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FrzS acts as a polar beacon to recruit SgmX, a central activator of type IV pili during Myxococcus xanthus motility.

Sarah Bautista, Victoria Schmidt, Annick Guiseppi, Emillia M. F Mauriello, Bouchra Attia, Latifa Elantak, Tâm Mignot and Romain Mercier
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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Thank you for submitting your manuscript for consideration by The EMBO Journal. I sincerely apologise for the protracted review process due to delays in review submission. We have now received two referee reports on your manuscript, which are included below for your information.

As you will see from the comments, both reviewers appreciate the work, while also indicating some points for further improvement of the study. Based on these positive assessments, I would like to invite you to address the concerns raised by the reviewers in a revised manuscript.

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, please contact me as soon as possible upon publication of any related work to discuss the appropriate course of action. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess. Please also see the attached instructions for further guidelines on preparation of the revised manuscript.

Please feel free to contact me if you have any further questions regarding the revision. I would be happy to discuss the revision in more detail via email or phone/videoconferencing.

Thank you for the opportunity to consider your work for publication. I look forward to receiving the revised manuscript.

Referee #1:

Bautista and coworkers describe in their paper "FrzS acts as a polar beacon to recruit sgmX, a central activator of type IV pili during Myxococcus xanthus motility." How SgmX is recruited to the cell pole of M. xanthus, thereby regulating twitching motility, was yet unknown. This generally well written paper uses elegant genetic experiments to show that the polar localization of the SgmX-TAD domain to cell poles is sufficient for motility. What makes this paper interesting for a wider audience (than those working on M Xantus and related bacteria) is how they showed this, by making use of a heterologous protein from Caulobacter crescentus PopZ, which has an inherit capacity to accumulate at cell poles in unrelated bacteria. By fusing SgmX-TAD to PopZ they were able to prove that the function of FrzS, and maybe yet other proteins involved in the process of polar SgmX

localization, is purely to recruit SgmX to the cell poles. Another interesting observation that could be interesting for a wide audience is the finding that the binding site on FrzS is at a different side of the protein than is normally observed for response regulators.

Aside for some minor remarks, listed below, the only main suggestion I have is to rewrite the opening paragraph of the Result section since it is written in a way that assume specialized knowledge of the system, which most readers will not have. It is very difficult to understand why previous research suggests that there is an alternative binding site occluded.

Minor remarks:

• The description of the system in the introduction is difficult to follow without a figure.
• Line 106, it is unclear from first reading to see why a binding site should be occluded. This paragraph should be better introduced.
• Line 178, when expressed together
• Line 186, depend on
• Line 188, Asp12, as well as (comma)
• Line 212-215, difficult sentence add line breaks/comma's
• Line 223, deletion strain
• Line 229, and/but
• Line 247, TPR formed? domain
• Line 251, remove "the"
• Line 276, Figure 5G is not clear
• Line 277, its regulation

Referee #2:

The Type IV pilus activated S-motility in Myxococcus have been shown to be primarily regulated through the MglA-dependent polar localization and activation of SgmX, a protein abundant with tetratrico peptide repeats. Nevertheless, the molecular mechanism that regulates the pole-specific localization and activation of SgmX resulting in Type IV pilus activation has remained a mystery. In this study, the authors, using a combination of genetic, imaging and interaction studies, have beautifully deciphered (i) the role of specific TPRs in SgmX for its localization and signaling, and (ii) teased out the involvement of the CheY-domain harboring protein, FrzS, in the pole-specific recruitment of SgmX. Using NMR analyses and interaction studies, they have shown that the CheY domain of FrzS might directly interact with SgmX to facilitate its recruitment to the cell pole. Furthermore, their results from localization and motility read-out indicate that the role of FrzS might be limited to the polar recruitment of SgmX. This well-conducted study, while solving an important aspect of Type IV pilus activated S-motility regulation in Myxococcus, has demonstrated a possible role for 'pseudo' CheY-like domains, that may have lost the canonical activity of a typical response regulator, in regulating diverse functions in bacteria. The results and the discussions to the results are well presented and the manuscript is easy to read. The below suggestions could help to further strengthen the manuscript.

Major suggestions:
Through the NMR analyses the authors have identified the possible residues that might facilitate the interaction between the CheY domain of FrzS and SgmX. In my opinion, these observations could get added value if authors could use mutational analyses to investigate the extent to which these residues are required for the localization of SgmX by FrzS. They could use the CheY-mNG-PopZ system for this purpose.

Could the bi-polar localization of FrzS-mCherry be a result of protein overabundance? Especially if authors' suggestion in the discussion that FrzS might have a curvature-dependent localization is a possibility.

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Line 597: The strain numbers are messed up. According to the strain list ΔfrzS ΔsgmX is RM437. Also, RMxx should be RM488.

Original Referees comments

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Authors' responses

Referee #1:

Minor remarks:

• The description of the system in the introduction is difficult to follow without a figure.
We thank the referee for the comment. To help the reader, we have prepared a figure that presents the model system described in the introduction section together with the principal findings presented in the manuscript. The figure could be added in the Appendix figures file. However, we think that the figure could be used as a synopsis figure.
• Line 106, it is unclear from first reading to see why a binding site should be occluded. This paragraph should be better introduced.
We have better introduced the paragraph to introduce the probable existence of a second polar targeting domain within SgmX (lines 106-113). The regulation by occlusion of the S-domain by the M-Domain, when not complexed with MglA, is also emphasised in the discussion section together with the working model.
• Line 178, when expressed together.
Amended
• Line 186, depend on.
Amended
• Line 188, Asp12, as well as (comma)
Amended
• Line 212-215, difficult sentence add line breaks/comma's
Amended
• Line 223, deletion strain
Amended
• Line 229, and/but
Amended
• Line 247, TPR formed? domain

Corrected by TPR domain

• Line 251, remove "the"

Removed

• Line 276, Figure 5G is not clear

We have expended our description of the figure 5G

• Line 277, its regulation

Amended

Referee #2:

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As suggested, we have extended our investigation of the interaction between the CheY domain of FrzS and SgmX to validate important residues involved. In that respect, we have generated three distinct point mutants of residues that showed important chemical shift perturbation upon SgmX interaction (Fig 4): $FrzS^{CheY}$-H92A, $FrzS^{CheY}$-G88A and $FrzS^{CheY}$-AHA(97-99)GGG.

To determine their involvement in the interaction, we have purified these mutants and used NMR to characterize the interaction with SgmX. The obtained results are presented in the Fig EV3 et described in the result section (lines 212-219). Two observations can be drawn from the results:

(i) The RMN profile of each mutant is similar to the wild type protein $FrzS^{CheY}$, showing that point mutations do not affect the overall protein structure (Fig EV3).

(ii) The RMN profile of each mutant no longer shows chemical shift perturbation upon SgmX interaction, as observed with the wild type protein $FrzS^{CheY}$ (Fig EV3).

In conclusion, the newly added results further validated the characterised interface of interaction interface between the CheY domain of FrzS and SgmX by pointing key residues involved.

Could the bi-polar localization of FrzS-mCherry be a result of protein overabundance? Especially if authors' suggestion in the discussion that FrzS might have a curvature-dependent localization is a possibility.

We thank the referee for his comment on the FrzS polar localization within cells. We can definitively not exclude that the intrinsic molecular mechanism that tethers FrzS at cell poles also relies on the amount of the protein in the cell. However, we have lines of evidence which suggest that the bipolar localization of FrzS is not linked to its fusion to fluorescent proteins, like mcherry. A protein fusion which could in turn change the intracellular concentration of FrzS.

First, FrzS the bipolar localization of FrzS has been shown to depend on an intrinsic Ct-terminal motif that when mutated makes FrzS unipolar (Mignot et al. 2007). Second, FrzS is bi-polar asymmetric, which changes when cells revers (Mignot et al., 2005). Last, the polar localization of $SgmX^{mimB}$, that lacks the M-Domain, only relies on an interaction with the FrzS protein. Thus, $SgmX^{mimB}$ can be used as the proxy to follow the FrzS localization in cells. As presented in Figure 1B-2, $SgmX^{mimB}$ showed a bipolar localisation profile like FrzS protein (Fig

2) (Mercier et al, 2020). This is specific because in the absence of FrzS, SgmX^{mimB} no longer localised at cell poles (Fig2).

Minor suggestions:
Line 539: Differently coloured 'cylinders'
Amended
Line 597: The strain numbers are messed up. According to the strain list ΔfrzS ΔsgmX is RM437. Also, RMxx should be RM488.
We have changed and checked strain numbers

Thank you for submitting a revised version of your manuscript. Your study has now been seen by one of the original referees, who finds that their previous concerns have been addressed and now recommends publication of the manuscript. There remain only a few editorial issues that have to be solved before I can extend formal acceptance of the manuscript.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal. I look forward to receiving the final version.

Referee #2:

The authors have satisfactorily addressed the concerns of the reviewers. I only have one minor comment:

Line 223: "Therefore, these amino acids are involved in the interaction" - The NMR experiments suggest the involvement of the residues in its interaction with SgmX in vitro. However, further studies are required to validate the in vivo significance. Therefore, the authors need to modify this sentence to a suggestion rather than a definite statement.

The authors performed the requested editorial changes.

Thank you for addressing the final minor issues in the manuscript. I am now pleased to inform you that your manuscript has been accepted for publication in The EMBO Journal.

EMBO Press Author Checklist

Corresponding Author Name: Romain Mercier and Tam Mignot
Journal Submitted to: Embo Journal
Manuscript Number: 2022-111661R

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This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.

Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:

  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Not Applicable	

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Not Applicable	

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Appendix File Tables 3

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Not Applicable	
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Not Applicable	

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Not Applicable	
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail **housing and husbandry conditions**.	Not Applicable	

Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Yes	Appendix File Tables

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgements

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Not Applicable	
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
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Include a statement about **sample size** estimate even if no statistical methods were used.	Not Applicable	
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Not Applicable	
Include a statement about **blinding** even if no blinding was done.	Not Applicable	
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Not Applicable	
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Not Applicable	

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	Materials and Methods
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Materials and Methods

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	
Studies involving **human participants**: Include a statement confirming that **informed consent** was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Not Applicable	
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Not Applicable	
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	

Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	

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The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

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State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided.	Not Applicable	
For **tumor marker prognostic studies,** we recommend that you follow the **REMARK** reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not Applicable	
For **phase II and III randomized controlled trials**, please refer to the **CONSORT** flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Not Applicable	
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	