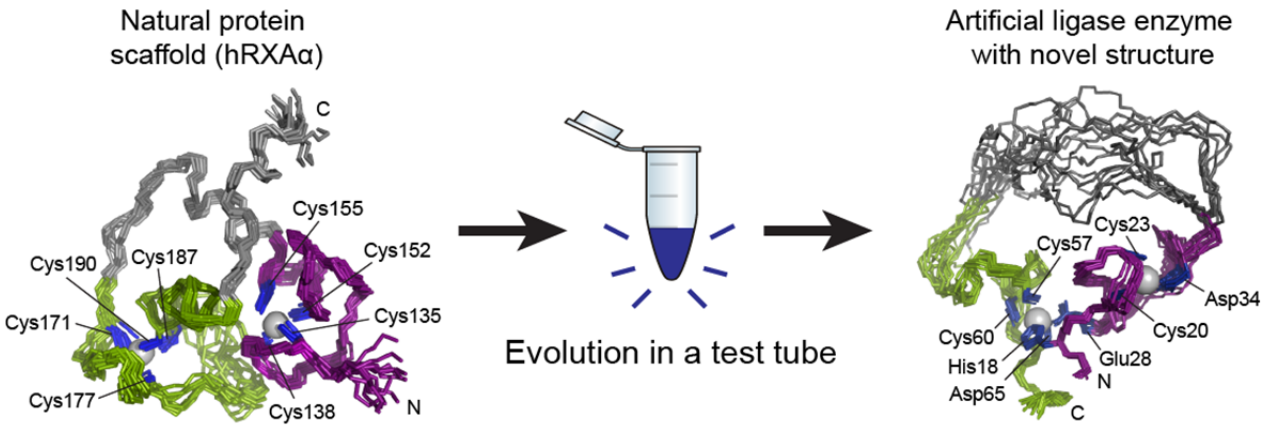
Unusual Structure and Dynamics of an Artificial Enzyme Created in a Test Tube

Enzymes catalyze an impressive range of chemical reactions, many of which can be quite challenging to accomplish through chemical methods alone. In recent years, enzymes have gained an important role in industry as cheap and environmentally friendly alternatives to traditional chemical catalysts. To this end, the creation of enzymes from scratch is necessary to provide biocatalysts for the wealth of non-natural reaction chemistries and substrates that have emerged over the last century. But thus far, this has only been achieved when extensive knowledge of the mechanism of the reaction is at hand[1].

Seelig, et al. recently used a clever *in vitro* strategy to synthesize an artificial RNA ligase enzyme starting from a non-catalytic small-protein domain consisting of two zinc finger motifs from a DNA-binding domain[2; 3]. The RNA ligase catalyzes the joining of a 5′-triphosphorylated RNA to the 3′-hydroxyl group of a second RNA for which no known natural enzyme catalysts have been found. Although zinc finger proteins are common structural motifs, they are not known to take part in catalysis in natural proteins. In contrast, the zinc finger RNA ligase (termed 10C) showed catalytic rate accelerations of more than two-million-fold. Thus, Seelig, et al. were able to obtain a biologically unknown catalytic activity from a protein not associated with catalysis.

Recently, Seelig, et al. used NMR and synchrotron-based Zn K-edge EXAFS to solve the structure of this novel biocatalyst. Data were measured on the biological x-ray absorption spectroscopy Beam Line 9-3 at



Changes in3D structure upon directed evolution of the hRXRα scaffold to the artificial RNA ligase enzyme 10C. Zinc ions are shown as gray spheres and zinc-coordinating residues are labeled and shown in blue. Although both structures contain two zinc coordination centers each (green and purple), the overall structures of the two proteins are fundamentally different.

SSRL. Results show that the structure contains two zinc sites coordinated by the protein and solvent molecules. The NMR studies show that relative to the initial non-catalytic protein hRXRα, the RNA ligase 10C has increased flexibility (compare the variability in the gray region of the two proteins in the figure). This flexible and completely different structure emerged simultaneously with the novel catalytic function. As this new structure has not been subjected to billions of years of natural evolution that shaped contemporary enzymes it can therefore be considered an early or primordial catalytic fold. The simplified environment of *in vitro* evolution allows the generation of model systems and the study of basic principles of complex natural evolution.

In summary, this *in vitro* directed evolution method has potential to generate novel biocatalysts for a multitude of applications, and the unique structure of the artificial ligase enzyme demonstrates that this methodology can successfully generate artificial enzymes without being limited to known biological folds.

This work was partly supported by the US National Aeronautics and Space Administration (NASA) Agreement NNX09AH70A issued through the NASA Astrobiology Institute–Ames Research Center (to F.-A.C., A.M., L.C. and B.S.); the Minnesota Medical Foundation (to B.S.) and the US National Institutes of Health (NIH) (T32 GM08347 to J.C.H., T32 DE007288 to L.R.M. and GM100310 to G.V.).

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Publication

Chao, F.-A., Morelli, A., Haugner III, J. C., Churchfield, L., Hagmann, L. N., Shi, L., Masterson, L. R., Sarangi, R., Veglia, G. & Seelig, B., "Structure and dynamics of a primordial catalytic fold generated by *in vitro* evolution" *Nat. Chem. Biol*. published online: 9 Dec. 2012, doi: 10.1038/nchembio.1138.

Authors

Burckhard Seelig, University of Minnesota and Ritimukta Sarangi, SSRL

SSRL is primarily supported by the DOE Offices of Basic Energy Sciences and Biological and Environmental Research, with additional support from the National Institutes of Health, National Institute of General Medical Sciences and the National Center for Research Resources.