–2), 88–93.
- (21) Robison, B. H.; Reisenbichler, K. R.; Sherlock, R. E. Giant Larvacean Houses: Rapid Carbon Transport to the Deep Sea Floor. *Science* **2005**, *308* (5728), 1609–1611.

(22) Mayer, G.; Oliveira, I. S.; Baer, A.; Hammel, J. U.; Gallant, J.; Hochberg, R. Capture of Prey, Feeding, and Functional Anatomy of the Jaws in Velvet Worms (Onychophora). *Integr. Comp. Biol.* **2015**, *55*, 217–227.

(23) Johansson, M. E.; Hansson, G. C. Immunological Aspects of Intestinal Mucus and Mucins. *Nat. Rev. Immunol.* **2016**, *16* (10), 639–649.

(24) Subramanian, S.; MacKinnon, S. L.; Ross, N. W. A Comparative Study on Innate Immune Parameters in the Epidermal Mucus of Various Fish Species. *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* **2007**, *148* (3), 256–263.

(25) Pelaseyed, T.; Bergström, J. H.; Gustafsson, J. K.; Ermund, A.; Birchenough, G. M.; Schütte, A.; van der Post, S.; Svensson, F.; Rodríguez-Piñeiro, A. M.; Nyström, E. E. The Mucus and Mucins of the Goblet Cells and Enterocytes Provide the First Defense Line of the Gastrointestinal Tract and Interact with the Immune System. *Immunological reviews* **2014**, *260* (1), 8–20.

(26) Cone, R. A. Barrier properties of mucus. *Adv. Drug Delivery Rev.* **2009**, *61* (2), 75–85.

(27) Li, J.; Celiz, A.; Yang, J.; Yang, Q.; Wamala, I.; Whyte, W.; Seo, B.; Vasilyev, N.; Vlassak, J.; Suo, Z. Tough Adhesives for Diverse Wet Surfaces. *Science* **2017**, *357* (6349), 378–381.

(28) Winkeljann, B.; Bauer, M. G.; Marcynski, M.; Rauh, T.; Sieber, S. A.; Lieleg, O. Covalent Mucin Coatings Form Stable Anti-Biofouling Layers on a Broad Range of Medical Polymer Materials. *Adv. Mater. Interfaces* **2020**, *7* (4), 1902069.

(29) Shi, S.-C.; Wu, J.-Y.; Huang, T.-F.; Peng, Y.-Q. Improving the Tribological Performance of Biopolymer Coating with MoS<sub>2</sub> Additive. *Surf. Coat. Technol.* **2016**, *303*, 250–255.

(30) Liu, J.; Sun, L.; Xu, W.; Wang, Q.; Yu, S.; Sun, J. Current Advances and Future Perspectives of 3D Printing Natural-Derived Biopolymers. *Carbohydr. Polym.* **2019**, *207*, 297–316.

(31) Andreeßen, C.; Steinbüchel, A. Recent Developments in Non-Biodegradable Biopolymers: Precursors, Production Processes, and Future Perspectives. *Appl. Microbiol. Biotechnol.* **2019**, *103* (1), 143–157.

(32) Makarovskiy, D.; Fadeev, L.; Salam, B. B.; Zelinger, E.; Matan, O.; Inbar, J.; Jurkewitch, E.; Gozin, M.; Burdman, S. Silver Nanoparticles Complexed with Bovine Submaxillary Mucin Possess Strong Antibacterial Activity and Protect Against Seedling Infection. *Appl. Environ. Microbiol.* **2018**, *84* (4), e02212–17.

(33) DuBuc, T. Q.; Traylor-Knowles, N.; Martindale, M. Q. Initiating a regenerative response; cellular and molecular features of wound healing in the cnidarian Nematostella vectensis. *BMC Biol.* **2014**, *12* (1), 1–21.

(34) Patwa, A.; Thiéry, A.; Lombard, F.; Lilley, M. K.; Boisset, C.; Bramard, J.-F.; Bottero, J.-Y.; Barthélémy, P. Accumulation of nanoparticles in “jellyfish” mucus: a bio-inspired route to decontamination of nano-waste. *Sci. Rep.* **2015**, *5*, 11387.

(35) Sharma, A.; Thakur, M.; Bhattacharya, M.; Mandal, T.; Goswami, S. Commercial application of cellulose nano-composites—A review. *Biotechnology Reports* **2019**, *21*, e00316.

(36) Agate, S.; Joyce, M.; Lucia, L.; Pal, L. Cellulose and nanocellulose-based flexible-hybrid printed electronics and conductive composites—a review. *Carbohydr. Polym.* **2018**, *198*, 249–260.

(37) Yang, J.; Li, J. Self-assembled cellulose materials for biomedicine: A review. *Carbohydr. Polym.* **2018**, *181*, 264–274.

(38) Castillo, R. R.; Baeza, A.; Vallet-Regí, M. Recent applications of the combination of mesoporous silica nanoparticles with nucleic acids: development of bioresponsive devices, carriers and sensors. *Biomater. Sci.* **2017**, *5* (3), 353–377.

(39) Pugh, G. C.; Burns, J. R.; Howorka, S. Comparing proteins and nucleic acids for next-generation biomolecular engineering. *Nature Reviews Chemistry* **2018**, *2* (7), 113–130.

(40) López Barreiro, D.; Yeo, J.; Tarakanova, A.; Martin-Martinez, F. J.; Buehler, M. J. Multiscale Modeling of Silk and Silk-Based Biomaterials—A Review. *Macromol. Biosci.* **2019**, *19* (3), 1800253.

(41) Holland, C.; Numata, K.; Rnjak-Kovacina, J.; Seib, F. P. The Biomedical Use of Silk: Past, Present, Future. *Adv. Healthcare Mater.* **2019**, *8* (1), 1800465.

(42) Mehrotra, S.; Chouhan, D.; Konwarh, R.; Kumar, M.; Jadi, P. K.; Mandal, B. B. Comprehensive review on silk at nanoscale for regenerative medicine and allied applications. *ACS Biomater. Sci. Eng.* **2019**, *5* (5), 2054–2078.

(43) Bansil, R.; Stanley, E.; LaMont, J. T. Mucin Biophysics. *Annu. Rev. Physiol.* **1995**, *57* (1), 635–657.

(44) Lai, S. K.; Wang, Y.-Y.; Wirtz, D.; Hanes, J. Micro-and macrorheology of mucus. *Adv. Drug Delivery Rev.* **2009**, *61* (2), 86–100.

(45) Bansil, R.; Turner, B. S. The biology of mucus: Composition, synthesis and organization. *Adv. Drug Delivery Rev.* **2018**, *124*, 3–15.

(46) Demouveau, B.; Gouyer, V.; Gottrand, F.; Narita, T.; Desseyn, J.-L. Gel-forming mucin interactome drives mucus viscoelasticity. *Adv. Colloid Interface Sci.* **2018**, *252*, 69–82.

(47) Lee, S.; Spencer, N. D. Sweet, hairy, soft, and slippery. *Science* **2008**, *319* (5863), 575.

(48) Pitenis, A. A.; Urueña, J. M.; Hormel, T. T.; Bhattacharjee, T.; Niemi, S. R.; Marshall, S. L.; Hart, S. M.; Schulze, K. D.; Angelini, T. E.; Sawyer, W. G. Corneal cell friction: Survival, lubricity, tear films, and mucin production over extended duration in vitro studies. *Biotribology* **2017**, *11*, 77–83.

(49) Spencer, N. D. *Aqueous lubrication: natural and biomimetic approaches*; World Scientific: Singapore, 2014.

(50) Yakubov, G. E.; McColl, J.; Bongaerts, J. H.; Ramsden, J. J. Viscous boundary lubrication of hydrophobic surfaces by mucin. *Langmuir* **2009**, *25* (4), 2313–2321.

(51) Button, B.; Goodell, H. P.; Atieh, E.; Chen, Y.-C.; Williams, R.; Shenoy, S.; Lackey, E.; Shenkute, N. T.; Cai, L.-H.; Dennis, R. G.; Boucher, R. C.; Rubinstein, M. Roles of mucus adhesion and cohesion in cough clearance. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (49), 12501–12506.

(52) Davidovich-Pinhas, M.; Bianco-Peled, H. Mucoadhesion: a review of characterization techniques. *Expert Opin. Drug Delivery* **2010**, *7* (2), 259–271.

(53) Rossi, S.; Vigani, B.; Bonferoni, M. C.; Sandri, G.; Caramella, C.; Ferrari, F. Rheological analysis and mucoadhesion: A 30 year-old and still active combination. *J. Pharm. Biomed. Anal.* **2018**, *156*, 232–238.

(54) Jiang, Z.; Applegate, T. J.; Lossie, A. C. Cloning, Annotation and Developmental Expression of the Chicken Intestinal MUC2 Gene. *PLoS One* **2013**, *8* (1), e53781–e53781.

(55) Offner, G.; Troxler, R. Heterogeneity of High-Molecular-Weight Human Salivary Mucins. *Advances in Dental Research* **2000**, *14* (1), 69–75.

(56) Perez-Vilar, J.; Hill, R. L. The Structure and Assembly of Secreted Mucins. *J. Biol. Chem.* **1999**, *274* (45), 31751–31754.

(57) Gendler, S. J.; Spicer, A. Epithelial Mucin Genes. *Annu. Rev. Physiol.* **1995**, *57* (1), 607–634.

(58) Desseyn, J.-L.; Tetaert, D.; Gouyer, V. Architecture of the Large Membrane-Bound Mucins. *Gene* **2008**, *410* (2), 215–222.

(59) Jonckheere, N.; Skrypek, N.; Frénois, F.; Van Seuningen, I. Membrane-bound Mucin Modular Domains: From Structure to Function. *Biochimie* **2013**, *95* (6), 1077–1086.

(60) Dhanisha, S. S.; Guruvayoorappan, C.; Drishya, S.; Abeesh, P. Mucins: Structural Diversity, Biosynthesis, Its Role In Pathogenesis And As Possible Therapeutic Targets. *Critical Reviews in Oncology/Hematology* **2018**, *122*, 98–122.

(61) Jin, C.; Kenny, D. T.; Skoog, E. C.; Padra, M.; Adamczyk, B.; Vitizeva, V.; Thorell, A.; Venkatakrishnan, V.; Lindén, S. K.; Karlsson, N. G. Structural diversity of human gastric mucin glycans. *Mol. Cell. Proteomics* **2017**, *16* (5), 743–758.

(62) Bergstrom, K. S.; Xia, L. Mucin-type O-glycans and their roles in intestinal homeostasis. *Glycobiology* **2013**, *23* (9), 1026–1037.

(63) Remmers, N.; Anderson, J. M.; Linde, E. M.; DiMaio, D. J.; Lazenby, A. J.; Wandall, H. H.; Mandel, U.; Clausen, H.; Yu, F.; Hollingsworth, M. A. Aberrant expression of mucin core proteins and

o-linked glycans associated with progression of pancreatic cancer. *Clin. Cancer Res.* **2013**, *19* (8), 1981–1993.

(64) Brockhausen, I. Mucin-type O-glycans in human colon and breast cancer: glycodynamics and functions. *EMBO Rep.* **2006**, *7* (6), 599–604.

(65) Martín, S. P.; Seeberger, P. H.; Silva, D. V., Mucins and Pathogenic Mucin-Like Molecules Are Immunomodulators During Infection and Targets for Diagnostics and Vaccines. *Front. Chem.* **2019**, *7*. DOI: 10.3389/fchem.2019.000710

(66) Bell, S. L.; Xu, G.; Forstner, J. F. Role of the Cystine-Knot Motif at the C-Terminus of Rat Mucin Protein Muc2 in Dimer Formation and Secretion. *Biochem. J.* **2001**, *357* (1), 203–209.

(67) O'Leary, J. M.; Hamilton, J. M.; Deane, C. M.; Valev, N. V.; Sandell, L. J.; Downing, A. K. Solution Structure and Dynamics of a Prototypical Chordin-like Cysteine-rich Repeat (von Willebrand Factor type C module) From Collagen IIA. *J. Biol. Chem.* **2004**, *279* (51), 53857–53866.

(68) Zhang, J.-L.; Huang, Y.; Qiu, L.-Y.; Nickel, J.; Sebald, W. von Willebrand Factor Type C Domain-Containing Proteins Regulate Bone Morphogenetic Protein Signaling Through Different Recognition Mechanisms. *J. Biol. Chem.* **2007**, *282* (27), 20002–20014.

(69) Zhou, Y.-F.; Eng, E. T.; Zhu, J.; Lu, C.; Walz, T.; Springer, T. A. Sequence and Structure Relationships Within von Willebrand Factor. *Blood* **2012**, *120* (2), 449–458.

(70) Nilsson, H. E.; Ambort, D.; Bäckström, M.; Thomsson, E.; Koeck, P. J.; Hansson, G. C.; Hebert, H. Intestinal MUC2Mucin Supramolecular Topology by Packing and Release Resting on D3 Domain Assembly. *J. Mol. Biol.* **2014**, *426* (14), 2567–2579.

(71) Ambort, D.; van der Post, S.; Johansson, M. E.; MacKenzie, J.; Thomsson, E.; Krengel, U.; Hansson, G. C. Function of the CysD Domain of the Gel-forming MUC2Mucin. *Biochem. J.* **2011**, *436* (1), 61–70.

(72) Demouveaux, B.; Gouyer, V.; Robbe-Masselot, C.; Gottrand, F.; Narita, T.; Desseyn, J.-L. Mucin CYS Domain Stiffens the Mucus Gel Hindering Bacteria and Spermatozoa. *Sci. Rep.* **2019**, *9* (1), 1–11.

(73) Parry, S.; Hanisch, F. G.; Leir, S.-H.; Sutton-Smith, M.; Morris, H. R.; Dell, A.; Harris, A. N-Glycosylation of the MUC1Mucin in Epithelial cells and Secretions. *Glycobiology* **2006**, *16* (7), 623–634.

(74) Staudacher, E. Mucin-type O-glycosylation In Invertebrates. *Molecules* **2015**, *20* (6), 10622–10640.

(75) Tailford, L. E.; Crost, E. H.; Kavanaugh, D.; Juge, N. Mucin Glycan Foraging in the Human Gut Microbiome. *Front. Genet.* **2015**, *6*, 81.

(76) Hong, Z.; Chasan, B.; Bansil, R.; Turner, B. S.; Bhaskar, K. R.; Afdhal, N. H. Atomic Force Microscopy Reveals Aggregation of Gastric Mucin at Low pH. *Biomacromolecules* **2005**, *6* (6), 3458–3466.

(77) Biesbroek, A. R.; Bobek, L. A.; Levine, M. J. MUC7 Gene Expression and Genetic Polymorphism. *Glycoconjugate J.* **1997**, *14* (4), 415–422.

(78) Chaudhury, N. M.; Proctor, G. B.; Karlsson, N. G.; Carpenter, G. H.; Flowers, S. A. Reduced Mucin-7 (Muc7) Sialylation and Altered Saliva Rheology in Sjögren's Syndrome Associated Oral Dryness. *Mol. Cell. Proteomics* **2016**, *15* (3), 1048–1059.

(79) Janicka-Klos, A.; Janek, T.; Burger, J.; Czapor-Irzabek, H. Human Salivary MUC7Mucin Fragment and Its Analogues. Coordination and Biological Studies. *J. Inorg. Biochem.* **2020**, *203*, 110923.

(80) Jumblatt, M. M.; McKenzie, R. W.; Steele, P. S.; Emberts, C. G.; Jumblatt, J. E. MUC7 Expression in the Human Lacrimal Gland and Conjunctiva. *Cornea* **2003**, *22* (1), 41–45.

(81) Wei, G.-X.; Campagna, A. N.; Bobek, L. A. Effect of MUC7 Peptides on the Growth of Bacteria and on *Streptococcus mutans* Biofilm. *J. Antimicrob. Chemother.* **2006**, *57* (6), 1100–1109.

(82) Xu, D.; Pavlidis, P.; Taskent, R. O.; Alachiotis, N.; Flanagan, C.; DeGiorgio, M.; Blekhman, R.; Ruhl, S.; Gokcumen, O. Archaic Hominin Introgression in Africa Contributes to Functional Salivary MUC7 Genetic Variation. *Mol. Biol. Evol.* **2017**, *34* (10), 2704–2715.

(83) Xu, D.; Pavlidis, P.; Thamadilok, S.; Redwood, E.; Fox, S.; Blekhman, R.; Ruhl, S.; Gokcumen, O. Recent Evolution of the Salivary Mucin MUC7. *Sci. Rep.* **2016**, *6* (1), 1–11.

(84) Melino, S.; Santone, C.; Di Nardo, P.; Sarkar, B. Histatins: Salivary Peptides with Copper (II)-and Zinc (II)-Binding Motifs: Perspectives For Biomedical Applications. *FEBS J.* **2014**, *281* (3), 657–672.

(85) Satyanarayana, J.; Situ, H.; Narasimhamurthy, S.; Bhayani, N.; Bobek, L.; Levine, M. Divergent Solid-Phase Synthesis and Candidacidal Activity of MUC7 D1, a 51-Residue Histidine-Rich N-Terminal Domain of Human Salivary Mucin MUC7. *J. Pept. Res.* **2000**, *56* (5), 275–282.

(86) Lang, T.; Klasson, S.; Larsson, E.; Johansson, M. E.; Hansson, G. C.; Samuelsson, T. Searching the Evolutionary Origin of Epithelial Mucus Protein Components—Mucins and FCGBP. *Mol. Biol. Evol.* **2016**, *33* (8), 1921–1936.

(87) Ostedgaard, L. S.; Moninger, T. O.; McMenimen, J. D.; Sawin, N. M.; Parker, C. P.; Thornell, I. M.; Powers, L. S.; Gansemer, N. D.; Bouzek, D. C.; Cook, D. P. Gel-Forming Mucins Form Distinct Morphologic Structures in Airways. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (26), 6842–6847.

(88) Bonser, L. R.; Erle, D. J. Airway mucus and asthma: the role of MUC5AC and MUC5B. *J. Clin. Med.* **2017**, *6* (12), 112.

(89) Ramos-Silva, P.; Kaandorp, J.; Huisman, L.; Marie, B.; Zanella-Cléon, I.; Guichard, N.; Miller, D. J.; Marin, F. The Skeletal Proteome of the Coral *Acropora millepora*: The Evolution of Calcification by Co-option and Domain Shuffling. *Mol. Biol. Evol.* **2013**, *30* (9), 2099–2112.

(90) Karunanithi, S.; Vadaie, N.; Chavel, C. A.; Birkaya, B.; Joshi, J.; Grell, L.; Cullen, P. J. Shedding of the Mucin-like Flocculin Flo11p Reveals a New Aspect of Fungal Adhesion Regulation. *Curr. Biol.* **2010**, *20* (15), 1389–1395.

(91) Benktander, J.; Venkatakrishnan, V.; Padra, J. T.; Sundh, H.; Sundell, K.; Murugan, A. V.; Maynard, B.; Linden, S. K. Effects of size and geographical origin on Atlantic salmon, *Salmo salar*, mucin O-glycan repertoire. *Mol. Cell. Proteomics* **2019**, *18* (6), 1183–1196.

(92) Moschini, R.; Gini, F.; Cappiello, M.; Balestri, F.; Falcone, G.; Boldrini, E.; Mura, U.; Del-Corso, A. Interaction of Arabinogalactan with Mucins. *Int. J. Biol. Macromol.* **2014**, *67*, 446–451.

(93) Darula, Z.; Sarnyai, F.; Medzihradzky, K. F. O-Glycosylation Sites Identified From Mucin Core-1 type Glycopeptides From Human Serum. *Glycoconjugate J.* **2016**, *33* (3), 435–445.

(94) Kim, J.; Ryu, C.; Ha, J.; Lee, J.; Kim, D.; Ji, M.; Park, C. S.; Lee, J.; Kim, D. K.; Kim, H. H. Structural and Quantitative Characterization of Mucin-Type O-Glycans and the Identification of O-Glycosylation Sites in Bovine Submaxillary Mucin. *Biomolecules* **2020**, *10* (4), 636.

(95) Dias, R.; Cardoso, C.; Pimentel, A. C.; Damasceno, T. F.; Ferreira, C.; Terra, W. R. The Roles of Mucus-Forming Mucins, Peritrophins and Peritrophins with Mucin Domains in the Insect Midgut. *Insect Mol. Biol.* **2018**, *27* (1), 46–60.

(96) Pales Espinosa, E.; Corre, E.; Allam, B. Pallial Mucus of the Oyster *Crassostrea virginica* Regulates the Expression of Putative Virulence Genes of Its Pathogen *Perkinsus marinus*. *Int. J. Parasitol.* **2014**, *44* (5), 305–317.

(97) Baer, A.; Schmidt, S.; Mayer, G.; Harrington, M. J. Fibers on the Fly: Multiscale Mechanisms of Fiber Formation in the Capture Slime of Velvet Worms. *Integrative and Comparative Biology* **2019**, icz048.

(98) Zhong, J.; Wang, W.; Yang, X.; Yan, X.; Liu, R. A Novel Cysteine-Rich Antimicrobial Peptide from the Mucus of the Snail of *Achatina fulica*. *Peptides* **2013**, *39*, 1–5.

(99) Gould, J.; Valdez, J. W.; Upton, R. Adhesive Defence Mucus Secretions in the Red Triangle Slug (*Triboniophorus graeffei*) can Incapacitate Adult Frogs. *Ethology* **2019**. DOI: 10.1111/eth.12875

(100) Barnes, W. J. P.; Goodwyn, P. J. P.; Nokhbatolfoghai, M.; Gorb, S. N. Elastic Modulus of Tree Frog Adhesive Toe Pads. *J. Comp. Physiol. A* **2011**, *197* (10), 969.

(101) Zamzow, J. P.; Losey, G. S. Ultraviolet Radiation Absorbance by Coral Reef Fish Mucus: Photo-Protection and Visual Communication. *Environ. Biol. Fishes* **2002**, *63* (1), 41–47.

(102) Branchini, B. R.; Behney, C. E.; Southworth, T. L.; Rawat, R.; Deheyn, D. D. Chemical Analysis of the Luminous Slime Secreted by the Marine Worm *Chaetopterus* (Annelida, Polychaeta). *Photochem. Photobiol.* **2014**, *90* (1), 247–251.

(103) Shah, D. U.; Vollrath, F.; Stires, J.; Deheyn, D. D. The Biocomposite Tube of a *Chaetopterid* Marine Worm Constructed with Highly-Controlled Orientation of Nanofilaments. *Mater. Sci. Eng., C* **2015**, *48*, 408–415.

(104) Fudge, D. S.; Levy, N.; Chiu, S.; Gosline, J. M. Composition, Morphology and Mechanics of Hagfish Slime. *J. Exp. Biol.* **2005**, *208* (24), 4613–4625.

(105) Brown, B. E.; Bythell, J. C. Perspectives on Mucus Secretion in Reef Corals. *Mar. Ecol.: Prog. Ser.* **2005**, *296*, 291–309.

(106) Hohn, S.; Reymond, C. E. Coral calcification, mucus, and the origin of skeletal organic molecules. *Coral Reefs* **2019**, *38* (5), 973–984.

(107) Palmer, C. V.; Traylor-Knowles, N. Towards an Integrated Network of Coral Immune Mechanisms. *Proc. R. Soc. London, Ser. B* **2012**, *279*, 4106–4114.

(108) Ritchie, K. B. Regulation of Microbial Populations by Coral Surface Mucus and Mucus-Associated Bacteria. *Mar. Ecol.: Prog. Ser.* **2006**, *322*, 1–14.

(109) Wild, C.; Huettel, M.; Klueter, A.; Kremb, S. G.; Rasheed, M. Y. M.; Jørgensen, B. B. Coral Mucus Functions as an Energy Carrier and Particle Trap in the Reef Ecosystem. *Nature* **2004**, *428* (6978), 66–70.

(110) Krediet, C. J.; Ritchie, K. B.; Paul, V. J.; Teplitzki, M. Coral-Associated Micro-Organisms and Their Roles in Promoting Coral Health and Thwarting Diseases. *Proc. R. Soc. London, Ser. B* **2013**, *280*, 20122328–20122328.

(111) Teplitzki, M.; Krediet, C. J.; Meyer, J. L.; Ritchie, K. B. Microbial Interactions on Coral Surfaces and within the Coral Holobiont. *The Cnidaria, Past, Present and Future* **2016**, 1–855.

(112) Zaneveld, J. R.; Burkepile, D. E.; Shantz, A. A.; Pritchard, C. E.; McMinds, R.; Payet, J. P.; Welsh, R.; Correa, A. M. S.; Lemoine, N. P.; Rosales, S.; Fuchs, C.; Maynard, J. A.; Vega Thurber, R. Overfishing and Nutrient Pollution Interact with Temperature to Disrupt Coral Reefs Down to Microbial Scales. *Nat. Commun.* **2016**, *7*, DOI: 10.1038/ncomms11833.

(113) Wright, R. M.; Strader, M. E.; Genuine, H. M.; Matz, M. Effects of thermal stress on amount, composition, and antibacterial properties of coral mucus. *PeerJ* **2019**, *7*, e6849.

(114) Hadaidi, G.; Röthig, T.; Yum, L. K.; Ziegler, M.; Arif, C.; Roder, C.; Burt, J.; Voolstra, C. R. Stable Mucus-Associated Bacterial Communities in Bleached and Healthy Corals of *Porites lobata* from the Arabian Seas. *Sci. Rep.* **2017**, *7*, 1–11.

(115) Pollock, F. J.; McMinds, R.; Smith, S.; Bourne, D. G.; Willis, B. L.; Medina, M.; Thurber, R. V.; Zaneveld, J. R. Coral-Associated Bacteria Demonstrate Phyllosymbiosis and Cophylogeny. *Nat. Commun.* **2018**, *9* (1), 4921–4921.

(116) Hadaidi, G.; Gegner, H. M.; Ziegler, M.; Voolstra, C. R. Carbohydrate composition of mucus from scleractinian corals from the central Red Sea. *Coral Reefs* **2019**, *38* (1), 21–27.

(117) Jatkar, A. A.; Brown, B. E.; Bythell, J. C.; Guppy, R.; Morris, N. J.; Pearson, J. P. Coral Mucus: The Properties of Its Constituent Mucins. *Biomacromolecules* **2010**, *11* (4), 883–888.

(118) Ramos-Silva, P.; Kaandorp, J.; Herbst, F. e.; Plasseraud, L.; Alcaraz, G.; Stern, C.; Corneillat, M.; Guichard, N.; Durlet, C.; Luquet, G.; Marin, F. e. The Skeleton of the Staghorn Coral *Acropora millepora*: Molecular and Structural Characterization. *PLoS One* **2014**, *9* (6), e97454.

(119) Hemond, E. M.; Vollmer, S. V. Diurnal and Nocturnal Transcriptomic Variation in the Caribbean Staghorn Coral, *Acropora cervicornis*. *Mol. Ecol.* **2015**, *24* (17), 4460–4473.

(120) Kitchen, S. A.; Ratan, A.; Bedoya-Reina, O. C.; Burhans, R.; Fogarty, N. D.; Miller, W.; Baums, I. B. Genomic Variants Among Threatened *Acropora* Corals. *G3: Genes, Genomes, Genet.* **2019**, *9* (5), 1633–1646.

(121) Takeuchi, T.; Yamada, L.; Shinzato, C.; Sawada, H.; Satoh, N. Stepwise evolution of coral biomineralization revealed with genome-wide proteomics and transcriptomics. *PLoS One* **2016**, *11* (6), e0156424.

(122) Avila-Magaña, V.; Kamel, B.; DeSalvo, M.; Gómez-Campo, K.; Enríquez, S.; Kitano, H.; Rohlfs, R.; Iglesias-Prieto, R.; Medina, M. Elucidating the adaptive potential of coral holobionts under thermal stress. In revision.

(123) Horricks, R. A.; Herbinger, C. M.; Lillie, B. N.; Taylor, P.; Lumsden, J. S. Differential Protein Abundance During the First Month of Regeneration of the Caribbean Star Coral *Montastraea cavernosa*. *Coral Reefs* **2019**, *38* (1), 45–61.

(124) Ohdera, A. H.; Abrams, M. J.; Ames, C. L.; Baker, D. M.; Suescún-Bolívar, L. P.; Collins, A. G.; Freeman, C. J.; Gamero-Mora, E.; Goulet, T. L.; Hofmann, D. K. Upside-down but Headed in the Right Direction: Review of the Highly Versatile *Cassiopea xamachana* System. *Frontiers in Ecology and Evolution* **2018**, *6*, 35.

(125) Ames, C. L.; Klompen, A. M. L.; Badhiwala, K.; Muffett, K.; Reft, A. J.; Kumar, M.; Janssen, J. D.; Schultzhaus, J. N.; Field, L. D.; Muroski, M. E.; Bezio, N.; Robinson, J. T.; Leary, D. H.; Cartwright, P.; Collins, A. G.; Vora, G. J. Cassiosomes are Stinging-Cell Structures in the Mucus of the Upside-Down Jellyfish *Cassiopea xamachana*. *Communications Biology* **2020**, *3*, 1–15.

(126) Crawshay, L. R. Possible Bearing of a Luminous Syllid on the Question of the Landfall of Columbus; Nature Publishing Group: 1935.

(127) Deheyn, D. D.; Latz, M. I. Internal and Secreted Bioluminescence of the Marine Polychaete *Odontosyllis phosphorea* (Syllidae). *Invertebrate Biology* **2009**, *128* (1), 31–45.

(128) Kotlobay, A. A.; Dubinnyi, M. A.; Purtov, K. V.; Guglya, E. B.; Rodionova, N. S.; Petushkov, V. N.; Bolt, Y. V.; Kublitski, V. S.; Kaskova, Z. M.; Ziganshin, R. H. Bioluminescence Chemistry of Fireworm *Odontosyllis*. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (38), 18911–18916.

(129) Mehr, S.; Verdes, A.; DeSalle, R.; Sparks, J.; Pieribone, V.; Gruber, D. F. Transcriptome Sequencing and Annotation of the Polychaete *Hermodice carunculata* (Annelida, Amphinomidae). *BMC Genomics* **2015**, *16* (1), 445.

(130) Schultz, D. T.; Kotlobay, A. A.; Ziganshin, R.; Bannikov, A.; Markina, N. M.; Chepurnyh, T. V.; Shakhova, E. S.; Palkina, K.; Haddock, S. H.; Yampolsky, I. V. Luciferase of the Japanese Syllid Polychaete *Odontosyllis umdecimdonta*. *Biochem. Biophys. Res. Commun.* **2018**, *502* (3), 318–323.

(131) Verdes, A.; Alvarez-Campos, P.; Nygren, A.; San Martin, G.; Rouse, G.; Deheyn, D. D.; Gruber, D. F.; Holford, M. Molecular Phylogeny of *Odontosyllis* (Annelida, Syllidae): A Recent and Rapid Radiation of Marine Bioluminescent Worms. *bioRxiv* **2018**, 241570.

(132) Gaston, G. R.; Hall, J. Lunar Periodicity and Bioluminescence of Swarming *Odontosyllis luminosa* (Polychaeta: Syllidae) in Belize. *Gulf and Caribbean Research* **2000**, *12* (1), 47–51.

(133) Tsuji, F. I.; Hill, E. Repetitive Cycles of Bioluminescence and Spawning in the Polychaete, *Odontosyllis phosphorea*. *Biol. Bull.* **1983**, *165* (2), 444–449.

(134) Fischer, A.; Fischer, U. On the Life-Style and Life-Cycle of the Luminescent Polychaete *Odontosyllis enopla* (Annelida: Polychaeta). *Invertebrate Biology* **1995**, *114*, 236–247.

(135) Barnes, R. D. Tube-Building and Feeding in the Chaetopterid Polychaete, *Spiochaetopterus oculatus*. *Biol. Bull.* **1964**, *127* (3), 397–412.

(136) Enders, H. E. In *Observations on the Formation and Enlargement of the Tubes of the Marine Annelid, (Chaetopterus variopedatus)*, Proceedings of the Indiana Academy of Science, 1907; pp 128–135.

(137) Shah, D. U.; Vollrath, F.; Porter, D.; Stires, J.; Deheyn, D. D. Housing Tubes From the Marine Worm *Chaetopterus* sp.: Biomaterials with Exceptionally Broad Thermomechanical Properties. *J. R. Soc., Interface* **2014**, *11* (98), 20140525.

(138) Joo, M. S.; Suh, D. W.; Bhadeshia, H. K. D. H. Mechanical Anisotropy in Steels for Pipelines. *ISIJ Int.* **2013**, *53* (8), 1305–1314.

(139) De Meulenaere, E.; Puzzanghera, C.; Dehenn, D. D. Self-powered bioluminescence in a marine tube worm. *FASEB J.* **2020**, *34* (S1), 1–1.

(140) Dehenn, D. D.; Enzor, L. A.; Dubowitz, A.; Urbach, J. S.; Blair, D. Optical and Physicochemical Characterization of the Luminous Mucous Secreted by the Marine Worm *Chaetopterus Sp.* *Physiol. Biochem. Zool.* **2013**, *86* (6), 702–715.

(141) Rawat, R.; Dehenn, D. D. Evidence that Ferritin is Associated with Light Production in the Mucus of the Marine Worm *Chaetopterus*. *Sci. Rep.* **2016**, *6* (1), 1–14.

(142) De Meulenaere, E.; Bailey, J. B.; Tezcan, F. A.; Dehenn, D. D. First Biochemical and Crystallographic Characterization of a Fast-Performing Ferritin From a Marine Invertebrate. *Biochem. J.* **2017**, *474* (24), 4193–4206.

(143) Weigand, W.; Messmore, A.; Tu, J.; Morales-Sanz, A.; Blair, D.; Dehenn, D.; Urbach, J.; Robertson-Anderson, R. Active Micro-rheology Determines Scale-Dependent Material Properties of *Chaetopterus* Mucus. *PLoS One* **2017**, *12* (5), e0176732.

(144) Martin, N.; Anctil, M. Luminescence Control in the Tube-Worm *Chaetopterus variopedatus*: Role of Nerve Cord and Photogenic Gland. *Biol. Bull.* **1984**, *166* (3), 583–593.

(145) Enders, H. E. *A Study of the Life History and Habits of Chaetopterus variopedatus*; Johns Hopkins University: 1906.

(146) Flood, P.; Fiala-Médioni, A. Structure of the Mucous Feeding Filter of *Chaetopterus variopedatus* (Polychaeta). *Mar. Biol.* **1982**, *72* (1), 27–33.

(147) Lau, E. S.; Oakley, T. H. Multi-Level Convergence of Complex Traits and the Evolution of Bioluminescence. *Biol. Rev.* **2020**. In review.

(148) Stabili, L.; Licciano, M.; Giangrande, A.; Gerardi, C.; De Pascali, S. A.; Fanizzi, F. P. First insight on the mucus of the annelid *Myxicola infundibulum* (Polychaeta, Sabellidae) as a potential prospect for drug discovery. *Mar. Drugs* **2019**, *17* (7), 396.

(149) Runnegar, B. Early Evolution of the Mollusca: The Fossil Record. In *Origin and Evolutionary Radiation of the Mollusca* Taylor, J., Ed., Oxford University Press: Oxford, 1996; pp 77–87.

(150) Barnes, R. S. K.; Calow, P.; Olive, P. J. W. *The Invertebrates: A New Synthesis*; Blackwell Scientific Publications Ltd: Oxford, 1993; p viii + 488 pp.

(151) Tacon, A. G. Trends in global aquaculture and aquafeed production: 2000–2017. *Reviews in Fisheries Science & Aquaculture* **2020**, *28* (1), 43–56.

(152) Newell, R. I. Ecosystem Influences of Natural and Cultivated Populations of Suspension-Feeding Bivalve Molluscs: A Review. *Journal of Shellfish Research* **2004**, *23* (1), 51–62.

(153) Robledo, J. A. F.; Yadavalli, R.; Allam, B.; Pales-Espinosa, E.; Gerdol, M.; Greco, S.; Stevick, R. J.; Gómez-Chiarri, M.; Zhang, Y.; Heil, C. A. From the Raw Bar to the Bench: Bivalves as Models for Human Health. *Dev. Comp. Immunol.* **2019**, *92*, 260.

(154) Simkiss, K. In *The Molluscan Epidermis and Its Secretions*, Symposium of the Zoological Society of London, 1977; pp 35–76.

(155) Braun, P. C.; Brousseau, D. J.; Leclair, G. R. Microbial Inhibition by Bacteria Isolated from Pallial Cavity Fluids and Associated Mucus of the Eastern Oyster *Crassostrea virginica* (Gmelin). *J. Shellfish Res.* **2019**, *38* (3), 565–527.

(156) Fernández-Boo, S.; Gervais, O.; Prado-Alvarez, M.; Chollet, B.; Claverol, S.; Lecadet, C.; Dubreuil, C.; Arzul, I. Is pallial mucus involved in *Ostrea edulis* defenses against the parasite *Bonamia ostreae*? *J. Invertebr. Pathol.* **2020**, *169*, 107259.

(157) Allam, B.; Espinosa, E. P. 12 - Mucosal Immunity in Mollusks. In *Mucosal Health in Aquaculture*, Beck, B. H.; Peatman, E., Eds.; Academic Press: San Diego, 2015; pp 325–370.

(158) Davies, M. S.; Hawkins, S., Mucus From Marine Molluscs. In *Advances in Marine Biology*; Elsevier: 1998; Vol. 34, pp 1–71.

(159) Mardones-Toledo, D. A.; Montory, J. A.; Joyce, A.; Thompson, R. J.; Diederich, C. M.; Pechenik, J. A.; Mardones, M. L.; Chaparro, O. R. Brooding in the Chilean Oyster *Ostrea chilensis*: Unexpected Complexity in the Movements of Brooded Offspring within the Mantle Cavity. *PLoS One* **2015**, *10* (4), e0122859.

(160) Lau, Y.-T.; Sussman, L.; Espinosa, E. P.; Katalay, S.; Allam, B. Characterization of Hemocytes from Different Body Fluids of the Eastern Oyster *Crassostrea virginica*. *Fish Shellfish Immunol.* **2017**, *71*, 372–379.

(161) Beninger, P. G.; St-Jean, S.; Poussart, Y.; Ward, J. E. Gill Function and Mucocyte Distribution in *Placopecten magellanicus* and *Mytilus edulis* (Mollusca: Bivalvia): The Role of Mucus in Particle Transport. *Mar. Ecol.: Prog. Ser.* **1993**, *98* (3), 275–282.

(162) Barillé, L.; Cognie, B. Revival Capacity of Diatoms in Bivalve Pseudofaeces and Faeces. *Diatom Research* **2000**, *15* (1), 11–17.

(163) Urrutia, M.; Navarro, E.; Ibarrola, I.; Iglesias, J. Preingestive Selection Processes in the Cockle *Cerastoderma edule*: Mucus Production Related to Rejection of Pseudofaeces. *Mar. Ecol.: Prog. Ser.* **2001**, *209*, 177–187.

(164) Pales Espinosa, E.; Allam, B. Reverse Genetics Demonstrate the Role of Mucosal C-Type Lectins in Food Particle Selection in the Oyster *Crassostrea virginica*. *J. Exp. Biol.* **2018**, *221* (6), jeb174094.

(165) Pales Espinosa, E.; Perrigault, M.; Ward, J. E.; Shumway, S. E.; Allam, B. Lectins Associated with the Feeding Organs of the Oyster *Crassostrea virginica* Can Mediate Particle Selection. *Biol. Bull.* **2009**, *217* (2), 130–141.

(166) Pales Espinosa, E.; Perrigault, M.; Allam, B. Identification and Molecular Characterization of a Mucosal Lectin (MeML) from the Blue Mussel *Mytilus edulis* and its Potential Role in Particle Capture. *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.* **2010**, *156* (4), 495–501.

(167) Pales Espinosa, E.; Hassan, D.; Ward, J. E.; Shumway, S. E.; Allam, B. Role of Epicellular Molecules in the Selection of Particles by the Blue Mussel, *Mytilus edulis*. *Biol. Bull.* **2010**, *219* (1), 50–60.

(168) Pales Espinosa, E.; Koller, A.; Allam, B. Proteomic Characterization of Mucosal Secretions in the Eastern Oyster, *Crassostrea virginica*. *J. Proteomics* **2016**, *132*, 63–76.

(169) McDermott, A. J.; Huffnagle, G. B. The Microbiome and Regulation of Mucosal Immunity. *Immunology* **2014**, *142* (1), 24–31.

(170) Vaquer, C. D. A.; Lam-Höai, T.; Rougier, C.; Mazouni, N.; Lautier, J.; Collos, Y.; Le Gall, S. Feeding Rate of the Oyster *Crassostrea gigas* in a Natural Planktonic Community of the Mediterranean Thau Lagoon. *Mar. Ecol.: Prog. Ser.* **2000**, *205*, 171–184.

(171) Burreson, E. M.; Ford, S. E. A Review of Recent Information on the *Haplosporidia*, with Special Reference to Haplosporidium nelsoni (MSX Disease). *Aquat. Living Resour.* **2004**, *17* (4), 499–517.

(172) Villalba, A.; Reece, K. S.; Ordás, M. C.; Casas, S. M.; Figueras, A. Perkinsosis in Molluscs: A Review. *Aquat. Living Resour.* **2004**, *17* (4), 411–432.

(173) Dubilier, N.; Bergin, C.; Lott, C. Symbiotic Diversity in Marine Animals: The Art of Harnessing Chemosynthesis. *Nat. Rev. Microbiol.* **2008**, *6* (10), 725.

(174) Dahl, S. F.; Thiel, J.; Allam, B. Field Performance and QPX Disease Progress in Cultured and Wild-Type Strains of *Mercenaria mercenaria* in New York Waters. *J. Shellfish Res.* **2010**, *29* (1), 83–91.

(175) Allam, B.; Carden, W. E.; Ward, J. E.; Ralph, G.; Winnicki, S.; Espinosa, E. P. Early Host-Pathogen Interactions in Marine Bivalves: Evidence that the Alveolate Parasite *Perkinsus marinus* Infects Through the Oyster Mantle During Rejection of Pseudofaeces. *J. Invertebr. Pathol.* **2013**, *113* (1), 26–34.

(176) Pales Espinosa, E.; Winnicki, S.; Allam, B. Early Host-Pathogen Interactions in Marine Bivalves: Pallial Mucus of *Crassostrea virginica* Modulates the Growth and Virulence of Its Pathogen *Perkinsus marinus*. *Dis. Aquat. Org.* **2013**, *104* (3), 237–247.

(177) Barr, J. J.; Auro, R.; Furlan, M.; Whiteson, K. L.; Erb, M. L.; Pogliano, J.; Stotland, A.; Wolkowicz, R.; Cutting, A. S.; Doran, K. S.; Salamon, P.; Youle, M.; Rohwer, F. Bacteriophage Adhering to Mucus Provide a Non-Host-Derived Immunity. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (26), 10771–10776.

(178) Russell, M. W.; Mestecky, J.; Strober, W.; Lambrecht, B. N.; Kelsall, B. L.; Cheroutre, H. Overview: the mucosal immune system. In *Mucosal Immunology*; Academic Press: Cambridge, 2015; pp 3–8.

(179) Foulon, V.; Boudry, P.; Artigaud, S.; Guérard, F.; Hellio, C. In silico analysis of Pacific oyster (*Crassostrea gigas*) transcriptome over developmental stages reveals candidate genes for larval settlement. *Int. J. Mol. Sci.* **2019**, *20* (1), 197.

(180) Harada, N.; Iijima, S.; Kobayashi, K.; Yoshida, T.; Brown, W. R.; Hibi, T.; Oshima, A.; Morikawa, M. Human IgGFc binding protein (FcγBP) in colonic epithelial cells exhibits mucin-like structure. *J. Biol. Chem.* **1997**, *272* (24), 15232–15241.

(181) Petrou, G.; Crouzier, T. Mucins as Multifunctional Building Blocks of Biomaterials. *Biomater. Sci.* **2018**, *6* (9), 2282–2297.

(182) Gorson, J.; Ramrattan, G.; Verdes, A.; Wright, E. M.; Kantor, Y.; Rajaram Srinivasan, R.; Musunuri, R.; Packer, D.; Albano, G.; Qiu, W.-G. Molecular Diversity and Gene Evolution of the Venom Arsenal of Terebridae Predatory Marine Snails. *Genome Biol. Evol.* **2015**, *7* (6), 1761–1778.

(183) Robinson, S. D.; Safavi-Hemami, H.; McIntosh, L. D.; Purcell, A. W.; Norton, R. S.; Papenfuss, A. T. Diversity of Conotoxin Gene Superfamilies in the Venomous Snail, *Conus victoriae*. *PLoS One* **2014**, *9* (2), e87648.

(184) Daniel, J. T.; Clark, R. J. G-Protein Coupled Receptors Targeted by Analgesic Venom Peptides. *Toxins* **2017**, *9* (11), 372.

(185) Verdes, A.; Anand, P.; Gorson, J.; Jannetti, S.; Kelly, P.; Leffler, A.; Simpson, D.; Ramrattan, G.; Holford, M. From Mollusks to Medicine: A Venomics Approach for the Discovery and Characterization of Therapeutics from Terebridae Peptide Toxins. *Toxins* **2016**, *8* (4), 117.

(186) Miljanich, G. Ziconotide: Neuronal Calcium Channel Blocker for Treating Severe Chronic Pain. *Curr. Med. Chem.* **2004**, *11* (23), 3029–3040.

(187) Barrientos, Z., Demography of the Land Snail *Tikoconus* (*Tikoconus*) *costarricanus* (Stylommatophora: Euconulidae) in Tropical Low Montane and Premontane Forests, Costa Rica. *Rev. Biol. Trop.* **2019**, *67* (6). DOI: 10.15517/rbt.v67i6.36201

(188) Liu, C.; Ren, Y.; Li, Z.; Hu, Q.; Yin, L.; Qiao, X.; Zhang, Y.; Xing, L.; Xi, Y.; Jiang, F., Giant African snail genomes provide insights into molluscan whole-genome duplication and aquatic-terrestrial transition. *bioRxiv* **2020**.

(189) Co, J. Y.; Cárcamo-Oyarce, G.; Billings, N.; Wheeler, K. M.; Grindy, S. C.; Holten-Andersen, N.; Ribbeck, K. Mucins Trigger Dispersal of *Pseudomonas aeruginosa* Biofilms. *NPJ. Biofilms and Microbiomes* **2018**, *4* (1), 1–8.

(190) Etim, L. B.; Aleruchi, C.; Obande, G. A. Antibacterial Properties of Snail Mucus on Bacteria Isolated from Patients with Wound Infection. *Br. Microbiol. Res. J.* **2016**, *11*, 1–9.

(191) Ganesan, D. S.; Mohamed, M. A. R.; Lhanzin, P.; Neelan, K.; Vadakkuvaselvi, L.; Subramani, S.; Chinnasamy, A. Antiproliferative Effect of Crude Venom from *Conus virgo* on Human Lung Cancer Cell Line and Toxicity Assessment on Adult Zebra Fish (*Danio rerio*). *Indian J. Pharm. Educ.* **2019**, *54* (1), 85–94.

(192) Holford, M.; Ogunwobi, O.; Huaman, J.; Filipenko, P.; Lyudmer, M.; Hossain, M.; Santamaría, C.; Huang, K.; Anand, P. Antitumor Effects of Tv1 Venom Peptide in Liver Cancer. *bioRxiv* **2019**, 518340.

(193) Dickson, I. Snail Venom for Gut Pain? *Nat. Rev. Gastroenterol. Hepatol.* **2016**, *13* (4), 189–190.

(194) Safavi-Hemami, H.; Brogan, S. E.; Olivera, B. M. Pain Therapeutics from Cone Snail Venoms: From Ziconotide to Novel Non-Opioid Pathways. *J. Proteomics* **2019**, *190*, 12–20.

(195) Clulow, S.; Gould, J.; James, H.; Stockwell, M.; Clulow, J.; Mahony, M. Elevated Salinity Blocks Pathogen Transmission and Improves Host Survival from the Global Amphibian Chytrid Pandemic: Implications for Translocations. *J. Appl. Ecol.* **2018**, *55* (2), 830–840.

(196) Hayward, M. W.; Scanlon, R. J.; Callen, A.; Howell, L. G.; Klop-Toker, K. L.; Di Blanco, Y.; Balkenhol, N.; Bugir, C. K.; Campbell, L.; Caravaggi, A.; Chalmers, A. C.; Clulow, J.; Clulow, S.; Cross, P.; Gould, J. A.; Griffin, A. S.; Heurich, M.; Howe, B. K.; Jachowski, D. S.; Jhala, Y. V.; Krishnamurthy, R.; Kowalczyk, R.; Lenga, D. J.; Linnell, J. D. C.; Marnewick, K. A.; Moehrenschlager, A.; Montgomery, R. A.; Osipova, L.; Peneaux, C.; Rodger, J. C.; Sales, L. P.; Seeto, R. G. Y.; Shuttleworth, C. M.; Somers, M. J.; Tamessar, C. T.; Upton, R. M. O.; Weise, F. J. Reintroducing Rewilding to Restoration – Rejecting the Search for Novelty. *Biological Conservation* **2019**, *233*, 255–259.

(197) Humbert, A. Études Sur Quelques Mollusques Terrestres Nouveaux Ou Peu Connus. *Mémoires de la Société de Physique Et d'Histoire Naturelle de Genève* **1863**, *17*, 109–128.

(198) McMichael, D. F.; Iredale, T. The Land and Freshwater Mollusca of Australia. In *Biogeography and Ecology in Australia*; Springer: 1959; pp 224–245.

(199) Burton, D. Anatomical Studies on Australian, New Zealand, and Subantarctic Athonacophoridae (Gastropoda: Pulmonata). *N. Z. J. Zool.* **1980**, *7* (2), 173–198.

(200) Martin, A.; Deyrup-Olsen, I. Function of the Epithelial Channel Cells of the Body Wall of a Terrestrial Slug, *Ariolimax columbianus*. *Journal of Experimental Biology* **1986**, *121* (1), 301–314.

(201) Deyrup-Olsen, I.; Luchtel, D.; Martin, A. Components of Mucus of Terrestrial Slugs (Gastropoda). *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **1983**, *245* (3), R448–R452.

(202) Wilks, A. M.; Rabice, S. R.; Garbacz, H. S.; Harro, C. C.; Smith, A. M. Double Network Gels and the Toughness of Terrestrial Slug Glue. *J. Exp. Biol.* **2015**, *218*, 3128.

(203) South, A. *Terrestrial Slugs: Biology, Ecology and Control*; Springer Science & Business Media: London, 1992.

(204) Drewes, R. C.; Roth, B. Snail-Eating Frogs from the Ethiopian Highlands: A New Anuran Specialization. *Zoological Journal of the Linnean Society* **1981**, *72* (3), 267–287.

(205) von Byern, J.; Cyran, N.; Klepal, W.; Nödl, M. T.; Klinger, L. Characterization of the Adhesive Dermal Secretion of *Euprymna scolopes* Berry, 1913 (Cephalopoda). *Zoology* **2017**, *120*, 73–82.

(206) Gong, J. P. Why are Double Network Hydrogels so Tough? *Soft Matter* **2010**, *6* (12), 2583–2590.

(207) Haque, M. A.; Kurokawa, T.; Gong, J. P. Super Tough Double Network Hydrogels and Their Application as Biomaterials. *Polymer* **2012**, *53* (9), 1805–1822.

(208) Webber, R. E.; Creton, C.; Brown, H. R.; Gong, J. P. Large Strain Hysteresis and Mullins Effect of Tough Double-Network Hydrogels. *Macromolecules* **2007**, *40* (8), 2919–2927.

(209) Braun, M.; Menges, M.; Opoku, F.; Smith, A. M. The Relative Contribution of Calcium, Zinc and Oxidation-Based Cross-Links to the Stiffness of *Arion subfuscus* Glue. *J. Exp. Biol.* **2013**, *216* (8), 1475–1483.

(210) Smith, A. M. Multiple Metal-Based Cross-Links: Protein Oxidation and Metal Coordination in a Biological Glue. *Biological and Biomimetic Adhesives: Challenges and Opportunities* **2013**, 3–15.

(211) Foltan, P. Influence of Slug Defence Mechanisms on the Prey Preferences of the Carabid Predator *Pterostichus melanarius* (Coleoptera: Carabidae). *Eur. J. Entomol.* **2004**, *101* (3), 359–364.

(212) Smith, A. M. Gastropod Secretory Glands and Adhesive Gels. In *Biological Adhesive Systems: From Nature to Technical and Medical Application*; von Byern, J.; Grunwald, I., Eds.; Springer Vienna: Vienna, 2010; pp 41–51.

(213) Ou, Q.; Shu, D.; Mayer, G. *Cambrian lobopodians* and Extant Onychophorans Provide New Insights into Early Cephalization in Panarthropoda. *Nat. Commun.* **2012**, *3* (1), 1–7.

(214) Rota-Stabelli, O.; Daley, A. C.; Pisani, D. Molecular Timetrees Reveal a Cambrian Colonization of Land and a New Scenario for Ecdysozoan Evolution. *Curr. Biol.* **2013**, *23* (5), 392–398.

(215) Oliveira, I. S.; Read, V. M. S. J.; Mayer, G. A World Checklist of Onychophora (Velvet Worms), with Notes on Nomenclature and Status of Names. *ZooKeys* **2012**, *211*, 1–70.

(216) Baer, A.; Schmidt, S.; Haensch, S.; Eder, M.; Mayer, G.; Harrington, M. J. Mechanoresponsive Lipid-Protein Nanoglobules

Facilitate Reversible Fibre Formation in Velvet Worm Slime. *Nat. Commun.* **2017**, *8* (1), 974.

(217) Bursell, E.; Ewer, D. W. On the Reactions to Humidity of *Peripatopsis moseleyi* (Wood-Mason). *Journal of Experimental Biology* **1950**, *26* (4), 335–353.

(218) Concha, A.; Mellado, P.; Morera-Brenes, B.; Sampaio Costa, C.; Mahadevan, L.; Monge-Nájera, J. Oscillation of the Velvet Worm Slime Jet by Passive Hydrodynamic Instability. *Nat. Commun.* **2015**, *6*, 6292.

(219) Ghiselin, M. T. *Peripatus* as a Living Fossil. In *Living Fossils*; Eldredge, N., Stanley, S. M., Eds.; Springer Verlag: New York, 1984; pp 214–217.

(220) Hamer, M. L.; Samways, M. J.; Ruhberg, H. A Review of the Onychophora of South Africa, with Discussion of Their Conservation. *Annals of the Natal Museum* **1997**, *38*, 283–312.

(221) Heatley, N. G. The Digestive Enzymes of the Onychophora (*Peripatopsis* spp.). *Journal of Experimental Biology* **1936**, *13* (3), 329–343.

(222) Manton, S. M.; Heatley, N. Y. Studies on the Onychophora. IV, II The Feeding, Digestion, Excretion and Food Storage of *Peripatopsis*. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **1937**, *227* (546), 441–464.

(223) Monge-Nájera, J.; Barrientos, Z.; Aguilar, F. Behavior of *Epiperipatus bilolleyi* (Onychophora: Peripatidae) Under Laboratory Conditions. *Revista de Biología Tropical* **1993**, *41* (3), 689–696.

(224) Read, V. M. S.; Hughes, R. N. Feeding Behavior and Prey Choice in *Macroperipatus torquatus* (Onychophora). *Proceedings of the Royal Society Series B-Biological Sciences* **1987**, *230* (1261), 483–506.

(225) Storch, V.; Ruhberg, H. Onychophora. In *Microscopic Anatomy of Invertebrates*, Harrison, F. W., Rice, M. E., Eds.; Wiley-Liss: New York, 1993; Vol. 12, pp 11–56.

(226) Reid, A. L. Review of the Peripatopsidae (Onychophora) in Australia, with Comments on Peripatopsid Relationships. *Invertebr. Syst.* **1996**, *10* (4), 663–936.

(227) Baer, A.; de Sena Oliveira, I.; Steinhagen, M.; Beck-Sickinger, A. G.; Mayer, G. Slime Protein Profiling: a Non-Invasive Tool for Species Identification in Onychophora (Velvet Worms). *Journal of Zoological Systematics and Evolutionary Research* **2014**, *52* (4), 265–272.

(228) Baer, A.; Hänsch, S.; Mayer, G.; Harrington, M. J.; Schmidt, S. Reversible Supramolecular Assembly of Velvet Worm Adhesive Fibers via Electrostatic Interactions of Charged Phosphoproteins. *Biomacromolecules* **2018**, *19* (10), 4034–4043.

(229) Baer, A.; Horbelt, N.; Nijemeisland, M.; Garcia, S. J.; Fratzl, P.; Schmidt, S.; Mayer, G.; Harrington, M. J. Shear-Induced  $\beta$ -Crystallite Unfolding in Condensed Phase Nanodroplets Promotes Fiber Formation in a Biological Adhesive. *ACS Nano* **2019**, *13* (5), 4992–5001.

(230) Benkendorff, K.; Beardmore, K.; Gooley, A. A.; Packer, N. H.; Tait, N. N. Characterisation of the Slime Gland Secretion from the Peripatus, *Euperipatoides kanangrensis* (Onychophora: Peripatopsidae). *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* **1999**, *124* (4), 457–465.

(231) Haritos, V. S.; Niranjane, A.; Weisman, S.; Trueman, H. E.; Sriskantha, A.; Sutherland, T. D. Harnessing Disorder: Onychophorans Use Highly Unstructured Proteins, Not Silks, for Prey Capture. *Proc. R. Soc. London, Ser. B* **2010**, *277* (1698), 3255–3263.

(232) Mora, M.; Herrera, A.; León, P. Análisis Electroforético de las Secreciones Adhesivas de Onicóforos del Género *Epiperipatus* (Onychophora: Peripatidae). *Revista de Biología Tropical* **1996**, *44* (1), 147–152.

(233) Röper, H. Analytical Investigations on the Defensive Secretions from *Peripatopsis moseleyi* (Onychophora). *Z. Naturforsch., C: J. Biosci.* **1977**, *32*, 57–60.

(234) Ruhberg, H.; Storch, V. Über Wehrdrüsen und Wehrsekret von *Peripatopsis moseleyi* (Onychophora). *Zoologischer Anzeiger* **1977**, *198*, 9–19.

(235) Toledo-Matus, X.; Rivera-Velázquez, G.; Monge-Nájera, J.; Morera-Brenes, B. An Undescribed Species of Velvet Worm from Chiapas, Mexico (Onychophora: Peripatidae). *Cuadernos de Investigación UNED* **2018**, *10* (1), 190–191.

(236) Morera-Brenes, B.; Monge-Nájera, J. A New Giant Species of Placented Worm and the Mechanism by which Onychophorans Weave Their Nets (Onychophora: Peripatidae). *Rev. Biol. Trop.* **2010**, *58* (4), 1127–1142.

(237) Morera-Brenes, B.; Monge-Nájera, J. *Epiperipatus hilkae*, n. sp. from Costa Rica (Onychophora: Peripatidae). *Revista de Biología Tropical* **1990**, *44* (4), 449–455.

(238) González, J. P. B.; Vega-Hidalgo, Á.; Monge-Nájera, J. Feeding Behavior of Costa Rican Velvet Worms: Food Hiding, Parental Feeding Investment and Ontogenetic Diet Shift (Onychophora: Peripatidae). *UNED Research Journal* **2019**, *11* (2), 85–88.

(239) Delsuc, F.; Brinkmann, H.; Chourrout, D.; Philippe, H. Tunicates and Not Cephalochordates are the Closest Living Relatives of Vertebrates. *Nature* **2006**, *439* (7079), 965–968.

(240) Hosp, J.; Sagane, Y.; Danks, G.; Thompson, E. M. The Evolving Proteome of a Complex Extracellular Matrix, the *Oikopleura* House. *PLoS One* **2012**, *7* (7), e40172.

(241) Katija, K.; Troni, G.; Daniels, J.; Lance, K.; Sherlock, R. E.; Sherman, A. D.; Robison, B. H. Revealing enigmatic mucus structures in the deep sea using DeepPIV. *Nature* **2020**, *583*, 1–5.

(242) Conley, K. R.; Lombard, F.; Sutherland, K. R. Mammoth grazers on the ocean's minuteness: a review of selective feeding using mucous meshes. *Proc. R. Soc. London, Ser. B* **2018**, *285* (1878), 20180056.

(243) Deibel, D. Feeding and Metabolism of Appendicularia. *Biology of Pelagic Tunicates* **1998**, 139–149.

(244) Sagane, Y.; Zech, K.; Bouquet, J.-M.; Schmid, M.; Bal, U.; Thompson, E. M. Functional Specialization of Cellulose Synthase Genes of Prokaryotic Origin in Chordate Larvaceans. *Development* **2010**, *137* (9), 1483–1492.

(245) Sagane, Y.; Hosp, J.; Zech, K.; Thompson, E. M. Cytoskeleton-mediated templating of complex cellulose-scaffolded extracellular structure and its association with oikosins in the urochordate *Oikopleura*. *Cell. Mol. Life Sci.* **2011**, *68* (9), 1611–1622.

(246) Lorenzo, P.; Bayliss, M. T.; Heinegård, D. A Novel Cartilage Protein (CILP) Present in the Mid-Zone of Human Articular Cartilage Increases with Age. *J. Biol. Chem.* **1998**, *273* (36), 23463–23468.

(247) Bayliss, M. T.; Venn, M.; Maroudas, A.; Ali, S. Y. Structure of Proteoglycans from Different Layers of Human Articular Cartilage. *Biochem. J.* **1983**, *209* (2), 387–400.

(248) Freedman, C. R.; Fudge, D. S. Hagfish Houdin: Biomechanics and Behavior of Squeezing Through Small Openings. *J. Exp. Biol.* **2017**, *220* (5), 822–827.

(249) Martini, F. H., The Ecology of Hagfishes. In *The Biology of Hagfishes*; Springer: 1998; pp 57–77.

(250) Newby, W. W. The Slime Glands and Thread Cells of the Hagfish, *Polistotrema stouti*. *J. Morphol.* **1946**, *78* (3), 397–409.

(251) Koch, E. A.; Spitzer, R. H.; Pithawalla, R. B.; Downing, S. W. Keratin-like Components of Gland Thread Cells Modulate the Properties of Mucus from Hagfish (*Eptatretus stoutii*). *Cell Tissue Res.* **1991**, *264* (1), 79–86.

(252) Knapp, L.; Mincarone, M. M.; Harwell, H.; Polidoro, B.; Sanciangco, J.; Carpenter, K. Conservation Status of the World's Hagfish Species and the Loss of Phylogenetic Diversity and Ecosystem Function. *Aquatic Conservation: Marine and Freshwater Ecosystems* **2011**, *21* (5), 401–411.

(253) Lim, J.; Fudge, D. S.; Levy, N.; Gosline, J. M. Hagfish Slime Ecomechanics: Testing the Gill-Clogging Hypothesis. *J. Exp. Biol.* **2006**, *209* (4), 702–710.

(254) Zintzen, V.; Roberts, C. D.; Anderson, M. J.; Stewart, A. L.; Struthers, C. D.; Harvey, E. S. Hagfish Predatory Behaviour and Slime Defence Mechanism. *Sci. Rep.* **2011**, *1* (1), 131.

(255) Fernholm, B. Thread Cells from the Slime Glands of Hagfish (Myxinidae). *Acta Zool.* **1981**, *62* (3), 137–145.

(256) Fudge, D. S.; Schorno, S. The Hagfish Gland Thread Cell: A Fiber-Producing Cell Involved in Predator Defense. *Cells* **2016**, *5* (2), 25.

(257) Koch, E. A.; Spitzer, R. H.; Pithawalla, R. B.; Parry, D. An Unusual Intermediate Filament Subunit from the Cytoskeletal Biopolymer Released Extracellularly into Seawater by the Primitive Hagfish (*Eptatretus stoutii*). *Journal of Cell Science* **1994**, *107* (11), 3133–3144.

(258) Koch, E. A.; Spitzer, R. H.; Pithawalla, R. B.; Castillos, F. A., III; Parry, D. A. Hagfish Biopolymer: A Type I/Type II Homologue of Epidermal Keratin Intermediate Filaments. *Int. J. Biol. Macromol.* **1995**, *17* (5), 283–292.

(259) Salo, W. L.; Downing, S. W.; Lidinsky, W. A.; Gallagher, W. H.; Spitzer, R. H.; Koch, E. A. Fractionation of Hagfish Slime Gland Secretions: Partial Characterization of the Mucous Vesicle Fraction. *Prep. Biochem.* **1983**, *13* (2), 103–135.

(260) Luchtel, D.; Martin, A.; Deyrup-Olsen, I. Ultrastructure and Permeability Characteristics of the Membranes of Mucous Granules of the Hagfish. *Tissue Cell* **1991**, *23* (6), 939–948.

(261) Wiens, J. J.; Graham, C. H.; Moen, D. S.; Smith, S. A.; Reeder, T. W. Evolutionary and Ecological Causes of the Latitudinal Diversity Gradient in Hylid Frogs: Treefrog Trees Unearth the Roots of High Tropical Diversity. *Am. Nat.* **2006**, *168* (5), 579–596.

(262) Clarke, B. T. The Natural History of Amphibian Skin Secretions, Their Normal Functioning and Potential Medical Applications. *Biol. Rev. Cambridge Philos. Soc.* **1997**, *72* (3), 365–379.

(263) Hill, I. D.; Dong, B.; Barnes, W. J. P.; Ji, A.; Endlein, T. The biomechanics of tree frogs climbing curved surfaces: a gripping problem. *J. Exp. Biol.* **2018**, *221* (5), jeb168179.

(264) Federle, W.; Barnes, W.; Baumgartner, W.; Drechsler, P.; Smith, J. Wet But Not Slippery: Boundary Friction in Tree Frog Adhesive Toe Pads. *J. R. Soc., Interface* **2006**, *3* (10), 689–697.

(265) Endlein, T.; Ji, A.; Samuel, D.; Yao, N.; Wang, Z.; Barnes, W. J. P.; Federle, W.; Kappl, M.; Dai, Z. Sticking Like Sticky Tape: Tree Frogs Use Friction Forces to Enhance Attachment on Overhanging Surfaces. *J. R. Soc., Interface* **2013**, *10* (80), 20120838.

(266) Langowski, J. K.; Singla, S.; Nyarko, A.; Schipper, H.; van den Berg, F. T.; Kaur, S.; Astley, H. C.; Gussekloo, S. W.; Dhinojwala, A.; van Leeuwen, J. L. Comparative and Functional Analysis of the Digital Mucus Glands and Secretions of Tree Frogs. *Front. Zool.* **2019**, *16* (1), 19.

(267) Khannoob, E.; Crawford, N.; Barnes, W. J. P.; Endlein, T., Tree Frog Adhesion to Surfaces Differing in Surface Energy. **2020**. In preparation.

(268) Hanna, G.; Barnes, W. J. P. Adhesion and Detachment of the Toe Pads of Tree Frogs. *Journal of Experimental Biology* **1991**, *155* (1), 103–125.

(269) Endlein, T.; Barnes, W. J. P.; Samuel, D. S.; Crawford, N. A.; Biaw, A. B.; Grafe, U. Sticking Under Wet Conditions: the Remarkable Attachment Abilities of the Torrent Frog, *Staurois guttatus*. *PLoS One* **2013**, *8* (9), e73810.

(270) Crawford, N.; Endlein, T.; Barnes, W. J. P. Self-Cleaning in Tree Frog Toe Pads; A Mechanism for Recovering From Contamination without the Need for Grooming. *J. Exp. Biol.* **2012**, *215* (22), 3965–3972.

(271) Barnes, W. J. P.; Oines, C.; Smith, J. M. Whole Animal Measurements of Shear and Adhesive Forces in Adult Tree Frogs: Insights into Underlying Mechanisms of Adhesion Obtained from Studying the Effects of Size and Scale. *J. Comp. Physiol., A* **2006**, *192* (11), 1179–1191.

(272) Scholz, I.; Barnes, W. J. P.; Smith, J. M.; Baumgartner, W. Ultrastructure and Physical Properties of an Adhesive Surface, the Toe Pad Epithelium of the Tree Frog, *Litoria caerulea* White. *J. Exp. Biol.* **2009**, *212* (2), 155–162.

(273) Bonduelle, C.; Lecommandoux, S. Synthetic Glycopolypeptides as Biomimetic Analogues of Natural Glycoproteins. *Biomacromolecules* **2013**, *14* (9), 2973–2983.

(274) Yang, Z.; Drew, D. P.; Jørgensen, B.; Mandel, U.; Bach, S. S.; Ulvskov, P.; Levery, S. B.; Bennett, E. P.; Clausen, H.; Petersen, B. L. Engineering Mammalian Mucin-Type O-Glycosylation in Plants. *J. Biol. Chem.* **2012**, *287* (15), 11911–11923.

(275) Javitt, G.; Khmelitsky, L.; Albert, L.; Elad, N.; Ilani, T.; Diskin, R.; Fass, D., Assembly Mechanism of Mucin and von Willebrand Factor Polymers. *bioRxiv* **2020**.

(276) Gouyer, V.; Dubuquoy, L.; Robbe-Masselot, C.; Neut, C.; Singer, E.; Plet, S.; Geboes, K.; Desreumaux, P.; Gottrand, F.; Desseyn, J.-L. Delivery of a Mucin Domain Enriched in Cysteine Residues Strengthens the Intestinal Mucus barrier. *Sci. Rep.* **2015**, *5* (1), 9577.

(277) Guyonnet Duperat, V.; Audie, J. P.; Debailleul, V.; Laine, A.; Buisine, M. P.; Galiegue-Zouitina, S.; Pigny, P.; Degand, P.; Aubert, J. P.; Porchet, N. Characterization of the Human Mucin Gene MUC5AC: A Consensus Cysteine-Rich Domain for 11p15 Mucin Genes? *Biochem. J.* **1995**, *305* (1), 211–219.

(278) Chen, G.; Tao, L.; Mantovani, G.; Geng, J.; Nyström, D.; Haddleton, D. M. A Modular Click Approach to Glycosylated Polymeric Beads: Design, Synthesis and Preliminary Lectin Recognition Studies. *Macromolecules* **2007**, *40* (21), 7513–7520.

(279) Thak, E. J.; Yoo, S. J.; Moon, H. Y.; Kang, H. A. Yeast Synthetic Biology for Designed Cell Factories Producing Secretory Recombinant Proteins. *FEMS Yeast Res.* **2020**, *20* (2). DOI: [10.1093/femsyr/foaa009](https://doi.org/10.1093/femsyr/foaa009)

(280) Wang, H. H.; Church, G. M. Multiplexed Genome Engineering and Genotyping Methods: Applications for Synthetic Biology and Metabolic Engineering. *Methods Enzymol.* **2011**, *498*, 409–426.

(281) Kai-Steffen Kranni, H. S. *Emerging Bioinspired Polymers: Glycopolypeptides*; Royal Society of Chemistry, 2014, 10 pp 4228–4235.

(282) Kasai, K.; Hashiguchi, K.; Takahashi, H.; Kasai, A.; Takeda, S.; Nakano, M.; Ishikawa, T.; Nakamura, T.; Miura, T. Recombinant Production and Evaluation of an Antibacterial L-Amino Acid Oxidase Derived from Flounder *Platichthys stellatus*. *Appl. Microbiol. Biotechnol.* **2015**, *99* (16), 6693–6703.

(283) Kramer, J. R.; Deming, T. J. Glycopolypeptides via Living Polymerization of Glycosylated-L-lysine N-Carboxyanhydrides. *J. Am. Chem. Soc.* **2010**, *132* (42), 15068–15071.

(284) Bäckström, M.; Ambort, D.; Thomsson, E.; Johansson, M. E. V.; Hansson, G. C. Increased Understanding of the Biochemistry and Biosynthesis of MUC2 and Other Gel-Forming Mucins Through the Recombinant Expression of Their Protein Domains. *Mol. Biotechnol.* **2013**, *54*, 250–256.

(285) Rabuka, D.; Forstner, M. B.; Groves, J. T.; Bertozzi, C. R. Noncovalent Cell Surface Engineering: Incorporation of Bioactive Synthetic Glycopolymers into Cellular Membranes. *J. Am. Chem. Soc.* **2008**, *130* (18), 5947–5953.

(286) Tsutsui, S.; Tasumi, S.; Suetake, H.; Kikuchi, K.; Suzuki, Y. Carbohydrate-Binding Site of a Novel Mannose-Specific Lectin from Fugu (*Takifugu rubripes*) Skin Mucus. *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* **2006**, *143* (4), 514–519.

(287) Ta, B. T. T.; Nguyen, D. L.; Jala, I.; Dontumprai, R.; Plumworasawat, S.; Aighewi, O.; Ong, E.; Shawley, A.; Potriquet, J.; Saichua, P.; van Diepen, A.; Sripa, B.; Hokke, C. H.; Suttiprapa, S. Identification, Recombinant Protein Production, and Functional Analysis of a M60-like Metallopeptidase, Secreted by the Liver Fluke *Opisthorchis viverrini*. *Parasitol. Int.* **2020**, *75*, 102050.

(288) Taus, C.; Lucini, C.; Sato, T.; Furukawa, K.; Grabherr, R.; Staudacher, E. Expression and Characterization of the First Snail-Derived UDP-N-Acetyl- $\alpha$ -D-Galactosamine:Polypeptide N-Acetylgalactosaminyltransferase. *Glycoconjugate J.* **2013**, *30* (9), 825–833.

(289) Wasano, K.; Hirakawa, Y. Recombinant Galectin-1 Recognizes Mucin and Epithelial Cell Surface Glycocalyces of Gastrointestinal Tract. *J. Histochem. Cytochem.* **1997**, *45* (2), 275–283.

(290) Young, J. D.; Tsuchiya, D.; Sandlin, D. E.; Holroyde, M. J. Enzymic O-Glycosylation of Synthetic Peptides from Sequences in Basic Myelin Protein. *Biochemistry* **1979**, *18* (20), 4444–4448.

(291) Crawley, S. C.; Gum, J. R., Jr.; Hicks, J. W.; Pratt, W. S.; Aubert, J.-P.; Swallow, D. M.; Kim, Y. S. Genomic Organization and

Structure of the 3' Region of Human MUC3: Alternative Splicing Predicts Membrane-Bound and Soluble Forms of the Mucin. *Biochem. Biophys. Res. Commun.* **1999**, *263* (3), 728–736.

(292) Moniau, N.; Escande, F.; Batra, S. K.; Porchet, N.; Laine, A.; Aubert, J. P. Alternative Splicing Generates a Family of Putative Secreted and Membrane-Associated MUC4Mucins. *Eur. J. Biochem.* **2000**, *267* (14), 4536–4544.

(293) Sternberg, L. R.; Byrd, J. C.; Hansson, G. C.; Liu, K.-F.; Bresalier, R. S. Alternative Splicing of the Human MUC2 Gene. *Arch. Biochem. Biophys.* **2004**, *421* (1), 21–33.

(294) Zhang, L.; Vlad, A.; Milcarek, C.; Finn, O. J. Human Mucin MUC1 RNA Undergoes Different Types of Alternative Splicing Resulting in Multiple Isoforms. *Cancer Immunol. Immunother.* **2013**, *62* (3), 423–435.

(295) Venkataraman, M.; Sasisekharan, R.; Raman, R. Glycan array data management at consortium for functional glycomics. In *Glycoinformatics*; Humana Press: New York, 2015; pp 181–190.

(296) Coordinators, N. R. Database Resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* **2016**, *44* (D1), D7–D19.

(297) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28* (1), 235–242.

(298) Guhlin, J.; Silverstein, K. A. T.; Zhou, P.; Tiffin, P.; Young, N. D. ODG: Omics Database Generator - A Tool for Generating, Querying, and Analyzing Multi-Omics Comparative Databases to Facilitate Biological Understanding. *BMC Bioinf.* **2017**, *18* (1), 367.

(299) Li, Z.; Chai, W. Mucin O-glycan microarrays. *Curr. Opin. Struct. Biol.* **2019**, *56*, 187–197.

(300) Werlang, C.; Cárcamo-Oyarce, G.; Ribbeck, K. Engineering mucus to study and influence the microbiome. *Nature Reviews Materials* **2019**, *4* (2), 134–145.

(301) Navarro, L. A.; French, D. L.; Zauscher, S. Advances in Mucin Mimic Synthesis and Applications in Surface Science. *Curr. Opin. Colloid Interface Sci.* **2018**, *38*, 122–134.

(302) Mahalingam, A.; Jay, J. I.; Langheinrich, K.; Shukair, S.; McRaven, M. D.; Rohan, L. C.; Herold, B. C.; Hope, T. J.; Kiser, P. F. Inhibition of the Transport of HIV in Vitro Using a pH-Responsive Synthetic Mucin-Like Polymer System. *Biomaterials* **2011**, *32* (33), 8343–8355.

(303) Hashimoto, R.; Fujitani, N.; Takegawa, Y.; Kuroguchi, M.; Matsushita, T.; Naruchi, K.; Ohyabu, N.; Hinou, H.; Gao, X. D.; Manri, N. An Efficient Approach for the Characterization of Mucin-Type Glycopeptides: The Effect of O-Glycosylation on the Conformation of Synthetic Mucin Peptides. *Chem. - Eur. J.* **2011**, *17* (8), 2393–2404.

(304) Authimoolam, S. P.; Vasilakes, A. L.; Shah, N. M.; Puleo, D. A.; Dziubla, T. D. Synthetic Oral Mucin Mimic from Polymer Micelle Networks. *Biomacromolecules* **2014**, *15* (8), 3099–3111.

(305) Authimoolam, S. P.; Dziubla, T. D. Biopolymeric Mucin and Synthetic Polymer Analogs: Their Structure, Function and Role in Biomedical Applications. *Polymers* **2016**, *8* (3), 71.

(306) Canton, I.; Warren, N. J.; Chahal, A.; Amps, K.; Wood, A.; Weightman, R.; Wang, E.; Moore, H.; Armes, S. P. Mucin-Inspired Thermoresponsive Synthetic Hydrogels Induce Stasis in Human Pluripotent Stem Cells and Human Embryos. *ACS Cent. Sci.* **2016**, *2* (2), 65–74.

(307) Artigas, G.; Hinou, H.; Garcia-Martin, F.; Gabius, H. J.; Nishimura, S. I. Synthetic Mucin-Like Glycopeptides as Versatile Tools to Measure Effects of Glycan Structure/Density/Position on the Interaction with Adhesion/Growth-Regulatory Galectins in Arrays. *Chem. - Asian J.* **2017**, *12* (1), 159–167.

(308) Stern, O.; Karageorgaki, C.; Zürcher, M.; Zürcher, S.; Scales, C. W.; Fadli, Z.; Spencer, N. D.; Tosatti, S. G. Reducing Friction in the Eye: A Comparative Study of Lubrication by Surface-Anchored Synthetic and Natural Ocular Mucin Analogs. *ACS Appl. Mater. Interfaces* **2017**, *9* (23), 20150–20160.

(309) Cohen, M.; Senaati, H. P.; Fisher, C. J.; Huang, M. L.; Gagneux, P.; Godula, K. Synthetic Mucus Nanobarriers for Identification of Glycan-Dependent Primary Influenza A Infection Inhibitors. *ACS Cent. Sci.* **2016**, *2* (10), 710–714.

(310) Kramer, J. R.; Onoa, B.; Bustamante, C.; Bertozzi, C. R. Chemically Tunable Mucin Chimeras Assembled on Living Cells. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112* (41), 12574–12579.

(311) Delaveris, C.; Webster, E.; Banik, S.; Boxer, S.; Bertozzi, C. Membrane-tethered mucin-like polypeptides sterically inhibit binding and slow fusion kinetics of influenza A virus. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117*, 12643.

(312) Godula, K.; Rabuka, D.; Nam, K. T.; Bertozzi, C. R. Synthesis and Microcontact Printing of Dual End-Functionalized Mucin-like Glycopolymers for Microarray Applications. *Angew. Chem., Int. Ed.* **2009**, *48* (27), 4973–4976.

(313) Godula, K.; Bertozzi, C. R. Density Variant Glycan Microarray for Evaluating Cross-Linking of Mucin-Like Glycoconjugates by Lectins. *J. Am. Chem. Soc.* **2012**, *134* (38), 15732–15742.

(314) Kesimer, M.; Ehre, C.; Burns, K. A.; Davis, C. W.; Sheehan, J. K.; Pickles, R. J. Molecular Organization of the Mucins and Glycocalyx Underlying Mucus Transport Over Mucosal Surfaces of the Airways. *Mucosal Immunol.* **2013**, *6* (2), 379–392.

(315) Holland, C.; Vollrath, F.; Ryan, A. J.; Mykhaylyk, O. O. Silk and Synthetic Polymers: Reconciling 100 Degrees of Separation. *Adv. Mater.* **2012**, *24* (1), 105–109.

(316) Shurer, C. R.; Wang, Y.; Feeney, E.; Head, S. E.; Zhang, V. X.; Su, J.; Cheng, Z.; Stark, M. A.; Bonassar, L. J.; Reesink, H. L. Stable Recombinant Production of Codon-Scrambled Lubricin and Mucin in Human Cells. *Biotechnol. Bioeng.* **2019**, *116* (6), 1292–1303.

(317) Shurer, C. R.; Colville, M. J.; Gupta, V. K.; Head, S. E.; Kai, F.; Lakins, J. N.; Paszek, M. J. Genetically Encoded Toolbox for Glycocalyx Engineering: Tunable Control of Cell Adhesion, Survival, and Cancer Cell Behaviors. *ACS Biomater. Sci. Eng.* **2018**, *4* (2), 388–399.

(318) Wheeler, K. M.; Cárcamo-Oyarce, G.; Turner, B. S.; Dellos-Nolan, S.; Co, J. Y.; Lehoux, S.; Cummings, R. D.; Wozniak, D. J.; Ribbeck, K. Mucin Glycans Attenuate the Virulence of *Pseudomonas aeruginosa* in Infection. *Nature Microbiology* **2019**, *4* (12), 2146–2154.

(319) Witten, J.; Samad, T.; Ribbeck, K. Molecular Characterization of Mucus Binding. *Biomacromolecules* **2019**, *20* (4), 1505–1513.

(320) Wang, B. X.; Wheeler, K. M.; Cady, K. C.; Lehoux, S.; Cummings, R. D.; Laub, M. T.; Ribbeck, K.; Mucin Glycans Signal Through the Sensor Kinase RetS to Inhibit Virulence-Associated Traits in *Pseudomonas aeruginosa*. *bioRxiv* **2020**.