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The BRCT domain from the large subunit of human Replication Factor C

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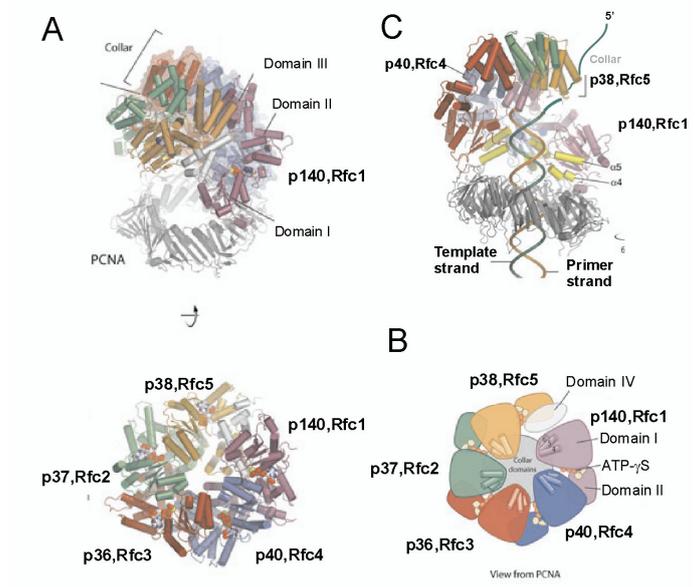
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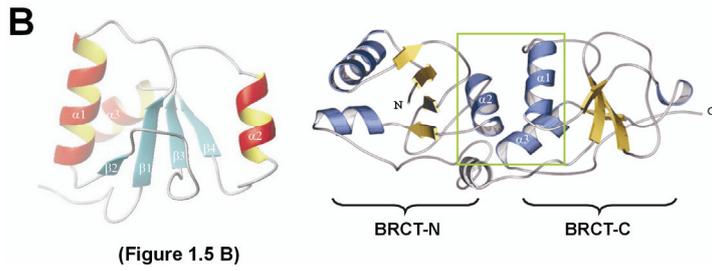
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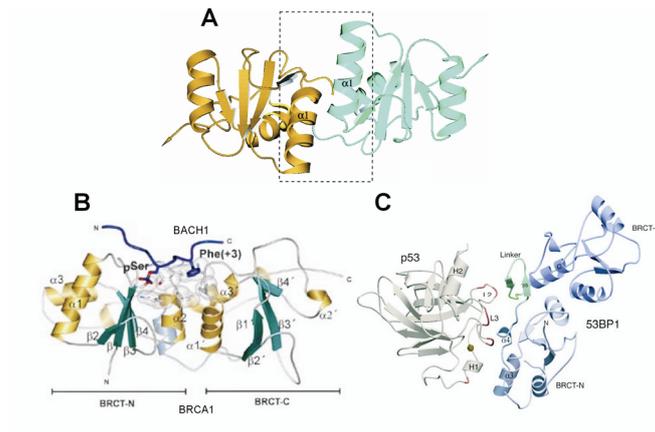
Appendices: Color Figures



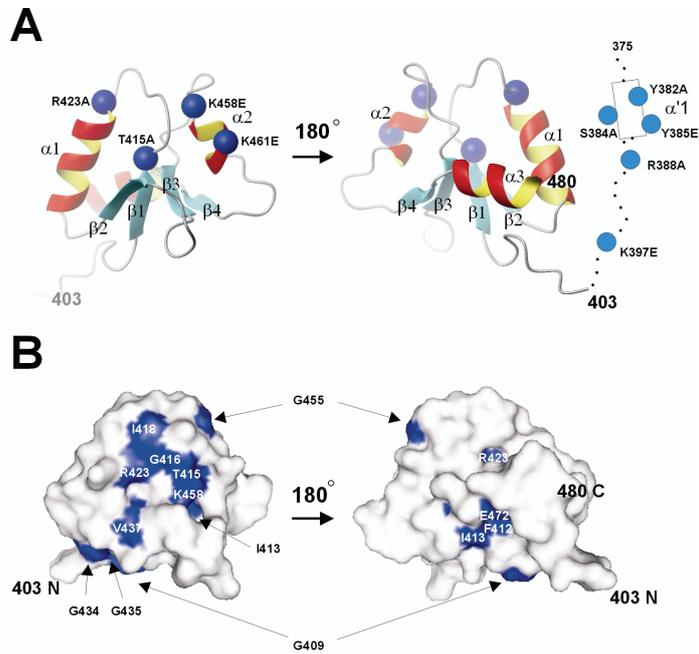
(Figure 1.3)



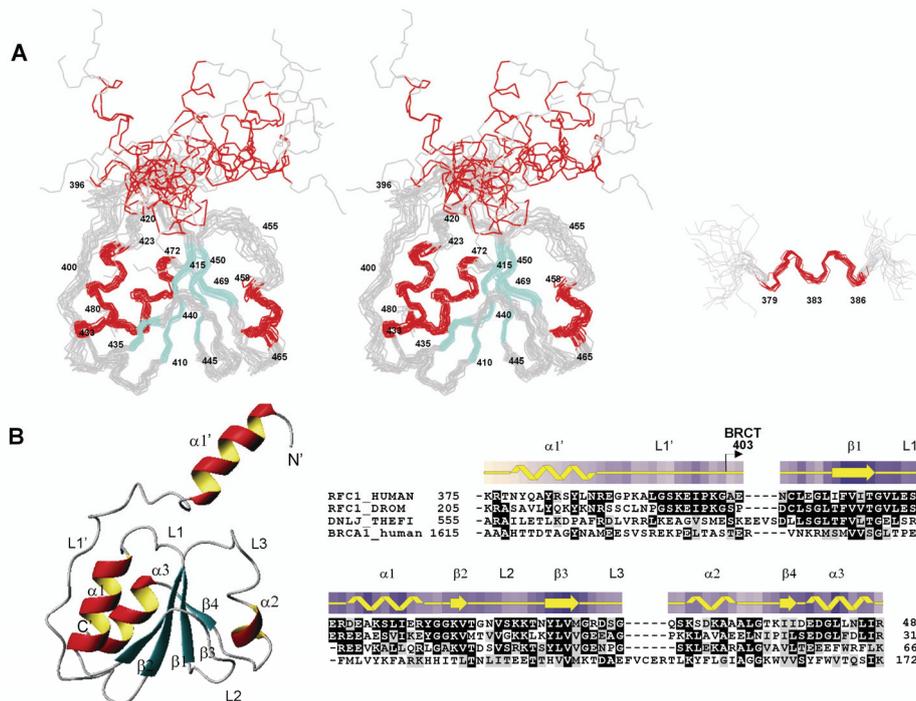
(Figure 1.5 B)



(Figure 1.6)



(Figure 3.2)



(Figure 5.2)

Figure Captions

(Figure 1.3) Yeast trRFC-PCNA complex. N-terminally truncated Rfc1 was used to form the trRFC complex. **(A)** Spiral assembly of AAA+ ATPase modules of RFC subunits (bottom). Each AAA+ ATPase module is formed by Domain I and II **(B)**. The ATP binding site (where ATP- γ S is bound) is located at the subunit interface which is comprised of the Walker A and B motifs of one subunit and the SRC motif of the adjacent subunit. **(C)** Domain III of one subunit packs against domain II from its neighbours to form a “Collar” (Top). **(C)** The proposed model of primer-template recognition by RFC. The 3' end of the primer strand (orange) is physically blocked by the “Collar” of RFC while the 5' template ssDNA (green) can escape through the wedge shaped gap between p38 and p140. Figures were adopted from the original publication by Bowman et al (31).

(Figure 1.5) **(B)** Ribbon representation of the XRCC1 C-terminal BRCT (left) and the tandem BRCT domains of BRCA1 (right). Each BRCT unit consists of a $\beta 1\alpha 1\beta 2\beta 3\alpha 2\beta 4\alpha 3$ topology. The interface between the tandem BRCT domains (-N and -C denoting N- and C-terminus) is highlighted with a box (right).

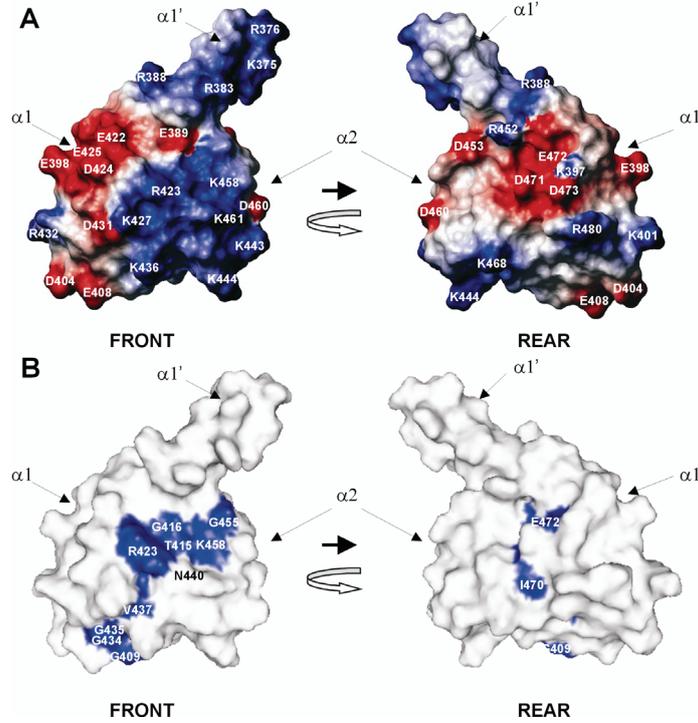
(Figure 3.2) Homology model of the BRCT domain from RFC p140 (403-480).

(A). Ribbon presentation of the model BRCT domain. The model was based on the NMR structure of the BRCT domain (PDB code:1IB7) from the NAD⁺ dependent DNA ligase which has 33 % amino acids identity with RFC p140 and was generated by 3D-JIGSAW (19). The residues where structural homology was not available are schematically indicated in the figure. The predicted helix between residues 379 and 386 is presented as a box and the loop (387-402) is presented as a dotted line. The blue balls represent the C α locations of the point mutations created in this study. **(B)**. Surface presentation of the model BRCT domain. The blue-shaded areas represent the accessible surface occupied by the conserved amino acids defined from the amino acid sequence alignment (Figure 3.1) using AMAS (17). The total accessible surface area (ASA) were calculated using MOLMOL (21).

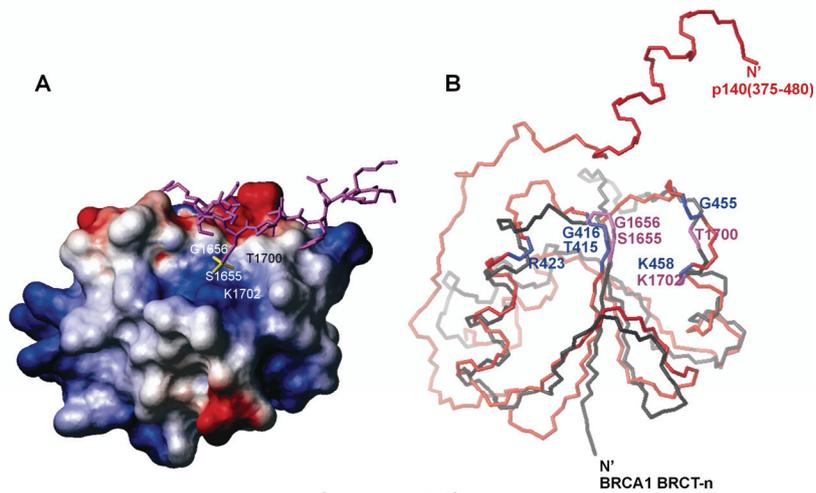
(Figure 5.2) Structure of RFC p140(375-480) when bound to dsDNA. **(A)** Left; Stereoview of a superposition of the backbone (N, C α and C') atoms for the 20 lowest-energy structures of RFC p140(375-480). α -helices and β -strands are colored in red and cyan respectively. Right; superposition of the backbone of residues 379-386 demonstrating the well defined helix $\alpha 1'$ the N-terminus. **(B)** On the left, sequence alignment of the p140(375-480) with the homologous region of RFC p140 from *Drosophila melanogaster* (RFC1_DROM), NAD⁺ dependent ligase of *Thermus filiformis* (DNLJ_THEF1) and human BRCA1 BRCT-n. The secondary structure of human RFC p140(375-480) was determined by PROCHECK-NMR(26) and the level of solvent accessibility of the residues is colored from light (accessible) to dark blue (not accessible). The loop regions are labeled as L1', L1, L2 and L3. On the left a ribbon diagram of the p140(375-480) structure with the lowest-energy is presented. The orientation is equivalent to (A). Figures (A) and (B) (right) were generated using MOLMOL (28)

Appendices: Color Figures

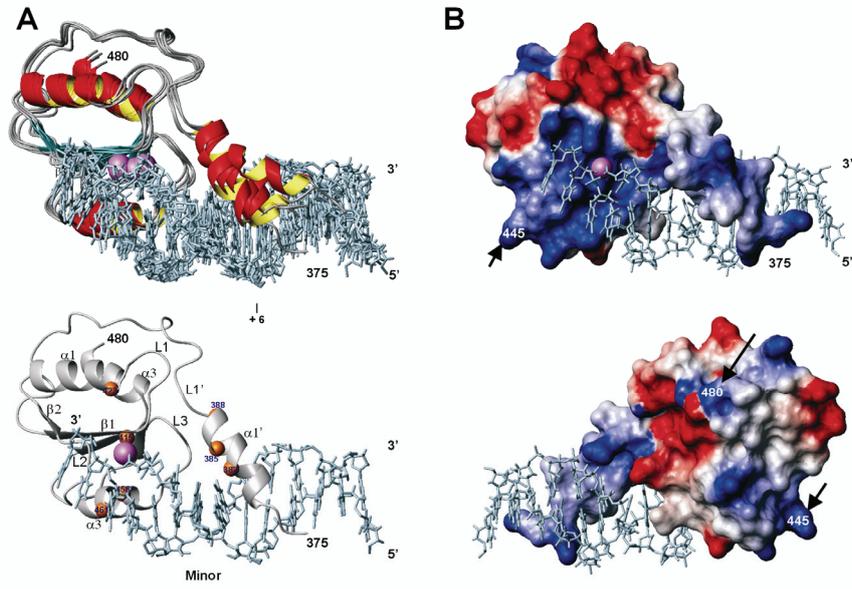
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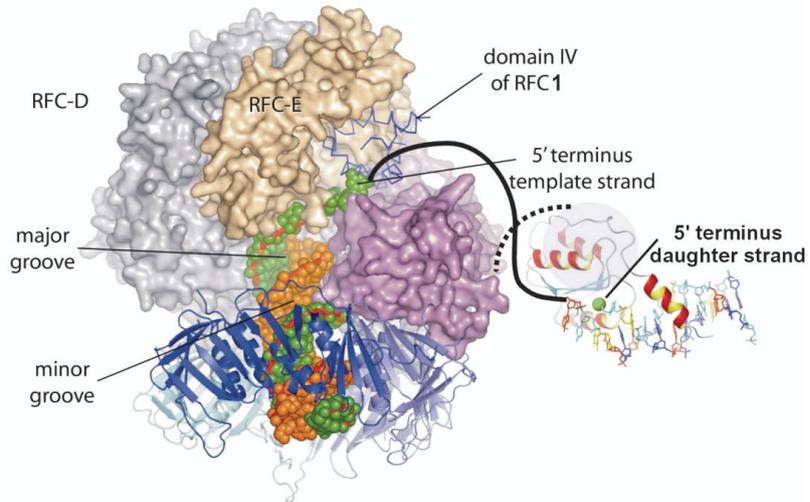
(Figure 5.3)



(Figure 5.4)



(Figure 5.5)



(Figure 6.2)

Figure Captions

(Figure 5.3) Surface potential of p140(375-480). **(A)** Electrostatic potential of the accessible surface of p140(375-480) is shown. Negative potential is colored in red and positive potential in blue. The residues contributing to the surface charge are indicated on the surface. **(B)** Surface accessible residues that are conserved (defined in Figure 3.1) are colored with blue onto the structure of p140 (375-480). N440 is not a conserved residue. An intermolecular NOE transfer between N440 and unassigned DNA proton was observed (Table 5.3). The “front” and “rear” images were generated by 180° rotation around the Z-axis. The electrostatic potential surface was calculated using MolMol (28) and the residue conservation map (B) was created with Pymol (33).

(Figure 5.4) Structure comparison between the BRCT domains

(A) Electrostatic surface presentation of the N-terminal BRCT (BRCT-n) of BRCA1 (PDB:1T29) in complex with a phosphoserine peptide (in magenta). The C-terminal BRCT domain is not directly involved in the phosphate binding and therefore has been deleted from this figure for clarity. Positive potential is shown in blue and negative potential in red. The amino acid residues forming the pocket that binds the phosphate moiety (in yellow) of phosphoserine are indicated on the surface. Phosphate is directly hydrogen bonded by S1655, G1656 and K1702. T1700 is a conserved residue which forms a hydrogen bond to the side chain oxygen of S1655 stabilizing the S1655 side chain conformation. **(B)** Backbone superposition of p140 (375-480) (red) and the BRCT-n from BRCA1 (black). The orientation of the BRCT-n is identical to that of A. The backbone C α , N and C' of the proteins are presented in red for p140(375-480) and in black for the BRCA1 BRCT-n. The conserved residues of p140(375-480) are presented in blue and the residues essential for the phosphate-moiety recognition of BRCA1 BRCT-n are presented in magenta (36-38). The overlay regions of p140 and (BRCA1) are 409-417(1649-1657), 421-452(1660-1691) and 453-479(1697-1723).

(Figure 5.5) Docking model of the p140 (375-480)-dsDNA complex generated by HADDOCK. **(A)** Ribbon representation of ensemble of the five lowest energy structures from the cluster 7. The 5'phosphopate is indicated by a magenta sphere. One of the ensemble structures in which the active residues defined in HADDOCK are presented in yellow spheres (bottom). **(B)** Electrostatic surface potential presentation of p140 (375-480) bound to dsDNA. The upper figure has the same orientation as in A while the lower has been rotated 180° around the Z-axis (bottom). The positive and negative charged surfaces are colored in blue and red respectively. Exposed hydrophobic residues are white or slightly colored **(C)** DNA binding activity of R480A and K445A. The mutations were designed as tests of the model of the Protein-dna complex. The wildtype DNA binding activity of the R480A and K445A mutants suggests that the “rear” side and the bottom of p140(375-480) (indicated with an arrow in **(B)**) do indeed not contact the DNA. **(D)** The sequence of the dsDNA used in the HADDOCK docking. The nucleotides with phosphates and bases in contact with the protein are boxed.

(Figure 6.2). Schematic model of the complete RFC-PCNA-DNA complex. The model is formed from our NMR structure of the RFC p140 BRCT region bound to 5' P dsDNA (right side ribbon view). The purple sphere behind it represents the 60 aa's C-terminal to the BRCT region we believe form a single domain. The broken line indicates the protein connection to the N-terminal portion of RFC1 in the crystal structure (purple surface). The template DNA strand (green) is schematically connected to the BRCT complex via the solid line. We estimate a minimum of 15 bases of ssDNA are required to bridge the 3' and 5' termini.