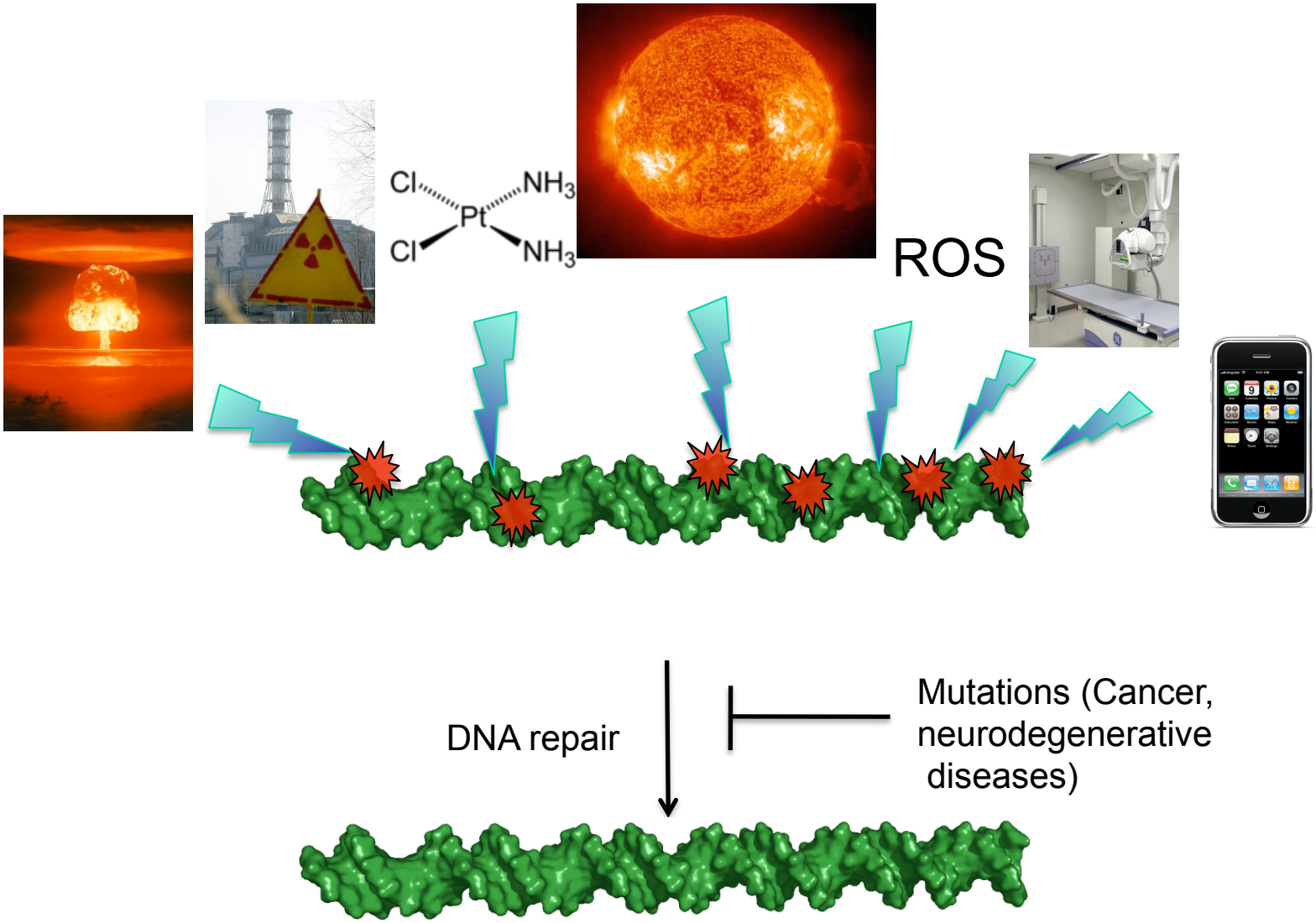
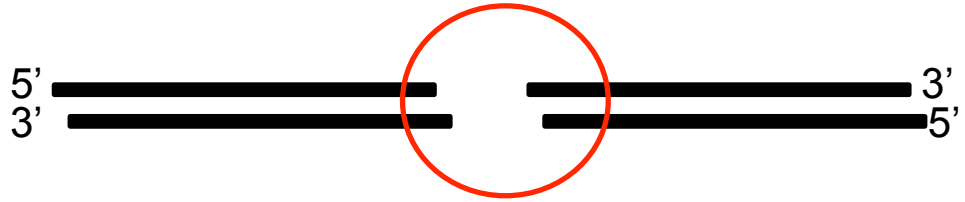
Structure and Function of BRCT Phosphoprotein Binding Domains in the DNA damage response

NIEHS
National Institute of Environmental Health Sciences


Cl
NH3
Pt
Cl
NH3
ROS
DNA repair
Mutations (Cancer, neurodegenerative diseases)

Genome Stability Structural Biology Group (LSB)

1. DNA double strand break repair (DSBR)



2. DNA end processing factors

- Structural analysis (eg. X-ray crystallography, SAXS) of DNA repair
- Mechanisms of repair factor inactivation in human diseases
- Targeted inhibition DNA repair factors

Overview

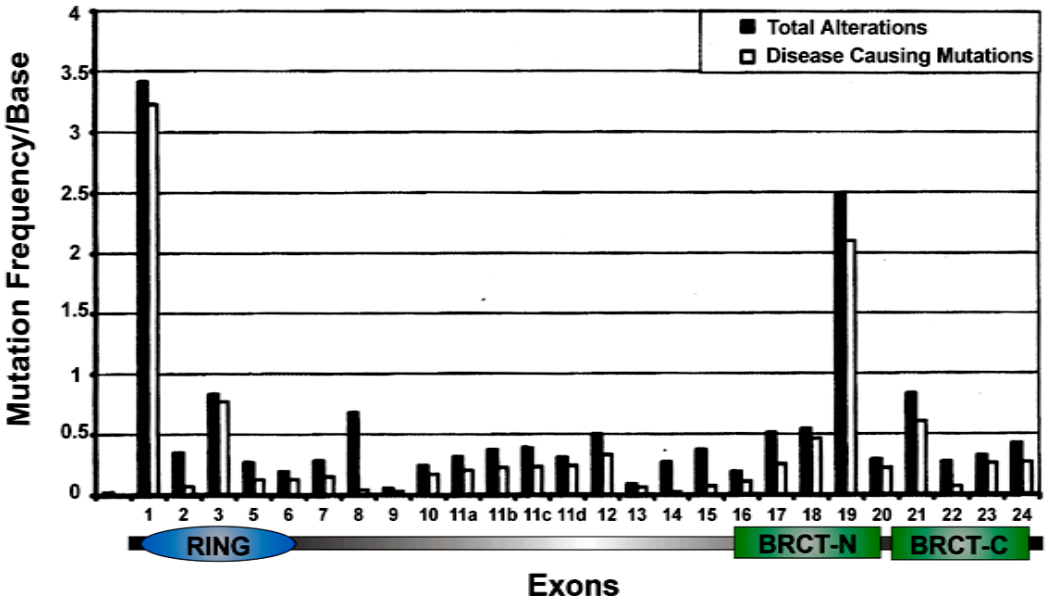
Structure and function of BRCT phosphoprotein binding
Domains in the DNA damage response

1. Background on BRCT domains

2. BRCT domain integration into multi-protein complexes
   - Structure/function of Nbs1, a DNA damage response signaling adaptor

3. Molecular basis for BRCT phosphoprotein binding and specificity
   - Structure/function of a fission yeast phospho-H2A binding BRCT protein, Brc1

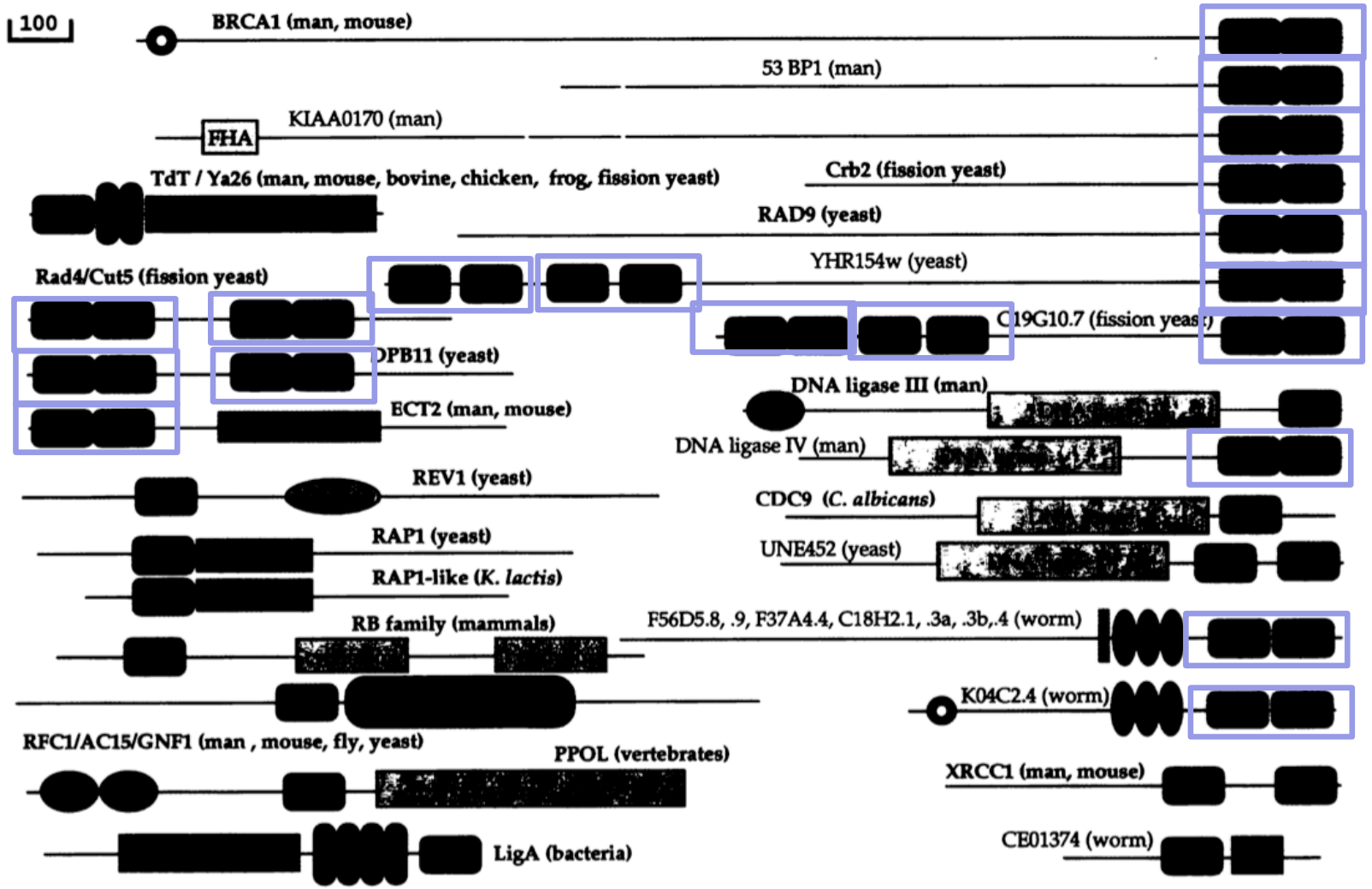
BRCA1 mutations



~80% of mutations lead to truncated protein products which delete all or > 25% of the BRCT repeat region

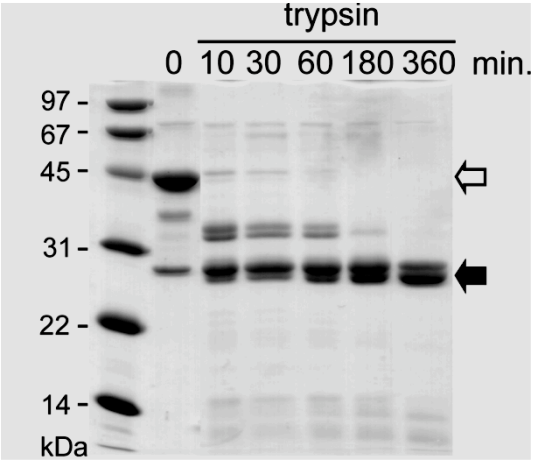
- All truncations are presumed to be cancer predisposing eg. 1853ter

BRCA1 **C**arboxyl **t**erminal (BRCT) Domains in DNA replication and repair proteins



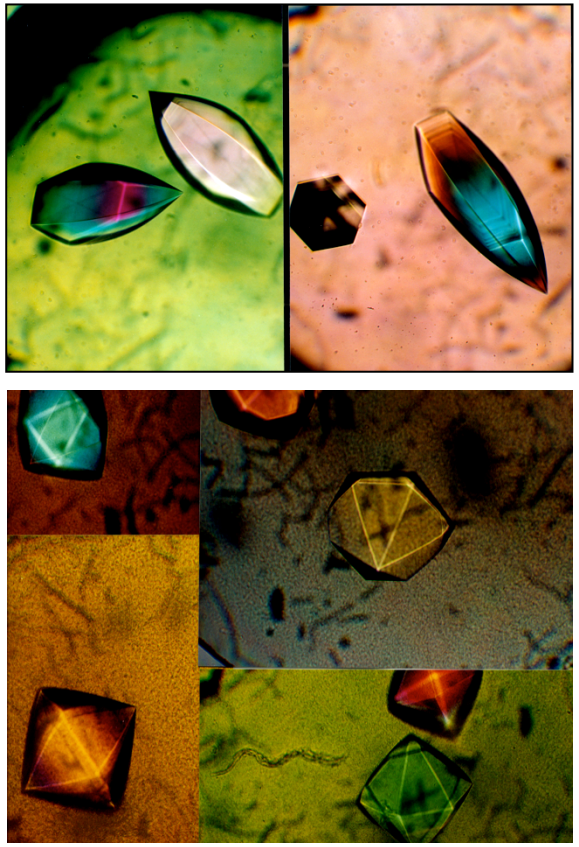
Koonin, Nat. genet. 1996, Faseb J., 1997

BRCT domains of hBRCA1 are an ordered tandem repeat



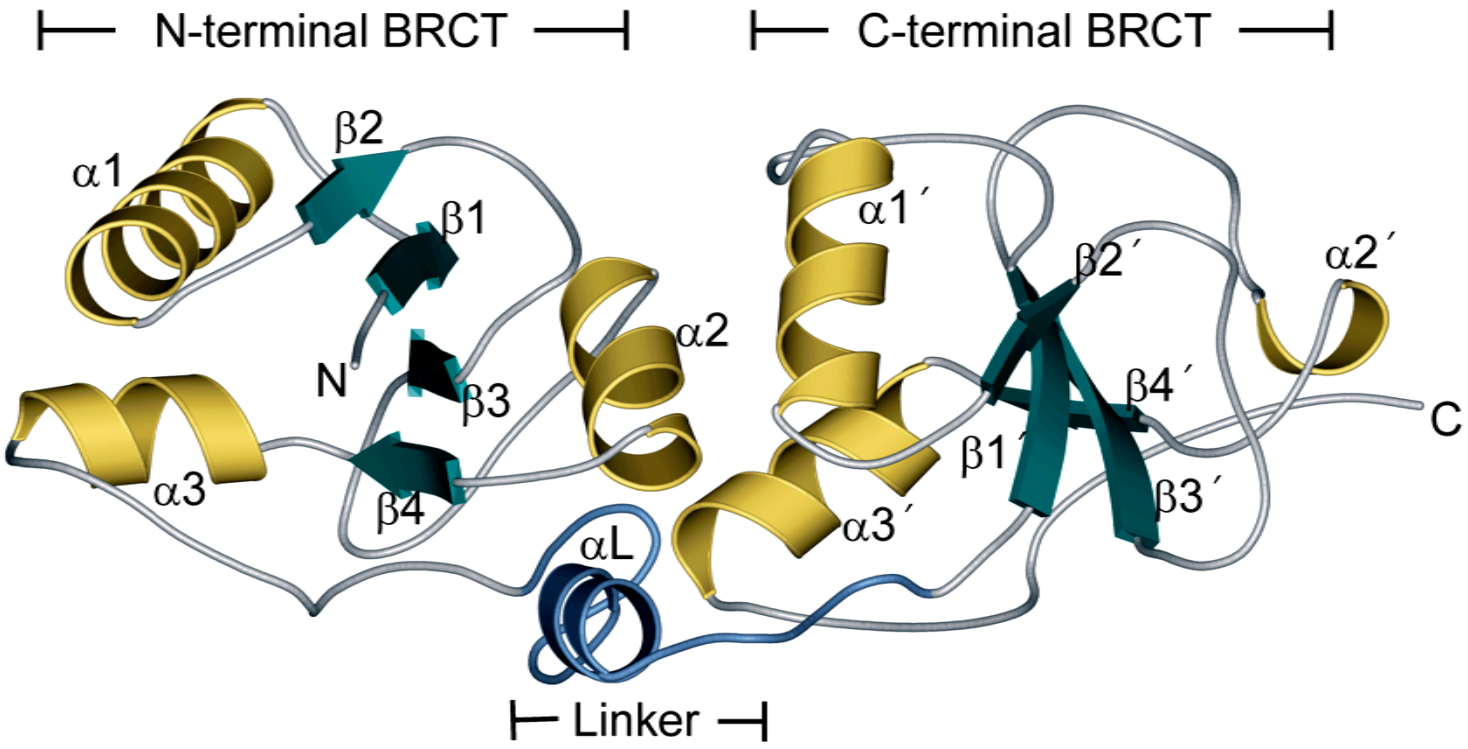
hBRCA1 1528-1863

hBRCA1 1646-1863



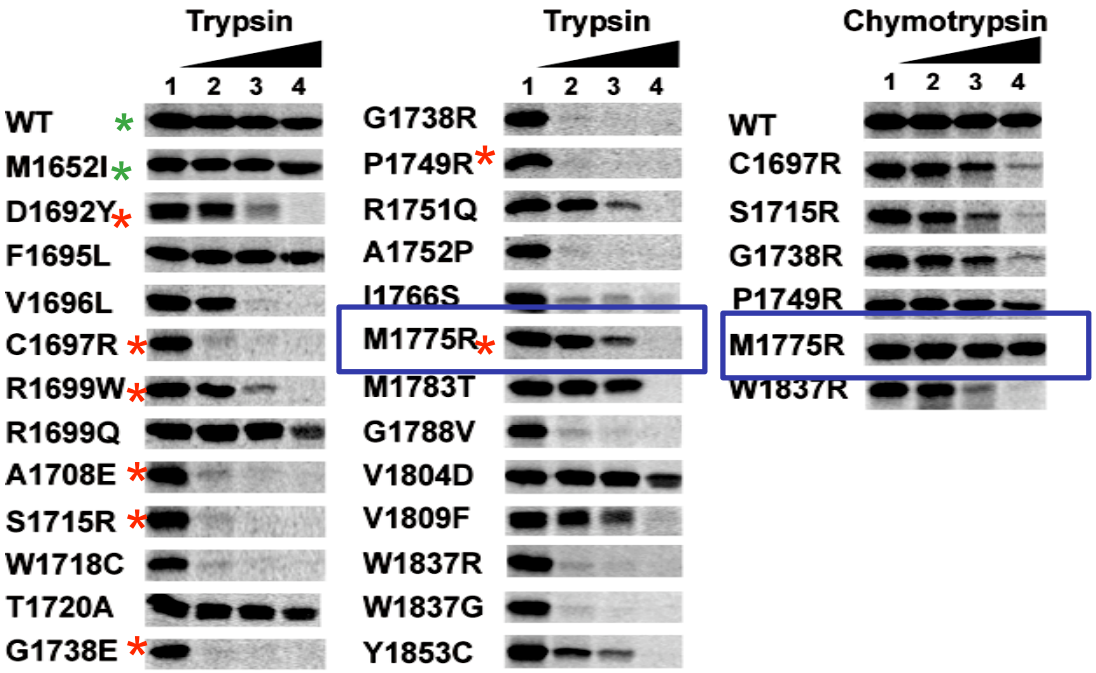
Williams et al. Nat struct. biol 2001

hBRCA1 tandem BRCT repeat Architecture



Williams et al. Nat struct. biol 2001

Most Patient BRCA1-BRCT mutations are destabilizing



* WT and Benign Polymorphisms are stable to proteolysis

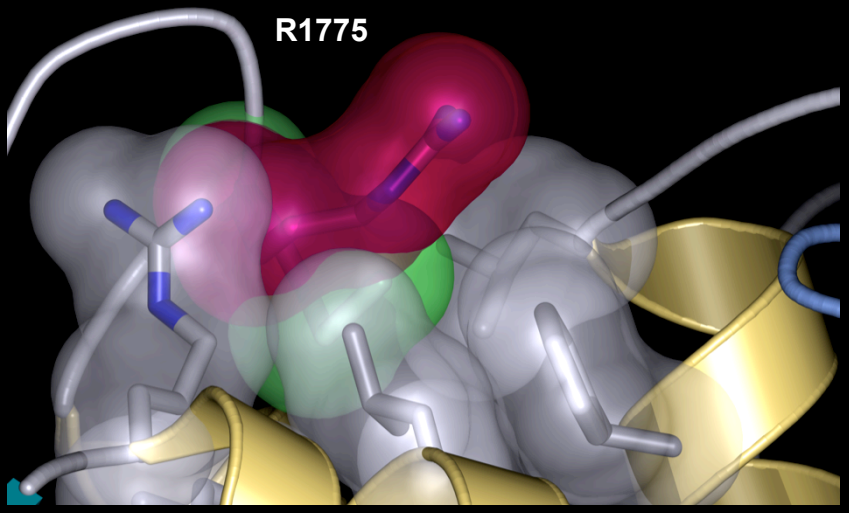
* Disease associated truncation and missense mutations destabilize the BRCT fold

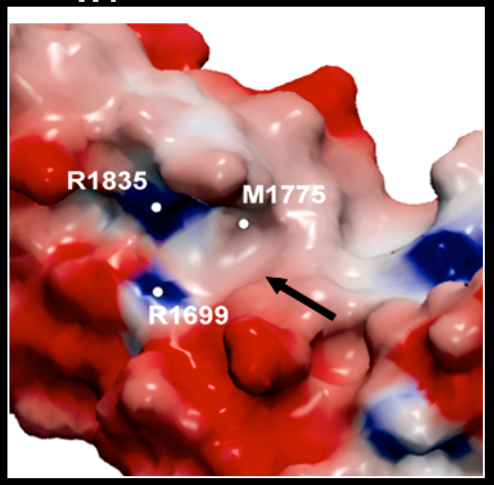
BRCA1 Cancer associated mutant M1775R

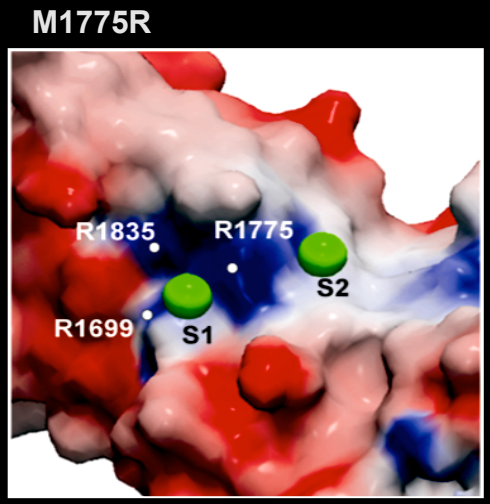
-Mutant BRCA1 allele encoding BRCA1-M1775R segregates with disease in families with breast and ovarian cancer (Futreal et al, 1994)

-Impaired kinetics of double strand DNA break repair

- Impaired protein binding to CtIP, histone deacetylase complexes and BACH1





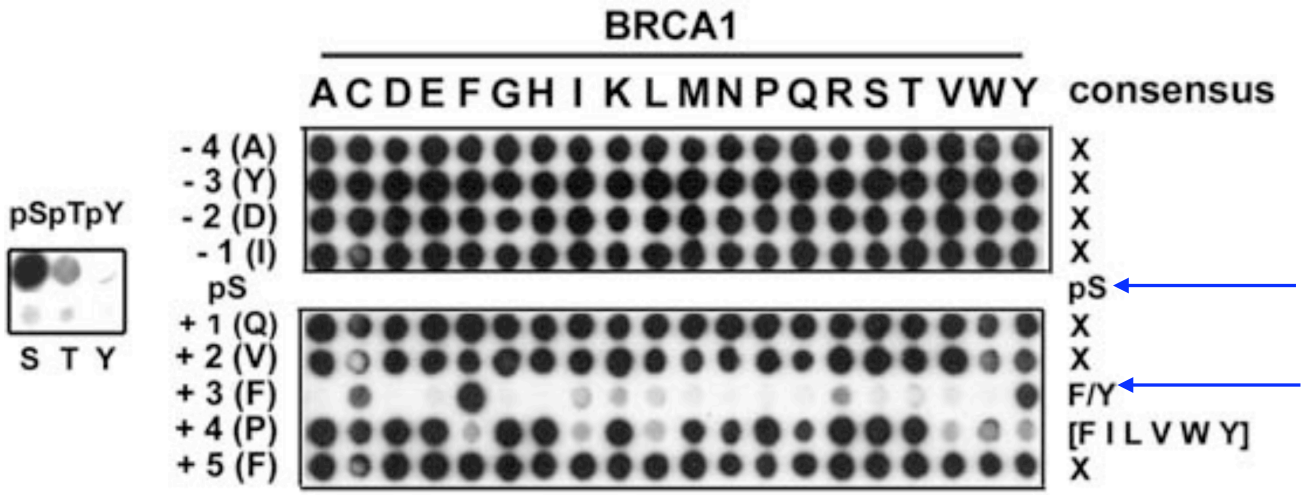


Protein stability or protein-ligand binding defect??

Tandem BRCT domains bind phosphoserine-modified proteins

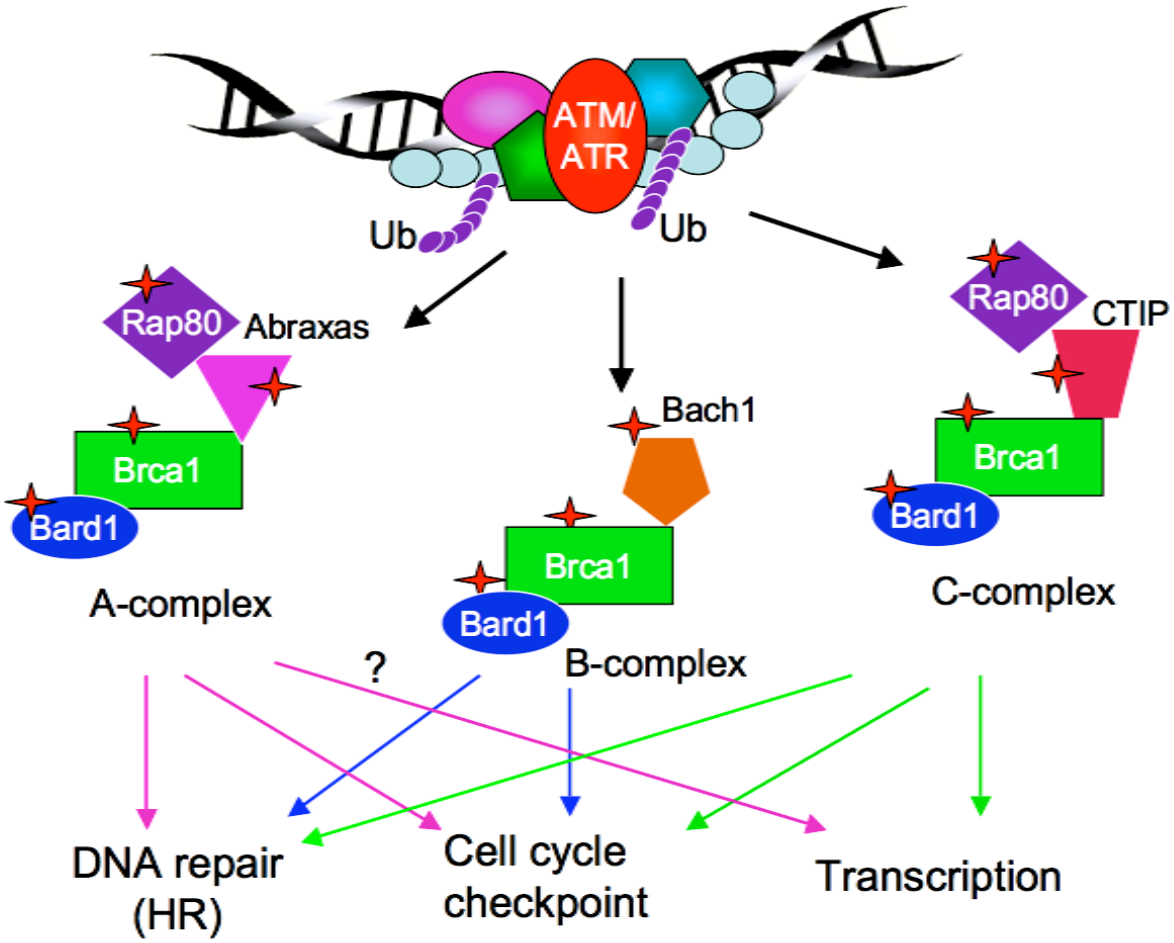
In vitro optimized phosphopeptides:
ATM kinase pSQ targets

- BRCTtide: …AYDI**pS**QVFPF…



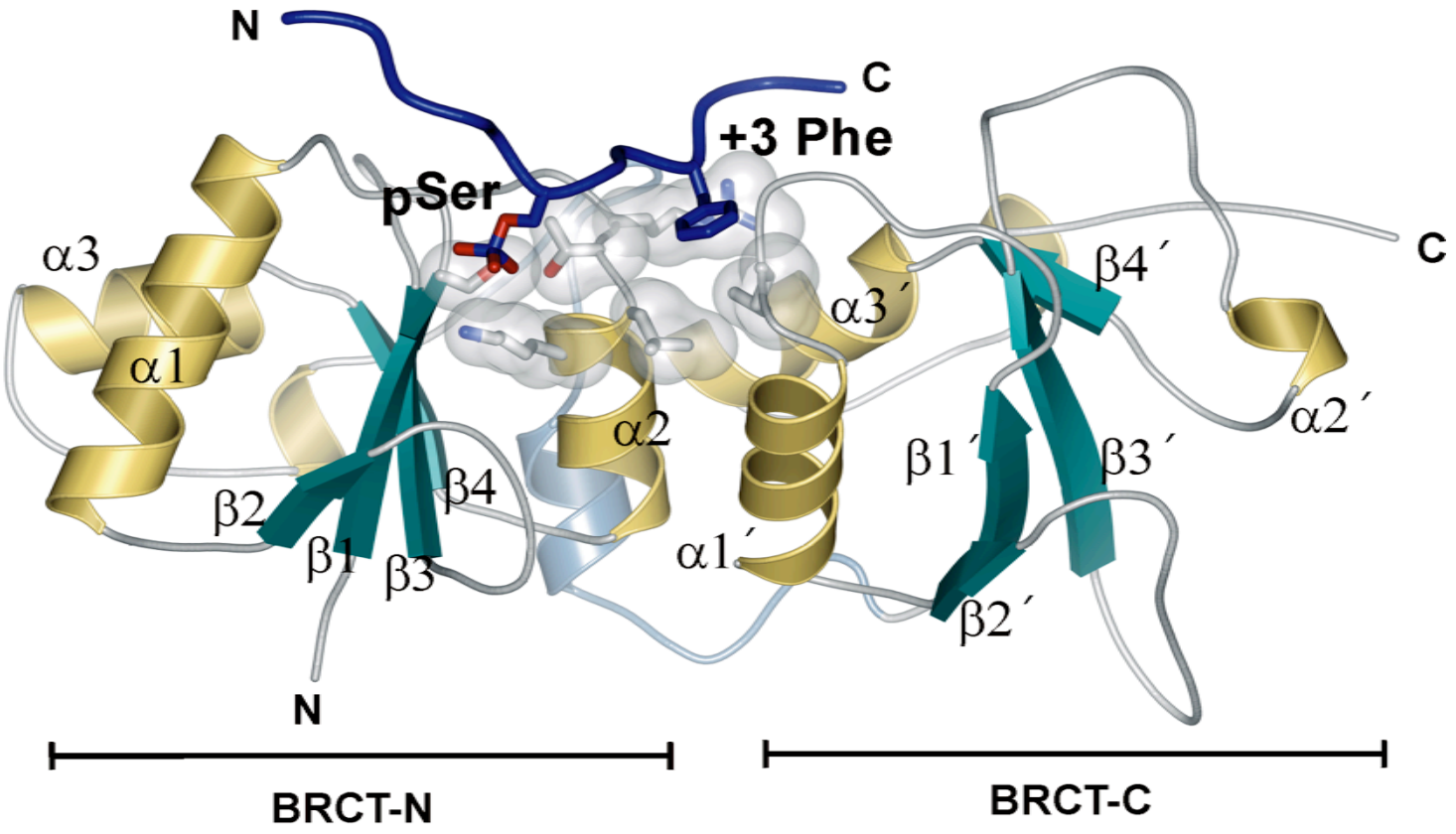
Manke et. al Science, 2003

BRCA1 BRCT interactions mediate BRCA1 integration into multiple protein complexes



Wang et al Science, 2007

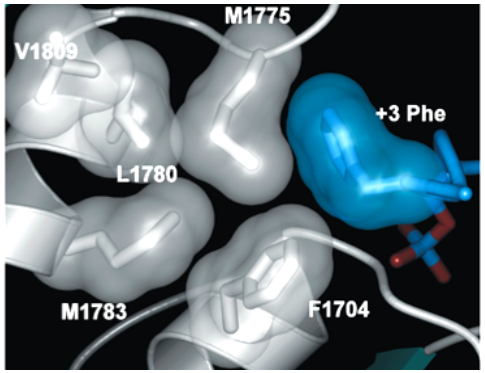
Structure of the hBRCA1-phosphoprotein complex



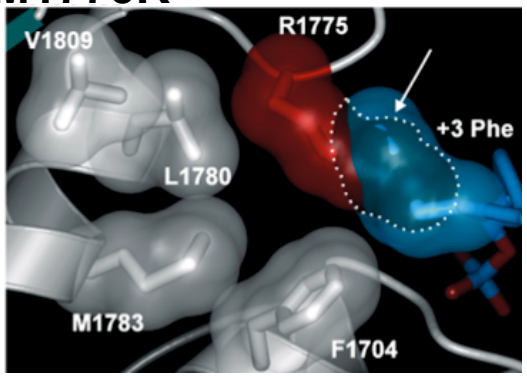
Nat Struct Mol Biol 2004

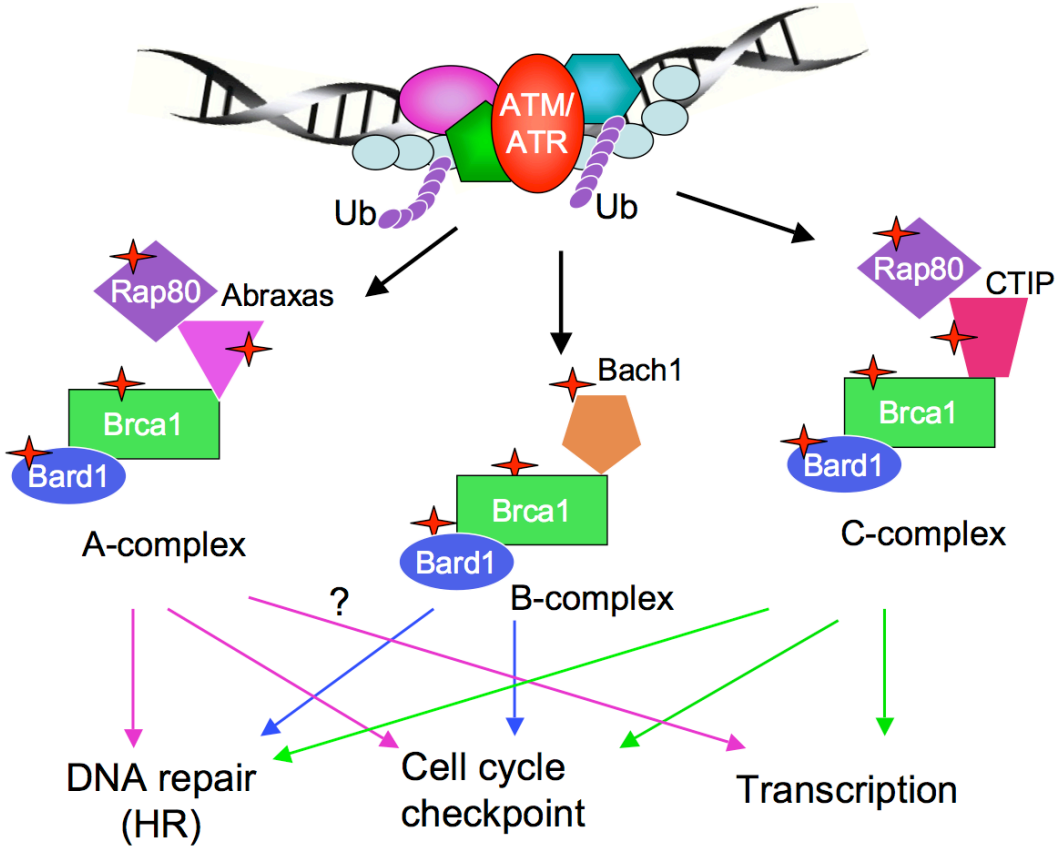
BRCA1 mutations occlude the phosphoprotein binding pocket

WT



M1775R





Wang et al Science, 2007

Williams et al, Nat. Struct. Mol. Biol. 2004

Questions:

Part 1.

1. How to BRCT domains integrate into multidomain/ multiprotein complexes?

2. Are BRCT phosphoprotein interactions regulated?

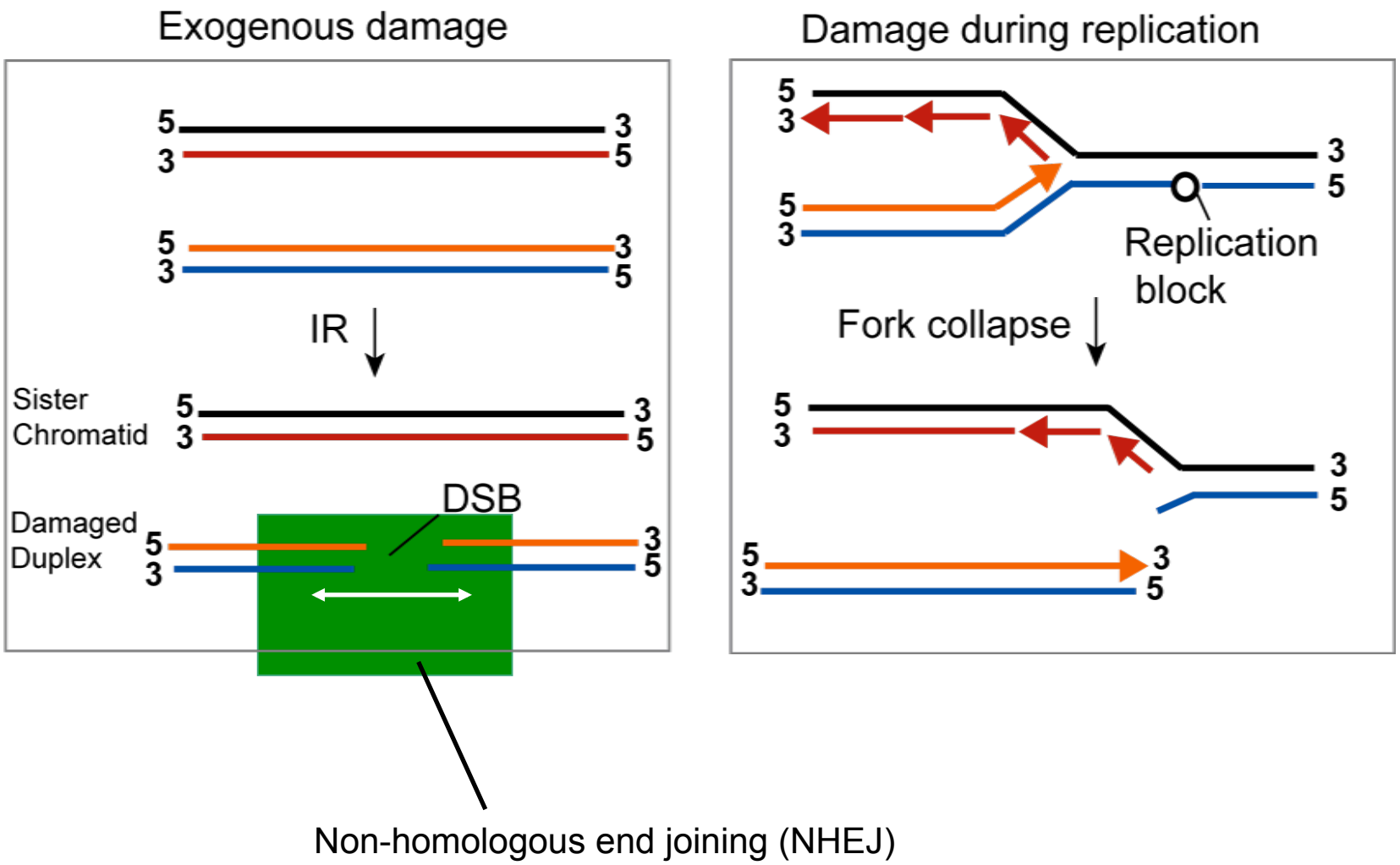
- Structure/function studies of Nijmegen breakage syndrome protein Nbs1

Part 2.

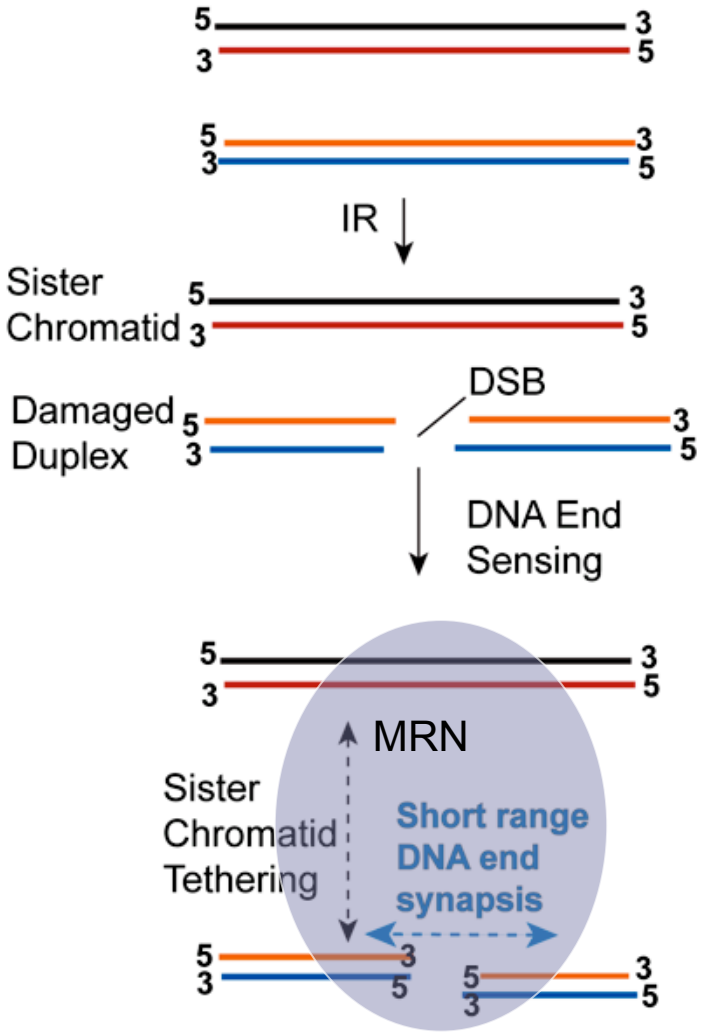
How do BRCT proteins recognize unique targets?

- Identification of a fission yeast S-phase phospho-H2A binding BRCT protein

DNA double strand breaks



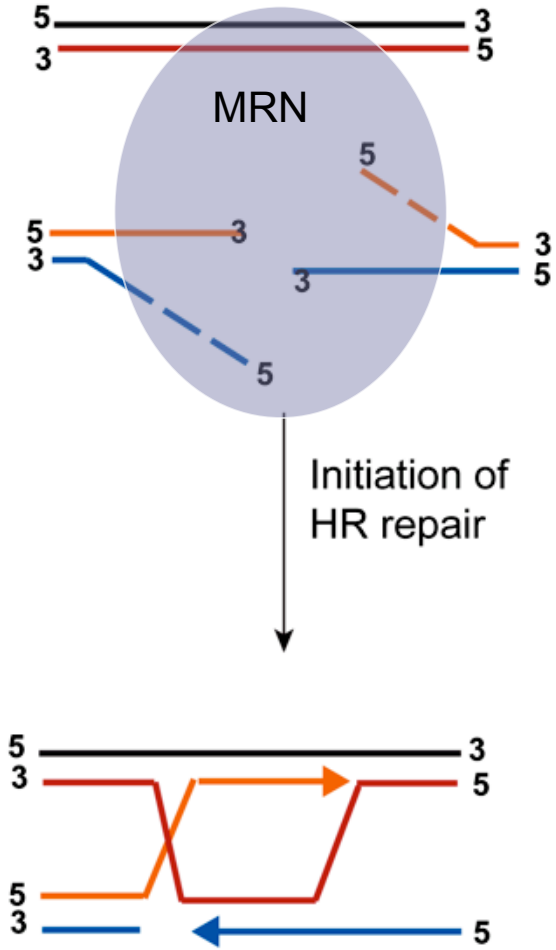
Homologous Recombination repair of DNA double strand breaks



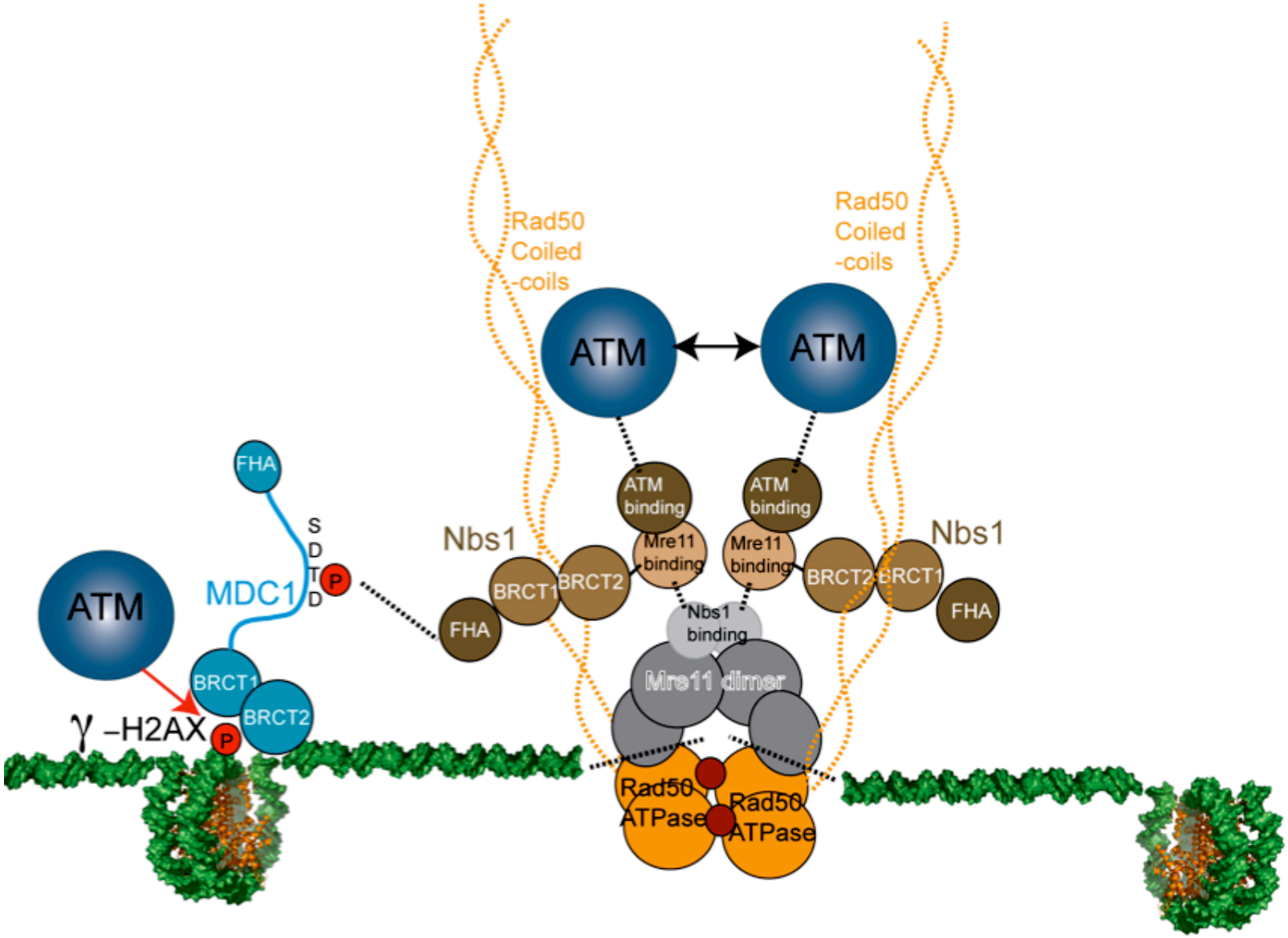
1. DNA Bridging Architecture

5'-3' resection

2. Catalysis



The Mre11/Rad50/Nbs1 complex integrates DNA double strand break sensing, repair and signaling



Composite Model of the Mre11-Rad50-DNA complex

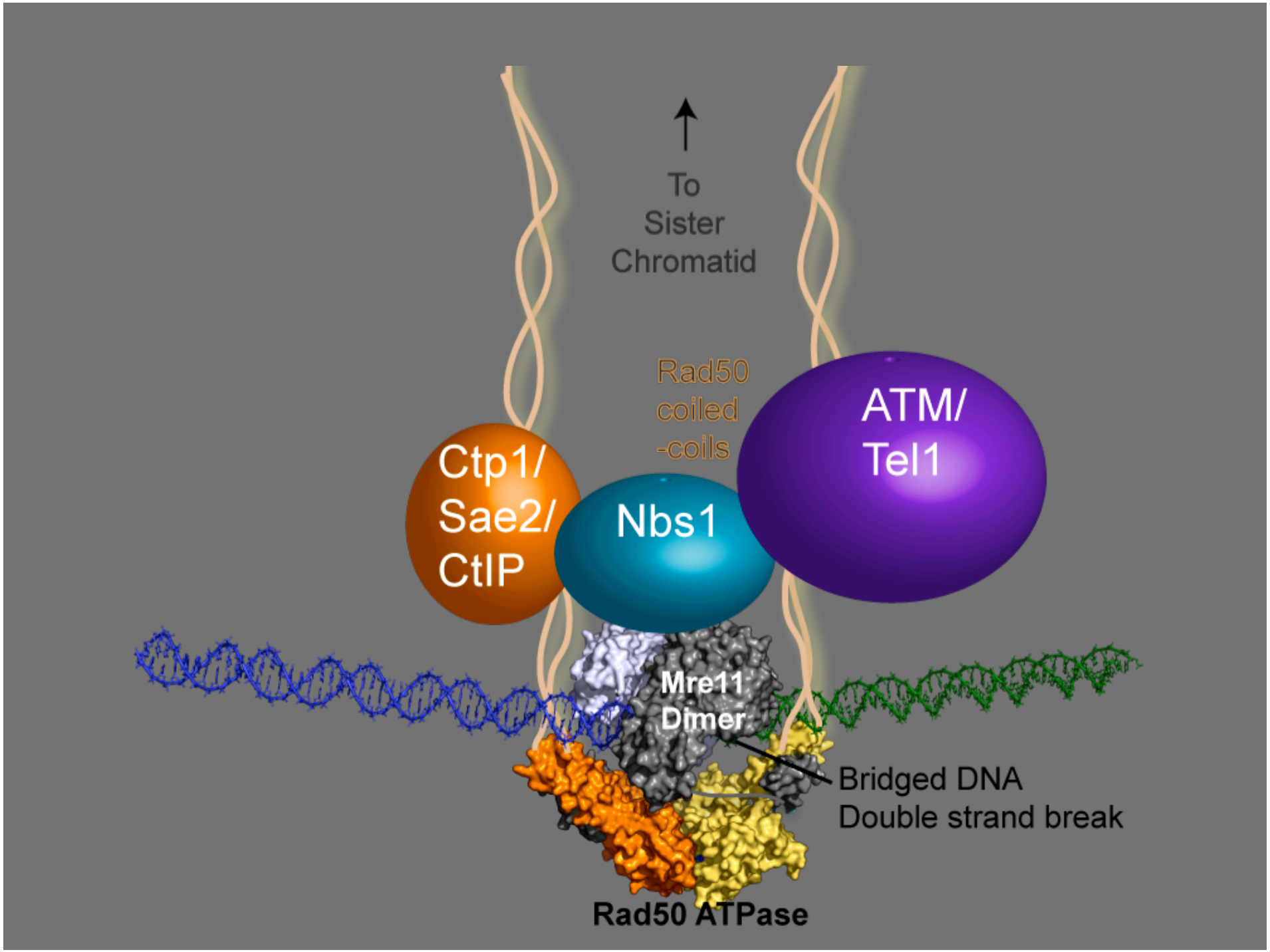
To
Sister
Chromatid

Rad50
coiled
-coils

Mre11
Dimer

Bridged DNA
Double strand break

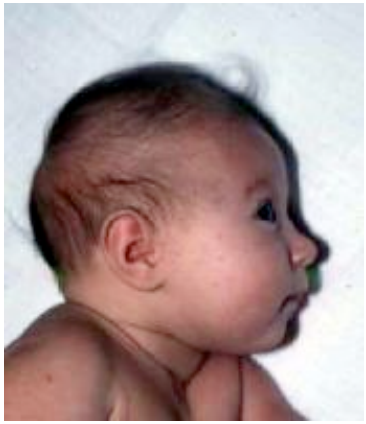
Rad50 ATPase


To
Sister
Chromatid
Rad50
coiled
-coils
ATM/
Tel1
Ctp1/
Sae2/
CtIP
Nbs1
Mre11
Dimer
Bridged DNA
Double strand break
Rad50 ATPase

Nijmegen Breakage Syndrome (NBS)

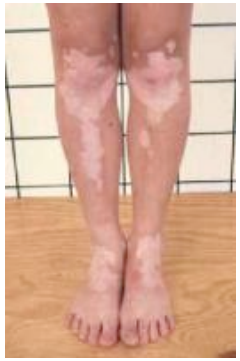
- Radiation sensitivity
- Immunodeficiency
- Strong predisposition to lymphoid malignancy
- Malignancies develop before patients are aged 20 years (mean age, 9 y).
- Cancer appears prior to the diagnosis of NBS in approximately 20-30% of patients.

Microcephaly

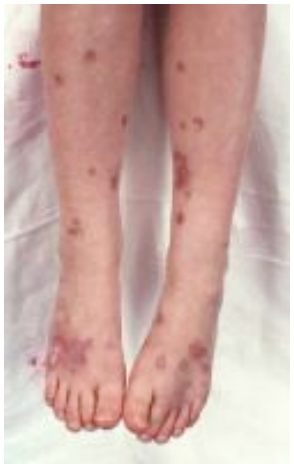


Preaxial polydactyly

Vitilego

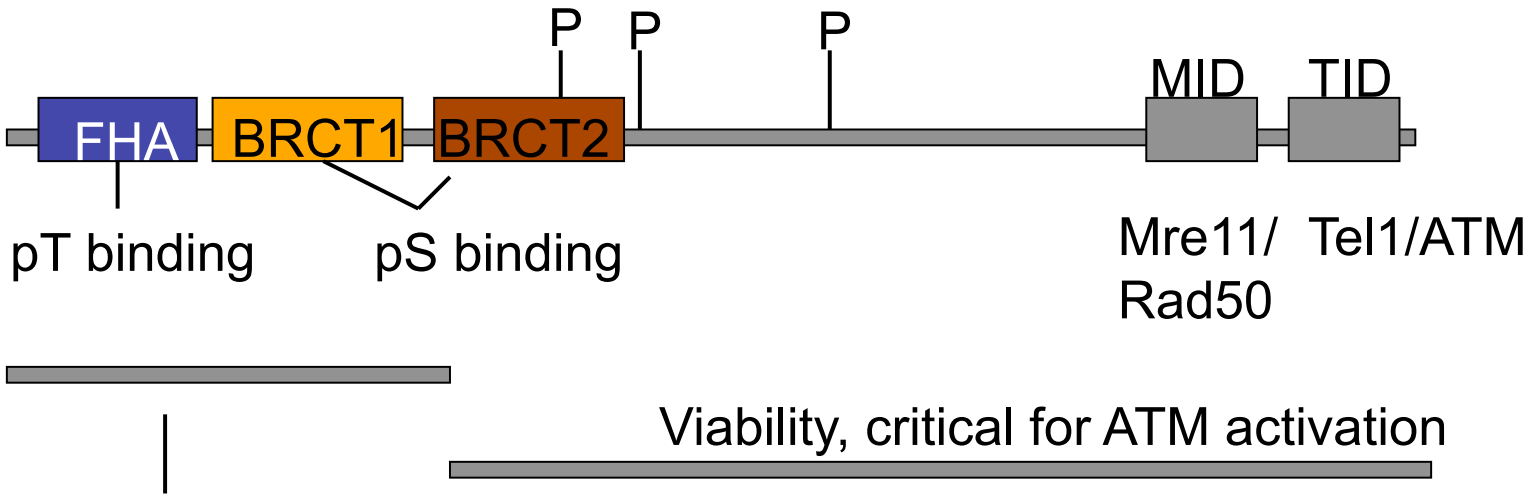


Cutaneous sarcoidosis



Cranio-facial abnormalities

Nbs1- domain architecture / interactions

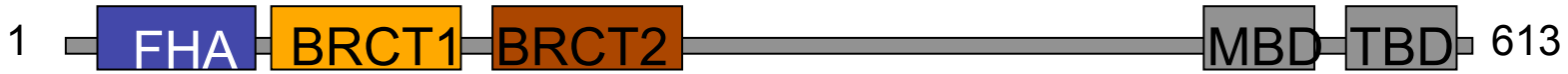


- T-cell, oocyte development,
- Efficient damage signaling
- IRIF formation

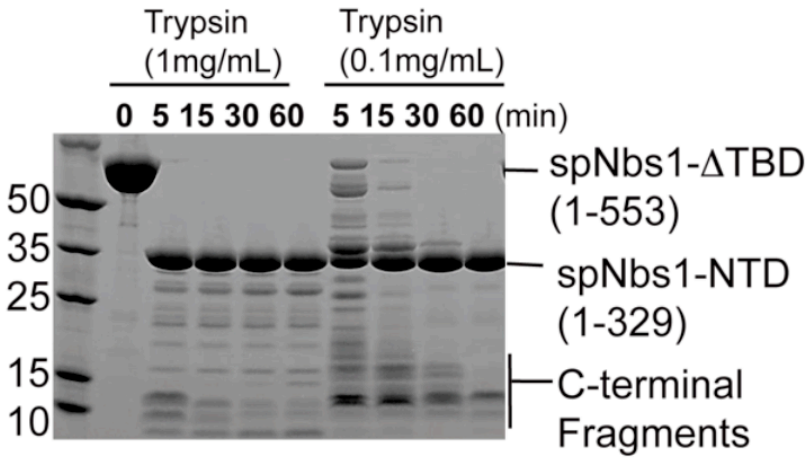
<u>FHA, BRCT Binding targets:</u>

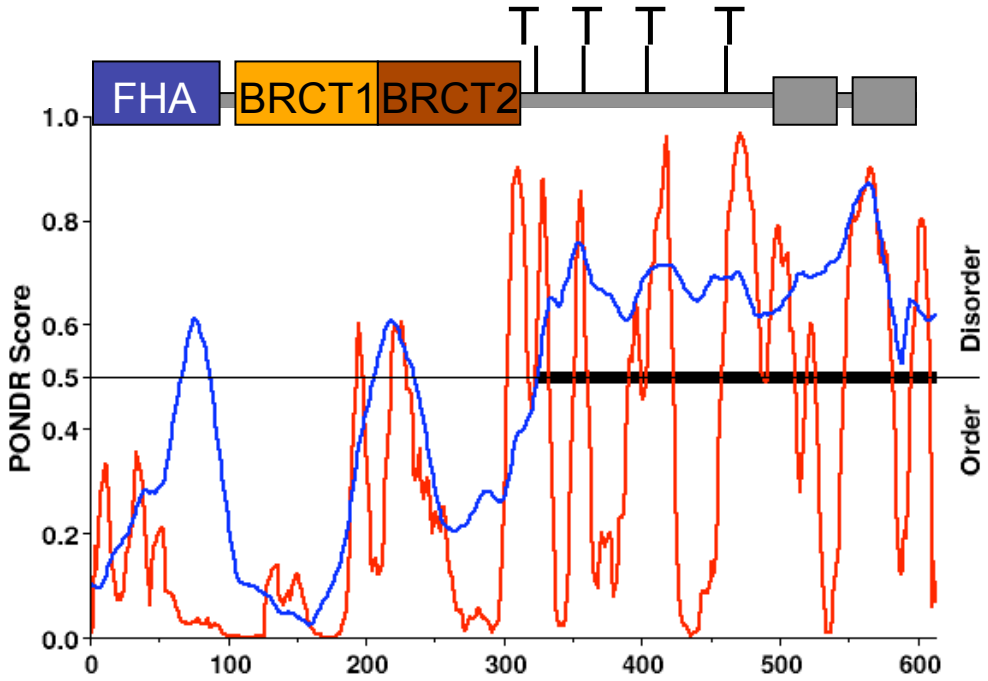
-MDC1, CtIP

Mapping structured domains in Nbs1

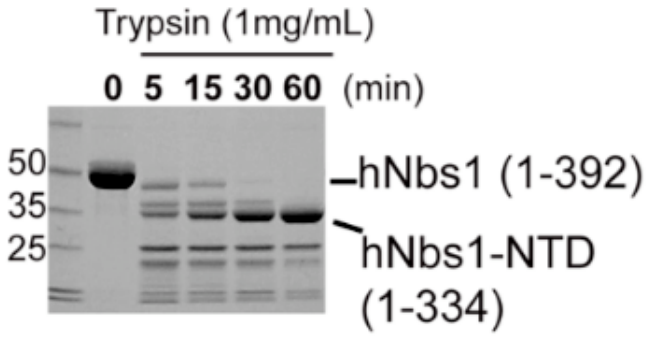


S. Pombe Nbs1

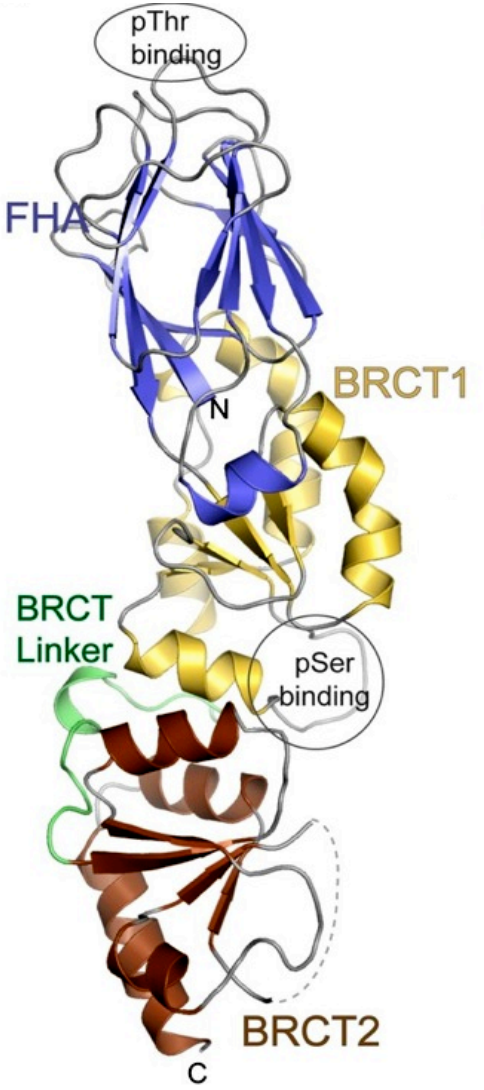


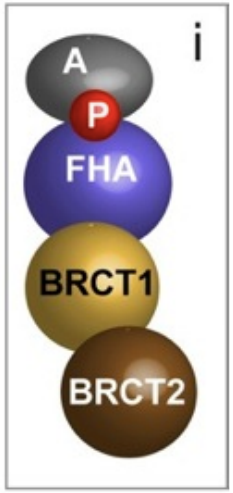


Human Nbs1



The Nbs1 N-terminus is a divalent phosphoprotein interaction scaffold





S. pombe FHA binding Targets???
hNbs1: hMDC1
scXrs2: Lif1

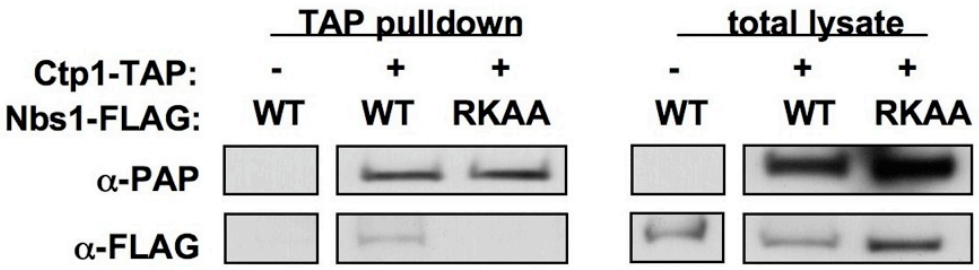
The Nbs1 FHA domain binds phosphorylated Ctp1

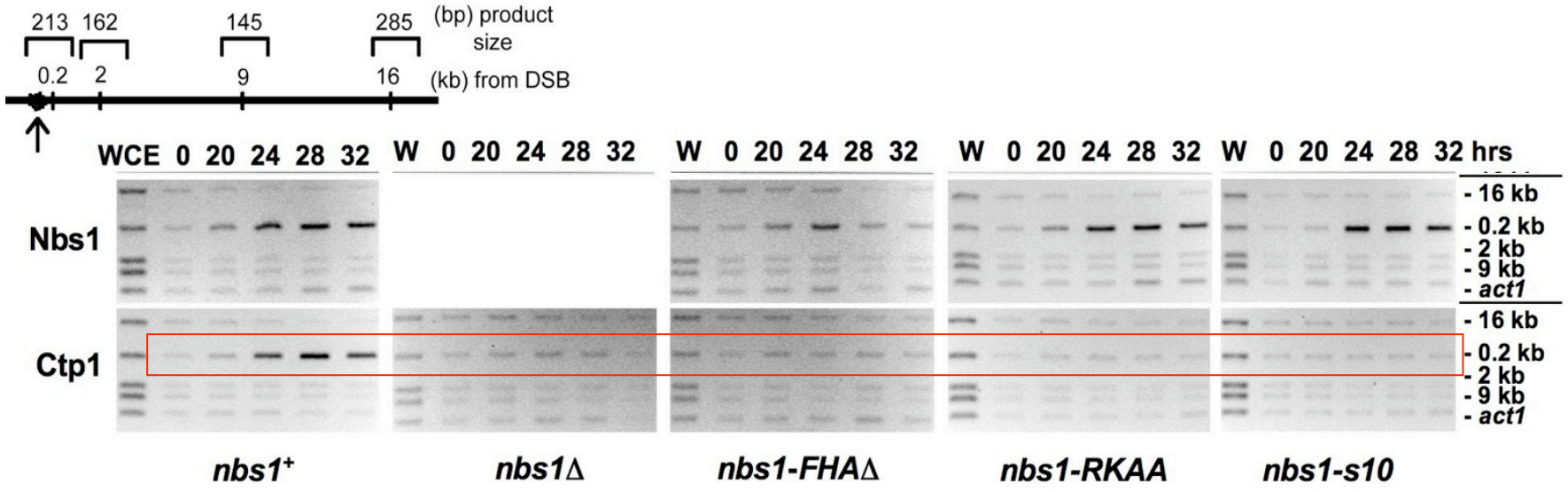
Ctp1-Nbs1

1. Ctp1 is a member of the MRN epistasis group.
2. Ctp1 is a high copy suppressor of Nbs1 FHA mutations
3. hCtIP co-IPs with MRN
4. Ctp1 is phosphorylated

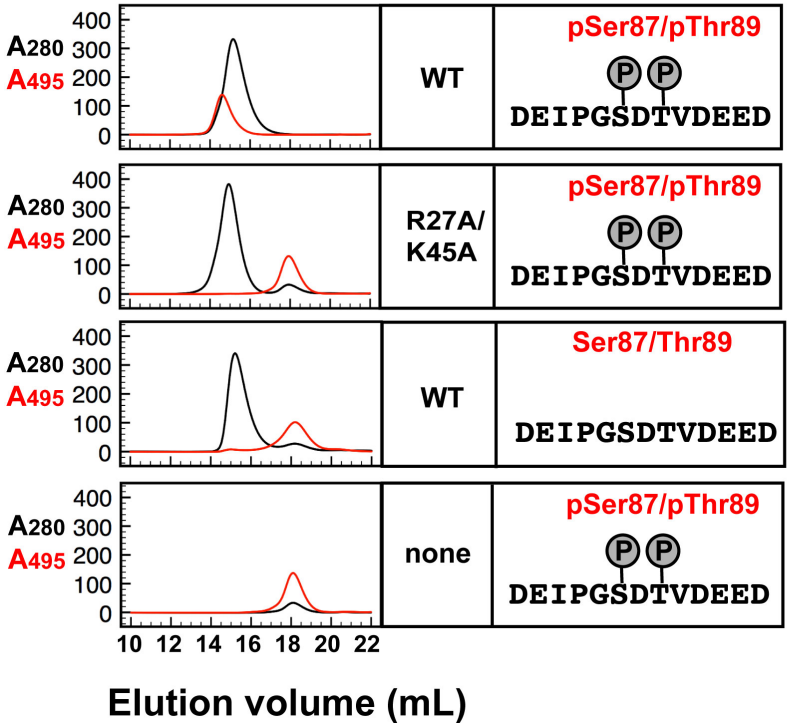

```
hMdc1    215-NLNSDTDVEE
hMdc1    296-GEDSDTDVDD
hMdc1    326-FIDSDTDAEE
hMdc1    373-QAGSDTDVEE
hMdc1    399-VINSDTDDEE
hMdc1    450-ERDSDTDVEE
spCtp1    74-ELDSTTDEDE
spCtp1    84-IPGSDTVDEE
scLif1   384-GSESETDASA
scLif1   414-QTESETDIET
```

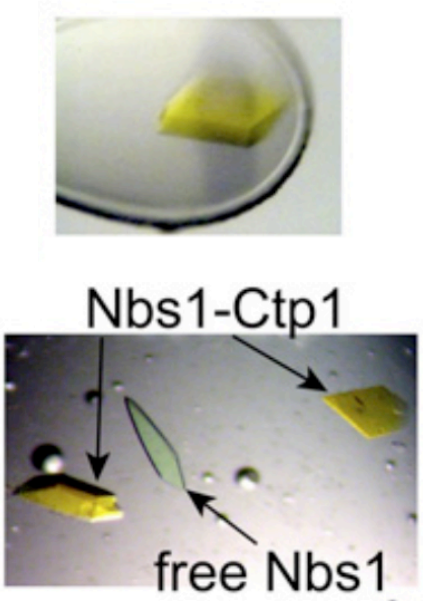
FHA domain mutations in Nbs1 impair Ctp1 recruitment to a DSB



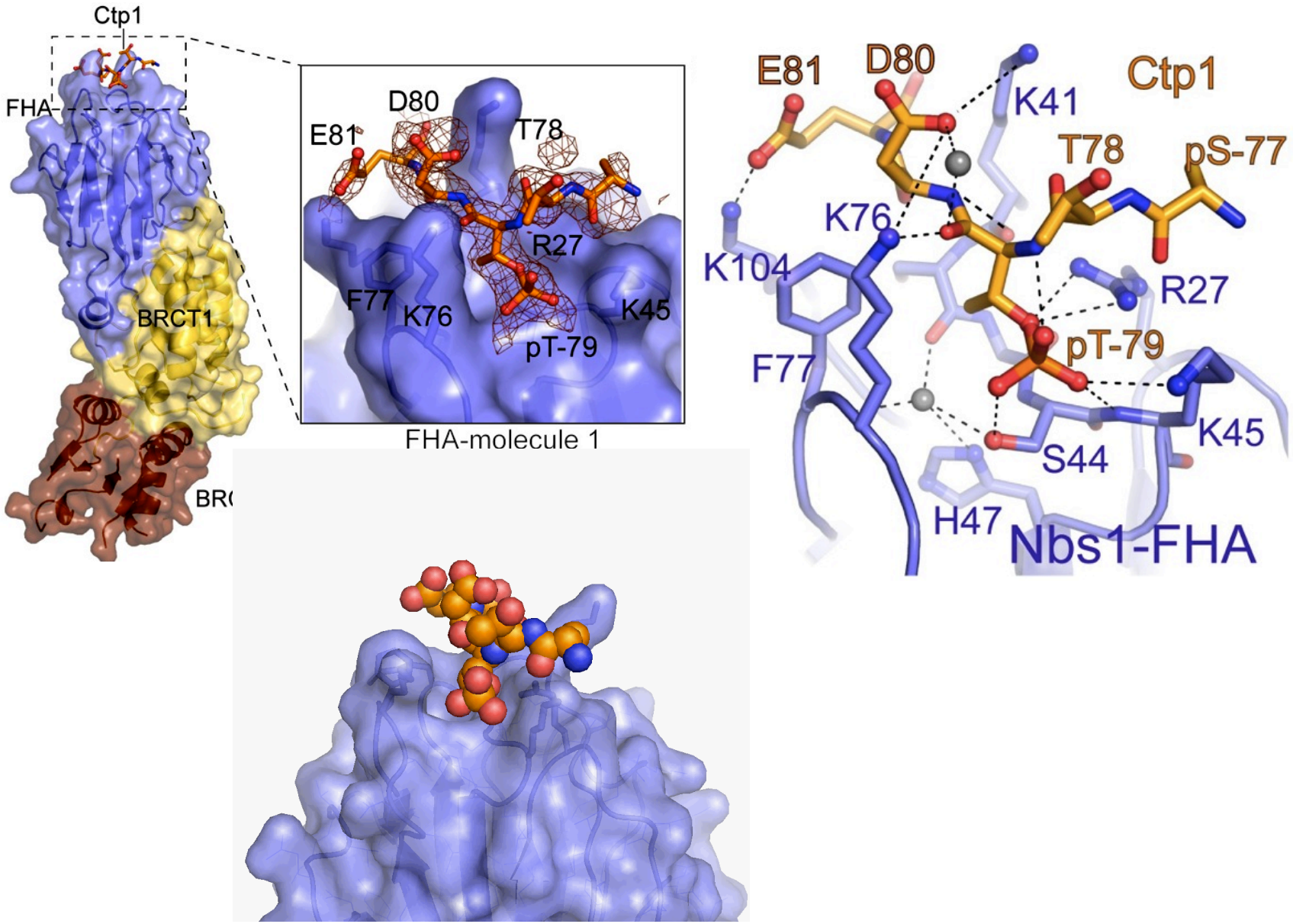


The Nbs1 FHA domain binds phosphorylated Ctp1

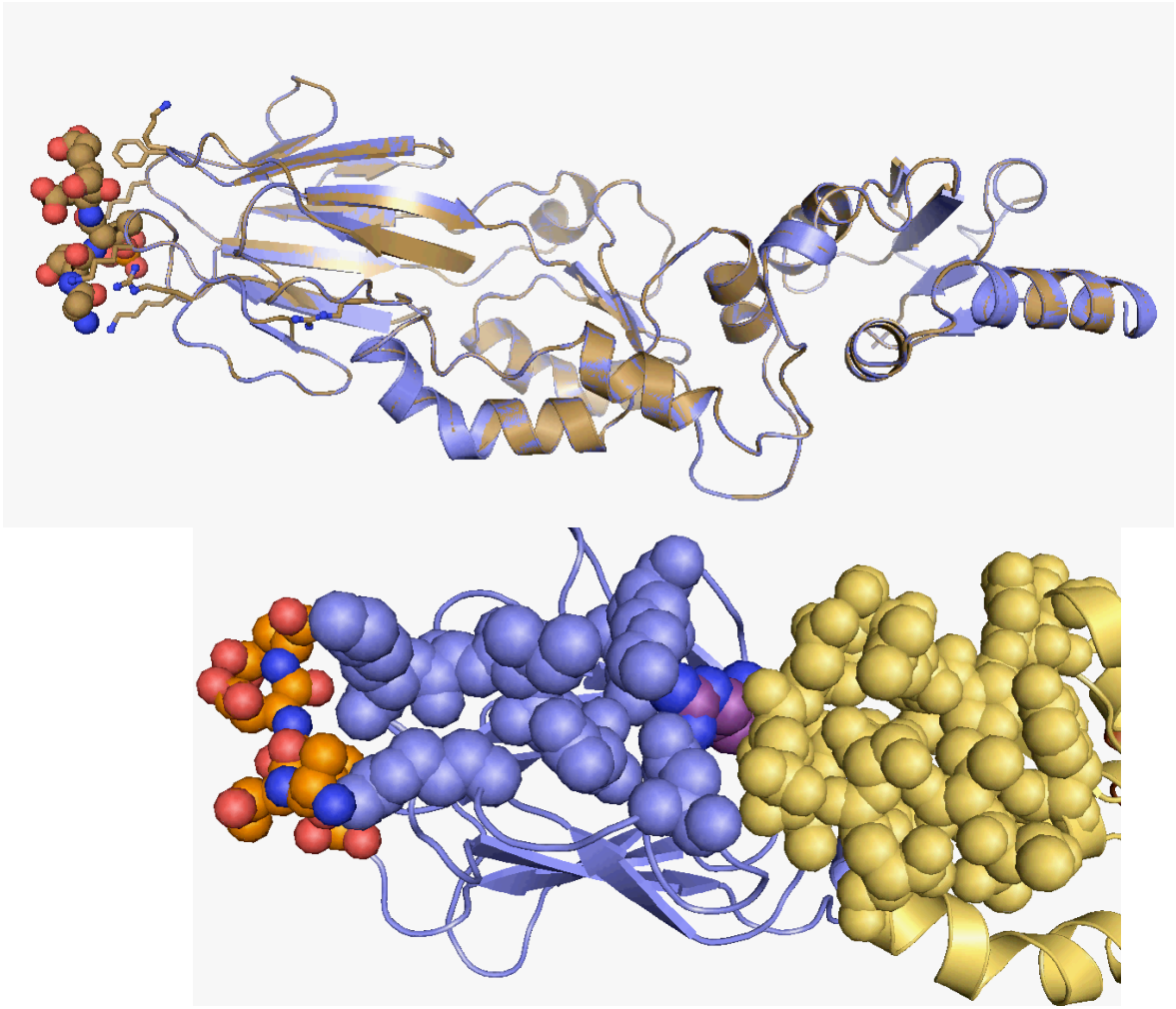




X-ray Structure of the Nbs1-Ctp1 complex

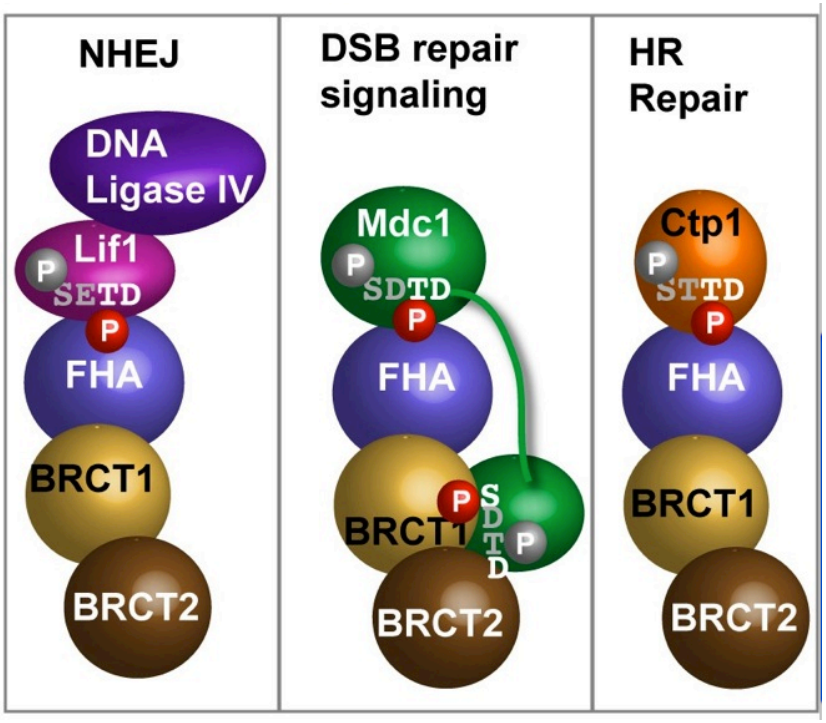


Ctp1-binding in the FHA modulates tandem BRCT conformations



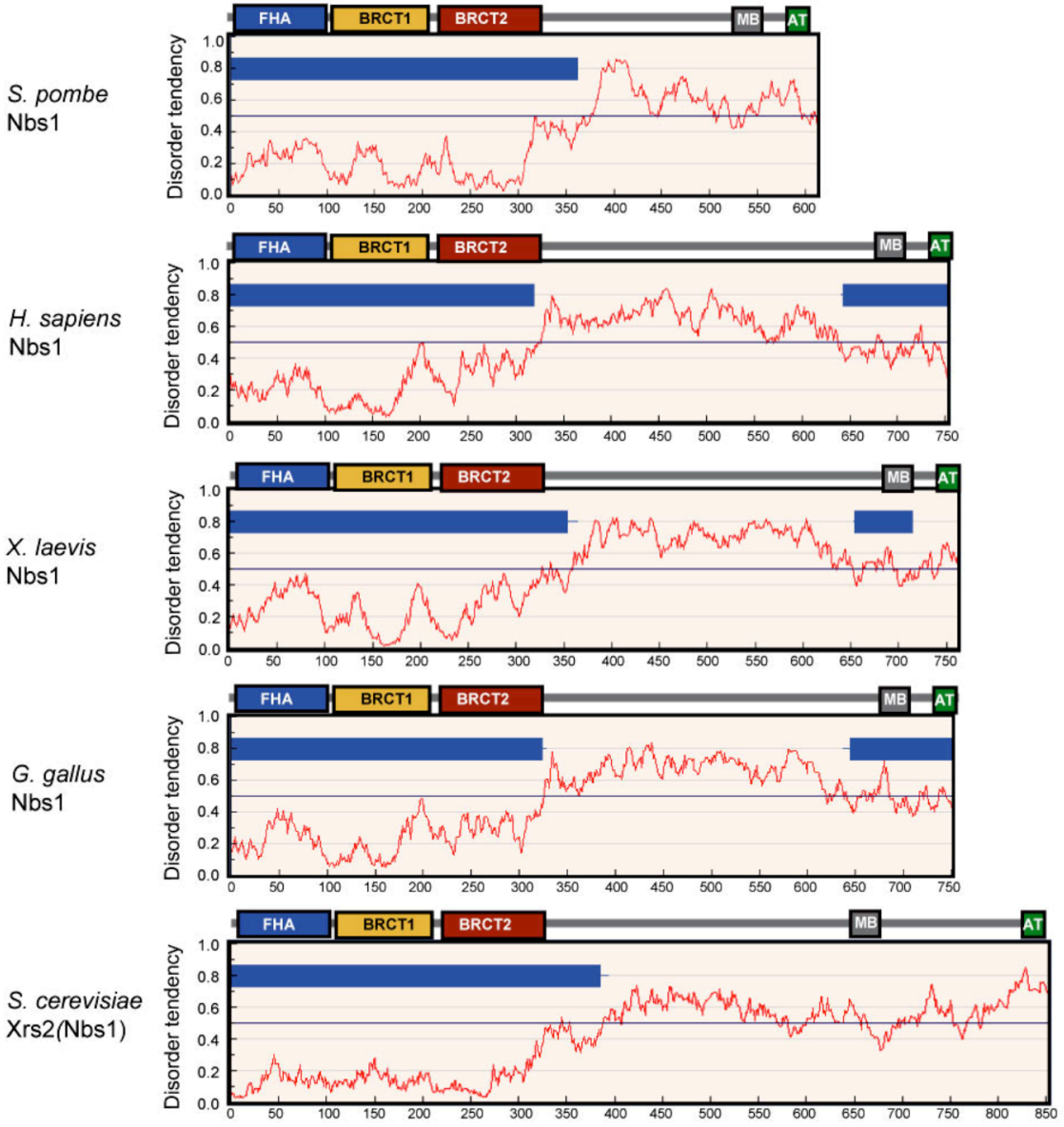
Hypothesis: FHA phosphoprotein interactions can allosterically regulate interactions in the BRCT domains of Nbs1

Nbs1 docks with multiple partners to regulate DSB repair

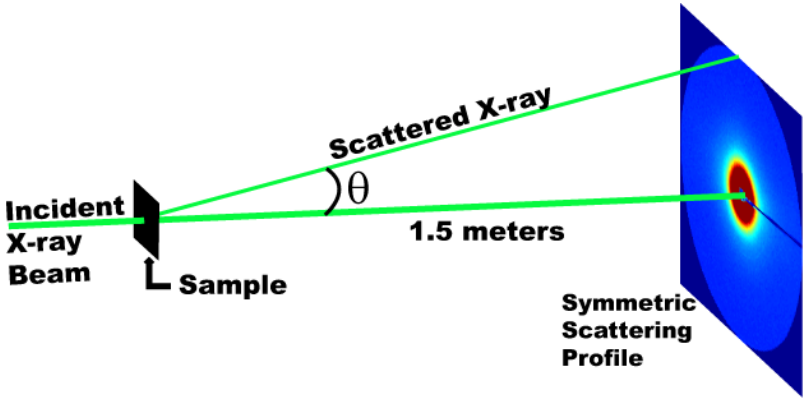


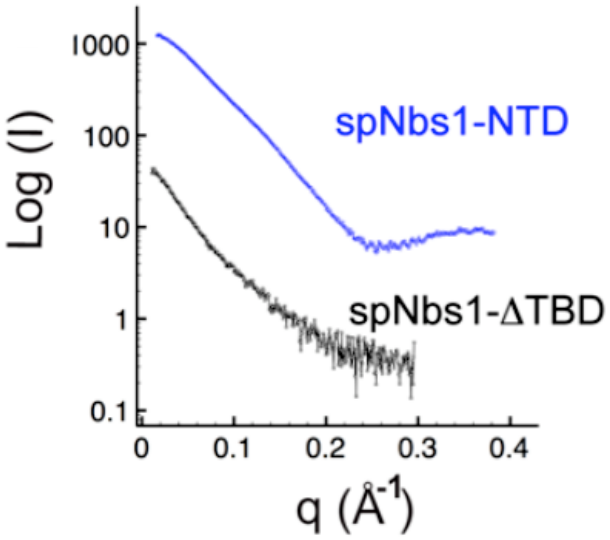
Partnering of Tandem BRCTs with an FHA expands the Nbs1 regulatory repertoire

Protein order/disorder predictions for Nbs1 homologs

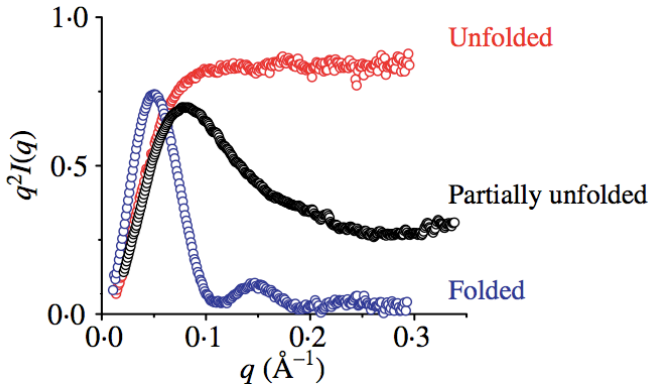


The Nbs1 C-terminal region is flexible

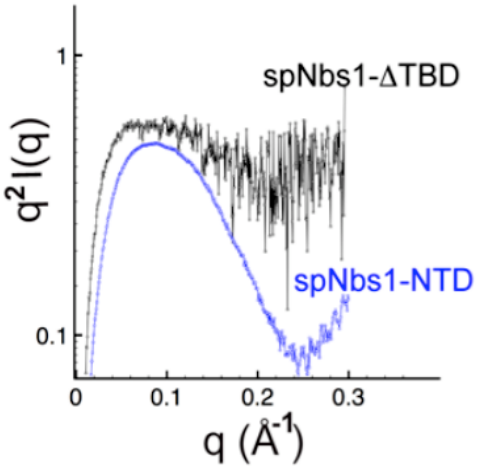




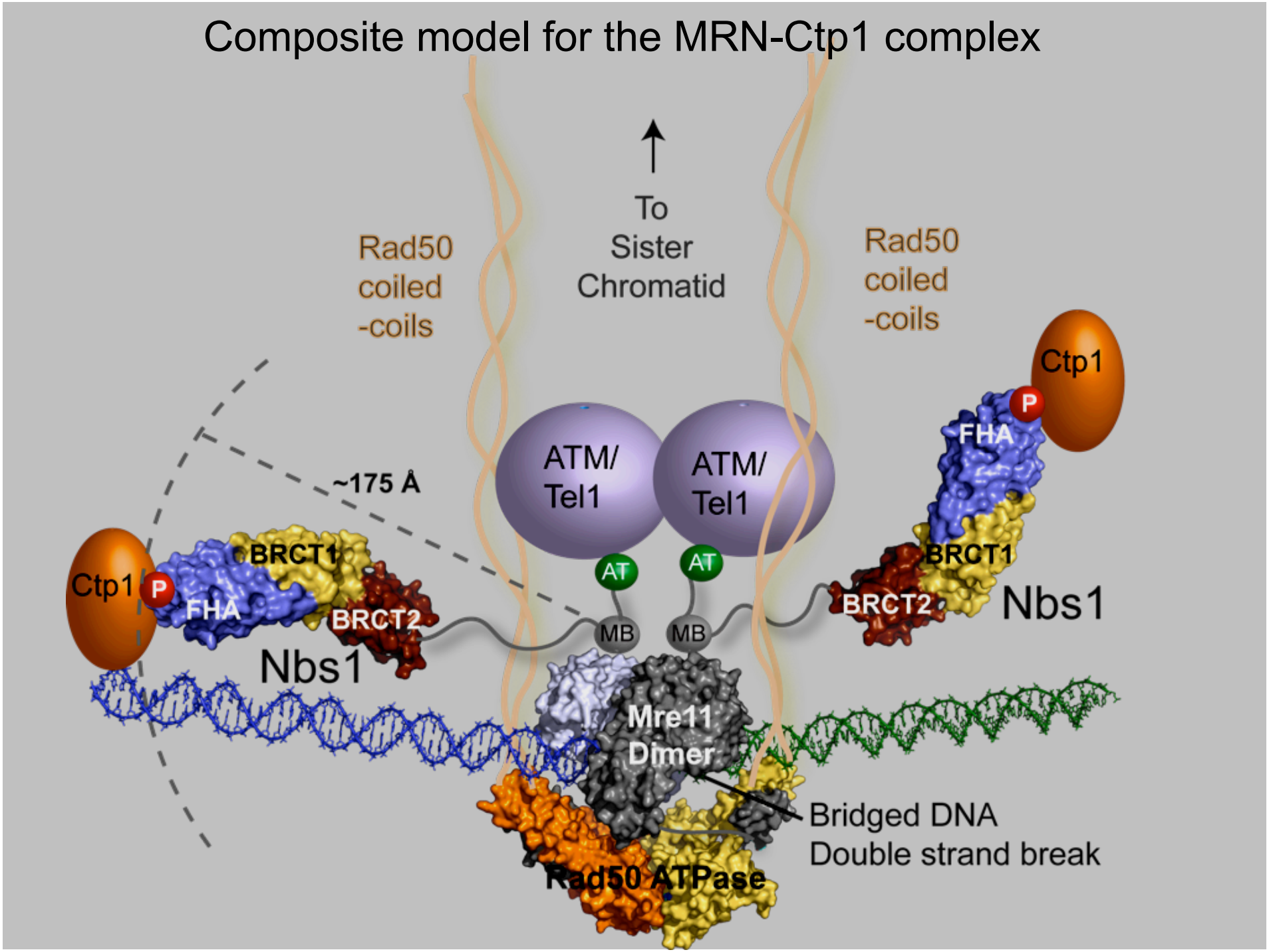
Kratky plot



Kratky plot



Composite model for the MRN-Ctp1 complex



Some answers to the Questions ...

Part 1.

A. How do BRCT domains integrate into multidomain/ multiprotein complexes?

- Fusion of BRCTs with an FHA expands the substrate binding repertoire of Nbs1
- FHA/BRCTs of Nbs1 flexibly link to enzymatic DSBR effectors

B. Are BRCT phosphoprotein interactions regulated?

- Possibly. The FHA-BRCT fusion provides a mechanism for allosteric regulation of phosphoprotein binding function ....

Part 2.

How do BRCT proteins recognize unique targets?

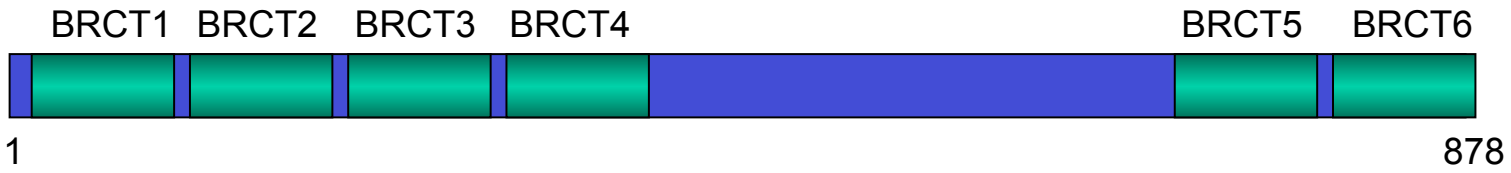
- Identification of a fission yeast S-phase phospho-H2A binding BRCT protein

hBRCA1(BACH1 CtIP)	hMDC1-γH2AX
pSxxF pSxxY	pSxxY(coo-)
NOT	NOT
pSxxL	pSxxF(coo-) pSxxL(coo-)

What about yeast γH2A BRCT effectors?

```
human H2AX              LPNIQAVLLPKKTSATVGPKAPSGGKKATQASQEY
mouse H2AX              LPNIQAVLLPKKSSATVGPKAPAVGKKASQASQEY
T.thermophila H2A.1     LPNINPMLLPSKSKKTESRG----------QASQDL
S.cerevisiae H2A.1      LPNIHQNLLPKKSAKATKA------------SQEL
S.cerevisiae H2A.2      LPNIHQNLLPKKSAKTAKA------------SQEL
S.pombe H2A.1           VPNINAHLLPKTSGRTGKP------------SQEL
S.pombe H2A.2           VPNINAHLLPKQSGK-GKP------------SQEL
```

S. pombe Brc1



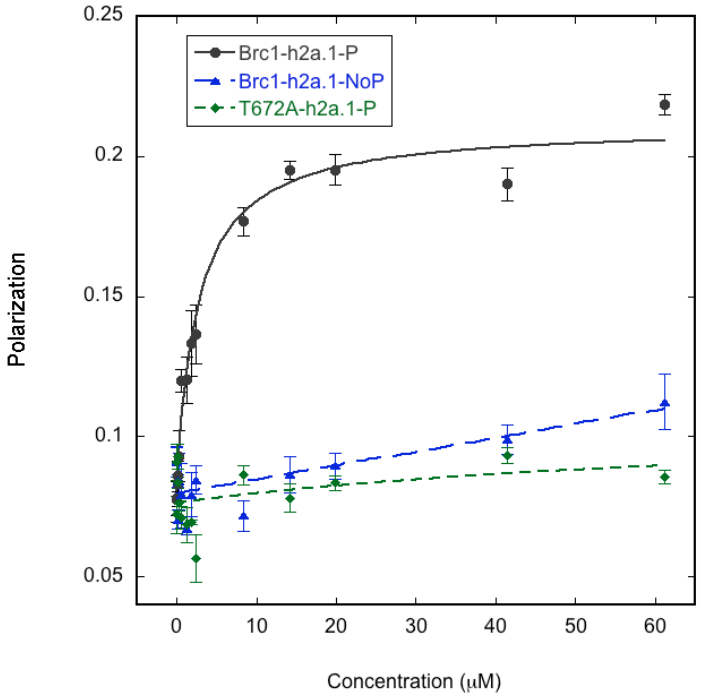
- Brc1$^{Rtt107/PTIP}$ forms spontaneous and DNA damage-induced nuclear foci by binding γH2A.
- Brc1 foci colocalize predominantly with ribosomal DNA repeats

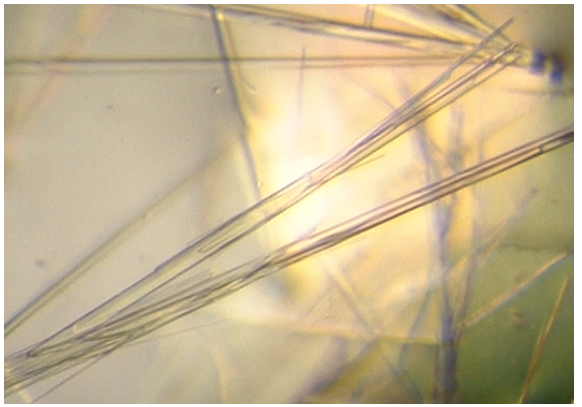
- DNA damage-induced foci colocalize with DSB response factors. The Brc1-γH2A interaction is critical for recovery from replication fork collapse.

- Brc1 docking to γH2A is a critical chromatin-specific response to replication-associated DNA damage.

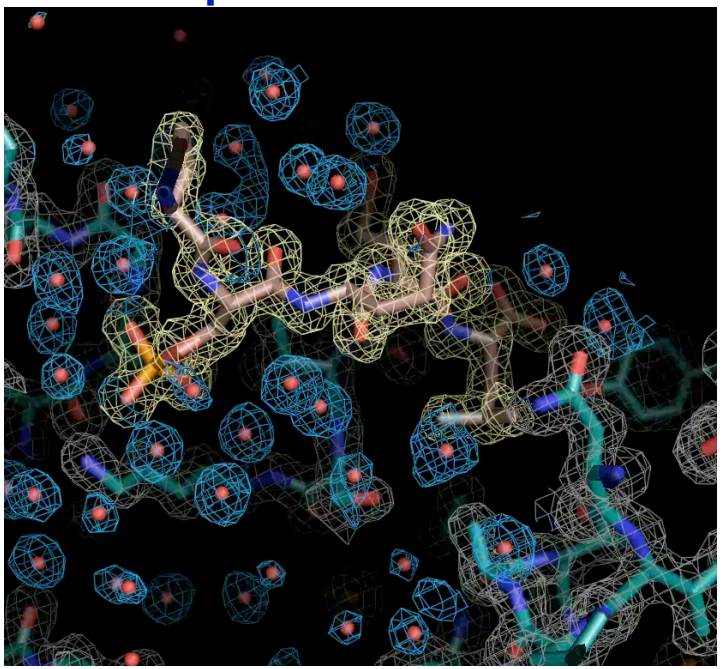
Williams et al EMBO J. 2010

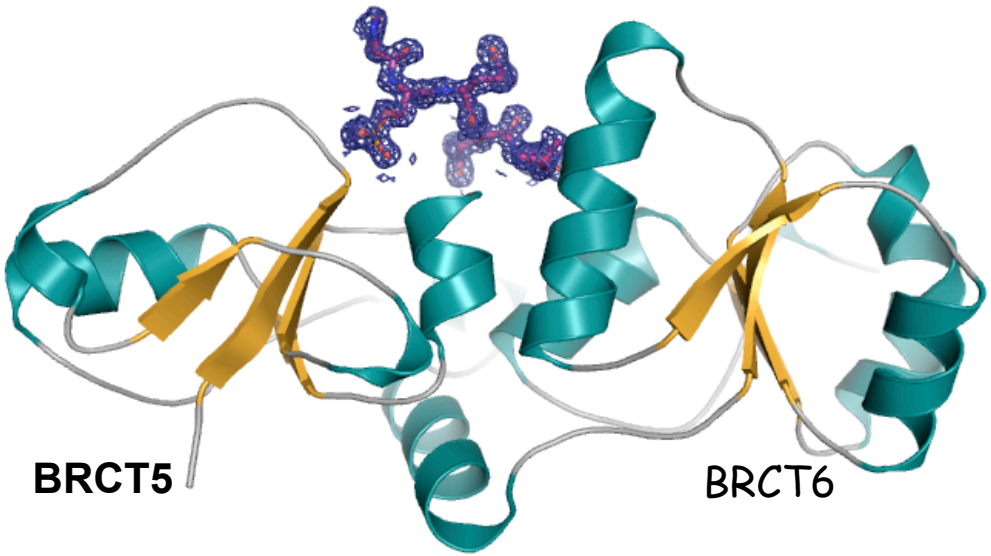
BRC1 directly binds the SpH2A.1 phosphorylated tail



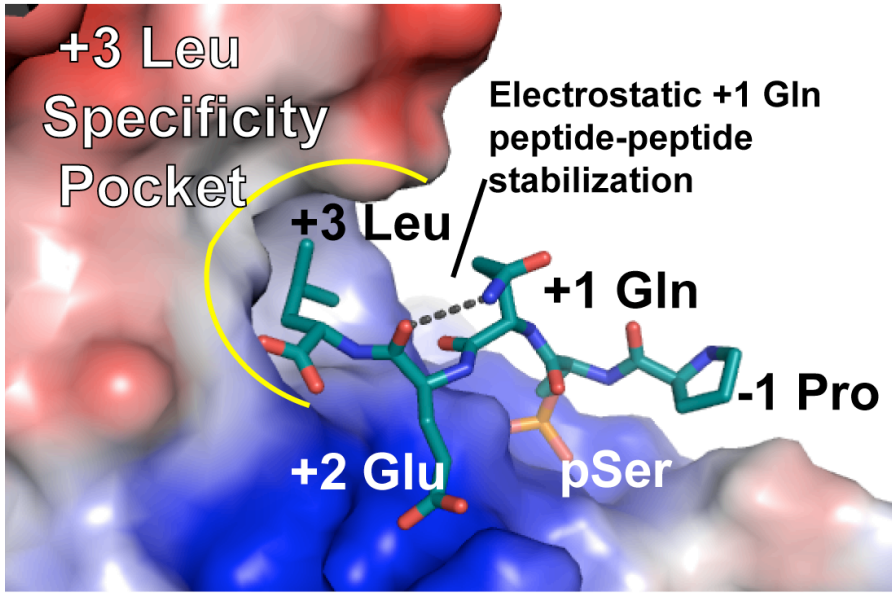


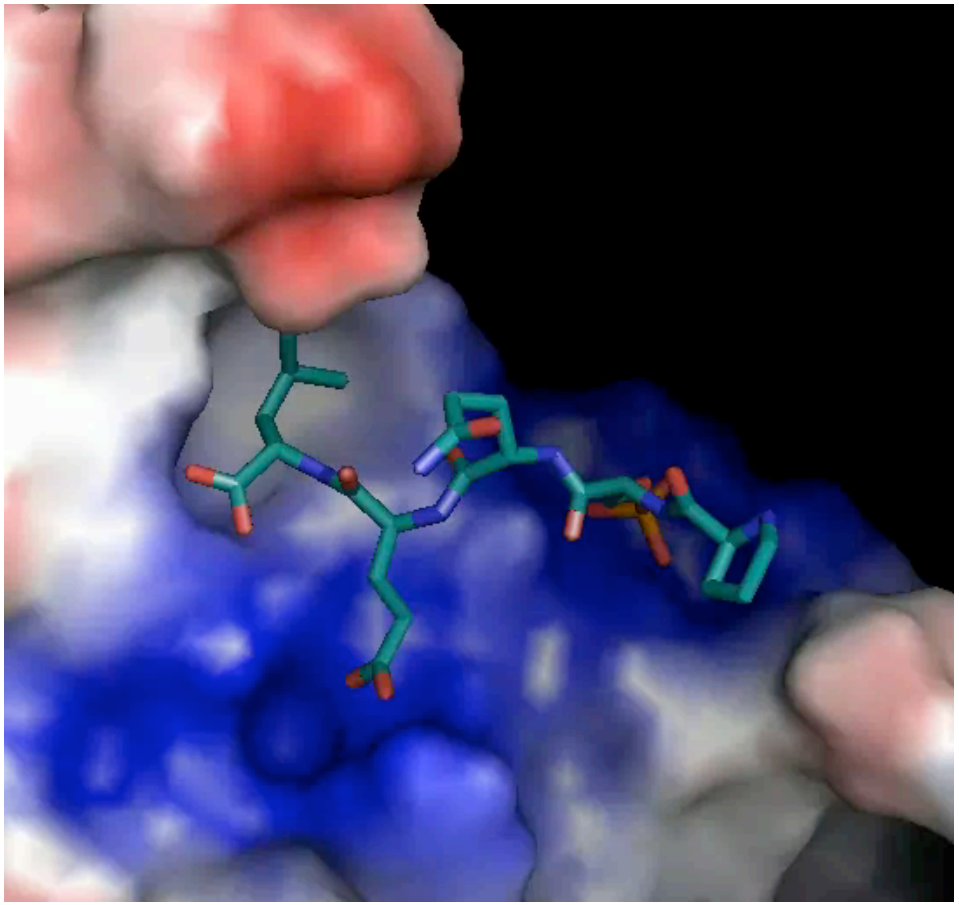
BRC1:phospho-H2A.1 complex at 1.45 Å





Specificity of the Brc1 BRCT5-6: phospho-H2A.1 complex

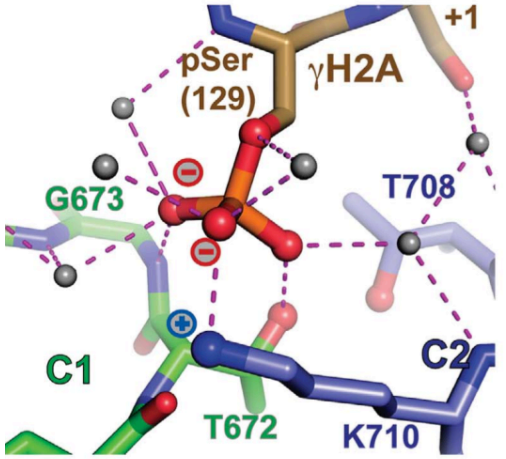


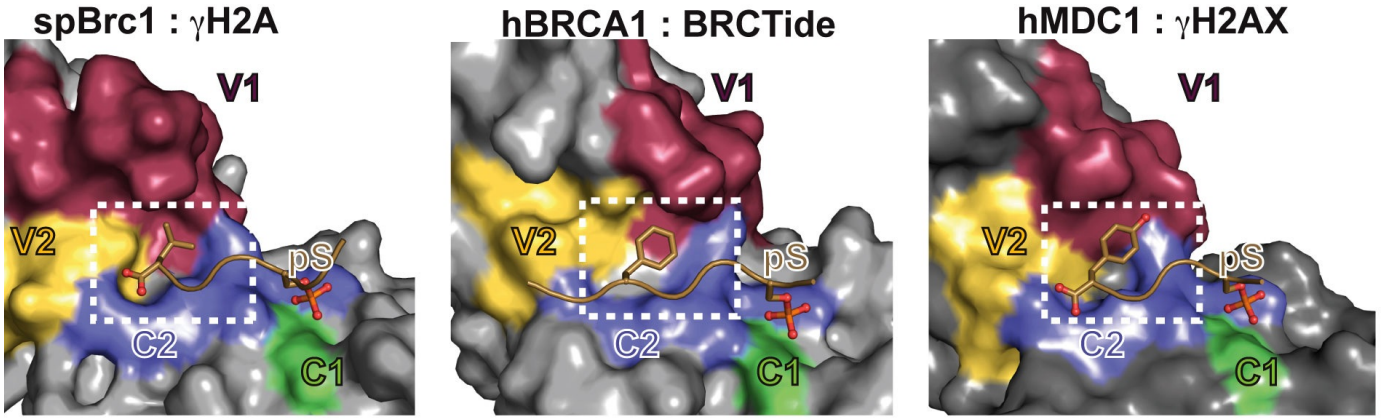


Constant regions bind pSer, Variable regions bind pSer +3

C1 C2

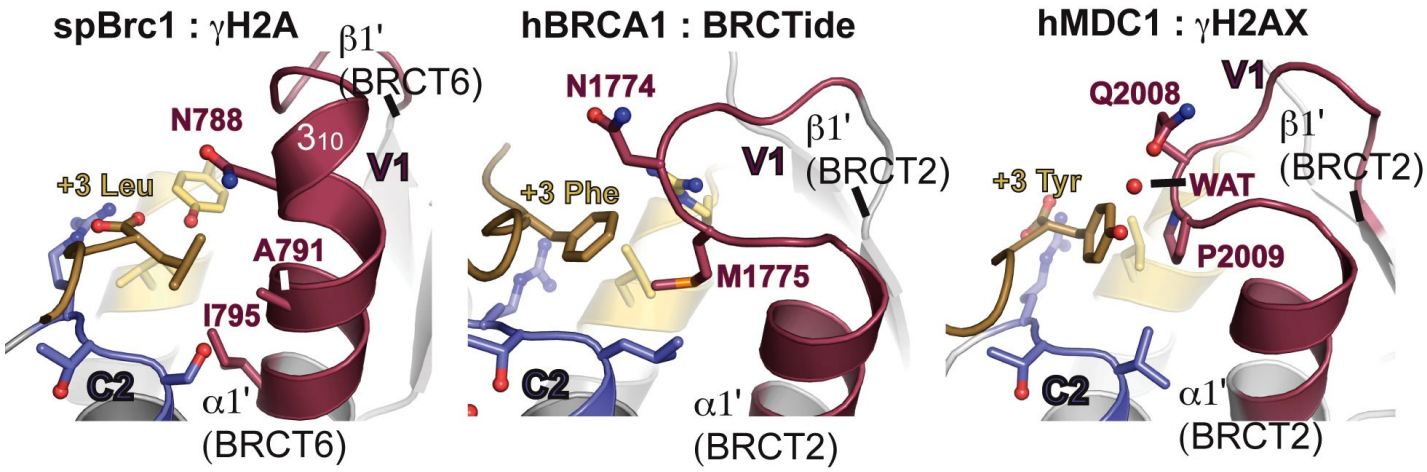
(S/T)G .....TXK

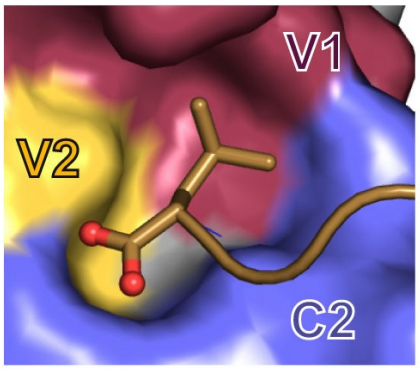




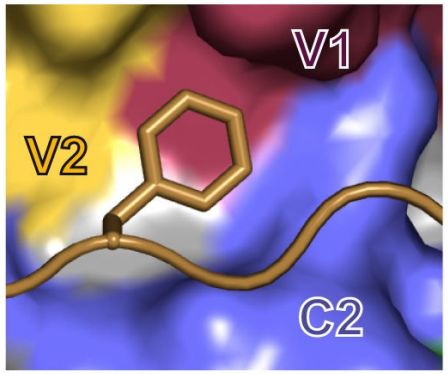
Williams et al EMBO J. 2010

Variability of V1 loops dictates pSer +3 binding specificity

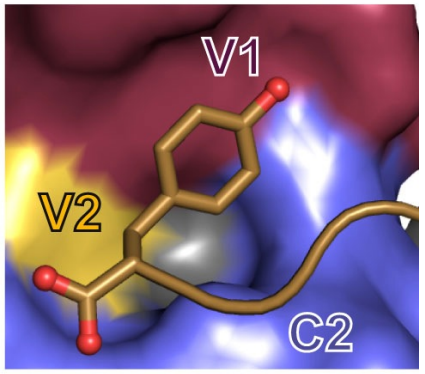




pSxxL (coo-)



....pSxxF ...



....pSxxY (coo-)

Some answers to the Questions ...

Part 1.

A. How do BRCT domains integrate into multidomain/ multiprotein complexes?

- Fusion of BRCTs with an FHA expands the substrate binding repertoire of Nbs1
- FHA/BRCTs of Nbs1 flexibly link to enzymatic DSBR effectors

B. Are BRCT phosphoprotein interactions regulated?

- Possibly. The FHA-BRCT fusion provides a mechanism for allosteric regulation of phosphoprotein binding function ....

Part 2.

A. How do BRCT proteins recognize unique targets?

- BRCTs utilize a two-point specificity
  - Two constant regions bind pSer
  - Variable sequence/structural insertions dictate binding specificity at pSer +3

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