Specificity Prediction and Design of Phosphoswitches

RosettaCon – August 5, 2009

Colin A. Smith, Kortemme Lab

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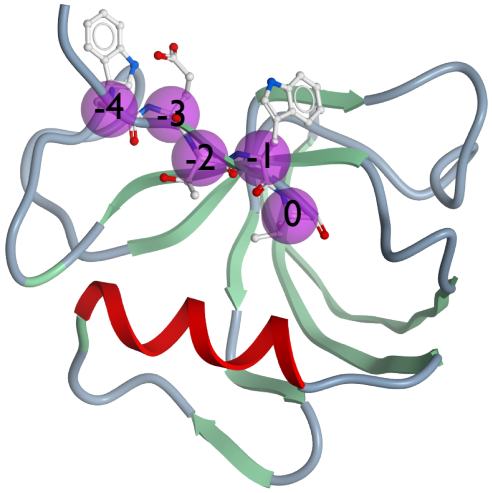
- Use PDZ domains as a model system for method development and structural understanding

PDZ domains recognize several positions in C-terminal peptides with high specificity



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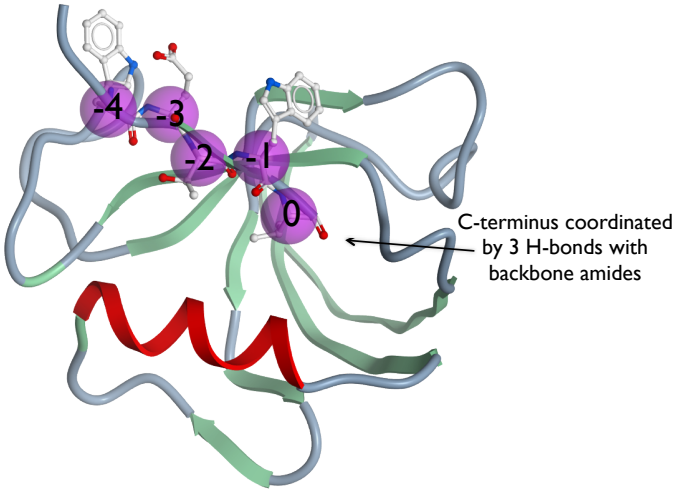

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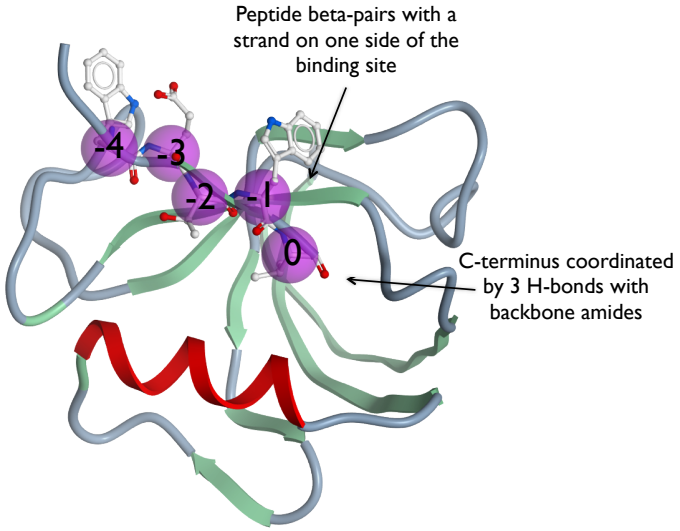

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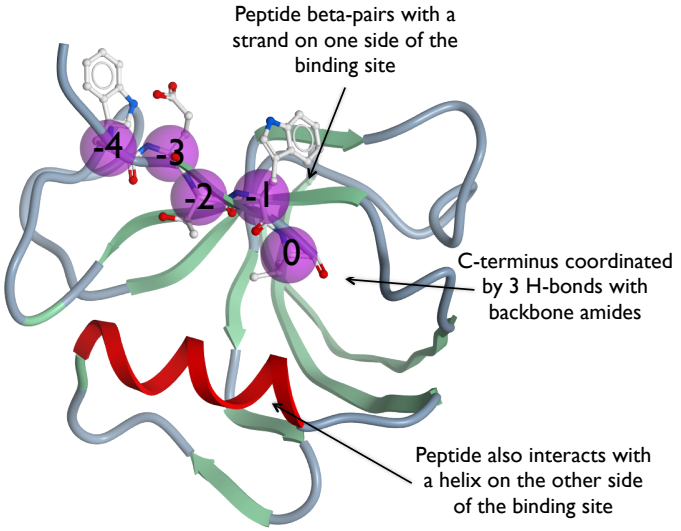
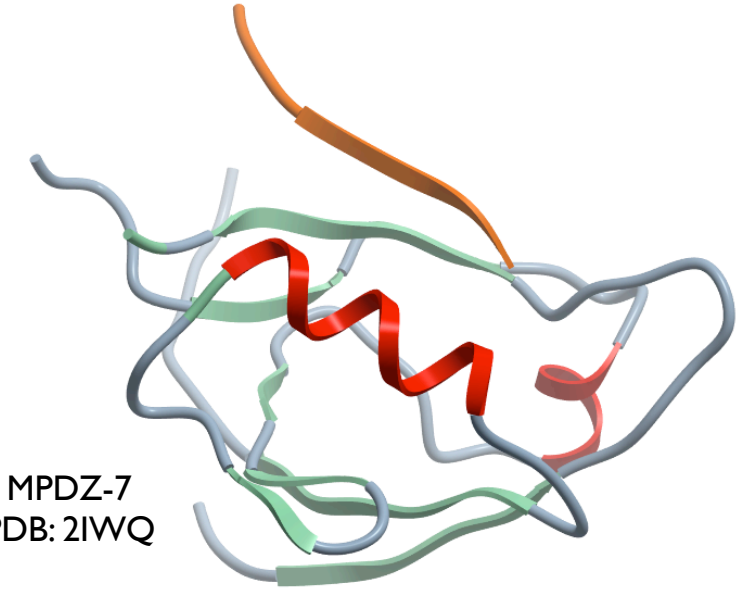
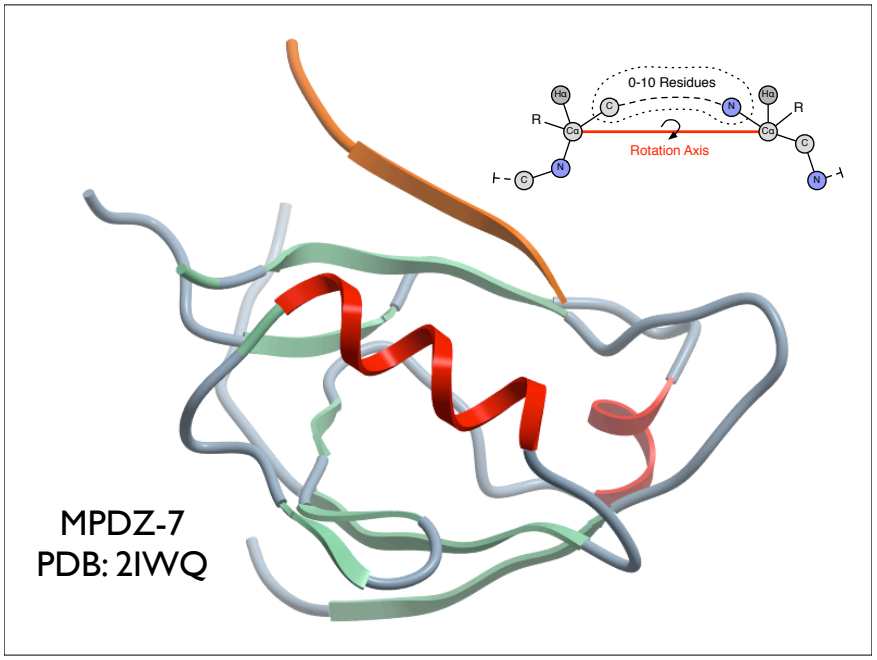


Phage Display Data Sets:

- 54 Human PDZ Domains
  (16 X-ray & 2 NMR complexes in PDB)
- 28 C. elegans PDZ Domains (none in PDB)
- Erbin PDZ Domain Mutants:
  - 91 Point Mutants
  - 61 5-8 Residue Mutants (E-1 to E-61)
  - 53 Enumerating a Mutational Pathway WT...E-14



MPDZ-7
PDB: 2IWQ


0-10 Residues
Rotation Axis
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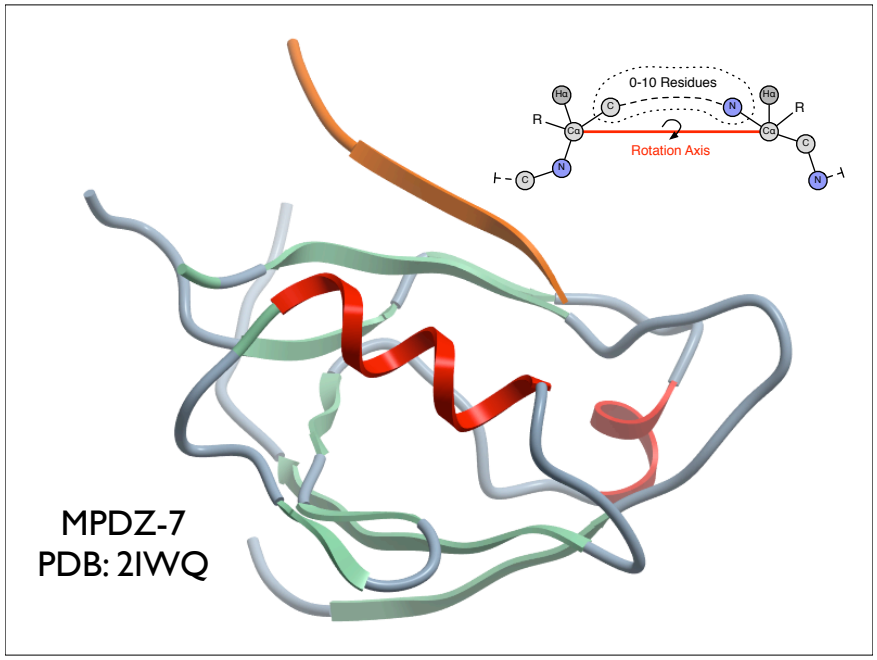
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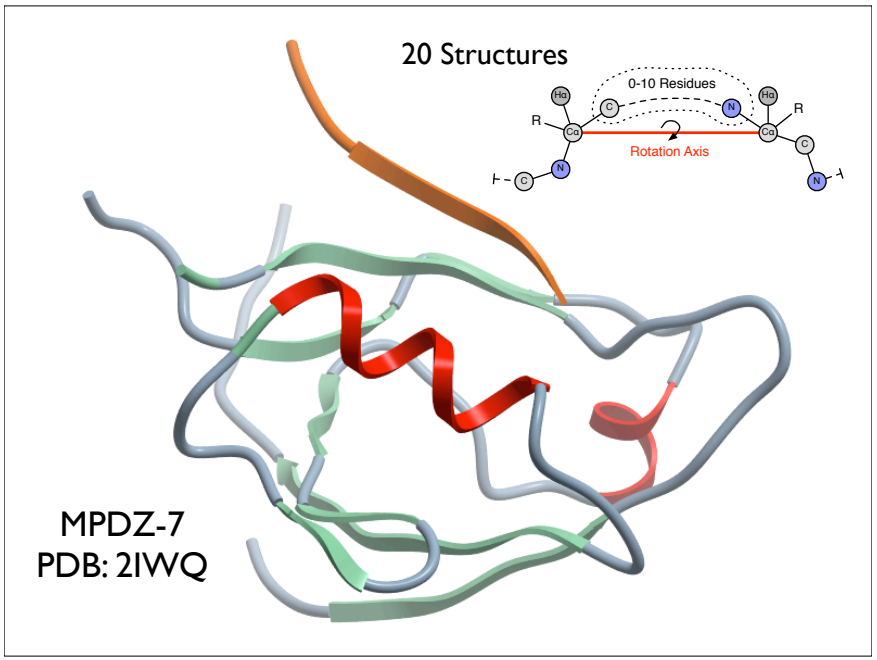
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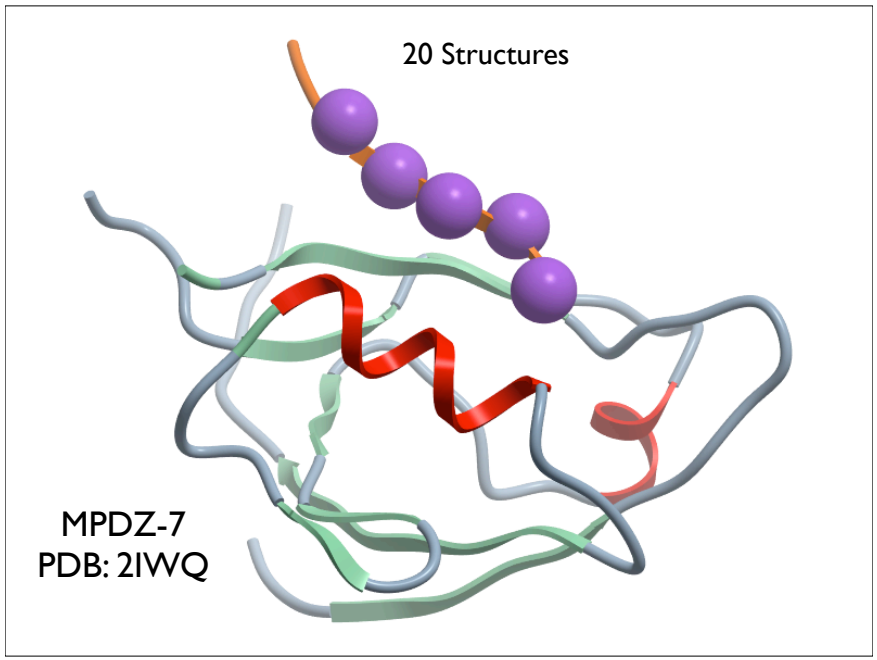
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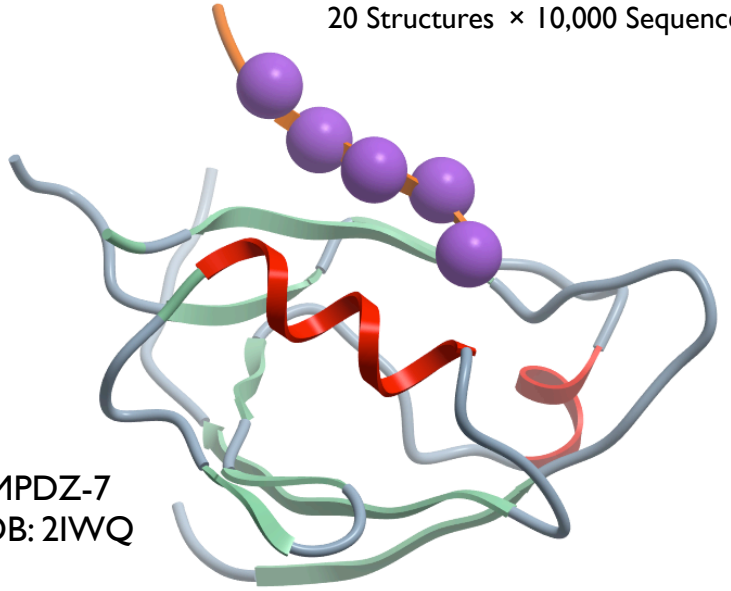
20 Structures × 10,000 Sequences



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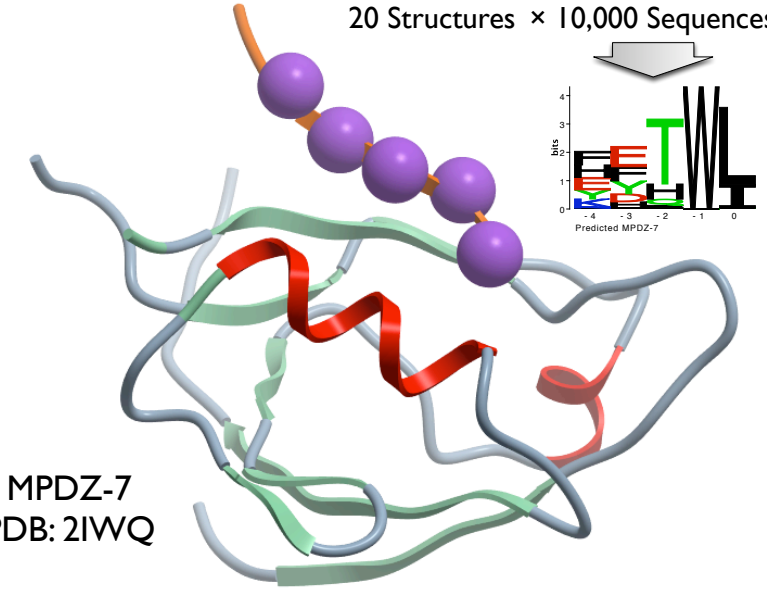

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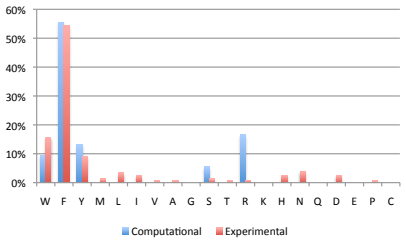
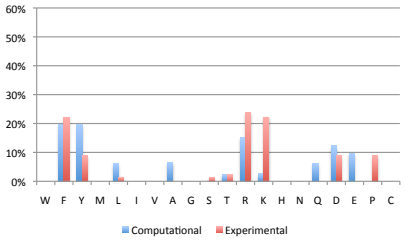
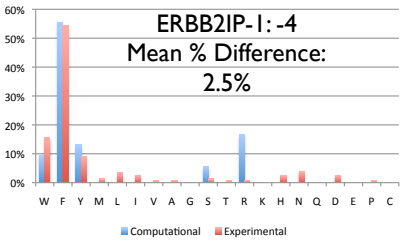
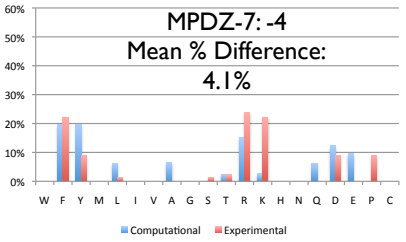
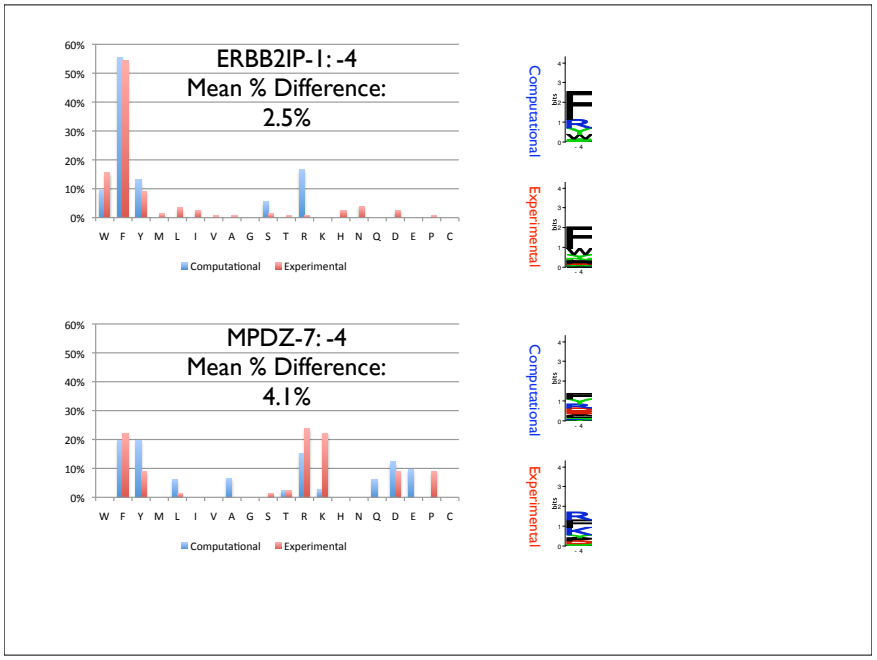
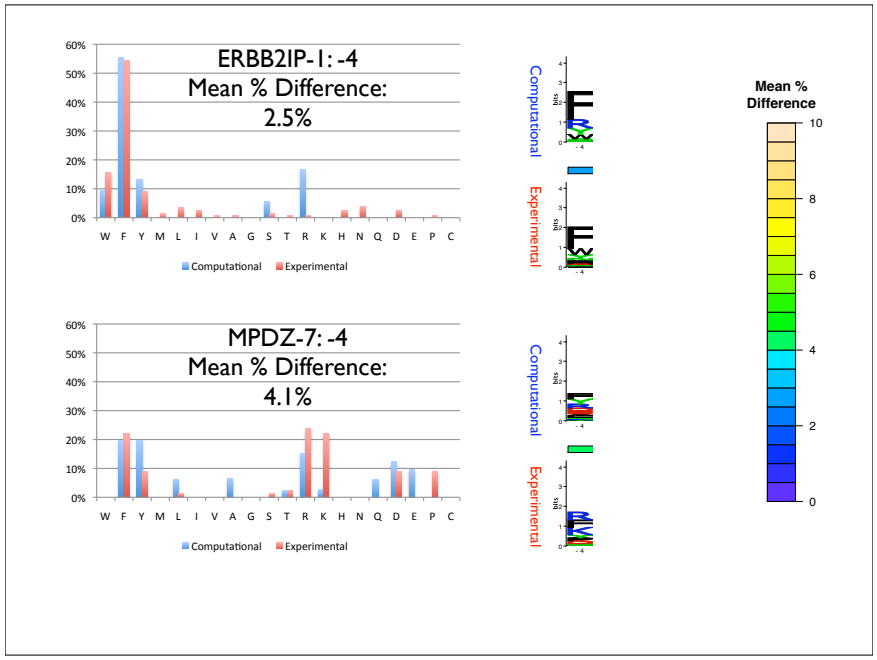









ERBB2IP-1: -4
Mean % Difference:
2.5%
W F Y M L I V A G S T R K H N Q D E P C
Computational
Experimental
MPDZ-7: -4
Mean % Difference:
4.1%
Computational
Experimental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MPDZ-7: -4
Mean % Difference:
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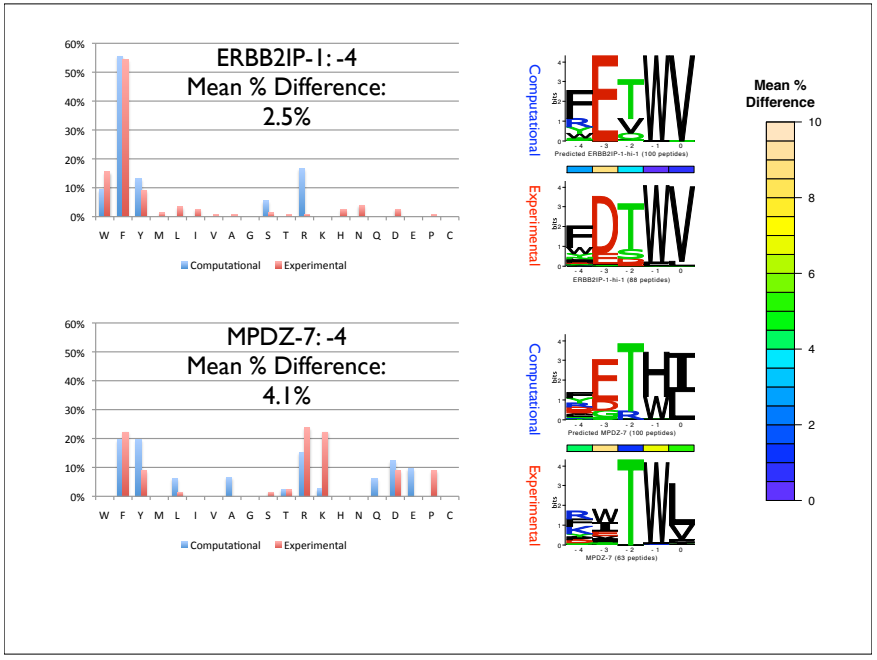
Mean %
Difference
10
8
6
4
2
0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Computational
Experimental
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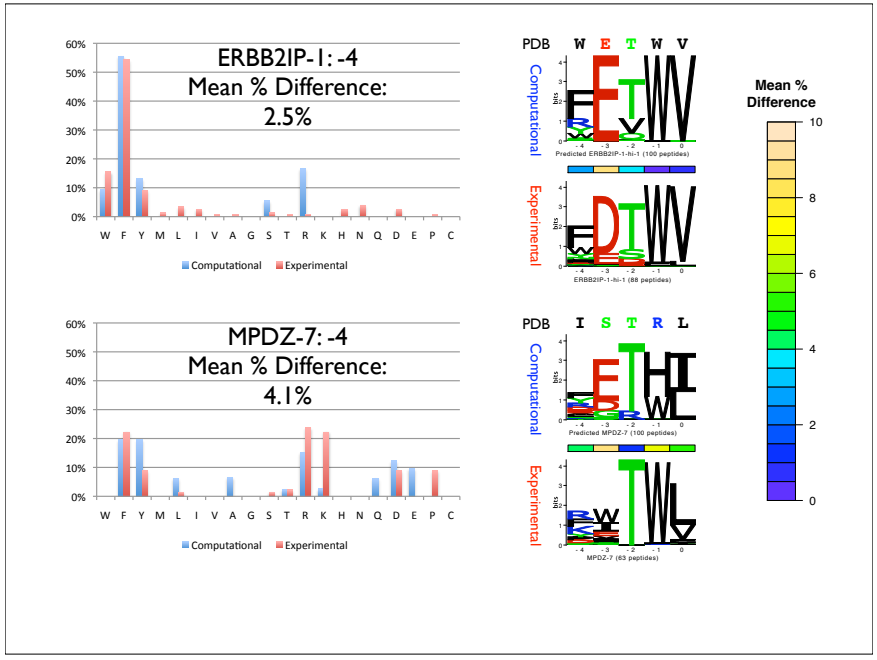
4.1%
Predicted ERBB2IP-1-hi-1 (100 peptides)
ERBB2IP-1-hi-1 (88 peptides)
Predicted MPDZ-7 (100 peptides)
MPDZ-7 (63 peptides)
Mean %
Difference
10
8
6
4
2
0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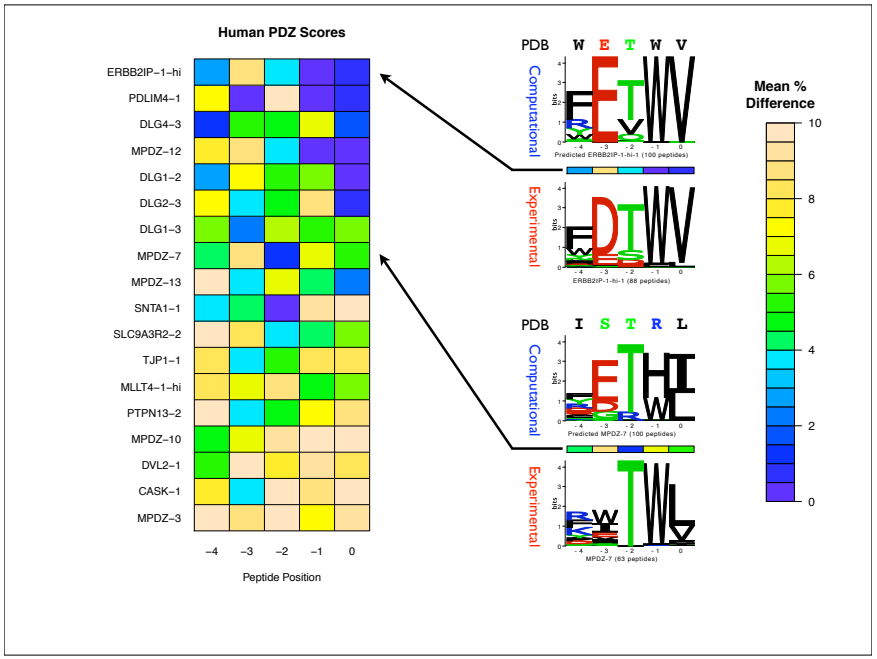
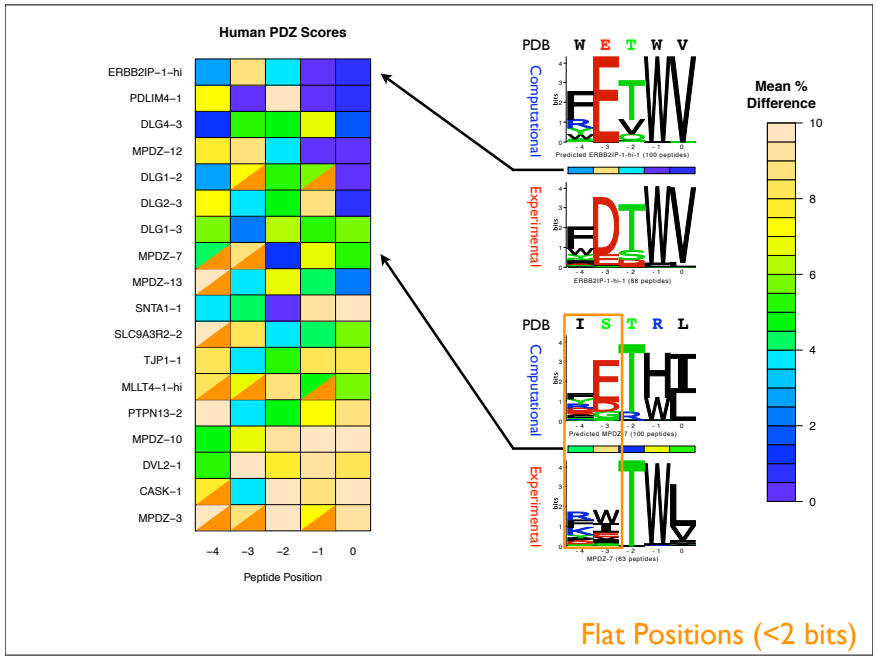
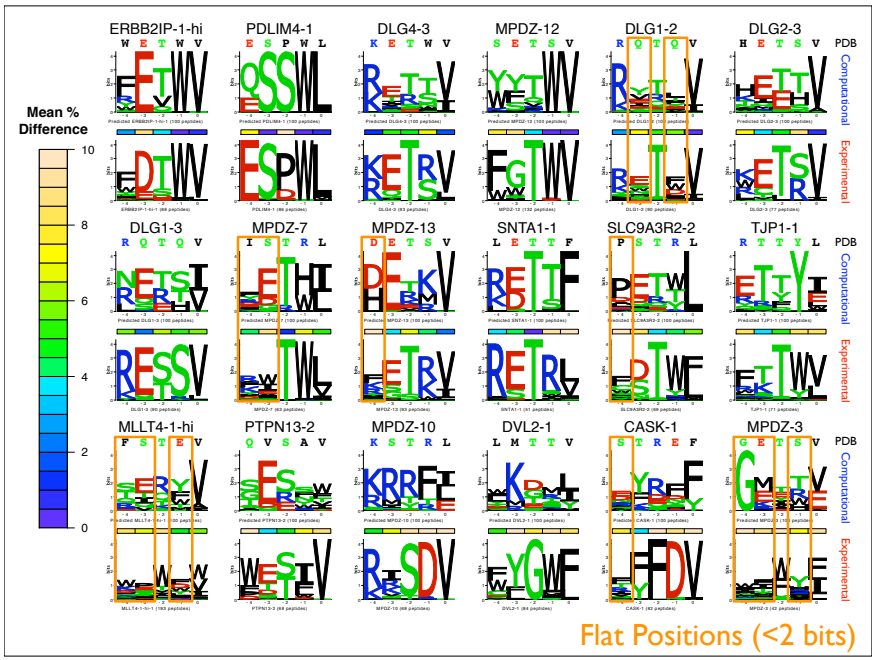




Flat Positions (<2 bits)



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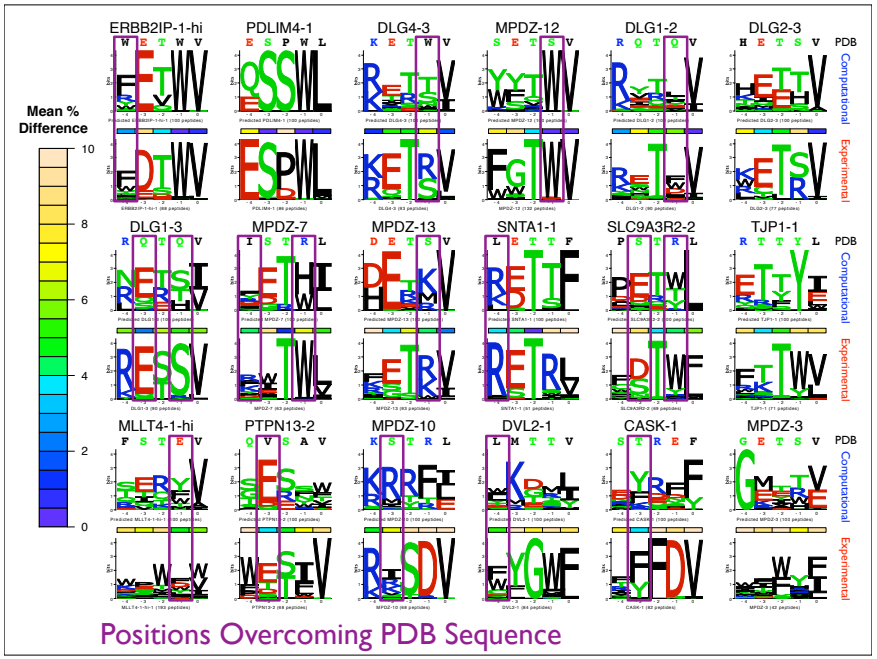

Method/Scoring Function Notes

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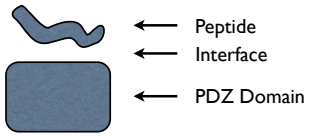

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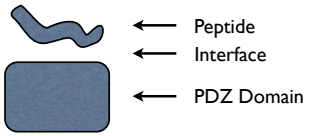

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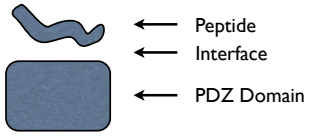

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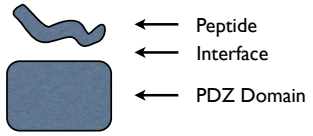
- NMR structures were proved just as effective as crystal structures (or more)



Future Directions/ Challenges

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- Better backbone sampling/ constrained motions/relax

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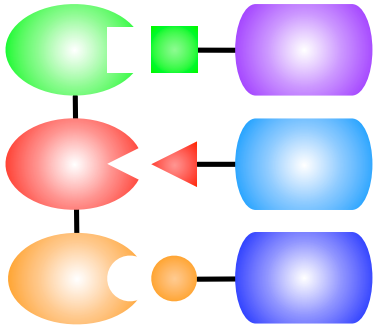
- Electrostatics:
  - Intricate hydrogen bond networks radius of convergence, demand high resolution
  - Surface salt bridges not weighted strongly enough in default score function

Regulated Scaffolding Project Goal

Engineering an adapter domain to have peptide binding and/or specificity regulated by phosphorylation



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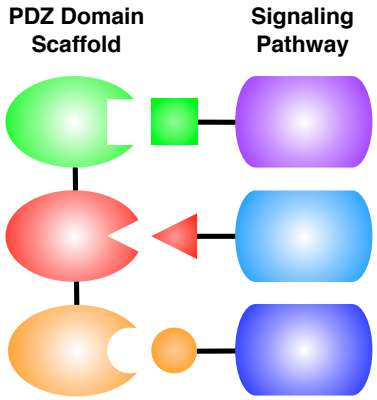

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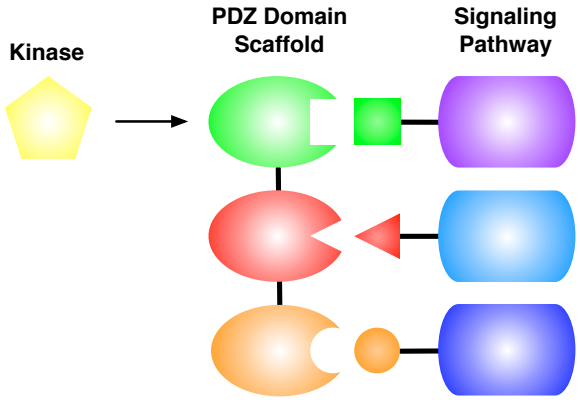

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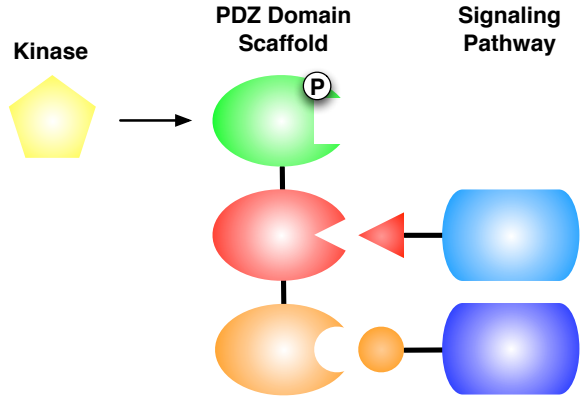

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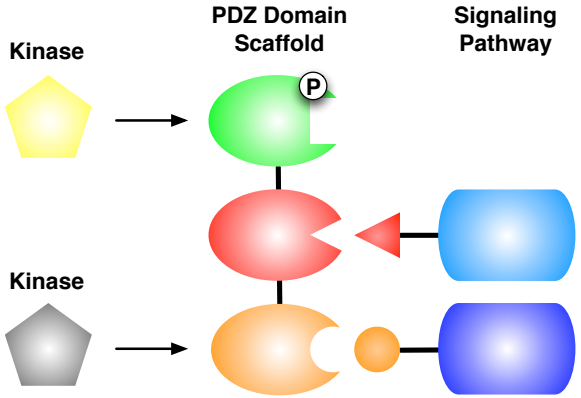

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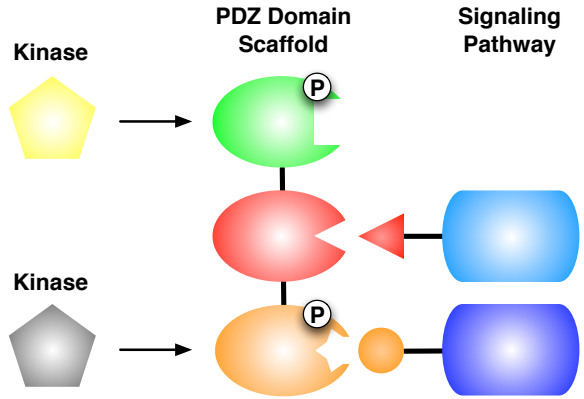

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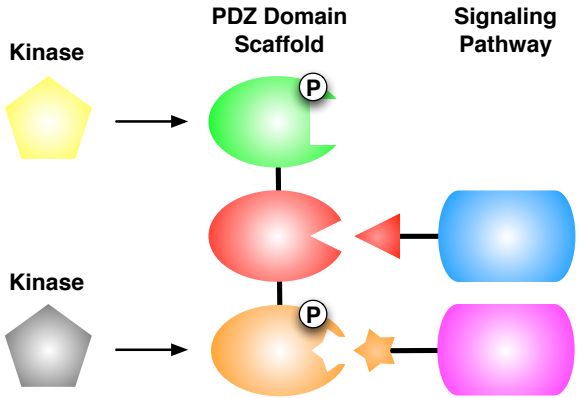

8 Sites Were Selected to Add a Kinase Recognition Motif

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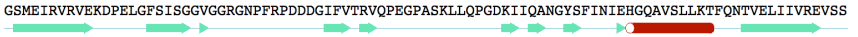

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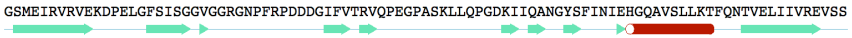

Protein Kinase A Recognition Motif: R R X S ϕ

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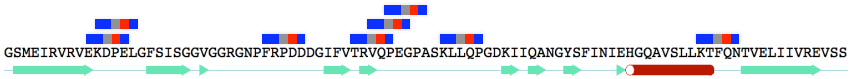
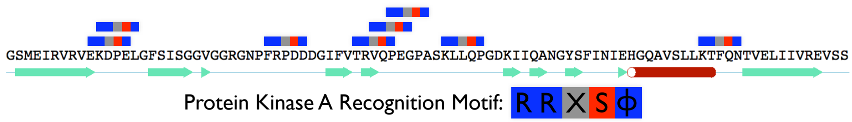


Protein Kinase A Recognition Motif: R R X S ϕ

Mutant	WT Sequence	Δ Rosetta Score	pkaPS Score
13 RXXSX	EKDPE	-5.4956	0.960
14 XRXSX	KDPEL	-1.5498	1.313
33 XXXSV	FRPDD	-0.4447	1.094
43 RRXSX	TRVQP	-0.1151	1.762
45 XRXSX	VQPEG	-3.1717	1.079
47 RRXSX	PEGPA	-0.7202	2.059
53 XRXSX	KLLQP	-0.5001	1.372
82 RRXSF	KTFQN	-0.5759	1.151

Designing Phosphoswitchable Protein Adapter Domains



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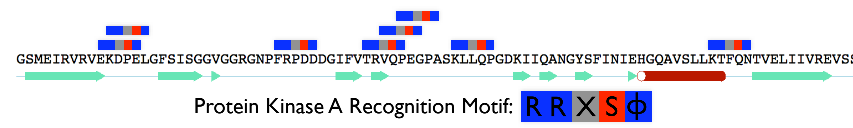

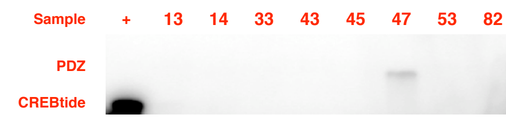

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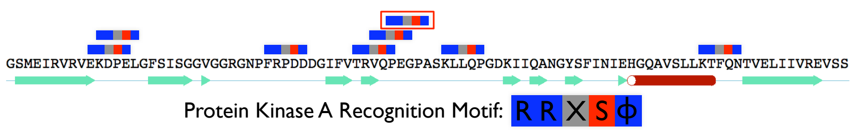

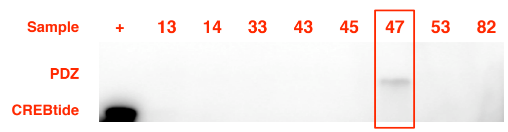

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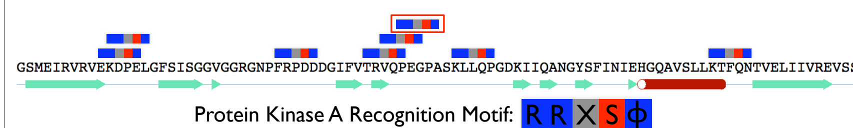
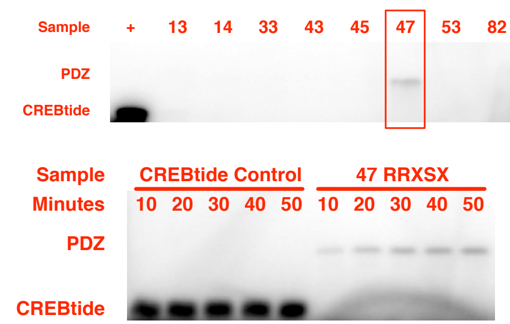
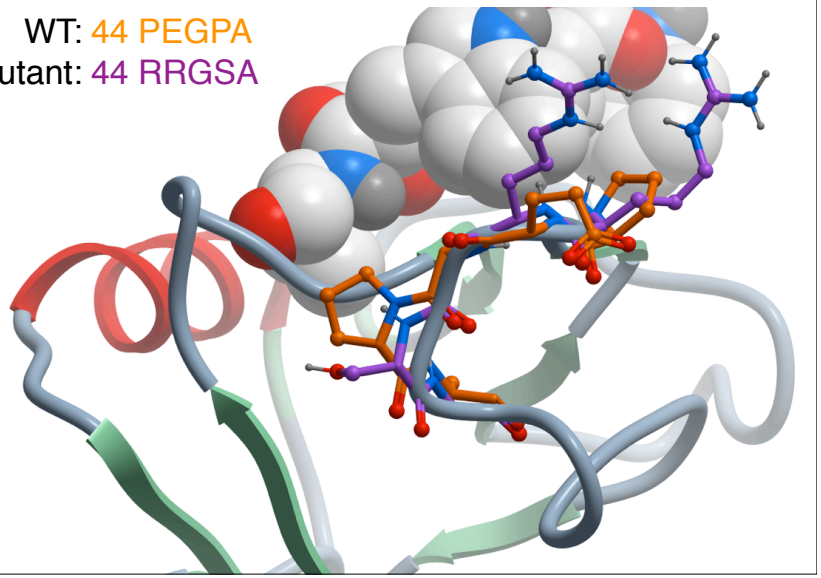




Predicted Structure of Position 44 Mutant

WT: 44 PEGPA
Mutant: 44 RRGSA



Next Step: Use computational design to test several models of phophoregulation

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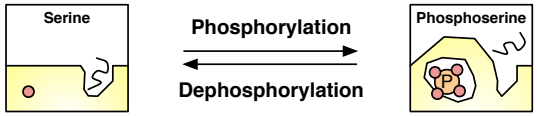
Destabilize Domain Structure



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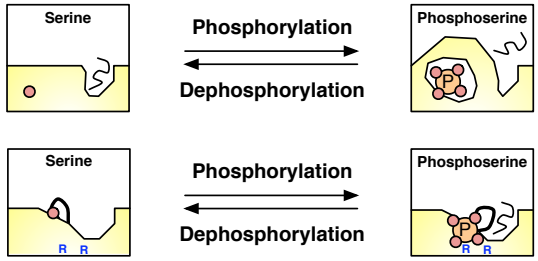
Destabilize Domain Structure

Restabilize Native Structure



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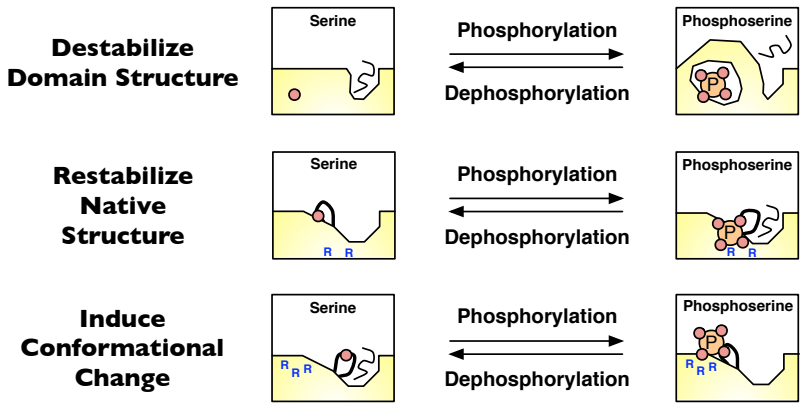

Acknowledgements

Tanja Kortemme
Elisabeth Humphris
Sachdev Sidhu
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