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Deciphering the Fluorous Effect in Protein Quaternary Assembly

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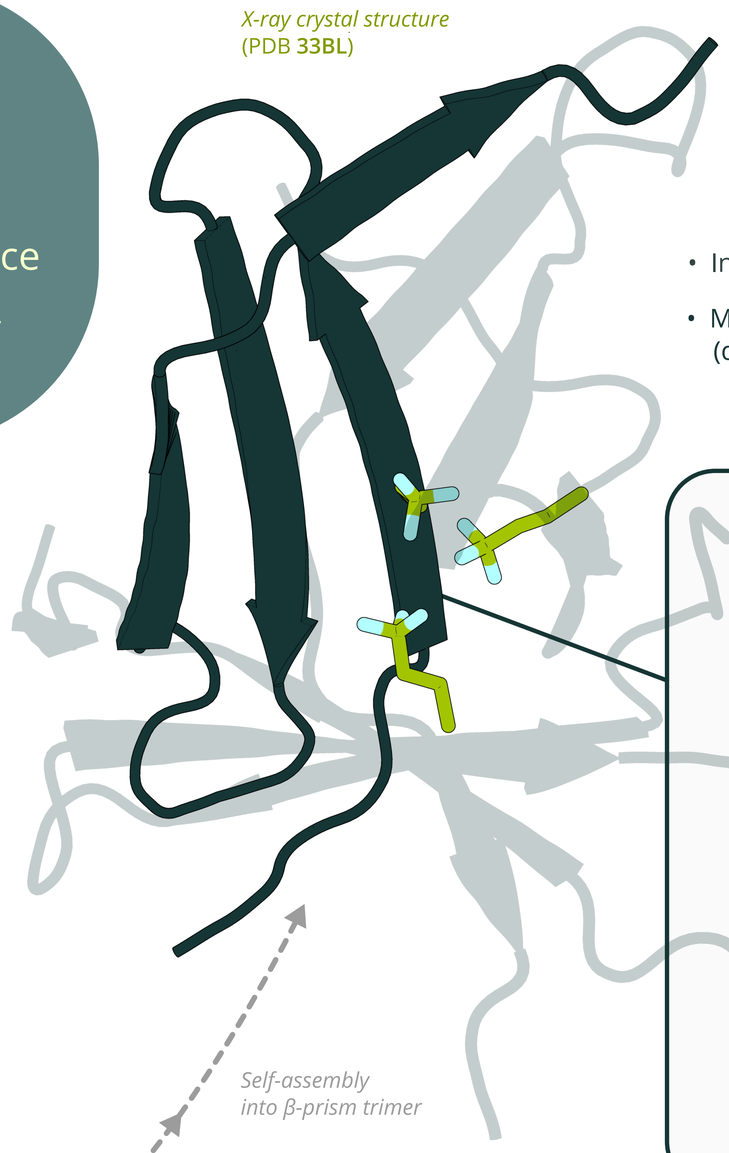
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Summary

- β-Sheet protein trimer, accessible by chemical synthesis
- Fluorine-substitution at trimer interface
- Structure/stability investigation (X-ray crystallography, NMR, native MS, CD)

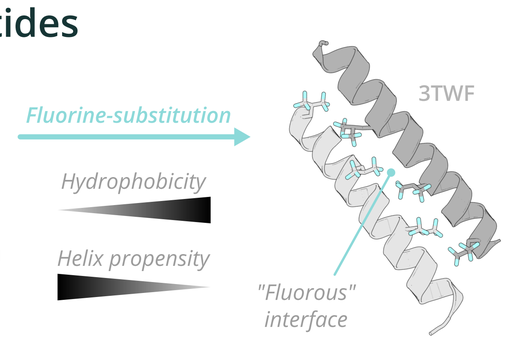
Highlights

- Defined quaternary strucuture with **fluorine cluster**
- Noncovalent, electrostatic **R–CF₃···F interaction**

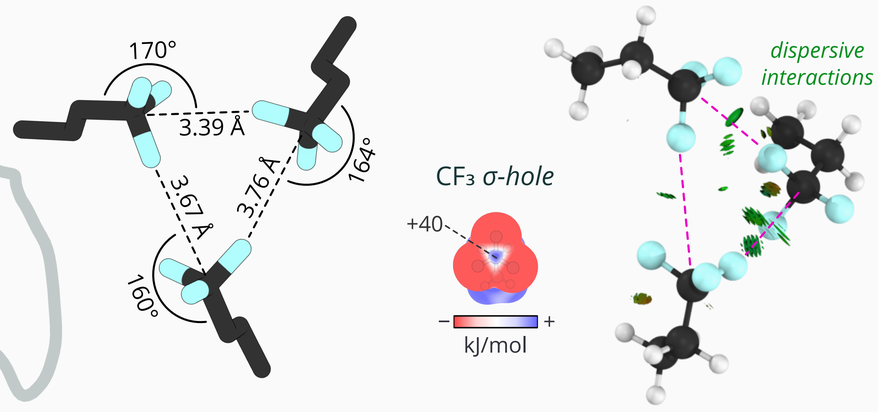


Previous studies focused mainly on coiled-coil peptides

- Increased coiled-coil stability[1 2]
- Modulation of cc oligomerization (dimer, trimer, etc.)[3]



Noncovalent R–CF₃···F Interaction

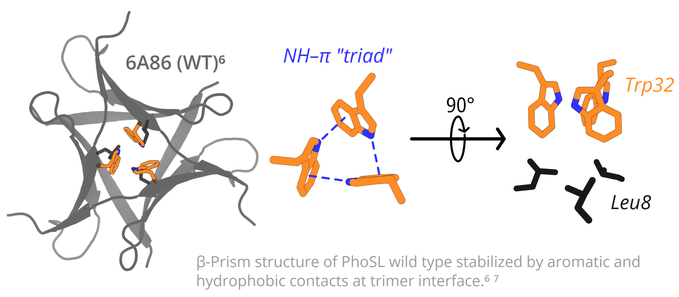


$$E_{int} = E_{ABC} - [E_A + E_B + E_C] = -3.4 \text{ kJ/mol}$$

Near-linear, σ-hole directed R–CF₃···F arrangement resembles tetrel bond.[4 5] Quantum chemical analysis (TPSSh-D3BJ/def2-TZVP) of truncated side chain model using crystal structure coordinates: Non-covalent, dispersive interactions characterized by NCI analysis are shown as green discs. Interaction energy of the trimer calculated at RI-MP2/def2-TZVP level.

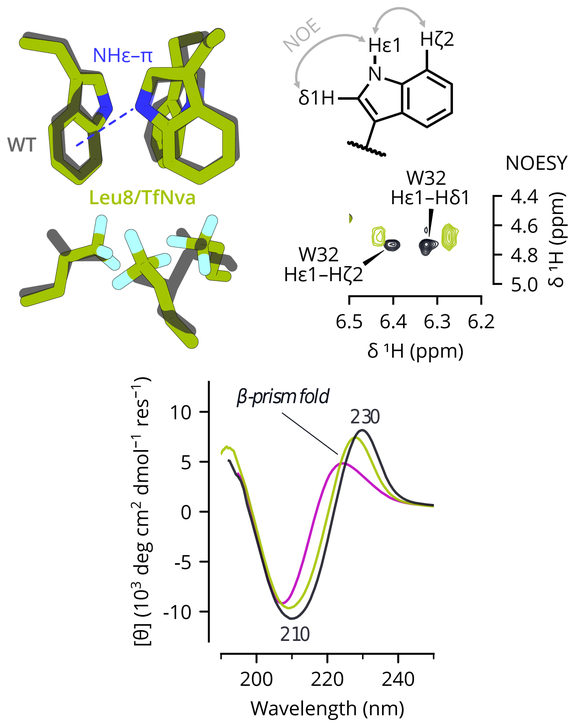
*A modular **β-strand scaffold** to investigate noncovalent **side chain interactions** in **protein self-assembly***

Pholiota squarrosa lectin (PhoSL)



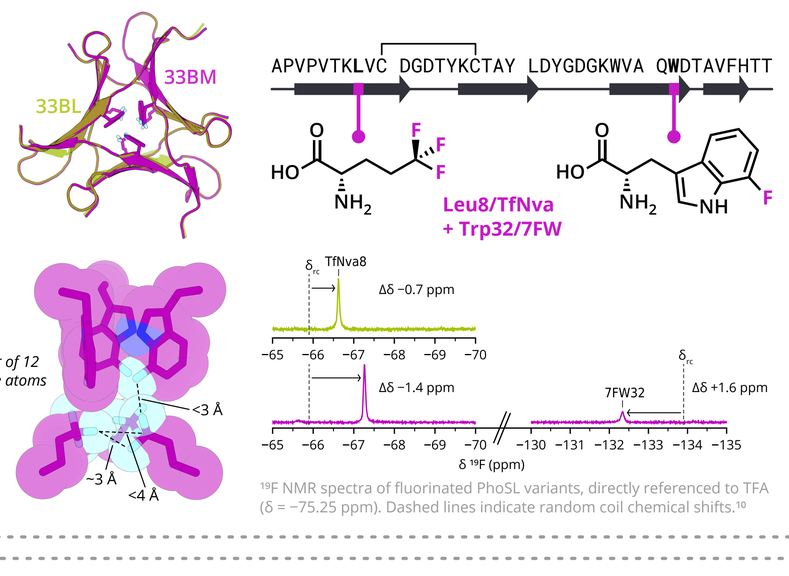
β-Prism structure of PhoSL wild type stabilized by aromatic and hydrophobic contacts at trimer interface.[6 7]

Interactions at trimer interface



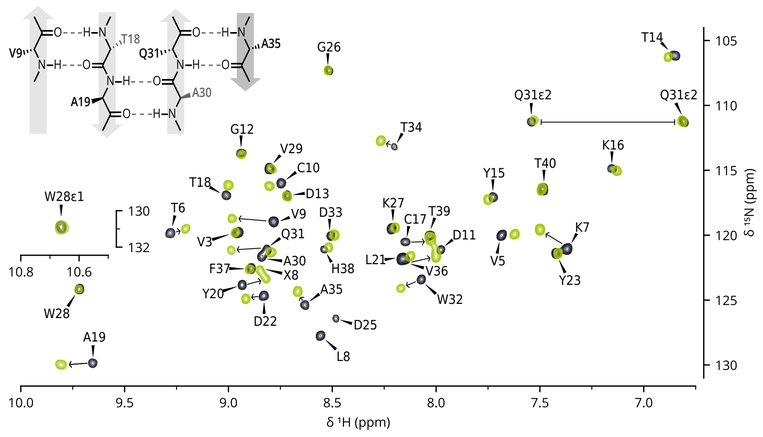
CD spectra of PhoSL variants (50 µM, pH 7.5, 20 °C)

High degree of fluorination tolerated



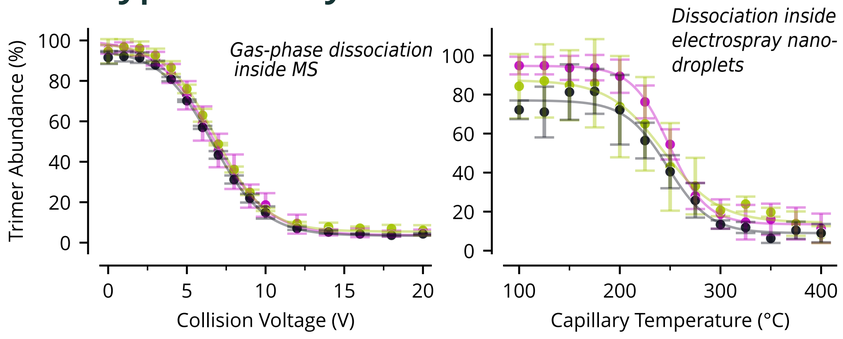
¹⁹F NMR spectra of fluorinated PhoSL variants, directly referenced to TFA (δ = −75.25 ppm). Dashed lines indicate random coil chemical shifts.[10]

Global protein structure conserved



¹H-¹⁵N HSQC spectra of wild type (gray) and Leu8/TfNva substituted PhoSL (lime) with scheme representing cross-strand hydrogen bond network.

Wild-type stability retained



Dissociation of PhoSL trimers in the gas-phase and in quasi-solution monitored by native MS.[8 9]

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Acknowledgements

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