

PROTEOMICS

Supporting Information for Proteomics

DOI 10.1002/pmic.200700688

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**Interactome and interface protocol (2IP): A novel strategy for high sensitivity
topology mapping of protein complexes**

Supplemental Figure 1

a

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E.coli RNAPβ'   1-928   not shown
T.aqu. RNAPβ'   1-1174 not shown

E.coli RNAPβ'   DLLEEN-SVDAVKVRSVVSCTDFGVCAHCYGRDLARGHIINKGEAIGVIAAQSIGEPGT  928
T.aqu. RNAPβ'   IKAAEAGEVREVPVRSPLTCQTRYGVCQKCYGYDLSMARPVSIGEAVGVVAAESIGEPGT 1234
                *  . * * ** ::*:* :*** :*** **: .: .: ***:**:**:*****

E.coli RNAPβ'   QLTMRTFHIGGAASRAAAESSIQVKNKGSIKLSNVKSVVNSSGKLVITSRNTLKLIDEF  988
T.aqu. RNAPβ'   QLTMRTFHTG----- 1244
                ***** *

E.coli RNAPβ'   GRTKESYKVPYGAVLAKGDGEQVAGGETVANWDPHTMPVITEVSGFVRFTDMIDGQTITR 1048
T.aqu. RNAPβ'   ----- 1244

E.coli RNAPβ'   QTDELTGLSSLVVLDSAERTAGGKDLRPALKIVDAQNDVLI PGTDMPAQYFLPGKAIVQ 1108
T.aqu. RNAPβ'   ----- 1244

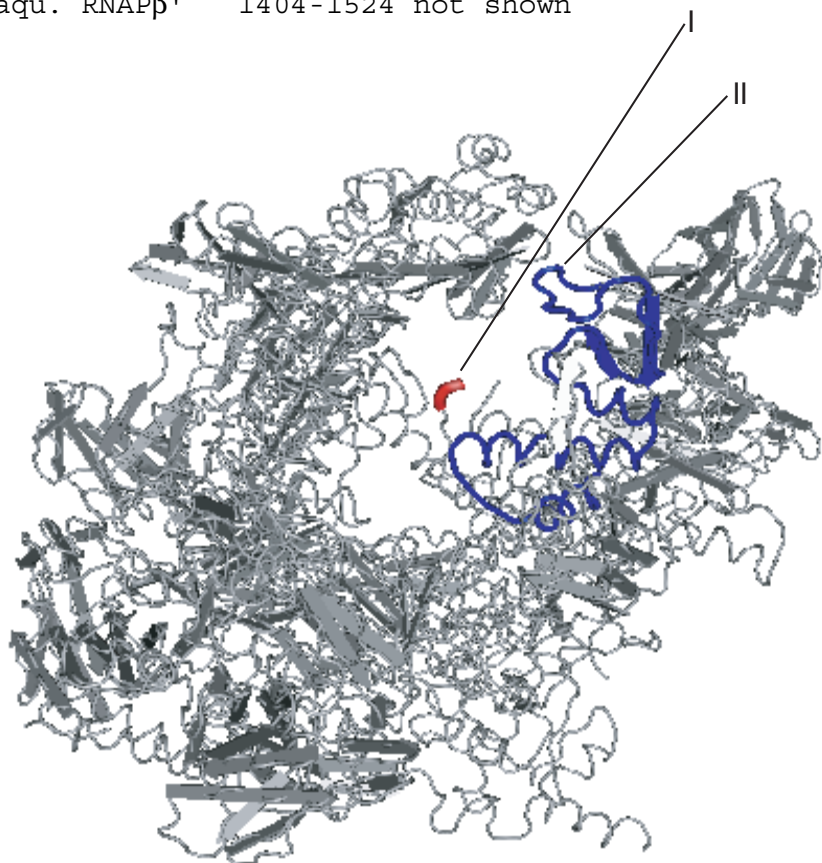
E.coli RNAPβ'   LEDGVQISSGDTLARIPQESGGTKDITGGLPRVADLFEARRPKEPAILAEISGIVSFGKE 1168
T.aqu. RNAPβ'   ---GVAVG-----TDITQGLPRVIELFEARRPKAKAVISEIDGVVRIE-- 1284
                ** :. .*** ***** :***** *::*.**.*:

E.coli RNAPβ'   TKGKRRLVITPVDGSDPYEEMI PKWRQLNVFEGERVERGDVISDGPEAPHDILRLRGVHA 1228
T.aqu. RNAPβ'   -EGEDRLSVFVESEGFSKEYKLPKDARLLVKGDYVEAGQPLTRGAIDPHQLLEAKGPEA 1343
                :*:* ** : . . . * :** :* * :*: ** * : :*. **::*. :* .*

E.coli RNAPβ'   VTRYIVNEVDVYRLOGVKINDKHIEIVRQMLRKATIVNAGSSDFLEGEQVEYSRVKIA 1288
T.aqu. RNAPβ'   VERYLVDEIQKVYRAQGVKLHDKHIEIVRQMLKYVEVTDPGDSRLLEGQVLEKWDVEAL 1403
                * **:*:*.*.*** *****:*****:*****: . :.:*.** :***: :* *:

E.coli RNAPβ'   1289-1407 not shown
T.aqu. RNAPβ'   1404-1524 not shown
    
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b

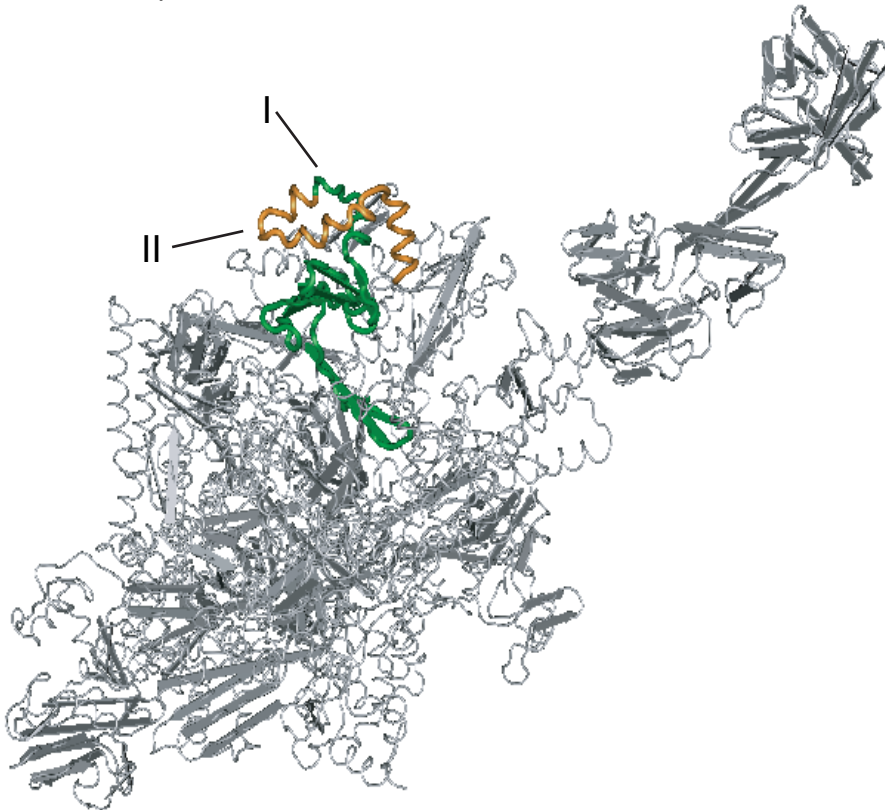


Supplemental Figure 2.

a

E.coli RNAPβ	M VYSYTEKKRIRKDFGKRQVLDVPYLLSIQLDSFQKFIEQDPEGQ----YGLEAAFRSV	56
T.aqu. RNAPβ	----- EIKR----FGRIREVIPLPPLTEIQVESYKKALQADVPPEKRENVGIQAAFKET	51
	* ** ** : * : * * . * : : * : * : * : * : * : *	
E.coli RNAPβ	FPIQSYS---GNSELQYVSYRLGEPVFDVQECQIRGVTYSAPLRVKLRLVIYEREAPGT	113
T.aqu. RNAPβ	FPIEEGDKGKGLVLDLFLEYRIGDPPFSQDECREKDLTYQAPLYARLQLHKD-----	104
	*** : . * . * : : * : * : * : * . : ** : : * : * . * : * : * : *	
E.coli RNAPβ	VKDIKEQEVY M GEIPL M FDNGTFVINGTER VIVS <u>QLHRSPGVFFDS</u> D KGKTHSSGKVLVN	173
T.aqu. RNAPβ	TGLIKEDEVFLGHLPL M TFEDGSFIINGADRVIVSQIHRSPGVYFTDPDPAR---PGRYIAS	161
	. *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *	
E.coli RNAPβ	<u>ARIIPYRGSWLDFF</u> F DPK N LFVRIDRRR KLPATIILRALNYTTEQILD FF EKV I FEIR	233
T.aqu. RNAPβ	IIPLPKRGPWIDLEVEASGV V IMKVNKR-KFPLVLLLRVLGYDQET-----	206
	: * ** . * : * : * : * : * . : : : : * * : * . : * : * . * * *	
E.coli RNAPβ	<u>DNKLO</u> M ELVPERLRGETASFIDIEANGKVYVEKGRRITARHIRQLEKDDVKLIEVPVEYIA	293
T.aqu. RNAPβ	-----	
E.coli RNAPβ	GKVVAKDYIDESTGELICAA N MELSLDLLAKLSOSGHKR IETLFTNDLDHG PY I SETLRV	353
T.aqu. RNAPβ	-----LVRELSAYG-----DLVQGLLDEAVLAM M	229
	* : ** * ** : * . * :	
E.coli RNAPβ	<u>DPTNDRLSALVEIYR</u> M MRPGEPPTREAAESLFENLFFSEDRYDLSAVGR M KFNRSLLREE	413
T.aqu. RNAPβ	RPE---E A M V RLFTLLRPGDPPKKDKALAYLFGLLADPKRYDLGEAGRYKAEEKLG-VG	284
	* . * : * : : * : * : * : * : * : * : * : * : * : * : * : *	
E.coli RNAPβ	IEGSGILSKDD-----IIDV M KKLIDIRNGKG--EVDDIDHLGNRRIRSVGEM A ENQF	464
T.aqu. RNAPβ	LSGRTLVRFEDGEFKDEVFLPTLRYLFALTAGVPGHEVDDIDHLGNRRIRTVGEL M A D QF	344
	: * : : * : : : * : * : * : * : * : * : * : * : * : * : * : *	
E.coli RNAPβ	465-1342 not shown	
T.aqu. RNAPβ	345-1119 not shown	

b



Supplemental Figure 1. Close spatial proximity of RNAP β prime subunit derived CNBr fragments identified from spot B. a) Sequence homology shown by alignment of *E. coli* RNAP β prime subunit (top, aa 929-1288, accession number: P0A8T7) and *Thermus aquaticus* (*T. aqu.*) RNAP β prime subunit (bottom, aa 1175-1403, accession number: Q9KWU6). CNBr cleavage sites on both sequences are highlighted. The two CNBr fragments identified in Spot B above are underlined and indicated with brackets on the side (I, II), while the peptides identified within these fragments by mass spectrometry analyses are indicated in bold italic font. b) Crystal structure of *T. aqu.* RNAP core complex (accession: 1YNN) depicting the intra-molecular interface identified in Spot B above. Regions of *T. aqu.* RNAP β prime subunit that are homologous to the two interacting CNBr fragments of *E. coli* RNAP β prime subunit identified in Spot B are highlighted for clarity (I: red and II: blue). Note that only six amino acid residues at the start of CNBr fragment I show homology between the two aligned sequences, and only two of those six amino acid residues are present in the available crystal structure of *T. aqu.* RNAP core complex.

Supplemental Figure 2. Topology information for RNAP β subunit derived from spot C is consistent with the high-resolution model for the bacterial RNAP core complex. a) Sequence homology depicted by alignment of *E. coli* RNAP β subunit (top, aa 1-464) and *Thermus aquaticus* (*T. aqu.*) RNAP β subunit (bottom, aa 1-344). CNBr cleavage sites on both sequences are highlighted. The two CNBr fragments identified in Spot C above are underlined and indicated with brackets on the side (I, II), while the peptides identified within these fragments by mass spectrometry analyses are indicated in bold italic font. b) Crystal structure of *T. aqu.* RNAP core complex (accession: 1YNN) depicting the intra-molecular interface identified in Spot C above. Regions of *T. aqu.* RNAP β subunit that are homologous to the two interacting CNBr fragments of *E. coli* RNAP β subunit identified in Spot C are highlighted for clarity (I: green and II: orange).

Supplemental Table 1. MALDI analysis of *E. coli* RNAP β' (P0A8T7) CNBr fragments contributing to Spot B on interface map (Fig. 3D)

#	CNBr Fragment ^a	M _r [kDa] Calc.	pI Calc.	[MH] ⁺ Observed	[MH] ⁺ Theoretical	Tryptic peptide sequence ^b	Start ^a	End	Missed	Mod. ^c	QqTOF method	Mascot Score
I	933-1025	9.9	9.6	1172.62	1172.62	RTFHIGGAASR	933	943	1	-	MS	N/A
				1016.54	1016.52	TFHIGGAASR	934	943	1	-	MS	N/A
				1424.71	1424.77	ESYKVPYGAVLAK	993	1005	1	-	MS	N/A
II	1190-1260	8.3	7	1475.72	1475.76	QLNVFEGERVER	1195	1206	1	-	MS/MS	35
				1690.83	1690.84	GDVISDGPEAPHDILR	1207	1222	0	-	MS	N/A
				1008.62	1008.60	LRGVHAVTR	1223	1231	1	-	MS	N/A
				1397.71	1397.70	YIVNEVQDVYR	1232	1242	0	-	MS	N/A
				1335.80	1335.77	INDKHIEVIVR	1248	1258	1	-	MS	N/A
I+II	Calculated total	18.2	9.4									
	Gelspot coordinates	17.5	7.8									

^aResidue number reflects position of N-terminal amino acid residue in open reading frame.

^bPeptide sequences are depicted in one-letter amino acid code. Tryptic cleavages occurred to the right of residues indicated in brackets.

^cModifications considered: Phospho-S, -T, -Y, phosphorylations of serine, threonine and tyrosine, respectively; Met-Ox: oxidation of methionine; carbamyl., carbamylation; 4-VP, 4-vinylpyridine alkylation.

Supplemental Table 2. MALDI analysis of *E. coli* RNAP β (P0A8V2) CNBr fragments contributing to Spot C on interface map (**Fig. 3D**)

#	CNBr Fragment ^a	M _r [kDa] Calc.	pI Calc.	[MH] ⁺ Observed	[MH] ⁺ Theoretical	Tryptic peptide sequence ^b	Start ^a	End	Missed	Mod. ^c	QqTOF method	Mascot Score
I	131-238	12.8	9.4	951.57	951.57	VIVSQLHR	144	151	0	-	MS	N/A
				1283.63	1283.63	SPGVFFDSDKGK	152	163	1	-	MS	N/A
				1983.02	1983.00	IIPYRGSWLDFFEDPK	176	191	1	-	MS	N/A
				1340.64	1340.61	GSWLDFFEDPK	181	191	0	-	MS	N/A
				1147.65	1147.62	DNLFVRIDR	192	200	1	-	MS	N/A
				1024.68	1024.69	KLPATIILR	203	211	1	-	MS	N/A
				1945.02	1945.00	ALNYTTEQILDFFEK	212	227	0	-	MS	N/A
				1133.66	1133.63	VIFEIRDNK	228	236	1	-	MS	N/A
II	316-369	6.2	5.1	2334.16	2334.16	IETLFTNDLDHGPIYSETLR	333	352	0	-	MS/MS	44
				3131.51	3131.52	IETLFTNDLDHGPIYSETLRVDPTNDR	333	359	1	-	MS	N/A
				1861.01	1860.98	VDPTNDRLSALVEIYR	353	368	1	-	MS	N/A
				1063.63	1063.62	LSALVEIYR	360	368	0	-	MS	N/A
I+II	Calculated total	19.0	6.9									
	Gelspot coordinates	15.5	7.2									

^aResidue number reflects position of N-terminal amino acid in open reading frame.

^bPeptide sequences are depicted in one-letter amino acid code. Tryptic cleavages occurred to the right of residues indicated in brackets.

^cModifications considered: Phospho-S, -T, -Y, phosphorylations of serine, threonine and tyrosine, respectively; Met-Ox: oxidation of methionine; carbamyl., carbamylation; 4-VP, 4-vinylpyridine alkylation.