Proteins and peptides: A view on their use in plant protection products

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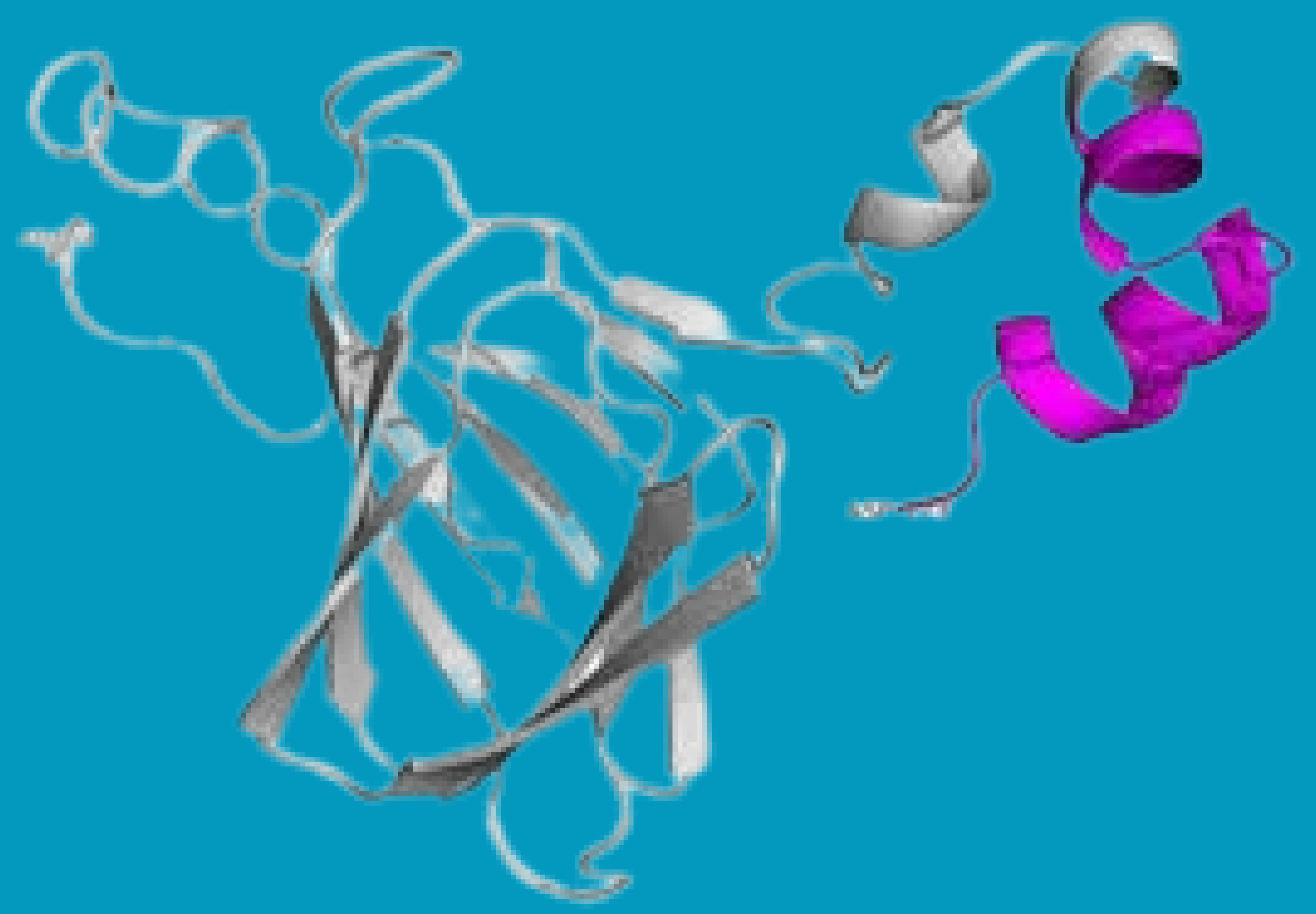

• WHAT ARE PEPTIDES?

- Proteins and peptides are both chains of amino acids linked by peptide bonds, differing mainly in size and complexity. This size difference results in important structural and functional distinctions.
  - They are used in the development of peptide-based drugs, vaccines, and targeted therapies for conditions such as cancer, diabetes, and infectious diseases.
  - In the food industry, peptides are used for their nutritional benefits, functional properties (e.g., improving texture or flavor), and health-promoting effects, such as antioxidant or antihypertensive activity.
- And new uses of proteins and peptides are now emerging for pest and disease control including:
  - Plant elicitor technology – eg Harpin proteins
  - Antibodies for pest and disease control eg Biotalys portfolio
  - Spider venom peptides for insect control eg Vestaron portfolio

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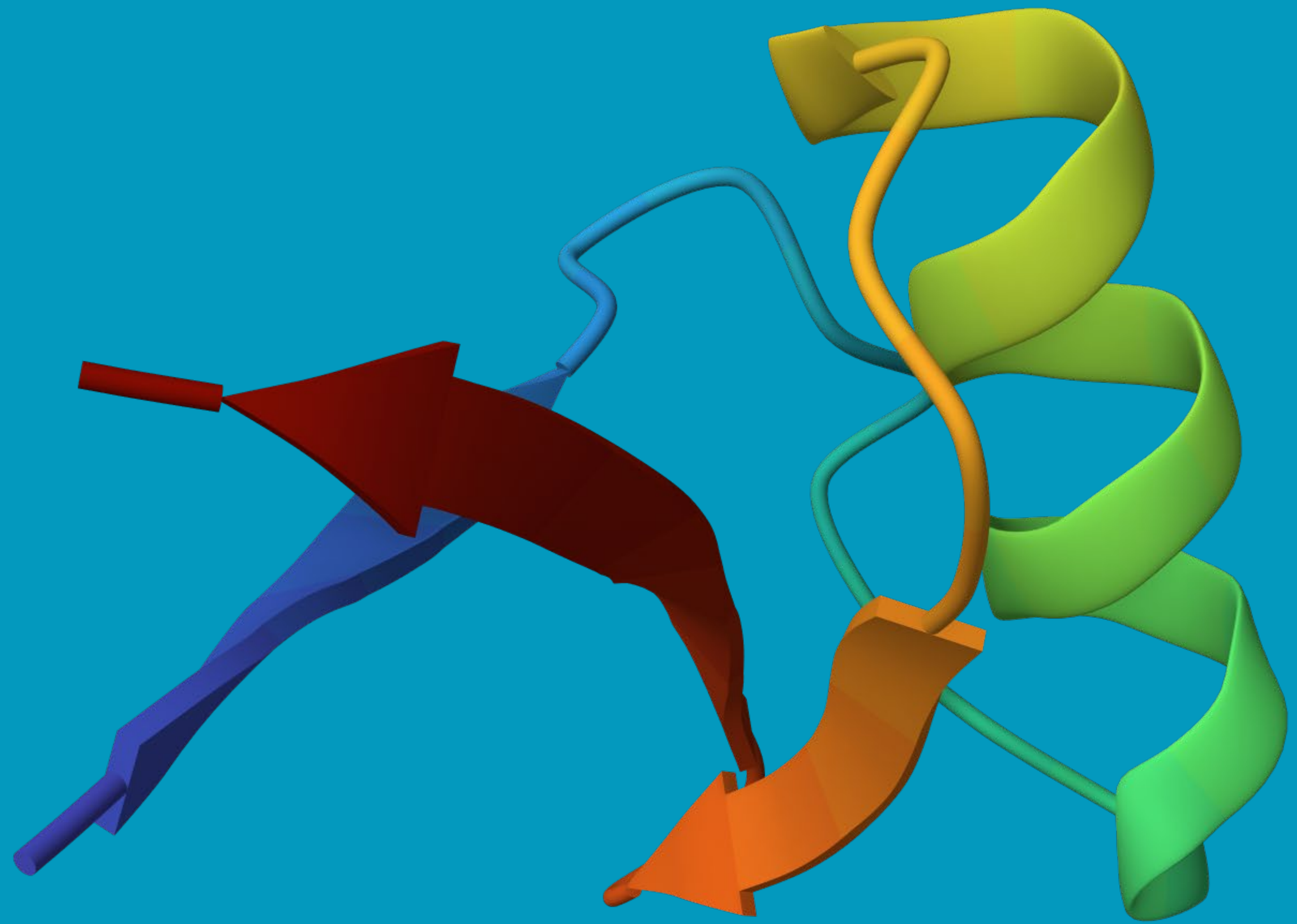
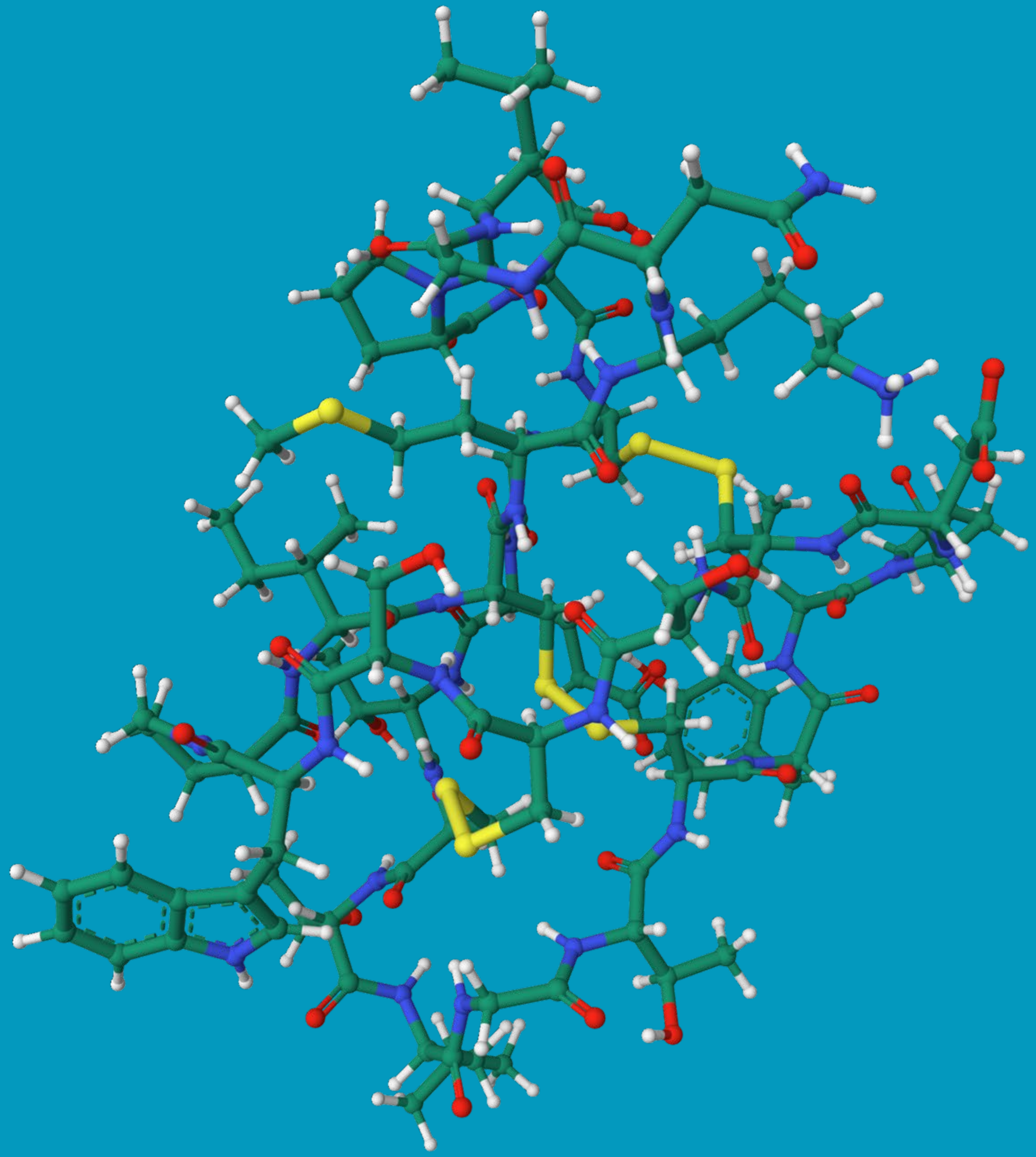


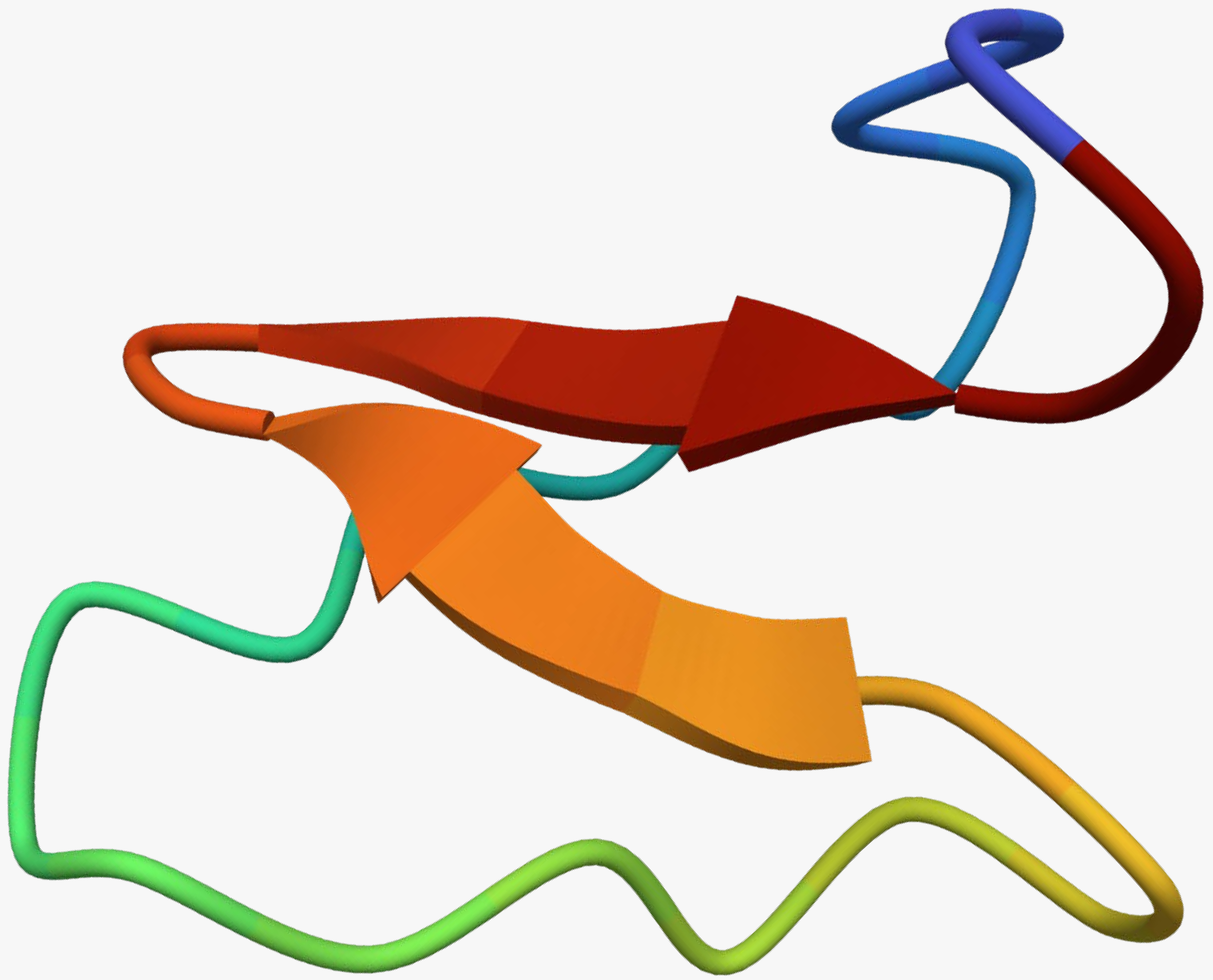
The future is now

Biocontrol innovation is shifting from discovery to design: from naturally occurring extracts and microbes to engineered peptides, bacteriophages, and RNA-based technologies. These products mimic or enhance nature’s mechanisms but are increasingly built through synthetic biology and precision design.



European institutions are starting to acknowledge this reality. The IBMA Framework Proposal (2025) calls for a dedicated *Biocontrol Regulation* and a *European Biocontrol Agency* – a “one market, one assessment” vision that could finally replace today’s fragmented zonal system. The European Commission and Parliament are exploring how to define biocontrol in law. These are important signals, but transformation takes time.

- In the meantime, innovators are moving faster than policy.
- New biologically inspired technologies challenge not only existing data requirements, but also our definitions of what counts as 'biological' versus 'biotechnological'.
- Future regulation will need to evolve beyond classification – towards *adaptive governance* that considers how these agents are designed, characterised, and understood.

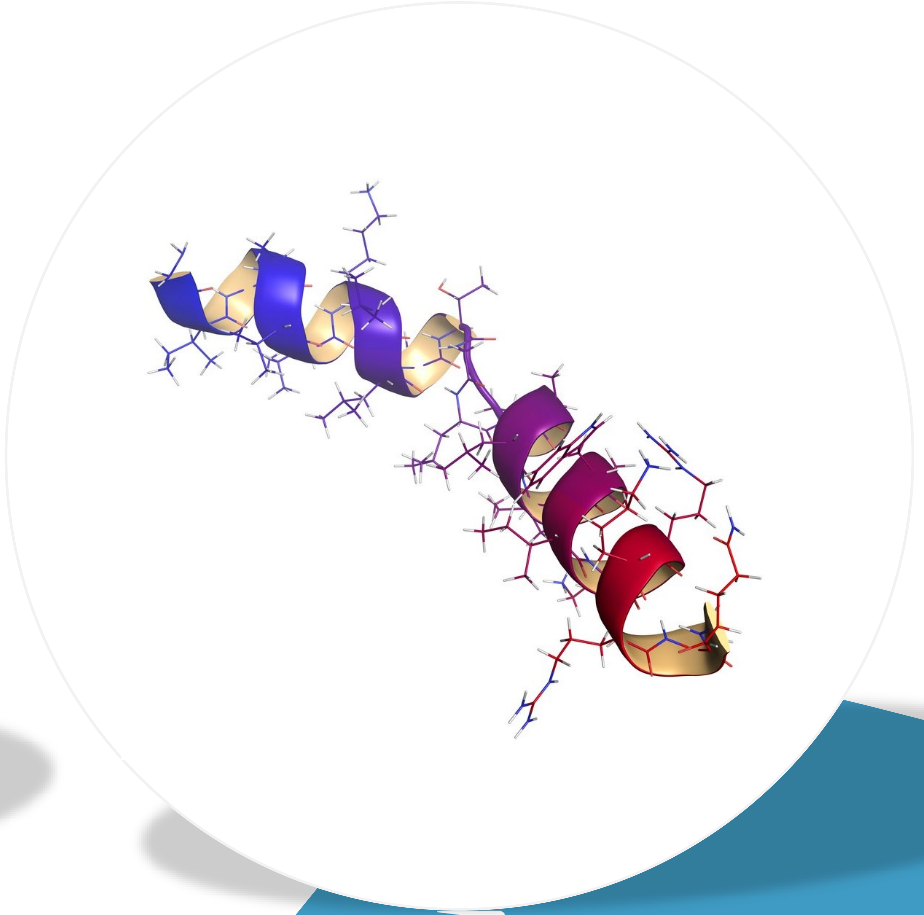


The 4 types of biocontrol: invertebrates, microbials, semiochemicals and natural substances which includes proteins and peptides

Invertebrates

Semiochemicals

Microbials



Natural substances

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IBMA Actions - Progressing Authorisation of Peptides/Proteins as PPP

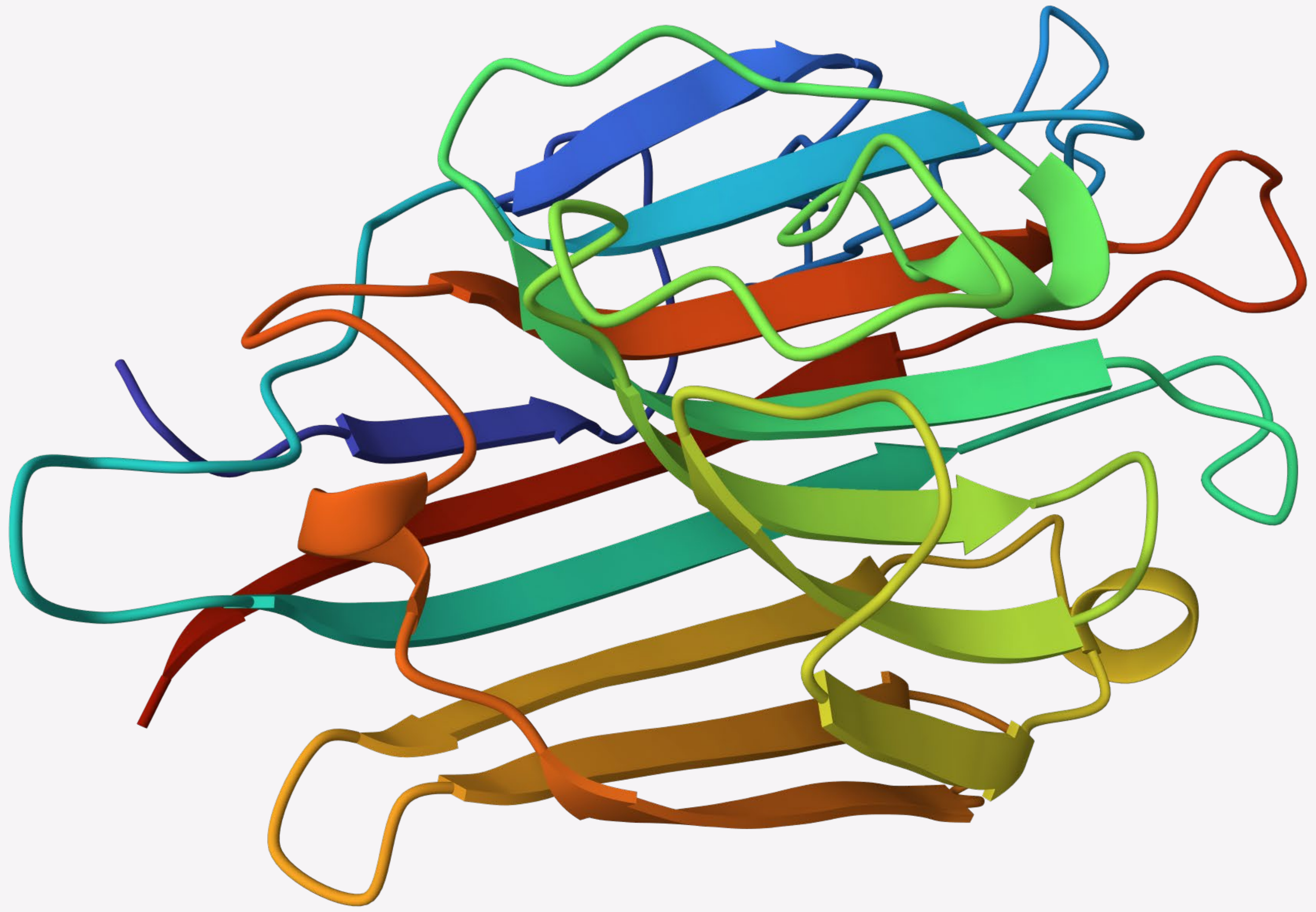
2023: Requests from IBMA members to consider regulatory aspects on proteins and peptides.

2023: Discussion on aspects related to proteins and peptide risk assessment with EU COM in the Biopesticides WG.

Q1 2024: OECD seminar on problem formulation, concluding with a request for more guidance on proteins and peptides at OECD level.

2024: IBMA proposal to OECD to organise an OECD EGBP seminar dealing with regulatory considerations for proteins and peptides in 2025.

Q3 2024: Adaptation of the IBMA definition allowing proteins and peptides to be considered as biocontrol with limited modifications (under certain conditions).



30 YEARS IBMA INTERNATIONAL BIOCONTROL MANUFACTURERS ASSOCIATION

Peptides and proteins: New biocontrol technologies leading to update IBMA definition for Natural Substances

IBMA Scoping Committee Conclusion: When certain conditions are met modified peptides/proteins are biocontrol

Natural substances consist of one or more components that originate from nature, including but not limited to: plants, algae/microalgae, animals, minerals, bacteria, fungi, protozoans, **peptides, proteins (e.g. enzymes, antibodies),** viruses, viroids, and mycoplasmas. They can either be **sourced from nature or are nature identical if synthetized.** This definition excludes semiochemicals and microbials, which have their own definition.

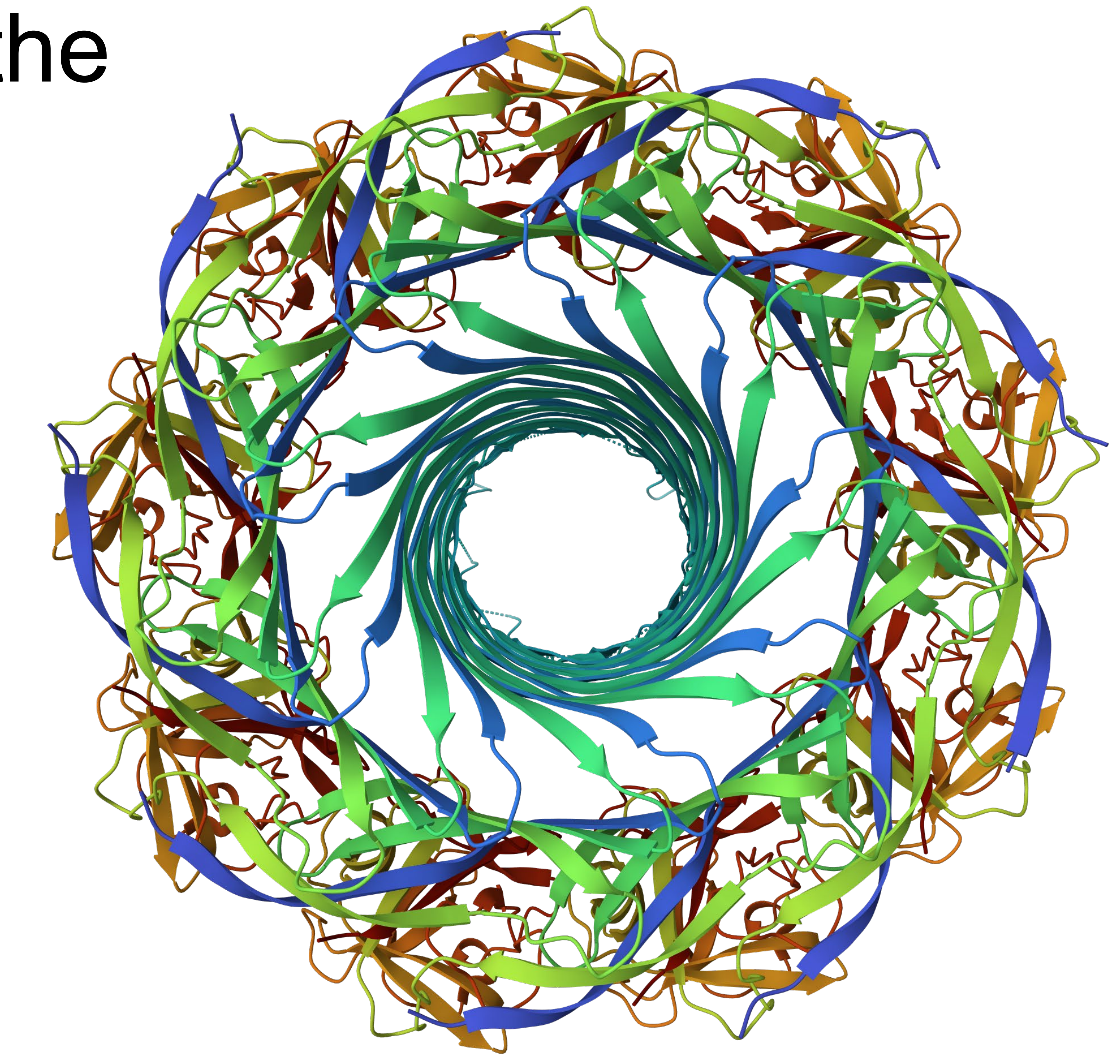
Among natural substances, IBMA considers that peptides and proteins containing sequence modifications of a peptide/protein sourced from nature are deemed nature-identical provided all of the following conditions are met

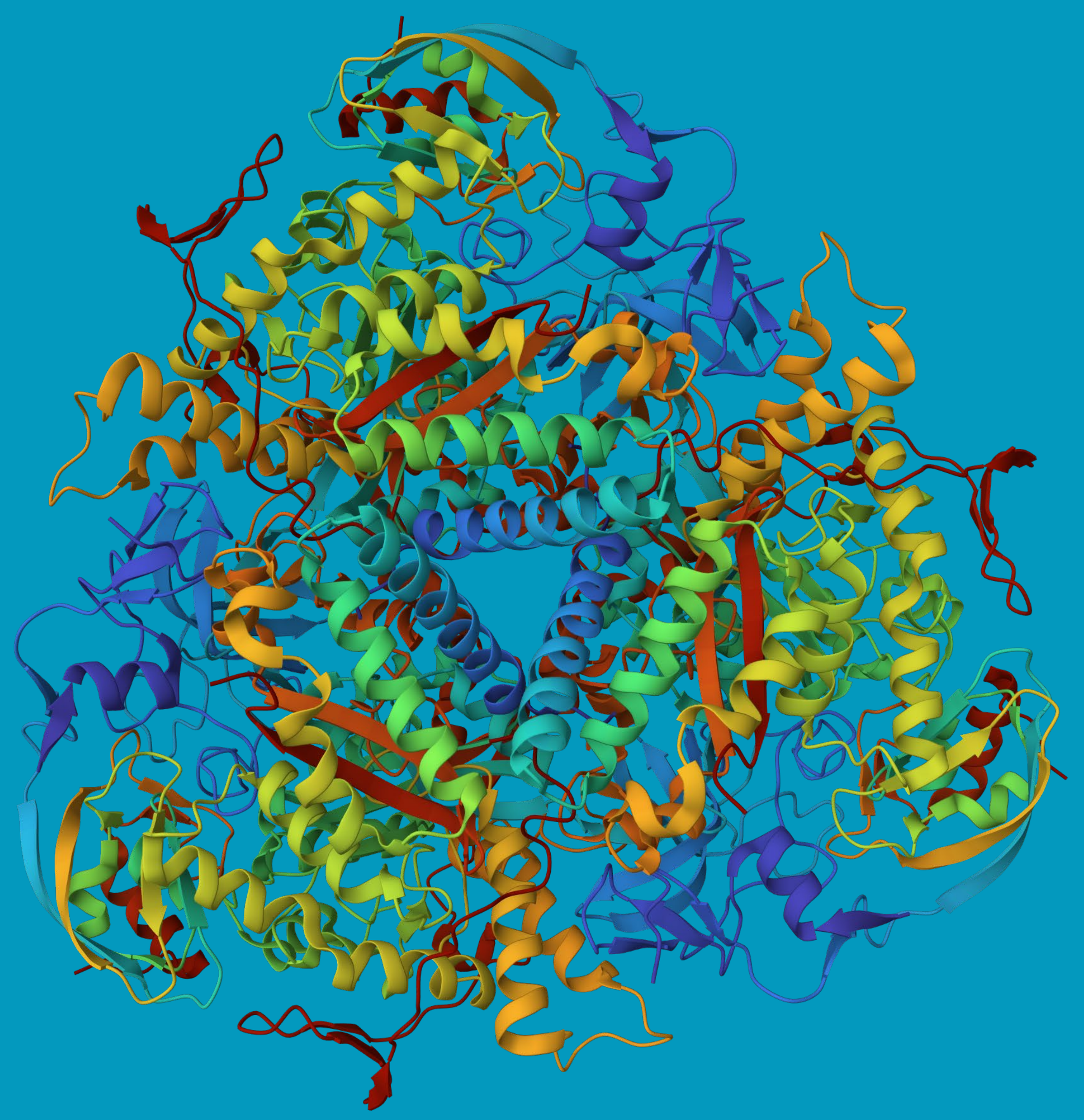
(1) **they contain only naturally occurring amino-acids**
(2) **such modifications do not change the 3-dimensional structure**
(3) **such modifications do not change the biological function and**
(4) **the biological breakdown occurs in a predicted way according to a natural pathway.**

Main messages from IBMA Proteins/Peptides Working Party

- Proteins and peptides are naturally occurring modern crop protection tools that fall within the biocontrol definition
- Peptides known today are the tip of the iceberg – many more to discover
- IBMA considers that peptides with small modifications can be deemed nature identical if certain structural and functional conditions are met
- Regulatory frameworks should follow a “need to know” decision tree approach enabling faster authorisation while ensuring a full safety evaluation
- Faster authorisation is necessary to bring more biocontrol to market to accelerate the transition

to a more sustainable agriculture



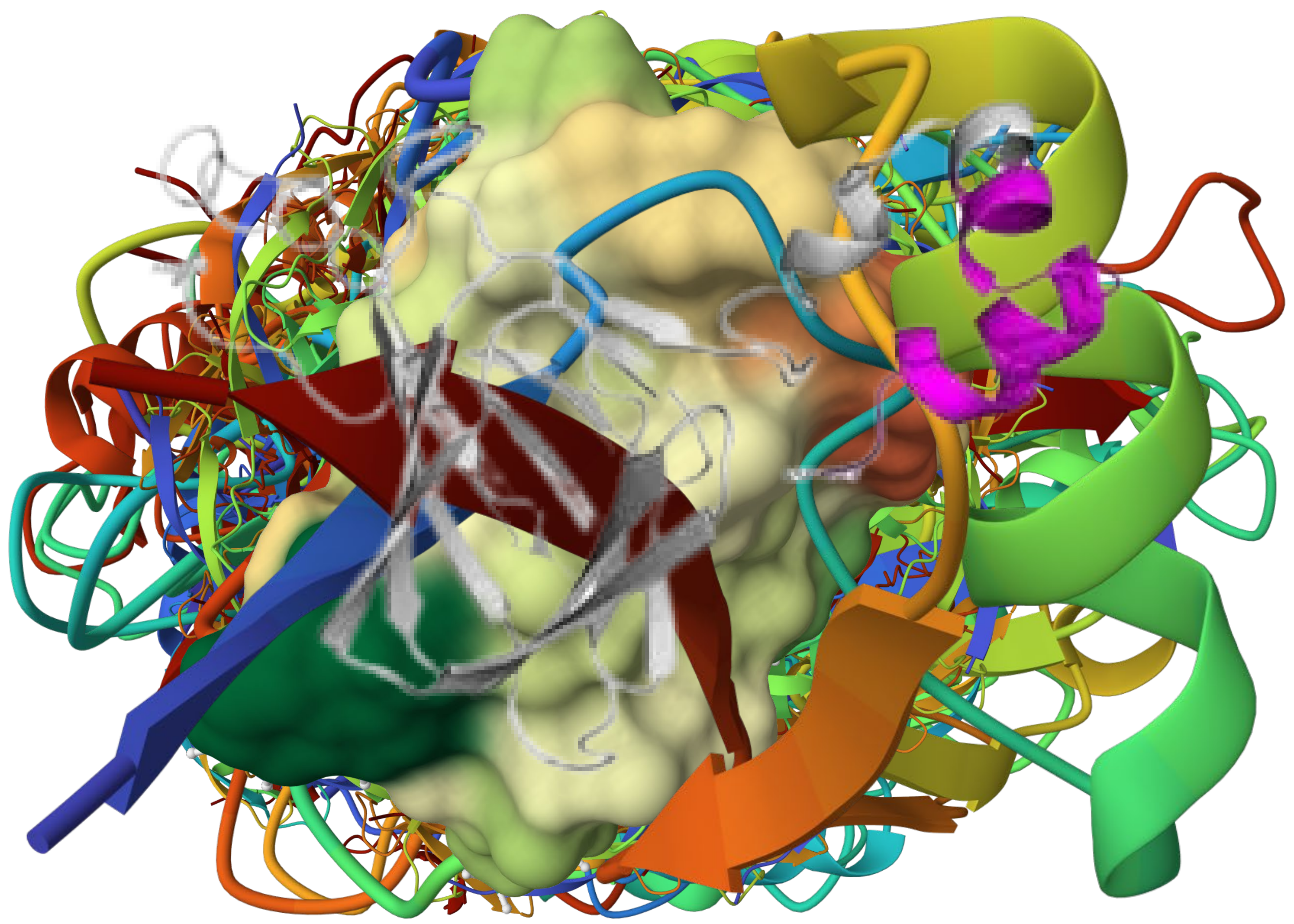


PEPTIDES

- Contribute to the adaptability and resilience of plants to different environmental conditions
- Regulate various plant processes, facilitating adaptation to the environment and enhancing defence responses
- Low concentrations are required to be effective and rapid degradation

Diversity of plant active defence peptides

- Defensin (pea, tomato - fungus)
- Ribosome inactivating proteins (e.g. pumpkin – fungus, virus, insects)
- Lectins (ricin, castor bean - insects)
- Plant protease inhibitors (cereals, squash, tuber –insects, nematodes)
- α-Amylase inhibitors (cereals, legumes - insects)
- Canatoxin-Like Proteins and Ureases (e.g. Jackbean - insects)
- Arcelin (e.g. beans - insects)
- Thionins (e.g. barley - microbes)
- Seed storage protein (lupin – fugus [approved 2021 low risk substance – RMS NL])
- Pore-Forming Toxins
- Cyclotides (*C. ternatea,* Cter M)
- A very few examples from 1000s of discovered…



Images created from the RCSB PDB (RCSB.org)

Mechanisms. Direct action against pathogens

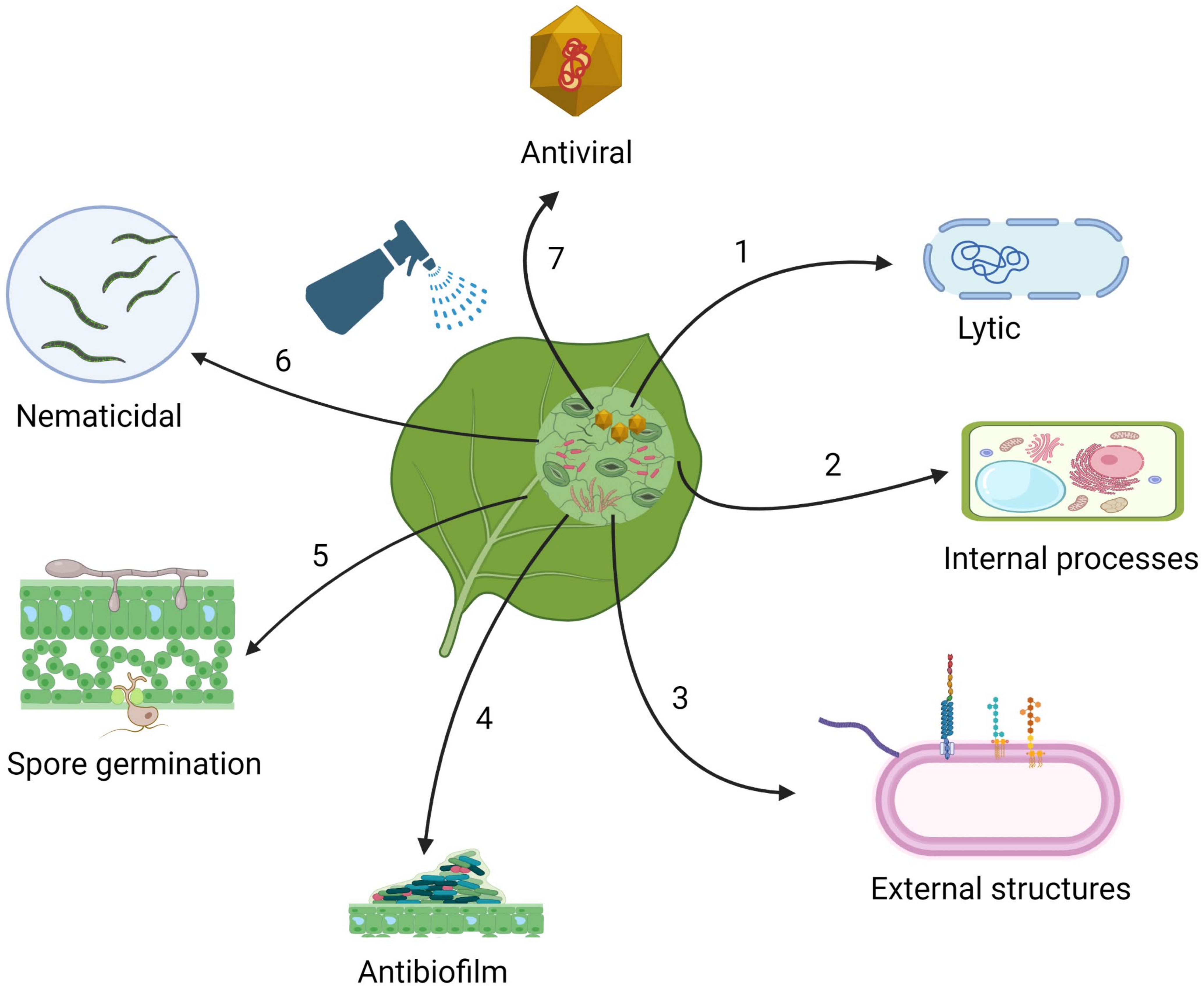


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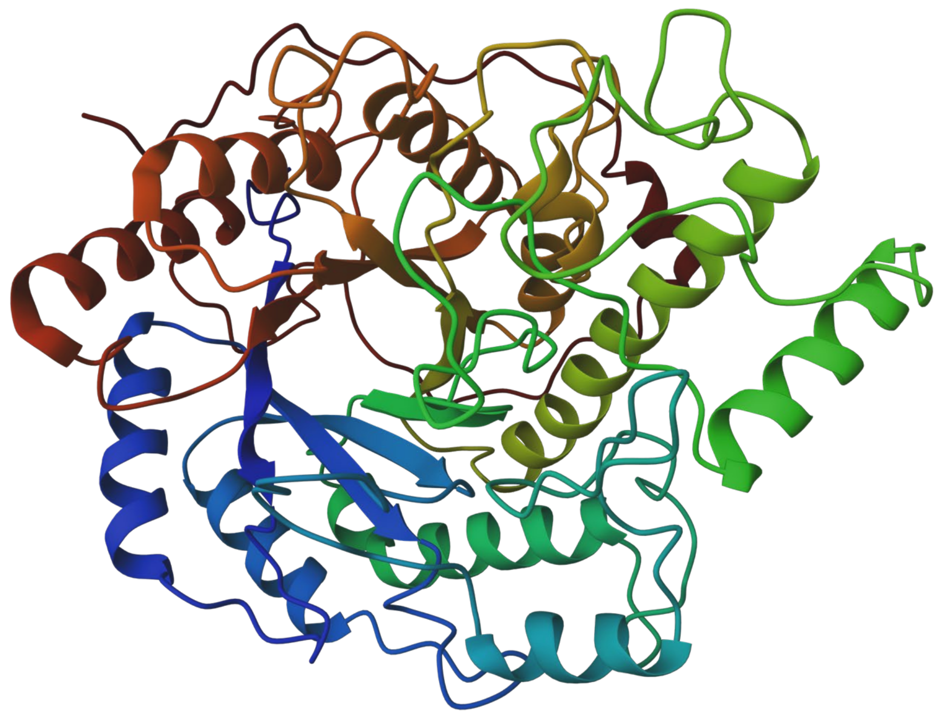
Figure from Montesinos, E., Annu.Rev. Phytopathol. (2023). Vol. 61.

Types of peptides used as plant protection products include:

- **Insecticidal peptides**
  - that target the nervous system, such as cysteine-rich venom peptides, and antimicrobial peptides (AMPs) that are part of an insect's immune response
  - cyclic peptides from plants, known as cyclotides, can be effective by disrupting cell membranes, and
  - other peptides target different physiological processes like growth and development

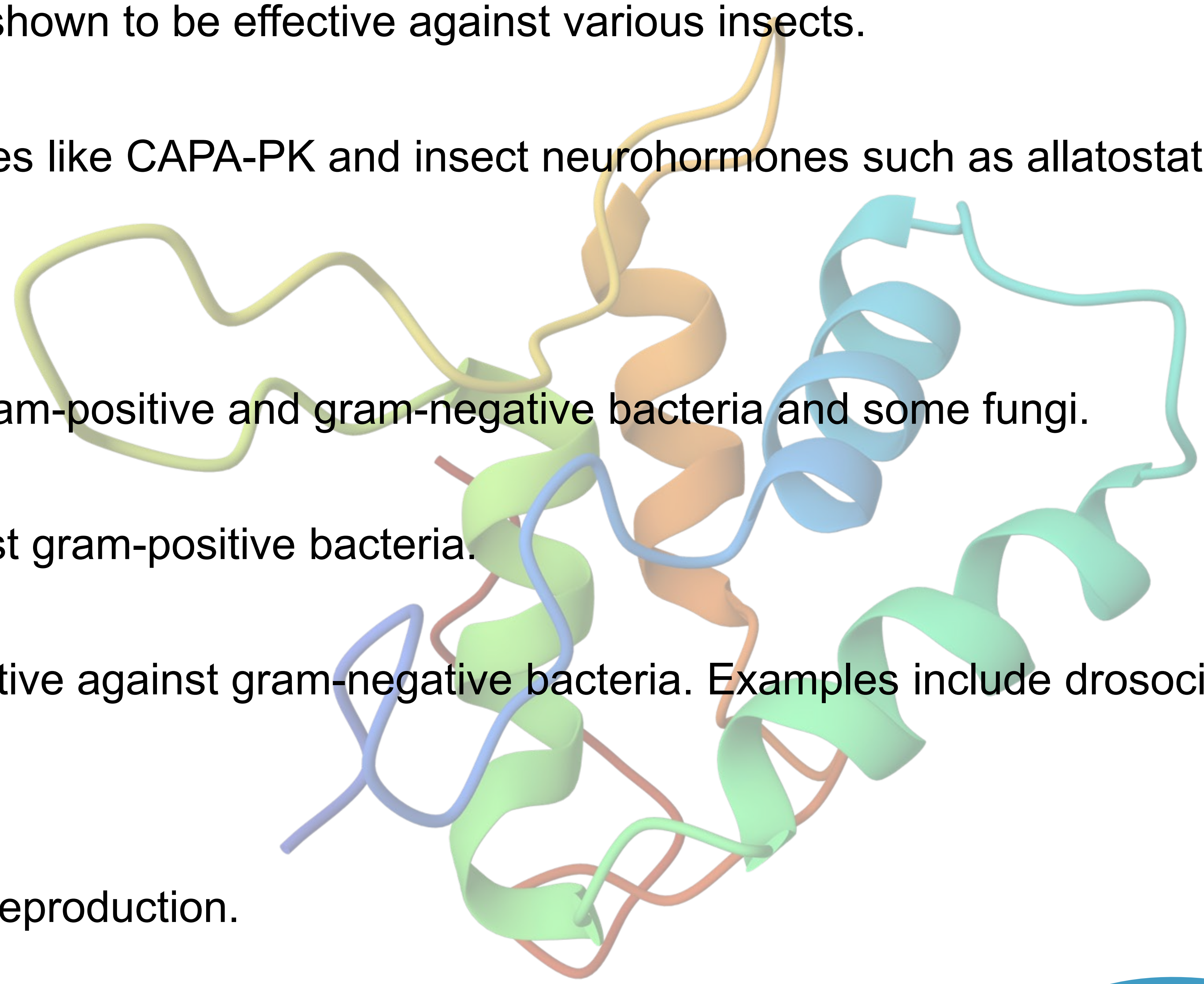


- **Fungicidal peptides**
  - Plant-derived peptides like defensins, thionins, and lipid transfer proteins, as well as P
  - Microorganism-derived peptides such as lipopeptides from Bacillus species and peptaibols from Trichoderma
  - Synthetic peptides, including those based on natural structures or randomly generated mixtures, are also used to target specific pathogens

- **Herbicidal peptides**
  - those derived from plants and microorganisms, such as cyclopeptides
  - linear peptides
  - those with specific structures like thaxtomin, tentoxin, and phosphinothricin derivatives
  - these peptides can be synthesized or naturally derived, and function through various mechanisms, often targeting specific biological pathways in weeds

Insecticidal peptides

- **Cysteine-rich venom peptides**:
  - These peptides, found in venoms from animals like spiders and scorpions, target ion channels in the insect nervous system.
- **Plant-derived peptides**:
  - Examples include cyclotides (like KalataB1) from plants that disrupt cell membranes, and peptides like BrD1 that are effective against specific pests.
- **Microorganism-derived peptides**:
  - Peptides from microbes, such as Beauveriolide I and Destruxins, have been shown to be effective against various insects.
- **Other types:**
  - Various other peptides are being studied, including analogues of neuropeptides like CAPA-PK and insect neurohormones such as allatostatins and allatotropins, which disrupt insect physiology.
- Antimicrobial peptides (AMPs)
- **Cecropins**:
  - Linear peptides with alpha-helical structures that are effective against both gram-positive and gram-negative bacteria and some fungi.
- **Defensins**:
  - Small peptides that are stabilized by disulfide bridges and are effective against gram-positive bacteria.
- **Other AMPs**:
  - This group includes peptides that are rich in glycine and proline, which are active against gram-negative bacteria. Examples include drosocin, attacins, and diptericins.
  - Peptides that target growth and development
- **Insulin-like peptides**:
  - These are important insect hormones that regulate metabolism, growth, and reproduction.
- **Pheromone biosynthesis activating neuropeptide (PBAN)**:
  - This neuropeptide regulates insect reproduction and can be a target for control.
- **Diapause hormone**:
  - This hormone plays a crucial role in the development of insects and can be a target for control.



Fungicidal peptides

- Plant-derived peptides
  - **<u>Defensins</u>:** Part of the plant's innate immune system, they have direct antimicrobial action.
  - **<u>Thionins</u>:** Both Type I and Type II thionins are basic peptides found in various plant tissues that can disrupt microbial membranes.
  - **<u>Lipid transfer proteins (LTPs)</u>:** These are found in plants and are involved in plant immunity.
  - **<u>Hevein-like peptides</u>:** These peptides can bind to chitin on fungal hyphae, leading to their disintegration.
  - **<u>Snakins</u>:** These are peptides that enhance plant immunity.
- Microorganism-derived peptides
  - **<u>Lipopeptides (surfactins, iturins, fengycins)</u>:**
    - Produced by Bacillus species, these peptides have both antimicrobial and plant growth-promoting effects.
  - **<u>Peptaibols</u>:**
    - These are peptides from fungi like Trichoderma that show antifungal activity and can induce systemic resistance in plants.
  - **<u>Bacteriocins</u>:**
    - Small peptides produced by bacteria that can inhibit other bacteria.
- **Other examples:**
    - Some microorganisms produce peptides that mimic plant hormones, which can have complex effects on plant-pathogen interactions.
    - Synthetic and rational design peptides
  - **<u>BP100</u>:**
    - A synthetic peptide effective against bacteria like Erwinia amylovora by disrupting the bacterial cell membrane.
  - **Random Peptide Mixtures (RPMs):**
    - Studies have shown that synthetic RPMs can have potent activity against plant pathogenic bacteria like Xanthomonas and Pseudomonas.
  - **Designed peptides:**
    - Peptides are rationally designed for specific functions, such as inhibiting bacterial growth, improving nutrient uptake, or influencing plant development.

Herbicidal peptides

- Types of **Cyclopeptides:**
  - These cyclic peptides, both natural and synthetic, have shown herbicidal activity.
- **Examples:** Synthesized cyclotetrapeptides and cyclopentapeptides have demonstrated phytotoxicity.
- **Linear peptides:**
  - These are peptides with a straight chain of amino acids and can be derived from plants or microorganisms.
- **Plant-derived:** Examples include Ala-Ala, Ala-Asn, and Gln-Gln from maize (Z. mays L.).
- **Microorganism-derived:** Examples include the Bialaphos/phosphinothricin family and compounds like Tentoxin.
- **Thaxtomin derivatives:**
  - These are a specific class of peptides produced by certain bacteria and have herbicidal properties.
- **Examples:** Thaxtomin A, Thaxtomin A o-isomer, and Thaxtomin B have been identified as herbicidal peptides.
- **Phosphinothricin derivatives:**
  - These are potent herbicides, with phosphinothricin itself (also known as Bialaphos) being an example.
- **Examples:** Bialaphos, Phosalacine, and Plumebmycin A are peptide-based herbicides in this group.
- **Other classes:**
  - Research has identified other specific peptides with herbicidal activity.
- **Examples:** Romidepsin, which targets a wide range of weeds, and Resormycin, which targets dicotyledonous weeds.
- How they work
- **Novel modes of action:**
  - Herbicidal peptides can act as inhibitors of essential enzymes, offering new modes of action for weed control.
- **Environmentally friendly:**
  - Peptides derived from natural sources are often considered more environmentally benign alternatives to traditional synthetic herbicides.
- **Targeted action:**
  - They can be designed to target specific biochemical pathways, making them potentially more selective and safer for crops.

Examples of Peptide Innovations

Harpinαβ and PRETec (Plant Response Elicitor Technologies) and PHC-91398 a growth regulator

Bialaphos a tripeptide herbicide

Peptide maSAMP for Huanglongbing

FLG22 induces the natural immune response

Computational biology & AI to ID micropeptides

Opportune™ herbicide

Agrobodies for pest and diseases: Evoca for Botrytis

Neuropeptide bioinsecticide Spear® derived from Spider venom

Antifungal peptide platform InnaLB™, InnaNema™, InnaHLB™

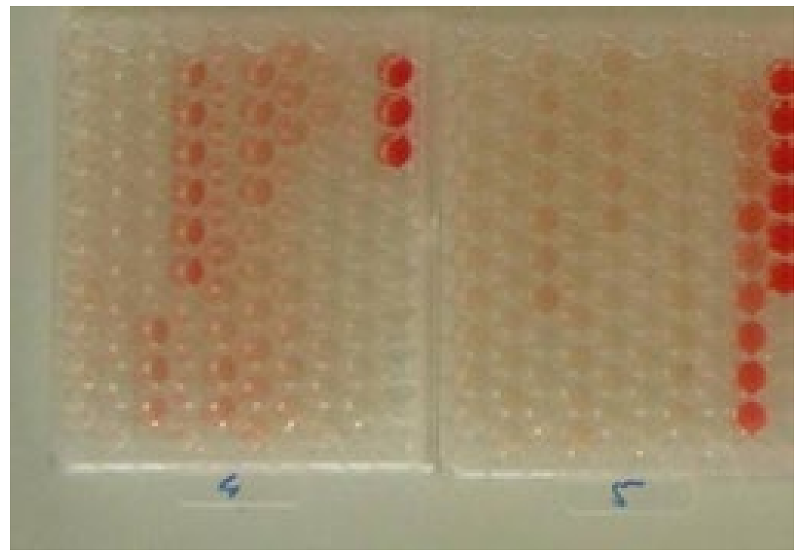
Problad Plus™ a biofungicide

Insect neuropeptides disturbing target physiology for pest kill

Antimicrobial peptide (AMP) technology

Modified from Pam Marrone

The challenges. Toxicity and non-target effects



Hemolytic activity

Phytotoxicity

Caenorhabditis elegans nematode

Mammalian toxicity

- Some peptides of natural origin are practically nontoxic, e.g., **piscidin**.
- CLPs such as **surfactin**, with biosurfactant activity, have low toxicity.
- **Iturin** and **fengycin** are less toxic than the detergent sodium dodecyl sulfate.
- **Peptaibols** are generally toxic (42).
- In synthetic peptides, it is possible to improve the toxicological profile by developing less-toxic analogs.

Other issues:

- biodegradability (protease susceptibility)
- range of action (non-target effects)

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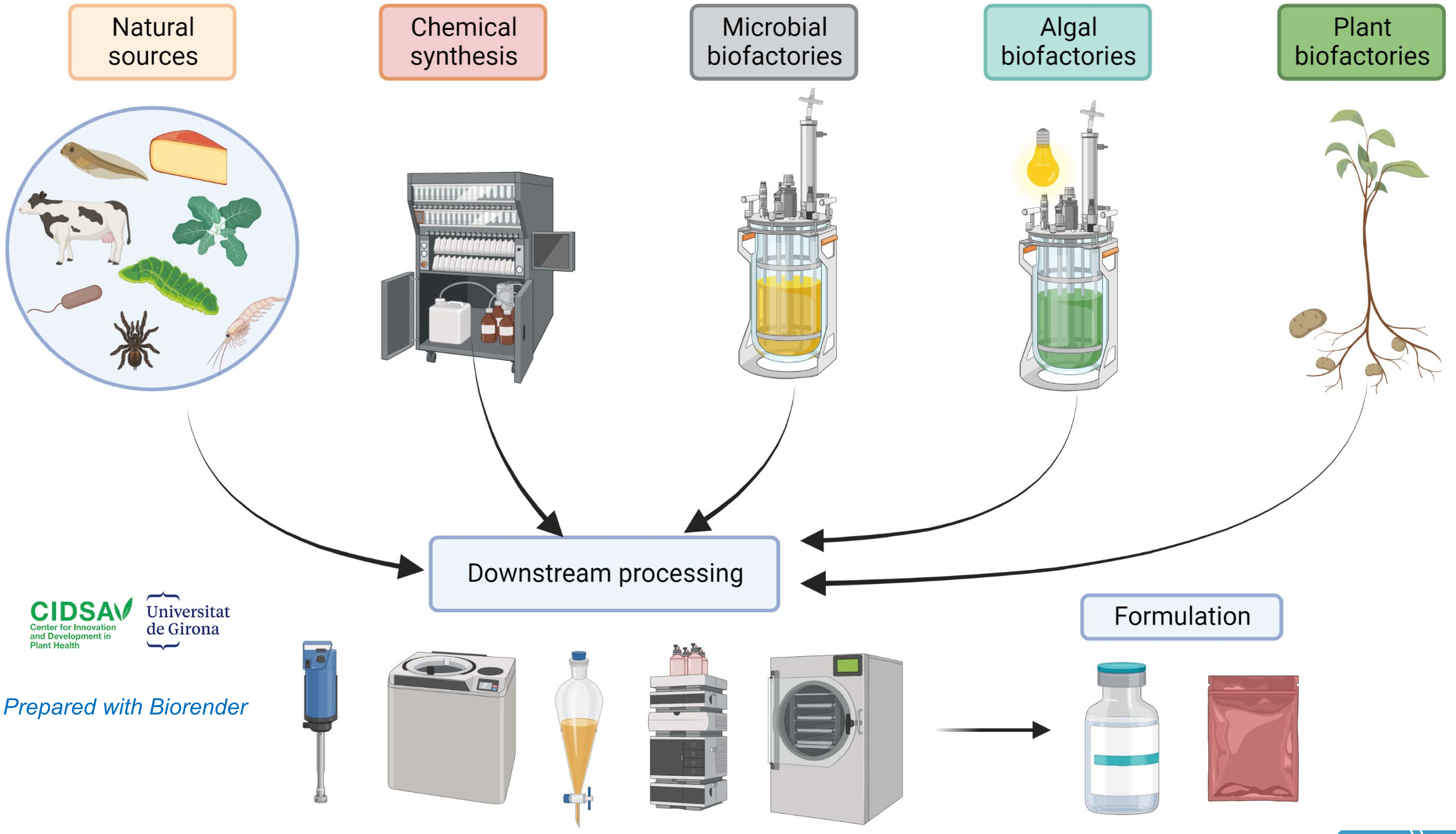
The challenges: Sources and production



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Is the EU legal framework ready for peptides?

It is already possible to take peptides to the EU market as biocontrol agents.

Under data requirements and uniform principles * Part A

*Reg (EU) No 282/2013, Reg (EU) No 546/2011

Credits

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