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10. Brief description of the proposal

The proposal aims to investigate the function of an important bacterial protein of which a large fraction is unstructured and able to produce a mechanical force in the presence of a specific environmental signal. The capture and import of scarce nutrients in Gram negative bacteria are carried out by a range of homologous outer membrane proteins. To allow cargo transit into the periplasm, a plug domain that occludes the lumen of each of these must be actively pulled out. It is now evident that the work is provided by a single protein resident in the inner membrane, called TonB, but its working mechanism remains elusive.
Preliminary data show that the intrinsically disordered linker domain of TonB, hitherto thought to play a passive role in the process, can switch from compact and mechanically stable to expanded and force labile structures upon changes in ionic strength, potentially driving the remodelling (i.e., the unplugging) of the outer membrane transporter. The aim of this proposal is to understand the physical basis of this remodeling, uncovering a novel function of intrinsically disordered proteins that impart mechanical signals by “functional collapse”.
The proposal is grounded on a wealth of preliminary data and aims to directly observe and measure the force exerted by the TonB protein at single molecule level using optical tweezers. Physical models and novel approaches to molecular simulation will be key to interpret experimental results, and predict modifications to the sequence of the protein that would affect the ability of bacteria to feed themselves. While the research carried out is fundamental in nature, our findings will provide information that can be directly used to devise alternatives to current anti-bacterial treatments, much needed given the steadily increasing antibiotics resistance. Also relevant for the development of novel antibacterial strategies is the fact that the nutrient import mechanism of Gram-negative bacteria, the central topic of this proposal, is hijacked by bacteriocins, killer proteins produced by competing bacteria.

11. Total cost of the research project identified by items

Associated Investigator	item A.1	item A.2.1	item B	item C	item D	item E	sub-total	Total
PACI Emanuele	37.244	56.483	56.236	5.000	0	7.250	162.213	**162.213**
CECCONI Ciro	18.096	67.780	51.526	0	0	5.700	143.102	**143.102**
Total	**55.340**	**124.263**	**107.762**	**5.000**	**0**	**12.950**	**305.315**	**305.315**

N.B. The Item B and TOTAL columns will be filled in automatically

- item A.1: enhancement of months/person of permanent employees
- item A.2.1: cost of contracts of non-employees, specifically to recruit
- item B: overhead (flat rate equal to 60% of the total personnel cost, A.1+A.2.1, for each research unit)
- item C: cost of equipment, tools and software products
- item D: cost of consulting and similar services
- item E: other operating costs

PART B

B.1

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1. State of the art

Intrinsically disordered proteins (IDPs) do not form a stable observable structure; yet exhibit biological activity. The flexibility and structural instability of IDPs, just like the three-dimensional shape of structured proteins, are uniquely determined by the amino acid sequence. Analogously, intrinsically disordered regions (IDRs) within proteins have diverse functional biological roles (1) that are facilitated by the structural adaptability: IDRs have been characterised that are flexible linkers (2), entropic springs (3), elastomers (4), native molten globules (5) and order–disorder-extension transitions (6).
A protein with a sizeable IDR plays a key role in the capture and import of scarce nutrients in Gram negative bacteria. Import occurs through homologous outer membrane proteins (OMPs) known as TonB dependent transporters (TBDTs). A plug domain that occludes the lumen of TBDTs must be partially or fully removed for nutrients to enter the cell. This selective ‘unplugging’ is carried out by a single protein complex in the inner membrane (TonB-ExbB-ExbD) making it an important drug target, but its mechanism remains elusive. We recently showed using force spectroscopy to measure the end- to-end length of single proteins (7) that the intrinsically disordered linker region of TonB, previously thought to play a passive role in the process, can switch between an extended and a compact shape upon changes in ionic strength. We believe that the process of “compaction” produces the force driving the unplugging of the outer membrane transporter.
The aim of this proposal is to investigate, using optical tweezers and physical models, this novel function of intrinsically disordered regions/proteins (IDR/Ps) using TonB as an example. This will reveal a novel mechanism for mechano-transduction, the mechanism of action of TonB (a paradigm for inside-out energy transduction in Gram negative bacteria) and, by targeting this process, a potential route to a novel antibacterial strategy.
Cecconi and Paci were among the first (8, 9), about two decades ago, to investigate the mechanical properties of individual proteins in two leading laboratories at UC Berkley and Harvard/Strasbourg, using optical tweezers and molecular modeling. Since, single molecule force spectroscopy (encompassing atomic force microscopy, optical and magnetic tweezers techniques) has become a key tool in investigating biomechanics at molecular level. Paci’s research group has studied protein mechanics and its relationship with protein folding in the context of biologically relevant systems, using the means of molecular simulation and theoretical models, in collaboration with world leaders in single molecule force spectroscopy techniques. Among these optical tweezers offer a unique opportunity to study

the response of proteins to small forces of biological relevance at molecular level due to the low stiffness of an optical trap. Cecconi has gained a long experience in the field of force spectroscopy studying the conformational equilibria of several proteins using optical tweezers. By manipulating one molecule at a time, he has intimately explored the conformational energy landscape of individual proteins and characterized the low energy barriers controlling the diffusion between different molecular states. This experimental method will be employed in this project to study the low-energy fluctuations and marginally stable structures expected in IDPs like TonB.

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2. Detailed description of the project: methodologies, objectives and results that the project aims to achieve and its interest for the advancement of knowledge, as well as methods of dissemination of the results achieved

Key to bacterial survival, is their ability to gather nutrients from their environment. In Gram-negative bacteria, proteins in the outer membrane surrounding the cell actively transport a variety of essential and rare nutrients such as iron chelates (FhuA), vitamin B12 (BtuB), oligopeptides and glycans (10) to the cell’s interior (i.e., the periplasm). The structure of many of these transport proteins have been determined. Such proteins are generically called TonB-dependent transporters (TBDTs) because their function is strictly related to that of inner membrane proteins called TonB. The lumen of TBDTs is occluded by a plug-domain, preserving the outer membrane’s function as a permeability barrier. Such a plug is opened selectively to let specific nutrients in, although it is also hijacked by bacteriocins, killer proteins produced by competing bacteria. Intriguingly, the outer membrane is not energised and the work necessary to unplugging the TBTD transporter is provided by the inner membrane protein TonB.
It is known that TonB binds to the plug-domain, recognising a pattern in the sequence of the plug-domain called “Ton box” (the handle). TonB grabs the handle and pulls off the plug, effectively unplugging the TBDT. We have previously shown (7), using a combination of atomic force spectroscopy and physical models, that the interaction is mechanically strong enough to drive plug unfolding prior to its dissociation, leaving a pore that is sufficiently large for nutrients to diffuse inside the cell. What is not yet known, is how the force necessary to unplug the TBDT is generated by the TonB.

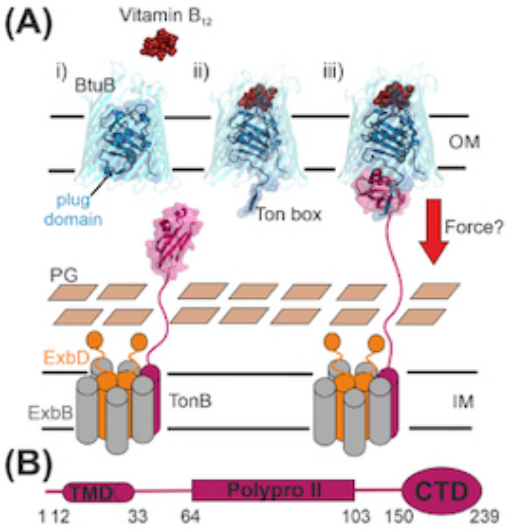

Figure 1. TonB-dependent import across the periplasm in Gram negative bacteria. (A) (i) in the absence of ligand, the lumen of the TBDT (BtuB here) is plugged. (ii) Upon ligand binding, the Ton box becomes unstructured in the periplasm, allowing engagement (iii) with TonB. (B) Ton B comprises of three structured components, separated by disordered regions. (C) Sequence of the intrinsically disordered region of TonB.

(C) ^{33}HQVIELPAPAQPISVTMVAPADLEPPQAVQPPPEPVV(EP)$_4$IPEPPKEAPVVIE(KP)$_6$
VKKVEQPKRDVKPVESRPASPFENTAPARPTSSTATAATSKPVTSVA^{150}S

In E. coli, TonB is a 239 aa protein comprising three regions: an N-terminal transmembrane alpha-helix domain (TMD,residues 12-32) is linked to the 89-residue globular CTD (residues 150- 239) via a largely disordered linker domain (the focus of this study).
The disordered linker comprises three regions – two disordered terminal regions of unknown function (~33-65 and ~101-149) separated by a proline-rich region (residues 66-100) that contains an unusual proline-rich amino acid sequence (in black in Figure 1C) shown to form a so-called polyproline II helix able to span lengths of ~14 nm (11). We previously showed by atomic force spectroscopy that the disordered region, astonishingly, requires large forces to be fully extended; also, such mechanical resilience is heavily modulated by the salt concentration (7): compact, mechanically resilient conformations are mostly seen at high salt concentration.

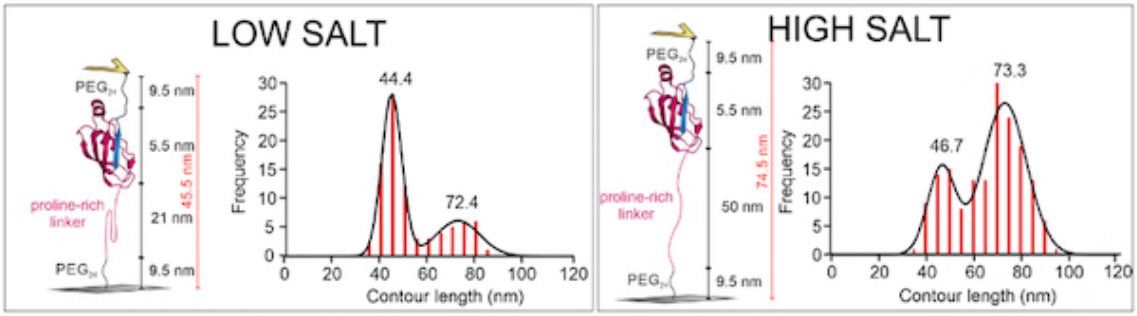

Figure 2: (A) The TonB linker domain populates two mechanical phenotypes: compact and force-resistant, evidenced by the 44.4 nm peak in the end-to-end contour length (Lc) frequency histogram (predominating at low [NaCl], left) and an extensible conformation (predominating at high [NaCl], right). Schematic (left) defines the Lc at rupture of the non-covalent TonB-Ton box complex linked to both the AFM tip and the surface by unstructured PEG linkers.

Unpublished small angle X ray scattering (SAXS) experiments also showed that at low salt, the IDR of TonB is collapsed, while it is expanded at high salt concentration. Simulations using atomistic molecular models also showed that, at low salt concentration, the IDR of TonB is mechanically resilient and collapsed at low salt, and mechanically weak and expanded at high salt concentration.

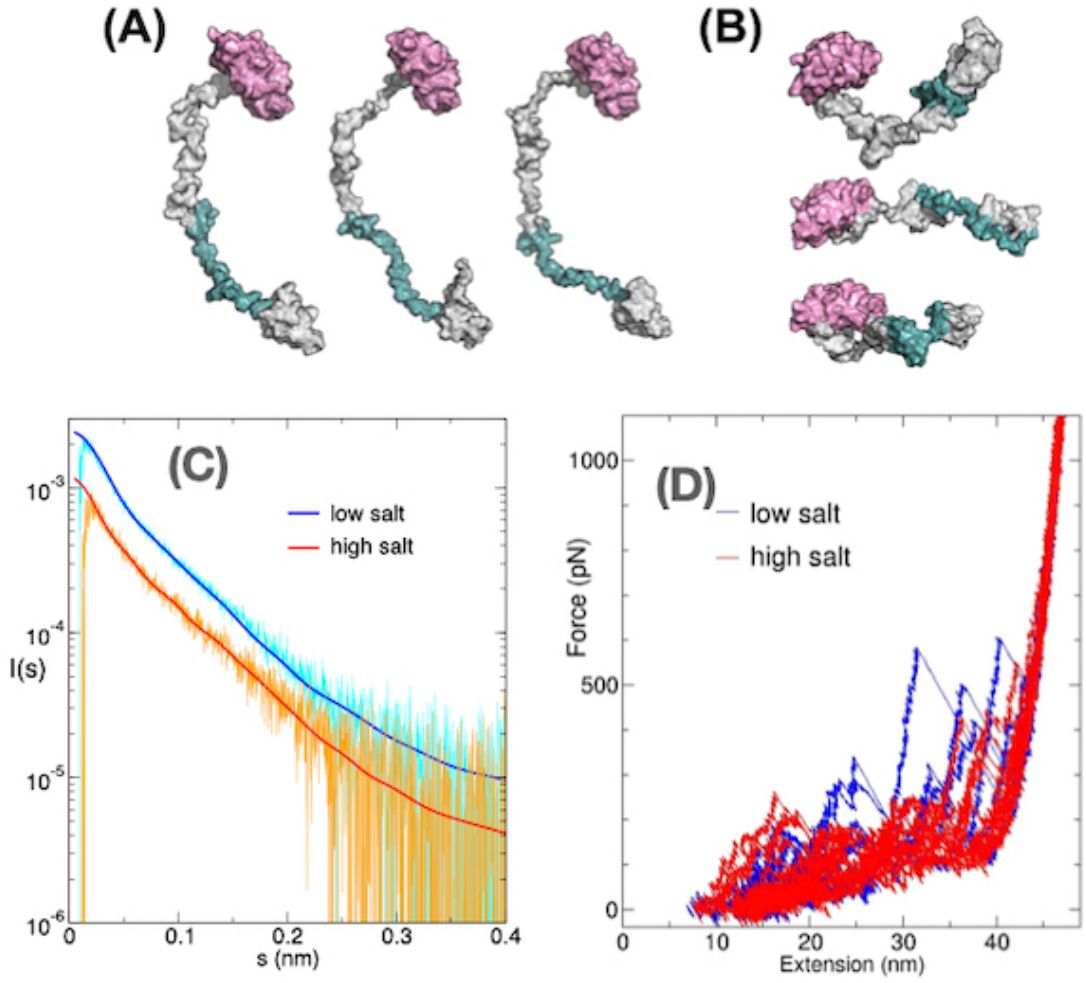

Figure 3:TonB populates strong and weak force phenotypes. Low free energy structures compatible with experimental SAXS in low (A) and high (B) [NaCl]. The protein is shown in surface representation and the structured CTD in pink and the PE-PK repeats region in teal. (C) SAXS intensities and average estimated intensity from models in A and B. D) Force-extension profiles from steered MD simulations of low and high salt models show that while compact, high salt structures are mechanically weak.

Atomic force microscopy and modelling, while showing that environmental changes modulate the mechanical and structural properties of the IDR of TonB (called IDR-TonB for short below), do not reveal the existence of specific interactions responsible for the work-producing collapse. Here we focus on exploiting optical tweezers to obtain information that techniques previously used could not provide.

The conformational equilibria of TonB will be characterized at single-molecule level using a force-measuring optical tweezers setup built by Cecconi at the University of Modena and Reggio Emilia (12, 13). Individual proteins will be manipulated with sub-nanometer precision using polystyrene beads and DNA molecular handles, as described in (14) and depicted in Figure 4, and their conformational changes will be monitored in real time with high temporal resolution (15). Optical tweezers will be used to produce traces of the transition between compact and extended conformations at forces within the physiological range (from a few to a few tens of pN).

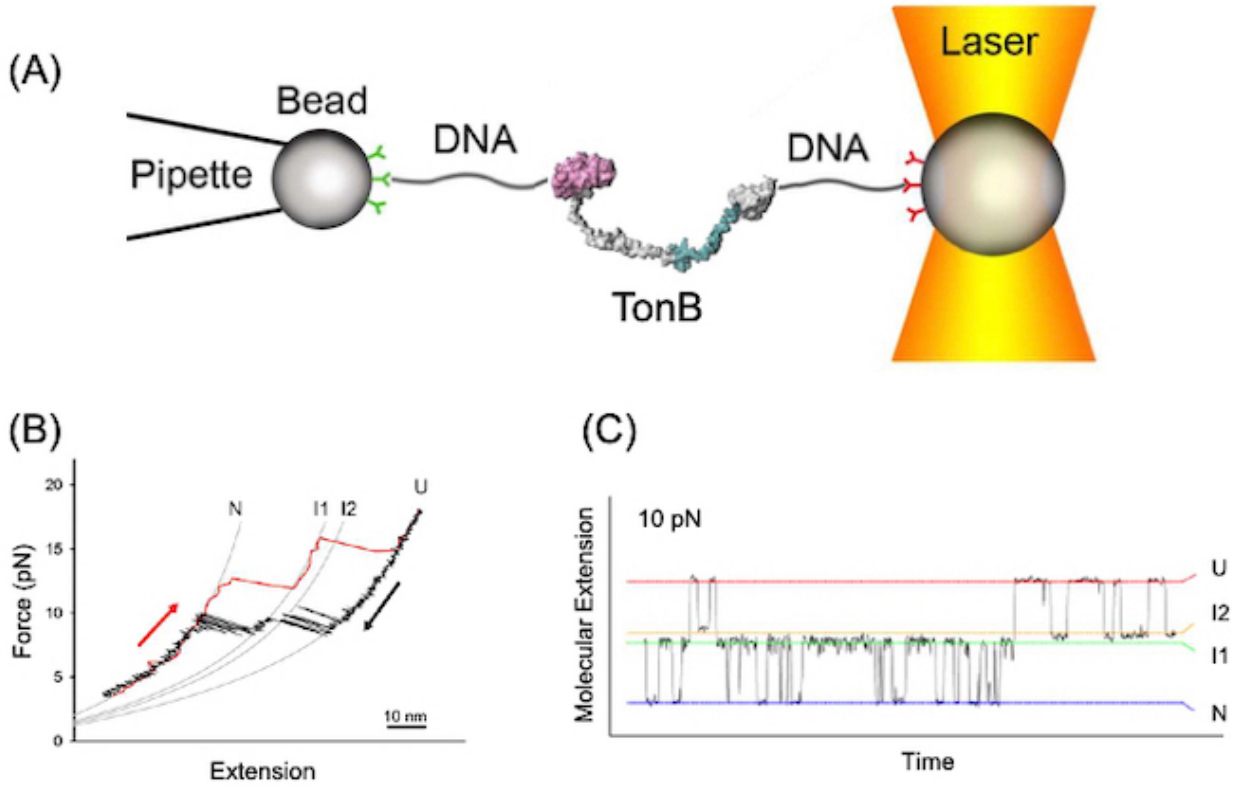

Figure 4: A) Schematic representation of the optical tweezers. Single proteins manipulated between two polystyrene beads by means of ~500 bp DNA molecules. B) In force-ramp experiments the protein is stretched and relaxed multiple times. C) In constant-force experiment the force applied on TonB is kept constant through a feedback mechanism: the protein fluctuates between different conformations. Examples here shown for a protein with a native and unfolded state and two on-pathway intermediates.

Experiments will be performed first in conditions in which the protein is “soft”. We expect to see a multiplicity of states stabilised by non-specific interactions. We also expect that the population of those force-bearing states will vary smoothly with the change in salt concentration. We seek the maximum sensitivity in the experiment to identify force-bearing states by their contour length.

Different approaches will be taken to manipulate individual TonB molecules (Figure 4A). In constant-velocity experiments (Figure 4B) the force applied on TonB will be increased and relaxed cyclically to induce the unfolding and refolding of the molecule, thus producing force-extension cycles (13). Under tension changes in molecular extension generate discontinuities (rips) in the recorded traces that allow us to follow in real-time the formation and loss of force-bearing states. Optical tweezers allow collection of many traces for the same molecule; hence a statistically relevant sample can be easily collected. Good statistics is very important within this project because distributions will be broad given the expected to be heterogeneous. The detected discontinuities can be characterized in terms of extension and forces. Applying the generalized Crooks fluctuation theorem to such non-equilibrium traces, has been shown before (16) that the corresponding change in free energy can be estimated.

In constant-force experiments (Figure 4C), the applied force will be kept constant through a feedback mechanism and changes in the extension of the molecule, as it fluctuates at equilibrium between different conformations (15), can be recorded and analysed in terms of occupancy of states and transition rate between states. Such experiment may not give results as clear as those relative to a protein with a well-defined native state and a small number of intermediate states, as shown in Figure 4C, but will be in all case useful to interpret the results of constant-velocity experiments.

To extract thermodynamic and kinetic information, the constant-force data will be analysed with an adapted Hidden Markov Model (HMM) algorithm (17, 18) that estimates the transition rates between the different molecular conformations, together with the mean value and standard deviation of the extension associated with them. In contrast to more traditional methods of analysis (i.e., discriminating the states based on a set of selected thresholds), the HMM approach can distinguish between states very similar in extension because it makes full use of the information about the time sequence of the observations. From the rates, the salient features of the free energy landscape (energy of the intermediate states, height, and position of the energy barriers between different molecular conformations) of TonB will be reconstructed. These experiments will be performed in a broad range of salt concentrations and in a broad range of pH to investigate the effect of these environment conditions on the conformational equilibrium of TonB. The effect of pH is of particular interest because salt concentration in the periplasm is about constant, while local variations of proton concentration occur.

These experiments pose clear challenges but have a good chance of success as several studies have shown that studying IDPs with optical tweezers is indeed possible. For example, exploiting the high temporal resolution and low stiffness of optical tweezers Neupane et al. were able to study the conformational equilibria of α-synuclein, a key driver of Parkinson’s disease (19). Multiple metastable states were detected at low forces and a reconstruction of the energy landscape revealed it to be flat but rough, representing a direct quantification of a proposed feature of IDP landscapes (20).
Optical tweezers data, even in the best-case scenario, are not sufficient to identify which specific interactions or interaction patterns are responsible for the “functional collapse” of TonB. Modelling and molecular dynamics simulations will be instrumental determining the ensemble of force-bearing structures that become populated in specific external conditions. Preliminary simulations have been performed with models that provided good agreement with the overall shape of the protein and the different response to applied forces, but they were limited by the quality of the model (not sufficiently detailed to accurately represent the effect of the environment) and by the limited statistics that models with such level of details could provide. Here we will use two very different molecular models. A coarse

grained one (21), which allows a thorough sampling of the conformation space of the polypeptide. In such a model each residue is represented by a single bead, a sequence-based statistical torsion potential is applied to all residues, and a screened Coulomb potential that is applied to all residues with non-zero charges. It has provided excellent predictions for the binding affinity of highly charged intrinsically disordered proteins and we believe it may, analogously, predict the collapsed structures of IDR-TonB, and their mechanical properties. We will also use a much more detailed model, so that the effect of the solvent, salt concentration and pH can be described as accurately as currently possible (22).

A common approach to generate structural ensembles at the molecular level is to use experimental observables as restraints in simulations or to extract compatible ensembles of structures. Both approaches are problematic if the experimental observables are sparse or correlated. Here, building on a workflow exemplified by our preliminary data (that obtained different mechanical phenotypes using SAXS data alone) we will use additional NMR and FRET data currently being collected at University of Leeds, to generate ensemble of structures whose properties are, on average, simultaneously in agreement with all experimental data.

We will initially use the computationally much more efficient coarse-grained model to sample the conformation space at different values of the electrostatic screening length (and adjustable parameter in the model) that mimics salt concentration. Simulations using the detailed, all-atom, constant-pH model will also be performed starting from structures representative of populated states of the coarse-grained model, which also agree with the experimental data mentioned above. The effect of external conditions (i.e. ionic strength and pH) on the heterogeneous ensembles of conformations sampled will be assessed using linear mixed models on a number of significative outcomes (microscopic observables, e.g. principal components of all amino acid pairwise distances). Such analysis will also provide the hierarchical structure of metastable states sampled in the simulations. Macroscopic experimental observables (SAXS intensities, FRET efficiencies, NMR chemical shifts, PREs and HX protection factors) for the states generated in silico will be calculated and compared to experimentally-derived values. Clustering of states sampled during the simulation (e.g. using Gaussian mixture models) will allow us to estimate the macroscopic observables of such states and find the best possible agreement. The subset of conformations that best reproduce the complete set of experimental properties will be investigated for common structural features and possibly motifs that may impart different mechanical properties to TonB under different salt concentrations and pH. Finally, the validity of each conformational subset will be assessed by measuring their mechanical properties using steered molecular dynamics simulations.

We expect, as preliminary results showed already, that steered molecular dynamics simulations will reproduce different mechanical properties observed by optical tweezers. The remaining challenge will consist in classifying pairwise intrachain force-bearing interactions and correlating them with patterns in the mechanical response. Integrating the outputs from experimental results to obtain a self-consistent model at molecular resolution would provide a step-change in the understanding of this process. Our predictions will be used to design TonB variants that modulate these phenotypes. Such variants will be expressed and purified in the laboratory Prof. David Brockwell (University of Leeds) and their mechanical properties measured in Cecconi’s laboratory. This may likely happen after the lifetime of the present project; we commit to find additional funding to continue the research, and find additional collaborators who will assess our predictions in vivo, a crucial step into developing molecules that mimic the modifications in the polypeptide chain of TonB that hinder the ability of E. coli to feed on specific nutrients, and provide leads for novel, much needed, antibacterial therapies.

Dissemination of the results will occur through the usual channels: publishing results in high profile specialised and generic journals and presenting results at international

conferences. We also consider crucial in the context of this specific funding, that our interdisciplinary biophysical methodology be shared with other research groups locally and further afield in Italy. Outreach is particularly important in this country where traditional separation of fields of knowledge is still customary. We will seek opportunity to provide demonstrations, also using virtual reality, of single molecule manipulation.

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3. Project development, with identification of the role of each research unit, with regards to related modalities of integration and collaboration

The project will be developed by two postdoctoral researchers. One, based in Cecconi's laboratory in Modena will perform the optical tweezers experiments. The other, based in Paci's computational group in Bologna, will perform the analysis of the data through the lenses of theoretical modelling and molecular simulation. The proposal involves a close integration of experiment, theory and computation.
The geographical proximity between the two sites will make possible regular in-person exchanges that will benefit the development of the two postdoctoral researchers and the advancement of the project. Key to the feasibility of the project is the ongoing collaboration between the Paci group and Brockwell laboratory in Leeds (UK). David Brockwell (University of Leeds, UK) will gift all the constructs needed for the experiments, so that optical tweezer measurements will be feasible as soon as the postdoctoral researchers are recruited. Brockwell (PI), Paci and Karamanos (co-I) have been recently awarded a three-years grant at University of Leeds, where Paci was previously employed, to study the phenomenon of bacterial import on nutrients from an orthogonal point of view: the project involves the use of NMR and single molecule fluorescence in vitro and in vivo. The integration of the two projects, as well as sharing data and resources, will be a major asset to the success of this highly interdisciplinary endeavour.

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4. Possible application potentialities and scientific and/or technological and/or social and/or economic impact

A steadily increasing number of proteins are discovered whose function is not dependent to them to have a well-defined three-dimensional structure. While technological (e.g., cryoEM) and computational methods (physical or data based) are providing plenty of protein structures or reliable models of them, their purpose is limited by the fact that they provide no useful information on intrinsically disordered proteins. Intrinsically disordered proteins are now at the centre of the attention because of their central role in many diseases, and an increasing number of biological phenomena such as the intriguing one we aim to study within this PRIN. We propose here to provide the broader community of scientists studying intrinsic protein disorder and function an approach that could be used in many different scenarios in which mechanical properties are a direct consequence of the modulation of the disorder at the single protein level. The specific project focuses on a protein essential for bacteria to absorb nutrients; molecular level understanding of its inner mechanisms would provide a novel, exploitable strategy to antibacterial therapy, which would have a dramatic social and economical impact.

5. Financial aspects: costs and funding for each research unit

nº		Total cost (euro)	Co-funding (item A.1) (euro)	MUR funding (other items) (euro)
1.	PACI Emanuele	162.213	37.244	124.969
2.	CECCONI Ciro	143.102	18.096	125.006
	Total	**305.315**	**55.340**	**249.975**

6. Bibliography

1. Oldfield, C. J., and A. K. Dunker. 2014. Intrinsically disordered proteins and intrinsically disordered protein regions. Annu Rev Biochem 83:553-584.
2. Qian, Y. Q., G. Otting, K. Furukubo-Tokunaga, M. Affolter, W. J. Gehring, and K. Wuthrich. 1992. NMR structure determination reveals that the homeodomain is connected through a flexible linker to the main body in the Drosophila Antennapedia protein. Proc Natl Acad Sci U S A 89:10738-10742.
3. Trombitas, K., M. Greaser, S. Labeit, J. P. Jin, M. Kellermayer, M. Helmes, and H. Granzier. 1998. Titin extensibility in situ: entropic elasticity of permanently folded and permanently unfolded molecular segments. J Cell Biol 140:853-859.
4. Rauscher, S., and R. Pomes. 2012. Structural disorder and protein elasticity. Adv Exp Med Biol 725:159-183.
5. Uversky, V. N., N. V. Narizhneva, T. V. Ivanova, M. D. Kirkitadze, and A. Tomashevski. 1997. Ligand-free form of human alpha-fetoprotein: evidence for the molten globule state. Febs Lett 410:280-284.
6. Dunker, A. K., and Z. Obradovic. 2001. The protein trinity--linking function and disorder. Nat Biotechnol 19:805-806.
7. Hickman, S. J., R. E. M. Cooper, L. Bellucci, E. Paci, and D. J. Brockwell. 2017. Gating of TonB-dependent transporters by substrate-specific forced remodelling. Nat Commun 8:14804.
8. Paci, E., and M. Karplus. 2000. Unfolding proteins by external forces and temperature: the importance of topology and energetics. Proc Natl Acad Sci U S A 97:6521-6526.
9. Cecconi, C., E. A. Shank, C. Bustamante, and S. Marqusee. 2005. Direct observation of the three-state folding of a single protein molecule. Science 309:2057-2060.
10. Noinaj, N., M. Guillier, T. J. Barnard, and S. K. Buchanan. 2010. TonB-Dependent Transporters: Regulation, Structure, and Function. Annu Rev Microbiol 64:43-60.
11. Kohler, S. D., A. Weber, S. P. Howard, W. Welte, and M. Drescher. 2010. The proline-rich domain of TonB possesses an extended polyproline II-like conformation of sufficient length to span the periplasm of Gram-negative bacteria. Protein Sci 19:625-630.
12. Heidarsson, P. O., I. Valpapuram, C. Camilloni, A. Imparato, G. Tiana, F. M. Poulsen, B. B. Kragelund, and C. Cecconi. 2012. A highly compliant protein native state with a spontaneous-like mechanical unfolding pathway. J Am Chem Soc 134:17068-17075.
13. Sonar, P., L. Bellucci, A. Mossa, P. O. Heidarsson, B. B. Kragelund, and C. Cecconi. 2020. Effects of Ligand Binding on the Energy Landscape of Acyl-CoA-Binding Protein. Biophys J 119:1821-1832.
14. Cecconi, C., E. A. Shank, S. Marqusee, and C. Bustamante. 2011. DNA molecular

handles for single-molecule protein-folding studies by optical tweezers. Methods Mol Biol 749:255-271.
15. Choudhary, D., B. B. Kragelund, P. O. Heidarsson, and C. Cecconi. 2018. The Complex Conformational Dynamics of Neuronal Calcium Sensor-1: A Single Molecule Perspective. Front Mol Neurosci 11:468.
16. Alemany, A., A. Mossa, I. Junier, and F. Ritort. 2012. Experimental free-energy measurements of kinetic molecular states using fluctuation theorems. Nature Physics 8:688-694.
17. Rabiner, L. R. 1989. A tutorial on hidden Markov models and selected applications in speech recognition. Proceedings of the IEEE 77:257-286.
18. Heidarsson, P. O., M. R. Otazo, L. Bellucci, A. Mossa, A. Imparato, E. Paci, S. Corni, R. Di Felice, B. B. Kragelund, and C. Cecconi. 2013. Single-molecule folding mechanism of an EF-hand neuronal calcium sensor. Structure 21:1812-1821.
19. Neupane, K., A. Solanki, I. Sosova, M. Belov, and M. T. Woodside. 2014. Diverse metastable structures formed by small oligomers of alpha-synuclein probed by force spectroscopy. Plos One 9:e86495.
20. Solanki, A., K. Neupane, and M. T. Woodside. 2014. Single-molecule force spectroscopy of rapidly fluctuating, marginally stable structures in the intrinsically disordered protein alpha-synuclein. Phys Rev Lett 112:158103.
21. Borgia, A., M. B. Borgia, K. Bugge, V. M. Kissling, P. O. Heidarsson, C. B. Fernandes, A. Sottini, A. Soranno, K. J. Buholzer, D. Nettels, B. B. Kragelund, R. B. Best, and B. Schuler. 2018. Extreme disorder in an ultrahigh-affinity protein complex. Nature 555:61-66.
22. Radak, B. K., C. Chipot, D. Suh, S. Jo, W. Jiang, J. C. Phillips, K. Schulten, and B. Roux. 2017. Constant-pH Molecular Dynamics Simulations for Large Biomolecular Systems. J Chem Theory Comput 13:5933-5944.

B.2

1. Scientific Curriculum of the Principal Investigator

Emanuele Paci
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Brief biography
I am a natural scientist whose career has focussed on how physical principles manifest themselves on the behaviour of complex molecular systems. I obtained a Laurea (MSc) summa cum laude in condensed matter physics from University La Sapienza (Rome) and a Doctorat (PhD) summa cum laude in physical chemistry University Pierre et Marie Curie (Paris) in 1996. Between 1996 and 2001 I was a Marie Curie and HFSP postdoctoral fellow under the joint supervision of Nobel laurate Martin Karplus (Strasbourg/Harvard) and Sir Chris Dobson (Oxford). During my postdoc I have pioneered the development of methods for integrated structural biology using sparse experimental data. Between 2001 and 2004 I was assistant professor at University of Zurich; between 2004 and 2021 I have been associate professor at University of Leeds; I am associate professor at University of Bologna since 2021. The central object of my research are the macromolecules involved in life sciences. I developed novel approaches to integrate biophysical experimental data using structural and mathematical models. With collaborators worldwide we predicted yet undiscovered mechanical properties of biomolecules relevant in health and disease. I have trained >20 postgraduate and postdoctoral researchers, published >120 peer reviewed articles cited >14,000 times (h-index 47) and delivered >100 lectures worldwide.

Academic qualifications
University Pierre et Marie Curie, Paris, France. Docteur ès Sciences (PhD) Summa cum Laude (1996)
University La Sapienza, Rome, Italy, Laurea in Fisica, Summa cum Laude (theoretical condensed matter physics) (1990)

Research topics
Soft matter theoretical physics, computational physics applied to macromolecules, protein dynamics, protein folding, protein-protein interactions, development of methods for path sampling and free energy calculation, proteomics, modelling and interpretation of biophysical experimental data (mass spectrometry, NMR, small angle X-ray scattering, crystallography, single molecule force spectroscopy, cryo electron microscopy, kinetics, mutagenesis)

Positions held
2021-: Università di Bologna. Professore associato. Dipartimento di Fisica e Astronomia
2004 - 2021: University of Leeds. Associate Professor (at School of Physics and Astronomy until 2007, then School of Molecular and Cellular Biology).
2001 - 2004: Institute of Biochemistry, University of Zurich, Switzerland. Oberassistent.
1996 - 2001: Institut le Bel, University of Strasbourg, France. Post-doctoral research fellow (with M. Karplus)
1996 - 2001: Oxford Centre for Molecular Sciences. Post-doctoral visiting research fellow (with C. Dobson)
1993 - 1996: Commisariat à l'Energie Atomique, Saclay, France. Post-graduate research associate (supervisors M. Marchi and P. Turq)
1991 - 1993: Centre Européen de Calcul Atomique et Moléculaire, Orsay, France. Post-graduate research associate (with G. Ciccotti)

Fellowships
1999 - 2001 Marie Curie Post-doctoral Fellowship
1997 - 1999 Human Frontier Science Programme Post-doctoral Fellowship

Selected recent invited talks
January 2018, Protein folding dynamics Gordon Research Conference, Galveston, TX, USA
June 2017, Computational Aspects of Biomolecular NMR Gordon Research Conference, Sunday River, ME, USA
February 2017, Biophysical Society Annual Meeting, New Orleans
December 2016, International Conference on Computational Science and Engineering, Ho-Chi-Minh, Vietnam.
November 2015, Deep Carbon Observatory "Molecular adaptation to life at extremes", Geophysical Laboratory of the Carnegie Institution of Washington, DC, USA
August 2015, CECAM workshop "Intrinsically Disordered Proteins - Bringing together Physics, Computation and Biology", ETH Zurich
June 2015, Proteins Gordon Research Conference Holderness, NH, USA
June 2014, SocBin2014, International Conference Bioinformatics 2014, Toruń, Poland
Organisation of conferences/workshops
October 29-November 1, 2017 "Computational approaches to investigating allostery" (with S. Wodak and N. Dokholyan), EPFL, Lausanne.
February 14-19, 2016, "Models for protein dynamics 1976-2016" (with D. Tildesley and B. Roux), EPFL, Lausanne
October 12-14, 2015, "Free energy landscapes for protein folding. Consensus or dissensus?" (with A Caflisch and S. Krivov), ETH-Zurich
April 12-15, 2011, "Multiscale computational biomechanics" (with F Gräter), ETH-Zurich
September 19-22, 2006 "Protein folding and misfolding: Bringing theory close to experiment" (with M. Vendruscolo), Lyon, France
Sept 26-29, 2006, "Theory of single molecule force experiments and simulations" (with P. Olmsted and R. Best), Lyon, France
Sept 9-12, 2003, "Protein folding: Bringing theory and experiment closer together" (with M. Vendruscolo), Lyon, France

Current grants
Does functional misfolding of TonB drive import across the outer membrane of Gram negative bacteria? (04/22-03/25) D. Brockwell (PI), E. Paci, T. Karamanos (Co-I) £600,000
Exploiting the SARS-CoV-2 nsp14 3′-5′-exoribonuclease as a target for antiviral chemotherapy, MRC-UKRI Covid-19 call. M. Harris (PI), E Paci (co-I) £260,000
Design of novel vectors for gene therapy, Innovate UK-Freeline Therapeutics Ltd E. Paci (PI) (01/20-12/23) £327,348
Rational design of dynamic molecules for enhanced multivalent binding, Leverhulme Trust, Paci E (PI), Turnbull B (co-I) (01/19-12/22) £248,699

Selected past grants
Phosphodependent helix switches in cellular signalling, BBSRC BB/S00730X/1 Bayliss R (PI), Paci E (co-I) (01/19-12/21) £640,001
Synthetic Glycobiology - new strategies to build and functionalise proto-cells and proto-tissues, ERASynBio ERA-Net: ERASYNBIO1-050, acronym: SynGlycTis, , Turnbull B (PI), Paci, E et al. (co-I), awarded (09/2014-08/2017), € 2,500,000
Single Alpha Helical Domains: Designing Artificial Levers for Biological Molecules, BBSRC, Peckham M (PI), Paci E et al. (co-I), 01/2012-12/2014, £ 900,000
The thermodynamics and kinetics of proteins under tension Wellcome Trust 4 years studentship for Gael Radou, 10/2012-09/2015, £130,000
Theoretical and experimental study of the molecular mechanism of protein folding, misfolding and aggregation and possible connection with disease Paci E , Vriend G, Finkelstein AV, Darinskii AL, Bichkova VI. INTAS project 05-1000004-7747 (€ 150,000)
The mechanism of amyloid formation in a model peptide Wales D, Dobson CM, Vendruscolo M, Paci E , BBSRC (BB/D000718/1) (£ 223,000)

Teaching
Current
Physics (85289, Laurea in "Genomics", FaBiT)
Physical methods of biology (87995, Second cycle degree in "Physics", DIFA)

Past
Undergraduate: Lecturing for the Biochemistry BSc programme, level 1, 2, 3 (topics: Bioinformatics, Thermodynamics, Protein dynamics). Supervision of BSc (level 3) and MSc (level 4) final projects. Module management for 15 level 3 modules (final projects and advanced topics). Graduate: lectures on a number of biophysical topics for the Wellcome Trust doctoral programme “The Molecular Basis of Biological Mechanisms”.
Programme leader and lecturer for the MSc Bioinformatics (2008-2013). Lecturing for the “Statistical Mechanics” module at the School of Physics and Astronomy, University of Leeds, leader of the postgraduate course “Biomolecular simulation” at the Department of Biochemistry, University of Zurich. I participated as lecturer to a number of summer schools across Europe.
Other activities
Regularly review manuscript for reputable journals in the field (including Nature, PNAS, JACS, PRL, JCP, JPCB, JMB, Proteins, Biophysical J, J Comp Chem, JCTC) and grant proposal for UK funding agencies (BBSRC, EPSRC, MRC, Wellcome, Leverhulme) and international funding agencies (Germany, Switzerland, Holland, France, USA, Ireland, Poland, EU). Member of the visiting committee evaluating the Structural Biology Department of Institut Pasteur, Paris in 2010 and Institut de Biologie Structurale, Grenoble in 2014. Member of the selection committee for a professorship at University of Barcelona. Academic editor of PLoS ONE, Biomedical Physics and Bioengineering Express (IOP) and PeerJ.

PhD supervised
• Molecular modelling of the GLP-1 receptor – a prototypic family B GPCR, Carla Gomez-Santiago, 2020
• Investigation of mechano-transduction across bacterial protein networks, Sam Hickman, 2016
• Helicase Functional Dynamics Probed with Low-Resolution Experimental Data and Simulation, Gael Radou, 2015
• In-silico investigation of ion-pumping rotatory A- and V-type ATPases, Konstantinos Papachristos, 2014
• Synthetic design capsid-like particles, James Ross, 2014
• High-dimensional models of protein folding, Supreecha Rimratchada, 2012
• Dynamics and Thermodynamics of Protein-Ligand Interactions, Richard Malham, 2011
• Enhancing the interpretation of dynamic force spectroscopy experiments, Zu Thur Yew, 2010
• Free energy landscapes of proteins: combining experimental data with numerical simulation, Lucy Allen, 2009
• The effects of local dynamics and geometry on protein mechanical unfolding behaviour, Dan West, 2006
• Computational studies of protein binding and peptide aggregation, Raffaele Curcio, 2006

Publications
Author of 123 peer reviewed articles and 3 book chapters
Google Scholar: h-index=47, i10-index=101, total citations=14,060 (21/03/2022)
Publons: h-index=38, total citations=10,122 (21/03/2022)
Web of Science ResearcherID: B-1893-2010
ORCID: 0000-0002-4891-2768

2. Scientific Curriculum of the associated investigators

1. CECCONI Ciro
Curriculum vitae of Ciro Cecconi

Professor Ciro Cecconi has gained a long experience in the field of force spectroscopy working for several years, first as a PhD student and then as a postdoctoral fellow, at the University of California, Berkeley, in the laboratory of Professor Carlos Bustamante, one of the world’s leading scientists in the field of single molecule biophysics. During his stay in Berkeley, Professor Cecconi has developed a new experimental method to manipulate single proteins with optical tweezers, which has made it possible to revisit protein folding with a completely new approach. By manipulating one molecule at a time in the low force regime of optical tweezers, Cecconi et al. monitored in real time unfolding and refolding transitions of the proteins RNase H and T4 Lysozyme and characterized these reactions with unprecedented detail, uncovering information inaccessible to more traditional bulk techniques. The results of these studies, which were published in Science and Nature, represented a breakthrough in single molecule biophysics and gave rise to a new field of research that has been undertaken by several laboratories around the world.
In 2006 Professor Cecconi was awarded a Marie Curie International Reintegration Grant (IRG) from the European Community to return to Europe and work on a project entitled “Single protein folding pathways”, at the University of Modena and Reggio Emilia (UNIMORE), Italy. The same year he won a competition named «Rientro dei Cervelli», issued by the Italian Ministry of Education, University and Research (MIUR), receiving funding to set up a single-molecule biophysics laboratory at UNIMORE, where he built a high-resolution force-measuring optical tweezers setup. In Modena, professor

Cecconi has started several lines of research in the field of single molecule biophysics, establishing collaborations with national and international esteemed scientists, and publishing papers in prestigious journals, such as Structure, JACS and PNAS. Over the years, Professor Cecconi has been the scientific supervisor of several postdoctoral fellows and PhD students and he has been awarded national and international grants. Currently his research activity is focused on: i) folding pathways and energy landscapes of single proteins, ii) protein misfolding and aggregation, iii) mechanisms of action of chaperones studied at single molecule level, and iv) protein-ligand interaction mechanisms. Additional information on the curriculum vitae of Professor Cecconi can be found at http://personale.unimore.it/rubrica/dettaglio/ccecconi.

PROFESSIONAL EXPERIENCE

2014-present Associate Professor
Department of Physics, Informatics and Mathematics
University of Modena and Reggio Emilia (UNIMORE)
Modena, Italy.

2010-2014 Group Leader
CNR Institute of Nanoscience S3
c/o University of Modena and Reggio Emilia
Modena, Italy.

2006-2010 Researcher
within the program “Rientro dei Cervelli - Scientific field: Applied Physics”
UNIMORE
Modena, Italy.

2004-2006 Postdoctoral fellow
Supervisor: Professor Carlos J. Bustamante
Lawrence Berkeley National Laboratory
Berkeley, CA – USA.

1996-2003 Graduate student
Supervisor: Professor Carlos J. Bustamante
Department of Physics and Molecular and Cell Biology
University of California, Berkeley – USA.

1995-1996 Visiting Scholar
NATO-CNR Advanced Fellowship - Scientific field: Physics
Supervisor: Professor Carlos J. Bustamante
University of Oregon, Eugene – USA.

HIGHEST ACADEMIC DEGREE

Doctor of Philosophy (Ph.D.)
University of California, Berkeley, USA - 2003
Supervisor: Prof. Carlos J. Bustamante, Department of Physics and Molecular & Cell Biology
Thesis Title: Studies of the Mechanical Unfolding and Refolding of RNase H and T4 lysozyme.

FELLOWSHIPS AND HONORS

• Rientro dei Cervelli - Scientific field: Applied Physics, 2006-2010.

• Marie Curie International Reintegration Grant (IRG), 2006-2008

• NATO-CNR Advanced Fellowship - Scientific field: Physics, 1995-1996.

• - First poster award of the 5th International Symposium on Optical Tweezers in Life Sciences, Berlin, Germany, 2013

• - Best oral communication of section IVb: Biophysics and Medical Physics, SIF-XCII National Congress - Turin, 2006

• The paper (Heidarsson et al., PNAS 2014) was the subject of a press release published in the CNR News and on the Bulletin Electronique du Service Scientifique de l'Ambassade de France à Rome with the title “Quand la protéine ne fait pas un pli ".

• The papers (Heidarsson et al, JACS 2012; Heidarsson et al, PNAS 2014; Choudhary et al, Frontiers in Molecular Neuroscience 2018) were selected to be published in the highlights of the Activity Reports of CNR NANO in 2013, 2015 and 2020 http://www.nano.cnr.it/?mod=men&id=511

RESEARCH SUPPORT

Current Research Support

• FAR – UNIMORE, 2021-2022 (partner)
• Grant RER "High competences for research and technology transfer", Emilia-Romagna Region, Italy, 2020-2021 (PI)
• PRIN, Italian Ministry of Education, University and Research (MIUR), 2019-2022 (partner)
Completed Research Support
• FAR – UNIMORE, 2017-2019 (partner)
• FAR – UNIMORE, 2015-2016 (PI)
• Lundbeck Foundation (DK), 2013–2014 (partner)
• Research Fellow Program for Training and Research in Italian laboratories (TRIL), ICTP, Trieste, Italy, 2011-2012, (PI)
• Marie Curie International Reintegration Grant (IRG) (Europe) - 2006-2008 (PI)
• Fellowship “Rientro dei Cervelli”, MIUR, 2006-2010 (PI)

SELECTED PUBLICATIONS MOST RELEVANT TO THIS TASK

1. Cecconi C and Heidarsson PO, “From folding to function: Complex macromolecular reactions unraveled one-by-one with optical tweezers“, Essays Biochem (2021) 65 (1): 129–142.
2. Sonar, P., Bellucci, L., Mossa, A., Heidarsson, P. O., Kragelund, B. B., and Cecconi, C., “Effects of ligand binding on the energy landscape of Acyl-CoA-binding protein”, Biophysical Journal 119, 1821–1832, 2020
3. Choudhary D., Mossa A., Jadhav M., and Cecconi C., “Bio-Molecular Applications of Recent Developments in Optical Tweezers”, Biomolecules, 9(1), 23, 2019
4. Choudhary D, Kragelund BB, P. O. Heidarsson PO and Cecconi C, “The Complex Conformational Dynamics of Neuronal Calcium Sensor-1: A Single Molecule Perspective”, Frontiers in Molecular Neuroscience 11 (468), 1-8, 2018
5. Alemany A, Rey-Serra B, Frutos S, Cecconi C, Ritort F, “Mechanical Folding and Unfolding of Protein Barnase at the Single-Molecule Level”, Biophysical Journal 110, 63-74, 2016
6. Naqvi M, Heidarsson PO, Otazo MR, Mossa A, Kragelund BB, Cecconi C, “Single-Molecule Folding Mechanisms of the apo- and Mg2D-Bound States of Human Neuronal Calcium Sensor-1”, Biophysical Journal 109, 113-123, 2015
7. Heidarsson PO, Naqvi MM, Otazo MR, Mossa A, Kragelund BB, and Cecconi C, “Direct single-molecule observation of calcium-dependent misfolding in human neuronal calcium sensor-1”, Proceedings of the National Academy of Sciences of the United States of America, 111 (36):13069-13074, 2014
8. Caldarini M, Sonar P, Valpapuram I, Tavella D, Volonté C, Pandini V, Vanoni MA, Aliverti A, Broglia RA, Tiana G, Cecconi C, “The complex folding behavior of HIV-1-protease monomer revealed by optical-tweezer single-molecule experiments and molecular dynamics simulations”, Biophysical Chemistry 195, 32–42, 2014
9. Heidarsson PO, Otazo MR, Bellucci L, Mossa A, Imparato A, Paci E, Corni S, Di Felice R, Kragelund BB, Cecconi C. “Single-Molecule Folding Mechanism of an EF-Hand Neuronal Calcium Sensor.” Structure, 21 (10), 1812-1821, 2013
10. Heidarsson PO, Valpapuram I, Camilloni C, Imparato M, Tiana G, Poulsen FM, Kragelund BB, Cecconi C, “A highly compliant protein native state with a spontaneous-like mechanical unfolding pathway”, J. Am. Chem. Soc. 134(41), 17068-17065, 2012
11. Shank, E., Cecconi, C., Dill, J., Marqusee, S., Bustamante, C., “The folding cooperativity of a protein is controlled by the topology of its polypeptide chain”, Nature 465 (7298), 637-641, 2010
12. Cecconi, C., Shank, E., Marqusee, S., Bustamante, C., “Protein-DNA chimeras for single molecule mechanical folding studies with the optical tweezers.” European Biophysics Journal, 37 (6), 729-738, 2008
13. Cecconi, C., Shank, E., Bustamante, C., Marqusee, S., “Direct Observation of the Three-State Folding of a Single Protein Molecule.” Science, 309, 2057-2060, 2005

BOOK CHAPTERS
1. Heidarsson PO, Naqvi M, Sonar P, Valpapuram I, Cecconi C, “Conformational dynamics of single protein molecules studied with direct mechanical manipulation”, Advances in Protein Chemistry and Structural Biology, 92, 93-133, 2013
2. Cecconi, C., Shank, E., Marqusee, S. and Bustamante, C. “DNA handles for single molecule protein folding studies by optical tweezers.” DNA Nanotechnology, 255-271, 2011

Main ERC field
LS1_6 Biophysics
PE3_19 Biophysics

Bibliometric Indexes
h-index 18 (Google Scholar)
i10-index 21
Citations 2180

CONFERENCES AND SCHOOLS

Professor Cecconi has attended many national and international Conferences and Schools, often by invitation

SUPERVISOR OF GRADUATE STUDENTS AND POSTDOCTORAL FELLOWS

Supervisor of 5 postdoctoral fellows and 4 graduate students at UNIMORE

Co-Supervisor of a graduate student at the University of Copenhagen, Denmark

PEER-REVIEWING ACTIVITY

Reviewer of International proposals and journals, including Nature Communications, Nature Chemical Biology, Journal of the American Chemical Society, ACS Nano

RECENT COLLABORATIONS

- Professor Sander Tans, FOM Institute for Atomic and Molecular Physics [AMOLF], Amsterdam, The Netherlands
- Professor Serena Carra, UNIMORE, Italy
- Professor Birthe B. Kragelund, University of Copenhagen, Denmark
- Professor Pétur O. Heidarsson, University of Iceland, Iceland

3. Main Principal Investigator's scientific publications (Max. 20)

1. Skinner S. P., Follmer A. H., Ubbink M., Poulos T. L., Houwing-Duistermaat J. J., Paci E. (2021). Partial Opening of Cytochrome P450cam (CYP101A1) Is Driven by Allostery and Putidaredoxin Binding. BIOCHEMISTRY, vol. 60, p. 2932-2942, ISSN: 0006-2960, doi: 10.1021/acs.biochem.1c00406 - **Articolo in rivista**
2. Batchelor M, Papachristos K, Stofella M, Yew Z, Paci E (2020). Protein mechanics probed using simple molecular models. BIOCHIMICA ET BIOPHYSICA ACTA-GENERAL SUBJECTS, vol. 1864, ISSN: 0304-4165, doi: 10.1016/j.bbagen.2020.129613 - **Articolo in rivista**
3. Ross JF, Wildsmith GC, Johnson M, Hurdiss DL, Hollingsworth K, Thompson RF, Mosayebi M, Trinh CH, Paci E, Pearson AR, Webb ME, Turnbull WB (2019). Directed Assembly of Homopentameric Cholera Toxin B-Subunit Proteins into Higher-Order Structures Using Coiled-Coil Appendages. JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 141, p. 5211-5219, ISSN: 0002-7863, doi: 10.1021/jacs.8b11480 - **Articolo in rivista**
4. Skinner S. P., Radou G., Tuma R., Houwing-Duistermaat J. J., Paci E. (2019). Estimating Constraints for Protection Factors from HDX-MS Data. BIOPHYSICAL JOURNAL, vol. 116, p. 1194-1203, ISSN: 0006-3495, doi: 10.1016/j.bpj.2019.02.024 - **Articolo in rivista**
5. Whelan F, Lafita A, Griffiths SC, Cooper REM, Whittingham JL, Turkenburg JP, Manfield IW, John ANS, Paci E, Bateman A, Potts JR (2019). Defining the remarkable structural malleability of a bacterial surface protein Rib domain implicated in infection. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 116, p. 26540-26548, ISSN: 0027-8424, doi: 10.1073/pnas.1911776116 - **Articolo in rivista**
6. Wodak SJ, Paci E, Dokholyan NV, Berezovsky IN, Horovitz A, Li J, Hilser VJ, Behar I, Karanicolas J, Stock G, Hamm P, Stote RH, Eberhardt J, Chebaro Y, Dejaegere A, Cecchini M, Changeux JP, Bolhuis PG, Vreede J, Faccioli P▪▪▪(2019). Allostery in Its Many Disguises: From Theory to Applications. STRUCTURE, vol. 27, p. 566-578, ISSN: 0969-2126, doi: 10.1016/j.str.2019.01.003 - **Articolo in rivista**
7. Batchelor M, Paci E (2018). Helical Polyampholyte Sequences Have Unique Thermodynamic Properties. JOURNAL OF PHYSICAL CHEMISTRY. B, CONDENSED MATTER, MATERIALS, SURFACES, INTERFACES & BIOPHYSICAL, vol. 122, p. 11784-11791, ISSN: 1520-6106, doi: 10.1021/acs.jpcb.8b08344 - **Articolo in rivista**
8. Radom F, Pluckthun A, Paci E (2018). Assessment of ab initio models of protein complexes by molecular dynamics. PLOS COMPUTATIONAL BIOLOGY, vol. 14, ISSN: 1553-7358, doi: 10.1371/journal.pcbi.1006182 - **Articolo in rivista**
9. Gowdy J, Batchelor M, Neelov I, Paci E (2017). Nonexponential Kinetics of Loop Formation in Proteins and Peptides: A Signature of Rugged Free Energy Landscapes?. JOURNAL OF PHYSICAL CHEMISTRY. B, CONDENSED MATTER, MATERIALS, SURFACES, INTERFACES & BIOPHYSICAL, vol. 121, p. 9518-9525, ISSN: 1520-6106, doi: 10.1021/acs.jpcb.7b07075 - **Articolo in rivista**
10. Papachristos K, Muench SP, Paci E (2016). Characterization of the flexibility of the peripheral stalk of prokaryotic rotary A-ATPases by atomistic simulations. PROTEINS, vol. 84, p. 1203-1212, ISSN: 0887-3585, doi: 10.1002/prot.25066 - **Articolo in rivista**

11. Tych KM, Batchelor M, Hoffmann T, Wilson MC, Hughes ML, Paci E, Brockwell DJ, Dougan L (2016). Differential Effects of Hydrophobic Core Packing Residues for Thermodynamic and Mechanical Stability of a Hyperthermophilic Protein. LANGMUIR, vol. 32, p. 7392-7402, ISSN: 0743-7463, doi: 10.1021/acs.langmuir.6b01550 - **Articolo in rivista**
12. Tych KM, Batchelor M, Hoffmann T, Wilson MC, Paci E, Brockwell DJ, Dougan L (2016). Tuning protein mechanics through an ionic cluster graft from an extremophilic protein. SOFT MATTER, vol. 12, p. 2688-2699, ISSN: 1744-683X, doi: 10.1039/c5sm02938d - **Articolo in rivista**
13. Farrance OE, Paci E, Radford SE, Brockwell DJ (2015). Extraction of Accurate Biomolecular Parameters from Single-Molecule Force Spectroscopy Experiments. ACS NANO, vol. 9, p. 1315-1324, ISSN: 1936-0851, doi: 10.1021/nn505135d - **Articolo in rivista**
14. Gruszka DT, Whelan F, Farrance OE, Fung HKH, Paci E, Jeffries CM, Svergun DI, Baldock C, Baumann CG, Brockwell DJ, Potts JR, Clarke J (2015). Cooperative folding of intrinsically disordered domains drives assembly of a strong elongated protein. NATURE COMMUNICATIONS, vol. 6, ISSN: 2041-1723, doi: 10.1038/ncomms8271 - **Articolo in rivista**
15. Hickman SJ, Ross JF, Paci E (2015). Prediction of stability changes upon mutation in an icosahedral capsid. PROTEINS, vol. 83, p. 1733-1741, ISSN: 0887-3585, doi: 10.1002/prot.24859 - **Articolo in rivista**
16. Heidarsson PO, Otazo MR, Bellucci L, Mossa A, Imparato A, Paci E, Corni S, Di Felice R, Kragelund BB, Cecconi C (2013). Single-Molecule Folding Mechanism of an EF-Hand Neuronal Calcium Sensor. STRUCTURE, vol. 21, p. 1812-1821, ISSN: 0969-2126, doi: 10.1016/j.str.2013.07.022 - **Articolo in rivista**
17. Schlierf M, Yew ZT, Rief M, Paci E (2010). Complex Unfolding Kinetics of Single-Domain Proteins in the Presence of Force. BIOPHYSICAL JOURNAL, vol. 99, p. 1620-1627, ISSN: 0006-3495, doi: 10.1016/j.bpj.2010.06.039 - **Articolo in rivista**
18. Yew ZT, Schlierf M, Rief M, Paci E (2010). Direct evidence of the multidimensionality of the free-energy landscapes of proteins revealed by mechanical probes. PHYSICAL REVIEW E, STATISTICAL, NONLINEAR, AND SOFT MATTER PHYSICS, vol. 81, ISSN: 1539-3755, doi: 10.1103/PhysRevE.81.031923 - **Articolo in rivista**
19. Paci E, Karplus M (2000). Unfolding proteins by external forces and temperature: The importance of topology and energetics. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 97, p. 6521-6526, ISSN: 0027-8424, doi: 10.1073/pnas.100124597 - **Articolo in rivista**
20. Paci E, Karplus M (1999). Forced unfolding of fibronectin type 3 modules: An analysis by biased molecular dynamics simulations. JOURNAL OF MOLECULAR BIOLOGY, vol. 288, p. 441-459, ISSN: 0022-2836, doi: 10.1006/jmbi.1999.2670 - **Articolo in rivista**

4. Main scientific publications of the associated investigators (Max. 20, for each research unit)

1. **CECCONI Ciro**

1. Heidarsson, Pétur O, Cecconi, Ciro (2021). From folding to function: complex macromolecular reactions unraveled one-by-one with optical tweezers. ESSAYS IN BIOCHEMISTRY, vol. 65, p. 129-142, ISSN: 0071-1365, doi: 10.1042/EBC20200024 - **Articolo in rivista**
2. Choudhary D., Mediani L., Carra S., Cecconi C. (2020). Studying heat shock proteins through single-molecule mechanical manipulation. CELL STRESS & CHAPERONES, vol. 25, p. 615-628, ISSN: 1355-8145, doi: 10.1007/s12192-020-01096-y - **Articolo in rivista**
3. Sonar P., Bellucci L., Mossa A., Heidarsson P. O., Kragelund B. B., Cecconi C. (2020). Effects of Ligand Binding on the Energy Landscape of Acyl-CoA-Binding Protein. BIOPHYSICAL JOURNAL, vol. 119, p. 1821-1832, ISSN: 0006-3495, doi: 10.1016/j.bpj.2020.09.016 - **Articolo in rivista**
4. CHOUDHARY, DHAWAL, MOSSA, ALESSANDRO, JADHAV, MILIND SURESH, Cecconi, Ciro (2019). Bio-molecular applications of recent developments in optical tweezers. BIOMOLECULES, vol. 9, p. 1-19, ISSN: 2218-273X, doi: 10.3390/biom9010023 - **Articolo in rivista**
5. Carra, Serena, Alberti, Simon, Benesch, Justin L. P., Boelens, Wilbert, Buchner, Johannes, Carver, John A., Cecconi, Ciro, Ecroyd, Heath, Gusev, Nikolai, Hightower, Lawrence E.**...**(2019). Small heat shock proteins: multifaceted proteins with important implications for life. CELL STRESS & CHAPERONES, vol. 24, p. 295-308, ISSN: 1355-8145, doi: 10.1007/s12192-019-00979-z - **Articolo in rivista**
6. Choudhary, Dhawal, Kragelund, Birthe B., Heidarsson, Pétur O., Cecconi, Ciro (2018). The complex conformational dynamics of neuronal calcium sensor-1: A single molecule perspective. FRONTIERS IN

MOLECULAR NEUROSCIENCE, vol. 11, p. 1-8, ISSN: 1662-5099, doi: 10.3389/fnmol.2018.00468 - **Articolo in rivista**

7. Alemany, Anna, Rey Serra, Blanca, Frutos, Silvia, CECCONI, CIRO, Ritort, Felix (2016). Mechanical Folding and Unfolding of Protein Barnase at the Single-Molecule Level. BIOPHYSICAL JOURNAL, vol. 110, p. 63-74, ISSN: 0006-3495, doi: 10.1016/j.bpj.2015.11.015 - **Articolo in rivista**
8. Marcoux, Julien, Mangione, P. Patrizia, Porcari, Riccardo, Degiacomi, Matteo T, Verona, Guglielmo, Taylor, Graham W, Giorgetti, Sofia, Raimondi, Sara, Sanglier Cianférani, Sarah, Benesch, Justin LP[...](2015). A novel mechano-enzymatic cleavage mechanism underlies transthyretin amyloidogenesis. EMBO MOLECULAR MEDICINE, vol. 7, p. 1337-1349, ISSN: 1757-4676, doi: 10.15252/emmm.201505357 - **Articolo in rivista**
9. Naqvi, Mohsin M, Heidarsson, Petur O, Otazo, Mariela R, Mossa, Alessandro, Kragelund, Birthe B, CECCONI, CIRO (2015). Single-Molecule Folding Mechanisms of the apo- and Mg(2+)-Bound States of Human Neuronal Calcium Sensor-1. BIOPHYSICAL JOURNAL, vol. 109, p. 113-123, ISSN: 0006-3495, doi: 10.1016/j.bpj.2015.05.028 - **Articolo in rivista**
10. Caldarini, M., SONAR, PUNAM SURESH, SAMIDASS, VALPAPURAM IMMANUEL, Tavella, D., Volonte, C., Pandini, V., Vanoni, M. A., Aliverti, A., Broglia, R. A., Tiana, G.[...](2014). The complex folding behavior of HIV-1-protease monomer revealed by optical-tweezer single-molecule experiments and molecular dynamics simulations. BIOPHYSICAL CHEMISTRY, vol. 195, p. 32-42, ISSN: 0301-4622, doi: 10.1016/j.bpc.2014.08.001 - **Articolo in rivista**
11. Heidarsson, Petur O., Naqvi, Mohsin M., Otazo, Mariela R., Mossa, Alessandro, Kragelund, Birthe B., CECCONI, CIRO (2014). Direct single-molecule observation of calcium-dependent misfolding in human neuronal calcium sensor-1. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 111, p. 13069-13074, ISSN: 0027-8424, doi: 10.1073/pnas.1401065111 - **Articolo in rivista**
12. Heidarsson, Petur O., Otazo, Mariela R., BELLUCCI, LUCA, Mossa, Alessandro, Imparato, Alberto, Paci, Emanuele, CORNI, STEFANO, DI FELICE, ROSA, Kragelund, Birthe B., CECCONI, CIRO (2013). Single-Molecule Folding Mechanism of an EF-Hand Neuronal Calcium Sensor. STRUCTURE, vol. 21, p. 1812-1821, ISSN: 0969-2126, doi: 10.1016/j.str.2013.07.022 - **Articolo in rivista**
13. Mangione, P. Patrizia, Esposito, Gennaro, Relini, Annalisa, Raimondi, Sara, Porcari, Riccardo, Giorgetti, Sofia, Corazza, Alessandra, Fogolari, Federico, Penco, Amanda, Goto, Yuji[...](2013). Structure, Folding Dynamics, and Amyloidogenesis of D76N beta(2)-Microglobulin ROLES OF SHEAR FLOW, HYDROPHOBIC SURFACES, AND alpha-CRYSTALLIN. THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 288, p. 30917-30930, ISSN: 0021-9258, doi: 10.1074/jbc.M113.498857 - **Articolo in rivista**
14. Heidarsson, Petur O., SAMIDASS, VALPAPURAM IMMANUEL, Camilloni, Carlo, Imparato, Alberto, Tiana, Guido, Poulsen, Flemming M., Kragelund, Birthe B., CECCONI, CIRO (2012). A Highly Compliant Protein Native State with a Spontaneous-like Mechanical Unfolding Pathway. JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 134, p. 17068-17075, ISSN: 0002-7863, doi: 10.1021/ja305862m - **Articolo in rivista**
15. Shank, Elizabeth A., CECCONI, CIRO, Dill, Jesse W., Marqusee, Susan, Bustamante, Carlos (2010). The folding cooperativity of a protein is controlled by its chain topology. NATURE, vol. 465, p. 637-U134, ISSN: 0028-0836, doi: 10.1038/nature09021 - **Articolo in rivista**
16. CECCONI, CIRO, Shank, Elizabeth A., Dahlquist, Frederick W., Marqusee, Susan, Bustamante, Carlos (2008). Protein-DNA chimeras for single molecule mechanical folding studies with the optical tweezers. EUROPEAN BIOPHYSICS JOURNAL WITH BIOPHYSICS LETTERS, vol. 37, p. 729-738, ISSN: 0175-7571, doi: 10.1007/s00249-007-0247-y - **Articolo in rivista**
17. Tang, Mei, CECCONI, CIRO, Bustamante, Carlos, Rio, Donald C. (2007). Analysis of P element transposase protein-DNA interactions during the early stages of transposition. THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 282, p. 29002-29012, ISSN: 0021-9258, doi: 10.1074/jbc.M704106200 - **Articolo in rivista**
18. Brown, Ta, CECCONI, CIRO, Tkachuk, An, Bustamante, C, Clayton, Da (2005). Replication of mitochondrial DNA occurs by strand displacement with alternative light-strand origins, not via a strand-coupled mechanism. GENES & DEVELOPMENT, vol. 19, p. 2466-2476, ISSN: 0890-9369, doi: 10.1101/gad.1352105 - **Articolo in rivista**
19. CECCONI, CIRO, Shank, Ea, Bustamante, C, Marqusee, S. (2005). Direct observation of the three-state folding of a single protein molecule. SCIENCE, vol. 309, p. 2057-2060, ISSN: 0036-8075, doi: 10.1126/science.1116702 - **Articolo in rivista**
20. Tang, M, CECCONI, CIRO, Kim, H, Bustamante, C, Rio, Dc (2005). Guanosine triphosphate acts as a cofactor to promote assembly of initial P-element transposase-DNA synaptic complexes. GENES & DEVELOPMENT, vol. 19, p. 1422-1425, ISSN: 0890-9369, doi: 10.1101/gad.1317605 - **Articolo in rivista**