Understanding principles of design of molecular machines: a structural biology perspective

TANWEER HUSSAIN
Molecular Reproduction, Development and Genetics (MRDG)
Indian Institute of Science (IISc)
Bangalore

Symposium on "Molecular Machines: a multidiscipline enterprise" 1st July 2017
28th mid-year meeting of Indian Academy of Sciences at IISc, Bangalore

Molecular machines in the cell

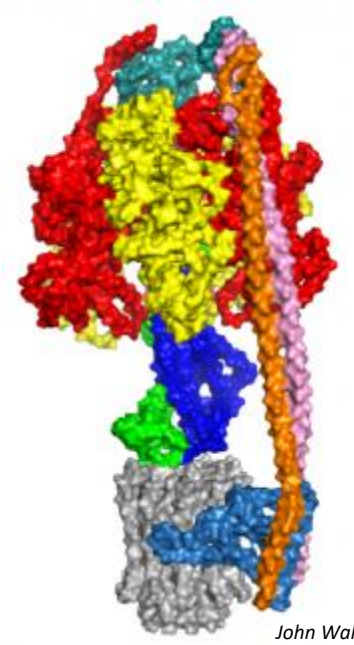
"*The entire cell can be viewed as a **factory** that contains an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein **machines**….*

Why do we call the large protein assemblies that underline cell function protein machines? Precisely because, like machines …, these protein assemblies contain highly coordinated moving parts"
Bruce Alberts, Cell 92, 291 (1998).

"Molecular Machinery of Life" animation by Harvard BioVisions at the Harvard University

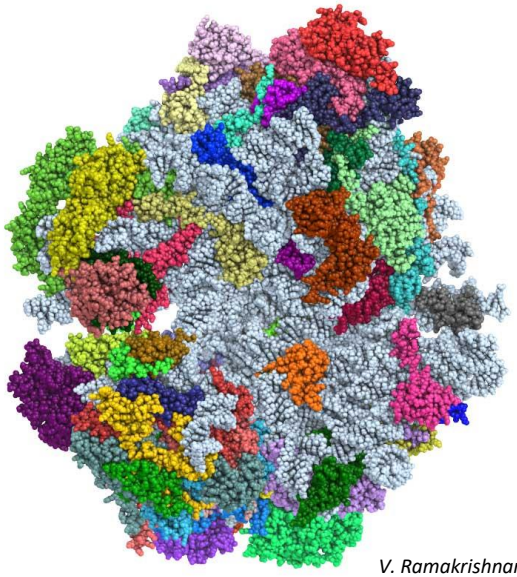
Macromolecular (biological) machines

Complex: multi-component integrated system



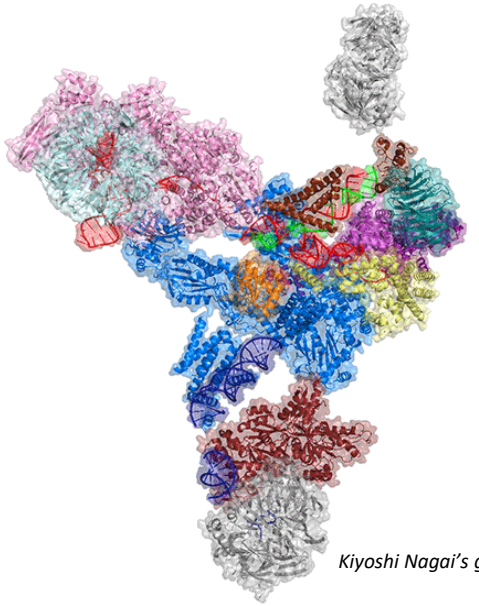
John Walker's group

ATP synthase



V. Ramakrishnan's group

Ribosome



Kiyoshi Nagai's group

Spliceosome

Operate under exquisite control (directional)

Translational and rotational movement with precision

Macromolecular (biological) machines

Complex: multi-component integrated system

Operate under exquisite control (directional)

Translational and rotational movement with precision

"Understanding and harnessing such phenomenal biological machines provides a strong incentive to design active nanostructures that can operate as molecular machines……..
…… it is conceivable that molecular motors and machines will play as prominent a role in the nanotechnological revolution of the twenty-first century as their macroscopic counterparts – the steam and internal combustion engines – played in catalysing the industrial revolution of the nineteenth century."
*Wesley R. Browne & **Ben L. Feringa*** *Nature Nanotechnology, Vol1, 2006*

Jean-Pierre Sauvage, Sir J. Fraser Stoddart and **Bernard L. Feringa** shared 2016 Noble Prize in Chemistry for their development of molecular machines.

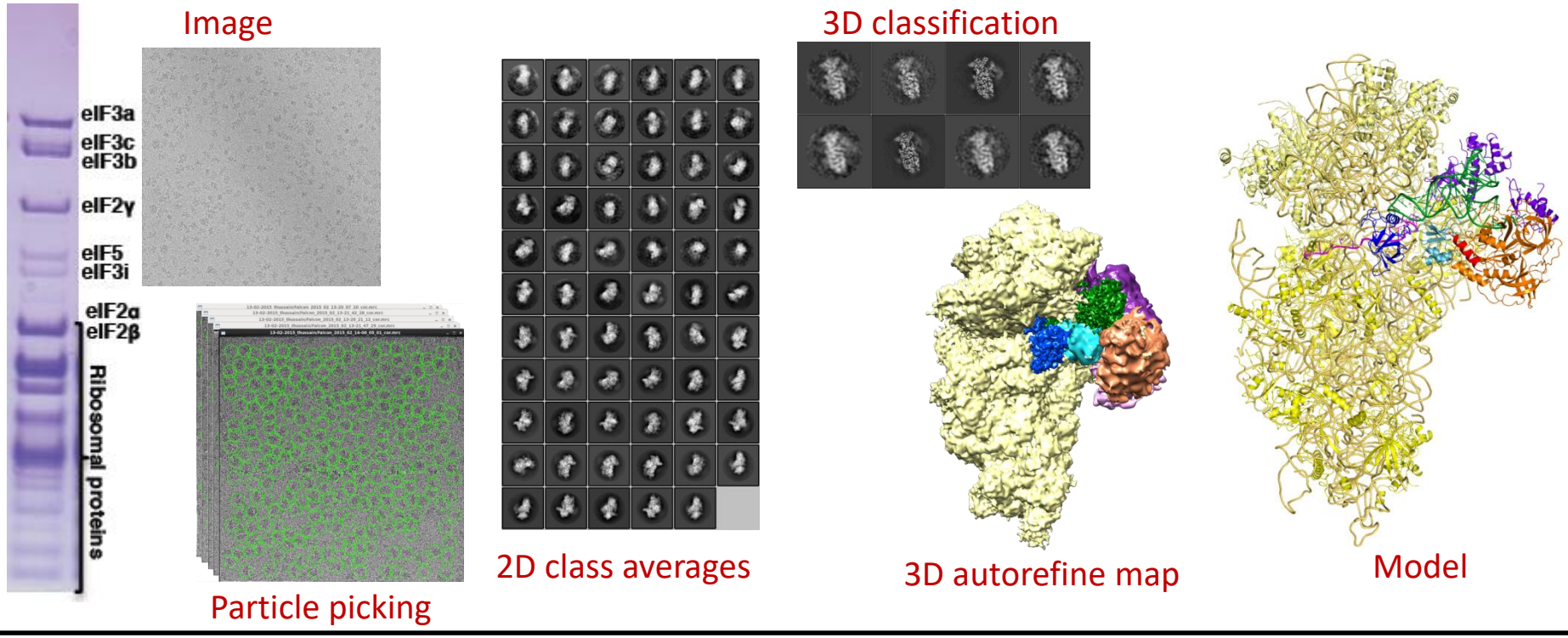
Structural biology of Molecular machines

Determine the three-dimensional structure of the macromolecular complexes

X-ray crystallography

Crystals → X-ray diffraction → Electron density → Model

Cryo-electron microscopy (cryo-EM)



Ribosome: a sophisticated molecular machine

reads the genetic code (nucleotide codons in the mRNA) to form the cognate polypeptide

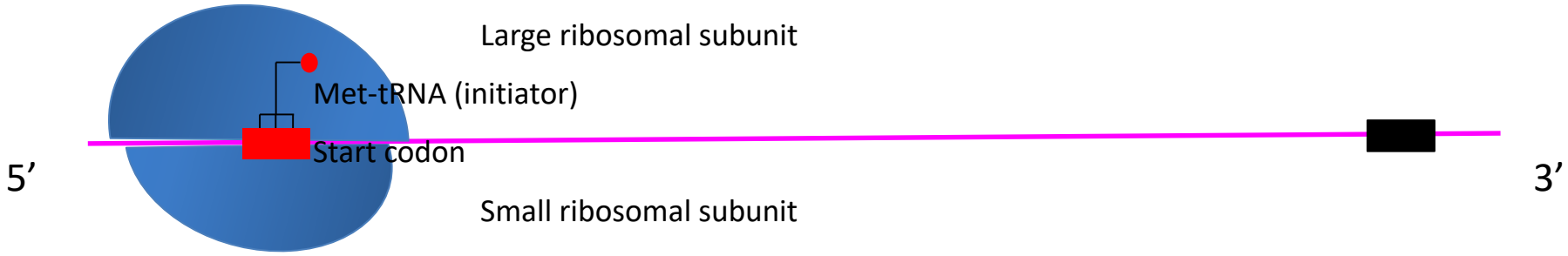
This require 3 distinct functions to be done at the same time:

1. Decode the codons to obtain the sequence of amino acids
   A decoding device: A site in small ribosomal subunit
2. Formation of peptide bonds between the amino acids
   A catalytic device: peptidyl transferase centre of large ribosomal subunit
3. Move along the mRNA
   Small and large subunit together (ratcheting movement)

Translation: polypeptide synthesis by ribosome

Three steps:

1. **Initiation**
2. Elongation
3. Termination (and recycling)

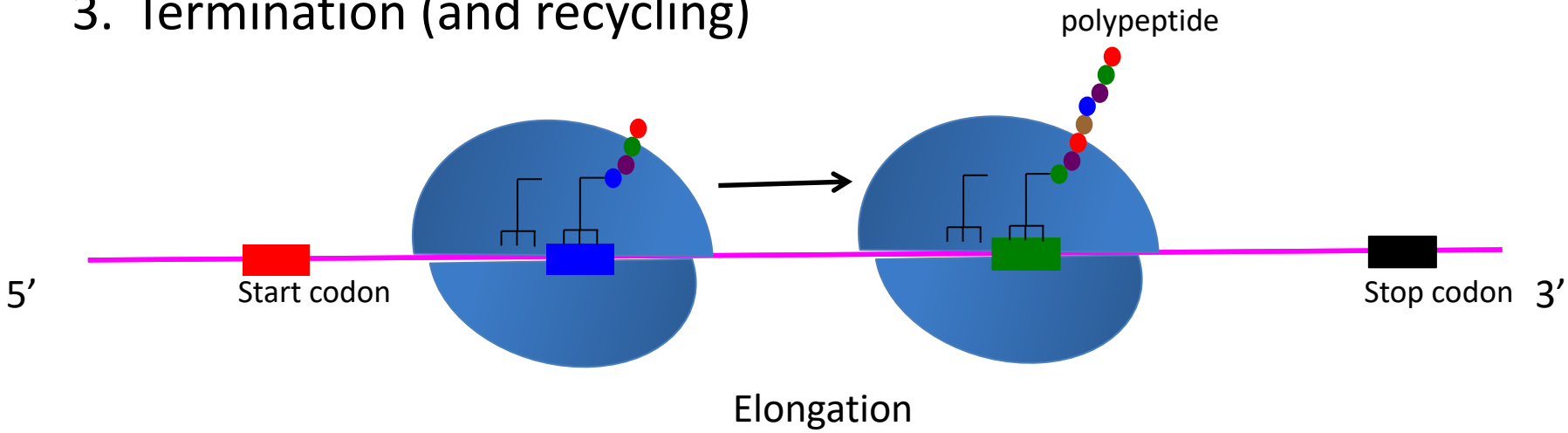


Initiation

Translation

Three steps:

1. Initiation
2. **Elongation**
3. Termination (and recycling)

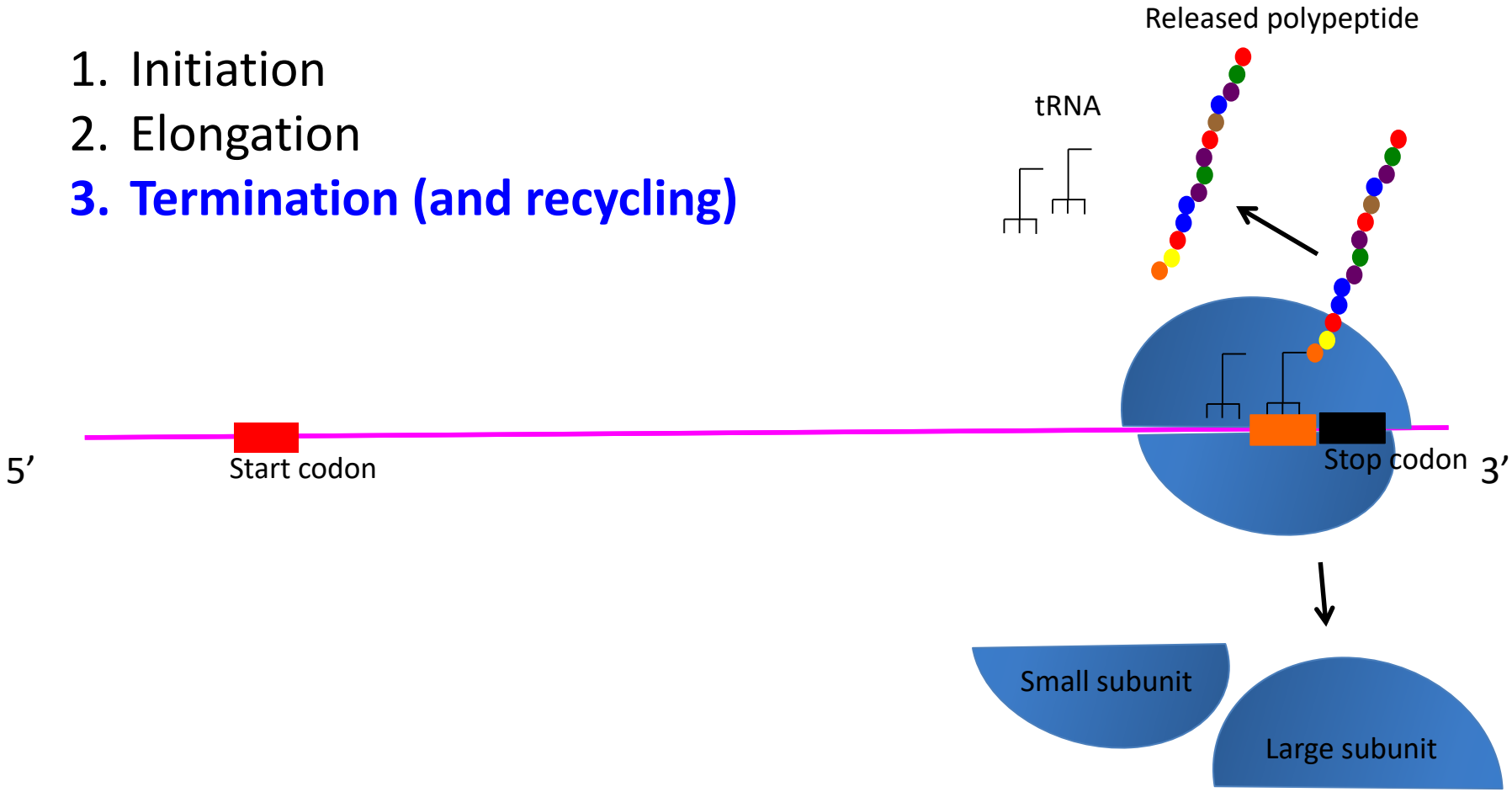


Elongation

Translation

Three steps:

1. Initiation
2. Elongation
3. **Termination (and recycling)**



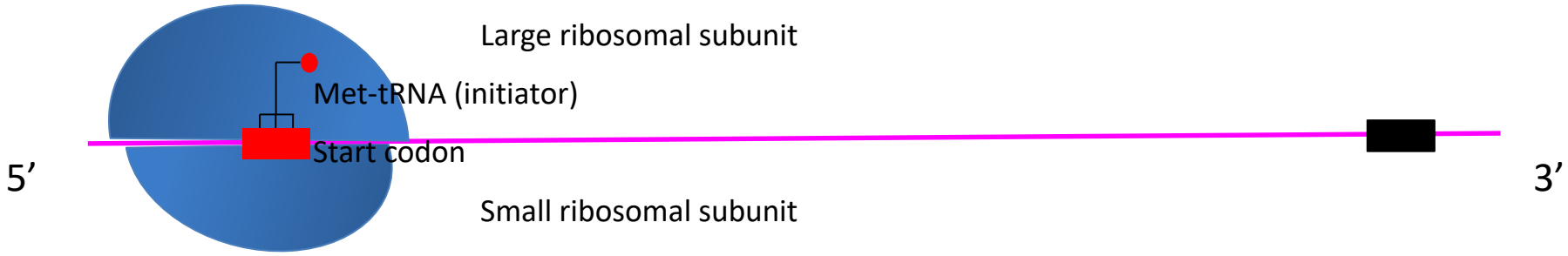
Animation of functioning of ribosome

Venki Ramakrishnan’s Lab

Translation

Three steps:

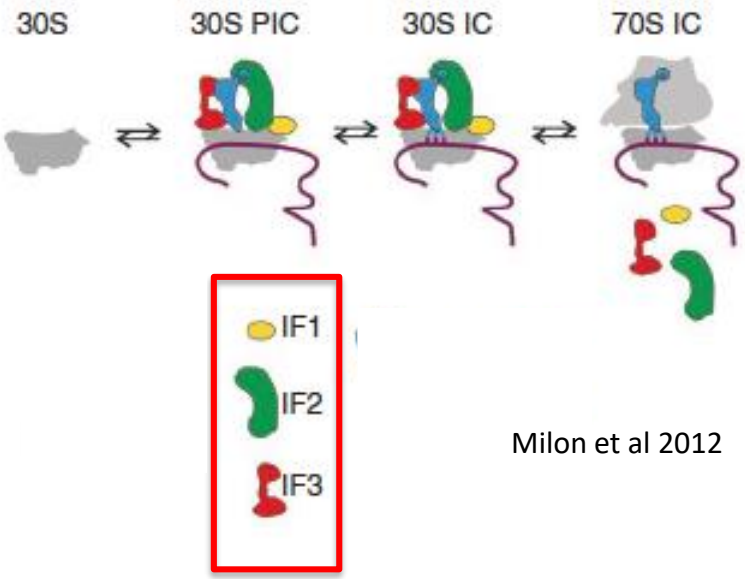
1. **Initiation**
2. Elongation
3. Termination (and recycling)



Initiation

Translation initiation

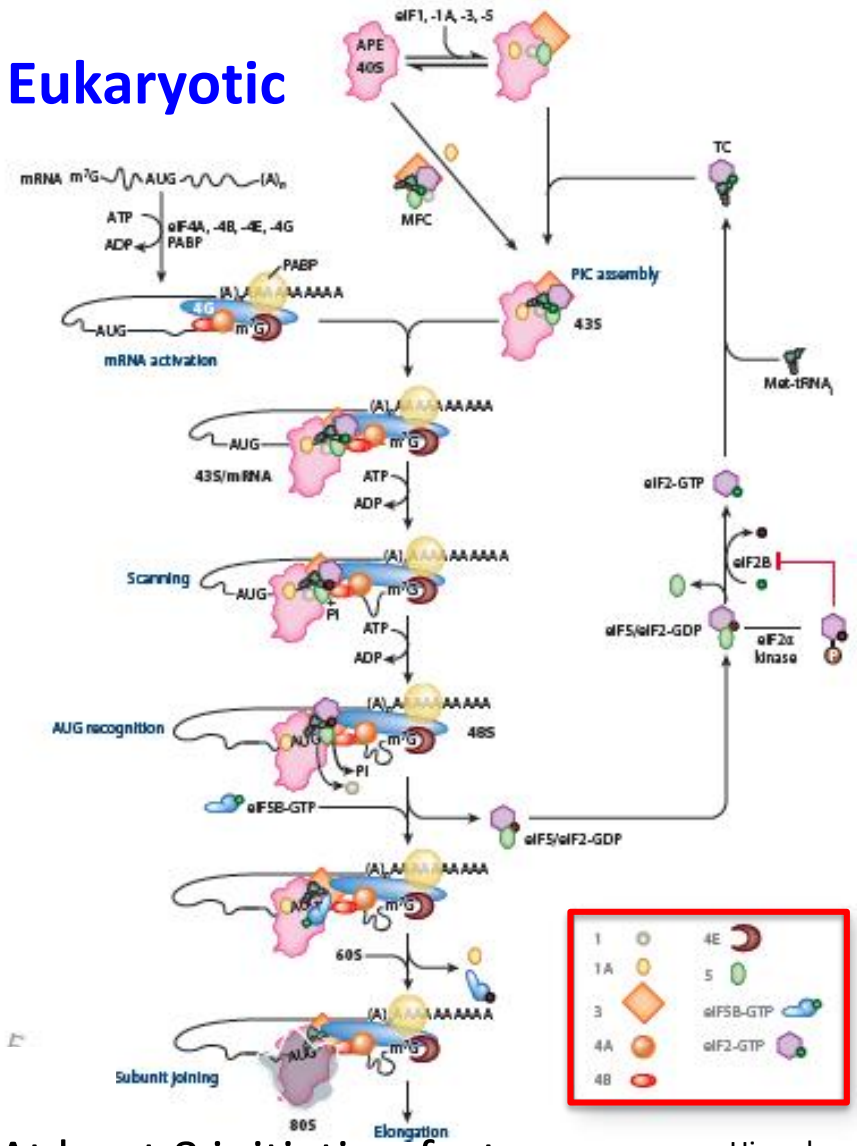
Prokaryotic



Milon et al 2012

Only 3 initiation factors
3 polypeptides

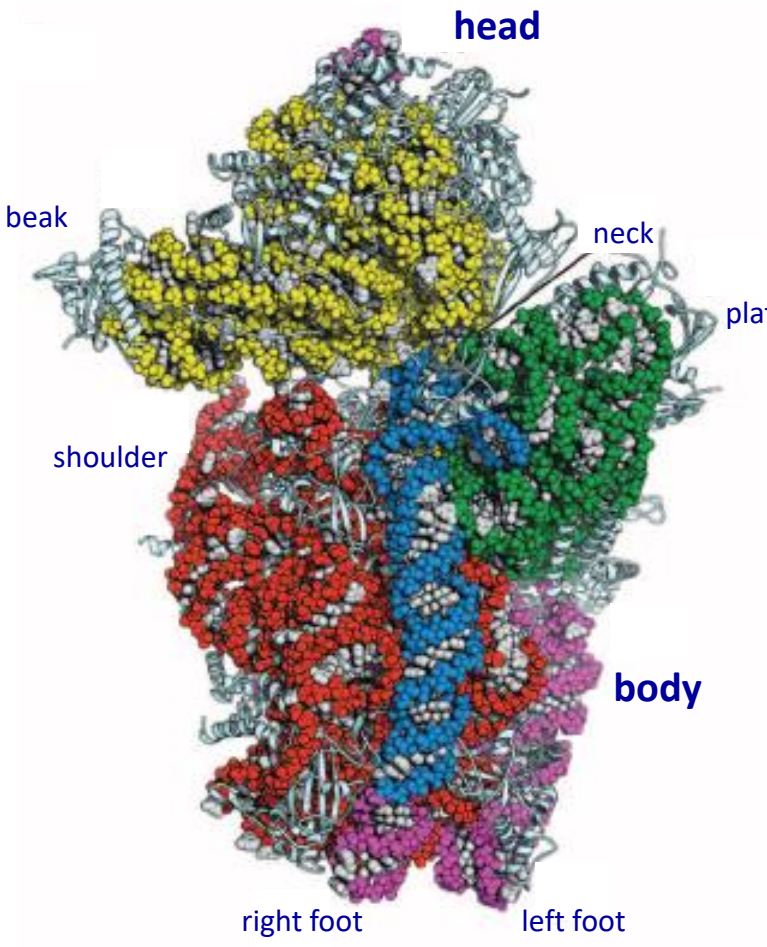
Eukaryotic



At least 8 initiation factors
18 - 25 polypeptides

Hinnebusch 2014

The overall structure of small ribosomal subunit

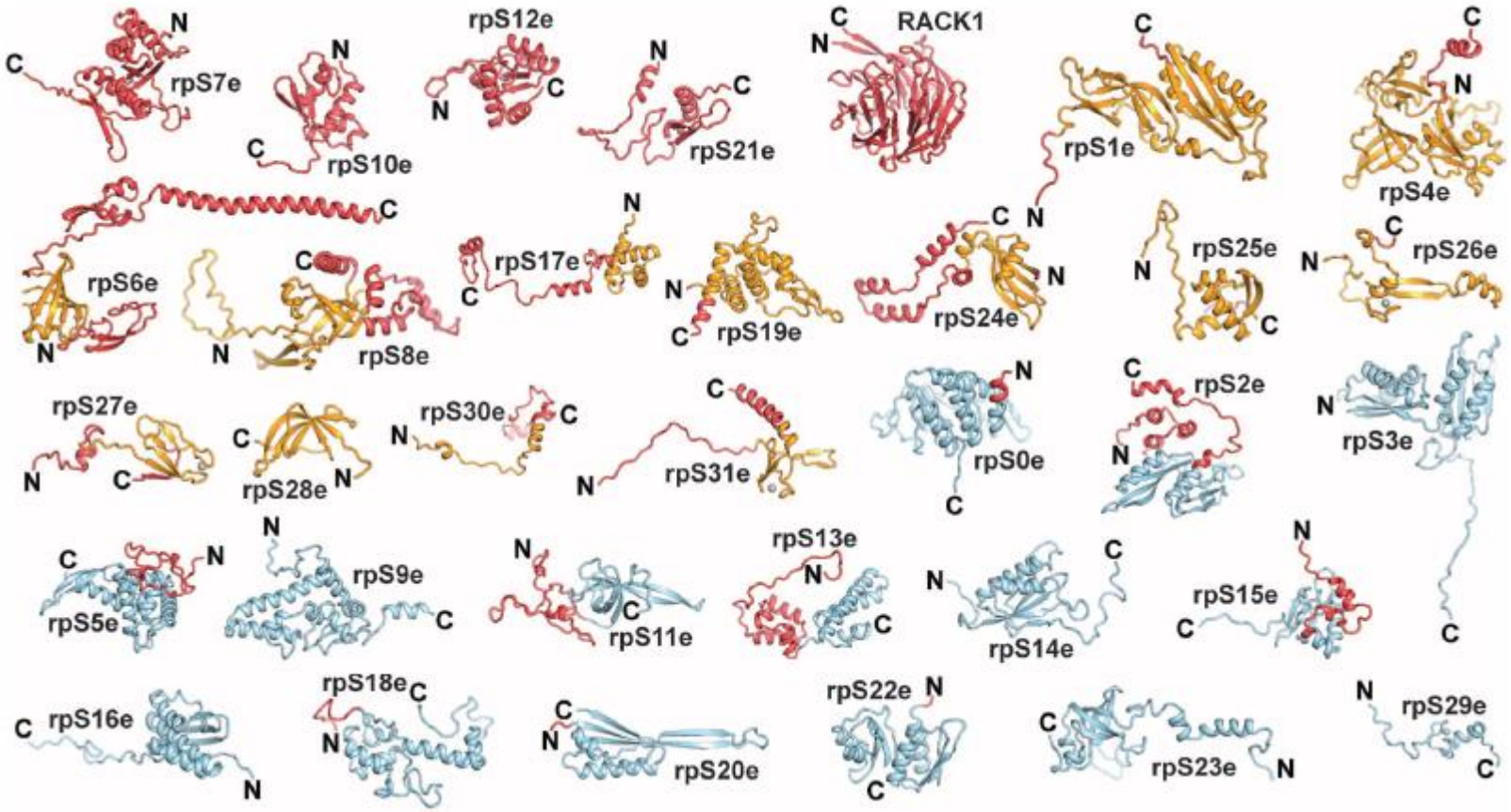


Rabl et al. Science 2011

40S small ribosomal subunit

http://www.duskyswondersite.com/animals/birds/

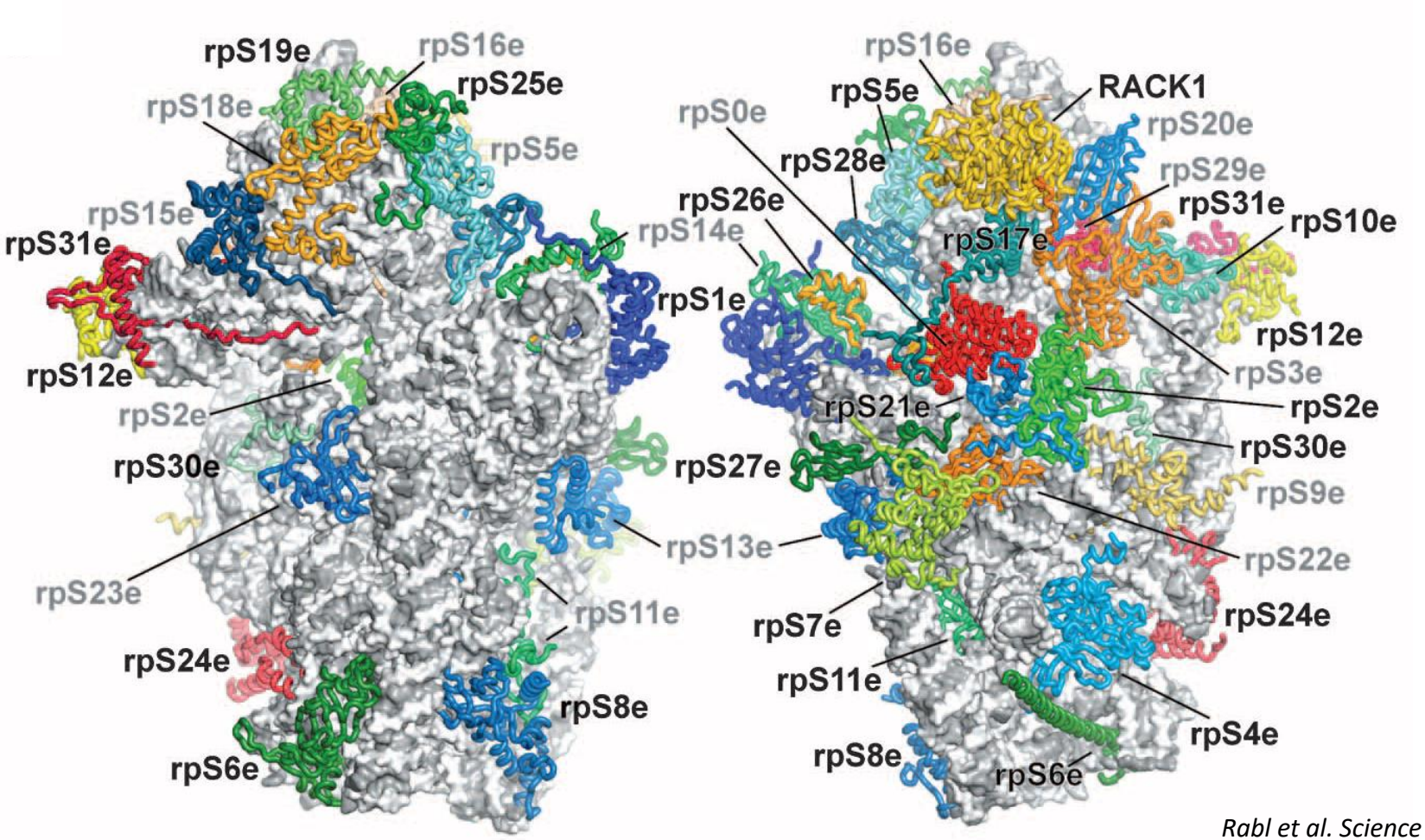
Multi-component molecular machine



Rabl et al. Science 2011

33 ribosomal proteins and one 18S rRNA (1798 nt) make up the 40S

Integrated multi-component system



Rabl et al. Science 2011

Front view **Back view**

40S small ribosomal subunit

- The structure reveals the overall design of the molecular machine
- It shows how the multiple components come together to form it
- The rigid and flexible regions of the molecular machines and the accessible surface/regions of the molecular machine
- It may give insights into the working of the molecular machine together with additional information from other studies

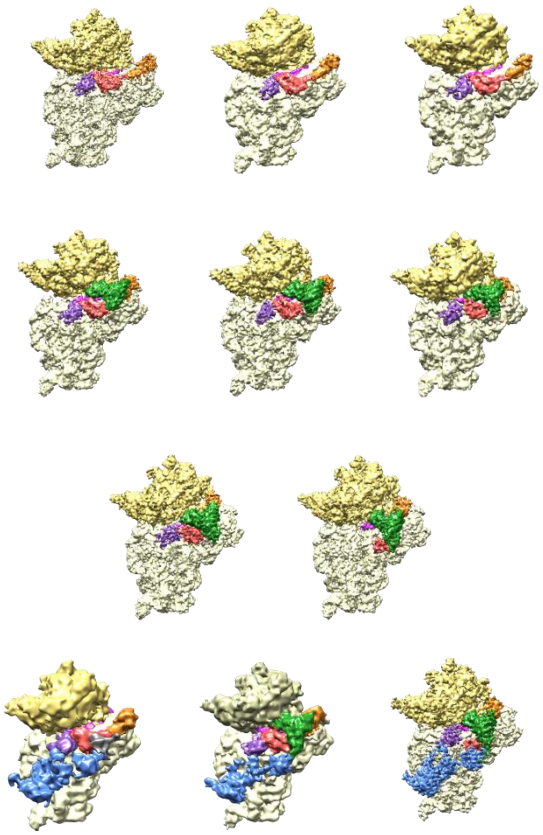
Catching the molecular machines in action….

(determine the structures of macromolecular complexes in multiple states of its activity)

Translation initiation (multiple states, cryo-EM maps)

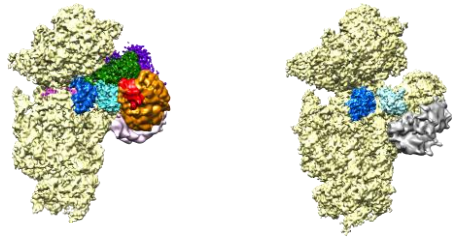
Venki Ramakrishnan's Lab

Bacterial initiation complexes



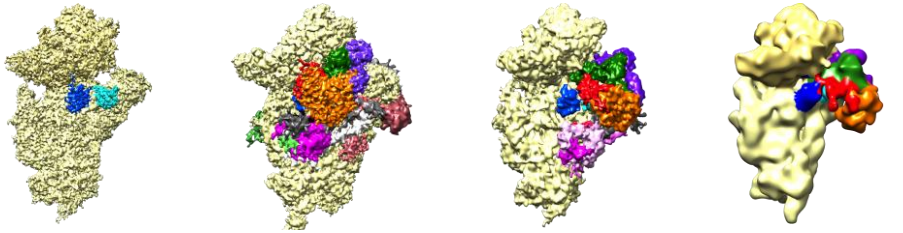
Hussain, Llacer* and Wimberly* et. at. (2016)*

Eukaryotic initiation complexes



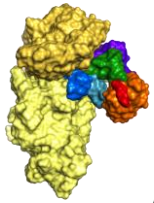
EMD-2763 EMD-2764

Hussain, Llacer* et. at. 2014*



EMD-3047 EMD-3048 EMD-3049 EMD-3050

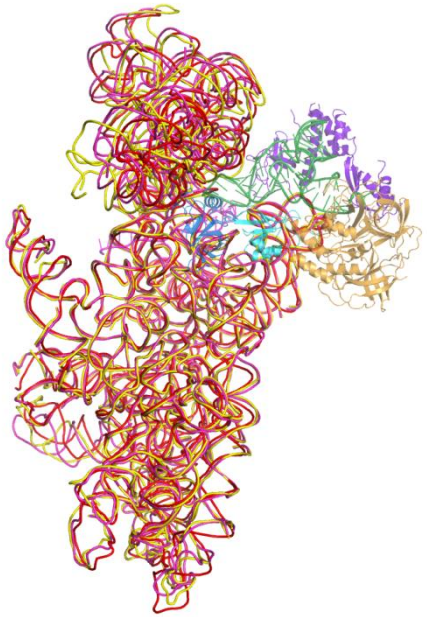
Llacer, Hussain* et. at. 2015*

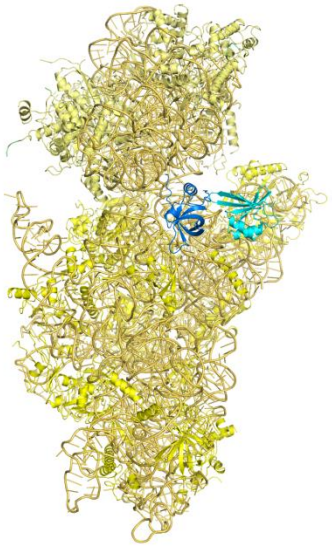


Llacer, Hussain* et. at. (unpublished)*

Initiation complexes

- The small ribosomal subunit are present in multiple conformations throughout the initiation pathway
- Initiation factors either bind to a certain conformation of the small ribosomal subunit or binding of these initiation factors shift the equilibrium towards one conformation





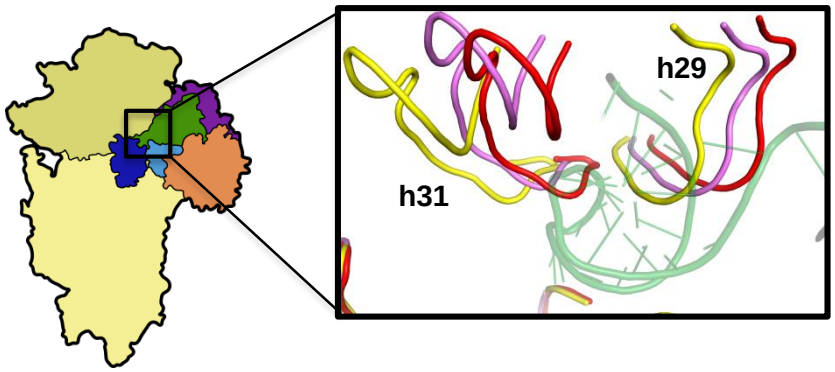
Hussain, Llacer* et. at. 2014*

Llacer, Hussain* et. at. 2015*

Hussain, Llacer* and Wimberly* et. at. 2016*

Initiation complexes

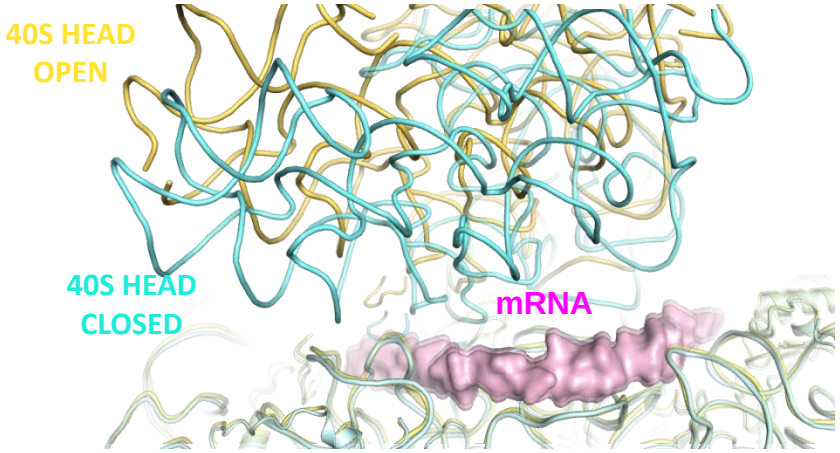
- The small ribosomal subunit are present in multiple conformation throughout the initiation pathway
- Initiation factors either bind to a certain conformation of the small ribosomal subunit or binding of these initiation factors shift the equilibrium towards one conformation
- Binding of initiation factors causes conformational changes at the P site which is essential for recruitment of initiator tRNA



Hussain, Llacer* et. at. 2014*
Llacer, Hussain* et. at. 2015*
Hussain, Llacer* and Wimberly* et. at. 2016*

Initiation complexes

- The small ribosomal subunit are present in multiple conformation throughout the initiation pathway
- Initiation factors either bind to a certain conformation of the small ribosomal subunit or binding of these initiation factors shift the equilibrium towards one conformation
- Binding of initiation factors causes conformational changes at the P site which is essential for recruitment of initiator tRNA
- Initial binding of initiator tRNA/mRNA would prefers open conformation of the small ribosomal subunit



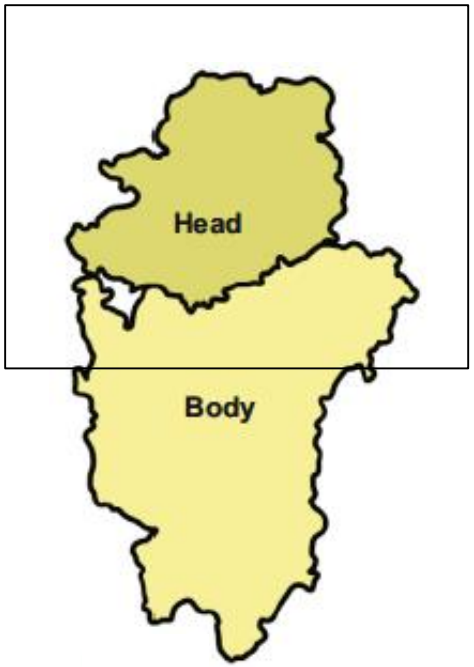
Hussain, Llacer* et. at. 2014*
Llacer, Hussain* et. at. 2015*
Hussain, Llacer* and Wimberly* et. at. 2016*

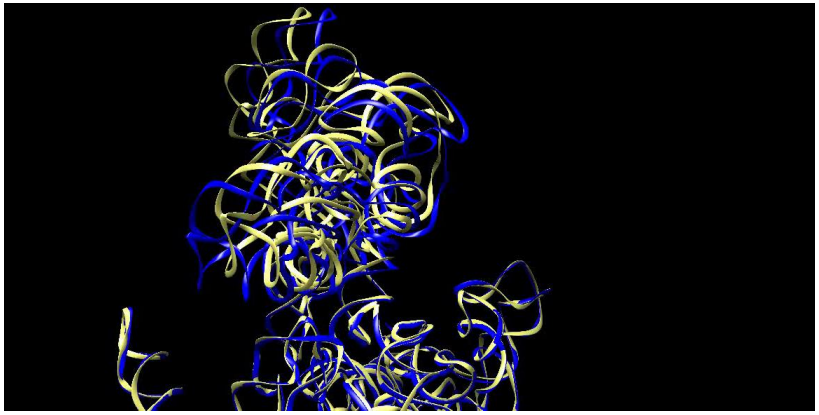
Initiation complexes

- The small ribosomal subunit are present in multiple conformation throughout the initiation pathway
- Initiation factors either bind to a certain conformation of the small ribosomal subunit or binding of these initiation factors shift the equilibrium towards one conformation
- Binding of initiation factors causes conformational changes at the P site which is essential for recruitment of initiator tRNA
- Initial binding of initiator tRNA/mRNA would prefers open conformation of the small ribosomal subunit
- The closed conformation of small ribosomal is essential for the fidelity of tRNA and start codon selection
- Multiple conformations of the initiation factors that may lead to its eventual release from the initiation complex

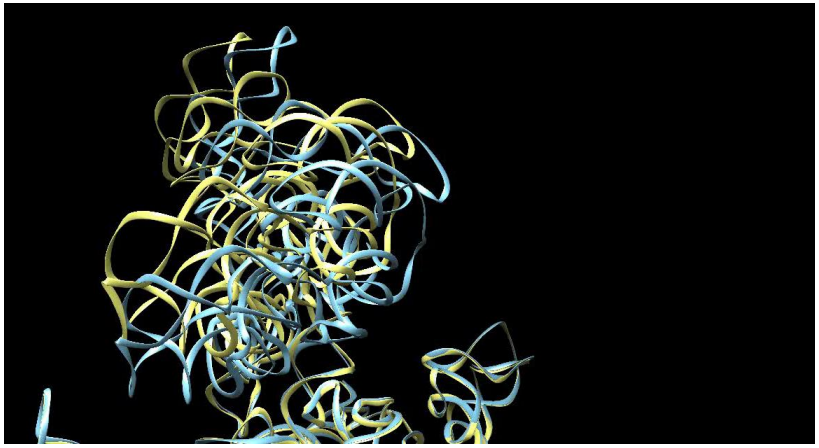
Hussain, Llacer* et. at. 2014*
Llacer, Hussain* et. at. 2015*
Hussain, Llacer* and Wimberly* et. at. 2016*

40S head movement in Eukaryotic initiation complex



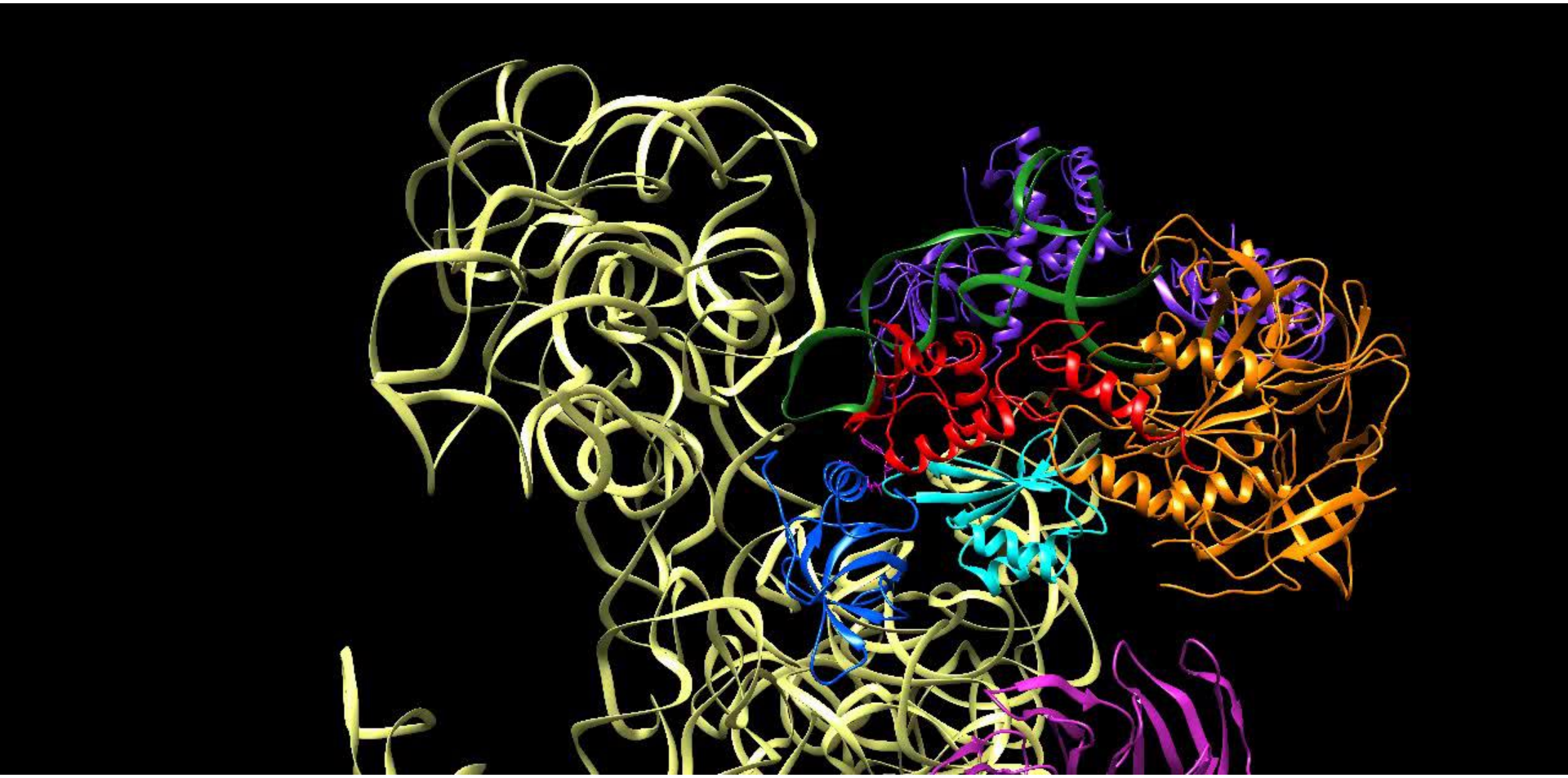


Head swivel



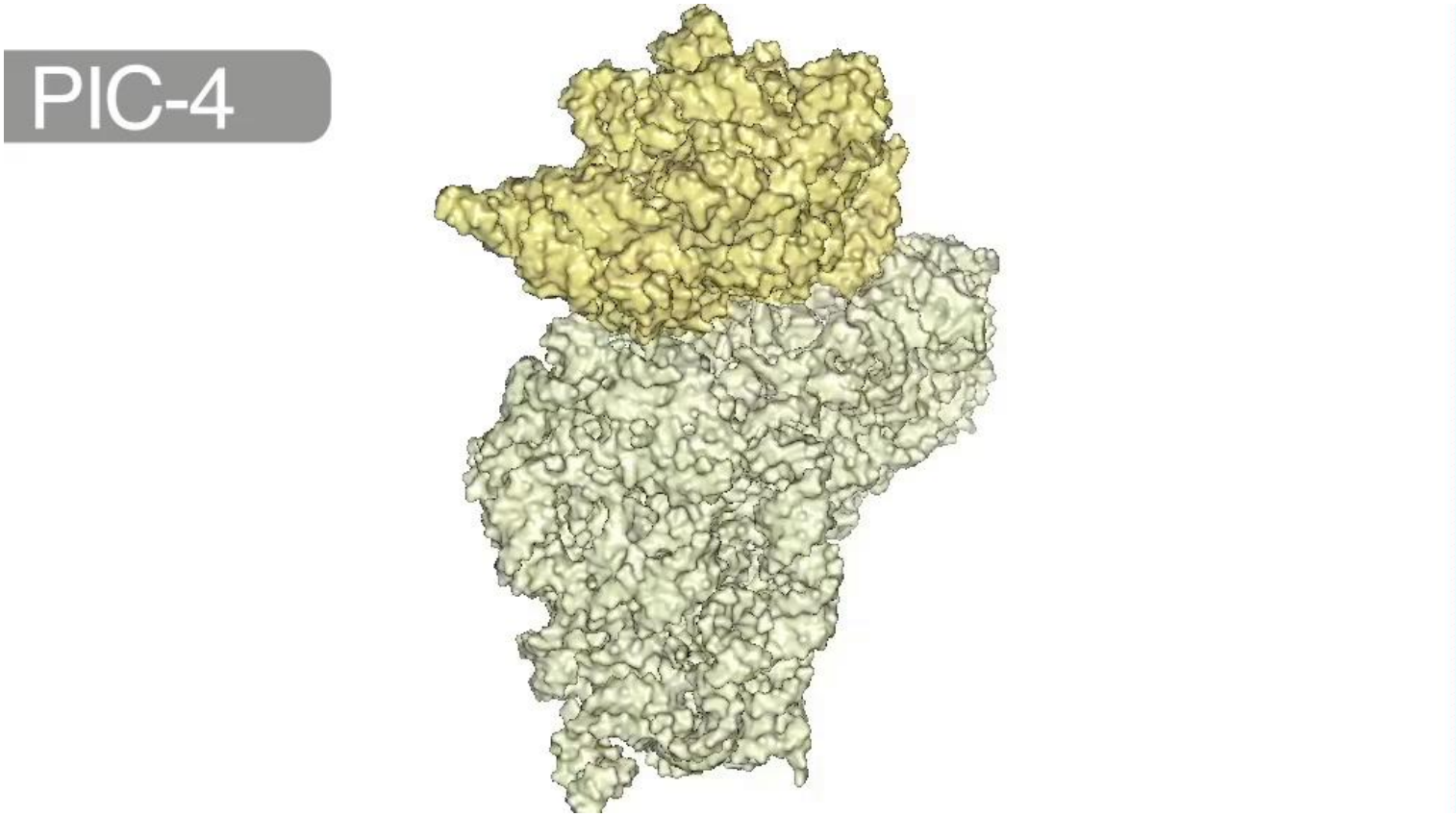
Head open
and closed
conformation

Conformational changes in eukaryotic initiation complex from open to closed conformation



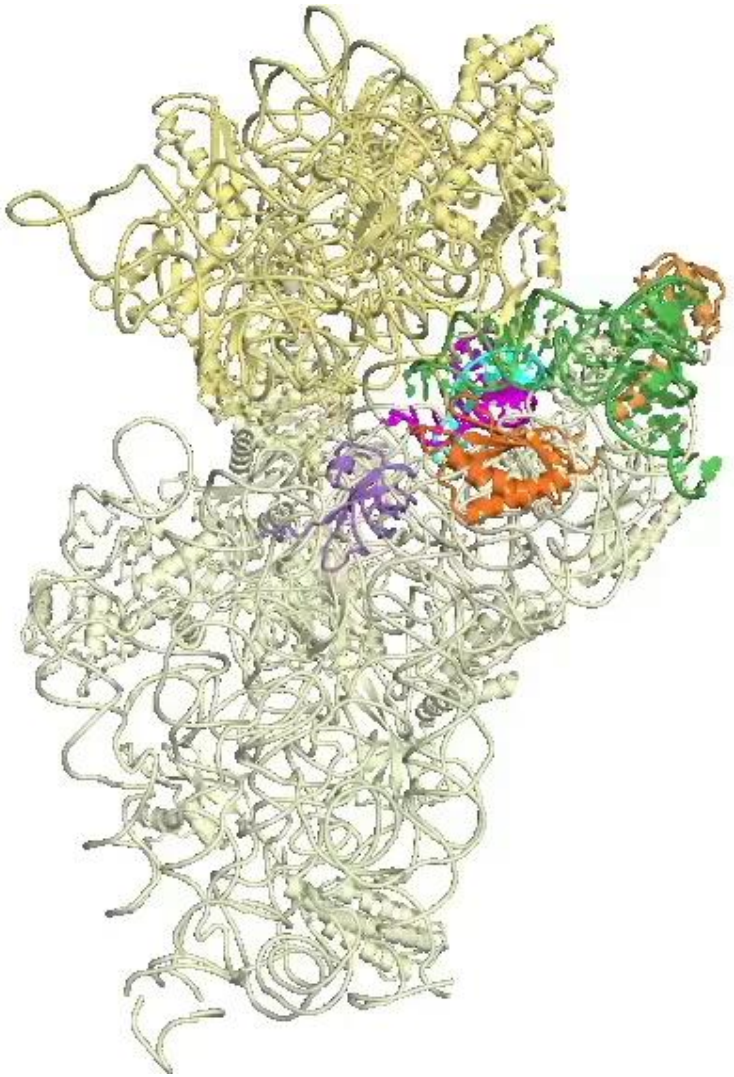
Conformational changes observed in 30S head

1. Rotation or swivel of the 30S head
2. Movement of 30S head up/down with respect to 30S body to open/close the mRNA latch



30S head movement drives initiator tRNA selection

PIC-2A



What we may infer towards design of molecular machines

- A loosely associated bulky head of small ribosomal subunit connected by a narrow neck to the body allows multiple conformations and hence motion

- Brownian motion seems sufficient to for swiveling and opening and closing movements. Binding of ligands to certain conformations may provide directionality to the motion. (Energy may be used as well to provide directionality)

- Flexibility and intramolecular movements may provide necessary conditions for the process of attachment and detachment of large ligands. So the binding as well dissociation may proceed via intermediate states with partially formed or disrupted binding contacts

Acknowledgement

Venki Ramakrishnan
and his lab members

Collaborators (at NIH)
Alan G. Hinnebusch
Jon R. Lorsch
(and their Lab members)

THANK YOU

EMBO

MRC | Laboratory of Molecular Biology