Non-Markovian modeling of protein folding

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friction term, is predominantly used. Such a Markovian theory yields many useful insights into protein-folding dynamics and culminated in the comparison of transition-path times and mean folding times (27, 28). However, the success of free energy folding theory on the Markovian level relies partly on the fact that the friction, which determines the prefactor of the Kramers folding time, is normally used as a fitting parameter. Even when the friction is allowed to vary with the reaction coordinate and is extracted from simulations, it is typically computed from folding or reconfiguration times, which by construction, leads to self-consistent predictions of the kinetics (29, 30). In fact, recent experiments revealed significant inconsistencies when comparing directly measured free energy barrier heights with those inferred from transition path and folding times (15), which were suggested to be due to memory effects (31, 32). The same inconsistencies are obtained when the friction of a reaction coordinate is not fitted to folding times but rather, extracted directly from simulation trajectories and used in the framework of Markovian theory, as we demonstrate here.

In our approach, instead of searching for a good reaction coordinate, we employ a standard one-dimensional coordinate that consists of the sum of the separations between native contacts. We use accurate tools for extracting all parameters of the GLE from molecular dynamic (MD) simulations for the helix-forming polypeptide Ala$_{16}$ in water. The free energy $U(q)$ shows multiple minima separated by low barriers, indicative of the sequential formation of the helix, while the longest decay time of the multiexponential memory function $\Gamma(t)$ is of the order of the unfolding time. These properties render $\Gamma(t)$ a very sensitive test of kinetic theory. We simulate the resulting GLE by Markovian embedding techniques. By comparison of the MD and GLE results for the mean folding and unfolding times, we demonstrate that the one-dimensional GLE is an accurate and practical tool for the description of protein-folding dynamics. On the other hand, the Markovian version of the overdamped GLE cannot describe the folding and unfolding kinetics of the polypeptide as long as the friction is not a fitting parameter but rather, taken as extracted from the MD simulations. This stays true even when the friction coefficient is allowed to depend on the reaction coordinate. As predicted by the Grote–Hynes theory, memory typically accelerates barrier crossing, where the acceleration magnitude depends primarily on the ratio of the memory time and the distance between the minimum and the barrier in reaction coordinate space (33–38). This memory-induced speedup of folding and unfolding is found to be accompanied by pronounced anomalous diffusion in reaction coordinate space. Our results are corroborated by a systematic Kramers–Moyal coefficient (KMC) analysis, which shows that higher-order quartic KMCs are negligible and that the linear and quadratic KMCs vanish in the short time limit, as expected in the presence of non-Markovian effects. This implies that the description of protein folding in terms of the Fokker–Planck equation is only valid above a certain timescale that needs to be suitably chosen. We also find that a spurious reaction coordinate–dependent friction profile arises when non-Markovian protein dynamics is described using a Markovian model.

**Results and Discussion**

**MD Simulations and GLE Parameter Extraction.** The effective GLE is constructed from a 10-μs-long MD trajectory for Ala$_{16}$ in water, which is the simplest polypeptide that forms an $\alpha$-helix (39) (Methods and SI Appendix, section 1 have details). As a reaction coordinate, we use the summed separations between the H-bond donor nitrogen of residue $n$ and the acceptor oxygen of residue $n + 4$,

$$q(t) = \frac{1}{3} \sum_{i=2}^{4} \left| \mathbf{r}_i(t) - \mathbf{r}_i^0(t) \right|,$$

which characterize the left-handed $\alpha$-helical conformation. In the $\alpha$-helical state, $q$ has a value around 0.3 nm, the mean H-bond length between nitrogen and oxygen. We will further also consider the end-to-end distance as an alternative reaction coordinate. The free energies $U(q)$ in Fig. 1A for different simulation lengths demonstrate that the simulation is fully converged after about 6 μs. The free energy displays several metastable states, which are also discernible in the trajectory in Fig. 1B and make this simple polypeptide challenging for theoretical description.

Using a generalization of earlier methods (40), we extract the running integral $G(t) = \int_0^t ds U(s)$ (SI Appendix, section 2 has details), from which the memory function $\Gamma(t)$ is obtained via a numerical derivative and fitted using least-square methods to a multiexponential of the form

$$\Gamma(t) = \sum_{n=1}^{5} \frac{\gamma_n}{\tau_n} e^{-t/\tau_n}.$$

The extracted $G(t)$ (gray line) is compared with the corresponding fit (red line) in Fig. 2A; no significant deviations can be discerned. The comparison of the extracted and fitted memory function $\Gamma(t)$ in Fig. 2B reveals oscillations below a picosecond, which are not reproduced by the exponential fit function but also do not play a role for the kinetics, as will be shown below. The fitted memory times $\tau_n$ and friction coefficients $\gamma_n$ are presented in Table 1; the typical reconfiguration time, which can be qualitatively inferred from the trajectory in Fig. 1B, is of the order of the longest decay time $\tau_5 \approx 5$ ns. This means that the reaction coordinate is not particularly good since it exhibits pronounced non-Markovian effects and thus, constitutes a suitable test of our methods.

The effective mass follows from the equipartition theorem according to $m = k_B T/\langle q^2 \rangle$ and turns out to be independent of $q$ and given by $m = 31.3$ u (SI Appendix, section 3). The motion described by the GLE is expected to become diffusive after the inertial time $\tau_m = m/\gamma_1$, where the total friction coefficient is given by $\gamma = \sum_n \gamma_n$ (Table 1). It follows that $\tau_m = 0.1$ is, even shorter than the MD integration time step; thus, inertial effects are completely negligible. Nevertheless, the
acceleration term in Eq. 1 is kept in the GLE simulations, as it stabilizes the numerical integration. In order to examine the importance of memory effects, the memory times τᵣ are compared with the diffusion timescale τD = β²L²/2 (36), which is the time it takes a free Brownian particle to diffuse over a length L in reaction coordinate space where β = 1/k BT is the inverse thermal energy. For L = 0.22 nm, the distance between the folded minimum at q = 0.32 nm and the barrier at q = 0.54 nm in Fig. 1, one obtains τD = 6.8 ns, which is of the order of the longest memory time τᵣ. This places the system in the so-called memory-acceleration regime, where memory effects are relevant and significantly accelerate barrier crossing (36–38).

Comparison of MD and GLE Simulations. Numerical integration of the GLE is straightforwardly achieved by Markovian embedding (i.e., by transforming the GLE into a system of linearly coupled LEs) (22) (SI Appendix, section 4). In Fig. 3, we show profiles of the first-passage time (MFPT) tMFPT(qs, qf) for unfolding (start position qs = qL = 0.32 nm; solid lines) and folding kinetics (start position qs = qR = 0.99 nm; broken lines) as a function of the final position qf. Statistical errors are determined accounting for data correlations (41) (SI Appendix, section 5) and are smaller than the line thickness. MD and GLE simulation results (blue and orange lines, respectively) agree nicely; this demonstrates that GLE-based non-Markovian modeling of protein folding is feasible and accurate. Even first-passage time distributions from GLE and MD simulations agree satisfactorily with each other, as shown in SI Appendix, section 6.

Beyond reproducing MD results, the GLE is a diagnostic tool that allows us to quantify the importance of memory effects. In order to modulate memory effects in the GLE, we rescale the memory times τᵣ → ατᵣ for n = 2, 3, 4, 5 while keeping the memory time τ₁ of the fastest exponential contribution fixed. Since τ₁ = 7 fs is above the simulation time step of 1 fs, this ensures that in the limit α → 0, we obtain a regularized model that, as we will show below, corresponds to the Markovian limit. In Fig. 3B, we show MFPTs between the three positions q₁ = 0.32 nm, q₀ = 0.54 nm, and q₉ = 0.99 nm as a function of the rescaling factor α from GLE simulations. The six different MFPTs are illustrated in Fig. 3C, Inset by filled and closed arrows and indicated in Fig. 3B by corresponding filled and open colored spheres. We see that reducing the memory time increases all MFPTs; in other words, memory accelerates barrier crossing (36). As expected, the GLE results approach the overdamped Markovian limit, denoted by the horizontal lines in the corresponding color and calculated from the exact expression in Eq. 10, without adjustable parameters as α tends to zero. Interestingly, for folding to the barrier (open green circles), the MFPT for α = 1 and the Markovian limit for α → 0 differ only by a factor of around 2.5. On the other hand, for unfolding to the barrier (filled red circles), the α → 0 and α = 1 MFPTs differ by a factor of around nine. This means that even when treating the total friction coefficient γ as a free parameter, the Markovian overdamped theory Eq. 10, because it is linear in the friction, can reproduce either the MD folding or unfolding times to the barrier but not both simultaneously. This is not due to inertial effects since the overdamped Markovian theory works perfectly for α → 0, as seen in Fig. 3B. Rather, memory effects influence the times of folding and unfolding to the barrier top differently. This is demonstrated by the plot of MFPT ratios as a function of α in Fig. 3C, where it is seen that the ratio of the folding and unfolding times to the barrier top tMFPT(qR, q₀)/tMFPT(qL, q₀), denoted by open green and filled red spheres, depends sensitively on α. In contrast, the ratios of reciprocal MFPTs (i.e., MFPTs with interchanged start and final positions), denoted by red, green, and blue lines with identically colored open and filled circles, do not depend on α, which shows that the memory dependence of ratios of MFPTs depends on the precise MFPT definition and by no means indicates a breakdown of the detailed balance or the law-of-mass action. In SI Appendix, section 6, we demonstrate that the memory-induced speedup is even more pronounced for transition-path times compared with folding and unfolding times, in agreement with previous findings (15, 31, 32).

The high accuracy of GLE simulations is furthermore reflected by the good agreement of the mean-square displacement (Δq(t)²) = ⟨(q(t + t) − q(t))²⟩ from MD and GLE simulations in Fig. 2C, which exhibits pronounced subdiffusive behavior with an exponent 0.4 for times between 1 ps and 1 ns. Anomalous diffusion is often modeled by fractional theories that allow to quantify the importance of memory effects. In order to modulate memory effects in the GLE, we rescale the memory times τᵣ → ατᵣ for n = 2, 3, 4, 5 while keeping the memory time τ₁ of the fastest exponential contribution fixed. Since τ₁ = 7 fs is above the simulation time step of 1 fs, this ensures that in the limit α → 0, we obtain a regularized model that, as we will show below, corresponds to the Markovian limit. In Fig. 3B, we show MFPTs between the three positions q₁ = 0.32 nm, q₀ = 0.54 nm, and q₉ = 0.99 nm as a function of the rescaling factor α from GLE simulations. The six different MFPTs are illustrated in Fig. 3C, Inset by filled and closed arrows and indicated in Fig. 3B by corresponding filled and open colored spheres. We see that reducing the memory time increases all MFPTs; in other words, memory accelerates barrier crossing (36). As expected, the GLE results approach the overdamped Markovian limit, denoted by the horizontal lines in the corresponding color and calculated from the exact expression in Eq. 10, without adjustable parameters as α tends to zero. Interestingly, for folding to the barrier (open green circles), the MFPT for α = 1 and the Markovian limit for α → 0 differ only by a factor of around 2.5. On the other hand, for unfolding to the barrier (filled red circles), the α → 0 and α = 1 MFPTs differ by a factor of around nine. This means that even when treating the total friction coefficient γ as a free parameter, the Markovian overdamped theory Eq. 10, because it is linear in the friction, can reproduce either the MD folding or unfolding times to the barrier but not both simultaneously. This is not due to inertial effects since the overdamped Markovian theory works perfectly for α → 0, as seen in Fig. 3B. Rather, memory effects influence the times of folding and unfolding to the barrier top differently. This is demonstrated by the plot of MFPT ratios as a function of α in Fig. 3C, where it is seen that the ratio of the folding and unfolding times to the barrier top tMFPT(qR, q₀)/tMFPT(qL, q₀), denoted by open green and filled red spheres, depends sensitively on α. In contrast, the ratios of reciprocal MFPTs (i.e., MFPTs with interchanged start and final positions), denoted by red, green, and blue lines with identically colored open and filled circles, do not depend on α, which shows that the memory dependence of ratios of MFPTs depends on the precise MFPT definition and by no means indicates a breakdown of the detailed balance or the law-of-mass action. In SI Appendix, section 6, we demonstrate that the memory-induced speedup is even more pronounced for transition-path times compared with folding and unfolding times, in agreement with previous findings (15, 31, 32).

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### Table 1. Fitted memory function parameters from Eq. 3

| n   | γn (u/ps) | τn (ps) |
|-----|-----------|---------|
| 1   | 2.2 × 10³ | 0.007   |
| 2   | 1.2 × 10⁴ | 4.6     |
| 3   | 4.2 × 10⁴ | 40.3    |
| 4   | 2.4 × 10⁵ | 4.399   |
| 5   | 5.7 × 10⁴ | 4.970   |

γ = ∑n γn
Fig. 3. (A) Comparison of unfolding and folding MFPTs $\tau_{MFPT}(q_s, q_f)$ from MD (blue) and GLE (orange) simulations as a function of the final position $q_f$ for start positions $q_s = q_L = 0.32$ nm (solid lines) and $q_s = q_R = 0.99$ nm (broken lines). The gray curve shows the folding free energy $U(q)$. (B) Dependence of different MFPTs from GLE simulations on the memory time rescaling factor $\alpha$; the corresponding start and final positions are illustrated in C, inset. Open and filled circles correspond to open and filled arrows, respectively, in C, inset. The colored horizontal lines denote corresponding results for the overdamped Markov limit from Eq. 10. (C) Ratios of the MFPTs do not depend on $\alpha$ (red, green, and blue lines that connect colored circles); only the ratio of the folding and unfolding times to the barrier top, $\tau_{MFPT}(q_s, q_f)/\tau_{MFPT}(q_l, q_a)$ (open green and filled red spheres), depends on $\alpha$.

(31, 42). Fig. 2C shows that it is accurately reproduced by multieponential memory and that it disappears when memory effects are eliminated, in line with recent theoretical analysis (43). The overall good agreement between MD and GLE simulation results shows that the GLE in the form of Eq. 1 describes the kinetics of $\text{Ala}_{10}$ very accurately. This is not due to our specific choice of reaction coordinate, as demonstrated in SI Appendix, section 7, where we present a similar GLE-based analysis using the $\text{Ala}_{10}$ end-to-end distance as reaction coordinate.

**Reaction Coordinate–Dependent Friction.** We so far demonstrated that the GLE in the form of Eq. 1 reproduces the MD simulation kinetics and that memory effects are significant. We now investigate whether reaction coordinate–dependent friction effects, which are not included in the GLE, are relevant. The Markovian LE that incorporates a friction function $\gamma(q)$ has been amply used to describe protein-folding dynamics (29, 30, 44). In the underdamped version, it reads

$$m\ddot{q}(t) = -U'(q) - \gamma(q)\dot{q}(t) + \sqrt{k_B T \gamma(q)} \eta(t),$$

which for general $U(q)$, unfortunately is analytically intractable. The overdamped version

$$0 = -U'(q) - \gamma(q)\dot{q}(t) - \frac{k_B T \gamma'(q)}{2} \dot{q}(t) + \sqrt{k_B T \gamma(q)} \eta(t)$$

is much more useful since the MFPTs can be calculated analytically. In these expressions, the random force $\eta(t)$ has vanishing mean, and its correlator is given by $\langle \eta(t) \eta(t') \rangle = 2\delta(t - t')$. For constant friction, the overdamped LE (Eq. 4) follows from Eq. 1 by neglecting the inertia term; the term proportional to the gradient $\gamma'(q)$ cancels a spurious drift term and follows by mapping on the Fokker–Planck equation (SI Appendix, section 9) (6). In fact, from the overdamped LE with constant friction, an arbitrary friction profile $\gamma(q)$ can be created by a nonlinear transformation of the reaction coordinate (29, 30) (SI Appendix, section 10); this suggests that spatially dependent friction is related to nonlinearities in the reaction coordinate that are not straightforwardly captured by the projection techniques used to derive the GLE Eq. 1.

Various methods to extract $\gamma(q)$ from experimental or simulated trajectories have been proposed; a systematic approach involves the KMCs, which for the overdamped case and for finite lag time $\Delta t$, read

$$D_k(q) = \frac{1}{k_B T} \left\langle \left( q(t + \Delta t) - q(t) \right)^k \right\rangle_{q(t)=q}.$$ 

The Fokker–Planck equation for the time-dependent probability distribution $P(q, t)$ in terms of the KMCs follows in the limit $\Delta t \to 0$ as (7)

$$\frac{\partial P(q, t)}{\partial t} = \sum_{k=1}^\infty \frac{\partial^k}{\partial q^k} \left[D_k(q) P(q, t)\right].$$

and the underdamped case is treated in SI Appendix, section 11. According to Pawula’s theorem, for a Markovian process, all KMCs with $k > 2$ vanish for $\Delta t \to 0$, and Eq. 7 takes the standard form of a second-order partial differential equation (7). For a non-Markovian process [i.e., if the memory function $\Gamma(t)$ in Eq. 1 has a finite range], all KMCs with $k > 1$ vanish for $\Delta t \to 0$, and thus, the stochastic properties of the process cannot be described by a partial differential equation for $P(q, t)$ at all (SI Appendix, section 12).

For the underdamped LE, the relation between the second-order velocity KMC $D_{vw}$ and the friction profile $\gamma_{UD}(q)$ reads (7)

$$D_{vw}(q) = \frac{1}{2\Delta t} \left\langle (v(t + \Delta t) - v(t))^2 \right\rangle_{v(t)=q} = k_B T \gamma_{UD}(q) m^2.$$ 

For the overdamped LE, $\gamma_{OD}(q)$ follows from the second-order position KMC $D_{qw}$ as

$$D_{qw}(q) = \frac{1}{2\Delta t} \left\langle (q(t + \Delta t) - q(t))^2 \right\rangle_{q(t)=q} = \frac{k_B T}{\gamma_{OD}(q)}.$$ 

(SI Appendix, section 9). For the numerical computation of the KMCs, we use kernel-density estimators (45) (SI Appendix, section 13). In Fig. 4A, we show the friction profiles $\gamma_{UD}(q)$ (circles) and $\gamma_{OD}(q)$ (lines) computed from the KMCs for different lag times $\Delta t$; a number of points are noteworthy. 1) We find no significant deviations between the friction profiles extracted from MD (solid lines and filled circles) and GLE (broken lines and open circles) trajectories; this reverberates that the GLE describes the protein dynamics very faithfully. 2) The underdamped and overdamped friction profiles $\gamma_{UD}(q)$ and $\gamma_{OD}(q)$
disagree for all lag times $\Delta t$, which very clearly demonstrates an inconsistency in the Markovian description of protein folding. In fact, in the limit $\Delta t \to 0$, both $D_{qy}$ and $D_{qv}$ vanish; thus, $\gamma_{OD}(q)$ diverges, while $\gamma_{UD}(q)$ goes to zero (SI Appendix, section 12). 3) While the underdamped friction $\gamma_{UD}(q)$ never reaches a realistic value close to $\tilde{\gamma}$, the overdamped friction $\gamma_{OD}(q)$ approaches $\tilde{\gamma}$ for $\Delta t \approx 1$ ns. This shows that lag times of the order of the longest memory time have to be used in order to generate realistic friction values. 4) The friction profiles extracted from the GLE simulations are position dependent, seen most clearly in $\gamma_{OD}(q)$ for $\Delta t = 1$ ns (purple broken line); this is clearly a spurious effect since the GLE has no position-dependent friction. We conclude that the mapping of a non-Markovian process onto a Markovian LE produces spurious position-dependent friction effects. Presumably, the effective friction of proteins will in general exhibit a dependence on the reaction coordinate, but the extraction of friction profiles would have to account for memory effects in order to avoid spurious effects. The capability of the GLE Eq. 1 to very accurately reproduce the MD simulation kinetics suggests that for the present case of Ala$_9$, the spatial dependence of friction is negligible.

An alternative way to determine a friction profile $\gamma(q)$ in the overdamped limit uses the one-to-one relation between the MFPT profiles in Fig. 3A and $\gamma(q)$. From the expressions for the folding and unfolding times (Eq. 10), $\gamma(q)$ follows by inversion according to Eq. 11 (30). In Fig. 4B, we show $\gamma_{unf}(q)$ and $\gamma_{fol}(q)$ computed from unfolding and folding MFPTs from MD simulations for start positions $q_{0u} = q_{0u}$ and $q_{0f} = q_{0f}$, respectively. Not surprisingly, the profiles $\gamma_{unf}(q)$ and $\gamma_{fol}(q)$ are rather close to $\tilde{\gamma}$ extracted from the MD simulations, which is shown as a gray horizontal line in Fig. 4B, but differ significantly from each other. This suggests that a single friction profile cannot describe folding and unfolding of Ala$_9$ simultaneously. In fact, the values of $\gamma_{unf}(q)$ and $\gamma_{fol}(q)$ go down as $q_F$ moves to the respective start positions (i.e., as the folding and unfolding times become shorter). This reflects that memory effects particularly accelerate fast transitions (36–38).

To demonstrate the limitations of the friction profiles in Fig. 4B, we show in Fig. 4C folding and unfolding MFPT profiles that are calculated according to Eq. 10 from $\gamma_{unf}(q)$ (filled circles) and $\gamma_{fol}(q)$ (open circles). By construction, the MFPTs using $\gamma_{unf}(q)$ reproduce the unfolding simulation data, while the MFPTs using $\gamma_{fol}(q)$ reproduce the folding simulation data. In contrast, the MFPTs using $\gamma_{unf}(q)$ fail to reproduce the simulated unfolding times, and the MFPTs using $\gamma_{fol}(q)$ fail to reproduce the simulated unfolding times, in particular when the folding/unfolding times become smaller than about 10 ns. In contrast, the GLE model (broken lines) reproduces both folding and unfolding MD dynamics (solid lines). This underlines that there is no consistent way of describing the complete folding/unfolding dynamics with a Markovian model.

**Conclusions**

By extracting the time-dependent friction from MD simulations for the polypeptide Ala$_9$ from explicit-water MD simulations, we demonstrate that the resulting GLE model can be straightforwardly integrated numerically and reproduces the folding and unfolding kinetics of the MD simulations very accurately. Our findings are not restricted to a reaction coordinate based on the summed distances between native H bonds. As we show in SI Appendix, section 7, the same analysis of the Ala$_9$ end-to-end distance leads to similar results. Decreasing the memory time in the GLE while keeping the friction coefficient (i.e., the integral over the memory function) constant, the folding kinetics changes significantly for folding and unfolding events. This shows that memory effects are important even for the formation kinetics of a single $\alpha$-helix.

In contrast, the Markovian LE cannot reproduce the full Ala$_9$ reconfiguration dynamics, even with a fitted friction profile; this follows from the comparison of the folding and unfolding kinetics, which would need to be modeled with different friction profiles in order to reproduce the MD simulation kinetics.

We have mostly used the GLE model as a diagnostic tool to understand and quantify non-Markovian effects; since non-Markovian simulations are rather inexpensive, they can also be used as an efficient tool to simulate the response of proteins to environmental changes (e.g., externally applied forces). In fact, our extraction technique for the memory function can in principle also be applied to trajectories from single-molecule experiments (13–15), which would enable us to perform non-Markovian GLE simulations on experimental systems directly, without the need of atomistic MD simulations. Because of the limited time resolution of typical
From MFPTs to Friction Profiles. The MFPT is defined as the mean time needed to reach the final position \( q_f \) for the first time when starting from a position \( q_i \). For the overdamped LE in Eq. 5, it reads for \( q_i < q_f \) (51),

\[
\tau_{\text{MFPT}}(q_i, q_f) = \int_{q_i}^{q_f} dq e^{-\beta U(q)} \int_{q_{\text{min}}}^{q_{\text{max}}} dq' e^{-\beta U(q')},
\]

and for \( q_i > q_f \),

\[
\tau_{\text{MFPT}}(q_i, q_f) = \int_{q_f}^{q_i} dq e^{-\beta U(q)} \int_{q_{\text{min}}}^{q_{\text{max}}} dq' e^{-\beta U(q')},
\]

Taking the derivative of Eq. 10 w.r.t. \( q_f \) gives the friction profile \( \gamma(q) \) as (30)

\[
\gamma_{\text{MFPT}}(q_f) = k_B T e^{-\beta U(q_f)} \frac{d\tau_{\text{MFPT}}}{dq_f},
\]

\[
\gamma(q_f) = k_B T e^{-\beta U(q_f)} \frac{d\tau_{\text{MFPT}}}{dq_f},
\]

Data Availability. Derivations that support the findings of this study are included in Section 1. Simulation input files data have been deposited in Institutional Repository (https://doi.org/10.17169/refubium-29935). Our codes for extracting the memory kernel, running GELE simulations, and for computing MFPTs are available in Github (https://github.com/lucastepper/memtools).

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