

1 **Machine learning based detection of genetic and drug class variant**
2 **impact on functionally conserved protein binding dynamics**

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27 Abstract

28 The application of statistical methods to comparatively framed questions about protein dynamics can
29 potentially enable investigations of biomolecular function beyond the current sequence and structural
30 methods in bioinformatics. However, chaotic behavior in single protein trajectories requires statistical
31 inference be obtained from large ensembles of molecular dynamic (MD) simulations representing the
32 comparative functional states of a given protein. Meaningful interpretation of such a complex form of
33 big data poses serious challenges to users of MD. Here, we announce DROIDS v3.0, a molecular dynamic
34 (MD) method + software package for comparative protein dynamics, incorporating many new features
35 including maxDemon v1.0, a multi-method machine learning application that trains on large ensemble
36 comparisons of concerted protein motions in opposing functional states and deploys learned
37 classifications of these states onto newly generated protein dynamic simulations. Local canonical
38 correlations in learning patterns generated from self-similar MD runs are used to identify regions of
39 functionally conserved protein dynamics. Subsequent impacts of genetic and drug class variants on
40 conserved dynamics can also be analyzed by deploying the classifiers on variant MD runs and
41 quantifying how often these altered protein systems display the opposing functional states. Here, we
42 present several case studies of complex changes in functional protein dynamics caused by temperature,
43 genetic mutation, and binding interaction with nucleic acids and small molecules. We studied the impact
44 of genetic variation on functionally conserved protein dynamics in ubiquitin and TATA binding protein
45 and demonstrate that our learning algorithm can properly identify regions of conserved dynamics. We
46 also report impacts to dynamics that correspond well with predicted disruptive effects of a variety of
47 genetic mutations. In addition, we studied the impact of drug class variation on the ATP binding region
48 of Hsp90, similarly identifying conserved dynamics and impacts that rank accordingly with how closely
49 various Hsp90 inhibitors mimic natural ATP binding.

50 Keywords

51 Molecular dynamics, machine learning, molecular evolution, pharmaceuticals, genetic variation, binding
52 interaction

53 Statement of significance

54 We propose a statistical method as well as offer a user-friendly graphical interfaced software pipeline
55 for comparing simulations of the complex motions (i.e. dynamics) of proteins in different functional
56 states. We also provide both method and software to apply artificial intelligence (i.e. machine learning
57 methods) that enable the computer to recognize complex functional differences in protein dynamics on
58 new simulations and report them to the user. This method can identify dynamics important for protein
59 function, as well as to quantify how the motions of molecular variants differ from these important
60 functional dynamic states. For the first time, this method of analysis allows the impacts of different
61 genetic backgrounds or drug classes to be examined within the context of functional motions of the
62 specific protein system under investigation.

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66 Introduction

67 The physicist Richard Feynman is said to have once famously quipped, ‘all biology is ultimately due to
68 the wiggling and jiggling of atoms’. Stated with more precision, Feynman’s conjecture would imply that
69 all biological function can ultimately be understood by analyzing rapid molecular motions in
70 biomolecular structures as they alter or shift their functional state(s). Many decades later, these
71 functional shifts in molecular dynamics are being illuminated by structural and computational biology.
72 Examples of functionally altered dynamics include the destabilization of inter-residue contacts, in both
73 disease malfunction and normal signal activation, as well as the stabilization of inter-residue contacts
74 during protein folding, the formation of larger complexes, and various other binding interactions to
75 small molecules. And while the functional role of rapid vibrations revealed by short term molecular
76 dynamic (MD) simulations has been debated in the past, more recent empirical and computational
77 studies have clearly demonstrated that differences in both rapid and directed vibrations can drive longer
78 term functional conformational change (1, 2). From a broader perspective, if Feynman’s conjecture is
79 true, then the specific details of a given protein system’s biomolecular dynamics will represent a
80 potentially large source of latent variability in our functional understanding of the genome; a problem
81 largely ignored by those disciplines currently generating the vast amounts of static forms of ‘omic’ type
82 data (i.e. DNA sequence, transcript level, and protein structure)(3). However, in the last decade,
83 simultaneous advances in the development of graphics hardware and biomolecular force fields has
84 elevated our ability to computationally simulate MD long enough to capture ns to μ s timescales for
85 moderately-sized proteins (4, 5), and finally ‘see’ some of their functionally relevant motions. And now,
86 the application of proper statistical comparisons of ensembles of short-timed framed MD simulation can
87 potentially enable meaningful interpretations of comparative questions about protein dynamics (6). But
88 due to the richly complex structure of data underlying the moving images generated by MD software,
89 functional interpretation of modern MD simulations poses a serious challenge to current users,
90 especially with comparatively-framed questions, where large ensembles of many production runs need
91 to be generated and subsequently analyzed. A potential solution to this problem exists with the
92 application of machine learning to the feature extraction and classification of the dynamic differences
93 between ensembles of MD runs. These ensembles can be designed to represent pair-wise functional
94 states of biomolecular systems (e.g. before/after chemical mutation or binding). Therefore, the high
95 performance accelerated computation used to generate simulated protein motions for comparison can
96 be effectively partnered with high performance methods for optimally extracting and learning the
97 underlying dynamic feature differences defining the different functional states of proteins. Although
98 machine learning has recently been applied to individual MD studies for a variety of specific tasks (7–9),
99 there is no current software platform for the general application of machine learning to comparative
100 protein dynamics.

101 In 2018, we released DROIDS v1.2 and v2.0 (Detecting Relative Outlier Impacts from molecular
102 Dynamic Simulation), a GPU accelerated software pipeline designed for calculating and visualizing
103 statistical comparisons of protein dynamics drawn from large repeated ensembles of short dynamic
104 simulations representing two protein states (6). This application allowed simple visual and statistical
105 comparison of protein MD ensembles set up in any way the user wanted to define them. Here, we
106 announce the release of DROIDS v3.0, which now offers multiple pipelines tailored for specific functional
107 comparisons of systems comprised of combinations of proteins, nucleic acids, and small ligand
108 molecules. Comparisons can include different temperatures, different protein binding states (i.e. to
109 DNA, drugs, toxins or natural ligands), or divergent genetic/epigenetic mutant states. We also include a
110 major new machine learning tool, maxDemon v1.0, a multi-machine learning post-processing application
111 for DROIDS that trains on the data representing the comparatively divergent functional dynamic states,

112 and subsequently identifies functionally conserved dynamics and genetic and/or drug class binding
113 variant effects when deployed on new MD simulations representing these variants of interest. Thus,
114 much like James Clerk Maxwell's mythical creature (10), maxDemon derives important information from
115 all atom resolution observation of dynamic motion. The three primary features/aims of our newly
116 expanded software is to (A) improve user experience in comparative protein dynamics, (B) enable the
117 local detection of functionally conserved protein dynamics, and to (C) enable the assessment of the local
118 dynamic impacts of both genetic and drug class variants within the functional context of protein system
119 of interest. Because the machine learning model we employ is trained on MD data representing normal
120 functioning dynamic states of a protein, this metric of impact is highly context dependent to how a given
121 mutation or drug impacts a specific protein. Thus, it potentially gives considerably more functional
122 relevance to the analysis of variants when compared to more general database-derived metrics of
123 mutational tolerance (e.g. SIFT, PolyPhen2 etc.). In Table 1, we list five primary methodological pipelines
124 in available in DROIDS 3.0+maxDemon to address functional questions in comparative protein dynamics.
125 In our results and discussion here, we present data on four case studies of functional protein dynamics
126 that include feature extraction and classification of (A) a simple temperature shift in ubiquitin dynamics,
127 (B) mutational impacts on ubiquitin binding dynamics, (C) mutation specific impacts on DNA binding of
128 TATA binding protein, and (D) comparison of binding dynamics of drug class variants that mimic ATP
129 binding in Hsp90.

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131 Materials and Methods

132 ***Overview of comparative dynamics and visualization with DROIDS v3.0***

133 Our DROIDS method/software leverages several important key concepts when making comparisons
134 between MD runs. The method utilizes structural alignment to restrict comparison of dynamics between
135 individual homologous amino acids. The method also restricts comparison averaged over atoms
136 common to all amino acids (i.e. backbone C, N, O and C α). The method also employs statistical
137 ensembling to make a robust comparison between protein dynamics in different functional states (6).
138 While this is computationally intensive, it is necessary because of the inherent chaotic nature and
139 unpredictability of single protein trajectory projections. This logic is analogous to the many storm tracks
140 repeatedly modeled by meteorologists to gain statistical confidence in a hurricane weather forecast,
141 where an ensemble of model runs all with slightly different initial conditions has far more predictive
142 power than any single simulation. In DROIDS, the user can decide how large the MD ensembles need to
143 be based upon the inherent stability of the protein under investigation. Generally, an ensemble size of
144 200 to 300 MD runs at 0.5-1 ns will suffice for most proteins. The dynamics is summarized by calculation
145 of root mean square fluctuations (*rmsf*) over constant time intervals represented by a constant number
146 of image frames defined by the user (thus allowing *rmsf* values to be sampled on repeatedly on an
147 identical and thus comparable scale). The default number of frames (*i*) in the software for a given time
148 slice is $n=50$ representing 0.01 ns of simulation time. The rmsf value is thus

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$$rmsf = \frac{1}{4} \sum_{i=1}^4 \sqrt{\frac{1}{n} * \sum_{j=1}^n (v_{jx} - w_x)^2 + (v_{jy} - w_y)^2 + (v_{jz} - w_z)^2}$$

150 where v represents the set of XYZ atom coordinates for i backbone atoms (C, N, O, and C $_{\alpha}$) for a given
151 amino acid residue over j time points and w represents the reference coordinate structure at the
152 beginning of each MD production run for a given ensemble. Therefore, $rmsf$ values as defined here
153 represent molecular dynamics at the resolution of a single amino acid backbone segment, and the same
154 resolution at which fine scale protein-level molecular evolution operates via amino acid replacement,
155 insertion and deletion. The $rmsf$ is also the most basic underlying functional quantity to extract from MD
156 simulation as its underpins all hierarchical levels of motion (1). Two ensembles of $rmsf$ values (a query
157 set and a reference set) are compared to calculate average delta $rmsf$ or $dRMSF$. The user can choose to
158 see the average angstrom difference between sets of values, or more preferably the user can calculate
159 the symmetric Kullback-Leibler divergence (i.e. relative entropy) between the two empirical statistical
160 distributions of $rmsf$. The KL divergence generally provides a richer more informative view of dynamic
161 differences with less loss of information than simple averaging. Thus $dRMSF$ comparing $rmsf$ values for
162 two ensembles of size m for a given amino acid is

$$163 \quad dRMSF_{avg} = \left(\sum_{i=1}^m rmsf \right)_{query} - \left(\sum_{i=1}^m rmsf \right)_{reference}$$

164 or

$$165 \quad dRMSF_{KL} = \frac{1}{2} * \left[\left(\sum_{i=1}^m p(rmsf_{query}) * \log \frac{p(rmsf_{query})}{p(rmsf_{reference})} \right) \right. \\ 166 \quad \left. + \left(\sum_{i=1}^m p(rmsf_{reference}) * \log \frac{p(rmsf_{reference})}{p(rmsf_{query})} \right) \right]$$

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168 The resulting dRMSF values are color mapped to either still structures or movie images of the dynamics
169 according to either a ‘temperature’ scale where (+) dRMSF = amplified vibration is red and (-) dRMSF =
170 dampened vibration is blue. A ‘stoplight’ scale where (+) dRMSF is green and (-) dRMSF is red is also
171 available. On both scales, neutral values are shaded towards white.

172 **Functional classification of new MD simulation with maxDemon v1.0**

173 While users can easily employ DROIDS 3.0 to examine ensemble differences between functional genetic
174 or binding states, the application of this knowledge to new MD simulation is nearly impossible due to
175 the inherent complexity of the moving protein behavior. Our new post-processing software, maxDemon
176 1.0, uses machine learning to label or classify the differences learned by a previous DROIDS
177 query/reference state comparison when subsequently applied to one or more new MD runs. The
178 machine learning-based detection of variant impacts on functional protein dynamics presented here is
179 outlined schematically in Figure 1. Similar to the statistics for comparative dynamics, the learning
180 algorithms are also applied individually to each amino acid backbone’s ensemble of $rmsf$ values. This
181 allows for similar single residue resolution in the results. Learners are also applied within the same user
182 defined time slices of $rmsf$ allowing for visualization of time resolution of classification of functional
183 dynamic behaviors as well. The learning performance is summarized by tallying the average
184 classification over all time slices for each amino acid. Thus, an average performance of 0.5 would
185 indicate that the learners are not finding the functional states defined by and trained by the initial

186 DROIDS comparative analysis. Canonical correlations in the positional performance plots are key in
187 detecting sequence encoded functionally conserved dynamics regions, as well as genetic and drug class
188 variant impacts to these functional regions as well. This is described with more formality below.

189 ***Machine learning training and validation***

190 The feature vectors (X) for machine learning are collections of *rmsf* values (x_i) labeled according
191 to a query (q) and reference state (r) defined by the DROIDS MD comparison (i.e. where labels y_i are $q =$
192 1 and $r = 0$).

$$193 \quad X = \{(x_i, y_i)\}_{i=1}^N$$

194 The length of the vector (N) is defined by the length of the MD production run chosen by the user and
195 the size of the ensemble of MD production runs taken. Thus if the user chooses an ensemble of 200 MD
196 production runs each at a time length of 0.5 ns (= 2500 frames) and uses the default time interval of 50
197 frames to calculate any given interval of *rmsf* then the resulting feature vector will contain 20,000 data
198 values for training (i.e. 10,000 values each for q and r).

199 Users create a ‘stacked model’ or meta-model containing up to seven different machine learning
200 classification algorithms including K-nearest neighbors, naïve Bayes, linear discriminant analysis,
201 quadratic discriminant analysis, random forest, adaptive boosting and support vector machine (with
202 kernel options including parameter tuned linear, polynomial, laplace and radial basis functions). R
203 packages employed here are KNN, MASS, kernlab, randomForest and ada. We restricted machine
204 learning to ‘shallow’ learning methods due to the relatively small datasets created when resolving
205 dynamics of protein systems to short slices of time over single amino acids and also because of the
206 robustness of the R packages when applied sequentially over time and structural space. Therefore, we
207 do not yet support implementation of deep learning neural networks. For methodologically robust
208 results on small proteins, we generally recommend users select all seven available methods. As real
209 features of dynamics should be detectable by any method of learning, the agreement of classification
210 obtained by the creation of a stacked model utilizing different learning methods makes the learning less
211 sensitive to methodological artifacts. Depending upon system resources, users can choose to include or
212 omit methods from four categories of learning (i.e. instance-based = KNN, probabilistic = NB / LDA /
213 QDA, black box = SVM, and ensemble learning = randomForest / adaboost. Users will want to use as
214 many as their system resources can handle, however for faster processing, a minimum of three of the
215 seven learning methods can be chosen. Currently, most methods run on single CPU cores, however, the
216 more CPU intensive methods of random forest and adaboost algorithms are programmed to use all
217 available CPU cores found on the system. SVM is often the slowest method for larger protein systems
218 and can be omitted when more than 300 residues are present in the protein simulation.

219 After learners are trained on the query and reference ensembles, they are validated on a new
220 MD run that matches the state of the reference MD runs during training. For example, when analyzing a
221 binding interaction where the reference ensemble of training runs are conducted in the unbound
222 protein state, a new run will be conducted in the unbound state and a line plot of the machine learning
223 performance (i.e. precision, recall and accuracy) will be generated for all positions on the protein. It
224 would be expected that if comparative differences in dynamics observed in the training set have a
225 genuine relation to function(s) defined during training, they will display repeated behavior in the new
226 reference run and be identified by the stacked learning model generating local peaks in learning

227 performance (i.e. accuracy) at functional regions (Figure 1D). Learner performance for a given machine
228 learning method is defined as

$$229 \quad 'performance' = \frac{TP + TN}{TP + TN + FP + FN} = \frac{TP + 0}{TP + 0 + FP + 0}$$

230 Where TP, TN, FP, and FN are true positive, true negative, false positive, and false negative
231 classifications resp. The zero value terms arise because the validation is conducted on simulation
232 representing just the reference state of the DROIDS comparison (where $y_i = 0$). Therefore, accuracy,
233 and precision are algebraically collapsed to a single equivalent performance metric while recall is always
234 equal to 1.

235 **Identifying regions of conserved dynamics**

236 Functionally conserved dynamics are defined as 'repeated or self-similar and sequence-dependent
237 dynamics' discovered after training machine learners on the functional state ensembles derived with
238 DROIDS. Conserved dynamics are detected via significant canonical correlations in position specific
239 learning performance patterns after the deployment of learners on new MD simulation runs that were
240 setup identically to the reference dynamic state defined by the MD ensemble training set. We expect
241 that functionally conserved dynamics will be sequence encoded and therefore should display a repeated
242 position dependent signature in our learned pattern profiles whenever MD runs are set up identically to
243 MD upon which learners were trained. Therefore, a significant local canonical correlation (i.e. Wilk's
244 lambda) between learning performance profiles of self-similar MD runs can be used to detect local
245 regions of conserved protein dynamics.

246 To detect functionally conserved dynamics after training and validation, an additional new MD
247 run matching the functional reference state is created (i.e. matching the MD validation run). The
248 learning performance of this run is compared to the MD validation run using a canonical correlation
249 analysis conducted using all selected learners (i.e. the stacked model) across both space and time (i.e.
250 fluctuations backbone atoms of individual amino acids over subdivided time intervals). Any sequence
251 dependent or 'functionally conserved' dynamics can be recognized through a significant canonical
252 correlation in the profile of the overall learning performance along the amino acid positions for the two
253 similar state runs. In effect, this metric defines dynamics that are functionally conserved by capturing a
254 signal of significant self-similarity in dynamics that co-localizes to a specific part of the protein backbone.

$$255 \quad conserved_{dynamics} = significant(CC_{self})$$

256 Significantly conserved regions calculated within a user defined sliding window (default value =20
257 residues with cutoff of $p < 0.01$) can be plotted upon the positional local correlational value profile (i.e. R
258 value) and also can be mapped to the reference structure of the protein, colored in dark gray on a light
259 background.

260 **Variant impact assessment**

261 By extension, mutational impacts of genetic or drug class variants on the functionally conserved
262 dynamics can be quantified by their effects that range significantly beyond that observed in the self-
263 similar reference runs. Thus when canonical correlations of variants differ significantly from the self-
264 correlation observed in functionally conserved regions, according to a bootstrap test, we can plot the

265 magnitude of impact defining how the variant's dynamics differs from the routine self-similar dynamics
266 of the normal functioning protein. The impacts of dissimilar states caused by altered amino acid
267 sequence or different binding partners are assessed through their local effect on the same canonical
268 correlation identifying conserved dynamics. We introduce a metric of relative entropy relating the
269 canonical correlations in both the self-similar and altered variant state. In essence, this is a metric of the
270 'impact' of a given genetic or drug class variant within the context of normal functioning dynamics. For
271 example, when trained on a natural binding interaction (e.g. DROIDS analysis comparing a DNA binding
272 protein in its bound and unbound states), novel MD simulations with a variety of amino acid
273 replacements can be deployed to see whether the learners can still recognize the functional dynamics in
274 the mutant forms. In this case, functionally tolerated mutations will result in functionally conserved
275 dynamics that do not vary outside of ± 3 standard deviation bounds of the self-similar runs, whereas
276 functionally intolerant mutations will result in significant deviations from self-similarity of motion. An
277 overall impact of a genetic and/or drug class binding variant on the conserved dynamic regions is
278 calculated by

$$279 \quad \text{variant}_{\text{impact}} = CC_{\text{self}} * \log \frac{CC_{\text{variant}}}{CC_{\text{self}}}$$

280 Comparative plots of local variant impacts outside of the 3 standard deviation bound determined by the
281 validation run are generated within a user defined sliding window. Thus, this variant impact metric is
282 designed to identify variant regions with dynamics that potentially alter disrupt conserved dynamic
283 features of the normal functioning protein system.

284 ***Four example applications (case studies)***

285 To demonstrate the performance and utility of DROIDS 3.0 + maxDemon 1.0, we ran the following four
286 comparative case studies using the PDB IDs mentioned below. Bound and unbound files were created by
287 deleting binding partners in UCSF Chimera and resaving PDBs (e.g. 3t0z_bound.pdb, 3t0z_unbound and
288 3t0z_ligand). Each MD run ensemble consisted of 200 production runs at 0.5ns each explicitly solvated
289 in a size 12 octahedral water box using TIP3P solvent model with constant temperature under an
290 Anderson thermostat. The models were charge neutralized with both Na⁺ and Cl⁻ ions. The heating and
291 equilibration runs prior to production were 0.3ns and 10ns respectively. Prior to heating 2000 steps of
292 energy minimization were also performed. All seven available machine learning classifiers were
293 trained on the functional MD ensembles and deployed upon new 5 ns production runs for each variant
294 analyzed.

295 Case study 1 (figure 2) – PDB ID = 1ubq – to analyze self-stability and effect of temperature shift in
296 ubiquitin

297 Case study 2 (figure 3)– PDB ID = 2oob – to analyze functional binding of ubiquitin to ubiquitin ligase and
298 impacts of several tolerance pre-classified genetic variants

299 Case study 3 (figure 4) – PDB ID = 1cdw – to analyze functional binding of TATA binding protein to DNA
300 and impacts of several genetic variants

301 Case study 4 (figure 5) – PDB ID = 3t0z – to analyze functional ATP binding in Hsp90 and subsequent
302 impacts of six inhibitor drug variants

303 ***Improvements and upgrades over previous versions***

304 To enhance the user experience and scientific utility, DROIDS v3.0 offers many new features beyond
305 earlier major release versions 1.2 and 2.0. These are summarized below.

- 306 - New GUI organization directs users to specific comparative tasks/applications in Table 1
- 307 - A new control file builder for managing path dependencies in Linux is included
- 308 - Amber16/18 support has been beta tested and is defined via paths.ctf file
- 309 - Single or dual GPU user options are available for faster analyses
- 310 - Automated structure prep (dry and reduce) via pdb4amber is now included in the GUI. The
311 'reduce' variable is optional allowing users to either setup their own protonation states ahead of
312 DROIDS, or simply allow DROIDS to hydrogenate the input structures entirely.
- 313 - Program/package dependency installer script named 'DROIDSinstaller.pl' is included. It will lead
314 users through all dependencies required after a fresh Linux build, including CUDA libraries and
315 tools required for Nvidia GPU accelerated Amber in the Linux environment
- 316 - KL divergence (= relative entropy) definition of dFLUX is now included as an option providing a
317 richer color mapping of dFLUX in images and movies than the simple averaging algorithm
318 offered in earlier DROIDS versions
- 319 - Binding interaction analysis for both protein-DNA and protein-ligand systems is now offered
320 with dedicated GUI for these comparisons. Protein-ligand system setup includes QMMM
321 preprocessing in Antechamber and SQM.
- 322 - LeAP control files for explicit solvent runs are now presented for advanced user modifications
323 (e.g. changing ion concentration, water model, water box dimension of volume).
- 324 - Dedicated GUI allowing genetic mutation placement (on DNA or AA) are included for setting up
325 variants to analyze
- 326 - Self-stability and temperature shift analysis has its own dedicated GUI, allowing users to copy
327 the input pdb file to compare MD ensembles generated on identical structures at the same of at
328 different temperatures
- 329 - MaxDemon 1.0 - machine learning based detection of functionally conserved dynamic regions
- 330 - MaxDemon 1.0 - machine learning based impact assessment of variants (genetic, structural or
331 binding)
- 332 - Dynamic visualization and movie rendering of machine learning classification performance
- 333 - Virtual reality and ChimeraX compatibility is also supported (additional information and
334 download code can be found here
335 <https://cxtoolshed.rbvi.ucsf.edu/apps/moleculardynamicsviewer>
336 https://github.com/kdiller713/ChimeraX_MolecularDynamicViewer

337 ***Basic implementation of DROIDS and maxDemon***

338 The first step of any DROIDS analysis is to find or create two homologous PDB file format structures that
339 represent the query and reference functional states of the protein system under investigation. Typically,
340 these would represent the same protein in a bound vs. unbound state, or in a mutant vs. wildtype state.
341 If the protein is interacting with a small ligand, and additional 'ligand only' PDB file should also be
342 created for subsequent quantum mechanical optimization and preparation by Ambergtools antechamber
343 program. These files should be placed within the DROIDS download folder. Upon implementation via the
344 command 'perl DROIDS.pl' launched from terminal within the DROIDS folder, the DROIDS graphical user
345 interface (GUI) will help the user write a control file for required working path directories on their
346 system (first use only) and then proceeds to a main GUI outlining the various types of comparisons that

347 can be generated (as detailed in Table 1) and the number of GPU available on the system. The next step
348 is provides a user-friendly GUI to control and schedule Amber16/18 GPU-accelerated MD simulation to
349 generate ensembles of short MD runs representing two functional protein states wanting to be
350 compared. These functional comparisons are not limited, but would typically entail the impact of
351 mutation (comparing dynamics before and after one or more amino acid replacements), the impact of
352 an environmental change (comparing two states of temperature of solvent set up), or the impact of a
353 molecular interaction (comparing bound to an unbound state). The DROIDS GUI will lead users through
354 the building of a structural alignment file using UCSF Chimera's MatchMaker and Match-Align tools. This
355 will be needed later by the graphics components of DROIDS to make sure that only homologous regions
356 of structures are being compared and analyzed. In this application, where the user is primarily
357 interested in genetic or drug class variant impacts on an interactive signaling function, the typical
358 training ensembles generated by DROIDS for further analysis with maxDemon should represent the
359 normal binding function of the wild-type protein and therefore the bound vs unbound comparison
360 would typically be used. A PDB file of the bound state can be the starting point and an unbound PDB
361 model can be saved after deleting chains in the original file. If a small molecule ligand interaction is
362 under study and requires application of an additional force fields such as GAFF, than an additional file
363 representing only the ligand should also be generated and saved for preparation with antechamber
364 software prior to building the solvent models using teLeap. The GUI will pop open the .bat files that
365 control more details of the simulation setup allowing advanced users to write more lines into the teLeap
366 modeling prep (e.g. to alter the water box dimensions, the water model itself, or to add additional ions
367 beyond simple charge neutralization). The user should read all warnings provided to the terminal at this
368 stage by the Amber software. Our GUI script will also double check the sizes of the files generated at this
369 stage and will supply a warning if teLeap failed altogether to set up the complete model system for
370 simulation. Upon successful setup the user can launch all the MD runs from the GUI. The requested jobs
371 are automatically scheduled to each GPU one at a time by our software. When finished, the user can
372 easily generate rmsf data by using the GUI to setup and launch cpptraj software provided in Ambertools.
373 Thus the total process from file preparation, MD production and post-processing for DROIDS analysis by
374 simply working down the buttons on each GUI from top to bottom and subsequently following the
375 directions on the main terminal. After MD simulation and post-processing, DROIDS will take users to a
376 second GUI for generating R plots and analyses for statistically comparing the dynamics, and then to a
377 third GIU for visualization and movie generation. We refer users to our user manual and previous
378 publication for more details. This third GUI has buttons to optionally launch our new machine learning
379 application maxDemon if users wish to go beyond simple comparative protein dynamics and investigate
380 novel simulations utilizing the DROIDS MD ensembles as a training set for subsequent machine learning.

381 More detailed instructions to users are included with our DROIDS 3.0+maxDemon 1.0 user
382 manual available in the GitHub repository.

383 **The main repository for DROIDS 3.0 and maxDemon 1.0 can be found here. Please follow the link to**
384 **"Releases" and download the latest release as .tar.gz or .zip file**

385 <https://github.com/gbabbitt/DROIDS-3.0-comparative-protein-dynamics>

386 and DOI: 10.5281/zenodo.3358976 concurrent with this publication

387 <https://zenodo.org/record/3358976#.XURVkhKiiM>

388 We also post various videos of examples using DROIDS, video tutorials, and ongoing projects here

389 <https://www.youtube.com/channel/UCJTbGq01pBCMDQikn566Kw>

390

391 Results and Discussion

392 To demonstrate the variety of comparative analyses that can be addressed with the new release of
393 DROIDS 3.0 and maxDemon 1.0, we chose four different case studies of comparative protein dynamics.
394 These included (A) an analysis of self-stability and temperature effects in single ubiquitin structure, (B) a
395 functional genetic variant analysis of ubiquitin and ubiquitin ligase binding interaction, (C) a functional
396 genetic variant analysis of DNA binding in TATA binding protein, and (D) a drug class variant analysis of
397 compounds targeting the Bergerat ATP binding region of Hsp90 heat shock protein.

398 ***Machine learning analysis of impacts due to simple environmental temperature shift***

399 We first ran a null comparison as a ‘sanity check’ by running a query and reference ubiquitin (11)
400 MD at the same temperatures (both 300K) and same solvent conditions. The DROIDS analysis (Figure 2A-
401 C) showed identical atom fluctuation profiles along the backbone and a random dFLUX profile indicative
402 of nonsignificant differences due to small random local thermal differences in the training sets. The
403 machine learning classification plots on new MD runs vary randomly around 0.5 reflecting the fact that
404 the learning algorithms effectively had no features to train on (Figure 2D). As expected, no significantly
405 conserved dynamics were identified either (Figure 2E). By contrast, a protein dynamic comparison run
406 with a 50K temperature difference (Figure 2 F-H) shows a much higher machine learner performance
407 upon deployment (i.e. 70-80% successful classification – Figure 2I). Because environmental temperature
408 shifts are not expected to reflect evolutionary conserved dynamics (i.e. are not position dependent in
409 their effect), they also subsequently do not result in significant canonical correlations in the learning
410 profiles (Figure J). Representative time slices of the positional classifications in each of these
411 experiments are shown in K and L resp and indicate that our machine learning is capable of extracting
412 and identifying simple differences in dynamics due to temperature. Another interesting observation
413 here was the slightly higher learning performance of the simpler machine learning methods QDA and
414 LDA over others at all sites in the temperature shifted example. We interpret this to be related to the
415 fact that underlying rmsf distributions are probably Gaussian, a critical assumption of these two models,
416 with unequal variances caused by steric hindrances on the backbone. This would predict that QDA might
417 outperform other learners in this situation and it appears that it does. We note that where more
418 complex functional dynamics are concerned, the more sophisticated learning methods such as support
419 vector machine and adaboost often perform slightly better than others. However, we also note that
420 these performance differences are usually quite small and that all learning methods generally come to
421 similar local conclusions about functional dynamics. We examine machine learning performance
422 regarding more functional binding dynamics in ubiquitin.

423

424 ***Machine learning analysis of impacts of genetic variants on a functional protein binding interaction***

425 To examine functional dynamics in ubiquitin, we conducted a DROIDS analysis comparing its two
426 functional states, bound and unbound to the ubiquitin associated binding (UBA) domain of ubiquitin

427 ligase (12)(Figure 3A-D). This binding domain is highly conserved among the many other proteins that
428 interact directly with ubiquitin. The binding interaction greatly reduces the atom fluctuation in ubiquitin
429 at 3 characteristic positions, two loop structures centered at LEU 8 and ALA 46 and a portion of beta
430 sheet at the C terminus (Figure 3C). These three regions also drive significant differences in dynamics
431 across the whole protein. In novel self-similar MD runs on the bound state, we successfully detect
432 significant canonical correlations indicating conserved dynamics in these three regions with a broad
433 expanse in conserved dynamics (Figure 3E and F) across the UBA region (Figure 3G). We tested a set of
434 24 mutations that included sites with the most and least tolerated effects on growth rate *in vivo* in yeast
435 according to a study by Roscoe et al. (13). In this study, nearly all mutations at E18 and G53 are tolerated
436 while nearly all mutations at K48 and R72 are not. Ultimately, the causes of tolerance in these variants
437 are not known, and do not necessarily invoke functional problems in dynamics. However, the impacts
438 that we did observe in simulation were on average twice as strong in the intolerant backgrounds when
439 compared to the mutation tolerant backgrounds. And, while we did not see large differences in the
440 number of mutational impacts on dynamics between tolerated and non-tolerated mutant groups, the 24
441 mutations analyzed all show a general trend of dynamic impact falling outside of most of the functional
442 binding region (Figure 3H-K), suggesting that ubiquitin may have evolved a tertiary structure that
443 allosterically translates dynamic impacts to less functional regions of the protein. Some interesting
444 exceptions to this rule were demonstrated by the very large impacts of K48L, K48W and R72D, centered
445 squarely in the functionally conserved binding regions of ubiquitin, and would obviously heavily disrupt
446 electrostatic charge interactions there as well.

447 ***Machine learning analysis of impacts of genetic variants on DNA binding interaction***

448 TATA binding protein (TBP) is a general transcription factor that binds DNA upstream in most
449 highly regulated eukaryotic gene promoter regions (14). While relatively small, it is a mechanically
450 dynamic protein with a C-clamp like structure that highly distorts the rigid DNA double helix by inserting
451 four phenylalanine side-chains between base pairs. It is thought that this bending allows TBP to be more
452 rapidly released from the TATA element, as opposed to TATA-less promoters, subsequently allowing
453 more highly controlled regulatory responses in TATA box genes (15). Due to its obvious symmetry and
454 ability to impart large forces during binding, we thought that it would represent a good candidate for
455 comparison of its dynamics during its binding interaction with DNA. We conducted a DROIDS analysis
456 comparing human TBP (16) in its functionally bound and unbound states (Figure 4A-C). TBP exhibits a
457 characteristic large signature of dampening of atom fluctuation throughout its entire structure with
458 most pronounced effects in two loop regions that interact with the minor groove of DNA (arrows in
459 Figure 4A and 4C). Canonical correlations in new self-similar MD runs marking increased performance in
460 classification were observed in these regions (Figure 4D) along with corresponding regions of conserved
461 dynamics identified by significant Wilk's lambda (Figure 4E). Conserved dynamics from these loop areas
462 are connected through the chains in the beta sheet region of TBP spanning the DNA major groove
463 contact. Mutational impacts of four variants affecting the binding loop most proximal to the C terminal
464 exhibited followed our expectation of increasing impact ordering from R192Q, R192K, R192polyD, and
465 R192polyW (Figure 4G and 4H). The polyD and polyW mutations incorporated 5 sequential ASP or TRP
466 residues centered at R192, both causing the loop region to become more rigid (causing increased
467 negative dFLUX). We expected the strong functional binding affect observed across nearly all residues in
468 this system would make it relatively highly tolerant to single amino acid substitutions, even when
469 located in the most functional binding loop. In accordance with our expectations, we found the most

470 impactful multiple mutation (i.e. R192polyW) significantly affected the dynamics of nearly 6 times more
471 local residues than the least impactful single substitution (i.e. R192Q).

472

473 ***Machine learning analysis of impacts of drug class variants targeting the ATP binding region of Hsp90***

474 In contrast to TBP, we wanted to use our method to examine a small molecule binding
475 interaction in a protein with potentially more complex impacts on molecular dynamics. Hsp90 is a well-
476 known chaperone protein that assists the folding of many proteins and thereby mitigating many
477 environmental stresses in the cell. Hsp90 even capacitates the evolutionary process by allowing
478 potential phenotypic variation exhibited under stress to be hidden from natural selection until needed in
479 response to environmental change (17). Hsp90 contains a highly conserved N-terminal domain where
480 ATP binding and activation occurs. The binding of ATP physically changes motions in this region creating
481 a ‘lid’ that closed during ATP binding and open when conversion to ADP occurs. Due to the role of Hsp90
482 in stress mitigation in most tumors, it is a common drug target for ATP inhibitors in many cancer
483 therapies (18, 19). The amino acid residues that interact with ATP in this region are well known and the
484 inhibitor geldanamycin is known to mimic nearly all the local ATP contacts as well (20). Other more
485 modern inhibitors interact with the ATP binding pocket quite differently (19, 21, 22), so we thought that
486 this system would be a good candidate for comparative analysis of drug class variants with our software.

487 We conducted a DROIDS analysis comparing the dynamics of Hsp90 chaperone, a common
488 drug target for inhibitors in many cancer therapies, in both its ATP bound and unbound states. The
489 binding of ATP was discovered to significantly destabilize three co-localized alpha helical regions of the
490 protein adjacent to and extending from the ATP binding site (Figure 5A-D). MaxDemon analysis
491 confirmed the dynamics of this region to be highly conserved in new MD runs (Figure 5D-G). We also
492 analyzed the impacts of the six drug class variants targeting the ATP site (20, 22, 21, 23, 24), but
493 interacting differently with residues in this region (Figure 5H). The contacts in the ATP binding site are
494 shown in Figure 5I. While the localized patterns of impacts of the drug variants were all quite similar to
495 ATP (Figure 5J), the drug variants that most closely mimicked the contacts of ATP (i.e. geldanamycin) had
496 far less impact on conserved dynamics than variants that interacted very differently with the binding
497 pocket (i.e. benzamide SNX1321 and inhibitor FJ1(Figure H-I)). We feel that this finding demonstrates not
498 only demonstrates the potential of our method/software quite well, but it also demonstrates that while
499 it is important to be able to target a druggable protein binding site (25), researchers should also consider
500 how these various small molecules might alter, or fail to alter, the natural dynamics of the system. In
501 situations where a drug might too closely mimic the dynamic effects of a natural activator like ATP, a
502 hyperactivation response might occur in non-tumor cells leading to secondary cancer (26–28).
503 Alternatively, other situations may require drug targeting that does not alter the natural dynamic
504 behavior too much, potentially activating proteolytic systems in the cell. Our software allows more
505 detailed investigations of these potential dynamic impacts of drug class variants.

506 ***Conclusion***

507 We provide a well demonstrated method and user-friendly software pipeline for conducting statistically
508 sound comparative studies of large ensembles of comparative protein dynamics. The method/software
509 also now provides machine learning based extrapolations of effects on novel MD simulations
510 representing various functional variants of interest to the user. While there currently is at least one

511 other software allowing users to connect sequence-based evolutionary metrics to protein dynamics (29),
512 our method/software is unique in that regions of functional conservation are identified by analyzing
513 self-similar features of dynamics themselves rather than relying upon marrying dynamics analysis to
514 traditional static sequence-based approaches, which do not necessarily assume that a conserved
515 function region has a strong dynamic component. By providing a systematic way of comparing protein
516 dynamics at single residue resolution, our method/software provides an important step beyond
517 traditional sequence-based bioinformatics, allowing investigators to gain a much more biophysically-
518 grounded view of functional and evolutionary change. Another advantage to our method/software is
519 that our functional impacts (i.e. mutational tolerance) are defined solely within the context of protein
520 dynamic system being simulated. This provides a much deeper look into protein specific function than
521 current genomic and proteomic database methods of predicting mutational tolerance (30, 31) currently
522 allow. As GPU technology continues to advance at a rapid pace over the next few years, our
523 method/software may have profound potential application to the development of precision and
524 personalized medicine, where understanding the detailed interaction between genetic and drug class
525 variants within the context of specific protein dynamic systems will be greatly needed.

526

527 Supporting Material

528 The main repository for DROIDS 3.0 and maxDemon 1.0 can be found at the GitHub repository link
529 below. Please follow the link to “Releases” and download the latest release as .tar.gz or .zip file

530 <https://github.com/gbabbitt/DROIDS-3.0-comparative-protein-dynamics>

531 We also post various videos of examples using DROIDS, video tutorials, and ongoing projects here

532 <https://www.youtube.com/channel/UCJTbGqGq01pBCMDQikn566Kw>

533 A ‘live version’ of the figures in this manuscript is also available on our YouTube channel.

534

535 Author Contribution

536 GAB and EPF conceived the project and method. All authors contributed to the code base. GAB and LEA
537 worked on beta testing and debugging.

538

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545

546 References

- 547 1. Henzler-Wildman, K.A., M. Lei, V. Thai, S.J. Kerns, M. Karplus, and D. Kern. 2007. A hierarchy of
548 timescales in protein dynamics is linked to enzyme catalysis. *Nature*. 450: 913–916.
- 549 2. Niessen, K.A., M. Xu, A. Paciaroni, A. Orecchini, E.H. Snell, and A.G. Markelz. 2017. Moving in the
550 Right Direction: Protein Vibrations Steering Function. *Biophys. J.* 112: 933–942.
- 551 3. Babbitt, G.A., E.E. Coppola, M.A. Alawad, and A.O. Hudson. 2016. Can all heritable biology really be
552 reduced to a single dimension? *Gene*. 578: 162–168.
- 553 4. Götz, A.W., M.J. Williamson, D. Xu, D. Poole, S. Le Grand, and R.C. Walker. 2012. Routine
554 Microsecond Molecular Dynamics Simulations with AMBER on GPUs. 1. Generalized Born. *J. Chem.*
555 *Theory Comput.* 8: 1542–1555.
- 556 5. Salomon-Ferrer, R., A.W. Götz, D. Poole, S. Le Grand, and R.C. Walker. 2013. Routine Microsecond
557 Molecular Dynamics Simulations with AMBER on GPUs. 2. Explicit Solvent Particle Mesh Ewald. *J.*
558 *Chem. Theory Comput.* 9: 3878–3888.
- 559 6. Babbitt, G.A., J.S. Mortensen, E.E. Coppola, L.E. Adams, and J.K. Liao. 2018. DROIDS 1.20: A GUI-
560 Based Pipeline for GPU-Accelerated Comparative Protein Dynamics. *Biophys. J.* 114: 1009–1017.
- 561 7. Díaz, Ó., J.A.R. Dalton, and J. Giraldo. 2019. Artificial Intelligence: A Novel Approach for Drug
562 Discovery. *Trends Pharmacol. Sci.* .
- 563 8. Plante, A., D.M. Shore, G. Morra, G. Khelashvili, and H. Weinstein. 2019. A Machine Learning
564 Approach for the Discovery of Ligand-Specific Functional Mechanisms of GPCRs. *Mol. Basel Switz.*
565 24.
- 566 9. Terayama, K., H. Iwata, M. Araki, Y. Okuno, and K. Tsuda. 2018. Machine learning accelerates MD-
567 based binding pose prediction between ligands and proteins. *Bioinforma. Oxf. Engl.* 34: 770–778.
- 568 10. Maxwell, J.C. 2001. *Theory of Heat*. 9 Reprint edition. Mineola, NY: Dover Publications.
- 569 11. Vijay-Kumar, S., C.E. Bugg, and W.J. Cook. 1987. Structure of ubiquitin refined at 1.8 Å
570 resolution. *J. Mol. Biol.* 194: 531–544.
- 571 12. Peschard, P., G. Kozlov, T. Lin, I.A. Mirza, A.M. Berghuis, S. Lipkowitz, M. Park, and K. Gehring.
572 2007. Structural basis for ubiquitin-mediated dimerization and activation of the ubiquitin protein
573 ligase Cbl-b. *Mol. Cell.* 27: 474–485.
- 574 13. Roscoe, B.P., K.M. Thayer, K.B. Zeldovich, D. Fushman, and D.N.A. Bolon. 2013. Analyses of the
575 effects of all ubiquitin point mutants on yeast growth rate. *J. Mol. Biol.* 425: 1363–1377.
- 576 14. Kornberg, R.D. 2007. The molecular basis of eukaryotic transcription. *Proc. Natl. Acad. Sci. U. S.*
577 *A.* 104: 12955–12961.
- 578 15. Tora, L., and H.T.M. Timmers. 2010. The TATA box regulates TATA-binding protein (TBP)
579 dynamics in vivo. *Trends Biochem. Sci.* 35: 309–314.

- 580 16. Nikolov, D.B., H. Chen, E.D. Halay, A. Hoffman, R.G. Roeder, and S.K. Burley. 1996. Crystal
581 structure of a human TATA box-binding protein/TATA element complex. *Proc. Natl. Acad. Sci. U. S.*
582 *A.* 93: 4862–4867.
- 583 17. Rutherford, S.L., and S. Lindquist. 1998. Hsp90 as a capacitor for morphological evolution.
584 *Nature.* 396: 336–342.
- 585 18. Neckers, L., and P. Workman. 2012. Hsp90 Molecular Chaperone Inhibitors: Are We There Yet?
586 *Clin. Cancer Res.* 18: 64–76.
- 587 19. Yuno, A., M.-J. Lee, S. Lee, Y. Tomita, D. Rekhtman, B. Moore, and J.B. Trepel. 2018. Clinical
588 Evaluation and Biomarker Profiling of Hsp90 Inhibitors. *Methods Mol. Biol. Clifton NJ.* 1709: 423–
589 441.
- 590 20. Stebbins, C.E., A.A. Russo, C. Schneider, N. Rosen, F.U. Hartl, and N.P. Pavletich. 1997. Crystal
591 structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor
592 agent. *Cell.* 89: 239–250.
- 593 21. Immormino, R.M., Y. Kang, G. Chiosis, and D.T. Gewirth. 2006. Structural and quantum chemical
594 studies of 8-aryl-sulfanyl adenine class Hsp90 inhibitors. *J. Med. Chem.* 49: 4953–4960.
- 595 22. Li, J., L. Sun, C. Xu, F. Yu, H. Zhou, Y. Zhao, J. Zhang, J. Cai, C. Mao, L. Tang, Y. Xu, and J. He. 2012.
596 Structure insights into mechanisms of ATP hydrolysis and the activation of human heat-shock
597 protein 90. *Acta Biochim. Biophys. Sin.* 44: 300–306.
- 598 23. Fadden, P., K.H. Huang, J.M. Veal, P.M. Steed, A.F. Barabasz, B. Foley, M. Hu, J.M. Partridge, J.
599 Rice, A. Scott, L.G. Dubois, T.A. Freed, M.A.R. Silinski, T.E. Barta, P.F. Hughes, A. Ommen, W. Ma,
600 E.D. Smith, A.W. Spangenberg, J. Eaves, G.J. Hanson, L. Hinkley, M. Jenks, M. Lewis, J. Otto, G.J.
601 Pronk, K. Verleysen, T.A. Haystead, and S.E. Hall. 2010. Application of chemoproteomics to drug
602 discovery: identification of a clinical candidate targeting hsp90. *Chem. Biol.* 17: 686–694.
- 603 24. Austin, C., S.N. Pettit, S.K. Magnolo, J. Sanvoisin, W. Chen, S.P. Wood, L.D. Freeman, R.J.
604 Pengelly, and D.E. Hughes. 2012. Fragment screening using capillary electrophoresis (CEfrag) for hit
605 identification of heat shock protein 90 ATPase inhibitors. *J. Biomol. Screen.* 17: 868–876.
- 606 25. Vajda, S., D. Beglov, A.E. Wakefield, M. Egbert, and A. Whitty. 2018. Cryptic binding sites on
607 proteins: definition, detection, and druggability. *Curr. Opin. Chem. Biol.* 44: 1–8.
- 608 26. Poulikakos, P.I., C. Zhang, G. Bollag, K.M. Shokat, and N. Rosen. 2010. RAF inhibitors
609 transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature.* 464: 427–430.
- 610 27. Hatzivassiliou, G., K. Song, I. Yen, B.J. Brandhuber, D.J. Anderson, R. Alvarado, M.J.C. Ludlam, D.
611 Stokoe, S.L. Gloor, G. Vigers, T. Morales, I. Aliagas, B. Liu, S. Sideris, K.P. Hoefflich, B.S. Jaiswal, S.
612 Seshagiri, H. Koeppen, M. Belvin, L.S. Friedman, and S. Malek. 2010. RAF inhibitors prime wild-type
613 RAF to activate the MAPK pathway and enhance growth. *Nature.* 464: 431–435.
- 614 28. Cichowski, K., and P.A. Jänne. 2010. Drug discovery: inhibitors that activate. *Nature.* 464: 358–
615 359.

- 616 29. Bakan, A., A. Dutta, W. Mao, Y. Liu, C. Chennubhotla, T.R. Lezon, and I. Bahar. 2014. Evol and
 617 ProDy for bridging protein sequence evolution and structural dynamics. *Bioinformatics*. 30: 2681–
 618 2683.
- 619 30. Kumar, P., S. Henikoff, and P.C. Ng. 2009. Predicting the effects of coding non-synonymous
 620 variants on protein function using the SIFT algorithm. *Nat. Protoc.* 4: 1073–1081.
- 621 31. Adzhubei, I., D.M. Jordan, and S.R. Sunyaev. 2013. Predicting functional effect of human
 622 missense mutations using PolyPhen-2. *Curr. Protoc. Hum. Genet.* Chapter 7: Unit7.20.

623 **Table 1. Common learner assisted comparative protein dynamic investigations enabled by**
 624 **DROIDS 3.0 + maxDemon 1.0.**

QUESTION	DROIDS 3.0 training comparison	Deployment of learners in maxDemon	Important notes
Measure dynamic tolerances of single protein to various genetic mutations	Two sets (ensembles) of MD on the same protein at the same temperature	MD run on one or more genetic mutant structures	Isolates MD impacts of mutation(s) from natural variability in self-similar dynamics
Measure dynamic tolerances of DNA binding interaction to genetic mutation(s)	MD ensembles comparing both the unbound and DNA bound protein	MD run on one or more unbound genetic mutant structures	Isolates MD impacts of mutation from natural binding function of the system
Measure dynamic tolerances of individual genetic differences to a given drug	MD ensembles comparing both the unbound and drug bound protein	MD run on one or more drug-bound genetic mutant structures	Isolates MD impacts of mutation from novel drug binding function of the system
Measure dynamic similarities of different drug candidates to natural ligand binding interaction	MD ensembles comparing both the unbound and ligand bound protein	MD run on one or more drug variant bound structures	Isolates MD impacts of drug candidates from the natural binding function of the ligand
Measure evolution of novel dynamics in paralog genes	MD ensembles comparing two ortholog proteins (i.e. same gene different species)	MD runs on one or more paralogs (i.e. duplicated genes in same species)	Isolates potential MD novelty in duplicated gene product from nonfunctional or neutral changes in different species

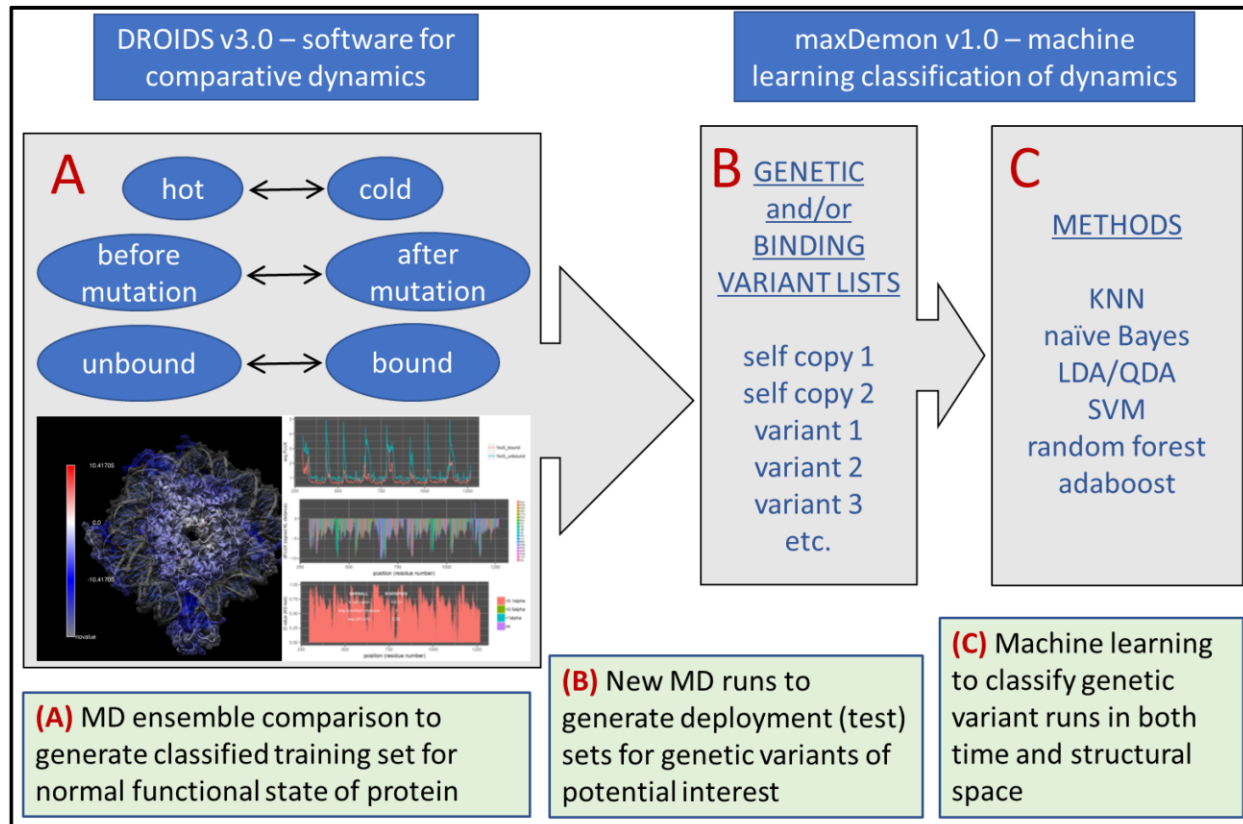
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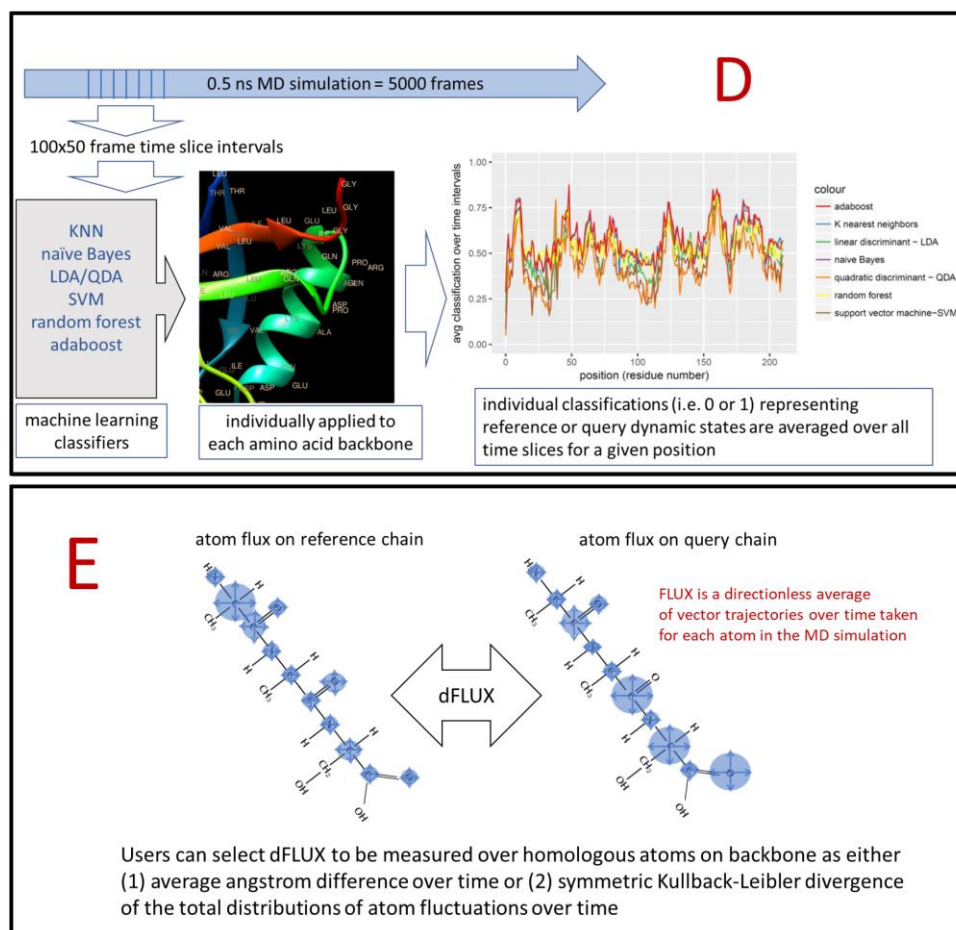
628 **Figures**

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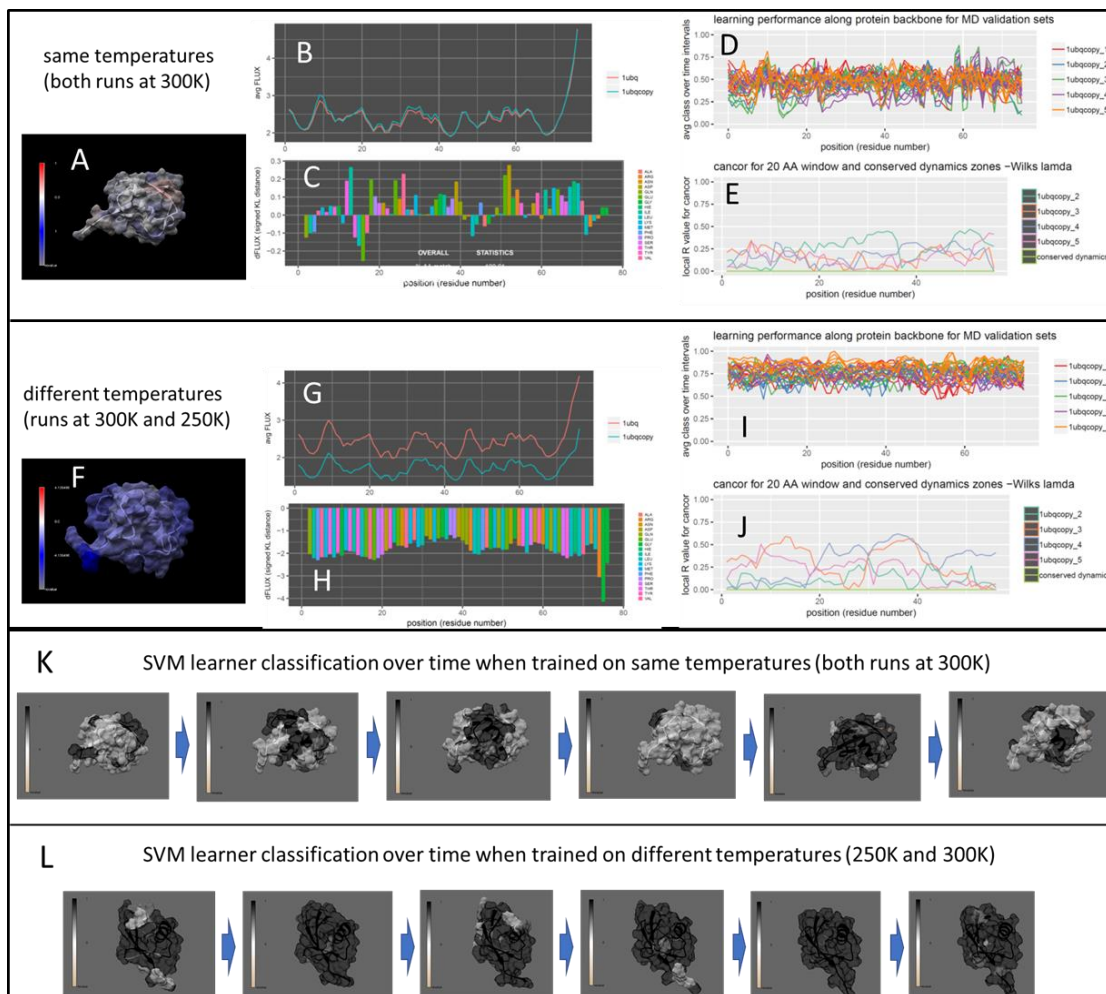
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633 **Figure 1. Schematic overview of DROIDS 3.0 + maxDemon 1.0 software for machine learning-based**
 634 **detection of variant impacts on functionally conserved protein dynamics.** The pipeline starts with (A)
 635 generation of two large ensembles of molecular dynamic (MD) simulations that represent a functional
 636 comparison of protein states (e.g. mutation, binding or environmental change). The root mean square
 637 fluctuations (rmsf) of protein backbone atoms in these ensembles are comparatively analyzed/visualized
 638 (i.e. using DROIDS) and are also later used as pre-classified training data sets for machine learning (i.e.
 639 using maxDemon). Note: in the pictured DROIDS analysis of nucleosome shows overall dampening of
 640 rmsf in the histone core with maximal dampening where the histone tails cross the DNA helix (B) New
 641 MD simulations are generated on two structures self-similar to the query state of training as well as a
 642 list of functional variants, and (C) up to seven machine learning methods are employed to classify the
 643 MD in the self-similar and variant runs according to the functional comparison defined by the initial
 644 training step. (D) The performance of learning is defined by average value of classification (i.e. 0 or 1)
 645 over 50 frame time slices for each amino acid position and regions of functionally conserved dynamics
 646 are later identified by significant canonical correlations in this learning efficiency (i.e. Wilk's lamda) in
 647 self-similar MD runs. The impacts of variants are defined by relative entropy of variant MD compared to
 648 the MD in the self-similar runs and plotted when this entropy is significantly different from the variation
 649 in self-similarity (i.e. bootstrapped z-test). (E) A visual representation of the difference in local rmsf
 650 (dFLUX) is typically calculated using symmetric Kullback-Leibler (KL) divergence between the two
 651 distributions of rmsf in the training MD ensembles.

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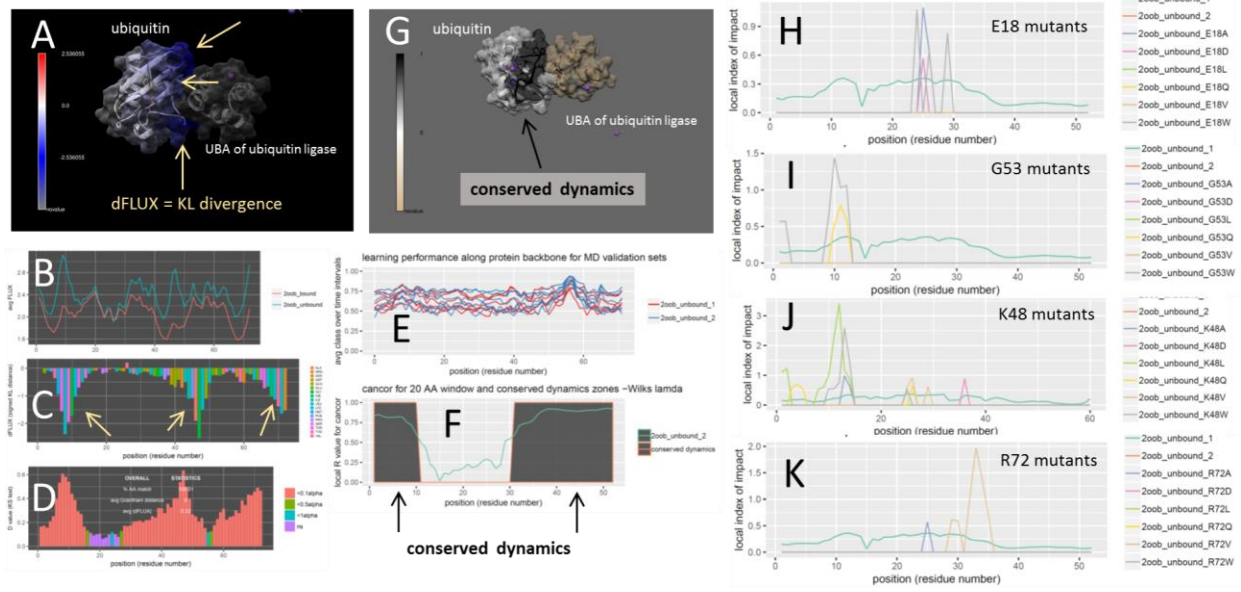
654 **Figure 2. Analysis of environmental temperature change on non-functional ubiquitin dynamics.**
 655 DROIDS image and analysis of random ubiquitin dynamics compared at the same (A-E) and different (F-J)
 656 temperatures. Note: blue color quantifies damped rmsf at temperature lowered by 50K. Note that
 657 performance is much higher when a temperature difference is modeled (D and I resp), however, as
 658 expected, neither comparison offers the machine learners a sequence-dependent profile by which to
 659 establish a signal of conserved dynamics (E or J). The learner classifications for the best performing
 660 learner in this case (quadratic discriminant function: QDA) is shown imaged on the ubiquitin structure
 661 over time in both the (K) random dynamics and (L) temperature dampened dynamics. (Movies of this
 662 can be observed in supplemental file A)

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668 **Figure 3. Analysis of mutational impact and tolerance on functional ubiquitin dynamics.** (A) DROIDS
 669 image and analysis of ubiquitin bound to the ubiquitin associated binding domain (UBA) of ubiquitin
 670 ligase. Note: blue color quantifies damped rmsf at binding interface. (i.e. negative dFLUX) also by the (B)
 671 respective rmsf profiles of bound and unbound training states and (C) the KL divergence or dFLUX profile
 672 colored by residue. Arrows indicate most prominent dampening of rmsf near loops at THR 9, ALA 46 and
 673 C terminus. (D) Significant differences in these rmsf profiles is determined by multiple-test corrected
 674 two sample KS test. (E) Local learning performance of each machine learning method in self-similar
 675 testing runs are shown color-coded by run and regions of functionally conserved dynamics, determined
 676 via significant local canonical correlation are shown in dark gray in both (F) traditional N to C terminal
 677 plot as well as (G) structural image. The mutational impacts of 24 genetic variants (H-K: six variants at
 678 each or four sites) are shown all demonstrating lack of impact in functionally conserved regions of the
 679 binding interaction.

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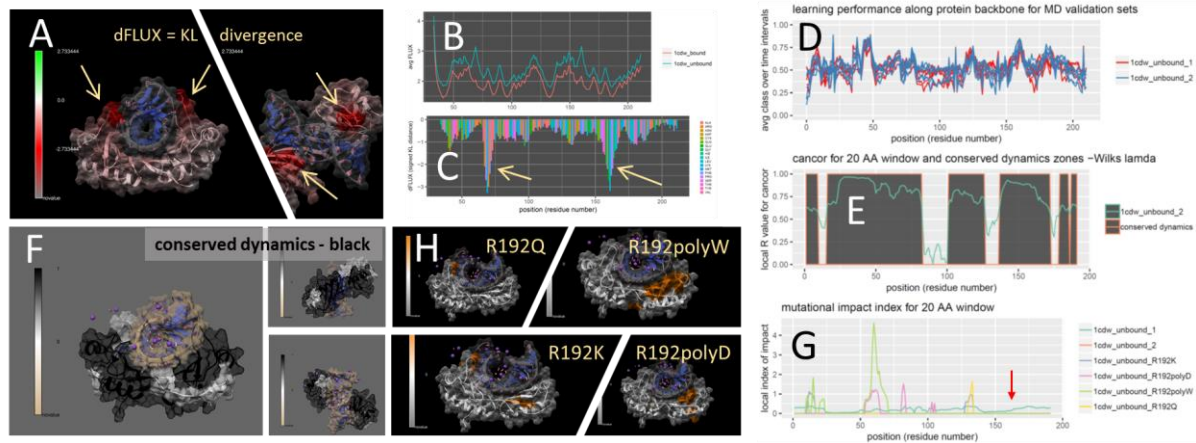
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693 **Figure 4. Analysis of mutational impact and tolerance on DNA binding in Tata Binding Protein (TBP).**
694 DRUIDS image and analysis of TBP in DNA-bound and unbound states showing (A) colored TBP structure,
695 (B) respective rmsf profiles and (C) KL divergence (dFLUX) plot. Note: arrows indicate functional binding
696 loops in the DNA minor groove red color indicates dampened rmsf. maxDemon analysis (D-E) identifying
697 conserved dynamics supporting both minor groove binding loops and (F) connecting them through the
698 central region of the beta sheet in the main body of TBP closest to the DNA. Mutational impacts of 4
699 genetic variants with increasing impact one of the functional loops are also shown (G) plotted and (H) on
700 the TBP structure. They are R192K, R192D, R192Q and polyW centered at R192 in 1cdw.pdb and and
701 position 161 (red arrow) in plots (Note: 31 position offset is due to DNA in the original file).

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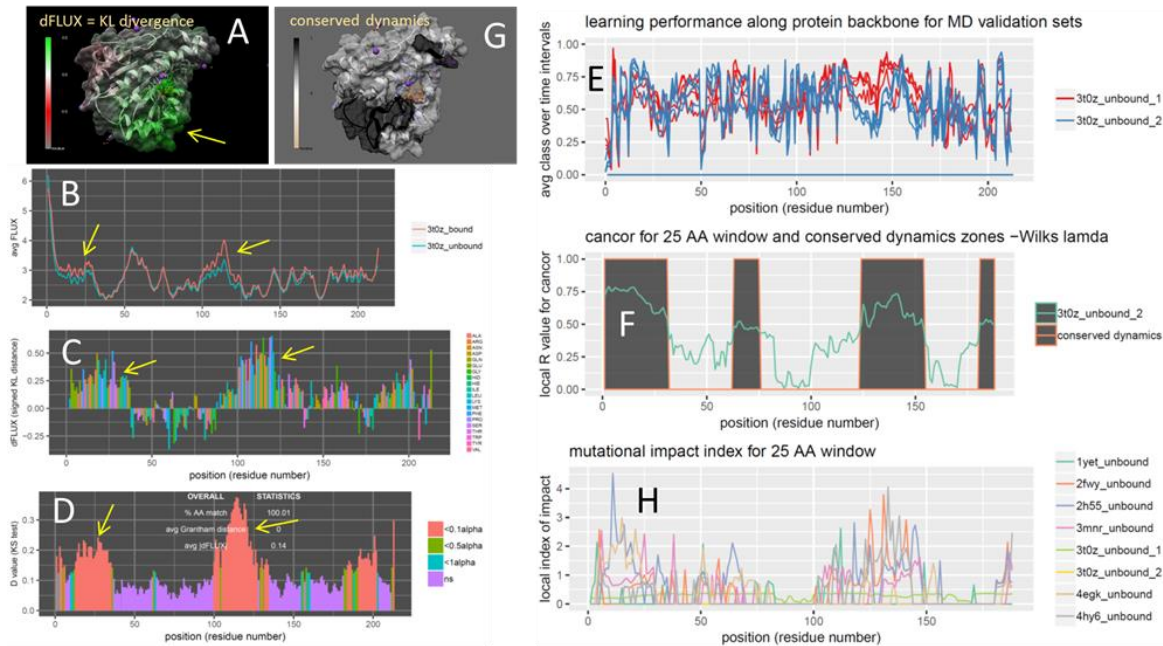
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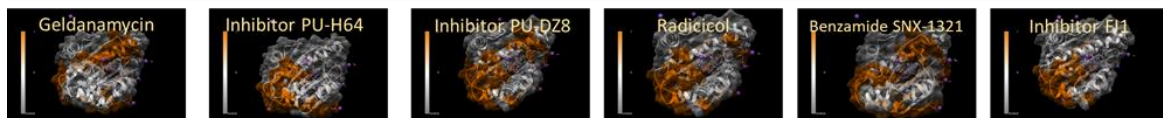
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I Ligand contacts of drug binding variants on protein dynamics of Hsp90 in the ATP binding pocket

ligand	PDB	% sim	Val 186	Thr 184	Trp 162	Tyr 139	Phe 138	Lys 112	Ala 111	Leu 107	Asn 106	Asp 102	Met 98	Gly 97	Ile 96	Asp 93	Lys 58	Ala 55	Asp 54	Asn 51
ATP	3t0z	100		x			x				x		x	x	x		x			x
Geldana mycin	1yet	87.5		x			x	x			x			x		x		x		x
Inhibitor PU-H64	2fwy	75		x	x		x			x			x			x		x		x
Inhibitor PU-DZ8	2h55	75		x	x		x			x			x		x	x	x	x		x
Radicalol	4egk	62.5	x	x			x			x						x	x	x	x	x
Benzamide SNX-1321	3mnr	37.5		x	x	x				x		x			x	x	x	x		
Inhibitor FJ1	4hy6	0			x	x			x											



J Impacts of drug binding variants on protein dynamics of Hsp90 that are significantly different than binding to ATP

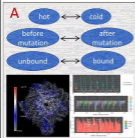
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716 **Figure 5. Analysis of drug class variant binding in the ATP-binding domain of Hsp90.** DROIDS image
 717 and analysis of Hsp90 in ATP-bound and unbound states showing (A) colored Hsp90 structure, (B)
 718 respective rmsf profiles and (C) KL divergence (dFLUX) plot and (D) significant differences in dynamics
 719 determined via the KS test. Note: arrows and green color indicate regions where rmsf is amplified in
 720 response to ATP binding. maxDemon analysis (E-G) identifying conserved dynamics connecting the ATP
 721 binding pocket and region of amplified rmsf. (H) Mutational impacts of 6 drug class variants targeting
 722 the ATP binding pocket of Hsp90 are plotted and (I) ordered by number of differences in structural
 723 contacts within the binding pocket. (J) Mutational impacts of these variants are demonstrated to
 724 predominantly impact the functionally conserved region of amplified rmsf thus mimicking the dynamic
 725 effect of functional ATP binding.

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DROIDS v3.0 – software for comparative dynamics

maxDemon v1.0 – machine learning classification of dynamics



(A) MD ensemble comparison to generate classified training set for normal functional state of protein

(B) New MD runs to generate deployment (test) sets for genetic variants of potential interest

(C) Machine learning to classify genetic variant runs in both time and structural space

