Chemical Approach to Biological Safety: Molecular-Level Control of an Integrated Zinc Finger Nuclease

Application of artificial nucleases (ANs) in genome editing is still hindered by their cytotoxicity related to off-target cleavages. This problem can be targeted by regulation of the nuclease domain. Here, we provide an experimental survey of computationally designed integrated zinc finger nucleases, constructed by linking the inactivated catalytic centre and the allosteric activator sequence of the colicin E7 nuclease domain to the two opposite termini of a zinc finger array. DNA specificity and metal binding were confirmed by electrophoretic mobility shift assays, synchrotron radiation circular dichroism spectroscopy, and nano-electrospray ionisation mass spectrometry. In situ intramolecular activation of the nuclease domain was observed, resulting in specific cleavage of DNA with moderate activity. This study represents a new approach to AN design through integrated nucleases consisting of three (regulator, DNA-binding, and nuclease) units, rather than simple chimera. The optimisation of such ANs could lead to safe gene editing enzymes.