Structural and Biochemical Studies to Assess Protein Interactions and (re)classify VUSs

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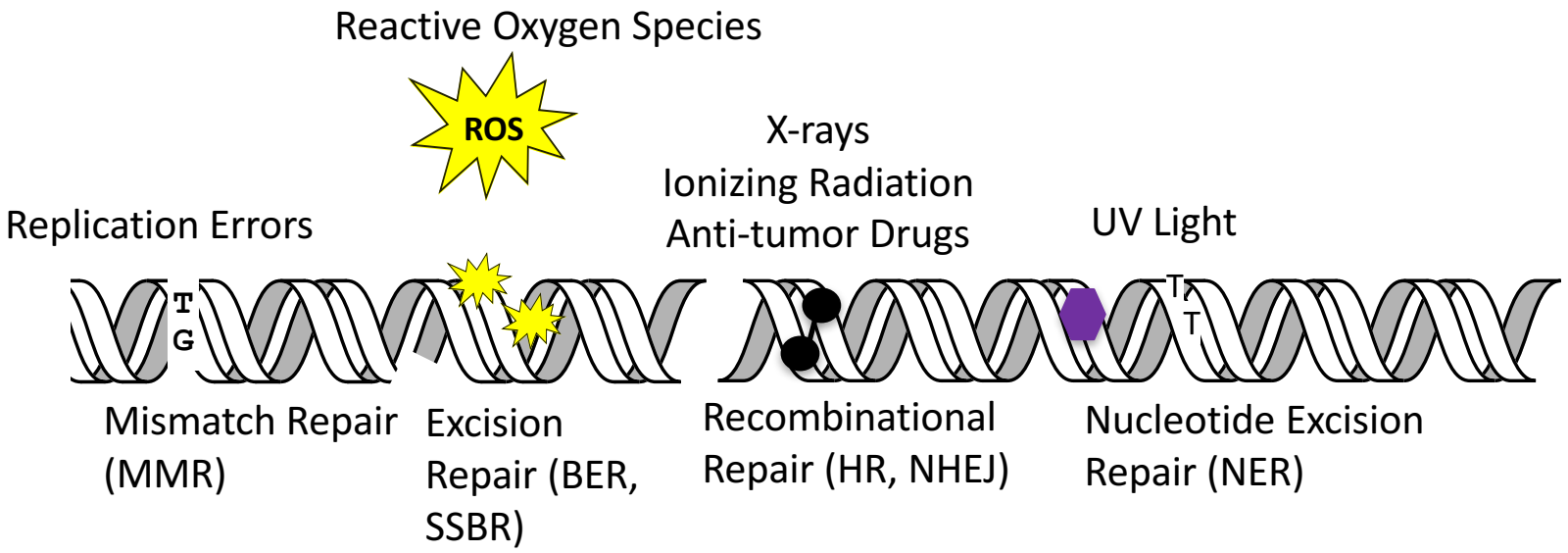
DNA Repair Video Conference
Feb 19th, 2019

Outline

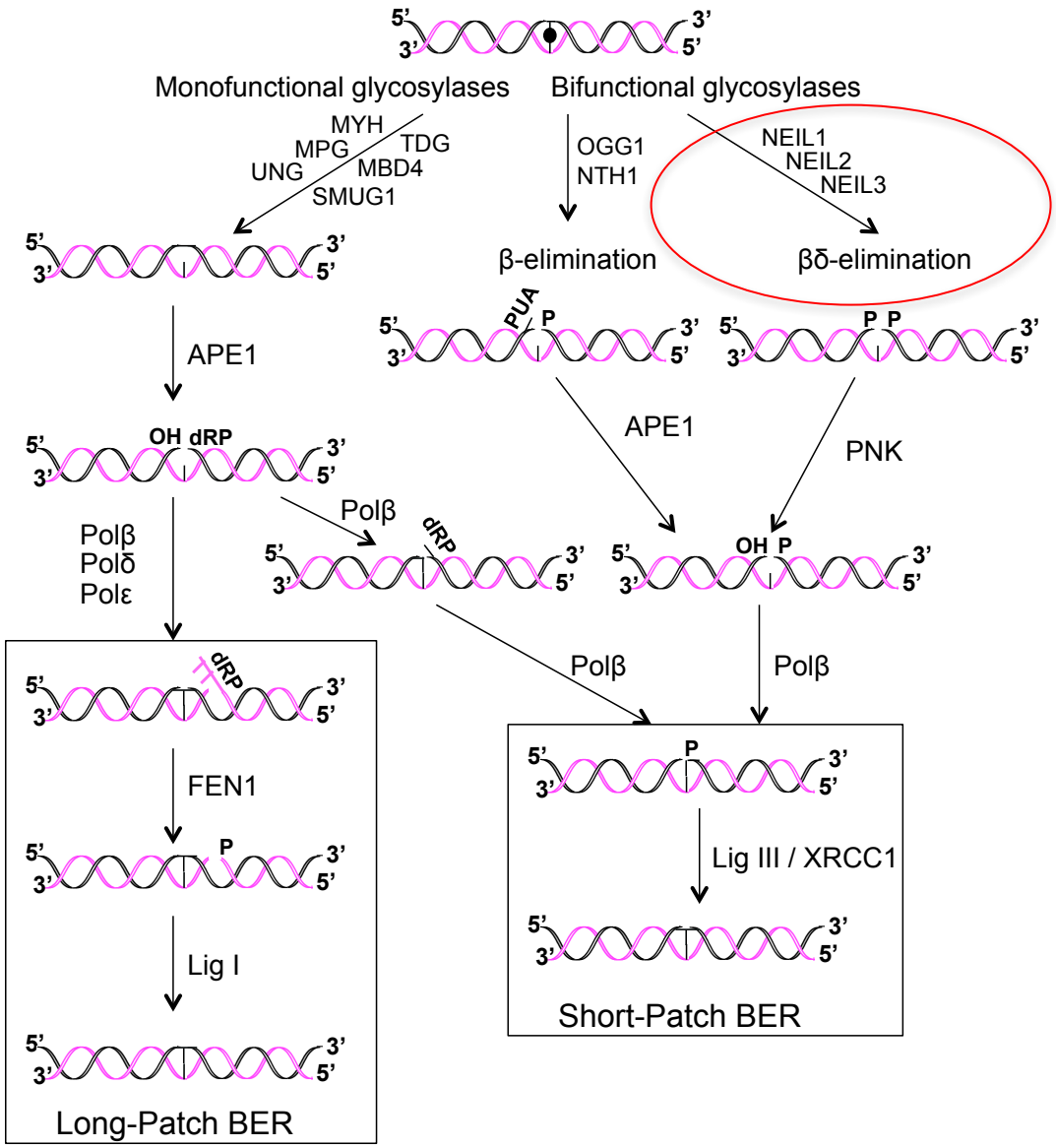
- **PART I: Specialized functions of the NEIL1 DNA Glycosylase**
  - BER & NEIL1
  - The interaction between NEIL1 and mitochondrial SSB, *in vitro*
  - Concluding remarks
- **PART II: (Re)Classification of Variants of Uncertain Significance in Lynch syndrome patients**
  - Identification of Variants of Uncertain Significance
  - Functional Characterization
  - Concluding remarks

DNA Damage and Repair Pathways



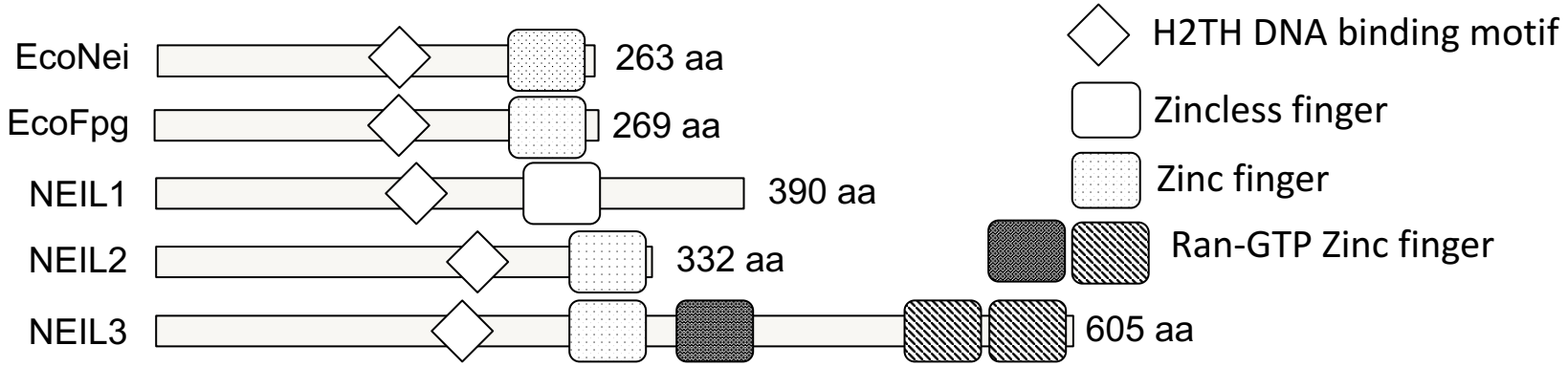
Adapted from Tubbs, Cell 2017;
https://diethylstilbestrol.co.uk/dna-repair-system/

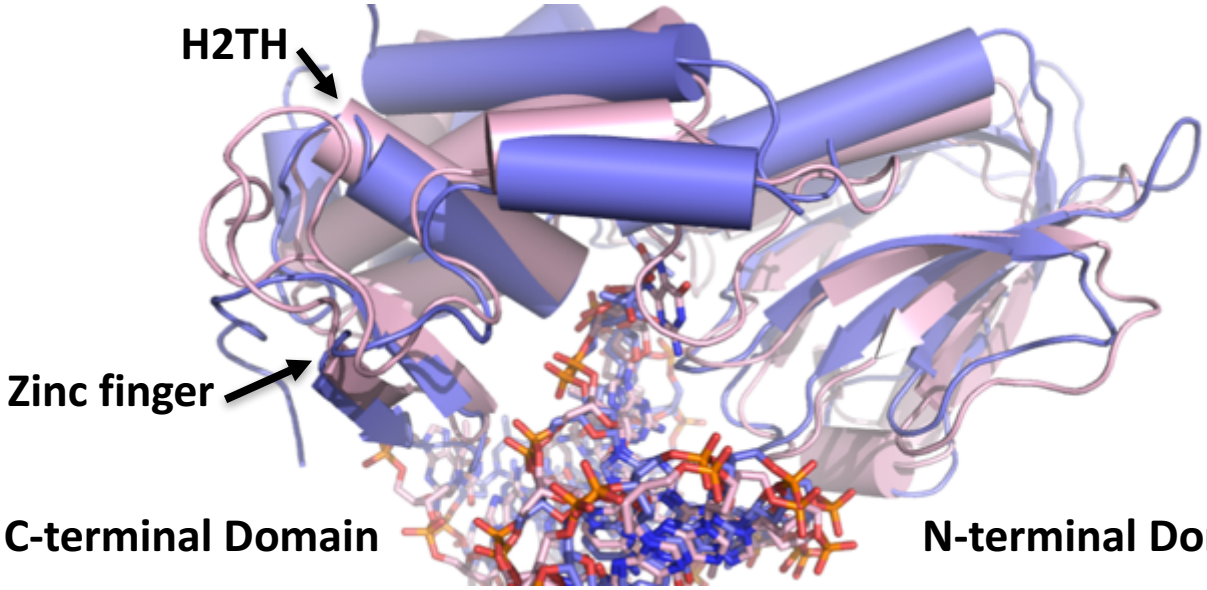
PART I: Base excision repair & NEIL1



Adapted from Hegde ML, Cell Research, 2008 and Duclos et al., 2011

Structural features of the Fpg/Nei DNA glycosylase family



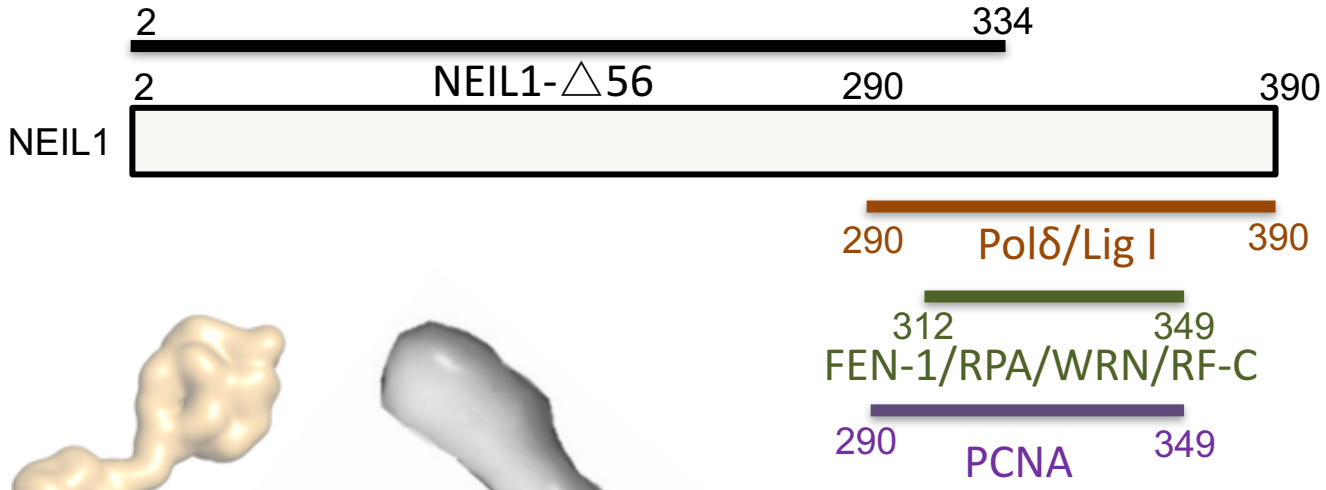


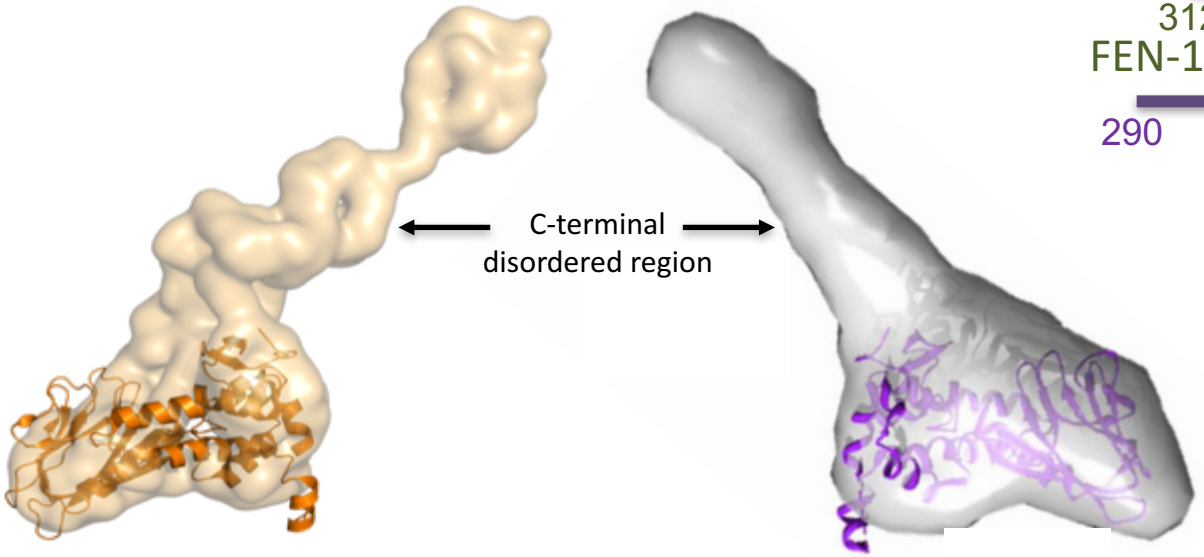
Blue – NEIL1
Pink - EcoFpg

PDB: 1R2Y
5ITX

Fromme, JC, Verdine, GL. (2003) J Biol Chem 278 51543-51548
Zhu, C. et. al. (2016) Proc.Natl.Acad.Sci.USA 113: 7792-7797

The C-terminal tail of NEIL1 is involved with protein interactions





Prakash A. et al., *NAR*. 2017
Sharma N. et al., *DNA Repair*. 2018
Doublié S. et al., PNAS. 2004

Hegde M. et al., *JMB*. 2013
Doublié S. et al., *PNAS*. 2004

Adapted from Rangaswamy et al., *Genes.* 2017

Role of NEIL1 in mitochondrial DNA repair

The metabolic syndrome resulting from a knockout of the NEIL1 DNA glycosylase

Vladimir Vartanian*, Brian Lowell*, Irina G. Minko*, Thomas G. Wood†, Jeffrey D. Ceci†, Shakeeta George‡, Scott W. Ballinger‡, Christopher L. Corless§, Amanda K. McCullough*, and R. Stephen Lloyd*¶

Repair of Formamidopyrimidines in DNA Involves Different Glycosylases

ROLE OF THE OGG1, NTH1, AND NEIL1 ENZYMES*

Received for publication, August 9, 2005, and in revised form, October 7, 2005 Published, JBC Papers in Press, October 11, 2005, DOI 10.1074/jbc.M508772200

Jingping Hu‡1, Nadja C. de Souza-Pinto‡1, Kazuhiro Haraguchi§, Barbara A. Hogue‡, Pawel Jaruga¶‖, Marc M. Greenberg§, Miral Dizdaroglu¶, and Vilhelm A. Bohr‡2

Differential age-related changes in mitochondrial DNA repair activities in mouse brain regions

Ricardo Gredilla,[a,1] Christian Garm,[a,1] Rikke Holm,[a] Vilhelm A. Bohr,[b] and Tinna Stevnsner[a,*]

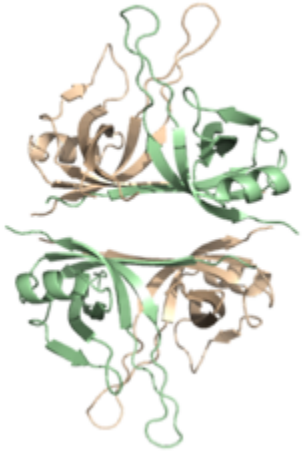
Nidhi Sharma

Question:

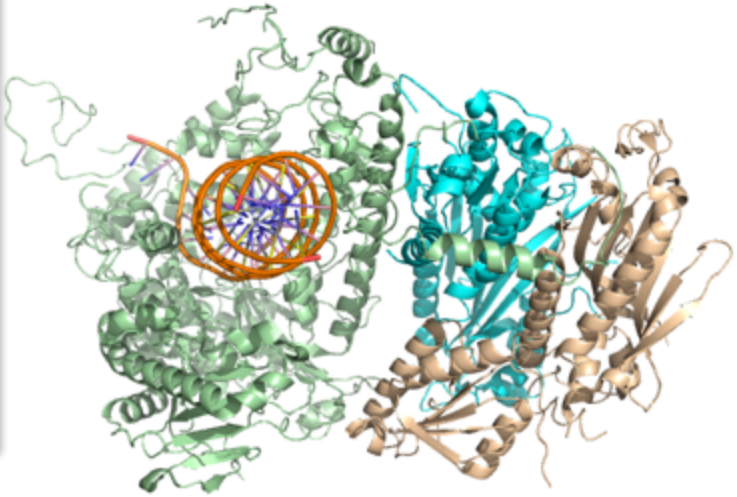
Does NEIL1 interact with proteins associated with mtDNA replication?

Some proteins involved with mitochondrial DNA maintenance

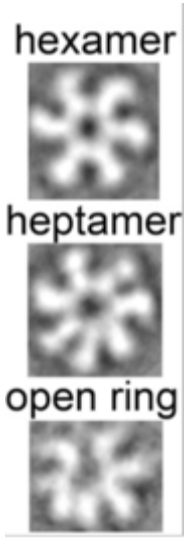
mtSSB, Homotetramer, PDB: 3ULL



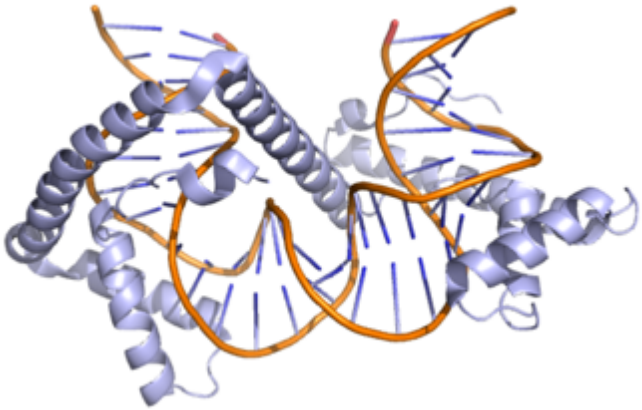
Polymerase Gamma, Heterotrimer, PDB: 5C51



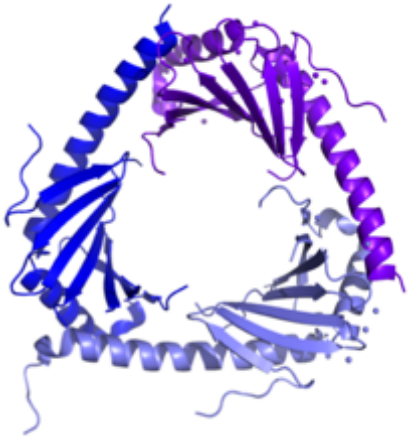
Twinkle helicase, Nucleic Acids Res. 2015 Apr 30; 43(8): 4284–4295.



Transcription factor A (TFAM), PDB: 3TMM



Complement Component 1, q subcomponent binding protein (C1QBP), homotrimer, PDB: 3RPX



Sharma N. et al. *DNA Repair*. 2018
Prakash Lab, unpublished. 2018

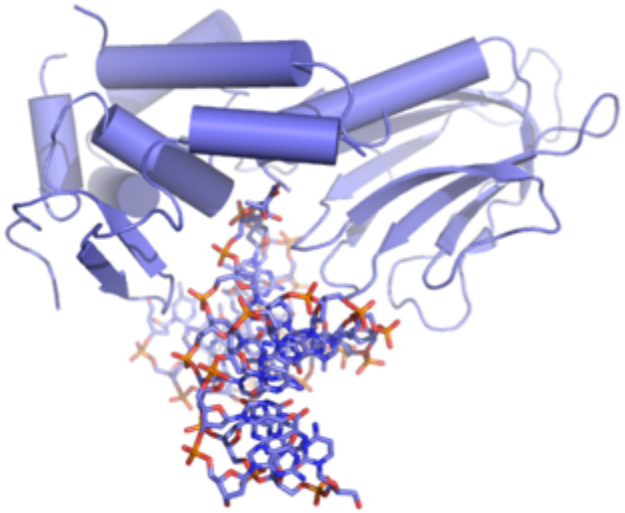
A combined approach to studying the interaction between NEIL1 and mtSSB

Structural Interactions between NEIL1 and mtSSB in solution

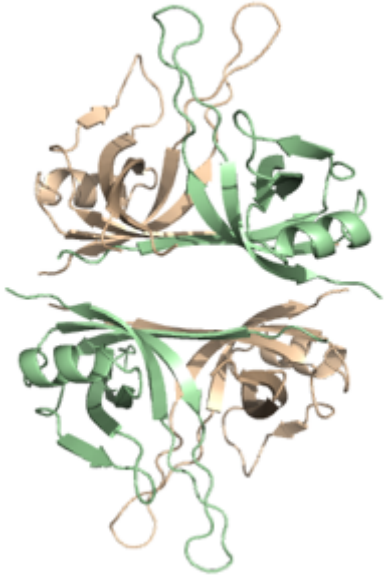
- Protein Painting & Far-western analysis
- Size-exclusion chromatography (SEC), Multi-Angle Light Scattering (MALS), & Small Angle X-ray Scattering (SAXS)

https://fineartamerica.com/featured/protein-structure-epstudio-design.html

NEIL1-DNA, PDB: 5ITX

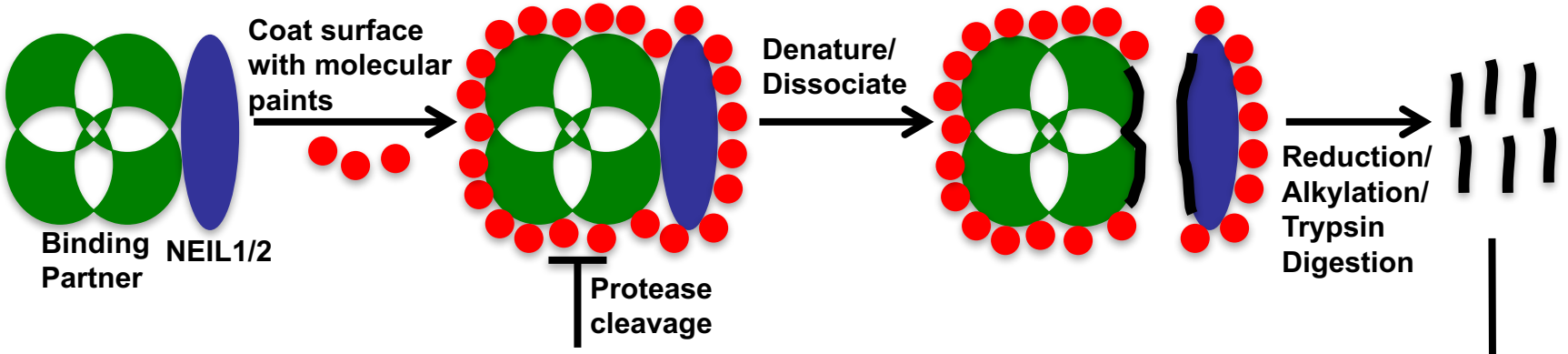


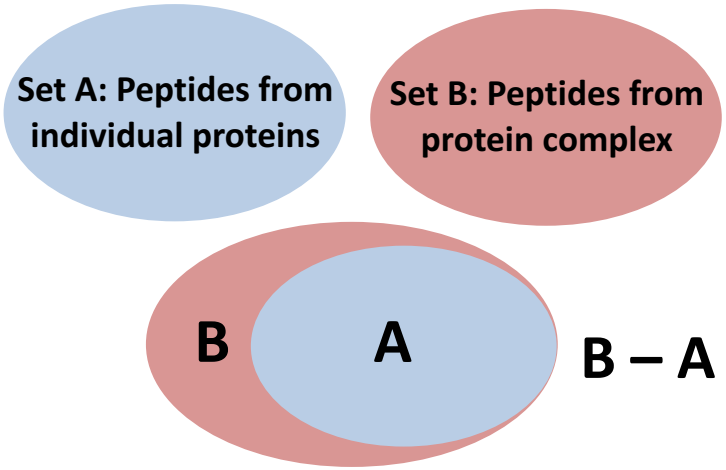
mtSSB Tetramer, PDB: 3ULL



Yang C. et. al., *Nat. Struct. Biol*. 1997
Zhu, C. et. al. *Proc.Natl.Acad.Sci.* 2016

Protein painting to determine interface contacts



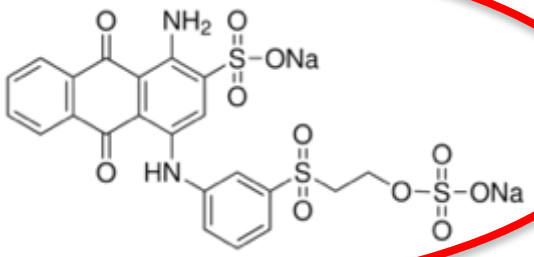


B – A = Interface contacts

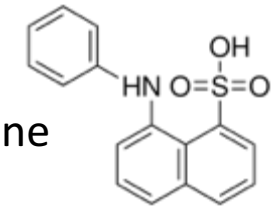
Adapted from Luchini A., et al. *Nature Communications* **5**, Article number: 4413 (2014)

Examples of molecular protein paints

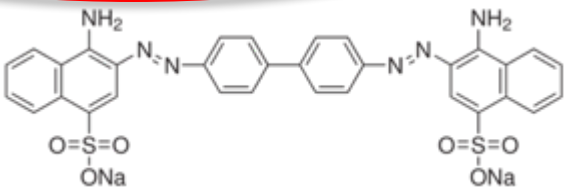
RBB,
Anthraquinone



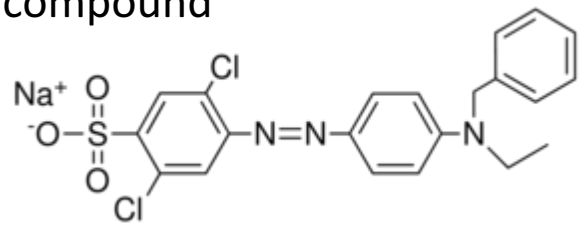
ANSA,
Naphthalene
Derivative



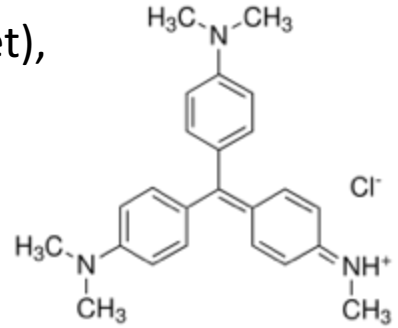
CR (Congo red),
Acryl azo
compound



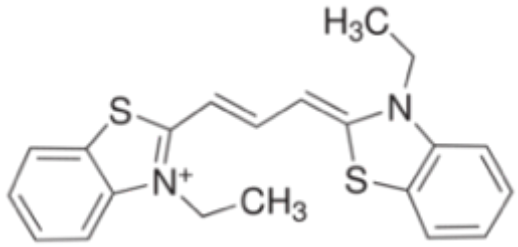
AO50 (Acid Orange 50),
Aryl azo compound



MV (Methyl violet),
Triarylmethane
compound



DECI,
Polymethine
compound



Adapted from Luchini A., et al. *Nature Communications* **5**, Article number: 4413 (2014)

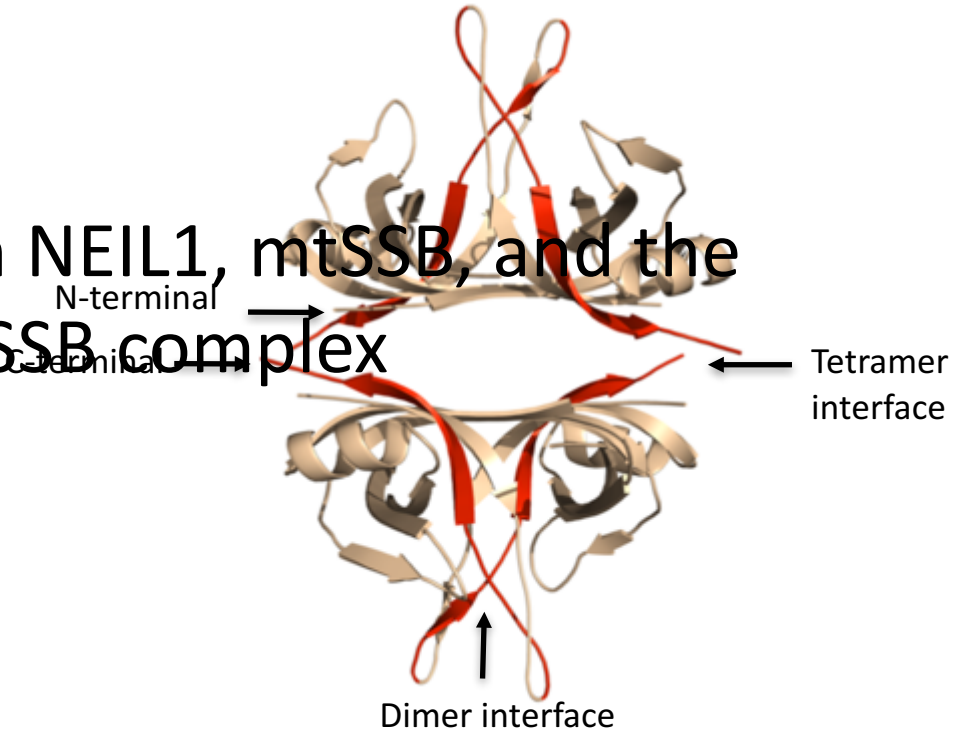
NEIL1 (Alone + RBB)

```
        10         20         30         40         50         60
MPEGPELHLA SQFVNEACRA LVFGGCVEKS SVSRNPEVPF ESSAYRISAS ARGKELRLIL

        70         80         90        100        110        120
SPLPGAQPQQ EPLALVFRFG MSGSFQLVPR EELPRHAHLR FYTAPPGPRL ALCFVDIRRF

       130        140        150        160        170        180
GRWDLGGKWQ PGRGPCVLQE YQQFRESVLR NLADKAFDRP ICEALLDQRF FNGIGNYLRA

       190        200        210        220        230        240
EILYRLKIPP FEKARSVLEA LQQHRPSPEL TLSQKIRTKL QNPDLLELCH SVPKEVVQLG

       250        260        270        280        290        300
GKGYGSESGE EDFAAFRAWL RCYGMPGMSS LQDRHGRTIW FQGDPGPLAP KGRKSRKKKS

       310        320        330        340        350        360
KATQLSPEDR VEDALPPSKA PSRTRRAKRD LPKRTATQRP EGTSLQQDPE APTVPKKGRR

       370        380        390
KGRQAASGHC RPRKVKADIP SLEPEGTSAS LE
```

SSB (Alone + RBB)

```
        10         20         30         40         50         60
ESETTTSLVL ERSLNRVHLL GRVGQDPVLR QVEGKNPVTI FSLATNEMWR SGDSEVYQLG

        70         80         90        100        110        120
DVSQKTTWHR ISVFRPGLRD VAYQYVKKGS RIYLEGKIDY GEYMDKNNVR RQATTIIADN

       130
IIFLSDQTKE KE
```

Protein Painting with NEIL1, mtSSB, and the NEIL1-mtSSB complex



mtSSB Structure (PDB ID: 3ULL)

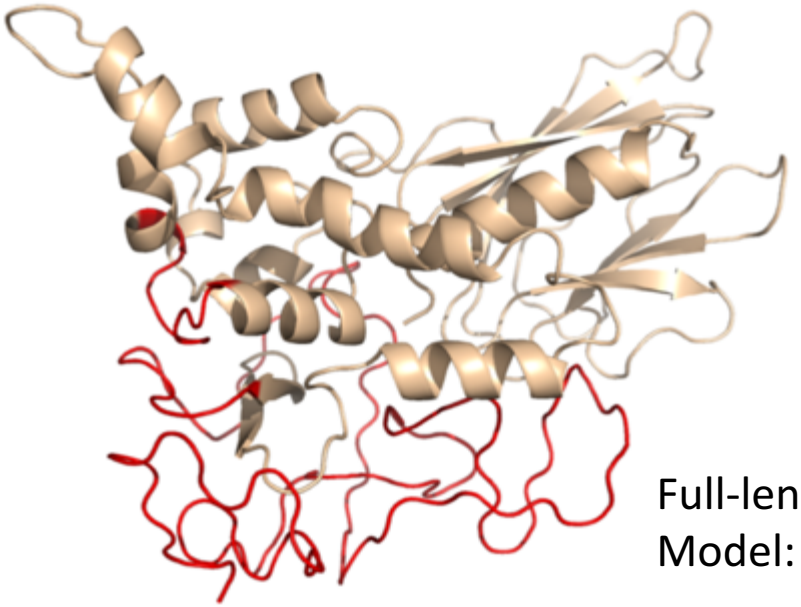
Protein painting with the NEIL1-mtSSB complex

NEIL1 (NEIL1-mtSSB Complex + RBB)

```
        10         20         30         40         50         60
MPEGPELHLA SQFVNEACRA LVFGGCVEKS SVSRNPEVPF ESSAYRISAS ARGKELRLIL

        70         80         90        100        110        120
SPLPGAQPQQ EPLALVFRFG MSGSFQLVPR EELPRHAHLR FYTAPPGPRL ALCFVDIRRF

       130        140        150        160        170        180
GRWDLGGKWQ PGRGPCVLQE YQQFRESVLR NLADKAFDRP ICEALLDQRF FNGIGNYLRA

       190        200        210        220        230        240
EILYRLKIPP FEKARSVLEA LQQHRPSPEL TLSQKIRTKL QNPDLLELCH SVPKEVVQLG

       250        260        270        280        290        300
GKGYGSESGE EDFAAFRAWL RCYGMPGMSS LQDRHGRTIW FQGDPGPLAP KGRKSRKKKS

       310        320        330        340        350        360
KATQLSPEDR VEDALPPSKA PSRTRRAKRD LPKRTATQRP EGTSLQQDPE APTVPKKGRR

       370        380        390
KGRQAASGHC RPRKVKADIP SLEPEGTSAS LE
```

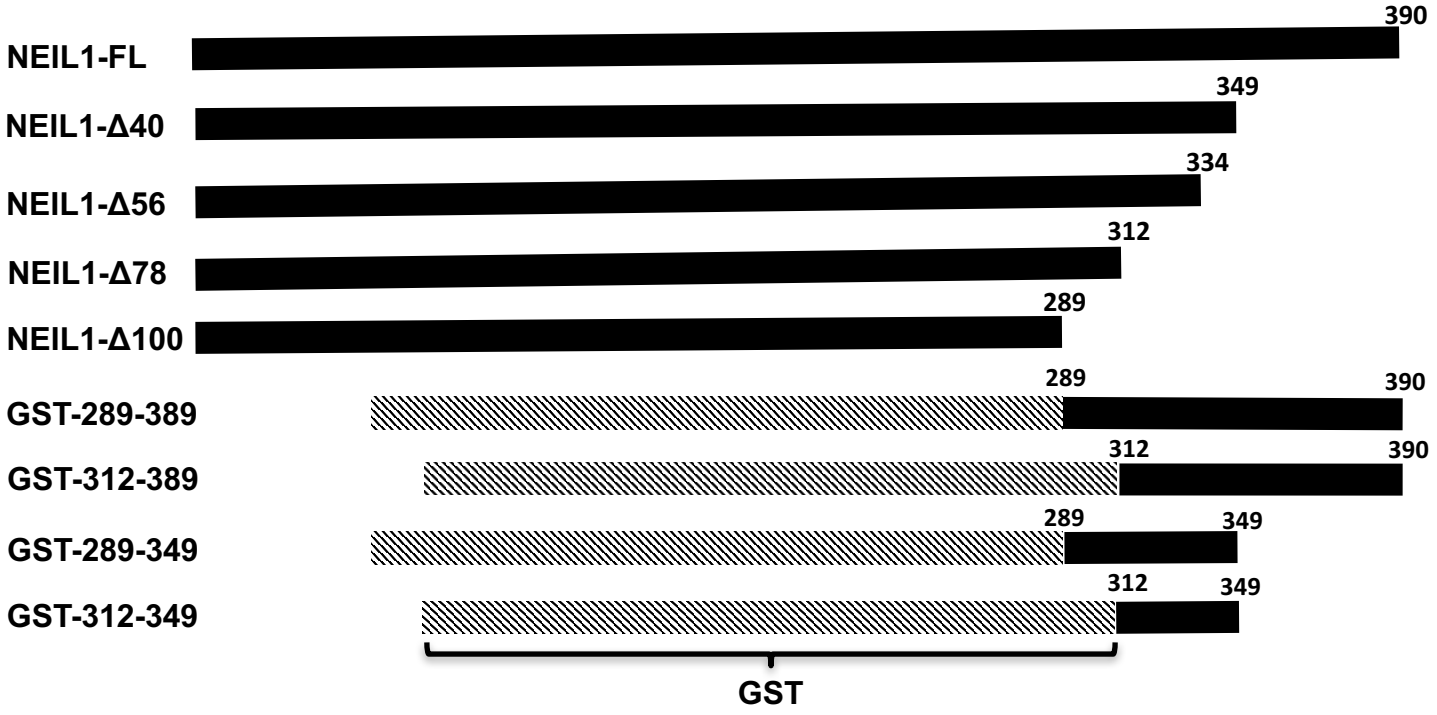
SSB (NEIL1-mtSSB Complex + RBB)

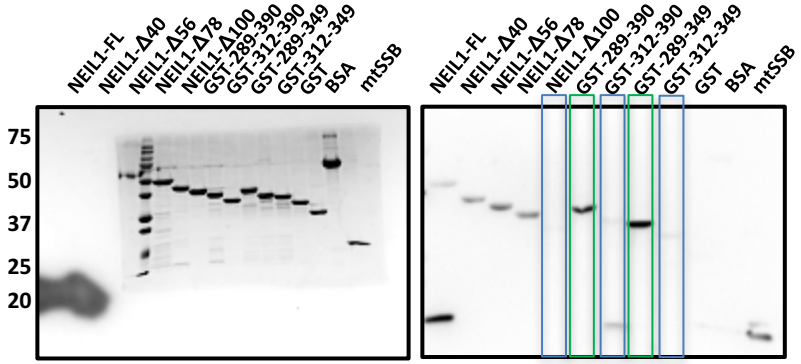
```
        10         20         30         40         50         60
ESETTTSLVL ERSLNRVHLL GRVGQDPVLR QVEGKNPVTI FSLATNEMWR SGDSEVYQLG

        70         80         90        100        110        120
DVSQKTTWHR ISVFRPGLRD VAYQYVKKGS RIYLEGKIDY GEYMDKNNVR RQATTIIADN

       130
IIFLSDQTKE KE
```



Full-length NEIL1
Model: RaptorX

NEIL1 residues 289 – 312 are required for an interaction with mtSSB





Coomassie stain

Far Western

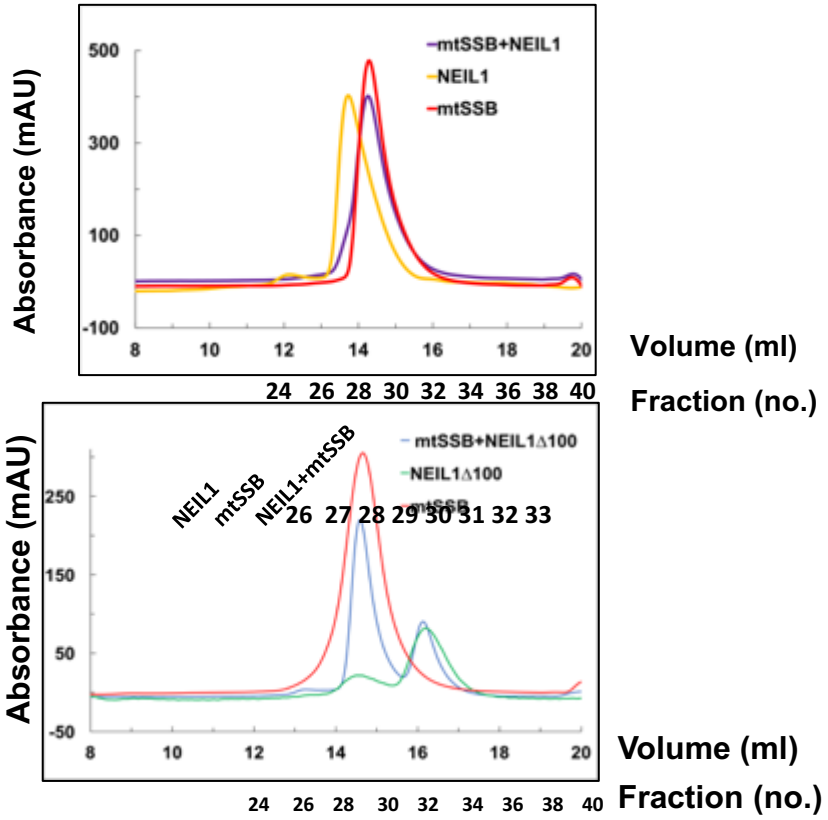
Sharma N. et al. *DNA Repair*. 2018

NEIL1:mtSSB complex formation via SEC

- Size exclusion chromatography (SEC) separates molecules based on molecular weight and shape or Stokes radius.
- Molecules with bigger Stokes radii elute first

✓ Complex formation between NEIL1-mtSSB in absence of DNA was confirmed as the molecule is eluted as single peak.

✓ We noted a smaller Stokes radius for the NEIL1-mtSSB complex.



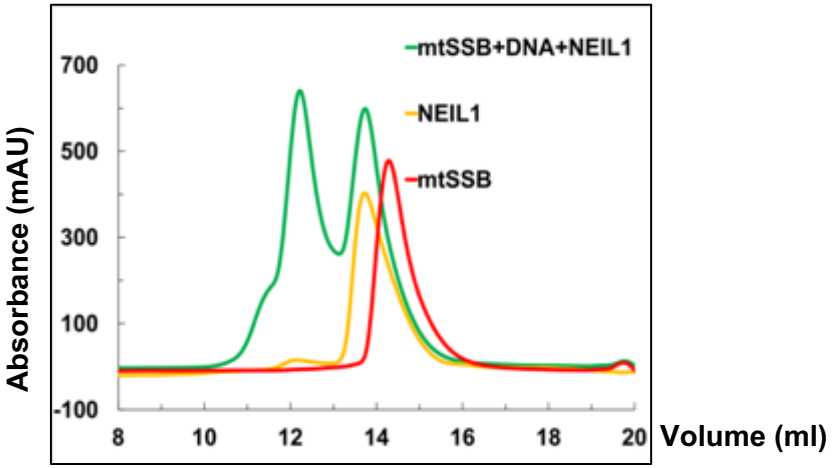
✓ Absence of the NEIL1 C-terminal 100 residues abolishes the interaction with mtSSB

Protein/complex	Theoretical molecular weight (kDa)	Elution Volume (ml)	Stokes radius (Å)
mtSSB	60.78	14.29	39.2
NEIL1	44.75	13.73	42.2
mtSSB-NEIL1	105	14.26	39.4

Sharma et. al. *DNA Repair*, 2018

Complex formation of NEIL1 and mtSSB in the presence of DNA

TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTAGACCTGGACG
CATCTGXACCTGC



Protein/complex	Theoretical molecular weight (kDa)	Elution Volume (ml)	Stokes radius (Å)
mtSSB	60.78	14.29	39.2
NEIL1	44.75	13.73	42.2
mtSSB-NEIL1-DNA	128	12.22	50.3

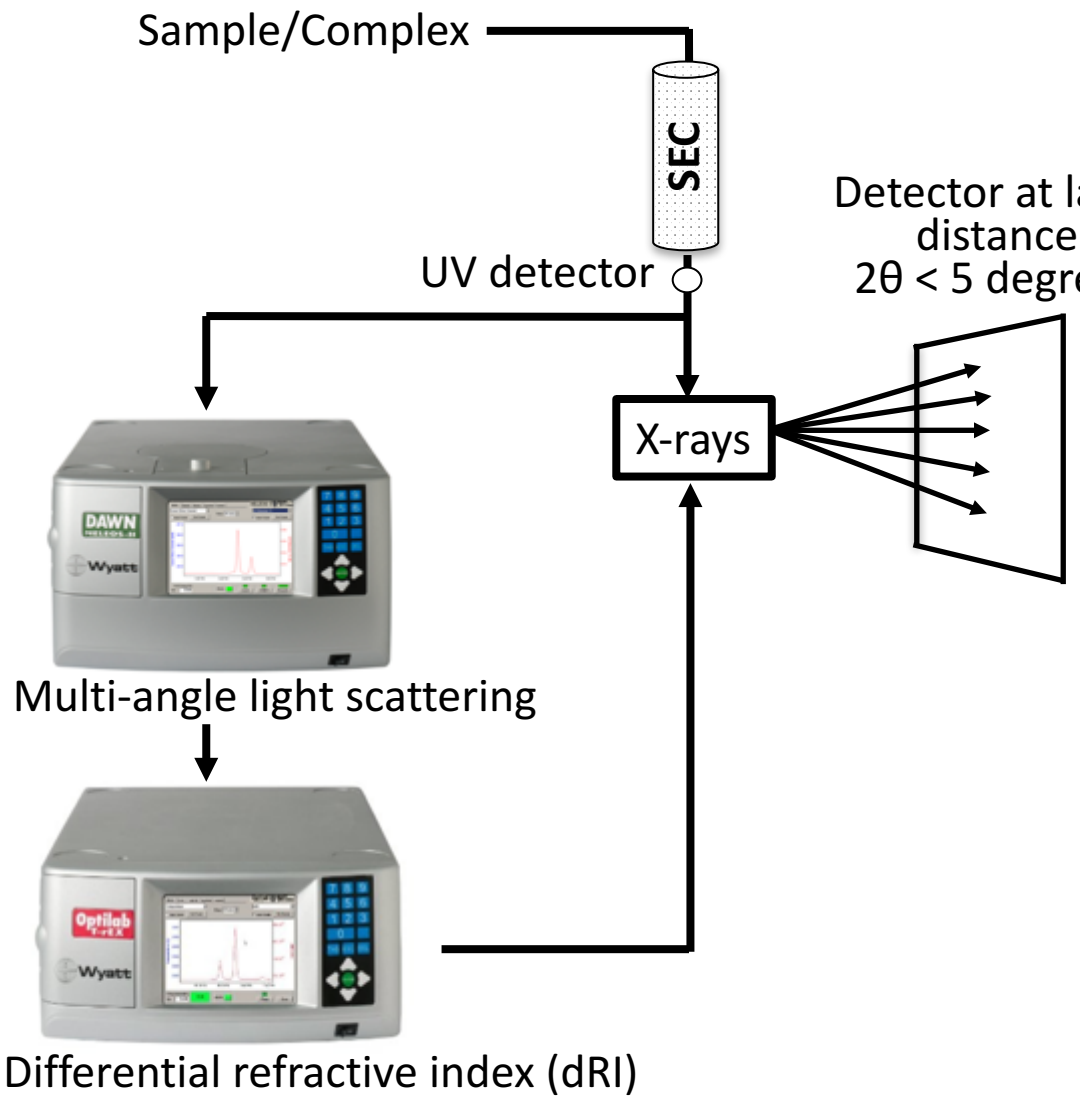
- ✓ Size exclusion chromatography indicated the formation of a larger ternary complex (NEIL1-DNA-mtSSB) in presence of DNA

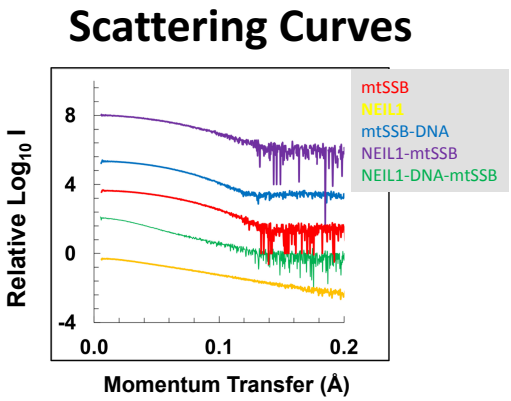
Sharma et. al. *DNA Repair*, 2018

Information obtained from SEC-MALS-SAXS

- Absolute molar mass and stoichiometry (MALS)
- Maximum particle dimension (Dmax; SAXS)
- Size and shape of molecule – P(r) function (SEC & SAXS)
- Flexibility/disorder (Kratky plot; SAXS)
- Estimation of molecular weight (MALS and SAXS)
- Oligomerization state and organization in solution (MALS & SAXS)
- Low resolution molecular envelopes (Blob-o-logy; SAXS)

SEC-SAXS/SEC-MALS-SAXS setup



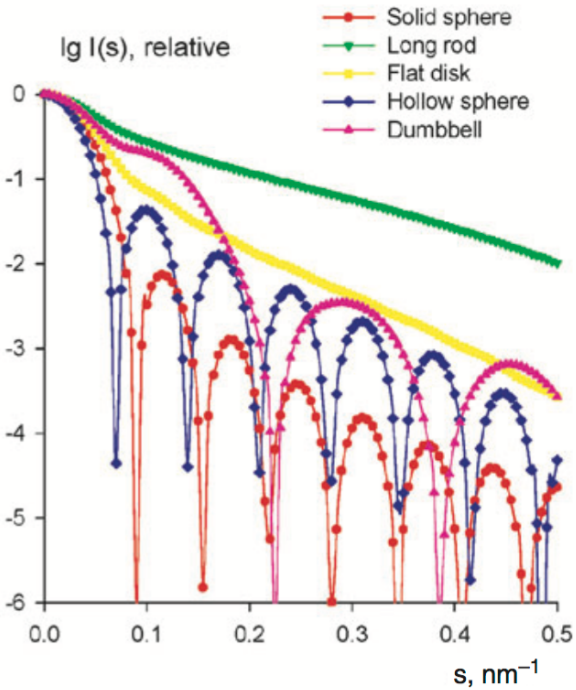


- **ATSAS Package/Scatter**
- **Data analysis- I_0, R_g, D_{max} , MW**
- **Ab-initio shape modeling**

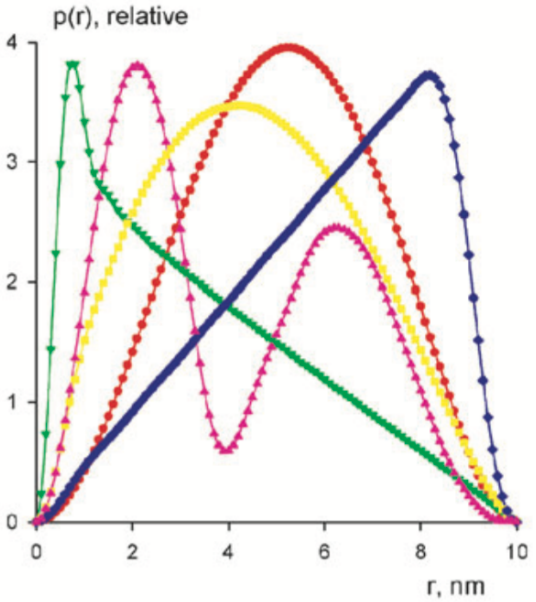
Srinivas Chakravarthy, PhD
BioCAT, Argonne National Labs

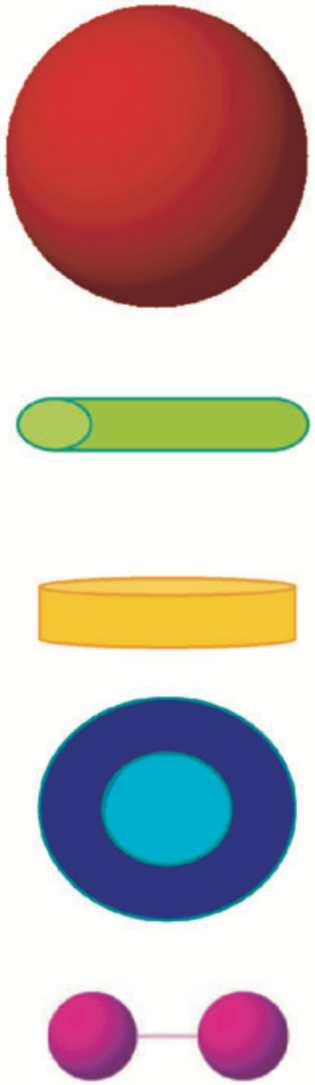
Shape I nalysis

2D Scattering Curves



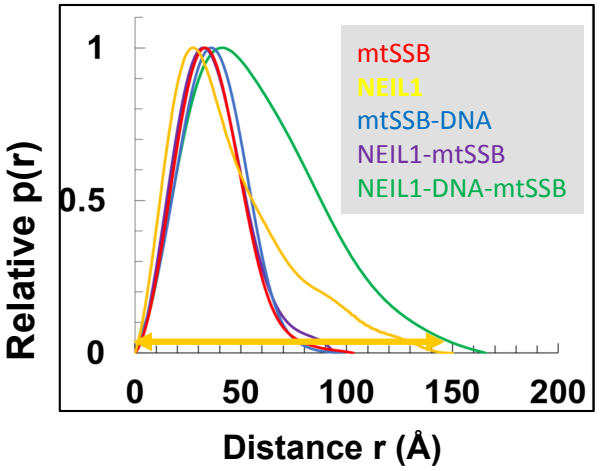
Distribution of inter-atomic distances, p(r)



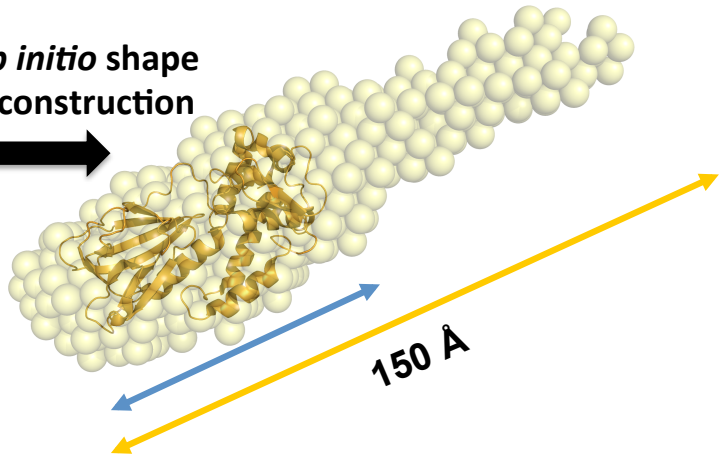


Svergun DI, Koch MHJ. Rep. Prog. Phys. **66** (2003) 1735–1782

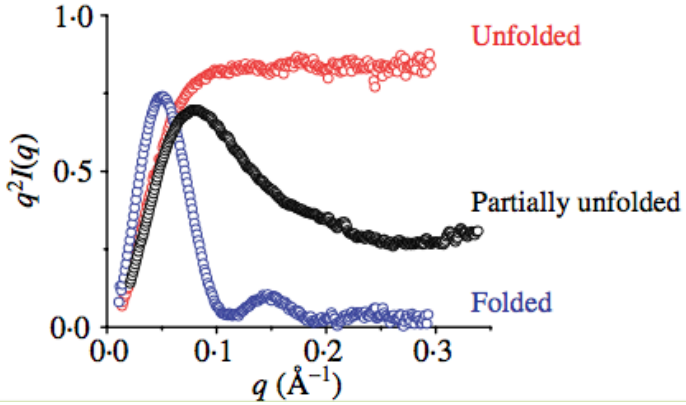
SEC-SAXS analysis for multimeric complexes



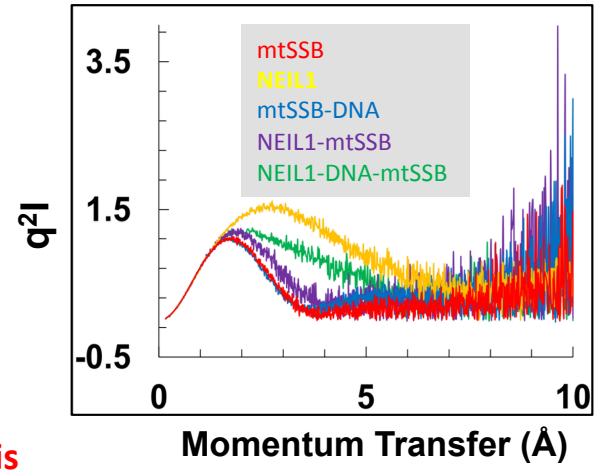
***Ab initio* shape reconstruction**



Pair-wise Distance Distribution
GNOM analysis



Kratky Analysis



Sharma et. al. *DNA Repair*, 2018
http://www.bioisis.net/tutorial/6

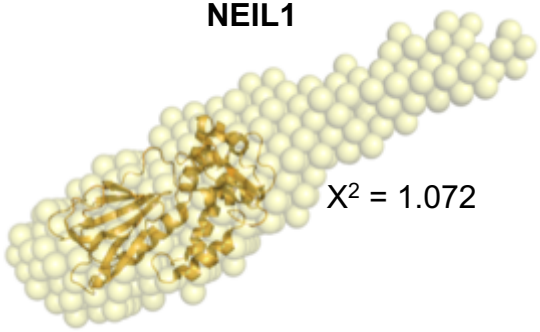
Summary of SAXS and MALS data for NEIL1, mtSSB, and complexes

	mtSSB (tetramer)	*NEIL1*	*mtSSB-DNA complex*	*NEIL1-mtSSB complex*	*NEIL1-DNA-mtSSB complex*
Structural parameters					
R_g (Å) from P(r)	27.52	37.15	28.18	28.12	46.86
R_g from Guinier	27.44±1.21	33.04±2.46	28.40±0.86	28.48±0.80	44.83±1.80
Dmax (Å)	103.21	149.96	98.74	94.32	165.72
Molecular weight determination (kDa)					
Theoretical	60.78	44.72	78	105.5	123
MW(V_p)	61	46	79	60.3	146.6
MW(V_c)	59	36	71	55.4	134.3
MW (MALS)	59.8±1.67%	48.5±2.23%	69±1.46%	58±1.54%	129.6±1.54%

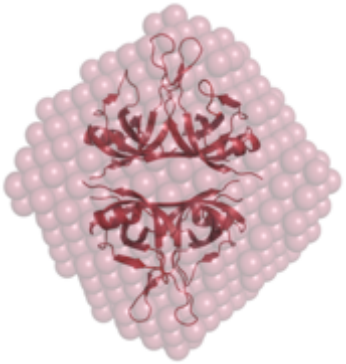
Sharma et. al. *DNA Repair*, 2018

Ab initio shape reconstruction of NEIL1, mtSSB, & complexes

NEIL1

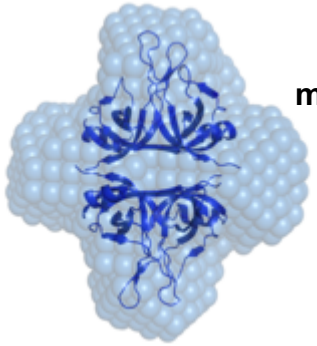


$X^2 = 1.072$



mtSSB

$X^2 = 1.041$



mtSSB-DNA

$X^2 = 0.945$

Sharma et. al. *DNA Repair*, 2018

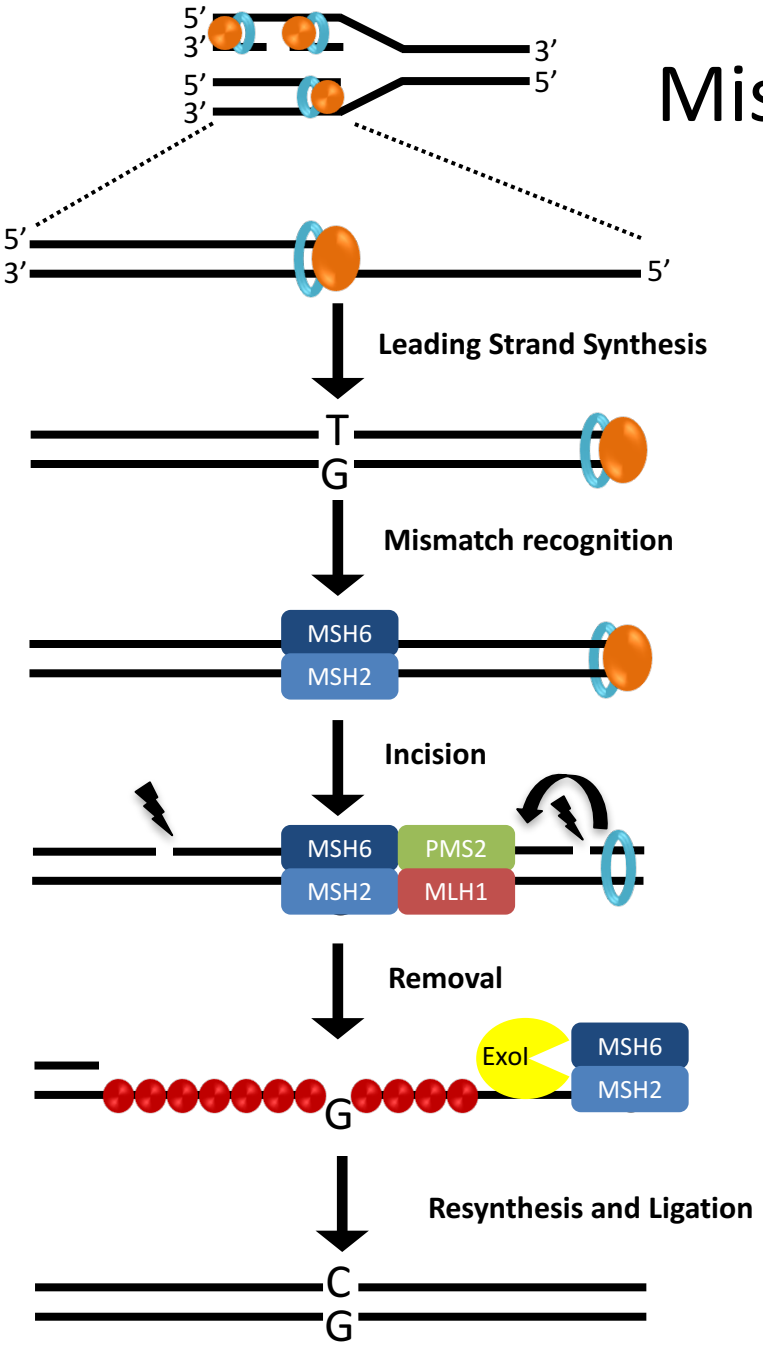
Conclusions and Significance

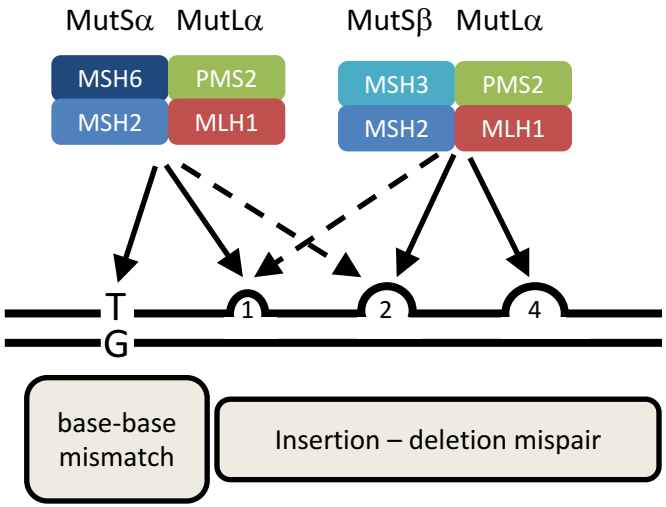
- NEIL1 interacts with mtSSB via its disordered C-terminal tail (protein painting, far western, SEC-MALS)
- Absolute molar mass values obtained via MALS indicate that NEIL1 interacts with a monomer of mtSSB
- The NEIL enzymes may have acquired specific functions during cellular processes such as replication and transcription
  - NEIL1-mediated disruption of replication proteins indicates a potential mechanistic switch between DNA replication and repair

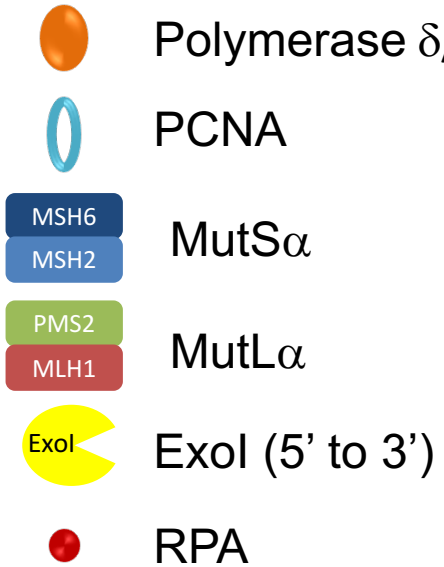
Outline

- **PART I: Specialized functions of the NEIL1 DNA Glycosylase**
  - BER & NEIL1
  - The interaction between NEIL1 and mitochondrial SSB, *in vitro*
  - Concluding remarks
- **PART II: (Re)Classification of Variants of Uncertain Significance in Lynch syndrome patients**
  - Identification of Variants of Uncertain Significance
  - Functional Characterization
  - Concluding remarks

Mismatch Repair (MMR) Overview







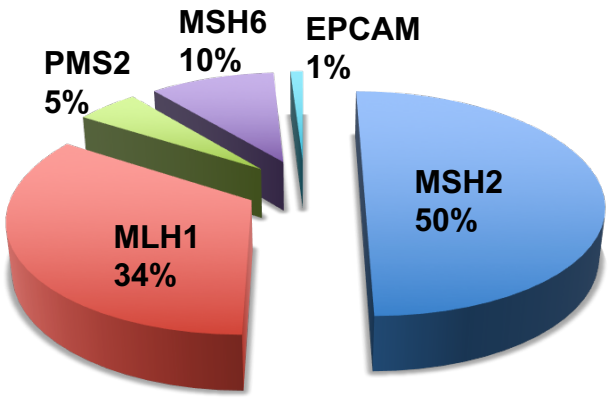
Brandon D’Arcy, Adapted from Hsieh P et al., 2017, and http://erielab.web.unc.edu/research/

Lynch syndrome and cancer

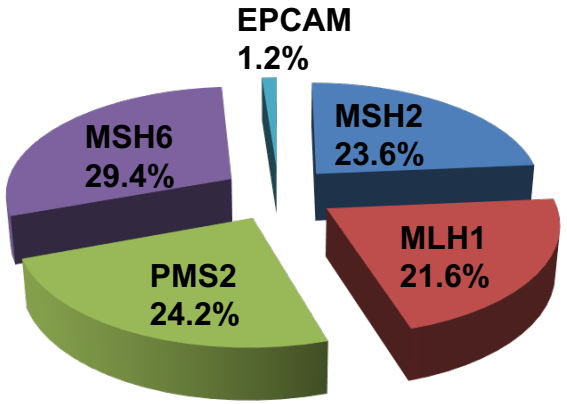
Heterozygous mutations in 1 of the 4 main MMR genes (MSH2, MSH6, MLH1, and PMS2) and EPCAM causes Lynch syndrome (LS).

LS is an autosomal dominant hereditary syndrome where individuals have a very high lifetime risk of developing CRC, endometrial cancer, ovarian, gastric, and others.

LS is one of the most common cancer predispositions, representing 2-7% of colorectal cancer cases in the US.



LS incidence based on meeting Amsterdam or Bethesda criteria



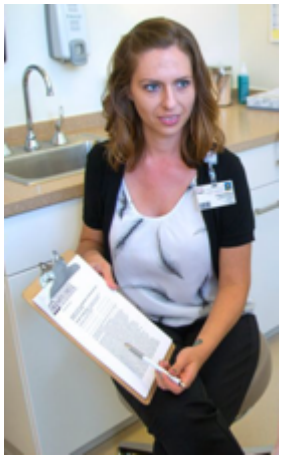
LS incidence using multigene panel testing regardless of family history

Blount and Prakash, *Clinical Genetics*, 2017

Variants of uncertain significance

- 20-30% of missense mutations identified in MMR genes are unclassified and are called VUSs

- A VUS result complicates the medical management of patients



Jessa Blount
(Genetic Counselor)

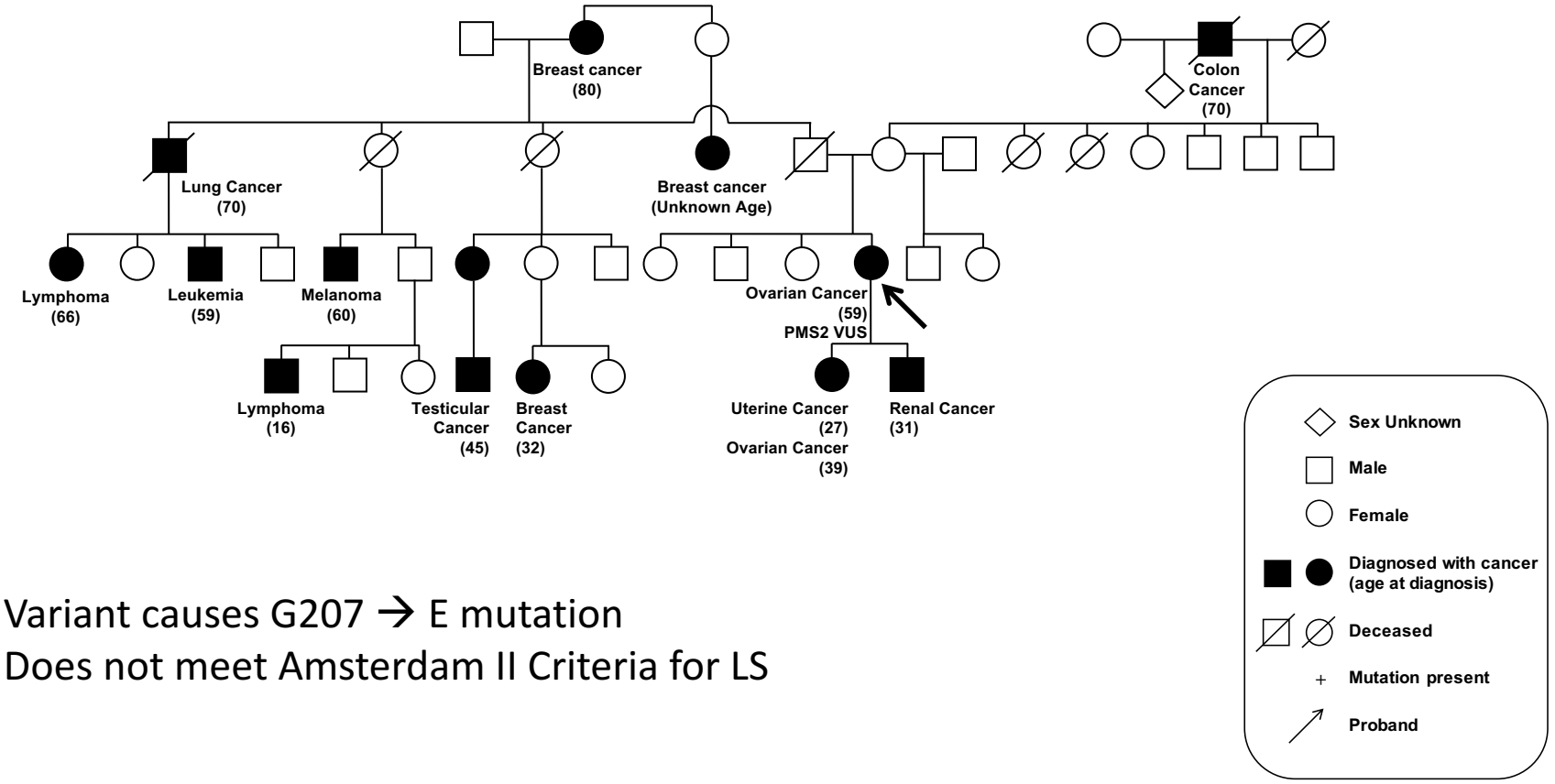
Brandon D'Arcy
(Post-doc)

Goal: Help with the assignment of a VUS as pathogenic or benign to assist with proper medical management.

Focus: PMS2. Of the 4 MMR genes, PMS2 has the highest incidence of VUSs.

- For this talk, we will focus on 2 PMS2 variants

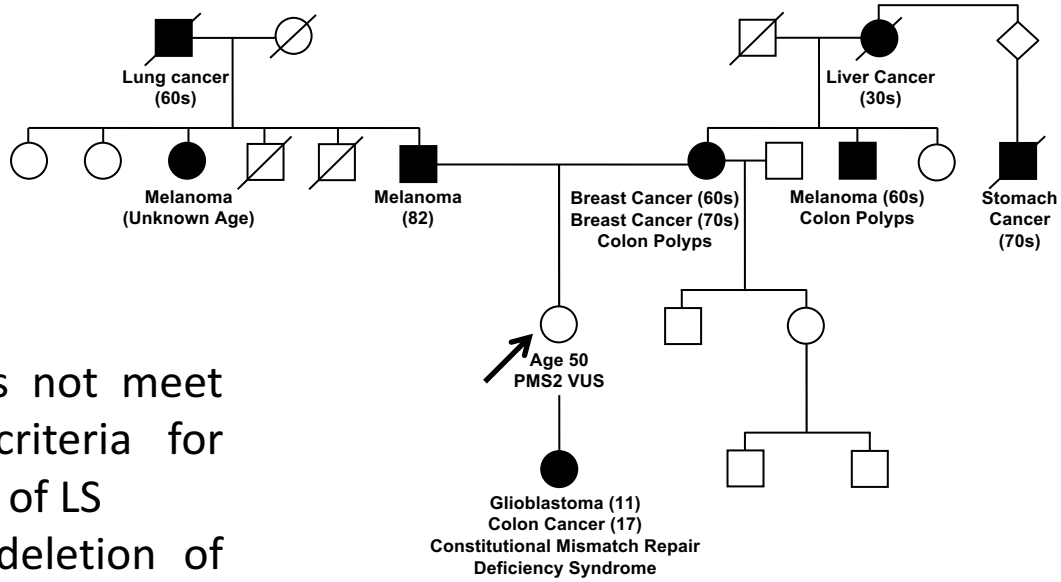
PMS2 Variant c.620G>A Family Pedigree



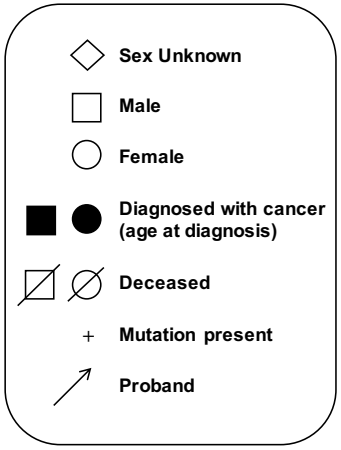
- Variant causes G207 → E mutation
- Does not meet Amsterdam II Criteria for LS

D'Arcy *et al.*, *Human Mutation*, 2019

PMS2 Variant c.123_131delGTTAGTAGA Family Pedigree

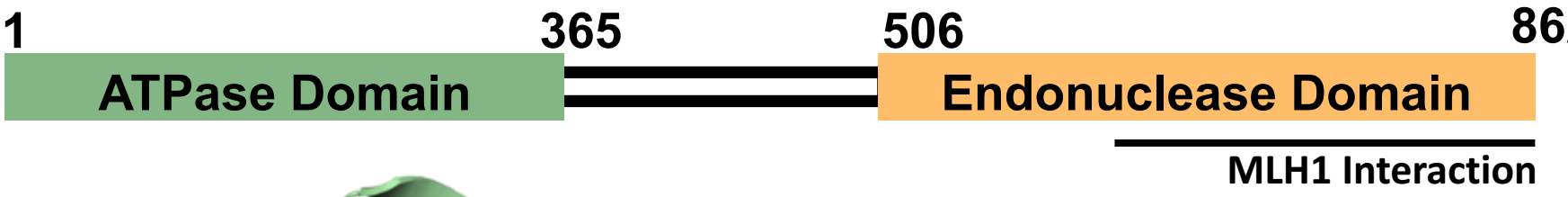


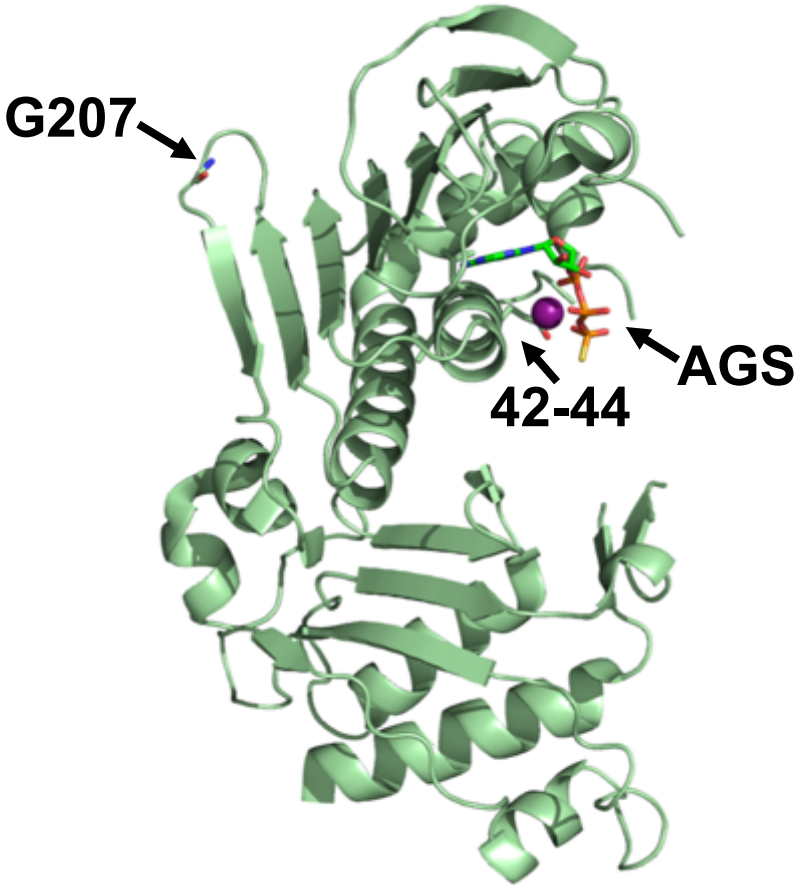
- This family does not meet Amsterdam II criteria for clinical diagnosis of LS
- Variant causes deletion of amino acids 42-44 (Δ42-44)
- CMMRD – rare autosomal recessive condition

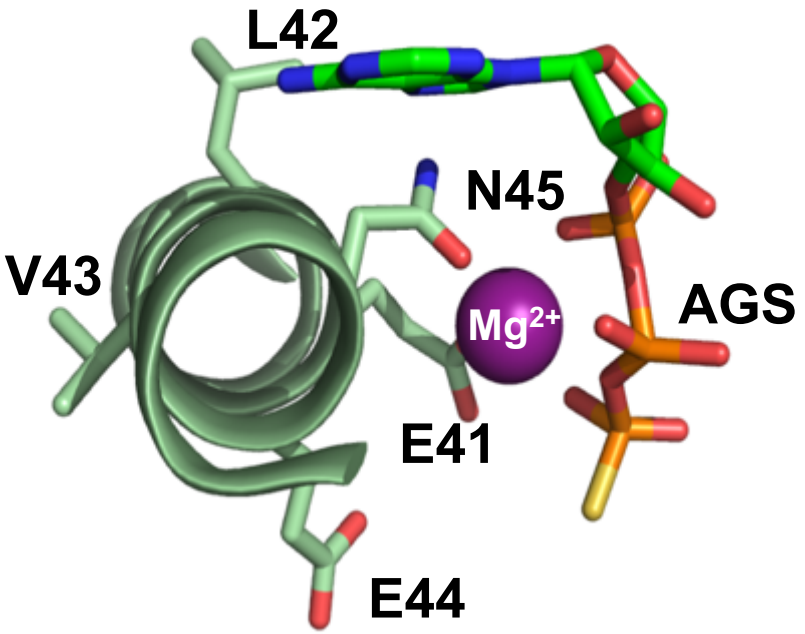


D'Arcy *et al.*, *Human Mutation*, 2019

Location of the PMS2 variants

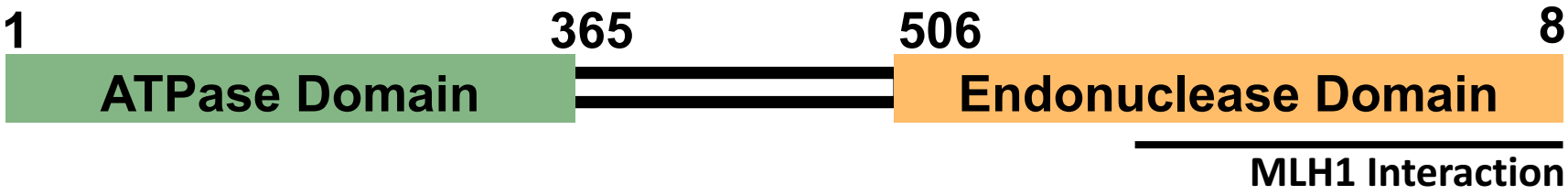


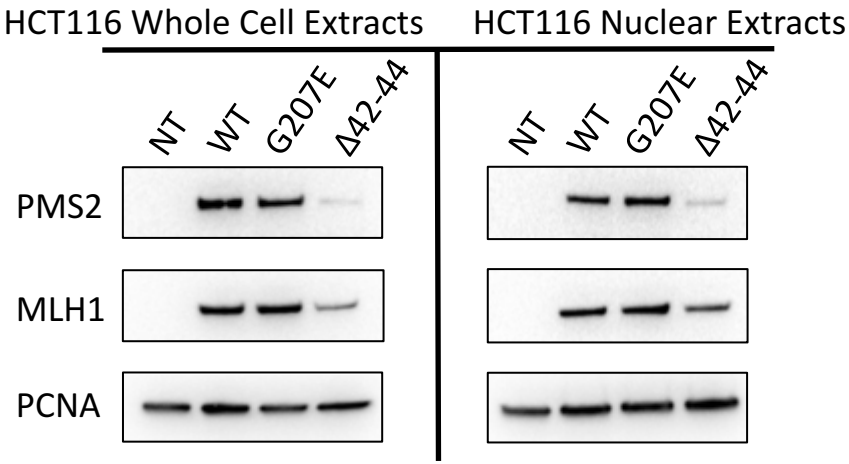


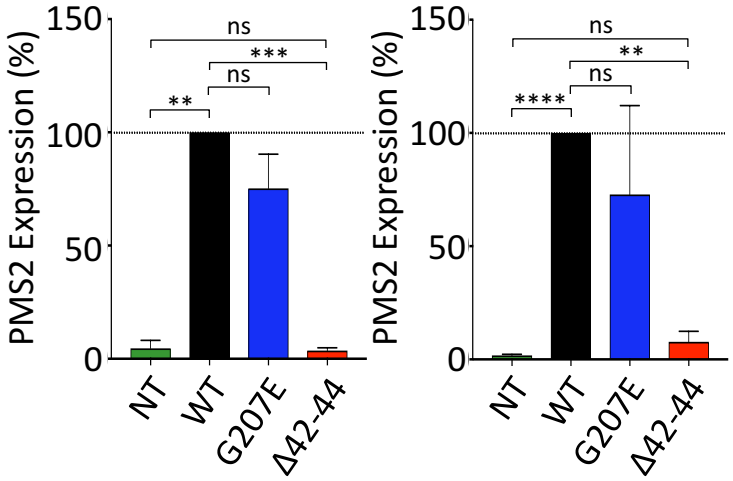


PDB ID: 1H7U, Guarne, A. *et al.*, 2001, Embo J

The Δ42-44 variant is unstable in cells

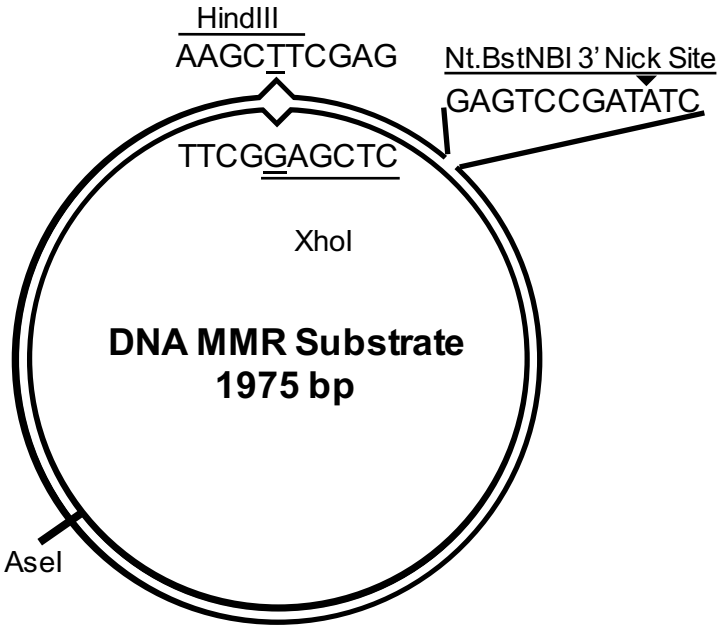


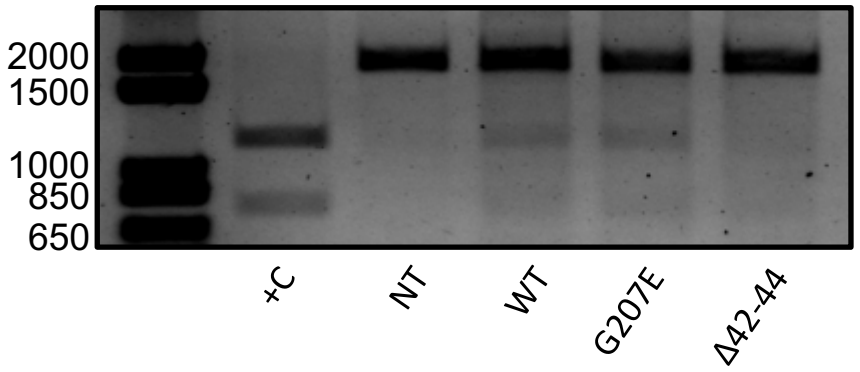


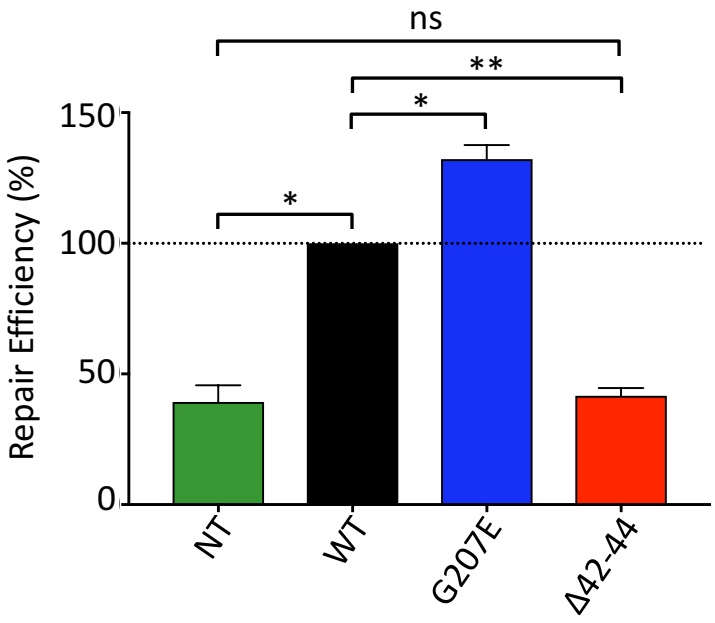


D'Arcy *et al.*, *Human Mutation*, 2019

The Δ42-44 variant is deficient in MMR

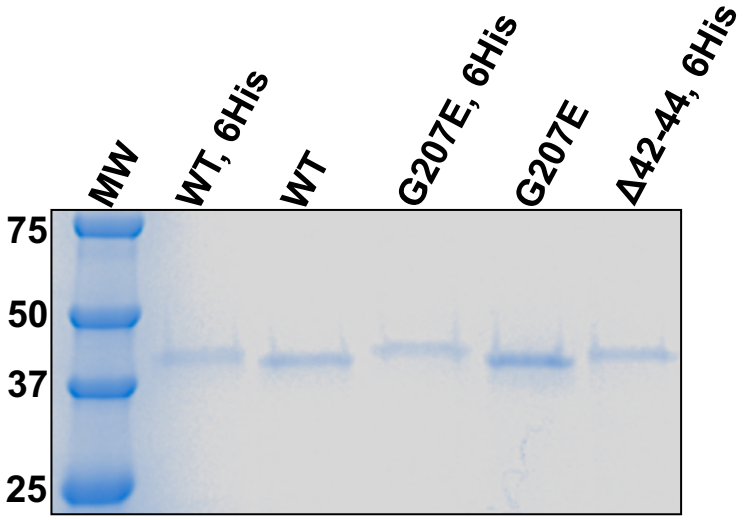






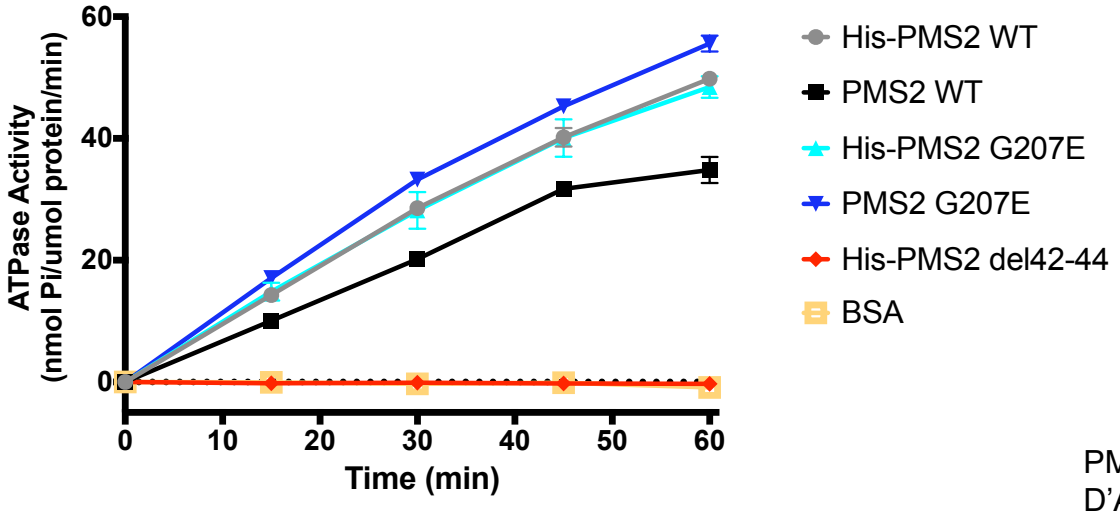
D'Arcy *et al.*, *Human Mutation*, 2019

PMS2 variants: purification and activity



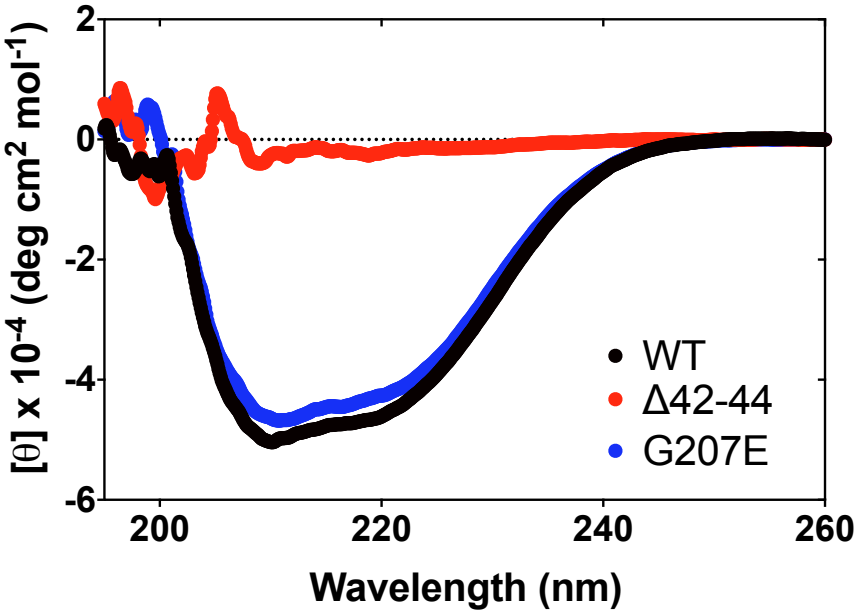
Coomassie Stained SDS-PAGE gel

- Δ42-44 was insoluble and found in the pellet
- Multiple attempts to purify this variant included different tags and refolding procedures



PMS2 ATPase domain construct, Wei Yang
D'Arcy *et al.*, *Human Mutation*, 2019

The G207E variant is properly folded whereas the Δ42-44 lacks alpha helical elements



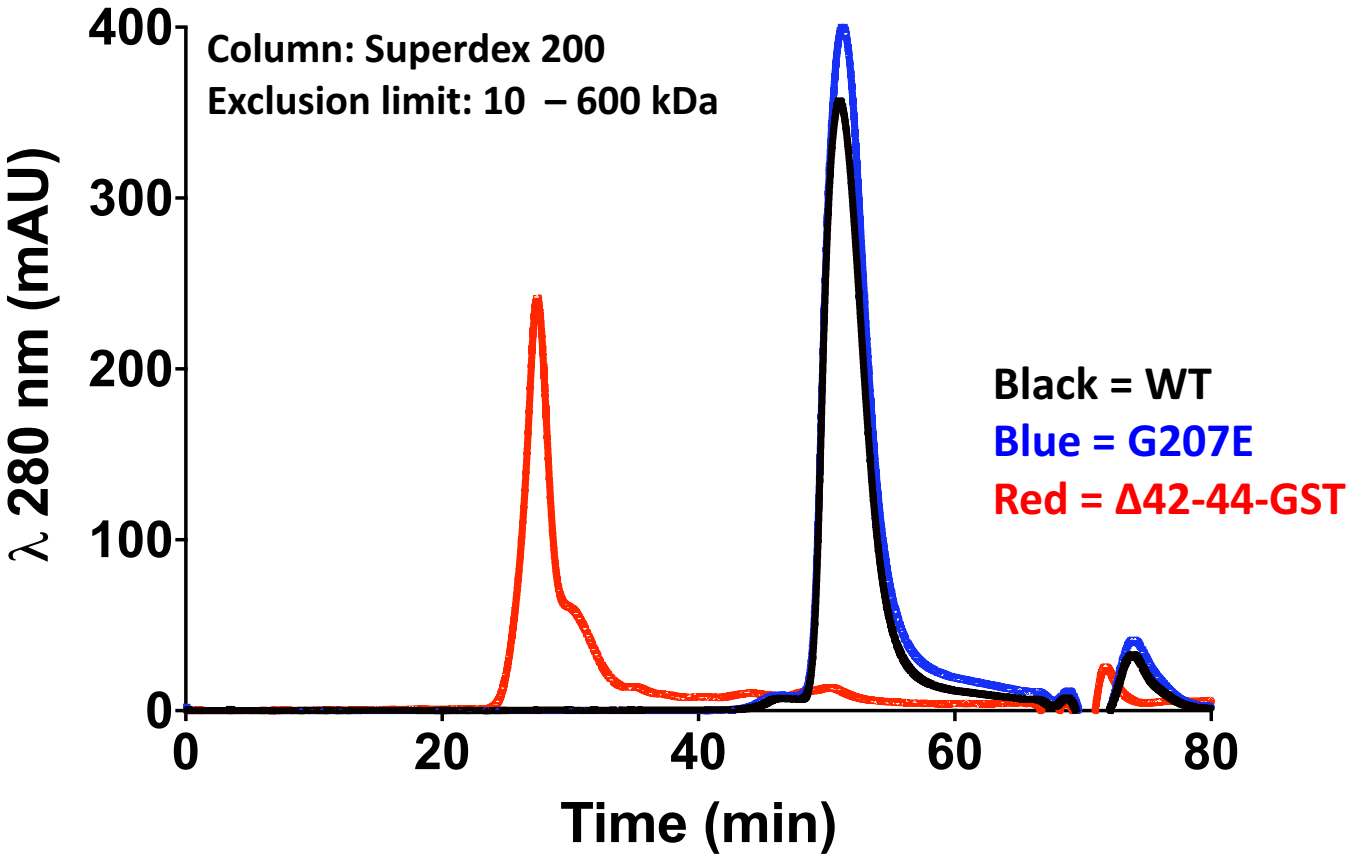
Enzyme	Helix 1	Helix 2	Strand 1	Strand 2	Turns	Unordered
WT	40%	8%	15%	11%	8%	18%
G207E	43%	10%	12%	9%	7%	18%
Δ42-44	0.2%	2.3%	11.3%	24.7%	17.6%	43.8%

Vijay Rangachari, USM
D'Arcy *et al.*, *Human Mutation*, 2019

Δ42-44 is likely an aggregated protein

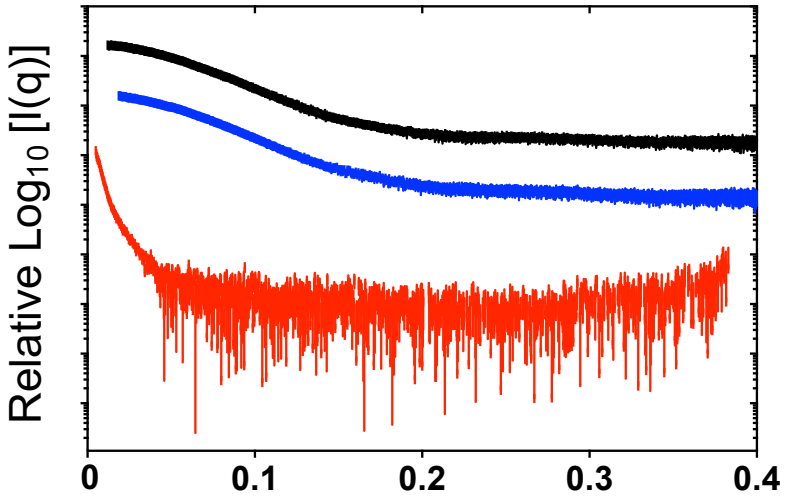
1 365

ATPase Domain ~40 kDa

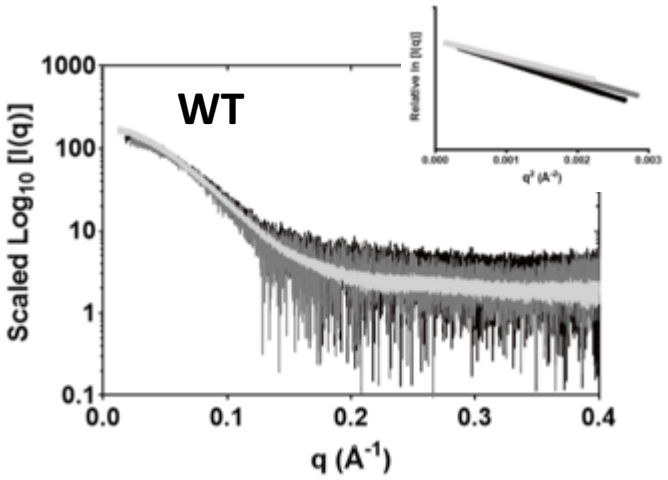


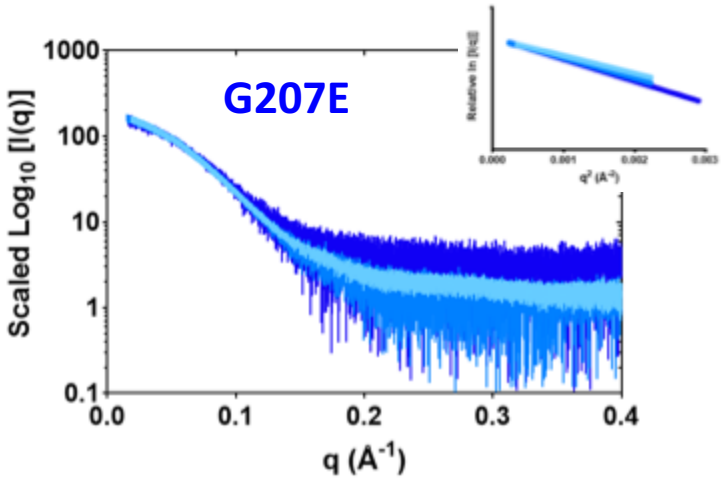
D'Arcy *et al.*, *Human Mutation*, 2019

SAXS data reveal that the Δ42-44 variant is aggregated



Black = WT
Blue = G207E
Red = Δ42-44-GST

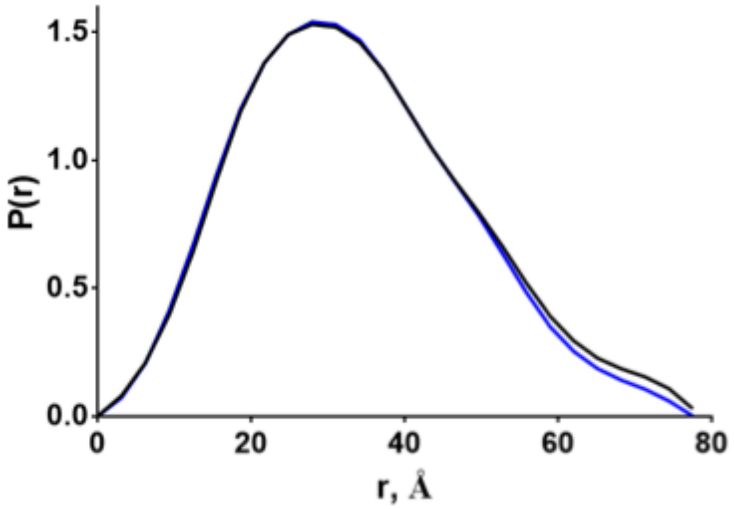




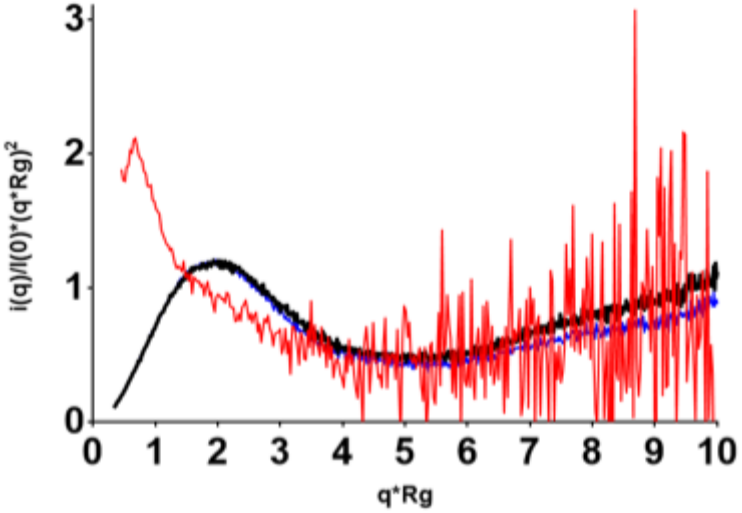
D'Arcy *et al.*, *Human Mutation*, 2019

SAXS data reveal that WT and G207E have similar properties in solution

Distance distribution function



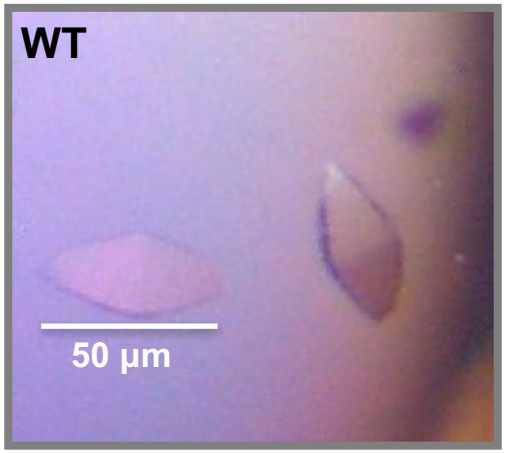
Kratky analysis

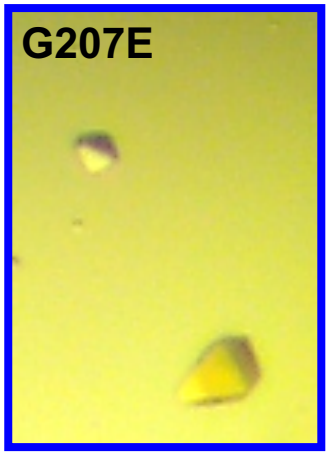


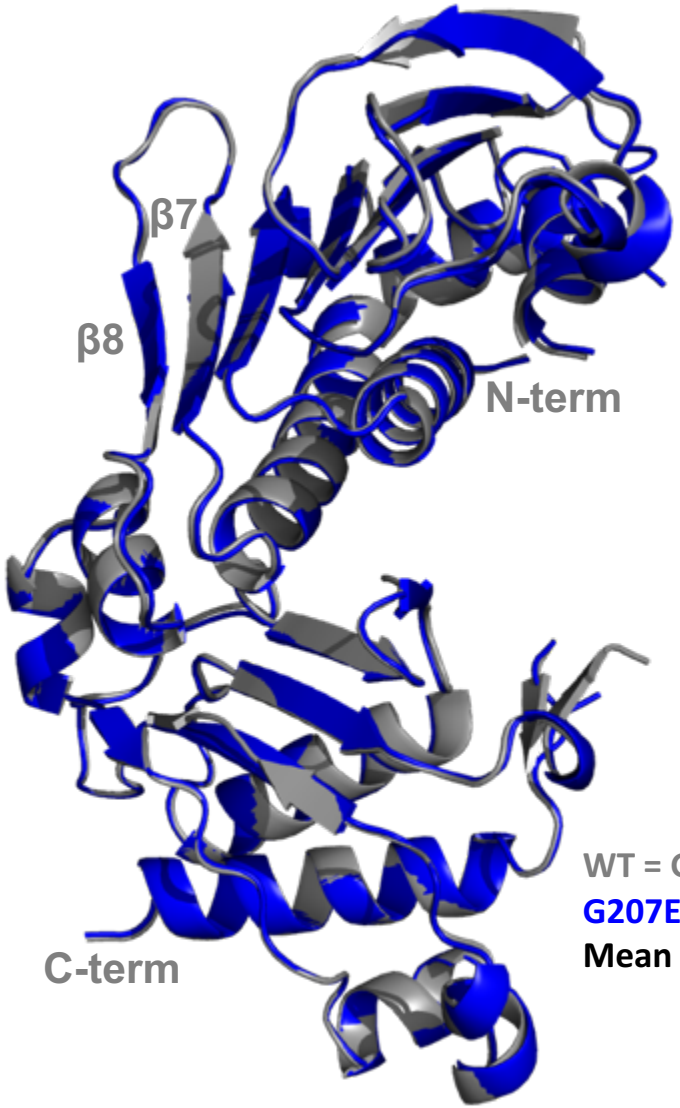
Black = WT
Blue = G207E
Red = Δ42-44

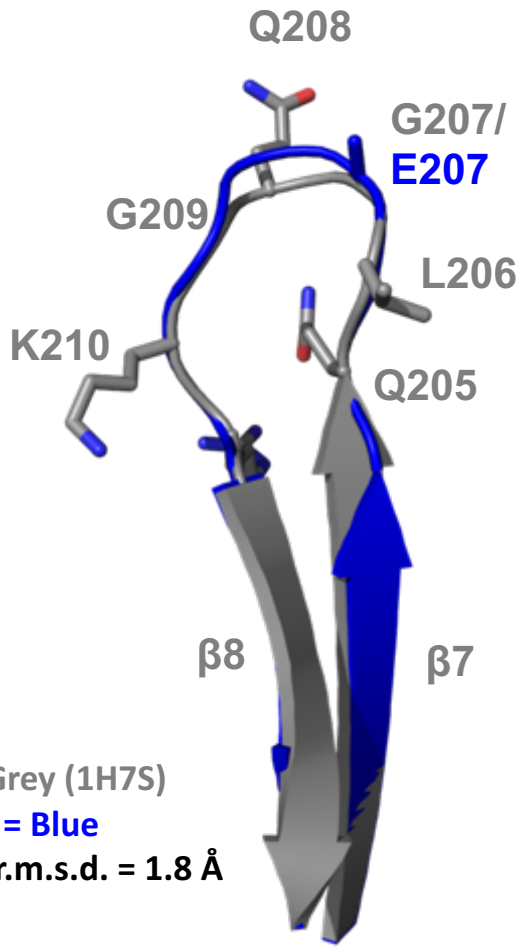
D'Arcy *et al.*, *Human Mutation*, 2019

Crystal structure of G207E









WT = Grey (1H7S)
G207E = Blue
Mean r.m.s.d. = 1.8 Å

G207E Structure
Twinned (k, h, -l)
SG: $P2_12_12_1$ (Orthorhombic)
Resolution: 2.6 Å (R-work = 23.06 %, R-free = 24.24%)

D'Arcy *et al.*, *Human Mutation*, 2019

Summary and Checklist

Goal of the study: Reclassify variants of uncertain significance as pathogenic or benign based on impact on protein function

Δ42-44 variant is:

- Unstable in cells and is MMR deficient
- An inactive ATPase
- An aggregated protein and lacks key secondary structure elements

The G207E is:

- MMR proficient
- An active ATPase
- Well folded and functions like WT enzyme

Summary and Checklist

Variant	American College of Medical Genetics and Genomics (ACMG) Standards & Guidelines		Pathogenicity
Δ42-44	Criteria	Implication	Likely Pathogenic (1 strong and 2 moderate criteria)
	PS3	Well-established *in vitro* or *in vivo* studies to support damaging effect	
	PM1	Located in a mutational hot spot and/or critical and well-established functional domain (active site)	
	PM2	Absent from controls (or at extremely low frequency in recessive)	
	PM3	For a recessive disorders, detected in *trans* with a pathogenic variant	
	PM4	Protein length changes as a result of deletion	
	PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product	
G207E	BS3	Well-established *in vitro* or *in vivo* studies show no damaging effect on protein function	Likely Benign (1 strong and 1 supporting criteria)
	BP4	Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, etc.)	

In the future:

- **We hope to study and assist with the classification of variants to help with medical management**
- **Develop high-throughput assays to assess pathogenicity**

D'Arcy *et al.*, *Human Mutation*, 2019

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Vijay Rangachari (USM)

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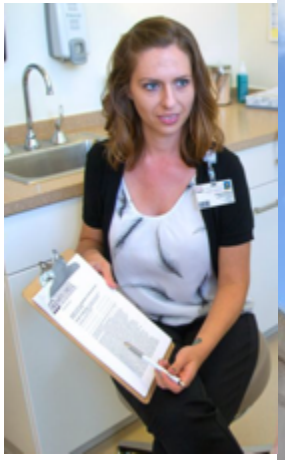
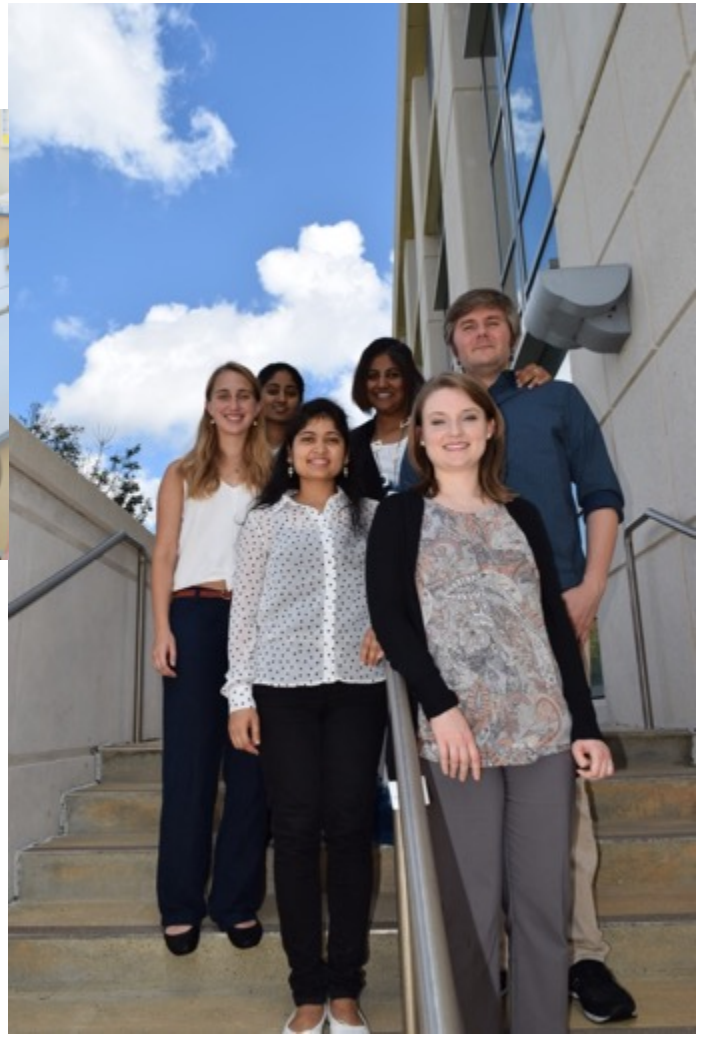
Advanced Photon Source: BioCAT

- Srinivas Chakravarthy, Ph.D.

Advanced Light Source: SIBYLS

- Susan Tsukatawa, Ph.D.





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Thank you & Questions