Allosteric regulation in CRISPR/Cas1-Cas2 protospacer acquisition mediated by DNA and Cas2

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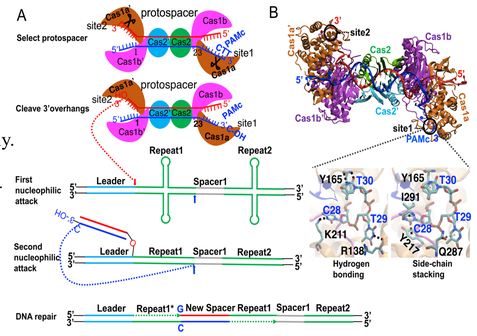
Introduction

Cas1 and Cas2 are highly conserved proteins across CRISPR-Cas systems and play a significant role in protospacer acquisition. Here we study the protospacer (or ps) DNA binding, recognition, and response to cleavage on the protospacer-adjacent-motif complementary sequence or PAMc by Cas1-Cas2, implementing all-atom molecular dynamics simulations. First, we noticed that two active sites of Cas1&1' bind asymmetrically to two identical PAMc in the simulation. For psDNA containing only one PAMc to be recognized, it is then found that the non-PAMc association site remains destabilized until after the bound PAMc being cleaved. Thus, correlation appears to exist between the two active sites, which can be allosterically mediated by psDNA and Cas2&2' in bridging.

To substantiate such findings, we further simulated Cas1-Cas2 in complex with synthesized psDNA sequences psL and psH, which have been measured with low and high efficiency in acquisition, respectively. Notably, such inter-site correlation becomes largely enhanced for Cas1-Cas2 in complex with psH, and remains low with psL. Hence, our studies demonstrate that PAMc recognition and cleavage in one active site of Cas1-Cas2 allosterically regulates non-PAMc association/reaction in the other site, and such allosteric regulation is mediated by non-catalytic Cas 2 and DNA protospacer in acquisition.



Methods

Molecular Dynamics simulations

We performed atomistic molecular dynamics simulations for CRISPR/Cas1-Cas2 with different protospacer DNA sequence by Gromacs 5.1.2 [1-2].

- **The protospacer DNA (psDNA) sequences and modification to psL/psH**

original seq	TTTTTCGTAGCTGAGGGCCTCAGCTACGTTTTCTT TTTTTTTGCATCGACTCCCGGAGTCGATGCTTTTT
psL seq	TTTTTTTGTACGACGGTATTAGAATTCATTTTCTT TTTTTTTAACATGCTGCCATAATCTTAAGTTTTTT
psH seq	TTTTTCATTGTTGTGCACGACGACATCATTTTCTT TTTTTTTGTAACAACACGTGCTGCTGTAGTTTTTT

- **Correlation calculation from the equilibrium MD simulation**

The correltion between each pair of residues is given by

$$C_{ij} = \frac{\langle (R_i - \langle R_i \rangle) \cdot (R_j - \langle R_j \rangle) \rangle}{\sqrt{\langle (R_i - \langle R_i \rangle)^2 (R_j - \langle R_j \rangle)^2 \rangle}}$$

where R_i and R_j are the positon vectors of residue *i* and *j*, taking at C_α atom of an aino acid.

The correlation between residue *i* and the active site1 (without including PAMc) as

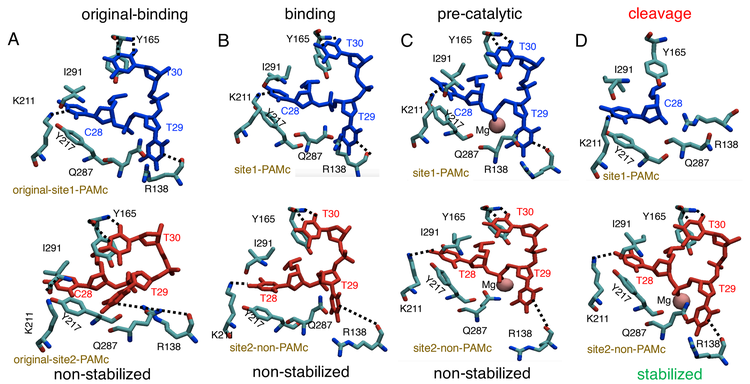
$$CR_i^{site1} = \sum_{j \in site1} C_{ij}^2$$

where residue *i* are counted for all C_α atoms from protein and the COM of nitrogen and phosphorous atoms in the psDNA [3-4].

Results

The equilibrium MD simulation for different state of Cas1-Cas2

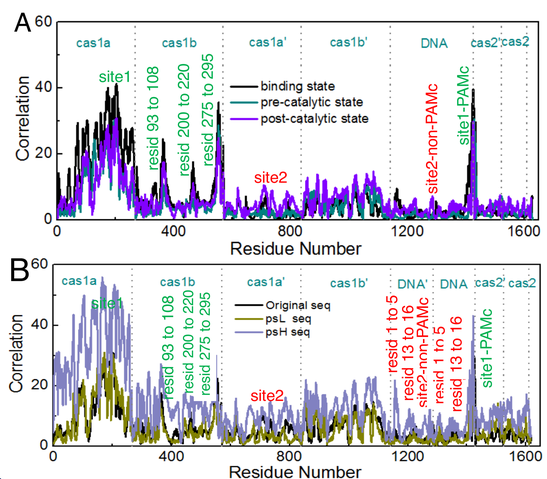
We first examined the original psDNA binding complex with two identical PAMc bound at both sites (site1 and site2); then we focused on the modified system, with one PAMc (CTT) bound at site1 and one non-PAMc (TTT) in association with site2. For such one-PAMc system, we examined not only the psDNA binding state, but also a pre-catalytic state (with catalytic magnesium ions bound to the active site1), and a half-site post-catalytic state (with PAMc cleaved at site1, as being catalyzed via an endonuclease reaction).



- ✓ Cas1-Cas2 bound with two identical PAMc are asymmetrically stabilized at one site and non-stabilized at the other site.
- ✓ The site2-non-PAMc cannot be stabilized until after the PAMc cleavage conducted at the site1.

Correlation calculation between active site1-PAMc and the rest part of the protein-DNA complex

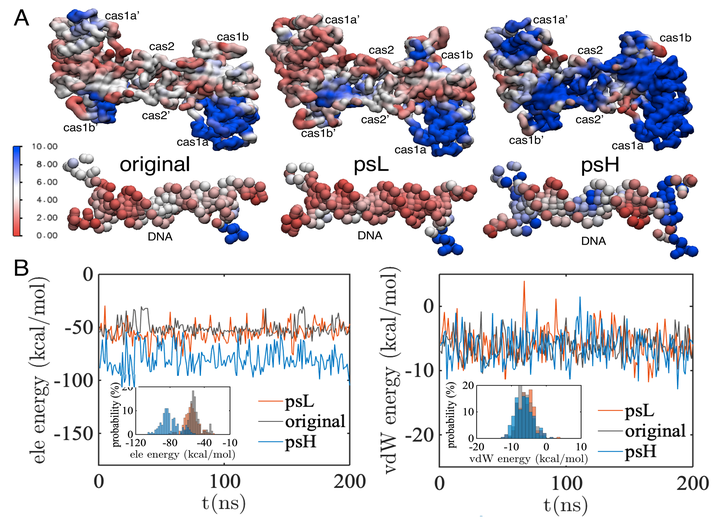
The correlation first calculated from binding to pre-catalytic and to post-catalytic state Cas1-Cas2-psDNA complex **(A)**. And then we calculated the protein internal correlations between the active site1 (with PAMc) and the rest part of the protein-DNA for both the psL and psH systems in post-catalytic state **(B)**.



- ✓ The allosteric propagation from the site1-PAMc to the site2-non-PAMc upon the site1-PAMc cleavage.
- ✓ Cas1-Cas2 in complex with psDNA of high acquisition efficiency (psH) shows the most prominent allosteric propagation upon the PAMc cleavage.

Correlation strength on Cas1-Cas2 and psDNA

The color maps of correlation strength between the active site1 (bound with PAMc) and the rest of the protein-DNA complex viewed on the structures with different psDNA sequences, in the post-catalytic state (A). And the electrostatic and vdW energies between Cas2&2' and dsDNA for various psDNA complexes: original/psL/psH(B).



- ✓ The psH system is highly correlated overall, and the allosteric propagation proceeds largely via Cas2&2' and dsDNA regions in the middle of complex.
- ✓ The electrostatic and vdW interactions between the Cas2&2' and DNA are also strongest in the psH system.

Conclusion

We conducted atomistic molecular dynamics simulations and correlation calculation from the equilibrium MD simulations to investigate the allosteric regulation in CRISPR/Cas1-Cas2 protospacer acquisition step.

I. The site2-non-PAMc cannot be stabilized until after the PAMc cleavage conducted at the site1.

II. Cas1-Cas2 in complex with psDNA of high acquisition efficiency (psH) shows the most prominent allosteric propagation upon the PAMc cleavage.

III. The psH system is highly correlated via Cas2&2' and dsDNA regions.

References and Acknowledgements

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