

Supplementary information: *Quantitative influence of macromolecular crowding on gene regulation kinetics*

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1 Macromolecular crowding on DNA

The concentration of NAPs per cell has been measured [1] in Luria-Bertani broth after 2.3h (it corresponds to the doubling time ~ 20 min [2] and $G = 3.8$ genome equivalents per cell [3]). Here we assumed, that DNA-binding proteins cover DNA on the length equal 2 times their hydrodynamic radius. Lrp forms an open ring structure, in which the DNA is wrapped around the octamer in a nucleosome-like structure [4]. Thus, we calculate that length $2\pi R_h$ of DNA is occupied by Lrp oligomers (similar mode is assumed for DPS). Oligomerisation levels of most NAPs is from [5]. The abundance of RNA polymerase holoenzyme and core complexes are taken from work by Bremer *et al.* [6] for *E. coli* growth rate 2.5 doublings per hour. The number of TFs are calculated from [7] and recalculated for expected number of TFs [8] (we assumed typical values of TF sizes). The number of MukB monomers per cell has been measured to be 400 ± 100 [9] for fast growing cells. Similar value (438 ± 180) has been recently obtained from single-molecule studies [10] but it appeared that only 20% are accumulated in discrete chromosome locations [10, 11]. Mean number of MukB monomers per cell participating in DNA compaction is 82 molecules. The number of cross-links is equal to $M_l = 2\text{MukB} - 1 + \text{SeqA} + \text{MatP}$. The number of cross-links for MukB comes from the following observation. In the presence of ATP, the globular domains of the homodimer associate with each other resulting in tight intramolecular compaction of two DNA chains. MukB dimers can oligomerise forming rosette-like or globular structures [12]. In this way the number of cross-links formed by MukB dimers is $2\text{MukB} - 1$. Recently, it has been recognized that non-classical NAPS such as SeqA, MatP, and SImA proteins play role in the organizing the chromosome [13]. The concentrations of all ncNAPs are unknown. The number of cross-links formed by the ncNAPs are anticipated from genome conformation capture experiment [14] in the exponentially growing cells. The high clustering between binding sites was attributed to single interaction between two MatP sites and 51 SeqA interaction between sites. Several SeqA loci were involved in interactions with more than single SeqA loci and it is impossible to determine from experiment whether these multiple interactions appears at the same time. The number of interactions between two loci is 34. No clustering was observed for SImA binding sites. For the proteins that forms cross-links we assume that they cover approximately 20 bp. MukB, SeqA, and MatP are elongated proteins of length below Kuhn length of DNA [15, 16, 17].

The volume of the cell is taken from [18] ($V_c = 4 \mu\text{m}^3$). The average number of LacI operators is $\langle O \rangle = 2^{(C(1-x)+T)/\tau}$ [3], where C is the time required to complete replication (42 min), T is time between completion of replication and division (23 min), τ is the doubling time (24 min), and x is the fractional position of LacI operator between the OriC site and terminus ($x=0.47$). Thus we get, $\langle O \rangle = 3.7$.

2 Calculation of LacI dimer sliding length

We use Monte Carlo simulations to determine the sliding length in the presence of NAPs from the study by Hammar *et al.* [19] on the *in vivo* kinetics of association of *lac* repressor. Hammar *et al.* measured rate constants of LacI binding to two identical operator sites positioned at center-to-center distances 25, 45, 65, 115, 203 bp. They demonstrated correlations for binding of LacI to two operators located at distances smaller than 65 bp. The fit of the experimental data to the model in the absence of NAPs gave value of the sliding length $s_L = \sqrt{2D_1/\Lambda} = 36 \pm 6$ bp. Here we determine the sliding length in the presence of NAPs.

The scheme of the algorithm is shown in Fig. S2. Following work by Berg *et al.* [20] we assume that TF after macroscopic dissociation can rebind to any location on the DNA with the same probability. The availability of the new nonspecific binding site is determined by the arrangement of NAPs in its vicinity. When the site is

obstructed by NAPs the TF can not bind and dissociates macroscopically. In the other case it binds nonspecifically and searches for operator by the sliding. Nonspecifically bound TF to DNA performs one of two exclusive steps: diffusive transition in either direction to the nearest base pair (sliding) or dissociation from DNA surface. The probability of the former is given by $P = 2D_1/(\Lambda + 2D) = 2s^2/(1 + 2s^2)$ and the latter $P_d = 1/(1 + s^2)$, where $s^2 = D_1/\Lambda$. Reflective boundary conditions are considered when TF encounters NAP during sliding.

The sliding length is calculated from the ratio of the mean number T_0 of macroscopic dissociations required for localisation of the single operator to the mean number of T dissociations needed to find one of two operators because it is equal to the ratio of experimentally measured association rates $k/k_0 = T_0/T$. The results of the simulations give the best fit to experimental data for $s^2 = 1000$. All simulations were performed using following parameter values: length of DNA $N = 20$ kbp with operator/operators placed at positions O_1 and O_2 , number of NAPs $M = 158$ (vacancy $f = 0.85$). 10^5 simulation realizations were performed to calculate mean number of macroscopic tries that lead to specific binding.

The full event-driven algorithm consists of the following steps:

1. *Initialise*: set s^2 , N , $T = 0$, M , O_1 , O_2
2. *Arrange NAPs*: for $i = 1$ to M :
 - (a) generate a random number $\rho \in (1, N)$:
 - (b) if any site from $[\rho, \rho + d]$ has been already occupied, go to step 2. (a)
 - (c) if all sites from $[\rho, \rho + d]$ are free, save new position of NAP
3. *Association of TF*: generate a random number $\rho \in (1, N)$
 - (a) if any site from $[\rho, \rho + r]$ is occupied by NAP, $T = T + 1$, go to step 2
 - (b) if all sites from $[\rho, \rho + r]$ are free, save current position of TF, POS= ρ
4. *Sliding*: if POS is equal to O_1 or O_2 , write T , exit; else draw a random number x from the uniform distribution in the unit interval, according to x choose one of three events:
 - (a) if POS+1 is free from NAP change the position to POS+1, else to POS-1; (with probability $P_+ = s^2/(1 + 2s^2)$), go to step 4
 - (b) if POS-1 is free from NAP change the position to POS-1, else to POS+1; (with probability $P_- = s^2/(1 + 2s^2)$), go to step 4
 - (c) *dissociation* (with probability $P_d = 1/(1 + 2s^2)$), $T = T + 1$, go to step 2

Table S1: *E. coli* nucleoid-associated proteins present in the early exponential phase of growth. NAP function: b - bending, l - linking of DNA chains, o - other. ncNAPs - non-classical NAPs.

Protein	oligomerisation	function	level (copies/genome)	Mw [kDa]	r_p [nm]	DNA covered (bp $\times 10^5$)
Rob	monomer	b	2650	33.145	3.04	0.474
Fis	dimer	b	7910	22.480	2.61	1.214
HU	dimer	b	7910	18.761	2.43	1.131
IHF	dimer	b	7250	22.005	2.59	1.104
Lrp	octamer[4]	b	82	151.096	5.52	0.084
H-NS	dimer	o	2650	30.694	2.95	0.460
StpA	dimer	o	3270	30.694	2.95	0.567
Dps	dodecamer[21]	b	133	224.340	6.43	0.158
DnaA	monomer	o	474	52.551	3.64	0.101
IciA	dimer	o	104	66.944	4.01	0.024
MukB	dimer [22]	l	11	-	-	0.005
ncNAPs		l	35-52			0.014-0.021
RNAP	holo		290	459	8.53	0.146
	core		2440	389	7.91	1.135
TF			1400	73	4.49	0.370

Table S2: Specification of *E. coli* transcription factors. List of TF is from *E. coli* EcoCyc [23] and RegulonDB [24] databases. Subscripts denote oligomerisation level of TFs. TFs are grouped according to families they belong to. Molecular weight (Mw, EcoCyc database) of TF is for its oligomeric form. D_3 , D_1 , and TF search time for single specific-site are calculated in this work. References correspond to works in which oligomerisation level of TF was determined or anticipated. TF with no information about oligomerisation level is assumed to oligomerise in a way that is the most common in its family.

TF	Mw (kDa)	TF family	D_3 ($\mu\text{m}^2/\text{s}$)	D_1 ($\mu\text{m}^2/\text{s}$)	Search time V_n/k (s)	Reference
MarA	15.184	AraC/XylS ¹	16.4	0.33	167	[25]
SoxS	12.911	AraC/XylS	18.7	0.40	157	[26]
Rob	33.145	AraC/XylS	8.2	0.11	240	[27]
AraC ₂	66.768	AraC/XylS	4.2	0.041	366	[28]
RhaS ₂	64.63	AraC/XylS	4.3	0.043	358	[29]
RhaR ₂	71.334	AraC/XylS	3.9	0.037	383	[30]
CaiF ₂	30.872	AraC/XylS	8.8	0.12	231	[31]
MelR ₂	69.856	AraC/XylS	4.0	0.038	378	[32]
XylR ₂	89.738	AraC/XylS	3.1	0.026	453	[33]
ChbR ₂	65.936	AraC/XylS	4.2	0.042	363	[34]
FeaR ₂	69.24	AraC/XylS	4.0	0.039	376	
YdeO ₂	57.45	AraC/XylS	4.8	0.051	331	
YqhC ₂	71.914	AraC/XylS	3.9	0.036	386	
YgiV ₂	35.654	AraC/XylS	7.7	0.10	249	
AdiY ₂	58.014	AraC/XylS	4.8	0.050	334	
GadW ₂	56.056	AraC/XylS	5.0	0.053	326	[35]
GadX ₂	63.124	AraC/XylS	4.4	0.044	353	[35]
GadW-GadX	59.59	AraC/XylS	4.7	0.048	339	[35]
AppY ₂	57.526	AraC/XylS	4.8	0.051	332	
EnvY ₂	58.038	AraC/XylS	4.8	0.050	334	
ZntR ₂	32.358	MerR ²	8.4	0.12	237	[36]
MlrA ₂	55.092	MerR	5.1	0.054	323	[37]
CueR ₂	30.47	MerR	8.9	0.13	229	[38]
SoxR ₂	34.3	MerR	7.9	0.11	244	[39]
YcgE ₂	56.528	MerR	4.9	0.052	328	
UidR ₂	43.598	TetR ³	6.3	0.077	279	[40]
NemR ₂	44.55	TetR	6.2	0.074	283	[41]
FabR ₂	53.106	TetR	5.2	0.057	315	[42]
AcrR ₂	49.534	TetR	5.6	0.064	302	[43]
BetI ₂	43.63	TetR	6.3	0.076	280	[44]
RutR ₂	47.376	TetR	5.9	0.068	294	[45]
EnvR ₂	50.396	TetR	5.5	0.062	305	
PaaX ₂	70.59	GntR ⁴	3.9	0.038	381	[46]
MngR ₂	56.546	GntR	4.9	0.052	328	
GlcC ₂	57.652	GntR	4.8	0.051	332	
McbR ₂	50.302	GntR	5.5	0.062	305	[47]
PdhR ₂	58.85	GntR	4.7	0.049	337	[48]
NanR ₂	59.048	GntR	4.7	0.049	337	[49]
CsiR ₂	49.982	GntR	5.6	0.063	303	

TF	M_w (kDa)	TF family	D_3 ($\mu\text{m}^2/\text{s}$)	D_1 ($\mu\text{m}^2/\text{s}$)	Search time V_n/k (s)	Reference
FadR ₂	53.938	GntR	5.2	0.056	318	[50]
LldR ₂	58.332	GntR	4.8	0.050	335	[48]
UxuR ₂	58.616	GntR	4.8	0.050	336	
ExuR ₂	59.672	GntR	4.7	0.048	340	
FNR ₂	55.934	CRP/FNR	5.0	0.053	326	[51]
CRP ₂	47.28	CRP/FNR	5.9	0.068	293	[52]
YeiL ₂	50.588	CRP/FNR	5.5	0.062	306	[53]
MarR ₂	32.13	MarR	8.4	0.12	236	
MprA ₂	41.126	MarR	6.7	0.083	270	[54]
SlyA ₂	32.706	MarR	8.3	0.12	238	[55]
Fur ₂	33.590	Fur	8.1	0.11	241	
Zur ₂	38.508	Fur	7.1	0.091	260	[56]
OmpR ₂	54.708	OmpR	5.1	0.055	321	[57, 58]
KdpE ₂	50.724	OmpR	5.5	0.061	306	[59]
TorR-TorI	33.911	OmpR	8.0	0.11	243	[60]
TorR ₂	52.466	OmpR	5.3	0.058	313	[59]
ArcA ₂	54.584	OmpR	5.1	0.055	321	[61]
BaeR ₂	55.312	OmpR	5.0	0.054	323	
CpxR ₂	52.624	OmpR	5.3	0.058	313	[62]
CreB ₂	52.250	OmpR	5.3	0.059	312	
PhoB ₂	52.866	OmpR	5.3	0.058	314	[63, 64, 65]
PhoP ₂	51.070	OmpR	5.4	0.061	308	[66]
RstA ₂	54.096	OmpR	5.1	0.056	319	
ArgP ₂	66.944	LysR	4.2	0.041	367	
DsdC ₂	70.664	LysR	3.9	0.037	381	
MetR ₂	71.258	LysR	3.9	0.037	383	[67]
ModE ₂	56.562	LysR	4.9	0.052	328	[68]
IlvY ₂	66.408	LysR	4.2	0.041	365	[25]
Dan ₂	70.630	LysR	3.9	0.037	381	
BirA ₂	70.624	LysR	3.9	0.037	381	[69]
CynR ₂	65.922	LysR	4.2	0.042	363	[70]
HcaR ₂	65.676	LysR	4.2	0.042	362	
AllS ₂	69.024	LysR	4.0	0.039	375	
NhaR ₂	68.568	LysR	4.1	0.039	373	
Cbl ₂	71.712	LysR	3.9	0.037	385	[71, 72]
Nac ₂	65.67	LysR	4.2	0.042	362	[73]
HdfR ₂	63.492	LysR	4.4	0.044	354	
GadE ₂	41.198	LysR	6.7	0.083	270	
LrhA ₂	69.188	LysR	4.0	0.039	375	
TdcA ₂	69.078	LysR	4.0	0.039	375	
GcvA ₂	68.804	LysR	4.1	0.039	374	[74]
GcvA-GcvR	55.171	LysR	5.0	0.054	323	[75]
LysR ₂	68.73	LysR	4.1	0.039	374	
LeuO ₂	71.39	LysR	3.9	0.037	384	
XapR ₂	67.254	LysR	4.1	0.040	368	

TF	M_w (kDa)	TF family	D_3 ($\mu\text{m}^2/\text{s}$)	D_1 ($\mu\text{m}^2/\text{s}$)	Search time V_n/k (s)	Reference
CysB ₄	144.6	LysR	1.9	0.012	669	[76, 77]
OxyR ₄	137.104	LysR	2.0	0.013	639	[78, 79]
CytR ₂	75.64	LacI/GalR ⁵	3.7	0.034	400	[80]
TreR ₂	69.062	LacI/GalR	4.0	0.039	375	[81]
PurR ₂	76.35	LacI/GalR	3.6	0.033	402	[82]
IdnR ₂	75.334	LacI/GalR	3.7	0.034	398	[83]
GalR ₂	74.188	LacI/GalR	3.8	0.035	394	[84]
GalS ₂	74.714	LacI/GalR	3.7	0.034	396	[85]
AscG ₂	73.888	LacI/GalR	3.8	0.035	393	
Mall ₂	73.25	LacI/GalR	3.8	0.035	391	
EbgR ₂	72.42	LacI/GalR	3.8	0.036	387	
RbsR ₂	73.224	LacI/GalR	3.8	0.035	390	
GntR ₂	72.884	LacI/GalR	3.8	0.036	389	
FruR ₄	151.996	LacI/GalR	1.8	0.011	700	[86]
LacI ₄	154.36	LacI/GalR	1.7	0.011	709	[87]
RcsB-GadE	44.27	LuxR/UhpA	6.2	0.075	282	[88]
RcsB ₂	47.342	LuxR/UhpA	5.9	0.068	294	[88]
BglJ-RcsB	47.156	LuxR/UhpA	5.9	0.068	293	[89]
RcsBRcsA	47.187	LuxR/UhpA	5.9	0.068	293	[88, 90]
EvgA ₂	45.38	LuxR/UhpA	6.1	0.072	286	
NarL ₂	47.854	LuxR/UhpA	5.8	0.067	295	[91, 92]
NarP ₂	47.150	LuxR/UhpA	5.9	0.068	293	[91, 92]
CsgD ₂	49.87	LuxR/UhpA	5.6	0.063	303	[93]
SdiA ₂	56.234	LuxR/UhpA	5.0	0.053	327	[94]
UhpA ₂	41.779	LuxR/UhpA	6.6	0.081	273	[95]
YoeB-YefM ₂	28.832	YefM	9.3	0.14	223	[96]
YefM ₂	18.616	YefM	13.7	0.25	182	[96]
RelE-RelB ₂	29.367	RelB/DinJ	9.2	0.13	225	[97]
RelB ₂	18.142	RelB/DinJ	14.1	0.26	180	[97]
HipB ₂ -HipA ₂	118.584	Cro/C1	2.3	0.017	565	[98]
HipB ₂	20.032	Cro/C1	12.9	0.23	188	[98]
MqsA ₂ MqsR ₂	51.87	Cro/C1	5.4	0.059	311	[99]
MqsA ₂	29.406	Cro/C1	9.2	0.13	225	[99]
NadR ₂	94.692	Cro/CI	2.9	0.024	472	[100]
MhpR ₂	62.644	IclR	4.5	0.045	351	[101]
KdgR ₂	60.058	IclR	4.6	0.048	341	[102]
IclR ₄	118.956	IclR	2.3	0.017	566	[103]
AllR ₄	117.08	IclR	2.3	0.017	559	[104]
YiaJ ₂	62.134	IclR	4.5	0.046	349	
DpiA ₂	50.906	CheY	5.5	0.061	307	
QseB ₂	49.356	CheY	5.6	0.064	301	
CusR ₂	50.788	CheY	5.5	0.061	306	
BasR ₂	50.062	CheY	5.6	0.063	304	
NagC ₂	89.082	NagC/XylR	3.1	0.026	450	[105]
DgsA ₂	89.632	NagC/XylR	3.1	0.026	452	[106]

TF	M_w (kDa)	TF family	D_3 ($\mu\text{m}^2/\text{s}$)	D_1 ($\mu\text{m}^2/\text{s}$)	Search time V_n/k (s)	Reference
IscR ₂	34.674	Rrf2	7.9	0.11	246	[107]
NsrR ₂	31.186	Rrf2	8.7	0.12	232	[108]
TdcR ₂	17.216	FIS	14.7	0.28	176	
GutM ₂	25.906	GutM	10.3	0.16	211	
TrpR ₂	24.71	TrpR	10.7	0.17	207	[109]
MetJ ₂	24.282	MetJ	10.9	0.17	205	[110]
MntR ₂	35.28	DtxR	7.7	0.10	248	[111]
DcuR ₂	54.977	CitAB	5.1	0.054	322	[112]
ArsR ₂	26.506	ArsR/SmtB	10.1	0.15	214	[113]
CdaR ₂	87.374	CdaR	3.2	0.027	444	
MtlR ₂	43.98	YggD	6.3	0.076	281	[114]
CadC ₂	115.626	ToxR	2.4	0.017	553	[115]
SgrR ₂	127.95	SgrR	2.1	0.015	602	
BolA ₂	23.988	BolA	11.0	0.18	204	
AccB ₂	33.374	biotin carboxylase	8.2	0.11	241	[116]
LexA ₂	44.716	peptidase s24	6.2	0.074	284	[117]
AlaS ₂	192.064	tRNA synthetase II	1.3	0.0075	871	[118]
PutA ₂	287.64	PRO dehydrogenase	0.8	0.0037	1328	[119, 120]
TyrR ₂	115.312	AAA ⁶	2.4	0.017	552	[121]
PrpR ₂	117.298	AAA	2.3	0.017	560	Tab S3
RtcR ₂	120.598	AAA	2.3	0.016	573	-
DhaR ₂	141.124	AAA	1.9	0.013	655	-
MalT	103.118	STAND	2.7	0.021	504	-
HyfR ₂	150.61	AAA	1.8	0.011	694	-
FhlA ₂	156.936	AAA	1.7	0.011	720	-
ZraR ₂	96.788	AAA	2.9	0.023	480	-
GlrR ₂	98.32	AAA	2.8	0.022	486	-
NorR ₂	110.472	AAA	2.5	0.019	533	-
PspF ₂	73.972	AAA	3.8	0.035	393	-
NtrC ₂	104.51	AAA	2.6	0.020	510	-
AtoC ₂	104.352	AAA	2.6	0.020	509	-
AgaR ₄	118.288	DeoR ⁷	2.3	0.017	563	[122]
UlaR ₄	104.408	DeoR	2.6	0.020	509	[123]
GatR ₄	113.008	DeoR	2.4	0.018	543	
FucR ₄	109.448	DeoR	2.5	0.019	529	
GutR ₄	112.944	DeoR	2.4	0.018	542	
GlpR ₄	112.192	DeoR	2.4	0.018	539	[124]
DeoR ₈	228.384	DeoR	1.1	0.0056	1037	[125]
MurR ₄	124.77	AlsR/YebK/YfhH ⁸	2.2	0.015	589	[126]
AlsR ₄	129.448	AlsR/YebK/YfhH	2.1	0.014	608	
RcnR ₄	40.536	RcnR	6.8	0.085	268	[127]
AidB ₄	242.36	acyl-CoA dehydrogenase	1.0	0.0050	1103	[128]
NikR ₄	60.376	CopG/NikR	4.6	0.047	342	[129, 130]
LsrR ₄	135.188	SorC	2.0	0.013	631	[131]
FlhD ₄ -FlhC ₂	96.396	FlhD/FlhC	2.9	0.023	478	[132]

TF	M_w (kDa)	TF family	D_3 ($\mu\text{m}^2/\text{s}$)	D_1 ($\mu\text{m}^2/\text{s}$)	Search time V_n/k (s)	Reference
ArgR ₆	101.96	ArgR	2.7	0.021	500	[133]
PepA ₆	329.28	peptidase M17	0.7	0.0029	1549	[134]
YqjI ₆	140.406	PadR	1.9	0.013	652	[135]
NrdR ₈	137.832	NrdR	2.0	0.013	642	[136]
AsnC ₈	135.104	AsnC	2.0	0.013	631	[137]
Lrp ₈	151.096	AsnC	1.8	0.011	696	[4]

¹*AraC*-type TFs contain bipartite HTH domains per subunit [138].

² All TFs from MerR family are dimers [38].

³ All TFs from TetR family are dimers [139].

⁴ All TFs from GntR family are dimers [140].

⁵ Leucine minizipper in the C-terminal region is required for tetramerization of TF from LacI/GalR family [141] (found only for FruR, LacI).

⁶ TFs from AAA family search as dimers [142] and oligomerize at an operator.

⁷ TFs from DeoR family are higher oligomers - tetramers, octamers with ability of DNA looping [143, 123].

⁸ Poorly characterized family of transcription factors, tetramer [126] MurR.

Table S3: AAA family of TFs. HyfR is assumed to be tetramer at operator due to high similarity to FhlA (46%)

TF	Operon	Number of UAS	Oligomerization level at operator	Reference
PrpR	prpR-prpB	2	hexamer	[144]
MalT	malZ	3	tetramer	[145]
	malP	4	tetramer	
	malS	3	tetramer	
	malE	2	tetramer	
	malK	3	tetramer	
HyfR	hyfA	1	tetramer	
FhlA	hycA	2	tetramer	[146]
	hypA	2	tetramer	
	fdhF	2	tetramer	
ZraR	zraS-zraP	2	hexamer	[147]
GlrR	glmY	3	hexamer	
NorR	norR-norV	3	hexamer	[148]
PspF	pspF-pspA	2	hexamer	[149]
NtrC	glnK,nac,glnL	1	hexamer	[142]
	ygiG	2	hexamer	
	astc	3	hexamer	
	glnH	4	hexamer	
	glnA	5	hexamer	
AtoC	atoD	2	hexamer	

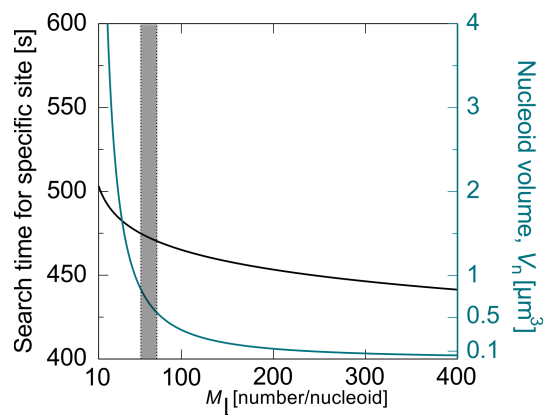


Figure S1: Changes of the LacI dimer searching time for specific site (left axis) and nucleoid volume (right axis) as a function of number of cross-links per nucleoid. The range marked in grey corresponds to the number of cross-links present in early exponential phase of growth [9].

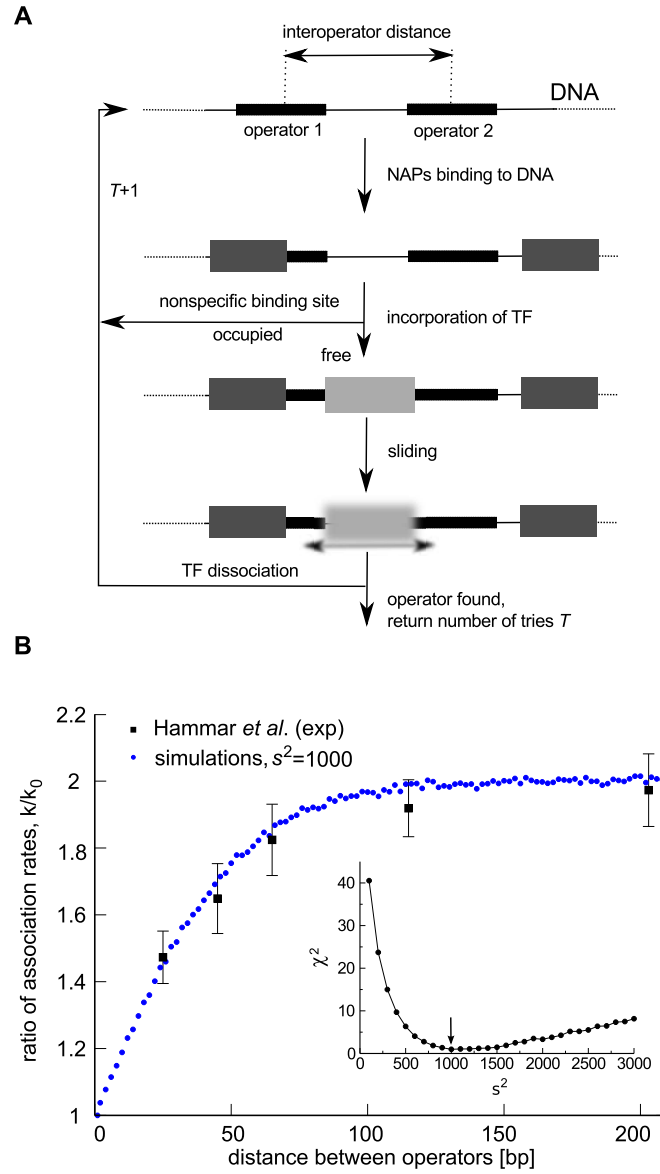


Figure S2: (A) Outline of the simulation algorithm with TF association and sliding in the presence of NAPs. Searching of the operator by TF requires many recurrent macroscopic associations T . Every such event lead to highly correlated scanning of nearby DNA sites by TF sliding. During sliding, TF samples contiguous DNA sites by performing one-dimensional random walk on DNA. (B) Interpretation of the results presented by Hammar *et al.* [19] for experiments with two identical operators (Fig. 2 in Ref. [19]) using model including random arrangement of NAPs on DNA. Ratio of the association rate to two operators to association rate with single operator on DNA as a function of the interoperator distance. Blue dots correspond to the best fit (inset shows χ^2 as a function of s^2 with minimum marked by an arrow) with squared capture distance $s^2 = D_1/\Lambda = 1000$ bp² from simulations.

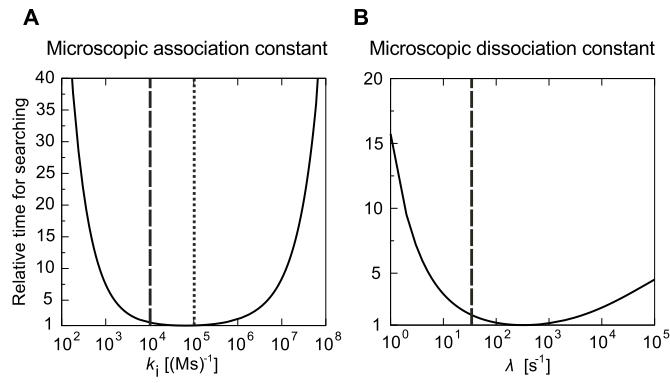


Figure S3: Relative changes of promoter searching by RNAP- σ^{70} complex. (A) Microscopic association constant is predicted to be optimised for fast target location. The times are given with respect to the minimal value. The dashed line corresponds to microscopic constant determined from equilibrium constant $K_{n.s}^{\text{RNAP}} = 300$ [150], while dotted one from the value 3000 M^{-1} [151]. (B) The minimum of searching time as a function of microscopic dissociation rate constant is shifted by one order of magnitude from determined value. The dashed line points to the determined value of microscopic dissociation rate constant.

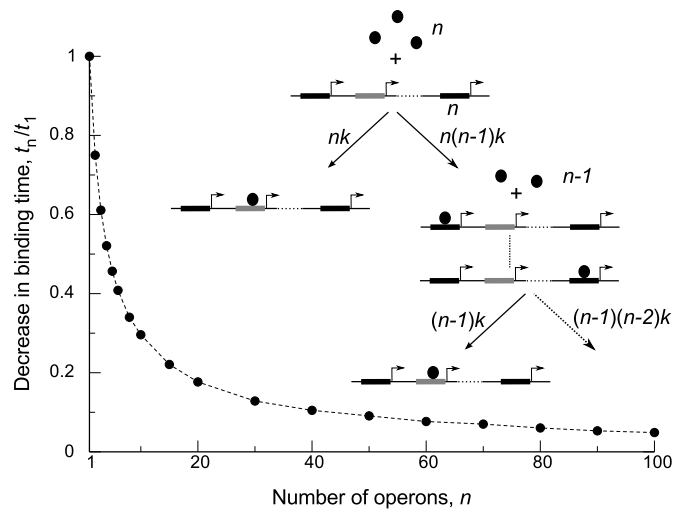


Figure S4: An average binding time to the operator of specific operon decreases with the regulon size. We consider the case in which the total number of TFs is equal to the number of regulated operons (n). Every operon contains single operator. The scheme shows all elementary events that finally lead to binding to the particular operator (grey box). At the first step two outcomes may occur: (1) binding to the specific operator with rate nk because there are n TFs and one specific operator or (2) binding to the rest $(n - 1)$ operators with rate $n(n - 1)k$. At the following steps the procedure is repeated until specific operator is bound with TF. We assume that unbinding of TF from operator is much slower than TF binding time. The binding time for the case in which $n = 1$ is $t_1 = 1/k$. Values of ratio t_n/t_1 are plotted as a function of the regulon size (number of regulated operons).

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