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Stoichiometry Determination for Mass-spectrometry Data: the Interval Case

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Abstract: In structural proteomics, given the individual masses of a set of protein types and the exact mass of a protein complex, the *exact stoichiometry determination problem (SD)*, also known as the money-change problem, consists of enumerating all the stoichiometries of these types which allow to recover the target mass. If the target mass suffers from experimental uncertainties, the *interval SD problem* consists of finding all the stoichiometry vectors compatible with a target mass within an interval.

We make contributions in two directions. From a theoretical standpoint, we present a constant-memory space algorithm (DIOPHANTINE) and an output sensitive dynamic programming based algorithm (DP++), both inherently addressing the interval SD problem. From an applied perspective, we raise three points. First, we show that DIOPHANTINE and DP++ yield an improvement from 3 to 4 orders of magnitude over state-of-the-art exact SD algorithms, for typical protein complexes facing uncertainties on the target mass in the range 0.1-1%. Second, we show that DIOPHANTINE behaves like an output-sensitive algorithm—especially when the interval width increases, albeit such a property cannot be expected in general. Third, from a biological perspective, using a panel of biological complexes (eukaryotic translation factor, yeast exosome, 19S proteasome sub-unit, nuclear pore complex), we stress the importance of enumeration, even at a null noise level. The programs accompanying this paper are available from <http://team.inria.fr/abs/addict/>.

Key-words: Protein complex, mass spectrometry, denumerant, dynamic programming, linear diophantine equation, enumeration, output sensitive algorithm.

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Sur la détermination de la stoechiométrie en spectrométrie de masse: détermination pour un intervalle de masses

Résumé : En protéomique structurale, étant données les masses exactes d'un ensemble de protéines et la masse exacte d'un complexe, le problème de la *détermination de la stoechiométrie* (SD), consiste à énumérer les stoechiométries de ces protéines permettant de reconstituer la masse du complexe. Si celle-ci présente une incertitude, généralement inférieure à 1%, il s'agit d'énumérer les stoechiométries compatibles avec une masse appartenant à un intervalle. Nous présentons des contributions dans deux registres.

D'un point de vue théorique et contrairement aux travaux antérieurs focalisés sur le problème exact, nous développons un algorithme travaillant à taille mémoire constante (DIOPHANTINE), ainsi qu'un algorithme sensible à la sortie basé sur la programmation dynamique (DP++), tous deux résolvant le problème de détermination de la stoechiométrie pour un intervalle. D'un point de vue appliqué, nous présentons trois résultats. Tout d'abord, nous montrons que DIOPHANTINE et DP++ sont plus rapides de 3 à 4 ordres de grandeur que l'algorithme état de l'art résolvant le problème exact, pour des complexes protéiques typiques présentant des incertitudes de l'ordre de 0.1% à 1%. D'autre part, nous montrons que DIOPHANTINE a un comportement d'autant plus *sensible à la sortie* que la taille de l'intervalle augmente. Enfin et d'un point de vue biologique, en étudiant plusieurs complexes protéiques (facteur de traduction eucaryote, *exosome* de la levure, sous-unité 19S du protéasome, pore nucléaire), nous soulignons l'importance des solutions multiples, même à un niveau d'incertitude nul.

Mots-clés : Complexes protéiques, spectrométrie de masse, dénumérant, programmation dynamique, équation diophantienne, énumération, algorithmes sensibles à la sortie.

1 Introduction

1.1 Structural Proteomics, Integer Partitions and Knapsack Problems

Mass spectrometry. Biology rests on multimeric assemblies, so that reconstructing models of such assemblies is a central problem in (computational) structural biology. While the structure determination of proteins and small complexes has increased at a fast pace thanks to structural proteomics projects [Jan07], that of larger complexes, which typically do not crystallize or are too big for nuclear magnetic resonance studies has remained elusive. Fortunately, mass spectrometry (MS), a technique initially developed in chemistry to infer the mass-to-charge ratio of molecules, is in the process of filling this gap, allowing to study complexes that vary in mass, size, solubility, and bound/unbound states [SR07, BR11]. In a nutshell, MS first requires isolating sample complexes from the cellular environment, a process typically resorting to Tandem Affinity Purification [ea01]. Operating on these samples in solution, a soft ionization technique such as electrospray ionization or matrix-assisted laser desorption/ionization allows ionizing the proteins or protein assemblies, while preserving non covalent interactions—a key feature to study proteins and complexes [SR07].

In classical MS, these primary ions are sorted by a mass analyzer according to their mass over charge (m/z) ratio, and the relative abundance of the samples are measured by a detector. Unfortunately, for a solution containing a mixture of complexes, different complexes with identical m/z ratio may contribute to the same peak in the mass spectrum. To disentangle such cases, tandem MS (denoted MS/MS) can be used. In this refined protocol, the precursor ions undergo a collision induced dissociation, which consists of removing highly charged peripheral sub-units thanks to gas collisions, so that the product ions can indeed be sorted by m/z ratio.

MS can also be used to gain information on the sub-complexes of an assembly [THS⁺08]. Denaturing solvent or high salt concentrations can be used to break the complex into pieces, which are then identified by MS or tandem MS. In extreme conditions, complete denaturation occurs, so that the masses of the individual components can be determined. In milder conditions, multiple overlapping complexes are generated, providing information on the building blocks.

Stoichiometry determination. Upon determining the masses (those of the complexes in a mixture, or those of the sub-complexes of an assembly), the stoichiometry determination (SD) problem consists of determining the stoichiometry of the protein types accounting for each target mass. Denoting p the number of protein types, w_i and s_i the mass and stoichiometry of the i th protein type respectively, one wishes to reconstruct the target mass M as follows:

$$\sum_{i=1,\dots,p} s_i w_i = M. \quad (1)$$

Prosaically, this problem is also known as the *money exchange* problem, for obvious reasons [BL05b]. Mathematically and since the masses are integers (expressed say in Daltons¹), SD is related to the theory of integer partitions [Com74], to linear diophantine equations [Sma98], and is also coupled to so-called knapsack and subset sum problems [Pis05].

Practically, two difficulties are faced in mass spectrometry. First, any mass experimentally determined is generally larger than the theoretical one, due to extra molecules (solvent and electro-spray buffer molecules) sticking to the molecule or complex analyzed. Second, because the proteins involved in different copies of a complex may have undergone different post-translational modifications² [Kel04], there might be a discrepancy between the masses of the sub-units. These

¹The unified atomic mass unit, also known as Dalton, is approximately equal to the mass of one neutron or one proton.

²The chemical modification of a protein after its bio-synthesis by the ribosome.

two problems explain why we are interested in an interval version of Eq. (1).

The interval problem. To account for the uncertainties on the masses just discussed, let ε be a positive integer corresponding to the mass discrepancy to be accommodated. Instead of solving Eq. (1), we are instead interested in the following enumeration problem

$$\text{Report all stoichiometry vectors } S = (s_1, \dots, s_p) \text{ such that } \left| \sum_{i=1, \dots, p} s_i w_i - M \right| \leq \varepsilon. \quad (2)$$

For the sake of conciseness, the previous problem uses symmetric errors terms $\pm\varepsilon$ with respect to the target mass M . However, an interval $[M - \varepsilon_1, M + \varepsilon_2]$ with $\varepsilon_1 \neq \varepsilon_2$ can be accommodated at the cost of trivial modifications of our algorithms.

1.2 Contributions

To the best of our knowledge, the question of mass accuracy for the complex composition to be determined has barely been touched upon in structural biology. In [THS⁺08], 100 hypothetical complexes from 6 to 14 sub-units have been created with masses in the range 10-50kD, and 100 dimers, trimers or tetramers were generated from these complexes. With an error rate of 1%, it is observed that no tetramer has a unique stoichiometry vector. Most importantly, the question of designing and analyzing algorithms solving this problem has not been addressed, to the best of our knowledge.

In this context, we make two contributions. First, we present a constant memory space algorithm (DIOPHANTINE), and an output sensitive dynamic programming based algorithm (DP++), both inherently addressing the interval SD problem. Second, we present a detailed experimental study on various biological and synthetic datasets, which results in three conclusions. The first observation is that for biological datasets, enumeration does matter even for a moderate (and sometimes even null) noise level. The second finding is that our algorithms DIOPHANTINE and DP++ outperform state-of-the art dynamic programming based approaches by three to four orders of magnitude, for a noise level in the range 0.1% to 1%. The last one is that DIOPHANTINE actually exhibits an output sensitive behavior.

2 Theory and Algorithms

In section 2.1, we recall the rich background of knapsack and integer partition problems. In section 2.2, we present classical counting results, and derive bound on the number of solutions for the SD problem.

2.1 Denumerants, Unbounded Knapsack and Subset-sum Problems

In the following, we consider a vector $\mathbf{W} = \{w_1, \dots, w_p\}$, of positive integers or real numbers. A stoichiometry vector is denoted $\mathbf{S} = \{s_1, \dots, s_p\}$. The vector is called positive if $s_i > 0$ for all i , and non-negative if $s_i \geq 0$ for all i .

Denumerants. Assume that the vector \mathbf{W} contains integers. In combinatorics, the number of non-negative integer solutions to Eq. (2) is known as the *denumerant* $D(M)$ of the target mass. It has been known since Bell [Bel43] that if $\text{lcm}(\mathbf{W})$ denotes the least common multiple

of the w_i s, then, for each $b \in \{0, \dots, \text{lcm}(\mathbf{W}) - 1\}$, if $M = m \text{lcm}(\mathbf{W}) + b$, the denominator is a polynomial in m of degree $p - 1$, that is:

$$D(m \text{lcm}(\mathbf{W}) + b) = \sum_{i=0, \dots, k-1} c_i m^i. \quad (3)$$

More generally, the number of solutions reads from the power series expansion of the generating function $1/\prod_i(1 - X^{w_i})$, but the difficulty precisely relies in extracting the coefficient of X^n in that expansion. In analytic combinatorics [FS09, Chapter IV], a classical result obtained by singularity analysis states that if $\text{gcd}(\mathbf{W}) = 1$, one asymptotically gets

$$D(M) \sim \frac{M^{p-1}}{(p-1)! w_1 \dots w_p}. \quad (4)$$

Upper and lower bounds on the denominator have also been obtained based on binomial coefficients solution of certain recurrence relations [Agn02]. However, these bounds are not of real interest for the SD problem for two reasons: first, the bounds concern a particular denominator rather than the solutions corresponding to an interval, as specified by Eq. (2); second, as we shall see with experiments, the bounds returned are not tight.

Frobenius numbers. The asymptotic behavior given by Eq. (4) apparently contradicts the existence of (small) masses which cannot be decomposed, this issue being related to the so-called Frobenius number and its generalizations. The Frobenius number $g_0(\mathbf{W})$ is defined as the largest integer which cannot be represented as a non-negative integer combination of the integers in \mathbf{W} [Alf05]. While explicit formula are known up to $M = 3$, only upper and lower bounds are known in general [Alf05]. Computing the Frobenius number is a NP-hard problem [RA96] for which pseudo polynomial time algorithms exist, such as the one by Round-Robin [BL05b]. (The running time of such an algorithm is polynomial with respect to the numerical values of the input data, but exponential in their bit-length.)

A related number is the positive Frobenius number $g_0^+(\mathbf{W})$, namely the largest integer which does not admit any positive integer solution. As observed in [BDF⁺10, Lemma 4], both numbers satisfy:

$$\text{Either } g_0^+(\mathbf{W}) = g_0(\mathbf{W}) = 0, \text{ or } g_0^+(\mathbf{W}) = g_0(\mathbf{W}) + \sum_{i=1, \dots, p} w_i. \quad (5)$$

Unbounded knapsack (UKP) and subset-sum (SSP). Assume that the weights in \mathbf{W} are real numbers coding the weights of p object types, and that each object also has a value $v_i \in \mathbb{R}^+$. Given a target mass M , the unbounded knapsack problem (UKP) consists of finding for each type the integral quantity $s_i \geq 0$, such that the corresponding sum of values is maximum while the corresponding sum of weights does not exceed M . This is formally defined by the following integer programming model:

$$\text{Maximize } \sum_{i=1}^p s_i v_i, \quad (6)$$

$$\text{Under the constraint } \sum_{i=1}^p s_i w_i \leq M. \quad (7)$$

The special case where $w_i = v_i$ holds is known as the subset-sum problem (SSP), and consists in finding the quantities s_i such that the corresponding sum of weights is the closest to M , without exceeding it. Changing Eq. (7) into an equality allows subset-sum to solve the exact SD problem of Eq.(1).

UKP and SSP: algorithms and complexity. The optimization version of SSP is one of the first problem proved to be NP-Hard [Kar72], but it is also known to be pseudo-polynomial. An example of such pseudo-polynomial time algorithms is well known Bellman recursion [Bel57], which solves UKP in $O(M \times p)$ time. More recently, an output sensitive algorithm solving SSP has been developed [BL05a]. Denoting w_1 the smallest mass, the algorithm has complexity $O(pw_1D(M))$, and relies on a data structure of size $O(pw_1)$. We shall use our implementation of this algorithm, called DECOMP, as main contender.

Other approaches have been developed to solve UKP, either based on dynamic programming, on branch and bound, or on a combination of both [PYA09]. Interested readers are referred to [PKP04] for detailed review on the different knapsack problems and on the different techniques used to solve them.

2.2 UKP and SSP: on the Number of Solutions

Non-negative solutions. The number of solutions $\#UKP(p, \mathbf{W}, M)$ to an unbounded knapsack problem or of a subset-sum problem that is constrained by Eq. (7) was first studied by Bege-Dov [Bd72], and the bounds were later refined by Padberg [Pad71] and Lambe [Lam74].

Denote $\#SD(p, \mathbf{W}, M, \varepsilon)$ the number of solutions to the SD problem defined by the vector \mathbf{W} , and by the target mass $M \pm \varepsilon$. The knowledge of this value is of interest in the context of stoichiometry determination, to avoid generating a large number of solutions — which would be impossible to analyze. From the exact number of solutions to an UKP problem, one gets

$$\#SD(p, \mathbf{W}, M, \varepsilon) = \#UKP(p, \mathbf{W}, M + \varepsilon) - \#UKP(p, \mathbf{W}, M - \varepsilon - 1). \quad (8)$$

However, the previous works [Bd72, Pad71, Lam74] only provide bounds. If $\#$ counts an exact number of solutions, denote $\underline{\#}$ and $\overline{\#}$ a lower and an upper bound on $\#$, respectively. Using upper and lower bounds on $\#UKP(p, \mathbf{W}, M)$, one gets the following lower bound

$$\#SD(p, \mathbf{W}, M, \varepsilon) \geq \underline{\#}UKP(p, \mathbf{W}, M + \varepsilon) - \overline{\#}UKP(p, \mathbf{W}, M - \varepsilon - 1), \quad (9)$$

together with the following upper bound

$$\#SD(p, \mathbf{W}, M, \varepsilon) \leq \overline{\#}UKP(p, \mathbf{W}, M + \varepsilon) - \underline{\#}UKP(p, \mathbf{W}, M - \varepsilon - 1). \quad (10)$$

In a nearby vein, a fully polynomial-time approximation scheme (FPTAS) has been derived to estimate the number of solutions of any knapsack problem [GKM⁺]. It provides an upper bound that is at most $(1 + \delta)\#$, where δ is an input parameter, and has a time complexity that is polynomial in M and in $1/\delta$. Using the properties of this upper bound, we can easily derive a lower bound and use them with Eqs. (9) and (10) to bounds SD. In the experiments section, we assess the ability of these strategies to estimate the number of solutions of real stoichiometry determination problems.

Positive solutions. In line with the definition of the shifted Frobenius number of Eq. (5), the question of estimating the number of positive solutions is also of interest, in particular for the biological cases where positive stoichiometries are prescribed for selected protein types. To estimate the number of such solutions, it is sufficient to compute the previous bounds with a target mass reduced by the minimal imposed stoichiometries.

3 The Interval Stoichiometry Determination Problem

3.1 Enumeration Based on Linear Diophantine Equations

The exact case. A pedestrian way to compute the denumerant consists of exhaustively trying all solutions [MHFH10, MR12]. Quite surprisingly, we shall derive a recursive algorithm solving the SD problem of Eq. (2) using the same principle. To see how, assume that the stoichiometry vectors are built incrementally—say from left to right, and that the stoichiometry vector \mathbf{S} has been computed up to index i . Under this assumption, we define the remaining mass to be accounted for by the $p - i$ proteins whose stoichiometry has to be determined by

$$M_0^-[\mathbf{S}] = M, \text{ and } M_i^-[\mathbf{S}] = M - \sum_{j=1, \dots, i} s_j w_j \text{ for } i = 1, \dots, p-1, M_i^-[\mathbf{S}] \geq 0. \quad (11)$$

Then, denoting $a \mid b$ the fact that the integer a divides the integer b , the naive counting strategy for the denumerant consists of computing the following nested sum:

$$\sum_{s_1=0}^{\lfloor M/w_1 \rfloor} \sum_{s_2=0}^{\lfloor M_1^-[\mathbf{S}]/w_2 \rfloor} \cdots \sum_{s_{p-1}=0}^{\lfloor M_{p-2}^-[\mathbf{S}]/w_{p-1} \rfloor} I(w_p : s_1, s_2, s_3 \dots s_{p-1}) \quad (12)$$

with

$$I(w_p : s_1, s_2, s_3 \dots s_{p-1}) = \begin{cases} 1 & w_p \mid M_{p-1}^-[\mathbf{S}] \\ 0 & \text{otherwise} \end{cases} \quad (13)$$

The interval case. To solve Eq. (2) using the generalized sum of Eq. (12), observe that upon reaching the last sigma, the following condition holds:

$$M - \sum_{j=1, \dots, p-1} s_j w_j - \varepsilon \leq s_p w_p \leq M - \sum_{j=1, \dots, p-1} s_j w_j + \varepsilon, \quad (14)$$

or equivalently

$$\left\lceil \frac{M - \sum_{j=1, \dots, p-1} s_j w_j - \varepsilon}{w_p} \right\rceil \leq s_p \leq \left\lfloor \frac{M - \sum_{j=1, \dots, p-1} s_j w_j + \varepsilon}{w_p} \right\rfloor \quad (15)$$

Therefore, denoting $\#(\cdot)$ the length of an integer interval, counting the number of stoichiometry vectors solving the SD problem is done by

$$\sum_{s_1=0}^{\lfloor (M+\varepsilon)/w_1 \rfloor} \sum_{s_2=0}^{\lfloor (M+\varepsilon)_1^-[\mathbf{S}]/w_2 \rfloor} \cdots \sum_{s_{p-1}=0}^{\lfloor (M+\varepsilon)_{p-2}^-[\mathbf{S}]/w_{p-1} \rfloor} I'(w_p : s_1, s_2, s_3 \dots s_{p-1}) \quad (16)$$

with

$$I'(w_p : s_1, s_2, s_3 \dots s_{p-1}) = \#_{s_p}(\lceil (M - \varepsilon)_{p-1}^-[\mathbf{S}]/w_p \rceil \leq s_p \leq \lfloor (M + \varepsilon)_{p-1}^-[\mathbf{S}]/w_p \rfloor) \quad (17)$$

Note that when $\varepsilon = 0$, the equations (16-17) and (12-13) coincide.

Remark 1. For the sake of conciseness, given a stoichiometry vector \mathbf{S} , let $\sum_{\mathbf{S}} = \sum_{s_i \in \mathbf{S}} s_i w_i$. Also, let the remaining mass associated to \mathbf{S} be defined as $m = M + \varepsilon - \sum_{\mathbf{S}}$. One has:

$$M - \varepsilon \leq \sum_{\mathbf{S}} \leq M + \varepsilon \Leftrightarrow 0 \leq \sum_{\mathbf{S}} - M + \varepsilon \leq 2\varepsilon \Leftrightarrow 0 \leq m \leq \sum_{\mathbf{S}} \leq 2\varepsilon. \quad (18)$$

These equivalent conditions characterize a valid stoichiometry vector.

Algorithm DIOPHANTINE. To turn Eq. (16-17) into an algorithm enumerating the solutions, observe that the $i + 1$ th Σ consists of finding the stoichiometry vector indices in the range $i + 1, \dots, d$, which accounts for the remaining mass $(M + \varepsilon)_i^-[\mathbf{S}]$. Algorithm 1 presents DIOPHANTINE, the corresponding recursive procedure. The recursion tree explored by this algorithm is presented on Fig. 1. Its size, defined as its number of edges, represents the cost of the execution. A leaf is called fertile if it yields a solution, and sterile otherwise. Likewise, an edge is called fertile if it leads to at least one fertile leaf, and sterile otherwise. We note in passing that when the masses are sorted in descending order, one case of output-sensitivity is easily detected. Indeed, if

$$w_p = w_{min} \leq 2 \times \varepsilon, \quad (19)$$

all leaves are fertile—the length of the interval of Eq. (14) is 2ε so that it contains at least one solution for s_p .

Regarding the memory footprint, because the stoichiometry vector \mathbf{S} is passed by reference, a unique vector is used along the whole recursion tree, and we have:

Observation. 1. *Algorithm DIOPHANTINE takes $\Theta(p)$ storage.*

Remark 2 (On positive versus non-negative solutions.). *As seen from the sigmas of Eq. (16), algorithm DIOPHANTINE generates all non-negative solutions. If minimal stoichiometries for the proteins are imposed, compliant solutions can be generated by starting the summations at these stoichiometries—in particular, starting at 1 rather than 0 yields positive solutions. Such solutions can also be generated by subtracting the mass corresponding to these constraints from the target mass, and seeking non-negative solutions.*

On the sortedness of weights. The cost of a particular enumeration problem is provided by the number of edges of the recursion tree explored by DIOPHANTINE, which actually depends on the sortedness of the vector \mathbf{W} of weights:

Theorem. 1. *The size of the recursion tree of algorithm DIOPHANTINE is minimized when the vector of weights is sorted in descending order.*

Proof. The proof is divided into three steps. First, we prove that swapping two consecutive weights w_i and w_{i+1} only changes the number of node at depth i . Second, that swapping w_i and w_{i+1} results in a smaller number of node at depth i only if after reordering, $w_i \geq w_{i+1}$. Third, that iteratively applying this results leads to the optimal weight reordering $w_1 \geq w_2 \geq \dots \geq w_p$.

Point 1: Consider the upper bound of Eq. (14). Without reordering, a node of the exploration tree at depth $k < p$ is a solution to $\sum_{i=1}^k s_i \times w_i \leq M + \varepsilon$, and the number of such node is equal to the corresponding number of such solutions (which we denote here by $\#_k$). For depth p , $\#_p$ is the number of solution as specified by Eq. (17). Thus, the number of tree node is $\#_1 + \#_2 + \dots + \#_p$. Let now swap w_i and w_{i+1} . The numbers of nodes at depth 1 to $i - 1$ do not depend on w_i nor on w_{i+1} , and thus do not change. $\#_{i'}$, the new number of nodes at depth i is now the number of solutions to $\sum_{j=1}^{i-1} (s_j \times w_j) + s_{i+1} \times w_{i+1} \leq M + \varepsilon$, which is different from $\#_i$. The number of nodes at depth $i + 1$ is the number of solutions to $\sum_{j=1}^{i-1} (s_j \times w_j) + s_{i+1} \times w_{i+1} + s_i \times w_i \leq M + \varepsilon$, which is the same as $\#_{i+1}$, and the same holds for larger depths.

Point 2: For any branch defined by a sub-solution $\mathbf{S} = (s_1, \dots, s_{i-1})$, the number of nodes at depth i is $\lfloor (M + \varepsilon)_{i-1}^-[\mathbf{S}] / w_i \rfloor + 1$. This number is smaller for larger values of w_i , and is thus

Algorithm 1 Algorithm DIOPHANTINE. The algorithm stores the stoichiometry vectors in a set Sol . The recursive function takes four arguments: the stoichiometry vector \mathbf{S} under construction, passed by reference as indicated by the ampersand & (C++ convention); the remaining mass $m = M_{i-1}[\mathbf{S}]$ of Eq. (11); the error threshold ε ; and the index $i \in [1, \dots, p]$ of the protein type to be processed by the current recursive call. The initial call is $DIOPHANTINE(\mathbf{S}_0, M + \varepsilon, \varepsilon, 1)$, where \mathbf{S}_0 is a stoichiometry vector whose p entries are set to 0, M is the target mass, and ε is the allowed error. The sub-routine `keep_value` checks whether the stoichiometry j of the i th protein is admissible—as one may impose a lower and/or upper bound on the stoichiometry of that type.

```
DIOPHANTINE(Stoi_vector & S, unsigned m, unsigned  $\varepsilon$ , unsigned i)
```

```
// Stop the recursion if we are on the last protein type
```

```
if  $i == p$  then
```

```
     $q_{min} = \lceil (m - 2\varepsilon) / w_p \rceil$  //  $p$ -th type: min stoichiometry
```

```
     $q_{max} = \lfloor m / w_p \rfloor$  //  $p$ -th type: max stoichiometry
```

```
    for  $j = q_{min}$  to  $q_{max}$  do
```

```
        if keep_value( $p, j$ ) then
```

```
             $S[p] = j$ 
```

```
            Insert  $\mathbf{S}$  into  $Sol$ 
```

```
    // Recurse to set the remaining stoichiometries
```

```
else
```

```
     $quotient = \lfloor m / w_i \rfloor$  //  $i$ -th type: max stoichiometry
```

```
    for  $j = 0$  to  $quotient$  do
```

```
        if keep_value( $i, j$ ) then
```

```
             $S[i] = j$  //Set the  $i$ -th stoichiometry
```

```
            DIOPHANTINE( $\mathbf{S}, m - (j w_i), \varepsilon, i + 1$ ) // Recursion
```

minimum if we reorder w_i and w_{i+1} so that $w_i \geq w_{i+1}$

Point 3: The optimal weights reordering is then by decreasing order of weights (i.e. when $w_1 \geq w_2 \geq \dots \geq w_p$). This is proved easily by remarking that using any other weights reordering suppose that there exist a i for which $w_i < w_{i+1}$, implying that the number of nodes in the corresponding exploration trees can be decreased by swapping protein types i and $i + 1$. \square

3.2 Enumeration Based on Dynamic Programming

The classical solution to the money changing problem based on dynamic programming is well known [BL05a]. It uses a binary table stating whether the target mass is decomposable using a subset of proteins, this table being used by the backtracking algorithm reporting all solutions. In the sequel, we show that a slight modification of the construction of the binary table based on Eq. (15) helps in accommodating the interval case. The enumeration algorithm using this table shall be denoted DP++, and is called as DP++($M + \varepsilon, p$).

Assume that the weights w_i are sorted by increasing value. Along the course of the backtracking, denote m the mass remaining from $M + \varepsilon$ once a set of protein instances have been used. That is, given a the stoichiometry vector \mathbf{S} , one has $m = M + \varepsilon - \sum_{s_i \in \mathbf{S}} s_i w_i$. We wish to build a binary table whose semantics is the following:

$$B[m, i] = 1 \quad \Leftrightarrow \quad \exists \text{ a mass } x \text{ in the interval } [max(0, m - 2\varepsilon), \dots, m] \\ \text{such that } x \text{ is decomposable over } \{w_1, w_2, \dots, w_i\}.$$

Denote $\#s_1$ the number of non-negative integer values satisfying $\lceil (m - 2\varepsilon)/w_1 \rceil \leq s_1 \leq \lfloor m/w_1 \rfloor$. For $i = 1, \dots, M + \varepsilon$ and $i = 1, \dots, p$, the binary table $B[i, m]$ as follows:

$$\text{For } i = 1 : B[1, m] = 1 \text{ if } \#s_1 \geq 0, \text{ and } 0 \text{ otherwise} \quad (20)$$

$$\text{For } i > 1 : B[i, m] = \begin{cases} B[i - 1, m] & \text{if } m < a_i \\ B[i - 1, m] \vee B[i, m - a_i] & \text{otherwise.} \end{cases} \quad (21)$$

DP++ consists of backtracking as usual using this binary table. DP++ is also output sensitive, a property inherited from the original algorithm. The number of backtracking steps for each solution being equal to $(M + \varepsilon)/w_1$ in the worst case, if $D(M, \varepsilon)$ denotes the total number of solutions, the enumeration runs in time $O((M + \varepsilon) D(M, \varepsilon)/w_1)$.

4 Material and Methods

In this section, we sketch the software developed, and introduce several datasets used in our experiments, namely **Data-Bio** (10 instances), **Data-Pseudo-Bio10** (200 instances), **Data-Prime10** (200 instances).

4.1 Implementation sketch

Algorithms. Four algorithms were implemented in (generic) C++, and are made available within the program ADDICT, available from <http://team.inria.fr/abs/addict/>:

- the algorithms DIOPHANTINE and DP++ from section 3 to solve the interval SD;

- the algorithm of [BL05a] to solve SSP, called **DECOMP**. Recall that this algorithm has a pre-processing step consisting of computing the so-called *extended residue table* (ERT), followed by the backtracking step which decomposes a particular mass. To solve the interval SD problem, we compute the ERT once, and call the decomposition procedure for every mass in the interval.
- the FPTAS of [GKM⁺] to estimate the number of solutions of any knapsack problem;
- the algorithm of [BL05b] to compute Frobenius numbers.

To avoid overflows, unsigned integers coded on 64 rather than 32 bits were used. Such number types respectively accommodate integers up to $2^{64} - 1 \sim 0.18 \times 10^{20}$ and $2^{32} - 1 \sim 0.42 \times 10^{10}$, and instances with more than 10^{10} solutions are commonly faced—see Experiments. We note that on Linux operating systems, the type `unsigned long long` is always coded on 8 byte, be the system 32 or 64 bits, see e.g. <http://en.cppreference.com/w/cpp/language/types>.

Running times. Practically, the experiments were conducted on a DELL PRECISION T7400 computer equipped with 8Go of RAM, running Fedora Core 14. For all programs, a cut-time of three hours was set, in order to handle the case of enumeration problems yielding an astronomical number of solutions. For such cases, we estimated the number of solutions using the FPTAS strategy of [GKM⁺]. In fact, whenever the 3 hours time limit was hit, we obtained a lower bound on the number of solutions by taking the maximum value of the number of solutions computed by DIOPHANTINE upon hitting the cut-time, and the lower bound computed from the FPTAS.

4.2 Biological Dataset

In the following, we briefly present the 10 biological complexes processed, whose masses span the range 321,274 – 5,276,467 Da (Table 1). This table lists experimental molecular weights, except for NPC (Y-ring and single spoke), where theoretical molecular weights were computed from the known stoichiometries. The reader is referred to the supplemental section 8.1 for more details.

Yeast 19S Proteasome lid. Proteasomes are protein assemblies involved in the elimination of damaged or misfolded proteins, and the degradation of short-lived regulatory proteins. The most common form of proteasome is the 26S, which involves two filtering caps (the 19S), each cap involving a peripheral lid, composed of 9 distinct protein types each with unit stoichiometry [STA⁺06].

COP9 Signalosome. The COP9 Signalosome is a multi-functional complex primarily involved in ubiquitin mediated proteolysis linked to diverse cellular activities such as signal transduction, cell cycle progression and transcriptional regulation. It is composed of 8 protein protein types each with unit stoichiometry.

Eukaryotic Translation factor EIF3. Eukaryotic initiation factors (eIF) are proteins involved in the initiation phase of the eukaryotic translation. They form a complex with the 40S ribosomal subunit, initiating the ribosomal scanning of mRNA. Among them, EIF3 consists of 13 different protein types each with unit stoichiometry.

Yeast Exosome. The exosome is a 3'-5' exonuclease complex involved in RNA processing and degradation. The yeast exosome is composed of 10 different protein types with unit stoichiometry [HDT⁺06].

Rotary ATPases. Rotary ATPases are membrane associated molecular machines involved in energy conversion by coupling ATP hydrolysis (or synthesis) with proton (or Na^+) translocation across biological membranes. Practically, we investigate the intact TtATPase, and four sub-complexes of EhATPase.

Yeast Nuclear Pore Complex (NPC). The NPC, which is the largest protein assembly known to date in the eukaryotic cell [WR10, DH08], is a protein assembly anchored in the nuclear envelope, regulating the nucleo-cytoplasmic transport. It is composed of ~ 30 distinct proteins types each present in multiple copies. It has eight-fold radial symmetry and consists of eight spokes, each containing 57 protein instances. One particular complex, the *Y*-complex, involving 7 protein types with unit stoichiometry, is present in two sets of 8 copies. Each such set presumably forms one ring (the *Y*-ring) involving 56 protein instances [FMPS⁺12, DDC12].

4.3 Artificial Datasets

Prime numbers instances: the Data-Prime10 dataset. The use of prime number weights prevents relative multiplicity of individual weights, and therefore avoids outright hardness of the instance stemming from so-called spanner sets [Pis05]. To generate the k th synthetic complex, with $k \in 1, \dots, 10$, p prime numbers were chosen randomly from the list of prime numbers from 7,000 to 70,000, in accordance with the weights (in Daltons) of individual proteins involved in the biological complexes of Data-Bio. Moreover, 20 instances for each k th weight vector (W_k) were generated by using 20 target masses uniformly spaced in the range $[\sum w_i, 2g_0^+(\mathbf{W}_k)]$. These 200 instances are referred to as Data-Prime10-i-j.

Random biological instances: the Data-Pseudo-Bio10 dataset. To generate synthetic complexes using the masses of real proteins, we replaced the set of prime numbers by the masses of ~ 6700 YEAST proteins retrieved from the non-redundant UNIPROT protein database, see <http://www.uniprot.org/help/about>. The theoretical molecular weight of proteins were calculated by adding up the weight of constituent amino acids in the proteins. (To be consistent with Data-Prime10, only masses beyond 7 kDa were retained.) As above, 20 vector of masses were picked, yielding 200 instances denoted Data-Pseudo-Bio10-i-j.

5 Experimental Results

The following comments are in order. First, since in structural proteomics, the protein types involved in a given protein complex can generally be determined by mass spectrometry—using denaturing conditions, Table 1 is about positive solutions. But all the remaining experiments have been conducted with non negative solutions. Second, when three series of numbers are reported, they refer to the three datasets Data-Bio, Data-Pseudo-Bio10 and Data-Prime10, enumerated in this order. Third, three error levels typical in structural proteomics were used, namely 0%, 0.1% and 1% — the value ε of Eq. (2) being set to the error level times the target mass. Fourth, the median of a list L of values, i.e. a list of ratios of running times, say $L = \{t_{\text{DECOMP}}/t_{\text{DIO}}\}$, is denoted $\text{median}(L)$.

5.1 Biological Examples: Enumeration Matters Even at Null Noise Level

The formula recalled in Eq. (4) shows an asymptotic polynomial growth of the number of solutions, yet, are situations with multiple solutions commonplace in biology? Table 1 answers this question positively, with multiple solutions even at null noise level in some cases. The case of the NPC is interesting. The case of one full spoke shows that the stoichiometry determination problem in this case is ill-posed, with an astronomical number of solutions. The same holds for the NPC-Y-ring system, which consists of 8 copies of the Y-complex, since a total of 788 solutions are obtained.

The last column of this table also shows that a large value M/g_0^+ is a good hint at a large number of solutions.

5.2 Counting Solutions and Convergence to the Denumerant

Rationale. As noticed in section 2, counting solutions ahead of computing them is of interest to avoid generating a large number of (useless) solutions. We compared the accuracy of the estimations provided in [Pad71, Bd72, Lam74] against those of the FPTAS algorithm of [GKM⁺].

One comment is also in order about *hard instances*, i.e. instances such that DIOPHANTINE does not terminate within a prescribed time limit—three hours in this work. According to [Pis05], two parameters lead to hard UKP and SSP instances: the magnitude of weights, and the linear redundancy between the weights. Intuitively, if a given weight is a linear combination of other weights, additional solutions can be generated. However, beyond the Frobenius number and in the asymptotic regime, the number of solutions of SSP is given by Eq. 4. By taking a large enough mass, one is therefore guaranteed to find instances with a number of solutions precluding execution in any fixed finite time. Therefore, instead of using a target mass proportional to the sum of masses, as in [Pis05], we systematically explored ranges of target masses expressed in units of Frobenius number.

Results: counting solutions. The biological examples just discussed show that estimating the number of solutions is important to detect ill posed problems.

Table 2 compares various strategies to estimate the number of solutions to a SD problem. First, we plugged the upper and lower bounds provided by [Pad71], [Bd72], and [Lam74] into Eqs. (9-10). For each biological system, we retained the best bounds obtained—those yielding the tighter interval (Table 2, columns 2-4). Second, we also computed estimates using the FPTAS of [GKM⁺] (Table 2, columns 5-7).

On the one hand, the combinatorial bounds are not of real interest, since at least two orders of magnitude of difference between $\#UKP(p, \mathbf{W}, M)$ and $\overline{\#UKP}(p, \mathbf{W}, M)$, yielding a null lower bound. On the other hand, the bounds from the FPTAS are much tighter.

Results: convergence towards the denumerant. Due to the rapid rise of the number of solutions, an interesting problem is the speed of the convergence of the number of solutions of a SD problem to the denumerant.

To study this convergence, a toy dataset called **Data-Quad4** consisting of the quadruplet $\{10, 15, 32, 48\}$ from [SS11] was used. As for the three main datasets, a total of 100 instances uniformly distributed in the range $[\sum w_i, 20g_0^+]$ was used, the j th instance being denoted **Data-Quad4**— j .

Such results are presented on a toy system and on a real biological instance on Fig. 2 and 3, the target mass being expressed in unites of the Frobenius numbers. In both cases, a fast

convergence is observed.

5.3 Running Times: DECOMP, DIOPHANTINE, DP++

Rationale. To compare the three running times t_{DIO} , t_{DECOMP} , $t_{\text{DP++}}$, we processed our three datasets at the noise levels 0%, 0.1%, and 1% (Fig. 4).

Results. While DIOPHANTINE and DP++ completed all instances within the imparted time, regardless of the noise level, on a per-dataset basis, DECOMP finished on 10/10, 200/200, 200/200 cases at zero noise level, 9/10, 104/200, 93/200 cases at 0.1% noise level, and 8/10, 56/200, 51/200 cases at 1% noise level.

At null noise level, one gets $\text{median}(\{t_{\text{DECOMP}}/t_{\text{DIO}}\}) = 1.4$, $\text{median}(\{t_{\text{DIO}}/t_{\text{DP++}}\}) = 13.66$, $\text{median}(\{t_{\text{DECOMP}}/t_{\text{DP++}}\}) = 16.02$. That is, DP++ is faster than DECOMP and DIOPHANTINE by about one order of magnitude.

At 0.1% and 1% noise level, DECOMP is not competitive anymore, since it is outperformed by three to four orders of magnitude. Clearly, the wider the interval $[M - \varepsilon, \dots, M + \varepsilon]$, the more challenging the instances for DECOMP. This owes to the fact that DIOPHANTINE and DP++ make a single call with target mass $M + \varepsilon$, while DECOMP runs the exact algorithm on every mass in $[M - \varepsilon, \dots, M + \varepsilon]$. In presence of noise, the two competitors are thus DIOPHANTINE and DP++. At 0.1% and 1% respectively, one has $\text{median}(\{t_{\text{DP++}}/t_{\text{DIO}}\}) = 1.07$, $\text{median}(\{t_{\text{DP++}}/t_{\text{DIO}}\}) = 1.2$. More interestingly, DIOPHANTINE is never twice slower than DP++, while in the worst case, DP++ is 17 (resp. 20) times slower than DIOPHANTINE at 0.1% (resp. 1%). Thus, DIOPHANTINE is more stable. We also notice that asymptotically when the target mass increases, the ratio of running times converges to one, hinting at an output sensitive behavior of DIOPHANTINE—recall that DP++ is output sensitive.

5.4 Output sensitivity of DIOPHANTINE

Rationale. An output sensitive algorithm performs a number of operations depending upon the output size. In the exact case, DIOPHANTINE can only be output sensitive for a target mass beyond the Frobenius number, since any call with a non representable mass necessarily results in useless operations.

Results: running time versus number of non-negative solutions. We first computed the Pearson correlation between t_{DIO} and the number of solutions (Fig. 5) With values equal to 0.97, 0.89 and 0.97 for the three datasets at 0.1% noise level, and to 0.97, 0.89 and 0.97 at 1% noise level, this correlation is excellent.

Results: running time versus the recursion tree size. The correlation between t_{DIO} and the recursion tree size is even better (Fig. 6), with Pearson correlation coefficients of 0.99, 0.99 and 0.99 at 0.1% noise level, and of 0.99, 0.95 and 0.98 at 1% noise level.

We further plotted the proportion of fertile edges as a function of the target mass (Fig. 7). Note that this proportion is 1 when the condition of Eq. (19) holds, which is the case for 0/410 and 338/410 instances at 0.1% and 1% respectively. As expected, this proportion increases with the error level: at 1%, all the instances which do not meet the condition of Eq. (19) have a ratio above 0.6, while no instance has such a high ratio at 0.1%.

Results: on the expected size of the recursion tree. One can also compare the recursion tree size against the expected size given that the instance processed has say Δ solutions. More precisely, the smallest and largest recursion trees yielding Δ solutions without any sterile leaf respectively have sizes

$$e_{min} = p - 1 + \Delta, \text{ and } e_{max} = p \times \Delta. \quad (22)$$

Checking whether the recursion tree size lies in the interval $[e_{min}, e_{max}]$ is thus of interest, although this criterion does not guarantee that the tree does not have any sterile leaf.

At 1% noise level, it actually turns out that the criterion of Eq. (22) is satisfied on nearly all instances processed: 10/10 for **Data-Bio**, 200/200 for **Data-Pseudo-Bio10**, 200/200 for **Data-Prime10** and 96/100 for **Data-Quad4** (see also Fig. 8).

6 Conclusion and Outlook

Mass spectrometry is playing an increasing role to investigate large macro-molecular assemblies, thus complementing more classical techniques such as X ray crystallography or nuclear magnetic resonance, which are better suited for smaller complexes. In this context, the stoichiometry determination (SD) problem, which consists of determining how many copies of each sub-unit are required to account for the observed mass, is the first one to be addressed, before investigating the geometry of the contacts between these sub-units. In this work, we develop a constant memory space enumeration algorithm (**DIOPHANTINE**), and an output sensitive dynamic programming based algorithm (**DP++**), which are shown to outperform state-of-the-art SD algorithms by several (three to four) orders of magnitude. These two algorithms exhibit comparable performances for typical noise levels in the range 0.1% to 1%, which is remarkable since **DIOPHANTINE** does not use any pre-processing and has constant memory footprint. Both algorithms performed to satisfaction on all the biological cases processed, which are the most challenging ones currently investigated in structural biology. It is noticeable that enumeration does matter, since systems with numerous solutions are observed at a moderate (sometimes even null) noise level. Also, the coupling between **DIOPHANTINE** (or **DP++**) and the fully polynomial-time approximation scheme (**FPTAS**) of **[GKM⁺]** provides a powerful way to get a lower bound on the number of solutions, and thus to identify ill-posed SD problems—which admit a too large number of solutions.

From a theoretical standpoint, two outstanding questions remain open. The first one is related to the output sensitivity of **DIOPHANTINE**, which is observed in practice, but cannot be guaranteed in general. Getting finer insights on this phenomenon may allow developing more efficient enumeration schemes—by pruning sterile portions of the recursion tree. The second one is reminiscent from phase transitions, a well known phenomenon for selected hard optimization problems—such as 3-SAT for example. While the SD problem is about enumeration rather than optimization, linear redundancies within protein masses (one can trade a set of proteins against another set) have a direct impact on the rise of the number of solutions. A challenge is therefore to gain insights on the role of linear relationships and the total number of solutions.

References

- [ACL⁺02] P. Aloy, F.D. Ciccarelli, C. Leutwein, A.C. Gavin, G. Superti-Furga, P. Bork, B. Böttcher, and R.B. Russell. A complex prediction: three-dimensional model of the yeast exosome. *EMBO reports*, 3(7):628–635, 2002.
- [Agn02] G. Agnarsson. On the sylvester denumerants for general restricted partitions. *Congressus numerantium*, pages 49–60, 2002.

- [Alf05] J.L. Ramírez Alfonsín. *The Diophantine Frobenius Problem*. Oxford University Press, 2005.
- [Bd72] A.G. Begeed-dov. Lower and upper bounds for the number of lattice points in a simplex. *SIAM Journal on Applied Mathematics*, 22(1):106–108, 1972.
- [BDF⁺10] A. Brown, E. Dannenberg, J. Fox, J. Hanna, K. Keck, A. Moore, Z. Robbins, B. Samples, and J. Stankewicz. On a generalization of the frobenius number. *Journal of Integer Sequences*, 13(2):3, 2010.
- [Bel43] E.T. Bell. Interpolated denumerants and lambert series. *American Journal of Mathematics*, 65(3):382–386, 1943.
- [Bel57] R.E. Bellman. *Dynamic Programming*. Princeton University Press, 1957.
- [BL05a] S. Bocker and Z. Liptak. Efficient mass decomposition. In ACM, editor, *ACM Symposium on Applied Computing*, 2005.
- [BL05b] S. Böcker and Z. Lipták. The money changing problem revisited: Computing the frobenius number in time $o(ka1)^*$. In Lusheng Wang, editor, *Computing and Combinatorics*, volume 3595 of *Lecture Notes in Computer Science*, pages 965–974. Springer Berlin / Heidelberg, 2005.
- [BR11] J.L.P. Benesch and B.T. Ruotolo. Mass spectrometry: come of age for structural and dynamical biology. *Current opinion in structural biology*, 21:641–649, 2011.
- [Com74] L. Comtet. *Advanced Combinatorics: The art of finite and infinite expansions*. Not Avail, 1974.
- [DDC12] T. Dreyfus, V. Doye, and F. Cazals. Assessing the reconstruction of macro-molecular assemblies with tolerated models. *Proteins: structure, function, and bioinformatics*, 80(9):2125–2136, 2012.
- [DFZ⁺07] E. Damoc, C.S. Fraser, M. Zhou, H. Videler, G.L. Mayeur, J.W.B. Hershey, J.A. Doudna, C.V. Robinson, and J.A. Leary. Structural characterization of the human eukaryotic initiation factor 3 protein complex by mass spectrometry. *Molecular & Cellular Proteomics*, 6(7):1135–1146, 2007.
- [DH08] M.A. D’Angelo and M.W. Hetzer. Structure, Dynamics and Function of Nuclear Pore Complexes. *Trends Cell Biology*, 18:456–522, 2008.
- [ea01] O. Puig et al. The tandem affinity purification method: A general procedure of protein complex purification. *Methods*, 24:218–229, 2001.
- [FMPS⁺12] J. Fernandez-Martinez, J. Phillips, M.D. Sekedat, R. Diaz-Avalos, J. Velazquez-Muriel, J.D. Franke, R. Williams, D.L. Stokes, B.T. Chait, A. Sali, and M.P. Rout. Structure–function mapping of a heptameric module in the nuclear pore complex. *The Journal of Cell Biology*, 2012.
- [FS09] P. Flajolet and R. Sedgewick. *Analytic combinatorics*. Cambridge Univ Pr, 2009.
- [GKM⁺] P. Gopalan, A. Klivans, R. Meka, D. Stefankovic, S. Vempala, and E. Vigoda. An FPTAS for # knapsack and related counting problems. In *IEEE Foundations of Computer Science*.

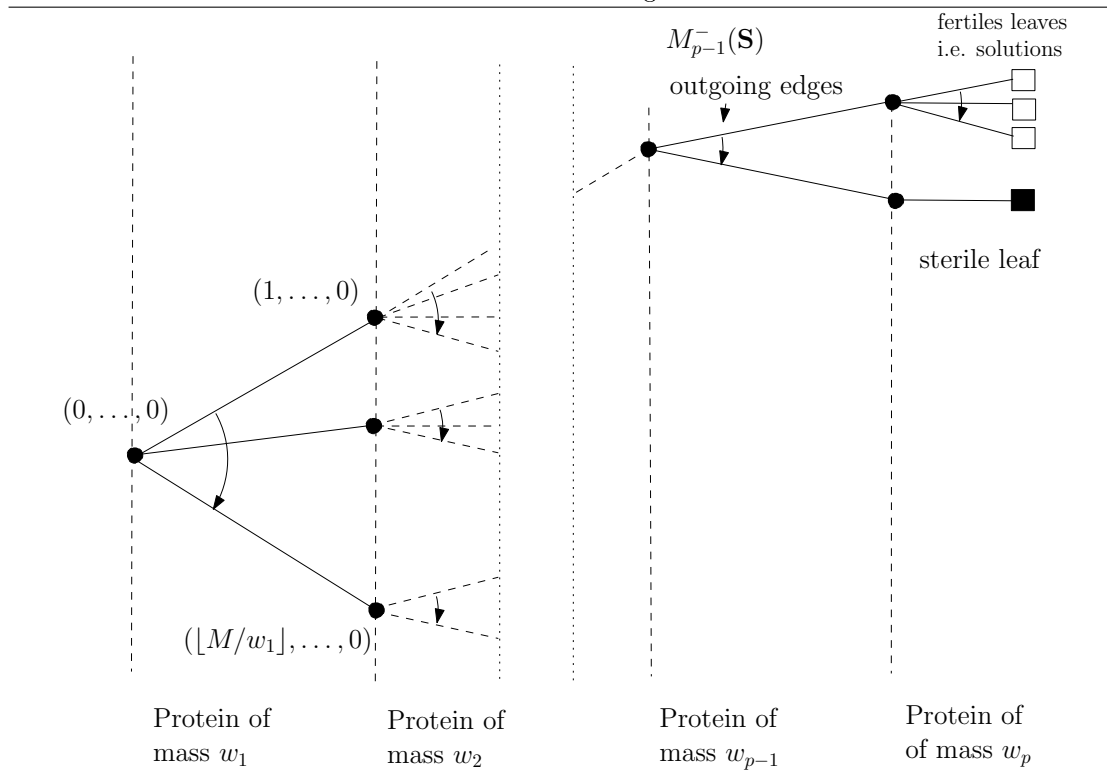
- [HDT⁺06] H. Hernández, A. Dziembowski, T. Taverner, B. Séraphin, and C.V. Robinson. Subunit architecture of multimeric complexes isolated directly from cells. *EMBO reports*, 7(6):605–610, 2006.
- [Jan07] J. Janin. structural genomics: winning the second half of the game. *Structure*, 15:1347–1349, 2007.
- [JHP10] R.J. Jackson, C.U.T. Hellen, and T.V. Pestova. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nature Reviews Molecular Cell Biology*, 11(2):113–127, 2010.
- [Kar72] R.M. Karp. Reducibility among combinatorial problems. *Complexity of Computer Computations.*, 6:85–103, 06 1972.
- [Kel04] N.L. Kelleher. Peer reviewed: Top-down proteomics. *Analytical chemistry*, 76(11):196–203, 2004.
- [Lam74] T.A. Lambe. Bounds on the number of feasible solutions to a knapsack problem. *SIAM Journal on Applied Mathematics*, 26(2):302–305, 1974.
- [MHFH10] R. Mahmoudvand, H. Hassani, A. Farzaneh, and G. Howell. The exact number of nonnegative integer solutions for a linear diophantine inequality. *IAENG International Journal of Applied Mathematics*, 40(1), 2010.
- [MR12] N. Morgner and C.V. Robinson. Massign: An assignment strategy for maximising information from the mass spectra of heterogeneous protein assemblies. *Analytical Chemistry*, 84(6):2939–2948, 2012.
- [Pad71] M.W. Padberg. A remark on “an inequality for the number of lattice points in a simplex”. *SIAM Journal on Applied Mathematics*, 20(4):638–641, 1971.
- [Pis05] D. Pisinger. Where are the hard knapsack problems? *Computers and Operations research*, 32:2271–2284, 2005.
- [PKP04] U. Pferschy, H. Kellerer, and D. Pisinger. *Knapsack Problems*. Springer, 2004.
- [PYA09] V. Poirriez, N. Yanev, and R. Andonov. A hybrid algorithm for the unbounded knapsack problem. *Discrete Optimization*, 6(1):110 – 124, 2009.
- [RA96] J. L. Ramírez-Alfonsín. Complexity of the frobenius problem. *Combinatorica*, 16:143–147, 1996. 10.1007/BF01300131.
- [Sma98] N.P. Smart. *The algorithmic resolution of Diophantine equations*, volume 41. Cambridge Univ Pr, 1998.
- [SMBE⁺09] M. Sharon, H. Mao, E. Boeri Erba, E. Stephens, N. Zheng, and C.V. Robinson. Symmetrical modularity of the cop9 signalosome complex suggests its multifunctionality. *Structure*, 17(1):31–40, 2009.
- [SR07] M. Sharon and C.V. Robinson. The role of mass spectrometry in structure elucidation of dynamic protein complexes. *Annu. Rev. Biochem.*, 76:167–193, 2007.
- [SS11] J. Shallit and J. Stankewicz. Unbounded discrepancy in frobenius numbers. *Integers*, 11:1–8, 2011.

- [STA⁺06] M. Sharon, T. Taverner, X.I. Ambroggio, R.J. Deshaies, and C.V. Robinson. Structural organization of the 19s proteasome lid: insights from ms of intact complexes. *PLoS biology*, 4(8):e267, 2006.
- [THS⁺08] T. Taverner, H. Hernández, M. Sharon, B.T. Ruotolo, D. Matak-Vinkovic, D. Devos, R.B. Russell, and C.V. Robinson. Subunit architecture of intact protein complexes from mass spectrometry and homology modeling. *Accounts of chemical research*, 41(5):617–627, 2008.
- [WR10] S.R. Wentz and M.P. Rout. The Nuclear Pore Complex and Nuclear Transport. *Colde Spring Harbor Perspectives in Biology*, 2(10):a000562, 2010.
- [ZMB⁺11] M. Zhou, N. Morgner, N.P. Barrera, A. Politis, S.C. Isaacson, D. Matak-Vinković, T. Murata, R.A. Bernal, D. Stock, and C.V. Robinson. Mass spectrometry of intact v-type atpases reveals bound lipids and the effects of nucleotide binding. *Science*, 334(6054):380–385, 2011.
- [ZSF⁺08] Min Zhou, Alan M. Sandercock, Christopher S. Fraser, Gabriela Ridlova, Elaine Stephens, Matthew R. Schenauer, Theresa Yokoi-Fong, Daniel Barsky, Julie A. Leary, John W. Hershey, Jennifer A. Doudna, and Carol V. Robinson. Mass spectrometry reveals modularity and a complete subunit interaction map of the eukaryotic translation factor eif3. *Proceedings of the National Academy of Sciences*, 105(47):18139–18144, 2008.

7 Artwork

7.1 Algorithms

Figure 1 The recursion tree explored by algorithm DIOPHANTINE. A leaf is called fertile if Eq. (17) has at least one solution, and sterile otherwise. Similarly, any edge of the tree is termed fertile if at least one fertile leaf is found downstream the tree, and sterile otherwise. The size of the recursion tree is defined as its number of edges.



7.2 Stoichiometry Determination for Biological Examples: Enumeration Matters

Table 1 Biological instances can lead to multiple positive solutions, even with null noise level. For the NPC-1-spoke, lower and upper bounds were obtained using UKP-FPTAS of [GKM⁺] with an error factor of $\delta = 0.1$.

Complex	#sol, 0% noise	#sol, 0.1% noise	#sol, 1% noise	M (Da)	g_0^+	#sol(1% noise)/2 ϵ	M/g_0^+
COP9	0	1	1	321,274 \pm 35	961,855	3.12×10^{-4}	0.33
Y-19S-lid	0	0	1	376,151 \pm 369	921,712	1.87×10^{-4}	0.41
EhATPase-sub-5	0	4	39	387,356 \pm 230	682,901	5.03×10^{-3}	0.57
Y-exosome	0	13	149	402,708 \pm 68	649,185	1.85×10^{-2}	0.62
EhATPase-sub-4	0	23	203	424,441 \pm 148	682,901	2.38×10^{-2}	0.62
EhATPase-sub-3	0	77	751	461,674 \pm 324	682,901	8.10×10^{-2}	0.68
EhATPase-sub-2	0	234	2,333	500,178 \pm 294	682,901	2.32×10^{-1}	0.73
TtATPase	21	24,487	246,242	659,202 \pm 131	607,304	18.7	1.08
EIF3	0	0	1	797,999 \pm 180	1,257,629	8.05×10^{-5}	0.63
NPC-Y-ring	788	6,900,664	69,042,257	4,603,280	2,282,543	750	2.02
NPC-1-spoke	[1.72×10^{16} , 1.73×10^{16}]	[1.72×10^{16} , 4.77×10^{16}]	[2.71×10^{17} , 3.44×10^{17}]	5,276,467	3,169,210	$\geq 2.57 \times 10^{12}$	1.66

7.3 Counting Solutions and Convergence to the Denumerant

Table 2 Estimating the number of positive solutions for five biological systems. Columns 2 to 4: upper and lower bounds obtained by plugging the upper and lower bounds of [Pad71], [Bd72], and [Lam74] into Eqs. (9-10). Columns 5 to 7: upper and lower bounds on the number of positive solutions obtained using the FPTAS of [GKM⁺], with $\delta = 0.1$.

Complex	# solutions	$M \pm 1\%$		$M \pm 1\%$	
		#SD	#SD	#SD	#SD
COP9	1	0	8	1	1
Y19-lid	1	0	10	1	1
Y-exosome	149	0	486	101	183
EIF3	1	0	14	1	1
NPC-1-Spoke	Unknown	0	1.06×10^{22}	2.71×10^{17}	3.44×10^{17}

Figure 2 Number of non-negative solutions vs. asymptotic behavior of the denumerant (Eq. (4)) for the quadruplet $W = \{10, 15, 32, 48\}$. The target mass is expressed in units of the Frobenius number.

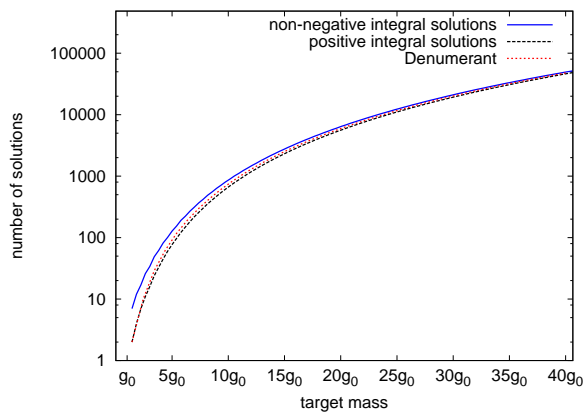
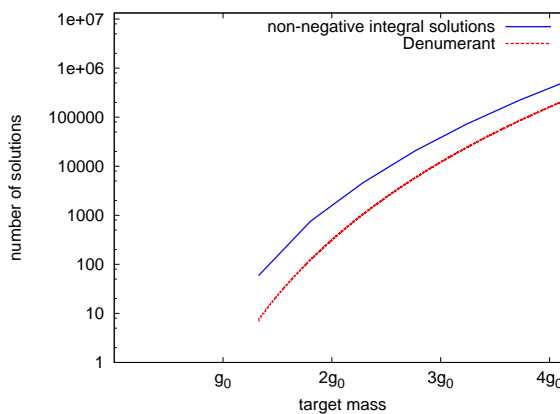


Figure 3 Number of non-negative solutions vs. asymptotic behavior of the denumerant (Eq. (4)) for Yeast Exosome. The target mass is expressed in units of the Frobenius number. Instances with a target mass beyond $5g_0$ did not terminate within the time limit.



7.4 Output sensitivity Analysis of Algorithm DIOPHANTINE

Figure 5 Running time t_{DIO} as a function of the number of solutions (Left) Noise level of 0.1% (Right) Noise level of 1%

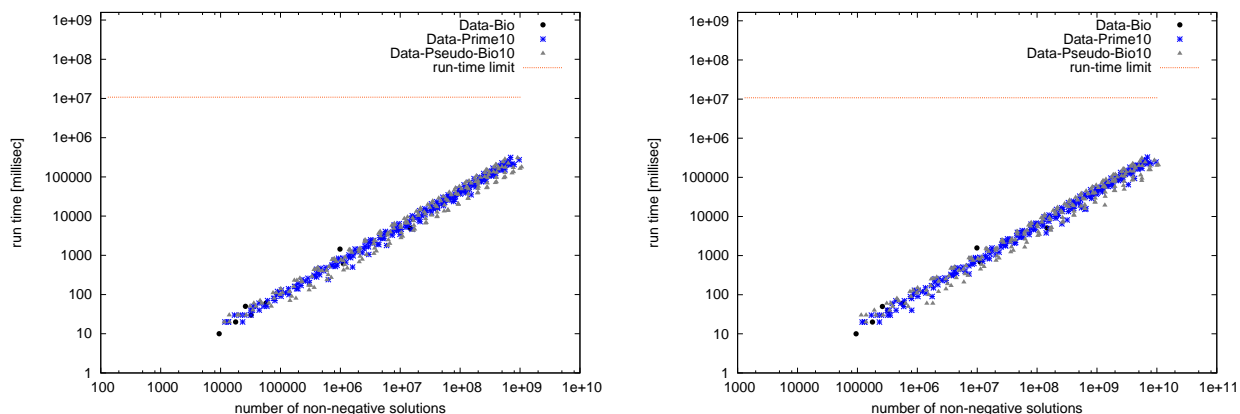


Figure 4 Running time as a function of the target mass at different noise levels: DIOPHANTINE vs. DECOMP vs. DP++. The 3 rows respectively correspond to the noise levels 0%, 0.1%, and 1%. Left column: comparing t_{DIO} and t_{DECOMP} Right column: comparing t_{DIO} and $t_{\text{DP++}}$ (y -axis: log scale).

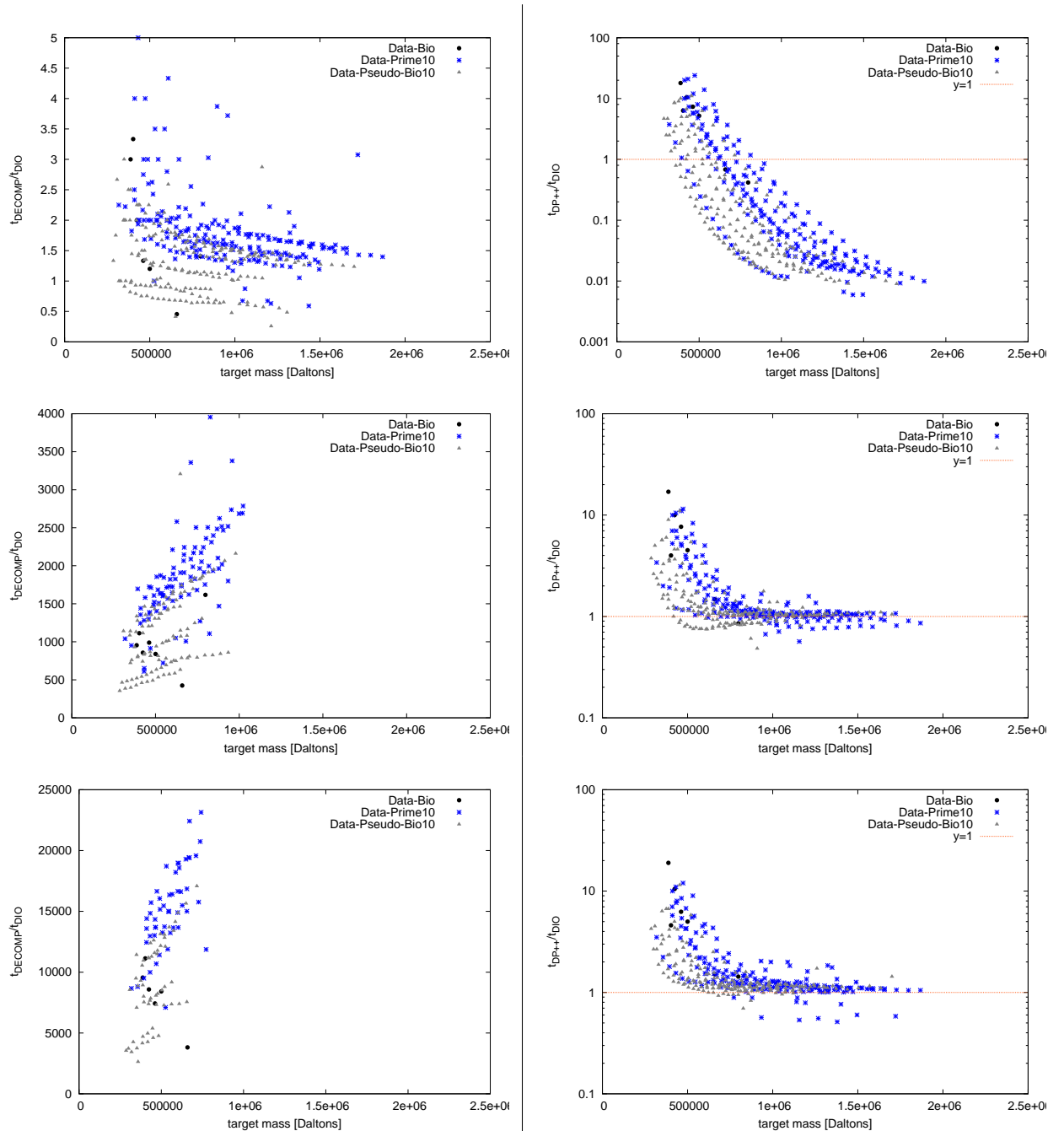


Figure 6 Running time t_{D10} as a function of the recursion tree size (Left) Noise level of 0.1% (Right) Noise level of 1%

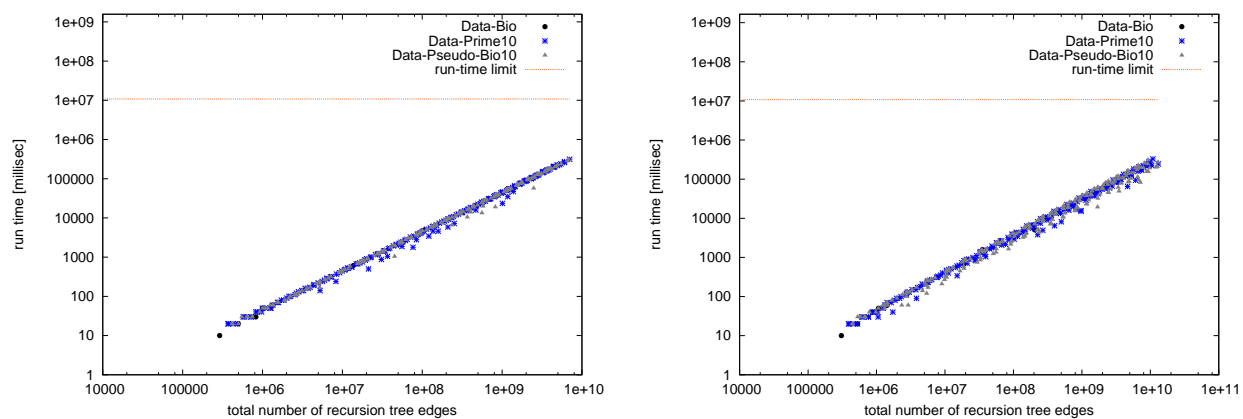


Figure 7 Fraction of fertile edges at three noise levels, 0% (Top-Left), 0.1% (Top-Right), 1% (Bottom-Left). Last figure pertains to those instances with 1% noise, which do not meet with the output sensitivity criterion stated in Eq. (19).

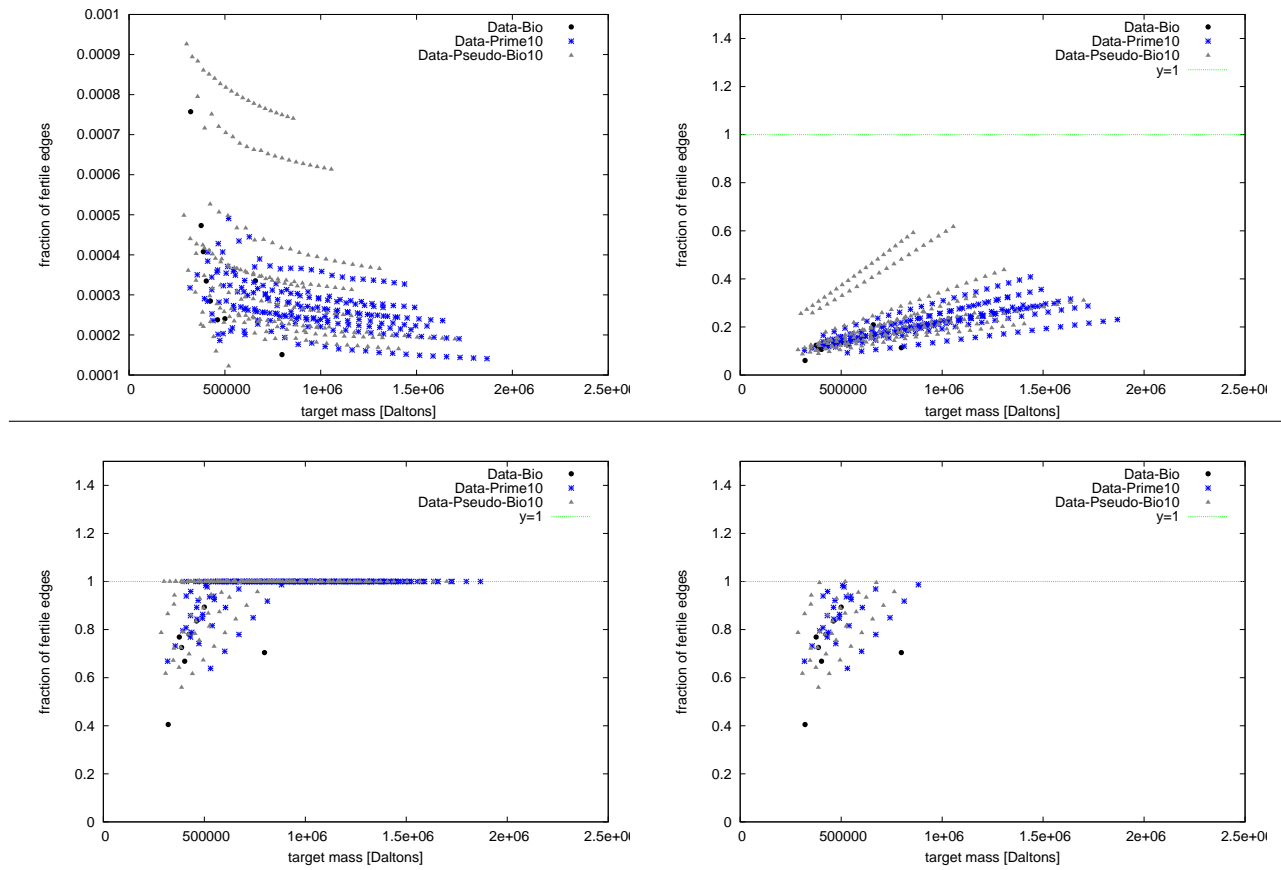
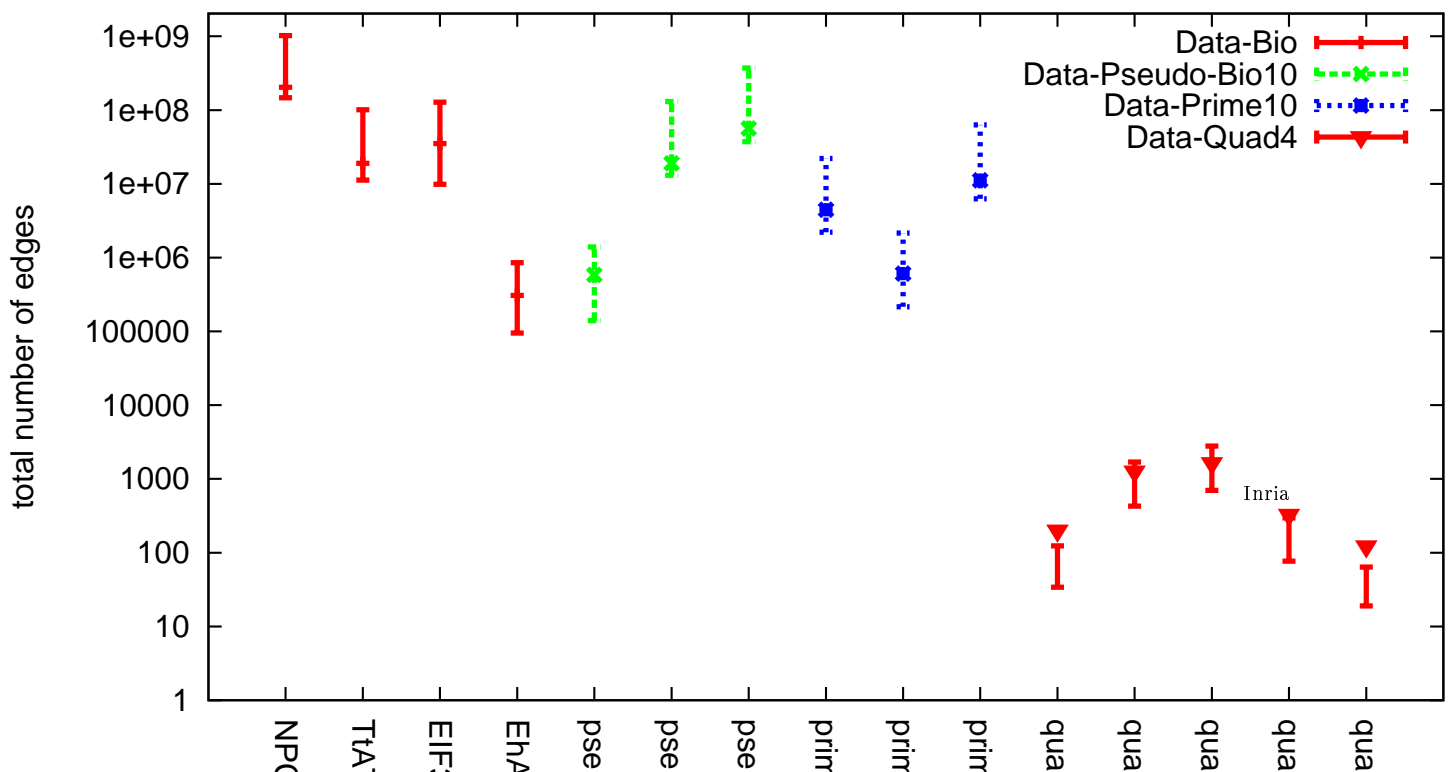


Figure 8 Number of edges in the Diophantine tree versus the upper and lower bounds of Eq. (22). Note: all cases are concerned with non negative solutions, even the Bio ones.



8 Supporting Information

8.1 Detailed Description of Biological Complexes

Yeast 19S Proteasome lid. Proteasomes are protein assemblies involved in elimination of damaged or misfolded proteins and the degradation of short-lived regulatory proteins. They are found in all eukaryotic cells and archaea, and also in selected bacteria. The most common form of the proteasome is 26S, named after its sedimentation coefficient—expressed in Svedberg.

The 26S Proteasome, consists of one core particle corresponding to the degradation chamber (the 20S) and of two regulatory caps filtering the entry of the proteins (the 19S). The 19S sub-complex itself subdivides into two other subcomplexes, the base that binds directly to the 20S core particle and a peripheral lid. The latter is composed of 9 different protein types with a single copy for each type. It is involved in recognition of the polyubiquitin chain of the substrate and followed by its deubiquitination. This substrate is then unfolded and translocated to the core particle for further degradation [STA⁺06].

For yeast, the measured mass of the intact 19S Proteasome lid complex is $376,151 \pm 369$ Da. Summing the theoretical weights of the protein types amounts to 374,576 Da, i.e. the error in the measurement is 0.42%.

COP9 Signalosome. The COP9 Signalosome is a multifunctional complex primarily involved in ubiquitin mediated proteolysis linked to diverse cellular activities such as signal transduction, cell cycle progression and transcriptional regulation. It is composed of 8 protein types with a single copy for each type and shares remarkable homology with Yeast 19S proteasome lid complex.

An Electrospray MS experiment conducted on human COP9 signalosome reconstituted by coexpression in *E. coli* reveals the molecular weight for an intact complex to be $321,274 \pm 35$ Da. Summing the theoretical weights of the protein types amounts to 321,270 Da, i.e. the error in the measurement is 0.0012% [SMBE⁺09].

Eukaryotic Translation factor EIF3. Eukaryotic translation initiation factors (EIFs) are the proteins involved in assembling of elongation competent 80S ribosome to initiate the translation process. There are atleast nine EIFs involved in the initiation process. They carry out their function in two steps: formation of 48S complex with established codon-anticodon base pairing in the P-site of 40S ribosomal subunits, and the joining of 48S complex with 60S subunits [JHP10].

Among them, EIF3 binds to the 40S subunit of the ribosome to initiate protein synthesis followed by recruitment of messenger RNAs. It also promotes attachment of 43S complexes to mRNA. It has 13 different protein types with a single copy for each type. These 13 protein types have been unambiguously identified when MS/MS spectra, and were scanned against UniProt/Swiss-Prot and NCBI nr database using the MASCOT search engine [DFZ⁺07]. The measured mass of the intact Yeast EIF3 complex is $797,999 \pm 180$ Da. Summing the theoretical weights of the protein types amounts to 793,558 Da, i.e. error w.r.t. to the theoretical weights is 0.56% [ZSF⁺08].

Yeast Exosome. The exosome is a 3'-5' exonuclease complex involved in RNA processing and degradation. In eukaryotes it is present in the cytoplasm and nucleolus and therefore reacts with different substrates in respective compartments. It is composed of 10 different protein types each with unit stoichiometry [HDT⁺06]. The yeast exosome complex is known to contain domains

homologous to ribonucleases e.g. RNase PH and RNase II and others, e.g. S1, KH, PINc and HRDC [ACL⁺02].

The measured mass of the intact Yeast exosome complex is $397,860 \pm 99$ Da. Summing the theoretical weights of the protein types amounts to 397,881 Da, i.e. error w.r.t. to the theoretical weights is 0.005%.

Rotary ATPases. Rotary ATPases are membrane associated molecular machines involved in energy conversion by coupling ATP hydrolysis (or synthesis) with proton (or Na⁺) translocation across biological membranes. There are primarily two types of ATPases, F-type and V-type complexes each having two domains F_0, F_1 and V_0, V_1 . The membrane embedded domains F_0/V_0 mediate proton (or Na⁺) translocation and F_1/V_1 are involved in ATP production or consumption respectively.

An electrospray mass spectrum is recorded for rotary ATPases from *E. hirae* (EhATPase) and *E. thermus* (TtATPase). Each of these complexes have nine different protein types but, the membrane embedded rotor for EhATPase is larger because each K type contains four transmembrane helices as compared to two transmembrane helices in corresponding L type in TtATPase.

MS experiment data was retrieved from [MR12]. EhATPase was undergone controlled disassembly resulting in formation of sub-complexes in gas phase and solution phase using collision induced dissociation (CID) and partial denaturation by manipulating the ionic strength, respectively. We choose four sub-complexes – 2,3,4 and 5 formed in the solution phase, and assumed that each subcomplex is composed of all the 9 types of proteins as does the intact complex. Measured molecular weights of subcomplexes and percentage error in measurement are shown in table 3.

Table 3 Measured molecular weights (in Da) and percentage error in measurement w.r.t. weighted sum of the theoretical masses of individual protein types complying with their known stoichiometries. Refer to fig. 6 of [MR12] and Table S1A and S1B of supplementary information of [ZMB⁺11]

Complex	Stoichiometries	Measured Mass (Da)	$\sum s_i w_i^t$	%error in measurement
EhATPase-sub-2	$A_3B_3CDE_2F_2G$	$500,178 \pm 294$	499,131	0.21
EhATPase-sub-3	$A_3B_3DE_2F_2G$	$461,674 \pm 324$	460,968	0.15
EhATPase-sub-4	A_3B_3DEFG	$424,441 \pm 148$	423,813	0.15
EhATPase-sub-5	A_3B_3DG	$387,356 \pm 230$	386,658	0.18
TtATPase	$A_3B_3CDE_2G_2FIL_{12}$	$659,202 \pm 131$	657,979	0.19

Yeast Nuclear Pore Complex (NPC). The NPC is a protein assembly anchored in the nuclear envelope, regulating the nucleo-cytoplasmic transport. It is composed of ~ 30 distinct proteins types each present in multiple copies, and is the largest protein assembly known to date in the eukaryotic cell [WR10, DH08]. It has eight-fold radial symmetry and consists of eight spokes, each with a cytoplasmic and a nuclear side.

While we are not aware of mass spectrometry experiments on the whole NPC, a number of sub-systems have been studied in detail. One of them is an heptameric sub-complex known as the Y-complex [FMPS⁺12, DDC12], which participates to the formation of the scaffold of the NPC, as one finds one copy of the Y-complex per half-spoke.

In the sequel, we simulated mass spectrometry data for two complexes. The first one is a complete spoke, which contains a total of 57 proteins instances of 30 different types. The second

one is the Y-ring complex, containing 8 copies of the Y-complex—that is 56 proteins of 7 types. For each complex, noise levels of 0%, 0.1% and 1% were applied to the exact mass of the complex computed from the masses of the individual proteins.

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