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eLIFE Advances

Beam-induced motion correction for sub-megadalton cryo-EM particles

Sjors HW Scheres

Medical Research Council Laboratory of Molecular Biology, United Kingdom

DOI: http://dx.doi.org/10.7554/eLife.03665
Published August 13, 2014
Cite as eLife 2014;3:e03665

Abstract

In electron cryo-microscopy (cryo-EM), the electron beam that is used for imaging also causes the sample to move. This motion blurs the images and limits the resolution attainable by single-particle analysis. In a previous Research article (Bai et al., 2013) we showed that correcting for this motion by processing movies from fast direct-electron detectors allowed structure determination to near-atomic resolution from 35,000 ribosome particles. In this Research advance article, we show that an improved movie processing algorithm is applicable to a much wider range of specimens. The new algorithm estimates straight movement tracks by considering multiple particles that are close to each other in the field of view, and models the fall-off of high-resolution information content by radiation damage in a dose-dependent manner. Application of the new algorithm to four data sets illustrates its potential for significantly improving cryo-EM structures, even for particles that are smaller than 200 kDa.

DOI: http://dx.doi.org/10.7554/eLife.03665.001

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Builds upon

Ribosome structures to near-atomic resolution from thirty thousand cryo-EM particles

Xiao-chen Bai, Israel S Fernandez, Greg McMullan, Sjors HW Scheres

A combination of direct-electron detectors and statistical movie processing allows ribosome cryo-EM structures to be determined to resolutions that were previously only attainable by X-ray crystallography.

eLife 2013;2:e00461
DOI: 10.7554/eLife.00461

Ribosome structures to near-atomic resolution from thirty thousand cryo-EM particles

Xiao-chen Bai, Israel S Fernandez, Greg McMullan, Sjors HW Scheres

Medical Research Council Laboratory of Molecular Biology, United Kingdom

DOI: http://dx.doi.org/10.7554/eLife.00461
Published February 19, 2013
Cite as eLife 2013;2:e00461

Abstract

Although electron cryo-microscopy (cryo-EM) single-particle analysis has become an important tool for structural biology of large and flexible macro-molecular assemblies, the technique has not yet reached its full potential. Besides fundamental limits imposed by radiation damage, poor detectors and beam-induced sample movement have been shown to degrade attainable resolutions. A new generation of direct electron detectors may ameliorate both effects. Apart from exhibiting improved signal-to-noise performance, these cameras are also fast enough to follow particle movements during electron irradiation. Here, we assess the potentials of this technology for cryo-EM structure determination. Using a newly developed statistical movie processing approach to compensate for beam-induced movement, we show that ribosome reconstructions with unprecedented resolutions may be calculated from almost two orders of magnitude fewer particles than used previously. Therefore, this methodology may expand the scope of high-resolution cryo-EM to a broad range of biological specimens.

DOI: http://dx.doi.org/10.7554/eLife.00461.001

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Built upon by

Beam-induced motion correction for sub-megadalton cryo-EM particles

Sjors HW Scheres

Building on previous work (Bai et al., 2013), we describe an algorithm that allows cryo-EM structure determination to near-atomic resolution for protein complexes as small as 170 kDa.

eLife 2014;3:e03665
DOI: 10.7554/eLife.03665

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Cdc48/p97 promotes degradation of aberrant nascent polypeptides bound to the ribosome

Rati Verma, Robert S Oania, Natalie J Kolawa, Raymond J Deshaies

California Institute of Technology, United States; Howard Hughes Medical Institute, California Institute of Technology, United States

DOI: http://dx.doi.org/10.7554/eLife.00308

[-] Abstract

Ubiquitin-dependent proteolysis can initiate at ribosomes for myriad reasons including misfolding of a nascent chain or stalling of the ribosome during translation of mRNA. Clearance of a stalled complex is required to recycle the ribosome for future use. Here we show that the ubiquitin (Ub) pathway segregase Cdc48/p97 and its adaptors Ufd1-Npl4 participate in ribosome-associated degradation (RAD) by mediating the clearance of ubiquitinated, tRNA-linked nascent peptides from ribosomes. Through characterization of both endogenously-generated and heterologous model substrates for the RAD pathway, we conclude that budding yeast Cdc48 functions downstream of the Ub ligases Ltn1 and Ubr1 to release nascent proteins from the ribosome so that they can be degraded by the proteasome. Defective RAD could contribute to the pathophysiology of human diseases caused by mutations in p97.

DOI: http://dx.doi.org/10.7554/eLife.00308.001

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Decision letter

Ivan Dikic, Reviewing editor, Goethe University, Germany

Thank you for choosing to send your work entitled "Cdc48/p97 promotes degradation of aberrant nascent polypeptides bound to the ribosome" for consideration at *eLife*. Your submission has been evaluated by three reviewers, two of whom are members of our Board of Reviewing Editors, and the decision has been discussed further with one of *eLife*'s Senior Editors. The following reviewer wants to reveal his identity: Roy Parker.

General assessment and substantive concerns: This manuscript addresses the role of Cdc48 in targeting nascent aberrant polypeptides for degradation. The main conclusion is that the Cdc48 complex plays a role in disassembling tRNA-peptide-ubiquitin conjugates from the ribosome. Cdc48 has been previously characterized as a "seggregase" to remodel certain ubiquitinated substrate complexes prior to their proteasomal degradation, but these findings are relevant because they describe a new role for Cdc48 in ribosome-associated protein quality control pathways. All of the reviewers have credited the quality of the work but they also indicated that mechanistic understanding of this new pathway is missing. For example, no evidence is presented that the Cdc48 role in this process is direct and it remains possible that Cdc48 affects other aspects of the cell that indirectly lead to this phenotype. Further detailed analyses of the pathway and a better mechanistic understanding of the process are needed for publication in *eLife*.

DOI: http://dx.doi.org/10.7554/eLife.00308.017

Decision letter

Ivan Dikic, Reviewing editor, Goethe University, Germany

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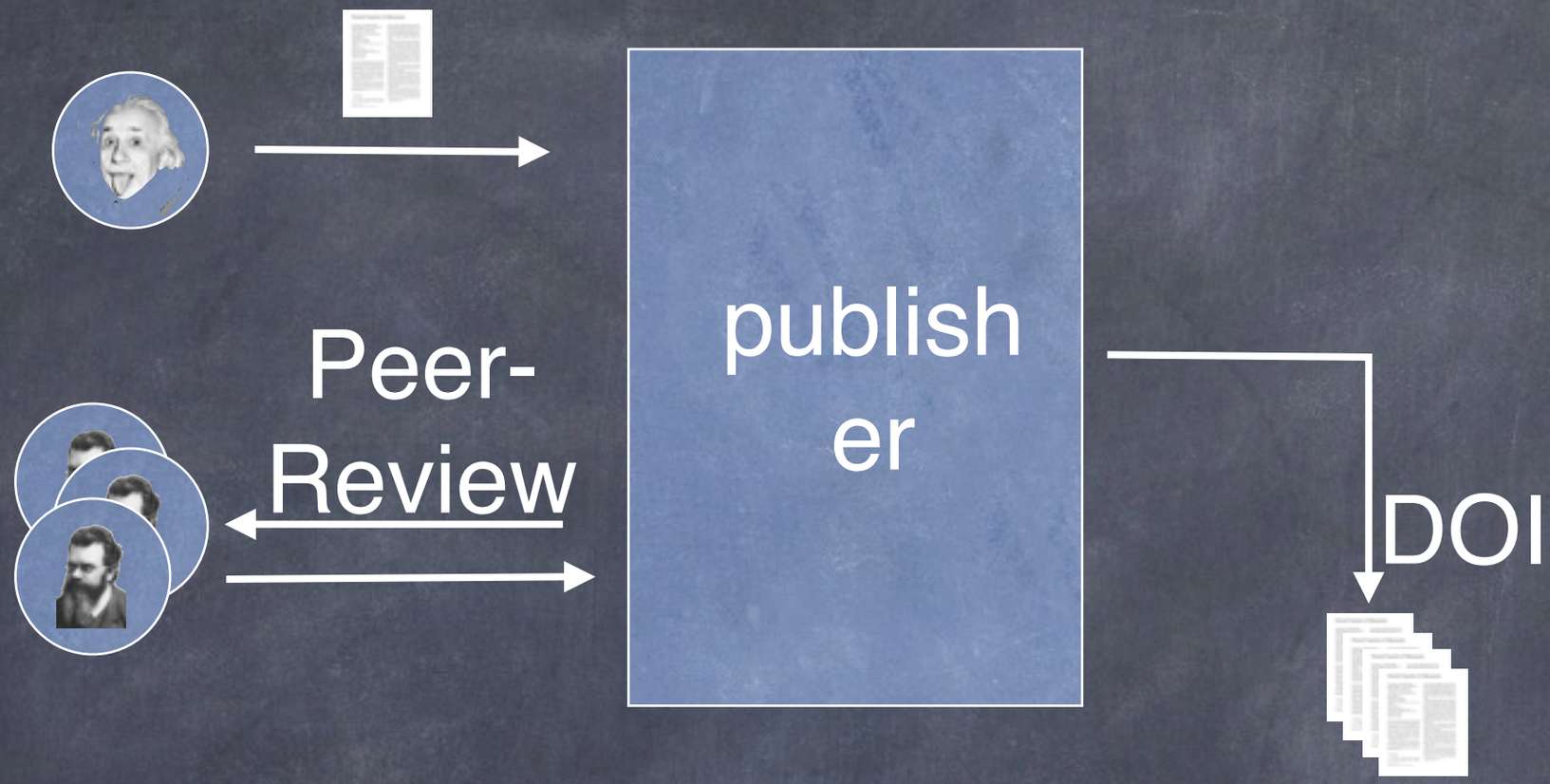
Author response

The main changes from the original submission are as follows:

1. The main criticism of the original submission was that there was not sufficient mechanistic insight into how Cdc48–Ufd1–Npl4 promotes degradation of tUNCs and non-stop decay (NSD) pathway substrates that are translated from messages that lack a stop codon. In particular, we provided no evidence to indicate that the role of Cdc48–Ufd1–Npl4 in this process is direct. In a series of email and telephone discussions with the editors, we were advised that providing evidence to support a direct role for Cdc48–Ufd1–Npl4 in RAD might suffice to address the concern that the reviewers raised about mechanism. We have addressed this criticism by showing in the revised manuscript that Cdc48 and Ufd1 were associated specifically with stringently-washed, affinity-purified ribosomes (Figure 1D). We also show that Cdc48 and Ufd1 bound the NSD substrate GFH^{NS}, but not the control GFH^{Stop} (Figure 3E), despite the latter being present at much higher levels than the NS reporter.

2. In addition to the new figure panels described above (Figures 1D and 3E), we have added the following additional data panels to address the other criticisms made by the reviewers: (i) input controls for Figure 1B, (ii) evidence that accumulation of tUNCs in *cdc48-3* cells is reversed by expression of wild type Cdc48 but not the ATPase-deficient Q2 mutant (Figure 1C), (iii) an expanded Figure 2B to include a control in which RNAse was added to cell lysate prior to isolation of ribosomes, (iv) a new version of Figure 3A showing the effect of *ubr1Δ* and *ltn1Δ* on accumulation of tUNCs in *cdc48-3* mutants, (v) a new version of Figure 3B showing the effect of Cdc48 pathway mutations on accumulation of the NSD substrate GFH^{NS}, including an anti-tubulin loading control, (vi) degradation assays for GFH^{NS} in wild type, *ltn1Δ*, and *ufd1-2* cells and GFH^{Stop} in wild type cells (Figure 3C), (vii) high-resolution sucrose gradient fractionation of lysates of *ltn1Δ* and *cdc48-3* cells expressing PrA^{NS} (Figure 4B; these data replace the sucrose gradient fractionations of GFH^{NS} that were previously shown in Figure 3D), (viii) levels of $Gnd1^{PTC}$ in total cell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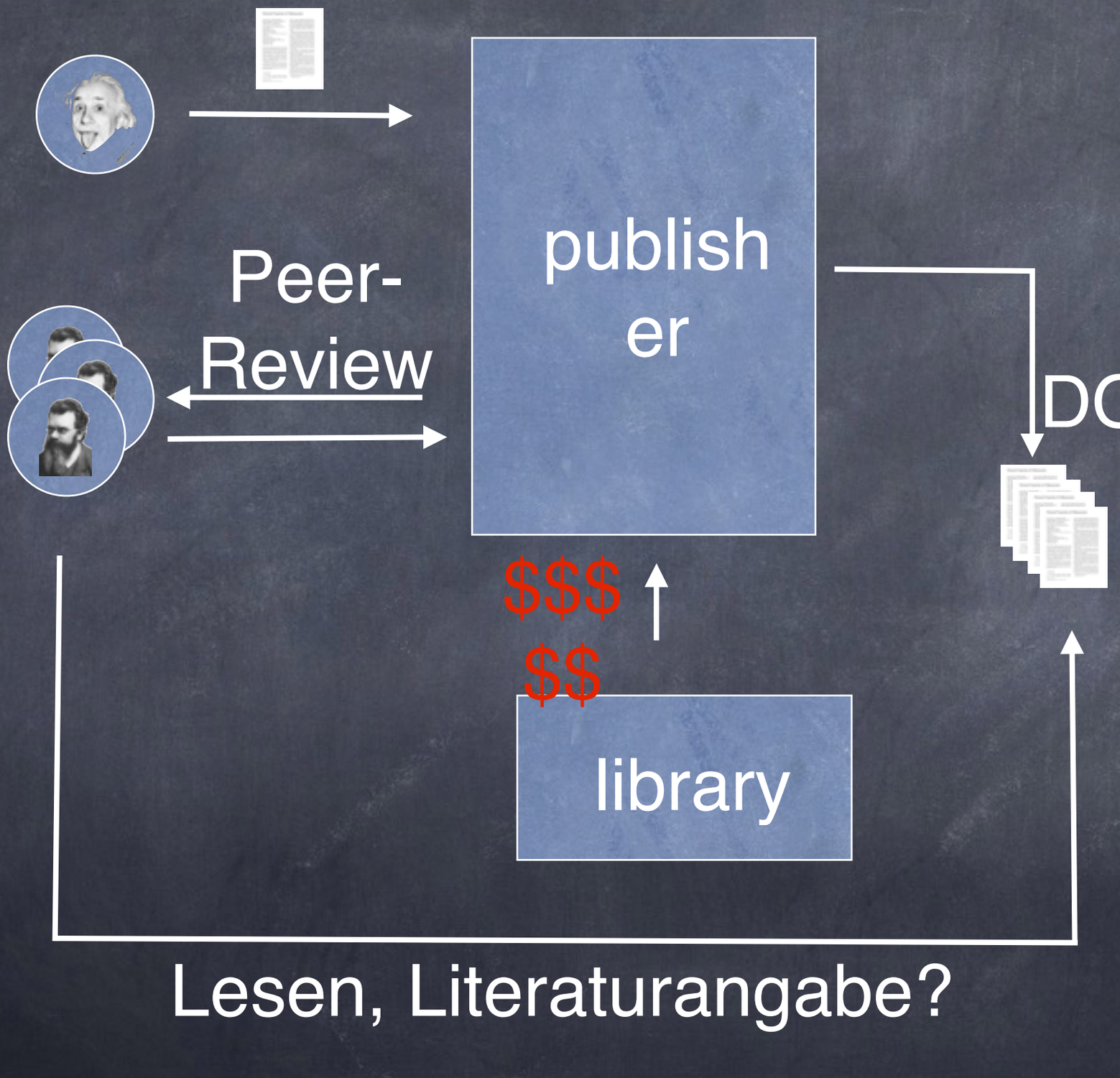
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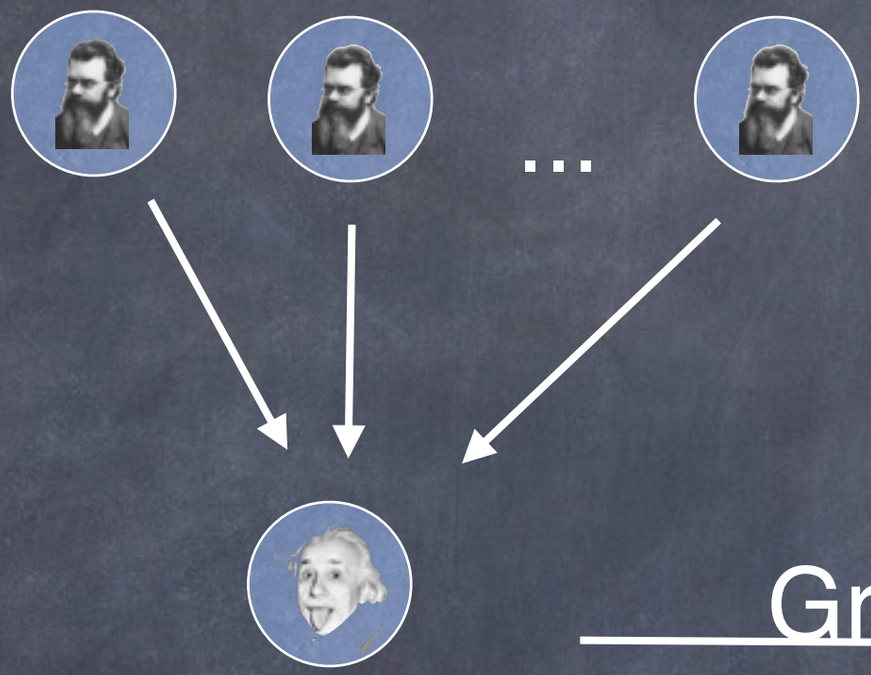

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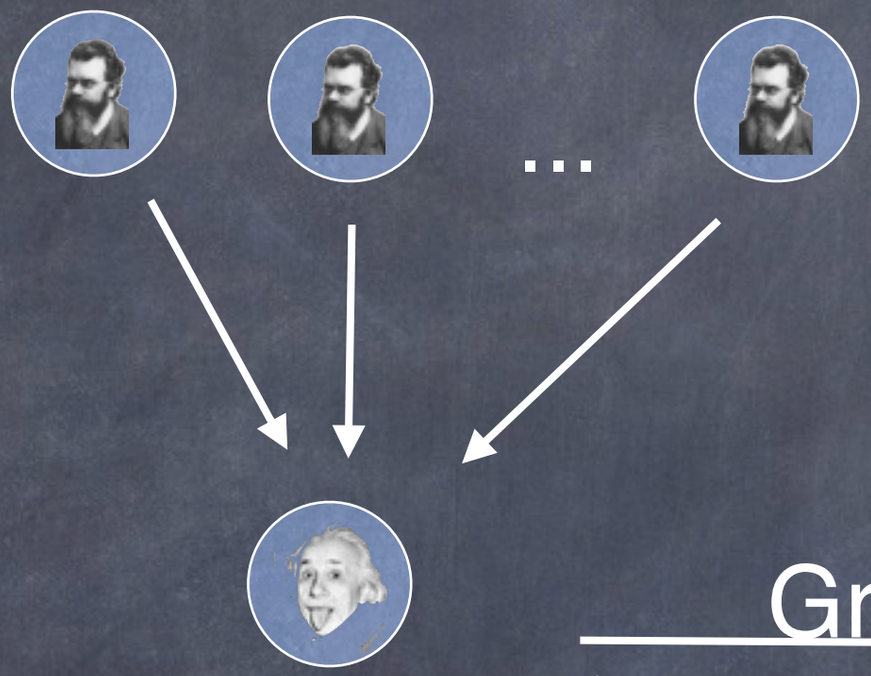

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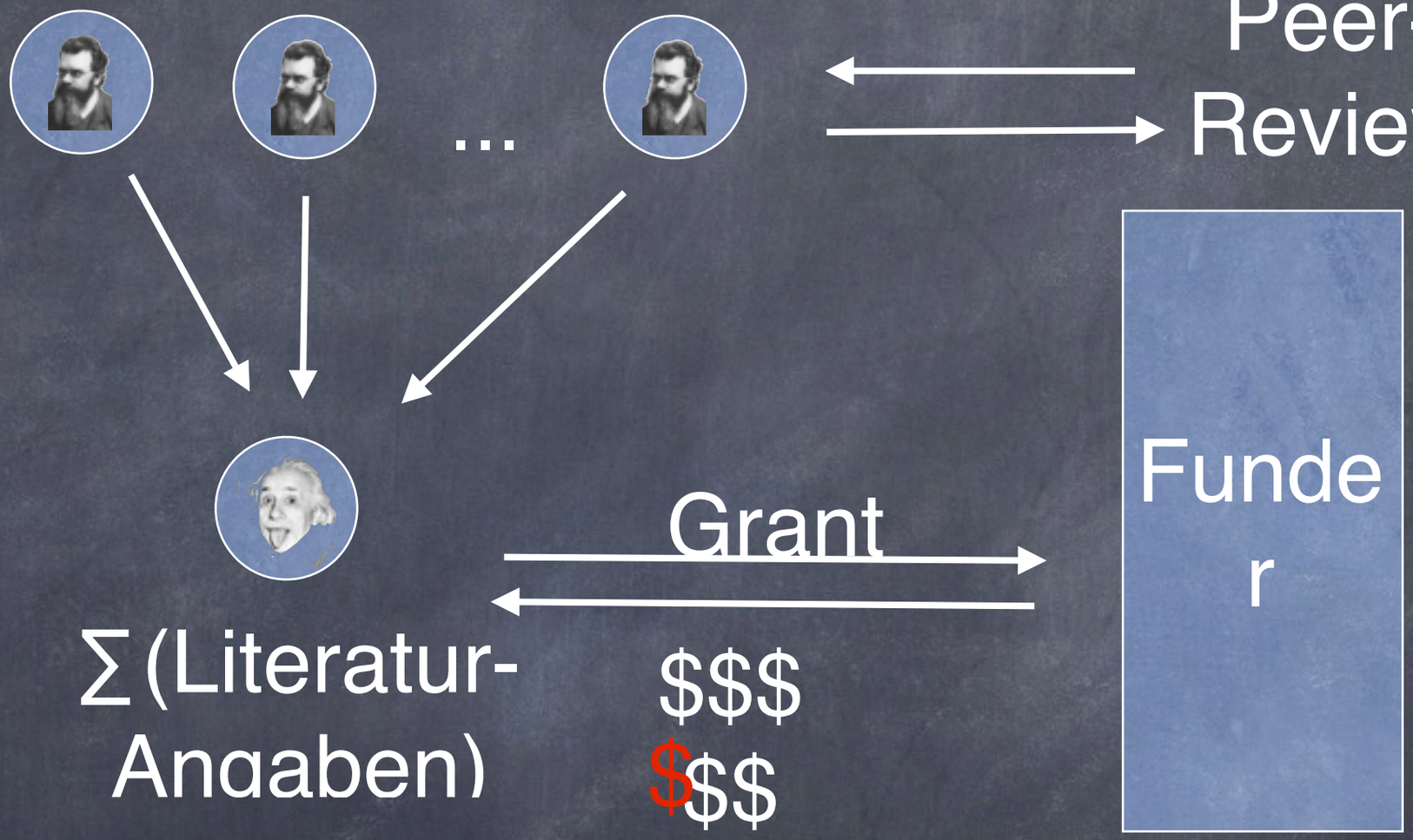
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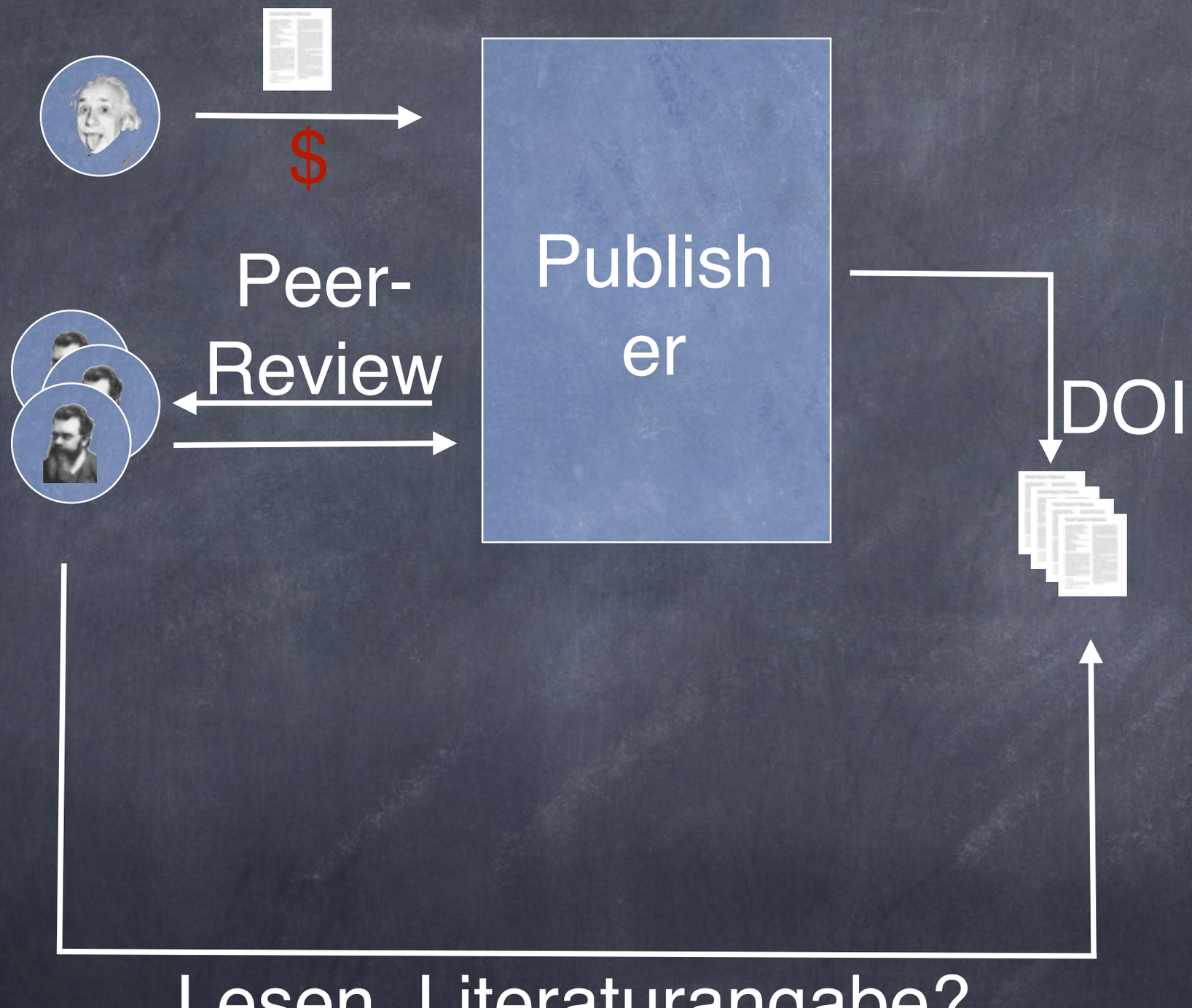
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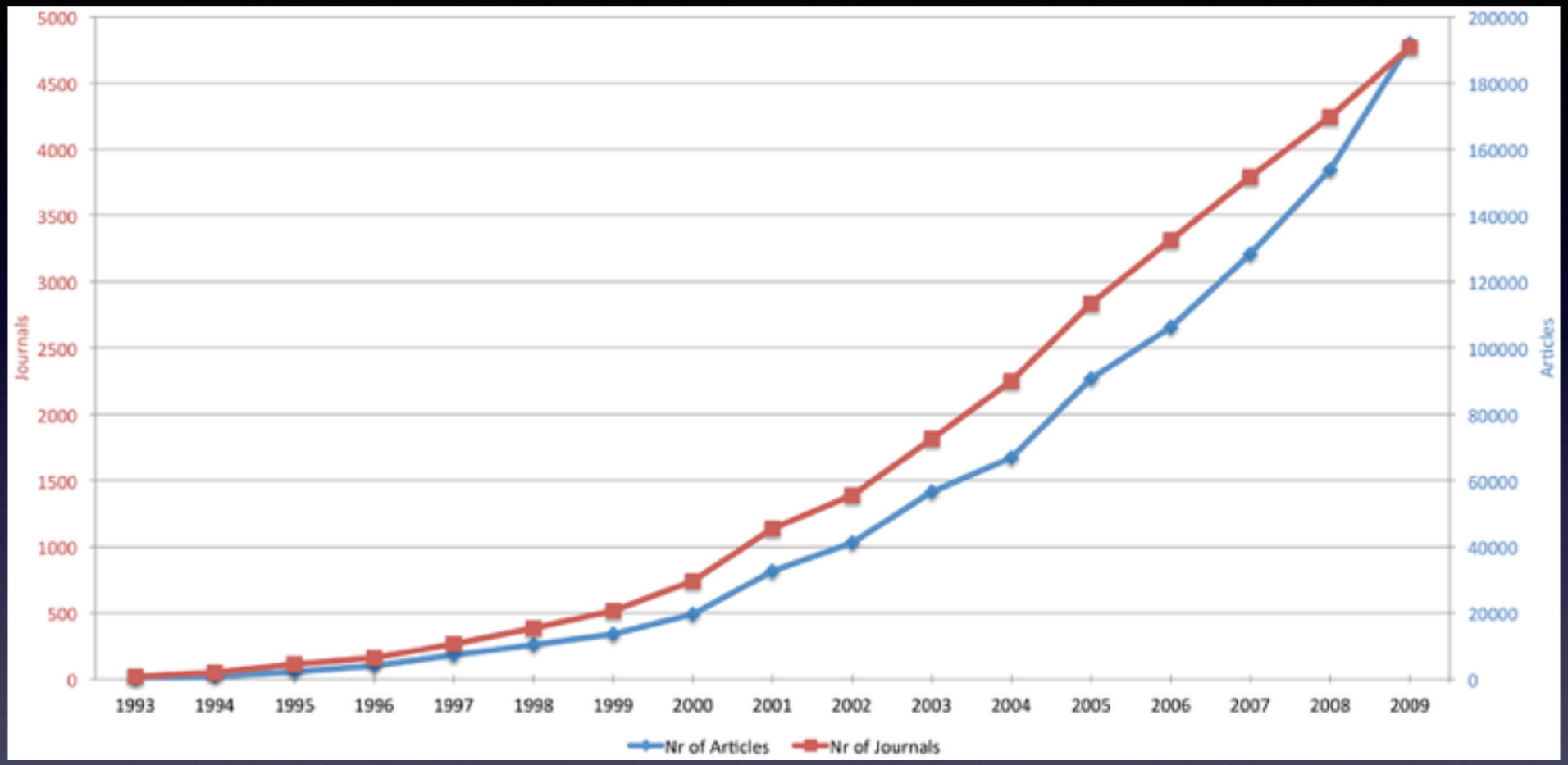
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Proportion of people that could use your work/ that create work you could use

$$P_{\text{(helping someone) (getting help)}} = \frac{Interest}{Effort^{n}} \times N$$

Ease of use and access of your work

Number of people that you reach

via Cameron Neylon

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Maximise

$$P = \frac{Interest}{n} \times N$$

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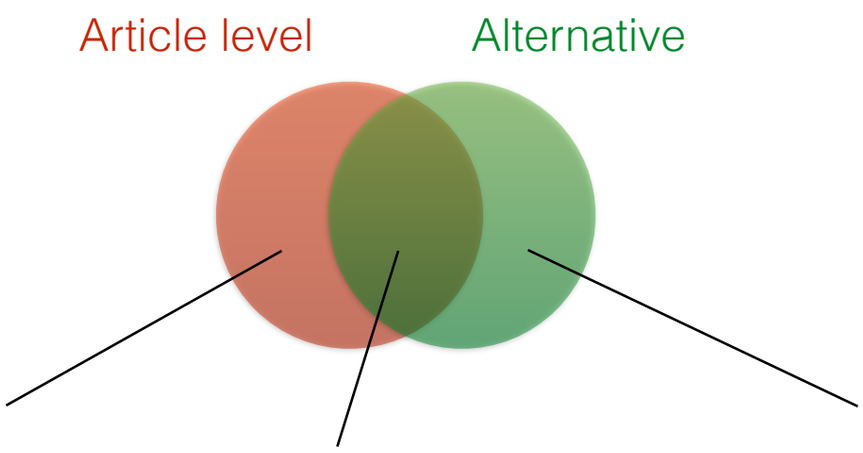

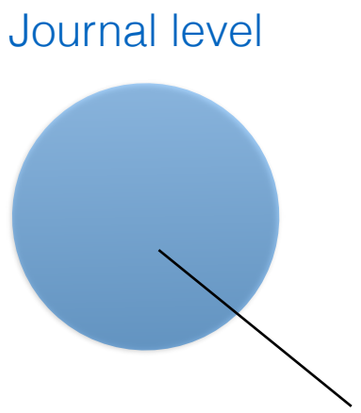

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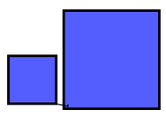

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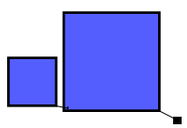

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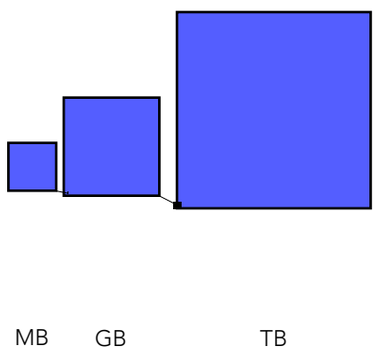
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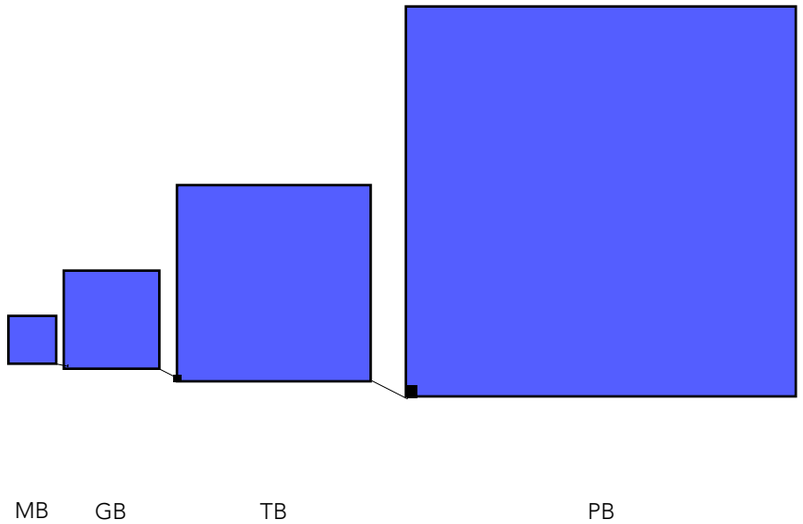
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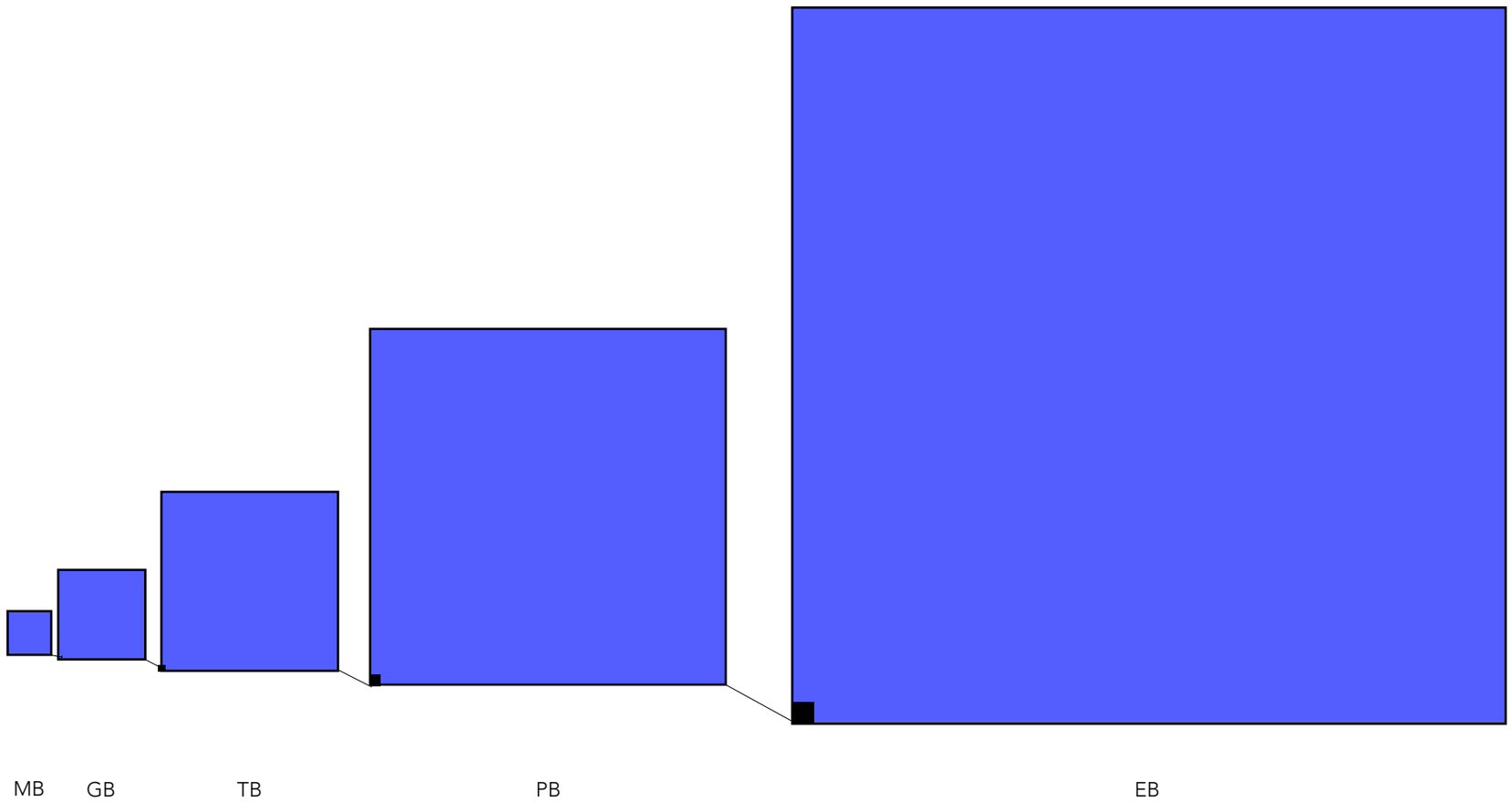
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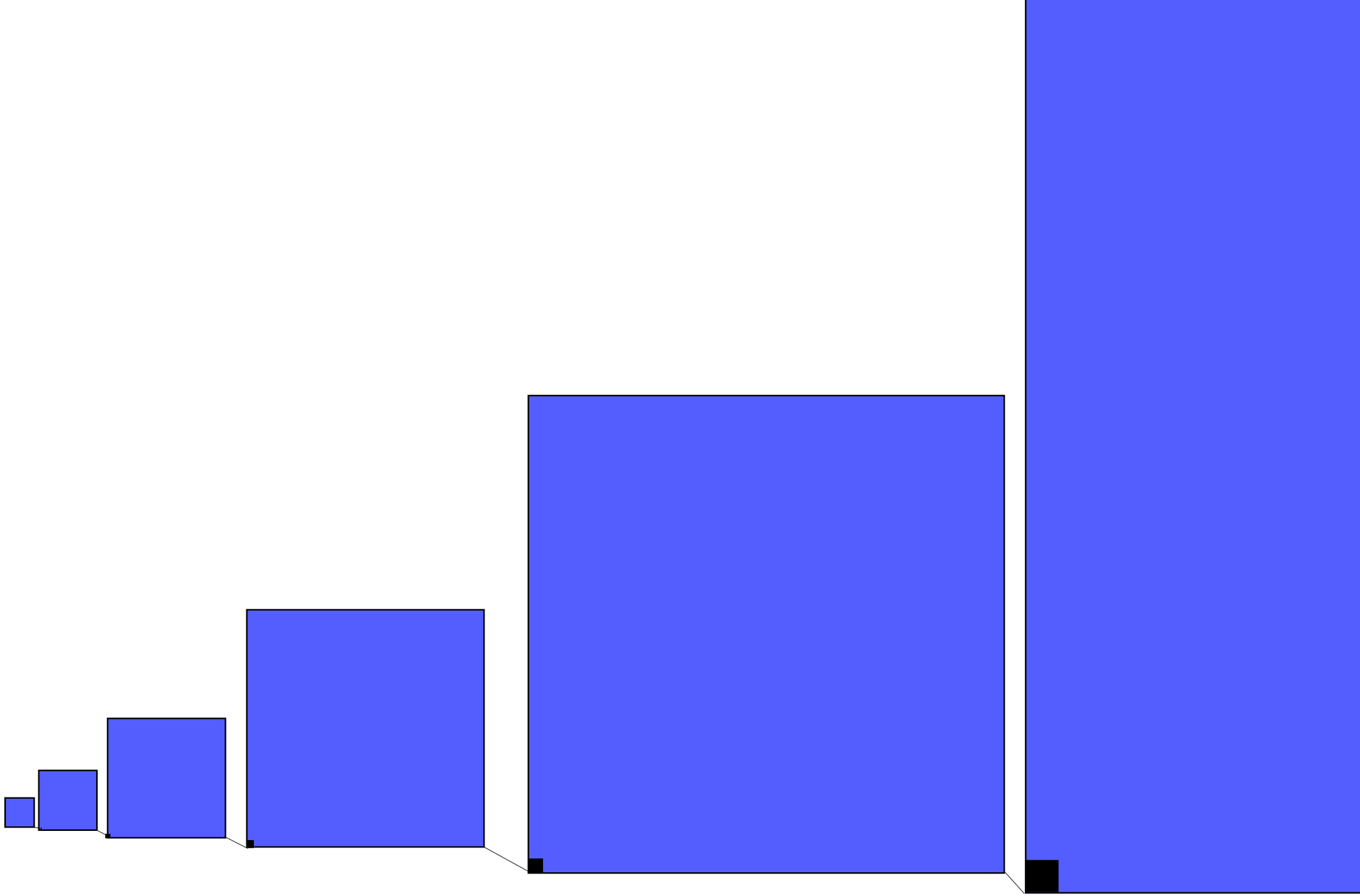
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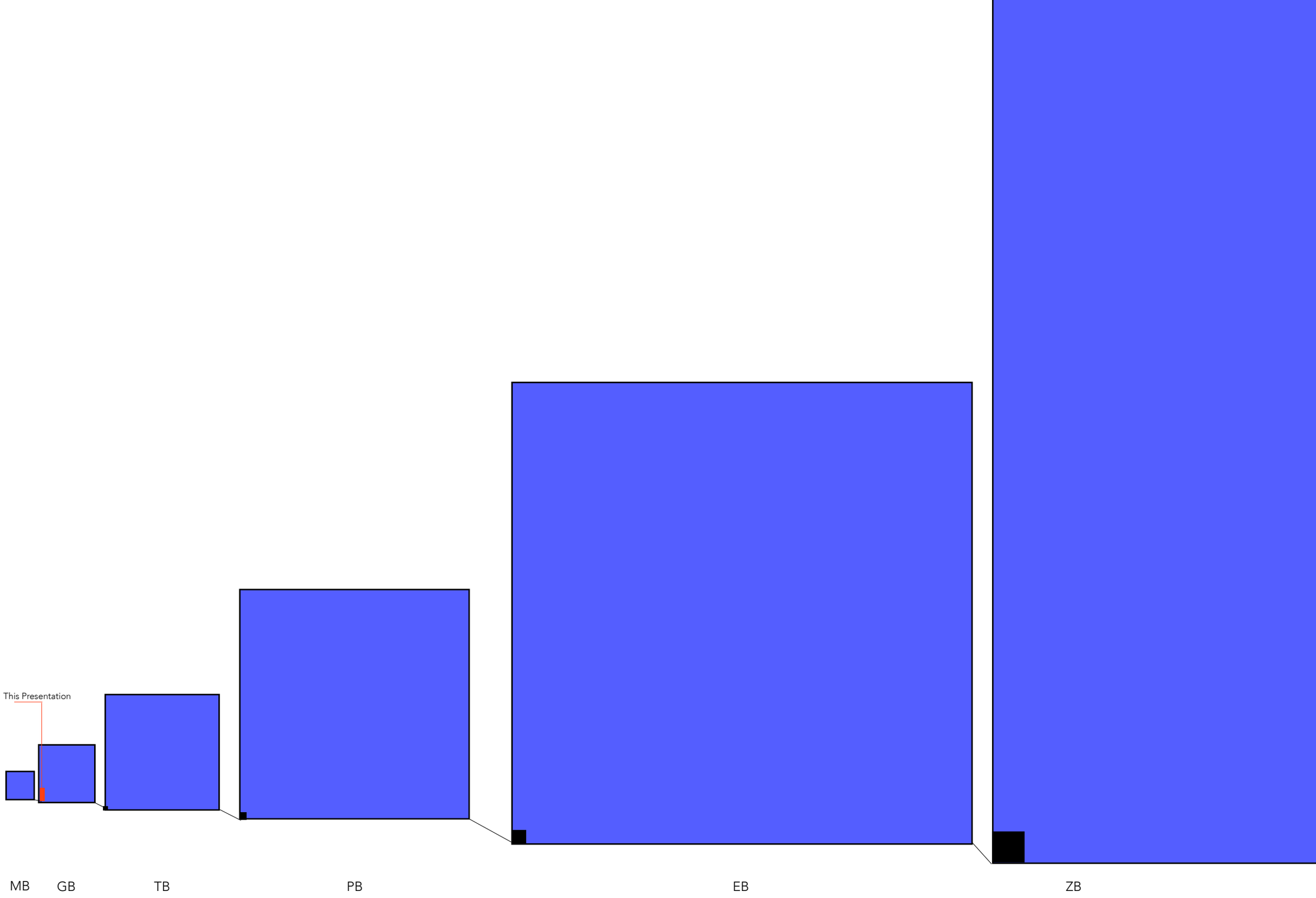
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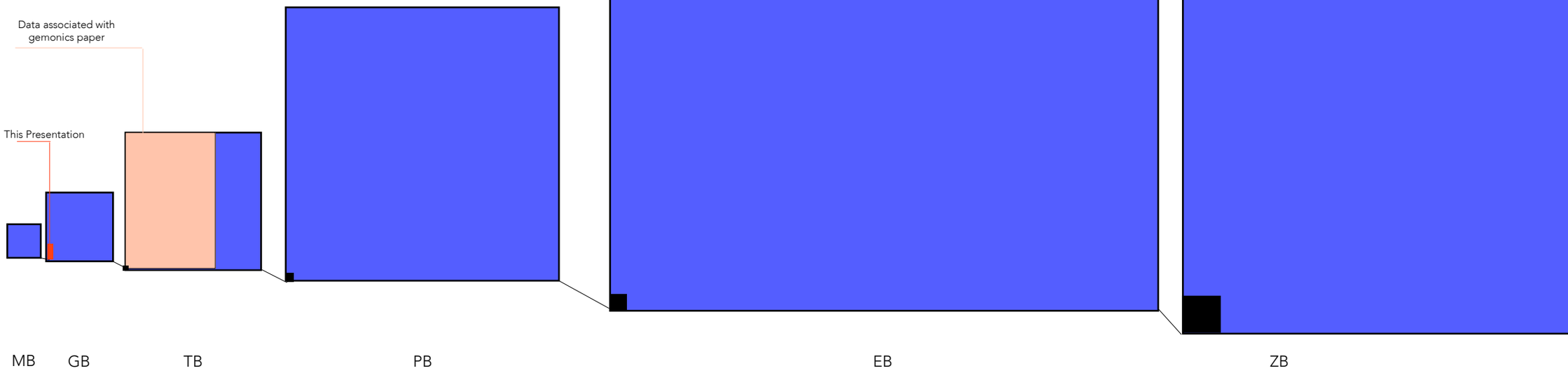
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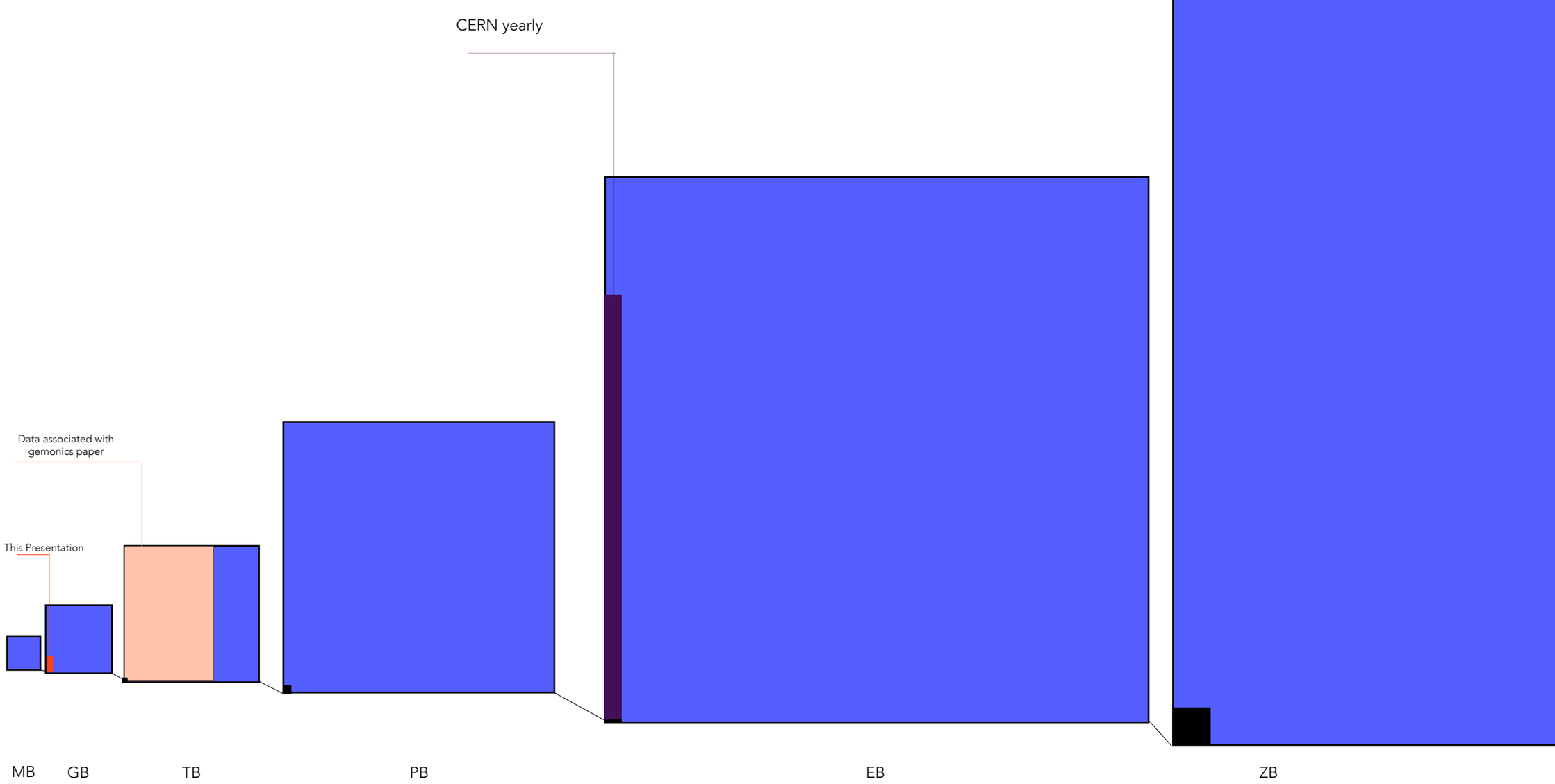
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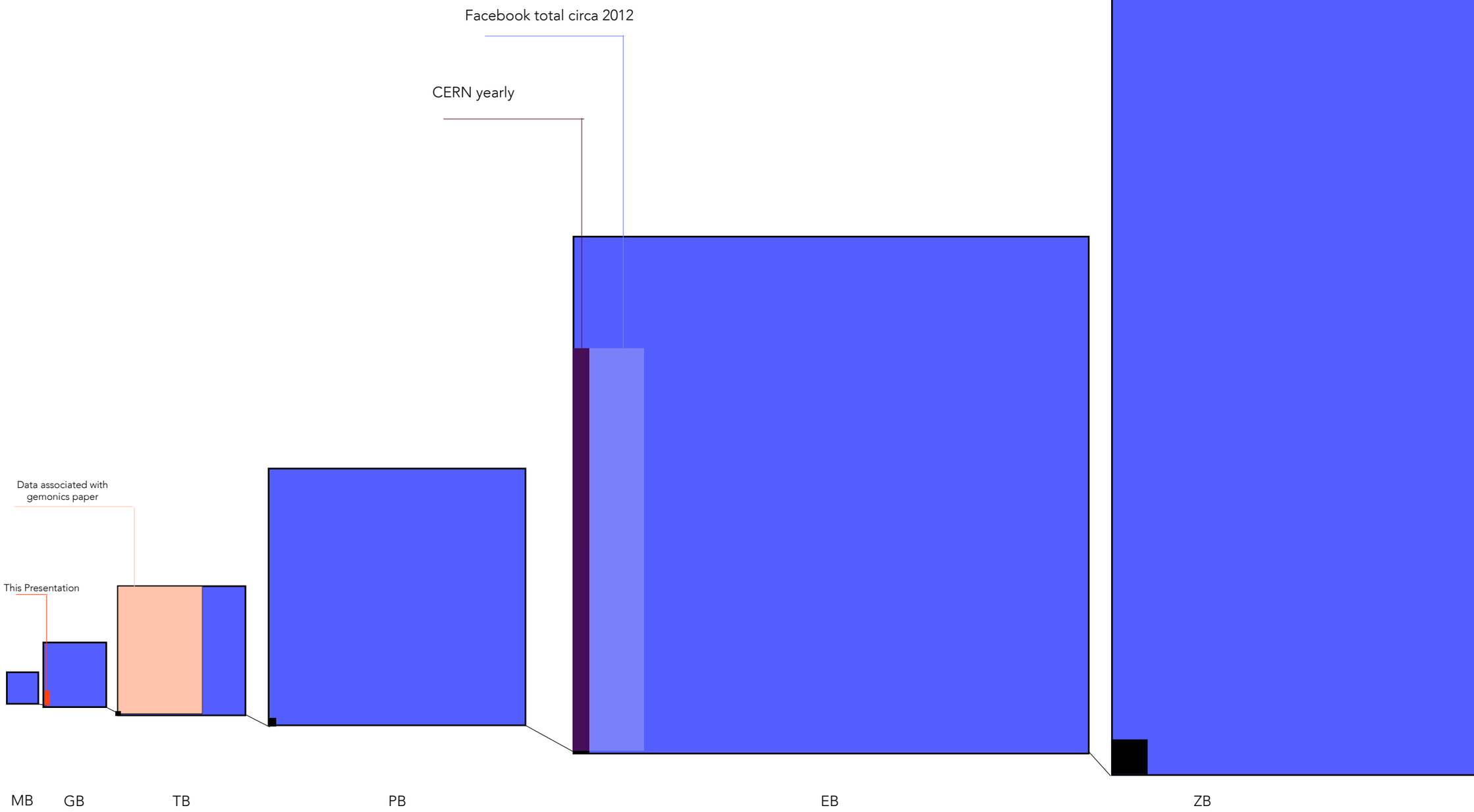
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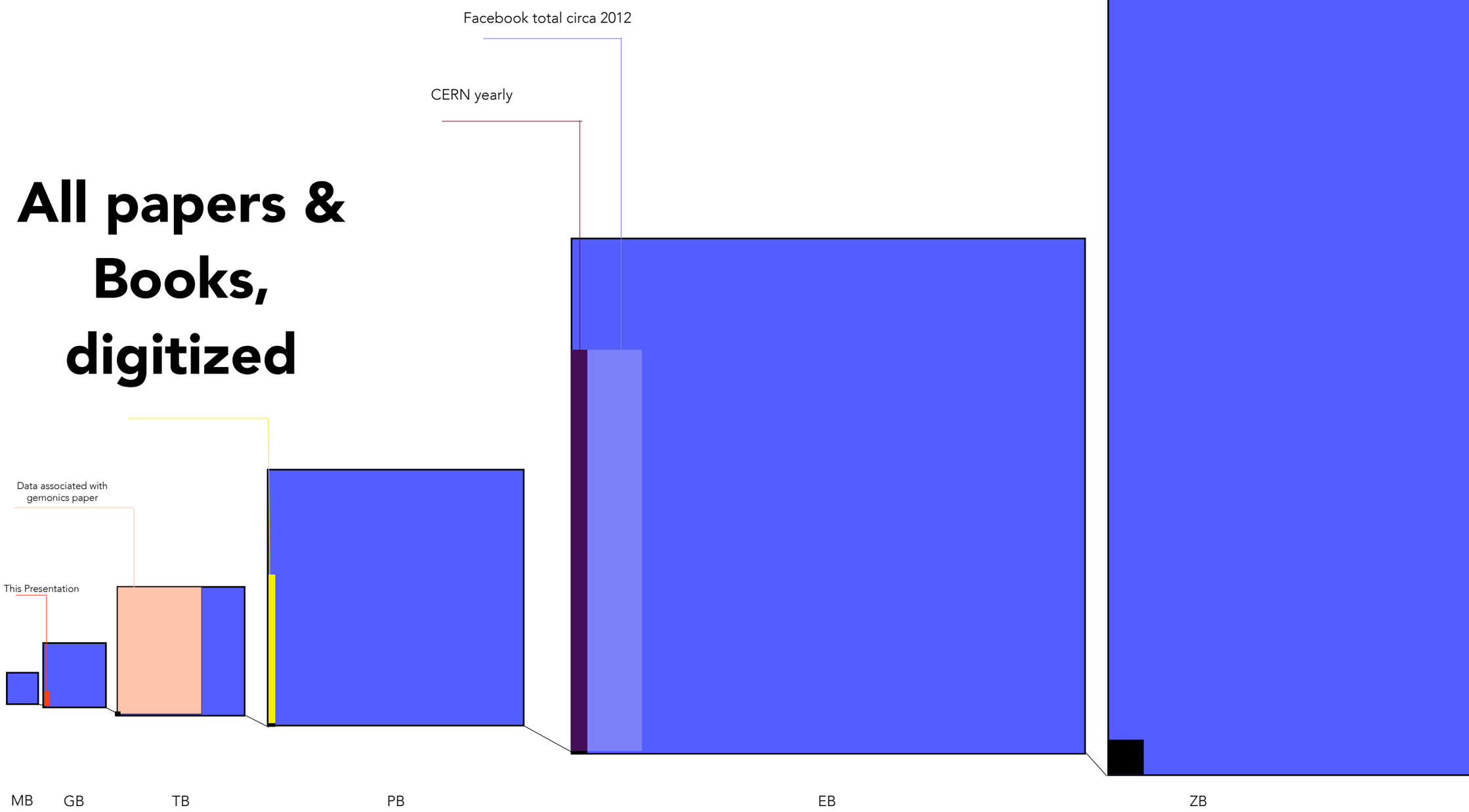

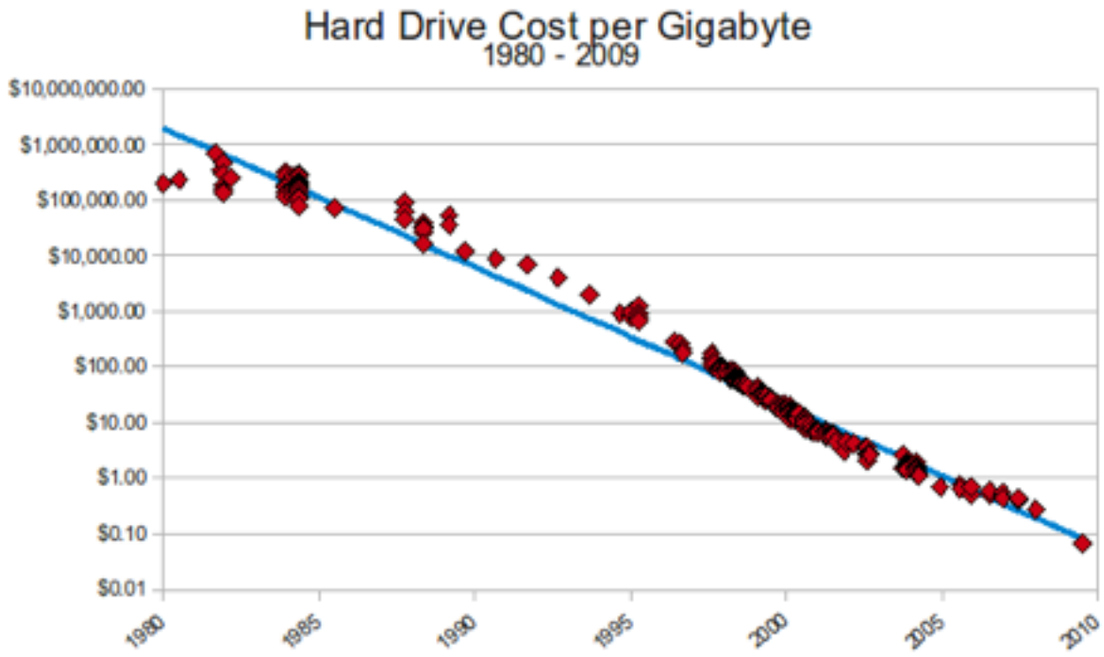
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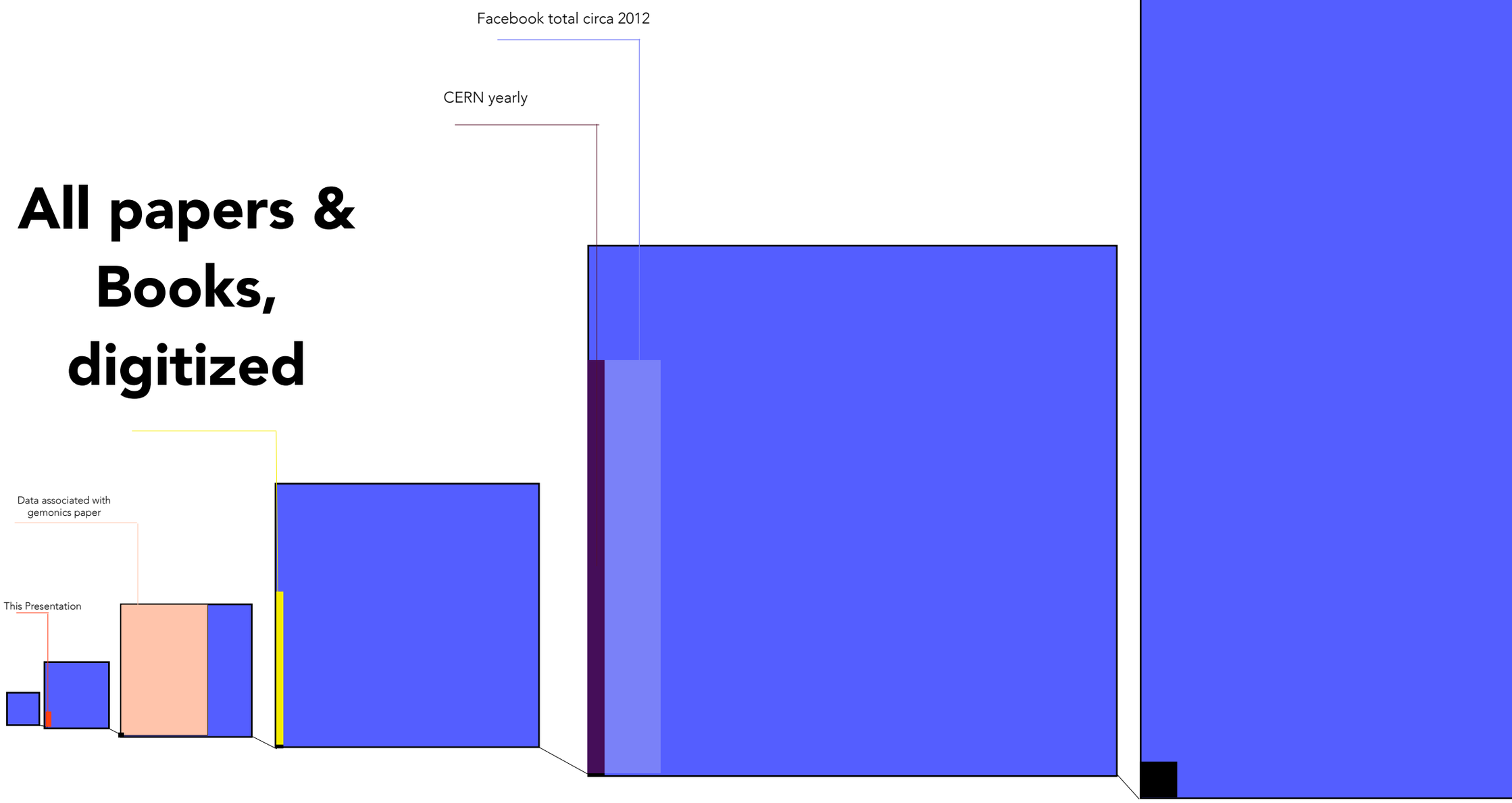

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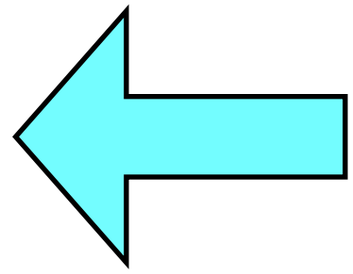

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