# From Quarks to Drugs: A Cross-Disciplinary Journey in Technology Transfer


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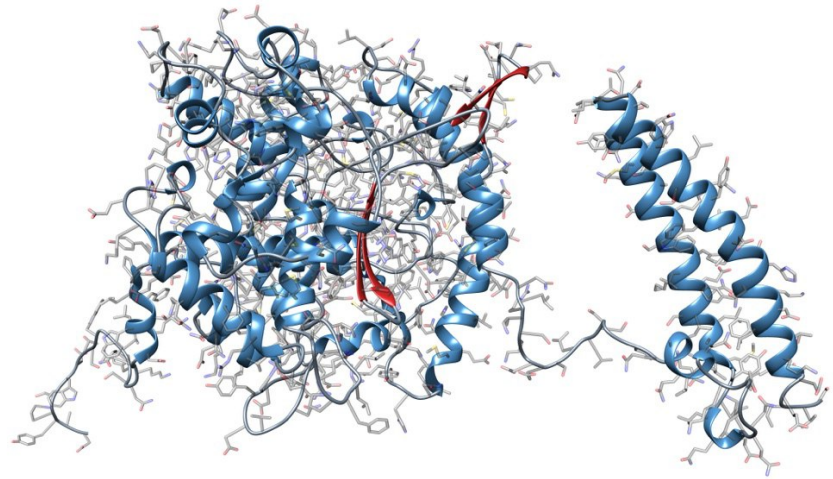
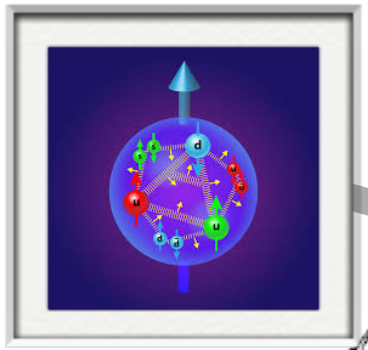


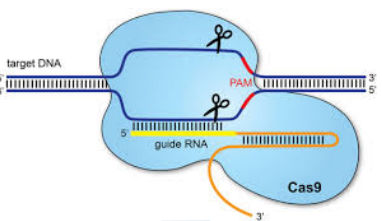
# A SCIENTIFIC JOURNEY



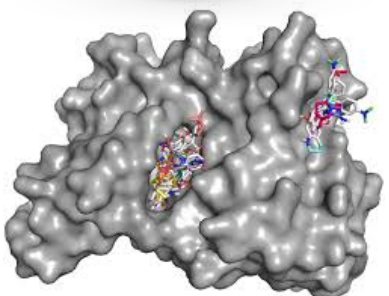
# FUNDAMENTAL DOGMA OF MOLECULAR BIOLOGY

patent file # 102018000007535 (with E. Biasini)

DNA → mRNA → Ribosome → Protein folding → Biological function




RNA silencing

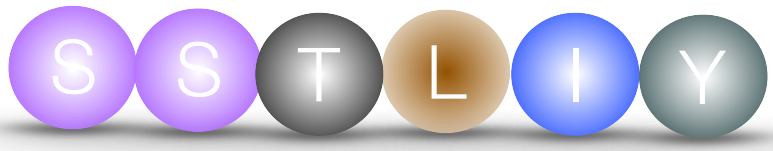


Eliminating protein from genome

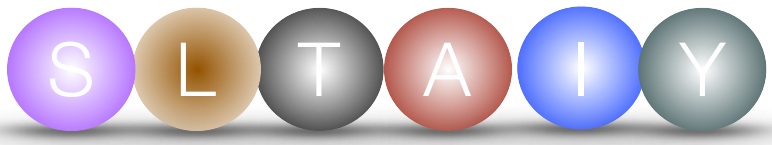
Suppressing transcription

Imparing function

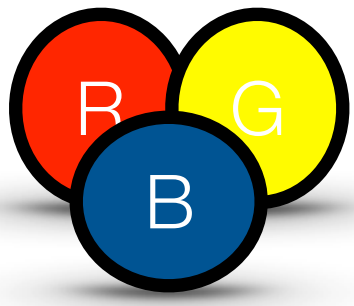
# PROTEINS AND HADRONS ARE VERY SPECIAL



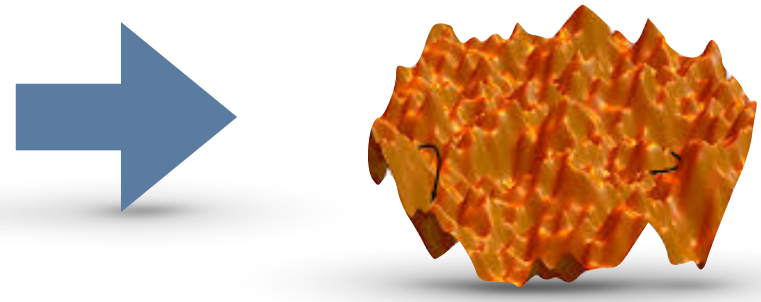

Random polypeptide

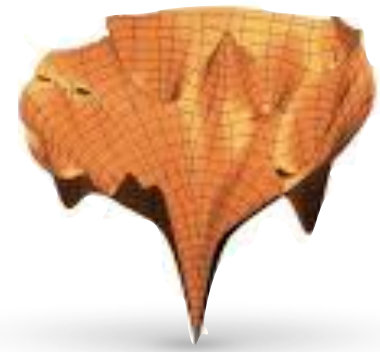


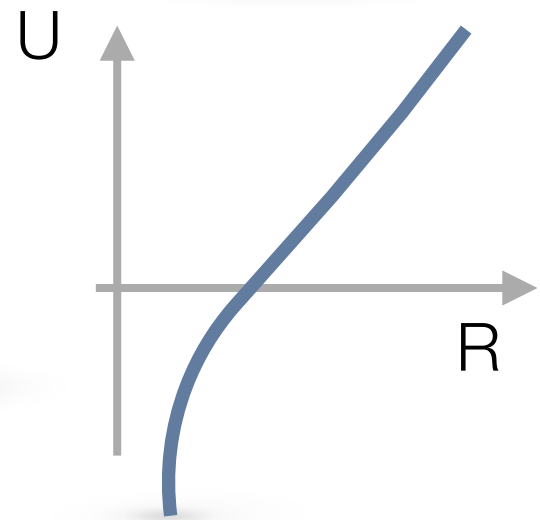

Protein



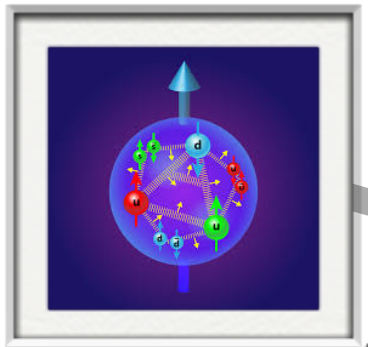

Baryon

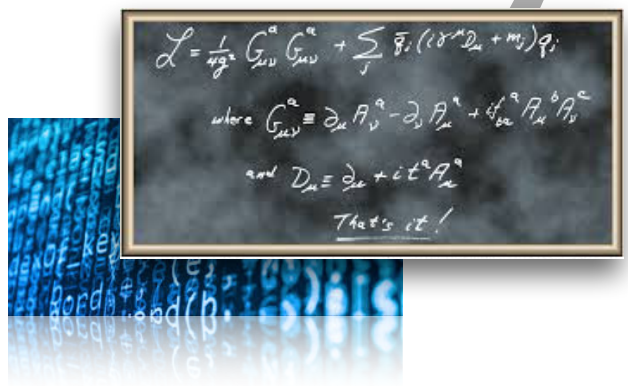




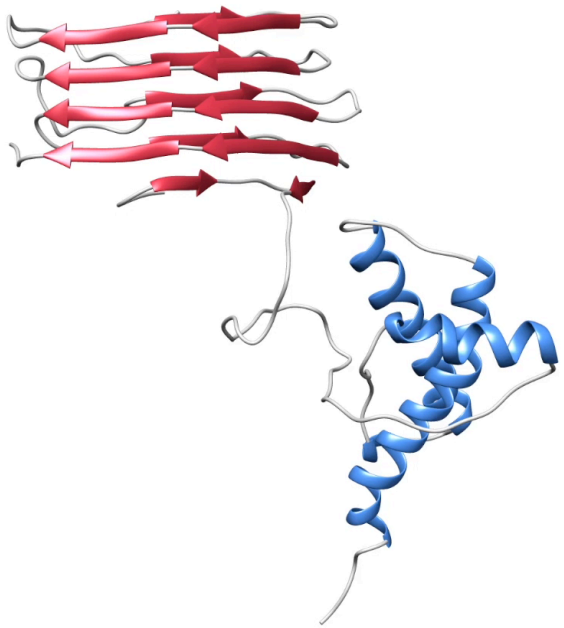


# PHASE 1: MATHEMATICAL FORMALISM & HIGH PERFORMANCE COMPUTING





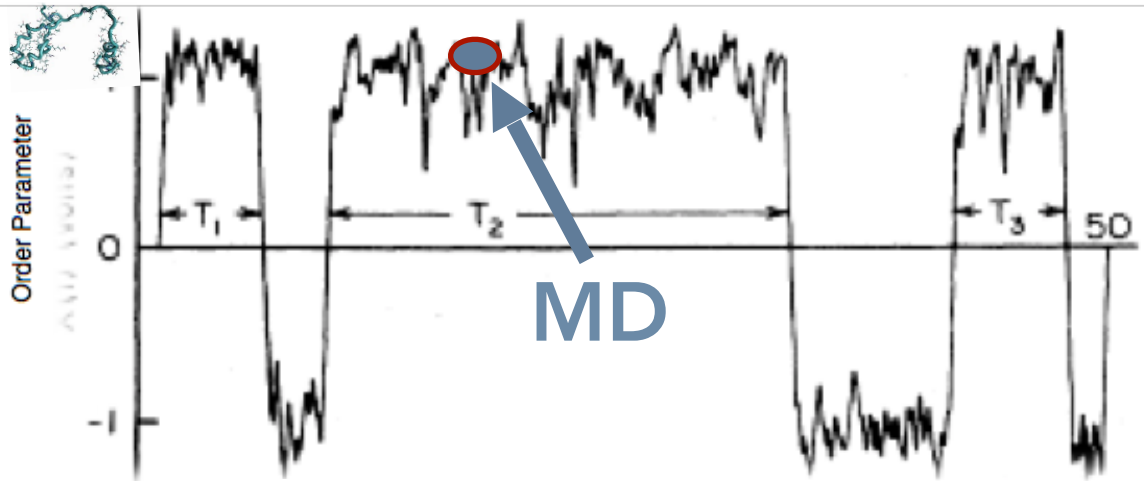

# REDUCTIONIST'S APPROACH TO MOLECULAR BIOLOGY

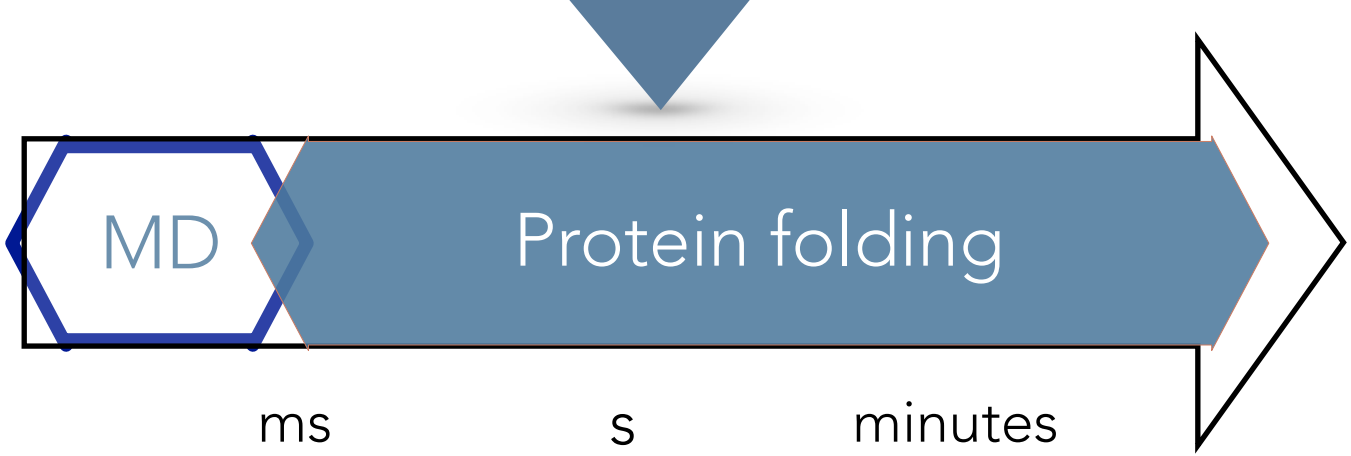


**Challenge:**

Integrate ~$10^6$ coupled Newton-type equations looking for **extremely rare events**

# RARE EVENT PROBLEMS

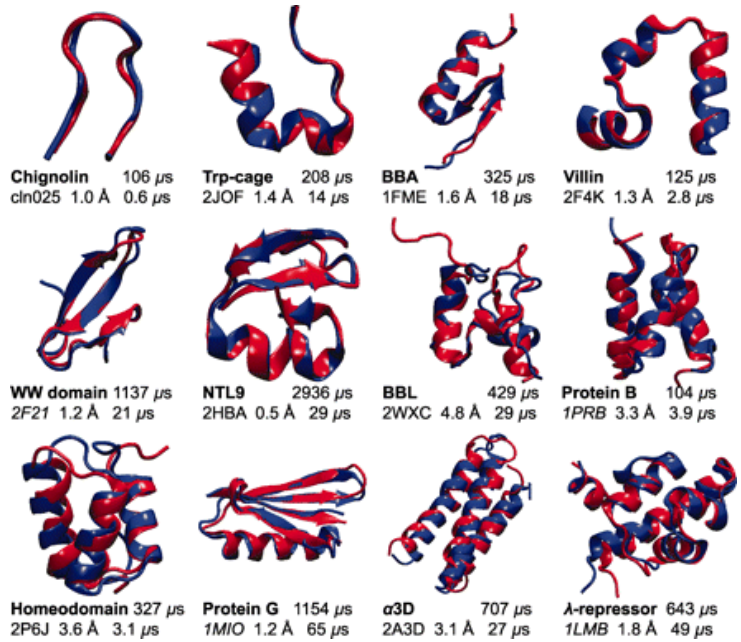


# MD YIELDS CORRECT PROTEIN NATIVE STATES

Anton supercomputer
(DES Research)

MD




Science
AAAS

**Atomic-Level Characterization of the Structural Dynamics of Proteins**
David E. Shaw, *et al.*
*Science* **330**, 341 (2010);
DOI: 10.1126/science.1187409

**How Fast-Folding Proteins Fold**

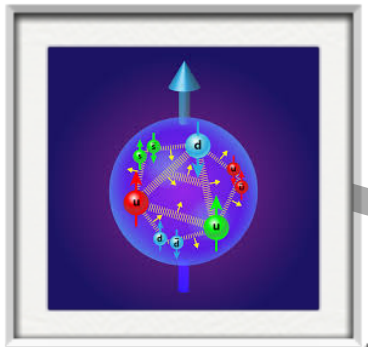
Kresten Lindorff-Larsen,[1*†] Stefano Piana,[1*†] Ron O. Dror,[1] David E. Shaw[1,2†]

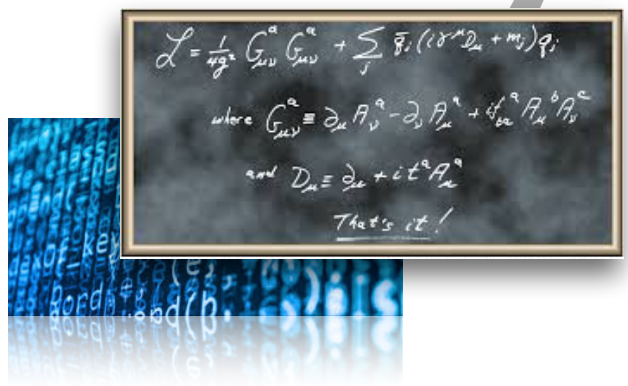
# ZOOLOGY OF ENHANCED SAMPLING METHODS

Markov State Models, Milestoning, Transition Path Sampling, Transition Interface Sampling, Forward Flux Sampling, Temperature Accelerated Molecular Dynamics, Metadynamics, Umbrella Sampling, Blue Moon Sampling, String Method,Stochastic Difference, … [and counting]

They are **all too computationally demanding** for many biologically relevant problems.

# PHASE 1: MATHEMATICAL FORMALISM & HIGH PERFORMANCE COMPUTING

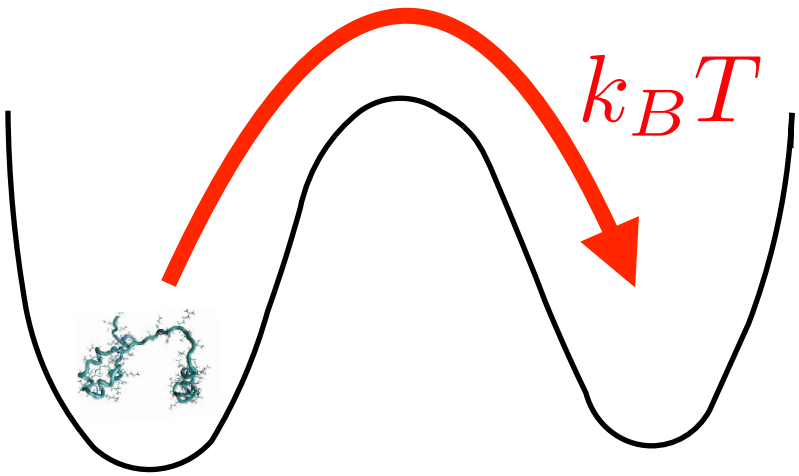





# A USEFUL ANALOGY

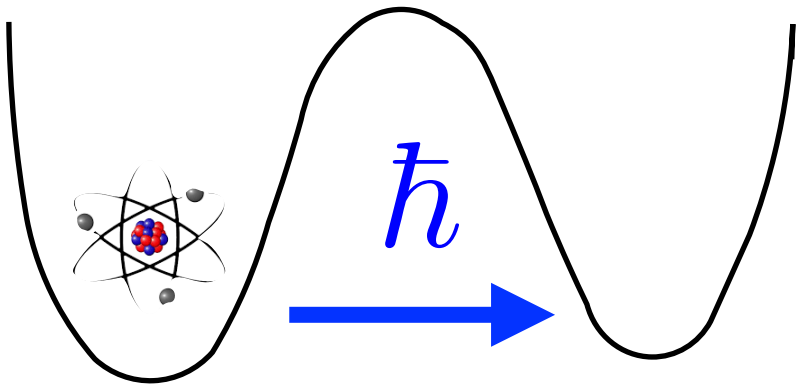
## Thermal activation

$( \beta = (K_B T)^{-1} )$



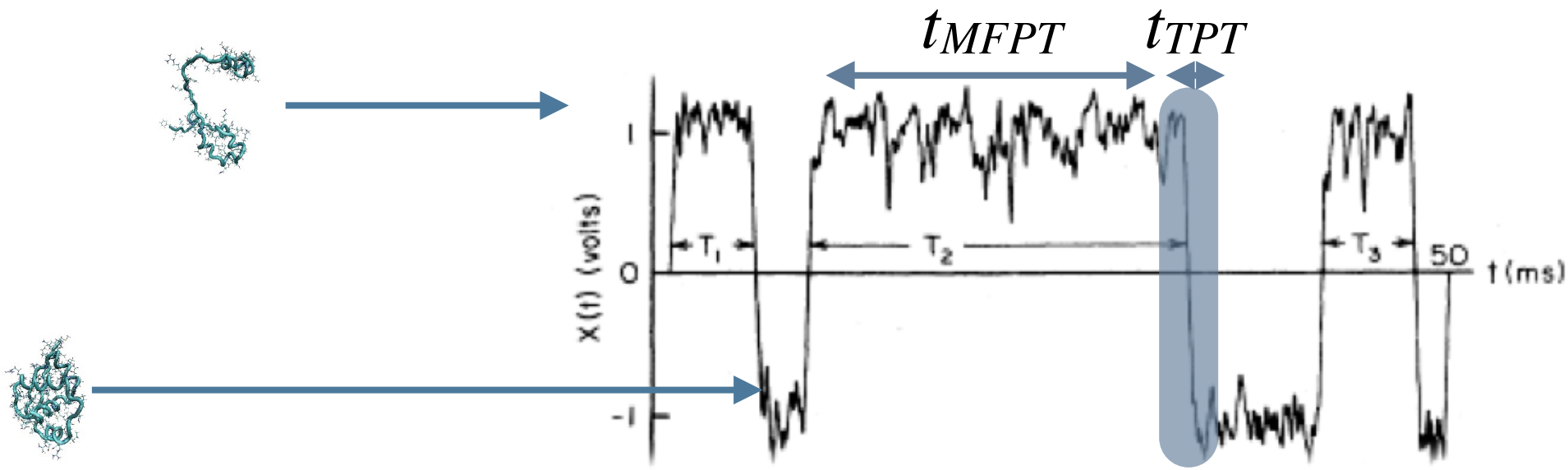

$$P(x_f, t|x_i) = \int_{x_i}^{x_f} \mathcal{D}q \; e^{-\frac{\beta}{4M\gamma} \int_0^t d\tau (M\ddot{q} + M\gamma\dot{q} + \nabla U(q))^2}$$

## Quantum tunneling



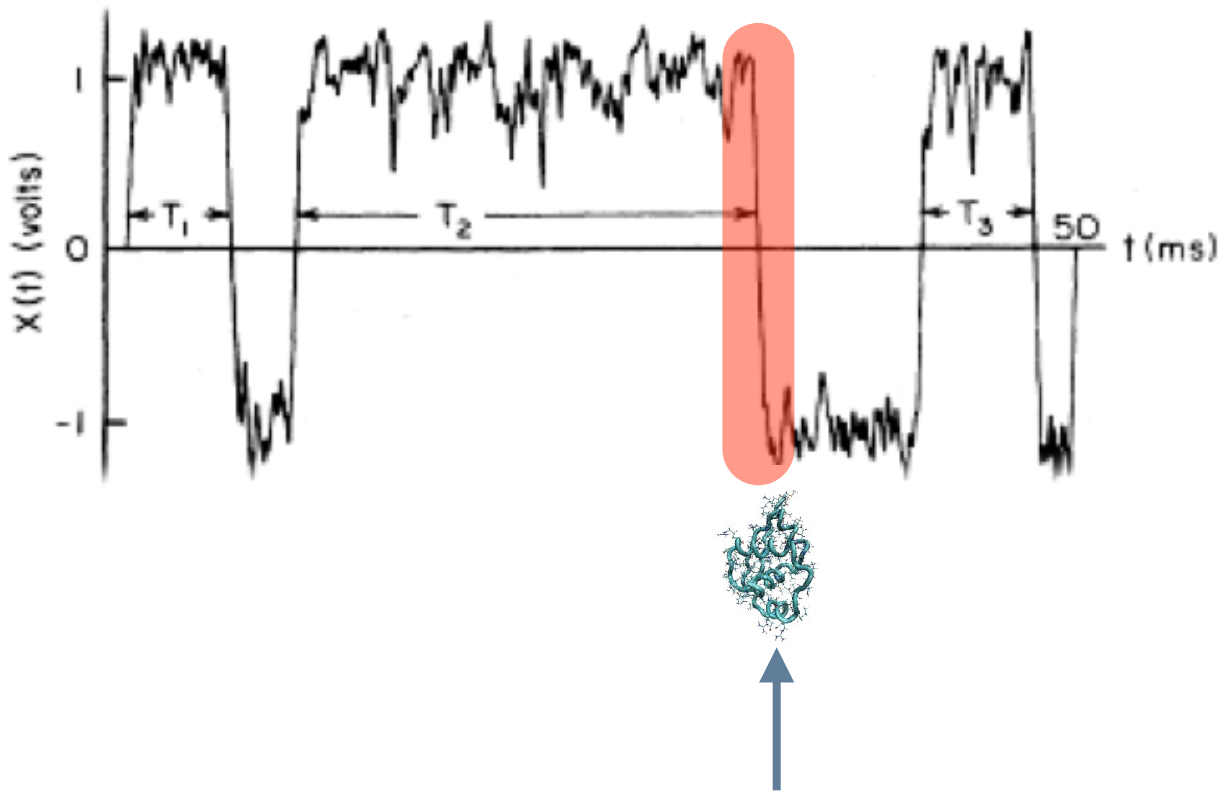

$$K_E(x_f, t|x_i) = \int_{x_i}^{x_f} \mathcal{D}q \; e^{-\frac{1}{\hbar} \int_0^t d\tau \left( \frac{M}{2}\dot{q}^2 + U(q) \right)}$$

# ADVANTAGES




$$t_{TPT} \sim \tau_0 \log\left[\log\left(\frac{t_{MFPT}}{\tau_0}\right)\right]$$

# IS THIS A "FREE LUNCH"?




All atom 3D structure of the native state **are given in input,** not predicted

# FULLY EXPLOITING THEORETICAL PHYSICS TOOLS



between the Gibbs distribution and the SCR estimate forward- and backward-committors, as in Eq. (A3). Introducing the distribution

$$P^{(P)}(x,t) \equiv \int dx_i\, P^{(P)}(x,t|x_i,0)\, \rho_0(x_i), \qquad (23)$$

the density in Eq. (22) reads

$$m_{SCR}(x) = \frac{1}{t_f - \tau_0} \int_{\tau_0}^{t_f} dt\; Q^{(R)}(x, t_f - t) P^{(P)}(x,t).$$

Using the detailed balance condition, we find $P^{(P)} = e^{-\beta U(x)} \frac{1}{Z_R} Q^{(P)}(x,t)$. Then, inserting this result into Eq. we find

$$m_{SCR}(x) = \frac{e^{-\beta U(x)}}{Z_R\,(t_f - \tau_0)} \int_{\tau_0}^{t_f} dt Q^{(R)}(x, t_f - t) Q^{(P)}(x,t).$$

Finally, recalling that $Q^{(R)}(x,t)$ and $Q^{(P)}(x,t)$ are n time-independent in the SCR and using Eqs. (17) and we recover a fundamental result of TPT [cf. Eq. (A Appendix A],

$$m_{SCR}(x) \propto e^{-\beta U(x)}\, q^{+}_{SCR}(x)\,(1 - q^{+}_{SCR}(x)\,).$$

Within the same framework, it is possible to d the reactive current in the SCR in complete analogy Eq. (22),

$$J^{i}_{SCR}(x) = \frac{-D}{t_f - \tau_0} \int_{\tau_0}^{t_f} dt Q^{(R)}(x, t_f - t) \times (\overrightarrow{\nabla} - \overleftarrow{\nabla} + \beta \nabla U(x))\, P^{(P)}(x,t).$$



$$\mathcal{P}_{\rm bias}[X] = \int \mathcal{D}Y e^{-S_{\rm bias}[X,Y] - U(X_i,Y_i)/k_BT}, \qquad (3)$$

where the functional $S_{\rm bias}[X,Y]$ is defined as

$$S_{\rm bias} \equiv \frac{1}{4k_BT} \int_0^t d\tau \Big[ \sum_{i=1}^{N} \frac{1}{\gamma_i m_i} (m_i \ddot{\mathbf{x}}_i + m_i\gamma_i \dot{\mathbf{x}}_i + \nabla_i U - \mathbf{F}^{\rm bias}_i)^2 + \sum_{j=1}^{N'} \frac{1}{\gamma_j m_j} (m_j \ddot{\mathbf{y}}_i + m_j\gamma_j \dot{\mathbf{y}}_j + \nabla_j U)^2 \Big]. \qquad (4)$$

The Onsager-Machlup functional $S_{\rm OM}[X,Y]$ entering Eq. (2) is recovered, setting $\mathbf{F}^{\rm bias}_i = 0$ in Eq. (4).

Let us now return to the problem of computing the reaction pathways in the *unbiased* Langevin dynamics [Eq. (1)]. Using the standard reweighting trick we can write the variational condition $(\delta/\delta X)\mathcal{P}[X] = 0$ as

$$\frac{\delta}{\delta X} [\mathcal{P}_{\rm bias}[X] \langle e^{-(S_{\rm OM}[X,Y] - S_{\rm bias}[X,Y;t])} \rangle_{\rm bias}] = 0. \qquad (5)$$

We now introduce our main approximation, by restricting the search for the optimum path $X(\tau)$ within an ensemble of trajectories generated by integrating the *biased* Langevin equation. By definition, these paths have a large statistical weight in the biased dynamics; i.e., they lie in the functional vicinity of some path $\bar{X}(\tau)$ which satisfies $(\delta/\delta\bar{X})\mathcal{P}[\bar{X}] = 0$. Thus, the typical biased paths approx-

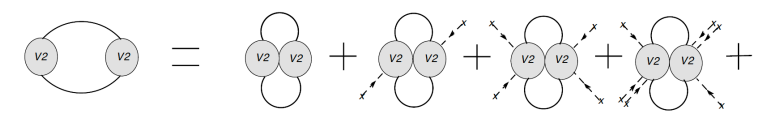



FIG. 3: Diagrammatic representation of the local time-derivative expansion of a non-local diagram —Eq. (49)—. Solid lines are fast-mode propagators, while dashed lines represent a single time derivative acting on the corresponding vertex function.

Notice that each term in the perturbative expansion (35) generates a new vertex, with an increasing power of the $x_>(\tau)$ field. The couplings to the fast modes depend implicitly on the time $\tau$, through the slow modes $x_<(\tau)$.

By Wick theorem, each term in the series (34) can be related to a Feynman graph with vertexes given by (36) and propagators given by —see appendix A —:

$$\langle x^i_>(\tau_1)\; x^j_>(\tau_2)\rangle_0 = \sum_{|\omega_m|,|\omega_n| \in S_b} G^{0\;ij}_>(\omega_n,\omega_m)\; e^{i(\omega_m\tau_1 + \omega_n\tau_2)} = \sum_{|\omega_n| \in S_b} \delta_{ij}\, \frac{2}{\beta\;\gamma\;t\;\omega_n^2}\; e^{i\omega_m(\tau_2 - \tau_1)}. \qquad (37)$$

The expansion (34) can be re-organized as the exponent of the sum performed over only connected diagrams:

$$e^{-\beta\; S_>[x<(\tau)]} = e^{\sum \text{(all connected diagrams)}}. \qquad (38)$$

Hence, the path integral (26) for the slow modes can be given the following exact diagrammatic representation

$$Z(t) \equiv \oint \mathcal{D}x_<\; e^{-\beta\; S_{eff}[x_<(t)]] + \sum \text{(all connected diagrams)}}. \qquad (39)$$

Below we give a classification of all the connected diagrams that may give a contribution to the expansion above



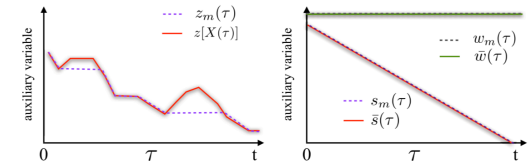



FIG. 1. Illustrative representation of the dynamics of the auxiliary variables introduced in the path integral representation of rMD (left panel) and in the derivation self-consistent path sampling algorithm (right panel).

of such a variable is frozen any time $z_m$ becomes smaller than $z(X)$ and any time the collective coordinate $z(X)$ is increasing. Its time derivative is otherwise set equal to $\dot{z}(X)$. Therefore, by choosing the initial conditions $z_m(0) = z(X(0))$, $z_m(\tau)$ is identically set equal to the minimum value attained by the collective coordinate $z$ until time $\tau$ (see left panel of Fig. 1).

The functional $S_{rMD}[X, z_m]$ in the exponent of Eq. (8) coincides with an OM action with the addition of the unphysical biasing force $\mathbf{F}_i$,

$$S_{rMD} = \sum_{i=1}^{N} \Gamma_i \int_0^t d\tau\, [m_i \ddot{\mathbf{x}}_i + m_i\gamma_i \dot{\mathbf{x}}_i + \nabla_i U - \mathbf{F}_i]^2. \qquad (9)$$

In Eq. (8), $\Phi[z_m, X]$ denotes a Jacobian factor that needs to be introduced in order to ensure that the statistical weight of the paths is not affected by the measure of the $\int \mathcal{D}z_m$ integral, i.e.,

$$\int \mathcal{D}z_m \Phi[z_m, X]\; \delta\Big[z_m(\tau) - \int_0^\tau d\tau' \dot{z}[X(\tau')]\theta(-\dot{z}[X(\tau')])$$

## III. SELF-CONSISTENT PATH SAMPLING

Let us now introduce our new algorithm, which provides major improvement with respect to the rMD and BF schemes discussed in Sec. II A. Indeed, it follows directly from the unbiased Langevin equation and allows us to remove the systematic errors associated to the choice of biasing coordinate.

Our starting point is path integral representation of the *unbiased* Langevin dynamics (2). We introduce two dumb auxiliary variables $w_m(\tau)$ and $s_m(\tau)$ into this path integral by means of appropriate functional Dirac deltas,

$$p(X_N, t|X_U) = \int_{X_U}^{X_N} \mathcal{D}X \cdot e^{-S[X]} \int_{\bar{s}(0)} \mathcal{D}s_m \int_{\bar{w}(0)} \mathcal{D}w_m \cdot \delta\Big[w_m(\tau) - \int_0^\tau d\tau' \dot{\bar{w}}(\tau')\,\theta(-\dot{\bar{w}}(\tau'))\,\theta(w_m(\tau') - \bar{w}(\tau'))\Big] \cdot \delta\Big[s_m(\tau) - \int_0^\tau d\tau' \dot{\bar{s}}(\tau')\,\theta(-\dot{\bar{s}}(\tau'))\,\theta(s_m(\tau') - \bar{s}(\tau'))\Big], \qquad (12)$$

where $\bar{s}(\tau)$ and $\bar{w}(\tau)$ are two external time-dependent functions to be defined below. In analogy with the path integral repre-

$$V^R_{eff}(\mathbf{X}) \simeq \frac{D_0\,(1-b)}{\pi\, b\Omega} \nabla^2 V_{eff}(\mathbf{X}) + \frac{1}{2}\left(\frac{D_0(1-b)}{\pi\, b\Omega}\right)^2 \nabla^4 V_{eff}(\mathbf{X}) + \frac{1}{6}\left(\frac{D_0\,(1-b)}{\pi b\Omega}\right)^3 \nabla^6 V_{eff}(\mathbf{X}) - \frac{D_0^2(1-b^3)}{3\pi\,(b\Omega)^3}\left(\partial_i\partial_j V_{eff}(\mathbf{X})\right)^2. \qquad (24)$$

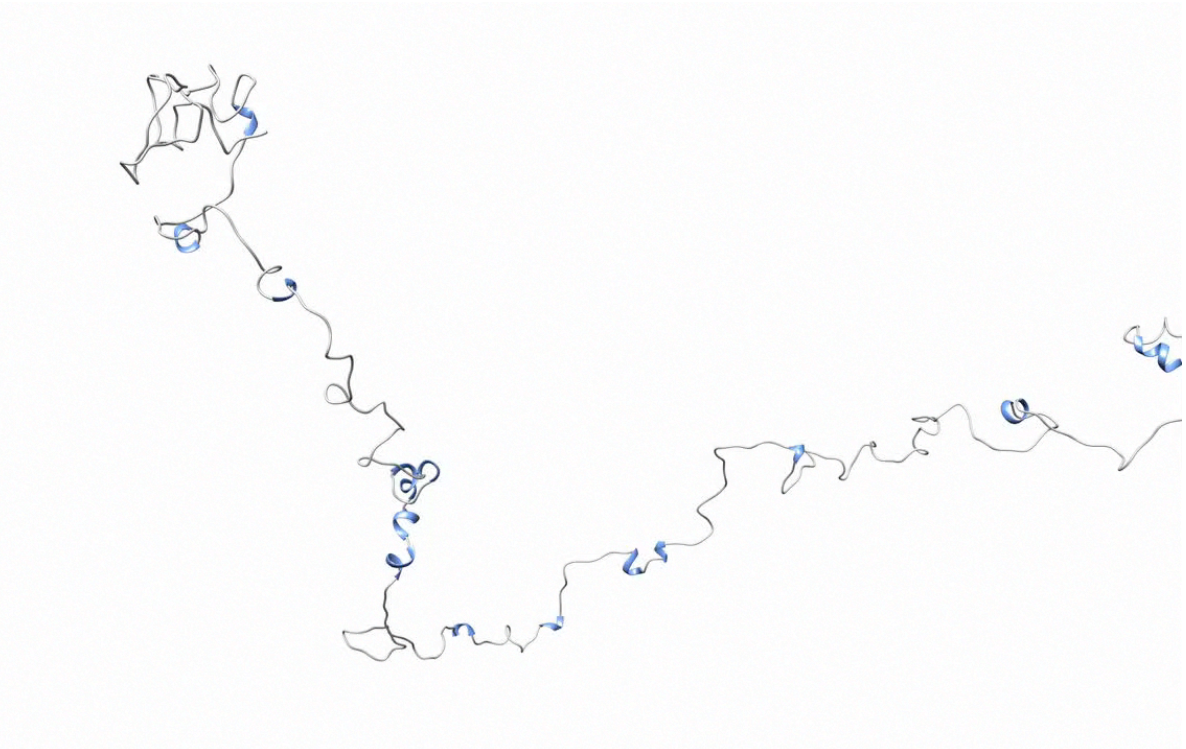
Note that the first line is the leading order term (i.e. $L = 1$) , while the second and third lines display the order $L = 2$ and $L = 3$ corrections, respectively.

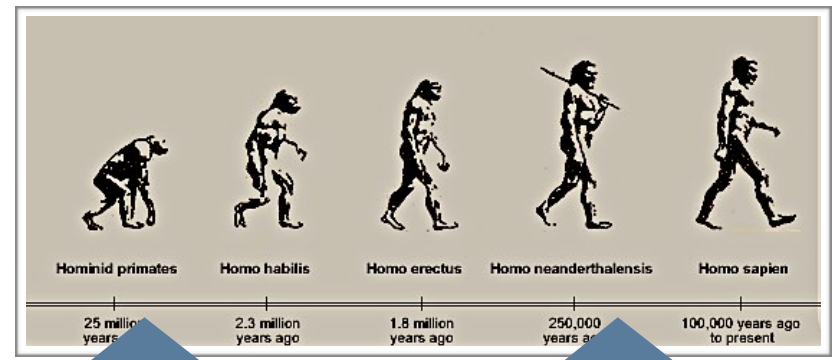
We emphasize that the result of the EST construction is a new expression for the *same* path integral (15), in which the UV cutoff been lowered from $\Omega$ to $b\Omega$. Equivalently, the path integral is discretized according to a larger elementary time step, $\Delta t \to \Delta t/b$:

$$Z^{\Delta t}(t) \equiv \oint_{\Delta t} \mathcal{D}\mathbf{X}\, e^{-S_{eff}[\mathbf{X}]} \propto \oint_{\Delta t/b} \mathcal{D}\mathbf{X}\, e^{-S_{eff}[\mathbf{X}] - \int_0^t d\tau\, V^R_{eff}[\mathbf{X}(\tau)]} \equiv Z^{\Delta t/b}_{EST}(t) \qquad (25)$$

In these expressions, the symbol $\oint_{\Delta t}$ denotes the fact that the path integral is discretized according to an elementary time step $\Delta t$ and we have suppressed the subscript "<", in the paths. It can be shown that the proportionality factor between $Z^{\Delta t}(t)$ and

# HUGE COMPUTATIONAL GAIN

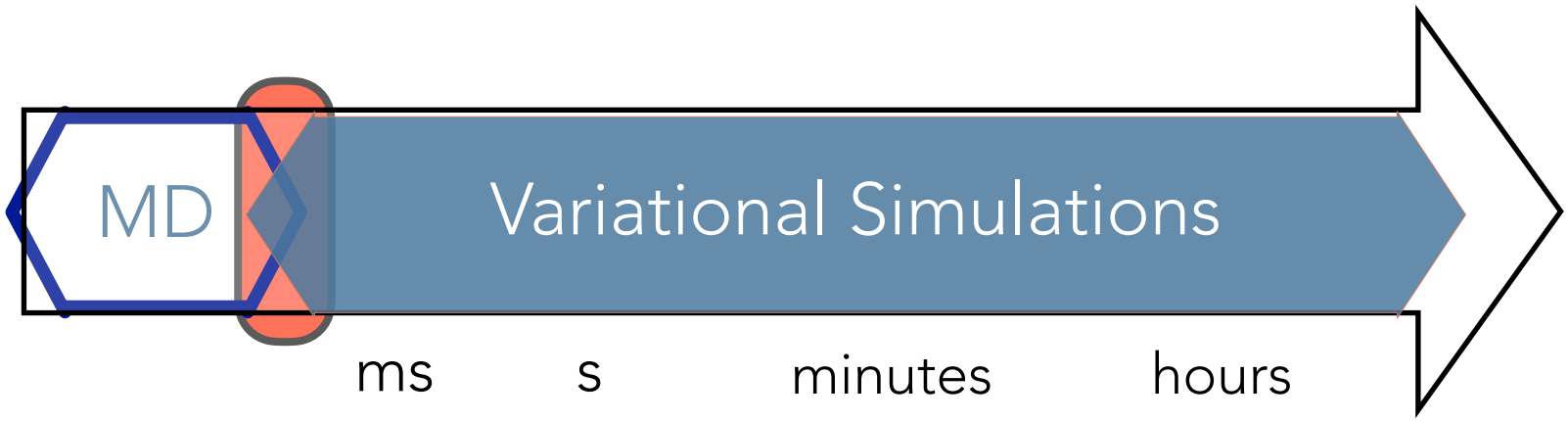


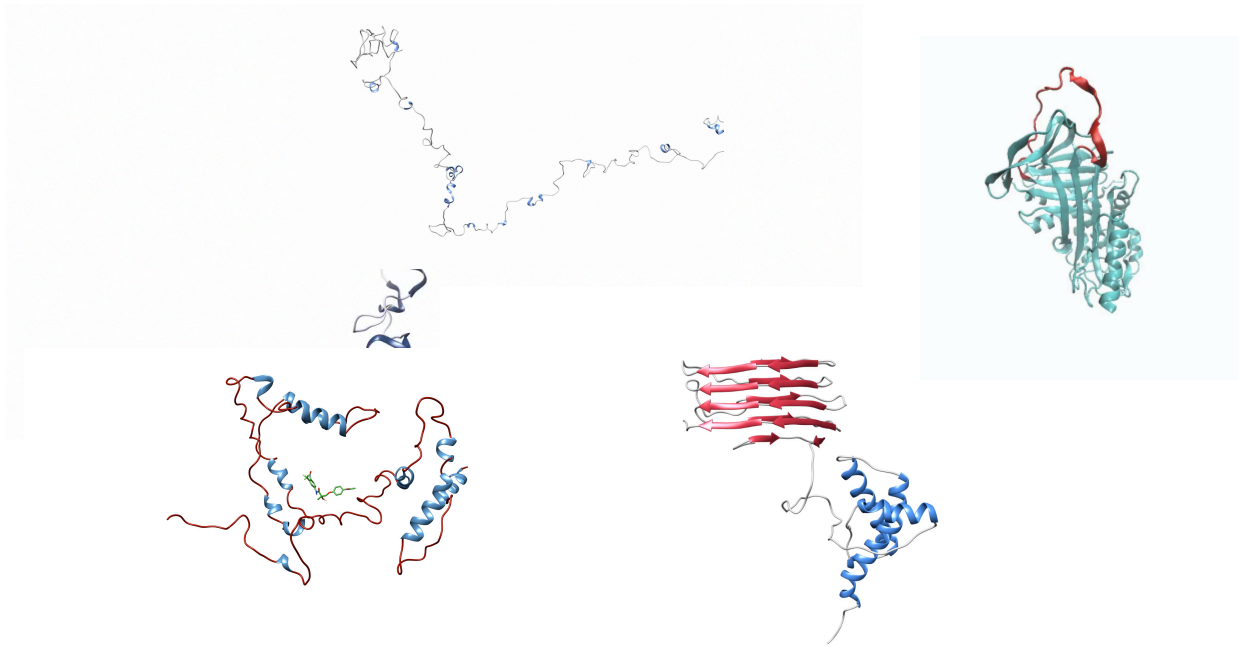



Using top all-purpose supercomputers

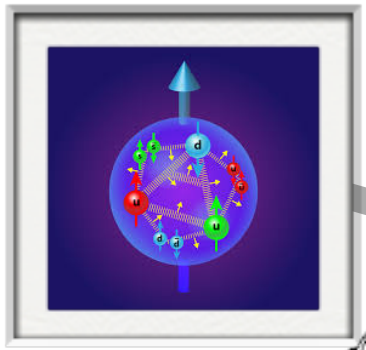
Using top special-purpose supercomputer

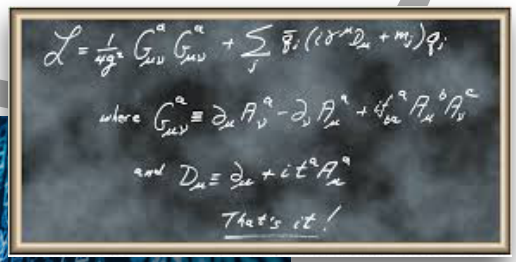
# VENTURING INTO THE BIO-ZONE

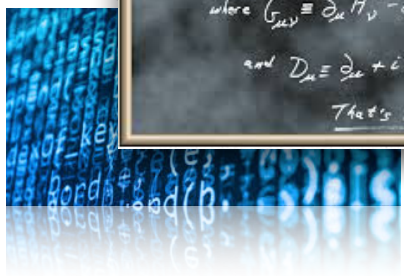


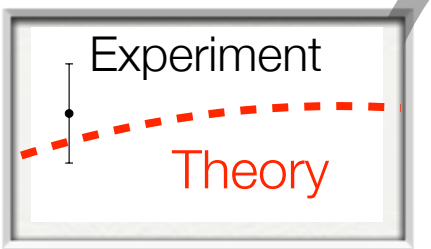



# PHASE 2: VALIDATION

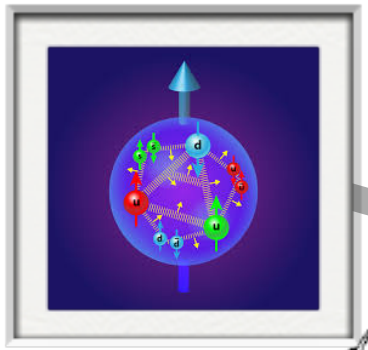


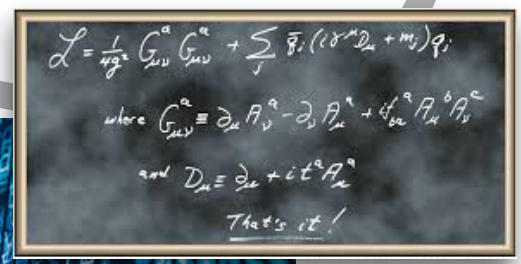


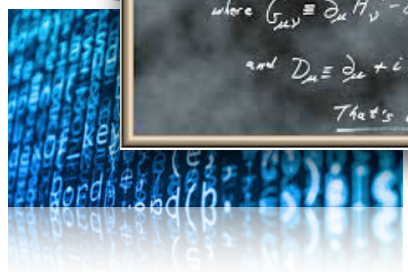



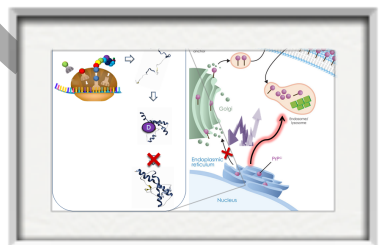


# PHASE 3: EXPLOITATION IN MOLECULAR BIOLOGY





# EXPLORING BIOLOGICAL PROCESSES

PNAS

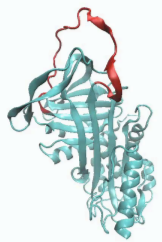
## Serpin latency transition at atomic resolution

Giorgia Cazzolli[a,b], Fang Wang[c], Silvio a Beccara[b,d], Anne Gershenson[e], Pietro Faccioli[a,b,1], and Patrick L. Wintrode[c,1]

[a]Dipartimento di Fisica, Università degli Studi di Trento, 38100 Povo (Trento), Italy; [b]Trento Institute for Fundamental Physics and Applications, 38123 Povo (Trento), Italy; [c]Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD 21201; [d]Interdisciplinary Laboratory for Computational Science, Fondazione Bruno Kessler, 38123 Povo (Trento), Italy; and [e]Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst, MA 01003

Edited by David E. Shaw, D. E. Shaw Research, New York, NY, and approved September 12, 2014 (received for review April 24, 2014)

**Protease inhibition by serpins requires a large conformational transition from an active, metastable state to an inactive, stable** for polypeptide chains consisting of nearly 100 amino acids (6), which are considerably smaller than PAI-1. Additionally, the



Biophysical Journal

Article

Biophysical Society

## All-Atom Simulations Reveal How Single-Point Mutations Promote Serpin Misfolding

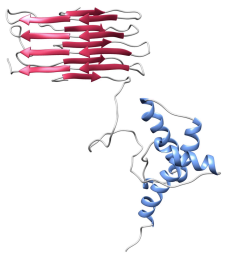
Fang Wang,[1] Simone Orioli,[2,3] Alan Ianeselli,[2,3] Giovanni Spagnolli,[2,3] Silvio a Beccara,[2,3] Anne Gershenson,[4,*] Pietro Faccioli[2,3,*] and Patrick L. Wintrode[1,*]

PLOS | PATHOGENS

RESEARCH ARTICLE

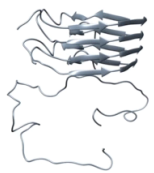
## Full atomistic model of prion structure and conversion

**Giovanni Spagnolli[1]*, Marta Rigoli[1,2], Simone Orioli[2,3], Alejandro M. Sevillano[4], Pietro Faccioli[2,3], Holger Wille[5], Emiliano Biasini[1]*, Jesús R. Requena[6]***



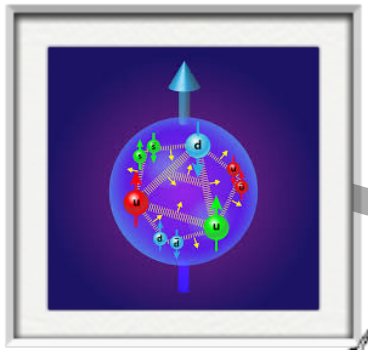
## All-Atom Simulation of the HET-s Prion Replication

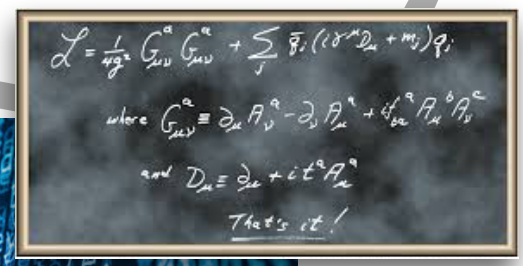
Luca Terruzzi[1,2]*, Giovanni Spagnolli[2,3]*#, Alberto Boldrini[1,2], Jesús R. Requena[4], Emiliano Biasini[2,3]# and Pietro Faccioli[5,6]#

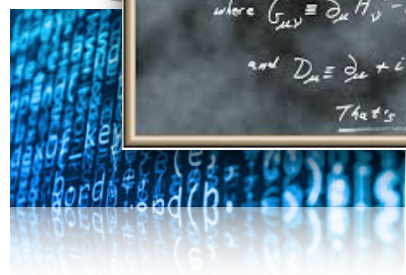


Teaming up with
**E. Biasini**'s lab (DICIBIO)

# PHASE 4: PHARMACOLOGICAL RESEARCH

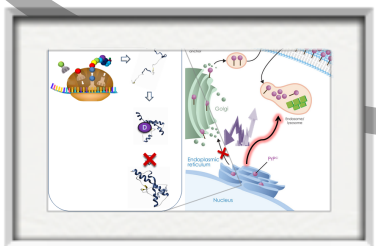




Experiment

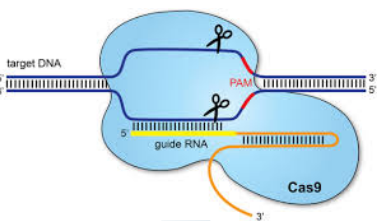
Theory



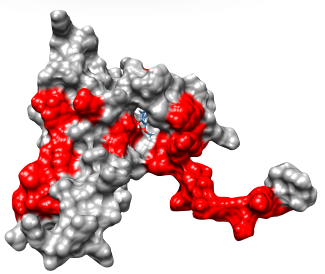
# PHARMACOLOGICAL PROTEIN INACTIVATION BY FOLDING INTERMEDIATE TARGETING

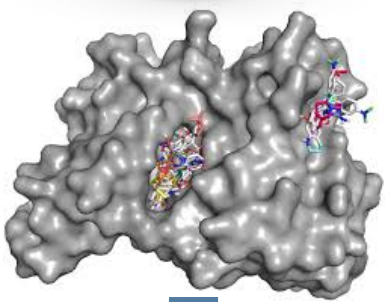
patent file # 102018000007535 (with E. Biasini)

DNA → mRNA → Ribosome → Protein folding → Protein function




RNA silencing





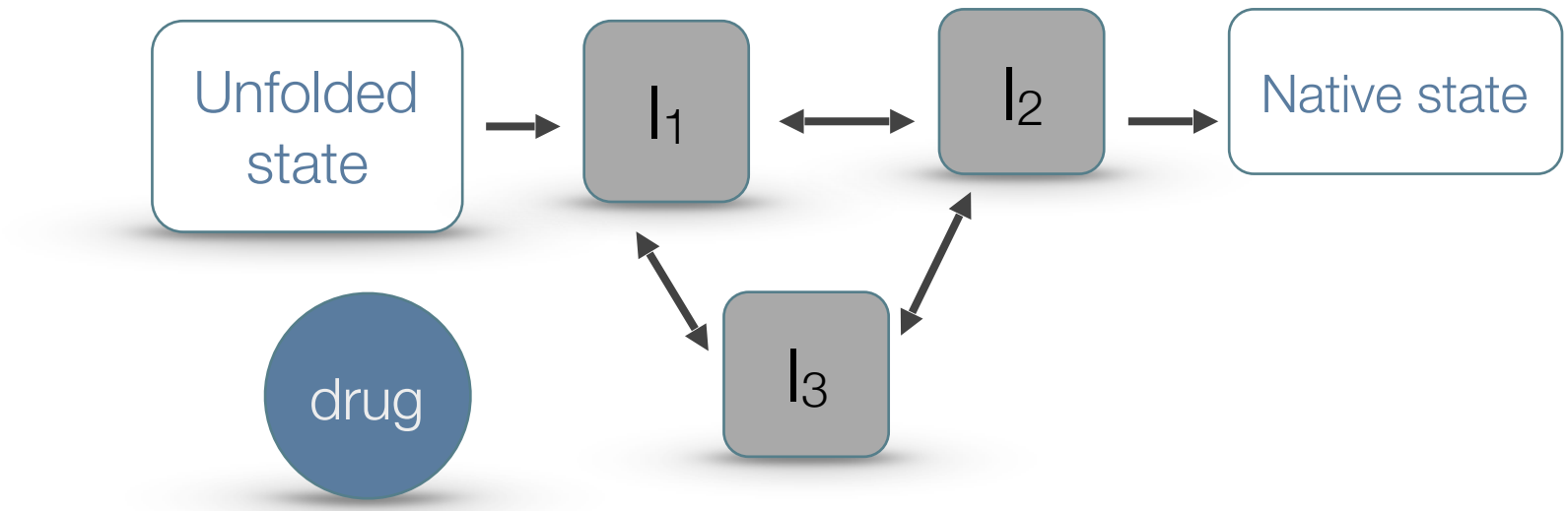
Eliminating protein from genome

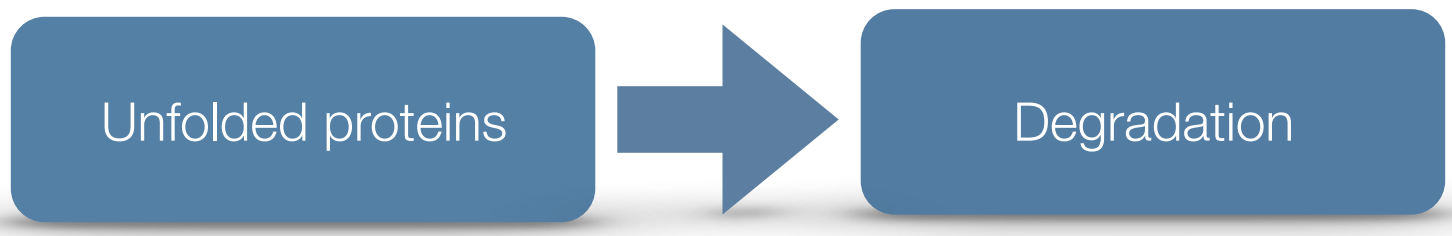
Suppressing transcription

Suppressing folding

Imparing function

# PHARMACOLOGICAL PROTEIN INACTIVATION BY FOLDING INTERMEDIATE TARGETING



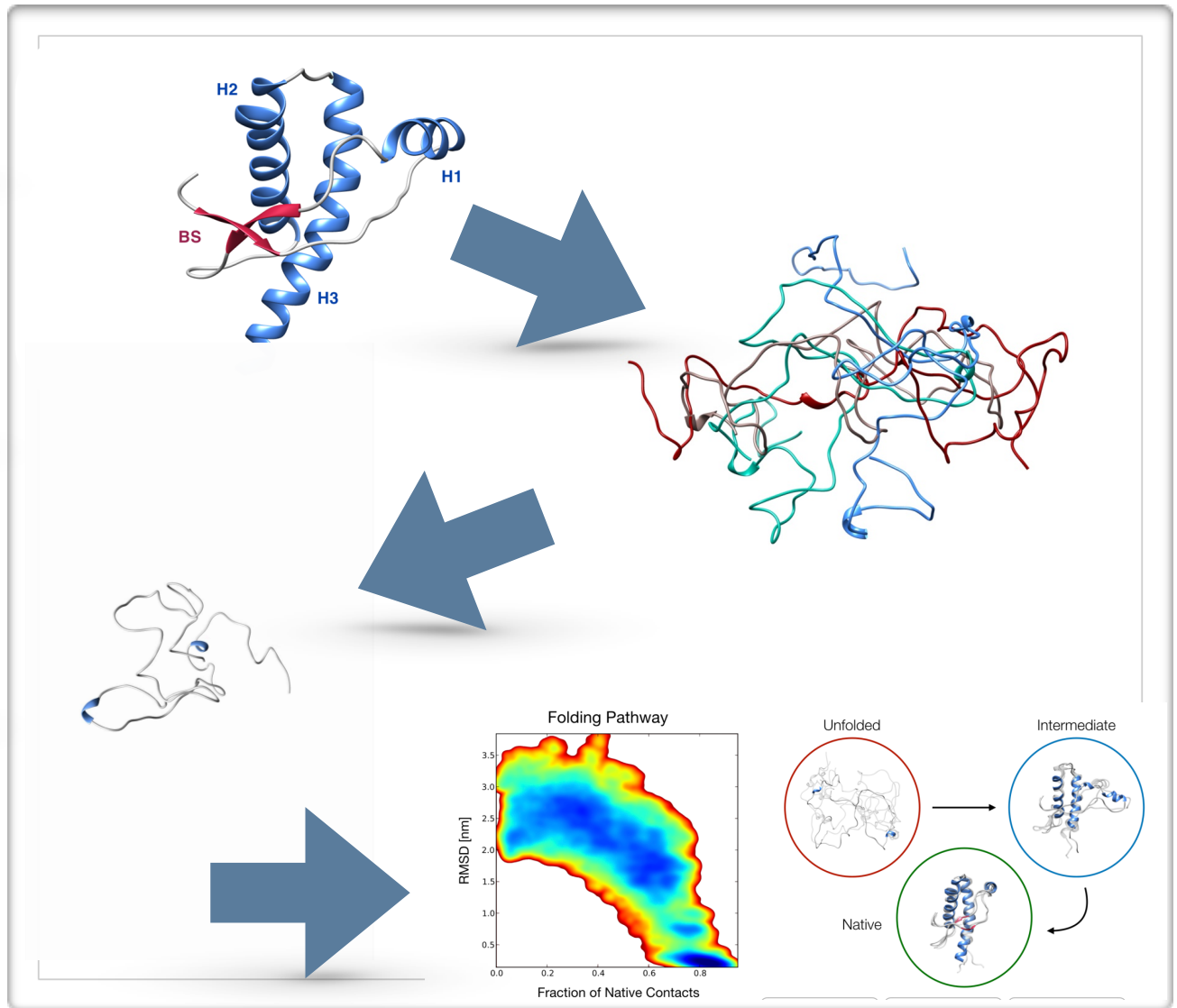



# PPI-FIT PIPELINE

Folding pathway characterization

Virtual screening on folding Intermediate

Biochemical Validation



# PPI-FIT PIPELINE

Folding pathway characterization

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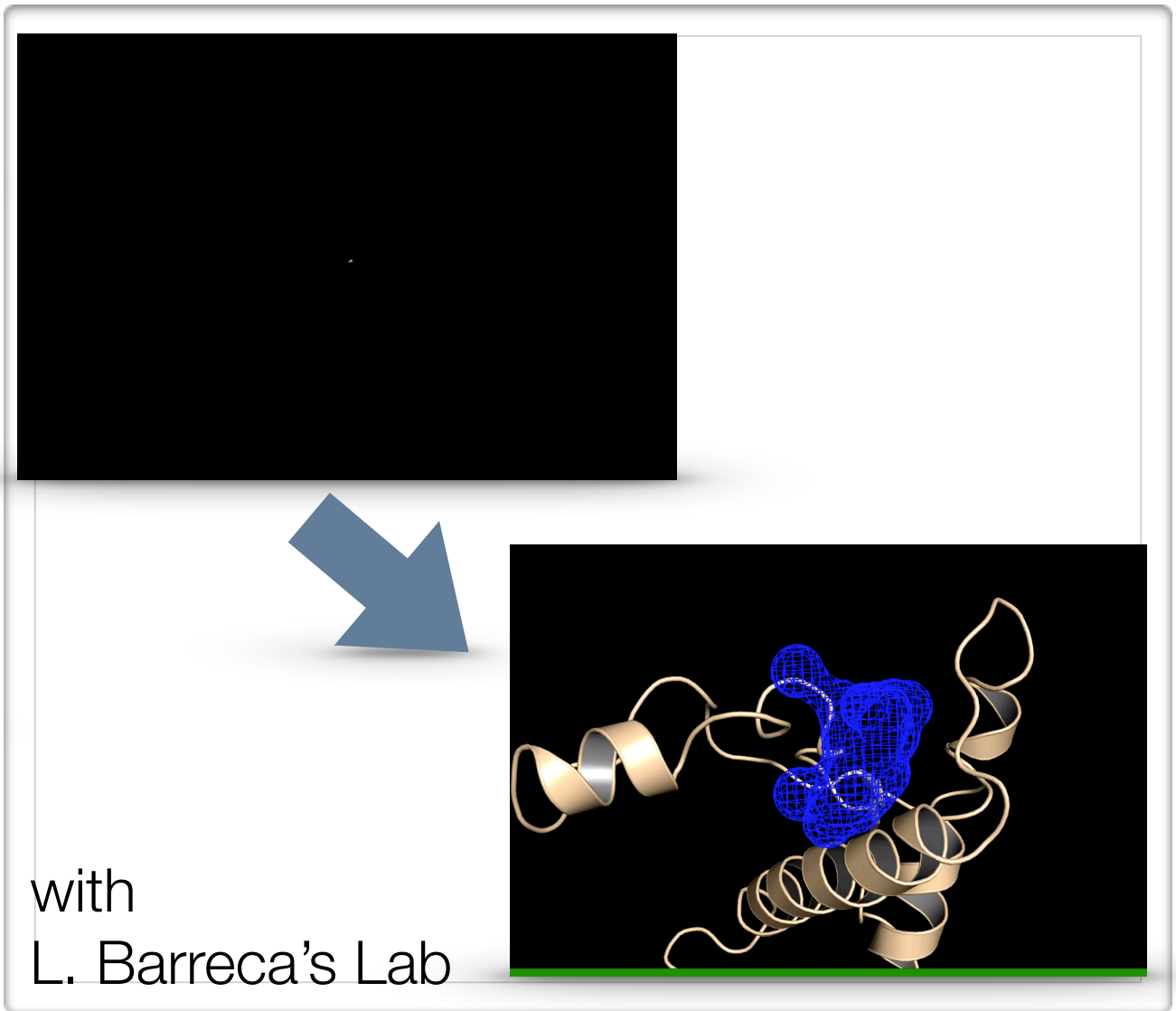
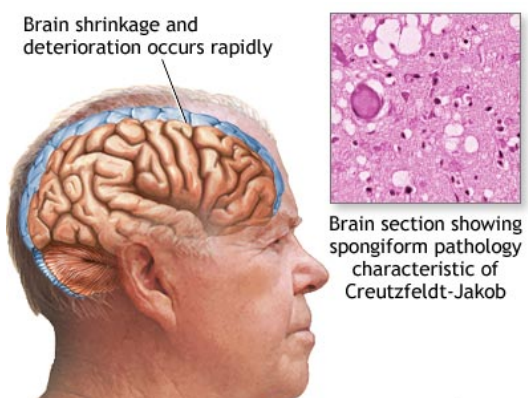


with
L. Barreca's Lab

# A FIRST VALIDATION

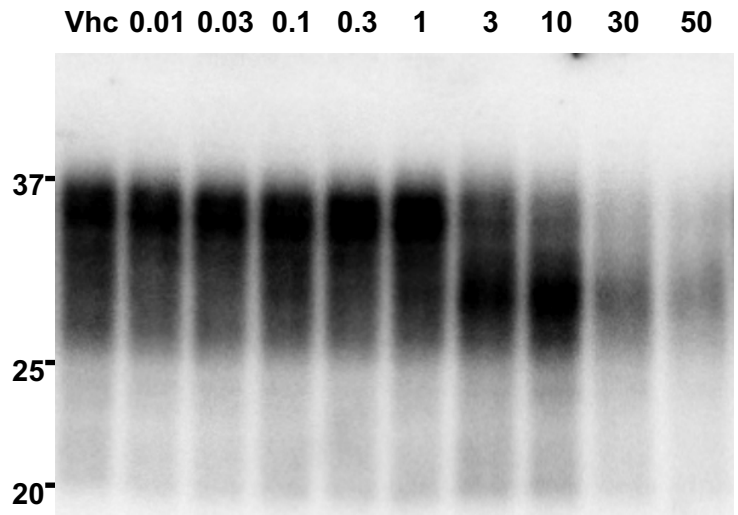
## Inactivation of Cellular Prion protein

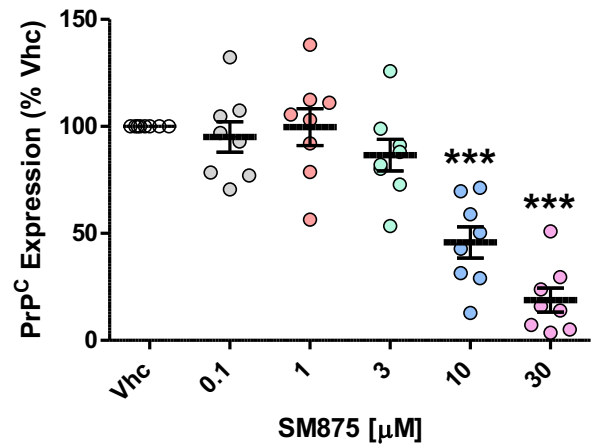


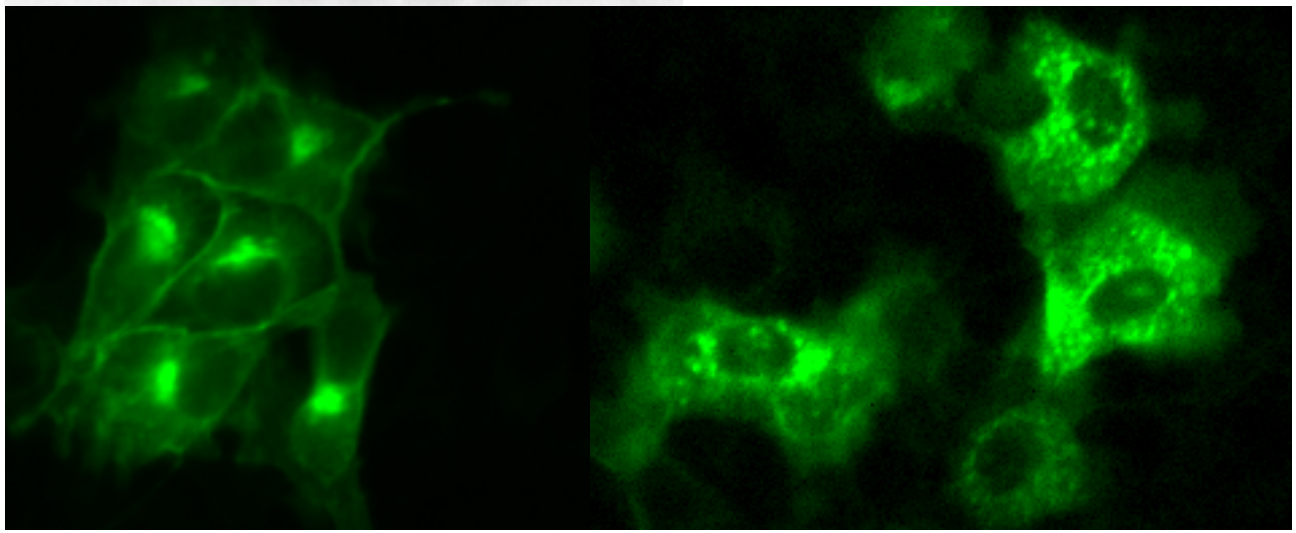

**PHARMACOLOGICAL PROTEIN INACTIVATION BY TARGETING FOLDING INTERMEDIATES**

Giovanni Spagnolli, Tania Massignan, Andrea Astolfi, Silvia Biggi, Paolo Brunelli, Michela Libergoli, Alan Ianeselli, Simone Orioli, Alberto Boldrini, Luca Terruzzi, Giulia Maietta, Marta Rigoli, Nuria Lopez Lorenzo, Leticia C. Fernandez, Laura Tosatto, Luise Linsenmeier, Beatrice Vignoli, Gianluca Petris, Dino Gasparotto, Maria Pennuto, Graziano Guella, Marco Canossa, Hermann Clemens Altmeppen, Graziano Lolli, Stefano Biressi, Manuel Martin Pastor, Jesús R. Requena, Ines Mancini, Maria Letizia Barreca, Pietro Faccioli, Emiliano Biasini

**doi:** https://doi.org/10.1101/2020.03.31.018069



SIBYLLA®

Fold the future

A few facts:

* Founded in 2017 & funded with 2.4 MEUR by VERTIS SCR in 2019.
* Spinoff of U.Trento, U.Perugia and INFN
* Exclusive license of the PPI-FIT technology
* Developing internal pipelines as well R&D service with a major Pharma
* International academic partnerships in USA and Europe

**CEO**:
Dr. Lidia Pieri

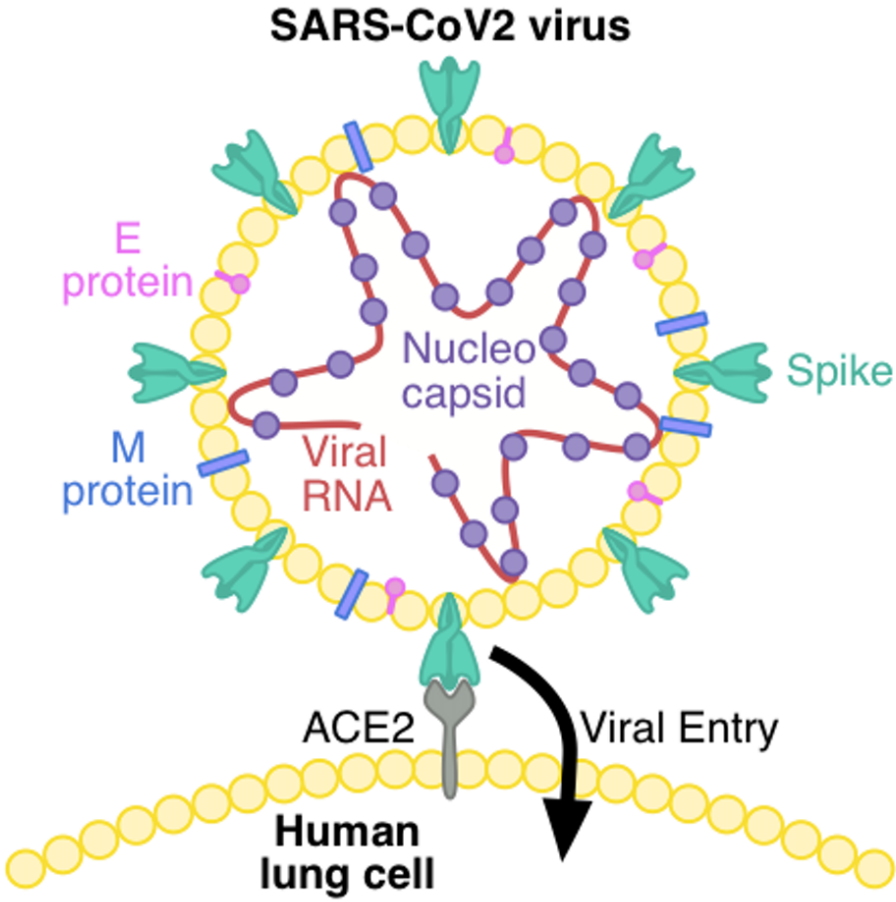
**Chief Scientists:**
Dr. Giovanni Spagnolli, Dr. Tania Massignan

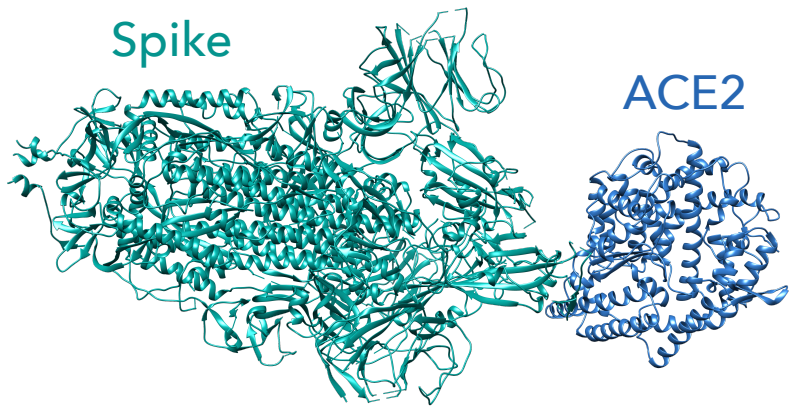
**Business Developer:**
Dr. Federica Mantovani

**Scientific Staff:**
Dott. Alberto Boldrin, Dott. LucaTerruzzi, Dott. Enrica Colasurdo, Dr. Serena Bonifati , Dr. Andrea Astolfi

**Scientific Consultant:**
Prof. Vincenzo Summa,

# SARS-CoV-2 Replication



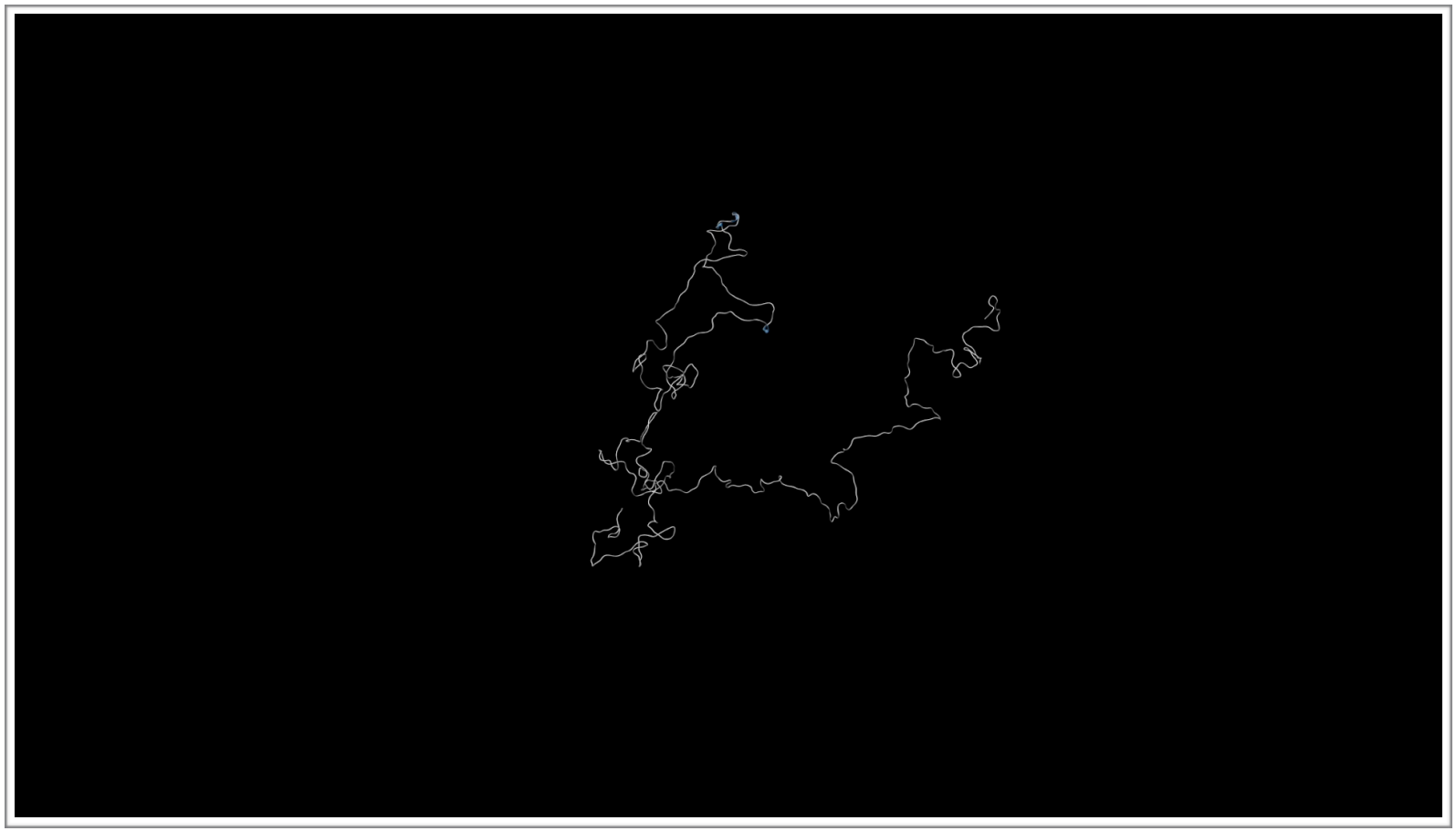

Goals:

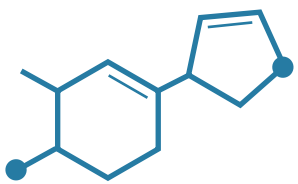
**Repurposing** of approved drugs!

Looking for suppressors of ACE2 expression levels

Figure taken from:
https://theconversation.com/where-are-we-at-with-developing-a-vaccine-for-coronavirus-134784

# PPI-FIT ON ACE2

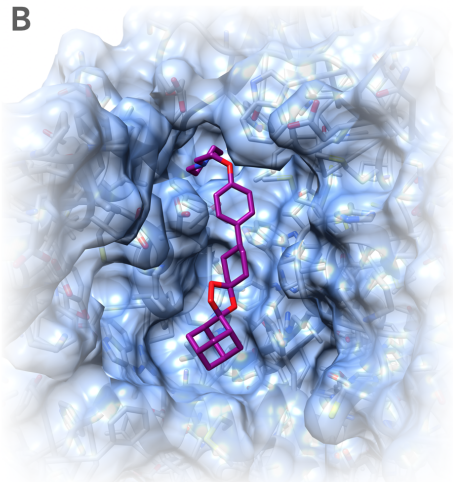


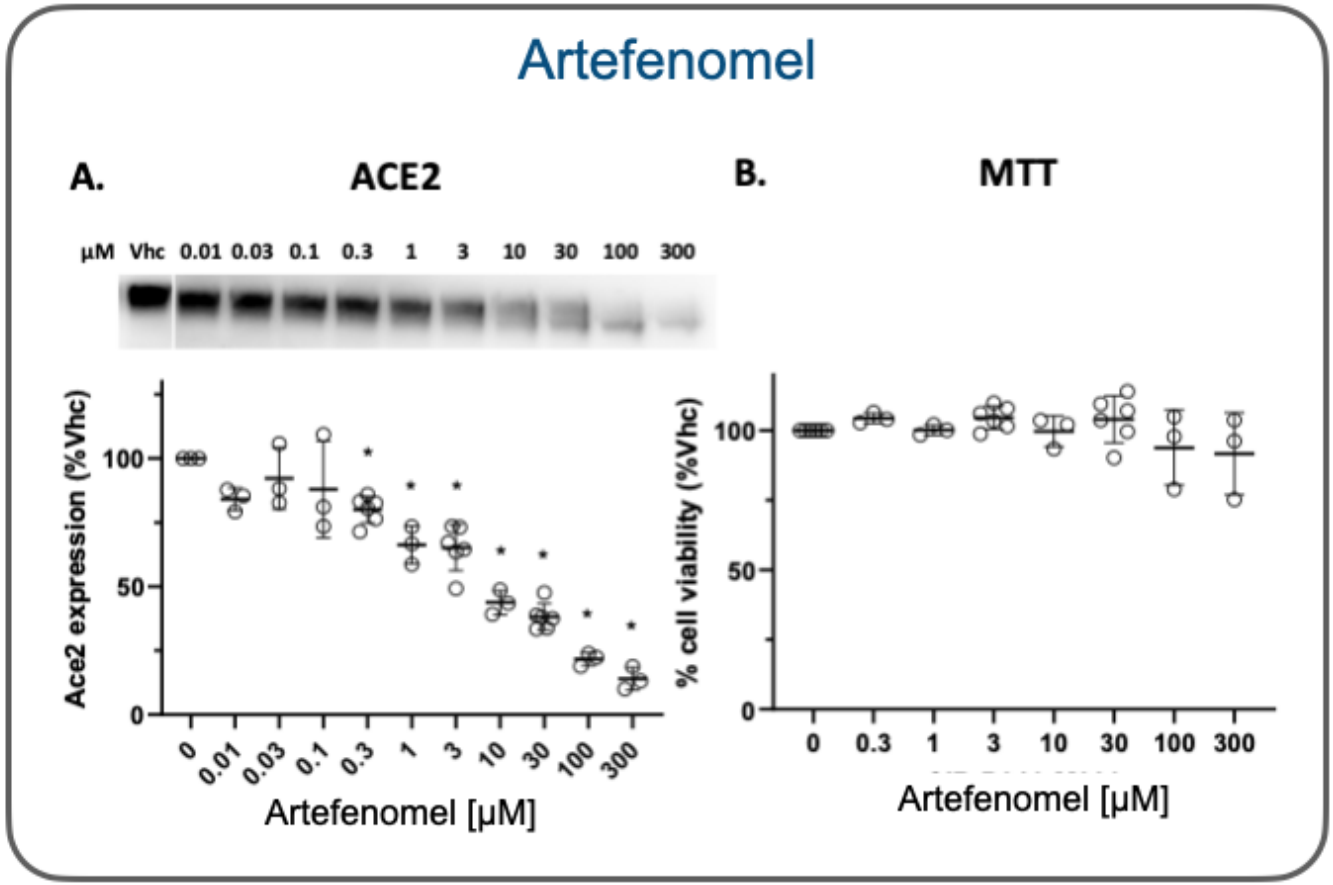


Out of 9000 candidates, we found 35 molecules binding in-silico the intermediate. Validation experiments on cellular bio-assays are ongoing.

# DOSE-DEPENDENT RESPONSE

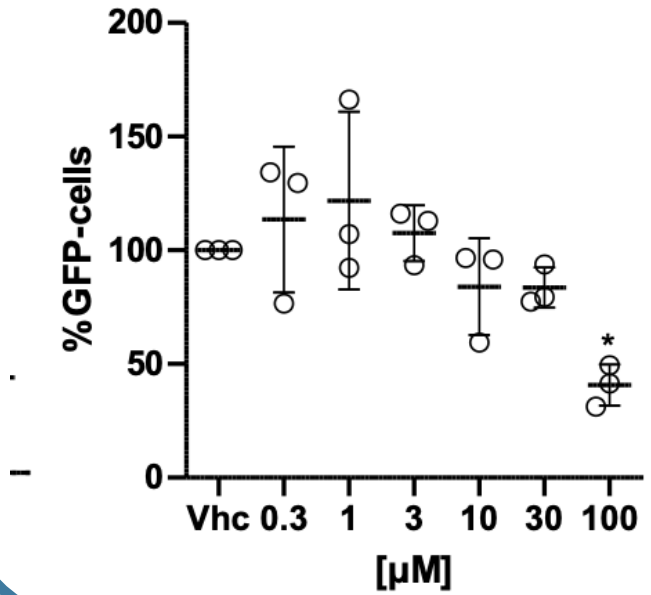
B



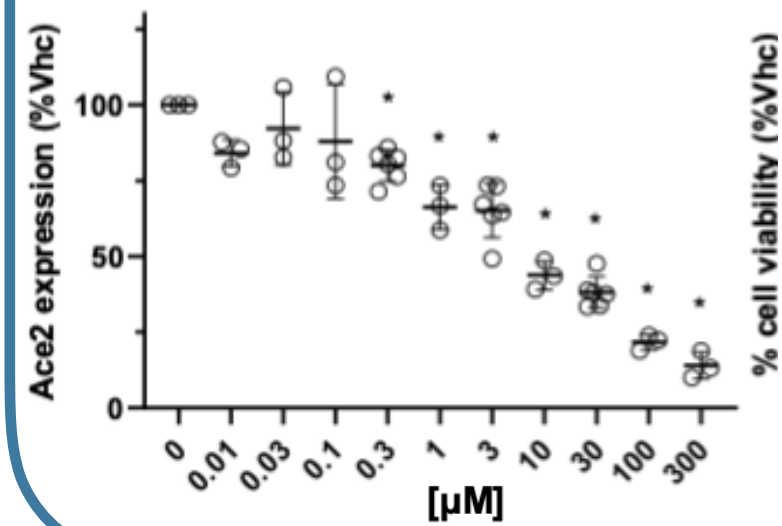


# ACTION ON A PSEUDOVIRAL VECTOR

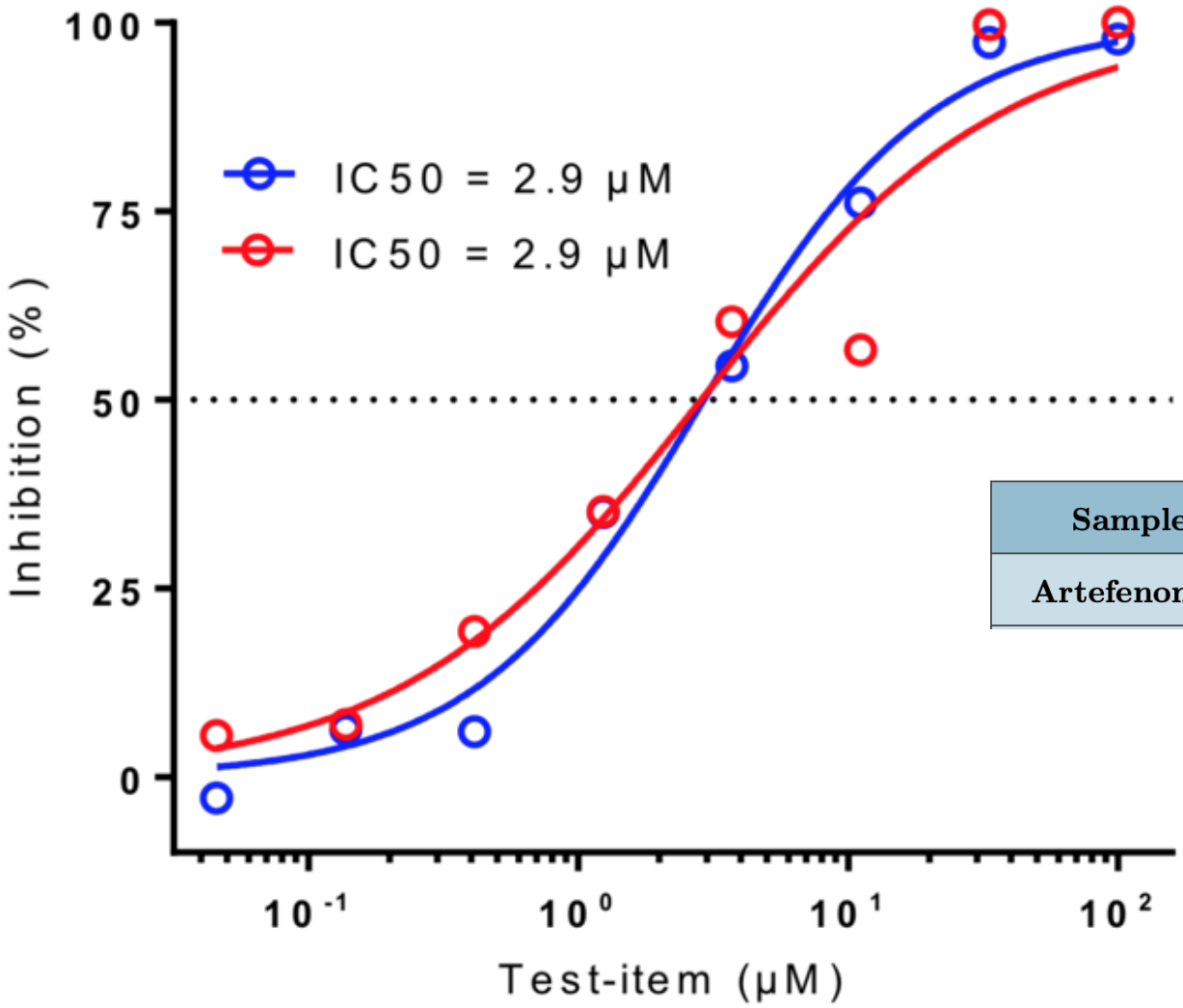
Pseudo-virus Transduction Efficiency




ACE-2 cellular expression level



# ANTI-VIRAL ACTIVITY AGAINST LIVE SARS-COV2




Concentration at which it becomes active

| Sample | IC50 [µM] | CC50 [µM] |
|---|---|---|
| Artefenomel | 2.9 | > 100 |

Concentration at which it becomes toxic

This value is *in principle* compatible with the maximum tolerated dose in humans. More to follow…

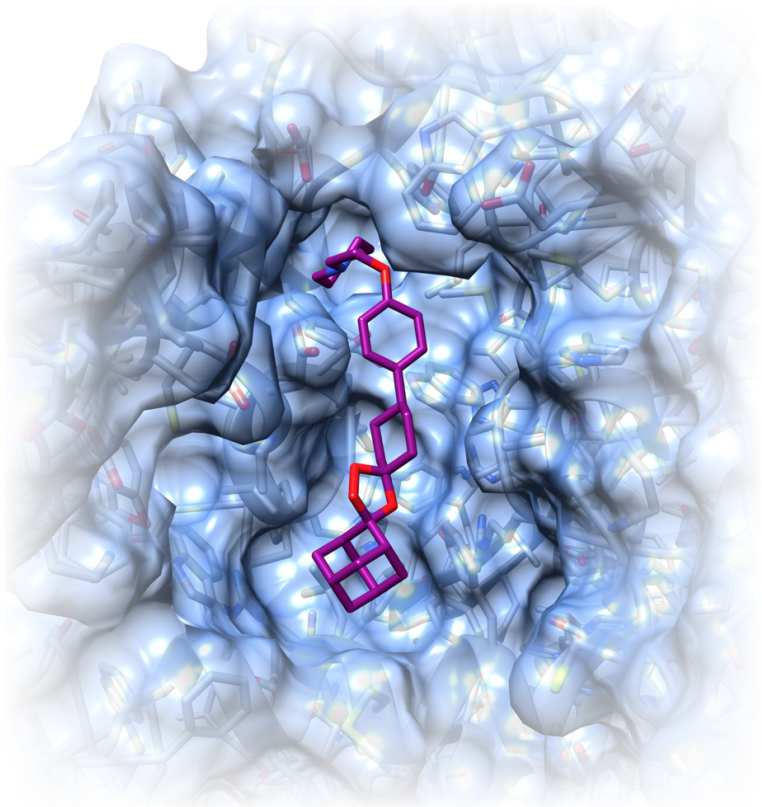
# NEW DIRECTIONS

# SPACE IS THE NEXT FRONTIER!


**Partners**:

U. Trento, Space Pharma, CJD Foundation (Israel), U. Tel Aviv, U. Santiago de Compostela, INFN

# A MAIN LIMITING FACTOR



Impossible to crystallize
folding intermediates
on Earth

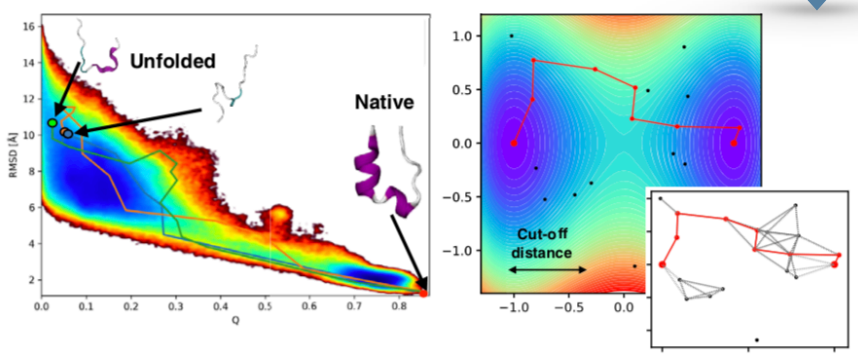
**Microgravity conditions** may provide the solution!

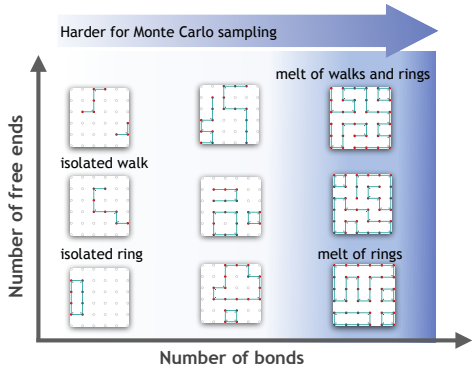
# Towards the next generation of molecular simulations

Quantum Computing

Theoretical Physics

Artificial Intelligence



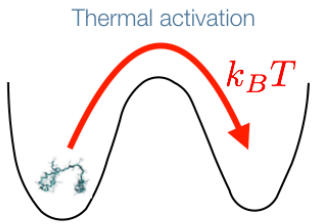




**Partners**:

U. Trento, Q@Trento, INFN, SISSA, BEC-CNR

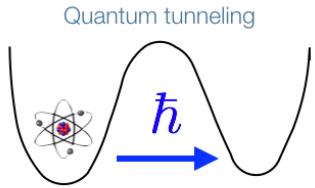
# Take home messages

1. Fundamental science breeds innovation




$( \beta = (K_B T)^{-1} )$

$$P(x_f, t|x_i) = \int_{x_i}^{x_f} \mathcal{D}q \; e^{-\frac{\beta}{4M\gamma} \int_0^t d\tau (M\ddot{q} + M\gamma\dot{q} + \nabla U(q))^2}$$




$$K_E(x_f, t|x_i) = \int_{x_i}^{x_f} \mathcal{D}q \; e^{-\frac{1}{\hbar} \int_0^t d\tau \left( \frac{M}{2} \dot{q}^2 + U(q) \right)}$$

2. Cross-disciplinarity is key to tackle complexity.
But in return it requires to redefine expectations

3. The importance of swimming in muddy waters

# Acknowledgments

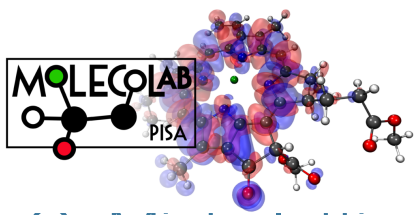

TIFPA Trento Institute for Fundamental Physics and Applications

FONDAZIONE Telethon

## Italy:

**Trento**: *E. Biasini*, A. Ianeselli (2014-2017), G. Spagnolli S. A Beccara (2009-2017), S. Orioli (2014-2018), E. Schneider (2012-2015), M. Carli (2017), M. Turelli (2018), F. Mascherpa (2014), *G. Garberoglio, F. Pederiva, M. Sega*, R. Covino (2012-2015),

**Pisa**: B. Mennucci, L. Cupellini, S. Jurinovich

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