The evolution of allosteric networks: How do polypeptides function?

Laboratory for Dynamic Structural Biology
Giorgos Gouridis

IMBB-FORTH-CRETE

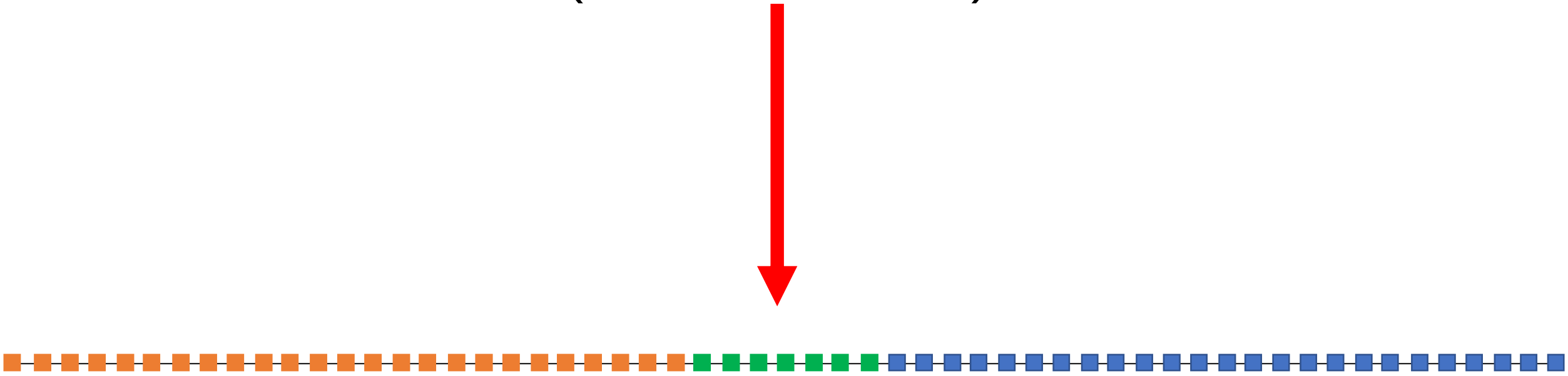
16-07-22
FORTH, CRETE, GR

The team

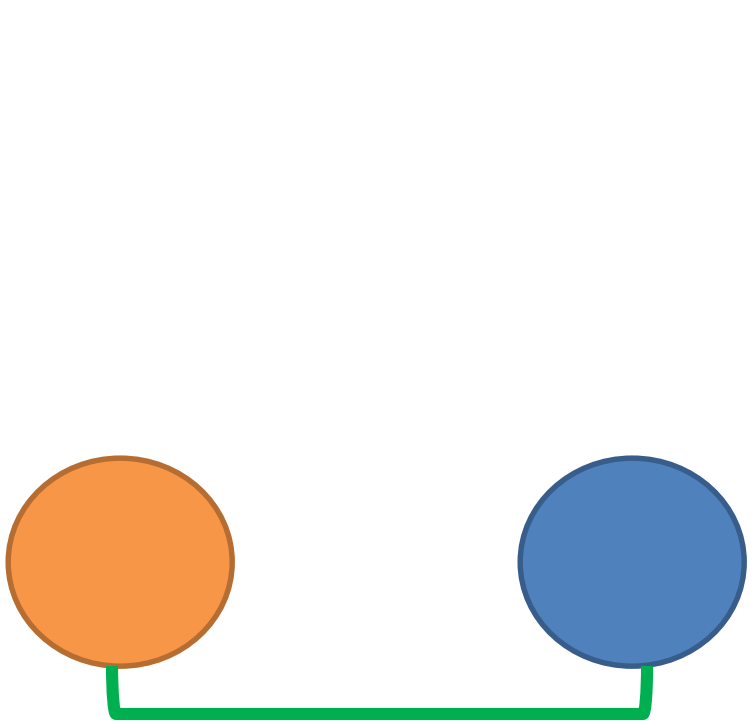
- Mary Providaki
- Dr. Yusran Muthahari
- Dr. Ruixue Xu
- PhD Chara Sarafoglou
- MSc Aliki Fotiadi
- BSc Eirini Triantou

The polypeptide biopolymer

Genetic code
(first secret of life)

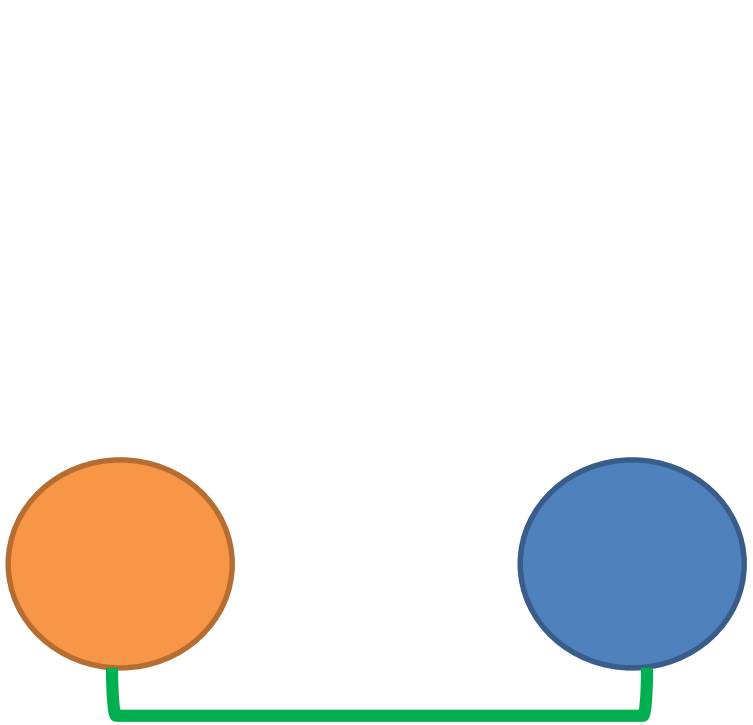


The properties of polypeptides

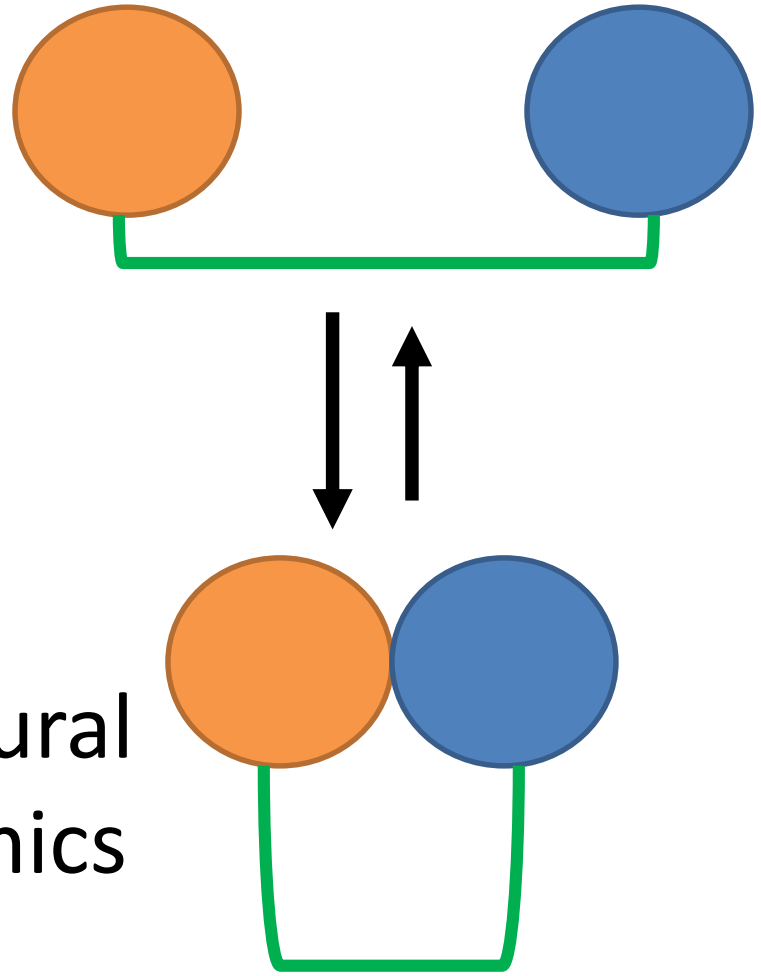


Folding dynamics

The properties of polypeptides



Folding dynamics



Native
Structural
Dynamics

Why Single-molecule approaches are essential to unravel structural dynamics??

100 μl 1 μM

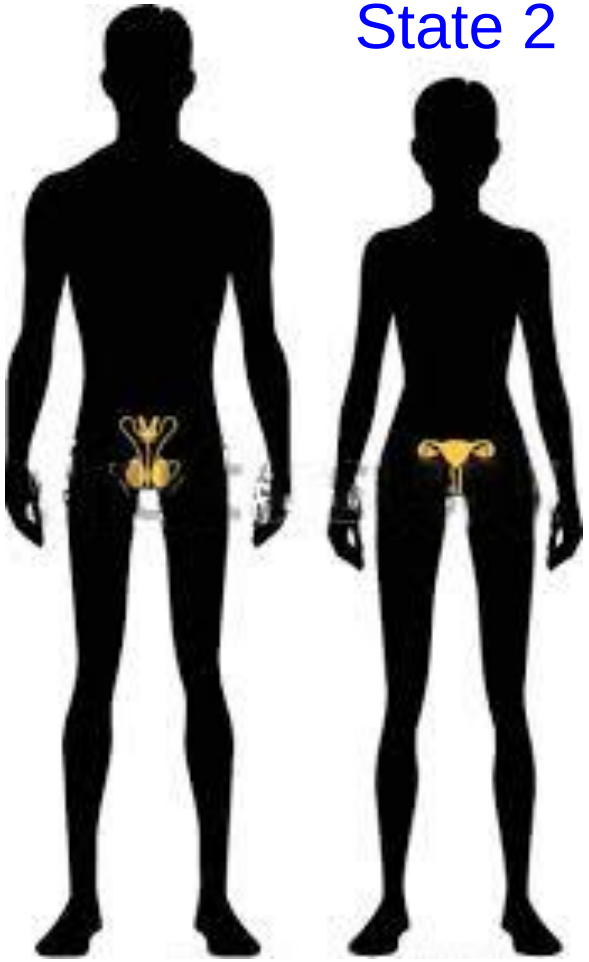
10^{13} =10 trillions protein structures

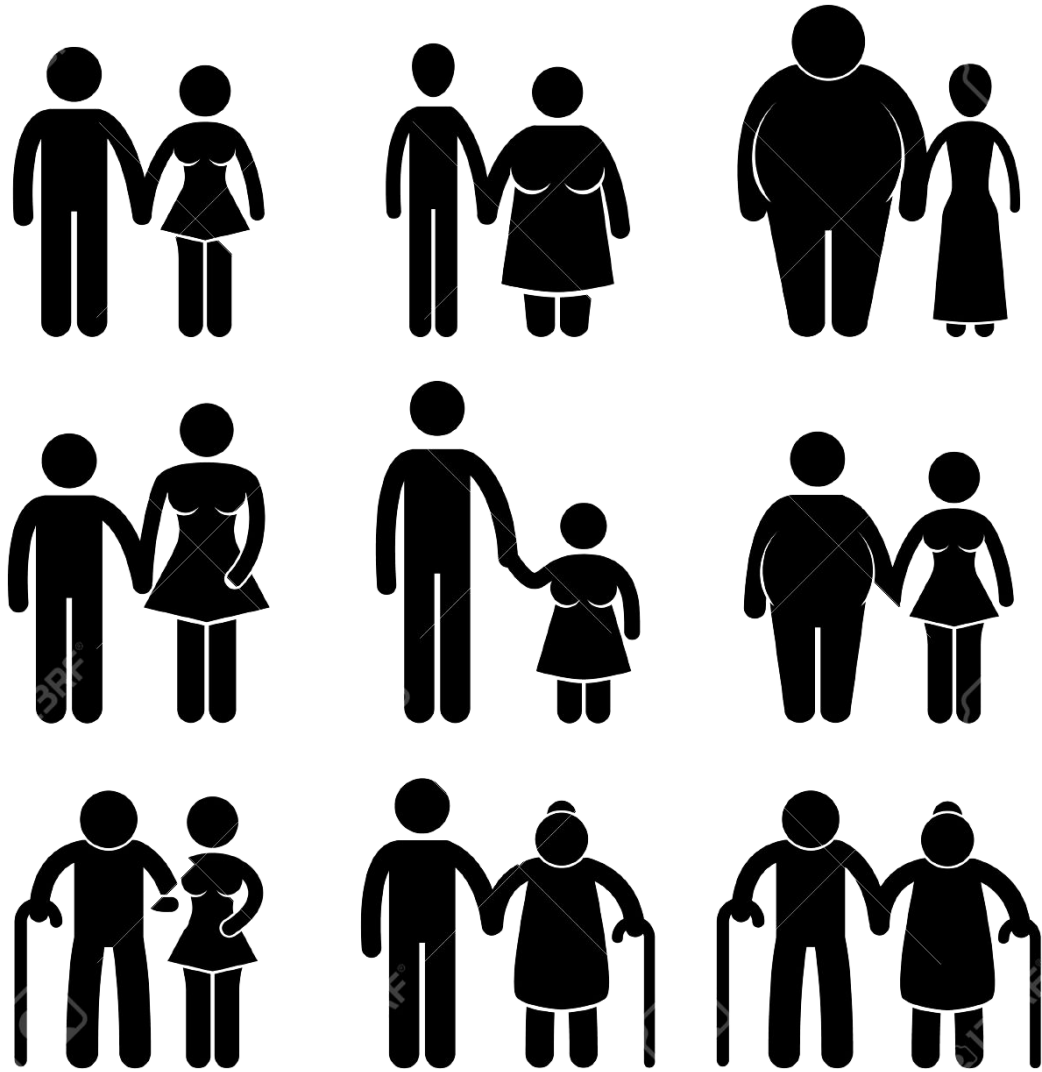
The ensemble average of human structure

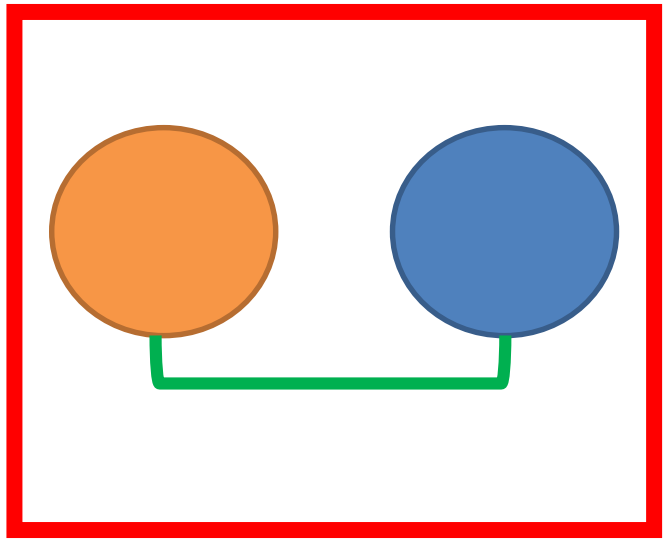
The ensemble average of human structure with single molecule resolution

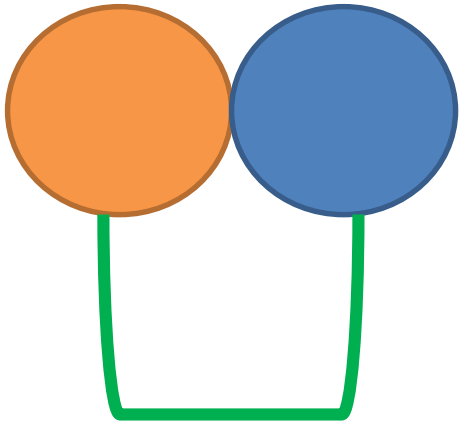
State 1

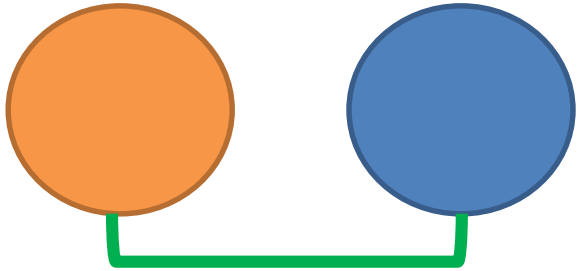
State 2

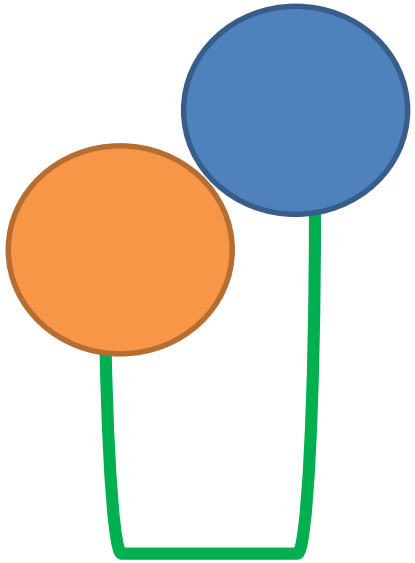


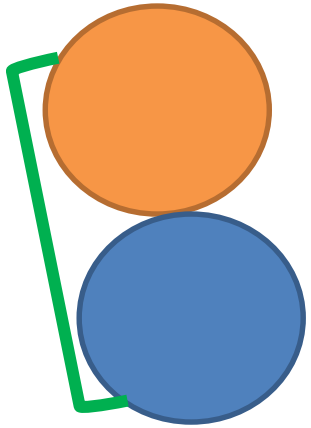


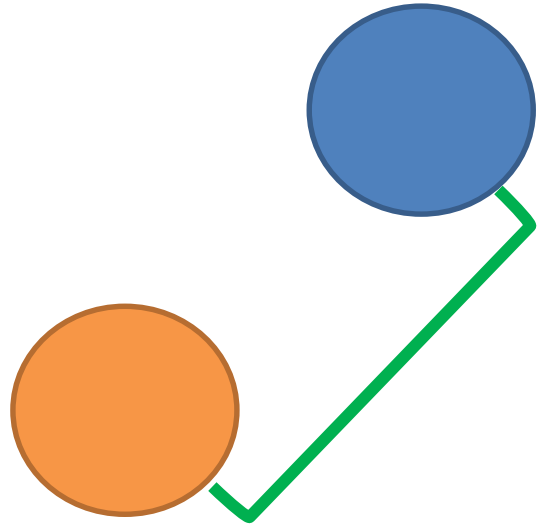




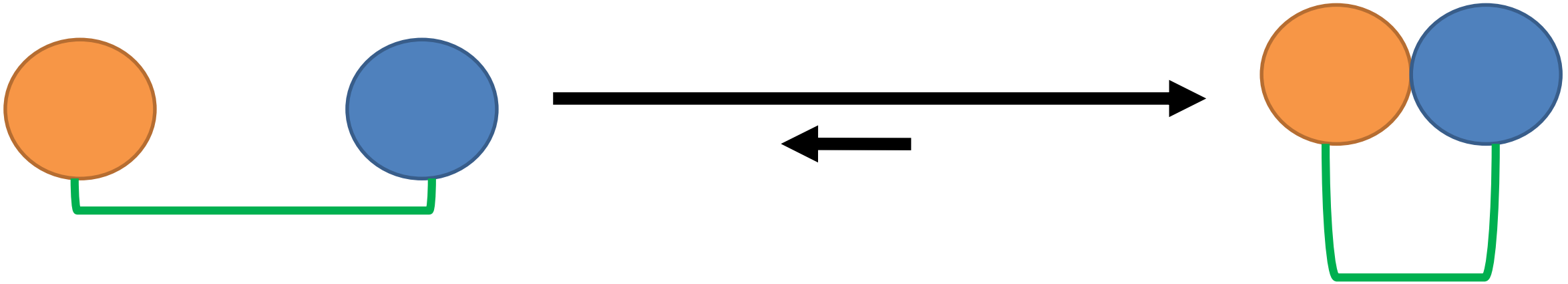




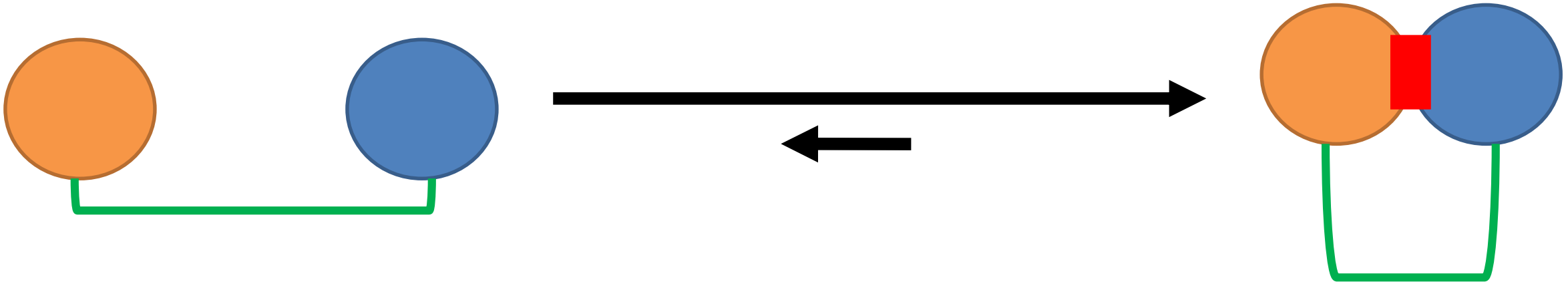




Native Structural Dynamics

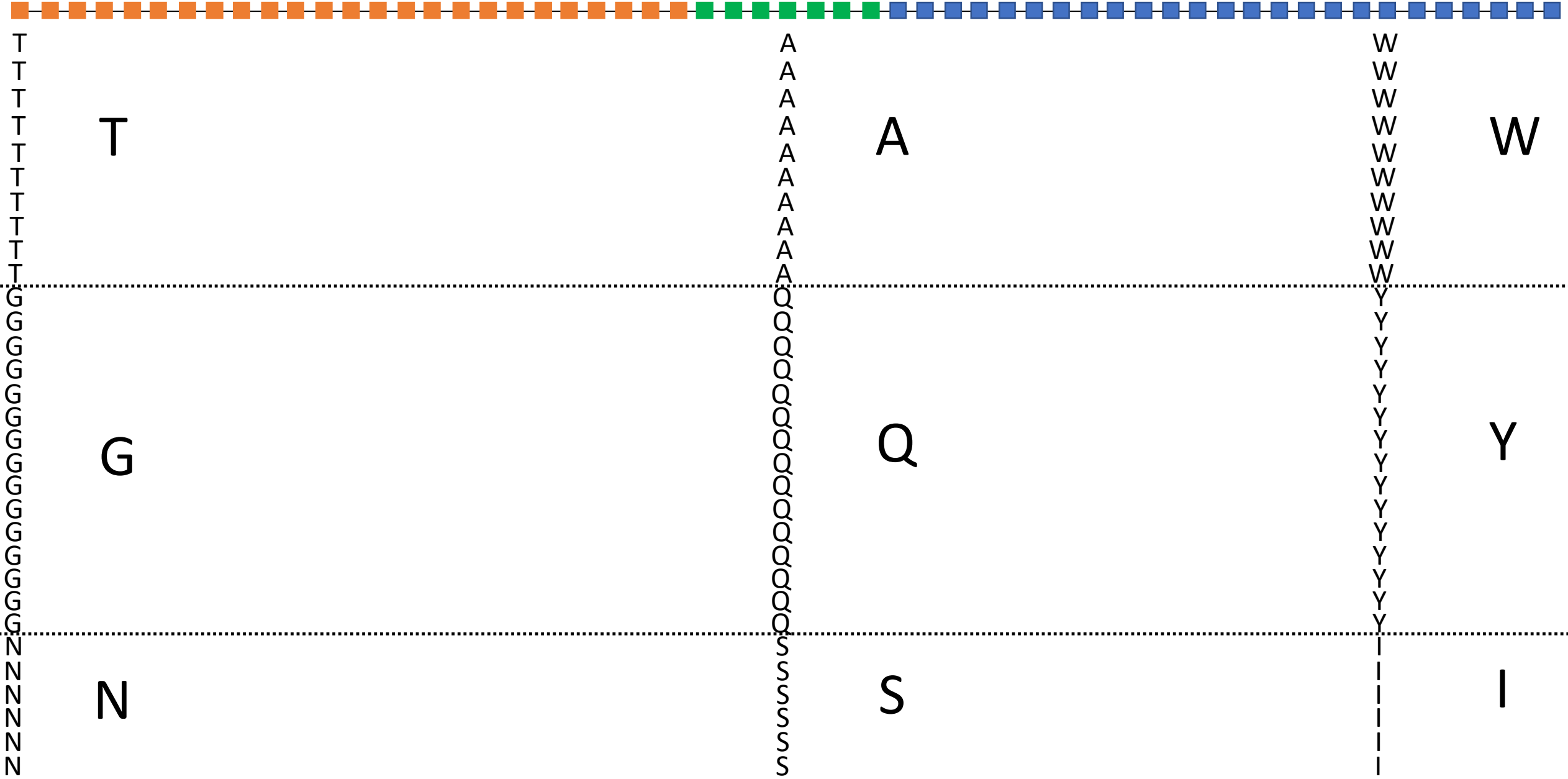


Native Structural Dynamics



Allostery. The second secret of life

Statistical Coupling Analysis
(Ernesto Freire & Rama Ranganathan)

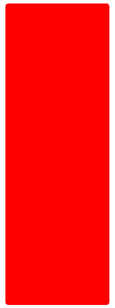


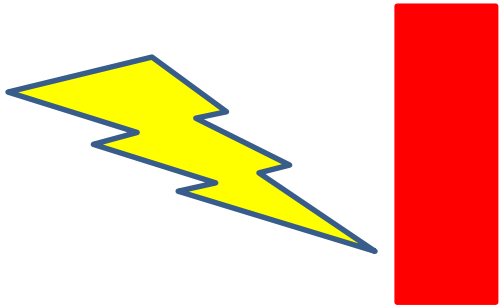
Allosteric network

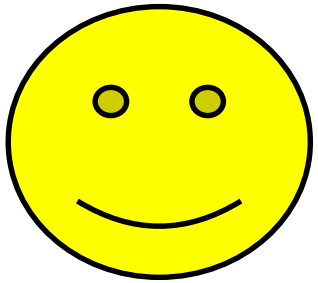
Allosteric network

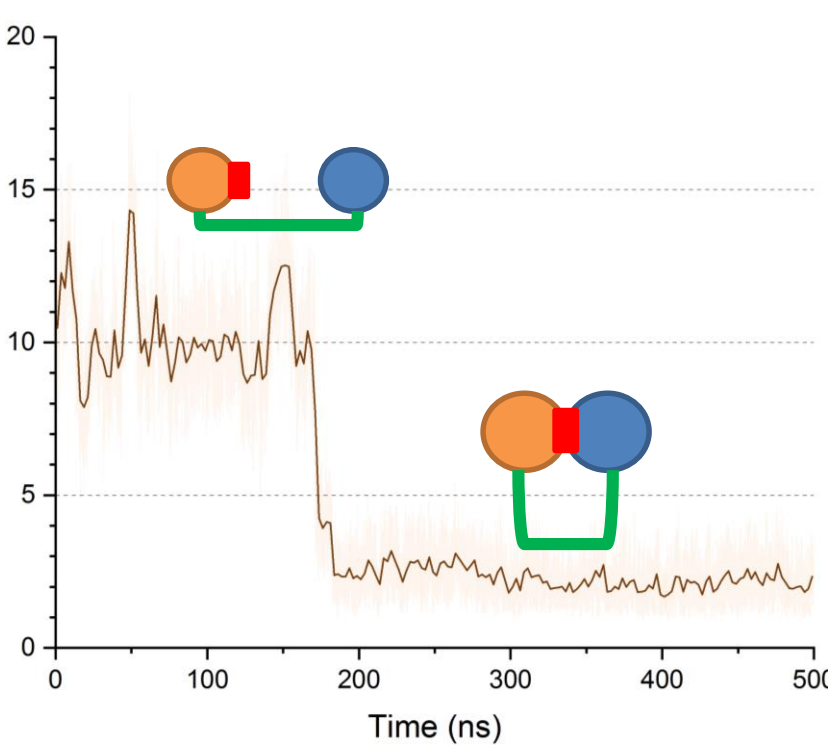
Allosteric network

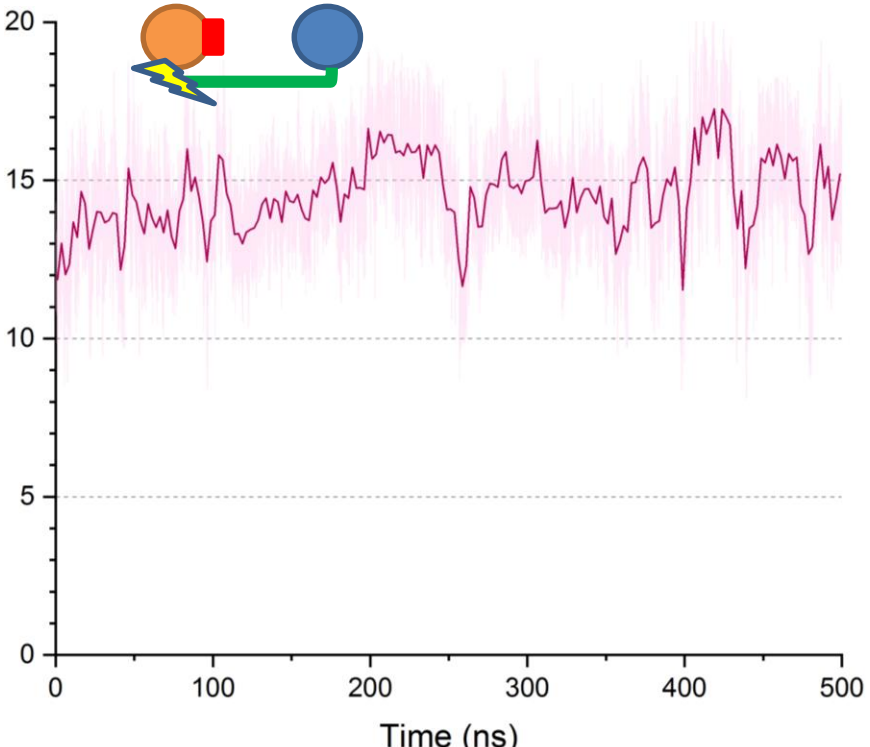
MD simulations

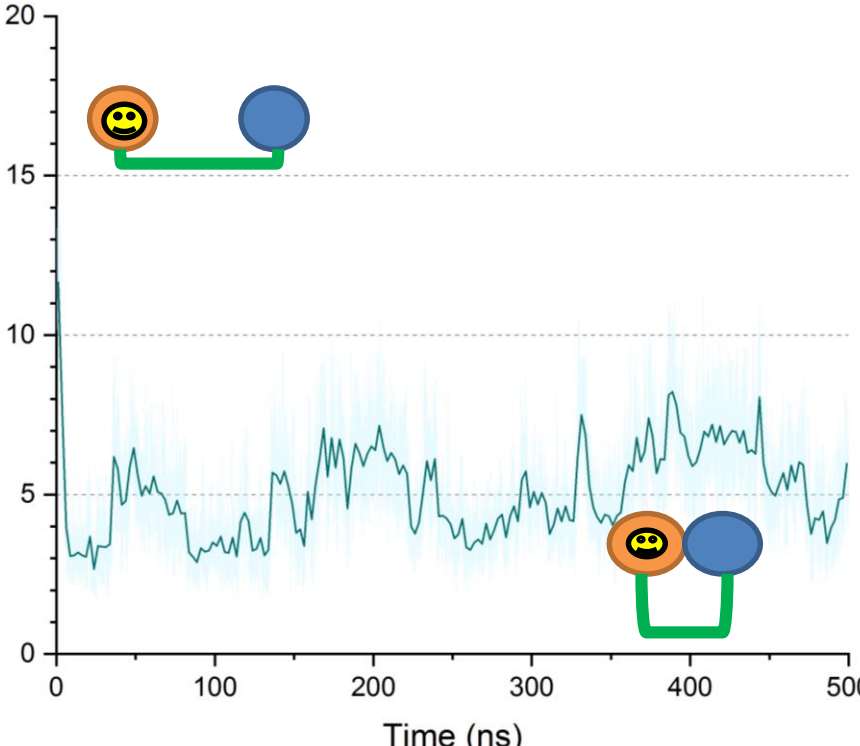












PNAS Proceedings of the National Academy of Sciences of the United States of America

Structural dynamics in the evolution of a bilobed protein scaffold

Giorgos Gouridis[a,b,c,d,1,2], Yusran A. Muthahari[a,c,d,1], Marijn de Boer[a], Douglas A. Griffith[b], Alexandra Tsirigotaki[c], Konstantinos Tassis[a], Niels Zijlstra[b], Ruixue Xu[c,e], Nikolaos Eleftheriadis[a], Yovin Sugijo[a], Martin Zacharias[f], Alexander Dömling[e], Spyridoula Karamanou[c], Charalambos Pozidis[d], Anastassios Economou[c], and Thorben Cordes[a,b,2]

[a]Molecular Microscopy Research Group, Zernike Institute for Advanced Materials, University of Groningen, Groningen 9747 AG, The Netherlands; [b]Physical and Synthetic Biology, Faculty of Biology, Ludwig Maximilians-Universität München, Planegg-Martinsried 82152, Germany; [c]Laboratory of Molecular Bacteriology, Department of Microbiology and Immunology, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven 3000, Belgium; [d]Structural Biology Division, Institute of Molecular Biology and Biotechnology, Heraklion-Crete 70013, Greece; [e]Drug Design Group, Department of Pharmacy, University of Groningen, Groningen 9713 AV, The Netherlands; and [f]Physics Department, Theoretical Biophysics, Technical University of Munich, Garching 85748, Germany

Edited by Martin Gruebele, University of Illinois at Urbana–Champaign, Urbana, IL, and approved October 28, 2021 (received for review January 4, 2021)

Novel biophysical tools allow the structural dynamics of proteins and the regulation of such dynamics by binding partners to be explored in unprecedented detail. Although this has provided critical insights into protein function, the means by which structural dynamics direct protein evolution remain poorly understood. Here, we investigated how proteins with a bilobed structure, composed of two related domains from the periplasmic-binding protein–like II domain family, have undergone divergent evolution, leading to adaptation of their structural dynamics. We performed a structural analysis on ~600 bilobed proteins with a common primordial structural core, which we complemented with biophysical studies to explore the structural dynamics of selected examples by single-molecule Förster resonance energy transfer and Hydrogen–Deuterium exchange mass spectrometry. We show that evolutionary modifications of the structural core, largely at its termini, enable distinct structural dynamics, allowing the diversification of these proteins into transcription factors, enzymes, and extracytoplasmic transport-related proteins. Structural embellishments of the core created interdomain interactions that stabilized structural states, reshaping the active site geometry, and ultimately altered substrate specificity. Our findings reveal an as-yet-unrecognized mechanism for the emergence of functional promiscuity during long periods of evolution and are applicable to a large number of domain architectures.

biophysics | evolution | structural dynamics | protein structure | ligand binding

Proteins drive and maintain all fundamental cellular processes (1) by interactions with small molecules and/or other coupling of the latter to structural changes enables proteins to perform a diverse range of functions.

Tier-0 dynamics were observed and characterized in various settings (e.g., in motor proteins, in which they are used in propelling movement along filaments) (16), in the transport of molecules or biopolymers across biological membranes (17–21), or in the activity of proteins that perform mechanical work (22). Tier-1 dynamics drive the actions of various signaling proteins for transmission of signals (23–25). Structural and biochemical data indicate that enzymes also show varying degrees of structural dynamics (26), although it is not well understood what precise role this plays for catalytic activity. The current belief is that extensive structural dynamics in enzymes are not necessarily required for catalysis (27) but rather enact in regulation. For instance, many protein kinases exploit Tier-0 dynamics to generate active or inactive structural states (28). Tier-2 dynamics have been shown to be important for the evolution of enzymatic function (29). In addition to domain motions occurring within a structure, protein

Significance

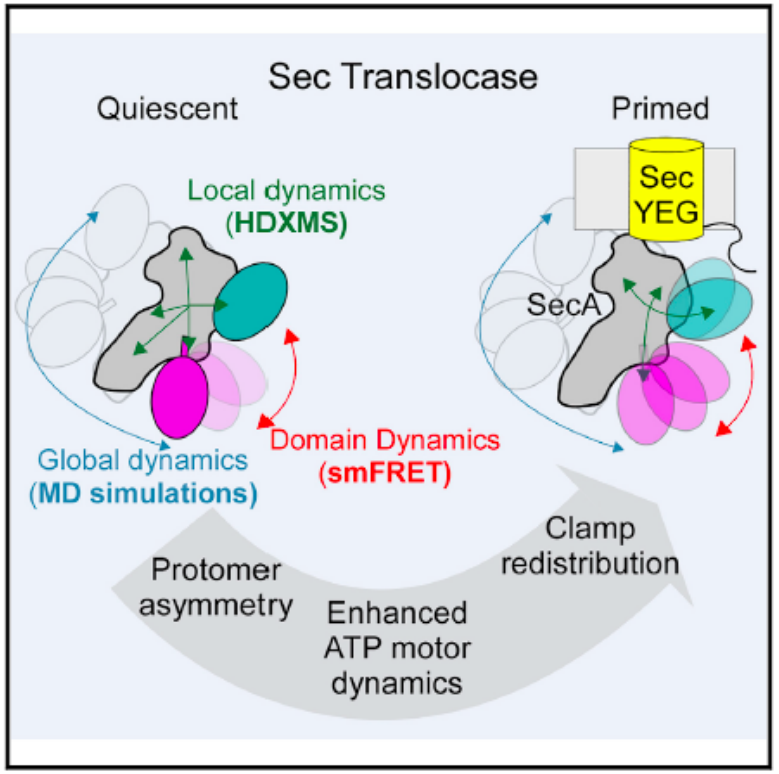
Proteins conduct numerous complex biological functions by use of tailored structural dynamics. The molecular details of how these emerged from ancestral peptides remains mysterious. How does nature utilize the same repertoire of folds to diversify function? To shed light on this, we analyzed bilobed proteins with a common structural core, which is spread throughout the tree of life and is involved in diverse

Structure

Article

A nexus of intrinsic dynamics underlies translocase priming

Graphical abstract



Highlights

- SecA exhibits global, domain, and local intrinsic dynamics that control its activity
- Cognate ligands alter dynamics nexuses to regulate dynamics and function

Authors

Srinath Krishnamurthy, Nikolaos Eleftheriadis, Konstantina Karathanou, ..., Ana-Nicoleta Bondar, Giorgos Gouridis, Anastassios Economou

Correspondence

g.gouridis@imbb.forth.gr (G.G.), tassos.economou@kuleuven.be (A.E.)

In brief

Krishnamurthy et al. dissect the bacterial Sec preprotein translocase using a combination of biophysical tools and reveal a nexus of multi-level intrinsic dynamics that regulates its activity and function. These data reveal the structural dynamics basis of translocase priming for high-affinity binding of multiple clients and ADP release.

Ras is a major drug target

1. 30% of human cancers
2. Psychiatric/developmental disorders
3. 10 mil$/year (NIH Ras Initiative)

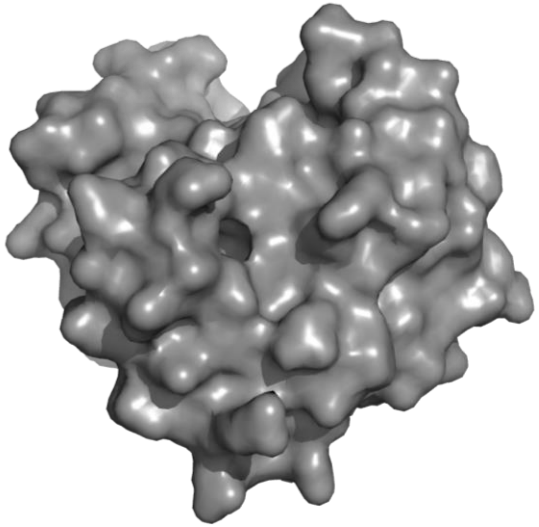
Why is Ras still undruggable?

Why is Ras still undruggable?

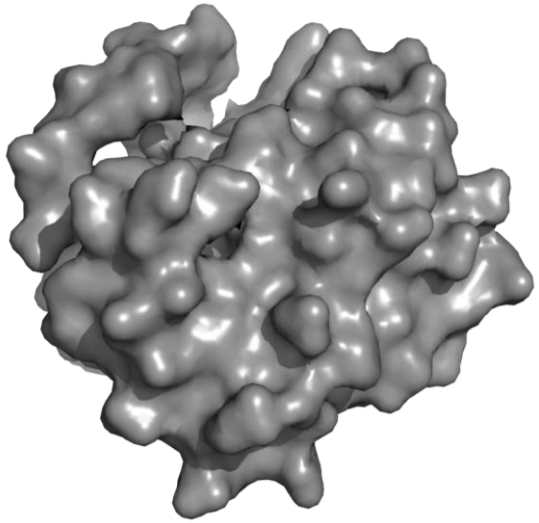
27 states!

Multiple states, dynamic pockets

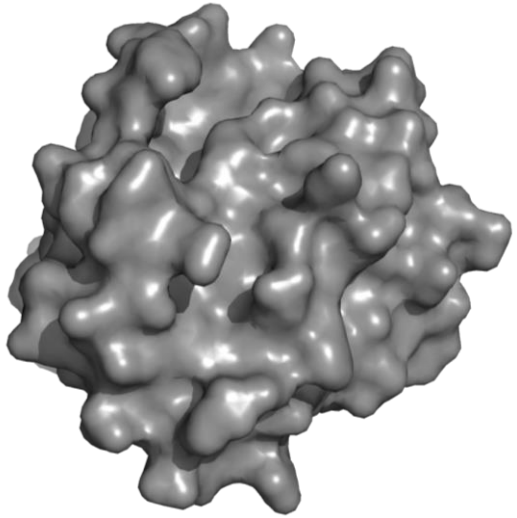
State 1



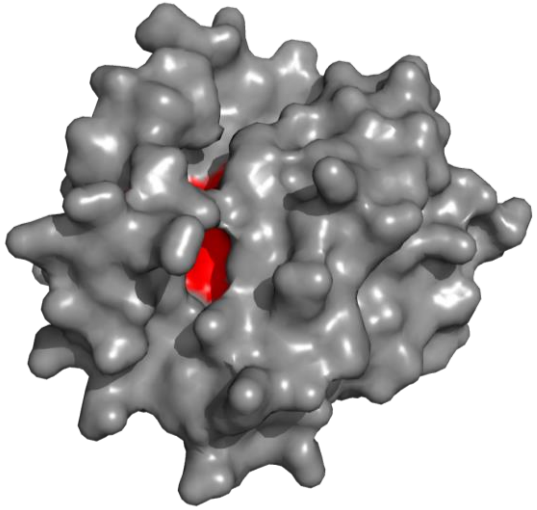
State 2



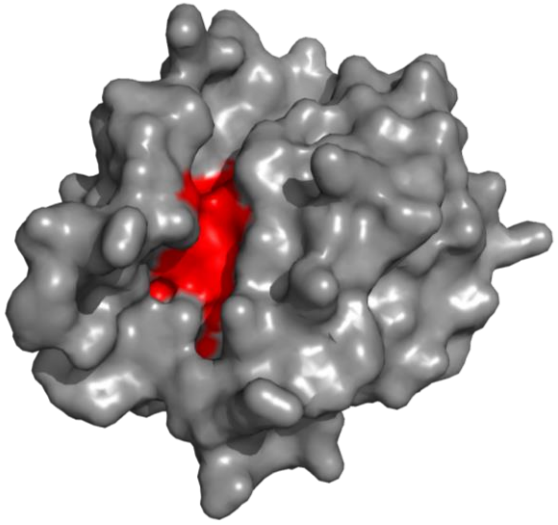
State 3



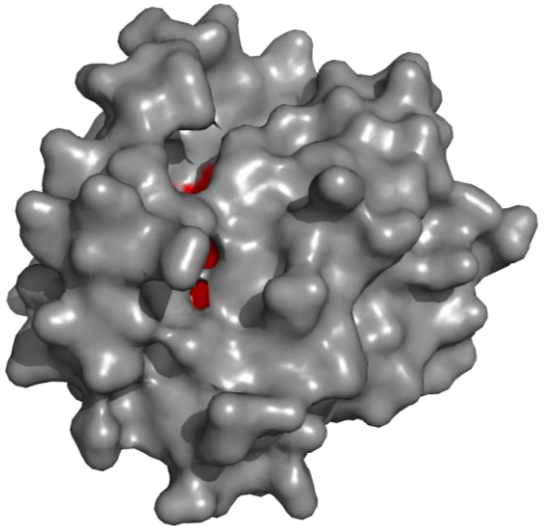
State 4



State 5



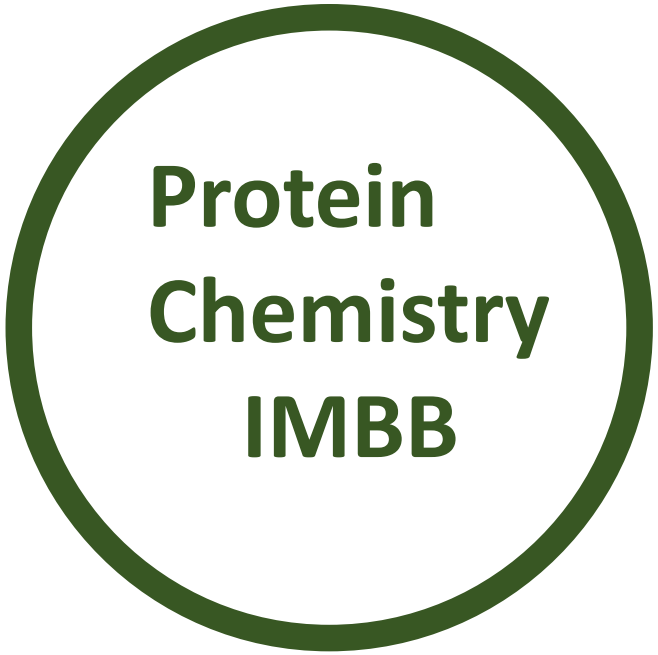
State 6



Protein Biophysics at FORTH

What is needed for protein biophysics

Prof. Kokkinidis
Emeritus
Structural Biology



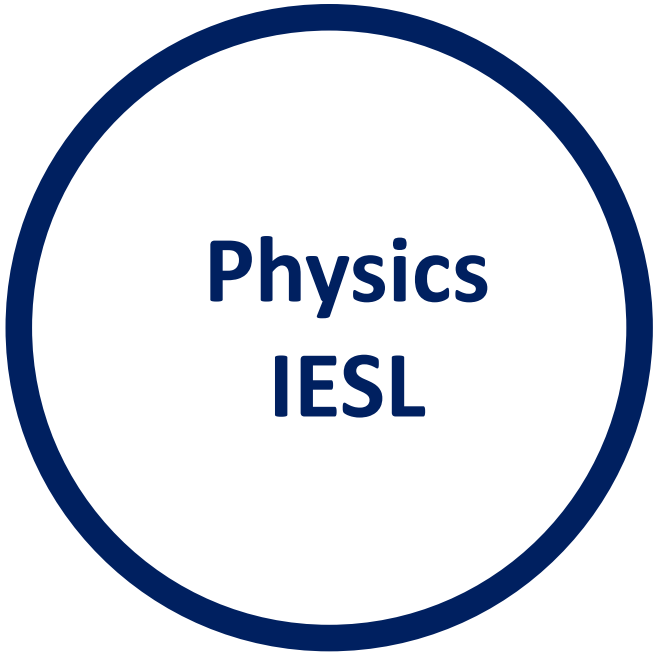
Protein Purification Facility (Pozidis)

Protein Biophysics at FORTH

What is needed for protein biophysics

Dr. Zacharakis
Researcher
Biophotonics/
Molecular
Imaging

Custom-made smFRET
Confocal set-up



Protein Biophysics at FORTH

What is needed for protein biophysics

Dr. Pantazis
Researcher
Statistical & Machine Learning

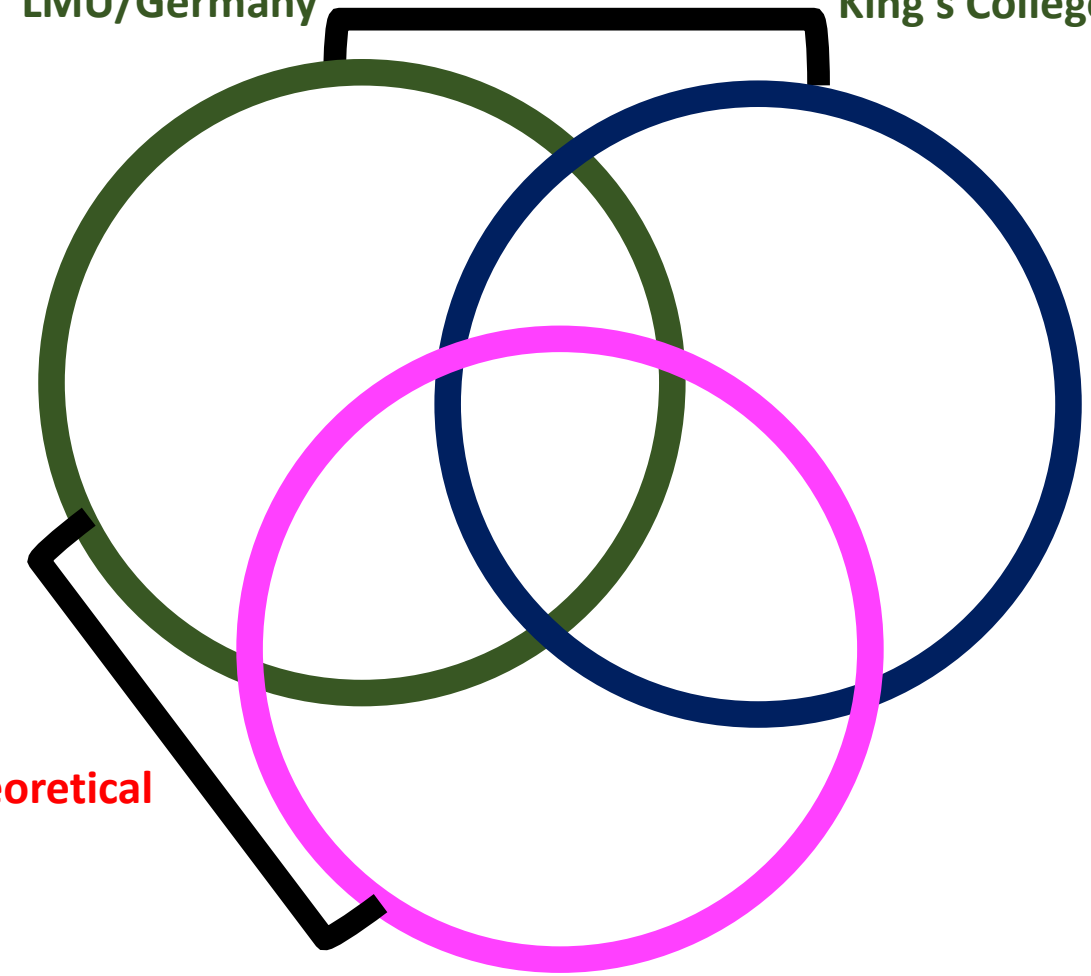
Statistics
Machine
Learning
IACM, ICS

- **Statistical Coupling Analysis**
- **AlphaFold (google)**
- **smFRET analysis tools**
- **smFRET/HDX-MS/MD data amalgamation**

Protein Biophysics at FORTH

Prof. Thorben Cordes
Single-molecule spectroscopy
LMU/Germany

Dr. Argyris Politis
Structural Mass-Spectrometry
King's College London/UK



Prof. Cecilia Clementi
Computational and Theoretical Biophysics
FUB/Germany