NMR structure of human restriction factor APOBEC3A reveals substrate binding and enzyme specificity

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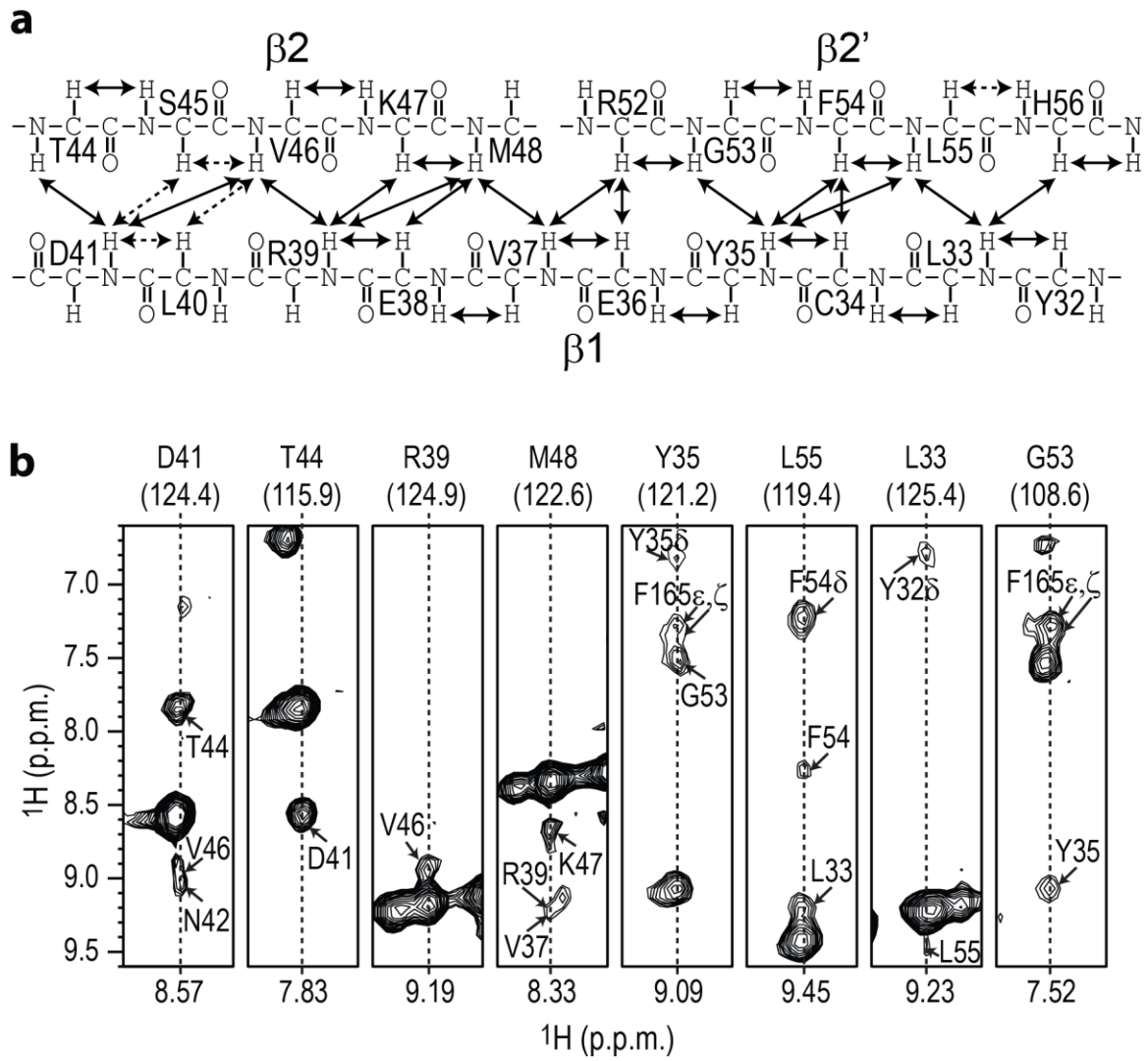
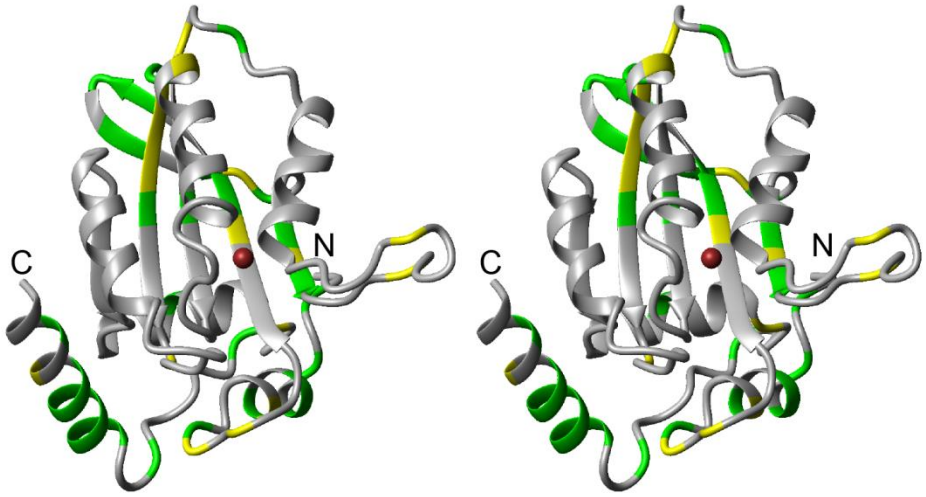
Supplementary Information

Supplementary Figures S1-S3, Supplementary Table S1 and Supplementary References

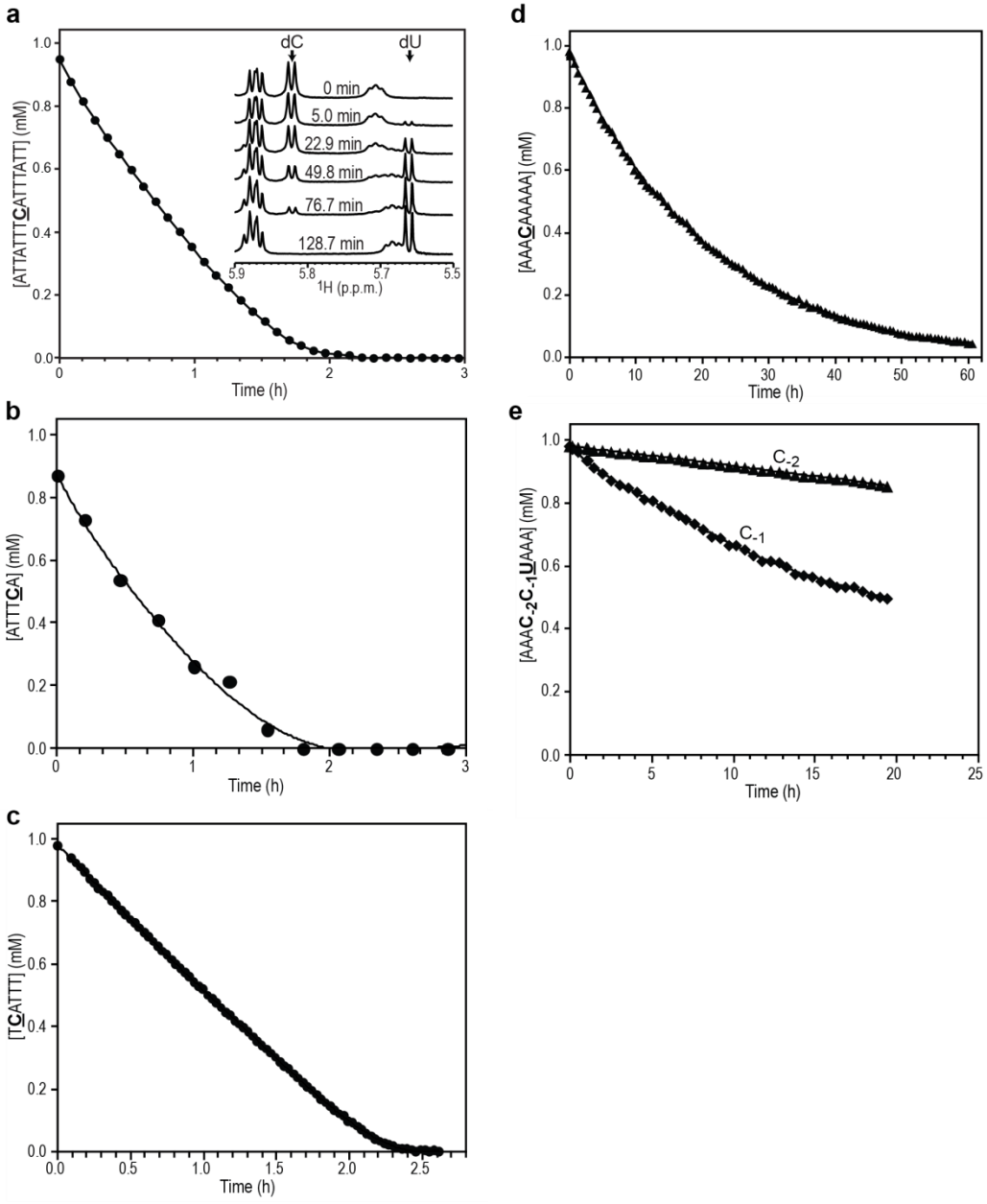
Supplementary Figures, Tables and References



Supplementary Figure S1. Observed inter-strand NOEs to identify the β2-bulge-β2' structure. **(a)** The NOEs that were assigned from 2D NOESY of an unlabeled sample and/or 3D $^{13}C,^{15}N$-edited NOESY data of a variety of isotope-labeled A3A samples (see RESULTS) are marked using double-pointed arrows. The NOEs involving Hα resonances whose chemical shifts are very similar to the H_2O signal are shown with dashed arrows. **(b)** Strip plots of selected regions of 3D ^{15}N-edited NOESY at 900 MHz, 25 °C, were obtained using a 0.17 mM $^{2}H/^{13}C/^{15}N$-labeled protein sample with selective protonations at the side chains of Tyr, Phe and Ile residues (25 mM sodium phosphate, pH 6.5, 200 mM NaCl). The inter-strand HN-HN NOEs are marked by arrows and labeled with residue names. Additional NOEs involving aromatic protons are also labeled. The residue names as well as amide ^{15}N and ^{1}H frequencies (in p.p.m.) are shown at the top and bottom of each strip, respectively.



Supplementary Figure S2. Non-specific binding sites on A3A upon addition of the 15-nt ssDNA, ATTATTTUATTTATT. Stereo-view of the A3A ribbon structure is shown to locate the non-specific binding sites. The ^{1}H,^{15}N-combined chemical shift changes are extracted from the ^{1}H-^{15}N HSQC spectra and calculated using $\sqrt{\Delta\delta_{HN}{}^{2} + (\Delta\delta_{N}/6)^{2}}$, where $\Delta\delta_{HN}$ and $\Delta\delta_{N}$ are the ^{1}HN and ^{15}N chemical shift differences, respectively, between the A3A samples (~0.1mM) containing low (0.440 mM, where A3A is barely affected by non-specific binding) and high (3.051 mM, where the non-specific binding sites of A3A are significantly affected) DNA concentrations. The resonances that undergo large chemical shift change are shown in green (>0.05p.p.m.) and yellow (0.028-0.050 p.p.m.).



Supplementary Figure S3. A plot of the concentration of the unreacted cytidine in substrates 15-nt ATTATTTCATTTATT **(a)**, 6-nt ATTTCA (**b**), 6-nt TCATTT (**c**), 9-nt AAACAAAAA **(d),** or 9-nt AAAC$_{-2}$C$_{-1}$UAAA **(e)** vs. incubation time. The concentrations of the remaining substrate and end product were measured from the peak intensities of the ^{1}H-5 (**a**, **c**-**d** from 1D) or ^{13}C-5-^{1}H (**b** from 2D) resonances (900 MHz, 25° C) of the cytosine and uracil bases in oligodeoxynucleotides, as a function of time after adding A3A (final concentration, 0.197 μM) to the ssDNA solutions. Six representative 1D NMR spectra acquired at the indicated times are shown in the **inset** in (**a**).

Supplementary Table S1. Structural comparison of APOBEC proteins

Protein	PDB ID	Method (resolution)[a]	Backbone r.m.s. differences (Å)[b]	reference
hA3A full-length (1-199)	XXXX	NMR	0	This study
hA3G-CTD (191-384) 2K3A mutant (L234K/C243A/F310K/C321A/C356A)	3IR2	X-ray (2.25Å)	1.86	35
hA3G-CTD (191-380) 2K2A mutant (L234K/C243A/F310K/C356A)	3V4K	X-ray (1.38Å)	1.87	59
hA3C full-length (1-190)	3VOW	X-ray (2.15Å)	2.30	36
hA3G-CTD (197-380)	3IQS	X-ray (2.30Å)	2.36	34
hA3G-CTD (197-380)	3E1U	X-ray (2.30Å)	2.41	34
hA3G-CTD (191-384) 2K3A mutant (L234K/C243A/F310K/C321A/C356A)	2KEM	NMR	3.36	33
mA2 (46-224) (monomer)	2RPZ	NMR	3.38	unpublished
hA2 (41-224) (monomer)	not deposited	CS-Rosetta model	3.35	37
hA2 (41-224) (tetramer)	2NYT	X-ray (2.50Å)	4.31	60
hA3G-CTD (193-384)	2KBO	NMR	4.37	32
hA3G-CTD (198-384) 2K3A mutant (L234K/C243A/F310K/C321A/C356A)	2JYW	NMR	4.99	31

[a] final refinement of the X-ray structures.
[b] Pairwise backbone atomic (N, Cα and C') r.m.s. differences (excluding loop 3) from the A3A lowest energy structure.

Supplementary References

59. Li, M. et al. First-in-class small molecule inhibitors of the single-strand DNA cytosine deaminase APOBEC3G. *ACS Chem Biol* **7**, 506-17 (2012).
60. Prochnow, C., Bransteitter, R., Klein, M.G., Goodman, M.F. & Chen, X.S. The APOBEC-2 crystal structure and functional implications for the deaminase AID. *Nature* **445**, 447-51 (2007).