

## FRET Mapping: A New Methodology to Elucidate Global Structural Information

### Supplemental Information: Pymol Script

**Mapping of sites within the SecA-SecYEG complex using FRET distances.** The overlap regions between the SecA-SecYEG structure (PDBID: 3DIN) and the FRET dataset (see Table 1) were determined from the intersection of the three spherical shells generated from the FRET distances for a specific labeled position on the SecA-PhoA chimera protein and the selected atoms of the SecA-SecYEG structure (Figs. 2 and 3). The thickness of the shell was defined by the maximum and minimum distances determined for that FRET pair based on the measured error. The shells were generated using standard selection algebra commands in the program Pymol (Schrodinger, LLC) ([https://pymolwiki.org/index.php/Selection\\_Algebra](https://pymolwiki.org/index.php/Selection_Algebra))<sup>1</sup>. For each shell, we made use of the "around" command for the maximum distance and "beyond" commands for the minimum distance and the Boolean "and" logical operator to create the set of atoms. A sample script for identifying the overlapping region would be as follows (adapted from Zhang et al.<sup>2</sup>:

```
PyMOL>select a37PhoA2-1, chain A and resi 30 around 82
Selector: selection "a37PhoA2-1" defined with 14843 atoms.
PyMOL>select a37PhoA2-2, a37PhoA2-1 beyond 52 of chain A and resi 30
Selector: selection "a37PhoA2-2" defined with 9699 atoms.
PyMOL>select a321PhoA2-1, chain A and resi 346 around 64
Selector: selection "a321PhoA2-1" defined with 11367 atoms.
PyMOL>select a321PhoA2-2, a321PhoA2-1 beyond 42 of chain A and resi 346
Selector: selection "a321PhoA2-2" defined with 5614 atoms.
PyMOL>select y292PhoA2-1, chain C and resi 288 around 83
Selector: selection "y292PhoA2-1" defined with 8822 atoms.
PyMOL>select y292PhoA2-2, y292PhoA2-1 beyond 53 of chain C and resi 288
Selector: selection "y292PhoA2-2" defined with 4932 atoms.
PyMOL>select PhoA2res, a37PhoA2-2 and a321PhoA2-2 and y292PhoA2-2
Selector: selection "PhoA2res" defined with 442 atoms.
```

#### References:

- 1 The PyMOL Molecular Graphics System, Version 2.4 (Schrodinger, LLC, New York, 2021).
- 2 Zhang, Q. *et al.* Alignment of the protein substrate hairpin along the SecA two-helix finger primes protein transport in *Escherichia coli*. *Proc Natl Acad Sci U S A.* **114** (35), 9343-9348, (2017).