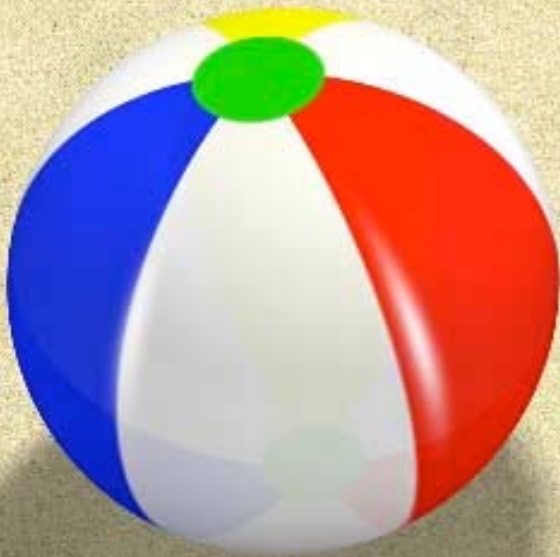


Structure prediction of zinc-binding proteins

Chu Wang
2009 RosettaCON



One year ago ...

A Jedi apprentice was about to finish his training from the academy ... He was summoned to his master in 2008 Annual Jedi Congress ...

Master: I sensed the disturbance in the force when metal binds to protein. Your are the chosen one to uncover its mystery ...

Apprentice: I've got a very bad feeling about this.

Master: I find your lack of faith disturbing.

Apprentice: But how?

Master: Use the force!

Apprentice: The force ?

Master: Yes. The Force is what gives a Jedi his power. It's an energy field created by all living things. It surrounds us and penetrates us. It binds the galaxy together. May the force be with you!

The apprentice set out to uncover the mystery, but he was lost in a galaxy far far away. One year later, he found his way back, thanks to the summons of the force, with his findings...

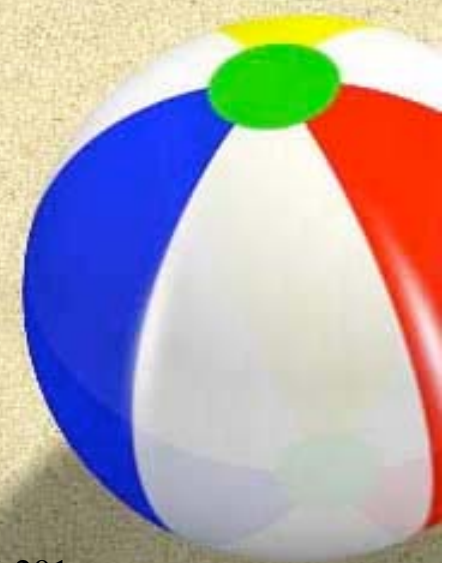


“Disturbance” of zinc-binding

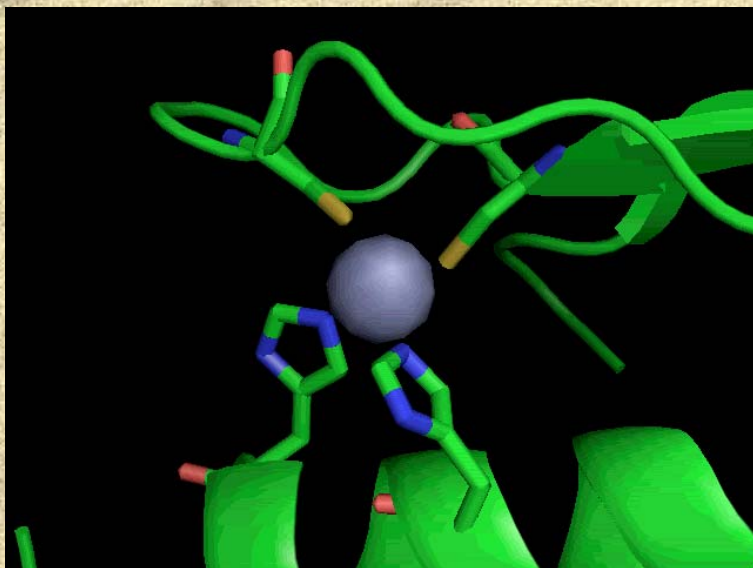
- ✻ “Zinc-finger” in DNA replication and transcription.
- ✻ “Zinc-metalloproteases” in cell differentiation, apoptosis and host defense etc.
- ✻ About 2800 potential zinc-binding proteins estimated by genome analysis, corresponding to 10% of human genome.

Andreini, C., Banci, L., Bertini, I. & Rosato, A. (2006).

Counting the zinc-proteins encoded in the human genome. *J Proteome Res* **5**, 196-201.

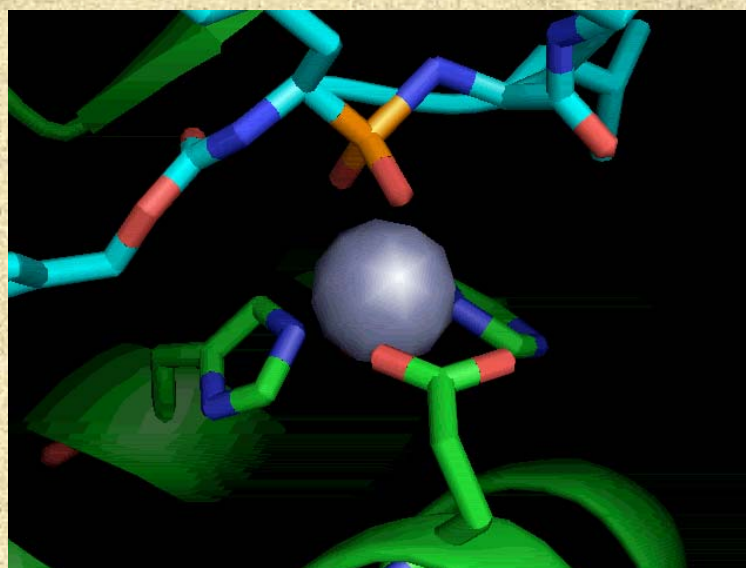


“Dark nature” of zinc-binding



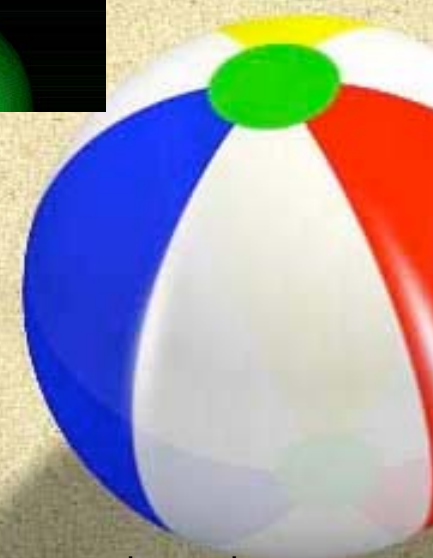
“Structural” zinc

- Tetrahedral coordination
- Four liganding residues
- Cys/His dominant



“Catalytic” zinc

- Tetrahedral coordination
- Three liganding residues
- His/Glu/Asp dominant



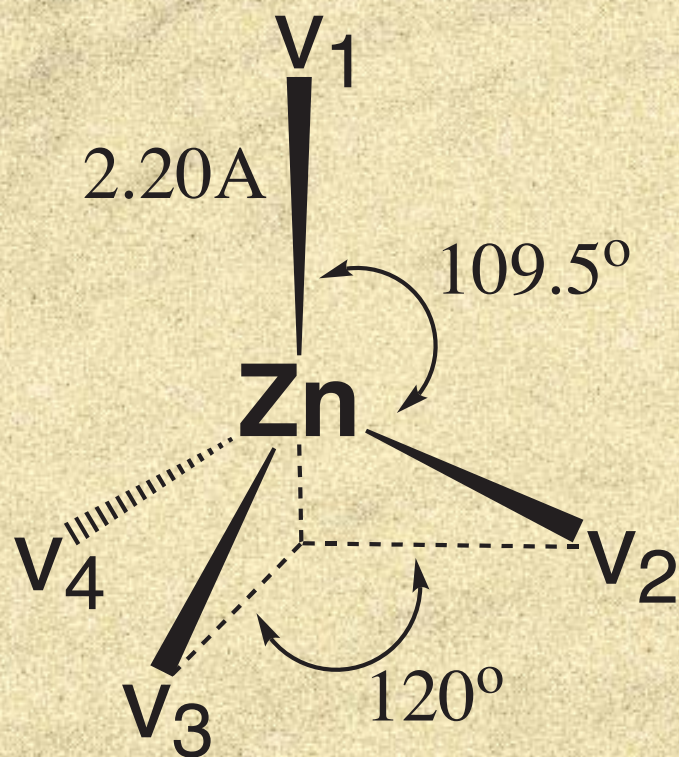
Challenging to model zinc coordination as potentials are no longer pair-additive and distance-dependent.

“Strong force” of Rosetta 3.0

- ✱ OOP design of chemical layer for easy residue creation.
 - ✱ new residue & variants: ZN, CYZ, HIS_D/E
 - ✱ Annotated fasta: chemical signature
- ✱ “Atom-tree” kinematics to integrate all DOFs.
 - ✱ Protein folding with zinc binding
 - ✱ Torsion & Jump fragment library
- ✱ OOP design of scoring engine for easy energy creation and evaluation, e.g., constraints.
 - ✱ Enforce tetrahedral zinc-coordination geometry with multi-constraints
- ✱ OOP design of protocol layer for easy protocol sharing
 - ✱ *ab initio* folding & loop modeling: from normal protein to zinc-metalloprotein



"Use the force" (I)-- zinc residue



- ✱ Perfect tetrahedron
- ✱ Zinc atom center
- ✱ Four virtual atom vertexes
- ✱ Zn-V distance of 2.20 Å
- ✱ Virtual atoms
 - ✱ No interaction energy
 - ✱ To define rigid-body orientation of zinc residue
 - ✱ To define tetrahedral zinc-coordination constraints



“Use the force” (II) -- annotated fasta sequence

- Standard fasta

- PPGLCPRCKKGYHWKSECKSKFDKDGNPLPP

- Annotated fasta

- P[PRO_p:NtermProteinFull]PGLC[CYZ]PRC[CYZ]KKGYP[HIS_D]WKSEC[CYZ]KSKFDKDGNPLPP[PRO_p:CtermProteinFull]
Z[ZN]

- Zinc-coordinating residues need to be pre-defined.

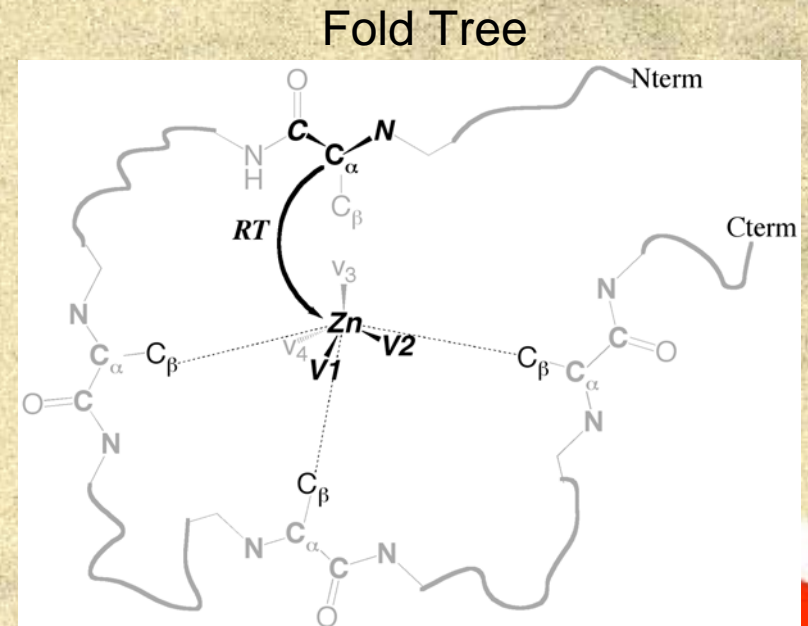
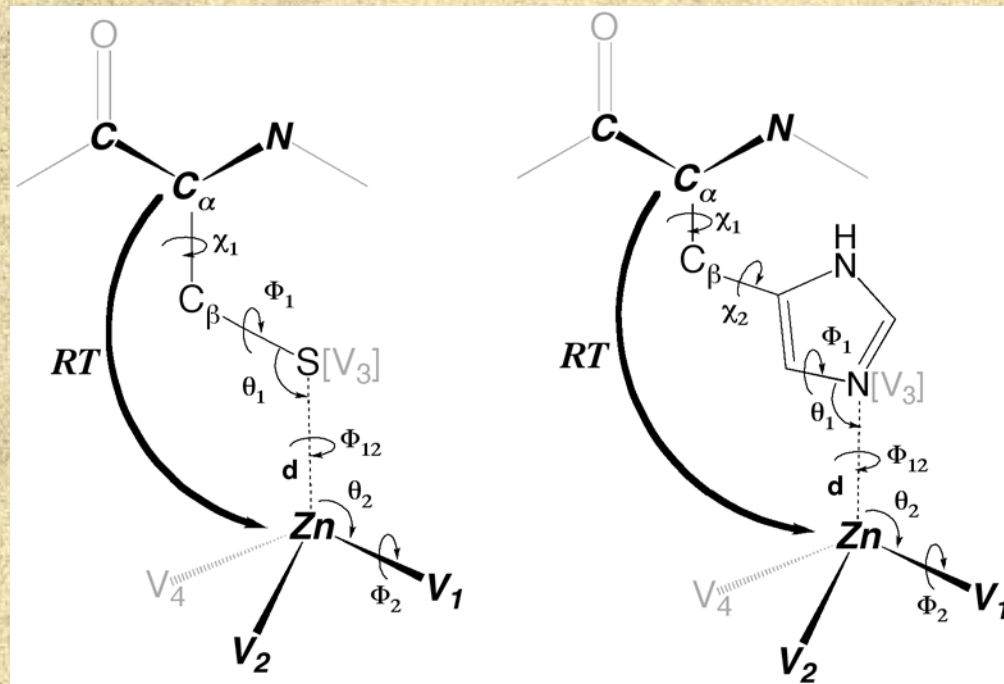
- From sequence alignment

- From chemical shift data

- As protein chemical signature to be included in general Rosetta silent IO.

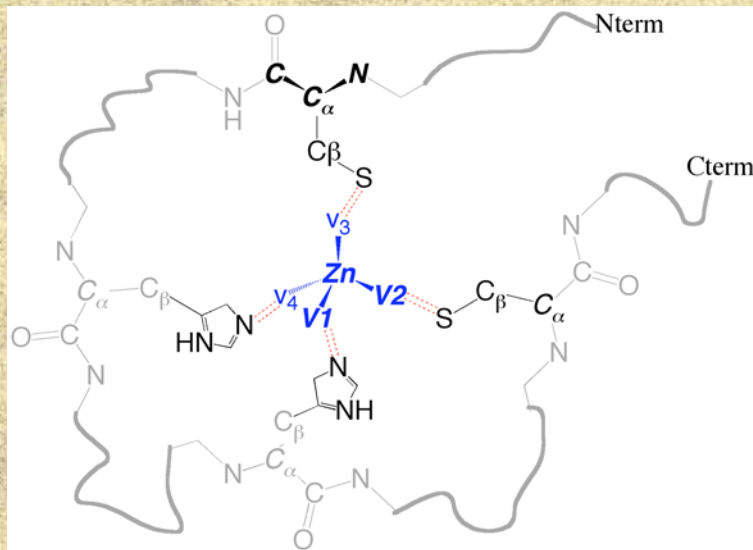


“Use the force” (III) -- Jump library for zinc

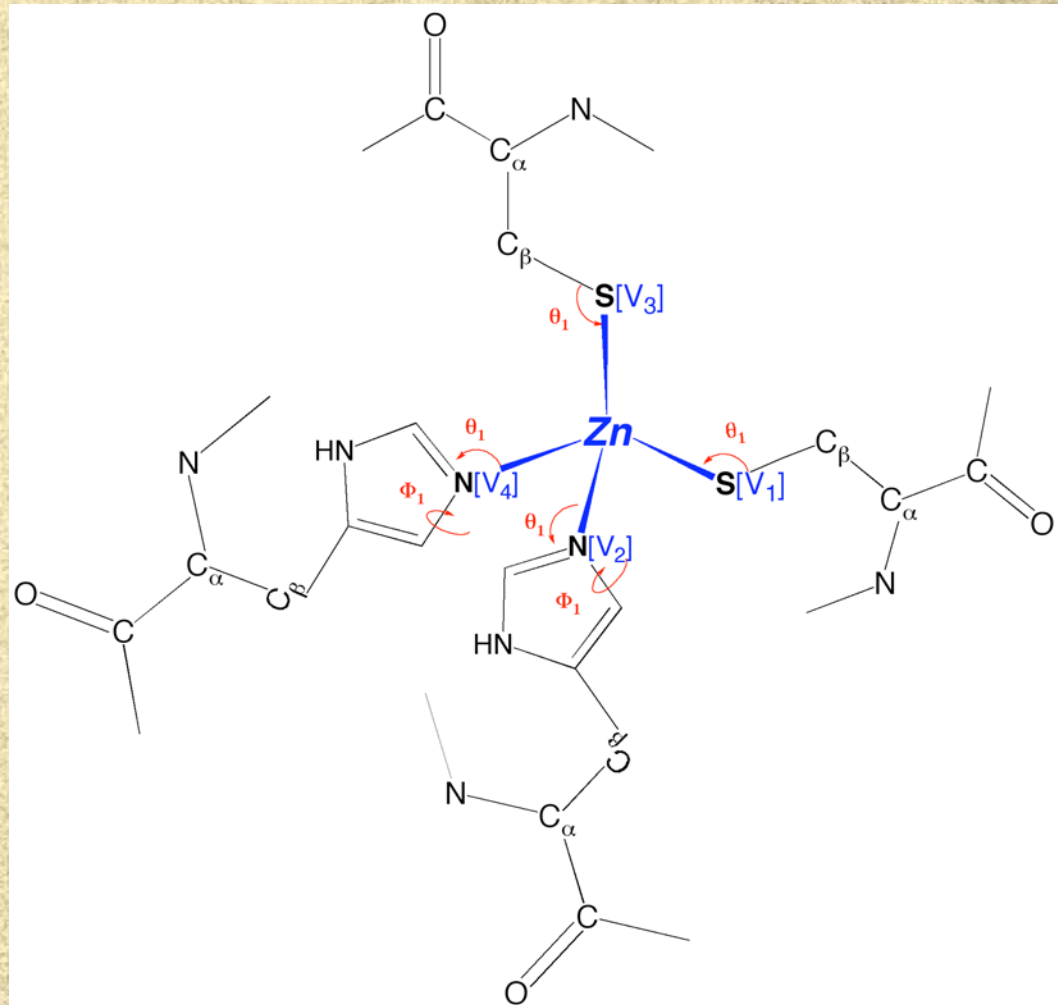


Residue-Zn	d	θ_1	θ_2	Φ_1	Φ_2	Φ_{12}
Cys - Zn	S _γ -Zn	C _β -S _γ -Zn	S _γ -Zn-V ₁	C _α -C _β -S _γ -Zn	C _β -S _γ -Zn-V ₁	S _γ -Zn-V ₁ -V ₂
	2.20Å	112.0°	109.5°	-180°:180°:30°	-180°:180°:30°	120.0°
His(D) - Zn	N _{δ1} -Zn	C _γ -N _{δ1} -Zn	N _{δ1} -Zn-V ₁	C _β -C _γ -N _{δ1} -Zn	C _γ -N _{δ1} -Zn-V ₁	N _{δ1} -Zn-V ₁ -V ₂
	2.20Å	120.0°	109.5°	0.0°	-180°:180°:30°	120.0°
His(E) - Zn	N _{ε2} -Zn	C _{δ2} -N _{ε2} -Zn	N _{ε2} -Zn-V ₁	C _γ -C _{δ2} -N _{ε2} -Zn	C _{δ2} -N _{ε2} -Zn-V ₁	N _{ε2} -Zn-V ₁ -V ₂
	2.20Å	120.0°	109.5°	180.0°	-180°:180°:30°	120.0°

“Use the force” (VI) -- full-atom constraints for zinc-coordination

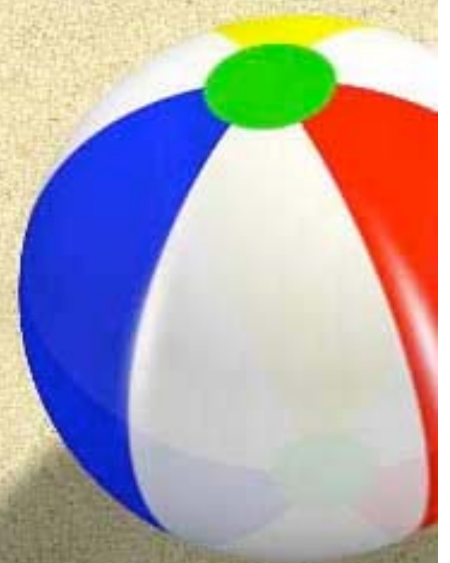


- distance constraints to virtual atoms
 - zero distance tether
 - force tetrahedral
- angular constraints
- dihedral constraints
 - in His ring
- multi constraints
 - one for each zinc-residue pair
- ambiguous constraints
 - handle chirality

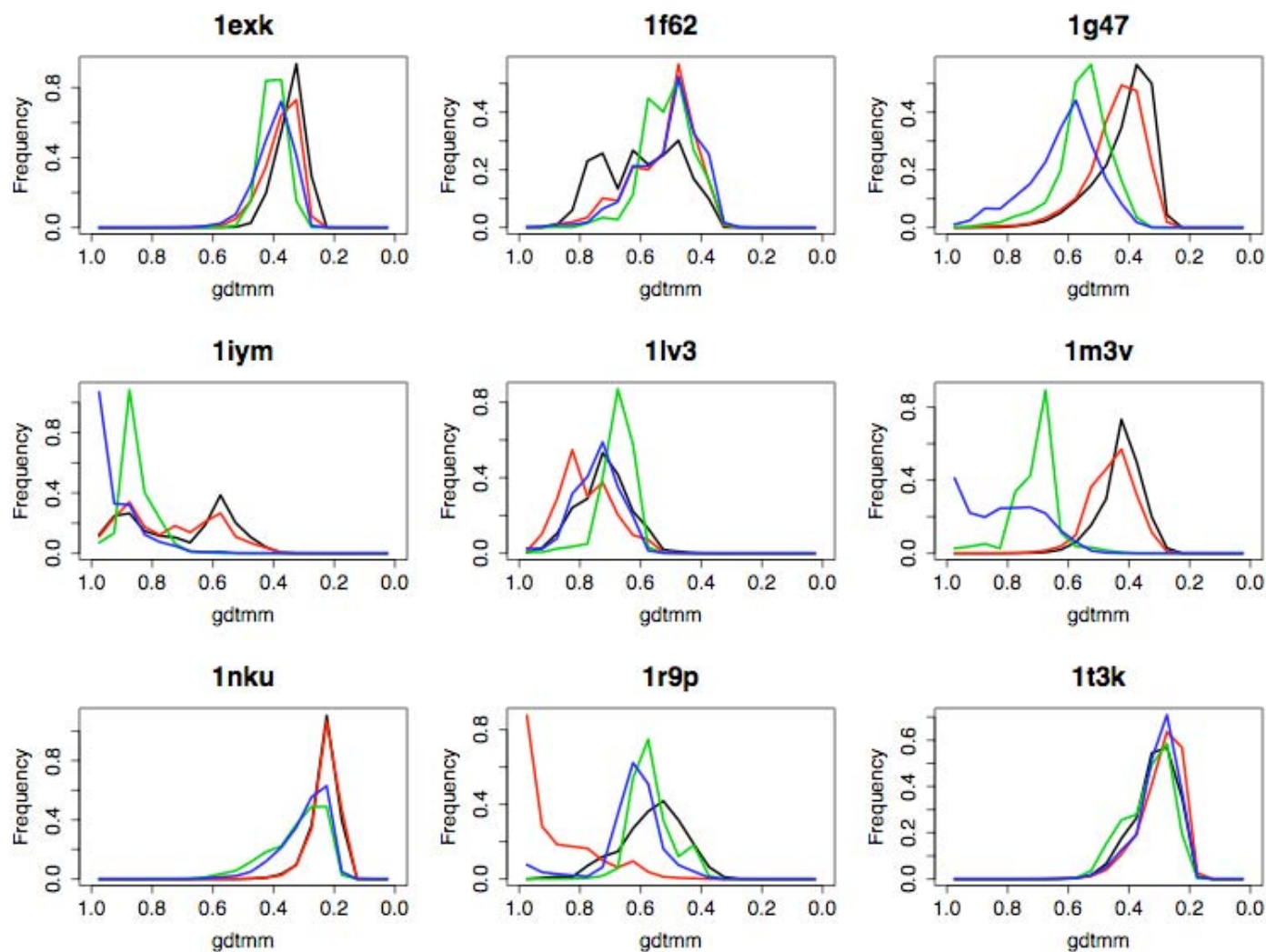


"Use the force" (V) -- *ab initio* folding & loop modeling

- ☀ Reuse existing protocols
- ☀ Define an annotated sequence with Zinc
- ☀ Define a fold tree and a jump library of Zinc
- ☀ Define centroid and all-atom constraints
- ☀ Hope that the force is with you



"Force Balance" -- Abrelax



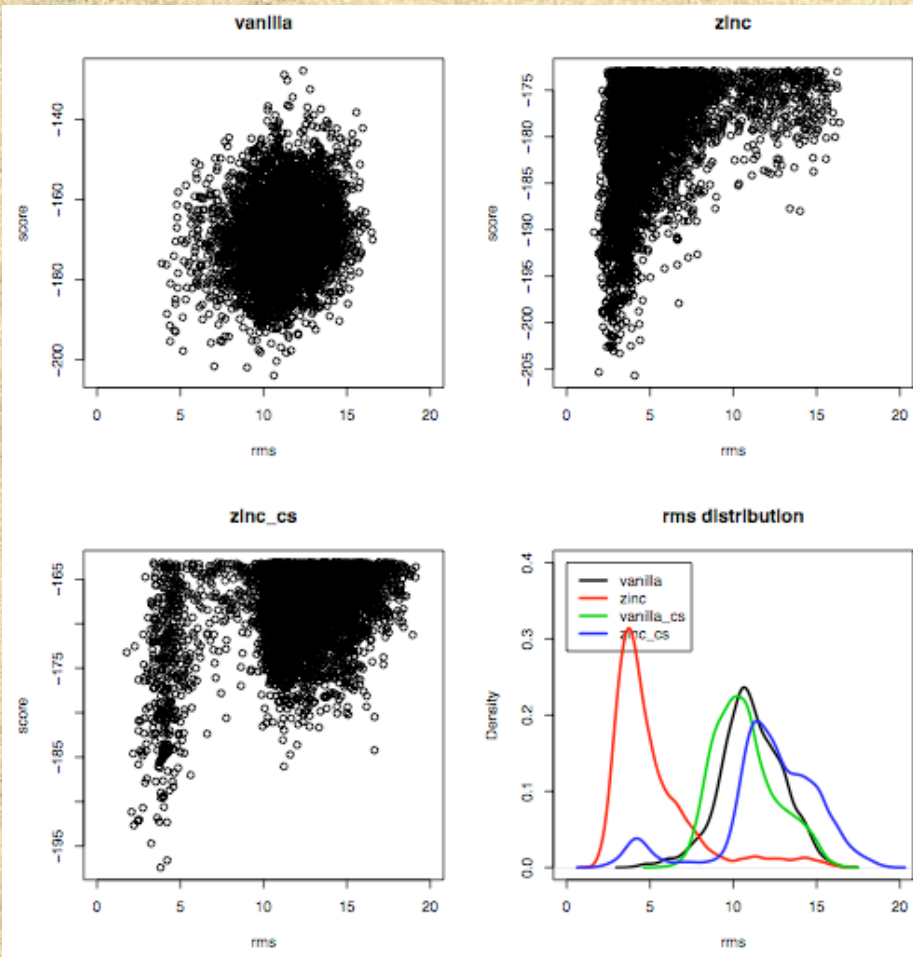
5 positive
1 negative
3 neutral



Kornhaber, G. J., Snyder, D., Moseley, H. N. & Montelione, G. T. (2006). Identification of zinc-ligated cysteine residues based on $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ chemical shift data. *J Biomol NMR* **34**, 259-69.

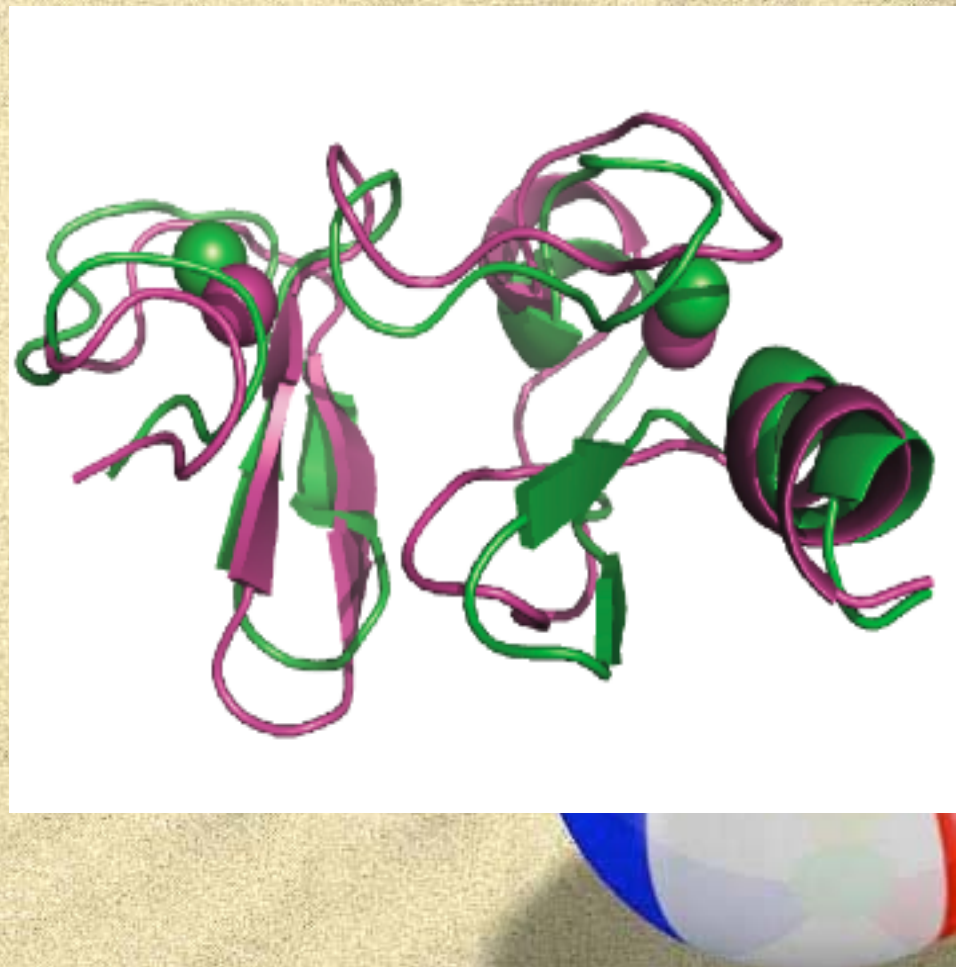
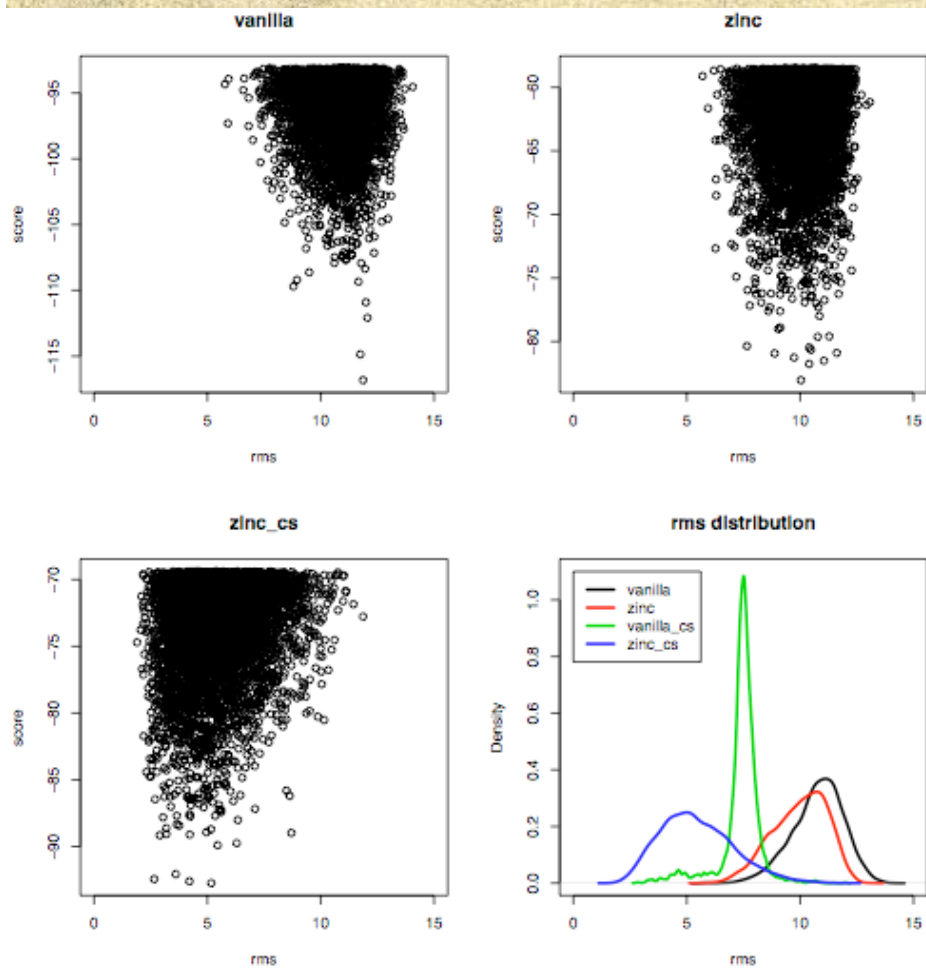
“When The force is with me” -- 1r9p

96 residues; 1.26Å rmsd

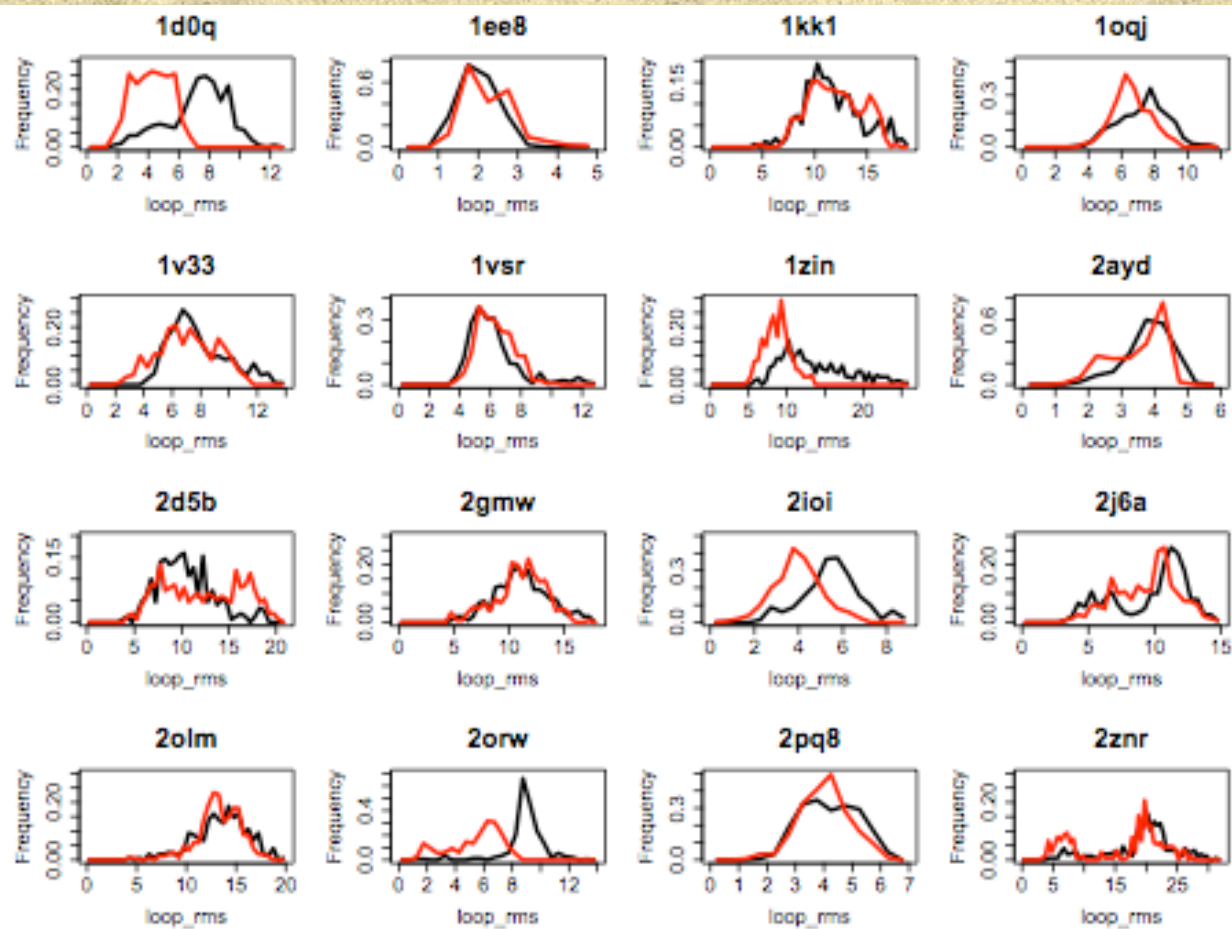


“When The force is with me” -- 1m3v

60 residues; 2 zincs; 1.96Å rmsd

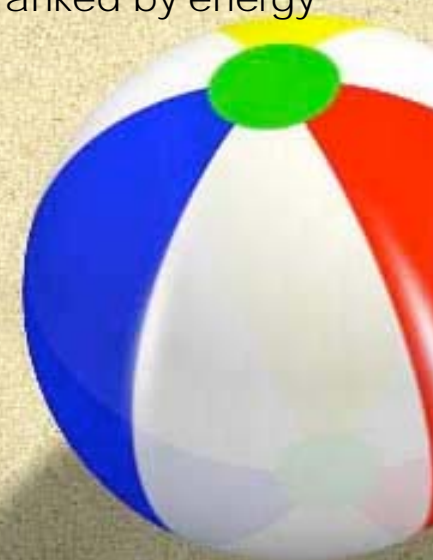


“Force Balance” -- Loop Modeling

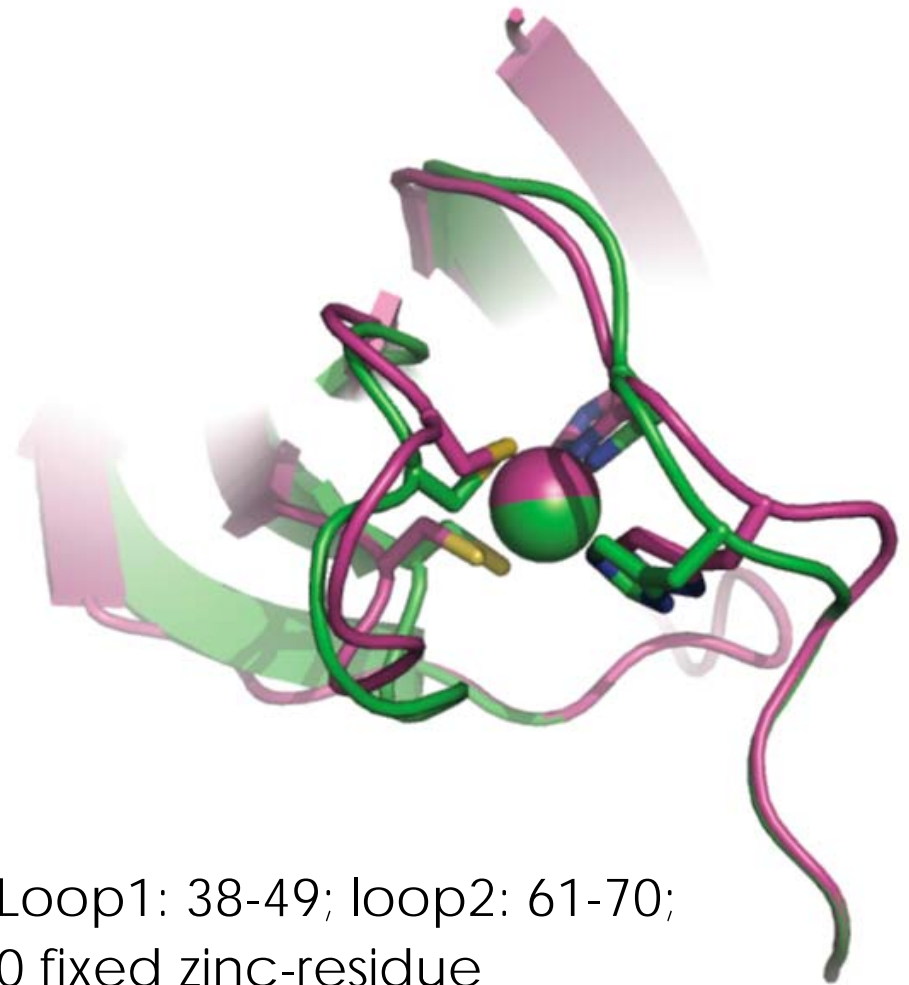
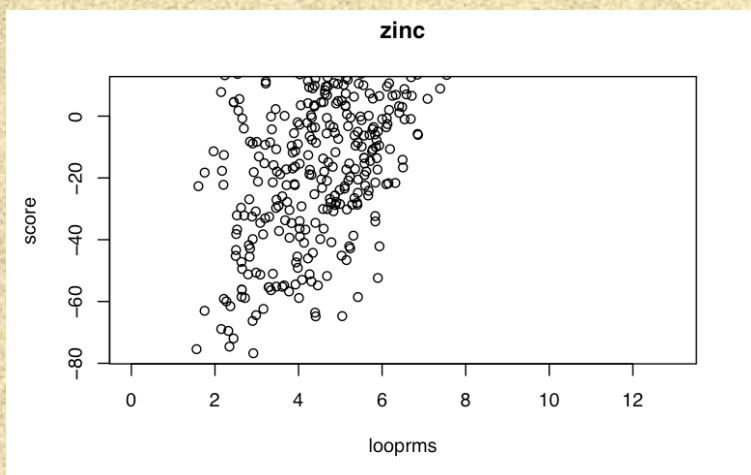
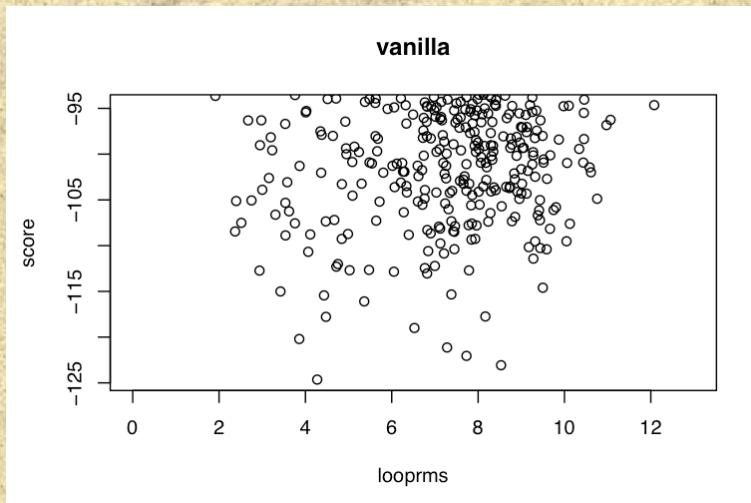


BL5	Vanilla	Zinc
<2	2	5
2-5	4	5
>5	10	6

BL5: best loop rms
of the top5 models
ranked by energy

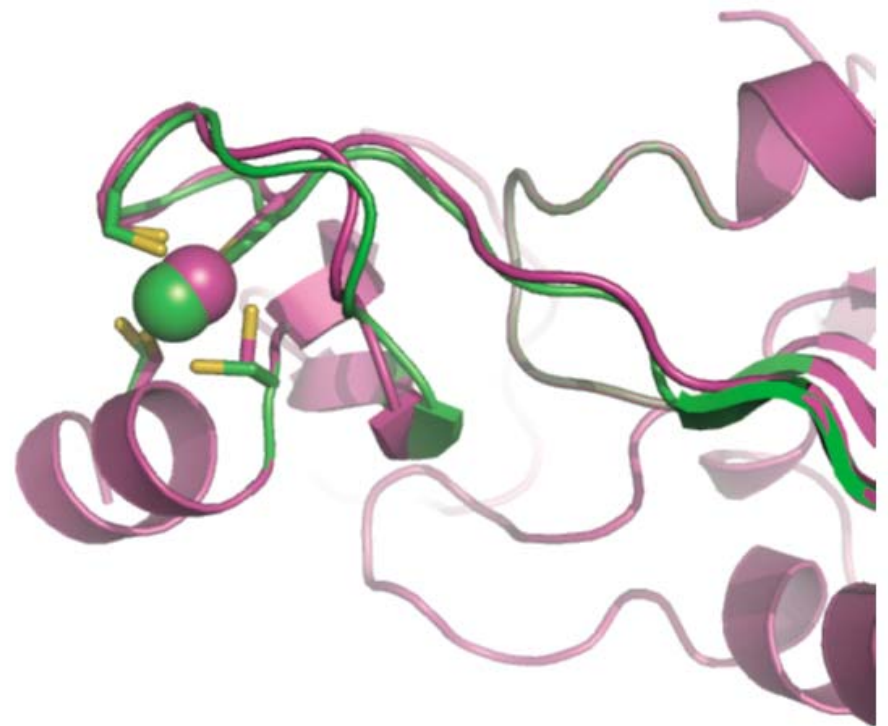
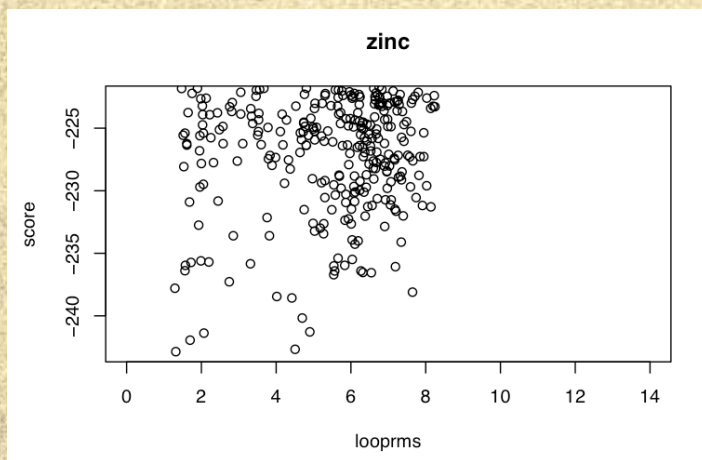
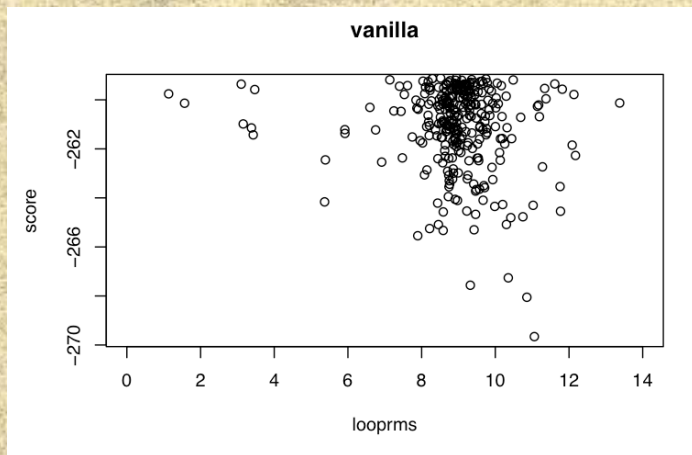


“When The force is with me” -- 1d0q



Loop1: 38-49; loop2: 61-70;
0 fixed zinc-residue

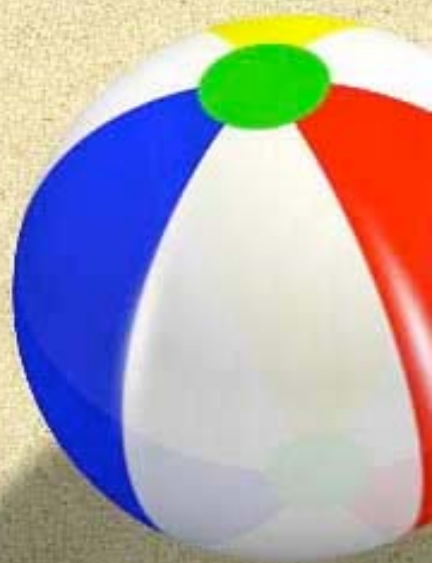
“When The force is with me” -- 2orw



Loop1: 135-49; 2 fixed zinc-residues

New insights of “force”

- ✱ Folding of proteins with other metals or small ligands
- ✱ De Novo Design of proteins with metals or small ligands.



Breaking News: Change we can!

August 5th, Leavenworth, WA

www.news-A-long-time-ago-in-a-galaxy-far-far-away.com

Last night, in the 2009 annual Jedi congress, the Grand Master delivered his key-note speech in front of all delegates...

Master: I start to sense more disturbances in the force.

Master: Rumors like "structures are not important any more" are being spread

Master: The menace of Molecular Dynamics is emerging

Master: Trays which we use to collect energy source were taken away, but most disturbing of all,

Master: Documented the secretes of using the force we have not. With more and more interest from the public, our young Jedi apprentices may be distracted from their training to become "bounty" hunters, I am afraid.

Master: So change we must have! And change we can!
May the force be with you!

According to a high-rank council member who wants to remain anonymous, new plan for change will be discussed at the Jedi council meeting soon



Salute to

- ☀ "Grand Master" Baker for training me from a droid into a knight
- ☀ "Knight" Lange for mini Abrelax
- ☀ "Knight" Tyka for mini Loops
- ☀ "Apprentice" Vernon for cs_frag
- ☀ "Master" Bradley and Leaver-Fay for leading general mini development
- ☀ "Master" Bradley and Schueler-Furman for organizing this congress

**Droids, younglings, apprentices, knights and masters,
may the force be with you!**

