

The solution structure of the
dimeric carboxy-terminal domain
of Hexim1 and the
characterization of its interaction
with Cyclin T1 by NMR

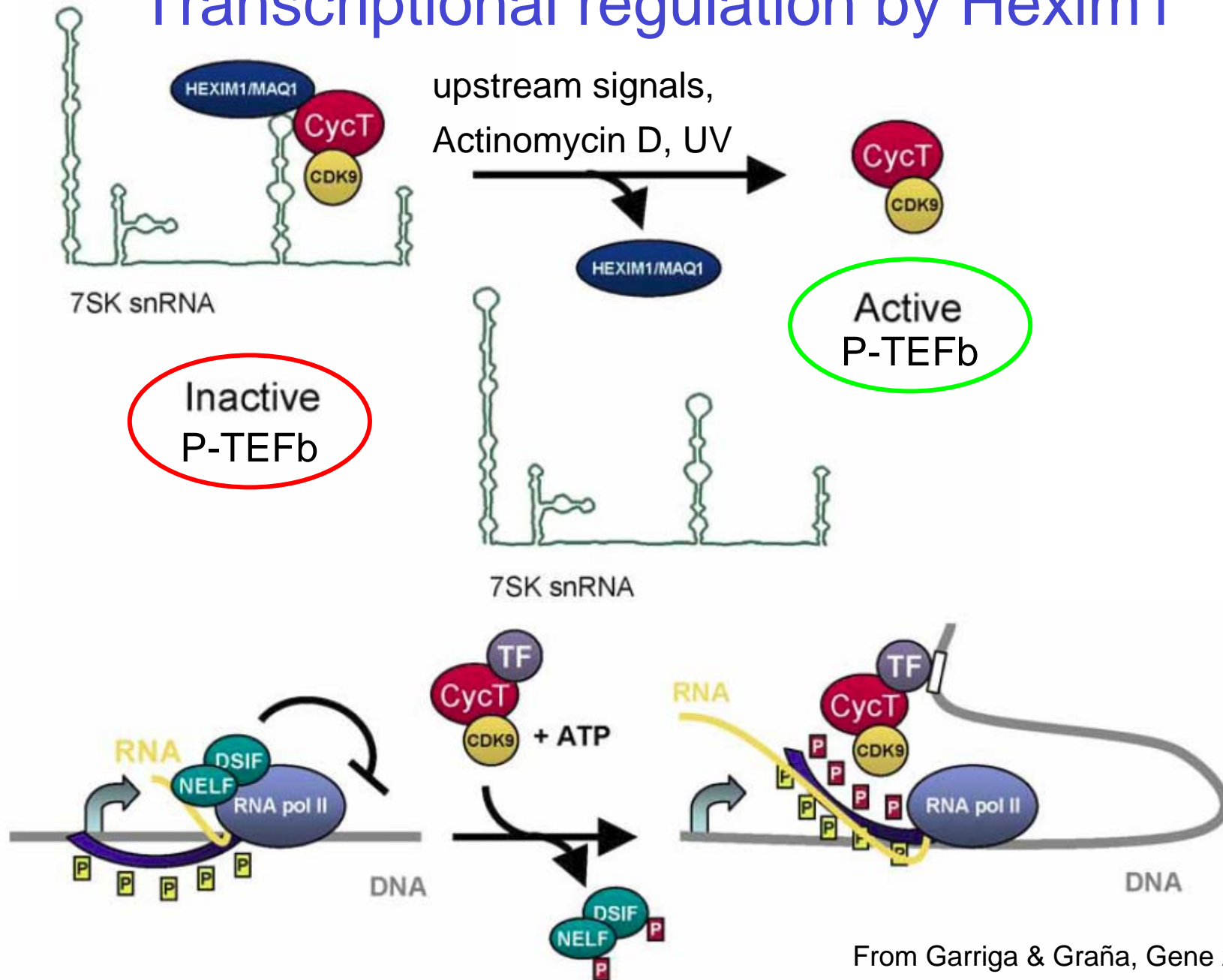


Sonja Alexandra Dames, University of Vienna, 28.06.2006

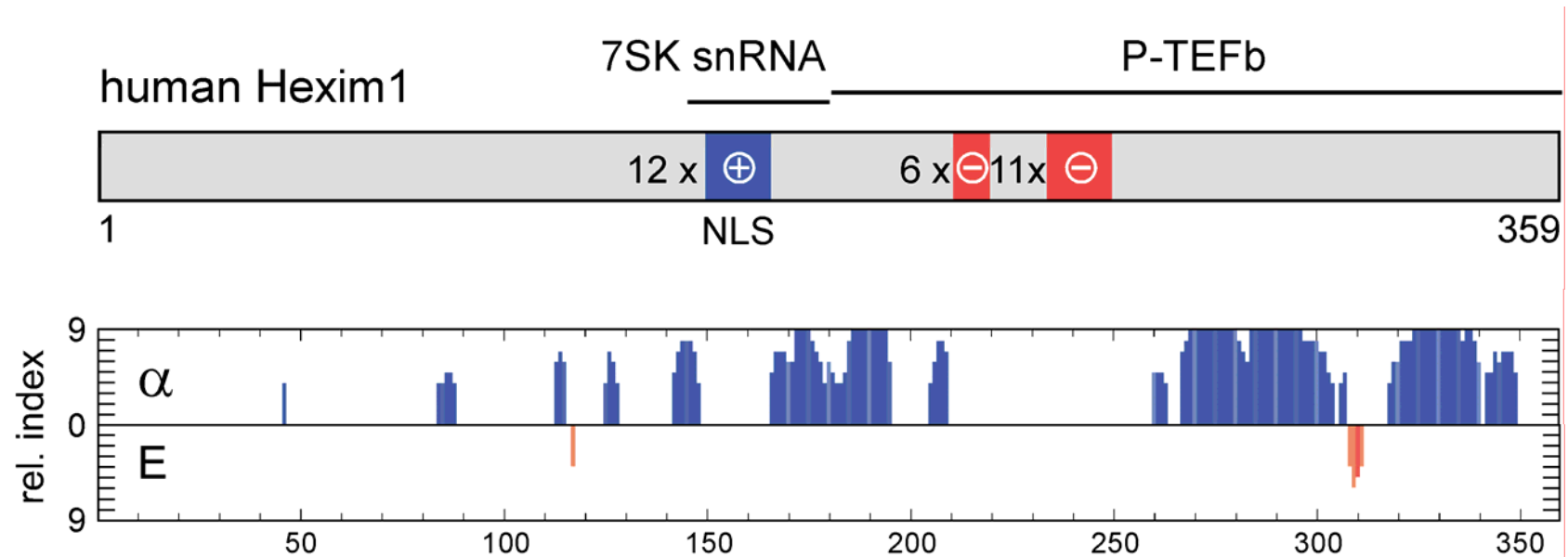
Many names - one protein: the discovery of Hexim1

- **Hexim1** (HMBA-inducible protein 1) - molecular target of Hexamethylene bis-acetamide in the cell
- **MAQ1** (ménage à quatre)
- **CLP-1** (cardiac lineage protein-1)
- **EDG1** (estrogen down regulated gene 1)

Transcriptional regulation by Hexim1



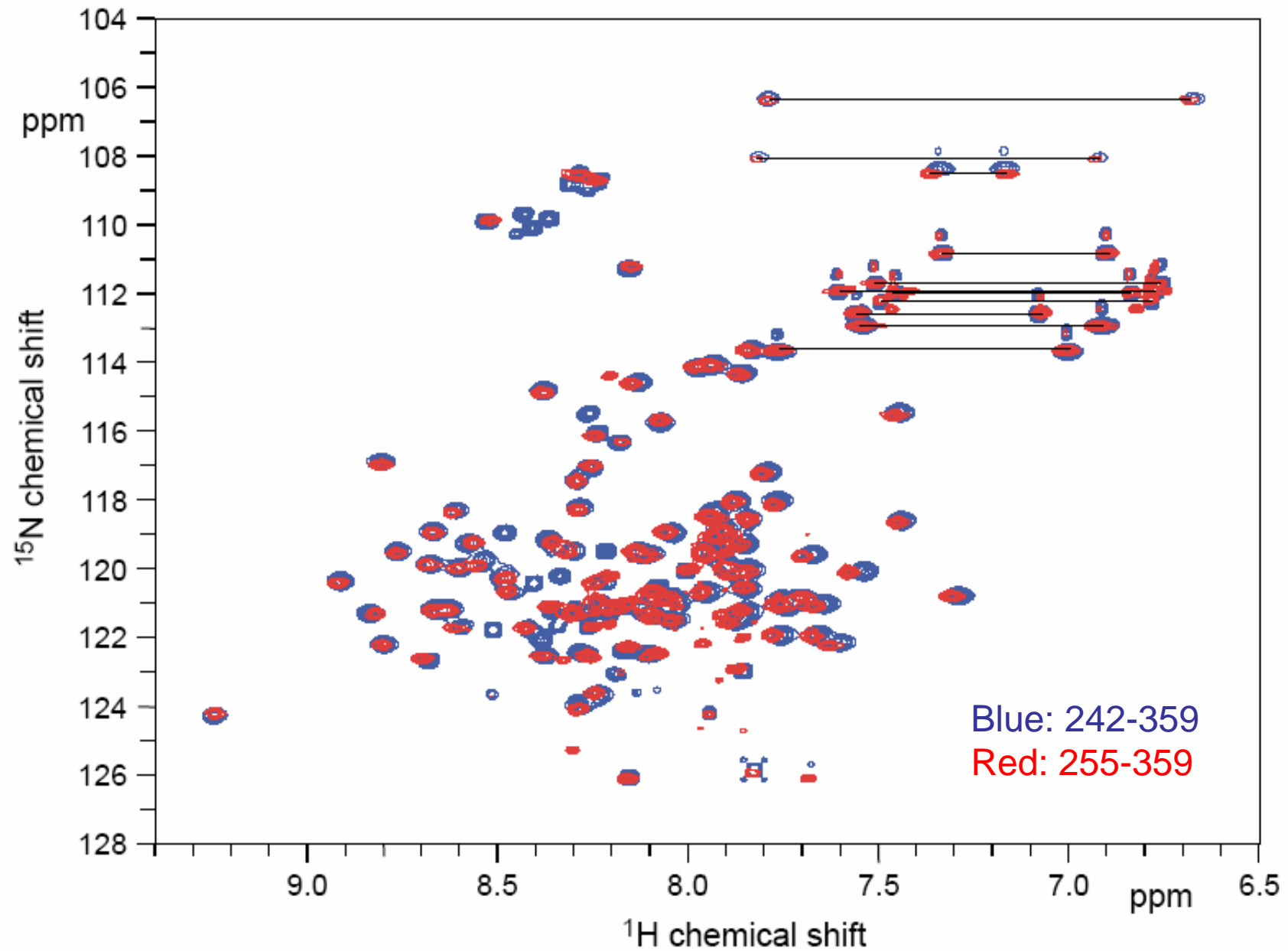
The domain structure of Hexim1



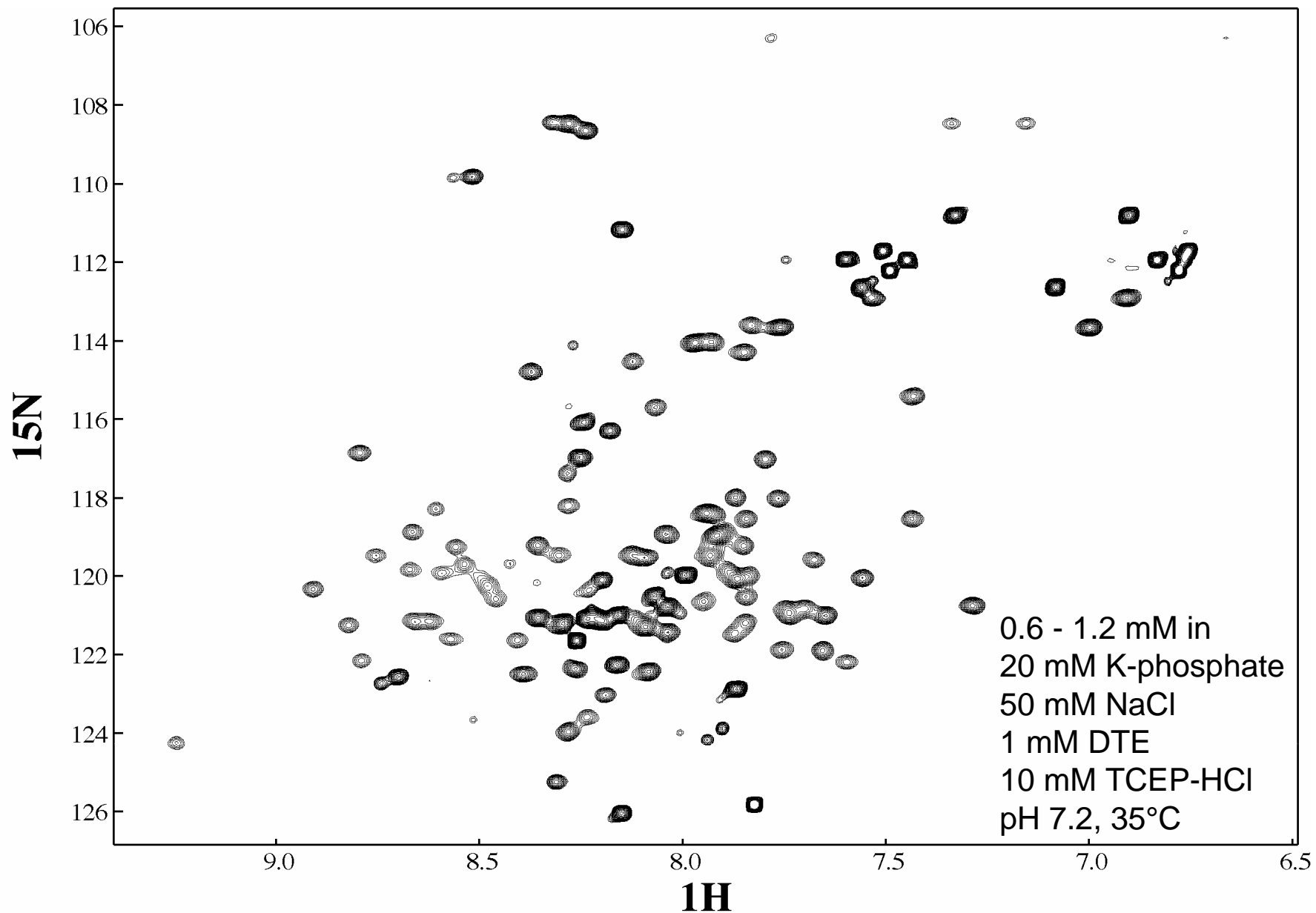
our construct: 255-359
= CTD

also tested: 242-359

^{15}N -HSQC spectra of different Hexim1 constructs



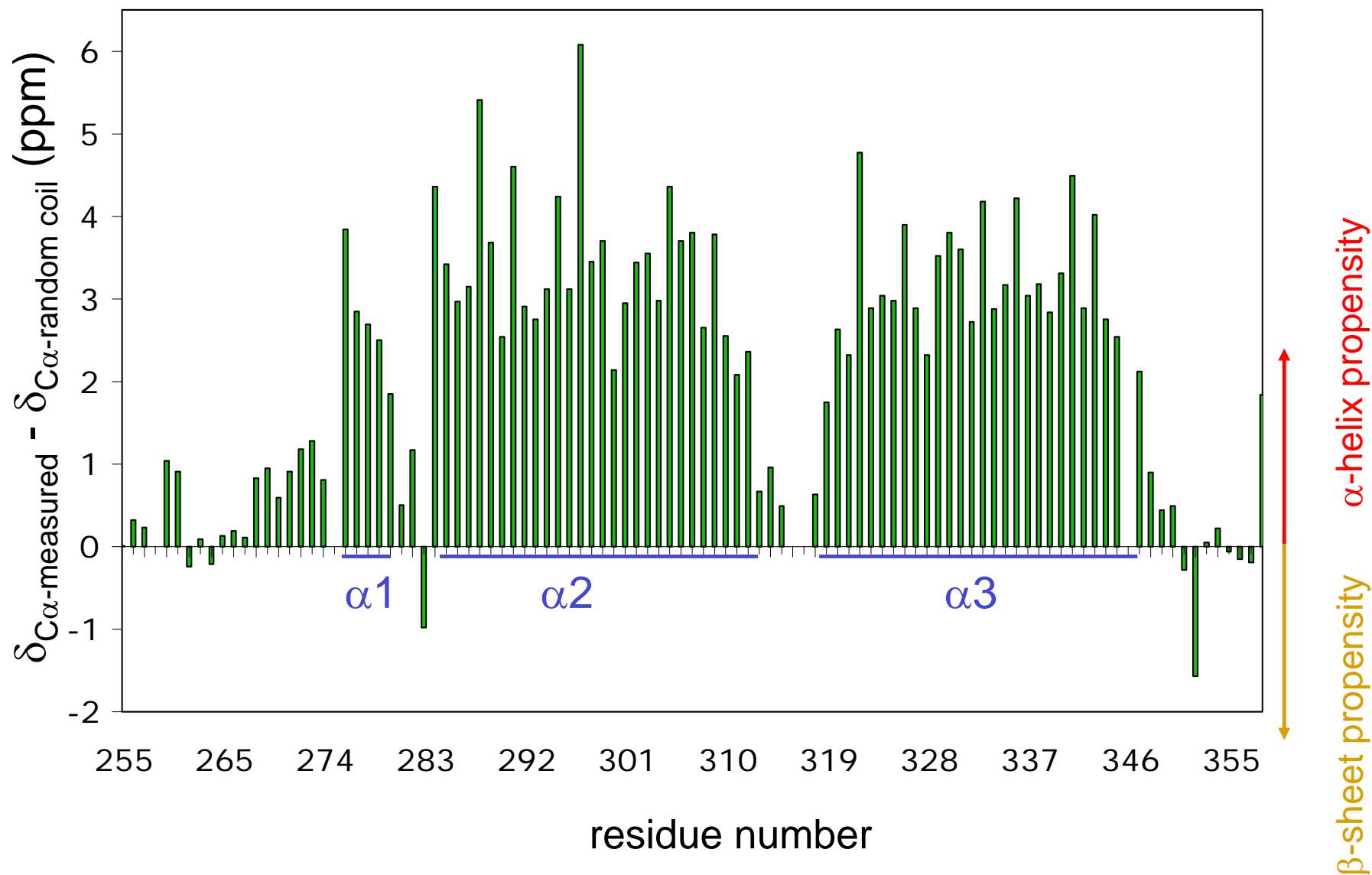
^{15}N -HSQC spectrum of Hexim1 (255-359)



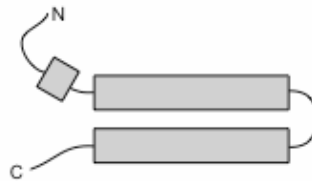
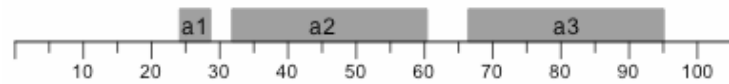
Hexim1 (255-359) NMR Data:

Fingerprints	2D ^{15}N -HSQC, 2D ^{13}C -HSQC
Sequential assignments	3D HNCA, 3D ^{15}N -NOESY, 3D CBCA(CO)NH, 3D CBCANH, 3D HNCO
Side chain assignments	3D HBHA(CBCACO)NH, 3D HCCH-TOCSY, 3D CCONH-TOCSY, 3D ^{15}N - & ^{13}C -NOESY
Stereo-specific assignments & dihedral angle information for backbone ϕ and side chain χ_1	3D-HACAHB-COSY, 3D-HNHA, 3D-HNHB, ^{13}C - $\{^{13}\text{C}_\gamma\}$ -SED- and ^{15}N - $\{^{13}\text{C}_\gamma\}$ -SED- ^{15}N - ^1H -HSQC, ^{13}C -HSQC of a 10% ^{13}C sample
Distance restraints	^{15}N - and ^{13}C -edited NOESY, ^{13}C -filtered NOESY (intermolecular contacts)
Residual dipolar coupling restraints	^{15}N - ^1H IPAP-HSQC, 3D HNCOCA (sample aligned with PF1 phages)
Dynamic information	^{15}N -T1, -T2, and $\{^1\text{H}\}$ - ^{15}N NOE

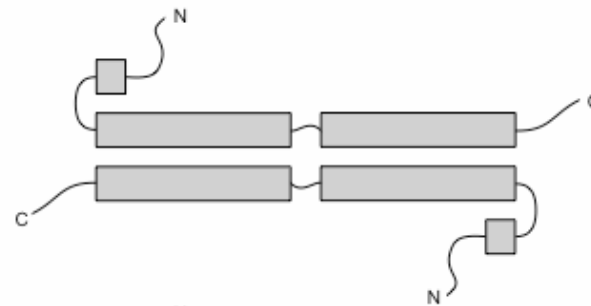
Secondary Structure information from the determined chemical shifts



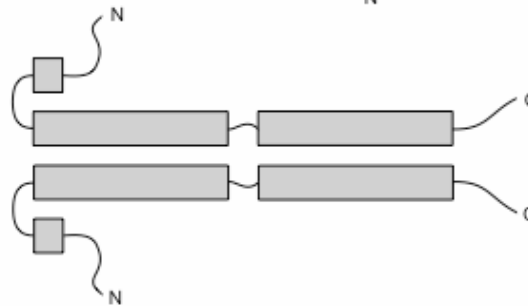
Models for the packing of helices in Hexim1 (255-359)



12.5 kD

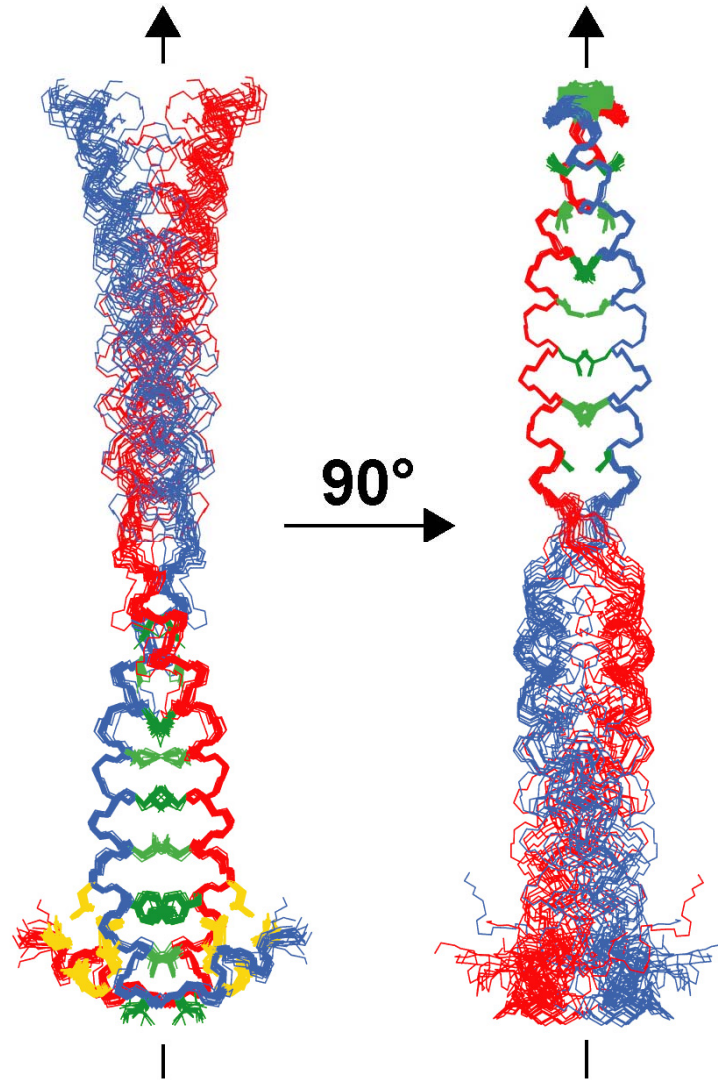


25 kD



Superposition of the 20 lowest energy Hexim1(255-359) NMR structures

Procheck
 most favored 96.3%
 add. allowed 3.3%
 gen. allowed 0.4%
 disallowed 0%



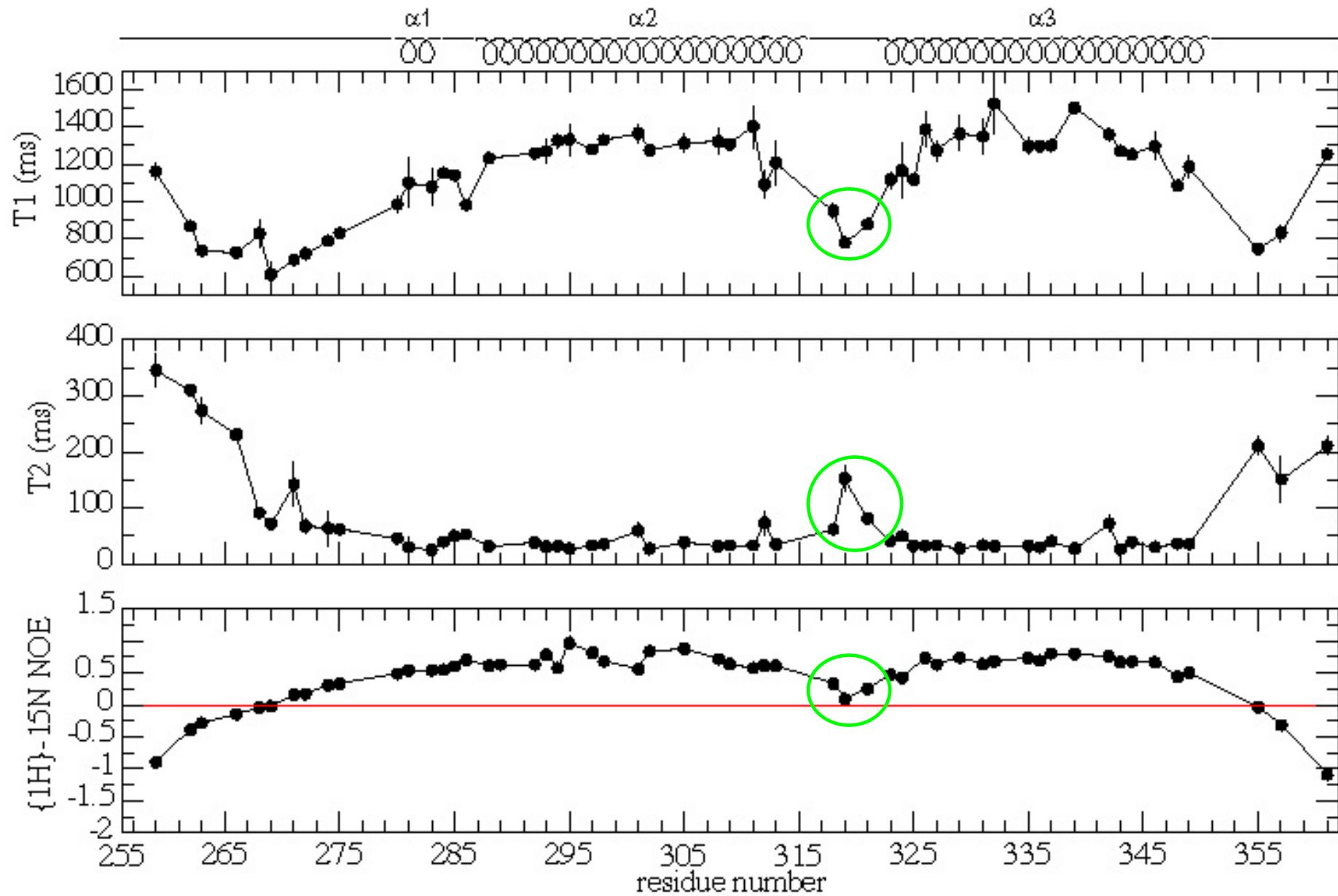
region superimposed
 rmsd bb/heavy (Å)

276-312
 0.38/0.94

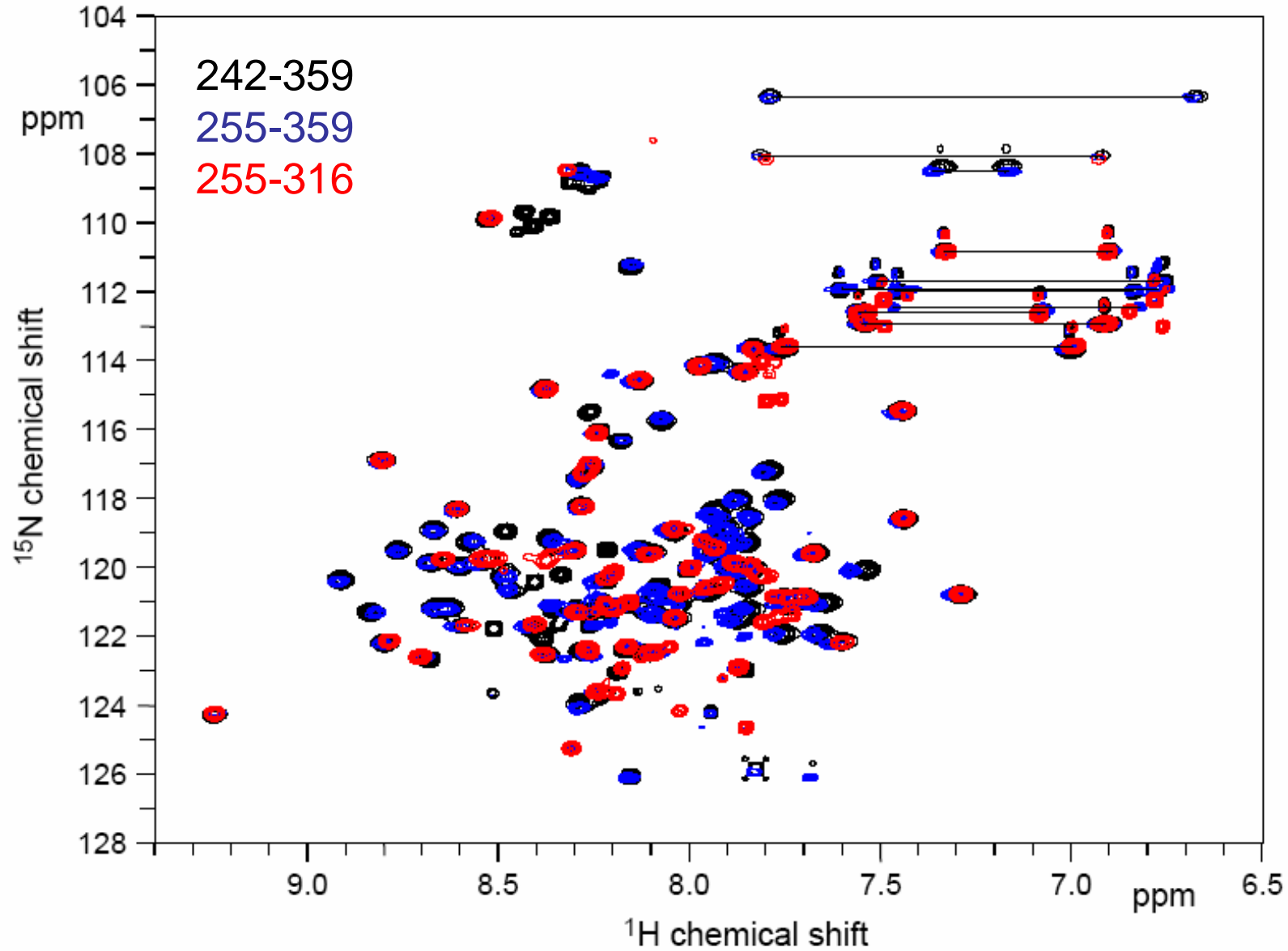
320-347
 0.45/1.1

276-347
 3.3/3.9

^{15}N -relaxation data of Hexim1 (255-359)



Comparison of the ^{15}N -HSQC spectra of Hexim1 255-359 and 255-316

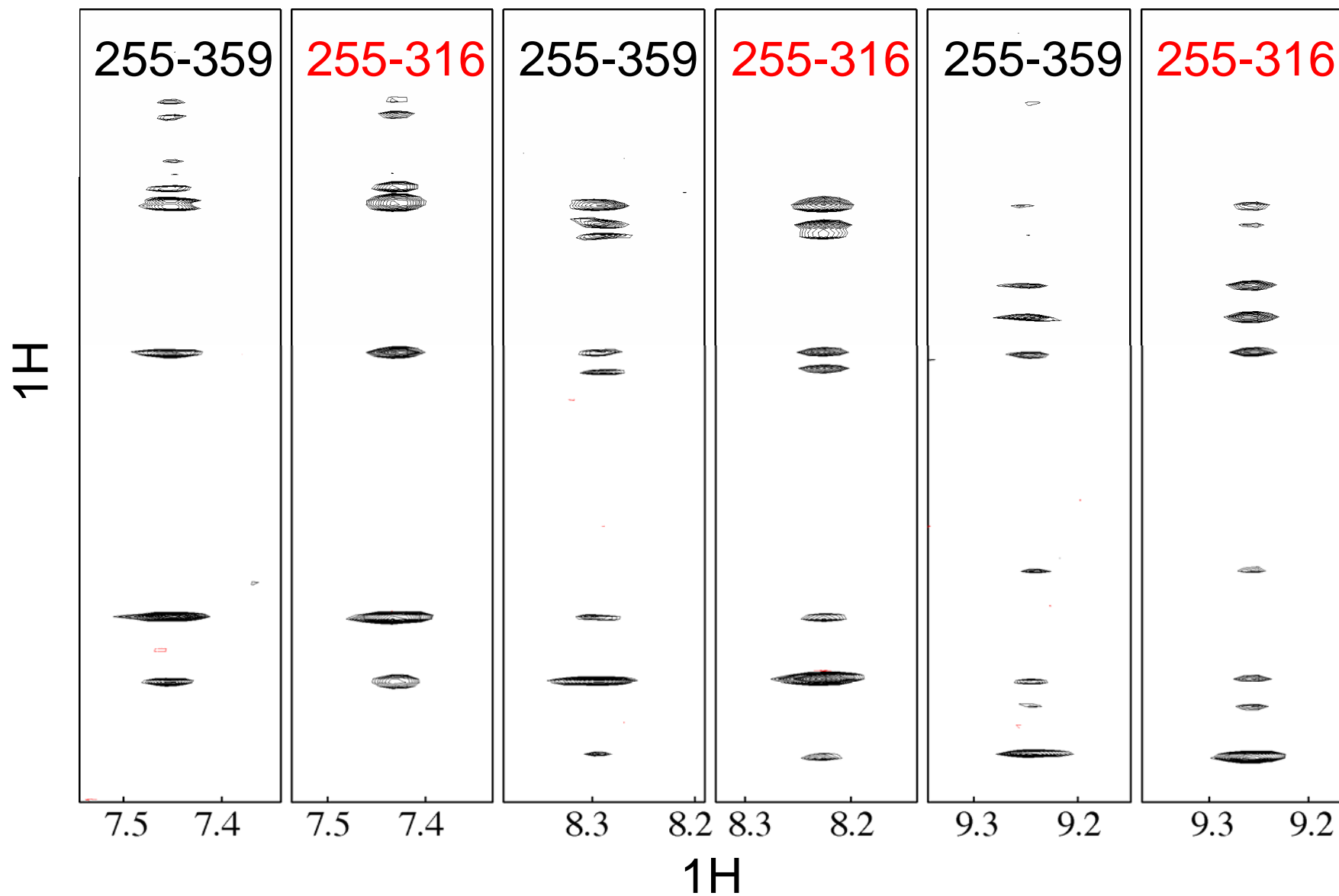


^{15}N -NOESY data of Hexim1 (255-359) & (255-316)

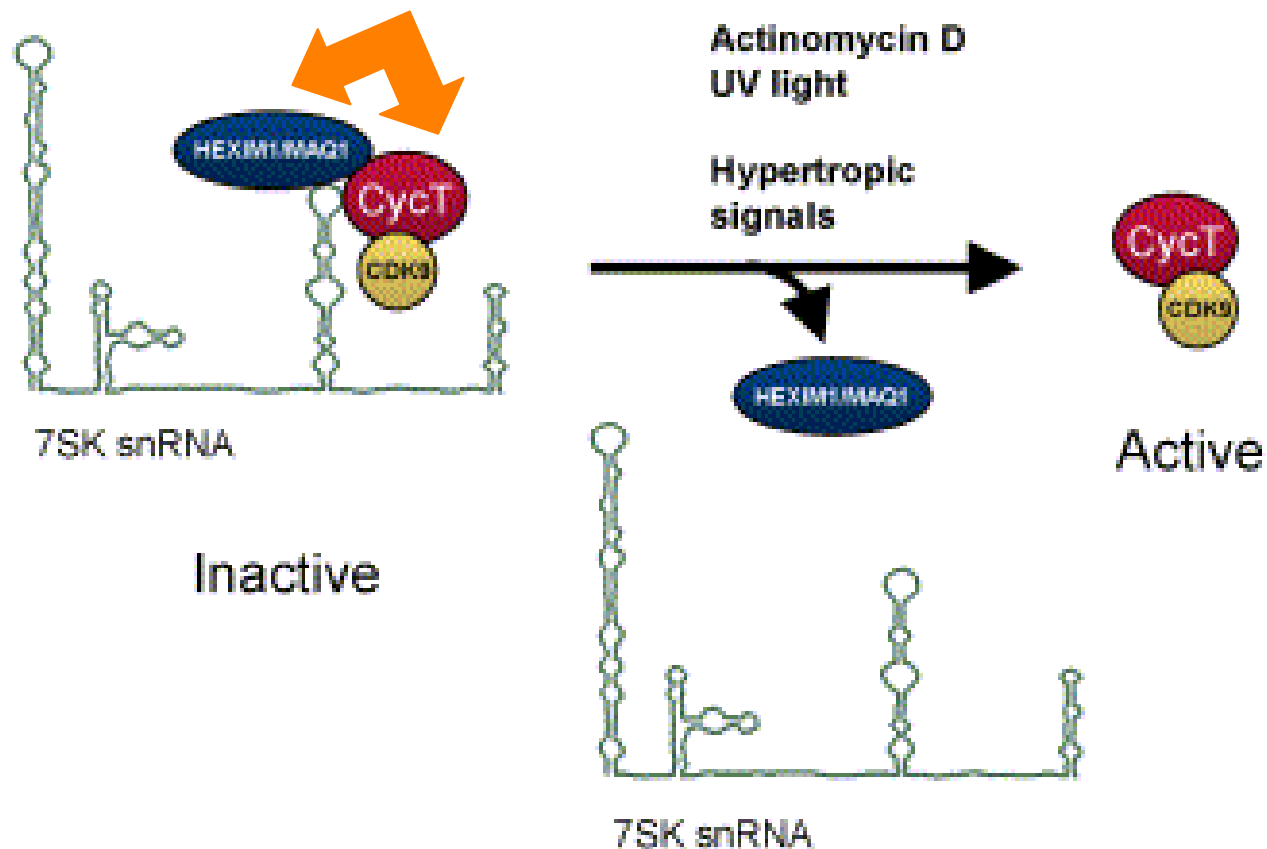
K289

E290

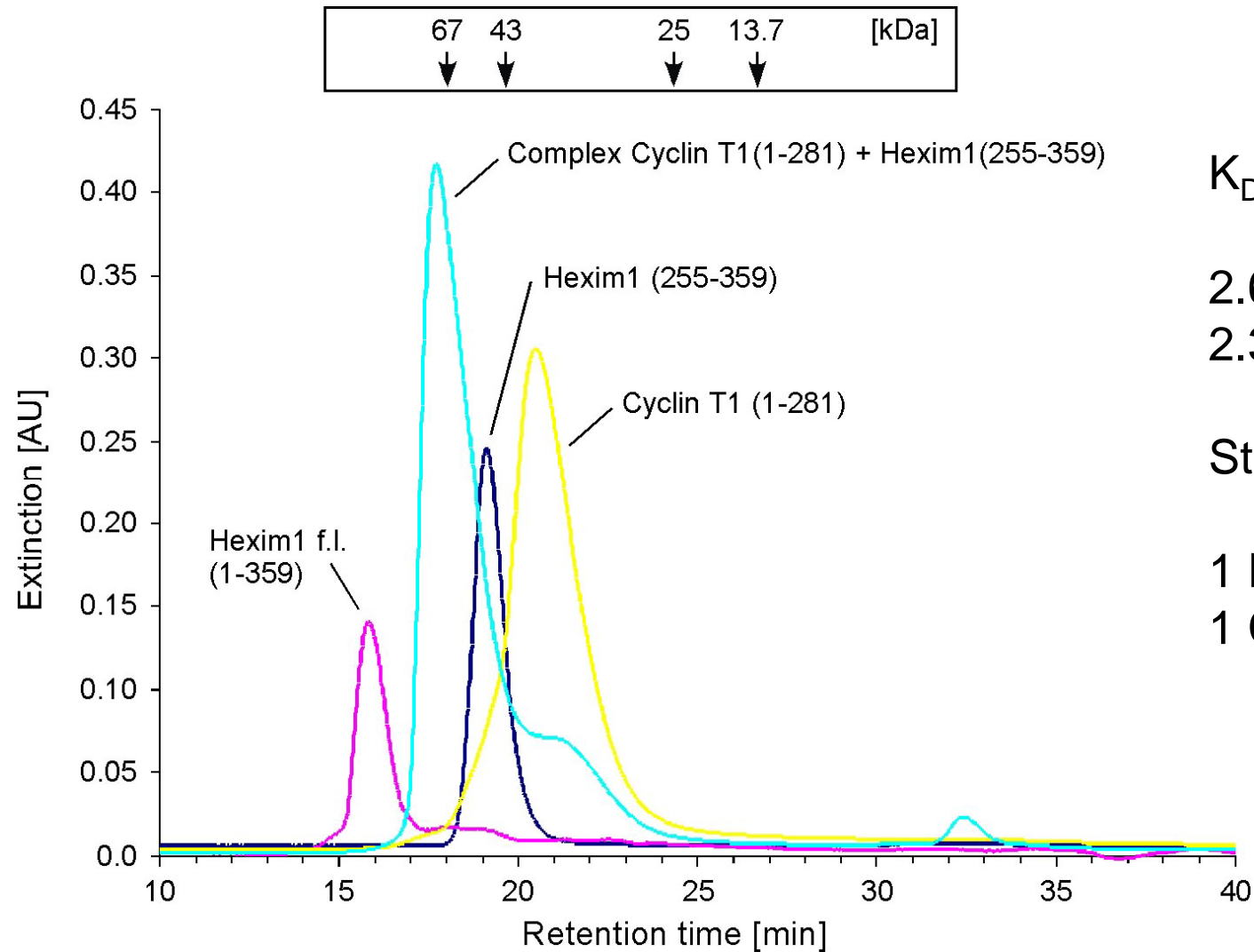
Y291



How does Hexim1 inhibit P-TEFb: analysis of the interaction between Hexim1 with CyclinT1



Gelfiltration experiments on the complex formation between Hexim1 and Cyclin T1



K_D :

2.6 μM (fluo)

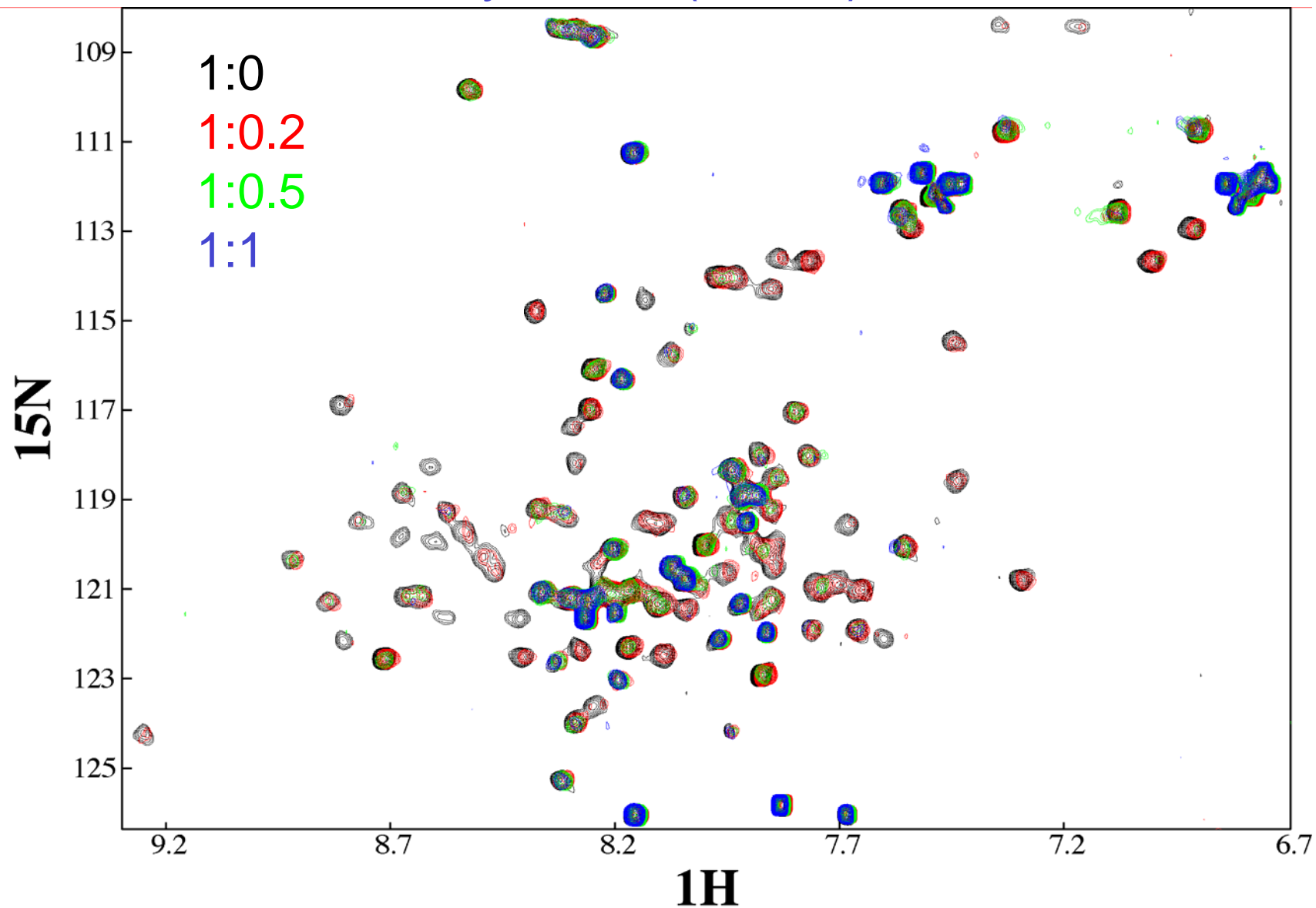
2.3 μM (ITC)

Stoichiometry:

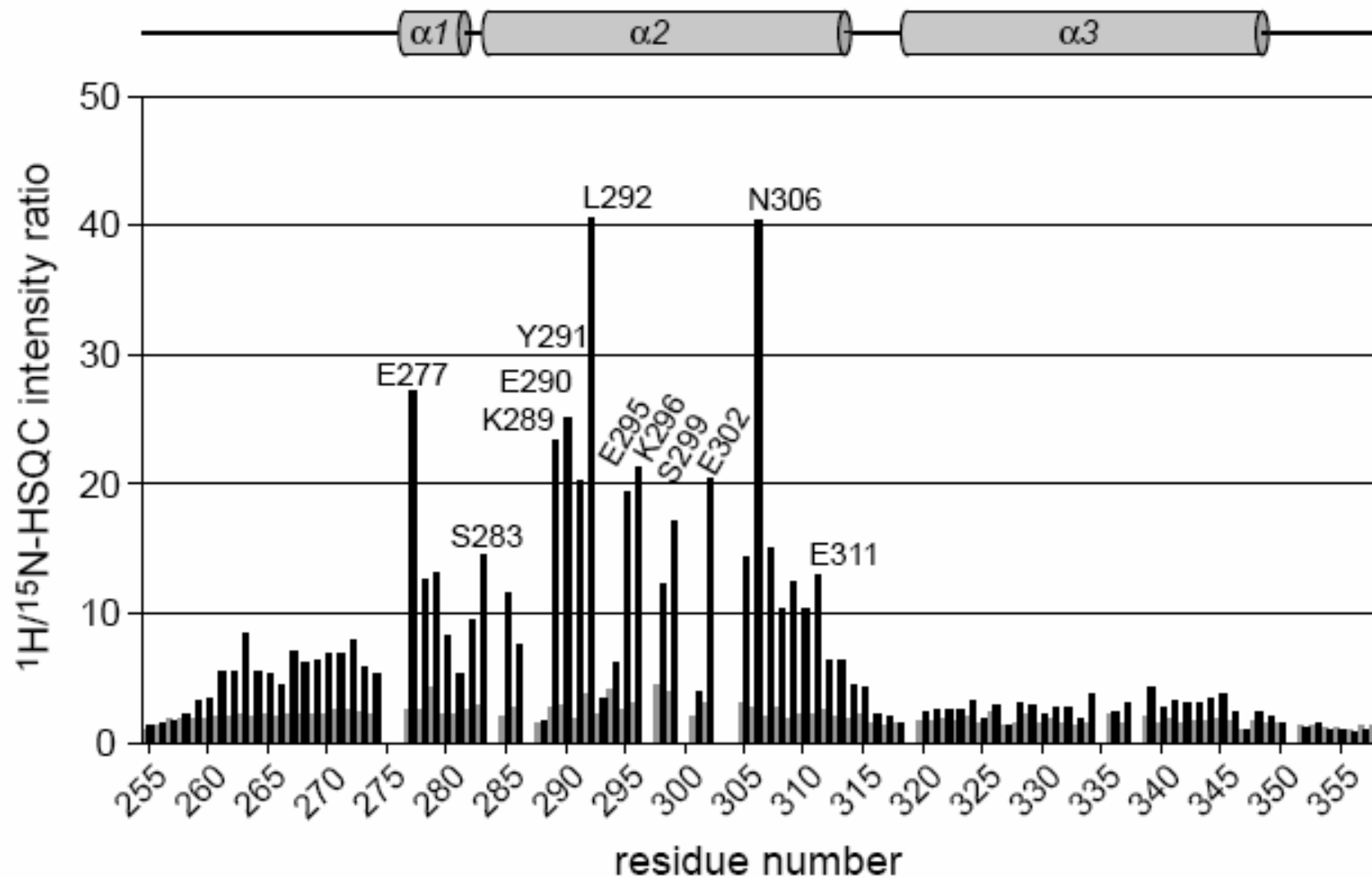
1 Hexim-Dimer :

1 Cyclin T1

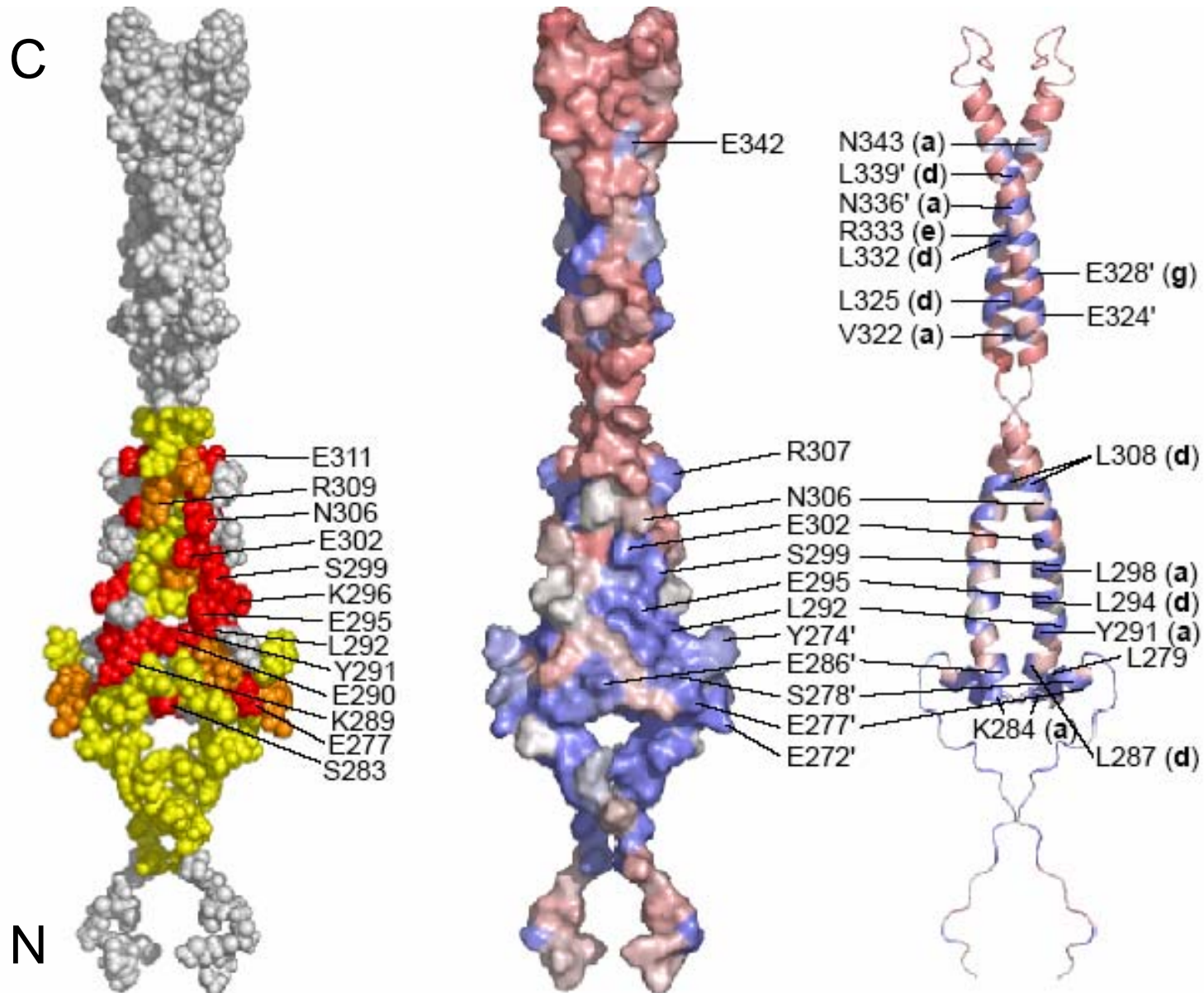
Titration of ^{15}N -Hexim1 (255-359) with unlabelled Cyclin T1(1-281)



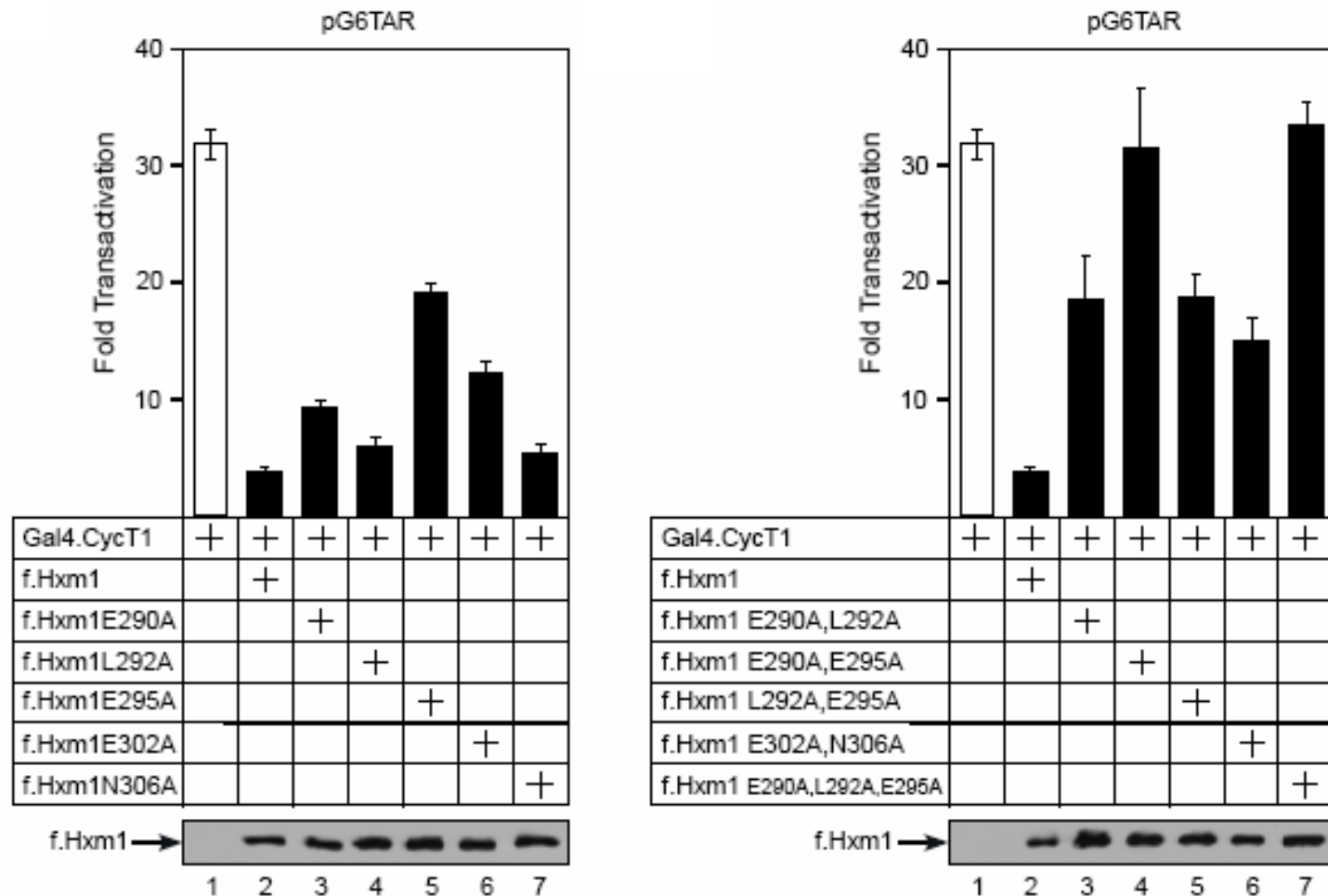
Analysis of the titration of ^{15}N -Hexim1 (255-359) with unlabelled Cyclin T1(1-281)



Mapping of the residues of Hexim1 (255-359) that changed most upon binding of Cyclin T1(1-281)



Functional analysis of mutant Hexim1 (255-359) proteins in cells



Replacement of multiple and mostly negatively charged residues in the CTD of Hexim1 distract its inhibitory function on P-TEFb (= CycT1/Cdk9)

Hexim1 CTD - Conclusions

Hexim1 255-359 is dimeric

Structure:

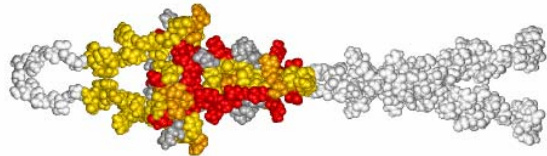
- ✓ 2 consecutive parallel coiled-coil regions and a short N-terminal α -helix that associates with the first
- ✓ Flexible regions: N-terminus, coiled-coil linker, C-terminus
- ✓ The 1st and the 2nd coiled-coil region are structurally independent

Hexim1 CTD - Cyclin T1 - Conclusions

- ✓ Binding to Cyclin T1 involves only the 1st coiled-coil and part of the flexible region N-terminal of it
- ✓ Stoichiometry: 1 Cyclin T1 : 1 Hexim1 dimer
- ✓ The association is largely driven by electrostatic interactions between a negative surface patch on the Hexim1 CTD and a positive region in the cyclin-box of Cyclin T1 (data not shown)

Thanks

Prof. Stephan Grzesiek

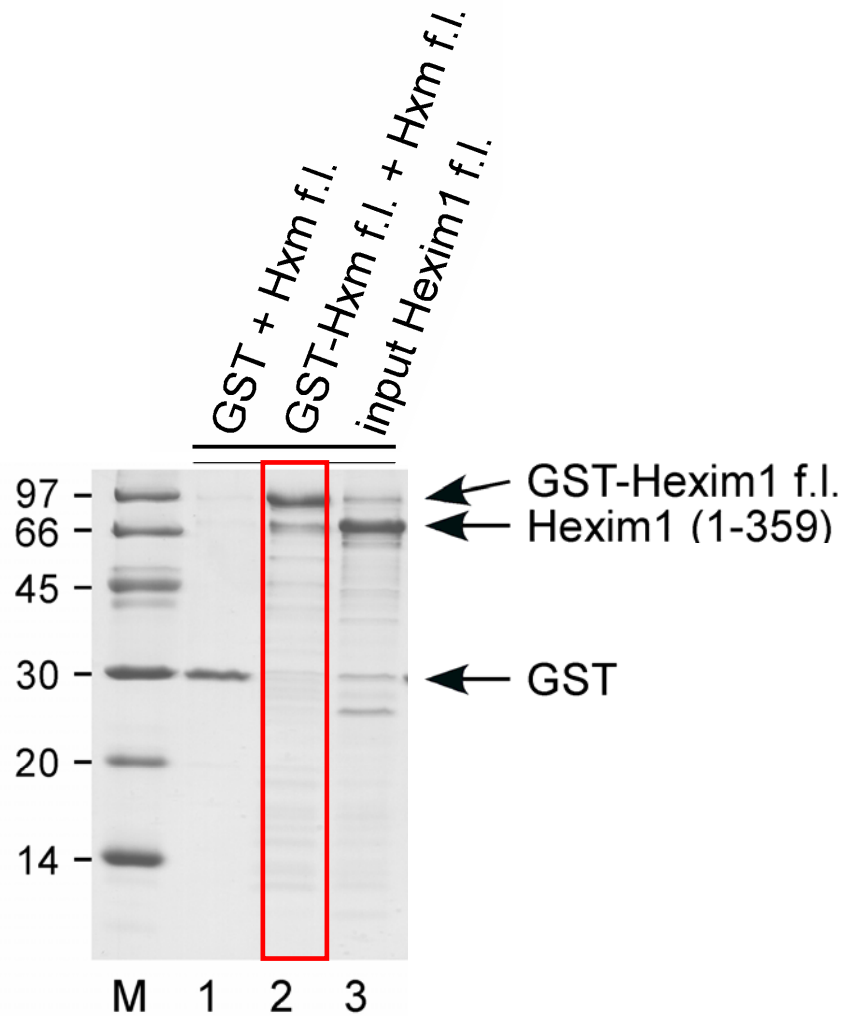


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SNF



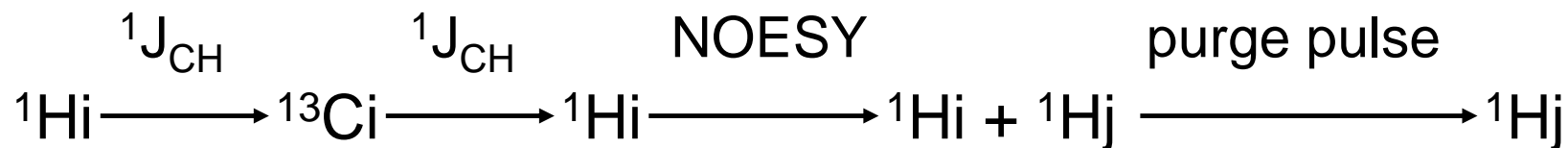
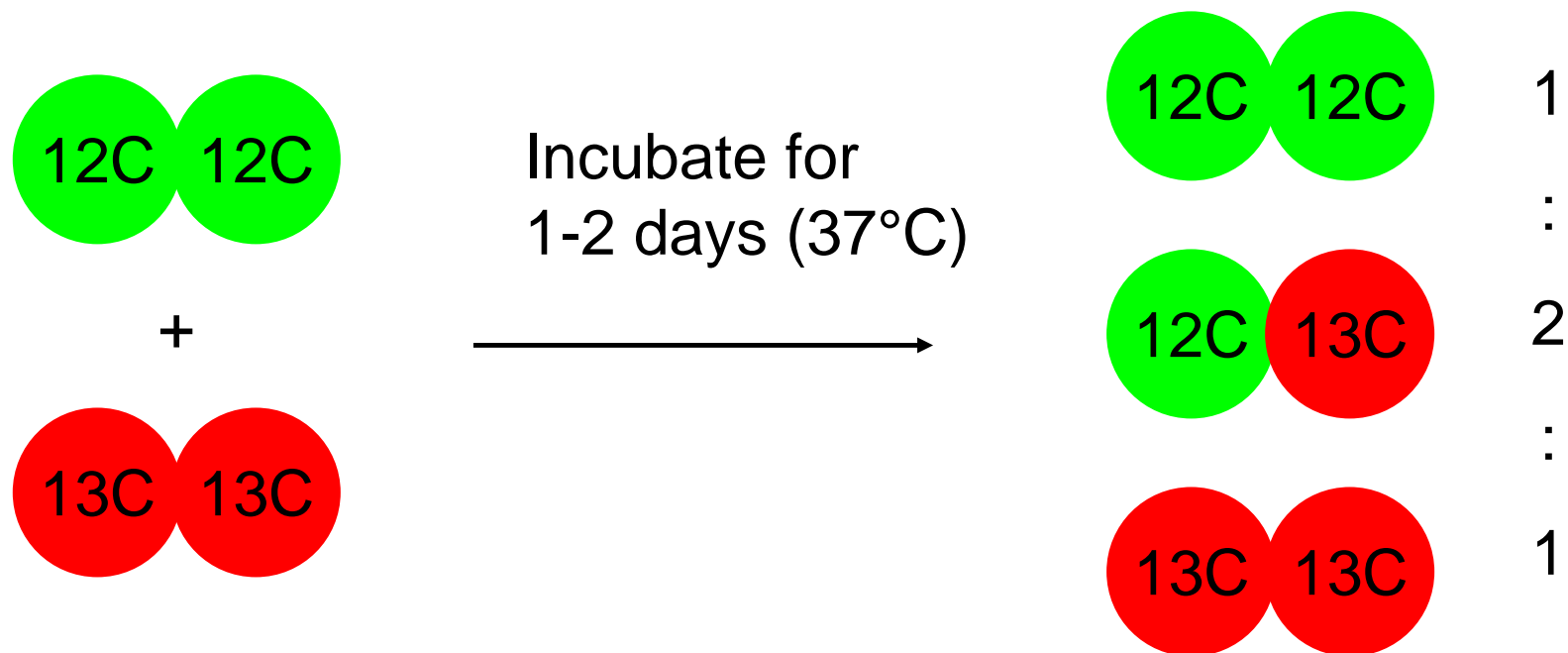
Hexim1 (255-359) forms a homodimer



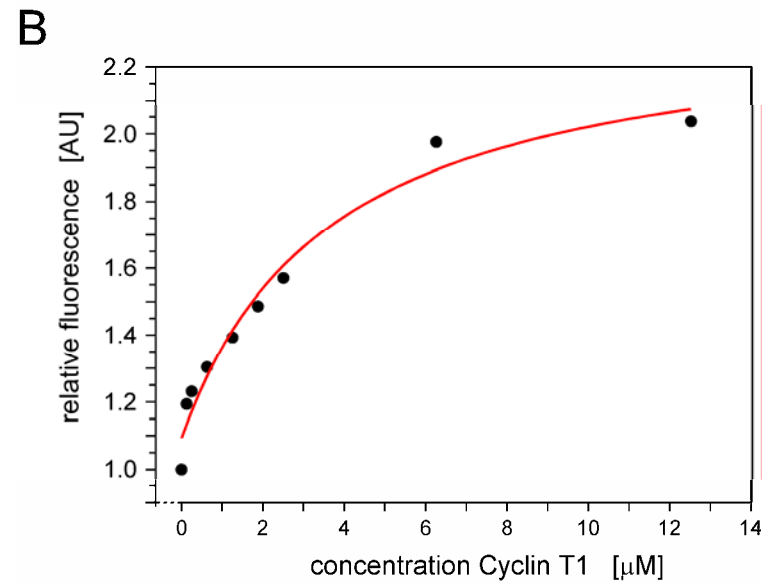
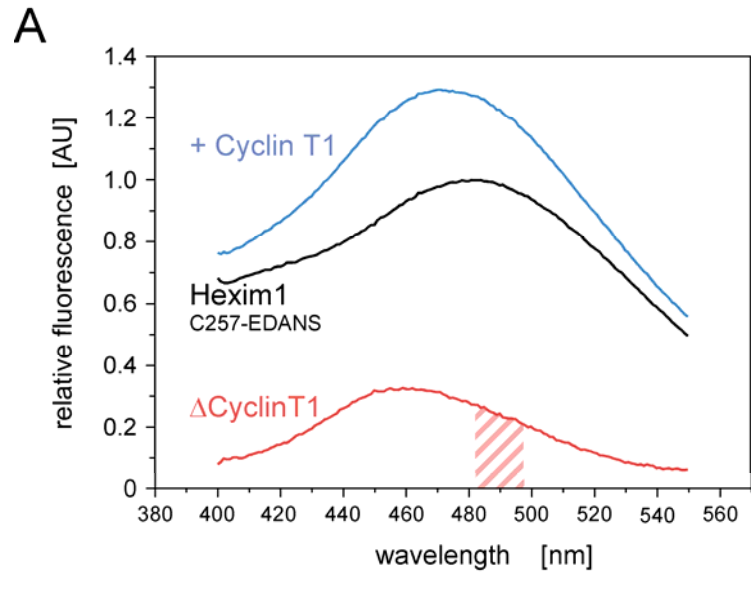
And:

- NMR relaxation data
- Chemical cross-link exp.
- Coexpression of flag-tagged and His-tagged Hexim constructs of different length

Intermolecular Distance Restraints from ^{13}C -filtered NOESY data

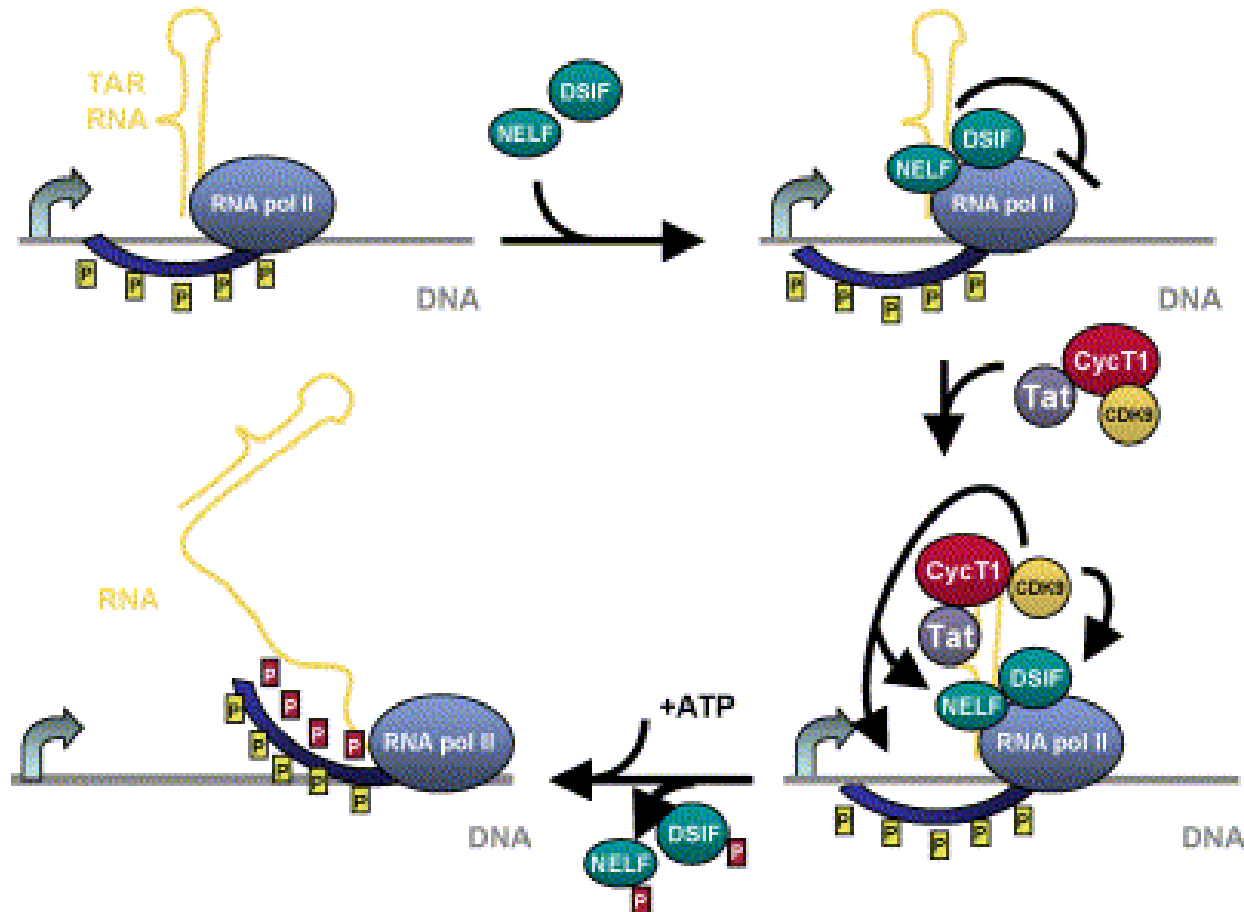


Flourescence data of Hexim1 and Cyclin T1



K_d 2.6 μ M (= k_{off}/k_{on} = 1.47 s^{-1} / 2.59 μ M $^{-1}$ s^{-1})

P-TEFb and HIV



Isothermal titration calorimetry of Hexim1 added to Cyclin T1

Syringe: 0,46 mM Hexim1 255-316
 Cell: 0,041 mM Cyclin T1 1-272
 06.06.05
 Temperature: 15°C

0,43 mM Hexim1 255-359
 0.043 mM Cyclin T1 1-272
 07.06.05
 15°C

Buffer:
 20 mM Hepes pH 7.5
 100 mM KCl
 1 mM MgCl₂

