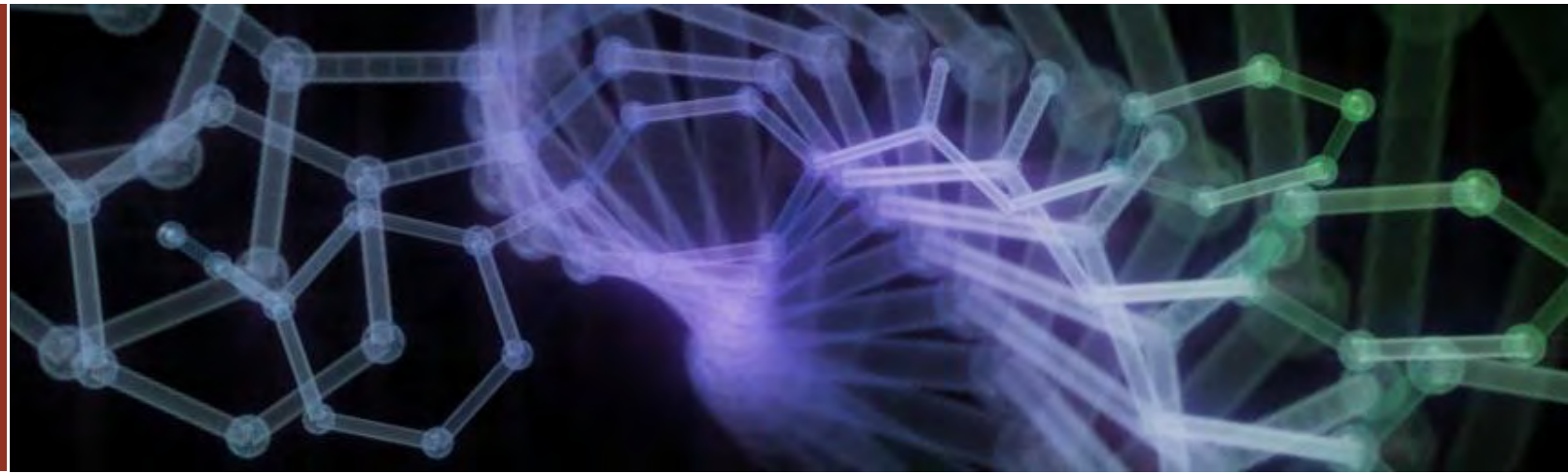

The ABC of kinase conformations

Interplay of conformation, sequence and ligand binding

Henrik Möbitz

Protein Kinase 2012

Accelrys, Cambridge Science Park, Cambridge, 21. May 2012

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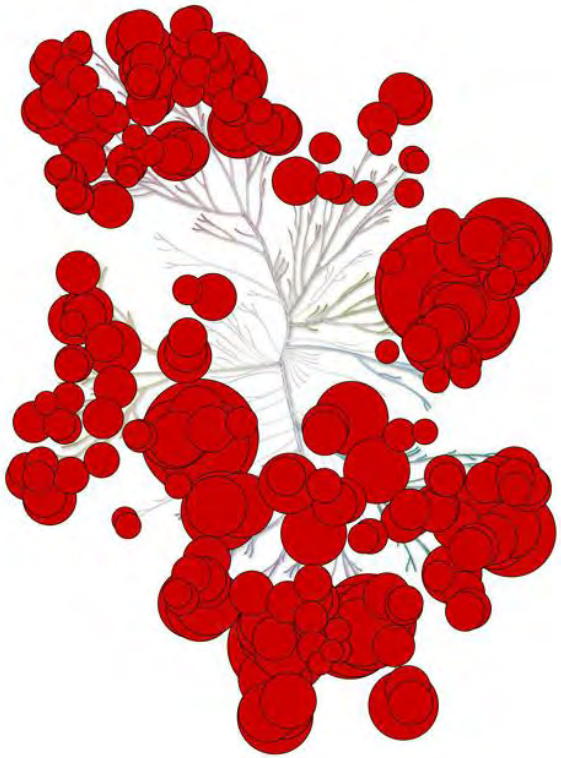
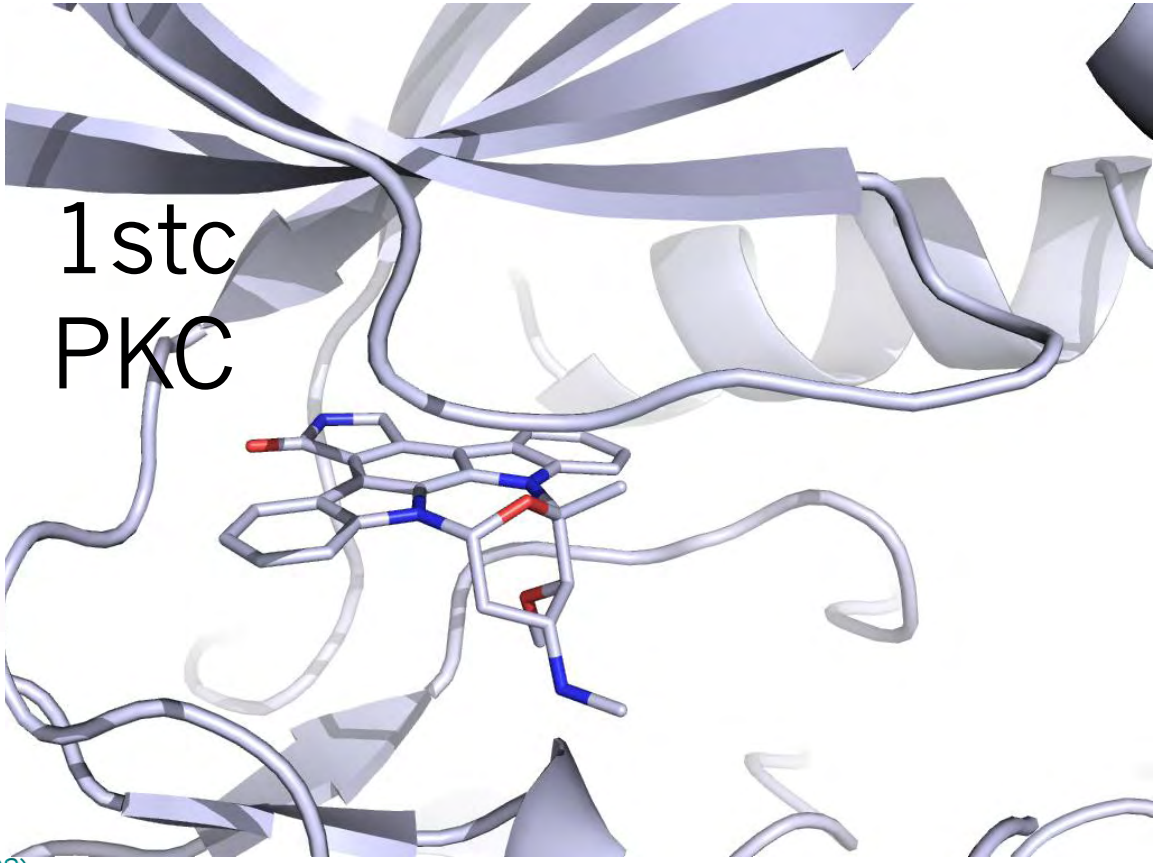
STU's journey through the kinaverse

...or the inherent flexibility of kinases





Karaman *et al.*, Nature Biotechnology 26, p.127 (2008)

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Universal residue nomenclature & alignment

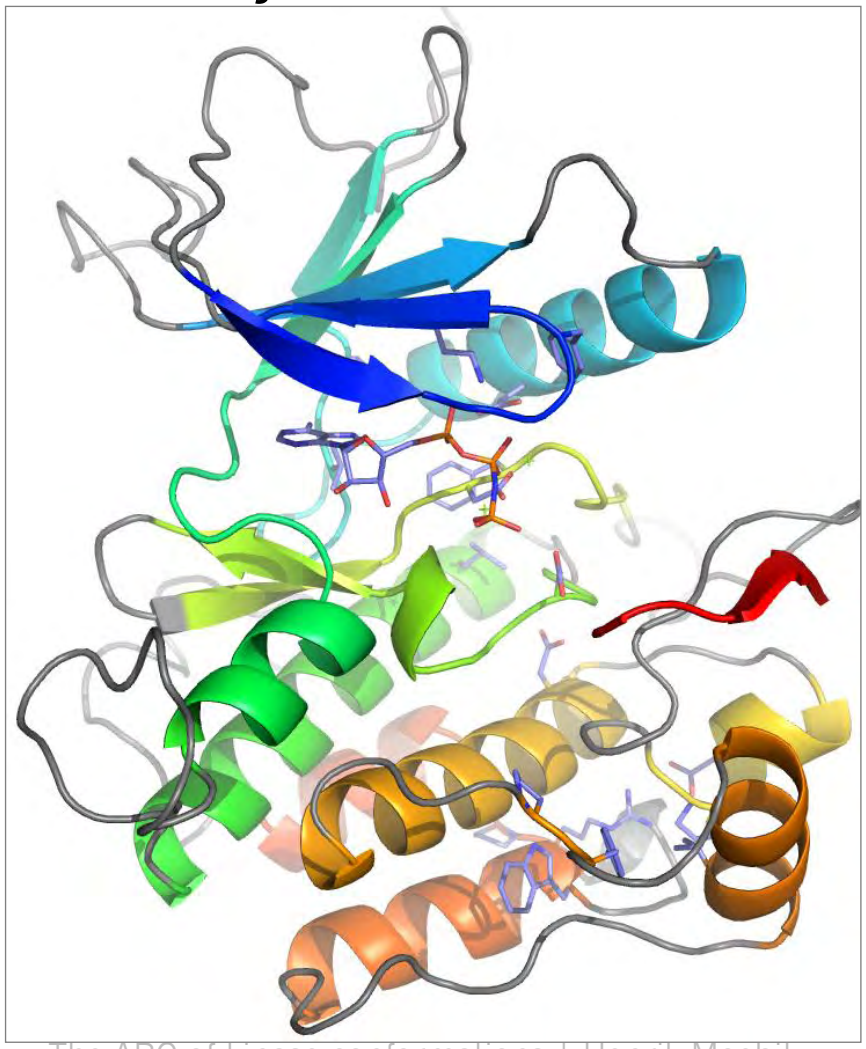
The ABC builds upon a structure based sequence alignment, in which every residue is named by distance to the closest anchor point

structurally conserved

N-term

C-term

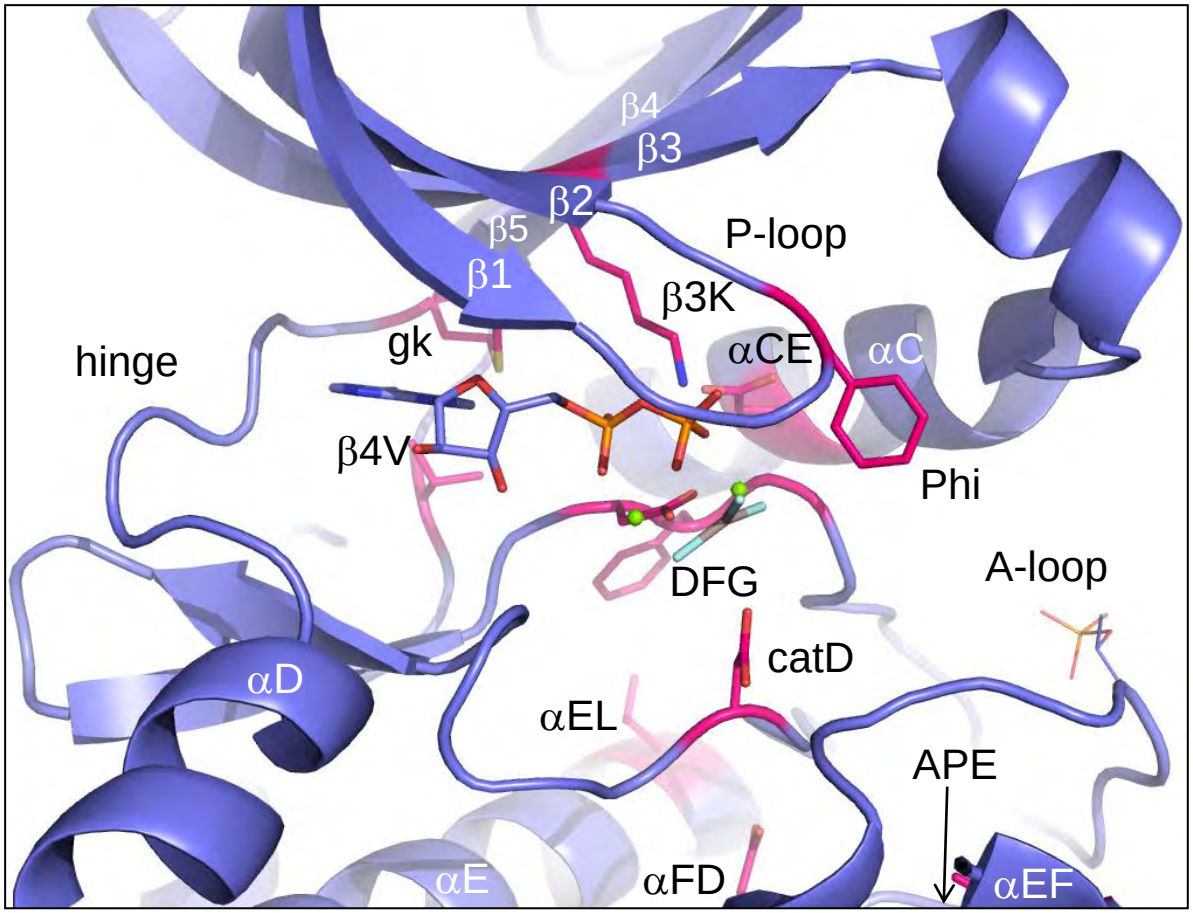
variable loops/inserts



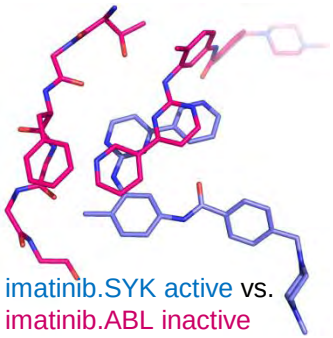
Universal residue nomenclature & alignment

The ABC builds upon a structure based sequence alignment, in which every residue is named by distance to the closest anchor point

subdomaine	anchor	residue PKAa
P-loop	Phi	54
β3	β3-Lys	72
αC	αC-Glu	91
β4	β4-Val	104
β5/hinge	gk	120
αE	αE-Leu	157
catalytic loop	catD/N	166/171
start A-loop	DFG	184-186
end A-loop	APE	206-208
αF	αF-Asp	220
	PY	237/238
αG	αG-Ile	250
αH	αH-Trp	273
	RP	280/281



Conformational bias governs key aspects of kinase biology



imatinib.SYK active vs.
imatinib.ABL inactive

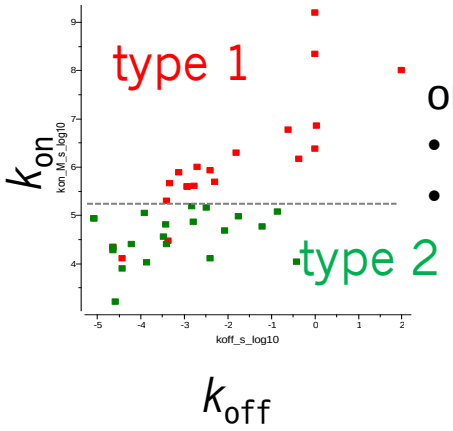
binding mode /conformation

"How much energy does the target conformation cost?"

ΔG_{conf}

$\Delta\Delta G_{conf}$

"How similar/accessible is the target conformation in other kinases?"



on- target effects:

- potency
- mode of action: kinetics, ATP competition, mutant susceptibility

off-target effects:

- kinase selectivity
- ph4-properites (e.g., hERG, IP, solubility)

imatinib Ambit panel

Conformational bias

physiologic

Inhibition

negative regulation
allosterism (e.g. myristate pocket)

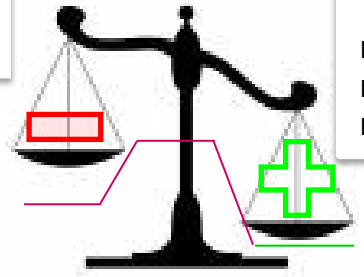
Activation

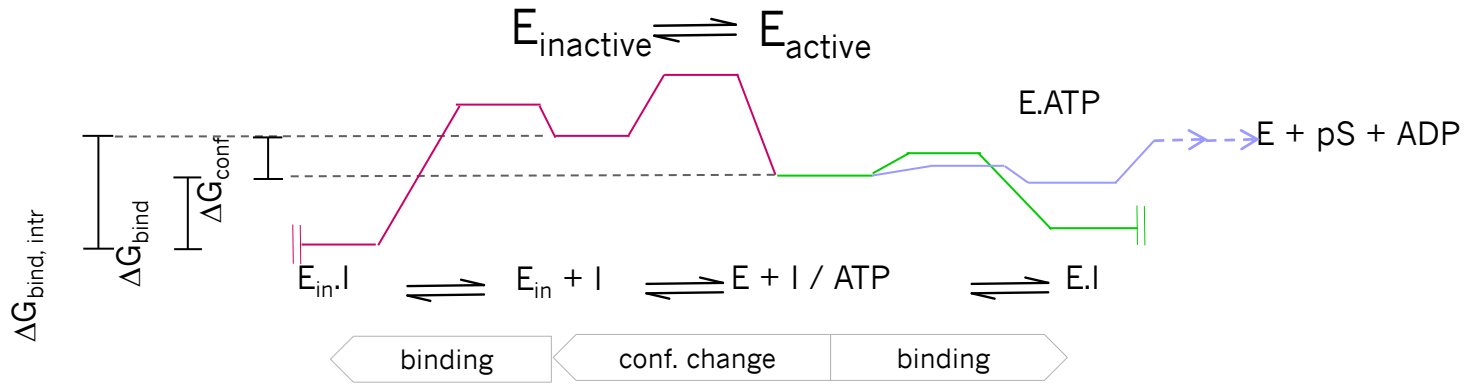
repression of negative regulation
positive regulation / effector binding (e.g. Cyclin)
phosphorylation
dimerization

pathologic

inhibitor binding

mutation
resistance mutation, helix C insertions/deletions
nucleotide, inhibitor binding (active conformation





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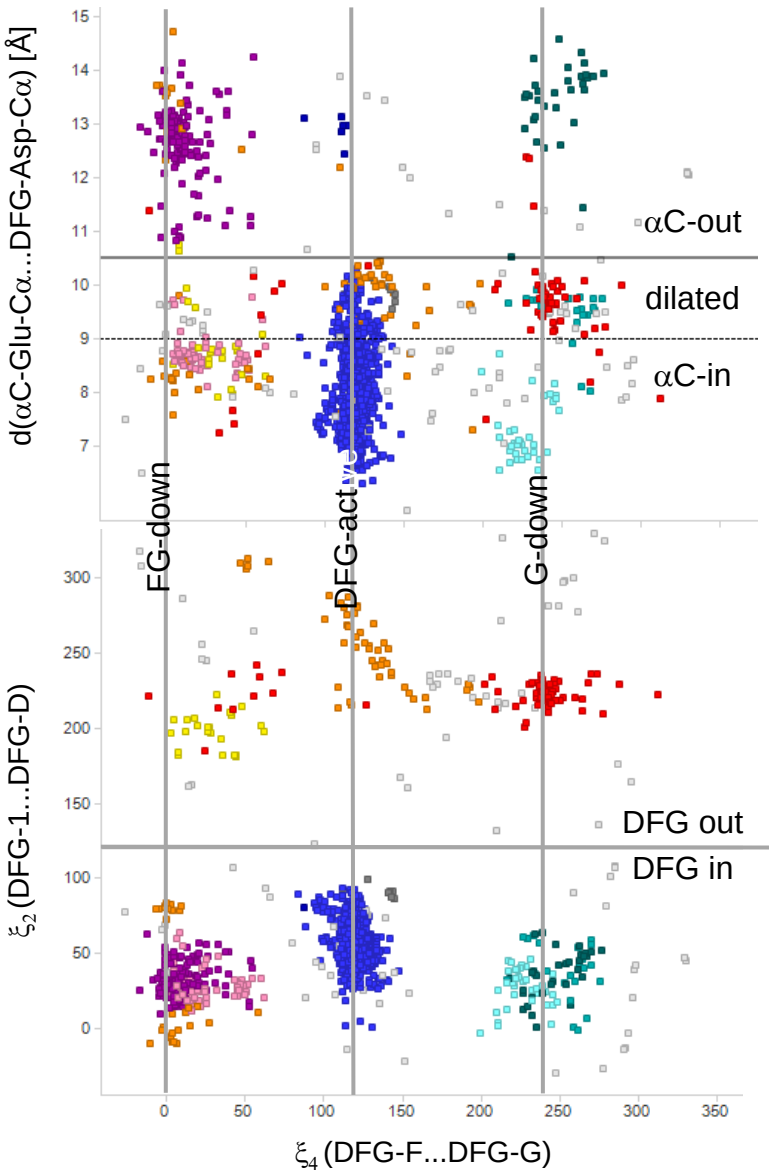
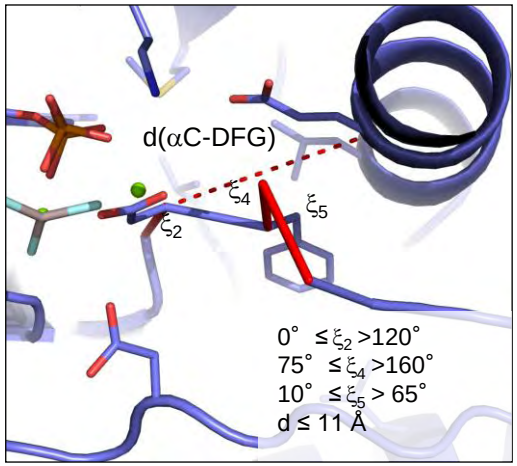
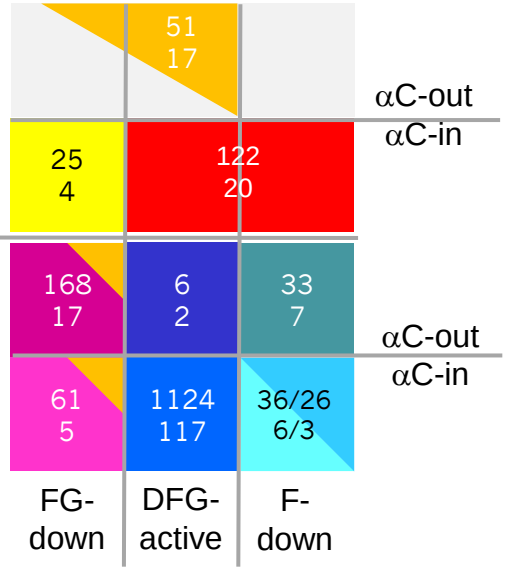
The London tube maps of conformational space







- A-helix
- AuP BRAF
- AuP FAK
- AuP FMS
- AuP MARK
- AuP MET
- DF flipped
- DFG out
- FG down
- G down C-helix out
- G down JNK
- G down P38
- active
- disordered
- other

Major conformations of kinases

Conformation	n(chains)	n(unique kinases)	%	Template	helix-αC	DFG
active	1124	117	58.7	1l3r, 1ir3	in	active
DFG-active αC-out	6	2	0.3	1k2p	out	active
FG-down	61	5	3.2	3dt1	in	in
FG-down αC-out	168	17	8.8	1hck	out	in
G-down JNK	36	6	1.9	1jnk	in	in
G-down p38	23	3	1.2	1p38	dilated	in
G-down αC-out	33	7	1.7	1s9j	out	in
DFG-flipped	25	4	1.3	1opk	in – dilated	out
DFG-out type 2	122	20	6.4	1iep	in – dilated	out
A-under-P BRAF	28	7	1.5	1uwh	dilated	out
A-under-P FAK	5	2	0.3	2jko		out
A-under-P FMS	18	8	0.9	1t46	in – dilated	out
A-under-P MARK	18	4	0.9	2hak	in	in
A-under-P MET	12	1	0.6	3ccn	out	in
other	98	30	5.1			
disordered	137		7.1			

pdb, June 2010

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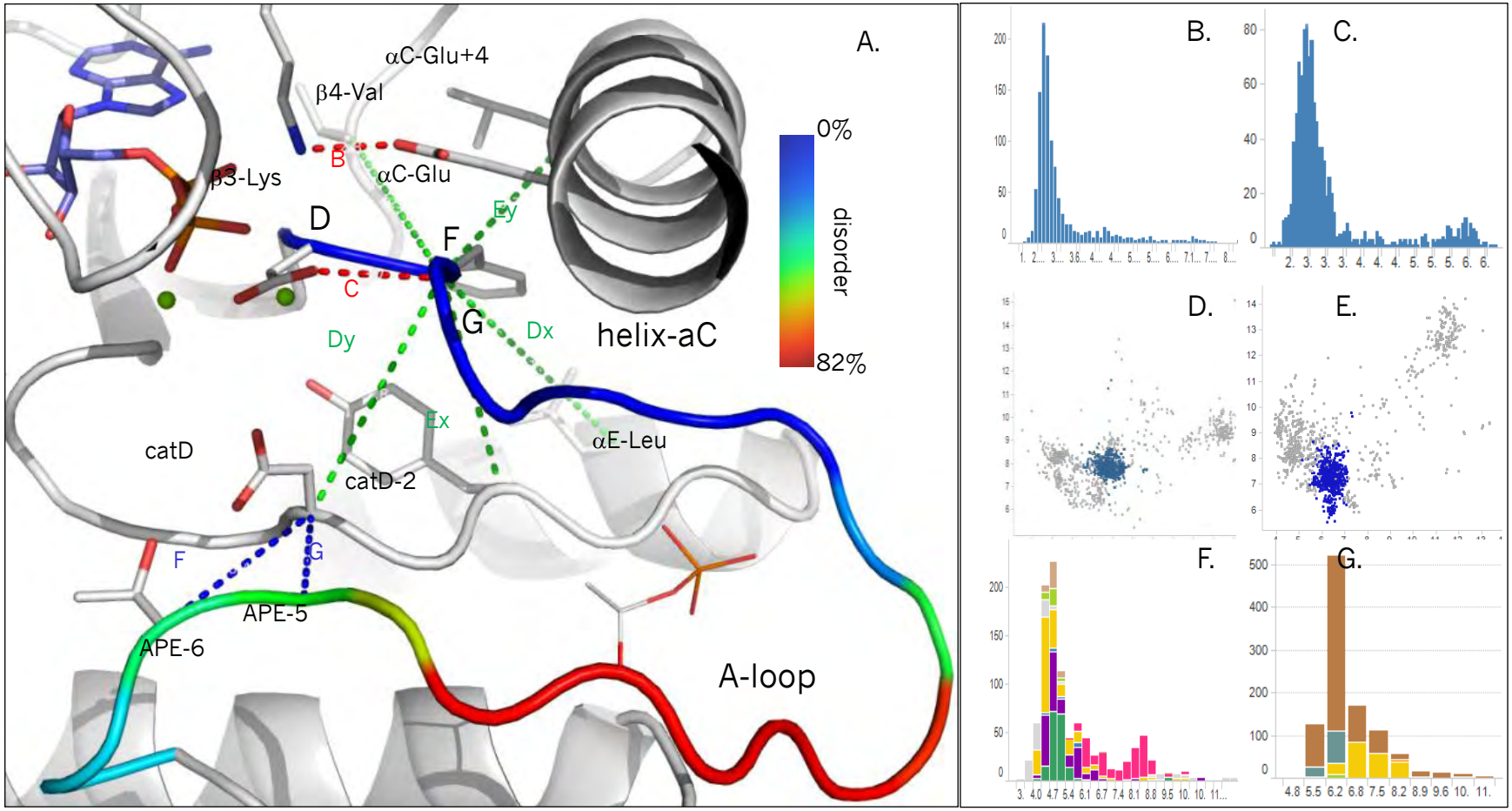
The DFG-helix-αC interaction organizes all key features of the active conformation



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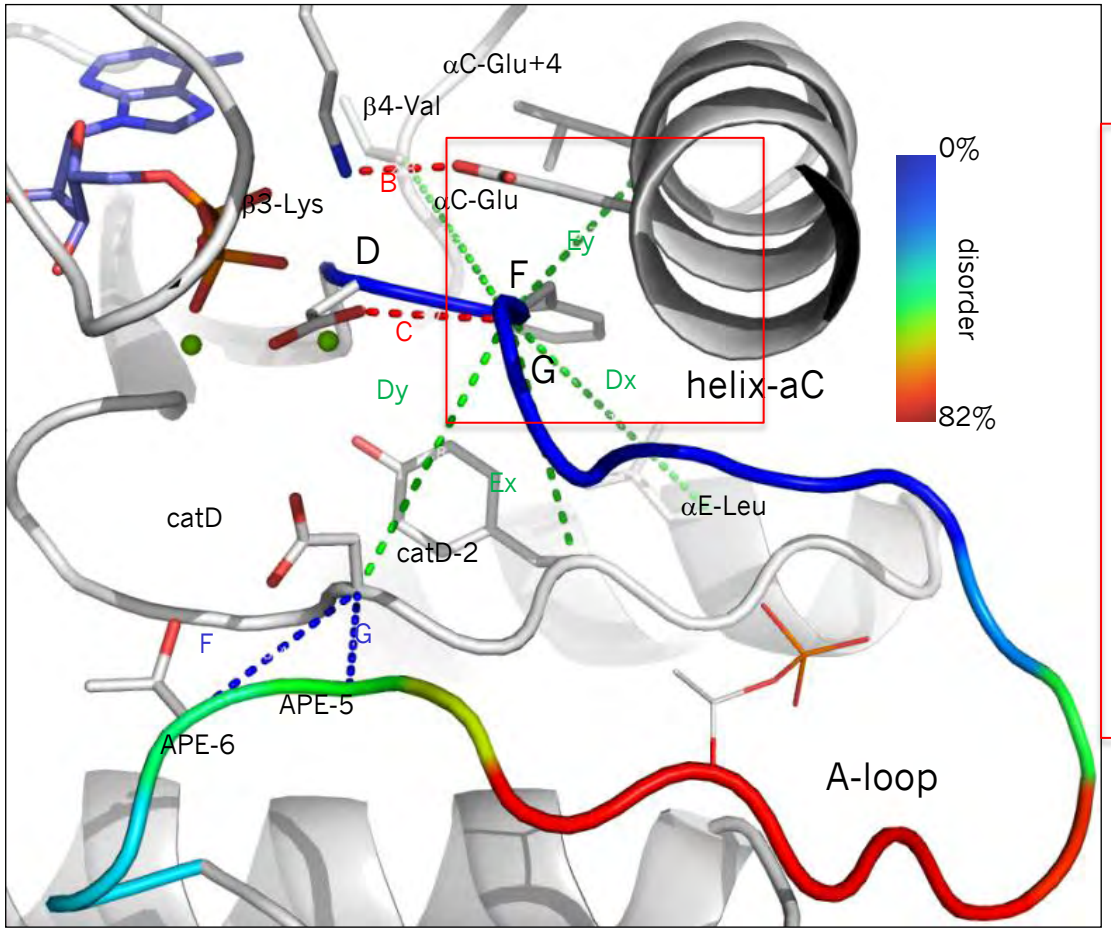
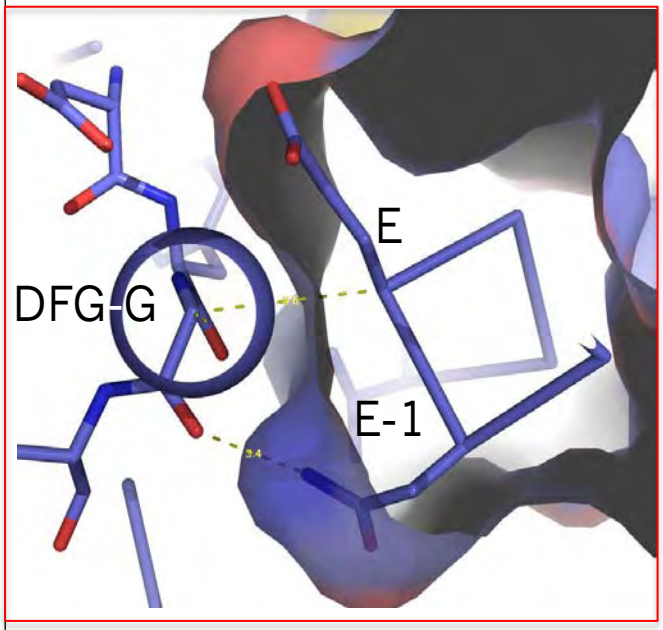
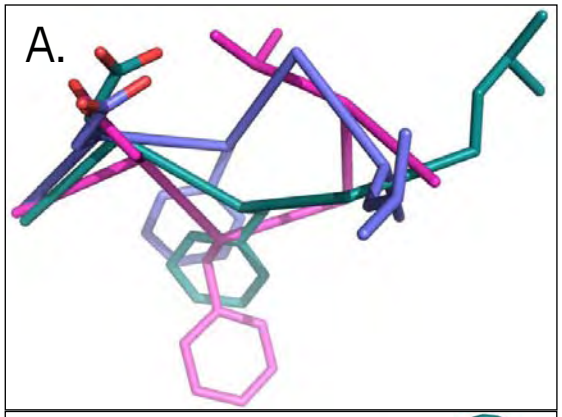
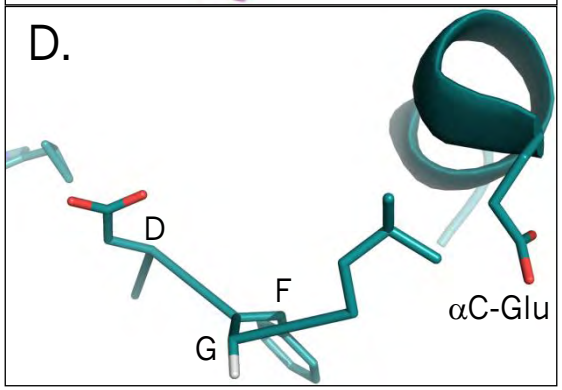
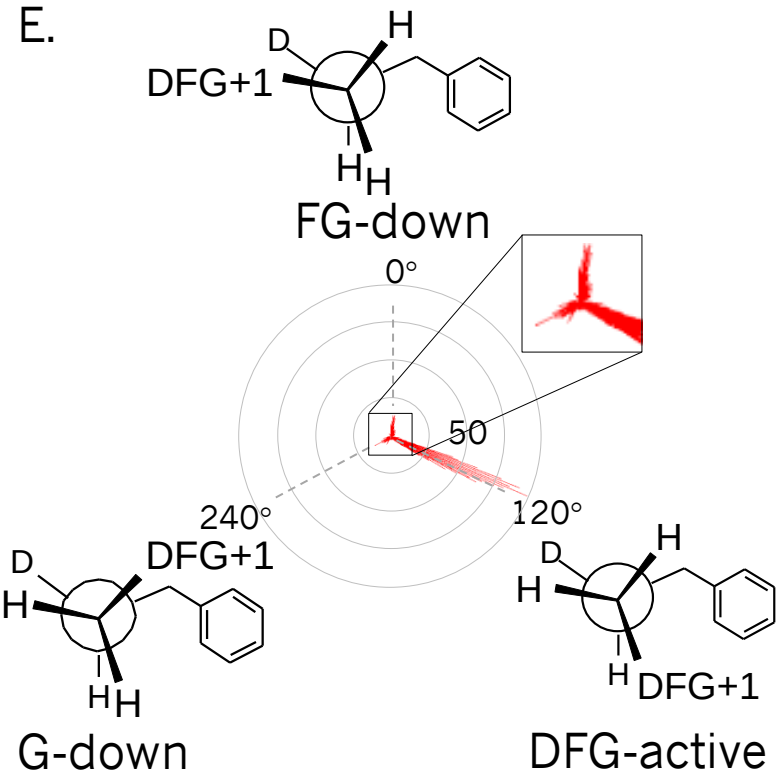


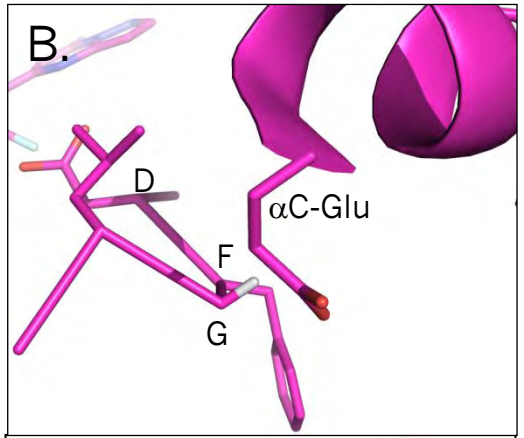


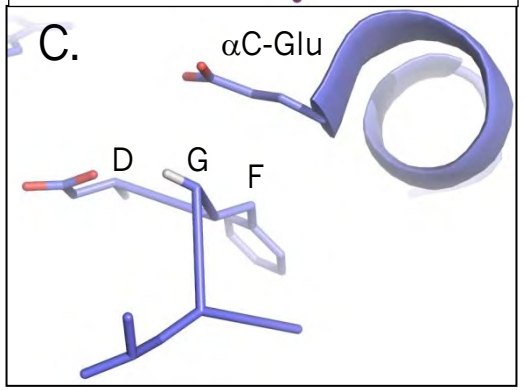
DFG-helix αC interaction weakened in F/FG-down







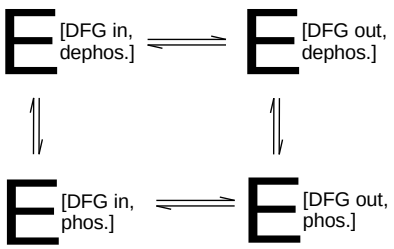


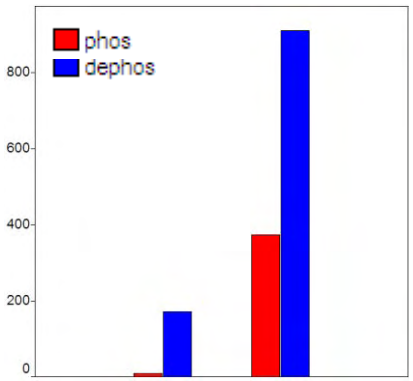


	FG-down	DFG-active	F-down
αC-out	168 17	6 2	33 7
αC-in	61 5	1124 117	36/2 6 6/3

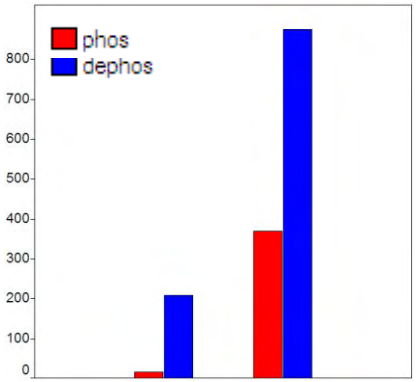
More conformational biases

ΔΔG = -1.0 kcal/mol



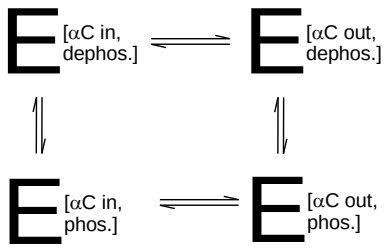


DFG out – in



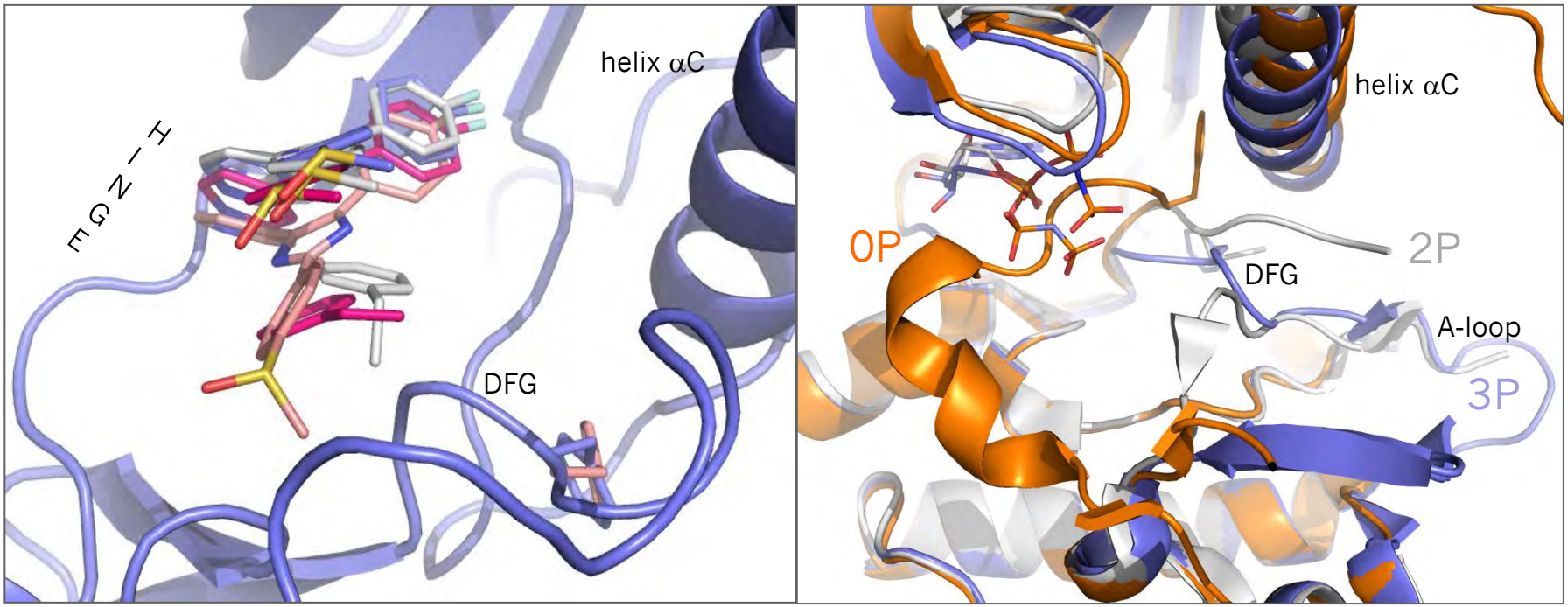
helix C out – in

ΔΔG = -0.9 kcal/mol



bias	population	n(all/bias)	$\Delta\Delta G_{conf}$ (DFG in-out)	$\Delta\Delta G_{conf}$ (helixC in-out)
phosphorylation	all	1885/463	-0.9	-1
cyclin	CDKs	438/136	+0.1	-3
nucleotide binding	nucleotide vs. apo	634/246	-0.3	+0.1
type 1 inhibitor binding	type 1 bound vs. apo	1436/1048	-0.3	+0.2
type 2 inhibitor binding	type 2 bound vs. apo	539/151	+2.5	-0.2
DFG-active	DFG-active DFG-in	1130	NA	+3.8
FG-down	FG-down DFG-in	229	NA	-0.5
G-down	G-down DFG-in	92	NA	0

Conformational equilibrium and bias at work



	pdb	Conformation DFG
p38a.SB203580	1a9u	DFG-in
p38a.SB203580	2ewa	DFG-in
p38a.SB203580	2ewa	DFG-out
p38a.SB203580	3gcp	DFG-out

	pdb	Conformation	V_{max}/K_m
IGF1R-3P	1k3a	active (DFG-in)	197
IGF1R-2P	1jqh	other (DFG-in)	118
IGF1R-0P	1p4o	A-under-P (DFG-out)	1.6

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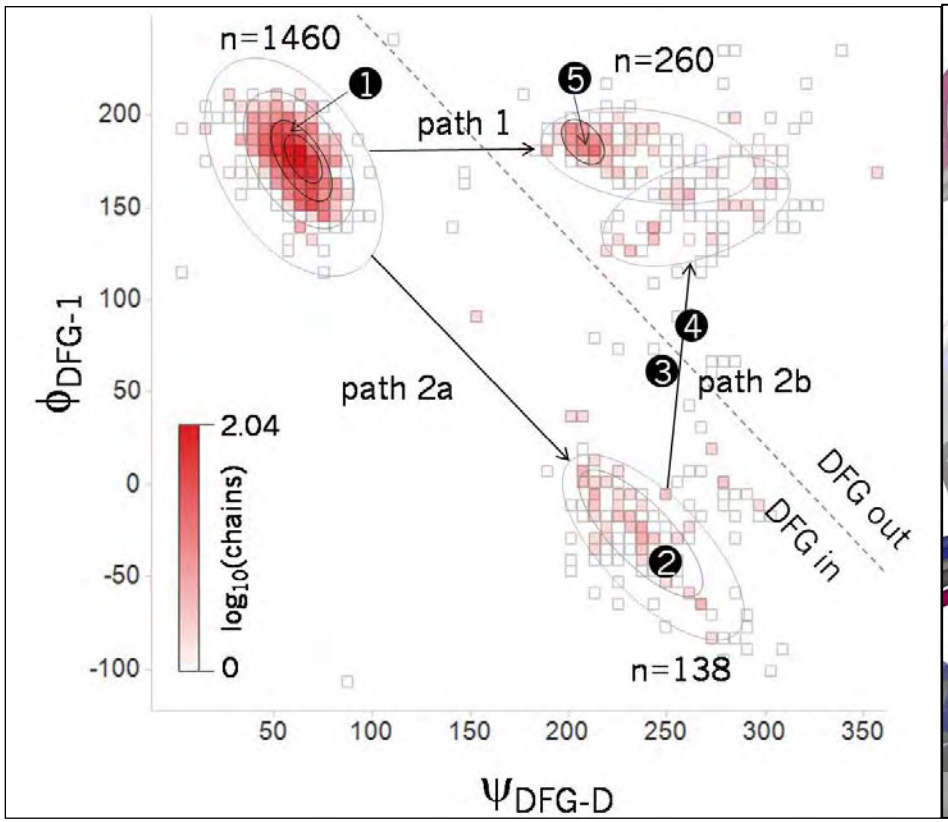
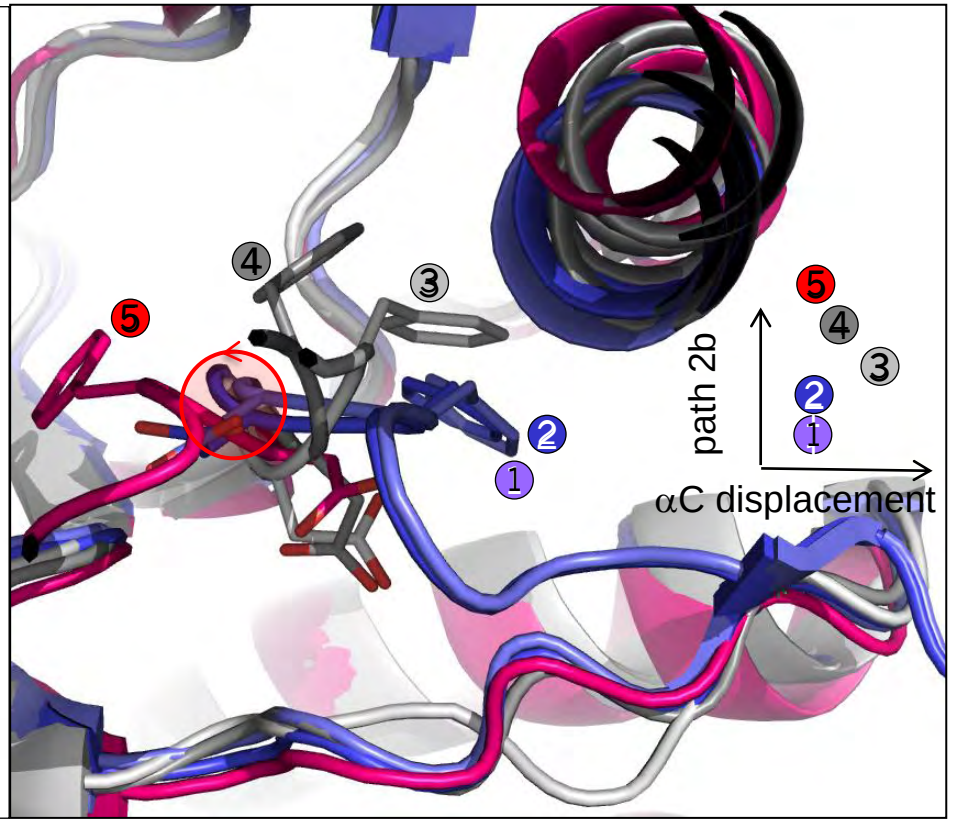
Mechanism of DFG out transition

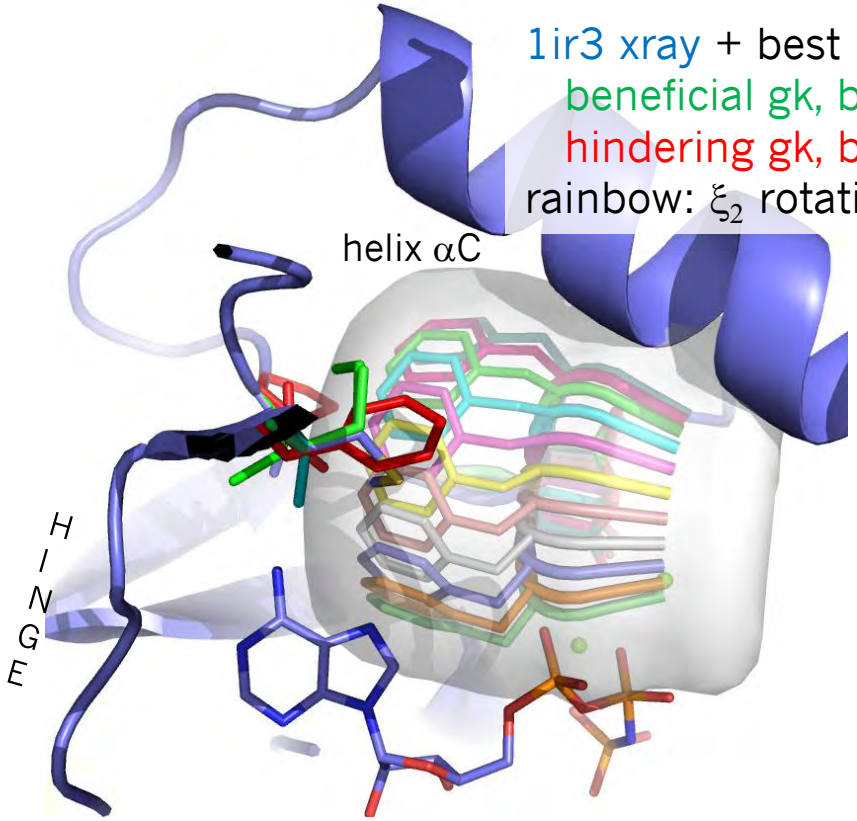
A side chain rotation between DFG-1 and DFG that requires a temporary displacement of helix-αC.





Sequence bias of DFG out transition

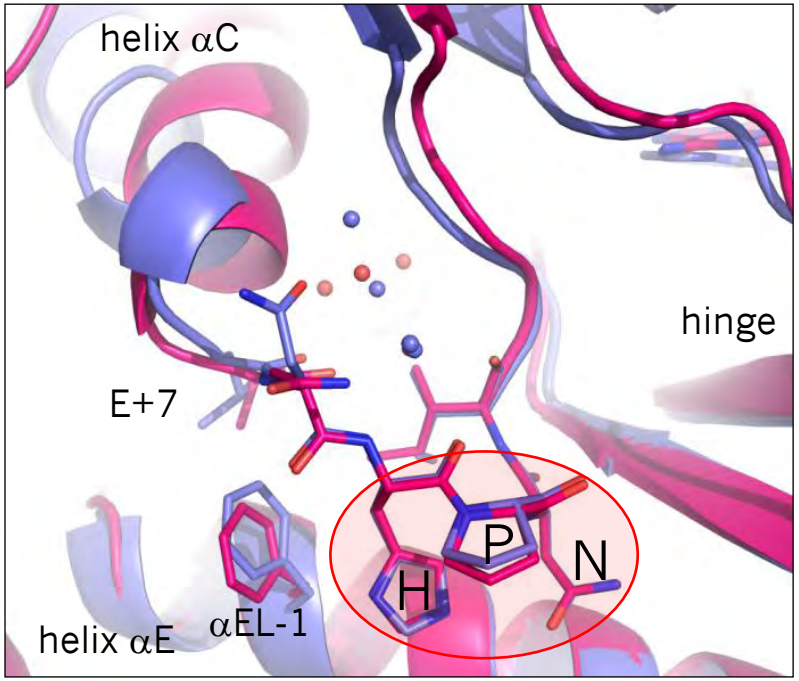
hydrophobic, flexible residues lubricate transition
polar residues hinder transition – HBs need to be broken
small, hydrophobic DFG-1 (A,G,C,V) stabilize DFG out
rotamers need to be compatible with DFG out switch
helix-αC flexibility strictly required to allow DFG out rotation

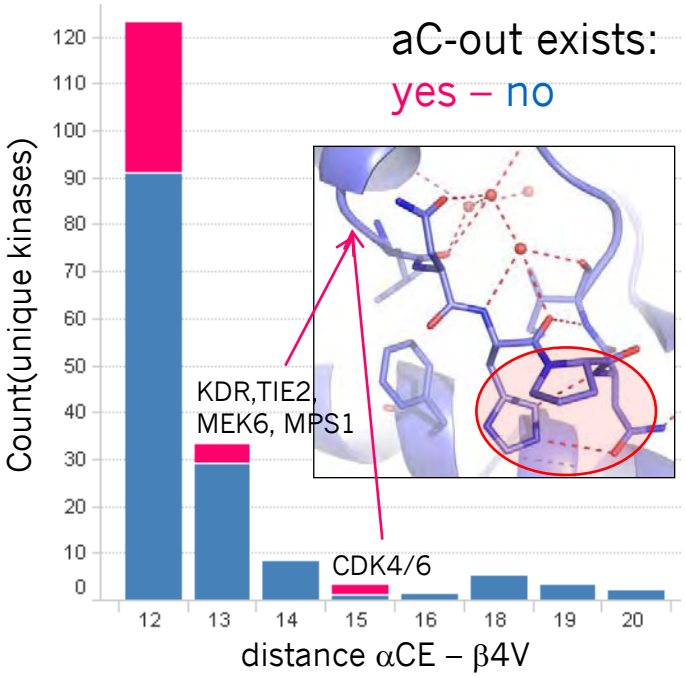


Mechanism of helix C out transition

rigid HPN hairpin between helix αC and β4V pivots transition
inserts at αC N-term and HPN hairpin (after αC+7) disable transition
more end states and lower conservation ⇒ sequence bias less significant
simultaneous bend and twist caused by improper shifts in HPN region

2cch CDK2 active
2r3i CDK2 FG down helix C out





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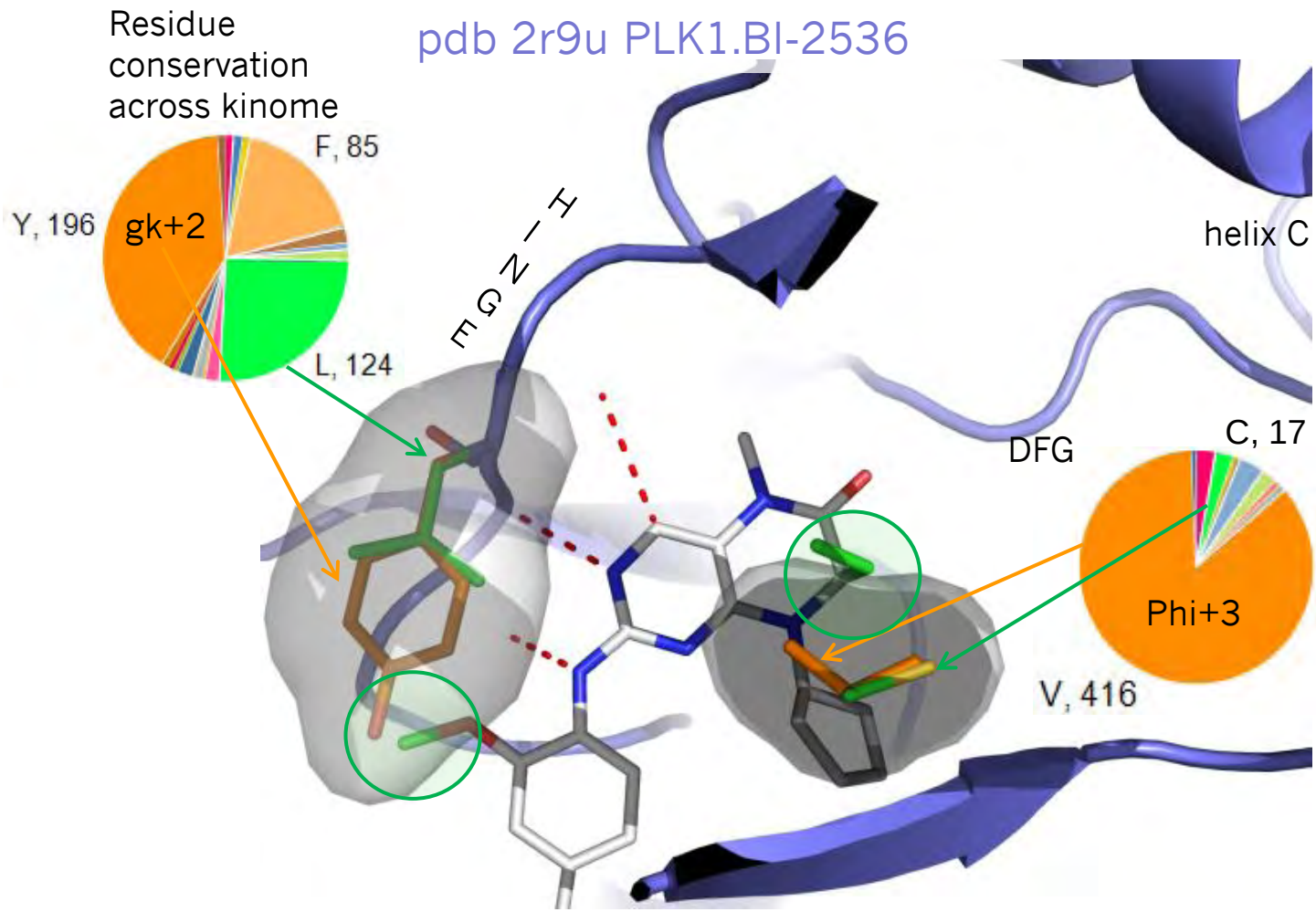
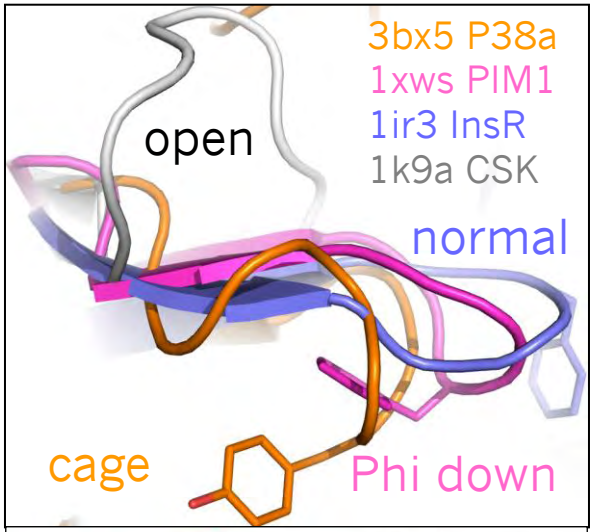
Interaction based selectivity

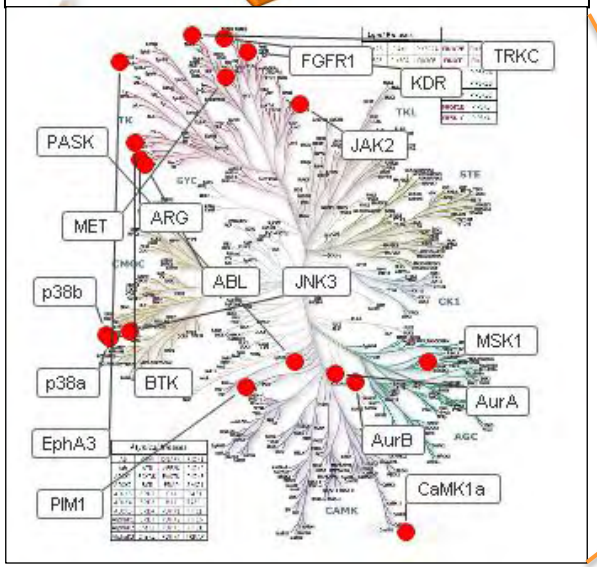
Exploiting residue conservation in (standard) binding mode

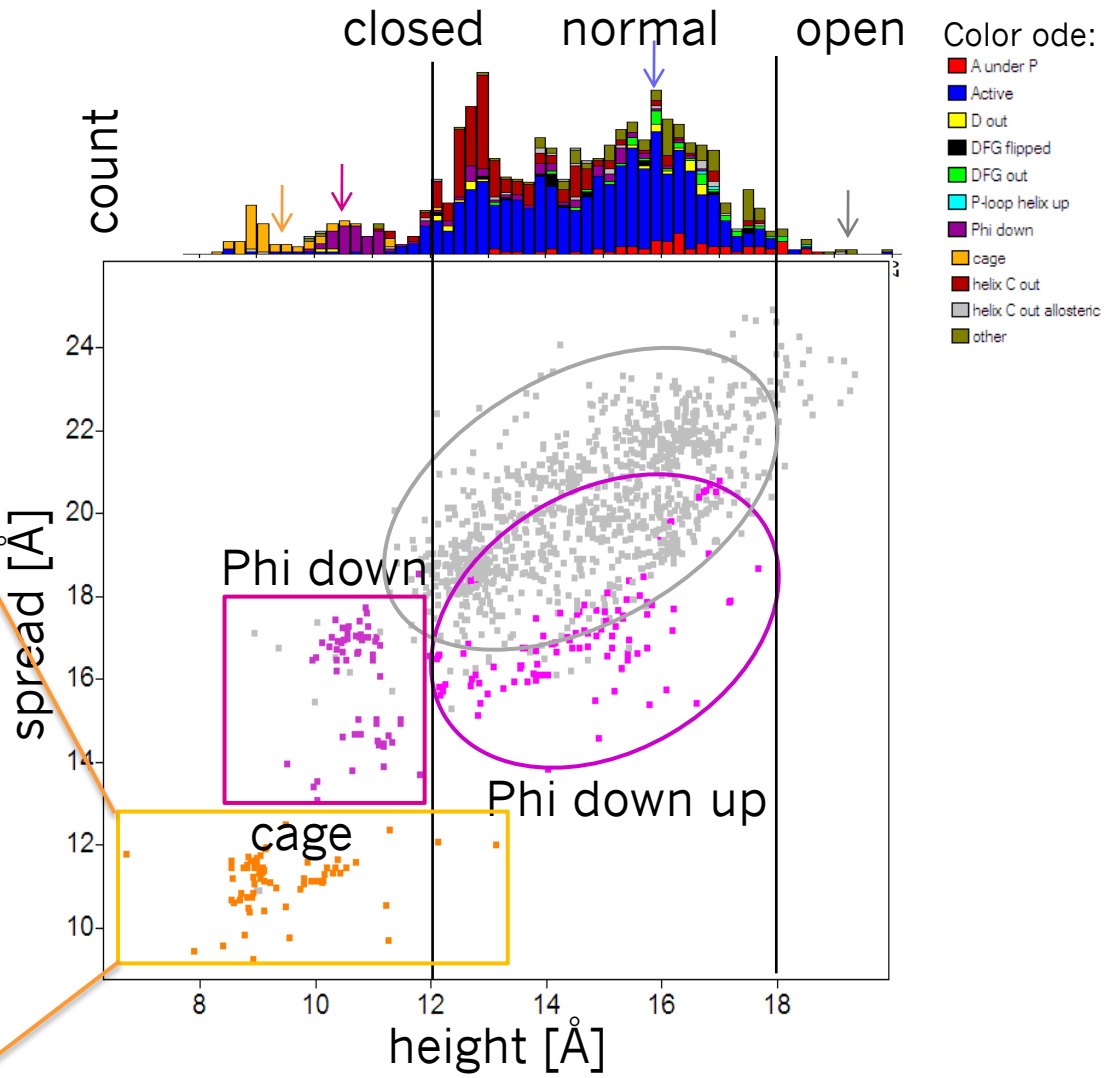


Ligand efficiency-based selectivity

High intrinsic LE due to buriedness: closed P-loop conformations

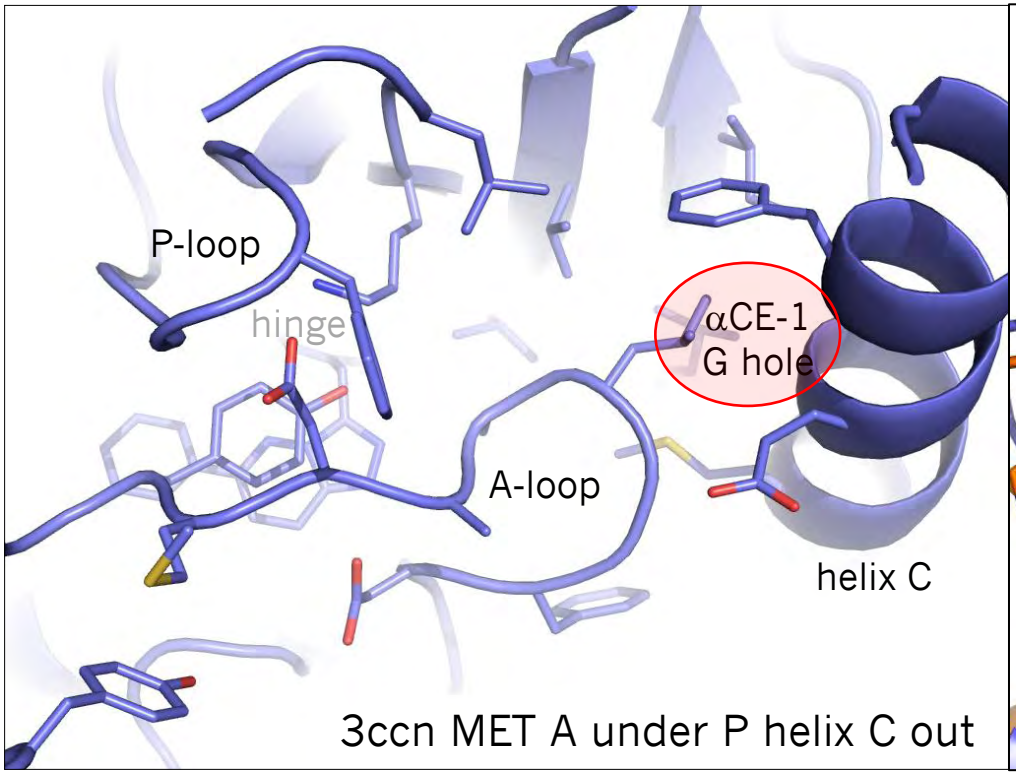


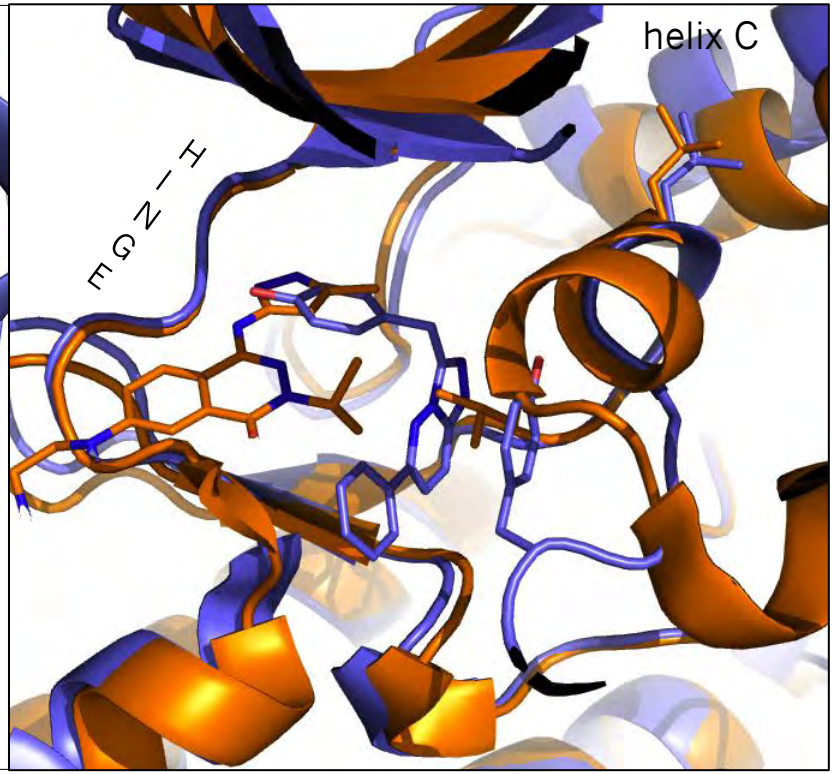




Selectivity from conformational penalty

Unique conformations stabilized by non-conserved residues

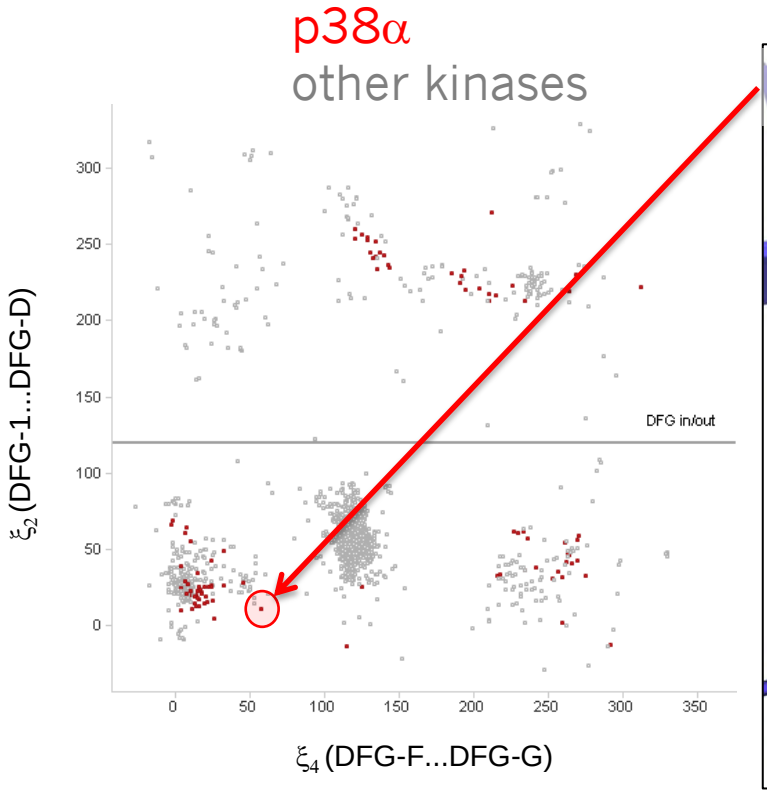


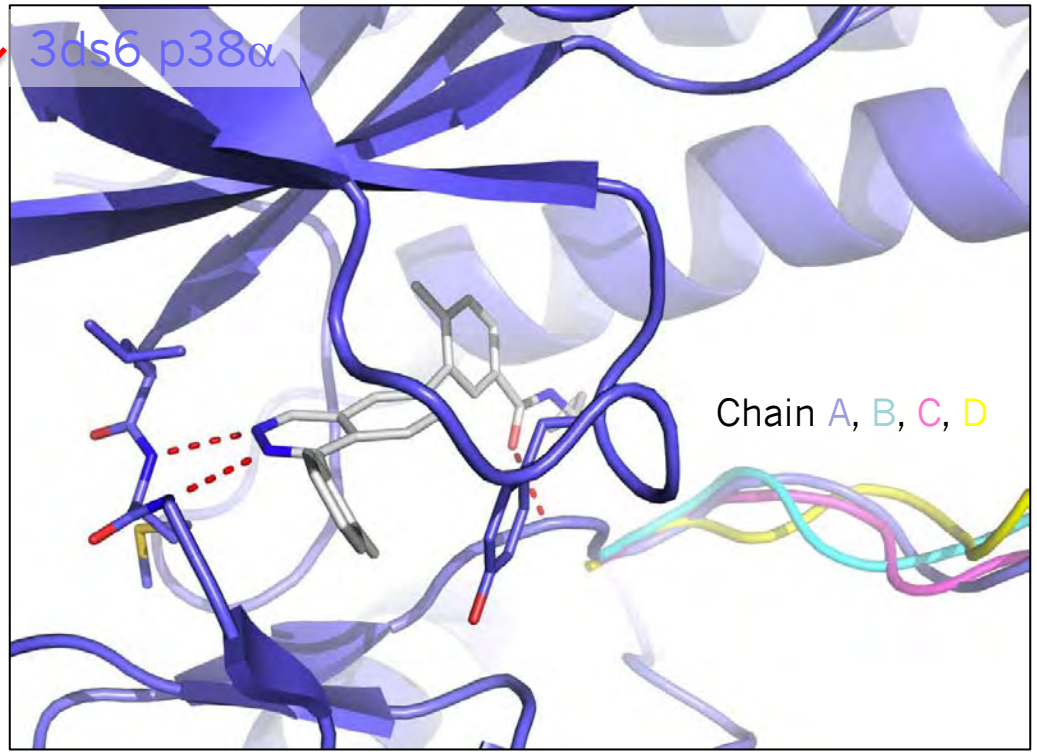


3pix BTK
3ccn MET

Selectivity from conformational penalty

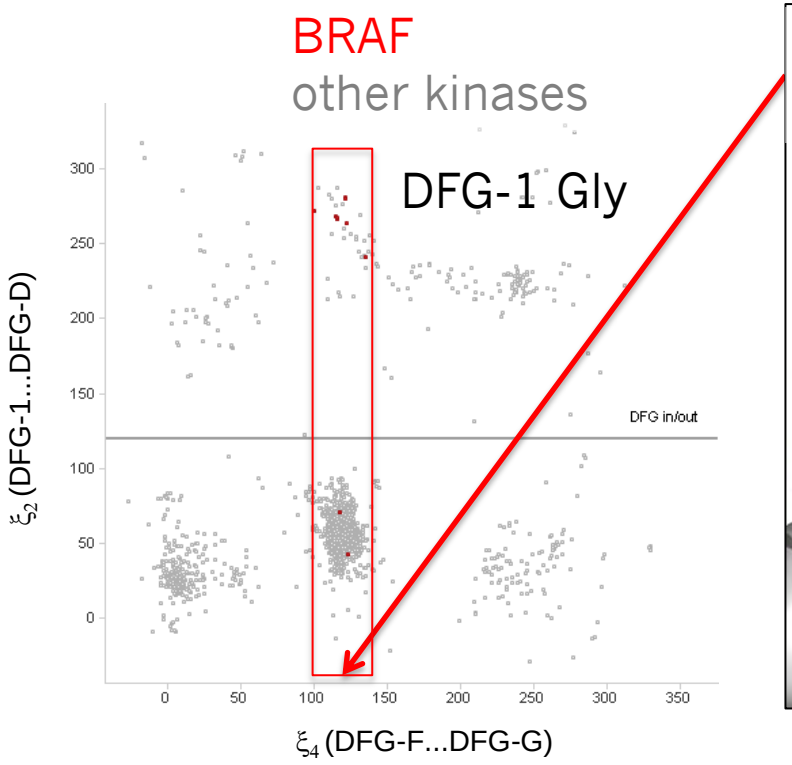
High energy conformations accessible in highly flexible kinases

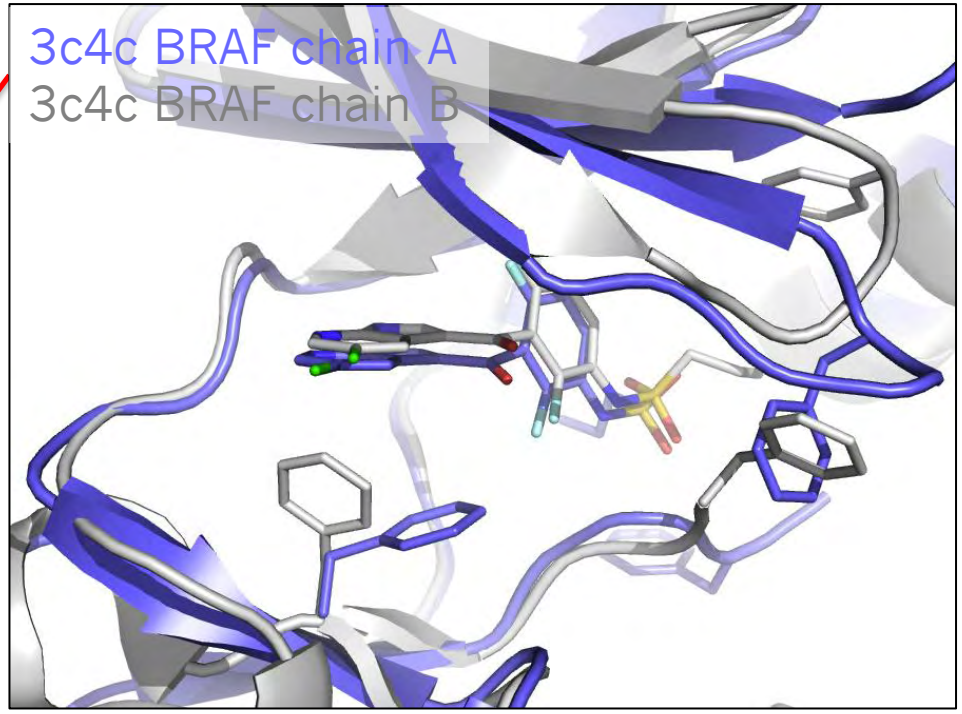




Selectivity from conformational penalty

High energy conformations accessible in flexible kinases





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