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Article

The Markov chain of Hidden Markov Model of interaction of the K-RAS4B proteins in catalytic environment with lipid membranes has a Hilbert measure

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Abstract: A Hidden Markov Model containing both stationary Markov processes and time-varying Markov processes is considered: the corresponding Markov chain is newly proven to be one whose transition operators are on a space with Hilbert metric (whose measure exists and is unique). The Markov chain is therefore newly proven to be one with bounded moments. The further mathematical developments are envisaged. Applications are newly given for the analytical expressions of description of allosteric systems. The model of interaction of the K-RAS4B proteins with lipid membranes is newly considered accordingly; new drug design is explained.

Keywords: Markov chains; Hidden Markov Models; Hilbert metrics; existence and uniqueness of measure

1. Introduction

The processes of interaction of the K-RAS4B proteins with the lipid membranes are considered: they are known to consist of two different states of a Markov Model, i.e. a Hiddden Markov Model, where two states are identified as the 'before-transition' state and the 'pre-transition' state, of which the former is a stationary Markov process, and the latter is a time-varying Markov process.

The Hidden Markov Model (HidMM) is obtained after some clustering techniques from the (experimentally-observed) Markov-State Model (MSM).

The Markov chain corresponding to the MSM from which the clusters of the HidMM are issued is newly analytically identified (from the experimental data) as one whose transition operators are on a space with Hilbert metrics whose measure is newly proven to exists and to be unique.

The results are therefore newly applied to the analytical expressions of description of the experiment of the interaction of the K-RAS4B proteins with lipid membranes. The new application to drug design are envisaged.

The paper is organised as follows.

In Section 3, the experiments observing the dynamics of K-RAS4B proteins with membranes are recalled.

In Section 4, the experiments demonstrating the qualities of the interaction of the K-RAS4B proteins with lipid membranes are reviewed; the corresponding HidMM's are described.

In Section 5, the elements apt to the identification of the corresponding Markov chain and of its probability space are gathered.

In Section 6, the clustering methods by means of which the HidMM's are issued from the MSM's are recapitulated.

In Section 7, the partitions of the corresponding Markov chain are proven to be Sinai; the Markov chain corresponding to the analysed HidMM is newly proven to be one with Hilbert metrics.

The Discussion is provided with the Markov chain, which is commented as being one with bounded moments. New drug design are discussed accordingly.

2. Aims and scope

The Chain from which the HidMM's of KRAS4-B rpoteins interaction with lipidic membranes are issued is analytically proven to be one with Hilbert metric.

To this purpose, the proerties of the HidMM's were analysed to demonstrated that the number of the states depend on the number of critical points in the time sequence (within the experimental data). The Baum-Welch algorithm and the Bakis model were compared; for the comparison, the numbers of states of the possible (within propagation of experimental error) HidMM's are chosen as those which are not varying in time: the motion capture data are described in the next Section.

A generic Markov Chain $\tilde{\mathcal{X}}$ is one whose states are defined on a Borel (sub-)set, endowed with its σ-algebra: these properties are here upgraded to a Chain with a Hilbert metric.

For further purposes, which can find application in both the improvement of the data analysis and theoretical developments, the originating Chain is commented to be one with bounded moments.

3. The dynamics of K-RAS4B proteins with membranes

From López et al. [1], the dynamics of the KRAS4B proteins with lipid membranes is schematised after data analysis and numerical simulation.

The behaviour of the KRAS4B proteins in solutions and in membranes was tried after numerical simulations in Prakash and Gorfe [2].

The data analysed are those related to the mechanisms of the cell growth and those of the cell differentiation: the synamics of the proteins is scrutinised at the cell-scale point of view.

Various schemes of membranes were taken into account, such as i.e. the 'binary fluids mixtures' and the 'heterogeneous raft mimics'.

The analysis in López et al. [1] applies the long time scale coarse graining discretisation method in order to define the Markov state models where the proper eigenvalue scheme is not explained ibidem. In the following, a dominant-egeinvalue technique will be newly followed.

The dynamics of the proteins is specified as 'modulated' after the presence of anyonic lipids and the activation is attributed to the 'nucleotide state'.

As a partial result from López et al. [1], we outline here the description of the hyper-viable region as induced of 'preferential partitioning the domains of the membranes, from which the signaling originates.

The study of the conformational-dynamics-dependent orientations has gained relevant focus after NMR spectroscopy and conformational-dynaics simulations techniques [3].

Two main orientations of the membranes were discovered to be associated with two main configurations, one orthogonal with respect to the membrane, and one parallel [4–7]: the experimental evidence gathered in these items of bibliography is in the present paper given a systematic analytical description.

Numerical simulations issued from the experimental data are available in a wide range of contests [8–13]: all these previous experimental results are in the present work framed within the suitable Markov model.

The dynamics of K-RAS4B proteins is studied in regulating signaling pathways which determine the cell growth and the cell differentiation in some authors' studies [14–17].

The allosteric behaviour of the K-RAs4B proteins in the molecular dynamics with respect to lipid membranes is proposed to be described in López et al. [1] as within the dynamics of Hidden Markov Model (HidMM).

More in details, the determination of the states of the HidMM is regulated after the Hyper-Viable-Region molecular dynamics , of which the description of the transition is path-signaling. The dynamics of the processes which determine the conditions for the transition in the Markov landscape are defined as:

$i)$ a 'before-transition state', which is defined after a stationary Markov process; and

$i)$ a 'pre-transition state', which is described as a time-varying Markov process.

The critical states of complex dynamics systems are the pre-transition states.

From Cholewa and Gomb [18], the methods to establish the number of states of the HidMM can follow form the number of critical points of the motion capture data.

The recognition of patterns in the data sequences is applied to time sequences. The number of states of the HidMM is predicted such as the number of states does not change with the time evolution.

The results can be achieved after the Baum-Welch algorithm of wrt the Bakis model.

From Cholewa and Gomb [19], the number of states of the HidMM is estimated after the number of critical points in the time sequence. As a perspective study, it is worth remarking that pioneering analyses of the dynamics at time scale which are not resolved after the experimental available technique are are reported in Prakash and Gorfe [20].

4. The molecular dynamics of K-RAS4B proteins within lipids membranes

The properties of the molecular dynamics of K-RAS4B proteins within the framework of interaction with lipids membranes are analysed in López et al. [1] from both an optimistic molecular-dynamics point of view and a coarse-grained molecular dynamics. The technique is placed among the study of force-field parameters for non-conventional amino-acids and that of post-transactionally lipid-modified amino-acids. The models are resolved after numerical simulation.

The Markov models were selected as follows, as chosen in López et al. [1].

The transition related to the orientation of the lipidic membrane are described after coarse-graining approximation.

The MSM and the HiddMSM are considered.

The use of the coarse-graining method is justified after the analysis of the protein data bank PDB 4GON which provides one with the coordinates of the KRAS4B proteins.

The meta-stable states within the coarse-graining method at the implied time scales are therefore selected. The free-energy barriers of the Markov states space are accounted for as modifying the Gaussian heights; a bias term is considered.

The MSM can be considered in order to account for the metastable states within the implies time scales.

The Markov-State model is one consisting of $n = 400$ microstates by use of k-means clustering algorithm as from Hartigan and Wong [21]. The algorithm [21] is based on searching all the points of one cluster which are characterised such that the movement form one cluster to another one does not reduce the 'within-cluster' sum of squares.

The microstates are selected according to the time scales inferred after the eigenvalues as a function of the lag time.

The observed differences of the lag timescale described after the presentation of M metastable states.

The implied time-scale exhibits a 'plateau' trend at $450ns$.

The Perron 'cluster-cluster' analysis from Deuflhard and Weber [22] is used: the almost-invariant sets of molecular dynamical systems are selected for time-discretised Markov opera-

tors: the matrix representation is constituted after the pertinent eigenvalue-problem.

The Perron 'cluster-cluster' analysis allows to select 4 metastable states M. The allosteric properties of the KRAS-4B proteins with respect to some lipid membranes is validated to be analysed within the Markov approaches after Unbiased Molecular Dynamics Simulations in Castelli [23].

The phenomenon of selective membrane localization and clustering into microdomains of KRAS-4B proteins is described in Weise [24].

5. Measure-theoretical developments from the probability spaces of the Markov chains

Finite-states Markov chains are used for the analysis in Petrie [25].

Let $\hat{A}$ be the matrix which generates a stationary Markov process $\{X_t\}$, according to the entries

$$a_{ij} = P[X_t = j \mid X_t = i]. \tag{1}$$

Let $\hat{B}$ be the matrix that generates the stationary Markov process $\{Y_t\}$ according to the entries

$$b_{jk} = P[Y_t = k \mid X_t = j]. \tag{2}$$

Let R be the set of integers $R = 1, 2, , ..., r$; R^{∞} is defined as

$$R^{\infty} = \Pi_{t-1}^{\infty} R_t. \tag{3}$$

where R_t is a point $Y \in R^{\infty}$ with coordinates Y_t; $\hat{A}$ and $\hat{B}$ define a measure on R^{∞} for $k_i \in R$ after the stationary absolute distribution for $\hat{A}$. The process $\{Y_t\}$ is the probabilistic function of the Markov process $\{X_t\}$.

From Baum et al. [26], the maximisation techniques which are necessitated in the statistical analysis of probabilistic functions of Markov chains are studied.

The given $\hat{A}$ is therefore a stochastic matrix; one requests $\hat{A}$ to be an $s \times s$ matrix, from which the a_i, i.e. the probability distributions are defined; the probability densities f_i are normalised as $\int f_i(y)dy = 1$.

Given a the stationary distribution of the matrix $\hat{A}$, the process $\{Y_t\}$ is the probabilistic function of the process $\{X_t\}$ determined after $\hat{A}$. The triple $(\hat{A}, a, f)$ therefore identifies a probability space. It is therefore needed to define the measure of the probability space. For this purpose, let Λ be an open subset of the Euclidean space: the following properties holds:

$$\forall \lambda \in \Lambda \exists \lambda : \lambda \to (\hat{A}(\lambda), a(\lambda), f(\lambda)) \tag{4}$$

with $f_i(y)$ being define the density in y $\forall$ fixed y; $f_i(y)$ is a smooth function of λ: therefore, $\exists y_1, y_2, ..., y_T$ which defines

$$P_{y_1,y_2,...,y_T}(\lambda) = P_{y_1,y_2,...,y_T}(\hat{A}(\lambda).a(\lambda), f(\lambda)) \tag{5}$$

$P_{y_1,y_2,...,y_T}(\hat{A}(\lambda), a(\lambda), f(\lambda))$ is a smooth function of λ.

The problem to be solved is one to maximalise P over λ. It is worth recalling that the measure is here chosen; therefore, the probabiities are to be maximalised according to the chosen measure, the procedure being complementary to that described in Jenkinson [27] as, even if a HiddMM is to be chosen, the state space can be requested to be scanned,i.e. the free-energy barriers between states are requested to be not infinite among the accessible chosen states. For

this purpose, one remarks that there exists a transformation $\mathcal{T}$, $\mathcal{T} : \Lambda \to \Lambda$, and such that

$$P_{y_1,y_2,\ldots,y_T}(\mathcal{T}(\lambda)) = P_{y_1,y_2,\ldots,y_T}(\lambda) \tag{6}$$

unless λ is a critical point. The critical points will be examined in the following Section 7. It is the present purpose to define the measure of the probability space $(\hat{A}, a, f)$.

Let P_λ be

$$P_\lambda = \int_X p(x,\lambda) d\mu(x), \tag{7}$$

and let $Q_{\lambda,\lambda'}$ be

$$Q_{\lambda,\lambda'} = \int_X P_\lambda(x,\lambda) log(p(x,\lambda')) d\mu(x); \tag{8}$$

the probability measure $d\nu_\lambda$ is therefore defined as

$$d\nu_\lambda \equiv \frac{p(x,\lambda)}{P(\lambda)} d\mu(x). \tag{9}$$

Furthermore, $\forall \mathcal{T}$ continuous map, $\mathcal{T}(\lambda)$ is a critical point of $\mathcal{Q}(\lambda,\lambda')$ as a function of λ'.

Moreover, $\forall f(u)$ $\exists$ a two-parameter family $f_{m,\rho}(u)$ which qualify $f(u)$ according to m the location and to $\rho > 0$ the scale parameter as

$$f_{m,\rho}(u) = \rho^{-1} f(u-m)\rho. \tag{10}$$

$P_{m,\rho}$ is expressed according to the measure $\mu(x) > 0$.

The corresponding expression of Q becomes

$$Q_i(m,\rho,m',\rho') \equiv \sum_{i=1}^{1=s} Q_i(m,\rho,m',\rho') \tag{11}$$

The request that $f_i(u)$ be strictly log-concave is expressed.

6. The clustering methods

As from Hartigan and Wong [28], in Hartigan [29] the K-means clustering algorithm is explained as aimed at dividing the n point (i.e. the states of the MSM) into M clusters (i.e. the states of the HidMM) in the way such that the 'within-cluster' sum of squares is minimised. Instead of the optimisation within the states of the MSM, the condition is given, that there exists no movement of a point form one cluster M to another cluster M' to reduce the 'within-cluster' sum of squares.

From Di Masi and Stettner [30], the condition for existence and uniqueness of the measure of the HidMM from the probability space corresponding to the processes is studied.

The probability space of the HidMM is one consisting of the triple $(\Omega, \mathcal{F}, \mathcal{P})$, being the triple $(state, obsrevation, filter)$ of the states $Omega$ of the partition, the observation operator $\mathcal{F}$ and the filter $\mathcal{P}$, respectively.

From the probability space, a normed space with Borel measure is obtained; the transition Kernel of the Markov process is therefore one with Borel measure.

The existence and uniqueness of the invariant measure for the pair $(filter, observation)$ is ensured after the existence and uniqueness of that of the probability space.

Ergodicity of the pair $(filter, observation)$ is ensured after the uniqueness of invariant measures for the transition operator of the Markov process.

The asymptotic stability of the $filter$ allows one to infer that the metric on the probability is that of probability measures defined in Liverani [31], where, for the Markov partitions, the

metrics are Hilbert.

As from Cholewa and Gomb [19], the mode of estimation of the number of states of the HidMM can be selected after the Akaike Information Criterion [32] (AIC) of search of the minimum AIC value of the likelihood function. Its effect is differently for different data-acquisition systems.

7. The measure of the chain

Within the enforcement of the Hilbert metric from Liverani [31] for the methods of Di Masi and Stettner [30], the ergodicity of the Markov processes described allows one to ensure that the partitions are Sinai [33].

From Sinai [33], the measure for the partition is the invariant measure of the σ-algebra of all the Borel subsets.

From Sinai [33], the measure of the partitioned manifold of the Markov chain is the Lebesgue measure.

In the present work, the existence and uniqueness of a Hilbert measure for the partitions from which the HidMM is obtained is newly proven by construction.

8. Discussion

The main purpose of the present work is the study of the originating Chain of the HiddMM of KRAS4B proteins in lipidic membranes environment. More in detail, the originating Chain is proven to be one with Hilbert metric. The achievement is to upgrade from the measures issued after the σ-algebra of the Borel (sub-)sets of the generic Markov Chain definition to a Hilbert measure.

In the present paper, the HiddMM's are considered, which contain both stationary Markov processes and time-varying Markov processes. The MSM's from which the HidMM's are issued are analysed. The corresponding Markov chain is newly proven by construction to be one with Hilbert metrics. In the present work, the allosteric behaviour of certain types of proteins with respect to membranes is newly considered.

More in detail, analytical expressions of the behaviour of K-RAS4B proteins with respect of lipid-membranes environments are newly taken into account.

In the study of Banerjee et al. [34], the hypervariable region of the KRAS4B proteins is demonstrated to be disordered; the oncogenic mutations are recalled to modulate the binding in lipidic environment: as a result, the interaction is depicted as determined after both the protein features and the environment. For this reason, in the study of Chen et al. [35], the phase transitions are identiifed, according to which a Hidden Markov Model is able to identify the stationary before-transition state to the time-varying pre-transition state.

The spatiotemporal distribution of the path-signaling region is studied in the study of Shrestha et al. [36]; the interaction with the phospholipids of cellular plasma membranes is focused about: the non-Brownian features are examined in the study of Metzler et al. [37].

The atomic interaction of the KRAS mutants with particular enzymes are investigated in the study of Khaled and Gorfe [38] after 'all-atom molecular dynamics simulations', by means of which corresponding Markov state models can be constructed; the catalytic domain and the hyper variable region are investigated according to the conformational changes which are aside of the binding mechanisms with membranes and with further-related proteins: the phosphorylation of specific aminoacids is anew investigated.

Application to new drug design in envisaged after Lu et al. [39] and after Lu et al. [40]. More in detail, in Lu et al. [39], the interaction of the K-RAS4B proteins with Phosphodiesterase δ is studied. Furthermore, in Lu et al. [40], the designs of new drugs for targeting a broader class of oncogens is examined. The new techniques developed in the present work allow one

to newly broaden the analyses from the new perspective as far as the stationary processes and the non-stationary processes are concerned.

The finding of the existence and of the uniqueness of the Hilbert measure is crucial to be applied to the modelisations described in the above as far as the statistical analyses of the experimental data are concerned for the processes descried in the above, as well as for several different applications.

The study of the moments of the chains is a longstanding problem.

The study of moments of the originating chain is crucial for the perspective studies here presented.

The convergence of movements in stationary Markov Chains was studied in Holewijn and Hordijk [41]; more in detail, the necessary conditions and the sufficient ones for the convergence of the first moment is studied.

In Bertail and Clémencon [42], the tails of functionals of Markov chains are proven to have sharp bounds.

In Dobrushin [43], the version of the central limit theorem of non-stationary Markov chains is studied.

The convergence of moments in a Markov-chain central limit theorem is proven in Steinsaltz [44].

In Kartashov [45], some of the properties of time-inohogeneous Markov chains are started to being investigated.

From Naor et al. [46], the Markov chain with Hilbert measure is one with bounded moments.

All these properties, and in particular, those which lead to the understanding of the central limit theorem on the chosen chain allow one to implement the least-square method after new finding of the corresponding the Tchebys'ev inequalities for the study of the maximal likeli-hood of the propagated error in the data analysis of the experiments: these purposes are uninvestigated yet. Nevertheless, already the upgrade to a normed space is wished.

Further aspects of the behaviour of the K-RAS4B proteins in lipid membranes can be taken into account.

The dynamics of the K-RAS4b proteins in lipid environment is studied to depend on the lipid composition of the medium after NMR techniques [47].

Nanoclusters are reported to be observed in X-ray cristallogrphy in Shrestha et al. [48]; the lifetime of the nanoclusters is calculated in Sarkar and Goswami [49].

The proteins conformations are analysed to undergo the effect of the lipid membranes as far as their interactions is concerned; the topology of the mechanism is described in Shree [50].

The role of electrostatics in the behaviour of the proteins in lipid membranes is depicted in Ki-Young et al. [47] after NMR experiments as far as the orientation of the proteins is concerned, and in Lee [51]. The spatio-temporal evolution of the orientation is analysed in numerical simulations of the dynamics in Van [52]; the quantitative paramagnetic effects are investigated in Gu et al. [53].

Potential applications of the mathematical features of the studied phenomenon to new drugs design is studied in the study of authors [54–56].

Data availability: The data used in the analysis of this manuscript is taken from ATC, Ghana. They usually mount the mast for MTN-Ghana.

Conflict of interest: The author declares no conflict of interest.

References

1. López CA, Agarwal A, Van QN, et al. Unveiling the Dynamics of KRAS4b on Lipid Model Membranes. The Journal of Membrane Biology. 2012; 254: 201.
2. Prakash P, Gorfe AA. Lessons from computer simulations of RAS proteins in solution and in membrane, Biochim. Biophys. Acta 2013; 1830, 211-5218.
3. Cao S, Chung S, Kim SJ, et al. K-Ras G-domain binding with signaling lipid phosphatidylinositol (4,5)-phosphate (PIP2): membrane association, protein orientation, and function. J. Biol. Chem. 2019; 294: 7068-7084.
4. Erwin N, Patra S, Dwivedi M, et al. Influence of isoform-specific RAS lipidation motifs on protein partitioning and dynamics in model membrane systems of various complexity. Biol. Chem. 2017; 398: 547-563.
5. Kapoor S, Weise K, Erlkamp M, et al. The role of G-domain orientation and nucleotide state on the RAS isoform-specific membrane interaction. Eur. Biophys. J. 2012; 41: 801-813.
6. Weise K, Kapoor S, Denter C, et al. Membranemediated induction and sorting of K-RAS microdomain signaling platforms. J. Am. Chem. Soc. 2011; 133: 880-887.
7. Werkmuller A, Triola G, Waldmann H, et al. Rotational and translational dynamics of RAS proteins upon binding to model membrane systems. Chem. Phys. Chem. 2013; 14: 3698-3705.
8. Chakrabarti M, Jang H, Nussinov R. Comparison of the conformations of KRAS isoforms, K-RAS4A and K-RAS4B, points to similarities and significant differences. J. Phys. Chem. B. 2016; 120: 667-679.
9. Li S, Jang H, Zhang J, et al. Raf-1 cysteine-rich domain increases the affinity of K-RAS/Raf at the membrane, promoting MAPK signaling. Structure 2018; 26: 513-525e2.
10. Mazhab-Jafari MT, Marshall CB, Smith MJ, et al. Oncogenic and RASopathy-associated K-RAS mutations relieve membrane-dependent occlusion of the effector-binding site. Proc. Natl. Acad. Sci. 2015; 112: 6625-6630.
11. McCarthy M, Prakash P, Gorfe AA. Computational allosteric ligand binding site identification on RAS proteins, Acta Biochim. Biophys. Sin. 2016; 48: 3-10.
12. Prakash P, Zhou Y, Liang H, et al. Oncogenic K-RAS binds to an anionic membrane in two distinct orientations: a molecular dynamics analysis. Biophys. J. 2016; 110: 1125-1138.
13. Travers T, Lopez CA, Van QN, et al. Molecular recognition of RAS/RAF complex at the membrane: role of RAF cysteine-rich domain. Sci. Rep. 2018; 8: 8461.
14. Hancock JF. RAS proteins: different signals from different locations. Nat. Rev. Mol. Cell. Biol 2003; 4: 73-385.
15. Hobbs GA, Der CJ, Rossman KL. RAS isoforms and mutations in cancer at a glance. J. Cell. Sci. 2016; 129 (7): 1287-1292.
16. Ntai I, Fornelli L, DeHart CJ, et al. Precise characterization of KRAS4B proteoforms in human colorectal cells and tumors reveals mutation/ modification cross-talk. Proc Natl Acad Sci USA. 2018; 115(16): 4140-4145.
17. Nussinov R, Tsai CJ, Jang H. Oncogenic RAS isoforms signaling specificity at the membrane. Cancer Res 2018; 78: 593–602.
18. Cholewa M, Gomb P. Deciding of HMM parameters based on number of critical points for gesture recognition from motion capture data. Available online: https://arxiv.org/pdf/1110.6287.pdf. (accessed on 2 March 2024).
19. Cholewa M, Gomb MP. Estimation of the number of states for gesture recognition with Hidden Markov Models based on the number of critical points in time sequence. Pattern Recognition Letters 2013; 34: 574-579.
20. Prakash, P, Gorfe, AA. Probing the conformational and energy landscapes of KRAS membrane orientation. J. Phys. Chem. B. 2019; 123: 8644-8652.
21. Hartigan JA, Wong MA. Algorithm AS 136: a K-means clustering algorithm. J. R. Stat. Soc. 1979; 28: 100–108.
22. Deuflhard P, Weber M. Robust Perron cluster analysis in conformation dynamics. Linear Algebra Appl. 2005; 398: 161–184.
23. Castelli M. Decrypting Allostery in Membrane-Bound K-Ras4B Using Complementary 'In Silico' Approaches Based on Unbiased Molecular Dynamics Simulations. J. Am. Chem. Soc. 2023; 146: 901-919.
24. Weise K. Dissociation of the K-Ras4B/PDEδ Complex upon Contact with Lipid Membranes: Membrane Delivery Instead of Extraction. J. Am. Chem. Soc. 2012; 134: 11503.
25. Petrie T. Probabilistic functions of finite-state Markov chains. PNAS 1967; 57(3): 580-581.
26. Baum LE, Petrie T, Soules G, et al. A Maximization Technique Occurring in the Statistical Analysis of Probabilistic Functions of Markov Chains. The Annals of Mathematical Statistics. 1970; 41: 164-171.
27. Jenkinson O. Ergodic optimization. Discrete and Continuous Dynamical Systems. AIMS 2006; 15: 197-224.

28. Hartigan JA, Wong MA. A K-Means Clustering Algorithm: Journal of the Royal Statistical Society. Series C (Applied Statistics). 1979; 28: 100-108.
29. Hartigan JA. Clustering Algorithms. New York: Wiley; 1975.
30. Di Masi GB, Stettner LH. Ergodicity of hidden Markov models. Control Signals Systems. Spring publishing; 2005; 17: 269-296.
31. Liverani C. Decay of Correlations. Annals of Mathematics Second Serie. 1995; 142(2): 239-301.
32. Akaike H. Information theory and an extension of the maximum likelihood principle. In: Petrov BN, Csáki F (editors). Proceedings of 2nd International Symposium on Information Theory; 2–8 September 1971; Tsahkadsor, Armenia. Springer-Verlag; 1992: 610-624.
33. Sinai Ya.G. Construction of Markov partitions. Functional Analysis and Its Applications. Springer publishing; 1968; 2: 61-82.
34. Banerjee A, Jang H, Nussinov R, et al. The disordered hypervariable region and the folded catalytic domain of oncogenic K-Ras4B partner in phospholipid binding. Curr. Opin. Struct. Biol. 2016; 36: 10-17.
35. Chen P, Liu R, Li Y, et al. Detecting critical state before phase transition of complex biological systems by hidden Markov model. Bioinformatics. 2016; 32(14): 2143.
36. Shrestha R, Chen D, Frank P, et al. Recapitulation of cell-like KRAS4b membrane dynamics on complex biomimetic membranes. Science 2021; 25(1): 103608
37. Metzler R, Jeon JH, Cherstvy AG. Non-Brownian diffusion in lipid membranes: Experiments and simulations. Biochim Biophys Acta. 2016; 1858(10): 2451.
38. Khaled M, Gorfe A. Sayyed-Ahmad A. Conformational and Dynamical Effects of Tyr32 Phosphorylation in K-Ras: Molecular Dynamics Simulation and Markov State Models Analysis. J. Phys. Chem. B. 2019; 123(36): 7667-7675.
39. Lu HX, Hu ZY, Faraudo J, et al. Discovery of a novel drug using lipid-based formulation targeting G12D-mutated KRAS4B through non-covalent bonds. Available online: https://doi.org/10.1101/2023.04.05.535682.
40. Lu HX, Hu ZY, Faraudo J, et al. In silico design of a lipid-like compound targeting KRAS4B-G12D through non-covalent bonds. Nanoscale. 2023; 15, 19359-19368.
41. Holewijn PJ, Hordijk A. On the convergence of moments in stationary Markov chains. Stochastic Processes and Their Applications. 1975; 3: 55-64.
42. Bertail P, Clémencon S. Sharp bounds for the tails of functionals of Markov chains. Theory of Probability and its Applications. 2010; 54: 505–515.
43. Dobrushin R.L. Central limit theorem for nonstationary Markov chains. I, Theory of Probability & and Its Applications. SIAM Publishing; 1956.
44. Steinsaltz D. Convergence of moments in a Markov-chain central limit theorem, Indagationes Mathematicae Publishing; 2001.
45. Kartashov M. The asymptotic behavior of rare Markov moments defined on time inhomogeneous Markov chains. Theory of Probability and Mathematical Statistics Publishing; 2014.
46. Naor A, Rao S, Regev O. Concentration of Markov chains with bounded moments, Ann. Inst. H. Poincaré Probab. Statist. 2020; 56: 2270-2280.
47. Ki-Young L, Mitsuhiko I, Marshall C.B. The Self-Association of the KRAS4b Protein is Altered by Lipid-Bilayer Composition and Electrostatics. Angewantde Chimie International Edition Publishing. 2023; 62: e202218698.
48. Shrestha, R, Carpenter, TS, Van, QN, et al. Membrane lipids drive formation of KRAS4b-RAF1 RBDCRD nanoclusters on the membrane. Commun Biol 2024; 7: 242.
49. Sarkar S, Goswami D. Lifetime of actin-dependent protein nanoclusters. Biophysical Journal. 2023; 122(2): 290-300.
50. Shree S. Revealing KRas4b topology on the membrane surface. Biochemical and Biophysical Research Communications. 2023; 678: 122-127.
51. Lee KY. Two Distinct Structures of Membrane-Associated Homodimers of GTP- and GDP-Bound KRAS4B Revealed by Paramagnetic Relaxation Enhancement. Angew Chem Int Ed Engl. 2020; 59: 11037-11045.
52. Ngo VA, Sarkar S, Neale C, et al. How Anionic Lipids Affect Spatiotemporal Properties of KRAS4B on Model Membranes. J. Phys. Chem. B. 2020; 124: 5434-5453.
53. Gu X, Liu D, Yu YK, et al. Quantitative Paramagnetic NMR-Based Analysis of Protein Orientational Dynamics on Membranes: Dissecting the KRas4B-Membrane Interactions. J. Am. Chem. Soc. 2023; 145: 10295-10303.
54. Lu H. In silico design of a lipid-like compound targeting KRAS4B-G12D through non-covalent bonds. Nanoscale. 2023; 15: 19359-19368.
55. Angela P, Sofia C, Alessandra M. Molecular dynamics simulations for the structure-based drug design: targeting

small-GTPases proteins. Expert Opinion on Drug Discovery 2024; 6: 1-21.
56. Lu HX, Hu ZY, Faraudo J, et al. Discovery of a novel drug using lipid-based formulation targeting G12D-mutated KRAS4B through non-covalent bonds e-print. BioRxiv; 2023. doi:10.1101/2023.04.05.535682