Méthodes d'Etude des Complexes

Technologies for studying macromolecular complexes

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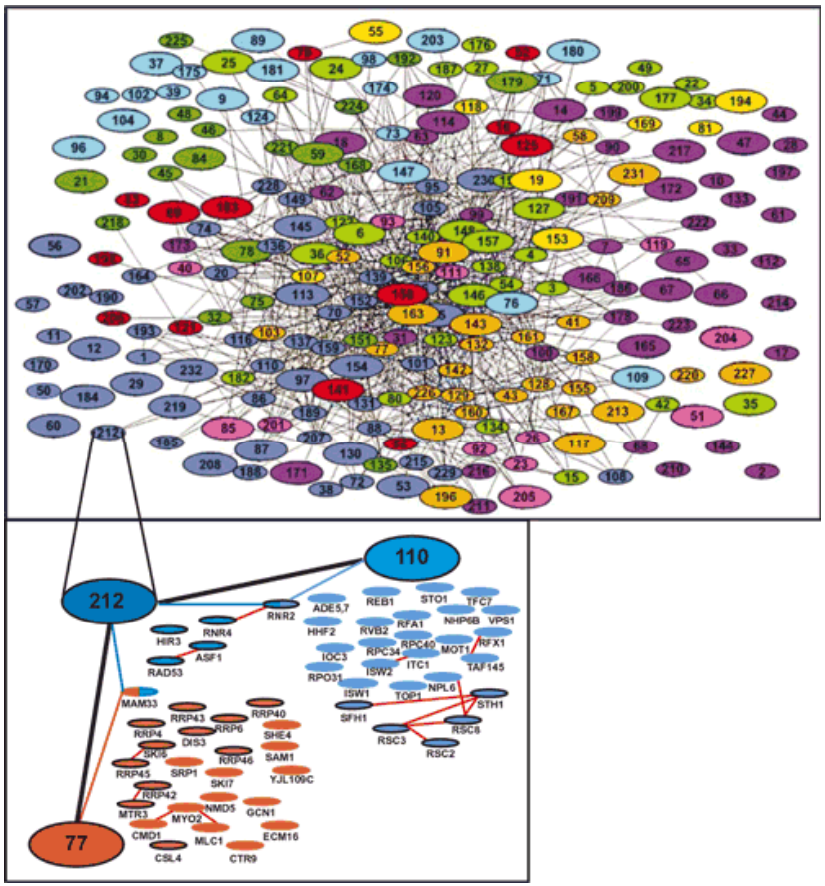
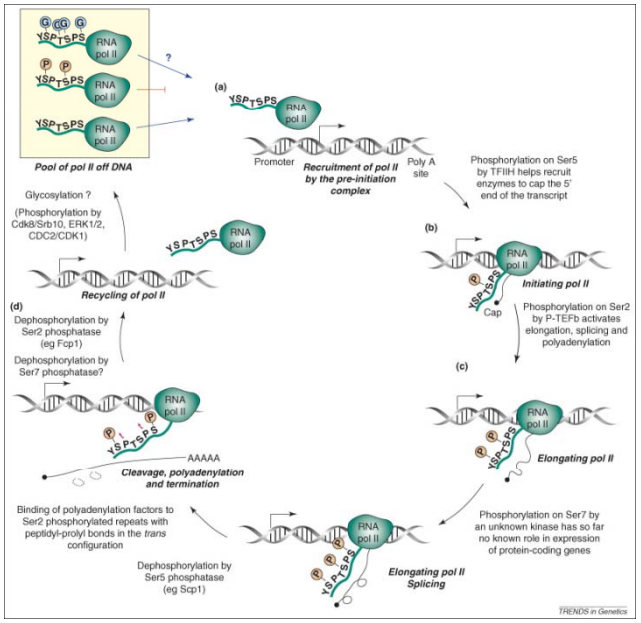
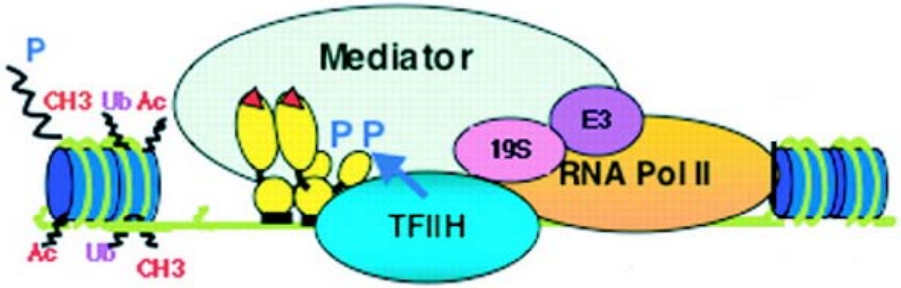
Most proteins do not function as isolated particules…

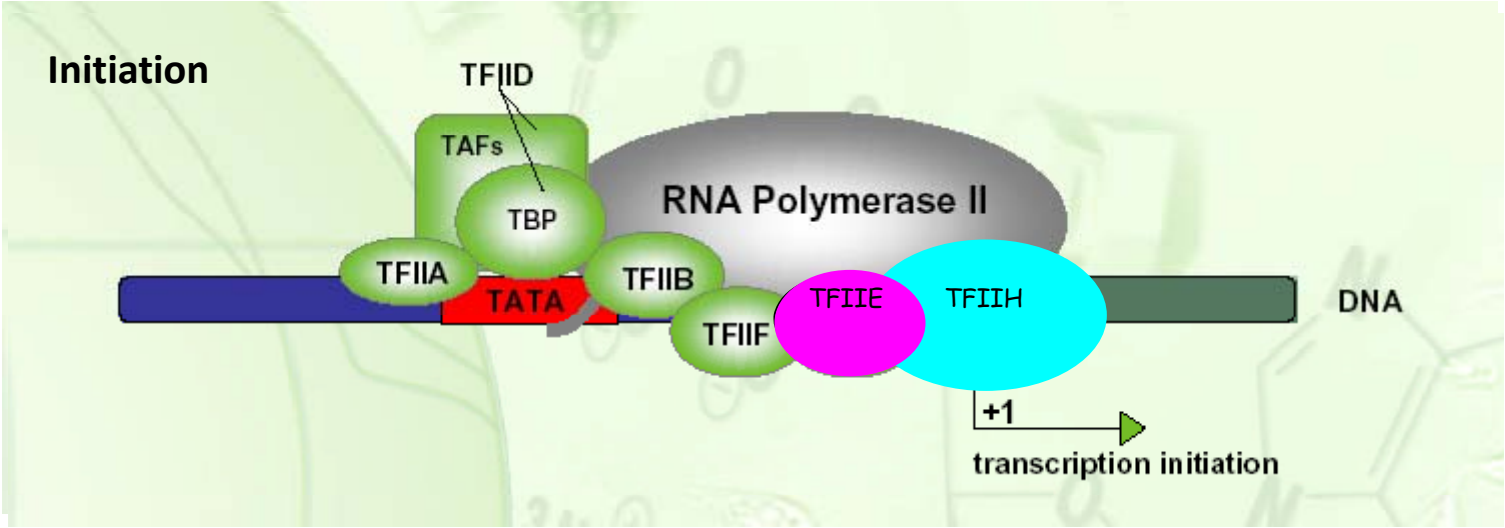
…but interact with partners to fullfill their fonction.



Transcription in Eukaryotes







Types of complexes

distinct types

Composition and structure

Protein-protein, protein-nucleic acid, protein-ligand

Homo- and hetero oligomeric complexes

Non obligate and obligate

Protomers are not found as stable structures in vivo

Subunits exist independantly

Lifetime of complexes

Permanant interactions: stable/only exist in complexed state

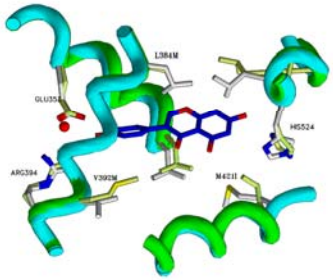
Transient interactions associate and dissociate in vivo
- weak: dynamic equilibrium in solution
- strong: molecular trigger to switch on and off

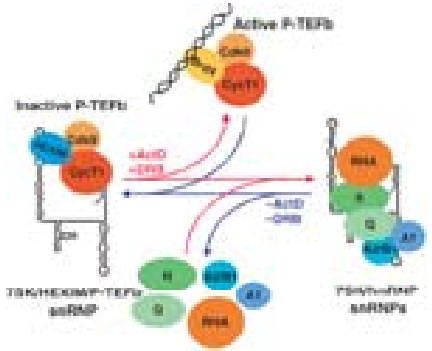
Types of complexes

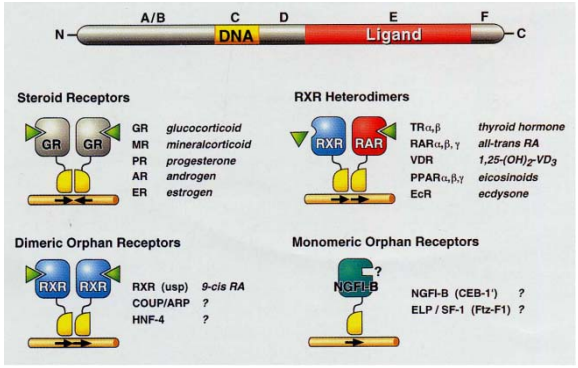
Composition and structure

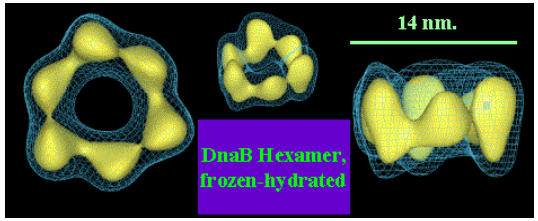
Protein-protein, protein-nucleic acid, protein-ligand

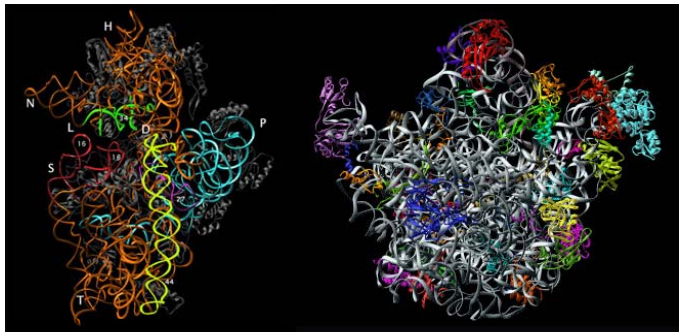
Homo- and hetero oligomeric complexes











aaRS, ribosome,

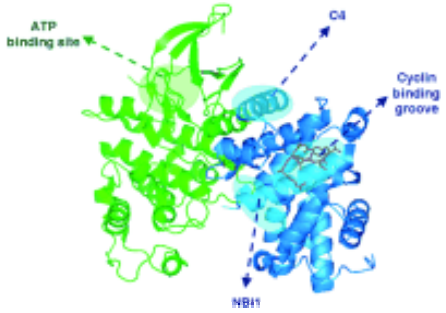
Types of complexes

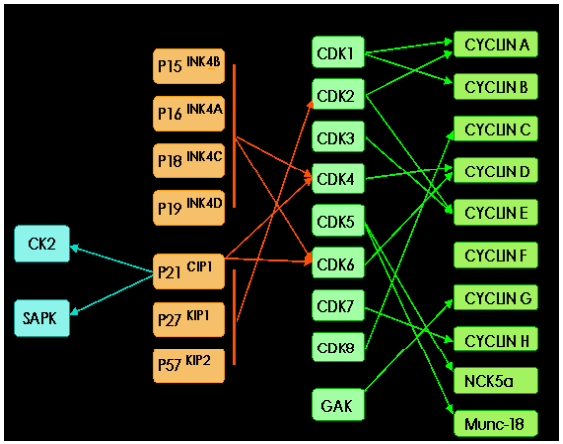
Obligate and non-obligate

Protomers are not found
as stable structures in vivo

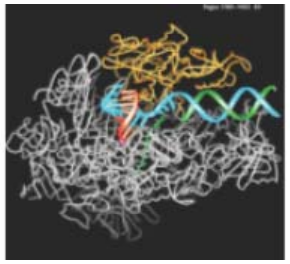
Subunits exist independantly

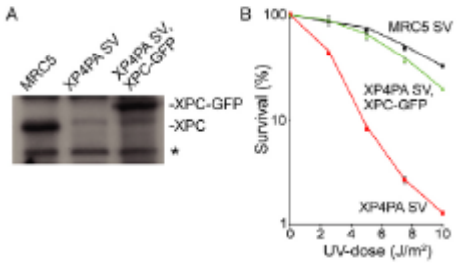
- Purification of isolated subunits
- In cell imaging

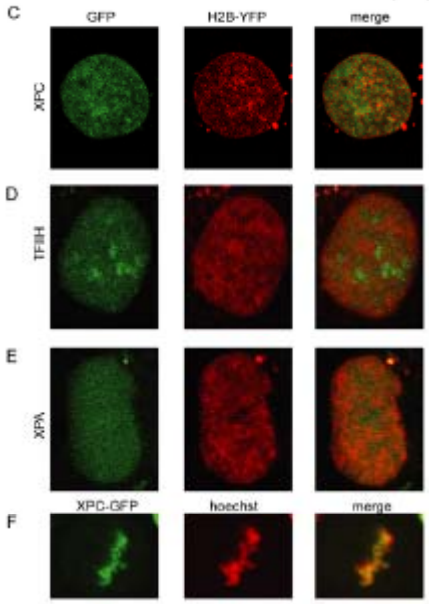




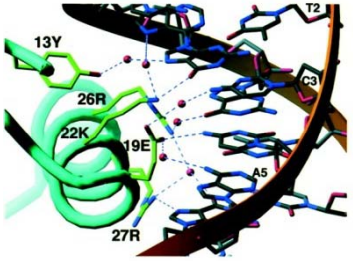
Subunits of RNA Pol II GFTs

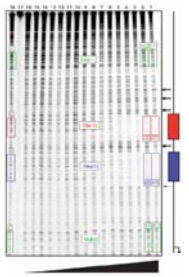






Types of complexes





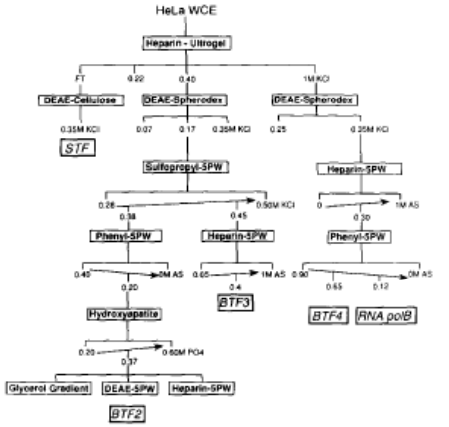


FIG. 1. **Scheme of purification of human RNA polymerase B and general transcription factors.** Abbreviations: *WCE*, whole cell extract; *RNA pol B*, RNA polymerase B; *STF*, stimulatory transcription factor; *BTF*, RNA polymerase B general transcription factor; *AS*, ammonium sulfate; *PO4*, phosphate buffer. The endogeneous BTF1/TFIID factor was mainly present in the heparin-Ultrogel 0.40 M and in the subsequent DEAE-Spherodex 0.35 M KCl eluted fractions.

Lifetime of complexes

Permanant interactions: stable/only exist in complexed state:
operational definition: that can be purified

Transient interactions associate and dissociate in vivo
- weak: dynamic equilibrium in solution
- strong: molecular trigger to switch on and off

Weak

(Tx, DNA repair electron transport complexes)

K_d mM-μM

Intermediate

(antibody-antigen, TCR-MHC-peptide, signal transduction PPI), K_d μM-nM

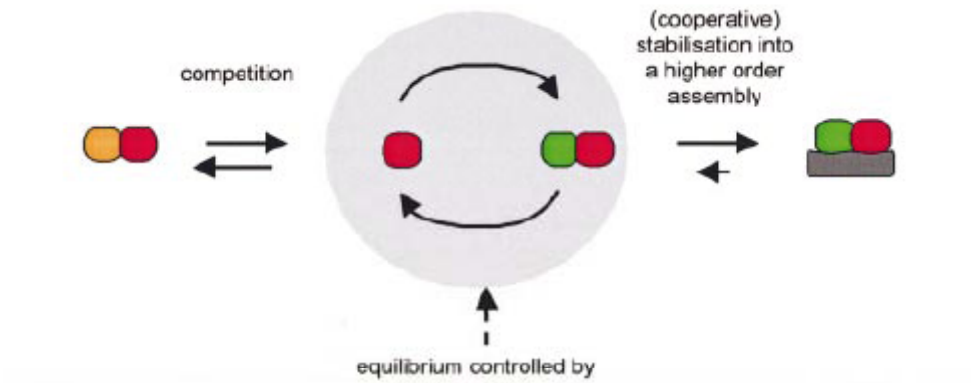
Strong

(require a molecular trigger to shift the oligomeric equilibrium)

K_d nM-fM

Control of assembly: protein oligomerization

(Nooren & Thornton, EMBO 2003)



Localisation and protein concentration(s)

Co-expression, subcellular localisation

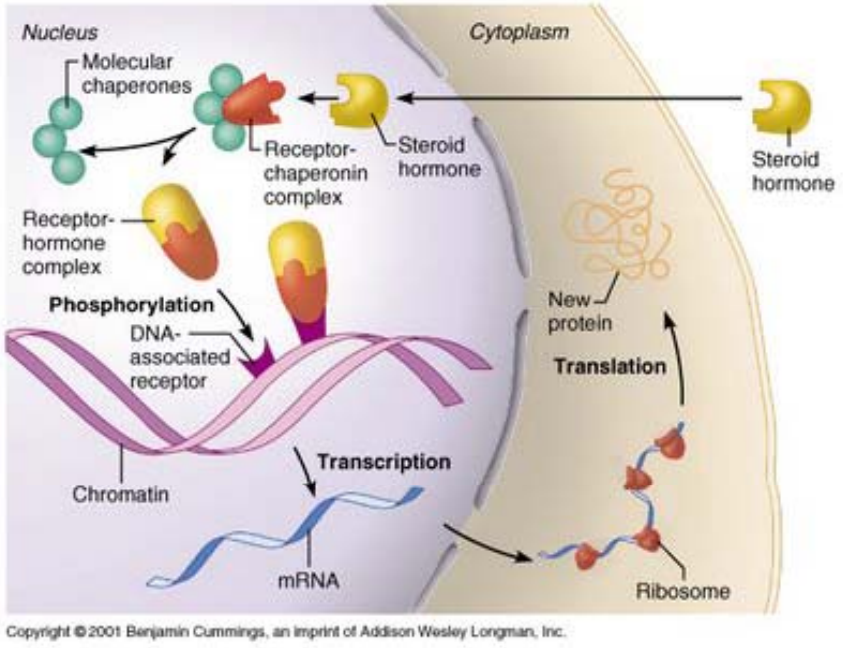
Level of gene expression/secretion, degradation
Temporary storage, local molecular environment,
Diffusion or viscosity

Binding energy ΔG

pH, temperature, ionic strengh

Molecular (cooperativity/allosteric) binding:
Concentration of metabolite (hormone), protein
(co-activators) or covalent modification (phosph)

Control of assembly: protein oligomerization



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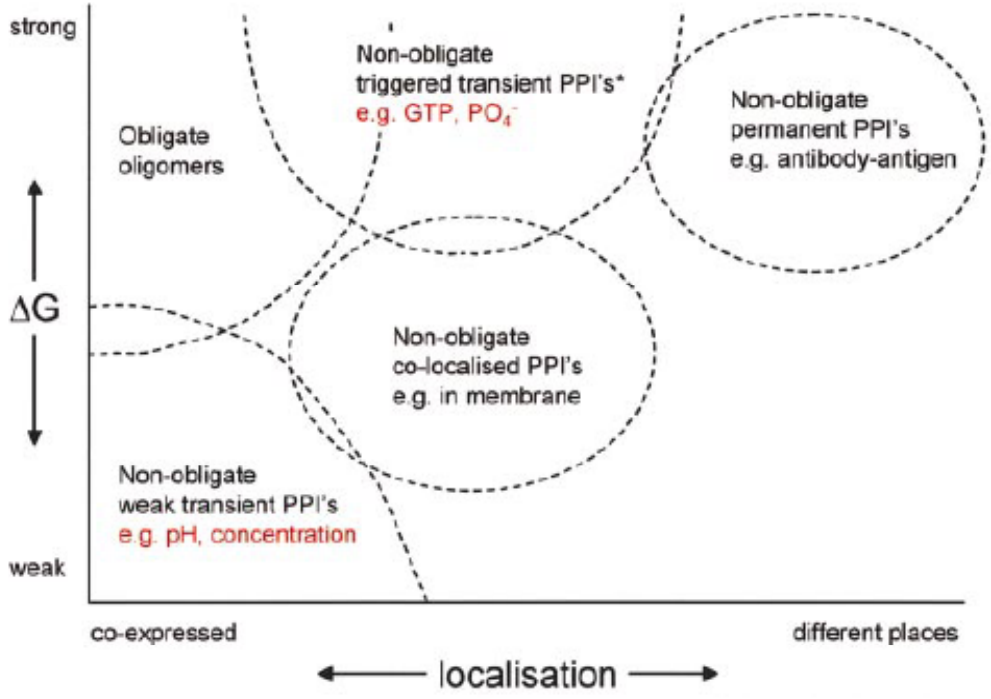
Molecular (cooperativity/allosteric) binding:
Concentration of metabolite (hormone), protein (co-activators) or covalent modification (phosph)

Relation between types of protein-protein interactions their binding affinity and cellular localization

Obligate PPI

usually permanent

the protomers are **not** found as stable structures on their own *in vivo*



Non Obligate PPI

permanent

the protomers can be found as stable structures on their own *in* vivo but form stable assemblies

Non Obligate PPI

transient

Intermediate

(antibody-antigen, TCR-MHC-peptide, ligand receptor), Kd μM-nM

Weak

(Tx, DNA repair electron transport complexes)

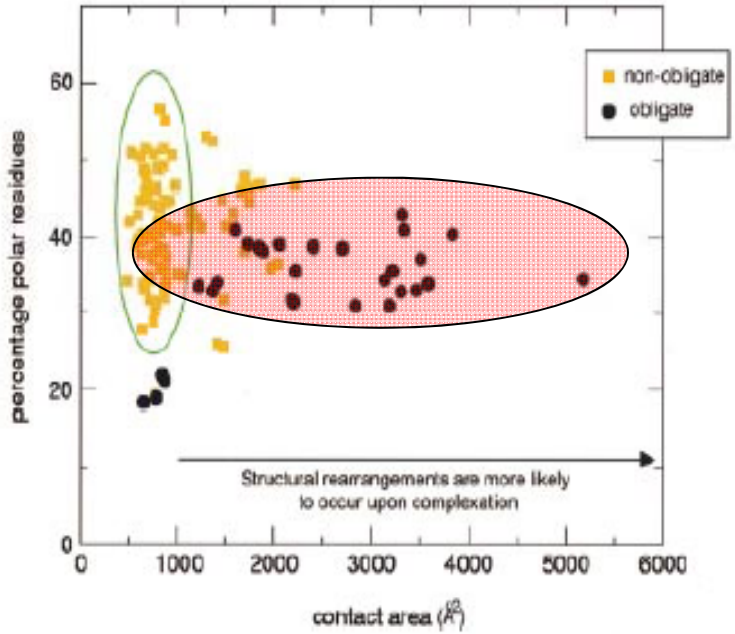
Kd mM-μM

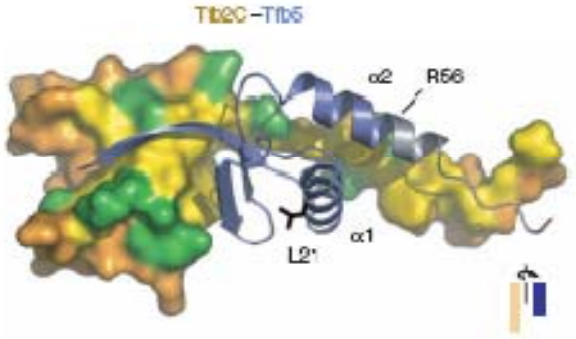
Strong

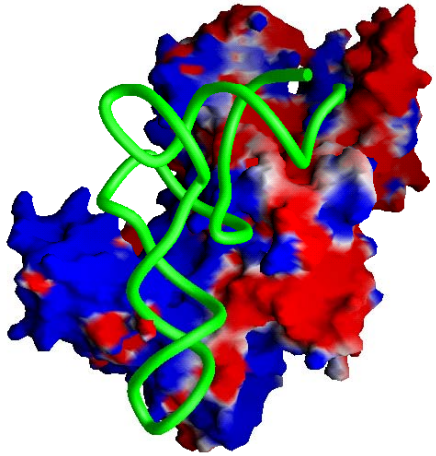
(require a molecular trigger to shift the oligomeric equilibrium)

Kd nM-fM

Structural characteristics of protein-protein interfaces







Contact area and polarity of various non obligate and obligate complexes. Thevertical ellipse denotes the aera-polarity space of weak transient interactions

The interfaces in obligate complexes) are generally larger and more hydrophobic than non-obligate associations.

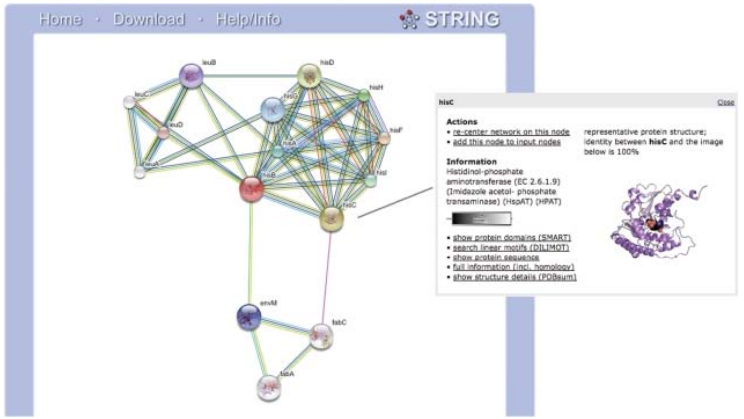
Databases

BIND (Biomolecular Interaction Network Database)

*www.**bind**.ca/*

DIP (Database of Interacting Proteins

***dip**.doe-mbi.ucla.edu/*



MINT (Molecular INTeraction database)

***mint**.bio.uniroma2.it/**mint**/*

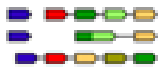
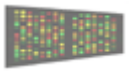
STRING (Search Tool for the Retrieval of Interacting Genes/Proteins)

***string**.embl.de/*

Search Tool for the Retrieval of Interacting Proteins

What it does ...

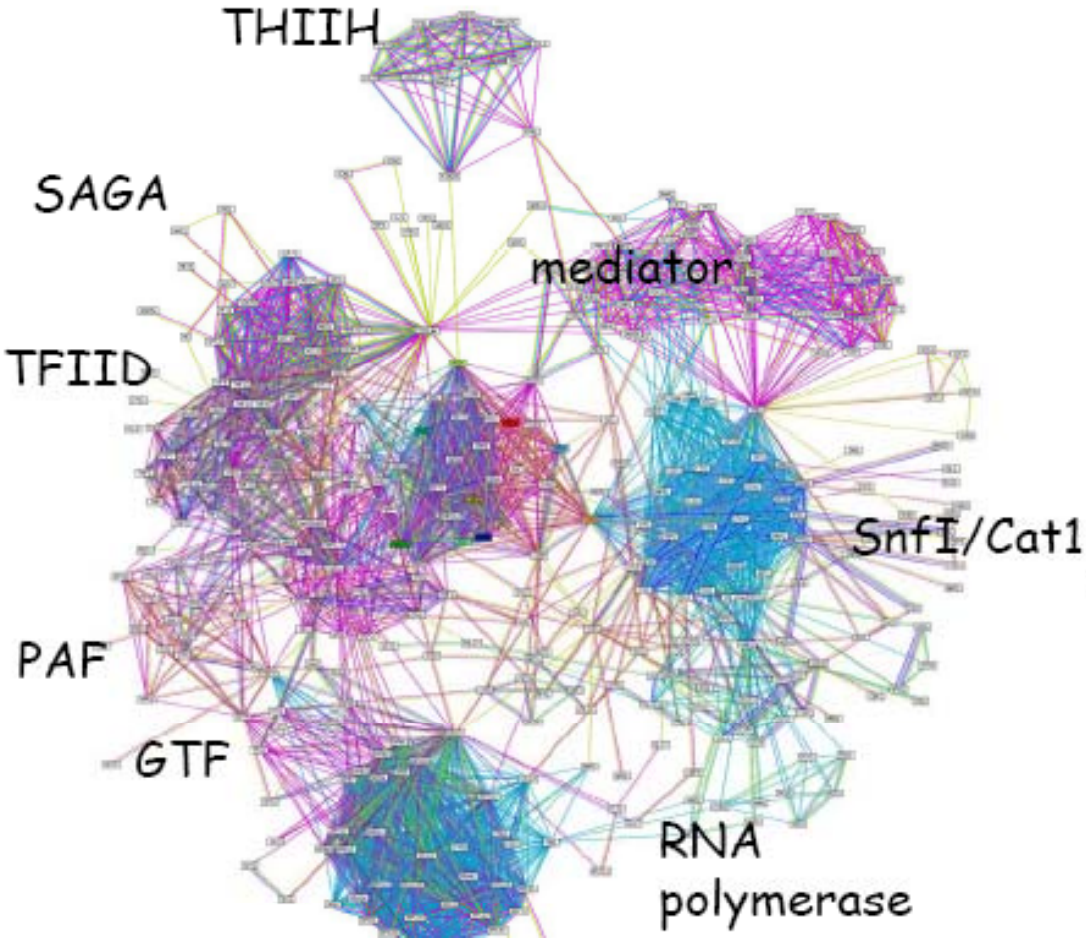
STRING is a database of known and predicted protein interactions.The interactions include direct (physical) and indirect (functional) associations; they are derived from four sources:

Genomic Context	High-throughput Experiments	(Conserved) Coexpression	Previous Knowledge
			

STRING quantitatively integrates interaction data from these sources for a large number of organisms, and transfers information between these organisms where applicable. The database currently covers 2,483,276 proteins from 630 organisms.

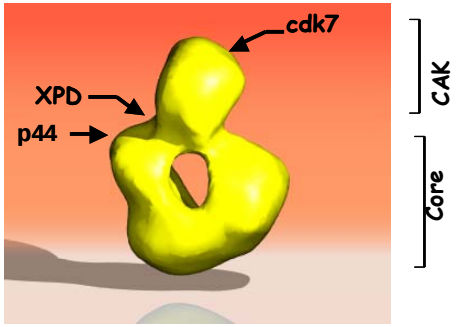
Interactions are scored
One can choose the number of interactors that will be represented

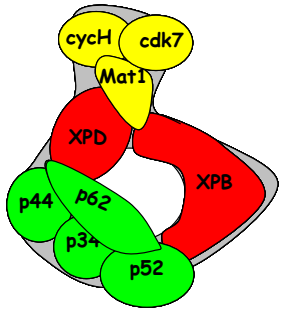
Complexes involved in transcription:
Yeast interaction networks derived from String
centered around the mediator complex Med1



- Stable complexes:
  - TFIIH
  - mediator
  - RNA polII
  - TFIID
  - SAGA
- Transient complexes:
  - Associated proteins
  - Shared subunits: TFIID/SAGA

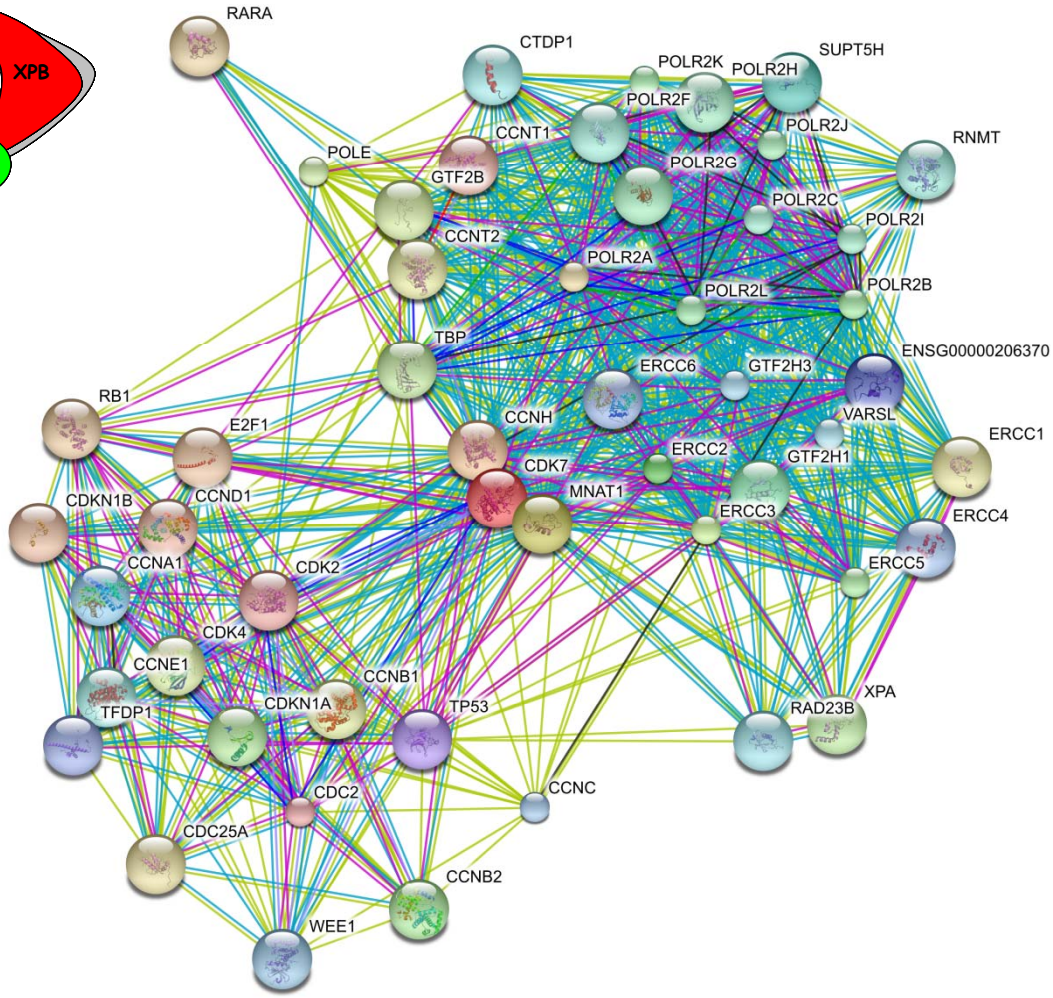
Physical and functional interactions: TFIIH: a 10-subunit complex





Model of molecular organisation for human TFIIH

		MW	Function/Characteristics
CAK	Cdk7/kin28	40kDa	Kinase
	Cycline H/ccl1	36kDa	Associated Cyclin to Cdk7
	MAT1/Tfb3	32kDa	
	XPD/rad3	80kDa	ATP dependant 5'-3' DNA helicase
core	XPB/Ssl2	89kDa	ATP dependant 3'-5' DNA helicase
	p62/Tfb1	62kDa	
	p52/Tfb2	52kDa	
	p44/Ssl1	44kDa	
	p34/Tfb4	34kDa	
	p8/Tfb5	8kDa	DNA repair specific subunit



Size is not the main problem: Mega-dalton complexes can analyzed at the atomic scale

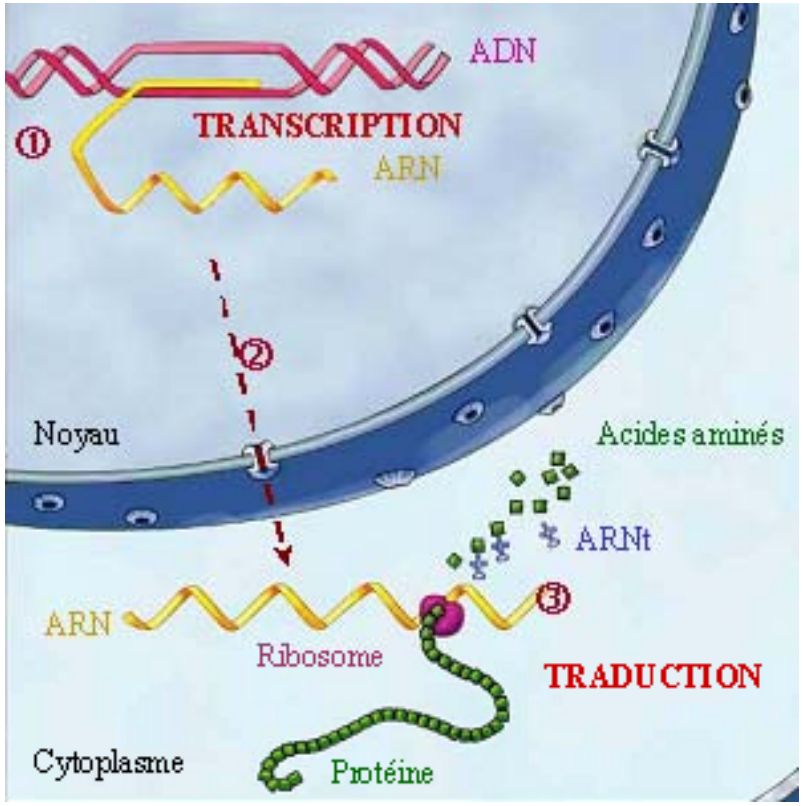
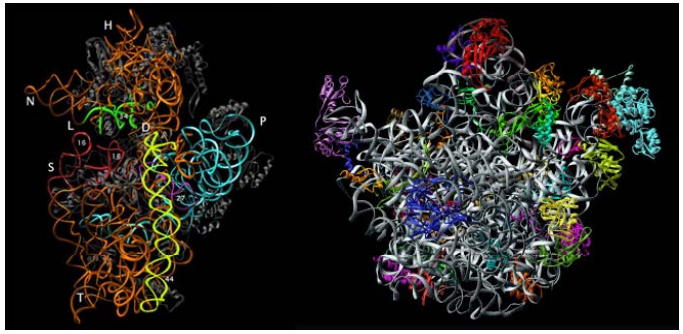


Fig. 1. The structure of RNA polymerase II (left) and RNA polymerase II in the act of transcription (right) featured on covers of *Science* Magazine. During transcription, a template strand of DNA (shown in blue on the right) is unwinding just before the active center. Newly formed RNA is shown in red.



Sample

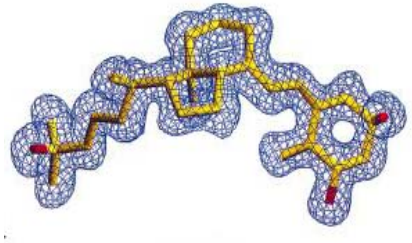
Bio-informatics

Biophysical approaches

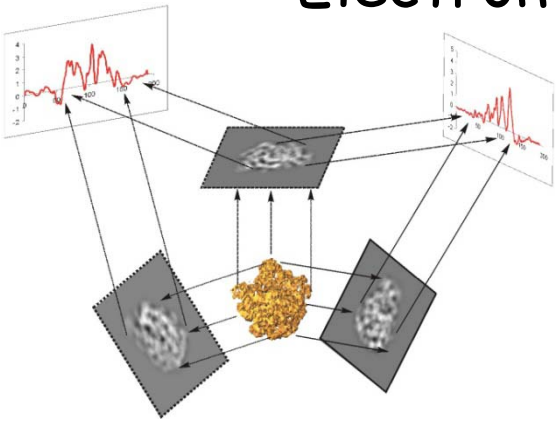
NMR

X-ray diffraction

Mass Spectrometry



Electron Microscopy



- overview and summary
- thermodynamics: the essentials (AP)

Identification, production

- In vivo approaches (BS)
- Protéomics: TAP tag/MS foot-printing (SS)
- Recombinant technologies (AP)

Purification and Biochemical characterization

- Biochemical methods I (purification strategies,) (DK)
- Biochemical methods II (equilibrium binding,.EMSA,...) (DK)

Biophysical approaches

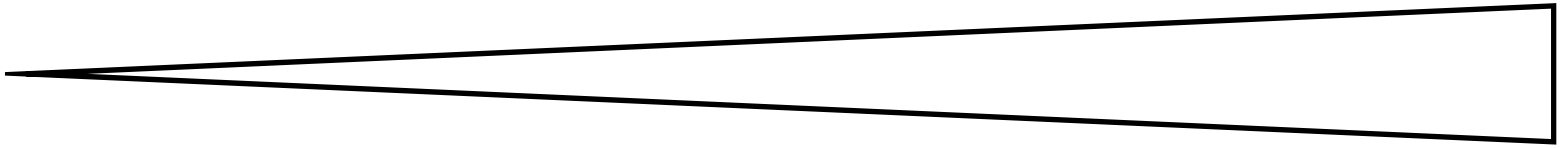
- spectroscopic methods/mass spectrometry of complexes (SB)
- microcalorimetry/thermofluor (EE, VC)
- surface plasmon resonnance (LC)
- analytical ultracentrifugation/DLS (CB, VC)
- electron microscopy/SAXS and SANS (PS)

Expression levels

x < 100 µg/L	Hopeless (for stuctural studies but not for function assays)
100 µg/L < x < 500 µg/L 500 µg/L < x < 2 mg/L	Micro-methods (Em, nanodrop crystallization, microfluidics)
2 mg/L < x	Standard approches

endogenous, recombinant

quantity

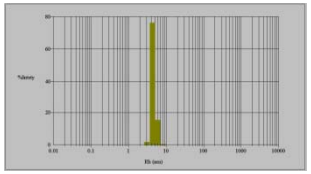


µg mg g

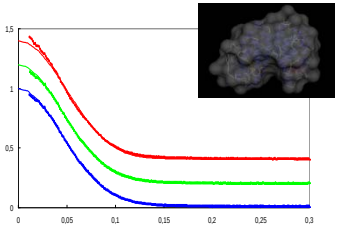
Poor expressors Difficult to handle proteins

EMSA

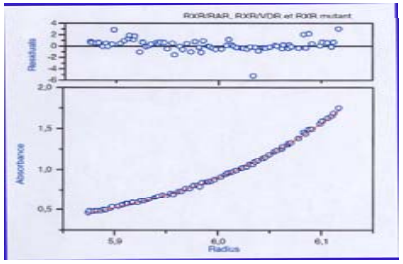
Dynamic Light Scattering



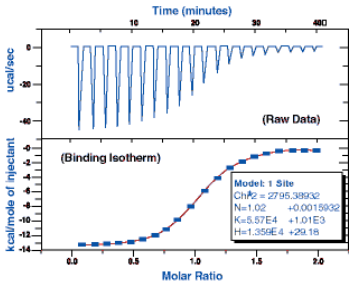
SAXS



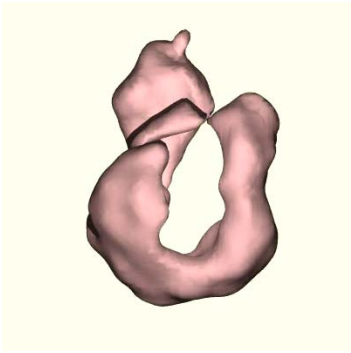
AUC



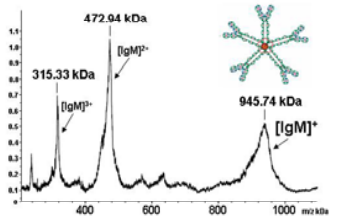
ITC/DSC



Electron microscopy



Mass Spectrometry



Sample requirements

In addition to chemical purity

Native Gels, EMSA: μg

DLS: # 1 mg/ml, 12μl/assay

thermofluor: # a few μg

ITC, DSC: # a few mg

AUC: equilibrium OD 0.2-0.5, 120μl/assay

AUC: velocity OD 0.2-0.5, 300μl/assay

EM neg staining: # 50 ng/ml, 5μl/assay

EM cryo: # 0.5 mg/ml, 5μl/assay

SAXS: # 1 mg/ml, 100μl/assay

MS:denaturing: 0.2 n mole
MS: native: 5.0 n mole
de-salted sample

LC-MS: denaturing: 1 n mole

Characterization Strategies for obligatory complexes

Purity ?	SDS page/coomassie or silver staining MS in denaturing conditions
Are the subunits associated ?	Pull down Native gel eletroporesis Gel filtration Native MS
Homogeneity/Monodispersity?	DLS, gel filtration, EM, AUC, DSC…..
Conformational homogeneity ?	EM, SAXS, SANS, AUC, DSC…..

Characterization Strategies for non-obligatory complexes

Isolated components are avalible and are pure/homogenous

Association ? (biochemical analysis)	Pull down Native gel eletroporesis, EMSA Gel filtration
Association ? (biophysical analysis)	AUC, SAXS, SANS Native MS, EM
Thermodynamic parameters ?	EMSA, ITC, spectroscopy, AUC, SPR…..
Conformational homogeneity ?	EM, SAXS, SANS, AUC, DSC…..

http://lbgs.u-strasbg.fr/news/MEC/

					Cours	TP/TD	
25/01/2010	08:10		sem3	AP	Intro, Thermochimie		
01/02/2010	08:10		sem4	AP	Production		
15/02/2010	08:10		sem5	DK	Methodes Biochimiques		
22/02/2010	08:10		sem6	DK	Methodes Biochimiques		
01/03/2010	08:10		sem7	BS	Methodes In vivo		
08/03/2010	08:10	10:12	sem8	CB	Ultracentrifugation	DLS+Ultra	VC
15/03/2010	08:10	10:12	sem9	PS	Microcal	Thermofluor/Interactions	VC
22/03/2010	08:10	10:12	sem10	EE	ME	ARC2	VC
29/03/2010	08:10	10:12	sem11	SB	Fluorescence	Fit/Fluo	VC/BS
12/04/2010	08:10	10:12	sem12	SS	Mass spect	MS+	VC
19/04/2010	08:10	10:12	sem13	LC	Biacore		

contrôle continu + contrôle terminal