double helix, proposed by Sternberg *et al.*[6] to be a Brownian ratchet motion that forms the R-loop structure present in the active form of the Cas9 complex (**Fig. 1d**).

Competition assays using DNAs of varying sequence and length show that the PAM is required not only for stable Cas9-DNA association but also for efficient DNA cleavage. A single-stranded DNA target is cleaved much more slowly than a double-stranded DNA substrate. In a hybrid substrate containing a short double-stranded sequence adjacent to the single-stranded target, the presence of a PAM sequence in the noncomplementary strand activates the target for cleavage. This is an important observation as it suggests that there might be a second PAM-mediated checkpoint in target specificity. It also highlights the need for further work to achieve a high-resolution structural analysis of the whole Cas9-DNA-RNA complex, including the PAM-containing strand, to visualize whether a PAM-dependent rearrangement is required for activation.

Although some tolerance for target mismatch is no doubt desirable in the context of phage resistance, the opposite is true for gene editing or gene modification applications. The three new studies provide the functional and structural foundation for analyzing and optimizing the specificity of Cas9 targeting. Given the requirement of the PAM for both targeting and activation, swapping individual PAM interacting motifs among different Cas9 proteins is one possible approach. Another is fusing Cas9 to other DNA binding domains as a means of enhancing specificity, similar to what has been done for homing endonucleases[11]. As these examples indicate, there are many exciting directions for engineering Cas9 to modulate the specificity of DNA recognition and cleavage.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Cong, L. *et al. Science* **339**, 819–823 (2013).
2. Mali, P. *et al. Science* **339**, 823–826 (2013).
3. Wang, H. *et al. Cell* **153**, 910–918 (2013).
4. Nishimasu, H. *et al. Cell* **156**, 935–949 (2014).
5. Jinek, M. *et al. Science* doi:10.1126/science.1247997 (6 February 2014).
6. Sternberg, S.H. *et al. Nature* **507**, 62–67 (2014).
7. Wiedenheft, B., Sternberg, S.H. & Doudna, J.A. *Nature* **482**, 331–338 (2012).
8. Deltcheva, E. *et al. Nature* **471**, 602–607 (2011).
9. Jinek, M. *et al. Science* **337**, 816–821 (2012).
10. Sapranauskas, R. *et al. Nucleic Acids Res.* **39**, 9275–9282 (2011).
11. Boissel, S. *et al. Nucleic Acids Res.* **42**, 2591–2601 (2014).

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