Laboratoire de Chimie et Biologie Structurales

Versatility of functions involving intrinsically disordered proteins

-

Deciphering the sequence function relationship of intrinsically disordered proteins: the case of WH2 domains that regulate the polymerization of actin

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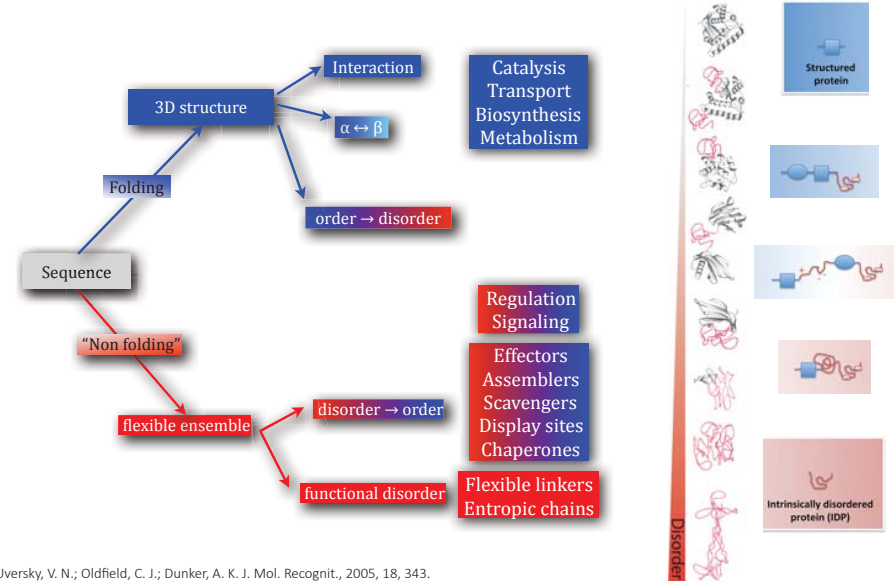
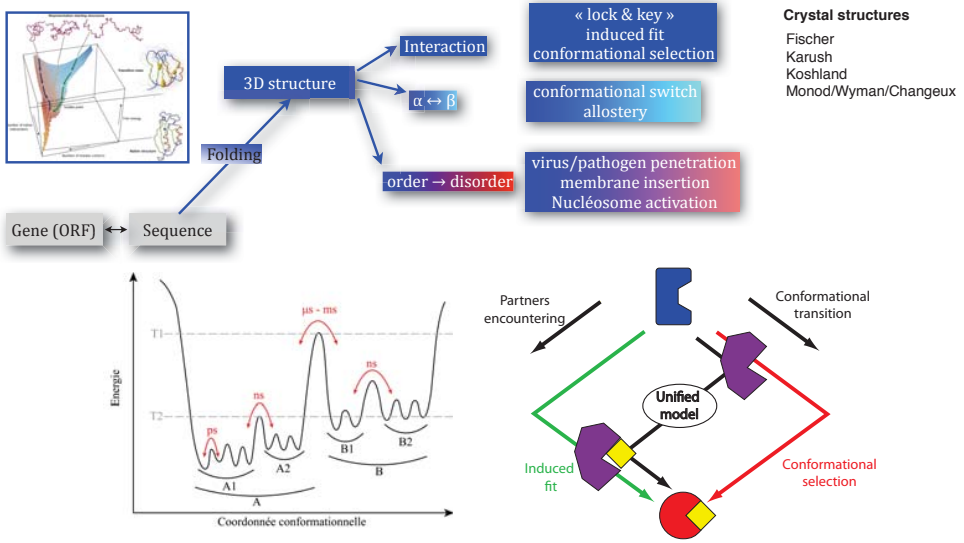
The challenge : understanding the multiple states of proteins a continuum from order to disorder



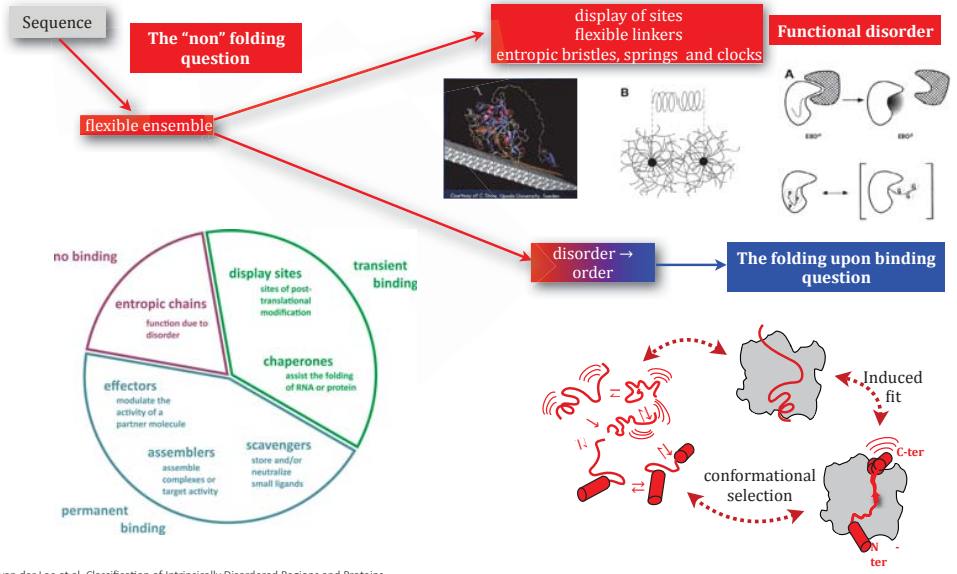
Uversky, V. N.; Oldfield, C. J.; Dunker, A. K. J. Mol. Recognit., 2005, 18, 343.
Habchi, Tompa, Longhi & Uversky, Chemical reviews 2014, introducing protein intrinsic disorder
van der Lee et al., Chemical reviews 2014, classification of IDRs and IDPs

The challenge : understanding the multiple states of proteins From the "folded path" ...



- Proteins are complex thermodynamical ensembles : multiple states in exchange
- Different state ensembles can be functionally different.

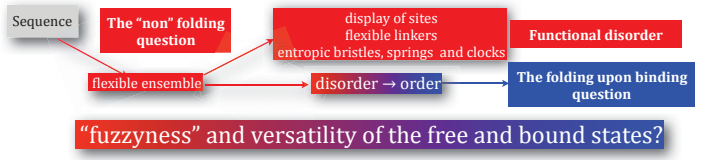
The challenge : understanding the multiple states of proteins From the "folded path" ... to the "intrinsically unfolded path"



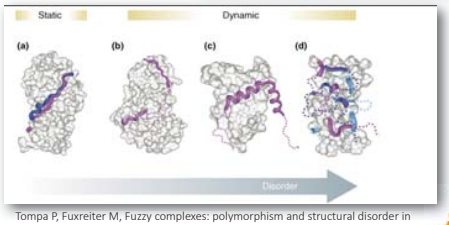
van der Lee et al. Classification of Intrinsically Disordered Regions and Proteins. Chemical Reviews, 114(13):6589–6631,2014.

Jan H. Hoh, "Functional Protein Domains From the Thermally Driven Motion of Polypeptide Chains: A Proposal", PROTEINS: Structure, Function, and Genetics 32:223–228 (1998)

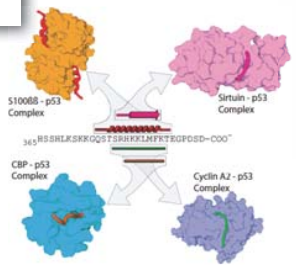
The challenge : understanding the multiple states of proteins
From the "folded path" … to the "intrinsically unfolded path"



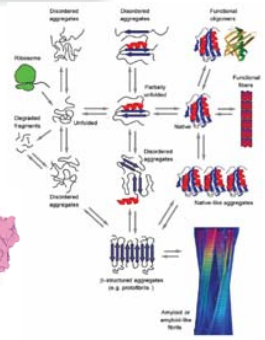
"fuzzyness" and versatility of the free and bound states?



Tompa P, Fuxreiter M, Fuzzy complexes: polymorphism and structural disorder in protein-protein interactions. Trends Biochem. Sci. 2008, 33, 2--8.

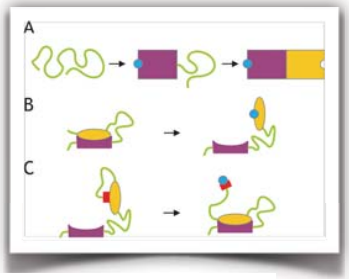


A chameleon sequence (the C-terminal region of p53) in complex with four different partners and exhibiting four different structural forms (Oldfield et al. BMC Genomics 2008, 9)



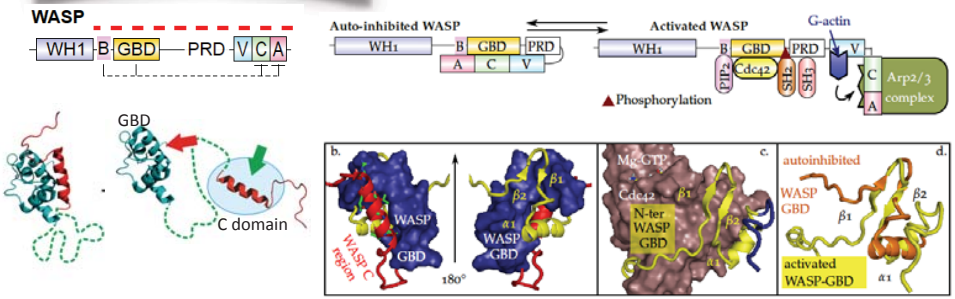
Fabrizio Chiti and Christopher M. Dobson, Protein Misfolding, Functional Amyloid, and Human Disease, Annu. Rev. Biochem. 2006. 75:333–66

"Intrinsically disordered" proteins/regions and interactions
Generalized concept of allostery in multi-domain proteins



Variable arrangement of globular domains, disordered linker regions and short recognition elements
- Complex conformational energy landscapes
- Population remodeling in the conformational ensemble, driven by ligand binding or changes in conditions (pH, salt, localization, concentrations, expression level, etc.)

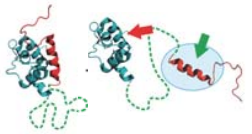
➡ Intrasteric regulation, autoinhibition, intramolecular regulation, active-site directed regulation, signaling function, motif exposure, etc…

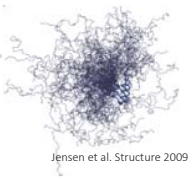


•Tompa, Chem. Rev. 2014, 114, 6715–6732. Multisteric Regulation by Structural Disorder in Modular Signaling Proteins: An Extension of the Concept of Allostery.
•Renault, Deville, van Heijenoort, Cytoskeleton 2013, 70:686–705. Structural Features and Interfacial Properties of WH2,β-Thymosin Domains and Other Intrinsically Disordered Domains in the Regulation of Actin Cytoskeleton Dynamics

? "Intrinsically disordered" ?

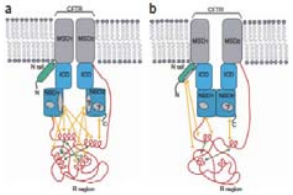
- Secondary/tertiary/quaternary structure?
- Conformers ensemble(s)
- Flexibility?
  - Timescales?
    - Spacial extension/restriction
    - exchange between conformers/folding/unfolding
- Thermodynamics/kinetics
  - Affinities?
  - Kinetics of binding?
  - Specificity?
- Trajectories/spatial exploration?



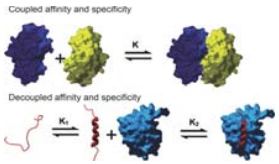


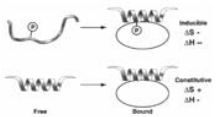
Jensen et al. Structure 2009

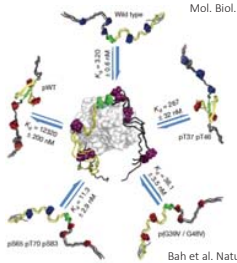
Saio, et al., science 2014



Baker et al. Nat. Struct. Mol. Biol. 2007







Bah et al. Nature 2015

? "Intrinsically disordered" ?

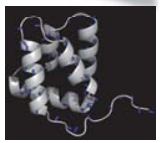
- Secondary/tertiary/quaternary structure? NMR CD/IR
- Conformers ensemble(s) NMR Simulation
- Flexibility? NMR ESR XRay/EM Fluorescence, FRET,etc. Protease digestion rates Stokes radius (SAXS, size exclusion chromatography) Single molecule experiments, AFM Simulation
  - Timescales?
    - Spacial extension/restriction
    - exchange between conformers/folding/unfolding
- Thermodynamics/kinetics
  - Affinities? NMR Biophysical techniques ITC, SPR, fluorescence, etc. Biochemistry
  - Kinetics of binding?
  - Specificity?
- Trajectories/spatial exploration? Simulation

Disorder seen by NMR

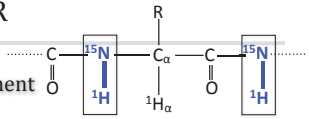
- Secondary/tertiary/quaternary structure? **NMR** Chemical Shifts
- Conformers ensemble(s) **NMR** Chemical Shifts, RDCs, Spin relaxation, PRE
- Flexibility? **NMR** Chemical Shifts, RDCs, Relaxation rates, Spin relaxation, PRE
  - Timescales?
    - Spacial extension/restriction
    - exchange between conformers/folding/unfolding
- Thermodynamics/kinetics
  - Affinities? **NMR** Titration experiments: Chemical Shifts, RDCs, Relaxation rates, Spin relaxation, PRE
  - Kinetics of binding?
  - Specificity?
- Trajectories/spatial exploration?

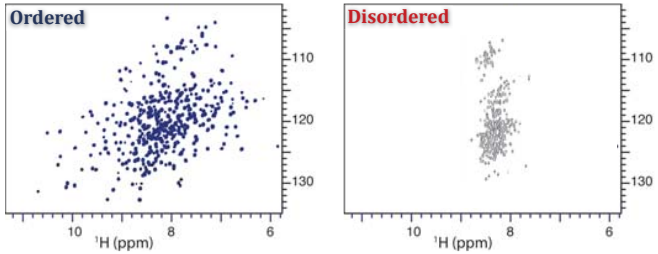
Disorder seen by NMR

NMR
- Chemical Shifts
- RDCs
- Relaxation rates
- Spin relaxation
- PRE

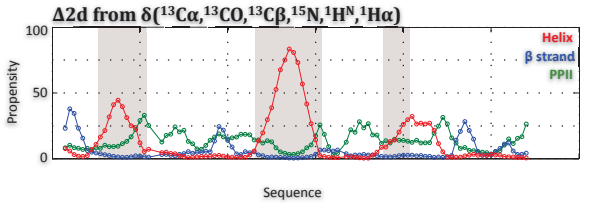


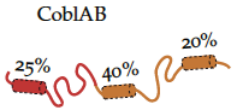
- HSQC spectra <-> electronic environment





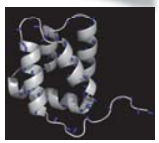
- Secondary shifts : $\delta^{(measured)}$-$\delta^{(random\ coil)}$ <-> Secondary Structure Propensities



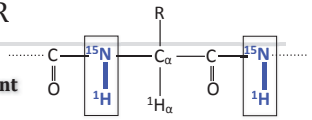


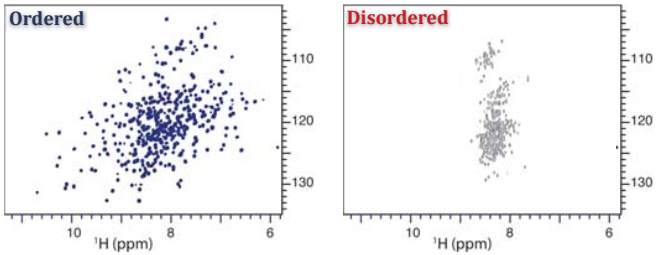
Disorder seen by NMR

NMR
- Chemical Shifts
- RDCs
- Relaxation rates
- Spin relaxation
- PRE

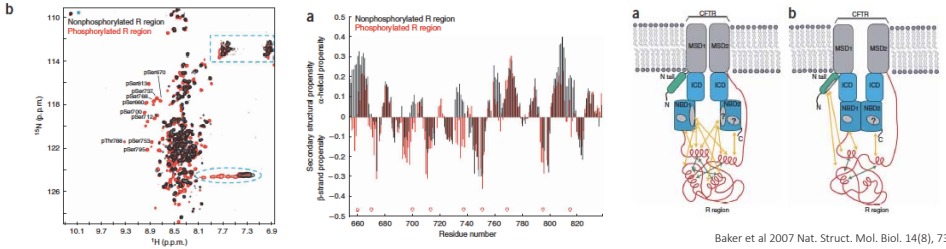


- HSQC spectra <-> electronic environment





- Secondary shifts <-> Secondary Structure Propensities

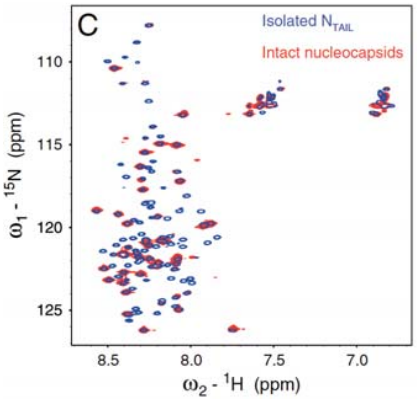


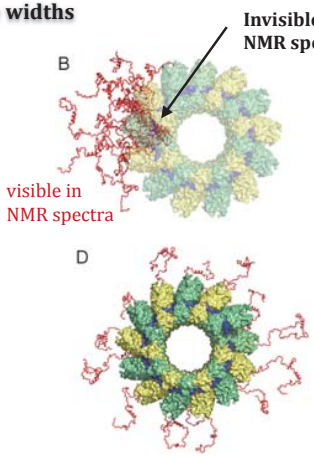
Baker et al 2007 Nat. Struct. Mol. Biol. 14(8), 738

Disorder seen by NMR

NMR
- Chemical Shifts
- RDCs
- Relaxation rates
- Spin relaxation
- PRE

- Relaxation rates <-> line widths





- Combined NMR, EM

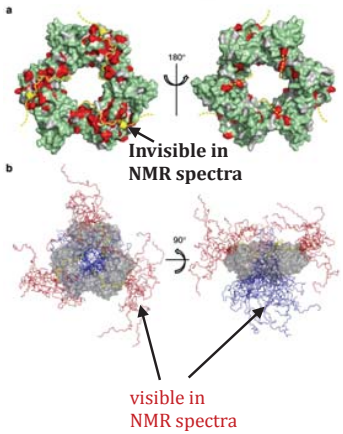
Jensen, M.R et al.,(2011) PNAS 108,24

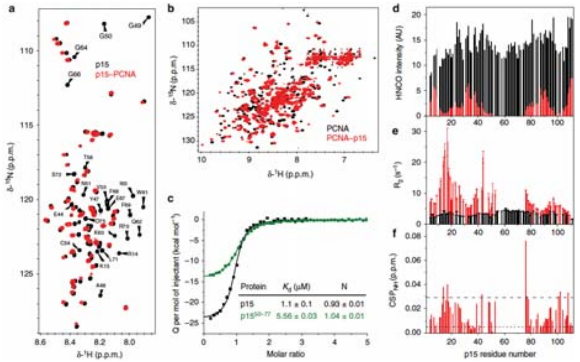
NMR

- Chemical Shifts
- RDCs
- **Relaxation rates**
- Spin relaxation
- PRE

Disorder seen by NMR

- **Relaxation rates <-> line widths**





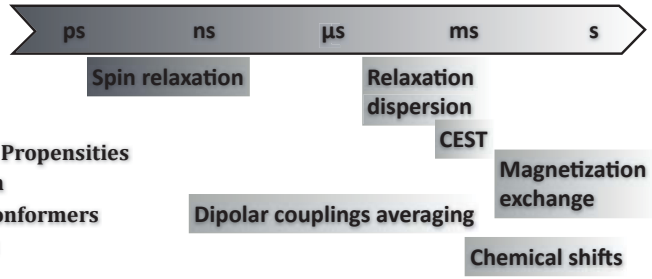
- **Combined NMR, XRay, ITC**

De Biasio et al., NATURE COMMUNICATIONS 2015

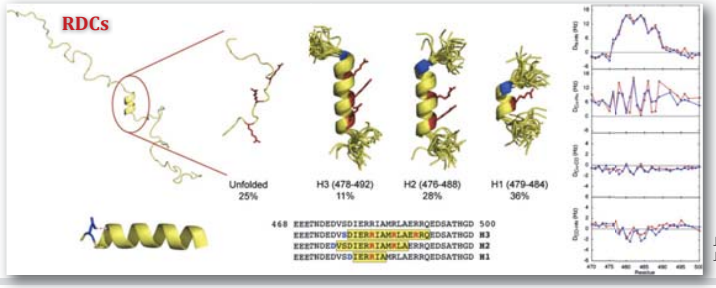
NMR

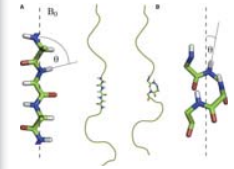
- Chemical Shifts
- **RDCs**
- **Relaxation rates**
- **Spin relaxation**
- PRE

Disorder seen by NMR



- **Secondary Structure Propensities**
- **Restriction of motion**
- **Exchange between conformers**
- **➡ Different timescales**





Jensen, M.R et al.,(2008)
J. Am. Chem. Soc. 130 ; (2009) structure 17.

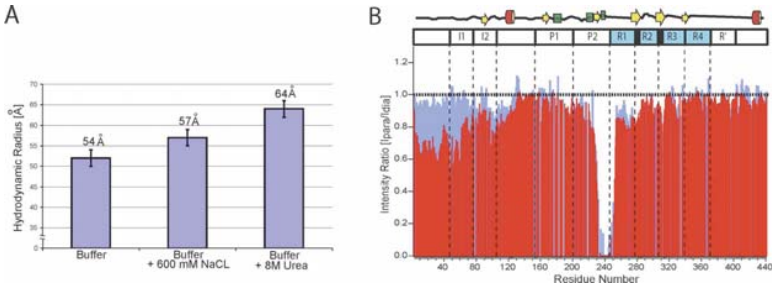
NMR

- Chemical Shifts
- RDCs
- Relaxation rates
- Spin relaxation
- **PRE**

Disorder seen by NMR

➡Long range contacts

➡contact => intensity decrease



Influence of Ionic Strength and Urea on Intramolecular Long-Range Interactions
(A) Hydrodynamic radius values of htau40 in buffer (99.9 % D2O, 50 mM phosphate buffer [pH 6.9]), upon addition of 600 mM NaCl and in the presence of 8 M urea.
(B) PRE profiles of amide protons in spin-labelled htau40 in buffer (red bars) and in the presence of 600 mM NaCl (blue bars). The single-cysteine mutant A239C was labelled with MTSL. Intensity ratios were averaged over a three-residue window.

Structural Polymorphism of 441-Residue Tau at Single Residue Resolution.
Mukrasch et al., PloS Biol. 2009, Vol 7, Issue 2

NMR

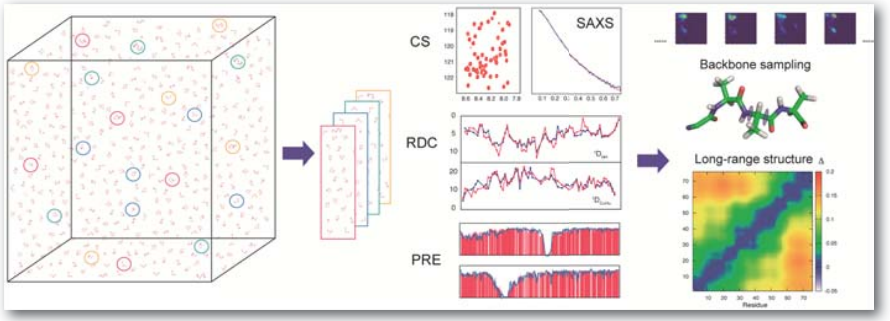
- **Chemical Shifts**
- **RDCs**
- **Relaxation rates**
- **Spin relaxation**
- **PRE**

Disorder seen by NMR

✓ Combination of NMR methods
- ➡ Disordered segments
- ➡ Secondary structure propensities
- ➡ Timescales of conformers exchange
- ➡ Long range contacts

✓ Combination of techniques
- ➡ Back-Calculation of parameters

➡ Modeling of conformers ensembles



Jensen, Zweckstetter, Huang &Blackledge, Chemical reviews 2014, special issues on IDPs
Exploring Free-Energy Landscapes of Intrinsically Disordered Proteins at Atomic Resolution Using NMR Spectroscopy

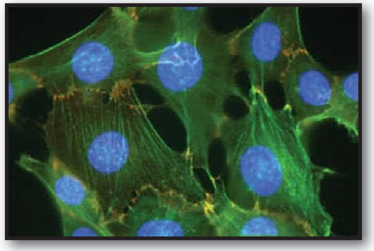
The case of intrinsically disordered domains that regulate actin polymerization

Actin

☞ **Major actor of**

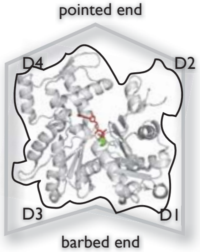
- motile processes
  (cell division, synaptic plasticity, endocytosis)
- morphogenetic processes
  (polarity of the embryo / right left asymetry)
- cellular motility
  (lamellipodia extension / cellular adhesion)

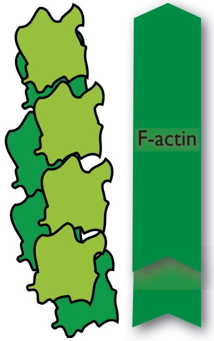
☞ **Assembly/disassembly of actin filaments**



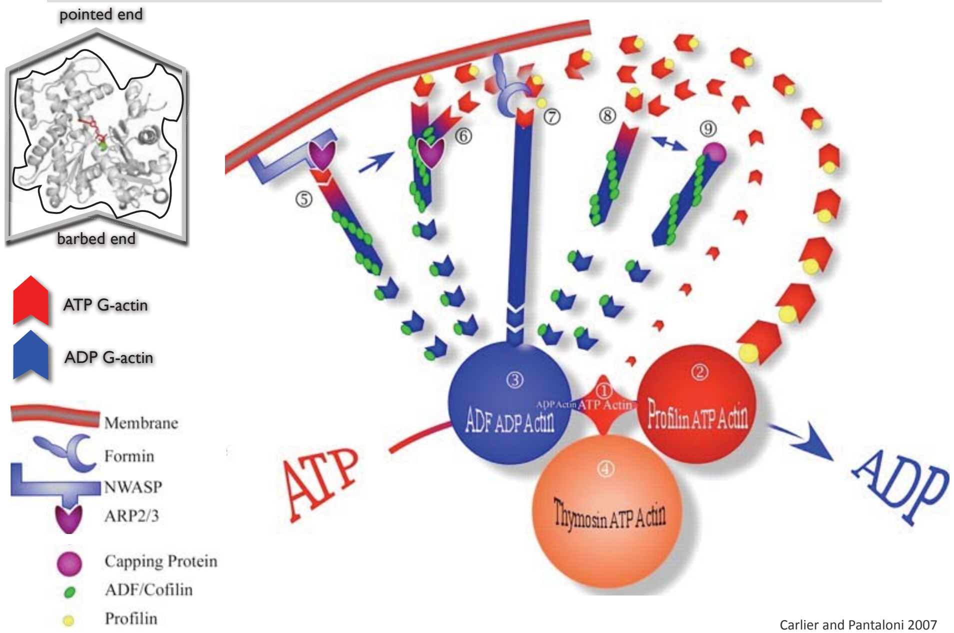
☞ Monomeric G-actin :

43 KDa ; 4 domains
Mg^{2+}; ATP





Dynamics of actin filaments, a highly regulated process



Carlier and Pantaloni 2007

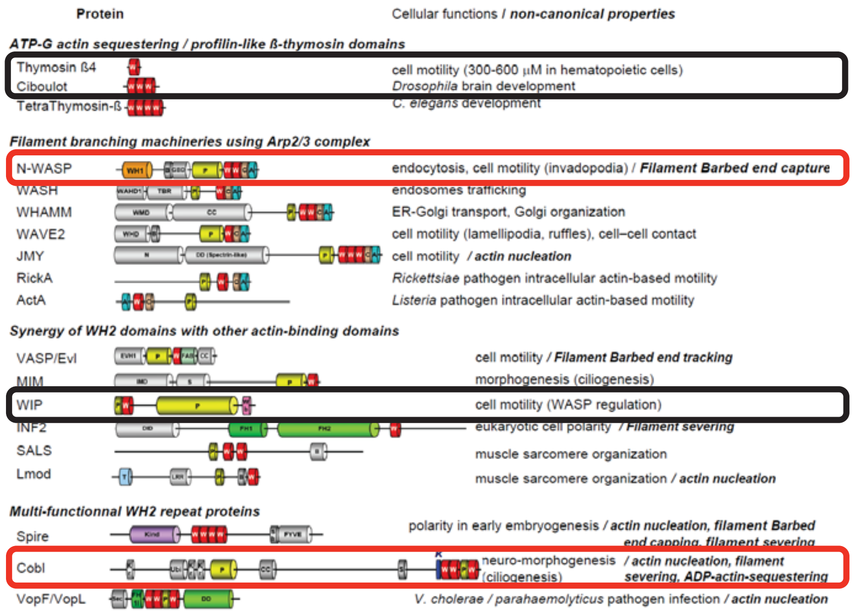
WH2/Tβ domains

Table I. Multiple Interactions and Roles of IDRs in Proteins Representative of Different Activities in Actin Self-Assembly Dynamics

Proteins	IDR(s)	Functions related to the IDR(s)	Role of disorder	Refs
Tβ4, *d. melanogaster* Ciboulot, WASP, WAVE, MIM, WIP, VASP,...	Single G-actin-binding WH2/ βT	sequester G-actin-ATP or promote its unidirectional assembly on barbed-end (profilin-like activity)	Fuzzy complexes: variable local dynamics in G-actin-bound states control variable association properties with barbed-end and can be tuned with a point mutation/modification	[Didry et al., 2012]
NPFs: WASP, N-WASP, WAVE, ...	Middle B-GBD or mea[nder-B] regions, C-terminal V/WH2-CA	B-GBD: binding to $PIP_{2/3}$, Rho-GTPase, kinase/SH2, (V)CA VCA: binding to B-GBD, G-actin-ATP, Arp2/3	signal integrator, molecular hub, mutually exclusive interactions, cooperative interdomain middle-to-tail autoinhibition, cooperative allosteric activations	[Kim et al., 2000; Chen et al., 2010]
mDia1–3, INF2, FMNL3, or FMN1–2 subfamilies of formins	C-terminal DAD/WH2-DAD or FSI	N-term. DID binding, cooperativity/synergy for FH2-mediated G-actin nucleation or FH1-FH2-mediated filament elongation, WH2-DAD: filament severing in association with FH2, FSI: Spire-KIND binding	signal integrator, mutually exclusive interactions, interdomain head-to-tail autoinhibition, dynamic binding with G- and/or F-actin, FSI: dynamic tuning of FH2 activity with Spire	[Li and Higgs, 2005; Chhabra et al., 2009; Gould et al., 2011; Vizcarra et al., 2011; Bor et al., 2012]
CP (α:β heterodimer)	β-subunit C-term. tentacle (β-tentacle)	Folding & binding to barbed-end terminal actin required for tight capping of CP	optimal and dynamic binding of CP α:β heterodimer to filament barbed end	[Takeda et al., 2010]
CD2AP, CKIP, CARMIL,...	CARMIL-peptide	uncap CP α:β heterodimer bound to barbed-end	allosteric mechanism disturbing CP α:β bending/twisting bound to barbed-end	[Takeda et al., 2011; Kim et al., 2012]
Formins (FH1-FH2 domains)	FH1 domain: repeats of proline-rich motifs binding to profilin	Recruit and feed the FH2-bound barbed end with profilin:G-actin-ATP	FH1 disorder state offers a larger capture radius for profilin:G-actin, and with its non-redundant repeats, speeds the elongation rate of barbed-ends associated with FH2-Cterminal domains	[Shoemaker et al., 2000; Paul and Pollard, 2008; Courtemanche and Pollard, 2012]
Spire, Cobl, VopF/VopL, JMY, N-WASP	WH2/βT repeats	sequester or nucleate G-actin-ATP/ADP, or promote G-actin unidirectional assembly, sever filament, cap barbed-end,...N-WASP, JMY: increase efficiency of branching mediated by NPF-Arp2/3	cooperative & dynamic binding with different actin tertiary & quaternary structures, repeats promote interactions with several actin subunits of filament or may assemble variable/dynamic G-actin oligomers	[Quinlan et al., 2005; Ahuja et al., 2007; Bosch et al., 2007; Husson et al., 2011; Gaucher et al., 2012]

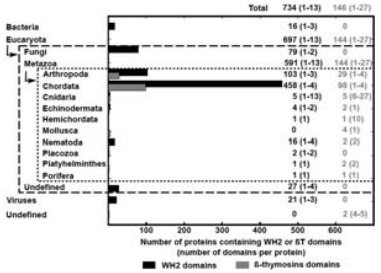
Renault, Deville, van Heijenoort, Cytoskeleton 2013, 70:686–705

WH2/Tβ domains

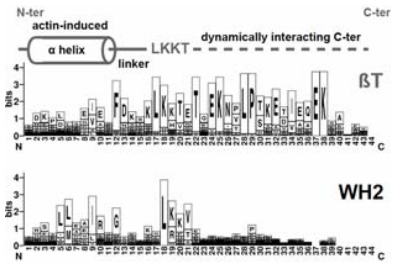


WH2/Tβ domains

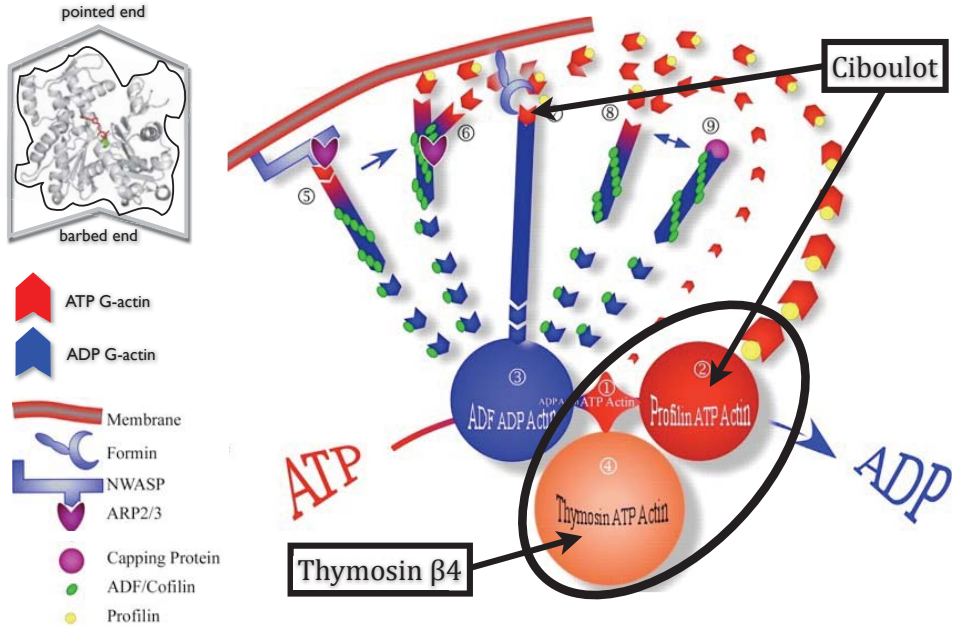
- ~850 putative WH2/Tβ domains in eukaryotic systems (SMART database)
- Isolated or inserted in large modular proteins
- Usually several repeats, up to 27



- Low sequence identity, variable length (25-55)
- 40% among tβ domains, 20% among WH2 domains, <15% for tβ/WH2
- N-ter helical propensity, LKKT/V, variable C-ter length



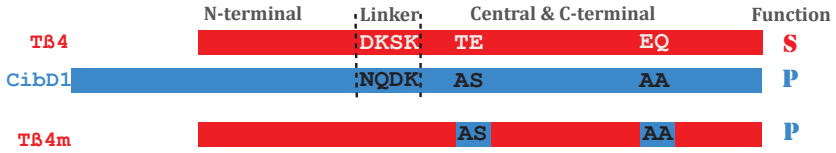
Dynamics of actin filaments, a highly regulated process



Sequestration vs Promotion of polymerization at barbed end of filaments

```
                                  αααααα linker   - central -        ---- C-terminal ---
Tβ4           1-------------MSDKPDMAEIEKFDKSKLKKTETQEKNPLPSKETIEQEKQAGES--44
Ciboulot_D1   1-MAAPAPALKDLPKVAENLKSQLEGFNQDKLKNASTQEKIILPTAEDVAAEKTQ-----53
                              αααααααααααααα
```

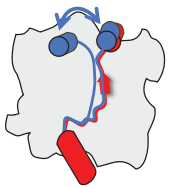
Tß4 : sequesters G-actin
Domain 1 of ciboulot (CibD1): promotes actin polymerization at barbed end of filaments



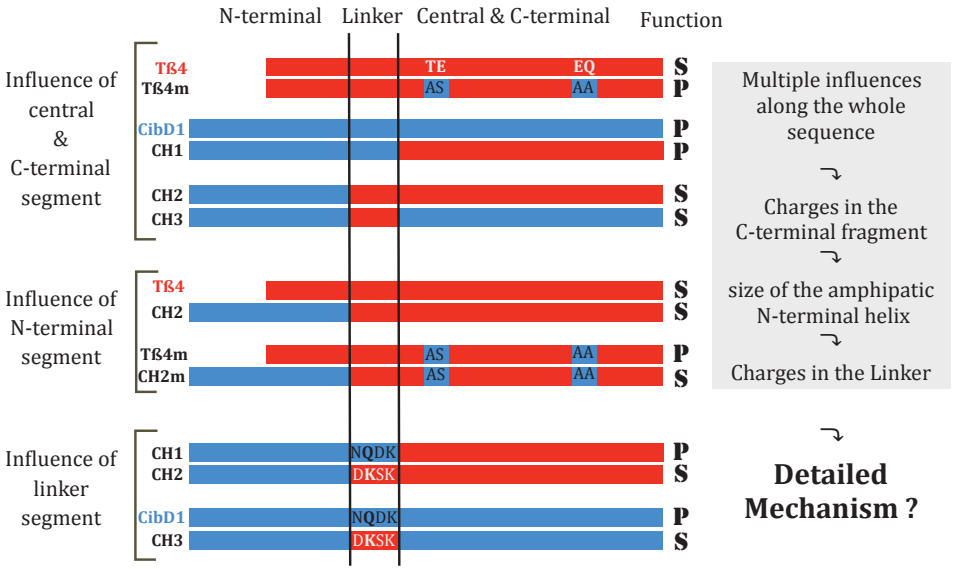
- Role of charges in the central and C-terminal fragment

  Domanski et al. JBC 2004, Hertzog et al. Cell 2004

- Design of mutants and chimera of Tβ4/CibD1

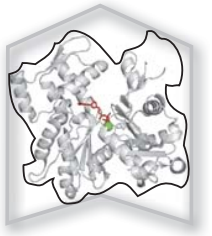


Sequestration vs Promotion of polymerization at barbed end of filaments



Domanski et al., J. Biol. Chem. 2004 ; Hertzog et al. Cell 2004; Carlier et al., An.N.Y.Ac.Sci. 2007, 2010; Cantrelle, Didry et al. EMBO J. 2012

Structural characterization of IDPs involved in actin regulation
A challenging task !



Actin polymerization (60µM @ 4°C)

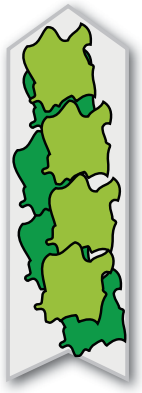
Low stability of G-actin

Actin mostly extracted from rabbit muscle

High flexibility of free WH2 domains

Local flexibility in the complexes

Low stability of complexes

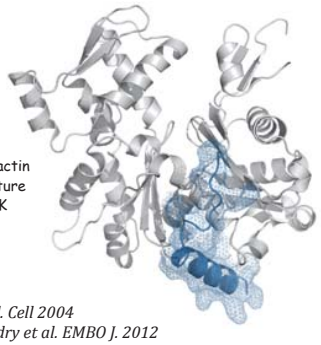


☞ **Combination of biochemical and structural techniques**

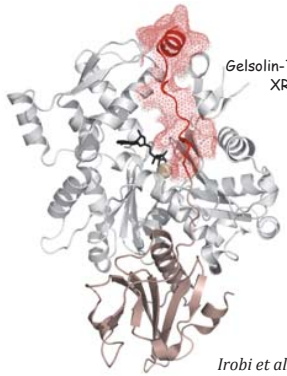
XRay diffraction, NMR, SAXS

Crystal structures of the bound states

	difficulties	informations
XRay diffraction	cristallization conditions dynamics / heterogeneity	**Partial view of the complex** Detailed view of the interface



Ciboulot/G-actin
XRay structure
pdb 1SQK



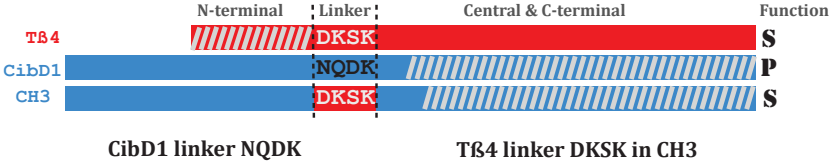
Gelsolin-Tβ4(C-ter)/G-actin
XRay structure
pdb 1T44

Hertzog et al. Cell 2004
Cantrelle, Didry et al. EMBO J. 2012

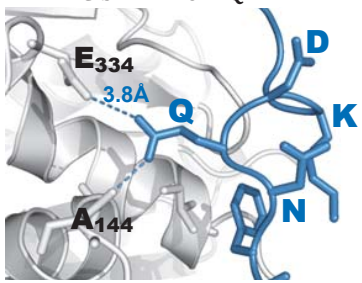
Irobi et al. EMBO J. 2004

Crystal structures of the bound states

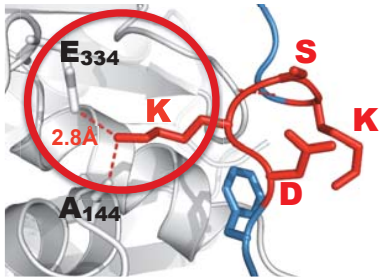
	difficulties	informations
XRay diffraction	cristallization conditions dynamics / heterogeneity	Partial view of the complex **Detailed view of the interface**



CibD1 linker NQDK

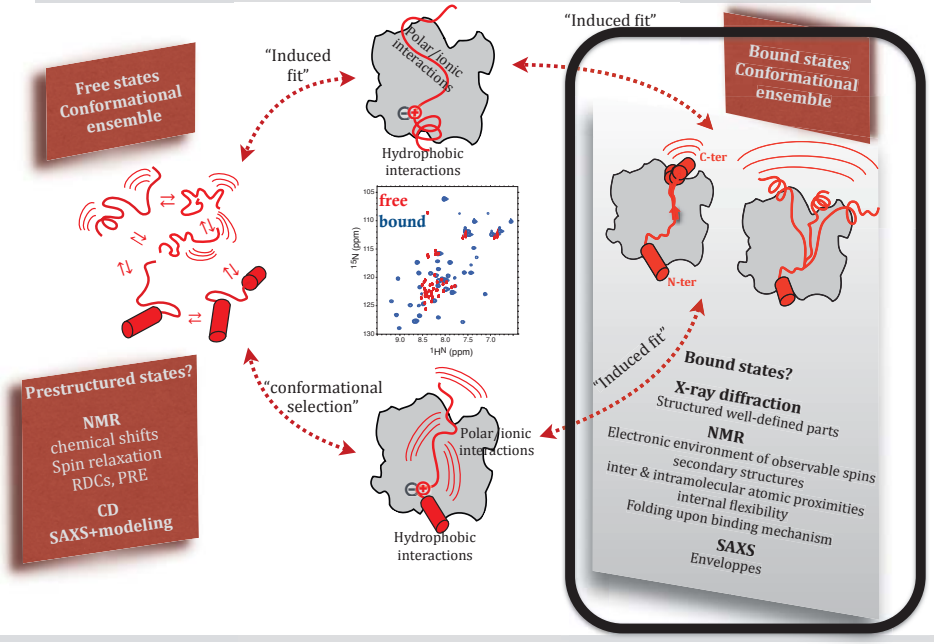


Tß4 linker DKSK in CH3



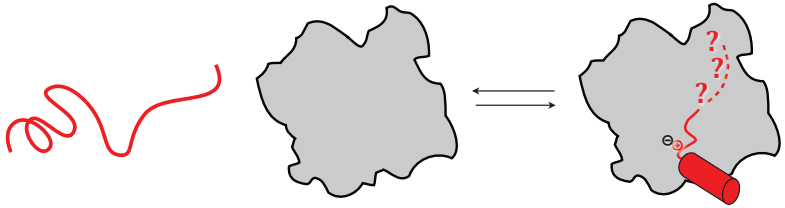
Salt bridge GLU^{actine} ---LYS^{linker,WH2}

What kind of questions can we tackle by NMR?



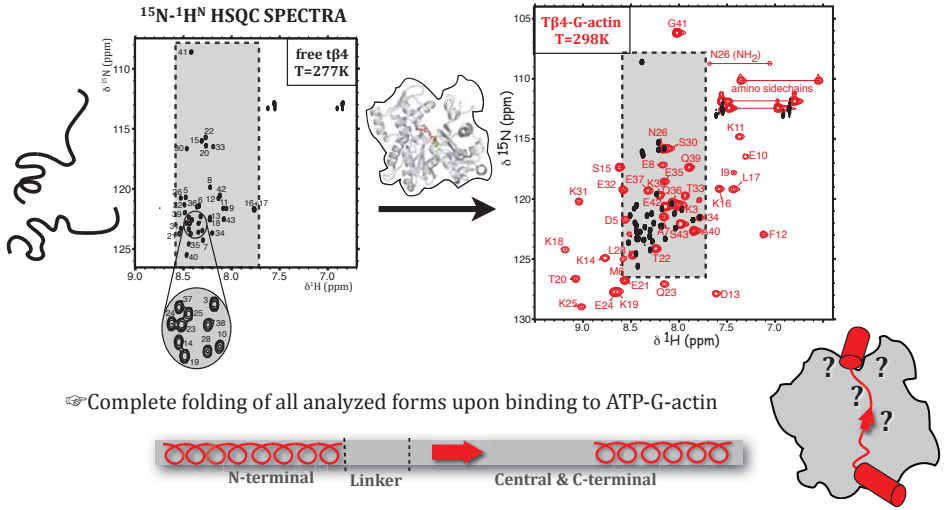
Detailed analysis of actin-Tß/WH2 domains interactions

	difficulties	informations
XRay diffraction	cristallization conditions dynamics / heterogeneity	**Detailed view of the interface** **Partial view of the complex**



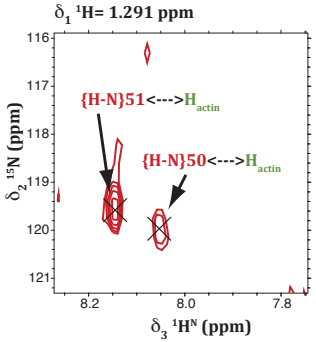
	difficulties	informations
NMR	Stability of the complex **No exploitable data on actin**	Detailed analysis of free and bound WH2 domains • Folding upon binding process • Global and local dynamics along the whole domain • Kinetics of binding • Variation of the physicochemical conditions

Folding upon binding process by NMR



☞Complete folding of all analyzed forms upon binding to ATP-G-actin

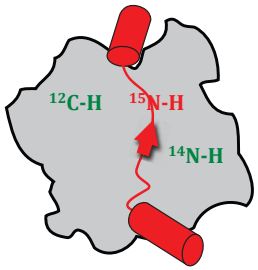
NMR structures of the bound states



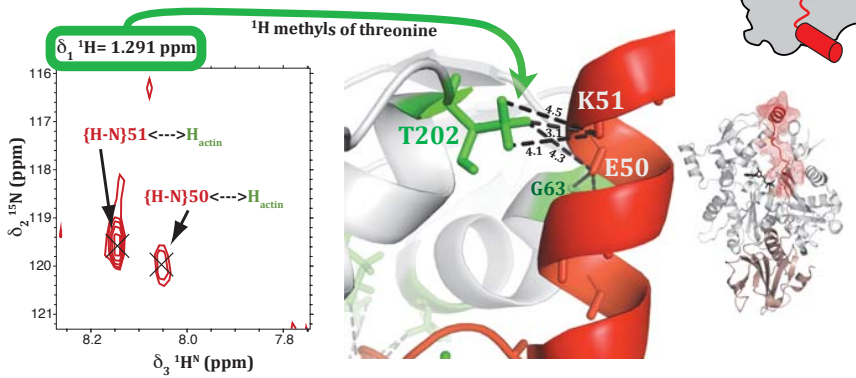
☞ **Filtered NOESY experiments**

***U*-[^{2}H,^{15}N] WH2 domain**

-> proximities between H{^{15}N} of the WH2 domain and H{^{14}N/^{12}C} of actin.



NMR structures of the bound states

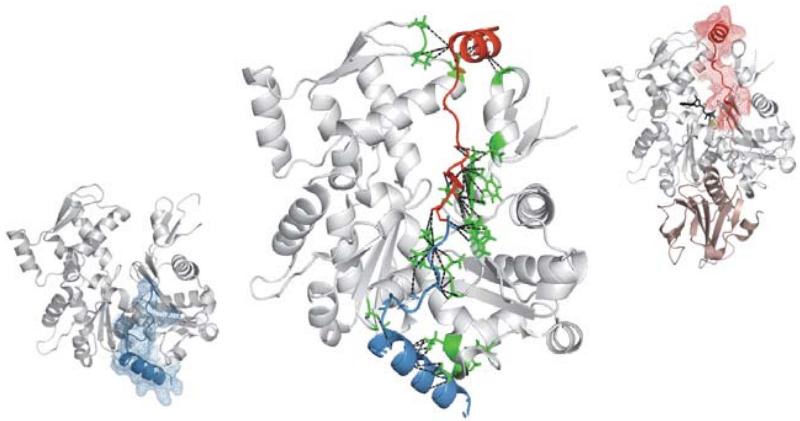


☞ The C-terminal end is in contact with G-actin

☞ nOe contacts are compatible with the proximities observed in the bound fragment of Tß4 in the crystal structure

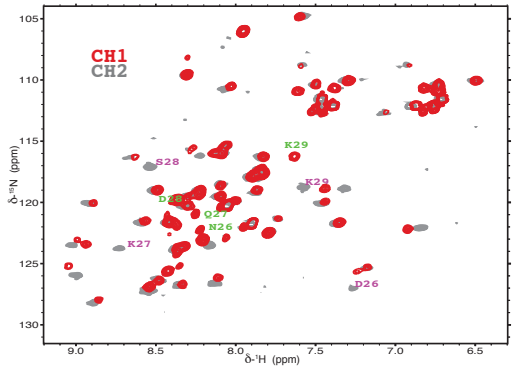
NMR structures of the bound states

☞ NOESY experiments: The whole WH2 sequence is in contact with G-actin
☞ nOe contacts are all compatible with the observed bound fragments of Ciboulot, chimeras & Tß4 in crystal structures



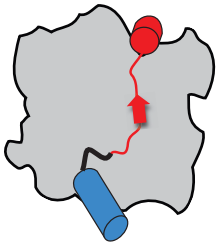
NMR structures of the bound states

	linker	- central -	---- C-terminal ---	Function
Ciboulot_D1	GPLGSLKDLPKVAENLKSQLEGFNQDKLKNASTQEKIILPTAEDVAAEKTQ			P
CH1	GPLGSLKDLPKVAENLKSQLEGFNQDKLKKTETQEKNPLPSKETIEQEKQAGES			P
CH2	GPLGSLKDLPKVAENLKSQLEGFDKSKLKKTETQEKNPLPSKETIEQEKQAGES			S
Tβ4	MSDKPDMAEIEKFDKSKLKKTETQEKNPLPSKETIEQEKQAGES			S

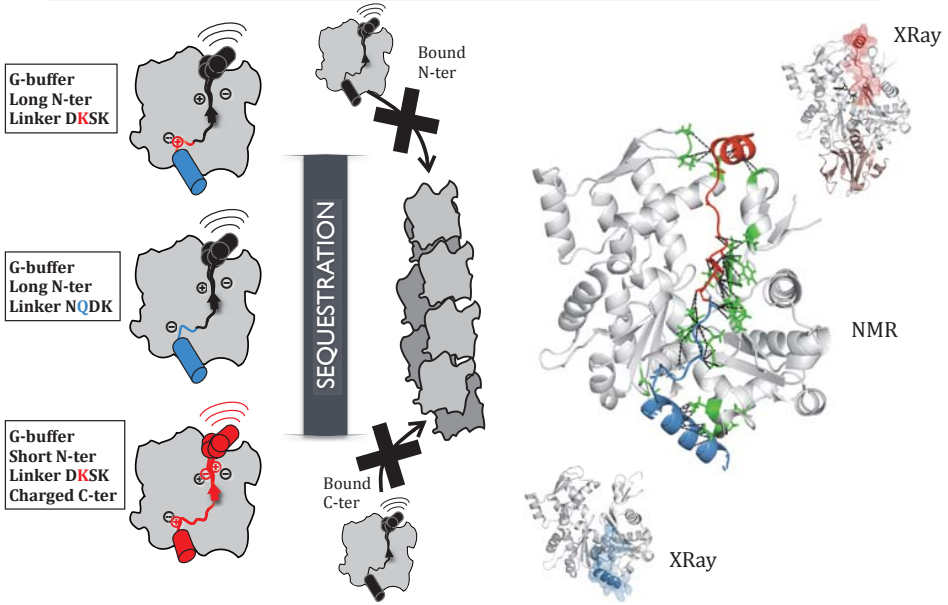


☞ Whatever the function:

Same positioning & structure for identical segments on actin



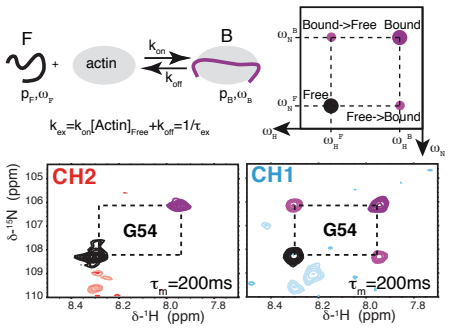
Isolated WH2/Tβ domains
G-buffer : sequestration

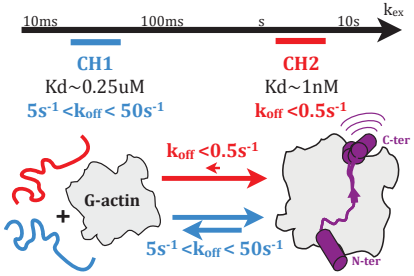


Cantrelle, Didry et al. EMBO J. 2012

kinetics of the binding process

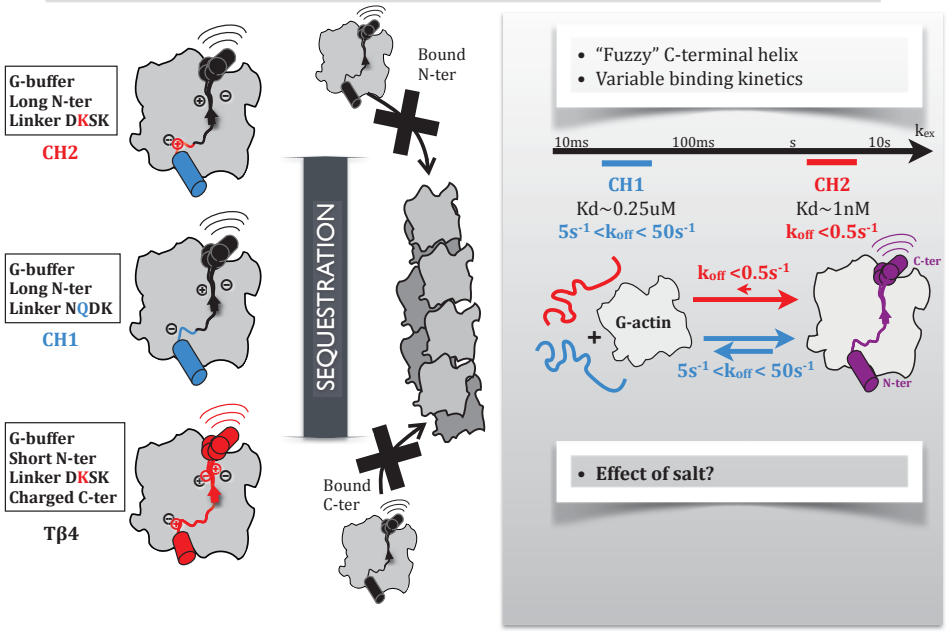
- NMR $^{15}N_{zz}$ exchange & ^{15}N R_2
- Kd measurements



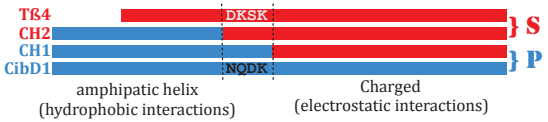


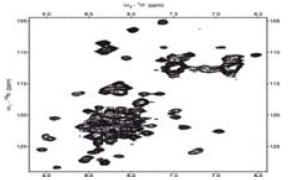
➤ Highly different binding kinetics between CH2 (sequesterer) and CH1 (Promotor of polymerization)

Isolated WH2/Tβ domains
G-buffer (low salt) : sequestration



Variation of physico-chemical conditions : effect of salt
G-buffer (no salt) ---------> F-buffer (150mM KCl)

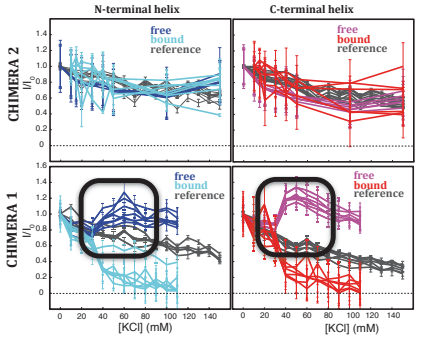




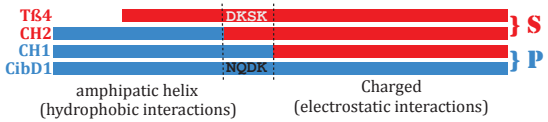
Effect of ionic strength on affinities

- **CH2**
  - Very large salt effect on Kd
    (Kd 1nM[G-buffer] --->0.5µM[F-buffer])
  - behavior at 150mM KCl shifts towards the one of CH1 without salt
- **CH1**
  - Moderate salt effect on Kd
    (Kd 0.25µM[G-buffer] --->3.5µM[F-buffer])
  - Complete release of the bound form at ~50mM KCl

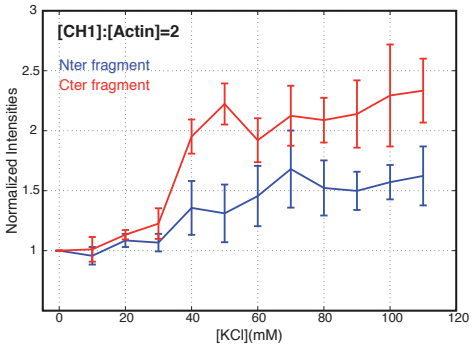
Evolution of intensities of HSQC peaks for free and bound states (WH2:actin 2:1) upon increasing [KCl]



Variation of physico-chemical conditions : effect of salt
G-buffer (no salt) ---------> F-buffer (150mM KCl)

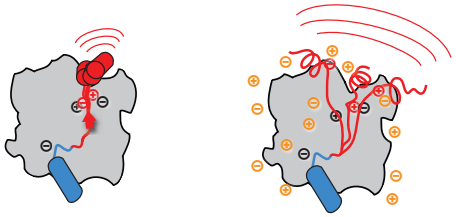


Evolution of intensities of HSQC peaks for free and bound states (WH2:actin 2:1) upon increasing [KCl]



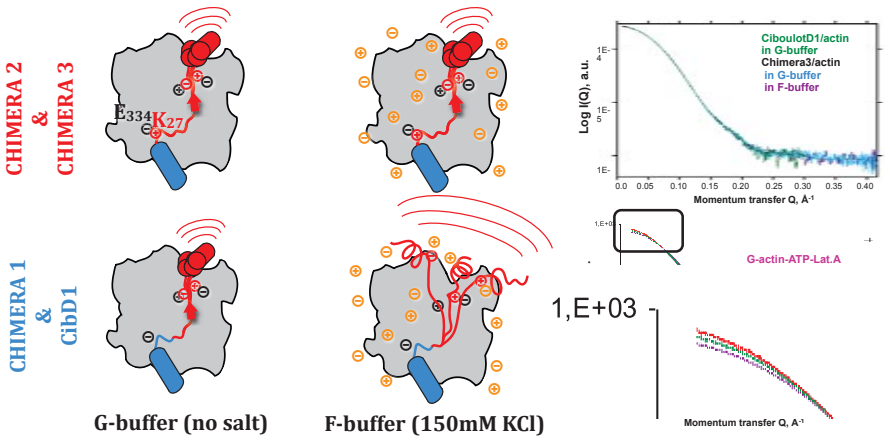
CH1 (promotes polymerization)

Prior release of the C-terminal segment upon KCl concentration increase.



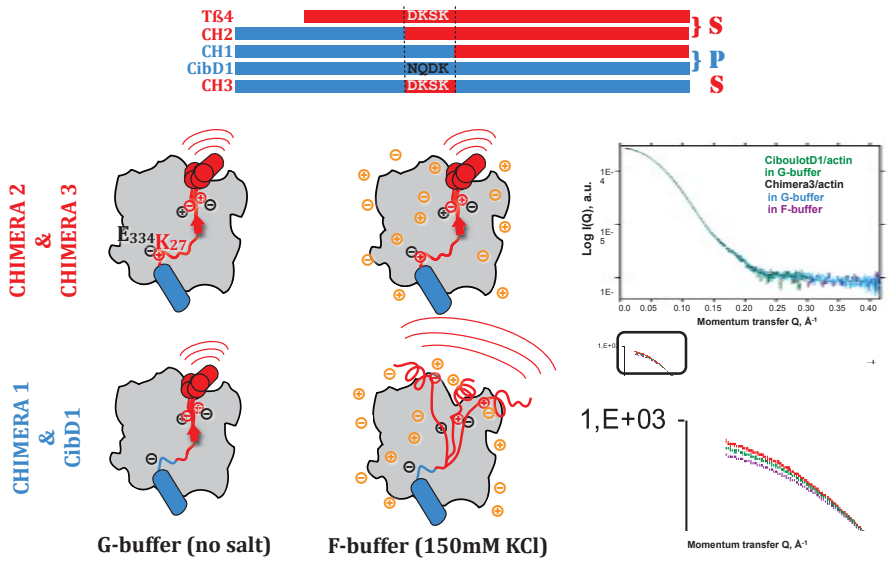
G-buffer (no salt) **F-buffer (150mM KCl)**

fuzziness of the bound states - NMR & SAXS

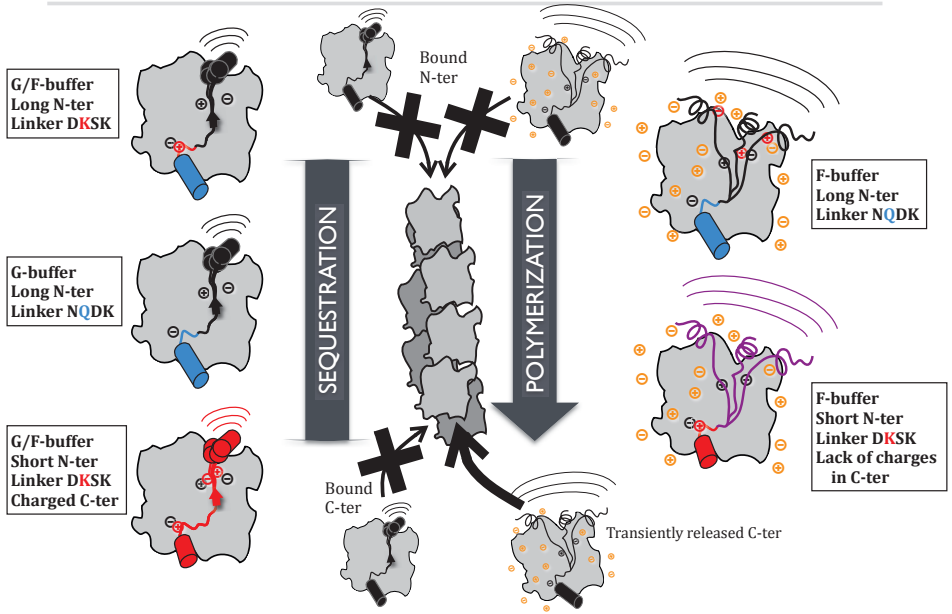


G-buffer (no salt) **F-buffer (150mM KCl)**

fuzziness of the bound states - NMR & SAXS



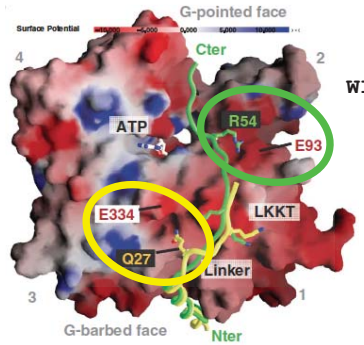
Isolated WH2/Tβ domains
G-buffer : sequestration / F-buffer : variable function



Cantrelle, Didry et al. EMBO J. 2012

Isolated WH2/Tβ domains
How scarce sequence elements control the dynamics and the function

```
                                  αααααα  linker  - central -      ---- C-terminal ---
Tβ4          1-------------MSDKPDMAEIEKFDKSKLKKTETQEKNPLPSKETIEQEKQAGES--44
Ciboulot_D1  1-MAAPAPALKDLPKVAENLKSQLEGFNQDKLKNASTQEKIILPTAEDVAAEKTQ-----53
HUMAN WIP     ----------EQAGRNALLSDISKG-K-KLKKTVTNDRSAPILDKPKGAG--------
                        αααααααααααααα
```



Sequestration

WIP EQAGRNALLSDISKG-K-KLKKTVTNDRSAPILDKPKGAG

N

Barbed end elongation

Sequestration

```
                                  αααααα linker  - central -      ---- C-terminal ---
Tβ4          1-------------MSDKPDMAEIEKFDKSKLKKTETQEKNPLPSKETIEQEKQAGES--44
Ciboulot_D1  1-MAAPAPALKDLPKVAENLKSQLEGFNQDKLKNASTQEKIILPTAEDVAAEKTQ-----53
```

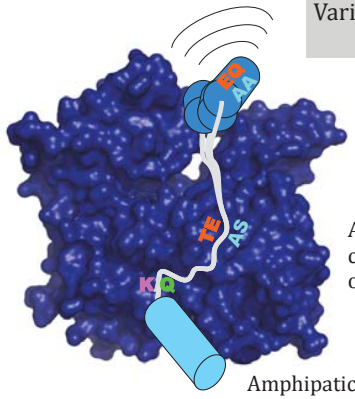
K

Q

Barbed end elongation

Isolated WH2/Tβ domains : "fuzziness" determines the function

One target & alternative functions
Variation of key sequence elements along the whole sequence control the dynamics and the function



ionic/polar interactions modulate local flexibility in the bound state

A key ionic/polar interaction can control the flexibility/anchorage of a fragment of the IDP on its target

Salt dependent

Control of dynamics & function

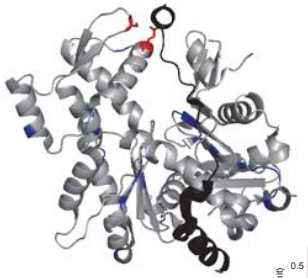
Amphipatic helix - hydrophobic interactions
Control of anchorage and modulation of global interaction strength

Fine tuning to obtain moderate affinities and versatile functions, compatible with a dynamic actin polymerization/depolymerization processes

Looking from the actin side

Producing actin

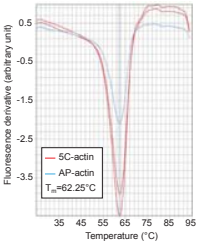
- recombinant 5C actin in insect cells
- non polymerizable actin A204E, P243K (AP-actin)



Rabbit actin/5C actin	
E2-	M176L
D3E	V201T
T5V	N225Q
T6A	T260A
C10V	I267L
L16M	S271A
V17C	A272C
I76V	I287V
T103V	N297T
V129T	M299L
T160S	T358S
N162T	A365S

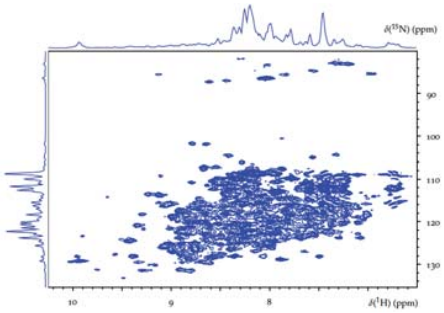
Thermal Shift Assays:
Same thermal stability

G-buffer
50mM Tris pH-7,4 ; 0,2mM ATP;
0,5mM CaCl2



Producing labeled actin

- ^{15}N-AP-actin, 100µM, 298K, 950MHz



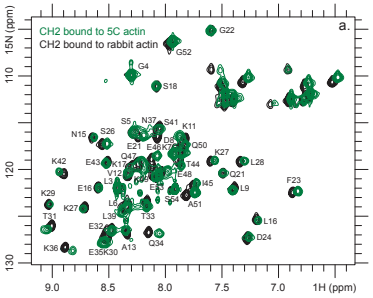
A. Meola et al., *Journal of Structural Biology* 188 (2014) 71–78

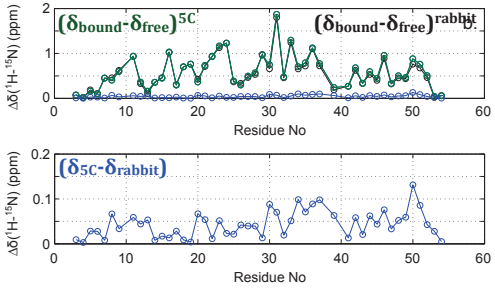
To go further :
^{15}N,^{2}H-actin

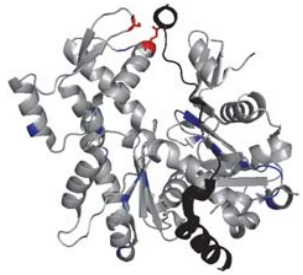
Question: On the structured target side?

Looking from the actin side

- Is recombinant 5C actin suitable to analyze WH2 interactions?





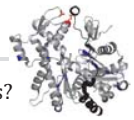


- recombinant 5C actin or rabbit muscle actin : Similar interaction of the CH2 domain

Carine van Heijenoort ReNaFoBis - Oléron - 2016 Mai 26th

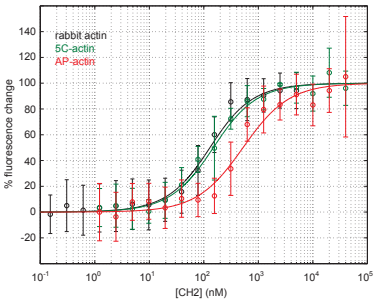
Question: On the structured target side?

Looking from the actin side



- Is recombinant non polymerizable AP-actin suitable to analyze WH2 interactions?

Microscale Thermophoresis (MST)



[actin]=50nM

Protein	K_d(nM)
rabbit actin	104±29
5C-actin	124±32
AP-actin	500±120

- recombinant AP-actin vs 5C/rabbit actin : Different interaction of the CH2 domain ?

- NB. Kd of 1nM measured from competition with Tβ4 using AEDANS-labeled α-skeletal G-actin

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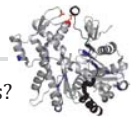
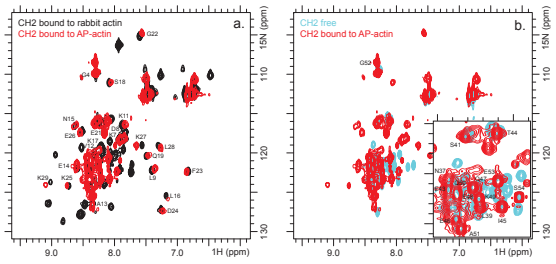
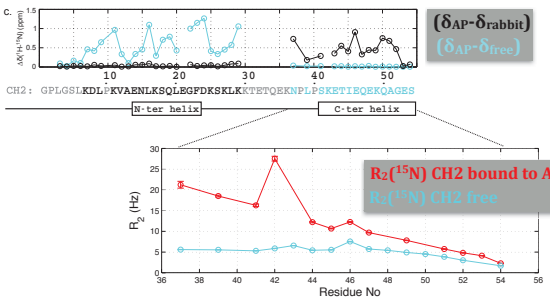
Question: On the structured target side?

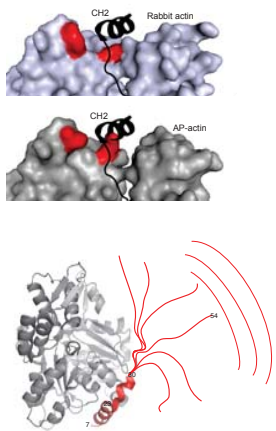
Looking from the actin side



- Is recombinant non polymerizable AP-actin suitable to analyze WH2 interactions?







- NO !

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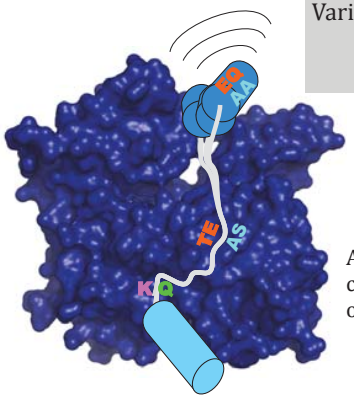
Isolated WH2/Tβ domains : "fuzziness" and actin state determines the function

One target & alternative functions
Variation of key sequence elements along the whole sequence control the dynamics and the function
+ Actin state



ionic/polar interactions modulate local flexibility in the bound state

A key ionic/polar interaction can control the flexibility/anchorage of a fragment of the IDP on its target

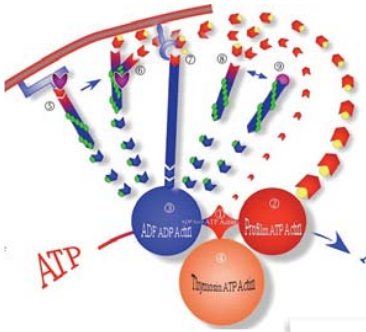
Salt dependent

Control of dynamics & function

Amphipatic helix - hydrophobic interactions
Control of anchorage and modulation of global interaction strength

⇀ Fine tuning to obtain moderate affinities and versatile functions, compatible with a dynamic actin polymerization/depolymerization processes

WH2/Tβ domains



- Sequestration of monomeric actin (Isolated WH2/Tβ)
- Promotion of polymerization at barbed end (Isolated WH2/Tβ)
- Nucleation (Formins, WH2/Tβ repeats, WASP, N-WASP, WAVE, …)
- Filaments branching (WASP, N-WASP, WAVE, …)
- Filaments capping (CP, CARMIL)
- Filaments severing (WH2/Tβ repeats)
- Inactivation/Activation of signaling cascades (WASP, WAVE, …)

- Usually moderate affinities (μM)
- Tunable specificities modulated by
  - key residues spread along the sequence
    - versatility of folding upon binding processes
    - variable flexibility in the complexes with actin
  - post-transcriptional modifications
  - expression levels of partners
    - Cooperative / antagonist key interactions
    - Allosteric processes

What about more complex systems?

Filament branching machineries using Arp2/3 complex

N-WASP — endocytosis, cell motility (invadopodia) / ***Filament Barbed end capture***

- Interaction of the tandem WH2 (V2) domain repeat with actin? mechanism? Cooperativity?

Fluorescence

$$V2 \xrightarrow[k_1=0.02\mu M]{+A} V2A \xrightarrow[k_2=0.3\mu M]{+A} V2A_2$$

Gel filtration

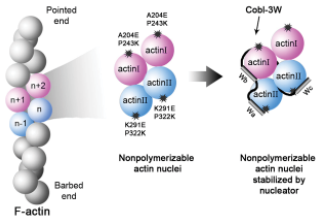
Crystal structure: 1:1 complex

Gaucher et al. J. Biol; Chem 2012

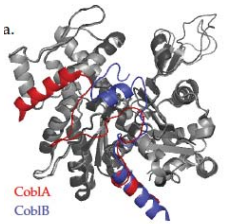
Multi-functionnal WH2 repeat proteins

Cobl — neuro-morphogenesis (ciliogenesis) / *actin nucleation, filament severing, ADP-actin-sequestering*

- Interaction of the tandem WH2 (CoblKAB) domain repeat with actin? mechanism? Cooperativity?



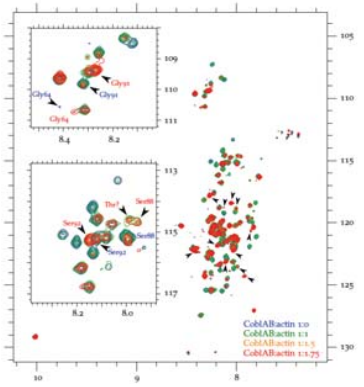
Crystal structure:
- 2 actin mutants
- Unusual electrostatic interaction of the amphipathic helix of the first domain
- Unusual actin/actin interface



Chen et al. Cell Reports, 2013.

Interaction CoblAB - Actin

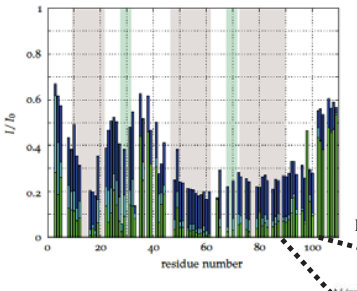
[^{15}N, ^{13}C, ^{2}H]-CoblAB



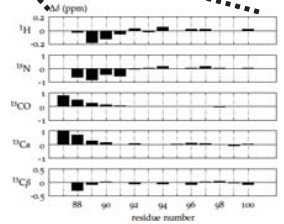
950MHz, 298K, pH7

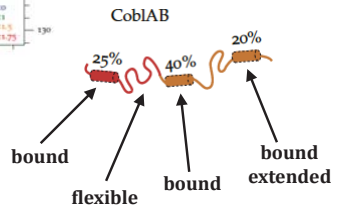
~30 new peaks

CoblAB:actin 1:1; 1:1.5 ; 1:1.75

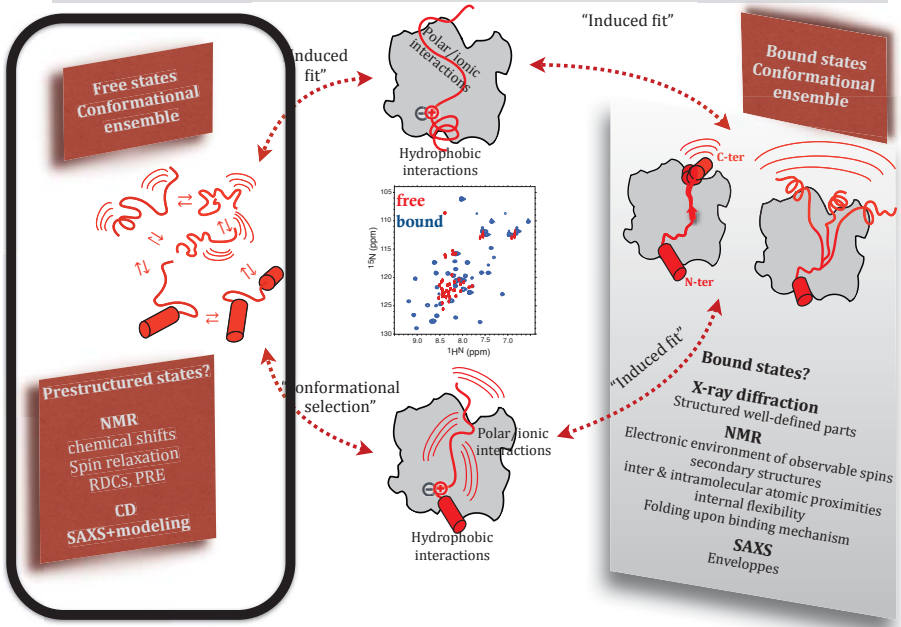


Extended C-terminal helix in the bound form

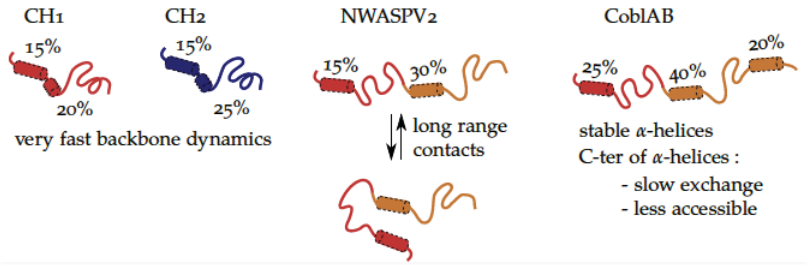




Analyzing the free states



What about more complex systems?



Highly variable and complex dynamics of the free states
Question - What is possible to do in molecular dynamics / simulation studies?

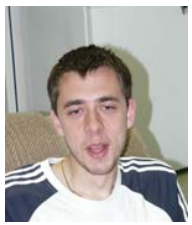
K_d(actin-CH2)
0.001µM /0.1µM
K_d(actin-CH1)
0.3µM / 3µM

K_d(actin-NWASPV2)
0.02µM ; 0.3µM

K_d(actin-CoblA)=0.5µM
K_d(actin-CoblB)=0.05µM

The relationship between pre-structuration and affinity is not straightforward!

Laboratoire de Chimie et Biologie Structurales



François-Xavier Cantrelle

Célia Deville

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