

Papers of the Week

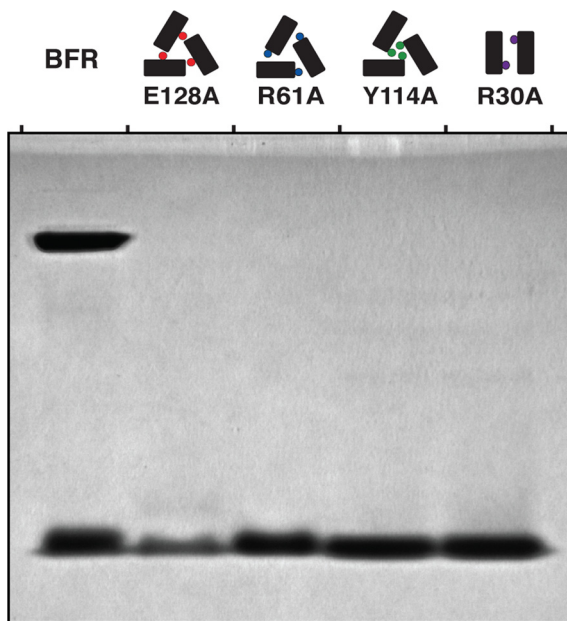
A Very Close Alanine Shave ♦

♦ See referenced article, *J. Biol. Chem.* 2010, **285**, 12078–12086

Alanine-shaving Mutagenesis to Determine Key Interfacial Residues Governing the Assembly of a Nano-cage Maxi-ferritin

Many proteins self-assemble into larger multisubunit complexes held together by protein-protein interactions. Although the interfaces between subunits are often quite large, the binding energies are usually localized to a few “hot spot” residues, thus providing key pharmaceutical targets. In this Paper of the Week, Yu Zhang and colleagues used *Escherichia coli* bacterioferritin (BFR), a nano-scale cage protein composed of 24 subunits, as a model to identify key amino acid residues that control self-assembly and protein stability. They first identified nine potential hot spot residues by inspecting the BFR crystal structure and then designed, expressed, and purified alanine mutants at these sites for a shaving mutagenesis study. Four of the residues, Arg-30, Arg-61, Tyr-114, and Glu-128, completely shut down formation of the 24-mer complex when mutated and instead led to the formation of a cooperatively folded dimer. This suggests that these residues are crucial “switch residues” that promote higher order assembly. In addition, the Arg-30 and Arg-61 alanine mutants resulted in a protein that was more thermodynamically stable than the native form, revealing some insights into the energetics of bacterioferritin. These findings provide an excellent starting point for future work analyzing how structure relates to function in supramolecular proteins, as well as for the design of drugs to disrupt protein self-assembly or even novel protein nano-structures.

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Native PAGE

Native gel electrophoresis shows that bacterioferritin mutants R30A, R61A, Y114A, and E128A exhibit no detectable 24-mers, indicating that these amino acids may be critical oligomerization “switch residues.”