### Title

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# Resurrected Ancestors Reveal Origins of Metamorphism in XCL1

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**In a recent study, Dishman *et al.* resurrected ancestors of the metamorphic chemokine, XCL1, inferred through phylogenetics, and found that metamorphism arose in the XCL1 lineage ~150 million years ago. A zigzagging evolutionary path suggests that the metamorphic properties are adaptive and reveals three design principles that could be used for technological applications.**

Proteins have essential roles in processes such as stimulus detection, signal transduction, DNA replication, gene expression, protein synthesis and turnover, timekeeping, and metabolism. Under physiological conditions, proteins are not static and often undergo changes in structure that involve repositioning of motifs, domains, and subunits that regulate function. However, the overall 3D arrangement of secondary structures (i.e., architecture) and the path taken by the polypeptide chain through the structure (i.e., topology) [1] remain more or less the same, consistent with the one-sequence one-fold paradigm; this is largely attributed to Christian Anfinsen, who was awarded the 1972 Nobel Prize in Chemistry[i] for his work 'concerning the connection between the amino acid sequence and the biologically active conformation'.

There are rare exceptions to this paradigm, where so-called 'metamorphic proteins' adopt more than one fold, reversibly, under native conditions [2]. However, the extent to which the proteome is populated with metamorphic proteins remains an actively studied question because computational methodologies with which to identify them are only now emerging [3–5] and high-throughput experimental techniques for this purpose are not yet well established. Thus, at present, metamorphic properties of a protein are discovered serendipitously, as happened in 2001–2002 by the Volkman laboratory when optimizing NMR solution conditions for the chemokine XCL1 [6].

The Volkman laboratory found that XCL1 reversibly adopts two folds under near-physiological conditions in equal proportions: a monomeric chemokine-like fold and a dimeric all-β sheet structure (Figure 1) [7]. By contrast, other chemokines, of which there are nearly 50, appear to be monomorphic (i.e., adopting only a single fold, the chemokine fold). Intriguingly, the chemokine fold supports two completely different types of dimer, mediated by different amino acids in each, suggesting that single-fold chemokines have evolved some functional flexibility under different conditions [8]. How metamorphism arose in XCL1, and whether it evolved as an adaptation to support multiple functions, is another highly relevant question. In the 2021 *Science* paper by Dishman *et al.* [9], the Volkman laboratory provided an explanation by using NMR to study the structures of XCL1 ancestors, the inferred amino acid sequences of which were resurrected through phylogenetics.

Dishman *et al.* discovered that the oldest deduced XCL1 ancestors were monomorphic, solely adopting the canonical chemokine fold. The first metamorphic ancestor along the evolutionary path toward XCL1, which they named Anc.3, appeared ~150 million years ago, and was followed by the also-metamorphic Anc.4. Like an evolutionary pendulum, Anc.3 prefers the chemokine fold, Anc.4 prefers the all-β sheet fold, and the modern XCL1 populates both folds in equal proportions. This back-and-forth path suggests that the metamorphic properties of XCL1 are evolutionary adaptations, according to Dishman *et al.* By comparing resurrected amino acid sequences and structures and using site-directed mutagenesis to perturb the metamorphic properties of the inferred ancestral proteins, the authors proposed that metamorphosis can arise from monomorphic proteins through concerted changes in (i) interaction interfaces; (ii) structural flexibility and strain; and (iii) noncovalent contact networks. In addition, they suggested that these three principles can be used to guide the rational design and engineering of novel metamorphic proteins. For example, metamorphic proteins have potential as reversible biosensors, bioswitches, and biosentries, and as components of synthetic oscillators with customized frequencies and outputs.

This latest paper by the Volkman laboratory also opens questions for future studies. For example, they point out that it is unclear why only XCL1 appears to be metamorphic in the chemokine family. Also, can it be determined *in vivo* the extent to which the metamorphic properties of XCL1 are advantageous? Dishman *et al.* suggest that the role of switching folds in XCL1 is to tune its function to the physiological situation: (i) at the site of an infection, the dimeric alternate fold would be used to combat bacteria; and (ii) the monomeric chemokine fold would be utilized to activate leukocytes bearing the XCR1 receptor without cells needing to produce a separate protein. The extent to which the metamorphic property of XCL1 provides added benefits to the host organism above and beyond its monomorphic cousins remains to be tested.

In other metamorphic proteins, the biological importance of their fold-switching abilities is perhaps better established. For example, the circadian clock protein KaiB adopts one fold needed at night and a distinctly different fold needed during the day [10]. Each fold has a different role and

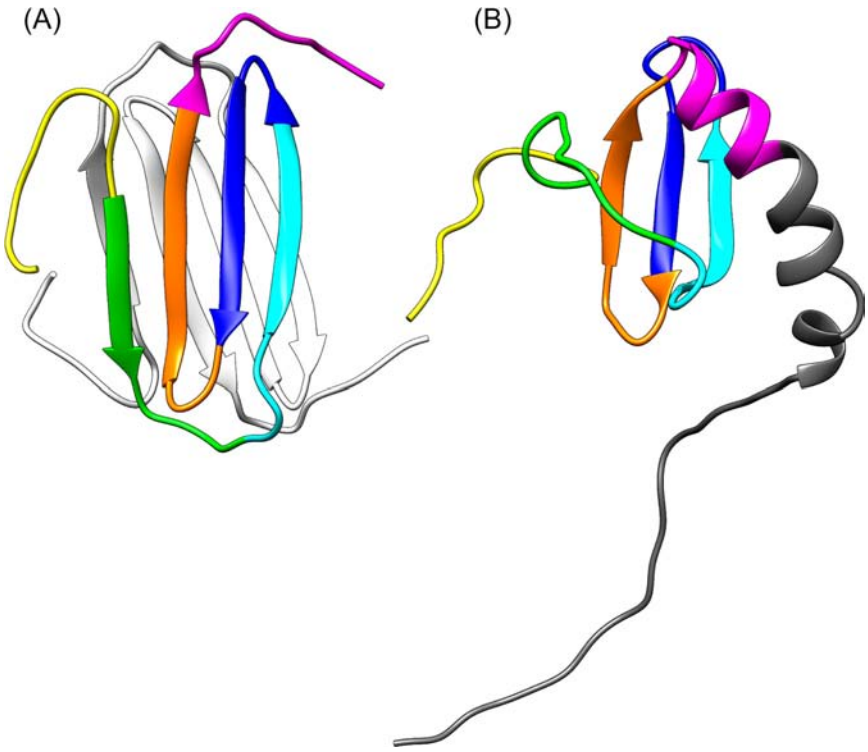


Figure 1. XCL1 Reversibly Adopts a Dimeric All-β Sheet Fold (A) (PDB ID 2JP1) and a Monomeric chemokine-like Fold (B) (PDB ID 1J9O). Corresponding ten-residue segments in both structures are rendered in the same colors. Residues rendered light gray in (A) and dark gray in (B) are not present in the other structure. The molecular modeling program University of California San Francisco (UCSF) Chimera[ii] was used to generate this figure.

perturbing the equilibrium between them disrupts clock function *in vitro* and *in vivo*. As the 'metamorphome' becomes more and more populated through a quickening pace of discovery using improved search methodologies, we are optimistic that metamorphism will be found to be responsible for a significant portion of the expansive repertoire of protein functions, and that this property can be harnessed, perhaps by implementing the three design principles of the Volkman laboratory, to enable new and important technological applications.

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## Declaration of Interests

None declared by authors.

## Resources

[i]www.nobelprize.org/prizes/chemistry/1972/anfinsen/biographical/

[ii]www.rbvi.ucsf.edu/chimera

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