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Engineering a Miniature Protein in a Visible Light-Powered Fully Artificial Electron Transport Chain

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The advancement of de novo design of metal-binding proteins has made remarkable progress, aiming to tailor proteins for specific applications. Over the last years, also supported by the recent advances in computational protein design, we have designed several custom-made enzymes, setting several milestones in the field [1,2]. By different approaches, we designed small, yet functional, models bearing various metal sites [3,4]. In this study, we address a particularly intriguing and extensively studied case to benchmark our understanding. We present the successful design of a single-chain linear protein, comprising only 28 residues, that folds and functions akin to a natural Rubredoxin (FeCys4 site) [5]. Notably, we achieve the first characterization of the crystal structure of a de novo protein featuring a tetrathiolate metal cluster. The structure exhibits remarkable agreement with the intended design, despite the absence of any sequence correlation with known Rubredoxins. Furthermore, we intentionally program a higher reduction potential in comparison to natural and designed FeCys4-containing proteins, by wisely introducing a set of hydrogen bonds in the second sphere. Leveraging this achievement, we harness the protein as a terminal electron acceptor in a fully artificial electron transport chain, powered by visible light. Our findings not only shed light on the unique structural arrangement of this miniaturized protein but also pave the way for its potential applications in the realm of artificial photosystems and electron transfer processes.

Keywords: *de novo protein design, metalloproteins, iron-sulfur proteins, electron transport*

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References

[1] Lombardi, A., Pirro, F., Maglio, O., Chino, M. & DeGrado, W. F. *Acc. Chem. Res.* **52,** 1148–1159 (2019).
[2] Leone, L., Chino, M., Nastri, F., Maglio, O., Pavone, V. & Lombardi, A. *Biotech. Appl. Biochem.* **67,** 495–515 (2020).
[3] Pirro, F., Schmidt, N., Lincoff, J., Widel, Z. X., Polizzi, N. F., Liu, L., Therien, M. J., Grabe, M., Chino, M., Lombardi, A. & DeGrado, W. F. *Proc. Natl. Acad. Sci.* **117,** 33246–33253 (2020).
[4] Pirro, F., La Gatta, S., Arrigoni, F., Famulari, A., Maglio, O., Del Vecchio, P., Chiesa, M., De Gioia, L., Bertini, L., Chino, M., Nastri, F. & Lombardi, A. *Angew. Chem. Int. Ed.* **62,** e202211552 (2023).
[5] Chino, M., Di Costanzo, L. F., Leone, L., La Gatta, S., Famulari, A., Chiesa, M., Lombardi, A. & Pavone, V. *Nat. Commun.* **14,** 2368 (2023).