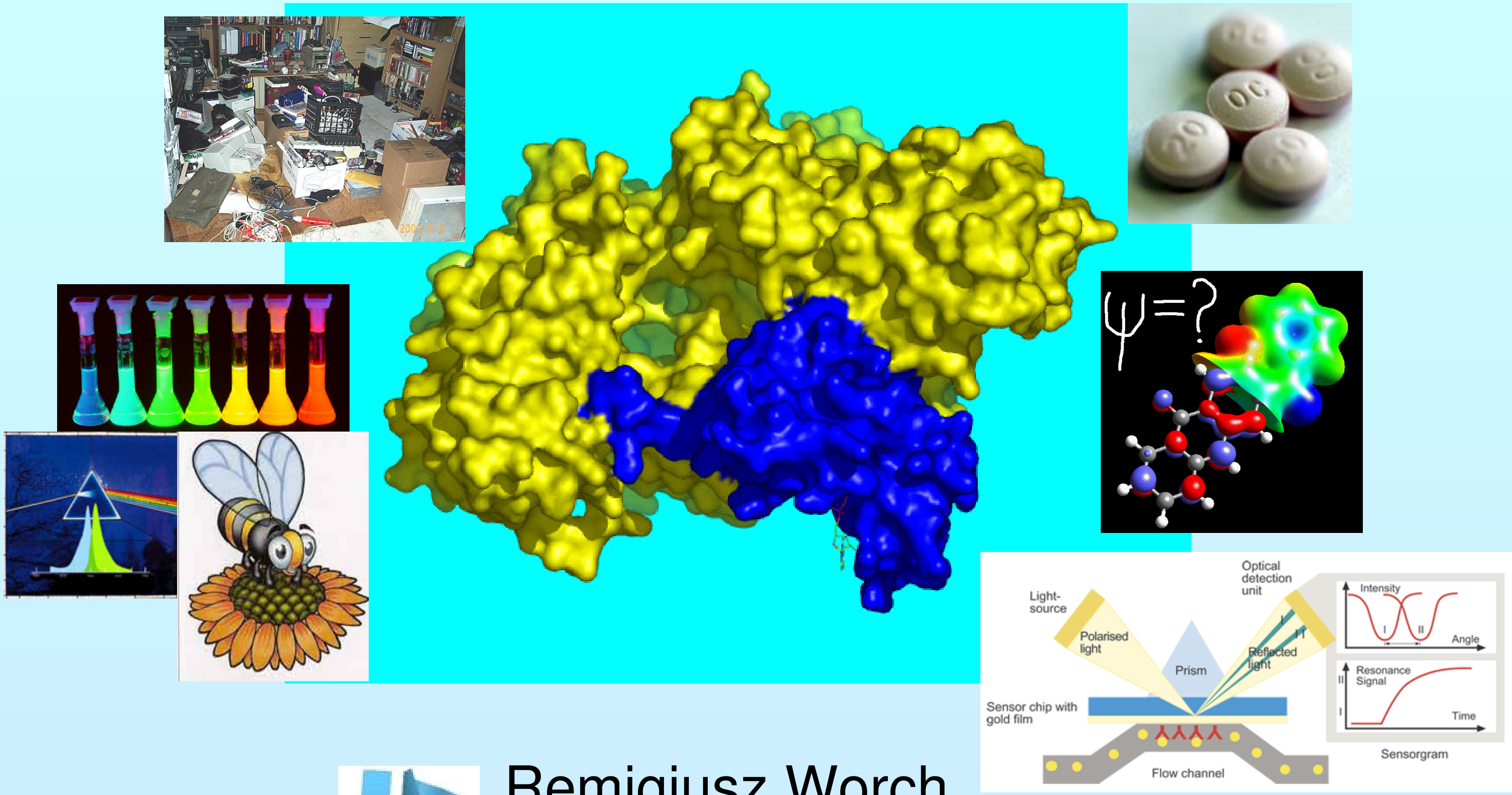
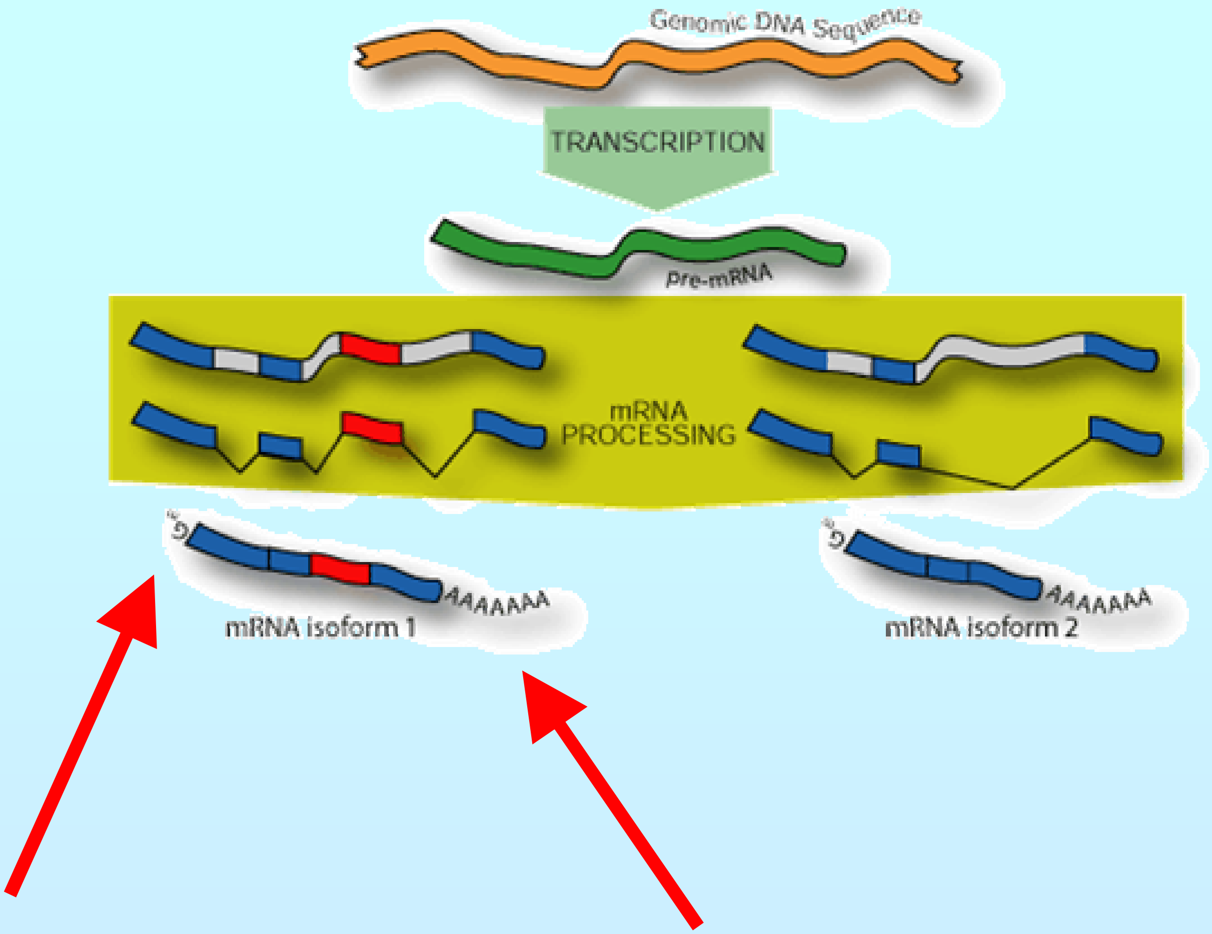
Around the CBC protein



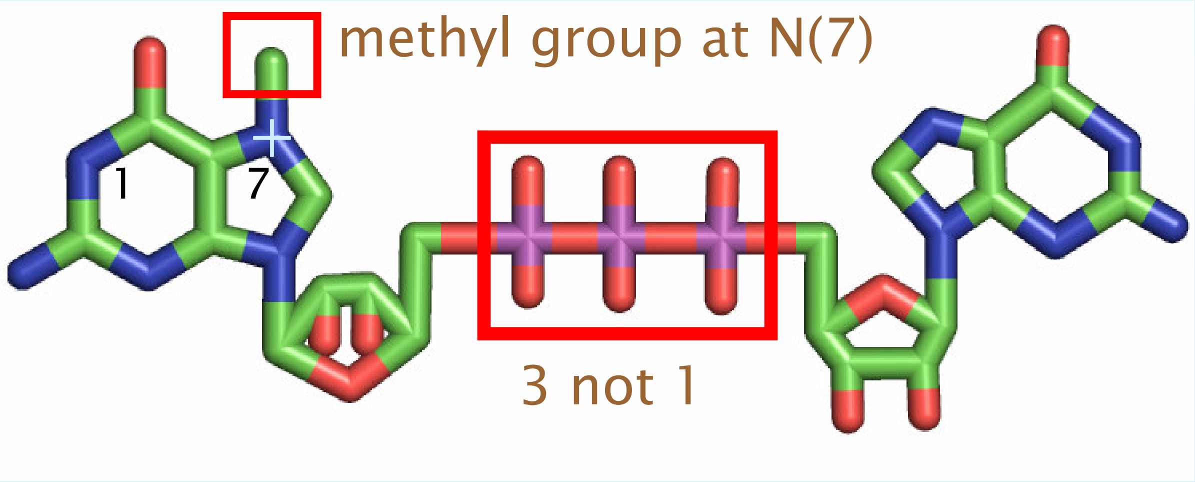
Remigiusz Worch
Biophysics Department
Warsaw University

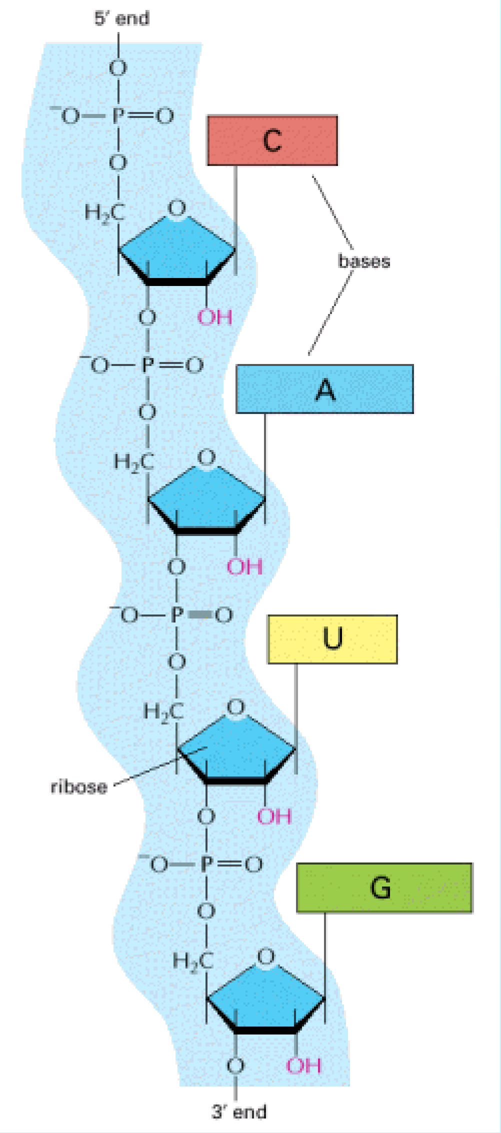


Każdy kij ma dwa końce.

mRNA chemistry

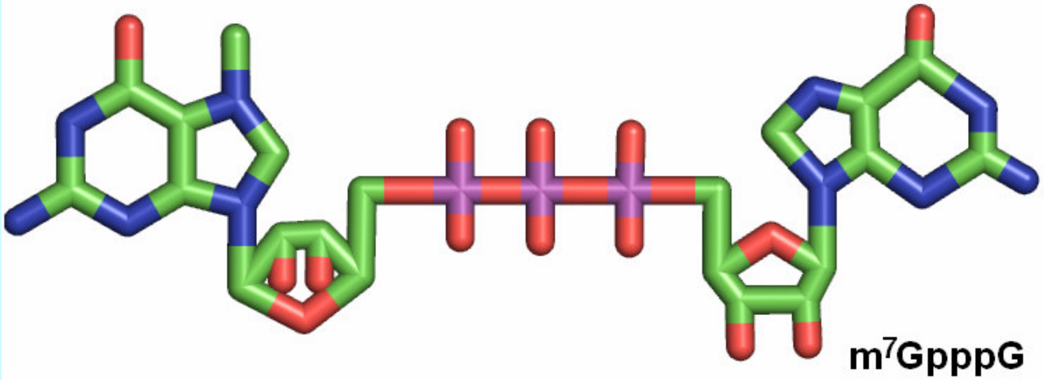
5’ cap



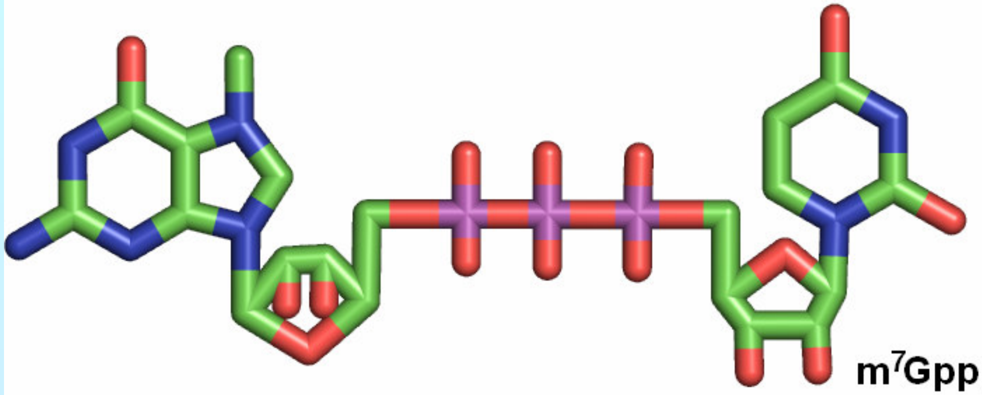


first transcribed nucleoside

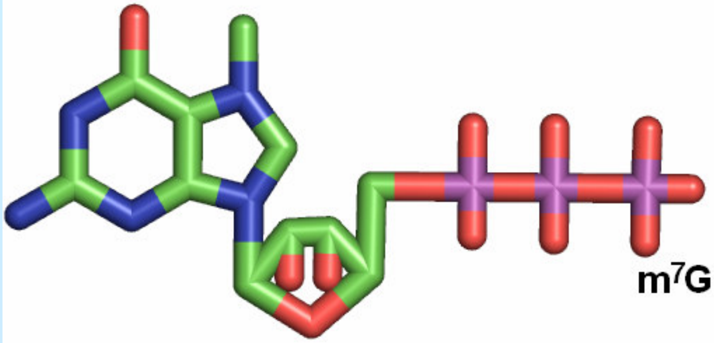
Cap analogues



m^7GpppG



m^7GpppU

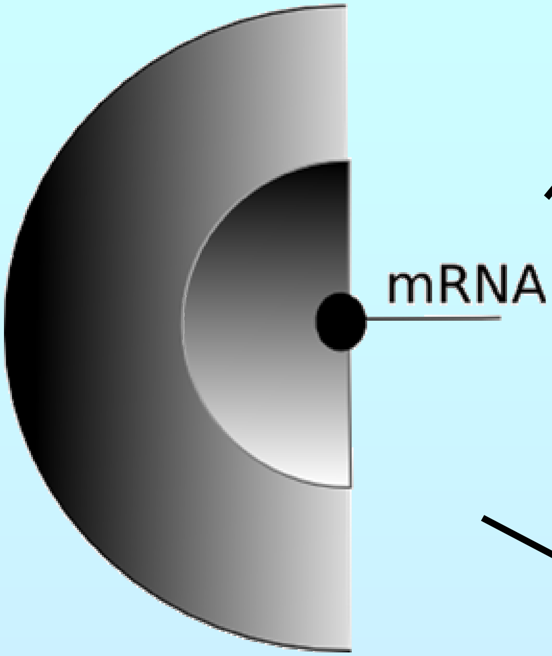


m^7GTP

If the cap fits

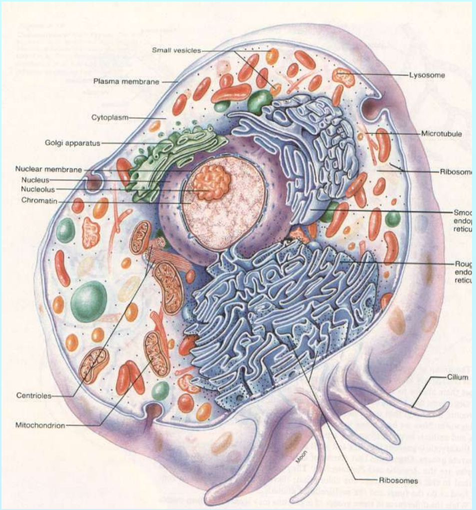
wear it!

CBC



pre mRNA splicing

mRNA and snRNA
nuclear export

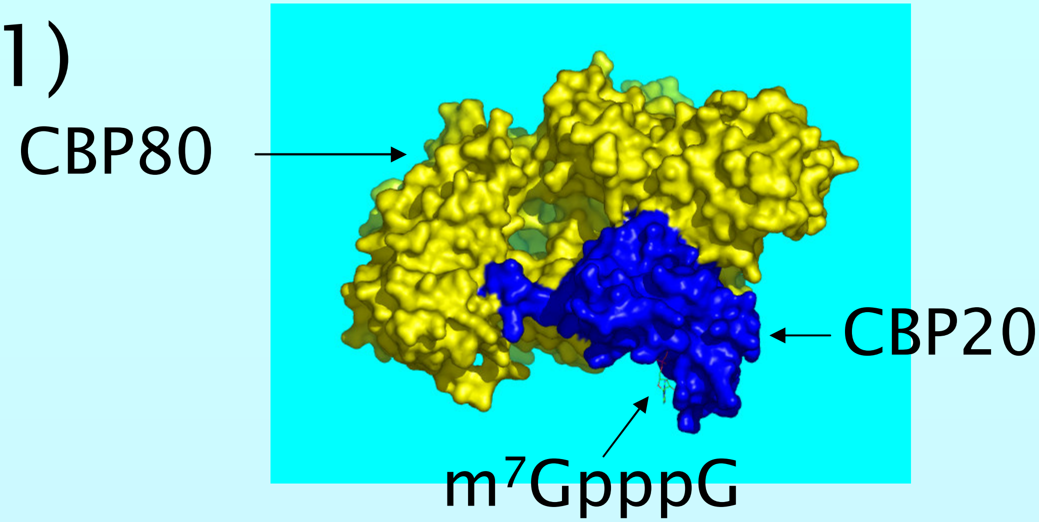


3’ mRNA terminus
processing

translation initiation

nonsense mediated decay (NMD)

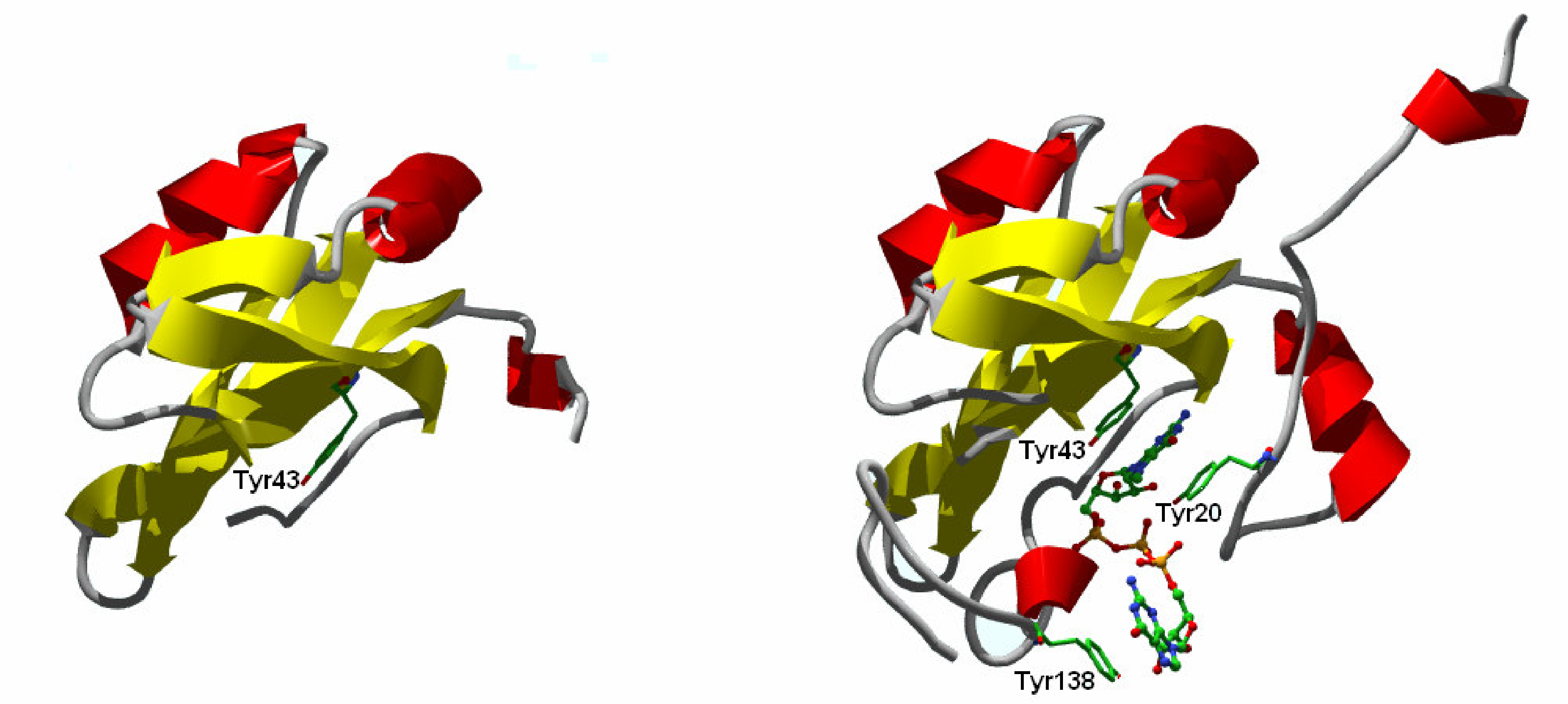
CBC structure (1)


CBP80

CBP20

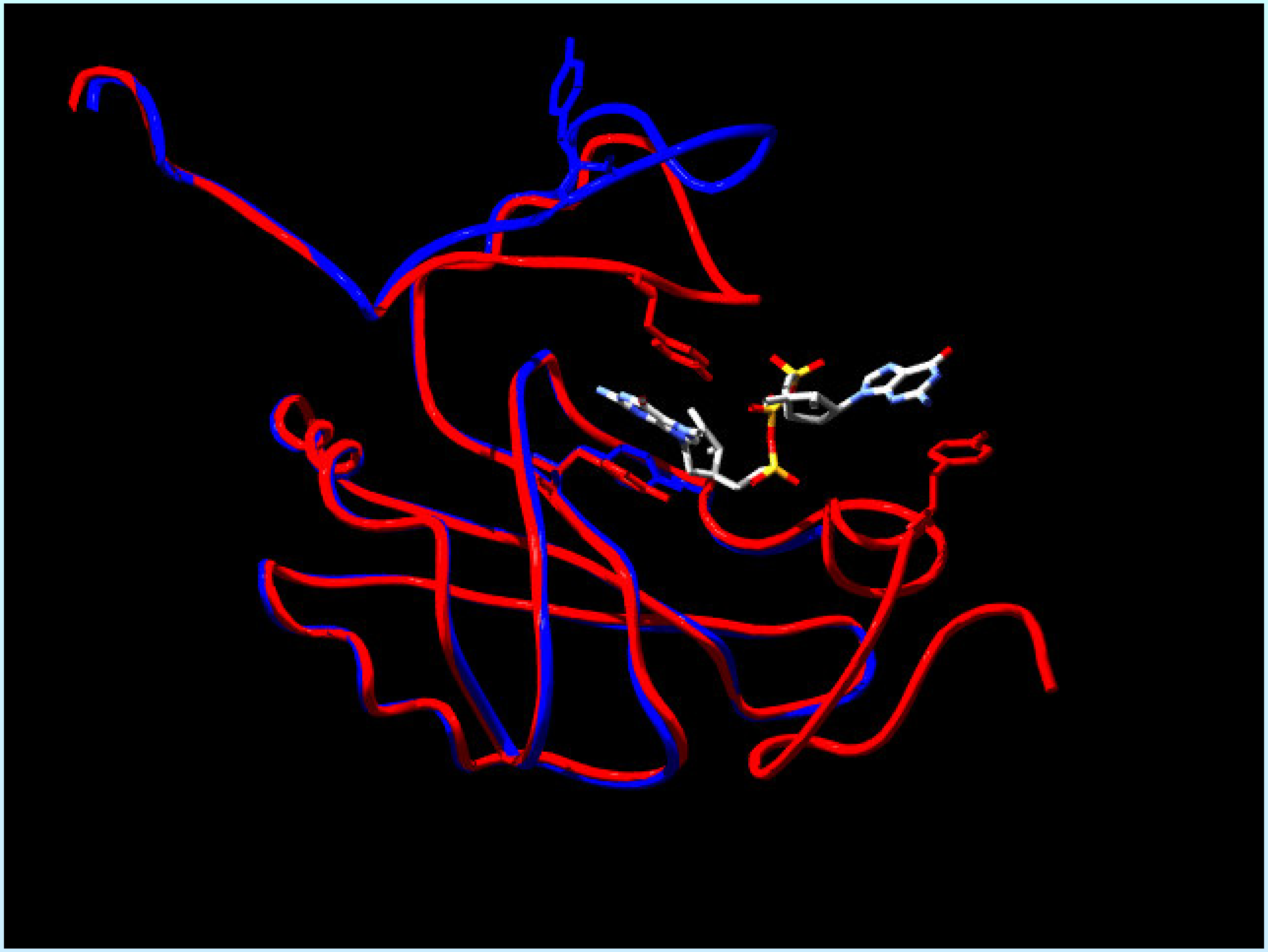
m^7GpppG

Induced fit of CBP20

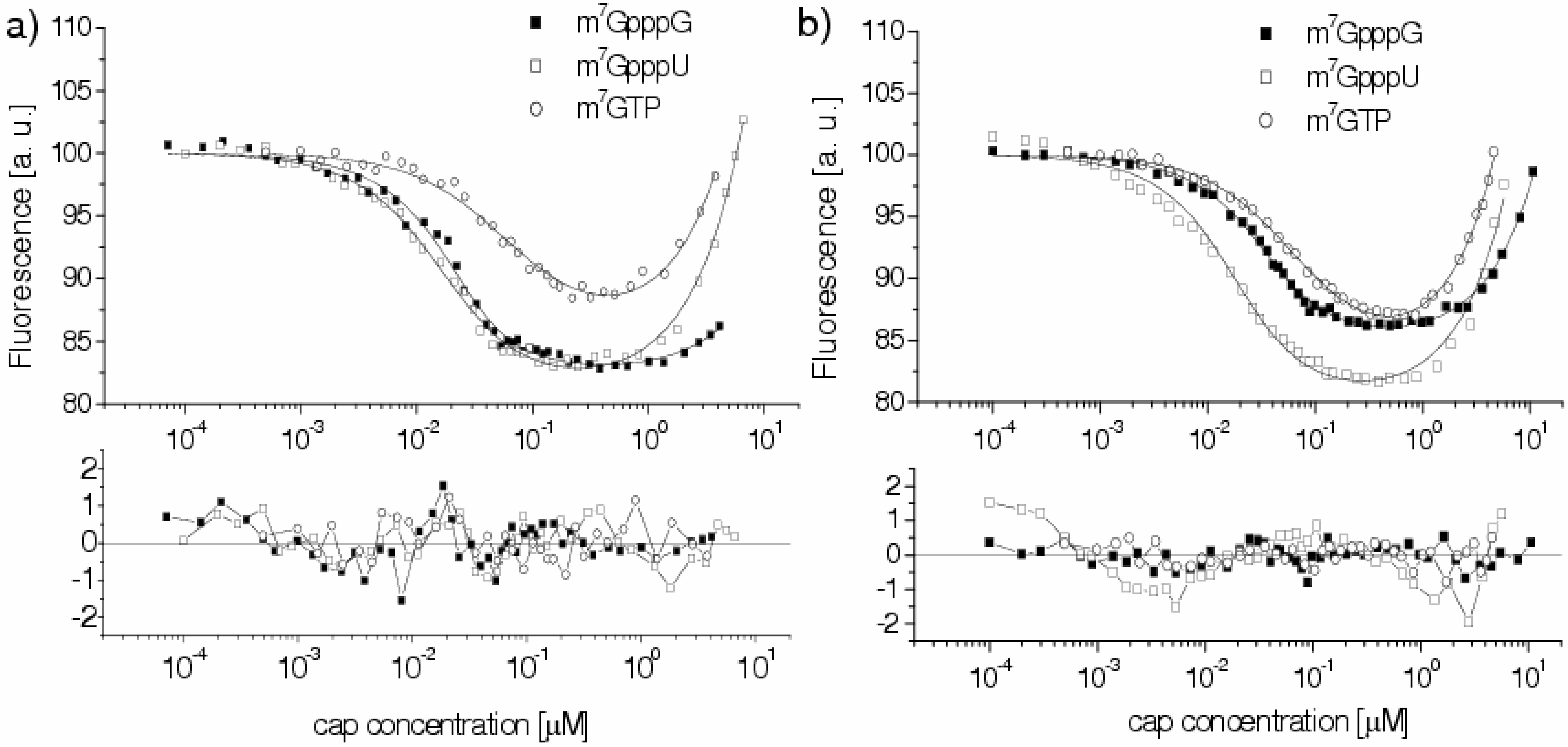


C. Mazza *et al.*, The EMBO Journal 2002

CBC structure (2)



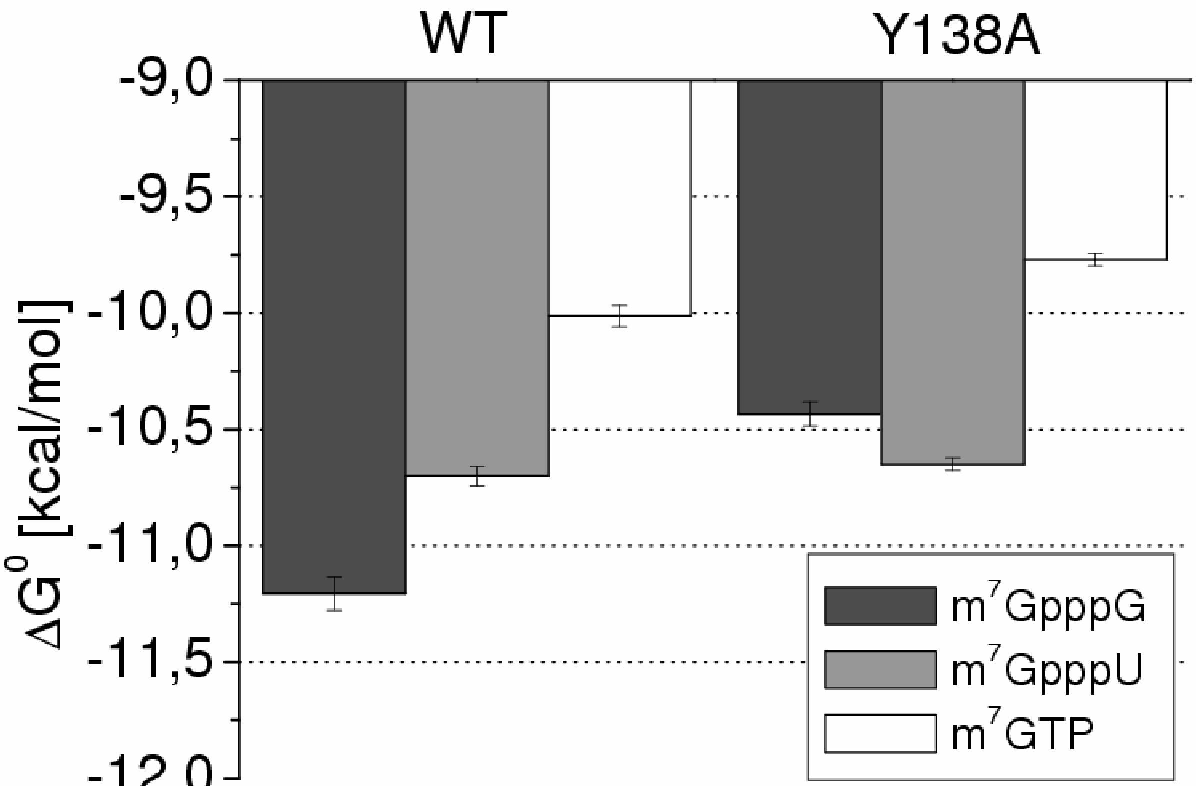
Calero, G *at al.*, Nature Struct. Biol., 2002

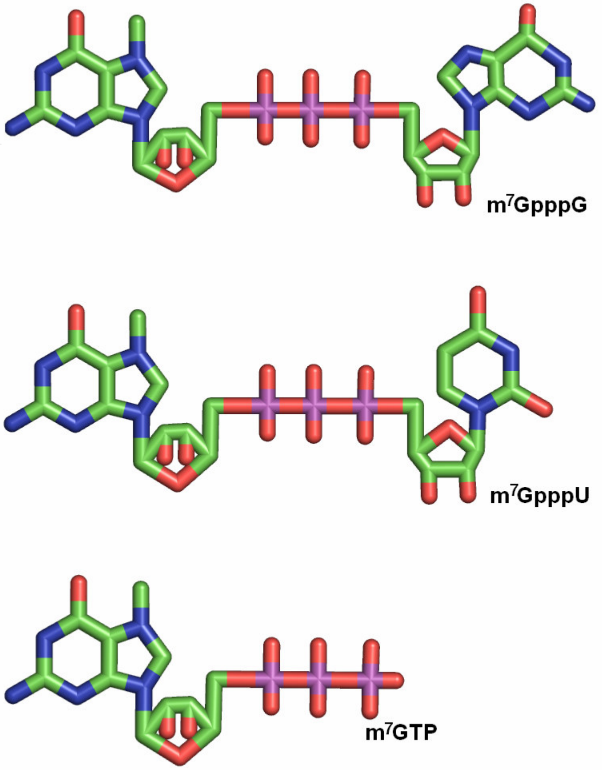


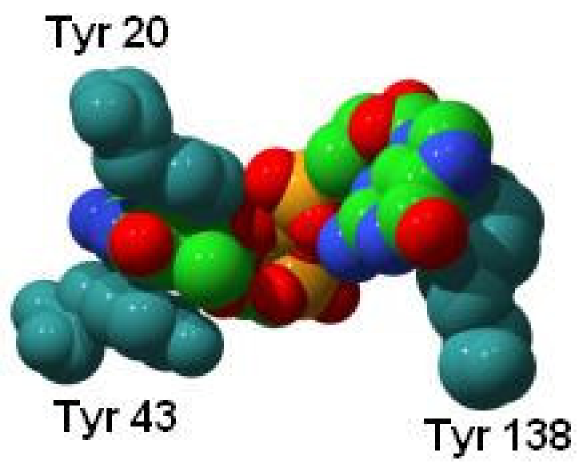
WT

Y138A

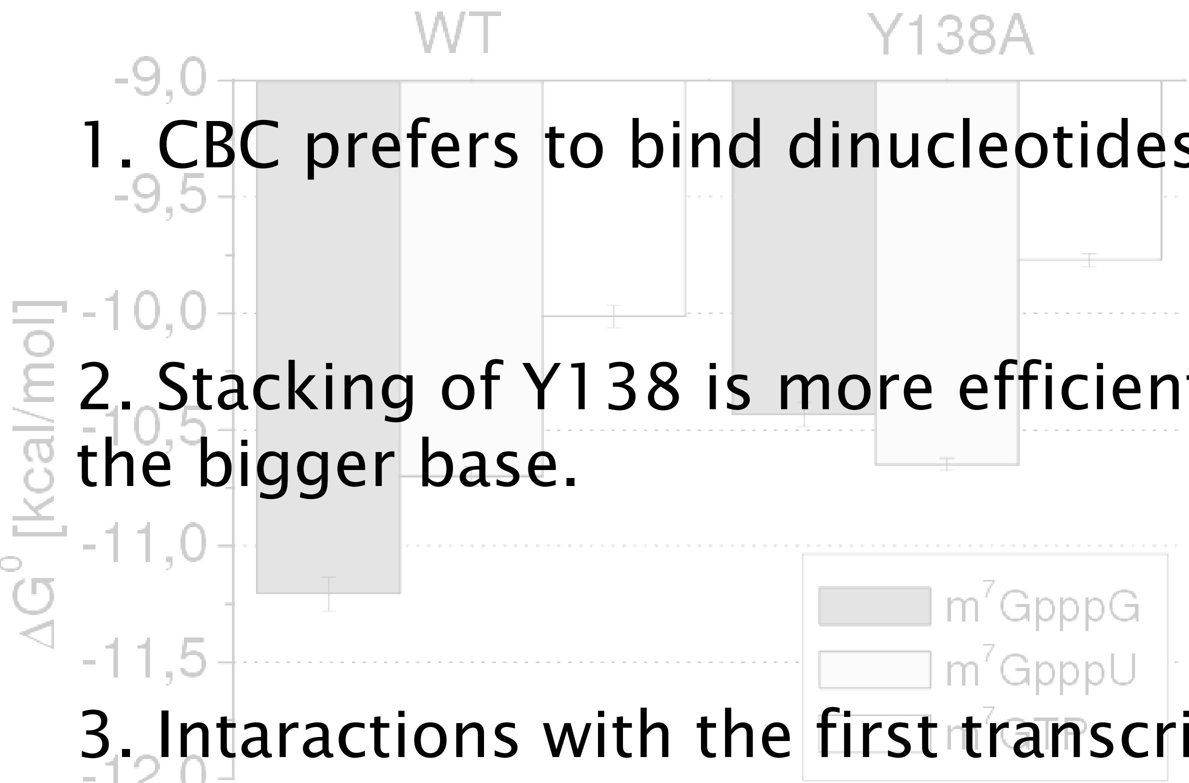
Worch, R. *et al.*, Journal of Physics: Condensed Matter *in press*

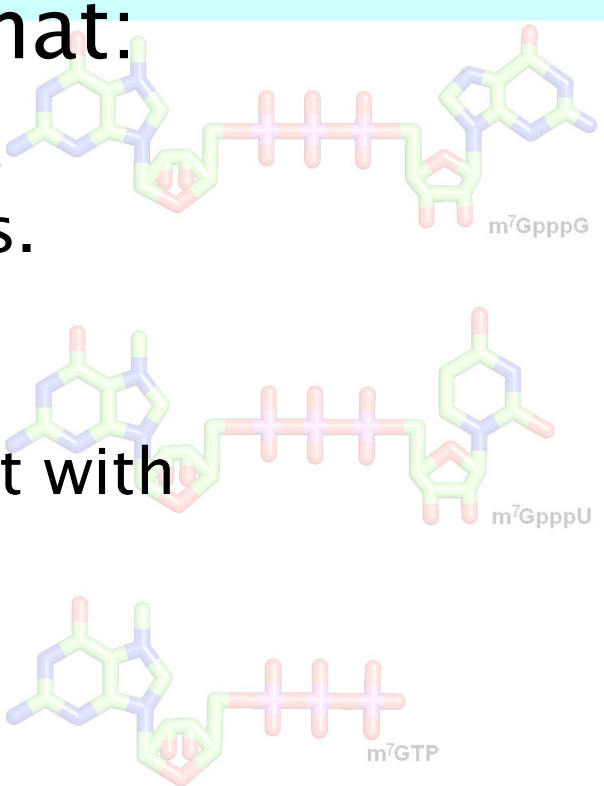

WT
Y138A
-9,0
-9,5
-10,0
-10,5
-11,0
-11,5
-12,0
ΔG^0 [kcal/mol]
m^7GpppG
m^7GpppU
m^7GTP

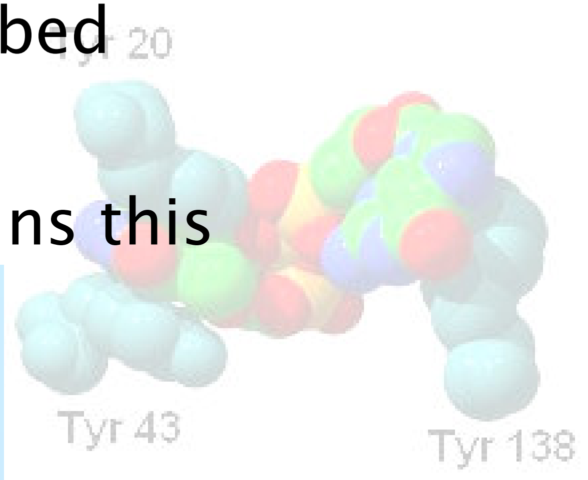

m^7GpppG
m^7GpppU
m^7GTP


Tyr 20
Tyr 43
Tyr 138

Gibbs free energy told us, that:







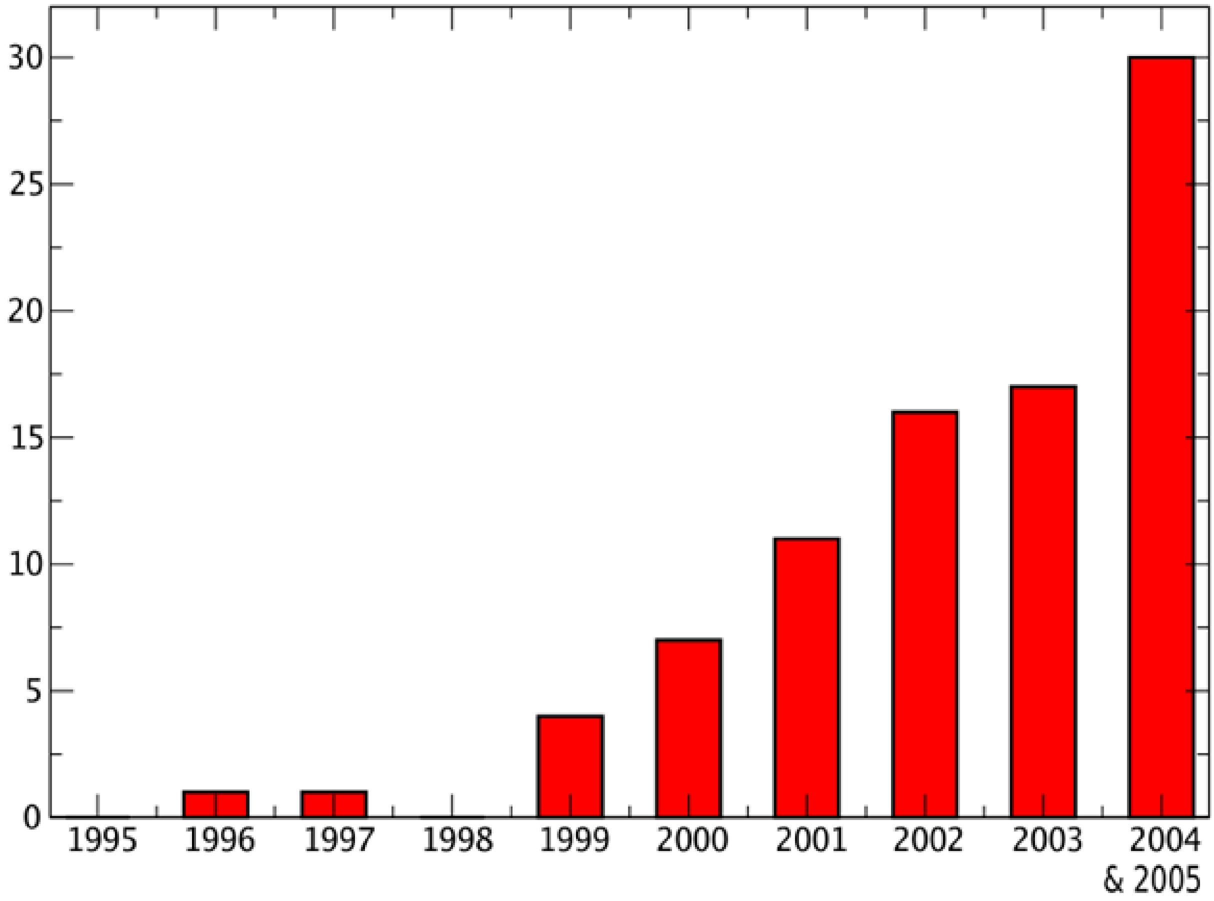
1. CBC prefers to bind dinucleotides.

2. Stacking of Y138 is more efficient with the bigger base.

3. Intaractions with the first transcribed nucleoside plays a key role.

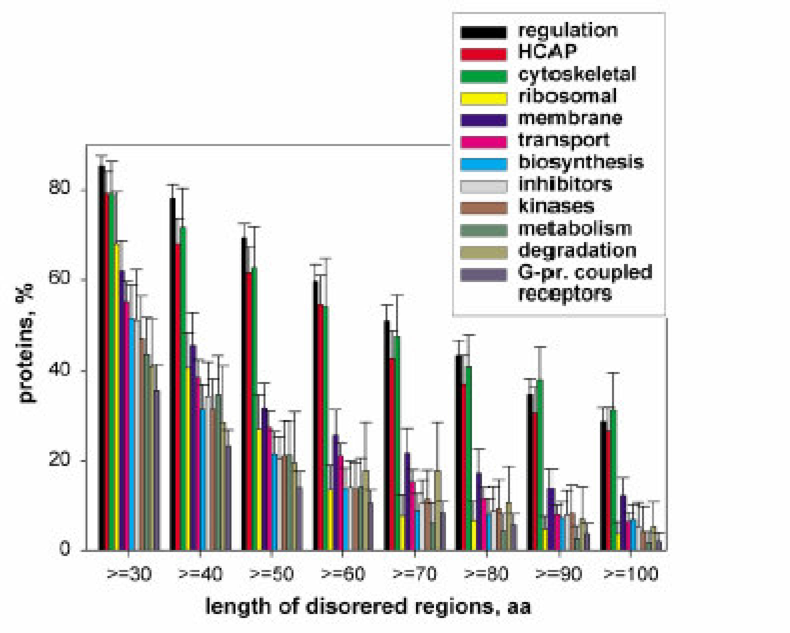
Among all known cap binding proteins this CBC feature is a unique one.

‘Natively unfolded’ proteins in the journals:



>30 % of eukaryotic proteins have disordered regions of lenght > 50 residues

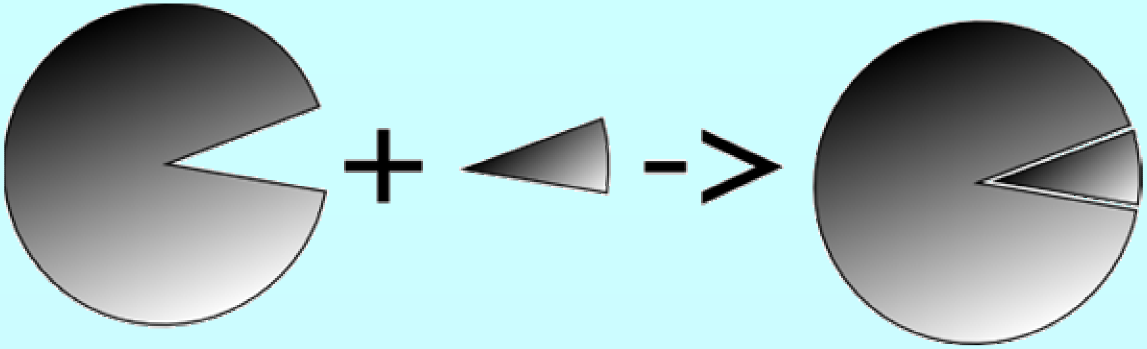
Dunker *et al.,* J. of Mol. Graph. and Modelling 2001



Iakoucheva, LM *at al.,* JMB 2002

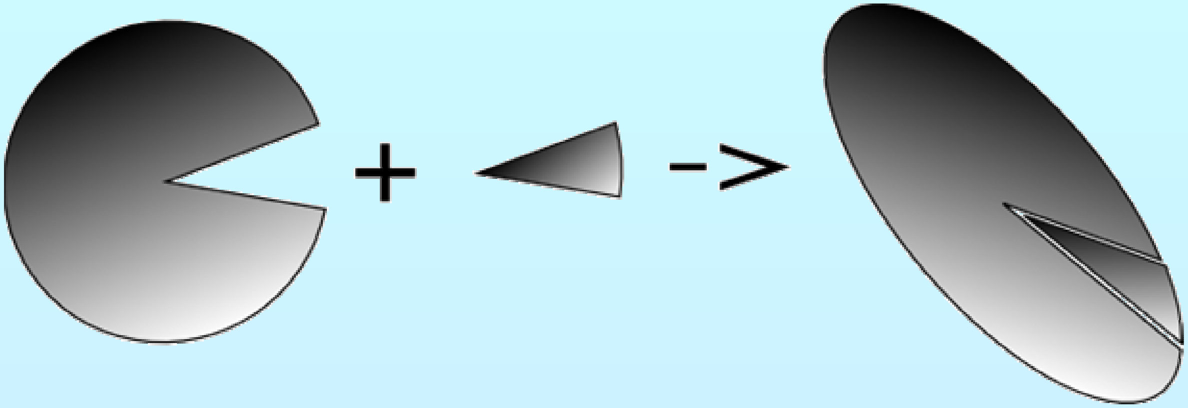
-> Coupling of folding and binding for unstructured proteins

Dyson & Wright, Curr. Opinion in Struct. Biol. 2002



Rigid

ΔS_{rigid}



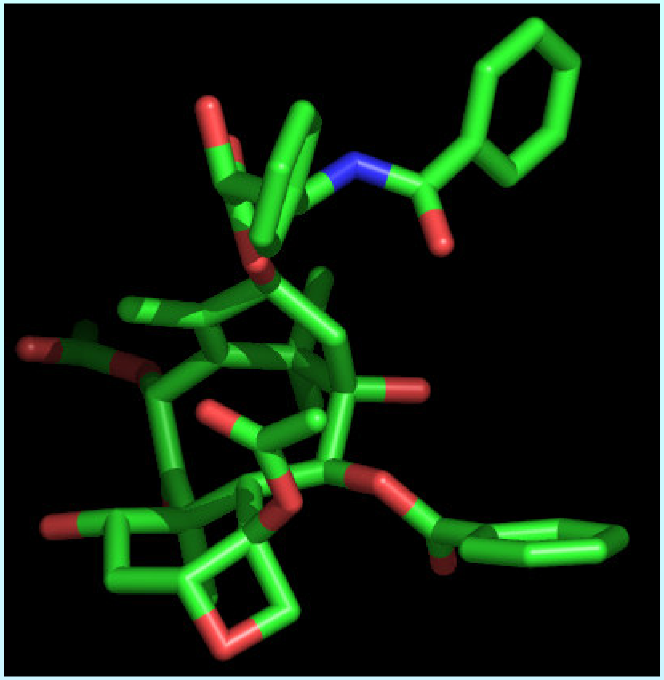
Flexible

ΔS_{flex}

ΔS_{rigid}, $\Delta S_{flex} < 0$ and $|\Delta S_{flex}| > |\Delta S_{rigid}|$

Because $\Delta G = \Delta H - T\Delta S$, assuming $\Delta H_{rigid} = \Delta H_{flex}$

$=> \Delta G_{rigid} < \Delta G_{flex}$



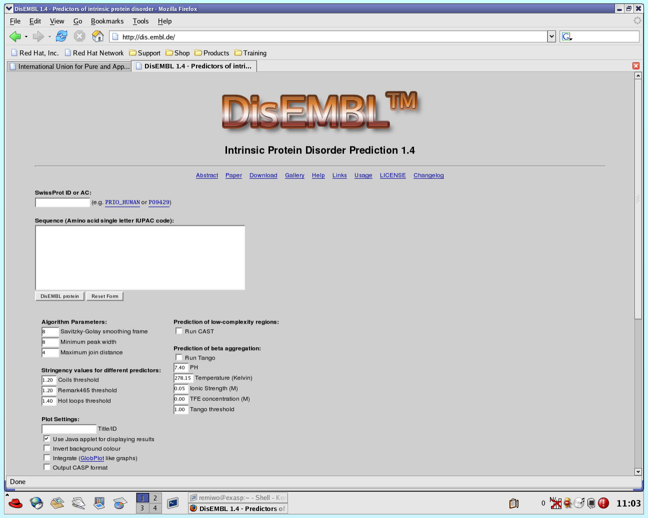
Taxol

interacts with an intrinsically disordered region of Bcl-2 protein

Wallace & Janes, Biochem. Soc. Transactions 2003

and is commonly used as anti-tumor drug

Disorder prediction



Intrinsic Protein Disorder Prediction 1.4

SwissProt ID or AC:
(e.g. PRIO_HUMAN or P09429)

Sequence (Amino acid single letter IUPAC code):

Algorithm Parameters:
8 Savitzky-Golay smoothing frame
8 Minimum peak width
4 Maximum join distance

Stringency values for different predictors:
1.20 Coils threshold
1.20 Remark465 threshold
1.40 Hot loops threshold

Plot Settings:
Title/ID
☑ Use Java applet for displaying results
☐ Invert background colour
☐ Integrate (GlobPlot like graphs)
☐ Output CASP format

Prediction of low-complexity regions:
☐ Run CAST

Prediction of beta aggregation:
☐ Run Tango
7.40 PH
278.15 Temperature (Kelvin)
0.05 Ionic Strength (M)
0.00 TFE concentration (M)
1.00 Tango threshold

DisEMBL

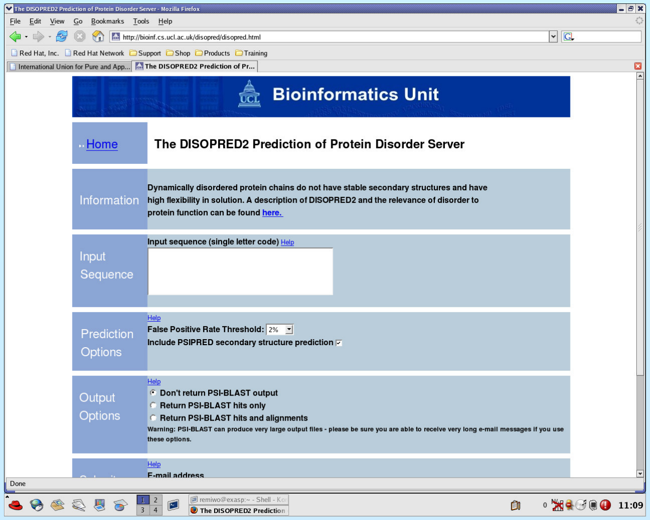
`http://dis.embl.de`

Lindig R. *et al.*, Structure 2003

DISOPRED2

`http://bioinf.cs.ucl.ac.uk/disopred/`

Ward JJ. *et al.*, JMB 2004



The DISOPRED2 Prediction of Protein Disorder Server

Information: Dynamically disordered protein chains do not have stable secondary structures and have high flexibility in solution. A description of DISOPRED2 and the relevance of disorder to protein function can be found here.

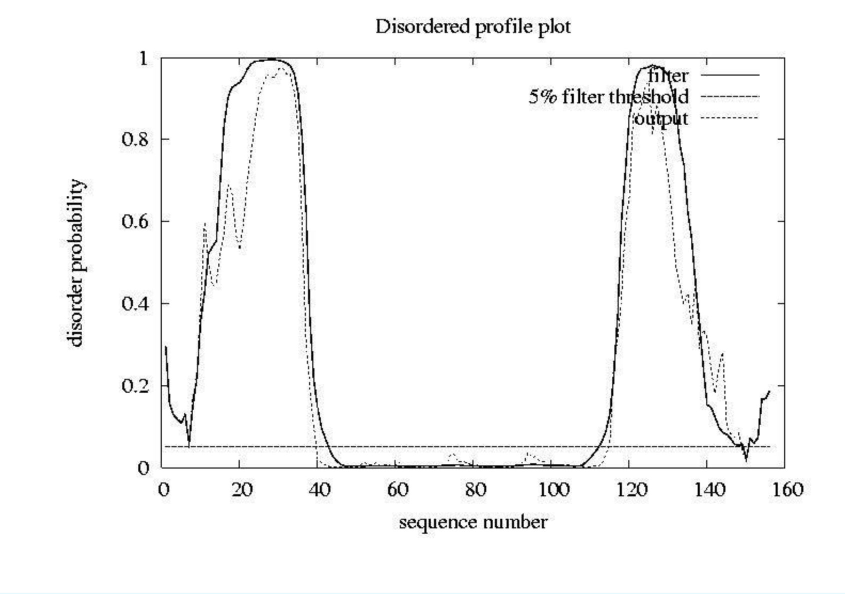
Input Sequence: Input sequence (single letter code)

Prediction Options:
False Positive Rate Threshold: 2%
Include PSIPRED secondary structure prediction ☑

Output Options:
● Don't return PSI-BLAST output
○ Return PSI-BLAST hits only
○ Return PSI-BLAST hits and alignments
Warning: PSI-BLAST can produce very large output files - please be sure you are able to receive very long e-mail messages if you use these options.

E-mail address

DISOPRED2 results for CBP20 sequence



Worch, R. *et al.*, Journal of Physics: Condensed Matter *in press*

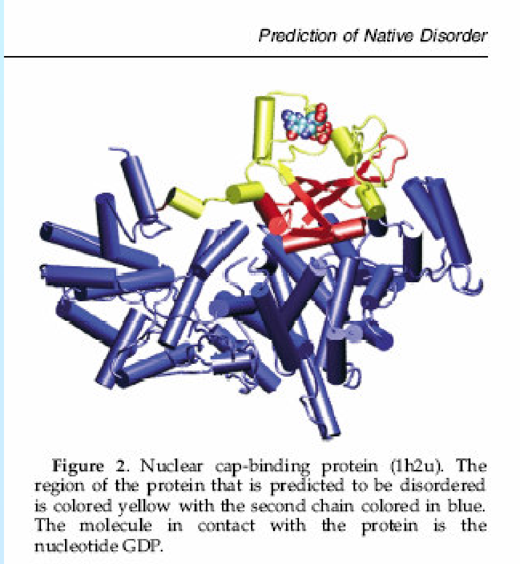
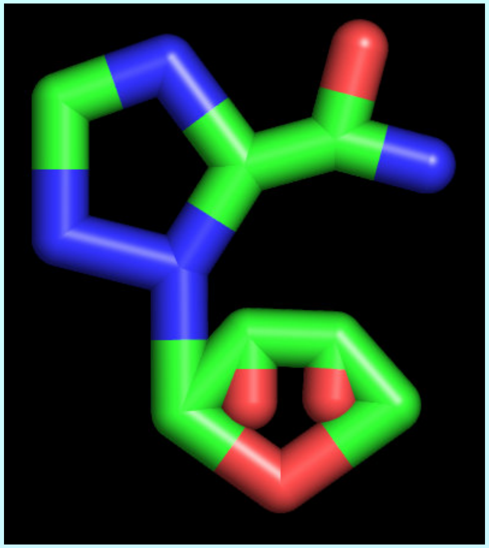

Prediction of Native Disorder

Figure 2. Nuclear cap-binding protein (1h2u). The region of the protein that is predicted to be disordered is colored yellow with the second chain colored in blue. The molecule in contact with the protein is the nucleotide GDP.

Ward JJ, *et al.*, JMB 2004

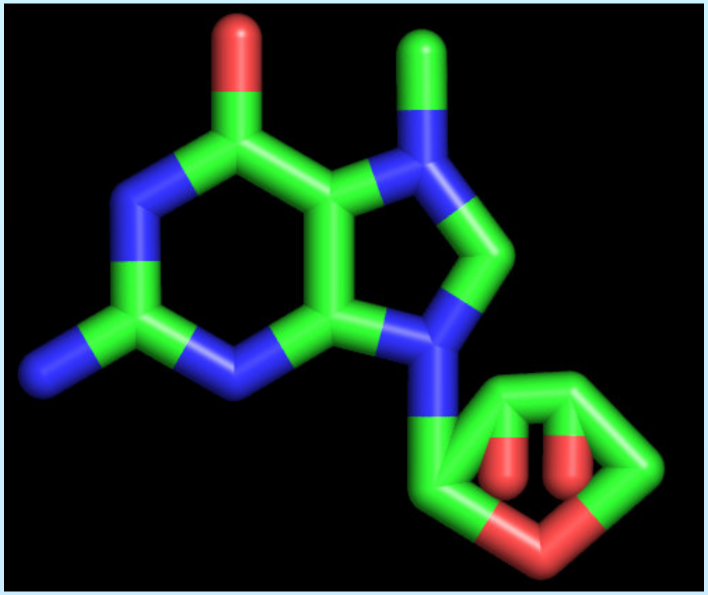


Ribavirin

Is a anti-virial drug used against polio and HCV

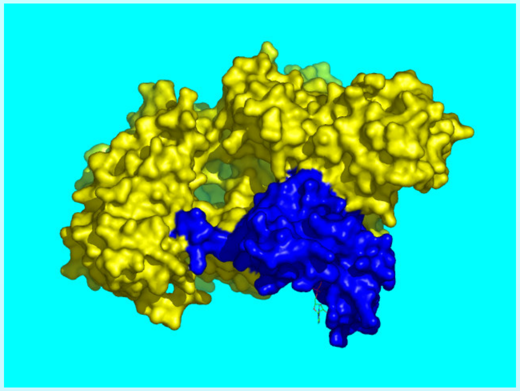
It appeared to inract with eIF4E as efficient as m^7GTP

Kentnis A. *et al.*, PNAS 2004

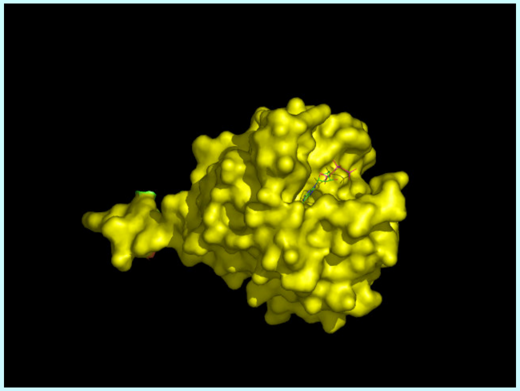


How does it interact with CBC?

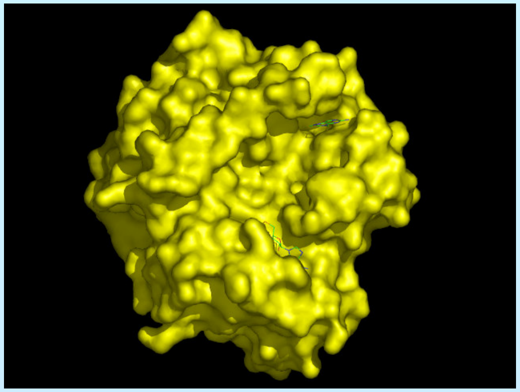
Crystalized cap-binding proteins…



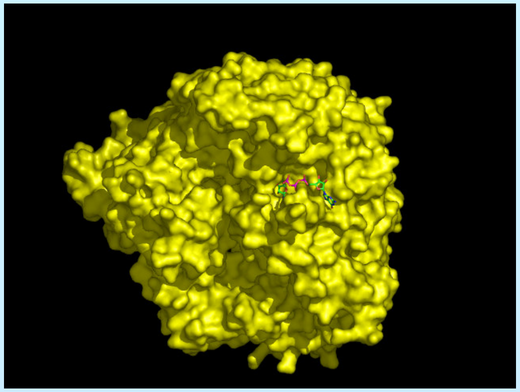
CBC



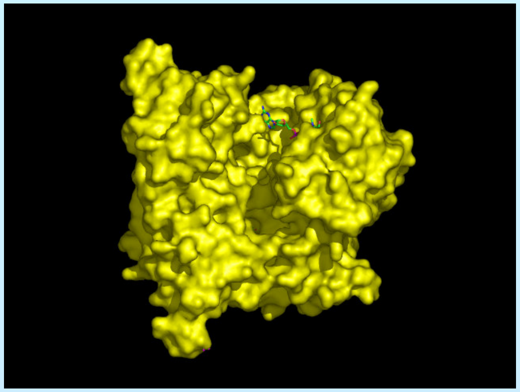
eIF4E



VP39
methyltransferase

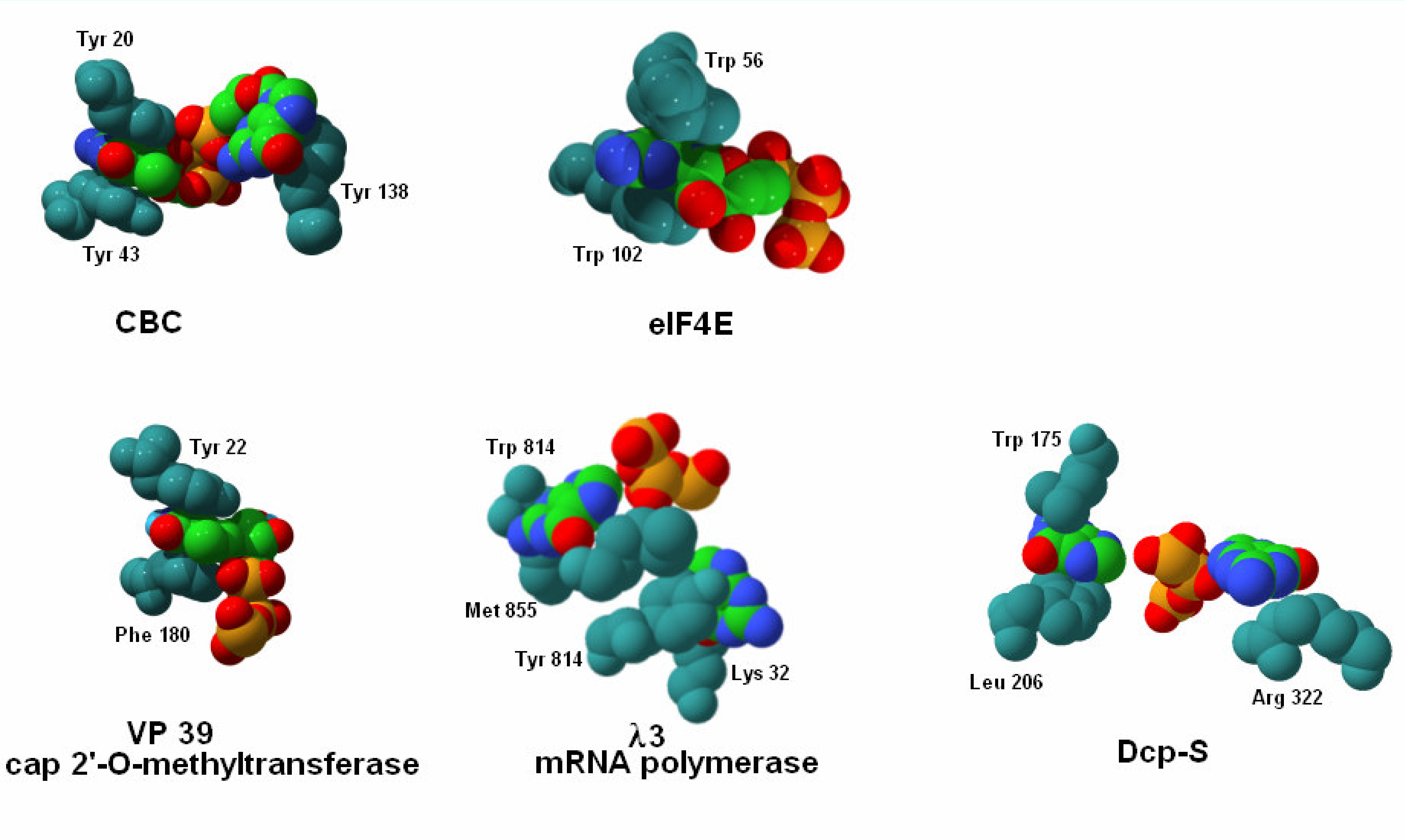


λ3 mRNA
polymerase

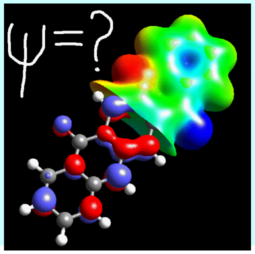


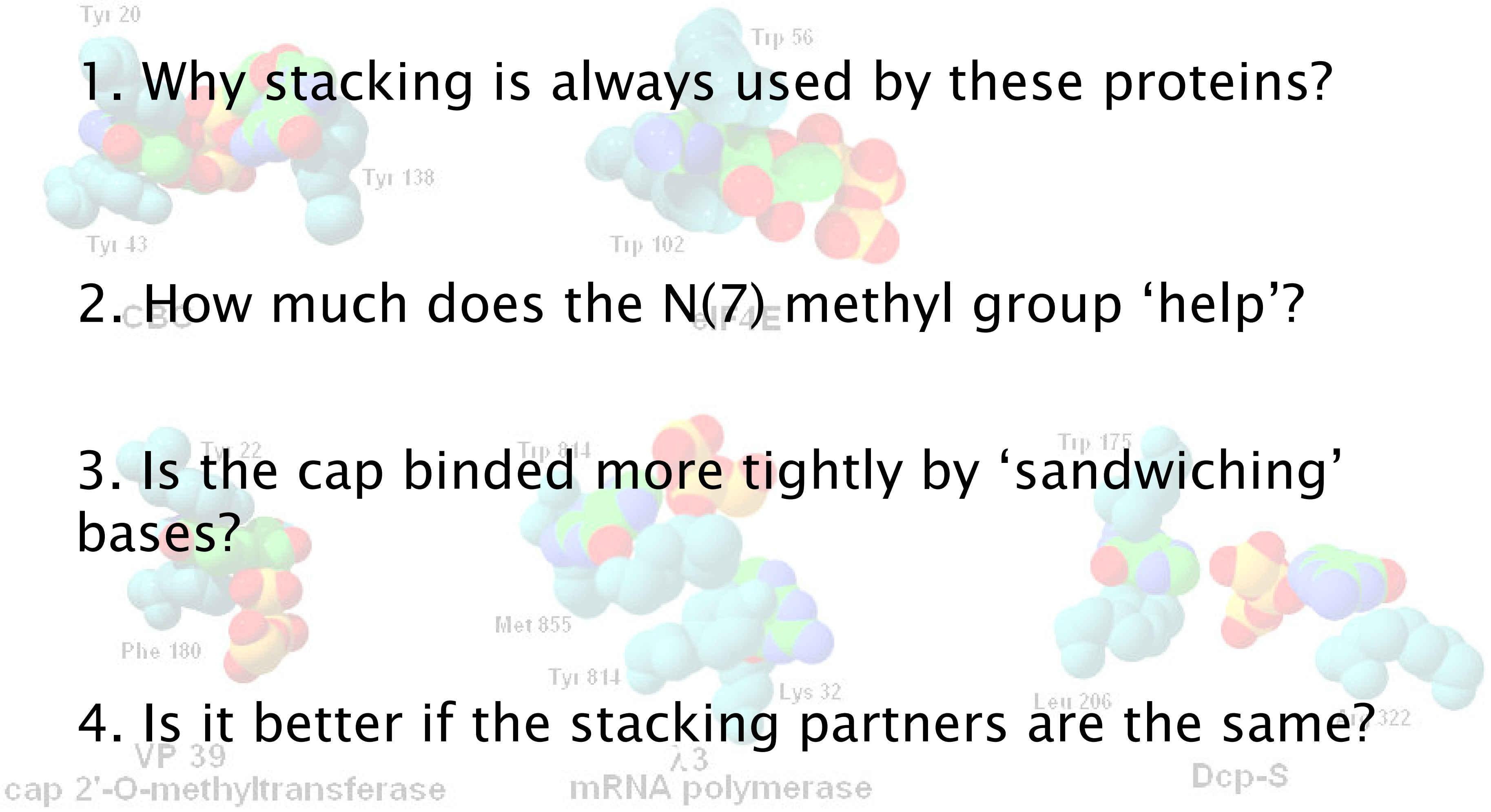
Dcp-S

…have something in common



Ab initio quantum calculations of the interaction energy





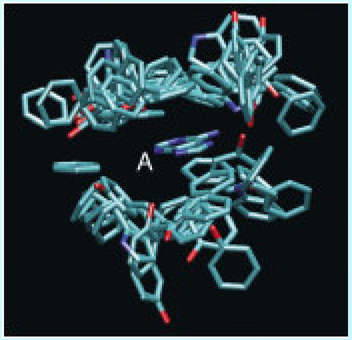
1. Why stacking is always used by these proteins?

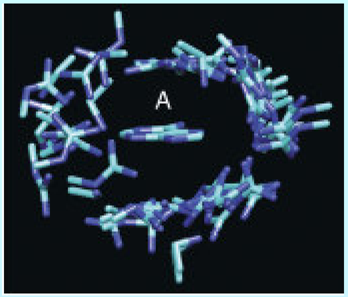
2. How much does the N(7) methyl group ‘help’?

3. Is the cap binded more tightly by ‘sandwiching’ bases?

4. Is it better if the stacking partners are the same?

Stacking and cation-π interactions in ATP-binding proteins

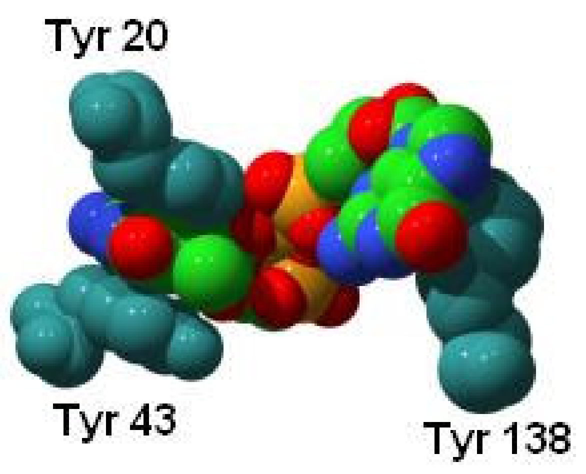




Ma L, *et al.,* JMB 2004

To describe the interactions more properly one has to use Moller-Plesset 2nd order perturbation calculation in 6-311G*+ basis set…

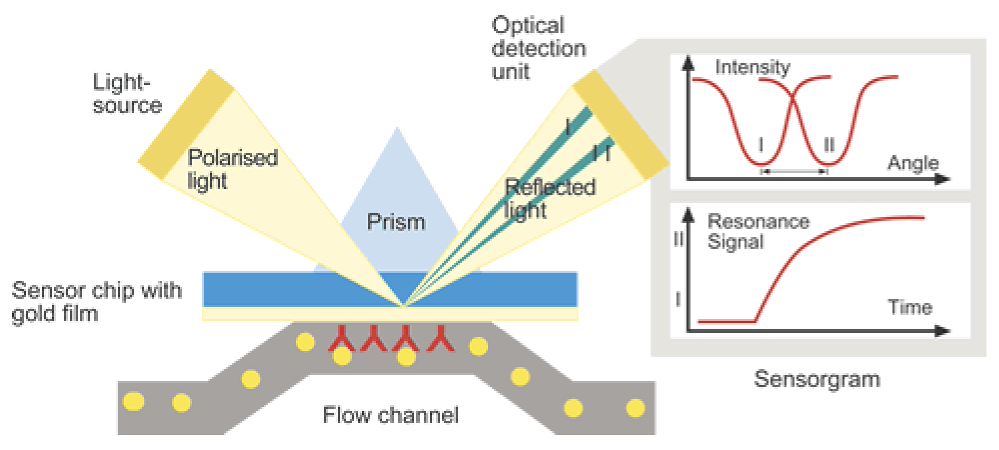
Does CBC have ‘talking’ tyrosines?



Preliminary results of CBC mutants binding studies suggest the cooperativity of between the two centers

It would be great to perform some kinetic experiments…

Surface Plasmon Resonance (SPR)



Molecular biology

Stephen Cusack

Catherine Mazza

Delphine Guilligay

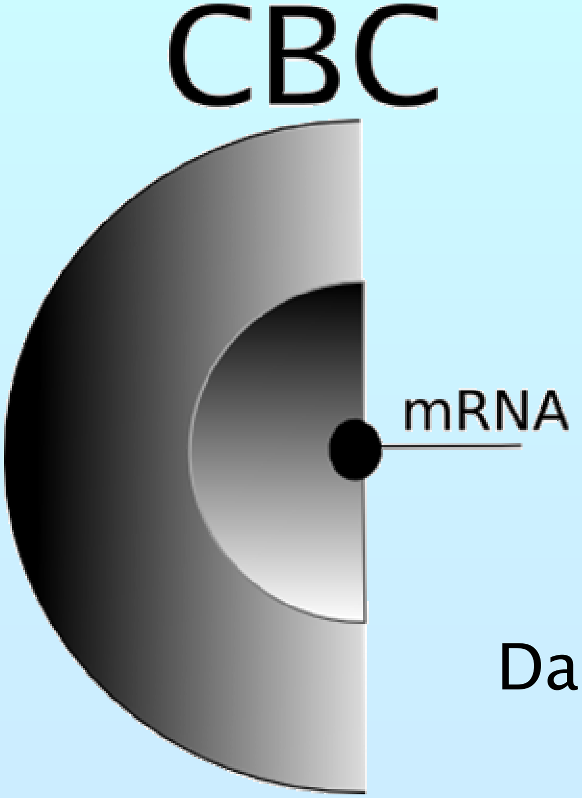
EMBL, Grenoble

Biophysics

Ryszard Stolarski

Anna Niedźwiecka

me



Chemistry

Edward Darżynkiewicz

and his group