A UT/ORNL PARTNERSHIP
NATIONAL INSTITUTE FOR COMPUTATIONAL SCIENCES
NICS

Structural Mechanism Associated with Domain Opening in GOF Mutations in SHP2 Phosphatase

Eva Darian
University of Maryland
Department of Pharmaceutical Sciences

UNIVERSITY OF MARYLAND
SCHOOL OF PHARMACY

Background

- SHP2 is a widely expressed Src homology 2 (SH2) domain-containing protein tyrosine phosphatase (PTP) that is important for normal cell development
- Its activity is regulated by the intermolecular interaction between the N-SH2 domain (red) and the PTP domain (blue), which contains the catalytic site (orange "Surf" representation). This interaction leads to a "closed" or auto-inhibited conformation of the SHP2 protein (Figure 1)
- Binding of phosphopeptides to both N-SH2 and C-SH2 (pink) domains leads to an "open" or active state of the protein (Figure 2)
- Number of gain-of-function (GOF) mutations in SHP2 such as D61G, E76K, and N308D cause hyperactivation of its catalytic activity. These mutations, implicated in Noonan's Syndrome and childhood leukemias, are thought to facilitate opening

NICS

Closed state of SHP2 protein from crystal structure

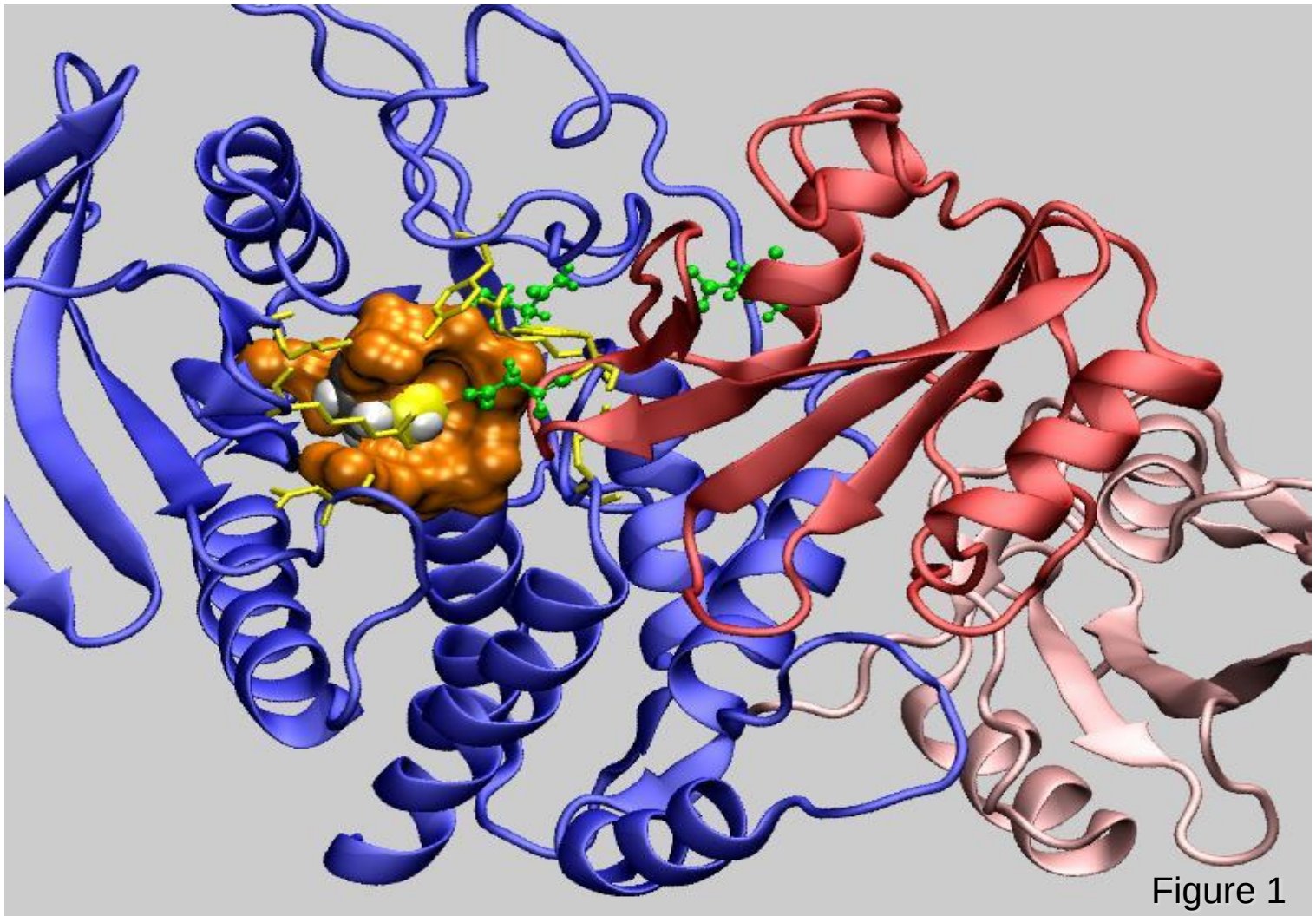


Figure 1

Open state of SHP2 protein obtained from PMF calculation

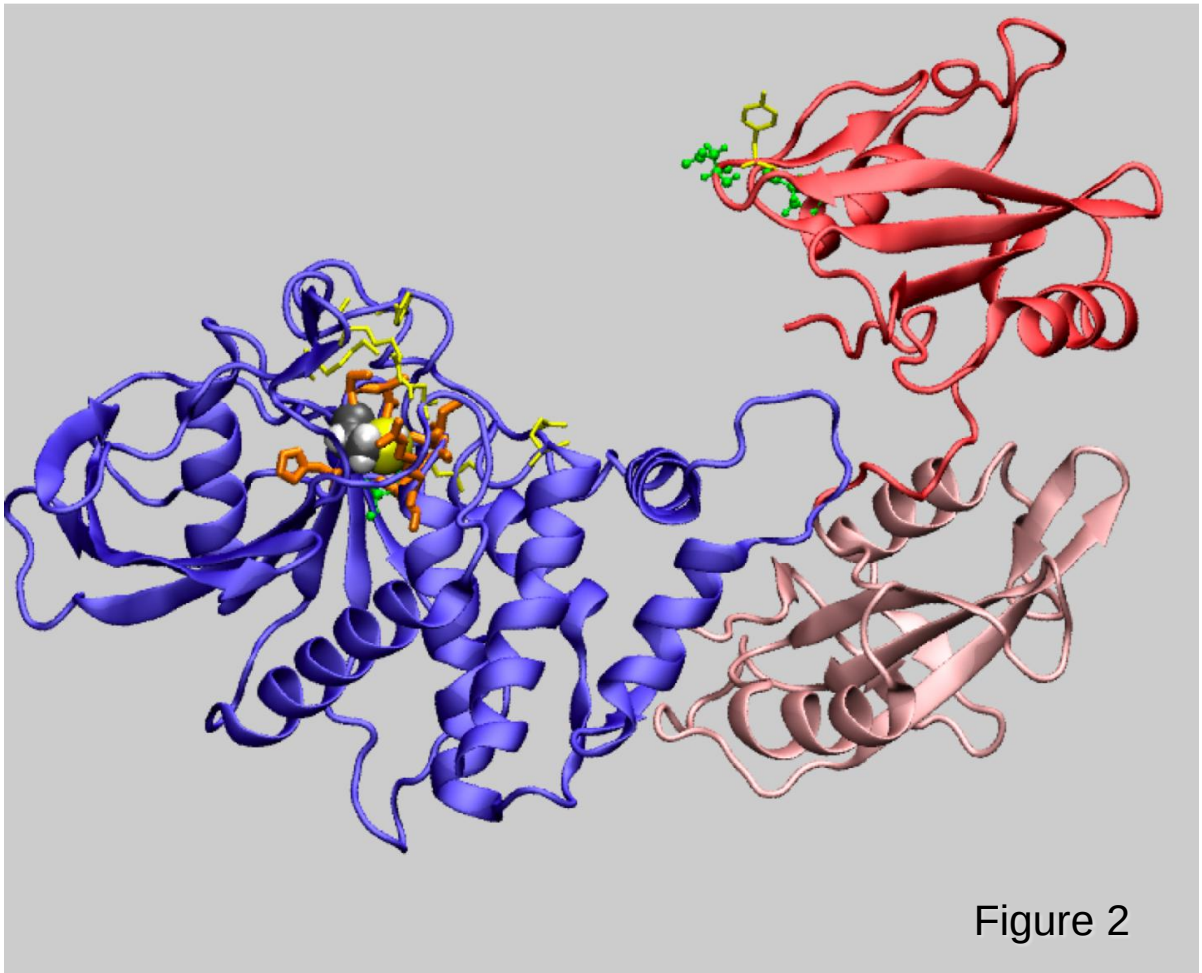


Figure 2

SHP2 domains shown:

PTP – blue
N-SH2 – red
C-SH2 – pink

Residues shown:

D61G, E76K, and N308D mutations are in green ball and stick

Catalytic Cys459 – VDW representation

Catalytic site residues – orange thick licorice

Other important residues – yellow thin licorice

NICS

Conclusions

- We have used a combination of computational and experimental methods to investigate the structural mechanism of opening of SHP2 and the impact of these GOF mutants on the opening mechanism
- The opening pathway analyzed from the PMF calculations suggests a role for the C-SH2 domain in stabilizing the open state of SHP2, when the N-SH2 domain undergoes sliding motion away from PTP
- Calculated free energies of opening (Figure 3) of WT, D61G, E76K, and N308D PMF suggest that spontaneous opening of SHP2 does not occur in either of proteins. They indicate that it is most likely facilitated by the protein substrates or other activator molecules
- Analysis of the present results indicates that Arg362 may play a role in initial recognition of substrate proteins (Figure 4), which in turn may enhance interactions of SHP2 with its substrate proteins and thereby aid opening
- In addition, D61G and E76K mutants alter direct interactions between the N-SH2 and PTP domains, and facilitate binding of phosphopeptides to the N-SH2 domain, further favoring the open state of the SHP2 protein

NICS

Free energy ΔG (kcal/mol) of opening between PTP and N-SH2 domains

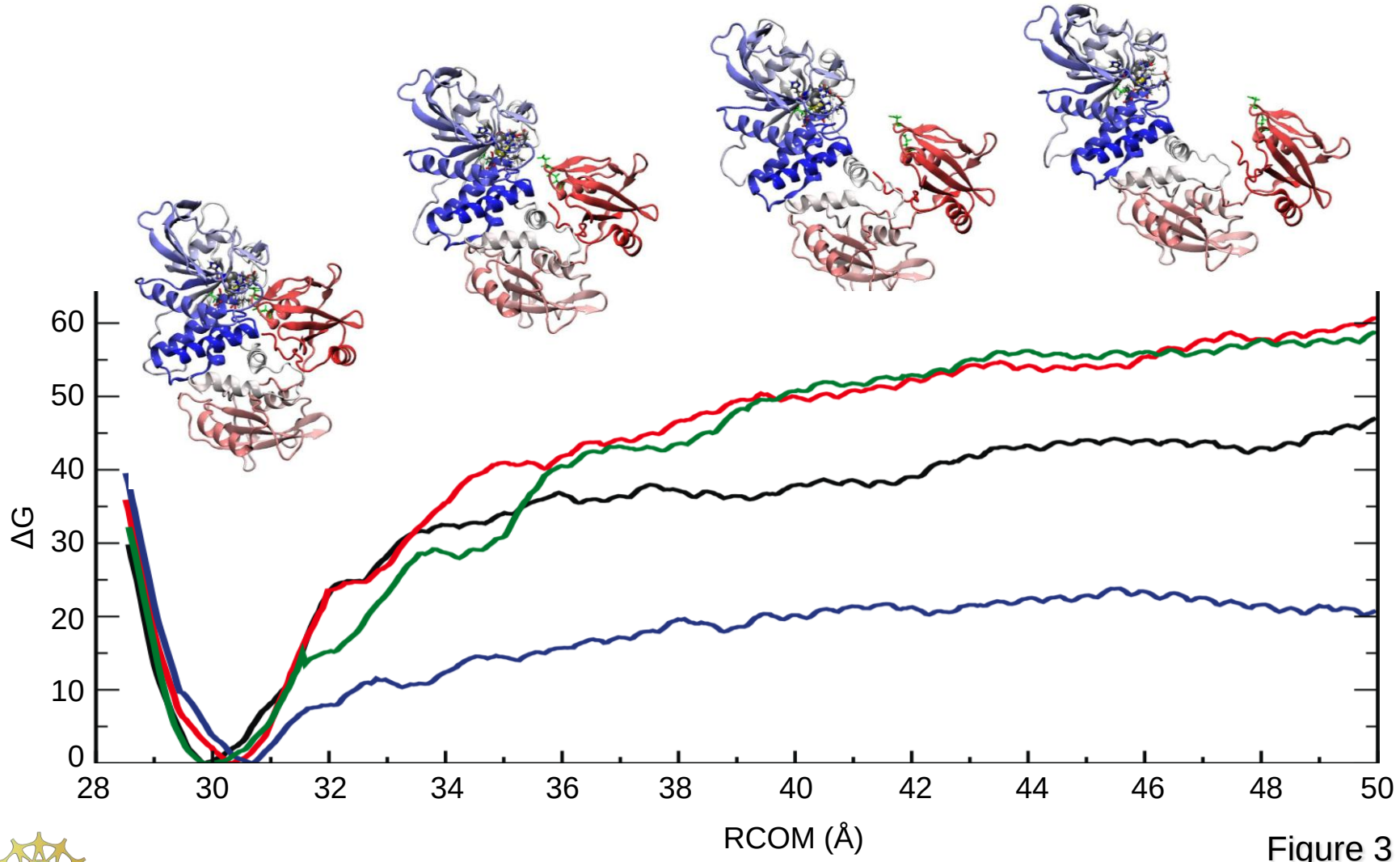
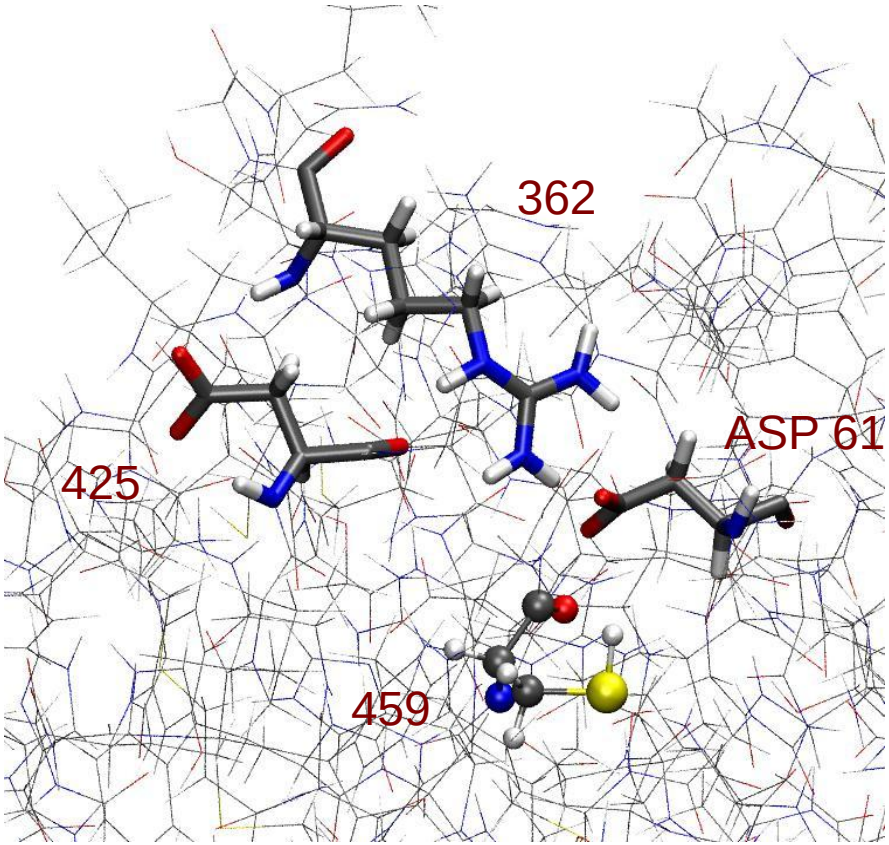


Figure 3

Position of ARG 362 with Asp 425 and Cys 459 in WT and D61G mutated protein

WT



D61G

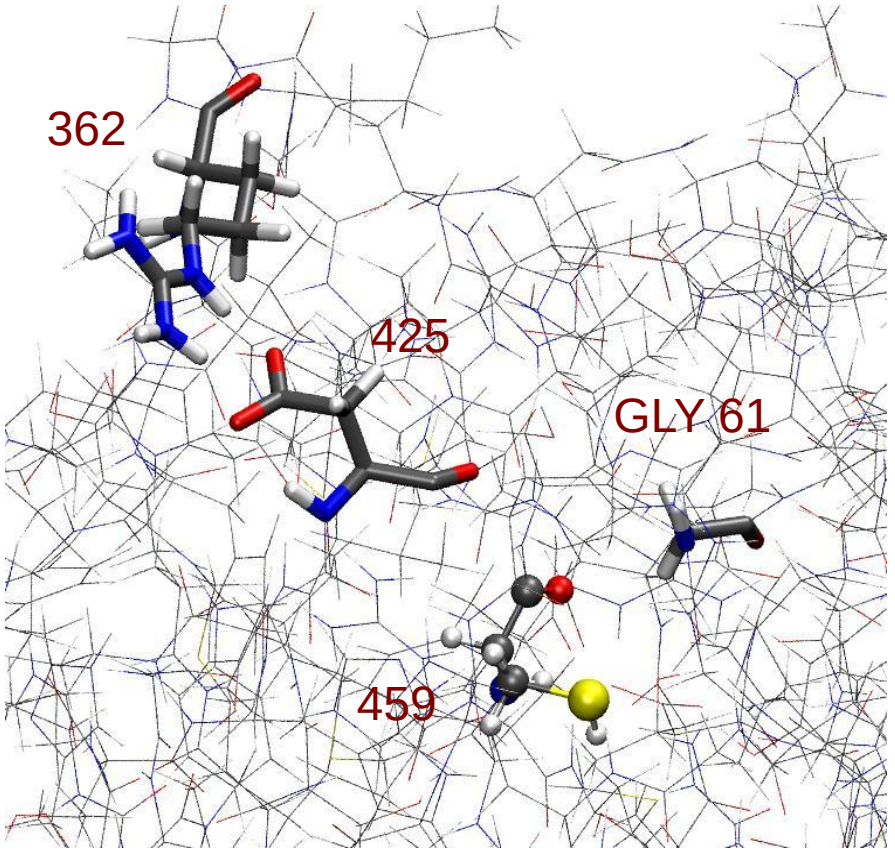


Figure 4

NICS

Contact

Eva Darian

University of Maryland
Department of Pharmaceutical Sciences
edarian@outerbanks.umaryland.edu