Science
AAAS

Chemical Genomic Profiling for Antimalarial Therapies, Response Signatures, and Molecular Targets

Jing Yuan *et al.*

Science **333**, 724 (2011);
DOI: 10.1126/science.1205216

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by clicking here.

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines here.

The following resources related to this article are available online at www.sciencemag.org (this information is current as of August 27, 2014):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/content/333/6043/724.full.html

Supporting Online Material can be found at:
http://www.sciencemag.org/content/suppl/2011/08/03/333.6043.724.DC1.html

A list of selected additional articles on the Science Web sites **related to this article** can be found at:
http://www.sciencemag.org/content/333/6043/724.full.html#related

This article **cites 39 articles**, 13 of which can be accessed free:
http://www.sciencemag.org/content/333/6043/724.full.html#ref-list-1

This article has been **cited by** 18 articles hosted by HighWire Press; see:
http://www.sciencemag.org/content/333/6043/724.full.html#related-urls

This article appears in the following **subject collections:**
Microbiology
http://www.sciencemag.org/cgi/collection/microbio

Downloaded from www.sciencemag.org on August 27, 2014

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. Copyright 2011 by the American Association for the Advancement of Science; all rights reserved. The title *Science* is a registered trademark of AAAS.

Chemical Genomic Profiling for Antimalarial Therapies, Response Signatures, and Molecular Targets

Jing Yuan,[1]* Ken Chih-Chien Cheng,[2]* Ronald L. Johnson,[2]† Ruili Huang,[2] Sittiporn Pattaradilokrat,[1] Anna Liu,[1] Rajarshi Guha,[2] David A. Fidock,[3] James Inglese,[2] Thomas E. Wellems,[1] Christopher P. Austin,[2]‡ Xin-zhuan Su[1]‡

Malaria remains a devastating disease largely because of widespread drug resistance. New drugs and a better understanding of the mechanisms of drug action and resistance are essential for fulfilling the promise of eradicating malaria. Using high-throughput chemical screening and genome-wide association analysis, we identified 32 highly active compounds and genetic loci associated with differential chemical phenotypes (DCPs), defined as greater than or equal to fivefold differences in half-maximum inhibitor concentration (IC_{50}) between parasite lines. Chromosomal loci associated with 49 DCPs were confirmed by linkage analysis and tests of genetically modified parasites, including three genes that were linked to 96% of the DCPs. Drugs whose responses mapped to wild-type or mutant *pfcrt* alleles were tested in combination in vitro and in vivo, which yielded promising new leads for antimalarial treatments.

The deployment of artemisinin (ART) and its derivatives against *Plasmodium falciparum* malaria parasites has been effective, and ART-based combination therapies (ACTs) are currently the recommended treatment in most endemic regions (*1*). The choice of partner drug is critical; an ideal partner drug should have pharmacokinetic and pharmacodynamic properties compatible with ART, employ a mode of action different from that of ART, retain efficacy against existing populations of drug-resistant parasites, and have no adverse pharmacologic interactions or additional toxicity (*2*). Unfortunately, parasites resistant to ART and its current partner drugs have been reported (*3*–*5*). New drugs or combinations are therefore urgently needed. Indeed, some promising leads have recently been identified through large-scale screening (*6*–*12*). Combinations of new or existing drugs that are synergistic or act on variant forms of parasite targets may mitigate the emergence of drug resistance. Here we have used quantitative high-throughput screening and genome-wide association and linkage analyses to identify new candidate antimalarial drugs with complementary or distinct response signatures for effective combination therapies. We show that many of the responses to a diverse collection of compounds are determined by a surprisingly limited number of genes, a finding that has broad implications for antimalarial drug development.

Chemical library screens for inhibitors and DCPs. Sixty-one parasite lines (table S1) were screened against the NIH Chemical Genomics Center Pharmaceutical Collection containing 2816 compounds registered or approved for human or animal use (*13*). The compounds were tested at eight fivefold serial dilutions (from 29 μM to 0.5 nM) using a parasite growth inhibition assay (*9*, *14*) to obtain dose-response curves and half-maximum inhibitor concentration (IC_{50}) determinations for each compound. From 171,776 drug assays that generated ~1.4 million data points, we identified 32 highly active compounds that inhibited the growth of at least 45 parasite lines with IC_{50} values ≤1 μM (Table 1). Among these pan-active compounds, seven (ecteinascidin 743, gramicidin, artenimol, decoquinate, epothilone B, atovaquone, and actinomycin D) yielded mean IC_{50} values lower than that of ART (IC_{50} < 10 nM) and, to our knowledge, 10 have not been reported to have antimalarial activity. Pairwise comparison of the IC_{50} values among 61 parasite lines identified 72,538 DCPs from 689 compounds, including 161 compounds that elicited DCPs between the parents of three *P. falciparum* laboratory crosses (7G8×GB4, Dd2×HB3, and 3D7×HB3) (table S2). As most of these compounds have been approved for human use, their ability to inhibit parasite growth at nanomolar levels makes them promising candidates for developing new antimalarial drugs or drug combinations. These DCPs can be investigated by using genome-wide association studies (GWAS) or linkage analysis to identify genetic determinants of susceptibility and study mechanisms of differential drug sensitivity.

Compounds with correlated responses and response signature groups. Compounds with positively correlated response patterns may act on common pathways or targets within the parasite. To search for compounds with correlated response patterns, we performed pairwise comparisons of 492 compounds (table S3) that were active [see supporting online material (SOM) for active compound definition] against at least one-third of the 61 lines profiled and identified 2082 pairs of compounds with highly correlated responses [correlation coefficient (CC) > 0.7] among these lines using methods described (*21*). For example, responses to 52 compounds were highly correlated with that of ART, 40 with that of mefloquine, and 25 with both ART and mefloquine. The 25 compounds included halofantrine, lumefantrine, dihydroergocristine, ergotamine, rifapentine, and bromocriptine. Correlations of parasite responses to ART, mefloquine, halofantrine, and lumefantrine have been documented in studies of culture-adapted parasites and in vivo patient isolates (*15*–*17*). The observations suggest that the compounds with highly correlated responses may share common features of drug action and/or resistance.

To investigate this idea further, we clustered the compounds into groups of common chemical response signatures using K-means and Dunn's index algorithms (*18*, *19*). The 492 compounds were grouped into 44 clusters, with some having relatively high activity indices (AIs, ranging from 0 to 1, with 1 being 100% correlation) that suggest potential common mechanisms of response (*19*) (Fig. 1A and table S4). Although the compound library was screened against 61 parasite lines, the numbers of clusters reached a plateau with just 10 lines (Fig. 1A). This result suggests that parasite responses to the compounds are restricted to a few common pathways. On the basis of their response patterns, parasite lines generally clustered according to their geographic origins (Fig. 1B). Distinct groups included one from South America, two from Cambodia, and a cluster of Africa–Central America–Thailand lines that could represent a recent parasite expansion from Africa. One of the Cambodian clusters exhibited high IC_{50} values to most of the compounds, reminiscent of an earlier description of Southeast Asian parasites with a phenotype showing an accelerated resistance to multiple drugs (*20*), and might represent an emerging ART-resistant population. These patterns of chemical response may reflect the population separation of Cambodian parasites that has been proposed on the basis of genome-wide microsatellite (MS) and single-nucleotide polymorphism (SNP) data (*17*, *21*) and suggest that antimalarial drugs have played an important role in the recent evolution and population structure of *P. falciparum*.

The compounds within most clusters generally showed strongly correlated responses and, in some cases, had similar structures as measured by a structure index (SI; values ranging from 0 to 1, with higher values having more similar structures) (*22*). For example, cluster 26 (AI =

[1]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA. [2]NIH Chemical Genomics Center, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20895, USA. [3]Departments of Microbiology and Immunology and of Medicine, Columbia University Medical Center, New York, NY 10032, USA.

*These authors contributed equally to this work.

†Present address: Division of Cancer Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20895, USA.

‡To whom correspondence should be addressed. E-mail: xsu@niaid.nih.gov (X.-z.S.); austinc@mail.nih.gov (C.P.A.)

0.55 and SI = 0.4) comprises 10 compounds, 8 of which contain a quinoline core, including chloroquine (CQ), cinchonine, quinacrine, quinidine, and quinocidum (fig. S1 and table S4). *P. falciparum* responses to various quinolines have been associated with the *P. falciparum* CQ resistance transporter (PfCRT) and multidrug resistance 1 protein (PfMDR1, also named P-glycoprotein homolog 1), both of which are proposed to function as transporters (*23*–*25*). Clusters 28 and 40 were characterized by high AI values (0.73) but relatively low SI values (0.15 and 0.2, respectively), which suggested highly correlated responses among compounds of diverse structures. Further investigation of these clusters and their targets may reveal how parasites respond to structurally diverse compounds.

Mutations enabling resistance to one compound may render parasites more sensitive to other compounds; in cases of negative correlation, pairs of such compounds may be good candidates for drug combination therapy, particularly if they are complementary in their actions on wild-type and mutant forms of a parasite target. Pairwise comparison of the 492 active compounds identified 1250 pairs that were negatively correlated, with CC ranging from −0.26 to −0.90 (table S5). In particular, 43 compounds were negatively correlated with CQ, including ciprofloxacin and clindamycin, each of which has been reported to have antimalarial activity alone (*26*, *27*). The combination of clindamycin and CQ (CC = −0.27) substantially improves patient cure rate as compared with CQ alone (94% compared with 32%) (*26*), and ciprofloxacin (CC = −0.30) significantly enhances CQ activity in vitro (*27*). Tylosin, a veterinary drug used to treat bacterial infections, is negatively correlated with CQ as well (CC = −0.43) and synergizes with it to inhibit *P. falciparum* in vitro (*28*). Further investigation of the compounds negatively correlated with CQ response could lead to new drug combinations for treating resistant parasites (also see below).

Tocainide and etonogestrel showed marked negative correlations with responses to ART or mefloquine (CC = −0.52 to −0.67) (table S5), warranting further evaluation as candidates for new classes of ACTs. These compounds were also negatively correlated with quinine, although to a somewhat lesser extent (CC = −0.34 and −0.42, respectively), which suggests potential shared mechanisms in response to quinine, ART, and mefloquine.

Impact of antimalarial drugs on parasite populations. To investigate how antimalarial drugs influence parasite evolution and population structure, we analyzed parasite responses to 134 compounds that produced an IC_{50} value with a good-quality dose-response curve (curve

Table 1. Compounds highly active against multiple *Plasmodium falciparum* isolates. CN, the cluster numbers in table S4; Lines active, numbers of parasite lines for which a compound is active with an IC_{50} value < 1 μM; Mean IC_{50}, mean half-maximum inhibitory concentrations determined from the numbers of parasite lines that produced good response data (indicated in the previous column); Chiral, compounds with chiral centers; NP/Syn, natural product or synthetic. Some of the compounds may contain mixtures of chiral isomers, but specific chiral information for each compound is not available. Nineteen known antimalarial drugs that meet the activity cutoff criteria but are not listed in the table include artemetero, ART, artemisininum, artenimol, amodiaquine, atovaquone, cinchonine, clindamycin, ethidium bromide, halofantrine, lumefantrine, mefloquine, methylene blue, methyl violet, quinacrine, quinidine gluconate, quinaldine blue, quinine scorbate, and trimetrexate glucuronate. NA, not applicable.

Name	CN	Lines active	Mean IC_{50} (M)	Annotations	Chiral	NP/Syn
Ecteinascidin 743	0	46	7.15E-10	Antineoplastic, alkylating	Yes	NP
Gramicidin*	0	51	1.52E-09	Anti-infective; antibiotics	Yes	NP
Decoquinate	9	47	2.40E-09	Anti-infective; antiparasitic, anticoccidian	No	Syn
Epothilone B†	11	61	3.22E-09	Antineoplastic; tubulin modulators	Yes	NP
Actinomycin D*	NA	57	4.89E-09	Antineoplastic; nucleic acid synthesis inhibitors	Yes	NP
Homoharringtonine*	22	61	2.27E-08	Antineoplastic, phytogenic; angiogenesis inhibitors	Yes	NP
Monensin sodium salt*	5	61	3.29E-08	Anti-infective; antiparasitic	Yes	NP
Docetaxel*	11	58	5.18E-08	Anti-infective; antiparasitic	Yes	NP‡
Buquinolate	5	59	1.03E-07	Antimicrobials; anticoccidiosis	No	Syn
Narasin*	1	61	1.12E-07	Anti-infective; antibiotics	Yes	NP‡
Bortezomib*	37	61	1.50E-07	Antineoplastic; protease inhibitors	Yes	Syn
Mupirocin*	6	58	1.88E-07	Antibacterial; anti-infective; antibiotics	Yes	NP
Fumagillin*	38	45	2.03E-07	Anti-infective; antibiotics; growth inhibitors	Yes	NP
Alazanine triclofenate	38	61	2.36E-07	Antiparasitic	No	Syn
Aclarubicin hydrochloride*	38	61	3.00E-07	Antineoplastic; topoisomerase II inhibitor	Yes	NP
Emetine*	20	60	3.28E-07	Protein synthesis inhibitors; anti-infective	Yes	NP
Lestaurtinib	23	60	3.41E-07	FLT3-selective tyrosine kinase inhibitor	Yes	NP‡
Deserpidine	39	61	3.50E-07	Cardiovascular; antihypertensive	Yes	NP‡
Dextroamphetamine saccharate	43	60	3.52E-07	Peripheral nervous system; sympathomimetics	Yes	Syn
Plicamycin*	38	51	3.52E-07	Antineoplastic; nucleic acid synthesis inhibitors	Yes	NP‡
Demecarium bromide*	11	59	3.90E-07	Parasympathomimetic; acetylcholinesterase inhibitor	No	Syn
Paclitaxel*	11	59	5.29E-07	Antineoplastic; antineoplastic, phytogenic	Yes	NP
Acriflavinium hydrochloride*	20	60	6.72E-07	Antiseptic; dye	No	Syn
Zinc pyrithione†	18	60	6.82E-07	Antifungal and antibacterial	No	Syn
Suberoylanilide*	31	58	7.30E-07	Anti-inflammatory, nonsteroidal	No	Syn
Orlistat	30	46	7.43E-07	Anti-obesity; lipoprotein lipase (LPL) inhibitor	Yes	NP‡
Lasalocid sodium*	23	60	8.18E-07	Anti-infective; antibiotics	Yes	NP
Clotrimazole*	29	54	8.20E-07	Ca^{2+}-activated K^+ channel inhibitors	No	Syn
Salinomycin *	1	57	8.83E-07	Anti-infective; antibiotics	Yes	NP
Puromycin hydrochloride*	NA	60	8.92E-07	Anti-infective; protein synthesis inhibitors	Yes	NP
Lauryl isoquinolinium bromide	11	57	9.26E-07	Detergent	No	Syn
Ciclesonide	30	50	9.36E-07	Antiallergic	Yes	NP

*Compounds with IC_{50} values reported previously. †Compounds with antimalarial activity identified previously, but IC_{50} values were not determined. (See SOM for additional explanations.) ‡Natural product derivative.

class 1.1, 1.2, and 2.1, see SOM) in each of the 61 parasite lines. Consistent with the geographic clustering, principal component analysis (PCA) separated the American parasites from the African and Asian parasites by component 2 and the Asian parasites from the African parasites by component 5 (Fig. 1C). Compounds contributing most positively to component 5 were predominantly the quinoline antimalarial drugs in cluster 26; compounds contributing negatively to this component included mibefradil, vinorelbine, and homoharringtonine (table S6). These results suggest that CQ and the other quinoline drugs have played a substantial role in the evolution of these two parasite populations. Similarly, the compounds contributing most negatively to component 2, which separates the New and Old World parasites, included dihydroergotamine, dihydroergocristine, and reserpine; responses to these drugs were mapped to *pfmdr1* in the progeny of the *P. falciparum* crosses (see below). Compounds contributing most positively to this component included docetaxel and clobetasone. Although these compounds have not been used to treat malaria, they could be linked to (mutant) alleles of parasite molecules (possibly *pfmdr1*) selected by other antimalarial drugs. These data suggest that *pfmdr1* played a substantial role in shaping the *P. falciparum* populations in America. The two groups of compounds showing positive or negative contributions to component 2 (eigenvector values <0.14 and those >0.14) were themselves positively correlated in response patterns within each group but were largely negatively correlated between the groups (Fig. 1D). Similar correlations were observed for the compounds in component 5, for example, CQ and mibefradil (Fig. 1E). The compounds with negatively correlated responses provide additional starting points for novel combination therapies.

GWAS of parasite responses with SNPs. To identify genes that may associate with the differences in responses to the active compounds, we performed GWAS using 3354 SNPs collected previously to search for SNPs associated with differential drug responses (*17*). First, we investigated whether known resistance determinants could be identified using this method. Indeed, mutations in *pfcrt* were significantly (corrected $P < 0.01$) associated with responses to more than 200 compounds, including the expected quinolines, such as hydroxychloroquine, CQ, quinine, and quinacrine (table S7). Similarly, responses to dihydrofolate reductase (DHFR) inhibitors, such as trimethoprim, trimetrexate, and triamterene, were significantly (corrected $P < 0.005$) associated with mutations in *pfdhfr* (table S8). Compounds associated with mutations in *pfmdr1* included dihydroergotamine and lumefantrine, consistent with previous reports (*9*, *29*) (table S9). Additionally, the major compounds contributing to the separation by PCA component 2 of the South American population from those of Asia and Africa were also associated with polymorphisms of *pfmdr1* (table S9). These results show that known mutations strongly influencing parasite drug responses can be identified using GWAS.

Fifteen genes were significantly (corrected P values < 0.005) associated with responses to both ART and mefloquine, consistent with the presence of these drugs in cluster 23 (tables S4, S10, and S11). The top six genes associated with responses to ART and its derivatives were MAL13P1.268 (*Plasmodium* conserved protein), PF11_0188 (heat shock protein 90), PFE0565w (conserved *Plasmodium* protein), PF08_0130 (ribosomal RNA processing WD-repeat protein), PFA0655w (SURFIN), and PFI0355c (adenosine triphosphate–dependent heat shock protein). In addition, the majority of the genes associated with ART response were also associated with responses to derivatives such as artemisininum, artenimol, and artemetero (table S10). Parasite response to primaquine was also significantly associated with MAL13P1.268 (corrected P value = 1.74E-04) (table S12). Because MAL13P1.268 was associated with responses to primaquine, ART, mefloquine, dihydroergotamine, and dihydroergocristine, the role of this gene in drug resistance should be studied further. Association analysis of 26 PCA-corrected parasites from the Thai-Cambodian border linked many SNPs with different drug responses; however, the genes associated with responses to CQ, mefloquine, ART, and antifolate drugs were not evident in this analysis because all or the majority of the parasites were resistant to these drugs (table S13). Although some of the associated genes identified

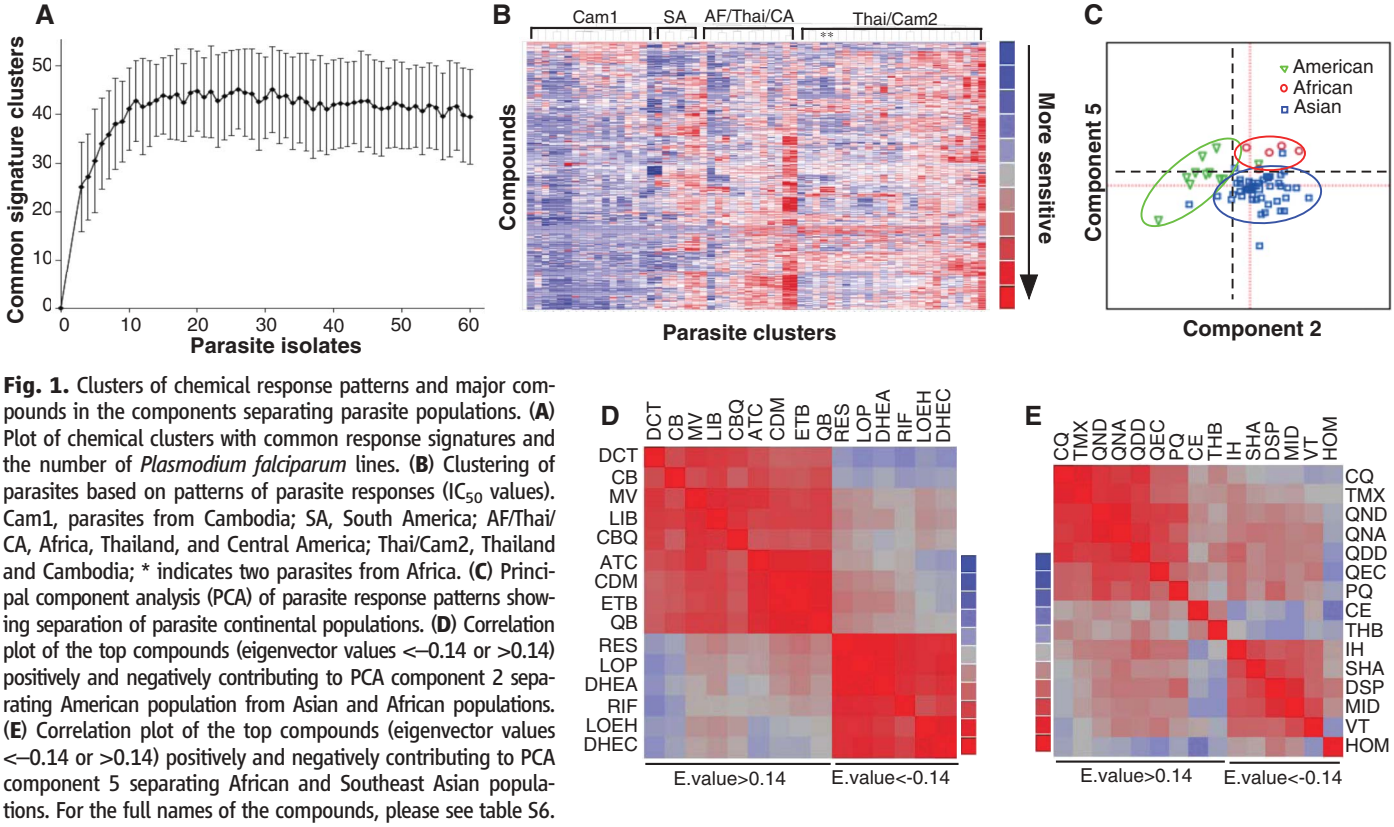

Fig. 1. Clusters of chemical response patterns and major compounds in the components separating parasite populations. (**A**) Plot of chemical clusters with common response signatures and the number of *Plasmodium falciparum* lines. (**B**) Clustering of parasites based on patterns of parasite responses (IC_{50} values). Cam1, parasites from Cambodia; SA, South America; AF/Thai/CA, Africa, Thailand, and Central America; Thai/Cam2, Thailand and Cambodia; * indicates two parasites from Africa. (**C**) Principal component analysis (PCA) of parasite response patterns showing separation of parasite continental populations. (**D**) Correlation plot of the top compounds (eigenvector values <–0.14 or >0.14) positively and negatively contributing to PCA component 2 separating American population from Asian and African populations. (**E**) Correlation plot of the top compounds (eigenvector values <–0.14 or >0.14) positively and negatively contributing to PCA component 5 separating African and Southeast Asian populations. For the full names of the compounds, please see table S6.

in this study could be false positives, these studies provide a starting point for further functional investigations of the genes and their roles in antimalarial drug resistance.

Three genetic loci linked to the majority of DCPs. To identify the genetic determinants that contribute to the DCPs and to further investigate some of the compounds associated with mutations in *pfcrt* and *pfmdr1*, we tested 128 DCP compounds on 33 recombinant progeny of the *P. falciparum* 7G8×GB4 cross and 98 DCP compounds on 34 recombinant progeny of the Dd2×HB3 cross (tables S1 and S14). Using quantitative trait loci analysis, we mapped 49 DCPs to 57 chromosomal loci with a logarithm (base 10) of odds (LOD) score ≥ 3.0. Remarkably, 47 of the 49 (96%) DCPs were mapped to three loci containing *pfdhfr* (chromosome 4), *pfmdr1* (chromosome 5), or *pfcrt* (chromosome 7) (Fig. 2 and table S14). These results indicate that *pfdhfr*, *pfmdr1*, and *pfcrt* dominate the parasite's differential response to many drugs. Additionally, eight DCPs were mapped to loci on chromosomes 3, 7, 8, 12, and 14, with DNA segments ranging from 55 to 193 kb (tables S14 and S15 and SOM text). Further studies are needed to identify the mutations conferring these DCPs.

The linkage analyses also confirmed many differential compound sensitivities associated with *pfcrt*, *pfmdr1*, or *pfdhfr* by GWAS. Responses to 11 compounds associated with *pfcrt*, three antifolate drugs with *pfdhfr*, and 15 compounds with *pfmdr1* were confirmed by linkage analysis and/or testing of parasites with genetically modified *pfcrt* or *pfmdr1* (tables S7 to S9 and S14) (also see below). These results demonstrate the usefulness of GWAS for detecting mutations mediating drug resistance in malaria parasites.

Confirmation of PfDHFR as the target of trimethoprim and triamterene by using parasites with different mutant *pfdhfr* alleles has been reported (*9*). To determine whether *pfcrt* and *pfmdr1* were indeed responsible for the DCPs that map to their respective loci, we tested the DCP compounds against parasites with genetically modified *pfmdr1* or *pfcrt* genes (tables S14 and S16). There are five common amino acid polymorphisms in PfMDR1 (*30*), and changes at positions S1034C, N1042D, and D1246Y have been shown to confer differential sensitivity to some drugs (*9*). In two progeny of the Dd2×HB3 cross, the CQ-sensitive GC03 parasite and the CQ-resistant 3BA6 parasite, conversion of the SDD *pfmdr1* allele (encoding amino acids S1034, D1042, and D1246) to a SND allele decreased sensitivity to some antimalarial agents, whereas conversion to a CDY allele resulted in increased sensitivity. For the eight DCP responses mapped to *pfmdr1* in Dd2×HB3 and 7G8×GB4 crosses, the IC_{50} values were 3 to 23 times those for parasites carrying the SND allele versus those carrying the CDY allele. As expected, control parasites in which the allelic exchange produced no change from *pfmdr1* SDD resulted in less than twofold changes in IC_{50} values for all DCPs mapped to the *pfmdr1* locus (table S14). Comparison of IC_{50} ratios from a genetically modified parasite receiving the parental SND *pfmdr1* allele over the one receiving the CDY allele showed significantly higher mean ratios (unpaired *t* test, $P < 0.001$) for the DCP compounds that mapped to *pfmdr1* than those that did not (table S14). These particular amino acid substitutions therefore mediate the DCPs that map to this locus.

Among the 23 DCPs linked to chromosome 7, twofold or greater changes in IC_{50} value were seen for 19 DCPs in the GC03 parasites engineered to carry the mutant *pfcrt* alleles of Dd2 or 7G8 ($GC03^{Dd2}$, $GC03^{7G8}$) (table S14). Similarly, the mean IC_{50} ratios for compounds mapped to *pfcrt* were significantly higher than those not mapped to *pfcrt* ($P = 0.002$) in the recombinant $GC03^{Dd2}$ parasites. In the genetically modified parasites, IC_{50} values were elevated for 4 compounds and decreased for 15, which suggests specific interactions with wild-type or mutant *pfcrt* alleles, respectively (table S14). Responses to many compounds—such as perhexiline, lopinavir, lumefantrine, carbetapentane, memantine, and duloxetine that did not map to any loci—were also altered in the *pfcrt*- or *pfmdr1*-modified parasites, which suggests that these compounds target or are transported by PfCRT or PfMDR1, both of which reside on the membrane of the intraerythrocytic parasite's digestive vacuole, the site of hemoglobin degradation (*23*, *31*).

The compounds generating the DCPs linked to *pfcrt* have amine groups that could become protonated at physiologic or lower pH (fig. S2). A plot of the parasite response patterns showed two groups of compounds with positive correlation in responses within each group, but negative correlation between groups (Fig. 3A). Except for the presence of a basic amine, however, there was no consistent structural basis for separating the two groups of compounds whose responses correlated positively or negatively with that of CQ (fig. S2). Although agents with structures similar to some of these compounds have been tested for "reversal" of CQ resistance (*32*–*35*), our data provide genetic evidence showing that the mechanism of this reversal is specific to mutant PfCRT, consistent with results from a recent study (*36*). These compounds may act as blockers of the PfCRT drug pore, as they all carry a basic nitrogen group (fig. S2) that may impede the mutant PfCRT-mediated flux of CQ out of the digestive vacuole (*37*). As expected, the DCPs linked to *pfmdr1* also included compounds with diverse structures, including dihydroergotamine, dihydroergocristine, miconazole, and rifampin in cluster 40; ergotamine and zeaxanthin in cluster 41; and reserpine, LOE 908, and sorafenib in cluster 36.

Drug combinations targeting PfCRT. CQ is no longer effective in treating *P. falciparum* infections because of mutations in *pfcrt* (*38*). Identification of compounds that interact with either wild-type or mutant forms of PfCRT

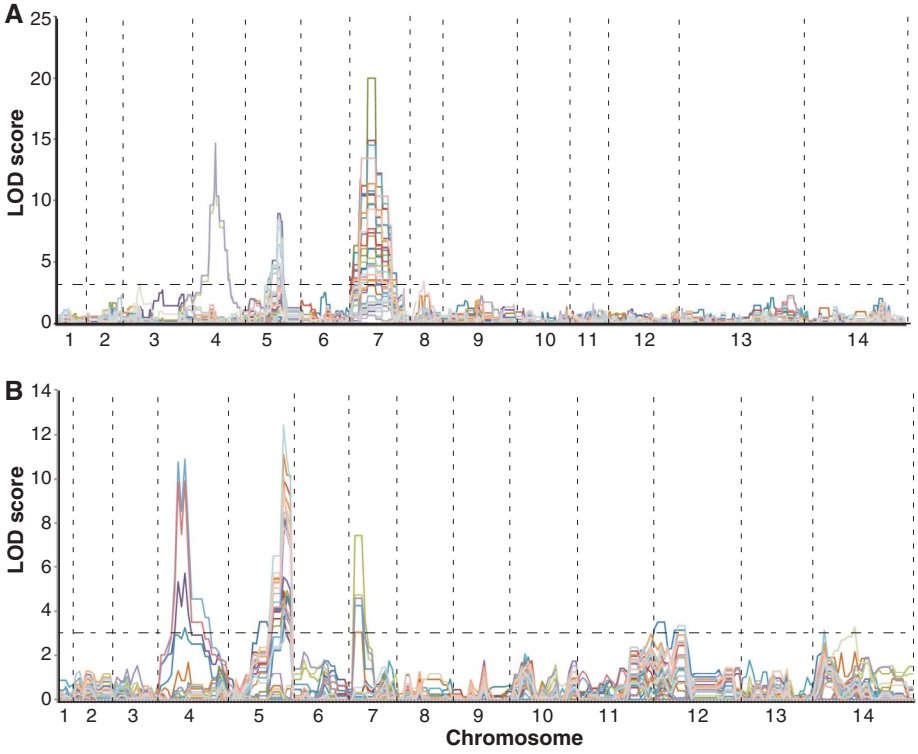

Fig. 2. Genetic loci linked to differential chemical phenotypes determined using progeny from two genetic crosses. (**A**) Plots of quantitative trait loci from compounds with a LOD score >3.0 in the Dd2×HB3 cross. (**B**) Plot of quantitative trait loci from compounds with a LOD score >3.0 in the 7G8×GB4 cross.

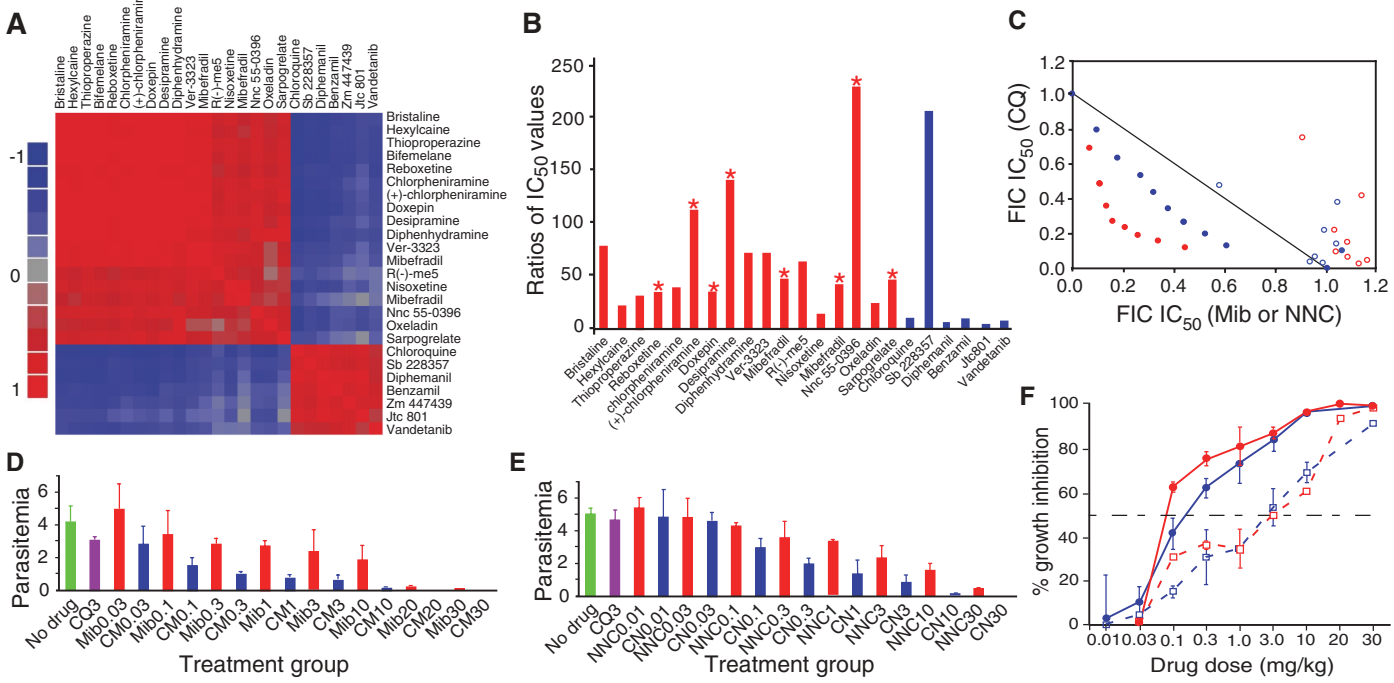

Fig. 3. Correlated responses among the progeny of the Dd2×HB3 cross and changes in parasite sensitivity against compounds mapped to *pfcrt* in the presence of low-dose chloroquine in vitro and in vivo. CQ, chloroquine; Mib, mibefradil; NNC, NNC55-0396. (**A**) Positive and negative correlations of compounds mapped to *pfcrt*; the color bar indicates correlation coefficient values from 1 (100% positive correlation, red) to −1 (100% negative correlation, blue). (**B**) Ratios of IC_{50} values from drug treatments with or without IC_{15} level of CQ for the Dd2 line. Red and blue bars indicate ratios for compounds whose responses are negatively and positively correlated with CQ, respectively. *Compounds with IC_{50} < 100 nM in the presence of IC_{15} levels of CQ. (**C**) Isobolograms showing synergistic interactions of CQ with NNC (blue dots) or Mib (red dots) in the CQ-resistant Dd2, but slightly antagonistic in the CQ-sensitive HB3 parasite (red circles, Mib; blue circles, NNC); FIC IC_{50}, fractional inhibitory concentration. Shown are representative plots of two independent experiments for each combination. (**D** and **E**) Mean parasitemia and standard deviations from groups of three to six mice treated with various doses of Mib (D) or NNC (E) in the presence (blue bars) or absence (red bars) of 3 mg/kg CQ. CQ3, 3 mg/kg CQ. The numbers after Mib (NNC) are Mib (NNC) doses in mg/kg, and the numbers after CM (CN) are Mib (NNC) doses plus 3 mg/kg CQ. (**F**) Plots of mean growth inhibition with standard errors from (D) and (E) to estimate IC_{50} values; blue lines, NNC; red lines, Mib; dashed lines, without CQ; solid lines, with 3 mg/kg CQ.

may allow the development of novel combination therapies to help overcome CQ resistance. Mibefradil, NNC55-0396 [(1*S*,2*S*)-2-(2-(N-[(3-Benzimidazol-2-yl)propyl]-N-methylamino)ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl cyclopropanecarboxylate dihydrochloride], and other compounds whose response profiles negatively correlated with CQ (Fig. 3A and tables S5, S6, and S7) could be combined with CQ for treating both CQ-resistant and CQ-sensitive parasites. Indeed, when tested with a low concentration of CQ (IC_{15}) in vitro, these combinations proved to be highly potent against the CQ-resistant line Dd2 (Fig. 3B and table S14). Among the 23 compounds that mapped to the *pfcrt* locus, 17 showed reduced IC_{50} values (by a factor of 21 to 229) in the presence of a low dose of CQ (IC_{15}), including six with IC_{50} values that dropped to below 100 nM. Such combinations offer a promising approach for treating CQ-resistant parasites and could prevent new resistance mutations because of structural and functional constraints on PfCRT. Similar strategies can be used to develop combinations targeting PfMDR1.

To further investigate the interactions of compounds negatively associated with CQ, we performed isobologram analysis of the combinations of CQ with either mibefradil or NNC55-0396 against the CQ-resistant line Dd2 and the CQ-sensitive line HB3. The actions of mibefradil and NNC55-0396 (both T- and L-type Ca^{++} channel blockers) were synergistic with that of CQ against Dd2 (Fig. 3C). Verapamil, an L-type Ca^{++} channel blocker and a known CQ resistance–reversal agent (*36*), is synergistic with CQ in Dd2, but antagonistic in HB3 (fig. S3). However, the IC_{50} of verapamil against Dd2 is three to four times that of mibefradil and NNC55-0396 (1.3 μM for verapamil versus ~200 nM for NNC55-0396 and ~300 nM for mibefradil). These results suggest that either mibefradil or NNC55-0396 may be more effective than verapamil in combination with CQ in treating CQ-resistant parasites.

We also tested these combinations against a CQ-resistant strain of *Plasmodium chabaudi* in vivo and showed that inclusion of a low dose of CQ (3 mg CQ per kg of body weight) with NNC55-0396 or mibefradil greatly increased the effect of these drugs and led to a reduction of IC_{50} by a factor of ~15 and ~40 in the treatment concentrations of NNC55-0396 and mibefradil, respectively (Fig. 3, D to F). Mibefradil is rapidly absorbed in vivo, reaches peak plasma concentration within 1 to 2 hours, and has an elimination half-life of 17 to 25 hours (*39*) (SOM), which indicates that this drug may have a suitable pharmacokinetic profile for combination treatments. The results are consistent with the in vitro data from *P. falciparum* and suggest that the *P. chabaudi* homolog of *pfcrt* may be involved in CQ resistance in this rodent model but requires further investigation.

Our analyses show that the majority of differential sensitivity of current *P. falciparum* populations to many compounds is linked to mutations in *pfdhfr*, *pfmdr1*, or *pfcrt*, which suggests that the number of parasite genes that contribute to drug responses may be limited. Analyses of DCPs and genome-wide MS and SNP genotypes from the progeny of two genetic crosses and field isolates identified many compounds interacting with wild-type or mutant *pfcrt* or *pfmdr1*, which were confirmed using genetically modified parasites and drug combinations. In addition to the 32 highly potent pan-active compounds, the compounds active against wild-type or mutant forms of PfCRT or PfMDR1 offer novel strategies for antimalarial therapies, and the loci and genes associated with drug responses provide a genetic basis to better delineate the nature of drug resistance in malaria.

References and Notes

1. N. J. White, *Science* **320**, 330 (2008).
2. N. J. White, P. L. Olliaro, *Parasitol. Today* **12**, 399 (1996).
3. H. Noedl *et al.*, *N. Engl. J. Med.* **359**, 2619 (2008).
4. M. Bonnet *et al.*, *Malar. J.* **8**, 192 (2009).
5. A. M. Dondorp *et al.*, *N. Engl. J. Med.* **361**, 455 (2009).
6. C. R. Chong, X. Chen, L. Shi, J. O. Liu, D. J. Sullivan Jr., *Nat. Chem. Biol.* **2**, 415 (2006).
7. J. L. Weisman *et al.*, *Chem. Biol. Drug Des.* **67**, 409 (2006).
8. N. Kato *et al.*, *Nat. Chem. Biol.* **4**, 347 (2008).
9. J. Yuan *et al.*, *Nat. Chem. Biol.* **5**, 765 (2009).
10. W. A. Guiguemde *et al.*, *Nature* **465**, 311 (2010).
11. F. J. Gamo *et al.*, *Nature* **465**, 305 (2010).
12. M. Rottmann *et al.*, *Science* **329**, 1175 (2010).
13. R. Huang *et al.*, *Sci. Transl. Med.* **3**, ps16 (2011).
14. J. Inglese *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 11473 (2006).
15. L. K. Basco, J. Le Bras, *Am. J. Trop. Med. Hyg.* **49**, 301 (1993).
16. A. Brockman *et al.*, *Trans. R. Soc. Trop. Med. Hyg.* **94**, 537 (2000).
17. J. Mu *et al.*, *Nat. Genet.* **42**, 268 (2010).
18. J. Seed *et al.*, *Crit. Rev. Toxicol.* **35**, 663 (2005).
19. R. Huang *et al.*, *Chem. Res. Toxicol.* **21**, 659 (2008).
20. P. K. Rathod, T. McErlean, P. C. Lee, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9389 (1997).
21. J. C. Wootton *et al.*, *Nature* **418**, 320 (2002).
22. M. Randić, *J. Chem. Inf. Comput. Sci.* **37**, 672 (1997).
23. D. A. Fidock *et al.*, *Mol. Cell* **6**, 861 (2000).
24. M. T. Ferdig *et al.*, *Mol. Microbiol.* **52**, 985 (2004).
25. J. M. Sá *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 18883 (2009).
26. B. Lell, P. G. Kremsner, *Antimicrob. Agents Chemother.* **46**, 2315 (2002).
27. O. J. Kazzim, O. M. Adegbolagun, O. Osho, C. I. Anumudu, *Ann. Trop. Med. Parasitol.* **100**, 579 (2006).
28. P. Gershon, R. E. Howells, *Trans. R. Soc. Trop. Med. Hyg.* **80**, 753 (1986).
29. M. Mungthin *et al.*, *Am. J. Trop. Med. Hyg.* **83**, 1005 (2010).
30. S. J. Foote *et al.*, *Nature* **345**, 255 (1990).
31. A. F. Cowman, S. Karcz, D. Galatis, J. G. Culvenor, *J. Cell Biol.* **113**, 1033 (1991).
32. D. E. Kyle, W. K. Milhous, R. N. Rossan, *Am. J. Trop. Med. Hyg.* **48**, 126 (1993).
33. A. Sowunmi, *Ann. Trop. Med. Parasitol.* **97**, 103 (2003).
34. N. Singh, S. K. Puri, *Acta Trop.* **77**, 185 (2000).
35. S. J. Burgess *et al.*, *J. Med. Chem.* **53**, 6477 (2010).
36. J. J. Patel *et al.*, *Mol. Microbiol.* **78**, 770 (2010).
37. T. E. Wellems, *Nat. Med.* **10**, 1169 (2004).
38. K. Hayton, X. Z. Su, *Curr. Genet.* **54**, 223 (2008).
39. S. J. Billups, B. L. Carter, *Ann. Pharmacother.* **32**, 659 (1998).

Acknowledgments: We thank P. Shinn and D. Van Leer for compound management, J. Wichterman for assay assistance, R. Eastman and C. O'Brien for comments on the manuscript, and NIAID intramural editor B. R. Marshall for assistance. The raw data from our chemical screening has been deposited at PubChem with accession no. 504749 (http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=504749). Because all authors except D.A.F. are government employees and this is a government work, the work is in the public domain in the United States. Notwithstanding any other agreements, the NIH reserves the right to provide the work to PubMedCentral for display and use by the public, and PubMedCentral may tag or modify the work consistent with its customary practices. Rights outside of the United States can be established subject to a government use license. **Funding:** This work was supported by the Intramural Research Program of the Division of Intramural Research at the National Institute of Allergy and Infectious Diseases (NIAID), the National Human Genome Research Institute, and the Director's Challenge Award Program, all at NIH. Funding for the studies from D.A.F. was provided by R01 AI50234 and the Burroughs Wellcome Fund. **Author contributions:** J.Y. performed quantitative high-throughput screening, parasite culture, and data analysis; K.C.-C.C. and R.G. carried out quantitative high-throughput screening and data analysis; R.L.J. and R.H. performed data analysis and writing; S.P. performed in vivo tests of drug combinations; A.L. performed isobologram analyses; R.G. performed the chemical library; D.A.F. transfected parasites and assisted with writing; T.E.W. provided progeny and writing; C.P.A. and J.I. carried out project planning, support, and writing; and X.-z.S. provided project conception, data analysis, and writing. **Competing interests:** The authors declare that they do not have any competing financial interests.

Supporting Online Material

www.sciencemag.org/cgi/content/full/333/6043/724/DC1
Materials and Methods
SOM Text
Figs S1 to S3
Tables S1 to S16
References

7 March 2011; accepted 13 June 2011
10.1126/science.1205216

REPORTS

Nonreciprocal Light Propagation in a Silicon Photonic Circuit

Liang Feng,[1,2,4]*† Maurice Ayache,[3]* Jingqing Huang,[1,4]* Ye-Long Xu,[2] Ming-Hui Lu,[2] Yan-Feng Chen,[2]† Yeshaiahu Fainman,[3] Axel Scherer[1,4]†

Optical communications and computing require on-chip nonreciprocal light propagation to isolate and stabilize different chip-scale optical components. We have designed and fabricated a metallic-silicon waveguide system in which the optical potential is modulated along the length of the waveguide such that nonreciprocal light propagation is obtained on a silicon photonic chip. Nonreciprocal light transport and one-way photonic mode conversion are demonstrated at the wavelength of 1.55 micrometers in both simulations and experiments. Our system is compatible with conventional complementary metal-oxide-semiconductor processing, providing a way to chip-scale optical isolators for optical communications and computing.

An example of nonreciprocal physical response, associated with the breaking of time-reversal symmetry, is the electrical diode (*1*). Stimulated by the vast application of this one-way propagation of electric current, considerable effort has been dedicated to the study of nonreciprocal propagation of light. The breaking of time-reversal symmetry of light is typically achieved with magneto-optical materials that introduce a set of antisymmetric off-diagonal dielectric tensor elements (*2*–*4*) or by involving nonlinear optical activities (*5*, *6*). However, practical applications of these approaches are limited for the rapidly growing field of silicon (Si) photonics because of their incompatibility with conventional complementary metal-oxide-semiconductor (CMOS) processing. Si optical chips have demonstrated integrated capabilities of generating (*7*–*11*), modulating (*12*), processing (*13*) and detecting (*14*) light signals for next-generation optical communications but require on-chip nonreciprocal light propagation to enable optical isolation in Si photonics.

Parity-time (PT) symmetry is crucial in quantum mechanics. In contrast to conventional quantum mechanics, it has been proposed that non-Hermitian Hamiltonians where $\hat{H}^{+} \neq \hat{H}$ can still have an entirely real spectrum with respect to the PT symmetry (*15*, *16*). Due to the equivalence between the Schrödinger equation in quantum mechanics and the wave equation in optics, PT symmetry has been studied in the realm of optics with non-Hermitian optical potentials (*17*–*19*). The breaking of PT symmetry has recently been experimentally observed, showing asymmetric characteristics transverse to light propagation above the PT threshold (*20*, *21*). Here, we have designed a Si waveguide integrated with complex optical potentials that have a thresholdless broken PT symmetry along the direction of light propagation, thus creating on-chip nonreciprocal light propagation.

On a Si-on-insulator (SOI) platform, the designed two-mode Si waveguide is 200 nm thick and 800 nm wide, allowing a fundamental symmetric quasi-TE mode with a wave vector of $k_1 = 2.59k_0$ and a higher-order antisymmetric mode with a wave vector of $k_2 = 2k_0$ at the wavelength of 1.55 μm. Periodically arranged optical potentials are implemented in the Si waveguide and occupy half of the waveguide width in the x direction (Fig. 1A). The optical potentials have a complex modulation in their

[1]Department of Electrical Engineering, California Institute of Technology, Pasadena, CA 91125, USA. [2]Nanjing National Laboratory of Microstructures, Nanjing University, Nanjing, Jiangsu 210093, China. [3]Department of Electrical and Computer Engineering, University of California, San Diego, La Jolla, CA 92093, USA. [4]Kavli Nanoscience Institute, California Institute of Technology, Pasadena, CA 91125, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: lfeng@caltech.edu (L.F.); yfchen@nju.edu.cn (Y.F.C.); etcher@caltech.edu. (A.S.)