Supplementary Table S1. Spectral and biophysical properties of selected GECIs.

The parameters listed are peak absorbance wavelength (λabs), extinction coefficient (EC), peak emission wavelength (λem), quantum yield (QY), brightness (the product of EC and QY), dissociation constant (*K*d), Hill coefficient (nH), *in vitro* Ca2+-dependent responses (∆*R*/*R*0 for ratiometric-pericam, GEM-GECO1, GEX-GECO1, and REX-GECO1; ∆*F*/*F*0 for others; negative numbers indicate inverse indicators), fluorescence response for a single action potential, and apparent acid dissociation constant (p*K*a). Some parameters are provided for both the Ca2+-free and Ca2+-bound states. NR, not reported.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| name | Ca2+ | λabs (nm) | EC (M-1 cm-1) | λem (nm) | QY | Brightness (mM-1cm-1) | *K*d (nM) | nH | *in vitro* ∆*F*/*F*0 or ∆*R*/*R*0[[1]](#footnote-1) | ∆*F*/*F*0 for 1 AP | p*K*a | References |
| Camgaroo | - | 400/490 | NR | ~520 | NR | NR | 7000 | 1.6 | 6 | NR | 10.1 | (Baird et al., 1999, 199) |
| + | NR | NR | NR | 8.9 |
| Flash-pericam | - | 403 | 26800 | 514 | 0.04 | 1.07 | 700 | 0.7 | 7 | NR | NR | (Nagai et al., 2001) |
| 488 | 6300 | NR | NR | NR |
| + | 410 | 21200 | 514 | 0.2 | 4.24 | NR |
| 494 | 16900 | NR | NR | NR |
| Ratiometric-pericam | - | 418 | 24100 | 511 | 0.3 | 7.23 | 1700 | 1.1 | 9 | NR | NR | (Nagai et al., 2001) |
| 494 | 4100 | NR | NR | NR |
| + | 415 | 20500 | 517 | 0.18 | 3.69 | NR |
| 494 | 10300 | NR | NR | NR |
| Inverse-pericam | - | 503 | 59000 | 515 | 0.64 | 37.76 | 200 | 1 | -0.49 | NR | NR | (Nagai et al., 2001) |
| + | 490 | 44000 | 513 | 0.44 | 19.36 | NR |
| G-CaMP | − | 409 | 1100 | NR | NR | NR | 235 | 3.3 | 3.5 | NR | 8.1 | (Nakai et al., 2001) |
| 488 | 570 | 510 | 0.03 | 0.02 |
| + | 410 | 690 | NR | NR | NR | 7.1 |
| 487 | 1400 | 510 | 0.05 | 0.07 |
| G-CaMP1.6 | − | 404 | 5800 | NR | NR | NR | 146 | 3.8 | 3.9 | NR | 8.8 | (Ohkura et al., 2005) |
| 489 | 1100 | 510 | 0.56 | 0.62 |
| + | 403 | 5200 | NR | NR | NR | 8.2 |
| 488 | 3800 | 509 | 0.79 | 3.00 |
| G-CaMP2 | − | 400 | 11100 | NR | NR | NR | 146 | NR | ~4 | NR | NR | (Tallini et al., 2006) |
| 491 | 5200 | 511 | 0.7 | 3.64 |
| + | 401 | 5800 | NR | NR | NR | NR |
| 487 | 19000 | 508 | 0.93 | 17.67 |
| GCaMP3 | - | 399 | 36000 | NR | NR | NR | 405 | 2.1 | 11.3 | 0.20 | 8.4 | (Tian et al., 2009; Dana et al., 2019) |
| 496 | 11000 | 513 | 0.2 | 2.20 |
| + | 399 | 20000 | NR | NR | NR | 7.0 |
| 496 | 50000 | 513 | 0.44 | 22.00 |
| GCaMP5A | - | NR | NR | NR | NR | NR | 307 | 2.7 | 16.4 | NR | 8.7 | (Akerboom et al., 2012) |
| + | 497 | 56100 | NR | 0.65 | 36.47 | 6.8 |
| GCaMP5D | - | NR | NR | NR | NR | NR | 730 | 2.5 | 21.0 | NR | 8.9 | (Akerboom et al., 2012) |
| + | 497 | 25300 | NR | 0.67 | 16.95 | 7.4 |
| GCaMP5G | - | NR | NR | NR | NR | NR | 460 | 2.5 | 31.7 | 0.37 | 9.1 | (Akerboom et al., 2012; Dana et al., 2019) |
| + | 497 | 49300 | NR | 0.67 | 33.03 | 7.0 |
| G-GECO1.2 | - | 402 | 37000 | NR | NR | NR | 1150 | 2.1 | 22.0 | NR | 10.4 | (Zhao et al., 2011) |
| 498 | 2000 | 513 | 0.25 | 0.50 |
| + | 402 | 22000 | NR | NR | NR | 7.2 |
| 498 | 33000 | 513 | 0.36 | 11.90 |
| GEM-GECO1 | - | 397 | 34000 | 511 | 0.31 | 10.20 | 340 | 2.9 | 109.0 | NR | 6.2 | (Zhao et al., 2011) |
| + | 390 | 36000 | 455 | 0.18 | 6.50 |
| GEX-GECO1 | - | 392 | 32000 | NR | NR | NR | 318 | 2.8 | 25.0 | 1.21 | 6.7 | (Zhao et al., 2011; Dana et al., 2019) |
| 482 | 0 | 512 | 0.21 | 6.70 |
| + | 390 | 32000 | 506 | 0.19 | 6.08 | 6.1 |
| 482 | 2500 | 506 | 0.39 | 0.98 |
| B-GECO0.1 | - | 378 | 22000 | 446 | 0.02 | 0.44 | 164 | 2.6 | 6.0 | NR | 5.0 | (Zhao et al., 2011) |
| + | 378 | 23000 | 446 | 0.18 | 4.10 | 5.6 |
| GCaMP6s | - | 505 | 2910 | NR | NR | NR | 144 | 2.9 | 62.2 | 0.23 | 9.8 | (Chen et al., 2013) |
| + | 497 | 68500 | 515 | 0.61 | 41.79 | 6.2 |
| GCaMP6m | - | 505 | 20700 | NR | NR | NR | 167 | 3.0 | 37.1 | 0.13 | 8.7 | (Chen et al., 2013) |
| + | 497 | 38200 | 515 | 0.61 | 23.30 | 6.9 |
| GCaMP6f | - | 505 | 2800 | NR | NR | 1.65 | 375 | 2.3 | 50.8 | 0.19 | 8.8 | (Chen et al., 2013) |
| + | 497 | 61900 | 515 | 0.59 | 36.52 | 6.3 |
| jGCaMP7s | - | NR | 5554 | NR | 0.58 | 3.22 | 68 | 2.5 | 39.4 | 0.657 | 7.7 | (Dana et al., 2019) |
| + | NR | 70117 | NR | 0.65 | 45.58 | 6.4 |
| jGCaMP7f | - | NR | 2358 | NR | 0.47 | 1.11 | 174 | 2.3 | 29.2 | 0.316 | 7.9 | (Dana et al., 2019) |
| + | NR | 56028 | NR | 0.59 | 33.06 | 6.5 |
| jGCaMP7b | - | NR | 5668 | NR | 0.59 | 3.34 | 82 | 3.1 | 21.1 | 0.406 | 7.8 | (Dana et al., 2019) |
| + | NR | 56562 | NR | 0.6 | 33.94 | 6.4 |
| jGCaMP7c | - | NR | 1541 | NR | 0.5 | 0.77 | 298 | 2.4 | 144.6 | 0.223 | 8.7 | (Dana et al., 2019) |
| + | NR | 49566 | NR | 0.59 | 29.24 | 6.7 |
| XCaMP-G | - | 398 | 50100 | 512 | 0.14 | 1.8 | 200 | 1.8 | 10.1 | 1.97 | 7.2 | (Inoue et al., 2019; Zhang et al., 2021) |
| 487 | 13200 |
| + | 399 | 36600 | 514 | 0.39 | 19.9 | 8.4 |
| 487 | 51400 |
| XCaMP-Gf | - | 401 | 97280 | 514 | 0.18 | 0.9 | 115 | 1.4 | 11.7 | 2.05 | 6.1 | (Inoue et al., 2019; Zhang et al., 2021) |
| 492 | 5220 |
| + | 394 | 82390 | 514 | 0.5 | 11.4 | 8.7 |
| 492 | 22530 |
| XCaMP-Y | - | 418 | 47300 | 525 | 0.11 | 1.7 | 81 | 1.5 | 6.2 | NR | 7.5 | (Inoue et al., 2019) |
| 498 | 16000 |
| + | 415 | 34500 | 527 | 0.26 | 12.3 | 9.4 |
| 503 | 47400 |
| jGCaMP8s | - | 496 | 2120 | 513 | NR | NR | 46 | 2.2 | 49.5 | NR | 7.7 | (Zhang et al., 2021) |
| + | 57000 | NR | NR | 6.5 |
| jGCaMP8f | - | 496 | 1930 | 512 | NR | NR | 334 | 2.1 | 78.8 | 3.1 | 7.7 | (Zhang et al., 2021) |
| + | 51000 | NR | NR | 6.7 |
| jGCaMP8m | - | 496 | 2250 | 512 | NR | NR | 108 | 1.9 | 45.7 | 5.0 | 7.4 | (Zhang et al., 2021) |
| + | 50000 | NR | NR | 6.7 |
| mNG-GECO1 | + | 496 | 102000 | 513 | 0.69 | 70.38 | 807 | NR | 44 | NR | NR | (Zarowny et al., 2020) |
| NCaMP7 | - | 402/406 | 46600 | 520 | 0.048 | 2.2368 | 96 | 2.2 | 88 | NR | 5.4/6.6 | (Subach et al., 2020) |
| + | 509/512 | 110000 | 522 | 0.52 | 57.2 | NR | 6.18 |
| R-GECO1 | - | 445 | 22000 | NR | NR | NR | 482 | 2.06 | 15.0 | NR | 8.9 | (Zhao et al., 2011) |
| 577 | 15000 | 600 | 0.06 | 0.72 |
| + | 445 | 9000 | NR | NR | NR | 6.6 |
| 561 | 51000 | 589 | 0.2 | 10.20 |
| R-GECO1.2 | - | 564 | 2800 | 595 | 0.16 | 0.45 | 1200 | 2.79 | 32.0 | NR | 8.9 | (Wu et al., 2013) |
| + | 556 | 52000 | 585 | 0.29 | 15.10 | 6.0 |
| O-GECO1 | - | 545 | 1400 | 570 | 0.07 | 0.10 | 1500 | 2.06 | 145.0 | NR | 9.4 | (Wu et al., 2013) |
| + | 543 | 65000 | 564 | 0.22 | 14.30 | 6.1 |
| CAR-GECO1 | - | 565 | 2500 | 620 | 0.11 | 0.28 | 490 | 2.01 | 26.0 | NR | 9.1 | (Wu et al., 2013) |
| + | 560 | 36000 | 609 | 0.21 | 7.60 | 5.7 |
| REX-GECO1 | - | 582 | 26000 | 600 | 0.06 | 1.60 | 240 | 1.8 | 99.0 | NR | 6.5 | (Wu et al., 2014) |
| + | 480 | 28000 | 585 | 0.23 | 6.40 |
| RCaMP1f | - | 574 | 17400 | 597 | 0.11 | 1.91 | 1900 | 2.80 | 11.3 | NR | 4.6 | (Akerboom et al., 2013) |
| + | 572 | 58900 | 592 | 0.48 | 28.27 | 5.3 |
| RCaMP1h | - | 575 | 18700 | 602 | 0.14 | 2.62 | 1300 | 2.50 | 9.0 | NR | 3.2/4.7/6.5 | (Akerboom et al., 2013) |
| + | 571 | 65100 | 594 | 0.51 | 33.20 | 4.9 |
| RCaMP2 | - | 445 | 28288 | NR | NR | NR | 69 | 1.2 | 3.8 | 0.09 | 8.7 | (Inoue et al., 2015) |
| 576 | 14200 | 591 | 0.11 | 1.59 |
| + | 445 | 16600 | NR | NR | NR | 6.1 |
| 563 | 48100 | 583 | 0.23 | 11.2 |
| jRCaMP1a | - | ~575 | 33800 | ~600 | NR | NR | 214 | 0.86 | 2.2 | 0.15 | 5.6 | (Dana et al., 2016) |
| + | ~570 | 54100 | ~595 | NR | NR | 6.4 |
| jRCaMP1b | - | ~575 | 25300 | ~600 | NR | NR | 712 | 1.6 | 6.2 | 0.09 | 6.4 | (Dana et al., 2016) |
| + | ~570 | 53400 | ~595 | NR | NR | 5.5 |
| jRGECO1a | - | ~580 | 61800 | ~600 | NR | NR | 148 | 1.9 | 10.6 | 0.3 | 8.6 | (Dana et al., 2016) |
| + | ~570 | 53300 | ~590 | NR | NR | 6.3 |
| XCaMP-R | - | 444 | 51600 | 598 | 0.14 | 3.3 | 97 | 1.1 | 5.6 | NR | 6.1 | (Inoue et al., 2019) |
| 574 | 37700 |
| + | 452 | 37900 | 593 | 0.28 | 21.7 | 8.7 |
| 561 | 76300 |
| K-GECO1 | - | 568 | 19000 | 594 | 0.12 | 2.28 | 165 | 1.12 | 11 | NR | NR | (Shen et al., 2018) |
| + | 565 | 61000 | 590 | 0.45 | 27.45 | NR |
| NIR-GECO1 | - | 678 | 62000 | 704 | 0.063 | 3.906 | 885 | 1.03 | -0.90 | -0.045 | 6.03 | (Qian et al., 2019) |
| + | 678 | 20000 | 704 | 0.019 | 0.38 | 4.68 |
| NIR-GECO2 | - | NR | 67000 | NR | 0.059 | 3.953 | 331 | 0.94 | -0.94 | -0.16 | 5.26 | (Qian et al., 2020) |
| + | NR | 18000 | NR | 0.014 | 0.252 | 4.85 |
| NIR-GECO2G | - | NR | 74000 | NR | 0.061 | 4.514 | 480 | 0.78 | -0.90 | -0.17 | 5.34 | (Qian et al., 2020) |
| + | NR | 21000 | NR | 0.021 | 0.441 | 4.84 |

Supplementary Table S2. Physical properties of selected small molecule-based Mg2+ indicators.

The parameters listed are peak absorbance wavelength (λabs), extinction coefficient (EC), peak emission wavelength (λem), and apparent dissociation constant (*K*d) for Mg2+ and Ca2+. Some parameters were provided at both Mg2+-free and Mg2+-bound states.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| name | Mg2+ | λabs (nm) | EC (M-1cm-1) | λem (nm) | *K*d for Mg2+ (mM) | *K*d for Ca2+ (µM) | References |
| mag-fura-2 | - | 369 | 22,000 | 511 | 1.5 | 53 | (Raju et al., 1989, 1; Meuwis et al., 1998) |
| + | 330 | 24,000 | 491 |
| mag-fura-5 | - | 369 | 23,000 | 505 | 2.3 | 28 | (Illner et al., 1992; Claflin et al., 1994) |
| + | 332 | 25,000 | 482 |
| mag-indo-1 | - | 349 | 38,000 | 480 | 2.7 | 35 | (Csernoch et al., 1998) |
| + | 330 | 33,000 | 417 |
| mag-fluo-4 | - | 490 | 74,000 | NR | 4.7 | 22 | (Zhao et al., 1996; Lee et al., 2009) |
| + | 493 | 75,000 | 517 |
| Magnesium Green | - | 506 | 77,000 | 531 | 1.0 | 6 | (Zhao et al., 1996; Shmigol et al., 2001) |
| + | 506 | 75,000 | 531 |
| + | 578 | 82,000 | 603 |
| KMG-104-AsH | - | 520 | NR | 540 | 1.7 | 100 | (Fujii et al., 2014) |
| + | 521 | NR | 540 |

Supplementary Table S3. Key properties of selected small molecule-based and genetically encodable pH indicators

The parameters listed are peak absorbance wavelength (λabs), extinction coefficient (EC), peak emission wavelength (λem), quantum yield (QY), isosbestic wavelength (λiso), dissociation constant (*K*d), Hill coefficient (nH), and *in vitro* fluorescent responses (∆*R*/*R*0 as (*R*basic-*R*acidic)/*R*acidic for r-pHluorin, where *R* is calculated as *F*475nm/*F*395nm, or ∆*F*/*F*0 as (*F*basic-*F*acidic)/*F*acidic for others). Some parameters are provided for both acidic and basic conditions.NR, not reported. NA, not applicable.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| name | pH | λabs (nm) | EC (M-1cm-1) | λem (nm) | QY | λiso (nm) | p*K*a | nH | ∆*F*/*F*0  or ∆*R*/*R*0[[2]](#footnote-2) | References |
| **Small molecule-based pH indicators** | | | | | | | | | | |
| BCECF | acidic | 470 | NR | NR | NR | 440 | 6.98 | NR | NR | (Rink et al., 1982) |
| 505 | NR | 535 | NR |
| basic | 470 | NR | NR | NR |
| 505 | NR | 520 | NR |
| SNARF-1 | acidic | 515 | 17700 | 583 | 0.03 | 521 | 7.62 | NR | NR | (Whitaker et al., 1991) |
| 544 | 21600 |
| basic | 573 | 44100 | 631 | 0.09 |
| SNARF-2 | acidic | 518 | 20500 | 584 | 0.03 | 530 | 7.43, 7.50 | NR | NR | (Whitaker et al., 1991) |
| 550 | 24400 |
| basic | 576 | 46400 | 633 | 0.16 |
| C.SNARF-1 | acidic | 518 | 23200 | 585 | 0.047 | 534 | 7.6 | NR | NR | (Whitaker et al., 1991) |
| 548 | 25700 |
| basic | 575 | 44900 | 637 | 0.09 |
| C.SNARF-2 | acidic | 516 | 26200 | 585 | 0.02 | 524 | 7.70, 7.78 | NR | NR | (Whitaker et al., 1991) |
| 550 | 29800 |
| basic | 577 | 51500 | 635 | 0.11 |
| **Genetically encodable pH indicators** | | | | | | | | | | |
| EGFP | NA | 480 | NR | 510 | NR | NR | 6.15 | 0.7 | NR | (Kneen et al., 1998) |
| EYFP | NA | 514 | NR | 527 | NR | NR | 7.1 | 1.1 | NR | (Llopis et al., 1998) |
| mNecterine | NA | 558 | NR | 578 | NR | NR | 6.9 | 0.78 | NR | (Johnson et al., 2009) |
| E2GFP | acidic | 424 | 31560 | 510 | 0.22 | NR | 7 | NR | NR | (Bizzarri et al., 2006) |
| basic | 401 | 28180 | 523 | 0.91 |  |
| 515 | 22400 | 527 | NR |  |
| dEGFP1 | acidic | 400 | 28700 | NR | NR | NR | 8.02 | NR | NR | (Hanson et al., 2002) |
| basic | 504 | 54800 | 516 | 0.49 |  |
| dEGFP2 | acidic | 398 | 21700 | NR | NR | NR | 7.25 | NR | NR | (Hanson et al., 2002) |
| basic | 496 | 38200 | 517 | 0.55 |  |
| dEGFP3 | acidic | 396 | 26700 | NR | NR | NR | 6.86 | NR | NR | (Hanson et al., 2002) |
| basic | 508 | 45900 | 518 | 0.57 |  |
| dEGFP4 | acidic | 400 | 26900 | NR | NR | NR | 7.37 | NR | NR | (Hanson et al., 2002) |
| basic | 509 | 50800 | 518 | 0.27 |  |
| r-pHluorin | NA | 395 | NR | 508 | NR | NR | NR | NR | -0.8 (pH 5.5–7.5) | (Miesenböck et al., 1998) |
| 475 | NR |  |
| e-pHluorin | NA | 395 | NR | 508 | NR | NR | NR | NR | 5 (pH 6–7.5) | (Miesenböck et al., 1998) |
| pHRed | NA | 440 | NR | 610 | NR | NR | 6.5 | NR | >9 | (Tantama et al., 2011) |
| pHTomato | NA | 550 | NR | 580 | NR | NR | 7.8 | NR | >2 (pH 7.5–9.8) | (Li and Tsien, 2012) |
| pHuji | NA | 556 | 31000 | 598 | 0.22 | NR | 7.7 | NR | 21 (pH 5.5–7.5) | (Shen et al., 2014; Liu et al., 2021) |
| SE-pHluorin | NA | 495 | 45000 | 512 | 0.52 | NR | 7.2 | 1.90 | 49 (pH 5.5–7.5) | (Sankaranarayanan et al., 2000; Shen et al., 2014; Liu et al., 2021) |
| pHoran1 | NA | 547 | NR | 564 | NR | NR | 6.7 | 0.87 | 9 (pH 5.5–7.5) | (Shen et al., 2014) |
| pHoran2 | NA | 549 | NR | 563 | NR | NR | 7.0 | 0.89 | 11 (pH 5.5–7.5) | (Shen et al., 2014) |
| pHoran3 | NA | 551 | NR | 566 | NR | NR | 7.4 | 0.87 | 14 (pH 5.5–7.5) | (Shen et al., 2014) |
| pHoran4 | NA | 547 | NR | 561 | NR | NR | 7.5 | 0.92 | 16 (pH 5.5–7.5) | (Shen et al., 2014) |
| pHmScarlet | NA | 562 | 85000 | 585 | 0.47 | NR | 7.4 | 1.1 | 26 (pH 5.5–7.5) | (Liu et al., 2021) |

Supplementary Table S4. Physical properties of genetically encodable Cl- indicators.

The parameters listed are peak absorbance wavelength (λabs), extinction coefficient (EC), peak emission wavelength (λem), quantum yield (QY), brightness (the product of EC and QY), dissociation constant (*K*d), Hill coefficient (nH), *in vitro* dynamic range (∆*R*/*R*0 for Clomeleon and SuperClomeleon, and ∆*F*/*F*0 for others; negative sign indicates inverse indicators), and acid dissociation constant (p*K*a). Some parameters were provided at both Cl--free and Cl--rich states.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| name | Cl- | λabs (nm) | EC (M-1cm-1) | λem (nm) | QY | brightness (mM-1cm-1) | *K*d (mM) | nH | ∆*F*/*F*0  or ∆*R*/*R*0[[3]](#footnote-3) | p*K*a | References |
| mNeonGreen | - | 400 | NR | NR | NR | NR | 9.8 | 0.72 | 20 | 5.7 | (Tutol et al., 2019a) |
| 505 | 10021 | 520 | 0.18 | 1.80 |
| + | 400 | NR | NR | NR | NR | 4.7 |
| 505 | 26884 | 520 | 0.4 | 10.75 |
| YFP | / | 514 | NR | 527 | NR | NR | 777 | NR | -0.4 | NR | (Wachter and James Remington, 1999) |
| Clomeleon | / | NR | NR | NR | NR | NR | 167 | NR | -0.8 | NR | (Kuner and Augustine, 2000, 200) |
| SuperClomeleon | / | NR | NR | NR | NR | NR | 8.1 | NR | -0.9 | NR | (Grimley et al., 2013) |
| YFP-H148Q | / | NR | 71000 | NR | 0.59 | NR | 100 | NR | -0.5 | 6.7 | (Jayaraman et al., 2000) |
| YFP-H148Q/I152L | / | NR | 52200 | NR | 0.6 | NR | 85 | NR | -0.5 | 6.92 | (Galietta et al., 2001) |
| phi-YFP | - | 480 | NR | 540 | 0.44 | NR | 384 | 0.9 | 2.5 | 4.9 | (Tutol et al., 2019b) |
| 400 | NR | 540 | 0.02 | NR |
| + | 480 | NR | 540 | 0.49 | NR | 5.4 |
| 400 | NR | 540 | 0.06 | NR |

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1. ∆*R*/*R*0for ratiometric-pericam, GEM-GECO1, GEX-GECO1, and REX-GECO1 [↑](#footnote-ref-1)
2. ∆*R*/*R*0 for r-pHluorin [↑](#footnote-ref-2)
3. ∆*R*/*R*0 for Clomeleon and SuperClomeleon [↑](#footnote-ref-3)