A Theoretical Exploration of Single-Molecule Mixture Through Combinatorial Method

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Abstract: Single molecule science and techniques have received increasing attention in recent years. A very interesting subject in this field is “single-molecule mixture”, which contains a mixture of molecules that have molecularly different structures, that surprisingly remain unexplored both theoretically and experimentally. A major barrier to investigate this form of molecules lies in how to generate a structural space that contain sufficient huge number of chemical structures to enable single-molecule mixtures to exist in macroscopic quantities and how to efficiently prepare it. In this article, a theoretical approach that combined model construction, thought experiment, and mathematical analysis was developed to study this elusive form of molecules. A possible route for the preparation of single molecule mixture was also provided.

There exist two extreme forms of molecules: pure form and single-molecule mixture. As is shown in Figure 1. Pure form contains only one kind of molecule. Ultrapure substances, such as ultrapure water, have find wide applications, however, “absolute pure” form of molecules can hardly be reached experimentally. Single-molecule mixture, on the other hand, contains a mixture of molecules that have molecularly different structures. In a given amount of single-molecule mixture sample, the number of molecular structures equals the number of molecules. In nature, at cell levels, each cell is different, at molecular levels, single-molecule mixture, in that each molecule has different structure, to the best of our knowledge, has not yet been explored, both theoretically and experimentally. In this paper, I wish to report a primary theoretical exploration of this form of molecules and provide a possible route for its preparation.
Two extreme forms of molecules

![Diagram of pure form, mixtures, and single-molecule mixture](image)

- Pure form
- Mixtures
- Single-molecule mixture

Figure 1. Two extreme forms of molecules: pure form and single-molecule mixture

To start our exploration, the first problem we met is how to generate a structural space that contains sufficient huge number of chemical structures\(^1\)\(^-\)\(^5\) to enable single-molecule mixtures to exist in macroscopic quantities, for example, at gram scale. Our goal is to generate a “synthesizable low-molecular-weight structural space”, in that this space is conveniently accessible from a synthetic point of view, not just a purely theoretical model. After many attempts, we choose substituted oligo-D-mannitol as a model system; the structures of the related substances are shown in Figure 2. The –OH groups in the chain of oligo-D-mannitol are substituted by substituted benzyl group, in which the substitutes are selected 30 isomers of decanyl group (–C\(_{10}\)H\(_{21}\)).
Figure 2. The model system of the present study: substituted oligo-D-mannitol

The overall numbers of possible structures of the substituted benzyl group were calculated to be $1.395 \times 10^4$. Thus, the number of possible structures of the tetra-substituted monomer unit of oligo-D-mannitol are $(1.395 \times 10^4)^4$, namely, about $3.787 \times 10^{16}$. Based on these calculated results, the numbers of possible structures of oligo-D-mannitol ($n = 2-6$) in the present model system, together with their molecular weight, were calculated, and the results are shown in Table 1.

Table 1. Calculated numbers of possible structures and molecular weight of the oligo-D-mannitol ($n = 2-6$) in the present model system.
| Oligomer | Number of Structures | Molecular Weight |
|----------|----------------------|------------------|
| H[OMe]1 | $3.787 \times 10^{16}$ | 2431.9           |
| H[OMe]2 | $1.434 \times 10^{33}$ | 4831.8           |
| H[OMe]3 | $5.431 \times 10^{49}$ | 7231.7           |
| H[OMe]4 | $2.057 \times 10^{66}$ | 9631.6           |
| H[OMe]5 | $7.789 \times 10^{82}$ | 12031.5          |
| H[OMe]6 | $2.950 \times 10^{99}$ | 14431.4          |

From these results we can see that simply by introducing 30 minimally different alkyl substitute group into the backbone of the oligomer structures generate a huge structural space for each oligomer, in that as many as $2.950 \times 10^{99}$ possible structures exist in the structural space of the hexamers.

A very interesting question occurs: if we take a given number ($n$) of structures for the above structural space (overall number of structures = $m$) individually and randomly (which means that the same structure may be taken more than once), what’s the probability ($P$) to get single-molecule mixture, in that each structure we take are different?

This problem can be solved through probability calculation.

$$P = \frac{c^n_{m-n}}{c^m_n} = \frac{(m-n)(m-n-1)(m-n-2)\cdots(m-n-n)}{m(m-1)(m-2)\cdots(m-n)}$$

$$= \left(\frac{n}{m}\right)\left(\frac{n}{m-1}\right)\left(\frac{n}{m-2}\right)\cdots\left(\frac{n}{m-n}\right)$$

If $m > n^2$, then $P > (1 - \frac{n}{m-n})^n > 1 - n\times \frac{n}{m-n}$

If $n^2 > m > 2n$, then $P < (1 - \frac{n}{m})^n$

In our case, we choose $n = N_A$ as the given number of molecules taken from the structural space. The calculated results are summarized in Table 2.
Table 2. Calculated probabilities to get single-molecule mixture from each model system, the number of molecules taken from each structure space are $n = N_A$

| Oligomer | $P$ |
|----------|-----|
| $\text{H} - \text{OMe}_1$ | 0 |
| $\text{H} - \text{OMe}_2$ | $< (1-4.20 \times 10^{-10}) N_A$ |
| $\text{H} - \text{OMe}_3$ | $> 0.999$ |
| $\text{H} - \text{OMe}_4$ | $> 1-1.76 \times 10^{-20}$ |
| $\text{H} - \text{OMe}_5$ | $> 1-4.64 \times 10^{-37}$ |
| $\text{H} - \text{OMe}_6$ | $> 1-1.23 \times 10^{-53}$ |

From these calculated results we can see that, for monomers and dimers, the probabilities to get single-molecule mixture corresponds to 0 (for monomers) or nearly 0 (for dimers). However, for trimers, the probability is larger than 0.999, implying that if we individually and randomly take $n = N_A$ molecules from the trimer structure space, we have larger than 0.999 probability to get single-molecule mixture. For higher oligomer structures - tetramers, pentamers and hexamers, the probabilities to get single-molecule mixtures even close to 1. A schematic diagram showing the single-molecule mixtures of the substituted oligo-d-mannitol system is illustrated in Figure 3.
Figure 3. Schematic diagram showing single-molecule mixtures of substituted oligo-D-mannitol system

Another interesting question arise is how to understand the macroscopic structure of the single-molecule mixture obtained from the above thought experiment. Although the structure of each molecules is different, in that the R group in each substitute site varied, they come randomly from the same structure space, thus, macroscopically, the R group can be viewed as a hybrid structure of the 30 possible isomers of the decanyl group (−C_{10}H_{21}). Based on this analysis, we can expect that some physical proprieties of the single-molecule mixture sample obtained from the trimer (or higher oligomer) space, such as density, will be consistent, just like in pure substance.

A possible route for the preparation of single-molecule mixture obtained from the above structural space is provided in Figure 4. This route starts from readily available methyl galloate and D-mannitol and 30 equal mixtures of alkyl bromide 2. Since the substitutes changes at bromide 2 is only minimal at remote position, these bromides are expected to have essentially the same reactivity and polarity. Thus, the synthetic and purification procedure are expected to be similar to handling pure materials^6-9.
Figure 4. A Possible Route for The Preparation of Single-Molecule Mixture

In the above model system, as much as 30 isomers of decanyl group are needed to
generate a structural space that contain sufficient huge number of chemical structures, which from synthetic point of view still not a conveniently accessible system. We next further consider the possibility of using only 2 different alkyl group to build a structural space that enable single-molecule mixtures to exist in macroscopic quantities. Consider a model chain molecule system illustrated in figure 5, this system contains $n$ substitutional sites, each site is randomly substituted by one of the two possible alkyl groups, $R^1$ or $R^2$, thus, the overall number of the structural isomers of in this structural space are $2^n$. As $n$ increases, the overall number of isomers increases exponentially. If $n = 200$, then the overall number of isomers of this system are $2^{200}$, namely, $1.60693804 \times 10^{60}$. This number is sufficient huge for single-molecule mixtures to exist in macroscopic quantities.

**Figure 5.** A model chain molecule system

Based on this consideration, a 24 mer of $O$-propyl substituted D-mannitol system was built, as illustrated in Figure 6. This system contains 192 phenolic hydroxyl group, which were randomly substituted by $n$-propyl or $i$-propyl group. The molecular weight of this system is 23775.1700 Da, and the number of structural isomers are $2^{192}$, namely, $6.277 \times 10^{57}$. This number, as calculated above, is sufficient huge for single-molecule mixtures to exist in **multi-ton scales**.

**Figure 6.** A 24 mer of $O$-propyl substituted D-mannitol model system.
Figure 7. A Synthetic Route of the 24 mer of O-propyl substituted α-mannitol model system.

A synthetic route of the above 24 mer is illustrated in figure 7. In this route, 4 equal mixtures of substituted benzyl bromide 12 are used, which are expected to have essentially the same reactivity and polarity. Thus, the synthetic and purification procedure are expected to be similar to synthesizing pure single-isomer products.\(^6\)
Figure 8. Hybrid Structure of Single-Molecule Mixture Sample 27

In order to better understand the structure of single-molecule mixture sample 27, inspired by the concept of orbital hybridization, if we define Pr* as a hybrid structure of n-Pr and i-Pr, then single-molecule mixture sample 27 can be viewed as a “pure sample”, in that each phenolic hydroxyl group is substituted by an unprecedented hybridized Pr* group, as illustrated in Figure 8. Thus, the physical proprieties of single-molecule mixture sample 27 will to some extent like a pure substance.

It is noted that in many realistic systems, including synthetic polymers systems and natural biopolymers systems, partial random substitution of the parent molecule by a few substituent groups (for example, random partial substitution of the hydrogen atom by a methyl group or bromine atom) frequently occurred, as illustrated in Figure 9. This substitution process will generate a large number of isomers with equal probabilities. In order to calculate the exact number of potential isomers, we constructed two additional model systems.

Figure 9. The process of partial random substitution of a parent molecule containing m substitutable sites by n substituent groups.

If the overall number of substitutable sites in the parent molecule are m, the number of substituted sites are n, the number of possible substitute groups in each randomly
substituted site is $s$, then the overall number of potential isomers from this structural space $r$ can be calculated as:

\[ r = C_m^n \times s^n \]

In model system A, we choose $m = 1000, s = 1$, implying that the parent molecule contains 1000 substitutable sites, a few of these sites are randomly substituted by another group ($R^0 \rightarrow R^1$), the overall number of potential isomers can be calculated as:

\[ r = C_{1000}^n \]

the calculated results are listed in Table 3.

**Table 3.** Calculated results of model system A.

| $n$ | overall substitute rate (%) | Overall number of potential isomers |
|-----|----------------------------|-------------------------------------|
| 0   | 0                          | 1                                   |
| 1   | 0.1                        | 1000                                |
| 2   | 0.2                        | $4.995 \times 10^5$                 |
| 3   | 0.3                        | $1.66 \times 10^8$                 |
| 4   | 0.4                        | $4.14 \times 10^{10}$              |
| 5   | 0.5                        | $8.25 \times 10^{12}$              |
| 6   | 0.6                        | $1.37 \times 10^{15}$              |
| 7   | 0.7                        | $1.94 \times 10^{17}$              |
| 8   | 0.8                        | $2.41 \times 10^{19}$              |
| 9   | 0.9                        | $2.66 \times 10^{21}$              |
| 10  | 1.0                        | $2.63 \times 10^{23}$              |
| 11  | 1.1                        | $2.37 \times 10^{25}$              |
| 12  | 1.2                        | $1.95 \times 10^{27}$              |
| 13  | 1.3                        | $1.48 \times 10^{29}$              |
| 14  | 1.4                        | $1.05 \times 10^{31}$              |
| 15  | 1.5                        | $6.88 \times 10^{32}$              |
| 16  | 1.6                        | $4.24 \times 10^{34}$              |
| n  | overall substitute rate (%) | Overall number of potential isomers |
|----|-----------------------------|-------------------------------------|
| 17 | 1.7                         | 2.45×10^{36}                        |
| 18 | 1.8                         | 1.34×10^{38}                        |
| 19 | 1.9                         | 6.92×10^{39}                        |
| 20 | 2.0                         | 3.39×10^{41}                        |
| 21 | 2.1                         | 1.58×10^{43}                        |
| 22 | 2.2                         | 7.05×10^{44}                        |
| 23 | 2.3                         | 3.00×10^{46}                        |
| 24 | 2.4                         | 1.22×10^{48}                        |
| 25 | 2.5                         | 4.76×10^{49}                        |

From Table 3 we can see that for this model system, as the number of substituted site increases, the overall number of potential isomers increases exponentially. If \( n = 25 \), then the overall number of potential isomers of this structural space are 4.76×10^{49}. This number is sufficient huge for single-molecule mixtures to exist in submolar-scale (10). Thus, we can conclude that if a polymer molecule contains 1000 substitutable sites, 2.5% of these sites are randomly substituted by another group, then the potential isomers exceed 4.76×10^{49}, if this substitution process proceed at sub-molar-scale, then the products are most likely existing as single-molecule mixture state!

In model system B, we choose \( m = 100, s = 10 \), implying that the parent molecule contains 100 substitutable sites, a few of these sites are randomly substituted by one of the ten possible R group \([R^0 \rightarrow (R^{1 \text{ to } 10})]\), the overall number of potential isomers can be calculated as:

\[
r = C_{100}^n \times 10^a
\]

the calculated results are listed in Table 4.

**Table 4.** Calculated results of model system B.
|   |   |    |
|---|---|---|
| 1 | 1 | 1000 |
| 2 | 2 | $4.95 \times 10^5$ |
| 3 | 3 | $1.62 \times 10^8$ |
| 4 | 4 | $3.92 \times 10^{10}$ |
| 5 | 5 | $7.53 \times 10^{12}$ |
| 6 | 6 | $1.19 \times 10^{15}$ |
| 7 | 7 | $1.60 \times 10^{17}$ |
| 8 | 8 | $1.86 \times 10^{19}$ |
| 9 | 9 | $1.90 \times 10^{21}$ |
|10 |10 | $1.73 \times 10^{23}$ |
|11 |11 | $1.41 \times 10^{25}$ |
|12 |12 | $1.05 \times 10^{27}$ |
|13 |13 | $7.11 \times 10^{28}$ |
|14 |14 | $4.42 \times 10^{30}$ |
|15 |15 | $2.53 \times 10^{32}$ |
|16 |16 | $1.35 \times 10^{34}$ |
|17 |17 | $6.65 \times 10^{35}$ |
|18 |18 | $3.07 \times 10^{37}$ |
|19 |19 | $1.32 \times 10^{39}$ |
|20 |20 | $5.36 \times 10^{40}$ |
|21 |21 | $2.04 \times 10^{42}$ |
|22 |22 | $7.33 \times 10^{43}$ |
|23 |23 | $2.49 \times 10^{45}$ |
|24 |24 | $7.98 \times 10^{46}$ |
|25 |25 | $2.43 \times 10^{48}$ |

From Table 4 we can see that for this model system, as the number of substituted site increases, the overall number of potential isomers also increases exponentially. If $n = 25$, then the overall number of potential isomers of this structural space are
2.43×10^6. This number is also sufficient huge for single-molecule mixtures to exist in submolar-scale. Thus, if this substitution process proceed at submolar-scale, then the products are also most likely existing as single-molecule mixture state.

In summary, single-molecule mixture, the opposite extreme form of molecules compared to “absolute pure” form, have been theoretically studied by a combination of model construction, thought experiment, and mathematical analysis, and some interesting results were obtained from this study. A possible route for its preparation is also provided. It is expected that this form of matters will exhibit unique, perhaps unprecedented, physical properties. This article also reveals that single-molecule mixture state may also exist in realistic synthetic or natural polymer system. It is hoped that this study will inspire further exploration of this intriguing and new area.

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**Competing interests**
The author declares no competing financial interest.

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10. “molar scale” is actually inaccurate to describe single-molecule mixture samples since in single-molecule samples every single-molecule structure is different.