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### **Pierced Lasso Proteins**

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Entanglement and knots are naturally occurring, where, in the microscopic world, knots in DNA and homopolymers are well characterized. The most complex knots are observed in proteins which are harder to investigate, as proteins are heteropolymers composed of a combination of 20 different amino acids with different individual biophysical properties. As new-knotted topologies and new proteins containing knots continue to be discovered and characterized, the investigation of knots in proteins has gained intense interest. Thus far, the principle focus has been on the evolutionary origin of tying a knot, with questions of how a protein chain ‘self-ties’ into a knot, what the mechanism(s) are that contribute to threading, and the biological relevance and functional implication of a knotted topology in vivo gaining the most insight. Efforts to study the fully untied and unfolded chain indicate that the knot is highly stable, remaining intact in the unfolded state orders of magnitude longer than first anticipated. The persistence of “stable” knots in the unfolded state, together with the challenge of defining an unfolded and untied chain from an unfolded and knotted chain, complicates the study of fully untied protein in vitro. Our discovery of a new class of knotted proteins, the Pierced Lassos (PL) loop topology, simplifies the knotting approach. While PLs are not easily recognizable by the naked eye, they have now been identified in many proteins in the PDB through the use of computation tools. PL topologies are diverse proteins found in all kingdoms of life, performing a large variety of biological responses such as cell signaling, immune responses, transporters and inhibitors (<http://lassoprot.cent.uw.edu.pl/>). Many of these PL topologies are secreted proteins, extracellular proteins, as well as, redox sensors, enzymes and metal and co-factor binding proteins; all of which provide a favorable environment for the formation of the disulphide bridge. In the PL topologies, the threaded topology is formed by a covalent loop where part of the polypeptide chain is threaded through, forming what we term a PL. The advantage of a PL topology for fundamental studies, compared to other knotted proteins, is that the threaded topology can easily be manipulated to yield an unknotted state. Exploiting the oxidative state of the cysteines, the building blocks that form the disulphide bridge generating the covalent loop, through altering the chemical environment, and thereby controlling the formation of the covalent loop, easily generates unknotted protein. The biological advantage, we have found, is that the PL can exert allosteric control through this on/off mechanism in a target protein. Most significantly, as the disulphide bridge acts as an on/off switch in knotting, the biophysical investigation of PL topologies can provide a new tool to steer folding and function in proteins, as disulphide bridges are commonly used in protein engineering and therapeutics.