

## Specificity in Protein-Protein Interactions: High-Throughput Characterization of Rationally Designed and Naturally Evolved Coiled-Coil Networks



**Abstract.** Proteins must interact specifically with certain partners to recapitulate the biology that constitutes life, but understanding the biochemical and evolutionary determinants of how proteins do so is challenging as existing techniques are limited in throughput and ability to pinpoint sequences of interest. Here, we create a high-throughput two-hybrid assay that marries gene synthesis with a next-generation sequencing readout, allowing us to quantitatively characterize tens of thousands of programmed interactions at once. We use this system to investigate specificity in de novo designed coiled-coils—small alpha-helical proteins which exhibit a high-degree of specificity. In an iterative design-build-test loop we screen more than 26,000 interactions, and use our data to improve coiled-coil design algorithms while finding the largest sets of orthogonal proteins to date. We also use our system to investigate the evolutionary origins of specificity—in particular, the coiled-coil domain of the PAR/E4BP4 family—whose extant paralogs do not heterodimerize though their common ancestor was a homodimer. Using ancestral sequence reconstruction we find specificity appears after most gene duplications, and while the process begins immediately it takes substantial time to resolve. Finally, we find that after specificity is acquired interactions are not regained and that the process is not driven by direct selection.

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*from*

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12:30 pm

[https://ucla.zoom.us/j/99253467228?  
pwd=eTNRMFpjaEdKM1RLTDdVdXdaWHRqQT09](https://ucla.zoom.us/j/99253467228?pwd=eTNRMFpjaEdKM1RLTDdVdXdaWHRqQT09)

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