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[Continued on next page]

(54) Title: METHODS OF MEASURING PROTEIN STABILITY

FIG. 9A

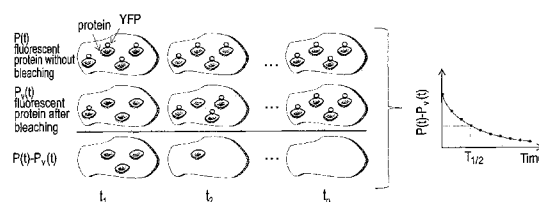


FIG. 9B

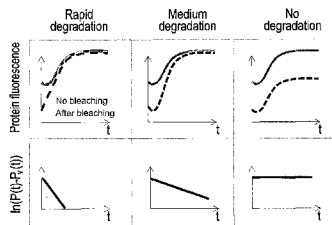
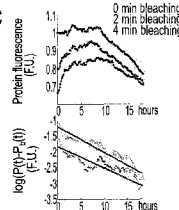


FIG. 9C



(57) **Abstract:** A method of determining a degradation rate of a fluorescently labeled polypeptide of interest in a population of cells is provided. The method comprises: (a) obtaining two identical populations of cells; (b) determining fluorescence levels of the fluorescently labeled polypeptide of interest in a first of the at least two populations of cells at at least two different points in time; (c) at least partially reducing a fluorescence of the fluorescently labeled polypeptide of interest in a second of the at least two populations of cells; (d) determining fluorescence levels of the reduced fluorescently labeled polypeptide of interest in the second of said at least two populations of cells at said at least two different points in time; (e) comparing fluorescence levels in the first and the second populations; and (f) based on the comparing, determining a degradation rate of the fluorescently labeled polypeptide of interest.



— with sequence listing part of description (Rule 5.2(a))

METHODS OF MEASURING PROTEIN STABILITY

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to a method of measuring protein stability inside a cell and system capable of same.

Protein molecules are continuously synthesized and degraded in all living organisms. The concentration of individual cellular proteins is determined by a balance between the rates of synthesis and degradation, which in turn are controlled by a series of regulated biochemical mechanisms. Differences in the rates of protein synthesis and breakdown result in cellular and tissue atrophy (loss of proteins from cells) and hypertrophy (increase in protein content of cells).

The dynamics of proteins are controlled at several levels, including transcription translation and degradation. In bacteria and single celled eukaryotes, transcription is probably the most important determinant of protein levels [1-7]. Although translation and degradation control are important in these organisms, much information can be gleaned from transcription assays. In contrast, in organisms such as mammals, the correlation between mRNA levels and protein levels is less clear. Snapshots of mRNA and proteins at a single time point suggests low correlation across different genes in humans and mouse [8-13]. Current technology makes it difficult to go beyond snapshots, especially due to the challenge of accurate proteomics.

Analysis of post-transcription control in mammalian cells is challenging. In the classic cell-cycle experiment that proved the existence of cyclins, Hunt et al., [Evans, T. *et al. Cell* 33, 389-396 (1983)] labeled sea urchin eggs with radioactive methionine and used pulse-chase analysis to show periodic destruction of a mysterious band at distinct time points after fertilization. Similar pulse-chase experiments with and without protein-synthesis inhibitors are now commonly used to measure the stability of individual proteins, but these assays are not amenable to high-throughput characterization of mammalian cells. Mass spectrometry and stable isotope-labeling approaches have been used to characterize protein turnover on a larger scale but have suffered from insufficient dynamic range and the confounding effects of cell growth and ongoing protein synthesis [19]. In yeast, high-throughput studies have defined half-lives for >3,750 proteins using a comprehensive library of genetically tagged strains and Western

blot analysis [Belle, A. et al. Proc. Natl. Acad. Sci. USA 103, 13004–13009]. However, the effects of epitope tags on transcription and translation remain as confounding factors, and no such integrated expression library exists for mammalian cells. A limitation common to all these methods is that protein stability is measured at the population level rather than in individual cells.

Yen et al [20] teach a method for determining global protein stability (GPS) profiling that couples flow cytometry with microarray technology. The method comprises delivery of a retroviral, dual-fluorescent reporter system that measures the abundance of target proteins while simultaneously controlling for experimental variability and cell-to-cell differences in transcription and translation.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a method of determining a degradation rate of a fluorescently labeled polypeptide of interest in a population of cells, the method comprising:

(a) obtaining two identical populations of cells;
(b) determining fluorescence levels of the fluorescently labeled polypeptide of interest in a first of the at least two populations of cells at at least two different points in time;

(c) at least partially reducing a fluorescence of the fluorescently labeled polypeptide of interest in a second of the at least two populations of cells;

(d) determining fluorescence levels of the reduced fluorescently labeled polypeptide of interest in the second of the at least two population of cells at the at least two different points in time;

(e) comparing fluorescence levels in the first and the second populations;
and

(f) based on the comparing, determining a degradation rate of the fluorescently labeled polypeptide of interest.

According to some embodiments of the invention, step (f) is effected by calculating the ratio of the (natural log (ln) of the difference in fluorescence of the polypeptide of interest in the first population of cells and fluorescence of the polypeptide of interest in the second population of cells at a first point in time): (ln of

the difference in fluorescence of the polypeptide of interest in the first population of cells and fluorescence of the polypeptide of interest in the second population of cells at a second point in time) divided by the difference in time between the two points in time.

According to some embodiments of the invention, step (f) is effected by
5 determining a slope of a graph, wherein a y axis of the graph indicates the $\ln(P(t) - P_v(t))$ and an x axis of the graph indicates time, wherein $P(t)$ is the amount of fluorescence of the polypeptide of interest at point t in time in the first population of cells and $(P_v(t))$ is the amount of fluorescence of the polypeptide of interest at point t in time in the second population of cells.

10 According to some embodiments of the invention, step (c) is effected with a mercury fluorescent lamp.

According to some embodiments of the invention, step (c) is effected for 1-8 minutes.

According to some embodiments of the invention, the population of cells
15 comprises an additional fluorescently labeled polypeptide, the additional fluorescently labeled polypeptide having a higher nuclear: cytoplasm expression ratio and the additional fluorescently labeled polypeptide being distinguishable from the fluorescently labeled polypeptide of interest.

According to some embodiments of the invention, the polypeptide of interest is
20 transcribed from its native location in the population of cells.

According to an aspect of some embodiments of the present invention there is provided a method of analyzing the effect of an agent on a degradation rate of a polypeptide of interest in a population of cells, the method comprising:

(a) contacting a population of cells with the agent;
25 (b) determining the degradation rate of the fluorescently labeled polypeptide of interest in the population of cells according to the method of the present invention; and

(c) comparing the degradation rate obtained in step (b) with a degradation rate of the polypeptide in an absence of the agent, thereby analyzing the effect of the
30 agent on the degradation rate of the polypeptide of interest.

According to an aspect of some embodiments of the present invention there is provided a system for determining a degradation rate of a fluorescently labeled

polypeptide of interest in a cell comprising a processing unit, the processing unit executing a software application configured for converting change in fluorescence levels over a period of time to a degradation rate.

According to some embodiments of the invention, the degradation rate is
5 determined according to the method of the present invention.

According to some embodiments of the invention, the system further comprises an imaging system.

According to some embodiments of the invention, the imaging system comprises an image capture apparatus.

10 According to some embodiments of the invention, the image capture apparatus is capable of capturing an image of the fluorescently labeled polypeptide over the period of time.

According to some embodiments of the invention, the imaging system further comprises a data processor for calculating a fluorescence level for each picture element
15 of the image.

According to some embodiments of the invention, the image capture apparatus is a fluorescent image capture apparatus.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which
20 the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

25 Implementation of the method and/or system of embodiments of the invention can involve performing or completing selected tasks manually, automatically, or a combination thereof. Moreover, according to actual instrumentation and equipment of embodiments of the method and/or system of the invention, several selected tasks could be implemented by hardware, by software or by firmware or by a combination thereof
30 using an operating system.

For example, hardware for performing selected tasks according to embodiments of the invention could be implemented as a chip or a circuit. As software, selected tasks

according to embodiments of the invention could be implemented as a plurality of software instructions being executed by a computer using any suitable operating system. In an exemplary embodiment of the invention, one or more tasks according to exemplary embodiments of method and/or system as described herein are performed by a data processor, such as a computing platform for executing a plurality of instructions. Optionally, the data processor includes a volatile memory for storing instructions and/or data and/or a non-volatile storage, for example, a magnetic hard-disk and/or removable media, for storing instructions and/or data. Optionally, a network connection is provided as well. A display and/or a user input device such as a keyboard or mouse are optionally provided as well.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying images. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-K are graphs and spectra illustrating that anti-cancer drug, CPT induces waves of time-dependant mRNA changes across different groups of functionally related genes. GO enrichment analysis was applied in order to indentify groups of genes sharing the same function, process or localization that also respond similarly to the drug. To this end, genes were ranked according to their level of expression at each time point and given as input to the Gorilla software which identifies and visualizes the enriched GO terms (for details see Method). Many cellular processes and functions were identified that responded in concert to the drug in a statistically significant manner ($P < 10^{-4}$ after multiple hypothesis correction) thus revealing different affects of the drug. In the figure, each enriched GO-term is depicted by three subplots. The top subplot is a 'GO-Gram' that shows the enrichment log P-value of the GO-term as a function of time with positive and negative values indicating whether the enrichment was attained in over-expressed or under expressed genes (values are

truncated at ± 20). The middle and bottom subplots show the average and the individual mRNA dynamics of all the genes associated with the GO-term. (A) Histones and genes responsible for nucleosome assembly are dramatically down regulated immediately upon drug addition ($P < 10^{-60}$) and then gradually recover to about half of the initial levels. (B) Transcription factors that control expression prior to drug addition are shut-down immediately upon drug addition followed by rise of an alternative set of transcription factors after 1.5 hours. (C) Surprisingly, stress related genes and DNA repair genes are significantly down-regulated 1.5 – 30 hours after the drug followed by a recovery. (D) Cell division related genes and DNA replication are down regulation ~5 hours after drug addition. This is in line with observations that the cells stop dividing after the drug and reach a complete halt after ~5 hours. Interestingly, an over-expression spike is observed of a few genes regulating cell proliferation just before the decrease in mitosis related genes begins. (E) RNA processing declines in response to the drug. (F) The drug induces a spike of kinase regulating genes, followed by a decrease of serine/threonine kinases. (G) MHC-I related genes exhibit a time-dependant increase in response to the drug. This finding is consistent with the role of MHC-I in other stress responses [1]. (H - K) Additional significant response include: increased expression of energy and metabolite generation related genes, down regulation of cytoskeletal related genes that is correlated in time with our measured decrease in cell motility, a transient decrease and then recovery of ubiquitine dependant catabolic processes and an increase in lysosomal genes.

FIG. 2 is a bar graph illustrating mRNA-protein temporal correlation distribution in real and shuffled data. The correlation distribution of mRNA and protein temporal drug responses is indicated by the gray bars. This was compared to the correlation distribution generated by pairing of the protein profiles with mRNA profiles that were randomly picked (blue bar). Note that the actual profiles were not shuffled, only the pairing. The empirical distribution showed about 15 % more positive correlations than in the random pairing distribution ($KS P < 10^{-6}$).

FIG. 3 are photographs and readout illustrating that the co-dynamics of 540 proteins and their respective mRNAs transcripts were measured for 48 hours after the addition of the anti-cancer drug, CPT. Levels of 540 endogenously YFP tagged proteins were measured for 48 hours (images were taken every 20 minutes) in human cancer cells

using time laps fluorescence microscopy. Custom made software was used for automatic video analysis including automatic cell tracking, phenotype detection and protein information retrieval. Additionally, the mRNA levels of all human transcripts were measured under the same conditions using Affymetrix GeneChip Human Gene 1.0 ST arrays at time points 0, 1.5, 3, 6, 9, 12, 23, 33, 48 hours after drug addition. Proteins were coupled to their respective mRNA resulting in a dataset of 540 protein-mRNA co-dynamic profiles.

FIG. 4 is a workflow of PhenoTrack – a system for automatic cell tracking and phenotype retrieval from time-laps movies. PhenoTrack receives as input 3 videos: phase contrast, protein tagged with YFP and cherry tag for image analysis. The output includes information on the individual cell including: protein levels, cell sizes, velocities, mitosis rate, time of death (in case cell dies) etc. Information is stored in a database and can be visualized.

FIGs. 5A-B are graphs illustrating day to day mRNA level reproducibility error is better than 15%. (A) the CV (std/mean) of all genes over all time points were computed using at least three independent experiments. The attained CV distribution has a median of less than 15% indicating that changes in the dynamics that are larger than 20 % can be detected. (B) Examples of mRNA fold change and the corresponding standard error ($std/\sqrt{n}$) over day to day repetitions. It can be seen that the measured mRNA dynamics is substantially larger than the experimental error.

FIG. 6 are graphs illustrating mRNA measurements obtained by microarrays were validated using qPCR. The drug response of 10 genes were measured using qPCR at times 0, 2, 9, 23 33 and 48 hours after drug addition (with at least three biological replicates at each time point) and compared to the measurements obtained using microarrays. The mRNA levels were normalized to levels before drug addition ($t = 0$) with blue and red lines indicate the qPCR and microarray mRNA measurements respectively. The control house keeping gene GAPDH, retained constant levels and hence there was no need to use it for normalization. The profiles obtained by the qPCR and the microarray are highly similar (median correlation $r = 0.83$).

FIG. 7 are graphs illustrating that mRNA and protein pairs fall into major co-dynamic clusters. The temporal responses of mRNA and protein pairs were

concatenated and clustered using k-means algorithm with 4 seeds. A few distinct general patterns were observed: (i) pairs in which the mRNA level is increases and the protein levels decrease in an anti correlated fashion; (ii) pairs in which mRNAs decreases and protein is constant or increases. (iii) degrading proteins whose mRNA
 5 first degrades and then starts to build up and (iv) correlated mRNA and proteins both of which have a short period of increase followed by a decrease.

FIGs. 8A-I are graphs illustrating that the response of most of the transcriptome and proteome is dis-correlated in time. (A) The distribution of the temporal correlation of 540 protein-mRNA pairs in response to a drug is shown. Approximately 30 % of the
 10 pairs showed a strong positive temporal correlation ($\rho > 0.6$); 15% were negatively correlated ($\rho < -0.6$) and the remaining 55% showed a weak or no correlation. (B) Examples of protein-mRNA pairs showing different type of behaviors: positive correlation (MYH9), time shift positive correlation (CKS2), no-correlations (EIF4A1, HDAC) and negative correlations (NCBP2, ENO1). (C - F) Functional groups of genes
 15 show a systematic dis-correlation or correlation between their proteins and mRNAs. (C, D) Ribosomal proteins and their mRNAs are dis-correlated in a systematic manner. Each row corresponds to a different ribosomal protein and its respective mRNA with yellow/red and red/green indicating high and low levels respectively. Protein and mRNA levels of each pair were linearly scaled between -1 and 1. While the ribosomal
 20 proteins decay in response to the drug their respective mRNAs increase in the first 12 hours and then begin to decrease after 24 hours. (E) Average response of ribosomal proteins and mRNAs (indicated by blue and red lines respectively) is weakly correlated ($r = 0.37$). Protein averages were normalized to $t = 0$ hours. (F, G) The dynamics of most cytoskeletal associated proteins are synchronized with their mRNA dynamics. (H)
 25 Average response of cytoskeletal associated proteins and their mRNAs is highly correlated ($r = 0.86$). (I) mRNA levels do not correlate well with relative protein levels across different genes. Each dot represents a pair of mRNA and protein measurements at a single time point. Pearson correlation across all mRNAs and protein abundances was found to be weak ($r = 0.13$) yet significant ($P < 10^{-9}$).

30 FIGs. 9A-C are graphs and drawings illustrating the difference between bleached and non-bleached protein fluorescence decays in time at a rate that is exponential in the protein degradation rate. (A) The bleach-chase protocol is illustrated:

protein fluorescent levels are measured at different time-points in bleached and non-bleached cells. The levels of the non-fluorescent proteins in the bleached cells are indirectly measured by subtracting the two profiles. Their decay in time reveals the protein degradation rate and half life. (B) The top panel illustrates three proteins that respond to the same stimulus given at $t = 0$ without bleaching (blue lines) and with bleaching (red broken lines). The log rate at which the bleached and un-bleached protein levels converge in each of the examples is indicated in the lower panel. The slope reveals the degradation rate according to the following equation:

$$\ln(P(t) - P_v(t)) = \ln(P_0 - P_{0v}) - \alpha \cdot t, \text{ where } P \text{ and } P_v \text{ are the protein levels with and without bleaching and } \alpha \text{ is the degradation rate.}$$

Hence, a bleached and non-bleached response that converges rapidly (upper left box) indicates rapid degradation where as parallel dynamics (upper right box) indicate no degradation at all. Note that the rate at which the dynamics converge does not depend on the production rate. (C) Degradation rate measurements using bleach-chase are invariant to the amount of bleaching. The CPT drug response of the ribosomal protein RPS3A was monitored after 0, 2 and 4 minutes of bleaching for 20 hours (indicated by blue, red and green lines in the upper box). The log convergence between 0 and 2 as well as 0 and 4 minutes of bleaching is shown in the lower box (green and red lines are the respective linear fits) both of which yield similar slopes and hence similar degradations (0.08 and 0.09 1 / hour).

FIGs. 10A-D are graphs illustrating that protein half-lives increase upon drug addition: the larger the half life, the larger the increase. (A) A comparison of the degradation rates before and after drug addition revealed that most proteins either decrease or retain the same level of degradation. (B) Examples of protein degradation after drug addition. Top panel shows four proteins (RPS3A, EEF2, DDX5 and LMNA) as they respond to the drug with and without bleaching indicated by pairs of red and blue lines respectively. Fluorescent levels were normalized to the unbleached levels at $t = 0$. Bottom panel shows the log difference between the bleached and unbleached responses as a function of time. The slope of the linear fit, α , is the protein degradation rate in 1/hours units. (C) The half-lives of most proteins after drug addition either increase or remain constant compared to their levels before drug addition. The increase in half-lives is stronger the longer the pre-drug lifetime of the protein is (as illustrated in

the inner box). Blue line is the predicted shift in half-lives due to growth arrest. It is obtained by setting T_{cc} to 20 hours (the cell-cycle duration in the present cells), k to 0 (since drug halts cell-cycle) and applying Eq. 6. The predicted behavior captures more than 40% of the variance in the data. (D) Increase in protein stability due to growth arrest is predicted to be inversely proportional to the cell cycle duration. Cell cycle duration, T_{cc} , is set to 4 hours, 1 day, 1 week and infinity (i.e. no division) and the corresponding half-life behaviors before and after growth arrest are plotted by applying Eq. 6. The model predicts that the faster cells divide prior to drug addition, the stronger the global increase in half-lives.

FIGs. 11A-E are graphs illustrating how protein translation rates decrease in response to the drug. (A-B) Average translation rate and individual protein translation rates are shown. Translation rates were normalized to levels prior to drug addition. Most proteins show a decrease in translation in response to the drug, with a mean response that is about half of the initial level. (C) Average ribosomal protein drug response shows a decrease that is synchronized with timing of translation decay. (D) Changes in half-life and translation rates before and 48 hours after drug addition are shown for each of the proteins (indicated by the trajectory connecting each pair of blue and red dots). The drug induces two opposing effects: a global increase in protein half-lives (indicated by the global shift from left to right) and simultaneous decrease in translation rates (indicated by the global shift from top to bottom). The balance between these counter effects varies considerably across different proteins and strongly affects protein dynamics. (E) Examples of four protein dynamics with dis-correlated and even anti-correlated mRNA responses. The proteins exhibit a decrease in translation and an increase in half-lives the balance of which determines protein behavior, thus resolving the dis-correlation between the mRNA and protein profiles in these proteins.

FIG. 12 are graphs illustrating an integrated view of protein, mRNA, half-life and translation rate dynamics in response to the drug. The time dependant changes 0 – 48 hours after drug addition in four different dimension are shown (i) protein fluorescence dynamics normalized to $t = 0$; (ii) mRNA dynamics normalized to $t = 0$; (iii) changes in protein half-lives measured in hours and (iv) changes in translation rates normalized to $t = 0$. Proteins are ordered according to increasing levels of post drug half-lives. It can be seen that most proteins show a time dependant decrease in their

translation rate and an increase in half-life. Proteins that degraded tend to have a small or no increase in their half-lives whereas proteins that retained a constant level or increased tend to show larger post-drug half-lives (e.g. ENO1, NASP, H2AFV and LMNA) indicating that half-lives after drug addition play a major role in dictating protein dynamics.

FIG. 13 are graphs of protein profiles in response to the translation inhibitor CHX. The dynamic profiles of 11 proteins were measured in response to 10 μ M of the translation inhibitor CHX. Colored lines and back line indicate the response of individual cells and the average response respectively. All values were normalized to the average level at $t = 0$.

FIG. 14 is a graph illustrating global increase in half-lives due to intra-cellular degradation inhibition does not fit the data. Protein half-lives before and after drug addition are shown. Predicted increase in half-lives due to of intra-cellular degradation inhibition is shown as well, where different k 's dictate the degree of inhibition according to Eq. S9. It can be seen that although this model predicts a global increase in protein half-lives, it does not account for the observation that stable proteins become increasingly more stable. On the contrary, stable proteins are expected to be the least affected, since their half-lives are largely determined by cell-division rather than intra-cellular degradation.

FIGs. 15A-B are examples of autoradiographs pulse-chase results for MYH9 (Figure 15B) and DDX5 (Figure 15A). The H1299 cells expressing the YFP tagged protein of interest were pulse-labeled with [35S] methionine/cysteine followed by cold chase as described in the Methods. At the times indicated, the cells were collected and whole cell lysates were prepared. The proteins of interest were recovered from 200 μ g of cell lysates by immunoprecipitation (IP). Anti-GFP antibody was used to precipitate the YFP tagged proteins; protein-specific Abs were used to precipitate both the YFP tagged and the wild type forms of the proteins; non-specific rabbit or mouse IgG was used as a negative control. The immunoprecipitated proteins were resolved on SDS-PAGE and visualized by autoradiography (the left panels). Parallel gels were blotted onto nitrocellulose membrane and probed with the indicated antibodies (the right panels) to ensure correct identification of the protein bands of interest. About 20-40 μ g of cell lysates (10-20% of the amount used for IP) were loaded on the gels as positive

controls (Input). Each experiment was done with at least two biological replicates. To determine protein removal rates, intensities of the relevant protein bands were quantified from the autoradiographs using a custom Matlab software. Levels of the radioactive labeled protein were plotted on a semi-logarithmic scale. The slope of the linear fit is the removal rate.

FIGs. 16A-B are graphs illustrating that removal rate measurements are invariant to the amount of bleaching. The CPT response of RPS3A was measured without bleaching (blue dots) and after two and four minutes of bleaching (red and green dots respectively). The convergence rate in both cases yielded similar removal rates (0.08 ± 0.01 and 0.09 ± 0.01 1 / hour).

FIGs. 17A-D are graphs illustrating that the balance between degradation and dilution under normal growth varies widely between proteins. Distribution of 100 protein removal rates (A) and half-lives (B) under normal growth. α ranges between 0.03 and 0.82 with average 0.1 ± 0.09 (1/h). (C) The balance between degradation and dilution of 100 proteins. (D) Proteins with similar functions or localizations tend to share similar half-lives.

FIGs. 18A-B are graphs illustrating protein half-lives at $t=0-24$ and $t=24-48$ hours after addition remain roughly constant or increase. (A, B) The half-lives of 10 proteins at $t = 0 - 24$ and $t = 24 - 48$ hours after drug addition are compared. Most half-lives remained approximately constant or slightly increased. CALM2 and K- α -1 showed a substantial increase in their half-lives. Blue line indicates the predicted increase due to growth arrest.

FIGs. 19A-G are graphs illustrating that protein half-lives increase in response to stress: the longer the half-life, the larger the increase. (A) Half-lives in CPT compared to normal growth. Blue line is the predicted half-lives due to growth arrest (Eq. 5), with $T_{cc} = 22.5h$ (measured cell-cycle duration) and $k_2 = 0$ (growth arrest). White dots indicate proteins that significantly deviate from line. (B) A model with reduced degradation (Eq. 2-3) does not account for the observed half-lives, whereas a model with reduced dilution (Eq. 4-5) does (C). (D-G) Changes in protein half-lives, comparing normal growth to different stresses, are captured by the reduced dilution model (blue lines), with k_2 equal to the measured ratio between post and pre-stress growth rates.

FIG. 20 is a graph illustrating that the expected increase in half-lives due to degradation inhibition does not account for the observed response to the drug CPT. Reducing degradation is expected to increase protein half-lives such that the longer the half-life, the smaller the increase. Protein half-lives before and after CPT addition are shown. The curved lines illustrate the predicted increase in half-lives due to reduced degradation. They are obtained by setting k_1 to 100%, 50%, 20% and 10% (reflecting the fold reduction in degradation) and applying Eq. 3 in 0. This pattern does not account for observed half-life increase in response to the drug.

FIG. 21 is a bar graph illustrating the null distribution generated by computing the absolute sum of differences over multiple shuffle rounds is shown, yielding a $P < 10^{-4}$. The observed differential increase in half-lives is statistically significant. The present inventors hypothesized that the longer the half-life of a protein, the larger its increase due to growth arrest. To assess the statistical significance of this assertion the following Monte-carlo shuffling test was observed. First, the deviation (absolute sum of differences) of the measured half-lives after drug from the predicted half-lives due to growth arrest was computed. Then, the *changes* in half-lives ($T_{1/2}$ after drug) – ($T_{1/2}$ before drug) were shuffled, and each of them was added to the pre-drug half-lives of a randomly selected protein. The null distribution generated by computing the absolute sum of differences over multiple shuffle rounds is shown, yielding a $P < 10^{-4}$.

FIG. 22 is a graph illustrating the effect of CPT of various polypeptides. A few proteins increased their removal in response to the drug CPT. The present inventors sought to identify proteins whose half-lives after the drug CPT deviated from the growth arrest model (depicted in 09A). To this end the $\log_2$ ratio was computed between the expected and observed post-drug half-lives. The present inventors subtracted or added the measurement error from the observed half-life of each protein so as to minimize this ratio. This correction procedure yields conservative estimations of the ratio (lower bound). The three proteins that showed the most significant deviation, CD44, DDX18 and RPS3A, increased their removal rates in response to the drug, suggesting protein specific removal regulation.

FIGs. 23A-C are graphs illustrating that the faster cells divide, the larger the expected half-life increase due to growth arrest. (A) Measured changes in half-lives (pre- and 24h post-stress) are positively correlated with changes in the corresponding

protein levels. The fold-increases in protein levels compared to half-lives are smaller, possibly due to decreased production rates and the fact that protein levels have not reached steady-state. (B) The expected half-life increase is steeper the faster cells divide prior to drug arrest. Curves are for different T_{cc} and $k_2=0$ (Eq. 5). (C) The longer the
5 protein half-life, the more sensitive it is to fluctuations in growth rate (changes in k_2 , Eq. 5 – Figure 19C).

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to a method of
10 measuring protein stability inside a cell and system capable of same.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

15 Analysis of post-transcription control in mammalian cells is challenging. Two methods are commonly used to determine a protein's half-life, namely radioactive pulse-chase analysis and cycloheximide chase. Pulse-chase analysis provides minimal distortion of normal cell physiology. The main disadvantages of this method are its laboriousness and necessity for radiolabeling. In contrast to pulse-chase analysis,
20 cycloheximide chase strongly affects cellular metabolism. Importantly, both methods do not allow real-time measurements at the single cell level.

The present inventors have devised a new method for measuring the time-dependant changes of protein degradation and translation rates. This method is based on a mild bleaching of fluorescent proteins, and is simple and robust. It does not require
25 radioactive labeling and can be scaled to measure multiple proteins at high temporal resolution in living cells.

It is important to note that this method applies regardless of how protein production rate changes in time, and does not require halting production.

Using this method, the present inventors were able to analyze the effect of a
30 particular anti-cancer agent on the degradation rate and production rate of a variety of proteins, gleaned an abundance of information regarding target proteins. Such

information may be useful in the design of novel drug therapies and the search for novel biomarkers.

Thus, according to one aspect of the present invention there is provided a method of determining a degradation rate of a fluorescently labeled polypeptide of interest in a population of cells, the method comprising:

- (a) obtaining two identical populations of cells;
- (b) determining fluorescence levels of the fluorescently labeled polypeptide of interest in a first of the at least two populations of cells at at least two different points in time;
- (c) at least partially reducing a fluorescence of the fluorescently labeled polypeptide of interest in a second of the at least two populations of cells;
- (d) determining fluorescence levels of the reduced fluorescently labeled polypeptide of interest in the second population of cells at the at least two different points in time;
- (e) comparing fluorescence levels in the first and the second populations; and
- (f) based on the comparing, determining a degradation rate of the fluorescently labeled polypeptide of interest.

As used herein, the phrase "degradation rate" refers to the sum of two underlying processes: intra cellular degradation (e.g. due to proteosome activity) and dilution due to cell growth that effectively reduces the protein amount by 50% every one cell generation.

The degradation rate can be mathematically calculated from the half life of a protein ($t_{1/2}$) and is equal to $\ln 2 / t_{1/2}$.

The term "cell" as used herein, refers to a biological cell, e.g. eukaryotic, such as of mammalian origin (e.g. human). The cell may be diseased (e.g. cancerous) or healthy, taken directly from a living organism or part of a cell line, immortalized or non-immortalized.

According to one embodiment, the cell is viable.

The present invention contemplates determining the degradation rate of all types of polypeptides. According to one embodiment, the polypeptide is one which is naturally expressed in the cell population.

According to one embodiment, the polypeptide is not constitutively degraded at a rate faster than fluorophore maturation time, since the polypeptide would not be observable. It will be appreciated that the temporal resolution of bleach-chase is limited by the rate of the fluorescent tag folding and maturation, which ranges between a few minutes and two hours, depending on the type of fluorophore and environmental condition. Proteins that are constitutively degraded at a rate faster than fluorophore maturation time would therefore not be observable using the present assay.

The fluorescently labeled polypeptides of the present invention typically comprise a fluorescent moiety. The fluorescent moieties are typically attached covalently to the polypeptides directly (i.e. via peptide bonds), although indirect attachment via linker peptides is also contemplated.

According to an embodiment of this aspect of the invention, the fluorescent moiety is not attached to the N terminus of the polypeptide so as not to interfere with naturally occurring degradation signals.

According to one embodiment, the polypeptide maintains wild type functionality (i.e., of non-tagged protein) and further has a similar cellular degradation pattern both prior to and following attachment of the fluorescent polypeptide. It will be appreciated however, that the method of the present invention does not require for the proteins to be functional.

Examples of suitable fluorescent moieties include, but are not limited to, phycoerythrin (PE), fluorescein isothiocyanate (FITC), Cy-chrome, rhodamine, Texas red, PE-Cy5, green fluorescent protein, the yellow fluorescent protein, the cyan fluorescent protein and the red fluorescent protein as well as their enhanced derivatives.

Table 1 below provides examples of sequences of identifiable moieties.

Table 1

<i>Identifiable Moiety</i>	<i>Amino Acid sequence (Genebank Accession No.)</i>	<i>Nucleic Acid sequence (Genebank Accession No.)</i>
Green Fluorescent protein	AAL33912	AF435427
orange fluorescent protein	AAL33917	AF435432
Fluorescein isothiocyanate	AAF22695	AF098239

For additional guidance regarding fluorophore selection, methods of linking fluorophores to various types of molecules see Richard P. Haugland, "Molecular Probes: Handbook of Fluorescent Probes and Research Chemicals 1992–1994", 5th ed., Molecular Probes, Inc. (1994); U.S. Pat. No. 6,037,137 to Oncoimmunin Inc.;
5 Hermanson, "Bioconjugate Techniques", Academic Press New York, N.Y. (1995); Kay M. *et al.*, 1995. *Biochemistry* 34:293; Stubbs *et al.*, 1996. *Biochemistry* 35:937; Gakamsky D. *et al.*, "Evaluating Receptor Stoichiometry by Fluorescence Resonance Energy Transfer," in "Receptors: A Practical Approach," 2nd ed., Stanford C. and Horton R. (eds.), Oxford University Press, UK. (2001); U.S. Pat. No. 6,350,466 to
10 Targesome, Inc.].

Methods of generating fluorescently labeled polypeptides in cells are known in the art. For example, polypeptides may be expressed as fusion proteins in cells using recombinant DNA technology. The DNA sequence encoding the fusion proteins is inserted into nucleic acid constructs and cells are transfected using methods commonly
15 known in the art as described further herein below.

Examples of mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1(+/-), pGL3, pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pSinRep5, DH26S, DHBB, pNMT1, pNMT41, and pNMT81, which are available from Invitrogen, pCI which is available from Promega,
20 pMbac, pPbac, pBK-RSV and pBK-CMV, which are available from Strategene, pTRES which is available from Clontech, and their derivatives.

According to one embodiment the fluorescently labeled polypeptide is transcribed from its native chromosomal location (i.e. it is endogenous to the cell).

Various methods are contemplated for inserting a fluorescent moiety into the
25 genome of the host cell in order to allow transcription of the polypeptide from its native chromosomal location. Such methods include for example homologous recombination, site-specific recombination non-homologous recombination.

As used herein, the phrase "homologous recombination" refers to the process in which nucleic acid molecules with similar nucleotide sequences associate and exchange
30 nucleotide strands. A nucleotide sequence of a first nucleic acid molecule that is effective for engaging in homologous recombination at a predefined position of a second nucleic acid molecule will therefore have a nucleotide sequence that facilitates the

exchange of nucleotide strands between the first nucleic acid molecule and a defined position of the second nucleic acid molecule. Thus, the first nucleic acid will generally have a nucleotide sequence that is sufficiently complementary to a portion of the second nucleic

5 As used herein, the phrase "site-specific recombinase" refers to a type of recombinase that typically has at least the following four activities (or combinations thereof): (1) recognition of specific nucleic acid sequences; (2) cleavage of the sequence or sequences; (3) topoisomerase activity involved in strand exchange; and (4) ligase activity to reseal the cleaved strands of nucleic acid (see Sauer, B., Current Opinions in
10 Biotechnology 5:521-527 (1994)). Conservative site-specific recombination is distinguished from homologous recombination and transposition by a high degree of sequence specificity for both partners. The strand exchange mechanism involves the cleavage and rejoining of specific nucleic acid sequences in the absence of DNA synthesis (Landy, A. (1989) Ann. Rev. Biochem. 58:913-949).

15 Nucleic acid constructs (also referred to herein as "*expression vectors*") capable of insertion in a directed manner typically comprise one or more functionally compatible recognition site for a site-specific recombination enzyme.

As used herein, the phrase "functionally compatible recognition sites for a site-specific recombination enzyme" refers to specific nucleic acid sequences which are
20 recognized by a site-specific recombination enzyme to allow site-specific DNA recombination (*i.e.*, a crossover event between homologous sequences). An example of a site-specific recombination enzyme is the Cre recombinase (e.g., GenBank Accession No. YP_006472), which is capable of performing DNA recombination between two loxP sites. Cre recombinase can be obtained from various suppliers such as the New
25 England BioLabs, Inc, Beverly, MA, or it can be expressed from a nucleic acid construct in which the Cre coding sequence is under the transcriptional control of an inducible promoter (e.g., the galactose-inducible promoter) as in plasmid pSH47.

The phrase, "non-homologous recombination" as used herein refers to the joining (exchange or redistribution) of genetic material through a mechanism that does not
30 involve homologous recombination (e.g., recombination directed by sequence homology) and that does not involve site-specific recombination (e.g., recombination directed by site-specific recombination signals and a corresponding site-specific

recombinase). Examples of non-homologous recombination include integration of exogenous DNA into chromosomes at non-homologous sites, chromosomal translocations and deletions, DNA end joining, double strand break repair, bridge-break-fusion, concatemerization of transfected polynucleotides, retroviral insertion, and
5 transposition.

Retroviral vectors integrate into eukaryotic genomes by a distinct mechanism of non-homologous recombination that is catalyzed by the action of the virally encoded integrase enzyme, and the mechanism of viral integration, replication and infection has been well described [see for example Retroviruses. Coffin, J M.; Hughes, S H.; Varmus,
10 H E. Plainview (NY): Cold Spring Harbor Laboratory Press; c1997; Use of wildtype retroviruses as mutagens]. The mutagenic ability of retroviruses and retroviral vectors and their ability to enable the rapid identification of mutated genes through the linkage of retroviral tag sequences within the transcripts of mutagenized genes are well known in the art (Friedrich G, Soriano P. Methods Enzymol. 1993;225:681-701; 3: Gossler A,
15 et al., Science. Apr. 28, 1989;244(4903):463-5; Friedrich G, Soriano P. Genes Dev. September 1991;5(9):1513-23; 5: von Melchner H, et al Genes Dev. June 1992;6(6):919-27].

Retroviral constructs of the present invention may contain retroviral LTRs, packaging signals, and any other sequences that facilitate creation of infectious
20 retroviral vectors. Retroviral LTRs and packaging signals allow the reporter polypeptides of the invention to be packaged into infectious particles and delivered to the cell by viral infection. Methods for making recombinant retroviral vectors are well known in the art (see for example, Brenner et al., PNAS 86:5517-5512 (1989); Xiong et al., Developmental Dynamics 212:181-197 (1998) and references therein; each
25 incorporated herein by reference). In preferred embodiments, the retroviral vectors used in the invention comprise splice acceptor (SA) and splice donor (SD) sequences flanking the sequence encoding the reporter polypeptide. Typically, the constructs of the present invention do not comprise a promoter, a start codon or a polyA signal. In this way, if the virus inserts into an actively transcribed gene, the reporter sequence is
30 retained as a new exon after splicing of the mRNA. Owing to the large size of the first intron and viral preference for integration sites near the start of genes, the first intron is

the most common point of insertion. The tagged mRNA translates to an internally labeled protein, with the reporter polypeptide usually near the N terminus.

Retroviral LTRs and packaging signals can be selected according to the intended host cell to be infected. Examples of retroviral sequences useful in the present invention include those derived from Murine Moloney Leukemia Virus (MMLV), Avian Leukemia Virus (ALV), Avian Sarcoma Leukosis Virus (ASLV), Feline Leukemia Virus (FLV), and Human Immunodeficiency Virus (HIV). Other viruses known in the art are also useful in the present invention and therefore will be familiar to the ordinarily skilled artisan.

Like retroviruses, transposons and transposon vectors can also be used to integrate sequences in a non-directed fashion into the chromosome of the cell. Also like retroviruses, transposons integrate by enzymatically catalyzed non-homologous recombination in which transposase enzymes catalyze the genomic integration and transposition of transposon DNA.

Numerous transposons have been characterized that function in mammals. In particular, the TC1/mariner derivative transposon, Sleeping Beauty, has been demonstrated to integrate efficiently in mammals.

When the fluorescent polypeptide is inserted into the genome in a non-directed fashion, the fluorescent moiety may be identified, such as by 3'RACE, using a nested PCR reaction that amplifies the section between the reporter polypeptide and the polyA tail of the mRNA of the host gene. The PCR product may be sequenced directly and aligned to the genome, thereby identifying the polypeptide.

Exemplary oligonucleotide primers that may be used for 3'RACE and sequencing are listed in Table 2 herein below.

Table 2

Primer name	Use	Sequence	Alignment in YFP or mCherry
AP first-strand	First-strand cDNA synthesis	GGCCACGCGTCGACTAGTAC(T) ¹⁷ (SEQ ID NO: 23)	
AP 92	RACE first and nested reaction 3' primer	GGCCACGCGTCGACTAGTAC (SEQ ID NO:24)	
YFP 90	RACE first reaction 5' primer for YFP-tagged	GCAGAAGAACGGCATCAAGG (SEQ ID NO: 25)	Bases 471–490

	genes		
YFP 85	RACE-nested reaction 5' primer for YFP-tagged genes	CGCGATCACATGGTCCTGCTG (SEQ ID NO: 26)	Bases 646–666
Cherry 45	RACE first reaction 5' primer for mCherry-tagged genes	GTGGTGACCGTGACCCAGGA (SEQ ID NO: 27)	Bases 322–341
Cherry 46	RACE-nested reaction 5' primer for mCherry-tagged genes	GCGGATGTACCCCGAGGACG (SEQ ID NO: 28)	Bases 456–475
Cherry 56	Sequencing of mCherry RACE product	GACTACACCATCGTGGAACA (SEQ ID NO: 29)	Bases 586–605
YFP 906	Sequencing of YFP RACE product	GGATCACTCTCGGCATGGAC (SEQ ID NO: 30)	Bases 686–705

The constructs of the present invention can be introduced into a cell and integrated into DNA by any method known in the art. In one embodiment, they are introduced by transfection. Methods of transfection include, but are not limited to, electroporation, particle bombardment, calcium phosphate precipitation, lipid-mediated transfection (e.g., using cationic lipids), micro-injection, DEAE-mediated transfection, polybrene mediated transfection, naked DNA uptake, and receptor mediated endocytosis.

Typically the introduction of the constructs of the present invention is effected whilst the cells are being cultured in a medium which supports well-being and propagation. The medium is typically selected according to the cell being transfected/infected.

According to one embodiment, the constructs of the present invention are introduced into the cell by viral transduction or infection. Suitable viral vectors useful in the present invention include, but are not limited to, adeno-associated virus, adenovirus vectors, alpha-herpesvirus vectors, pseudorabies virus vectors, herpes simplex virus vectors and retroviral vectors (including lentiviral vectors).

In order to carry out the method of the present invention, two identical populations of cells are required each expressing the fluorescently labeled polypeptide to the same extent.

The phrase "identical cell populations" refers to homogeneous cell populations (i.e. of the same cell type) and being under the same conditions (e.g. culturing

conditions), wherein the proteins in each of the populations are identically labeled. Thus for example one population of cells may be obtained which expresses the fluorescently labeled polypeptide which is then divided into at least two sub-populations.

As mentioned, the method of the present invention is effected by
5 determining the change in fluorescence levels of the fluorescently labeled polypeptide over time in both the cell populations. The measurements may be effected simultaneously, or one following the other in any order.

Methods of analyzing fluorescence levels are known in the art. Preferably, the analysis does not affect the viability or function of the cell. Fluorescence levels may be
10 measured using a fluorescent confocal microscope or using flow cytometry at particular time points. Alternatively, fluorescence levels may be measured in real-time using long period time-lapse microscopy. Time-lapse movies may be obtained as described by Sigal *et al.* (Sigal, Milo et al. 2006, supra) with for example an automated, incubated (including humidity and CO₂ control) inverted fluorescence microscope (e.g. Leica
15 DMIRE2) and a CCD camera (e.g. ORCA ER- Hamamatsu Photonics).

According to one embodiment the measurements are taken over a period of an hour, over a period of 6 hours, over a period of 12 hours, over a period of 18 hours, over a period of 24 hours or over a period of 48 hours. The number of measurements is not limited by typically are in the range of 2-20.

20 Prior to measuring fluorescence in the second cell population, the cells are treated in order to reduce fluorescence. According to one embodiment the reducing transforms a fraction of the fluorescent proteins into non-fluorescent. This action is referred to herein as bleaching.

According to one embodiment, the bleaching is such that there is a 10 – 100 %
25 reduction, more preferably a 10-90 % reduction, more preferably a 10-80 % reduction, more preferably a 10-70 % reduction, more preferably a 10-60 % reduction, more preferably a 10-50 % reduction, in the level of fluorescence.

Preferably, the bleaching is irreversible, or at least irreversible over the time over which measurements are recorded.

30 According to another embodiment, the bleaching does not alter cell-viability, motility, mitosis rate or morphology.

An exemplary method of bleaching includes shining pulses of light on the cells. The pulses of light can last from 15 seconds to 30 minutes (e.g. 1-8 minutes).

Following fluorescence measurements, the change in fluorescence levels in the bleached cell is compared to the change in fluorescence levels in the non-bleached
5 cells.

The present inventors have shown that the degradation rate is equal to the ratio of (the natural log (ln) of the difference in fluorescence of the polypeptide in the non-bleached cells and fluorescence of the polypeptide in the bleach cells at a first point in time) : (natural log (ln) of the difference in fluorescence of the polypeptide in the non-bleached cells and fluorescence of the polypeptide in the bleached cells at a second
10 point in time) divided by the difference in time between the two points in time.

For a more accurate determination of the degradation rate fluorescence levels may be measured over a number of time points. A graph can then be plotted, with the y axis indicating the $\ln (P(t) - P_v(t))$ and the x axis graph indicating time, wherein $P(t)$ is
15 the amount of fluorescence of the polypeptide at point t in time in the non-bleached cells and $(P_v(t))$ is the amount of fluorescence of polypeptide at point t in time in the bleached population of cells. The slope of the graph indicates the degradation rate.

It will be appreciated that in the case where the degradation rate is not constant it may be retrieved by using the following formula.

20

$$\ln (P(t) - P_v(t)) = \ln (P_0 - P_{0v}) - \int_0^t \alpha(t') dt'$$

where P and P0 are two measurements of the polypeptide without the bleaching at two different time points. Pv and Pv0 are two measurements of the polypeptide with
25 the bleaching at two different time points.

$$\int_0^t \alpha(t') dt'$$

is the integral over the two different time points.

30

It will further be appreciated that once the degradation rate of a polypeptide is calculated, it is also possible to calculate the production rate. Measurement of production rate is only possible when the fluorescently labeled polypeptide is transcribed from its native chromosomal location, as described herein above.

Thus according to another aspect of the present invention, there is provided a method of determining a production rate of the polypeptide. The production rate may be determined according to the following equation:

5 Eq. (2) $\beta(t) = dP(t)/dt + \alpha(t) \cdot P(t)$

where beta is the production rate at a given time point (t). $dP(t)/dt$ is equal to the change in protein in time, alpha is the degradation rate at a given point in time and P is the protein level at a given point in time.

10 Various methods of measuring protein levels are known in the art including quantitative immunoblots, Bradford assay etc.

According to one embodiment, protein levels are measured by causing the cells to express an additional fluorescently labeled polypeptide in order to eliminate variations in lamp intensities across experiments. According to an embodiment of this aspect of the present invention the additional fluorescently labeled polypeptide has a higher nuclear: cytoplasm expression ratio and further is distinguishable from the fluorescently labeled polypeptide of interest. Thus, fluorescent moieties for example may be selected such that each emits light of a distinguishable wavelength and therefore color when excited by light.

20 Once protein levels $P(t)$ are measured at different time points, one can retrieve $dP(t)/dt$ by subtracting the protein levels in consecutive times points (i.e. $dP_i/dt = P_{i+1} - P_i$).

The method of this aspect of the present invention may be adapted in order to test the effect of a particular agent on the degradation rate of the cells. Thus, according to another aspect of the invention there is provided a method of analyzing the effect of an agent on a degradation rate of a polypeptide of interest in a population of cells, the method comprising:

- (a) contacting a population of cells with the agent;
 - (b) determining the degradation rate of the fluorescently labeled polypeptide of interest in the population of cells according to the method of claim 1; and
- 30

(c) comparing the degradation rate obtained in step (b) with a degradation rate of the polypeptide in an absence of the agent, thereby analyzing the effect of the agent on the degradation rate of the polypeptide of interest.

According to this aspect of the present invention the degradation rate of the polypeptide in the absence of the agent may be known in the literature or ascertained using any of the methods known in the art.

It will be appreciated that the method of the present invention may also be adapted to compare the degradation rate of a polypeptide in diseased and healthy cells.

It will be further appreciated that the method disclosed herein can be used to study protein degradation in different cellular compartments (e.g. nucleus versus cytoplasm) because bleach-chase allows a microscopy-based assay of protein removal. Additionally, bleach-chase facilitates measurements of degradation rates as a function of cell-cycle stage, without the need for chemical or physical synchronization. This can be achieved by applying bleach-chase to a population of unsynchronized cells, followed by in-silico synchronization.

The conversion from rate of change in fluorescence of a polypeptide under bleached and non-bleached conditions to degradation rate and/or production rate of the polypeptide may be effected manually or using a computer system comprising a processing unit executing a software application configured for converting a difference in rate of change of fluorescence between a bleached and non-bleached population of cells to degradation rate and/or production rate.

As used herein, the phrase "processing unit" refers to a data processor, e.g., a computer.

The software application can be embodied in a tangible medium such as, but not limited to, a floppy disk, a CD-ROM, a hard drive of a computer, and a memory medium (e.g., RAM, ROM, EEPROM, flash memory, etc.). The software can be run by loading the software into the execution memory of the processing unit, configuring the processing unit to act in accordance with the instructions of the software. All these operations are well-known to those skilled in the art of computer systems.

According to an embodiment of this aspect of the present invention, the system may further comprise an imaging system.

Such an imaging system typically comprises an illuminating device for the purposes of measuring fluorescence. The illuminating device may also comprise a white light source which may be required for delineating the borders of the sample being analyzed. Alternatively or additionally, the illuminating device may comprise
5 light sources of different wavelengths such that it is capable of detecting more than one type of fluorescent moiety.

The imaging system of the present invention further comprises an image capture apparatus. The image capture apparatus typically includes means to acquire the image (e.g. a CCD) by translating the light into electronic impulses and transmits the
10 image to a display device. The image capture apparatus may also include means for magnifying the image (e.g., a microscope). The CCD may be any suitable photosensitive device, including but not limited to a photomultiplier tube (PMT), a phototransistor, or a photodiode.

Examples of picture elements include, but are not limited to a pixel or a group of
15 pixels.

According to a preferred embodiment of this aspect of the present invention, the display device of the system displays the degradation rate and/or the production rate. The display may be in numerical form and/or graphical form.

According to an additional embodiment of this aspect of the present invention,
20 the system further comprises a time recording device.

According to a preferred embodiment of this aspect of the present invention, the image capture apparatus is calibrated for capturing an image of the fluorescently labeled polypeptide. The calibrations typically take into account the cell population (e.g. number of cells, size of cells) and the particular fluorescent moiety used.

25 As used herein the term "about" refers to $\pm 10\%$

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of means "including and limited to".

The term "consisting essentially of" means that the composition, method or
30 structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

5 Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as
10 individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

15 Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all
20 the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical,
25 pharmacological, biological, biochemical and medical arts.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided
30 separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various

embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find both calculated and
5 experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

10 Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed.
15 (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998);
20 methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th
25 Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345;
30 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins

S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

GENERAL MATERIALS AND METHODS

Fluorescently-tagged protein clones: Clones used in this study were taken from the LARC library, in which proteins were fluorescently-tagged under their endogenous regulation by CD-tagging as previously described [14, 17-18, 30-31]. Briefly, Clones are based on H1299 non small cell lung carcinoma where each clone contains two tags: The first, common to all clones, is a red fluorescent protein mCherry that creates a pattern, which is strong in the nucleus and weaker in the cytoplasm and is used for image analysis purposes. The second is a yellow tag (eYFP or Venus) of the protein of interest. The CD-tagging scheme used to generate the clones tends to preserve protein functionality and localization [15, 18, 27-29]. Note, however that the purpose of this study does not require the proteins to be functional but merely reliable reporters of the endogenous protein dynamics. Additional information regarding the LARC library is published elsewhere [18].

Tissue culture media: Cells were grown in RPMI 1640 supplied with (+) L-Glutamine (GIBCO, cat. No. 21875) medium supplemented with 10 % Fetal Calf Serum (certified fetal bovine serum, membrane filtered, Biological Industries, 04-001-1A) and 0.05 % Penicillin-Streptomycin antibiotics (Biological Industries Cat. No. 03-031-1B). Cells were grown in incubators at 37 °C and 8 % CO₂.

CPT drug addition: Camptothecin (CPT, C9911 Sigma) was dissolved in DMSO giving a stock solution of 10 mM. In each experiment, the drug was diluted to the desired concentration in transparent growth medium (RPMI 1640, 0.05 % Penicillin-

Streptomycin antibiotics, 10 % FCS, with L-Glutamine, lacking riboflavin and phenol red, Bet Haemek, Biological Industries Cat. No.06-1100-26-1A). Normal transparent growth medium (2 ml, without the drug) was replaced by the diluted drug (2 ml medium with 10 μ M CPT diluted 1:1000 from stock) under the microscope after at least one round without the drug.

Time-lapse microscopy: Time-lapse movies were obtained at 20x magnification. Three automated microscopes were used, based on inverted fluorescence microscopes from Leica (DMIRE2 and DMI6000B). All microscopes included live cell environmental incubators maintaining 37 °C (37-2 digital and Heating unit, PeCon, Germany, Leica #15531719) humidity and 8 % CO₂ (PeCon, GmbH, Germany #0506.000-230, Leica #11521733) and automated stage movement control (Corvus, ITK, GmbH, Germany); stage was surrounded by a custom enclosure to maintain constant temperature, CO₂ concentration, and humidity. Transmitted and fluorescent light paths were controlled by electronic shutters (Uniblitz, model VMM-D1, Rochester, NY); Fluorescent light sources were Mercury short arc lamp HXP and Mercury HBO100 (OSRAM, Germany). Cooled 12 and 14 bit CCD cameras were used QImaging, (RETIGA-S&V, Fast 1394, RET-SRV-F-M-12-C, Canada); CoolSNAP, (Roper Scientific HQ, photometrics); ORCA-ER (C4742-95-12ERG, and Hamamatsu photonics K.K, Japan). The filters used were from Chroma Technology Corp: single channel filters were YFP: (500/20 nm excitation, 515 nm dichroic splitter, and 535/30 nm emission, Chroma #41028) and mCherry Red: (575/50 nm excitation, 610 nm dichroic splitter, and 640/50nm emission, Chroma #41043); The hardware was controlled by ImagePro5 Plus software (Media Cybernetics) with integrated time-lapse acquisition, stage movement, and software based auto-focus.

Cells were grown and visualized in 12-well optical glass-bottom plates (MatTek cultureware, Microwell plates-uncoated, part No. P12G-0-14-F, Lot No. TK0289) coated with 10 μ M fibronectin 0.1% (solution from bovine plasma, Sigma, Cat. No. F1141) diluted 1:100 in Dulbecco's Phosphate Buffered Saline, PBS (Sigma, Cat. No. D8537). In order to reduce background fluorescence, cells were grown in RPMI 1640 lacking riboflavin and phenol red (Beit Haemek, Biological Industries Cat. No.06-1100-26-1A), supplemented with L-Glutamine, 10% Fetal Calf Serum (certified fetal bovine serum, membrane filtered, Biological Industries, 04-001-1A) and 0.05% Penicillin-

Streptomycin antibiotics (Biological Industries Cat. No. 03-031-1B). For each well, time-lapse movies were obtained at four fields of view. Each movie was taken at a time resolution of 20 minutes and was filmed for at least two days (over 140 time points). Each time point included transmitted light image (phase
 5 contrast), and two fluorescent channels (red and yellow). No significant bleaching was observed (on average less than 3 % over the duration of the experiment)

Image analysis of time lapse movies: Custom computer vision software was built to automatically extract quantitative measurements of protein dynamics from time-lapse movies. The main modules in the software include background normalization, cell
 10 segmentation, cell tracking and automated identification of various cellular phenotypes (e.g. mitosis and cell death). Cell and nuclei segmentation was based on the patterns generated by the red-tagging common to all clones. An LDA classification approach [32] is first used to identify cell nucleus according to intensity and texture followed by seeded watershed segmentation in order to identify cell boundaries [33]. The next step,
 15 cell tracking, links each cell in a given frame to the appropriate cell in the preceding and following frames. To this end the following scheme was applied: each triplet of cells (i.e. a cell in frame $i - 1$ and $i + 1$) generates a trajectory whose cost depends on its velocity consistency (large fluctuations in velocity increase the cost) angle smoothness (abrupt changes in trajectory angle increase the cost) as well as the similarity between
 20 the size and appearance of the connected cells. The problem of tracking is then reduced to finding a non-overlapping assignment of trajectories which minimizes the global trajectory cost [34]. Lastly, the software identifies different cellular phenotypes such as apoptosis and mitosis using a machine learning approach [18].

Protein Calibration protocol: A method of protein calibration was used to
 25 compare levels across different proteins. The cherry fluorescence marker was used, common to all the clones used in this study, as a common reference in order to eliminate variations in lamp intensities across experiments. The levels of both yellow and red fluorescence measurements are described by the following two equations:

$$\text{Eq. (7)} \quad F_y = P_y \cdot T_y \cdot I$$

$$\text{Eq. (8)} \quad F_r = P_r \cdot T_r \cdot I$$

where P_x is the number of tagged protein copies, T_x is the exposure time, I is the intensity of the lamp and sub-index y and r denote YFP and cherry respectively. It follows that the lamp intensity is:

$$\text{Eq. (9)} \quad I = F_r / P_r \cdot T_r$$

5

By applying Eq. 9 on Eq. 7 and 8 and omitting P_r (since it is constant in all clones) one finds:

$$\text{Eq. (10)} \quad P_y = F_y \cdot T_r / F_r \cdot T_y$$

Eq. 10 is used to retrieve the levels of the YFP-tagged protein. Briefly the reproducibility of this approach was tested on 13 proteins spanning a wide range of expression levels (over 500 fold ratio between lowest and highest levels) and found it better than 30% (std/mean). This approach was further tested by comparing protein fluorescence levels to quantitative immunoblots with protein standards (no. of replicates ≥ 3). Measurements obtained by the two techniques are in good agreement ($r > 0.8$, $P < 0.001$).

15

Microarray experiments: Cells were plated in 10 cm plates (1.6×10^4 cells per plate) with at least three replicates for each time point. CPT (10 μ M) was added 48 hours after plating. At each time point (0, 0.5, 1.5, 3, 6, 9, 12, 15, 18, 23, 28, 33, 38, 43 and 48 hours after drug addition) cells were trypsinized and counted followed by lyses of 4×10^4 and total RNA was purification using RNeasy kit (QIAGEN), with elution in 30 μ l of RNase-free water. A total of 10 μ l of 200 ng/3 μ l (66.67 ng/ μ l) RNA were transferred to microarray chip hybridization. Amplified cRNA was prepared from 200 ng total RNA using the WT cDNA Synthesis and WT cDNA Amplification Kits (900672, Affymetrix). Biotinylated single-stranded cDNA was generated from the amplified cRNA and then fragmented and labeled with the WT Terminal Labeling Kit (Affymetrix), following manufacturer protocol. Samples were hybridized to Human Gene 1.0 ST Arrays (Affymetrix) and scanned at the Weizmann Institute Microarray Core Facility using the Affymetrix GeneChip Scanner 3000 7G. Raw data is given in the SOM. Partek Genomic Suite software was then used to extract raw data, perform mean probe summarization, RMA and quintile normalization and GC content correction [35].

30

Quantitative real-time PCR: To validate the microarray measurements we selected 10 genes that exhibited different drug response profiles and measured their mRNA using qPCR (TOP1, PSMB4, AIP, CKS, JAGN1, DDX5, ENO1, NCBP2, MYH9 and GAPDH). To this end, cells were plated at 1.6×10^4 cells in a 10cm plate with five replicates at each time point. 48 hours after plating, $10 \mu\text{M}$ of Camptothecin(CPT) was added. Cells were harvested at different time points after CPT addition (0, 2, 9, 23, 33 and 48 hours). Then, 5×10^4 cells were lysed and total RNA was purified using the RNeasy kit (QIAGEN), followed by quantification using the NanoDrop machine (ND-1000 Spectrophotometer). RNA samples were then reverse transcribed by Omniscript RT kit (Qiagen) and random hexamers. Real-Time PCR reactions were performed in a $20 \mu\text{l}$ mixture containing 1/10 volume of cDNA ($2 \mu\text{l}$), and $18 \mu\text{l}$ of Real-time PCR reaction mix containing $10 \mu\text{l}$ SYBR Green buffer (PE Applied Biosystems, Foster City, CA, USA), $0.2 \mu\text{M}$ of each primers (forward and reverse of each gene), and $7.6 \mu\text{l}$ DDW. QRT-PCR reactions were performed on a qRT-PCR Strategene Mx 3000P machine. Primer sequences used for the qPCR are provided in Table 3, herein below.

Table 3

Gene symbol	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
TOP1	TCCGGAACCAGTATCGAGAAGA (SEQ ID NO: 1)	CCTCCTTTTCATTGCCTGCTC (SEQ ID NO: 2)
PSMB4	ACGCGGACCCAGAACCCCAT (SEQ ID NO: 3)	GCGTAGTCGCCAGAGGCACC (SEQ ID NO: 4)
AIP	GCACTGCTGCGGTGTTGCAC (SEQ ID NO: 5)	TGCCAGGGCTCTCCACCTTCA (SEQ ID NO: 6)
GAPDH	AGGCTGGGGCTCATTTGCAGG (SEQ ID NO: 7)	TGACCTTGGCCAGGGGTGCT (SEQ ID NO: 8)
CKS2	GTTGCCTGGGCTGGACGTGG (SEQ ID NO: 9)	AACATGCCGGTACTCGTAGTGTTCG (SEQ ID NO: 10)
JAGN1	TCTGGGCAGGCACAATGGCG (SEQ ID NO: 11)	AGCCTCAGGTGTCCCACGCT (SEQ ID NO: 12)
PPIA	GCCAAGACTGAGTGGTTGGATGGC (SEQ ID NO: 13)	TGGGGTGGAGGGGTGCTCTC (SEQ ID NO: 14)

DDX5	GGAGTGCGACTTGGCCAAAAGA (SEQ ID NO: 15)	GGCAGGCCACCCATCTCTCCT (SEQ ID NO: 16)
ENO1	TGGTGTCTATCGAAGATCCCTT (SEQ ID NO: 17)	CACTGTGAGATCATCCCCAC (SEQ ID NO: 18)
NCBP2	TGCCTTGACATGAGGACCAGCA (SEQ ID NO: 19)	GGCATCTGCTGCAGGAACCTT (SEQ ID NO: 20)
MYH9	AAGCAGGCGTGCGTGCTCAT (SEQ ID NO: 21)	CTGCAGCAGGCCTGGAACCC (SEQ ID NO: 22)

GO Enrichment analysis: GO enrichment analysis was applied in order to identify groups of genes exhibiting similar dynamic profiles in response to the drug that share a common function, process or localization [36]. To this end Gorilla was applied, a software for automatic identification and visualization of enriched GO terms in ranked lists of genes [37]. For each time point, genes were ranked according to their level of expression (over-expressed at the top and under-expressed at the bottom of list). This was given as input to GOrilla, which identifies sets of genes sharing a common function, process or localization that are enriched at the top of the list. The determination of the exact cutoff is done automatically, using an optimization scheme called mHG which produces an enrichment P-value [37-38]. Enrichment was considered significant if a term showed a $P < 10^{-4}$ after multiple hypothesis correction due to the multiple time points and GO terms. The same procedure was then repeated on the inverse lists identifying sets of genes sharing similar functions that were down regulated at some time point in response to the drug. A summary of enriched GO terms as a function of time after drug addition is shown in Figures 1A-K.

Time shift correlations: To allow for effects of delay between mRNA and protein, the temporal protein profiles were shifted with respect to the mRNA profiles. Using a sliding window ranging from -10 to +10 hours with 20 minutes resolution the Pearson correlation was recorded at each time point. Alignment of mRNA and protein profiles was determined according to the time point at which maximal correlation was attained.

Pulse chase protocol for measuring protein half-lives: Approximately 2×10^6 cells were plated in 10 cm dishes and cultured for 24 hours as described above. To perform the pulse-chase, cell monolayers were rinsed twice with warm sterile PBS and

starved of methionine and cysteine by incubation for 1 hour at 37 °C in 5 ml of Pulse-chase Medium: methionine/cysteine-free RPMI 1640 (Sigma, R-7513) supplemented with 10 % dialyzed FBS (Biological Industries Beit Haemek) and 2 mM L-glycine. Following starvation, the cells were labeled for 1 hour at 37 °C with 4 ml of Pulse-chase medium containing 4 mCi (1mCi/mL) of [35S]methionine/cysteine (EasyTagTMExpre³⁵S³⁵S Protein Labeling Mix, #PENEG772, PerkinElmer). The radioactive medium was then removed, the cells rinsed three times with PBS, re-fed with Pulse-chase medium supplemented with 2.5 mM L-Methionine and 2.5 mM L-Cysteine Hydrochloride Hydrate (Biological Industries Beit Haemek), and incubated for the indicated times. The cells were then collected and lysed with modified RIPA buffer (50 mM, Tris-HCl, pH 7.4; 150 mM NaCl; 1 mM EDTA; 1 % NP-40; 0.25 % Na-deoxycholate; 1 mM PMSF and 1 µg/ml of each aprotinin, leupeptin and pepstatin). The protein concentrations were determined by BCA protein assay kit (Thermo scientific). The proteins of interest were recovered from 200 µg of cell lysates by immunoprecipitation using anti-GFP or protein-specific antibodies listed below. The immunoprecipitates were subjected to SDS-PAGE, and protein bands were detected by autoradiography. Parallel gels were blotted onto nitrocellulose membrane and probed with protein-specific antibodies to ensure correct identification of the protein bands of interest.

It is assumed that radioactive protein, $P(t)$, decays in time at a rate that is exponential in the degradation rate, α :

$$\text{Eq. (11)} \quad P(t) = P_0 e^{-\alpha t},$$

where P_0 is protein levels at $t = 0$ and hence one can retrieve α by applying a linear regression to the following equation:

$$\text{Eq. (12)} \quad \ln(P(t)) = \ln(P_0) - \alpha(t) \cdot t.$$

Applying the translation inhibitor CHX for measuring protein half-lives:

Cyclohexamide (CHX, C4859 – Ready-made solution, 100mg/ml in DMSO, Sigma) was diluted in transparent growth medium to 10 µM. Normal transparent growth medium (2ml, without CHX) was replaced by the 2ml diluted CHX under the microscope after at least one round without the CHX. The dynamic profiles of the proteins were then measured for 20 hours (20 minutes resolution) using time-laps

microscopy as described in previous sections. It was found that about 10 % of the cells underwent apoptosis after 20 hours as a result of CHX addition and most of the cells stopped dividing.

Measurements were done on 11 proteins (DDX5, CKS2, ENO1, JAGN1, DNCH1, RPS3, LMNA, MAP2K2, NCBP2, PSMB4 and RPL22) with at least two replicates. One usually assumes that the translation inhibitor CHX halts protein production and induces a time dependant exponential decay according to Eq. 11. However, the present inventors observed different behaviors: Most proteins decayed to a new steady state (instead of decaying to zero) and one protein, ENO1, showed an increase in response to CHX (Figure 2). About half of the tested protein tested showed dynamics that did not fit an exponential decay to zero (e.g. CKS2), suggesting that translation inhibition was incomplete. Increasing the dosage of CHX did not eliminate this effect (Figure 11).

Taken together, these measurements suggest that in the present human cells CHX does not halt translation but only reduces it (increasing CHX dosage by 10 fold gave similar protein profiles). Hence, retrieving degradation rates from the present measurements requires a model that allows for both production and degradation. The present inventors tested several models but found that the estimated degradation rate can change considerably (factor of 10) according to the choice of model. Overall, the present inventors were unable to obtain reliable degradation rates in the present human cells using a CHX based approach.

Bleach chase: theory and experimental protocol: This section describes how bleach-chase works by deriving Eq. 2 used for degradation rate measurement and the experimental protocol. Assume a fluorescently tagged protein whose degradation rate, α , is constant in time (later we show how to retrieve degradation in case α changes in time). By exposing the cells to a brief pulse of light one can bleach a fraction of the proteins inside the cells effectively transforming them from fluorescently tagged into non-fluorescent. One can therefore think of the total protein, P , as the sum of two cohorts of proteins, one visible to fluorescent microscopy, P_v , and another which is invisible, $\bar{P}$:

$$\text{Eq. (13)} \quad P(t) = P_v(t) + \bar{P}(t).$$

The invisible protein $\tilde{P}$ is produced only during the pulse, after which it starts to degrade according to the following equation:

$$\text{Eq. (14)} \quad \frac{d\tilde{P}(t)}{dt} = -\alpha * \tilde{P}(t).$$

Hence, measurement of how $\tilde{P}$ changes in time would enable the retrieval of the degradation rate. But, since $\tilde{P}$ is invisible it cannot be measured directly. The solution is to retrieve it indirectly by measuring P and P_v and applying Eq. 13 and 14:

$$\text{Eq. (15)} \quad \frac{d(P(t) - P_v(t))}{dt} = -\alpha * (P(t) - P_v(t)).$$

The solution to this equation means that the difference between the bleached and non-bleached experiment decays exponentially in time at a rate that depends solely on the degradation rate of the protein under study:

$$\text{Eq. (16)} \quad (P(t) - P_v(t)) = (P_0(t) - P_{v0}(t))e^{-\alpha t}.$$

and hence:

$$\text{Eq. (17)} \quad \ln(P(t) - P_v(t)) = \ln(P_0(t) - P_{v0}(t)) - \alpha t.$$

One can relax the assumption that the degradation rate is constant in time by applying $\alpha(t)$ instead of α in Eq. 17 and deriving a new set of equations.

Protein fluorescence was measured with and without the drug in 12 well plates using multiple fields of view under time lapse microscopy as described in previous sections. However, in bleach-chase two concurrent measurements are done: without and with the bleaching. The latter was done using a pulse of light given one round prior to drug addition. In the rest of the time-lapse normal exposure times are used that are sufficient to detect the protein under study. We tested a wide range of pulse durations (1 - 8 minutes) using a mercury fluorescent lamp (120 W) resulting in different fractions of the protein fluorescence bleaching (10 % - 60 % drop). The measured degradation rates were roughly insensitive to the degree of bleaching (CV < 0.1).

A simple model: changes in growth-rate induce global changes in half-lives

In this section the present inventors derive Eq. 6, that captures the half-life after a change in growth rate, $\frac{T_1^*}{2}$, as a function of the half-life prior to the change, $\frac{T_1}{2}$, the

cell cycle duration, T_{cc} , and the fold change in growth rate, k . By applying Eq. 3, it follows that:

$$Eq.(18) \quad T_{\frac{1}{2}} = \frac{\ln(2)}{\alpha_{deg} + \alpha_{dil}} ,$$

$$Eq.(19) \quad T_{\frac{1}{2}}^* = \frac{\ln(2)}{\alpha_{deg} + k\alpha_{dil}} ,$$

$$Eq.(20) \quad T_{cc} = \frac{\ln(2)}{\alpha_{dil}} .$$

5

Applying Eq. 18 and 20 to Eq. 19 gives:

$$T_{1/2}^* = \frac{\ln(2)}{\alpha_{deg} + \alpha_{dil} + (k-1) \cdot \alpha_{dil}} = \frac{\ln(2)}{\frac{\ln(2)}{T_{1/2}} + (k-1) \frac{\ln(2)}{T_{cc}}} ,$$

and hence we get Eq. 6:

$$T_{1/2}^* = \frac{T_{1/2}}{1 + (k-1) \frac{T_{1/2}}{T_{cc}}}$$

10

EXAMPLE 1

Parallel measurement of Protein and mRNA dynamics in human cancer cells

Results

The dynamics of 540 proteins and their mRNAs were followed for 48 hours as cancer cells responded to the drug CPT (experiment summarized in Figure 3). These genes represent a wide range of biological functions and processes (Table 4).

Table 4

Entrez Gene	Description
23	ATP-binding cassette, sub-family F (GCN20), member 1
11332	acyl-CoA thioesterase 7
71	actin, gamma 1
81	actinin, alpha 4
10121	ARP1 actin-related protein 1 homolog A, centractin alpha (yeast)

98	acylphosphatase 2, muscle type
128	alcohol dehydrogenase 5 (class III), chi polypeptide
132	adenosine kinase
9049	aryl hydrocarbon receptor interacting protein
339803	hypothetical protein LOC339803
26993	A kinase (PRKA) anchor protein 8-like
51321	archaemetzincins-2
51129	angiopoietin-like 4
54443	anillin, actin binding protein
10541	acidic (leucine-rich) nuclear phosphoprotein 32 family, member B
311	annexin A11
653145	annexin A8
313	acyloxyacyl hydrolase (neutrophil)
51074	APAF1 interacting protein
334	amyloid beta (A4) precursor-like protein 2
378	ADP-ribosylation factor 4
57492	AT rich interactive domain 1B (SWI1-like)
403	ADP-ribosylation factor-like 3
23204	ADP-ribosylation factor-like 6 interacting protein 1
84071	armadillo repeat containing 2
10552	actin related protein 2/3 complex, subunit 1A, 41kDa
10109	actin related protein 2/3 complex, subunit 2, 34kDa
10093	actin related protein 2/3 complex, subunit 4, 20kDa
29028	ATPase family, AAA domain containing 2
466	activating transcription factor 1
9551	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit F2
567	beta-2-microglobulin
573	BCL2-associated athanogene
9532	BCL2-associated athanogene 2
9531	BCL2-associated athanogene 3
55971	BAI1-associated protein 2-like 1
55024	B-cell scaffold protein with ankyrin repeats 1
580	BRCA1 associated RING domain 1
7918	HLA-B associated transcript 4
56647	BRCA2 and CDKN1A interacting protein
144453	bestrophin 3
552900	bolA homolog 2 (E. coli)
25855	breast cancer metastasis suppressor 1
682	basigin (Ok blood group)
689	basic transcription factor 3
695	Bruton agammaglobulinemia tyrosine kinase
708	complement component 1, q subcomponent binding protein
781	calcium channel, voltage-dependent, alpha 2/delta subunit 1

805	calmodulin 2 (phosphorylase kinase, delta)
830	capping protein (actin filament) muscle Z-line, alpha 2
831	calpastatin
857	caveolin 1, caveolae protein, 22kDa
220869	COBW domain containing 5
23468	chromobox homolog 5 (HP1 alpha homolog, Drosophila)
151903	coiled-coil domain containing 12
374969	coiled-coil domain containing 23
152137	coiled-coil domain containing 50
51372	coiled-coil domain containing 72
338657	coiled-coil domain containing 84
908	chaperonin containing TCP1, subunit 6A (zeta 1)
8763	CD164 molecule, sialomucin
23607	CD2-associated protein
960	CD44 molecule (Indian blood group)
967	CD63 molecule
928	CD9 molecule
81602	cytidine and dCMP deaminase domain containing 1
11140	cell division cycle 37 homolog (S. cerevisiae)
1033	[1x82 char]
8837	CASP8 and FADD-like apoptosis regulator
51142	coiled-coil-helix-coiled-coil-helix domain containing 2
84303	coiled-coil-helix-coiled-coil-helix domain containing 6
51287	coiled-coil-helix-coiled-coil-helix domain containing 8
51550	cyclin-dependent kinase 2-interacting protein
84916	cirrrosis, autosomal recessive 1A (cirhin)
1164	CDC28 protein kinase regulatory subunit 2
1182	chloride channel 3
9685	clathrin interactor 1
1209	cleft lip and palate associated transmembrane protein 1
1266	calponin 3, acidic
149951	COMM domain containing 7
10980	COP9 constitutive photomorphogenic homolog subunit 6 (Arabidopsis)
22818	coatamer protein complex, subunit zeta 1
23603	coronin, actin binding protein, 1C
23406	coactosin-like 1 (Dictyostelium)
10328	COX4 neighbor
1350	cytochrome c oxidase subunit VIIc
1351	cytochrome c oxidase subunit 8A (ubiquitous)
1399	v-crk sarcoma virus CT10 oncogene homolog (avian)-like
7812	cold shock domain containing E1, RNA-binding
1452	casein kinase 1, alpha 1
1479	cleavage stimulation factor, 3 pre-RNA, subunit 3, 77kDa

1485	cancer/testis antigen 1B
8452	cullin 3
1611	death-associated protein
9802	DAZ associated protein 2
131566	discoidin, CUB and LCCL domain containing 2
8853	development and differentiation enhancing factor 2
8886	DEAD (Asp-Glu-Ala-Asp) box polypeptide 18
9188	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21
9879	DEAD (Asp-Glu-Ala-Asp) box polypeptide 46
1655	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5
7913	DEK oncogene (DNA binding)
1665	DEAH (Asp-Glu-Ala-His) box polypeptide 15
27120	dickkopf-like 1 (soggy)
9229	discs, large (Drosophila) homolog-associated protein 1
9988	cyclin D binding myb-like transcription factor 1
3301	DnaJ (Hsp40) homolog, subfamily A, member 1
23234	DnaJ (Hsp40) homolog, subfamily C, member 9
1786	DNA (cytosine-5-)-methyltransferase 1
1809	dihydropyrimidinase-like 3
8624	Down syndrome critical region gene 2
11034	destrin (actin depolymerizing factor)
150290	dual specificity phosphatase 18
1778	dynein, cytoplasmic 1, heavy chain 1
83658	dynein, light chain, roadblock-type 1
9521	eukaryotic translation elongation factor 1 epsilon 1
1937	eukaryotic translation elongation factor 1 gamma
1938	eukaryotic translation elongation factor 2
1964	eukaryotic translation initiation factor 1A, X-linked
1968	eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa
8661	eukaryotic translation initiation factor 3, subunit A
1973	eukaryotic translation initiation factor 4A, isoform 1
1977	eukaryotic translation initiation factor 4E
9470	eukaryotic translation initiation factor 4E family member 2
1978	eukaryotic translation initiation factor 4E binding protein 1
8672	eukaryotic translation initiation factor 4 gamma, 3
7458	eukaryotic translation initiation factor 4H
9669	eukaryotic translation initiation factor 5B
2014	epithelial membrane protein 3
2023	enolase 1, (alpha)
2029	endosulfine alpha
2079	enhancer of rudimentary homolog (Drosophila)
2109	electron-transfer-flavoprotein, beta polypeptide
653784	family with sequence similarity 128, member A
259282	family with sequence similarity 44, member A

196483	family with sequence similarity 86, member A
26268	F-box protein 9
26509	fer-1-like 3, myoferlin (<i>C. elegans</i>)
9637	fasciculation and elongation protein zeta 2 (zygin II)
2275	four and a half LIM domains 3
55137	fidgetin
80308	FAD1 flavin adenine dinucleotide synthetase homolog (<i>S. cerevisiae</i>)
55082	hypothetical protein FLJ10154
55110	mago-nashi homolog 2
201283	hypothetical protein FLJ32065
649446	hypothetical protein LOC649446 (FLJ35776), mRNA
2317	filamin B, beta (actin binding protein 278)
2332	fragile X mental retardation 1
23048	formin binding protein 1
8061	FOS-like antigen 1
6624	fascin homolog 1, actin-bundling protein (<i>Strongylocentrotus purpuratus</i>)
8880	far upstream element (FUSE) binding protein 1
84248	forty-two-three domain containing 1
11337	GABA(A) receptor-associated protein
2590	[1x94 char]
2597	glyceraldehyde-3-phosphate dehydrogenase
2617	glycyl-tRNA synthetase
2644	GTP cyclohydrolase I feedback regulator
2739	glyoxalase I
51053	geminin, DNA replication inhibitor
10399	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1
2791	guanine nucleotide binding protein (G protein), gamma 11
2879	glutathione peroxidase 4 (phospholipid hydroperoxidase)
2885	growth factor receptor-bound protein 2
80273	GrpE-like 1, mitochondrial (<i>E. coli</i>)
2935	G1 to S phase transition 1
2963	general transcription factor IIF, polypeptide 2, 30kDa
94239	H2A histone family, member V
8520	histone acetyltransferase 1
3066	histone deacetylase 2
3068	hepatoma-derived growth factor (high-mobility group protein 1-like)
164045	HFM1, ATP-dependent DNA helicase homolog (<i>S. cerevisiae</i>)
3099	hexokinase 2
10042	high-mobility group protein 2-like 1
8091	high mobility group AT-hook 2
3150	high-mobility group nucleosome binding domain 1
3151	high-mobility group nucleosomal binding domain 2

9324	high mobility group nucleosomal binding domain 3
51155	hematological and neurological expressed 1
3181	heterogeneous nuclear ribonucleoprotein A2/B1
220988	heterogeneous nuclear ribonucleoprotein A3
3182	heterogeneous nuclear ribonucleoprotein A/B
3184	[1x88 char]
4670	heterogeneous nuclear ribonucleoprotein M
3251	hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)
3320	heat shock protein 90kDa alpha (cytosolic), class A member 1
3326	heat shock protein 90kDa alpha (cytosolic), class B member 1
22824	heat shock 70kDa protein 4-like
3315	heat shock 27kDa protein 1
3460	interferon gamma receptor 2 (interferon gamma transducer 1)
121457	IKK interacting protein
3563	interleukin 3 receptor, alpha (low affinity)
3608	interleukin enhancer binding factor 2, 45kDa
8821	inositol polyphosphate-4-phosphatase, type II, 105kDa
10527	importin 7
124152	IQ motif containing K
51015	isochorismatase domain containing 1
84522	jagunal homolog 1 (Drosophila)
11104	katanin p60 (ATPase-containing) subunit A 1
25948	kelch repeat and BTB (POZ) domain containing 2
9710	KIAA0355
23255	KIAA0802 (KIAA0802), mRNA
401265	kelch-like 31 (Drosophila)
148930	kinocilin
889	KRIT1, ankyrin repeat containing
3856	keratin 8
23367	La ribonucleoprotein domain family, member 1
3939	lactate dehydrogenase A
3958	lectin, galactoside-binding, soluble, 3
51474	LIM domain and actin binding 1
4000	lamin A/C
130074	p20
541471	hypothetical LOC541471
79836	LON peptidase N-terminal domain and ring finger 3
9663	lipin 2
123872	leucine rich repeat containing 50
55379	leucine rich repeat containing 59
9208	leucine rich repeat (in FLII) interacting protein 1
124801	LSM12 homolog (S. cerevisiae)
27258	LSM3 homolog, U6 small nuclear RNA associated (S. cerevisiae)
51631	LUC7-like 2 (S. cerevisiae)

55646	Ly1 antibody reactive homolog (mouse)
57226	LYR motif containing 2
5605	mitogen-activated protein kinase kinase 2
28956	mitogen-activated protein-binding protein-interacting protein
5594	mitogen-activated protein kinase 1
79109	mitogen-activated protein kinase associated protein 1
10150	muscleblind-like 2 (Drosophila)
84693	methylmalonyl CoA epimerase
10988	methionyl aminopeptidase 2
64863	methyltransferase like 4
57020	esophageal cancer associated protein
440400	similar to DNA segment, Chr 11, Brigham & Womens Genetics 0434 expressed
389538	MGC72080 pseudogene
84365	MKI67 (FHA domain) interacting nucleolar phosphoprotein
23609	makorin, ring finger protein, 2
4301	[1x94 char]
10627	myosin regulatory light chain MRCL3
103910	myosin regulatory light chain MRLC2
51253	mitochondrial ribosomal protein L37
51154	mRNA turnover 4 homolog (S. cerevisiae)
2956	mutS homolog 6 (E. coli)
4478	moesin
4482	methionine sulfoxide reductase A
4502	metallothionein 2A
92140	metadherin
441024	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2-like
26579	[1x79 char]
4627	myosin, heavy chain 9, non-muscle.
4666	nascent polypeptide-associated complex alpha subunit
4678	nuclear autoantigenic sperm protein (histone-binding)
80218	N-acetyltransferase 13
22916	nuclear cap binding protein subunit 2, 20kDa
4691	nucleolin
4701	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 7, 14.5kDa
54539	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 11, 17.3kDa
4713	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa
4717	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1, 6kDa
4724	[1x81 char]
23327	neural precursor cell expressed, developmentally down-regulated 4-like
91624	nexilin (F actin binding protein)
4780	nuclear factor (erythroid-derived 2)-like 2
51335	neugrin, neurite outgrowth associated (NGRN), transcript variant 1,

	mRNA
4809	NHP2 non-histone chromosome protein 2-like 1 (<i>S. cerevisiae</i>)
4836	N-myristoyltransferase 1
644928	hCG2033311
27077	B9 protein
51406	nucleolar protein 7, 27kDa
4863	nuclear protein, ataxia-telangiectasia locus
9520	aminopeptidase puromycin sensitive
4869	nucleophosmin (nucleolar phosphoprotein B23, numatrin)
10360	nucleophosmin/nucleoplasmin, 3
1728	NAD(P)H dehydrogenase, quinone 1
54780	non-SMC element 4 homolog A (<i>S. cerevisiae</i>)
123803	N-terminal asparagine amidase
64710	nuclear casein kinase and cyclin-dependent kinase substrate 1
84955	NudC domain containing 1
134492	NudC domain containing 2
11165	nudix (nucleoside diphosphate linked moiety X)-type motif 3
9972	nucleoporin 153kDa
9688	nucleoporin 93kDa
114883	oxysterol binding protein-like 9
5036	proliferation-associated 2G4, 38kDa
8106	poly(A) binding protein, nuclear 1
8712	P antigen family, member 1 (prostate associated)
5062	p21 (CDKN1A)-activated kinase 2
5074	PRKC, apoptosis, WT1, regulator
5090	pre-B-cell leukemia homeobox 3
55251	[1x76 char]
9141	programmed cell death 5
10954	protein disulfide isomerase family A, member 5
5202	prefoldin subunit 2
5204	prefoldin subunit 5
5216	profilin 1
5230	phosphoglycerate kinase 1
90102	pleckstrin homology-like domain, family B, member 2
29085	phosphohistidine phosphatase 1
5303	[1x75 char]
5585	protein kinase N1
5358	plastin 3 (T isoform)
5411	pinin, desmosome associated protein
51082	polymerase (RNA) I polypeptide D, 16kDa
5435	polymerase (RNA) II (DNA directed) polypeptide F
5436	polymerase (RNA) II (DNA directed) polypeptide G
84265	polymerase (RNA) III (DNA directed) polypeptide G (32kD)-like
5445	paraoxonase 2

5447	P450 (cytochrome) oxidoreductase
8611	phosphatidic acid phosphatase type 2A
8496	PTPRF interacting protein, binding protein 1 (liprin beta 1)
5514	protein phosphatase 1, regulatory (inhibitor) subunit 10
94274	protein phosphatase 1, regulatory (inhibitor) subunit 14A
26472	protein phosphatase 1, regulatory (inhibitor) subunit 14B
5504	protein phosphatase 1, regulatory (inhibitor) subunit 2
9055	protein regulator of cytokinesis 1
25824	peroxiredoxin 5
9581	prolyl endopeptidase-like
3276	protein arginine methyltransferase 1
10544	protein C receptor, endothelial (EPCR)
8899	PRP4 pre-mRNA processing factor 4 homolog B (yeast)
5396	paired related homeobox 1
11168	PC4 and SFRS1 interacting protein 1
5682	proteasome (prosome, macropain) subunit, alpha type, 1
5684	proteasome (prosome, macropain) subunit, alpha type, 3
5688	proteasome (prosome, macropain) subunit, alpha type, 7
5689	proteasome (prosome, macropain) subunit, beta type, 1
5692	proteasome (prosome, macropain) subunit, beta type, 4
5694	proteasome (prosome, macropain) subunit, beta type, 6
5695	proteasome (prosome, macropain) subunit, beta type, 7
5700	proteasome (prosome, macropain) 26S subunit, ATPase, 1
5704	proteasome (prosome, macropain) 26S subunit, ATPase, 4
5707	proteasome (prosome, macropain) 26S subunit, non-ATPase, 1
5718	proteasome (prosome, macropain) 26S subunit, non-ATPase, 12
9861	proteasome (prosome, macropain) 26S subunit, non-ATPase, 6
5714	proteasome (prosome, macropain) 26S subunit, non-ATPase, 8
9491	proteasome (prosome, macropain) inhibitor subunit 1 (PI31)
5757	prothymosin, alpha (gene sequence 28)
5763	parathymosin
51495	protein tyrosine phosphatase-like A domain containing 1
5770	protein tyrosine phosphatase, non-receptor type 1
754	pituitary tumor-transforming 1 interacting protein
54899	PX domain containing serine/threonine kinase
5829	paxillin
8766	RAB11A, member RAS oncogene family
5861	RAB1A, member RAS oncogene family
11031	RAB31, member RAS oncogene family
5876	Rab geranylgeranyltransferase, beta subunit
5879	[1x87 char]
5902	RAN binding protein 1
3843	RAN binding protein 5
54922	Ras interacting protein 1

125950	ribonucleoprotein, PTB-binding 1
389677	RNA binding motif protein 12B
55285	RNA binding motif protein 41
9939	RNA binding motif protein 8A (RBM8A), mRNA
9978	ring-box 1
5981	replication factor C (activator 1) 1, 145kDa
64864	regulatory factor X domain containing 2
6001	regulator of G-protein signaling 10
6007	Rh blood group, D antigen
6118	replication protein A2, 32kDa
6135	ribosomal protein L11
23521	ribosomal protein L13a
6141	ribosomal protein L18
6146	ribosomal protein L22
6147	ribosomal protein L23a
6152	ribosomal protein L24
6155	ribosomal protein L27
6159	ribosomal protein L29
25873	ribosomal protein L36
6167	ribosomal protein L37
6170	ribosomal protein L39
6124	ribosomal protein L4
6171	ribosomal protein L41
6128	ribosomal protein L6
6129	ribosomal protein L7
6130	ribosomal protein L7a
6132	ribosomal protein L8
6175	ribosomal protein, large, P0
6176	ribosomal protein, large, P1
6181	ribosomal protein, large, P2
10799	ribonuclease P/MRP 40kDa subunit
6205	ribosomal protein S11
6206	ribosomal protein S12
6207	ribosomal protein S13
6209	ribosomal protein S15
6210	ribosomal protein S15a
6217	ribosomal protein S16
6218	ribosomal protein S17
6222	ribosomal protein S18
91582	ribosomal protein S19 binding protein 1
6224	ribosomal protein S20
6228	ribosomal protein S23
6229	ribosomal protein S24
6230	ribosomal protein S25

6234	ribosomal protein S28
6188	ribosomal protein S3
6189	ribosomal protein S3A
6194	ribosomal protein S6
6201	ribosomal protein S7
6237	related RAS viral (r-ras) oncogene homolog
51018	ribosomal RNA processing 15 homolog (<i>S. cerevisiae</i>)
26156	ribosomal L1 domain containing 1
65117	arginine/serine-rich coiled-coil 2
57142	reticulon 4
11017	putative nucleic acid binding protein RY-1
6281	S100 calcium binding protein A10
6282	S100 calcium binding protein A11
9667	scaffold attachment factor B2
29115	SAP30 binding protein
10066	secretory carrier membrane protein 2
6396	SEC13 homolog (<i>S. cerevisiae</i>)
140606	selenoprotein M
55752	septin 11
10169	small EDRK-rich factor 2
5269	serpin peptidase inhibitor, clade B (ovalbumin), member 6
6418	SET translocation (myeloid leukemia-associated)
6419	SET domain and mariner transposase fusion gene
23451	splicing factor 3b, subunit 1, 155kDa
51639	splicing factor 3B, 14 kDa subunit
6434	[1x76 char]
6455	SH3-domain GRB2-like 1
30011	SH3-domain kinase binding protein 1
7979	split hand/foot malformation (ectrodactyly) type 1
10572	SIVA1, apoptosis-inducing factor
7884	stem-loop (histone) binding protein
6575	solute carrier family 20 (phosphate transporter), member 2
5003	[1x74 char]
51312	solute carrier family 25, member 37
6515	solute carrier family 2 (facilitated glucose transporter), member 3
6534	[1x75 char]
9368	solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1
79811	SAFB-like, transcription modulator
11042	glucuronidase, beta pseudogene
4088	SMAD family member 3
6611	spermine synthase
6628	small nuclear ribonucleoprotein polypeptides B and B1
6632	small nuclear ribonucleoprotein D1 polypeptide 16kDa

6636	small nuclear ribonucleoprotein polypeptide F
6637	small nuclear ribonucleoprotein polypeptide G
10073	snurportin 1
8724	sorting nexin 3
58533	sorting nexin 6
6647	superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))
65244	spermatogenesis associated, serine-rich 2
28972	signal peptidase complex subunit 1 homolog (<i>S. cerevisiae</i>)
23111	spastic paraplegia 20 (Troyer syndrome)
6711	spectrin, beta, non-erythrocytic 1
144108	SPT2, Suppressor of Ty, domain containing 1 (<i>S. cerevisiae</i>)
23350	U2-associated SR140 protein
10250	serine/arginine repetitive matrix 1
23524	serine/arginine repetitive matrix 2
10274	stromal antigen 1
10963	stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizing protein)
6789	serine/threonine kinase 4
3925	stathmin 1/oncoprotein 18
7341	SMT3 suppressor of mif two 3 homolog 1 (<i>S. cerevisiae</i>)
6613	SMT3 suppressor of mif two 3 homolog 2 (<i>S. cerevisiae</i>)
8887	Tax1 (human T-cell leukemia virus type I) binding protein 1
64786	TBC1 domain family, member 15
1155	tubulin folding cofactor B
22980	transcription factor 25 (basic helix-loop-helix)
6950	t-complex 1
6996	thymine-DNA glycosylase
26136	testis derived transcript (3 LIM domains)
7019	transcription factor A, mitochondrial
29844	TCF3 (E2A) fusion partner (in childhood Leukemia)
80764	THAP domain containing 7
10189	THO complex 4
7077	TIMP metalloproteinase inhibitor 2
7082	tight junction protein 1 (zona occludens 1)
116238	TLC domain containing 1
55002	transmembrane and coiled-coil domains 3
114908	transmembrane protein 123
121256	transmembrane protein 132D (TMEM132D), mRNA
81671	transmembrane protein 49
7112	thymopoietin
7125	troponin C type 2 (fast)
54543	translocase of outer mitochondrial membrane 7 homolog (yeast)
9868	translocase of outer mitochondrial membrane 70 homolog A (<i>S. cerevisiae</i>)

7150	topoisomerase (DNA) I
7168	tropomyosin 1 (alpha)
7170	tropomyosin 3
7171	tropomyosin 4
1200	tripeptidyl peptidase I
29896	transformer-2 alpha
79090	trafficking protein particle complex 6A
7706	tripartite motif-containing 25
203062	t-SNARE domain containing 1
79989	tetratricopeptide repeat domain 26
10376	tubulin, alpha 1b
84790	tubulin, alpha 1c
81027	tubulin, beta 1
10383	tubulin, beta 2C
7295	thioredoxin
9352	thioredoxin-like 1
7296	thioredoxin reductase 1
10587	thioredoxin reductase 2
7307	U2 small nuclear RNA auxiliary factor 1
6675	UDP-N-acetylglucosamine pyrophosphorylase 1
7322	ubiquitin-conjugating enzyme E2D 2 (UBC4/5 homolog, yeast)
7332	ubiquitin-conjugating enzyme E2L 3
7334	ubiquitin-conjugating enzyme E2N (UBC13 homolog, yeast)
92912	ubiquitin-conjugating enzyme E2Q (putative) 2
84993	ubiquitin-like 7 (bone marrow stromal cell-derived)
7357	UDP-glucose ceramide glucosyltransferase
7360	UDP-glucose pyrophosphorylase 2
26019	UPF2 regulator of nonsense transcripts homolog (yeast)
7381	ubiquinol-cytochrome c reductase binding protein
7386	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
9100	ubiquitin specific peptidase 10
219333	ubiquitin specific peptidase 12
9097	ubiquitin specific peptidase 14 (tRNA-guanine transglycosylase)
51118	UTP11-like, U3 small nucleolar ribonucleoprotein, (yeast)
7402	utrophin
9218	VAMP (vesicle-associated membrane protein)-associated protein A, 33kDa
7408	vasodilator-stimulated phosphoprotein
7411	von Hippel-Lindau binding protein 1
7414	vinculin
7430	villin 2 (ezrin)
7431	vimentin
9559	vacuolar protein sorting 26 homolog A (S. pombe)
9525	vacuolar protein sorting 4 homolog B (S. cerevisiae)

7453	tryptophanyl-tRNA synthetase
55759	WD repeat domain 12
144406	WD repeat domain 66
339005	WAS protein homology region 2 domain containing 1-like 1
9503	X antigen family, member 1D
7520	[1x125 char]
2547	[1x94 char]
10138	YY1 associated factor 2
10413	Yes-associated protein 1, 65kDa
4904	Y box binding protein 1
91746	YTH domain containing 1
7531	[1x91 char]
7534	[1x88 char]
7528	YY1 transcription factor
9839	zinc finger E-box binding homeobox 2
51663	zinc finger RNA binding protein
23613	zinc finger, MYND-type containing 8
9726	zinc finger protein 646
7784	zona pellucida glycoprotein 3 (sperm receptor)
9183	ZW10, kinetochore associated, homolog (Drosophila)

The proteome response was measured using a dynamic proteomics approach [14, 16-18]. A library of human lung cancer clones (H1299 cell line) was used, where each clone has a different endogenous protein fused to a yellow fluorescent protein (YFP) that is used as a reporter. The YFP was inserted as an artificial exon at the natural chromosomal loci of the gene, resulting in a full length protein that is under its native regulation. Comparisons to immunoblots indicated that 80 % of the tagged proteins are accurate markers for the endogenous protein dynamics [18]. All the clones in the library contain an additional red fluorescent marker that enables accurate automated image analysis and tracking of individual cells. Analysis was performed automatically using custom software (Methods and Figure 4)

Protein dynamics were measured at high temporal resolution (every 20 minutes, 144 time points) using time lapse microscopy under incubated conditions in multi-well plates as described [18]. Experiments were done in two repetitions on average with four fields of views taken from each well. For each protein, the dynamics were obtained by automatically tracking 300 – 600 individual cells and averaging their profiles. After 24 h of growth, the topoisomerase-1 poison CPT [21] was added at 10 μ M. All cells

stopped dividing 5-7 hours after drug addition and about 25 % underwent apoptosis after 48 hours. Assay reproducibility in day to day repeats was better than 20 % (std/mean). Thus changes larger than 20 % in a tagged protein intensity can be reliably detected using the present assay.

5 Dynamic proteomics has previously been used to measure the temporal changes of proteins compared to a reference time-point. Here dynamic proteomics was extended to enable comparisons across different proteins. For this purpose a calibration was developed that converts protein fluorescent units into relative protein abundance. The calibration employs the red fluorescence marker common to all the clones as a reference
10 for normalizing the different proteins (details in Methods). This calibration yielded excellent agreement with quantitative immunoblots on selected proteins.

 mRNA dynamics was measured using Affymetrix GeneChip Human Gene 1.0 ST arrays that comprehensively cover known human transcripts. Cells were grown in 10 cm plates in the same conditions as the protein experiments and harvested at nine
15 different time points before and after drug addition (0, 1.5, 3, 6, 9, 12, 23, 33 and 48 hours) with at least three replicates per time point. Data was normalized using standard RMA background correction and GC content normalization. Reproducibility was better than 15 % (std/mean) (Figures 5A-B). To further test the microarray measurements, qPCR was performed on 10 genes that showed diverse dynamics. The measurements
20 obtained by the two assays yielded very similar profiles with a median correlation of 0.83 (Figure 6). Time-dependant gene ontology (GO) enrichment analysis revealed temporal waves of functionally related genes that responded in concert to the drug: some falling together (e.g. histone and nucleosome assembly constituents $P < 10^{-60}$, cell division related genes $P < 10^{-13}$ and snoRNAs $P < 10^{-15}$) and other rising (e.g. MHC-I
25 genes $P < 10^{-8}$ and lysosomal genes $P < 10^{-8}$) (for details see Figures 1A-K).

 Finally, the protein and mRNA sets were intersected to create a dataset of 540 protein and mRNA pairs of co-dynamic responses (Table 4, Figure 7).

EXAMPLE 2***Dis-correlation between dynamics of mRNAs and their corresponding proteins in response to a drug***

The present inventors asked whether changes over time in a given protein are related to the changes in that protein's mRNA levels. To examine this the temporal dynamics of 540 proteins were compared to their corresponding mRNAs. This revealed a wide range of co-dynamic behaviors. About 30% of the mRNA-protein pairs exhibited strong positive correlations in their temporal profiles ($\rho > 0.6$). Strikingly, strong anti-correlations ($\rho < -0.6$) were found in 15 % of the genes in response to the drug. The rest of the genes (about 55 %) showed weak or no correlation to their mRNA ($-0.6 < \rho < 0.6$) (see Figures 8A-B).

The significance of the observed correlations was tested by a data shuffling test, in which the identities of mRNAs and proteins were randomly shuffled. It was found that the dynamics of only about 15 % of the proteins exhibited a positive correlation that is more than expected in shuffled data ($P < 10^{-6}$, Figure 2). This suggests that under the present conditions only a small fraction of the mRNA variations over time determine the variations in protein levels.

Time-shift correlation analysis was also performed, in which the temporal protein profiles were shifted with respect to the mRNA profiles, to test for effects of delay between mRNA and protein. It was found that time shift did not yield more positive correlations than observed in shuffled data ($P \approx 0.5$). This suggests that time delay in protein production is not a major factor causing the observed dis-correlation.

It was found that sets of genes sharing similar functions tended to share similar correlations or lack of correlations between their mRNA and protein dynamics. For example, ribosomal proteins were dis-correlated to their mRNAs in a systematic manner: whereas most ribosomal proteins decay in response to the drug (GO enrichment $P < 10^{-4}$) their corresponding mRNAs all showed a synchronized increase in the first 8 hours and then a slow decrease (Figures 8C-E). In contrast, most cytoskeleton related proteins showed a correlated decay in both protein and mRNA that was synchronized with loss of cell motility (Figures 8F-H).

In addition to following the temporal correlation between pairs of mRNA and protein over time, the correlation across different genes using snapshots of the

transcriptome and proteome at single time points was also tested. The present data has a dynamic range of about 500 fold between the least and most abundant protein. The present inventors asked whether these differences in protein levels are compatible with variations in their mRNAs levels. It was found that relative mRNA levels do not
 5 correlate well with relative protein levels across different genes ($r = 0.13$, $P < 10^{-9}$), Figure 2I. Correlation remained roughly constant in snapshots taken at different times after drug addition ($r = 0.1 - 0.15$). This result is in agreement with previous studies that compared mRNA and protein snapshots in human cells [8-13].

Overall, it was found that snapshots as well as time dependant changes in pairs
 10 of proteins and mRNAs are uncorrelated and often anti-correlated in human cancer cells under the present condition. This suggests that post-transcriptional control is a key factor in determining protein dynamics in response to the drug.

The present inventors next asked how protein translation and degradation rates combine to produce the observed dynamics. It was found that kinetic models with
 15 constant translation and degradation rates poorly fitted the data, whereas models with time varying translation and degradation were not constrained enough to explain the data: changes in either production or degradation could result in the same dynamic profile of protein levels. Hence, to proceed, direct measurements of protein dynamic degradation and translation rates were measured.

20

EXAMPLE 3

Bleach-chase: a novel method for accurate measurements of protein degradation rate dynamics

Protein dynamics is dictated by production of new proteins and their removal.
 25 The latter is the sum of two underlying processes: intra cellular degradation (e.g. due to proteosome activity) and dilution due to cell growth that effectively reduces the protein amount by 50% every one cell generation [22]. For clarity the term degradation rate, α , will refer to the sum effect of both intra cellular degradation, α_{deg} , and the dilution rate, α_{dil} :

30 Eq. (1) $\alpha = \alpha_{deg} + \alpha_{dil}$

Then protein levels, $P(t)$, then follows the simple equation [22]:

$$\text{Eq. (2)} \quad \frac{dP(t)}{dt} = \beta(t) - \alpha(t) \cdot P(t),$$

where $\beta(t)$ is the production rate and $\alpha(t)$ the degradation rate (note that production and degradation may also implicitly depend on mRNA and protein levels).

5 The present goal in this section is to measure the production and degradation rates $\beta(t)$ and $\alpha(t)$ as a function of time.

The present inventors first attempted to measure protein degradation rates using a standard translation inhibition protocol [23]. In this approach protein levels are measured at different times after the addition of the translation inhibitor Cyclohexamide (CHX). It is expected that protein production will halt and that the protein dynamics will gradually decay to zero in a rate that is exponential in the degradation rate. The present inventors followed this protocol and added the inhibitor CHX, with and without the drug CPT, at different concentrations and measured the levels of 11 proteins with our dynamic proteomics assay (see Methods). This method did not yield satisfactory results. The present inventors observed one protein that increased its level rather than decreased. Furthermore, about half of the proteins tested showed dynamics that did not fit an exponential decay to zero levels, suggesting that translation inhibition was incomplete (increasing the dosage of CHX did not eliminate this effect) (Figure 2). Additionally, about 10 % of the cells underwent cell-death and most cell stopped dividing due to the CHX addition. Therefore, extracting meaningful degradation rates for many of the tested proteins was not possible using this approach.

An alternative method for measuring protein degradation rates is ‘pulse-chase’ in which proteins are radioactively labeled over a brief period (the pulse) and then the decay in radioactivity is measured in time (the chase) and used to infer protein half life. This assay requires a different antibody for each protein of interest in order to pull it down. Despite its accuracy, it is difficult to scale this method up to more than a few proteins and time points.

In order to address these limitations and measure protein degradation and production rates of multiple proteins at high temporal resolution without the need for translation inhibitors or radio-active labeling, the present inventors developed an assay called ‘bleach-chase’. In bleach-chase, one bleaches the fluorophore of the tagged

protein using a brief pulse of light thus irreversibly turning it non-fluorescent (it is sufficient to bleach only a small fraction of the tagged proteins). Following the cells over time shows that protein fluorescence recovers over hours to days, due to production of new tagged proteins. The bleaching did not alter cell-viability, motility, mitosis rate or morphology. This is in agreement with the general observation that GFP-fused proteins are relatively non-phototoxic under fluorescence microscopy and retain protein activity under mild bleaching [24-25]. This method has been termed 'bleach-chase', to highlight the analogy with the classical 'pulse-chase' method. In bleach-chase cells are subjected to a mild pulse of light, which transforms a fraction of their fluorescent proteins into non-fluorescent. The decay of the non-fluorescent protein fraction (the difference between the fluorescence of the bleached and unbleached cells) is then chased in time to reveal the protein half-life: this difference decays in time according to the degradation rate, as described by the equation (obtained by subtracting and solving Eq. 1 for the bleached and unbleached cells, see Methods):

$$\text{Eq (3)} \quad \ln(P(t)-P_v(t)) = \ln(P_0-P_{0v}) - \alpha \cdot t,$$

where $P(t)$ and $P_v(t)$ are the fluorescence levels of the unbleached and bleached cells, respectively; P_0 and P_{0v} are the fluorescence levels of the unbleached and bleached at an initial time point, respectively. If the degradation rate, α , varies with time

the same equation applies, but α is replaced with $\int_0^t \alpha(t') dt'$. The formula is derived in the Methods.

To understand this method, note that the profiles of the bleached and unbleached cells begin at different initial levels and eventually converge to the same dynamics (Figures 9A-C). The rate at which they converge reveals the degradation rate: rapid convergence means rapid degradation and slow convergence means slow degradation. It is important to note that bleach-chase applies regardless of how protein production rate changes in time, nor does it require halting production. The intuition behind this result is that the production of the bleached and non-bleached cells is the same (bleaching does not eliminate the protein, only inactivates its tag making it invisible to fluorescent microscopy) and thus one can cancel-out the effects of production by subtracting the two dynamics.

The day to day error of bleach-chase is about 0.4 (std/mean). Hence, the present inventors averaged of over 3 - 4 replicates to obtain accurate measurements. The present inventors also performed radioactive pulse-chase experiments and compared the measurements to those obtained using bleach-chase. The two methods yielded highly similar measurements with average deviation less than 15% (see Table 5, herein below).

Table 5

Gene Symbol	Bleach-chase $T_{1/2}$ (hours)	Pulse-chase $T_{1/2}$ (hours)	Deviation (%)
RPS3A	5.9 ± 0.6	6.6 ± 0.3	12 %
MYH9	6.4 ± 0.6	5.7 ± 0.6	12 %
DDX5	9.2 ± 1.4	$10.3 \pm$	8 %
K- α -1	17.1 ± 3.0	14.5 ± 1.8	17 %

The present inventors also compared the degradation rate of the YFP-tagged protein to the non-tagged endogenous protein expressed from the untagged allele in the same cells and found them to be very similar (average deviation less than 10 %, Table 6).

Table 6

Gene Symbol	Untagged protein $T_{1/2}$ (hours)	YFP-tagged protein $T_{1/2}$ (hours)
MYH9	5.2 ± 0.3	5.7 ± 0.6
DDX5	$9.1 \pm$	$10.3 \pm$
K- α -1	$18.3 \pm$	14.5 ± 1.8

This result is in line with previous studies that found good agreement between half-lives and dynamics of fluorescently tagged endogenous proteins and untagged ones. As a final test of the bleach-chase method, bleaching at different intensities was applied. The measured degradation rates remained similar (CV < 0.2), regardless of bleaching intensity (Figure 9C).

These tests suggest that bleach-chase is an accurate method for estimating protein degradation rates.

EXAMPLE 4

5 *Protein half-lives increase in response to the drug*

In this example, bleach chase is used to investigate the reasons for the dis-
correlation between transcript and protein dynamics. To address this, 20 proteins were
selected spanning different cellular localizations and functions bleach-chase was used to
measure their degradation rates 0 - 48 hours after drug addition at high temporal
10 resolution (every 10 minutes) with 4 – 6 repeats. The degradation rates in cells were
cultured without the drug (4 – 6 repeats) was measured and the results compared.
Strikingly, a global shift in protein degradation rates was observed due to the drug: most
proteins either decreased their degradation (14 out of 20) or retained the same level (5
out of 20) as depicted in Figure 10A.

15 Overall, it was found that upon drug addition, degradation rates drop and then
retain roughly constant levels (Figure 10B).

EXAMPLE 5

The longer the protein half-life, the larger the increase due to growth arrest

20 This example investigates possible mechanisms that may explain the global
effect described in Example 4.

A protein's half-life, $T_{1/2}$, given a constant degradation rate, α , is given by:

$$Eq. (4) \quad T_{1/2} = \frac{\ln(2)}{\alpha}.$$

Therefore, the observed global decrease in degradation rates is tantamount to an
25 increase in protein half-lives. The present inventors found that the increase showed the
following trend: the larger the protein half-life, the larger its increase after drug
addition. In other words, stable proteins become increasingly more stable whereas
unstable proteins remain largely unaffected by the drug. This phenomenon is shown in
Figure 10C.

30 One plausible explanation for the observed systematic increase in protein half-
lives is a global down-regulation of the protein degradation machinery (e.g. inhibition

of ubiquitin-proteasome mediated proteolysis). However, this explanation does not account for the observation that stable proteins become increasingly more stable. On the contrary, stable proteins are expected to be the least affected, since their half-lives are largely determined by dilution due to cell-division rather than intra-cellular degradation.

5 Next, the present inventors turned to focus on an alternative simple model that accounts for the observed global half-life behavior. It assumes that the drug induces cellular growth arrest. This assumption was tested by obtaining quantitative measurements of the mitosis rates at different time points before and after drug addition using the image analysis software. It was found that mitosis rate drops upon drug
10 addition and reaches a complete stop after 5 - 7 hours.

To see why growth arrest is sufficient to produce the observed half-life behavior, one must return to Eq. 1 that describes protein degradation as the sum of intra-cellular degradation and dilution due to cell growth. A decrease in growth rate thus affects the degradation rates of all proteins as follows:

15 Eq. (5) $\alpha = \alpha_{\text{deg}} + k \cdot \alpha_{\text{dil}}$

where $0 \leq k \leq 1$ defines the degree of growth arrest. Note, that although the decrease in dilution rate is the same across all proteins, the relative change in α_{tot} may vary considerably. Unstable proteins have rapid intra-cellular degradation ($\alpha_{\text{deg}} \gg \alpha_{\text{dil}}$) and thus their α_{tot} remains unaffected even when dilution rate drops to zero. This is in
20 agreement with our observation that proteins with short half-lives are less affected by growth arrest. Stable proteins, on the other hand, have slow intra-cellular degradation ($\alpha_{\text{deg}} \ll \alpha_{\text{dil}}$), their half life is mainly determined by the rate of cell division, and hence its arrest is expected to significantly increase protein half-life. This is also in agreement with our measurements.

25 Next, the present inventors tested whether the growth arrest model can quantitatively explain the measured changes in half life. One can predict a protein's half-life after growth arrest, $T_{1/2}^*$, based on its half-life prior to the arrest, $T_{1/2}$, and the average cell cycle time, T_{cc} using the following equation (based on Eq. 3 and 4, see Methods):

$$\text{Eq. (6)} \quad T_{1/2}^* = \frac{T_{1/2}}{1 + (k - 1) \frac{T_{1/2}}{T_{cc}}},$$

The human lung cancer cells studied here have an average cell cycle time of 20 hours ($T_{cc} = 20 \pm 3$ hours). Applying this information with the observation that cell cycle stops after the drug addition ($k = 0$) to Eq. 6 yields the predicted half-life behavior depicted by the blue line in Figure 10C ($R^2 = 0.4$). Deviations from the line are due to protein specific regulation.

One interesting property that emerges from the model is that the increase in protein stability due to growth arrest is inversely proportional to the cell cycle duration, which means that the faster cells divide prior to the drug addition, the stronger the affect as illustrated in Figure 10D.

Overall, it was found that protein lifetime increased after drug addition, an effect that is stronger the longer the pre-drug lifetime of the protein is. The present analysis suggests that this effect is largely due to cell-growth arrest.

15

EXAMPLE 6

Protein translation gradually decreases in response to the drug

Next, the dynamics of the translation rates in response to the drug were tested. To this end the production rate $\beta(t)$ in Eq. 1 was estimated, which accounts for both transcriptional and post transcriptional effects influencing protein production. The production rate for these proteins can be determined using the measured degradation dynamics (i.e. applying Eq. 1). The translation rate dynamics, $\gamma(t)$, is then determined using the production rate and the measured mRNA dynamics, $M(t)$, according to the following equation:

$$\text{Eq. (7)} \quad \beta(t) = \gamma(t) \cdot M(t).$$

Note, that $\gamma(t)$ accounts for all post transcriptional effects influencing protein production.

It was found that for most of the proteins, translation decreases gradually to less than half of the initial level (Figure 11A, B). The decay in translation is similar in timing for most proteins in this study, suggesting a common cause. This is in line with the observation that CPT causes a decrease in ribosomal proteins on a similar timescale

30

(Figure 11C, $\rho = 0.8$). These results taken together with the half-life measurements indicate that the present drug has two opposing effects: a global increase in protein half-lives and simultaneous decrease in translation rates. The balance between these counter effects significantly changes in response to the drug, a change which varies considerably across different proteins, as depicted in Figure 11D. The change in balance between half-lives and translation rates, rather than changes in mRNA dynamics, is in many instances the dominant factor controlling the protein response (Figure 12). Figure 11E, shows examples of proteins whose temporal response is dis-correlated and even anti-correlated to their mRNAs dynamics but in accordance with their relative increase in half-lives.

EXAMPLE 7

Additional information

1. *A kinetic model with constant translation and degradation rates does not explain the data*

It was asked whether a simple kinetic model that assumes constant translation and degradation rates may explain the observed dis-correlation between mRNA and protein. To address this, the present inventors used the following model:

$$\text{Eq. (S1)} \quad \frac{dP(t)}{dt} = \gamma \cdot M(t) - \alpha \cdot P(t),$$

where γ is the translation rate (note that γ accounts for all post-transcriptional production), α is the degradation rate, $P(t)$ is protein levels and $M(t)$ mRNA levels. Both $M(t)$ and $P(t)$ were normalized to their levels at $t = 0$ prior to drug addition. $M(t)$ was then interpolated to match the sampling frequency of $P(t)$ yielding two vectors with 144 time points. Hence, for each pair of mRNA and protein profiles an over-determined linear set of equations (143 equations and two parameters) is obtained. Translation and degradation rates were then estimated by identifying the two values that minimize the sum of square errors over all equations (other estimation procedures such as minimization on the integral solution yielded similar results). One can then use the

estimations of translation and degradation rates together with mRNA measurements to predict protein levels using the integral solution of Eq. S1:

$$\text{Eq. (S2)} \quad P(t) = P_0 e^{-\alpha t} + \int_0^t \gamma \cdot M(k) e^{-\alpha(t-k)} dk$$

Next, the predicted protein profiles were compared to the empirical protein
 5 measurements and found that in about half of the cases the difference between the predicted and empirical protein levels was greater than the present experimental measurement error (CV < 20 %) indicating that the above model is inadequate for capturing the dynamics of many of the proteins. This suggested that the model assumption, that translation and degradation are roughly constant in response to the
 10 drug, is incorrect and that at least one of these components is significantly changing in time.

2. Kinetic models with time varying translation and degradation rates yield non-unique predictions

15 The present inventors therefore, turned to investigate models that allow for temporal changes in the behavior of the translation and degradation. To this end the following more general model was used:

$$\text{Eq. (S3)} \quad \frac{dP(t)}{dt} = \gamma(t) \cdot M(t) - \alpha(t) \cdot P(t).$$

Note that since this model allows both translation and production to change in
 20 time, there are more parameters than equations. To circumvent this problem of over-fitting two approaches were tested: The first was to penalize for non-smooth profiles of $\gamma(t)$ and $\alpha(t)$ thus restricting the rate of temporal change. The second was to use a piecewise linear approach where both translation and degradation profiles are modeled using 2 – 5 connected lines. While both methods were able to accurately capture most
 25 protein behaviors (i.e. error of fit was better than measurement error) the parameter estimations were non-unique: different profiles of production or degradation could

result in the same dynamic profile of protein levels. Overall, the present inventors were unable to indirectly retrieve reliable translation and degradation rates from mRNA and protein dynamics alone. We therefore turned to direct measurements of protein degradation and translation rate dynamics.

5

3. A decrease in intra-cellular degradation is expected to induce a global change in half-lives

This section discusses a simple model for predicting the shift in half-lives due to a global down-regulation of intra-cellular degradation (e.g. inhibition of ubiquitin-proteosome mediated proteolysis). This model suggests that the half-lives of all proteins is expected to increase but that stable proteins will be the least affected. The rationale is that their half-lives are largely determined by cell-division rather than intra-cellular degradation.

First, the model assumes that the process of protein degradation is a sum of two underlying processes: (i) intra-cellular active degradation, α_{deg} , (e.g. due to proteosome activity) and (ii) protein dilution rate due to cell growth, α_{dil} , which effectively reduces the protein amount by 50 % after one cell generation time. A protein's effective degradation rate, α_{tot} , can therefore be captured by the following equation:

20

$$Eq.(S4) \quad \alpha = \alpha_{deg} + \alpha_{dil}$$

A global inactivation of intra-cellular degradation can be assumed (due to down regulation of one of the essential components) that can be captured according to the following equation:

$$Eq.(S5) \quad \alpha = k \cdot \alpha_{deg} + \alpha_{dil}$$

25

where $0 \leq k \leq 1$ defines the degree of reduction in intra-cellular degradation.

The goal now is to derive the new protein half-life after, $\frac{T_1^*}{2}$, based on its half-life prior to the inactivation, $\frac{T_1}{2}$, and the average cell cycle time, T_{cc} . Using Eq. 3, S4 and S5 one can derive the following relations:

$$Eq.(S6) \quad \frac{T_1}{2} = \frac{\ln(2)}{\alpha_{deg} + \alpha_{dil}}$$

$$Eq.(S7) \quad \frac{T_1^*}{2} = \frac{\ln(2)}{k \cdot \alpha_{deg} + \alpha_{dil}}$$

$$Eq.(S8) \quad T_{cc} = \frac{\ln(2)}{\alpha_{dil}}$$

Applying Eq. S6 and S8 to S7 gives:

$$T_{1/2}^* = \frac{\ln(2)}{k(\alpha_{deg} + \alpha_{dil}) + (1-k)\alpha_{dil}} = \frac{\ln(2)}{k \frac{\ln(2)}{T_{1/2}} + (1-k) \frac{\ln(2)}{T_{cc}}},$$

resulting in:

$$Eq.(S9) \quad T_{1/2}^* = \frac{T_{1/2}}{k + (1-k) \frac{T_{1/2}}{T_{cc}}}$$

This model suggests that when intra-cellular degradation decreases the result is a global increase in half lives but stable proteins are expected to be the least affected. This point is illustrated in Figure 14, where the predicted increase for different k's are shown given a $T_{cc} = 20$ hours (as measured in the cells used in this study).

Discussion

This study provides an integrated view of the dynamics of the transcriptome, proteome, protein degradation and translation rates as human cancer cells respond in

time to a drug. The present inventors asked how well are temporal changes in the proteome correlated with changes in the transcriptome. Measurements of 540 protein dynamics and their respective mRNAs in response to the drug revealed poor correlations and even anti-correlations in 70 % of the pairs. Time shift correlations for capturing potential delays between mRNA and protein as well as kinetic models did not resolve the observed discrepancy, suggesting that post-transcriptional control plays an important role in fine tuning the response to the drug CPT. Whether the temporal dis-correlation between mRNA and protein observed in the present condition applies to other non-drug like stimuli in human cells is still an open question.

Next, the present inventors asked how temporal changes in post transcriptional control, namely protein translation and degradation, combine to produce the observed protein dynamics. Current methods for measuring protein degradation rates have several limitations: pulse-chase is accurate but requires radioactive labeling, protein specific antibodies and is laborious, limiting its usage to the measurements of few proteins at a time; the translation inhibitor CHX is non-radioactive and can be used to measure multiple proteins but is highly perturbative to the cells and may alter the native degradation rates due to translation inhibition of ubiquitine related proteins. To overcome these limitations the present inventors have devised a novel method for accurate measurements of protein half-lives in living cells - bleach-chase. It is non-radioactive, simple to apply, and can be readily scaled to measure multiple proteins at high temporal resolution. The mild pulse of bleaching is also significantly less perturbative than translation inhibition. The main limitation of bleach-chase is the requirement that the protein under study be fluorescently tagged. While, tagged proteins tend to preserve the same dynamics and half-lives as untagged ones (Table 6) their availability is still limited to a few organisms (e.g. the yeast GFP tagged protein library [29] and the human LARC library [18]). Broad usage of bleach-chase will become more feasible with the expected generation of additional tagged protein libraries in other organisms and cell-lines. One future application of bleach-chase is to study how localization changes affect protein half-life dynamics in living cells.

The present inventors used bleach-chase to measure protein degradation dynamics in response to the drug. A global increase in the half-lives of most proteins was observed. Notably, the larger the protein half-life prior to drug addition, the larger

the increase in half-life after addition (Figure 10C). To explain the global increase in life-times, a simple quantitative model may be proposed, which assumes that the drug induces cellular growth arrest. It was found that cells stop dividing after drug addition and that the model accurately reproduces the observed trend. Several properties emerge from this model. First, it suggests that the increase in life time may be a general property of cells that slow down their growth. Second, it predicts that the faster the growth prior to growth arrest, the steeper the increase in half-lives. This implies that rapidly dividing cells (e.g. cancer cells or hair follicles) will experience a larger disequilibrium in their half-lives compared to slowly dividing cells (e.g. neurons) as a result growth arrest (Figure 10D) thus creating an imbalance in protein levels in the cell, with stable proteins rising in levels more than unstable ones. This might be related to the ability of growth arrest drugs to differentially harm fast dividing cells such as tumor cells. Third, an increase in cell-growth is expected to induce the opposite effect: a global decrease in half-lives in which stable proteins undergo a large decrease and unstable proteins remain largely unaffected. These predictions are now readably testable in multiple conditions and proteins using bleach-chase.

Finally, the temporal changes in translation rates were determined in response to the drug. A gradual decrease in translation rates was observed, whose timing agrees with the decrease of ribosomal proteins. Taken together with the measurements of half-lives, it was found that the drug induces two global, opposing effects: a decrease in translation and an increase in half-lives. The balance of these effects varies widely across different proteins. This balance was found to be a dominant factor in explaining protein dynamics in response to the drug resolving the observed dis-correlation between the proteome and transcriptome. The present approach opens the way for a global study of the time-dependant interplay between the transcriptome, translation and protein degradation rates and how they shape the dynamic response of the proteome to different stimuli.

EXAMPLE 8

Further studies using Bleach chase

The present inventors compared the bleach-chase method for analyzing half-lives as described herein with radioactive pulse-chase experiments, the gold standard,

on additional proteins (above those analyzed in Example 3) and showed similar half-lives for tagged proteins (11% median difference, seven proteins) as illustrated in Table 7 and Figures 15A-B.

The difference between the two methods was assessed using the following formula. Average difference was 10 %.

$$\frac{\left| T_{\frac{1}{2}}^{\text{bleach-chase}} - T_{\frac{1}{2}}^{\text{pulse-chase}} \right|}{T_{\frac{1}{2}}^{\text{bleach-chase}} + T_{\frac{1}{2}}^{\text{pulse-chase}}} \times 2$$

Table 7

<i>Gene Symbol</i>	<i>Bleach-chase T_{1/2} (hours)</i>	<i>Pulse-chase T_{1/2} (hours)</i>	<i>Difference (%)</i>
STMN1	3.9 ± 0.6	3.7 ± 0.5	5%
MYH9	6.4 ± 0.6	5.7 ± 0.6	12%
RPS3A	6.7 ± 0.9	6.6 ± 0.3	1%
TXN1	8.6 ± 1.6	9.8 ± 0.5	13%
DDX5	8.7 ± 1.0	9.6 ± 0.4	10%
K-α-1	15.3 ± 2.4	18.9 ± 4.5	21%
DDX18	22.5 ± 1.4	>22.5	NA

The present inventors compared additional tagged proteins (above those analyzed in Example 3) to their untagged counterparts (16% median difference, six proteins), as illustrated in Table 8 and 015A-B.

In 5 out of 6 cases it was found that the YFP-tagged and its corresponding native protein showed similar half-lives (average difference of 14%).

Table 8

Gene Symbol	Untagged protein $T_{1/2}$ (hours)	YFP-tagged protein $T_{1/2}$ (hours)	Difference (%)
MYH9	5.2 ± 0.3	5.7 ± 0.6	9%
DDX5	8.2 ± 0.9	9.6 ± 0.4	16%
TXN1	13.0 ± 0.7	9.8 ± 0.51	28%
K- α -1	19.7 ± 3.8	18.9 ± 4.5	4%
DDX18	>22.5	>22.5	NA
STMN1*	7.8 ± 0.4	3.7 ± 0.5	71%

** immunoblots showed a missing 17KD in the YFP tagged protein possibly due to a truncation caused by the tagging. This may account for the observed discrepancy between the half-life of tagged and untagged STMN1.*

As a further test of the method, bleaching was applied at different intensities. The measured removal rates remained similar ($CV < 0.2$), regardless of bleaching intensity (Figures 16A-B). Thus, bleach-chase seems to accurately measure protein removal rates in living cells.

The present inventors next asked which process, degradation or dilution, dominates protein removal. Initial experiments were conducted using growth conditions in which the cells vigorously divided, and bleach-chase was used to assay 100 proteins spanning different cellular localizations and functions for 24 hours (every 20 minutes) with 3-4 day-to-day repeats. A broad protein half-life distribution was observed ranging between 45 minutes to 22.5 hours, with mean 9.0 ± 4.6 h (Figure 17B and Table 9, herein below).

Table 9

Gene symbol	Description	$T_{1/2}$ under normal growth (hours)		
ANXA2	annexin A2 isoform 1	6.1	$\pm$	0.5
BAG1	BCL2-associated athanogene.	5.3	$\pm$	1.6
C1QBP	complement component 1, q subcomponent binding	5.7	$\pm$	2.5

CALM2	calmodulin 2	6.7	±	1.1
CD44	CD44 antigen isoform 1 precursor	20.8	±	1.9
CIRBP	cold inducible RNA binding protein	6.9	±	2.1
CKS2	CDC28 protein kinase 2	2.6	±	1.6
COPS6	COP9 signalosome subunit 6	5.4	±	1.0
COTL1	coactesin-like 1	8.4	±	1.4
COX7C	cytochrome c oxidase subunit VIIc precursor	22.5	±	5.1
DDX18	DEAD (Asp-Glu-Ala-Asp) box polypeptide 18	20.3	±	1.4
DDX46	DEAD (Asp-Glu-Ala-Asp) box polypeptide 46	7.9	±	2.2
DDX5	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	8.7	±	1.0
DNMT1	DNA (cytosine-5-)-methyltransferase 1	6.9	±	2.0
DYNC1H1	dynein, cytoplasmic, heavy polypeptide 1	13.8	±	4.3
EEF1A1	eukaryotic translation elongation factor 1 alpha	9.9	±	0.6
EEF1E1	eukaryotic translation elongation factor 1	2.7	±	0.7
EEF2	eukaryotic translation elongation factor 2	8.8	±	0.7
EIF2S2	eukaryotic translation initiation factor 2 beta	8.3	±	1.5
EIF2S3	eukaryotic translation initiation factor 2,	10.5	±	2.5
EIF4A1	eukaryotic translation initiation factor 4A	7.3	±	1.2
EIF4E	eukaryotic translation initiation factor 4E	11.7	±	0.9
EIF4EBP1	eukaryotic translation initiation factor 4E	8.5	±	2.3
EIF4G3	eukaryotic translation initiation factor 4	8.8	±	1.1
EIF4H	eukaryotic translation initiation factor 4H	16.3	±	6.2
EIF5B	eukaryotic translation initiation factor 5B	8.9	±	2.8
ENO1	enolase 1	8.0	±	3.5
FAU	ubiquitin-like protein fubi and ribosomal	12.2	±	1.2
FSCN1	fascin 1	5.8	±	0.2
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	0.8	±	0.8
H2AFV	H2A histone family, member V isoform 2	13.9	±	1.8
HAT1	histone acetyltransferase 1 isoform a	8.5	±	1.2
HDAC2	histone deacetylase 2	4.5	±	2.4
HMGA1	high mobility group AT-hook 1 isoform a	11.1	±	1.5
HSP90AA1	heat shock protein 90kDa alpha (cytosolic),	4.2	±	1.8
HSP90AB1	heat shock 90kDa protein 1, beta	8.8	±	2.4
HSPA4L	heat shock 70kDa protein 4-like	10.6	±	2.7
HSPH1	heat shock 105kD	16.5	±	4.2
ILF2	interleukin enhancer binding factor 2	16.5	±	3.5
K- α -1	tubulin, alpha 1b	15.3	±	2.4
LMNA	lamin A/C isoform 2	12.8	±	2.2
MYH9	myosin, heavy polypeptide 9, non-muscle	6.4	±	0.6
NASP	nuclear autoantigenic sperm protein isoform 1	16.3	±	2.3
NCL	nucleolin	13.9	±	2.8
NDUFAF2	NADH dehydrogenase (ubiquinone) 1 alpha	6.6	±	1.4

NDUFB11	NADH dehydrogenase (ubiquinone) 1 beta	10.8	±	1.2
NPM1	nucleophosmin 1 isoform 1	5.2	±	0.1
PLEC1	plectin 1 isoform 6	11.7	±	4.6
POLR2F	DNA directed RNA polymerase II polypeptide F	5.4	±	1.8
POLR2L	DNA directed RNA polymerase II polypeptide L	10.5	±	2.6
POLR3GL	polymerase (RNA) III (DNA directed) polypeptide	3.5	±	0.0
POMP	proteasome maturation protein	7.0	±	2.9
PRDX5	peroxiredoxin 5 precursor, isoform a	9.4	±	2.6
PSMA1	proteasome alpha 1 subunit isoform 2	8.8	±	3.1
PSMA3	proteasome alpha 3 subunit isoform 1	4.0	±	4.4
PSMA7	proteasome alpha 7 subunit	9.0	±	2.6
PSMB1	proteasome beta 1 subunit	6.7	±	0.1
PSMB4	proteasome beta 4 subunit	7.3	±	0.8
PSMB6	proteasome beta 6 subunit	8.5	±	4.7
PSMB7	proteasome beta 7 subunit proprotein	5.1	±	0.7
PSMC1	proteasome 26S ATPase subunit 1	5.2	±	1.5
PSMC4	proteasome 26S ATPase subunit 4 isoform 1	4.4	±	1.2
PSMD12	proteasome 26S non-ATPase subunit 12 isoform 1	4.0	±	2.0
RFC1	replication factor C large subunit	9.3	±	1.5
RPA2	replication protein A2, 32kDa	11.2	±	2.0
RPL11	ribosomal protein L11	14.9	±	2.3
RPL18	ribosomal protein L18	9.2	±	2.0
RPL22	ribosomal protein L22 proprotein	11.1	±	2.8
RPL27	ribosomal protein L27	15.4	±	3.1
RPL27A	ribosomal protein L27a	4.2	±	1.9
RPL29	ribosomal protein L29	4.8	±	1.8
RPL30	ribosomal protein L30	6.1	±	0.6
RPL39	ribosomal protein L39	3.4	±	0.5
RPL6	ribosomal protein L6	9.3	±	1.6
RPS19BP1	S19 binding protein	21.8	±	0.9
RPS2	ribosomal protein S2	19.7	±	3.5
RPS3	ribosomal protein S3	5.5	±	2.4
RPS3A	ribosomal protein S3a	6.7	±	1.0
RPS6	ribosomal protein S6	14.4	±	3.5
RPS7	ribosomal protein S7	4.3	±	1.3
RPS8	ribosomal protein S8	3.0	±	0.2
SLBP	histone stem-loop binding protein	8.2	±	1.1
SSBP1	single-stranded DNA binding protein 1	6.9	±	0.3
STMN1	stathmin 1	3.9	±	0.6
TARS	threonyl-tRNA synthetase	6.6	±	1.1
TBCA	tubulin-specific chaperone a	2.4	±	0.0

TFAM	transcription factor A, mitochondrial	16.3	±	3.7
TIMM23	translocase of inner mitochondrial membrane 23	7.8	±	1.5
TOMM70A	translocase of outer mitochondrial membrane 70	6.5	±	1.3
TUBA1C	tubulin alpha 6	8.2	±	8.6
TUBB2C	tubulin, beta, 2	5.1	±	0.5
TXN	thioredoxin	6.9	±	1.6
TXNRD1	thioredoxin reductase 1	6.6	±	0.2
UBA52	ubiquitin and ribosomal protein L40 precursor	10.8	±	2.4
UBE2K	ubiquitin-conjugating enzyme E2-25K isoform 1	6.3	±	0.8
UBE2N	ubiquitin-conjugating enzyme E2N	14.9	±	2.3
UBE2V2	ubiquitin-conjugating enzyme E2 variant 2	5.6	±	1.4
VCL	vinculin isoform meta-VCL	11.7	±	2.1
VIL2	villin 2	11.1	±	3.1
VIM	vimentin	4.9	±	0.1

Degradation rates were obtained by subtracting the dilution rate ($\alpha_{dil}=0.03\pm0.004$ 1/h, from the removal rates. Degradation was dominant for 45 % of the proteins, dilution was dominant for 12 %, and the two were comparable for 43 % (017C). Thus, the dependence of protein removal on degradation or dilution varies widely between proteins. We tested whether this correlates with function and localization (0). Proteins localized to the cytoplasm had higher degradation rates than expected by chance (mHG $P<10^{-4}$), as did members of the anaphase promoting complex (mHG $P<10^{-2}$) and cell-cycle regulating proteins (mHG $P<10^{-3}$). In contrast, proteins of the translation-initiation complex tended to be degraded more slowly (mHG $P<10^{-2}$). Thus, proteins with similar function or localization seemed to rely similarly on either dilution or degradation for their removal.

The present inventors used the anti-cancer drug camptothecin (CPT), a topoisomerase-1 poison, and measured the half-life of additional proteins (above those tested in Example 4) for 24 hours after drug addition.

The results are illustrated in Table 10 herein below.

Table 10

<i>Gene symbol</i>	<i>T1/2 under normal growth (hours)</i>			<i>T1/2 after CPT addition (hours)</i>		
BAG1	5.3	±	1.6	9.5	±	1.5
CKS2	2.6	±	1.6	1.3	±	0.8

DDX5	8.7	±	1.0		12.9	±	3.5
DYNC1H1	13.8	±	4.3		25.9	±	3.5
EEF2	8.8	±	0.7		7.9	±	1.8
ENO1	8.0	±	3.5		26.3	±	7.3
GAPDH	0.8	±	0.2		1.7	±	0.4
LMNA	12.8	±	2.2		31.0	±	7.0
MYH9	6.4	±	0.6		13.2	±	3.6
PSMB4	7.3	±	0.8		10.9	±	3.6
RPS3A	6.7	±	1.0		3.2	±	0.6
STMN1	3.9	±	0.6		11.3	±	2.0
FSCN1	5.8	±	0.2		13.1	±	4.0
H2AFV	13.9	±	1.8		>48	±	NA
K- -1	15.3	±	2.4		30.9	±	8.1
NASP	16.3	±	2.3		>48	±	NA
RPS3	5.5	±	2.4		4.2	±	1.6
RPS7	4.3	±	1.3		4.4	±	2.1
VCL	11.7	±	2.1		38.5	±	8.0
VIL2	11.1	±	3.1		23.0	±	4.0
VIM	4.9	±	0.1		16.1	±	3.9
CALM2	6.7	±	1.1		11.5	±	2.9
CD44	20.8	±	1.9		16.0	±	5.0
COTL1	8.4	±	1.4		9.5	±	1.0
DDX18	20.3	±	1.4		22.5	±	4.2
ILF2	16.5	±	3.5		34.1	±	8.1
RFC1	9.3	±	1.5		18.4	±	4.6
RPA2	11.2	±	2.0		21.9	±	4.1
RPL22	11.1	±	2.8		14.9	±	2.3
TARS	6.6	±	1.1		11.6	±	1.4
VPS26A	10.6	±	2.3		15.3	±	6.9
RPS6	14.4	±	3.5		21.3	±	7.8

Comparing protein removal with and without the drug revealed a global increase in half-lives: most proteins (22/32) increased their half-life or retained the same half-life (9/32), and only one showed a decrease. Mean half-life doubled from 9.0 ± 4.6 h to 18.8 ± 14.8 h (paired t-test $P < 10^{-5}$). This effect also persisted 24-48 hours after drug addition (Figures 18A-B).

Notably, the increase in half-lives showed the following pattern: Long-lived proteins became longer-lived in response to the drug, whereas short-lived proteins remained largely unaffected.

The systemic increase in half-lives means that protein removal rates were globally reduced by the drug. One mechanism that could cause this decrease is down-regulation of the degradation machinery (e.g. inhibition of ubiquitin-proteasome mediated proteolysis). However, this would not account for the differential half-life increase of long-lived proteins. On the contrary, long-lived proteins would be expected to be the least affected, because their half-lives are largely determined by dilution due to cell-growth rather than degradation (Figure 19B, Figure 20).

An alternative explanation is that the drug stopped cell growth, thus reducing the dilution rate. Indeed mitosis rate dropped immediately upon drug addition and halted after 5-7h. To see why growth arrest is sufficient to produce the observed half-life effect note that growth arrest eliminates dilution, which differentially affects the protein removal rate α . Since $\alpha = \alpha_{deg} + \alpha_{dil}$, proteins with slow degradation have a larger relative reduction in α than proteins with fast degradation (described by Eq. 4-5 in Figure 19C).

This reasoning can quantitatively predict a protein's half-life after growth arrest, $T_{1/2}^*$, based on its half-life prior to the arrest, $T_{1/2}$, and the average cell-cycle duration, T_{cc} (Eq. 5 in Figure 19C). Using the measured cell-cycle duration, $T_{cc} = 22.5 \pm 2.6$ hours and $k_2 = 0$, because cell-cycle stops after drug addition, generates predictions for the half-lives after drug addition. Note, that since the model parameters are all measured, no parameter fitting is required. The predictions (blue line in Figure 9B), capture the measured behavior reasonably well ($P < 10^{-4}$, Figure 21). Three proteins (CD44, DDX18 and RPS3A) deviated from the general trend with degradation rates increasing in response to the drug, indicating specific degradation regulation (Figure 22).

To further test the generality of this effect, half-life changes were measured in response to four additional stresses: serum-starvation, the transcription inhibitor actinomycin-D, and the anti-cancer drugs paclitaxel and cisplatin, which reduced cell-division rates to 15%, 10%, 0% and 85% of pre-drug rates, respectively. It was found

the same effect as in CPT: Long-lived proteins became longer-lived whereas short-lived proteins remained less affected (0 19D-G, Tables 11-14), in a way that was quantitatively predictable using the appropriately reduced dilution rates.

Table 11 illustrates protein half-lives following addition of the drug paclitaxel.

5

Table 11

<i>Gene symbol</i>	<i>T1/2 after paclitaxel addition (hours)</i>		
BAG1	11.5	±	2.6
CKS2	3.5	±	1.4
DDX5	16.4	±	1.6
H2AFV	39.2	±	5.7
LMNA	>48	±	NA
NASP	>48	±	NA
RPS7	2.5	±	0.9
VIL2	>48	±	NA

Table 12 illustrates protein half-lives during serum starvation.

Table 12

<i>Gene symbol</i>	<i>T1/2 after starvation (hours)</i>		
BAG1	12.7	±	0.3
CKS2	5.0	±	1.2
DDX5	14.9	±	5.0
H2AFV	>48	±	NA
LMNA	25.5	±	3.0
NASP	33.0	±	3.5
RPS7	4.6	±	0.3
VIL2	31.3	±	5.0

10

Table 13 illustrates protein half-lives following addition of the drug cisplatin.

Table 13

<i>Gene symbol</i>	<i>T1/2 after cisplatin addition (hours)</i>		
BAG1	5.6	±	0.9
CKS2	0.9	±	1.3
DDX5	6.1	±	1.4

H2AFV	20.5	±	1.6
LMNA	8.0	±	2.1
NASP	15.8	±	2.3
RPS7	2.7	±	0.2
VIL2	14.5	±	6.1

Table 14 illustrated protein half-lives after addition transcription inhibitor actinomycin-D

Table 14

<i>Gene symbol</i>	<i>T_{1/2} after actinomycin-D addition</i>		
BAG1	14.9	±	12.0
CKS2	3.2	±	1.3
DDX5	9.9	±	1.9
H2AFV	34.6	±	15.1
NASP	36.2	±	11.8

5

One unexpected finding is that the present cells do not appear to compensate for changes in growth-rate by correspondingly altering protein degradation rates. Consequentially, changes in growth-rate directly affect protein half-lives, resulting in corresponding changes in protein levels (023A). Thus, drugs that change growth-rate can cause a global imbalance in proteome levels. This imbalance is expected to be larger the faster the cells grow prior to the drug (0B). Proteome unbalancing may cause lethality, and therefore might be a mechanism for differential killing of rapidly growing cell types, such as tumors, by means of growth-arresting drugs.

10

The present analysis also highlights the inherent sensitivity of long-lived proteins to fluctuations in cellular growth (023C), suggesting that one way to preserve robust levels is by maintaining proteins short-lived. Furthermore, to preserve stoichiometry, it helps to provide proteins in the same complex or system with similar degradation rates, so that fluctuations in dilution would not affect the ratio of their levels.

15

20

Elucidating the principles that govern protein removal is important for understanding how cells dynamically control their proteome. This study presents an accurate assay of protein removal, and a principle by which one can understand and

predict how different stresses, including chemotherapeutic drugs, affect protein removal in living human cancer cells.

5 Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

10 All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission
15 that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

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WHAT IS CLAIMED IS:

1. A method of determining a degradation rate of a fluorescently labeled polypeptide of interest in a population of cells, the method comprising:

- (a) obtaining two identical populations of cells;
- (b) determining fluorescence levels of the fluorescently labeled polypeptide of interest in a first of said at least two populations of cells at at least two different points in time;
- (c) at least partially reducing a fluorescence of the fluorescently labeled polypeptide of interest in a second of said at least two populations of cells;
- (d) determining fluorescence levels of said reduced fluorescently labeled polypeptide of interest in said second of said at least two populations of cells at said at least two different points in time;
- (e) comparing fluorescence levels in said first and said second populations; and
- (f) based on said comparing, determining a degradation rate of the fluorescently labeled polypeptide of interest.

2. The method of claim 1, wherein step (f) is effected by calculating the ratio of the (natural log (ln) of the difference in fluorescence of said polypeptide of interest in said first population of cells and fluorescence of said polypeptide of interest in said second population of cells at a first point in time) : (ln of the difference in fluorescence of said polypeptide of interest in said first population of cells and fluorescence of said polypeptide of interest in said second population of cells at a second point in time) divided by the difference in time between said two points in time.

3. The method of claim 1, wherein step (f) is effected by determining a slope of a graph, wherein a y axis of said graph indicates the $\ln(P(t) - P_v(t))$ and an x axis of said graph indicates time, wherein $P(t)$ is the amount of fluorescence of said polypeptide of interest at point t in time in said first population of cells and $P_v(t)$ is the amount of fluorescence of said polypeptide of interest at point t in time in said second population of cells.

4. The method of claim 1, wherein step (c) is effected with a mercury fluorescent lamp.

5. The method of claim 1, wherein step (c) is effected for 1-8 minutes.

6. The method of claim 1, wherein said population of cells comprises an additional fluorescently labeled polypeptide, said additional fluorescently labeled polypeptide having a higher nuclear: cytoplasm expression ratio and said additional fluorescently labeled polypeptide being distinguishable from said fluorescently labeled polypeptide of interest.

7. The method of claim 1, wherein the polypeptide of interest is transcribed from its native location in said population of cells.

8. A method of analyzing the effect of an agent on a degradation rate of a polypeptide of interest in a population of cells, the method comprising:

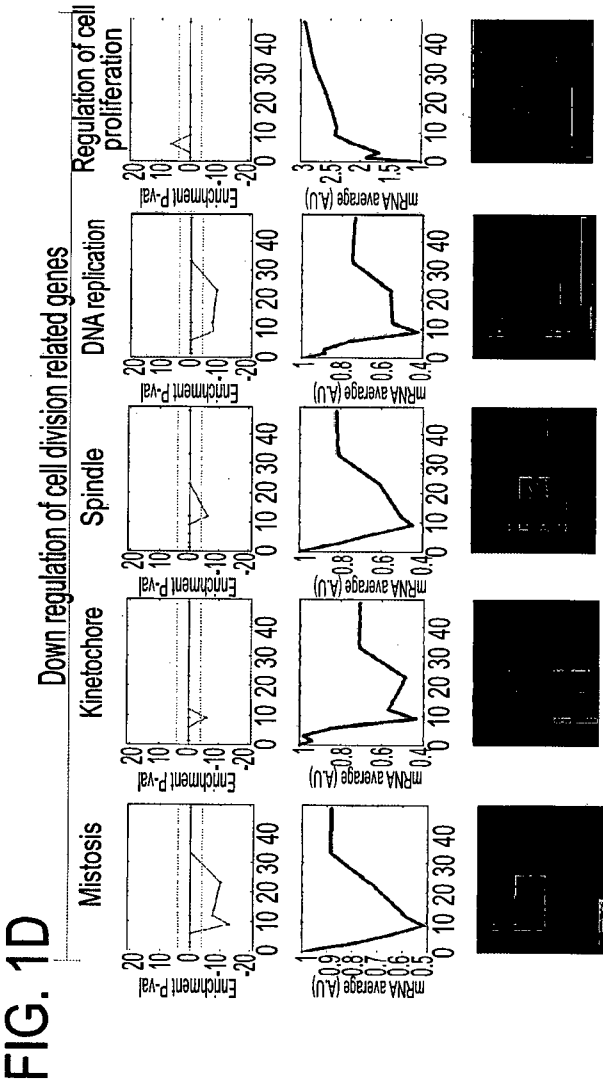
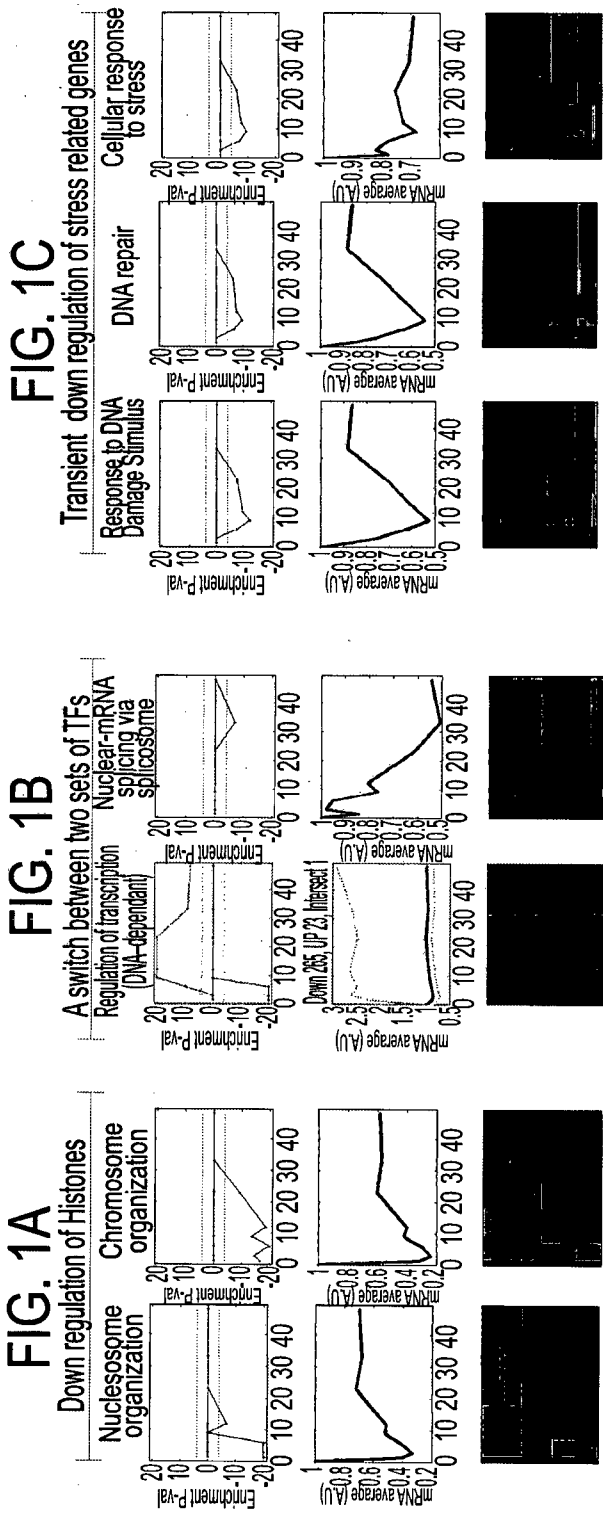
(a) contacting a population of cells with the agent;
(b) determining the degradation rate of said fluorescently labeled polypeptide of interest in said population of cells according to the method of claim 1;
and

(c) comparing the degradation rate obtained in step (b) with a degradation rate of the polypeptide in an absence of the agent, thereby analyzing the effect of the agent on the degradation rate of the polypeptide of interest.

9. A system for determining a degradation rate of a fluorescently labeled polypeptide of interest in a cell comprising a processing unit, said processing unit executing a software application configured for converting change in fluorescence levels over a period of time to a degradation rate.

10. The system of claim 9, said degradation rate is determined according to the method of any of the claims 1-7.

11. The system of claim 9, further comprising an imaging system.
12. The system of claim 11, wherein said imaging system comprises an image capture apparatus.
13. The system of claim 12, wherein said image capture apparatus is capable of capturing an image of said fluorescently labeled polypeptide over said period of time.
14. The system of claim 11, wherein said imaging system further comprises a data processor for calculating a fluorescence level for each picture element of said image.
15. The system of claim 12, wherein said image capture apparatus is a fluorescent image capture apparatus.



Log fold change
(compared to levels
before drug addition)

-3 0 3

FIG. 1E

Down regulation of RNA processing

FIG. 1F

Down regulation of kinase activity

FIG. 1G

FIG. 1H

FIG. 1I

FIG. 1J

FIG. 1K

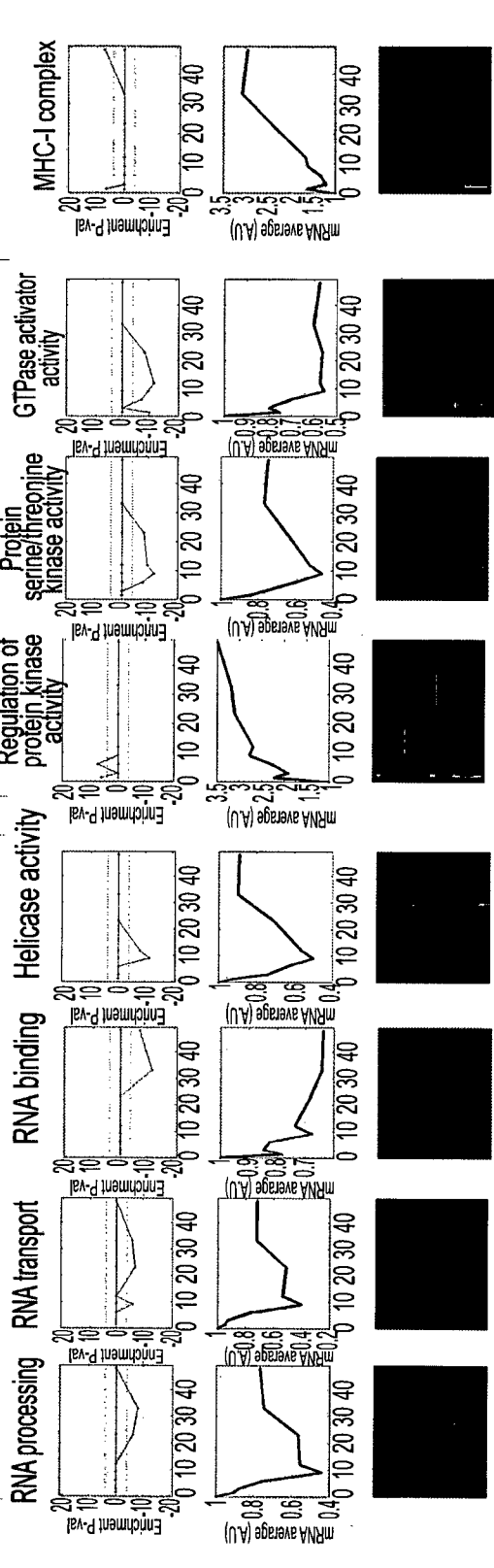


FIG. 1H Increased expression of energy and metabolite generation related genes

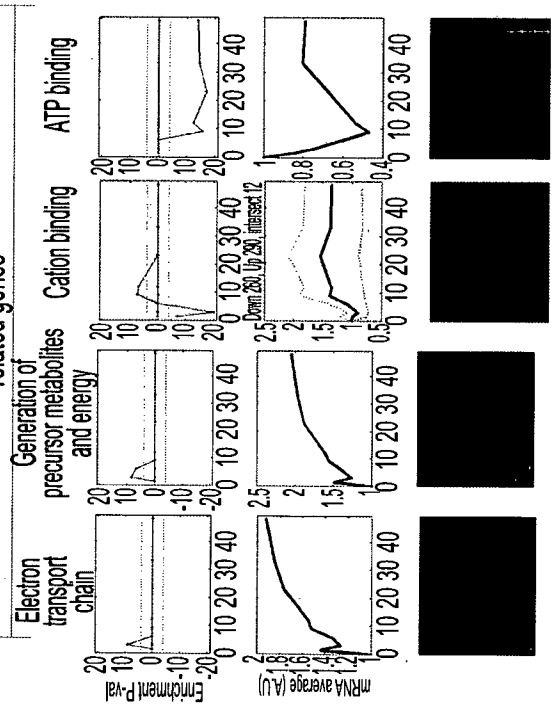


FIG. 1J

FIG. 1K

FIG. 1L

FIG. 1M

FIG. 1N

FIG. 1O

FIG. 1P

FIG. 1Q

FIG. 1R

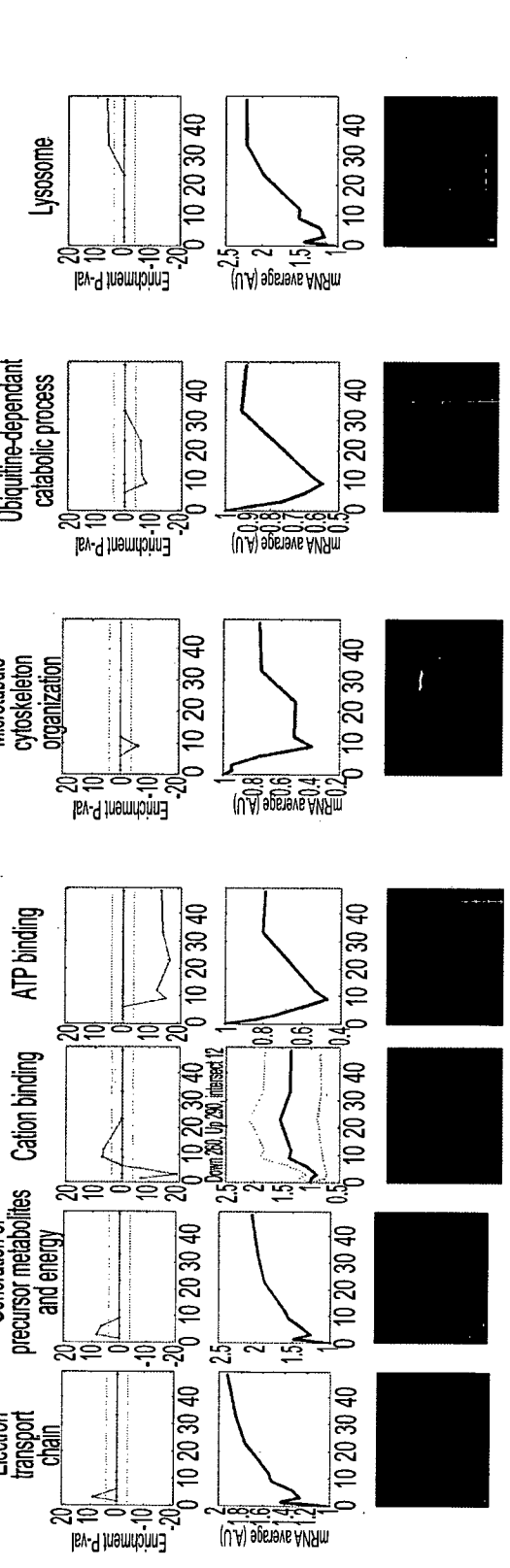
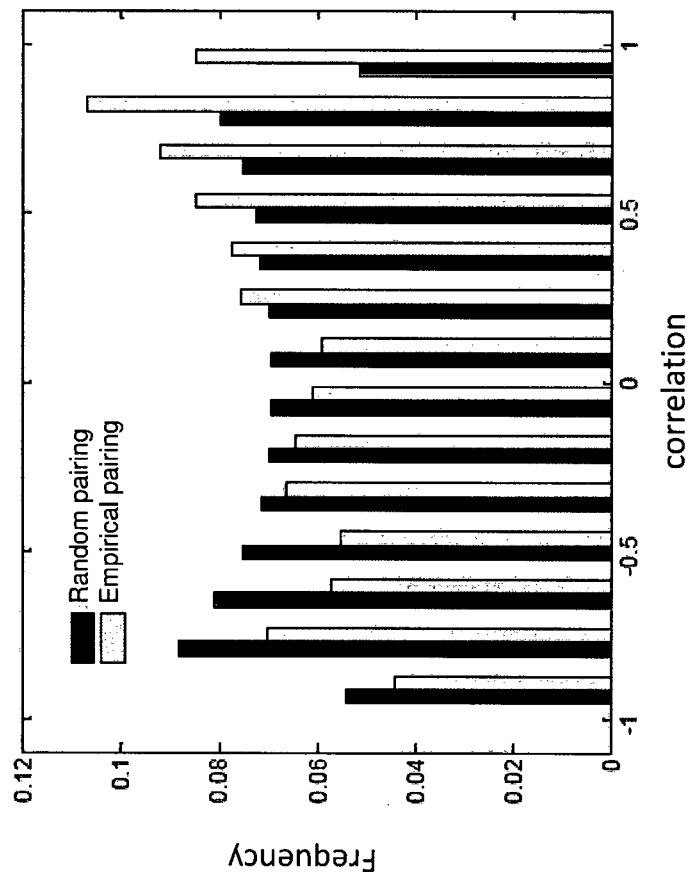


FIG. 2

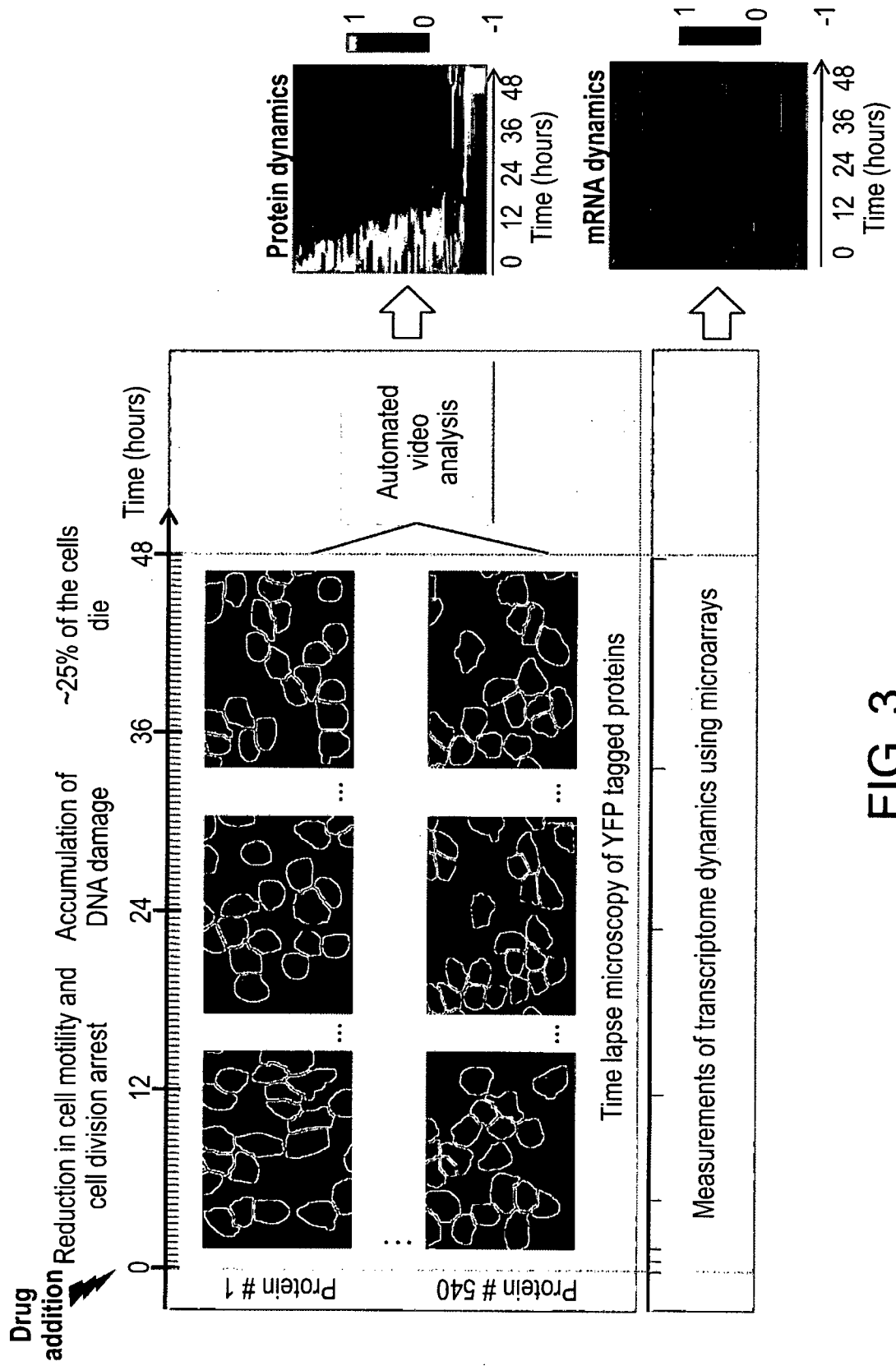


FIG. 3

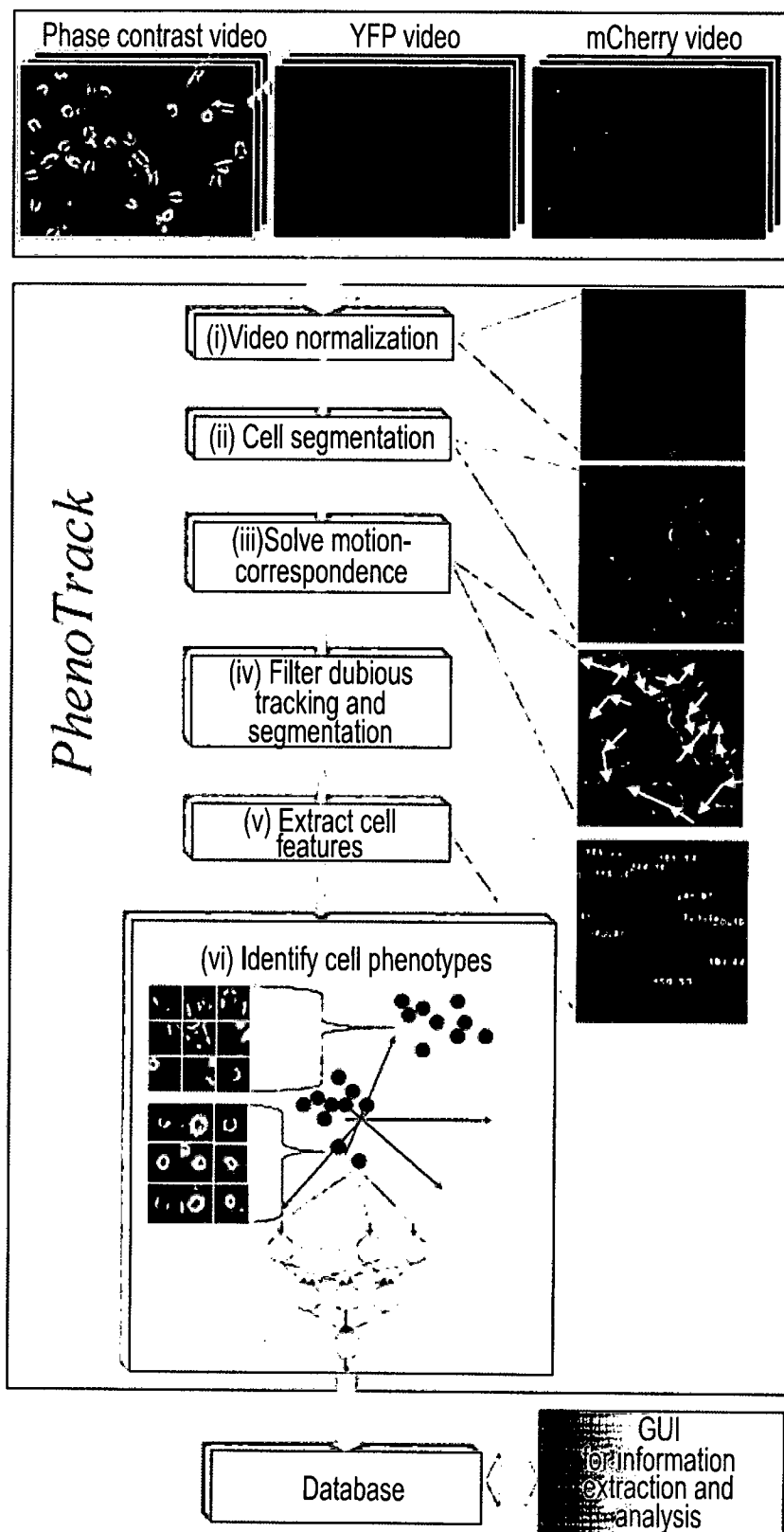


FIG. 4

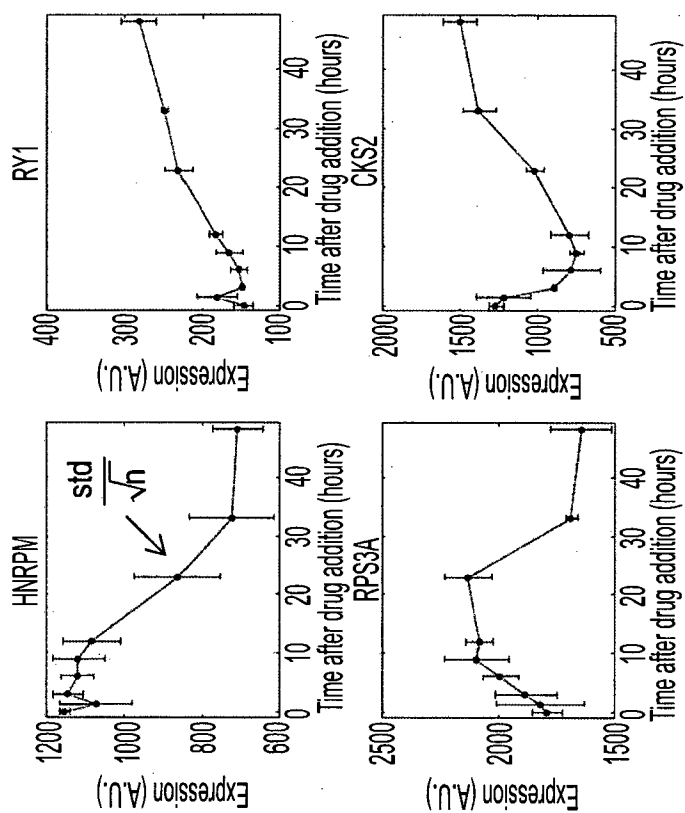


FIG. 5B

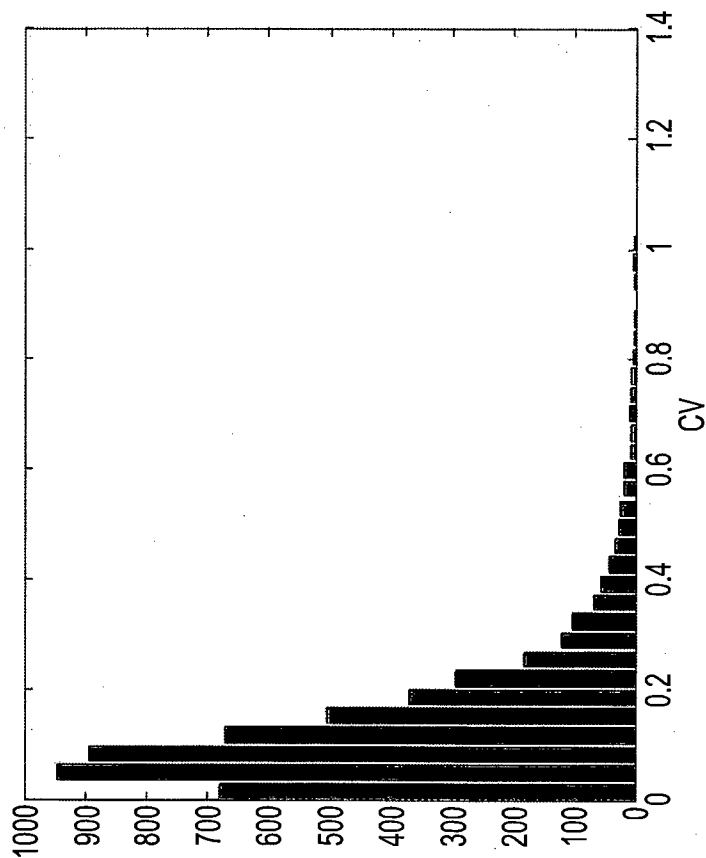


FIG. 5A

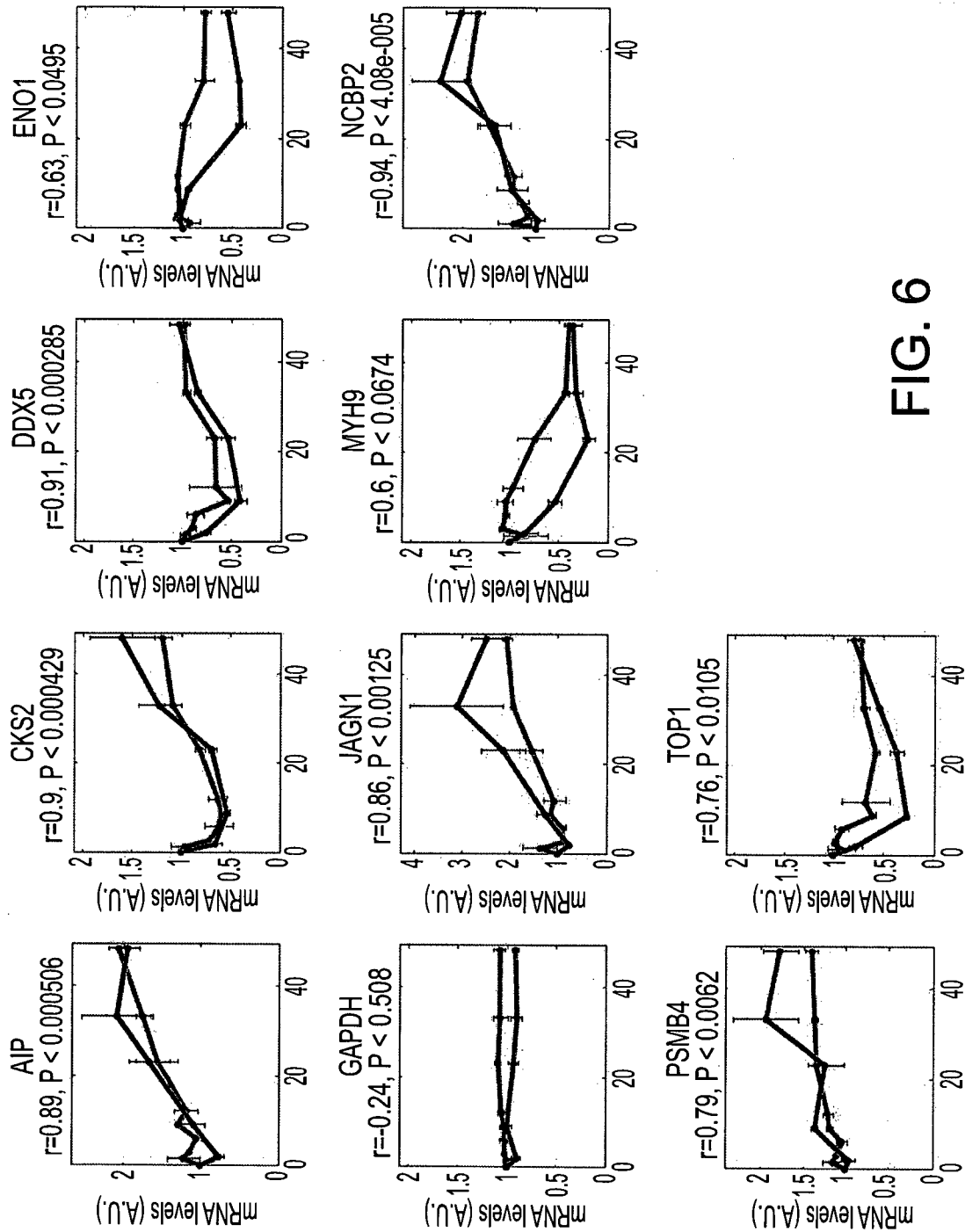
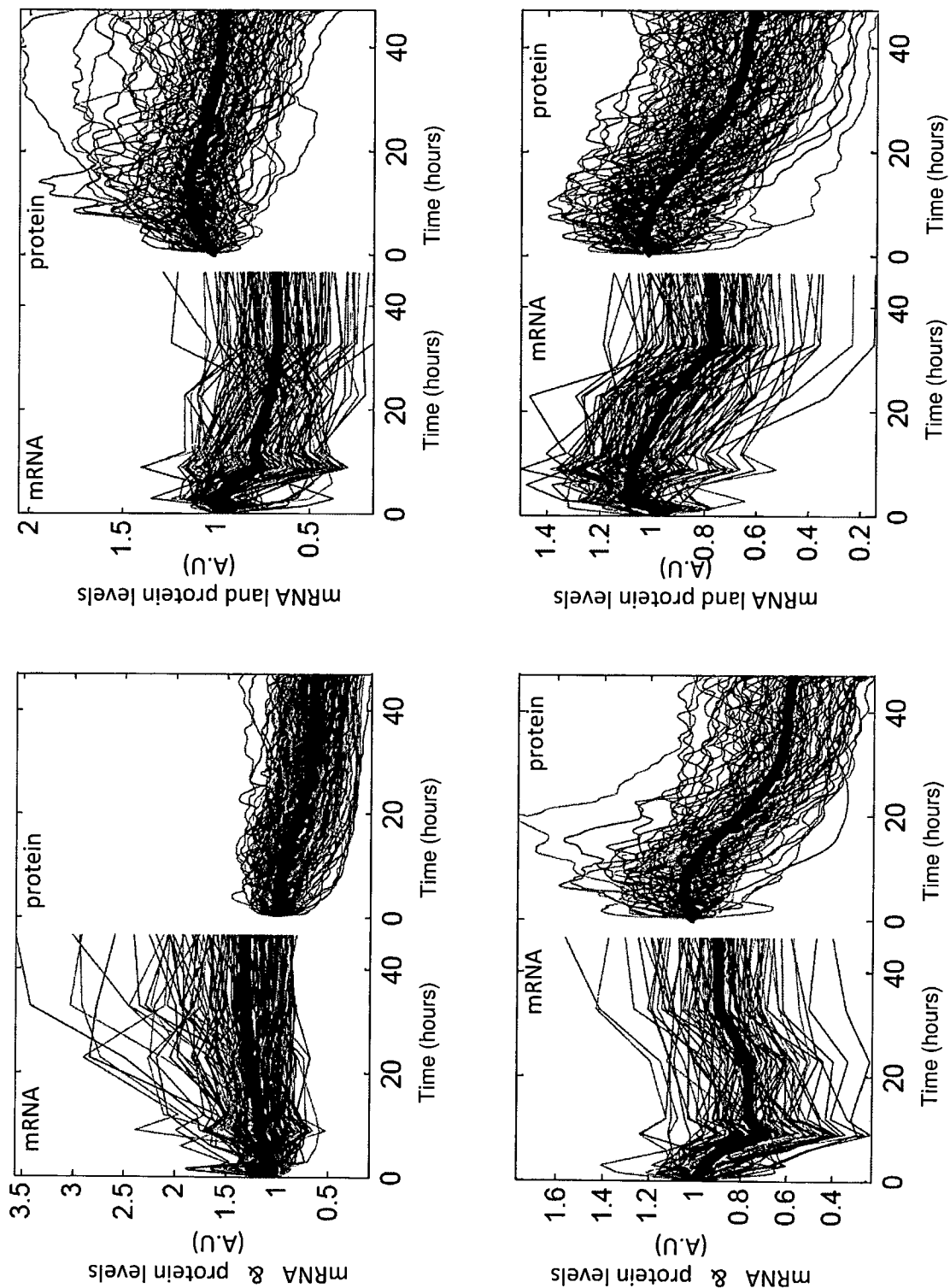


FIG. 6

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FIG. 7



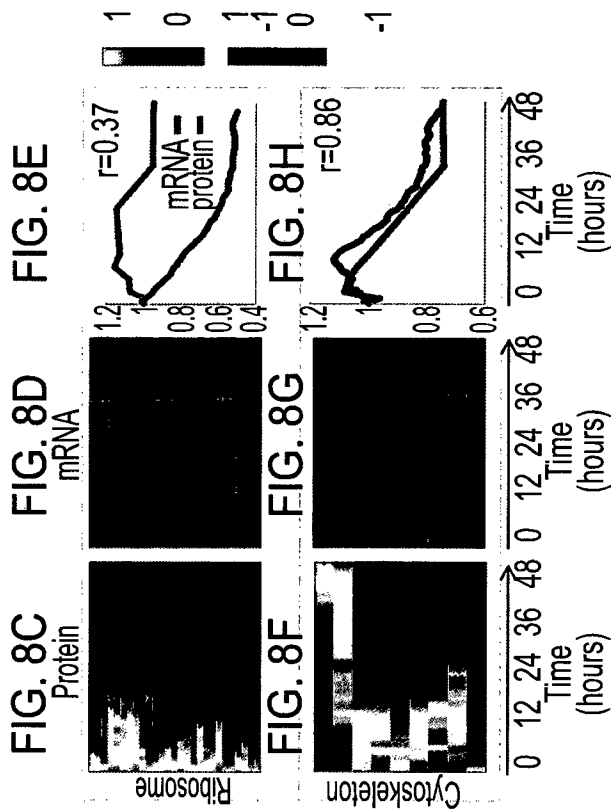
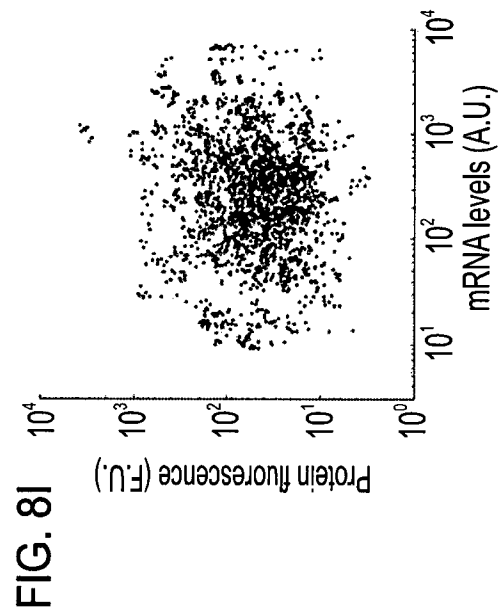
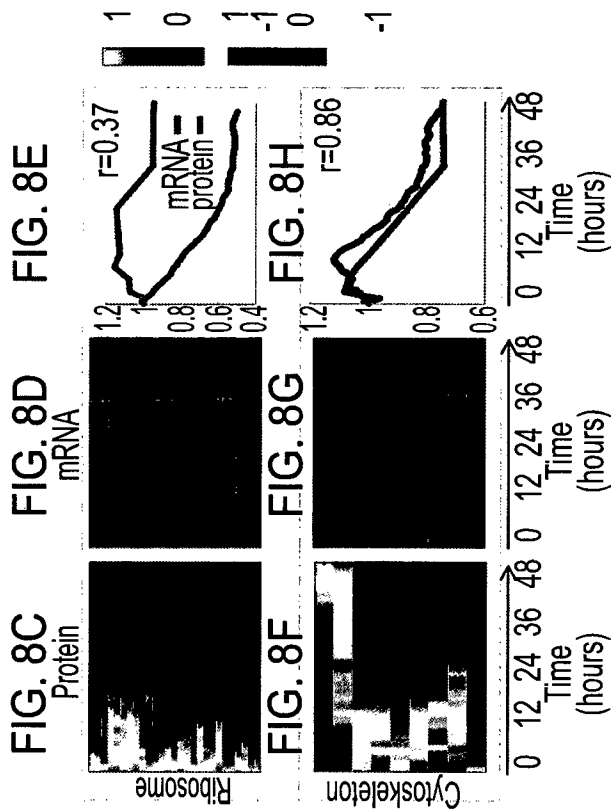
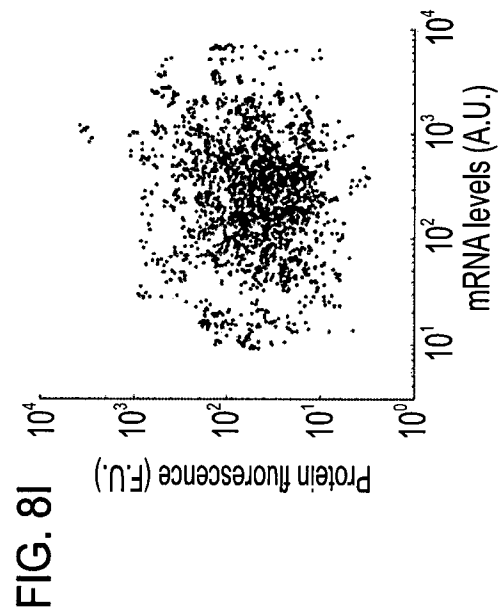
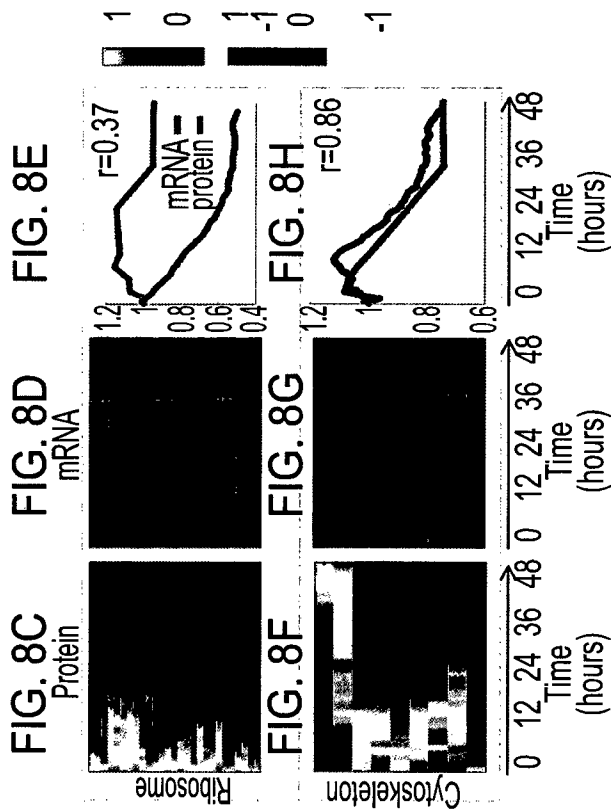
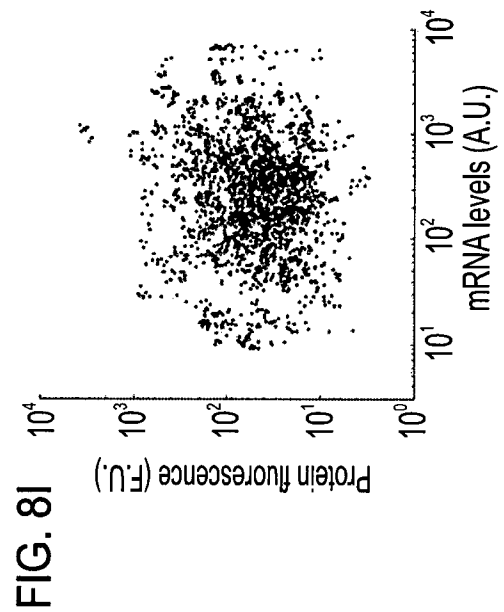
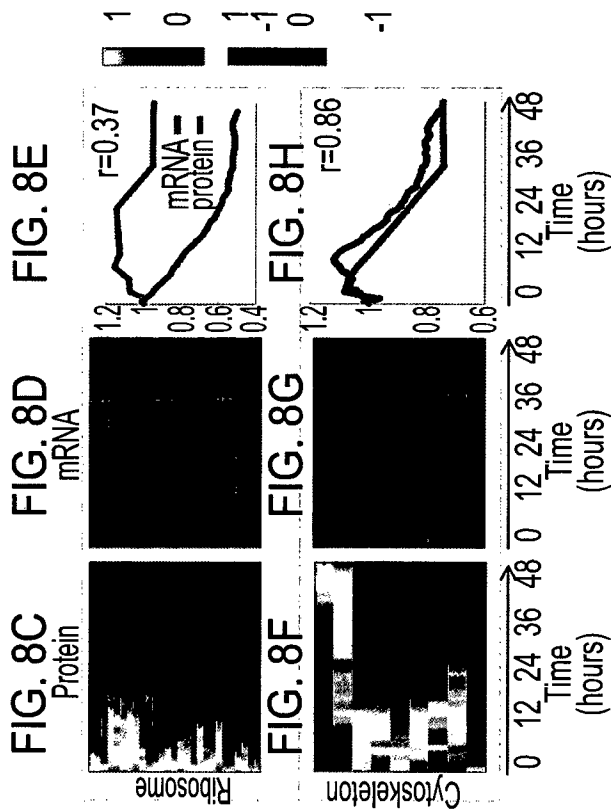
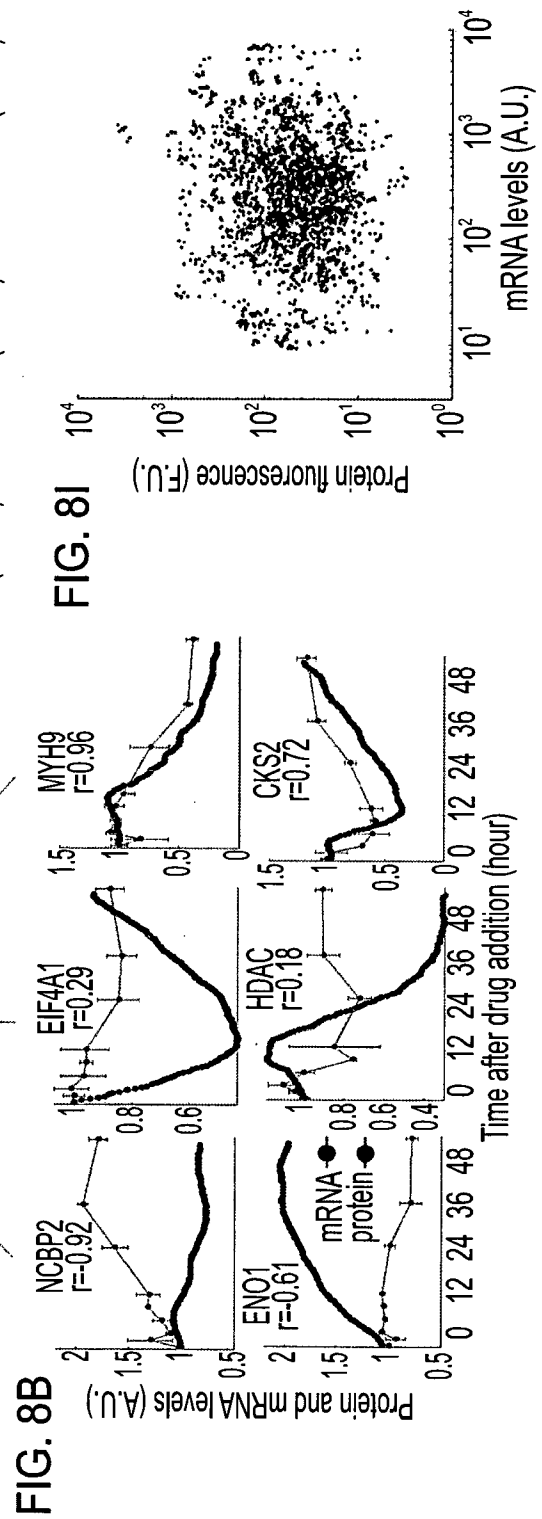
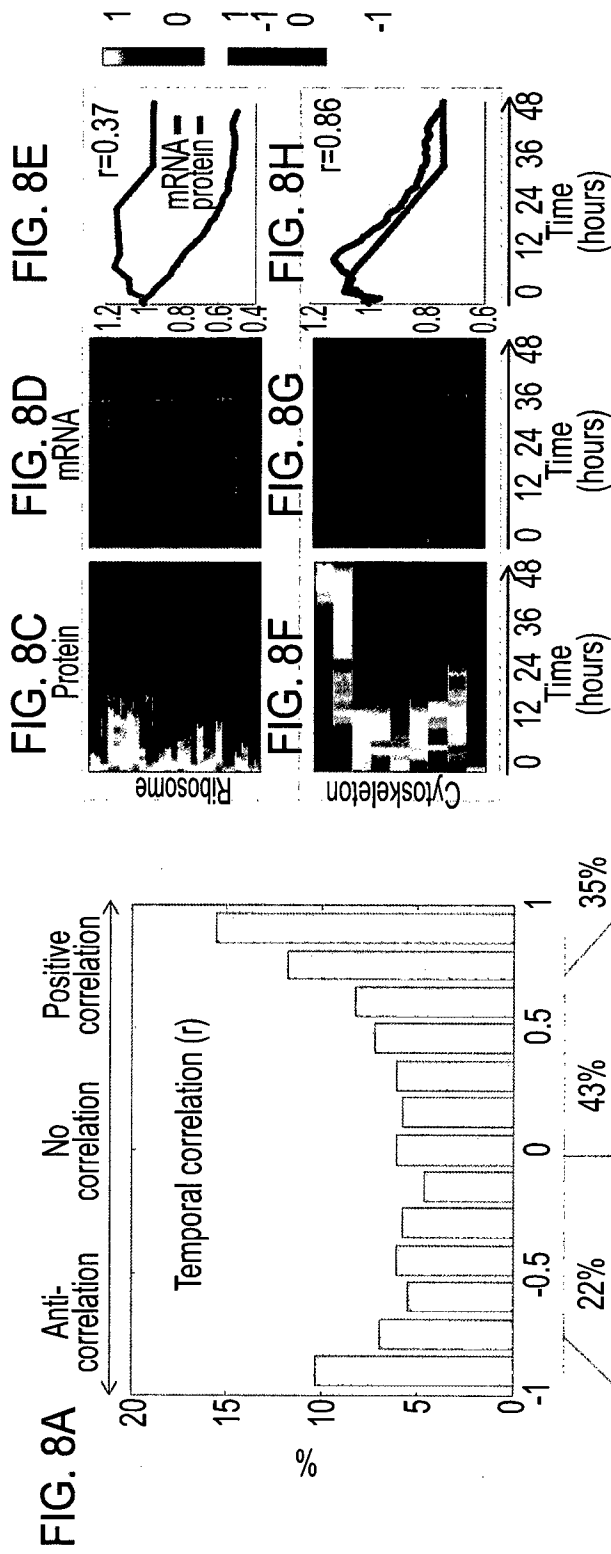


FIG. 9A

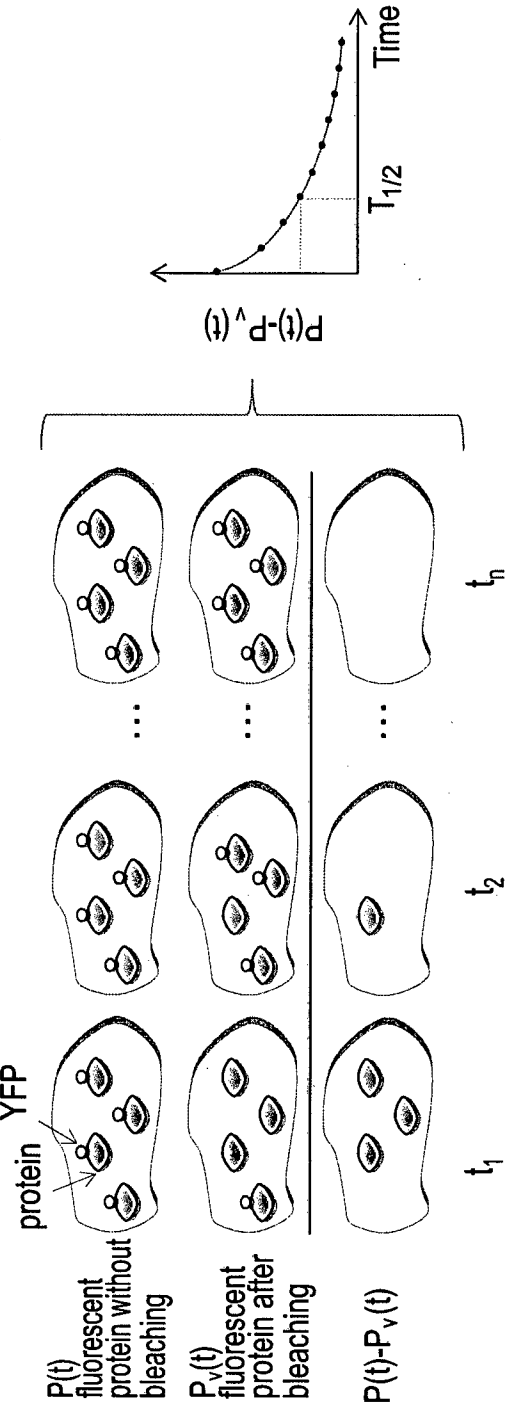


FIG. 9B

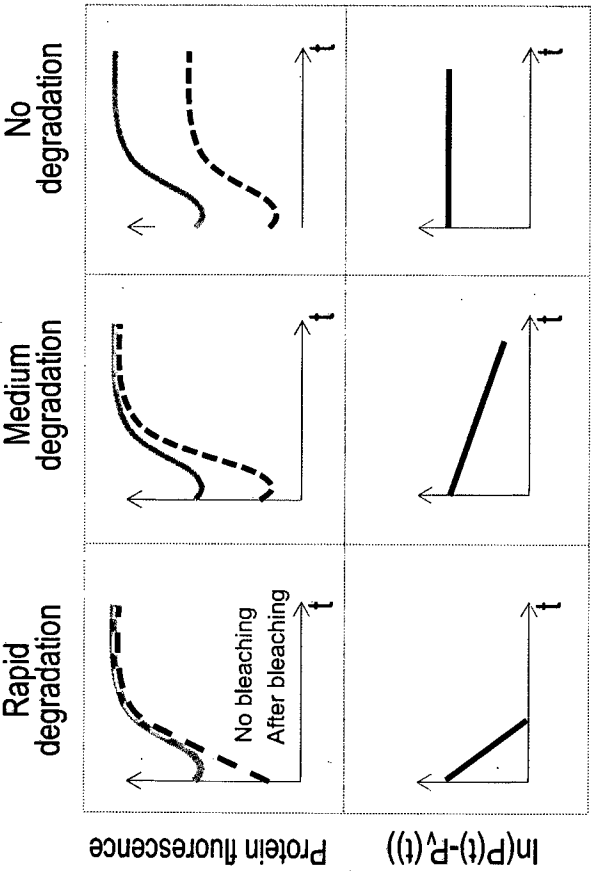


FIG. 9C

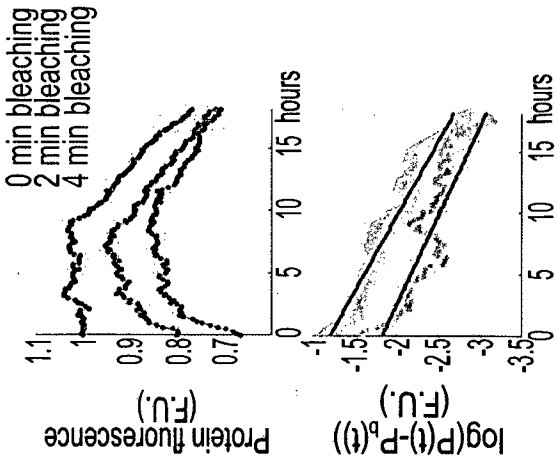


FIG. 10A

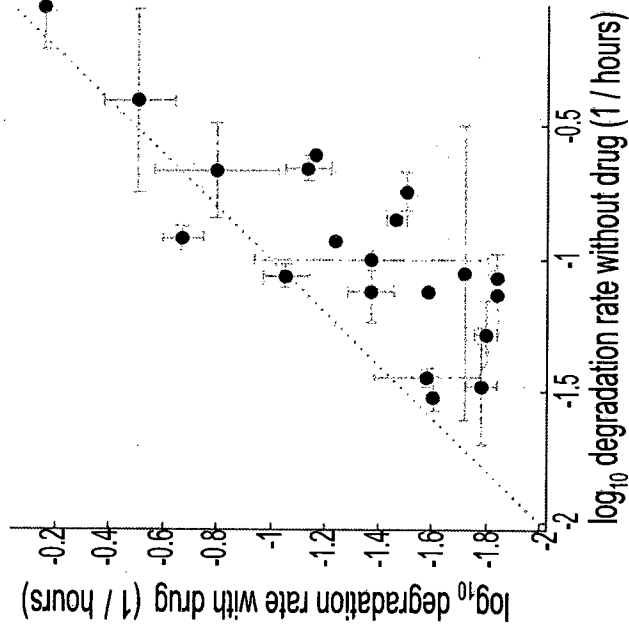


FIG. 10C

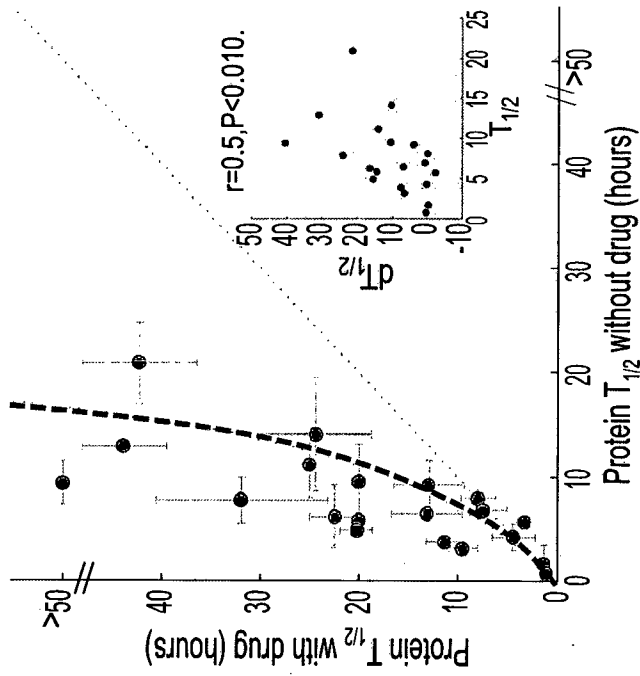


FIG. 10B

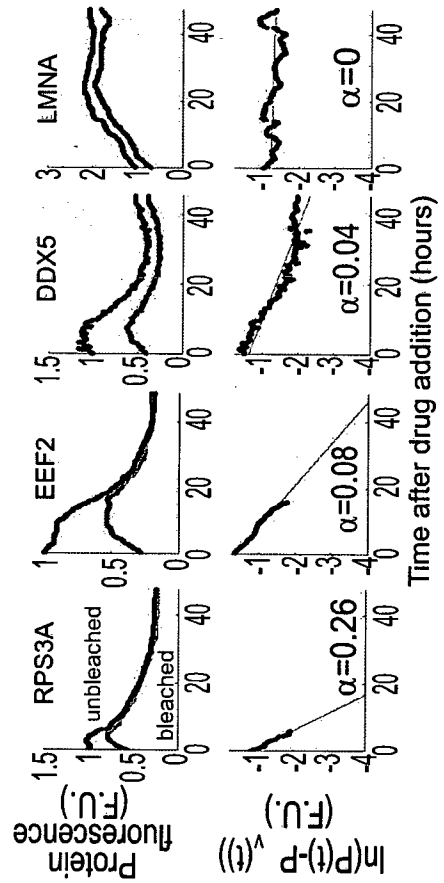
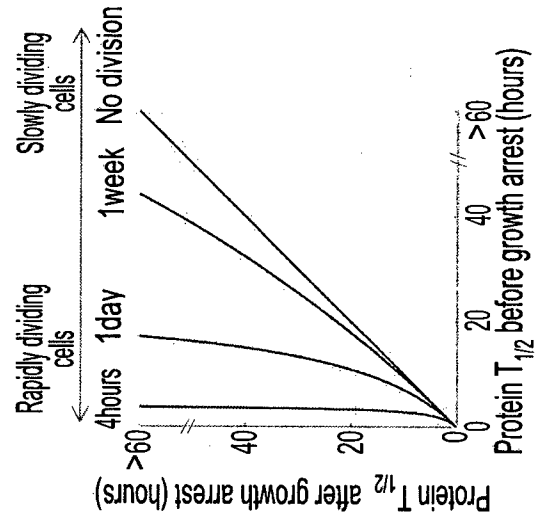


FIG. 10D



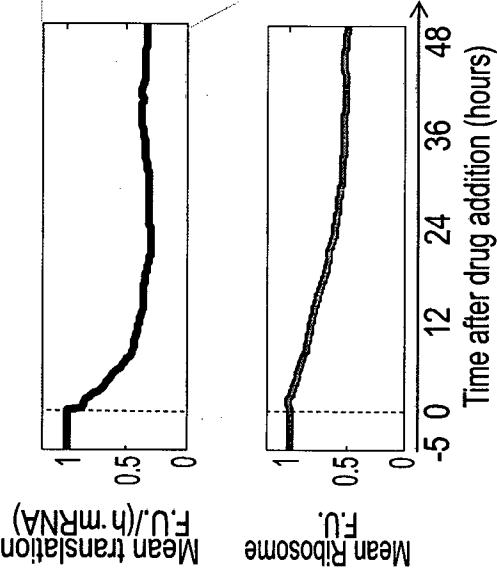
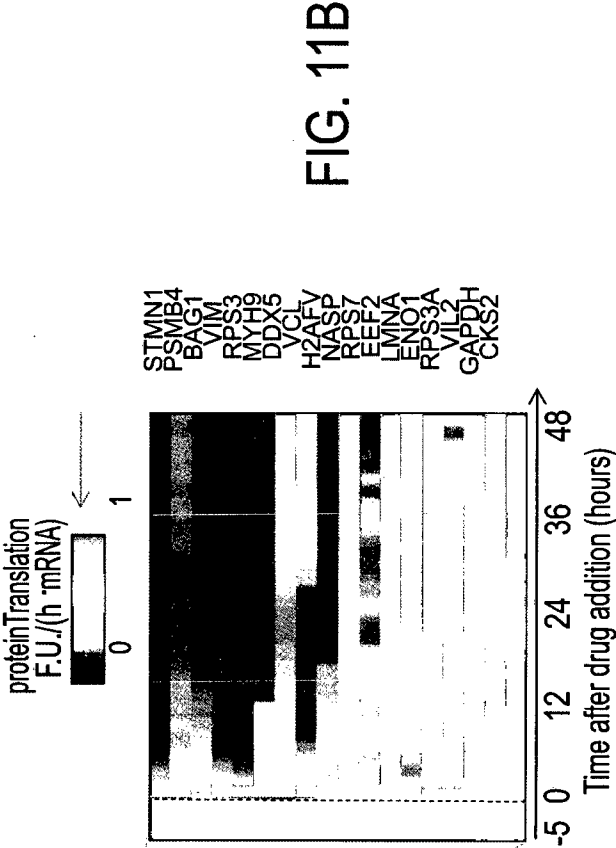


FIG. 11C

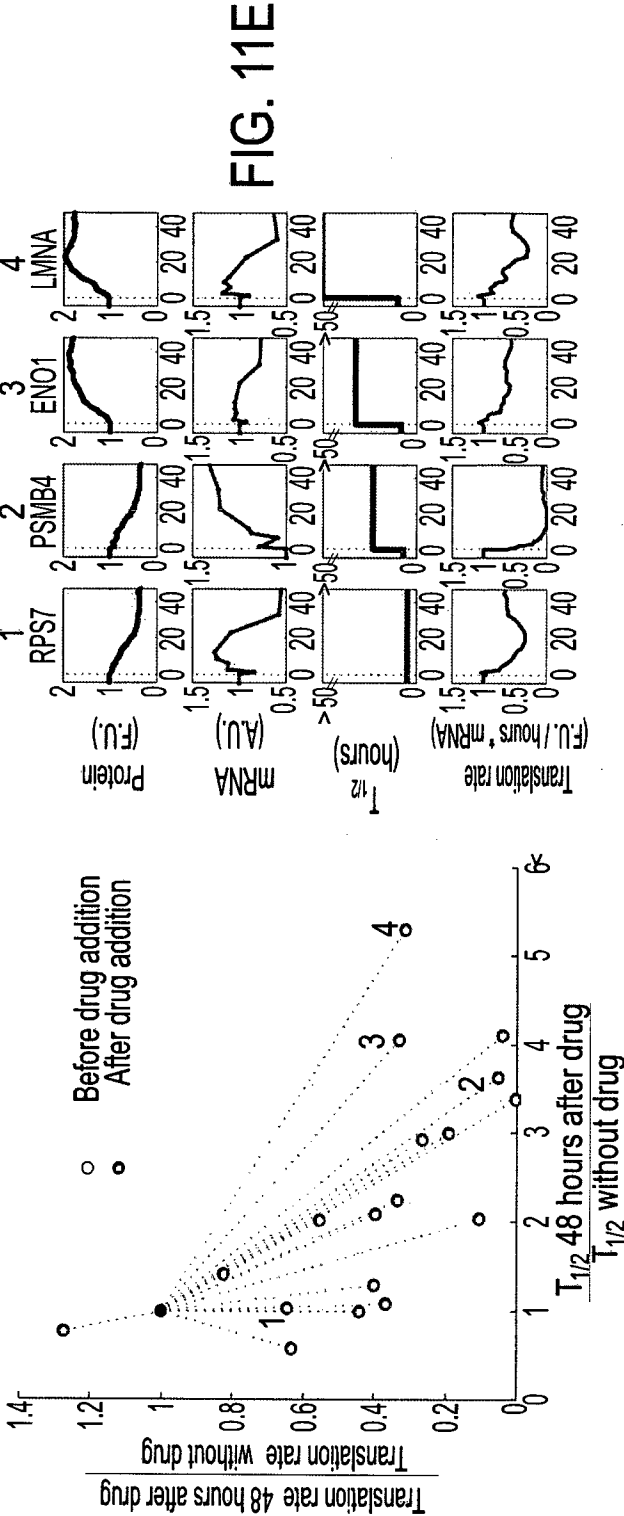


FIG. 11E

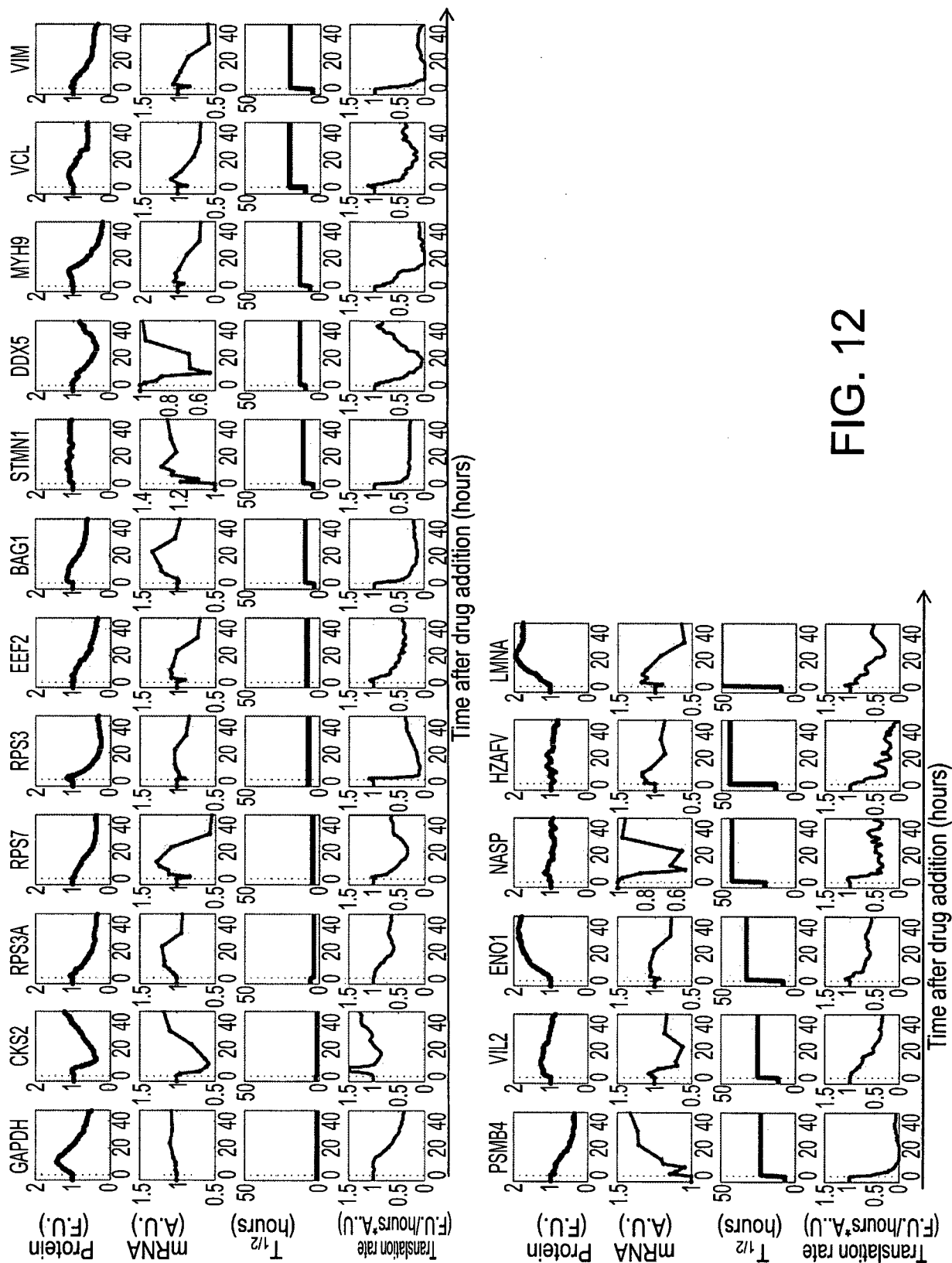


FIG. 12

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FIG. 13

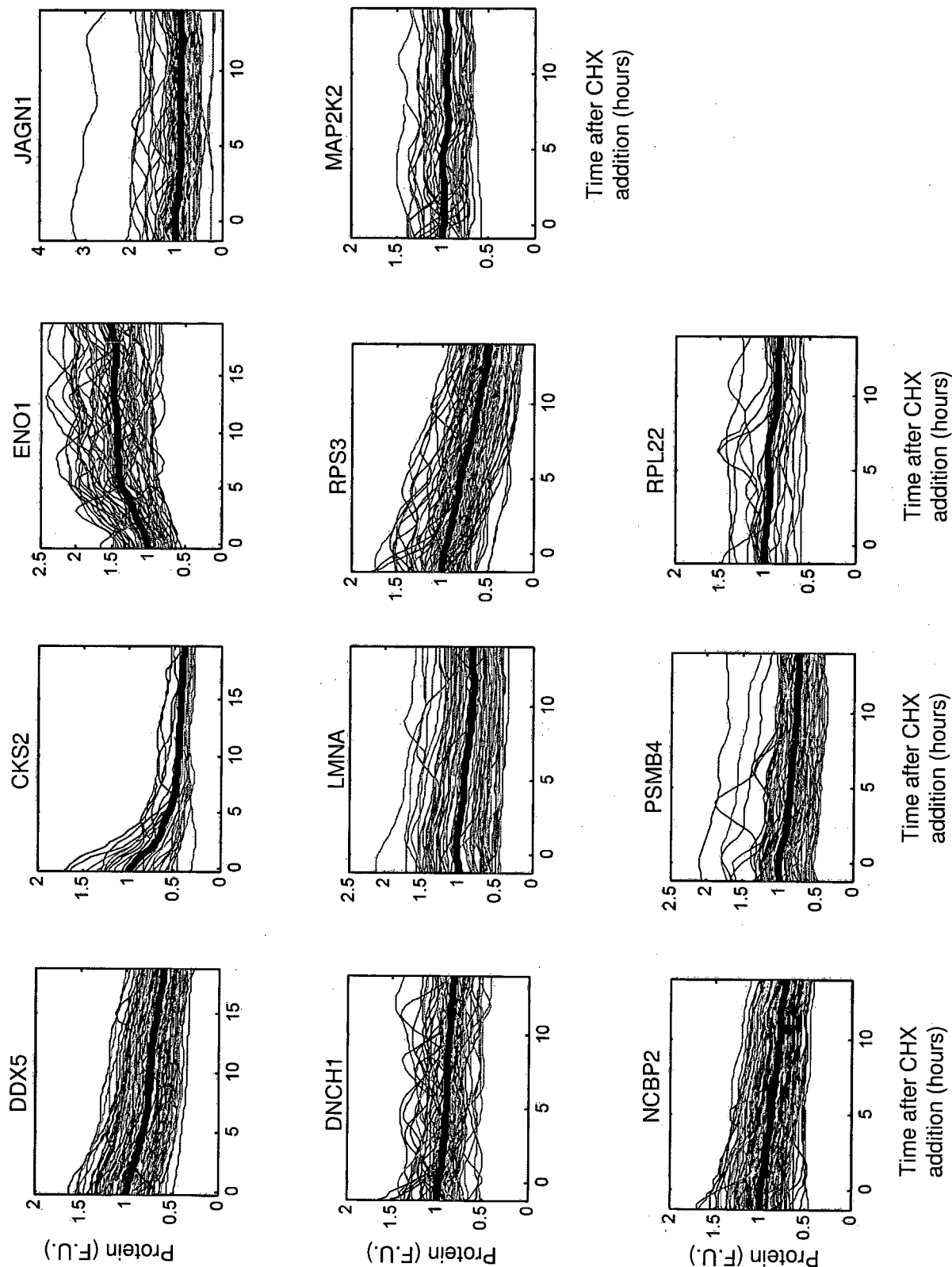
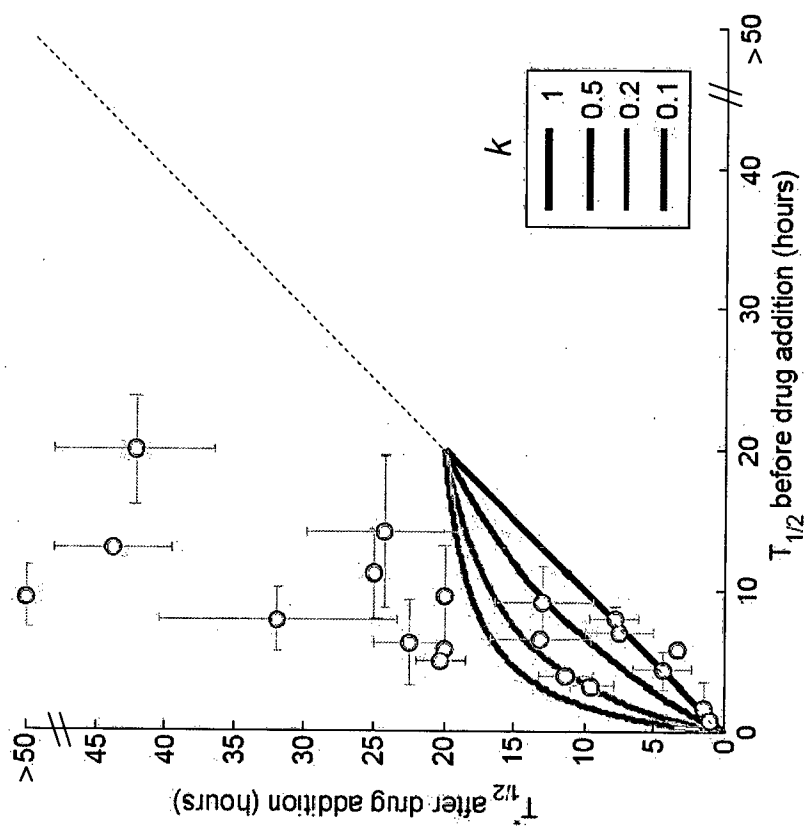
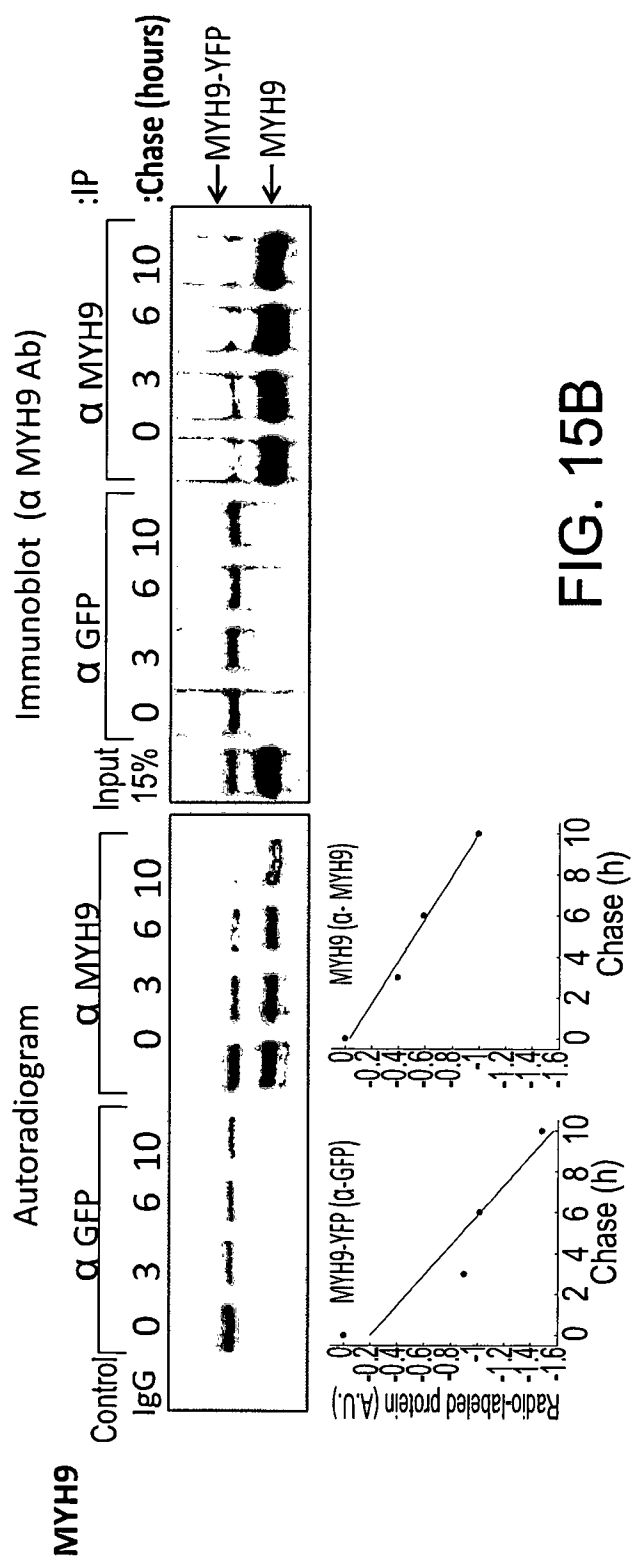
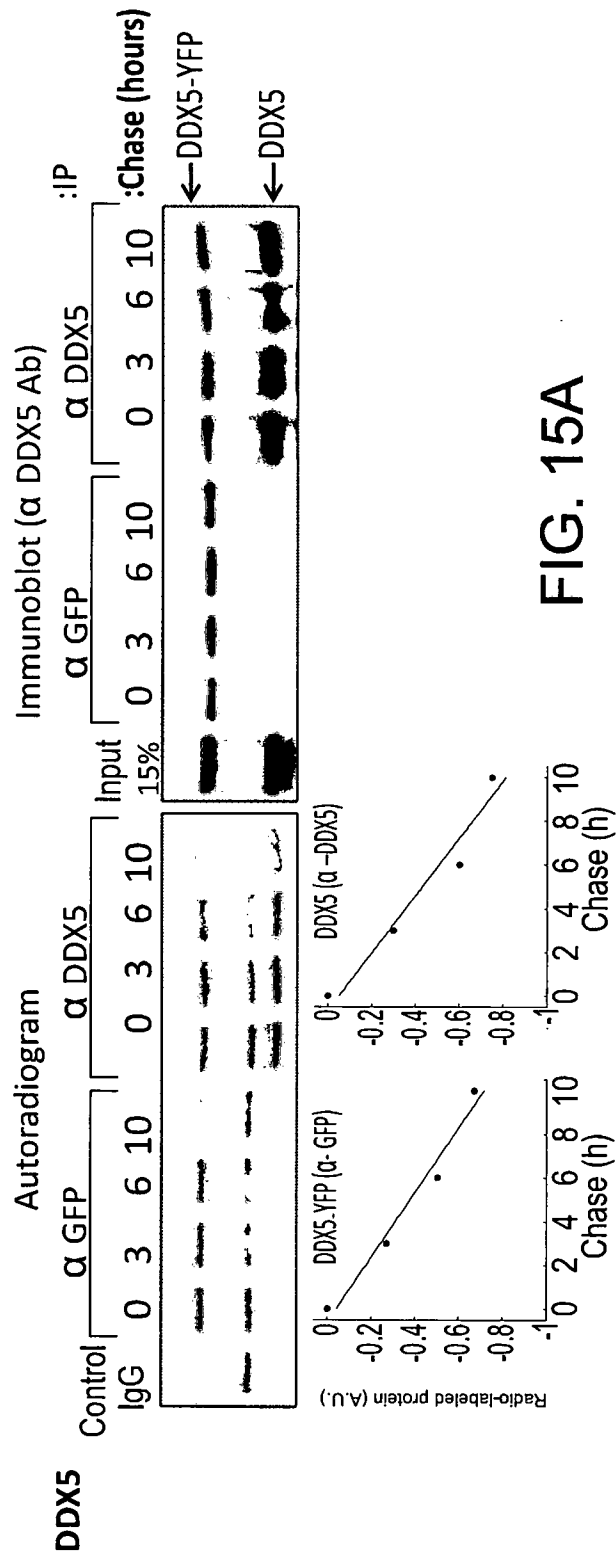


FIG. 14





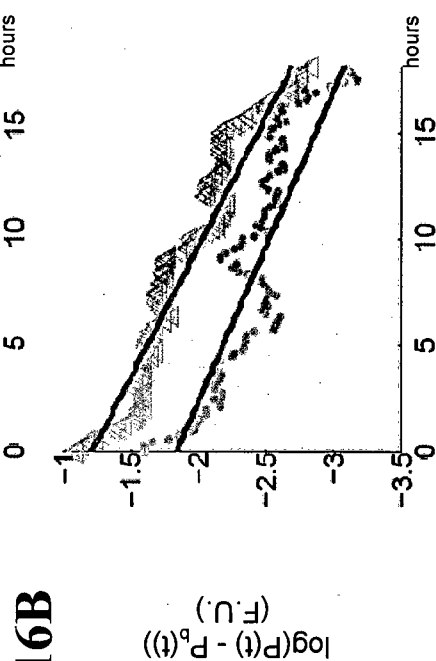
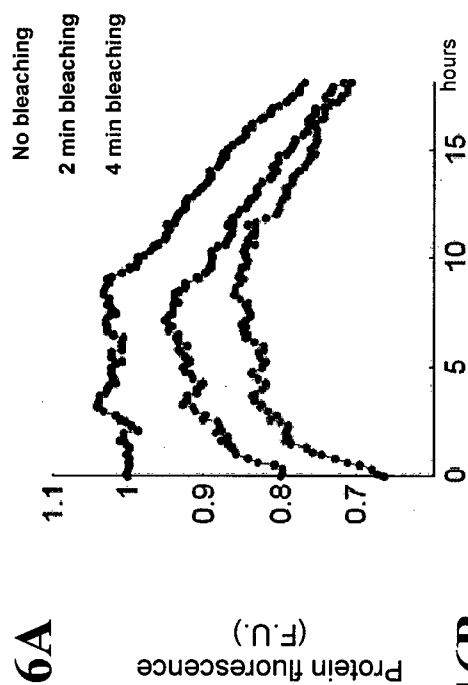


FIG. 17A

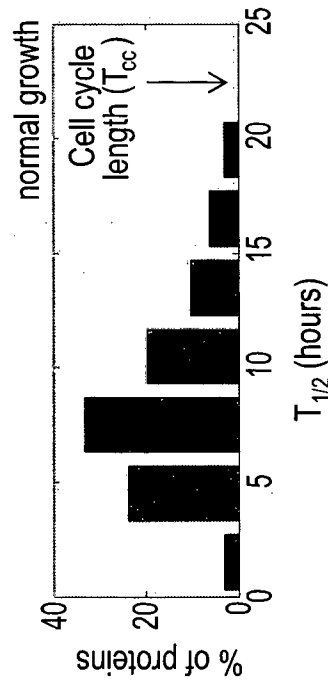
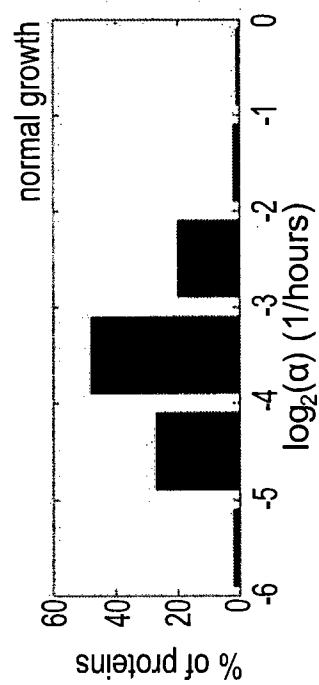


FIG. 17B

FIG. 17C

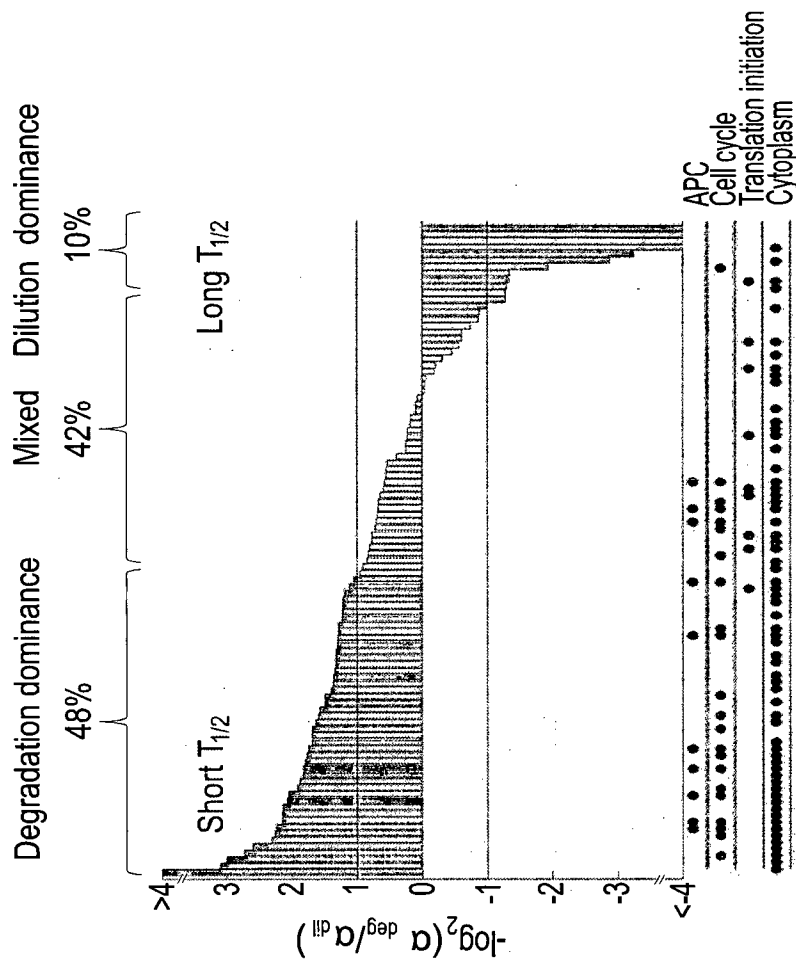
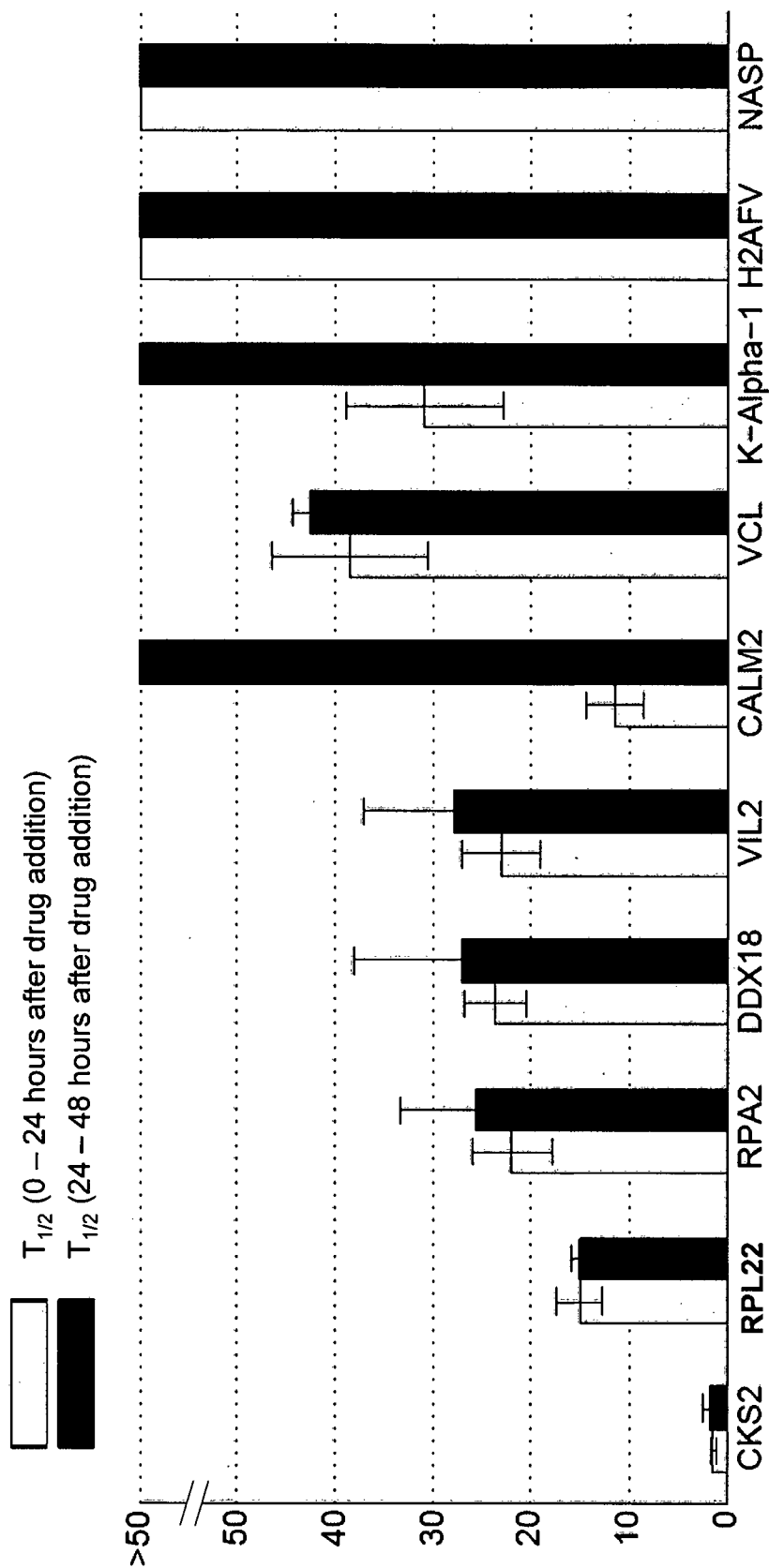


FIG. 17D

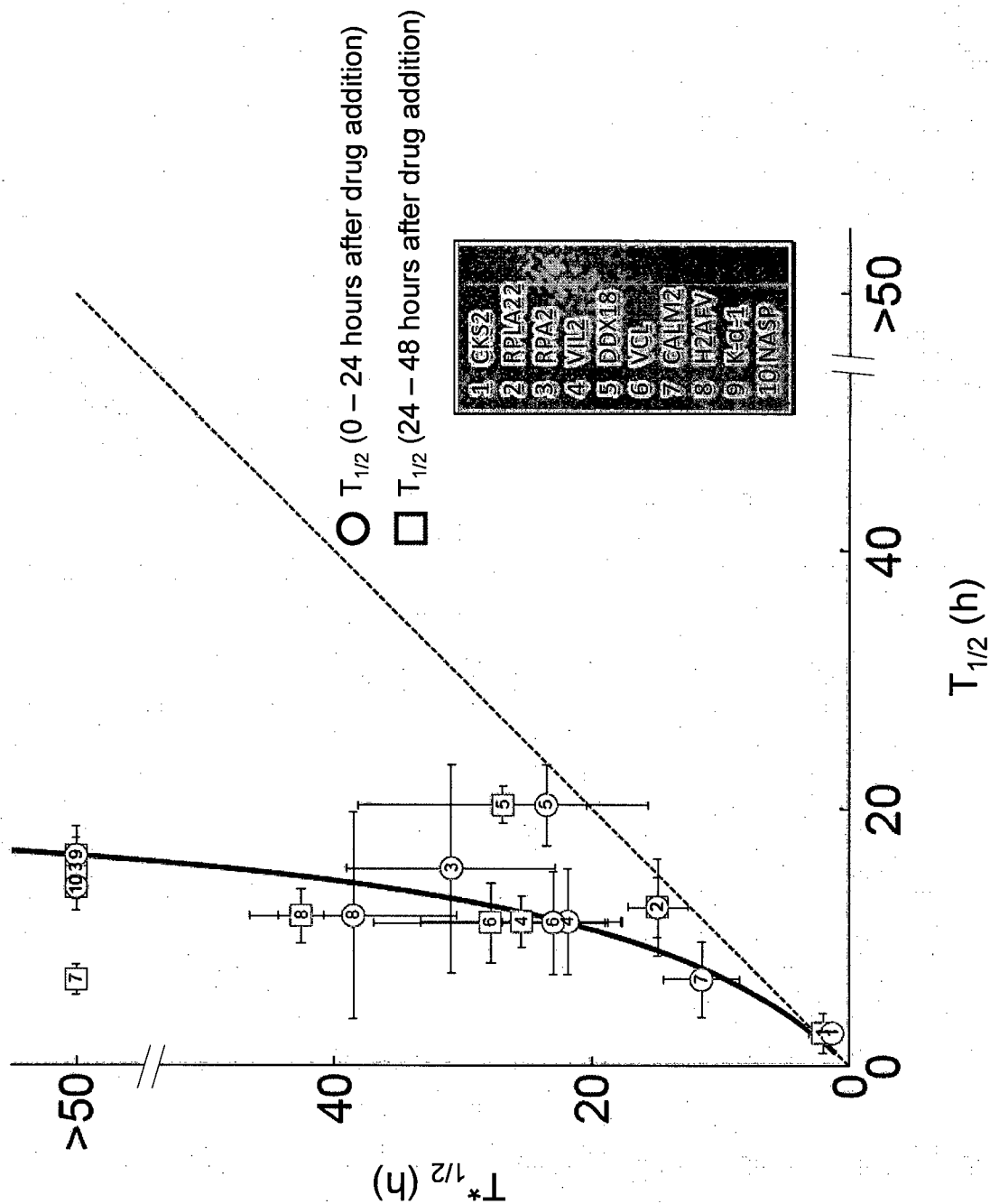
19/25

FIG.18A



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FIG. 18B



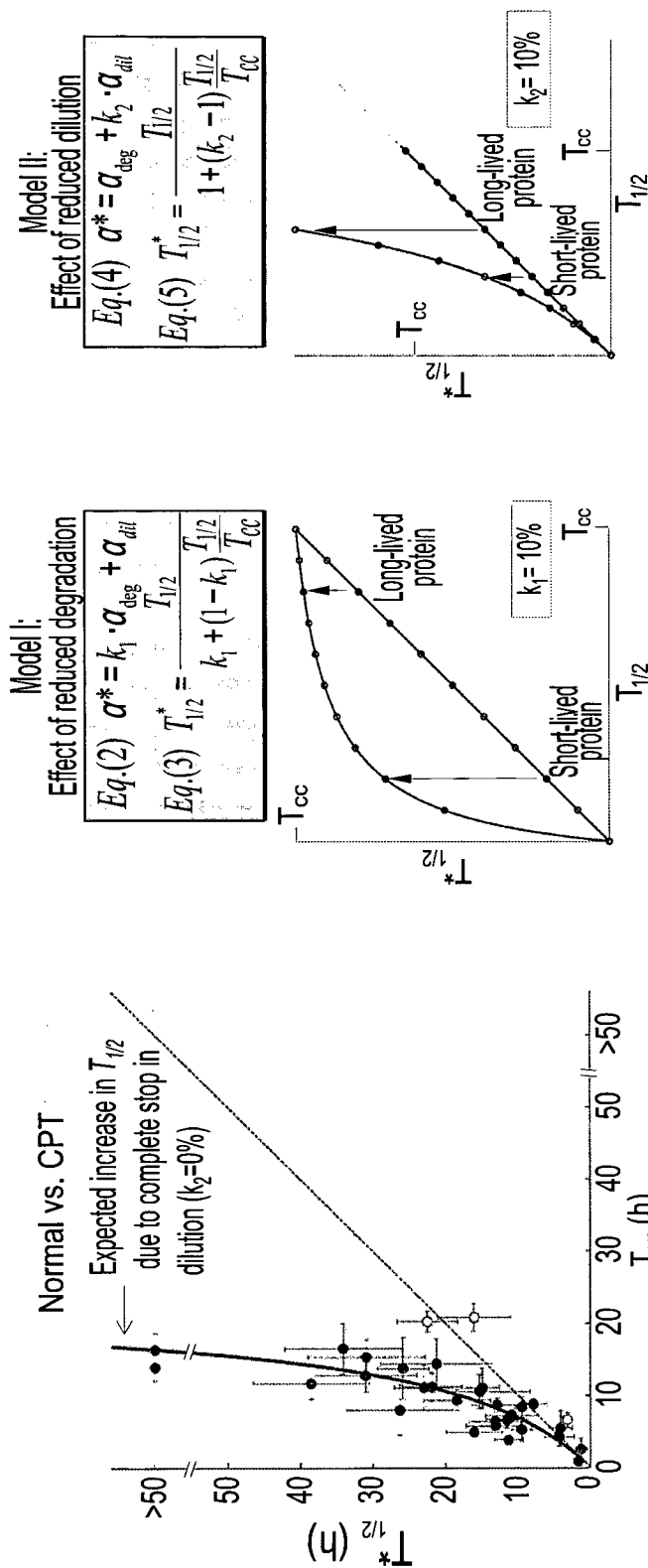


FIG. 19B

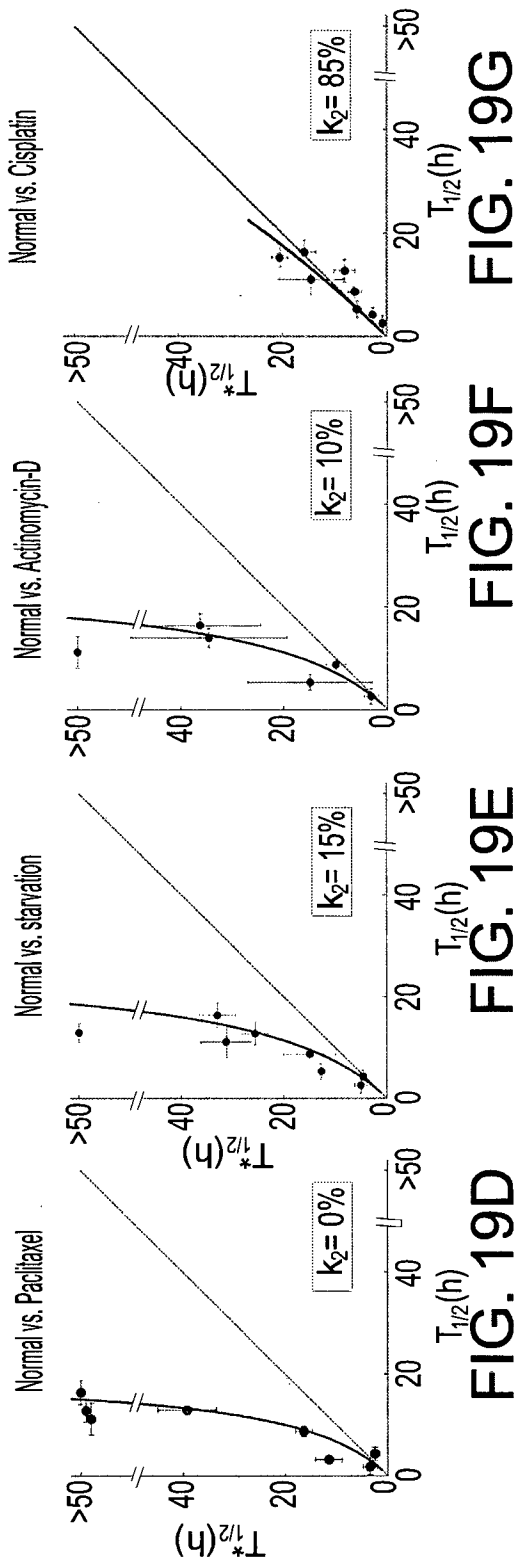


FIG. 20

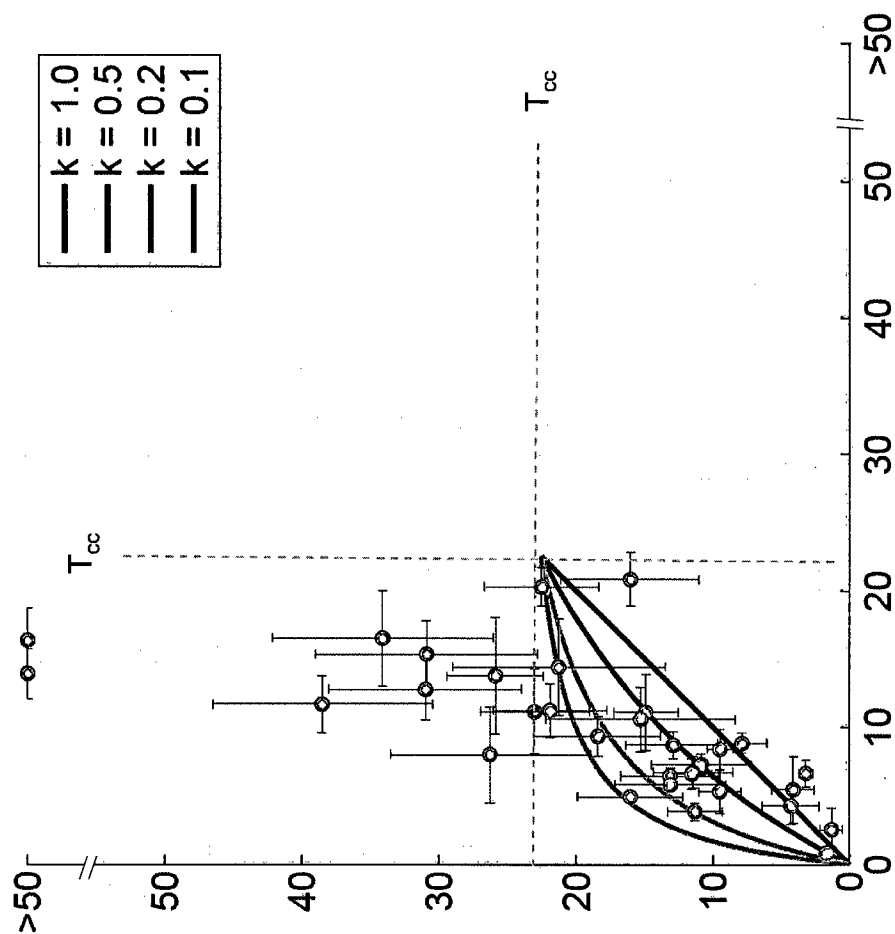


FIG. 21

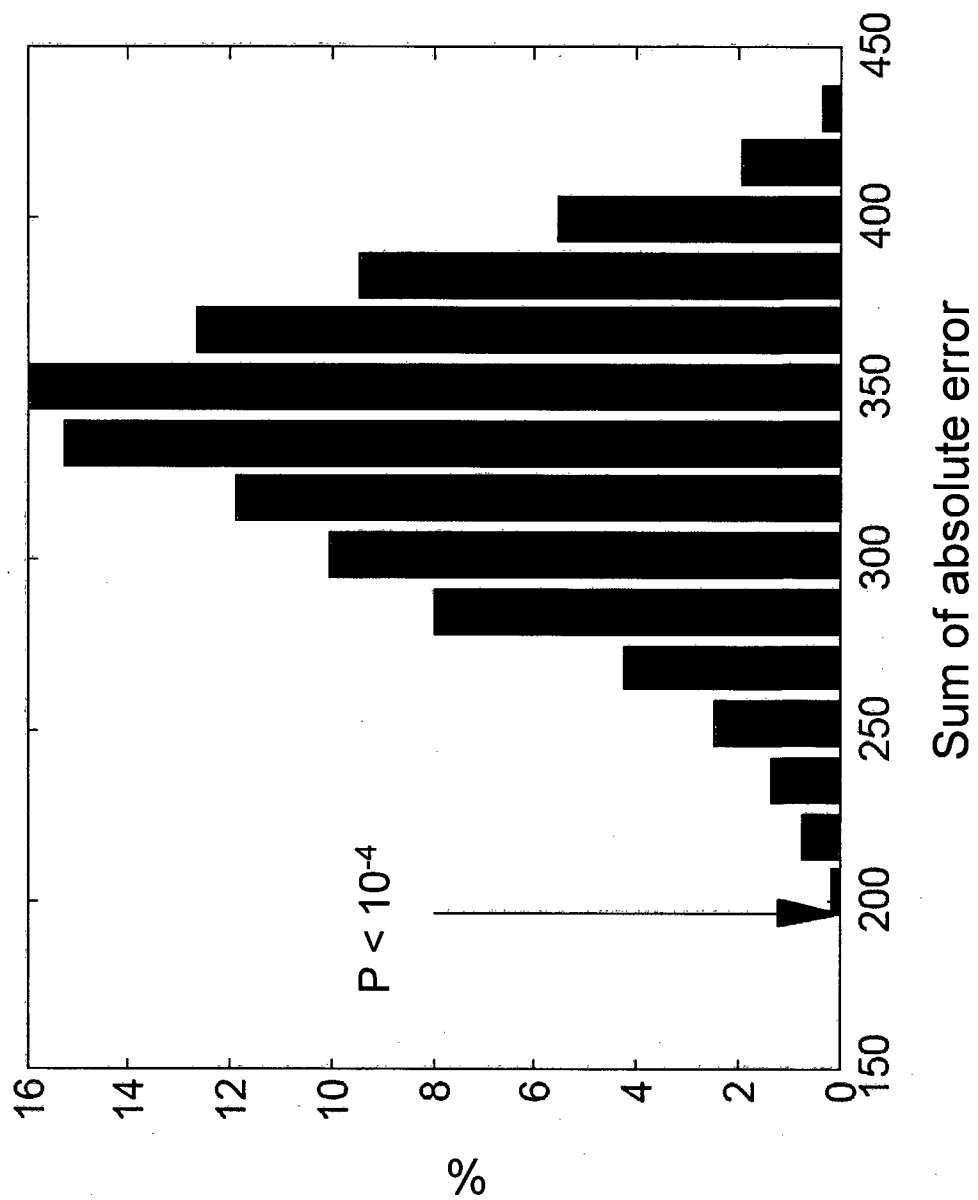
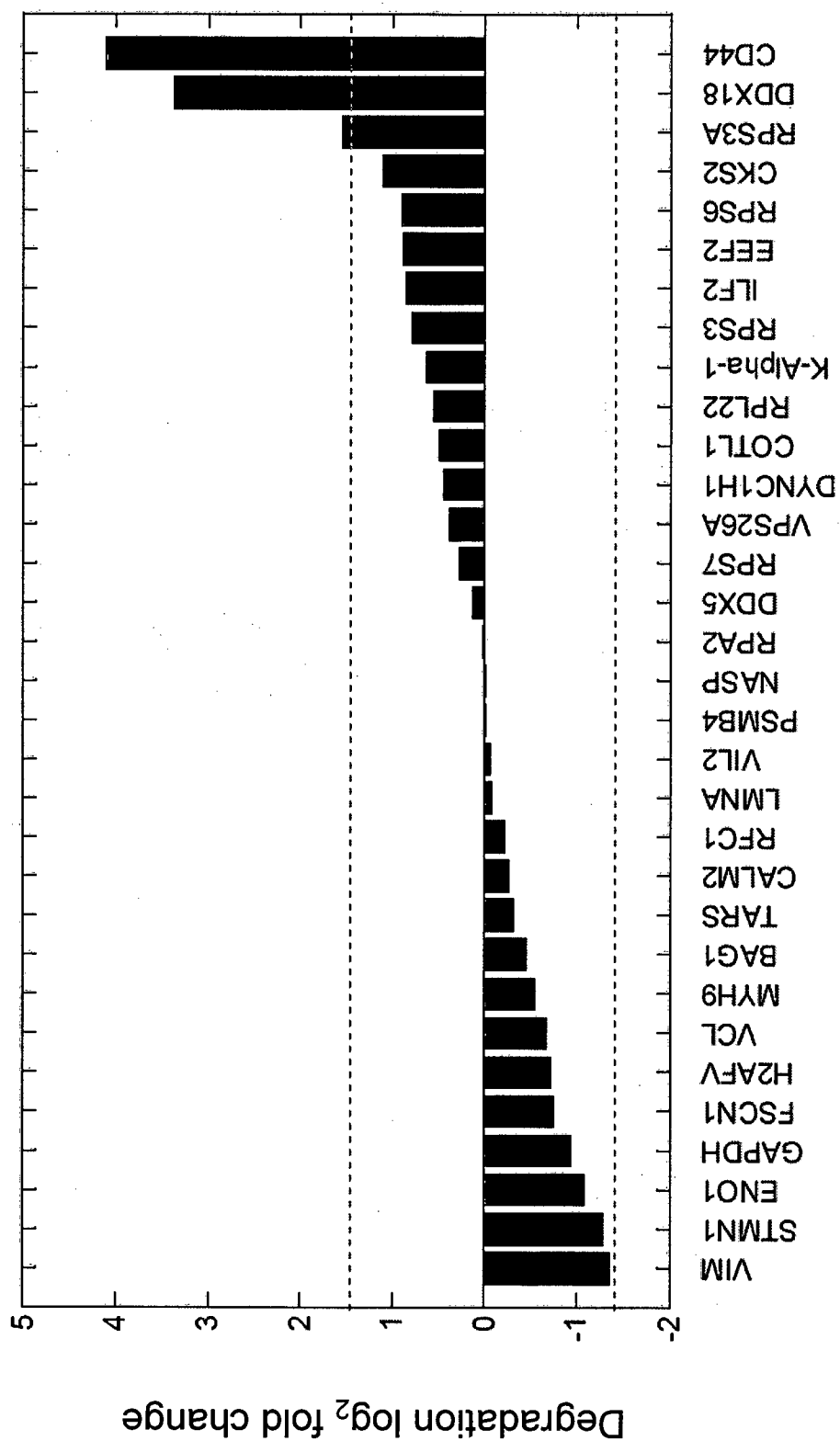


FIG. 22



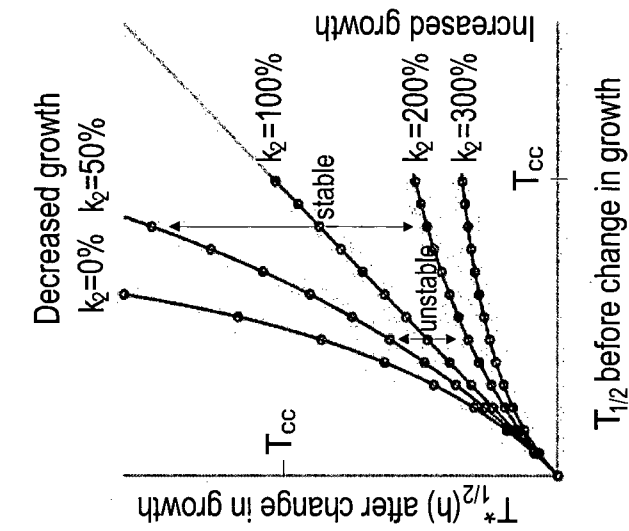


FIG. 23A

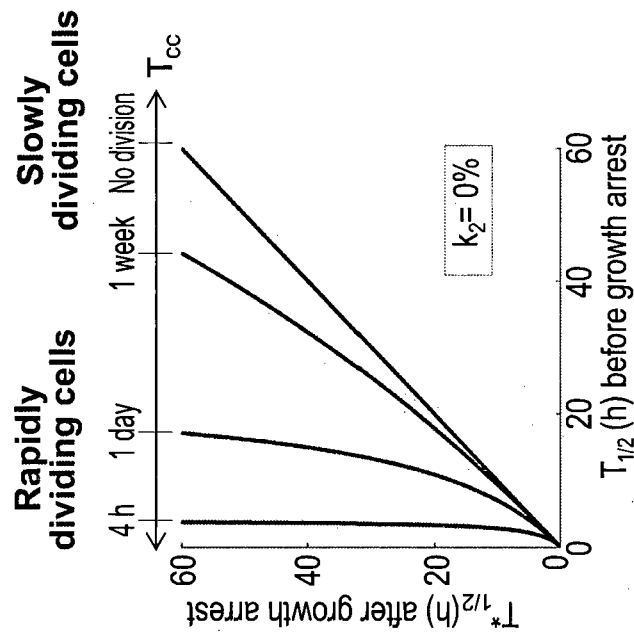


FIG. 23B

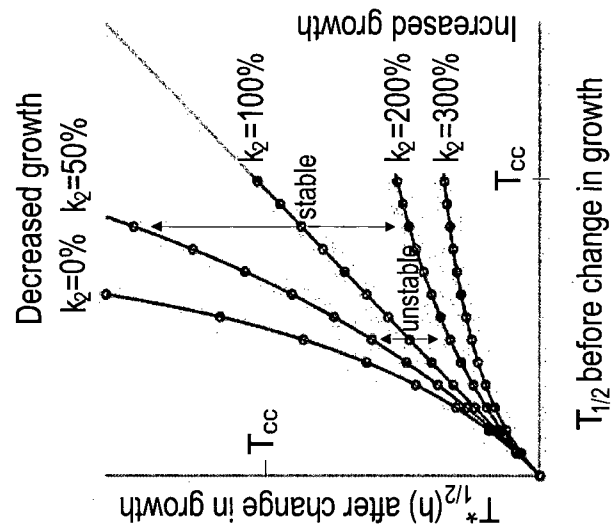


FIG. 23C

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2011/000213

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/50

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, FSTA, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A. KICHEVA ET AL: "Kinetics of Morphogen Gradient Formation", SCIENCE, vol. 315, no. 5811, 26 January 2007 (2007-01-26), pages 521-525, XP55001477, ISSN: 0036-8075, DOI: 10.1126/science.1135774 abstract page 523 - page 524; figure 2 -----	1-15
X	ARIEL AHARON COHEN ET AL: "Protein Dynamics in Individual Human Cells: Experiment and Theory", PLOS ONE, vol. 4, no. 4, 17 April 2009 (2009-04-17), page E4901, XP55001476, DOI: 10.1371/journal.pone.0004901 abstract; figure 1 -----	9-15



Further documents are listed in the continuation of Box C.



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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search

28 June 2011

Date of mailing of the international search report

11/07/2011

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