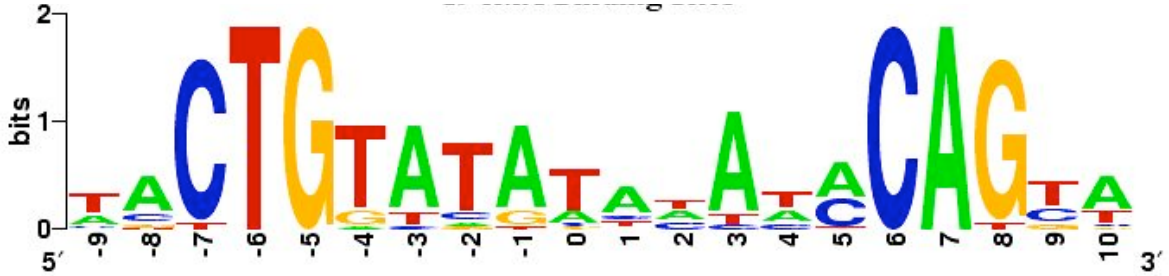
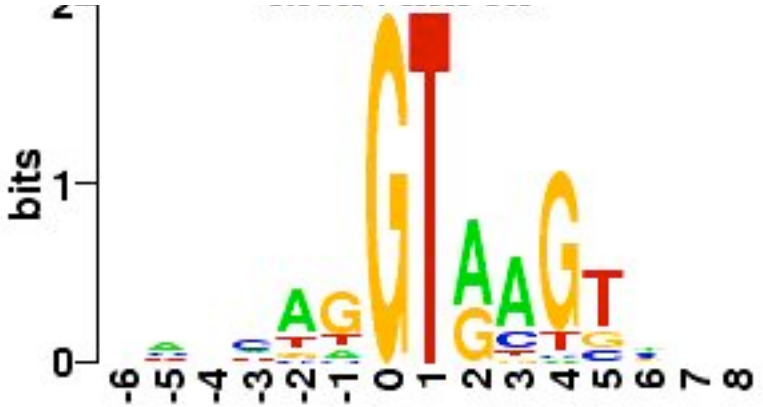
Predicting the specificity of motif-mediated interactions

Many proteins recognize short linear motifs in the sequences of their polymer partners

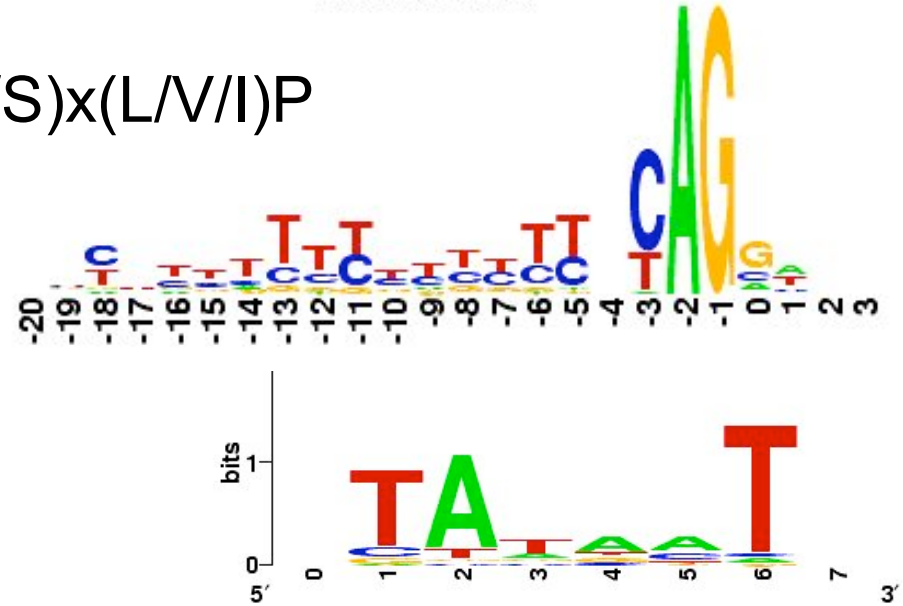


PxxP

PPxY

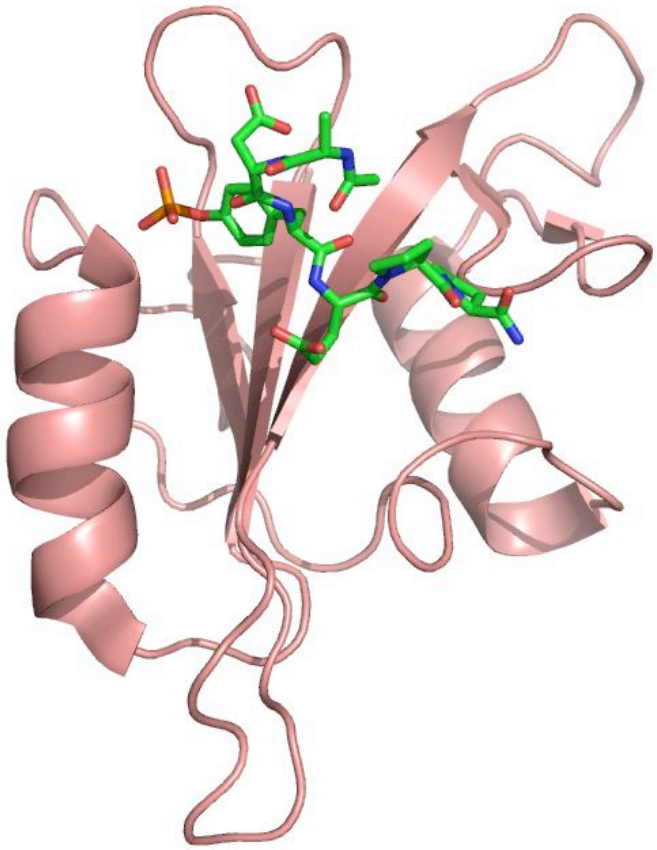


P(T/S)x(L/V/I)P

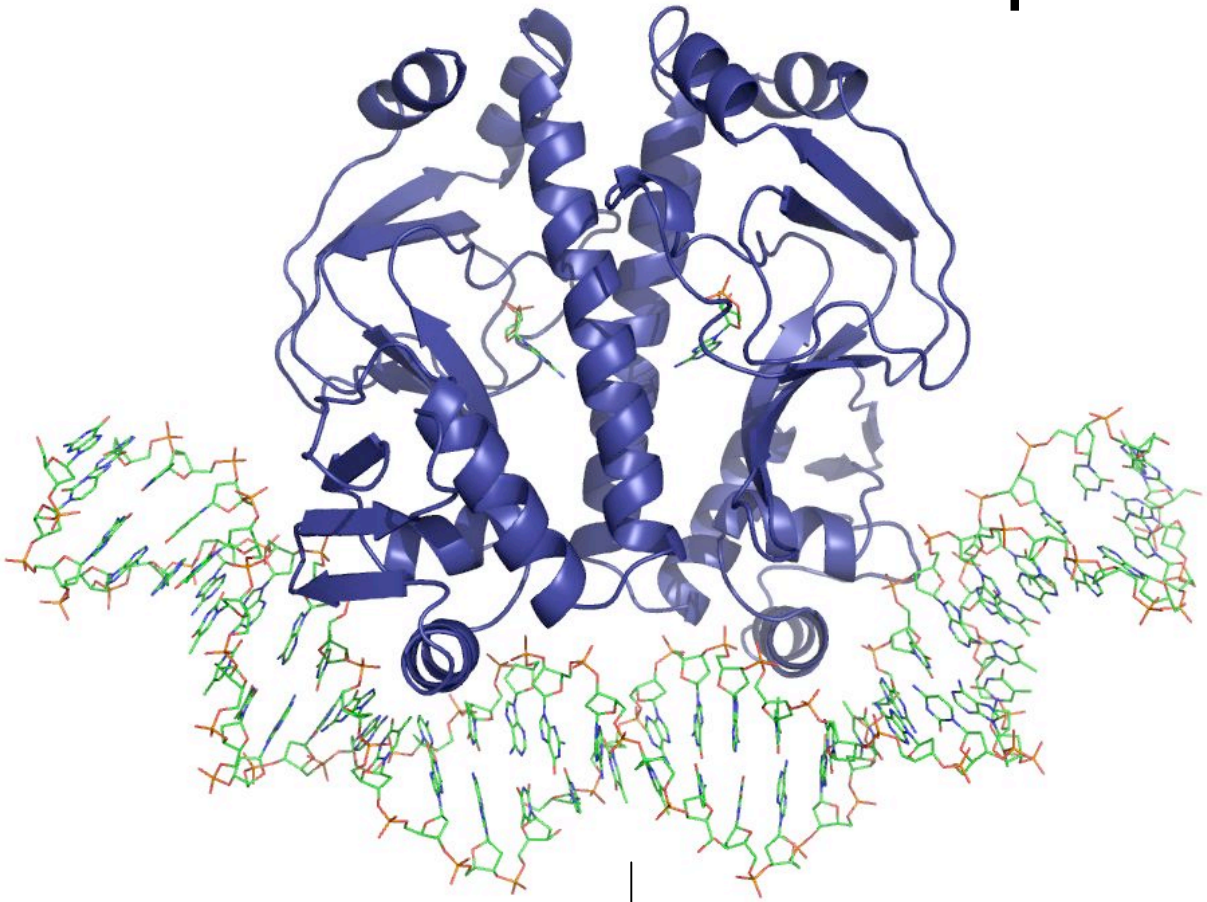


Structure-based prediction of interaction specificity

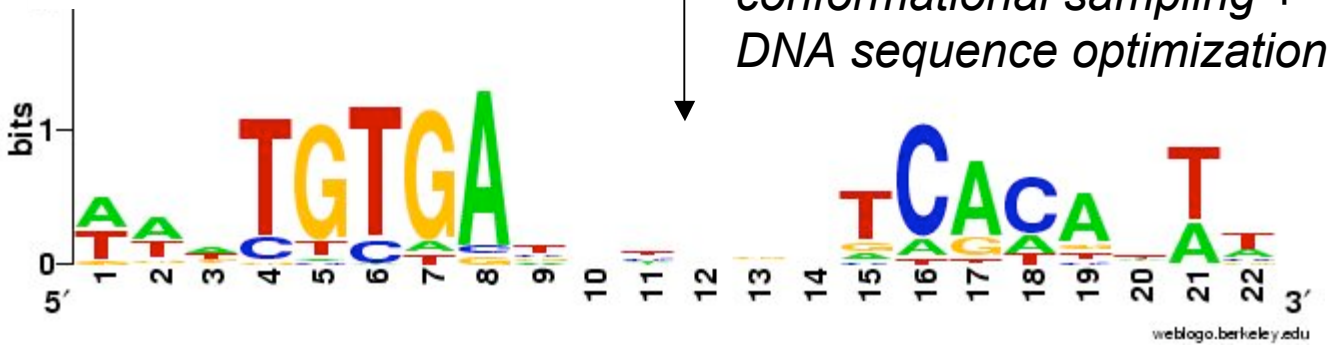
- General problem: given a structural model of a protein-{peptide,DNA,RNA} complex, calculate relative affinities for alternate sequences of the partner
- Together with homology modeling, could be used to predict approximate binding specificities for uncharacterized transcription factors, peptide-binding domains, etc, in high-throughput fashion.



Protein-DNA interaction specificity



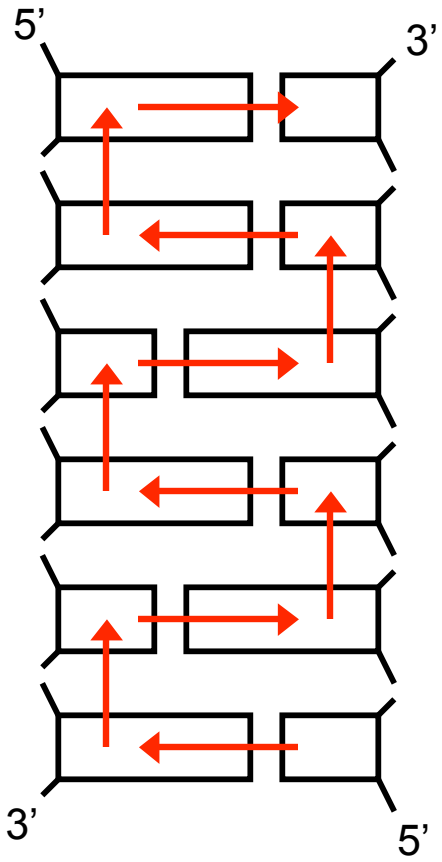
conformational sampling +
DNA sequence optimization



protein-DNA methods

- DNA flexibility
  - wriggling (for small torsion angle changes)
  - double-fragment closure
  - DNA rotamer library
  - double-helical fragments
  - DNA “design” preserving WC base pairing
  - base-centric foldtree
- centroid env and pair for protein-DNA
- waters

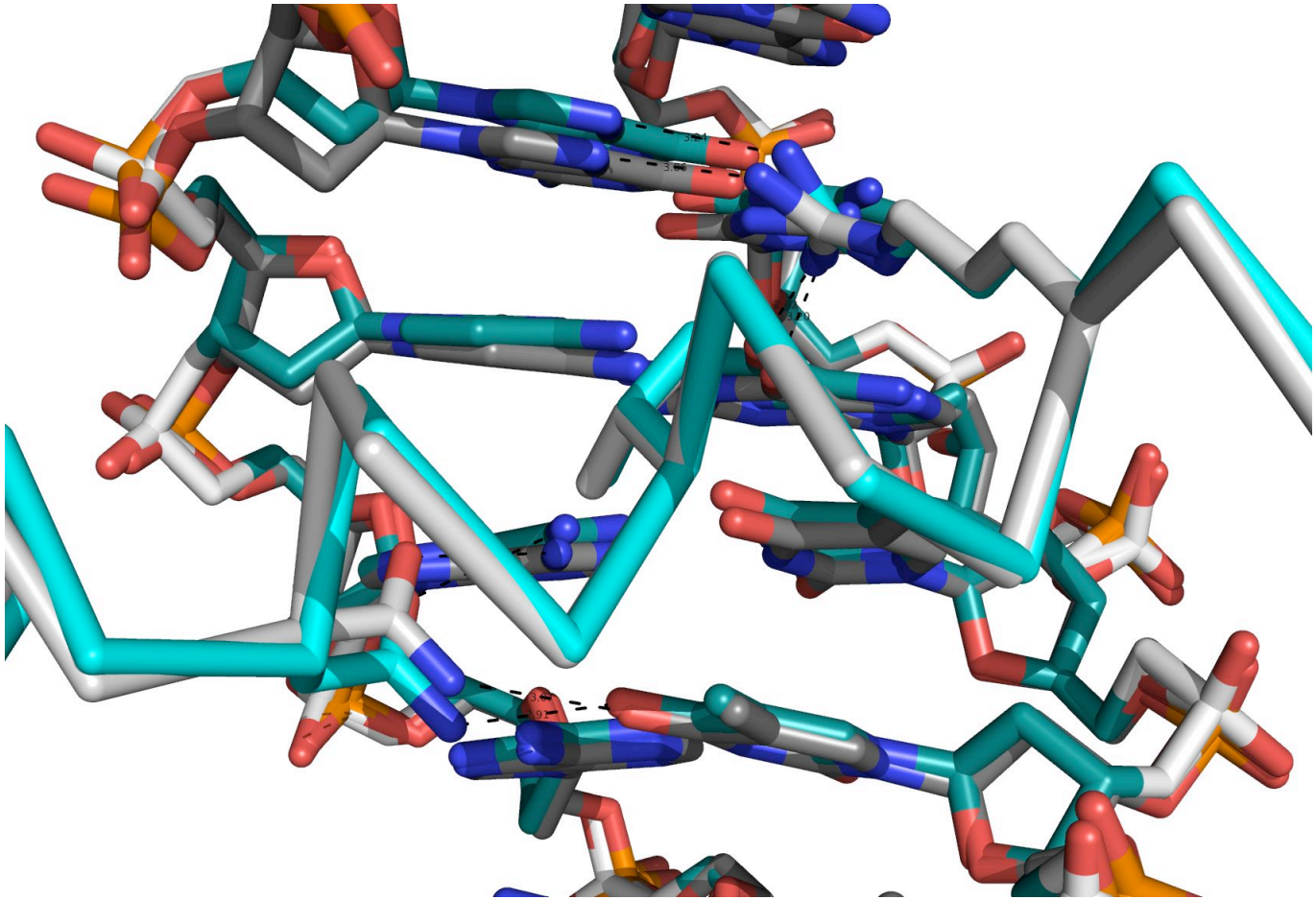
Base-centric kinematics



Rigid-body “jump”
(6 DOFs)

go to centroid-level fragment simulation

lowest energy model w/ native

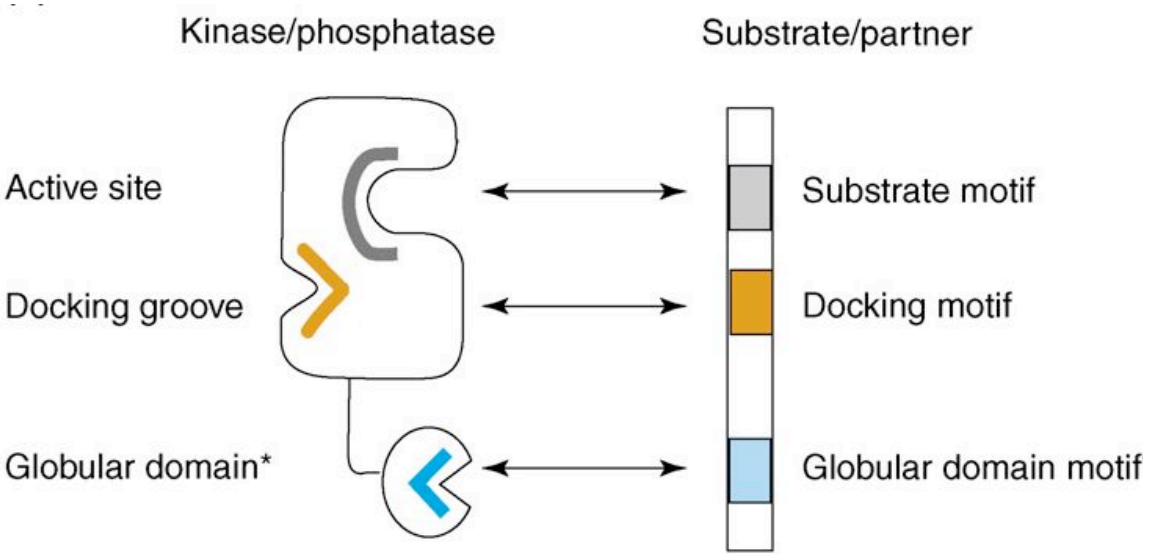


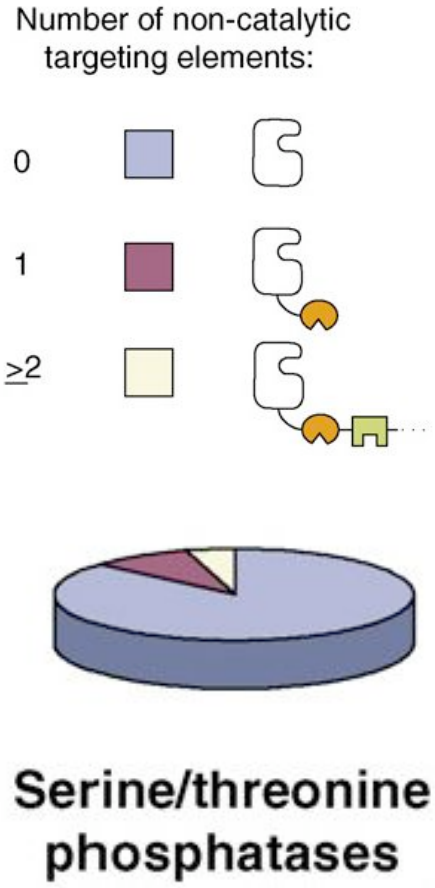
waters in pymol

Kinase Specificity

- Globular Domain Motifs (SH2, SH3, PDZ…)
- Linear Docking Motifs (…XXRRXSLXX…)

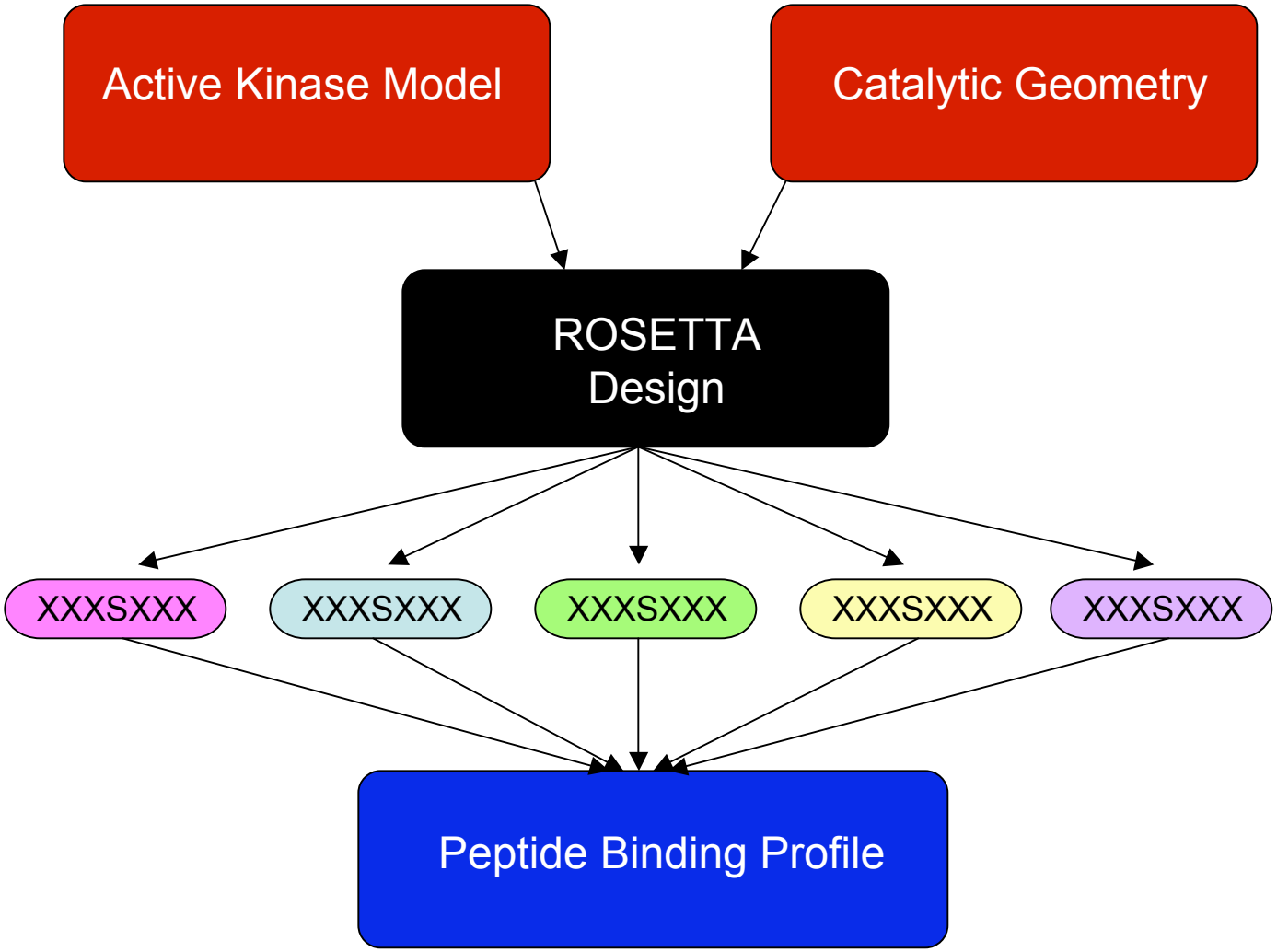
- <u>Most Thr/Ser kinases use Linear Docking Motifs</u>



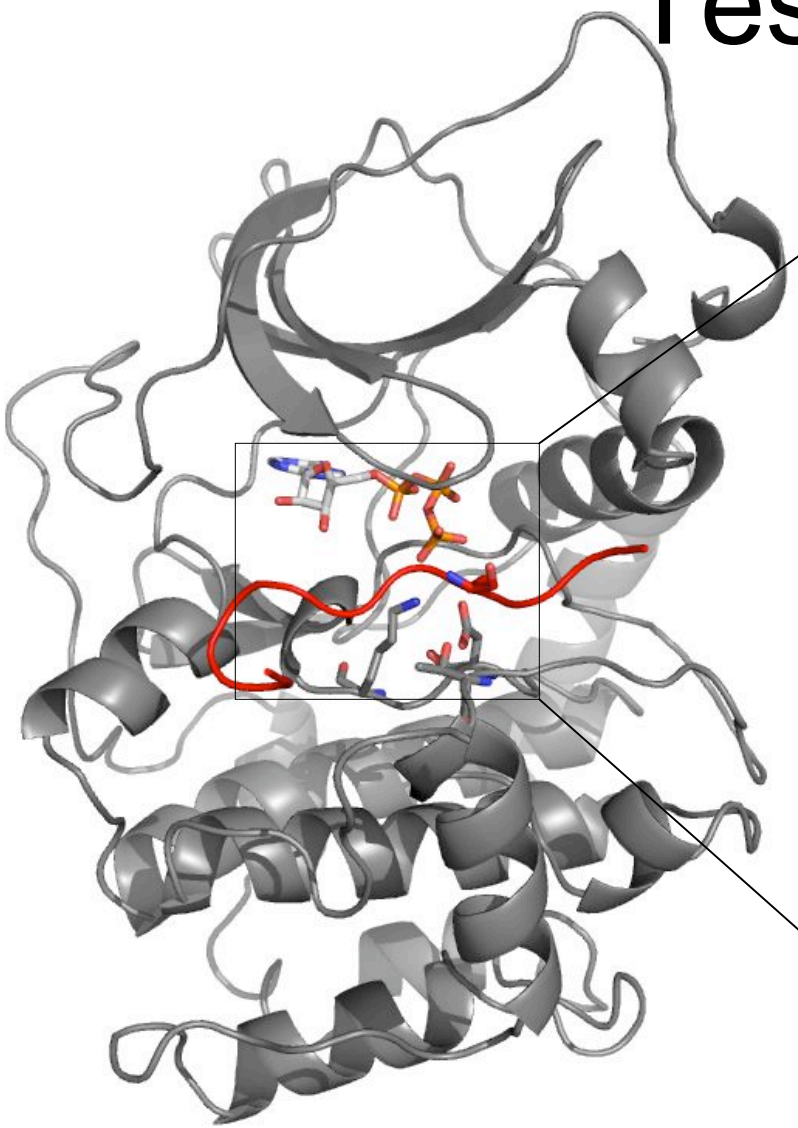


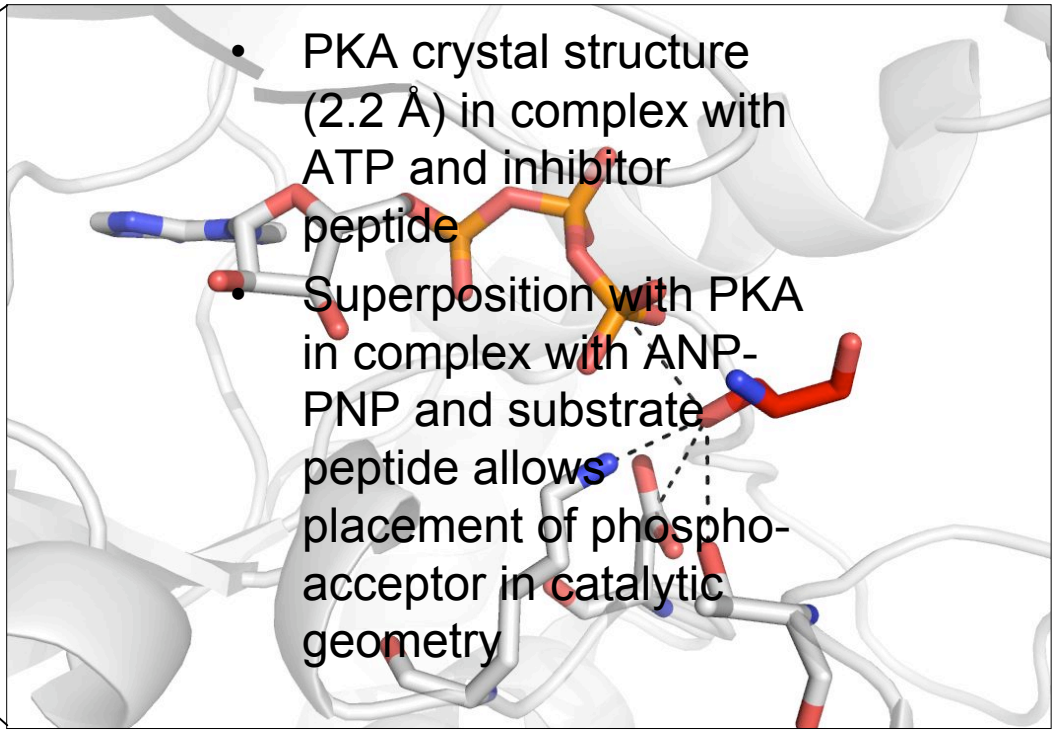
Remenyi et al. *Curr. Opin. Struct. Biol.* 2006, **16**:676

Goal

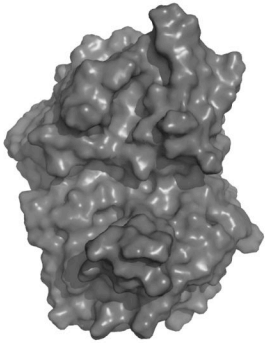

Active Kinase Model
Catalytic Geometry
ROSETTA
Design
XXXSXXX
XXXSXXX
XXXSXXX
XXXSXXX
XXXSXXX
Peptide Binding Profile

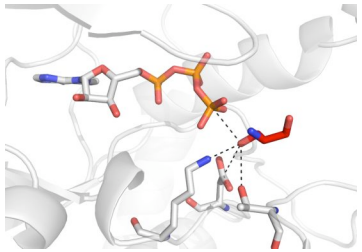
Test System





- PKA crystal structure (2.2 Å) in complex with ATP and inhibitor peptide
- Superposition with PKA in complex with ANP-PNP and substrate peptide allows placement of phospho-acceptor in catalytic geometry

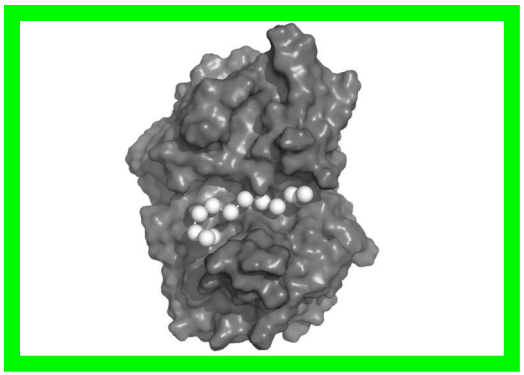




Step 1.

Generate

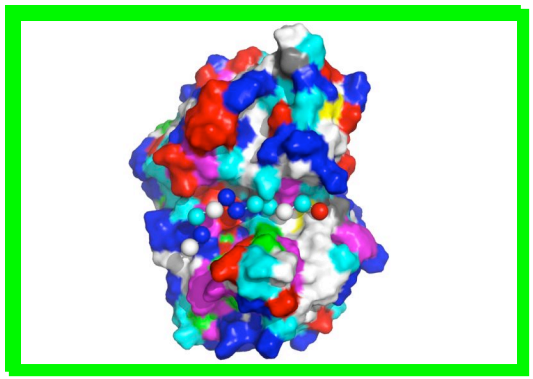
IN Backbone OUT



Step 2.

Refine

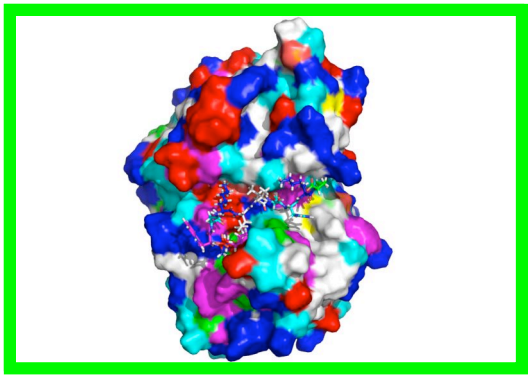
IN Backbone OUT



Step 3.

Design

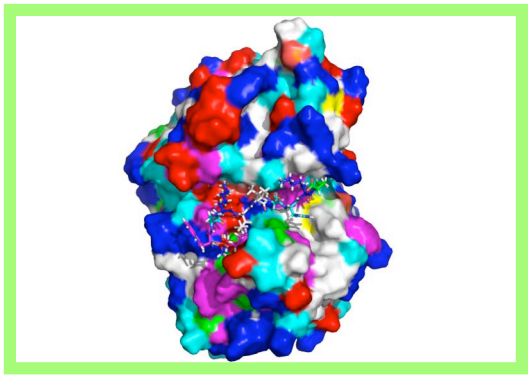
IN Sequence OUT



Step 4.

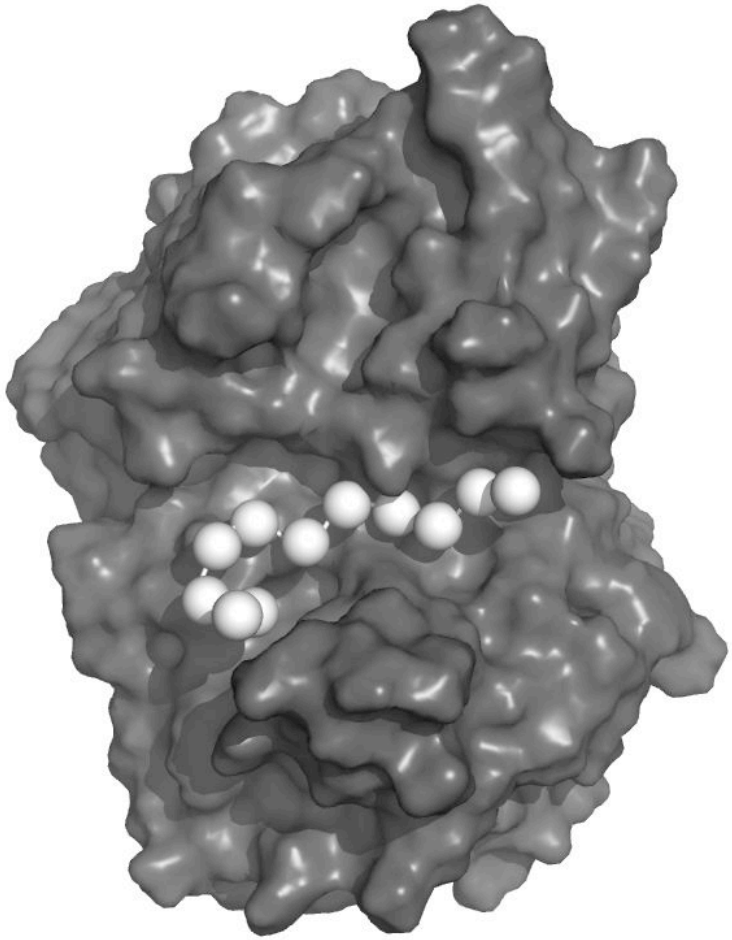
Sequence Motif

Predicted Peptides	Score
RKFSIVH	7
GRKSPPG	14
RGSSPPI	3
HILSGED	21
SGRSKRI	9



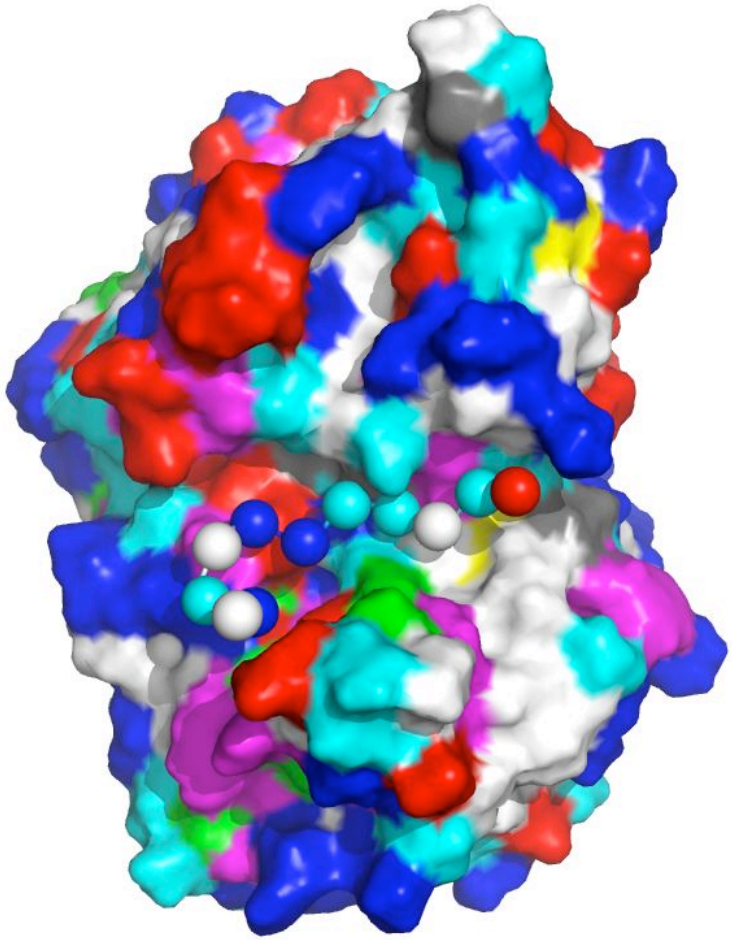
1. Generate Backbone

- Start with known phospho-acceptor conformation
- Generate ghost peptides via fragment insertion
- Scoring function penalizes clashes and backbone solvent accessibility



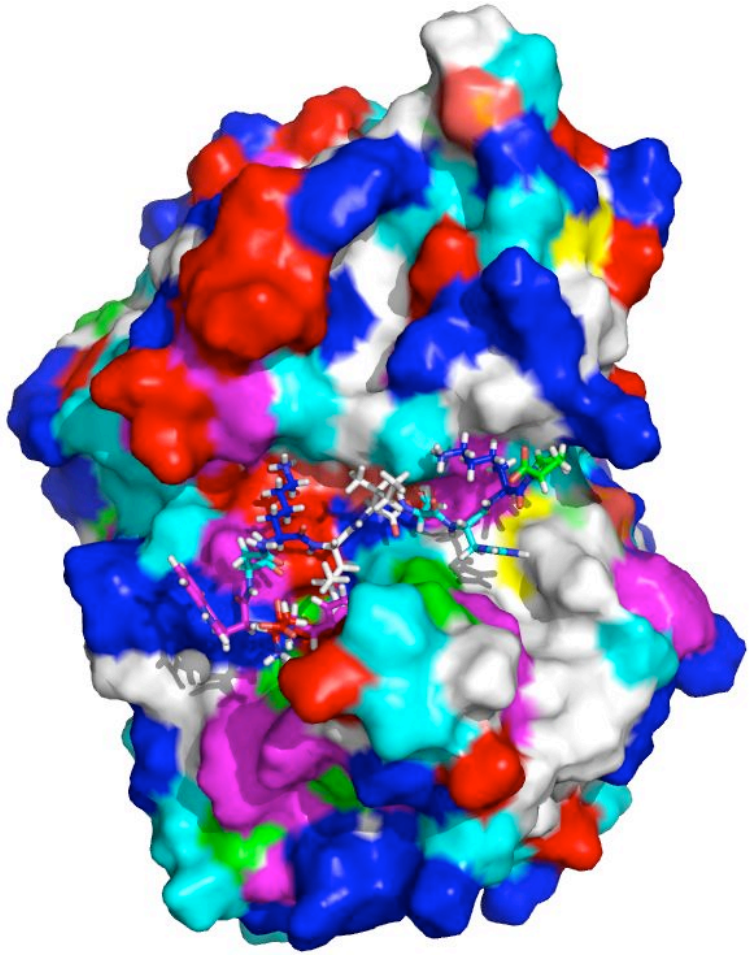
2. Refine Backbone

- Assign random sequence and turn on sequence-specific scoring terms
- Optimize backbone structure with small torsional rotations



3. Design Sequence

- Switch to all-atom representation and design optimal sequence
- Refine backbone structure with tiny backbone moves and gradient minimization
- Scoring function uses all-atom terms with soft-core VdW potential



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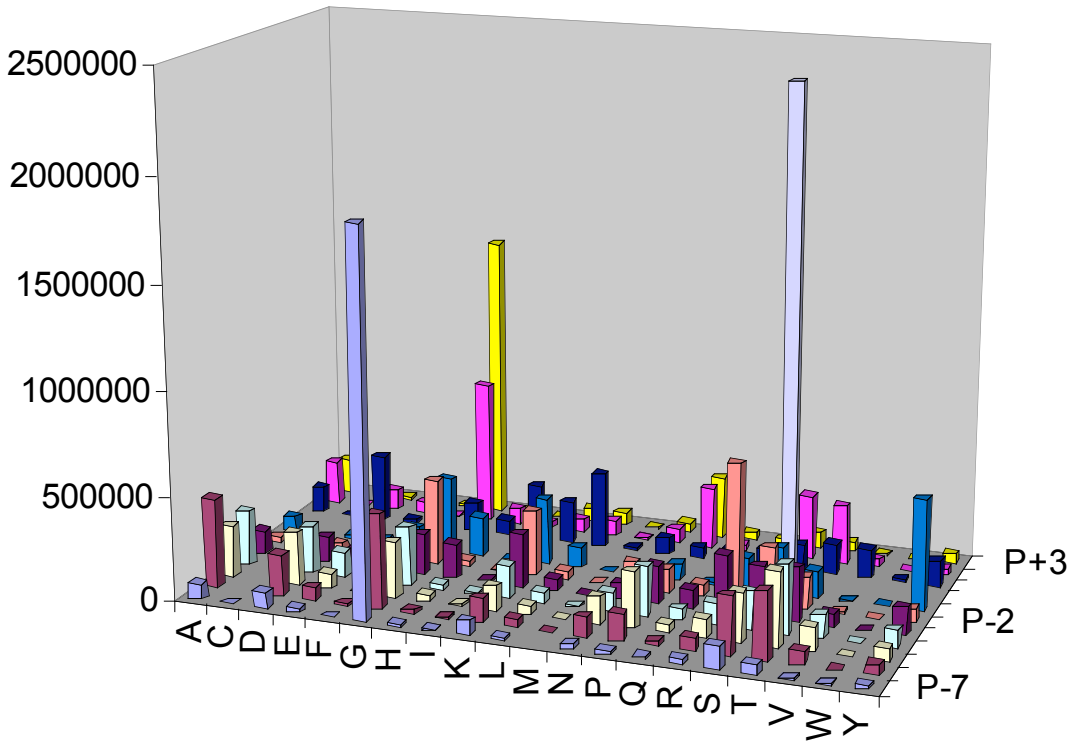
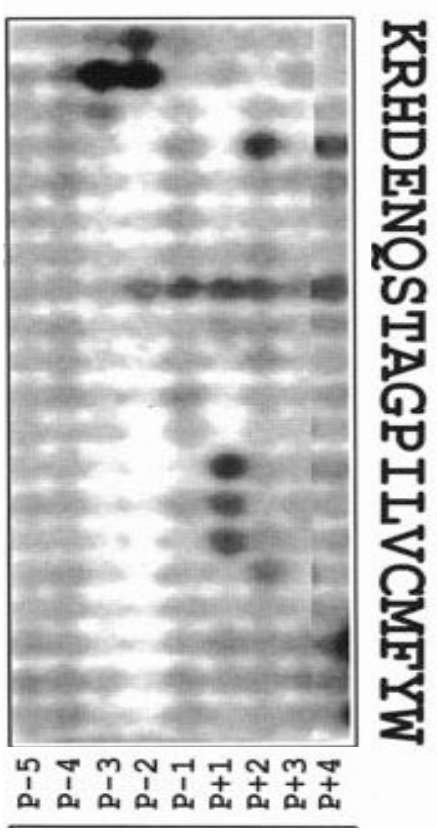


Experimental Verification

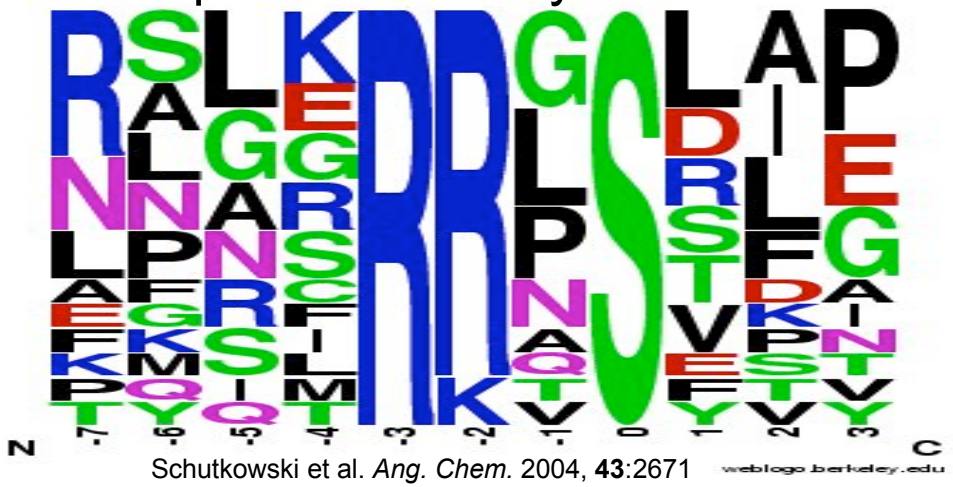
- Peptide Microarray



Motif R[RK]X[ST][ILVFY][DCX]XD

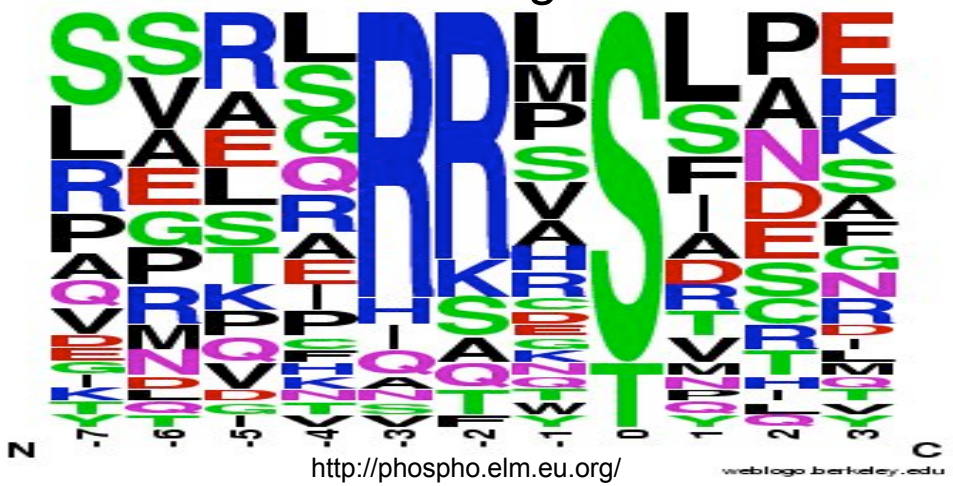
Rodriguez et al. *J. Biol. Chem.* 2004, **279**: 8802

- Peptide Microarray



Schutkowski et al. *Ang. Chem.* 2004, **43**:2671

- *In vivo* kinase target database



http://phospho.elm.eu.org/

- ROSETTA prediction
- Peptide Microarray
- *In vivo* kinase target database