Instabilities in complex mixtures with a large number of components

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Inside living cells are complex mixtures of thousands of components. It is hopeless to try to characterise all the individual interactions in these mixtures. Thus, we develop a statistical approach to approximating them, and examine the conditions under which the mixtures phase separate. The approach approximates the matrix of second virial coefficients of the mixture by a random matrix, and determines the stability of the mixture from the spectrum of such random matrices.

Mixtures are not always simple, well-characterised and made up of 2 or 3 components. The mixtures of biomacromolecules inside living organisms contain thousands of different macromolecules, and the oil extracted from wells by the petroleum industry may also contain many different hydrocarbons and related compounds. This gives us two main problems: i) the number of components is so large that the data we have is inadequate to characterise all the components, ii) even if we were able to precisely characterise each component then comprehending, and calculating with, this mass of data would be difficult. The sheer complexity of the mixture overwhelms our ability to comprehend the mixture and to predict its properties. An analogous problem afflicted nuclear physics 50 years ago. Large nuclei, such as \textsuperscript{235}U, are complex many-body systems with complex spectra. Nuclear physicists were faced with energy spectra with so many energy levels that comprehending and predicting them directly was impossible. Starting with Wigner \textsuperscript{1,2,3,4}, they resorted to statistical methods, and replaced the complex and unknown Hamiltonian matrix of a nucleus with a Hamiltonian matrix whose elements were random variables. A drastic approximation but one which worked. They were able to reproduce the statistical properties of energy spectra. By statistical properties, we mean properties such as the probability distribution of, unknown, second virial coefficients of some mixture, by a random matrix. For large \(N\), the eigenvalues of this matrix depend on two variables only: the mean and standard deviation of its elements, which we can vary to fit a specific mixture or just vary to explore the generic features of the phase behaviour of such a mixture. Once we are using random matrices, the fact that we have \(N \gg 1\) components is a help not a hindrance.

The Helmholtz free energy per unit volume, \(f\), of an \(N\) component mixture, truncated after the second-virial coefficient terms is

\[
f = \sum_{i=1}^{N} \rho_i (\ln \rho_i - 1) + \frac{1}{2} \sum_{i=1}^{N} \sum_{j=1}^{N} \rho_i B_{ij} \rho_j,
\]

where \(\rho_i\) is the number density of component \(i\). The \(N\) densities form a row matrix \(\rho = (\rho_1, \rho_2, \ldots, \rho_N)\). We use units such that the thermal energy \(k_B T = 1\). Here, we will not calculate complete phase diagrams with the densities and compositions of coexisting phases. We will calculate the limits of stability, spinodals, where the system becomes unstable with respect to density perturbations. Thus, we will only be able to determine qualitative features of the phase behaviour, such as whether or not a phase transition occurs and whether the transition is demixing of the components or their condensation. Stability of the mixture requires that \(f\) be convex. Convexity requires that the second order term, \(\delta^2 f\), in an expansion of \(f\) in powers of \(\delta \rho\), be positive for any small perturbation \(\delta \rho\). For our \(N\) component mixtures \(\delta \rho\) is a row matrix of length \(N\). Now, \(\delta^2 f\) is given by

\[
\delta^2 f = \frac{1}{2} \delta \rho (P + \mathbf{B}) \delta \rho^T,
\]

where \(\mathbf{B}\) is the matrix of second virial coefficients and \(P\) is a diagonal matrix with the \(i\)th diagonal element equal to \(1/\rho_i\); it contains the ideal or perfect gas contributions.
to the free energy change. Of course, any $\delta \rho$ can be expressed as sum of eigenvectors of $P + B$ and so the requirement $\delta^2 f > 0$ implies that all eigenvalues of $P + B$ must be positive. The mixture becomes locally unstable when an eigenvalue, the lowest obviously, becomes zero. Stability is determined only by the lowest eigenvalue. However, if each component has more-or-less the same mobility, then the decay of small density modulations can be described as the decay of the set of eigenvectors of $P + B$, with each eigenvector component of the density modulation decaying at a rate proportional to its eigenvalue.

The eigenvalues of $P + B$ form the row matrix $\gamma$. For simplicity we will assume that in the mixture all components are present in equal amounts $\rho_i = \rho_T/N$, $i = 1, \ldots, N$, where $\rho_T$ is the total density. Then $P = (N/\rho_T)I$, where $I$ is the $N$ by $N$ unit or identity matrix, and the eigenvectors of $P + B$ are equal to those of $B$. The eigenvalues of $P + B$, are those of $B$, which form a row matrix $\lambda$, shifted by $N/\rho_T$, i.e.,

$$\gamma = \lambda + (N/\rho_T)u$$  \hspace{1cm} (3)

where $u = (1, \ldots, 1)$.

Stability is determined by the sign of the lowest eigenvalue of $P + B$, $\gamma_{\text{min}}$. It requires $\gamma_{\text{min}} > 0$, and the spinodal is reached when $\gamma_{\text{min}} = 0$. From Eq. (3) it follows that the total density $\rho_T$ at the spinodal is $\rho_{\text{sp}} = -N/\lambda_{\text{min}}$, with $\lambda_{\text{min}}$ the lowest eigenvalue of $B$.

Now, $B$ is a random matrix, and for simplicity we choose its elements $B_{ij}$, $i \leq j$, as independent random variables with mean $b$ and standard deviation $\sigma$ (we actually choose a Gaussian distribution, but the particular choice of this distribution is irrelevant for large $N$). Since Wigner’s pioneering work [1,2], the problem of characterising the spectrum of such a random matrix has received a great deal of attention (see [6] for a review). In our particular case, there are two theorems that fully describe the spectrum of $B$.

The first theorem is due to Arnold [3,4], and states that the density of rescaled eigenvalues, $x \equiv \lambda/2\sigma N^{1/2}$, of $B$ converges in probability, as $N \to \infty$, to

$$W(x) = \begin{cases} \frac{2}{\pi} \sqrt{1-x^2} & \text{if } |x| \leq 1, \\ 0 & \text{if } |x| > 1. \end{cases}$$  \hspace{1cm} (4)

This is known in the literature as Wigner’s semicircle law. It also holds if the diagonal elements, $B_{ii}$, have a mean $b' \neq b$.

The second theorem, by Füredi and Komlós [5], refines this result a little. Under the further assumption that $|B_{ij}| \leq K$ for all $i, j = 1, \ldots, N$, if $b > 0$ ($b < 0$) then the highest (lowest) eigenvalue is asymptotically distributed with a Gaussian of mean $Nb + (b'-b) + \sigma^2/b + O(N^{-1/2})$ and variance $2\sigma^2$, and the remaining $N-1$ eigenvalues follow Wigner’s semicircle law. If $b = 0$ the highest (lowest) eigenvalue is also within the semicircle.

All this can be seen in numerically calculated spectra, for $N = 25$, in Fig. 1. For $|b| \gtrless \sigma/N^{1/2}$ the probability density function, $p(\lambda)$, for the eigenvalues clearly exhibits a lone, Gaussian distributed, eigenvalue, and for all $b$ there is a clear semicircle.

The two theorems above permit us to describe, for large $N$, the lowest eigenvalue of $B$ and hence the spinodal instability of our mixture. Let us define rescaled variables $\beta \equiv N^{1/2}b/\sigma$ and $\xi_{\text{sp}} \equiv \rho_{\text{sp}} \sigma/N^{1/2}$. For $\beta \lesssim -1$, the (rescaled) lowest eigenvalue, $x_{\text{min}}$, which determines the spinodal is due to a lone eigenvalue, see the bottom curve in Fig. 1. This eigenvalue has a mean value $(\beta + \beta^{-1})/2$ and standard deviation $1/\sqrt{2N}$. As the latter goes to zero when $N \to \infty$, $x_{\text{min}}$ is a self-averaging quantity. So for large $N$ we can take [10]

$$\langle \xi_{\text{sp}} \rangle = \left\langle \frac{-1}{2x_{\text{min}}} \right\rangle$$  \hspace{1cm} (5)

for the spinodal. Replacing $x_{\text{min}}$ by its mean value,

$$\langle \xi_{\text{sp}} \rangle = \frac{-1}{\beta + \beta^{-1}}.$$  \hspace{1cm} (6)

The nature of the instability is described by the corresponding eigenvector. Füredi and Komlós show that this eigenvector is almost parallel to $u$ [3,4], so it is a condensation instability, as the densities of all the components increase (or decrease) together according to the eigenvector: the instability looks like the incipient formation of one phase enriched in all the components coexisting with a phase depleted in all the components.

Conversely, if $\beta \gtrsim -1$, then $x_{\text{min}}$ and hence the spinodal, is determined by the semicircle. The lowest eigenvalue will be near the lower end of the semicircle. We
probability density.) As \( X = \int \) (Notice that \( X \) using Eq. (4), we obtain an equation for \( N \) roughly distributed according to \( \beta < \). Eq. (6) for \( m \) with \( N \) values of \( N \) by \( \langle \xi_{sp} \rangle = 2 + 1\) for \( \beta > -1 \), is plotted as a long-dashed curve. For \( \beta < -1 \), it lies on top of the numerically calculated mean density of the spinodal for \( N = 25, 100 \) and \( 400 \) (from top to bottom). The short-dashed horizontal lines are the predictions of Eq. (11) for these values of \( N \) (again from top to bottom). The \( N \to \infty \) limit of Eq. (3) for \( \beta < -1 \), and of Eq. (11) \( (\langle \xi_{sp} \rangle = 1/2) \) for \( \beta > -1 \), is compared with the results of numerical calculations in Fig. 2, for \( \beta < -1 \). For \( \beta < -1 \), it lies on top of the numerically calculated values and so is not visible. The crossover between condensation and demixing at \( \beta = -1 \) is marked by a vertical dotted line.

then estimate the distribution of the lowest eigenvalue as follows.

Given the semicircle law, the expected number of (rescaled) eigenvalues between \( -1 \) and \( X \) will be given by \( N \int_{-1}^{X} W(x) \, dx \), so \( x_{min} \) will be in the interval \([-1, X(N)] \), where \( X(N) \) is defined by

\[
N \int_{-1}^{X(N)} W(x) \, dx = 1.
\]

Using Eq. (6), we obtain an equation for \( X \)

\[
\frac{1}{2} + \frac{1}{\pi} \arcsin X + \frac{1}{\pi} X \sqrt{1 - X^2} = \frac{1}{N},
\]

which yields

\[
X(N) = -1 + m + \frac{1}{10} m^2 + \frac{11}{350} m^3 + O(m^4),
\]

with \( m \equiv (1/2)(3\pi/2N)^{2/3} \). Now, for a given (large) \( N \), \( x_{min} \) will be roughly roughly roughly roughly distributed according to

\[
p_N(x_{min}) = \begin{cases} 
NW(x_{min}) & \text{if } -1 \leq x_{min} \leq X(N), \\
0 & \text{otherwise.}
\end{cases}
\]

(Notice that \( \int_{-\infty}^{\infty} p_N(x) \, dx = 1 \), so it is a well defined probability density.) As \( X(N) \to -1 \) when \( N \to \infty \), \( x_{min} \) is self-averaging in this case as well. We can then make use of Eq. (5) to determine the spinodal. Thus from Eqs. (9) and (10),

\[
(\langle \xi_{sp} \rangle ) = \frac{1}{2} + \frac{3}{10} m + \frac{33}{140} m^2 + O(m^3).
\]  

As for the eigenvector, we know that when there is a lone eigenvalue its corresponding eigenvector is almost parallel to \( u \), so the eigenvector of any eigenvalue of the semicircle must be almost orthogonal to \( u \) (the matrix is symmetric). By continuity this holds for \( x_{min} \), as well when there is no lone eigenvalue. Then, roughly half of the components of the eigenvector are of one sign while the rest are of the opposite sign. The instability is with respect to a density modulation in which about half the components are separating from the other half: the instability looks like demixing into two phases, each one enriched in some components and depleted in others.

The predictions of theory in both regimes, Eqs. (6) and (11), are compared with the results of numerical calculations in Fig. 2, for \( N = 25, 100 \) and \( 400 \). The crossover between demixing and condensation occurs for \( \beta \sim -1 \) in all cases. This crossover can also be seen by looking at the angle, \( \theta \), the eigenvector of \( x_{min} \) makes with \( u \). In Fig. 3 we have plotted the mean and standard deviation of \( |\cos(\theta)| \), as a function of \( \beta \), for \( N = 25 \). At around \( \beta = -1 \) the cosine drops and the standard deviation peaks, indicating that the instability eigenvector is switching over from being nearly parallel to \( u \), to being nearly perpendicular.

For \( \beta < -1 \), as \( N \to \infty \), the instability, which is with respect to condensation, approaches that of a single component system with second virial coefficient \( b \). Such a sys-
tem becomes unstable at a spinodal density \( \rho_{sp} \) is equal to \(-1\), and Eq. \(6\) yields this result as \( N \to \infty \). For \( \beta > -1 \), \( \langle \xi_D \rangle \to 1/2 \), as \( N \to \infty \), and so the total number density at the spinodal diverges as \( N^{1/2} \). The mixture becomes stable with respect to demixing at all densities as \( N \to \infty \)—also consistent with the mixture behaving as a single component system. For finite but large \( N \), demixing requires either high densities or a broad distribution of the second virial coefficients, i.e., large \( \sigma \). In Fig. \(2\) we see that even for the smallest \( N \) of 25, the prediction of our theory for demixing (\( \beta > -1 \)) is quite accurate. We also see that for condensation, our theoretical prediction is on top of the numerical curves for all 3 values of \( N \), and that the density of the condensation instability is insensitive to \( N \).

Many properties of random matrices, such as the distribution of their eigenvalues (properly scaled), are self-averaging \(\text{\footnotesize{\cite{2,3,4,5}}}\). A property \( R \) of an \( N \) by \( N \) matrix \( A_N \) is said to be self-averaging if \( \langle R(A_N) \rangle \) converges in probability, as \( N \to \infty \), to \( \langle R(A) \rangle \), where \( \langle \rangle \) denotes an average over some ensemble of matrices \( A_N \). This simply means that, for large \( N \), almost all matrices \( A_N \) have the same value of \( R \). In particular, the lowest (scaled) eigenvalue \( x_{\min} \) of our random matrices \( B \) is a self-averaging property of these matrices and hence so is the value of the density at the spinodal. The distribution function of the spacing of the eigenvalues of random matrices is also self-averaging, and experimental data on nuclei show that the spectra of nuclei far from their ground state are, approximately, self-averaging \(\text{\footnotesize{\cite{6}}}\). The good agreement between theory and experiment in the study of nuclei \(\text{\footnotesize{\cite{6}}}\) on both the model and the experimental system having a self-averaging property. As emphasised by Dyson and others \(\text{\footnotesize{\cite{7}}}\), the theoretical prediction is obtained via statistical methods but this is then compared to the results for a single experimental system, e.g., a \(^{235}\text{U} \) nucleus.

So, are the thermodynamic properties of complex mixtures, such as those found inside living cells, self-averaging? This can only be determined by experimental measurements of these properties, and we are not aware of data that could decide one way or the other. We can only say that if they are self-averaging, then statistical theories like that presented here will be an effective way of predicting and understanding their properties, while if they are not self-averaging then their properties will be sensitive to small details and very difficult to predict.

The analysis we have just carried out in this Letter relies on several simplifying assumptions. We have employed a second virial coefficient approximation, have studied a mixture of components with equal densities, and have taken the virial coefficients to be independent random variables. Correlations were neglected so that \( \langle B_{ij}B_{ik} \rangle = \langle B_{ij} \rangle^2 \) for \( j \neq k \). All three assumptions are simple, minimal assumptions which can be relaxed in a more elaborate, but still of course statistical, model.

Quite generally, to develop theories for very complex systems, specified by very large numbers of parameters, there seems little alternative to statistical approaches. By statistical approaches we mean those that have parameters which instead of being definite numbers which are put into the model, are random variables taken from a probability distribution function which is put into the model. The cytoplasm of bacteria such as \( E. coli \) is a very complex system: it is a mixture of thousands of different types of rather complex bio-macromolecules, mostly protein but also RNA, DNA, polysaccharides, etc.\(\text{\footnotesize{\cite{11,12}}}\). We can neither obtain nor want details of all the interactions of these molecules, but we do want to understand and to be able to predict the collective properties of this mixture, such as its osmotic pressure, where it becomes unstable and so on. In these circumstances, statistical approaches, such as the one described here, are the only means of making predictions. They rely on self-averaging occurring in the experimental system, and so, in order to establish their validity, we need to know whether or not the complex mixtures found inside living cells have self-averaging thermodynamic properties, something that experiments will have to assess.

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