Redesign of Non-specific Endonucleases

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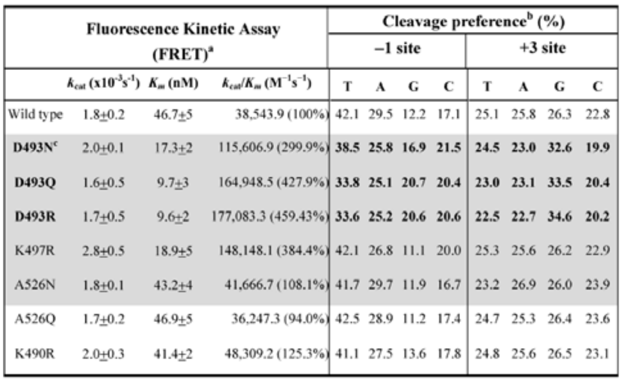
It is of crucial importance to elucidate the underlying principles that govern the binding affinity and selectivity between proteins and DNA. Here we use the nuclease domain of Colicin E7 (nColE7) as a model system to generate redesigned nucleases with improved DNÃbinding affinities and altered sequence cleavage preferences. ColE7 is an Escherichia coli toxin, bearing a nonspecific endonuclease domain with a preference for hydrolyzing DNA phosphodiester bonds at the 3'O-side after thymine.

Using systematic computational screening, six nColE7 mutants were predicted to fold and bind DNA. Fluorescence kinetic assays confirmed that all of the six designed mutants were well-folded active enzymes, and three of the six mutants, D493N, D493Q and K497R, had 3 to 5̃fold higher DNA binding affinity than wildt̃ype nColE7. Footprint assays further showed that D493N and D493Q digested DNA with an increased preference for guanine at -1 and +3 sites, as compared to the wild-type enzyme (Table 1).



Fluorescence Kinetic Assay (FRET)[a]				Cleavage preference[b] (%)							
				−1 site				+3 site			
	k_{cat} (x10^{-3}s^{-1})	K_m (nM)	k_{cat}/K_m (M^{-1}s^{-1})	T	A	G	C	T	A	G	C
Wild type	1.8±0.2	46.7±5	38,543.9 (100%)	42.1	29.5	12.2	17.1	25.1	25.8	26.3	22.8
D493N[c]	2.0±0.1	17.3±2	115,606.9 (299.9%)	**38.5**	**25.8**	**16.9**	**21.5**	**24.5**	**23.0**	**32.6**	**19.9**
D493Q	1.6±0.5	9.7±3	164,948.5 (427.9%)	**33.8**	**25.1**	**20.7**	**20.4**	**23.0**	**23.1**	**33.5**	**20.4**
D493R	1.7±0.5	9.6±2	177,083.3 (459.43%)	**33.6**	**25.2**	**20.6**	**20.6**	**22.5**	**22.7**	**34.6**	**20.2**
K497R	2.8±0.5	18.9±5	148,148.1 (384.4%)	42.1	26.8	11.1	20.0	25.3	25.6	26.2	22.9
A526N	1.8±0.1	43.2±4	41,666.7 (108.1%)	41.7	29.7	11.9	16.7	23.2	26.9	26.0	23.9
A526Q	1.7±0.2	46.9±5	36,247.3 (94.0%)	42.5	28.9	11.2	17.4	24.7	25.3	26.4	23.6
K490R	2.0±0.3	41.4±2	48,309.2 (125.3%)	41.1	27.5	13.6	17.8	24.8	25.6	26.5	23.1

Table 1: The DNA binding affinity, catalytic efficiency and cleavage preference of nColE7 proteins measured by FRET and foot printing assays.

To elucidate the observed higher DNÃbinding affinity and altered sequence preference in cleavage, the crystal structure of the nColE7 mutant, D493Q, in complex with an 18-bp DNA was determined, and the X-ray diffraction data were collected at the beamline 13C1 and 13B1 of the NSRRC

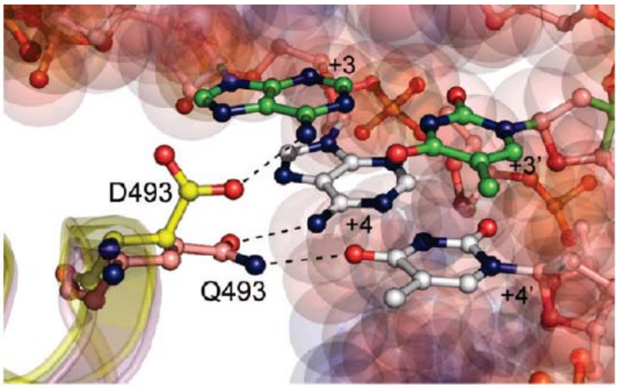


Fig. 1: Protein-DNA interactions between D493Q nColE7 mutant and DNA. Superposition of the D493Q-DNA complex structure with the H545Q-DNA complex structure (PDB entry: 2IVH) shows that the D493 side chain in the H545Q-DNA complex (in yellow) made a hydrogen bond with Ade13 N6 (+3 site), whereas the Q493 side chain in the D493Q-DNA complex (in pink) changed its conformation and made two hydrogen bonds with Ade14 N6 (+4 site) and Thy23 O4 (+4' site).

Analyses of the D493Q-DNA complex crystal structure and the DNA-binding free energies provide a physical basis for the observed improved affinity of the nColE7 mutant D493Q for DNA. Both analyses show that the Q493 side chain hydrogen bonds directly to the DNA. The crystal structure of the D493Q-DNA complex confirmed that the hydrogenbonding network within nColE7 and between nColE7 and DNA is preserved(Fig. 1), whereas the free energy decompositions further show that unfavorable electrostatic interactions with the phosphate backbone are eliminated, thus contributing to the observed enhanced affinity. In general, for a nonspecific DNA-binding protein, replacing an acidic residue at the protein-DNA interface with a polar or positively charged residue such as Asn, Gln, and Arg that can maintain the native hydrogen-bonding network would likely improve the protein-DNA binding affinity.

In summary, these results show that a combination of crystal structural analysis and computational screening is a useful means for generating redesigned nucleases with higher DNÃbinding affinities and altered sequence preferences in DNA cleavage.