

A genome-wide, multi-layered, network-based approach for personalised medicine

CENIIT Final Report (2022-02-28)

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Summary of the most important scientific results

The strategy developed in the project aimed to implement, standardize, and make maximal use of three key network biology concepts (Figure 1) and thereby establish a generally applicable data mining pipeline for personalized medicine in complex diseases. These concepts were based on Gustafsson's previous findings between 2006-2015 in the field of systems biology. The CENIIT project was performed in direct collaboration with Astra Zeneca, but also other biotechnical partners like BioControl and Merck have been closely connected to the project with expert user input and usage.

Key results of the projects are:

- 1) Establishment of system biology R and Python packages implementing the concepts in Figure 1.
- 2) Benchmarking different module and hub methods for systems medicine which allows also for other researchers to assess performance of the improvements.
- 3) Creation of a stand-alone graphical interface combining several modules and hubs in the pipeline for data-mining new gene groups and making it easy to use through a docker solution.

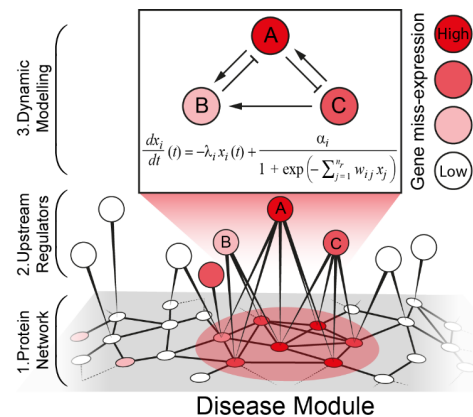


Figure 1. Project outline. (1) Molecular disease modules are identified as densely connected miss expressed groups of genes, by combining the protein interaction network with data of miss expression in diseases. (2) The most important upstream regulators of the disease modules using a gene regulatory network (3) Dynamical modelling of those upstream regulators and their genome-wide downstream effects.

Summary of the degrees and promotions the project has contributed to

Assoc Professorship (Bitr Professor) of Mika Gustafsson (2021) in Bioinformatics

Ph D of Ramsus Magnusson (2020) in Bioinformatics

Docentship of Mika Gustafsson (2016) in Bioinformatics

Summary of the master thesis works that have been performed within the project

Julia Åkesson (2018) in Technical Biology

Hendrik de Weerd (2017) in Systems Biology

Mattias Köpsen (2016) in Technical Biology

Persons funded by the project

CENIIT has funded 660 kkr salary for Ph D student Ramsus Magnusson, and 660 kkr for Post Doc Andreas Tjärnberg (2016-2018), which has worked full time within the CENIIT project (detailed in Table 1). CENIIT has covered approximately 30% of their salaries, and the remaining parts (3080 kkr) was been taken from my IFM start-up and VR-grants. Tjärnberg moved New York and Magnusson graduated in May 2020 and are now not financed by CENIIT. For 2019-2021 CENIIT covered the salary of D Martinez (1340 kkr) who worked to implement our tools in the pipelines of AstraZeneca.

Remaining parts of D Martinez salary has been covered through teaching and a new VR grant. Their salaries have so far been critical for supervising master students, establishing the industrial collaborations and implementing the ideas as open source software.

| Year | Tjärnberg [kkkr] | Magnusson [kkkr] | Martinez [kkkr] | Sum [kkkr] |
|-------|------------------|------------------|-----------------|------------|
| 2016 | 440 | | | 440 |
| 2017 | 110 | 330 | | 440 |
| 2018 | 110 | 330 | | 440 |
| 2019 | | | 440 | 440 |
| 2020 | | | 450 | 450 |
| 2021 | | | 450 | 450 |
| Total | 660 | 660 | 890 | 2660 |

Table 1: Annual funding distribution of CENIIT project in thousands of crowns (kkkr).

Industrial connections

The CENIIT project started from an interest in our generally applicable coarse-graining strategy for personalized medicine from Dr Daniel Muthas (head of Bioinformatics unit) at the pharmaceutical company Astra Zeneca and the German Biotech Company Biocontrol by 2015. Since 2017 three new companies had interest in the proposed strategy, namely MultiD, TATAA and MERCK, and through a collaboration with Dr Zelmina Lubovac at Högskolan i Skövde received 8 MSEK from KK-stiftesen. The strategy for industrial collaboration in the project is to continue the work within clinically aimed sub-projects, together with Astra Zeneca for coarse-grain data mining of asthma, using (1-3). In parallel to these projects, we developed software tools that care tested by Astra Zeneca and will be re-used for analysis of other complex diseases. To make these readily used within the community we will further teach these software tools at multiple levels. To further increase the implementation of the tools created within the CENIIT-project Gustafsson has twice presented the project work for National Bioinformatic Life Science Support (NBIS).

Creation of a new research group

The project has enabled Gustafsson to start his group and he has already received several grants for related ideas to this project. In detail, he has as main applicant received three grants from Åke Wiberg (650.000 SEK), two from Neuroförbundet (106.000 SEK), three from Swedish Research Council (6.900 000 SEK), one from Swedish Strategic Research (29.000.000 SEK) and one from KAW/DDLS initiative (3 800.000 SEK). In addition, he has also been co-applicant for four funded VR grant applications and is currently on two by the University prioritized large KAW applications. He has established and supervised continuously held a group of 2-5 PhD students, 1-2 Post Docs, one technician, and 1-2 master students. Gustafsson has no common grants with his previous supervisors M Hörnquist (PhD supervisor at ITN) and M Benson (Post Doc supervisor, IKE) and leads his group and research line completely independent from these two. He has built up a rich international clinical collaboration network with researchers from Stockholm, USA, Saudi Arabia, Australia, Germany, and Finland. Moreover, he has developed three new undergraduate and Ph D courses in Bioinformatics and Systems Biology as well as being in a TEMA group for the new Bsc in Industrial and Experimental Biomedicine (fully responsible for Bioinformatics and Systems Biology courses). He became a docent by 2016, “Biträdande Professor” 2021 and is currently externally evaluated for a full professorship.

References and list of publications 2016-2022 (18 items)

- [1] Köpsen Mattias, *A network based approach for identification of robust disease modules in complex diseases*, master thesis LITH-x-EX.12/3131—SE, 2016
- [2] Hellberg S, Eklund D, Gawel D R, Köpsen M, Zhang H, Nestor C E, Kockum I, Olsson T, Skogh T, Kastbom A, Sjöwall C, Vrethem M, Håkansson I, Benson M, Jenmalm M C, **Gustafsson M**^{#*}, and Ernerudh J[#], **#shared last and * lead corresponding author**. *Gene Expression Profiling of Resting and Activated CD4+ T Cells in Patients with Multiple Sclerosis*, Cell Reports **16**, 1-12, Sept 2016, IF 8.
- [3] Vlaic S, Tokarski-Schnelle C, **Gustafsson M**, Dahmen U, Guthke R, & Schuster S. ModuleDiscoverer: Identification of regulatory modules in protein-protein interaction networks. Scientific Reports **8** No. 433, 2018, IF 4.
- [4] de Weerd D, supervised by **Gustafsson M**. *Disease modules: Comparing and integrating inference methods*. Msc thesis Höskolan i Skövde, june 2017.
- [5] Magnusson R, Köpsén M, Lövfors W, Gawel D R, Nordling T, Schulze S, Nestor C, Cedersund G, Benson M, Tjärnberg A, and **Gustafsson M**. *LASSIM –a network inference toolbox for genome-wide mechanistic modelling*. a) ICSB conference 2016-9 and, b) PLoS Computational Biology, 2017-06, IF 5, c) <https://gitlab.com/Gustafsson-lab/> .
- [6] Martinez D, supervised by Muthas D (*Astra Zeneca*) & **Gustafsson M**. *Identification of personalized multi-omic disease modules in asthma*. Msc thesis at Höskolan i Skövde , 2018-6
- [7] MODifier: an Ensemble R Package for Inference of Disease Modules from Transcriptomics Networks published as:
a) de Weerd H A, Badam T V S, Martinez-Enguita D, Åkesson J, Muthas D, **Gustafsson M**[#] & Lubovac Z[#], **#shared last** Bioinformatics 2020 Jun 1;36(12):3918-3919, IF 6
b) An (R-package) <https://gitlab.com/Gustafsson-lab/MODifier>
c) A webtool available at <http://transbioinfo.liu.se>
d) Badam T V S , de Weerd H A, Martinez D, Åkesson J, Muthas D, Alfredsson L, Olsson T, Jagodic M, Lubovac Z, **Gustafsson M**, BMC Genomics, **22** (1), pp 1-17, 2021.
- [8] Åkesson J, Lubovac Z, Magnusson R & **Gustafsson M**, *ComHub: Community predictions of hubs in gene regulatory networks*. Published as:
a) BMC Bioinformatics **22** (1), pp 1-13, 2021.
b) Msc thesis at Linköping University, 2018-10,
c) Tool available at see also <https://gitlab.com/Gustafsson-lab/comhub>
- [9] Magnusson R, & **Gustafsson M**, *LiPLike: Towards gene regulatory network predictions of high-certainty*
a) Bioinformatics, vol 36-8, 2020, pp 2522–2529, IF = 6
b) ICSB/RECOMB conference 2018-12 in New York,
c) Tool available at <https://gitlab.com/Gustafsson-lab/liplike>
- [11] de Weerd HA, Åkesson J, Guala D, **Gustafsson M**[#] & Lubovac Z[#], **#shared last**. *MODalyseR-a novel software for inference of disease module hub regulators identified a putative Multiple Sclerosis regulator supported by independent eQTL data*
a) Bioinformatics Advances, Vol. 2 (1) 2022
b) Tool available at <https://gustafsson-lab.gitlab.io/MODalyseR/>

List of all peer-reviewed publications since project start (*publications directly associated with CENIIT project)

1. * de Weerd HA, Åkesson J, Guala D, **Gustafsson M[#]** & Lubovac Z[#], **#shared last**. *MODalyseR-a novel software for inference of disease module hub regulators identified a putative Multiple Sclerosis regulator supported by independent eQTL data*. *Bioinformatics Advances*, Vol. 2 (1) 2022
2. Rundquist O, Nestor C E, Jenmalm M J, Hellberg S, & **Gustafsson M**. Progesterone inhibits the establishment of activation-associated chromatin during TH1 differentiation. *Frontiers in Immunology* Feb 02. (2022) IF 7
3. Badam T V, Hellberg S, Mehta R B, Lechner-Schott J, Lea R A, Tost J, Mariette X, Svensson-Arvelund J, Nestor C E, Benson M, Berg G, Jenmalm M C, Gustafsson M[#] & Ernerudh J[#], **#shared last authorship**. CD4+ T-cell DNA methylation changes during pregnancy significantly correlate with disease-associated methylation changes in autoimmune diseases. *Epigenetics*. Sep 15, 2021. IF=5.
4. Zenere A, Rundquist O, Gustafsson M, & Altafini C, Using high-throughput multi-omics data to investigate structural balance in elementary gene regulatory network motifs. *Bioinformatics*, btab577, <https://doi.org/10.1093/bioinformatics/btab577>, 2022.
5. * Badam TV, de Weerd HA, Martínez-Enguita D, Lubovac Z[#] & **Gustafsson M[#]**, **#shared last authorship**. A validated generally applicable approach using the systematic assessment of disease modules by GWAS reveals a multi-omic module strongly associated with risk factors in multiple sclerosis. *BMC Genomics* **22**, 631 (2021). IF 4.
6. Hellberg S, Raffetseder J, Rundquist O, Magnusson R, Papapavlou G, Jenmalm M C, Ernerudh J & **Gustafsson M**, Progesterone Dampens Immune Responses in In Vitro Activated CD4+ T Cells and Affects Genes Associated With Autoimmune Diseases That Improve During Pregnancy. *Front. Immunology*, 12, 1726, 2021. IF 6
7. *Åkesson J, Lubovac-Pilav Z, Magnusson R, & **Gustafsson M**, ComHub: Community predictions of hubs in gene regulatory networks. *BMC bioinformatics* 22 (1), 1-12, 2021. IF 3
8. Herrgårdh T, Madai VI, Kelleher JD, Magnusson R, **Gustafsson M**, Milani L, Gennemark P & Cedersund G. Hybrid modelling for stroke care: Review and suggestions of new approaches for risk assessment and simulation of scenarios. *Neuroimage: Clinical*, Vol 21, 102693, 2021 IF 4
9. Papapavlou, G., Hellberg, S., Raffetseder, J., Brynhildsen, J., Gustafsson, M., Jenmalm, M.C. & Ernerudh, J. Differential effects of estradiol and progesterone on human T cell activation *in vitro*. *Eur. J. Immunol.* <https://doi.org/10.1002/eji.202049144>, 2021. IF 4.
10. Bensberg M, Rundquist O, Selimović A, Lagerwall C, Benson M, Gustafsson M, Vogt H, Lentini A, & Nestor CE, TET2 as a tumor suppressor and therapeutic target in T-cell acute lymphoblastic leukemia. *Proc of the Nat Acad of Sci (PNAS)* 118 (34), 2021. IF 9.
11. * Huoman J, Martinez-Enguita D, Olsson E, Ernerudh J, Nilsson L, Duchon K, **Gustafsson M***, & Jenmalm M C* **#shared senior authors**. Combined prenatal Lactobacillus reuteri and ω-3 supplementation synergistically modulates DNA methylation in neonatal T helper cells. *Clinical Epigenetics*, 2021 Jun 30;13(1):135. IF 5
12. Dwivedi, S.K., Tjärnberg, A., Tegnér, J. & **Gustafsson M**, Deriving disease modules from the compressed transcriptional space embedded in a deep autoencoder. *Nature Communications* 11 (1), 1-10, 2020. IF 12
13. *Magnusson R & **Gustafsson M**. LiPLike: Towards gene regulatory network predictions of high certainty. *Bioinformatics* 2020, Jan 6, IF 6
14. *de Weerd HA, Badam TV, Martínez-Enguita D, Åkesson J, Muthas D, **Gustafsson M[#]** & Lubovac Z[#], **#shared last authorship**. *MODifieR: an ensemble R package for inference of disease modules from transcriptomics networks* *Bioinformatics* 2020 Jun 1;36(12):3918-3919, IF 6.
15. Björn N, Badam TV, Spalinskas R, Brandén E, Koyi H, Lewensohn R, Petris, Lubovac Z, Sahlén P, Lundeberg J, **Gustafsson M***, Gréen H*. **#shared senior authors**. *Whole-genome sequencing and gene network modules predict gemcitabine/carboplatin-induced myelosuppression in non-small cell lung cancer patients* *Nature Publishing Journal Systems biology and applications* 2020 6 (1), 1-15. IF 4

16. Forsberg A, Huoman J, Söderholm S, Mehta R B, Nilsson L, Abrahamsson T R, Ernerudh J, **Gustafsson M***, & Jenmalm M C***_ *shared senior authors** . Pre- and postnatal *Lactobacillus reuteri* treatment alters DNA methylation of infant T helper cells *Pediatric Allergy and Immunology*, 2020 31(5). IF 4
17. *Gawel D, Serra-Musach J, Lilja S, Aagesen J, Arenas A, Asking B, Bengner M, Björkander J, Biggs S, Ernerudh J, Hjortswang H, Karlsson J, Köpsen M, Lee EJ, Lentini A, Li X, Magnusson M, Martinez-Enguita D, Matussek A, Nestor CE, Schäfer S, Seifert O, Sonmez C, Stjernman H, Tjärnberg A, Wu S, Åkesson K, Shalek AK, Stenmarker M, Zhang H, **Gustafsson M***, & Benson M***, *shared senior authors**. A validated single-cell-based strategy to identify diagnostic and therapeutic targets in complex diseases *Genome medicine*, 11(1):47-, 2019. IF 11
18. Björnsson B, Borrebaeck C, Elander N, Gasslander T, Gawel DR, **Gustafsson M**, Jörnsten R, Lee EJ, Li X, Lilja S, Martínez-Enguita D, Matussek A, Sandström P, Schäfer S, Stenmarker M, Sun XF, Sysoev P, Zhang H, Benson M. Digital twins to personalize medicine. *Genome medicine*, 2019/12 (1), 1. IF=11
19. Carlstrom K, Ewing E, Granqvist M , Gyllenberg A, Aeinehband S, Enoksson S L, Checa A, Venkata T B, Huang J, Gomez-Cabrero D, Gustafsson M, Nimer F, Wheelock C, Kockum I, Olsson T, Jagodic M, Piehl F Therapeutic efficacy of dimethyl fumarate in relapsing-remitting multiple sclerosis is linked with monocytic oxidative responses, *Nature communications*, 10, (1), 1-13, 2019, IF 12
20. Das J , Verma D, **Gustafsson M #**, Lerm M # , **#corresponding authors**. Identification of DNA methylation patterns predisposing for an efficient response to BCG vaccination in healthy BCG-naïve subjects. *Epigenetics* 14 (6), 589-601, 2019. IF=5
21. *Magnusson M, Mariotti G P, Köpsén M, Lövfors W, Gawel D R, Jörnsten R, Linde J, Nordling T, Nyman E, Schulze S, Nestor C E, Zhang H, Cedersund G, Benson M, Tjärnberg A & **Gustafsson M**. LASSIM –a network inference toolbox for genome-wide mechanistic modelling. *PLoS Comp Biol*, 2017 Jun, 13(6): IF 5
22. *Vlaic S, Tokarski-Schnelle C, **Gustafsson M**, Dahmen U, Guthke R, & Schuster S. ModuleDiscoverer: Identification of regulatory modules in protein-protein interaction networks. *Scientific Reports*. 2018 8 (1) pp 1-11, IF 4.
23. Nestor C, Lentini A, **Gustafsson M**, Mattson L, Meehan R, Zhang H, Benson M. A multi-layered, time-series genomics approach reveals TET1-mediated DNA demethylation as a master regulator of human T-helper cell differentiation. *Cell Reports* , Vol. 16, Issue 2, p559–570, 2016, IF 8.
24. *Hellberg S, Eklund D, Zhang H, Nestor C, Vrethem M, Jenmalm M, Benson M, **Gustafsson M^{#+}**, and Ernerudh J[#], **#shared last and *lead corresponding author**. Gene Expression Profiling of Resting and Activated CD4⁺ T Cells in Patients with Multiple Sclerosis, *Cell Reports*, Vol. 16, Issue 11, p2928, 2016, IF 8.
25. Serra-Musach J , F Mateo , G Garibay , A Villanueva , E Capdevila , S Jaeger , Aloy , **M Gustafsson**, M Benson , H Heyn , M Vizoso , H Pérez , Á Cordero , E Gonzalez Suarez , M Esteller , X Yang , J Arribas , G Moreno-Beuno , C Lázaro , M Ferrer, M Pujana. Network activity predicts cancer therapeutic response and synergism. *Genome Med*, 8 (1), 88, 2016, IF 11.
26. *Magnusson R, **Gustafsson M**, Strålfors P, Cedersund G, Nyman E, Combined positive and negative crosstalk activates mTORC2 in insulin resistant adipocytes, *Bioscience Reports*, BSR20160514, 2016, IF 2.
27. Mattson L, Lentini A, Gawel D R, Badam T, Benson M, Ledin T, Nestor C E, **Gustafsson M**, Serra-Musach J, Björkander J, Xiang Z, & Zhang H, Potential Involvement of Type I Interferon Signaling in Immunotherapy in Seasonal Allergic Rhinitis, *Journal of Immunology Research* 2016, Article ID 5153184, IF 3.
28. *Schleicher J, Conrad T, **Gustafsson M**, Cedersund G, Guthke R, Linde J. Facing the challenges of multiscale modelling of bacterial and fungal pathogen-host interactions. *Briefings in Functional Genomics*, 2016. Feb 8, IF 4.