Towards Understanding the Role of Disulfide Bonds in Non-Specific Lipid Transfer Proteins

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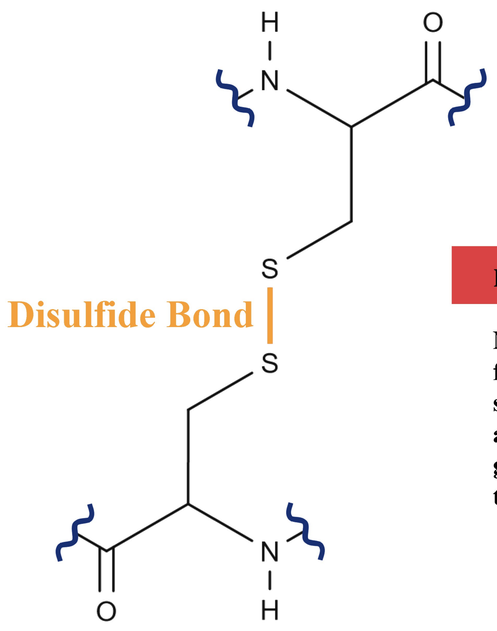
Disulfide Bonds



The role of proteins in nature is mainly determined by their structure, which is influenced by several factors: amino-acid sequence, presence of secondary-structure elements, such as α-helices and β-sheets, their ability to interact with each other to form tertiary and higher-level structures, and environment. Out of 20(21) standard amino acids, only cysteine thiol groups can be oxidized and form a covalent bond of inter- or intramolecular origin, called disulfide bond, linking two distant parts of proteins.

nsLTP

Non-specific Lipid Transfer Proteins (nsLTPs) are members of a protein family found in terrestrial plants. NsLTPs have been reported to be crucial to membrane stabilization, cell wall organization, signal transduction, resistance to biotic and abiotic stresses, plant growth and development, as well as seed development and germination [4]. They consist of four or five helices and four disulfide bonds forming the eight-Cys motif (8CM) (Fig. 1.), which is a recognisable feature of those proteins.

TESTED CONDITIONS

DEFAULT VALUES ARE UNDERLINED

TEMPERATURE

300K 325K 350K

SALINITY

0M 0.15M 0.6M

pH

2 4 6 11

PRESSURE

1 100 1 000 10 000 bars

MEMBRANE

W/O 5:1 POPC:POPS

LIGAND

W/O (PDB: 2MAL)
PGM (PDB: 5LQV)

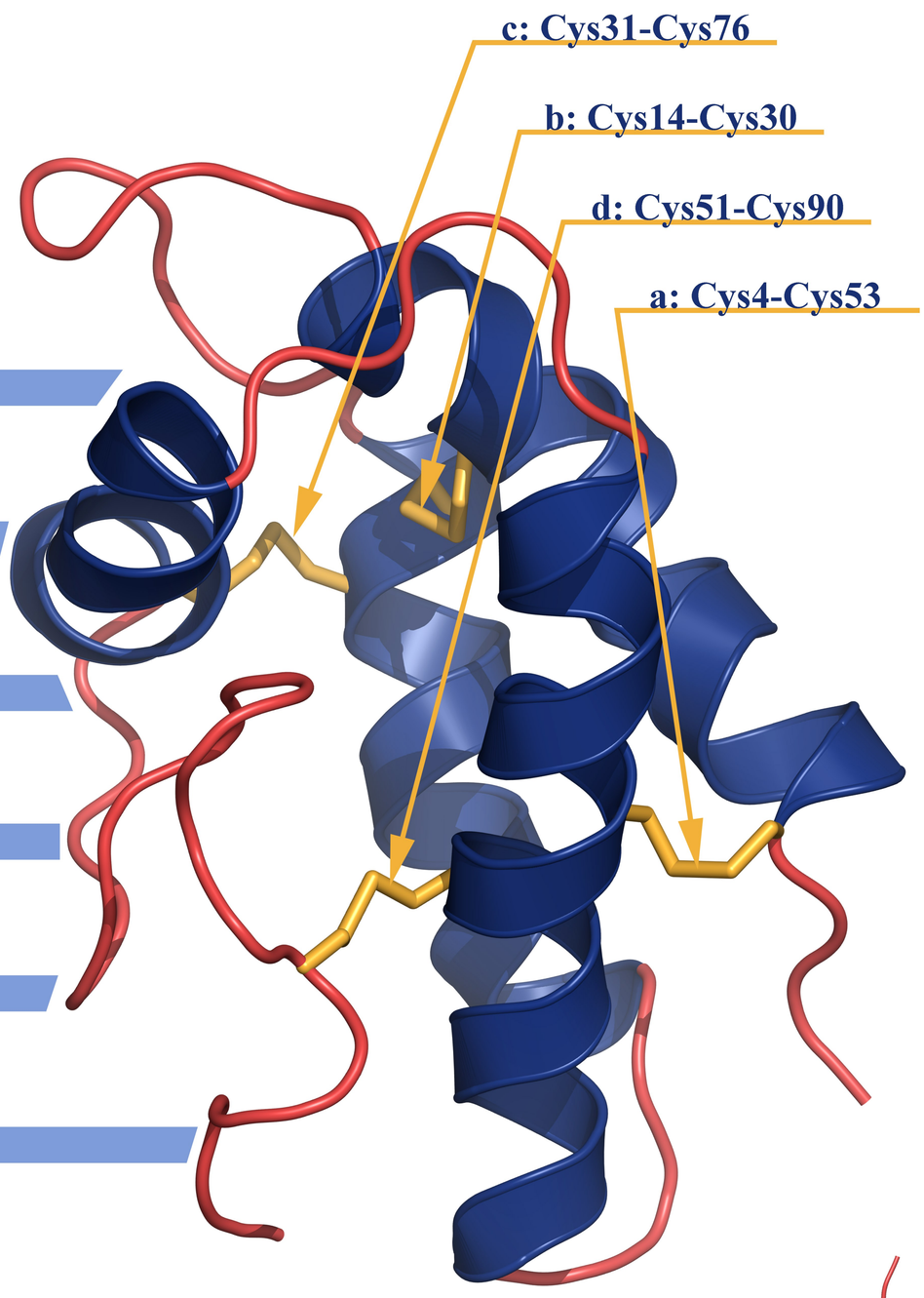


Fig 1. Cartoon representation of the nsLTP from Pea Pisum Sativum. PDB code: 2N81.

Why Study Disulfide Bonds?

Disulfide bonds are present in more than 20% of known proteins, yet their function is still not fully understood. Disulfide bonds were believed to be responsible mostly for the structural stability of proteins, but recent studies showed that this is not always true [1] and the introduction of an additional disulfide bond may not increase stability [2], or lack of one of native disulfides bonds may not reduce it [3]. On the other hand, the presence of disulfide bonds can prevent enzymatic proteolysis, influence amyloidosis, be used as a method to control biochemical processes or impact viral efficiency.

Methodology

This study employed all-atom and coarse-grained molecular dynamics (MD) simulations, utilizing AMBER ff19SB, CHARMM36m, SIRAH2.0, and UNRES force fields, to explore the impact of various disulfide-bond combinations on nsLTP behavior. Disulfide-bond names are named by single letters from the alphabet (a-d) based on the order of appearance of the first cysteine residues forming a bond in a native structure of nsLTPs, for convenience.

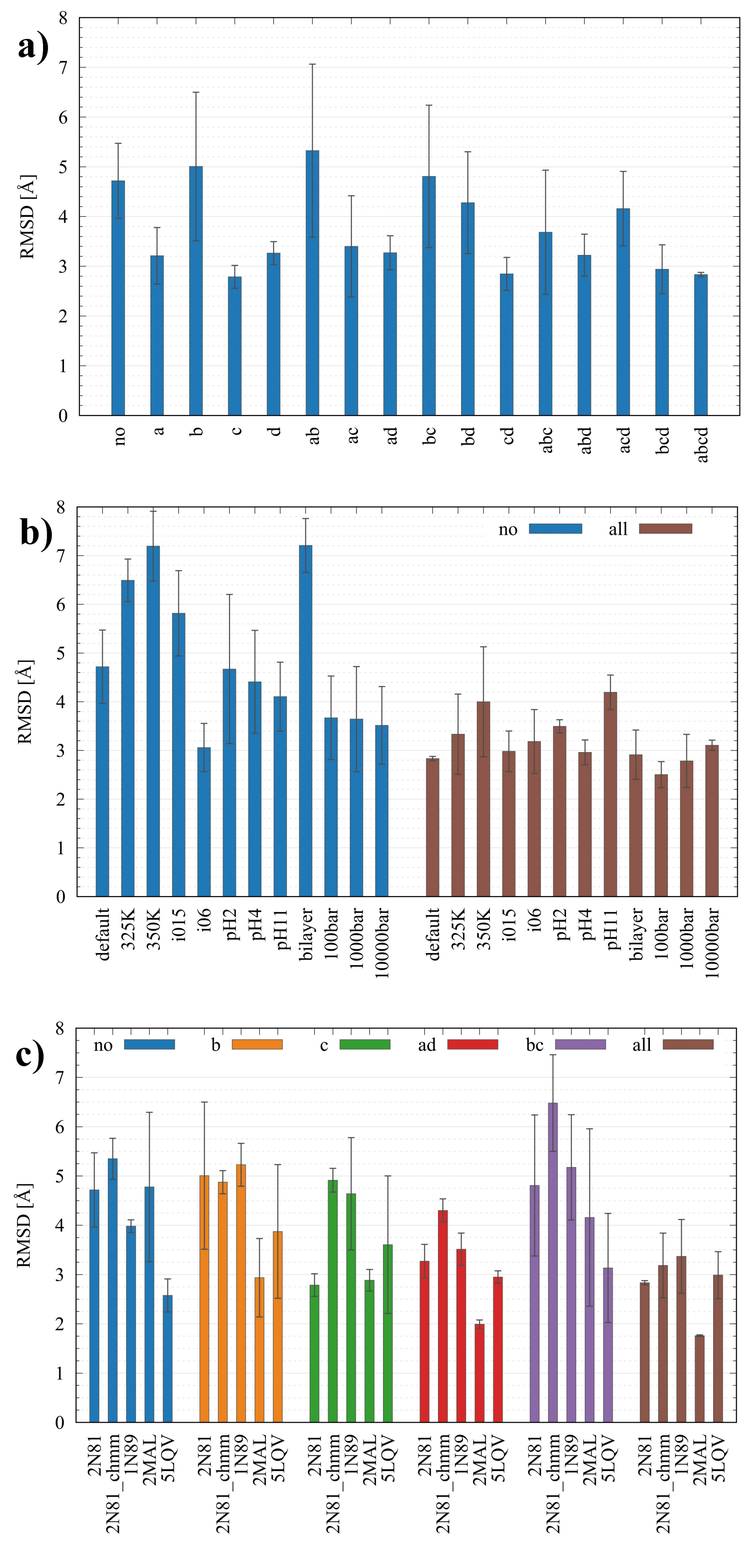


Fig 3. RMSD values calculated from simulations a) with all combinations of disulfide bonds present for nsLTP from pdb 2N81, b) with all and without any (no) disulfide bonds in varing environment conditons for nsLTP from pdb 2N81, and c) with ff19SB (2N81) and CHARMM36m (2N81_chmm) force fields and for nsLTP from pdb 2N81, 1N89, 2MAL and 5LQV.

Disscusion & Conclusions

- In the absence of disulfide bonds, the nsLTP protein maintains its overall shape (radius of gyration (rg) is comparable for all variations).

- Without disulfide bonds that are bonding termini, there is a noticeable increase in the flexibility of these parts of the protein (Fig. 2).

- Single disulfide bonds can provide greater structural stabilization than certain combinations of two or even three disulfide bonds.

- Some of the disulfide bonds can destabilize nsLTP by imposition of internal tension of non-local protein fragments, especially Cys14-Cys30, which are located between the helices forming a cavity and can cause internal stress that lowers stability (Fig. 3a).

- MD simulations conducted under challenging conditions, highlight a more pronounced influence of disulfide bonds on nsLTP stability (Fig. 3b), indicating a closer association with protein destabilization and enzymatic degradation during stress rather than the sole maintenance of stability within typical physiological contexts.

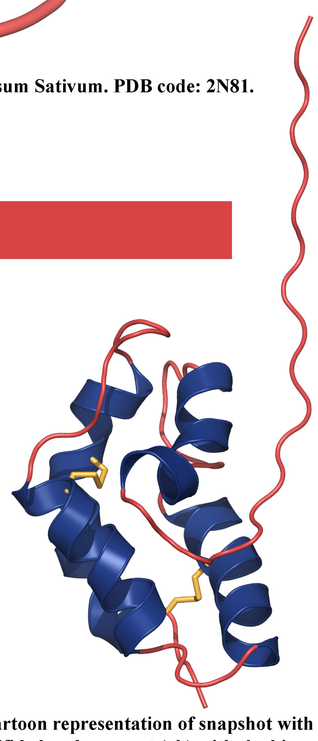


Fig 2. Cartoon representation of snapshot with two disulfide bonds present (ab) with the biggest RG value. Lack of disulfide d (Cys51-Cys90) allows flexible movement of C-terminus.

What's next?

We plan to study how influence of disulfide bonds:

- impacts nsLTP stability in the presence of various lipids to mimic the natural environment in which they are present,
- impacts nsLTP stability and resistance to mechanical stress,
- impacts nsLTP stability in water-lipid interface.

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[2] Zavodszky, M.; Chen, C.-W.; Huang, J.-K.; Zolkiewski, M.; et al.. Disulfide Bond Effects on Protein Stability: Designed Variants of Cucurbita Maxima Trypsin Inhibitor-V. Protein Sci. 2001, 10, 149– 160
[3] Liu, H.; Schittny, V.; Nash, M. A. Removal of a Conserved Disulfide Bond Does Not Compromise Mechanical Stability of a VHH Antibody Complex. Nano Lett. 2019, 19, 5524– 5529
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