Supporting Information

Cuneo et al. 10.1073/pnas.0914077107

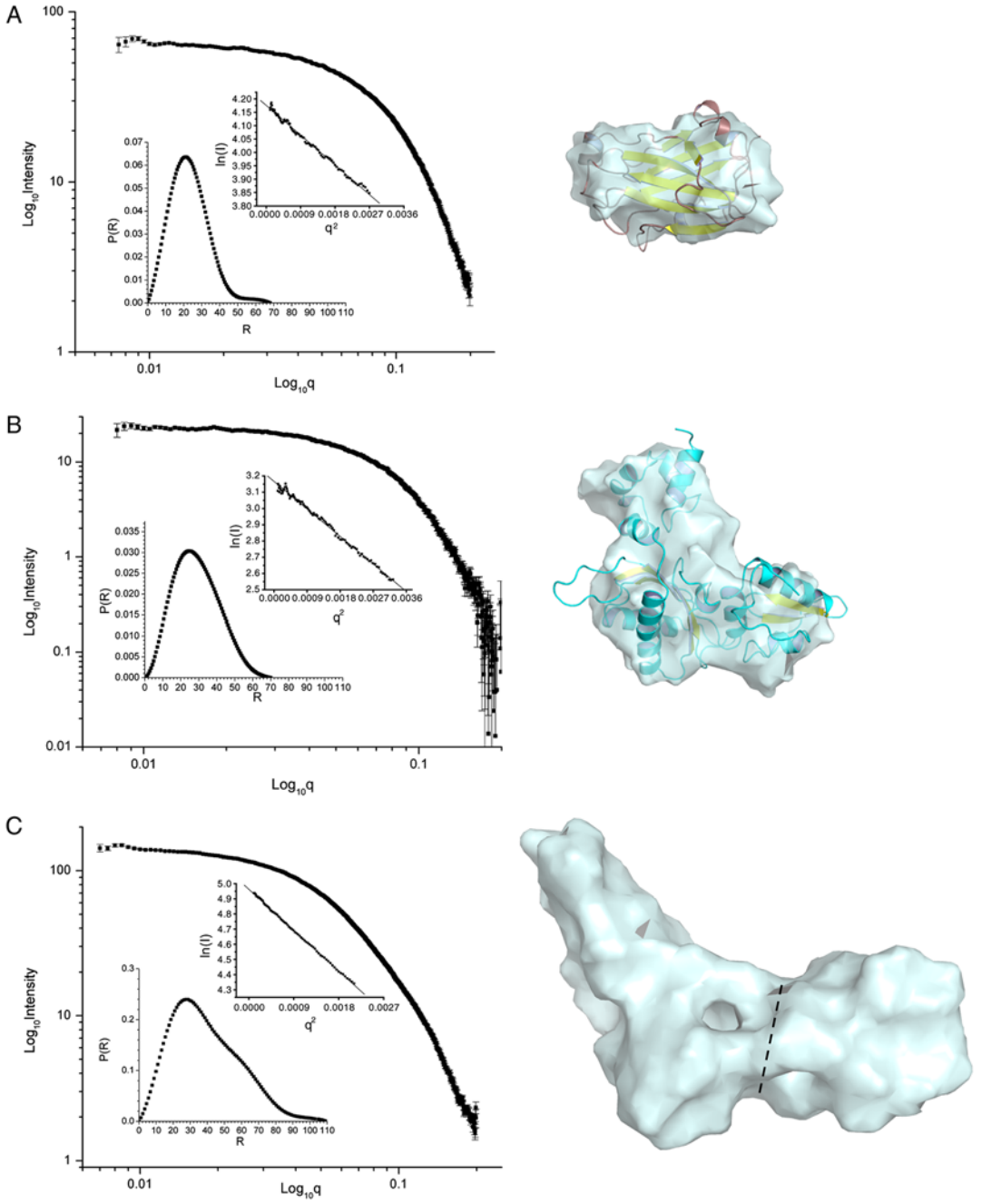

Fig. S1. Small-angle x-ray scattering (SAXS) of XRCC1-N-terminal domain (NTD) and Pol β CD. (*A*) XRCC1-NTD alone, reduced form (13.7 mg/mL), (*B*) Pol β CD alone (3.0 mg/mL), and (*C*) XRCC1-NTD in the reduced form complexed with Pol β CD (12.3 mg/mL). (*Left*) SAXS intensity $I(q)$ data inset with pairwise vector length distribution curve and Guinier fit of the $I(q)$ scattering data; (*Right*) ab initio models derived from SAXS intensity data (surface representation) superimposed with crystal structures. The model in *C* has been divided into two regions of shape and size that are similar to the respective SAXS and crystal structure models of the two component proteins (black dashed line).

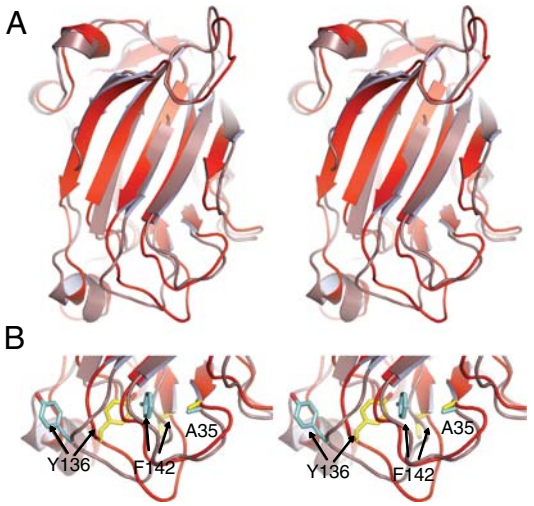

Fig. S2. Comparison of the x-ray crystal and NMR structures of XRCC1-NTD. The crystal structure is shown as a violet ribbon with gray side chains, and the NMR structure as a red ribbon with yellow side chains. (*A*) Stereoview of the x-ray crystal structure of XRCC1-NTD superimposed with the NMR structure. (*B*) Close-up stereoview of the region of the models showing significant variation in structure and indicating the difference in relative orientation of Ala35 and Phe142. The position of Y135 on the surface of the molecule allows it to interact directly with Pol β in the complex, consistent with mutagenesis data of Marintchev et al. (1).

1 Marintchev A, Gryk MR, Mullen GP (2003) Site-directed mutagenesis analysis of the structural interaction of the single-strand-break repair protein, x-ray cross-complementing group 1, with DNA polymerase beta. *Nucleic Acids Res* 31:580–588.

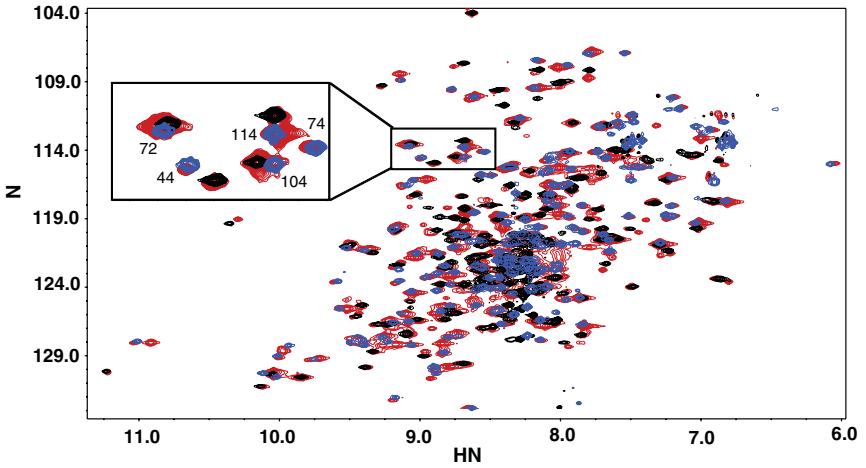

Fig. S3. ^{1}H-^{15}N heteronuclear single quantum coherence NMR spectra of XRCC1-NTD. Wild-type XRCC1-NTD (blue); XRCC1 I4D (black); wild-type XRCC1-NTD treated with 5 mM H_2O_2 (red). Inset shows an expanded region of the spectra with residue assignments of the wild-type XRCC1-NTD, demonstrating that the H_2O_2-treated spectrum is composed of a mixture of reduced protein, protein that coresonates with the I4D mutant, as well as some additional resonances. In many cases, resonances arising from residues positioned far from the disulfide-induced structural changes are not significantly shifted.

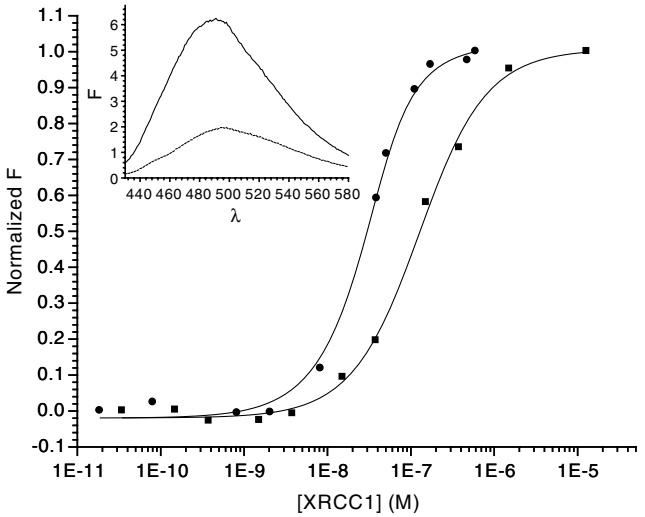

Fig. S4. Determination of Pol β CD/XRCC1 dissociation constant. Titration of aminosulfonylbenzofurazan (ABD)-labeled Pol β CD P300C mutant with XRCC1-NTD (■) and XRCC1 I4D (●). Solid lines are a fit to a two-state binding isotherm. An intensity increase of ~300% is observed (*Inset*) when saturating amounts of XRCC1-NTD are added.

Table S1. SAXS data analysis

Sample	I_0/C		MW, kDa		CRSYOL χ	$R_{g(exp)}$, Å	$R_{g(calc)}$, Å	D_{max}, Å
	Observed	Expected	Calculated	Expected				
Lysozyme	4.3 ± 0.4			14.3	1.3	15.8 ± 0.07 *	15.4	43
XRCC1tr-NTD (residues 1–151)	5.0 ± 0.1	5.4	16.6	17.9	2.0	17.4 ± 0.04 *	16.7	68
Pol β catalytic domain (residues 91–335)	8.6 ± 0.2	8.7	28.8	29.0	2.5	22.8 ± 0.2	23.2	70
Complex	12.7 ± 0.3	14.1	42.6	46.9	2.1	30.7 ± 0.4	28.8	110

*R_g was extrapolated to zero concentration.

Table S2. Data collection and refinement statistics

	Oxidized complex	Reduced complex	XRCC1-NTD
Resolution range, Å	30.0–2.35	25.0–2.95	30.0–2.6
Unique reflections	17,765	19,123	32,108
Redundancy	10.3 (7.1)	3.6 (3.6)	2.8 (2.7)
Mean I/σ (I) *	19.0 (4.20)	17.7 (4.3)	8.8 (3.8)
R_{merged}, % *	11.7 (50.3)	7.4 (33.9)	7.4 (33.9)
Completeness, % *	99.8 (99.0)	98.7 (99.6)	98.0 (95.6)
Unit Cell			
Dimensions, Å	$a/b = 75.2$	$a = 140.8$	$a = 64.5$
	$c = 126.2$	$b = 44.1$	$b = 89.3$
		$c = 152.8$	$c = 93.2$
Angles, °		$\beta = 107.2$	$\beta = 90.3$
Space group	$P3_121$	C2	$P2_1$
Non-hydrogen atoms in refinement			
Protein	2,739	6,349	9,370
Water	234	0	122
R_{cryst}/R_{free}, % †	18.9/25.0	24.2/29.5	20.0/23.7
rmsd from ideal			
Bond lengths, Å	0.006	0.005	0.007
Bond angles, °	0.92	0.842	0.972
B-factors, Å^2			
XRCC1	Chain $A = 27.4$	Chain $B = 48.3$	Average of all eight chains = 39.3
		Chain $C = 64.5$	
Pol β	Chain $B = 23.3$	Chain $D = 46.3$	
		Chain $E = 51.9$	
Water	25.4		19.5

*Numbers in parentheses represent values in the highest resolution shell.
†R_{free} is the R factor based on 5% of data excluded from refinement.

Table S3. Interprotein hydrogen bonding and salt bridge interactions

Direct hydrogen bonds—reduced XRCC1			Direct hydrogen bonds—oxidized XRCC1		
Pol β CD	Dist., Å*	XRCC1	Pol β PT	Dist., Å	XRCC1
GLU 309[OE1]	3.4/3.2	ARG 109[NH1]	GLU 309[OE1]	2.7	SER 92[N]
GLU 309[OE2]	3.2/3.8	ARG 109[NH2]	GLU 309[OE1]	2.7	ARG 109[NH1]
ASP 321[OD2]	3.6/4.4	ARG 100[NH2]	ASP 321[OD1]	3.0	ARG 100[NH1]
ASP 321[OD2]	3.7/4.6	SER 97[OG]	ASP 321[OD2]	3.0	ARG 100[NH2]
TYR 322[O]	2.9/2.9	TYR 136[OH]	TYR 322[O]	2.6	TYR 136[OH]
VAL 303[N]	2.7/3.0	GLU 69[OE2]	VAL 303[N]	3.0	GLU 69[OE2]
GLU 309[N]	3.7/3.2	THR 90[O]	GLU 309[N]	3.1	THR 90[O]
GLN 324[NE2]	2.4/3.3	PRO 135[O]	GLN 324[NE2]	3.4	PRO135[O]
GLU 309[OE1]	3.1/2.8	SER 92[N]	GLU 309[OE2]	3.0	ARG 109[NH2]

*Values shown are for molecules CE/BD.