Happy baby having dreams fights against the biofilms

A novel approach to the biofilm treatment

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...and Iakov Bogantcev

SMTB

ZIMIN FOUNDATION

Bacterial Bakery Lab - 2020

Candidate proteins that might affect the biofilm formation

Known major regulators that are involved in the biofilm formation

H-NS, CRP, Lrp, CsgD, OmpR, Fis, IHF, FNR

Candidate proteins that we selected based on our preliminary data

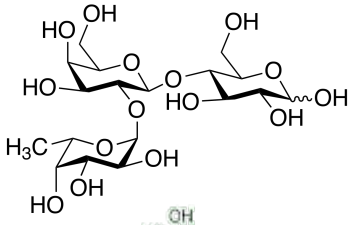
UxuR, YjjM, ExuR, Dps

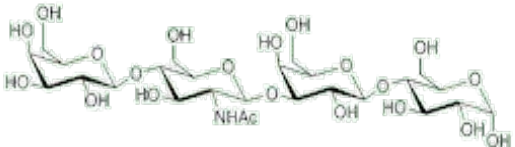
UxuR and YjjM are slightly different in pathogenic EPEC and in Nissle 1917 strains

Tested candidate ligands

Sugars from the human gut

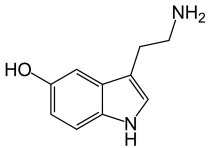
D-glucose
D-glucuronate (acid form)
D-galacturonate
Gulonic acid (acid and lactone forms)
Lactose
Galactose
Mannose
Acetate
Glycerol
Succinate
Maltose
Fructose
Sucrose

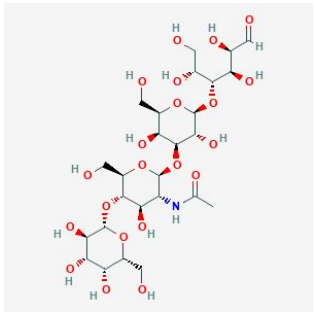


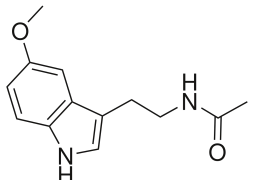


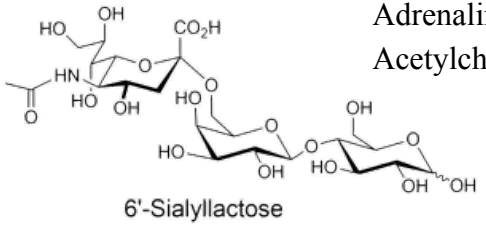
Sugars from breast milk

2′-fucosyl lactose
6′-sialyl lactose
Lacto-N-neotetraose
Sialic acid









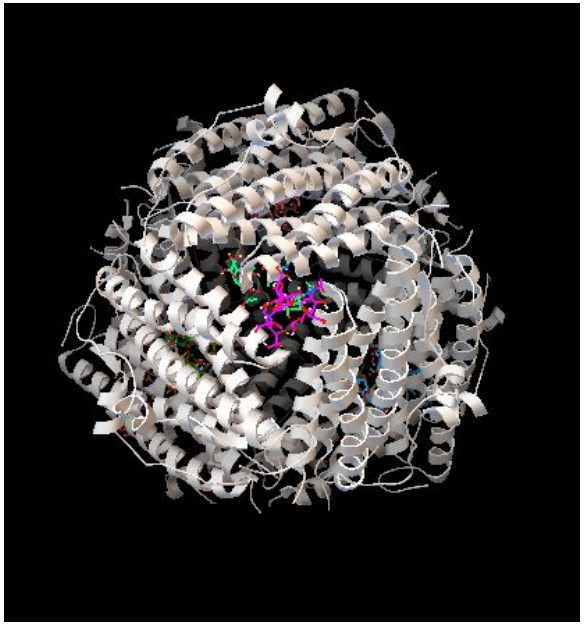
6'-Sialyllactose

Neuromediators

L-glutamic acid
D-glutamic acid
Dopamine hydrochloride
Serotonin
Melatonin
Adrenalin
Acetylcholine

Docking results

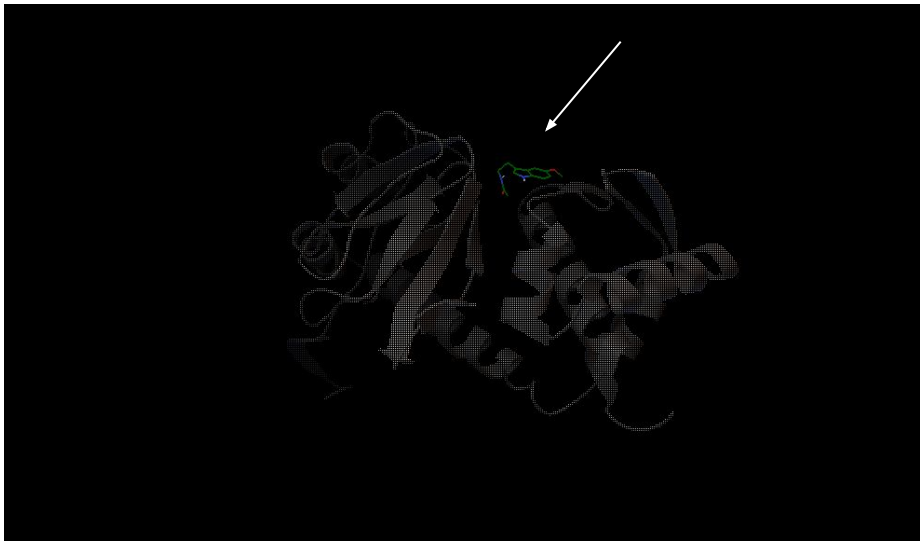
PROTEIN	LIGAND	MINIMAL ENERGY (kcal/mol)
IHF	Lacto-N-neotetraose	-8.0
	Fucosyl lactose	-8.0
	Lactose	-7.9
	Sialyl lactose	-7.9
	Maltose	-7.8
HNS	Sialyl lactose	-6.7
CsgD	Sialyl lactose	-7.3
	fructose	-6.6
	Serotonin	-6.5
	Melatonin	-6.4
	D-glucuronic acid	-6.2
YjjM normal	Lacto-N-neotetraose	-6.8
	Maltose	-6.4
	Sialyl lactose	-6.4
	Lactose	-6.2
YjjM EPEC	Lacto-N-neotetraose	-7.8
	Sialyl lactose	-7.4
	Lactose	-6.3
UxuR normal	D-glucuronic acid	-6.2
	D-galacturonic acid	-6.1
UxuR EPEC	Melatonin	-6.0
	Serotonin	-5.8
	Maltose	-5.7



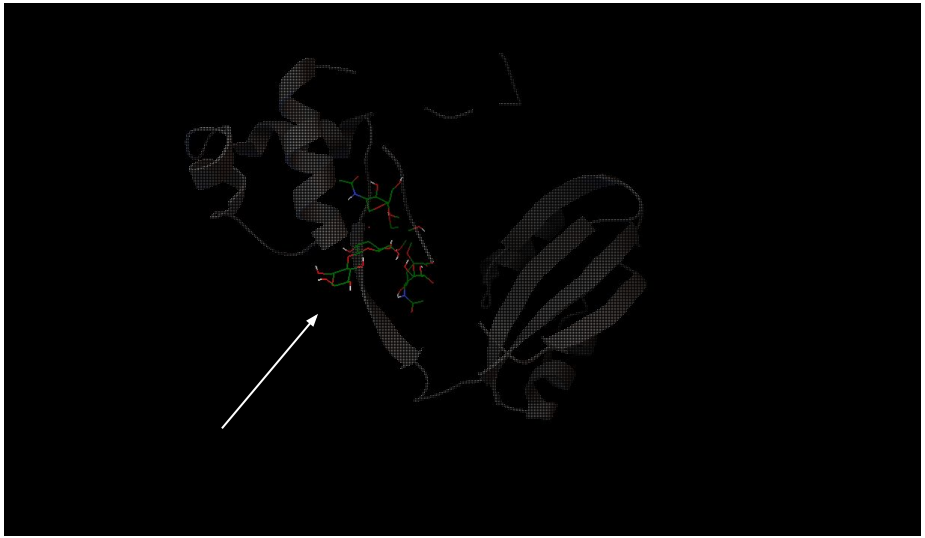
Lacto-N-neotetraose binding the Intersubunit space of the Dps dodecamer with high free energy, might prevent dodecamer assembly or promote its disassembly

PROTEIN	LIGAND	MINIMAL ENERGY (kcal/mol)
Dps	Lacto-N-neotetraose	-9.0
	Sialyl lactose	-7.0
OmpR	Maltose	-6.3
	Lactose	-6.3
	Sucrose	-6.0
	Sialic acid	-5.8
	Melatonin	-5.5
Lrp	Lacto-N-neotetraose	-8.3
	Sialyl lactose	-7.1
	Lactose	-6.0
	Maltose	-6.0
	Melatonin	-6.0
	Serotonin	-6.0
FNR	Lactose	-7,1
	Sucrose	-6.9
	Maltose	-6.7
	Melatonin	-6.5
CRP	Lacto-N-neotetraose	-7.9
	Sucrose	-6.3
	Lactose	-6.4

Some other nice results from docking



Melatonin in the FNR pocket

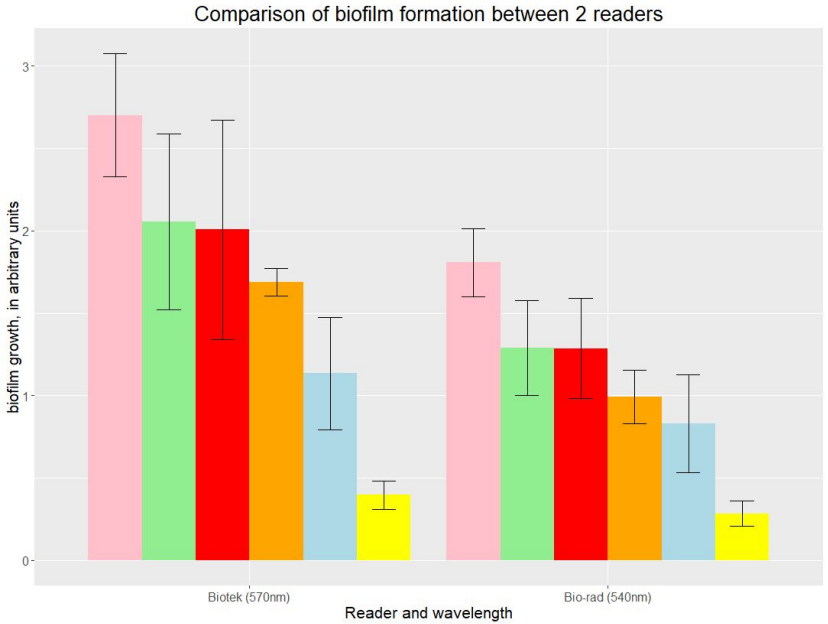


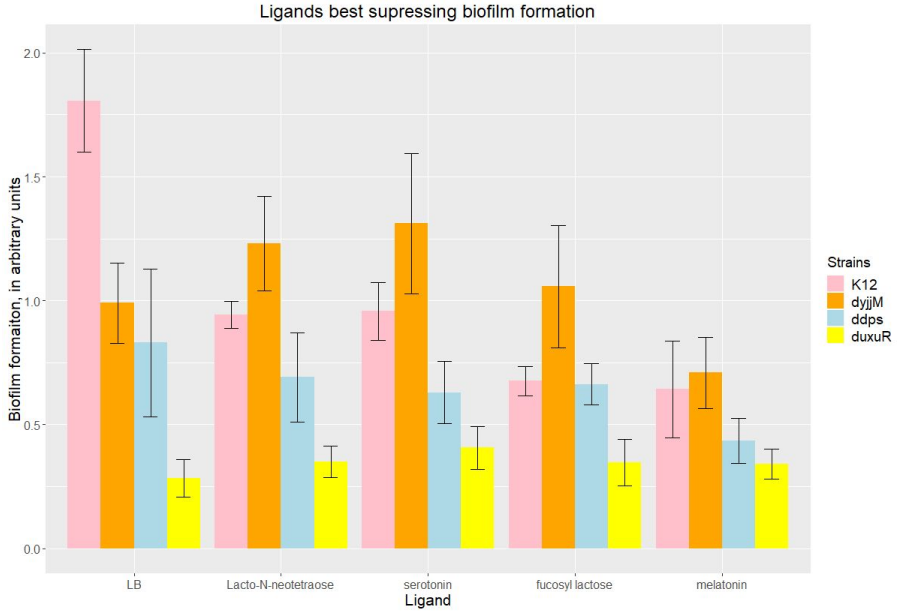
Lacto-N-neotetraose in the Lrp pocket

Observations:

- ➔ almost the same ligands (sugars from breast milk, melatonin, serotonin and lactose) were found to be optimal for all tested regulators
- ➔ Fis has no suitable ligands from the tested set

Ability of different *E. coli* strains to form biofilms and the effects of selected candidate ligands

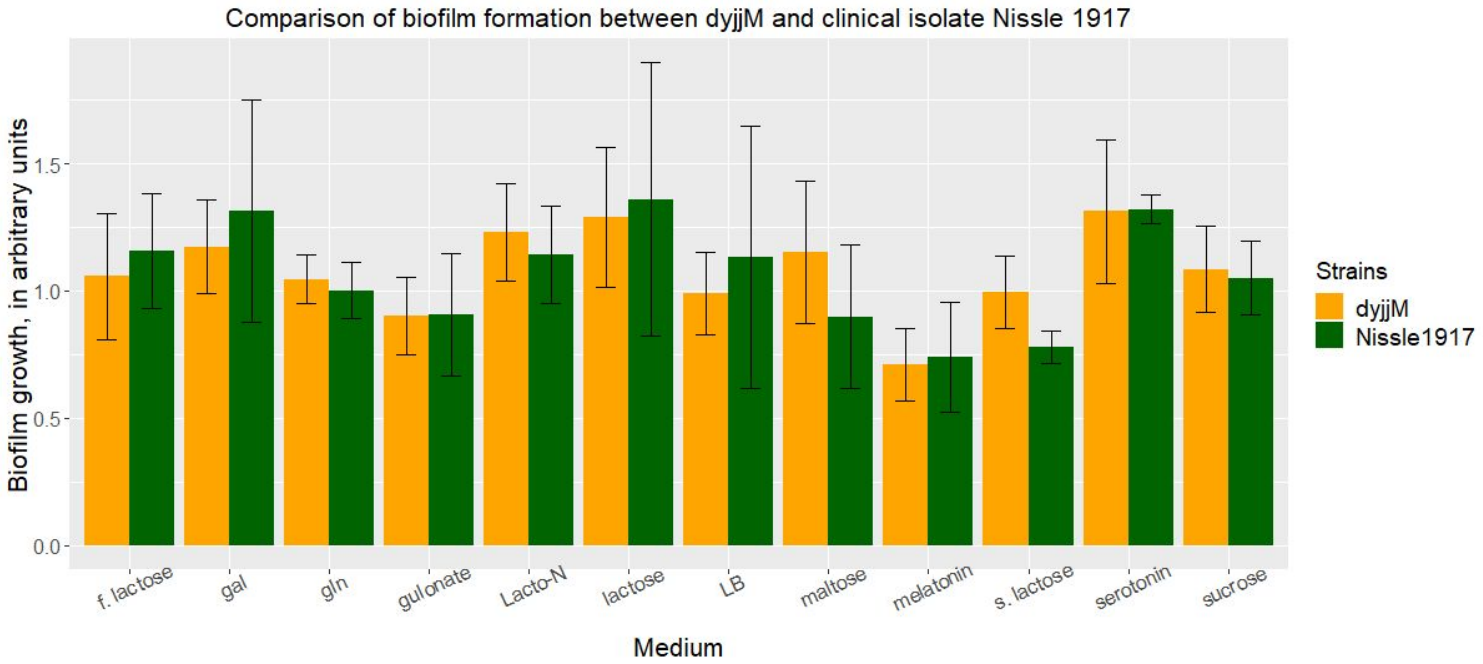




- ➔ UxuR is crucial for the biofilm formation
- ➔ Dps is involved in the regulation of this process but not to the same extent
- ➔ CRP and H-NS have almost no effect

- ➔ Sugars from the breast milk, melatonin and serotonin are the most prospective candidates for further tests

YjjM might be a key player in modulation of the biofilm formation



➔ The ability of Nissle 1917 where YjjM is mutated to form biofilms is equal to the K-12 strain with deleted *yjjM*.

➔ No effect of any candidate ligand was detected meaning that functional YjjM is necessary for the biofilm regulation

What happened to YjjM in the clinical isolates?

```
EPEC5_nucleotides_PRJNA528578:E4    MSRSQNLRHSVMNQVIEDMASGNIPSPLPSQSGLAEMYNISRTTVRHMLQHLSACGVLTL
K12_reference                       MSRSQNLRHNVINQVIDDMARGHIPSPLPSQSALAEMYNISRTTVRHILSHLRECGVLTQ
                                    *********.*:****:*** *:*********.**************:*.**  *****

EPEC5_nucleotides_PRJNA528578:E4    VGKNYVIARKPEQNDGFDCITASLKEQNRLFEQAFFTMINLRQLRAGESFSELQLARAAS
K12_reference                       VGNDYVIARKPDHDDGFACTTASMSEQNKVFEQAFFTMINQRQLRPGETFSELQLARAAG
                                    **::*******:::*** * ***:.***.:********** ****.**:**********.

EPEC5_nucleotides_PRJNA528578:E4    VSPVVVREYLLKFGRYNLIQSEKRGQWSMKQFDQSYAEQLFELREMLETHSLQHFLNLPD
K12_reference                       VSPVVVREYLLKFGRYNLIHSEKRGQWSMKQFDQSYAEQLFELREMLETHSLQHFLNLPD
                                    ******************:****************************************

EPEC5_nucleotides_PRJNA528578:E4    DDPRWLQAKTMLERHRTLRDSIGSNFRMFSQLDRDFHAMLLSAADNIFFNQSLEIMSVIF
K12_reference                       HDPRWLQAKTMLERHRLLRDNIGNSFRMFSQLDRDFHSLLLSAADNIFFDQSLEIISVIF
                                     ************** ***.**..***********::**********:*****:****

EPEC5_nucleotides_PRJNA528578:E4    HFHYQWDESDLKQRNIIAVDEHMTILSALICRSDLDAMTALRNHLDTAKQSMIRSISQHH
K12_reference                       HFHYQWDESDLKQRNIIAVDEHMTILSALICRSDLDATLALRNHLNSAKQSMIRSINENT
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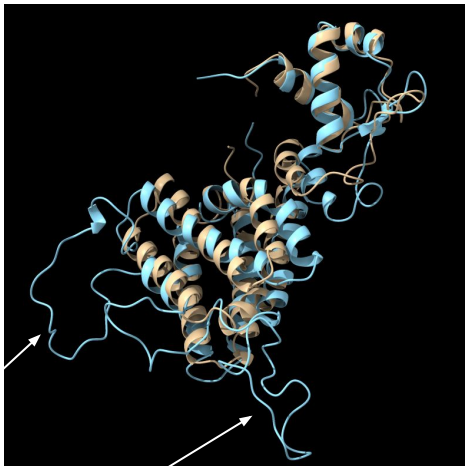
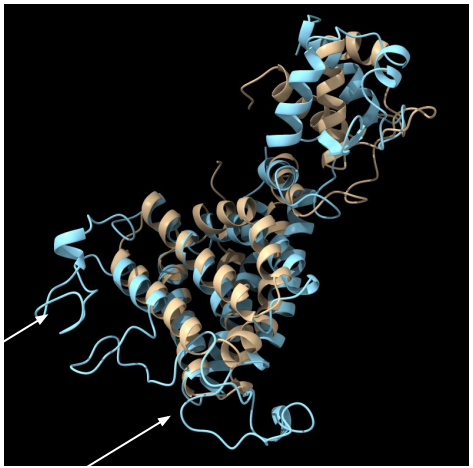
Substitutions broke the alpha spirals leading to significant structural rearrangements



150 ns m.d.

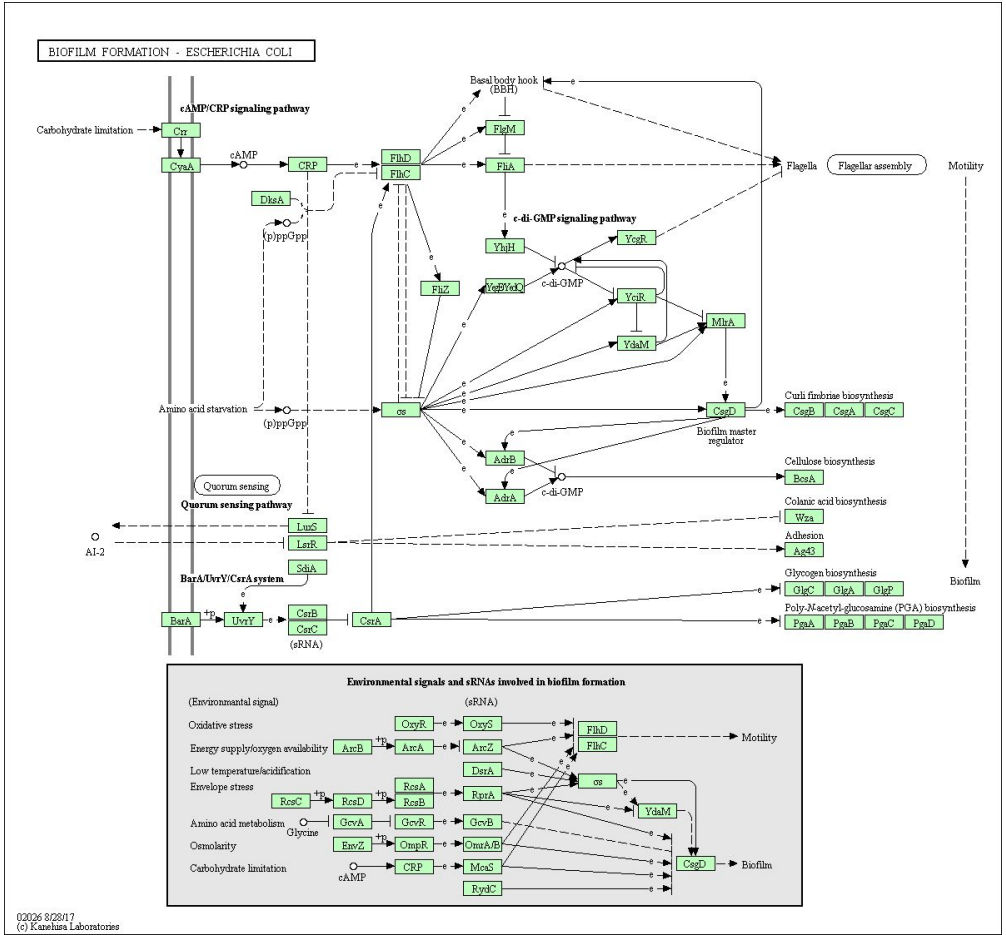


Predicted 3D-structures of YjjM from clinical isolates (teal) and from K-12 (beige), after several rounds of molecular dynamics at 80 ns

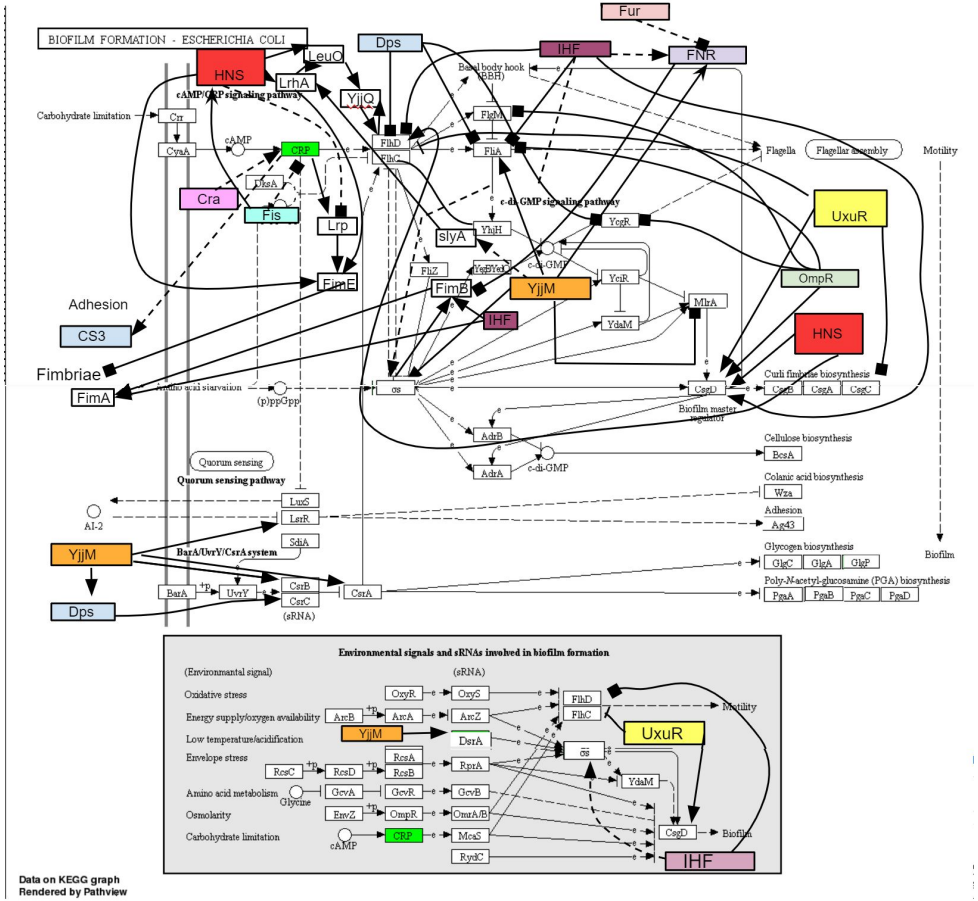
Flexible parts forming big pockets are marked with arrows

8000 ns m.d.

Regulation of the biofilm formation was like this

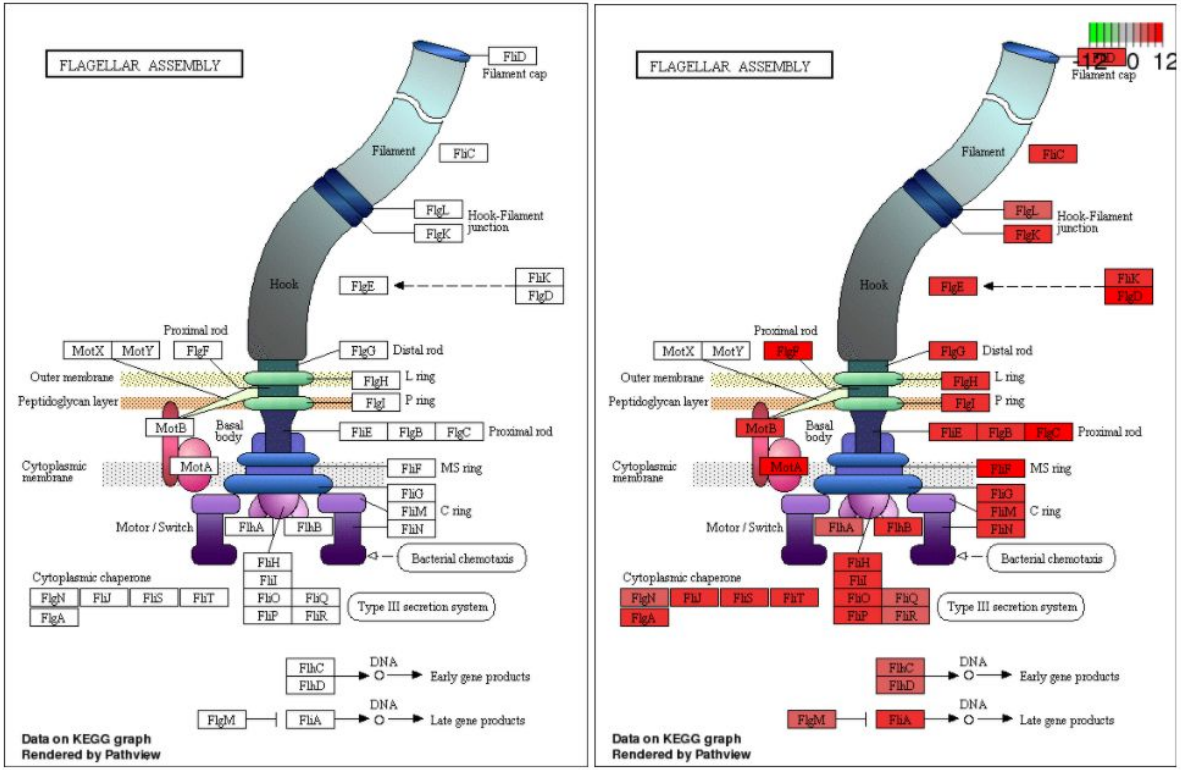


...and after 8 days in the lab became this!



E. coli strain	Effects on the biofilm formation and motility
ΔompR, ΔyjjM, ΔuxuR, ΔexuR, Δdps	Biofilm formation is reduced Increased flagella formation Increased production of proteins related to chemotaxis.
Δhns, Δcrp	Biofilm formation increased or didn't change. Decrease flagella formation Decreased production of proteins related to chemotaxis.

Effects of selected regulators on flagella assembly as revealed by RNA-seq analysis



Comparison of transcriptomic data of WT strain to Δ*crp* on glucose (left) and Δ*uxuR* on glucose (right). The same flagella upregulation shown in the right can be said for the strains: *ΔyjjM* on glucuronate and glucose, Δ*uxuR* on glucuronate, Δ*exuR* on glucuronate and glucose, Δ*ompR*, and Δ*dps* at 4 hours. Meanwhile, the strains: *ΔyjjM* on gulonate, *Δcrp* on glucuronate, and *Δhns* have the same expression profile as the left diagram.

Conclusions

(1) Best (and previously unknown) ligands that significantly decrease biofilm formation in the *E. coli* strains: **serotonin**, **melatonin** and **breast milk sugars** (fucosyl-lactose and lacto-N-neotetraose)

(2) Studied transcriptional factors (OmpR, Dps, Yjjm, ExuR, UxuR, CRP, HNS) all turned out to be involved in regulation of biofilm formation / opposite processes (e.g. motility). **YjjM, Dps** and **UxuR** seem to be the most important (nobody knew of this either).

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Molecular dynamics

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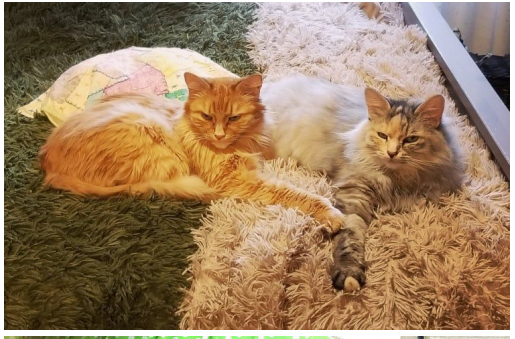

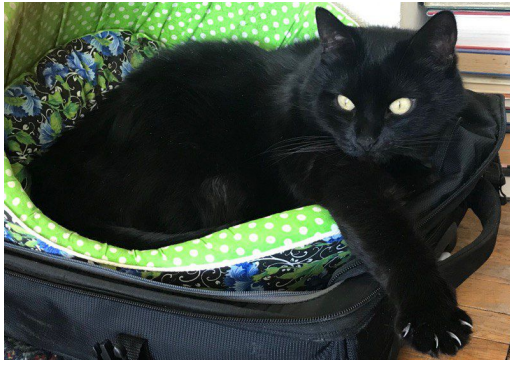

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