Quantitative analysis of intra-Golgi transport shows intercisternal exchange for all cargo

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The mechanisms controlling the transport of proteins through the Golgi stack of mammalian and plant cells is the subject of intense debate, with two models, cisternal progression and intercisternal exchange, emerging as major contenders. A variety of transport experiments have claimed support for each of these models. We reevaluate these experiments using a single quantitative coarse-grained framework of intra-Golgi transport that accounts for both transport models and their many variants. Our analysis makes a definitive case for the existence of intercisternal exchange both for small membrane proteins and large protein complexes—this implies that membrane structures larger than the typical protein-coated vesicles must be involved in transport. Notwithstanding, we find that current observations on protein transport cannot rule out cisternal progression as contributing significantly to the transport process. To discriminate between the different models of intra-Golgi transport, we suggest experiments and an analysis based on our extended theoretical framework that compare the dynamics of transiting and resident proteins.

Golgi apparatus | secretory pathway | quantitative transport model | resident golgi enzymes | convection-diffusion

The Golgi apparatus, a complex cellular organelle responsible for lipid and protein maturation and sorting, has attracted a great deal of attention, with many conflicting viewpoints regarding its mechanisms of transport. The Golgi of plant and animal cells consists of a stack of 5–20 cisternae (1), possibly interconnected by membrane tubules (2), which exchange material by vesicle budding and fusion (3, 4) (Fig. 1). Each cisterna has a distinct chemical identity, allowing progressive protein maturation from the cis to the trans face (5). There is a long-standing argument about the way proteins are transported through the Golgi, an issue intimately tied to the structure and dynamics of the organelle itself. The Golgi could be a rather static structure, in which cisternae keep constant positions and identities, and exchange proteins by vesicular transport. Alternatively, cisternae could progress from the cis end to the trans end without exchanging their cargo (6). Biochemical maturation of individual cisterna is known to occur in yeast (Saccharomyces cerevisiae) Golgi, which is not stacked but made of dispersed cisternae (7, 8). The cisternal progression model posits that this maturation translates into a physical progression of the cisternae (and their content) along the stack. It is supported by the observation that large molecules such as procollagen aggregates, presumably unable to enter conventional transport vesicles, nonetheless progress through the stack, suggesting that cisternae are created at the cis face and destroyed at the trans face (9). This picture was recently challenged (10) by the observation that proteins do not exit the Golgi linearly with time (as a model purely based on cisternal progression would predict) but exponentially, as can be explained by intercisternal exchange. These are however two extreme models, and cisternal progression and intercisternal exchange could act concomitantly. This is clearly in the cisternal progression model, which requires that resident Golgi enzymes (which are found in particular location in the Golgi stack) undergo specific retrograde (vesicular) transport.

Existing quantitative models are often tailored to support (11) or disprove (10) the cisternal progression model, and their comparison with quantitative data involves a large number of fitting parameters (10).

However, the relevance of each mode of transport can only be identified by an unbiased quantitative model based on the general formalism of transport phenomena (12). We report here that all available quantitative data on a variety of cargo, including large procollagen aggregates, can be reproduced by a combination of (i) global protein translation from the cis- to the trans-Golgi, (ii) diffusive-like protein exchange between cisternae, and (iii) protein exit throughout the stack. As shown below, the diffusive component implies that intercisternal exchange is not restricted to small protein-coated vesicles, and involves large transport carriers. We rigorously establish that transport data based on tagging a single molecular species can be argued to be consistent with many different models of transport and therefore cannot provide an unequivocal picture of intra-Golgi transport. To reach this goal, we propose experimental strategies based on dynamical correlations between transiting and resident Golgi proteins. A useful virtue of our formalism is that it can include the influence of the local biochemical and physical environment within the different cisternae as an energy landscape through which proteins diffuse, and thus permits a description of transiting proteins and resident Golgi enzymes within the same mathematical framework.

Model

Transport Equations for Intercisternal Exchange. Treating the Golgi stack as composed of distinct cisternae, we analyze protein transport data based on tagging a single molecular species can be argued to be consistent with many different models of transport and therefore cannot provide an unequivocal picture of intra-Golgi transport. To reach this goal, we propose experimental strategies based on dynamical correlations between transiting and resident Golgi proteins. A useful virtue of our formalism is that it can include the influence of the local biochemical and physical environment within the different cisternae as an energy landscape through which proteins diffuse, and thus permits a description of transiting proteins and resident Golgi enzymes within the same mathematical framework.

Significance

Two models compete to explain the way proteins transit through the Golgi, a cellular organelle consisting of stacked membrane-bound compartments (cisternae) and responsible for protein maturation and sorting. The cisternal maturation model proposes that cisternae created de novo move through the stack, carrying their content with them. The vesicular transport model views cisternae as static structures between which proteins are exchanged by vesicular transport. We have developed a transport model that can quantify the importance of intercisternal exchange by analyzing the spatiotemporal evolution of a protein distribution within the Golgi. Intercisternal exchange is confirmed both for small membrane proteins and large protein complexes. This suggests the involvement of membrane carriers much larger than typical protein-coated vesicles.
transport along its axis of polarity (the cis–trans axis), for which the cisterna number \( n \), varying between 1 (the cis-most) and \( N \) (the trans-most) plays the role of a discrete spatial coordinate. The distribution of a chemical species \( A \) within the Golgi may be characterized by its concentration \( A_n(t) \) in cisterna \( n \) at time \( t \). Intercisternal exchange is restricted to “jumps” between adjacent cisternae (with rates \( k_n \) for \( n \to n+1 \) and \( k_n' \) for \( n \to n-1 \); Fig. 1). We emphasize that the rates \( k_n, k_n' \), and \( r_e \) characterizing the coarse-grained dynamics may be used regardless of the microscopic details of the exchange process. For vesicular transport, they are the product of the rates of fusion, translocation, and fusion of vesicles carrying \( A \), and include the waiting time of \( A \) within a cisterna. They are not restricted to processes involving protein-coated vesicles, and may include transport through connecting membrane tubules and contributions from any fragment that detaches from one cisterna and fuses with a neighboring cisterna. These rates may depend on the local concentration \( A_n \). A master equation (12) can be written for the concentration \( A_n(t) :\)

\[
\frac{\partial A_n}{\partial t} = k_{n-1}A_{n-1} - k'_nA_n - (k_nA_n - k'_nA_{n+1}).
\]

A straightforward generalization of the model could include transport between distant cisternae. This however does not bring new insight, nor does it improve the comparison with available experimental data on transiting proteins.

We will rewrite Eq. 1 in a continuous formalism, because this allows for a better description of cisternal progression. The coordinate \( n \) (the cisterna number) can be written as a continuous variable, and spatial variations are then written as a derivative: \( \partial_n A_n = (A_{n+1} - A_{n-1})/2 \), with distances normalized by the intercisternal distance (the connection between the discrete and continuous models is described in detail in SI Appendix). If the different exchange rates do not depend too drastically on position (\( k_n \sim k_0 \)), Eq. 1 can be transformed into a Fokker-Planck equation (12). In this continuous description, intercisternal exchange amounts to an effective translation with velocity \( v_t \), combined with an effective diffusion with a diffusion constant \( D_t \) :}

\[
\frac{\partial A_n}{\partial t} = -v_t \frac{\partial A_n}{\partial n} + \frac{1}{2} \frac{\partial^2 A_n}{\partial n^2}.
\]

This illustrates that intercisternal exchange always yields an effective diffusion coefficient, even if all transport steps are anterograde (\( k_n > 0, k_n' = 0 \)), as we discuss below.

**Including Cisternal Progression and External Fluxes.** Proteins may be transported toward the Golgi face by cisternal progression, defined as the process by which the entire content of a cisterna moves from position \( n \) to position \( n + 1 \) in the stack over a time \( \Delta t \). The progression velocity is thus defined as \( v_p \equiv 1/\Delta t \), and is the same for all cisternae. Furthermore, the species \( A \) may in principle be imported to or exported from any cisterna along the stack. These processes, which include direct recycling to the endoplasmic reticulum (ER), may be expressed as an external flux \( J^e = J_0^n - r_eA_n \) composed of an influx \( J_0^n \) to cisterna \( n \), that could come from outside the Golgi or from distant cisternae, and a rate of exit \( r_e \) from cisterna \( n \). Eq. 2 becomes

\[
\frac{\partial A_n}{\partial t} = -v_t \frac{\partial A_n}{\partial n} + \frac{1}{2} \frac{\partial^2 A_n}{\partial n^2} - v_p A_n + \left( J_0^n - r_eA_n \right) + \left( v_t + v_p \right) A_n.
\]

The influx is not expected to contribute significantly to the dynamics of transiting proteins coming from the ER. It is ignored for now but is reintroduced in Results, where we derive the distribution of resident Golgi enzymes. Fluxes entering at the cis face and exiting from the trans face of the stack are included in the model as boundary fluxes (see below).

Eq. 3 illustrates three fundamental mechanisms governing the temporal evolution of a protein distribution within the Golgi: \( i \) protein exchange between neighboring cisternae introduces an effective diffusion of the concentration along the Golgi stack, characterized by a diffusion coefficient \( D_t \); \( ii \) directed protein transport from the cis- to the trans-Golgi leads to protein translation at a velocity \( v_t = v_0 + v_p \); this accounts both for cisternal progression (at velocity \( v_p \)) and for a bias for anterograde \( v_0 > 0 \) or retrograde \( v_0 < 0 \) cisternal exchange, and \( iii \) proteins may in principle exit from any Golgi cisterna to join other organelles (the ER or lysosomes) at a rate \( r_e \), which may be zero. Note that because the spatial coordinate is a dimensionless number, all three parameters have units of rates (\( \text{min}^{-1} \)).

Because it does not depend on the microscopic processes responsible for transport, Eq. 3 constitutes the most rigorous quantification of an arbitrary transport process, and should be used as a first approach to characterize Golgi transport. The impact of the three main parameters on the distribution of proteins throughout the Golgi is best seen when analyzing the propagation of an initially localized protein distribution (pulse-chase experiments, Fig. 1). The translation velocity displaces the concentration peak (linearly in time if \( v_t \) is constant), diffusion broadens the peak (its width increases as the square root of time if \( D_t \) is constant), and protein exit decreases the total protein concentration (exponentially with time if \( r_e \) is constant). The various rates could vary for different proteins, possibly transported by different mechanisms, and should in particular be very different for transiting proteins and resident Golgi enzymes.

Cisternal progression only affects the translation velocity in Eq. 3, whereas anterograde intercisternal exchange affects both the velocity and the diffusion coefficient. Our formalism thus readily shows a fundamental qualitative difference between the two contending models. Within the cisternal progression model, the movement of transiting proteins may occur in the absence of
intercisternal exchange, thus \( v_J > 0, k_n = k_n' = 0 \). This amounts to a perfect translation, without broadening, of a peak of concentration, i.e., \( D_J = 0 \). Intercisternal exchange, on the other hand, necessarily involves some broadening, with an apparent diffusion coefficient directly related to the translation velocity \( D_J = v_J / 2 \) in the absence of retrograde transport, i.e., when \( k_n = 0 \), and \( D_I > v_I / 2 \) if \( k_n \neq 0 \). This immediately leads to a powerful conclusion: if the analysis of the pulse-chase data using Eq. 3 suggests that \( v_I > 2D_I \), then we can unambiguously conclude that the data are incompatible with a transport based purely on intercisternal exchange and must allow for some cisternal progression. This illustrates how a quantitative analysis based on generic transport equations may shed light on the nature of intra-Golgi transport, without requiring the knowledge of microscopic details of individual transport steps. We show in the next section that all available data for transiting proteins are well fitted by assuming constant exchange rates. Spatial variations of the transport rates are then introduced to study the dynamics of resident Golgi enzymes.

**Boundary Fluxes.** Eq. 3 must be supplemented by boundary conditions at the cis \((n = 1)\) and trans \((n = N)\) faces of the stack. At the cis face, the influx \( J_n^{\text{in}} \) of material from the ER is taken as a parameter (possibly varying with time), imposed by the experimental procedure (e.g., in pulse-chase or incoming wave protocols, see below). The rate of protein exit at the cis face is taken as a fitting parameter \( k^c (= k_n') \). The outflux of material at the trans face \( J_n^{\text{out}} \) includes contributions both from vesicles secreted at the trans-Golgi and from the maturation of the trans cisterna: \( J_n^{\text{out}} = (v_J + k_n') A_N \). As can be seen from Eq. 3, these two contributions may not be easily distinguished, as the net flux throughout the Golgi involves the net velocity \( v = v_n + v_J \). We thus write the exit flux \( J_n^{\text{out}} = (v + k^t) A_N \), where \( k^t = k_N - v_I \) is the fitting parameter of trans-Golgi exit. In addition to the transport parameters \( (v \text{ and } D) \) and the exit rate \( r \), there are thus two additional boundary parameters \( k^- \) and \( k^+ \) in the model. Boundary conditions do affect the spatiotemporal distribution of proteins inside the Golgi, but we show below that the (bulk) parameters \( D \) and \( r \), which control the actual transport through the Golgi, can nevertheless be determined with reasonable accuracy.

**Results**

**Confrontation with Experimental Data on Transiting Proteins.** Our theoretical framework was used to analyze different experimental observations, collectively illustrated in Fig. 2. Fluorescence recovery after photobleaching experiments (FRAP) performed on the whole Golgi gives access to the total concentration of tagged proteins inside the Golgi. An exponential recovery dynamics is reported in ref. 10, both for small membrane proteins (glycoprotein of vesicular stomatitis virus, VSVG) and for large cytosolic protein complexes (procollagen). This was used as an argument against pure cisternal progression, for which a linear recovery dynamics is expected.

Our analysis shows (Fig. 2A) that the recovery profile is rather insensitive to the mode of intra-Golgi transport, and in particular to the effective diffusion coefficient \( D_c \), the only parameter that solely depends on intercisternal exchange. We fit the data with a single exponential decay of characteristic time 16 min, which could be accounted for by any one of the following: protein exit throughout the Golgi (parameter \( r \)), early exit from the cis face (parameter \( k^- \)), late exit via the trans face (parameter \( k^+ \)), or any combination of the three. The dynamics of small inert soluble cargo molecule reported in ref. 10 follows a similar, although slightly faster, exponential recovery, with similar conclusions regarding its means of transport. When fluorescent VSVG proteins were only allowed to enter the Golgi for a short time, the

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**Fig. 2.** Quantitative analysis of data from different experimental protocols using a numerical solution of Eq. 3. (A and B) Optical microscopy assays. A whole Golgi FRAP experiment probing the exit of tagged proteins from the Golgi following (A) a steady influx, abruptly stopped at \( t = 0 \), of a small transmembrane protein (VSVG) and a large soluble protein aggregate (procollagen), and (B) a short influx, starting at \( t = 0 \) and stopping at \( t = 5 \) min, of VSVG (10). \( k^- \) was set to zero in the fits because it does not influence the early relaxation. (C and D) EM assays. (C) Pulse-chase experiment for VSVG (13). Setting either convection (gray curve) or diffusion (dashed curve) to zero cannot reproduce the data. Fits are constrained so that the total protein concentration matches the data at \( t = 14 \) min. \( k^- \) was set to zero because it has the same effect as \( r \). (D) Evolution of the concentration of procollagen aggregates in the cis (black) and trans (gray) face of the Golgi upon sudden blockage of ER secretion (exiting wave experiment) (9). Data are in percentage of the concentration in normal conditions (steady ER secretion), and are not sensitive to exit rate. More information on the fitting procedure and experimental uncertainty is given in SI Appendix.
exponential recovery started immediately after the cessation of the fluorescence influx (Fig. 2B). This shows that proteins do not need to reach the trans face to exit the Golgi, because recovery would otherwise show a delay (gray curve in Fig. 2B), and suggest that proteins can exit at the cis face (parameter $k_c$) or throughout the stack (parameter $r$). Although these experiments give important information concerning the rate at which proteins are exported from the Golgi, such average measures of Golgi dynamics do not yield any clear-cut conclusion on the dominant means of transport across the Golgi stack. For instance, the exponential fluorescence decay of both FRAP experiments is consistent with a transport solely based on cisternal progression ($D_t=0$), provided proteins are allowed to exit throughout the Golgi at a sufficient rate ($r \gg v/N$). A quantitative assessment of intra-Golgi transport, which is tantamount to obtaining numerical values for $v$ and $D_t$, requires the knowledge of the protein distribution inside the entire organelle.

Following the transport of a pulse of protein (pulse-chase protocol, Fig. 1C), or the evolution of the protein distribution across the Golgi after ER secretion has been suddenly blocked (exiting wave protocol), could in principle yield independent measurements of the various parameters. Our analysis of pulse-chase data on small membrane proteins (VSVG, Fig. 2C) (13) clearly shows a combination of translation ($v \neq 0$), broadening ($D_t \neq 0$), and decay (at least one nonvanishing parameter among $\{r, k_c, k_t\}$) of the peaked concentration distribution. The best fit (black curve in Fig. 2C) suggests that all transport rates have similar values ($v - D_t \sim k_t \approx 0.2 - 0.3$ min$^{-1}$). The value of the velocity corresponds to a transit time across the Golgi of order $\sim 2$ min, the value of the concentration differences is rather surprising for such large protein complexes and of fundamental significance.

Experimental limitations, such as variability within and between cells or the finite amount of time needed to set up transport block, could be argued to sharpen concentration gradients in a way similar to intercisternal exchange. We show in SI Appendix that given the experimental error (below 10% for data of Fig. 2D, ref. 9), a finite diffusion coefficient must be invoked to explain the procollagen exiting wave data provided ER export ceases within 10 min of the initiation of the block. For a 5-min block, we find $D_t \approx v/2$ for procollagen (SI Appendix).

The analysis of Fig. 2C and D provides compelling evidence that the two cargo molecules studied undergo retrograde transport during their journey through the Golgi apparatus. Indeed, our formalism enables us to determine the average number of intercisternal exchange steps experienced by a protein. In a stack with $N$ cisternae, it is equal to $k + k'$ times the average time ($N/v$) spent in the Golgi, or equivalently to $2Nv/v$. We thus predict an average of $2N \approx 10$ exchange steps for VSVG, and $5 - 10$ steps for procollagen. Because $v > v_p$ and using $k = D_t + v^2/2$ and $k' = D_t - v^2/2$, we find that at least one-fourth of these transport steps is backward (toward the ER).

### Protein Retention Inside the Golgi

We now apply our formalism to resident Golgi proteins (e.g., glycosylation enzymes) that define the identity and function of specific cisternae and thus must remain in particular locations along the stack. Scenarios for protein retention in the Golgi usually involve either fast recycling of proteins by transport vesicles and/or localization by interaction with the surrounding membrane environment. A popular mechanism for the latter is the hydrophobic mismatch (14, 15), in which proteins are sorted by the span of their transmembrane domains compared with bilayer thickness.

In our framework (Eq. 3), localization by recycling corresponds to an influx of protein targeted to a particular cisterna $n_0$ combined with protein exit at every cisterna ($\Delta I_m = I_m(6(n - n_0)$ and $r > 0$). The stationary protein distribution along the stack is the stationary solution to Eq. 3. (see SI Appendix):

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**Fig. 3.** Localization of Golgi resident proteins. (A) Fast recycling of proteins imported at specific Golgi location leads to a peaked protein distribution around the import location. The steady-state distribution profile is shown for parameter values corresponding to VSVG (not a resident protein, $v = D_t = 0.3$, $r = v/10$, gray curve) and for a recycling 10 times faster (black curve). (B) Local variation of the transport rates $k$ and $k'$ can be converted into energy landscapes $E_n$ and related to physical mechanisms, such as hydrophobic mismatch. The example shows a quadratic landscape $E_n = k/2(n - n_0)^2$ and the corresponding rates. The steady-state distribution shows a peak where the net velocity $v - k - k' + v_p$ vanishes. (C) Pulse-chase experiment on resident proteins in a quadratic energy landscape, showing the evolution of a protein distribution initially localized at the cis face at $t = 0$, and the variation of the total protein content with time. Variation of the progression velocity strongly influences the protein distribution and lifetime in the Golgi. Larger $v_p$ (black curve) displaces the peaks toward the trans face and promotes protein exit.

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The protein distribution is peaked at $n_0$ and is asymmetric, $A_n$ corresponding to $n > n_0$ and $A_n$ to $n < n_0$. It is spread over $1/A_n$ (respectively, $1/n_0$) cisternae toward the trans (respectively, cis) Golgi face, and is broader toward the trans face due to protein convection. Accurate protein localization requires $\Delta v \geq v$, as illustrated in Fig. 3A, a much faster rate than the one we measured for the transiting protein VSVG ($\Delta v \approx \sqrt{v})$. The stationary distribution (Eq. 4 and Fig. 3A) requires that the influx is balanced by the outflux $J_n = D_n \delta \langle v \rangle$, but is not sensitive to details of the recycling pathway. Whether proteins leaving the Golgi are recycled to cisterna $n_0$ directly or via a more complex pathway (e.g., involving the ER or lysosomes) does not modify the steady-state profile.

The effect of the biochemical environment on protein retention corresponds to a variation of the transport coefficients $v_i$ and $D_i$ along the stack. Generically, protein movement in the Golgi can be written as a diffusion in an effective energy landscape $E(n)$ characterizing the protein's energy in the different cisternae, supplemented by an activation energy $\Delta E(n)$ associated with transport intermediates. In Appendix, we show that

$$D = \frac{k_B}{2} \left( 1 + \frac{e^{\Delta E}}{K} \right) \quad \text{and} \quad v = k_r \left( 1 - \frac{e^{\Delta E}}{K} \right),$$

with $k_0 = k_0 e^{-\Delta E(n)}$ and $k_{n+1} = k_0 e^{\Delta E(n)}$.

A landscape that promotes localization near a particular cisterna $n_0$ can locally be written as a quadratic potential: $E(n) = \frac{1}{2} K (n-n_0)^2$, where $K$ is the coupling strength. About half the proteins moving through such a landscape would be localized at or near the minimum $n_0$ with a spread $\Delta n = 1/\sqrt{K}$ cisternae. A bulk flow (e.g., due to cisternal progression) with velocity $v_p$ displaces the energy minimum by an amount $\delta n = v_p (K \Delta t)$ (Fig. 3B and SI Appendix). Thus, precise and robust localization require a large coupling strength $K \geq v_p/D_t$.

The landscape approach allows us to test the relevance of the hydrophobic mismatch mechanism, for which the energy $E(n)$ can be computed. The membrane thickness of organelles is known to continuously increase along the secretory pathway from about 3.7 nm in the ER to 4.2 nm at the plasma membrane (16), and proteins could be confined to membranes that best match the length of their hydrophobic domains. The energy of hydrophobic mismatch leads to a quadratic energy landscape with $K = \alpha K (\Delta h)^2 \approx 0.25 K \Delta h$ (17) ($\Delta h \approx 0.1$ nm is the mismatch between adjacent cisternae and $K_0 \approx 0.1$ J/m$^2$ is the bilayer stretching modulus). Hydrophobic mismatch can thus in principle localize proteins against thermal fluctuations with an accuracy of about $\Delta n \approx 1/\sqrt{K}$ cisternae, and protein localization is indeed known to be affected by the length of its transmembrane domain (18, 19). It is however not robust against variation of the anterograde flux because $K < v/D_t \approx 1$, consistent with the observation that the transmembrane domain length was not the sole factor affecting protein localization in the Golgi (18).

The two mechanisms above (localization by recycling and by an energy landscape) were used to analyze the distribution of the resident enzyme Man I in Arabidopsis thaliana Golgi stacks; SI Appendix, Fig. S2. This enzyme is localized to cisternae 3 and 4 of the stack with a 90% accuracy (20). Such strong confinement requires either fast recycling ($\Delta \approx 2.6 \mu s$) or a deep energy well ($K \approx 2.2$). Such large value of $K$ is inconsistent with retention solely based on hydrophobic mismatch ($K \approx 0.25$).

**Discussion**

**Cisternal Progression or Vesicular Transport.** Our framework produces two strong predictions: (i) the level of intercisternal exchange (although not its directionality) can be directly quantified by measuring the coarse-grained diffusion coefficient $D_t$, and (ii) measuring a convection velocity $v > 2D_t$ would necessarily imply some level of cisternal progression. We stress that cisternal progression cannot be disproved in case $v < 2D_t$, because this could correspond to progression combined with retrograde vesicular transport. Our analysis of the data clearly shows the existence of some degree of diffusion, including significant backward transport steps, both for the small membrane protein VSVG and for the large protein complex procollagen (Fig. 2). Furthermore, we find that $v \approx D_t$ for both species. This implies that (i) intercisternal exchange is confirmed in both cases, and (ii) cisternal progression cannot be proved or disproved by the existing transport data. We emphasize that this follows from a strict application of general transport principles, and reflects the inadequacy of the existing experimental data to be more discriminating. Detailed microscopic models used to interpret coarse-grained experimental transport data (10) should be viewed with caution, confirming both the utility and necessity of our coarse-grained approach.

Our analysis shows that procollagen is exchanged between cisternae despite its size. This is at odds with packaging and transport in conventional small protein-coated transport vesicles, and implies that transport is at least partly mediated by large "pleiomorphic membrane carriers" (PMCs), as sketched in Fig. 4. PMCs containing procollagen aggregates could take the form of "megavesicles" such as those involved in the transport of large (engineered) protein complexes (21), or of large tubulo-vesicular connections such as those connecting the Golgi to surrounding organelles (22). Such large transport intermediates have not yet been seen, but recent experiments suggest indirectly that large cargo can indeed be exchanged between cisternae (23). A mechanism based on lateral segregation in the cisternal membranes caused by a Rab cascade, the cisternal progenitor model (24), has recently linked the formation of large intra-Golgi transport carriers to the maturation of membrane components. The present work directly infers their involvement in intra-Golgi transport from quantitative transport data. Note that intercisternal exchange could be quite fast, so a given procollagen aggregate could only spend a very short fraction of its transit time outside cisternae. If an exchange step takes $\approx 1$ s and there are 10 such steps for a transit time of $\approx 15$ min, an aggregate spends about 99% of its time inside cisternae. This suggests that the formation of megavesicles could be a rare event.

Finally, it is intriguing that diffusion and convection are found to occur at similar rates ($v \approx D_t$) for both cargoes. This could indicate that these two processes share the same underlying mechanisms and/or that one process is coupled to the other, as
suggested by the cisternal progenitor model (24). More insight could be gained by comparing values for v and D; in different organisms. Scale-forming algae such as Scherffelia davia have a regularly stacked Golgi of 15–20 cisternae. Proteoglycan scales readily identifiable by EM and too large to fit in conventional transport vesicles transit through the stack without ever being seen outside cisterna (20). The absence of scale-containing megavesicles would imply that these scales undergo pure convection in the Golgi. A quantitative incoming wave experiment, yet unavailable to our knowledge, should produce data along the dashed lines of Fig. 2D, corresponding to the absence of diffusion.

**Experimental Proposal.** We have shown that all available data on the transport of two very different types of cargo through the Golgi (the small membrane protein VSVG and the large collagen complex procollagen) are reproduced by a model of intra-Golgi transport involving constant anterograde and retrograde transport rates, corresponding to a net constant velocity v and constant effective diffusion coefficient D. Models involving more than these two or equivalent parameters for intra-Golgi transport are not falsifiable by current transport experiments and should be treated with caution.

Our analysis shows that diffusion, a signature of intercisternal exchange, contributes to the transport of both types of cargo. This is rather surprising for the large protein complex procollagen and should therefore be confirmed by additional transport data with high statistical significance, and using a fast (<10 min) transport block protocol. We advocate the use of high-resolution microscopy instead of low resolution optical assays (FRAP), because the latter are dominated by the boundary conditions (Fig. 2A and B) and do not give sufficient insight into the intra-Golgi dynamics.

Direct evidence for cisternal progression may be obtained only if \( v > 2D \); however, our analysis of the transport data showed \( v \approx D \) for both types of cargo. More information on the nature of protein transport could be gained by studying correlation in the transport dynamics of different protein species. A promising technique is the newly developed Retention Using Selective Hooks method (25), which allows one to precisely control the release of proteins from the ER into the Golgi, following which their progression and export can be monitored by optical or electron microscopy.

More insight on the interplay between progression and exchange could be gained by comparing the dynamics of transiting and resident Golgi proteins. Monitoring the distribution and dynamics of resident proteins under conditions that affect the transport of transiting proteins could be a promising strategy, as the localization of resident proteins is affected by cisternal progression. One first needs to identify the mechanism by which particular resident proteins are localized, fast recycling (Fig. 3A) and localized retrograde transport related to an energy landscape (Fig. 3B) being the two generic ones. The distribution of resident proteins within the stack should then be determined, by high-resolution microscopy, under conditions affecting the transit time of proteins putatively transported by cisternal progression, such as drugs targeting the cytoskeleton. According to our predictions, particular distributions of transiting and resident proteins localized by retrograde transport should be displaced (and not broaden) toward the trans-Golgi face, under conditions that decrease the Golgi transit time (Fig. 3). Finding such correlations would bring support to the cisternal progression mechanism.

We close by recalling that transport solely based on cisternal progression cannot be reconciled with existing transport data (10). Exchange mediated by large membrane structures (the PMCs) seems the most reasonable compromise, and can be linked to bio-chemical maturation by the cisternal progenitor model (24). In fact, the distinction between cisternal progression and intercisternal exchange becomes less clear if transport involves large PMCs (Fig. 4) of size possibly close to the cisterna size, that undergo frequent scission and fusion. A more crucial question is whether there exists a bulk anterograde flow of material in the Golgi, or whether transport is mainly protein-specific. Dynamical correlation between different transiting proteins could inform us of the extent to which they use the same carrier. It would in particular be very interesting if the transport of VSVG, for instance, was increased by the presence of procollagen. That would suggest that procollagen can create its PMCs and that VSVG can be exchanged between cisterna by riding along these structures.

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Supplementary Information for: ‘Quantitative analysis of intra-Golgi transport reveals inter-cisternal exchange for all cargo’

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S1 Continuous approximation of the Master equation

In our article, we start from a Master equation written with a discrete spatial variable and convert it to a Fokker-Planck equation with a continuous spatial variable. While the spatially discrete structure of the Golgi apparatus leads to a spatially discrete transport equation, the continuous version is far more tractable and intuitive. We have checked numerically that this conversion only introduces small quantitative error.

The correspondence between discrete and continuous spatial variables has been abundantly discussed as it is a necessary step for the numerical analysis of many systems [1,2]. The well established Fokker-Planck equation:

\[ \partial_t A = \partial_n (D \partial_n A - vA) \]  

is obtained by expressing the spatial variations of a given quantity \( X \) around a position \( n \) using the symmetric spatial derivative:

\[ \frac{\partial X}{\partial n} \bigg|_n = \frac{X_{n+1} - X_{n-1}}{2\delta x} \]  

with \( \delta x \) the distance between two cisternae. Let use define \( X_{n+1/2} \) and write alternatively the derivative of \( X \):

\[ X_{n+1/2} = \frac{X_n + X_{n+1}}{2} \]

\[ \frac{\partial X}{\partial n} \bigg|_n = \frac{X_{n+1/2} - X_{n-1/2}}{\delta x} \]  

Let us note that Eq.(S3) yields the usual formulation for the second derivative:

\[ \frac{\partial^2 X}{\partial n^2} \bigg|_n = \frac{X_{n+1} + X_{n-1} - 2X_n}{\delta x^2} \]  

The discrete transport equation for inter-cisternal exchange reads

\[ \partial_t A_n = -(k_n + k'_n)A_n + k'_{n+1}A_{n+1} + k_{n-1}A_{n-1} \]  

Discretizing the Fokker-Planck equation (Eq.(S1)) using Eq.(S3) leads to:

\[ \partial_t A_n = D_{n+1/2} (A_{n+1} - A_n) - D_{n-1/2} (A_n - A_{n-1}) - \frac{1}{2} v_{n+1/2} (A_{n+1} + A_n) + \frac{1}{2} v_{n-1/2} (A_n + A_{n-1}) \]  

Identifying Eq.(S6) and Eq.(S5) yields:

\[ D_{n+1/2} = \frac{\delta x^2}{2} \left( k_n + k'_{n+1} \right) \]  

\[ v_{n+1/2} = \delta x \left( k_n - k'_{n+1} \right) \]  

In the following, we will normalize all distances by \( \delta x \) so as to have the normalized velocity and diffusion coefficient as rates in \( \text{min}^{-1} \). We will call them \( v_t \) and \( D_t \) respectively, as they stem from inter-cisternal transport. We allow for protein exit throughout the Golgi stack at a rate \( r_n \) for
cisterna \( n \). This may correspond to direct export toward lysosomes, or recycling to the E.R., and is written: \( \partial_t A_n = -r_n A_n \). There can also be localized influx of material, which we can write \( J_{in}^n \).

Adding a putative cisternal progression at a velocity \( v_p \), we find:

\[
\partial_t A = \partial_n (D_t \partial_n A - vA) - rA + J_{in}^n, \quad v = v_t + v_p
\]

(S9)

The in-flux \( J_{in}^n \) of a particular molecular species could be targeted at particular location of the Golgi stack other than the \( cis \) cisterna (particularly for \( medial \) and \( trans \) resident enzymes, see below). A flux targeted to cisterna \( n_0 \) can be written \( J_{in}^n = J_{in} \delta(n - n_0) \).

**S2 Solution of the Fokker-Planck equation**

There are many approaches to solving Eq.(S9). To solve this equation, we need to establish initial conditions (the concentration at \( t = 0 \)) and boundary conditions (conditions on the fluxes or the concentrations at \( n = 1 \) (\( cis \)-end) and \( n = N \) (\( trans \)-end)). At the \( trans \) boundary, molecules exit due to cisterna progression with a flux \( v_p A_N \), in which \( v_p \) is the progression velocity. In addition to this convective exit, there can be a diffusive exit from the first and last compartment, with a rate \( k_1' \) and \( k_N \) respectively. So-called \textit{reflective} boundary condition corresponds to setting this rates to zero, whereas any other value leads to a \textit{permeable} boundary.

For now, we assume that molecules only enter the Golgi stack through its \( cis \) face, at a rate \( J_{in}^1 = J_{in} \), we can write generic boundary conditions as:

\[
J^1 = J_{in} - k^- A_1 \quad J^N = v_{off} A_N
\]

with \( v_{off} = v + k^+ \), \( k^- = k_1' \)

(S10)

in which \( k^+ = k_N - v_t \). Stationary solutions can be found analytically, and the time-dependent solutions can be found numerically with any choice of boundary conditions in order to reproduce experimental results.

**S2.1 Stationary solutions**

The stationary solution of Eq.(S9) for the protein distribution in the Golgi under a constant in-flux \( J^1 \) at the \( cis \) boundary and an exiting flux \( J^N = v_{off} A_N \) may be found by setting \( \partial_t A = 0 \) for all \( n \), leading to:

\[
A(n) = \alpha \left( e^{\lambda_+ n} + \beta e^{\lambda_- n} \right)
\]

\[
\lambda_{\pm} = \frac{v}{2D_t} \left( 1 \pm \sqrt{1 + \frac{4rD_t}{v^2}} \right)
\]

(S11)

In which \( \alpha \) and \( \beta \) are found by applying the boundary conditions (S10). Some limiting cases are of particular interest.
- In case of large convection velocity: $v^2 \gg 4rD_t$, the asymptotic behavior of the two characteristic decay parameters are $1/\lambda_- \simeq -v/r$ and $1/\lambda_+ \simeq v/D_t$. The stationary protein distribution of proteins joining the Golgi at the cis face is thus exponential localized within a region $1/\lambda_- = v/r$ of the cis Golgi.

- In the absence of exit: $r = 0$, we find:

$$A(n) = J^1 \left( \frac{1}{v_{\text{off}}} - \frac{1}{v} \right) e^{\frac{x}{v_{\text{off}}}(n-N)} + \frac{1}{v}$$

(S12)

Which leads to:

$$A_N - A_0 = J^1 \left( \frac{1}{v_{\text{off}}} - \frac{1}{v} \right) \left[ 1 - e^{-\frac{v}{v_{\text{off}}}} \right]$$

(S13)

We find $A_N > A_0$ (resp. $A_N < A_0$) if $v > v_{\text{off}}$ (resp. $v < v_{\text{off}}$). Concentration gradients are mostly located near the trans end of the stack, within a region of size of order $\delta n = (A_N - A_0)/\partial_n A|_N$, with:

$$\delta n = \frac{D_t}{v} \left( 1 - e^{-Nv/D_t} \right)$$

(S14)

With $v \simeq D_t$, this size is of the order of (or bigger than) the size of a cisterna. Therefore, the solution to the continuous equation is quite close to the numerical solution of the discrete equation, which validates our choice of boundary conditions, and confirms that the continuous equation is a good device even for a 7-compartment system.

S2.2 Numerical simulations

To describe the experiments, we use a numerical simulation of the Fokker-Planck equation. In the particular case in which the convection speed is constant, we can use a moving frame to model convection, and a simple explicit Euler implementation of the diffusion in order to have an accurate simulation [1].

Let us introduce $dx$, the unit spatial step, $dt$, the time step for diffusion, and $\Delta t$, the time step for convection. Those parameters are linked by the relations:

$$v\Delta t = dx$$

(S15)

$$n = D_t \frac{dt}{dx^2} \quad n \gg 1$$

(S16)

$$N = \frac{\Delta t}{dt} \quad N \gg 1$$

(S17)

The algorithm can be written as follows:

while $t < T_f$:

$t = t + \Delta t$


$A[0] = J_{\text{on}}$

$k = 0$
for k=1 to N:
    AL = A[1]
    AR = A[Lx−1]
    A[0]=A[0]∗(1−1/n−kL/n)+AL/n+kL∗Jon∗dt
    A[Lx]=A[Lx]∗(1−1/n−kR/n)+AR/n

We can run this simulation over a wide range of parameters and find the best fitting set of parameters. As mentioned in the main text, fits of the FRAP experiments (Fig.2b-c, main text) are quite degenerate. Several sets of parameters can reproduce experimental results with reasonable accuracy. Fitting the protein distribution across the stack (Electron Microscopy assays) yields a more quantitative conclusion, in every case supporting the existence of inter-cisternal exchange ($D_t \neq 0$, see Fig. 2c-d, main text). Variability within cells across space and time and between different cells could smoothen concentration gradients in a way qualitatively similar to inter-cisternal exchange. In the case of the procollagen exiting wave experiment (Fig.2d main text), experimental error bars are below 10% [4], which give high confidence to our conclusions. Transport blocks are not instantaneous, but require a finite time to be established, which may also smoothen concentration gradients. To mimic this, the time-dependent influx was made to vary smoothly from its physiological value $J_{max}$ to a small value $J_{min}$ over a “block time” $\tau$: $J_{in}^{1} = J_{min} + (J_{max} - J_{min})e^{-t/\tau}$. The fit given in the main text, Fig.2d, corresponds to $\tau \to 0$ (perfect block). Fig.S1 shows the equivalent fits for $\tau = 5$ min and $\tau = 11$ min. A non-zero diffusion coefficient, supporting the occurrence of inter-cisternal exchange of procollagen complexes, must be invoked to explain the data provided the block time is faster than $\approx 10$ min, which seems a quite reasonable assumption.

S3 The Golgi stack as an energy landscape

Each cisterna along the stack is characterized by a specific biochemical and physical identity which can affect protein dynamics, and one may expect the transport rates $k$ and $k'$ appearing in Eq.(S5) (and their continuous counterparts $D_t$ and $v = v_p + v_t$ in Eq.(S9)) to vary along the Golgi stack. Location-dependent transport rates, which reflects the preference of a resident protein to a particular cisterna $n$, can have two distinct origins; variations of the local environment within cisternae, which directly influences the local protein’s energy (called $E(n)$), and variation the protein energy state in transport intermediates between cisternae, which can formally be accounted for by an activation energy (called $\Delta E(n)$ for the $n \to n+1$ step). Adopting an Arrhenius’ formalism for the exchange rates [5], one may generically write

$$k_n = k_0 e^{-\Delta E(n)} \quad k'_{n+1} = k_n e^{\delta_n E(n)}.$$  \hspace{1cm} (S18)

The two energy functions are defined with respect to the reference energy available from the environment to perform the transition. That is the thermal energy $k_B T$ for thermally activated processes, and of order $20k_B T$ for processes optimally utilizing the hydrolysis energy of one ATP molecule. The typical rate $k_0$ at which a given protein encounters the energy barrier is limited by diffusion within individual cisternae. For a membrane protein like VSVG with a lateral diffusion coefficient $D_m (\approx 0.15 \mu m^2/s)$ [6,7], diffusing in a cisterna of lateral size $R (\approx 0.5 \mu m)$, one expects $k_0 \sim D_m/(\pi R^2) \approx 10/\text{min}$ (see Section S5.1). This is about 50 times faster than the exchange rates $0.2–0.3/\text{min}$ inferred from the VSVG data, which suggests the presence of an energy barrier.
of order $\Delta E = 3 - 4$, independent of $n$, associated to VSVG inter-cisternal exchange. Possible microscopic models for slow transport rate are discussed in Section S5.1.

Inserting Eq.(S18) into Eq.(S9), we find:

$$D_t = k_n \left( 1 + e^{\partial_n E} \right) / 2 \simeq k_n (1 + \partial_n E / 2)$$

$$v_t = k_n \left( 1 - e^{\partial_n E} \right) \simeq -D_t \partial_n E$$

(S19)

The velocity $v_t$ is directly related to the diffusion coefficient $D_t$, as they result from similar processes (transport between cisternae), but unlike $D_t$, $v_t$ is entirely controlled by the gradient of cisternal properties. Cisternal progression can be included in this formalism by defining an effective energy landscape $\tilde{E}_n = E_n - v_p n / D_t$, where the progression velocity $v_p$ is the same for all protein species and independent of $n$. The global convection velocity $v$ accessible to experiments is then $v = -D_t \partial_n \tilde{E}$.

Though the exchange rates can always be expressed by Eq.(S18), $E$ and $\Delta E$ can be seen as physical energies only if the inter-cisternal exchange satisfies detailed balance, which is not required for non-thermal processes. Eq.(S18) shows that the energy barrier $\Delta E$ sets the time scale of inter-cisternal exchange, and Eq.(S19) shows that gradient of either $E$ or $\Delta E$ correspond to a bias for anterograde or retrograde transport rates (gradients of $\Delta E$ lead to gradients of the diffusion constant $D_t$, which result in a net displacement of proteins along the stack even if $v = 0$). This extended formalism can describe both resident and cargo proteins, whether they are transported by cisternal progression, asymmetrical vesicular exchange or combinations thereof, with the same generic equation. Protein specificity only influences the values of transport rates, and not the form of the transport equation. In addition to its practical interest for directly comparing the transport of different species, our formalism also permits to quantitatively test the different mechanisms that have been proposed for protein retention within a single framework, as we show below.

## S4 Resident proteins

We are interested in knowing how proteins can be localized in a region of the Golgi apparatus despite the diffusion $D_t$ and the velocity $v$. There are basically two ways to localize proteins in such a case: either by quick exit (with a rate $r$) of these proteins, or by the existence of a well in the energy landscape. Since we now focus on resident Golgi proteins, we are interested in a quasi-stationary distribution (in opposition with the transient distribution of a pulse).

### S4.1 Localization by recycling

If the influx of protein is targeted to a particular cisterna $n_0$ instead of the cis face, it can be written: $J_{in}(n) = J_{in} \delta(n - n_0)$, and Eq.(S9) becomes

$$\partial_t A = \partial_n (D_t \partial_n A - v A) - r A + J_{in} \delta(n - n_0)$$

(S20)

One has to solve this equation left and right of $n_0$, with $A$ being continuous at $n_0$ and with aforementioned boundary conditions at the cis and trans faces. If $n_0$ is sufficiently distant from the
cis and trans cisternae (compared to $\lambda_{\pm}$), the stationary protein distribution $A_{\text{stat}}$ is not affected by Golgi boundaries. This can be seen in experiments on the localization of the mannosidase manI in *Arabidopsis*, where the concentration on the first and last cisternae is flat and close to zero.

Therefore, using Eq.(S11) with the stationary condition $\int_0^N A_{\text{stat}}dn = J_{\text{in}}/r$, we find:

$$A_{\text{stat}, \pm} = \frac{J_{\text{in}}}{\sqrt{v^2 + 4rD_t}} e^{\lambda_{\pm}(n - n_0)} \quad , \quad \lambda_{\pm} = \frac{v}{2D_t} \left( 1 \pm \sqrt{1 + \frac{4rD_t}{v^2}} \right)$$

(S21)

where $A_{\text{stat}, +}$ is valid when $n < n_0$ and $A_{\text{stat}, -}$ when $n > n_0$. The stationary distribution is asymmetric, as discussed in the main text (see Fig.4a).

S4.2 Localization by a potential energy

If protein localization is promoted by its interaction with the local environment of a cisterna, its transport amounts to a diffusion through an energy landscape $E(n)$ (see Eq.(S19)), which is described by the Fokker-Planck equation:

$$\partial_t A = \partial_n (D_t \partial_n A - vA) \quad , \quad v = D_t \partial_n E$$

(S22)

We consider the simplest form of a potential well, i.e. a quadratic energy:

$$E(n) = \frac{1}{2} K(n - n_0)^2$$

(S23)

Solving the stationary concentration of $A$ requires the knowledge of the flux leaving the Golgi, as a few molecules can escape the potential well if they reach the borders by diffusion. However, if this outward flux is small enough, the concentration in the Golgi is quasistatic, i.e. it is close to the stationary solution. At first order the stationary distribution of proteins is:

$$A = A_0 e^{-\frac{K(n - n_0)^2}{2}}$$

(S24)

Where $A_0$ is proportional to the total concentration $A$. At first order, for strong enough potential, $A_0 \simeq A\sqrt{\frac{K}{2\pi}}$. The spread of the protein distribution is $1/\sqrt{K}$ and therefore a coupling strength $K \propto 1/\Delta n^2$ is required to localize proteins in a zone of size $\Delta n$. This computation yields information on the spread in the localization of Golgi enzymes subjected to an energy landscape, but we can also be interested in the robustness of this localization, for enzymes undergoing fluctuations of, for instance, the velocity $v$.

A change $\delta v$ in the velocity can be added as a linear term in the energy (as is clear from Eq.(S22)) and the effective energy can be written $\tilde{E} = E - \delta vn/D_t$. Therefore, the minimum of the energy potential is shifted from $n_0$ to $n_0 + \delta n$, with :

$$\delta n = \frac{\delta v}{K D_t}$$

(S25)

Therefore, the localization of a resident enzyme is robust only if $\sqrt{\langle \delta v^2 \rangle} \leq KD_t$, in which $\sqrt{\langle \delta v^2 \rangle}$ represent the root mean square fluctuations of the velocity in the Golgi. Note that since the
curvature of the energy landscape, which determines the spread of the protein distribution, is
independent of the progression velocity: $\partial^2_n \tilde{E} = \partial^2_n E$, the width of the distribution is not affected
by cisternal progression.

**S4.3 Measuring the control parameters**

Both localization mechanisms above result in a localized stationary protein distribution in the Golgi
(Eqs. S21,S24). The two profiles are distinct; recycling produces an asymmetric profile, while an
potential wells produces a Gaussian profile (see Fig.4, main text). This difference might however not be
sufficient to discriminate between the two strategies based on experimental measurements due to
low resolution or low statistics (see Fig.S2). The value of the control parameters (the exit rate $r$
or the potential strength $K$) may however give insight on whether a particular localisation mechanism
is realistic. Fig.S2 shows experimental data on the resident protein Man I in *Arabidopsis* [8] and
concentration profiles obtained with the two schemes described here (localization by recycling and
localization by an energy landscape). We find that localization by recycling requires $r \gtrsim 2.6v$
(assuming $D_t \simeq v$), and that localization in an energy landscape requires $K > 2.2$. As discussed in
the text, such strong value of $K$ cannot be explained by energetic considerations such as hydrophobic
mismatch. It could however stem from an asymmetry of the transport transport rates $k_n$ and $k'_n$. In
the absence of cisternal progression ($v_p = 0$), we may use Eq.(S18) to conclude that the asymmetry
of the exchange rates that would amount to such potential is of order $k_2/k'_3 \simeq k'_2/k_4 \simeq e^K \simeq 10$. The
anterograde transport rate would thus needs to be about ten times larger (respectively smaller) than
the retrograde transport rate at the *cis* (respectively *trans*) Golgi side of the cisterna of residence.

**S4.4 Residence times of Golgi enzymes**

We can use the previous results to compute the residence times of Golgi enzymes (i.e. the time
spent in the Golgi before being recycled).

- Protein localized by fast recycling outside the Golgi only spend a short time in the Golgi, and
  their residency time is simply $1/r$. The slower the recycling is ($r < v/N$), the weaker protein
  localization, and proteins may also exit by the *trans* Golgi face, reducing their residence time. Note
  however that the proteins exiting a given cistern with the rate $r$ may be directly recycled to another
  Golgi cisterna, as discussed in the main text. In this case, the total protein content of the Golgi
  would remain constant in time, and the recycling dynamics would not be seen of coarse-grained
  optical measurement such as the FRAP protocol of [11].

- Enzymes localized by a well in the energy landscape exit because they diffuse in this well and
  may reach the Golgi borders. If $J_{\text{out}} = -v_{\text{off}} A(t,L)$, then we find a characteristic residence time
  $\tau_0 = A/J_{\text{out}}$ to be

  $$\tau_0 = \frac{1}{v_{\text{off}}} \sqrt{\frac{2\pi K e^{K} N - n_0}}{2}$$

  (S26)

  It is interesting to compute the influence of fluctuations of the velocity on the residence time, as
  we did concerning the robustness of localization. In the presence of an additional velocity $\delta v$, the
  energy landscape is changed to $\tilde{E} = E - \delta vn/D_t$ and the position of the minimum is shifted by
\[ \delta n = \delta v/KD_t. \] One can thus easily compare the average residency time with and without flux. As long as \( \delta n \ll N \), we find:

\[ \frac{\tau_v}{\tau_0} \simeq e^{-K\delta n(N-n_0)} \simeq e^{-\frac{\delta v}{D_t}(N-n_0)} \quad (S27) \]

This is an interesting result: the ratio of residence times does not depend upon the stiffness \( K \) of the potential well, and only depends on the variations of the Peclet number \((Pe = vN/D_t)\). The residence time may thus be expected to decrease exponentially with increasing convection velocity.

**S5 Connecting coarse-grain parameters to microscopic processes**

**S5.1 Microscopic model for the effective diffusion coefficient**

The typical exchange rates discussed in the main text may be written \( k = k_0e^{-\Delta E} \), where \( k_0 \) is the typical rate of encounter between a diffusing protein and a particular target where transport can take place (a tubule or a maturing protein-coated pit) and the exponential factor expresses the possible existence of an energy barrier \( \Delta E \) associated to the transport. The diffusive motion in search of a target on the cisterna membrane is itself characterized by a diffusion constant \( D_m \); a 2-dimensional diffusion for membrane proteins and protein aggregates larger than the cisterna thickness, and a 3-dimensional diffusion for small cytosolic proteins. \( 1/k_0 \) is the average time needed to find the target, starting anywhere on the cisterna (which has two faces). Since one face of a cisterna has an area \( \pi R^2 \), where \( R \) is the radius of a cisterna, one can expect the scaling \( k_0 \sim D_m/(\pi R^2) \).

The fact that a protein coming on one side of a cisternae must first cross its edges to find a tubule on the other side, or a vesiculation spot near the cisterna edges, introduces a logarithmic correction of order \( \log \frac{R^2}{a^2} \) to the rate of encounter \( k_0 \), where \( a \) is the size of the target \([9]\). We have checked by numerical simulations that the expression \( D_t = k_0 = D_m/(\pi R^2) \) produces a good approximation for the diffusive transport through a stacked structure of circular compartments with tubular connections, in the absence of an energy barrier to enter the connections.

**S5.2 Various scenarios for slow inter-cisternal exchange**

In the main text, we provided evidence that small membrane proteins (VSVG), but also large protein complexes (PC) are exchanged between cisternae with typical rates of order \( 0.2 - 0.3/\text{min} \). These rates are limited either by the rate of physical transport of material between cisternae, or by the rate of biochemical maturation within cisternae (i.e. a protein needs to undergo all the maturation steps before being exported).

Physical transport between cisternae is thought to involve either vesicular transport or tubular connections \([10]\) (Fig.1 - main text). Both mechanisms involve the diffusive search for scarce “hot spots”; the entrance of a tubule or a spot of vesicular secretion. As discussed above, one expects that transport rates are of order \( k_0 \sim D_m/(\pi R^2) \), where \( R \simeq 0.5 \mu m \) is the lateral size of a cisterna and \( D_m \) is the lateral diffusion coefficient (of order \( D_m \sim 0.15 \mu m^2/s \) for membrane proteins like VSVG, \([6, 7]\)). One finds \( k_0 \simeq 10/\text{min}, \) which is about 50 times faster than the exchange rates.
inferred from data, which could suggest that biochemical maturation is the rate-limiting step for Golgi trafficking. Several other factors could however slow down the exchange process:

i) Drawing inspiration from activated processes [5], the rate \( k \) could be hindered by an energy barrier \( \Delta E \), yielding \( k \sim k_0 e^{-\Delta E} \). An energy barrier of order \( \Delta E = 3 - 4 \) (in units of \( k_B T \) if transport is thermally activated) would lead to exchange rates similar to the observations. Such a barrier could exist if transport requires either to create or to diffuse through membrane regions of high curvature \( C \). One would then expects \( \Delta E \sim \kappa sC^2 \), in which \( \kappa \simeq 5 - 20k_B T \) is the membrane bending rigidity and \( s \) the protein area. Such energy barriers would not hinder the diffusion of small membrane proteins such as VSVG (\( \Delta E \sim 0.15k_B T \) for \( s = 5 \text{ nm}^2 \) and \( C = 2/(50 \text{ nm}) \)), but could considerably reduce the transport of protein complexes like PC that may need to create their own membranous transport intermediates (\( \Delta E \sim \kappa \) for \( sC^2 \sim 1 \)).

ii) Not invoking the existence of an energy barrier, protein diffusion might be slower than expected. A large fraction of VSVG (about 95%) appears rather static in Golgi membranes [11, 6], possibly because of its segregation within membrane domains and/or membrane-cytoskeleton interaction. Protein diffusion is only effective in the mobile state, so the effective diffusion coefficient (for all proteins) could be of only about 5% of the microscopic one, leading to an inter-cisternal transport rate of \( 0.5/\text{min} \), close to the fitted value for VSVG.

iii) Finally, inter-cisternal exchange could be limited by the growth kinetics of membrane domains (for instance Rab in the cisternae progenitor model [12]) which need to reach the size \( l_c \) of a transport intermediate. Domain growth is a slow collective process and the domain size usually increases as a power law of time: \( l(t) \propto t^\alpha \). In some circumstances, the Lifshitz-Slyosov-Wagner theory [13] predicts \( l(t) = (At)^{1/3} \) with \( A \simeq D_m \phi s / k_B T \), where \( \gamma \) is the domain line tension, \( \phi \) is the surface fraction and \( s \) the area of the molecules undergoing aggregation. In this model, the exchange rate is very sensitive to the size \( l_c \) of the carrier. Using \( D = 0.15 \mu\text{m}^2/\text{s} \), \( \gamma = 1 \text{ pN} \), \( \phi = 5\% \) and \( s = 5 \text{ nm}^2 \), one finds \( k \simeq 5/\text{min} \) with \( l_c = 50 \text{ nm} \) and \( k \simeq 0.5/\text{min} \) for \( l_c = 200 \text{ nm} \).

References


  Schwartz. High-density mapping of single-molecule trajectories with photoactivated localiza-


  Cis-golgi cisternal assembly and biosynthetic activation occur sequentially in plants and algae.


   at different levels of the golgi complex in glucose-stimulated mouse islet beta cells. *Proc Natl

    Transport through the golgi apparatus by rapid partitioning within a two-phase membrane


Figure Captions

Figure S1: Fitting electron microscopy assay of an exiting procollagen wave, assuming constant \( v \) and \( D_t \), for different block timescales \( \tau \). For \( \tau < 10 \) minutes, a non-zero diffusion coefficient is required.

Figure S2: localization of the resident Golgi protein mannosidase I (ManI) in Golgi stacks of Arabidopsis (red dots, [8]). The best fit with the localization by recycling scheme is shown in gray (using Eq.(S21)), and using the energy landscape scheme is shown in black (using Eq.(S24)). Other transport parameters were chosen to match the value obtained from transit proteins (\( v = D_t = 0.3/\text{min} \), Fig.2 main text), although these were obtained in a different organism.