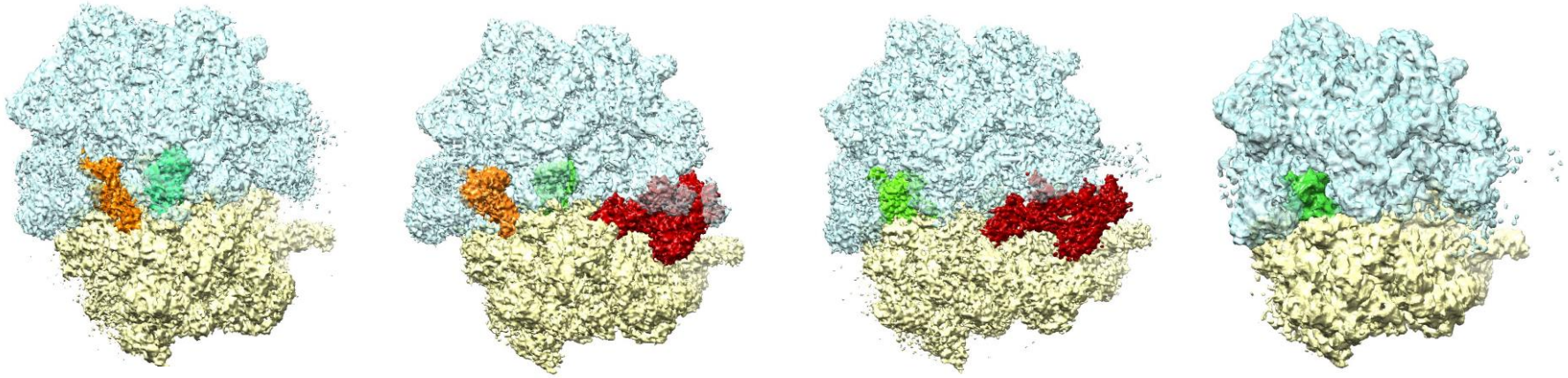
Recovering continuous conformations and reconstructing the energy landscape of a molecular machine

Ali Dashti, Peter Schwander, Abbas Ourmazd, A. Hosseinizadeh, U. of Wisconsin -- the algorithm
Robert Langlois and Hstau Liao, Columbia – handshaking with cryo-EM formats and conventions
Jesper Pallesen, Nesh Sharma, Joachim Frank, Columbia – cryo-EM
Vera Stupina, Jon Dinman, Anne Simon, U. of Maryland – ribosomes purified from yeast
PNAS, in press.

MULTIPLE STATES COEXIST IN THE IN VITRO TRANSLATION SYSTEM

nr 70S--P-E nr 70S--EF-G—P-E r 70S—EF-G—P/E r 70S—P/E



Maps	Annotation of States: (r70S: rotated) (nr70: non-rotated)	Resolution(Å)	Number of particles
1	nr70S-PtRNA-EtRNA	4.0	~50K
2	nr70S-PtRNA-EtRNA-EFG	3.6	~90K
3	r70S-P/EtRNA-EFG	4.2	~35K
4	r70S-P/EtRNA	5.7	~15K

What about continuous changes?

Do we impose the model assumption of discrete states, due to limited computation power?

Most applications of Relion rarely use more than K=10

Continuous distribution of states, if they exist, will be artificially chopped into discrete clusters

Treat classification as a two-step process

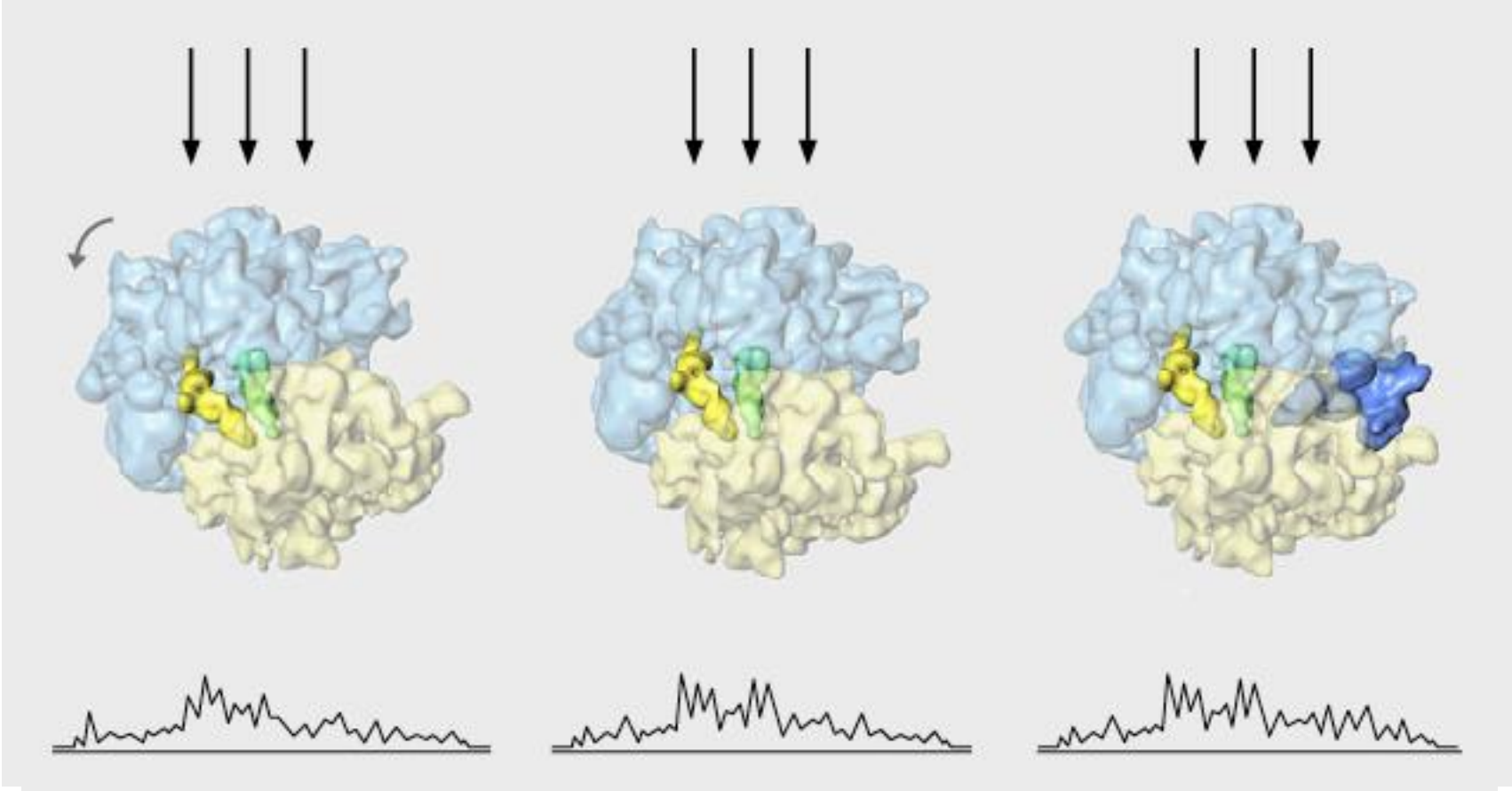
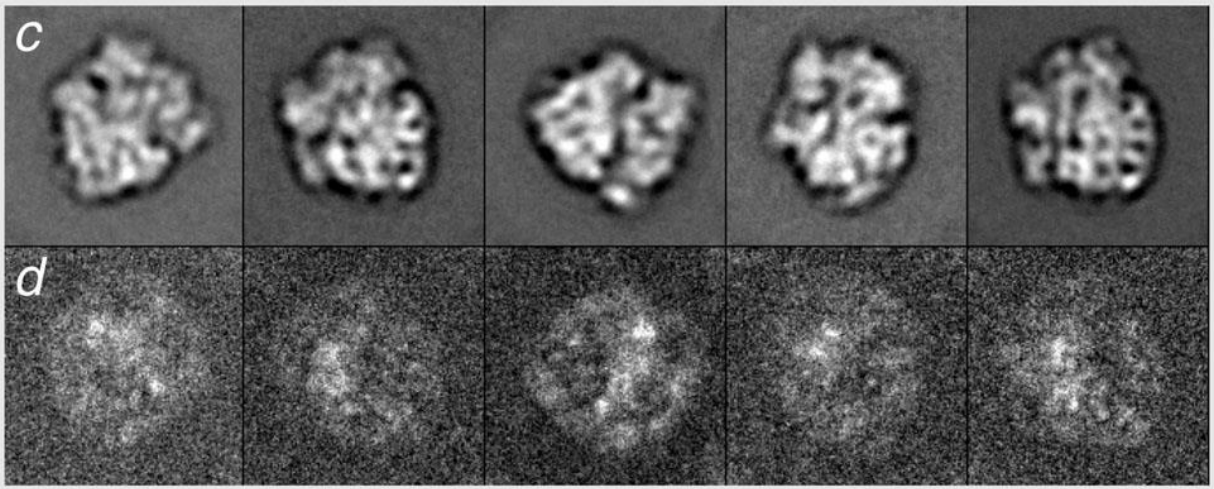


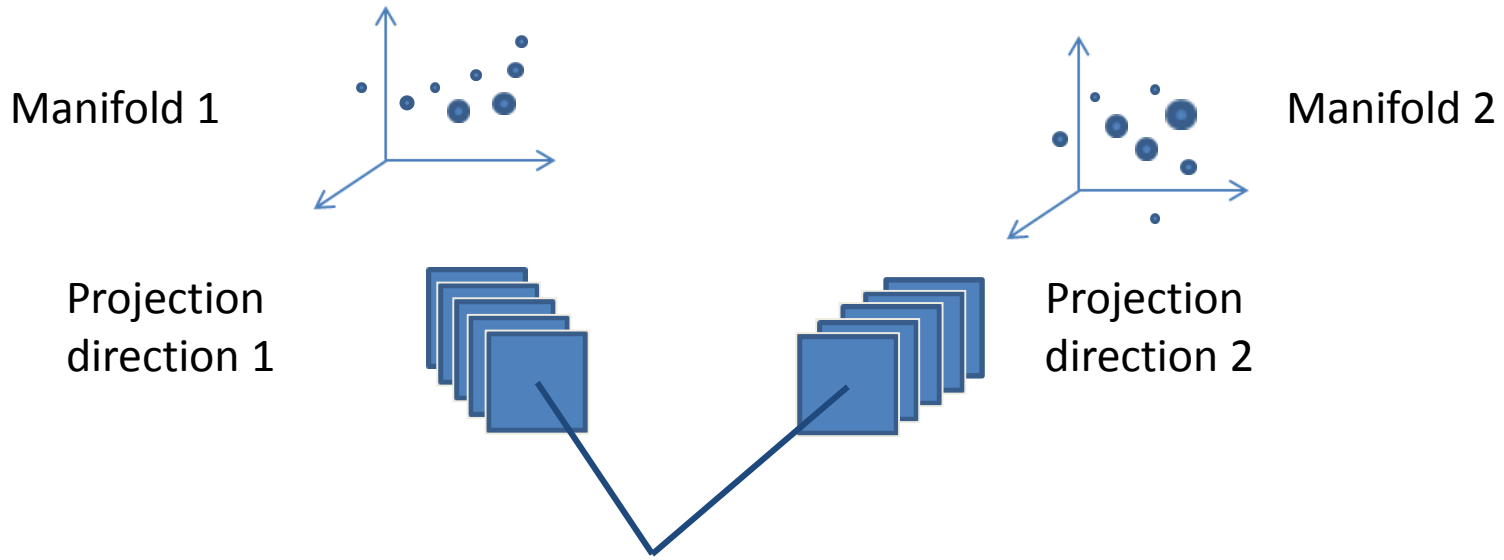
Image variations due to change in viewing angle are quite large compared to image variations due to conformational changes/binding states.



2D variances related to 5 projection directions

Classification of a continuum of states, and mapping of the energy landscape

Joachim Frank (Columbia), Peter Schwander and Abbas Ourmazd (U. of Wisconsin)

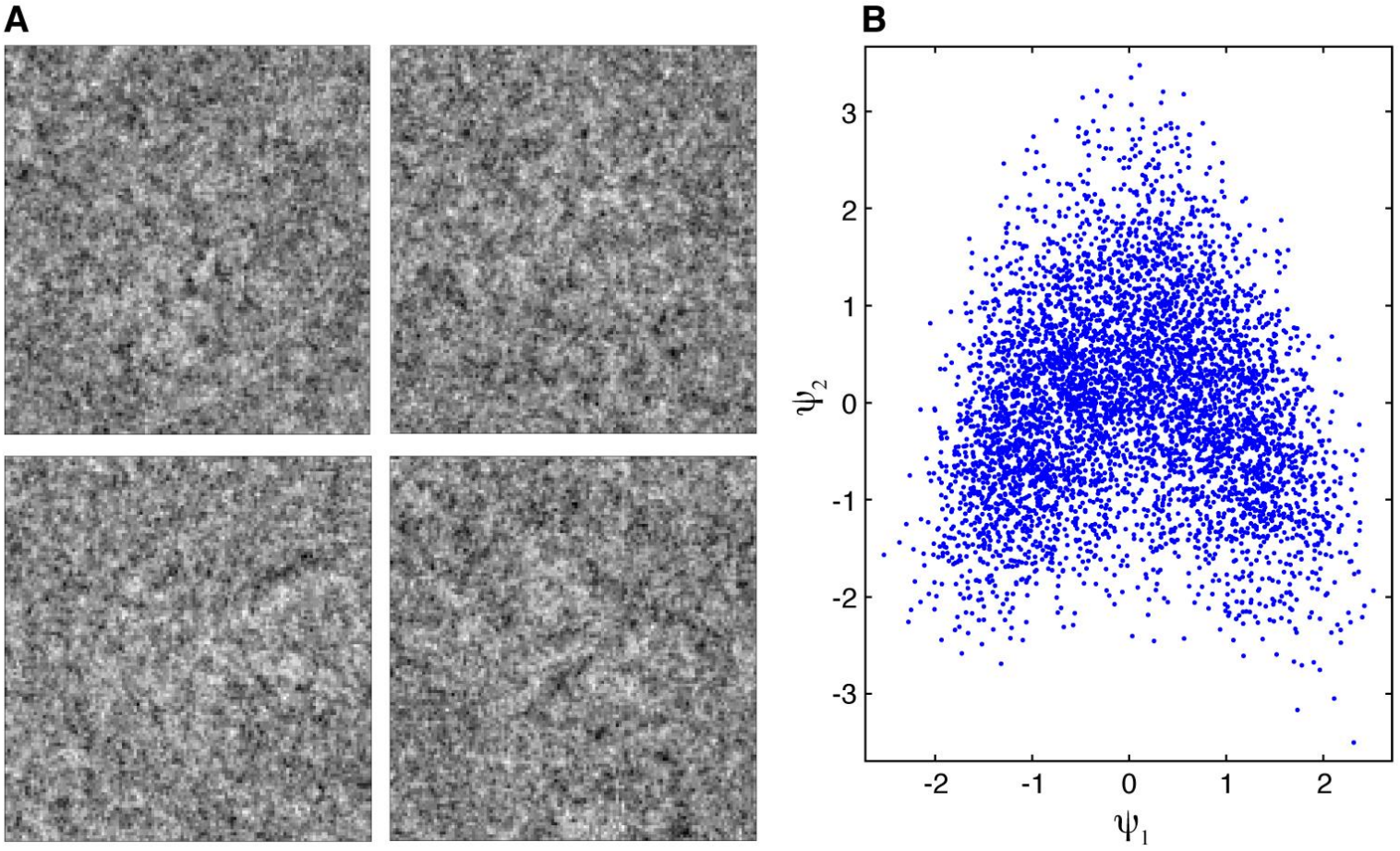


Premise: variation of particle image due to conf. changes is small compared to its variation due to changes in projection direction. Step 1: sort particles by orientation.

Set of projections in direction 1 forms an N-dim. manifold where N is the number of degrees of freedom.
Set of projections in direction 2 forms another N-dim manifold that is quite different since conf. variations manifest themselves differently in different projection directions.

*How are the two manifolds related to one another? More generally, is there a mapping operation (a "synchronization") that allows us to "collect" **all** particle snapshots, from **all** directions, that originate from particles in the same conformational state? And then do the same thing for all conformations encountered?*

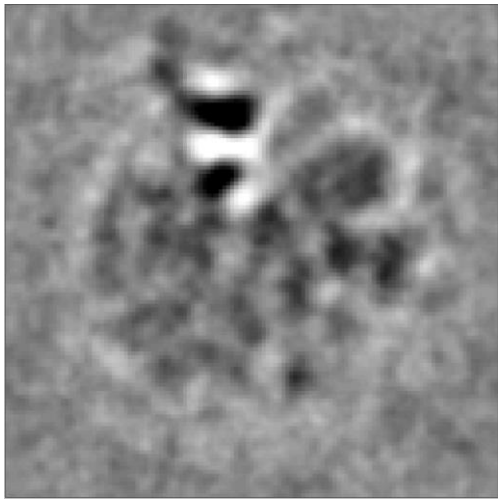
- 80S ribosomes, cell extract from yeast
- 1,100,000 -- > 850,000 images
- Polara recorded on Tietz 4k x 4k CCD camera
- 1.5 A/pixel

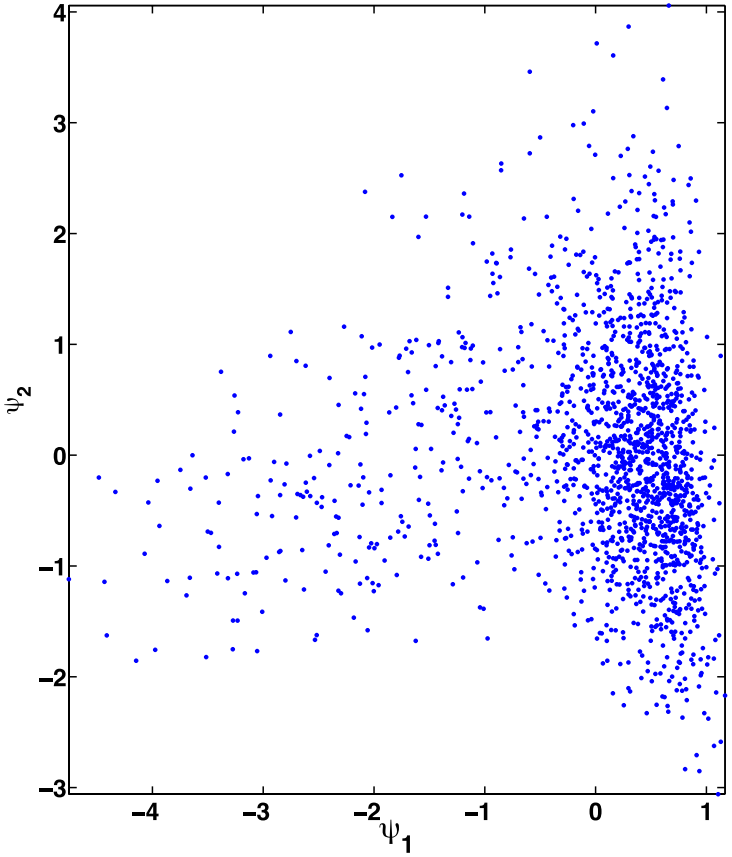


5000 images in a selected viewing direction

Diffusion map embedding: description of the curved manifold in terms of the orthogonal eigenfunctions of the Laplace-Beltrami operator
5 degrees of freedom found

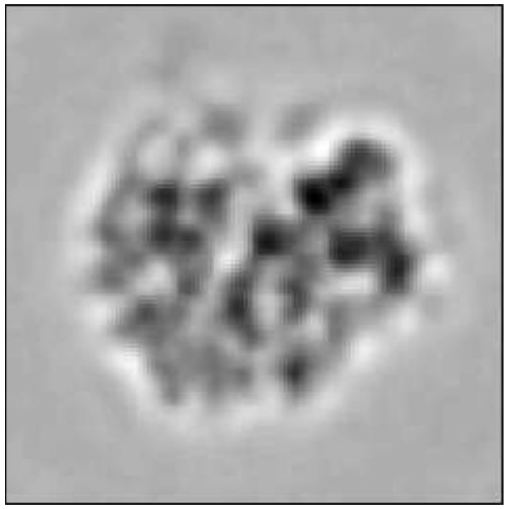
Topo2 along ψ_2

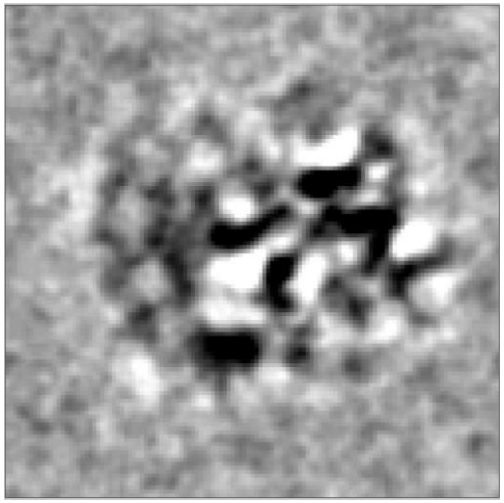




Projection's Euler angles:
Φ: 59.41
θ: 77.66
Ψ: 300.58

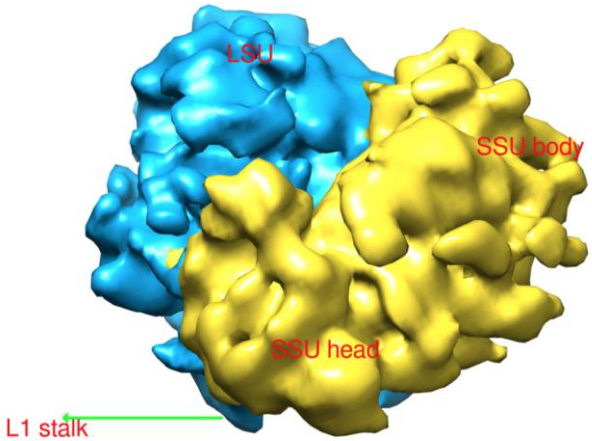
2D projection view





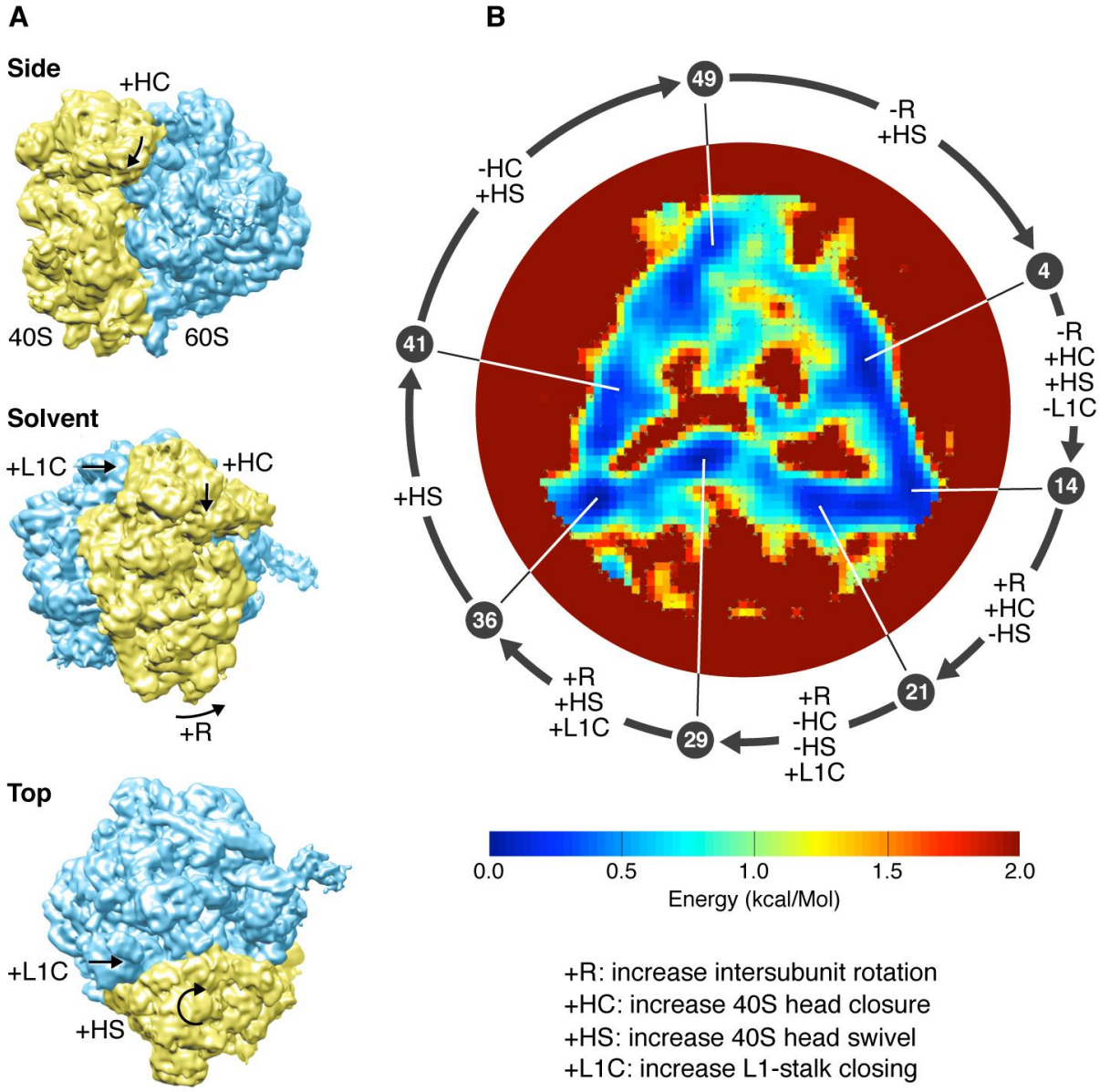
Topo2 along ψ_1

3D projection view



- I omitted some slides, and all movie links, since the publication is imminent.
- Look for Dashti et al., PNAS 2014.

50 distinct states along triangular closed path.
Conformational changes reminiscent of elongation cycle



Conclusions

- Continuous range of conformations can be explored
- Number of degrees of freedom of a molecular machine
- Construction of the free-energy landscape
- Exploration of the way ligand binding, temperature, buffer conditions, change the landscape
- The ribosome assumes a wide range of conformations even in the absence of functional ligands
- These conformational changes are reminiscent of those used in the elongation cycle