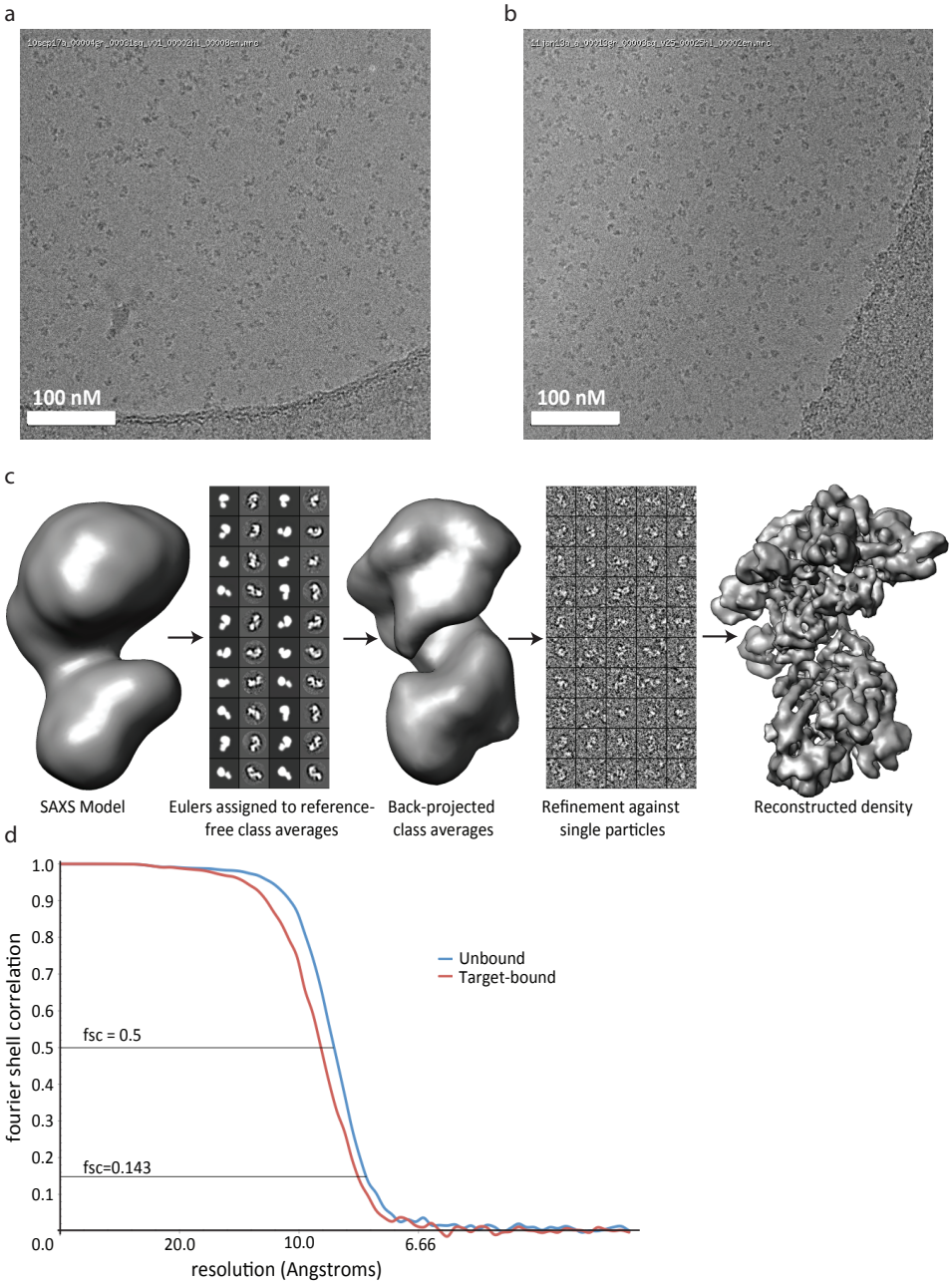
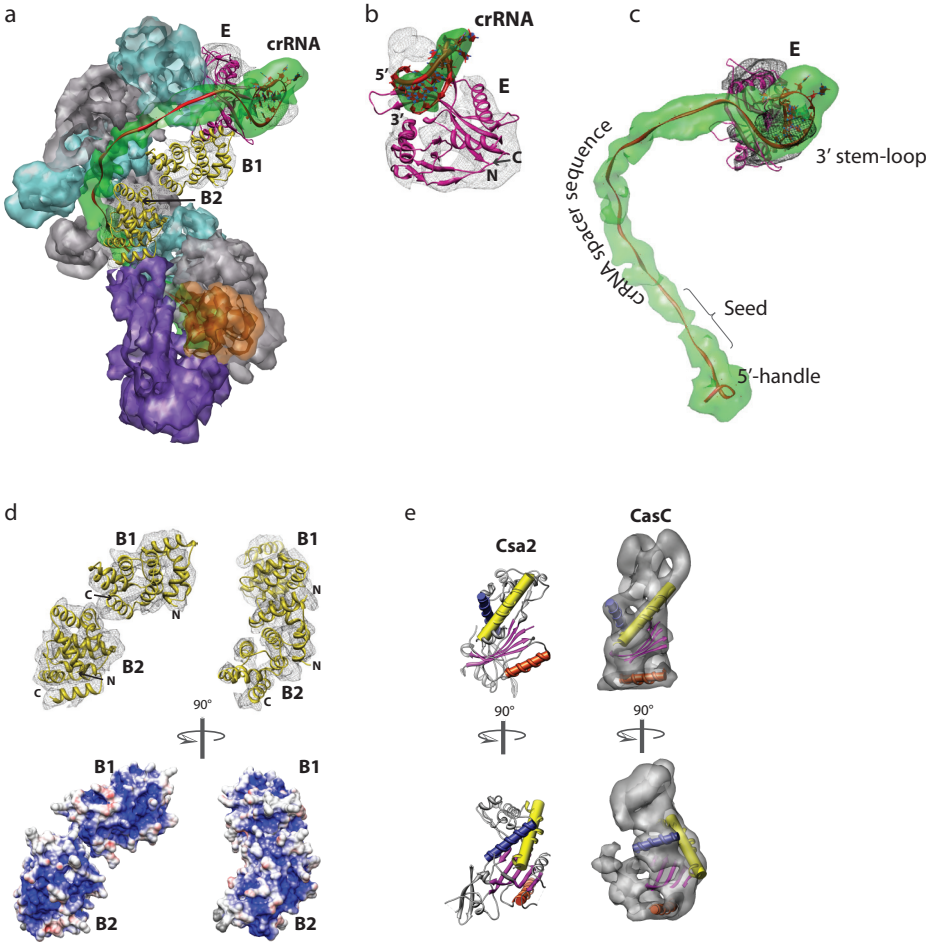
doi:10.1038/nature10402

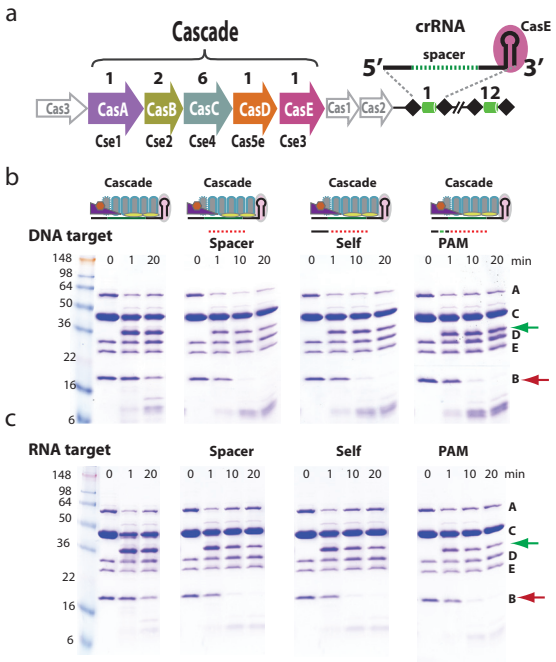


Supplementary Figure 1 | Cryo-electron microscopy and three-dimensional reconstruction. Images of the unbound **(a)** and RNA bound Cascade complex **(b)** collected at 100,000X magnification. **c,** Initial models for three-dimensional reconstruction were determined using a low-resolution SAXS reconstruction[14]. The SAXS reconstruction was low-pass filtered to 60Å resolution, forward-projected at an angular increment of 15 degrees, and a multi-reference alignment was performed

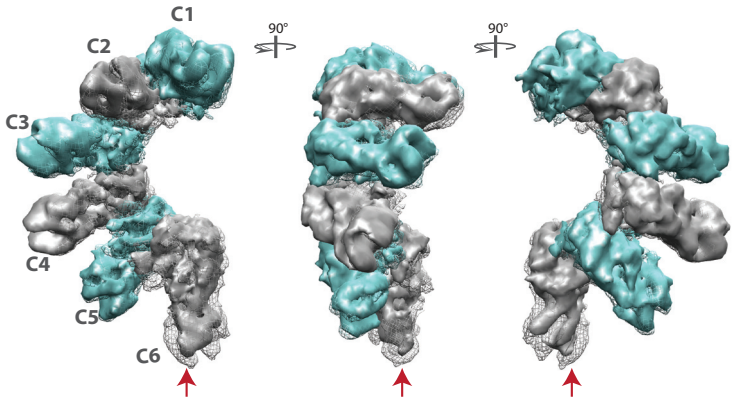
using 5,000 reference-free class averages of each of the Cascade complexes. The aligned class averages were back-projected to generate a new density, which was then used for another iteration of projection-matching. The unbound and target-bound datasets were processed separately, each using their corresponding initial model. Three-dimensional refinements of the starting densities were performed using an iterative projection-matching and Fourier reconstruction approach using libraries from the EMAN2 and SPARX software packages[24,25]. **d,** The resolution of each structure was estimated using Fourier shell correlations[32]. Resolutions reported in the paper were made using a conservative 0.5 Fourier shell correlation criterion.



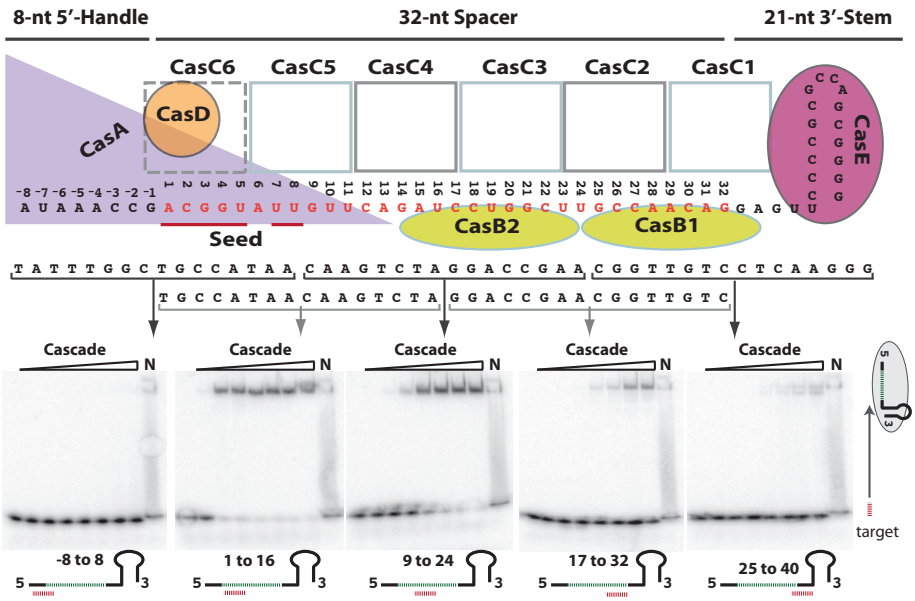
Supplementary Figure 2 | Three-dimensional docking of crystal structures into Cascade. a, Atomic coordinates for homologous structures of CasE, the crRNA stem-loop and CasB from *T. thermophilus* HB8 were docked in the ~8 Å cryo-EM map. **b,** Crystal structure of a CRISPR-specific endoribonuclease (magenta) and the crRNA stem-loop (red) docked in the segmented density for CasE (gray mesh) and the crRNA (green surface) (PDB: 2Y8W)[11]. **c,** crRNA sequence (61nt) modeled into the crRNA density. **d,** Crystal structure of a CasB homolog (Cse2) form *T. thermophilus* (yellow) docked in the segmented density for CasB (gray mesh) (PDB: 2ZCA)[33]. Surface charge representation of the *E. coli* CasB sequence threaded onto the Cse2 crystal structure. **e,** Crystal structure of a distant CasC homolog from the P2 strain of *Sulfolobus solfataricus* (PDB: 3PS0)[34]. The Csa2 structure is significantly different form the CasC density and the two structures do not superimpose with high fidelity. However, shared secondary structure elements are detectable (blue, yellow, magenta and orange) and slight modifications to the pitch or angle of these elements reveal a similar architecture.



Supplementary Figure 3 | Structural interrogation of Cascade by limited proteolysis. a, Schematic of the CRISPR/Cas locus in the *E. coli* K12 genome. Cascade assembles from an unequal number of Cas proteins and a crRNA ($CasA_1B_2C_6D_1E_1crRNA_1$). b-c) Limited trypsin digestion of Cascade bound to a nucleic acid substrate complementary to the spacer, the spacer plus the 5' handle (self) or to an oligo that contains a protospacer adjacent motif (PAM). A proteolytic product appears at the first time point (green arrow) in all samples. The CasB subunits are sensitive to proteolysis 20 minutes after binding to DNA or RNA targets (red arrow).



Supplementary Figure 4 | The CasC subunits are superimposable between the target bound and unbound structures. The CasC hexamer from the target bound (surface) and unbound structures (mesh) of Cascade. The red arrow indicates additional density for C6 in the unbound structure.



Supplementary Figure 5 | Molecular recognition of foreign nucleic acids by Cascade. Top, Schematic of the Cascade architecture highlighting the relative position of each Cas protein and the three distinct segments of the mature crRNA (8-nt 5' handle / 32-nt spacer / 21-nt 3' stem-loop). The spacer sequence is shown in red and the protein subunits are colored according to Fig 1. **Bottom,** Target binding affinities at discrete regions along the crRNA were determined by electrophoretic mobility shift assays with increasing concentrations of Cascade (0-1000 nM) in each panel. Sixteen-nucleotide ssDNA oligos that tile along the length of the crRNA were 5' end labeled with 32P. The last lane in each panel includes 500 nM Cascade and a ssDNA oligo that is not complementary to the crRNA (N). Cascade makes low affinity, non-sequence specific interactions with DNA[14].

Supplemental references

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