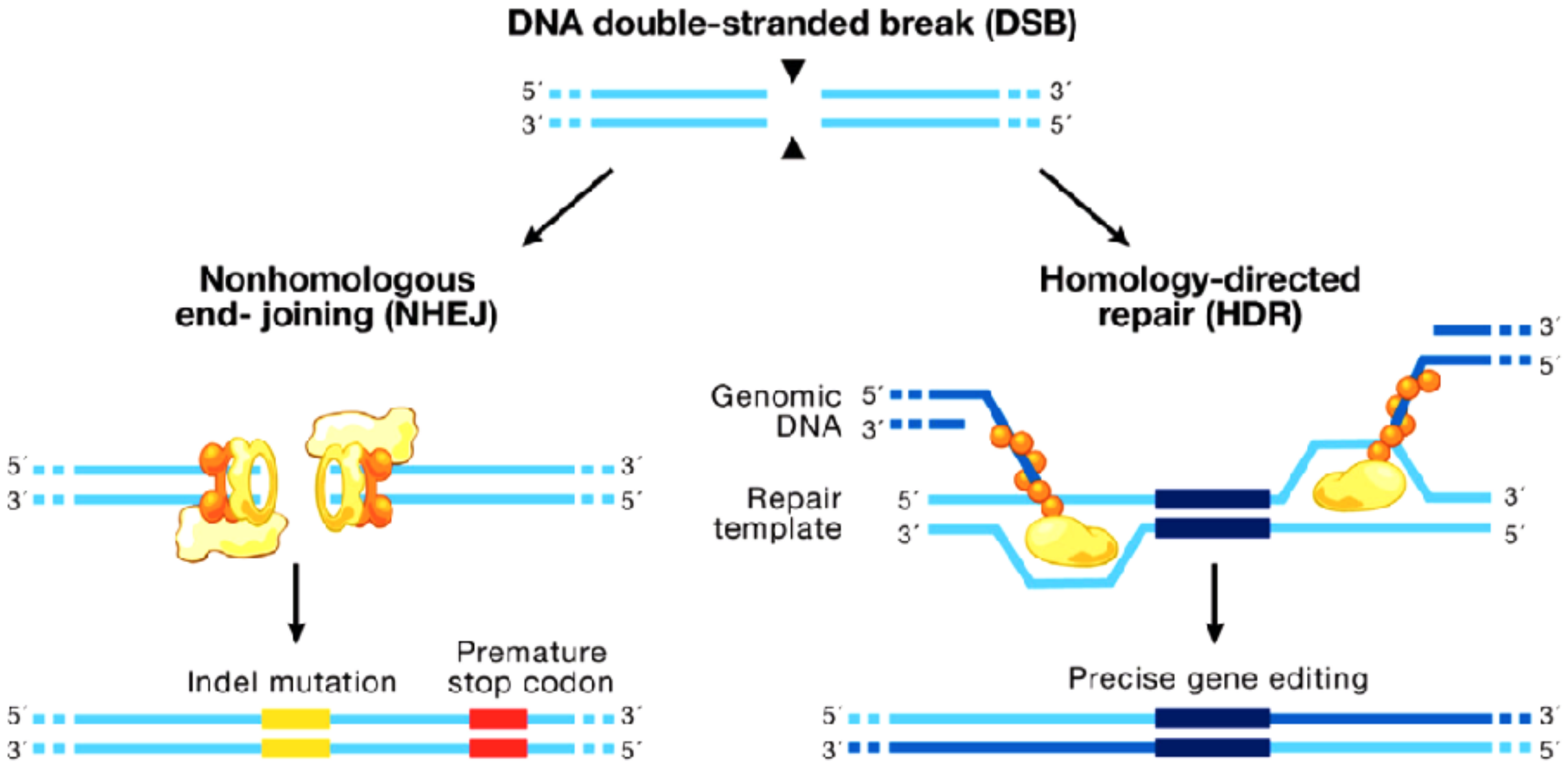
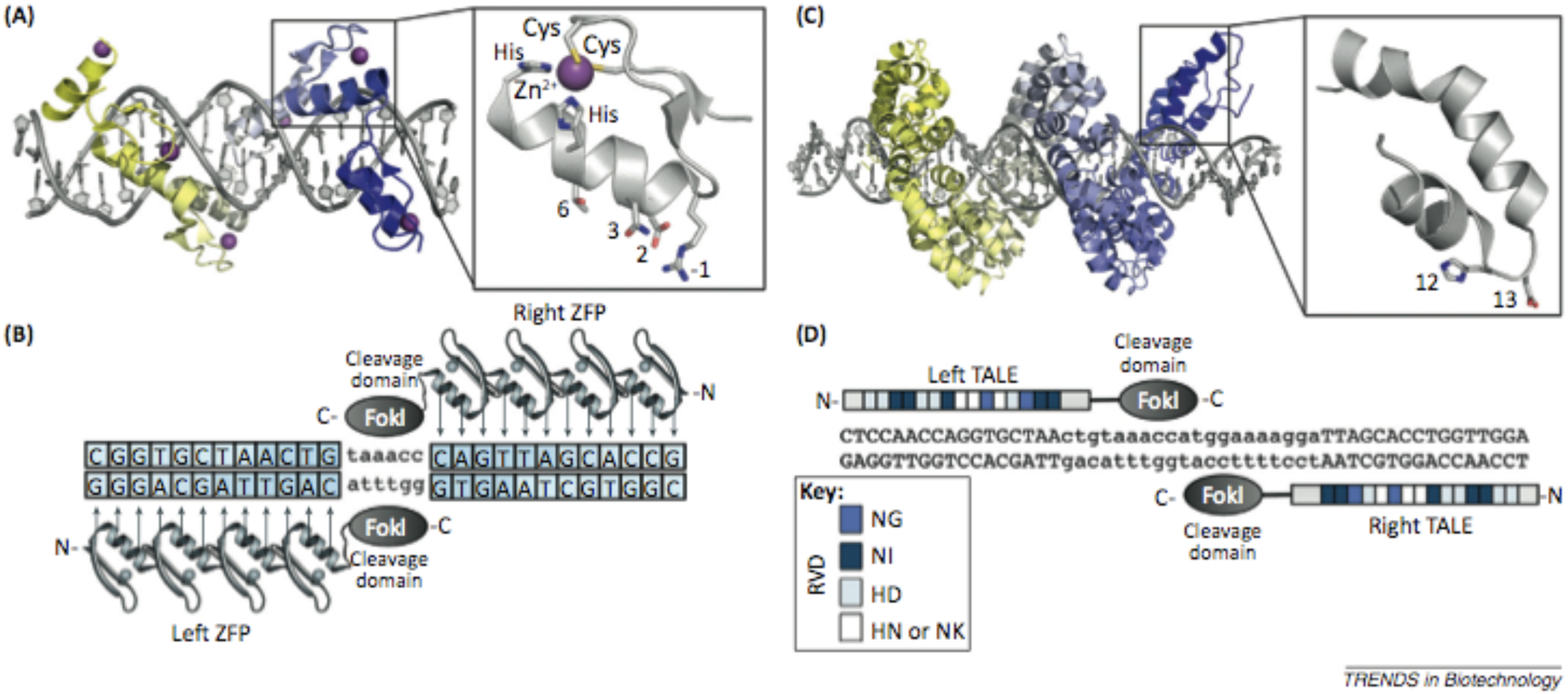
DNA damage repair pathways



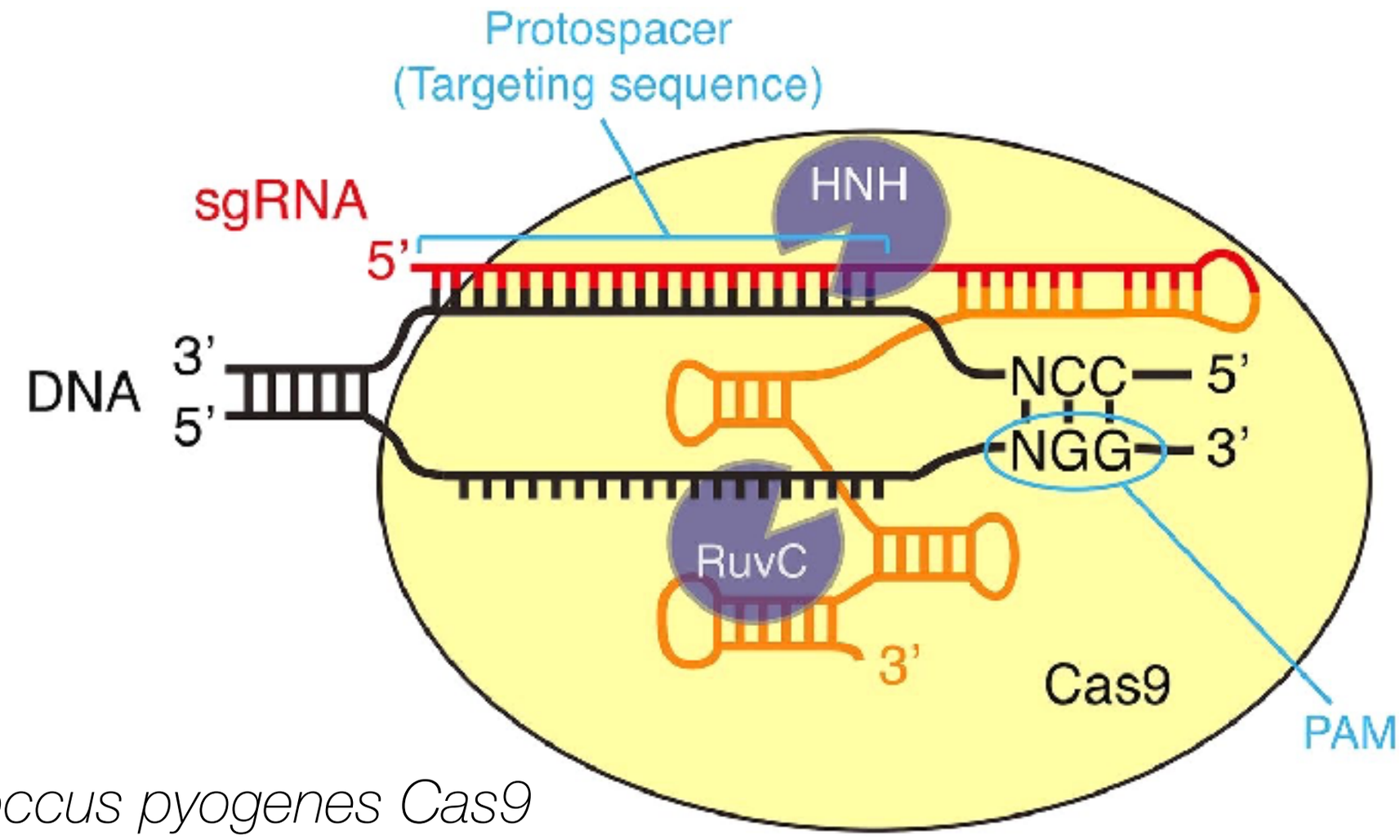
Patrick D. Hsu, Eric S. Lander, Feng Zhang

Genome editing with ZnFs and TALENS



TRENDS in Biotechnology

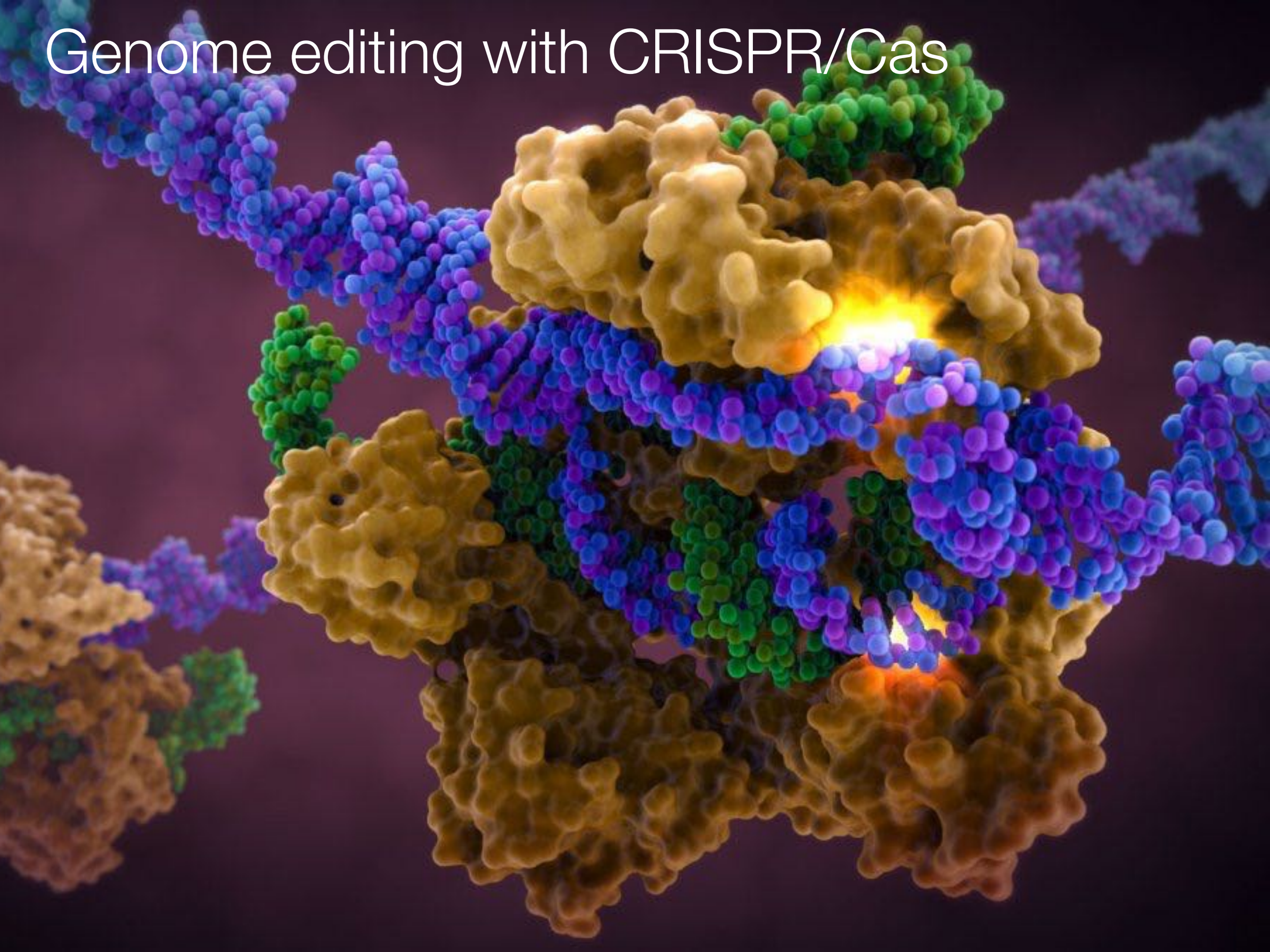
Genome editing with CRISPR/Cas



Streptococcus pyogenes Cas9

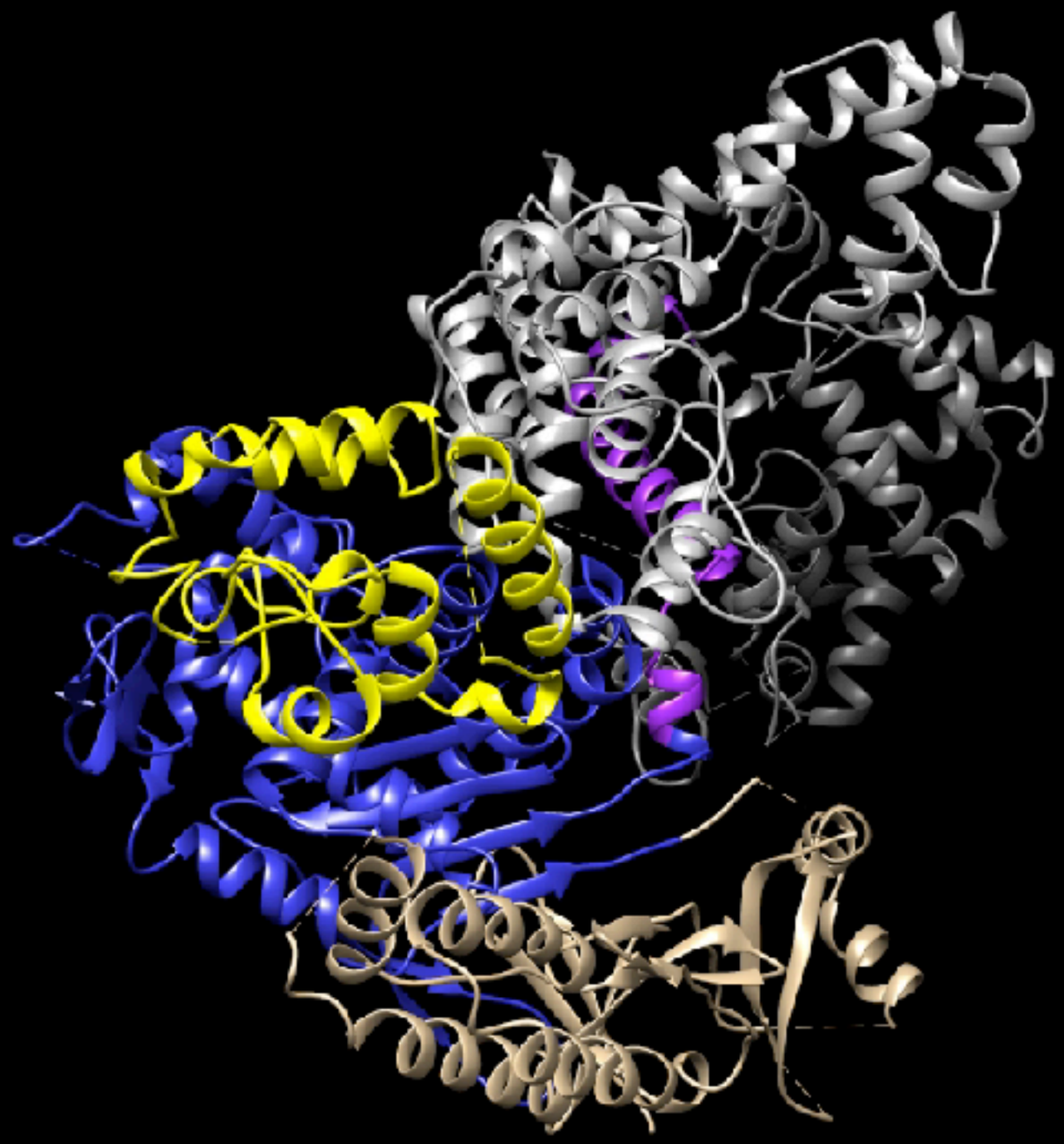
- Incredibly easy to program for any specific site
- HNH and RuvC can be mutated to create strand specific nickases or a non-cutting but still binding enzyme

Genome editing with CRISPR/Cas



Cas9 Conformational Dynamics

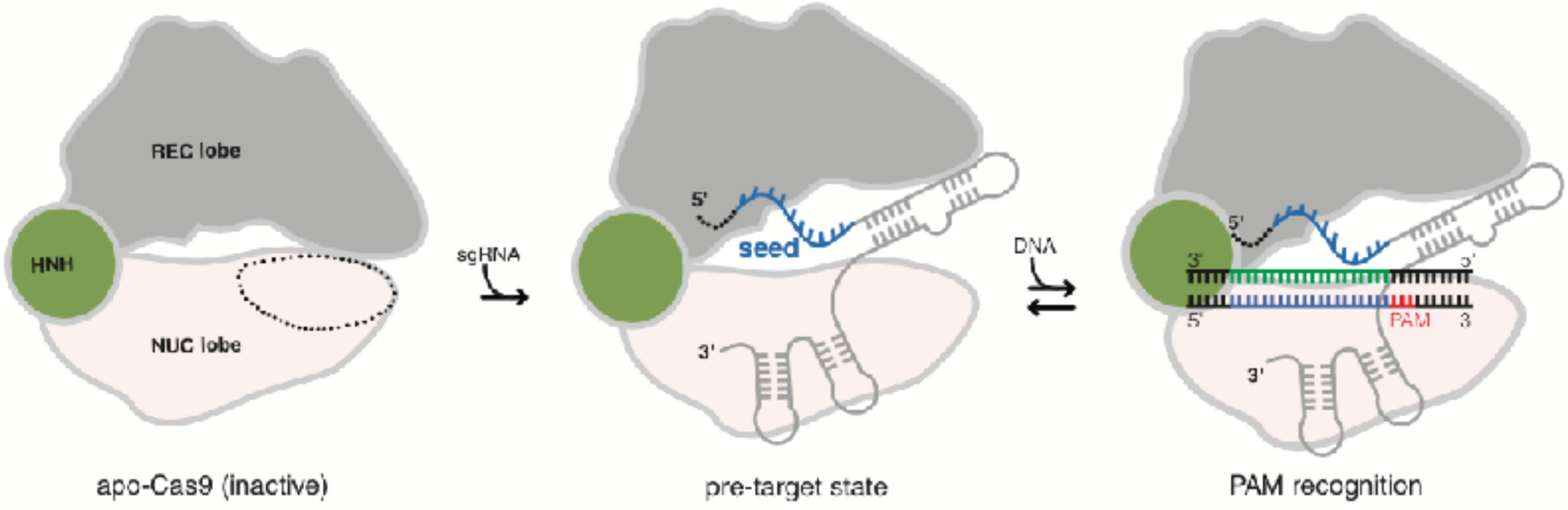
Apo (4CMP) → sgRNA-bound (4ZT0)

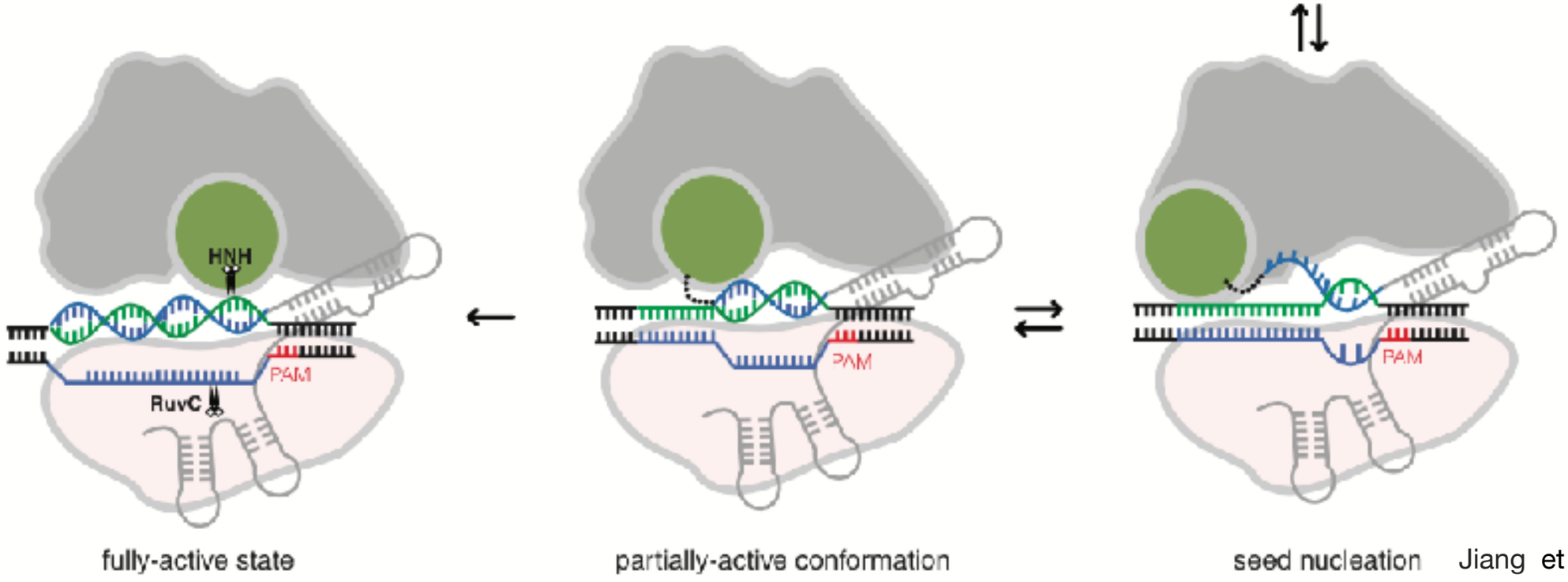


PI

Doudna lab

Cas9 cutting has multiple checkpoints

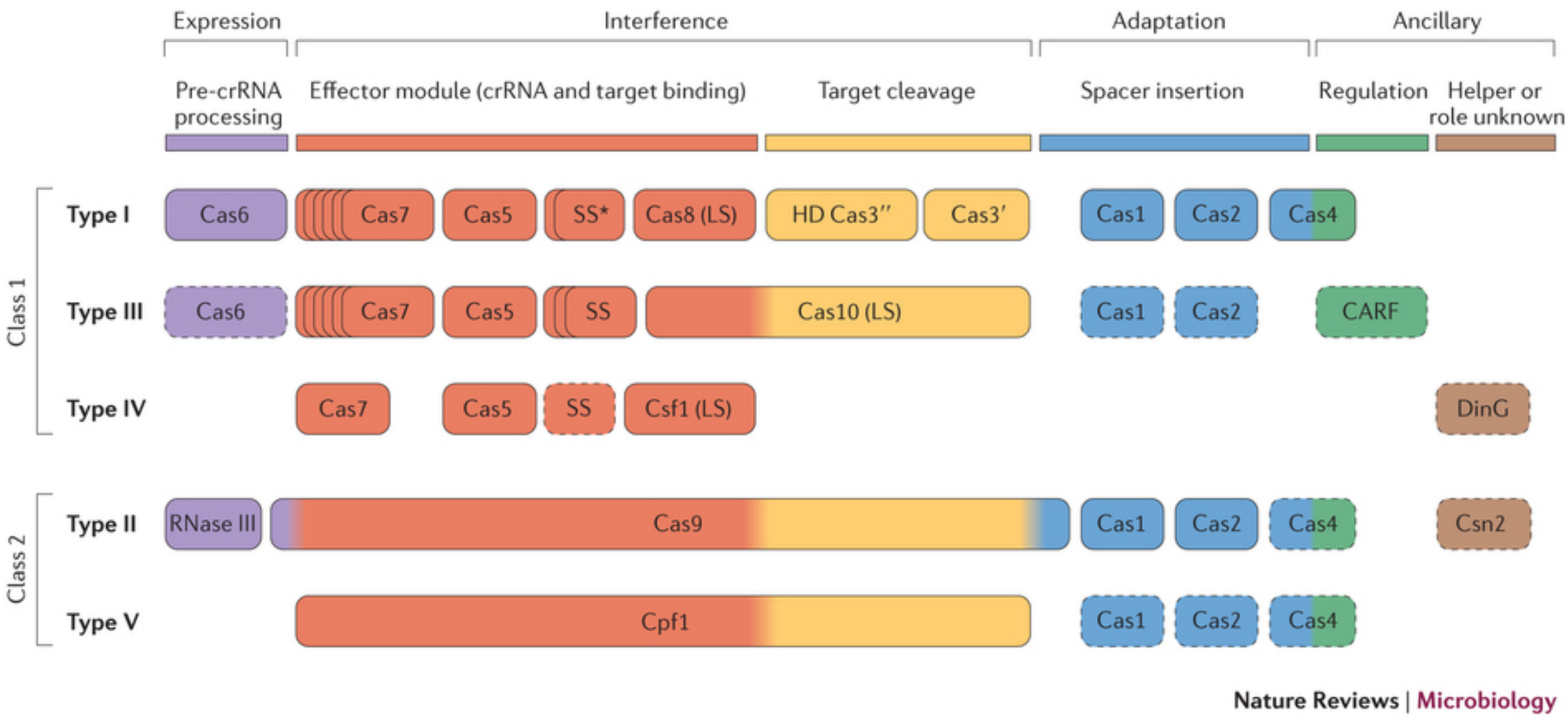




Jiang et al 2015

Fig. 4. Proposed mechanism for Cas9-mediated DNA targeting and cleavage. When Cas9 is in the apo state, its PAM-interacting cleft (dotted circle) is largely disordered. In the pretarget state, the PAM-interacting domain and seed sequence from guide RNA are preorganized for PAM recognition, followed by dsDNA melting next to PAM. The nonseed region is disordered and indicated as a dotted line. Base pairing between the seed sequence and the target DNA drives Cas9 into a near-active conformation; complete base pairing between the full guide segment and the target DNA strand enables Cas9 to reach a fully active state.

Classes of CRISPR



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