Exploring RAD51 Structural Plasticity Through Molecular Simulations and Integrative Structural Biology

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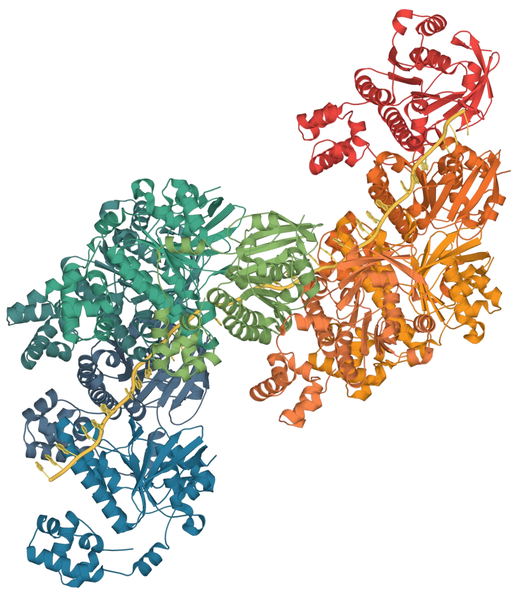
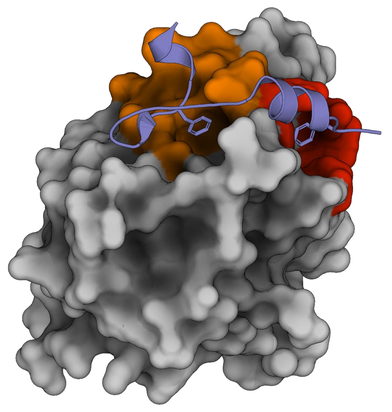
RAD51 is the central recombinase of **homologous recombination** (HR), assembling into **nucleoprotein** filaments that drive homology search and strand exchange.[1]

Its conserved ATPase core is complemented by **flexible regions** that shape RAD51 interactions and conformational plasticity.[2]

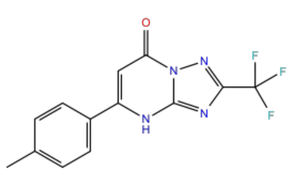




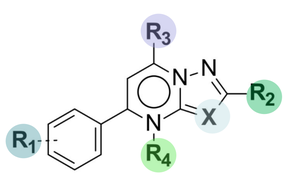
BRCA2 engages RAD51 through two adjacent interaction regions: **Site I**, which accommodates the FxxA motif and overlaps with the RAD51 oligomerization interface, and **Site II**, which recognizes the LFDE motif.

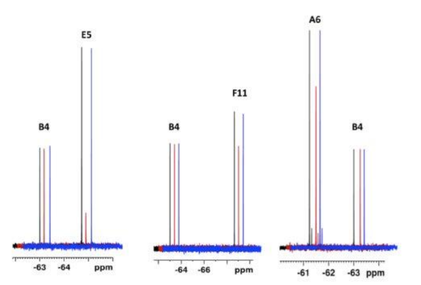
Deciphering E5 Binding to RAD51

A ^{19}F NMR fragment-based screening identified **E5**, but its binding mode remained unclear.[3] Guided by biological evidence, we used **Boltz-2** to investigate E5 and selected derivatives.



E5





Fragment alone
Fragment + RAD51 WT 1 μM
Fragment + RAD51 WT 1 μM + 20 μM BRC4

Substituents:

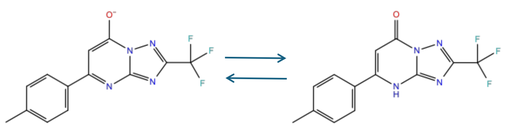
R_1 = H, F, Cl, Br, Me, CF_3

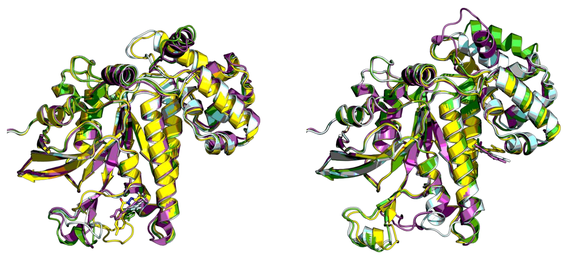
R_2 = Alkyl, Cycloalkyl

R_3 = Cl, =O, Amine, Ether, Alkenyl

R_4 = Alkyl, H

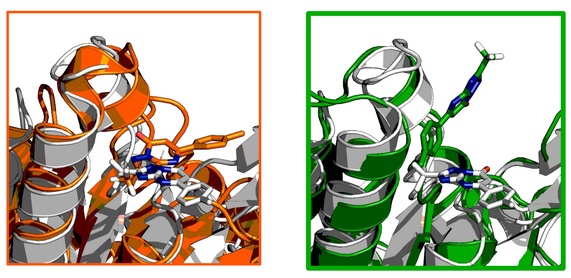
Alternative scaffold (X=N or CH)

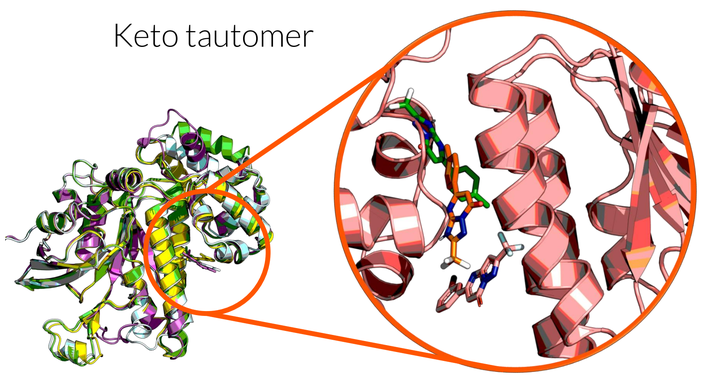




Enol tautomer

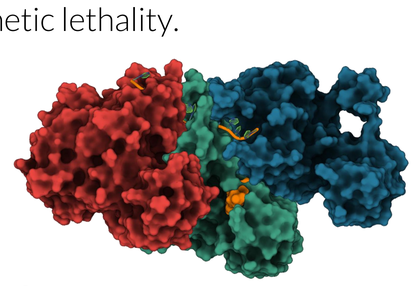
Keto tautomer





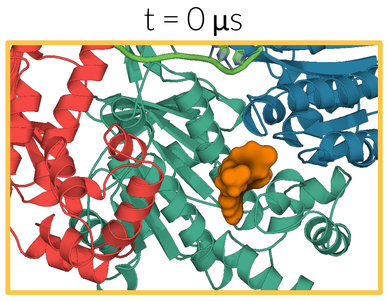
An Unconventional Route to Synthetic Lethality

- Minor modifications of **E5** and **35d** derivatives[4] unexpectedly **switched HR inhibition into enhancement**, reaching ~200% of basal activity.
- Excessive HR stimulation reduced cell viability, suggesting a potential alternative route to synthetic lethality.

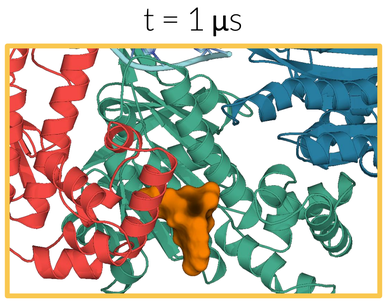


Monomer A
Monomer B
Monomer C
Small molecule (surface)

t = 0 μs



t = 1 μs



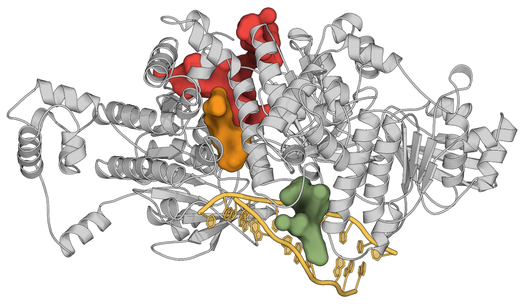
Searching for Alternative RAD51 Binding Sites

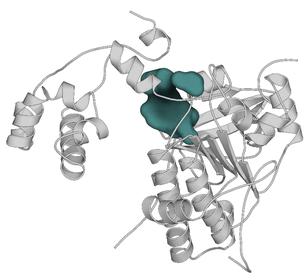
ADMET liabilities of Site I/II ligands motivated the search for alternative RAD51 pockets. Experimental structures and refined ensembles[5] are screened to identify new modulatory sites.

25 PDB structures + ensemble representatives of RAD51-BRC2

SiteMap

Structural knowledge

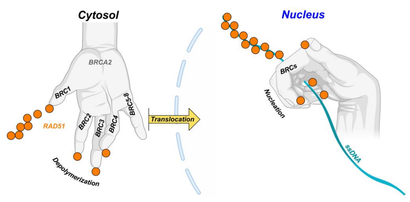




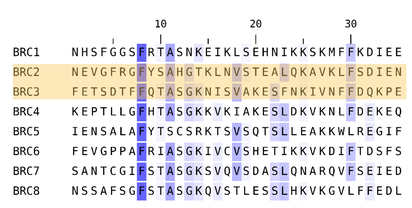
Docking/IF → MD simulations → Work in progress: Biological evaluation

An Integrative Structural Biology Approach to the RAD51-BRCA2 Interaction

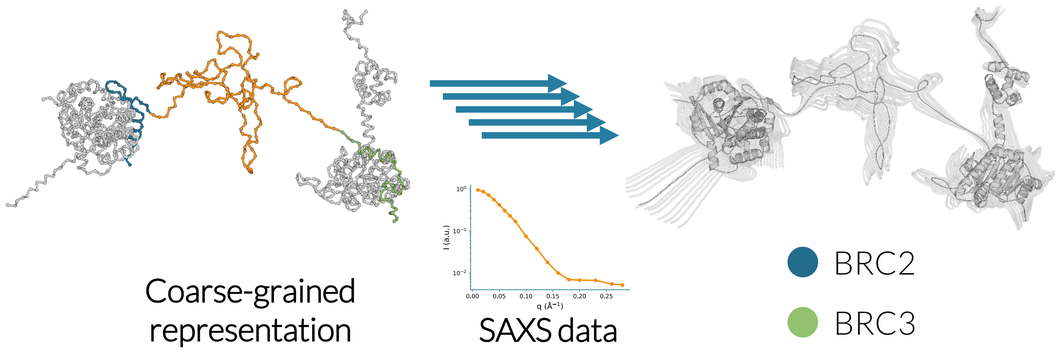
BRCA2 BRC repeats bind RAD51 with distinct affinities and regulate its assembly at DNA damage sites.[6]



We are **reconstructing RAD51-BRC2/BRC3 conformational ensembles** to characterize their structural heterogeneity.



Metainference



Coarse-grained representation

SAXS data

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[1] Thorslund, Tina, et al. *Nature structural & molecular biology* 17.10 (2010): 1263-1265.
[2] Rinaldi, Francesco, et al. *Angewandte Chemie* 135.51 (2023): e202312517.
[3] Previtali, Viola, et al. *Journal of medicinal chemistry* 67.14 (2024): 11488.
[4] Bagnolini, Greta, et al. *ACS Medicinal Chemistry Letters* 13.8 (2022): 1262.
[5] Bresciani, Veronica, et al. *Journal of Chemical Information and Modeling* 65.21 (2025): 11965.
[6] Rinaldi, Francesco, et al. *bioRxiv* (2025): 2025-11.